SPECTRAL-LIFETIME UNMIXING ALGORITHMS FOR BIOLOGICAL FLUORESCENCE IMAGING

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ABSTRACT

Recent advances in molecular imaging technology have enabled simultaneous measurement of the emission spectra and lifetime of fluorescent molecules. Multispectral imaging enables the multiplexed labeling of tissue features for biological research, allowing individual biomarker distributions to be characterized spectrally and separated using unmixing algorithms. However, there exist several physiologically relevant factors whose changes have little or no effect on the fluorophore spectra, such as the binding state of the fluorophore, pH of the microenvironment, and metabolic activity. Combined spectral-lifetime imaging provides additional independent information along the temporal dimension, helping to further characterize the dyes to improve unmixing with respect to such factors. Current unmixing algorithms, however, are limited to multispectral imaging. Two analytical methods are proposed for unmixing dye contributions using both spectral and temporal information. A robust global analysis using a biexponential fluorescence decay model was formulated to solve the spectral and temporal variables simultaneously. Additionally, an iterative approach was developed to handle larger datasets, in which segmentation was based on lifetime, after which spectral fitting was performed on each region.

The two methods were tested on both calibrated experimental data and simulations, both of which consisted of 16 wavelengths and 25ps time bins. We show that unmixing algorithms that consider both spectral and temporal dimensions achieve more accurate unmixing than traditional multispectral unmixing techniques. Unmixing the real data showed less than 7% error of the known dye fractional contributions using the more robust global analysis, and less than 9% error with the iterative technique. Additional experiments on simulated data in which SNR and spectral separation between dyes were varied showed the iterative technique as achieving 10% higher accuracy compared with traditional unmixing techniques for spectral shifts less than 4nm and maximum photon counts of less than 200. Although our study was limited to microscopy data, we anticipate the extension of this work to other fluorescence imaging modalities, such as Diffuse Optical Tomography.