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IN VIVO LIGHT MICROSCOPY OF ORGANS

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Abstract

High resolution, brightfield and fluorescence, light microscopic methods have been developed for examining living organs in situ. The methods permit study of the rate, duration, magnitude and direction of dynamic histologic, pathologic, physiologic and pharmacologic events. In addition, morphometric analyses of such living preparations can provide basic information needed to evaluate alterations induced by fixation and processing of these organs for electron microscopy. Most organs are amenable to such investigations. In anesthetized animals, the selected organ is trans-and/or epi-illuminated with selected wavelengths of monochromatic light, imaged with water immersion objectives and the resulting monochromatic optical images televised using silicon or silicon intensified target (SIT) vidicon cameras. Quantitation is obtained by interfacing appropriate analog and/or digital instrumentation with the electro-optical system. Under optimal conditions the resolution is 0.3 µm, essentially the maximum obtainable by light microscopy. As a result, imaging is possible of most intra- and extravascular cell types, their nuclei, nucleoli as well as some cytoplasmic organelles and inclusions. The use of vital dyes and fluorescent tracers along or in combination with physiologic and pharmacologic stimuli provides information about structural-functional relationships in these intact living organs during both health and disease.

KEY WORDS: In vivo microscopy, light microscopy, organs, electronic imaging

Introduction

High resolution, brightfield and fluorescence, light microsocpic methods have been developed during the past two decades for examining living organs. Most organs and tissues are amenable to study. Use of these methods has resulted in a better understanding of the dynamic structure and function of several organs since the rate, duration, magnitude and direction of histologic, pathologic, physiologic and pharmacologic events can be monitored (4-14,16-19). While most studies have been of intact organs in situ, recent results indicate that isolated, perfused organs also are suitable candidates for in vivo microscopy (19).

The basic methods that have been used to study organs in vivo include: (a) examination of surgically exposed organs in situ or as isolated, perfused preparations; (b) examination of organs in situ through windows implanted in the body wall; (c) and examination of grafts of organs contained within chambers implanted in ectopic sites. Each method has advantages and disadvantages (for review, see ref. 7). Since only the first option ((a), above) consistently permits high resolution studies at the cellular level, this paper will be restricted to reviewing its methodology as applied to abdominal organs.

Methods

A standard compound binocular microscope (Leitz Panphot) has been modified for in vivo microscopy (Fig. 1) and is equipped for trans-illumination and/or epi-illumination. After the animal is anesthetized, the organ to be studied is gently exteriorized through an appropriate abdominal incision and positioned over a window of optical grade glass or mica mounted in a specially designed microscope stage having provision for drainage of irrigating fluids (Figs. 2 and 3). The window overlies a long working distance condenser. The organ is covered by a piece of Saran (Dow Chemical Co.) which for some studies is cemented to a movable "U"-shaped metal or plastic frame (Figs. 2 and 3). The Saran holds the organ in position (Fig. 3) and limits movements induced by respiration, the heart and intestines, yet it is flexible enough to avoid compression of the underlying microvasculature in the organ.

Homeostasis is maintained by constant irrigation of Ringer's solution over the surface of the organ. The temperature of the Ringer's is maintained at body temperature automatically by electronically controlled, proportional regulating heaters. Using this system, the surface of the organ is maintained at rectal temperature + 0.3°C.

Transillumination

The organ is transilluminated with white light or with monochromatic light (400-800 nm) obtained from a Leitz prism monochromater equipped with a xenon lamp (XBO-150) through the long working distance condenser. The microscopic images of the microvasculature and its surrounding tissue are secured at magnifications of 100-1500X using Leitz 10X, 22X, 50X, 55X, 75X, 80X, 90X, or 100X water immersion objectives with appropriate oculars. The resulting optical images are either studied and photographed directly or are projected onto the photocathode of a silicon or silicon intensified target (SIT) vidicon television system using a projection ocular (1.6-5.0X). Video images are either taped using a 1.87 cm (3/4") U-matic video tape recorder (Sony) or kine-recorded at 30 fps from a 42.5 cm (17") video monitor using a 16 mm Arriflex 16-S motion picture camera equipped with a special motor to synchronize the framing of the video and photographic images. Kodak Tri-X or Plus-X Reversal film is used. Figure 1 illustrates some of the optical and electronic equipment.

