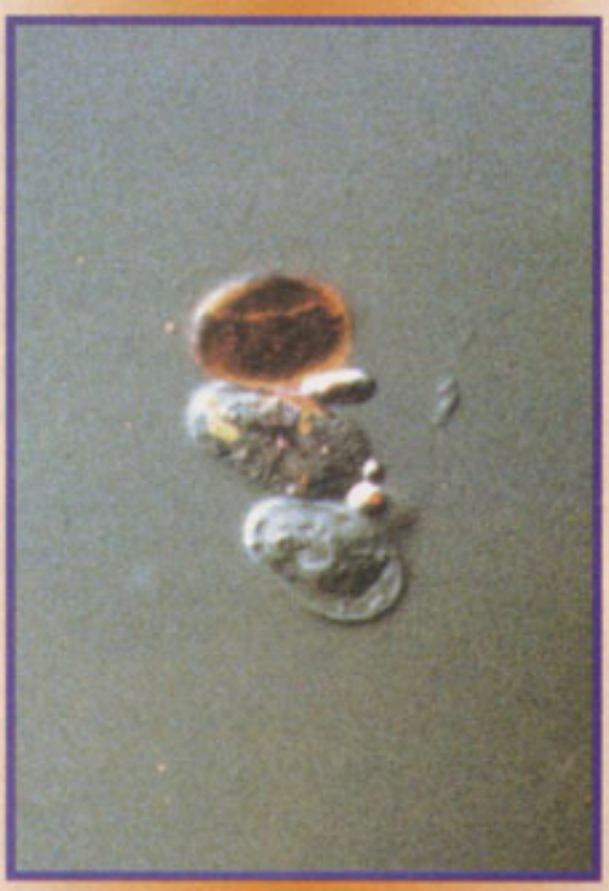
A laser trap aids study of infectious protozoans

Each year, the protozoans *Giardia* and *Cryptosporidium* sicken thousands of people, some fatally. They prey especially on the old, the young and anyone with a compromised immune system. *Cryptosporidium* outbreaks have occurred in Milwaukee, Las Vegas and other cities in the US and abroad. Because these parasites are hard to detect and to kill, researchers at the University of Southern California in Los Angeles and the Beckman Laser Institute in Irvine, Calif., have been working on better ways to study them.

"The infective agents of giardiasis or cryptosporidiosis are the cysts and oocysts, respectively — cellular resting stages," explained the university's Rodolfo Iturriaga. "The microscopic cysts are thick-walled and highly resistant to environmental conditions and chemical treatment. Epidemic outbreaks of giardiasis or cryptosporidiosis have been recognized worldwide. In most cases, the municipal water supply has been the vehicle of transmission."

The parasites live in the intestines until they reach a point in their life cycle when they are ready to spread to another host. They do this by becoming cysts. Like a plant seed, the cyst is a tough shell designed to protect the organism. It is excreted by the host and enters the water supply. The enzymes and acids in a host's digestive tract help dissolve the cyst and reactivate the organism. Because water plays a crucial role in how the cysts are transmitted, Iturriaga and his colleagues have been working on a method for studying the cysts in water using laser traps, epifluorescence microscopy and microphotometry.



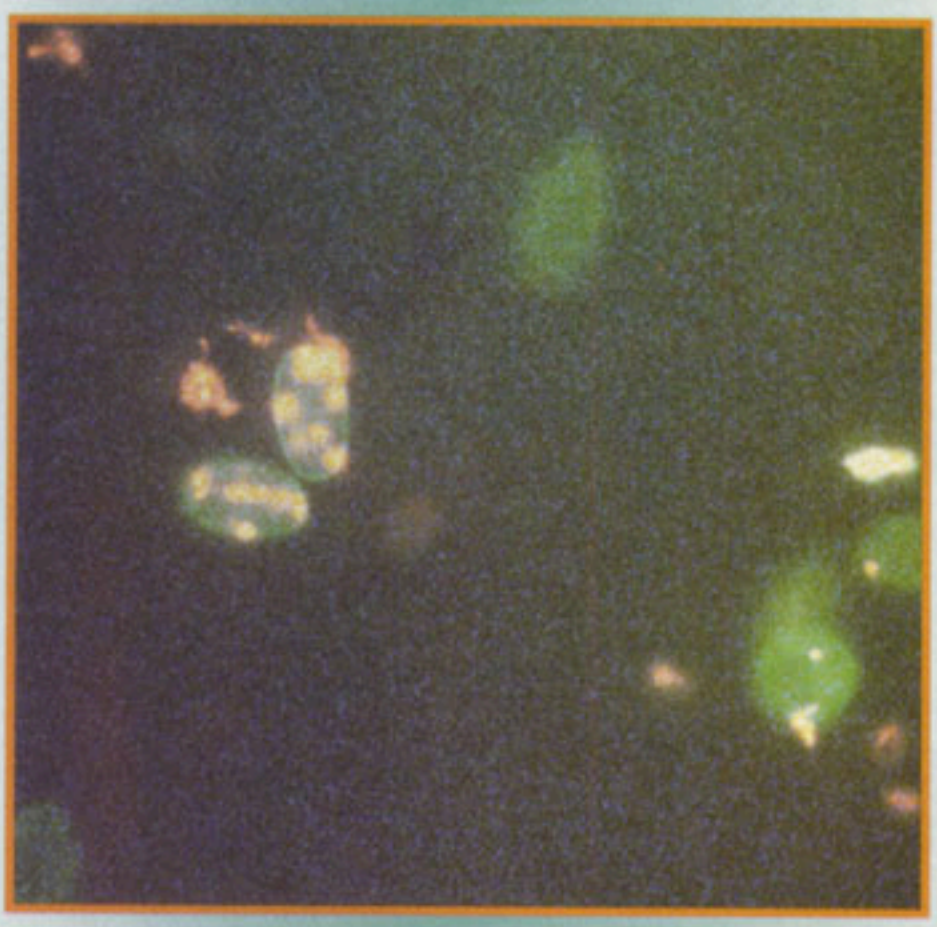
Giardia cysts, with only redox dye CTC, were observed under epifluorescence and Nomarski optics microscopy. Photos reprinted from* Journal of Microbiological Methods, *Vol. 46:1, pp. 19-29.

