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TECHNICAL NOTE

Complete one-to-one correspondence between magnifying endoscopic and histopathologic images: the KOTO method II

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Abstract

Recent advances in magnifying endoscopy with narrow-band imaging/blue laser imaging have aided in the diagnosis of gastrointestinal lesions. However, it requires knowledge of the relationship between magnifying endoscopic and histopathological images. We propose a novel method which makes possible a complete correspondence between magnifying endoscopic and histopathological images at the single glandular duct level. The KOTO method II enables three-dimensional visualization of the correlation between the endoscopic surface pattern of the mucosa and histopathological images. This method may be helpful in the development of diagnosis using magnifying endoscopy.

Keywords Magnifying endoscopy · Histopathology

Introduction

Recent advances in magnifying endoscopy with narrow-band imaging (NBI) [1]/blue laser imaging (BLI) [2] have allowed observation of the gastrointestinal mucosal structure at a single glandular duct level with high-definition images, which has contributed to improved endoscopic diagnosis of gastrointestinal lesions [3]. Previous studies have reported that endoscopic observation using magnifying endoscopy is useful for diagnosis of gastric cancer [4–6]; however, histopathology is still the gold standard. As the surface pattern observed using magnifying endoscopy with NBI/BLI reflects the histopathological image, understanding the relationship between magnifying endoscopic and histopathological images, which are obtained from the same mucosal structure from different viewing directions, would be helpful in the development of diagnosis using magnifying endoscopy. Therefore, knowledge of the relationship between endoscopic and histopathological images is indispensable.

We previously described a method of adjusting endoscopic and histopathological images (the KOTO method) [7]. The histopathological image was correlated with stereomicroscopic images depending on the mucosal cleavage line of the resected specimen. However, a complete adjustment between these two images has been difficult because the process of making histopathological specimens involves shaving surfaces of paraffin blocks (cut mucosal surfaces).

We propose a modification of the KOTO method, which allows a complete correspondence between endoscopic and histopathological images at the single glandular duct level. This method, the KOTO method II, can aid in improving endoscopic diagnosis and help us to better understand the correlation between endoscopic and histopathological findings.

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Materials and methods

Materials

Gastric specimens were obtained by endoscopic submucosal dissection (ESD) [8] after imaging the region of interest using magnifying endoscopy with NBI/BLI (Fig. 1). The specimens were fixed overnight with 10% neutral buffered formalin. The mucosal cleavage specimen was prepared as previously reported [7] and stained with crystal violet to emphasize the surface pattern (Fig. 2). After imaging the region of interest using a stereomicroscope, the mucosal cleavage was completely sectioned. The sectioned specimens were embedded in paraffin blocks after dehydration and delipidation, sliced into 3 µm sections (the 3-µm preparation), and thin sections were stained with hematoxylin and eosin (H&E) for examination and diagnosis according to the usual protocol.

Reversing procedure

After thin sectioning for H&E staining, as mentioned in "Materials", the paraffin block was dewaxed using heat and xylene treatment. Then, the dewaxed specimen was treated with 100% alcohol for 1 h and hydrated with a graded series of alcohol solutions for a short time, after which the paraffin-embedded specimen was reversed from the previous orientation, prior to being embedded in paraffin.

Adjustment procedure

Step 1: adjusting the stereomicroscopic and magnifying endoscopic image (Fig. 3).

The corresponding characteristic surface patterns near the marking were identified in both the stereomicroscopic and magnifying endoscopic images. Crystal violet staining of the resected gastric specimen emphasized its surface pattern (Fig. 2), helping us identify the corresponding characteristic surface pattern in the endoscopic image.

Step 2: identification of the sectioning line for the hematoxylin and eosin (H&E) section on the stereomicroscopic image before thin sectioning (Fig. 4).

The reversed specimen, which is after the 3-µm preparation, was stained with crystal violet, and the characteristic patterns both in the stereomicroscopic image before and after the 3-µm preparation were identified. The sectioning line for the H&E section on the stereomicroscopic image before the 3-µm preparation was identified based on the matching characteristic pattern of these two images.

