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An Inexpensive, Automated Instrument for Laser Irradiation of Cultured Cells

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ABSTRACT

Objective: **Laser irradiation of cultured cells is a valuable technique for elucidating the mechanisms of low-level laser therapy, but is often tedious because of the need to manually change the position of the laser beam. Consequently, we developed a computer-based system that automatically moves a cell culture plate over a laser beam and times the exposure.** ***Background Data:*** **There are presently no commercial devices available for automated laser irradiation of cultured cells. Many investigators thus manually aim and time laser exposure, a time-consuming task that is prone to errors.** ***Materials and Methods:*** **We used outdated, surplus computer components to construct a system for automated laser exposure of cultured cells. This design strategy makes the system quite inexpensive.** ***Results:*** **Construction and operation of the system is described and an example of its use is presented. Alternate means of accomplishing automated laser irradiation are also presented.** ***Conclusion:*** **Inexpensive and relatively simple devices can be constructed for automated laser irradiation of cultured cells. These devices can eliminate the tedium and errors of manual laser exposure.**

Ed: Added # before intro head to base align columns.

INTRODUCTION

LASER IRRADIATION of cultured cells has become an important method for elucidating the photochemical and cellular mechanisms underlying low-level laser therapy.[1–4] A major drawback of this method is the necessity of manually aiming the laser beam on a succession of cell culture dishes and manually timing each exposure. Although this method is invaluable, it can be tedious and prone to exposure errors. These difficulties encouraged us to design and construct a simple computer-based instrument that automatically irradiates a series of cell culture wells. A key feature of our design is the use of outdated, unused, but serviceable computer equipment that accumulates due to rapidly changing computer technology.

The overall design of the automated irradiation system is straightforward. A computer with an Analog-to-Digital (A/D) converter produces two voltage signals that control the X and Y axes of an X-Y plotter. The X-Y plotter movement is mechanically connected to a frame that holds a 24-well cell culture plate over a fixed laser beam. A BASIC program controls the X and Y positions of the 24-well plate over the laser beam and times the exposure.

MATERIALS AND METHODS

X-Y plotter and plate mover

The X-Y plotter moves a 24-well cell culture plate over a stationary laser beam (Fig. 1). We used a Hewlett-Packard X-Y plotter that was previously used to plot on 21.5 × 27.8 cm (8.5 × 11 inch) paper. A rectangular frame for holding the cell culture plate was connected to the X-Y plotter by a length of lightweight (1.875 mm [1⁄16 in]) aluminum. The end of this aluminum piece was attached to the plotter pen-holder with small nuts and bolts (Fig. 1). The frame rested on a 1.0-cm (⅜ in) thick flat plastic panel that was supported four metal posts attached to a base plate. A 2.0-cm (¾ in) diameter hole was drilled in the center of the plastic supporting plate to allow the laser beam to be shone upwards onto the bottom of the cell culture wells.

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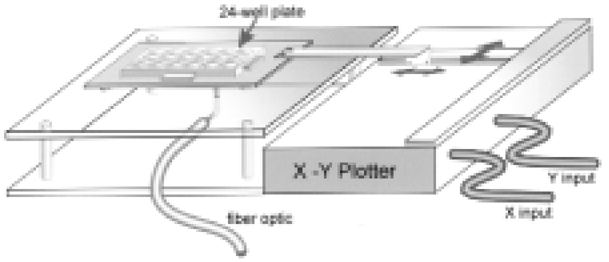

FIG. 1. Drawing of the instrument for moving a 24-well cell culture plate over a stationary laser beam. Computer commands activate the horizontal (X) and vertical (Y) movement of the X-Y plotter. The X-Y plotter is mechanically coupled to a platform on rollers that holds the cell-culture plate.

The frame for holding the 24-well cell culture plate was constructed by removing a central, rectangular area of the aluminum so the wells can be exposed to the laser beam. The cell-culture plate is held in place by the four rectangular pieces of plastic attached to the aluminum frame with double-stick tape. Since the frame must move smoothly over the supporting plastic plate, four rollers were installed at the corners of the frame. We simply used small (~6 mm [¼ in]) bearing balls that rested in small holes drilled into the frame.

