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## SHORT COMMUNICATION

# DOUBLE-FOCAL VIDEOMICROSCOPY: A SIMPLE VIDEO SYSTEM FOR ANALYZING THE DYNAMICS OF CELL MOTILITY

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Three-dimensional information is important for studying cellular organization and function, and many approaches have been used over the years to reconstruct the three-dimensional structure of microscopic objects (Gaunt and Gaunt, 1978; Inoué, 1986). However, most of them are limited to the analysis of static objects. We have developed a simple and inexpensive method of obtaining three-dimensional information on dynamic processes in living cells. This method, which we have named 'double-focal videomicroscopy', yields images at two different focal planes simultaneously, making it possible to obtain information along the axis of observation. This method is especially useful for studying rapid cell movement, such as sperm motility.

Although the focus control is usually used when looking at different focal planes, such images may also be brought into focus by changing the mechanical tube length of the microscope. This principle is applied in the case of double-focal microscopy. By using the microscope with two viewing tubes and by slightly changing their relative mechanical tube lengths, images at two different focal planes of a single object may be obtained at the same time. The difference in the mechanical tube length is within the limits of the mechanical tube length tolerance, in the range where defocusing spherical aberration is so small that it may be negligible yet the tolerance is large enough to observe cells of normal size. In coupling the video camera and the microscope, two methods are used: a camera lens is mounted on a video camera when using eyepieces; alternatively, a projection lens is mounted on a video camera when eyepieces are not used (Fig. 1), as is usually the case in videomicroscopy. In the latter case, strictly speaking, images at two focal planes are produced not by changing the mechanical tube length but by changing the distance between the object and the primary image for the two viewing tubes (Doi in Fig. 1). The common principle in both cases is

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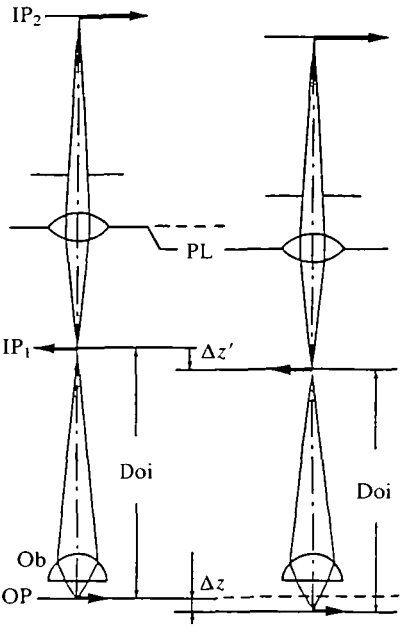


Fig. 1. Schematic optical system of double-focal microscopy. Ob, objective; PL, projection lens; OP, optical plane; $IP_1$, primary image plane; $IP_2$, secondary image plane; Doi, object to primary image distance; $\Delta z$, difference in object planes; $\Delta z'$, difference in primary image planes.

that the difference in object planes ($\Delta z$) is brought about by the difference in primary image planes ($\Delta z'$).

The double-focal videomicroscope consists of a microscope for producing double-focal images and a video system for recording these images. The simplified diagram of the double-focal videomicroscope is shown in Fig. 2. We used a Nikon Optiphot microscope equipped with a phase-contrast objective (plan 40× BM or plan 20× BM) or a differential interference contrast objective (plan 40× DIC or plan 20× DIC). The projection lenses consisted of Nikon zoom lenses mounted on two video cameras (WJ-1300A, Matsushita Communication Industrial Co., Ltd, Yokohama; AVC-1550, Sony Corporation, Tokyo). Varying the position of one zoom lens relative to that of the other yields images of an object at two different focal planes. Although this procedure produces a difference in the objective magnification of two images, the difference is so slight as to be negligible. Moreover, the zoom lens may be used for correcting it. The outputs of the two video cameras were montaged using a special-effects generator (WJ-545, National), and were then recorded onto video tape with a National time-lapse video tape recorder (NV-8030) through a time–date generator (VTG-33, For-A Corporation, Tokyo). The special-effects generator allows video signals from two video cameras to be montaged synchronously on a single video monitor. Images were processed by dividing a monitor screen so that half was occupied by one

