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Phase-contrast microscopy of the primate retina

James D. Fix
Department of Anatomy, Marshall University School of Medicine, Huntington, West Virginia 25701, USA

Summary. The study of hematoxylin and eosin stained thick sections (15 µm) of the primate retina with the phase-contrast microscope provided a means for the selective demonstration of many cellular structures that could not be resolved with the same degree of detail which was possible when bright-field microscopy was used, or when phase-contrast microscopy was employed to examine unstained material. The H & E-stain greatly enhanced the phase-contrast image, so that cytoplasmic structure, fiber trajectories, and gross synaptic detail of the retina could be demonstrated to better advantage.

Key words: Primate- Retina

Introduction

Since the discovery of phase-contrast microscopy by the Dutch physicist Zernike (Zernike, 1935) in the early 1930's, and its subsequent development by Koehler and Loos (Koehler and Loos, 1941) in the early 1940's, its principal use in biology has been the examination of thin unstained histological material as well as living and dead microorganisms, although there has been some investigations performed on stained and supravitally stained material (Barer, 1947, 1948; Shanklin and Azzam, 1965; Muro, 1966). Fix pointed to the advantage of using phase-contrast in examining stained thick sections of the central and peripheral nervous systems (Fix, 1970, 1975).

Offprint requests to: Prof. James D. Fix, Department of Anatomy, Marshall University School of Medicine, Huntington, West Virginia 25701, USA

The primary object of this study was to study H & E-stained thick sections of the primate retina with phase-contrast microscopy. It was anticipated that differences in the refractive indices of various cellular organelles and their surrounding retinopil would render visible structures that could not or only unsatisfactorily be resolved by the use of bright-field microscopy.

