The benefits of excitation based hyperspectral microscopy

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Czerski, J., Colomb, W., Cannataro, F., and Sarkar, S.K. (2017). Spectroscopic identification of individual fluorophores using photoluminescence excitation spectra. Journal of Microscopy. (Pending Review)

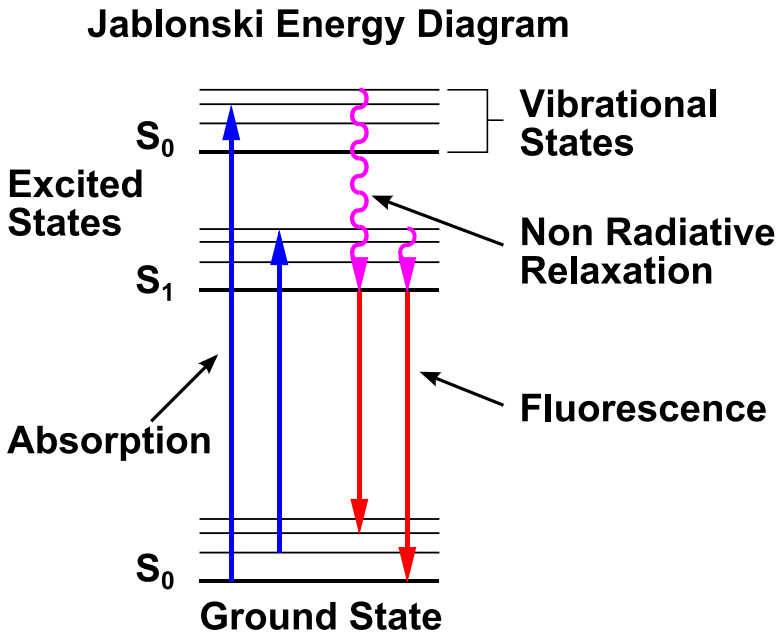
A quick introduction

The Single Molecule Biophysics Lab

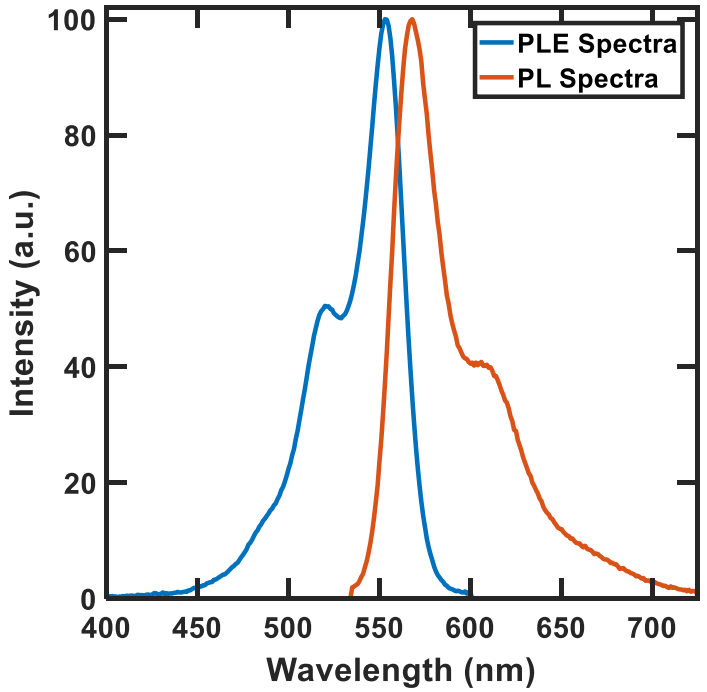
- Collagen/MMP dynamics and structure
- Advanced biomedical imaging
- Single molecule measurement and simulation

