# Machine learning algorithm to classify multiphoton microscopy images of pancreatic neuroendocrine tumors

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## Background

Surgery is the preferred method of treatment for most pancreatic neuroendocrine tumors (PNETs), particularly functional PNETs or those greater than 2 cm in largest dimension. Existing techniques include intraoperative ultrasound and manual palpation, both of which have inherent disadvantages such as poor resolution and low contrast against normal pancreatic tissue (Fig. 1). This results in surgeons performing more demolitive resections, such as the Whipple procedure, when they may not be strictly necessary in order to ensure total removal of tumors [1]. Therefore, improving surgical localization methods could greatly improve patient outcome and quality of life.


Fig 1. (A) Intraoperative laparoscopic sonography and (B) intraoperative sonography of gastrinomas [1].

Multiphoton microscopy (MPM) is an optical imaging technique capable of visualizing intrinsic biomarkers through two-photon fluorescence (Fig. 2), and collagen through second harmonic generation (SHG), notably without the aid of exogenous labels and with increased penetration depth compared to conventional microscopy [2].

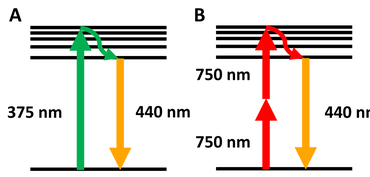


Fig 2. (A) Traditional microscopy using single photon excitation. (B) Multiphoton microscopy using two photon excitation [2].

## Objective

*Our work tests the use of MPM as a new method of microscopic tumor localization by applying this data to novel machine learning algorithms designed to automatically classify tissue types. Previous work has shown that fixed frozen samples are able to be classified accurately using this method; this work further probes whether sample preparation affects our results.*

## Methods

Formalin-fixed paraffin-embedded PNET (n=27) and normal pancreas (n=21) samples were imaged with a multiphoton microscope at five excitation and emission wavelengths corresponding to four endogenous fluorophore and SHG signals (Fig. 3A). Images covered an area approximately 4 mm by 4 mm. Texture features were then extracted using Haralick's method [3], and a computer model trained to classify the samples using linear discriminant analysis [4]. Sets of one to six features were tested, and models assessed using a leave-one-out-approach. Accuracy of classifiers was evaluated as the ratio of the number of correctly identified samples (Fig. 3B).

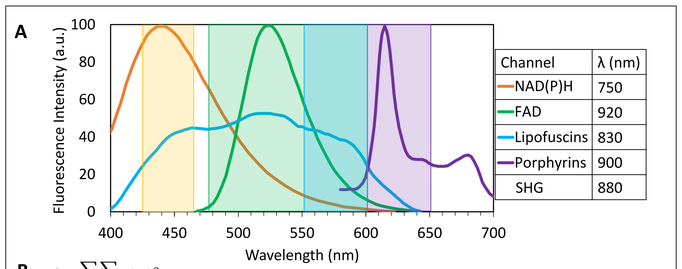


Fig 3. (A) Emission spectra for the four endogenous fluorophores that dominate four of the five MPM channels for an excitation wavelength of 344 nm [5]. Actual excitation wavelengths used for all five MPM channels are listed in the legend. Colored overlays correspond to detection wavelength ranges for each channel. (B) The method used to determine optimal texture features for classification.

## Results

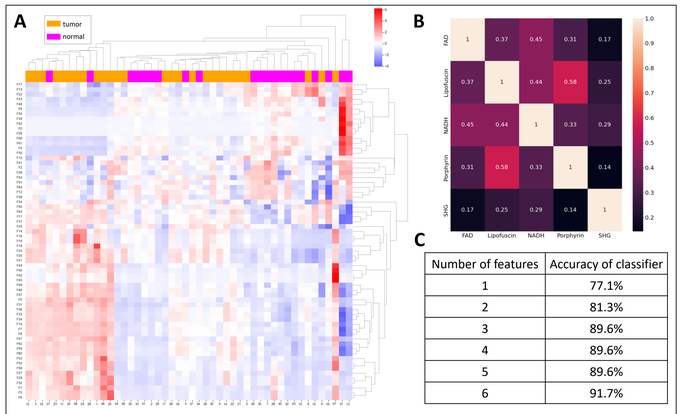


Fig 4. Visualization of features and correlation between imaging channels. (A) Z-score dendrogram of features for each sample, and (B) a heat matrix showing average correlation between imaging channels for all samples. (C) Accuracy of classifiers defined as number of correctly labeled samples out of total samples.

