ANTICANCER RESEARCH *39*: 2519-2525 (2019)
doi:10.21873/anticanres.13373

The Utility of Optical Instrument "ORALOOK®" in the Early Detection of High-risk Oral Mucosal Lesions

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Abstract. *Background/Aim: Oral cancer screening is important for early detection and early treatment, which help improve survival rates. Biopsy is invasive and painful, while fluorescence visualization is non-invasive, convenient, and real-time, and examinations can be repeated using optical instruments. The purpose of this study was to clarify the usefulness of an optical instrument in oral screening. Patients and Methods: A total of 201 patients, who were examined using an optical instrument in our Department between 2017 and 2018, were enrolled in this study. Fluorescence visualization images were analyzed using subjective and objective evaluations. Results: Subjective evaluations for detecting oral cancer and oral epithelial dysplasia offered 83.3% sensitivity and 75.7% specificity. Regarding the objective evaluations for detecting oral cancer and oral epithelial dysplasia, sensitivity and specificity were 47.4% and 72.4% for luminance value, 94.7% and 79.6% for luminance ratio, and 100.0% and 68.0% coefficient of variation. Conclusion: Fluorescence visualization using optical instruments is useful for oral cancer screening.*

Oral and pharyngeal cancers represent a global health challenge, with an estimated incidence of about 660,000 newly diagnosed and 330,000 fatal cases in recently estimated annual rates worldwide (1). More than 90% of oral cancers in Japan are oral squamous cell carcinomas (SCCs) (2). Delayed diagnosis accounts for poor quality of life and high mortality rate; since nearly half of SCC cases are at an advanced stage at the time of initial diagnosis (3). The 5-year overall survival rate is 40-60% for cases detected at an advanced stage. Early detection of SCC improves morbidity-accompanying treatment and 5-year overall survival rate (4). Therefore, early detection and early treatment are crucial to help improve the survival rate of SCC (3, 4).

SCC may develop from oral potentially malignant disorder (OPMD), such as leuko- or erythroplakia or oral lichen planus (OLP). Prevalence of OPMDs ranges from 1-5% worldwide (5). It has been reported that early detection and management of oral epithelial dysplasia (OED) in OPMD is an important preventative step against malignant transformation (6). It seems, therefore, reasonable that early evaluation of OPMDs can have a dramatic impact on oral cancer mortality rates.

Although surgical biopsy is regarded as the golden standard, this process is invasive, time-consuming and painful (7). Various methods for diagnosing oral cancer have emerged (8). Screening for OSCC can involve cytology (9), vital staining (10, 11), and fluorescence visualization (FV) (12). FV is non-invasive, convenient, and real-time, and the examinations can be repeated using optical instruments (OI) (12, 13). More specifically FV uses blue light (400-460 nm) to visualize collagen cross-links (CCL) or flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH) (13). A selective filter allows the viewer to directly visualize the apple-green autofluorescence emitted from normal tissue (FV retention, FVR). On the other hand, abnormal tissues such as those seen in OSCC, OED, and inflammatory diseases exhibit decreased autofluorescence and appear as dark-brown areas in comparison with the green surrounding normal tissue. Such dark-brown areas are referred to as FV loss (FVL) (12). FVL is caused by the absorption of a specific wavelength of blue light due to the breakdown of CCL and decreases in FAD and NADH, and angiogenesis. However, the evaluation of FV has been visual and subjective. Therefore, definitive results have been lacking (12, 13).

The purpose of this study was to clarify the utility of subjective and objective evaluations in the discrimination of high-risk oral mucosal lesions (HRL) from other lesions using OI for oral screening.

