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Qualitative and quantitative comparison of colonic microendoscopy image features to histopathology

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Abstract

Colorectal cancer is the second leading cause of cancer deaths in the United States, affecting more than 130,000 Americans every year¹. Determining tumor margins prior to surgical resection is essential to providing optimal treatment and reducing recurrence rates. Colorectal cancer recurrence can occur in up to 20% of cases, commonly within three years after curative treatment. Typically, when colorectal cancers are resected, a margin of normal tissue on both sides of the tumor is required. The minimum margin required for colon cancer is 5 cm and for the lower rectum 2 cm. However, usually more normal tissue is taken on both sides of the tumor because the blood supply to the entire segment is removed with the surgery and therefore the entire segment must be removed. Anastomotic recurrences may result from inadequate margins. Pathologists look at the margins to ensure that there is no residual tumor and this is usually documented in the pathology report.

We have developed a portable, point-of-care fiber bundle microendoscopy imaging system for detection of abnormalities in colonic epithelial microstructure. The system comprises a laptop, a modified fiber bundle image guide with a 1mm active area diameter and custom Lab VIEW interface, and is approved for imaging surgically resected colon tissue at the University of Arkansas for Medical Sciences. The microendoscopy probe provides high-resolution images of superficial epithelial histology in real-time to assist surgical guidance and to localize occult regions of dysplasia which may not be visible.

Microendoscopy images of freshly resected human colonic epithelium were acquired using the microendoscopy device and subsequently mosaicked using custom post-processing software. Architectural changes in the glands were mapped to histopathology H&E slides taken from the precise location of the microendoscopy images. Qualitatively, glandular distortion and placement of image guide was used to map normal and dysplastic areas of the colonic tumor and surrounding region from microendoscopy images to H&E slides. Quantitative metrics for correlating images

were also explored and were obtained by analyzing glandular diameter and spatial distribution as well as image texture.

Keywords

colorectal cancer; microendoscopy; histopathology; proflavine; image analysis; clinical

1. INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths in the United States, with more than 130,000 Americans diagnosed and over 50,000 deaths per year^{1,2}. The standard for intraoperative decisions on how much tissue to excise is 2cm margins for rectal tumors, and 6cm for colonic tumors. If the blood flow is removed from a larger section of colon, margins of up to 20cm are realistic. Removal of blood flow to portions of the colon may play a significant role in determining the size of the margin excised, beyond what may appear necessary for a given tumor size³.

Fiber bundle microendoscopy may serve as an intraoperative monitoring tool to better delineate tumor margins, by providing instant feedback as well as a rapid method to inspect a wider area. The results in this manuscript present *ex vivo* microendoscopic image data of colorectal tissue and aim to identify the differences in glandular structure between normal and dysplastic epithelium. An understanding of how colorectal epithelium changes as it transitions from normal tissue into invasive carcinomas is a necessary step in utilizing microendoscopy for early detection. Our custom microendoscopy system works by topically staining the *ex vivo* tissue with 0.01% (w/v) proflavine, bringing the custom fiber bundle into contact with the epithelial surface, and exciting the vital dye with blue epi-fluorescent light. The fiber bundle has a 1mm active area with 4μm center-to-center spacing of individual fibers, providing cellular-level resolution for distinguishing abnormalities in epithelial glandular structure, which could provide the physician an additional resource in making an informed decision. Image texture analysis could provide quantitative data to complement visual analysis, and image mosaicking techniques would provide a broader field of view to offset the probe-size limitations. To date, we have analyzed eight freshly resected colorectal cancer tissue specimens to confirm our system's ability to distinguish tumors accurately.

2. METHODS

2.1 Fiber bundle microscopy system

The microscopy system used has been previously described^{4,5}, and consists of a blue LED light source (455nm, Philips, USA), a filter set (Chroma Tech, USA) comprising a 525nm/40nm emission bandpass filter, a 460nm shortpass filter, and a dichroic mirror with a cutoff wavelength of 475nm, a camera (Flea 3 (USB), Point Grey Research Inc., CA), a 10× objective (NA 0.25, Olympus, Japan), and a customized fiber bundle image guide with a 1mm active area diameter (FIGH-50-1100N fiber, Myriad Fiber Imaging Tech., Inc., USA). The fiber bundle image guide has a custom SMA connectors on both ends, on the proximal

end for connecting to the main microendoscopic system, and on the proximal end a modified SMA connector whose rounded edges design reduces the friction between the fiber bundle and the epithelial tissue during imaging, as well as protects the delicate glass surface of the distal tip from chipping.

