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optimal use of photomultipliers for
chemiluminescence and bioluminescence
applications

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abstract

Photomultipliers are the preferred detectors for quantifying weak or short-lived light emissions. The quality of signal recovery has an upper limit imposed by the statistical nature of light detection. It is shown that the photon counting method can provide performance close to ideal, whereas performance in the current measuring method is degraded by noise in the multiplier gain process and by the nature of the dark current. Performance variations because of changes in temperature, shock, vibration and magnetic field effects are significantly reduced when using the photon counting method. The effects of dead-time can be corrected making photon counting superior to current measuring with regard to dynamic range. The benefits of using photon counting packages are presented.

1 introduction

The photon counting method for making low light level measurements has been known for over fifty years. Astronomers were amongst the first to realise the power of the technique for detecting and quantifying faint objects. The paper of Rodman and Smith¹ in 1963 is typical of literature at that time when the primary concern was one of how to select photomultipliers from manufacturer's general production suitable for photon counting, since even the manufacturers had a poor grasp of the subject at that time.

Over the period 1965 to 1969 some classic papers covering the statistics of signal recovery in both the current measuring and photon counting methods were presented²⁻⁴, not without a measure of controversy. The statistical methods used in the present publication and the theoretical arguments in favour of photon counting are discussed in the above publications as are sources of background, correlated pulses and the benefits of cooling.

The review papers of Poultney⁵ in 1972 and Candy⁶ in 1985 provide a useful bibliography on many aspects of photon counting. The purpose of this paper is to highlight the present capability of photon counting photomultipliers with regard to the needs of instrument manufacturers - which are different from those of the research environment. Stability and quality of performance close to theoretical predictions can be realised with the proper choice of photomultiplier and operating condition.

2 choice of photocathode

Bioluminescence and chemiluminescence emissions lie typically within the range 350 – 700 nm. Aequorin for example peaks at 470 nm while firefly luciferase is characterised by a peak emission at about 565 nm. There is a further complication in that the emission spectrum may be sensitive to pH⁷ and undoubtedly to temperature. Since photocathodes do not have a flat spectral response, as illustrated in **figure 1**, measured count rates will be sensitive to any changes in the light emission spectrum.

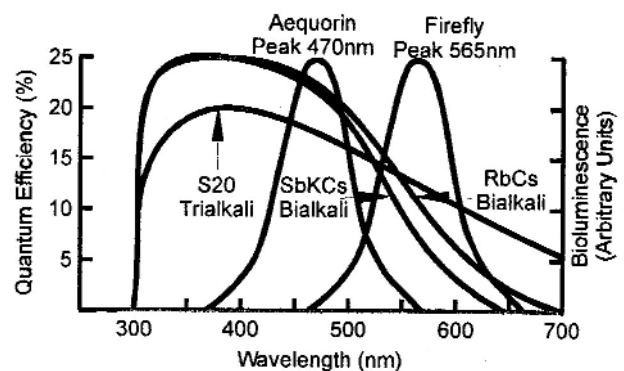


figure 1 photocathode spectral response characteristics and two emission spectra common in bioluminescence.

The most appropriate photocathode for a stated application is arrived at by folding the photocathode response with the light emission spectrum and noting that the area under the resulting curve is directly proportional to the expected response. This will indicate which photocathode will provide the maximum sensitivity in terms of counts per second. However, other considerations, such as cost (photomultipliers with S20 cathodes tend to cost 2-3 times those with bialkali cathodes) and dark counts, discussed later in this paper, also need to be considered.

3 statistical considerations in the detection of light

3.1 noise in signal

Light is detected by photosensitive devices through the generation of photocurrent. This is a noisy process because light and charge are quantised. The majority of light emission processes of commercial interest can be described by Poisson statistics. Assuming this, consider a steady light flux incident on a photocathode producing m photoelectrons per second. Over any period of one second we expect on average m photoelectrons with a standard deviation of $m^{1/2}$. The signal-to-noise ratio is:

$$S/N = m/m^{1/2} = m^{1/2} \quad \dots(1)$$

Individual photoelectron events can be counted with a detector of sufficient gain but the precision of any measurement can never be better than the limit imposed by equation 1.

