Photodetector Characteristics—Considerations for Multicolor Flow Cytometry

Background

Flow cytometry is a widely used technique that provides both qualitative and quantitative information about individual cells by detecting light scatter and fluorescence signals from cells stained with fluorescent markers. A key factor in the design of flow cytometers is careful consideration of the type of photodetector used, because accurate identification of dim signals from less abundant populations, or populations stained by fluorochromes that are lower in brightness, is a requirement in many flow cytometry applications. Detecting these cell populations is increasing in importance with the advent of high parameter flow cytometry and single cell multi-omics analyses for the discovery of biological mechanisms, enabling effective study of disease conditions and development of lifesaving therapeutics.

In this article we shall describe the factors that are important for optimal photodetection and review the types of photodetector devices commonly used in flow cytometry instrumentation. We shall also consider which detection device provides the best results for obtaining the highest data quality and reproducibility.

Variables associated with photodetection in flow cytometers

The main variables that define a photodetector's ability to clearly resolve fluorescent populations are sensitivity, signal-to-noise ratio, dynamic range, and linearity.

Detection **sensitivity** is the ability of a photodetector to convert incident light into a usable signal that is easily resolvable from other signals (pulses coming from each interrogated event). The sensitivity of a photodetector depends on its **quantum efficiency** and **intrinsic gain**. **Quantum efficiency** is the probability that a photoelectron will be generated from a single photon, and is a function of wavelength, because the energy of a photon is inversely proportional to its wavelength. **Intrinsic gain** is how much electrical signal can be generated from a photoelectron when a voltage is applied to a detector. Amplification of the photoelectrons using an external circuit may be required if the incident light is very low.

When measuring a dark sample, quantum effects cause the intensity of the detected signal to fluctuate according to a Poisson distribution. This is called shot noise. To achieve stable micro-output, it is important to know how much light emitted from the sample enters the detector. Knowing how efficiently light entering the photodetector can be converted into an electrical signal and amplified without increasing noise determines the effectiveness of detection sensitivity.

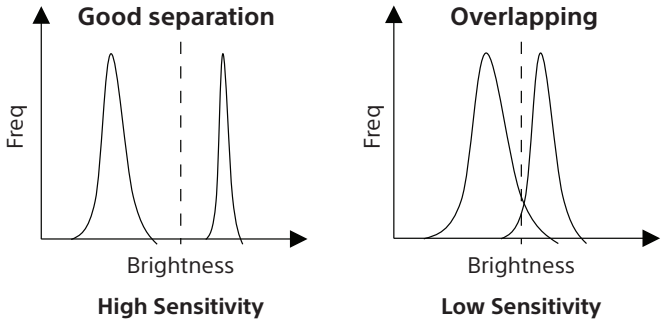

Figure 1. When a detector has good sensitivity (high quantum efficiency and intrinsic gain), two individual pulses or events can be easily resolved.

Signal-to-noise ratio is the ability of a photodetector to amplify the intended signal without also producing excess noise. Since the amount of incident light received by a photodetector is typically very small, the incoming signal must be amplified to produce enough signal output for detection. This is referred to as the **intrinsic gain**.

Dynamic range is the ratio of the maximum and minimum light levels that can be measured. If the dynamic range is large, samples with different signal intensities can be easily measured without the dim signals being buried in noise and the bright signals being saturated. In a large percentage of flow cytometry experiments, a four-log scale is usually required .

Linearity is a simple linear regression calculation across the dynamic range of the detector. If the range is linear, the correlation coefficient R^2 will be greater than 99%.

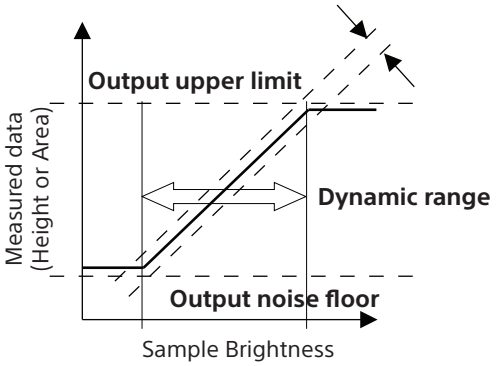

Figure 2. Relationship between the linearity and dynamic range of a photodetector. For a detector to have good linearity, it must have a linear regression R^2 of greater than 99% across its dynamic range.