Patients and Methods

Patients. A total of 201 patients were enrolled by the Department of Oral and Maxillofacial Surgery at Tokyo Dental College from March 2017 to February 2018. The inclusion criteria were: 1) examined using OI before treatment, 2) provided informed consent

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Key Words: Oral squamous cell carcinoma, oral epithelial dysplasia, oral potentially malignant disorder, optical instrument, fluorescence visualization loss.

to participate in this study, 3) confirmed diagnosis was obtained by biopsy, except for stomatitis and normal tissue. All patients with OSCC were staged using the 8th edition of the TNM staging system by the Union for International Cancer Control (UICC) (7). HRL cases were classified as SCC and OED, and comparisons were made between HRL and other diseases (Others). The study was approved by the institutional review board and performed in accordance with the requirements of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). This study was approved by the Tokyo Dental College Ethics Committee (Authorization Number 740).

Evaluation. As examination protocol, the OI used in this study was ORALOOK® (HITS PLAN, Tokyo, Japan). The OI allowed FV images to be taken and saved to the camera as digital data. FV images of the lesion can be observed in real time because the OI have their own monitor. These lightweight instruments can be used with one hand. FV images were taken in a darkened room as much as possible, with a distance between the lesion and OI of about 10 cm. The irradiating light was set perpendicular to the lesion and the irradiation range was set to about 10 cm (14).

In subjective evaluations, oral photographs and FV images were compared to evaluate FVR or FVL in lesions (Figure 1A and B). FV images were analyzed using Image J software version 1.5 (National Institutes of Health, Bethesda, Maryland, USA). A region of interest (ROI) for the lesion and control area was established in FV images (14, 15).

The ROI of the lesion was defined as the area of FVL. The control ROI was set in a same sub-site of the oral cavity in normal mucosa in front of the lesion without FVL (Figure 1C). The state of the lesion ROI was expressed as a surface plot. In addition, color mapping was performed for the lesion ROI as a surface plot (Figure 1D). The surface plot was used as a reference for subject evaluation (14, 15).

In objective evaluations, mean area of the ROI (measured in pixels), luminance value (LV), standard deviation of luminance (SD), coefficient of variation of luminance (CV), and luminance ratio (LRatio) were calculated. CV was defined as the ratio of the SD to mean luminance. LRatio was defined as the ratio of the luminance of the lesion ROI to the luminance of the control ROI: [LRatio=(ROI of lesion/ROI of control) ×100%] (14, 15).

Statistical analysis. For differential diagnosis of oral mucosal diseases, the Chi-square test, Mann-Whitney U test, and Fisher's exact test were used as statistical analyses. Cut-off values were set using a receiver operating characteristic (ROC) curve for detecting HRL. Area under the curve (AUC), sensitivity and specificity, accuracy were calculated by ROC analysis. And multivariate analysis was performed using the logistic regression analysis. Forward stepwise algorithms were used, with the rejection of variables that did not show a relevant fit to the model. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. All statistical analyses were performed using SPSS version 25.0 (IBM, Tokyo, Japan). Values of $p<0.05$ were deemed statistically significant.

Table I. *Patient characteristics*.

	(n=201)
Gender	
Male/Female	113/88
Age, mean, (range)	65.9 (28-91)
Lesion site	
Tongue	94
Buccal mucosa	51
Lower gingiva	24
Upper gingiva	32
Disease	
SCC	20
OED	4
Leukoplakia (without OED)	21
Oral lichen planus	36
Stomatitis	15
Benign tumor	12
Hemangioma	5
Other disease	26
Normal mucosa	62

SCC, Squamous cell carcinoma; OED, oral epithelial dysplasia.

Table II. *Deafferentation of HRL and others*.

	HRL (n=24)	Others (n=177)
Gender, male/female	13/11	100/77
Age, mean, (range)	66.1 (36-88)	65.8 (28-91)
Lesion site		
Tongue	16	78
Buccal mucosa	2	22
Lower gingiva	1	50
Upper gingiva	4	28
Subject evaluation		
FVR/FVL, (FVL rate, %)	3/21 (83.3)	134/43 (24.3)
Object evaluation, mean, (range)		
Area, pixels	567,691 (77,582-1732,183)	365,108 (13,956-246,686)
LV, cd/m^2	66.7 (31.9-94.5)	78.6 (35.6-130.2)
SD	15.8 (8.7-37.8)	5.9 (2.1-10.5)
CV	0.23 (0.11-0.28)	0.08 (0.04-0.21)
LRatio, %	69.0 (41.0-98.5)	94.8 (53.6-136.5)

HRL, High risk lesion; FVR, fluorescence visualization retention; FVL, fluorescence visualization loss; LV, luminance value; SD, standard deviation of luminance; CV, coefficient of variation of luminance; LRatio, luminance ratio.

