表　　題　　早期胃癌の存在診断における Linked Color Imaging の優位性の検証試験

(Improvement of early gastric cancer detection with linked color imaging)

論文の区分　　博士課程

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ABSTRACT

Most early gastric cancers are challenging to detect because of subtle morphological or color differences with surrounding atrophic mucosa and intestinal metaplasia. Linked color imaging (LCI) enhances the mucosal color difference, making it easier to detect early gastric cancers (EGCs). The main aim of this thesis was to clarify the clinical significance of LCI and its superiority over conventional white light imaging (WLI) in the early detection of gastric cancer. For this purpose, we conducted two separate studies with different designs.

Study I assessed the advantages and disadvantages of LCI for diagnosis of EGCs retrospectively in a large series. The visibility of a total of 550 EGC lesions was evaluated by six endoscopists. The results showed that the detection visibility scores using LCI were significantly higher than those using WLI regardless of lesion characteristics including location, size, histological type, depth of invasion, and *Helicobacter pylori* status. The detection score improved in 46.4% cases and deteriorated in 4.9% when the modality changed from WLI to LCI. A multivariate logistic regression analysis showed that use of LCI (odds ratio [OR] 2.57), elevated type (OR 1.92), and invasion to the submucosa (OR 2.18) were significantly associated with improved visibility of EGC.

Study II aimed to clarify whether LCI with ultrathin endoscopy facilitates detection of EGC despite its lower resolution compared with high-resolution WLI with standard endoscopy. This is a retrospective analysis with prospectively collected video including 166 consecutive cases with EGC or gastric atrophy alone. Ninety seconds of screening video was collected using standard and ultrathin endoscopes with both WLI and LCI for each case. Three expert endoscopists assessed each video and the sensitivity of detecting EGC calculated. Color difference calculations were performed. Results showed that sensitivities using ultrathin WLI,

ultrathin LCI, standard WLI, and standard LCI for the identification of cancer were 66.0%, 80.3%, 69.9%, and 84.0%, respectively. The color difference between malignant lesions and the surrounding mucosa with ultrathin LCI and standard LCI were significantly higher than using ultrathin WLI or standard WLI, supported subjectively by the visibility score. Ultrathin LCI color difference and visibility score were significantly higher than those obtained with standard WLI.

In conclusion, the results of studies described in this thesis indicate that LCI is a clinically useful method to improve the detection of EGCs by increasing the color contrast between malignant lesion and its surrounding mucosa and improving lesion visibility regardless of their characteristics, even with a lower resolution endoscope.

ABBREVIATIONS

CIELAB	International Commission on Illumination
CagA	Cytotoxin-associated gene A
DNA	Deoxyribonucleic acid
EGC	Early gastric cancer
ESD	Endoscopic submucosal dissection
FAP	Familial adenomatous polyposis
FICE	Flexible spectral imaging color enhancement
HDGC	Hereditary diffuse gastric cancer
HM	Horizontal margin
IEE	Image enhanced endoscopy
JGES	Japan Gastroenterological Endoscopy Society
LCI	Linked color imaging
LED	Light Emitting Diode
M	Invasion to muscularis mucosa as M
NBI	Narrow band imaging
OR	Odds ratio
ROI	Region of interest
SD	Standard deviation
SM1	Tumor invasion within 0.5 mm of the muscularis mucosae
SM2	Tumor invasion of 0.5 mm or more deep into the muscularis mucosae
UMIN	University Hospital Medical Information Network
USAF	United States Air Force
VacA	Vacuolating cytotoxin
WHO	World Health Organization
WLI	White light imaging

1 INTRODUCTION

Gastric cancer is the fifth most common cancer and the fourth common cause of cancer-related death [1]. The incidences and mortality rates of this disease vary across the globe, and the highest were observed in South Korea, followed by Mongolia and Japan [1]. Results of the South Korean screening program for gastric cancer revealed a decrease in mortality rates from gastric cancer [2]. Several case-controlled studies in Japan have also reported a decreased mortality rate from gastric cancer in patients who underwent endoscopic screening [3, 4]. Therefore, endoscopic screening plays an essential role in controlling the disease.

Gastric cancer is asymptomatic in the early stage, and in advanced stages patients have dysphagia, indigestion, weight loss, early satiety, and anemia. Ninety-five percent of malignant neoplasms of the stomach are adenocarcinomas, followed by primary gastric lymphoma. Gastric adenocarcinoma arises from the glandular epithelium of the gastric mucosa and is classified based on location, histology, and stage. The etiology of gastric cancer varies among races and is associated with dietary and non-dietary factors. In the Asian population, it is mostly associated with *Helicobacter pylor*i infection which causes transformation of normal gastric mucosa through stages of chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia, and cancer [5-7]. Pathogenic protein CagA and vacuolating toxin VacA of the *Helicobacter pylori* are reported to be strongly associated with the development of gastric malignancies [8-10]. Hereditary diseases such as hereditary diffuse gastric cancer (HDGC), Lynch syndrome, familial adenomatous polyposis (FAP), and Peutz-Jeghers syndrome increase the risk of developing gastric cancer. The oncogenesis of gastric cancer is a complex, multistep process including various genetic and epigenetic alterations of tumor suppressor genes, oncogenes, cell cycle regulators, signaling molecules and DNA repair genes [5].

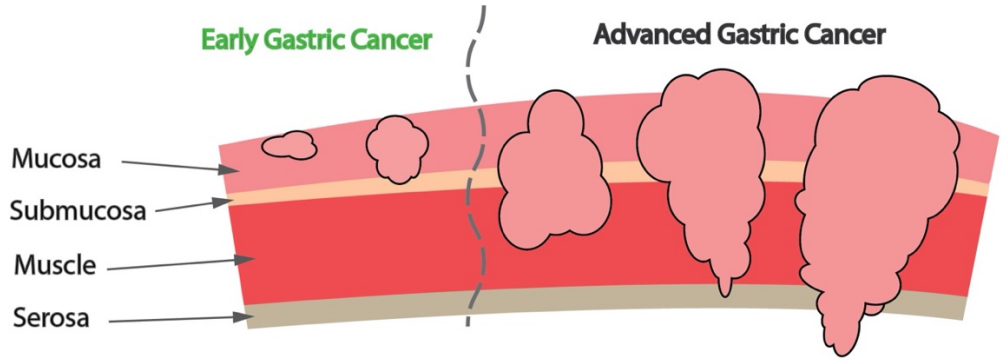

Figure 1. Early gastric cancer and advanced gastric cancer. (Drawing by the author ©).

1.2 Early gastric cancer

Early gastric cancer (EGC) was defined as one limited to the mucosa or submucosa regardless of the presence of lymph node metastases (Figure 1) [11]. According to initial reports of early gastric cancer, approximately19.5 % of gastric cancers were found at an early stage with barium contrast radiography and the 5-year survival rate for patients with gastric cancer was 36.7% in Japan in the 1960s [11]. Advances in early endoscopic diagnosis and treatment of gastric cancer have improved the 5-year survival rates up to 95%.

As reported by the Japanese classification of gastric carcinoma, gross tumor morphology is divided into superficial (Type 0) or advanced type [12]. Type 0 is classified into: Type 0-I (polypoid, elevated more than 3mm); type 0-IIa (slightly elevated, less than 3mm); type 0-IIb (flat); type 0-IIc (slightly depressed); type 0-III (deeply depressed) [12]. The histopathological types of EGC are distinguished by biopsy results taken from the lesion and pathological examination of the resected specimen. Lauren`s classification was the main histologic classification that divides gastric cancers into intestinal and diffuse types. Recently, the WHO classification and the Japanese Gastric Cancer Association classifications are commonly used worldwide. Tubular and papillary histological subtypes are regarded as differentiated and are associated with a considerably better prognosis. Poorly cohesive, signet-

ring cell subtypes are recognized as undifferentiated and are reported to be associated with a poor prognosis [12].

1.2.1 Endoscopic detection and diagnosis of EGC

Endoscopy has been proven to be the standard method for the detection and diagnosis of EGC [13]. In recent decades, endoscopic imaging technology has improved markedly and is divided into conventional white light imaging (WLI), image-enhanced endoscopy (IEE), magnifying, microscopic, and tomographic types [14]. Although magnifying endoscopy was developed earlier and helped to evaluate the characteristics of EGC, it was not efficient in areas with a wide lumen such as the stomach because of inadequate optical resolution [15]. Later, optical IEE technologies such as narrow band imaging (NBI) (Olympus Medical System, Tokyo, Japan), and blue light or laser imaging (BLI) (Fujifilm, Tokyo, Japan) combined with magnification had a significant impact on the evaluation and detailed endoscopic diagnosis of EGC. These technologies were developed based on high absorption of narrow band light by hemoglobin and decreased penetration depth which allows observation of superficial vessels and structures more clearly [16, 17]. NBI and BLI technologies were ideal for characterization of EGC but not convenient for its screening because of insufficient light to observe the entire stomach in a short amount of time. The most recently developed type of IEE, linked color imaging (LCI), is reported to enhance the visibility of EGC with enough light to illuminate the entire gastric cavity [18].

The diagnosis of EGC consists of detection and differentiation between malignant and non-malignant lesions [19]. Detection of EGC requires the endoscopist to have technique and knowledge. The stomach must be well prepared and the endoscopist then screens the entire stomach avoiding blind spots. Except for obvious polypoid or ulcerative lesions, the key signs of EGC on endoscopy are color changes and subtle changes of the surface mucosa [19]. After

finding a suspicious lesion, BLI/NBI techniques are useful to differentiate it from non-malignant changes. Basic characteristics of EGC include the presence of a demarcation line between malignant and non-malignant mucosa, irregular microvessels and an irregular surface pattern. Following the differentiation, a target biopsy is taken from the lesion [20].

1.2.2 Treatment of EGC

Endoscopic resection is considered to be the primary treatment modality for EGC. The absolute indication for endoscopic submucosal dissection (ESD) is defined as following: 1) a differentiated-type adenocarcinoma without ulcerative findings, and depth of invasion T1a, regardless of lesion size, 2) a differentiated-type adenocarcinoma with ulcerative findings, depth of invasion of T1a, and less than 3cm in size, 3) an undifferentiated-type adenocarcinoma without ulcerative findings (UL0) with a depth of T1a and less than 2cm in size[21]. Endoscopic treatment success and future follow-up are decided based on complete primary tumor resection and the likelihood of lymph node metastases. Recently, an endoscopic resection curability scoring system has been developed and used to predict lymph node metastasis and determine the treatment strategy after ESD for EGC [22]. Endoscopic curability A (eCuraA) is defined as a resected cancer without ulcerative findings (UL0), with an *en bloc* resection, any tumor size, histologically differentiated type-dominant, pT1a, negative horizontal margin (HM0), negative vertical margin (VM0) and no lympho-vascular infiltration. If the lesion has ulcerative findings and less than 3cm in size, it is considered as eCuraA. Also, the resection is classified as eCuraA for undifferentiated type-dominant cancer UL0, an *en bloc* resection, pT1a, HM0, VM0, Ly0, V0 and tumor size ≤ 2 cm. On the other hand, if the cancer is histologically differentiated type-dominant, pT1b1 (SM1) (< 500 μm from the muscularis mucosae), HM0, VM0, Ly0, V0, tumor size ≤ 3 cm, it is also considered as eCuraB. The eCuraC1 includes differentiated type EGC resections that were not resected en bloc or with

positive horizontal margin. The resection is classified as eCuraC2 when it does not fulfill the conditions described. Annual or biannual endoscopy is recommended after an eCuraA resection and additional abdominal ultrasonography or computed tomography (CT) scan for surveillance for metastases is recommended after an eCuraB resection [23]. For eCuraC1, observation can be chosen but for eCuraC-2 additional surgery is considered as standard treatment [23].

1.3 Linked color imaging

LCI is an IEE that produces images bright enough to screen the entire stomach with its large lumen and provides high color contrast between various lesions and the surrounding mucosa. A prospective multi-center study revealed that LCI could detect upper gastrointestinal cancers that were missed using WLI [24].

