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Abstract. Actinic cheilitis (AC) is a disease caused by prolonged and cumulative sun exposure that mostly affects the lower lip, which can progress to a lip squamous cell carcinoma. Routine diagnosis relies on clinician experience and training. We investigated the diagnostic efficacy of wide-field fluorescence imaging coupled to an automated algorithm for AC recognition. Fluorescence images were acquired from 57 patients with confirmed AC and 46 normal volunteers. Three different algorithms were employed: two based on the emission characteristics of local heterogeneity, entropy and intensity range, and one based on the number of objects after K-mean clustering. A classification model was obtained using a fivefold cross correlation algorithm. Sensitivity and specificity rates were 86% and 89.1%, respectively. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: [10.1117/1.JMI.3.4.044004](https://doi.org/10.1117/1.JMI.3.4.044004)]

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1 Introduction

Actinic cheilitis (AC) is a potentially malignant disorder of the lip that is mainly caused by cumulative sunlight exposure. The lower lip is the most frequently affected site, and the disease is mainly presented as a multifocal and heterogeneous lesion in fair-skinned men.^{1–3} It may coexist with and progress to a lip squamous cell carcinoma, a malignancy commonly associated with clinical and/or microscopic features of AC.^{4,5}

Due to the heterogeneous clinical appearance of AC, selecting the best area to be biopsied, especially targeting areas with the most representative histological changes, is a complex task. Thus, the use of auxiliary diagnostic tools, such as autofluorescence imaging⁶ and spectroscopy,⁷ have the potential for improving the visual contrast of tissue alterations.

When biological tissue is illuminated with UV radiation, light diffuses into the tissue, and photons undergo both scattering and absorption phenomena. Absorbed photons can result in vibration of the excited molecules, producing heat, or in light re-emission (luminescence). Molecules responsible for luminescence are called fluorophores. The main fluorophores in human oral tissue are keratin, collagen, elastin, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD). NADH and FAD fluorescence signals have been demonstrated to be good sources for cell metabolism.⁸ The signal arising from elastin and collagen can give information on the stroma conditions, while a lack of such fluorescence can arise from a snag in the connective tissue due to cancer cell infiltration.⁹

Wide-field fluorescence imaging is an optical technique that uses a simple technology and provides *in situ* visualization

of tissue biomorphological alterations. Most of the reported fluorescence systems use a commercial semiprofessional camera coupled to a UV light that provides the illumination of the investigated tissue.^{6,10–20} A longpass filter is placed before eye visualization or image registration to remove backscattered excitation photons. Fluorescence light can be detected by a CCD camera providing a three-scalar spectral image of the tissue. As the fluorescence intensity and spectra are highly dependent on tissue organization and chemical composition, this technique is capable of providing biochemical and macromorphological information of the tissue.^{9,21}

Wide-field fluorescence imaging was already demonstrated to be a valid tool for oral cancer diagnosis. By screening the oral cavity with a hand-held imaging device, premalignant lesions appear dark-brownish, in comparison to the pale green color of healthy tissue. This feature probably arises from a lack of signal from connective tissue whose integrity has been affected by cancer cell infiltration.^{9,21} Several studies have reported high sensitivity and specificity in oral cancer assessment,^{10,14,17} as well as in detecting hidden areas and enhancing the visualization of lesion borders for complete removal.^{10,11,14,16,17} However, tissue discrimination between cancer and potentially malignant disorders presents lower diagnostic resolution, mainly due to false-positive areas that have low fluorescence associated with inflammation and its high light absorption pattern of the hemoglobin content.^{15,16} Fluorescence imaging has been approved by the U.S. Food and Drug Administration for autofluorescence-based oral mucosal screening. Examples of commercial devices are already available in the USA.^{22–27} Nowadays, new devices are proposing

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a combination of fluorescence with more techniques such as reflectance,¹⁹ optical coherent tomography,²⁸ and fluorescence lifetime imaging.²⁹

