

Time Lapse Cinephotomicrography of Tissue Cultures^{1 2}

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Cinephotomicrography is here interpreted to mean the recording for movie projection of objects viewed through a microscope. Time lapse recording, and the increased contrast in photographic images over that apparent by direct observation, greatly enhance the appreciation of the morphology and cytophysiology of cells growing in tissue culture. At the same time the characteristics of tissue cultures require special techniques and raise special problems in addition to those encountered in ordinary cinematography. Three principal characteristics are: (A) the small size and low contrast of the objects; (B) their slow rates of activity; (C) the complexity of simultaneously occurring processes. The problems raised by each will be briefly considered.

The small size and lack of contrast of cellular constituents, such as mitochondria and the various components of the nucleus, require the highest possible resolving power. At the histological level resolving power is not critical, for patterns of cell masses can be followed at low magnification. But when cytophysiological studies are undertaken the highest possible resolving power is needed. This means that culture chambers used for photography must be very thin to make use of condensers and objective lenses of high numerical aperture and consequent short working distance. Both sides of the culture chamber should be made of absolutely clean coverglasses, without scratches or other distorting aberrations. Curved refracting surfaces in the culture system must be avoided. Cultures which are growing in a plasma clot present a convex surface unless fluid is added to make contact between the clot and the opposite coverglass. Elimination of the air space directly adjacent to the culture may have injurious effects on the cells but many cells can tolerate such conditions for short intervals without obvious damage. A means of avoiding this has been suggested by Hughes and Fell (6), who cut an annular mote in the plasma clot just peripheral to the explant. The serum exuding from the clot filled the mote, with a flat surface across the top. Cells which subsequently grew out on the glass in the fluid of this region were selected for photography.

Phase difference optics have lately made possible a major increase in resolving power and particularly in contrast. Structures previously poorly visualized have been photographed with great clarity by this method. The techniques of phase optics, however, impose limitations in addition to those encountered in "bright field" microscopy. The absence of curved surfaces in the culture is particularly critical and extremely thin objects are the best for study. A single layer of cells, or if possible

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² Editor's Note. This paper and the succeeding papers in this division are part of a symposium on tissue culture studies.

isolated cells, without closely adjacent neighbors, are required for best work, and the medium in which cells are growing should be structureless (i.e., clear fluid). Thus the cells under observation may be removed from a very important environmental influence, that of closely adjacent cells. In interpreting results it must be remembered that the vast majority of cells in the culture have been excluded on an arbitrary basis. In many cases more value may be obtained from pictures taken by standard "bright field" technique rather than by phase contrast, for a greater portion of the culture may be studied by this means.

Cell processes, even in the actively amoeboid blood cells, are so slow that direct observation and analysis is an extremely tedious business. Much has been learned by direct observation, but time lapse photography makes it possible to decrease tremendously the time spent in observation. The dynamics of some cell processes cannot be clearly appreciated, and others may be entirely missed unless the time scale is sufficiently condensed that the observer can encompass the entire activity within a reasonably short time interval. More important, however, is the permanent record that can be shown repeatedly, and projected frame by frame if necessary, to analyze the activity. This becomes of major value when a number of processes are proceeding at once, as in mitotic division.

When we consider the complexity of activities proceeding simultaneously, a compromise must be made between a field taking in enough cells to record the complexity of the activity, and a resolution sufficient for the details within individual cells. It may be a matter of chance whether or not the cells in focus exhibit the hoped for activity. It is often not possible to choose the correct field by direct observation because the significance of the activity may not be apparent until the movie, with the activity speeded up, is viewed. This difficulty can be met in part by photographing with an objective of high resolving power and introducing very little further magnification. It may be profitable to replace the eye piece with a negative lens to reduce the image size. This is particularly true with the very small frames of 16 mm movie film. While 35 mm film has the advantage of permitting larger field size with the same magnification, it is much more expensive to use. As image size is reduced the grain of the film becomes the limiting factor in the resolving power of the system.

The required apparatus varies somewhat with the specific problems but should include a camera (no lens required) which has a single frame shaft, one revolution of which takes one picture, to be activated by a driving motor (not using the spring drive of the camera). The motor should be able to operate the camera continuously or to start and stop it at regular intervals, each start resulting in one picture. There must be a device between the microscope and the camera to permit focusing during photography.

