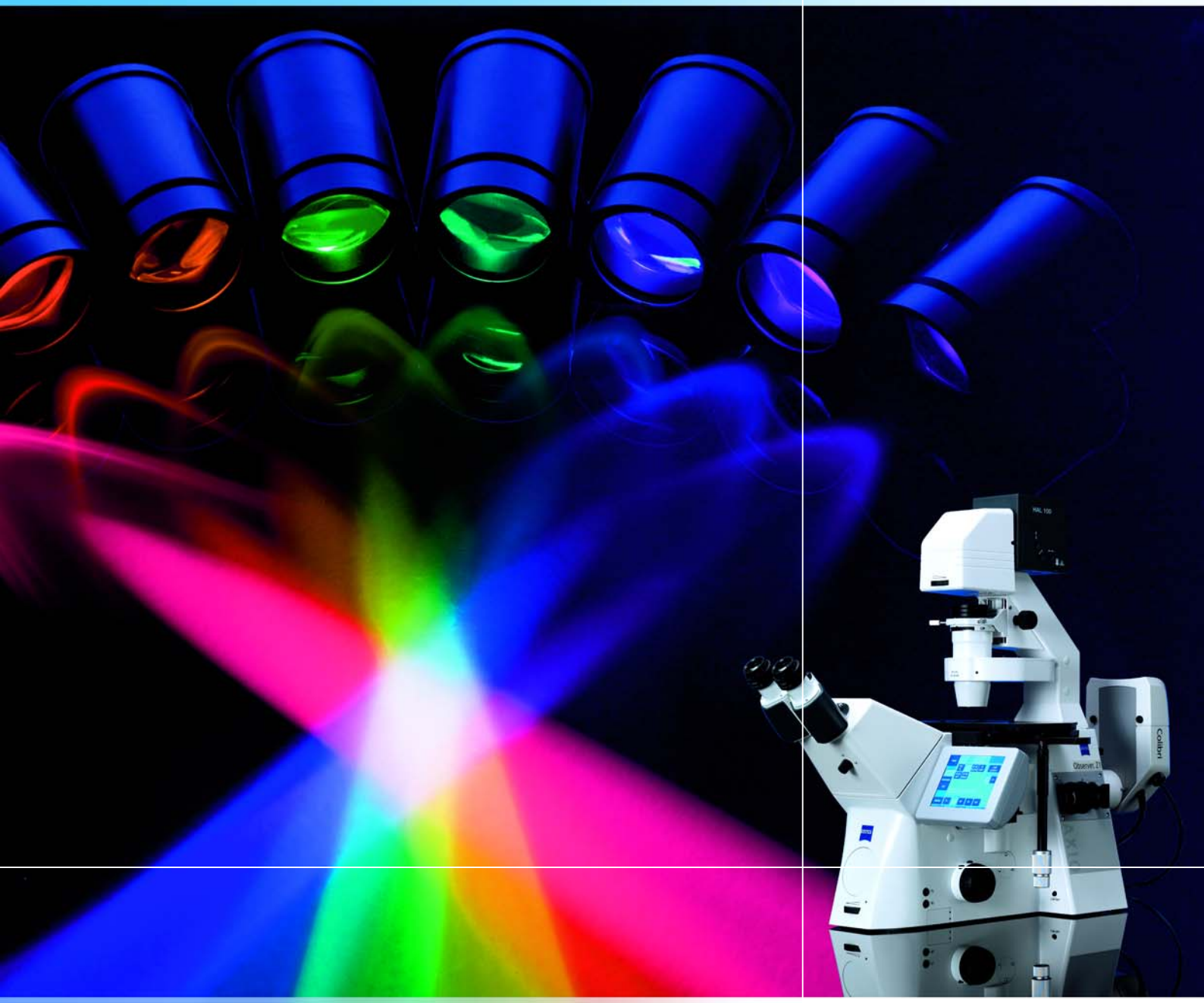
Colibri
Highlights for Fluorescence Microscopy



The LED Light Source for Research And Routine

Vilber EX1054

ZEISS
We make it visible.

New Light in Fluorescence

High-performance LEDs (Light Emitting Diodes) taking the place of a white light source – with this innovation Carl Zeiss is breaking new ground in fluorescence microscopy. Colibri is the first light source that enables you to take full advantage of the countless benefits offered by LEDs for fluorescence applications. Each individual LED delivers only a precisely defined range of the spectrum. No undesired light is emitted, which means that there is also no need to suppress it. As a result, you benefit from extremely high-contrast images with a high dynamic range – images that will enable you to achieve meaningful scientific results in tasks ranging from simple routine applications through to complex applications in Live Cell Imaging.

