Discrimination of Benign and Neoplastic Mucosa with a High-Resolution Microendoscope (HRME) in Head and Neck Cancer

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SYNOPSIS

The efficacy of surgical treatment for head and neck squamous cell carcinoma (HNSCC) depends critically on obtaining negative margins. Optical imaging has the potential to improve their accuracy and reduce frozen section utilization. We sought to determine accuracy and inter-rater reliability of the interpretation of high-resolution microendoscopic (HRME) images.

ABSTRACT

Background: The efficacy of ablative surgery for head and neck squamous cell carcinoma (HNSCC) depends critically on obtaining negative margins. While intraoperative "frozen section" analysis of margins is a valuable adjunct, it is expensive, time-consuming, and highly dependent on pathologist expertise. Optical imaging has potential to improve the accuracy of margins by identifying cancerous tissue in real time. Our aim was to determine the accuracy and inter-rater reliability of head and neck cancer specialists using high-resolution microendoscopic (HRME) images to discriminate between cancerous and benign mucosa.

Methods: Thirty-eight patients diagnosed with HNSCC were enrolled in this single-center study. HRME was used to image each specimen after application of proflavine, with concurrent standard histopathologic analysis. Images were evaluated for quality control, and a training set containing representative images of benign and neoplastic tissue was assembled. After viewing training images, seven head and neck cancer specialists with no prior HRME experience reviewed 37 test images and were asked to classify each.

Results: The mean accuracy of all reviewers in correctly diagnosing neoplastic mucosa was 97 percent (95% CI = 94-99%). The mean sensitivity and specificity were 98 percent (97-100%) and 92 percent (87-98%), respectively. The Fleiss kappa statistic for inter-rater reliability was 0.84 (0.77-0.91).

Conclusions: Medical professionals can be quickly trained to use HRME to discriminate between benign and neoplastic mucosa in the head and neck. With further development, the HRME shows promise as a method of real-time margin determination at the point of care.

INTRODUCTION

Oncologic surgery in the head and neck requires striking a difficult balance between removing all malignant tissue while simultaneously preserving as much healthy tissue as possible. Extensive resection can leave patients with serious functional and aesthetic deficits, compromising their ability to perform vital daily functions such as chewing, swallowing, or speaking. However, if the tumor margins are not accurately defined, and the diseased tissue is not completely removed, cancer is more likely to persist or recur (1, 2). Thus, the ability to define the margins of the tumor with a high degree of accuracy is critical for maximizing the efficacy of surgical treatment and the patient's subsequent quality of life.

While intraoperative "frozen section" analysis of surgical margins is a valuable adjunct to oncologic surgery, the method is expensive, time-consuming, and highly dependent on the experience and expertise of both the pathologist and the surgeon (3, 4). Image-guided surgery, the use of imaging technology during surgical resection, is a novel approach that has the potential to avoid some of these issues inherent with the use of frozen sections. By providing spatial resolution of structural changes in the epithelium that indicate neoplastic progression in real time (5, 6), optical imaging technologies have the potential to ensure that as little normal tissue as possible is removed during the course of a complete resection. In addition, availability of point-of-care imaging may also reduce the number of frozen section analyses needed to accurately define tumor margins.

In this paper, we describe the use of a probe-based imaging device, the high-resolution microendoscope (HRME) (7, 8), for the detection of squamous cell carcinoma of the head and neck. The HRME uses a flexible fiber-optic bundle to obtain images *in situ* and in real-time,

allowing the user to visualize neoplastic changes in epithelium. The aim of this study was to determine the accuracy and inter-rater reliability of head and neck cancer specialists in interpreting HRME images of neoplastic and non-neoplastic mucosa in the head and neck.

METHODS

The study protocol was approved by both the Mount Sinai School of Medicine (GCO #09-0945) and Rice University (09-166E) Institutional Review Boards. All participants in the study had squamous cell carcinoma of the head and neck (HNSCC) diagnosed by prior biopsy and were scheduled for surgical resection of their primary tumor. Patients were prospectively enrolled in this study, and written informed consent was obtained from each patient prior to surgery.

