ApoTome
Suddenly Everything Looks Different

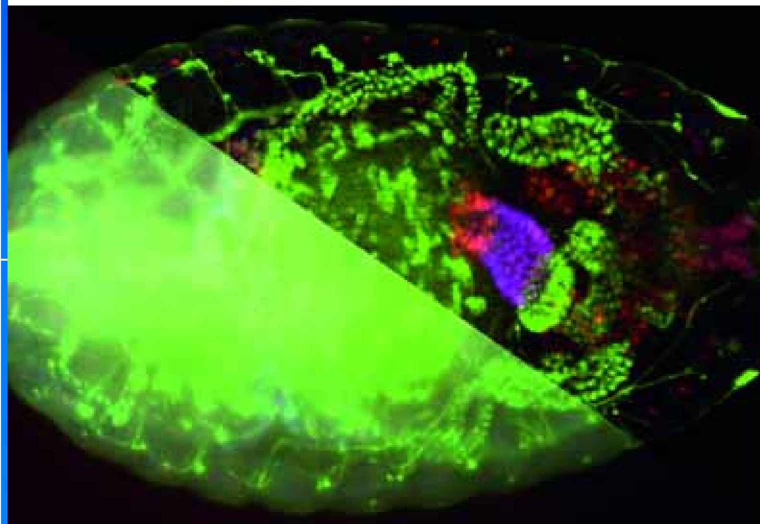
The standard for brilliant images
in 3D fluorescence imaging

We make it visible.

Carl Zeiss: FluoresScience

Fluorescence is the basis of a number of modern methods in the field of Life Sciences. Today, new and differentiated fluorescence applications are constantly being developed to enable us to monitor the molecular relationships inside cells. The demands on the corresponding microscope systems are increasing.

Their development is a task to which we at Carl Zeiss are devoting all of our commitment and know-how. After all, when you are working at the limits of visibility, only the very best tools count: those that offer optimum efficiency and the most innovative technologies, the most powerful imaging systems such as the ApoTome, which generates deblured optical sections of fluorescence samples. Our focus on the key method used for research into life has been given a name – Carl Zeiss: FluoresScience.



Triple fluorescence labeling of a drosophila embryo,
Bottom left: Conventional fluorescence image,
Top right: Optical section using ApoTome,
Frank Josten and Michael Hoch, University of Bonn, Germany

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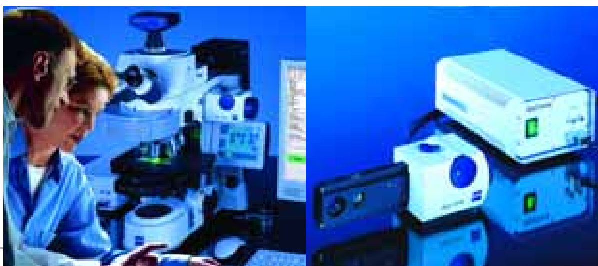
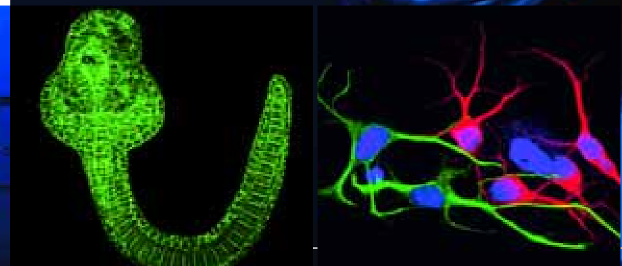
The Quality Factor: Where the Exceptional is Standard

Innovation in 3D imaging is here: ApoTome. With the launch of this product, Carl Zeiss triggered a minor revolution in fluorescence microscopy and since then everything has looked different. ApoTome has now been established as a standard method in advanced biological research in the field of life sciences. Since the introduction of ApoTome, the problems associated with stray light from other focal planes have become a concern of the past. Carl Zeiss now offers outstanding technology for the generation of optical sections in 2D and 3D fluorescence imaging. Exceptional performance has become the standard.

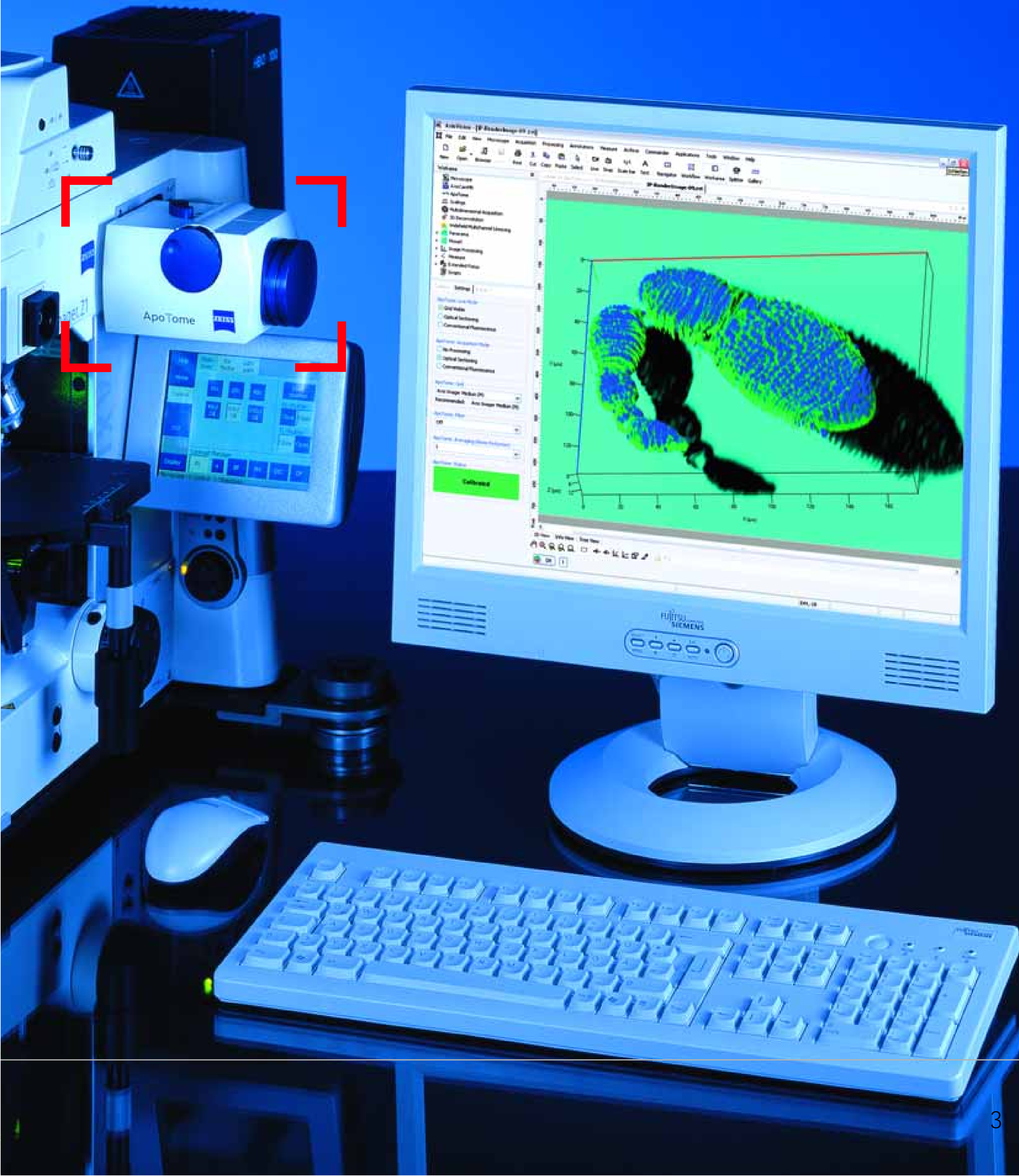
- Exceptional contrast, image quality and resolution – with an optical section thickness of one Rayleigh unit
- Exceptional ease of operation – extremely simple and flexible
- Exceptional seamless integration – easy to implement in existing Carl Zeiss system solutions

Carl Zeiss has raised the level of performance once again. With ApoTome for Axio Imager, the new generation of light microscopes has set a new standard in 3D imaging. Optical sections even in complex applications. For fascinating images, brilliant contrast and undreamt-of possibilities for scientific analysis.



