The best of two worlds, now in a single instrument

Leica MacroFluo™ – the new dimension in fluorescence macroscopy

Leica
MICROSYSTEMS

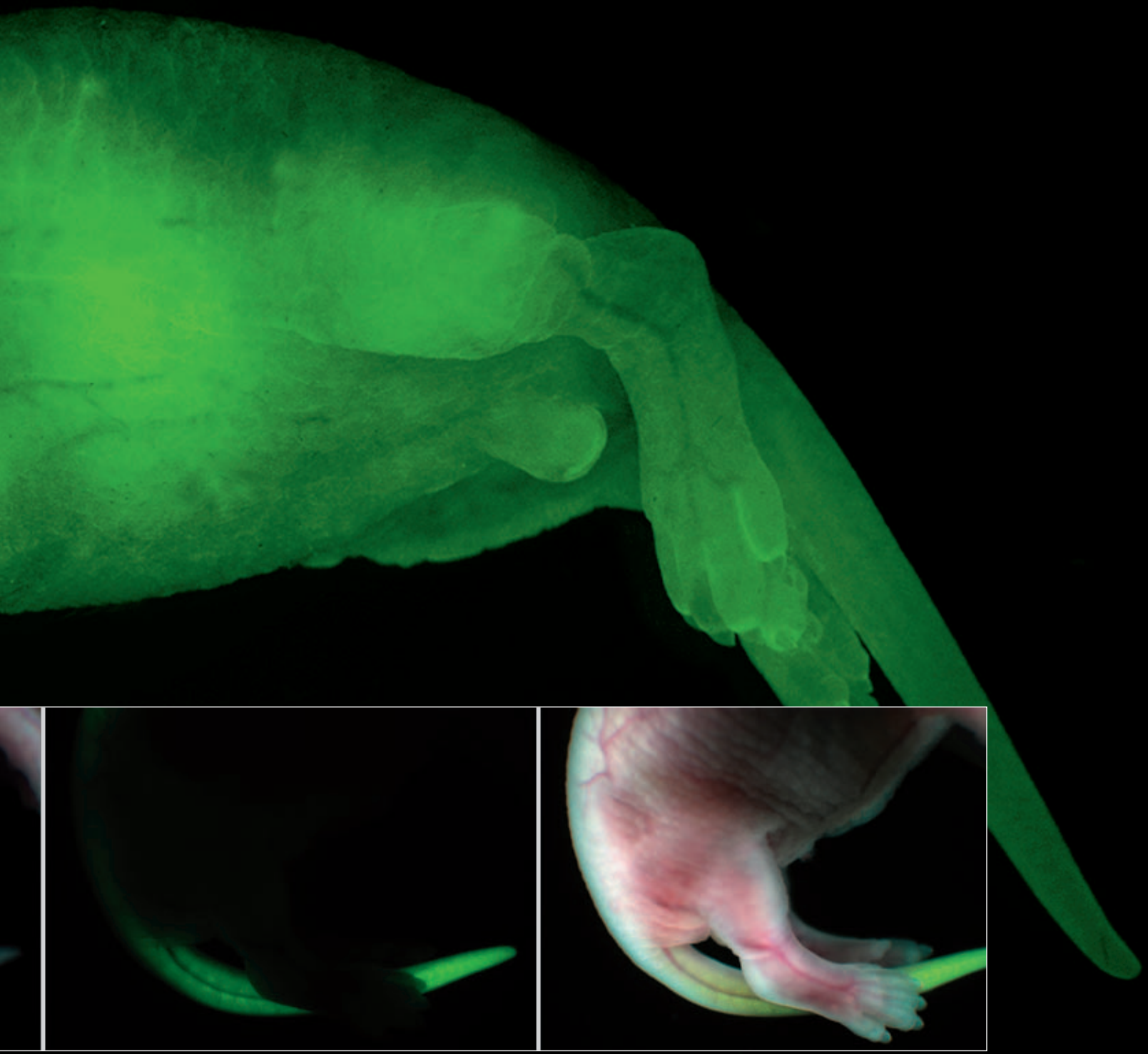
Studying nature's clues

Of the genes of humans and mice, around 95 percent match. Mice suffer from the same diseases as humans – hypertension, heart attacks and strokes; cancer, Alzheimer's, Parkinson's and obesity – and genetic defects are always responsible. The mouse is therefore an ideal biological model for in-vivo studies exploring the causes of disease in humans. These, in turn, are a precondition for the development of new approaches in gene therapy.

Leica Microsystems presents the only worldwide macro documentation systems for fluorescences: the new Leica MacroFluo™ systems with 6.3:1 and 16:1 zoom. The MacroFluo™ concept combines the advantages of macroscopy – large object fields, large working distances, parallax-free and precise imaging – using fluorescence technology (even multi-color fluorescences) at high resolution. Leica MacroFluo™ systems are the right choice for observing and digitally recording the effects of genetic defects in living transgenic models the size of a whole mouse with the highest precision and resolution in an intensely fluorescent field.

Transgenic mouse expressing green fluorescent protein (GFP, also cover image). GFP filter, size of mouse: 2.5cm. Group of Dr. Daniel Metzger, Prof. Pierre Chambon and Imaging Centre of IGBMC

"We are interested in applying mouse genetics to study development and to connect to human disease."
Nagy Lab, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada



Expression of green fluorescence in an EGFP (Enhanced Green Fluorescent Protein) transgenic mouse. Images contributed by Dr. Massimo Pasqualetti, University of Pisa and Dr. Filippo Rijli, IGBMC and the Imaging Centre of IGBMC

When excellence meets brilliance

Leica MacroFluo™ introduces the world of fluorescence to macroscopy and vice versa – and lets you experience a new dimension of brilliant images of the highest possible precision. For this purpose, we combined the excellent optics of our apochromatic Leica Z6 APO (6.3:1) and Z16 APO (16:1) zoom systems with brilliant Leica fluorescence technology. The result: the first and only macro documentation systems for fluorescence – and images of a unique sharpness, precision and depth of information.