Step 3: identification of the sectioning line for H&E section on the magnifying endoscopic image (Fig. 5).

The sectioning line for the H&E stained section on the magnifying endoscopic image was identified based on the

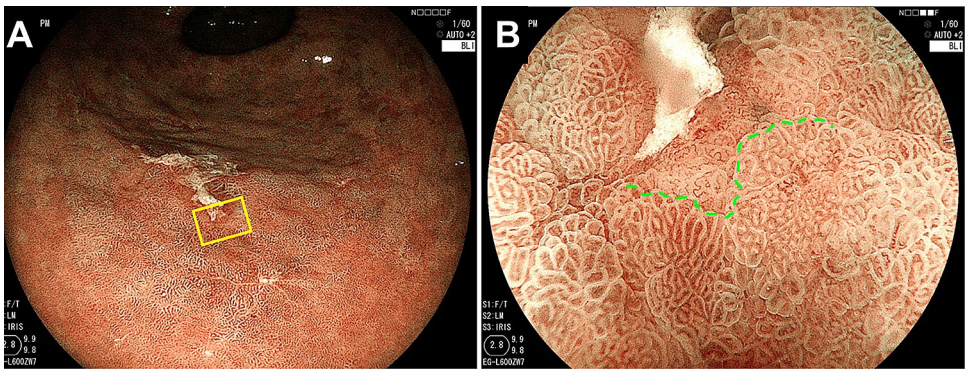

Fig. 1 Detailed observation of the target lesion. Endoscopic image of the target lesion using blue laser imaging (BLI) (**A**). The area surrounded the yellow box is the region of interest. Magnifying endoscopic image of the interest region using BLI (**B**). The surface pattern is different across the dotted green line

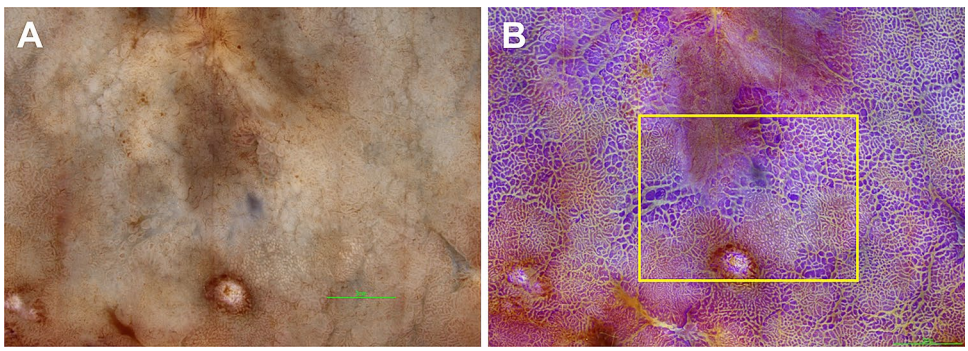

Fig. 2 Crystal violet staining of the resected specimen fixed by formalin. Stereomicroscopic image after fixation (**A**) and after crystal violet staining (**B**). The surface pattern of the fixed specimen is emphasized after crystal violet staining. Marking is made endoscopically for easy identification of the interested region (yellow box)

