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Auto-Fluorescence Imaging is useful to assess the activity of ulcerative colitis

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Auto-Fluorescence Imaging is useful to assess the activity of ulcerative colitis

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Running title: Usefulness of AFI in ulcerative colitis

Key words

AFI, ulcerative colitis, histological severity, image quantification

Abstract: The efficacy of AFI for assessing UC activity was reviewed. AFI images of UC lesions could be classified into 4 categories; Green, Green with Purple Spots, Purple with Green Spots, Purple. The strength of purple in AFI images of UC lesions is related to histologic severity, thus this classification is helpful to assess the activity of UC. We also quantified the auto-fluorescence of AFI images using color analysis software. Active lesions showed significantly low auto-fluorescence / reflex ration rather than inactive area in UC patients. The ratio is inversely proportional to histologic severity. When defining the cut-off value as 0.9, the accuracy to predict histologic activity by AFI is 92%.

Auto-Fluorescence Imaging (AFI) is a novel technique to detect the auto fluorescence emitted from the intestinal tissues, which mainly released from collagen-I in submucosal layer, in real time. Recent advances of endoscopic technology have developed high-operability and -resolution colonoscopy with AFI and enabled them to be appropriate for clinical application. It has been shown that AFI contributed to detect gastric¹ or colonic neoplasms² but still unknown the efficacy of AFI in Ulcerative colitis (UC). We here report our preliminary study that AFI is useful for predicting the histologic severity of UC and can quantify the activity of UC in real time.

1. AFI features in UC patients and their correlation to hitologic severity

AFI images of colonic mucosa were reproducible and stable when observing under filling sufficient air. However, insufficient extension of colon made AFI images unstable and diverse, suggesting the importance of intestinal condition when taking AFI images assessed.

Obvious ulcerations as well as erosions were detected as clear purple (**Figure 1**). Either blood or vessels were colored as dark green or black. Atrophic regenerative mucosa that receive damages due to strong inflammation revealed as faint purple with green spots

(Figure 2). Almost normal mucosa with no inflammation showed green area with vascular network which seen in dark green **(Figure 3)**. Granular mucosa with slight redness showed various patterns of AFI images. In order to simplify the findings of mucosa, we classified AFI images into 4 categories as follows; Green, Green with Purple Spots, Purple with Green Spots, Purple **(Figure 4-7)**. And then, we retrospectively examined relationship between our classification and histologic severity of biopsy specimens obtained from the area assessed by AFI.

Inactive inflammation was detected in histologic specimens obtained from the lesions classified into Green (n=7). Just 2 of 10 lesions classified into Green with Purple Spots showed active inflammation in biopsy specimens corresponded. In contrast, All 14 lesions in the groups of Purple or Purple with Green Spots indicated the active inflammation in histologic specimens **(Table)**. Thus, the stronger the color purple in AFI images, the more severe the degree of histologic inflammation.

2. Quantification of AFI images and its implication in histologic severity

We also try to quantify fluorescence color (green) of AFI images using special software which can calculate the fluorescence / reflex ratio. Briefly, here picture of AFI images of UC

lesions were analyzed with the software shown above and the ratio were compared with histologic severity of biopsy specimens obtained from the mucosa which corresponds to the area assessed by AFI.

The fluorescence / reflex ratio was in the range of 0.84 to 2.43 (1.28 ± 0.10) in the area without active inflammation, although the ratio was from 0.65-0.96 (0.75 ± 0.06) in mild active lesions and 0.48-0.75 (0.56 ± 0.04) in moderate to severe inflammation. The active lesions showed significantly low ratio rather than inactive area in UC patients. Thus, the calculated ratio appears to be inversely proportional to histologic severity. When defining the cut-off value of fluorescence / reflex ratio as 0.9, the accuracy that predicts the activity of UC lesions using AFI is 92%.

3. Perspective of AFI in inflammatory bowel disease

Auto-fluorescence is mostly emitted from collagen I in submucosal layer in intestinal tissues. Therefore, auto-fluorescence signal is known to be decreased by either damaged submucosal layer or thicken mucosal layer in some manner. Evaluating UC activity with AFI is seemed to be reasonable because auto-flourescence should be diminished in active inflammation due to the thick mucosal layer and/or impaired sumucosal layer with the

infiltration of inflammatory cells. Our preliminary data support the usefulness of AFI to assess the activity of UC. Further analysis is needed to determine if AFI is truly efficient for evaluating the activity of UC as well as predicting the non-relapsing periods.

AFI is possibly useful to detect dysplasia utilizing the ability to evaluate mucosal alterations with diminished auto-fluorescence. High cell-density in dysplasia is regarded to alter the permeability of auto-fluorescence, which results the change of AFI signal. AFI can be efficient for the surveillance study of UC. The multi-center study is on going to determine how AFI contributes to IBD examination and treatment.

Reference

1. Ohkawa A, et al. Ogihara T, Sato N, et al. Diagnostic performance of light-induced fluorescence endoscopy for gastric neoplasms. *Endoscopy* 36; 515-521, 2004.
2. Nakaniwa N, Namihisa A, Ogihara T, et al. Newly developed autofluorescence imaging videoscope system for the detection of colonic neoplasms. *Digestive Endoscopy* 17; 235-240, 2005.

Figure legends

Figure 1

Small ulcerations are seen in localized area with conventional colonoscopy (a). AFI detects clear purple lesions in corresponded site (b).

Figure 2

Atrophic mucosa with inflammatory polyps was observed with conventional colonoscopy (a). Purple area which corresponds to atrophic mucosa was detected with AFI. Polypoid lesions were seen as white or light green spots with AFI (b).

Figure 3

Conventional colonoscopy reveals the fine network pattern (a). AFI image shows green area with dark green network which corresponds to vessels (b).

Figure 4

Quiescent mucosa with dense vascular network was seen with conventional colonoscopy (a). AFI image classified into 'Green' describes dense vascular as dark green (b).