The use of a monochromater permits the selection of wavelengths of light which are selectively absorbed or transmitted by specific tissue and cellular components. When such differences of absorption are sensed by the television tube and converted into an electronic image, the contrast between tissue and cellular components can be enhanced further by readjustment of the brightness and contrast controls on the video monitor. Thus, the images of a particular structure can be enhanced or suppressed depending upon the wavelength of light selected and the adjustments of the television system. For studying highly vascular organs such as the spleen or liver, it is useful to transilluminate the organ at wavelengths of light between 575 and 750nm to eliminate the absorption of light by the hemoglobin contained in the numerous erythrocytes flowing or sequestered in the microvasculature. This not only increases the amount of light transmitted through the organ, but also enhances the definition of the endothelium and other cellular components. When such images are televised using a silicon vidicon having a peak spectral response between 600 and 800 nm, the following can be observed in most organs at very low light levels (10^{-2} fc): differentiation of the microvasculature into arterioles, capillaries or sinusoids, and venules; patterns of blood flow through these vessels; the shape and deformation of individual blood cells; the endothelium of most vessels; and identification of most extravascular cellular types and some of the cytoplasmic and nuclear detail in these cells (e.g., secretory granules, fat droplets, mitochondria, and lysosomes, nucleoli) (Figs. 4-8). The measured resolution of this system under optimal conditions is 0.3-0.5 µm when using 80-100X objectives. It should be noted, that the use of phase contrast and differential interference contrast optics has not yet been useful in the study of most trans-silluminated living organs since the thickness of the tissue precludes obtaining resolvable phase differences.

Epi-illumination and Fluorescence.

In addition to transillumination, the organ can be epi-illuminated through the objective lens using a Leitz Ploempak illuminater containing a variety of selectable excitation and barrier filters. Either a mercury (HBO-200) or xenon (XBO-150) light source is used depending upon the desired spectral illumination. While epi-illumination is most useful for studying the patterns and distribution of fluorescent probes (Figs. 9 and 10), it also can be used to visualize the natural fluorescence of intracellular substances (e.g., Vitamin A). However, the definition obtained using this method is never as good as that obtained by transillumination (Fig. 10). For intensely fluorescing materials, epi- and trans-illumination may be combined to provide improved definition of the cellular localization of a variety of fluorescent probes (Fig. 9). Alternatively, weakly fluorescing probes may first be imaged and recorded by epi-illumination and their localization subsequently identified by transillumination.

Image processing.

For many weakly fluorescing substances, the use of intensified (SIT, ISIT) video cameras is necessary with the result that considerable electronic noise is present in the video image (Fig. 10). In such cases,the use of digital image processing techniques and filtering can significantly improve the image quality and extraction of the desired information. Digital image processing techniques also permit quantification of information contained in the video images (3,19). For example, it is now possible to morphometrically evaluate organs under living conditions to determine the effects of preparation and fixation for LM, TEM and SEM procedures.

Conclusion

In conclusion, high resolution electro-optical techniques now permit quantitative evaluation of intact, living organs at the cellular levels *in situ*. Responses to pharmacodynamic substances,

Figure 5. *In vivo* photomicrograph of sinusoid lining cells in rat liver. S, sinusoid; KC, Kupffer cell which has phagocytosed 1 µm latex particles (arrows); E, endothelial cells; FSC, process of fat storing cells. Bar=10µm.

Figure 6. *In vivo* photomicrograph of sinus (S) in rat spleen with erythrocytes (RBC) penetrating the endothelial lining (E). Bar=10 µm.

Figure 7. *In vivo* photomicrograph of higher magnification showing erythrocytes (RBC) penetrating endothelial lining (E) of sinus (S) in rat spleen. Arrows indicate direction of flow. Bar= 10 µm.

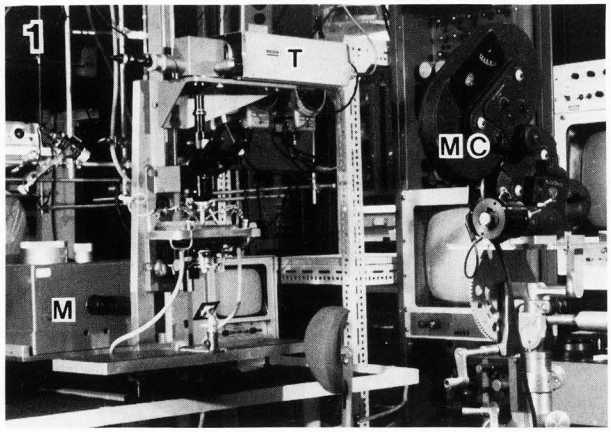


Figure 1. Light microscope modified for high resolution in vivo microscopy, using monochromatic light source (M) and television (T) imaging. MC, motion picture camera modified for recording video images.

