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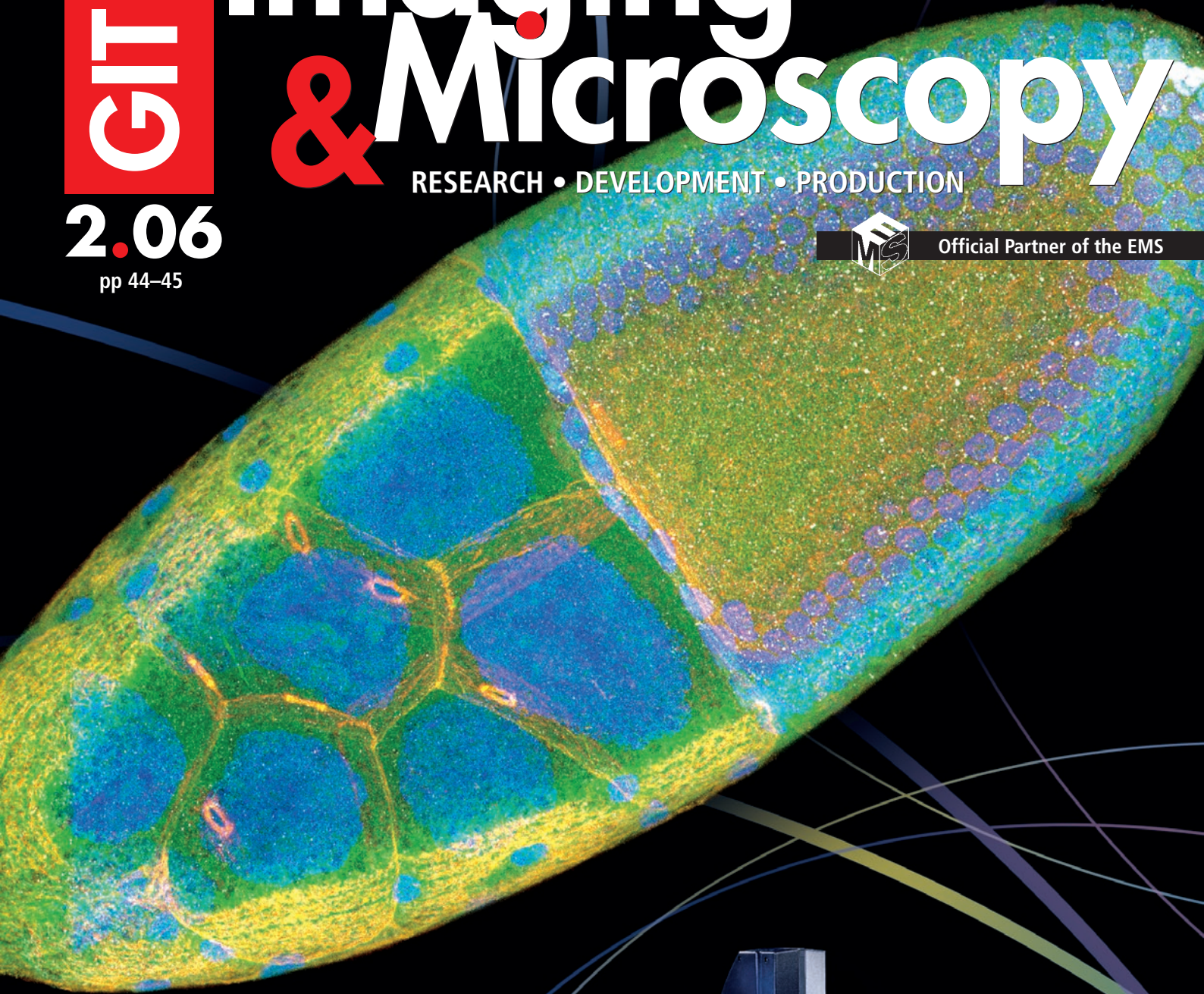
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# Imaging & Microscopy

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VERENA NICKL  
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**True Confocal Microscopy**  
Affordable Excellence for  
Everyday Research



