

The new LSM 700 from Carl Zeiss Olaf Selchow, Bernhard Goetze

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Company Profile / tech feature by Carl Zeiss MicroImaging GmbH

Product: The new LSM 700 confocal microscope by Carl Zeiss

Authors: Dr. Olaf Selchow and Dr. Bernhard Götze

Key words: confocal, laser scanning microscope, variable secondary dichroic

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Suitability for publication in BTJ: The profile article was invited. Also: The imaging special issue "Imaging host-pathogen interactions" targets our major customer group.

All authors concur with the manuscript.

The new LSM 700 from Carl Zeiss.

The new LSM 700 confocal microscope is a member of the seventh generation of confocal microscopes from Carl Zeiss – a product family that is characterized by a wealth of genuinely innovative ideas and technologies. It is perfectly suited for a range of established advanced fluorescence microscopy applications like FRAP (Fluorescence Recovery after Photobleaching), FRET (Förster Resonance Energy Transfer) and Spectral Imaging. At the same time it also serves as a reliable and most sensitive workhorse for routine multicolor fluorescence and live-cell confocal imaging. The LSM 700 concept combines Carl Zeiss quality and exceptional ease of operation at an excellent price-performance ratio.

It can be equipped with up to 4 diode lasers (405 or 445, 488, 555, 639 nm) to cover the excitation spectra of the vast majority of commonly used fluorescence labels. Inside the scanning module the LSM 700 hardware is based on the same high end components as the Carl Zeiss Premium Class confocal microscope LSM 710. Examples are the patented PCT Laser coupling concept between laser fibers and scanning module, the beam combining optics as well as the solid state pinhole mechanics. Also scanners, PMT detectors, detection electronics and the *Fixed Gate* main beam splitter match the high end standards of Carl Zeiss premium systems. Together with a straightforward and impressively light-efficient beam path these components ensure the extraordinary detection sensitivity of the LSM 700. Even demanding applications with faintly stained fluorescent samples can be imaged with great image quality using only minimal excitation laser power (Figure 1).

The LSM 700 is available in a one or two channel version and the scanning module can be fitted to almost any Carl Zeiss high end scientific microscope. The system is operated by the next generation of ZEN, the user-friendly and popular Carl Zeiss LSM software platform. The ergonomically designed user interface as well as dedicated features like, e.g., the *Smart Setup* tool contributes to make the LSM 700 an extremely easy to use microscope system. Altogether, the LSM 700 is geared to cover all standard LSM applications with an outstanding performance.

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At the heart of the LSM 700, the new "Variable Secondary Dichroic" (VSD) splits the emission between two detectors (in the 2 channel configuration only). The VSD is a substrate coated with a special dichroic gradient layer. Embedded in a new detection beam path design the VSD is mounted on a motorized slider, and depending on its position, it can be a short pass/long reflectance dichroic at any wavelength between 420 and 630 nm (Figure 3). Thus, the user of the LSM 700 can freely choose the exact wavelength for the dichroic split. For example: With the VSD set to 527 nm, all the wavelengths shorter than 527nm will pass to channel 1, and all the wavelengths longer than 527 nm will be reflected to channel 2. The full integration of the VSD in the ZEN 2009 software allows switching the split from one scan to the next to ensure optimal fluorescence signal separation in the 2 detectors (Figure 4). Combining VSD position and emission filters, the user can freely adjust the active spectral detection window. The LSM 700 is thus prepared for any fluorescent dye combination and with the line-switching scanning mode 4 dyes can easily be imaged simultaneously at liveimaging frame rates. In addition to optimized channel separation, this unique detection beam path design also guarantees highest efficiency with regard to photon yield: No photon is ever lost since all the signal is directed either to detector 1 or 2.

Moved in defined steps between each scan, the VSD further permits the acquisition of spectrally resolved image series known as lambda stacks. These are prerequisite for getting optimal results in the separation of overlapping fluorescence signals by means of spectral unmixing. This technique allows the separation of even strongly overlapping fluorescent spectra like the signals from, e.g., GFP and YFP. Due to the unique detection beam path design and the properties of the VSD, each pair of images of a lambda stack represents the complete emission light. The spectral information is encoded in the relative changes of the image intensities with respect to the splitting wavelength. This property of the LSM 700 is the basis of a unique new way of lamda stack image acquisition and spectral unmixing.

The general theme of the development of the LSM 700 was to provide the user with a confocal microscope at no compromise in image quality and reliability: "Whatever this system does it does it extremely well". The technical concept to guarantee outstanding image quality, reliability and

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reproducibility of the acquired data includes, for example, an internal laser power calibration that guarantees constant light exposure during long time-lapse imaging or when re-using imaging parameters after months or years. Furthermore, a user-accessible software tool for automated calibration and alignment helps ensuring permanent perfect alignment and informs the user about the status of the system.