- **Precise adjustment of intensity for optimum protection of samples**
- **Switching times in the microsecond range**
- **Long life, high economic efficiency**
- **Stand-alone operation and full integration into the AxioVision system software**

The task of developing increasingly differentiated fluorescence techniques to decode the secrets of life is an area to which Carl Zeiss is devoting particular attention. And we have given our work in this field a name: FluoresScience. We are placing the emphasis here on research involving living cells and aim to make the best tools available for both research and routine tasks, now and in the future. We are therefore constantly driving forward the development of innovative solutions – one example being a high-quality light source that performs at the limits of what is technically possible today.

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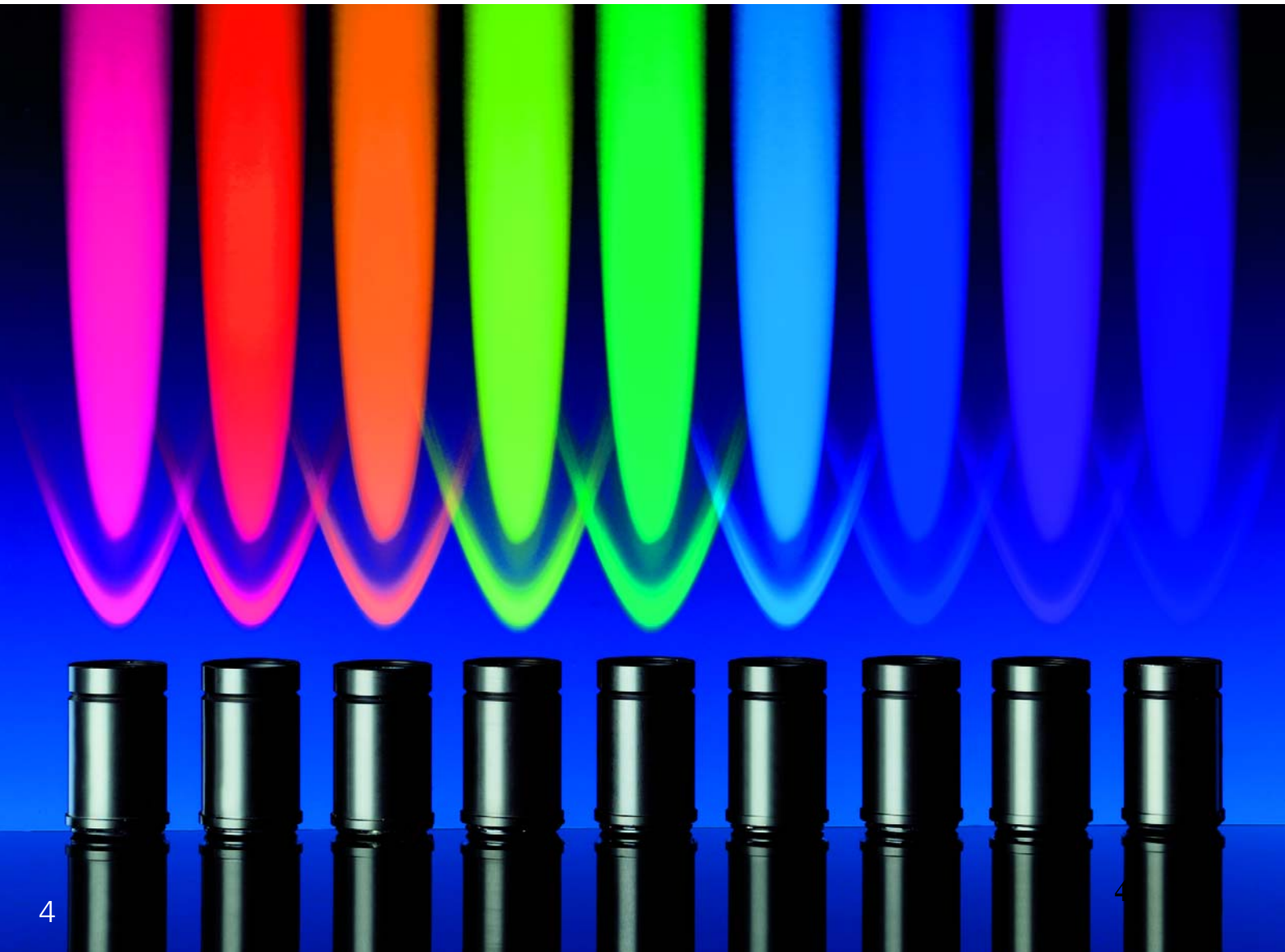
Designed for The Highest Speeds: Switching Times Enter A New Dimension

Colibri's super-fast control makes it a convenient and ideal solution for all high-speed applications. Each LED can be switched on and off and adjusted in the micro-second range. For complex applications in particular, the possibility of combining wavelengths flexibly and of coupling an external white light source opens up a wide range of possible uses.