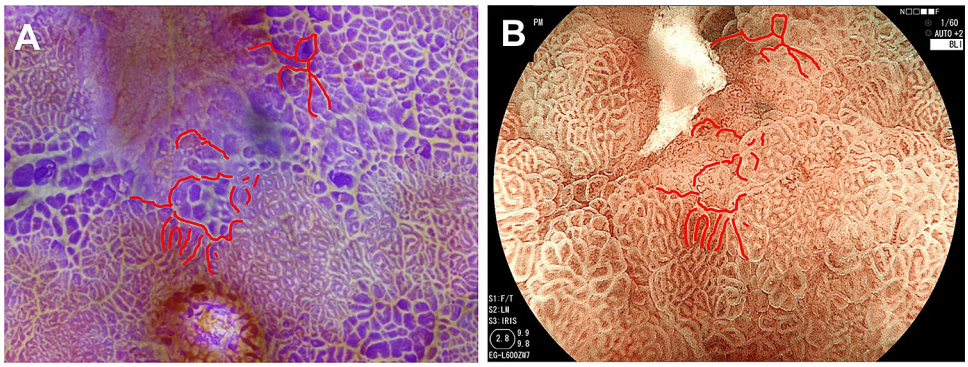

Fig. 3 Step 1: one-to-one correspondence between stereomicroscopic and magnifying endoscopic images. Stereomicroscopic image of fixed specimen stained by crystal violet (**A**) and magnifying endoscopic image using blue laser imaging (**B**) of the interested region. The same characteristic patterns (red lines) can be observed in both images

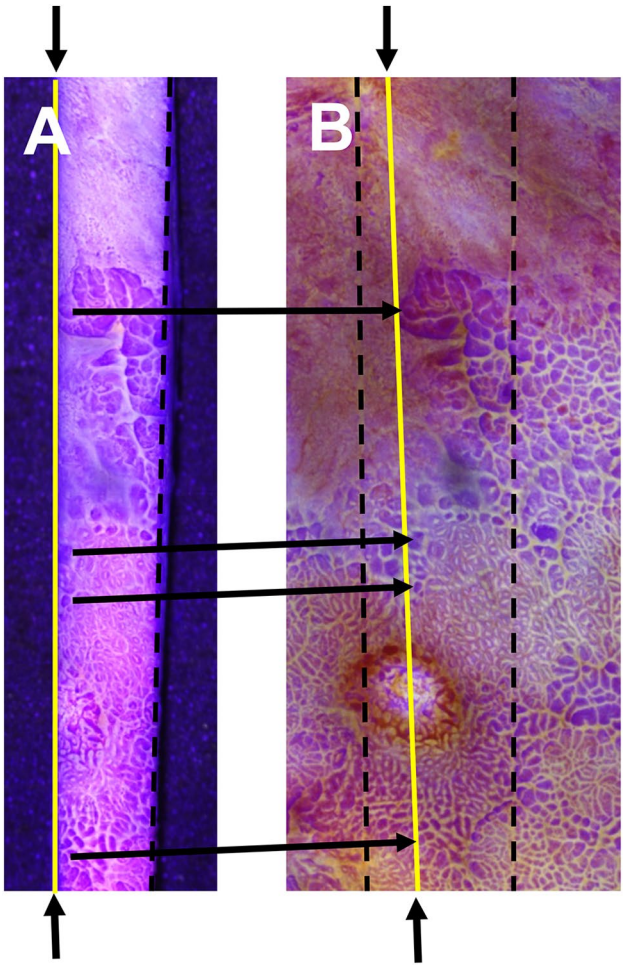

Fig. 4 Step 2: identification of the sectioning line for the hematoxylin and eosin (H&E) stained section in the stereomicroscopic image before 3 μm preparation. Reversed specimen including the interested region stained by crystal violet (**A**). Stereomicroscopic imaging including the interested region before 3 μm preparation (**B**). The sectioning line for the H&E stained section (yellow line) was identified by adjusting these two images depending on the same characteristic surface pattern. Dotted line represents mucosal cleavage line of resected specimen

matching pattern of stereomicroscopic and magnifying endoscopic images.

Step 4: matching magnifying endoscopic and histopathological images with stereoscopic images (Fig. 6).

Crystal violet staining of the reversed specimen enabled us to observe not only the surface pattern but also the lateral structure, which was the same as the histopathological image stained with H&E. As the reversed specimen is like a strip of paper with some thickness, it can be imaged from different angles using a stereomicroscope by fixing both ends with pins and tilting. Reversed specimens, especially those made from deep depressed lesions, sometimes tear in the process; therefore, careful handling is required. The surface patterns and lateral structures of the reversed specimen could be observed simultaneously, viewed obliquely, which enabled us to match the vertical stereomicroscopic and lateral histopathological images appropriately and helped us to understand the relationship between the surface pattern and lateral structure. The histopathological and magnifying endoscopic images could also be matched with the stereomicroscopic image, because the magnifying endoscopic and stereomicroscopic images were matched in step 3.

