Characterizing the Optical Fingerprint of Duodenal Gastrinoma Using Quantitative Multi-Photon Autofluorescence Microscopy

Thomas G. Knapp1, Suzann Duan2, Juanita L. Merchant2, Travis W. Sawyer1,2,3
Department of Biomedical Engineering,2 College of Medicine,2 and College of Optical Sciences,3 University of Arizona

Background and Motivation
Duodenal gastrinoma tumors (DGasts) secrete the hormone gastrin which results in the over-production of stomach acid, a condition termed Zollinger-Ellison Syndrome (ZES). ZES symptomology includes chronic diarrhea, stomach ulcers, tissue adhesions with increased acid secretion, and over-production of stomach acid, a condition termed Zollinger-Ellison Syndrome (ZES). DGasts typically develop as small, diffuse, lesions within the submucosa of the proximal small intestine (Figure 1). The submucosa of the proximal small intestine typically develop as small, diffuse, lesions within the submucosa of the proximal small intestine (Figure 1). The submucosa of the proximal small intestine typically develop as small, diffuse, lesions within the submucosa of the proximal small intestine (Figure 1).

Methods
Endogenous fluorescence was measured in formalin-fixed and paraffinized (FFPE) DGast samples from 12 patients using two-photon microscopy (Zeiss LSM 880 NLO microscope). Excitation wavelengths and detection bands (Figure 3) were chosen to collect fluorescence from molecules related to common markers of cancer:
- NADH: excitation: 700 nm; emission: 425 - 455 nm
- FAD: excitation: 925 nm; emission: 465 - 600 nm
- lipofuscin: excitation: 830 nm; emission: 550 - 600 nm
- porphyrin: excitation: 800 nm; emission: 590 - 625 nm

The optical redox ratio is a measure of tissue metabolic state determined by the ratio of NADH to FAD fluorescence.[2,3] Second-harmonic generation (SHG) is a light-scattering phenomena that is elicited by collagen structures. SHG was measured with 880 nm excitation and a 430 - 450 nm detection band.

Difficult resection of DGasts shifts the focus of treatment towards management of symptoms (e.g., PPI to reduce acid production), which does not mitigate the potential for tumor metastasis. Aggressive surgical resection, such as the Whipple procedure (Figure 2) is often recommended for patients at high-risk for spread of the cancer.

Hypothesis
Differences in the composition between DGasts and normal duodenal tissue will provide contrast with endogenous fluorescence optical imaging, laying the groundwork for augmented diagnostic and targeted resection tools.

Results

Conclusions
Significant differences in signal intensity of endogenous fluorophores is measurable in FFPE samples of DGast using two-photon microscopy. This suggests that endogenous fluorescence can be used as a label-free approach for distinguishing DGasts from healthy tissue due to differences in the relative abundances of fluorophores between tissue types. Specifically, our findings show fluorescence signals related to tissue metabolism, cell senescence, and local blood concentration.(6) These findings suggest different values when measured in DGast tumors and normal tissue of the duodenum.

Future Directions
Current efforts are focused on image texture analysis using Haralick feature extraction of the fluorescence and SHG images, which provides statistics on the spatial relationship of pixel gray-level values. Discriminant analyses (Example Figure 7) from each of the five image channels will provide insights into the type and number of features required for adequate discrimination of the DGast tumors from their surrounding environment. This information will inform further work in the development of imaging systems used for in-vivo detection of DGasts and other neuroendocrine tumors.

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References