Parallax-free fluorescence macroscopy
The unique feature of the MacroFluo™ concept is the combination of large working distances and object fields of a stereomicroscope with the vertical optical path typical of microscopes. Unlike stereomicroscopy with two convergent optical paths, it guarantees absolutely parallax-free imaging and maximum precision when examining whole, living models. The result is much greater accuracy in digital image processing, analysis and measurements. That applies especially to processing with multifocus, overlay and deconvolution programs.

Large specimens – high resolution
Leica MacroFluo™ can be equipped with an apochromatically corrected zoom system of your choice: Leica Z6 APO with 6.3:1 zoom or Leica Z16 APO with 16:1 zoom. Magnifications, field diameters and working distances can be adapted to the task at hand with planapochromatic 1×, 2×, 0.8×, 0.5× or 5× objectives.

Comfortable motorization for fast and safe application
The Leica Z6 APO A and Leica Z16 APO A zoom systems provide motorized zoom, iris diaphragm and fine focus. This allows for automating future documentation tasks. It proves to be extremely time-saving and useful for many applications since images can be calibrated automatically and integrated into a database.

Macro and micro
With the planapochromatic 5× HR (high resolution) microscope objective, the MacroFluo™ systems deliver a previously unattainable wealth of information. Magnifications of up to 225× (6:1 zoom) or 575× (16:1 zoom) can be achieved with a resolution of 1500 Lp/mm. For manual zoom systems, the optional fine focusing drive is recommended for precise focusing at such high magnifications. For the automated zoom systems Z6 APO A and Z16 APO A, the motorized fine focus is integrated and can be operated via handheld controller or PC.

Intense luminosity at all zoom settings
The coaxial fluorescence illuminator with five position reflector disk provides extremely bright, homogeneous fluorescence of outstanding quality, even at low magnifications. The field automatically adjusts to match the object field when zooming.

Harmonized from the stand to the DFC camera
The apochromatic 6.3:1 and 16:1 zoom systems are harmonized with the utmost Leica precision to HC (harmonic component) optical system components such as eyepieces, trinocular HC photo tubes and photo/TV adapters. As a result, the corrective effect of the system as a whole is maximized to create optimal conditions for precise measurements, analyses and image processing.

"Studying diseases while ignoring genes is like a bad detective wanting to solve a murder without looking for the murderer. We're all the victims." James Watson, American biochemist, winner of the Nobel Prize for medicine in 1962 together with Francis Crick and Maurice Wilkins for determining the structure of DNA. Their discovery of the "rope ladder of life" rang in the age of genetics.

Leica wishes to extend its thanks to Jean-Luc Vonesch and Didier Hentsch of the Imaging Centre IGBMC Strasbourg for the development of this innovative assembly.

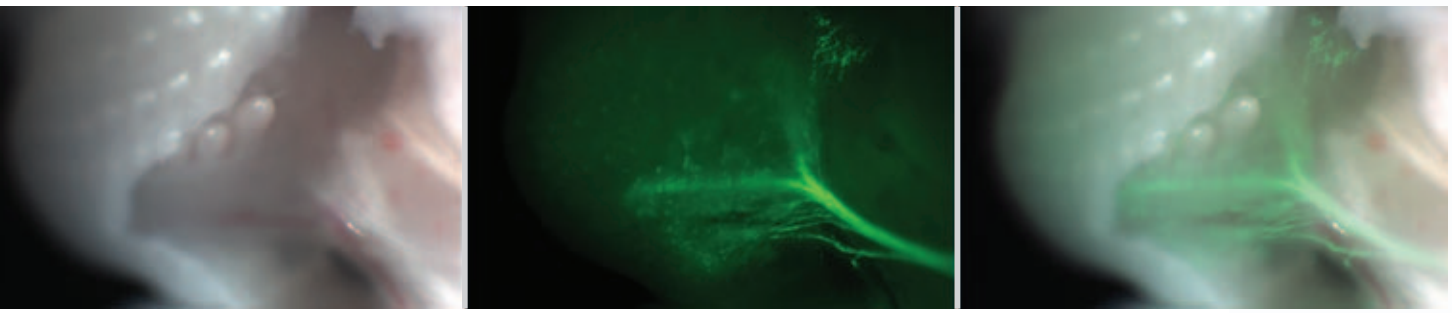
Leica MacroFluo™ with motorized 16:1 zoom
Leica Z16 APO A and UMC handheld controller

Leica Design by Christophe Apothéloz

As unique as your DNA: the optics

Fluorescence technology is constantly opening new, interesting possibilities for the visualization and analysis of gene expression in chicken embryos, mice, zebrafish and Xenopus, as well as in transgenic virus and bacteria concentrations. It permits the study of soil samples, cells and surfaces of plants. Development processes in cells can be visualized, as can capillary flow, nerve tracts and tumor growth in mice.

Geometrically precise overviews which are high in contrast and rich in detail at low magnifications and strong fluorescence signals are required when observing connections in large models. On the other hand, further-reaching analysis and digital image processing call for high magnification and resolution extending into the domain of light microscopy. The Leica MacroFluo™ system is a modular design that can be equipped with two different zoom systems and optical accessories for a range of different tasks.



Leica MacroFluo™ 6.3:1, manual or motorized:
highest macro resolution and powerful fluorescence for large specimens
The Leica MacroFluo™ 6.3:1 is the best choice for experimental documentation at low magnification, for depicting overviews of whole mice, rats or soil samples in a brilliant, precise manner suitable for analysis. The apochromatic 6.3:1 zoom delivers images with the world's highest macro resolutions of 351 Lp/mm (Planapo 1× objective), 702 Lp/mm (Planapo 2× objective) and 1500 Lp/mm with the 5× HR planapo micro objective. The extremely high numerical aperture – 0.117 nA with the Planapo 1× objective – also benefits the power of the fluorescence signal.

