The new hyperspectral microscopic system for cancer diagnosis

Yao-Fang Hsieh[*1], Ou-Yang Mang[2], Jin-Chern Chiou[2], Yung-Jiun Lin[2], Ming-Hsui Tsai[3], Da-Tian Bau[3], Chang-Fang Chiu[3], Guan-Chin Teseng[3], Nai-Wen Chang[3], Wen-Chung Kao[4], Shun-De Wu[5]
[1]Dept. of Optics and Photonics, National Central Univ., Jhongli City, Taoyuan County , Taiwan
[2]Dept. of Electrical Engineering, National Chiao-Tung Univ., Hsihchu city, Taiwan
[3]School of Medicine, China Medical Univ., Taichung City, Taiwan
[4]Dept. of Applied Electronics Technology, Taiwan Normal Univ., Taipei City, Taiwan
[5]Dept. of Mechatronic Technology, Taiwan Normal Univ., Taipei City, Taiwan

ABSTRACT

Until now, 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 research aims to develop a new system and a human-machine interface to control the hyper-spectral microscope. The interface can control the moving speed of motor, the exposure-time of hyper-spectrum, real-time focus, image of fluorescence, and record the data of spectral intensity and position.

Keywords: hyperspectral image, fluorescent image, cancer, diagnosis

1. INTRODUCTION

As the Willoughby et al. defined, the hyperspectral image had tens or hundreds of spectral band and the order of spectral resolution was 0.01 [1]. The hyperspectral image is an image which contains the spatial and spectral information (x, y and λ). In this study, the y axis is parallel to the slit direction, x axis is the scanning direction and the λ axis is the wavelength axis. The hyperspectral image provides the spectrum of each pixel. The information of hyperspectral-image is stored as 3D data set. Also, the hyperspectral image can regard as the collection of many images which are measured at different wavelength individually. The hyperspectral image can apply to macroscopic-technical examination and documentation of objects of artistic and historic analysis and also can apply to analyze biomedical fluorescence image [2-3]. Up to now there are four scanning methods for hyper-spectral image [4] which are wavelength-scan, spatial-scan, time-scan and compromise-scan. In this article, the novel hyper-spectroscopic imaging system uses the spatial-scan method. Resulting from a complex scanning mechanism of the relative movement, we propose that an internal relay lenses would scan on the extra image plane to achieve a hyperspectral images with a better image quality. In recent years, the biomedical diagnostic technique utilizes the fluorescent spectrum to diagnosis the cancer cell. The technique utilizes the difference of fluorescent spectrum of photosensitizer in the human body to diagnose the pathological cell. As the Fig.1 Shows, the normal oral cell and the abnormal oral has the different fluorescent spectrum at red band (600nm).

Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues IX, edited by Daniel L. Farkas, Dan V. Nicolau, Robert C. Leif, Proc. of SPIE Vol. 7902, 79020J · © 2011 SPIE · CCC code: 1605-7422/11/$18 · doi: 10.1117/12.874843

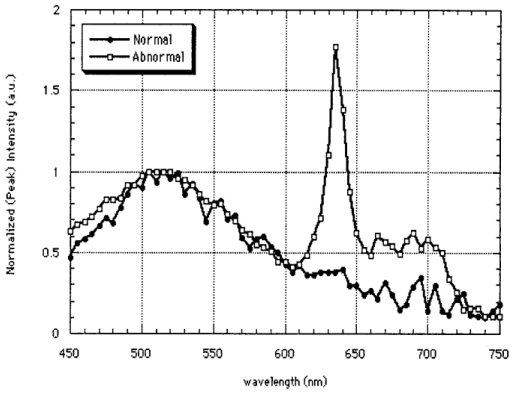

Figure 1. The fluorescent spectrum of normal and abnormal cells.

The diagnostic techniques of fluorescent spectrum can be classified to the autofluorescence and the dye fluorescence. The autofluorescence mainly utilizes the structural changes of pathological cell to differentiate the changes of fluorescent spectrum, such as NADH, Collagen, Elastin. The dye fluorescence utilizes the adding photosensitizer to produce fluorescent spectrum. In the past, the moving stage of hyperspectral microscopic system always moved the sample or the hyper-spectroscope to achieve the scanning purpose. But, the size of cells was nano-scale, so the moving stage must be moved in nano-scale. So the error of measurement was big and the cost was expensive. In this study, we use the scanning relay-lenses to replace the moving stage, as Fig.2 and Fig.3 showes.