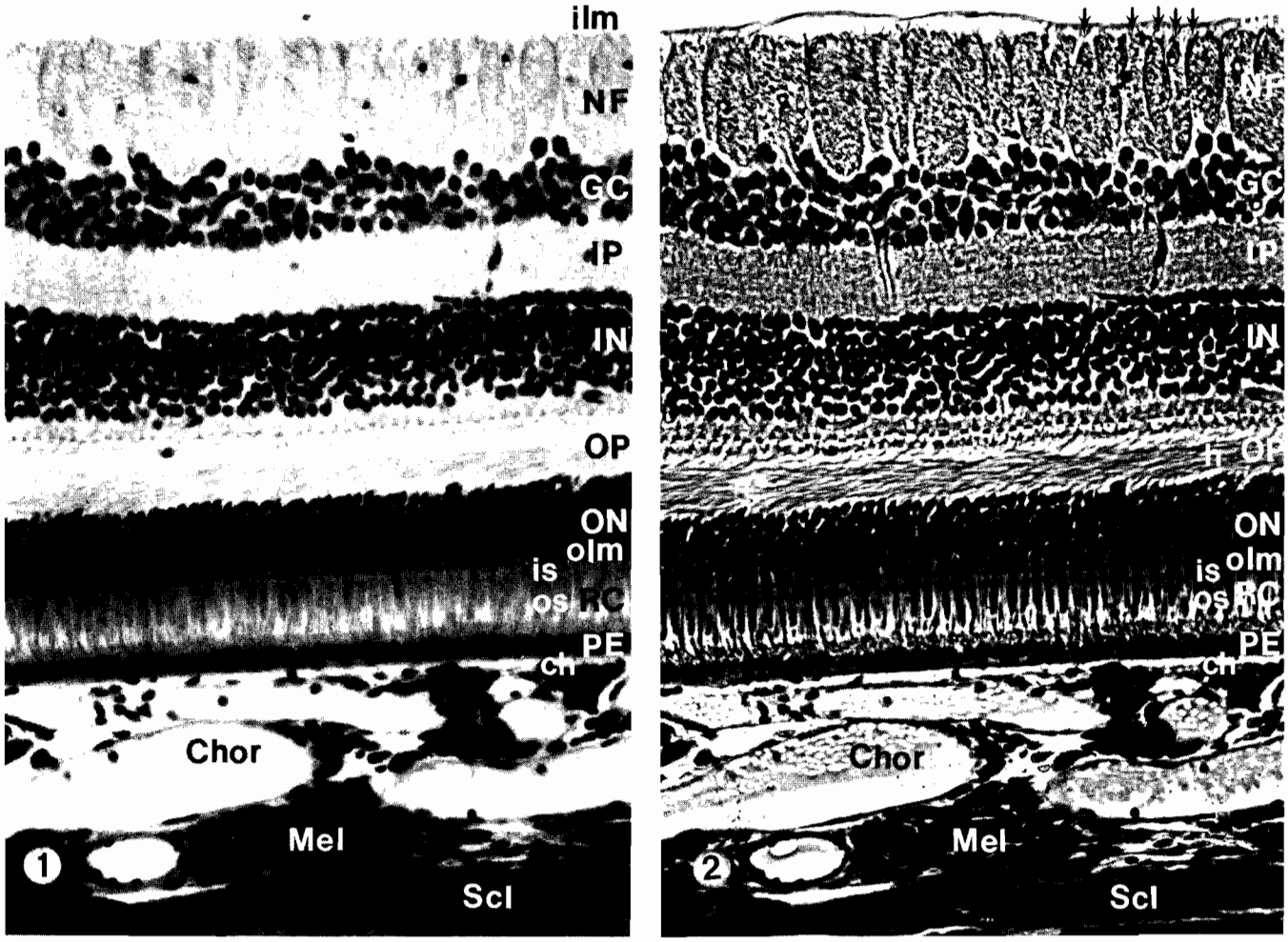
Materials and methods

The eyes of Rhesus monkeys, *Macacus rhesus,* were enucleated from anesthetized animals. The globes were injected with Bouin's solution to insure immediate infiltration of the retina, and then left in the same fixative for 48-72 hours. The entire globe was embedded in celloidin. Thick serial sections (15µm) were cut in the vertical plane and stained with hematoxylin and eosin.

All observations and microphotographs were made with a Zeiss photomicroscope II equippend with the following phase-contrast and bright-field optics: 1) 10.8mm Neofluar 16/0.40, 2) 7.1mm Neofluar 25/0.60, 3) 1.6mm plan-apochromat 100/1.3 oil, and 4) an acromatic-aplanatic phase-contrast condensor, N.A. 1.4. Kodak Panatomic-X film with a green filter was used.

Results

Figure 1 represents a hematoxylin and eosin section of the parafoveal retina located 1.27mm from the fovea in the inferior hemiretina of *Macacus rhesus,* and photographed with bright-fields optics. Although the classic ten layers of the retina are visible in bright-field, only the pigment epithelial layer (PE), the outer nuclear layer (ON), the inner nuclear layer (IN), and the ganglion cell layer (GC) are seen to good advantage. The non-cellular layers of the retina, with the exception of endothelial cells, glial elements,



Figs. 1 and 2. Parafoveal retina of *Macacus rhesus.* Section 15μm thick. Stained with hematoxylin and eosin. Located 1.27 mm from the fovea in the inferior hemiretina. 1. Bright field microphotograph, 2. Phase contrast microphotograph of the same field. Compare the definition of the fibrous layers in particular. Note the microvasculature of the outer four layers (to and including the inner nuclear layer). The inclination and trajectory of Henle's layer can be used to determine the orientation of the section will reference to the fovea. In this case the fovea is to the left. Ilm, inner limiting membrane; NF, optic nerve fiber layer; GC, ganglion cell layer; IP, inner plexiform layer; IN, inner nuclear layer; OP, outer plexiform layer; h, Henle's layer; ON, outer nuclear layer; olm, outer limiting membrane; RC, layer of rods and cones; is, layer of inner segments; os, layer of outer segments; PE, pigment epithelial layer; ch, choriocapillaris, Chor, choroid; Mel, melanocytes; Scl, sclera.

and an occasional aberrant or ectopic perikaryon, are devoid of structure. The oblique trajectory of Henle's fibers and the postsynaptic cone-pedicle zone of the outer plexiform layer (OP) are poorly demonstrated. The layer of rods and cones (RC) is not clearly differentiated into a layer of outer segments (os), and a layer of inner segments (is). Rod and cone segments are not distinguishable from each other, as they are, for example, when using Mallory's triple stain (see Wolff, 1970).

Figure 2 is a photomicrograph of the identical parafoveal retinal field shown in figure 1, but using phase-contrast optics. The **inner limiting membrane** (ilm) is clearly seen as partially dehisced from the optic nerve fiber layer (NF). Müller cell processes (and astrocytic processes from the optic nerve layer) are shown in contact with the ilm. The optic nerve fiber layer layer (NF) contains many oval and spindle-shaped fascicles of axons, cut primarily in cross-section. The radial fibers of Müller cells package these nerve fiber bundles. The ganglion cell layer (GC) is four to five cells thick. Well defined capillaries can be followed throughout this layer. Prima vista the **inner plexiform layer** (IP) appears amorphous. Closer inspection shows that this layer can be subdivided into a number of longitudinal sublayers. These lines are best seen by turning