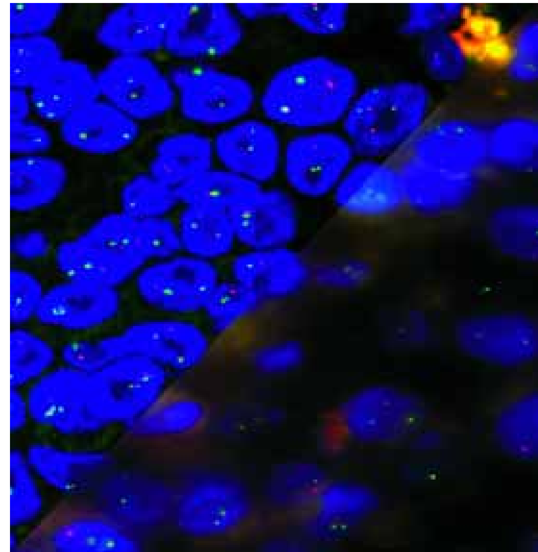


ApoTome



The Performance Factor
One Module – Numerous Advantages

ApoTome is more than just the sum of its advantages. Advantages that include brilliant image quality in classical fluorescence microscopy, performance and speed, and an impressive spectrum of fluorescence dyes. Together with ease of operation and practice-driven design, all these benefits add up to a clear leap forward in performance of your digital imaging workstation.



Multiple myelom, triple fluorescence, C-Apochromat 63x/1.. Peter Hutzler and Kar Bink, GSF Neuherberg Germany

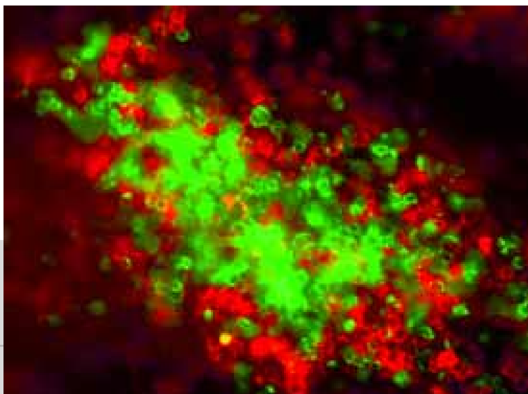
1A: Image quality

One Rayleigh unit optical section depth – this value represents high optical resolution with an excellent signal-to-noise ratio. In the z-direction, visible resolution is increased in comparison to conventional fluorescence microscopy by a factor of 2. As a result optical sections and 3D image reconstruction can be obtained from the sample. This advantage is particularly crucial for thick specimens where it is also necessary to achieve optimal resolution and contrast in the xy plane. The optimized algorithm for precise, artifact-free images is yet another benefit. In a nutshell, you get maximum contrast, excellent image quality, deblurred image planes – and, consequently, more visible information for your analysis, documentation and publications.

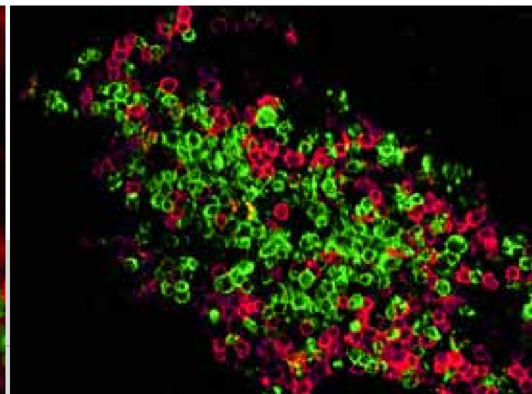
Optimized: The speed

Three raw images are acquired for each optical section. The results are calculated online, taking less than 80 msec – depending on the size of the image used with the resulting image immediately displayed. Multichannel fluorescence and Z-stack images are controlled automatically. Using the Axio Imager, acquisition is up to 30% faster; utilizing a reflector revolver speed of 250 msec and optimized electronics.

Conventional



ApoTome mode



Mouse omentum (peritoneal reflection), double fluorescence, Plan-Apochromat 20x/0.75, Mike Tighe, Kim Kusser and Troy Randall, Trudeau Institute, New York, USA

ApoTome

Position 1: Iris diaphragm

Included: The iris diaphragm

Designed as a slider for the field diaphragm plane of the reflected light beam path, ApoTome, with its two positions, offers the essential prerequisites for sophisticated digital imaging – without time-consuming exchange of the slider. Position 1: the iris diaphragm – crucial in the Köhler beam path for image quality and for presetting the system. Position 2: ApoTome – the revolutionary technique for optical sections and brilliant image quality.

Position 2: ApoTome mode

No limits: The fluorescence dyes

Yet another significant advantage: using the ApoTome, you have almost unlimited freedom in your choice of fluorochromes. So whether you decide to work with DAPI, FITC, Rhodamine or with living dyes such as GFP or YFP – it's up to you.

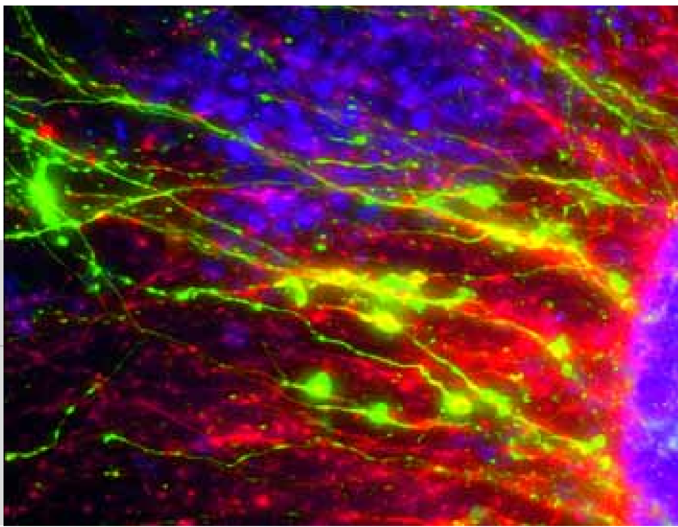
Plug and go: Ease of operation

Simply slide ApoTome into the field diaphragm plane of the reflected light beam path of your microscope. It's just as easy to use as it is to insert. Set ApoTome to the appropriate position – iris diaphragm or ApoTome mode, depending on what your application demands.

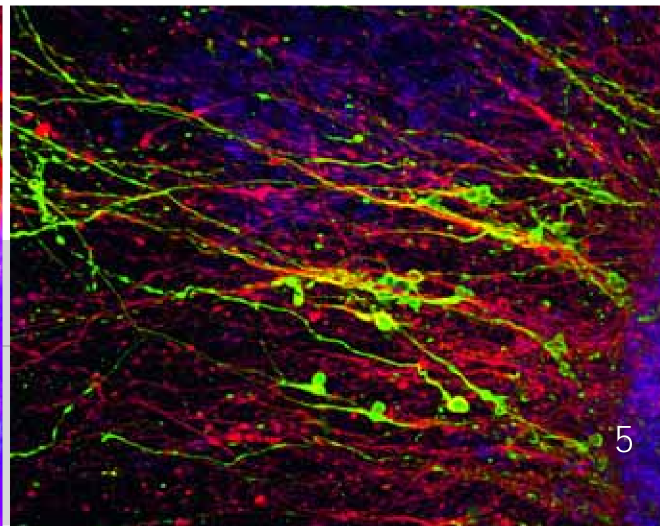
Reliable: Safe and secure

ApoTome for Axio Imager can be locked in the reflected light beam path, ensuring that your individual application is protected against unintended access. Additionally, potential vibrations in the stand that could impair the quality of the image are virtually eliminated and the grid is precisely aligned in the light path. After calibration of the system, the grids in the ApoTome slider are focused in the specimen plane depending on the fluorescence wavelength used. During this procedure, the grids remain permanently inside the slider for maximum handling safety.