The basics of fluorescence

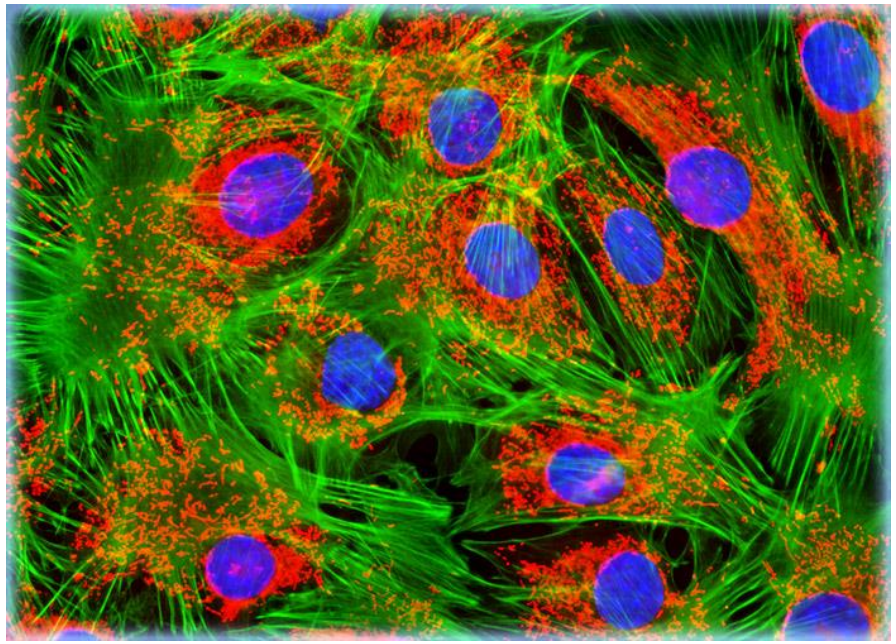
- Fluorescent molecules must absorb and emit



- Their PLE and PL spectra are unique



The benefits of fluorescence microscopes



Embryonic Swiss Mouse Fibroblast cells Curtesy of Olympus Microscopy Resource Center

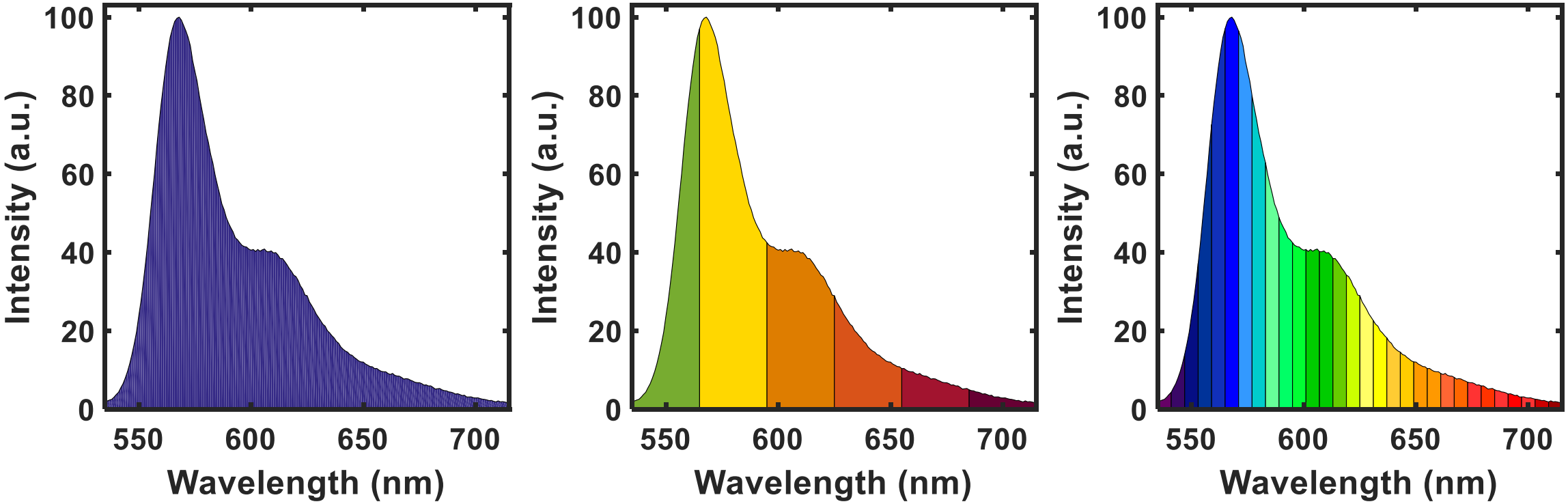
- Signals only from labeled samples (mostly)
- Multiple labels can be used.
- sub-diffraction localization is possible