Evaluating types of photodetectors for fluorescent light detection in flow cytometry experiments

The types of photodetectors commonly used in flow cytometry systems are photomultiplier tubes (PMTs), avalanche photodiodes (APDs), and photodiodes (PDs). PDs are typically used for forward scatter detection, so for the purpose of evaluating fluorescence signal detection, we will focus on and highlight the differences between PMTs and APDs.

Sensitivity

The main differences between PMTs and APDs are in the areas of **quantum efficiency** and **intrinsic gain**. The **quantum efficiency** of PMTs is about 10%–30%, while the intrinsic gain can be as large as 10^6. This produces a maximum of 100,000 to 300,000 electrons for a single incident photon. PMTs also have a high **signal-to-noise ratio**, which allows for large output without increasing noise when the detected light level is very low or dim.

In general, PMTs are more sensitive at shorter wavelengths, and APDs are more sensitive at longer wavelengths. Wavelength detection sensitivity characteristics of PMTs vary depending on the material used to produce the photocathodes, which convert photons into the electrical signal. Most PMTs with a bi-alkali photocathode deliver the highest sensitivity in the range of 300–600 nm. PMTs with multi-alkali photocathodes can maintain sensitivity up to the near-infrared region, about 800 nm. For even longer wavelengths above 900 nm, specialized PMTs with InGaAs or GaAs photocathodes can be used to capture infrared signals.

While APDs have a **high quantum efficiency** of about 90%, their **intrinsic gain** is only about 10^2. This produces a maximum of 90 electrons for a single incident photon. APDs have a low signal-to-noise ratio, which means in a low light setting the noise is also amplified with an external circuit to raise the signal level.

Noise

There are several types of noise that affect photodetectors.

- One type of noise is caused by gain variation. Increasing the gain of a detector also increases the noise. The amount of noise produced is dependent on the signal-to-noise ratio.
- A second type of noise is shot Poisson noise, which can come from the signal itself or from dark current that resides within the photodetector. Shot noise from the signal is most evident due to dispersion in the signal when the detected light level is low.

APDs with higher quantum efficiency can overcome the effect of noise for certain light levels. However, as the gain of APD is modulated to detect even lower light levels, the noise is increased, and the signal cannot be differentiated from noise. PMTs have improved capability for detection in a low light setting because the signal can be amplified without increasing the noise. In both PMTs and APDs, as the temperature rises, the dark current increases and the intrinsic gain of the photodetector changes. With PMTs, the change in intrinsic gain is limited. With APDs, the gain may drop significantly with a 10°C rise in temperature, which can affect experimental results. To avoid this, it is critical to control the temperature to mitigate the dark current shot noise

Dynamic range

Since a variety of signal intensities must be measured in flow cytometry, a photodetector needs to be able to accurately detect photons from both bright and dim fluorescent markers. When the light intensity is high, the signal can become saturated, which affects the linearity of the detector. This issue can be resolved by setting the gain of the detector to the appropriate level for the incident light. Adjusting the gain maintains the linearity while maximizing the dynamic range and signal-to-noise ratio of the detector. The dynamic range for PMTs and APDs is greater than four logs. A PMT's gain can be increased with minimal effect on the signal-to-noise ratio, maintaining a larger dynamic range. However, this is not the case with APDs, to which only so much gain can be applied

before the noise overcomes the signal, shrinking the useful dynamic range of the detector.

Standardization in photodetector selection

Due to variations in the characteristics of excitation sources and detection optics in flow cytometers, when a sample is measured across multiple instruments, the output signal will differ, even if the same detector type and same instrument setup are used. The factors that cause the variation need to be managed through system standardization to ensure that results remain the same over time and between instruments.