Alternatively, the photocurrent can be taken as a measure of the incident light signal. The noise associated with the photocurrent I_k , taking the system bandwidth, B , into account is given by the shot noise formula:

$$S/N = I_k/(2eI_kB)^{1/2} \quad \dots(2)$$

where e is the electronic charge. The forms of equations 1 and 2 are similar since they refer to the same phenomenon and naturally they predict the same result. In equation 1 the signal is treated in a digital way and in equation 2 it is taken as a continuous variable, the photocurrent.

3.2 noise in gain

A practical detector is one which offers sufficient gain to amplify signals to the level of sensitivity available from commercial electronics. The gain of any active device is a statistical quantity and associated with the mean gain $\langle g \rangle$ there is an associated noise. This is described in terms of a noise factor F which is defined quite generally as:

$$F = 1 + \text{var}(g)/\langle g \rangle^2 \quad \dots(3)$$

Where $\text{var}(g)$ is the square of the standard deviation σ_g .

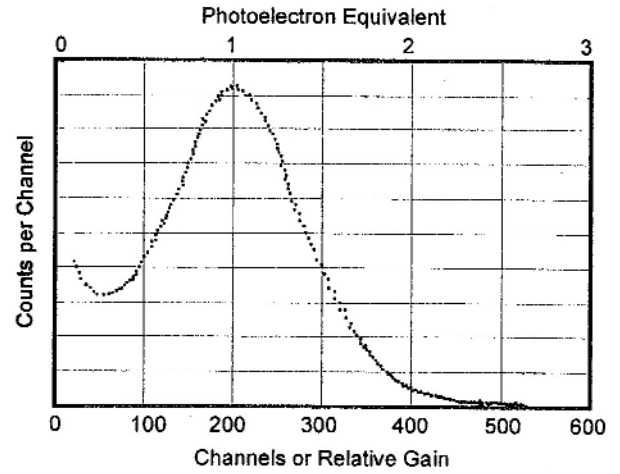


figure 2 pulse height distribution for the output of a photon counting photomultiplier when excited with single photons. The peak is taken as one photoelectron equivalent.

For signal, F_s may be determined for a photomultiplier by computation using the single electron response, SER. That is, the output pulse height distribution derived from the illumination of the photocathode with single photons. An example of such a distribution, recorded with a multichannel analyser, is shown in figure 2. The scale on the x axis may be variously expressed as: channel number, which is proportional to pulse height; photomultiplier gain, or photoelectron equivalent pulse height. The significance of the SER and methods for gain determination are treated full in⁸. F_s ranges from 1.2 for a photon counting quality photomultiplier to about 2 for unselected tubes.

The effects of noise in gain are minimal in photon counting, where the method is one of counting all output pulses which exceed a fixed threshold. There is, however, a contribution from threshold jitter, but this is insignificant in properly designed discriminators. Equation 1 provides a faithful description of the statistical nature of a random light source under photon counting conditions, whereas for current detection (measured at the anode), equation 2 must be modified to include the noise term F_s .

$$\begin{aligned} S/N &= \langle g \rangle I_k / (2e \langle g \rangle^2 I_k F_s B)^{1/2} \\ &= I_k / (2e I_k F_s B)^{1/2} \end{aligned} \quad \dots(4)$$

3.3 the effect of background on low light level measurements

All practical light detectors produce output signal in the absence of light stimulation. This unwanted signal, called background, is referred to as dark counts or dark current, depending on the application. Considerable confusion exists in the scientific

