

TECHNOLOGY
HOW IT WORKS

The Confocal Microscope

By Aileen Constans

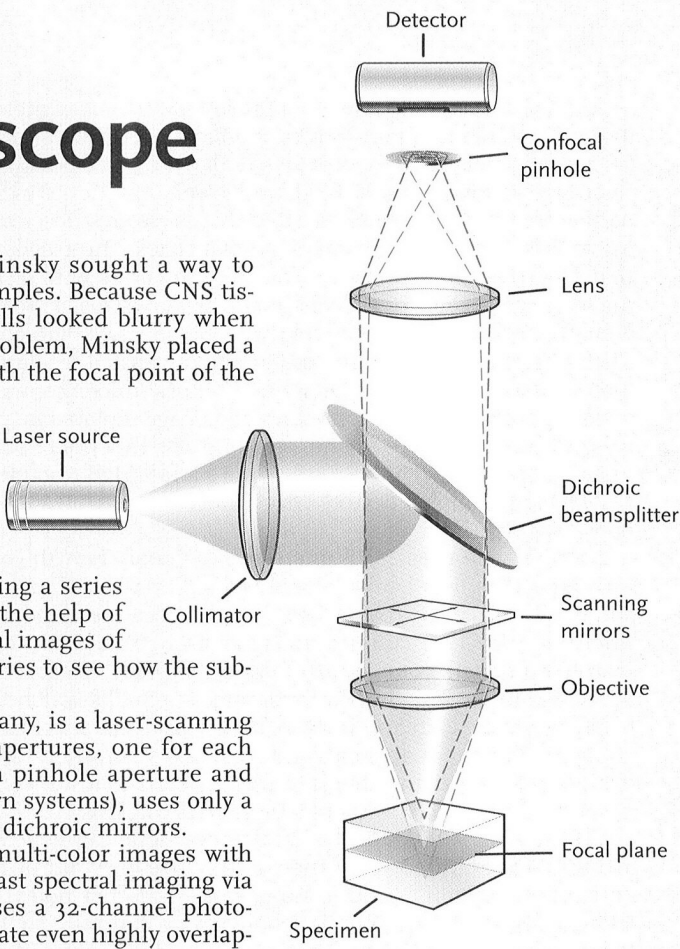
In the mid-1950s, Princeton University researcher Marvin Minsky sought a way to increase signal-to-noise when imaging central nervous system samples. Because CNS tissue is very dense and scatters light, fluorescently dyed brain cells looked blurry when viewed under a conventional widefield microscope. To counter this problem, Minsky placed a pinhole aperture at the emission side of the objective. Conjugated with the focal point of the lens (hence, “confocal”), the pinhole allowed in-focus light to reach the detector while blocking light emanating from regions above and below the focal plane (see box 1). In essence, it allowed him to view virtual “optical slices” through the haze of thick tissue.

But the resulting image, however sharp, represented just a small piece of a single optical slice. To image a complete slice, the entire plane had to be scanned. Today there are two primary approaches: laser scanning and spinning disk (see box 2). By collecting a series of such planes (a so-called “z-stack”) researchers can create, with the help of sophisticated computer algorithms, high-resolution three-dimensional images of the sample. Some researchers go even further, collecting temporal series to see how the subtle architecture of bone or other tissue changes with time.

The LSM 510 META pictured here, from Carl Zeiss of Jena, Germany, is a laser-scanning confocal microscope with up to four separate, adjustable pinhole apertures, one for each detection channel. Unlike Minsky’s original setup, which placed a pinhole aperture and objective on each side of the sample, the 510 META (like most modern systems), uses only a single objective lens and separates excitation and emission light with dichroic mirrors.

The system has two acquisition modes. In addition to collecting multi-color images with filters and point detectors (photomultiplier tubes), it also allows for fast spectral imaging via a proprietary technology called Emission Fingerprinting (EF). EF uses a 32-channel photomultiplier tube (the META detector) and specialized software to separate even highly overlapping emission spectra of up to eight different fluorescent dyes.

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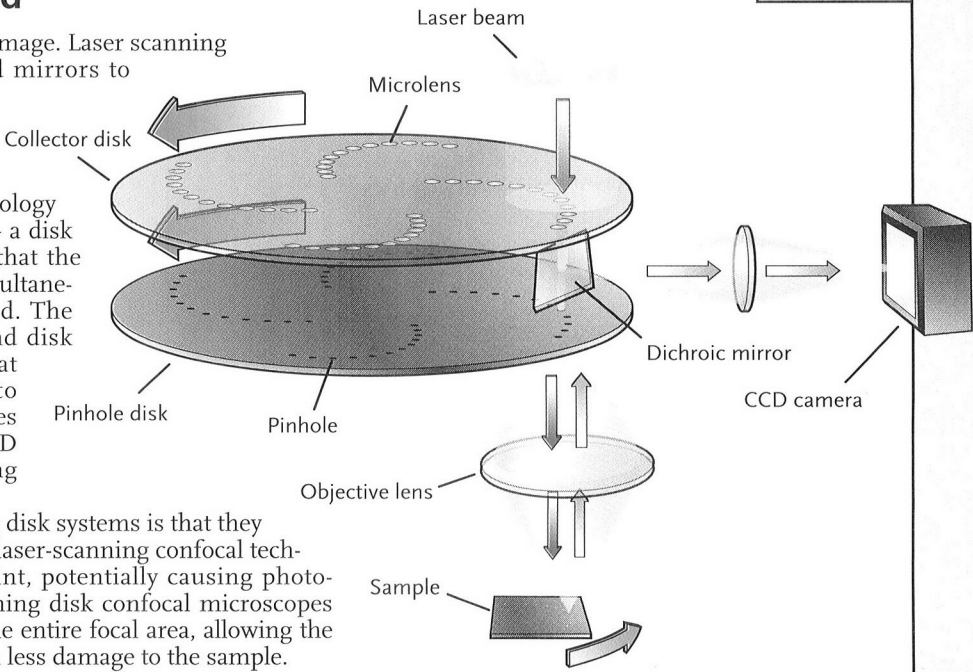


