An automated system for the KIRAMS microbeam

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Summary
An electron microbeam system has been developed to investigate the biological effect of cells by irradiating cell-nuclei with low-energy and low-flux electrons. 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 ㎛² should be moved more than 500 times for irradiating more than 10,000 cells per an hour. 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.

Key words:
Microbeam, cell irradiation, electron beam, target tracking

1. Introduction

There are burgeoning subjects in the field of life sciences, such as gene expression, cell cycle, DNA damage, chromosomal changes, and cell-to-cell communication [1–8]. In 1986, Yu et al. firstly reported the biological effects induced by ion beam implantation in seeds and microorganisms followed by applications in mutation breeding [1–3]. Recently, ion beam implantation has been widely used in DNA modification, DNA fragment transfer, gene mutation, and chemical origin and evolution of life. However, the mechanisms responsible for these biological effects are poorly understood, especially the different biological end points induced by either nuclear irradiation or cytoplasmic irradiation.

The biological interest in microbeams stems from the potential for energy absorption, mass deposition and charge exchange of energetic ions, resulting in bio-effects [1–8]. The use of microirradiation techniques in radiation biology dates back to the 1950s to the work of Zirkle and Bloom [8]. However, we are now able to take advantage of recent developments in particle delivery, focusing and detection, image processing and recognition and computer control, coupled with the benefits of new assays of individual cellular response.

Automated beam positing is required for the single electron hit technique for the individual irradiation of large number of cells. 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. Microbeam

An electron microbeam system has been developed to investigate the biological effect of cells by irradiating cell-nuclei with low-energy and low-flux electrons. The Korea Institute of Radiological and Medical Sciences (KIRAMS) electron microbeam system is designed to enable the target cells in vitro to be selectively irradiated with low-energy, low-current electrons.

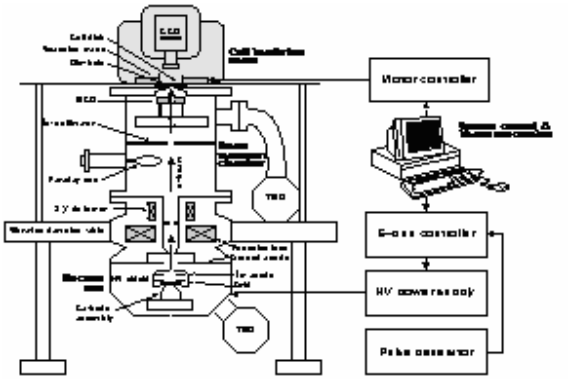

Fig. 1. Schematic diagram of the electron microbeam cell-irradiation system.

Though the basic scheme of the KIRAMS electron microbeam system is similar to that of other charged-particle microbeam systems, it must overcome additional difficulties, such as controlling an electron path under the

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influence of the environmental magnetic field, delivering low-energy electrons to the target cells through a scattering medium, and maintaining a stable, low-current electron beam. Fig. 1 presents the schematic diagram of the KIRAMS electron microbeam system. The system comprises (1) an electron gun, (2) a beam transport chamber, and (3) a cell image acquisition and positioning stage. The design requirements of an electron microbeam system to be used for studying low-dose radiation effects have been described in previous publications [9].

The computer-controlled unit for cell image acquisition and positioning is composed of a Carl Zeiss Axiotech100 microscope, a CCD camera, and an image grabber. The IMAQ Vision 7.0 is used for cell image processing. The cells are cultured in a Mylar-bottom dish, which is specially designed to minimize the degradation of the low-energy electron beam. After the cell dish is placed in the dish holder, the CCD camera captures the image of the cells attached to the dish bottom. The relative positions of the cells to the beam exit are registered by pre-processing of the cell image data. The target cells, assigned by the operator, are moved individually to the beam exit. For micro-precision target cell positioning, an XY stage (65 50 mm) and an MCU-28 motor controller are mounted on the cell irradiation stage. The LabView 7.1 is employed to operate the MCU-28 motor controller and to control the electron source beam generation.

3. 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.

