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Multi-scale optical imaging techniques for early cancer detection in the gastrointestinal tract

by

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ABSTRACT

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Gastrointestinal (GI) cancers impose an enormous burden on the society worldwide and a significant proportion of this burden can be prevented through early cancer detection and treatment. Current screening and surveillance protocols rely primarily on conventional white light endoscopy, the accuracy and efficacy of which need to be improved. The main objective of this research is to develop and optimize novel multi-scale optical imaging modalities to improve detection of GI cancers with enhanced imaging performance and increased clinical ease of use at a low cost.

A modular video endoscope (MVE) was developed to combine widefield with high-resolution imaging modalities. Trimodal imaging, including standard white light imaging (WLI), vital-dye fluorescence imaging (VFI) and high-resolution microendoscopy (HRME), was enabled in a single endoscopic insertion. A pilot in vivo clinical trial showed that glandular architectural dysregulation, as visualized in VFI and HRME, was associated with cancer progression in Barrett’s esophagus (BE). The MVE/HRME platform was further evaluated for gastric cancer detection. In both ex vivo and in vivo pilot studies, early cancers were found to be highlighted by
alterations in glandular patterns and nuclear morphology in VFI and HRME. Preliminary data in the in vivo trial showed that the platform may be useful to detect additional advanced lesions, but suggested that the specificity needs to be improved.

A low-cost confocal HRME was developed to improve the axial performance of HRME with optical sectioning. By synchronizing a digital light projector (DLP) with the rolling shutter of a CMOS sensor, line-scanning confocal imaging was enabled in a compact design. Initial ex vivo validation in imaging squamous and columnar epithelium of mouse specimens demonstrated that optical sectioning improved the visualization of nuclear morphometry, especially in crowded regions with degraded image quality using a conventional HRME.

Automated analysis of HRME images was also explored to facilitate its clinical applications. In 58 in vivo colorectal HRME images, a set of clinically relevant features were quantified. A 3-feature model was developed through linear discriminant analysis to achieve a sensitivity and specificity of 91% and 89%, and an AUC of 0.94 in classification of neoplastic from non-neoplastic polyps.

The unique contributions of this research are the development of multi-scale imaging modalities with enhanced imaging performance and improved clinical ease of use. Computer-aided interpretation of clinical data was also investigated. These results can potentially contribute to improved early GI cancer detection, especially in community and low-resource settings.
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Contents

Acknowledgments ........................................................................................................ iv

Contents .................................................................................................................. vi

List of Figures .......................................................................................................... x

List of Tables .............................................................................................................. xvi

List of Equations ...................................................................................................... xvii

Nomenclature .......................................................................................................... xviii

Introduction .............................................................................................................. 1
  1.1. Overview ........................................................................................................ 1
  1.2. Specific aims .................................................................................................. 3
    1.2.1. Specific aim 1 .................................................................................... 3
    1.2.2. Specific aim 2 .................................................................................... 4
    1.2.3. Specific aim 3 .................................................................................... 4
    1.2.4. Specific aim 4 .................................................................................... 4
  1.3. Chapter summaries ...................................................................................... 5

Background ............................................................................................................ 9
  2.1. Motivation and significance ......................................................................... 9
  2.2. Esophageal adenocarcinoma ....................................................................... 10
    2.2.1. Clinical significance and needs ....................................................... 10
    2.2.2. Current optical imaging technologies for screening and surveillance .... 11
      2.2.2.1. Chromoendoscopy ................................................................ 12
      2.2.2.2. Narrow band imaging ............................................................. 13
      2.2.2.3. Autofluorescence imaging ......................................................... 13
      2.2.2.4. Confocal laser endomicroscopy ............................................... 14
  2.3. Gastric cancer ............................................................................................... 15
    2.3.1. Clinical significance and needs ....................................................... 15
    2.3.2. Current optical imaging technologies for screening and surveillance .... 16
      2.3.2.1. Chromoendoscopy ................................................................ 17
      2.3.2.2. Narrow band imaging ............................................................. 17
      2.3.2.3. Autofluorescence imaging ......................................................... 18
2.3.2.4. Confocal laser endomicroscopy .................................................. 18
2.4. Colorectal cancer ........................................................................... 18
2.4.1. Clinical significance and needs ..................................................... 18
2.4.2. Current optical imaging technologies for screening and surveillance ... 19
  2.4.2.1. Narrow band imaging ............................................................. 20
  2.4.2.2. Confocal laser endomicroscopy .............................................. 20
2.5. Discussion ..................................................................................... 21

Modular video endoscopy for in vivo detection of Barrett’s-associated neoplasia ..... 23
3.1. Introduction ..................................................................................... 23
3.2. Materials and methods .................................................................. 25
  3.2.1. MVE instrumentation ................................................................. 25
    3.2.1.1. White balance in WLI ......................................................... 28
    3.2.1.2. Contrast enhancement in VFI ............................................. 29
    3.2.1.3. System performance ......................................................... 31
  3.2.2. High resolution imaging ............................................................ 31
  3.2.3. Pilot study .................................................................................. 32
    3.2.3.1. Imaging procedure ............................................................. 32
    3.2.3.2. Contrast quantification and evaluation ................................... 34
3.3. Results .......................................................................................... 35
  3.3.1. System performance ................................................................. 35
    3.3.1.1. White balance in WLI ......................................................... 35
    3.3.1.2. Contrast enhancement in VFI ............................................. 37
  3.3.2. In vivo pilot study: representative images ...................................... 38
3.4. Discussion ..................................................................................... 40

Multimodal imaging for in vivo detection of gastric cancer and precursors .......... 43
4.1. Introduction ..................................................................................... 43
4.2. Materials and methods .................................................................. 44
  4.2.1. MVE optimization ................................................................. 44
  4.2.2. Ex vivo pilot study ................................................................. 46
  4.2.3. In vivo pilot study ................................................................. 47
    4.2.3.1. Imaging protocol ............................................................. 47
    4.2.3.2. Diagnostic performance ................................................ 49
4.3. Results .......................................................................................... 50
4.3.1. G2 MVE optimization ......................................................................................... 50
4.3.2. Ex vivo pilot study: representative images .......................................................... 51
4.3.3. In vivo pilot study: representative images ........................................................... 53
4.3.4. In vivo pilot study: diagnostic performance ....................................................... 55
4.4. Discussion .................................................................................................................. 57

Line-scanning confocal microendoscope for nuclear morphometry imaging ............ 60
5.1. Introduction ............................................................................................................... 60
5.2. Materials and methods ........................................................................................... 62
  5.2.1. Optical setup ...................................................................................................... 62
  5.2.2. Synchronization and confocal imaging .............................................................. 64
  5.2.3. Characterization of axial sectioning performance ............................................. 67
  5.2.4. 2D phantom validation and ex vivo imaging .................................................... 68
  5.2.5. Real-time confocal imaging .............................................................................. 69
5.3. Results ...................................................................................................................... 70
  5.3.1. Axial sectioning performance .......................................................................... 70
  5.3.2. 2D phantom and ex vivo validation .................................................................. 72
  5.3.3. Real-time confocal imaging ............................................................................. 76
5.4. Discussion and conclusion ...................................................................................... 78

Quantitative analysis of high-resolution microendoscopic images in discrimination of colorectal neoplasia ................................................................. 80
6.1. Introduction ............................................................................................................... 80
6.2. Materials and methods ........................................................................................... 82
  6.2.1. Patient enrollment and imaging procedure ...................................................... 82
  6.2.2. Imaging system ................................................................................................ 83
  6.2.3. Quantitative image analysis ............................................................................ 83
  6.2.4. Algorithm development ................................................................................... 86
6.3. Results ...................................................................................................................... 87
  6.3.1. Patient enrollment: sites and images ............................................................... 87
  6.3.2. Algorithm development: model selection and performance ......................... 89
6.4. Discussion ................................................................................................................ 91

Conclusion .................................................................................................................... 95
7.1. Summary and research contributions ................................................................. 95
7.2. Future research directions ................................................................................ 98
References .................................................................................................................. 101
List of Figures

Figure 3-1. Overview of G2 MVE. (A) A custom-coated 435 nm long-pass filter placed in front of the endoscope CCD. (B) The long-pass filter is installed in the steel housing and secured by a Halo cap. (C) In the G2 MVE, the excitation wavelength is shifted from 455 to 405 nm. A 435 nm long-pass filter is used in VFI mode as the emission filter, as well as in conventional WLI with white balance.................................................................26

Figure 3-2. Comparison of imaging protocols in G1 and G2 MVE. In the G1 flowchart, the VFI module is attached between the first and second scope insertion. On the bottom panel, in the G2 MVE both WLI and VFI images are acquired in a single scope insertion.................................................................33

Figure 3-3. Verification of white balance in the G2 MVE. Ex vivo images of a mouse stomach (A) without the VFI module or color balance (original), (B) with the G1 VFI module but no color balance (G1 uncorrected), (C) with the G1 VFI module and color balance (G1 corrected), (D) with the G2 VFI module but no color balance (G2 uncorrected), (E) with the G2 VFI module and color balance (G2 corrected). The probability mass function (PMF) of the selected ROI (box in (A)) for each image is compared. The available color correction failed to restore the original color due to the almost complete elimination of blue light by the G1 emission filter, while the PMF of the G2 corrected image is similar to the original.................................................................36

Figure 3-4. Performance of the contrast enhancement in vivo. Original (A) and enhanced (B) images of a Barrett’s esophagus island (white arrow) surrounded by the squamous epithelium. (B) Sharpened edges of glands are apparent without introducing artifacts in the homogeneous squamous epithelium. (C) Boxplot of unsharp mask intensities in two types of epithelium as shown in (B), with columnar epithelium indicated by the yellow box and squamous epithelium by the white box.................................................................38

Figure 3-5. In vivo images of normal BE, HGD and carcinoma in WLI, VFI and HRME with the corresponding histopathology, and mean Lab variances of selected ROIs in each widefield image. From normal BE (A) to HGD (F) in WLI, glandular patterns appear more distorted and disrupted. The loss of glandular architecture is highlighted in VFI with enhanced contrast (G). Progressing to carcinoma, the WLI image (K) indicates glandular effacement and abnormal
vascularization; the VFI image (L) shows further distortion (yellow solid box) and effacement of glands (white solid box). HRME image (C), as well as histopathology of normal BE (D) shows regular and intact glands with presence of goblet cells (black arrows); absence of goblet cells is observed in HGD (I), which also reveals disruption of glandular patterns in HRME (H). This glandular pattern is effaced in carcinoma in HRME (M), which is confirmed by the histopathology (N). The mean Lab variances of selected ROIs (white dashed boxes) are higher in all VFI images than their corresponding WLI images, indicating that VFI presents enhanced contrast than WLI. The error bars show the standard deviation.

Figure 4-1. Overview of an external laser module to provide additional illumination for VFI. (A) The portable laser module can stay on the biopsy channel entrance without interrupting the routine endoscopy procedure. (B) An endoscopic illumination lens was secured at the fiber distal end with medical grade tubing to diverge the illumination. (C) The fiber was inserted through the working channel to deliver illumination. (D) The lens assembly was epoxied and secured with hypodermic tubing.

Figure 4-2. Multimodal imaging protocol for gastric cancer detection. The filter is attached prior to endoscopy. Trimodal imaging can be performed in a single endoscopic insertion. “Suspicious” areas were identified in WLI and VFI, and further interrogated using HRME. Additional HRME images were also acquired in areas identified as “unsuspicious” by widefield modalities. Finally, biopsies were obtained from imaged areas and control areas.

Figure 4-3. Irradiance that can be achieved in the G2 MVE and updated G2 MVE in VFI mode. Irradiance provided by the internal laser diode is almost doubled in the updated G2 VFI mode. When needed, additional illumination can be provided with the portable laser module through the biopsy channel.

Figure 4-4. Hyperplasia, high grade dysplasia and adenocarcinoma in WLI, VFI and HRME. Glandular patterns were apparent and intact in WLI and VFI images of the hyperplastic biopsy. The corresponding HRME image showed round-shaped glands throughout the image, while the sizes were slightly varied. From hyperplasia to HGD, the glandular patterns became irregular and disconnected in WLI and VFI; HRME showed near effacement of glandular architecture with few fractions of glands visible in the background. In adenocarcinoma, the glandular patterns were absent in all imaging modalities and nuclear crowding was prominent in HRME.
Figure 4-5. WLI, VFI, HRME and pathology images of a site diagnosed as oxyntic mucosa. When compared to WLI, VFI highlights the intact and regular glandular patterns in normal gastric mucosa. The HRME image also shows the round-shaped glands consistent with histopathology.................................................................54

Figure 4-6. WLI, VFI, HRME and pathology images of a site diagnosed as intestinal metaplasia. The precursor lesion was clearly defined in VFI. HRME image of this site revealed linear crypts and the histology image showed presence of goblet cells as a hallmark of metaplasia (white arrows)..................54

Figure 4-7. WLI, VFI, HRME and pathology images of a malignant lesion. Both WLI and VFI showed total effacement of glands and obvious bleeding. HRME and histopathology images confirmed the absence of glandular patterns and nuclear crowding. ..................................................................................................................55

Figure 4-8. Diagnostic performance of WLI, and MVE/HRME in detection of premalignant and malignant lesions. MVE/HRME detected all carcinomas while WLI missed 1 out of 9. For detection of premalignant lesions, WLI lacked the capability to differentiate IM; MVE/HRME successfully identified 10 of 19 IMs, but also misclassified 17 of 38 negative controls as premalignant. ..............57

Figure 5-1. Schematics of the line-scanning confocal HRME platform. Solid arrows show directions of scanning on the DLP, CMOS and fiber surface; dashed arrows show the data flow between the laptop, DLP and camera. The dashed box indicates the optical system enclosure.................................................................64

Figure 5-2. Construction of a confocal image by averaging two complementary images. The green and red arrows in (A) and (C) mark the rows where exposure starts and ends, respectively. The rolling shutter aperture, shown as a clear window on the CMOS, contains D rows under exposure concurrently; the size of the illumination patterns matches that of the detection aperture. During image acquisition, the patterns are projected in a non-overlapping manner while synced with the rolling shutter, resulting in a non-uniform exposure distribution. A confocal image without imaging artifacts can be constructed by averaging two complementary frames, as shown in (B), (D) and (E). The rolling shutter aperture, illumination pattern and CMOS sensor were not drawn to scale. Lens paper was imaged as a target.................................................................67

Figure 5-3. Optical sectioning profiles of the standard HRME, confocal HRME, standard microscope and confocal microscope. The line scanning confocal configurations improve the axial resolution of the standard HRME and
microscope significantly. With the slit size increased, the axial performance transits from the confocal to non-confocal regime. Comparing the standard HRME with the standard microscope, the former outperforms the latter due to the added optical sectioning provided by the fixed spatial pattern of the fiber bundle. When the scanning detection slit is introduced, however, the microscope configurations demonstrate better sectioning than their fiber-optic counterparts; the irregularity of fibers within the bundle and crosstalk among neighboring fibers are the major contributing factors that compromise confocality and axial performance. A 20 pixel slit on the CMOS sensor is about 16.5 µm on the fiber surface.

Figure 5-4. 2D phantom images with varied slit widths. Images acquired at distances of 0 and 100 µm are shown for the standard HRME and two confocal configurations (60-pixel and 20-pixel slit). Compared with the image at 0 µm, the corresponding image at 100 µm revealed varied levels of signal loss and indicated rejection of background signal at this depth. The signal loss was most significant using the 20-pixel slit, suggesting that a smaller detection slit in the confocal configuration resulted in improved optical sectioning capability.

Figure 5-5. Ex vivo images of mouse squamous epithelium using the standard (A) and confocal (B) HRME. The confocal HRME resolved the nuclei with enhanced contrast, especially in regions indicated by the arrows. The normalized intensities of nuclear and cytoplasmic regions are shown in (C); the error bars show the intensity standard deviation in these regions. The resulting ratio of the nuclear-to-cytoplasmic signal in the standard and confocal HRME was 1.19 and 1.51, respectively.

Figure 5-6. Ex vivo images of mouse columnar epithelium using the standard (A) and confocal (B) HRME. Profiles are shown for the line scans on the left (C) and right (D). The brackets indicate the glandular walls and lumens. The resulting gland to lumen ratio was 101±26% higher in the confocal than the standard HRME (1.57±0.25 in standard and 3.20±0.84 in confocal; p < 0.0001).

