A System for Automatic Control of the KIRAMS Electron Microbeam System

S.W. Park[1,3], D.H. Lee[2], G. M. Sun[1], K.B. Song[1], E.H. Kim[1]*, S.W. Bae[2], S.H. Hong[3]
[1] *Laboratory of Cyclotron Application, Korea Institute of Radiological and Medical Science, Seoul, Korea*
[2] *Laboratory of Radiation Effect, Korea Institute of Radiological and Medical Science, Seoul, Korea*
[3] *School of Electronic Engineering, Inha University, Incheon, Korea*
**corresponding to ekim@kcch.re.kr*

1. Introduction

An electron microbeam system is under development to investigate the biological effect of cells by irradiating cell-nuclei with low-energy and low-flux electrons[1]. It is essential to discern the cell nucleus from its cytoplasm and the culture medium and to locate it exactly onto the beam exit. The irradiation speed at more than 10,000 cells per hour is another requisite for the observations on cellular response to have good statistics. Long-time labor with patience and high concentration is needed since the frames of 320 ㎛ × 240 ㎛ should be moved more than 500 times for irradiating more than 10,000 cells per an hour.

The system consists of an electron gun, a beam transport chamber, a cell-irradiation stage with a cell image acquisition and cell position control system. This paper describes the electron microbeam system with a focus on the user interfaces concerning the process of automatically recognizing the cell nuclei and injecting electron beam into the target cell nuclei at the irradiation speed of more than 10,000 cell nuclei per hour.

2. System

The cell irradiation stage of the electron microbeam system consists of the image acquisition part and the cell position control part. The center coordinates of the cell nuclei are tracked with the accuracy of micro-meter scale[2] by utilizing the cell image acquired through a Carl Zeiss Axioskop 40 microscope, a CCD camera and an image grabber. An MCU-28 motor controller and an XY scanning stage (65×50mm) have eased the image tracking and positioning of the cell nuclei. The cell positioning is controlled either automatically by a user interface software or manually by a joystick. The user interface program was written in IMAQ Vision 7.0 for cell-image acquisition and processing, and LabView ver. 7.1 for the control of MCU-28 motor and the whole irradiation procedure.

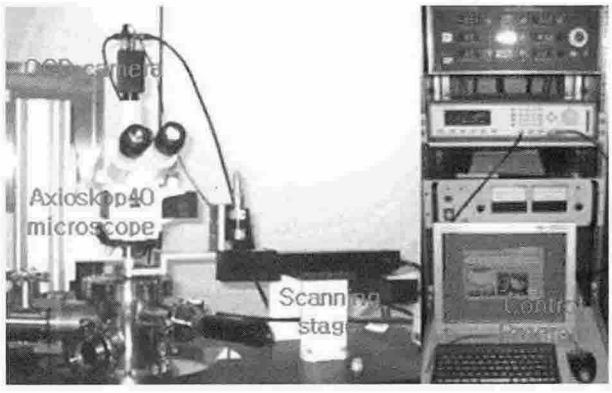


Figure 1. Cell image acquisition and target cell positioning part of the electron microbeam system.

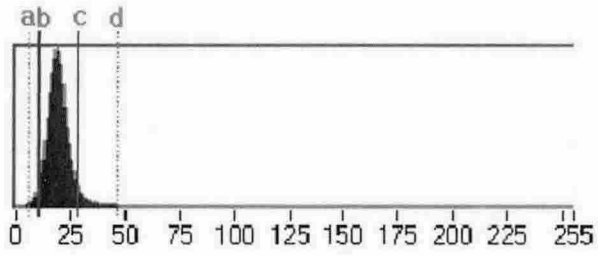
3. Methods and Results

First practice of cell image acquisition was made with L929 mouse fibroblast cells. Those cells were dyed with DAPI (4′6-diamidino-2-phenylindole, 0.33 ㎍/㎖ to observe with fluorescent microscopes using 50-watt mercury lamp as a UV light source. The analogue image magnified by 200 times in the microscope is digitized in the image grabber to 8-bit gray image with a resolution of 640 × 480 pixels. The size and the coordinates of the cell image were calibrated using a ruler with a unit of 10㎛

3.1 Automatic recognition of cell nucleus

The whole region of the cultured cells is divided into several hundred submilli-scale frames. The gray images of cells in each frame are gained through the image acquisition devices. The images are smoothed by using 3×3 median filter to eliminate noise and are convoluted by using highlight details. Afterward, the final images of cell nuclei are abstracted by applying the thresholding technique. Thresholding is one of the powerful methods for image segmentation; it is useful in discriminating objects from the background in many classes of the intensity histogram. In our case, the contour image is revealed as two parts, that is, cell nuclei and their background (including cytoplasm). The centric coordinates of the cell nuclei are extracted using some mathematical techniques and then registered in the buffer.