Results

Patient characteristics are shown in Table I. There were 113 male and 88 female patients. Mean age was 65.9 years (range=28-91 years). The most common lesion site was tongue (n=94), followed by buccal mucosa (n=51), lower gingiva (n=24), and upper gingiva (n=32 patients). As for oral mucosa disease and condition, 20 patients had SCC, 4 OED, 21 leukoplakia without OED, 36 OLP, 15 stomatitis, 12 benign tumor, 5 hemangioma, 62 normal mucosa, and

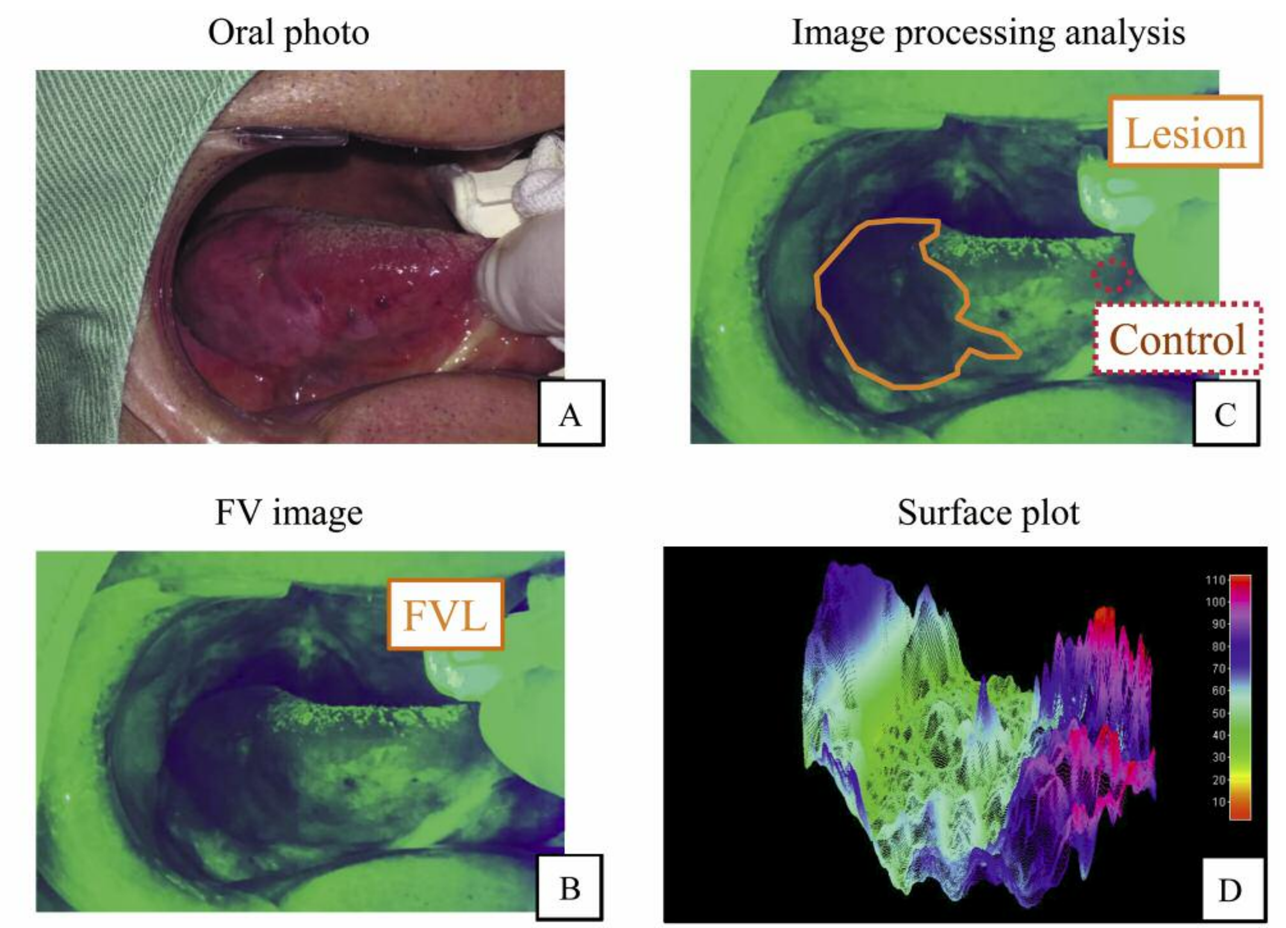


Figure 1. *In subjective evaluations, oral photographs and Fluorescence visualization (FV) images are compared (A), to evaluate FV retention or FV loss (FVL) in lesions (B). The region of interest (ROI) of the lesion is defined as the area of FVL. The control ROI is set in a sub-site of the same oral cavity in the normal mucosa in front of the lesion and without FVL (C). The state of the lesion ROI is expressed as a surface plot (D).*

26 other diseases. Eleven patients had stage I disease and 9 had stage II. The mean pathological depth was 2.3 mm (range=0.5-5.0). OED low- and high grade were 1 and 3. Therefore, HRL were 24, Others were 177 patients.

Typical cases with SCC are shown in Figure 2. The representative case of SCC involved a 65-years-old female with leukoplakia lesion on the right tongue. In clinical finding, non-uniform leukoplakia lesion on right tongue was observed, it wasn't palpated the surrounding induration on lesion. On surface