Figure 5

Conventional colonoscopy shows granular mucosa with diffuse redness (a). The AFI image is classified into 'Green with Purple Spots.' Histologic finding of corresponded area reveals

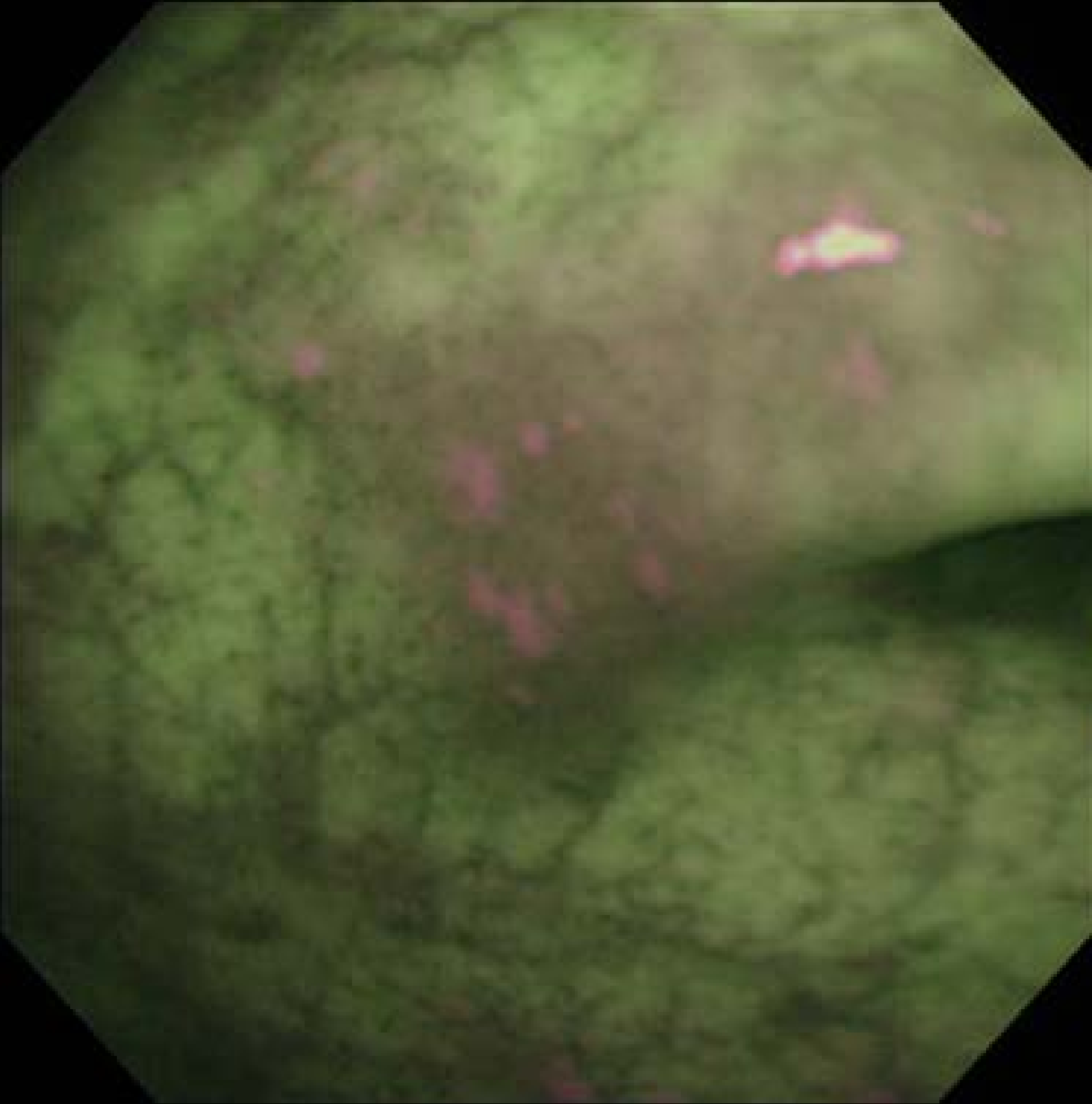
Figure 6

Granular mucosa with red spots and irregular vascular pattern is observed with conventional colonoscopy (a). AFI image shows dominant purple area with partial green, which is classified into 'Purple with Green Spots' (b).

