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Usefulness of objective evaluations by fluorescence visualization device for distinguishing between superficial Oral Squamous Cell Carcinoma and Oral Lichen Planus

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Abstract

Objective: There are various diseases in the oral mucosa, which makes diagnosis difficult. Fluorescence visualization (FV) device are non-invasive for oral squamous cell carcinoma (OSCC) screening. Although its usefulness has been reported in subject evaluation. In this study, it was aimed to clarify a utility as the screening of the oral mucosal disease using FV device in subject and objective evaluations.

Patients and methods: Fifty patients were selected with superficial OSCC or oral lichen planus (OLP) at Tokyo Dental College between April 2016 and March 2018. Subject evaluation was discriminated FV Retention or FV loss (FVL), objective evaluation was calculated a luminance value and luminance rate [LR: (luminance of the lesion/control)], a border change rete (BCR) using image processing software, and calculated AUC and sensitivity, specificity using receiver operating characteristic (ROC) analysis.

Results: In subjective evaluation, FVL was observed in all cases (p = 1.000). In objective evaluation, luminance of OSCC was higher than OLP, but it was not significant (p = 0.08). LR of OSCC was significantly higher than OLP (p = 0.00002). And BCR of OSCC was significantly lower than OLP (p = 0.000001). The results of ROC analysis were Luminance value and LR, BCR; AUC were 0.613 and 0.864, 0.913,: cut off value were 97.0 and 69.0, 27.2,: sensitivity were 85.0% and 90.0%, 85.5%,: specificity were 36.7% and 70.0%, 93.3%.

Conclusions: It was suggested that performing object evaluations such as LR and BCR, by using the FV device are useful for discriminating between OSCC and OLP.

Keyword:

fluorescence visualization device, autofluorescence, oral potentially malignant disorders, oral squamous cell carcinoma, oral lichen planus

1. Introduction

Oral squamous cell carcinoma (OSCC) is a major health problem worldwide, with 300,373 new cases and 145,000 deaths annually [1]. Delated diagnosis accounts for the high morbidity and mortality, since half of OSCC cases are advanced at the time of initial diagnosis, resulting in dysfunction and poor quality of life [2]. Early detection and early treatment of OSCC are very important for improving prognosis. Various oral mucosal diseases, such as oral potentially malignant disorders (OPMD) and OSCC, along with other mucosal diseases, can develop in the oral cavity, and diagnosis can thus be difficult. While the clinical presentations might show some similarities, OPMD more often develops into OSCC. Oral lichen planus (OLP) is one of the major diseases comprising OPMD, and correct diagnosis is important because the malignant transformation rate of OLP had been reported to be 0 to 3.5% [3]. Biopsy is required to obtain a definitive diagnosis, but is surgically invasive. Screenings for OSCC include vital staining, cytology, and fluorescence visualization (FV). Vital staining with iodine solution (IS) in the oral cavity is easily observed and real-time. With OSCC, and low- and high-grade oral epithelial dysplasia (OED), glycogen in the granule cell layer is low, and is unstained by iodine. However, IS cannot be used in patients with iodine allergy [4]. IS is useful for movable mucosa such as the tongue, oral floor, buccal mucosa, and soft palate, but is difficult to use on keratinized mucosa such as the gingiva and hard palate [5]. Cytology is easy and simple, but is somewhat invasive and sufficient specimens cannot be

collected from keratinized lesions or necrotic tissues [6].

FV devices have been reported as useful in OSCC screening [7]. se of such devices is non-invasive, and examinations can be repeated. FV uses a blue light (wavelength, 400-460 nm) to illuminate collagen cross-links (CCLs) or flavin adenine dinucleotides (FADs). A selective filter allows the viewer to directly visualize the apple-green autofluorescence given off by normal tissue, defined as FV retention (FVR). On the other hand, abnormal tissues such as OSCC and OED, and inflammatory diseases, show decreased autofluorescence and appear as dark-brown areas in comparison with the surrounding green normal tissues. This dark-brown area is called FV loss (FVL) [8] and is caused by absorption of a specific blue light due to the breakdown of CCLs and decreases in FADs and hemoglobin. However, evaluation of FV is visual and subjective. Outcomes have thus differed depending on the report [8 - 14]. In this study, we conducted subjective and objective evaluations using an FV device to distinguish between OSCC and OLP.

2. Patients and Methods

2.1. *Patients*

A total 50 patients (30 patients with superficial OSCC, 20 patients with OLP) were selected by the Department of Oral Medicine, Oral and Maxillofacial Surgery and Oral Cancer Center at Tokyo Dental College from April 2016 to March 2018. All patients

underwent pathological diagnoses. Clinical appearance types followed the General Rules for Clinical and Pathological Studies on Oral Cancer [15] and the Eisen's report in OLP [16]. Patients with OSCC were re-staged using the Union for International Cancer Control (UICC) 8 th edition of the TNM staging system [17].

2.2. *Observation protocol*

The FV device used was an Illumscan® (SHOFU, Kyoto, Japan). This device takes FV images and saves the digital data to the camera. The observation methods of this study are shown.; At first, with full observations of the oral cavity performed in a state of natural light.; Next, with the device positioned 10 cm from the lesion to irradiate it perpendicularly in a room darkened as much as possible [18].

