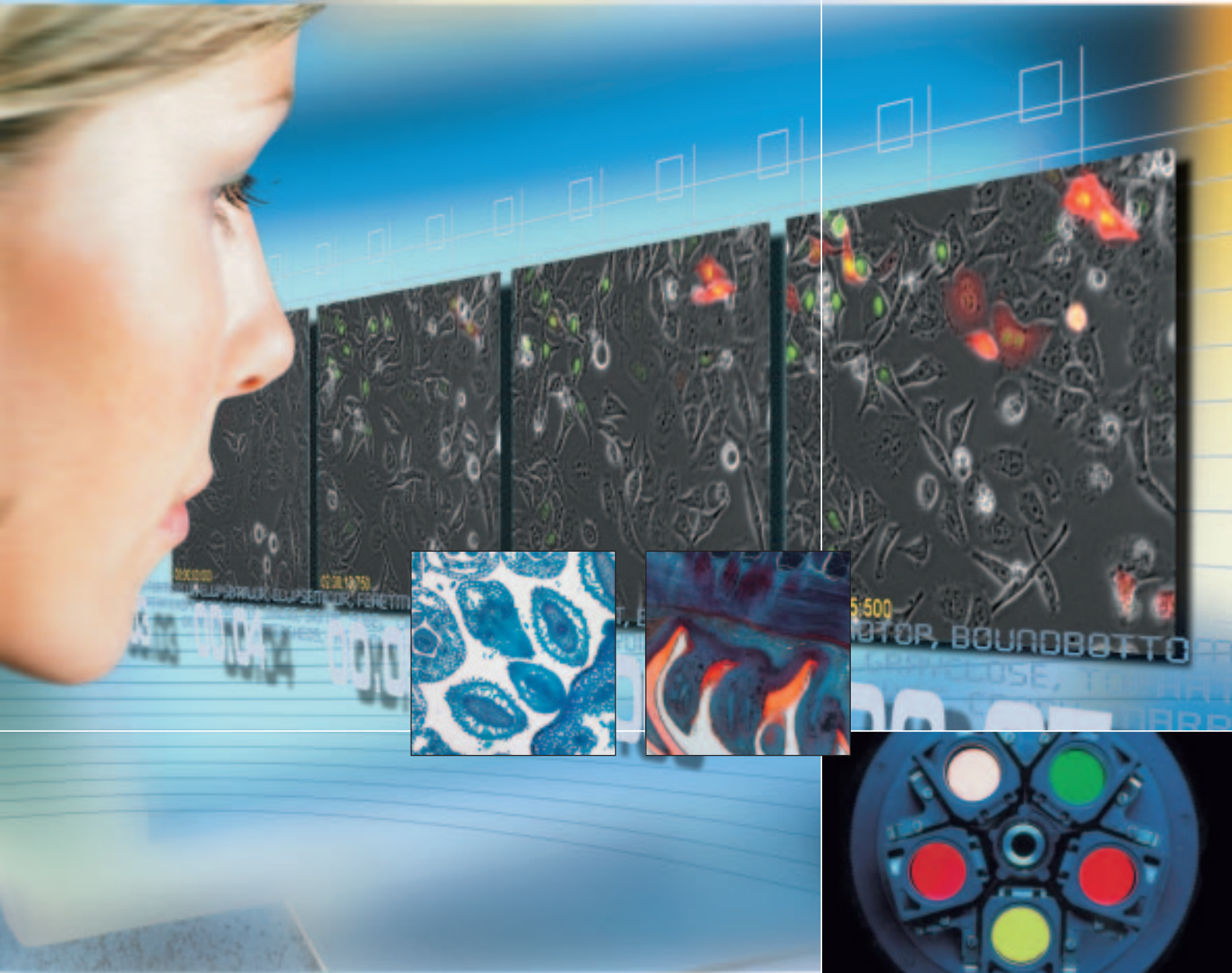


AxioVision

Perform to Perfection



New dimensions in digital image processing for the life sciences



We make it visible.

A New Way of Thinking

The requirements of biomedical sciences such as histology, pathology, neuro sciences, cell biology and pharmacology are growing ever more complex, thus making the application of digital microscope software increasingly valuable. Carl Zeiss is driving this process with new solutions that are continually setting new standards. A major component is AxioVision, the microscope software designed by the microscope specialists. Thanks to its unique modular architecture, this software is equally suited for both newcomers and advanced users. Software packages such as AxioVision applications offer complete solutions for defined requirements. The philosophy behind AxioVision is uncompromising: the highest possible performance, easy operation, extreme flexibility, and seamless integration into Carl Zeiss microscope and camera systems. A homogeneous solution with functions that are 100% effective – right from the start.



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Mark&Find

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- Transformation of color models

MosaiX*

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Image acquisition

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Image analysis

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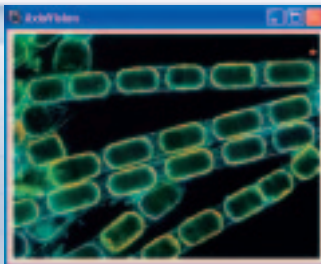


Image acquisition

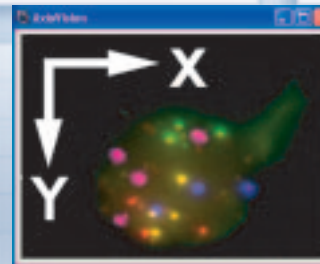
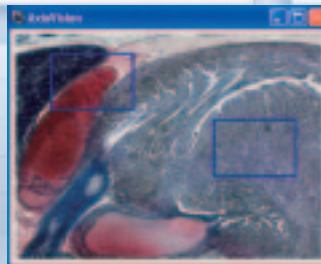


Image processing

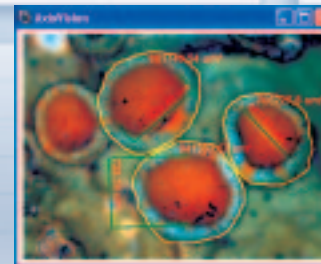


Image analysis

00.03

00.04

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00

Basic program

AxioVision

The decision for the basic program from AxioVision is a decision for a sound investment in digital imaging. For a 100% compatible system solution that can be adapted at any time to your changing requirements and demands. A decision that not only protects your investment but guarantees you enormous flexibility.

Growing possibilities

The world of life sciences is constantly changing and evolving, which means it requires a software package that can change and evolve with it. AxioVision is very flexible in its design. Because with every update and every expansion, Carl Zeiss is at the cutting edge of innovative software developments. In addition, the user interface is customizable. This gives users the ability to make it easy to understand and use for their specific applications. The functions of the basic modules – imaging, processing, annotations, archiving, reporting, and microscope control – can be quickly expanded to meet your growing needs by inserting additional modules. Moreover, new solutions for specific applications are continually being developed. They include additional functions for image processing, interactive measuring, and automated image analysis as well as control modules for filter wheel shutters and motorized stages.

Cumulus Workgroup*
Image cataloging and archiving in network

VBA*
Programming environment

Cumulus Single User
Image cataloging and archiving

Commander*
Recording and automatic execution of all steps

Documentation
Image archiving and reporting

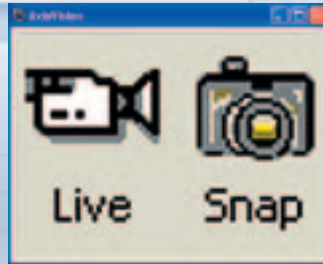
Configuration
Customization of user interface

AxioVision FRET
Measurement of molecule interaction

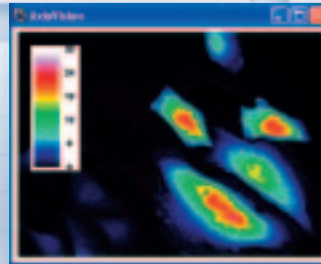
KS ELISPOT
Exact immune reaction measurement



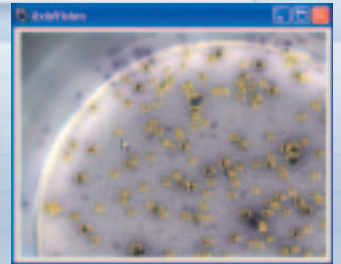
Documentation



Configuration



FRET



ELISPOT

Application kits

Extensive Basics

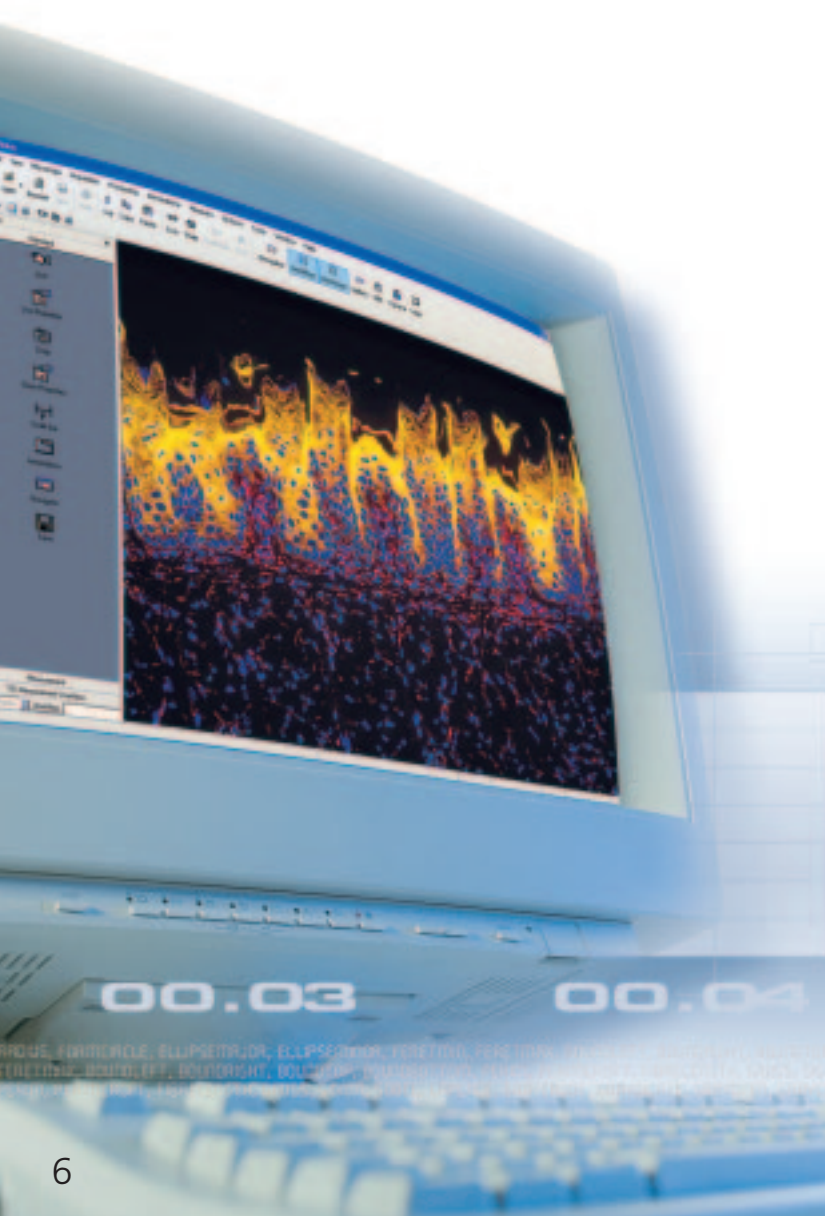
The entry-level version of AxioVision provides a highly powerful image processing and analysis system for modern microscopy. With a wealth of functions ranging from microscope control to convenient image archiving, it is guaranteed to meet the high demands placed on state-of-the-art digital microscopy.

