

ADVANCED METHODS FOR SPECTRAL IMAGING AND LINEAR UNMIXING: IMAGE ACQUISITION, IMAGE PROCESSING AND QUALITY CONTROL

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An increasing number of fluorochromes in combination with Laser Scanning Microscopy provide a powerful tool to directly visualize the complex relationships and interactions of cell components and molecules in their natural context. To use the full capacity of various fluorescent dyes and labelling techniques it is essential to clearly identify and separate their emission signals. This is often complicated by similar excitation and emission spectra. As a consequence, conventional imaging approaches using filters do not provide an accurate identification of fluorescent components with similar spectral properties.

Emission Fingerprinting as an acquisition mode introduced with the LSM 510 META to remedy this problem has opened the opportunity to distinguish even highly similar fluorochromes.

This concept has been taken a step further through the innovative changes in the new design of the QUASAR Detector in the LSM 710. The arrangement of a 32-Array photomultiplier tube (PMT) and two flanking single PMTs now allows fast spectral detection into a 34-channel simultaneous read out.

The excellent sensitivity of this calibrated and linearized detector unit (the QUASAR detector) and its outstanding suppression of noise provides the basis for advanced processing techniques. Based on knowledge on the fluorochromes in the sample the Smart Setup function of the ZEN 2008 software guides the user towards imaging configurations suitable to obtain the most accurate data from the unmixing experiment. Advanced algorithms such as weighted linear unmixing greatly enhance the accuracy of the unmixing result. Finally, as an internal quality control of the unmixing procedure, the ZEN 2008 software provides images representing confidence intervals of the pixel intensities in the unmixed image. .

Overall, the linear unmixing technology coming with the LSM 710 results in both improved precision and signal strength of the resulting crosstalk-free images.