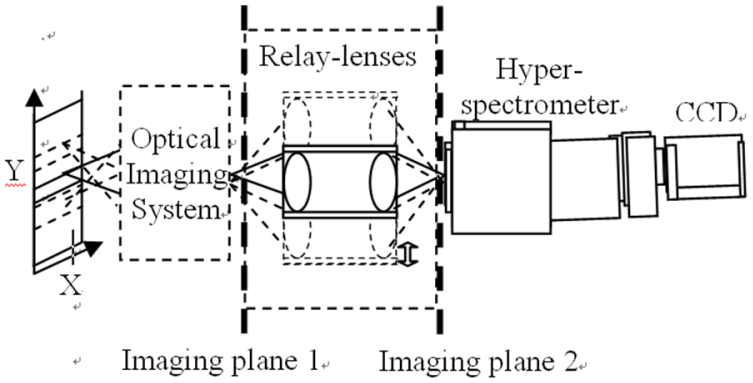

Fig. 2: The conceptual figure of relay lenses scanning method.

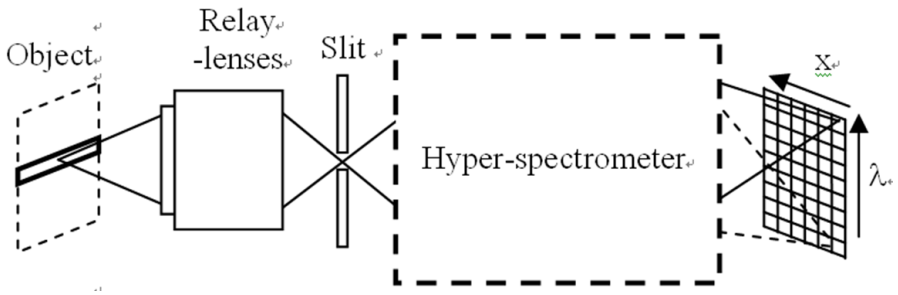

Fig. 3: The conceptual figure of CCD recording.

The scanning relay lenses module is composed of relay lenses and stepping motor. The relay lenses would be similar as a finite conjugate optical module with unit transverse magnification. Therefore while a light passes through the system in an angle, the light would leave the system in the same angle. The finite conjugate optical module consists of two symmetric infinite conjugate lenses with the same focus point in order to cancel itself aberration. Because the system can form an extra image of real objects at a finite distance, the relay lenses system can carry an image through a distance but in the limited diameter of the lenses. The relay lenses system is also a telecentric system. The telecentric system means the exit pupil of optical system at infinity and the image size didn't change with the variation of focus. Because of telecentricity, the off axis image can the same as the central image and slight focus change didn't affect the image size. So, the distortion of image is very small. The new hyperspectral microscopic system consists of EMCCD, scanning systems, the microscope and hyper-spectroscope, as shown in Fig.4.

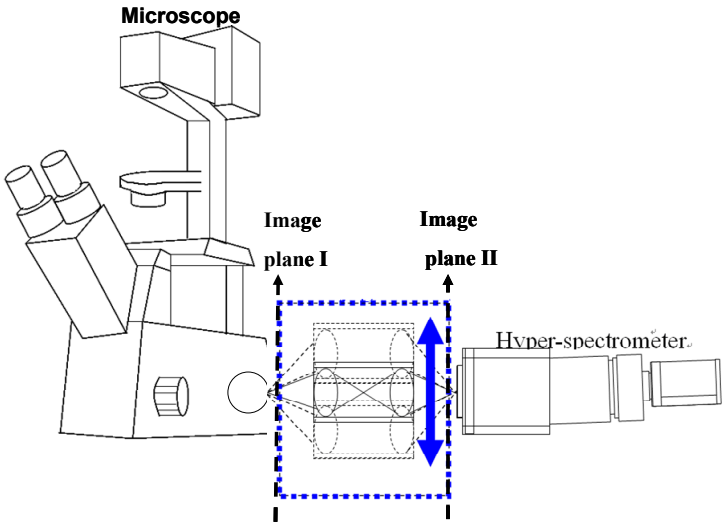

Figure 4. The conceptual figure of the new hyperspectral microscopic system.