LASEREO, an endoscopic system (LL-4450/VP-4450/L7000) with a gastrointestinal endoscope (EG-L590ZW, EG-L590WR, EGD-L600ZW, EG-L600WR), a laser source and ELUXEO 7000 systems with a LED source enable examinations with the LCI mode. The initial laser system emits two wavelengths of light using laser, including a white light (450±10 nm) for white-light illumination, and 410 nm short wavelengths. The key color contrast is created by 410 nm narrow band light, which is absorbed by capillaries and the structures on the mucosal surface, creating the color difference between normal mucosa, inflammation, and malignant lesions. LCI enhances subtle differences in the red color tone by advanced post-processing steps and the effect of the 410 nm violet light, which is absorbed by hemoglobin in superficial capillaries. In neoplastic lesions, dilated microvascular vessels and abundant glandular neoplastic cells are accumulated in the shallow layer of the mucosa, and 410 nm violet light is absorbed by vessels. Therefore, neoplastic mucosa usually appears with orange or orange-red color on LCI imaging. In inflammatory mucosa, dilated micro-vessels and glandular cells are mainly accumulated in the deeper layer of mucosa, and 410 nm violet light is reflected without

absorption, thus creating a violet color on the mucosal surface [17].

1.4 Conventional WLI and significance of LCI in detecting EGC

EGC of the elevated type and/or with submucosal invasion can be found with relative ease. However, many early cancers have subtle morphological or color features and are surrounded by atrophy and intestinal metaplasia. Therefore, they might be easily by conventional WLI, resulting in a possible delay in diagnosis for several years ultimately necessitating surgical resection [25, 26]. Moreover, recent reports reveal that the risk of gastric cancer remains even after *H. pylori* eradication due to atrophy and intestinal metaplasia in the background mucosa [27]. Detecting EGC in a post-eradication stomach is challenging due to non-neoplastic epithelium covering the malignant tissue, making the cancer border indistinct and diminishes the obvious characteristics of cancer, especially when using conventional WLI[28] [29].

Rapid advances in IEE improved the detection of early malignancies in the gastrointestinal tract. Comparative studies showed the inferiority of WLI for both detecting and characterizing EGC compared to IEE methods [30, 31]. The combination of the narrow band technique and bright illumination of LCI improves detection of EGC. The high color contrast between malignant lesions and the surrounding mucosa enables better visibility of flat lesions and post eradication EGCs [32, 33].

However, in the latest guidelines on the detection of EGC from the Japan Gastroenterological Endoscopy Society (JGES), the usefulness of IEE for the detection of EGC such as LCI are reported as unclear, whereas the efficacy of magnifying endoscopy with BLI/NBI is recommended.

1.5 Screening of EGC with ultrathin endoscopy

Ultrathin endoscopy (≤ 6 mm diameter) has been used mainly via the transnasal route since the early 2000s because of minimal pain and gag reflex induced during the examination [34] [35]. It is well tolerated without sedation and is less costly [36-38]. It is considered as a safer procedure with less effect on the cardiopulmonary function of elderly patients including blood pressure and pulse rate [35, 39, 40]. Therefore, ultrathin endoscopy is used mainly in private clinics and for routine health evaluations in Japan [41]. However, there is a trade-off between the resolution of the images and the smaller caliber endoscope using WLI. The diagnostic accuracy using an ultrathin endoscope and WLI is lower than with a standard endoscope and WLI for detecting gastric neoplasms [42]. There is concern that lower quality images may result in the inability to diagnose malignant lesions of the upper gastrointestinal tract in routine outpatient practice, which has resulted in hesitation to use this modality [41, 43-46]. However, there are no reports regarding the efficacy of ultrathin endoscopy together with the high color contrast produced by LCI.

2 AIMS

The overall aim of the studies described in this thesis was to assess the improvement of EGC detection and visibility with LCI and clarify its clinical significance. The specific aims of the two studies were:

Study I. To evaluate the advantages and any possible disadvantages of using LCI for detecting obscure EGCs. We aimed to investigate how significant LCI is among the various factors that affect detection of EGC and to examine what types of cancers become more or less visible by LCI compared with WLI.

Study II. To evaluate the efficacy of LCI with ultrathin endoscopy for the detection of EGC compared with standard WLI endoscopy and determine which is more important during the short amount of time allotted to screening endoscopy, high resolution imaging by standard WLI endoscopy or high color contrast obtained by ultrathin LCI endoscopy.

3 MATERIALS AND METHODS

3.1 Study I

3.1.1 Study design and patients

We retrospectively analyzed 665 EGCs resected by ESD at Jichi Medical University Hospital between January 2015 and April 2018, since the introduction of LCI into clinical practice. A laser endoscopic system (LL-4450/VP-4450) with a gastrointestinal endoscope (EG-L590ZW, EG-L590WR, EGD-L600ZW, EG-L600WR) and a laser light source was used. Inclusion criteria were patients aged over 18 years, diagnosed with EGC, who underwent ESD, having appropriate imaging by both WLI and LCI before ESD. We consecutively collected endoscopic images taken at a distant view from the same location without magnification in both modes. One hundred fifteen lesions were excluded due to inadequate images for evaluation or the presence of synchronous lesions in the same resected specimen (Figure 2). Both WLI and LCI images of 550 histologically proven lesions were prepared and evaluated by three non-expert and three expert endoscopists. A non-expert endoscopist is defined as one with less than three years of endoscopic experience and a basic knowledge of using LCI, and an expert endoscopist is defined as one with more than ten years of endoscopic experience and more than two years of LCI experience.

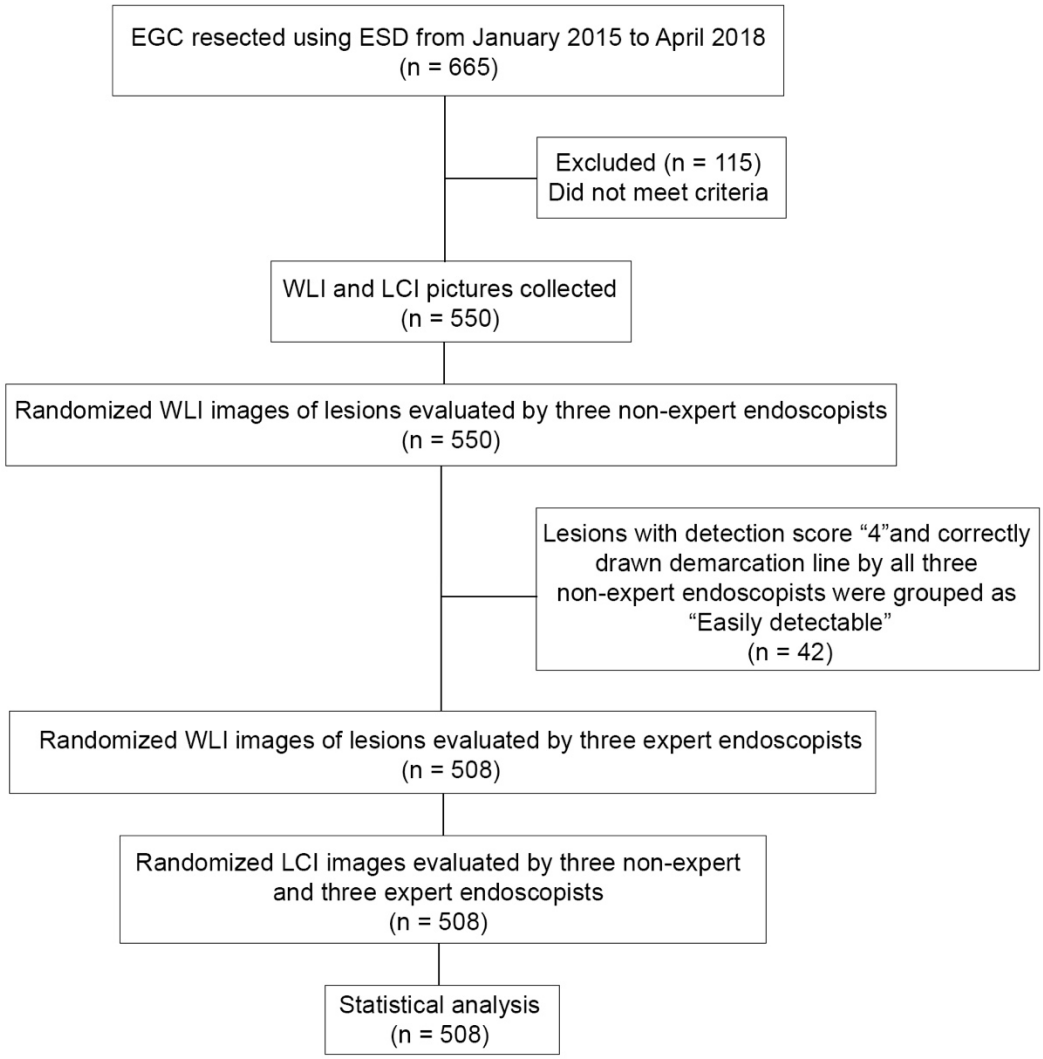

Figure 2. Study I flowchart. EGC, early gastric cancer; WLI, white light imaging; LCI, linked color imaging; ESD, endoscopic submucosal dissection.

3.1.2 Evaluation and scoring

Endoscopic images were assessed by endoscopists in random order with a wash-out period so that LCI and WLI images of the same lesion were evaluated at different times. Endoscopists scored detection of the lesions based with a visibility scale: score 4, excellent (easily detectable); score 3, good (detectable with careful observation); score 2, fair (hardly detectable without careful examination); score 1, poor (not detectable) as previously described[47]. Endoscopists were also asked to determine the visibility score for the lesion's extent, that is, the demarcation line between the lesion and the surrounding mucosa from 1 (demarcation

cannot be identified) to 4 (easily identified). In cases where the visibility score for extent was "4", assuming the endoscopist could clearly see the delineation, a demarcation line was drawn. The correct demarcation line between the lesion and surrounding mucosa was carefully prepared based on the pathology report specimen marking, ESD marking and other available endoscopic images including with magnification. In order to investigate the accuracy of the scores, endoscopists were asked to identify the suspected lesion and were double-checked with corresponding correct answer. If the lesion was identified incorrectly, both scores were set at a score of 1, poor (not detectable and no identifiable line of demarcation). Detection visibility score was used for the main analysis. A representative image for each score is shown in Figure 3. Endoscopists were additionally asked to choose whether color or shape contributed to the good visibility (score 3 and 4) evaluation.

Obviously elevated malignant lesions are easily visualized and diagnosed regardless of the imaging modalities. To investigate the effect of LCI and WLI for the detection of obscure early gastric cancer, a total of 42 lesions scored "4" both for detection and extent, accompanied by correctly drawn demarcation lines by all three non-expert endoscopists were classified as "Easily detectable" and were not included in the main analysis (Figure 4).

Endoscopic findings of malignant lesions were defined based on the Japanese gastric cancer treatment guidelines and the Japanese Classification of Gastric Carcinoma. Well- and moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were classified as differentiated type, and poorly differentiated adenocarcinoma and signet ring cell carcinoma as undifferentiated type. The locations were classified according to the trisected portions of the stomach: upper, middle, and lower portions. Successful eradication was determined by the patient`s history of undergoing Helicobacter pylori eradication and confirmed either by immunoglobulin level and/or stool antigen test.