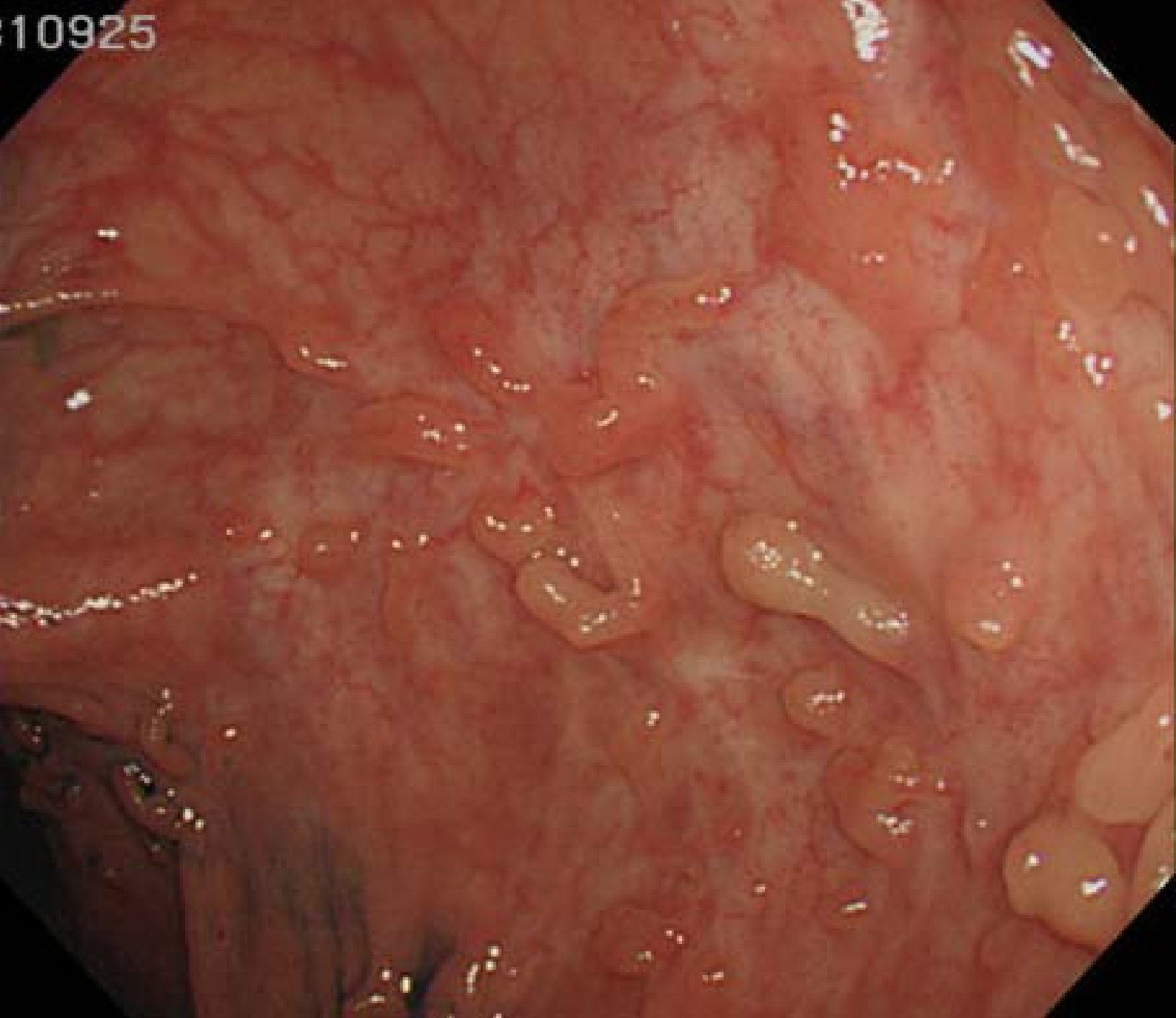
Figure 7

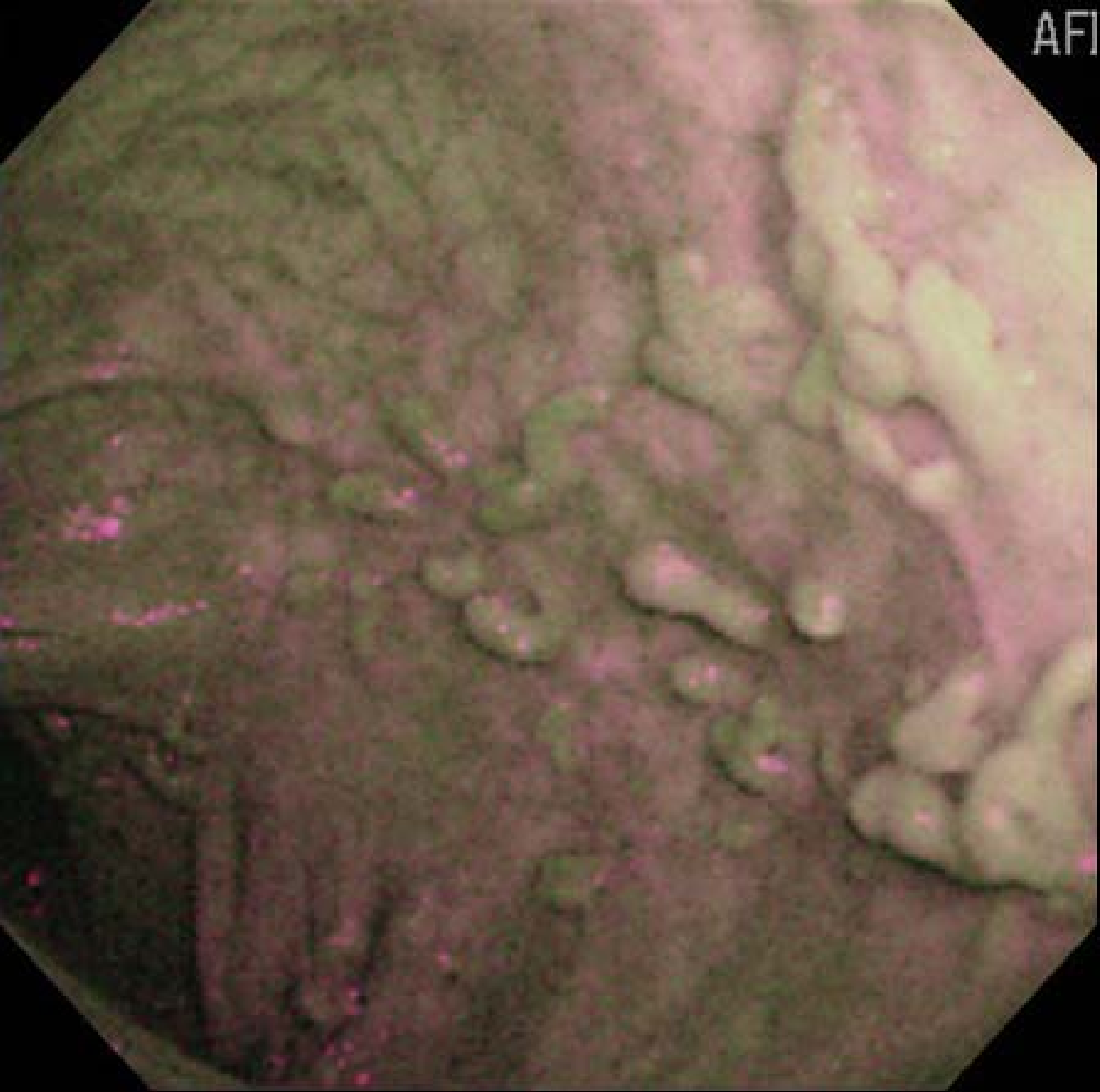
Edematous mucosa with small ulcerations and bleeding is revealed with conventional colonoscopy (a). AFI describes almost all area as purple with black spots which corresponds to bleeding (b).

