## Letter to the Editor

[The Editor is not responsible for the views expressed by the correspondents]

### **Fascinating Journey of Confocal Microscopy**

SIR, - The letter describes the fascinating progression of the confocal microscope from its discovery to its most recent developments. Marvin Minsky came up with the first idea for a confocal scanning microscope in 1957, which involved moving the stage to scan the illumination point in the focus plane. Due to the most likely lack of the powerful light sources required for imaging and the computing capacity needed to handle vast volumes of data, Minsky's idea went mostly undetected. His ambitions to view biological processes as they take place in living tissue (in vivo) was a major factor in the creation of the confocal technique, and Minsky wanted to image neural networks in unstained preparations of living brains. Several researchers developed functional laser scanning confocal microscope designs in the years that followed Minsky's discovery. In 1979, Dutch physicist G Fred Brakenhoff created a scanning confocal microscope, and almost simultaneously, Colin Sheppard added an image creation theory to the technology. The idea was cultivated by Tony Wilson, Brad Amos, and John White, who later (during the late 1980s) showed the value of confocal imaging in the analysis of fluorescent biological material.

In 1987, the first commercial instruments were released. More powerful and stable lasers, high-efficiency scanning mirror systems, high-throughput fiber optics, better thin film dielectric coatings, and detectors with improved noise characteristics were made possible by improvements in optics and electronics during the 1990s. One of the most important developments in optical microscopy is laser scanning confocal microscopy. Visual sections of small structures that would be challenging to physically segment can be seen, and the images obtained can be used to create 3D structures. In order to create a 3D reconstruction, the technique essentially scans specimen point-by-point with a focussed laser beam.

Modern confocal microscopes can be thought of as fully integrated electronic systems where the optical microscope plays a central role in a configuration that includes several laser systems combined with wavelength selection devices and a beam scanning assembly, one or more electronic detectors, and a computer (for image display, processing, output, and storage). Modern confocal microscopes can be thought of as fully integrated electronic systems, with the optical microscope serving as the primary component of a setup that includes one or more electronic detectors, a computer (for image display, processing, output, and storage), and a number of laser systems coupled with wavelength selection devices and a beam scanning assembly.

With the rapid development of precision instrument manufacturing and semiconductor processing industry, the observation and measurement of micro-structure surface profile has become an important orientation of scientific research. Confocal microscopy has become a crucial tool for biological research across many areas during the past 30 years. Even with the most recent developments in light sheet and field synthesis microscopy, confocal microscopy will continue to play a significant role in biological imaging for many years to come due to its ease of use and universal accessibility. Confocal microscopy-based approaches continue to be the most straightforward way for biologists with little familiarity with imaging to address fundamental concerns because these more advanced technologies still require significant expertise to establish and follow through to analysis.

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Preliminary confocal Microscope 10.1002/sca.4950100403

# Scanning Microscope 710

#### References

- Minsky M Memoir on inventing the confocal scanning microscope. Scanning 1988; 10: 128-38. https://doi.org/10.1002/ sca.4950100403
- 2 Song L, Varma CA, Verhoeven JW, Tanke HJ Influence of the Triplet Excited State on the Photobleaching Kinetics of Fluorescein in Microscopy. *Biophys J* 1996; **70**: 2959-68.
- 3 Methods in Cell Biology Cell Biological Applications of Confocal Microscopy VOLUME 70 Second Edition. Edited by Brian Matsumoto.
- 4 Pawley J B (ed), Handbook of Biological Confocal Microscopy, *New York: Plenum Press*, [1995].
- 5 Bagc C, Sever M, Belder N, Adam P Bennett, Erdener S and Dalkaraa T — Overview of extracellular vesicle characterization techniques and introduction to combined reflectance and fluorescence confocal microscopy to distinguish extracellular vesicle subpopulations *Neurophotonics* 2022; 9(2): 021903.
- 6 Zeidan, Adel, Dvir Yelin Reflectance confocal microscopy of red blood cells: simulation and experiment. *Biomedical optics express* 2015; 6(11): 4335-43.
- 7 Skvara H, Plut U, Schmid JA, Jonak C Combining in vivo reflectance with fluorescence confocal microscopy provides additive information on skin morphology. *Dermatol Pract Concept* 2012; **2(1)**: 3-12.
- 8 Paddock SW (ed.) Confocal Microscopy: Methods and Protocols, Totowa, New Jersey: Humana Press, [1999].
- 9 Chiu LD, Su L, Reichelt S, Amos WB Use of a white light supercontinuum laser for confocal interference-reflection microscopy. J Microsc 2012; 246(2): 153-9.
- 10 Centonze V and Pawley J Tutorial on Practical Confocal Microscopy and use of the Confocal Test Specimen. *Hand*book of Biological Confocal Microscopy, New York: Plenum Press 1995; 549-70.
- Lopez J, Fujii S, Doi A, Utsunomiya H Microscopy/Image Processing: A deconvolution revolution for confocal image enhancement. Optics Jan 2019; 2.
- 12 Foltánková V, Matula P, Sorokin D, Kozubek S, Bártová E Hybrid Detectors Improved Time-Lapse Confocal Microscopy of PML and 53BP1 Nuclear Body Colocalization in DNA Lesions *Microsc. Microanal* 2013; **19:** 360-9.
- 13 Bruschini AC, Charbon E Dynamic range extension for photon counting arrays. Opt Express 2018; 26: 22234-48.
- 14 Becker W, Su B, Holub O, Weisshart K FLIM and FCS detection in laser-scanning microscopes: Increased efficiency by GaAsP hybrid detectors. *Microsc Res Tech* 2011; **74**: 804-11.

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