MAKARA, KESEHATAN, VOL. 13, NO. 2, DESEMBER 2009: 79-82

CLASSIFICATION OF SEVERAL SKIN CANCER TYPES BASED ON AUTOFLUORESCENCE INTENSITY OF VISIBLE LIGHT TO NEAR INFRARED RATIO

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Abstract

Skin cancer is a malignant growth on the skin caused by many factors. The most common skin cancers are Basal Cell Cancer (BCC) and Squamous Cell Cancer (SCC). This research uses a discriminant analysis to classify some tissues of skin cancer based on criterion number of independent variables. An independent variable is variation of excitation light sources (LED lamp), filters, and sensors to measure autofluorescence intensity (IAF) of visible light to near infrared (VIS/NIR) ratio of paraffin embedded tissue biopsy from BCC, SCC, and Lipoma. From the result of discriminant analysis, it is known that the discriminant function is determined by 4 (four) independent variables i.e., blue LED-red filter, blue LED-yellow filter, UV LED-blue filter, and UV LED-yellow filter. The accuracy of discriminant in classifying the analysis of three skin cancer tissues is 100%.

Keywords: autofluorescence, multiexcitation, skin cancer

Introduction

Skin cancer is one type of a serious cancer. Although the case of skin cancer like melanoma malignant is not prevalent in Indonesia, these cancers grow faster than other types of cancers. Early diagnosis of cancer is important because the possibility to be cured at an early stage is very high.

Skin cancer is a disease characterized by the growth of skin cells that are not controlled, can damage surrounding tissues, and can spread to the other parts of the body. Because the skin consists of several types of cells, skin cancers also vary according to the type of cells affected. Cancers that are most often found are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanoma (MM). BCC and SCC are often classified into non-melanoma skin cancer.[1]

The standard method to ensure the type of skin cancer is to perform clinical and histopathology tests by taking a sample of the skin which is suspected as a cancerous tissue to be studied under a microscope. Another technique is to analyze the image of skin cancer by using macroscopic and dermatoscopic. Recent studies indicate a diagnose by using a dermatoscopic technique has a level accuracy of 75% - 97%, while the use of a diagnose by using macroscopic has a level accuracy of 65% - 80%.[2,3]

Another method which can also be used to detect skin cancers or other skin disorders is using a spectroscopic technique. Tissue classification by analyzing the first derivative of function of reflectance percentage to the wavelength of near infrared (NIR) to visible light (VIS) region has been carried out to determine the type of a lesion in skin.[4]

One type of optical spectroscopy widely used for cancer diagnose is fluorescence spectroscopy. In the literature, the fluorescence spectroscopy is known to rapidly distinguish normal and tumor tissues from various epithelialized organs[5] However, although this technique is known to have high sensitivity in detecting tumor tissues, specificity in differentiating tumor and normal tissues in the early stages of tumor is still quite low. [6] This is a constraint in the application of a fluorescence spectroscopy technique as an early diagnostic tool for tumors.

The low specificity can occur because the observation of the fluorescence spectrum of tissues is usually performed only at one excitation wavelength range. In fact, the process of pathogenesis and metastatic

carcinoma is a process with many stages and is controlled by many genes and many other factors as well as involving many compounds. Therefore, the fluorescence spectroscopy method that uses a range of excitation wavelengths or multiexcitation is a method that can be developed as the basis for tissue classification.

The purpose of this study is to test the sensitivity and specificity of spectrofluorometer instrument that is being developed to detect the presence of cancerous tissues, especially skin cancer. This research is expected to provide benefits in the development of a cancer diagnostic tool, a simple, relatively inexpensive, easy to use, but quite sensitive, both for early detection systems and to see progression and prognosis of cancer.

Methods

The working principle tool developed in this study can be explained as follows (Figure 1): The source of light in the form of light emitting diode (LED) is focused through lens (L) and inserted into the collimator lens. Then, it is imposed on the beam dividing lenses (dichroic-1) and deflected into the sample. Reflection of light from the sample is transmitted in a parallel form through the dichroic-1, and the outcome is imposed on dichroic-2. One laser beam is deflected into the detector (D2) and the other beam passes through the detector (D1). In front of both detectors, filters are installed so that they can forward the fluorescence light with a wavelength greater than wavelength of light sources.

