Advanced Open-Source Machine Learning Tool for Hyperspectral Fluorescence Imaging in Ophthalmology

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Introduction: The eye, a complex optical system, consists of multiple layers, each with unique optical properties, including the cornea, sclera, uvea, and retina. These layers exhibit autofluorescence, observable through fluorescence microscopy, which is often considered as background noise when detecting signals from exogenous fluorescent probes targeting specific molecules. By integrating fluorescence microscopy with hyperspectral imaging technology, we have been able to acquire datasets with enhanced spectral and spatial resolutions. Recent advancements have demonstrated that the distinct autofluorescence spectral signatures of eye layers can be utilized for segmentation purposes. Nevertheless, the visualization and analysis of Hyperspectral Fluorescence Microscopy Imaging (HFMI) data present significant challenges. Our objective is to develop an open-source desktop application designed for the preprocessing, visualization, semantic segmentation, and boundary detection of hyperspectral images of eye tissue sections

Methods: This platform was developed utilizing hyperspectral datacubes (210 X 210 X 54), derived from frozen sections of pigmented and albino mouse eyes, captured using a snapshot Image Mapping Spectrometer imager (54 wavelengths from 528 to 836 nm) mounted on a fluorescence microscope. The aim is to devise segmentation and boundary detection algorithms for examining the distribution of biomolecules exhibiting endogenous fluorescence within specific layers of eye tissue. The algorithms, including Spectral Information Divergence Spectral Angle Mapper (SIDSAM) with an optional unmixing feature, and Spatial Fuzzy C-means (FCM) clustering integrated with a Sobel edge detector, have been incorporated into the application. This app provides a comprehensive suite of functionalities for data preprocessing—such as normalization, denoising, and superpixel generation—alongside visualization tools. These tools enable 2D and 3D spectral-based interactive exploration, selection of regions of interest (ROI), calculation and display of average spectral curves, and the preliminary identification of eye layer signatures through segmentation tools.

Results: The segmentation algorithms and additional tools integrated into the application offer an effective approach for analyzing Hyperspectral Fluorescence Microscopy Imaging (HFMI) across different environments. This integration facilitates the unsupervised, label-free segmentation of eye layers, including the retina, choroid, and sclera, significantly diminishing the time required for experts to label these layers for subsequent quantitative analysis.

Conclusions: The described desktop application, featuring label-free segmentation algorithms, enables users to derive insights from and add significance to complex biomedical hyperspectral data. Intended for release as an open-source tool, it is adaptable for use with various tissues beyond its initial scope. Ultimately, this application is poised to expedite research efforts aimed at validating clinical imaging techniques through hyperspectral imaging.