

Innovations in Scanning Electron and Confocal Microscopy

Highest Versatility in eXtended Variable Pressure (XVP) Microscopy

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Knowledge based enterprises based upon insulating materials face a range of imaging challenges to provide high quality information allowing them to reach their own targets on advancing technology, productivity, and ultimate profitability. The analysis of insulators has always been a challenge for the high vacuum (HV) scanning electron microscopes (SEM) as the accumulation of electrons within the surfaces of the non conductive samples leads to imaging artefacts referred to as “charging”. As a partial solution to this problem, the relatively recent introduction of NTS eXtended Variable Pressure (XVP) EVO series that introduce a controlled gas atmosphere into the specimen chamber has largely overcome the charging problem. Here, a special pumping technique in combination with a pressure limiting aperture is used to keep the column under high vacuum condition to protect the tungsten or lanthanum hexaboride filament whilst the pressure inside the specimen chamber is adjustable in the range 10 Pa to 750 Pa. A further click in the NTS SmartSEM software returns the system back to high chamber vacuum should your sample examinations require it.

On an insulating sample, the surface charge that would normally build up and cause imaging artefacts is neutralised by the ions formed during the primary electron beam collision with the introduced gas molecules. The secondary electrons generated as a result of incident electron beam interaction migrate away from the sample surface. These low energy electrons interact with the molecules of gas introduced to the chamber in a collision zone above the specimen surface. The collision produce further electrons and ions. This provides the local environment that dissipates the charge build up from the specimen. In addition to ions, SE collision with the gas molecules also produces photons. These photons are collected by the VPSE detector and amplified using a high gain photomultiplier to produce a true SE image. Because of this, the EVO series offers not only the highest performance from a state of the art conventional microscope but also the flexibility to image insulating materials, without charging artefacts quickly and easily. It also improves productivity thanks to reduced or zero sample preparation and the convenience of non-destructive artefact-free imaging and analysis of samples in their natural state. Imaging with backscattered electrons in XVP mode provides slightly different and complementary information to the classical secondary electron detector since information comes from deeper within the material. Most insulating samples can be imaged charge-free at a chamber pressure between 10 and 100 Pa. Nevertheless the EVO XVP mode offers the possibility of increasing the chamber pressure up to 750 Pa enabling the investigation of strong out-gassing and very porous samples. When a cooling stage and water vapour from the water container are used, the XVP mode can also keep samples humid or wet. In addition, in-situ dynamic experiments are possible to improve the characterization and analysis of materials and speed up research and development.

High Speed Confocal Scanning and MultiPhoton Excitation

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Over the last years, new dyes and lasers as well as new tools for fast focus and ultra high resolution Z-steps and other application dependent accessories have helped to adapt the confocal technique to ever more important but highly specialist fields of research.

Conventional confocal microscopy with point scanners is too slow to visualize living cells, fluid motion, specialized cell processes and other fast processes like metabolic pathways, growth processes or action potentials. With a unique combination: superior scanning rate, outstanding image quality and exceptional sensitivity, the **LSM 5 LIVE** collect up to 120 confocal images per second at a resolution of 512 x 512 pixels, scans about 20 times faster than any other confocal system. In this way, motion trajectories of all objects of interest may be investigated reliably and quantitatively. The entire optical concept has been consistently tailored to biomedical applications. With its precise optics, creative beam splitter design and innovative beam delivery, the LSM 5 LIVE opens up new horizons in life sciences with fluorescence yield at the limits of what is technically possible. By reducing the scan field, up to 60,000 lines/sec can be made visible.

Another limitation to be overcome is the depth of penetration, which for biological tissue and blue laser light (380nm) barely exceeds 50 µm. Also the photo bleaching effect caused by ultraviolet excitation light has limited research particularly in molecular interaction and live cell imaging. Both problems can be addressed by the multiphoton technology, where the rapid interaction of two photons with one molecule provoke twice the energy than input than the laser wavelength would normally allow for. The excitation of blue or green emitting dyes with infrared light allows for up to 15 times deeper penetration without damaging out of focus molecules. This is mainly due to two factors: the longer wavelength of the femtosecond laser used, and the restriction of fluorescence excitation to the focal spot.

The LSM 510 Meta NLO combines the advantage of optimal multiphoton excitation (150 femtosecond pulses and restriction of fluorescence excitation to the focal spot) with a single line Photomultiplier array and a multipinhole system. This allows the combination of parallel multichannel imaging with multiphoton excitation and Fluorescence fingerprinting to create complete three-dimensional reconstructions of fluorescent structures, delivering valuable information about the architecture of cells and tissues. It is possible to define a number of ROIs (Region of interest) for manipulations with living cells such as the bleaching of fluorescent proteins or the photochemical uncaging of biologically active substances, choose planes for the optical manipulation of varied sizes and with any outline, or select one or several of them and the laser light will only irradiate the areas selected. The META detector supplies spectral information. There are up to four channels in the non-descanned mode, one of which can also be used as a transmitted-light channel

High optical precision and innovative technology in conjunction with intelligent scanning procedures allow low-damaging image acquisition from all kinds of specimens.