2.3. *Subject and objective evaluation*

Subject evaluation discriminated between FVR and FVL (Fig.1A). Regions of interest (ROI) in the lesion area and control area were set on the FV image using image processing software version 006 (SHOFU, Kyoto, Japan) [19]. The ROI of the lesion was defined as the area of FVL. The ROI of the control area was set in the same oral cavity sub-site in the normal mucosa in front of the lesion part on the straight line. For objective evaluation, mean luminance value of the ROI and luminance ratio (LR: luminance of the

lesion/luminance of the control) were calculated (Fig. 1B). Fig 2A was shown the boundary area of FVL. The boundary change rate (BCR) was a vector and can express by the composition of X-axis direction ingredient and the Y-axis direction ingredient, and it is guaranteed that a law of Pythagoras is applicable mathematically. And it is calculated by the following equation. The D in the denominator is the distance that is determined by the software. Since it is set up and down or left and right from the pixel of interest, it is doubled. (Fig. 2B) [20].

$$\text{Luminance change rate} = \frac{(\text{Luminance of Point}) - (\text{Luminance of another point})}{[\text{Spacing of two points (in pixels)}]}$$

$$X = \frac{(Px2)-(Px1)}{2d},\ \mathrm{Y} = \frac{(Py2)-(Py1)}{2d}$$

$$\mathrm{BCR} = \sqrt{X^{2} + Y^{2}}$$

2.4 *Statistical analysis*

Differences between OSCC and OLP were examined using the Mann-Whitney U test. Comparisons of clinical appearance types were examined using the Kruskal-Wallis test. The cut-off value was set using a receiver operating characteristic (ROC) curve. Statistical analysis was performed using EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Values of $p < 0.05$ were considered to indicate a significant difference.

3. Result

3.1. *Patients with superficial OSCC*

Characteristics of all patients are shown in Table 1. Mean age of patients with superficial OSCC was 57 years (range, 35 - 88 years). There were 16 males and 14 females. The lesion site was the tongue in 27 patients, and the buccal mucosa in 3 patients. According to UICC 8 th edition criteria, 17 patients were stage 1, and 13 patients were stage 2. Mean pathological depth was 1.6mm, (range 0.1 - 5.0 mm). In terms of clinical appearance, leukoplakia type was present in 20 patients, ulcerative type in 10 patients, erosive type in 5 patients and granular type in 3 patients. A typical case with OSCC is shown in Fig. 3.

As subjective evaluations, all patients with OSCC showed FVL. In terms of objective evaluation, mean luminance value was 94 cd/m^2 in all OSCC cases (range, 37 - 157 cd/m^2). By clinical appearance type, mean luminance value was 105 cd/m^2 for leukoplakia type (range, 69 - 157 cd/m^2), 73 cd/m^2 for ulcerative type (range, 37 - 92 cd/m^2), 89 cd/m^2 for erosive type (range, 74 - 1299 cd/m^2), and 93 cd/m^2 for granular type (range, 89 - 104 cd/m^2). Significant differences were evident between clinical appearance types in luminance value (p = 0.00294).

Mean LR was 78.1% (range, 37.1 - 113.4%) in all OSCC cases. By clinical appearance type, mean LR was 83.9% for leukoplakia type (range, 58.8 - 111.3%), 67.7% for ulcerative type (range, 37.8 - 93.9%), 74.6% for erosive type (range, 57.1 - 86.7%), and

86.5% for granular type (range, 79.5 - 95.2%). Significant differences were seen between clinical appearance types in LR ($p = 0.04816$).

Mean BCR was 18.4% (range, 12.8 - 36.5%) in all OSCC cases. By clinical appearance types, BCR was 18.4% for leukoplakia type (range, 13.2 - 26.6%), 19.6% for ulcerative type (range, 13.6 - 36.5%), 20.8% for erosive type (range, 14.9 - 29.9%), and 20.7% for granular type (range, 17.6 - 23.5%). No significant differences were seen by clinical appearance type in BCR ($p = 0.726$).

3.2. *Patients with OLP*

Mean age of patients with OLP was 65 years (range, 48 - 74 years). There were 6 males and 14 females. The lesion site was the buccal mucosa in 19 patients, and the tongue in one. Clinical appearance was reticular type in 16 patients, erythematous type in 2 patients, and erosive type in 2 patients. A typical case with OLP is shown in Fig. 4.

As subjective evaluation, all patients with OLP showed FVL. As objective evaluation, mean luminance value was 83 cd/m^2 in all OLP cases (range, 38 - 137 cd/m^2). By clinical appearance, mean luminance value was 85 cd/m^2 for reticular type (range, 38 - 137 cd/m^2), 70 cd/m^2 for erythematous type (range, 63 - 77 cd/m^2), and 81 cd/m^2 for erosive type (range, 68 - 94 cd/m^2). No significant differences by clinical appearance type were identified in luminance value ($p = 0.378$).

Mean LR was 57.8% in all OLP cases (range, 35.8 - 83.1%). Clinical appearance was reticular type in 59.4% (range, 35.8 - 83.2%), erythematous type in 49.0% (range, 47.4 - 50.7%), and erosive type in 54.2% (range, 48.6 - 59.9%). No significant differences were seen by clinical appearance type in LR ($p = 0.187$).

Mean BCR was 32.4% (range, 16.3 - 49.3%) in all OLP cases. In terms of clinical appearance, mean BCR was 32.8% (range, 16.3 - 49.3%) for reticular type, 29.4% (range, 21.1 - 37.7%) for erythematous type, and 32.0% (range, 28.2 - 35.8%) for erosive type. No significant difference was seen by clinical appearance type ($p = 0.928$).

3.3. *Differentiation between OSCC and OLP*

Results of evaluation with FV are shown in Table 2.

FVL was observed in all cases, and differentiation by subjective evaluation was difficult. Sensitivity and specificity of subjective evaluation were 100.0% and 0.0% in this study ($p = 1.000$).

In objective evaluation, mean luminance tended to be higher for OSCC than for OLP, but this difference was not significant ($p = 0.08$). LR of OSCC was significantly higher than OLP ($p = 0.00002$). BCR was significantly lower for OSCC than for OLP ($p = 0.000001$) (Fig. 5).