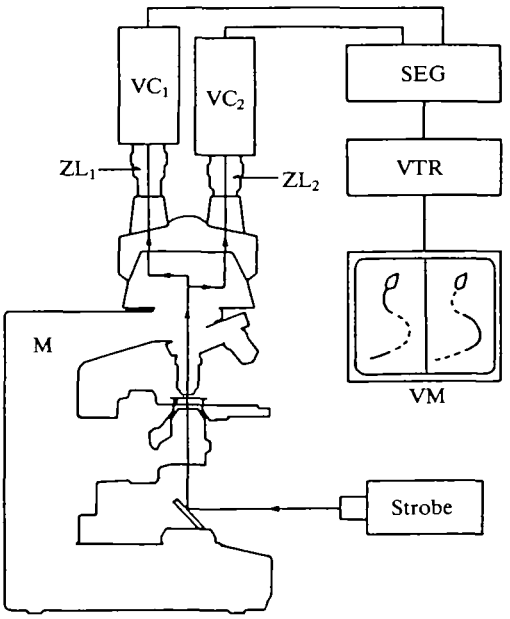


Fig. 2. Schematic diagram of the double-focal videomicroscope. M, microscope; $ZL_1$, $ZL_2$, zoom lenses; $VC_1$, $VC_2$, video cameras; SEG, special-effects generator; VTR, video tape recorder; VM, video monitor; Strobe, stroboscopic illumination.

image and the remainder by the other (Fig. 2). When using a microscope with three viewing tubes or more, images at multi-focal planes are produced at the same time. Combining this system with stroboscopic illumination and/or a high-speed video system makes it possible to observe and record more rapid movements, such as cilia and flagellar movement.

It is well-known that spermatozoa swim along spiral paths (Gray, 1955; Hiramoto and Baba, 1978). Double-focal videomicroscopy was applied to determine the direction of rotation of this spiral movement of spermatozoa. Fig. 3A,B is a pair of micrographs of the sea urchin *Scaphechinus mirabilis* spermatozoon yielded by double-focal videomicroscopy. The upper (Fig. 3A) and lower (Fig. 3B) halves of the videomicrographs are the successive images focused at the upper and lower focal planes, respectively. From these images, the left-handed spiral path of a swimming spermatozoon was reconstructed (Fig. 3C). The rotation of the spiral path was left-handed in most sea urchin spermatozoa; however, a few specimens swam along right-handed spiral paths (Table 1). The direction of the spiral path was also examined in starfish and golden hamster spermatozoa. As in sea urchins, a left-handed spiral path was more common than a right-handed one in starfish spermatozoa, and *vice versa* in golden hamster (Table 1).

Double-focal videomicroscopy has also been employed to determine the rolling direction of spermatozoa. The rotational direction of the spiral path obtained from the present study corresponded closely to the rolling direction of spermatozoa in

