Preliminary Result of Non-stained Cell Imaging for the KIRAMS Electron Microbeam Cell-Irradiation System

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1. Introduction

The KIRAMS electron microbeam Cell-Irradition System (KEMCI) provides a well-defined electron beam for single-cell irradiation [1]. The cell dish is located directly on the beam exit, therefore any cell-imaging techniques adopted must use reflective microscopy. Cell imaging becomes a critical subject when reflective microscope should be used for the nearly transparent target like cell. Fluorescent reflective microscopy has obvious merit that the cell images are easily obtained but has demeri that chemicals like fluorescent dye usually combined with ultaviolet can cause the undesirable cellular response in direct or indirect ways. For this, the cell imaging and processing routine have been already developed based on the threshold method and applied to the stained-cells [2]. For non-stained cells, there are various optical tricks which can be used to obtaine an image. These include Nomarsky differential interference contrast (DIC) technique [3] and phase contrast technique [4]. Recent improvement of quantitative phase microscopy is also worthy of notice [5] and another phase-based technique using Mirau interferometric objective lens was also developed for the Columbia University microbeam facility [6].

They both require special optical elements in the light path before it reaches the cells. We now try somewhat easier techniques to our optical system. One is the pseudo transmission technique. Another is the use of the reflector slider with ultraviolet and visible light from mercury arc lamp(HBO lamp) without dyeing.

2. Methods and Results

In this section some of the techniques used to snap the cell mage are described, which are normal visible light reflective microscopy, pseudo transmission microscopy, ultraviolet and visible light reflection microscopy and fluorescent reflective microscopy.

For test, human lung cancer cells were used as the sample. Images in various methods were obtained using an Achroplan 20x/0.50wPh2 objective lens and a W-PI 10x/23 eye lens. The image magnified by 200 times in the microscope is digitized in the image grabber to gray image with a resolution of 1300 × 1030 pixels. The image was processed using the National Instruments VisionAssistant v.7.0 and appropriate image processing method was applied for each image type.

Figure 1 shows an image obtained by normal visible light reflection microscopy using a halogen lamp and a reflector slider. The cell boundaries are not clear and the spots in the background are prominently shown. The pattern matching method was applied to the cell-recognition but the result was not so good and obscurity of the cell boundary and the prominent spots lead to the mis-recognition.

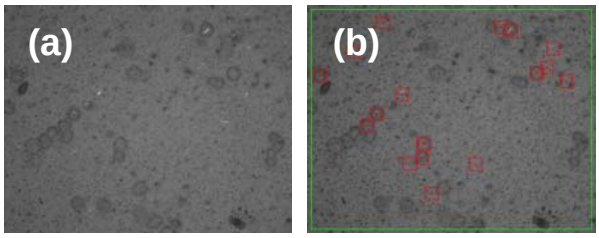

Fig. 1. Image obtained by normal visible light reflection microsocopy. The cell boundaries are not clear and the spots in the background are promently shown. (a) original image and (b) processed image and cell-recognition.

Figure 2 shows an image obtained by pseudo transmission microscopy, in which the cell boundaries are more clearly visible and the spots in the background are degraded. This was realized by covering the Mylar foil as vacuum window on the pin-hole, which enhanced the light reflected back into the cell dish. The cell image was processed using the eroding and patten matching method. This preliminary result was encouraging. The correctness for cell-recognition was better than that of normal visible reflective microscopy. The reflected light from the vacuum window material underneath the cell-dish must be more enhanced to establish the clear-cut boundaries of the cells and their culture medium.

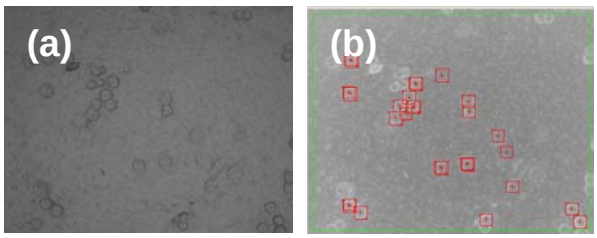

Fig. 2. Image obtained by the pseudo transmission microscopy, in which the cell boundaries are more clearly visible. (a) original image, (b) processed image and cell-recognition

Figure 3 shows an image obtained by ultraviolet and visible light reflection microscopy using a reflector slider and a mercury arc lamp. The image seems more realistically solid. Image processing was done using pattern matching and the cell-recognition rate was higher than those of normal visible reflective

microscopy and pseudo transmission microscopy. If the time for cell imaging is shortened as possible as we can to reduce the ultraviolet influence, this method can be also taken in consideration.

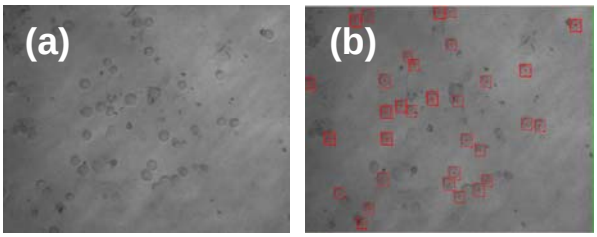

Figure 3. Image obtained by ultraviolet and visible light reflective microscopy, which seems more realistically solid. (a) original image, (b) processed image and cell-recognition.

Figure 4 shows a fluorescent image of the cells dyed with Hoechste for comparison of methods. Image was smoothed by using 3×3 median filter to eliminate noise and are convoluted by using highlight details. Afterward, the final image was abstracted by applying the threshold technique and the cell-recognition was done using the circle-searching technique, which shows the recognition rate of above 90 %.

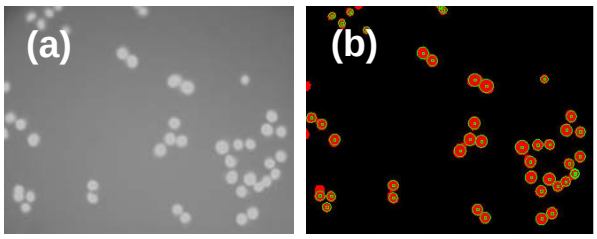

Figure 4. Fluorescent image of the cells dyed with Hoechste for comparison of techniques. (a) original image, (b) processed imaged and cell-recognition.

3. Conclusion

The preliminary images obtained by using the easily accessible methods for the non-stained cells and the image for the stained cell were demonstated for comparision. The results by pseudo transmission method and ultraviolet and visible light reflection method seem encouraging for non-stained cell imaging and these methods will be further tested.

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