CINEMICROGRAPHY
IN
CELL BIOLOGY

Edited by

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Preface

Although not to be considered a general textbook of cinemicrography, this volume brings together significant perspectives of this analytical approach from eighteen laboratories in Italy, Germany, Russia, Poland, England, Mexico, and the United States. In this way a great diversity of technique has been accumulated which will afford reviewers a panorama of the potential available through cinemicrographic methods. Perhaps such diversity as well as the multiplicity of techniques peculiar to biological studies has dissuaded the production of books on this subject previously. This work, then, is a first attempt to organize into book form data from many disciplines of biology which have already made use of cinemicrography. It contains (1) factual information on the cine apparatus and techniques, (2) special methods of analysis possible only with the use of cine equipment, and (3) a broad array of data on tissues and cells derived from the permanent records of film strips.

It will be apparent from the various dissertations of the authors that cinemicrography, like all other procedures which collect and assimilate biological information, does not stand alone as an all-encompassing means for cellular understanding. It has its limitations, but, unlike many other techniques, the data derived from it cannot be obtained with other methods. This unique dimension, therefore, reserves for cinemicrography a place in biological research which is not likely to become obsolete in the near future. We can confidently look forward to a continuing series of advances and technical refinements. For instance, the sophisticated achievements with ultraviolet microscopy already well established (Chapters 4 and 5) and the combination of cine analysis with autoradiography (Chapter 7) are two significant trends to specialization.

Cinemicrography uniquely combines time-lapse or other variable speed components (Chapters 1 and 3) with its inherent capacity for maintaining a continuing surveillance of biological specimens. Life events may be condensed or expanded as required to perceive and assimilate the biological structure, function, and response. Moreover, cine records are permanent and reversible and may be dissected and measured through a variety of techniques. Each author has found cinemicrography to have a special usefulness to his particular research.

Their means of extracting data vary widely with respect to subject material, cultivation procedures, microscopic power, and timing sequences.

Cinemicrography was born in the laboratory of W. H. Lewis. Though one never can be certain of the source of the germ which gives rise to an idea, Dr. Lewis's brilliant work on pinocytosis in the early 1930's certainly marks the beginning of the cinemicrographic era for biology. Zernike's valuable development of the phase-contrast microscope, which when integrated with the time-lapse cine equipment, produced the new and exciting technique of cellular analysis, now often called cine phase, was of gigantic importance in the historical development of this approach. It would be inappropriate not to mention the vigor and enthusiastic support given the union of this pair of tools by C. M. Pomerat. It was largely through his immediate grasp of the significance of cine phase with time-lapse techniques and his subsequent dissemination of its usefulness through motion-picture documented teaching and lecturing to biomedical groups around the world that cinemicrography in cell biology has advanced so rapidly to its present stature. The editor feels especially responsible and grateful to these three men.

I am also deeply grateful to the many authors who so willingly submitted their time and talents to the writing of the various chapters and to my secretary, B. J. Trammell, and technician, M. K. Peterson, for their excellent support.

GEORGE G. ROSE

July 1963

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PART I: TECHNIQUES

Modular Design for Time-Lapse Cinemicrography

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I. INTRODUCTION

Ease of operation and versatility are key words in the design of a cinemicrographic time-lapse unit which will be adequate for making records of the activity of living cells in tissue culture.

In the course of several years' experience, it became obvious that it would be highly desirable to construct a reliable cine unit at reasonable cost, which might serve graduate students or independent workers. Such a system is made up of the following components: camera, timer and camera drive, stand, microscope, light source and filters, incubator, film, meter, and clock systems.

The first section of this chapter, therefore, is concerned with a general description of these basic components which enter into the design of more complex models. Special features will be detailed in the remainder of the chapter.

II. COMPONENTS, GENERAL DESCRIPTION

A. Camera

The camera should always be tested for abnormal vertical or lateral film movement before purchase. This is done by mounting it on a sturdy tripod and making a record of a stationary object. When the film is viewed, the frame adjustment of the projector should be positioned so as to allow visual inspection of the film-frame line. The register should be perfect. If any frame-line movement is noted, the camera should be sent back to the factory for adjustment with the test film included to illustrate the problem. One should be certain that the projector used for this test normally projects a steady image.

Cameras of any type can be adapted for time-lapse cine work. What needs to be modified, if anything, depends upon the type of timer incorporated. An electric drive has a definite advantage over use of the camera-spring motor as a source of power, its best feature being the availability of power at all times. This avoids the necessity of having to wind the spring motor to keep the unit functioning during long periods of study.

A camera with a reflex viewer incorporating cross hairs is highly desirable for making microscopic records. The light can be easily centered and allowed to fill the area of the film frame. Ideal optical contrast is obtained in this manner. An interchangeable film chamber is not essential; however, it does afford flexible use of various types of film without sacrificing footage. Chamber removal exposes a level relatively close to the camera-film plane so that a reasonably accurate meter reading can be made.

A reflex or lens focus camera does not allow access to the actual film plane, but it is possible to center the light to the actual film field and a meter reading can be obtained at the eyepiece. It also permits the interchange of films with slight footage loss. The advantage of having only one reading for all optical conditions will be discussed in more detail under Section II, H.

B. Timer and Camera Drive

Various companies in the United States manufacture time-lapse equipment.[1] Most foreign optical concerns, such as Zeiss, Reichert, and

[1] Kodak Pamphlet No. N-2, p. 8, Rochester, New York, 1961.

Leitz, which manufacture microscopes, also have units available for time-lapse work. One can build a unit according to a very simple design at a minimal cost.[2] It is important to consider many factors in terms of one's particular research goals before making a choice from several possible combinations. For example, for some cameras, will a spring-motor drive suffice? How many frames per second, minute, or hour will be needed? Will the camera shutter remain open and stationary during light exposure? Do you plan to employ a light which would remain on constantly as is required with the use of an arc lamp? If so, can the timer be equipped to synchronize another shutter for the light source so that illumination reaches the cell object only during exposure or while focusing? Will stroboscopic technique ever be essential?

In our installation (Fig. 1) only a limited amount of space was available for the positioning of five cine time-lapse units. This was in the corner of a room against two walls, 8 and 10 ft. in length. The timer components, as seen in Fig. 1, were mounted on wall shelves within easy access. In order to be able to reposition the components, pieces of plywood measuring 30 inches $\times$ 3/4 inch were attached along the course of each wall. Strips of electrical outlets with 12 inch centers were affixed onto the plywood at a level of 47 inches from the floor.

Since several Cine Kodak Special II 16-mm cameras were already available, timers and drives were needed. These cameras were equipped with a single-frame shaft which opened and closed the shutter at each revolution and which also advanced the film. The most efficient drive should turn the shaft 180°, at that point opening the shutter and stopping while the light is on, thus avoiding movement during the actual exposure. The shutter should then rotate another 180°, close, and advance one film frame.

It is desirable that the instrumentation be capable of variable exposure times. Since living cells are injured by light, the exposures should be of minimal duration. However, if for some reason it is impossible to obtain the correct intensity of illumination for a given object, the exposure time should be adjustable.

While investigators have not yet agreed on the standardization of exposure to facilitate the interpretation of each other's results, our experience, covering more than a decade, was built on the use of consistent taking rates. In our opinion the most useful for time-lapse work are 1, 2, 4, 8, and 16 frames per minute. Certain phenomena require unusual photographic recording such as a very slowly growing organotypic culture or an object which may incur acute light damage. Exposures of one frame

[2] PMI-Photo Methods for Industry, NPD Corp., Vol. 3, No. 4, p. 38, New York, New York, 1960.