Zero-Cost Modification of Bright Field Microscopes for Imaging Phase Gradient on Cells: Schlieren Optics

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Abstract

A simple, zero-cost, reversible modification of a bright field microscope permits visualization of phase gradients in cells by transmitted illumination, yielding a Nomarski-like effect. This modification, based on schlieren optics, is simultaneously compatible with high-aperture epi-illumination fluorescence excitation. For many objectives that are intended for use in fluorescence work, but are unavailable in phase contrast versions, the modification provides a simple means for locating cells in culture with good image contrast and resolution.

Index Entries: microscope, bright field; optics, schlieren; schlieren optics; fluorescence microscopy, of cell phase gradients; phase gradients, of cells by microscopy; cell culture, schlieren microscopic imaging of.

Introduction

Examination of cells by epi-illumination (i.e., through the objective) fluorescence excitation is greatly facilitated by high-aperture liquid-immersion microscope objectives. Unfortunately, most such high aperture objectives intended for epi-fluorescence are compatible only with transmitted illumination in bright field mode; they are often not commercially available with any means for phase-sensitive observation, such as phase contrast, to visualize unstained cells. This paper discusses a simple modification of ordinary bright field objectives that allows imaging of phase gradients in microscopic samples. This modification can be performed reversibly in a few minutes on either high- or low-aperture and magnification objectives, and at virtually zero cost with only two pieces of black masking tape. The modification is completely compatible with simultaneous

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use of the objective for epi-illumination fluorescence or bright field illumination.

Similar microscope systems were originally described by Abbe (*1*) in 1873 for creating relief contrast in microscopic images, and by Saylor (*2*) in 1935 for accurately measuring the refractive index of crystals immersed in fluids of closely matched refractive indecies. Although the optical system described here is only slightly different from Saylor's, its potential application for visualizing biological cells with high contrast through bright field/epifluorescence objectives is not well known among cell biologists and deserves attention.

The physical principle is based on schlieren optics (*3*), whereby a ray of light passing through a sample with a phase gradient is bent toward or away from an opaque barrier positioned behind the objective. Previous versions of schlieren microscopes (*4–6*), other than Saylor's (*2*), require extra lenses and significant alteration of the conventional microscope's sample mounting system. Modulation contrast microscopy (*7*), which is also based on the schlieren principle, is somewhat similar to the system discussed here, except that modulation contrast requires installation of a somewhat more sophisticated (and expensive) plate of graded opacity behind the objective. All forms of schlieren microscope optics produce image intensity variations sensitive to phase gradients in the sample. The resulting "three-dimensional" effect qualitatively resembles that of the much more expensive Nomarski differential interference contrast system, which is particularly popular for viewing biological cells.

Method

Figure 1 shows a simple schlieren modification of a bright field microscope with Köhler illumination. An opaque edge stop (e.g., a piece of black masking tape) is positioned in the front focal plane of the condenser so that it blocks about 4/5 of the condenser diameter (the exact fraction is not critical). Another opaque edge stop (again, black masking tape is sufficient) is inserted at the back focal plane of the objective. The two edge stops must be oriented with their edges parallel so that a narrow band of illumination light can propagate unimpeded between the edge stops. The relative positioning of these opaque edge stops can be easily checked by looking down through the microscope eyepiece tube with the ocular removed; both the edge stop in the objective's back focal plane and an image of the edge stop in the condenser's front focal plane should appear as shown in Fig. 1. A flat sample with zero phase gradient will not bend the illumination light and the narrow band of transmitted illuminated light will have a width w_0 as it passes the objective edge stop. A sample with a constant nonzero phase gradient normal to the edge stops will bend the illumination light so that the transmitted band will have an altered width w_+ ($>w_0$) or w_- ($<w_0$) depending on the sign of the gradient. The width of the transmitted band determines the brightness of the sample image. Images of samples with variegated phase retardation will appear bright where the phase gradient is "positive", dim where the phase gradient is "negative", and intermediate elsewhere. Contrast

