**Bulletin of the American Physical Society**

**APS March Meeting 2023**
**Las Vegas, Nevada (March 5-10)**
**Virtual (March 20-22); Time Zone: Pacific Time**

**Session F17: Spectroscopy in Space and Time III; Super-resolution and Advanced Imaging**
8:00 AM–11:00 AM, Tuesday, March 7, 2023
Room: Room 209

Sponsoring Unit: DCP
Chair: David Osborn, Sandia National Laboratories

## Abstract: F17.00004 : Blinking-Based Multiplexing (BBM): Harnessing Molecular Photophysics for Single-Emitter Classification*

9:00 AM–9:36 AM

← Abstract →

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Single-molecule fluorescence approaches have revolutionized biological and materials microscopy. However, many questions can only be addressed by multicolor imaging of multiple targets, a capability that is limited by the small subset of available, well-performing, and spectrally-distinct fluorescent probes. We recently introduced an alternative single-molecule multiplexing approach termed blinking-based multiplexing (BBM), wherein individual molecules are classified on the basis of their intrinsic blinking dynamics. We demonstrate accurate (>93.5%) binary classification of spectrally-overlapped rhodamine and quantum dot emitters using BBM, even when substantial blinking heterogeneity is observed. Classification can be accomplished using change point detection (CPD) analysis of blinking dynamics or a deep learning (DL) algorithm, the latter of which provides up to 96.6% accuracy. Here, we use CPD and DL algorithms to probe the excitation power, environmental, and molecular dependence of BBM. In addition to providing new opportunities in single-molecule spectroscopy and imaging, BBM represents a new take on single-molecule research, where blinking dynamics can be harnessed for more than just traditional localization or nanoreporting.

*This work was supported by the National Science Foundation (CHE-2102099).