Analyze fluorescent characteristic of cancer cell using hyperspectroscopic imaging system (HIS)

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ABSTRACT

Currently, the cancer was examined by diagnosing the pathological changes of tumor. If the examination of cancer can diagnose the tumor before the cell occur the pathological changes, the cure rate of cancer will increase. This research develops a human-machine interface for hyper-spectral microscope. The hyper-spectral microscope can scan the specific area of cell and records the data of spectrum and intensity. These data is helpful to diagnose tumor. This study finds the hyper-spectral imaging have two higher intensity points at 550nm and 700nm, and one lower point at 640nm between the two higher points. For analyzing the hyper-spectral imaging, the intensity at the 550nm peak divided by the intensity at 700nm peak. Finally, we determine the accuracy of detection by Gaussian distribution. The accuracy of detecting normal cells achieves 89%, and the accuracy of cancer cells achieves 81%.

Keywords: hyperspectral, microscope, diagnosis, chacteristic, fluorescence, cancer

1. INTRODUCTION

The field of spectral imaging can be divided into techniques called multi-spectral image, optical image, hyper-spectral image and ultra-spectral optical image. As Willoughby [1] et al. defined, the hyper-spectral image included spectral and spatial information (λ, x and y). The λ axis was the wavelength, the x axis was the scanning direction of the slit, and the y axis was the pixel on the slit. Moreover, there were tens or hundreds of spectral band in one hyper-spectral image, and the spectral resolution was 0.01. Because of the three axes, we could get a 3D image in the hyper-spectral. As Y. Garini [2] proposed, time-scan, spatial-scan, wavelength-scan, and compromise-scan were the mainly scanning method in hyper-spectral image. Hyper-spectrometers capture an array of optical images at a time, and to spectrally scan the entire target, it is required to move the spectrometer or the optics located in front of the spectrometer, and this is quite inconvenient for design and usage. Furthermore, moving the spectrometer or the optics arbitrarily would cause the problem of optical path difference and thus would degrade the quality of the optical image obtained. In view of the foregoing, there is a

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need in the related field to provide suitable apparatuses and methods for scanning hyper-spectral image [3-5]. Also, the conventional method of diagnosing cancer cells used the naked eyes, so the accuracy was limited by the wavelength (400nm to 700nm). This paper proposes a new mechanism of hyperspectral microscopic system. As Fig. 1 shows, the sample is put on a stage of microscope, and the imaging is transferred from the port of microscope to the hyper-spectrometer (from 400nm to 1000nm) by the relay lens. This study uses 330nm to 385nm wavelength to be the fluorescent excitation analyzes the hyper-spectral imaging by the wavelength from 400nm to 1000nm. As Reblyer et al. [6] proposed, when using the 365nm fluorescent excitation, the spectral curve of the normal cells had a high peak at 475nm, and the high peak of cancer cells at 460nm. Furthermore, the intensity of normal peak was higher than the cancer peak. As Schwarz et al. [7] research, the normal cells had higher intensity in the spectral than cancer cells at 350nm fluorescent excitation. Koh et al. [8] research, when the 567nm excitation peak was divided by the peak of 515nm fluorescent excitation, the cancer cells had higher scores of 567/515nm ratio. This article wants to try different methods for detecting the sample, and also expects the higher accuracy.

