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Complimentary Fluorescence Lifetime and Raman Microscopy Methods for Cellular Imaging

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Raman microscopy and fluorescence lifetime microscopy are two complimentary techniques used as contrast methods for a biologically interesting cellular image. Often enough, the optical phenomena of fluorescence and Raman interfere with each other depending on the molecular and optical properties of a cellular sample. Instead of avoiding and Raman signals in fluorescence microscopy and avoiding fluorescence signals in Raman microscopy, the two are used as complimentary techniques to extract molecular information from a cellular sample. Exciting this particular sample with NIR light and collecting the image using a Raman microscope gives information about molecular structures around the different parts of the cell. Different contrast information in a cell can be used by fluorescence microscopy. With excitation in the visible wavelength range, the fluorescence signal from the same sample is enhanced and the image shows differences in molecular fluorescence by the cellular structures, lending different variations in the image. In addition, the fluorescence lifetime image is taken using the same fluorescence microscope on the same sample, supporting a third method of differentiation in the microscope image.

All Raman images were measured on an XploRa Raman microscope with a 785 laser for excitation. All fluorescence images were measured with a DynaMyc fluorescence lifetime microscope with a 450 nm, 50 MHz pulsed laser diode and both a fluorescence camera and a photomultiplier tube (PMT). Dichroic filters were selected for emission collection on both microscopes. Difficulties in using both methods here include high amounts of scattered light, and liquid media. While highly scattering samples is optically preferred for Raman signals, this phenomenon can cause artifacts in fluorescence signals. On the reverse side, Raman signals are usually very weak when using liquid media due to the lack of scattering signal. By utilizing optimal filters and excitation sources, these two issues can be overcome by optimization of the spectroscopic and microscopy instrumentation and methodologies presented here.

With fast scanning of the lifetime decays using time-correlated single photon counting, both fluorescence intensity and lifetime images, are easy for looking at a single image with different contrast methods. Raman microscopy lends the third level of contrast with information on molecular structure and environment with a cell.