Why we care about spectral resolution

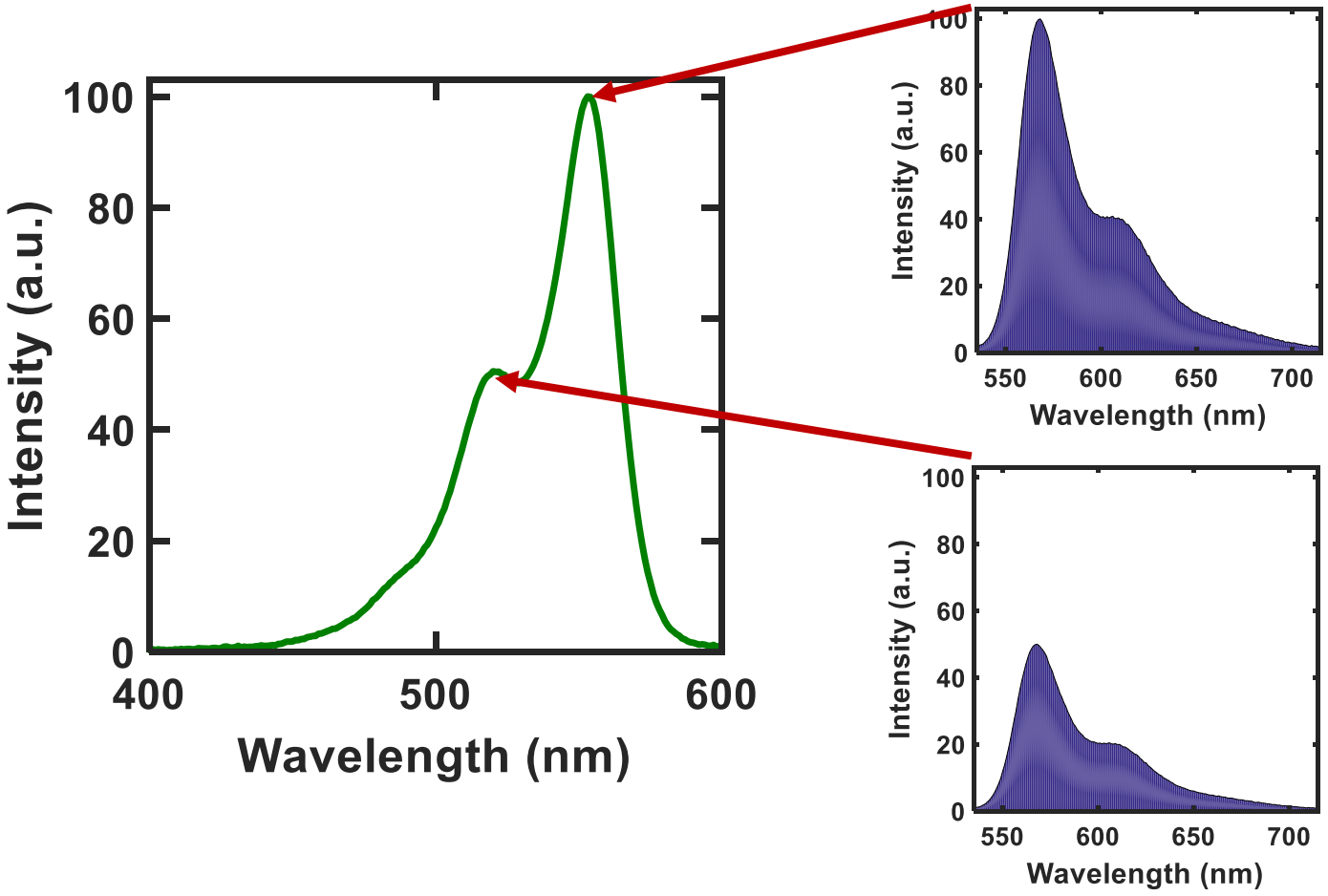
- It is required for quantitative identification
- Accurate localization for co-localized fluorophores
- In the presence of overlapping spectra, noise, and unidentified fluorescence It’s necessary.

Emission Filtering

As I break my emission into channels, the number of photons being collected in each channel decreases.