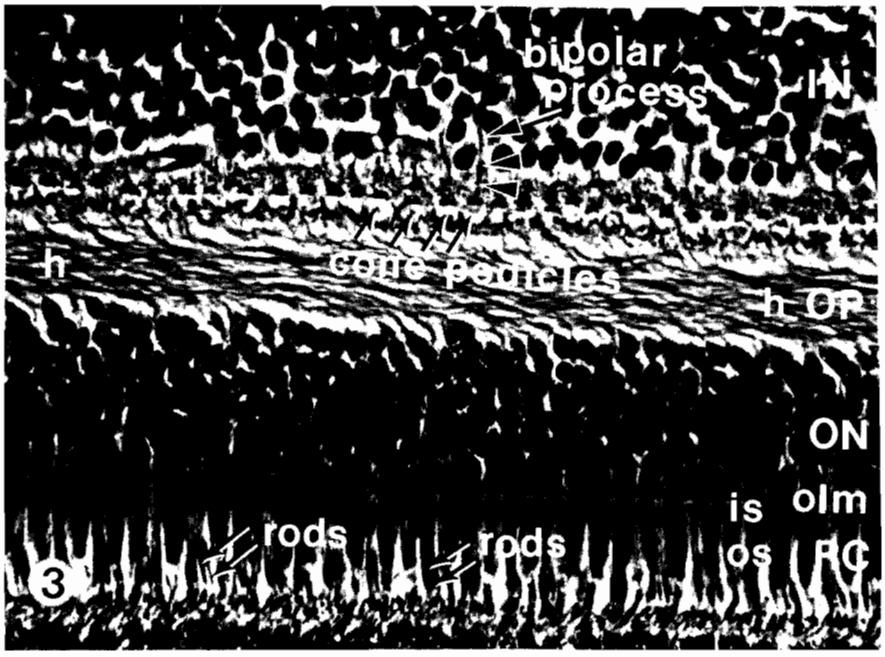


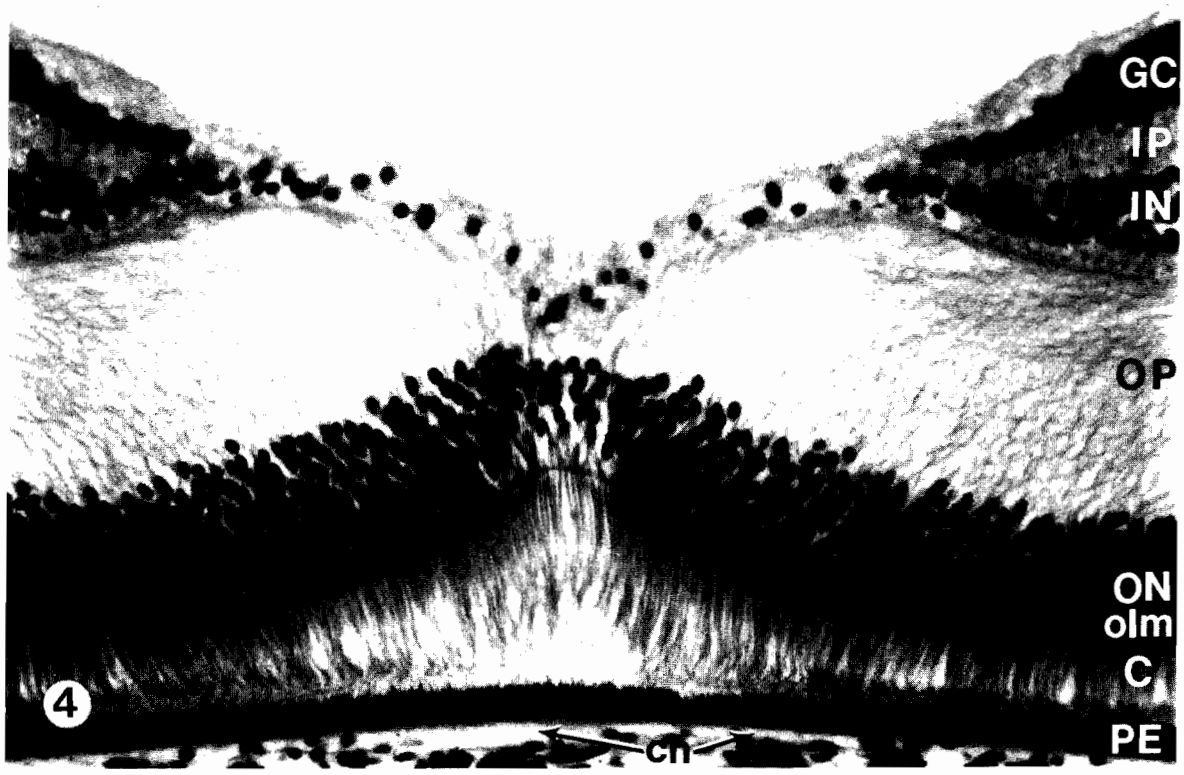
Fig. 3. Parafoveal retina at 1.6mm from the fovea in the same plane of section as figures 1 an 2. Magnification has been increased to give more detail of the outer plexiform layer, and the layer of rods and cones. The trajectory of the axons of Henle's layer is well defined. Their course is away from the fovea. The bipolar processes can be traced to the dark staining synaptic zones which are in "contact" with the cone pedicles. In the layer of rods and cones note a clear stratification of dark-light zones especially in the cone outer segments. Slender rod outer segments can be differentiated from the plumper cone outer segments.