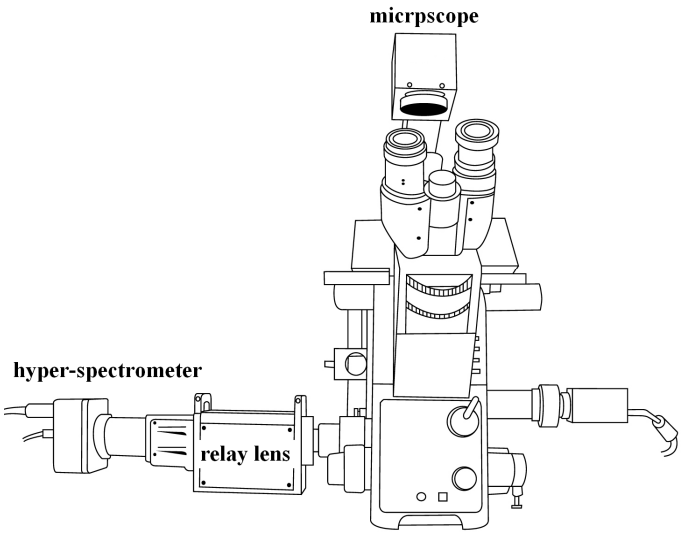

Fig. 1: The new hyper-spectral microscopic system

2. METHODOLOGY

The relay-lenses are tele-centric imaging system and can transfer image to another imaging plane. As the Fig. 2 shows, the relay lenses can transfer the microscopic image from image plane I to image plane II. This novel setup can obtain the scanning microscopic image and doesn't need to move the sample stage of microscope or hyperspectrum. Figure 3 is the finished product of our system. The CCD of right side can preview the sample to search the location where doctor wants to analyze. The relay lenses scans the interested region to obtain the spectrum and intensity information. The scanning procedure of fluorescent image takes only 5 minutes. The novel microscope can diagnose the spectrum of cell and its shape, simultaneously. The doctor diagnosed the cancer by watching the shape of cell. This way would have some error for judging the cell. So, the novel setup is very useful to help the doctor to diagnose the cancer cell.

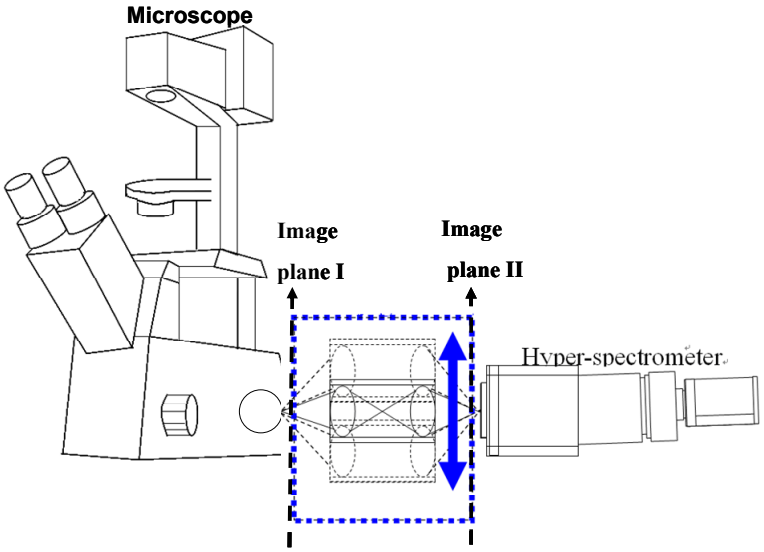

Fig. 2: The conceptual figure of the hyper-spectral microscopic system.

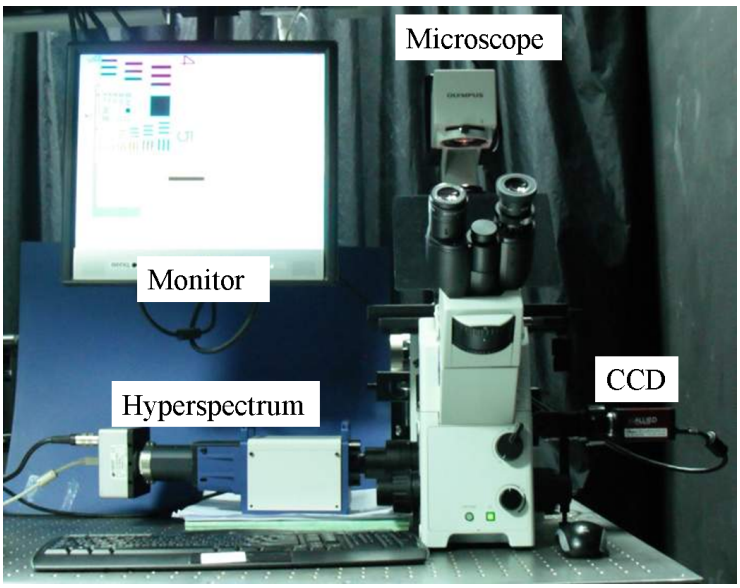

Fig. 3: The finished product of the hyper-spectral microscopic system.

