APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1976, p. 605-608
Vol. 31, No. 4

Continuously Variable Amplitude Contrast Microscopy for the Detection and Study of Microorganisms in Soil[1]

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Received for publication 11 November 1975

A new type of phase microscope was used to detect and observe the microflora in soil and to differentiate cells and spores from soil debris. This microscope provides a continuous variation in the amplitude ratios between undeviated and deviated beams of light, and the microbial cells, spores, and debris in soil change in appearance to a differing degree in response to the changes in amplitude ratio.

There are several procedures and types of microscopes available for detecting and observing the microbial flora in soil (1-30). The relative merits of several of these were evaluated and discussed by Casida (6). The phase microscope has not been generally used for this purpose because of the interacting problems of image deterioration due to halation occurring around cells and soil debris, light refraction caused by minerals, and a general inability to specifically differentiate between vegetative bacterial cells, spores, mineral fragments in the size range of microorganisms, and organic debris. These are not solved by using various of the above techniques in conjunction with phase microscopy; for example, color infrared photomicrography is not usable with phase microscopy (8).

The present study presents a method for the detection and observation of indigenous microorganisms in soil by the use of a new type of phase microscope; habitats other than soil were not examined. This microscope was recently introduced by the American Optical Corp. (Buffalo, N.Y.), and it is designated as the Polanret Microscope, Continuously Variable Phase and Amplitude Contrast System. It provides the capabilities of continuous variation of the phase difference between the undeviated and the deviated (diffracted) beams of light, and of continuous variation of the amplitude ratio (or light intensity ratio) between the undeviated and the deviated beams of light. In the present study variations in phase difference were tested, but these did not improve results; therefore, the phase-shift knob was set and left at 90° ($\lambda/4$ phase retardation). Variations made in the amplitude ratio in the dark-contrast range, however, made bacterial cells, spores, etc., appear with varying degrees of darkness against a range of from dark to light backgrounds; therefore, amplitude values of 8° to 60° were evaluated. A conventional phase microscope has an amplitude value of 24°. The bright-contrast range (imaging of a particle as a bright object against a dark background) was available on this scope, but it did not provide differentiation of microbial cells from soil and other debris. For either range, the tangent of the amplitude setting gives the amplitude ratio between the undeviated and the deviated beams of light.

The microbial and soil preparations observed as aqueous mounts with this microscope (oil bridge to the condenser) were as follows:

(i) soil blended in 0.1% sodium pyrophosphate and then centrifuged at 23,000 × *g* for 20 min (the upper organic layer, the middle layer composed of small mineral fragments, and the bottom layer composed of larger mineral fragments were separated and observed); (ii) mixed cell populations scraped from the surfaces of soil counting plates; (iii) Rossi-Cholodny buried slides; (iv) soil smears; (v) pure laboratory cultures of *Arthrobacter globiformis*, *Bacillus licheniformis* (spores and vegetative cells), and *Streptomyces* species (hyphae and conidia); and (vi) smears composed of soil mixed with the *Bacillus* spores and the soil-cell mixture.

Visual observations were made and photographs were taken at amplitude knob settings of 8, 15, 30, 45, 53, and 60. Black-and-white photography used Kodak High Contrast Copy, Plus X, and Photomicrography Monochrome SO-410 films; color photographs were made with Kodak High Speed Ektachrome (tungsten) and Ektachrome Infrared films. A summary of results for all microscopic preparations is presented in Table 1. All appearances of the cells, spores, minerals, etc., as observed vis-

[1] Paper no. 4962 in the journal series of the Pennsylvania Agricultural Experiment Station.

