Multispectral endoscopic imaging of colorectal dysplasia in vivo

Bishnu P. Joshi¹, Sharon J. Miller¹, Cameron M. Lee², Eric J. Seibel², and Thomas D. Wang¹,³

¹Department of Internal Medicine, University of Michigan, Ann Arbor, MI USA
²Department of Mechanical Engineering, University of Washington, Seattle, WA USA
³Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

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Light provides a broad range of colors in the visible and near-infrared (400–900 nm) spectrum that can be used to transmit information about molecular expression in normal and diseased tissues. The absorbance spectrum of hemoglobin, the primary chromophore in tissue, is shown in Fig. 1A. Genetic changes that occur in cancer transformation can be heterogeneous, and several signaling pathways may be activated concurrently.¹ Moreover, molecular activity levels may vary between individual patients, at different time points, and within the tumor. Thus, a single genetic mutation is unlikely to characterize most disease processes over a general population. Unfortunately, most endoscopic imaging methods are sensitive to only one molecular parameter.² Thus, a methodology that can image multiple molecular targets simultaneously is better suited to address the heterogeneity of cancer and achieve disease detection with high sensitivity and specificity desired for efficient surveillance. In addition, these multiplexed strategies can potentially visualize the interactions between signaling pathways and allow for better understanding of disease pathogenesis on a systems level.

Recently, significant progress has been made in advancing new methods of endoscopic imaging to target disease in the digestive tract, including the development of novel instruments and specific contrast agents.³ In this video journal, we aim to demonstrate the application of a novel, multispectral endoscope that uses a scanning fiber to image multiple targets at the same time. Fluorescence imaging can be achieved with high target-to-background ratios, and allow us to see the different target expression patterns in an Apc-mutation dependent mouse model of spontaneous colorectal adenomas using multiple peptides as targeting ligands.⁴

Author Contributions:
- Designed research – BPJ, SJM, EJS, TDW
- Performed research – BPJ, SJM, CML
- Contributed new reagents or analytic tools – BPJ, SJM
- Analyzed data – BPJ, SJM, TDW
- Wrote the paper – BPJ, TDW

Competing Interests: University of Michigan has filed a provisional patent on behalf of BPJ, SJM, and TDW on the peptides presented in this study.
**Description of Technology**

A CPC;Apc mouse model of spontaneous colorectal adenomas development was used under approval from the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. A scanning fiber endoscope was used that has a 1.6 mm outer diameter at the distal tip, and passes easily into the rectum, Fig. 1B, scale bar 1 mm. The three laser sources ($\lambda_{ex} = 440, 532,$ and 635 nm) are delivered simultaneously into a single mode optical fiber (arrowhead) that is scanned in spiral pattern, Fig. 1C. The three colors that come out of the fiber are focused onto the same point on the illumination plane using a custom lens assembly at the distal tip of the endoscope. The laser output power is $<2$ mW for each channel, a level that has been determined to be a non-significant risk (NSR) by the FDA (21 CFR 812). This property of the imaging system is important for future human clinical studies. Fluorescence is collected by a ring of optical fibers (arrow) with numerical aperture 0.63 and outer diameter 250 $\mu$m located around the periphery of the instrument, Fig. 1C. A set of longpass and notch filters are used to reject any reflected laser excitation that emerges from the collection fibers. The fluorescence emission is deflected into three separate channels using dichroic beamsplitters, and an additive (RGB) set of dichroics filters is used to narrow the spectra of the individual fluorescence beams, which are focused onto separate photomultiplier tubes for detection.