2. METHODLOGY

As the Fig. 4 shows, the microscope can collect the radiation from the object and form an image on the image plane 1 (ImP1). The relay lenses can relay the image from Image plane 1 (ImP1) to image plane 2 (ImP2), which is the slit of hyper-spectroscope. The slit of hyper-spectroscope is in the y axis. The width of slit is 30 μm. The wavelength range of hyper-spectroscopic is from 400nm to 1000nm. The spectral resolution is 2.8nm. The spatial resolution is smaller than 9 μm. The F/number is 2.4. A narrow slit of the hyper-spectroscope allows for one line of ImP2 to be imaged on the electron multiplying charge coupled device (EMCCD). Although the relay lenses can relay the full image from ImP1, the image only has the size of slit after passing through the slit. The collimating optics (CO) can collimate the image to the dispersive (D) structure, and then the dispersive structure disperses the image. The dispersive element of hyper-spectroscope is prism-grating-prism (PGP) structure. The PGP structure can disperse more wavelength than the wavelength-scan method and compromise-scan method. Finally, the image is focused on the EMCCD by the focusing optics (FO). So, while the relay lenses is static, the image of slit size and its spectrum can be recorded on the EMCCD. The pixel size is 8 μm×8 μm. The frame rate is 12.4 frames per second. The digitization is 14 bit. In order to get the corrective spectral information, we also calibrate the spectrum and response of the EMCCD before using the novel system. While the stepping motor (SM) scans along the x axis, individual line image is recorded in y-λ□plane on EMCCD. The stepping motor moves one step in the x axis to acquire next line image and its spectrum. Each y- λ□ image is recorded as a single y- λ file for each row along the object corresponding the radiation collection region which maps through the hyper-spectroscope to the EMCCD. After all the line images are acquired, the data cube of all the y-λ□files is loaded to memory. The finished product is showed in Fig.3.

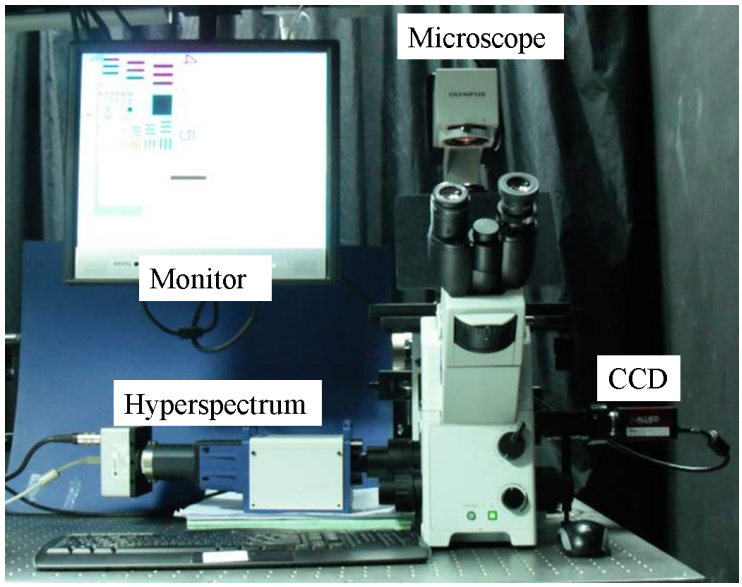

Figure 5. The finished product of the hyper-spectral microscopic system.

3. RESULTS

3.1 The measurement of normal oral cell

We design the human-machine interface of this new system. The interface can change the exposure time, the moving speed of motor, the position of motor and the trigger mode of EMCCD. The Fig.6 is the hyperspectral image of normal oral cell. There also capture the fluorescent image in order to analyze the spectral image.

