Combined multiphoton microscopy and somatostatin receptor type 2 imaging of pancreatic neuroendocrine tumors

Noelle Daigle[1], Thomas Knapp[2], Suzann Duan[3], David W. Jones Jr. [3], Ali Azhdarinia[4], Sukhen C. Ghosh[4], Solmaz AghaAmiri[4], Naruhiko Ikoma[5], Jeannelyn Estrella[5], Martin J. Schnermann[6], Juanita L. Merchant[3], Travis W. Sawyer[1,2,3]

Wyant College of Optical Sciences[1], Department of Biomedical Engineering[2], College of Medicine[3], University of Arizona
Health Science Center at Houston[4], MD Anderson Cancer Center[5], The University of Texas
National Cancer Institute[6]

Biomedical Optics & Optical Measurement

THE UNIVERSITY OF ARIZONA

Background

Pancreatic neuroendocrine tumors (PNETs) are a rare but increasingly more prevalent cancer, with diagnoses increasing six-fold over the last two decades. Surgery is the preferred method of treatment for the majority of PNETs, yet existing surgical localization methods provide poor contrast and resolution (Fig. 1), resulting in incomplete resections and pushing surgeons to perform more demolitive surgeries than necessary [1]. Better intraoperative localization techniques are needed to improve survival and quality of life.

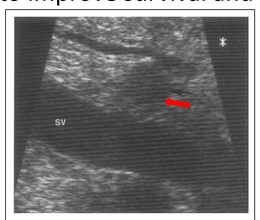

Fig 1. A PNET located in the body of the pancreas using intraoperative ultrasound [3].

Multiphoton microscopy (MPM) is a fast-growing optical imaging technique that provides label-Free tissue contrast at cellular-level resolutions. However, MPM is limited to small, sub-mm fields of view, and necessarily would require a secondary imaging technique capable of macroscopic surveillance to direct sampling over a large area [2]. Notably, somatostatin receptor type 2 (SSTR2) is overexpressed in >80% of PNETs [1], indicating fluorescently-tagged SSTR2 imaging (Fig. 2) could be used for wide-field localization to complement the inherent small field of view of MPM.

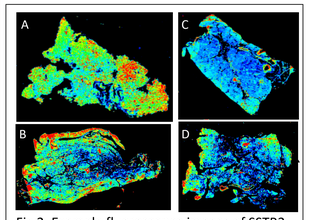

Fig 2. Example fluorescence images of SSTR2-labeled (A,B) PNET and (C,D) normal tissue. Red is high concentration, blue is low.

Objective

Our work tests the suitability of combined SSTR2 imaging and MPM for localizing PNETs, combining the labeled technique of SSTR2 fluorescence imaging with the label-free technique of MPM for enhanced contrast.

Methods

We imaged 12 fixed frozen patient samples with MPM and fluorescence imaging. Six samples were tumor and six normal pancreatic tissue. Five wavelength channels were obtained using MPM, selected to probe four endogenous fluorophores that are common biomarkers of disease (FAD, NADH, lipofuscin, and porphyrin) and second harmonic generation (SHG), a light scattering event exhibited by non-centrosymmetric molecules, predominantly collagen. Fig. 3 shows selected excitation and detection wavelengths.

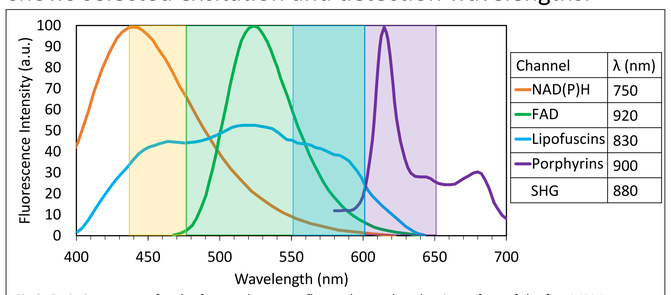

Fig 3. Emission spectra for the four endogenous fluorophores that dominate four of the five MPM channels for an excitation wavelength of 344 nm [4]. Actual excitation wavelengths used for all five MPM channels are listed in the legend. Colored overlays correspond to detection wavelength ranges for each channel.