Previously, the peptides KCCFPAQ-GGGSK; AKPGYLS-GGGSK; LTTHYKL-GGGSK were identified using phage display, and synthesized using solid phase synthesis. The peptides were labeled separately with the fluorophores DEAC ($\lambda_{ex}=432$ nm, $\lambda_{em}=472$ nm), 5-TAMRA ($\lambda_{ex}=541$ nm, $\lambda_{em}=568$ nm) and CF633 ($\lambda_{ex}=630$ nm, $\lambda_{em}=650$ nm), respectively, Fig. 1. The fluorescence emission spectra of the three dyes do not overlap. These fluorophores were chosen to match the excitation wavelength $\lambda_{ex}$ of each laser source. Mice were anesthetized, and sedation was maintained with 1.5% isoflurane via a nose cone during endoscopy. The colon of each animal was first prepped by delivering tap water rectally using a transfer pipet to remove debris and to identify the presence of adenomas. A small animal endoscope (Karl Storz Veterinary Endoscopy, Goleta, CA) with 0 degree viewing angle and a 3 Fr instrument channel was used to collect the white light videos. Each peptide at 100 $\mu$M (1.5 mL in PBS) was administered to the distal colon through the instrument channel of the endoscope and incubated for 5 minutes. The unbound peptides were then rinsed away with tap water, and fluorescence videos were captured at 30 frames/sec. Adenomas (arrows) in the distal colon are identified on white light images, Fig. 2A–D. Shown below each white light image is the corresponding fluorescence image, demonstrating targeted detection using separate fluorophore-labeled peptides E) KCCFPAQ-DEAC, F) AKPGYLS-TAMRA, and G) LTTHYKL-CF633. Administration of KCCFPAQ-DEAC and AKPGYLS-TAMRA together results in a mixed blue and green spatial pattern representing binding to different cell surface targets, Fig. 2H. There is incomplete registration between the white light (Figs. 2A–D) and fluorescence images (Figs. 2E–H) because of colonic motion from peristalsis that occurs in between collection of these separate sets of images.

**Video Description**

Fluorescence images were collected from droplets of KCCFPAQ-DEAC, AKPGYLS-TAMRA, LTTHYKL-CF633, and an unlabeled peptide (1 $\mu$M) on a cover glass to demonstrate the ability of the multispectral endoscope to simultaneously detect multiple peptides labeled with different fluorophores over the visible spectrum. A real time white light video collected in vivo from adenoma(s) in the distal colon is shown prior to peptide application, and is followed by the corresponding fluorescence video collected from the same adenoma(s) after staining with a fluorophore-labeled peptide. White light and

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fluorescence videos are shown for each peptide KCCFPAQ-DEAC (blue), AKPGYLS-TAMRA (green), and LTTHYKL-CF633 (red) in sequence. The last video segment shows white light and fluorescence images from a colonic adenoma stained with two peptides at the same time (KCCFPAQ-DEAC and AKPGYLS-TAMRA), demonstrating a multiplexed binding pattern of two different cell surface targets. In all channels, the fluorescence intensity from the adenoma was much higher than that from the neighboring normal-appearing colonic mucosa, and the lesion margins were sharp. Some autofluorescence background can be seen from normal mucosa.

**Take Home Message**

Multispectral fluorescence imaging of colorectal dysplasia can be achieved in real time in vivo using a novel scanning fiber endoscope by employing three unique fluorescent-labeled peptides. This integrated methodology has the potential to advance methods for early cancer detection and image-guided therapy in the clinic by simultaneously visualizing multiple targets that are overexpressed in pre-malignant or malignant lesions. The small size of this instrument allows for endoscope-compatibility and for generalizing this imaging strategy for future use in other hollow organs such as biliary tract, esophagus, stomach, small bowel, and pancreatic duct. Using this technology, simultaneous diagnosis of multiple targets on tumor cells may be possible in vivo, which could improve both early detection and tissue characterization at the molecular level imaging in this manner can provide unique gene signatures for individual patients.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**

A) The visible and near-infrared spectrum of light provides a broad range of colors to generate molecular images from digestive tract mucosa that can be collected with a B) 1.6 mm diameter multispectral scanning fiber endoscope. C) Excitation is delivered through a single fiber (arrowhead) that scans in a spiral fashion, and fluorescence is collected by a ring of optical fibers (arrow) located around the periphery of the instrument. Peptides can be labeled with fluorophores that have non-overlapping spectra, such as DEAC, TAMRA, and CF633 to multiplex the images.
Figure 2.
A–D) Adenomas (arrows) in the distal colon are identified on white light images collected endoscopically in an Apc-mutation dependent mouse model of colorectal cancer. Shown below each white light image is the corresponding fluorescence image, representing targeted detection of adenomas using separate fluorophore-labeled peptides E) KCCFPAQ-DEAC, F) AKPGYLS-TAMRA, and G) LTTHYKL-CF633. H) Administration of KCCFPAQ-DEAC and AKPGYLS-TAMRA together results in a mixed blue and green spatial pattern representing binding to different cell surface targets.