Advanced endoscopic imaging of esophageal neoplasia; old looks and new visions

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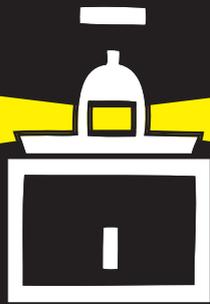
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VOLUMETRIC LASER ENDOMICROSCOPY IN BARRETT'S ESOPHAGUS: A STUDY ON HISTOLOGICAL CORRELATION

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WORK IN PROGRESS

ABSTRACT

BACKGROUND: Volumetric laser endomicroscopy (VLE) is a novel balloon based optical coherence tomography (OCT) imaging technique. It provides a 6-cm long circumferential volumetric scan of the esophageal wall layers to a depth of 3 mm with a resolution that is comparable to low-power microscopy. VLE has the potential for detection and delineation of early neoplastic lesions in Barrett's esophagus (BE). In order to investigate this, it is important that structures identified on VLE can be correlated with histology and -vice versa- that of areas containing early neoplasia on histology the corresponding VLE features can be studied. Most previous OCT studies lack such a direct correlation between histology and OCT images.

AIM: To investigate the optimal approach for one-to-one correlation of VLE images with histology.

METHODS: BE patients with and without early neoplasia underwent endoscopic resection (ER) of areas marked in-vivo with electrocoagulation markers (ECM). Subsequently ER specimens underwent additional ex-vivo marking with several different markers (ink, pin, ECM) followed by ex-vivo VLE scanning. Tissue blocks were carefully sectioned guided by the placed markers. After further histological processing a histopathology slide was sectioned from each block. When necessary, extensive sectioning of tissue blocks was performed in order to visualize all markers that were included in the tissue block on histology. All histopathology and VLE slides were evaluated by 2 researchers and considered a match if a) ≥ 2 markers were visible on both modalities and b) mucosal patterns aside from these markers matched on both histology and VLE. All slides were evaluated by an expert BE pathologist.

RESULTS: From 16 ER specimens (overall diagnosis: 7 non-dysplastic BE, 9 dysplastic BE (1 LGD, 4 HGD, 4 EAC)) 120 tissue blocks were sectioned of which 57 contained multiple markers and thus could potentially be matched with VLE. Based on several combinations of these markers in total 14 histology-VLE matches could ultimately be constructed. Markers that achieved the best yield of matches respectively were: in-vivo placed ECMs (8 matches with 12 markers), pins (7 with 11), and ink (4 with 5). Histopathological evaluation was not hindered by marker use. In this pilot study the last 6 ER specimens yielded 9/14 matches demonstrating a clear learning curve due to methodological improvements in marker placement and tissue block sectioning.

CONCLUSION: One-to-one correlation of VLE and histology is complex but feasible. The groundwork laid in this study will provide high-quality histology-VLE correlations that will allow further research on VLE structures and VLE features of early neoplasia in BE.

INTRODUCTION

Incidence of esophageal adenocarcinoma (EAC) has been rising steeply over the past decades(1,2). When patients are diagnosed at an advanced stage, EAC often has a dismal prognosis, however when diagnosed at an early stage prognosis is excellent. A known precursor lesion of EAC is Barrett's esophagus (BE). Most BE patients therefore undergo regular endoscopic surveillance for early detection of EAC. Early neoplastic lesions (*i.e.* high-grade dysplasia (HGD) a/o intramucosal cancer) are often difficult to distinguish within the Barrett's mucosa. Random biopsies are associated with sampling error and pose significant burden on the patient and health care costs.

Optical coherence tomography (OCT) is an imaging technique that may improve the efficacy of BE surveillance. OCT works analogous to ultrasound utilizing light waves instead of sound waves to form two-dimensional images based on differences in optical scattering of tissue structures. OCT is capable of generating cross-sectional images of tissues with an axial-resolution of up to 10 μm, which is comparable to low-power microscopy. Previous studies have suggested that OCT may differentiate between normal squamous mucosa, Barrett's epithelium and HGD/EAC(3–6). The clinical utility of first generation OCT systems, however, was hampered by slow acquisition rates and small scanning areas. With the development of second-generation OCT, termed optical frequency domain imaging (OFDI), it is now possible to perform high resolution, high-speed acquisition of large luminal surfaces (7).