2.2 Image acquisition

Microendoscopic images were acquired of freshly resected human colorectal tissue in collaboration with the University of Arkansas Medical Sciences (UAMS) in Little Rock, Arkansas, under an IRB approved protocol (IRB#202224). To date, a total of eight patients who underwent colorectal surgery to resect an existing tumor have been imaged under this protocol. The freshly resected tissue was sent to the pathology lab, where the tissue was washed and anatomically stained for orientation once sectioned, longitudinally sections the tissue to expose the lumen, and pins the tissue down onto a paraffin block for later submersion in formalin. Cases four through eight were marked with pins (see Figure 1) to denote the endpoints of our imaging and facilitate correlation to histopathology. The tissue was then placed on a separate bench with all the necessary equipment and supplies for microendoscopic imaging. Just prior to imaging, the tissue was stained by dipping a cotton swab in proflavine (0.01% w/v in 1x PBS) and rolling the cotton swab over the area of interest. Occasionally dye was re-applied during imaging to ensure adequate signal.

During microendoscopy image acquisition, a DSLR camera was used to document and match each position where microendoscopic images were taken. A ruler was also included adjacent to the tissue in the DSLR images to aid in subsequent correlation. The average time allotted for imaging was twenty minutes, with about 20–30 1mm regions imaged for each case. The microendoscopy camera was usually set to an exposure of 100–150ms, and a gain of 0–5dB. Video (on average 10–15 seconds) was acquired in .avi format, using the Point Grey software, by slowly dragging the distal tip of the fiber bundle over the epithelial surface of the tissue.

A custom acrylic positioner was designed and used for precise targeting and registration of image locations and stabilization to reduce image blur. The prototype consisted of overlapping circles with 3mm center to center spacing, drilled into a 6"×2"×0.25" clear acrylic piece epoxied onto two posts that allowed for height adjustment (Figure 3).

2.3 Correlation of microendoscopic images to histopathology

DSLR images were taken of each position on the gross tissue where microendoscopic images were taken, for rough correlation (see Figure 5a). More precise correlation was achieved by matching the edge of the tissue on the histopathology slide with the position of the pin on the tissue that marked the endpoint for our imaging. Knowing the scale of both microendoscopic images and the histopathology slides aids in correlation, as well as the awareness that tissue that has been placed in formalin has the tendency to shrink⁶.

Images of H&E slides, along with histopathology diagnoses at various points, were provided by Keith Lai, M.D., at UAMS. He was consulted on matching H&E images positions to gross positions (DSLR images) as well. These matched positions were then matched to our microendoscopic image positions (see Figures 4 and 5).

2.4 Image feature analysis

Quantitative data was extracted from images by measuring gland size and circularity, as well as analyzing image texture features (standard deviation and entropy). We used ImageJ to calculate gland sizes and circularity in microendoscopic images of both normal and abnormal tissue. The glands were manually traced in ImageJ. Image texture features were measured using the MATLAB Image Analysis toolbox for standard deviation of images and entropy calculation. Images were normalized (the histogram stretch so that the highest pixel intensity on the image was mapped to 255, and the rest of the pixels were adjusted accordingly) in MATLAB for more accurate comparisons of standard deviation and entropy between images. The standard deviation of pixel intensities across entire images were tabulated and grouped into Normal or Abnormal classifications based on the histopathology at each site. Normal histopathology was categorized as non-invasive, and Abnormal histopathology was classified as invasive which included tubular adenoma, invasive rectal adenocarcinoma and invasive colonic adenocarcinoma. Entropy values, a measure of pixel intensity randomness, of entire images were also tabulated and grouped into Normal and Abnormal classifications.