Conventional



ApoTome mode



Axons of a dorsal root ganglia explant, triple fluorescence, Plan-Apochromat 20x/0.75

The Image Quality Factor: The Visible Difference

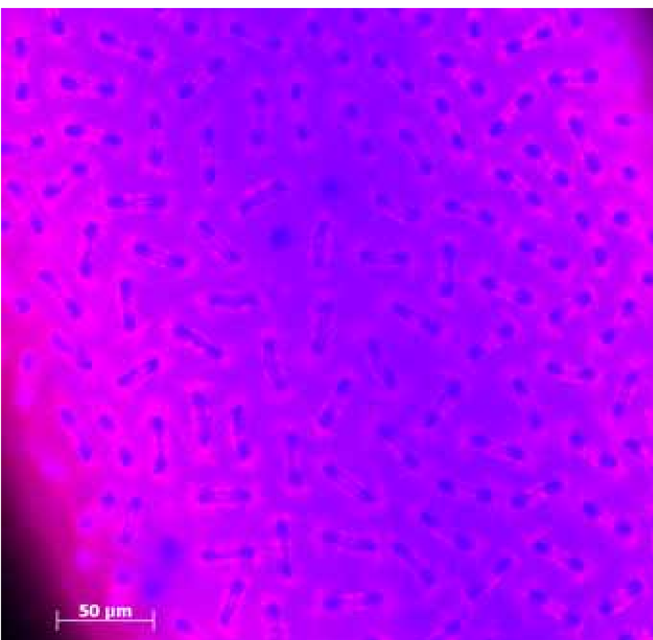
Experience the difference: ApoTome brings fluorescence imaging to a new level of performance – with excellent image quality, fascinating images and new perspectives for science and research.

Decoding gene functions

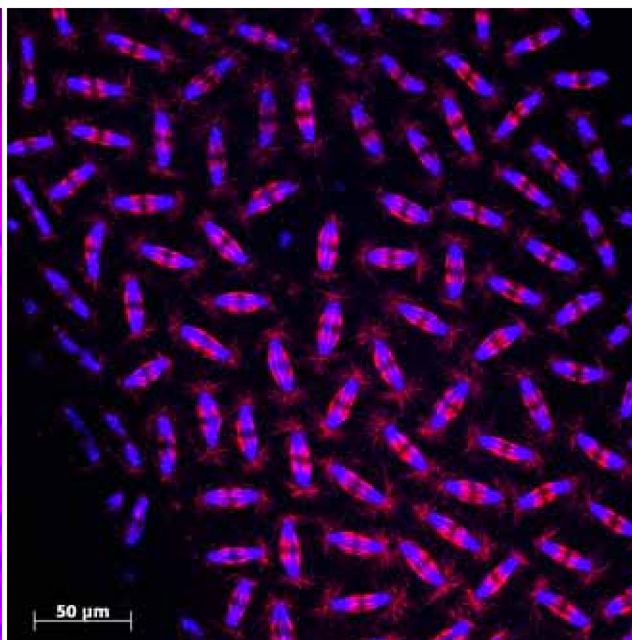
In Drosophila research, wild type and mutant embryos are frequently compared with each other in various stages of development. In the case of the mutants, genes playing a specific role in developing or maintaining bodily functions have been manipulated. The comparative study allows conclusions to be drawn about gene functions in certain developmental processes. The images below show the development of Drosophila melanogaster embryos in a relatively early stage following fertilization. All the cells are in the same cycle of division. In the ApoTome mode, the spindle microtubules (red), which separate the chromosomes (blue), can be clearly identified.

Research into neuronal connections

Studies in developmental neurobiology include the examination of how nerve cells developing in a certain part of the body connect with cells in a completely different part of the body. The connection is formed through long cell processes, known as axons, which, by exploring their environment, e.g. along a chemical gradient, find the way through the tissue to their target cells. This

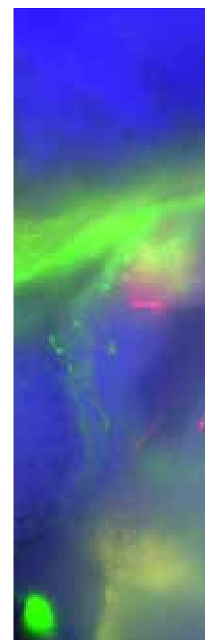


Conventional

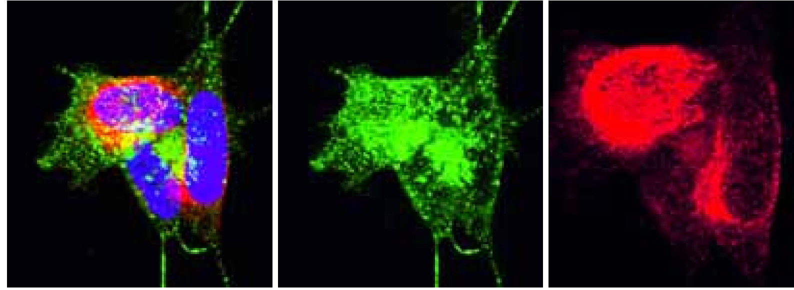


ApoTome mode

Drosophila melanogaster embryo, double fluorescence, EC Plan-Neofluar 40x/1.3, George Scaria, University of Illinois, Chicago Medical School, USA



Conventional



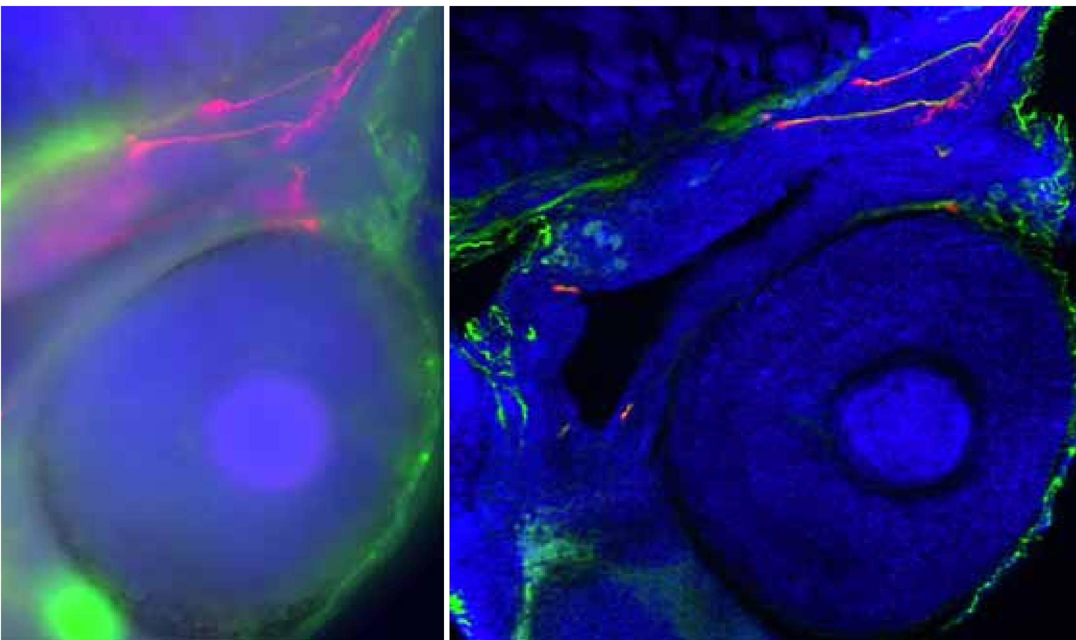
process takes place primarily during the embryonic development of an organism. In order to decode these highly specific and extremely complex mechanisms, zebrafish research also uses mutants. These are compared with wild type individuals to identify the genes that are crucial for this connection. These findings have a major significance for clinical questions.