For both PMTs and APDs, the number of amplified electrons can vary between instruments, even if the applied gain or voltage remains constant. When a sample with the same fluorescence intensity is measured with Detector A and Detector B, the relationship between the gain settings can be defined using a gold standard fluorescent particle during a QC process and then corrected so that there is minimal difference between device output for an incoming signal.

Sony Biotechnology has developed a process to perform this type of standardization through a daily quality control process that uses AlignCheck beads to allow Sony spectral cell analyzers to be run in a standardization mode. This mode uses a correlation coefficient to define the relationship between the signal intensity and PMT voltage for each detector. In the standardization mode, the signal amplification is performed using a standard value (ST) instead of a percent voltage to standardize the system across detectors. Because a PMT's intrinsic gain is very large at about 10^6, it is wide enough to cover the correction to standardize each detector and still detect the vast range of signal intensities common in flow cytometry experiments. The intrinsic gain of APDs is limited at about 10^2. Due to this limitation, it is difficult to standardize the signal output of APDs.

In summary, PMTs may be expensive to produce and may be larger in size than APD or multi-pixel APD (SPM), and PMTs may also show variation in performance characteristics. But PMTs have specific and unique characteristics that other photodetectors cannot overcome in terms of large gain scalability, which make them valuable for multicolor experiments. PMT leads the field in delivering higher level performance and potential for enabling inter-instrument standardization.

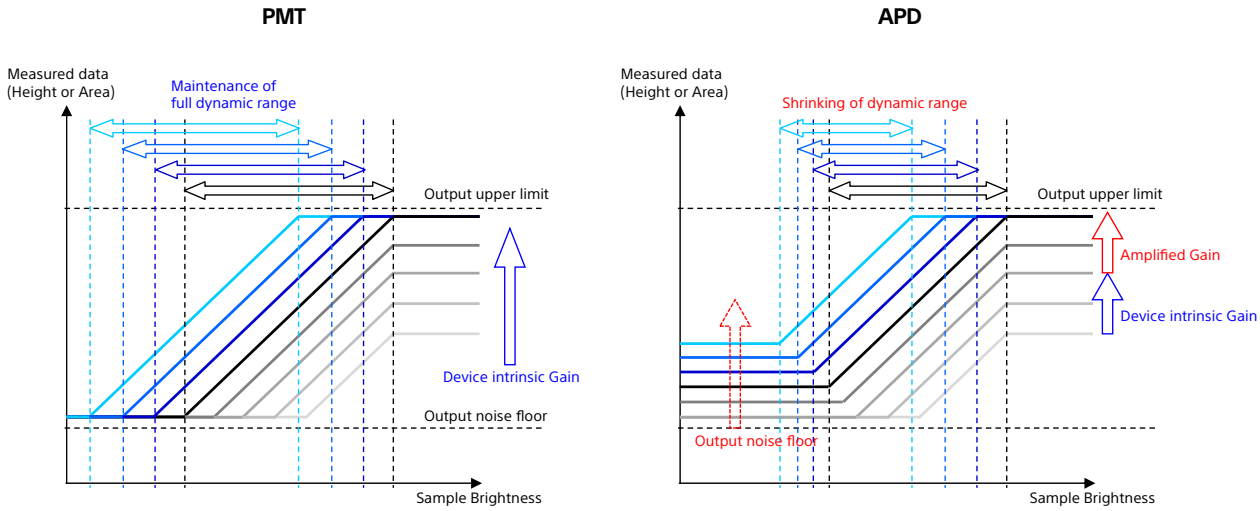

Figure 3. When detecting variable light levels, the gain of a photodetector must be modulated to prevent signals with high intensity from saturating the detector. PMTs have a high intrinsic gain and a high signal-to-noise ratio, so that gains can be modulated to account for signal intensity variance without limiting the dynamic range of the detector. This, however, is not the case for APDs. As gains are modified, the noise is amplified, which shrinks the useful dynamic range.

7.02.052621.0