

# 3D Topography and Fluorescence Measurements

## Bringing High-end Confocal Laser Scanning Technology to the Masses

●▶ Confocal Microscopy has evolved from a technique used by specialists only, to a technique of every day use. In biomedical research, the use of biomarkers like the Green Fluorescent Protein (GFP) has pushed confocal microscopy from the hands of a few into numerous labs of biologists doing basic and applied biomedical research. The combination of fluorescence and non-tactile surface measurements has proven to be the method of choice for many confocal applications in materials research. Changing demands for applications in both fields have lead to the development of new instruments enabling researchers to perform increasingly complex experiments.

Three scientists have been awarded the Nobel Prize for chemistry in 2008 for the discovery and development of GFP (Osamu Shimomura, Martin Chalfie, Roger Tsien). This protein acts like a little lamp when attached to natural proteins inside of living cells – enabling researchers to see and follow those cellular proteins in living cells and organisms. This discovery has been one of the milestones in fluorescence microscopy and lead to a variety of applications and groundbreaking discoveries over the past 20 years. Today, the wave of new applications is without cease and the second generation of those fluorescent proteins is leading to new technologies like high resolution microscopy.

In the same time frame as pure biomedical research has discovered fluorescent proteins to boost research, another area has emerged, using those techniques as well: Biomaterials. Bioactive materials are in use in various applications like e.g. implants for surgical interventions in the medical field or research on computer – brain interfaces in biotechnology. For the development processes of those products, the combination of confocal fluorescence microscopy and non-tactile surface measurements is a key technique.

### THE AUTHORS

#### OLAF SELCHOW

Dr. Olaf Selchow studied Physics at the Technical Universities of Darmstadt and Munich and received his PhD from the University of Kaiserslautern and the EMBL Heidelberg. He then specialized in microscopy and took over a core facility at the University of Stuttgart. Before joining the Product Management Team at Carl Zeiss MicroImaging in 2007 he worked as an Application Specialist for 2 Photon Microscopy for a German company. At Zeiss his responsibilities cover Spectral Imaging, special aspects of Confocal and 2 Photon Microscopy as well as Light Sheet based Microscopy and thick specimen imaging.



#### BERNHARD GOETZE

Dr. Bernhard Götz has studied Biology at the University of Hohenheim. He did his PhD in Neuroscience at the Max Planck Institute for Developmental Biology in Tuebingen, where his focus was to reveal the molecular mechanisms of learning and memory in the mammalian brain. (During his research he was using confocal, widefield and electron microscopy techniques to investigate nerve cells at the microscopic and molecular level.) He joined the Product Management team of Carl Zeiss MicroImaging in 2006 and is responsible for mid-range confocal microscopes and software.



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All the above mentioned applications at some point require the use of 3D Imaging – thus optical sectioning. Although other techniques are emerging, Laser Scanning Confocal Microscopes have been the most flexible instruments to do this. With the applications becoming more common, the instruments have undergone changes as well. Whereas the first confocal microscopes required specialized personnel to operate them, today's systems are designed to be used by researchers without special education in physics or microscopy. Like most advanced technologies, Laser Scanning Microscopy has also become more affordable with time. Therefore today new instruments are

used even in small research groups to perform routine tasks like live cell and 3D multicolor imaging. Laboratories as well as companies are requesting such cost-effective instruments in order to fit both their application needs and budgets. The need for precision and quality of the instrument however is still one of the deciding factors for purchase decisions. To meet those application and financial needs, Carl Zeiss has developed a new confocal microscope system:

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 concept of the system combines both quality and exceptional ease of operation at an excellent price – performance ratio. Moreover, this system can be equipped to combine a materials research microscope system for the assessment of surface qualities with a high performance fluorescence microscope system – a combination most useful e.g. for forensic research laboratories.