The results of ROC analysis were showed in Fig 6. AUC of luminance value and LR,

BCR were 0.613 and 0.864, 0.913. Cut-off value of luminance value, LR, and BCR were set at 97.0 cd/m^2, 69.0%, and 27.2%. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 85.0%, 36.7%, 66.8% and 62.0% for luminance value, 90.0%, 70.0%, 81.8% and 82.4% for LR, and 85.5% and 93.3%, 95.0% and 81.1% for BCR, respectively.

4. Discussion

World Health Organization (WHO) classification in 2017 proposed the concept of OPMD, and included leukoplakia, erythroplakia and OLP [17]. OLP are chronic inflammatory disease, and it exacerbates, it is difficult to distinguish it from OSCC. Screening of OSCC is performed using IS, cytology, and FV. Epstein have reported that IS offers 87.5% sensitivity and 84.2% specificity [5]. Cytology is a simple and easy examination, but is mildly invasive [6]. In the conventional Papanicolaou classification, sensitivity has been reported as 80.8%, and specificity as 83.6% [21]. However, screening should be less invasive, repeatable and inexpensive.

On the other hand, the FV device allows non-invasive examinations that can be repeatedly performed. FV can also be applied to the gingiva and palate where IS is difficult. Illumscan® irradiates the target with blue light at an excitation wavelength of about 425 nm, and is a device capable of observing only excited green fluorescence (470 - 580 nm). Because

a filter cutting wavelengths ≤470 nm and ≥580 nm is used, red excited by the porphyrin produced by bacteria of the fur is not observed [14]. Therefore, because the Illumscan® is adaptable to all areas of the oral mucosa, and luminance can be analyzed, this study used it.

The organic information that can be distinguished with the naked eye differs from that obtained using fluorescent wavelengths. This is because normal tissues and diseases each show different absorption and scattering of the light. Typical autologous fluorescence is seen with FAD, CCL, and hemoglobin. FAD is an important cellular metabolic index and an oxidative increase in glucose causes a decrease in FAD. In other words, because metabolism is active in OSCC and OPMD, FAD decreases rather than normal cells and shows decreased intensity of fluorescence [22]. Fluorescent substances are involved in glycolysis, the tricarboxylic acid cycle, and the electron transport chain of cellular metabolism. Especially in OSCC, anaerobic metabolism is known to be enhanced more than aerobic metabolism, representing the Warburg effect. Also, with respect to the CCL present in the interstitium, OSCC is destroyed and fluorescence lost by the reduced cross-linking structure of the cell-binding ability [23]. In inflammatory disease without such destruction, angiogenesis and blood vessel density are increased. Hemoglobin is involved as a factor in the identification of FVL. In inflammatory diseases such as OLP, hemoglobin causes the expansion of blood vessels. Because the blood has a characteristic of absorbing blue light, blue excitation light is strongly absorbed by hemoglobin in the capillary, so the intensity of the excitation light is

weakened, and a dark appearance result [24]. As a result, it was observed as an indirect FVL due to increased absorption.

In this study, we conducted subjective and objective evaluations using an FV device for distinguishing between OSCC and OLP. All cases showed FVL, and differentiation was difficult using subjective evaluations. In the objective evaluation, no significant difference was seen between OSCC and OLP in luminance value, and OSCC was higher than OLP. Observation conditions and environmental factors influenced the luminance value, but these influences can be excluded by taking the ratio compared to the control [15, 25], a and OSCC was significantly higher than OLP in LR. As a reason, OSCC showed uneven luminance and included various types of clinical appearance, such as the leukoplakia type. Conversely, OLP showed uniform declines in luminance. Various studies have reported the utility of using this LR in investigations of leukoplakia [15] and OED [25, 26].

No previous reports appear to have focused on BCR yet. OSCC was significantly lower than OLP in BCR. When a surgeon tries to remove an superficial OSCC, the area of OED is not easily distinguished from the normal-appearing area surrounding the OSCC. Leaving this unresected OED can often lead to local recurrence or second primary malignant tumors. OED surrounding OSCC has been termed "field cancerization" [27]. In OSCC, boundary luminance with FVL and the normal part decreased moderately, and the existence of OED around the cancer was suggested to be involved. This was considered to represent "field

cancerization". Also, as a principle of FV device, OSCC involves several factors such as FAD decrease, CCL destruction, angiogenesis. Whereas OLP is angiogenesis mainly because OLP is chronic inflammation. Therefore, it was considered that irradiation light was absorbed and BCR was increased in OLP. Nomura reported that FV devices can delineate various types of OED, while delineation of surgical margins is the same or more than in IS [28]. One problem with FV is that luminance is easily changed by scan distance, irradiation angle, and differences in natural light. However, using the BCR noted in this study, it is less likely to be affected by the shooting distance and angle. By setting this ratio, the influence of natural light was able to be reduced, and BCR that was not influenced by clinical appearance type proved useful.

Several reports have described screening of OSCC using the FV device, but such evaluation is subjective, and the rate of accurate diagnosis varies. Lane et al. showed 98% sensitivity and 100% specificity when discriminating normal mucosa from OED and OSCC [8]. Rana et al. showed 100% sensitivity and 74% specificity, but noted that FV devices were likely to lead to over-diagnosis [11]. Awan reported a high sensitivity of 84.1% and a low specificity of 15.3% when discriminating benign lesions from OED, and noted that thus was inadequate for early diagnosis [10]. We therefore considered that new methods of objective evaluation are necessary to analyze images. The present study achieved high sensitivity and high specificity with LR and BCR, suggesting these as useful items for screening for OSCC.