Recently, a balloon-based system incorporating OFDI was introduced: volumetric laser endomicroscopy (VLE) (NVision, NinePoint Medical, Boston, USA). This system provides a 6-cm long circumferential scan of the esophagus in 96 seconds, with a resolution comparable to low-power microscopy.

To investigate if VLE may allow detection of early neoplasia in BE, VLE images need to be correlated with histology. Previous studies endeavoured to accomplish direct correlation of OCT images with histology. However, a reliable correlation between distinct OCT features and histology was never made.

The aim of this study was to investigate the optimal approach for correlation of VLE-images with histology.

METHODS
Setting

This study was conducted at the department of Gastroenterology of the Academic Medical Center (AMC), Amsterdam, Netherlands, a tertiary-care referral centre for endoscopic treatment of early Barrett's neoplasia, in collaboration with the department of Biomedical Engineering and Physics of the AMC. This study was approved by the medical ethical committee of the AMC (trial nr NTR4055, registered at www.trialregister.nl).

Patients

Patients undergoing surveillance of non-dysplastic (ND)BE, or referred for work-up and treatment of early neoplasia (HGD a/o EAC) were eligible for this study.

Inclusion criteria:

- Age $\geq$ 18 – 80 years.
- Known BE defined as columnar lined epithelium of the esophagus containing intestinal metaplasia, and/or;
- A visible lesion suspicious for early neoplasia, amendable for ER;
- Signed informed consent.

Exclusion criteria:

- Inability to undergo ER and/or obtain biopsies (*e.g.* due to anticoagulation, coagulation disorders, varices).
- Presence of erosive esophagitis (Los Angeles classification ≥A).
- Unable to provide signed informed consent.

Nvision VLE™ Imaging System

For more comprehensive technical details of OFDI which is used in the Nvision VLE™ Imaging System, we refer to previous publications (8–10). In short, the VLE system consists of a disposable catheter containing the optical probe and an imaging console. The imaging console consists of a display with user interface, a swept light source, optical receiver and interferometer, and a data acquisition computer. The light source generates near infrared light (bandwidth range 1250 to 1350 nm) and transmits the light into the catheter. At the distal end of the catheter is a polymer, noncompliant balloon (25 mm diameter) which centers the probe in the esophageal lumen for in-vivo imaging. During an automatic pullback of the optical probe through the catheter, a 6-cm long segment of the esophagus is scanned with VLE. This is a circumferential scan that reaches a depth of 3 mm, with an axial-resolution of up to 10 μm.

Endoscopic procedure

Endoscopic procedures were performed by two expert endoscopists (JB and BW) with extensive experience in advanced imaging techniques and endoscopic treatment of early Barrett's neoplasia.

Patients were sedated by intravenous administration of Propofol or Midazolam (2.5-15 mg) supplemented with Fentanyl (0.1-0.2 mg) or Pethidine (25-50 mg) if necessary. The esophagus was first examined in overview with white light endoscopy using a high-definition HDTV endoscopy system (GIF-HQ190 endoscope and Exera-III endoscopy system, Olympus Endoscopy, Hamburg, Germany). The length of the Barrett's segment was recorded according to the Prague-classification(11).

Inspection with white light and narrow band imaging was performed to detect macroscopic lesions within the Barrett's segment. Overview and detailed images of detected lesions were obtained. For all lesions the location (distance from the incisors and endoscopic quadrant), diameter and lesion type according to the Paris classification(12) were recorded. In case a lesion was detected, it was delineated with electrocoagulation markers (ECMs), using the tip of a snare with pure coagulation current (forced coagulation effect 2, 40W, ERBE Vio 200D,

Erbe Elektromedizin GmbH, Tuebingen, Germany). Within the delineated area, four to five reference ECMs were placed in predefined position (see figure 1A). An endoscopic picture of the lesion was taken after marking. In case of patients with NDBE a random area was selected at the discretion of the endoscopist, and delineated and marked as described above.