Texture features were extracted using Haralick's method for all five MPM images. For the SSTR2 images, 10 regions of interest were selected and a simple average intensity was calculated. Images were classified using linear discriminant analysis (LDA), and a leave-one-out approach used to determine LDA classifier accuracy [5].

Results

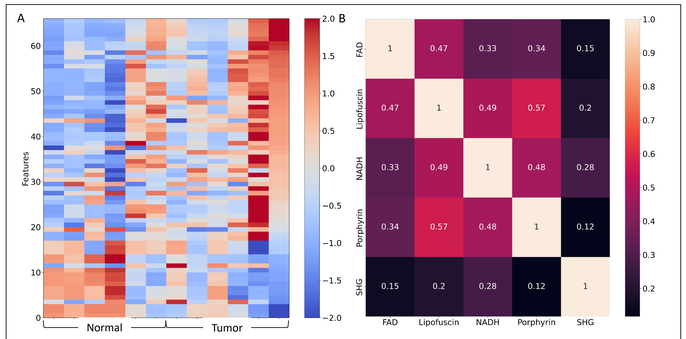

Fig 4. Visualization of features and correlation between imaging channels. (A) Z-scores of features for each sample, and (B) heat matrices showing average correlation between imaging channels for all samples.

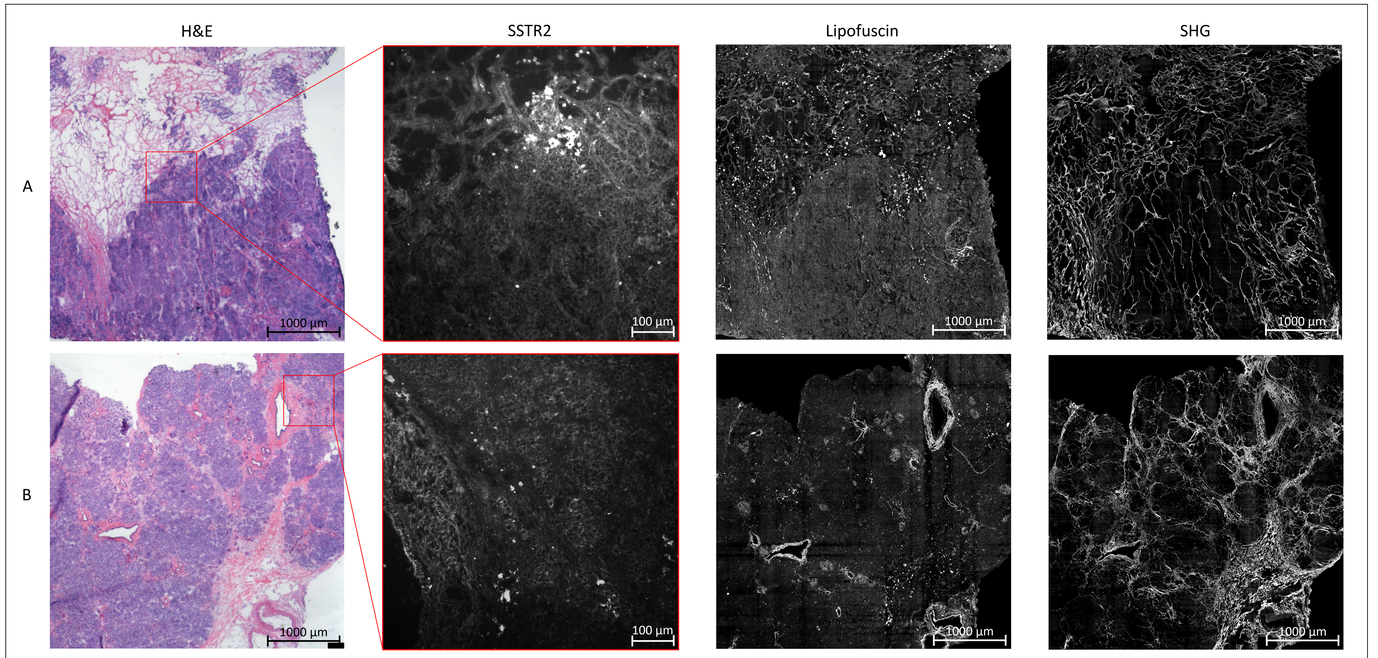

Fig 5. Selected MPM and SSTR2 images for one tumor (A) and normal (B) sample. Note the SSTR2 images are one region of interest chosen from the set of 10 used for analysis. Hematoxylin and eosin (H&E) images were obtained for all samples for ground truth validation. Images were brightened for viewing.

Conclusions

We demonstrate that 4 features are sufficient to classify tumor and normal tissue with 100% accuracy using both MPM and SSTR2 images. Using only MPM images, we can obtain the same accuracy with the same number of features, indicating MPM could be used to determine surgical margins with high sensitivity and specificity. Using only SSTR2 images and 1 feature, we obtain an accuracy of 66.6%, indicating that SSTR2 provides sensitivity to disease, but the addition of MPM can improve sensitivity and specificity (Fig. 6).

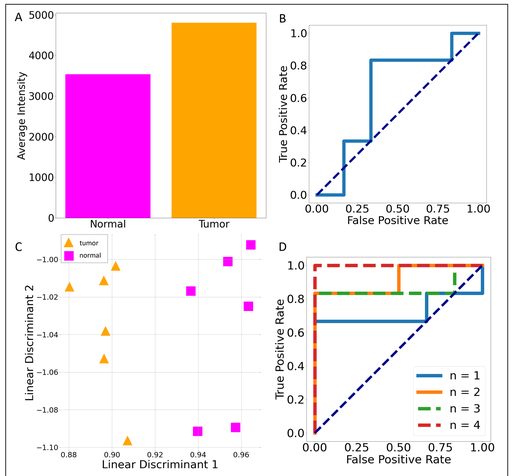

