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## Confocal Analysis of Microstructures and Micromaterials

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# Confocal Analysis of Microstructures and Micromaterials

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This is the age of microsystems and nanotechnology, of new and biocompatible materials, microtools and bio-MEMS. An age that challenges materials testing and analysis to look for new approaches. Confocal microscopy is one of the most promising methods because it provides a fast optical and non-contact 3D analysis of such structures and materials. With the LSM 5 PASCAL confocal laser scanning microscope Carl Zeiss has risen to the challenge – with multimode analysis, innovative scanning strategies, unrivaled optics and comprehensive measurement functions.

## Confocal Principle

Laser light coupled into the microscope hits the sample at the objective focus. The light reflected or emitted by the sample surface passes the objective and is collected by a tube lens. In this way, the laser beam converges at a second focal point, which is optically conjugate to the first. A pinhole arranged in this confocal plane ensures that only light from

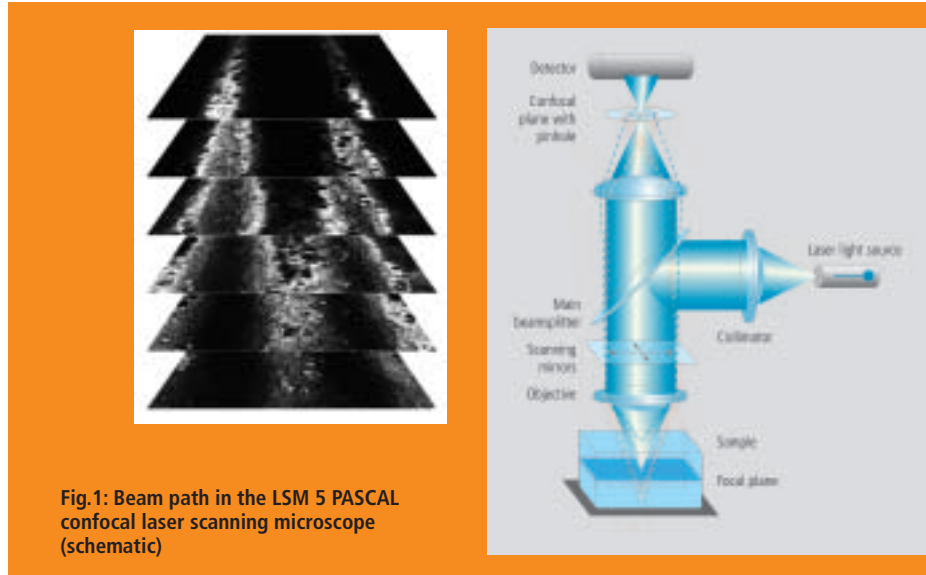


Fig.1: Beam path in the LSM 5 PASCAL confocal laser scanning microscope (schematic)

the focal plane reaches the detector, whereas light reflected or emitted by regions above or below is dramatically reduced. This enhances contrast and improves lateral and axial resolution.

## Optical Section – Image Stack – Topography

The laser beam is made to scan the sample in X and Y directions - point-by-point, and line-by-line. As the laser focus trav-

els along the X and Y coordinates, it generates an optical section of the sample. After the sample has been shifted along the optical axis (Z), the laser beam creates another optical section of a different sample plane. With the Z position changed successively, a three-dimensional stack of digital images is generated. It contains the digital brightness levels (intensity values) for each individual point defined by the laser focus coordinates  $X_i$ ,  $Y_j$ ,  $Z_k$ . From this data

