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COMPUTER-BASED TRACKING OF LIVING CELLS

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SUMMARY

A computer-based tracking technique has been developed to follow the movement of living cells and keep them centered in the field of view of an optical microscope. With the use of an image-processing system, the video image of a cell can be sufficiently processed to allow computer-recognition of the cell boundaries. Determination of the location of the center of the cell enables comparison of successive cell positions and correction for any cell movement. In order to illustrate the versatility of this technique, patterns of movement were obtained of cancerous and non-cancerous cells in an effort to determine the difference in motility between the two cell types. After examination of the data gathered, it was found that there is no difference in the motility between the two cell types over 1-h periods.

The need to observe a given cell over an extended period of time is a frequent problem confronting the cell biologist. Not only is it necessary to keep a cell centered and in focus in the field of view, but also it may be desirable to obtain a graph showing the cell's actual movement with time. Although methods have been developed to obtain a record of a cell's movement [2, 6], they are tedious to do, and may also result in damage to the cell [2]. In addition, it has been virtually impossible to keep a cell centered in the field of view under high magnification over an extended period of time. Recent advances in the study of cell motility make the need to record cell movements over time even more important [5].

In order to track cells, a technique must be developed that can recognize a cell, determine if it has moved, and move the microscope stage to correct for the movement; it must also display numerical and graphical data of the movement. Finally, all this must be done without damaging the cell. Whereas this may be beyond the

means of conventional equipment in microscopy, it is not out of reach of high speed digital image processing. It should be possible to use a computer-controlled image processing system to enhance an image of a living cell and make a determination of its position.

The purpose of this paper is to report the development of a computer-based tracking technique that is capable of determining the center of a cell, detecting a change in its position, correcting for the change, and displaying a graph of the movement. In order to illustrate the usefulness of the technique, cells from a non-malignant mouse cell line (3T3) and a related cell line with malignant potential (3T12) were tracked to see if there was any difference between the movement of non-tumorigenic and tumorigenic cells [1].

MATERIALS AND METHODS

Optics

Optical images are produced by an inverted Zeiss AXIOMAT microscope using phase contrast optics.

All images are obtained with a $\times 25$ Neofluar phase 2 objective and further magnified $\times 1.4$ with the variable zoom control on the AXIOMAT. The image is further magnified another $\times 1.6$, producing a final magnification of $\times 56$. Microscope images can be viewed through the oculars and projected onto the video camera simultaneously. The stage of the microscope is driven in the X-Y plane by two $0.5\text{-}\mu\text{m}$ step motors which can be moved manually or under the control of the computer. Additionally, a Zeiss autofocus device is used to maintain a proper focus on the specimen.

The video camera (model LST-1, Sierra Scientific Products, Mountain View, Calif.) consists of a 1-inch Newvicon video tube. The output video signal can be modified with external controls for gain, d.c. offset, and contrast. The video signal is generated in a 525 line format at a rate of 30 frames/sec. Output from the camera can be directed simultaneously to a 13-inch black and white monitor, to a video tape recorder, and to the input of the image processor.

Image processor

At the input of the image processor, a video digitizer converts each video line into 512 sequential picture elements (pixels). Each pixel may range in value from 0 (black) to 255 (white). The result is that the image is converted to a 512×512 array of pixels which can be stored in memory with each pixel taking an integer intensity from 0 to 255.

The image processor (Model IP5500, De Anza Systems, San Jose, Calif.) was originally designed for analysis of LANDSAT images and has been adapted for use with the microscope. The processor contains three separate 512×512 memory channels that correspond to the red, green, and blue primary images of a color picture; however, they may be used independently of each other to store and manipulate images. The processor itself can perform one of 96 different arithmetic or logical operations on any combination of two inputs from the three memory channels or the live digitized video signal. Additional hardware features allow for the definition of a specific region of interest by generating a cursor-defined box controlled by an external joystick. The box may be made any size up to the full field and moved in the X-Y plane. Finally, image manipulation can be obtained through the use of predefined look-up tables which substitute different intensity values in the 0-255 range. The processed image is converted back to video format and displayed on a 19-inch color monitor.

Computer

An LSI-11/23 microprocessor (Digital Equipment Corporation) controls the operation of the image processor by performing read/write operations to registers that control the processor operation. FORTRAN and MACRO language programs that direct the read/write operations are stored on floppy disks and an RLO1 hard copy disk.

Program

The purposes of the program developed and reported here are: enhance the edge of the cell, locate the center

from the enhanced edges, determine if the center has moved, move the stage in the direction opposite of the movement, and display all the movements in graphical and numerical form.