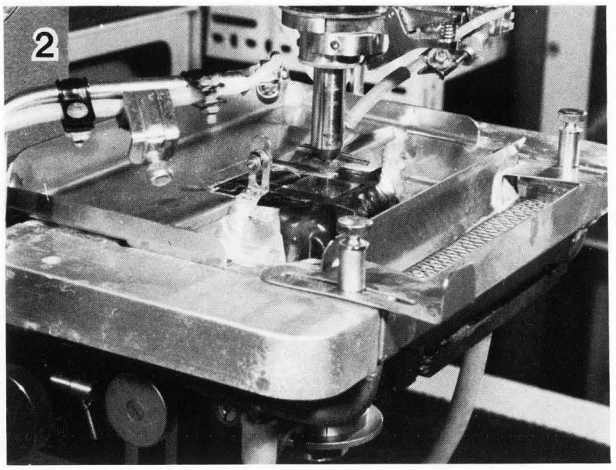


Figure 2. Microscope stage modified to hold small laboratory animals for in vivo microscopy.

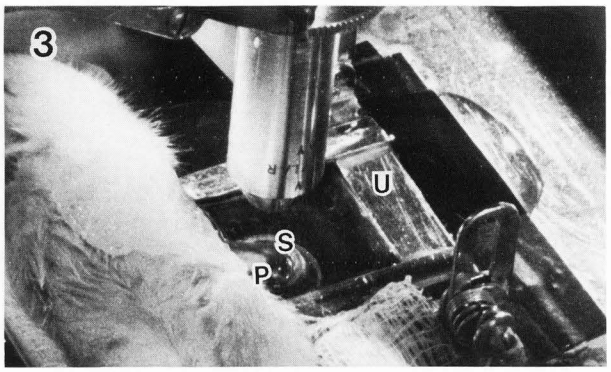


Figure 3. Exteriorized spleen (S) and pancreas (P) of anesthetized mouse. To reduce movements, the organ is stabilized with Saran held in a "U" frame (U).

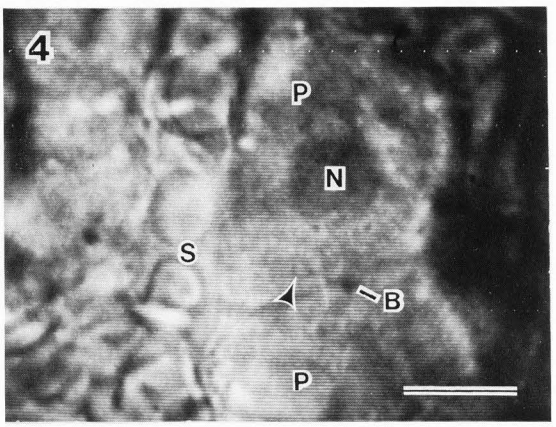
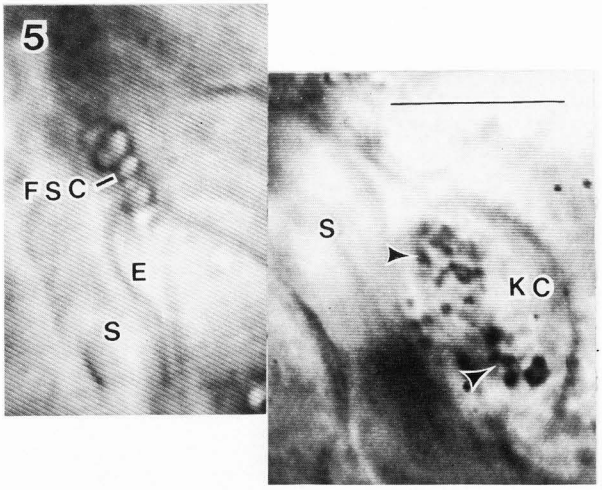
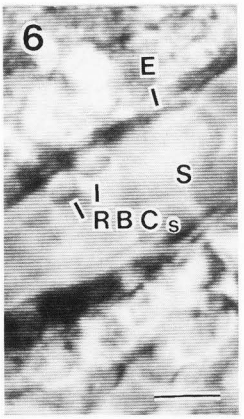
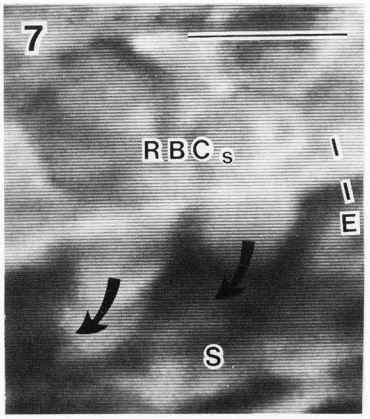


