

## Scanning Slit Confocal Microscopy of the Living Eye

Barry D. Masters, Ph.D., Uniformed Services University of the Health Sciences, Bethesda, MD

The living human eye is in constant motion. The cornea, which is the transparent front surface of the eyeball, is a formidable specimen for microscopy. How can we use a microscope to obtain sufficient contrast in order to observe cellular and subcellular details on a moving, transparent specimen? Although the normal human cornea is free of blood vessels, there are many nerves within the 500  $\mu\text{m}$  thickness of this tissue. How can we observe these nerves in the living human eye?

To accomplish these aims, use a new real-time, scanning slit confocal microscope that was developed by Dr. A. Thaeer for imaging the *in vivo* human cornea. The optical design of the real-time, scanning slit confocal microscope is shown as follows in Figure 1. The confocal microscope is a modification of the real-time, scanning slit confocal microscope based on an oscillating two-sided mirror (bi-lateral scanning) which was designed and first constructed in 1969 by G. M. Svishchev in Leningrad. The design considerations of this confocal microscope were as follows:

- ◆ The use of nonapplanating, high numerical aperture, water immersion microscope objectives, Leitz 50X and Leitz 100X microscope objectives.
- ◆ The microscope objective would use a drop of Physiological Ringer's solution, or a methylcellulose gel to optically couple the tip of the microscope objective to the cornea. There was no applanation or direct physical contact, which deforms the cornea, between the objective and the surface of the cornea.
- ◆ One half of the numerical aperture was used for illumination, and one half of the numerical aperture was used for collection of the reflected and fluorescence light.
- ◆ Optical sectioning in the plane of the cornea was obtained with two sets of conjugate slits. The slit heights are variable and adjustable.
- ◆ An oscillating, two-sided mirror (bi-lateral scanning) was used for scanning the image of the slit over the back focal plane of the microscope objective, and for descanning the reflected and back scattered light collected by the microscope objective from the focal plane in the specimen.

- ◆ The light source is a 12 volt halogen lamp. For fluorescence studies a mercury arc lamp or a xenon arc lamp can be used.
- ◆ The scanning was synchronized with the read-out of an interline CCD camera in order that the full vertical resolution of the intensified CCD camera could be utilized.

This new real-time *in vivo* confocal microscope is in clinical investigations in several eye clinics in Europe. We have observed in single video frames, without the need for any image processing, the following: superficial epithelial cells, wing cells, basal epithelial cells, stromal nerves, stromal keratocyte cell bodies and cellular processes, and endothelial cells. **A unique feature of this new confocal microscope is that every video frame is recorded without any image blur.** Other video recording systems on real-time confocal microscope developed for ocular imaging record the eye movements as blurred images.

This new real-time *in-vivo* confocal microscope is being used to diagnose corneal pathology and investigate the cellular responses to laser refractive surgery and wound healing. Other active clinical applications are the cellular responses of the cornea to contact lens wear.

Microscopy is alive and booming. It is an exciting time to be involved in this exploding field of technology.

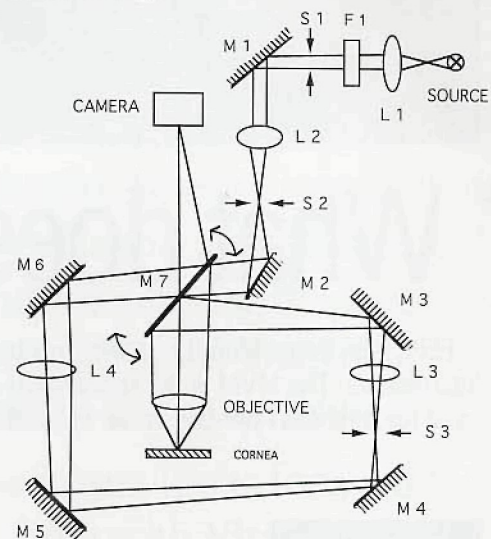


Figure 1: Schematic diagram of the bilateral scanning mirror *in vivo* confocal microscope

### Front Page Images: Relating to above article on Scanning Slit Confocal Microscopy

The upper slide shows the three-dimensional volume reconstruction of the full-thickness of a freshly enucleated rabbit eye. A stack of optical sections, obtained from reflection mode confocal microscopy, was reconstructed to form this volume visualization. The cornea is 400  $\mu\text{m}$  thick. The epithelium is on the top of the reconstruction and the endothelium is on the bottom. Within the stroma a large nerve is shown.

The lower slide shows the three-dimensional reconstruction of the *in situ* lenticular fibers of the ocular lens from a freshly enucleated rabbit eye. A stack of optical sections, obtained from reflection mode confocal microscopy, was reconstructed to form this volume visualization. The width of the individual lenticular fibers is 7  $\mu\text{m}$ .