A rate of exposure ranging from 2 to 120 times per minute is sufficient for nearly all situations in tissue culture. This results in a speeding up of the activity from 720 to 12 times, respectively. Activity of rapidly amoeboid blood cells can be recorded fairly well at 120 exposures per minute and it is rarely necessary to follow cells for longer than 24 hours of continuous photography. At two exposures per minute, 33 hours of activity can be recorded on 100 feet of film. The timer which starts and

stops the motor and the speed of the motor are best arranged so that the exposure time is the same regardless of the interval between exposures, when operated intermittently. A convenient speed of rotation of the motor for intermittent exposure is 15 revolutions per minute or 4 seconds per revolution. Since the shutter is open about half the time of each revolution, this results in an exposure of about two seconds, which has certain advantages: it minimizes the harmfulness of slight intermittent vibrations and permits quite low intensity of light to be used. While intermittent vibration in the camera, if not too severe, can be tolerated because of the slow exposure, a vibration in the camera lasting for the entire exposure interval will, of course, result in blurred images. Any vibration of the microscope on the other hand must be avoided because of the great magnification by the lens system of any vibratory movement. For this reason camera and microscope should not be in direct contact, and the microscope should be mounted on a very solid support, insulated from sources of vibration. For continuous operation at 30, 60, and 120 times per minute, the exposure time will be proportionately shortened. The light source should also be controlled by the timer so that the culture is not illuminated between exposures.

My own apparatus consists of a synchronous motor, connected through a variable gear box to one plate of a clutch. The other side of the clutch is connected to a single frame shaft on the camera. The standard operating speed is 15 revolutions per minute but with the gear box this can be changed to 30, 60 or 120 per minute. A pin is placed to arrest the revolution of this drive shaft on the camera side of the clutch. While the pin is in place the motor continues but the clutch slips and the camera does not move. A solenoid when activated by a microswitch removes the pin momentarily, allowing the camera shaft to make one revolution. The microswitch is activated by removable pins on a disc driven at one turn per minute by a simple clock motor. The number of pins can be adjusted to give 1, 2, 4 or 8 equally spaced intervals producing a corresponding number of exposures per minute. One revolution of the camera takes 4 seconds, during about half of which the shutter is open. If the pin is continuously retracted the exposures are continuous at 15 per minute. The revolution of the camera shaft activates the light switch so that it is on during the interval when the shutter is open.

For tissues of warm blooded animals the microscope is enclosed in an incubator to maintain an even temperature. Variations in temperature must be small (0.5°) to prevent loss of focus by expansion and contraction effects.

This system has worked well except that the continuously dragging clutch puts a heavy load on the motor when exposures are being made intermittently. An excellent description of a satisfactory device has been made recently by Hughes (4) and another device is being tested at the Audio-Visual Laboratory at Indiana University, which may be superior to this one.

A large number of investigators in this country and abroad are using time lapse photography to study cells in tissue culture. The few investigations mentioned here will serve to indicate several types of problems which lend themselves to this technique.

Lewis (8) has studied a great variety of cells by this method. DeBruyn (2) analyzed the amoeboid activity of blood cells in tissue culture and showed, among other things, that variations in the type of movement were related to mechanical influences within the culture. Firor and Gey (3) have compared the activity of normal and malignant cells in tissue culture. Bloom and Zirkle (1) are investigating the effects of ionizing radiations on cells and parts of cells. It may be possible to irradiate a single chromosome or mitochondrion and note the effects. This requires a very delicate system of registration whereby the exact point irradiated can be accurately determined. Michel (9) has studied the mitotic process in spermatocytes. Hughes and Swann (7) have studied the mitotic process in certain cells using polarized light images compared on alternate frames with phase contrast images. This requires an ingenious process termed bi-frame recording and makes possible certain quantitative measurements. For example, they measured the speed of movement of chromosomes as related to the rate of change in length of the spindle. Hughes and Fell (6) and Hughes (5) have studied the effects of numerous chemical agents on cells in tissue culture. The author (10) has studied cell differentiation in tissue culture by photographing cells of a pure strain. Rapid and direct transformations of certain cells into cells of an apparently different type were seen to occur at the moment of mitotic division. The details of this phenomenon are under further investigation. Weiss (11) is engaged in photographing certain tissues infected with virus particles in the hope of following the changes that occur in the cells and in the virus colonies. The success of such photographs depends in part, in this case, on the fact that the particular virus he is using (feline pneumonitis virus) is large enough to be resolved by phase contrast optical systems.

In conclusion, the method of time lapse cinephotomicrography can give information about certain problems which is not available by other means, with apparatus which is not too expensive or difficult to operate. Interpretation of results, however, must take account of certain important reservations: (A) results may be significantly influenced by choice of cells to be photographed; (B) added to the already numerous unknown factors affecting cells *in vitro* are additional unknown effects peculiar to the conditions of light, mechanics and nutrition under which time lapse cinephotomicrographs are made.

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