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# True Confocal Microscopy

Affordable Excellence for Everyday Research

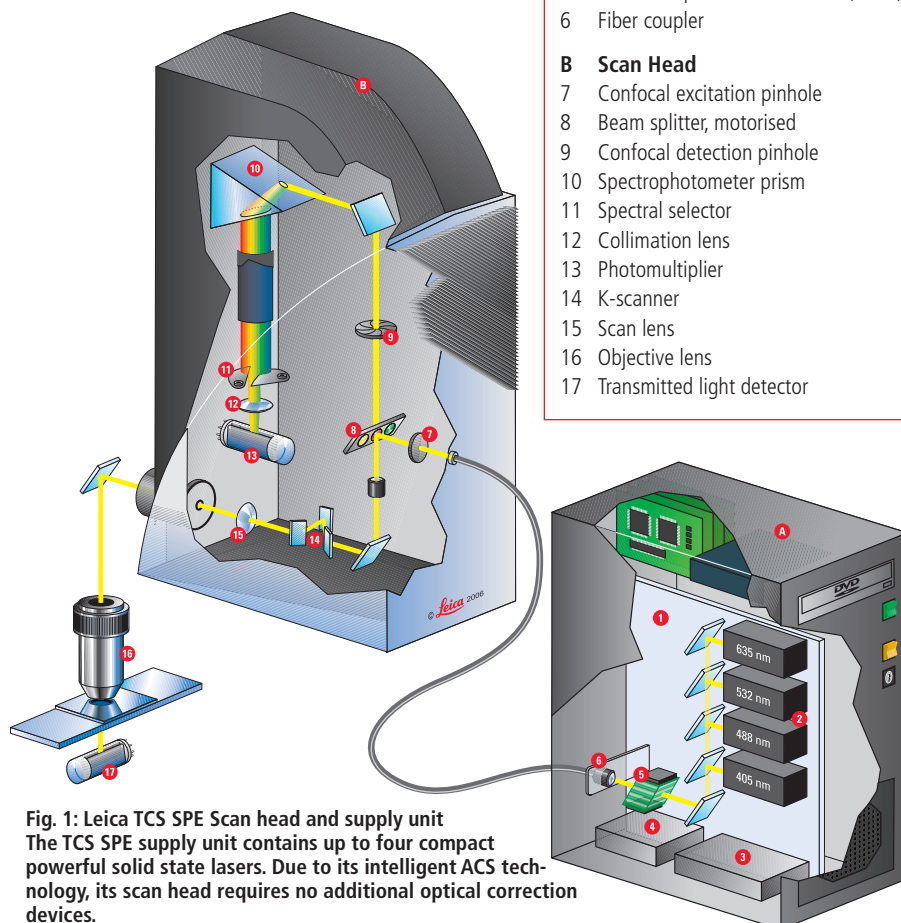
The application range in confocal microscopy has increased tremendously throughout the last decade: High quality fluorescent images have been an important key to new discoveries in biomedical research. Morphological studies, representing multiparameter fluorescence in fixed samples, are a major area of confocal microscopy. These applications require noise-free, high resolution imaging in order to see structural details, interconnections of organelles or proteins. A growing interest in confocal microscopy can be seen in clinical research, for example in pathology. High-resolution 3D confocal imaging may also give deeper insights and lead to new discoveries in pharmaceutical and biotechnological research.

## Efficient and Economic Entry-level System with Innovative Concepts

In order to make confocal technology available to a wide range of users in their daily research, Leica Microsystems has developed a compact, easy-to-use and robust system in the entry-level class of confocal microscopes, the Leica TCS SPE. The new system covers the target applications in research and routine laboratory work. It requires minimal training effort and even confocal newcomers are able to quickly produce spectacular 3D images.

Despite its compactness, the Leica TCS SPE confocal microscope features a range of innovative concepts and standards without compromise in optical quality.

The Leica TCS SPE is the only confocal system in its class with true spectral detection. It is based on a prism – spreading the light into its spectrum – and a detector device to select the spectral detection range. Thus, the system allows freely tunable spectral detection from 430–750 nm.



