

# HIGH-SPEED CONFOCAL FLUORESCENCE MICROSCOPY

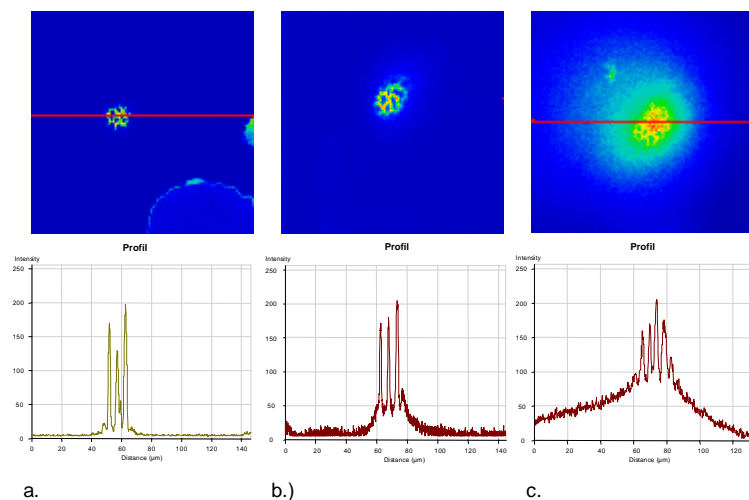
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## 1. REAL-TIME CONFOCAL MICROSCOPY

Research in the Life Sciences increasingly involves the investigation of fast dynamic processes at the cellular and sub-cellular level. For this task we introduce a fast fluorescence line scanner with image acquisition speeds in excess of 100 frames per second at 512 x 512 pixels and with a more than 10- fold increased sensitivity compared to point scanning confocal systems. Since the system preserves the capability for optical sectioning of confocal systems it allows to observe processes in three dimensions.

The microscope is based on scanning a line rather than a point over the sample. The capability for 3D-imaging is ensured by detecting the fluorescence light excited along the line with a slit-shaped line detector. Such a configuration has first been introduced by Brakenhoff et al. [1] and Corle et al. [2]. Carl Zeiss has realized a concept implementation yielding a device that is about 10 times faster and 10 times more sensitive than state-of-the-art point scanning



microscopes (LSM 5 LIVE). An entirely new solution to separate the light of the sample from excitation light is used that yields greatly improved sensitivity and flexibility. In regard to depth discrimination the real-time microscope is somewhat inferior to confocal point-scanning microscopes but clearly superior to other parallel confocal imaging systems as the Nipkow disk scanner (see Fig.1).

Figure 1: Imaging of pollen grains using a.) a point scanning LSM 510 META, b.) the LSM 5 LIVE and c.) Nipkow scanning system using a Zeiss Plan-Apochromat 63x/1.4 oil lens. For a.) and b.) the pinhole radius and slit half-width, respectively, was set to 1 Airy Unit.

## 2. APPLICATIONS

Based on its imaging properties the microscope is well suited for spatially resolved studies of dynamic processes in living biological samples. Application examples ranging from imaging of calcium dynamics in cardiac myocytes to investigations of the dynamics of blood flow in developing mouse embryos will be discussed.

[1] G. J. Brakenhoff and K. Visscher, "Confocal imaging with bilateral scanning and array detectors," *J. Microscopy* **165**, 139-146 (1993).

[2] T. Corle and G. Kino, Confocal Scanning Optical Microscopy and Related Systems (Academic, San Diego, 1996).