The laser beam is shone upwards onto the bottom of the cell culture wells. In some instances, a fiber optic is aimed upwards (Fig. 1), while in other instances, a 45° mirror is positioned beneath the hole in the supporting plate and the laser beam is reflected upward.

Computer and D/A converter

We used an Apple IIe computer with an external 5½ in floppy disk drive. A two-channel, eight-bit D/A converter was plugged into a spare slot on the computer's motherboard. Each channel of the D/A converter could produce 256 different voltage levels from 0 to 5 volts. One channel was connected to the horizontal (X) axis of the plotter and the other to the vertical (Y) axis. Thus, voltage signals from Channel 1 of the D/A controlled vertical movement of the cell culture plate and Channel 2 controlled horizontal movement.

Software

A BASIC program controls the voltages put out by the D/A converter and hence the position of the cell culture wells over the laser beam. The program (Appendix I) consists of five subroutines, described below. Figure 2 shows the numbering of the wells on the cell culture plate. Wells are exposed in numerical order.

1. Enter variables and set initial values (Appendix I, lines 10–50). The program requests the operator to enter the exposure duration (ED) and number of wells to be irradiated (N). The exposure clock (CL) and count of number of wells exposed (CNT) are set to 0. The memory address of the D/A converter (A) is set. (This address is unique to the particular D/A board we used. Other D/A boards will use different addresses.)

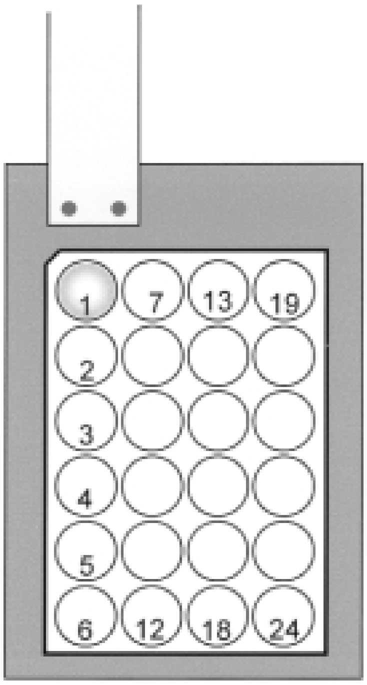

FIG. 2. Illustration of numbering of wells on the cell-culture plate.

2. D/A converter (Appendix I, lines 100–130). The core portion of the program enables the D/A converter to put out two voltages, one that controls the X-axis movement of the X-Y plotter, and the other that controls the Y-axis movement. Each channel of the D/A converter can produce 256 voltage steps, but only four are needed for the X direction and six for the Y direction since the cell-culture plate ahs 4 × 6 wells. Thus, a FOR-loop that controls X-axis movement (Appendix I, line 100) steps in units of 64 (i.e., 0, 64, 128, 192, and 256). (The 256 level is never used.) Similarly, the FOR-loop that controls the Y-axis movement (Appendix I, line 120) steps in units of 51 (i.e., 0, 51, 102, 153, 204, 255), producing six steps for the six wells. The "POKE" instructions in this section (Appendix I, lines 110, and 130) transfer the step number (e.g., 64) to the D/A converter.

3. Exposure timer (Appendix I, lines 150–210). The FOR loop executes for 1 sec. We determined the upper limit of the FOR loop empirically to produce 1-sec loop operation. When the loop is complete, the clock (CL) is incremented by 1 sec. An IF statement (Appendix I, line 190) tests to see if the clock has reached the desired exposure time (ED). If it has, the program goes to the next subroutine, if not, it executes the timer loop again.

4. Well count (Appendix I, lines 300–330). This subroutine keeps track of how many wells have been irradiated. Once the previous subroutine has completed irradiating a well, the well count is incremented by 1 and then the well count (CNT) is compared to the number of wells entered by the operator (N). If all wells have been irradiated (i.e., CNT = N), the program goes to the subroutine that returns the 24-well plate to the starting well (Appendix I, line 500). If all of the wells have not been irradiated, the D/A subordinate is activated and a new well is positioned over the laser beam.