longitudinal sublayers. These lines are best seen by turning the photograph 90° and looking down this layer from the side. Capillaries are seen to good advantage passing vertically through this layer. The **inner nuclear layer** (IN) has a high numerical cell density and contains a well defined capillary net, which is not found in the subsequently described layers. In the transition zone between the inner nuclear layer (IN) and the **outer plexiform layer** (OP), processes from cells in IN are seen passing to the dense zone of the OP. The **outer plexiform layer** (OP) is clearly divided into three zones: 1) a dense zone subjacent to the IN, 2) a synaptic layer consisting of a conspicuous line of dense staining dashes or dots, and a series of corresponding pale staining (or non-stained) circular profiles which represent the cone pedicles and 3) Henle's fiber layer. Henle's layer (h) runs obliquely in figures 2 and 3 and consists of the long distal processes of rods and cones which terminate as rod and cone pedicles respectively. The **outer nuclear layer** (ON) in this figure is densely packed. From the innermost cell layer of the ON, processes of the rods and cones can be seen projecting into the oblique Henle's fiber layer. The intermittent outer limiting membrane (olm) is sharply dilineated under phase-contrast optics. The **layer of rods and cones** (RC), also called the bacillary layer, is subdivided by optical path differences into numerous structural subdivisions. This stratification is much better defined in the wider cones than in the thinner rods. These are described as follows: 1. a narrow pale staining zone seen directly below the olm; this represent the myoid portion of the inner segments, 2. a thicker dark staining zone, representing the ellipsoid portion of the inner segments; the densely packed mitochondria are responsible for this dark staining band, 3. a thin lightstaining transitional zone between the densely packed mitochondria and the outer segments, 4. a dark broad staining band of the outer segments; especially of the cones - representing the stacked discs of EM; 5. a loose zone containing large artifactual spaces of fixation, the processes of the pigment epithelial cells, and the terminal tips of the outer segments of the rods and cones which are under going degradation; and 6. a dense bushy zone, consisting of the processes of the pigment epithelium; the circular nuclei of the pigment epithelium seem to rest on the well defined Bruch's membrane or *lamina vitria.*

