

## [54] TWO-PHOTON LASER MICROSCOPY

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250/423 P, 459.1; 356/318; 365/127, 106

## [56] References Cited

### U.S. PATENT DOCUMENTS

4,405,237	9/1983	Illanuccia et al. ....	365/301
4,407,008	9/1983	Schmidt et al. ....	358/93
4,466,080	8/1984	Swainson et al. ....	365/106
4,471,470	9/1984	Swainson et al. ....	365/127
4,631,581	12/1986	Carlsson ....	358/93
4,734,578	3/1988	Horikawa ....	250/234
4,786,170	11/1988	Groeblor ....	356/318
4,791,310	12/1988	Honig et al. ....	250/458.1
4,792,341	11/1988	Kozikowski et al. ....	8/103
4,827,125	5/1989	Goldstein ....	250/234
4,838,679	6/1989	Bille ....	351/205
4,863,226	9/1989	Haupt et al. ....	350/6.5
4,877,965	10/1989	Dandliker et al. ....	250/458.1
4,887,721	12/1989	Martin et al. ....	209/579

### OTHER PUBLICATIONS

Ohsawa et al., "On the Possibility of Gas Temperature Measurement Using Two Photon Excitation", Dept. of Applied Physics, Tokyo Univ. of Agriculture & Technology, 21-27 May 1979, pp. 523-528.

Fritzler et al., "A Spectrometer for Semiautomatic Two Photon Fluorescence Spectroscopy", Journal of Physics E: Scientific Inst., 1975, vol. 8, pp. 530-532.

Slomba et al., "A Laser Flying Spot Scanner for Use in Automated Fluorescence Antibody Instrumentation",

Journal of the Assoc. for the Advancement of Med. Instrumentation, vol. 6, No. 3, 1972, pp. 230-234.

"Three Dimensional Optical Storage Memory", Parthenopoulos Science, vol. 245, pp. 843-845, Aug. 25, 1989.

"Theory and Practice of Scanning Optical Microscopy", T. Wilson and C. Shepard, Academic Press, London, 1984, pp. 8 and 9.

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## [57] ABSTRACT

A laser scanning microscope produces molecular excitation in a target material by simultaneous absorption of two photons to thereby provide intrinsic three-dimensional resolution. Fluorophores having single photon absorption in the short (ultraviolet or visible) wavelength range are excited by a stream of strongly focused subpicosecond pulses of laser light of relatively long (red or infrared) wavelength range. The fluorophores absorb at about one half the laser wavelength to produce fluorescent images of living cells and other microscopic objects. The fluorescent emission from the fluorophores increases quadratically with the excitation intensity so that by strongly focusing the laser light, fluorescence as well as photobleaching are confined to the vicinity of the focal plane. This feature provides depth of field resolution comparable to that produced by confocal laser scanning microscopes, and in addition reduces photobleaching. Scanning of the laser beam, by a laser scanning microscope, allows construction of images by collecting two-photon excited fluorescence from each point in the scanned object while still satisfying the requirement for very high excitation intensity obtained by focusing the laser beam and by pulse time compressing the beam. The focused pulses also provide three-dimensional spatially resolved photochemistry which is particularly useful in photolytic release of caged effector molecules.

21 Claims, 7 Drawing Sheets