Efficient microscope control

AxioVision allows you to control all motorized microscopes from Carl Zeiss – both automatically and interactively. Of course, you can use manual standard microscopes as well. One of the advantages of software control is that you can store desired microscope parameters quickly and easily, ensuring repeatability from sample to sample. In addition, scaling factors and complex workflows like time lapse can be recalled during analysis, greatly increasing the speed at which measurements can be performed.

Flexible camera operation

Thanks to its interfaces for such standard technologies as framegrabber or TWAIN, AxioVision allows you to use all types of cameras, from basic video and digital cameras right up to scientific microscope cameras. This includes the AxioCam family of cameras from Carl Zeiss. The seamless integration of cameras into AxioVision software allows the user to generate multidimensional images at a mouse click, e.g. image stacks from different focus planes. The AxioCams from Carl Zeiss can provide significant advantages in the areas of



Digital camera



AxioCam



The complete range: from digital microphotography with standard digital cameras up to image analysis with high-resolution microscope cameras.

Basic program

speed and resolution, optimized live image, automatic adjustment of exposure time or image acquisition. All cameras in the AxioCam family are controlled by the same operational elements. If you want, you can also alter them to fit your individual applications.

Easy and clear interfaces for camera control and image acquisition.

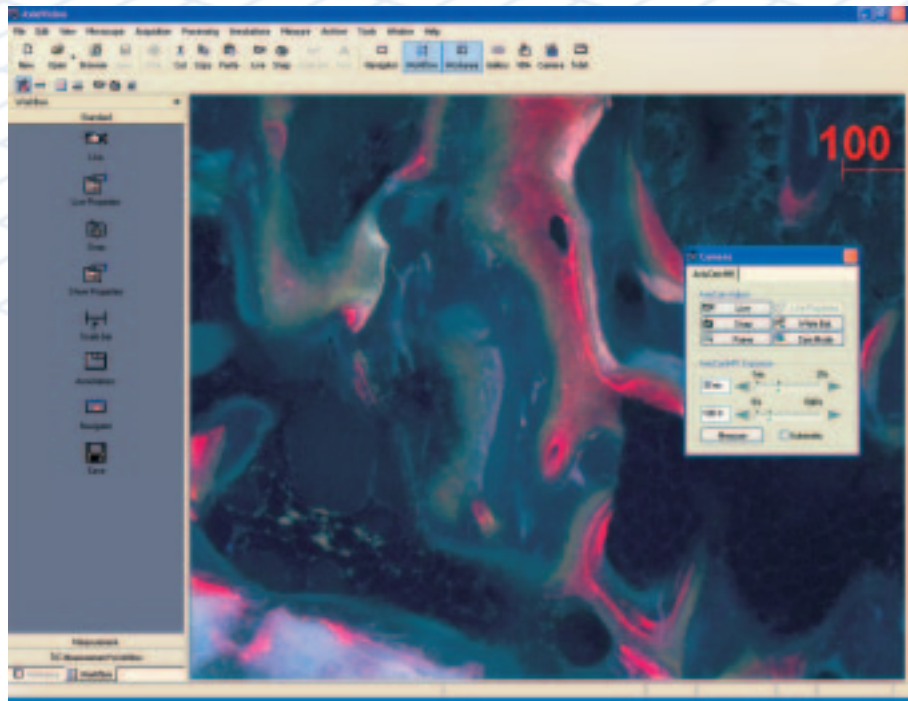
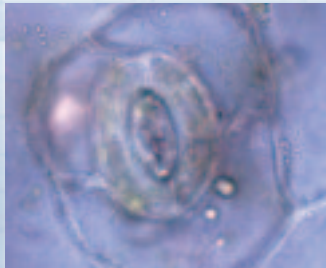
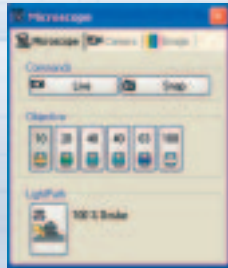


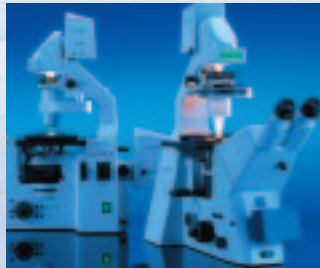
Image acquisition



Microscope control



Microscope (manual or motorized)



Rapid image processing

AxioVision offers you all the tools for:

- contrast, brightness, and color control
- noise suppression, smoothing, and contour enhancement
- improved sharpness and detail emphasis
- correction of illumination conditions and white balance control

Integration of text and graphic elements

From scale bars and color markings right up to text and graphic elements – with AxioVision you can add all important annotations to your images. Since everything is in one package, you don't have to use other software for image processing. The corresponding scale is stored with each image, and scale bars can be interactively added at any time.

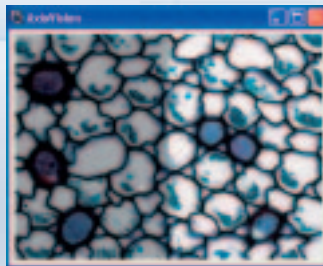
Precise image measurement

With the entry-level program, you can easily perform interactive measurements, such as length, area, and angles with the option of optimizing your workflow with the help of a measurement wizard. The measurement data are available in a list, which can be easily exported to most spreadsheet programs, such as Microsoft® Excel.

ZVI images

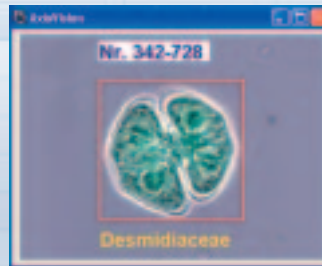
ZVI: the image format that stores your image data together with image number, acquisition date, microscope settings, exposure data, size and scale data, contrasting technique used etc. The advantages are obvious – the image information is available at any time. In addition, annotations are not permanently burned into the image, but are stored in a file together with the image data. The image can be reproduced even years later under identical conditions.

Image processing



Contrast optimization by histogram normalization (left: sub-optimal contrast, brightened and contrast enhanced image).

Text and graphic elements



Dividing algae, marked with sample number and name.

Image Measurement



Length measurement of diatoms.

Basic program

Efficient image archiving

Storing images in an electronic archive gives users an organized, space-efficient, and easy alternative to boxes of 35 mm slides. Digital data can be stored for years to come – with no loss in quality. In addition to images, you can store microscope parameters such as objective setting and filter position as well as annotations and comments directly in the image. The benefits of a digital archive are:

- fast, flexible search functions via image and specimen name, date, or other designations
- clear overview of all information recorded with the image
- use of CD, MOD etc. drives for large amount of data

Convincing documentation

Whether using individually formatted or predefined layouts, AxioVision gives you all the options you need to generate effective reports or presentations:

- predefined layouts for combining image and comments in various formats
- layouting functions

ZVI

Reports

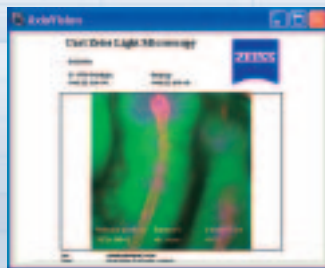
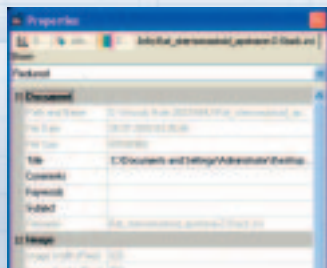


Image Acquisition Modules: Enhanced Performance

The results of your work are only as good as the quality of the information the images yield. AxioVision offers you specially designed modules to expand your image acquisition functions. These optional modules provide you with the added information that is crucial for high-quality images.

Mark&Find

This module is used to record, store, and automatically retrieve different positions on your specimen and culture dishes. It requires the use of motorized x/y stages. The positions on the sample are stored together with the recorded image and can be used to reposition the sample at a later stage. And it allows the easy scanning of multiwell plates. Lists of positions can also be imported. Your advantage: time saving, reliable documentation of the sample, while keeping statistical accuracy. Multiple datasets can be extracted from the same sample.

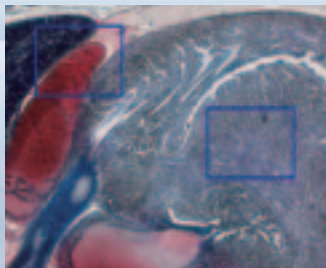
Autofocus

The Autofocus module calculates the optimal focal position for a sample in reflected light, transmitted light and fluorescence. The system is calibrated for each objective so that the software focuses accurately every time. In addition, with images that are recorded at different positions, the system automatically refocuses. The Autofocus module works with all cameras that are directly controlled by AxioVision, providing that a microscope with motorized focus drive is used.

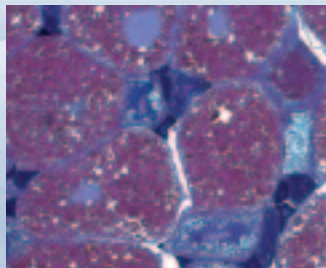
MosaiX*

Developed to analyze large surfaces, MosaiX permits the area of a specimen to be scanned in order to generate one single large image. This electronically created image can thus serve as a map to navigate the specimen and provides a basis for further analyses. In addition, the specimen only has to be scanned once.