"Since cysts are responsible for transmitting these infections, it is highly relevant to know their survivability in water," he said. "Despite this, few studies have been conducted with intact cysts and under near-in-situ conditions." The researchers' method enables them to study the metabolic activity or the respiratory potential of intact cysts while in conditions similar to a municipal water supply. An added benefit is that they can test the effects of chemical or pharmaceutical treatments on the cysts.

Until this study, researchers typically studied the cysts using a stain and an epifluorescence microscope. Iturriaga and his colleagues have used a fluorescent oxidation-reduction (redox) dye that enables them to visualize the respiration of the cyst. The details of the experiment are published in the *Journal of Microbiological Methods*, Vol. 46, No. 1.

The process by which a molecule or atom gains electrons is called a reduction. When the redox dye CTC is introduced to a sample, it decreases in the cell's respiratory chain. The result is that the cell creates a product of the reduction inside its wall. This product, called formazan in the case of CTC, fluoresces red.

Because the dye is picked up and transformed in the process of cell respiration, researchers can judge from the fluorescence how active a cell is. "Determining the respiratory potential or the metabolic activity of *Giardia* cysts or *Cryptosporidium* oocysts is highly relevant to assessing their periods of latency or reactivation under natural or altered conditions. [This] is all relevant to understanding their [ability



These Giardia cysts, with CTC and antibodies, were viewed under epifluorescence microscopy.

to] survive under different conditions," Iturriaga said.

Using CTC, he said, could represent a useful tool to study the reactivation stage. Because the cells' respiratory activity increases when they reactivate, the CTC reduction and, therefore, the fluorescence signal in the cyst increase too. The tough wall of the cysts, however, poses a minor challenge. The group had to soften up the cysts with a touch of dimethyl sulfoxide, which helps the dye permeate.

Imaging methods

The scientists studied several methods of examining the cysts. They used an epifluorescence microscope from Carl Zeiss and illumination from a mercury or xenon lamp. They also used a microphotometer system from Zeiss to perform spectral analyses of absorption, fluorescence and reflectance spectra from cysts transferred to microscope slides, and an Nd:YAG laser trap microphotometer system to study the cysts' fluorescence spectra in situ.

"Our preliminary task was to explore the presence of natural fluorochromes in the cysts that could have been used as natural markers of cell integrity or conditions," Iturriaga said. "However, the fluorescence emission signal as a function of different excitations, including UV, was too low to be used as a marker." He said the group recorded emission of the CTC fluorochrome and that of fluorescein antibody labels to help select the appropriate emission filters for cytofluorometric studies and to help detect any other vital staining that could be used with CTC and fluorescein.

"To date, most observations [of] *Giardia* or *Cryptosporidium* cysts have been performed on stained cysts under the epifluorescence microscope. In the case of respiratory enzymes, [studies] were performed on cyst homogenates," Iturriaga said. "The advantage of the laser trapping and microphotometric detection is the ability to stain the cysts with an antibody for their specific detection and at the same time to quantitatively determine their respiratory potential or metabolic activity on intact cysts."

The group studied *Giardia* cysts for 21 days after they had been shed from a host. For the first week after the cysts formed, respiratory activity could be detected, and the cysts gradually became dormant as the days passed. However, what distinguishes a viable cyst from a nonviable one remains unclear.

Iturriaga said the researchers are hopeful that their method will provide an additional way to study the respiration rates for cysts, as well as to gain quantitative information about the cysts' spectral characteristics. He said that they also are looking into new types of instrumentation and that adding a flow cytometer could offer scientists a way of processing a large number of samples in a short time.

"For these types of studies, or any other type of environmental research that requires assessing cellular processes under natural conditions, we have considered [developing] a fiber optic laser trap microphotometer system," he said. "This system would be capable of trapping cells or particles at a certain depth in an aquatic environment, allowing us to perform observations in real time under natural conditions." □

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