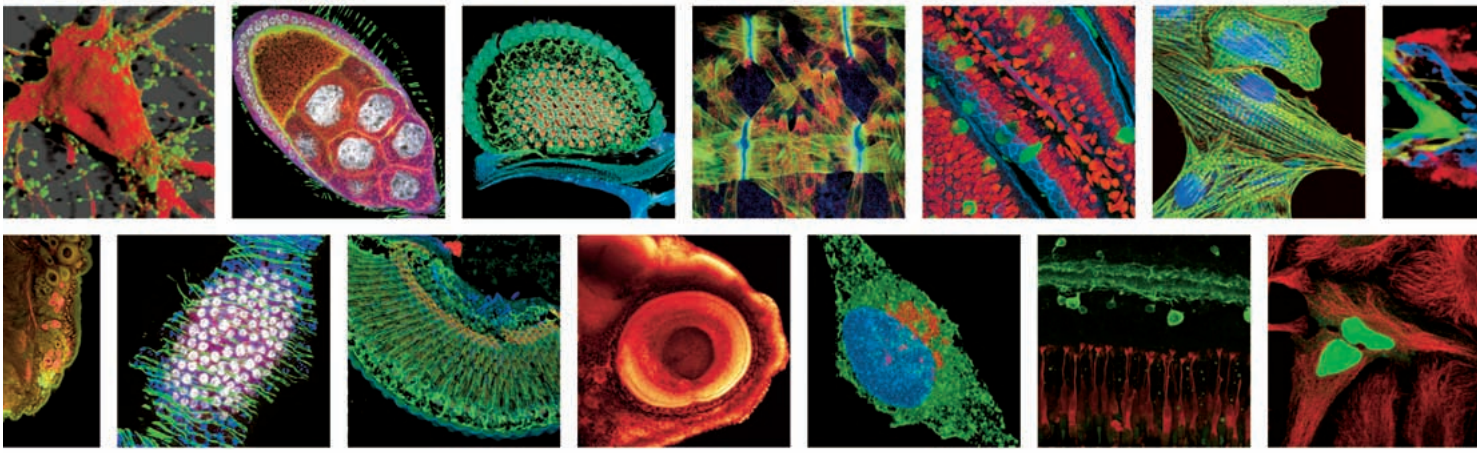
# AOBS®

Leica TCS SP5: The Broadband Confocal

High Sensitivity for High-Quality Results

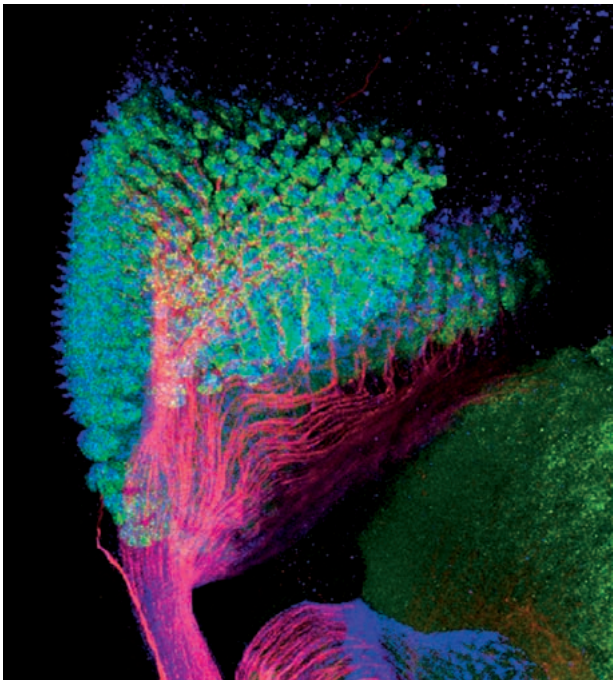
Leica
MICROSYSTEMS



**Thousands of applications**

The applications of fluorescence microscopy have enormously increased during the last five decades. The growing variety of different fluorochromes featuring different excitation and emission properties caused an increasing demand of new fluorescence filters and dichroics. The benefits of the numerous stains call for successful missions, but the numberless filters are rather inconvenient for researchers, too. Here, a broadband confocal microscope – covering all requirements by a single device – means a true relief.

