Time lapse movies of cell movement and mitosis

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Abstract

Using cultured mammalian cells, an inverted microscope, and a CCD video camera, it was possible to demonstrate motility of single cells and movements within cells. We looked at membrane ruffling, making and breaking cell-cell contacts, the action of trypsin on cellular adhesions, the identification of late G2 phase cells by characteristic movements of the nucleus, and mitosis.

Introduction

Photographing movements in cultured cells is about the most popular topic in my cell biology lab course. Movie-making is quite simple and it opens up a wide variety of inquiry-driven special projects.

Equipment and supplies

We use a 37°C CO_2-humid incubator, a liquid nitrogen freezer for storing cell lines, a 30-year old Nikon inverted phase contrast tissue culture microscope with 10x and 20x objectives, a COHU model 4915-2010 analog video camera, and a 7-year old "AV" Macintosh 7600 computer running the freeware Scion/NIH Image 1.62. We also have an old reconditioned laminar flow sterile hood but I think the hood is not essential if there is another available nearby for occasional use. The cells we use mostly are the human line SV80 and the mouse line 3T12 because they grow well in inexpensive medium, Dulbecco's modified Eagle's medium (DMEM) plus 5% adult bovine serum. I would be glad to discuss equipment, supplies, and procedures by e-mail and I would be glad to distribute the cell lines.

Tissue culture methods and microscopy in a lab class

We do tissue culture on the open bench in my class. We passage the cultures for a week or two after seeding them from a frozen vial but we prepare the frozen cells in a sterile hood. The sterile hood itself is much too small to teach a class of 15-25 students. Even when working on the open bench, we rarely get bacterial or fungal contamination, and if we do get contamination, we can thaw another vial of frozen cells and can get back in business in a day or two. In some motility studies, we can work at room temperature on the microscope stage, but in most others including mitosis, we need to have the cultures in the 37°C incubator. We can position a T-flash reproducibly on the microscope stage by abutting it against guides, two microscope slides taped on the stage at right angles to each other. Then we can photograph the same area of interest repeatedly and have the culture out of the incubator for no more than a minute.

Membrane ruffling and computer use

Many cell lines show membrane ruffling but the most actively ruffling cell line we have found so far is the human SV80 line derived from a Fanconi's anemia patient and immortalized by transformation with Simian Virus 40, a DNA tumor virus. (The integrated viral genome in SV80 is replication-defective and so SV80 is not a health hazard.) Isolated SV80 cells show constant and rapid membrane ruffling, which slows down when cells contact each other. To make a movie of ruffling SV80 membranes (or of any other cell mobility), we launch Scion/NIH Image, choose “Start Capturing” under the Special menu, focus the image on the computer screen, and draw a box around an image of cells using the area-selection tool. Under the Stacks menu, we choose Make Movie and choose 50 frames at 10 seconds per frame. The movie takes 500 seconds or 8.3 minutes to make. We give the movie a name (Save As…) and save it on the hard drive or on a student's Zip disk. We play it back by double-clicking on its icon and choosing Animate from the Stacks menu. Animate plays the movie over and over. We control the speed of playback by selecting a speed, digits 1-9 on the numeric keypad.