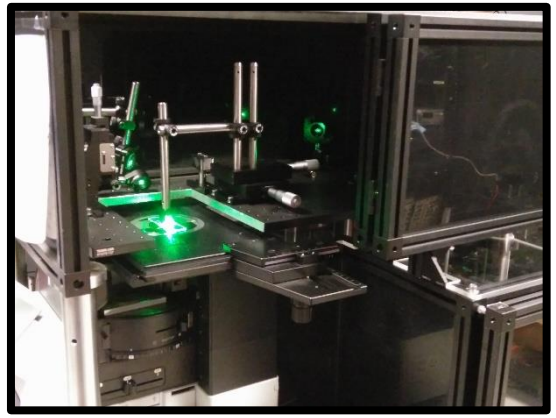


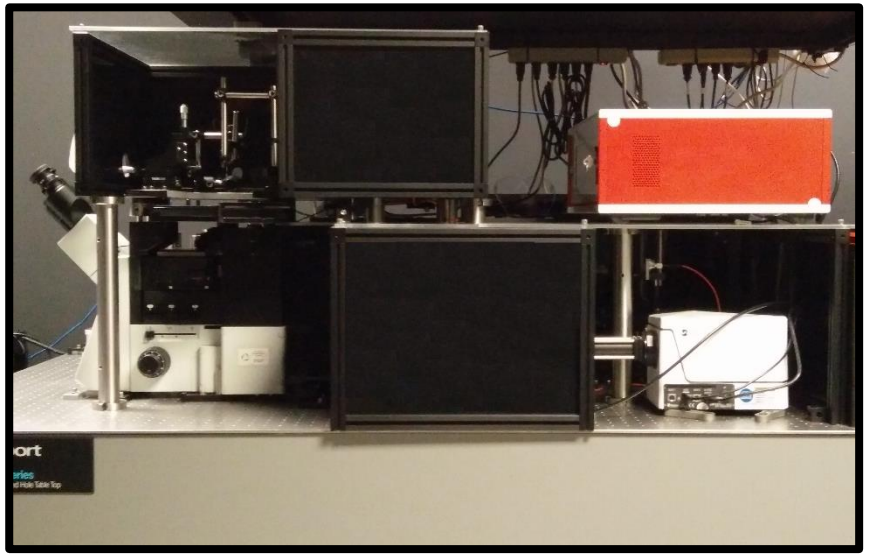
Excitation Filtering



With one good emission filter I collect all the emission regardless of the excitation wavelength

