

## THE MICROSCOPE PAST: 50 YEARS AGO

# Some Results of High Power Photomicrography of Rapidly Moving Living Objects with New Apparatus and Materials

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Many rapid changes accompanied by structural alterations take place in living cells and tissues under experimental conditions which it would be desirable to study while they are taking place rather than subsequent to the various histological methods of fixation, dehydration and embedding.

Detailed alterations can be studied and photographed by means of oil immersion phase contrast optics of high power provided the living cells are not too rapid motion or the changes are not too sudden; in general motion of living cells is sufficiently slow to allow the necessary few seconds exposure with the hitherto available photographic materials and microscope illuminants.

However, some cells and organisms had a motion too rapid to allow photomicrography to be used for the detection of changes taking place in the living cell even where these changes themselves were gradual; this was due to the limitations of photographic emulsion speed and microscope illumination. These limitations applied particularly to high power photomicrography of spermatozoa and also to some extent to ciliary activity, movements of blood cells in capillaries and reactions of various protozoa and some micro-organisms.

To arrest motion under these conditions electronic flash equipment as used in ordinary photography has

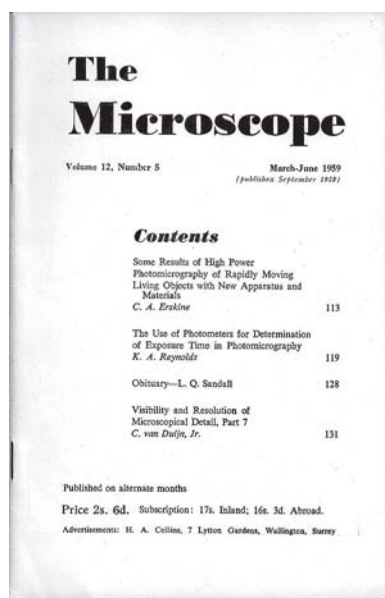
been adapted to the microscope from time to time for the past 10 to 15 years, but not with entirely satisfactory results. It is only in the past year or two that equipment designed specifically for effective flash photomicrography has become available.

### NEW PHOTOMICROGRAPHIC FLASH TUBES AND APPARATUS

Among the disadvantages for photomicrography of the hitherto available electronic flash equipment were lack of homogeneous light source, insufficient power for high power microscope objectives and for full use of the objective aperture, or too short a flash duration with high powers. Thanks to the researches of Michel (1) and the apparatus of Zeiss and Paffrath and Kemper, and to the designs of the firms of Leitz and Mannesmann, these problems have been successfully solved, including that of a suitably short flash duration (yet not short enough to introduce serious

factors of reciprocity failure).

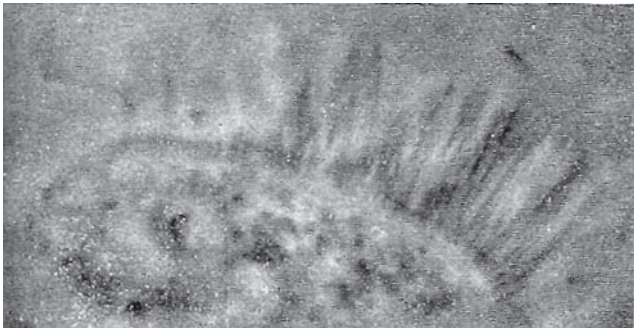
In the Zeiss equipment a homogeneous source was obtained by a meander design of flash tube and matched incandescent illumination (introduced into the ray path by means of a prism), to make convenient adjustment for Köhler illumination; in the Leitz-



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**Figure 1**



**Figure 2**

*Figures 1 and 2. Isolated living cells from pharynx of frog with irregular ciliated activity. Granules at attachment of cilia are seen in the top figure in optical section, and from the surface at the edge of the figure. Leitz Ph. Apo. x90; on print, x3600.*

Mannesmann equipment a somewhat more powerful tube of special design is brought directly beneath the Heine phase contrast condenser for maximum efficiency, the incandescent ray path for adjustment being passed through the flat and homogeneous flash aperture. These designs at once facilitate use and eliminate all chance of "hit or miss" methods.