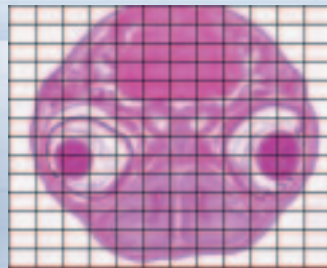
Mark&Find



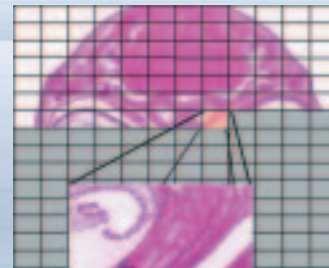
Autofocus



MosaiX



1.



2.

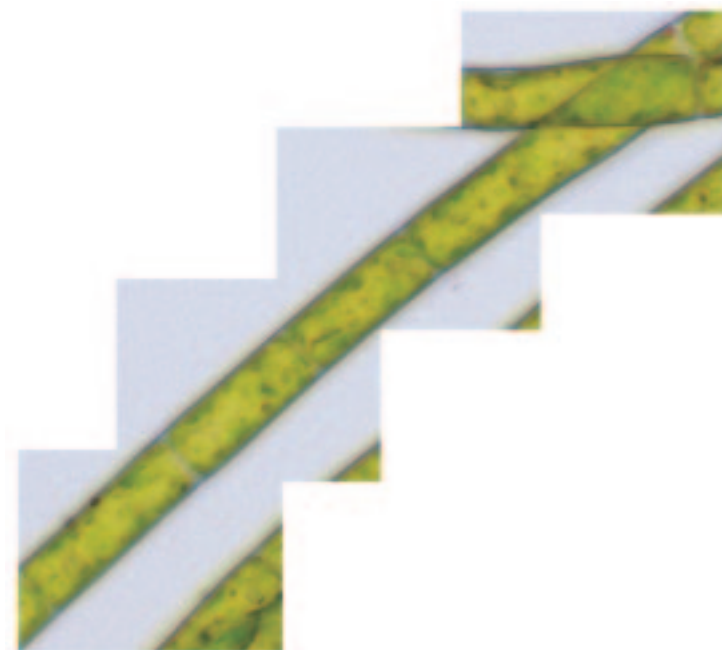
Figs. 1 and 2: Cross-section through the head of a Tupaia embryo.

The overview image permits easy navigation. The details are needle-sharp.

Image acquisition

Panorama*

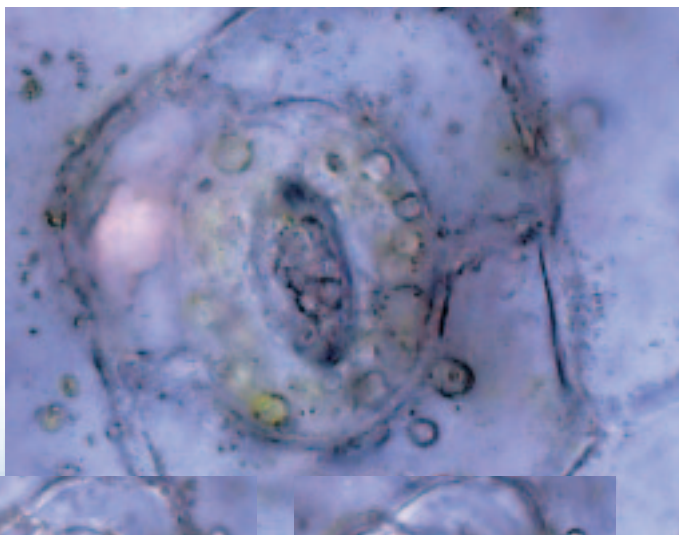
This module is ideal for specimens which do not fit into the image frame. High-resolution panorama or overview images can be formed with pixel accuracy from individual shots. Even overlapping images can be combined so precisely that all the important details of your specimen are recorded in a single image.



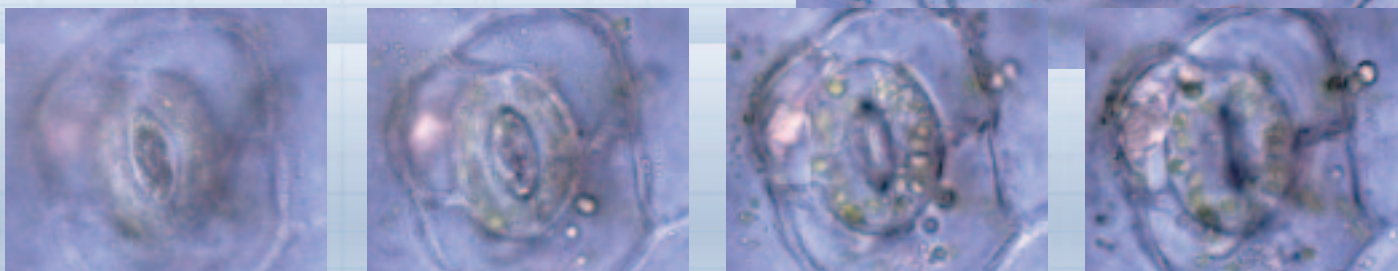
After forming the overview image out of four individual shots, it is easy to accurately and precisely interpret the sample.

Extended Focus

A microscope's depth of field is often not sufficient to obtain a single image which is sharp over the whole field. The software solution to this problem is the Extended Focus module. How it works is simple and ingenious. While focussing through your sample, you record a number of images at different focus positions. Then it extracts the sharp details from each individual shot to calculate a final image that is rich and sharp in every detail.



Extended Focus



Single shots from different focal planes of a stoma. With Extended Focus, users can achieve an image that is sharp across the whole field.

Image Acquisition Modules: Living Cells in Focus

Live cell imaging is a routine application for many laboratories, for example, in neuro- or cell biology. It is based on software solutions tailor-made for this application. Ranging from the acquisition of multichannel fluorescence images, Z-stack images or documentation of your time lapse experiments, these AxioVision modules are 100% practice-driven, highly flexible and easy to use.

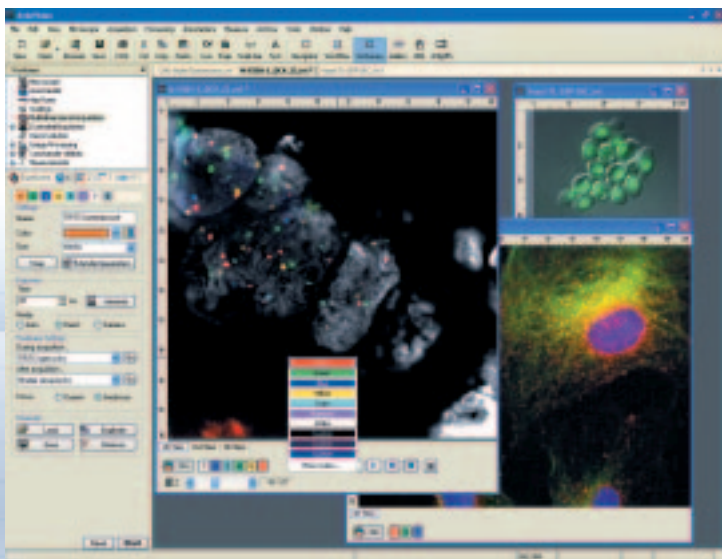
Multichannel Fluorescence

This module enables you to generate images with up to 8 channels. Various fluorescence channels can be freely combined with transmitted light images (e.g. phase contrast). A channel with optimal exposure time is selected for every excitation wavelength. The Reuse function allows acquisition parameters to be extracted from a multichannel image that has been saved. This enables further image acquisition under identical conditions – as a result, your data are more reliable. The advantage for you: unparalleled flexibility in presenting complex connections in biological specimens.

Z-Stack

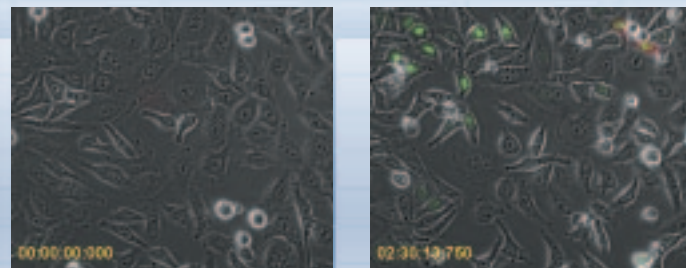
With this module you can generate Z-stacks. The software controls the Z-drive of a motorized microscope in exact steps, synchronizing it with the image. You can either determine the focusing steps yourself or have them automatically computed. The advantage of this module: optimal detection of information in the third dimension. In addition, with the OrthoView function, even the entry-level version of AxioVision provides you with a highly effective technique for the Z-stack analysis.

Multichannel Fluorescence



Complex but easy to operate: using a few simple controls, you have multichannel selection, color assigning, Z-stack and time lapse selection – even with very large images.

Time Lapse



Analysis of protein function with the help of digital microscopy: expression of a DsRed-coupled reporter protein (red fluorescence) in response to the expression of a GFP-coupled HIV protein (green fluorescence) in HeLa cells (phase contrast) – 3-channel time lapse image (Dr. Severine Demart, GSF-Institut for Molecular Virology, Neuherberg).

Image acquisition

Time Lapse

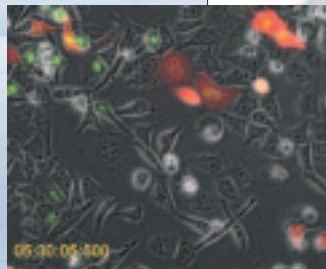
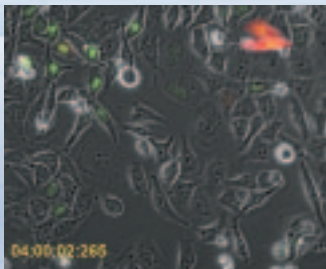
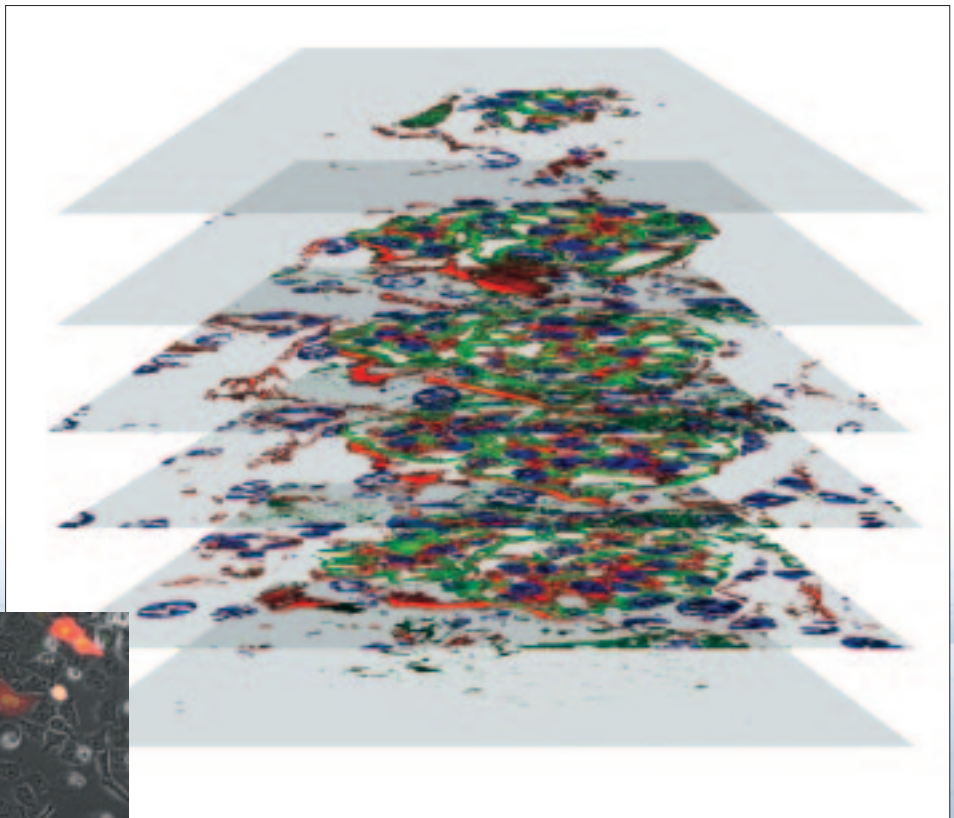
Observing living specimens, investigating changes over time, documenting results clearly – with the Time Lapse module, you can precisely control both image and microscope. Fast light control prevents damage to the specimen – an essential prerequisite for the quantitative analysis of life.