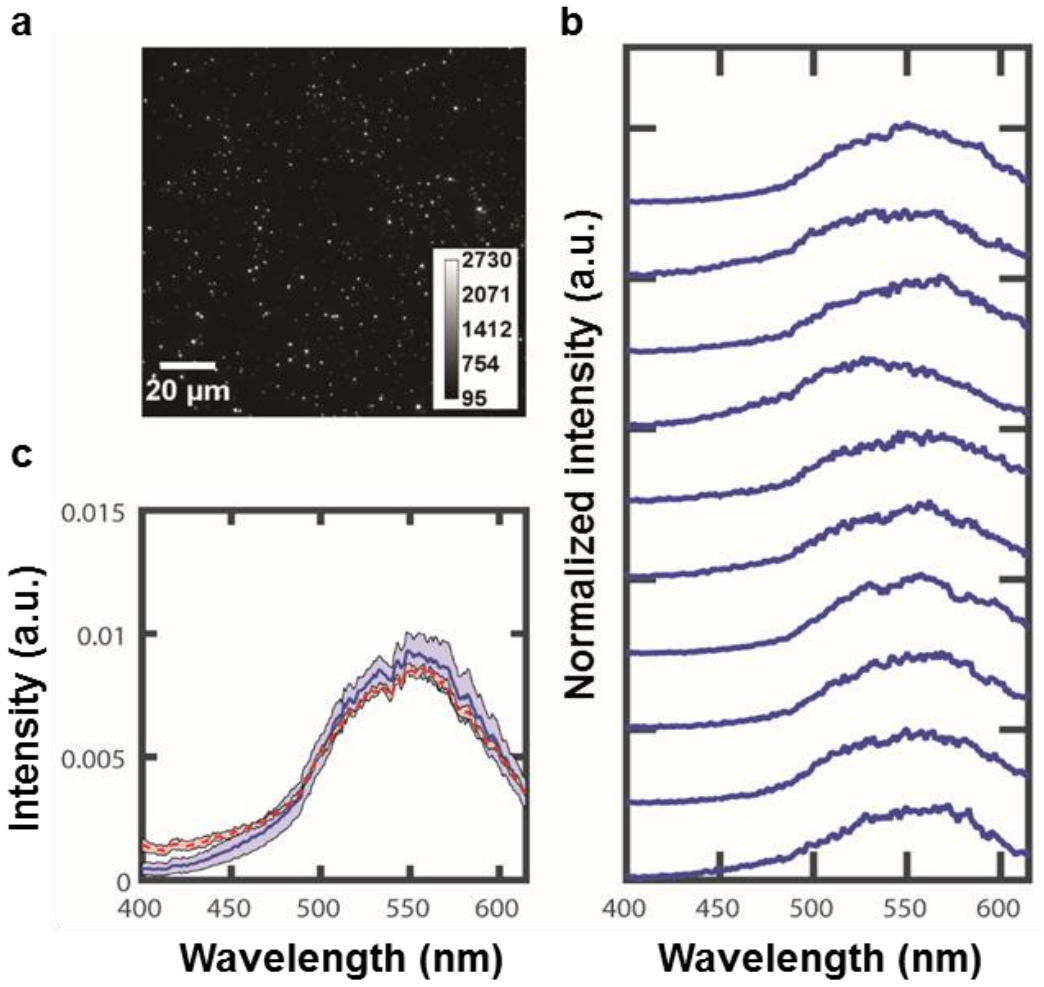
Our system





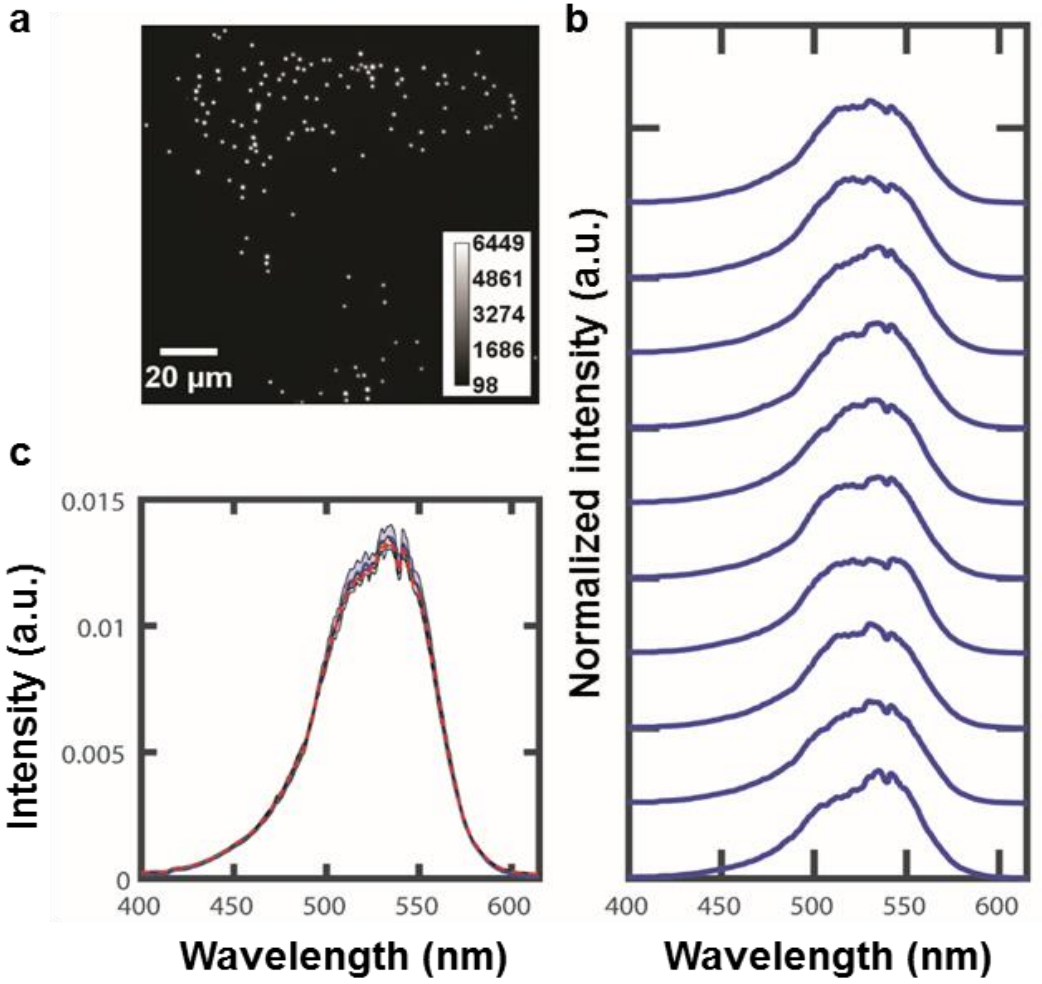
- Prizm/Objective type total internal reflection fluorescence microscope
- Built around Olympus IX73 inverted microscope
- Fianium supercontinuum laser with Photon Etc volume Bragg grating filter
- Andor IXon3 EMCCD camera