Expression of green fluorescence in an EGFP (Enhanced Green Fluorescent Protein) transgenic mouse. Images contributed by Dr. Massimo Pasqualetti, University of Pisa and Dr. Filippo Rijli, IGBMC and the Imaging Centre of IGBMC

Together with the 1.25× magnification factor of the FSA trinocular tube, the zoom range extends from 7.1× to 45× (with Planapo 1× objective, wide-field HC Plan 10×/25 eyepieces), to 90× with Planapo 2× objective, and a maximum of 225× with the 5× HR Planapo micro objective. The 1× Planapo objective provides a convenient 97mm working distance for manipulation, or 39mm with the 2× Planapo objective.

Leica MacroFluo™ 16:1, manual or motorized: Fluorescence analysis at the highest level
The Leica MacroFluo™ 16:1 is the first macroscope to permit the observation and documentation of fluorescent structure widths of 0.33 (5× HR Planapo objective) micron. The world's largest zoom permits the study of clearly contoured overviews of chicken embryos and neural systems, as well as selected details such as the expression of proteins, concentrations of viruses, or the migration of bacteria in Petri dishes at high magnifications and resolutions. Image sharpness remains constant over the entire zoom range from 7.1× to 115× (Planapo 1× objective, wide-field HC Plan 10×/25 eyepieces, FSA 1.25× trinocular tube).

With optical accessories (5× HR Planapo micro objective), objects can be viewed at magnifications of up to 575x and a resolution of 1500 Lp/mm.

The premier class objectives
Objectives have a decisive effect on image quality. That's why we combine the Leica MacroFluo™ with 1×, 2×, 0.5×, 0.8× and 5× HR planapochromats. This objective type features a range of additional lenses with varying refractive power and dispersion to correct errors such as chromatic and spherical aberration, field curvature, astigmatism and asymmetric optical aberration. The field of view, which consistently maintains its sharpness to the very edge, permits a quick overview to be gained of specimens without the need for focal corrections.

The 0.5× Planapo objective, with its 187mm working distance and intensively illuminated 70mm field of view, is ideal for studying living organisms such as whole mice, Xenopus (clawed frogs) or zebrafish. The depth of field can be adjusted with the integrated iris diaphragm.

Leica MacroFluo™ with manual Leica Z16 APO 16:1 zoom

"Experimentation with lower organisms will illuminate the meaning of the sequence in humans."
David Botstein, Ph.D., renowned pioneer of modern genetics, Professor of Genetics at Stanford University, member of National Academy of Sciences and the Institute of Medicine.

Powerful, even with weak fluorescence

The development of new fluorescence stains and staining methods is expanding the range of methods and fields of application for fluorescence technology in biomedical research, molecular genetics and biotechnology. Multiple labeling, for example, is opening completely new perspectives for the study of development stages, reactions, signal transduction and metabolic pathways. This involves selectively marking cell or tissue components with a variety of fluorescence stains and exposing them to short-wavelength exciter light of a specific bandwidth to cause them to fluoresce.

The Leica MacroFluo™ fluorescence illuminator features a filter turret for five filter blocks. Multicolor fluorescence can thus be observed in parallel or freely combined using Leica IM1000 Image Overlay software. The color differentiability of the emission spectra provides greater analysis reliability and reduces the number of experiments required. Transmitted-light methods can be used simultaneously or alternatively in order to clearly allocate fluorescent and non-fluorescent structures.

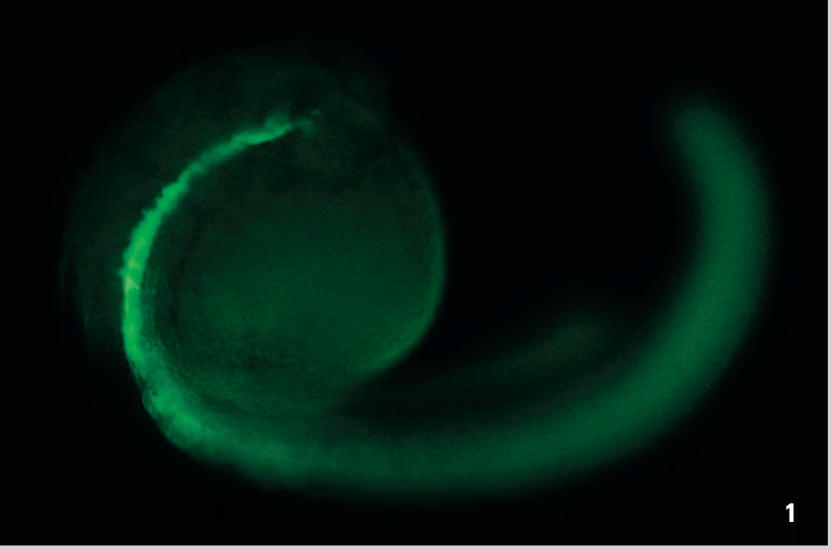
Leica fluorescence technology
The integrated fluorescence illuminator – also an HC component – ensures optimal light flux for maximum intensity and homogeneity. Up to five filter blocks can be snapped into place horizontally and replaced easily on the convenient filter disk. The patented Leica Zero Pixel Shift technology guarantees exact alignment when overlaying different fluorescent images. It prevents undesirable pixel shifts between the images of the individual spectral ranges when switching filter blocks, thus providing ideal preconditions for digital multicolor fluorescence experiments.

The centerable aperture diaphragm reduces reflections and protects the specimens. The field diaphragm can be used to increase contrast and reduce excitation to prevent the rapid fading of sensitive specimens.

The source for powerful fluorescence
The Hg 100W high-pressure mercury burner is the perfect light source for powerful fluorescent excitation in the specimen. It guarantees the highest intensity in the near-UV and short-wavelength visible spectral range. Additional design measures in the 106 z lamp housing ensure strong flow of light and an ideal light quality: the chromatically corrected four-lens collector, the focusable heat-absorbing filter, the focusable and centering reflector and the centering lamp mount.