## Keywords

Confocal microscopy, optical profilometry, roughness analysis

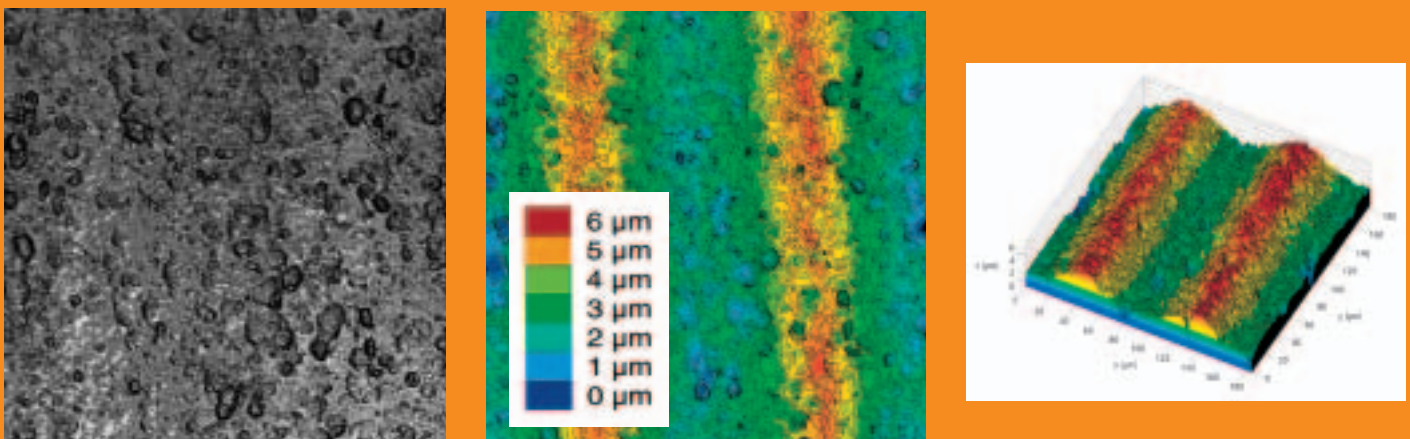


Fig. 2: From confocal section to topography: From a succession of optical slices, the software computes an intensity projection with extended depth of focus (a), a 2D topographic map (b), or a 3D surface topography (c).

record, the software quickly computes intensity projections with extended depth of focus, intensity or height profiles, topographic maps, or surface topographies.

### Fast Overview – Efficient Navigation – Highly Resolved Details

Besides confocal slices and 3D image stacks, a variety of innovative scanning

cumbersome sample reorientation, the scanning area and orientation can be matched to the structures under investigation. Changes of the sample in time can be observed with fully automated time series sequences – either after a definite „schedule“ or „guided“ by external trigger events. This saves valuable time and creates vacancies for further important tasks.

possible number of optical sections - up to a maximum of 2048, depending on the application.

Roughness and waviness measurements with the LSM 5 PASCAL can now be carried out with improved comparability: The traversing distance captured by the 10x objective is > 12.5 mm. The 20x objective covers > 4 mm, and with the 100x it is still an impressive >1.25