Imaging System

The high-resolution microendoscope (HRME) used in this study has been described previously by Muldoon *et al* (Figure 1) (7). The HRME probe consists of a 1-meter long fiber-optic probe with 30,000 individual two-micron fibers, allowing a circular field of view of 720 microns. A blue LED with output spectrum centered at 455 nm provides approximately 1 mW of illumination power at the imaging site. The HRME used in this study was configured for use with the contrast agent proflavine, a fluorescent nuclear stain (9). Proflavine is the predominant component of acriflavine, which has been tested and used extensively in Europe and Australia in *in vivo* studies of the gastrointestinal tract without any reported adverse events (10-13). A 0.01% solution of proflavine in buffered saline was applied topically to the epithelial surface.

After application of proflavine, the mucosa was rinsed with saline to remove any remaining unbound dye, and imaging was immediately performed in a laboratory in close proximity to the operating room. Images were displayed at a frame rate of 10 to 15 frames per second in real time. Image data were captured in video format (.avi), with individual image frames (.jpg) subsequently extracted from the video file for analysis.

Each specimen was imaged immediately after surgical resection at multiple sites of interest, including suspected tumor, adjacent benign appearing mucosa, and transition areas, with three to ten HRME videos of two to three seconds duration obtained from each site. 3-mm punch biopsies were also obtained after imaging to establish a gold-standard diagnosis with H&E histopathologic analysis for each site by a pathologist.

Image Quality Control

One representative movie was randomly selected from the three to ten movies available from each site. Selected movies were then reviewed for quality control, and were included in further analysis only if at least 50% of the frame contained nuclei for the majority of the movie, the image did not contain areas of oversaturation from residual proflavine, there was not significant motion artifact, and the histology showed either benign mucosa or invasive cancer (dysplasia was excluded). A representative still frame was subsequently extracted from each video. For each site imaged, the highest quality image was selected. Selected images were reviewed for quality control, and were included in further analysis only if at least 50% of the frame contained nuclei for the majority of the movie, and an appropriate quantity of proflavine had been applied to the specimen. Additionally, still images were excluded if the specimen was

too heavily inked by the pathologist to allow interpretation, or if histology showed hyperkeratosis, submucosal tumor spread, or severe inflammation as these may present pitfalls for HRME imaging. Contrast and brightness levels were digitally and uniformly adjusted for optimum image quality.

Training and Test Sets

A training set of images was assembled from data passing quality control in order to illustrate HRME image features characteristic of normal and neoplastic tissue. The training set included two representative images of histologically normal mucosa, one image of histologically dysplastic mucosa, and three images from histologically cancerous mucosa. A test set of separate images was assembled from the remaining data passing quality control. All remaining images passing quality control were used in the test set.

Measurement of Diagnostic Accuracy and Inter-rater Reliability of HRME Images

Training set images were presented a group of seven head and neck cancer specialists together with a summary of features associated with each diagnostic category; raters had an opportunity to ask questions about classification features during test set presentation. Raters were administered a test set of 36 images (11 benign, 25 cancer) after training with five representative images (two benign, three cancer), and asked to classify each image as benign mucosa vs. invasive cancer.

Table 1 lists the three main classifiers which raters were instructed to use in discriminating images and videos of benign mucosa from invasive cancer. These classifiers were

chosen *a priori*, based on principles of conventional histopathology, and verified by review of representative HRME/biopsy pairs from each anatomical site imaged (oral cavity, oropharynx, larynx/hypopharynx).

Statistical Analysis

Accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were calculated with Microsoft Excel. SAS version 9.2 (SAS Institute, Cary, NC) was used to calculate the inter-rater agreement between multiple raters, or Fleiss kappa statistic, using the MAGREE function. Two-tailed P values of < 0.05 were considered to be statistically significant.

RESULTS

Thirty-eight patients were enrolled between June 2009 and January 2011. From 1,070 still images from 117 imaging subsites (Table 2), we selected the highest quality image for each site, and then applied our exclusion criteria to create a bank of 41 high quality, representative images (Table 3). Correlation of nuclear morphology and distribution observed on HRME with conventional H&E histopathology is shown in Figure 2, which shows examples of representative images of benign mucosa and invasive cancer obtained with HRME. Overall, benign mucosa or invasive cancer from all anatomical sites demonstrated consistent imaging characteristics which permitted discrimination between the two (Figure 3).