BOX 1: Beam Path in Confocal LSM

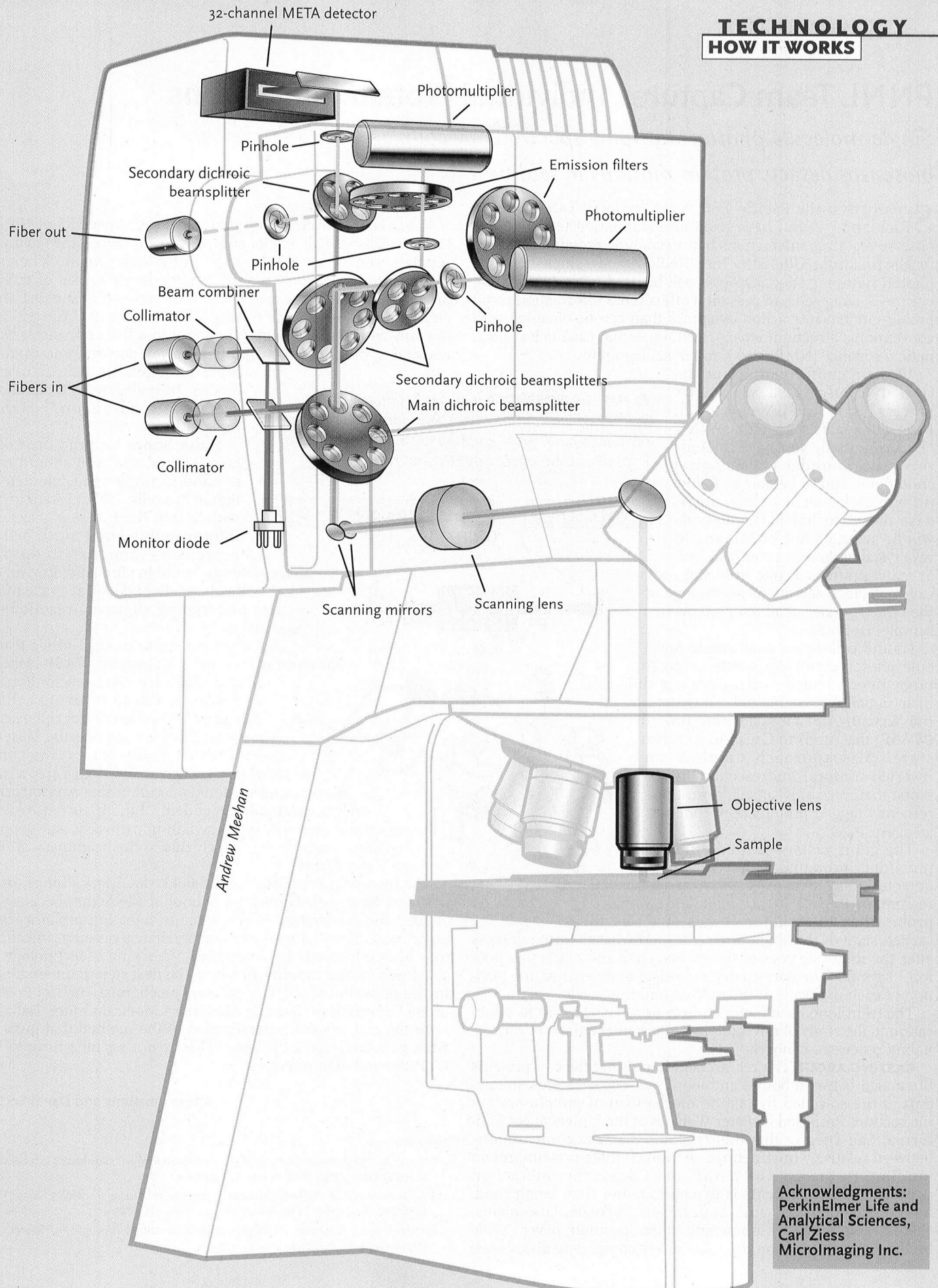
The Spinning Disk Method

There are two ways to obtain a confocal image. Laser scanning confocal microscopes use motorized mirrors to raster scan a single laser-produced spot across the sample, and use photomultiplier tubes (PMTs) to detect emitted light. In an alternative method – spinning disk confocal microscopy, the technology behind PerkinElmer’s UltraView system – a disk containing multiple pinholes rotates so that the sample is scanned at multiple points simultaneously at a rate of 1,000 scans per second. The UltraView microscope also uses a second disk containing thousands of microlenses that focus the light through the pinholes to ensure that enough excitation light reaches the sample. Spinning disk systems use CCD cameras rather than PMTs, thus enabling real-time detection.

One of the main advantages of spinning disk systems is that they can more easily image live cells. Standard laser-scanning confocal technology concentrates light at a single point, potentially causing photobleaching or cell death. In contrast, spinning disk confocal microscopes dissipate the same amount of light over the entire focal area, allowing the user to scan for longer periods of time with less damage to the sample.



BOX 2



Acknowledgments:
PerkinElmer Life and
Analytical Sciences,
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