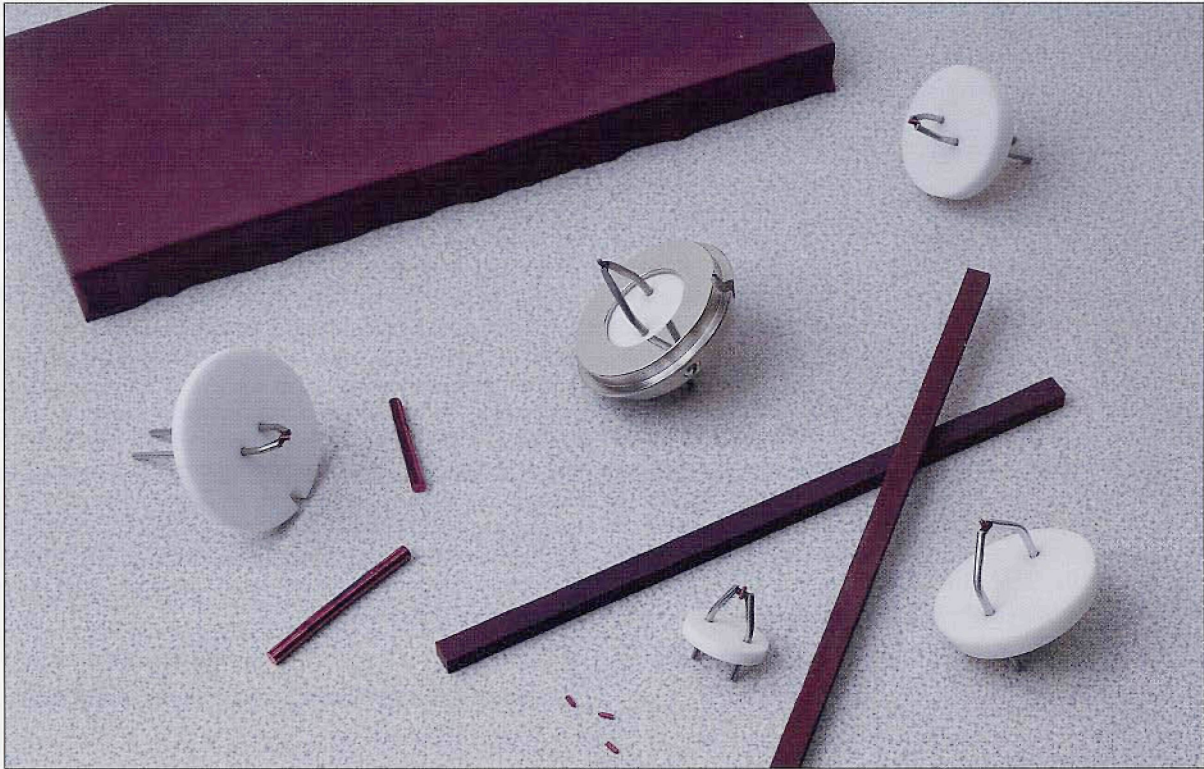
## MICROSCOPY TODAY

*A monthly newsletter dedicated to the unique interests in microscopy of, and mailed at no cost to, some 10,000 microscopists worldwide.*

PO Box 620122, Middleton, WI 53562 - Tel.: (608)836-1970 - Fax: 608)836-1969 - eMail: MicroToday@aol.com

Copyright in 1994 by Today Enterprises, All rights reserved

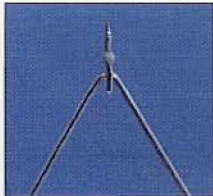
Don Grimes, Editor



LaB<sub>6</sub> Cathodes

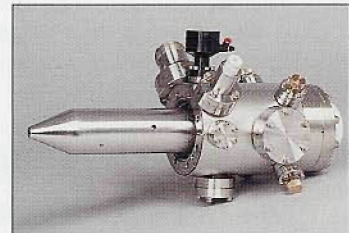
# What does fei stand for?

FEI's Mini Vogel Mount was the *first* true long-life, high stability LaB<sub>6</sub> cathode for all electron beam instruments. The MVM is the economical *emitter* choice, providing consistent high quality performance and the best cost-per-use value. FEI is the *innovator* in the application of field emission technology...



### Field Emission Cathodes

FEI supplies Schottky *field* emitters to EM manufacturers worldwide. Schottky *emission's* high current *intensity* has established it as the preferred electron source for high resolution SEM, TEM, Auger, ESCA, EDX, and lithography.



### UHV Electron and Ion Columns

FEI's high current density, UHV field emission *focusing* columns *easily install* on existing SIMS, Auger, and SEM instruments.



### FIB Workstations

*Focused* ion beam micromilling workstations range from the 8"-wafer compatible model to the *economical* 2" small sample model *ideal* for semiconductor, biological, TEM, and MEMS specimens.



FEI Company  
7451 NE Evergreen Parkway  
Hillsboro, OR 97124-5830  
(503) 640-7500 Fax (503) 640-7509  
email: dlh@feico.com

Now, when you think of FEI, you'll know we are the Specialists in Field Electron and Ion Technology.