5. Return wells to starting position (Appendix I, lines 500–550). This subroutine causes the D/A converter to put out zero volts in each channel, thus returning the 24-well plate to the upper-left (i.e., well 1). This subroutine is used initially to adjust the X-Y plotter offset controls to assure the well is centered over the laser beam. Once all of the wells have been irradiated, the plate is returned to this position so that the laser does not continue to expose the final well. We always leave the first well empty.

Calibration

Before using the system to expose cells, it is necessary to calibrate the X and Y movement of the cell-culture plate using the X-Y plotter gain and offset (zero) controls. Knobs on the X-Y plotter allow the operator to select the voltage range and the zero position of the pen. The calibration adjustment procedure is illustrated in Figure 3. Cell 1 is selected using an adjustment program (Appendix II) and the plotter's X and Y offset adjustments are used to center the cell over the laser beam. Cell 19 (upper-right) is then selected and the plotter moves. If the X-axis gain is too high, the cell culture plate will move too far as shown in Figure 3B. The X-axis gain is therefore reduced until the laser beam coincides with well 19. Cell 1 is again selected and the plotter offset adjusted as needed. This process is continued until the wells 1 and 19 are placed precisely over the laser beam. The same procedure is then carried out in the vertical (Y) direction using wells 1 and 6.

RESULTS

A photograph of our device is shown in Figure 4. We have used the instrument for irradiating cultured human dermal fibroblasts with a variety of lasers.

Experimental value of the autoirradiator

Since verifying the functionality and accuracy of the autoirradiator, we have used the device to irradiate cells with lasers and ultrasound. For example, in one of our earliest experiments, we used the device to deliver 0.2, 0.3, and 0.4 J cm^{-2} energy fluence of 904 nm Ga-As laser treatment to fibroblasts cultured in 24 well plates.[5] Given the output of the laser device, 7.0 mW, it was necessary to treat each well for 28.5 sec in order to obtain an energy fluence of 0.2 J cm^{-2}. Similarly, each well was treated for 42.85 sec or 57.15 sec to obtain 0.3 and 0.4 J cm^{-2} doses. Thus, in this experiment alone, it would have taken nearly 1 h to manually apply the three doses to the 24 wells per session (i.e., approximately 11.5, 17.1, and 22.9 min for the 0.2, 0.3, and 0.4 J cm^{-2} doses, respectively). Using the same parameters, about 3.0 h will be needed to manually apply treatment to a 72-well plate each time. Further examples of the time and labor savings afforded by the autoirradiator can be seen in Table 1, where we have summarized the amount of time needed to execute treatment in a few recent studies that provided enough information to computer time savings. The data underscore the labor-intensive nature of these experiments, as well as the cost savings and accuracy afforded by the autoirradiator.

DISCUSSION

We constructed the device described here because there is no commercially available instrument for this purpose. A central feature of the design is the use of "old," readily available computer equipment. Although our design used an Apple IIe computer, other types of computers should work as well. For example, IBM-compatibles with 386 and 486 processors usually have some form of BASIC available as part of the system. In addition, many older D/A boards work with these computers. The minimum D/A converter requirements are (1) two D/A channels, (2) eight-bit (i.e., 256 level) voltage output, and (3) simple BASIC instructions control the converters. We chose to use BASIC software for its simplicity, but other programming languages that are compatible with the D/A con-

