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TRENDS & TECHNIQUES IN LIFE SCIENCE RESEARCH

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The Leading Imaging System in Fluorescence Microscopy

With the Axio Imager, Carl Zeiss is presenting its new generation of upright imaging systems that have been specially tailored to the demands of the fluorescence techniques.

Demands are Increasing

Understanding the molecular details of development is a key to the treatment of illnesses and malformations. This means that in addition to the morphology of cells and tissue, the processes that lead to the phenotypes of interest also need to be identified and analyzed. Information about molecular details is obtained by conducting experiments in which the molecules concerned are specifically labeled, making it possible to analyze their spatial and temporal dynamics.

One approach towards this goal is the use of fluorescent proteins (FPs), boosting, fluorescence microscopy into one of

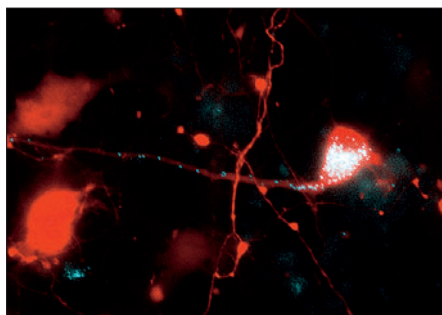


Fig. 2: Multichannel image – primary neurons (rat) in culture. Red: YFP-labeled cell bodies. Cyan: CFP-labeled peroxisomes. Objective: EC Plan-Neofluar 40x/0.75. Y. Okada, Dept. Cell Biol. & Anatomy, Grad.Sch.Med, Univ. Tokyo, Hongo, Tokyo, Japan.

the key technologies within life sciences and diagnostics.

The special interest shown in the results from the fact that they can be used as expression markers and fusion partners for other proteins.

Probably the most well-known FP is green fluorescent protein (GFP), which was originally isolated from the Atlantic jellyfish *Aequorea victoria*. The strong fluorescence emitted by FPs in the visible region of the spectrum makes them useful for detecting even extremely small quantities of protein molecules, and they are therefore also used successfully for simple molecule detection. More than 50 variants of GFP have now been described.



Fig. 1: Axio Imager: One of the system's innovations is the flexibility it offers. Eight stand variants are available to the user. There are stands well-suited to routine applications and high-end stands which are designed for changing specifications.

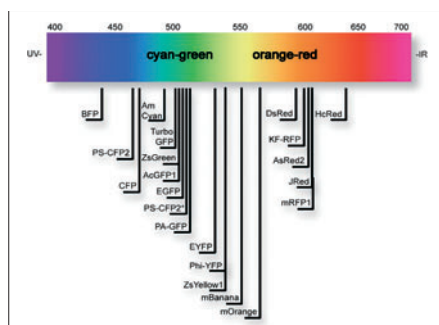


Fig. 3: Comparison of the emission spectra of known FPs. According to H. Wolff – GSF, Munich, Germany

To analyze developmental processes at molecular level, it is often necessary to label and simultaneously image several proteins (e.g. proteins interacting during the differentiation of a cell).

Depending on the application, documenting the FPs requires a high level of spatial and spectral resolution, sensitivity and speed, in various weightings. The imaging of fixed samples requires partic-

ularly high resolution, whilst live cell imaging demands high speed, high contrast and high spectral resolution. Single molecule detection, on the other hand, calls for high sensitivity and a high signal/noise ratio.

If we compare research with diagnostic applications in routine areas of work, other important aspects emerge, such as reproducible imaging (definable device and system parameters) and efficient and ergonomic operation. With automatic test procedures in particular, users want to be constantly informed about the status of the device. For longer, manual evaluation, a relaxed working position and an intelligent operating concept are indispensable.

Focus on Color

The most important feature of an imaging system for fluorescence is a color-corrected (apochromatic) reflected light beam path. Carl Zeiss has completely re-

designed this component for the Axio Imager. The result is incredibly homogeneous illumination of the observed specimen, from the UV range right through to the near infrared.

This significant benefit becomes obvious to the user in cases where several

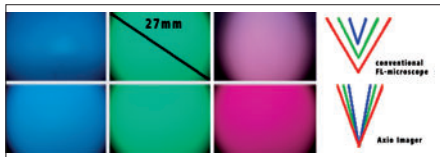


Fig. 4: The chromatic aberrations that occur with conventional fluorescence microscopes (top row) are eliminated by the newly designed beam path in the Axio Imager (bottom row). All colors are imaged in the specimen in the same plane.

colors with widely differing emission spectra are being observed. In the past the user had to readjust the illumination – a very time-consuming task when fluorescence signals were being imaged in several channels. With Axio Imager, Carl Zeiss is also introducing the new, adjustment-free HBO lamp. With the help of a patented technique, this automatically optimizes the burner position when the