Figure 5-7. Correction of illumination artifacts in the confocal HRME using a fluorescence target as a calibration tool. (A) A fluorescence target was used as a calibration tool and the confocal image of the target with illumination artifacts was acquired. For each column in the FOV, a bandpass filter was applied to locate the maxima and minima in the exposure time distribution. (B) shows the Fourier transform of the 500th column with the bandpass filter
to preserve the periodic distribution of $TE$. Compared to the original intensity plot of this column, the filtered signal showed accurate locations of exposure time maxima and minima in (C). Based on these locations for each column, a ratio map (D) was constructed to boost the intensities for pixels with partial exposure.

Figure 5-8. Real-time correction of illumination artifacts in the confocal HRME. The ratio map was applied to confocal images of lens paper (A) and oral mucosa of a normal volunteer (C). The tissue fibers and nuclei were clearly resolved in (B) and (D), respectively.

Figure 6-1. Lumen segmentation algorithm in two representative images (one diagnosed as normal and one as TA). Before segmentation, the fiber patterns were removed and image contrast was enhanced. The images were then converted into a binary image and small structures arising from noise in segmentation were removed. The next step consists of identifying lumens on the FOV border (outlined in red), and removing these partial lumens if they contain only a small fraction (grey lumens outlined in red). Partial lumens on the border containing a significant luminal area (white lumens outlines in red) are included in the final segmentation image.

Figure 6-2. Representative in vivo images and the corresponding pathology. The normal colorectal mucosa reveals uniform glandular distribution across the image. The luminal size and shape are slightly varied with occasional widening in the hyperplastic polyp. In contrast, both the TA and TVA images show linear crypts; villous structures were observed in TVA. HP, hyperplastic polyp; TA, tubular adenoma; TVA, tubulovillous adenoma.

Figure 6-3. LOOCV AUC and training AUC for k-feature models. The linear classifiers evaluated in the plot comprises 1 to 5 features. As expected, the training set AUC was improved with added features, while it almost plateaued when more than three features were used. In comparison, the LOOCV AUC was decreased when more than three features were used, suggesting risks of overfitting with increased model complexity.

Figure 6-4. Performance of the 3-feature model. The final predictive model offered a sensitivity and specificity of 91% and 89%, respectively. The AUC was 0.94. The posterior probability scatter plot showed four false positives (one diagnosed as colitis and three as HP) and two false negatives (both diagnosed as TA).
List of Tables

Table 2-1. Advanced optical imaging for BE and associated neoplasia. ..........12
Table 2-2. Advanced optical imaging for gastric cancer and precursors. .........16
Table 2-3. Advanced optical imaging for colorectal polyps and neoplasia. ......20
Table 3-1. Comparison of G1 and G2 VFI system...........................................28
Table 4-1. Site information in the post hoc analysis........................................56
Table 6-1. Description of features calculated for each colon HRME image. .....84
Table 6-2. Site information based on histopathology......................................87
Table 6-3. Features that show statistically significant differences between the neoplastic and non-neoplastic groups (p < 0.05). ..............................................89
List of Equations

Equation 3-1. Unsharp masking ................................................................. 30
Equation 3-2. Smoothing filter used to blur original VFI image .................. 30
Equation 5-1. The aperture size is proportional to the shutter time. ............. 65
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFI</td>
<td>Autofluorescence imaging</td>
</tr>
<tr>
<td>BE</td>
<td>Barrett’s esophagus</td>
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<tr>
<td>CLE</td>
<td>Confocal laser endomicroscopy</td>
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<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
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<tr>
<td>EAC</td>
<td>Esophageal adenocarcinoma</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>HGD</td>
<td>High grade dysplasia</td>
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<tr>
<td>HP</td>
<td>Hyperplastic polyp</td>
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<tr>
<td>HRME</td>
<td>High-resolution microendoscope</td>
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<tr>
<td>LGD</td>
<td>Low grade dysplasia</td>
</tr>
<tr>
<td>MVE</td>
<td>Modular video endoscope</td>
</tr>
<tr>
<td>NBI</td>
<td>Narrow band imaging</td>
</tr>
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<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>TA</td>
<td>Tubular adenoma</td>
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<td>TVA</td>
<td>Tubulovillous adenoma</td>
</tr>
<tr>
<td>VFI</td>
<td>Vital-dye fluorescence imaging</td>
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<td>WLE</td>
<td>White light endoscopy</td>
</tr>
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<td>WLI</td>
<td>White light imaging</td>
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</tbody>
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1.1. Overview

Based on GLOBOCAN estimates by the International Agency for Research on Cancer (IARC), 14.1 million new cancer cases were diagnosed and 8.2 million deaths occurred in 2012 worldwide. Cancers in the gastrointestinal (GI) tract constitute a substantial portion of this enormous and growing burden in developed and developing countries alike. In the lower GI tract, colorectal cancer is the third most common cancer in males and the second in females. Malignancies in the esophagus and stomach of the upper GI tract account for 13% of cancer related deaths globally.

Carcinogenesis in the GI tract involves multistep cascades and a significant proportion of the healthcare cost can be prevented through early cancer detection and treatment. The 5-year survival of patients with esophageal adenocarcinoma can
be as high as 81% if diagnosed at stage I, while the overall survival rate is only 13%. On a similar note, a 5-year survival rate above 70% has been reported for stage I and II gastric cancers, in stark contrast to an overall survival rate of about 20-30%. Evidence is also significant to support the role of colonoscopy as a widely accepted screening tool, with the mortality of colorectal cancer patients reduced by 53% due to colonoscopic removal of adenomatous polyps.

The current standard of care for cancer screening and surveillance in the GI tract depends primarily on conventional white light endoscopy; site-specific biopsy protocols are also developed in an attempt to maximize the opportunity for early detection of cancers and their precursors. However, the accuracy and efficacy of white light endoscopy need to be further improved, which motivates the development of novel advanced imaging technologies.

A variety of imaging modalities have been assessed to probe different aspects of cancer progression in vivo. Widefield imaging modalities such as narrow-band imaging and chromoendoscopy can take advantage of either endogenous contrast or exogenous dyes to rapidly survey the entire mucosa. However, they usually lack the resolution to resolve detailed cellular abnormalities; magnification endoscopy can be performed in conjunction but is usually limited to tertiary centers. High-resolution modalities such as confocal laser endomicroscopy offer subcellular interrogation, but only at the cost of a limited field of view.

Due to these limitations, the potential of bridging widefield and high-resolution modalities in a multimodal platform to increase the detection accuracy is
explored in this thesis. In this work, trimodal imaging at different scales was enabled in a single endoscopic insertion with a modular design that can be conveniently adapted to existing commercial systems. Specifically, vital-dye fluorescence imaging (VFI) using proflavine was developed to bridge white light endoscopy (WLE) with high-resolution interrogation. The capability of the high-resolution microendoscope (HRME) to resolve cellular morphology was also improved by enabling confocal imaging at a low cost. The platform performance was optimized and assessed for early cancer detection in the upper GI tract. Meanwhile, automated and objective analysis of microendoscopic colorectal images was explored to facilitate the applications of HRME in community and low-resource settings.

1.2. Specific aims

The overall goal of this thesis is to develop a multimodal and multi-scale imaging platform and evaluate its capability for early cancer detection in the esophagus, stomach and colon. The specific aims to accomplish this goal include:

1.2.1. Specific aim 1

Develop a modular video endoscope (MVE) to enable trimodal imaging in a single endoscopic insertion for detection of Barrett’s associated neoplasia. Using a modular design, multi-scale trimodal imaging was enabled to allow for vital-dye fluorescence imaging and high-resolution imaging in addition to standard white
light imaging. In a pilot study, *in vivo* images illustrating cancer progression in BE were acquired using the MVE/HRME platform.

**1.2.2. Specific aim 2**

**Optimize the modular video endoscope and evaluate its performance in characterization and detection of gastric cancers and precursors.** The working distance of the MVE in VFI mode was expanded to allow for rapid examination of the entire stomach. The performance of the MVE/HRME platform was first validated in an *ex vivo* pilot study and then evaluated *in vivo* in 35 consenting subjects at high risks for gastric cancer.

**1.2.3. Specific aim 3**

**Develop a low-cost line-scanning confocal microendoscope to improve visualization of nuclear morphometry with optical sectioning.** A line-scanning confocal microendoscope was developed by synchronizing a digital light projector to the rolling shutter of a CMOS sensor. Compared to the non-scanned microendoscope, the axial resolution was significantly improved. The optical sectioning performance was further validated by imaging 2D phantoms and *ex vivo* mouse specimens.

**1.2.4. Specific aim 4**

**Develop a computer-aided algorithm to discriminate neoplastic from non-neoplastic colorectal mucosa in high-resolution microendoscopic images.**
Using histopathology as the gold standard, quantitative analysis of 58 high-resolution microendoscopic images was performed to enable objective evaluation of clinical data. A three-feature model was constructed through leave-one-out cross-validation to achieve a sensitivity and specificity of 91% and 89%, respectively.

1.3. Chapter summaries

This dissertation describes the development of multi-scale optical imaging techniques and their evaluation for early cancer detection in the GI tract. Previously work has been done to develop and assess prototype devices combining widefield with high-resolution imaging modalities in Barrett’s esophagus.\textsuperscript{15,16} In this work, the clinical ease of use was improved by enabling trimodal imaging in a single endoscopic insertion. In addition, the utility of the platform was extended to early gastric cancer detection. At the same time, the imaging performance of HRME was improved by implementing the line-scanning confocal mechanism. Finally, an objective quantitative algorithm for diagnosis of colorectal adenomas in high-resolution microendoscopic images was developed to potentially enhance the applicability of HRME out of an academic setting.

Chapter 2 describes the motivation of this work by summarizing the clinical significance, highlighting the clinical needs and reviewing current advanced optical imaging modalities. Clinical background for early cancer detection in the esophagus, stomach and colon were provided. Current imaging strategies and novel advancement were also discussed, detailing their advantages and limitations.
Chapter 3 describes the development of a modular video endoscope to enable trimodal imaging for early cancer detection in Barrett’s esophagus.\(^{17}\) In a single endoscopic insertion, VFI and HRME can be performed following standard white light examination. In VFI mode, a real-time contrast enhancement algorithm allows improved visualization of glandular patterns in metaplasia and neoplasia compared to standard WLI. The ability to switch between VFI and standard WLI allows for convenient and accurate correlation between the two widefield imaging modalities. Moreover, images illustrating progression from metaplasia to dysplasia and cancer are acquired during *in vivo* esophageal endoscopy.

Chapter 4 extends the utility of MVE to characterization and detection of gastric cancer and precursors. Additional illumination was provided in VFI mode to allow for rapid examination of gastric mucosa with an expanded working distance. The performance was first validated in imaging *ex vivo* gastric specimens and then evaluated in an *in vivo* pilot study. The ability of MVE to resolve the glandular architecture was confirmed in both *ex vivo* and *in vivo* pilot studies. The architecture alterations, including distorted and effaced glands and nuclear crowding, were shown to be associated with cancer progression in the stomach. The diagnostic performance in early cancer detection was also analyzed in 35 patients in the *in vivo* trial.

Chapter 5 describes the development and initial validation of a low-cost and compact confocal microendoscope. In this chapter, the first demonstration of a line-scanning confocal microendoscope based on a digital light projector and a CMOS
camera was presented without the need for mechanical scanning. The axial 
performance was characterized in comparison with a non-scanned standard HRME. 
The optical sectioning capability was further validated by imaging a 2D phantom 
and nuclear morphometry in mouse squamous and columnar epithelium.

Chapter 6 presents quantitative analysis of high-resolution microendoscopic 
images to identify neoplastic colorectal polyps. Using histopathology as the gold 
standard, a computational algorithm was developed to discriminate neoplastic from 
non-neoplastic colorectal polyps in 58 in vivo HRME images. The computer-aided 
analysis may reduce observer variability and improve diagnostic accuracy, 
especially for low-resource and community-based settings in which expertise and 
training can be lacking.

In Chapter 7, the major conclusions of this work and their implications are 
discussed. This thesis focused on development and assessment of multi-scale 
endoscopic imaging techniques aiming to increase the accuracy of early cancer 
detection in vivo. The imaging capability of both widefield and high-resolution 
modalities, as well as the clinical ease of use, were improved. The system 
performance was evaluated both ex vivo and in vivo during pilot clinical studies. 
Computer-aided algorithms were also developed for quantitative analysis of clinical 
images to improve diagnostic accuracy and consistency.

Chapter 7 also addresses future directions of the optical imaging modalities 
developed in this work. A few steps are required to further clarify the clinical impact 
of the multimodal imaging platform. First, a larger study is required to establish the
diagnostic benefits of VFI and HRME in gastric cancer detection, especially for the identification of dysplasia. Second, future work will also be required to explore the potential benefits of optical sectioning of the confocal HRME in different pathological conditions. Finally, the real-time implementation of automated algorithms for colorectal neoplasia diagnosis can be further evaluated in a larger study and for low-resource settings.
Chapter 2

Background

2.1. Motivation and significance

Cancers in the gastrointestinal tract imposes an enormous burden on society both in the US and worldwide. In the lower GI tract, colorectal cancer is the third most common cancer in males and the second in females; this pattern is mirrored in the US, with colorectal cancer the second leading cause of cancer related deaths.\textsuperscript{1,2} Malignancies in the esophagus and stomach of the upper GI tract account for 13% of cancer related deaths globally, although they are less common in the US and many other western countries.\textsuperscript{1}

Early cancer detection and treatment is critical to alleviate this rising healthcare burden. While there exist striking disparities in the prevalence of GI cancers worldwide, screening is usually recommended for high-risk populations to
identify early cancers and precursors, based on which surveillance and intervention strategies can be determined.\textsuperscript{6,10,18} In this chapter, the clinical significance of esophageal adenocarcinoma, gastric and colorectal cancer will be reviewed highlighting early cancer progression and associated pathological alterations. Advances in optical imaging technologies aiming to address these clinical needs during GI cancer screening and surveillance will be discussed.

### 2.2. Esophageal adenocarcinoma

#### 2.2.1. Clinical significance and needs

Esophageal cancer is estimated to have been diagnosed in 16,940 cases in the United States in 2013, more than half of which have adenocarcinoma.\textsuperscript{19,20} Although it is an uncommon disease in western countries, esophageal adenocarcinoma (EAC) has been an increasing burden due to its drastically rising incidence rate.\textsuperscript{21,22} This is of particular concern, given the poor five-year survival rate of only 13%.\textsuperscript{3}

The most important risk factor for EAC is Barrett’s esophagus, a premalignant lesion in which metaplastic columnar epithelium replaces the stratified squamous epithelium that normally lines the distal esophagus.\textsuperscript{23} Compared with the general population, there is an approximately 30 times increase in the risk of EAC for BE patients.\textsuperscript{24} While direct evidence from randomized controlled studies to prove the benefit of surveillance of BE patients remains lacking,
a number of retrospective studies indicate that endoscopic surveillance is substantially associated with prolonged survival.\textsuperscript{25–32}

Endoscopic surveillance of BE patients aims at early identification of dysplasia and neoplasia at a curable stage. Surveillance endoscopy is performed following the Seattle protocol that comprises conventional white light endoscopy and random four-quadrant biopsies.\textsuperscript{10} Under conventional WLE, however, dysplasia and early neoplasia are often difficult to detect due to their similar appearance to normal mucosa and especially inflammation; it has been shown in previous studies that WLE can miss 43 – 57\% of early cancers.\textsuperscript{11} There is a critical need to improve the accuracy of early cancer detection in Barrett’s esophagus.

2.2.2. Current optical imaging technologies for screening and surveillance

Advanced optical imaging techniques are emerging to improve accuracy of endoscopic detection and enable guided biopsies. Most widely studied modalities for early cancer detection in BE include conventional and virtual chromoendoscopy, autofluorescence imaging and confocal laser endoscopy in Table 2-1.
Table 2-1. Advanced optical imaging for BE and associated neoplasia.