2.5 Image mosaicking

Image mosaicking was achieved by transforming video into a stack of uncompressed .tiff files in MATLAB, and using a semi-automated MATLAB algorithm⁷ that maps matching points on sequential images. Two points are selected on an image, and the matching two points were then selected on the sequential image, and the program rotates and adjusts the images as needed to overlap the matching regions. Each sequential image was stitched to the previous in this fashion. This was performed during post-processing.

3. RESULTS

3.1 Qualitative correlation of microendoscopic images to histopathology

See Table 2 for examples of microendoscopic images matched to H&E sites, grouped by the diagnoses provided by the pathologist: Normal tissue, and Abnormal tissue with the sub-classifications of Invasive colonic adenocarcinoma, Invasive rectal adenocarcinoma, and Tubular adenoma.

3.2 Qualitative results of mosaicking video acquisition

Three examples of image mosaicking from video files are shown in Figure 6. The top mosaic shows an example of primarily normal epithelium. The middle shows epithelium that has undergone adjunct treatment for cancer and is neither an example of healthy epithelium nor of cancerous epithelium. The bottom mosaic shows an example of occult dysplasia. The glandular structure suggests a hyperplastic polyp, according to pathology resident Amy Joiner at UAMS.

3.3 Quantitative analysis of image texture

Quantitative measurements of glandular size and circularity, as well as standard deviation and entropy of images, were grouped into Normal or Abnormal classifications and plotted

(see Figure 7). Our data showed statistical differences between glandular area and circularity in microendoscopic images of normal and abnormal (tubular adenoma, invasive rectal adenocarcinoma, invasive colonic adenocarcinoma) tissue. Glands were smaller, more uniform in size, and more circular in normal tissue. Normal tissue also contained more glands than abnormal tissue; 18 images of normal tissue yielded 288 measureable glands, and 19 images of abnormal tissue yielded 132 measureable glands. Entropy and standard deviation of images of normal tissue did not yield significantly different values.

4. DISCUSSION

Portable microendoscopic systems are a viable complement to traditional tumor margin detection procedures, allowing for rapid probing and analysis of wider regions of interest. We have shown that our fiber bundle microendoscopy system is capable of cellular resolution, and can provide information on glandular structure as well as image texture. The results of our qualitative correlation of microendoscopic images with histopathology show that there is a visible difference between the glandular structure of normal lumen and highly dysplastic tissue. Tumors have highly degenerate glands that are enlarged and non-circular, and at times show little sign of any glandular structure at all. Normal tissue shows homogenous glands that are roughly circular. This distinction is what we aim to apply to mapping tumor margins, and aid in determining where cancerous tissue transitions to normal epithelium.

The ability to mosaic images will provide a resource to overcome the limitation of a 1mm active area, by displaying a wide-field view of the imaged tissue. This would allow for a better overall view of what the tumor margin seems to be, as well, as help identify small occult lesions that might look normal to a surgeon under bright light (see Figure 6c). Also, a comparison of surrounding tissue to a certain microendoscopic image might show a change in the glandular structure that might not seem out-of-place if taken individually and out of context. The results of quantitative image analysis demonstrate that there is a measureable significant difference between the glandular structure and overall image texture of normal tissue as compared to adenocarcinomas. With a sufficient database of normal and abnormal glandular sizes and circularity, a threshold could be set to aid the surgeon in determining whether the glandular features fall within the normal range or seem to be abnormal.

Correlation of microendoscopic images to histopathology is not a trivial procedure, and we are continually adjusting our methodology to improve confidence in our correlation. Placing pins as endpoints for our imaging made correlation much smoother, as did improved communication with the pathology residents. Future improvements that will be implemented include using two stains to mark both exterior and distal orientations in the gross tissue, reducing imaging to 2cm sections that will fit in a single cassette for paraffin fixation, and beginning to collect and correlate samples of normal, transition, and tumor tissue at different regions in the gross tissue.

The depth limitation of our current microendoscopic is currently being targeted by Gage Greening, whose work is also being presented at SPIE BiOS 2015, by integrating this

system with a diffuse reflectance probe. Further work is also being done to automate image feature extraction, image mosaicking, and design a superior acrylic support template.