### Scan Head and Supply Unit (Fig. 1)

#### A Supply Unit

- 1 Internal optical bench
- 2 Solid state lasers (405 optional)
- 3 Laser driver board
- 4 Power supply
- 5 Acousto Optical Tunable Filter (AOTF)
- 6 Fiber coupler

#### B Scan Head

- 7 Confocal excitation pinhole
- 8 Beam splitter, motorised
- 9 Confocal detection pinhole
- 10 Spectrophotometer prism
- 11 Spectral selector
- 12 Collimation lens
- 13 Photomultiplier
- 14 K-scanner
- 15 Scan lens
- 16 Objective lens
- 17 Transmitted light detector

**Fig. 1: Leica TCS SPE Scan head and supply unit**  
The TCS SPE supply unit contains up to four compact powerful solid state lasers. Due to its intelligent ACS technology, its scan head requires no additional optical correction devices.

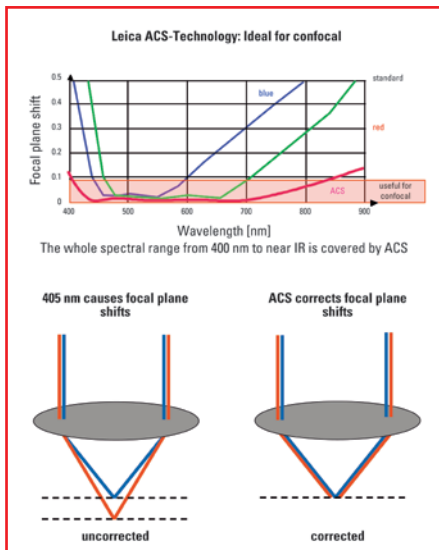


Fig. 2: Leica ACS Technology

An Acousto Optical Tunable Filter (AOTF) with 0–100% excitation power minimises exposure to light by individual tuning of the laser power for effective protection of fixed and living samples.

A new optical concept, the Advanced Correction System (ACS), especially designed for the Leica TCS SPE, allows perfect colocalisation and ensures the high quality of the images.

### Small But Powerful – A Highly Integrated System

The TCS SPE control box is no larger than a standard PC and contains up to four solid state lasers: 488, 532 and 635 nm, the standard excitation lines for most common dyes (fig. 1), and a 405 nm laser, which is optionally available, for nuclear staining. These lasers have the advantage of being very durable and, furthermore, do not need any external cooling device.

The Leica TCS SPE has no specific room requirements. An anti-vibration table for the microscope and an ordinary workbench are fully adequate to arrange the system. Due to its small size and few requirements it surely fits in any laboratory – good news for institutes and facilities where space is often rare and precious.

For high utilisation of the system, the control box contains an integrated PC with a DVD-writer, optimizing the system for multi-user environments, such as imaging facilities or shared resource laboratories. Individual user settings and results can be saved and reproduced later, while in the meantime other users may employ the system for other applications with their individual settings. The LAN connectivity of the TCS SPE and USB

sticks are additional possibilities of storing and transferring data.

### New Optics for Perfect Colocalisation

Leica developed the new optical concept ACS for super positioning of all laser foci at only one point in the focal plane – from excitation to detection (fig. 2). In order to avoid two different optical section planes when working in the UV range, the UV laser is usually coupled in by a separate optical light path. However, optical correction devices are redundant with the new Leica Advanced Correction System. This correction concept is – like the true spectral detection – unique in this class of confocal microscopes. With the ACS, the Leica TCS SPE achieves maximum transmission within the entire light band from 405 nm to infrared (fig. 1). As the system operates with only one AOTF and a shortened light path with single fiber coupling, it is a robust and reliable confocal that requires minimum maintenance.

### A New Software Platform for Easy True Confocal

An easy-to-use software interface inaugurates confocal microscopy to non-experienced users. With pre-defined system settings for defined dyes, confocal newcomers are able to acquire high quality images right from the start. The software is workflow-oriented and context sensitive to encourage error-free operation. On the other hand, the highly automated system is flexible for individual tuning and adapts to the needs of experienced users. Users also benefit from the integrated software Leica Application Suite – Advanced Fluorescence (LAS AF), which operates throughout all advanced fluorescence systems from Leica: the high-end broadband confocal system Leica TCS SP5 as well as Leica's wide-field camera solutions AF6000 and AF6000 LX.

### Tailored System Solution for Imaging Facilities

With its small footprint, its long-life solid state lasers, the easy-to-use software interface for confocal newcomers and experts and the innovative ACS optics, the Leica TCS SPE fits into modern imaging facilities. Optimised for target applications like morphological studies and live cell imaging in small research groups as well as in multi-user environments, the TCS SPE is therefore designed for relieving the workload of high-end imaging systems.