In summary, Carl Zeiss has set new standards by proving that uncompromised image quality and ease of use can come at an attractive price tag. The LSM 700 is a small, extremely sensitive and reliable versatile confocal microscope system which covers a vast range of applications like FRAP, FRET, spectral imaging, live-cell imaging and many more.

Figure captions:

Figure 1:

Human lymphocytes transmitting the HIV virus from cell to cell. Red: HIV (Gag), Alexa 546. Green: Actin-phalloidin-Alexa 488. Blue: Cytosol marker. Courtesy: D. Rudnika, N. Sol-Foulon and O. Schwartz, Institut Pasteur, Virus and Immunity Unit, Paris, France

Figure 2:

LSM 700 system overview and beam path of the scanning module

Figure 3:

The variable secondary dichroic (VSD) beam splitter. (illuminated with white light.)

Figure 4: Principle of spectral separation of the fluorescence signal with the **V**ariable **S**econdary **D**ichroic: a) the VSD is positioned so that the fluorescence light hits the one extreme end of the coated glass: all light is transmitted to PMT 1. b) in an intermediate position, the light with shorter wavelength than the VSD set-point is transmitted while all higher wavelengths are reflected to PMT

2. c) in the other extreme position, all light is reflected to PMT 2. The minimum step-width of the positioning is 1 nm.

Figure 5: The LSM 700 can be fitted to almost any Carl Zeiss high end scientific microscope.

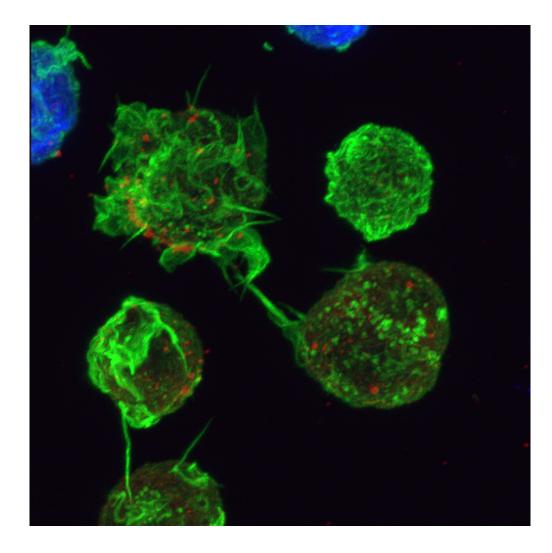
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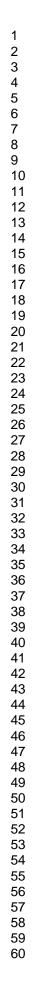
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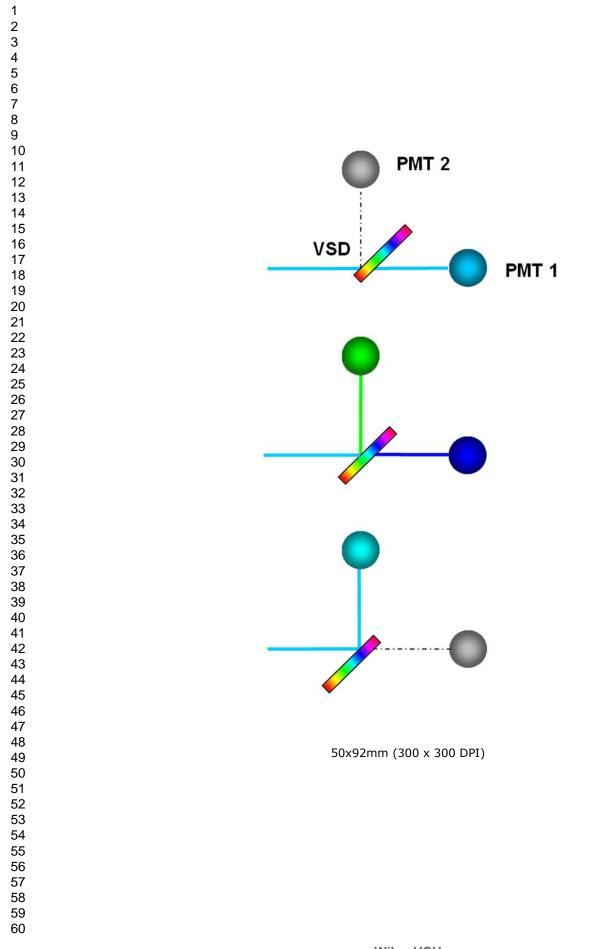


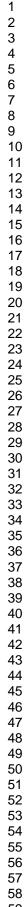
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