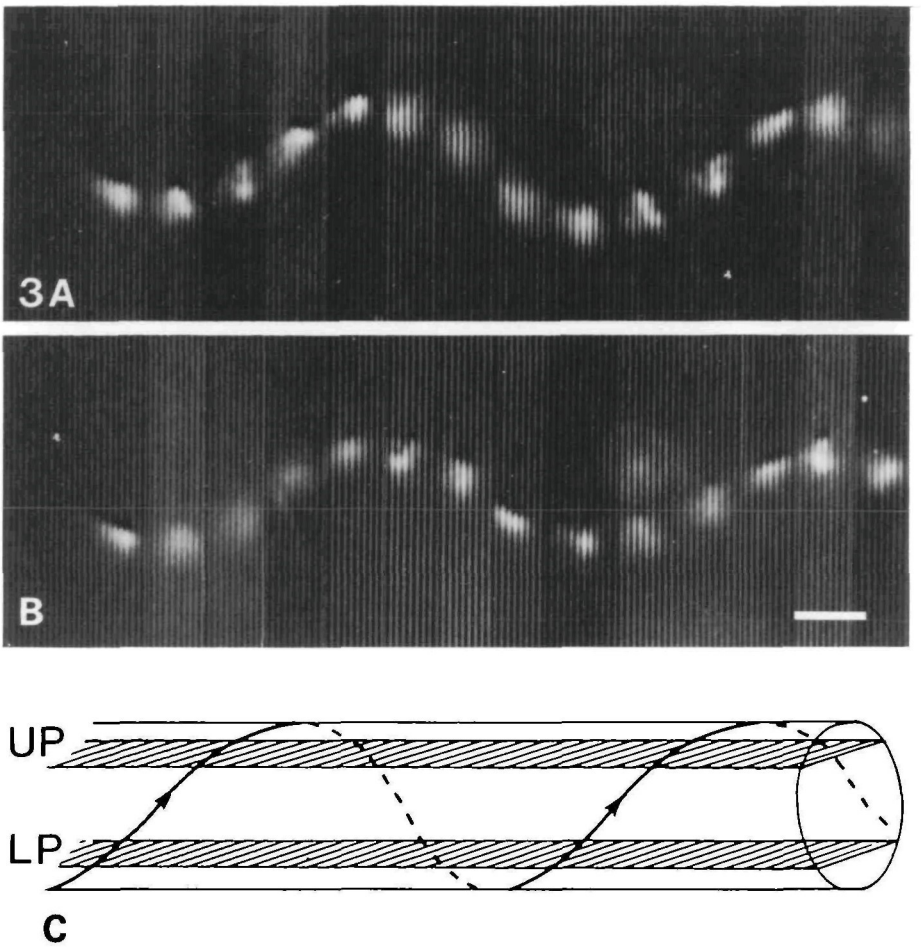


Fig. 3. Differential interference contrast videomicrographs of a spermatozoon of the sea urchin *Scaphechinus mirabilis* from double-focal videomicroscopy. (A,B) Images of the sperm head focused on the upper and lower focal planes, respectively. The difference between the two focal planes is adjusted to be 14 $\mu$m. The scale bar represents 10 $\mu$m. The time interval between successive images is 1/15 s. A schematic drawing of the spiral path reconstructed from these images is shown in C. UP and LP indicate the upper and lower focal planes, respectively.

the three species examined. Gray (1955) suggested that the handedness of the superhelix depends on the rolling direction; it is right-handed if the cell is rolling in an anticlockwise direction relative to an observer viewing the cell from its anterior part; and it is left-handed if the direction of roll relative to such an observer is clockwise. From the results of our two studies mentioned above, Gray's interpretation proved to be correct.

Table 1. *Handedness of spiral path of spermatozoa determined by double-focal videomicroscopy*

| Species | Percentage of handedness of spiral path | | *N* |
|---|---|---|---|
| | Right | Left | |
| Sea urchin (*Scaphechinus mirabilis*) | 8 | 92 | 25 |
| Starfish (*Asterina pectinifera*) | 32 | 68 | 25 |
| Golden hamster (*Mesocricetus auratus*) | 65 | 35 | 17 |

Measurements were carried out at 37°C for golden hamster spermatozoa and at room temperature (23°C) for sea urchin and starfish spermatozoa.
*N*, Number of spermatozoa measured.

Double-focal videomicroscopy could also be useful in studying interconnections between cellular components at different focal planes.

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## References

GAUNT, P. N. AND GAUNT, W. A. (1978). *Three Dimensional Reconstruction in Biology*. Kent: Pitman Medical Publishing, Co. Ltd.

GRAY, J. (1955). The movement of sea-urchin spermatozoa. *J. exp. Biol.* **32**, 775–801.

HIRAMOTO, Y. AND BABA, S. A. (1978). A quantitative analysis of flagellar movement in echinoderm spermatozoa. *J. exp. Biol.* **76**, 85–104.

INOUÉ, S. (1986). *Video Microscopy*. New York: Plenum Press.