This example shows the head area of a zebrafish embryo (Danio rerio). In the ApoTome mode, two different populations of neurons can be clearly distinguished by the red and green dye. The nuclei are labeled using DAPI.

New discoveries in neurobiology

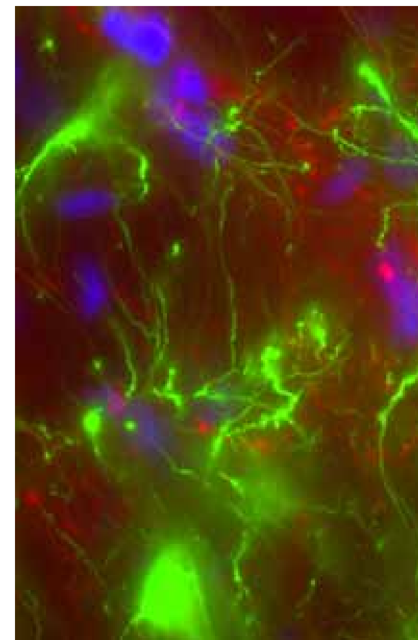
New research results in neurobiology are disproving the theory that all nerve cells are born during the embryonic phase and new cells can neither be born nor connected after this phase. In animals, it is possible to show that new neurons can also be formed in the hippocampus of the adult rat. Such research results are relevant for clinical issues such as depression, Parkinson's disease etc.

Two cell types can be identified in the two images: neurons (red) and neuroglia cells (green) which are important for maintaining the physiological processes in the brain. New-born cells in the hippocampus have been marked specifically (red fluorescence).



ApoTome mode

Zebrafish (Danio rerio),
triple fluorescence,
Plan-Apochromat 20x/0.75,
Monika Marx and Martin Bastmeyer, University of Karlsruhe, Germany



Conventional

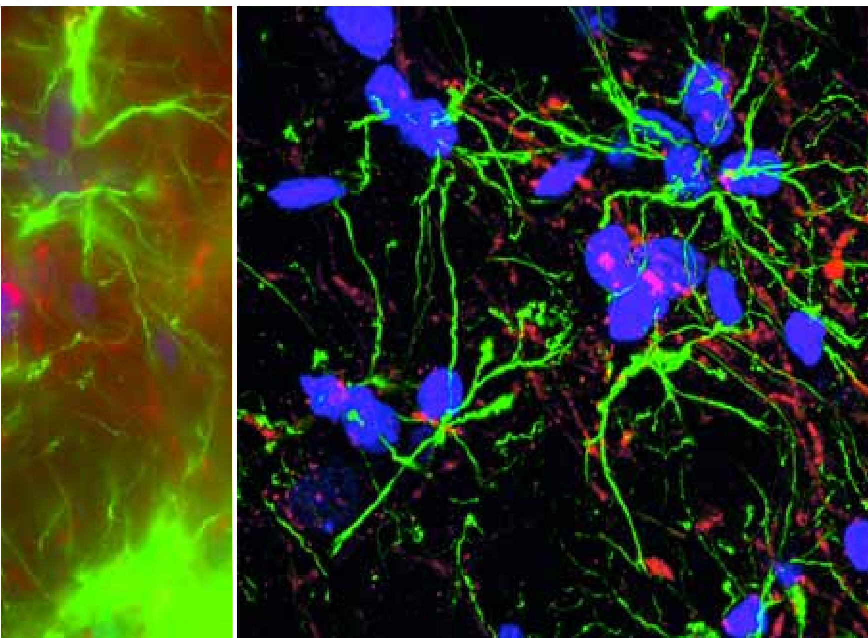
Hippocampus of a rat,
triple fluorescence, maximum projection of a 3D image stack,
Plan-Apochromat 63x/1.4,
Eberhard Fuchs and Susanne Bauch, DPZ Göttingen, Germany

Neuronal interactions in 3D reconstruction

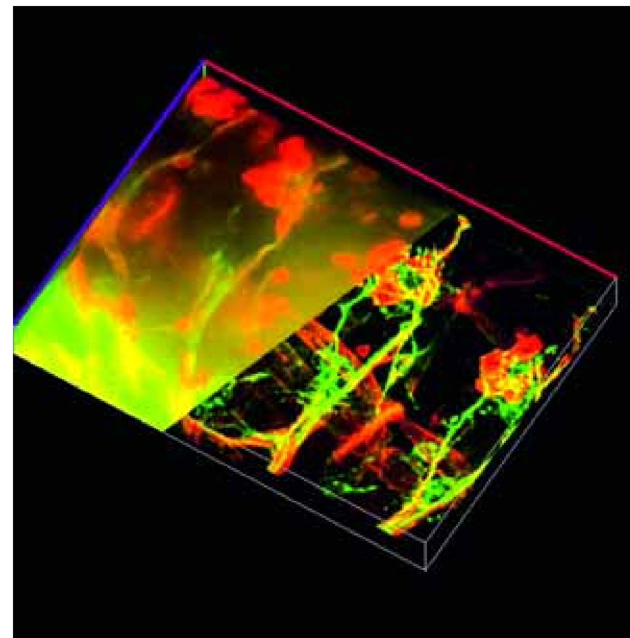
Every nerve tissue, such as the brain or spinal cord, has a three-dimensional structure. The neuronal connection process and nerve and neuroglia cell interactions also always take place in 3D. The only possible way to reconstruct and analyze these structures in research is to generate optical sections – just as ApoTome allows you to do.

The specimen is a Drosophila melanogaster embryo. A 3D image stack, which has been reconstructed using the Inside4D AxioVision software module, is shown. The nerve cells (green) and neuroglia cells (red) have been specifically marked using a fluorescence dye.

Further applications, images and additional movies can be found under:
www.zeiss.de/apotome



