Spectral Autofluorescence: Towards Quantitative Pathology

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Abstract

Cancer is the result of cells that have undergone abnormal changes in their programming, leading to uncontrolled growth and invasion into surrounding tissues. These cells propagate between healthy tissues and are characterized by subtle shape and color. Surgeons and pathologists train for years in recognizing these features, utilizing standard color cameras, white light and trained human eyes. Scientists at the Translational Imaging Center, developed technologies that probe the intrinsic signals in tissues utilizing modalities of imaging that overcome the limits of human vision: hyperspectral imaging and fluorescence microscopy. In previous work, we applied these technologies to image cancer in fixed human tissue samples. Utilizing advanced microscopes, we imaged fixed esophageal cancer biopsies from patients, mosaicing single-cell images across millimeter-scale samples and utilizing multiple laser illuminations. The product is a large, complex and information-rich database that spans across the dimensions of fluorescence emission and excitation, with corresponding datasets from the gold standard of pathology, labeled by expert pathologists.

This year, we applied the multi-dimensional database with analytical approaches to quantify the spectral signature of cancer with respect to the one of healthy tissues, referencing to the gold standard of pathology. Our work aimed to summarizing the results in a scientific publication.

Background

The current standard of pathology requires H&E staining of pathology slides, then technicians perform white light imaging of the sample after it is stained. A pathologist uses previous experience and training to look at the morphology, stain color, and bias. Importantly, a pathologist’s work is subjective, requiring 30-40 minutes for staining, and $70, and hiring a pathologist costs $311.2 billion industry where staining and imaging a slide costs $70, and hiring a pathologist costs $150/h. The process is time-intensive, requiring 30-40 minutes for staining, and up to an hour or more for imaging and analyzing, depending on sample complexity. Importantly, a pathologist’s view, subject to their training, isn’t quantitative, presenting potential for error or bias.

Objective

• The objective of this project is to investigate a faster, quantitative method to distinguish cancerous tissue from non-cancerous tissue using autofluorescence microscopy.

Approach

Slide Acquisition

Data Collection

Data Analysis

Pathology structure

Pathologist annotations

Spectral Autofluorescence Microscopy

Unstained cancer biopsy slide

Unstained cancer biopsy slide

Cancer

Cancer

Non-cancerous

Non-cancerous

Spectral range 410nm-692nm.

Fig 1c: White Light Imaging of Esophageal Cancer stained tissue with pathologist’s annotation.

Fig 1a: Esophageal Cancer Slide. Stained with H&E.

Fig 1b: Esophageal Cancer Slide, Unstained.

Fig 2a: SEER (Spectrally Encoded Enhanced Representations) technology offers a faster, less costly alternative, providing a quantitative reference for pathology, which can reduce diagnostic errors.

Results

• Collected 23 hyperspectral datasets of different patients from different esophageal cancer patients.

• Identified 7 different wavelengths (450nm, 495nm, 546nm, 561nm, 594nm, 633nm) detected wavelengths in the spectral range 410 nm-692 nm.

• Used SEER (Spectrally Encoded Enhanced Representations), which plots spectra onto a plot. The sine Fourier transform (S) is the y-axis and the cosine Fourier transform (G) is the x-axis.

• Applied 5 different color maps to each image (contour, gradient descent, gradient descent mass morph, radius phasor, and radius phase mass morph).

• Used Fij to mask the cancerous area and non-cancerous area to find the barycentric center of mass for the phaser plot, which was represented by the g and s coordinates.

• Found the average of all centers of massess and used it to find the average cancerous and non-cancerous areas on the phaser plot for each wavelength.

• Used the average area for the cancerous and non-cancerous area to plot a region of interest on the phaser plot, having the radius be the variance of the region.

Fig 6b: Table reporting the p-values for G and S phasor coordinates of cancerin-non-cancerous areas at different excitation values

Conclusions

• SEER analysis shows cancer and non-cancerous tissue have different spectra and can be visualized with different color.

• SEER images allow visual differentiation of cancerous and non-cancerous regions.

• Created a database of 10 samples of Esophageal Cancer with 7 separate EX wavelengths and each EX wavelength has 32 EM wavelengths.

• Tested multiple SEER maps and visually, Gradient Descent Mass Morph showed the most difference in color.

Summary

Our research work centers on the potential use of autofluorescence spectral imaging to address the inherent subjectivity and potential for error in traditional pathology. This technology offers a faster, less costly alternative, providing a quantitative reference for pathologists. The quantitative analysis can expose features possibly missed using conventional methods, without necessitating slide staining. This compares favorably to the more time-consuming and expensive H&E staining process. In addition, our work with the SEER1 technology has shown promising results, enabling rapid identification and differentiation between healthy and cancerous tissue. As we continue our research, we look forward to testing this technique on a broader range of cancers, such as skin cancer. We also aim to evaluate the efficacy of this approach on unstained slides without pathology annotations, with the ultimate goal of generalizing the technology and identifying cancerous areas without a pre-existing ground truth.