<table>
<thead>
<tr>
<th>Category</th>
<th>Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide-field</td>
<td>Chromoendoscopy</td>
<td>Improved image contrast and quality</td>
<td>Mixed results, time-consuming, contrast agent potentially harmful</td>
</tr>
<tr>
<td></td>
<td>NBI</td>
<td>No contrast agent, high accuracy for HGD</td>
<td>Low specificity for BE, criteria validation</td>
</tr>
<tr>
<td></td>
<td>AFI</td>
<td>High sensitivity, no contrast agent</td>
<td>Low specificity</td>
</tr>
<tr>
<td>High-resolution</td>
<td>CLE</td>
<td>High specificity with experts</td>
<td>Limited FOV, requires fluorescein, need for expertise</td>
</tr>
</tbody>
</table>

2.2.2.1. Chromoendoscopy

Chromoendoscopy depends on exogenous dyes used with conventional white light endoscopes to differentiate early neoplasia from benign BE. With several types of dyes clinically tested for more than a decade, methylene blue and indigo carmine are most commonly used in staining of BE.\textsuperscript{33,34}

Methylene blue is an absorptive dye that stains intestinal epithelium to enhance the contrast of BE and dysplasia. Mixed results have been reported in various studies, and a meta-analysis of 450 patients in 9 studies showed methylene blue is not superior to random biopsies in detection of dysplasia or carcinoma.\textsuperscript{35,36} In addition, there is also decreased enthusiasm due to the toxicity of methylene blue during endoscopy, which may expose the mucosa to potential DNA damage.\textsuperscript{37} Indigo carmine, on the other hand, highlight the surface topography and has been shown to help identify specific patterns associated with metaplasia and HGD in BE.\textsuperscript{38} While
chromoendoscopy presents higher image quality, the diagnostic benefit at the expense of exogenous dyes seems to be minimal.\textsuperscript{33}

\subsection*{2.2.2.2. Narrow band imaging}

Narrow-band imaging (NBI) is a virtual chromoendoscopy that highlights the surface pattern and vascular network of superficial mucosa using narrow spectral ranges of light for illumination. A recent meta-analysis of eight studies that include 446 patients with 2194 lesions showed a high sensitivity and specificity of 96\% and 94\% using NBI in diagnosis of HGD. However, a magnification endoscope was required to achieve the performance, and a low specificity of 65\% for BE detection was reported.\textsuperscript{39}

Like conventional chromoendoscopy, NBI is subject to observer variability and a moderate interobserver agreement was reported by Giachino et al.\textsuperscript{40} More recently, the Barrett's International NBI Group (BING) proposed an NBI classification and reported 85\% overall accuracy for dysplasia classification and a substantial interobserver agreement.\textsuperscript{41} This classification system remains to be externally validated in a larger study.

\subsection*{2.2.2.3. Autofluorescence imaging}

Autofluorescence imaging (AFI) enhances the contrast of BE-associated neoplasia by exciting endogenous fluorophores such as collagen, amino acids and flavins.\textsuperscript{42,43} Initial studies demonstrated a high sensitivity in detection of HGD/EAC, but a specificity of 43\% was reported.\textsuperscript{43} To overcome this drawback, AFI was more
recently used in conjunction with HD-WLE and NBI in a trimodal imaging system.\textsuperscript{44-46} In these studies, however, there was only a marginal reduction of false positives with addition of NBI and the benefits of AFI seemed limited.\textsuperscript{40}

2.2.2.4. Confocal laser endomicroscopy

Confocal laser endomicroscopy (CLE) uses an intravenous contrast agent (fluorescein sodium) to allow for real-time histological assessment of the esophageal lining. Two types of CLE systems are clinically available: the endoscope-based CLE (eCLE; Pentax, Tokyo, Japan) and probe-based CLE (pCLE; Mauna Kea Technologies, Paris, France).

The eCLE has a miniaturized confocal imaging window built into a conventional endoscope. In a study of 63 patients by Kiesslich et al., a high sensitivity and specificity of 92.9\% and 98.4\% was achieved to identify neoplasia \textit{in vivo}.\textsuperscript{47} More recently, however, Jayasekera et al. reported a relatively lower sensitivity and specificity of 75.7\% and 80.0\% for HGD/EAC detection based on a sample of 50 patients and argued that the benefit of eCLE is minimal when it is added to HD-WLE compared to NBI with HD-WLE.\textsuperscript{48} The difference may stem from the extent of training and expertise for accurate interpretation of confocal images, which will likely limit the potential wide clinical application of eCLE.

pCLE utilizes a fiber through the biopsy channel of a commercial endoscope for image acquisition. In a pilot study of 38 patients, Pohl et al. developed pCLE criteria for HGD/EAC in BE and achieved a sensitivity of 75.0\% and a specificity of 88.8\% - 91.0\% for two independent investigators.\textsuperscript{49} Using the same criteria, a more
recent larger study involving 101 patients showed consistently high specificity of
92.7% but a relatively low sensitivity of 62.5% for HGD/EAC detection.\textsuperscript{50} Gaddam et
al. developed different classification criteria and were able to achieve a sensitivity of
75.6% and specificity of 85.19\%.\textsuperscript{51} These results suggest CLE may facilitate real-
time decision-making with a high specificity, but effective classification criteria and
sufficient training are required.

2.3. Gastric cancer

2.3.1. Clinical significance and needs

Gastric cancer is the fifth most common cancer and the third leading cause of
cancer related mortality worldwide.\textsuperscript{1,2} Geographic and ethnic disparities are
remarkable in gastric cancer incidence and mortality. In the United States and other
western countries, the incidence is low and declining which leads to the unjustified
and controversial cost-effectiveness of screening programs.\textsuperscript{52-54} In contrast, mass
screening is implemented in certain high-incidence populations such as East Asians,
and evidence is significant to support the resulting benefits of early diagnosis and
treatment.\textsuperscript{6,7}

Screening for gastric cancer in high-risk populations focuses on early
detection and accurate diagnosis of premalignant and malignant lesions. In addition
to identification of dysplasia and neoplasia, patient stratification based on risk
factors is critical to determine surveillance and treatment strategies. One of the
most important risk factors for gastric cancer is intestinal metaplasia, a precursor lesion preceding dysplasia and adenocarcinoma in the gastric cancer cascade.\textsuperscript{55-59}

Macroscopic findings during conventional gastroscopy, however, are of limited value to reveal underlying pathological abnormalities and assist in clinical decision making.\textsuperscript{12,60-62}

2.3.2. Current optical imaging technologies for screening and surveillance

The advent of various advanced optical imaging modalities has been shown to improve the accuracy of early cancer detection of gastric neoplasia and its precursors. The most well studied modalities in Table 2-2 are discussed in details below.

<table>
<thead>
<tr>
<th>Category</th>
<th>Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide-field</td>
<td>Standard WLE</td>
<td>Widely available</td>
<td>Limited accuracy</td>
</tr>
<tr>
<td></td>
<td>Chromoendoscopy</td>
<td>Improved image quality and accuracy</td>
<td>Time-consuming, contrast agent potentially harmful, magnification is needed</td>
</tr>
<tr>
<td></td>
<td>NBI</td>
<td>No contrast agent, high accuracy</td>
<td>Criteria validation, impractical for community settings</td>
</tr>
<tr>
<td></td>
<td>AFI</td>
<td>High sensitivity, no contrast agent</td>
<td>Low specificity</td>
</tr>
<tr>
<td>High-resolution</td>
<td>CLE</td>
<td>High accuracy</td>
<td>Relatively few studies, small FOV, requires fluorescein, need for expertise</td>
</tr>
</tbody>
</table>
2.3.2.1. Chromoendoscopy

Chromoendoscopy with methylene blue, when used with magnification, has been shown to assist in identification of intestinal metaplasia and dysplasia with classification criteria proposed by Dinis-Riberio et al. More recently, a meta-analysis of seven prospective studies including 429 patients reported improved detection accuracy for cancer and precancer lesions using CE compared to WLI. The pooled sensitivity and specificity were 0.90 (95% CI 0.87 to 0.92) and 0.82 (95% CI 0.79 to 0.86), respectively; both indigo carmine and methylene blue were included in this meta-analysis.

2.3.2.2. Narrow band imaging

The utility of NBI in gastric lesion detection and characterization has been evaluated in multiple studies. A meta-analysis of 14 studies comprising 2171 patients demonstrated a pooled sensitivity and specificity of 0.86 (95% CI 0.83 to 0.89) and 0.96 (95% CI 0.95 to 0.97) for early gastric cancer detection, respectively. NBI has also been shown to be useful for detection of intestinal metaplasia, resulting in a sensitivity of 89% and specificity of 93%. Recently, a unified classification algorithm for early gastric cancer detection in magnifying NBI was proposed based on the microvascular and micro-surface patterns. The performance of the classification system needs to be independently validated in a larger prospective study.
2.3.2.3. Autofluorescence imaging

Similar to the findings in esophageal adenocarcinoma, the diagnostic utility of AFI in the stomach suffers from a high false positive rate.\textsuperscript{68} It was thus used as a red-flagging technique in multimodal imaging platforms combining AFI with NBI.\textsuperscript{69,70} While the performance seemed improved compared to conventional WLE, the contribution of AFI remains to be clarified.

2.3.2.4. Confocal laser endomicroscopy

The capability of CLE to visualize gastric pit patterns makes it potential useful to examine gastric lesions and assist in targeted biopsies. A meta-analysis of 6 studies revealed a pooled sensitivity and specificity of 94\% and 95\%, respectively.\textsuperscript{71} While similar results have been reported for the detection of neoplastic lesions,\textsuperscript{72} CLE has not been as widely performed as other wide field modalities in the stomach. This can be attributed to the need of dedicated equipment and dexterous operators with appropriate training, which may limit its widespread clinical application.

2.4. Colorectal cancer

2.4.1. Clinical significance and needs

Colorectal cancer is the third most common cancer in males and the second in females globally, with the highest incidence rates found in many western countries.\textsuperscript{1} In the United States, although the incidence has declined, colorectal
cancer remains the third most common cancer and second leading cause of cancer-related deaths. Several screening options for colorectal cancers are available for low- and high-resource settings, such as stool DNA test, CT colonography, fecal occult blood test and sigmoidoscopy. As the most commonly used screening procedure in many developed countries, colonoscopy allows for single-session detection and removal of neoplasms.

Adenomatous polyps are precursor lesions that are commonly found during screening, and their removal through polypectomy reduces cancer incidence and morbidity. However, routine examination under white light colonoscopy lacks the capability to differentiate neoplastic from benign lesions. Current guidelines recommend removal of all visible polyps followed by histopathologic diagnosis to determine surveillance intervals. While aiming at a complete removal of neoplastic polyps, the standard of care results in a low neoplasia detection rate, especially in diminutive (≤ 5 mm) and small (6-9 mm) polyps. In addition to high costs of resection and pathology evaluation, it increases the risk of associated complications such as perforation and post-polypectomy bleeding. Advanced imaging technologies have been introduced and evaluated to allow for a more selective biopsy approach during screening and surveillance.

2.4.2. Current optical imaging technologies for screening and surveillance

Advanced optical imaging technologies have been developed to improve the detection of neoplasia and potentially support a paradigm shift to a “diagnose-and-
leave” strategy, in which diminutive high-confidence hyperplastic polyps are identified and left without resection or biopsy.

Table 2-3. Advanced optical imaging for colorectal polyps and neoplasia.

<table>
<thead>
<tr>
<th>Category</th>
<th>Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide-field</td>
<td>NBI</td>
<td>No contrast agent, meets the ASGE threshold</td>
<td>Expertise and high confidence required, impractical for community settings</td>
</tr>
<tr>
<td>High-resolution</td>
<td>CLE</td>
<td>High accuracy</td>
<td>Relatively few studies, small FOV, fluorescein required, observer variability</td>
</tr>
</tbody>
</table>

2.4.2.1. Narrow band imaging

NBI is the most widely studied optical diagnosis modality for colon polyps and has dominated the literature in Western countries. A meta-analysis of 20 studies by the ASGE technology committee showed that optical biopsy with NBI exceeded the NPV threshold (90%) for adenomatous polyp detection and thus supported a “diagnose-and-leave” strategy. However, it should be noted that a high degree of heterogeneity was found among these studies, and recommendations were only made when the condition is diagnosed by experts with high confidence. Evidence exists in other studies to argue that the performance in academic and research settings may not be replicated in routine community practice. For other virtual chromoendoscopy technologies such as FICE and i-SCAN, high levels of heterogeneity were also observed in different studies and inferior performances were found.

2.4.2.2. Confocal laser endomicroscopy

Relatively few studies have been conducted using CLE for colorectal cancer. Qualitative criteria have previously been developed to diagnose neoplastic polyps in
the colon. The detection accuracy and observer variability have been investigated in both pCLE and eCLE. A moderate interobserver agreement \((k = .55)\) was reported with an overall accuracy of 75\% for three pCLE users by Gomez et al;\(^8\) similarly, Kuiper et al\(^8\) found a moderate interobserver agreement with an accuracy of 81\% in pCLE for five observers. In eCLE, a substantial interobserver agreements has been reported and the observer accuracy ranges from 85.6\% to 92.2\%.\(^8\) Given the variability present among the few studies using CLE, more evidence is required to support its widespread application.\(^1\)

### 2.5. Discussion

In conclusion, advancement in novel optical imaging techniques has demonstrated substantial potential to accurately diagnose early cancers in the GI tract. Different clinical needs are present for cancers in the esophagus, stomach and colon; this stems from the difference of the cancer development cascades and associated pathological alterations. As a result, although similar modalities have been applied throughout the upper and lower GI tract, their diagnostic performances are varied in the esophagus, gastric and colon.

Despite the advances in optical imaging methods, there exist barriers to endorse their widespread application during routine clinical practice. High accuracies have been reported for several widefield modalities, such as chromoendoscopy for gastric cancer detection and NBI for colorectal cancer detection. However, many studies require the use of a magnification scope, which can limit the widespread application of these modalities. Excellent diagnostic performance in the GI tract was also reported for CLE when performed by experts, but it lacks the capability to survey a large area of mucosa. In future studies, the
advantages of multiple modalities can be potentially combined to survey the GI mucosa at different scales.

Meanwhile, most of the trials to evaluate the advanced optical imaging techniques were conducted in a research and academic setting, and additional considerations are necessary for these methods to be more widely adopted. These advanced imaging modalities rely on delicate equipment that is usually limited to tertiary care centers, making widespread adoption prohibitively expensive. In addition, although diagnostic criteria have been proposed and validated in previous studies, unified and validated classification systems remain to be established. Finally, the interpretation of clinical data may rely on clinical expertise, which can be lacking in community settings without appropriate training.

Potential solutions to overcome these barriers include lower-cost technologies with comparable performance or technologies that can be readily adapted to existing systems. Objective and quantitative analysis of clinical data can also play a critical role in the dissemination of new technologies, which can be particularly useful for clinicians outside of academic or tertiary centers.
Chapter 3

Modular video endoscopy for \textit{in vivo} detection of Barrett’s-associated neoplasia

3.1. Introduction

The burden of esophageal adenocarcinoma (EAC) has increased at an alarming rate in western countries, due to a combination of rising incidence and poor outcomes\textsuperscript{3,20–22,86}. Since Barrett’s esophagus is the major risk factor for EAC, patients with BE are usually recommended to undergo surveillance endoscopy at regular intervals.\textsuperscript{87} However, dysplasia in BE can often be missed with standard white light imaging (WLI),\textsuperscript{11} and there is a critical need for new endoscopic techniques to improve the accuracy of early detection of EAC and its precursors.
A variety of imaging modalities have been developed to probe different aspects of cancer progression in BE. For example, narrow-band imaging targets endogenous hemoglobin absorption to highlight the vascular network and autofluorescence imaging excites endogenous fluorophores to detect BE-associated malignancies. The use of exogenous dyes, such as in Lugol’s chromoendoscopy or fluorescein targeted confocal laser endomicroscopy, can further enhance the contrast between neoplastic tissue and surrounding normal mucosa. Other sources of contrast include changes in sub-surface scattering measured via optical coherence tomography.

Recently, we developed vital-dye fluorescence imaging (VFI), a modality that highlights nuclear morphology and enhances glandular patterns by staining the epithelial nuclei to potentially red-flag abnormal lesions and bridge widefield imaging with high-resolution imaging. While images obtained with the initial prototype VFI system improved the ability to monitor changes in glandular morphology, the first generation system used an external cap for the VFI imaging which necessitated removal of the endoscope, placement of a cap and reinsertion for the transition from WLI to VFI. Moreover, the multiple insertions made it difficult to correlate regions imaged in the two modalities. To overcome these barriers, here we describe a second-generation modular video endoscope (G2 MVE) that incorporates WLI, VFI and high-resolution microendoscope (HRME) in a single endoscopic insertion. Both WLI and VFI are enabled with a removable cap module and the endoscopist can seamlessly switch between the two modalities throughout the
procedure. The removable cap design can readily expand the imaging capability of different existing endoscopes to include fluorescence imaging by installing a laser diode and attaching the cap. A contrast enhancement algorithm is also integrated in real time in VFI mode to further improve visualization of metaplastic glands associated with Barrett’s esophagus. Images illustrating progression from metaplasia to dysplasia and cancer are acquired during in vivo esophageal endoscopy in three modalities.