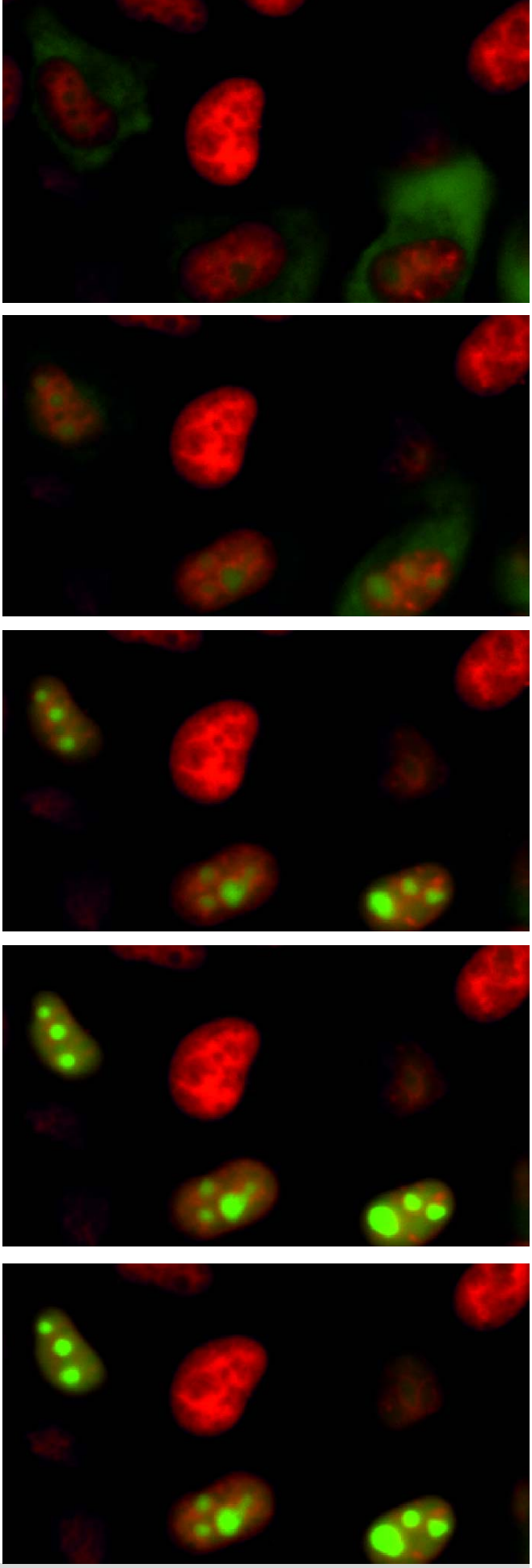
From UV to dark red in microseconds

With Colibri you have ten LEDs at your fingertips, ranging from UV through to dark red, via the full spectrum commonly used in fluorescence microscopy. Up to four LED modules can be used in parallel. You can set the desired intensity with precision in percentage steps for each individual LED using a control panel or with the help of the AxioVision system software. The illumination can be adjusted individually depending on the sensitivity of the sample. This prevents unnecessary bleaching and ensures your sample is optimally proteced.



Time lapse series: HeLa cells
red: 2B histones - DsRed; green: HIV-1 REV - mutant YFP
K. Hadyan and H. Wolff, GSF, Institute of Molecular Virology, Neuherberg, Germany

If export is blocked, the mutated HIV-1 REV protein is redistributed into the cell nucleus



Optoelectronics for faster switching

No switching mirrors, no mechanical shutters, no filter wheels – with Colibri switching takes place solely using optoelectronics. Nothing has to be moved – only the current is switched. Thanks to this sophisticated system employed by Colibri, switching times in the microsecond range can be achieved between the different LED modules and between the different intensity settings of a single LED.

Optimum protection for living samples

When it comes to analyzing rapid processes in living cells with high temporal resolution, Colibri's extremely high switching speeds make it the light source of choice. Thanks to the excellent signal-to-noise ratio and high dynamic range of the images, it is possible to identify the finest details despite low illumination intensity. The possibility of adjusting the intensity in fine steps ensures maximum protection for living samples and thereby enables you to achieve considerably longer observation times. With Colibri you will easily find the ideal compromise between illumination, phototoxicity and bleaching.

Greater options for fixed specimens

Speed and steplessly adjustable illumination intensity offer numerous advantages with fixed samples too. In particular, the possibility of freely adjusting the various wavelengths – which can also be employed simultaneously – opens up entirely new possibilities for scientific analysis. Experiments that were previously time-consuming and labor-intensive, such as searching for a small number of cells with a certain combination of differently labeled proteins, are now incredibly simple.

