# A New Microscopic Tool for Biology

## *Near-infrared microscopy increases contrast, improves light transmission, and aids study of photosensitive systems*

Microscopy in the near-infrared (NIR), long-neglected by the biological community, promises some new benefits for the study of living tissues and other organic materials. The two most important attributes of NIR light are the low energy of its photons and the changed transmission characteristics that occur during a shift from the visible to the NIR. In many cases, those different transmission characteristics mean that contrast is much greater in NIR light, eliminating the need for the dyes that are often used in visible-light microscopy to increase contrast. In some cases, objects that are opaque in visible light become transparent or translucent in the NIR. The energy of NIR photons is so low, furthermore, that it is rarely transferred to the specimen. This means that living cells are not killed or caused to mutate, as is often the case with x-ray or electron microscopy. More important, it means that photosensitive cellular systems are generally not activated, and therefore they can be studied in their resting state.

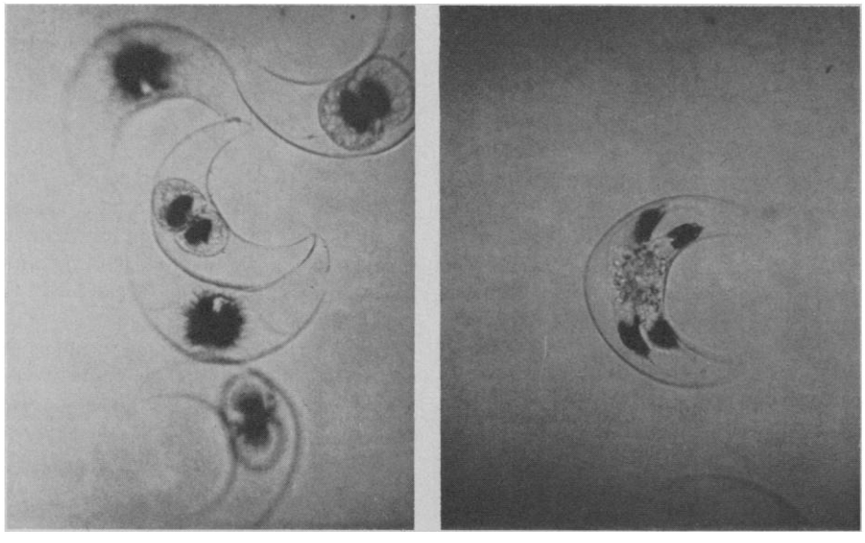
These capabilities have been recognized for some time by investigators in other fields. Forensic scientists, for example, routinely use NIR microscopy for examination of questioned documents for alterations, obliterations, and erasures; the increased contrast in the NIR range reveals even subtle changes. The technique can also be used for characterization of inks and pigments and for detection of handwriting or printing on burned or partially destroyed documents. The technique is also useful in the semiconductor industry. At wavelengths near 1100 nanometers, for example, materials such as silicon and gallium arsenide are transparent, and internal defects can be observed. Polarized NIR light can also reveal defects in bonding between components of a solid-state device.

Some materials of biological origin are also transparent to NIR light. For example, Lawrence A. Harris of the Oak Ridge National Laboratory is using NIR microscopy to study the structure of coal and coke. The ideal way to study coal structure is by transmission microscopy, Harris says, because transmitted light reveals the bulk properties of the sample better than reflected light. Transmission microscopy of coal with visible light, though, requires sections less than 0.5 micrometer thick, and preparation of such thin samples of coal is difficult. But Harris has found that many normally opaque constituents of coal are transparent to NIR light in sections as thick as 10 micrometers. Many fossilized plant components in coal, such as resinous bodies and spore exines, are also transparent or translucent in NIR light. Thus NIR microscopy can provide valuable information about the conditions under which a particular sample of coal was formed, Harris says. This information could be useful in determining the suitability of specific types of coal for conversion to synthetic fuels.