Fig 6. (A) Bar chart of average SSTR2 average intensity for tumor and normal tissue. (B) Receiver operator characteristic (ROC) curve for classifiers developed using only the SSTR2 feature. (C) LDA projection for n=4 features for classifiers developed using both MPM and SSTR2 features. (D) ROC curve for classifiers developed using both MPM and SSTR2 features.

Acknowledgements

We would like to thank the Imaging Core Marley and other members of the Merchant and Sawyer laboratories for their support at the University of Arizona, as well as our collaborators at the Azhdarinia laboratory for supplying the NIR SSTR2 dye, and at MD Anderson for clinical expertise and guidance.

References

[1] Bartolini, I., et al. (2018). Current Management of Pancreatic Neuroendocrine Tumors: From Demolitive Surgery to Observation. *Gastroenterology Research and Practice.* https://doi.org/10.1155/2018/9647247

[2] Borile, G., et al. (2021). Label-Free Multiphoton Microscopy: Much More Than Fancy Images. *International Journal of Molecular Sciences*, *22*(5), 2657. https://doi.org/10.3390/ijms22052657

[3] Gorman, B., & Reading, C. C. (1995). Imaging of Gastrointestinal Neuroendocrine Tumors. *Seminars in Ultrasound, CT, and MRI*, *16*(4), 331–341.

[4] Croce, A. C., & Bottiroli, G. (2014). Autofluorescence spectroscopy and imaging: A tool for biomedical research and diagnosis. *European Journal of Histochemistry*. https://doi.org/10.4081/ejh.2014.2461

[5] Knapp, T. G., Duan, S., Merchant, J. L., & Sawyer, T. W. (2022). Quantitative Characterization of Duodenal Gastrinoma Autofluorescence using Multi-photon Microscopy (p. 2022.05.19.492747). *bioRxiv*. https://doi.org/10.1101/2022.05.19.492747