It has to be pointed out that the majority of the studies performed until now still rely on clinical assessment by one or more doctors trained in this technique. The possibility to develop an automated algorithm capable of recognizing the lesion based on wide-field images acquired using fluorescence imaging could lead this technique to be more objective and less dependent on operator skills. Roblyer et al. developed an algorithm based on the intensity ratio of green to red fluorescence emission for oral cancer diagnosis. By means of this algorithm, the authors were able to diagnose premalignant lesions and invasive cancer from the normal oral mucosa, with 100% sensitivity and 91.4% specificity.¹⁰

In a previous study,³⁰ we examined the effectiveness of wide-field autofluorescence imaging for AC diagnosis. We proved the usefulness of this technique for clinical inspection: in 16 cases (28.1%), areas without any clinical evidence were found to have either loss or increase of fluorescence, hence, being potentially altered. Furthermore, a first algorithm involving only K-mean clustering was already able to score AC with a sensitivity of 80.7% and a specificity of 78.3%.

In this study, we expand and improve our algorithm, adding two new scores for assessing image inhomogeneity. The analysis was performed in fluorescence images from 57 patients with diagnosed AC and 46 normal volunteers. As described in our previous work under visual inspection, AC images present a spotted pattern and it has already been shown that this feature can be scored using the K-mean algorithm.³⁰ Moreover, lower lip fluorescence images appear highly inhomogeneous in patients with AC compared with the ones from healthy volunteers. We found that this characteristic could be enhanced by calculating the local intensity entropy and local intensity range. Their average values were then used as scores, together with the one coming from the K-mean algorithm. Finally, in the 3D space of the scores, a K-nearest neighbor (KNN) algorithm was used to classify, and therefore, potentially diagnose, lips affected by AC from the healthy ones. Final results for sensitivity and specificity were of 86% and 89.1%, respectively.

2 Materials and Methods

2.1 Patients

Fifty-seven patients were enrolled in this study with a clinical diagnosis of AC; 27 males (47.4%) and 30 females (52.6%) with ages ranging from 23 to 82 years (mean 58.3 ± 12.9 years). A control group included 46 subjects with clinically normal lips; 24 males (52.2%) and 22 females (47.8%) with ages ranging from 30 to 88 years (mean 50.6 ± 14.5 years). All subjects were evaluated at the School of Dentistry, Fluminense Federal University (Nova Friburgo, Rio de Janeiro, Brazil). The research protocol was approved the Ethics Committee Review Board of the School of Medicine, Antonio Pedro School Hospital, Fluminense Federal University (Niterói, Rio de Janeiro, Brazil).

2.2 Fluorescence Imaging

A custom-made wide-field fluorescence hand-held device (prototype developed by MMOptics, São Carlos, São Paulo, Brazil) was coupled to a semiprofessional camera (Canon XSi Obj.18-55 3.5-5.6 IS) through a macro lens (Canon 100 mm 2.8 AF

USM). The hand-held device consists of an LED, emitting in the range of 400–460 nm, coupled to a dichroic beam splitter mirror that directs the light to the sample. Part of the re-emitted light exiting from the tissue surface enters in the device, passes through the dichroic mirror, and a longpass filter cuts the wavelength at 475 nm. The final light that reaches the camera detector is mainly tissue autofluorescence. Fluorescence images from the lower lip were acquired in a dark room.

2.3 Image Analysis

2.3.1 Software

All image preprocessing and analysis algorithms were implemented on a Matlab® R2009a platform (The MathWorks Inc., Natick, Massachusetts, USA).

2.3.2 Image preprocessing

The area of interest, i.e., the lower lip, was handily selected and cut. The selected image was then placed on a black background image with dimensions of 4000×2100 pixels. For similar dimensions, all lip images were resized to a final length of 3500 pixels, preserving image proportions. Images were smoothed by applying a Gaussian filter and reduced by a factor of four in order to reduce the calculation time. Colored images were finally split into their RGB channels. Only the green component was used for further analysis, as it is the main contributor for tissue autofluorescence.

2.3.3 K-mean clustering

The spotted pattern of the images was enhanced using a K-means algorithm.^{31,32} Using this algorithm, it was possible to reduce the number of the gray scale from 256 to 4 clusters. Briefly, the algorithm assigns each pixel to a cluster and classification based on its mean intensity value, minimizing the difference between the pixel values with the cluster average. For this technique, the total number of closed objects belonging to the same cluster was considered as a score.