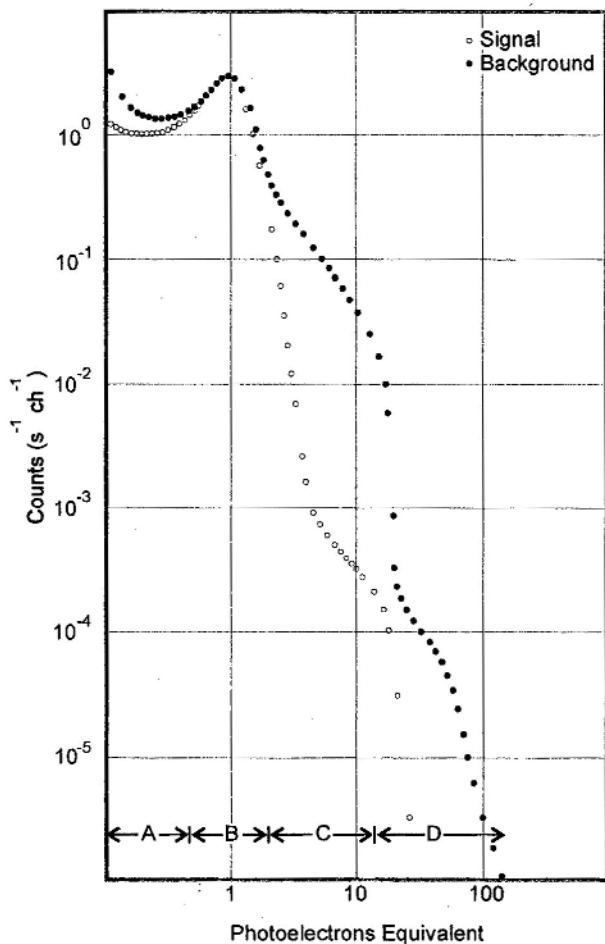


figure 3 pulse height distributions for signal and background normalized to show the higher proportion of small and large pulses in the background.

literature by the improper use of the term noise to describe dark current. It should be clear from the above considerations that noise refers to a statistical fluctuation on signal stemming from the quantised nature of light and charge. Noise is inherent in both light signal and in background.

4 pulse counting consideration

In all practical situations the signal rate relating to the phenomenon being observed is obtained by subtracting the background count rate from the measured signal plus background rate. Assigning of the correct precision is not straightforward, requiring a knowledge of the sources of background. Detailed measurements, based on a range of different photomultiplier types, have led to an understanding of the relative importance and contributions made by the various sources of background⁹. Pulse height distributions taken from this work are shown in **figure 3**.

The following generalisation applies to photomultipliers when comparing signal and background. Referring to the normalised curves of **figure 3**:

there is a higher proportion of undersized pulses (region A) and of large pulses (regions C and D) in the background spectrum. In the photon counting mode of operation, a window may be set corresponding to the lower and upper boundaries of Region B, thereby eliminating the contributions of background signals that are not found in the spectrum of the signal alone. Note that in photon counting all pulses, regardless of size, but within the upper and lower threshold levels, contribute with equal weighting to dark count.

Since the proportion of pulses in region C of the spectrum is of the order of 10% or less of the total background, window discriminator techniques are seldom used in practical photon counting systems. Performance of acceptable quality can invariably be obtained by the use of a single, low level discriminator.

5 current detection consideration

Dark current I_D , is the sum of a pulse component and a leakage component⁹. The pulse component is the integral of the background spectrum discussed above. Leakage is the major component of dark current at low gain but it always makes a contribution to dark current under low light level detection conditions, where the gain is high.

In the current detection mode all pulses in the spectrum contribute to dark current in proportion to their pulse heights. Those in region A make a reduced contribution whereas those in regions C and D contribute significantly to I_D because of their multi-photoelectron size.

6 background considerations: dark count vs dark current

Consider an ideal photomultiplier which has the same shape pulse height distribution for both signal and background. In other words all background pulses are derived from photocathode emission and the anode dark current, I_D , and the total dark count $N_D s^{-1}$ are related by:

$$I_D = N_D e \langle g \rangle \quad \dots(5)$$

Where e is the electronic charge and $\langle g \rangle$ is the mean multiplier gain. If $\langle g \rangle$ is known then an ideal photomultiplier, for which N_D is also independent of gain, is represented by a single point lying on the curve of $I_D / \langle g \rangle$ vs N_D . Results⁹ for 28 photomultipliers of different types are shown in **figure 4** where each point represents the performance of a single

photomultiplier operated at a gain of 5×10^6 ; the trajectory taken to the highest gain of 10^9 is indicated by the terminating arrow. We note that dark currents are generally a factor of two higher than the ideal straight line requires. Thus, **figure 4** illustrates in a practical way that the effect of dark current is roughly twice as severe in signal recovery terms in current detection compared with the effect of dark counts in photon counting. The primary reasons for this are those given under **pulse counting and current detection considerations**.