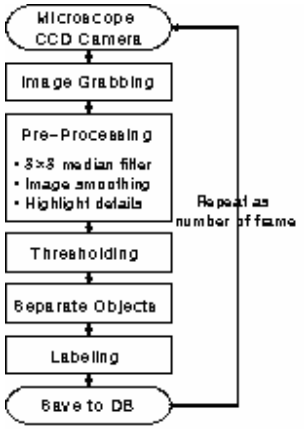
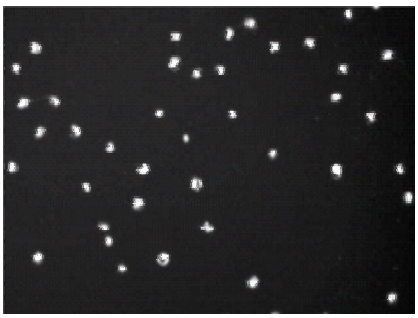
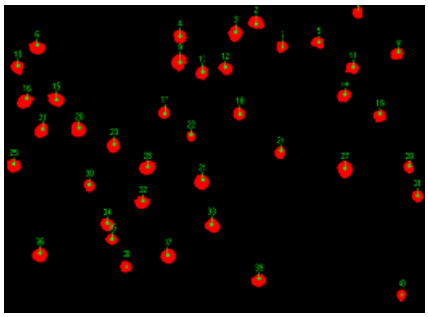


Fig. 2. A flow chart of the set actual positioning process.

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.



(a) Image of L929 mouse fibroblast cell



(a) Image of L929 mouse fibroblast cell after positioning

Fig.3 Auto positioning of L929 mouse fibroblast cell

They are automatically searched, still leaving the chance to be changed by the user. 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.

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.

4. Automatic 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 ㎛ as a sample and enhancing the accuracy of stage movement.

5. Cell experiment

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 ㎛.

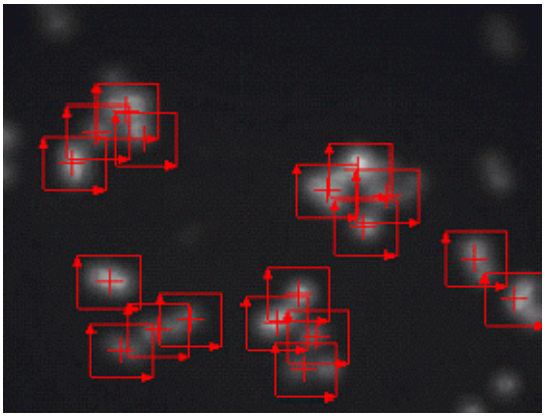


Fig. 4. The fluorescent image by UV after cell-irradiation.

We had irradiated to selected cell after image processing process. As a result, the target cells emitted light such as fig 4.

Table1 show the timing of the various steps in the microbeam irradiation protocol using L929 mouse fibroblast cell. It is to the exclusion of remove dish from incubator, aspirate medium, and mount on microbeam stage. The necessary time of image processing had measured using performance meter. The process divided four steps and necessary time measured using stopwatch. It takes 41 minutes at irradiation to 10,000 cells. It is suitable to the observations on cellular response to have good statistics.

Table. 1. Timing of the various steps in the microbeam Irradiation protocol.

Protocol step		Time
Step 1. Image processing of a frame	Image Grabbing: 20ms Smoothing-Medial: 9.0ms Highlight Details: 16.7ms Auto Threshold: 4.3ms Invert Binary Image : 1.7ms Remove small objects : 3.0ms Separate objects : 165.3ms Label objects: 5.7ms Save to DB: 100ms	325.7ms
Step 2 Visit each frame		300ms
(A) step1+ step2 each range of irradiation domain	A1. Range:1×1mm^2, Number of frames:20	12,214ms (12s)
	A2. Range:5×5mm^2, Number of frames:336	209,935.2ms (210s)
	A3. Range:5×10mm^2, Number of frames:672	420,170.4ms (420s)
	A4. Range:10×10mm^2, Number of frames: 1,344	840,640.8ms (840s)
Step 3. Irradiation after cell moving	Move of stage : average 150ms Irradiation: < 30ms	< 200ms
(B) The necessary time of step3 each range of irradiation domain	B1. Range:1×1mm^2, Number of cells: 300	60,000ms (1m)
	B2. Range:5×5mm^2, Number of cells: 5,040	1,008,000ms (17m)
	B3. Range:5×10mm^2, Number of cells: 10,080	2,016,000ms (34m)
	B4. Range:10×10mm^2, Number of cells: 20,160	4,032,000ms (67m)
Total time	stepA1+ stepB1	1.2m
	stepA2+ stepB2	20.5m
	stepA3+ stepB3	41.0m
	stepA4+ stepB4)	81.0m

6. 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.

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