Gaining an Understanding of Detector Gain

Image quality is determined by the number of photons recorded per pixel as well as pixel size relative to the magnified image of the sample. For a given fluorescent dye and microscope, the number of photons recorded is largely determined by the product of the detector **exposure time** and the **power** of the excitation light. Longer exposure times allow more photons to be collected and so increase image signal to noise ratio (SNR). Higher excitation powers cause more photons to be emitted, and so also increase SNR (at least until bleaching occurs).

At the detector, emitted photons interact with a semi-conducting material, where they are converted into free electrons (photo-electrons). However, this initial conversion is a 1:1 process, so the resulting small number (often 1,000s) of electrons must be electronically amplified into an electrical current large enough to be encoded and sent to the computer. This amplification process is termed **gain**. In order for the final current to remain proportional to the number of photons initially detected, the gain must be linear. In the case of linear gain, the gain is conceptually just a constant of proportionality that relates a given number of photons detected to a final electric current that is encoded for the computer.

What can make this simple process difficult to understand is that the range of possible currents used for encoding is fixed, say, between 1-10 volts. As a result, the gain must always be adjusted by the user, depending on the number of photons initially recorded. For a given gain, if many photons are recorded, the resulting current will exceed the upper end of this fixed range (10 volts). If such an excess occurs, the detector is said to be **over-saturated** and all quantitative information about the initial number of photons is lost. The gain must be decreased in this case. Conversely, if very few photons are recorded, the resulting current may not be large enough to exceed the minimum current level (1 volt). Here, weak signal may go undetected and the detector is said to be **under-saturated.** The gain must be increased in this case. **Both over and under saturation must always be avoided since quantitative information about the sample is lost!**

It is important to note that, to a first approximation, changing the gain does <u>not</u> change the SNR in the image (in the absence of saturation effects): It only changes the magnitude of the final currents produced. Larger currents will result in a brighter image as displayed on the computer screen, but do not provide any new information about the sample and do not impact shot noise, which is a function of the number of photons initially converted into free electrons. A high gain implies a small number of detected photons, and thus also poor image quality. **Good images are collected with low gain**, since less amplification is required when the number of photons initially detected is already large. Relatedly, high sensitivity detectors generally imply worse image quality since they are designed to record small numbers of photons.

Other factors can also impact SNR and saturation, but they are generally encountered only in special circumstances or are detector specific. For example, the process of converting photons into electrons can itself become saturated, especially when using a camera. If using a PMT, higher gains are likely to introduce other significant sources of electronic noise. Finally, a dye's ability to emit fluorescence can become saturated at the point of excitation, especially if using high (>100 uW) laser powers.

Please contact us (microscopy@umich.edu) to help you appropriately adjust your acquisition settings in order to capture the best possible quality images. We look forward to working with you!

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