The sensitivity and specificity of interpretation (benign vs. cancer) for blinded raters of HRME images was 98 percent (95% CI = 97-100) and 91 percent (95% CI = 85-97), respectively. The kappa statistic for inter-rater reliability was 0.84 (95% CI = 0.77-0.91) (Figure 4).

DISCUSSION

This study describes the use of a novel optical imaging tool that can be used to obtain high-resolution images of mucosal surfaces for discrimination between benign and cancerous mucosa in real time, and establishes the preliminary accuracy and interrater reliability of HRME image interpretation. We found that head and neck cancer specialists unfamiliar with the device could be quickly trained to accurately interpret HRME images obtained from *ex vivo* specimens of squamous cell carcinoma of the head and neck (sensitivity = 98.3 percent, specificity = 90.9 percent, kappa statistic = 0.84). These results support future *in vivo* studies to evaluate the potential of the HRME in the operative setting for treatment of patients with squamous cell carcinoma of the head and neck. With further development, point-of-care imaging with devices like the HRME may allow more judicious use of frozen section analysis, with concomitant decrease in surgical time and total procedure costs.

Our study has several strengths. Raters were able to classify pre-selected images as either normal (benign squamous mucosa) or abnormal (squamous cell carcinoma) with excellent accuracy and inter-rater reliability based on qualitative classifiers with minimal training. We also found we were able to successfully image benign and neoplastic mucosa from a wide variety of head and neck subsites using the HRME.

Although HRME is a promising tool, there are several limitations which highlight areas for further development. One issue is the strong affinity of the contrast agent, proflavine, for keratin. Images obtained from keratinized tissue display background artifact proportional to the density of keratinization, making them difficult or impossible to interpret. Heavy keratinization

limits effectiveness of proflavin-enhanced HRME in keratinized subsites of the oral cavity, including the hard palate and mucosa overlying the alveolar ridge. Conversely, ectopic keratinization can also be seen with HNSCC at typically non-keratinized sites; while this is a barrier to imaging the mucosa, it may also serve as a hallmark of potentially neoplastic mucosa. One area of future research is the testing of alternative contrast agents which do not have the disadvantage of affinity for keratin, particularly those which highlight specific tumor markers selectively overexpressed in squamous cell carcinomas of the head and neck. Targeted contrast agents with affinity for epidermal growth factor receptor (EGFR), for example, may allow for selective visualization of cancer cells with optical imaging technology (14, 15).

Another problem encountered during imaging is the limited depth of penetration, which makes it difficult to detect submucosal tumor spread. Because HRME imaging is limited to the superficial mucosa with a depth of penetration of roughly 50 micrometers, images may be wrongly classified as normal, when in fact tumor extends into the submucosa underneath normal superficial mucosa on histopathologic analysis. This problem may be addressed by development of strategies to permit greater depth of imaging (such as altering the excitation wavelength or interface of the probe with the mucosa), or submucosal delivery of the HRME probe.

CONCLUSION

High-resolution microendoscopic imaging provides non-invasive visualization of squamous epithelium in the upper aerodigestive tract in real time, and permits accurate discrimination of benign mucosa and invasive cancer. Keratinization and submucosal tumor

spread are diagnostic challenges, which may be addressed by development of strategies for submucosal delivery of the HRME probe. With further refinement, HRME and other optical imaging methods have the potential to enhance the rational selection of initial margins, and decrease operative time and expense by limiting the use of frozen section analysis.

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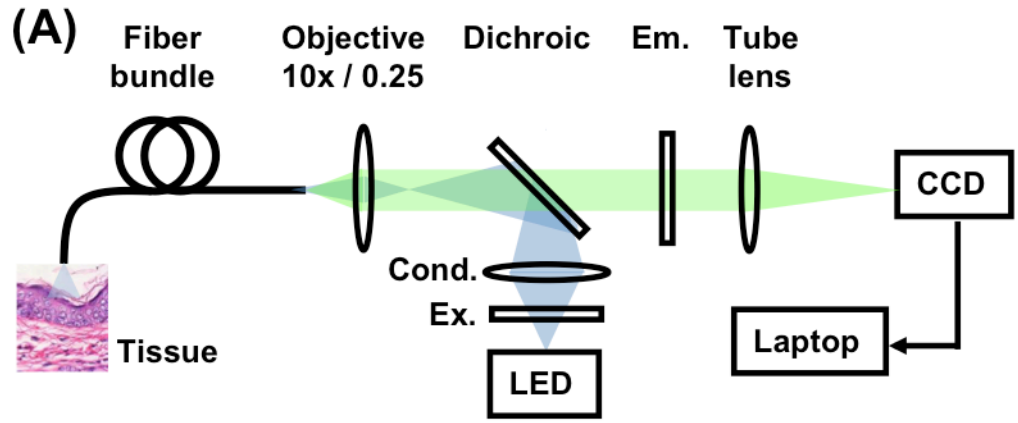
TABLES AND FIGURES