Filter block system
New and evolving examination methods, as well as new fluorescence stains in molecular medicine and genetic engineering regularly require new filter blocks for optimal coverage of the required spectral ranges. 26 fluorescence blocks are currently available for a variety of macro and multicolor applications (further filter blocks available on request).

A zebrafish embryo (20 hours old) carrying a green fluorescent protein (GFP) gene under control of sonic hedgehog gene regulatory sequences. GFP expression is detectable in the anterior floor plate and in the notochord. Anterior to the left and dorsal up. Refocalisation d'images. Filter L5. Prof. Dr. Uwe Straehle, Forschungszentrum Karlsruhe and Imaging Centre IGBMC, Cédric Vonesch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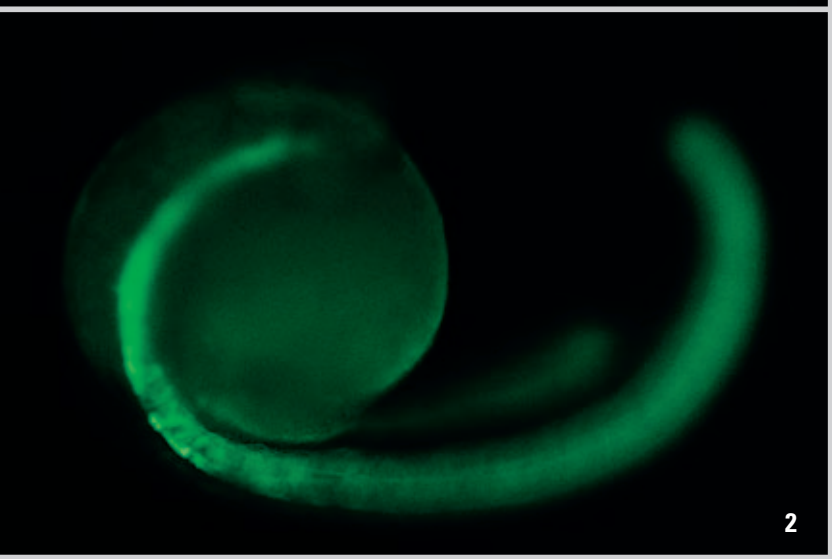
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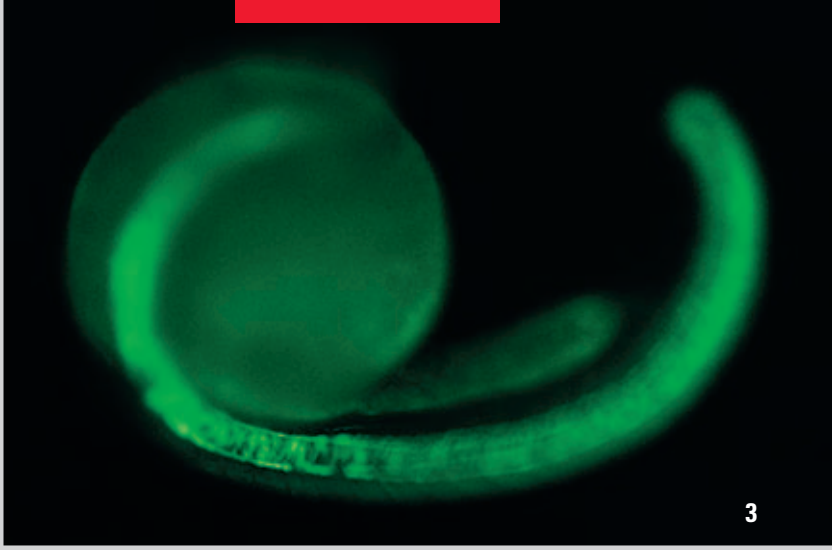
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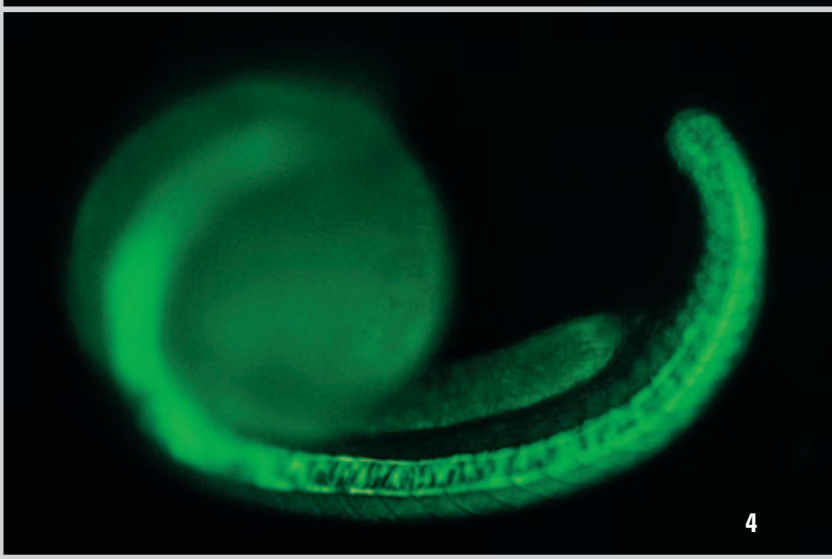
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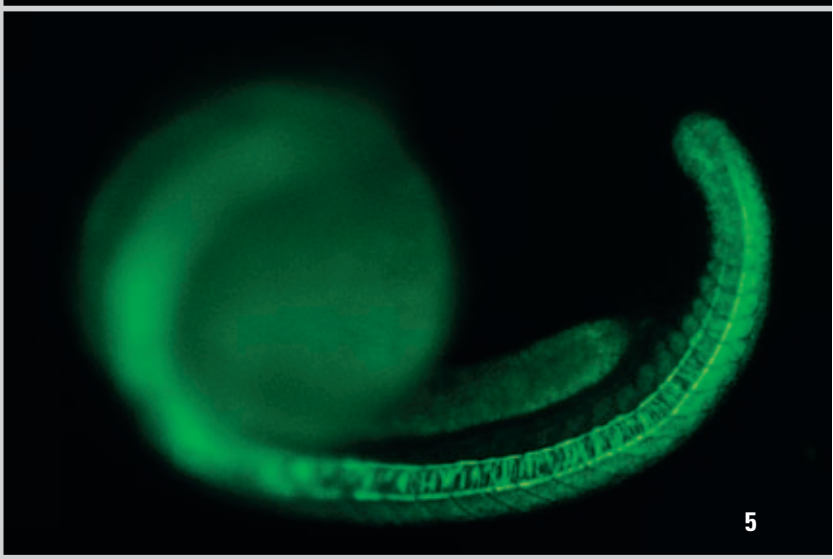
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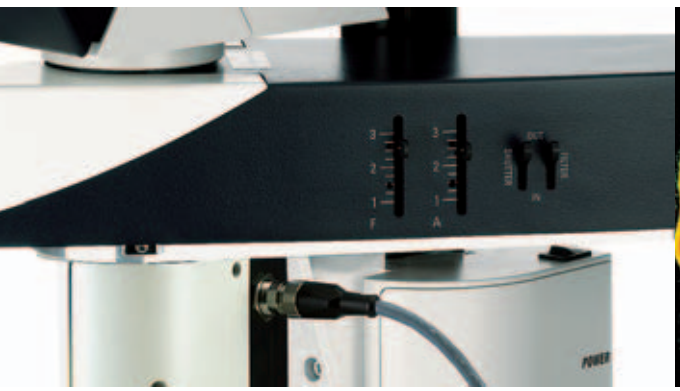
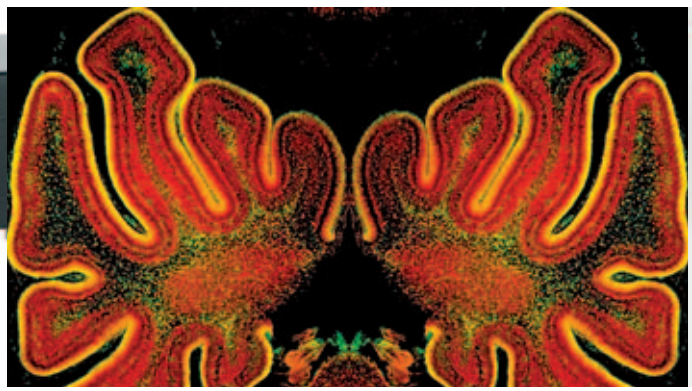
Harmonized overall system