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References

1. [Accessed 1/11/15] <http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf>.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014; 64:9–29. 2014; [PubMed: 24399786]
3. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, Sargent D. Guidelines 2000 for colon and rectal cancer surgery. *Journal of the National Cancer Institute*. 2001; 93(8):583–596. [PubMed: 11309435]
4. Prieto SP, Powless AJ, Majid A, Laryea JA, Mizell J, Sharma SG, Muldoon TJ. Fiber Bundle Microendoscopy for Characterization of Dysplastic Lesions in Colonic Epithelium. *Proc. Biomedical Optics* (pp.BT3A-14). Optical Society of America. 2014
5. Muldoon TJ, Thekkekk N, Roblyer D. Evaluation of quantitative image analysis criteria for the high-resolution microendoscopic detection of neoplasia in Barrett's esophagus. in *Journal of Biomedical Optics*. 2010; 15(2):26–27.
6. Hsu PK, Huang HC, Hsieh CC, Hsu HS, Wu YC, Huang MH, Hsu WH. Effect of formalin fixation on tumor size determination in stage I non-small cell lung cancer. *The Annals of thoracic surgery*. 2007; 84(6):1825–1829. [PubMed: 18036892]
7. <http://www.pages.drexel.edu/~sis26/MosaickingTutorial.htm>.

Figure 1. Microendoscopy System, (a) Enclosed system and fiber bundle image guide. (b,c) Distal end of fiber bundle image guide.

Figure 2. Distal end of fiber bundle in contact with freshly resected tissue. Pins mark the path of the imaging, along which the tissue is later sectioned and placed into cassettes. Yellow arrow points to one of the pins/endpoints. Ruler is in centimeters.

Figure 3.
Acrylic support template, (a) Example of fiber bundle inserted through acrylic, (b)
Schematic of acrylic support, posts, and fiber bundle.

Figure 4.

Example summary of fiber bundle positions. DSLR images of each site were taken and each position marked on the tissue with yellow ovals. Numbers denote the sequence of positions where microendoscopy images were taken. Ruler is in centimeters.

Figure 5.

Overview of correlation procedure from microendoscopic images to histopathology. (a,b) Positions on DSLR images of gross tissue are matched to histopathology, and approved by the pathologist. (c,d) Positions on gross tissue are marked with yellow ovals, as in Fig. 4, and combined with the approved matched positions in (a) and (b). (e) Positions of yellow ovals/microendoscopic image sites are then matched to H&E images. Scale bars are 1mm.

Figure 6.

Mosaics of videos taken with the microendoscope system, of three different specimens, (a) Mosaic of tumor (invasive colonic adenocarcinoma) epithelium, (b) Mosaic of non-cancerous tissue that had undergone adjunct treatment, (c) Mosaic of occult hyperplastic polyp in what visibly looked to be normal epithelium. Scale bar = 1mm.

Figure 7.

Bar plot comparisons of normal and abnormal human colon images. Entropy of microendoscopic images ($n=21$, $n=43$, respectively, $p=0.3$), measured using MATLAB. Standard Deviation of microendoscopic images ($n=21$, $n=43$, respectively, $p=0.3$), measured using MATLAB. Average Glandular Area ($n_{\text{images}}=18$, $p<<0.001$), measured using ImageJ. Average Glandular Circularity ($\text{Circularity}^* = (4\pi)(\text{area}/\text{perimeter}^2)$, $n_{\text{images}}=19$, $p<<0.001$), measured using ImageJ.

Table 1

Summary of cases and patient diagnoses

Case	Diagnosis
1	Invasive colonic adenocarcinoma
2	Invasive colonic adenocarcinoma
3	Invasive rectal adenocarcinoma
4	Invasive colonic adenocarcinoma
5	Invasive rectal adenocarcinoma
6	Tubular adenoma
7	Invasive colonic adenocarcinoma
8	Invasive colonic adenocarcinoma

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Table 2

Microendoscopic images matched to H&E image sites, grouped by diagnosis. Scale bars = 1 mm. Image brightness was adjusted for publication.

Diagnosis:	Normal	Invasive colonic adenocarcinoma	Invasive rectal adenocarcinoma	Tubular adenoma
Microendoscopic Image
H&E Image
Microendoscopic Image
H&E Image

Diagnosis:	Normal	Invasive colonic	Invasive rectal	Tubular adenoma
Microendoscopic Image
H&E Image