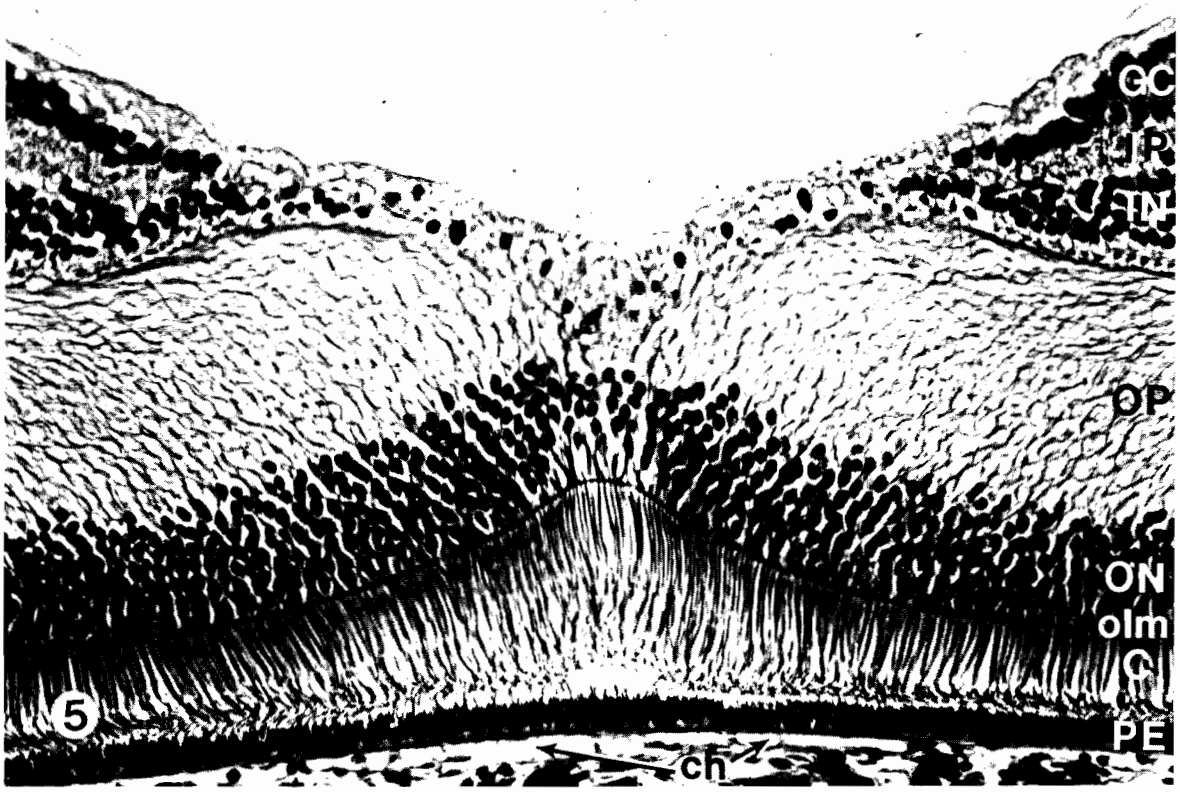
The choriocapillaris (ch) and the choroid show more structural definition with phase-contrast optics than bright-field optics.

Figure 3 was taken 1.6 mm from the fovea in the same plane of section as figures 1 and 2. The magnification is greater than figures 1 and 2 and gives better definition of the outer plexiform layer (OP) and the layer of rods and cones (RC) (bacillary layer). Processes from the inner nuclear layer appear to terminate in the dense staining dashes which are thought to represent synapses on the cone pedicles. The course of Henle's fibers are seen to good advantage. The stratification of the layer of rods and cones is well demonstrated. Cone outer segments can be differentiated from the thinner rod outer segments.

Figures 4 and 5 represent the fovea centralis, in bright-field and phase-contrast respectively. The cone peripheral processes (outer plexiform layer), the cone nuclei (outer nuclear layer), and the bacillary layer (in this case cones only) dominate the picture. The inner five layers have been pushed laterally and are seen in the clivus of the fovea.



Figs. 4 and 5. Fovea centralis of the retina. 4. Brigh field microphotograph. 5. Phase contrast microphotograph of the same field. Note the direction of the axons in Henle's layer, i.e., away from the fovea. The foveal retina is intrinsically avascular. The choriocapillaris is three times thicker in the fovea as in the parafoveal retina figs 1 and 2.



Capillaries are absent as would be expected. Note, however, that the choriocapillaris (ch) has expanded to a diameter of three red blood cells (Figs. 2, 3). The cone inner and outer segments are greatly elongated in the fovea. As in the parafoveal retina, the bacillary layer is subdivided into five alternating zones of light and dark, which, however, are not as distinct as in the parafoveal periphery. Note in figure 5 that the peripheral cone processes are deflected away from the fovea and course obliquely in Henle's layer of the outer plexiform layer. This holds true in the parafoveal retina (Figs. 1, 2, 3).

Discussion

The phase contrast microscope used in combination with H & E-stained material proved to be a valuable tool in studying the light microscopic structure of the retina. The angioarchitecture of the inner four layers of the retina is seen to excellent advantage. The structural detail of the fibrous layers seen with phase-contrast is vastly superior to that seen with bright-field optics. The outer plexiform layer provides a striking example of the periodicity of synaptic organization; the distal fibers of the rods and cones (Henle's fibers) can be traced to their zone of termination subjacent to the inner nuclear layer. Cone pedicles can be resolved without difficulty. A postsynaptic condensation of bipolar terminals is readily visible as a periodic line of intermittant dashes.

The use of this combination method, stained sections/phase-contrast optics, should prove valuable to the electron microscopist interested in the orientation of his tissue for thin sections. It is possible, for example, to determine the location of the tissue sample with respect to the fovea by observing the deflection of the fibers in Henle's layer. This method would be advantageous not only to the neuroanatomist interested in investigating cyto- and angioarchitecture, but also to the ocular pathologist studying changes in cell structure and the deranged angioarchitecture associated with disease.

This combination method should be used, or at least demonstrated, to all students who study the structure of the retina.

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