This study proposes a method to distinguish characteristics of oral cancer cell and normal cell. First, the 50 points of normal and cancer cells are marked in the nucleus, as showed in Fig. 4 (the region a. is the normal region and the region b. is the cancer region). Each one region is a square concluding 100 pixels totally because we want to eliminate the noise by average. In order to compare the hyper-spectral curve easily, we normalize the hyper-spectral data. After observe the normalized curve, as Fig. 4 shows, we can see there are two high peak at 550nm and 700nm.

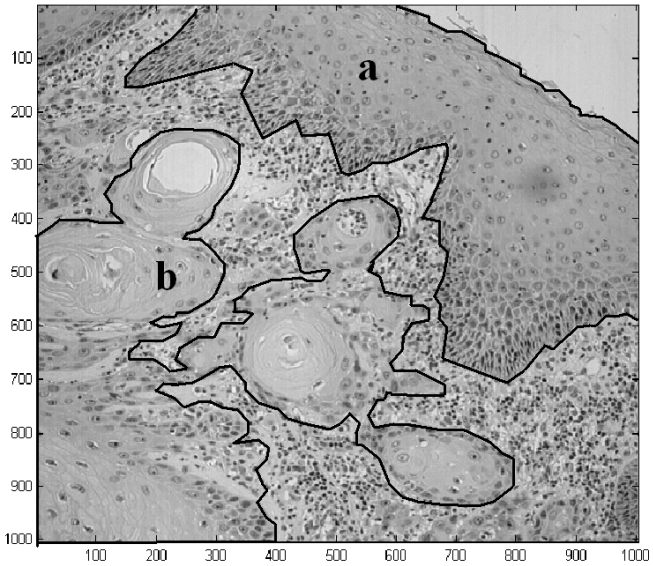

Fig. 4: a is the Region of normal cells, and b is the region of cancer cells

In order to strengthen the difference between two curves, we divide the intensity of the two high peak, and then we can get the ratio, as (1) shows:

$$R_{I1} = \frac{(\sum_{i=-n}^{n} I_{H1i})/(2n+1)}{(\sum_{i=-n}^{n} I_{H2i})/(2n+1)} \tag{1}$$

,IH10 is the value of the peak at 550nm,IH20 is the peak value at 700nm.Then we use Gaussian distribution to analyze the ratio value, as (2) shows:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \tag{2}$$

3. RESULTS

3.1 The measurement of normal oral cell

This results shows the scanning pictures of normal oral cell which is obtained by the novel hyperspectral microscopic system. This system has transmittance, fluorescence 1 and fluorescence 2 mode. The transmittance image can help doctor to diagnose oral cell by cell shape and choose the suspicious regions. The fluorescent image can analyze the characteristic of oral cell. In our study, the fluorescence 1 image has the helpful information for analysis.

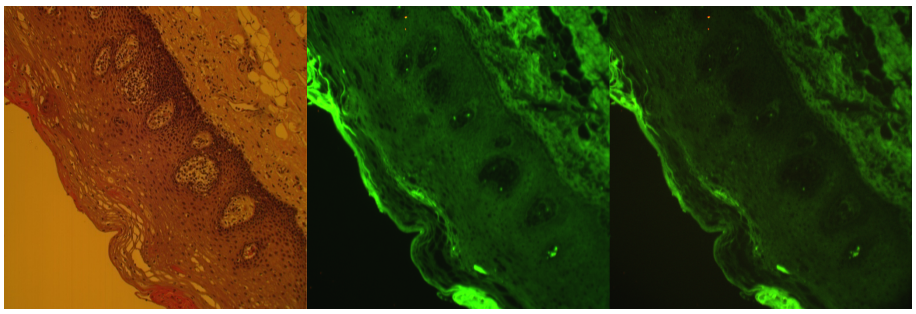

Fig. 5. (a) is the transmittance image. (b) and (c) are the fluorescence images. The fluorescence of (b) is 380nm. The fluorescence of (c) is 420nm. The different band of fluorescence can help us to discriminate the cell.

