This probably explains why lysozyme did not lyse completely our cell-wall preparations, some parts remaining undissolved even after a longer period of lysozyme digestion.

**ILLUMINATION OF MICROMANIPULATOR TOOLS IN ULTRAVIOLET-FLUORESCENCE MICROSCOPY**

L. E. CASIDA, Jr.

*The Pennsylvania State University, University Park, Pennsylvania*

Received for publication July 16, 1962

In recent years, the ultraviolet-fluorescence microscope has been used to identify microorganisms in cultures, tissues, and excretions (Beutner, Bacteriol. Rev. 25:49, 1961; Mellors, *Analytical Cytology*, McGraw Hill Book Co., Inc., New York, 1959), in soil (Schmidt and Bankole, Science 136:776, 1962), and in other materials. The living microorganisms of soil have been counted by staining the soil and its microflora with acridine orange before viewing with the ultraviolet-fluorescence microscope (Strugger, Can. J. Res. 26B:188, 1948). The soil particles, including clay and humus, and dead microorganisms fluoresce an orange-to-red, whereas the living microorganisms fluoresce a bluish-to-yellowish-green.

Casida (Can. J. Microbiol. 8:115, 1962) presented a technique by which individual living soil microorganisms stained with acridine orange could be viewed with the ultraviolet-fluorescence microscope, and then isolated by micromanipulation directly from the soil to a growth medium. The organisms could then be cultured for morphological and physiological investigations. A problem was encountered in this technique in that the micromanipulator tool, which did not fluoresce, was difficult to locate in ultraviolet light. This problem was solved by utilizing a nichrome wire tool that was opaque to ultraviolet and visual light. Thus, by lowering the condenser, the shadow of the tool could be seen for its initial location in the microscopic field, and the tool itself could be observed as it was brought close to the organism to be isolated. In the latter instance, the tool was seen because it blocked a portion of the weak light emitted by the fluorescing microorganisms and soil particles.

It now has been found that visual light can be transmitted through a glass manipulation tool so that the point is easily observed in a field of ultraviolet light. This is accomplished by butting one end of a fiber optic (NA 0.56) with the back end of the tool, so that the light traveling through the fiber optic will continue through the tool and will be emitted at a point where the tool has been fire-polished and drawn to a point. Tools made of solid glass carry more light to their points than do those of capillary glass, although the latter can be seen in an ultraviolet field.

The fiber optic for this study was provided by the American Optical Co., and was of a diameter (approximately 0.7 mm) which would just fit into the back end of the manipulator-tool holder. Light was introduced into the fiber optic by mounting an exposed end in a cork held in the aperture of the iris diaphragm of a microscope illuminator, so that the optic was a few mm forward of the front lens of the illuminator.

Although this technique was developed for micromanipulation of organisms stained with acridine orange, it also should find use in studies where microorganisms, etc., have been stained with fluorescent antibody.

This study was supported by a grant from the Medical Sciences Research Foundation at Stanford University.