Conclusions

We propose a novel method, the KOTO method II, which can demonstrate a complete one-to-one correspondence between magnifying endoscopic and histopathological images. Previously [7], we described the KOTO method, a procedure to achieve a detailed adjustment and correlation between these two images. In the KOTO method, the prepared section for H&E staining was considered to be made near the mucosal cleavage line of the gastric specimen. However, the mucosal cleavage line did not completely match the line on which the histopathological image was made because the prepared section for H&E staining was made several hundred microns forward (3 μm preparation) from the mucosal cleavage line. Therefore, another method to identify the line on which the prepared section for H&E staining was developed is required

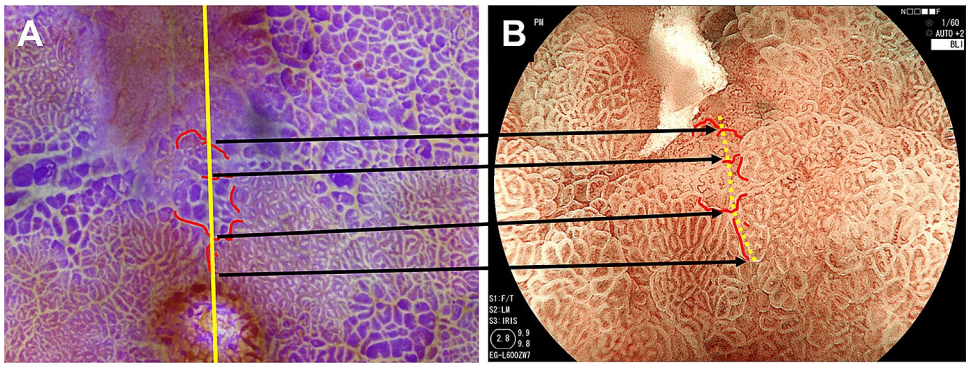

Fig. 5 Step 3: identification of the expected sectioning line for the hematoxylin and eosin (H&E) stained section in the magnifying endoscopic image. Stereomicroscopic image of resected specimen with the sectioning line for the H&E stained section (yellow line) (**A**). Magnifying endoscopic image of the interested region with the expected sectioning line for the H&E stained section (dotted yellow line) (**B**). The expected sectioning line can be drawn depending on the same characteristic surface patterns of stereomicroscopic image and magnifying endoscopic image

for complete adjustment between the magnifying endoscopic and histopathological images. We then considered that comparing the surface patterns before and after the 3-μm preparation could identify the accurate line on which the prepared section for H&E staining was developed. For this, the paraffin-embedded specimen after 3 μm preparation should be stained with crystal violet and the surface pattern produced should be imaged. In this method, we solved this problem using a reversing procedure. The reversed specimen can be stained with crystal violet, which enables the comparison of the surface patterns before and after the 3-μm preparation and identification of the accurate line on which the prepared section for H&E staining was made (step 2, Fig. 4).

We also discovered that not only the surface pattern but also the lateral structure of the reversed specimen could be stained with crystal violet, which help understanding the correlation of the surface pattern of the mucosa and the histopathological image three-dimensionally by observing the stained reversed specimen in an oblique view. Hence, staining with crystal violet is one of the most important processes in this method, and without it, precise one-to-one correspondence between magnifying endoscopic and histopathological images cannot be achieved. Materials were stained for a few minutes and times with a low concentration of crystal violet to avoid strong staining at our institute.