Fortunately, many techniques already exist for enhancing the edge of an object [4]. One technique consists of calculating a convolved or smoothed transformation of the image (see eq. 1), and subtracting it from the original unsmoothed image. This involves looking at a window around each pixel and calculating an average intensity according to eq. (1), and replacing the pixel at the center of the window with that value. A box of larger dimensions averages larger blocks of the image and smooths the image to a greater degree than smaller boxes.

If the image is a function $I_{(x,y)}$, then the smoothed image, $S_{(x,y)}$, is

$$S_{(x,y)} = \frac{\sum_{i=x-\frac{(N-1)}{2}}^{x+\frac{(N-1)}{2}} \sum_{j=y-\frac{(N-1)}{2}}^{y+\frac{(N-1)}{2}} \cos\left(\frac{2(i^2+j^2)^{1/2}}{N}\right) e^{\left(\frac{-4(i^2+j^2)^{1/2}}{N}\right)}}{N^2}, \quad (1)$$

where N = length of box edge which must be ≥ 3 and an odd number.

The edge enhanced image is $E_{(x,y)}$ and is equal to:

$$E_{(x,y)} = 255 \quad \text{if } I_{(x,y)} - S_{(x,y)} > \text{threshold} \\ E_{(x,y)} = 0 \quad \text{if } I_{(x,y)} - S_{(x,y)} \leq \text{threshold}$$

where the threshold is > 0 and < 255 .

The program reported here uses this technique to transform the entire image and stores the result in one of the three memory channels. This smoothing process most strongly affect the intensity of edges in the image. When the smoothed image is subtracted from the original image, the area with the greater changes (i.e., the edges) will be either lightest or darkest. The background will then drop to near-black, whereas edges will remain either darker or lighter than the background. A substitution of pure white for the edges and pure black for the rest of the field exactly defines the edges within the cell. Depending upon the choice of the window size, finer and finer edges can be dropped out, leaving only the major edges of the cell displayed (e.g., cytoplasm, nucleus, nucleoli).

The image is scanned for pure white pixels, and the total number of white pixels is counted. The size of the box surrounding the object is decreased in the X-direction by moving one side of the box a column at a time, and the number of white pixels is counted again. The process continues in this fashion until half the original total of white pixels is reached; the edge is the center in the X-axis. The process is repeated for the Y-axis. The center is calculated for each image. If the center obtained is different from the first center calculated, then the computer sends an electronic pulse to the microscope stage, moving it in the direction opposite that of the cell's movement. It also sends a pulse triggering the auto-focus mechanism.

If the program detects a movement, it will take in a new image immediately and repeat the process of locating the center in order to see if the previous move-

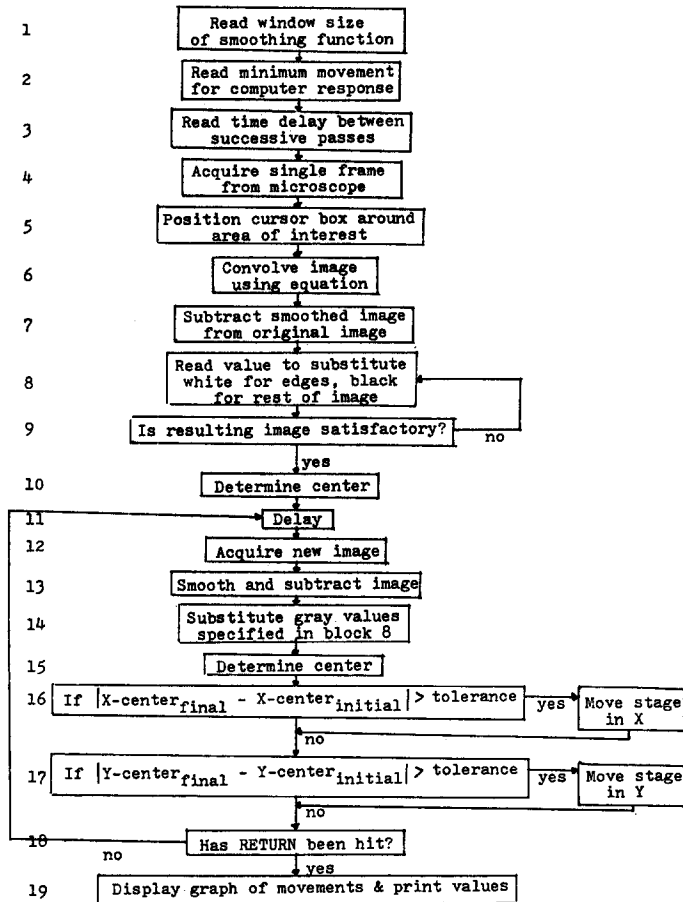


Fig. 1. Flowchart of program.

ment brought the cell back to the center of the field. If there was no movement, the program will wait a specified amount of time before repeating the process.