Takashi Hoshino of the Dokkyo University School of Medicine in Mibu, Japan, has found that sections of bone up to 1.5 centimeters thick are transparent to NIR light. He is using NIR microscopy to study the deposition of minerals in bone around plastic and metal prosthetic attachments to observe their effects on healthy bone and to learn how to strengthen the attachments. Similarly, the shells of crustaceans, snails, and other species have been shown to be transparent or translucent to NIR light, so that mating and other habits can be readily observed under NIR illumination.

Some of the potential applications of NIR microscopy in biology are still in very preliminary stages. Robert Fisher of the Eastman Kodak Company, for instance, has found that some strains of algae emit NIR light after they have been stimulated with visible light. He speculates that this might provide a means for counting algae. At Mobil Oil Corporation, a group of investigators headed by Richard Tonkyn has found some evidence that healthy plant cells can be distinguished from dead and dying cells by differences in their absorption of NIR; no useful differences appear in visible light. Neither group has undertaken to follow up on their original observations, however.

Tomas Hirschfeld of the Lawrence Livermore Laboratory is applying NIR microscopy to analytical cytology, especially to a technique known as flow cytometry. In this technique, individual cells are suspended in solution and dyed with appropriate preparations to emphasize specific characteristics. The dyed cells are then carried by the solution through a cell illuminated by a laser beam operating in the visible or ultraviolet range. By use of a computer attached to a light detector in the cell and fluid switching devices, cells that fluoresce when stimulated by the laser can be sep-



*Visible (left) and infrared (right) micrographs of the dinoflagellate* Pyrocystis lunula. *In visible light, the dark chloroplasts congregate in the center of the cell, obscuring the nucleus, but in infrared light, they remain on the periphery. [Source: Research Devices, Inc.]*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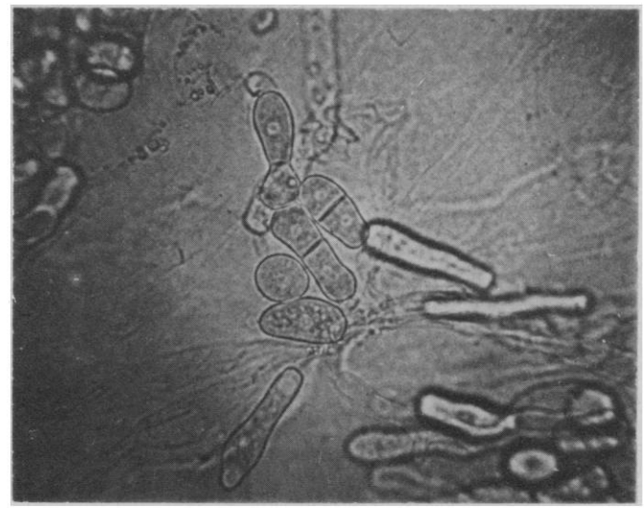
arated from those that do not, thus affording a greatly enriched concentration of one or the other. But, Hirschfeld says, two main problems are associated with this technique. Most work is done by illumination with blue, green, or ultraviolet light, and the lasers required to produce light in this part of the spectrum are relatively expensive. In those wavelength regions, furthermore, many of the cells of interest fluoresce even without being dyed, so that separation can be quite difficult.