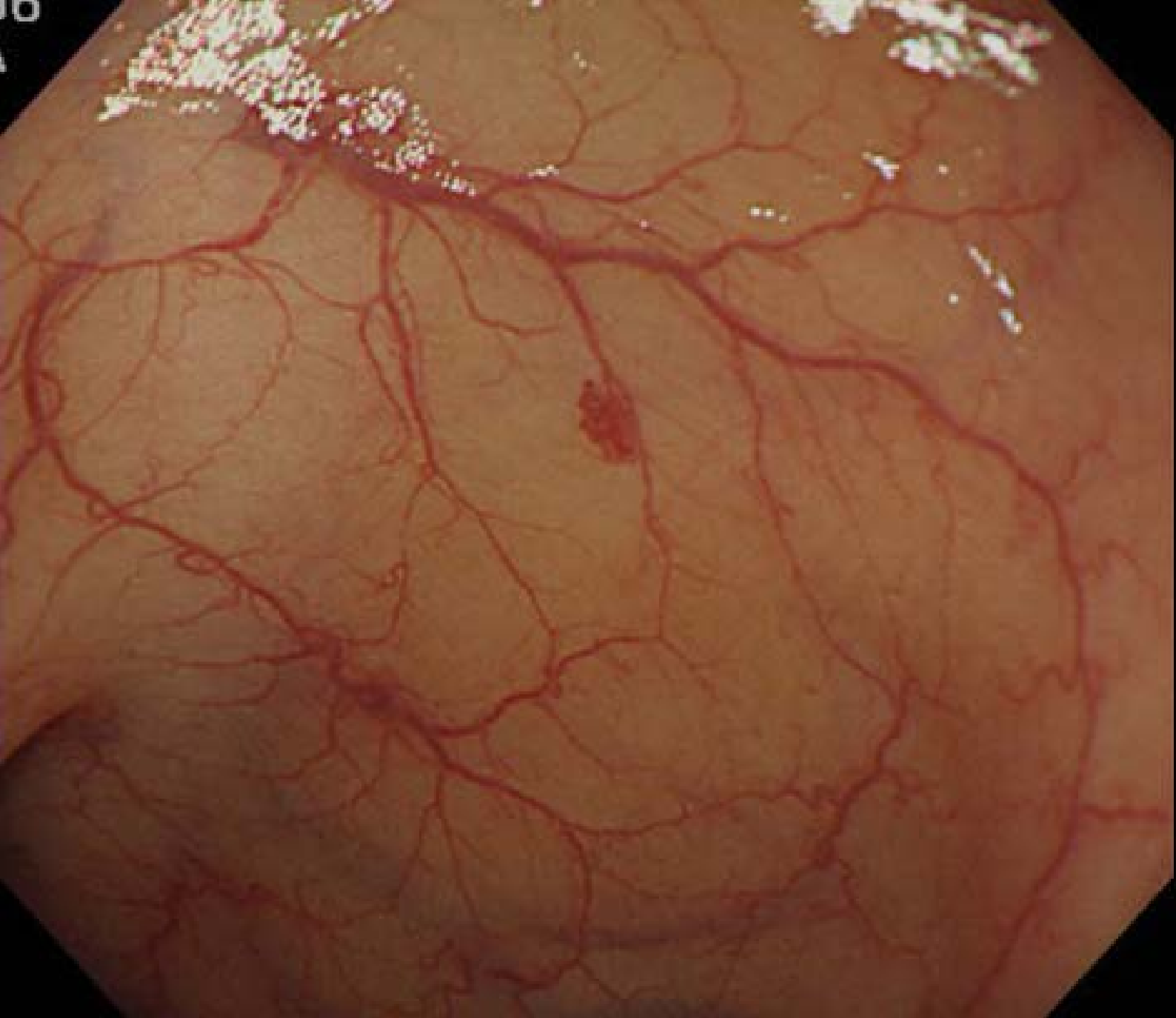


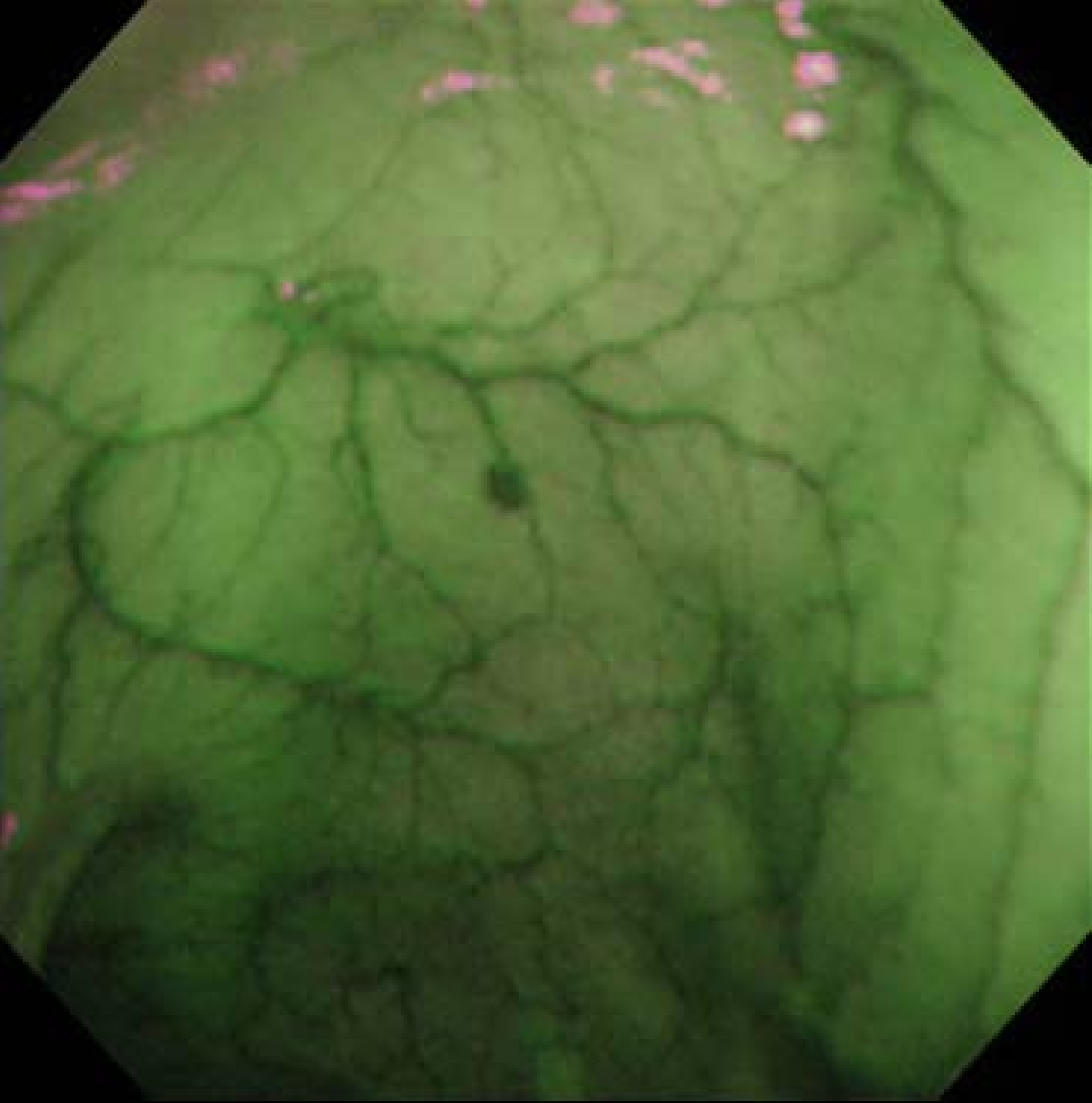


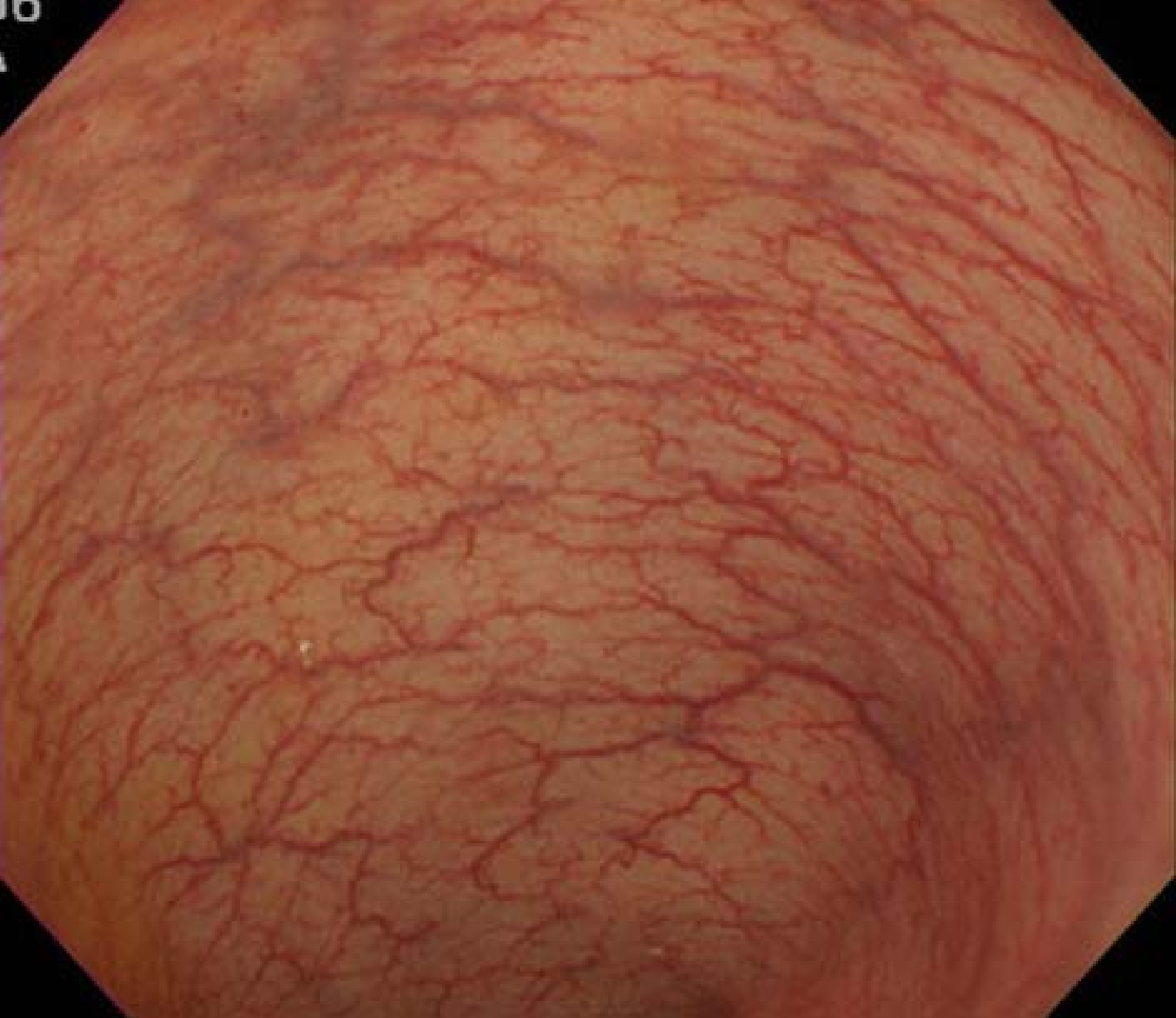