When it is desired to stop the program, one merely presses a key and enters a scale factor for the graph. The program will then display the graph of the cell's movement and print the actual coordinates.

Cells

In order to test the program, cells of two different types were tracked for a period of 1 h. The cultures were obtained from the University of California, Irvine, Cell Culture Laboratory (Department of Developmental and Cell Biology). Both cultures of cells were obtained from BALB mouse embryonic fibroblasts. One group, a non-transformed (non-cancerous) culture, designated 3T3 by the American Type Tissue Culture Collection (Washington, D.C.) was compared with the 3T12 cells, a culture transformed to the cancerous state. Both groups were suspended in minimal essential culture medium containing vitamins, essential and non-essential amino acids, 10% fetal calf

serum (FCS), and antibiotics. The culture was set up at a density of 1.3×10^4 cells/ml and maintained at a pH of 7.0–7.2. The cells were grown in rose culture chambers on no. 1 thickness glass coverslips and incubated in a 10% CO_2 incubator at 37°C for 1–2 days before tracking.

All cells chosen for tracking were selected on the basis of their morphology. The cells selected were always isolated from cell clusters and displayed spindle-like shapes with a flat, ruffled end and an elongated tail-end (fig. 2). At least eight cells were tracked from each cell type.

RESULTS

The first step in the tracking process is to develop a program to determine whether a cell has moved. The flowchart in fig. 1 illustrates the logic used in developing the program.

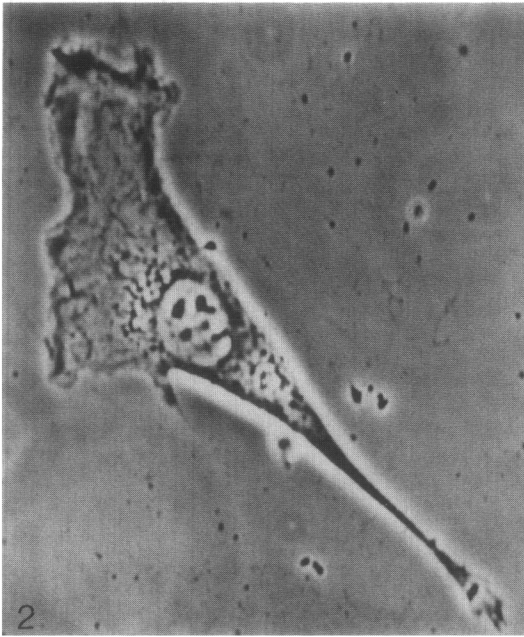


Fig. 2. An unenhanced image of a cell displaying morphology of cells tracked.



Fig. 3. The same cell after edge enhancement.

The first two blocks of the flowchart define parameters necessary for enhancing the image of the cell's edge and the minimum cell movement necessary for the computer to respond. Block 3 accepts a time delay factor. Blocks 4 and 5 acquire the image and

define the region of interest. Blocks 6-9 enhance the image and allow explicit defining of the edge through black and white substitutions. Block 10 determines the center of the cell. Block 11 provides for the specified time delay. Blocks 12-15 repeat the process of enhancing the image and determining the center of the cell. Block 16 and 17 decide if a correction for the cell's movement is needed. Block 18 looks for a 'carriage return', an indication to terminate the tracking process. If the process is terminated, a graph of the cell movement is displayed on the video monitor, and the set of coordinates is printed on a line printer.

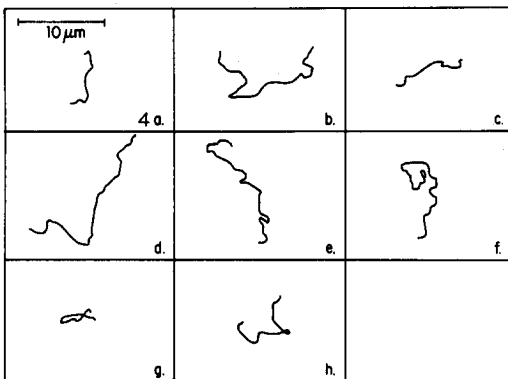


Fig. 4. Reproductions of 3T3 tracks.

The photograph in fig. 2, taken from the video monitor, shows an unenhanced image of a cell exhibiting the morphology typical of the cells tracked with the program. Fig. 3 is a photograph after the image of the cell has been edge-enhanced.

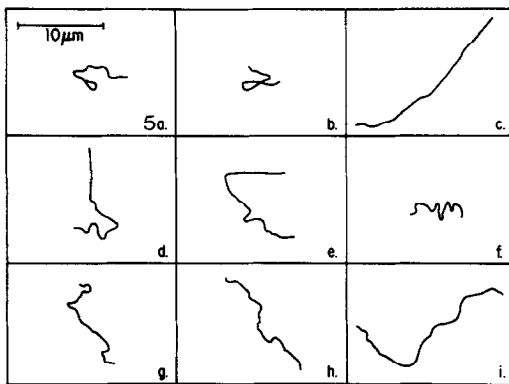


Fig. 5. Reproductions of 3T12 tracks.