### 3.2. Materials and methods

#### 3.2.1. MVE instrumentation

The first generation (G1) of the MVE consisted of a modified commercial Pentax endoscopy system (Pentax EPK-i processor, EG-2990i endoscope) and an interchangeable module to achieve vital-dye fluorescence imaging. The VFI module of the MVE system was designed for use with proflavine, a topically applied vital dye. The aminoacridine-derived dye preferentially stains DNA in cell nuclei, providing strong contrast between cell nuclei and surrounding cytoplasm. With a small size, the amphipathic molecule can easily cross cell membranes and thus permits rapid topical application. Proflavine has been used in the triple dye as an antibacterial agent on the newborn’s umbilical cord. Several in vivo studies also reported the dye can be safely delivered to image epithelium in the gastrointestinal tract, oral cavity and cervix without adverse effects.
As shown in Figure 3-1, proflavine has absorption and emission maxima at approximately 450 nm and 515 nm, respectively. To image proflavine fluorescence using the G1 MVE, a 455 nm laser diode was installed, replacing the auxiliary white light LED in the EPK-i processor and the VFI module included a 500 nm long-pass filter.
filter (LPF) that was placed in front of the CCD camera for fluorescence imaging. Because the 500 nm long-pass filter in the VFI module blocks blue light from reaching the CCD camera, the VFI module must be removed for white light imaging (WLI). Thus, the G1 MVE required two separate scope insertions for WLI and VFI.

In the G2 MVE, a 405-nm laser diode (Nichia Corporation, Tokyo, Japan) was installed to provide illumination in VFI mode. As in the first-generation system, the diode replaced the auxiliary white light LED in the EPK-i and was powered by a laser driver (Wavelength Electronics, Bozeman, Montana). Since the diode has an optical output of approximately 1.2 W (to our knowledge, it is the most powerful 405 nm diode laser at the time of system development), a customized aluminum heatsink was installed and air-cooled. Through mechanical control, the illumination source could be switched between a Xenon lamp for WLI and the laser diode for VFI. Although the absorption of proflavine is reduced at 405 nm, shifting the illumination to 405 nm allowed for the installation of a 435 nm long-pass filter (Schott North America, Duryea, Pennsylvania) in place of the original 500 nm long-pass filter, permitting more blue light to reach the CCD and allowing WLI without removal of the VFI module. As in the G1 MVE, the filter was installed in a customized stainless steel cap and secured to the distal endoscope tip by a commercially available halo cap (Covidien, Sunnyvale, California), as shown in Figure 3-1 (A) and (B). The long-pass filter combined a colored absorption glass (GG435, 4.6 mm x 4.8 mm x 0.5 mm thick) with a custom coating to accommodate angular FOVs up to +/-70°. At a typical working distance of 5 – 10 mm, the FOV ranges from 15 to 25 mm. The laser diode
excitation and the emission filter spectra of the G1 and G2 systems are measured using an Ocean USB2000 spectrometer (Ocean Optics, Dunedin, Florida) and a Cary 5000 spectrophotometer (Agilent Technologies, Santa Clara, California) respectively, and shown in Figure 3-1 (C). A detailed comparison of the two systems, including the excitation source, emission filter, light guide transmission and proflavine absorption, is shown in Table 3-1.

Table 3-1. Comparison of G1 and G2 VFI system

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation peak wavelength (nm)</td>
<td>455</td>
<td>401</td>
</tr>
<tr>
<td>Achievable irradiance at 5 mm working distance (mW/cm²)</td>
<td>14.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Achievable irradiance at 10 mm working distance (mW/cm²)</td>
<td>7.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Emission filter</td>
<td>500 nm LPF</td>
<td>435 nm LPF</td>
</tr>
<tr>
<td>Transmission of endoscope light guide at corresponding excitation wavelength</td>
<td>10.5%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Normalized proflavine absorption at corresponding excitation wavelength²⁷</td>
<td>85.1%</td>
<td>35.8%</td>
</tr>
<tr>
<td>SBR</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

3.2.1.1. White balance in WLI

The 435 nm long-pass filter in the VFI module of the G2 MVE still blocks blue light below 435 nm from reaching the CCD camera during WLI. To compensate for the light loss in the blue spectral range during WLI, white balance was performed in white light images with the EPK-i processor using color balance options provided on
the front panel for the red and blue components of the RGB image. For each component, 11 adjustment levels were available ranging from -5 to +5 and could be adjusted in real time. The optimal white balance levels were determined by imaging a white balance target (X-Rite, Grand Rapids, Michigan) with the long-pass filter removed and with the long-pass filter in place. The color difference between each image with the LP filter in or out of the optical path was calculated based on the CIEDE2000 formula, and the color balance levels yielding the minimum color difference were determined as the optimal white balance setting. To assess the performance of white balance, ex vivo white light images of a mouse stomach were acquired with and without the long-pass filter in place; probability mass functions (PMF) of the original WLI image were compared to these of the color-balanced image obtained with the long-pass filter in place.

3.2.1.2. Contrast enhancement in VFI

A contrast enhancement feature was implemented via Labview (Labview 2012, National Instruments) in VFI mode to highlight glandular patterns in BE, the distortion and effacement of which have been shown to be associated with cancer progression. This feature utilized an unsharp filter to enhance edges in the image. During unsharp masking, the original image was first smoothed by an average filter $F_s$. An unsharp mask $M$ was then obtained by subtracting the blurred image from the original; the resulting weighted mask was added to the original image to produce a sharpened image. The process is summarized in Equation 3-1.
\[ g(x, y) = f(x, y) + kM(x, y) = f(x, y) + k[f(x, y) - \overline{f(x, y)}] \]

Equation 3-1. Unsharp masking

with \( f(x, y), \overline{f(x, y)} \) and \( g(x, y) \) denoting the original, smoothed and enhanced images, respectively. In Labview, the smoothing filter used to blur the original VFI image is

\[
F_s = \frac{1}{13^2} \begin{bmatrix} 1 & \cdots & 1 \\ \vdots & \ddots & \vdots \\ 1 & \cdots & 1 \end{bmatrix}_{13 \times 13}
\]

Equation 3-2. Smoothing filter used to blur original VFI image

The level of enhancement was tuned by adjusting the constant \( k \). In this study an unsharp mask with \( k = 2 \) was used.

The enhanced VFI mode was initially tested with \textit{in vivo} VFI images. As indicated in Equation 3-1, the intensities of the unsharp mask \( M \) are proportional to the difference between the original and enhanced images. The variances of the unsharp mask pixel intensities in two regions of interest (ROIs), as defined in squamous and columnar epithelium respectively, were compared to verify more significant enhancement of glandular patterns in metaplastic areas. The algorithm was then implemented in real time and contrast-enhanced videos were displayed during endoscopy without compromising the frame rate.
3.2.1.3. System performance

Two sources of background were potentially present in the imaging system – leakage of excitation light and autofluorescence of optical components when illuminated by a 405-nm laser. To verify that any background signal was minimal compared to proflavine fluorescence in VFI mode, non-fluorescent frosted quartz was used as a negative control to approximate the amount of excitation light that would typically be backscattered into the collection optics, resulting in system autofluorescence. During ex vivo imaging, proflavine stained columnar epithelium of an excised mouse stomach was first imaged in VFI mode to obtain the fluorescence signal; the background signal was then measured by imaging the frosted quartz at the same working distance and compared to proflavine fluorescence. The signal to background ratio was above 10, indicating minimal excitation leakage in fluorescence images.

3.2.2. High resolution imaging

The HRME was developed and described in details elsewhere. In essence, the system couples a compact fluorescence microscope with an optical imaging fiber. The 1-mm HRME probe is a flexible coherent fiber bundle consisting of 30,000 individual fibers with a center-to-center spacing of approximately 4 µm and a circular field of view (FOV) of 720 µm (IGN-08/30, Sumitomo Electric Industries). An LED centered at 455 nm (M455L2, Thorlabs, Newton, New Jersey, USA) was used to provide illumination and a scientific CCD camera (Grasshopper 2, FLIR Integrated
Imaging Solutions Inc, Richmond, Canada) was used for fluorescence imaging. Real-time videos were recorded and displayed using a laptop computer at a rate of 12 frames per second.

3.2.3. Pilot study

An *in vivo* pilot study using the G2 MVE and HRME was conducted in a high-risk population of subjects with Barrett's esophagus at Mount Sinai Medical Center. Patients who had histologically confirmed Barrett’s metaplasia, dysplasia or EAC and were scheduled for routine surveillance or endoscopic treatment were eligible for enrollment. The study information was provided to eligible patients and written informed consent was obtained. This pilot study was IRB-approved at both Mount Sinai Medical Center and Rice University.

3.2.3.1. Imaging procedure

The G2 MVE was used together with HRME to examine the esophagus. Compared with the G1 MVE which requires two separate insertions, G2 MVE allows for a more convenient imaging protocol (Figure 3-2). Prior to imaging, 10ml of 0.01% proflavine solution is prepared by a pharmacist using proflavine hemisulfate salt hydrate powder (purity by titration >= 98%, Sigma-Aldrich, St. Louis, Missouri). The VFI module was attached before endoscopic insertion and white balance was set. Conventional WLI was performed followed by topical application of proflavine (5 – 10 ml, 0.01%) through a spray catheter (Olympus America, Center Valley, Pennsylvania) which was inserted through the biopsy channel of the endoscope.
Following proflavine application, the esophagus was rinsed with water, and VFI images were obtained with contrast enhancement. The HRME probe was then introduced via the instrument channel of the endoscope and placed the probe in gentle contact with the esophageal mucosa. “Suspicious” areas identified during widefield imaging were imaged with the probe; additional HRME images were obtained from sites considered “unsuspicious” by widefield modalities. The entire imaging procedure was performed by a single expert endoscopist.

Figure 3-2. Comparison of imaging protocols in G1 and G2 MVE. In the G1 flowchart, the VFI module is attached between the first and second scope insertion. On the bottom panel, in the G2 MVE both WLI and VFI images are acquired in a single scope insertion.

Previously developed imaging criteria were used during the endoscopy to identify abnormalities through the BE segment. Sites were considered
suspicious in VFI mode if they revealed disrupted or effaced glandular architecture. Notes on location information of each imaged site were taken: endoscope depth and quadrant, time stamps, and clinical landmarks (such as islands, ulceration or bleeding).

Suspicious regions in any of the imaging modalities were biopsied post-imaging; standard four-quadrant biopsies were acquired every 1 to 2 cm in the BE segment together with controls from nonsuspicious metaplastic areas. All biopsies were examined by a single pathologist. Images were extracted from videos in each modality and correlated for each imaged site. Extracted frames for each site were reviewed and compared to the histopathology results.

3.2.3.2. Contrast quantification and evaluation

The contrast of representative widefield images obtained in the pilot study was quantified. Lab variance, defined as the geometrical mean of the variance in each channel of the CIELAB color space, has been shown to correlate well with perceived contrast of color images showing different content. This metric was calculated to assess and compare the contrast of WLI and VFI images.

In each widefield image, three ROIs (100 x 100 pixels) showing representative features were selected; the Lab variance of selected ROIs was calculated and averaged to evaluate image contrast. The mean Lab variance in each VFI image was compared to that in the corresponding WLI image for different
diagnostic categories. The quantitative results were also compared with the qualitative findings in these images.

3.3. Results

3.3.1. System performance

3.3.1.1. White balance in WLI

The optimal white balance levels in the G2 MVE were determined by comparing images of a white balance target with and without the G2 VFI module in place. The CIE2000 color differences between the original WLI image (with the G2 VFI module removed) and images at available color balance levels (Blue -5 to +5, Red -5 to +5) with the G2 VFI module in place were calculated. The color balance levels yielding the minimum color difference were Blue +5 and Red +1, which were used as the optimal white balance setting for the G2 MVE during WLI. Similarly, for the G1 VFI module the optimal white balance levels available were Blue +5 and Red -1; however, the color difference at the optimal white balance levels in the G2 MVE was decreased by approximately one order of magnitude compared to the G1 MVE.
Figure 3-3. Verification of white balance in the G2 MVE. *Ex vivo* images of a mouse stomach (A) without the VFI module or color balance (original), (B) with the G1 VFI module but no color balance (G1 uncorrected), (C) with the G1 VFI module and color balance (G1 corrected), (D) with the G2 VFI module but no color balance (G2 uncorrected), (E) with the G2 VFI module and color balance (G2 corrected). The probability mass function (PMF) of the selected ROI (box in (A)) for each image is compared. The available color correction failed to restore the original color due to the almost complete elimination of blue light by the G1 emission filter, while the PMF of the G2 corrected image is similar to the original.

*Ex vivo* WLI images of an excised mouse stomach were obtained to verify the white balance performance. The stomach was imaged with and without the G1 or G2 VFI modules in place as shown in Figure 3-3 (A), (B), and (D). Corrected images were acquired with the VFI modules at the corresponding optimal white balance levels (i.e., Blue +5, Red -1 for G1, and Blue +5, Red +1 for G2). The available white balance levels failed to restore the original color in the G1 corrected image as shown in Figure 3-3 (C). In contrast, the G2 uncorrected image in Figure 3-3 (D) appeared significantly less yellow as compared to the G1 uncorrected image; the color was
further corrected using the optimal white balance levels, as shown in Figure 3-3 (E). The probability mass function of the blue channel in the same ROI (black box in Figure 3-3 (A)), as shown in Figure 3-3 (F), reveals nearly total elimination of blue light by the G1 VFI module, and only partial loss with the G2 VFI module, allowing for appropriate compensation using the optimal white balance levels.

### 3.3.1.2. Contrast enhancement in VFI

Since irregularity of the glandular architecture in BE was shown to be an indicator of dysplasia and cancer, a contrast enhancement feature was integrated to highlight the glands; its performance was assessed using in vivo VFI images. Figure 3-4 (A) shows the original VFI image of an island of Barrett’s esophagus surrounded by squamous epithelium. The BE island reveals regular and intact glandular patterns as normally seen in columnar epithelium; the squamous epithelium shows no apparent structures with a homogeneous appearance across the FOV. In the enhanced VFI image in Figure 3-4 (B), edges of glands on the BE island are sharpened while the appearance of squamous epithelium remains homogeneous. Two ROIs, as defined in Figure 3-4 (B), show the columnar epithelium enclosed by the yellow box and squamous epithelium by the white box respectively. Figure 3-4 (C) shows the boxplot of unsharp mask intensities (on a scale of 0 – 255) in two types of epithelium. An F-test shows the 95% CI of variance ratio in the two ROIs is 2.82 – 3.05 (p < 0.05), indicating that the unsharp filter highlights the glands while introducing minimal level of artifacts in the uniform squamous epithelium.
3.3. In vivo pilot study: representative images

*In vivo* endoscopy was performed on three patients. Images were acquired in WLI and VFI modes and biopsies were taken. Figure 3-5 shows representative images of sites diagnosed as normal BE, HGD and carcinoma with the corresponding histopathology. Both WLI and VFI images were contrast enhanced at the same enhancement level. In Figure 3-5 (A), normal BE is characterized in WLI by intact gland borders that are evenly spaced. In VFI mode, as shown in Figure 3-5 (B), the regular glandular architecture is highlighted with enhanced contrast. The glandular
patterns are apparent in the HRME image in Figure 3-5 (C), consistent with the corresponding histopathology that also shows the presence of goblet cells as a hallmark of normal BE (black arrows in Figure 3-5 (D)). From normal BE to HGD, the glandular patterns become distorted and disrupted in WLI. The distortion of borders is shown in VFI mode with sharpened edges; when compared to normal BE, some areas show near effacement of the glandular architecture. In carcinoma, the WLI image in Figure 3-5 (K) reveals glandular effacement with abnormal vascularization. The VFI image in Figure 3-5 (L) shows severe distortion and disruption of glands in the ROI enclosed by the yellow solid box, and almost total effacement of glandular architecture in the ROI enclosed by the white solid box. The corresponding HRME and pathology images, as revealed in Figure 3-5 (M) and (N), show total obliteration of glands and absence of goblet cells.

