PRO EXPERIMENTIS

A Microscopic Film of the Movements of Bull Sperm Cells in Dark-field Illumination

In the course of an investigation of the movements of bull spermatozoa, it became desirable to make films of sperm samples. Since the results yielded by these films had to be coordinated with other measurements taken simultaneously on the same samples, working with dark-field illumination could not be avoided.

In brief, the films were further required to give:

(1) Inter-frame-time $< {}^1/_{40}$ s; 50 frames per s were chosen, because of the convenience of synchronisation with the mains.

(2) Exposure time less than 1 ms.

(3) Magnification, taken from sample to emulsion, $\times$ 25 at least, in order not to be limited in resolving power by the grain of the emulsion.

(4) Total length of one film at least 30 s.

We decided to work with a low numerical aperture, which gives the advantage of great depth of focus.

The difficulty is to obtain proper illumination of the samples. From some experiments made with a small-aperture dark-field condenser (a single lens, the central part of which had been diaphragmed) and a incandescent lamp, it appeared that an energy of about 10 Ws per frame would be needed. The construction of a point-shaped light source capable of producing this 50 times per second is extremely difficult.

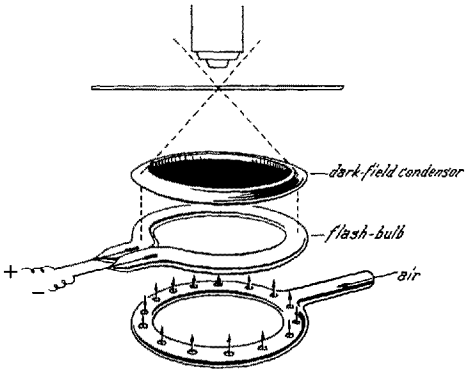

Abb. 1.

Obviously it is advantageous to use light sources with larger dimensions. We decided to use a ring-shaped electronic flash; the ring has the same dimensions as the opening of the condenser (see Fig. 1). This optical arrangement is in first approximation independent of the distance lamp condenser which implies that the adjustment is not at all critical, whilst at the same time, geometrically, a very satisfactory useful effect of light is obtained.

At our request NV Philips Eindhoven developed a suitable flash-bulb, made of quartz and air-cooled. The shape of the lamp makes it easy to do this latter very effectively.

The electrical circuit of the lamp is very simple: a single phase rectifier charges a capacitor battery of 15 μF in about ${}^1/_4$ of a period of the mains. In the following negative half period the bulb is triggered by a thyatron with a tesla coil. By putting a resistor in series with the capacitors, the peak voltages on the latter may be varied from 800 to 1800 V, giving an energy of 5 to 25 Ws.

At a power input of 0·3 kW, the lamp may be used for many hours continuously; at the maximum input of 1·3 kW, this is reduced to several minutes. Sufficient data as to life are not yet available. The flash-time was measured to be 100–250 μs, dependent on the energy used.

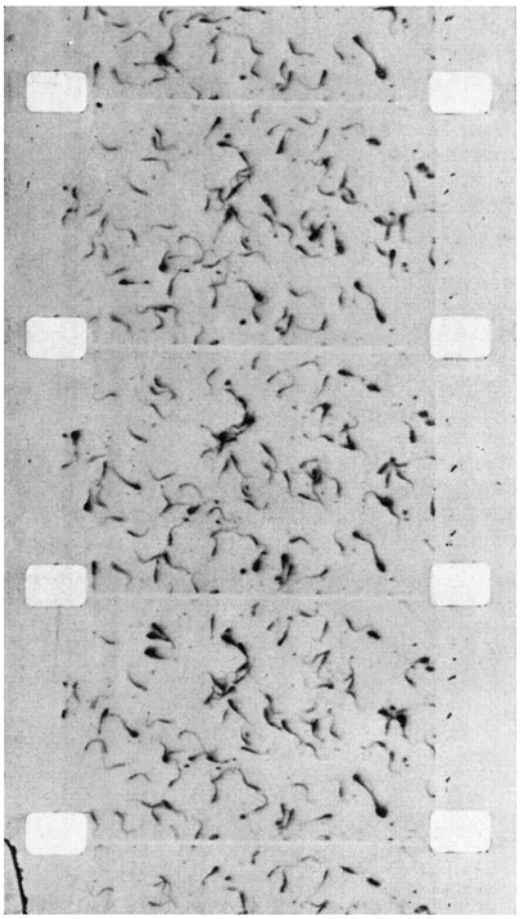

Abb. 2.

Some more details about the equipment:

(1) The image of the objective ($\times$ 10, A = 0·25) is magnified $\times$ 2·5 with a small (corrected) lens.

(2) A Kodak gun-camera, in this case driven by a 40 W synchronous motor.

(3) Emulsion: Kodak Tri-X, developed with high contrast developer.

Some films of sperm-samples have been made with this equipment. Figure 2 shows a number of frames taken at an energy of 15 Ws. The object in mind, namely the determination of the manner of movement of sperm cells, can obviously be achieved in this way.

In conclusion, we may say that the making of a microscopic moving picture in dark-field illumination has been realised with relatively simple means. The illumination employed seems to be quite suitable for phase-contrast microscopy also (where the quantity of light desired is much lower), by focussing the lamp on the ring-shaped opening of the condensers.

A more detailed description of the apparatus will be published later on.

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Research Institute for Animal Husbandry T.N.O., Utrecht, October 15, 1955.