PLE measurements from FNDs



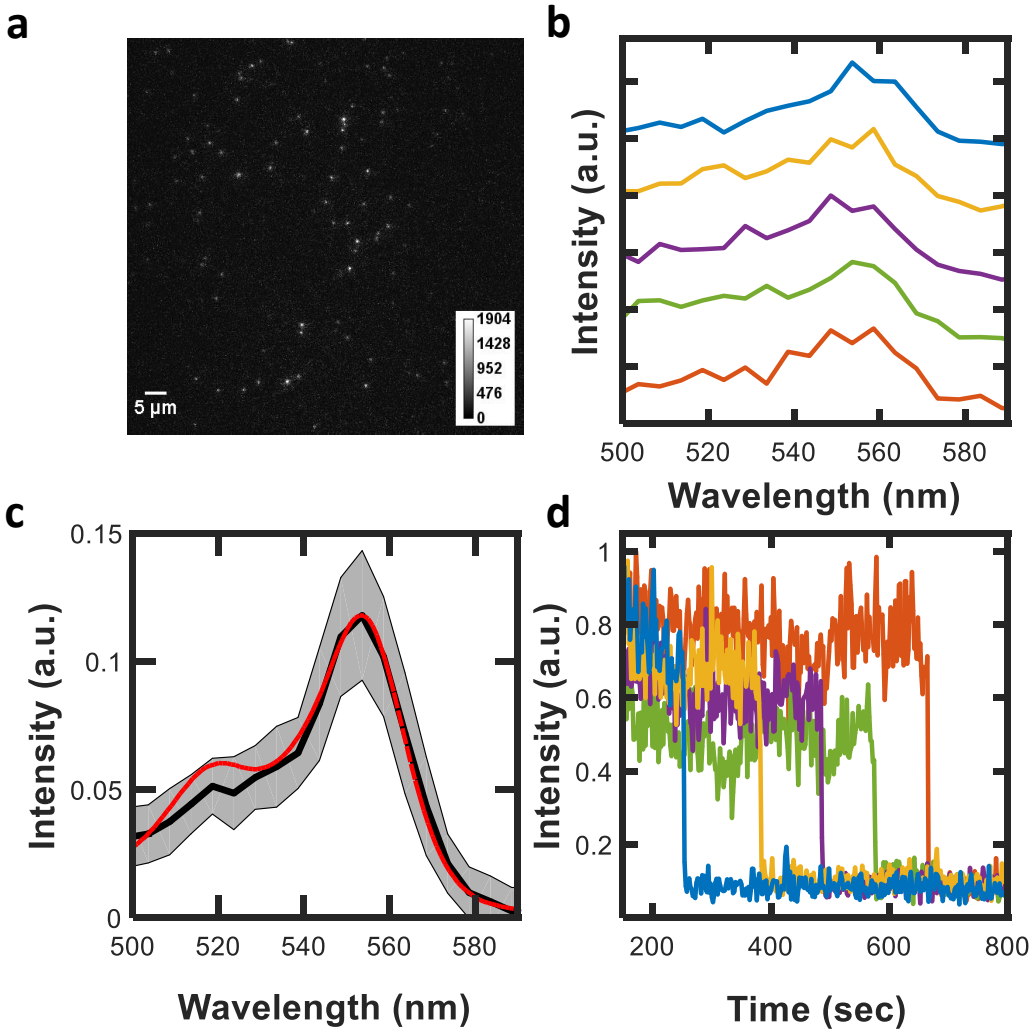
- We took PLE spectra of more than 100 individual FND
- Scans were over from 400 – 600 nm with 1 nm resolution

PLE measurements from beads



- We took PLE spectra of more than 100 dyed polystyrene beads
- Scans were over from 400 – 600 nm with 1 nm resolution

PLE measurements from Alexa Fluor 555



- Scans were over from 500 – 600 nm with 5 nm resolution
- Distinct one step photobleaching

So what's the big deal?

- One size fits all laser!
- Improved SNR!
- Single molecule sensitivity!

Questions?

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