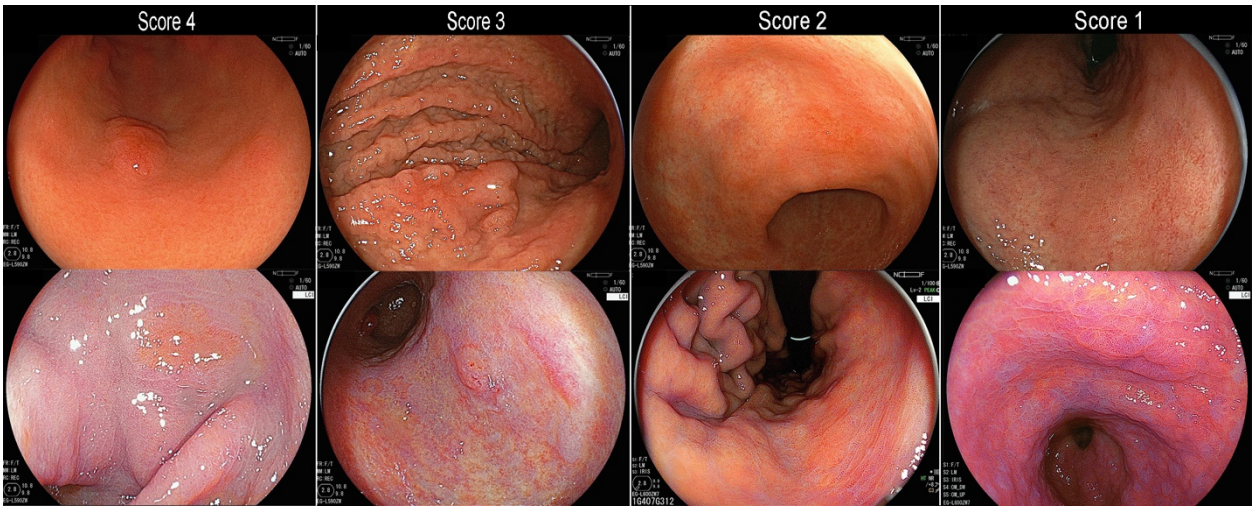

Figure 3. Representative images for detection visibility scores.

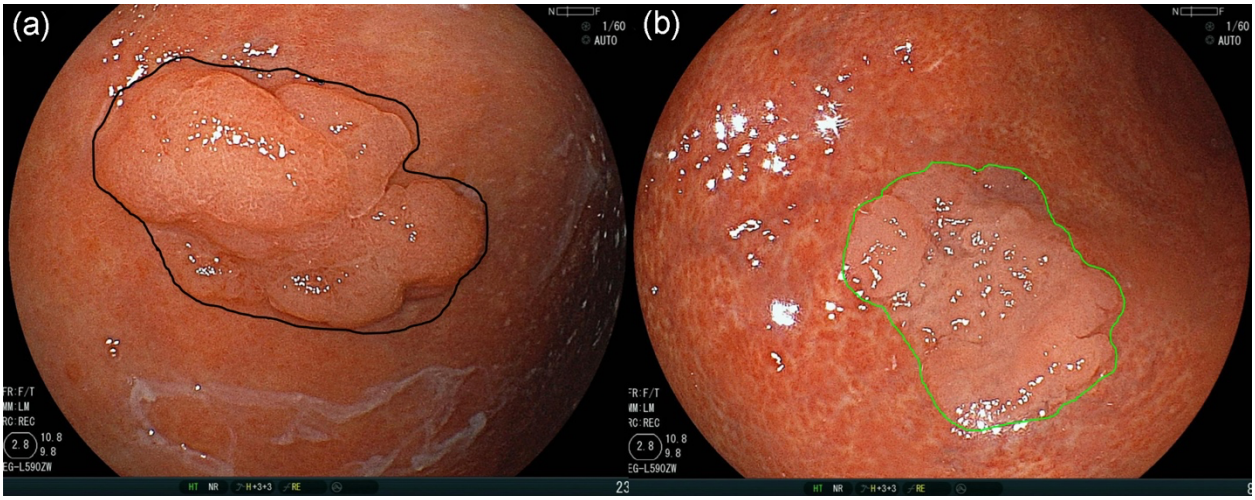

Figure 4. Representative images of lesions (total n=42) with a detection score "4" and correct demarcation line on white light imaging (WLI) by all three non-expert endoscopists.

3.1.3 Statistical analysis

Quantitative data are expressed as the mean and standard deviation (SD). The improvement rate of visibility between WLI and LCI was calculated by the paired t-test. WLI and LCI visibility score differences between depth groups and H. pylori infection status groups were performed by two-way ANOVA and Tukey's multiple comparison tests. The interobserver agreement was measured using the kappa statistics. The interobserver agreement was calculated at two levels: good visibility (score 3 and 4) and poor visibility (score 1 and 2)

among the three endoscopists of each group of expert endoscopists and non-expert endoscopists.

Multivariate mixed-effects logistic regression analyses with endoscopist-specific random effects were conducted to evaluate the associations between each potential predictor and good visibility of EGC[48]. Scores of 1 and 2 were considered to be poor visibility while scores 3 and 4 to be good visibility. Each image evaluation by each endoscopist was considered as independent data. Predictors included endoscopic modality (WLI vs LCI), morphology of the lesion (elevated vs non-elevated), size ($\leq$ 20mm vs $\geq$ 21mm), location (upper region as a reference vs middle and lower regions) depth (mucosa vs submucosa) and H. pylori infection status (positive vs negative) [49, 50]. Results were reported as odds ratios (ORs) with the corresponding 95% confidence intervals (CIs). Statistical significance was defined as $p < 0.05$. Statistical analyses were conducted using Stata 16.0 (Stata Co, TX, USA) and GraphPad Prism software (GraphPad software, La Jolla, CA, USA).

3.2 Study II

3.2.1 Study design

The current study was registered as a clinical trial (University Hospital Medical Information Network Clinical Trials Registry number UMIN 000028328). The study protocol and its revision (adding an author) were reviewed and approved by the Institutional Review Board of Jichi Medical University Hospital (Numbers A15-241 and A20-032, respectively). This was a retrospective analysis of prospectively collected video data including malignant gastric lesions with chronic gastritis and chronic gastritis alone from June 2016 to July 2017.

The purpose of this study was to evaluate LCI with ultrathin and standard endoscopy and its ability to facilitate the detection of EGCs compared with WLI endoscopy.

3.2.2 Study outcome and sample size

The primary endpoint of this study was the sensitivity of EGC detection using LCI with ultrathin and standard endoscopy. The secondary endpoints included: (1) the color difference between EGC and surrounding mucosa using WLI and LCI; (2) visibility differences for EGC comparing images from an ultrathin endoscope and a standard endoscope using WLI and LCI.

Based on expected 25% difference in the endoscopic detection of EGC with WLI versus LCI using G power ($\alpha = 0.05$, $\beta = 0.2$), we estimated that a sample size of 56 patients with EGC would be sufficient to demonstrate a significant difference using StatFlex version 6.0 software (Artech) [42]. Considering 30% synchronous lesions and 10% excluded cases we sought to collect 95 gastric cancer cases. There were 95 EGC cases accrued from June 2016 to July 2017. We collected consecutive screening videos taken with WLI and LCI including non-cancer cases.

3.2.3 Patients and endoscopic procedure

One hundred sixty-six consecutive patients requiring detailed upper gastrointestinal endoscopic examinations referred from smaller clinics or hospitals were enrolled except patients with a history of gastric surgery. Written informed consent was obtained from each patient before the procedure. Inclusion criteria included: (1) patients with a single EGC in the background mucosa with atrophy (2) patients with atrophic gastritis but without a malignant lesion in the stomach. We have excluded cases with synchronous cancers, advanced cancer, non-atrophic stomach, inadequate video and no report of ESD pathology.

Repeat informed consent was deemed necessary and eight participants could not be contacted or chose not to be included in the study. Finally, a total of 81 cases with 52 EGCs and 29 with atrophic gastritis alone were included in the final analysis (Figure 5).

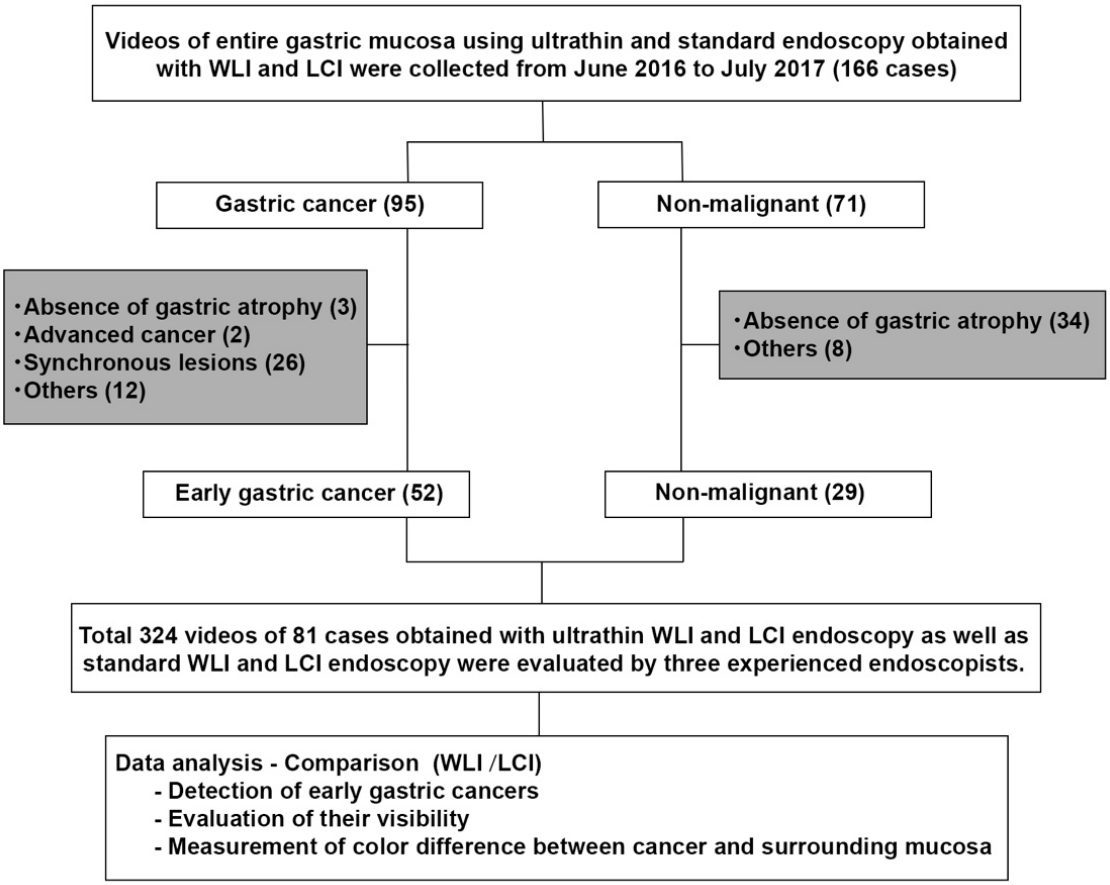

Figure 5. Study II flowchart. The process from collecting the video of gastric lesions using white light imaging (WLI) and linked color imaging (LCI) to data analysis evaluated by expert assessors.

Four experienced endoscopists (HO, YI, YM, and TT) performed gastric screening endoscopy under the same protocol with an ultrathin endoscope (EG-L580NW, 5.9mm in diameter), and a standard endoscope (EG-L590WR, 9.6mm). Videos were taken with standard WLI, standard LCI, ultrathin WLI and ultrathin LCI in order. First, endoscopists observed from the gastric body to the pyloric ring in an antegrade view followed by antrum to fornix and inversely in a retrograde view, and subsequently from antrum to upper body in an antegrade view by withdrawing the endoscope within 90 seconds. Still images were not taken to decrease the bias of highlighting lesions depending on the location [51].

Approximately ninety seconds of gastric screening videos in each mode were obtained without still images (a total of 4 videos/patient) to be reviewed later by expert assessors. Subsequently, precise endoscopic examinations were carried out with the equipment necessary for detailed diagnosis such as a magnifying endoscope and endoscopic ultrasound. The procedure was carried out under conscious sedation with intravenous midazolam and pethidine hydrochloride injection.

3.2.4 Evaluation of endoscopic videos

Endoscopic videos were arranged at random order with a wash-out period of 3 weeks for the same case. Three expert endoscopists with at least two-years of detailed endoscopic examination experience using laser endoscopy with no prior knowledge of the study cases evaluated the videos only once without a time limit with free review. They were asked to check whether early gastric cancer was present and complete a case report form. If they suspected or detected a malignant lesion, a visibility score was assigned as follows: score 3, excellent (video was viewed one time); score 2, good (video was viewed two times); score 1, fair (video was viewed three times or more) in reference to a previously described procedure for the evaluation of endoscopic videos [52]. To make the score descriptions accurate, endoscopists were asked to stop the video, record the time and draw the location of the lesion on the screen simulation area in the case report form. All suspected lesions were carefully double-checked with corresponding pathology reports and ESD reports. If the lesion was missed the visibility score was scored as "0".