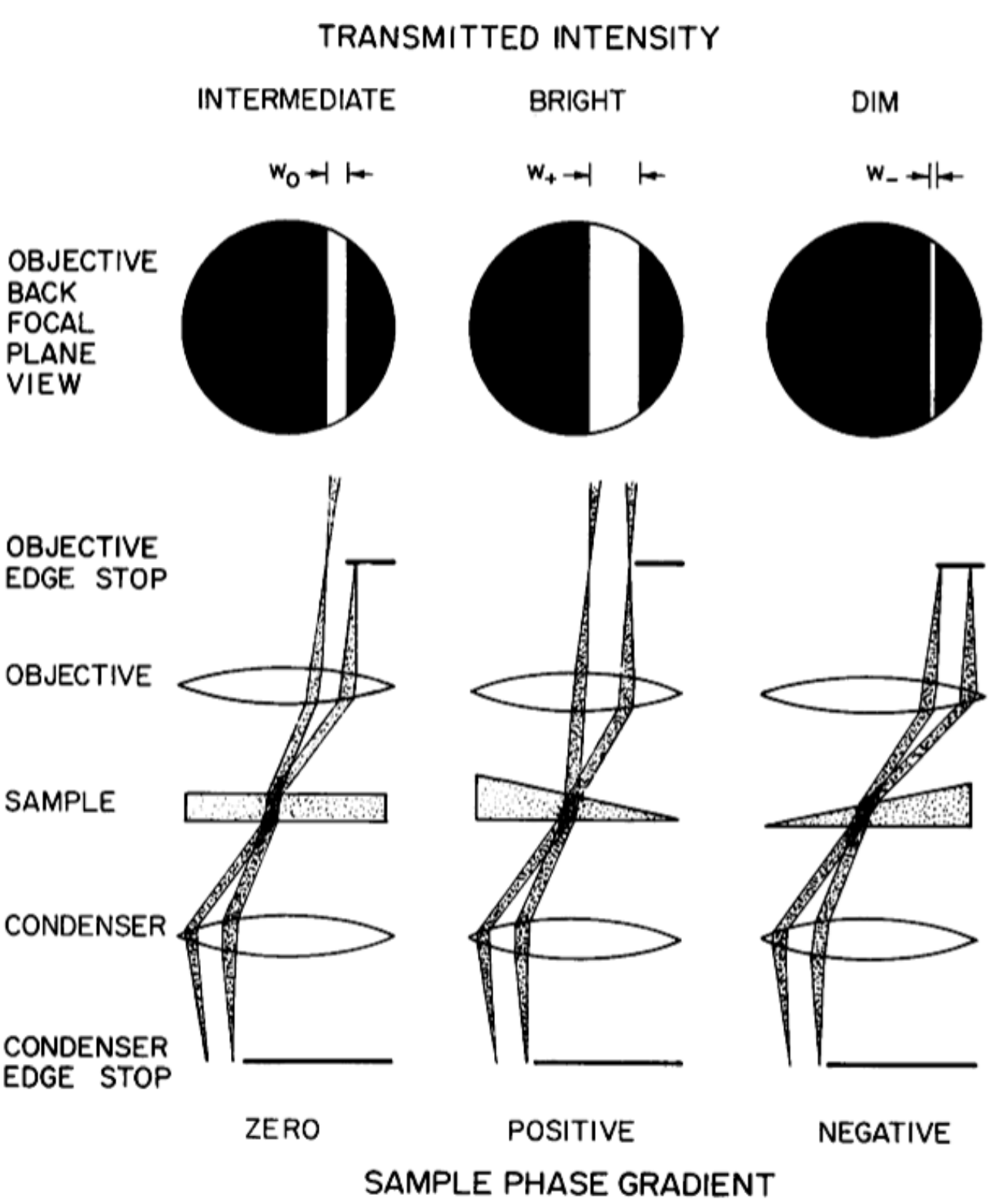

Fig. 1. The optical path of rays through the schlieren microscope system for three idealized samples of different phase retardation profiles: i.e., zero, positive, and negative phase gradients. Typical rays are shown schematically emanating from two points in the condenser front focal plane: one near the opaque edge stop and the other somewhat farther from the edge. (Actual transmitted illumination is a continuum). For the positive-phase gradient sample, both rays are shown to be transmitted past the objective edge stop; for the zero gradient, one ray is transmitted; for the negative gradient, neither ray is transmitted. The view of the objective back focal plane (seen directly with the ocular removed) shows the objective edge stop on the right side of each circular field and the inverted image of the condenser edge stop on the left side. For an actual sample, typically with a zero-average phase gradient, the condenser edge stop should be laterally positioned to give a back focal plane view similar to the zero phase gradient case shown here.

