

## Multiple Focus and Exposure Photomicroscopy of Nematodes for Increased Depth of Field

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Lack of depth of field is often a limiting factor in preparing photomicrographs of

nematodes suitable for publication. A narrow depth of field, usually not a problem in the observation of whole mounts of nematodes, is sometimes helpful because it optically sections the specimen. Photomicroscopy of one optical section, however, often produces a micrograph with some areas of the specimen in focus and others out of focus. For some specimens, addi-

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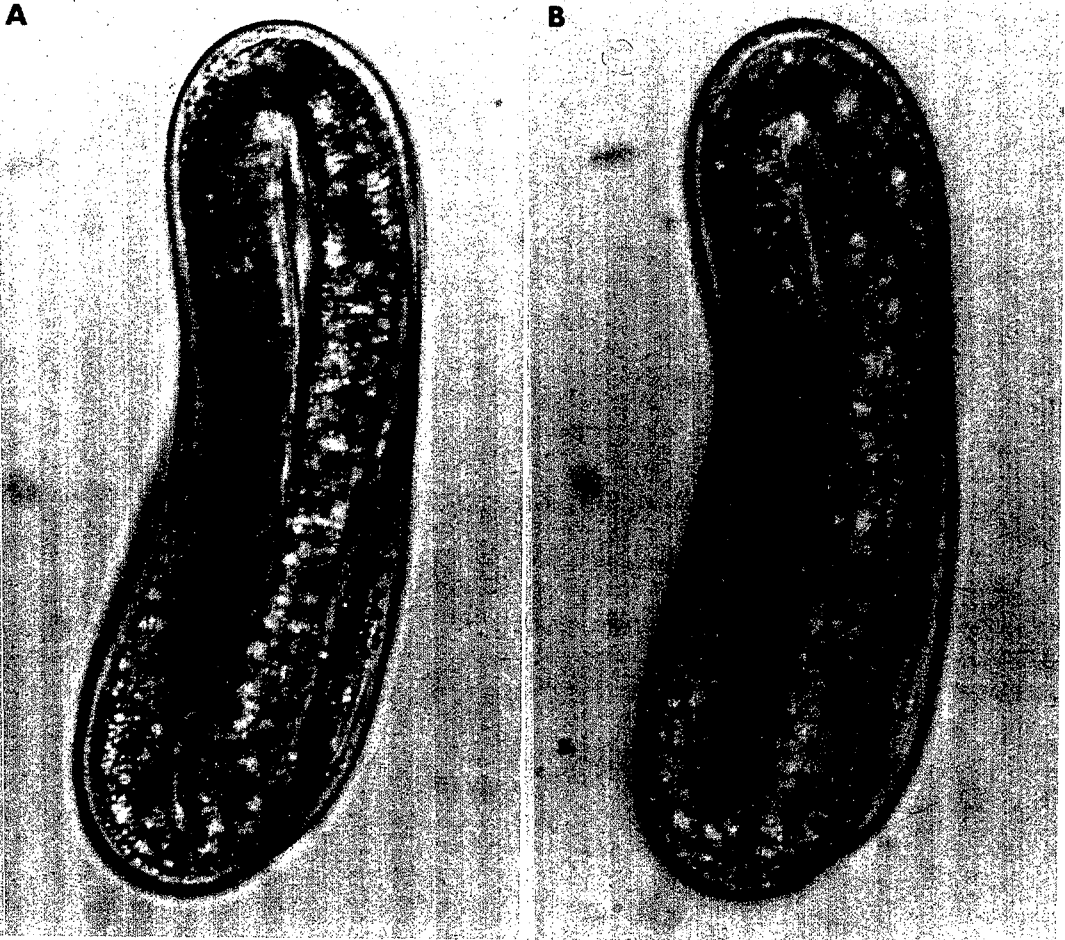


FIG. 1. Second-stage juvenile of an undescribed *Heterodera* sp. inside the egg shell. A) Photomicrograph recorded by a single exposure of 40 seconds through a 40 $\times$  objective. B) Photomicrograph recorded by four exposures of 10 seconds each at four different levels of focus with a 40 $\times$  objective. Note the increase in the amount of information in a single photograph made by multiple focus and exposures.

tional areas in focus would greatly clarify the image and show more detail than could be illustrated in the photograph otherwise.

A simple technique of multiple focus and multiple exposure photomicroscopy combines several optical sections to make one image with added depth (1). Several levels of focus are photographed as multiple exposures on a single sheet of film. The time required to make a correct exposure is divided by the number of exposures, and each level of focus is subsequently exposed for that portion of the total time (Fig. 1 legend). Care must be taken to prevent movement and vibrations of the specimen and camera system during and between exposures. Details of the procedures have been published previously (1).

Such methodology may have application in the study of nematodes. More information can be recorded in a single photograph by using multiple focus and exposures than with a single exposure. In

Figure 1A, some edges of the egg and droplets within the egg are in sharper focus than in Figure 1B; however, more information is presented in Figure 1B. The anterior end, the stylet, and the first fold in the body of the nematode are visible in Figure 1B but not in Figure 1A.

This method of photomicroscopy is most appropriate for specimens that are 10–20  $\mu\text{m}$  thick and nearly translucent. Generally, 2–5 exposures per frame are optimal, and the best results are obtained at magnifications of 40 $\times$  and higher. The technique also gives very good results with a 100 $\times$  oil immersion objective. Therefore, multiple focus and exposure photomicroscopy may have wide appeal to nematologists.

#### LITERATURE CITED

1. Singh, R. N. 1986. How to increase the effective depth of focus of the light microscope for photography. *Stain Technology* 61:17–20.