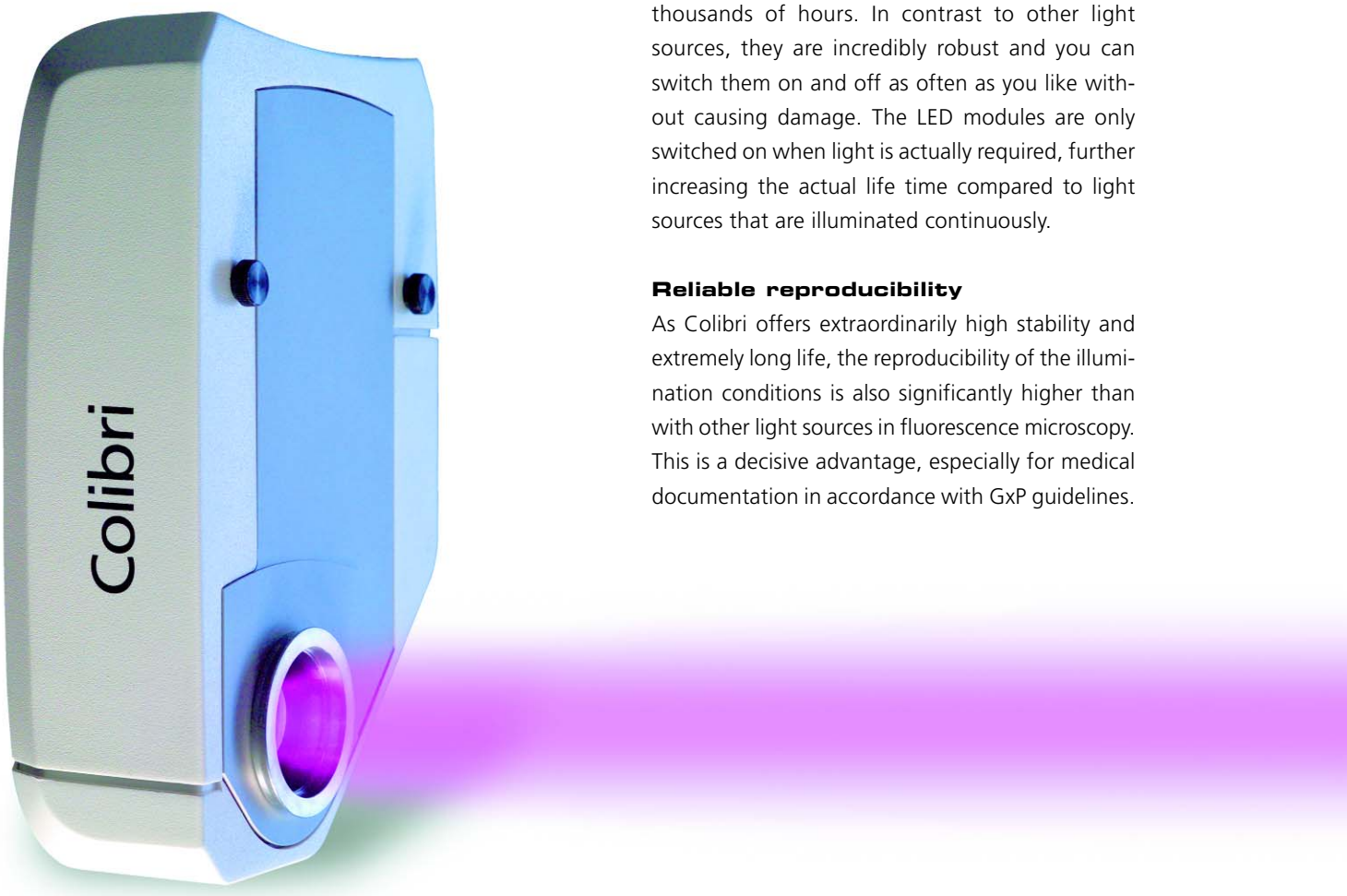
LED modules covering the available spectral range from UV through to dark red

Colibri's Highlights: Performance in Every Detail

What makes Colibri top-class technology is its consistent orientation toward users' requirements. Every technical detail has been tailored to provide the best possible support for your fluorescence applications – from routine through to high-end. With an efficient workflow, extremely simple operation and reliable reproducibility.

Steplessly adjustable intensity

The output of each individual LED can be adjusted precisely to the sample and application in percentage steps, depending on your particular requirements. It is possible to switch the LEDs individually, in sequence or simultaneously in any combination.



Thermal and mechanical stability

Colibri converts electricity into light highly efficiently, and only minimally into heat. The fact that so little heat is generated by the LEDs also means that Colibri does not burden the microscope with heat. And as the optoelectronics perform switching without any movement, there are no vibrations, noises or time delays.

Stable emission

The LEDs have very high emission stability. The fluctuations familiar with arc lamps are therefore avoided.

Long life

The LEDs have an extremely long life, lasting for thousands of hours. In contrast to other light sources, they are incredibly robust and you can switch them on and off as often as you like without causing damage. The LED modules are only switched on when light is actually required, further increasing the actual life time compared to light sources that are illuminated continuously.

Reliable reproducibility

As Colibri offers extraordinarily high stability and extremely long life, the reproducibility of the illumination conditions is also significantly higher than with other light sources in fluorescence microscopy. This is a decisive advantage, especially for medical documentation in accordance with GxP guidelines.