However, this method has several limitations. First, correspondence of characteristic surface patterns between stereomicroscopic and magnifying endoscopic images, which are obtained in step 1, might be difficult to achieve when magnifying endoscopic images are taken diagonally. As the stereomicroscopic image is taken vertically, the magnifying endoscopic image needs to be taken at the same angle. Therefore, it is difficult to adopt this method in cases where the location of the target lesion is difficult to approach with the endoscope vertically. Second, clear imaging using an endoscope and a stereomicroscope is difficult in cases where the target area has height differences because it is difficult to focus on the whole target area in one image. Third, while this method can provide precise correspondence in millimeter units for the targeted portion of the lesion, correspondence for a wide range of the target is difficult. However, we consider the correspondence of the target area in millimeter units as the greatest strength of this method, which is particularly effective in the case of gastric cancer, wherein the demarcation line is unclear, such as crowing type differentiated or undifferentiated type adenocarcinoma in clinical practice.

In conclusion, we can obtain a complete one-to-one correspondence between magnifying endoscopic and histopathological images using the KOTO method II, a powerful tool for understanding the correlation between magnifying endoscopic and histopathological findings.

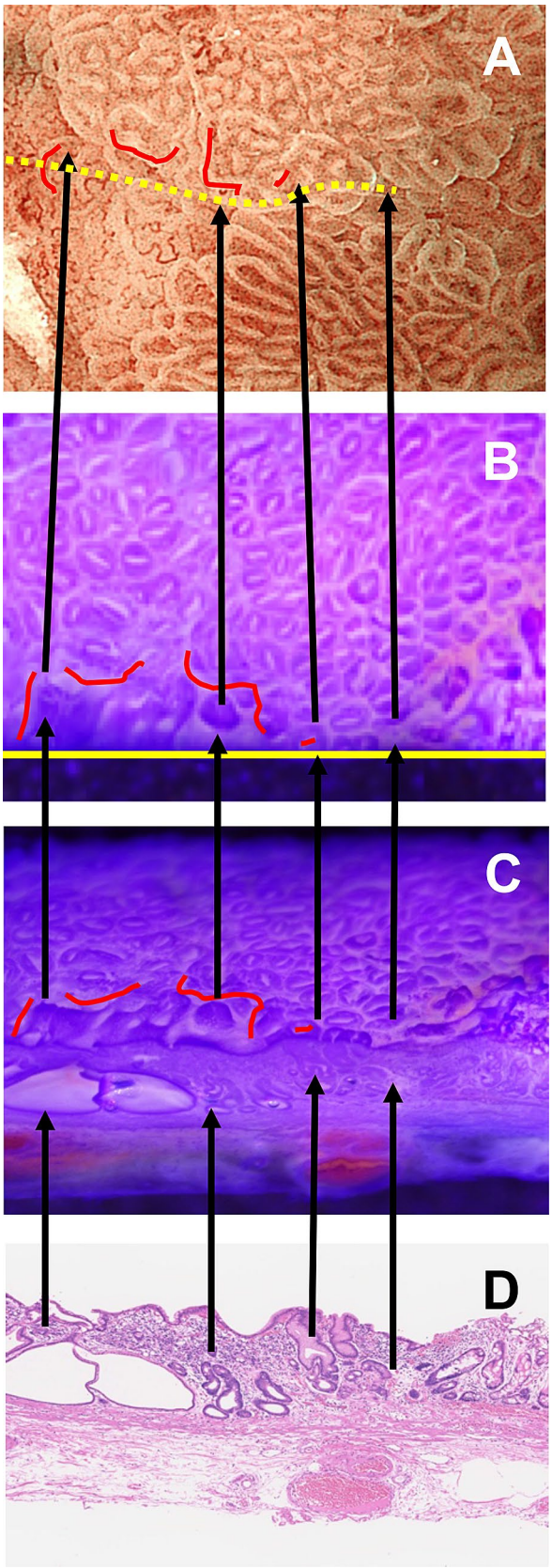

Fig. 6 Step 4: matching histopathological image and magnifying endoscopic image via stereomicroscopic image. Magnifying endoscopic image (**A**), stereomicroscopic image of reversed specimen viewed vertically (**B**) and diagonally (**C**), and histopathological image (**D**) including the interest region. These images are completely matched using the KOTO method II ▶

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

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