

Single-molecule imaging is widely used in biology and materials science, having several applications of importance to NASA. However, these studies are limited by the requirement to have spectrally distinct fluorescent probes. We recently developed blinking-based multiplexing (BBM), a technique to differentiate spectrally-overlapped single emitters based on their intrinsic pattern of emission over time. The study tested both an empirically-derived metric and deep learning algorithm, which both have significant drawbacks. Here, a multinomial logistic regression (LR) classification is applied to rhodamine 6G (R6G) and CdSe/ZnS quantum dots (QD) in various experimental conditions and environments (i.e., bin time and glass versus polymer). We showed that LR is rapid, readily generalizable, and classification accuracies are consistently achieved, even in complex polymer environments. This study provides significant versatility to BBM, opening the possibility for high-resolution, multicolored single-molecule imaging of complex materials.