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High contrast

The narrow-band emission of the LEDs means less stray light in the background of the images of your samples. The images have a higher dynamic range. Consequently, even the finest structures and the weakest signals can be detected more easily.

High investment security

Thanks to Colibri's modular structure, it will be possible to retrofit it at any time if new LED technologies become available.

Superior economic efficiency

The long life of the LEDs ensures that the cost of running Colibri remains very low. And with Colibri there is no need for the laborious process of changing and possibly adjusting super-pressure lamps (HBO/XBO/metal halide).

New degree of flexibility

The LED modules and beam combiners can be easily changed, meaning that Colibri can be adapted simply as the requirements of your applications evolve. Via the integrated interface, you also have the option of using a white light source, coupled using a light guide, for applications that are not yet covered optimally by LEDs.

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Convenient, Flexible, Easy to Operate: Perfect Interaction As Part of A System

Outstanding fluorescence, unparalleled optics, a huge range of possible applications – when integrated into a system and interacting perfectly with a microscope and the AxioVision software, Colibri really excels. Its simple operation is a particularly convenient aspect for the user. Colibri can be controlled in AxioVision or using a control panel. And the LEDs and beam combiners can be exchanged quickly with just a few operations to allow you to change wavelengths.

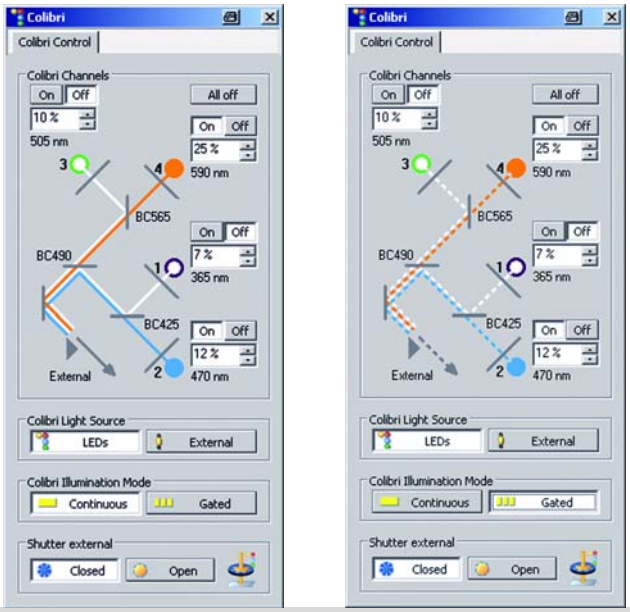
Intelligently integrated into AxioVision

When used in combination with the AxioVision system software, Colibri reaches its full potential. Convenient, fully automated processes can be created by integrating the LEDs, switching prism and shutter of the recommended HXP 120 white light source into hardware settings. Control of Colibri is integrated into AxioVision 4.6 and does not have to be purchased separately. All the components of the system are synchronized with a high degree of precision. Control of the LEDs is taken over directly by the digital cameras of the AxioCam family via a trigger signal. The advantage of this is that the LEDs can be operated in gated mode and sensitive samples are illuminated only during image acquisition. Optimum acquisition conditions are achieved, as each individual LED can be adjusted precisely and independently. This means that equidistant image acquisition can also be achieved with multichannel time lapse series, something that is particularly important for the quantitative analysis of the results.

Perfect complement to the Cell Observer® HS

Extremely high switching speeds and precise, highly reproducible control make Colibri the ideal light source for high-speed systems such as the Cell Observer® HS. All the benefits and possibilities are only utilized fully when the following components are used in combination to create a fully integrated total system:

- Colibri illumination system
- High-speed AxioCam HS camera
- Fast piezo focusing
- Fast data streaming
- Monitoring and control using AxioVision



Colibri's user interface
Left: LED modules in continuous operation mode
Right: in gated mode, where the LEDs are switched using trigger signals

Axio Observer.Z1 with Colibri and the external HXP 120 white light source; monitoring and control using AxioVision

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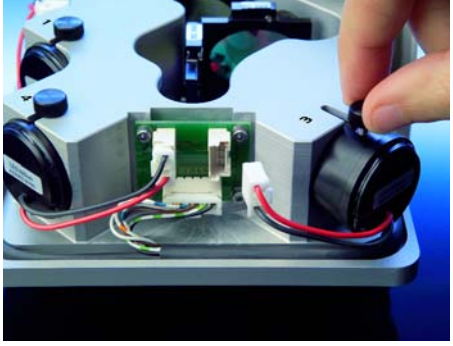
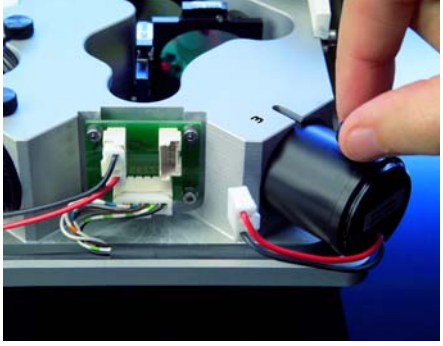
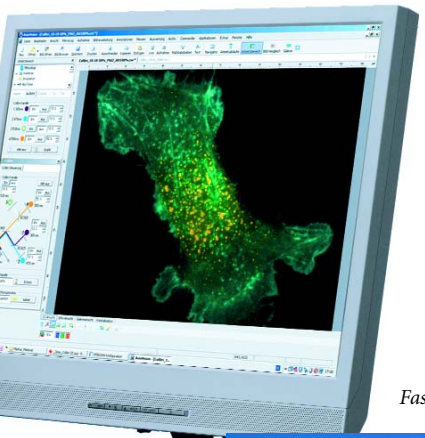