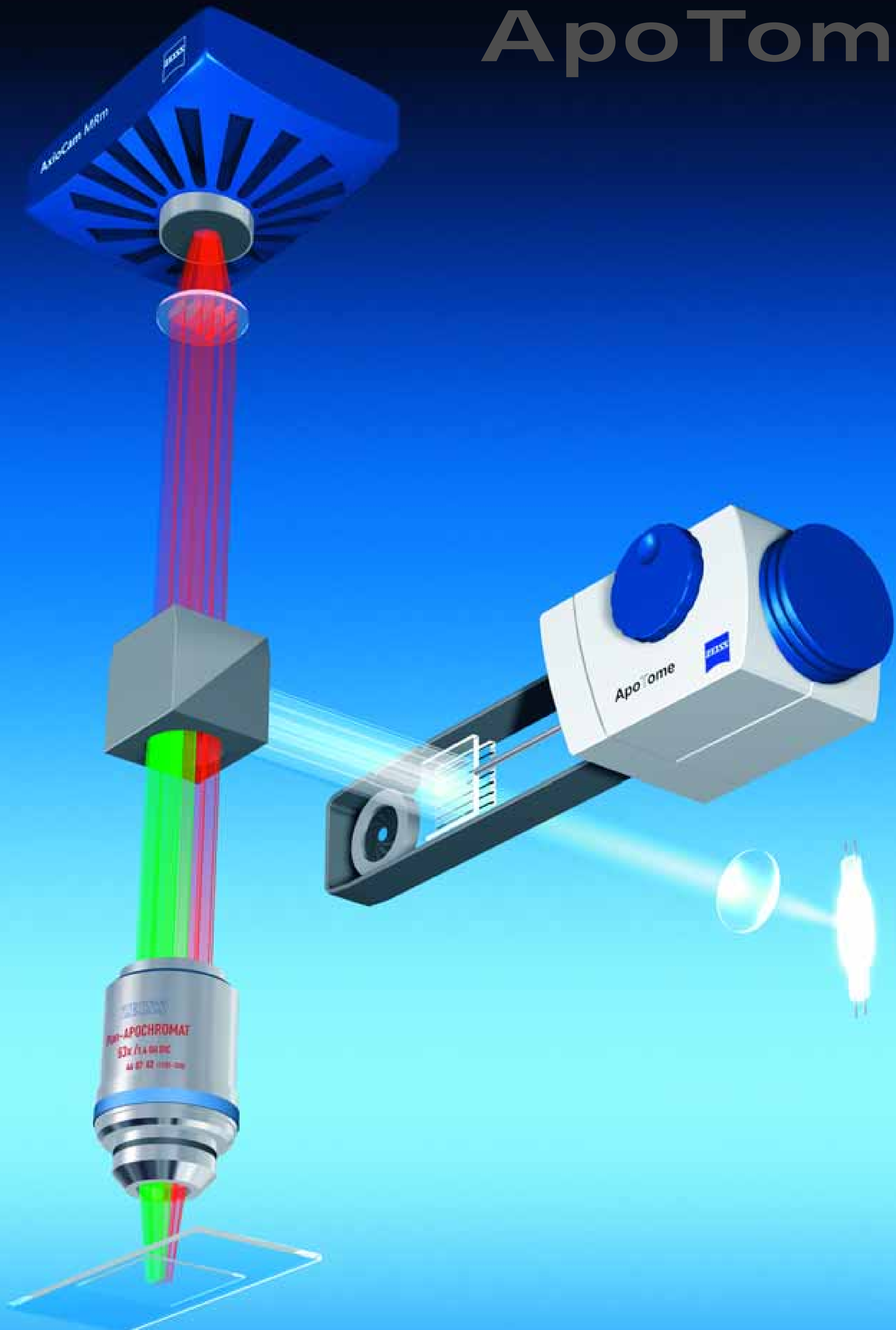
ApoTome mode



3D image stack

Drosophila melanogaster embryo,
double fluorescence,
Plan-Neofluar 40x/0.75,
Christian Klämbt, University of Münster, Germany

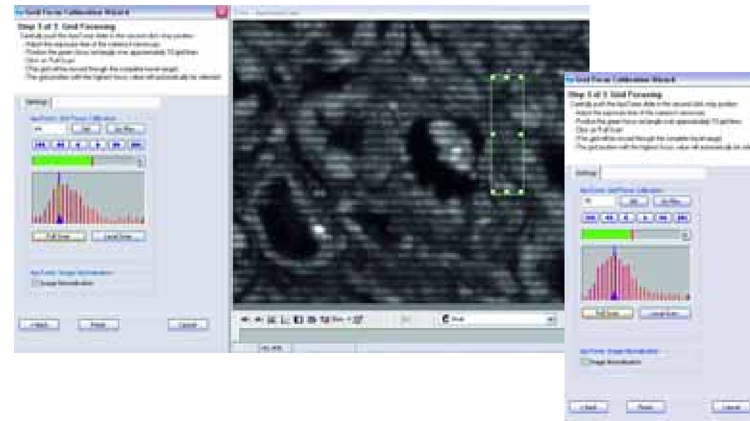
ApoTome



The beam path:
Fluorescence excitation light passes through two glass plates in the ApoTome slider. The grid pattern found on the first glass plate is "imprinted" in the excitation light. The second glass plate is tilted by a scanning mechanism, thereby slightly moving the beam path with the imprinted grid pattern up and down. The excitation light (green) with the grid pattern is then projected onto the specimen via a conventional fluorescence filter set. The emission light (red) is gathered by the objective and imaged to the camera sensor.

The Function Factor: Recognized for its Excellence

The realization of structured illumination by Carl Zeiss for the first time provides artifact free images and received numerous awards from experts in the field. And now with its integration to Axio Imager its performance is again elevated.

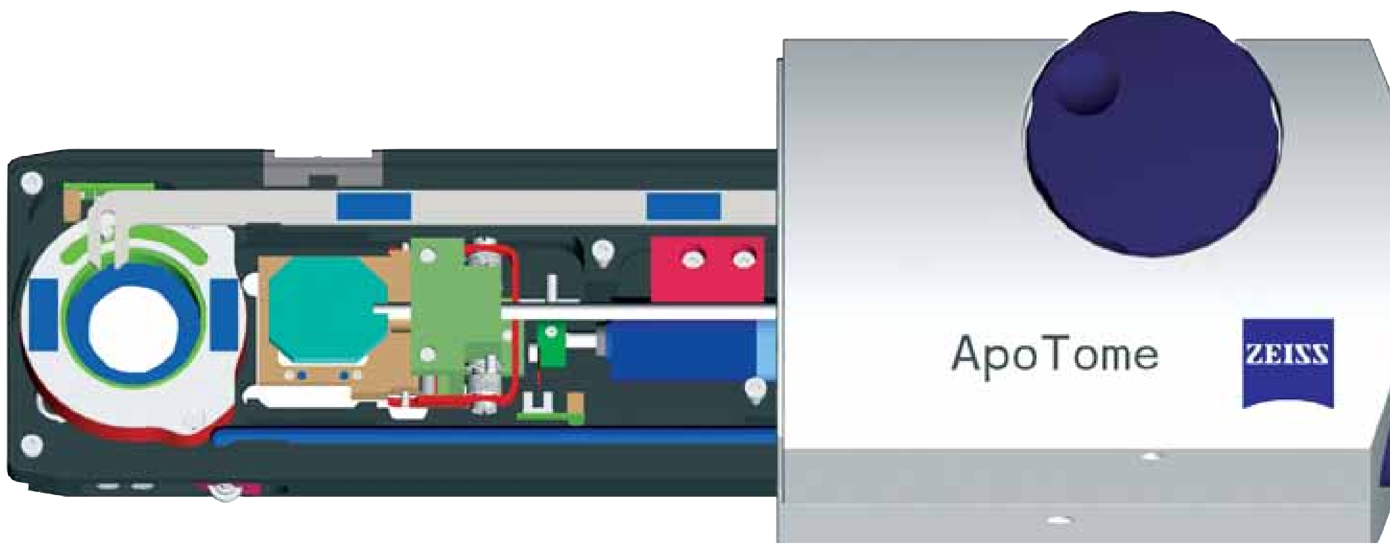


ApoTome: The grid pattern

With the help of ApoTome, image blurring is virtually eliminated. In the ApoTome mode, a grid is inserted into the field diaphragm plane of the reflected light beam path (p. 11, fig. 2A). The grid pattern is sharply projected into the object plane – and is clearly visible through the eyepiece and in the live image. In a second step, a high-precision scanning mechanism moves the grid pattern in defined steps in the sample plane. In the third step, images are acquired at each grid position (figs. 2A-C). Finally, a single resulting image is calculated using a fast mathematical algorithm (fig. 2D). The result: a precise optical section through the specimen. Thanks to this technique, it is possible to acquire information from a z-plane of the sample and display it with enhanced resolution and increased contrast – with no blurring and perfect for 3D reconstruction.

Precisely in focus: The calibration

A fundamental challenge in generating precise, deblurred optical sections using fringe projection is to focus the grid depending on the objective and fluorescence wavelength being used. A suitable grid is available for every magnification range. Using ApoTome, perfect calibration is executed with an AxioVision Wizard function. This assures full system optimization and unprecedented image quality. And with the apochromatic beam path of the Axio Imager, calibration is even easier since repositioning of the grid for multi wavelength imaging in the visible range is almost neglectable.



Background

In conventional fluorescence microscopy, the image always consists of the signal coming from the focused object plane and the structures found above and below it. The blurred structures are either perceived as being out of focus or – if you are clearly outside the focal plane – have the effect of brightening the image background, which reduces the contrast of the image.