Delineated areas were resected (including the reference markers inside) per standard clinical practice, using the ER-cap technique (13). For mucosal lifting saline without adrenaline was used and a hard oblique cap (13.8 mm diameter) was used in order to minimize (post-procedure) complications as bleeding risk. After inspection of the resection wound, the specimen was retrieved. An endoscopic picture of the lesion location post-ER was taken.

Ex-vivo ER specimen processing

All ER specimens were pinned on cork containing a 5 mm-squared grid for reference (see figure 1B). In addition to the in-vivo placed ECMs, ER specimens were marked ex-vivo in order to optimize visibility of markers on ex-vivo VLE images and histology. The following ex-vivo markers were used: a) Ink markers; injecting a drop of carbon black ink using a 25 gauge needle by puncturing through the ER specimen (see figure 1C), b) Inked ECMs; accentuating in-vivo placed ECMs with carbon black ink, c) GP markers; placing ex-vivo ECMs using a Gold Probe™ Bipolar Electrohemostasis catheter, 10 French (Boston Scientific, Boston, USA) and d) Pins (0.5 mm diameter); pin holes were created in the borders of the specimen when these were pinned down on cork. During ex-vivo marking it was attempted to place two or more markers on the same gridline.

After ex-vivo marking, the ER specimens were placed in a custom-designed tubular shaped fixture (see figure 1D). Subsequently, the VLE catheter (NinePoint Medical Inc., Boston, USA) was placed over the ER specimen, the balloon was inflated and ex-vivo VLE scanning was performed. All ex-vivo VLE scans were performed within 15 minutes after the ER. Subsequently, the specimen was placed in buffered formalin and fixated for 24 hours minimum. The first 5 ER specimens underwent an additional ex-vivo VLE scan after 24 hours of fixation in formalin to assess a possible shrinkage effect.

Histological evaluation

After fixation both lateral sides of the bottom of the specimen were stained in two different colours to allow correct orientation on the histopathology slides. Then the ER specimens were sectioned every 2-3 mm according to the gridlines, yielding 4-11 tissue blocks per specimen. All blocks were embedded in paraffin separately. Subsequently, 4 µm histopathology slides were sectioned from these tissue blocks. All histopathology slides were stained with hematoxylin and eosin (H&E) for histological analysis and evaluated by an expert gastroenterology pathologist (SM). When necessary, extensive sectioning of tissue blocks was performed in order to visualize all markers on histology that were included in the tissue block. For correlation with VLE images, histopathology slides containing markers were digitalized in high resolution tiff images (10x magnified) using an Olympus Slide system (vs 2.5 Tokyo, Japan). All histopathology slides that were considered a match with VLE images were annotated by the pathologist (blinded for VLE images) in a custom-designed software program with several marking functions. The following
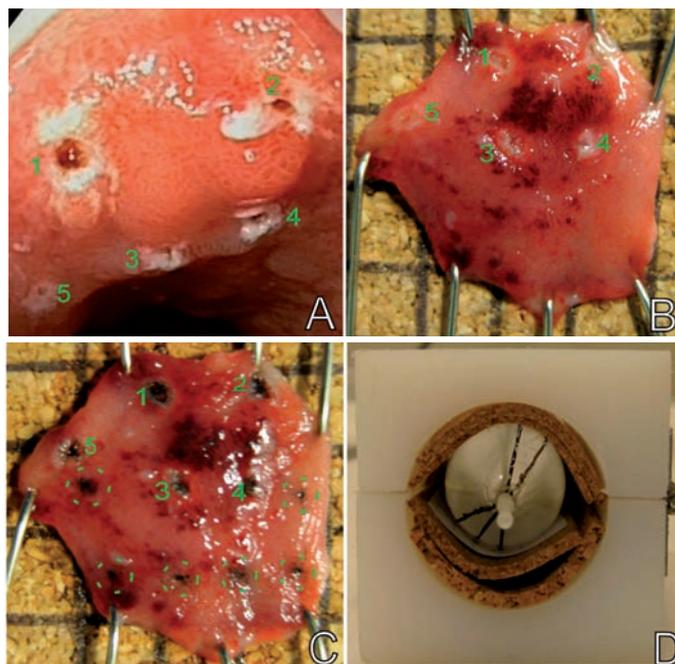
Figure 1. A. In-vivo placement of reference ECMs (numbered 1 to 4; 5 is delineation ECM), ECM 1 and 2 represent 12 o'clock position endoscopically. B. Ex-vivo specimen in in-vivo orientation pinned on gridded cork. C. In-vivo ECMs (1-5) accentuated with ink, dashed circles indicate ink markers placed with 25 gauge needle. D. Custom designed tubular shaped fixture for VLE scanning of ER specimen. ECM = electrocoagulation marker, VLE = volumetric laser endomicroscopy, ER = endoscopic resection.