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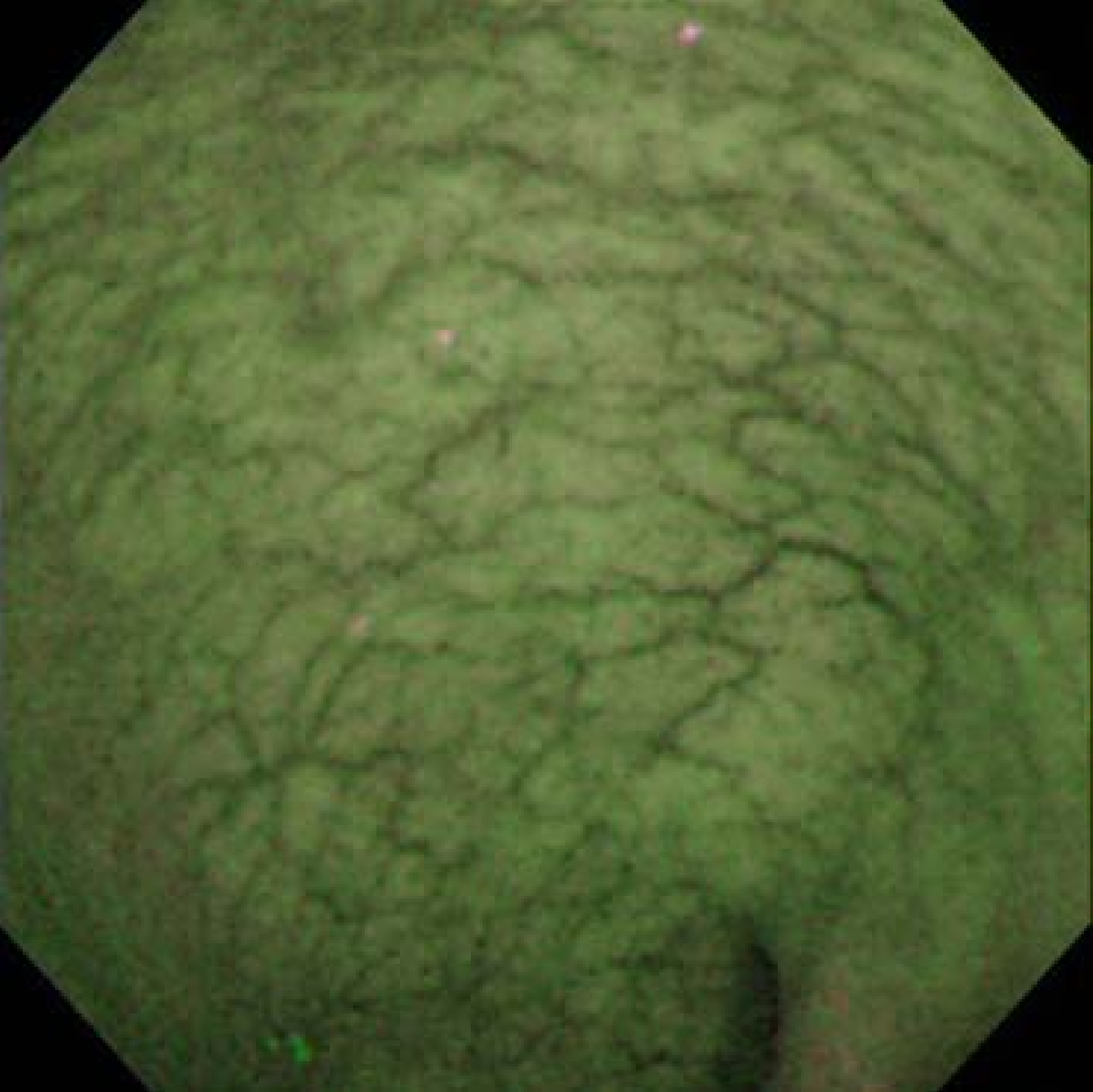