2.3.4 Local entropy

For image analysis, entropy is defined as the score of randomness of the pixel values.^{33,34} In our analysis, a processed image was created where each pixel value corresponds to the local entropy calculated on its neighborhood of 21 pixels. Image heterogeneity was scored by the weighted average on the lower lip area of the entropy values.

2.3.5 Local range

The intensity range of a pixel neighborhood is defined as the difference between its maximum and minimum values. As in the case of local entropy, a processed image was created.³⁴ Pixel values in the new image correspond to the local range value calculated in the neighborhood of 21 pixels. The weighted average of local range values was used as the last score for our analysis.

2.4 KNN Algorithm

KNN is a well-established algorithm used to classify an unknown sample based on its similarity with others from a database. The sample is classified according to the category of its

KNN, i.e., the most similar ones.³⁵ Usually, the similarity measurement is the Euclidean distance between a sample and the known samples in a database. Although the similarity measurement and the number K of neighbors can be modified, providing flexibility in the modeling process, such parameters must be chosen in order to achieve optimal discrimination and avoid bias. A cross validation method is used to minimize such errors in the modeling process. For KNN analysis, the WEKA program was used.³⁶

3 Results and Discussion

3.1 Fluorescence Imaging

Typical fluorescence images from a healthy volunteer and a patient with confirmed AC are shown in Fig. 1. From a clinical point of view, a healthy lip shows a homogeneous fluorescence, while the AC image shows a highly heterogeneous and spotted pattern. These features are reflecting the clinical evidence: atrophy, hyperplasia, elastosis, loss of lip contours, cracks, and ulcerations. The highly fluorescent areas could be correlated with strongly keratinized areas. Keratin is indeed a strong fluorescence emitter when excited at 405 nm. Areas characterized by a loss of fluorescence could be correlated to ulceration or pigmented regions.

3.2 K-mean Clustering

A K-mean clustering algorithm was applied to all the green component images to reduce the number of gray scale levels to four tones. Images were then simplified and the presence of a spotted pattern was enhanced and could be quantified, as can be observed in Fig. 2.

Again, a more homogeneous pattern from the normal lip is observed: areas belonging to the same cluster appear more regular in shape. Furthermore, closed objects belonging to the same cluster are present in a higher number in an AC lip. It is possible to note that objects corresponding to the highest intensity are more likely to appear at the center of the image. This is due to the nonflat anatomical conformation of the lower lip, where the center of the lip is closer to the illumination source.

As a final score, the total number of closed objects was chosen. To remove small objects, probably caused by noise, a threshold for an object area was set to 300 pixels. Spotted patterns were scored by counting the number of regions for each image.

3.3 Local Entropy

Image homogeneity can be assessed by means of a measure of its entropy, defined according to Ref. 33. One of the main concerns in our analysis was that the entropy of the whole image was affected by a high variation of pixel intensity along the entire lip. Therefore, homogeneity was measured locally, using a sliding window of 21×21 pixels with the exposure condition considered uniform. For each image, a new one was obtained with the local entropy values, as shown in Fig. 3.

As interfaces between the lip image and the black background are highly inhomogeneous, also due to the Gaussian blurring, local entropy lip border images were not included. In the image, higher values of local entropy are represented as red shifted, while low values are more shifted to the blue. It is possible to notice that images from a healthy volunteer present, on average, a green–blue color, while an AC image

Fig. 1 Fluorescence imaging (a) from the lower part of the lip of a healthy volunteer and (b) from a patient with AC. A high fluorescence signal from teeth is noted. Compared with normal lip, AC lip shows a more heterogeneous fluorescence characterized by a spotted pattern.

Fig. 2 K-mean clustered images (a) from the lower lip of a healthy volunteer and (b) from a patient with AC.

Fig. 3 Local entropy in neighborhood of 21×21 pixels image (a) from a healthy volunteer and (b) from a patient with AC. Red-shifted values show higher entropy, and blue values show lower entropy.