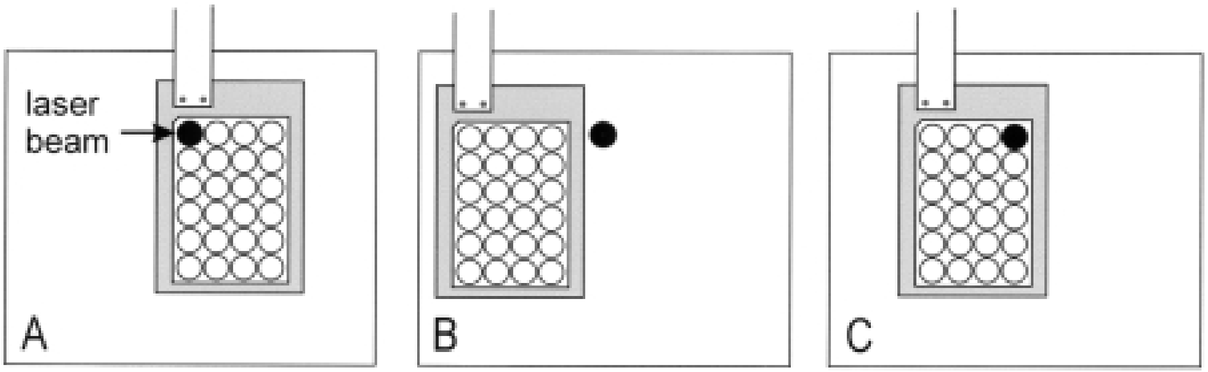

FIG. 3. Illustration of method for adjusting the X-Y plotter gain and offset so that wells are accurately exposed. **(A)** Well 1 centered over laser beam using offset controls. **(B)** Well 19 selected using program, but X-axis plotter gain is too high. **(C)** After reducing X-axis gain, well 19 is over the laser beam.

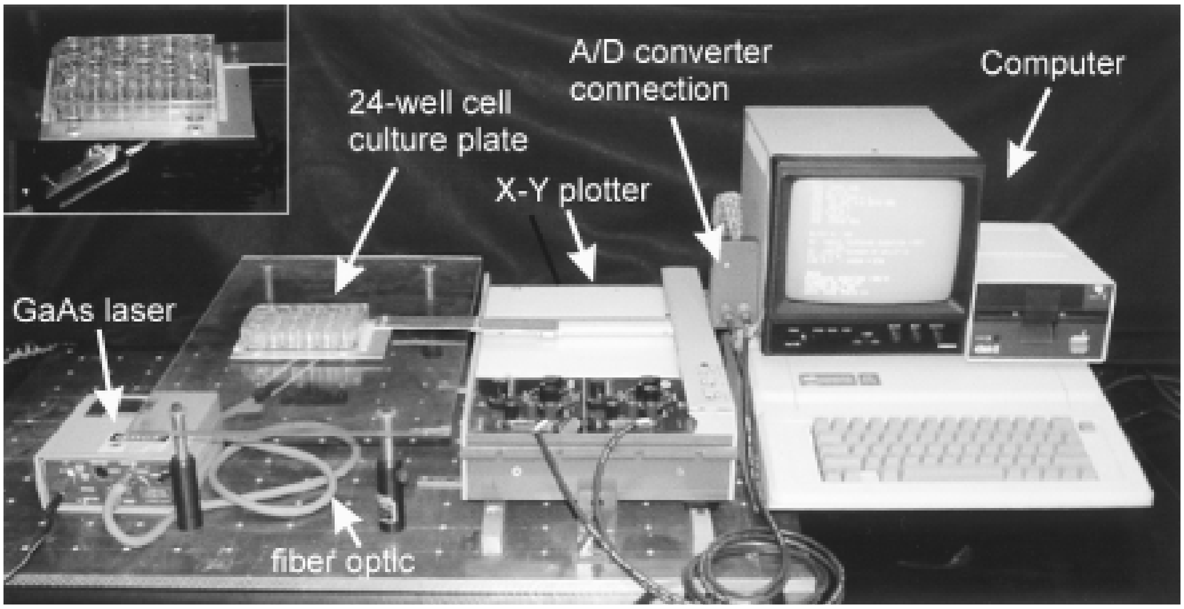

FIG. 4. Photograph of apparatus being used to expose cultured cells with a GaAs laser.