The Leica MacroFluo™ system delivers its full performance in the interaction of a full range of harmonized components. Investing in state-of-the-art optical technology pays off in terms of image precision and brilliance in documentation and image analysis.

Harmonized illumination and imaging optics
The correction effect is harmonized throughout the optical HC (harmonic component) system components such as the HC PLAN eyepieces, the trinocular HC L 3TP photo tube and the photo/TV adapters. As a result, the corrective effect of the system as a whole is improved significantly to create optimal conditions for modern camera adaptations.

Sharp contours in the finest structures
Simple optical systems cause distracting color fringes, especially when depicting fine and extremely fine structures. The physical reason: not all spectral colors are depicted identically when passed through glass. However, the apochromatic 6.3:1 and 16:1 zoom systems that we have combined with the Leica MacroFluo™ correct chromatic aberrations for images with ultrasharp, deep black contours and color fidelity. Minute gene expressions can be identified safely, quickly and reliably; measurements, analyses, and image processing become more precise.

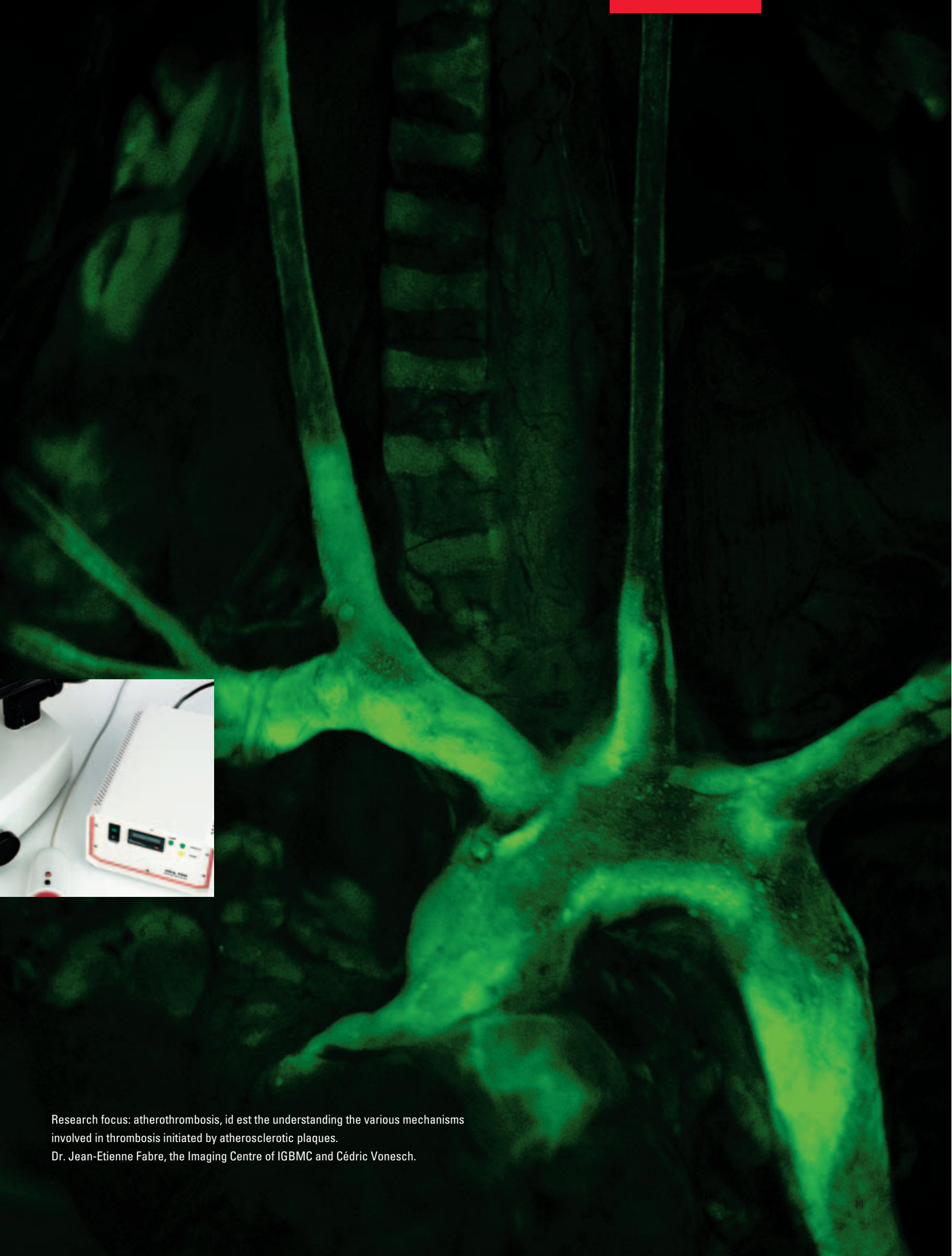




Gliding stage

Coupled zoom optics and illumination
The illumination of the instrument is coupled to the zoom optics. This unique innovation by Leica Microsystems guarantees precise illumination, maximum light utilization, and homogenous object fields at any zoom position. The additional engineering effort this involves is rewarded generously by intensively luminous, flare-free and richly detailed fluorescent images on a deep black background.

Double labeling to show cell reproduction in a mouse brain 6 days after birth. The green marking alexa 488 (filter L5) is an antibody against BrdU to render the cell reproduction visible. The red marking alexa 594 (filter TX) is a neuronal marking. The colocalization shows that the neuronal precursors of the external granular layer of the mouse brain are reproducing. Dr. Ludovic Collin, University College London, together with Dr. Emiliana Borrelli IGBMC.



Research focus: atherothrombosis, id est the understanding the various mechanisms involved in thrombosis initiated by atherosclerotic plaques.
Dr. Jean-Etienne Fabre, the Imaging Centre of IGBMC and Cédric Vonesch.

Investment with a future

From the stand to the camera, every Leica MacroFluo™ system can be customized for any research, educational or documentation task. Particularly in this regard, the MacroFluo™ system is an investment that will pay off in the long run. Thanks to its modular design, you can take advantage of a complete range of accessories such as manual and motorized 6.3:1 and 16:1 zoom systems, planapochromatic objectives, high-performance transmitted-light stands, a motor focus system, digital cameras and analysis software. The following section introduces a typical range of accessories for the MacroFluo™.