plot, it showed a reduction in non-uniform and uneven luminance. In FV images, non-uniform FVL was observed, and the boundaries of FVL were unclear. In objective evaluation, area was 200,306 pixels, LV was 60.5 cd/m^2, SD was 16.6, and CV was 0.27, and LRatio was 62.7%.

In subject evaluations, FVL rates for HRL and Others were 83.3% and 24.3% (p<0.001). Subjective evaluation for detecting HRL provided 83.3% sensitivity and 75.7% specificity, and 77.1% accuracy.

In objective evaluation, mean area for HRL and Others were 567,691 pixels (range=77,582-1732,183) and 365,108 pixels (range=13,956-246,686) (p=0.045), mean LV were 66.7 (range=31.9-94.5) and 78.6 cd/m^2 (range=35.6-130.2) (p=0.042), mean SD were 15.8 (range=8.7-37.8) and 5.9 (range=2.1-10.5) (p<0.001), and mean CV were 0.23 (range=0.11-0.28) and 0.08 (range=0.04-0.21) (p<0.001), LRatio were 69.0% (range=41.0-98.5) and 94.8% (range=53.6-136.5) (p<0.001).

Objective evaluations for detection of HRL by ROC analysis are shown in Figure 3. AUC was 0.621 for LV, 0.875 for LRatio, 0.848 for SD, and 0.894 for CV. Cut-off values were set at 60 cd/m^2 for LV, 82.8% for LRatio, 7.8 for SD, and CV for 0.123. Sensitivity and specificity, accuracy were 47.4% and 72.4%, 69.2% for LV, 94.7% and 79.6%, 82.1% for LRatio, 84.2% and 71.3%, 74.1% for SD, and 100.0% and 68.0%, 73.6% for CV, respectively.

Multivariate analysis showed that LV (p=0.038, OR=1.043, 95% CI=1.231-1.995), CV (p=0.011, OR=1.900,