TABLE 1. *Changes in appearances of cells, conidia, spores, and mineral fragments with changes in amplitude contrast*

Amplitude knob setting	Appearance						
	Bacterial cells, streptomycete mycelium, and conidia[a]			Bacterial spores	Mineral fragments		
					Spore size and shape[b]	Slightly larger particles	Larger particles
8	Black	Blue, dark rim, lighter blue central spot	White, dark rim	White, dark edge	White, blue edge	White, dark edge	White, blue edge
15	Black	As above, but with blackish cast	Medium blue, dark rim	White having slight bluish cast, dark edge	As above	White, blue rim	Blue center, then white; blue rim
30		Black or gray-black		Medium blue, black rim	As above	Blue, darker blue rim	As above, but less white
45		Blackish gray[c]		Black, some with small central blue area	Medium blue, dark rim	As above	As above, but less white
53		Blackish gray		Black		Blue, darker blue rim	
60		Blackish gray		Black		Gray-blue dark rim	

[a] The three different appearances are not correlated with cell types.
[b] Small particles having the size and shape of spores.
[c] Becoming progressively lighter, respectively, at the 45, 53, and 60 settings.

ually in these preparations were duplicated in the preparation containing soil with added cells and spores, and the results of the latter are presented in Fig. 1. It will be noted (Table 1) that, as the amplitude knob setting was changed, the relative darkness, brightness, and color of the various objects changed at a differing rate. Thus, vegetative cells of bacteria and streptomycetes, and streptomycete conidia, appeared dark at the lower values of amplitude knob settings, but then appeared progressively lighter as the values for the amplitude knob settings were increased. Organic debris showed a similar pattern but, per given amplitude knob setting, it appeared lighter (sometimes taking on a brownish cast) than the vegetative bacterial cells and streptomycete hyphae and conidia. Thus, microbial cells sitting on organic debris usually can be detected. Bacterial spores progressed from a white to a blue brightness as the amplitude knob value was changed from 8 to 30, but then assumed a dark appearance for amplitude knob values from 45 to 60. Soil mineral fragments, including those that were the size and shape of bacterial spores, had approximately the appearance of a bacterial spore at amplitude knob values from 8 to 30, but they did not undergo the darkening at amplitude knob values between 45 and 60; they remained as blue to gray-blue. Based on the above, a soil sample could be scanned for its microbial content at amplitude knob values of 30 or 45, and individual questionable objects could be identified by examining them at higher or lower amplitude knob values. The halo caused by light diffracted around objects could also be used to a certain extent in object identification. Vegetative bacterial cells, streptomycete hyphae and conidia, and organic debris showed relatively little halo regardless of amplitude knob setting (Fig. 1). Halation was present, however, for mineral fragments and spores. It was more pronounced for the mineral fragments, and it was most evident at an amplitude knob setting of 8. It progressively decreased with increased amplitude knob values so that little was evident at 45 through 60. Therefore, observations made in this range sometimes allowed spores and vegetative cells to be seen on the mineral surfaces.

The brightness noted above for spores and mineral fragments was observed visually as either white or degrees of blue coloration. Photographs made on properly exposed High Speed Ektachrome film showed all of the variations in amplitude contrast as noted in Table 1, and spores could be distinguished from mineral fragments in the pictures at the higher amplitude knob settings. Photography with black-and-white and with color infrared films was not as successful, however, because the spores and