Figure 3-5. In vivo images of normal BE, HGD and carcinoma in WLI, VFI and HRME with the
corresponding histopathology, and mean Lab variances of selected ROIs in each widefield image. From normal BE (A) to HGD (F) in WLI, glandular patterns appear more distorted and disrupted. The loss of glandular architecture is highlighted in VFI with enhanced contrast (G). Progressing to carcinoma, the WLI image (K) indicates glandular effacement and abnormal vascularization; the VFI image (L) shows further distortion (yellow solid box) and effacement of glands (white solid box). HRME image (C), as well as histopathology of normal BE (D) shows regular and intact glands with presence of goblet cells (black arrows); absence of goblet cells is observed in HGD (I), which also reveals disruption of glandular patterns in HRME (H). This glandular pattern is effaced in carcinoma in HRME (M), which is confirmed by the histopathology (N). The mean Lab variances of selected ROIs (white dashed boxes) are higher in all VFI images than their corresponding WLI images, indicating that VFI presents enhanced contrast than WLI. The error bars show the standard deviation.

The mean \( Lab \) variance of selected ROIs (white dashed boxes) with the standard deviation in each diagnostic category is shown in Figure 3-5 (E), (J) and (O). The \( Lab \) variance of VFI images in all diagnostic categories is higher than corresponding WLI images and show as much as a 10-fold enhancement for normal BE, indicating improved contrast in VFI when compared with WLI. Contrast in VFI images decreases with disease progression due to the distortion and effacement of glandular patterns associated with neoplastic progression. In contrast, contrast in WLI images increases with disease progression, due to increased vascular atypia during neoplastic progression. Nonetheless, average contrast in VFI images is higher than in corresponding WLI images for all disease categories. This is consistent with qualitative assessment of VFI images showing improved visualization of glandular patterns.

3.4. Discussion

In this research, we report the development and initial clinical use of a second-generation modular video endoscope. The novel system can perform both
white light endoscopy and vital-dye fluorescence imaging in a single endoscopic insertion. In VFI mode, a real-time contrast enhancement algorithm allows improved visualization of glandular patterns in metaplasia and neoplasia compared to standard WLI. The ability to switch between VFI and standard WLI allows for convenient and accurate correlation between the two imaging modalities.

Disruption and effacement of the glandular architecture is associated with cancer progression in BE; 15,102,103 these findings are verified in Figure 3-5. Visual assessment of WLI and VFI images of normal BE show that glands are more readily apparent in VFI than WLI images; the alterations of glandular patterns in HGD and EAC are also discerned more readily in VFI images. Using Lab variance as a measure of image contrast, the qualitative findings are confirmed, with VFI images showing higher contrast. Since VFI targets glandular architecture through nuclear staining, it facilitates the following high-resolution imaging and bridges investigation of the mucosa architecture at two different spatial scales. Results of this pilot trial suggest that additional studies are warranted to determine the overall accuracy of dysplasia/EAC detection using the MVE/HRME platform.

Multimodal imaging has been achieved in other advanced imaging platforms. For example, narrow-band imaging, autofluorescence imaging and chromoendoscopy can be performed in conjunction with standard white light imaging. Compared to these imaging modalities, VFI targets alterations in epithelial glandular patterns during cancer progression. Moreover, it is implemented using a
removable cap that can be readily adapted to existing commercial platforms with the installation of a laser diode.

With increased ease of use, the effectiveness of the G2 MVE can be further evaluated in various clinical settings. This imaging platform can also be extended to other gastrointestinal sites to study glandular alterations related to the progression of metaplasia to neoplasia, such as the stomach and colon. Other modalities, such as fiber-based optical coherence tomography and Raman spectroscopy, could also be used in conjunction to accomplish imaging at multiple resolutions. 
4.1. Introduction

The incidence and mortality rates of gastric cancer have been steadily declining in many developed countries including the US. However, it remains the fifth most common cancer and the third leading cause of cancer mortality worldwide.\textsuperscript{1,2} In countries with a high incidence rate such as Japan and Korean, mass screening is practiced and substantial benefits of early detection and treatment have been reported.\textsuperscript{5,7}

One of the most important risk factors for gastric cancer is intestinal metaplasia, a precursor lesion preceding dysplasia and adenocarcinoma in the
gastric cancer cascade.\textsuperscript{55–59} Macroscopic findings during conventional gastrosCOPY, however, are of limited value to reveal underlying pathological abnormalities associated with precursor lesions.\textsuperscript{12,60–62}

In this chapter, the MVE/HRME platform was optimized and evaluated in pilot clinical studies for early detection of gastric cancer and its precursor. The achievable working distance in VFI mode of the G2 MVE was expanded by providing additional illumination. The performance of MVE/HRME was first validated in an \textit{ex vivo} pilot study and then evaluated \textit{in vivo} in a population at high risk for gastric cancer.

4.2. Materials and methods

4.2.1. MVE optimization

The G2 MVE was previously developed to enable WLI and VFI in a single endoscopic insertion and offers compatibility with high-resolution imaging modalities such as HRME. By shifting the illumination to 405 nm in VFI, the working distance, however, was limited to about 20 mm. The limited working distance make it difficult to rapidly survey the entire stomach, especially occult regions such as the fundus. Two approaches were explored to expand the working distance in VFI mode.

First, illumination introduced through the internal light guides of the endoscope was increased. The modular design of the MVE allows for convenient
adoption of VFI mode in existing commercial platforms. In the updated G2 MVE, a high-definition EG-2990i endoscope was used with a Pentax i-5000 processor. Similarly, a 405-nm laser diode (Nichia Corporation, Tokyo, Japan) was installed to provide illumination in VFI and a removable module holding a 435 nm long-pass filter was used in both WLI and VFI. Compared to the EPK-i processor in the previous G2 MVE, the laser alignment was improved in the newer i-5000 processor and the resulting coupling efficiency was almost doubled.

In addition, an external laser module was assembled to provide additional laser excitation as needed through an optical fiber inserted in the working channel of the endoscope. The portable laser module is shown in Figure 4-1 (A) was attached to the outside of the biopsy channel, allowing it to remain fixed in place during the endoscopy procedure. A laser diode was installed in a customized copper heatsink and powered by a miniature laser driver (Micro Flex Drive V5, Nautilus Integration, Cleveland, Ohio). Two micro lenses (Thorlabs Inc, Newton, New Jersey) were used to couple the beam into a 2mm plastic optical fiber (Edmund Optics Inc, Barrington, New Jersey) in a SMA connector. At the distal end, the fiber was coupled to a miniaturized lens assembly (Ø2.20mm, Advanced Power Group Corp, Alhambra, California) designed to diverge the beam and accommodate the wide angular FOV of the endoscope. The distal end of the fiber and the lens assembly were enclosed and epoxied in a short section of hypodermic tubing (OD 2.41 mm, ID 2.29 mm, MicroGroup, Medway, Massachusetts) as shown in Figure 4-1 (D).
Figure 4-1. Overview of an external laser module to provide additional illumination for VFI. (A) The portable laser module that consists of the laser diode and battery attaches to the outside of the biopsy channel, allowing it to remain fixed in place without interrupting the routine endoscopy procedure. (B) An endoscopic illumination lens was secured at the fiber distal end with a short section of medical grade tubing to diverge the illumination. (C) The fiber was inserted through the working channel to deliver illumination. (D) The lens assembly was epoxied and secured with a short section of hypodermic tubing.

4.2.2. Ex vivo pilot study

An ex vivo pilot study was conducted in a high-risk population of patients at the Mount Sinai Medical Center to validate the capability of MVE/HRME in imaging gastric mucosa. Patients were eligible for enrollment if they had known or suspected gastric cancer and were scheduled for either endoscopy or surgical resection. Enrolled patients were provided with the study information and written informed consent was obtained. The pilot study was approved by the IRBs at both Mount Sinai Medical Center and Rice University.
11 eligible patients were enrolled in the study. For each patient, the endoscopic procedures were performed as routine. Following removal of resected specimens (biopsies or gastrectomy), imaging was performed immediately using the MVE and HRME in both normal and abnormal areas. 0.01% proflavine was topically applied prior to VFI and HRME imaging. The specimens were submitted for histology examination by an expert pathologist post-imaging.

### 4.2.3. In vivo pilot study

The performance of the trimodal platform was evaluated in an *in vivo* pilot study. Patients were eligible for enrollment if they had known or suspected gastric cancer and was scheduled for gastroscopy. Patients were recruited at Mount Sinai Hospital, New York and Hospital Evangelico in Siguatepeque, Honduras with written informed consent. The pilot study was approved by the IRBs at Mount Sinai Medical Center, Hospital Evangelico and Rice University.

#### 4.2.3.1. Imaging protocol

The imaging procedure is illustrated in Figure 4-2. The MVE module was attached and secured by a halo cap before the endoscope insertion. WLI was first performed with predetermined white balance settings. During WLI, each site was graded in real time by the endoscopist as malignant (gastric carcinoma or dysplasia), premalignant (intestinal metaplasia) and negative control (negative for neoplasia, dysplasia and intestinal metaplasia) based on the standard endoscopic criteria.
Following WLI, 0.01% proflavine (5 – 10 ml) was topically administered via a spray catheter (Olympus America, Center Valley, Pennsylvania) on the gastric mucosa and VFI was performed to scan the stomach. Sites were considered “suspicious” in VFI if dysregulation in gastric glandular architecture, or glandular obliteration was observed. The HRME probe was then inserted through the biopsy channel and “suspicious” areas identified by either widefield modality were imaged. Additional HRME images were also acquired in areas identified as “unsuspicious” by widefield modalities. Finally, biopsies were obtained from the imaged areas and 2 control areas. The whole procedure was performed by an expert endoscopist in a single endoscopic insertion. All biopsies were submitted for histologic examination by two expert pathologists and discrepancies were resolved through a consensus read.

Figure 4-2. Multimodal imaging protocol for gastric cancer detection. The filter is attached prior to
endoscopy. Trimodal imaging can be performed in a single endoscopic insertion. “Suspicious” areas were identified in WLI and VFI, and further interrogated using HRME. Additional HRME images were also acquired in areas identified as “unsuspicious” by widefield modalities. Finally, biopsies were obtained from imaged areas and control areas.

4.2.3.2. Diagnostic performance

The performance of the MVE/HRME platform in detection of malignant and premalignant lesions was evaluated in a post hoc fashion and compared to that of the real-time conventional WLI. The performance of MVE/HRME was evaluated using a two-step imaging protocol. First, widefield modalities of the MVE including WLI and VFI are performed to identify suspicious areas. HRME was then guided by the widefield findings to interrogate suspicious regions as the diagnostic technique.

Sites were selected for the post hoc analysis if images in three modalities were acquired and the histologic diagnosis was available. Images were subject to quality control (QC) by two independent researchers blinded to endoscopy and histology reads, and a single representative image was selected for each imaging modality per site. Using histology as the gold standard, sites were graded as malignant (gastric carcinoma or dysplasia), premalignant (intestinal metaplasia) or negative control (negative for neoplasia, dysplasia and intestinal metaplasia).

Images acquired in the three modalities were reviewed sequentially per site by an expert endoscopist with experience in MVE and HRME. Each site was graded as malignant, premalignant or negative control based on the MVE and HRME images following the two-step imaging protocol. During the review, WLI images were graded as “suspicious” or “unsuspicious” based on WLE criteria. VFI images were
considered “suspicious” if they revealed dysregulation in gastric glandular architecture, or if glandular obliteration was observed. Since HRME was guided by the MVE to investigate “suspicious” regions based on the two-step imaging protocol, sites identified as “unsuspicious” in both WLI and VFI were categorized as negative control. Regions red-flagged by at least one widefield modality were subject to further HRME interrogation following previously developed criteria. HRME images were considered malignant (cancer or dysplasia) if they showed distorted or effaced glands, heterogeneous luminal spacing, or nuclear crowding and enlargement. They were considered premalignant (intestinal metaplasia) if intact glands were present and appeared elongated. Sites diagnosed as negative for intestinal metaplasia, dysplasia or cancer based on the HRME images were categorized as negative control.

4.3. Results

4.3.1. G2 MVE optimization

The achievable irradiance was increased in VFI mode by improving the coupling efficiency of the internal diode and introducing external illumination with a portable laser module. As shown in Figure 4-3, irradiance provided by the internal laser diode was almost doubled in the updated G2 VFI mode. This was further improved by introducing the portable laser module through the biopsy channel. With the working distance expanded to 30 – 40 mm, the MVE takes better advantage of the wide angular FOV of the Pentax endoscope and allows for scanning the entire
stomach conveniently prior to examinations at a shorter working distance. This can be particularly useful for endoscopists to investigate occult regions such as the fundus. All the irradiance levels are below the threshold limit value (TLV) by the American Conference of Governmental Industrial Hygienists.

Figure 4-3. Irradiance that can be achieved in the G2 MVE and updated G2 MVE in VFI mode. Irradiance provided by the internal laser diode is almost doubled in the updated G2 VFI mode. When needed, additional illumination can be provided with the portable laser module through the biopsy channel.

4.3.2. Ex vivo pilot study: representative images

Ex vivo images of biopsies and gastrectomy specimens were acquired. Figure 4-4 shows images of hyperplasia, high grade dysplasia (HGD) and adenocarcinoma in WLI, VFI and HRME. Wide-field images were obtained at a typical working distance of 5 mm and the biopsies were about 3-5 mm in diameter. Glandular patterns were apparent and intact in WLI and VFI images of the hyperplastic biopsy. The corresponding HRME image showed round-shaped glands throughout the
image, while the sizes were slightly varied. From hyperplasia to HGD, the glandular patterns became irregular and disconnected in WLI and VFI; HRME images show near effacement of glandular architecture with a few fractions of glands visible in the background. In adenocarcinoma, the glandular patterns were absent in all imaging modalities and nuclear crowding was prominent in HRME images.
Figure 4-4. Hyperplasia, high grade dysplasia and adenocarcinoma in WLI, VFI and HRME. Glandular patterns were apparent and intact in WLI and VFI images of the hyperplastic biopsy. The corresponding HRME image showed round-shaped glands throughout the image, while the sizes were slightly varied. From hyperplasia to HGD, the glandular patterns became irregular and disconnected in WLI and VFI; HRME showed near effacement of glandular architecture with few fractions of glands visible in the background. In adenocarcinoma, the glandular patterns were absent in all imaging modalities and nuclear crowding was prominent in HRME.

4.3.3. *In vivo* pilot study: representative images

Figure 4-5 shows normal gastric mucosa in WLI, VFI and HRME modes and the corresponding histopathology. When compared with WLI, VFI demonstrated enhanced visualization of the glandular patterns. The glands were clearly defined showing a regular and uniform distribution. Consistent with previous *ex vivo* imaging, HRME images showed round-shaped lumens, which was further confirmed by the histopathology.
Figure 4-5. WLI, VFI, HRME and pathology images of a site diagnosed as oxyntic mucosa. When compared to WLI, VFI highlights the intact and regular glandular patterns in normal gastric mucosa. The HRME image also shows the round-shaped glands consistent with histopathology.

Images from an intestinal metaplastic site are shown in Figure 4-6. Like the findings in normal mucosa, VFI shows increased contrast of the polyp with the glands clearly visible. HRME image of this site revealed linear crypts and the histology image showed presence of goblet cells as a hallmark of metaplasia.

Figure 4-6. WLI, VFI, HRME and pathology images of a site diagnosed as intestinal metaplasia. The precursor lesion was clearly defined in VFI. HRME image of this site revealed linear crypts and the histology image showed presence of goblet cells as a hallmark of metaplasia (white arrows).

Figure 4-7 shows images from a site diagnosed as carcinoma. The absence of regular glands, together with the presence of severe bleeding on the nodule, suggested the cancer diagnosis in WLI. The VFI image confirmed the total effacement of glands with the bleeding region showing low fluorescence. Loss of
regular glands and nuclear crowding were prominent in HRME and the corresponding histology.

![Image](image_url)

**Figure 4-7.** WLI, VFI, HRME and pathology images of a malignant lesion. Both WLI and VFI showed total effacement of glands and obvious bleeding. HRME and histopathology images confirmed the absence of glandular patterns and nuclear crowding.