Fig. 4 Local range in neighborhood of 21×21 pixels images (a) from a healthy volunteer and (b) from a patient with AC. Lower range values are shown with blue color; and higher values are shown with red color.

appears more in the range of yellow–red. This indicates a higher local homogeneity in the case of a lower lip healthy image. For each image, the average, weighted on the lip image area, of the local entropy values was used as a first score of image homogeneity.

3.4 Local Range

Another method to score image homogeneity is implemented by measuring its range, where range is the average difference between its highest and lowest pixel values. As in the case of entropy, the range value was locally calculated. A typical range image from a healthy volunteer and from a patient with AC is presented in Fig. 4.

High heterogeneity is highlighted by a red shift in the image, representing higher local range values. The AC image shows a greater number of pixels in the yellow–red range and, consequently, a lower homogeneity. For each image, the weighted average of local range values was chosen as a second score for image homogeneity. Also in this case, it was necessary to not consider the lip borders.

3.5 Classification Algorithm

Using the three algorithms described above, three score values for each image were obtained. Looking at the plot in Fig. 5, where each point represents a patient (red for a healthy volunteer and black for AC), it is possible to observe how the two different groups occupy two different volumes in the space of the scores. A fivefold cross correlation algorithm was used for classifying

the images. A KNN algorithm was chosen as the classifier.³⁶ Final results were of 86% sensitivity and 89.1% specificity, values well above the ones obtained with the stand-alone K-mean algorithm shown in a previous study.³⁰ The better outcome of this analysis relies on the peculiar specificity of each algorithm introduced, giving complementary scores for different features. K-mean was found to be capable of detecting the presence of

Fig. 5 Scatter plot of score values: local entropy, local range, and number of objects. AC lips are represented by black dots, and normal lips as red dots.

spotty patterns inside the image. A local range algorithm enhances the abrupt changes in the image intensity mainly due to hyperkeratosis or atrophy. Finally, local entropy gives important information on the image homogeneity. In addition to increasing the sensitivity, the requirement of simultaneously having all these three features decreases the chances to have false positives and, hence, increases the final specificity.

High rates of sensitivity and specificity show the potential of the wide-field fluorescence imaging and the proposed algorithm as an auxiliary clinical tool for AC diagnosis. The diagnostic resolution is not as high as the ones obtained for oral cancer discrimination. This was not expected as AC lip is a potentially malignant disorder, and its biochemical and structural changes compared with normal histology are not as evident as the ones present in carcinoma, resulting in less fluorescence pattern changes.

Future analysis may include clinical information to improve algorithm performance. Other attempts using other color channels or the whole RGB image, have been tried, but in all cases, the diagnostic performances were lower (data not shown). Similar results regarding specificity and sensitivity have been obtained using other classification algorithms, as support vector machine and naïve Bayes, demonstrating the robustness of the three scores chosen in this study. Between all the presented scores, local entropy showed the highest resolution in image classification, but the addition of the other techniques always increases the algorithm performance. In the case of K-mean clustering, further improvements can be obtained by compensating for differences in tissue illumination and by considering other image features, such as spot areas, shape, and localization.

4 Conclusion

Fluorescence wide-field imaging is revealed to be a powerful tool for assisting AC clinical diagnosis. The fluorescence visualization, when compared with the standard white light examination, enhanced the discrimination of the AC lip heterogeneous pattern. Healthy lips presented a more homogeneous fluorescence. The weighted averages, at the lip area, of the values of local entropy and range, calculated on a neighborhood of 21×21 pixels was used as a score for image heterogeneity. Bright and dark objects were highlighted by a K-mean clustering ($K = 4$). The number of objects belonging to the same cluster was used as final score. Using the three scores values, a KNN classification algorithm was obtained for an automated recognition of AC. Algorithm performance was measured using a five-fold cross correlation method. The values of sensitivity and specificity obtained were of 86% and 89.1%, respectively. Therefore, fluorescence wide-field imaging coupled with an automated algorithm is a promising less subjective tool for assisting AC clinical diagnosis.

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