Simple changing of LED modules

Simply attach and start using your microscope

The way in which Colibri is operated is extremely simple and very much geared toward practical requirements. You simply attach the light source to the microscope's reflected light port and it is ready to use straight away. It is possible to control it either as a stand-alone unit or via AxioVision. This means that operation on manual microscopes as well as on fully automated systems is as simple as possible. The control panel is used to switch each LED module on and off and set its intensity in percentage steps. And thanks to the tactile control elements, you can operate the system reliably even while looking through the microscope. It is therefore possible to adjust the intensity of each LED optimally to the sample during observation. If required, a white light source can be coupled via the motorized switching unit, making it possible to use fluorescent dyes that cannot yet be optimally excited using today's LEDs. We recommend the HXP 120 white light source with a long-life metal halide lamp, a heat protection filter and a very fast triggerable shutter. The Colibri control panel is used to switch over to and control the shutter of the HXP 120.



Rapid changing and simple expansion

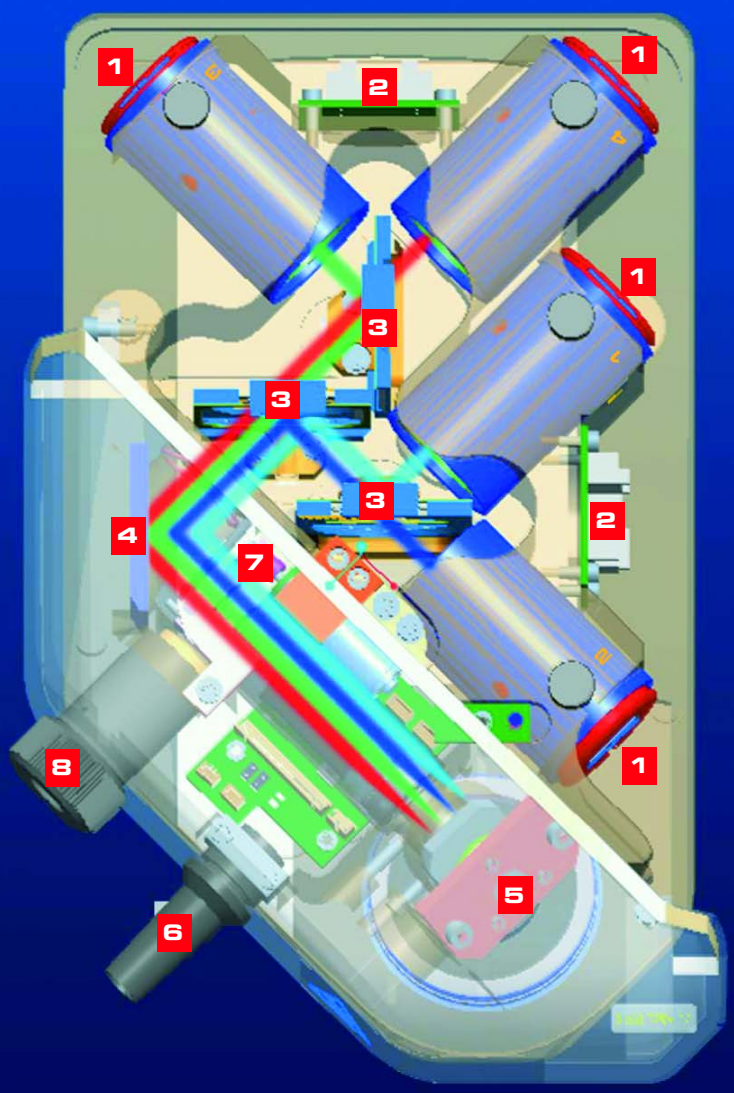
Changing the LED modules is a quick and simple task: remove the plug, undo the clamping screw, remove the LED module and insert a new LED. Exchanging the beam combiners is even easier: remove the mounting frame and insert a new frame. With Colibri there is no need at all for any laborious adjustment. In addition, each LED module is equipped with an ACR chip (Automatic Component Recognition) that enables the new module to be detected automatically. The ACR chip eliminates the possibility of incorrect operation and ensures that each module is correctly assigned to its position. Thanks to the modular structure, it is easy to expand the system if new applications demand additional wavelengths.