3.2 The measurement of cancer oral cell

This results shows the scanning pictures of cancer oral cell which is obtained by the novel hyperspectral microscopic system. This system has transmittance, fluorescence 1 and fluorescence 2 mode. The transmittance image can help doctor to diagnose oral cell by cell shape and choose the suspicious regions. The fluorescent image can analyze the characteristic of oral cell. In our study, the fluorescence 1 image has the helpful information for analysis.

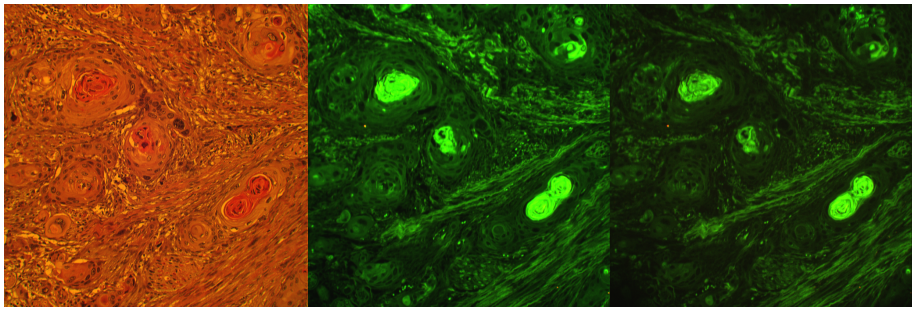

Fig. 6. (a) is the transmittance image. (b) and (c) are the fluorescence images. The fluorescence of (b) is 380nm. The fluorescence of (c) is 420nm. The different band of fluorescence can help us to discriminate the cell.

3.3 The results of analysis

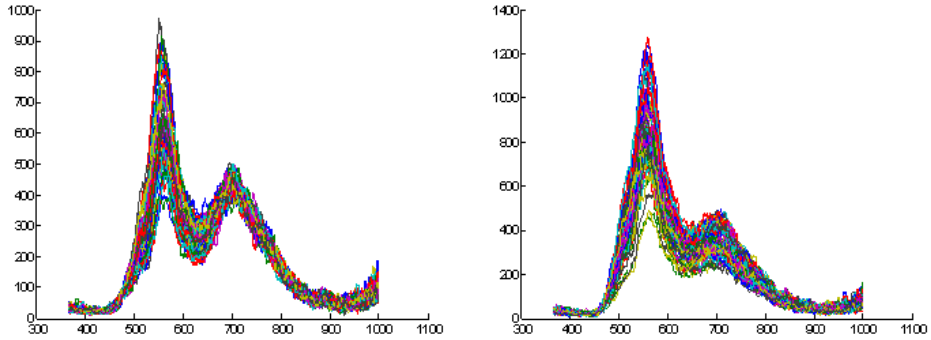

Fig. 7. (a) The result of normal oral cell (b) The result of cancer oral cell

4. CONCLUSION

This study finds the overlapped region in the normal and cancer cells curve are 6.83% and 21.43%. Then we calculate the accuracy of detecting normal and cancer cells. Generally, the accuracy of confidence index is 83%. The accuracy of our experiment's result in normal cells is 89%, and in cancer cells is81%. In the application of detecting cancerous cells, not only analyzing the spectral intensity, but also the analysis of image is an important way. When the cancerous lesions happen, the nuclear of cells will change. In the future, we will combine the analysis of fluorescence intensity and the analysis of imaging in order to improve the sensitivity and specificity.

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