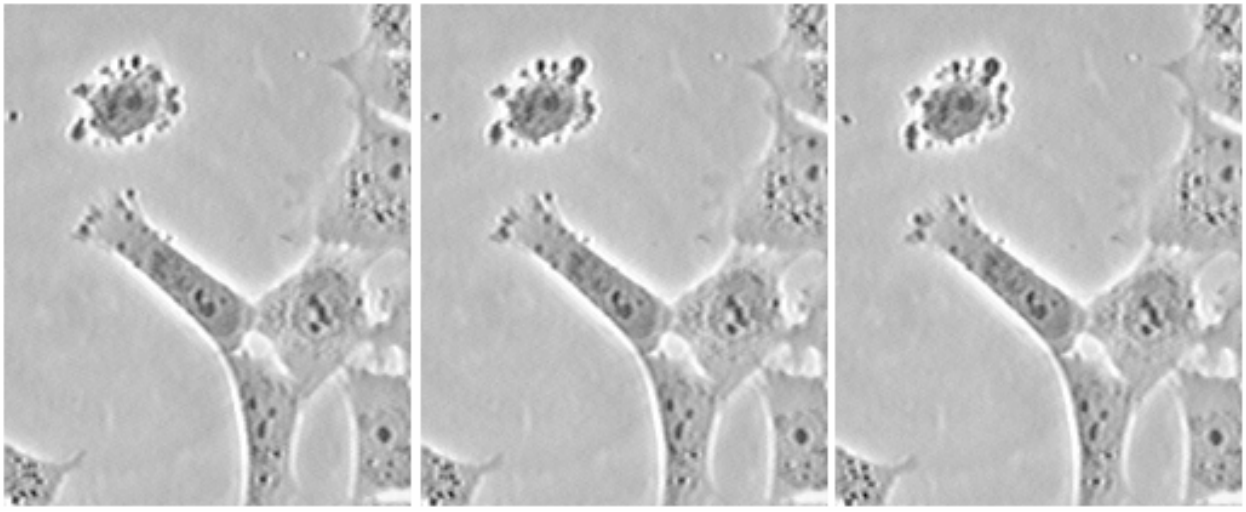


Figure 1. Membrane activity in isolated SV80 cells, 3 frames from a movie with 10 second intervals. Notice the variations in isolated cell and the extended ruffled membrane of the attached cell. The effect is much more dramatic by making continuous animation, as described.

Making and breaking contacts

Confluent areas of SV80 cultures are not totally inactive; they make and break contacts with each other continuously. In a longer movie (30-second intervals instead of 10-second intervals), we can see SV80 cells making and breaking contacts between its neighbors. Sometimes a cell will break away from a group and take off on its own.

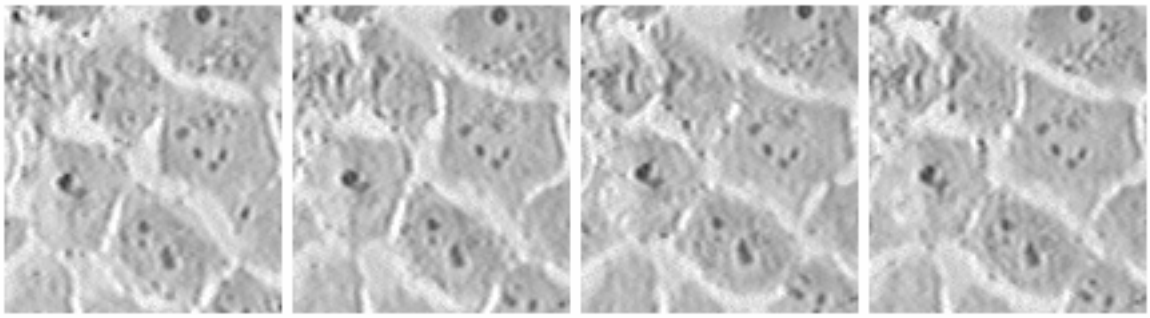


Figure 2: Making and breaking contacts in a confluent SV80 culture, 4 frames from a movie with 30-second intervals. Again, the effect is much more dramatic with continuous animation.

Trypsin action on monolayer cultures

The action of the protease trypsin demonstrates that proteins are probably required for attachments of cells to each other and to the culture substrate. First, the cell-cell contacts break, and eventually the cell-substrate contacts break, and the cells float away. Again, the action is much more vivid with animation. Notice how the nuclei become apparently smaller (in two dimensions) so we concluded that the attachments in monolayer culture are strong enough to flatten out the nucleus.

