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Hyperspectral Line Scanning Microscopy for High-Speed Multicolor Quantum Dot Tracking

***Short Abstract* — One of the fundamental goals in observing protein-protein interactions on the cell membrane is in achieving nanometer scale spatial resolution along with temporal resolutions sufficient to study live cell behavior. Traditional fluorescence microscopy methods have been unsuccessful in studying these interactions due to the diffraction limit with visible light. Single particle tracking techniques using quantum dots have provided single particle localizations to well below the diffraction limit, however, clustering of multiple particles limits the unique identification and thus tracking of individual particles throughout the (possibly dynamic) clustering process. This problem can be solved by tracking multiple quantum dot colors using a high-speed hyperspectral microscope. The biological goal, optical design details and layout, and simulation methods and results are discussed.**

I. Abstract

Interactions of membrane bound proteins play a fundamental role in cell signaling. Proteins interact at nanometer distances, and are sometimes transient within small fractions of a second. Traditional light microscopes are diffraction limited to resolutions on the order of approximately 200 nm, making it impossible for them to observe these protein interactions. Advancement in this field requires not only nanometer scale tagged protein localization, but also simultaneous identification of several different individual interacting proteins at a high imaging speed (~30 fps) [1].

One way that this goal can be accomplished is with an instrument that employs the nanometer scale localization of single quantum dots along with unambiguous tracking via spectral separation of emitted light from a multicolored set of quantum dots, and a high speed imaging design for the necessary temporal resolution. Here we describe the construction, characterization, and performance tuning of a hyperspectral line scanning microscope that will accomplish this objective.

The microscope design and layout will be an improvement upon one described by Sinclair, et al. [2]. A 488 nm laser will be scanned across the sample through the microscope objective via a galvanometer driven mirror. The emitted light from the quantum dots will then be descanned via the same mirror and focused through a confocal slit and into a prism spectrometer. Our design will use a line scanning system produced with a laser line generator lens [3] along with a fast EMCCD, providing an overall system design that is specifically tailored for sub-diffraction-limited, high-speed tracking of proteins labeled with spectrally distinct quantum dots.

The first application of this microscope will be in studying the dynamics of the FcεRI transmembrane receptor using quantum dot tagged IgE antibodies.

References

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