The macroscopic classification was as follows: 0-I and/or 0-IIa as elevated type, 0-IIb flat type, 0-IIc and/or 0-III depressed type. Successful eradication was determined by a history of *Helicobacter pylori* eradication and confirmed either by serum immunoglobulin level or stool antigen test. Depth is recorded based on the final pathology report of the resected specimen,

tumor confined to the mucosa or invasion into the muscularis mucosa as M, tumor invasion within 0.5 mm into the submucosa as SM1 and tumor invasion of 0.5 mm or more deep into the submucosa as SM2.

3.2.5 Color difference calculations

Similar images of EGCs were captured from the videos and analyzed objectively based on the L*a*b* (L* = light/dark; a* = red-green; b* = yellow-blue) color values in the CIELAB system using Adobe Photoshop CC2019 as previously reported [53] [30] [54]. The five regions of interest (ROI; 20x20 pixels) were selected at random from malignant lesions and then their adjacent surrounding mucosae from standard WLI, standard LCI, ultrathin WLI and ultrathin LCI images. To avoid selection bias as much as possible, these selections and calculations were performed by a single operator who can recognize malignant lesions on endoscopic images. The average of five median RGB values for five sample points was calculated in each region. The L*a*b* values were calculated from the average RGB values. The color difference ($\Delta E^*=[(\Delta L^*)^2+(\Delta a^*)^2+ (\Delta b^*)^2]^{1/2}$) of the pixel values was analyzed to evaluate color contrast between malignant lesions and surrounding mucosa using ultrathin WLI, ultrathin LCI, standard WLI and standard LCI, respectively. Color differences were classified based on the size of the malignant lesion, morphology, location, *H. pylori* status, histology and depth of lesions histologically evaluated using the resected specimens.

3.2.6 Resolution measurement of ultrathin and standard endoscopes

The resolution using ultrathin endoscopy compared with standard endoscopy has not been studied in detail. As an initial investigation, we compared the resolution between a standard endoscope (EG-L590WR) and an ultrathin endoscope (EG-L580NW) because the data associated with gastric screening have not been objectively reviewed. The standard industry

testing protocols for image resolution (United States Air Force-1951 test target) were used to confirm differences in resolution [55, 56]. The ratios of resolution were measured at a near view of 10mm, mid-distant view of 20mm and a far-distant view of 50mm from the resolution chart, simulating the distance between the endoscope and target gastric mucosa during screening endoscopy (Figure 6).

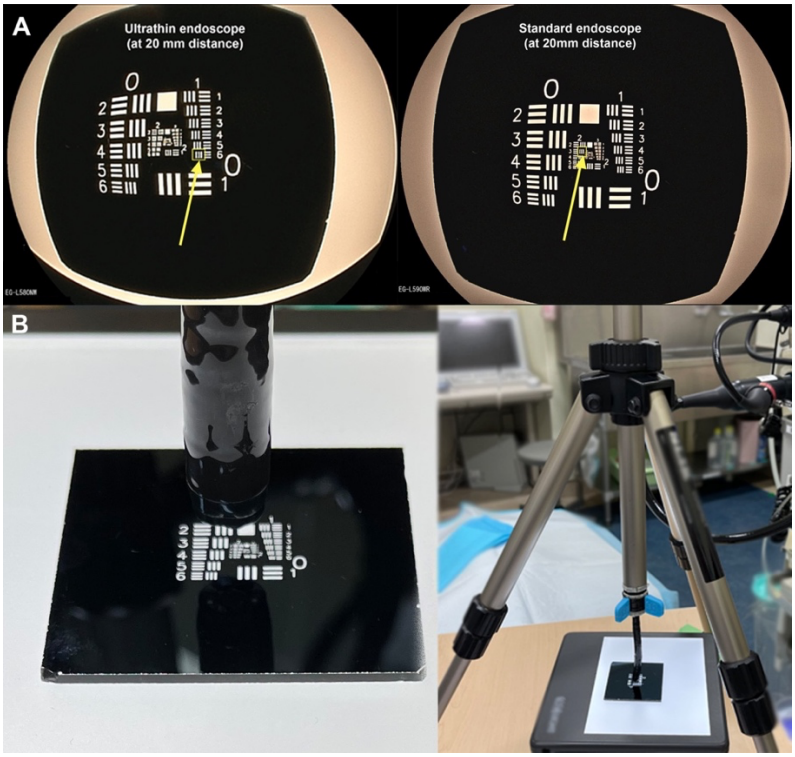

Figure 6. A. The arrows show the smallest group of elements from United States Air Force (USAF) resolution test card chart than can be clearly visualized at 20 mm of working distance for ultrathin endoscope and standard endoscope. B. The setting of endoscope and USAF 1951 chart for resolution calculation.

3.2.7 Statistical analysis

Statistical analyses were carried out using Stata 16 (version for Windows, StataCorp, TX, USA) and Graphpad Prism Version 9 software (Graphpad software, La Jolla, CA, U.S.A.). Levels of color differences and values of L*, a* and b* were expressed as the mean (± SD). Comparisons between 4 modes were made using the one-way Analysis of Variance (ANOVA)

with Bonferroni post-hoc test for significance between paired groups. Significant differences were assumed if P values of less than 0.05 were obtained. The distribution of visibility scores was compared between WLI and LCI using the linear-by linear chi-squared test. *P*-values < 0.05 were considered significant.

4 RESULTS

4.1 Study I

4.1.1 Visibility of malignant lesions

Baseline characteristics of 508 EGCs from 456 patients are shown in Table 1. Small EGC (≦10mm), those in a post-eradicated state, flat type and undifferentiated type accounted for 150 (30%), 205 (40%), 21 (4%) and 21 (4%) lesions, respectively.

The mean (± SD) detection visibility scores of lesions for all endoscopists were significantly higher for images obtained using LCI (3.31 ± 0.74) than for those using WLI (2.79 ± 0.87) ($p < 0.001$) (Table 2). Scores were significantly higher for both mucosal and submucosal cancers viewed using LCI than when using WLI. For differentiated cancers, the mean detection score with LCI (3.31 ± 0.73) was significantly higher than with WLI (2.8 ± 0.87). The detection score using LCI to image undifferentiated cancers was (2.98±0.88), which is significantly higher than when using WLI (2.59 ± 0.98). The current study evaluated as many as 150 lesions ≦10mm. LCI provided significantly higher visibility scores to detect the small cancers compared with WLI, similar to the scores for larger lesions (Table 2).

Table 1. Baseline characteristics of patients and early gastric cancer lesions

Number of patients/lesions, n	456/508
Age, years (range)	73 (36-89)
Gender, n (%)	
Male	398 (78%)
Female	110 (22%)
Tumor location, n (%)	
Upper	75 (15%)
Middle	231 (46%)
Lower	190 (37%)
Remnant stomach	12 (2%)
Macroscopic type, n (%)	
Elevated	166 (33%)
Flat	21 (4%)
Depressed	321 (63%)
Tumor size, n (%)	
≦ 10mm	150 (30%)
11 - 20mm	188 (37%)
21 - 30mm	91 (18%)
More than 31mm	79 (15%)
Helicobacter pylori infection status, n (%)	
Positive	213 (42%)
Eradicated	205 (40%)
Negative, but positive gastric atrophy	75 (15%)
Negative, without gastric atrophy	3 (0.6%)
Undetermined	12 (2.4%)
Histological type, n (%)	
Differentiated	487 (96%)
Undifferentiated	21 (4%)
Histological depth, n (%)	
M	432 (85%)
SM1	29 (6%)
SM2	47 (9%)

M, tumor confined to the muscularis mucosa or invasion into the muscularis mucosa;
SM1, tumor invasion within 0.5 mm into the submucosa;
SM2, tumor invasion of 0.5 mm or more deep into the submucosa.

Table 2. Mean detection visibility scores with WLI and LCI mode.

	WLI, Mean ± SD	LCI, Mean ± SD	ΔMean score	*p value*
Total	2.79 ± 0.87	3.31 ± 0.74	0.51	< 0.001*
Tumor location				
Upper	2.69 ± 0.81	3.24 ± 0.74	0.55	< 0.001*
Middle	2.76 ± 0.84	3.33 ± 0.67	0.57	< 0.001*
Lower	2.84 ± 0.93	3.28 ± 0.82	0.44	< 0.001*
Remnant stomach	3.21 ± 0.74	3.51 ± 0.56	0.31	0.1045
Macroscopic type				
Elevated	3.09 ± 0.8	3.48 ± 0.66	0.39	< 0.001*
Flat	1.71 ± 0.58	2.57 ± 0.65	0.86	< 0.001*
Depressed	2.70±0.85	3.26 ± 0.74	0.56	< 0.001*
Tumor size				
≦10mm	2.67 ± 0.89	3.12 ± 0.85	0.45	< 0.001*
11-20mm	2.80 ± 0.87	3.34 ± 0.72	0.54	< 0.001*
21-30mm	2.93 ± 0.83	3.47 ± 0.60	0.54	< 0.001*
More than 30mm	2.84 ± 0.82	3.38 ± 0.59	0.54	< 0.001*
Helicobacter pylori infection status				
Positive	2.98 ± 0.82	3.38 ± 0.70	0.41	< 0.001*
Eradicated	2.65 ± 0.91	3.23 ± 0.77	0.59	< 0.001*
Negative, but positive gastric atrophy	2.62 ± 0.79	3.27 ± 0.69	0.65	< 0.001*
Histological type				
Differentiated	2.8 ± 0.87	3.31 ± 0.73	0.52	< 0.001*
Undifferentiated	2.59 ± 0.98	2.98 ± 0.88	0.57	0.0017*
Histological depth				
M	2.72 ± 0.87	3.27 ± 0.75	0.55	< 0.001*
SM1	2.99 ± 0.85	3.55 ± 0.69	0.56	< 0.001*
SM2	3.3 ± 0.71	3.49 ± 0.57	0.19	0.0253*

Data are shown as mean ± standard deviation; WLI, white light imaging; LCI, linked color imaging.
M, tumor confined to the muscularis mucosa or invasion into the muscularis mucosa;
SM1, tumor invasion within 0.5 mm into the submucosa;
SM2, tumor invasion of 0.5 mm or more deep into the submucosa.
*Statistically significant.

The detection score of lesions with depth M was significantly lower than SM2 using WLI, while the detection score with LCI was higher than with WLI and not significantly different among M, SM1 and SM2 (Figure 7a). Similarly, the detection score was significantly higher in the *H. pylori* positive group using WLI, while with LCI there were no significant difference in *H. pylori* status subgroups (Figure 7b). The visibility of 411 differentiated cancers ≦ 30mm referring to absolute indications for ESD, were significantly higher when using LCI. The visibility of 13 undifferentiated cancers ≦ 20mm were also significantly higher using LCI compared to WLI (Figure 7c).

