Spectrally resolved FLIM for accurate lifetime investigation

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KEY WORDS: Fluorescence lifetime, Spectrally resolved, Tunable bandpass filter, TCSPC

Fluorescence lifetime imaging microscope (FLIM) is a good method to distinguish various fluorophores in bio-specimen because fluorescence lifetime is sensitive to local environment such as pH, charges, refractive index, and temperature [1]. However, it is difficult to measure lifetimes of a specific fluorophore if there are many fluorophores in bio-specimen. In that case, many emission spectra of fluorophores are overlapped [2] so that separations of each fluorophore’s spectrum are necessary to get more exact information of fluorescence lifetime.

In this paper, spectral FLIM system is implemented by using ‘Angle-tuned bandpass filters (ATBFs)’. Center wavelength penetrating ATBF is varying with incidence angle on ATBF. So transmission wavelength can be controlled simply by rotating an ATBF which has own spectral bandwidth. We chose one kind of this ATBF as product of Semrock’s 550/15 nm Versachrome® of which center wavelength is varying from 550 nm to 486.5 nm when AOI is changing from 0° to 60° and maintains 20 nm FWHM bandwidth all over the range. In addition, in order to control spectral bandwidth we set two ATBFs in series. If we adjust each ATBF’s angle slightly different relatively, spectral bandwidth can be narrower than before. In this experiment, we set spectral bandwidth to 10 nm and observed spectrally resolved FLIM images. We confirmed nuclei and membranes of lung cancer tissue (age 80, male) are well distinguished by spectrally resolved analysis in both images and lifetime distribution histograms.