TABLE 1. A FEW RECENT STUDIES IN WHICH TIME AND LABOR COULD HAVE BEEN SAVED USING AUTOIRRADIATION

Study	*(a) Power (mW)*	*(b) Energy fluence (Jcm^{-2})*	*(c) Number of wells/ culture*	*(d) Treatment time (sec) per well/culture*	*(e) Treatment time (sec) (c × d)*	*(f) Treatment sessions*	*(g) Total time (h) (Σe) × f*
Kreisler et al.[6]	10	0	22	0	0	2–3 per well	6.4–9.6 h
	10	1.96	22	75	1650		
	10	3.92	22	150	3300		
	10	7.84	22	300	6600		
Kipshidze et al.[7]	3.5	0.10	24	60	1440	1 per well	73.6 h
	3.5	0.31	24	180	4320		
	3.5	0.52	24	300	7200		
	3.5	1.05	24	600	14400		
	3.5	1.57	24	900	21600		
	3.5	2.10	24	1200	28800		
	3.5	3.15	24	1800	43200		
	3.5	4.20	24	2400	57600		
	3.5	6.30	24	3600	86400		
Thawer et al.[8]	30	0	12	0	0	3–5 per culture	5.1–8.5 h
	30	0.23	12	7.67	92		
	30	1.37	12	45.67	548		
	30	2.75	12	91.67	1100		
	30	2.75	12	91.67	1100		
	30	3.66	12	122.67	1464		
		4.58	12	152.67	1832		

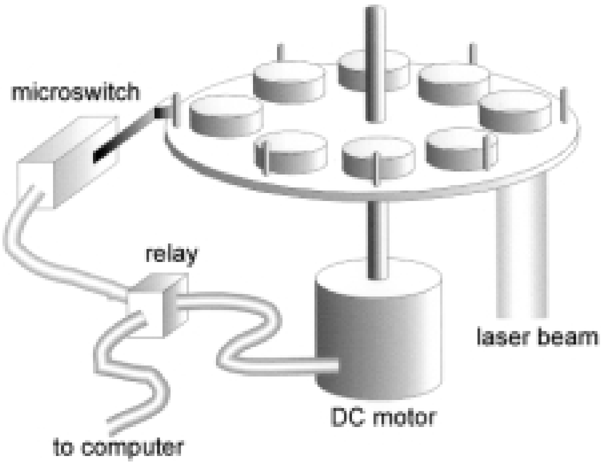

FIG. 5. Design of a rotary mechanism for exposing cultured cells.

verter could be used. It should also be possible to use “higher-order” programming for instrument control, such as that provided by LabView (National Instruments, Austin, TX) or HPV (Data Translation, Marlboro, MA).

The instrument design described here used an X-Y plotter as the “motor” to move the 24-well cell culture plate. Such plotters are often found unused because modern computer equipment and software provides faster, more convenient means of displaying graphical data. There are, however, other possible means of moving the cell culture dishes over the laser beam under computer control. A possible instrument, using rotary control, and individual cell-culture dishes is shown in Figure 5. A signal from a computer (or possibly a laboratory timer) activates a relay causing the motor to turn and bring a cell-culture dish over the laser beam. When the dish is in position, a small vertical post attached to the circular plate activates the microswitch that stops the motor. After irradiation is complete, another signal from the computer reactivates the motor and moves the next cell dish over the laser beam.

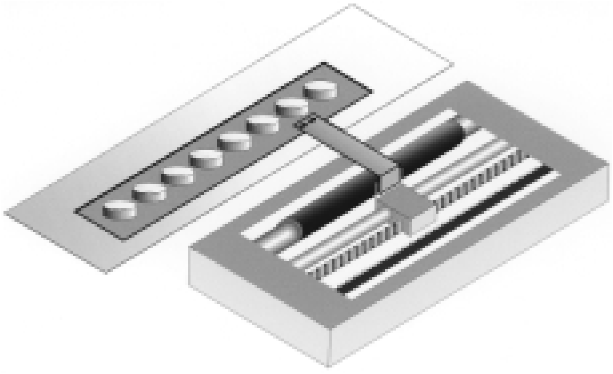

FIG. 6. Design of a mechanism for moving a row of cell culture dishes over a laser beam using a dot-matrix printer.