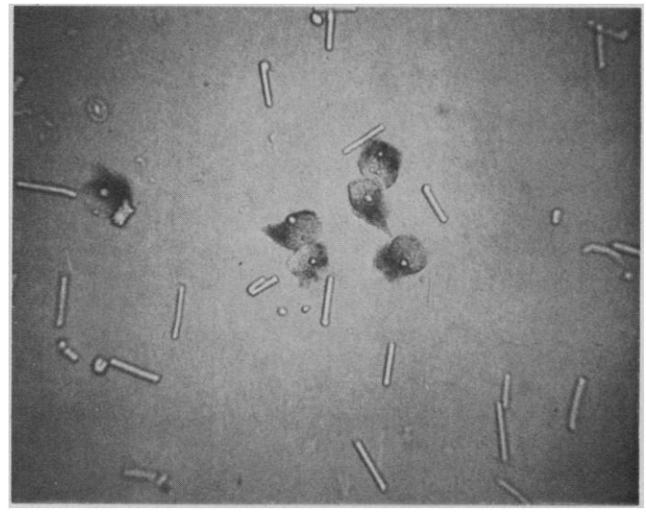
Hirschfeld has found, however, that cells illuminated with red light will fluoresce in the NIR region. Helium-neon lasers that emit red light are inexpensive, he says, and cells stimulated with red light exhibit much less autofluorescence. The main impediment now is that there has been very little development of dye systems for use with stimulation by red light. Hirschfeld is investigating such dye preparations with NIR microscopy and, once good systems have been developed, he says, the relatively low cost of flow cytometry in the NIR region should enable many more investigators to take advantage of it.

Perhaps the greatest potential of NIR microscopy in biology, however, is the study of photosensitive cells. Edward Meek of the University of Iowa, for example, is investigating retinal degeneration in the eye. In one aspect of the study, he is looking at the distribution of components and the metabolism of pigment epithelial cells, a group of cells that lie behind another layer of cells known as the neural layer. Illumination of the neural layer with visible light bleaches the cells so that their activity is altered. The neural cells are insensitive to NIR light so that the deep layer of choroid cells can be studied in an intact eye by NIR microscopy. Meek suggests that NIR microscopy could prove to be a valuable technique for studying retinal degeneration.

Ellis R. Loew and Ronald Riif of Cornell University are studying another form of retinal degeneration by attempting to monitor the deposition of small particles—which are probably photoreceptor cell fragments—in the pigment epithelium. They find that the number of particles present can be determined most effectively by measuring the scattering of NIR light, and that this can be accomplished without bleaching the photoreceptor cells. They have also found that certain types of lesions in diseased eyes, such as those due to vitamin E deficiencies, are more evident under NIR illumination than under visible. The Cornell group is thus studying ways for early diagnosis of eye disease in dogs by NIR illumination in hope that the techniques can be adapted for use in humans.



*Infrared micrograph of the fungus* Basidaboulus microsporus *without staining. The cell wall, nucleus, and nucleolus appear in high contrast because of the infrared illumination. [Source: Research Devices, Inc.]*



*Infrared micrograph of individual receptor cells from a frog retina. These cells can be observed in a completely dark-adapted condition because they do not respond to infrared light. [Source: Research Devices, Inc.]*

Such use may become increasingly important as a result of recent research which suggests that levels of visible light well below the intensity which causes thermal burns can damage the retina. Work by several investigators, says Jackie Lanum of Corpus Christi State University, suggests that the damage is localized primarily in the receptor. One potential cause of damage is the visible light source in the ophthalmoscopes and fundus cameras that are used in the diagnosis of eye diseases. This potential for hazard could be eliminated, some investigators argue, by the use of NIR light in such devices. Such a shift in illumination, though, will probably require considerable background work to ensure that practitioners will be able to relate what they see under NIR light to what they have previously observed under visible illumination.

Application of NIR microscopy in biology has been hindered by the lack of commercial instrumentation. Most biologists who have tried infrared microscopy have generally used a light microscope in conjunction with an image converter not unlike the sniperscope used for firing a rifle at night. Some resolution is lost in such homemade devices because the microscope lenses are optimized for visible light rather than infrared. Recently, however, Research Devices, Inc. of Berkeley Heights, New Jersey, has introduced a new NIR microscope that has been optimized for NIR wavelengths. The resolution of this $6900 instrument, say those who have used it, is about twice as good as that of adapted visible microscopes. The company has also begun manufacturing NIR adaptors for fundus cameras and ophthalmoscopes. With the new availability of such devices, the potential of NIR microscopy in biology should soon be much more thoroughly explored.—THOMAS H. MAUGH II