In generally, early detection of cancer improves the survival rate [29]. The advantage of FV is the lack of invasiveness and easy repetition. With specific training, clinicians beyond oral surgeons can use these devices. If oral mucosal examinations using an FV device are applied routinely in general dentistry clinics, OSCC and OPMD may be detected early. This modality can be used for regular observation because there is no invasiveness, so the burden on the patient can be greatly improved.

In the future, we need to accumulate more data and investigate the utility of FV. Visualization ability using this analysis should be validated with pathological investigations. Furthermore, we are planning to consider screening of other OPMD, and the effectiveness of treatment on OSCC [30].

5. Conclusion

Objective evaluations such as LR and BCR using the FV device during examinations of the oral mucosa appear useful for discriminating between OSCC and OLP.

Ethical approval

This retrospective study was reviewed and approved by the TDC Ethics Committee (No. 740).

Notes

This study was awarded the excellent poster award at the 63rd Congress of the Japanese Society of Oral and Maxillofacial Surgeons, in November 2018 at Chiba-city, Japan.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig 1. Methods of evaluation on Luminance and LR

Subjective evaluation discriminated FVR or FVL (A). Regions of interest (ROI) in the lesion area (red line) and control area (orange line) are set on the FV image using image processing software. The ROI of the lesion is defined as the area of FVL. The ROI for the control area is set in the same oral cavity sub-site in the normal mucous in front of the lesion part on the straight line (Fig 1B). For objective evaluation, mean luminance of the ROI and luminance ratio [LR: (luminance of the lesion/luminance of the control) are calculated.]

Fig 2. Methods of evaluation on BCR

Fig2A was showed boundary area of FVL. FVL shows a low luminance value. The boundary around the FVR shows the slope by the red line. For FVL, the boundary change rate (BCR) is calculated in the X and Y axis directions at the boundary of the FVL in the following equation from the vector (Fig. 2B).

Fig 3. Ulcerative type of OSCC

The case involved a 72-year-old woman. The right tongue finds tumors with ulcers. On FV images, the boundaries of the FVL are unclear, and the gradient of the boundary is steep. In a subjective evaluation, OSCC shows FVL. In an objective evaluation, the

luminance value is 95 cd/m^2, LR is 84.8%, and BCR is 17.6%.

Fig 4. Reticular type of OLP

This case involved a 46-year-old woman. The left buccal mucosa shows erythema with lace-like vitiligo. In a subjective evaluation, OLP shows FVL. In FV images, the boundaries of the FVL are clear, and the gradient of the boundary is gradual. In an objective evaluation, luminance value is 97 cd/m^2, LR is 56.0%, BCR is 49.5%.

Fig 5. Objective evaluation

With objective evaluations, mean luminance tends to be higher for OSCC than for OLP, but the difference is not significant ($p = 0.08$). LR is significantly higher for OSCC than for OLP ($p = 0.00002$). BCR is significantly lower for OSCC than for OLP ($p = 0.000001$)

Fig 6. ROC analysis

AUCs for luminance value, LR, and BCR were 0.613, 0.864, and 0.913, respectively. Optimal cut-offs for luminance value, LR, and BCR were set at 97.0 cd/m^2, 69.0%, and 27.2%. Sensitivity, specificity, PPV and NPV were 85.0%, 36.7%, 66.8% and 62.0% for luminance value, 90.0%, 70.0%, 81.8% and 82.4% for LR, and 85.5%, 93.3%, 95.0% and 81.1% for BCR, respectively.

Table 1. The patients' characteristics

OSCC is bserved in males and involving the tongue, while OLP is often found in females and involving the buccal mucosa.

Table 2. Subjective and objective evaluation

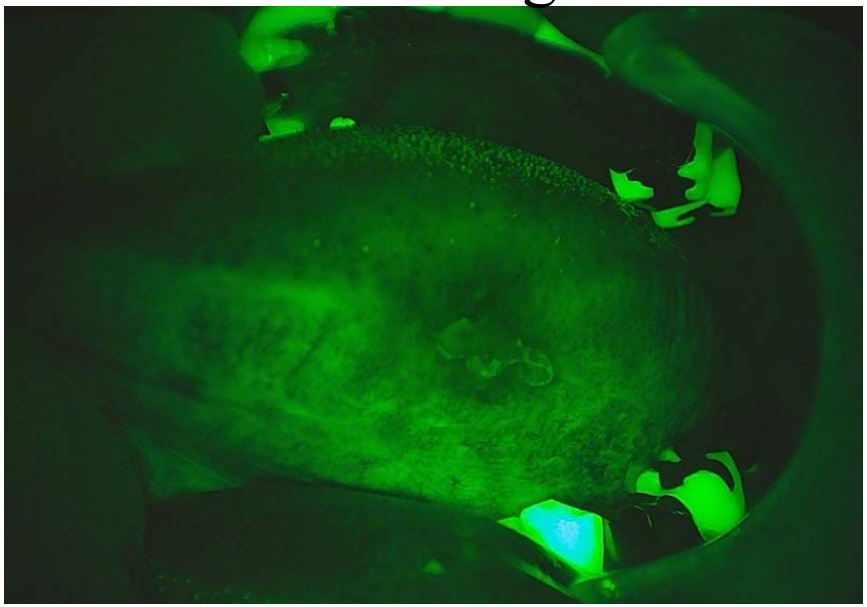
FVL was applied in all cases, and differentiation was difficult using subjective evaluations. In objective evaluations, LR and BCR differed significantly.