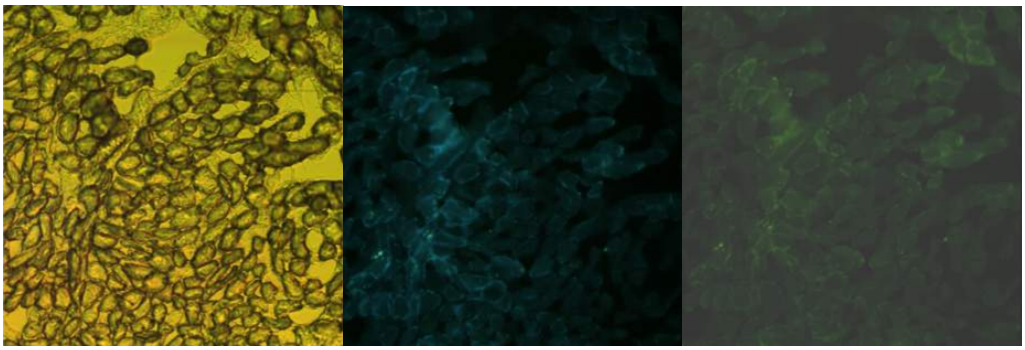

Fig. 6. (a) is the transmittance image. (b) and (c) are the fluorescence images. The fluorescence of (b) is 380nm. The fluorescence of (c) is 420nm. The different band of fluorescence can help us to discriminate the cell.

3.2 The measurement of oral cancer cell

The Fig.7 is the hyperspectral image of oral cancer cell. There also capture the fluorescent image in order to analyze the spectral image.

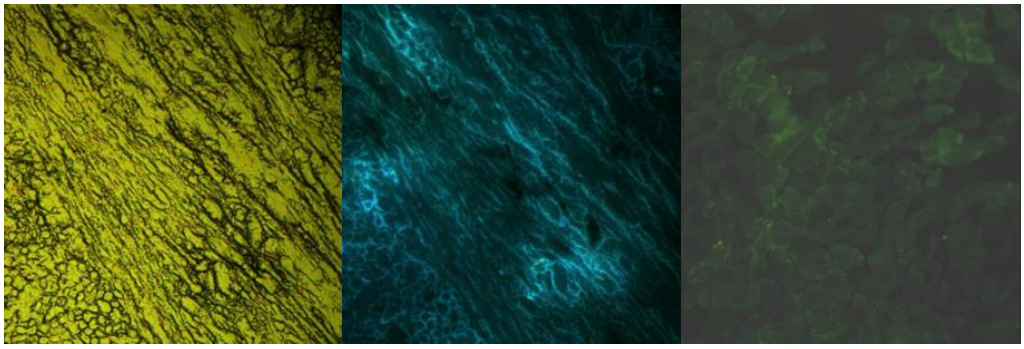

Fig. 7. (a) is the transmittance image. (b) and (c) are the fluorescence images. The fluorescence of (b) is 380nm. The fluorescence of (c) is 420nm. The different band of fluorescence can help us to discriminate the cell.

4. CONCLUSION

The relay-lenses scanning method was implemented in this article. The relay lenses replaced the traditional scanning part. The traditional method was very inconvenient for measurement and the cost was very expensive. This research was applied to measure the real oral cell and take their spectrum. We also calibrated the system for spectrum and radiometry. The calibration makes sure the correct of the hyperspectral imaging information.

5. REFERENCE

[1] C. T. Willoughby, M. A. Folkman, and M. A. Figueroa "Application of hyperspectral imaging spectrometer systems to industrial inspection," Proc. SPIE 2599, 264-272 (1996).

[2] C. Balas, V. Papadakis, N. Papadakis, A. Papadakis, E. Vazgiouraki, and G. Themelis, "A novel hyper-spectral imaging apparatus for the non-destructive analysis of objects of artistic and historic value," J. Cult. Herit. 4, 330s-337s (2003).

[3] M. B. Sinclair, D. M. Haaland, J. A. Timlin, and H. D. T. Jones, "Hyperspectral confocal microscope," Appl. Opt. 45, 6283-6291 (2006).

[4] Y. Garini, I. T. Young, and G. McNamara, "Spectral Imaging: Principles and Applications," Cytometry. 69A, 735-747 (2006).

6. ACKNOWLEDGEMENT

This work was supported by Nation Science Council of Taiwan under these two projects (NSC 99-2622-E-009-023-CC1 and NSC 99-2218-E-039-001).