The fluorescence behavior of spatially extended fine structures is usually examined with the help of small fluorescent beads which have a diameter of less than 0.5 µm. Through the acquisition of such a bead in a Z-stack, it is possible to visualize what is known as the point-spread function (PSF) of the optical system (fig. 1).

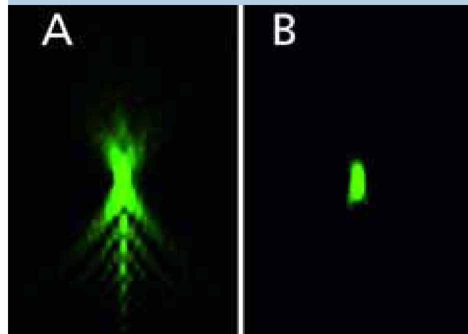


Fig. 1: Section in xz direction through the PSF (Plan-Apochromat 63x/1.4)
A: PSF conventional fluorescence
B: PSF structured illumination with ApoTome

The "structured illumination" principle

For the "structured illumination" process used in ApoTome, a grid is inserted into the field diaphragm plane of the fluorescence beam path. Superimposing the projected grid over the fluorescent sample generates an image of the specimen that has dark fringes running through it (fig. 2A). The projected grid is visible in the focal plane, as the grid covers parts of the sample in the form of fringes no fluorescence is excited at these points.

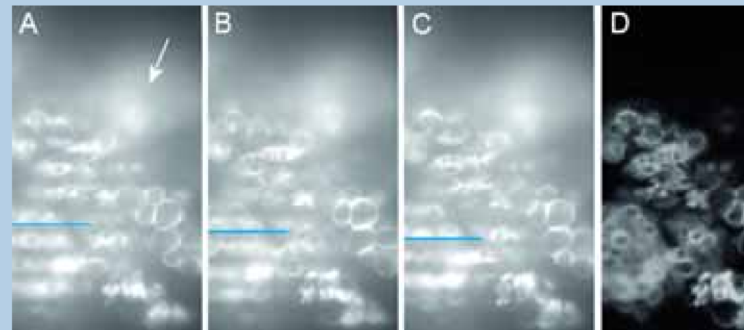


Fig. 2: Schematic representation of the grid projection in a fluorescence sample
A-C: Raw images with the grid in various positions
D: Optical section through the sample

If parts of the projected grid fall on specimen structures that are out of focus, the image of the grid can no longer be seen at these points (fig. 2A, arrow). This provides a criterion which makes it possible, using image analysis, to determine which areas of a camera image contain object structures that are located within the focus of the objective and which areas contain signals from the area that is out of focus. This can be achieved simply by determining the grid contrast depending on the location (xy) and can be evaluated pixel by pixel and combined to form an optical section through the specimen (fig. 2D and 1B).

In order to reconstruct all the image information, at least three raw images must be acquired with the grid projected in different positions (fig. 2 A-C).

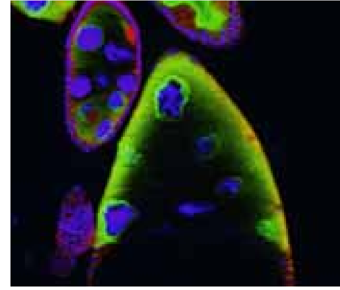
Optical section thickness* d for the Axio Imager (480 nm excitation wavelength):

Objective	Grid (Lines/mm)	d (µm)
20x/0.75	15.5	1.5
40x/0.75	7.5	1.6
40x/1.3	12.0	0.8
63x/1.4	7.5	0.7

*In accordance with the definition of the Rayleigh unit (distance from maximum to first minimum of the PSF)

Literature

Schäfer, L. et al.: Structured Illumination Microscopy: Artifact analysis and reduction utilizing a parameter optimization approach. J. Microsc. 216 (2004) pp.165



The System Factor: Where Every Detail Counts

Carl Zeiss offers a broad range of system components which all have one thing in common: when used in combination with ApoTome, they fully utilize all of the possibilities of fluorescence imaging.
All system components are coordinated with each other perfectly i.e. research microscopes using fluorescence technology, recognized as the very best, high resolution or high speed digital cameras, image acquisition and subsequent image processing using the AxioVision software for 2D and 3D.

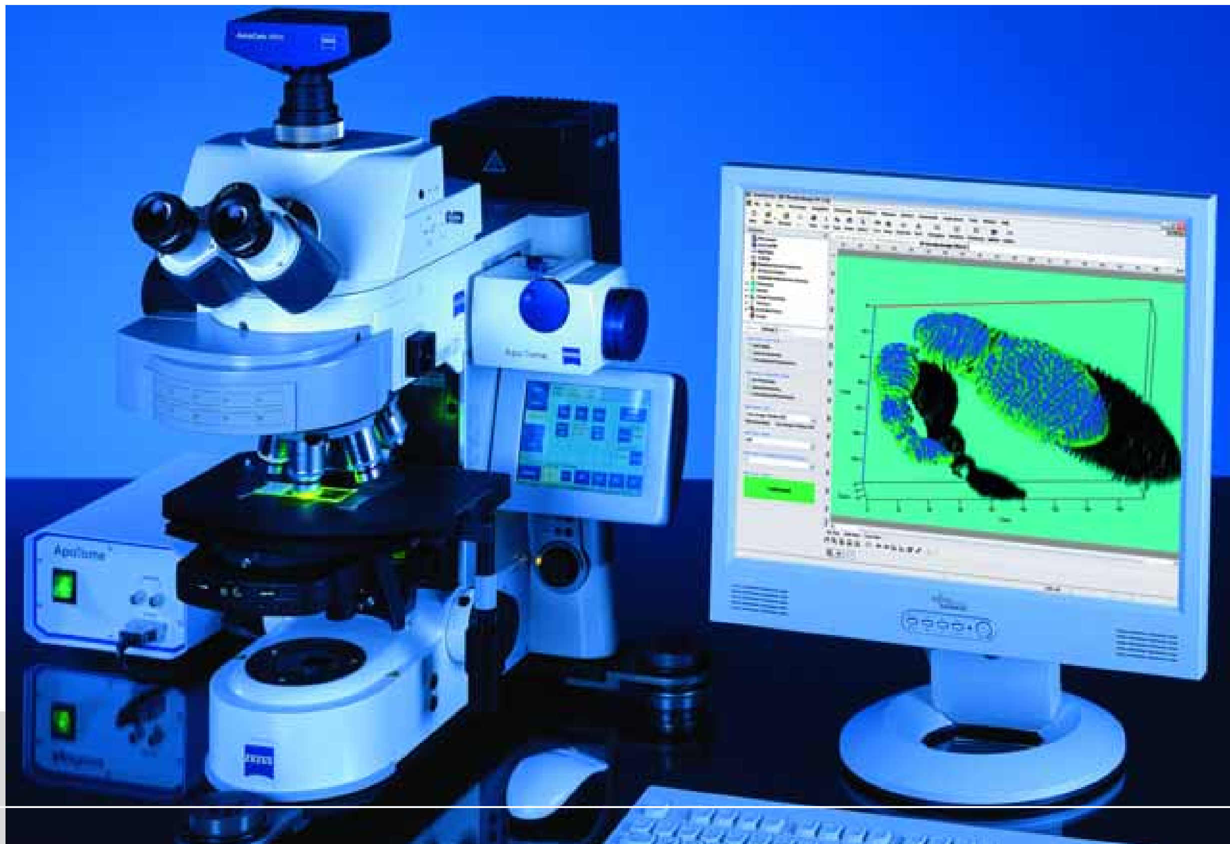
Optimized for fluorescence: Axiovert 200 and Axio Imager

The Axiovert 200 has proven its worth in fluorescence imaging.