Interface for digital knowledge exchange

The trinocular HCL tube contains a multiple-lens tube optical system to eliminate image faults. The observation and photo beam path can be switched to three positions. The optimal amount of light is thus available for every application: 50% for binocular observation, 100% for photography or observation through a single eyepiece. B and C mount adapters are available for a variety of camera types.

High-performance optics, camera and software – a perfect system

The frequently weak fluorescence signals place the highest demands on the digital camera and its control programs. Digital high-performance FireWire camera systems for scientific photography deliver image data suitable for analysis purposes – even of weak fluorescent and living objects. Intuitive, easy-to-learn frame grabbing and editing functions guarantee their convenience and versatile deployment. The Leica DC500 FireWire camera systems are compatible with PC and Mac, with Leica Image Manager image management software and Leica QWin or FW4000 analysis software. Thanks to their TWAIN interface, they can be integrated in common Windows programs (MS Office, Photoshop, etc.) with ease.

Focusing without effort

Frequently repeated, subtle motor tasks like focusing place high demands on the musculoskeletal system. The Leica motor focus system moves any equipment you may require effortlessly and with precision – via manual control, footswitch or computer. You can store specific focusing planes for your experiments with multifocus programs and quickly and precisely select them by pressing a button. The fine focus in the Leica Z6 APO A and Leica Z16 APO A zoom systems is motorized.

Stages for any purpose

Gliding stages ensure the necessary sensitivity when positioning specimens (see page 10 for image). Warmth is an important requirement for successful in-vivo examinations of temperature-sensitive living cells. The Leica MATS (microscope stage automatic thermocontrol system) thermocontrol system with its heatable stage made of optical glass ensures absolutely uniform temperatures over the entire stage surface, and reliably monitors and controls it. This temperature stability over long periods lets you perform time-lapse experiments with precision.

High-performance HL RC™ transmitted-light stand for excellent contrast

In the undyed state, living cells are nearly transparent and impose the highest demands on the imaging performance of stereomicroscope and transmitted illumination. The high-performance HL RC™ transmitted-light base is the ideal supplement to make these phase specimens visible with richness in contrast without artificial dyeing. The innovative Rottermann Contrast™ technology from Leica Microsystems delivers excellent images of transparent and semitransparent specimens in positive, inverted and dynamic relief contrast. This allows the new high-performance HL/RC™ transmitted-light base to offer ideal conditions for observation and documentation.