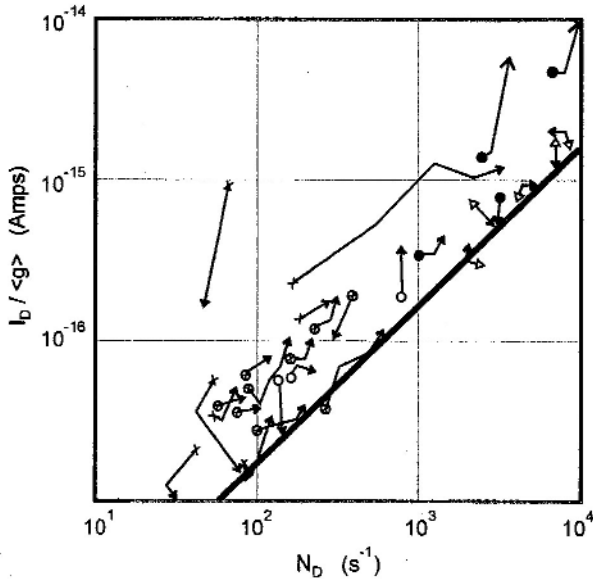


figure 4 the equivalent cathode dark current $I_D / \langle g \rangle$ measured over a range of multiplier gains from 5×10^6 to 10^9 . The trajectory taken by each of the 28 photomultipliers illustrated terminated in an arrow. N_D is the background count within the amplitude range 0.2 to 10 photoelectrons equivalent. The solid line represents equation 5.

7 comparison of photon counting and current detection with regard to S/B

The expected ratio of Signal to Background rates, S/B, is an indicator of both the accuracy and the precision in a low light level measurement. For the purpose of this discussion we can categorise low level in terms of S/B ratios that fall below the limit of $S/B \leq 1$ and in the extreme $S/B \leq 0.1$. In a practical measurement we arrive at an estimate of the signal rate, S, that is deduced from the difference between two measurements: (s + b) counts for signal plus background over time t_1 and b' over time t_2 for background count alone, with

$$S = (s + b)/t_1 - b'/t_2 \quad \dots(6)$$

The precision associated with equation 6 is more complicated to calculate than the simple form of the equation suggests.

8 signal to background considerations in photon counting

Certain sources in the background produce correlated events ^{3,9,10} and consequently the noise in a background measurement of b counts is always greater than $b^{1/2}$, as is predicted by equation 1, for signal. Background measurements for a 30 mm, bialkali, photomultiplier operated over a range of temperatures are shown in **figure 5**. The function k is the ratio of the measured to expected standard deviation based on Poisson statistics. At low temperature, where the background counts are in the region of 10 s^{-1} , the excess noise factor k exceeds

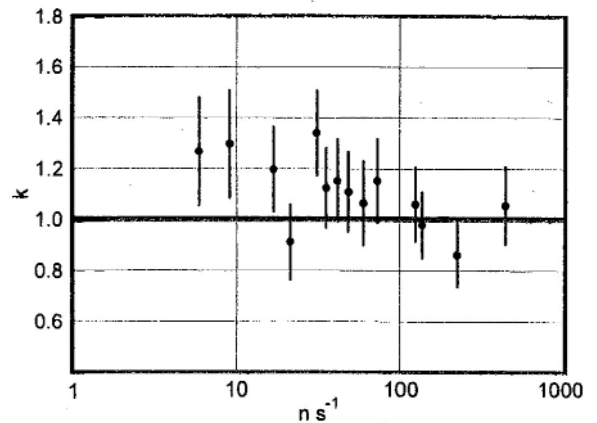


figure 5 noise measurements for a photomultiplier that is statistically well behaved. N is the count rate and k is defined in the text.

unity by about 20%, but at higher temperatures $K \rightarrow 1$ because statistically well behaved thermal background dominates the other sources of background. The experimental confirmation that $k \approx 1$ for a well behaved photomultiplier means that the signal recovery process may be described by Poisson statistics.

