Full Visible Spectrum, Multicolor Imaging of Single Molecules

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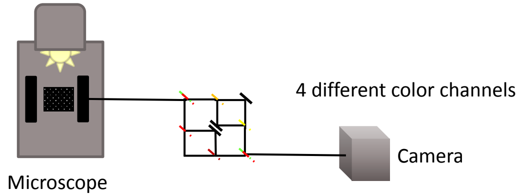
Introduction

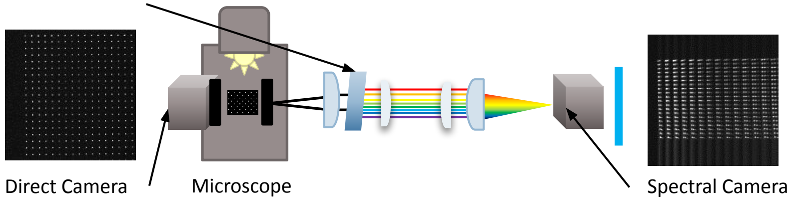
In conventional fluorescence imaging systems, determining color involves a myriad of optical elements. Differentiating each additional color may require light to be split through mirrors and filtered further. This process is limiting because intensity is crucial for single molecule imaging, and the loss accumulates as the path is split and filtered. Furthermore, the colors are limited to just 2-4 filtered ranges across the visible spectrum.

Zhang et. al. utilized a dispersive element[1], a prism, in order to simultaneously image and differentiate multiple far red dyes and further extract hyperspectral information. Our optical system involves a prism that yields linear dispersion, and with further characterization, hyperspectral information can be obtained without the use of filters.

Standard multicolor system
Dichroic mirrors and filters coupled

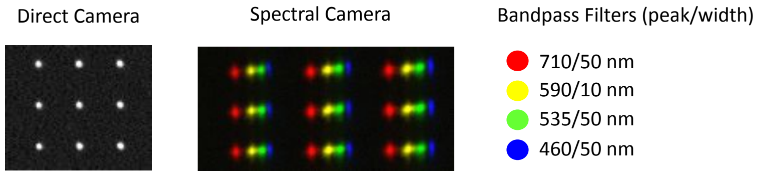


Hyperspectral system
Prism yields linear dispersion across visible spectrum

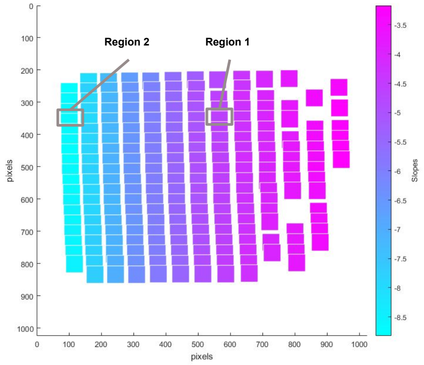


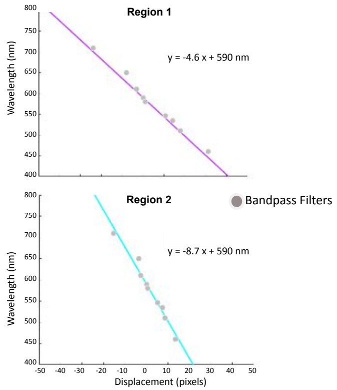
Implementing hyperspectral imaging

Imaging uniform nanogrid with bandpass filters across visible spectrum to quantify dispersion



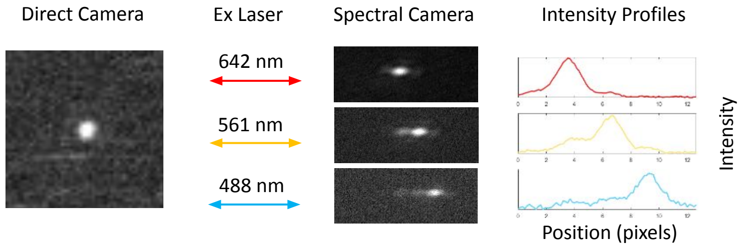
Linear dispersion achieved and mapped over image as slopes of wavelength vs. pixel displacement



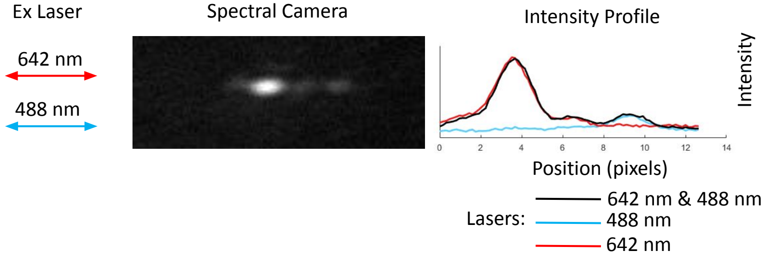


Fingerprinting dyes to separate overlapping spectral patterns

Building a library of spectral fingerprints by analyzing intensity profiles of single bead with three dyes



Using single dye library to separate spectral information of two dyes emitting from same location



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Citations

1. Zhang, Z., Kenny, S. J., Hauser, M., Li, W., & Xu, K. (2015). Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. Supplement. *Nature Methods*, *12*(10), 935–938.