A step towards Barrett's esophagus auto-fluorescence imaging using UV LEDs

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Summary
Screening of Barrett's Esophagus currently requires time-consuming biopsy and pathology. In a move towards a real-time imaging system for Barrett's morphology, 275nm and 365nm UV LEDs were used to excite tissue auto-fluorescence. The lining of porcine esophageal and duodenal tissues was used as a first step model for the changes apparent with the characteristic Barrett's transition to a more intestinal lining phenotype. Images showing easily visible differences in auto-fluorescence wavelength and intensity were captured using the iphone CMOS camera and analyzed.

Introduction
Barrett's Esophagus is a pre-cancerous condition where epithelial cells of the lower esophagus change morphology to resemble the epithelial cells of the small intestine. Early neoplasia can be difficult to detect using conventional white light endoscopy (1). As a first step towards real-time imaging of Barrett's, we use UV LEDs to assess intrinsic tissue auto-fluorescence of porcine esophageal lining and duodenal lining (a surrogate for the changes seen in Barrett's). The captured images are limited to visible wavelengths due to glass and filters on the CMOS, any UV light picked up is translated to blue. Images obtained using an Apple® iPhone® CMOS were analyzed for RGB differences.

Goals
- **Distinguish between esophageal and duodenal tissue on the basis of UV LED stimulated tissue auto-fluorescence**
- **Use a simple RGB intensity analysis to distinguish between tissue types**
- **Determine whether UV LED 275nm, UV LED 365nm, or combined UV LED illumination provides the most discrimination between esophageal and duodenal tissue types**

Experimental Methods
The lumens of esophageal and duodenal tissues were displayed side by side, allowing uniform illumination. Fresh surgical tissue samples were obtained from Legacy Research Institute (Portland, OR). Samples were slit to expose the lumen and washed prior to illumination. Tissues were examined within one hour of surgical removal.
Tissue samples were illuminated with 275nm, 365nm, or simultaneously with 275nm and 365nm. Irradiance at the tissue surface was 7.2 mW/cm² (275nm) and 206.5 mW/cm² (365nm). Light sources were 75mm from the tissue surface. Images were captured with an iphone CMOS camera. Tissues hydration was maintained with normal saline. Both tissues are included in each analyzed image. For each tissue type in an image, red/green/blue intensity histograms were constructed from three independent 256 x 256 pixel squares. The intensities were compared for the two tissue types.

Discussion
To date most tissue auto-fluorescent examination of Barrett's tissue has used excitation wavelengths in the visible region 400nm to 475nm, for example 425nm to 455nm (2). The result is an image where Barrett's and squamous tissue appear green while neoplasia appears magenta (3). These methods have in common a reliance on excitation wavelengths in the visible spectrum (1). UV LED illumination at 275nm showed an obvious average intensity shift in the blue channel from 129 RU (relative units) for duodenum and 244 RU for esophagus, easily allowing tissue discrimination. In comparison, the shift for 365nm was muted (275 RU esophagus, 174 RU duodenum) but with a larger distribution of intensities in duodenum. The CMOS camera images of the combined illumination also showed a high average intensity shift, 223 RU (esophagus) and 120 RU (duodenum).

Conclusions
A simple RGB image analysis of UV LED illuminated tissue lumen can provide a baseline for tissue discrimination between esophageal and duodenal tissue. While auto-fluorescent tissue discrimination is possible with 365nm excitation alone, including 275nm illumination is superior. Next steps will include examination of authentic Barrett's esophagus tissue samples.

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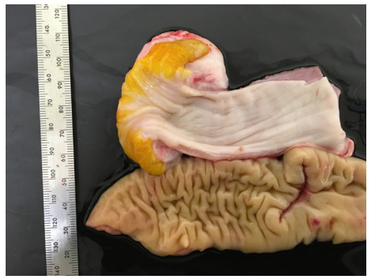
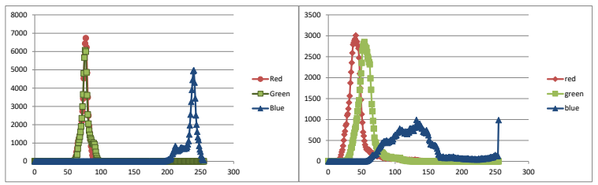


Figure 1: White light illumination of tissue samples
Top: Fresh surgical porcine esophageal tissue. Bottom: Fresh surgical porcine duodenal tissue. Tissue samples were sliced and rinsed in normal saline to expose the lumen. Photograph taken with An Apple® iPhone® CMOS camera.

References
1) Sturm and Wang (2015) Emerging optical methods of surveillance of Barrett's Oesophagus. Gut;64(11):1816-1823.
2) Niepsuj K, Niepsuj G, Cebula W, et al. (2003) Auto-fluorescence endoscopy for detection of high grade dysplasia in short segment Barrett's esophagus. Gatrointest Endosc 58(5):715-719.
3) Croce A.C. and Bottiroli G (2014) Auto-fluorescence spectroscopy and Imaging: a tool for biomedical research and diagnosis. Eur J Histochem 58(4):2461.
4) di Pietro M, Boerwinkel DF, Shariff MK, et al. (2015) The combination of auto-fluorescence endoscopy and molecular biomarkers is a novel diagnostic tool for dysplasia in Barrett's oesophagus. Gut;64:49-56.