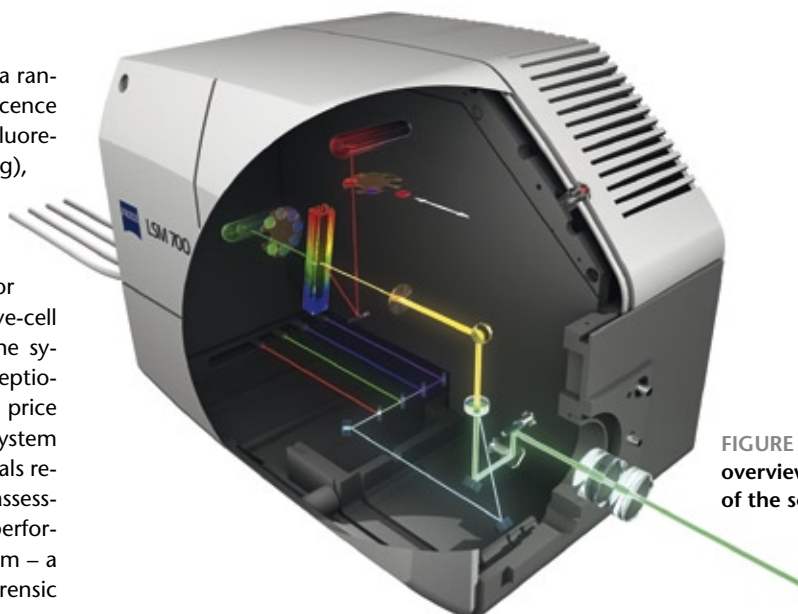


FIGURE 1: LSM 700 system overview and beam path of the scanning module.

### Image Quality, Sensitivity and Precision

A radically simple beam path design is the basis for the systems' excellent performance. The Fix-Gate main beam splitter suppresses laser light so efficiently, that the detection beam path could be designed filter free and continuously variable. This flexibility renders the system suitable for almost any kind of dye and dye combination – a feature most important for an instrument which has to be used in multi-user facilities for various applications. The simplicity of the beam path has additional effects which are beneficial for the user: The reduction of the number of optical elements to the absolute minimum is the key for excellent image quality, precision and sensitivity. The lower the number of lenses and mirrors present, the higher the light efficiency and optical performance of a system. Furthermore, the focus on a handful of those components makes the use of high-quality optical elements possible – a fact, that potentiates the positive effect of a simple beam path when it comes to sensitivity. In the scanning module, the hardware is based on the same high end components as the Carl Zeiss Premium Class confocal microscopes. 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 match the high end standards of Carl Zeiss premium systems. Therefore, even demanding applications with faintly stained fluorescent samples can be imaged with great image quality using only minimal excitation laser power.

The system can be equipped with up to 4 long-life diode lasers (405 or 445, 488,

555, 639 nm) to cover the excitation spectra of the vast majority of commonly used fluorescence labels (Figure1).

The system is available in a one or two channel version. The scanning module can be fitted to almost any Carl Zeiss high end scientific microscope. Altogether, it is geared to cover all standard applications with an outstanding performance. The system has a full upgrade path inbuilt, so the more the applications will grow the more the system can be upgraded with detection channels and lasers.

A special hybrid Fix-Gate main beam splitter has been developed to allow the combination of fluorescence imaging and reflection imaging for surface measurements; the shortest wavelength (405 nm) in the system can also be used for reflection imaging and is available for fluorescence applications together with the other laser lines as well. The combination of those two imaging techniques are beneficial for a growing number of applications like e.g. the assessment of print and paper quality (Figure 2) or the design of glass-protein surfaces to address the growth and adherence of nerve or cancer cells.

### Ease of use

Biologists and material researchers have one thing in common when they use confocal systems: No one wants to undergo a lengthy training before operating one of those systems. Ease of use is perhaps the most important factor when it comes to make the most out of a system. All confocal Microscope Systems are operated by ZEN the new confocal software from Carl Zeiss. The user interface is entirely application-orientated. Features like interactive Joysticks or graphical representations of the experi-