We note in equation 6 that the noise enters into the determination of S twice: firstly in the (s + b) measurement and then in the b' measurement. The well-known results for the optimal division of counting time between (s + b) and b' (see, for example³) prescribes that for $S < B$ the time should be split equally while for $S > B$ best precision is obtained by devoting a higher proportion of the counting time to the measurement of (s + b).

9 signal to background considerations in current detection

Noise in dark current derives from the combination of statistical fluctuation in the background spectrum and from the noise factor F_D acting on the amplification of the dark current. Note that F_D is always

greater than F_S since the variance of the background spectrum exceeds that for signal – this is because there are relatively more counts in regions A and C of the spectrum of **figure 3**.

To summarise, the case against current measuring for low light level determinations is made for the following considerations:

- 1 Noise factors F_S and F_D enter into the signal current and dark current respectively and increase the noise.
- 2 Dark current contains a leakage component
- 3 Multi-electron pulses contribute to dark current in proportion to their number and pulse height but only contribute to dark counts in proportion to their number.

10 the photon counting method – quality of performance

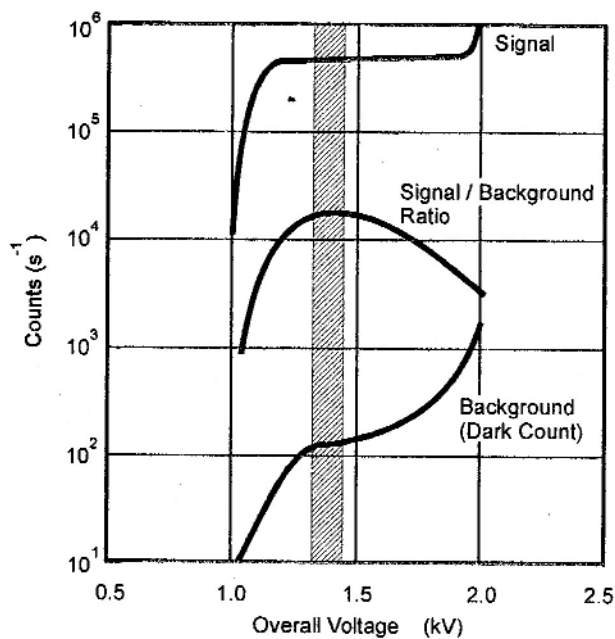


figure 6 photon counting plateau characteristics for single photons and for background. The plot of the ratio of the two curves suggests an optimal operating point within the hatched area.

11 plateau characteristic

In its simplest and most common form, practical photon counting instrumentation consists of a fast amplifier and a discriminator set to a low threshold of $\sim -2\text{mV}$, referred to the input. This threshold has been shown, from experience, to correspond to the optimal compromise between susceptibility to electrical pick-up and operating the photomultiplier at excessive gain.

The signal plateau characteristic of **figure 6**, for a light source of fixed intensity, is obtained by varying the high voltage to the photomultiplier whilst noting the counts above threshold. The background plateau characteristic is measured in the same way but with the light removed. The ratio of the signal and background curves for this example suggests a choice of operating point between 1.35 to 1.45 kV, where S/B counts reach a maximum. The precise operating voltage for the photomultiplier is best set with reference to the slope of the signal characteristic also. Electron Tubes Ltd. Set the operating point where the slope first becomes less than 0.1% per volt (at 1.40 kV in this example) which corresponds to counting all pulses which exceed a threshold of about $\frac{1}{4}$ photoelectron. Referring to **figure 2**, we see that about 90% of the single photon events are counting by selecting this threshold. A note of caution is appropriate: operation at higher voltages to enhance the counting efficiency is done at the cost of higher dark counts from Region A of the spectrum and increased afterpulses with consequent increase in k values.