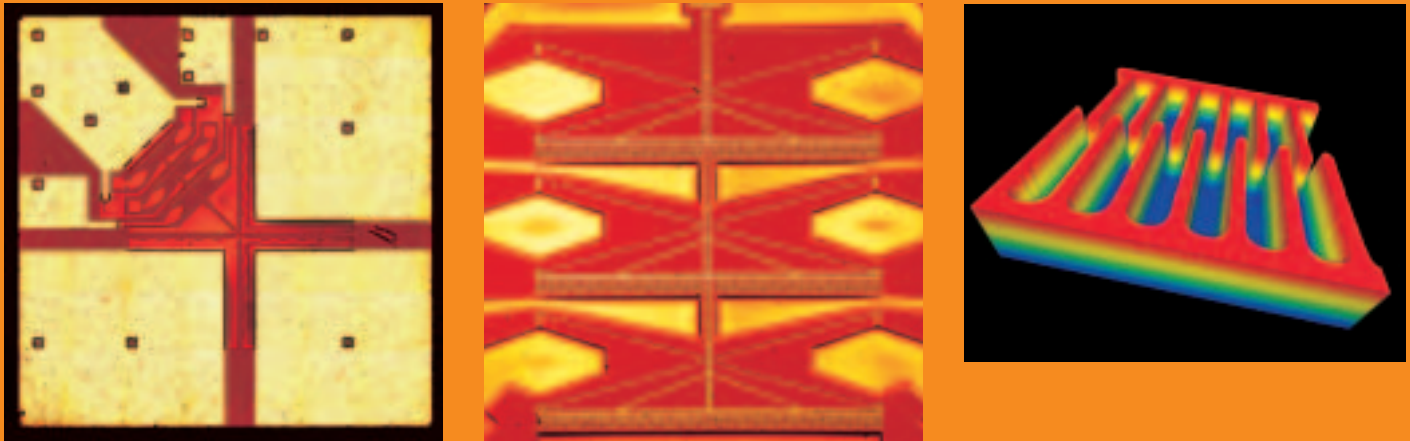


Fig.3: Microcomb drive (photoresist structure). Sample courtesy: Korean Institute of Science and Technology, Seoul, South Korea. a) Non-confocal overview image of a complete chip. Tile Scan assembled from 8x8 partial images. 14,497.1  $\mu\text{m}$  x 14,497.1  $\mu\text{m}$ , 4,096 x 4,096 pixels. b) Non-confocal sectional re-enlargement. Single exposure with rotated scanning field. 2,562.7  $\mu\text{m}$  x 2,562.7  $\mu\text{m}$ , 2,048 x 2,048 pixels. c) 3D surface topography from confocal stack. 115.5  $\mu\text{m}$  x 115.5  $\mu\text{m}$  x 26.7  $\mu\text{m}$ , 604 x 604 pixels x 100 sections.

modes can be performed with the LSM 5 PASCAL confocal laser scanning microscope from Carl Zeiss. Direct acquisition of single profiles – either along a straight line or a free hand tracking curve (Spline Scan) – mosaic-like large overview images (Tile Scan – Fig. 3a), free rotatable scan fields (Fig. 3b) and scanning fields of variable size and shape define a new state-of-the-art in laser scanning microscopy.

Only structures and features of interest are investigated. There is no need for

### Scan Field Size: XXL – Accuracy: Nano

In conjunction with the StitchArt option and a motorized XY scanning stage, the LSM 5 PASCAL also acquires assembled height profiles and image stack arrays. This allows to image large-area segments, to measure long distance profiles and so to broaden the microscope's horizon. In the Multiple Profile Mode up to 16,384 data points can be acquired. Higher axial resolution means a greater

mm. In Fourier terms, broadened microscopical horizon means the detection of very low spatial frequencies. The combination of the StitchArt and Topography options enables the LSM 5 PASCAL to perform also optical waviness analysis.

Confocal laser scanning microscopy is a highly beneficial complement to classical light microscopy or to scanning electron microscopy (SEM). It has become the method of choice for those applications that require fast, direct and accurate quantitative analysis of 3D microstructures. Equipped with alternative contrast techniques such as confocal fluorescence (see also image on back cover) and polarization imaging, a high degree of motorization and comfortable 3D display and processing software packages, the modern day confocal Laser Scanning Microscope has paved the way for new and exciting research as well as for routine applications in industry.

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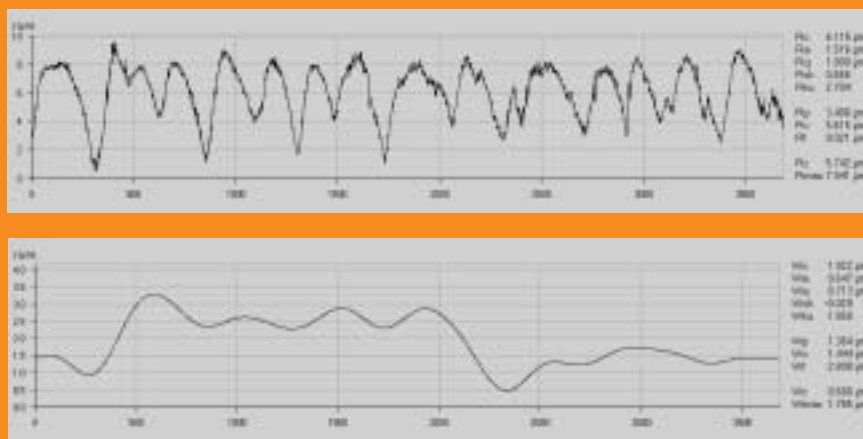