### THE COMPANY

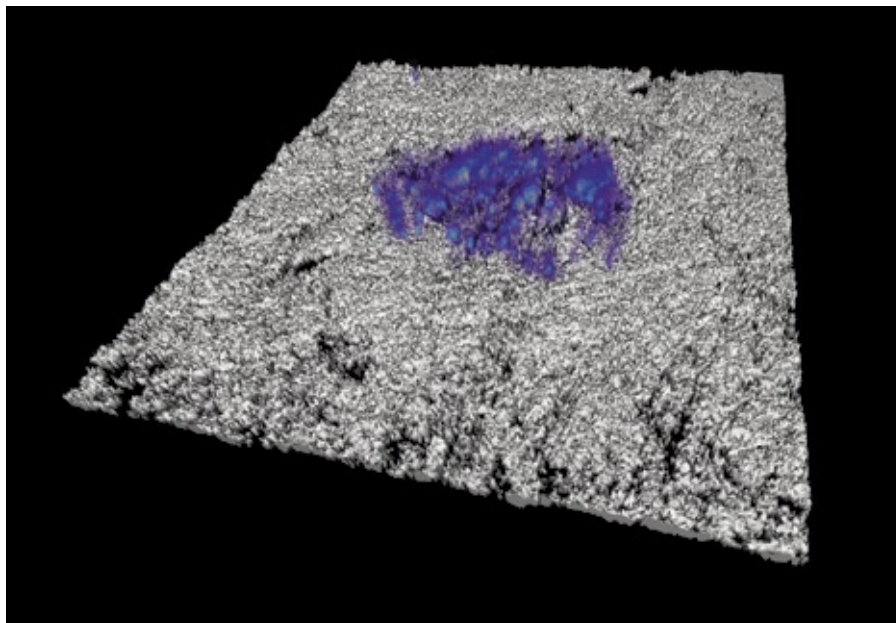
#### Carl Zeiss MicroImaging GmbH

Carl Zeiss MicroImaging GmbH offers microscopy solutions and systems for research, laboratories, routine and industrial applications, as well as spectral sensors for the analysis market. The spectrum of products ranges from traditional light and stereo microscopes to laser scanning systems and automated microscope systems, covering applications ranging from biotechnology, pharmaceutical research, health care and biomedicine to quality assurance and materials analysis. This microscopy portfolio is supplemented by a range of single components such as gratings, spectral sensors, spectrometer units and specific solutions in process analysis.

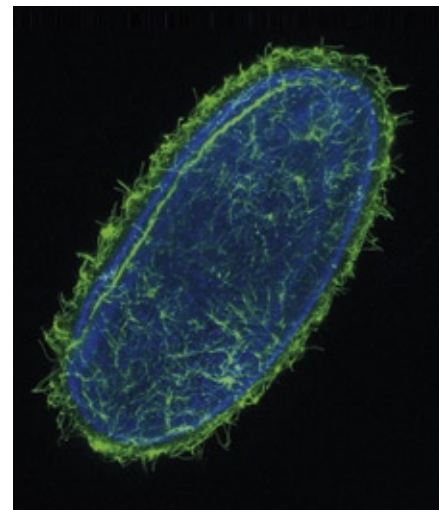
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ment type make the system not only easy to use but save precious time while setting up the experiments. The core functionality however can be operated by a function unique in fluorescence imaging. "Smart Setup" is taking care of all settings of the hardware once the user has entered the combination of dyes present in the sample. The algorithm behind this tool computes the most suitable hardware setting for the given dye combination. This feature is crucial since modern confocal systems can be tuned continuously on the detection side – confronting the user with literally millions of possible settings.

At the heart of the LSM 700, the new "Variable Secondary Dichroic" (VSD) flexibly splits the emission between two detec-



**FIGURE 2:** Fluorescent flake in paper, reflection/fluorescence excitation at 555 nm, 3D projection in mixed rendering mode.



**FIGURE 3:** Amphioxus *B. floridae*, neurula stage embryo, Staining: DAPI (chromatin), Alexa 488 (anti-tubulin, cilia) Jr-Kai Sky Yu, Ph. D. (jkyu@gate.sinica.edu.tw), Source: Institute of Cellular and Organismic Biology, Academia Sinica (Taipei, Taiwan).

tors or – in the one channel version – selects a detection band for the single channel. The VSD is a substrate coated with a special dichroic gradient layer. Embedded in the 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 1). Thus, the user can freely choose the exact wavelength for the dichroic split. For example: With the VSD set to 527 nm, all the wavelengths shorter than 527 nm 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. Combining VSD position and emission filters, the user can freely adjust the active spectral detection window. The system is thus prepared for any fluorescent dye combination and with the line-switching scanning mode 4 dyes can easily be imaged simultaneously at live-imaging frame rates.

### Spectral imaging

Since the discovery of GFP a whole basket full of fluorescent proteins has been developed by biologists to mark cellular structures of interest. In tissues or organisms the interaction of two or more molecules delivers insight into the function of those molecules. If the proteins of interest are tagged with different fluorescent markers those interactions can be observed on the micro-

scopic level in living cells via various techniques like FRET. The emission signals of those markers can overlap and therefore the separation of the emitted light is a prerequisite to draw correct scientific conclusions. The VSD can be moved in defined steps between each scan, and thus permits the acquisition of spectrally resolved image series known as lambda stacks. This leads to 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. The spectral information is encoded in the relative changes of the image intensities with respect to the splitting wavelength. This property of the system is the basis of a unique new way of lambda stack image acquisition and spectral unmixing. It renders spectral imaging not only more accurate due to more detection signal but also more sensitive since each pair of images of a lambda stack represents the complete emission signal of the sample.

### Conclusion

The general theme of the development of the system was to provide the user with a confocal microscope at no compromise in image quality and reliability. The technical concept to guarantee outstanding image quality, reliability and 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. Fur-

thermore, 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. Space in modern research laboratories is a valuable resource that is not easy to access in most of the cases. The system has therefore been designed to fit into small rooms: With its small footprint, integrated anti vibration system and no need for high voltage power supplies, the system can be setup virtually in every corner of the lab.

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.