13th Day of Microscopy

Workshop 3

The Perfect Image

Tips and Tricks when Using Confocal Laser Scanning Microscopes (LSM)

High end laser scanning microscopes (LSM) are often used in different scientific fields of modern life sciences. Beside the investigation of fixed samples, today's applications focus more and more on dynamic processes within living cells and tissues. To produce the desired fluorescent signals, the samples are illuminated with different wavelengths of light. The image acquisition itself should be as gentle as possible on the specimen under investigation to minimize any influences on the processes being studied. Adverse phototoxicity, bleaching, and specimen heating should be avoided. Depending on the scientific question to be answered, the suitable experimental imaging acquisition parameters must be defined by the user. Fixed samples, for example, allow for lower scan speeds so that less noisy images can be generated due to longer exposure (pixel dwell) times and higher photon numbers. On the other hand, to capture highly dynamic processes, the focus of live cell microscopy is rather on the acquisition speed. This requires shorter exposure times in combination with lower laser power which poses a challenging demand on the LSM's ability to capture the signal perfectly.. In addition to the detector sensitivity, a fundamental knowledge on how to optimize the image acquisition parameters is necessary to capture the perfect image.
This workshop presents the basic steps for creating a perfect confocal image and will enable you to reproduce these same steps on your own LSM.