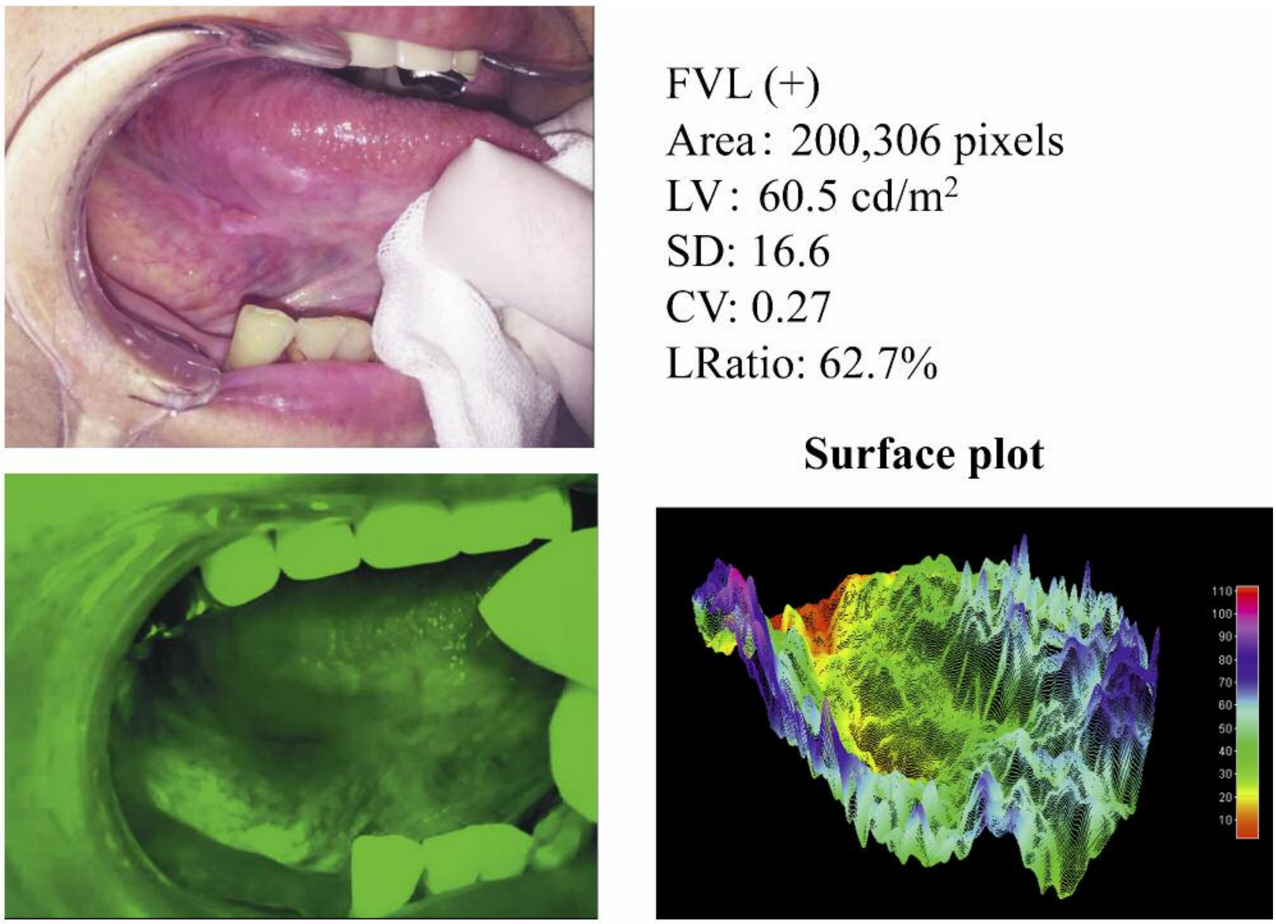


Figure 2. *The case of squamous cell carcinoma (SCC) of a 65-years-old female with leukoplakia lesion on the right tongue. In clinical finding, non-uniform leukoplakia lesion on right tongue is observed. On surface plot, a reduction in non-uniform and uneven luminance is shown. In FV images, non-uniform FVL is observed. In objective evaluation, area is 200,306 pixels, luminance value (LV) is 60.5 cd/m^2, standard deviation of luminance (SD) is 16.6, and coefficient of variation of luminance (CV) is 0.27, and luminance ratio (LRatio) is 62.7%.*

95% CI=1.420-9.110), LRatio (p=0.017, OR=1.753, 95% CI=1.719-7.991) were factors for detecting HRL (Table III).