Combinations

Mark&Find, Autofocus, Multichannel Fluorescence, Z-Stack, Time Lapse, ApoTome - all these modules can be freely combined with each other, creating system solutions capable of precisely meeting a wide range of demands. The result: the cost-effective adaptation of individual solutions to a specific application – with no unnecessary investments.

Z-Stack

Recognizing structural relationships in 3D:
cross-section of mouse kidney (glomeruli: green,
actin fibers: red, cell nuclei: blue).
Z-stack 3-channel fluorescence image acquired
with the ApoTome module.

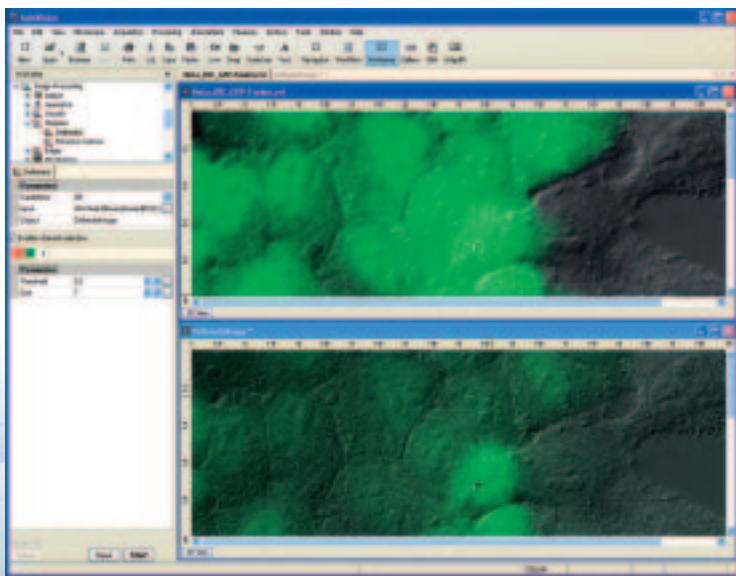


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Image Processing Modules: Added Insights

Seeing what your eyes wouldn't normally see: Imaging Plus offers you all the important digital image processing and analysis techniques you need in a single module. Each image is processed so that you attain the best analytical results from each step, e. g. measurement of cells.

Image enhancement



Precise edge detection with the Delineate function.

Imaging Plus

• Image enhancement

In addition to improving contrast, brightness, and color, this function compensates lighting deficiencies and shading. Included are filters for smoothing, sharpening, and edge detection as well as user-definable filter operators.

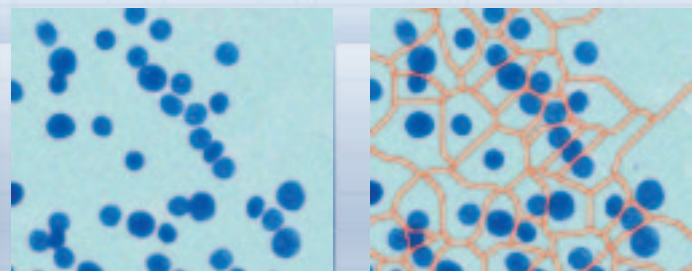
• Gray morphology

A number of functions enable the precise separation of joined structures such as individual cells in a cell agglomeration.

• Image arithmetics

The process of calculating a new image from existing images pixel-by-pixel: AxioVision Imaging Plus permits quantitative combination and comparison of images.

Gray morphology



Morphology functions permit the exact reconstruction of particle borders, thus preparing them for automatic measurement.

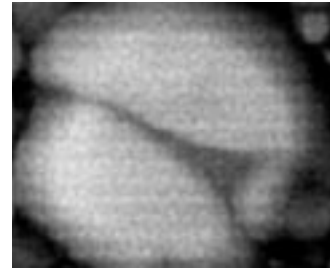
Image processing

- **Fourier transformation**

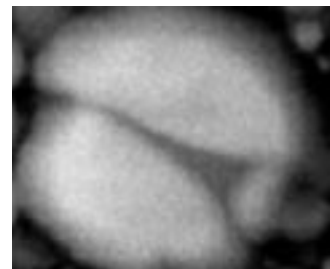
For efficient filtering and restoration of images. The module allows the customer to freely retain or eliminate parts of the images frequency spectrum.

- **Transformation of color models**

This functionality permits color discrimination for better separation of objects from their background as well as color independent image processing.



1.



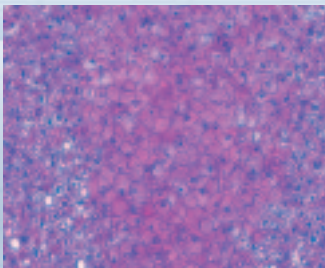
2.

Disturbances in the image such as sinusoidal artifacts (fig. 1) can be effectively eliminated through the use of Fourier transformation (fig. 2). Fig. 3 shows the parts of the spectrum, which were eliminated.

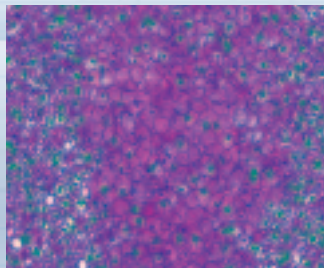


3.

Transformation of color models



1.



2.

Fig. 1: Live section with HE stained nuclei.

Fig. 2: Blue nuclei detected by color discrimination and marked green.

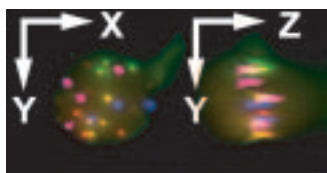
Thus, blue nuclei can clearly be separated from the background.

Image Processing Modules: See More - Identify More

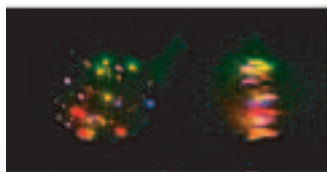
Microscopy in the third dimension – both a reality in laboratory routine application and a challenge. In fluorescence microscopy, reliable results are almost impossible without image-optimizing techniques. AxioVision provides you with powerful tools capable of meeting the demands of your applications and the technical parameters of your laboratory. Tools ranging from the established mathematical 3D Deconvolution technique to ApoTome, the innovative technique for creating optical sections with the help of structured illumination. In addition, you can present these high-contrast, deblurred results both in 3D and 4D with the visualization module Inside4D.

3D Deconvolution

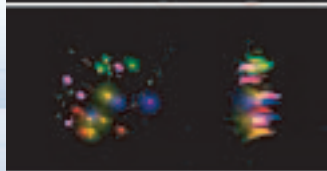
The quality of optical sections is frequently diminished by light scattered from areas above and below the focal plane, resulting in distortion of the image. Consequently, 3D fluorescence imaging and analysis are not possible without the support of image-optimizing systems. The 3D Deconvolution module from Carl Zeiss provides this support. Using the point spread function (PSF), this established mathematical technique restores the 3D image stack: light from above and below the focal plane is calculated back to its plane of origin to create a sharp image.



Original



Nearest Neighbor

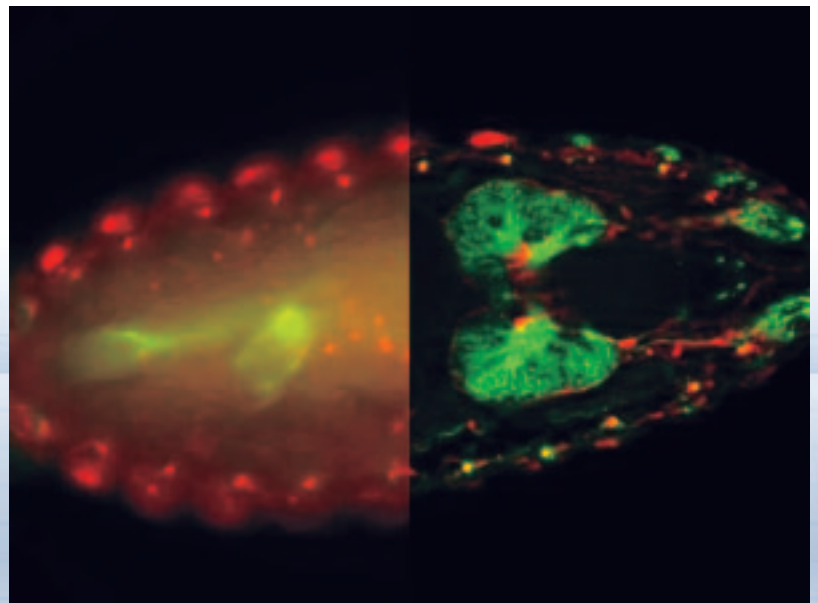


Regularized Inverse Filter



Iterative Maximum Likelihood

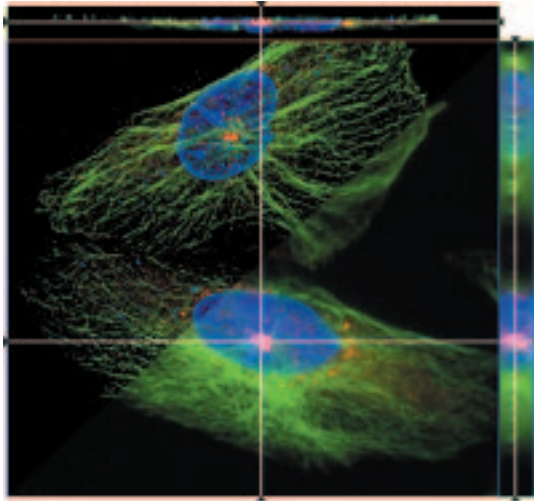
ApoTome



3D Deconvolution of a 6-channel Z-stack. Cell nuclei of an ovarian carcinoma hybridized with FISH probes specific for 5 chromosome types (Dr. Michael Speicher, Technical University, Munich).