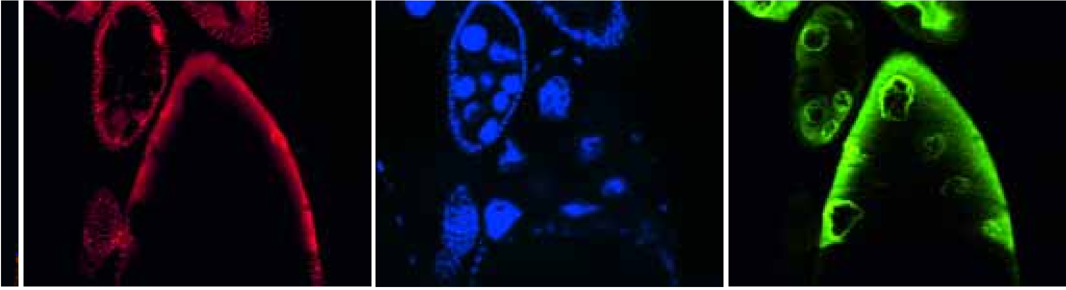
- Outstanding optics with optimized fluorescence
- Fully motorized microscope for high-end research
- Excellent stability
- Z-focus with a resolution of 25 nm
- Outstanding ergonomics for comfortable microscope use

The Axio Imager used in connection with ApoTome is the optimum platform for 3D imaging in particular.

- Stable Cell design: Maximum focus stability
- Z-drive with a resolution of 10 nm and typical accuracy of +/- 20 nm for maximum reproducibility and exact focus positions
- 30% more speed for multidimensional images through the newly developed electronics
- TFT display for optimum ease of operation



Axio Imager system with ApoTome, AxioCam and AxioVision



- Apochromatic fluorescence beam path ensuring brilliant image quality for multichannel images
- 10x reflector revolver for up to 10 different fluorescence filter sets

Powerful even in weak fluorescence: The cameras

AxioCam: the leading generation of Peltier-cooled digital cameras from Carl Zeiss. These cameras feature increased sensitivity, high 14 and 12 bit dynamics as well as maximum resolution and image quality. All these cameras are available in a monochrome version and have been carefully and rigorously tailored to your applications in fluorescence microscopy.

Efficient in research and routine applications: The microscope control

Using the Carl Zeiss AxioVision software platform, you are able to control all motorized microscope systems from Carl Zeiss interactively or fully automatically. The advantage: simple, quick operation and clarity of the user interface. The microscope's setting parameters can be saved as required and called up again at any time. AxioVision determines magnifications automatically and complex acquisition modes are easy to configure. In this way your results can be reliably reproduced.

Axiovert 200 system with ApoTome, AxioCam and AxioVision

The Intelligence Factor: New Perspectives for Life Sciences

ApoTome and AxioVision – a perfect combination. AxioVision is the software platform from Carl Zeiss for all requirements in fluorescence imaging – from two-dimensional imaging up to the sixth dimension. Any combination of x, y, z, lambda, time-lapse and different sample positions is possible for automatic image acquisition. And, by implementing ApoTome in AxioVision, optical sections are available in every mode.

Multichannel fluorescence

Using this module, in connection with ApoTome, you can acquire multichannel images automatically with high contrast and optimal quality. One channel is acquired using the optimum exposure time for each excitation wavelength. The grid is focused automatically for each fluorescence wavelength. Every channel of the optical section is calculated online. The advantage: you see the result immediately, for example, colocalization of fluorescence dyes in thick specimens.

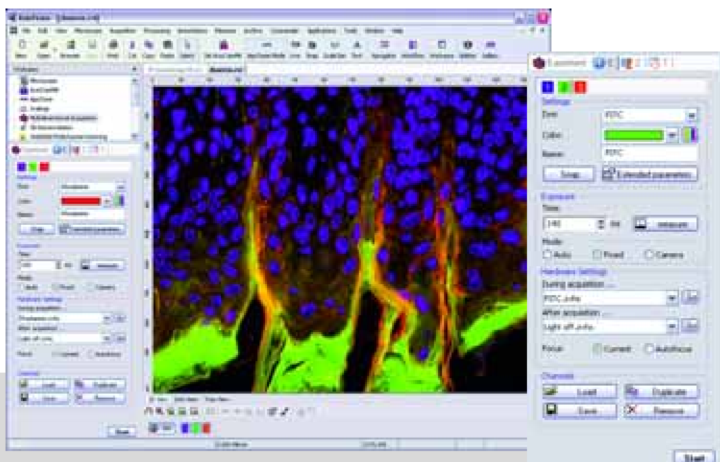
Z-stack

Using this module you can acquire and display different focal planes of a specimen as an image stack – with high precision and accurate reproduction. ApoTome controls the acquisition of the three raw images. The optical section is calculated online in every plane. This makes it possible for you to see the result quickly.

Time lapse

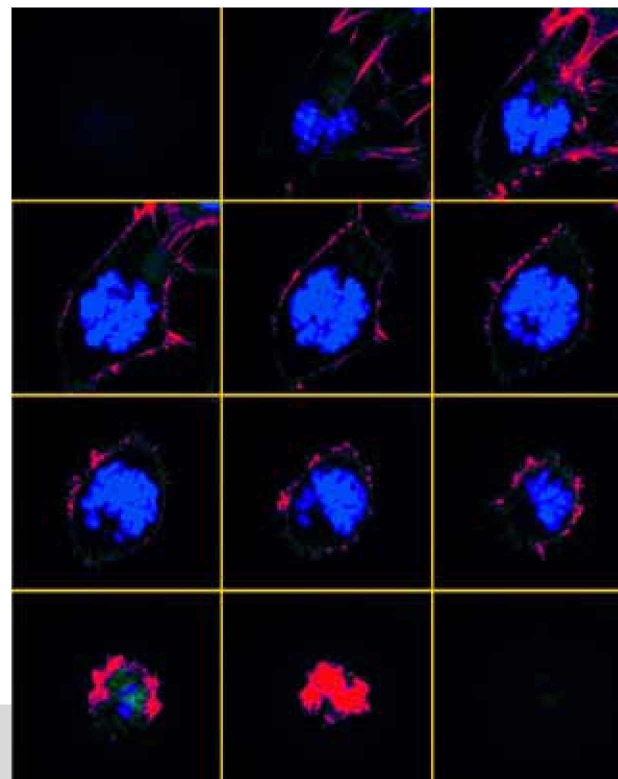
Observing specimens, studying and recording changes over time, documenting the results clearly – the time lapse module enables you to control acquisition and the microscope with precision.

Multichannel fluorescence



User interface for multichannel images

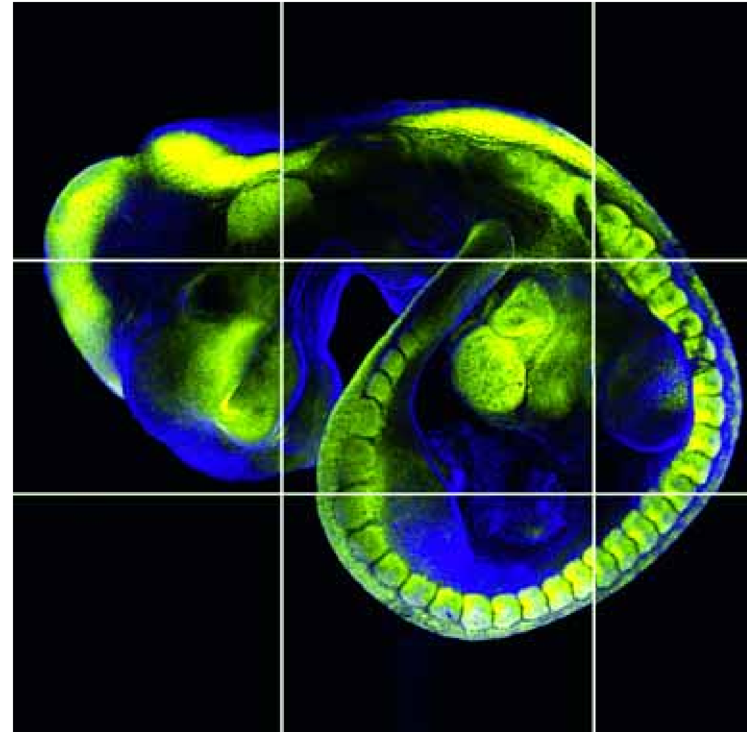
Z-stack



Gallery view of a Z-stack

Muscle-specific GFP labeling (yellow), nuclear labeling (blue), developmental stage of 10.5 days,
2-channel fluorescence Z-stack image in ApoTome mode,
Plan-Neofluar 5x/0.15,
Dr. Frederic Relaix and Emanuelle Perret, PFID, Pasteur Institute, Paris, France

MosaiX



MosaiX

Using this module and a motorized xy stage, even extremely large, extended specimens can be displayed with high resolution and rich contrast. The specimen is scanned extensively in order to generate a large composite image. This electronically generated image of the specimen serves as a map for navigating around the sample, and can be used as a basis for further analysis.

