PHOTOSWITCHING NOISE DISTORTS ALL FLUORESCENT IMAGES

Benjamin K. Cooper and Andrew G. York
Calico Life Sciences, LLC
1170 Veterans Blvd., South San Francisco, CA, 94080
Email: bkcooper@calicolabs.com, andrew.g.york+fom@gmail.com

It is well known that fluorescence image quality is limited by the presence of Poisson shot noise in photoemissions. What is often ignored is that stochastic fluctuations in fluorophore behavior (such as photobleaching, deactivation, or blinking) also manifest as image noise. Since these dynamics are ubiquitous, this additional noise source applies to essentially all fluorescence images. Unlike Poisson noise, photoswitching noise is not spectrally white and may be easily misinterpreted as signal (Figure 1). Using photobleaching as a concrete example, we show that while longer exposure times decrease the influence of Poisson noise, they strengthen the influence of photobleaching noise. Thus lengthening exposure time past a critical (and often surprisingly small) value actually degrades image quality. We derive where this crossover occurs for photobleaching noise and find a simple heuristic for the ideal exposure time. This rule of thumb depends only on the number of detected photoelectrons a typical fluorophore yields before bleaching, allowing easy practical recommendations for optimizing exposure. We discuss implications for advanced fluorescence imaging modalities such as STED or RESOLFT.

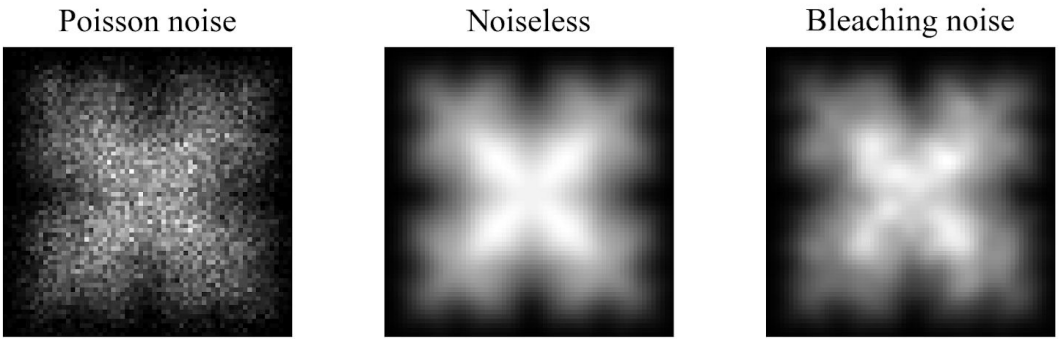

Figure 1: (center panel) an ideal noiseless measurement; on the sides are images corrupted by (left panel) Poisson and (right panel) photobleaching noise.