Figure 1. The high-resolution microendoscope (HRME), shown in a schematic (A) and fully assembled before imaging (B).



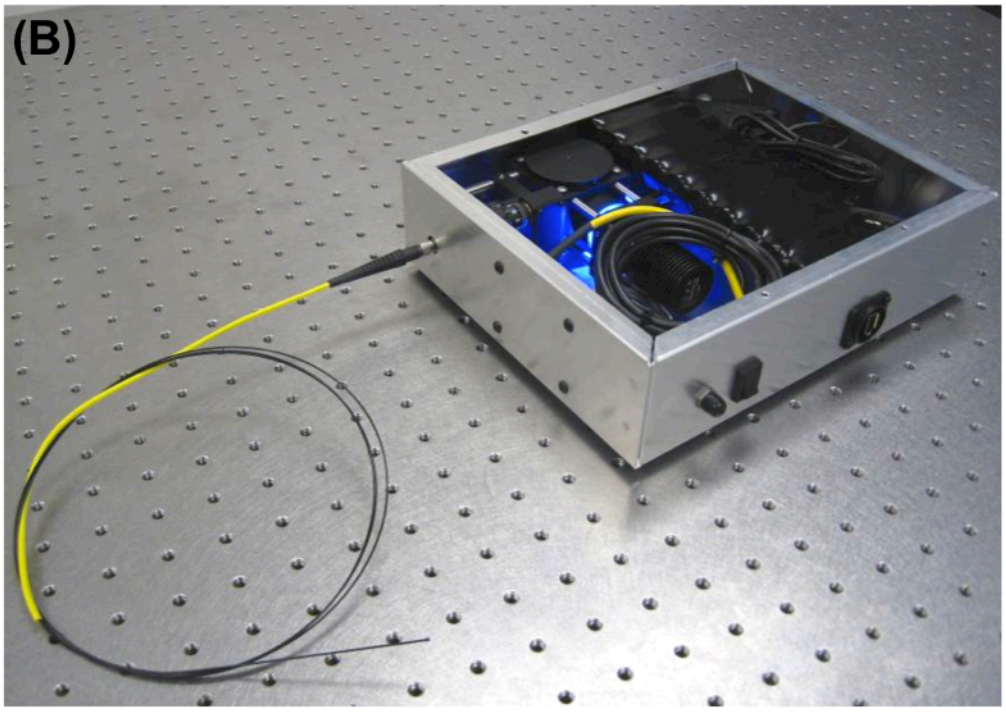


Table 1. HRME imaging characteristics associated with benign, dysplastic, and cancerous mucosa.

	Tissue Classification	
Cellular Feature	**Benign**	**Cancerous**
Nuclear Size	Normal	Enlarged
Nuclear-to-Cytoplasm Ratio	Normal	Increased
Overall Cellular Architecture	Regular and Symmetric	Absent

Table 2. Distribution of images obtained by site and diagnosis.

	HRME Images		
Site and Diagnosis	**Collected (# pts)**	**Unique Sites**	**Included in Test/Training (# pts)**
Oral Cavity			
Normal	117 (10)	15	7 (5)
Dysplasia	39 (4)	3	0 (0)
Cancer	320 (11)	35	9 (9)
Total	*437 (16)*	*50*	*16 (14)*
Oropharynx			
Normal	139 (10)	13	4 (4)
Dysplasia	42 (4)	4	0 (0)
Cancer	154 (11)	17	9 (9)
Total	*293 (21)*	*30*	*13 (13)*
Larynx			
Normal	63 (7)	12	2 (3)
Dysplasia	10 (1)	1	0 (0)
Cancer	196 (7)	17	10 (5)
Total	*259 (9)*	*29*	*12 (8)*
All Sites*	*989 (38)*	*109*	*41 (35)*
Normal	319 (27)	40	13 (12)
Dysplasia	91 (9)	8	0 (0)
Cancer	670 (29)	69	28 (23)

*Totals may not add up because a patient may have had varying pathology (normal and cancer).

