SPAD IMAGERS FOR CHARACTERIZATION OF ULTRA FAST DYES FOR SUPER RESOLUTION LOCALIZATION MICROSCOPY

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Super resolution localization microscopy has pushed the boundaries of acquisition times to video rate utilizing high frame rates provided by sCMOS imagers. However, when increasing the dye's blinking to the maximum rates that charge-accumulating imagers can support, even faster instrumentation is needed to accurately characterize blinking [2]. Considering multiple energy states, the molecule can indeed include several switching rates that lower the total molecule intensity (Fig. 1). Single-photon avalanche diode (SPAD) imagers such as SwissSPAD (Fig. 2) feature bit depths comparable to those of sCMOS imagers, but they are also capable of generating 1-bit frames of microsecond time duration without readout and clock induced noise, thus paving the way to an in-depth time-resolved analysis of the sample [1].

In this context, we investigated an imager's optimal frame rates, given a molecule intensity and background, when molecular blinking properties are known. Thanks to SwissSPAD's precisely controlled, highly uniform time gating, fluorophore statistics could be obtained with better than 200ps (FWHM) time accuracy and 6.4µs time resolution. We recorded the first super resolution localization microscopy results obtained with a SPAD pixel imager, with a localization uncertainty of 30 nm. We will also report the detailed methodology for blinking analysis of fluorescent dyes based on experimental data.

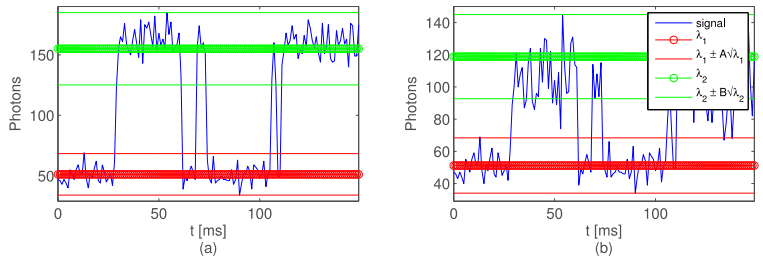

Figure 1: Blinking (a) without and (b) with additional fast blinking. The emission changes in (b) fall outside the emission band (green) estimated with Poisson statistics.

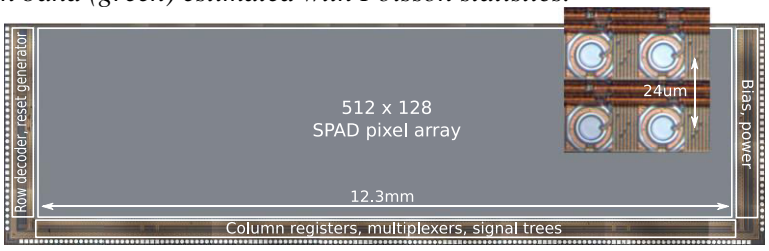

Figure 2: SwissSPAD: a 512x128 pixels, 6.4 µs frame time single-photon imager.

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[2] G. T. Dempsey, *et al.*, "Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging," Nat. Methods **8**, 1027–36 (2011).