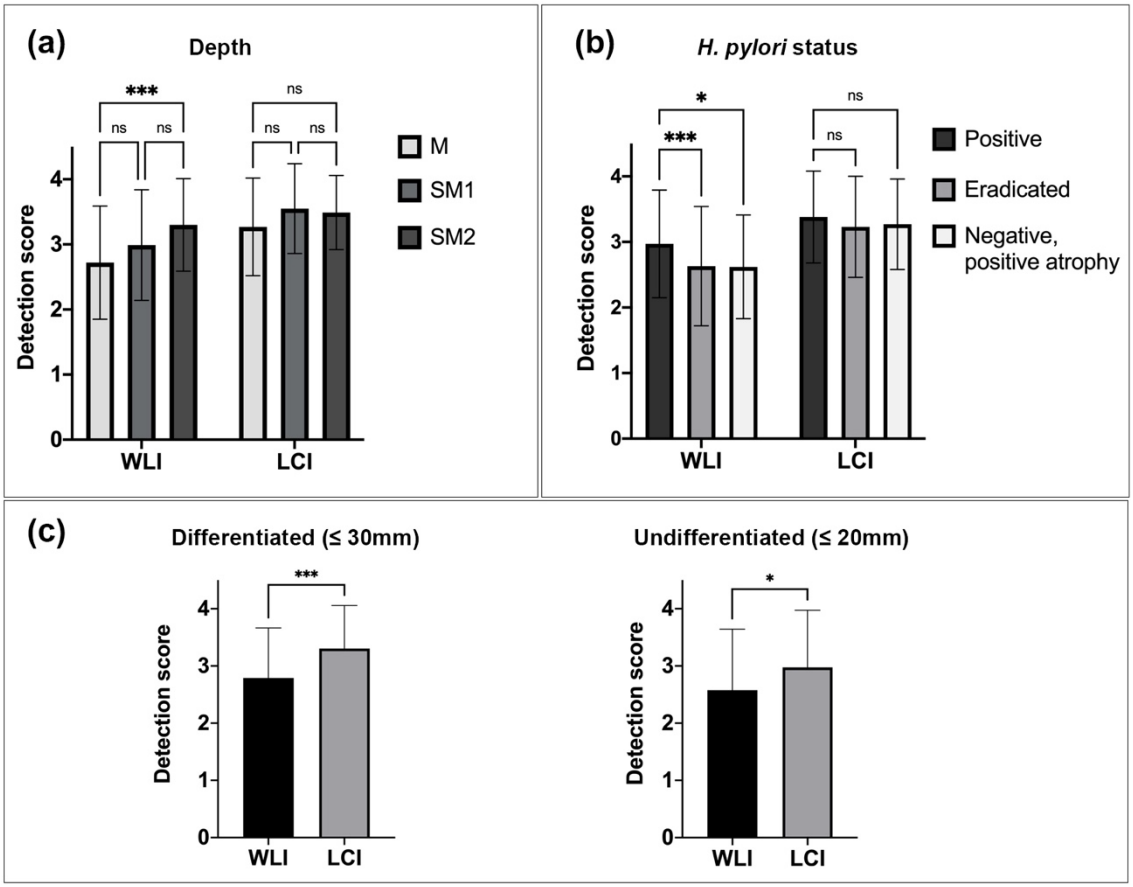

Figure 7 Subset analysis between groups. a. Linked color imaging (LCI) visibility score and white light imaging (WLI) visibility score difference between lesion depth groups; b. between *H. pylori* infection status groups. (Statistical significance was determined by two-way ANOVA and Tukey's multiple comparison tests; *** $p < 0.001$, ** 0.001 to 0.01, * 0.01 to 0.05, ns ≥ 0.05); c. WLI and LCI score differences for differentiated (n = 411) and undifferentiated (n=13) early gastric cancers depending on size.

Of a total of 3048 evaluations (508 lesions × 6 endoscopists), there were 2409 evaluations with good visibility with LCI and 1884 evaluations with WLI. The color of the lesions including surrounding mucosa contributed to the good visibility for 64% of evaluations with LCI and shape for 36%, while 35% and 65% with WLI respectively (Table 3). Of the evaluations where color was chosen, 52% were orange lesion surrounded by purple mucosa, 33% were lesions with darker orange color and 15% were purple colored lesion. Of the evaluations where shape was chosen, 80% were elevated and 20% were non-elevated lesions. Of the evaluations where color was chosen with WLI, 75% were red lesions and 25% were whitish lesions. Of the evaluations where shape was chosen, 71% were elevated and 29% were non-elevated type (Figure 8, 9). Interobserver agreement among expert endoscopists and non-expert endoscopists were 0.57 and 0.39 for WLI, while 0.30 and 0.38 for LCI and judged to have “fair to moderate agreement”.

Table 3. Contributing color and shape to good visibility for early gastric cancers by six endoscopists evaluation.

	n (%)
LCI	
Color	1541 (64%)
Orange surrounded by purple mucosa	797
Dark orange compared to surrounding mucosa	508
Purple	232
Others	4
Shape	868 (36%)
Elevated	691
Non-elevated	177
WLI	
Color	656 (35%)
Reddish	490
Whitish	165
Others	1
Shape	1228 (65%)
Elevated	877
Non-elevated	351

LCI: linked color imaging, WLI: white light imaging.

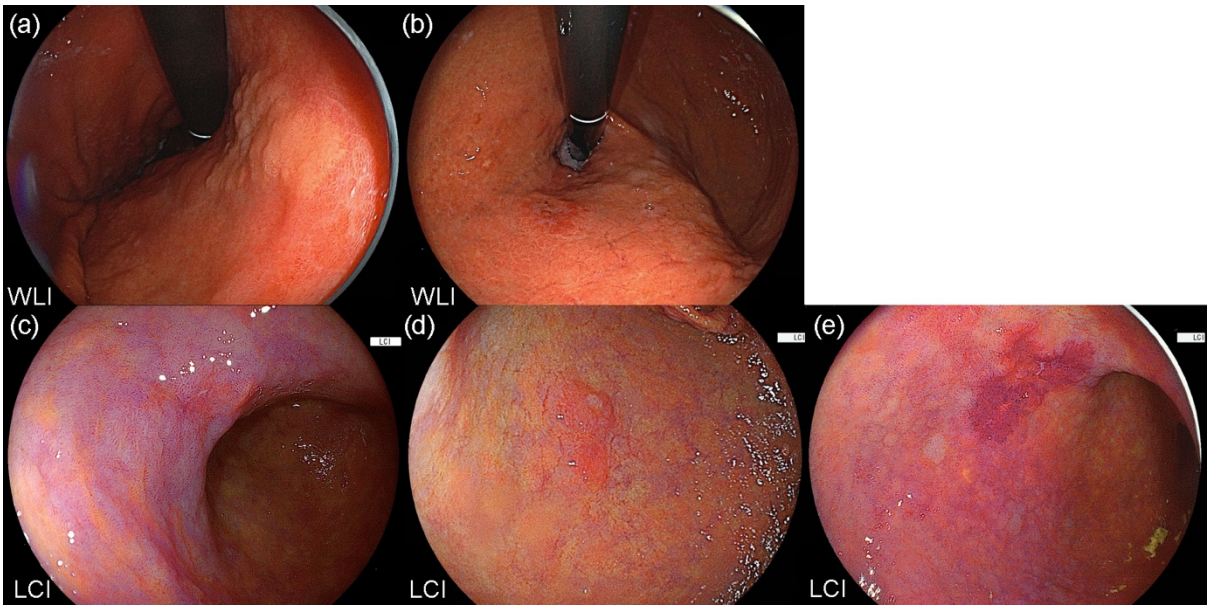

Figure 8. Representative images of early gastric cancers (EGC) where color contributed to good endoscopic visibility rather than shape according to six endoscopists evaluation. a. Whitish lesion with white light imaging (WLI); b. Reddish lesion with WLI; c. Orange lesion surrounded by purple mucosa with linked color imaging (LCI); d. Dark orange lesion compared to surrounding mucosa with LCI; e. Purple lesion with LCI.

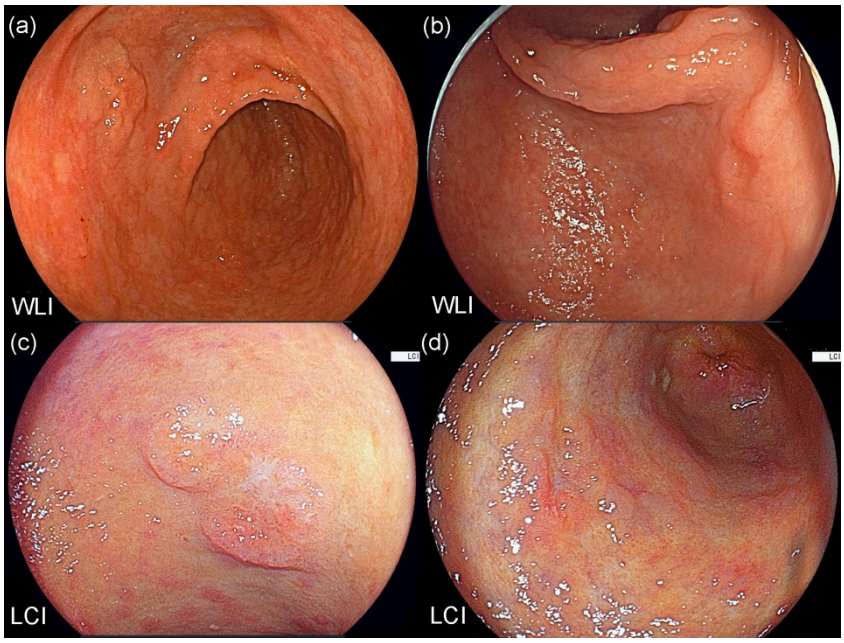

Figure 9. Representative images of early gastric cancers (EGC) where shape contributed to good endoscopic visibility rather than color according to six endoscopists evaluation. a. Elevated lesion with white light imaging (WLI); b. Non-elevated lesion with WLI; c. Elevated lesion with linked color imaging (LCI); d. Non-elevated lesion with LCI.

4.1.2 Multivariate analysis of factors significantly associated with improved visibility of early gastric cancer

Mixed-effects multivariate analysis revealed that LCI imaging (OR 2.57, 95% CI 2.27 - 2.9), elevated type (OR 1.92, 95% CI 1.67 - 2.22), invasion to submucosa (OR 2.18, 95% CI 1.78-2.66), differentiated histologic type (OR 1.57, 95% CI 1.17 - 2.11) and *H. pylori* positive (OR 1.36, 95% CI 1.2 - 1.54) were significantly associated with improved visibility of EGC (Table 4). Tumor location in lower gastric region (OR 1.43, 95% CI 1.2-1.72) and middle gastric region OR 1.26, 95% CI 1.05-1.51 were significantly associated with improved visibility of EGC compared to upper gastric region. Lesion size was not associated with an improved visibility score.

Table 4. Multivariate analysis of factors associated with good visibility of early gastric cancers

	Multivariate analysis	
Factor	OR (95% CI)	*p* value
LCI image (vs WLI image)	2.57 (2.27-2.9)	< 0.001*
Elevated (vs non-elevated)	1.92 (1.67-2.22)	< 0.001*
Depth within submucosa (vs mucosa)	2.18 (1.78-2.66)	< 0.001*
Differentiated (vs undifferentiated)	1.57 (1.17-2.11)	0.003*
Helicobacter pylori positive (vs negative)	1.36 (1.2-1.54)	< 0.001*
≥ 21mm (vs ≤ 20mm)	1.1 (0.96-1.26)	0.161
Upper region (reference)		
Middle region	1.26 (1.05-1.51)	0.011*
Lower region	1.43 (1.2-1.72)	< 0.001*

LCI: linked color imaging, WLI: white light imaging, OR: Odds ratio, CI: confidence interval, good visibility is defined as a visibility score of 3 or 4. *Statistically significant.

4.1.3 Improvement and deterioration of detection visibility scores changing from WLI to LCI

A total of 3048 evaluations were made by 6 endoscopists for all 508 lesions using WLI and LCI are shown in Figure 10. Overall, 38% of all lesions were poorly visible and 62% had good visibility using WLI. In contrast, 21% of all lesions were poorly visible and 79% had good visibility using LCI. When the score increased by 2 or more with a change from one modality to the other, it was defined as an "improvement". Of 1164 scored lesions evaluated as having poor visibility using WLI, the visibility of 540 lesions (46.4%) improved when using LCI. When the score dropped by 2 or more, it was defined as "deterioration". Of 1884 scored lesions having good visibility using WLI, the visibility score of 92 lesions (4.9%) deteriorated when using LCI. Eighteen lesions had a deteriorated score when evaluated by two to four endoscopists. Most of these lesions were red using WLI but purple when using LCI. Three lesions had a color similar to the surrounding atrophic mucosa using LCI, and 4 lesions had slight differences in angle and/or distance from the endoscope to the target area when comparing WLI and LCI.

