NOTES
Method for Detecting Mycoplasma and Bacterial L-Form Colonies in Relief with an Ordinary Light Microscope by Means of Oblique Light

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A simple method is described for obtaining images in relief of fresh preparations with an ordinary light microscope by lowering the condenser and displacing the objective at an "off-clicked" position.

The detection of the typical "fried-egg" colonies of mycoplasmas and L-forms grown in agar has become an obligatory first step for their recognition (2). For this purpose, their direct observation under low magnification through a stereoscopic microscope is widely used. If the source of light is obliquely transmitted, as recommended by Edward (1), the results are improved, since a relief view of anything growing on the agar surface can be more characteristic and identifiable.

Due to a chance observation, we found a very simple method for obtaining similar images in relief with an ordinary light microscope, by means of a special optic effect caused by oblique light entering the objective. While observing colonies of mycoplasma grown in agar, with the condenser kept in a low position to accentuate the contrast, colonies appeared as if seen in relief, due to a sort of lateral illumination. Upon further careful observation, this effect was found to be due to inadvertently positioning the revolving nosepiece to a slightly "off-clicked" position. This observation is explained as follows. When the condenser is kept low, it illuminates a wider area than in the normal, high position, and the laterally located objects in the area receive oblique light which causes the effect of relief. The laterally displaced nosepiece can then reach this obliquely illuminated area. To obtain this special illumination, two adjustments in the microscope should be made. (i) The condenser must be placed in a low position, sometimes in the lowest one possible, depending on the microscope. (ii) The body of the objective to be used should then be intentionally deviated from the correct "clicked" position. The relief increases as the deviation from the correct position increases, and the contrast of the illumination is accentuated. There is always, however, an optimal median point at which a satisfactory relief is obtained before definition becomes poorer and the contrast too strong. In addition, the position of the condenser, being always low, should be adjusted to the best point as determined by the image quality. Once this is found for each microscope, the results are always excellent and reproducible. Only magnifications of an approximate total of ×100 can be obtained with this method with a concomitant loss in definition, since the image is viewed through the periphery of the lens. However, with the larger diameter (×4 and ×10 objectives) commonly used when looking for mycoplasma or L-form colonies the quality of the image is not substantially impaired (Fig. 1–6).

The primary advantage of this method, apart from the general one of getting a stereoscopic view through an ordinary light microscope, is the possibility of taking consecutive pictures of a specific specimen under different kinds of light. Thus, the details of its surface can be observed first with the "off-clicked" position of the objective; then, when the nosepiece is clicked to the normal position, the inner features of the specimen can be easily studied with the transmitted light, either fresh or after...
Fig. 1. Lacy-like colony of *M. hominis* grown in agar. x100. Pictures (Fig. 1-6) were taken with an ordinary light microscope with the condenser kept low and the objective in an "off-clicked" position. Details are shown in relief. Figures 1 and 2 were taken with a Leitz, SM-F model microscope with an attached low-voltage lamp-illuminating system of 6-V, 15-W, achromatic 10× NA-0.25 objective and NA-0.90 condenser; exposure time, 0.25 s. The camera used was a Kodak Retina III-S. Figures 3-6 were taken with a Nikon, LUR-Ke model microscope with built-in low-voltage lamp of 6-V, 30-W, Plan achromatic 10×, NA-0.25 objective and achromatic NA-1.25 condenser; exposure time, 1 s. The camera used was a M-35 dark box with a semiautomatic Microflex EFM attachment. Film used for Fig. 1-4 was Kodak Panatomic X; for Fig. 5 Ilford Pan F was used, and for Fig. 6 Agfa Isopan was used. Positives were made in Agfa BEH-1 paper.

Fig. 2. Satellitism of *M. hominis* colonies in agar around a cluster of cells. x100. See legend to Fig. 1.
staining. Compensation for the shift in field of view, induced by moving the lens from the "off-clicked" to the "on-clicked" position, must be made visually through the ocular during the process to insure the correctness of the field being viewed.

We think that this simple method, or rather a trick, as one might call it, will be an aid to persons working with mycoplasma and L-forms as a means of distinguishing between colonies and artifacts (2, 3). It may also be useful to virologists using tissue cultures, since the characteristic cytopathogenic effect will take a tridimensional appearance (Fig. 6). In general, any observation of a surface permeable to light will benefit from the application of this
FIG. 5. Human embryo kidney cells in culture, unstained. x90. See legend to Fig. 1.

FIG. 6. Characteristic cytopathogenic effect of Varicella-Zoster virus in human fetal diploid fibroblasts, unstained. x90. See legend to Fig. 1.
method; although in our experience the examination of specimens on the agar surfaces of petri dishes is the most rewarding one.

To our knowledge, a simple method for obtaining special contrast in fresh preparations with accompanying sensation of relief with an ordinary light microscope is described here for the first time. Because of its simplicity, convenience, and the quality of the images that can be seen or photographed in a wide range of circumstances without extra cost or special attachments, this technique should gain wide use.

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