12 temperature instability

Cathode quantum efficiency, η , and multiplier gain, g , are both sensitive to changes in temperature. The multiplier gain changes by approximately $-0.2\% \text{ } ^\circ\text{C}^{-1}$ but the photocathode sensitivity depends on wavelength in the manner shown in **figure 7**. The count rate, n , of a photomultiplier will thus be sensitive to temperature. For a given photomultiplier:

$$n = n(g, \eta)$$

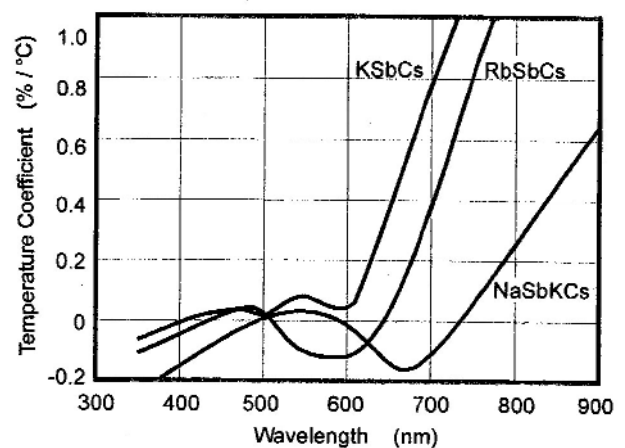


figure 7 the temperature coefficient for quantum efficiency, η , of various photocathode types. Note how the coefficient increases sharply at long wavelengths.

and the temperature coefficient of the count rate is

given by:

$$\begin{aligned} \frac{dn}{dT} &= \frac{\partial n}{\partial g} \frac{dg}{dT} + \frac{\partial n}{\partial \eta} \frac{d\eta}{dT} \\ &= \frac{\partial n}{\partial V} \frac{dV}{dg} \frac{dg}{dT} + \frac{n}{\eta} \frac{d\eta}{dT} \end{aligned}$$

The relative change in count rate is

$$\frac{1}{n} \frac{dn}{dT} = \left\{ \frac{1}{n} \frac{\partial n}{\partial V} \right\} \left\{ \frac{dV}{dg} g \right\} \left\{ \frac{1}{g} \frac{dg}{dT} \right\} + \frac{1}{\eta} \frac{d\eta}{dT} \dots(7)$$

Where:

$$\frac{1}{n} \frac{\partial n}{\partial V} = \text{relative plateau slope} = 10^{-3} \text{ per volt}$$

The relationship between gain and applied voltage follows the power law $g = aV^m$ where m is 8 for the photomultiplier of **figure 6**.

$$\frac{dV}{dg} g = \frac{V}{m} = \frac{1400}{8} \text{ Volts}$$

$$\frac{1}{g} \frac{dg}{dT} = -0.2 \times 10^{-2} \text{ } ^\circ\text{C}^{-1}$$

$$\frac{1}{\eta} \frac{d\eta}{dT} = \text{quantum efficiency temperature coefficient}$$

The temperature coefficient of the photocathode depends on the wavelength of interest, as shown in **figure 7**. It is typically $-0.1 \text{ \%}^\circ\text{C}^{-1}$ at 400 nm.

Combining the numerical values of all the terms in **equation 7**, we have

$$\begin{aligned} \frac{1}{n} \frac{dn}{dT} &= -0.035 = 0.1\%^\circ\text{C}^{-1} \\ &= -0.135\%^\circ\text{C}^{-1} \end{aligned}$$

In current mode of operation the temperature sensitivity is:

$$\begin{aligned} \frac{1}{I} \frac{dI}{dT} &= \frac{1}{g} \frac{dg}{dT} + \frac{1}{\eta} \frac{d\eta}{dT} \\ &= -0.2 - 0.1\%^\circ\text{C}^{-1} \dots(8) \\ &= -0.3\%^\circ\text{C}^{-1} \end{aligned}$$

the above treatment shows that the effect of gain change is much reduced in photon counting compared with current detection at 400 nm, for example. Note that at 500 nm, where the temperature coefficient of the photocathode is close to zero, the overall temperature coefficient is a factor of six dif-

ferent for the two methods.