Fluorescence of nerve and glia cells in a Drosophila embryo. Left: conventional fluorescence; right: optical section with ApoTome (Dr. Christian Klämbt, University of Muenster).

Image processing

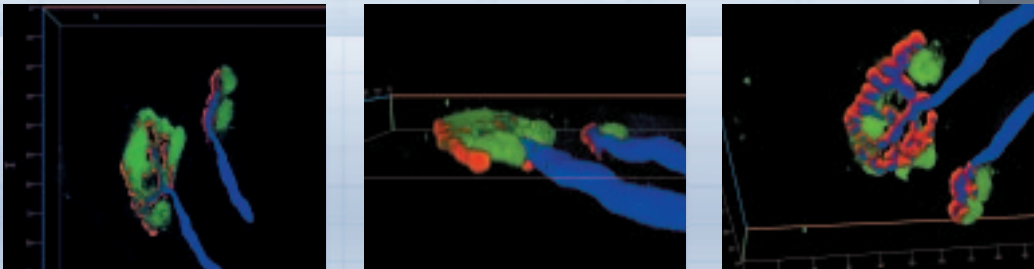


*Resolution of fine structures with the help of 3D Deconvolution:
3-channel fluorescence Z-stack image of endothelial cells
(BPAE, mitochondria: red, F-actin filaments: green, cell nuclei: blue).
View before and after 3D Deconvolution in the orthogonal section
(AQLM course, Woods Hole, MA, 2002).*

ApoTome

Tissues and other thick specimens are of particular challenge for fluorescence microscopy. Developed to provide deblurred optical sections, ApoTome offers considerably higher image quality, sharpness, contrast and optical resolution in the axial direction. In addition, it has further essential advantages for 3D fluorescence microscopy: greater speed, higher throughput, extensive flexibility in the use of fluorescence dyes, and easy handling. ApoTome is a slider for the plane of the field diaphragm of fluorescence illumination and is combined with special software. Designed for the research microscopes Axioplan 2 imaging and Axiovert 200, ApoTome raises the performance level of your digital imaging system to new and exceptional heights.

Inside4D



Neuromuscular synapses in rat sternomastoid muscle (stained with anti-S100: green, neurofilaments and synaptic vesicle: blue, rhodamine-bungatoxin: red) 3-channel fluorescence Z-stack imaged with the ApoTome module.

(Images acquired in the Neurobiology course, Woods Hole, 2003. Specimens from Dr. Le Tian and Dr. Wes Thompson, University of Texas at Austin).

Inside4D

3-dimensional visualization in space: using the Inside 4D module, you can easily display cells and tissue sections in space. All you need is a 3-dimensional image stack. With a simple mouse click, Inside4D computes a precisely scaled 3D view (rendering). Important – the original image data remains unchanged so that you can switch from 3D back to 2D within seconds for control and comparison. Inside 4D also enables you to present your time lapse images in the fourth dimension, time. Moreover, Inside4D is not only easy to use – it also provides you with an impressive spectrum of functions. With its four rendering techniques, a wealth of options, multichannel views, animation and movies in 3D and 4D, Inside4D is a powerful tool for the clear and convincing presentation of your data. It's fast and easy, too.

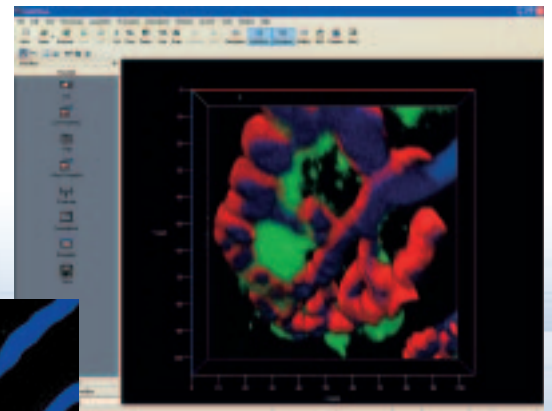


Image Analysis Modules: Uncompromising Precision

Utilizing all the information of an image: AxioVision offers you a powerful spectrum of additional modules for image analysis. Both interactive and automatic, they range from complex segmentation techniques to sophisticated measurement options. In addition, the measurement wizard allows you to create your individual measurement program. Fast and easy. The advantages of all AxioVision modules are standardized processes and uncompromising precision. This means significantly faster results, high efficiency and maximum reproducibility.

Interactive Measurement

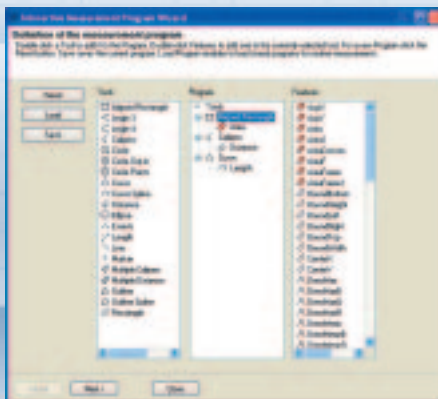
With this module, parameters describing the specimen can be determined interactively (e.g. size). A measurement program wizard allows users to exactly determine which measurements shall be taken. All parameters are then executed in the specified order. As a result, geometric and densi-

tometric parameters are presented in a straightforward measurement list, to be stored with the image in the archive. You can retrieve this information later at any time. In addition, all requested measurement values can be exported (e.g. into Excel).

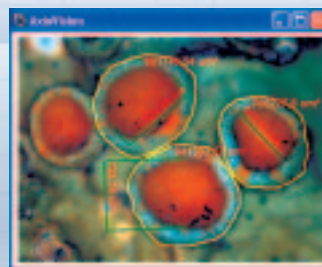
AutoMeasure

If you need to create automatic measuring routines yourself: with the AutoMeasure module you can rapidly obtain precise results – without any complicated programming. With the help of a measurement wizard, AutoMeasure enables you to carry out complicated measurements within a few minutes. Simply define the programs that you need and you can measure an unlimited number of images – while completely controlling the measuring process. You can determine which steps are to be conducted. Even automatic processes can be interrupted at any time and all parameters individually adjusted with the function dialog.

Measurement parameters



Interactive Measurement



Interactive measurement of lichen apothecia (*Xanthoria parietina*).

Measurement tables

#	Tool	Feature	Unit	Value
1	Length	Diameter	um	66,74
2	Outline Spline	Perimeter	um	66,67
3	Outline Spline	Perim	um	770,62
4	Outline Spline	Area	um ²	13719
5	Outline Spline	ExtentsMeanD	um	8437,11
6	Outline Spline	ExtentsHeight	um	255,7
7	Outline Spline	Extents	um	153,54
8	Outline Spline	ExtentsMin	um	800,08
9	Outline Spline	ExtentsMax	um	98,87
10	Outline Spline	Center X	um	401,6
11	Outline Spline	CenterY	um	1624,55
12	Outline Spline	CenterT	um	466,76
13	Outline Spline	ExtentsMinD	um	147,14

Measurement tables can be stored in Excel.

Image analysis

AutoMeasure Plus

Recording the entire structure of the image completely automatically – now possible in a single measurement step with this module. The result: fast, precise and reproducible quantitative analyses. Further advantages: the direct access to all functions via the menu and the option to combine them with the automatic processing module Commander*. It enables you to merge the results of repetitive work steps in a single command – ideal for the automatic processing and reproducibility of standard lab assignments. This module consists of three functionality groups:

- **Segmentation**

This function offers threshold operators for monochrome and color images necessary to identify the objects based on gray color values. The objects can also be quickly identified at a mouse click using "region growing". These methods are supplemented by complex methods for segmentation, including dynamic and automatically generated threshold as well as edge detection. Dynamic discrimination allows you to differentiate and

emphasize important details from the background. Valley detection makes dark lines visible (position and direction). Even weakly defined edges can be clearly identified using several detection techniques. The result is a binary image in which all specimen pixels are white and all background pixels black.

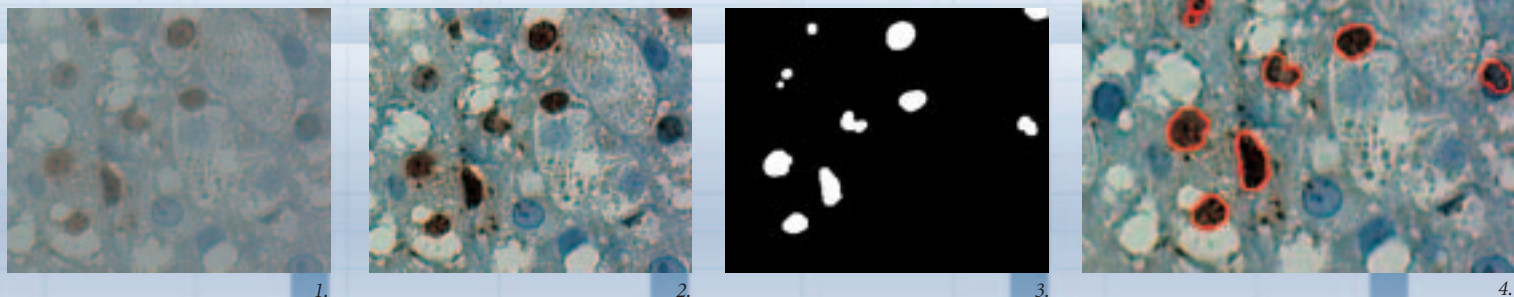
- **Binary image processing**

Numerous functions ensure that the binary image is optimally prepared for measurement. They include linking, masking, and filling voids. Artifacts can be removed easily and contours smoothed. During "skeletonizing" objects are thinned to a 1-Pixel-line or separated from each other using a background skeleton.

- **Automatic measurement**

Morphometric measurement parameters are generated by the contour of the specimen. The software uses the binary image as a mask to calculate geometric and densitometric parameters from the original image. Their export into Excel enables statistical information about specimen details.