Control panel in LED mode with simultaneous use of three wavelengths (left) and after switching over to external white light source (right)

Fast changing of beam combiners

High-tech in Detail

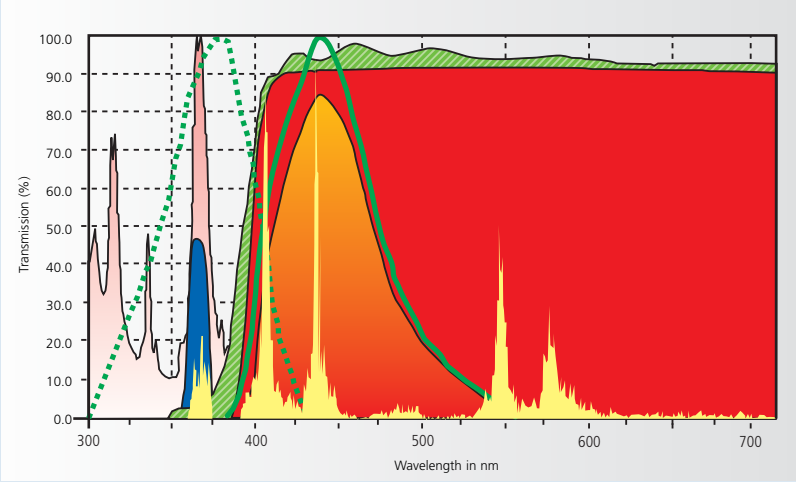
1 Positions for LED modules
2 Plug contacts for LED modules
3 Positions for beam combiners
4 Deflection mirror
5 Coupling to microscope
6 Connection for electronics
7 Motorized switching prism for changing between LED mode and white light source
8 Coupling for liquid light guide



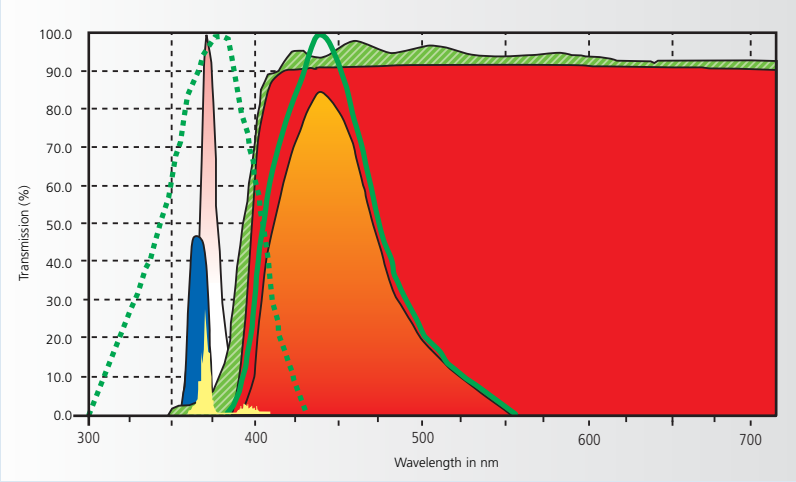
Schematic view of Colibri's design
After the upper section of the housing has been removed, the top part can be accessed to change LED modules and beam combiners. The lower section of the housing, shown here as transparent, mainly contains electronics and optical adjustment elements and is not accessible.

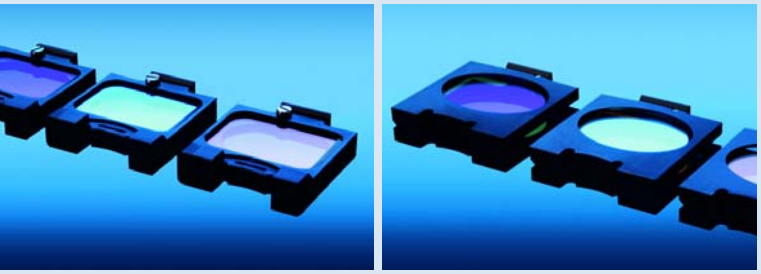
Background Information

Colibri's outstanding contrast behavior can be explained by its light source characteristics. A white light source emits light across the entire spectrum. For the excitation of a fluorescent dye, however, only a small part of the spectrum is required, which is determined by the excitation filter. The rest of the undesired light has to be suppressed as efficiently as possible, as otherwise it will be superimposed onto the fluorescence signal and will reduce the contrast. Even the best fluorescence filters available do not achieve absolute suppression of undesired wavelengths. And each photon that finds its way to the detector contributes to the background, therefore causing deterioration in the signal-to-noise ratio.



