PHYSICAL MATH SEMINAR

Breaking the Spectral Barrier: Novel Flux Enabled Multiplexing and FLIM in Single Molecule Localization Microscopy

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ABSTRACT:

Single Molecule Localization Microscopy (SMLM) offers nanometer scale resolution, however true multiplexed imaging remains limited by the need for multiple lasers, spectral separation, and complex detection hardware. This talk introduces a new class of flux-based strategies that overcome these limitations and enable both hardware minimal multiplexing and low-cost fluorescence lifetime imaging (FLIM).

A novel demixing approach is presented based on fluorescence flux, an intrinsic property of fluorophores that, when illumination and detection conditions are controlled, provides a species-specific signature independent of emission spectra. This enables simultaneous demixing of even spectrally overlapping fluorophores on a standard single camera SMLM setup, and can further enhance the performance of conventional spectral demixing systems.

Building on this, two new methods for flux based FLIM are introduced that eliminate the need for fast detectors or high end pulsed lasers. By operating fluorophores in the saturation regime, their emitted flux becomes directly linked to fluorescence lifetime. Using (a) microsecond gated continuous wave excitation and (b) a pulsed laser in a double pulse configuration with tunable delays, lifetime-based multiplexing is demonstrated, validating these concepts in proof of principle experiments. Together, these developments provide a path toward simpler, faster, and more accessible super resolution imaging, expanding the reach of SMLM and FLIM to a broader range of laboratories and biological applications.

Finally, the talk will briefly touch upon other applications of wave dynamics, including FRET-based studies of glucagon receptor signaling, a slingshot-based droplet generator for the tunable production of small, uniform airborne droplets, and the phenomena of damping-driven time reversal of waves.

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2:30 PM – 3:30 PM

Building 2, Room 449

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