features were annotated: type of mucosa (gastric, normal squamous epithelium, Barrett's epithelium and presence and grade of dysplasia ((low-grade dysplasia (LGD), HGD and EAC)), layering (epithelium, lamina propria, muscularis mucosa, submucosa, muscularis propria) and other structures ((ducts of) (sub)mucosal glands, dilated Barrett's glands, and blood vessels).

Histology-VLE correlation

Based on a macroscopic photo of the ER specimen showing the gridlines, mucosal landmarks (e.g. transition zone of Barrett's mucosa and normal squamous epithelium) and in- and ex-vivo placed markers, the exact location of the histological transection plane was determined per tissue block. Subsequently, the corresponding VLE plane was determined in the VLE-scan based on distance according to the gridlines and the markers. The visibility of the five different markers (in-vivo placed ECM, inked ECM, pin, ink and GP markers) in both histology and VLE-scan was recorded. All transection planes that contained visible markers on both VLE and histology were evaluated by two assessors (AS and WC) and were considered a match if a) ≥ 2 markers were visible on both modalities and b) mucosal patterns aside from these markers matched on both histology and VLE. For the second, benchmark OCT criteria according to Evans (3,14) were used.

RESULTS

Demographics

Fourteen patients (mean age 63 years; 12 males) were included. Nine patients were referred for work-up of early Barrett's neoplasia and 5 NDBE patients underwent standard surveillance endoscopy. The median circumferential extent of the BE segment was 1.5 cm (IQR 1-5.25), the median maximum extent of the BE segment was 5,5 cm (IQR 3.75-7.5) and all patients had a hiatal hernia with a median length of 3 cm (IQR 3.75). In total 16 ER specimens were obtained; 2 patients underwent a piecemeal resection yielding 2 specimens per patient. The worst histological outcome of the ER specimens was EAC in 4, HGD in 4, LGD in 1 and NDBE in 7 specimens. No complications were reported during or after the procedure.

Correlation markers

Table 1 shows the visibility on histology and VLE of the different markers. In general, markers showed acceptable to excellent visibility on histology and VLE; ranging from 44-100% on histology and 68-100% on VLE. Examples of the appearance of different markers on both modalities are shown in figure 2-5. In-vivo placed ECMs were in 31 out of 80 not visible after resection due to a superficial cautery effect and incorporation of markers in the cauterized resection margins. Of the remaining 49 that were macroscopically visible ex-vivo, 44 were sectioned during histological evaluation. Ink, inked ECMs and pins were easy to find on both modalities: they all showed 100% visibility on histology, inked ECMs also showed 100% visibility on VLE. However, in the ECMs that were accentuated with ink laceration of the coagulated surface caused a wide spread of ink into the mucosa and submucosa resulting in large scattering shadows on VLE. This compromised the VLE evaluation of large areas and was therefore abandoned (Figure 5). The Gold Probe created wide superficial markers which were difficult to see on histology and the wide surface hampered identification of the correct VLE plane.