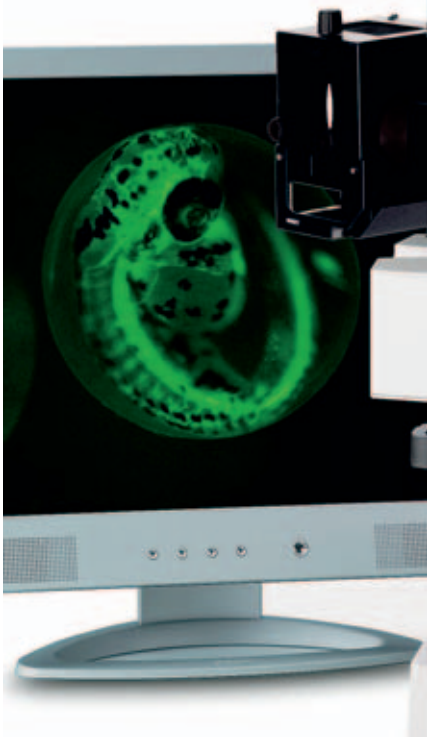
Specialists for digital image processing

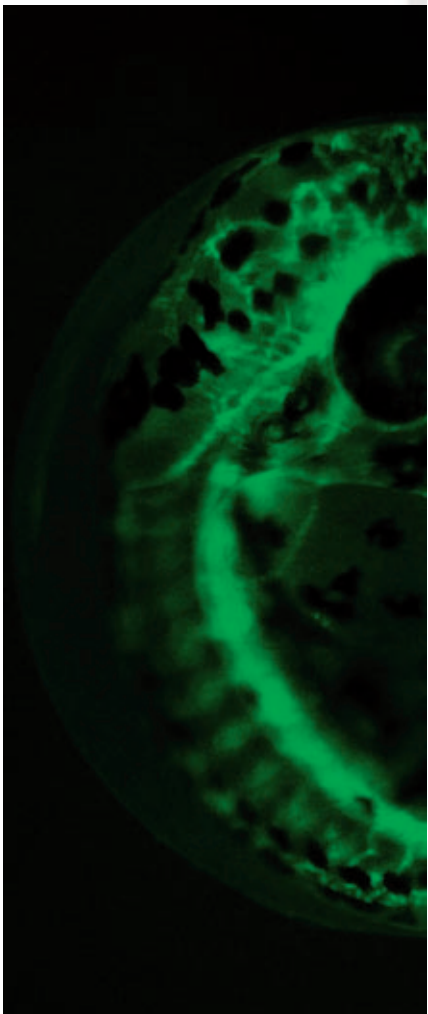
In fluorescence methods, selected object structures without primary fluorescence are treated with fluorescent stains and exposed to short-wavelength exciter light. The emitted fluorescence is frequently weak and places high demands on the performance of the documentation system as a whole. The innovative MacroFluo™ technology, high-resolution digital cameras and wide range of application software by Leica Microsystems is a perfect team for professional image capture, processing, archiving and subsequent analyses. Our program features a series of professional FireWire cameras for PC and Mac as well as image management and analysis software developed specifically for your fluorescence applications.

The Leica DFC350 FX monochrome FireWire camera system was developed especially for demanding imaging tasks in genetics, biotechnology and medicine. The Leica DFC350 FX captures vital cells, sequence of motions and quickly bleaching fluorescence specimens or particles – even at the lowest light intensities. The high sensitivity in the red and nearly infrared range guarantees reliable results, particularly for GFP and other low-light applications. The high sensitivity of the 2/3" Progressive Scan Interline Sensor permits quick photography of rapidly bleaching fluorescence specimens.

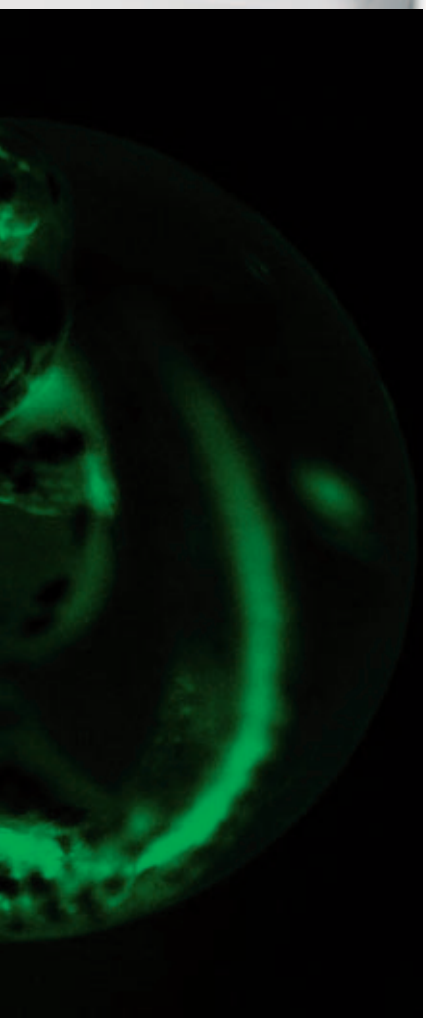
The Leica DFC300 FX color FireWire camera system satisfies the most stringent demands related to imaging in genetics, biotechnology and medicine. Thanks to the high sensitivity of the 2/3" Progressive Scan Interline Sensor, rapidly bleaching fluorescence specimens can be photographed quickly and the fluorescence excitation light can be significantly reduced to protect sensitive living cells. The active Peltier element cooling of the sensor elements guarantees noise-free images even at lowest light intensities.

The Leica DFC480 high-performance FireWire camera system furnishes images of highest resolution as well as color and detail fidelity – and is, therefore, best qualified for the highest demands in industry and science. Innovative data reading modes allow for the free selection of image transfer rates and scan methods. The quick readout of monochromatic images have made the high-resolution recording of fluorescence images a reality.





Pre-hatch zebrafish expressing flk1 vasculogenesis marker



The Leica DC500 12-megapixel high-performance FireWire camera system is the ultimate professional digital camera for analyses, measurements and further processing of quality image data. The Leica DC500 allows for unlimited use for all contrasting, bright light and dark light procedures in microscopy and particularly for extremely low-light specimens and weak fluorescence. Four selectable imaging modes/resolutions provide perfect true-color image quality for all applications.