AutoMeasure Plus



Automatic analysis of a histological section with brown stained nuclei: original image (fig. 1), with contrast enhancement (fig. 2), binary image with removed artifacts and separated nuclei (fig. 3). The result was superimposed in color on the original (fig. 4).

Archive Modules: Superior Data Management

AxioVision Cumulus – with this powerful software module you can manage your image, text and graphic data as digitally stored assets. Available in either a single user or network-compatible workgroup edition*. You can catalog your data and classify them according to category or keyword. You can add annotations and comments in addition to calling up metadata from the files in order to list, export, and further process them. The basis for compatibility with other databases is ODBC, Open Data Base Connectivity.

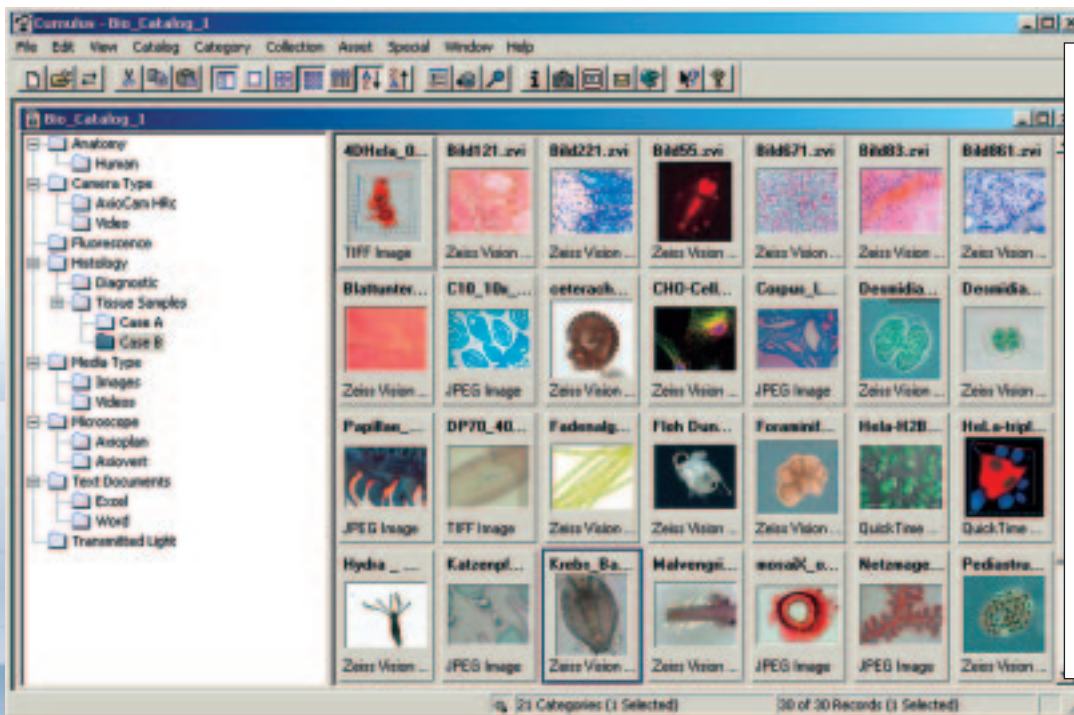
Easy searches

A tremendous advantage for you is that assets can be classified into several categories (e.g. type of sample or contrasting technique). A double click on one or more categories provides you with all relevant records. Efficient searches with more than one search condition are easy to conduct and can be stored, if desired.

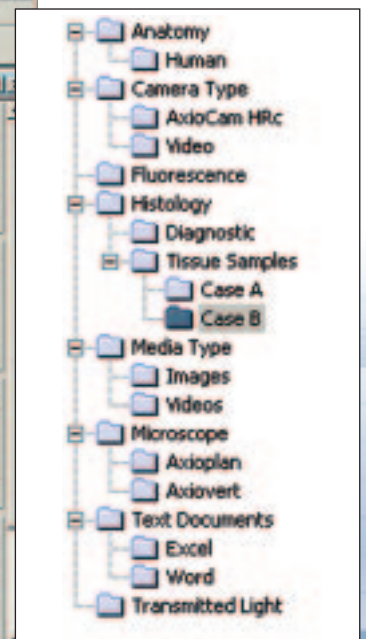
Managing assets

A module with advanced archiving capabilities, AxioVision Cumulus displays your assets on the monitor together with text and graphic elements of your images. Moreover, you can present your assets as a slideshow, together with audio notes and teaching material. AxioVision Cumulus facilitates the publication of your results on the

AxioVision Cumulus



Archiving



Creating individual database structure with categories and sub-categories is clear, interactive, and can be checked immediately.

Available in either a single user or network-compatible workgroup edition*, AxioVision Cumulus is a powerful software module for data management and archiving.

Internet (access via hyperlinks). Sending preview catalogs to your colleagues makes it easy for them to view your results. Additional modules permit the integration of further file formats (ppt, pdf) in catalogs or searches.

Cumulus Workgroup*

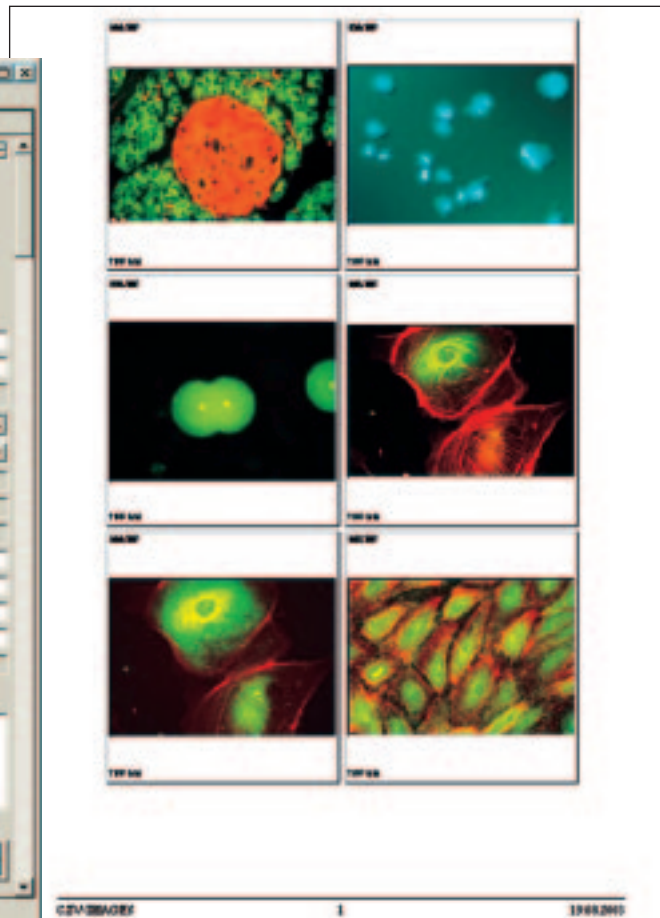
Cumulus Workgroup provides a client-server solution for image management, where data are stored centrally on a file server. Workgroup members

can access these systems efficiently and securely as "clients". For every catalog in the search path, access can be regulated according to user rights.

AxioVision Cumulus facilitates the management of images and data.

Printout

Information window



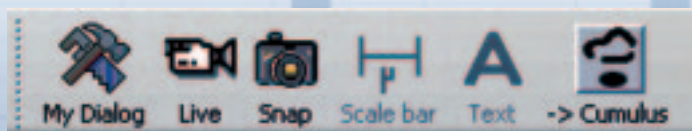
Configuration Modules: My AxioVision

AxioVision provides a unique range of options to create individualized interfaces and functions. This is ideal to simplify and optimize digital microscopy according to transparent workflows. Already the entry-level program of AxioVision gives you the freedom you need to create your own personal working environment. You can configure individual toolbars or combine operating elements necessary for camera and microscope control in new dialog boxes. Moreover, you can add or omit elements, depending on what you need for your work. The efficiency of these configuration possibilities available in the entry-level package can be further increased with the Commander* and VBA* module.

My Dialog



Toolbar



User-defined toolbar for image acquisition, addition of scale bar and comments, archiving.

Individual dialog to operate microscope and camera.

Configuration

Commander*

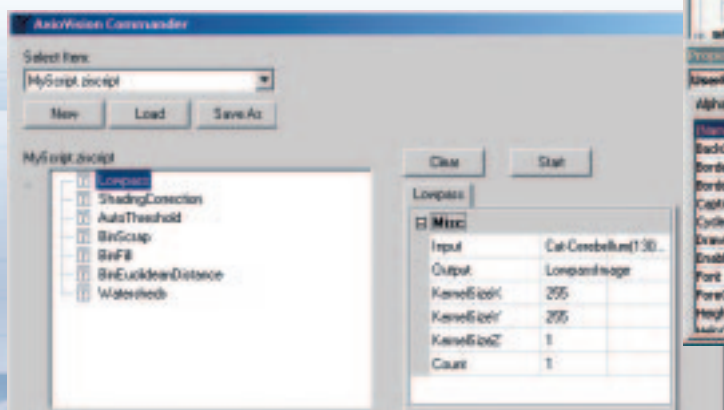
The Commander module allows you to record steps for image acquisition, processing, and measurement in a single command. The benefits are impressive: automatic processing of typical lab assignments and complete reproducibility of the results in addition to fast adaptation to new lab assignments.

VBA*

Perhaps you need more functions than the wide range that AxioVision 4 provides. In this case, it is possible to increase and extend the performance of Carl Zeiss software with VBA (Visual Basic for Applications), the programming language Carl Zeiss uses for AxioVision functions. VBA provides

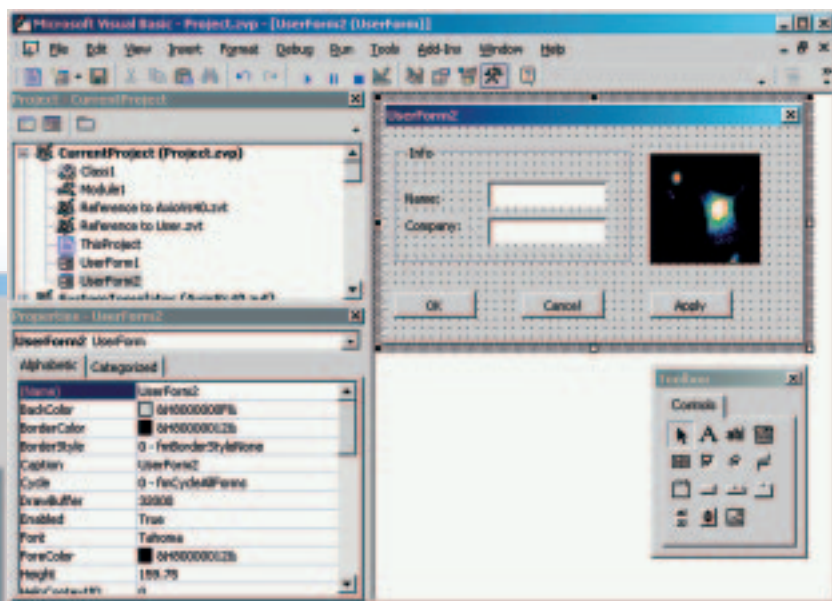
a completely integrated development environment that is familiar to programmers. Since VBA is directly integrated into the host application, it offers the advantages of fast internal cooperation as well as the opportunity to develop solutions without additional programs. The results look and act just like AxioVision. The big advantage: a minimum of training time for the users of individually developed software.