		Poor visibility		Good visibility	
		WLI score 1 (n=523)	WLI score 2 (n=641)	WLI score 3 (n=833)	WLI score 4 (n=1051)
Poor visibility	LCI score 1 (n=289)	5.7% (173)	2.1% (64)	**1.2% (36)**	**0.5% (16)**
	LCI score 2 (n=350)	4% (123)	3.8% (117)	2.3% (70)	**1.3% (40)**
Good visibility	LCI score 3 (n=549)	**3.2% (99)**	4.8% (147)	5.9% (179)	4.1% (124)
	LCI score 4 (n=1860)	**4.2% (128)**	**10.3% (313)**	18% (548)	28.6% (871)

Score of all 6 endoscopists are calculated.
Score 1 and 2 represents poor visibility; score 3 and score 4 represents good visibility.
Cases increased or decreeased by two scores are highlighted in box.

Figure 10. Score proportion for good visibility and poor visibility using white light imaging (WLI) and linked color imaging (LCI).

4.1.4 Visibility of extent of malignant lesions

The extent visibility score using LCI (2.88±0.73) was significantly higher than when using WLI (2.22±0.72) (p<0.001). The rates of score 1 were 29% and 15% using WLI and LCI, respectively, while those with a score 4 (excellent) were 10% and 36% using WLI and LCI, respectively, (Figure 11).

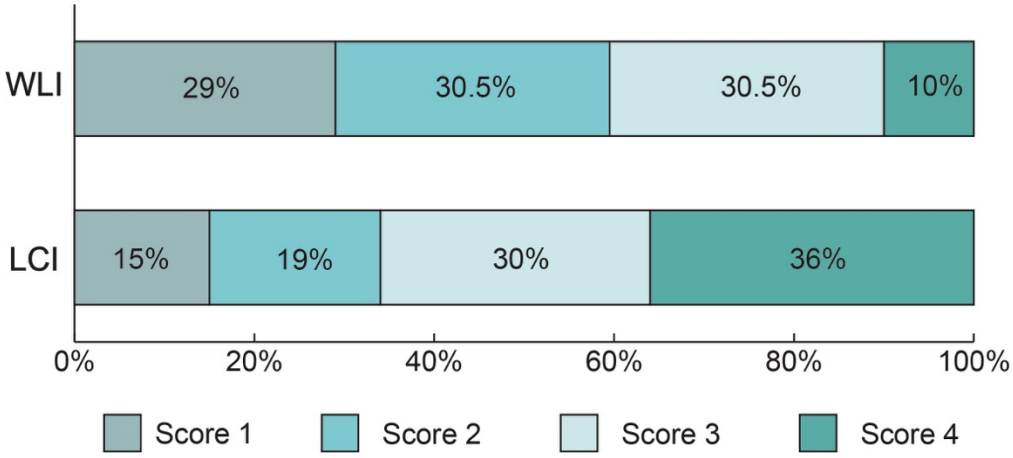

Figure 11. Distribution of the extent visibility scores of white light imaging (WLI) and linked color imaging (LCI).

4.2 Study II

4.2.1 Patients

Patient baseline and lesion characteristics are shown in Table 4. There were 52 patients with single EGC lesions with atrophic gastritis. Of the 29 patients without gastric malignancy but with gastric atrophy, eight had esophageal lesions, seven had duodenal lesions, six had gastric submucosal tumors and eight had atrophic gastritis alone.

Table 5. Baseline characteristics of study patients

Number of patients, n	81
Gender (male/female)	64/17
Age, median (range)	70 (44-89)
Early gastric cancer, n	52
Location	
Upper / Middle / Lower	5 / 14 / 33
Morphology	
Elevated / Flat/ Depressed	12 / 4/ 36
Size	
≦10mm / 11-20mm / 21-30mm / 30mm<	14 / 14 / 9 /15
H. pylori status	
Positive / Eradicated/ Unknown	32 / 14/ 6
Histology	
Differentiated / Undifferentiated	48 / 4
Depth	
M / SM1 / SM2	41 / 4 / 7
Non-malignant, n	29
Atrophic gastritis	8
Esophageal lesion	8
Duodenal lesion	7
Gastric submucosal tumor	6
Atrophy, n	
Closed/Open	14/67

M, tumor confined to the muscularis mucosa or invasion into the muscularis mucosa;
SM1, tumor invasion within 0.5 mm into the submucosa;
SM2, tumor invasion of 0.5 mm or more deep into the submucosa.

4.2.2 Sensitivities and specificities of LCI and WLI for detecting EGC

Ultrathin WLI, ultrathin LCI, standard WLI and standard LCI showed sensitivities of 66.0%, 80.3%, 69.9% and 84.0% and specificities of 67.8%, 59.3%, 59.8% and 50.6%, respectively for the detection of EGCs (Table 6). Sensitivity with ultrathin WLI was slightly lower that with standard WLI similar to a previous report [9]. Sensitivities with LCI were higher than those with WLI using both ultrathin and standard endoscopes for all three endoscopists. Sensitivities with ultrathin LCI were also higher than those with standard WLI for all three endoscopist. Specificities were lower for LCI than those with WLI and were different among the three endoscopists. Figure 12 shows representative images of EGCs using WLI and LCI, which are captured from the respective video recordings. The interobserver agreement was measured using the kappa statistics. The interobserver agreement for standard WLI was 0.51 for standard LCI was 0.28, for ultrathin WLI was 0.47 and for ultrathin LCI was 0.31 and judged to have "fair to moderate agreement".

Table 6. Sensitivity and specificity of white light imaging (WLI) and linked color imaging (LCI) with standard and ultrathin endoscopes

	Ultrathin WLI	Ultrathin LCI	Standard WLI	Standard LCI
Sensitivity (%)	66.0%	80.3%	69.9%	84.0%
Specificity (%)	67.8%	59.3%	59.8%	50.6%
Expert endoscopist 1				
Sensitivity (%)	75.0%	84.6%	78.8%	88.5%
Specificity (%)	55.2%	41.4%	48.3%	27.6%
Expert endoscopist 2				
Sensitivity (%)	65.4%	81.1%	71.2%	82.7%
Specificity (%)	58.6%	50.0%	44.8%	41.4%
Expert endoscopist 3				
Sensitivity (%)	57.7%	75.0%	59.6%	80.8%
Specificity (%)	89.7%	86.2%	86.2%	82.8%

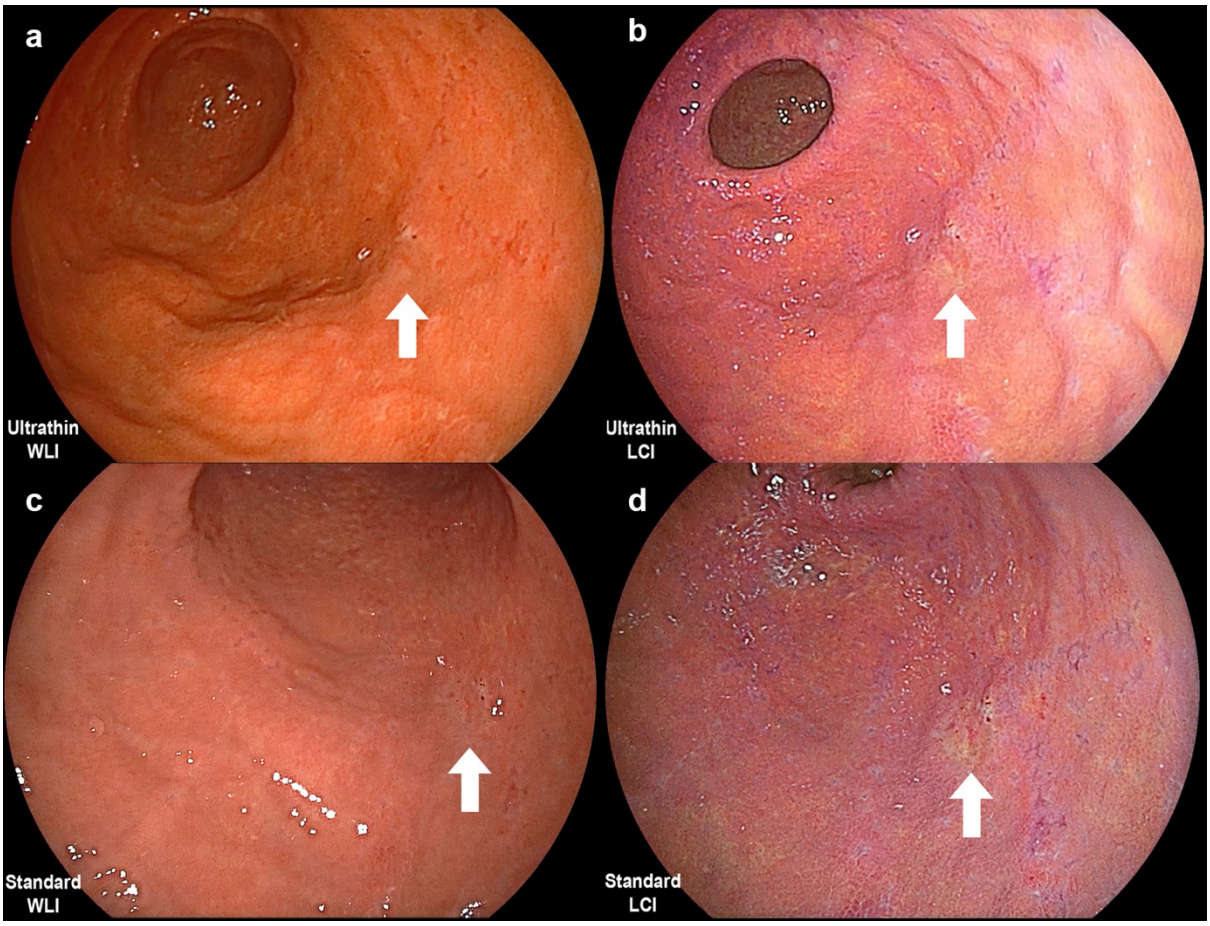

Figure 12 Representative images of early gastric cancers obtained using white light imaging (WLI) and linked color imaging (LCI). Compared with WLI (a) using an ultrathin endoscope, LCI (b) produces image with a high color contrast (white arrow) between the malignant lesion and the surrounding mucosa. Similar images are found using WLI (c) and LCI (d) using a standard endoscope.

4.2.3 Visibility scores for malignant lesions

Mean visibility scores for malignant lesions (n=52) were 1.76±1.15 and 2.32±0.98 for ultrathin WLI and LCI, and 1.94±1.09 and 2.49±0.84 for standard WLI and LCI, respectively. The distributions of visibility scores were compared using each mode and evaluated using the linear-by-linear association chi square test (Figure 13). Visibility scores were higher with LCI than with WLI for both ultrathin (P<0.001) and standard endoscopes (P<0001). LCI with an ultrathin endoscope resulted in a significantly higher visibility score than WLI with a standard endoscope (p=0.001).

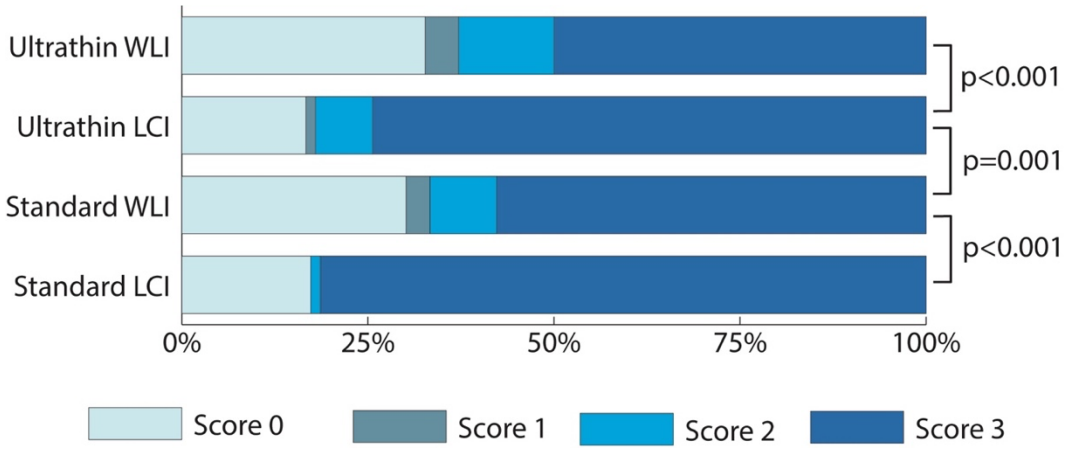

Figure 13. Distribution of visibility scores showing the superiority of linked color imaging (LCI) compared to white light imaging (WLI) as assessed by expert endoscopists. Scores from 0 to 3 indicate missed, fair, good, and excellent visibility, respectively. Statistical values are calculated by the linear-by-linear association chi square test.