Light reflection, as a signal of infrared (NIR) is forwarded to a detector (D2) and into the signal divider (Divider). Light that is deflected into the detector (D1), as a visible light beam (VIS), is also incorporated into the divider signals (Divider). The outcome is compared in the form of intensity by the following formula:

$$\left(\frac{I_{VIS}}{I_{NIR}} \right) \qquad (1)$$

Where : I_{NIR} = Intensity of near infrared
I_{Vis} = Intensity of visible light

Experiments were conducted at the Department of Anatomical Pathology, the Faculty of Medicine, the University of Indonesia. Experiments were carried out using tissues of skin cancer patients that were received from April 2008 to December 2008. Histopathology examination was performed on samples stained with haematoxylin-eosin and immunohistochemistry to determine the normal tissue or cancer. Then the IAF of

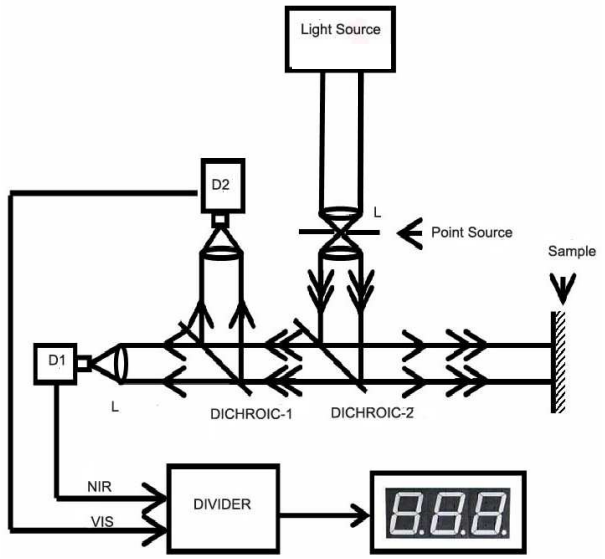

Figure 1. Measurement of the Fluorescence Emission Intensity on Tissue Section (Design Tools for Fluorescence Light Detection)

blind samples was measured by spectrofluorometer to determine the samples classification.

3. Results and Discussion

The VIS/NIR ratio of SCC, BCC, and lipoma (L) show a value of less than 1 (Table 1). This means in the NIR region molecules are much more excited than in the VIS region.

In a study by Zellweger et al., the same condition was found in carcinoma in situ (CIS).[7] The study concluded that the higher the IAF in the infrared region, the higher level of the transformation that occurs in the tissue which has led to the formation of cancerous tissue.

Although most of the endogenous fluorophores that have been identified as aromatic amino acids and proteins, pyridine nucleotide, flavin, and porphyrin have the energy emission at a wavelength range of 200-750 nm or the UV-VIS region,[8] in this study it can be proven that the energy of autofluorescence (AF) also appears in the NIR region.

However, it needs more to prove whether the AF energy that appears in the NIR region is specific for a particular fluorophore, or arises from the combination of several spectrums that are reflected by an object. This is because the combination of spectral emission from fluorescence molecules with the absorption spectrum, reflectance, and scattering produces a wide and unstructured spectrum.[9,10]

Table 1. The VIS/NIR Ratio of Sample Preparation on Skin Cancer Tissues

Types of tissue	code	VIS/NIR ratio*
SCC	1	0.599
	2	0.597
	3	0.592
BCC	1	0.616
	2	0.615
	3	0.613
L	1	0.572
	2	0.574
	3	0.563

* VIS : a blue LED light source, red filter, LDR sensors
NIR : red LED light source, without the red filter, sensor photodiode

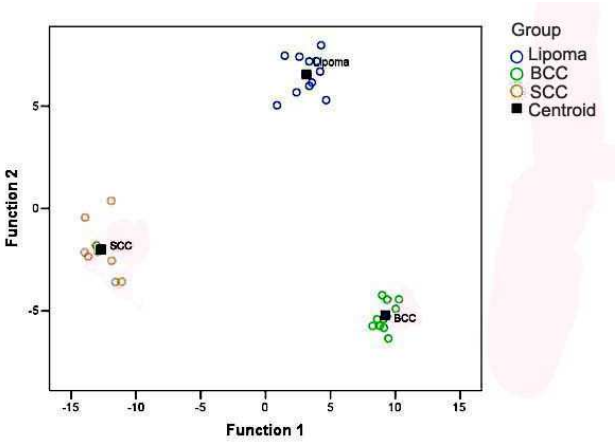

Figure 2. Discriminant Analysis of Skin Tissues

IAF measurement in this study is using several excitation wavelengths, or more commonly known by multiexcitation. Through this technique, the normal tissue and carcinoma are expected to be differentiated more significantly since the overall tissue specificity will be determined by the specific intensity that appears in the fluorescence emission spectrum of each natural fluorophore that is excited.

In this study, discriminant analysis is used to classify some skin cancer tissues based on the criteria of a number of independent variables.[11,12] The independent variables used are variations of the treatment of excitation light sources (LED), filters, and sensors used to measure the ratio of the VIS/NIR emission energy in an object. The results of discriminant analysis of BCC, SCC, and limpoma tissues are presented in Figure 2.