Commander



Recording work steps for automatic processing.

VBA



Programming environment to develop individual solutions in AxioVision.

Application Kits: Specialists for Special Applications

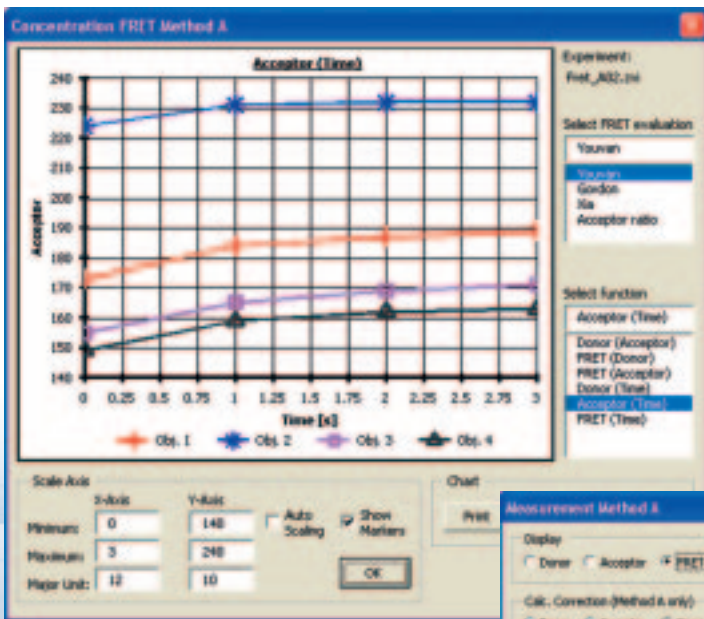
Practice-oriented know-how can best be seen in the development of technical solutions, which continually set new standards in microscopy. This is particularly true when new, complex applications are in question. Developing specialized solutions for these applications is a challenge that Carl Zeiss successfully meets time and again. The results? Tailor-made, complete solutions that set high standards in performance – and prove that solutions based on intelligent concepts make it possible to easily master even the most complex applications.

AxioVision FRET - the complete solution for quantitative FRET measurements

Fluorescence resonance energy transfer (FRET) is one of the latest techniques to identify the binding and interaction of proteins, lipids, enzymes, DNA and RNA in living cells. AxioVision FRET is the first complete system containing all techniques required to conduct FRET experiments. It includes, for example, complete functionalities for all necessary steps, such as image acquisition, microscope control, measurement, display, and presentation. In addition, it integrates five important correction and evaluation techniques, widely recognized in international research:

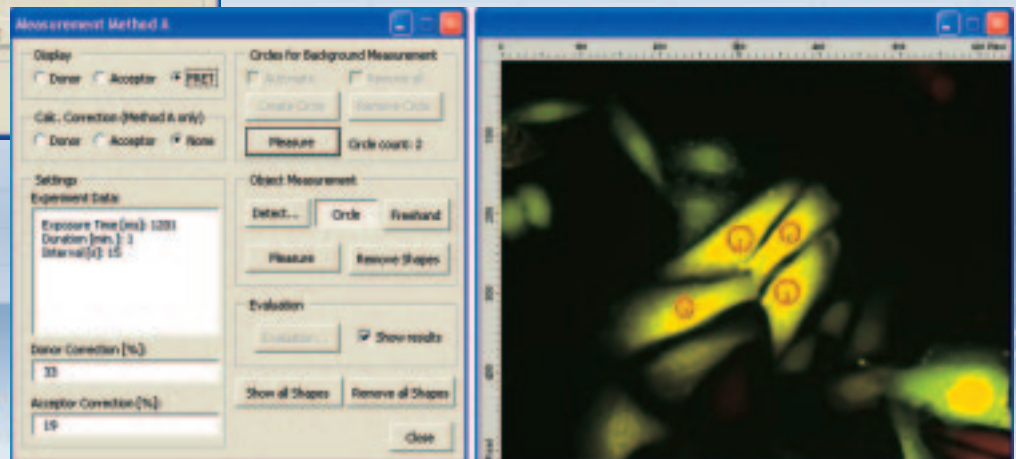
Youvan (1997): corrects measured values for the background and the cross-talk from the donor and the acceptor (basic technique for all correction measurements).

Gordon (1998): normalizes the Youvan value to the direct donor and acceptor signal.

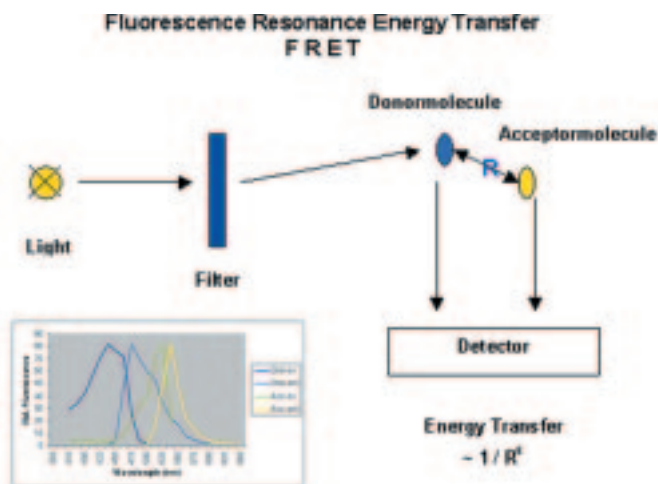


All settings for FRET measurements are displayed in a single window. AxioVision FRET provides the graphic tools to visualize the results in histograms.

FRET measurement



Application kits



Today GFP mutants such as CFP/YFP, which clearly overlap in the excitation and emission spectrum, are generally used in FRET experiments. This cross-talk must be corrected to achieve quantitatively precise evaluation.

KS ELISPOT - an expert system for immunology

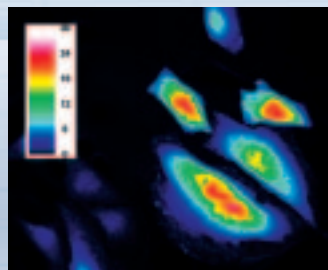
The KS ELISPOT technique determines the number of cells which produce a certain antigen. The results are highly significant for therapy control in tumor, vaccination and AIDS research. KS ELISPOT was especially developed as a complete system solution for the automated and easy evaluation of Elispot assays. Operation is user-friendly, intuitive and can be individually configured. All necessary settings can be carried out using only four buttons. Microscope, camera and correction techniques are configured in a single file. In the "Teach mode", system parameters for spot identification can be modified by a simple click of the mouse – a great advantage for the reliable reproducibility of your measurements. You can see the results of your analysis immediately on the monitor. The quantity, size and intensity of the individual spots can be printed out or exported into a file for further statistical evaluation. Moreover, standardized specimen evaluation allows data to be exchanged easily and efficiently.

Xia (2001): determines the FRET value on the basis of the Youvan result independently of donor and acceptor concentration.

Siegel – acceptor bleaching technique (2000): measures donor emission before and after selective photo bleaching of the acceptor.

Different histograms are available for each of these techniques (e.g. donor vs. acceptor) as well as the graphic tools necessary for clear and well-structured display in the histograms. AxioVision FRET offers easy, practice-driven operation combined with high flexibility – from individual definitions of ROIs (Regions of Interest), gray threshold values, circles or freehand right up to the configuration of individual user interfaces.

FRET evaluation



FRET according to Youvan's technique – cross-talk and background corrected.

ELISPOT evaluation



Examples of different type of evaluations of 96-well plates using KS Elispot (from left to right):

Fig. 1: Spots stained brown after HRPO staining of separated well membranes.



Fig. 2: Evaluation of HRPO spots (numbers indicate spot diameter).

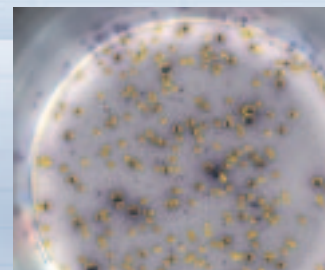


Fig. 3: In plate evaluation of blue (AP) spots.

Image Aquisition	Functions	Contents/Description
Image Formats:	<ul style="list-style-type: none"> Image import Image export 	bmp, tif, jpg, gif, tga, png, j2k, jp2, mac, msp, ras, pct, eps, wmf, psd, img, cmp, zvi, lsm, czi bmp, jpg, tif, tga, png, psd, cmp, avi, lsm, mov, j2k, jp2, pcx, tga, wmf, pcf
Camera Control*:	<ul style="list-style-type: none"> Exposure time adjustment Gray value scaling Digital gain White balance 3200 K Resolution Binning Histogram Color adjustment Frame Black reference White reference Image orientation Shutter control Live image frame rate Focus/exposure frame 	Manual adjustment, exposure time measurement, automatic mode Conversion to 8 bit dynamic range Adjustment of digital signal amplification Interactive or automatic adjustment of optimum neutral balance of the color channels Default value for white balance, optimized for halogen light source at 3200° K Selection of Microscanning resolution modes (AxioCam HR) Increased camera sensitivity by combining the signals of adjacent pixels Intensity distribution histogram for all three color channels Manual adjustment of the color reproduction of the white balance Interactive selection of an image sensor sub frame Image background optimization for low light level applications Correction of optical and illumination inhomogeneities in bright background Rotation and mirroring of image orientation for optimum image display Trigger of external shutter, synchronous to image acquisition Selection: slow/medium/fast Activation of measurement frame for focus and exposure information
Image Processing		
Annotate:	<ul style="list-style-type: none"> Annotation 	Addition of text, marking of elements (arrows, scale bars, etc.)
Adjust:	<ul style="list-style-type: none"> Brightness/contrast Color Balance 	Adjustment of brightness, contrast and gamma Manual adjustment and readjustment of color rendition
Lightness:	<ul style="list-style-type: none"> Hue/Lightness/Saturation Shading correction 	Adjustment of hue and saturation Correction of uneven illumination
Geometric		
Transformation:	<ul style="list-style-type: none"> Pixelshift OrthoView 	xyz-shift with subpixel-accuracy Display of orthogonal 3D image cub
Smooth:	<ul style="list-style-type: none"> Gauss, Sigma 	Image smoothing using gauss or sigma filter
Sharpen:	<ul style="list-style-type: none"> Enhance Contour 	Image sharpening using contour enhancement
Resample:	<ul style="list-style-type: none"> Resample 	Image zoom-out and zoom-in
Image Analysis		
Interactive measurement tools and parameters:		
	<ul style="list-style-type: none"> Scaling Length Outline 	Scaling in geometric units Distance between 2 points Measurement of diameter, area, perimeter, length and width of the circumscribing rectangle, radius, center of gravity, mean density, standard deviation of mean density of gray value
	<ul style="list-style-type: none"> Angle 3, Angle 4 Circle 	Definition through 3 or 4 points Measurement of diameter, area, perimeter, length and width of the circumscribing rectangle, radius, center of gravity, mean density, standard deviation of mean density of gray value
	<ul style="list-style-type: none"> Events Interactive Measurement Wizard 	Counting of events Directed interactive measurement
Documentation		
	<ul style="list-style-type: none"> Image gallery Search functionality Information window Printing of images/data Reports 	Clear presentation of loaded images as thumbnails Full text search in all information fields Display of all information on the image Print of images Creation of user definable reports
Configuration		
	<ul style="list-style-type: none"> Toolbars/Dialogs/Workflows Short-cuts 	Creating individual toolbars, dialogs and workflows Allocation of keyboard entries with AxioVision functions