In contrast to the above, an LED emits only a narrow spectral band, outside of which no light is given out. A comparison can be seen in the two graphs. They represent a simulation calculated on the basis of genuine filter set data for a typical UV filter set. The graphs compare the use of a white light source with typical mercury line spectrum (top) with the use of a UV LED (bottom). The light that could not be suppressed by the filter system is shown greatly amplified in pale yellow in each case. As the LED does not emit any light outside its narrow emission band, undesired light contributes only minimally to the background.



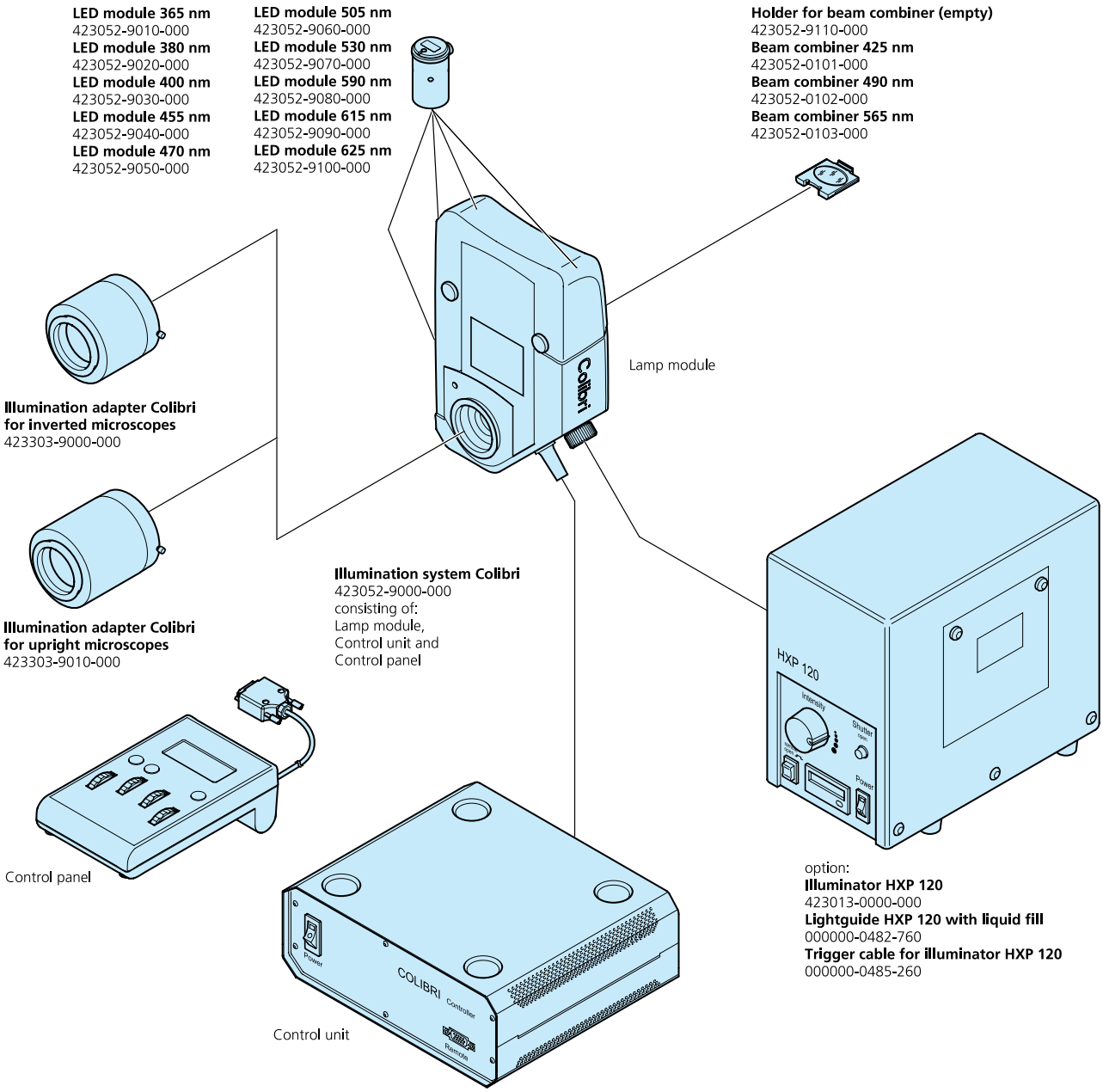


Beam combiners in mounting frames

- **Excitation spectrum of fluorescent dye**
- **Emission spectrum of fluorescent dye**
- **Transmission spectrum of excitation filter**
- **Transmission spectrum of beam splitter**
- **Transmission spectrum of emission filter**
- **Emission of light source**
- **Stray light components (shown amplified in relation to light emission)**

System Overview

Colibri is recommended for use with the Axio Observer, Axiovert 200, Axio Imager, Axioplan 2, Axioskop 2, Axioskop 2 FS and Axioskop 40



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Printed on environmentally-freindly paper, bleached without the use of chlorine. Subject to change.

46-0124 e 05.2007