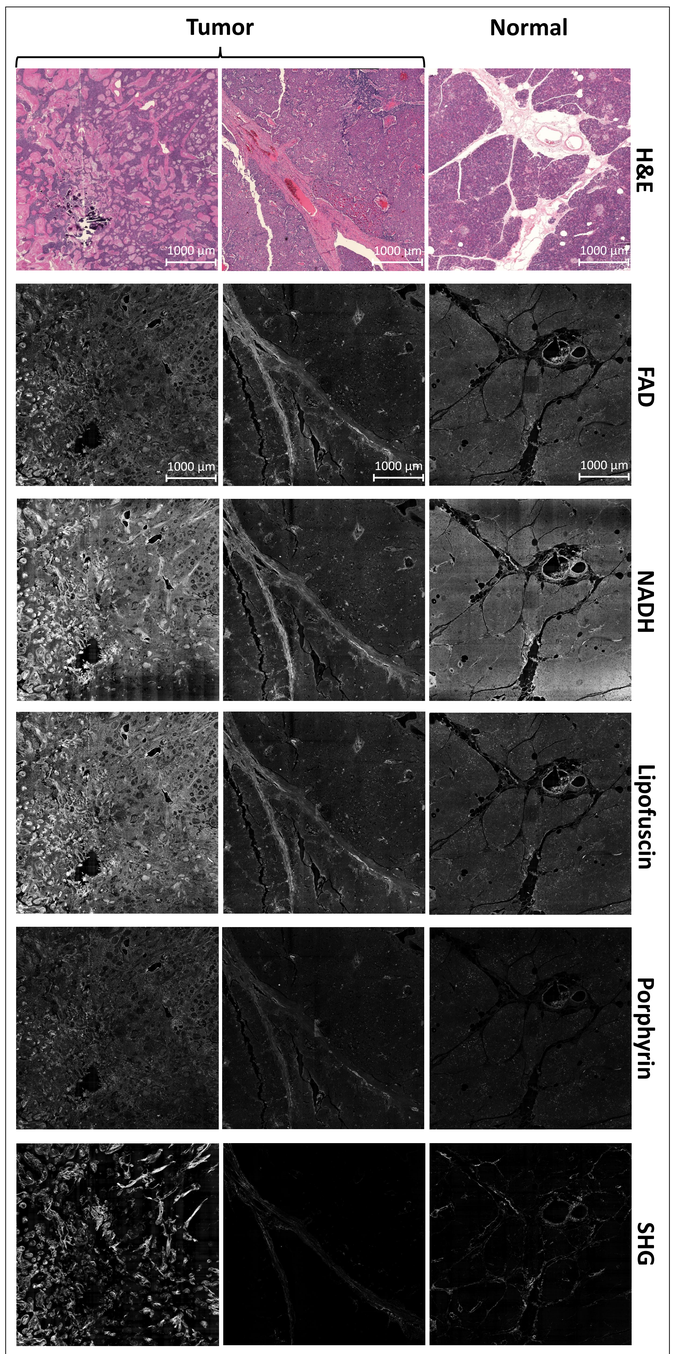


Fig 5. Selected MPM images for two tumor and one normal sample. Hematoxylin and eosin (H&E) images were obtained for samples for ground truth validation. Brightness was increased by 40% and contrast by 20% for MPM images for improved viewing.

## Conclusions

We have demonstrated that using texture analysis with MPM images, we are able to distinguish between PNETs and normal pancreatic tissue to 91.7% accuracy. By building this model, we can begin to test which imaging wavelengths and texture features are optimal for distinguishing the tissue types, which in turn provides guidelines for the development of new surgical guidance instruments. This supports the continued investigation of MPM as a clinical imaging technique and lays the groundwork for the integration of machine learning methods to real time imaging.

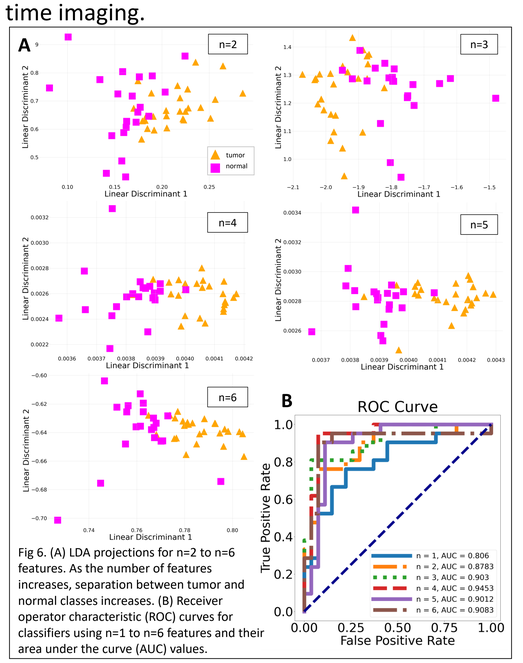


Fig 6. (A) LDA projections for n=2 to n=6 features. As the number of features increases, separation between tumor and normal classes increases. (B) Receiver operator characteristic (ROC) curves for classifiers using n=1 to n=6 features and their area under the curve (AUC) values.

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