Correlation of VLE images and histology

From 16 ER specimens 120 tissue blocks were sectioned of which 57 contained multiple markers and thus could potentially be matched with VLE. We found 14 corresponding histology and VLE slides that were considered a definite match according to our predefined criteria. Several combinations of markers were used in different matches. In figure 2-4 examples of histology-VLE matches are shown.

Table 1. Number of sectioned correlation markers and their visibility on histology and VLE.

	In-vivo placed ECMs	Ink	Inked ECMs	GP	Pins
Sectioned markers **	44/80 (55%)	19/23 (83%)	5/5 (100%)	9/9 (100%)	38/105 (36%)
Visibility on histology*	34/44 (77%)	12/19 (63%)	5/5 (100%)	4/9 (44%)	30/38 (78%)
Visibility on VLE *	30/44 (68%)	19/19 (100%)	5/5 (100%)	7/9 (78%)	38/38 (100%)
Visibility on histology and VLE*	23/44 (52%)	12/19 (63%)	5/5 (100%)	4/9 (44%)	30/38 (79%)

ECM = electrocoagulation marker, GP = Goldprobe marker, VLE = volumetric laser endomicroscopy.
**Number of histologically *sectioned* markers divided by total number of *placed* markers.
*Number of *visible* markers divided by the number of histologically *sectioned* markers.

Figure 2. Histology-VLE match with 2 pins. These are visible on histology as pin holes (black arrows) and create low-backscattering, well demarcable structures on VLE. Area 1: squamous epithelium, characterized on VLE by layering (top white arrow indicates transition of epithelium to lamina propria and bottom white arrow to muscularis mucosa). Area 2 and 3 are Barrett's mucosa, which is visible by loss of layering and dilated BE glands (arrowheads). Bold arrow indicates VLE balloon.

DISCUSSION

VLE is a new imaging modality that uses second generation OCT (OFDI) for rapid circumferential scanning of the esophagus over a length of 6 cm and up to a depth of 3 mm. Several studies have demonstrated that OCT can identify Barrett's epithelium and features of dysplasia in BE. Previous OCT systems used probes that could only scan a small area. The improved image acquisition rate of VLE allows cross-sectional imaging of generally the whole BE in a single scan. VLE may therefore be a promising technique for the detection of early neoplasia during BE surveillance. In addition, relevant lesions visible on VLE can be marked directly with laser (15).In order to investigate the ability of detection and delineation of early neoplastic lesions in BE, it is important that structures identified on VLE can be correlated with histology and -vice versa- that of areas containing early neoplasia on histology the corresponding VLE features can be studied. Several studies investigating OCT systems endeavoured to correlate OCT images with histology (3–5,14,16). However, one-to-one correlation of OCT images lacked in most studies, since using biopsies as histological counterparts is inevitably compromised by sampling error. In the current study we therefore evaluated a unique approach for one-to-one correlation that has, to our knowledge, not been performed before.