Discussion

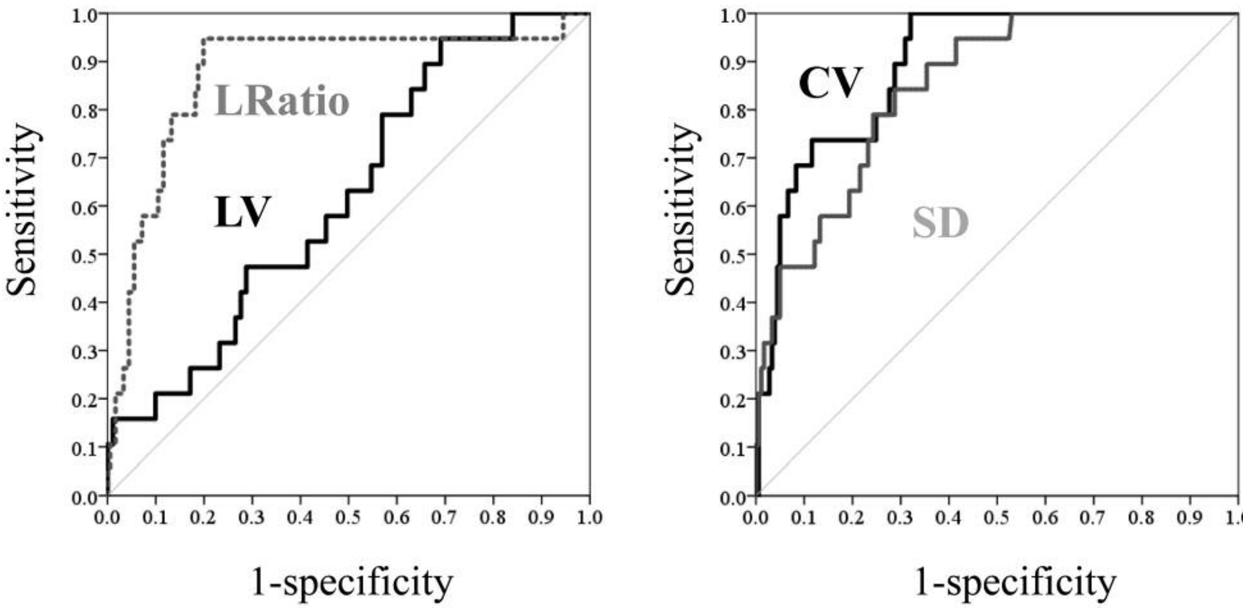
Oral screening is central to the discovery of SCC and OED because the oral cavity is readily accessible for ocular inspection and palpation (16). One major limitation is the difficulty in distinguishing between benign and high-risk lesions by general dentists, since early-stage SCC and OED may not present with typical features, and a wide variety of oral mucosal diseases can present in various ways in the oral cavity (8).

Screening should be minimally invasive, repeatable and inexpensive. Cytology (9), vital staining (10, 11), and FV (12-15) are simple and effective as methods of screening for HRL. Oral brush cytology (OBC) is a well-tolerated,

Table III. *Results of univariate and multivariate analysis.*

	Univariate	Multivariate		
	p-Value	*p*-Value	OR	95%CI
Gender, male/female	0.885			
Age, mean	0.924			
Lesion site,	0.170			
Subject evaluation	<0.001	0.470		
Object evaluation				
Area	0.045	0.056		
LV	0.042	0.038	1.043	1.231, 1.995
SD	<0.001	0.082		
CV	<0.001	0.011	1.900	1.420, 9.110
LRatio	<0.001	0.017	1.753	1.719, 7.993

OR, Odds ratio; CI, confidence interval; LV, luminance value; SD, standard deviation of luminance; CV, coefficient of variation of luminance; LRatio, luminance ratio.



