Hyperspectral imaging techniques have recently been applied to many biological applications to improve isolation of individual fluorophores in multilabel samples and identify fluorophores in the presence of highly autofluorescent tissue. Hyperspectral imaging is traditionally performed by collecting fluorescence emission over a broad wavelength range (emission scanning). However, significant light loss and long acquisition times can result from filtering the emission light.

Excitation scanning is a novel method of hyperspectral imaging that may provide higher sensitivity for detecting fluorophores than traditional emission-scanning techniques. Excitation scanning is performed by filtering the excitation light over many wavelengths, and subsequently collecting the emission at each excitation wavelength. This results in higher available signal, and shorter acquisition times.

Here, we report implementation of an excitation-scanning hyperspectral imaging microscope and preliminary results comparing excitation scanning to emission scanning. A comparative study was conducted using a model of lung injury featuring GFP-expressing pulmonary microvascular endothelial cells (PMVECs) in highly autofluorescent lung tissue. Our results indicate 1-2 orders of magnitude increased signal detection using excitation-scanning techniques compared to emission-scanning, and improved sensitivity for detection of GFP in autofluorescent lung tissue. Additionally, excitation scanning resulted in higher delineation of nuclear regions compared to emission scanning. Our future work will further test the efficacy of excitation scanning compared to emission scanning for applications in FRET detection, multi-label studies, and detection of changes in autofluorescence due to lung cancer.