13 improved performance by cooling

Figure 8 shows how dark counts vary with temperature in the three most common photocathode types. Although there is considerable variation amongst photomultipliers of the same type, the curves have a characteristic shape. For the KSbCs bialkali, for example, dark counts are substantially constant up to a temperature of 20°C, beyond which the counts double per 5°C temperature rise. Equation 6 was previously discussed in the context of precision of measurement but it is clear that the accuracy of determining S relies on the stability of b over the $s + b$ and over the b' measurement periods. Clearly if b varies because of temperature changes then the estimate of S will be inaccurate. If the background is a significant proportion of the signal, or indeed exceeds the signal, then taking a background measurement immediately before or after a signal plus background determination, helps in reducing this source of error. Best performance will always result whenever the photomultiplier is maintained at constant temperature. In very low light level measurements, further improvements in precision and accuracy can both be realized by maintaining the photomultiplier at a constant cooled temperature, as is clear from **figure 8**.

In some instances it may be necessary to compromise between the benefit of dark current reduction versus a possible reduction in cathode sensitivity. **Figure 7** illustrates that there is a serious loss in cathode sensitivity, in the S20 cathode for example, at wavelengths beyond 700 nm. Hence the recommendation: 'cool no more than necessary'.

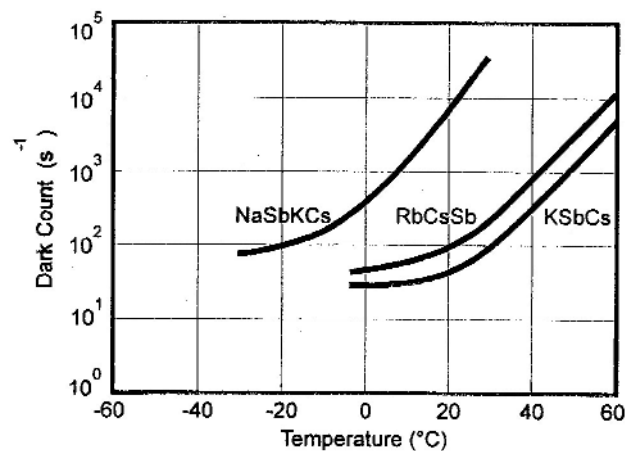


figure 8 variation of dark counts with temperature in 30 mm photomultipliers for the three most common photocathode types.

14 other sources of instability

Multiplier gain is sensitive to all of the following:

- photomultiplier ageing^{11a}
- power supply instability
- rate effects^{11b}
- magnetic fields^{11c}
- shock and vibration¹²

The same reasoning used in temperature sensitivity considerations tells us that photon counting will provide better performance than current detection against these sources of gain instability.

Magnetic fields affect the gain and the collection efficiency of a photomultiplier. A change in collection efficiency is equivalent to a change in effective quantum efficiency, η . Conventional, open ended, mu-metal shields are least effective against the field component directed along the axis of a photomultiplier.

Vibration caused by rotating equipment such as vacuum pumps and shock, caused by sudden impulses, are the main causes of microphonic effects in photomultipliers. Resonant vibrations in the dynode structure generate a measurable current analogue at the anode. The spectrum exhibits frequency components centred around 1kHz¹². In photon counting these signals can be discriminated against by suitable high pass filtering but no such solution is available in current mode.

linearity

This refers to the degree of linearity between the intensity of the light stimulus and the output of the photomultiplier. In photon counting, at sufficiently high light levels, the output rate will deviate from the photoelectron detection rate because of electronic dead-time effects caused by pulse pile-up. Pulses that arrive while the discriminator is busy are ignored. The type of discriminator used by Electron Tubes is the non-paralizable type where the measured count rate can be corrected according to the following formula.

$$N = n / (1 - n\tau) \quad \dots(9)$$

Where N is the true count rate corresponding to a measured rate of n and τ is the dead-time of the discriminator. The value of τ that gives the best linearity is that determined experimentally from a set of counting rates of known ratios. Figure 9 shows an example of a dead time correction that provides linear response to better than $\pm 0.5\%$ up to $20 \times 10^6 \text{ s}^{-1}$, based on $\tau = 23.5 \text{ ns}$.