The entire process of handling, marking, histological processing and analysis of the ER specimens was subjected to critical re-evaluation during this pilot-study. In the first ER specimens we found that in-vivo placed ECMs were often not visible after resection, which led to an insufficient number of ECMs on the same gridline in tissue blocks for matching. In the following cases, we
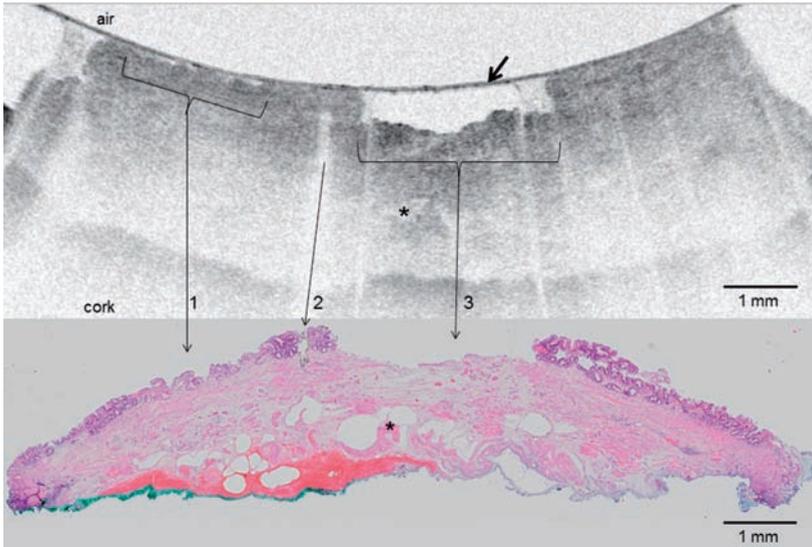
Figure 3. Histology-VLE match with 2 ECM's and 1 ink marker, containing Barrett's mucosa on the lateral sides. Accolades with arrow 1 and 3 indicate ECMs; ECM 1 is more subtle than ECM 3. Arrow 2 indicates ink marker which is visible as a puncture hole with black ink on histology and as low-backscattering narrow beam on VLE. Asterix indicates blood vessel. ECM = electrocoagulation marker, VLE = volumetric laser endomicroscopy.
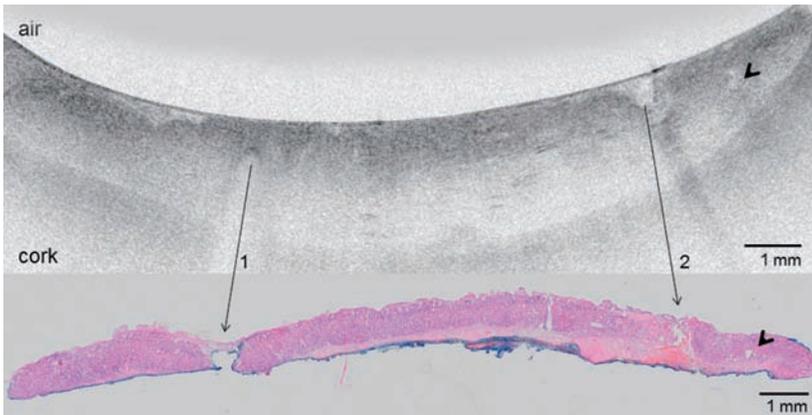

Figure 4. Histology-VLE match with 1 pin (arrow 1) and 1 ECM (arrow 2), containing gastric mucosa in between and on lateral sides. Asterix indicates dilated gastric gland. ECM=electrocoagulation marker, VLE= volumetric laser endomicroscopy.

made in-vivo ECMs more profound and placed additional in-vivo ECMs within the delineation. This proved to be an effective strategy. In addition, we accentuated several ECMs ex-vivo with ink prior to VLE scanning. However, the ink spread over the lacerated ECM surface causing large scattering shadows in the VLE images obscuring a valid VLE evaluation.
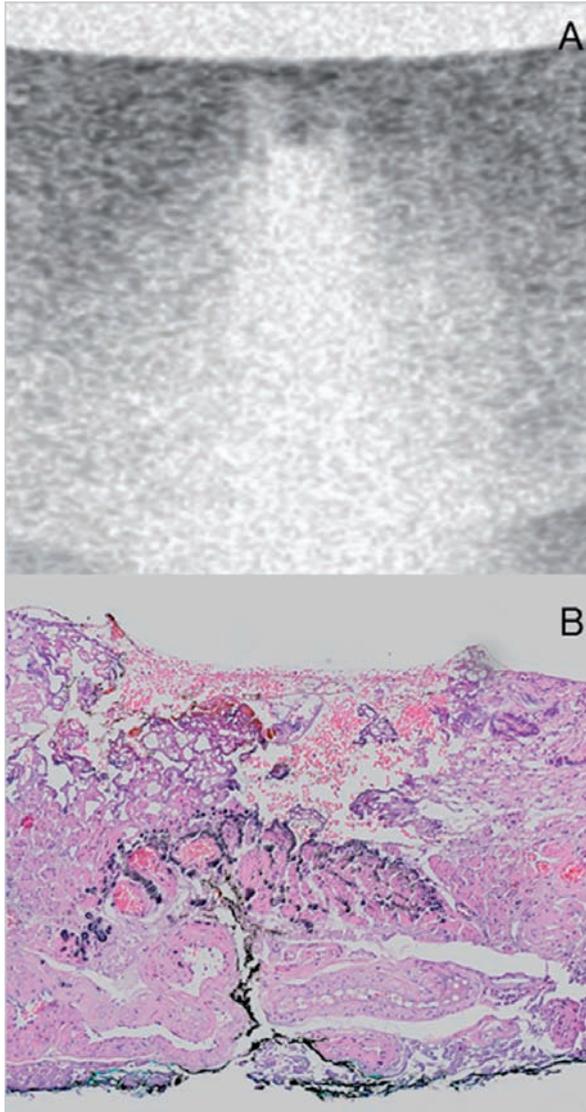
Figure 5. Appearance of inked ECM on VLE (A) and histology (B). ECM=electrocoagulation marker, VLE= volumetric laser endomicroscopy.