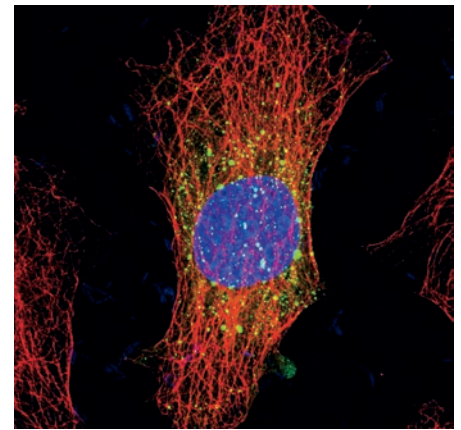


Fig. 3: COS cells; Green: uncharacterised protein; Red:  $\alpha$ -Tubulin, Cy3; Blue: Nuclei, DAPI. Courtesy of Prof. Wei Bian, Cell Research Center, Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai, China.

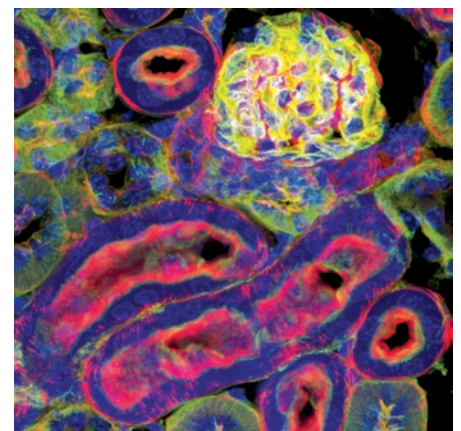


Fig. 4: Mouse kidney section; Green: glomeruli and convoluted tubules, Alexa 488 WGA; Red: F-Actin (prevalent in glomeruli and brush border); Blue: Nuclei, DAPI.

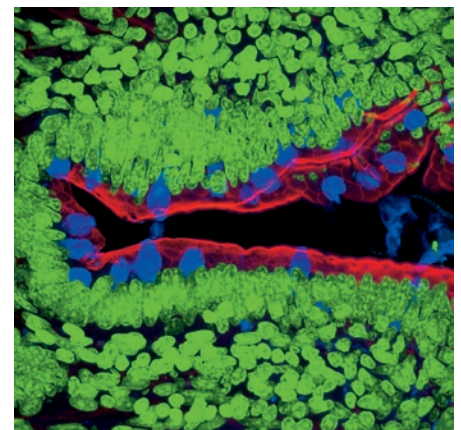


Fig. 5: Mouse Intestine Section; Green: Nuclei, Sytox, green; Red: F-Actin (prevalent in brush border) Alexa 568; Blue: mucus of goblet cells, Alexa 350 WG.

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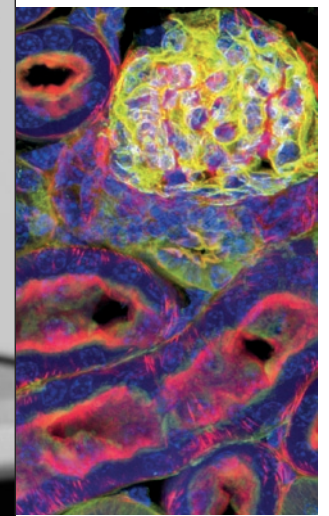
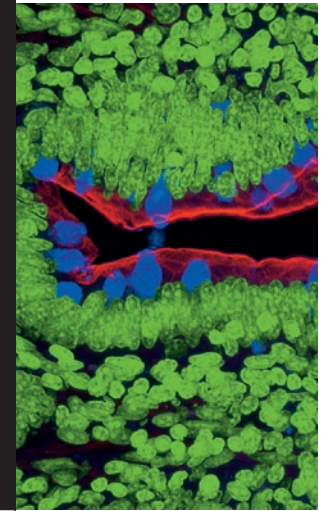
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# Spectacular Imaging! Leica TCS SPE

**High-Resolution Spectral Confocal – Affordable Excellence for Your Daily Research**

- Spectacular Imaging
- Easy to Achieve
- A Reliable System
- Affordable Excellence