**4.3.4. *In vivo* pilot study: diagnostic performance**

35 patients were enrolled in the *in vivo* pilot study (2 from Mount Sinai Medical Center and 33 from Hospital Evangelico). Images derived from 66 sites in 31 patients passed QC and were included in the post hoc analysis. Detailed site information based on histopathology is shown in Table 4-1. In this high-risk population, 19 sites were diagnosed as intestinal metaplasia and categorized as premalignant. All 9 malignant lesions were diagnosed as carcinoma and no dysplasia was identified. It should also be noted that sites in the negative control
group were found to predominantly harbor gastritis, with only 4 out of 38 diagnosed as normal mucosa without remarkable pathological alterations.

Table 4-1. Site information in the post hoc analysis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Histopathology Dx</th>
<th># of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>Normal Mucosa</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Gastritis</td>
<td>34</td>
</tr>
<tr>
<td>Premalignant</td>
<td>Intestinal Metaplasia*</td>
<td>19</td>
</tr>
<tr>
<td>Malignant</td>
<td>Carcinoma</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>66 (31 pts)</td>
</tr>
</tbody>
</table>

* 7 sites showed focal intestinal metaplasia

The diagnostic performance of MVE/HRME in the post hoc analysis, as well as that of the real-time conventional WLI, are summarized in Figure 4-8. For each histologic diagnosis category (malignant, premalignant or negative control), the sites were stratified based on the optical imaging diagnosis. Since three diagnostic categories exist, the sensitivity and specificity for detection of premalignant and malignant lesions were calculated using pairwise classification. On the one hand, as shown in the confusion matrix, WLI detected 8 of 9 gastric carcinomas with a sensitivity and specificity of 88.9% and 93.0%. However, it was of limited value in the detection of premalignant lesions, missing as many as 94.7% of metaplastic sites. On the other hand, MVE/HRME detected all carcinomas and the specificity was 96.5%. Regarding the detection of premalignant lesions, MVE/HRME successfully identified 10 of 19 IMs, resulting in a higher sensitivity compared to WLI. However,
it also misclassified 17 of 38 negative controls as premalignant. The results suggested that the MVE/HRME platform can be potentially of use to detect advanced lesions, but the specificity needs to be improved in future work.

<table>
<thead>
<tr>
<th>Path</th>
<th>WLI</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Premalignant</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Malignant</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Path</th>
<th>MVE/HRME</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Premalignant</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Malignant</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premalignant</td>
<td>5.3%</td>
<td>100.0%</td>
<td>52.6%</td>
<td>63.8%</td>
</tr>
<tr>
<td>Malignant</td>
<td>88.9%</td>
<td>93.0%</td>
<td>100.0%</td>
<td>96.5%</td>
</tr>
</tbody>
</table>

Figure 4-8. Diagnostic performance of WLI, and MVE/HRME in detection of premalignant and malignant lesions. MVE/HRME detected all carcinomas while WLI missed 1 out of 9. For detection of premalignant lesions, WLI lacked the capability to differentiate IM; MVE/HRME successfully identified 10 of 19 IMs, but also misclassified 17 of 38 negative controls as premalignant.

4.4. Discussion

In this chapter the MVE was optimized and evaluated for early cancer detection in the stomach. The working distance of VFI was expanded by improving the irradiance for fluorescence excitation. The performance of the MVE/HRME platform was first validated in imaging ex vivo gastric specimens and then evaluated in vivo in a pilot study. The capability of MVE/HRME to resolve the glandular
architecture was confirmed during both ex vivo and in vivo pilot studies. The architectural alterations, including distorted and effaced glands and nuclear crowding, were shown to be associated with cancer progression in the stomach. These findings were similar to previous results in esophageal adenocarcinoma.

The platform performance was assessed in vivo in a high-risk population of 35 subjects. Features of gastric mucosa and lesions were characterized, with VFI and HRME revealing structural and morphological information not visible in WLI. Due to the small sample size and the population, however, early cancers such as low and high grade dysplasia were not identified in this pilot trial. The platform performance for dysplasia characterization and detection, therefore, remains to be clarified in a larger study.

The in vivo clinical study also suggested that the accuracy of MVE/HRME for detection of intestinal metaplasia needs to be further improved. This can be potentially attributed to a few factors. First, while remarkable architectural alterations in dysplasia and carcinoma were observed in both HRME and histology, these changes can be minor in intestinal metaplasia. A histologic hallmark of intestinal metaplasia is goblet cells, but it can be challenging to resolve them with nuclear staining agents and HRME. Secondly, potentially confounding factors exist in the pathological spectrum of the subjects enrolled in this study. As shown here, MVE/HRME misclassified 8 of 19 intestinal metaplasia as negative for IM and 17 of 38 negative control sites as intestinal metaplasia. On one hand, it should be noted that 3 of 8 false negatives for IM classification harbored only focal IM; these foci can
be missed by HRME with a limited FOV, especially for endoscopists without previous experience. On the other hand, sites in the negative control group were predominantly gastritis which may promote a false positive diagnosis.

In conclusion, we optimized VFI and evaluated the MVE/HRME platform for early detection of gastric cancer and its precursors. Glandular alterations as visualized in VFI and HRME were demonstrated to be associated with gastric cancer progression. Further work is needed to characterize early cancers and precancerous lesions including intestinal metaplasia and dysplasia in these imaging modalities.
5.1. Introduction

Endomicroscopy in combination with molecular probes has provided clinicians a powerful tool to visualize tissue architecture and cellular morphology to investigate disease progression. In probe-based endomicroscopy, a coherent fiber bundle is used to enable microscopic imaging with sub-cellular resolution through the working channel of a standard endoscope. Due to its minimal invasiveness, it is widely applicable in the evaluation and management of many clinical conditions, such as early detection of neoplasia in the gastrointestinal tract,\textsuperscript{81,107-111} cervix,\textsuperscript{98} pancreas\textsuperscript{112} and lung.\textsuperscript{113} Existing commercial and research platforms, such as the Cellvizio Endomicroscopy System (Mauna Kea Technologies, Paris, France)\textsuperscript{114} and
the high-resolution microendoscope (HRME)\textsuperscript{92}, offer an opportunity to provide real-time histological information.

The coherent fiber bundle used in probe-based endomicroscopy serves as an optical image-relay that allows for external implementation of sophisticated opto-mechanical systems at its proximal end. Optical sectioning, for example, can be introduced to increase the axial resolution via structured illumination or confocal scanning.\textsuperscript{115,116} The resulting benefits, as demonstrated in a range of laboratory and clinical studies,\textsuperscript{117–122} are manifold. When used with topical staining, optical sectioning has been shown to reduce out-of-focus light and improve image contrast.\textsuperscript{117,120} This is particularly desirable in imaging highly scattering tissues with crowded nuclei, such as in regions of precancerous or cancer.\textsuperscript{117,118} The enhanced ability to resolve individual nuclei can also potentially facilitate development of automated algorithms to diagnose disease based on cell morphology such as nuclear size, density and nuclear to cytoplasmic area ratio.\textsuperscript{123,124} In addition, subsurface tissue imaging can be realized in confocal endomicroscopy by incorporating depth focusing objectives at the distal fiber end.\textsuperscript{114,122,125}

The implementation of optical sectioning, however, usually requires integration of opto-mechanical components to rapidly scan and descan individual fibers in the coherent bundle, which adds to system cost and complexity.\textsuperscript{126} Alternatively, a digital light projector (DLP) can be used as a spatial modulator to provide scanning illumination.\textsuperscript{127,128} For confocal detection, the rolling shutter of a CMOS sensor can be synchronized with the DLP to reject out-of-focus signal.\textsuperscript{129} By
tuning the spatiotemporal alignment between the DLP and CMOS, confocal imaging can be realized without the need for physical scanning while offering the versatility of an adjustable detection aperture.

In this work, we present the first demonstration of a line-scanning confocal microendoscope based on a DLP and a CMOS camera without the need for mechanical scanning. We characterized its axial performance in comparison with a non-scanned high-resolution microendoscope (or the standard HRME), and we validated its optical sectioning capability by imaging a 2D phantom and \textit{ex vivo} mouse esophageal and colon specimens. The system offers high resolution endoscopic imaging with optical sectioning and can be built into a compact enclosure at a low cost (~$5000).

5.2. Materials and methods

5.2.1. Optical setup

The standard HRME is described in detail elsewhere. Briefly, the HRME probe is a coherent fiber bundle consisting of 30,000 individual fibers with a core-to-core spacing of approximately 4 µm and a circular field of view (FOV) of 720 µm (IGN-08/30, Sumitomo Electric Industries). An LED centered at 455 nm (M455L2, Thorlabs, Newton, New Jersey, USA) was used to provide illumination and a scientific CCD camera (Grasshopper 2, FLIR Integrated Imaging Solutions Inc, Richmond, Canada) was used for fluorescence imaging.
Line-scanning confocal imaging can be integrated with HRME using a DLP to provide scanned illumination and a CMOS sensor for confocal detection. A schematic of the novel confocal HRME is shown in Figure 5-1. A digital light projector (DLP LightCrafter 4500, Texas Instrument, Dallas, Texas, USA) was used to program the illumination patterns. Through a collimation lens and a 10x objective (RMS10X, Thorlabs, Newton, New Jersey, USA), the projected patterns were focused on the proximal fiber end; scanning of the illumination across the fiber was achieved by the sequential projection of the illumination patterns. Fluorescence signal collected by the fiber was imaged with a scientific CMOS sensor (Firefly MV USB 2.0, FLIR Integrated Imaging Solutions Inc, Richmond, Canada). The optical setup at the proximal end, as shown in the solid box in Figure 5-1, was housed in a 16” x 14” x 6” enclosure.

A laptop was used to program the DLP pattern sequence and retrieve the camera images. During image acquisition, a transistor-transistor logic (TTL) signal was sent from the DLP to trigger the camera exposure; the rolling shutter was synchronized with the DLP sequence spatiotemporally to perform confocal imaging.
Figure 5.1. Schematic of the line-scanning confocal HRME platform. Solid arrows show directions of scanning on the DLP, CMOS and fiber surface; dashed arrows show the data flow between the laptop, DLP and camera. The dashed box indicates the optical system enclosure.

5.2.2. Synchronization and confocal imaging

The rolling shutter of the CMOS camera offers a versatile electrical slit for confocal detection. When the sensor is triggered, the exposure of each row is activated sequentially at a line frequency of 16231 Hz ($f_L$), as determined by the A/D converter speed. With 1048 rows on the CMOS, it takes approximately 64.6 ms to scan the entire sensor. After exposure starts, each row acquires photons for a predefined shutter time $T_s$ before the readout takes place. With a sufficiently small $T_s$, exposure of the bottom row on the sensor can be activated before readout of the topmost row starts, resulting in a slit aperture containing all rows under exposure concurrently. In Figure 5-2 (A), the locations of the activation and readout rows are marked with the green and red arrows, respectively; both arrows move in the
scanning direction at a line frequency $f_L$. The clear window between the two rows shows the rolling shutter aperture, which moves in the same direction while maintaining its size of $D$ rows. With a fixed $f_L$, the aperture size $D$ is determined by $T_s$ in Equation 5-1:

$$D = T_s \times f_L$$

Equation 5-1. The aperture size is proportional to the shutter time.

To synchronize the illumination with the rolling shutter aperture, the DLP is programmed to perform in the pattern sequence mode. An illumination pattern is saved as a 1-bit image consisting of 912 x 1140 pixels, each representing a micromirror. The pattern size and optical magnification were adjusted so that the illumination and detection widths were matched.

Within the time period of a single-frame acquisition (~64.6 ms), the DLP supports a continuous projection of 48 patterns stored as two 24-bit RGB images in its flash memory. To scan an active CMOS FOV of 960 rows with 48 patterns in a non-overlapping manner, the distance between the central rows of two adjacent patterns should be no less than 20 pixels. The discrete nature of DLP scanning introduces non-uniform exposure among the rows, and the correction for this is discussed in detail below.

The root of imaging artifacts is illustrated in Figure 5-2 (A). In imaging configuration I, as mentioned above, the green and red arrows indicate the activation and readout rows, respectively; the rolling shutter aperture is indicated
by a clear window between these two arrows and contains $D$ rows. When the camera is triggered, both arrows and the aperture are scanned vertically at a fixed line speed. The illumination patterns with a matching size are projected sequentially without overlap, each of them centered at row $D$, $2D$, $3D$, etc. At the beginning of a frame acquisition cycle, pattern 1 is projected when its central row (row $D$) is activated. The 1st pattern is replaced by pattern 2 when the readout of row $D$ starts; meanwhile the activation arrow moves on to row $2D$, the central row of pattern 2. Each of the following patterns is synchronized in a similar way so that the projection of a pattern spans between the start of activation and readout of its central row. The effective exposure time $T_E$, defined as the period during which the illumination overlaps with the exposure for each row, varies along the scanning direction. Specifically, it maximizes at the pattern central rows (i.e., row $D$, $2D$, $3D$, etc) and drops by half linearly on the pattern borders as shown in Figure 5-2 (A). This results in the imaging artifacts revealed in Figure 5-2 (B).
Figure 5-2. Construction of a confocal image by averaging two complementary images. The green and red arrows in (A) and (C) mark the rows where exposure starts and ends, respectively. The rolling shutter aperture, shown as a clear window on the CMOS, contains D rows under exposure concurrently; the size of the illumination patterns matches that of the detection aperture. During image acquisition, the patterns are projected in a non-overlapping manner while synced with the rolling shutter, resulting in a non-uniform exposure distribution. A confocal image without imaging artifacts can be constructed by averaging two complementary frames, as shown in (B), (D) and (E). The rolling shutter aperture, illumination pattern and CMOS sensor were not drawn to scale. Lens paper was imaged as a target.

To eliminate these artifacts, a complementary image can be acquired using imaging configuration II. In Figure 5-2 (C), the illumination patterns are physically shifted by $D/2$ rows while maintaining the temporal alignment to obtain a complementary $T_E$ distribution. By averaging the two frames in Figure 5-2 (B) and (D), a confocal image without imaging artifacts is constructed in Figure 5-2 (E).

Both imaging configurations were programmed in the DLP so that the complementary images can be obtained with two consecutive frames. The averaged images were displayed in a Matlab GUI (The MathWorks, Natick, Massachusetts, USA).

5.2.3. Characterization of axial sectioning performance

The optical sectioning capability of the confocal HRME was evaluated and compared with a standard HRME. The axial performance was also measured when the optical slit size was varied by tuning the shutter time and adjusting the DLP sequences.

To accurately evaluate the optical sectioning performance, a mirror (PF10-03-G01, Thorlabs, Newton, New Jersey, USA) was used as a target. The system was
converted into reflection mode to image the mirror by removing the emission filter. The mirror was mounted to a stepper motor (LSA10A-T4, Zaber Technologies, Vancouver, BC, Canada) and was initially placed in gentle contact with the fiber bundle surface. It was then moved away from the fiber with a 20 μm increment up to 400 μm. The image intensities were measured at each axial distance. The background signal, mainly from internal reflections, was determined with the mirror removed and subtracted from all measurements. This was repeated for slit widths of 20, 40 and 60 pixels, with the 20-pixel slit as the smallest aperture size to scan the entire FOV.

The fiber-optic system can also be used as a microscope by removing the fiber probe. The axial profiles of the microscope without the fiber were measured in a similar manner. By comparing the axial profiles with (HRME) and without (microscope) the fiber bundle, the impact of fiber bundle patterns on the system’s sectioning performance was evaluated.

5.2.4. 2D phantom validation and ex vivo imaging

The optical sectioning performance in fluorescence mode was first evaluated by imaging a 2D phantom. The fluorescence phantom was developed as previously described.\(^{120}\) 15 μL of 15 μm fluorescent polystyrene microspheres (F-21010, Thermo Fisher Scientific, Waltham, MA, USA) in solution was pipetted onto a glass slide, which was then left to dry for about 20 mins prior to imaging. Similar to the mirror, the 2D phantom was first imaged in gentle contact with the fiber and then
imaged each time the fiber was retreated by 20 μm. The same measurements were also repeated for 20, 40 and 60-pixel slits.

The confocal HRME was also evaluated by imaging excised *ex vivo* mouse tissue. Squamous epithelium in the esophagus and columnar epithelium of the colon were imaged after topically staining with proflavine (0.01% w/v in PBS). The image contrast of the standard and confocal HRME was compared. In squamous epithelium, the nuclear and cytoplasmic regions were manually segmented and the nuclear to cytoplasmic signal ratio was calculated. In columnar epithelium, the contrast between the glandular walls and lumens in line scans was quantified and compared.