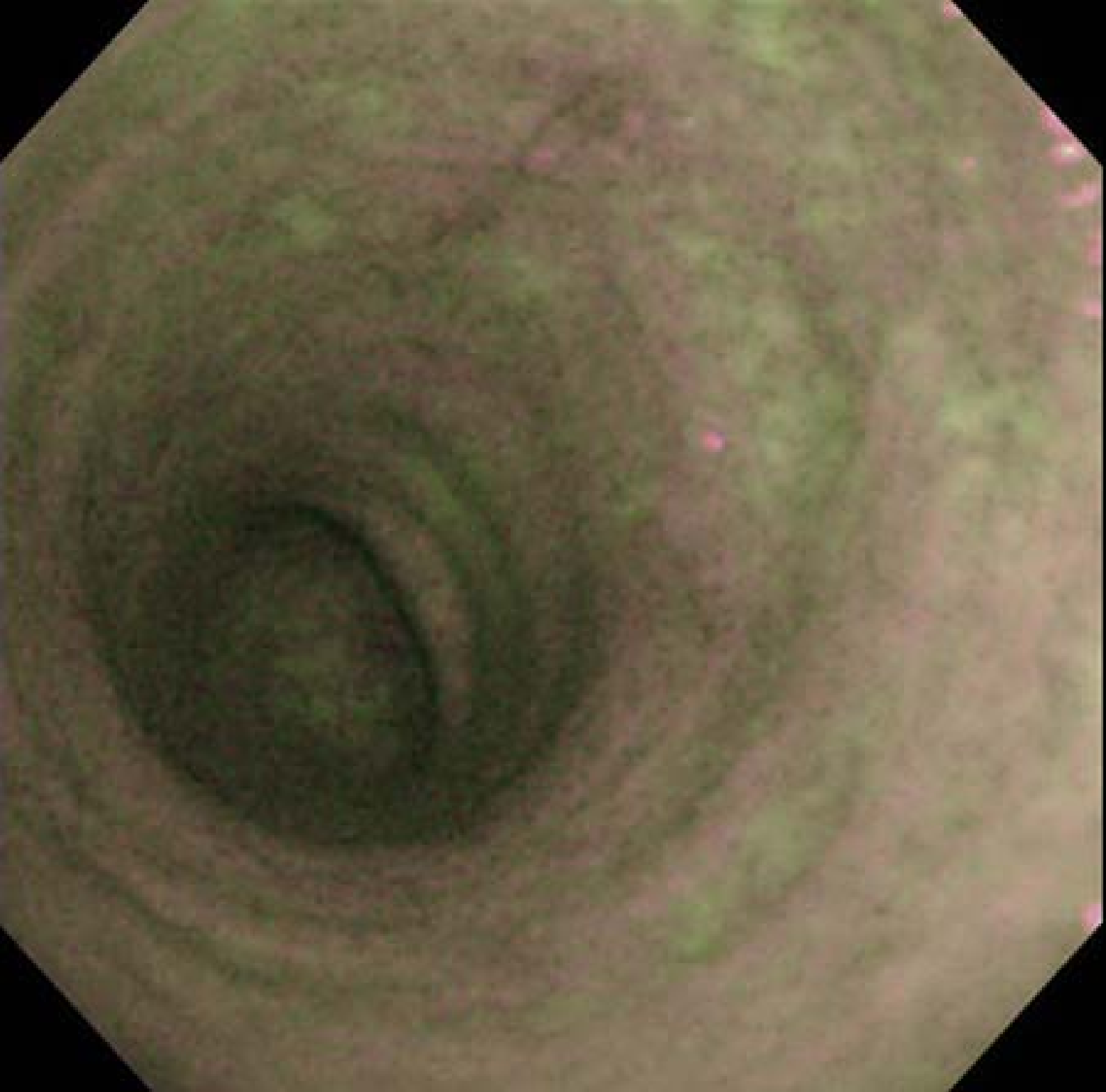




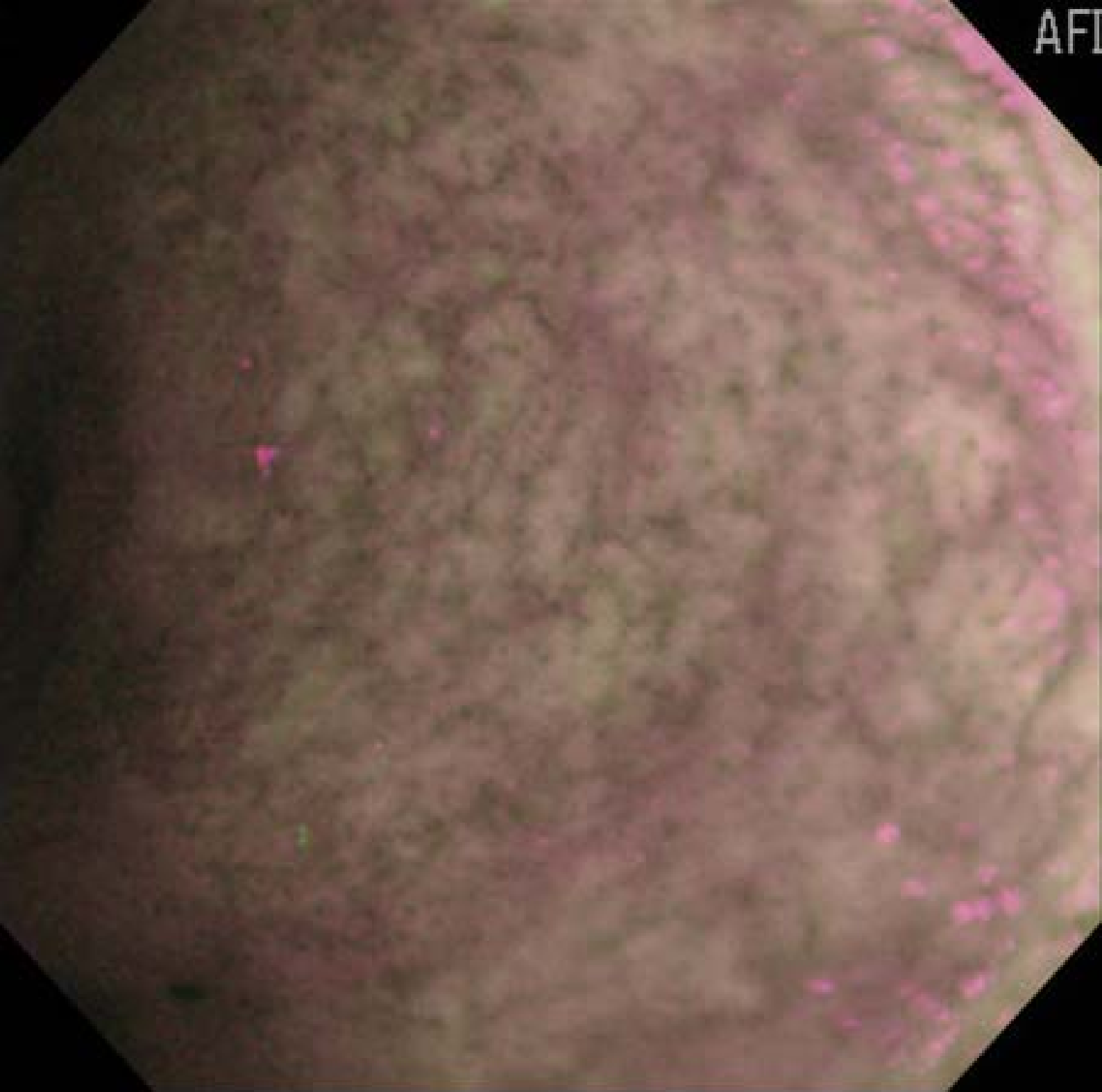


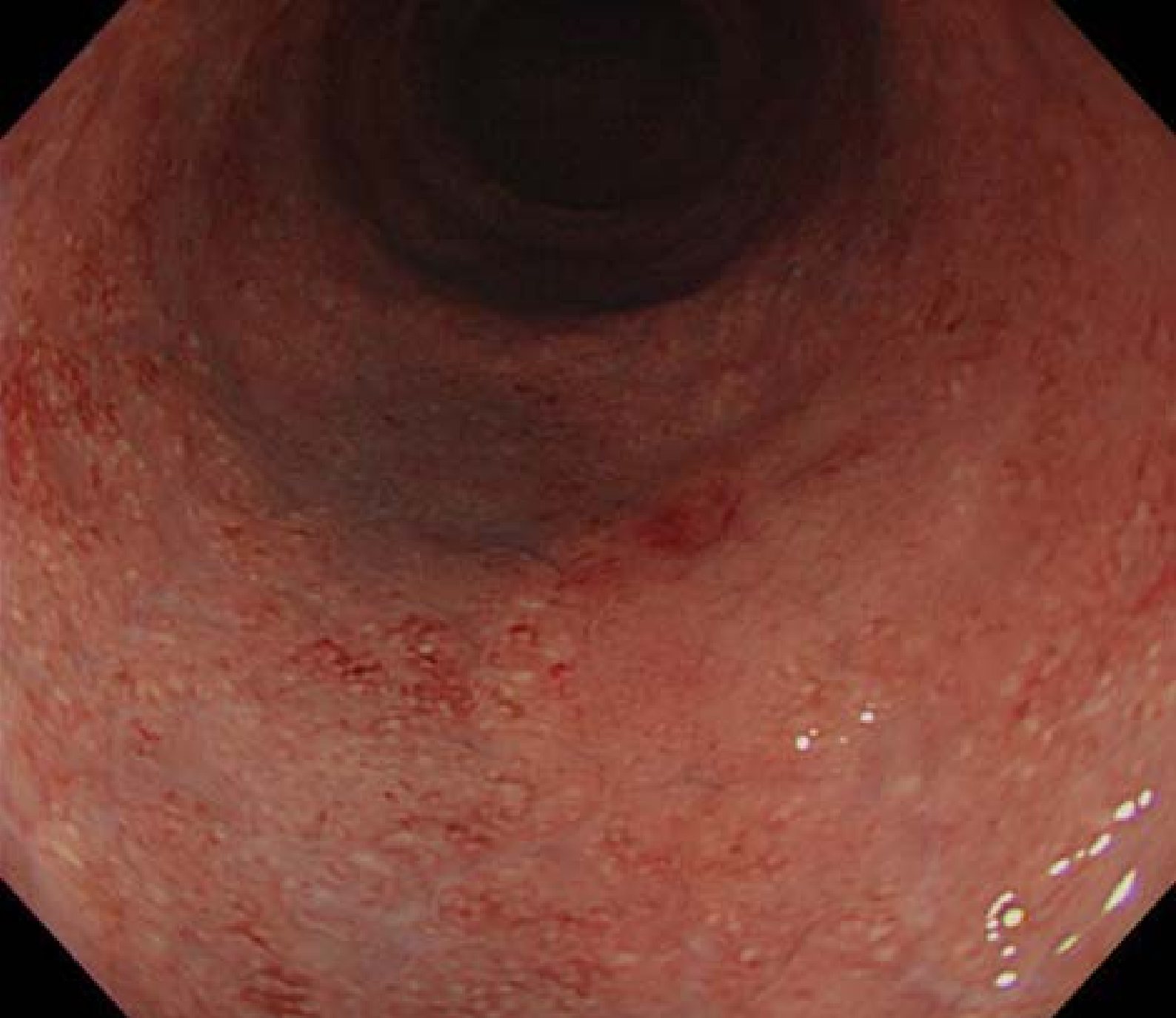