Fig 4: Multiple Profile Scan from 10 single profiles. 3685.5  $\mu\text{m}$  x 15.5  $\mu\text{m}$ , 8192 pixels x 155 sections. a) Profile, roughness-filtered, cutoff frequency 0.8 mm, b) Profile, waviness-filtered, cutoff frequency 0.8 mm

# 'Learn from Nature!'

The Microscopy Business Division of the Carl Zeiss Group staged the '7th Microscope Day at Zeiss' in Jena on 19th March with the motto 'Bionics – in the footsteps of nature'. With more than 400 visitors from the areas of research, technology and academia, the event achieved a new visitor record.

The programme had something to everyone, from those new to the field to experts. There was plenty of information on trends and innovations for visitors in lectures, workshops and expert discussions, and the latest technologies and software developments were presented in hands-on demonstrations with devices.

As guest speaker, Professor Andreas Offenhäuser of the Institute for Thin Films&Interfaces (ISG2) – Bio and Chemosensors, Jülich Research Center, reported amongst other things on the development of cell-based hybrid systems. The objective of these projects is the controlled formation of networks made of nerve cells, which are then used – together with electronics – to investigate neuronal information processes.

One focus of the event was the presentation of innovative microscope solutions for the material sciences, amongst other things, the optical imaging of uneven surfaces in the nanometre range, and the measurement of the depth of focus in quality-real time. This accelerates inspection procedures and also improves their quality.

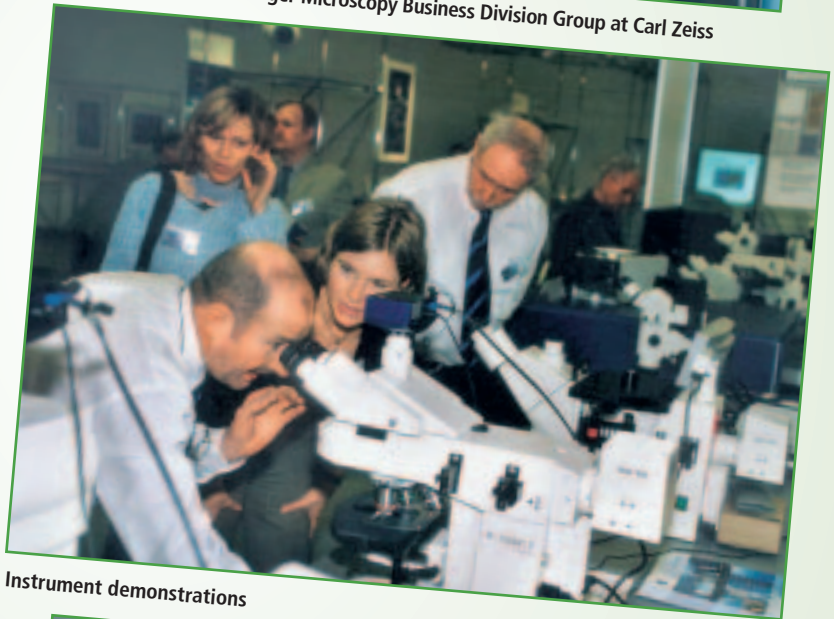
A new development in the area of fluorescence microscopy puts users in a position to obtain optical sections of biological fluorescence samples that are not masked. This increases the degree of contrast in the images captured and collocations of marked substances can be clearly demonstrated.

In the past business year, the Microscopy Business Division of the Carl Zeiss Group achieved an increase in turnover of 21% compared to the previous year. This meant that this division had the highest growth rate within the group for two years running.

Dr Martin Friedrich



Dr Ulrich Simon, General Manager Microscopy Business Division Group at Carl Zeiss



Instrument demonstrations



Hands-on session