5.2.5. **Real-time confocal imaging**

Confocal images above were constructed by averaging two complementary images to eliminate the illumination artifacts. Since the artifacts stem from the sawtooth $T_E$ distribution as shown in Figure 5-2 (A), there also exist opportunities to correct them in a single frame by boosting the gain for rows with partial exposure. A compensation algorithm described below was developed for real-time imaging without the need for averaging two complementary frames.

To compensate for the difference in $T_E$ in a single frame, the pattern central rows with $T_E$ maxima in Figure 5-2 (A) were first located. A fluorescence target was imaged to highlight the illumination non-uniformity. A bandpass filter was then applied in each column to extract the periodic illumination pattern. The maxima
were then located in the filtered line profile. For each pixel, a compensation ratio was calculated based on the local $T_E/T_s$ ratio and a ratio map was constructed for the entire FOV. The resulting ratio map was multiplied with the original image to alleviate the illumination artifacts.

The algorithm was implemented in real time in a Matlab GUI (The MathWorks, Natick, Massachusetts, USA). Preliminary results were obtained in free-hand imaging of lens paper and oral mucosa of a normal volunteer. Prior to real-time imaging, a fluorescence target was used as a calibration tool. A compensation ratio map was calculated based on an image of the fluorescence target and applied frame by frame during subsequent imaging to correct for the illumination non-uniformity.

5.3. Results

5.3.1. Axial sectioning performance

The sectioning profiles with and without the fiber probe are shown in Figure 5-3. Confocal imaging with 20, 40 and 60-pixel slits significantly improved the axial resolution of HRME without slit detection. As the confocal aperture size was increased from 20 to 60 pixels, the axial performance transited from the confocal to the non-confocal regime. The same trend was observed for the microscope without the fiber bundle.
Figure 5-3. Optical sectioning profiles of the standard HRME (no slit), confocal HRME (20, 40 and 60-pixel slit), standard microscope (no slit) and confocal microscope (20, 40 and 60-pixel slit). The line scanning confocal configurations improve the axial resolution of the standard HRME and microscope significantly. With the slit size increased, the axial performance transits from the confocal to non-confocal regime. Comparing the standard HRME with the standard microscope, the former outperforms the latter due to the added optical sectioning provided by the fixed spatial pattern of the fiber bundle. When the scanning detection slit is introduced, however, the microscope configurations demonstrate better sectioning than their fiber-optic counterparts; the irregularity of fibers within the bundle and crosstalk among neighboring fibers are the major contributing factors that compromise confocality and axial performance. A 20 pixel slit on the CMOS sensor is about 16.5 \( \mu \)m on the fiber surface.

Without line-scanning and slit detection, the standard HRME slightly outperformed the standard microscope in its axial sectioning capability. This was expected since the network of individual fibers on the probe functions as a fixed spatial pattern that provides optical sectioning. The improvement, however, was minimal when compared to that introduced by the line-scanning mechanism using a slit of 20 to 60 pixels (about 5 to 15 times of fiber core spacing); the optical sectioning was most prominent using a 20-pixel slit on the CMOS sensor, which corresponds to about 16.5 \( \mu \)m on the fiber surface. It was also observed that for each
slit width shown in Figure 5-3, there was better optical sectioning without than with the fiber. This can be attributed to the randomness of fiber patterns and crosstalk among neighboring fibers that compromised the line-scanning confocality.

5.3.2. 2D phantom and *ex vivo* validation

The findings above were validated by imaging a layer of 15 μm beads in fluorescence mode. In each column of Figure 5-4, the image at 0 μm was normalized to itself, and images at greater axial distances were adjusted equally. Compared to the image at 0 μm, the corresponding image at 100 μm appeared defocused and revealed intensity loss. Consistent with the imaging profiles in Figure 5-3, the signal loss was minimal using the standard HRME (no slit), and was most striking when a 20-pixel slit was used, confirming that a small detection slit resulted in improved optical sectioning capability.

![Figure 5-4. 2D phantom images with varied slit widths. Images acquired at distances of 0 and 100 μm are shown for the standard HRME and two confocal configurations (60-pixel and 20-pixel slit). Compared with the image at 0 μm, the corresponding image at 100 μm revealed varied levels of](image)
signal loss and indicated rejection of background signal at this depth. The signal loss was most significant using the 20-pixel slit, suggesting that a smaller detection slit in the confocal configuration resulted in improved optical sectioning capability.

Based on the results above, a 20-pixel slit was used in subsequent evaluation of the imaging performance in highly scattering tissue samples. Figure 5-5 shows images of mouse squamous epithelium acquired by the standard (A) and confocal (B) HRME. Both images were normalized for visual comparison. Compared with the standard HRME, the confocal image demonstrated improved rejection of out-of-focus signal and enhanced image contrast. The enhancement was most striking in regions indicated by the white boxes, with the confocal image showing clearly defined nuclei that were difficult to resolve in the standard HRME. The difference was quantified by manually segmenting the nuclear and the cytoplasmic regions; the average intensity of nuclear and cytoplasmic regions were normalized and shown in Figure 5-5 (C). The ratio between the nuclear and cytoplasmic signal in the standard and confocal HRME was 1.19 and 1.51, respectively.
Figure 5-5. Ex vivo images of mouse squamous epithelium using the standard (A) and confocal (B) HRME. The confocal HRME resolved the nuclei with enhanced contrast, especially in regions indicated by the arrows. The normalized intensities of nuclear and cytoplasmic regions are shown in (C); the error bars show the intensity standard deviation in these regions. The resulting ratio of the nuclear-to-cytoplasmic signal in the standard and confocal HRME was 1.19 and 1.51, respectively.

Images of ex vivo mouse colonic mucosa are shown in Figure 5-6. The confocal image revealed significant background rejection, which was most prominent in the lumens; in addition, individual nuclei on the glandular walls were more readily resolved. To evaluate this difference, intensity profiles were plotted for two line scans in Figure 5-6 (C) and (D) (corresponding to white lines on the left and right, respectively). Regions for the glandular walls and lumens were identified and
indicated by the brackets; the contrast was quantified by comparing the average intensity of the glandular wall (maxima) to the lumen (minima). The ratio between the two was calculated for 12 pairs of maxima and minima in two line scans. The resulting gland-to-lumen ratio was about one-fold higher in the confocal than the standard HRME (1.57±0.25 in standard and 3.20±0.84 in confocal; p < 0.0001).
Figure 5-6. *Ex vivo* images of mouse columnar epithelium using the standard (A) and confocal (B) HRME. Profiles are shown for the line scans on the left (C) and right (D). The brackets indicate the glandular walls and lumens. The resulting gland to lumen ratio was about one-fold higher in the confocal than the standard HRME (1.57±0.25 in standard and 3.20±0.84 in confocal; p < 0.0001).

5.3.3. Real-time confocal imaging

An image of a fluorescence target shows the uncorrected illumination artifacts in Figure 5-7 (A). While the vertically periodic illumination patterns were clear, a line scan of a single column (the 500th column) in Figure 5-7 (C) showed significant noise due to the fiber bundle patterns and debris. To eliminate the noise, a bandpass filter in the frequency domain was used. In Figure 5-7 (B), the logarithmic Fourier transform of column 500 revealed two peaks that corresponded to the periodic non-uniform illumination. After applying a bandpass filter, the line scan in the spatial domain in Figure 5-7 (C) revealed the periodic distribution of $T_E$. The compensation ratio map constructed based on the locations of $T_E$ maxima is shown in Figure 5-7 (D).
Figure 5-7. Correction of illumination artifacts in the confocal HRME using a fluorescence target as a calibration tool. (A) A fluorescence target was used as a calibration tool and the confocal image of the target with illumination artifacts was acquired. For each column in the FOV, a bandpass filter was applied to locate the maxima and minima in the exposure time distribution. (B) shows the Fourier transform of the 500th column with the bandpass filter to preserve the periodic distribution of $T_E$. Compared to the original intensity plot of this column, the filtered signal showed accurate locations of exposure time maxima and minima in (C). Based on these locations for each column, a ratio map (D) was constructed to boost the intensities for pixels with partial exposure.

The ratio map was used to correct for the illumination artifacts in free-hand imaging of lens paper and oral mucosa of a normal volunteer. Original frames with illumination artifacts are shown in Figure 5-8 (A) and (C). Corrected images revealed clearly defined paper fibers in Figure 5-8 (B) and nuclear structures in Figure 5-8 (D) with minimal residual artifacts.
Figure 5-8. Real-time correction of illumination artifacts in the confocal HRME. The ratio map was applied to confocal images of lens paper (A) and oral mucosa of a normal volunteer (C). The tissue fibers and nuclei were clearly resolved in (B) and (D), respectively.

5.4. Discussion and conclusion

In this chapter, I report the development and *ex vivo* validation of a confocal microendoscope that integrates a digital light modulator and a CMOS sensor in a compact design. The optical sectioning capability improved the visualization of cell architecture, especially in crowded regions with degraded image quality. In addition, quantitative analysis reveals enhancement in parameters such as the nuclear to cytoplasmic ratio, which can potentially contribute to the automated and objective diagnosis of diseases based on cell density and morphology.\textsuperscript{123,124}
Measured axial profiles in Figure 5-3 suggest that the system sectioning performance could be further improved by reducing the detection slit size. This can be realized in the future with a faster DLP supporting sequential projection of added patterns, although it may increase the system cost. Alternatively, if there is only minimal sample motion, the out-of-focus signal can be further reduced at the expense of a lower frame rate. For example, the residual background signal during line-scanning can be recorded with a detection slit offset, and an image subtraction offers an axial resolution comparable to a point-scanning confocal microscope. In a different approach, multifocal scanning can be introduced via the DLP and consecutive images can be multiplexed to reconstruct a confocal image with improved background rejection.

In conclusion, the work here presents an affordable (~$5000) and portable microendoscope capable of confocal imaging. The versatility of a digital illumination modulator and an electrical detection aperture permits convenient implementation and evaluation of varied optical arrangements. Future work will be needed to explore the potential benefits of optical sectioning in a variety of pathological conditions.
Quantitative analysis of high-resolution microendoscopic images in discrimination of colorectal neoplasia

6.1. Introduction

Colorectal cancer is the third most common cancer and second leading cause of cancer related deaths in the US.\textsuperscript{52} Adenomatous polyps are precursor lesions that are commonly found during screening, and their removal through polypectomy reduces cancer incidence and morbidity.\textsuperscript{9,74} As the most commonly used screening procedure, colonoscopy allows for single-session detection and removal of neoplasms.\textsuperscript{8,73} However, routine examination under white light colonoscopy lacks the capability to differentiate neoplastic from benign lesions.\textsuperscript{13} Current guidelines recommend removal of all visible polyps followed by histopathologic diagnosis to determine surveillance intervals.\textsuperscript{18} A significant portion of polypectomy, especially
the removal of diminutive (≤ 5 mm) and small (6-9 mm) polyps, can be potentially avoided since they do not harbor neoplasia.\textsuperscript{75,76} In addition to the rising cost of resection and pathology evaluation, it increases the risk of associated complications such as perforation and post-polypectomy bleeding.\textsuperscript{77}

Advanced imaging technologies have been developed to improve the detection of neoplasia and potentially support the adoption of a “diagnose-and-leave” strategy, in which diminutive polyps can be left without resection when diagnosed as hyperplastic with high confidence. While high diagnostic performance has been demonstrated using modalities such as narrow-band imaging (NBI) and confocal laser endomicroscopy (CLE), one of the major limiting factors is the need for dedicated and expensive equipment.\textsuperscript{14,133,134} High-resolution microendoscopy (HRME) is a novel lost-cost (<$3500) optical imaging tool that can resolve subcellular structures in real time. Recently, Chang et al\textsuperscript{81} and Parikh et al\textsuperscript{135} demonstrated the ability of HRME to discriminate neoplastic from nonneoplastic colorectal polyps. Qualitative classification criteria for neoplastic lesions in HRME images were developed.\textsuperscript{81} With appropriate training in three endoscopists, Parikh et al\textsuperscript{136} showed that HRME achieved a negative predictive value above 90% for adenomatous histology and met the thresholds established by the ASGE Preservation and Incorporation of Valuable endoscopic Innovations (PIVI) for real-time endoscopic assessment of diminutive polyps.\textsuperscript{137}

In this study, we performed quantitative analysis of high-resolution microendoscopic images to enable objective evaluation of clinical data. Using
histopathology as the gold standard, a computational algorithm was developed to
discriminate neoplastic from non-neoplastic colorectal polyps in *in vivo* HRME
images. The computer-aided analysis could facilitate the dissemination of HRME by
reducing the observer variability for novice users. With reproducible and reliable
interpretations, HRME offers an inexpensive alternative to confocal endomicroscopy
for low-resource and community-based settings.

### 6.2. Materials and methods

#### 6.2.1. Patient enrollment and imaging procedure

An *in vivo* clinical trial was conducted in patients undergoing routine
screening or surveillance colonoscopies at Mount Sinai Medical Center. The study
information was provided to eligible patients who were scheduled for screening or
routine polyp surveillance colonoscopy and written informed consent was obtained.
This study was IRB-approved at both Mount Sinai Medical Center and Rice
University. The clinical trial was registered on Clinicaltrials.gov (registration
number NCT01384240).

Colonoscopy was performed by an expert endoscopist using a high-definition
white light endoscope. Polyps identified during white light examination were
further interrogated with HRME in real time. Prior to HRME imaging, 1–4 ml of
proflavine (0.01%) were topically applied through a spray catheter (Olympus
America, Center Valley, Pennsylvania). The imaged polyps were removed and
submitted for histopathologic analysis post-imaging. All biopsies were diagnosed by an expert gastrointestinal pathologist using standard criteria. Based on the histology, the sites were graded as benign (normal, hyperplastic polyp and inflammatory polyp) or neoplastic (tubular adenoma, tubulovillous adenoma, sessile serrated adenoma, dysplasia and cancer).

6.2.2. Imaging system

The HRME was developed and is described in detail elsewhere. In essence, the system couples a compact fluorescence microscope with an optical imaging fiber. The 1-mm HRME probe is a coherent fiber bundle consisting of 30,000 individual fibers with a core-to-core spacing of approximately 4 \( \mu \text{m} \) and a circular field of view (FOV) of 720 \( \mu \text{m} \) (IGN-08/30, Sumitomo Electric Industries). An LED centered at 455 nm (M455L2, Thorlabs, Newton, New Jersey, USA) was used to provide illumination and a scientific CCD camera (Grasshopper 2, FLIR Integrated Imaging Solutions Inc, Richmond, Canada) was used for fluorescence imaging. Real-time videos were recorded and displayed using a laptop computer at a rate of 12 frames per second.

6.2.3. Quantitative image analysis

Original HRME videos and images were reviewed for quality control by reviewers blinded to HRME reads and histopathology. First, one reviewer selected representative frames from the recorded videos. Second, images were compiled by imaging site and reviewed by two independent researchers to select a single image
for each site. Images were discarded if 50% or more of the field of view was out of focus or showed presence of debris or motion artifacts.

A variety of quantitative features were calculated for the entire image set to evaluate their potential diagnostic ability (Table 6-1). It was previously shown that texture features can be used to extract important spatial information about the glandular architecture that may not appear apparent to human eyes.\textsuperscript{16,138–141} Since the colonic mucosa images show quasiperiodic 2D luminal patterns, frequency contents were calculated in the Fourier frequency space. A power spectrum was first calculated with a 2D Fourier transform and then divided into 10 partitions. Each partition represents a particular range of spatial frequency, and their relative contributions were calculated resulting in 10 distinct features.

Table 6-1. Description of features calculated for each colon HRME image.