The threshold values can be determined by gaining an intensity histogram of the cell image as shown in figure 2. They are automatically searched, still leaving the chance to be changed by the user. The thresholds can be taken at b for the minimum and at c for the maximum instead of the corresponding values a and d considering the noise of images. After unnecessary objects around the cell nucleus are eliminated, the segmented images are converted into binary images. Every single cell nucleus is differentiated from its neighboring cell nuclei by applying the separate objects technique and then given with its center coordinates.



. Figure 2. Intensity histogram of cell image.
a. min value b. min value with a noise considered
c. max value d. max value with a noise considered

By embodying the automatic recognition process, more than 95 % of cell nuclei in 30 frames covering the 1 mm ×1 mm area were successfully detected. In an extended experiment with 374 frames covering the scope of 5 mm × 5 mm, the percentage of detected cell nuclei decreased to below 80 %. This results from the blurring of the microscope in focus during its movement over many frames, which means that the coordinates should be corrected in each frame. The coordinates values and the width of each cell nucleus are stored in database for stage driving.

3.2 Stage control

The cell positioning stage is composed of two DC motors driven by PID control method and one motor controller. PID control method creates an optimized operational condition by adjusting the gains of each control variable through the combination of proportion(P), integral(I) and differential(D). Each order delivered to control the stage movement is encoded to be recognized by the program written in Labview 7.1.

The cumulative error in the movement of 10 mm has been decreased to below 5 ㎛ as a result of taking calibration measurement marked with the unit of 10㎛ a sample and enhancing the accuracy of stage movement.

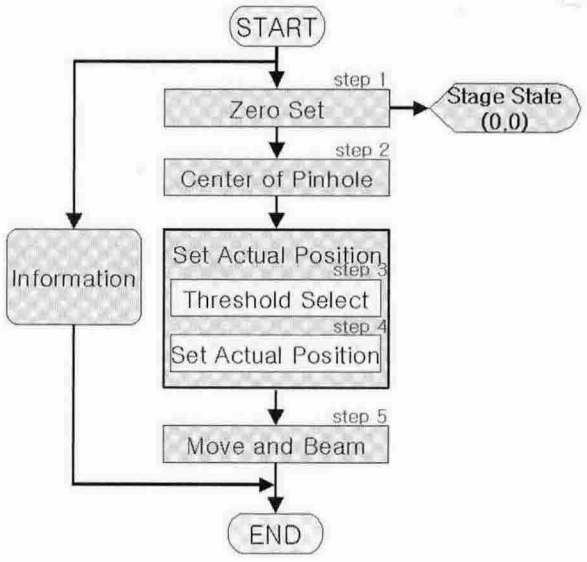


Figure 3. A flow chart of the system operation process.

4. Conclusion

An automatic control system for the cell image acquisition and cell positioning of the KIRAMS electron microbeam system has been demonstrated. With the L929 mouse fibroblast cells, more than 95 % of cell nuclei were recognized by image acquisition process. Regarding the accuracy in cell positioning, the cumulative error could be limited below 5㎛ for the movement of 10 mm by correcting the coordinates in each image frame. It is suggested that the irradiation scope of cell nuclei be limited to the 1 mm x 1 mm area due to the blurring in the focus of microscope during the movement over the wider area of the cell nuclei objects.

REFERENCES

[1] Myoung-Cheol Choi, Eun-Hee Kim, Ki-Baek Song, Dong-Hoon Lee and Seung-Woo Park, "Design and operation specification of an electron microbeam system for investing cellular radiation effect," Journal of Nuclear Science and Technology, in press.

[2] S.W. Park, D. H. Lee, M.C. Choi, E.H. Kim, S.H. Hong, "A Study of the Cell Image Acquisition and Target Tracking of Microbeam System for Microdosimetric Aspects of Low-Dose Radiation," IEEE EMBS Asian-Pacific conference on biomedical engineering, 2003

[3] Digital image processing, R.C. Gonzalez and R.E. Woods, Addison-Wisley, 2001.