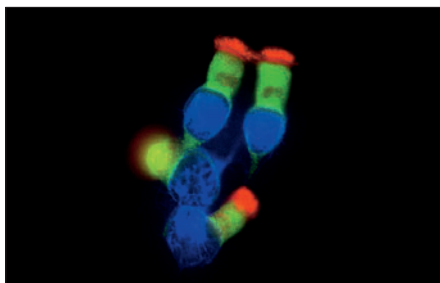


Fig. 5: Respiratory epithelial cells (human) – multichannel image. Red: cilia (Alexa Fluor 546). Green: ER (Alexa Fluor 488). Blue: cell nuclei (Hoechst 33342). Objective: Plan-Apochromat 63x/1.4. M. Fliegau, H. Olbrich, H. Omran, Center for Pediatrics, Freiburg University Hospital, Germany.

system is started, allowing the user to work under homogeneous illumination conditions at all times.

Focus on Contrast

Stray light in the illumination and imaging beam paths is the main negative influence on contrast in fluorescence imaging. The patented light trap in the reflector turret, has been further developed in a consistent way for the Axio Imager and expanded to include additional active shielding elements in both beam paths. Further improvements have also been achieved through the use of new fluorescence filter modules. Tilting the emission filter prevents reflections in the imaging beam path and increases the contrast in the image.

For Axio Imager, Carl Zeiss has designed new filter sets especially for the detection of FPs. These so-called HE (high-efficiency) filter sets for GFP, CFP, YFP and dsRed stand out due to their particularly high transmission and the

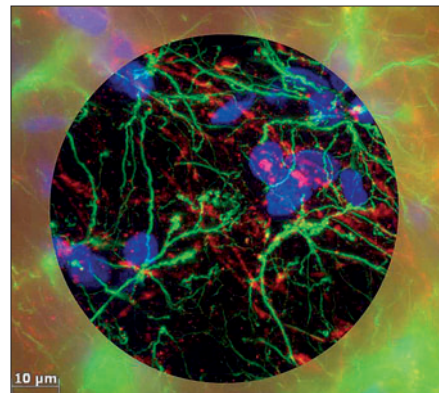


Fig. 6: Brain section (rat) – three-channel image with ApoTome (inside) and without ApoTome (outside). Green: astrocytes labeled with GFP. Blue: cell nuclei (DAPI). Red: TRITC (neurons). Objective: Plan-Apochromat 63x/1.4.

steeper slopes of the bandpass filters for excitation and emission. This makes it possible to reduce exposure times, and therefore damage to the sample, by around 50%.

The significantly improved EC (enhanced-contrast) Plan Neofluar objectives also help to minimize stray light and substantially improve contrast in the acquired image.

Even better contrast can be achieved (in the third dimension too), especially for tissue sections, using the improved ApoTome.

Focus on Precise Imaging

The “Imaging cell” is an integral component of all stand variants. As the stage support, z-guide and nosepiece are connected in an extremely stable way, external vibrations are virtually eliminated in the sample space. Together with the high precision z-motor (stepsize: 10 nm with reproducibility of +/- 10 nm in “closed loop” operation, with a travel speed of 8 mm/s), this creates the optimum conditions for fast and precise acquisition of z-stacks for deconvolution or for confocal sections with the LSM.

Fast shutters for reflected and transmitted light have been integrated into the stand and can be controlled directly by the AxioVision system software. For time-lapse analyses, e.g. of the development of the threadworm *Caenorhabditis elegans*, it is essential to restrict not only the reflected-light exposure, but also the transmitted-light exposure as efficiently as possible to the moments when image acquisition is actually taking place. Oth-

erwise the organism will be exposed to heat-shock or cytotoxic conditions due to the increase in temperature. Both the above influence normal development and lead to artifacts.

For complex imaging, additional illumination sources or detectors can be coupled via interfaces into the beam path. The fast and exchangeable 6x and 10x reflector turrets have been specially developed for the rapid imaging of numerous colors.

In developmental biology it is often desirable to assign fluorescence signals to the structures in question in differential interference contrast (DIC). To achieve this, the fluorescence signals and the DIC image are acquired in separate channels and automatically overlaid in AxioVision. The image shift caused by wedge errors in the analyzer can have a disruptive effect if the structures being observed are particularly small, e.g. proteins in cilia. With the Axio Imager, Carl Zeiss is now presenting a shift-free analyzer that virtually eliminates such effects.

Summary

- Carl Zeiss has given the quest for optimized solutions for fluorescence imaging a name: Carl Zeiss: FluoresScience.
- The Axio Imager is one of the innovative solutions developed in particular to meet the increasing demands in the field of life sciences for greater speed, sensitivity and resolution in up to six dimensions (x, y, z-stack, time-lapse, multi-color, numerous positions).
- Thanks to its incredible flexibility and user-friendliness, the Axio Imager is perfectly suited to the fluorescence techniques used in both routine and research applications.
- www.zeiss.de/FluoresScience

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