# Light – a precious matter



**Sensitivity tips the scales**

Efficiency is the key to success. Not only in science and economy – where it turns to profitability, but as well in research and routine laboratories, where productivity has always been a strong requirement. Efficiency strongly depends on the suitability and the capabilities of the instrumentation. What does this mean in terms of confocal fluorescence microscopy? Here, first and foremost, efficiency is determined by the sensitivity of the detection device. Of course, speed, flexibility and the ergonomics are important parameters, though but the principle purpose of the device is to guide the maximum number of photons, emitted by the fluorochromes in the sample, safely to the detector.

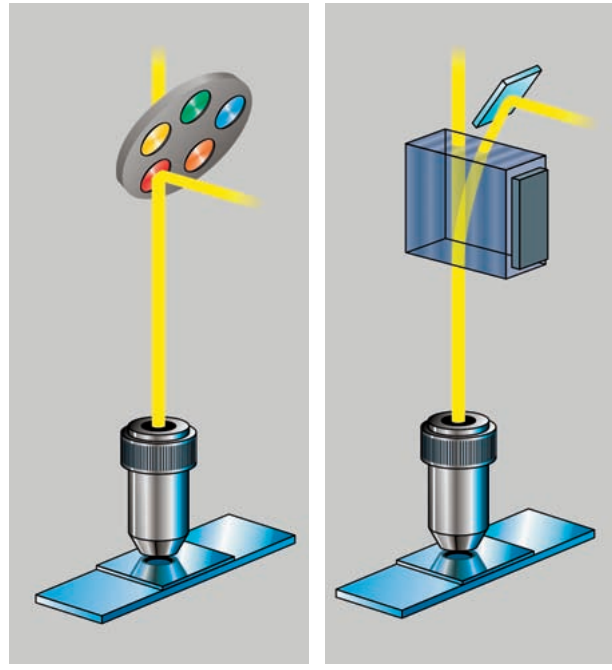
*Drosophila melanogaster larvae* (eye-disc)
Green: RNA binding protein (nuclei) Alexa 488
Red: Axons, Cy 3
Blue: Axon endings of MJ94-positive neurons, Cy 5
Courtesy of Dr. Christoph Melcher, Research Center Karlsruhe,
Institute for Toxikology and Genetics, Eggenstein-Leopoldshafen, Germany

**The technical task**

In fluorescence, it has become a standard to illuminate the sample and detect the image from the same side. A technique called „incident light microscopy", therefore, uses a device in the beam path, which decides which light goes where, very much like a three-way valve. For a singly FITC stained sample, blue light has to reach the sample and green light has to reach the detector. Classically, this is achieved by a color-discriminating mirror: in the FITC-case, it is a mirror for blue light, but a window for green light. For other fluorochromes, one has to insert other dichroics, meeting the different color requirements. So far, this is manageable – although not efficiently.

It becomes truly confusing, when multiple stainings from the same sample need to be recorded. You can easily calculate: if e.g. eight different excitations are needed, all possible permutations make 255 different dichroics.

A severe problem is the low transmission of dichroic mirrors, which is even worse for mirrors serving multiple stains (the so-called double and triple dichroics). Moreover, to insert them in the beam path, one needs a turret or slider: a quite slow solution and prone to misalignment and failure. Not to speak about the moment, when you need to exchange one of the mirrors for new dyes that you employ – a costly field service call is necessary in most cases.