4.2.4 Color differences between malignant lesion and surrounding mucosa

Color differences with LCI were significantly higher than those with WLI for both ultrathin and standard endoscopes (P<0.001) (Table 7). LCI with an ultrathin endoscope resulted in significantly higher color differences than WLI with a standard endoscope (P<0.001). Significantly higher color difference using ultrathin LCI were found regardless of H. pylori status and the size of the malignant lesion (Table 6). In the mid- and distal stomach, elevated and depressed type, differentiated type, depth within mucosa, the color difference with ultrathin LCI mode was significantly higher than with standard WLI (Table 6).

The purple surrounding mucosae, mainly corresponding to intestinal metaplasia, are considered to influence the color difference [17, 18] and we therefore evaluated the ratio of purple color to the entire circumference of the malignant lesion. The number of lesions with ratios ≤50%,50-75% and 75%≤ was 31, 3 and 18, respectively.

We assessed three cases with gastric cancers missed using standard WLI by more than two endoscopists but detected by ultrathin LCI (Figure 14). The first case had an inflammatory map-like redness near a small, depressed cancer on the lesser curvature of the gastric midbody. The second and the third case were flat and flat-elevated lesion, respectively. These malignant and benign lesions could not be identified or differentiated by WLI but were visualized as orange red malignant lesions and purple inflammatory lesions by LCI.

Table 7. Comparison of color differences between malignant lesions and the surrounding mucosa with White Light Imaging (WLI) and Linked Color Imaging (LCI) (n=51)

	Ultrathin WLI	Ultrathin LCI	Standard WLI	Standard LCI	*P value*	Ultrathin WLI vs Ultrathin LCI	Standard WLI vs Standard LCI	Ultrathin LCI vs Standard WLI
Total lesions (n = 51)								
ΔE	6.9(3.6)	11(4.5)	6.6(3.7)	12(4.9)	<0.001*	<0.001*	<0.001*	<0.001*
ΔL	-0.6(3.6)	-0.1(4.7)	-0.01(3.4)	-0.6(4.8)	ns			
Malignant lesion	143.5(21.1)	159.5(17.6)	138(18)	157.2(17.7)				
Surrounding lesion	145.1(20.5)	159.5(16.9)	138,4(18.4)	158.6(19.2)				
Δa	3.2(3.9)	4.2(6.3)	2.8(4.4)	5.6(6.3)	<0.001*	ns	<0.001*	ns
Malignant lesion	169.6(6.1)	165.1(6.3)	165.9(6.2)	162.7(6.9)				
Surrounding lesion	166.4(6.1)	161(6.2)	163.1(5.7)	157.1(7.4)				
Δb	2.5(4.3)	1.3(8.4)	2.7(3.3)	2.1(8.3)	ns			
Malignant lesion	175.5(7.4)	151.6(7.6)	163.1(7.5)	147.5(7.3)				
Surrounding lesion	173(7)	150.2(6.7)	160.3(7.9)	145.4(7.3)				
Location ΔE								
Upper (n = 5)	10.6(2.5)	13.1(2.8)	9.6(2.6)	14.1(6.8)	ns			
Middle (n = 13)	6.4(3.9)	11.3(4.4)	6.9(4.6)	11.1(4.2)	0.0002*	0.0032*	0.007*	0.0158*
Lower (n= 33)	6.6(3.4)	11(4.8)	6(3.3)	11.9(4.9)	<0.001*	<0.001*	<0.001*	<0.001*
Morphology ΔE								
Elevated (n = 12)	6.5(3.7)	11.2(3.9)	7(4.5)	12.7(4.6)	0.0002*	0.0265*	0.0052*	0.0287*
Flat (n = 4)	7.1(5.7)	13.3(5.2)	6.2(3.7)	14.2(4.5)	ns			
Depressed (n= 35)	7.1(3.4)	11.1(4.7)	6.4(3.5)	11.4(5)	<0.001*	<0.001*	<0.001*	<0.001*
Size ΔE								
≦10mm (n = 13)	7.3(3.4)	11.8(4.8)	6.4(2.9)	12.3(5.3)	0.0002*	0.0009*	0.0023*	0.0006*
11-20mm (n = 14)	7.1(4.5)	9.7(3.9)	6(3.8)	10.2(3.8)	0.0015*	0.039*	0.0075*	0.0288*
21-30mm (n = 9)	6.1(2.7)	11.3(5.3)	6.4(4.8)	12.2(5)	0.0006*	ns	0.02*	0.0441*
30mm< (n = 15)	7.1(3.5)	12.4(4.4)	7.4(3.8)	13(5.4)	<0.001*	0.0013*	0.004*	0.0008*
H. pylori status ΔE								
Positive (n = 32)	6.7(3.5)	11.5(4.9)	6.5(4)	12.5(5.5)	<0.001*	<0.001*	<0.001*	<0.001*
Eradicated (n = 13)	7.6(4.2)	10.9(4.6)	6.6(3.7)	11.2(3.8)	0.0004*	0.0139*	0.0027*	0.0019*
Histology ΔE								
Differentiated (n = 47)	6.9(3.5)	11.4(4.6)	6.6(3.7)	12.2(4.8)	<0.001*	<0.001*	<0.001*	<0.001*
Undifferentiated (n = 4)	7.5(5.5)	9.9(4.9)	6.6(5.0)	8.0(4.3)	0.0447*	ns	ns	ns
Depth ΔE								
M (n = 40)	6.4(3.5)	10.7(4.6)	5.9(3.4)	11.5(5)	<0.001*	<0.001*	<0.001*	<0.001*
SM1 (n = 4)	7.3(4.4)	13.6(4.7)	8.0(3.2)	14.7(1.9)	ns			
SM2 (n = 7)	9.7(2.5)	13.4(3.6)	9.6(4.3)	12.5(5.2)	0.0265*	ns	ns	0.0258*

One lesion was excluded from analysis because of minute size. Data are shown as mean (standard deviation). ⊿E* shows color difference and is calculated from the following formula: $[(⊿L^*)^2+(⊿a^*)^2+ (⊿b^*)^2]^{1/2}$. *L** is defined as lightness, *a** as the red–green component and *b** as the yellow-blue component. ⊿L is obtained from a formula: (absolute L of malignant lesion - absolute L of surrounding mucosa) x100/255. Values (⊿a, ⊿b) were obtained by subtracting the value for the surrounding mucosa from the value for the malignant lesion. WLI, white light image. LCI, linked color imaging. ns, not significant. Comparisons between 4 modes were made using the one-way Analysis of Variance (ANOVA) with Bonferroni post-hoc test for significance between paired groups.*Statistically significant.

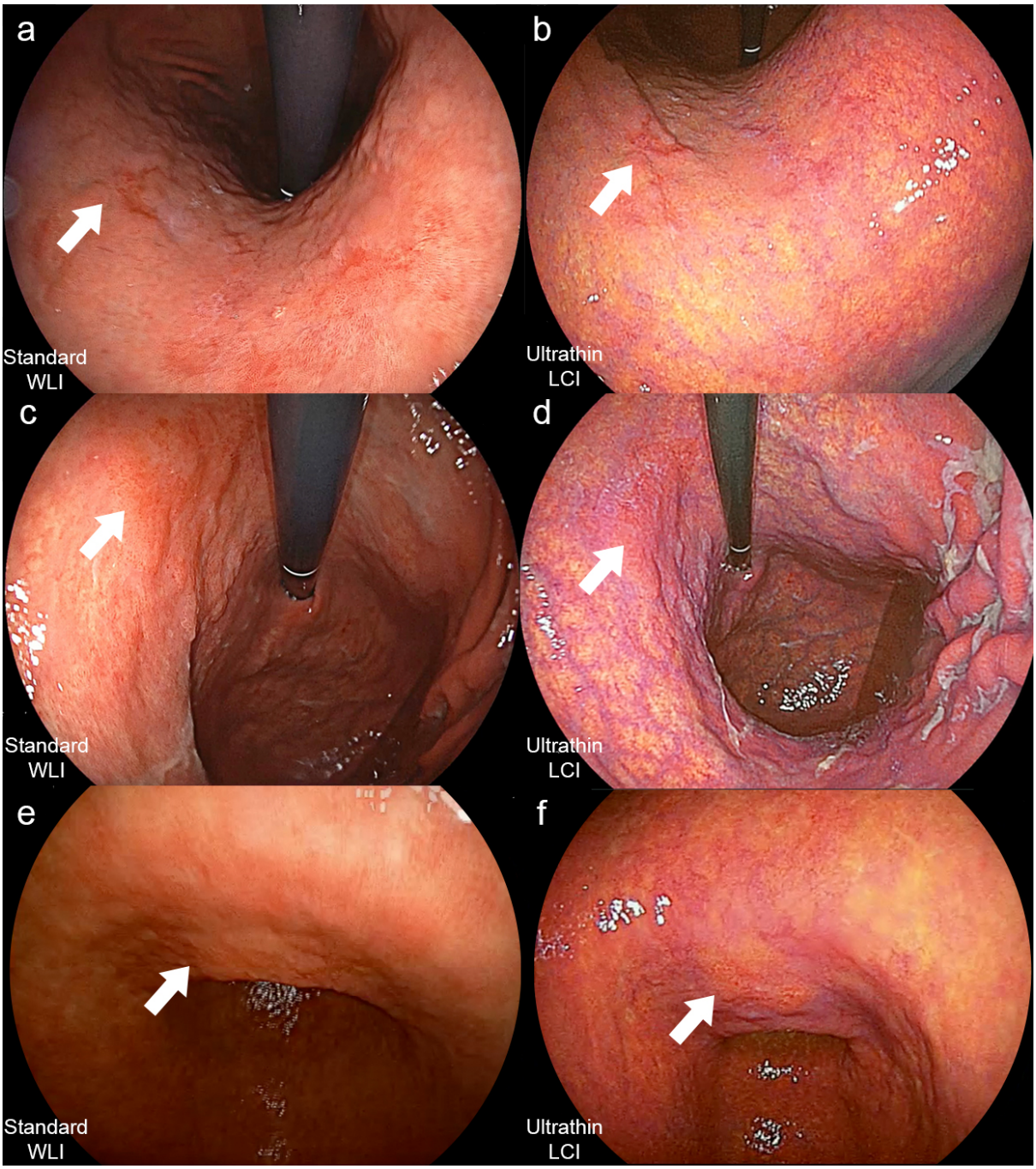

Figure 14. Early gastric cancers missed using standard white light imaging (WLI) by more than two endoscopists but detected by ultrathin linked color imaging (LCI). (a) Small, depressed cancer (white arrow) near inflammatory map-like redness using standard endoscope, WLI (b) ultrathin endoscope, LCI. (c), (e) Standard WLI; (d), (f) ultrathin LCI of flat-elevated and flat lesion respectively.

4.2.5 Difference of resolution between standard and ultrathin endoscope

The resolution using an ultrathin endoscope was compared with that of a standard endoscope and expressed as a ratio to the value obtained using the standard endoscope (arbitrary units). The resolution using the ultrathin endoscope was the same as that using the standard endoscope at a 10mm view from the resolution chart but was lower at 20mm (0.71) and 50mm (0.89) views simulating the distance between the endoscopic tip and target gastric mucosa during screening (Figure 15).