	AUC	*p*-value	Cut-off	Sensitivity	Specificity	Accuracy
LV	0.621	0.044	60	47.4	72.4	69.2
LRatio	0.875	< 0.001	82.8%	94.7	79.6	82.1
SD	0.848	< 0.001	7.8	84.2	71.3	74.1
CV	0.894	< 0.001	0.123	100.0	68.0	73.6

Figure 3. *Cut-off values were set using a receiver operating characteristic (ROC) curve for detecting High risk lesion (HRL). Area under the curve (AUC), sensitivity and specificity, accuracy were calculated by ROC analysis. AUC for luminance value (LV) was 0.621, 0.875 for luminance ratio (LRatio), 0.848 for standard deviation of luminance (SD), and 0.894 for variation of luminance (CV).*

mildly invasive, safe approach for harvesting cells from the oral mucosa (9). This method offers 60-100% sensitivity and 23.5-100% specificity, while the results are obtained within several days (9). Vital staining with iodine solution (IS) in the oral cavity allows an easy observation of the results in real time. With OSCC or low- and high-grade OED, little glycogen is present in the granule cell layer, resulting in a relative lack of iodine staining. However, IS is mildly invasive and it cannot be used for patients with iodine allergy (10). In addition, the adaptation site is limited to the movable mucosa (11), and the method offers 71.0-87.5% sensitivity and 84.2% specificity (11, 17, 18).

FV with OI is non-invasive, convenient, real-time, and examinations can be repeated (12, 13). ORALOOK® is a lightweight and simple instrument; it has a built-in filter, while FV images and oral photographs are captured using one unit. This OI irradiates the target with blue light at an excitation wavelength of about 410 nm, while it can detect only apple-green fluorescence *via* a filter (520-560 nm). Hence, ORALOOK® was selected in this study because it is managed as a single green fluorescence color and image processing analysis is simple. Subjective evaluation for the detection of HRL showed a high sensitivity and specificity in this study. The effectiveness of such evaluations in HRL screening has also been reported in previous studies (12, 13). However, visual evaluation for oral cancer screening is difficult since FVL is caused also in inflammatory diseases, such as OLP and stomatitis (15). The past evaluation of FV was subjective, varying depending on the report (12), therefore we considered necessary to use a new objective evaluation method.

On the other hand, concerning objective evaluation, LV was significantly lower in HRL than in Others according to multivariate analysis. In this study, although the protocol was kept constant, some degree of influence from environmental factors cannot be ruled out (14). However, using LRatio, the influence of environmental factors was reduced; thus, HRL was significantly lower than Others by multivariate analysis on LRatio.

Furthermore, SD and CV were used for the evaluation of internal properties. Particularly with CV, Others showed constant values, whereas fluctuations in HRL were significantly greater. Also, in surface plots, SCC showed a less uniform condition compared to Others. In recent years, SCC has been reported to show intratumoral heterogeneity (19), suggesting that CV is indicative of intratumoral heterogeneity (14). Each objective evaluation showed significant results, suggesting their effectiveness. Semi-quantitative methods can eliminate differences between institutions and can facilitate a more uniform medical treatment (14, 15).

This study had several limitations. First, it was retrospective and was conducted in a single institution with a limited number of cases. Moreover, there was no comparison with other screening methods; thus, a future prospective study is planned. Furthermore, we are planning to consider screening of HRL compared to screening methods.

In our Department, we introduced the Oral Cancer Navigation System (Navi-System) to Japan in 2012. This Navi-System allows medical cooperation *via* the Internet (20). Also, in recent years, many reports have described the potential application of artificial intelligence (AI) to medical fields (21). We are considering next-generation oral cancer screening with medical AI by accumulating cases and using objective evaluations in the future.

This study revealed the utility of objective evaluation in oral cancer screening using OI, suggesting that future research could focus on the development of a HRL screening system using OI combined with medical AI.

Conflicts of Interest

The Authors report no conflicts of interest.

Authors' Contributions

Morikawa T. conceived and planned the study and wrote the manuscript. Kosugi A. carried out the analysis. Shibahara T. contributed to this study as professor of Tokyo Dental college.

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Received March 11, 2019
Revised April 15, 2019
Accepted April 18, 2019