Table 3. Reasons for excluding images from the final test set.

Reasons for Exclusion	**Number of HRME Images Excluded**	**% of Total Set (n=117)**
Uninterpretable due to excessive artifact or poor imaging technique	**36**	**30.8%**
Diagnostic pitfalls	**19**	**16.2%**
Submucosal tumor	11	9.4%
Inflammation	2	1.7%
Keratin	6	5.1%
Not target pathology (histology showed dysplasia and not invasive cancer or benign mucosa)	**6**	**5.1%**
Pathology coordination issues (slides lost, unable to correlate image with biopsy, correct image not available)	**13**	**11.1%**
Total	74	63.2%

Figure 2. Representative HRME Images from the oropharynx of (A) benign mucosa and (B) squamous cell carcinoma, with corresponding H+E histopathologic images. Note that HRME images are obtained parallel to the tissue surface, while the H+E sections are in cross-section.

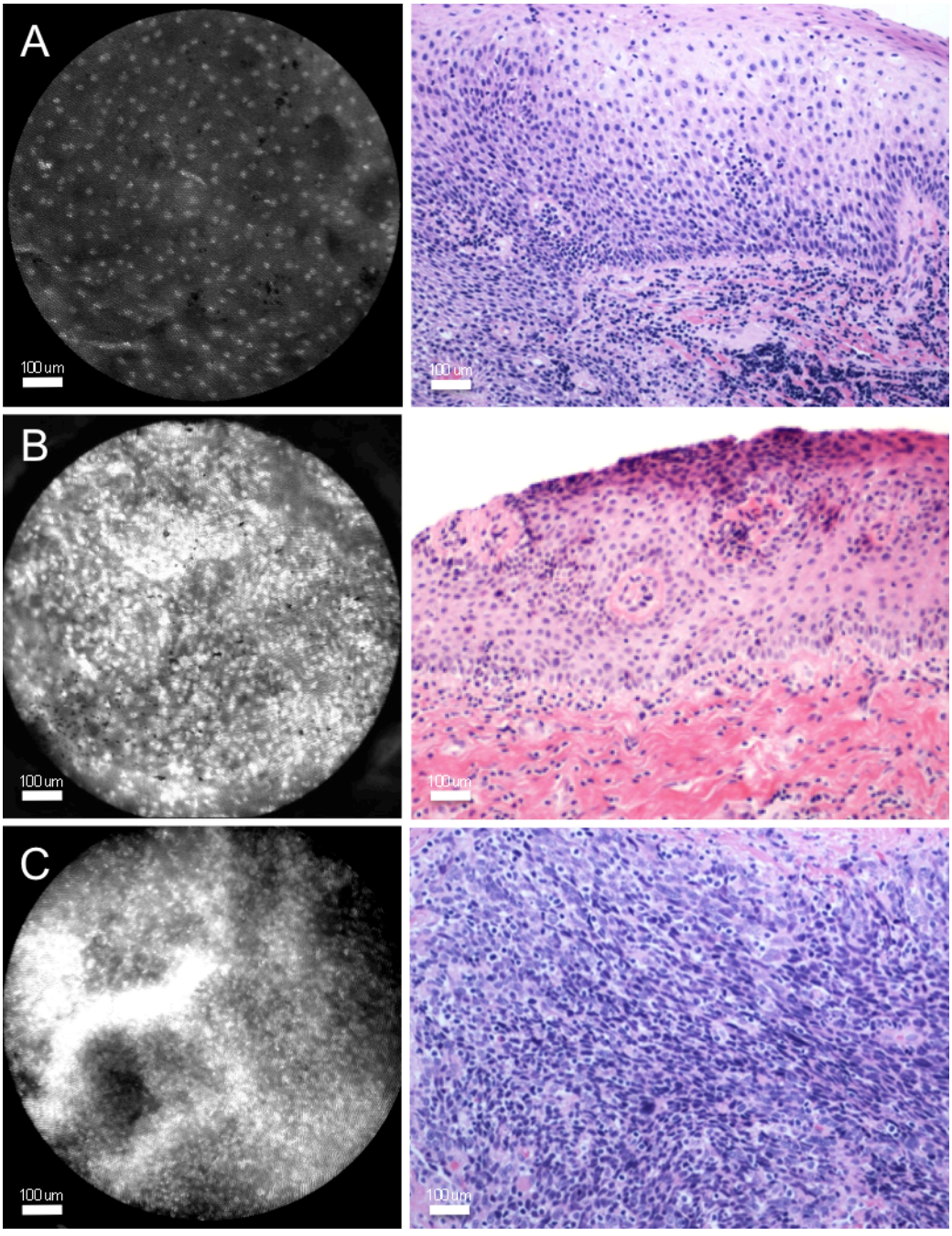


Figure 3. Representative HRME Images from various sites of benign (top) and cancerous (bottom) mucosa in the (A) oral cavity, (B) oropharynx, and (C) larynx.

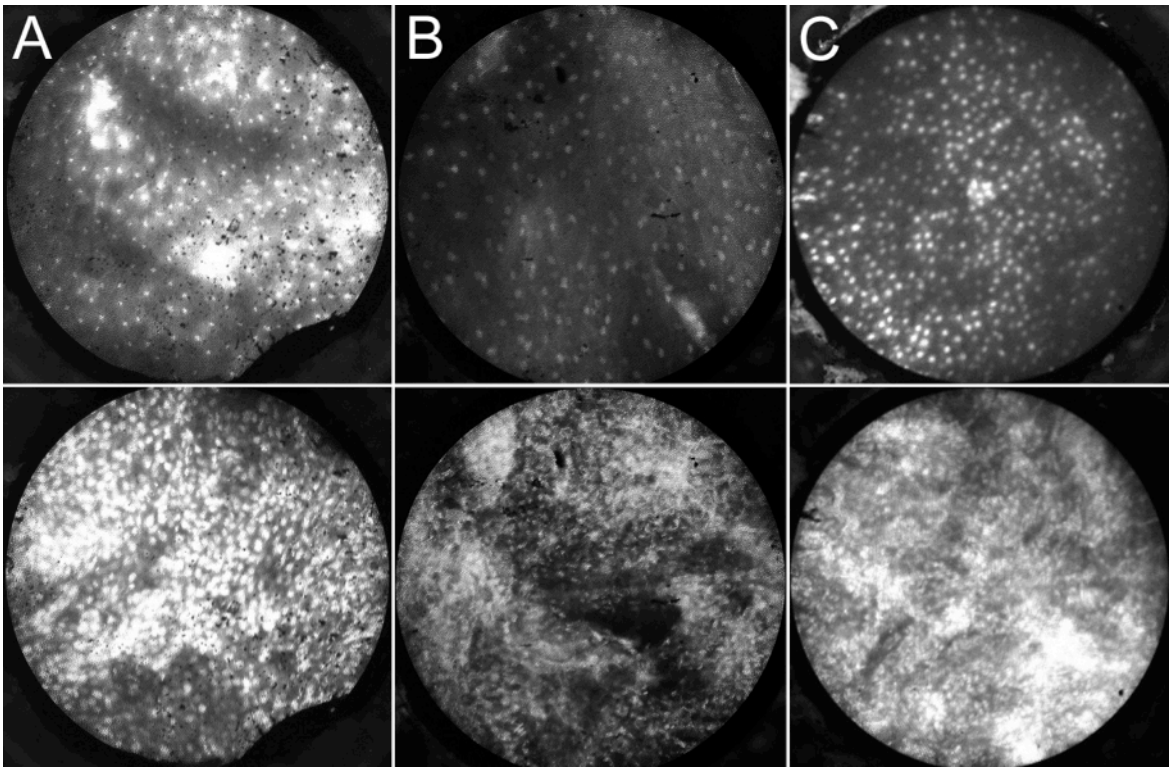


Figure 4. Results of the interpretation of HRME images of benign mucosa vs. invasive cancer for representative still images.