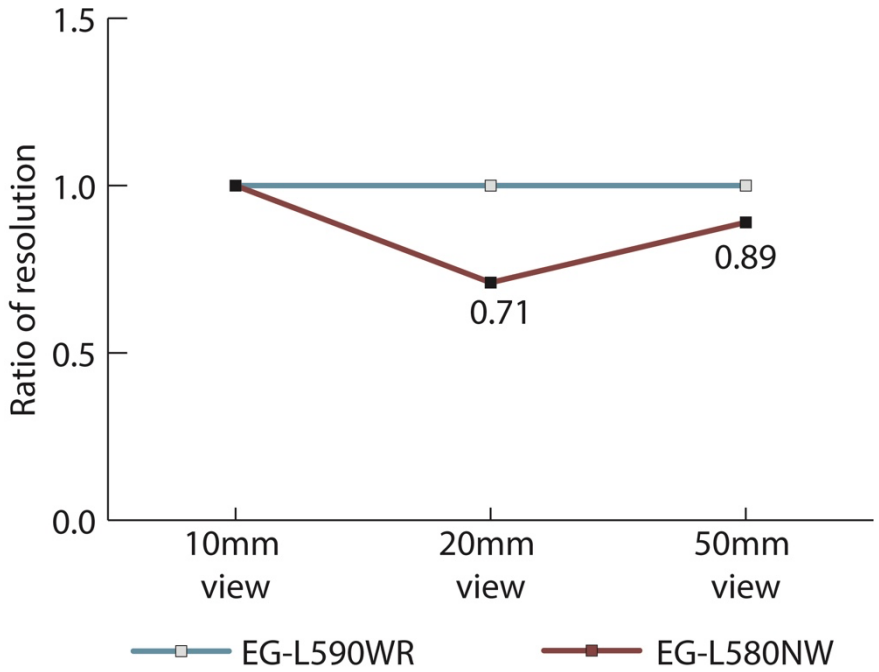

Figure 15. Ratio of resolution of the ultrathin endoscope (EG-L580NW) to the standard endoscope (EG-L590WR) at each distance between the endoscope tip and the resolution chart. The resolution using the ultrathin endoscope is lower than when using a standard endoscope at the 20mm and 50mm distances, simulating the distance between the endoscope tip and the target gastric mucosa at screening.

5 GENERAL DISCUSSION

5.1 Study I

This study demonstrates that LCI improves the visibility of obscure EGC at a distant view regardless of lesion characteristics including location, depth, pathology, size and *H. pylori* status. Even undifferentiated and small cancers are more visible using LCI compared with WLI. Multivariate analysis reveals that the use of LCI had the highest odds ratio among the factors evaluated when assessing visibility of EGCs. This is the first report that analyzes the factors which influence the detection of EGC in a large series and evaluates both the advantages and disadvantages of LCI.

Early gastric neoplasms of both the polypoid and ulcerative types are diagnosed easily when using WLI[57] but many EGCs only have obscure endoscopic findings. Detection or missed detection of EGCs may be strongly associated with not only morphological changes but other factors that influence the visibility of the lesion, although this issue has not been previously described. The multivariate analysis in the present study clarifies several factors which affect the visibility of EGC. Interestingly, LCI had the highest odds ratio among those factors such as elevated morphology and submucosal invasion. Several studies reported high color contrast between EGC and the surrounding mucosa using LCI[30]. The color difference between a lesion and the surrounding mucosa was closely associated with the visual score [30, 53], [58]. Additionally, the results of the present study suggest that high color contrast contributed to good visibility evaluation rather than the shape with LCI, while opposite with WLI.

It is unknown how many EGCs that are poorly visible using WLI can be recognized with LCI, despite reports describing improved visibility for EGCs compared with other modalities [59, 60]. In a recent randomized clinical trial of the detection of neoplastic lesions in the upper gastrointestinal tract, the miss rate of neoplasms was 39% with WLI and 7% with LCI. [24] In

the present study, the improved rate of visibility (46.4%) of EGCs is much higher than the rate of deteriorated visibility (4.9%) when the endoscopic modality changed from WLI to LCI. Poor visibility of EGCs missed by WLI can be improved using LCI, while the visibility of a small number EGCs might be decreased when using LCI. We have previously reported that malignant lesions exhibiting a redder color than the surrounding mucosa using WLI become purple when using LCI[30]. However, most endoscopic courses and lectures about LCI focus on the major EGC type which has a similar color to the surrounding mucosa and are poorly visible using WLI but turn orange using LCI and are easier to detect. When a malignant lesion is purple using LCI, the endoscopist might not recognize it as a malignant lesion despite its irregular surface. This implies that additional education about the significance of observing purple color using LCI is necessary. Nevertheless, endoscopists should be aware that using LCI has greater advantages and few disadvantages regarding the visibility of malignant lesions.

Despite its promising effect for detection of EGC, LCI has not been evaluated regarding its main endoscopic treatment. Considering the indications, difficulty of therapeutic procedures and delays, it is ideal to detect malignant lesions when they are as small as possible, leading to successful resection and reduction of mortality in the long term [2],[20]. The results of the present study show three advantages of LCI: 1) better visibility of lesions with a size within the endoscopic treatment range, including those ≦ 10mm, 2) similarly high visibility regardless of the depth of the EGC, suggesting utility for recognition of mucosal cancers, and 3) better visibility of undifferentiated lesions which are amenable to endoscopic resection compared with WLI. These results imply that using LCI is recommended from the beginning of endoscopic screening procedures for gastric lesions.

The presence of a demarcation line is considered to be one of the main endoscopic characteristics of EGC[19] and is recommended to be observed using magnified blue light imaging or narrow band imaging[61]. However, during screening endoscopy there is a limited

amount of time when the endoscopist suspects a lesion and has to make a decision. The present findings show that LCI improves the recognition of extent of a cancer for the endoscopist, also suggesting a possible explanation for the improved visibility of EGCs.

There are several limitations to this study. First, this study has a retrospective design and was conducted at a single hospital. Second, although we tried to exclude images that might lead to bias, images from the exact same angle and distance might not be available for both WLI and LCI due to patient movement, gastric motility, and gas insufflation. Third, the visual scores were subjectively made by endoscopists. The present study also has important strengths. First, as many as 508 LCI images of EGC resected endoscopically were evaluated and compared with images obtained using WLI. Second, EGCs with low incidence could be assessed including undifferentiated and small lesions. Third, multivariate analysis showed that LCI has a high odds ratio for providing good visibility compared with WLI.

5.2 Study II

This is the first report to demonstrate that the color contrast between a malignant lesion and its surrounding mucosa is more important than high resolution images when screening for EGCs. These results show both ultrathin LCI and standard LCI improve the ability to detect EGCs compared with ultrathin WLI and standard WLI, respectively. Ultrathin LCI had a higher diagnostic sensitivity, significantly higher visibility scores and color difference than standard WLI. This suggests that color contrast is more important than resolution for the identification of EGC. The introduction of ultrathin LCI seems to be suitable for EGC screening in clinical practice including routine health examinations.

Ultrathin endoscopy is generally considered to yield low resolution images compared with standard endoscopy. Our test of resolution using industry standard testing protocols showed that ultrathin endoscopy results in images with a lower resolution at a distant view. However,

the sensitivity for the detection of EGCs was highest using standard LCI, followed by ultrathin LCI, standard WLI, and ultrathin WLI. This order implies that endoscopists are aware of the color contrast between malignant lesions and the surrounding mucosa as previously reported using ultrathin endoscopy with flexible spectral imaging color enhancement (FICE) [62] [63]. LCI accelerates the ability for the early detection of gastric cancers, with the superiority of ultrathin LCI compared to standard WLI.

The specificity of LCI was lower than WLI both with ultrathin and standard endoscopes. Most non-malignant gastric lesions such as intestinal metaplasia, erosions and regenerative epithelium exhibit mucosal changes with lower color contrast to the surrounding mucosa on WLI, but with high color contrast on LCI, which may result in lower specificity of LCI compared with WLI. Using LCI, suspicious lesions may increase but blue light imaging (BLI) allows endoscopists to differentiate the malignant lesion due to better visualization of surface patterns even without magnification. The final diagnosis is made by target biopsy. In our experience, we use LCI in routine clinical practice as the optimal mode for detection of EGC, but not as the final endoscopic diagnosis tool.

Older age groups have a high risk for gastric cancer even after *H. pylori* eradication due to atrophy and intestinal metaplasia in the background mucosa [27]. However, establishing this diagnosis is challenging due to non-neoplastic epithelium covering the malignant tissue which makes the cancer border indistinct and diminishes the obvious characteristics of cancer[29]. The current data shows that color differences between malignant lesions and the surrounding mucosa of EGC is significantly higher with ultrathin LCI than standard WLI regardless of *H. pylori* infection status. Ultrathin endoscopy reduces pain and panic during the procedure and is advantageous especially for elderly patients and/or high-risk with cardiopulmonary dysfunction [39, 40]. Together with the previously reported superiority of LCI for screening

in the upper gastrointestinal tract [24], ultrathin LCI can be suggested as the first choice for gastric screening in such patients.

Ultrathin endoscopy has been shown to result in poor visibility of malignant lesions in the proximal stomach using a xenon endoscope [42] but not using laser endoscopy, although it has good visibility around the lesser curvature of the angle [64, 65]. In this study, ultrathin LCI showed high visibility scores and significantly higher color differences in the proximal stomach compared with standard WLI. Of five malignant lesions in the lesser curvature near the angle, at least two assessors missed malignant lesions using standard WLI whereas all assessors identified all lesions with ultrathin LCI. Ultrathin endoscopy has advantages such as allowing direct visualization of these areas due to a shorter radius at the tip and has the potential to observe the entire stomach with fewer blind spots. However, all assessors identified all these lesions even with standard LCI endoscopy, which may suggest the true efficacy of LCI rather than the physical flexibility of the ultrathin endoscope. Additional studies are necessary to conclusively evaluate this matter.

We have previously reported that LCI provides images with high color contrast to the surrounding mucosa for EGC [30]. LCI increased the a* value in the red-green component and/or b* values in the yellow-blue component in the color space when evaluating color differences between malignant lesions and the surrounding mucosa [30, 53]. In this study, most malignant lesions were surrounded by purple mucosa only in a partial circumferential area (or not at all). The malignant areas and surrounding mucosa were mostly orange-red and light orange, respectively, resulting in the possibility to influence a* value in the red-green component rather than b* values related to purple.

Recent advances in endoscopic treatment such as endoscopic submucosal dissection improves the prognosis of patients with EGCs and allows patients to maintain a high quality of life after therapy. It is beneficial to detect cancers when they are as small as possible to

allow the use of endoscopic therapy. The current study showed that the color difference with ultrathin LCI is higher than with standard WLI even with lesions at a diameter $\leq$10mm, suggesting that the advantage was found regardless of lesion size.

This study has several acknowledged limitations. First, this is a single-center study with a small number of assessors. Second, the evaluated videos may not be representative of live endoscopic screening for gastric cancer. Third, the endoscopists who performed EGC to create the videos were not blinded to patient data or the type of endoscopes. Multicenter prospective clinical trials are needed to confirm these results. Fourth, diagnosing EGC in 90 seconds is challenging and thus only expert endoscopists participated.

6 CONCLUSIONS

Detecting gastric cancer earlier saves many lives, missing might lead to surgeries, chemotherapies and even death. Current studies were able to demonstrate the superiority of LCI for early detection of gastric cancer with both subjective and objective methods using large number of EGC cases.

Study I showed that LCI, the color contrast is an important factor to improve endoscopic visibility of obscure EGCs, which may explain the improved detection rate of EGC and the decreased miss rate previously reported.

Study II showed that LCI facilitates the early detection of gastric cancers by providing high color contrast to the surrounding mucosa with either a standard or ultrathin endoscope. LCI with a low-resolution ultrathin endoscope is superior to WLI with a high-resolution standard endoscope for gastric cancer screening. This suggests that the color contrast between malignant lesions and the surrounding mucosa is more important than high resolution imaging.

Both studies proved the crucial role of high color contrast between a lesion and surrounding normal mucosa for detecting EGC. In conclusion, LCI is strongly expected to become an IEE useful in screening for gastric cancer in clinical practice and shall be recommended in the future guidelines.

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