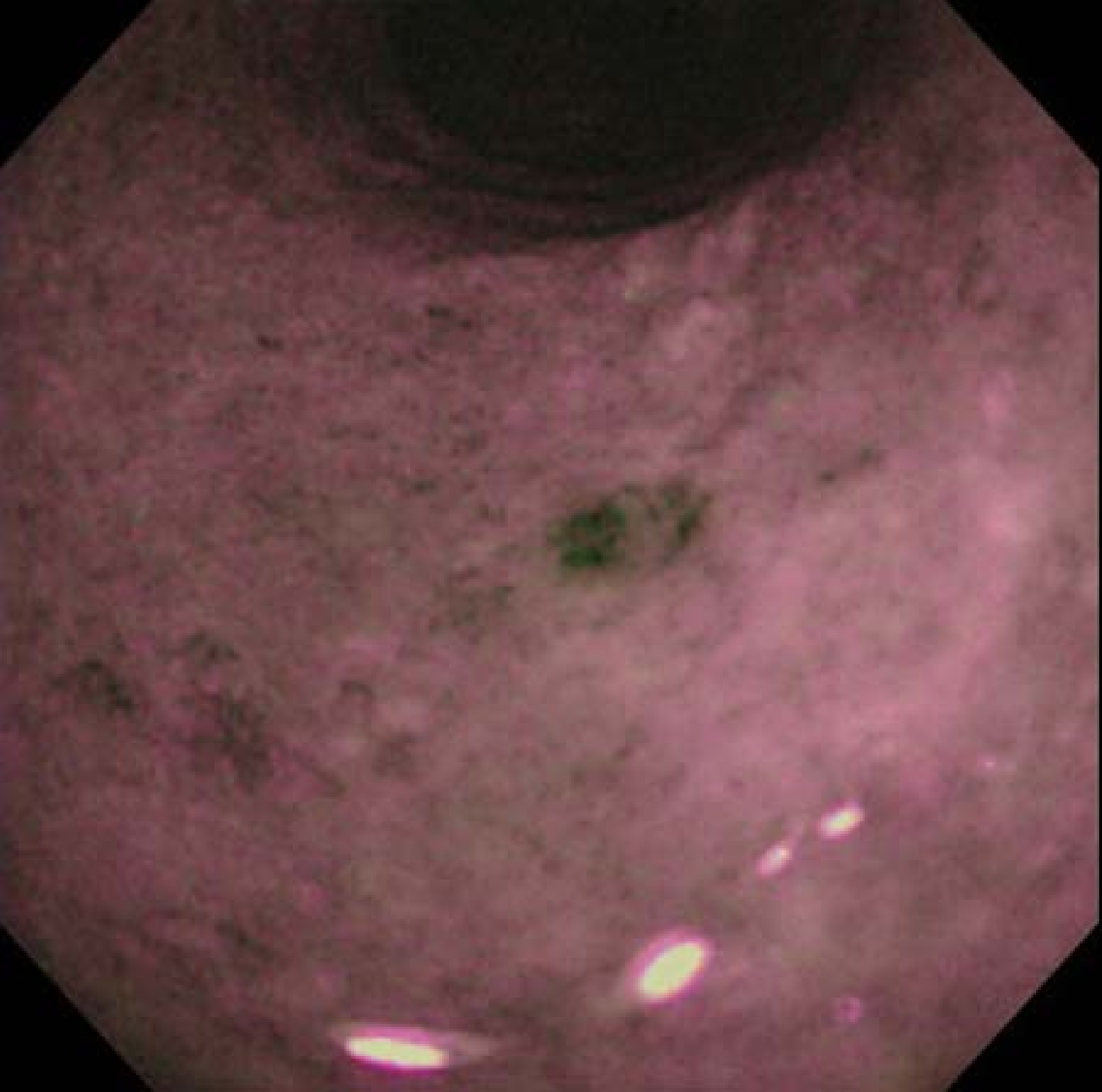


Table Relationship between AFI images and histologic severity

AFI images	Histologic Severity			Total
	1	2	3 or more	
Green	7			7
Green with Purple Spots	8	2		10
Purple with Green Spots		4	1	5
Purple			9	9
Total	15	6	10	31