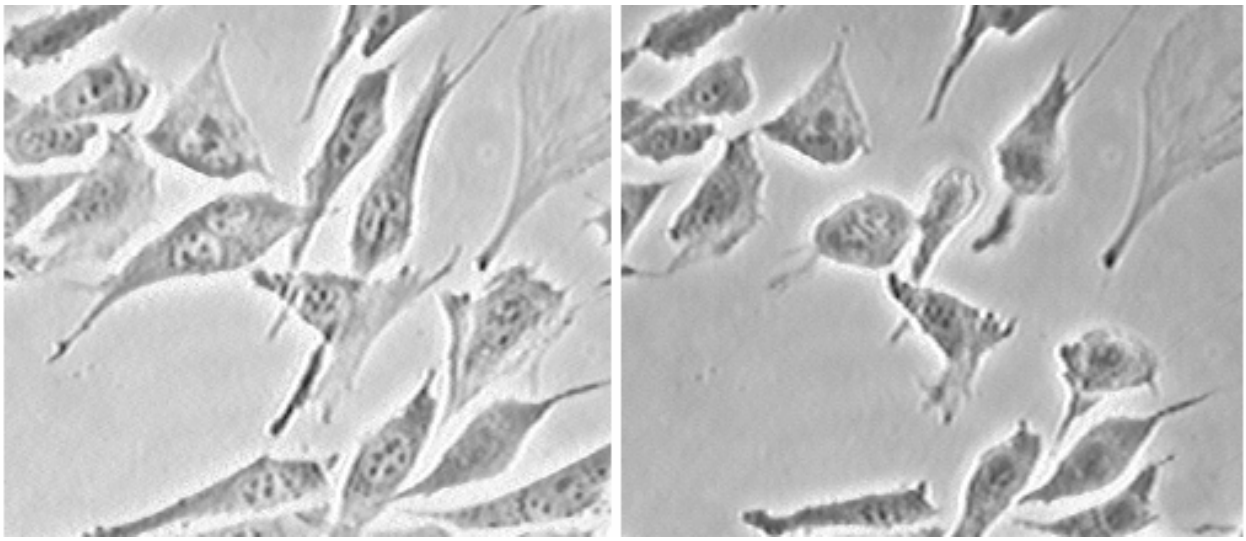


Figure 3: Two frames from a movie of 3T12 cells being "trypsinized" at room temperature, 30 second intervals.

G2 identification

To identify the G2 part of the cell cycle by the appearance of the interphase nucleus, we took several series of random pictures of the same field of cells every 30 minutes and looked for

mitotic figures. After finding a mitotic figure we tracked back 2-3 hours before mitosis to see what that same cell had looked like shortly before mitosis. In this study, the cells were in the incubator most of the time, and we took them out for less than a minute every 30 minutes to photograph them again, using guides on the microscope stage to position the T-flask reproducibly. A good cell line for this study was mouse Balb 3T12 which flattened out nicely and which had a reasonably high mitotic index in sparse culture. The conclusion was that nucleus seemed to rotate within the cell during G2, shortly before mitosis.

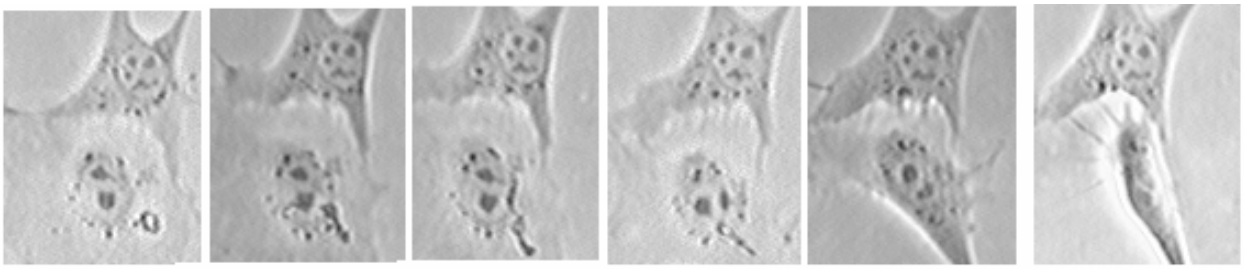
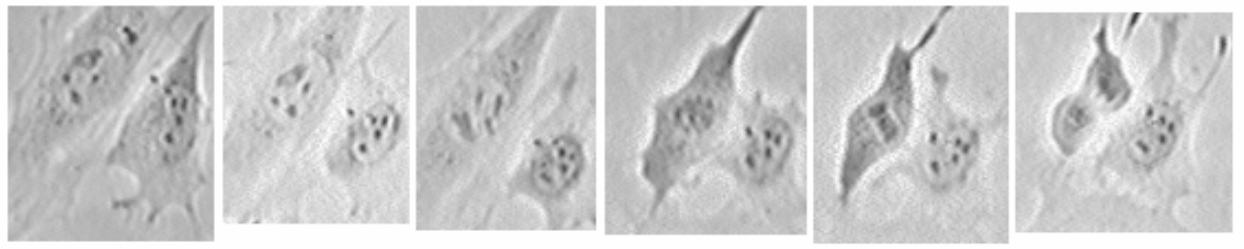


Figure 4: Six still frames taken every 30 minutes of two 3T12 cells. The frame on the right is mitotic (prophase). From the positions of the nucleoli the nucleus seemed to rotate about 150° in late G2, about an hour before entering mitosis.

Mitosis

We used the same technique of random photos of 3T12 cells and reproducible positioning of a T-flask to look at the stages of mitosis. The cells tended to stop at room temperature in prophase. Then we put the cultures back in the incubator to allow the cells to proceed through the other stages. The cells stayed in metaphase for a few minutes, zipped very quickly through anaphase, and tended to become arrested again in telophase. Once initiated at 37°C, the action



stages, anaphase and cytokinesis, were observable at room temperature.

Figure 5. Six still frames taken every 30 minutes of 3T12 cells. At room temperature, mitosis in SV80 and 3T12 became arrested in prophase (4th frame) or telophase (6th frame). To get cells to proceed through metaphase and anaphase (5th frame) it was necessary to incubate the cultures at 37°. In this series, as in the previous one (Fig. 4), the nucleus rotated (3rd frame) in late G2 phase shortly before entering mitosis.