The principle of discriminant function is a kind of regression equation in the output; its discriminant analysis can be seen in Table 2. In Table 2 it seems that from some variations of the light source and filters, the discrimination function is determined from 4 (four) variables. Based on that information, it can be formed into discriminant functions as follows:

Discriminant Function 1
Z Score_1 = -334.930 + (23.828 blue-red filter) - (74.941 blue-yellow filter) + (249.969 UV- blue filter) + (148.130 UV-yellow filter)

Discriminant Function 2
Z Score_2 = -207.771 - (61.085 blue-red filter) + (19.205 blue-yellow filter) + (40.720 UV- blue filter) + (207.771 UV-yellow filters)

Discriminant functions 1 and 2 are created in territorial map with function 1 as X axis and Y axis functions as the second (Figure 3) for determining the classification of each object to be measured.

Once created and discriminant function classification process carried out, the next that will be seen is how accurate the classification is made or what percentage of classification errors is. This can be seen in Table 3.

Table 2. Coefficients of Discriminant Function

	Coefficient function	
	1	2
Blue LED-red filter-paraffin*	23.828	-61.085
Blue LED-yellow filter-paraffin	-74.941	19.205
UV LED-blue filter-paraffin	249.969	40.720
UV LED-yellow filter-paraffin	148.130	207.701
Constant	-334.930	-207.771

Note: * Source blue LED and red filters
Measurements on samples of paraffin blocks

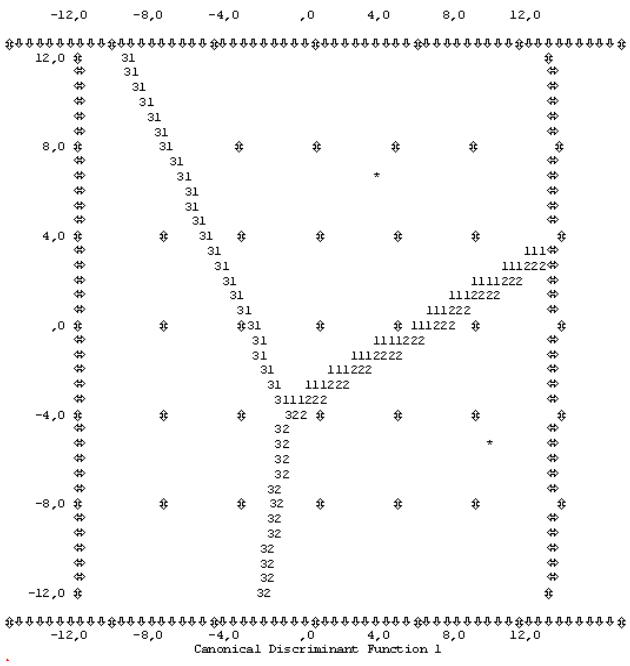

Figure 3. Territorial Map of the Object Graph which is formed by Discriminated Function (1. Lipoma, 2. BCC, 3. SCC, * Group of *Centroid*)

Table 3. Classification Accuracy with Discriminant Analysis

Group		Group Predicted			Total
		Lipoma	BCC	SCC	
Original	Lipoma	11	0	0	11
	BCC	0	10	0	10
	SCC	0	0	10	10
Cross-validated	Lipoma	11	0	0	11
	BCC	0	10	0	10
	SCC	0	0	10	10

Table 4. Accuracy of Data Classification and Data Training

Tissue	Accuracy (%)			
	Testing Data	Training Data	Testing Data	Training Data
Lipoma	3	11	100.00	100.00
BCC	3	10	100.00	100.00
SCC	3	10	100.00	100.00

Table 3 shows that all tissue samples at the beginning of the data (after histopathology examination) are classified as lipoma (n = 11) of the prediction results of classification and remain in the group of lipoma. The same thing happens in the entire samples of BCC and SCC. Thus the accuracy of prediction with this model is 100%, which means that the results of discrimination function are able to predict the classification of all objects measured with precision.

Having proven that the discriminant function has a high predictive accuracy, then the resulting discriminant function can be used to predict the classification of a case. In this case as many as 9 (nine) or blind samples (testing data) are used to view the classification accuracy. Classification accuracy, the result of the nine samples, is compared with the training data (Table 4).

Table 4 shows that all blind samples can be classified correctly. This indicates that the independent variables can be used to classify lipoma, BCC, and SCC very well.

Conclusion

Fluorescence spectrophotometer, developed in this study, is able to distinguish limpoma, BCC, and SCC in the preparation of paraffin biopsy with 100% accuracy. Discriminant function, resulting from the measurement of VIS/NIR intensity of autofluorescence (IAF), distinguishes lipomas, BCC, and SCC tissues and is determined by four independent variables: Blue LED-filter red, blue LED-yellow filter, UV LED-blue filter, and UV LED-yellow filter.

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