<table>
<thead>
<tr>
<th>Metric</th>
<th># of features</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency content</td>
<td>10</td>
<td>Frequency distribution of pixel values in each of the 10 partitions of the power spectrum</td>
</tr>
<tr>
<td>Lumen segmentation</td>
<td>12</td>
<td>Mean, CV, skewness &amp; kurtosis of lumen eccentricity, diameter, perimeter</td>
</tr>
</tbody>
</table>

The luminal morphology such as the caliber, shape and size distribution has been shown to be critical in differentiation of adenomatous from benign polyps.\textsuperscript{81} To quantitatively evaluate the glandular and luminal patterns, we developed an automated algorithm to segment the lumens in HRME images as illustrated in Figure 6-1. For each image, the fiber patterns were removed with a low-pass filter and the
image contrast was enhanced. An initial segmentation was then performed using a threshold identified based on the image histogram. The segmentation noise was then removed through morphologic processing such as image opening and closing. The next step entailed identifying the lumens that were only partially imaged by the probe as outlined in red. Luminal fractions that were smaller than 1% of the image size were removed (gray with red outlines) and preserving lumens were included in the final segmentation image (white in red outlines). Finally, the average eccentricity, diameter (as the equivalent diameter of a circle with the same area) and perimeter were calculated. To evaluate the variations of these parameters, the coefficient of variation, skewness and kurtosis of these luminal parameters were also included in the analysis.
Figure 6-1. Lumen segmentation algorithm in two representative images (one diagnosed as normal and one as TA). Before segmentation, the fiber patterns were removed and image contrast was enhanced. The images were then converted into a binary image and small structures arising from noise in segmentation were removed. The next step consists of identifying lumens on the FOV border (outlined in red), and removing these partial lumens if they contain only a small fraction (grey lumens outlined in red). Partial lumens on the border containing a significant luminal area (white lumens outlines in red) are included in the final segmentation image.

6.2.4. Algorithm development

A set of 22 features was used for the linear discriminant analysis to classify neoplastic (tubular adenoma, tubulovillous adenoma and sessile serrated adenoma) from non-neoplastic (normal mucosa, inflammatory polyp, hyperplastic polyp) polyps. In a univariate analysis, the classification potential of each of the 22 features was evaluated with a Student’s t-test. The mean values of a single input feature in two groups were compared and its significance level was determined.

Multivariate analysis was performed to build a two-class classifier based on a subset of up to 5 features. In each of the following models, a subset was selected via an exhaustive search in the entire set of 22 features to compute the posterior probability through linear discriminant analysis. A receiver operating characteristic (ROC) curve was constructed with histopathology as the gold standard and the area under the curve (AUC) was used to monitor the model performance. Initially, a best performing subset of up to 5 features was selected when the classifiers were trained and tested in all images. To limit the variance associated with high model complexity, the number of features was optimized through cross-validation based model selection. For a k-feature model, leave-one-out cross validation (LOOCV) was performed to compute the LOOCV accuracy. In each fold, the model was developed
using all images but one as the training set and the posterior probability was computed for the remaining image. The resulting ROC curve was constructed and the LOOCV AUC was calculated. The number of features in the final predictive model was determined to maximize the LOOCV performance.

6.3. Results

6.3.1. Patient enrollment: sites and images

Images were derived from original videos for 70 sites in 52 patients with corresponding histopathology. Images for 12 sites failed to pass the quality control and consensus was reached for the remaining 58 sites in 46 patients for subsequent analysis. In this 58-image set, 36 sites were diagnosed as non-neoplastic (normal mucosa, inflammatory polyp, hyperplastic polyp) and 22 were diagnosed as neoplastic (tubular adenoma, tubulovillous adenoma and sessile serrated adenoma). The detailed histopathologic diagnosis was shown in Table 6-2.

Table 6-2. Site information based on histopathology.

<table>
<thead>
<tr>
<th>Category</th>
<th>Histopathology Dx</th>
<th># of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-neoplastic</td>
<td>Normal mucosa</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Hyperplastic Polyp</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Inflammatory Polyp</td>
<td>6</td>
</tr>
<tr>
<td>Neoplastic</td>
<td>Tubular Adenoma</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Tubulovillous Adenoma</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Serrated Adenoma</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>58 (46 pts)</td>
</tr>
</tbody>
</table>
Figure 6-2 shows representative images for sites diagnosed as normal mucosa, hyperplastic polyp, tubular adenoma and tubulovillous adenoma with the corresponding histopathology. Normal colonic mucosa was characterized by the uniform glandular distribution across the entire image; round or oval openings were present with similar shapes and sizes. The lumens became slightly distorted in the hyperplastic polyp; while some lumens appeared elongated, they were confined by intact glandular borders. Both tubular adenoma and tubulovillous adenoma presented larger and more linear gland openings. Similar structural alterations were also prominent and confirmed in the corresponding histopathology images.
Figure 6-2. Representative in vivo images and the corresponding pathology. The normal colorectal mucosa reveals uniform glandular distribution across the image. The luminal size and shape are slightly varied with occasional widening in the hyperplastic polyp. In contrast, both the TA and TVA images show linear crypts; villous structures were observed in TVA. HP, hyperplastic polyp; TA, tubular adenoma; TVA, tubulovillous adenoma.

6.3.2. Algorithm development: model selection and performance

Table 6-3 lists features that show statistically significant differences between the neoplastic and non-neoplastic groups (p < 0.05). The features were shown in a descending order based on the p value. Differences in the mean values were found statistically significant in 7 of the 22 features.

Table 6-3. Features that show statistically significant differences between the neoplastic and non-neoplastic groups (p < 0.05).

<table>
<thead>
<tr>
<th>Feature</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency content 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frequency content 9</td>
<td>0.002</td>
</tr>
<tr>
<td>Skewness of luminal diameter</td>
<td>0.004</td>
</tr>
<tr>
<td>CV of luminal diameter</td>
<td>0.024</td>
</tr>
<tr>
<td>Frequency content 4</td>
<td>0.029</td>
</tr>
<tr>
<td>Skewness of luminal perimeter</td>
<td>0.037</td>
</tr>
<tr>
<td>CV of luminal perimeter</td>
<td>0.049</td>
</tr>
</tbody>
</table>

The performance of k-feature models was monitored with the training AUC and LOOCV AUC in Figure 6-3. When the entire image set was used for training and
testing, the performance increased for up to 3 features and then began to plateau. The training AUC was always increased with added features, which was anticipated because the same image set was used as the training and test set. In comparison, the LOOCV AUC was maximized with 3 features, suggesting potential overtraining with more than 3 features. A three-feature model revealed both high training and LOOCV AUC, and was selected as the final predictive model.

![Graph showing LOOCV AUC and training AUC for k-feature models.](image)

**Figure 6-3.** LOOCV AUC and training AUC for k-feature models. The linear classifiers evaluated in the plot comprises 1 to 5 features. As expected, the training set AUC was improved with added features, while it almost plateaued when more than three features were used. In comparison, the LOOCV AUC was decreased when more than three features were used, suggesting risks of overfitting with increased model complexity.

In each fold of the LOOCV, the features consistently selected for the 3-feature model were frequency content 7, skewness of luminal diameter, and mean of luminal perimeter. Therefore, a final predictive model was constructed using these three features. Based on the LOOCV posterior probability, the ROC curve in Figure
6-4 showed an AUC of 0.94 in classification of neoplastic from non-neoplastic images. The sensitivity and specificity were 91% and 89% at the Q-point, respectively. A scatter plot of the posterior probability is shown in Figure 6-4 with the sites grouped by the histopathologic diagnosis. Of four false positives identified by the 3-feature model, one was diagnosed as an inflammatory polyp and three were hyperplastic. Both false negatives were diagnosed as tubular adenomas.

![Figure 6-4. Performance of the 3-feature model. The final predictive model offered a sensitivity and specificity of 91% and 89%, respectively. The AUC was 0.94. The posterior probability scatter plot showed four false positives (one diagnosed as colitis and three as HP) and two false negatives (both diagnosed as TA).](image)

### 6.4. Discussion

In this research, we report a computer-aided algorithm to discriminate neoplastic from non-neoplastic colorectal mucosa in high-resolution microendoscopic images. The algorithm was created through two-class linear discriminant analysis and a three-feature model demonstrated a sensitivity and specificity of 91% and 89%. Among the three features, two were statistically
different between the two groups. The first feature was frequency content in the 7th partition of the power spectrum. This partition belongs to the high frequency range and its relative contribution was significantly lower in neoplastic images, which can be associated with the loss of small structures and widening of lumens. The other two features were skewness of luminal diameter and mean of luminal perimeter, confirming the discrimination power of luminal morphometry.

Qualitative criteria have previously been developed for endomicroscopy to diagnose neoplastic polyps. The observer variability and learning curves of these criteria have also been examined in probe- and endoscope-based laser confocal endoscopy (pCLE and eCLE), as well as in HRME. A moderate interobserver agreement ($k = .55$) was reported with a sensitivity and specificity of 76% and 72% for three pCLE users by Gomez et al; similarly, Kuiper et al found a moderate interobserver agreement with a sensitivity and specificity of 66% and 83% in pCLE for five observers. In eCLE, a substantial interobserver agreements has been reported and the accuracy of three observers ranges from 85.6% to 95.6%. As an inexpensive alternative to the commercial platforms, HRME demonstrated a sensitivity and specificity of 70% and 94% with a substantial agreement. Significant evidence is also emerging to support the use of other advanced imaging technologies such as conventional or virtual chromoendoscopy. However, due to the subjective nature of visual interpretation, observer variability can occur even among experts. In addition, most of these studies were conducted in academic and
research settings and similar performance characteristics may not be achieved in routine community practice.\textsuperscript{79,80}

The quantitative classification algorithm presented here demonstrates an accuracy of detection that is comparable to previous studies based on visual inspections.\textsuperscript{81,83,84} The automated and objective framework offers the potential to improve detection consistency and accuracy. This may be especially desirable in low-resource settings where comprehensive training in new imaging techniques may not be adequately provided. In addition, HRME is an inexpensive alternative to the costly confocal endomicroscopy platforms that are usually available only in tertiary-care referral centers.

There are several potential limitations to this study. First, a larger sample size will be required to optimize and evaluate the algorithm performance in a separate validation and test set. For this study, we employed cross-validation to evaluate and guard against the potential risk of overtraining. Secondly, the FOV of HRME is inherently limited by the diameter of the probe (720 \(\mu\)m). As demonstrated in Figure 6-1, some lumens can only be partially imaged in a single image. The segmentation algorithm was optimized to exclude fractions of lumens in the FOV which may skew the distribution of parameters such as luminal area. In the future, a mosaicking algorithm can be potentially incorporated to expand the FOV and increase the sampling size.\textsuperscript{143}
The image processing algorithm presented here provides a means for objective interpretation of clinical images with a high accuracy. The use of software-based image analysis may overcome barriers of training and expertise in low-resource settings and allow dissemination of HRME as an inexpensive high-resolution imaging technology. Future studies are warranted to evaluate and optimize its performance to assist in real-time diagnosis.
Chapter 7

Conclusion

7.1. Summary and research contributions

Advanced imaging modalities offer clinicians powerful new tools to probe different aspects of disease and cancer progression in the upper and lower GI tract. Both endogenous and exogenous contrast have been investigated to highlight abnormalities associated with biomolecular, architectural or vascular alterations. While the key clinical needs vary in the esophagus, stomach and colon, similar technologies have been widely evaluated and promising results have been demonstrated. Wide field modalities have been developed to scan the mucosa macroscopically, while high-resolution techniques provide clinicians real-time histological information.
With encouraging results from various clinical trials, there exists a substantial potential to combine the strengths of widefield and high-resolution modalities to further improve the diagnostic accuracy. At the same time, the wide dissemination of current advanced technologies is usually limited by the cost of equipment and the need for clinical expertise. To facilitate the translation and application of these novel tools, there is a critical need to increase the clinical availability and ease of use at a low cost.

The first contribution of the dissertation addresses this need by developing a multi-scale imaging platform that combines widefield imaging modalities with a high-resolution imaging probe. In a potential two-step imaging protocol, widefield modalities are used to survey the entire mucosa and suspicious macroscopic sites are further interrogated with HRME. VFI was used to bridge conventional WLI with high-resolution imaging, and the clinical ease of use was improved by enabling trimodal imaging in a single endoscopic insertion. Pilot clinical trials in BE in Chapter 3 showed that glandular architecture dysregulation is a hallmark for cancer progression and can be visualized in VFI and HRME with improved contrast.

The modular design allows for convenient adoption of VFI and HRME in existing commercial systems. In Chapter 4, VFI was optimized in a newer Pentax system and the MVE/HRME platform was evaluated for gastric cancer detection. Consistent with the findings in BE, alterations in glandular structure and nuclear morphometry were found to be associated with early cancers in the stomach. Preliminary clinical data suggest that MVE/HRME can be useful to detect additional
advanced lesions while the specificity needs to be improved, and future work is needed to assess its performance in detection of dysplasia in a larger study.

The second contribution of this thesis is to enable confocal imaging in HRME and enhance its axial resolution. In Chapter 5, the optical sectioning capability of the previously developed HRME was significantly improved through confocal imaging while maintaining a low cost and a compact design. *Ex vivo* imaging of mouse squamous and columnar epithelium demonstrated that the confocal HRME can better resolve nuclear morphometry; enhancement in quantitative parameters such as the nuclear to cytoplasmic ratio can potentially facilitate development of quantitative analysis of optical biopsy images. At a fraction of the cost, it offers an affordable alternative to existing confocal microendoscopic systems that are usually limited to tertiary referral centers.

The third contribution includes the development of an automated algorithm to classify neoplastic colorectal lesions in *in vivo* HRME images. In a set of 58 images from 46 patients, the algorithm showed an AUC of 0.94 in classification of neoplastic from non-neoplastic images. The sensitivity and specificity were 91% and 89% at the Q-point, respectively. The accuracy of detection demonstrated in the computer-aided interpretation is comparable to previous studies based on visual inspections by clinicians when provided with adequate training.\textsuperscript{81,83,84} The automated and objective framework offers the potential to improve detection consistency and accuracy. This may be especially desirable in low-resource settings in which
comprehensive training in new imaging techniques may not be adequately provided.

7.2. Future research directions

Future work is required to translate this multimodal imaging scheme in pilot trials to clinical practice. Technical development of the MVE/HRME platform has improved the imaging capability and clinical ease of use with the modular design at a low cost. Its clinical effectiveness and applicability remains to be further clarified in various steps.

First, the diagnostic performance of the MVE/HRME platform needs to be assessed and established in a larger clinical trial. In both the upper and lower GI tract, HRME were found to be capable of visualizing glandular and nuclear architecture, the alterations in which are associated with cancer progression. However, due to the relatively small sample size in pilot trials, its performance in detection of gastric dysplasia remains to be evaluated. A larger trial will also be required to understand the benefits of adding VFI as an intermediate step to connect WLI with HRME. Meanwhile, there is a critical need to establish diagnostic criteria for these novel modalities to effectively and consistently classify neoplastic and precursor lesions, such as intestinal metaplasia and dysplasia in the stomach.

Secondly, the clinical benefits of confocal imaging in the confocal HRME can be explored in various pathological conditions. With a potential to assist in
qualitative interpretation of clinical data with enhanced image contrast, the confocal HRME may also facilitate quantitative analysis of cancer-associated features. For instance, diagnostic performance of previously developed classification algorithms based on parameters such as nuclear to cytoplasmic area ratio can be evaluated for the confocal system and compared to the standard HRME. Since confocal imaging is enabled, there also exist opportunities to use other contrast agents such as fluorescein to highlight different aspects of disease progression. On the instrumentation end, other imaging techniques can be potentially implemented by taking advantage of the versatility of the digital spatial light modulator and the rolling shutter. For example, the residual background signal during line-scanning can be recorded with a detection slit offset, and an image subtraction offers an axial resolution comparable to a point-scanning confocal microscope. In a different approach, multifocal scanning can be introduced via the DLP and consecutive images can be multiplexed to reconstruct a confocal image with improved background rejection.

Finally, the classification algorithm for colorectal HRME images needs to be further assessed to assist in real-time decision making. A larger sample size will be required to optimize and evaluate the algorithm performance in a separate validation and test set. For this study, we employed cross-validation to evaluate and guard against the potential risk of overtraining. Meanwhile, the FOV of HRME is inherently limited by the diameter of the probe (720 µm). The segmentation algorithm was optimized to exclude fractions of lumens in the FOV which may skew
the distribution of parameters such as luminal area. In the future, a mosaicking algorithm can be potentially incorporated to expand the FOV and increase sampling size.\textsuperscript{143}

Various technical, clinical and economical aspects need considerations for new technologies to be adopted in the routine practice. This dissertation aims to develop novel optical endoscopic systems with an emphasis on the clinical ease of use and affordability. Multiple steps as detailed in this chapter are still required before their potential widespread clinical adoption. If successful, these modalities can potentially contribute to improved early GI cancer detection in a larger population in community and low-resource settings.
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