The graphs in figs 4 and 5 are reproductions of tracings of 1-h cell tracks made directly from the video monitor. The data in tables 1 and 2 show the total distance traversed by each cell, the net displacement between the final and initial positions, and the ratio of total distances to net displacement. Also included in tables 1 and 2 are means, variances, and standard deviations.

Table 3 gives the data from a *t*-test performed on the two populations [3]. It shows the standard error of the differences between the means of the two cell groups, the

Table 1. Movement data for 3T3 cells

Cell	Total distance (μm)	Net displacement (μm)	Total/net
a	16.0	7.65	2.09
b	26.0	10.6	2.46
c	15.8	7.60	2.07
d	30.0	20.7	1.45
e	25.4	12.3	2.07
f	20.9	6.20	3.37
g	15.0	1.12	13.4
h	17.1	5.30	3.21
Mean	20.8	8.94	3.77
$\sigma^2(N-1$ weighting)	32.6	34.0	15.6
$\sqrt{\sigma^2}$	5.71	5.83	3.94

Table 2. Movement data for 3T12 cells

Cell	Total distance (μm)	Net displacement (μm)	Total/net
a	12.4	3.50	3.53
b	12.3	4.75	2.58
c	26.3	24.3	1.08
d	22.3	11.8	1.89
e	23.3	9.00	2.58
f	10.4	5.00	2.06
g	17.4	9.95	1.75
h	19.9	17.4	1.14
i	25.4	19.9	1.27
Mean	18.9	11.7	1.99
$\sigma^2(N-1$ weighting)	36.2	47.7	0.651
$\sqrt{\sigma^2}$	6.01	6.91	0.807

values of *t*, and the approximate value of $t_{0.05}$ required for a significant difference between the means with 95% confidence. The value of *t* must be equal to or greater than $t_{0.05}$ before significance can be claimed.

As the *t*-test indicates, none of the calculated *t*s exceed the required *t* at the 95% level. One can then conclude that the two populations of cells have no significant difference between their means in any of the three areas evaluated: total movement, net displacement, and the ratio of total movement to net displacement.

DISCUSSION

Although no difference was observed between cancerous and non-cancerous cells in the area of cell motility, the computer-technique developed proved successful. The 1-h tracks obtained indicate the computer's ability to follow a cell over an extended period of time (subsequent investigations have involved cell tracking for up to 72 h). Stray particles entering the field of view often caused errors. They were enhanced with the rest of the image

Table 3. *t*-Test data

	Total distance	Net displacement	Total/net
$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{\frac{\Sigma(x - \bar{x}_1)^2}{N_1(N_1 - 1)} + \frac{\Sigma(x - \bar{x}_2)^2}{N_2(N_2 - 1)}}$	2.85	3.19	1.42
$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1 - \bar{x}_2}}$	0.667	0.865	1.25
$t_{0.05} = \frac{S_{\bar{x}_1}^2 t_1 + S_{\bar{x}_2}^2 t_2}{S_{\bar{x}_1}^2 + S_{\bar{x}_2}^2}$	2.34	2.33	2.37

and tended to cause errors in the location of the center. By selecting large box sizes for the convolving function, the effect of small particles can be held to a minimum. It is important to realize that previous techniques of cell tracking [2, 6] have shown to be applicable only in limited situations. The technique reported here not only displays the cell's movements graphically but keeps the cell centered in the field of view. This allows experiments to be performed on highly motile cells under high or low magnification without the cells moving out of the field of view.

Having demonstrated that the program tracked cells successfully, the results of the experiment must be interpreted. The failure to show a significant difference between the motility of cancerous and non-cancerous cells in this experiment does not mean that the difference does not exist. It was hoped that differences would occur in the areas of total distance moved or net displacement. The ratio of these two values gave an indication of the straightness of the tracks, a straighter track having a value closer to 1 than a curved track. It was hoped that one of the groups would display less direct tracks than the other.

The inability to find a difference in any of the three areas tested only indicates that 3T3 and 3T12 cells displaying similar morphology move similarly over 1-h time periods. Before concluding that there is no difference between the motility of the two cell lines, one must determine if there is any difference in movements over longer periods of time, or whether cells displaying the morphology of the cells tested are more abundant in one group than in the other. It must be understood that the question of the difference in motility is not simple; many questions must be answered before conclusions can be drawn. The experiment reported here merely serves as a starting point and demonstrates the effectiveness of the computer-tracking technique developed. This technique can now be applied to many different problems in cell biology that involve cell movement.

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