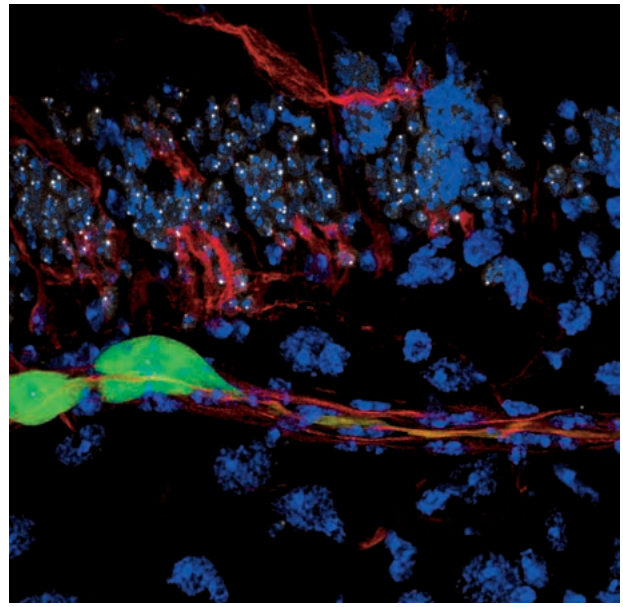


Left: conventional beam splitting by dichroic mirrors requires many optical elements with fixed properties.
Right: the AOBS® is electronically adaptable to all tasks.

# Our Solution

**Acousto Optical Beam Splitter AOBS®**

To make the researcher's life easier, Leica has introduced a revolutionary technology in confocal microscopy, which overcomes all drawbacks of dichroic mirrors described above: the acousto optical beam splitter, AOBS®. In brief, the new device is not a specially coated mirror, but a switching valve for light, which is tunable to channel any laser line onto the sample and simultaneously transmit very efficiently the emitted light to the detector. It consists of an acousto optical crystal, known as tunable deflection device. The clever bit: we operate the crystal in reverse mode. For details, you may want to consult the suggested readings.



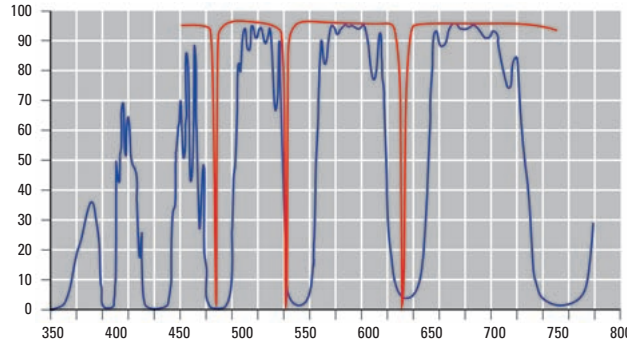
*Drosophila melanogaster*
Green: Feb211 positive neurons and their axons, Alexa 488
Red: Fibrous part of the cns (i.e. all axons), Cy3
Blue: Nuclei, DAPI
Grey: Nuclei of neurons, Alexa 594

**The benefits of AOBS®**

How does the AOBS® improve your scientific work?

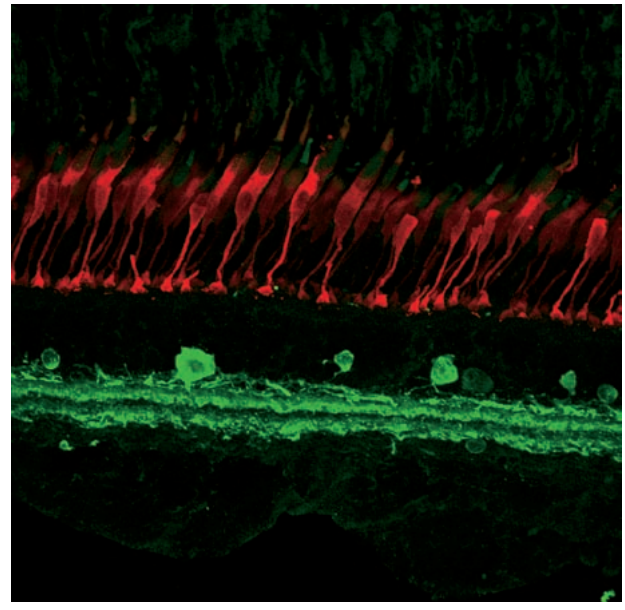
Here is a convincing list of beneficial features:

1. Clear, low noise imaging needs high transmission. The sample bleaching results from high numbers of averaging. The transmission of the AOBS® is superior to most dichroic mirrors over the full visible spectrum. Consequently, less averaging is necessary. The sample will live much longer.
2. Bright and crisp images require wide emission bands as provided by the AOBS®. This is important to channel as much photons as possible from the sample to the detector – again improving the image quality.
3. Low bleaching during image acquisition is important to protect the sample from fading and to protect living specimen from toxic chemicals that accumulate on photolysis of fluorochromes. The AOBS® has very steep slopes allowing collecting emission very closely to the excitation band.
4. Any visible-range dye can be excited, as the position of the reflection-pins can be tuned individually.
5. Multiparameter fluorescence is solved: up to eight laser lines programmable, leaving still sufficient space for emission collection – and the frequencies are tunable!
6. Ratio dyes, like excitation ratio metabolite-probes, e.g. for $Ca^{2+}$, membrane potential, pH or chloride expect fast switching in sequential scanning. The AOBS® has switch times of only few microseconds.
7. Reflected light imaging as another option. The very strong suppression of the excitation can be reduced individually, if necessary for reflection imaging.
8. ROI-scanning is improved as well: different excitation patterns are possible for different regions during a single scan.
9. Large 3D volume recording, in sequential mode will benefit as well from fast switching devices, as speed improves dramatically the system efficiency.
10. Fluorescence correlation spectroscopy (FCS) requires very low background and stray-light. Only the AOBS® sufficiently blocks close co-emitted lines, e.g. from Ar-lasers.
11. Spectral recording (lambda scan) supply correct spectra, as the transmission of the AOBS® is "white", which means, that is does not alter the emission spectra – a common problem, if spectral scanning is done in a dichroic-mirror system.
12. True confocal optical sectioning requires point-shaped illumination and emission. The AOBS® fits to point-scanning confocal devices.
13. Multiphoton and UV-imaging can be done in parallel without any drawbacks or restrictions. The AOBS does not alter the excitation of non-visible lasers, and the emission is not modified.




Transmission curves
Blue: triple dichroic, blue, green, red
Red: AOBS® tuned to 488, 543,594, 633 nm
Higher transmission, wider bands and steeper slopes with AOBS®

*Cyprinus carpio* (retina)
Green: Amacrincells, FITC
Red: red and green cones, Cy3
Courtesy of Dr. Konrad Schultz, Carl-von-Ossietzky University Oldenburg, Neurobiology, Oldenburg, Germany



14. No maloperation is possible, as the AOBS® is directly controlled together with the excitation control via AOTF. If an excitation line is selected, the AOBS® is programmed accordingly. No decision has to be taken by the operator – it is always correct and automatic.
15. No misalignment is introduced by mechanical turrets or sliders, as there are no moving parts. The crystal is firmly mounted and the programming is purely electronics.
16. No expensive accessories like filter-cubes, dichroic-sliders etc. are necessary. And will consequently save expensive field service calls for mounting new planar optical parts.

No doubt: a true broadband confocal needs an AOBS® to meet all the expectations from future-oriented research in the biomedical field. And it is a must in multi-user environments, the most challenging being imaging facilities in large institutions.

# Perfect Fit

**How the AOBS fits the future**

The AOBS® is only one out of three ingenious improvements Leica invented for confocal microscopes. The Leica SP® overcomes the restrictions of classical filter cascades for emission splitting. It is a series of tunable elements at very high transmission, allowing selecting any wavelength band for emission collection. Up to five such bands simultaneously! Tunable emission bands fit perfectly to tunable dichroics: the combination of these two technologies will not leave open any application requirement.

Suggested reading:


1. V. Seyfried, H. Birk, R. Storz and H. Ulrich: Advances in multispectral confocal imaging. Progress in Biomedical optics and imaging. Vol 5139, 22 - 23 June, pp 146 ... 157
2. R. Borlinghaus: The AOBS: Acousto Optical Beam Splitter – colorful brightness in confocal microscopy. Imaging and Microscopy 3/2002, pp 10 ... 12.


**Acousto-Optical Beam Splitter**

- Adaptable to any new dye
- 8 lines simultaneously
- Reflected light imaging
- High transmission
- Truly confocal – real optical sectioning
- Fast switching
- Freely tunable
- FCS with multi-line lasers

www.confocal-microscopy.com

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