

ONE AND TWO PHOTON HYPERSPECTRAL FLUORESCENCE LIFETIME IMAGING

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KEY WORDS: Fluorescence lifetime imaging, hyperspectral imaging.

Measurement of fluorescence excitation and emission spectra and the temporal fluorescence decay (lifetime) may provide valuable information on the biochemical and structural properties of molecular tissue components and protein interactions in cells. In practice, however, most imaging instrumentation operates primarily in the spectral or the time domain. We report the development of two spectro-temporal microscope systems that measure the fluorescence lifetime as a function of wavelength at every spatially resolved position in the sample. In addition, we can use a novel tunable continuum source (TCS) to make *in-situ* measurements of relative excitation spectra and may thus record the time-evolution of the full excitation-emission matrix. We believe this approach will be useful to optimize and understand the origin of fluorescence contrast and are applying it to tissue autofluorescence.

This spectro-temporal imaging may be implemented using single or multi-photon excitation. For each case we use line excitation from an ultrafast laser and relay the resulting fluorescence to an imaging spectrograph, providing the full spectrum of the line image. Temporal resolution is achieved using a wide-field time-gated optical intensifier and stage scanning of the sample across the excitation line allows this hyperspectral fluorescence lifetime data set to be acquired across two spatial dimensions of the sample. We are using the TCS to optimise the excitation wavelength and to obtain the full lifetime excitation-emission matrix of the sample. These instruments are being applied to the study of the endogenous fluorescence in cartilage in an attempt to find a fluorescence-based molecular signature of disease. As an example of hyperspectral-temporal imaging, we have analysed the spectral FLIM data after exciting a sample of untreated porcine cartilage excited at 400 nm on the single-photon microscope. Figure 1 shows that the fluorescence intensity and the histograms of the FLIM maps of the specimen over the whole spectrum and over two different spectral regions (450 to 550nm) and (550 to 650 nm). These histograms show that different lifetimes are measured for each spectral window. Given this information, lifetime contrast can be enhanced by appropriate choice of the spectral window.

We note that spectro-temporal measurements are well established for cuvette studies but are not usually applied in an imaging modality. Appropriate spectral and temporal sampling permits convenient studies of tissue autofluorescence and may be applicable to live cell imaging. In general, exploring the full excitation-emission-lifetime parameter space offers new opportunities to maximise contrast, hopefully allowing the detection of diseased states in tissue with higher specificity and sensitivity.

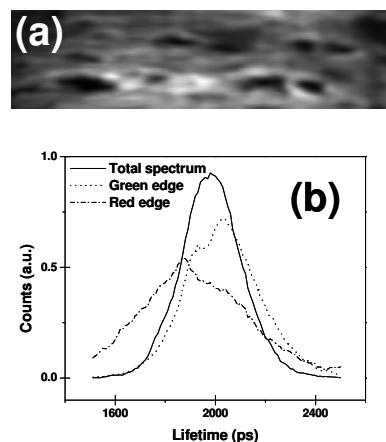


Figure 1: Lifetimes of a sample of cartilage (a) analysed over different spectral windows (b)