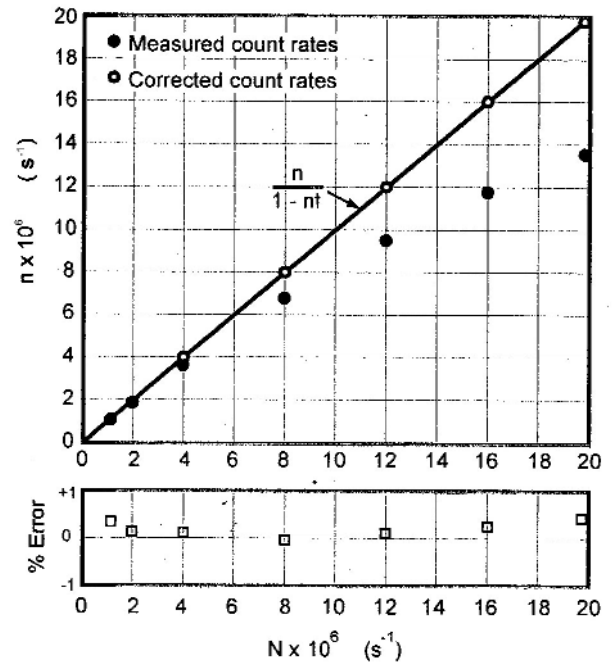


figure 9 showing an example of dead-time correction applied to the measured count rate, n , extending linear performance up to $20 \times 10^6 \text{ s}^{-1}$ to within $\pm 0.5\%$.

Anode current is the integral of pulse height and rate and is unaffected by pulse pile-up. However, it is easily verified by substitution into the signal analogue of equation 5 that operation at gain of 10^7 , required for photon counting, implies an anode current of $32 \mu\text{A}$ at a count rate of $20 \times 10^6 \text{ s}^{-1}$. The deviation from current linearity caused by the rate effect (11b) can be of the order of a few percent at anode current levels beyond $10 \mu\text{A}$. Poor voltage divider design is often the major cause but there is also a contribution from the photomultiplier itself. This is a complex topic and further work needs to be done before this aspect of photomultiplier performance is fully understood.

A point that does not appear to be generally appreciated is that current measurement at fixed high voltage does not offer a wider dynamic range than photon counting using fast counting electronics. Operating a photomultiplier at a continuous anode current in excess of $100 \mu\text{A}$ causes loss of gain^{11a} and this is essentially the upper level any instrument manufacturer should accept. However, as mentioned above, signal non-linearity can set in well before this limit. Correction is possible but it must be done for each photomultiplier individually. Of course, in the current method, gain can be reduced for operation at high light levels thereby extending the dynamic range, but this is not without practical difficulties.

At the low count rate end of the dynamic range the arguments already given show that photon counting can offer another decade of performance over current detection through superior signal to back-

ground performance.

photon counting modules

Commercially available photon counting modules containing a selected photomultiplier, electromagnetic screening, high voltage supply, and a fast amplifier discriminator are aimed primarily at the OEM customer. The benefits of using a photon counting package are:

- complete, rugged, integrated system
- compact, lightweight, low power consumption
- fully enclosed high voltage
- simplicity of installation – only low voltage input and signal output to connect
- simplicity of operation – no set-up or adjustment
- preset operating conditions for optimized performance
- can be customised to suit specific applications

15 discussion

The signal recovery performance offered by photon counting has been shown to be superior to the current detection method over the entire dynamic range of photomultiplier operation. In photon counting terms this corresponds to rates of a few photons per second to $\sim 10^8$ per second, equivalent to ~ 1 pA to 100 μ A in output current.

Photon counting is preferred over current detection because

- the gain process is noisy and introduces a factor F into current detection measurements.
- dark current in current detection always exceeds the dark current equivalent of dark counts in photon counting because of leakage. Also, no component of the dark current can be eliminated by discrimination as in photon counting.
- it is less sensitive to temperature effects, ageing, high voltage stability, rate effects, magnetic effects and microphonics.
- the dynamic range is superior at fixed gain.
- the photon counting method preserves the temporal structure of the signal.

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