Yet another possible design uses a dot-matrix printer (Fig. 6). The print head of dot-matrix printers is advanced in fixed units by simple computer commands (e.g., PRINT “-”). A specially constructed linear arrangement of cell-culture wells could be moved by connecting it to the printer pen carriage. This design does not require a D/A converter and would need only simple programming.

CONCLUSION

In summary, the device we have described eliminates the tedium often associated with laser irradiation of multiple cell samples. The device has been used for irradiation of cultured cells with several different lasers. In addition, we have used it for ultrasound irradiation by positioning the ultrasound head above the cell culture well plate using ring stands and clamps. With minor adaptations, the system can be used to treat cells or tissue samples with electrical stimulators, capacitors, or electromagnetic fields. There are many possible designs of an automated irradiation device. Our description here is intended primarily to provide the interested investigator with the overall concept for constructing an instrument for automated cell laser irradiation.

APPENDIX I: COMPUTER PROGRAM FOR MOVING CELL CULTURE PLATE

```
 10   INPUT “EXPOSURE (SEC)”: ED     Input exposure duration (ED).
 20   INPUT “NUMBER OF WELLS”; N     Input number of wells to be irradiated (N).
 30   CL = 0                         Set exposure clock timer to zero.
 40   A = 16384 + 256                Set D/A converter initial value.
 50   CNT = 0                        Set well count to 0.

 60   GOSUB 500                      Subroutine to center first well over laser beam.

100   FOR K = 0 to 255 STEP 64       Loop to set X well position. Four steps.
110   POKE A+0, K                    Set D/A voltage for X well position.
120   FOR I = 0 to 255 STEP 51       Loop to set vertical well position. Six steps.
130   POKE A+1, I                    Set D/A voltage for Y well position.
```

APPENDIX I: COMPUTER PROGRAM FOR MOVING CELL CULTURE PLATE(CONT’D)

```
150   FOR J = 0 TO 302              Exposure timer loop. Time 1 second.
160   D = J                         D is a dummy variable
170   NEXT J
180   CL = CL + 1                   Increment timer by 1 second
190   IF CL = ED GOTO 210           Test if clock (CL) has reached ED.
200   GOTO 150                      Clock has not reached ED, continue loop.
210   CL = 0                        Exposure duration achieved, reset clock.

300   CNT = CNT + 1                 Increment well count.
310   IF CNT = N GOTO 500           All wells exposed, return to well 0,0.
320   NEXT I                        Not all wells exposed, go to next vertical well.
330   NEXT K                        Not all wells exposed, go to next horizontal well.

500   PRINT “CENTER WELL 0”         Subroutine for adjusting well 0, 0 over laser beam
510   POKE A + 0, 0                 Set X D/A voltage to 0.
520   POKE A + 1, 0                 SET Y D/A voltage to 0.
530   INPUT “WHEN DONE PRESS 1”; Z  Press “1” when done adjusting zero
540   IF Z = 1 GOTO 100             Begin irradiation.
550   GOTO 530                      Continue if “1” not pressed.
```

APPENDIX II: COMPUTER PROGRAM FOR ADJUSTING X-Y PLOT MOVEMENT

```
 10   PRINT “ENTER 1 FOR H, 2 FOR V”; AX
 20   IF AX = 1 GOTO 100
 30   GOTO 300

100   PRINT “ENTER WELL 1 OR 19”; HW
110   IF HW = 1 GOTO 150
120   IF HW = 19 GOTO 170
130   GOTO 100
150   POKE A + 0, 0
160   GOTO 180
170   POKE A + 0, 256
180   PRINT “PRESS 4 TO CONTINUE H ADJ”; CN
190   IF CN = 4 GOTO 100
200   GOTO 10

300   PRINT “ENTER WELL 1 OR 6”; VW
310   IF VW = 1 GOTO 350
320   IF VW = 6 GOTO 370
330   GOTO 300
350   POKE A + 1, 0
360   GOTO 380
370   POKE A + 1, 256
380   PRINT “PRESS 4 TO CONTINUE H ADJ”; CN
389   IF CN = 4 GOTO 300
400   GOTO 10
```

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