Washington University in St. Louis
School of Medicine

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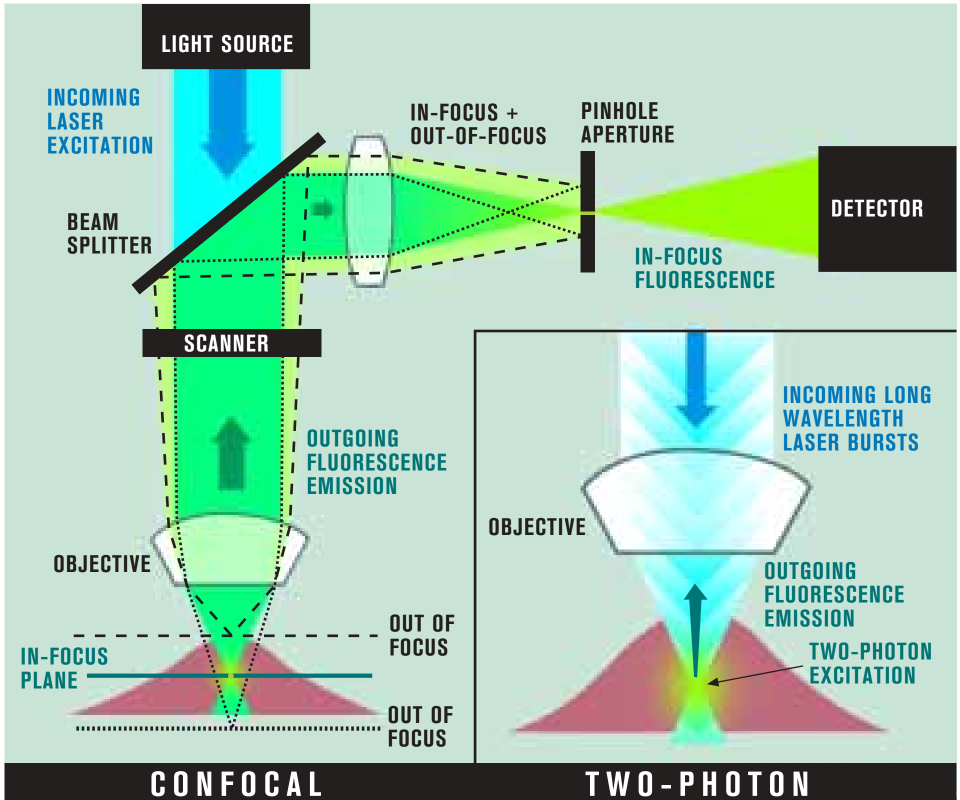

Neurons in a new light

"Trying to view neural elements deep in brain tissue with a standard microscope is like trying to see an object below the surface of a muddy pond by shining a flashlight into the water," says Jeff W. Lichtman, MD, PhD. Now, two groundbreaking techniques are helping to clear the water.

Not great-grandpa's microscope

Standard microscopes reveal too much of a good thing: Light scatters throughout the specimen, degrading the image. The late 20th century development of confocal microscopy changed all that by "pinpointing" the center of interest, delivering a "planely" better image.

Rather than allow the illumination to spread, the confocal system aims the waist of an intense, hourglass-shaped light beam at a thin plane of focus. Light fluorescing off this point is refined one step further by means of a pinhole aperture, eliminating nearly all out-of-focus light.

The result is an extraordinarily clear, though tiny, image. Assembling rows of these snapshots in a computer, along with successive layers above and below within the specimen, gleans three dimensions worth of data.

Less is more

Restricting the light a little helps a lot. Control the light particles still more and the story gets even better. Another enhancement, two-photon microscopy, is like the wedding album of two photons that chanced to meet at the focal plane.

Fluorescent molecules turn "on" when hit by one photon of the correct wavelength. Yet two near-simultaneous, half-strength photons work just as well. This time the laser beam pulsates; once again the high density of light at the waist of the hourglass-shaped beam works its magic: It's now the likeliest spot for a single half-strength photon to meet another at a fluorescent molecule.

This process creates no out-of-focus light and "sees" deeper into living tissue as the laser pulses 100 million times per second. If not pulsed, says Lichtman, the beam would require tremendous energy —and burn through anything in its path.

BY ERIC YOUNG