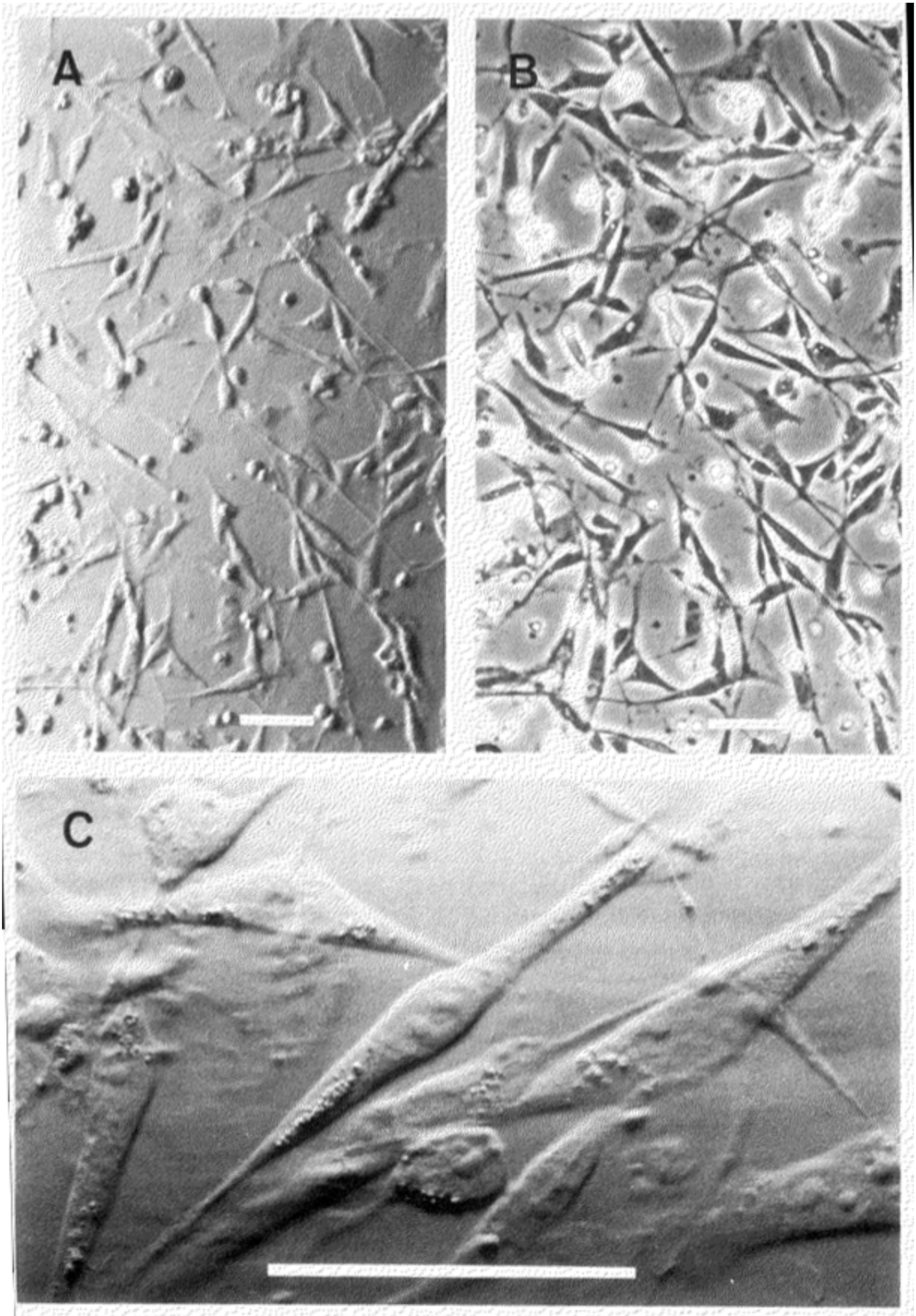

Fig. 2. A, cells in a primary culture of dissociated rat muscle tissue (*8*), viewed by schlieren optics with a 10×, NA = 0.25 Leitz achromat objective and a NA = 0.60

Fig. 3. "Optical sectioning" on a human cheek epithelial cell viewed by schlieren optics with a 100×, NA = 1.25 oil-immersion Leitz achromat objective and a NA = 0.90 Leitz condenser. Both A and B show the same field of view, but their planes of focus differ by about 5 μm. The nucleus is prominent in A whereas cytoplasmic granules are prominent in B. Bar = 25 μm.

magnification objectives (≤20 ×) is less critical; if an insert tube is not feasible, the masking tape can be placed at the back surface of the objective. Nonideal longitudinal positioning of the edge stops causes parallax between the edge stops as viewed down the eyepiece tube and a smooth gradation of background intensity from one side of the field of view to the other. However, the schlieren effect is often preserved over most of the field of view.

The two opaque edge stops in the schlieren technique are analogous to the condenser slit and the graded density modulator plate in the modulation contrast technique (*7*). Both techniques are sensitive to a preferred direction of phase gradients; both are free of halos characteristic of phase contrast; and both show little confusion from slightly out-of-focus phase gradients ("optical sectioning"; see Fig. 3). The schlieren technique has the advantages that it can be installed nonprofessionally, requiring no specially manufactured modulator plate and that the transmitting portion of the objective aperture is entirely unaltered.

The schlieren modification discussed here is completely compatible with si-

jective's aperture clear. [In Saylor's schlieren system (*2*), the objective obscures approximately half of the aperture.] This offset is of particular advantage in the epi-illumination fluorescence where high objective apertures are desirable.

Acknowledgment

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