Fig 1. Methods of evaluation on Luminance and LR

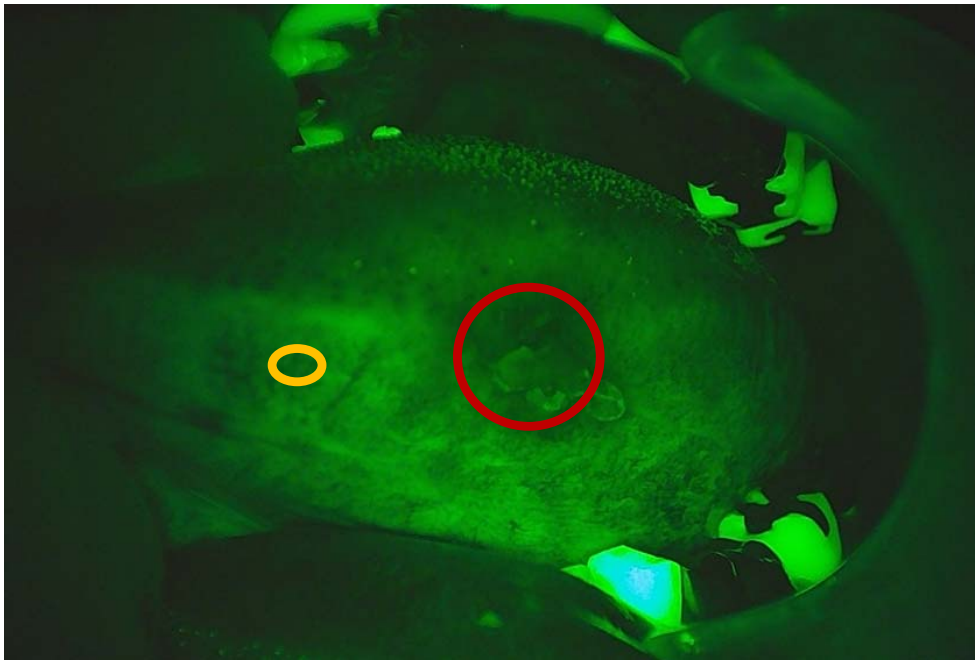
A

Oral Photograph

FV Image



B



lesion **control**

Luminance Ratio (LR)

= Luminance of the lesion / control

Fig 2. Methods of evaluation on BCR

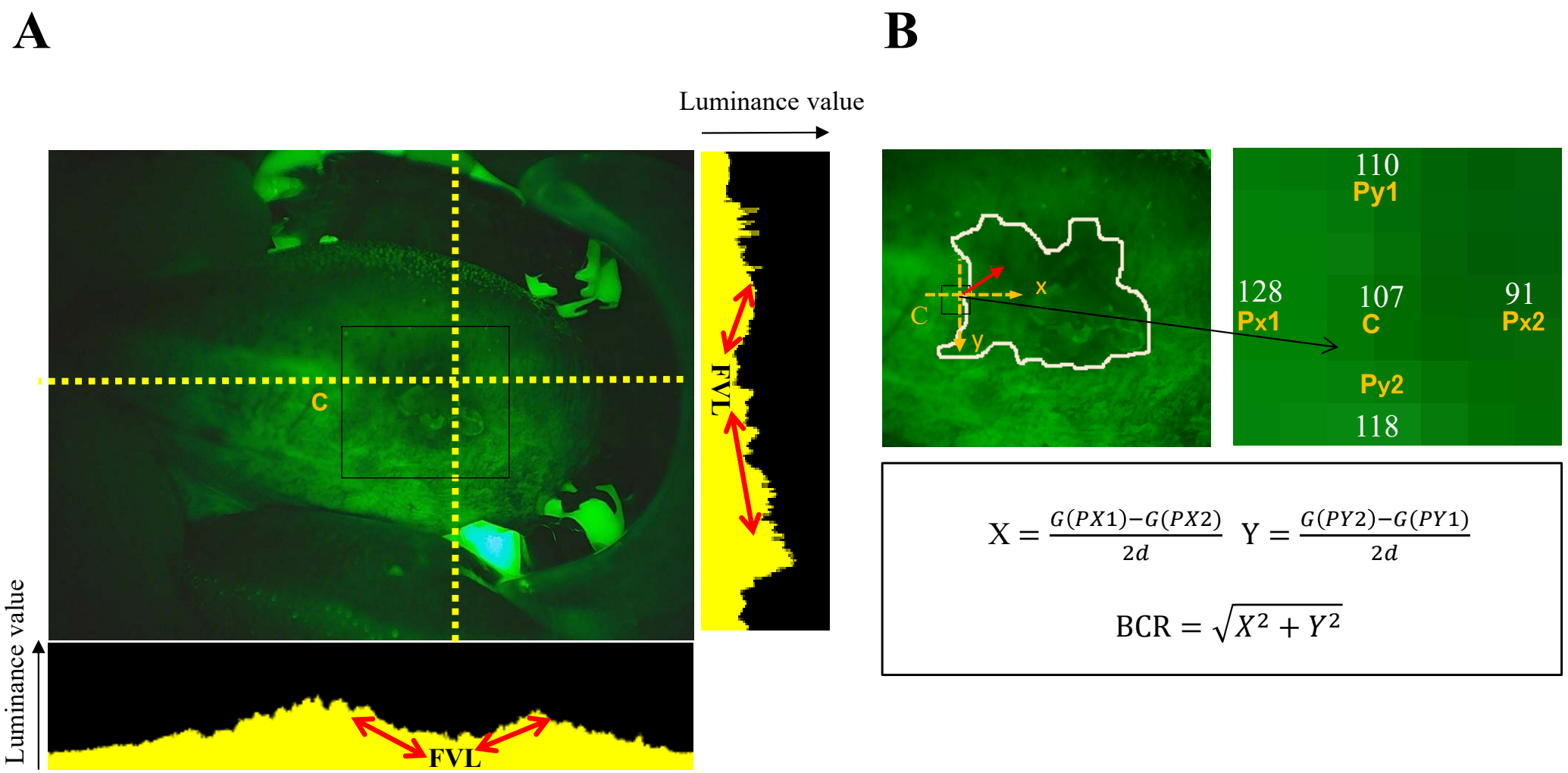


Fig 3. Ulcerative type of OSCC

Subject evaluation:

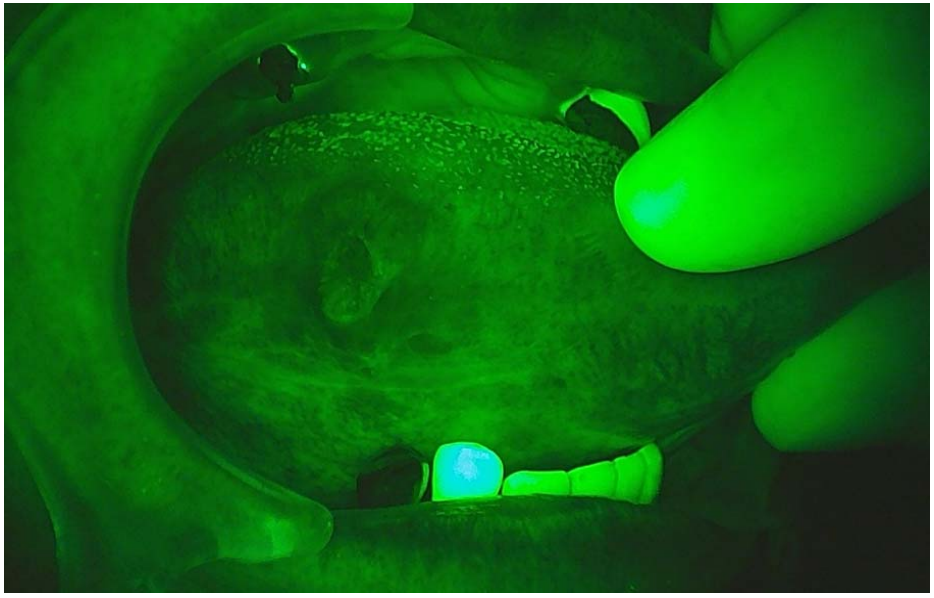
FVL (+)

Object evaluation:

Luminance value: 95 cd / m^2

LR: 84.8%

BCR: 17.6%



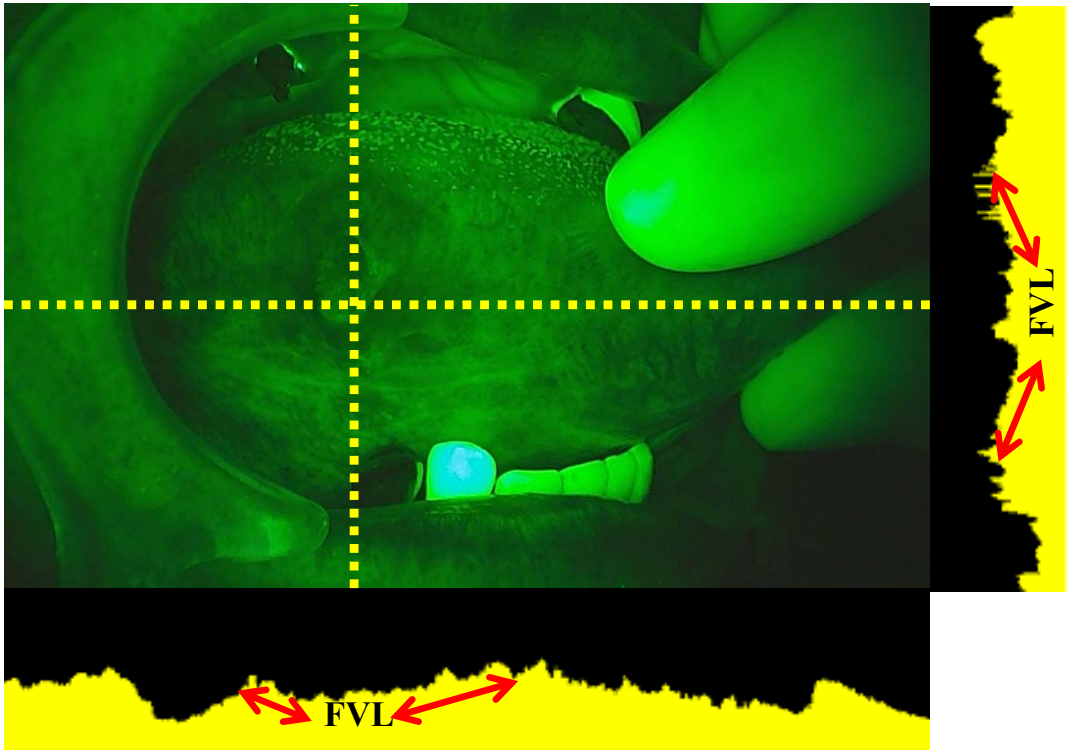


Fig 4. Reticular type of OLP

Subject evaluation:

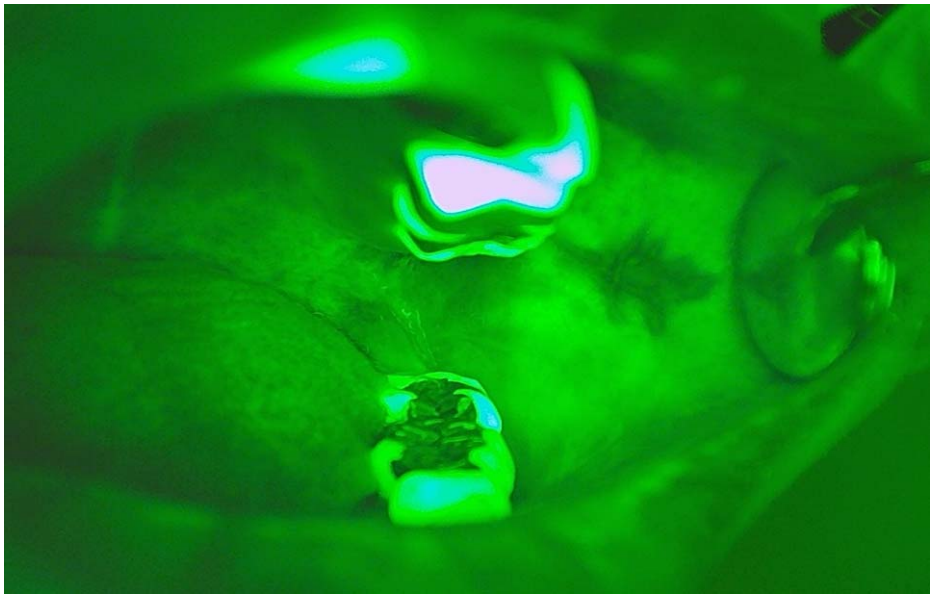
FVL (+)

Object evaluation:

Luminance value: 97 cd / m^2

LR: 56.0%

BCR: 49.5%



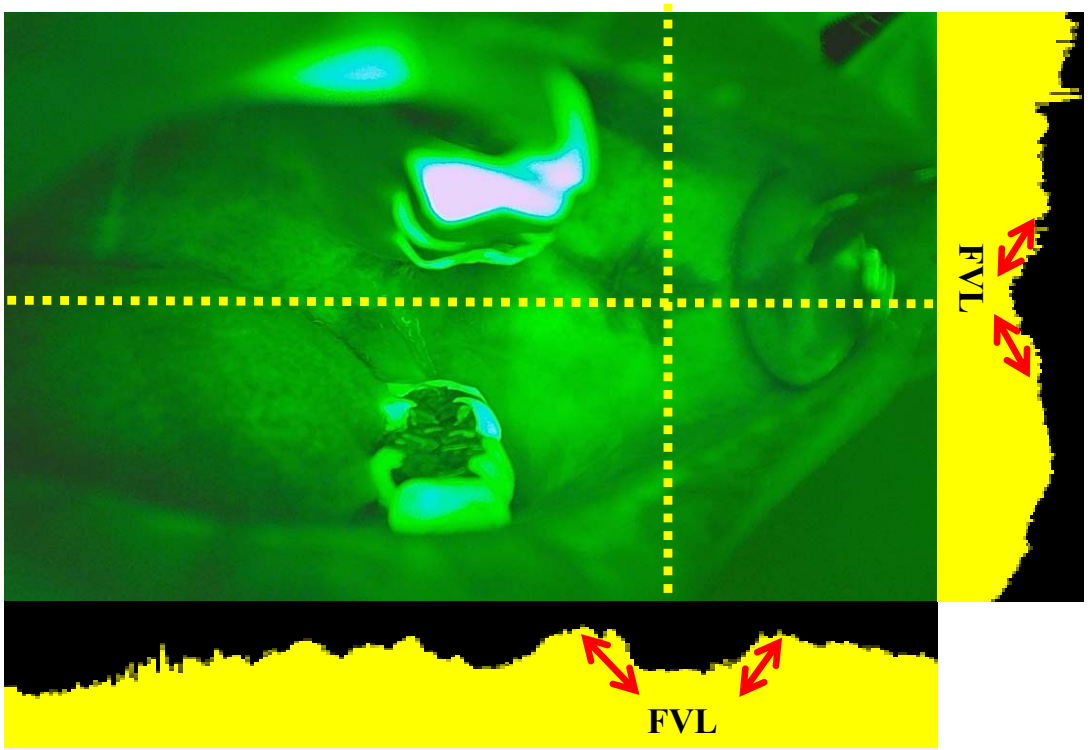


Fig 5. Object evaluation

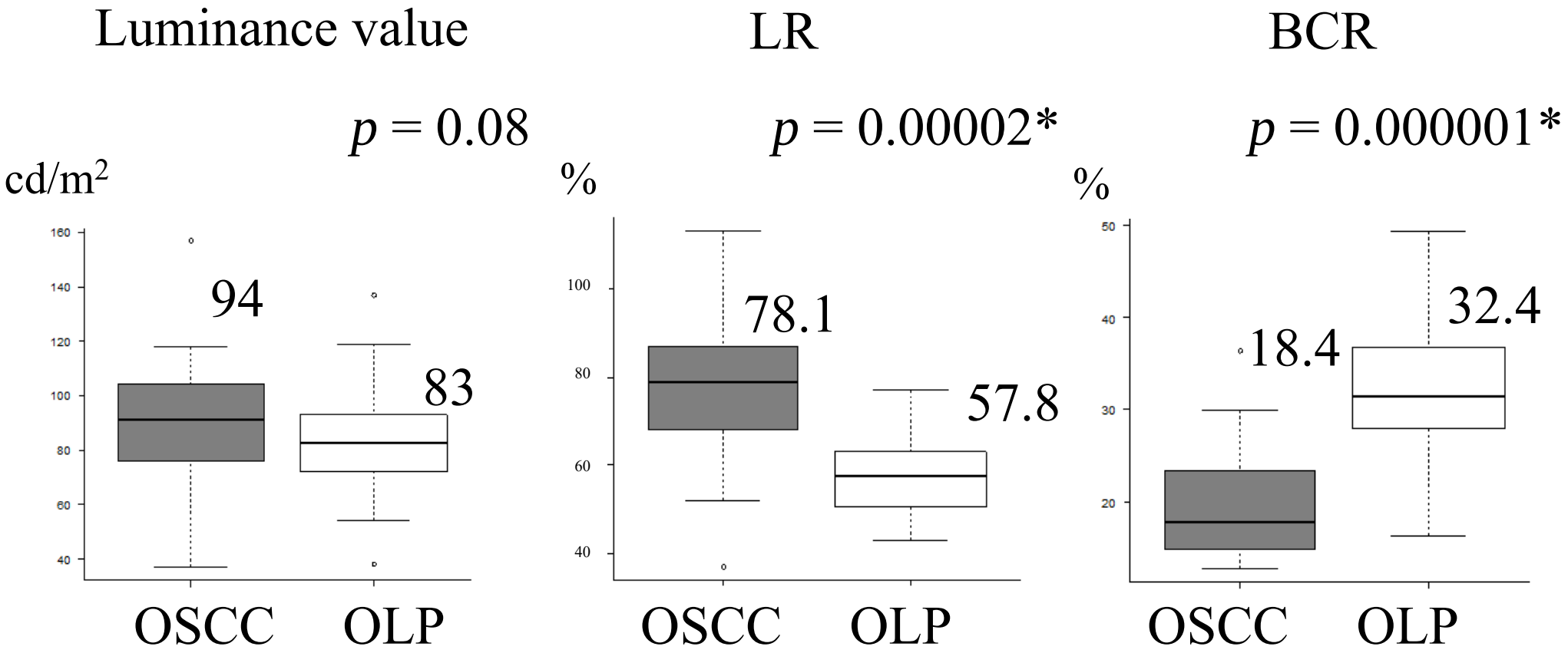
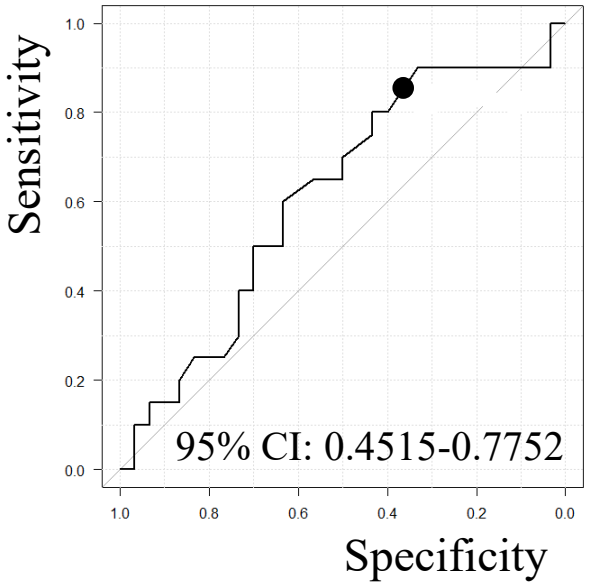


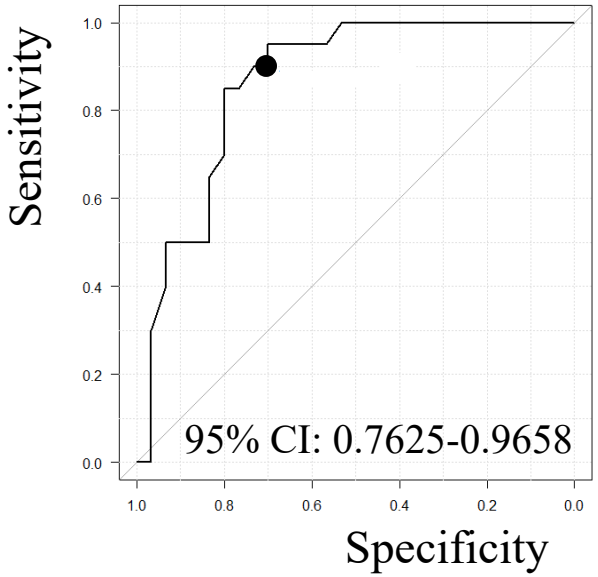
Fig 6. ROC analysis

● Cut off point

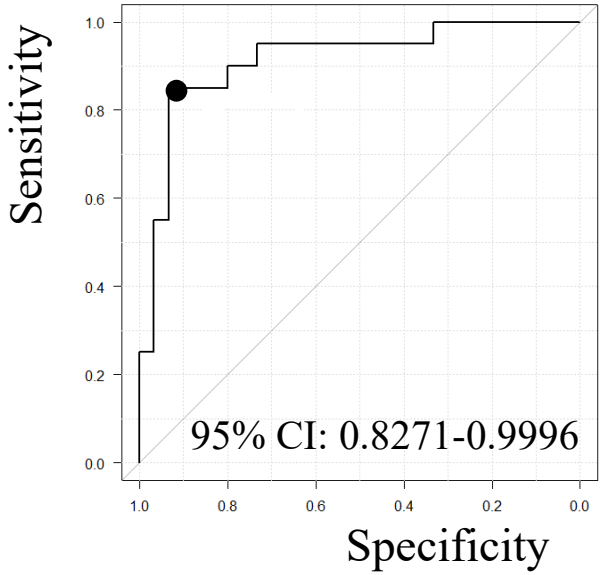
Luminance value



LR



BCR



	AUC	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Luminance value	0.613	97 cd /m^2	85.0	36.7	66.8	62.0
LR	0.864	69.0%	90.0	70.0	81.8	82.4
BCR	0.913	27.2%	85.5	93.3	95.0	81.1

Table 1. The patients' characteristics

	OSCC (N = 30)	OLP (N = 20)
Age, mean, (range)	57 (35 - 88)	65 (48 - 74)
Sex, male / female	16 / 14	6 / 14
Site, tongue / buccal mucosa	27 / 3	1 / 19
Clinical types,		
leukoplakia / ulcerative erosive / granular	12 / 10 / 5 / 3	-
reticular / erythematous / erosive	-	16 / 2 / 2
Stage, I / II	17 / 13	-