## NEW PHOTOGRAPHIC EMULSIONS

Developments in flash technique would still remain insufficient for the requirements of photomicrography of rapidly moving living objects with oil immersion objectives of the highest power combined with optimum filtration if they were used with the hitherto available speed films.

<sup>1</sup> Observation has shown that phase contrast objectives used in photomicrography can reveal useful detail at magnifications considerably greater than the theoretical  $1,000 \times \text{N.A.}$

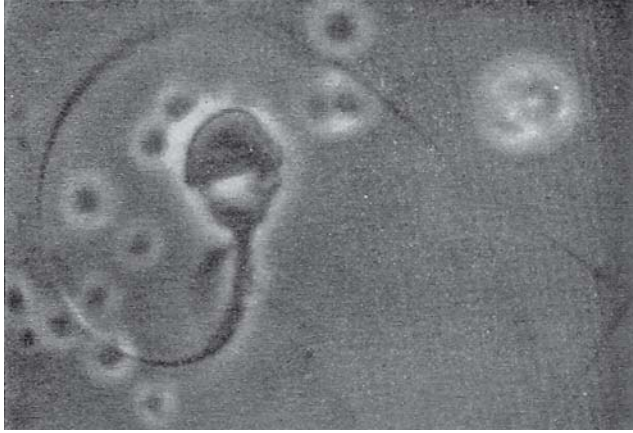
Flash photomicrography of spermatozoa reproduced by Smith (2) in 1946 demonstrated the fact that relatively low powers determined by the film speeds then available precluded detail in any way comparable to the fixed and stained specimens, and a recent example of photography of this subject in 1958 by Needham (3) shows lack of detail which may be presumed to be due to the use of earlier film material. Other examples of various living protozoa used to demonstrate the use of photomicrographic flash equipment three or four years ago show that the materials available limit the optics to dry objectives of  $\times 40$ .

Parallel with the development of effective flash equipment the recent revolution in film speeds and quality makes possible the use of oil immersion phase contrast objectives combined with suitable filtration to provide the best possible image with the new flash tubes. The new materials have speeds generally 10 times greater than those formerly available; the first of these materials, Kodak Royal X Pan, has been available for some time as a sheet film. This very fast Kodak material has been matched by the 35 mm Agfa Isopan Rekord introduced last autumn at the Cologne PhotoKina.

Satisfactory resolution at over 2,000 ASA is provided by this film when it is suitably processed; grain, contrast and resolution are acceptable up to 4,000 ASA and more. Practical monochromatic illumination by filtration can be obtained, and magnification of  $2,000 \times^1$  objectives in motion (which can be stopped at about  $1/800$  second) becomes possible. It should be pointed out that this speed is not sufficient to arrest motion at the terminal parts of active spermatozoa, though it is adequate for head and body parts. Experiments in processing show that the new Agfa material still has a reserve of speed which would probably allow flash durations of  $1/2000$  to  $1/3000$  second at 300 watt/seconds.

With the use of a Zeiss split image eyepiece to keep the object under continuous observation and interference band filters to supply monochromatic illumination, structural details in ciliated cells (Figures 1, 2) and in living spermatozoa (Figures 3, 4) can be revealed; serial phase contrast photomicrographs of such living specimens provides an opportunity to study directly changes which take place in the organism under experimental conditions.

At present, research on immune reactions in spermatozoa [Erskine (4)] by means of these materials and equipment have provided new details on structural alterations and the site of changes in parts of the living spermatozoa.



**Figure 3**



**Figure 4**

Figures 3 and 4. Living active spermatozoa of a guinea pig. Figure 3 shows the arrest of movement of head and body parts in rapid swinging motion; part of tail is not resolved since lashing motion is too rapid. Structural changes are seen in the body. Figure 4 reveals the internal fibrous structure of the body. Leitz Ph. Apo. x90; on print, x3600.

#### REFERENCES

1. Michel, K. *Photographie und Forschung*, April, 5, No. 5, 1953.
2. Smith, A. (ed.). *Medical Research*. Philadelphia: Lippincott (article by Bohrod and Gibson), 1946.
3. Needham, G.H. *The Practical Use of the Microscope*. Springfield: Charles C. Thomas, 1958.
4. Erskine, C.A. *Proc. Soc. Study Fertil.* (to be published), 1959.