Zusammenfassung

Eine Anordnung zur kinematographischen Aufnahme von Spermien von Musterbullen in Dunkelfeldbeleuchtung wird beschrieben. Die Bildfolge von 50 Aufnahmen je Sekunde erfordert eine Lichtquelle von etwa 0,8 kW, welche von einer Spezial-Elektronenblitzlampe geliefert wird.

PRO EXPERIMENTIS

A Method for the Isolation of the Parts of Ciliates[1]

Methods have been devised in this laboratory for isolating quantities of the parts of the infraciliature of *Tetrahymena*, in order that the structure and biochemistry of the ciliary apparatus may be studied. In view of the interest shown by some workers, it has been thought advisable to indicate something of the nature of the method, the kind of results obtainable, and the possibilities of using the method for physiological and morphological analysis of the parts of ciliates.

The method now in use is an adaptation of the method originated by Mazia and Dan[2] and modified by Mazia[3] for the isolation of the mitotic apparatus of dividing sea urchin eggs. In general, it consists of killing the cells by immersion in aqueous-ethanol at sub-zero temperatures, and subsequently transferring them to an aqueous solution of digitonin. The digitonin solution solubilizes much of the cytoplasm of the cell, releasing the nonsolubilized components of the cell so that they are found free in the medium, whereupon they can be collected by differential centrifugation. In the present work, the ciliate *Tetrahymena pyriformis* strain *W* has been grown axenically in 2% proteose-peptone. The cells are concentrated by centrifugation and washed three times with distilled water. The cells are then suspended in 40% ethanol at $-10°$C, and stored at $-10°$ for a few hours. The alcohol suspension is then centrifuged at $-10°$ to collect the cells, the alcohol is decanted, and the sedimented cells are suspended in a 1% aqueous solution of digitonin. The alcohol treatment must never take place above 0°C or the cytoplasm will coagulate and be rendered resistant to the solubilizing action of the digitonin. The solubilization of the cells in digitonin can be followed by observation with phase contrast microscopy.

It has been observed that, when the digitonin suspension is salt-free, all the components of the cell are disrupted and solubilize except the cilia, the kinetosomes, and certain other fibrous structures. The endoplasm, pellicle, and nucleus are dispersed. The kinetosomes forming the membranelles around the mouth are held intact by connecting fibers, and can be isolated *en masse* along with the attached gullet fibers. The somatic kinetosomes (those not in the oral apparatus) do not hold together, except in the anterior polar region where cross-connecting fibers appear to be present. With the dissolution of the pellicle, the cilia are detached from their respective basal granules and float free. Figure 1 is a high-power phase contrast photomicrograph of such a preparation that has been allowed to solubilize under a coverslip so that the kinetosomes retain their natural positions.

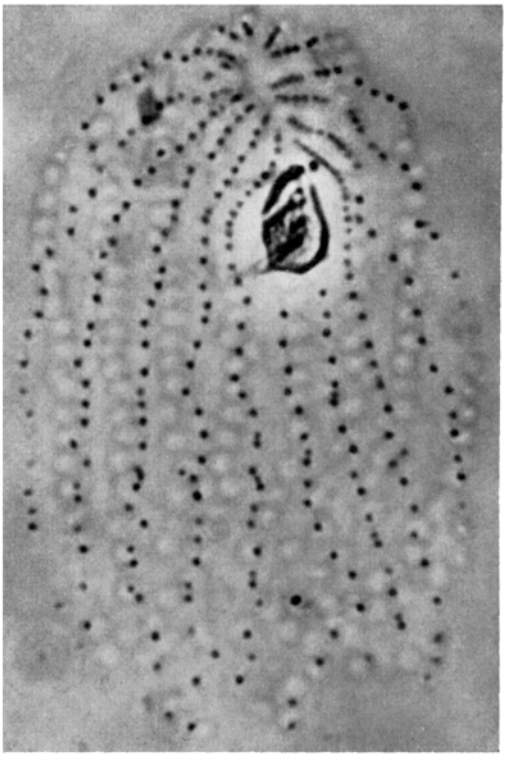

Fig. 1.–Phase contrast photomicrograph of a "solubilized" *Tetrahymena*, showing the kinetosomes and oral apparatus. × 2000.

The structure and arrangement of the infraciliature, especially the associations of kinetosomes and fibers of the oral apparatus, can be seen in such "solubilized" preparations under phase contrast microscopy with a clarity which is approached only by the more tedious methods of silver staining and the "sonicated" preparations used by Metz and coworkers[4]. In addition, these latter methods render the material itself unsuitable for biochemical investigation. Preparations of other protozoa, by the alcohol-digitonin method, have yielded material of further interest. The hypermastigote flagellate *Trichonympha*, obtained from the gut of the termite *Zootermopsis*, has given strikingly clear preparations of isolated anterior ectoplasmic cones with the flagella attached. Pitelka has used this method to produce preparations of opalinid ciliates which have been favorable for electron microscopy and will be described in a later publication.

The dissolution of such structures as the nucleus, pellicle, and mitochondria can be prevented by carefully using a digitonin-KCl solution for solubilization. The nucleus can be prevented from being solubilized if the digitonin solution contains some salt, up to 0·05 *M* KCl.

[1] This investigation was supported by the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[2] D. Mazia and K. Dan, Proc. nat. Acad. Sci. *38*, 826 (1952).

[3] D. Mazia in: *Symposium of the Society for Experimental Biology 9*, 335 (1955).

[4] C. B. Metz, D. R. Pitelka, and J. A. Westfall, Biol. Bull. *104*, 408 (1953). – C. B. Metz and J. A. Westfall, Biol. Bull. *107*, 106 (1954).