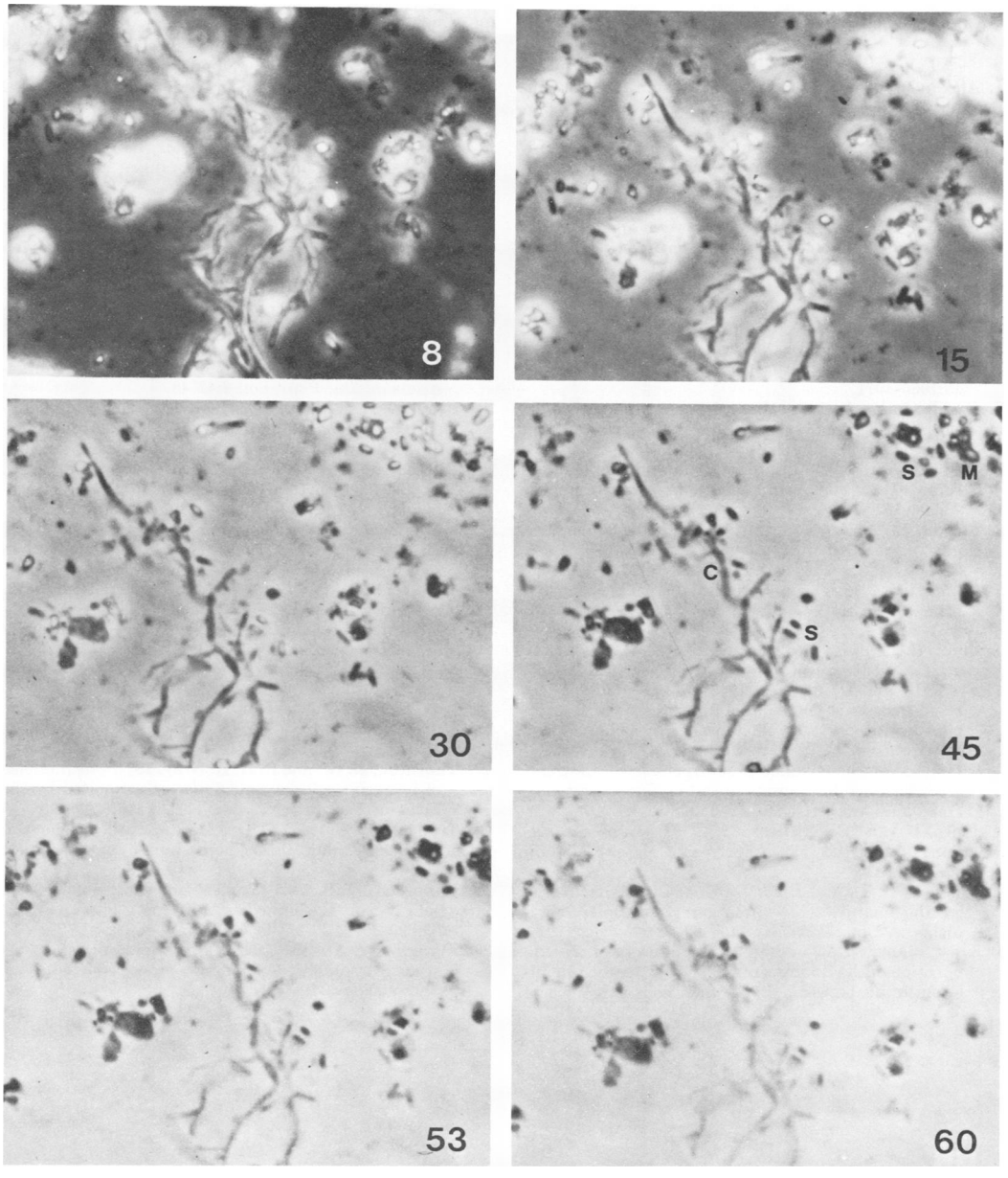

FIG. 1. *Changes in appearances of cells, spores, and mineral fragments with changes in amplitude contrast. The values 8, 15, 30, 45, 53, and 60 refer, respectively, to the amplitude knob settings. Cells (C), bacterial spores (S), and mineral fragments (M) that are the size and shape of bacterial spores are shown in the amplitude knob setting 45 print. Magnification ×1,184.*

mineral fragments could not be differentiated from each other in the photographs. Thus, at amplitude knob settings from 45 to 60, visual observation and High Speed Ektachrome film could differentiate the blue to gray-blue color of mineral fragments from the dark appearance of spores, but the black-and-white and color infrared films were unable to do so. Optical filtration with green, blue, and yellow filters during black-and-white photography did not correct this. The black-and-white photographs in Fig. 1 were obtained by rephotographing colored images that had been recorded on High Speed Ektachrome film.

This work was supported by contract NGR 39-009-180 with the National Aeronautics and Space Administration.

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