A FUNDAMENTAL IMPROVEMENT TO FLUORESCENCE EFFICIENCY IN A CONFOCAL SYSTEM: AOBS - PRINCIPLES, RECENT ADVANCES AND APPLICATIONS

Martin Hoppe, Ph.D.,
Leica Microsystems Heidelberg GmbH,
Am Friedensplatz 3, D-68165 Mannheim, Germany
E-mail: Martin.Hoppe@leica-microsystems.com

KEY WORDS: Confocal, AOBS, Acousto-Optical Beam Splitter, Spectral Imaging, FRAP, FCS

The principle of programmable Acousto-Optical Beam Splitters (AOBS) has been introduced by Leica in 2002. As the central optical element of a fluorescence system, this device offers a range of advantages over conventional color/interference beam splitters, such as: extremely narrow bandwidth on the excitation side which ideally fits laser line bandwidths, and a very large bandwidth and high transmission for the emitted fluorescence light, thus maximising sensitivity of the confocal system for low-level detection. In addition, the AOBS can be re-programmed to fit any laser line combination, providing ideal preconditions for multi-labelled specimens. The degree of suppression of each excitation line can be continuously adjusted to e.g. superimpose reflection and fluorescence imaging or to maximise the dynamic range for fluorescence detection.

With these advantages in a key optical element in the fluorescence system, the AOBS is ideally complementing highly-sensitive Multi-Band Spectral Imaging (SP detector, Leica 1997). The sensitivity improvement is especially valuable for dynamic measurements, such as FRAP (Fluorescence Recovery After Photobleaching) and is generating improved results with analytical methods, like FCS (Fluorescence Correlation Spectroscopy). Recent Advances and Applications are discussed.

.