High-quality data for analysis

Leica digital cameras are compatible with the Leica IM1000 image management system and image analysis programs such as Leica QWin and FW4000. The Image Overlay software module for Leica IM1000 provides optimum results for multiple fluorescence photography in cell biology, genetics, viviology (human and animal physiology), botany, and pharmacology. Ask your Leica representative about our software solutions for interactive image archiving, processing and analysis.

The modular Leica FW4000 fluorescence imaging system is a user-friendly solution for all microscopic fluorescence applications. Leica FW4000 offers various software options for imaging, processing, measurement and documentation of microscopic fluorescence images.

The professional Leica IM1000 Image Manager application satisfies the most stringent demands with regard to professional, customer-specific solutions for digital data management. Leica IM1000 is a modular image management package for image acquisition, processing, measurement and output as well as for exchange and storage of data. A broad selection of application modules is available for Leica IM1000, such as measuring, multifocus, image correlation, time-lapse, presentation, and many more. Image Overlay assigns the correct color to each received wavelength and merges up to 8 fluorescence images into a single composition.

"We're investigating the functions of genes using fish as our model system. Vertebrates are so similar genetically that there's a 90% to 95% probability that a gene that we identify in a fish will have the same function in humans." Dr. Stefan Schulte-Merker, Head of Research, Artemis Pharmaceuticals GmbH.

Leica MacroFluo™ – Features, accessory modules

Designation	Leica MacroFluo™ with 6.3 zoom: 1	Leica MacroFluo™ with 16: 1 zoom
Microscope type	Macroscope with vertical beam path, apochromatic zoom system, incident illuminator, fluorescence illumination coupled with the zoom optical system, and trinocular tube	
Zoom, manual or motorized	Apochromatic zoom 6.3 : 1 (zoom factor 0.57 – 3.6), lead-free Apochromatic zoom 16 : 1 (zoom factor 0.57 – 9.2), lead-free	
Tubes	FSA HC L 3TP trinocular tube, photo/TV port 100% : 0% / 50% : 50% / 0% : 100%, viewing angle 30° / HL L 2 TU trinocular tube	
Built-in iris diaphragm	For continuous adjustment of the depth of field Motorized iris diaphragm for Z6 APO A and Z16 APO A	
Fluorescence illuminator		
Type	LRF 4/22 coaxial incident illuminator and carrier for incident-light fluorescence and incident-light bright field with 5 position reflector disk, 0-pixel technology, centerable aperture and field diaphragm, blue filter BG 38, UV slot for light stop	
Fluorescence filters	A, + A4, D, E4, H3, I3, K3, + L5, M2, N2.1, + N3, + TX2, + Y3, + Y5, + Y7, + GFP, + YFP, + Red GFP, + CGFP and many more, filter combinations according to customer specifications	
Light source	106 z lamp housing with 100 W high-pressure mercury burner, chromatically corrected collector, focusable	
Safety measures	UV antiglare shield, UV light stop, stray-light protection for lamp housing	
Visual data	with Planapo 1× objective / 10× /25 eyepieces / FSA HC L 3TP trinocular tube (tube factor 1.25×)	
– Magnification	7.1× – 45×	7.1× – 115×
– Resolution	60 – 351 Lp/mm	51 – 336 Lp/mm
– Finest visible structure width	1.4 µm	1.49 µm
– Numerical aperture	0.02 – 0.117 nA	0.017 – 0.112 nA
– Object field ∅	5.6mm – 35mm	2.2mm – 35mm
– Depth of field	3.1mm – 0.08mm	3.8mm – 0.05mm
Visual data	with Planapo 5× objective / 10× /25 eyepieces / FSA HC L 3TP trinocular tube (tube factor 1.25×)	
– Magnification	36× – 225×	36× – 575×
– Resolution	300 – 1500 Lp/mm	255 – 1500 Lp/mm
– Finest visible structure width	0.33 µm	0.33 µm
– Numerical aperture	0.1 – 0.5 nA	0.09 – 0.5 nA
– Object field ∅	1.1 – 7mm	0.4 – 7mm
Data with Leica DFC300 FX digital camera / Planapo 1× objective / FSA HC L 3TP trinocular tube (tube factor 1.25×) / video objective 0.63×		
– Magnification chip: – object	0.45× – 2.8×	0.45× – 7.25×
– Digital resolution	17.4 – 110 Lp/mm	17.4 – 281 Lp/mm
– Object field projected onto chip	20mm × 14.9mm / 3.2mm × 2.4mm	20mm × 14.9mm / 1.24mm × 0.93mm
– Depth of field	1.41mm – 0.04mm	1.8mm – 0.03mm
Optical accessories		
Objectives	Planapochromatic 1× (97mm), 0.8× (112mm), 2× (39mm), 0.5× (187mm)	
Micro objective	Planapochromatic HR 5×/0.45, working distance 19mm	
Fine focusing	10-mm travel, 1 micron resolution, integrated and motorized for Z6 APO A and Z16 APO A	
Eyepieces for eyeglass wearers	Wide-field HC Plan 10×/22, 10×/25 eyepieces	
Imaging		
Image acquisition, storage and processing	Digital FireWire camera systems for fluorescence	
Software for image archiving, analysis and processing	Leica Image Manager with Image Overlay and Multifocus modules, FW4000, QWin, LAS	
Stands, illuminations		
Transmitted-light stands	High-performance HL RC base with innovative contrast methods, high-performance HL base, stands for bright field and bright/dark field	
Incident-light stand	with stage plate, black and white, ∅ 120mm	
Motor focus system	with 500mm column, control via handswitch, footswitch or PC	
Manual focusing drive	Coarse/fine focus, adjustable torque, with 500mm column	
Stages	Gliding stage, heating stage Leica MATS thermocontrol system, polarization	

Detailed technical specifications and data can be found in the brochure M1-416-5 and on our Homepage: **www.stereomicroscopy.com**

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MICROSYSTEMS

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