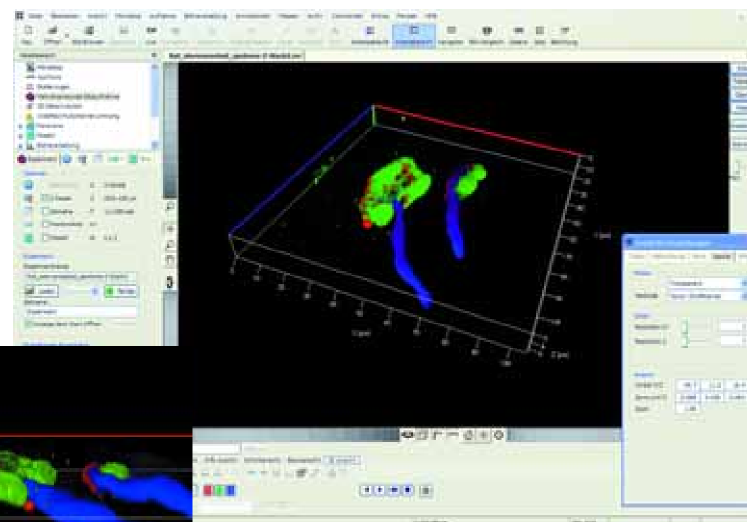
Inside4D

In combination with ApoTome, Inside4D makes multidimensional visualization and presentation of cells and tissue sections exceptionally easy. ApoTome generates the optical sections online. You can then visualize the data within Inside4D immediately. 3D rendering can be performed at any angle. Inside4D is ideal for comparing the original and processed image. Using this module you will be able to detect structural details in the sample. In addition, you have an excellent tool for generating movies – for the best possible presentation of your 3D images on the internet, using Powerpoint™, and much more.

Combinations

ApoTome, multichannel fluorescence, Z-stacks, time lapse – all these modules can be freely combined with each other and form system solutions, which meet the widest range of requirements – to the letter. The result: cost-effective adaptation of the solution to suit your applications without having to make unnecessary investments.

Inside4D



Neuromuscular synapses in the sternomastoid muscle of a rat,
3-channel fluorescence Z-stack,
Dr. Le Tian, Dr. Wes Thompson, University of Texas, Austin, USA

3D Imaging Solutions from Carl Zeiss

No matter how complex your application in 3D imaging is – Carl Zeiss has the solution. ApoTome has enhanced Carl Zeiss' 3D imaging solutions to include an excellent method for generating optical sections.

1. LSM 5 product family

- Confocal laser scanning microscopes
- Light from the focal plane is selected via a pinhole diaphragm
- Precise control of specimen illumination, e.g. for FRAP and uncaging experiments
- LSM 5 product family for spectral analysis solutions (separating overlapping fluorescence dyes), 2-photon microscopy and the acquisition of rapid processes

2. ApoTome

- Combination of grid projection system and mathematical approach to displaying an optical section through the specimen
- Can be used in conventional fluorescence imaging
- Enhanced image quality

ApoTome for Axio Imager

- Possibility of locking the slider in the beam path
- Fast acquisition speed
- Available as a system solution and also as an upgrade for Axiovert 200, Axio Imager.D1 and Axio Imager.Z1
- Available as an upgrade for Axioplan 2 imaging*

	AxioVision 3D Deconvolution	ApoTome	LSM 5 product family
Technical features			
Light source	HBO dye excitation in range from approx. 340 – 700 nm	HBO dye excitation in range from approx. 340 – 700 nm	Laser excitation lines LSM 5 PASCAL: 405, 458, 488, 514, 543, 633 nm; LSM 510: 351 nm - NIR
Image format during acquisition	Always 3D	2D or 3D	From spot to 3D
Thickness of optical section	Cannot be determined	Determined by variable grid patterns used	Adaptable to specimen requirements (signal intensity, necessary resolution) by adjustable pinhole
Exposure ROI	no	no	yes
Image quality	++	++	+++
Multichannel image acquisition	Sequential	Sequential	Sequential or parallel in up to 8 channels
Digital zoom and image rotation	no	no	yes
Online optical sectioning	––	+	++
Applications			
Single dye of fixed specimens	++	++	+++
Multiple dyes of fixed specimens	++	++	+++
2D optical sections of living specimens	–	+ slow processes (e.g. cell migration, division)	++ slow to extremely fast processes by adapting the image geometry or line scan
3D reconstruction of living specimens	+	–	+++
Local exposure for FRAP, uncaging	–	–	+++

High resolution microscopy camera AxioCam MRm Rev. 2
000000-0445-554

Camera adapter T2-C 1.0x
426104-0000-000

Adapter 60N-T2 1.0x
426103-0000-000

Control box ApoTome
included in 423660-0000-000

Axio Imager.Z1 or Axio Imager.D1

Anti-vibration plate Axio Imager
000000-1311-079

Transmission grid H1 ApoTome
423662-0000-000
Transmission grid M ApoTome
423663-0000-000
Transmission grid L1 ApoTome
423664-0000-000

Slider system ApoTome for Axio Imager
423660-0000-000

High resolution microscopy camera AxioCam MRm Rev. 2
000000-0445-554

Camera adapter 60 C 1" 1.0x
456105-9901-000

Control box ApoTome
included in 000000-1189-776

Axiovert 200

Anti-vibration plate Axiovert 200
000000-1209-889

Transmission grid VL ApoTome
000000-1151-004
Transmission grid VH ApoTome
000000-1151-002

Slider system ApoTome for Axiovert 200
000000-1189-776

Systems	ApoTome	
	New	Can be upgraded
Axio Imager	x	x
Axiovert 200	x	x
Axioplan 2 imaging*		x

3. AxioVision 3D Deconvolution

- Mathematical method for processing and improving the quality of image stacks
- Prerequisite is the acquisition of a 3D stack
- On the basis of the point-spread function, the light above and below the focal plane is calculated back to its plane of origin

The Clarity Factor: Where Advantages Add up to Power

The name	ApoTome – innovation in 3D imaging
The module	Slider technology for the Carl Zeiss Axiovert 200, Axio Imager.D1, Axio Imager.Z1 and Axioplan 2 imaging* microscopes
The performance	New image quality and enhanced contrast in conventional fluorescence microscopy Deblurred 2D planes, even with thick specimens
The price	Affordable leap forward into an exceptional category of digital imaging
The principle	Sophisticated technique for artifact-free structured illumination
The positions	Proven 2-in-1 slider for conventional fluorescence (position 1) and the ApoTome mode (position 2) for demanding digital imaging
The handling	Quickly integrated Easy to use
The fluorescence	Flexible for the entire spectrum of potential fluorochromes
The system	Perfectly matched, high-performance components: ApoTome – microscope – camera – software – PC
The versions	Matches your budget, either as a complete system or an upgrade for your existing system

*All Axioplan 2 imaging microscopes delivered after February 2000. Serial numbers: from 35 11 000001; from 35 10 000001; from 35 02 000001.

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