

What Makes a 'Good' Image? Part I: Signal to Noise Ratio

Though biologists most often qualify an image based on how the sample 'looks', imaging scientists define 'good' based on the quantitative and sample-independent criteria of sampling rate and signal to noise ratio (SNR). Here, we focus on the importance of signal to noise ratio.

Given a reasonably modern microscope, the SNR in an image is most fundamentally determined by the fluorophores used to label the sample. Each dye molecule has an inherent **photon budget**, which is the number of photons, on average, that it can emit before becoming non-fluorescent (often due to photo-bleaching). Importantly, a dye's brightness (brightness = extinction coefficient x quantum yield) does not necessarily equate to photon budget: For example, a bright dye could bleach very rapidly. Thus, **photo-stability** (resistance to bleaching) is often a more important parameter, yet difficult to quantify, since it depends on the dye's local chemical environment. Photon budgets are usually in the range of 10,000-100,000 photons. Given a budget of 10,000 photons, and 1000 dye molecules per 'brightest' image pixel area, the total emissions in that pixel will be around 100,000 photons – *on average*.

The exact number of photons emitted cannot be known in advance, because fluorescence is a random process, that follows Poisson statistics. The resulting random variations in light levels are termed **shot noise**. These Poisson statistics state that the average number of emissions (N) over many replicate measurements is equal to the variability across the measurements (thus also N). So, if the fluorophore concentration is the same everywhere in an image, the SNR is defined as $N/\sqrt{N} = \sqrt{N}$. Note that as more photons are emitted on average, the SNR (\sqrt{N}) increases, resulting in a better image. This definition is not straight forward to apply to real images, where the fluorophore concentration itself is also variable. While a full mathematical treatment of a more realistic case is beyond the scope of this summary, the basic idea still holds – more photons equals a better image.

The number photons emitted is not the only important factor for image quality since photons 'don't count' unless they have been detected. To collect the emitted photons, the instrument light path, detector efficiency, and acquisition parameters are also important. Wide-field detection with a high numerical aperture objective and high quantum efficiency camera is the most efficient design and might record 25% of all photons emitted (>50% of all photons immediately travel away from the objective). **Compared to a wide-field design, the best confocals are ~25x less efficient** due to the complexity of the light path and PMT detectors. A 'bright' wide-field image pixel may record 25,000 photons (SNR = 158) while a 'bright' confocal pixel perhaps 1,000 (SNR = 31). Perhaps surprisingly, the electronic noise produced by modern detectors is usually negligible compared to the shot noise.

The above argument implies that a wide-field microscope produces a better image than a confocal. This is true, but only when the dye is restricted exclusively to the focal plane (often <1 μm). For thicker samples, out-of-focus fluorescence emissions also contribute shot noise that combines additively with emissions from the focal plane. Thus, for thick samples, confocal imaging becomes an advantage, as its ability to block out-of-focus shot noise offsets its relatively less efficient ability to detect in-focus emissions.

Choosing high photon budget dyes and the right microscope can easily increase SNR by a factor of 2x-5x fold. Please contact us (microscopy@umich.edu) to help you design and troubleshoot your fluorescence imaging experiments. We look forward to working with you!