Two ex-vivo markers proved to be useful in histology-VLE correlation: Ink applied with a narrow diameter needle and pin holes created by the pins that were used to pin down the ER specimens on cork. Both demonstrated an excellent visibility on VLE and histology and made a great contribution when used in combination with ECMs.

For correct correlation it is important that the transection planes of the VLE image and the histopathology slide are identical. To achieve this ER specimens were placed on a grid to

allow histological sectioning and VLE scanning to be oriented in the same plane. Histological sectioning was done manually, hence the sectioning plane may slightly differ from the VLE plane. In order to visualize all markers in the same histological plane, it was sometimes necessary to perform extensive sectioning of the tissue blocks. Given the limitations of this experimental set-up small differences between the histology and VLE planes cannot be avoided. A methodological improvement would be to obtain a three-dimensional histology-VLE match by rendering the VLE data in 3D and obtain serial histopathology slides with markers that contain one match. Hereby a match would be better visualized and additionally small differences between matched histology and VLE planes would be overcome.

By optimizing all different steps (*e.g.* marker placement, grid orientation, histological sectioning) in the correlation process we were able to improve the yield of histology-VLE matches throughout the pilot study. This was demonstrated by the observed learning curve which can be depicted by the increasing yield of matches: 5 matches in the first 10 specimens and 9 matches in the last 6 specimens.

A limitation of the current study was that only ex-vivo VLE imaging was performed, therefore our VLE images may not exactly represent the in-vivo situation. Given the difficulties we experienced regarding localization of markers and orientation of the right plane, a direct comparison of in-vivo images with ER specimen histology is at this stage not feasible. In the current set-up, we were able to control orientation on both the histological specimen and VLE scanning under optimal circumstances. For the correlation studies of VLE images and histology, ex-vivo VLE after optimizing marker placement is in our opinion the first step.

Since all ER specimens were embedded in formalin for at least 24 hours after VLE imaging, fixation artefacts may have influenced the histology-VLE evaluation. In order to assess shrinking and formalin artefacts, we performed an additional VLE scan after fixation in formalin in the first 5 specimens included. Analysis of length, width and depth measurements of the specimens showed that differences before and after fixation were diminutive and thus considered negligible (data not shown). We can, however, not exclude the possibility that the histological processing may have altered the structures (*i.e.* collapsing of vessels/glands or change of architecture) and therefore may have incorporated errors in our evaluation. In addition, we experienced several artefacts due to histological processing that made certain histopathology slides (partly) not eligible for evaluation, such as tissue folds, tears and denudation of the mucosa.

Histological evaluation was done by a single pathologist with extensive experience in Barrett's neoplasia. For grading of early Barrett's neoplasia a panel of pathologists is preferable given the inter-observer variability amongst pathologists. Since this study was not aimed at providing a VLE classification of early Barrett's neoplasia but at optimizing the matching process, a second pathology review was not deemed necessary at this stage.

In conclusion, we have demonstrated that one-to-one correlation of histology and VLE is feasible but challenging. The ground-work laid in the current study provides a methodological roadmap for correlation studies of histology and VLE.

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