Figure 4. In vivo photomicrograph of rat hepatic parenchymal cells (P) and sinusoid(S) containing erythrocytes. Note nucleus (N), bile canaliculus (B) and organelles (arrow) in the parenchymal cells. Bar=10 µm.







(Captions for figures 5, 6, and 7 are on the facing page).

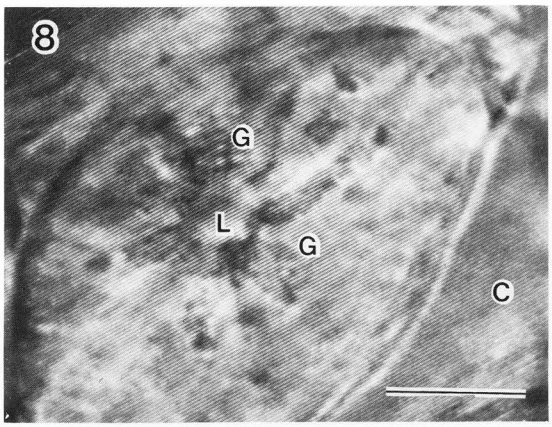


Figure 8. In vivo photomicrograph of base of intestinal gland in the mouse. C, capillary; G, secretory granules; L, lumen of gland. Bar=10 μm.

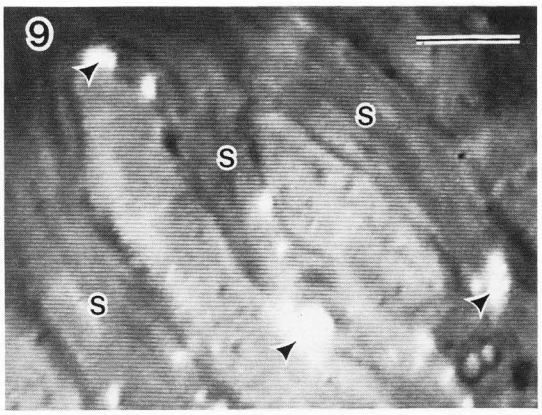


Figure 9. In vivo photomicrograph of sinusoids (S) in rat liver. Both transmitted and epi-illumination were used to simultaneously image some cellular detail and FITC-labelled latex particles (arrows) by Kupffer cells. Bar=10 μm.

physiologic stimuli and pathological conditions (5-14,16-19) can be evaluated in a quantitative manner, as can biological processes such as transvascular exchange of solutes (19), cellular secretory phenomena (8) and endocytotic mechanisms (see report by Wisse and McCuskey in these proceedings). While many of the above are just now being studied in intact living organs, similar studies in isolated cells and tissues have been ongoing for a number of years (1,2,15,20,21). In short, many of the techniques developed for investigations in cell biology are adaptable for in vivo microscopic studies of such cells in intact organs where their normal microenvironment is preserved. Thus, the possibilities of obtaining new information are exciting and limited only by limitations of personnel, space, time and financial support.

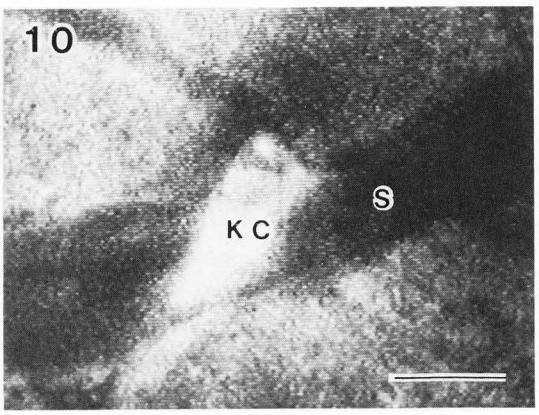


Figure 10. In vivo photomicrograph of Kupffer cell (KC) in sinusoid (S) of mouse liver. Epi-fluorescence of endocytosed FITC-endotoxin imaged with SIT vidicon; note electronic noise and lack of cellular detail. Bar=10 μm.

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