Figure 2: UV LED 275nm illumination
The graphs show a comparison of the pixel intensity distributions for esophagus and duodenum. Each graph represents an independent measure corresponding to one of the target areas indicated on the photograph below. The vertical axes are number of pixels and the horizontal axes are intensity. For each target area, a representative graph of intensities is shown for the red, green and blue channels. In the photograph the esophageal tissue is above the duodenal tissue.



Esophagus		Duodenum	
Average blue intensity	244.7	Average blue intensity	129.3
Red/green to blue shift	162.0	Red/green to blue shift	81.7

Blue intensity difference E to D	115.3
Red/green to blue shift difference E to D	80.3

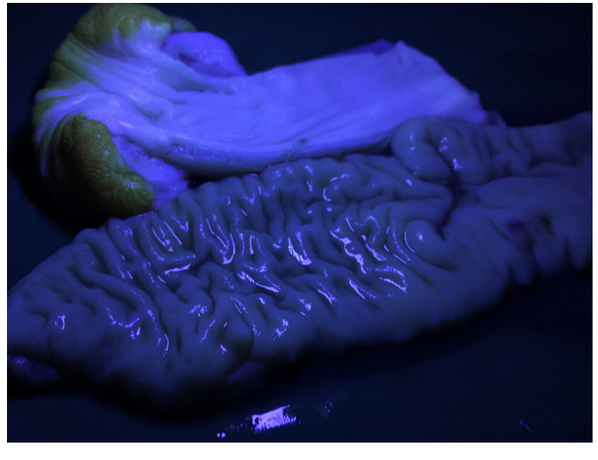
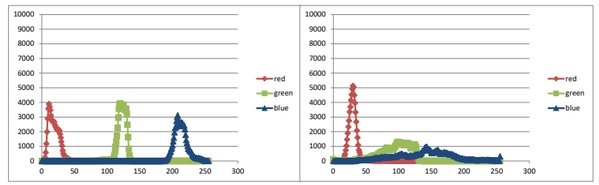


Figure 3: UV LED 365nm illumination
The graphs show a comparison of the pixel intensity distributions for esophagus and duodenum. Each graph represents an independent measure corresponding to one of the target areas indicated on the photograph below. The vertical axes are number of pixels and the horizontal axes are intensity. For each target area, a representative graph of intensities is shown for the red, green and blue channels. In the photograph the esophageal tissue is above the duodenal tissue.



Esophagus		Duodenum	
Average blue intensity	225.0	Average blue intensity	174.1
Red/green to blue shift	142.9	Red/green to blue shift	89.2

Blue intensity difference E to D	51.0
Red/green to blue shift difference E to D	53.6

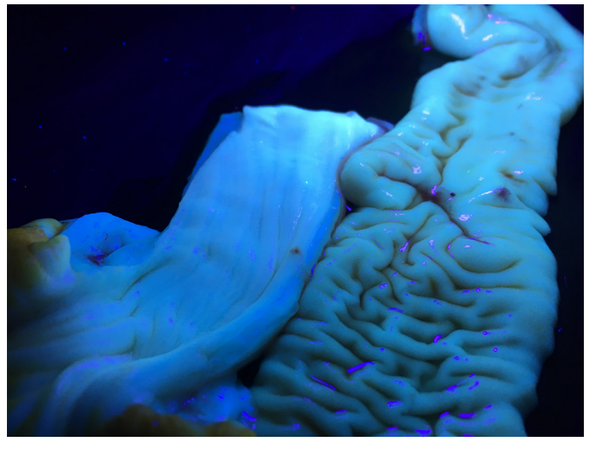
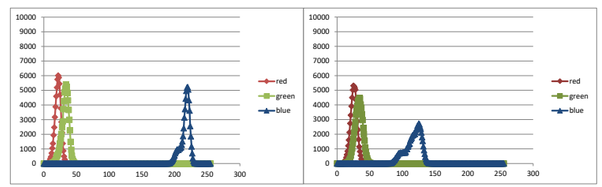


Figure 4: UV LED 275nm and 365nm simultaneous illumination
The graphs show a comparison of the pixel intensity distributions for esophagus and duodenum. Each graph represents an independent measure corresponding to one of the target areas indicated on the photograph below. The vertical axes are number of pixels and the horizontal axes are intensity. For each target area, a representative graph of intensities is shown for the red, green and blue channels. In the photograph the esophageal tissue is above the duodenal tissue.



Esophagus		Duodenum	
Average blue intensity	223.7	Average blue intensity	120.3
Red/green to blue shift	187.2	Red/green to blue shift	85.6

Blue intensity difference E to D	103.4
Red/green to blue shift difference E to D	101.6