Module	Functions	Contents/Description
Mark&Find	Recording and retrieving of specimen	
	• Database	Management of projects and samples in a database
	• Mark interactively	Color assignment of sample positions in the database
	• Visualize	Visualization of the selected points. Relocation by clicking on the colored marker
	• Focus position	Relocation with optional use of stored focus position
	• Calibrate	Calibration using a "Home slide"
Autofocus	Automatic Focusing	
	• Calibrate	Calibration by specifying the optimum focus position, usage of current microscope settings with motorized microscopes
	• Focus	Automatic calculation of the focus plane. Suited for transmitted light, reflected light as well as bright field, dark field, fluorescence
Extended Focus	Calculation of sharp images from several focus positions	
	• Execute	Capture of an extended depth of focus image by combining images of different focus positions
MosaiX	Automatic scanning of large surfaces	
	• Execute	Scanning of entire sample (motorized stage required)
Panorama	Formation of overview images	
	• Execute	Seamless composition of images
Multichannel Fluorescence	Image acquisition in several fluorescence channels	
	• 8 channels	Simultaneous acquisition of up to 8 independent channels per image
	• Channel configuration	Adjustment of exposure time and microscope settings independent for each channel
	• Optimal display	Channel display as pseudo colored merge image or monochrome display of every single channel
	• Color coding	Free assignment of pseudo colors to channels with easy choice from list
	• Extended parameters	Listing of all channels in a spreadsheet format with extended parameter settings
	• Dye selection	Choice of most commonly used fluorescence dyes from list
	• Focus position	Assignment of different focus positions to individual channels with correct aberrations
	• Channel pool	Storing of channel configurations in a channel pool for easy recombination into other experiments
	• Image information	Display of channel specific information as annotation
	• Experiment	Saving of channel configurations as experiment for exact reproduction of experimental set up
	• ReUse	Extraction of channel settings from previously acquired images for the exact reproduction of an experimental set up
Z-Stack	Acquisition of image series from different focus positions	
	• Focus control	Automatic adaptation of the minimal possible step size according to microscope type
	• Z-stack configuration	Definition of start and stop position (or center position) as well as desired interval between individual Z-planes
	• Nyquist criterion	Automatic calculation of the optimal Z-interval for 3D Deconvolution or ApoTome
	• Navigation	Precise stepwise navigation through defined Z-stack or to the start, stop or center position
	• Experiment	Saving of Z-stack definitions as experiment for exact reproduction of the experimental set up
	• ReUse	Extraction of Z-stack definitions from previously acquired images for the exact reproduction of an experimental set up
Time Lapse	Acquisition of image series over time	
	• Time configuration	Definition of interval as well as number of cycles or total time
	• Exposure time	Automatic measurement of the correct exposure time for the first timepoint
	• Display	Easy control of the experiment through continuous display of most recently acquired image
	• Image information	Time of acquisition as annotation in image
	• Autosave	High data security during long time lapse acquisitions due to autosave-function

Module	Functions	Contents/Description
	• Experiment	Saving of time lapse configurations as experiment for exact reproduction of experimental set up
	• ReUse	Extraction of time lapse settings from previously acquired images for the exact reproduction of an experimental set up
	• Image size	Acquisition of images as large as required by experimental conditions (limited only by hard disk capacity)
3D Deconvolution	Restoration of Z-stack images	
	• Automatic PSF Calculation	Automatic extraction of all necessary microscope parameters from the ZVI image for calculation of an optimized "Point Spread Function" (PSF)
	• Nearest Neighbor	Method for rapid contrast improvement and blur removal from all Z-stack images
	• Regularized Inverse Filter	Filter for rapid 3D restoration of Z-stacks
	• Constrained Iterative	Iterative filter for the quantitative 3D restoration of Z-stacks
	• Preview function	Deconvolution within a user definable small region of interest for fast preview
	• Optimal noise treatment	Automatic calculation of the optimal strength of restoration by determination of image noise levels through "General Cross Validation"
	• Auto-Stop	Iterations stop automatically upon reaching optimal image improvement
	• Display	Three normalization methods for individual adaptation of result images (Clip, AutoLinear, MatchInput)
ApoTome	Optical sectioning of fluorescent samples with "structured illumination"	
	• Image acquisition	Automated acquisition of three temporary images followed by online processing to an optical section
	• Scanner control	Automatic and precise shift of grid pattern in the object plane
	• Grid calibration	Calibration of grid focus for correct acquisition of varying fluorescence wavelengths
	• Correction algorithms	Automatic correction of fluctuations in illumination as well as signal degradation due to bleaching
Inside4D	Visualization in 3D	
	• Volume display	Volume display of Z-stack fluorescence images with up to 8 channels with selective switching between different channels or view in merged pseudo color mode
	• Shadow projection	Creation of animations with strong sense for spatial conditions
	• Transparency rendering	Presentation of transparent tissues or cultures
	• Surface rendering	Enhancement of individual structures
	• Maximum projection	Ideal for prints and publication
	• Spatial interaction	Free positioning of the 3D volume in space with free choice of angles for x,y and z, lateral position and zoom factor
	• 3D inside view	Orientation within a volume
	• Annotations	Optional display of volume edges, color coding and scaling of axes
	• Animations	Generation of animations as rendered image series with export options in popular video formats (avi, QuickTime)
	• Maximum rendering speed	Acceleration of rendering methods through modern graphic boards (support of OpenGL-standard)
Imaging Plus	Processing, Gray Morphology, Fourier Transformation, Color Transformation	
	• Adjust	
	– Contrast	Contrast enhancement using interactive/automatic histogram adaptation
	– Negative	Calculation of inverted image (negative)
	– Gray transformation	Adjustment of gray values using transformation tables
	• Geometric Transformations	
	– Rotate, Shift	Rotation around an axis
	– Mirror	Mirror along horizontal or vertical axis
	– Alignment	Alignment of two images using reference points
	• Smooth	
	– Lowpass	Lowpass filter (floating average value)
	– Median	Median filter (non-linear method)

Module	Functions	Contents/Description
	– Rank	General rank operator
	• Sharpen	
	– Delineate	Enhancement of edges
	• Edges	
	– Sobel	Edge detection using Sobel filter
	– Laplace	Laplace filter
	– Highpass	Highpass filter
	• Morphology	
	– Gray Erode, Gray Dilate	Erosion or dilation of objects
	– Gray Open, Gray Close	Erosion followed by dilation or dilation followed by erosion
	– Tophat White	Removal of bright regions
	– Tophat Black	Accentuation of dark regions
	– Gray Gradient	Morphological gradient to detect contours
	– Watersheds	Watersheds – algorithm for separation/reconstruction
	• Arithmetics	
	– Add, Subtract	Addition or subtraction of two images
	– Add Constant	Addition of a constant value
	– Multiply, Divide	Multiplication or division of two images
	– Multiply Constant	Multiplication with a constant value
	– Average	Average of two images
	– Maximum, Minimum	Maximum or minimum of two images
	– Square, SquareRoot	Square or squareroot of an image
	– Logarithm, Exponential	Logarithm or exponent of an image
	– Combine	Linear combination of two images
	• Fourier Transformation	
	– Transform	Fourier transformation on an image
	– Spectrum	Calculation of power or phase-spectrum
	– Filter	Filtering in the frequency domain using a defined filter
	– Inverse	Inverse Fourier transformation
	• Utilities	
	– Copy Region	Copying of image regions
	– Color Model	Transformation of RGB color space into HLS color space and vice versa
	– Split Channels	Split RGB image into single color channels
	– Combine Channels	Combine single color channels to a color image
	– Convert Pixel Format	Conversion of pixel formats (e.g. "8 bit integer" to "float")
	– User Filter	Filtering an image with used defined filter matrix
Interactive Measurement	Expanded interactive measurement techniques	
	– Distance, Line, Calipers	Measurement of length
	– Multiple Calipers	Measurement of the length of multiple lines, perpendicular to a base line
	– Multiple Distance	Measurement of distance between multiple parallel lines and a base line
	– Curve, Curve (Spline)	Measurement of length of the drawn curve
	– Aligned rectangle or free orientation, outline, outline (spline), circle	Measurement of geometric and densitometric object features to the center
	– Circle	Drawing of a circle from the contour to the center, clicking on contour points
	– Marker	x- and y- coordinates of a point
	– Interactive Measurement Program Wizard	Guided generation of a program for interactive measurement

Module	Functions	Contents/Description
AutoMeasure	Creation of easy measurement programs with a measurement wizard	
Creation of measurement programs	<ul style="list-style-type: none"> • Automatic Measurement Program Wizard – Image enhancement – Segmentation – Binary image clean-up – Automatic object separation – Interactive editing of the measurement mask • Selection of measurement parameters – Definition of measurement conditions ("objectfilter") – Definition of a measurement frame – Measurement – Documentation – Data storage 	<p>Guided generation of a program for automatic measurement</p> <p>Contrast, brightness, Gamma, noise reduction (Sigma), shading correction, improvement of edges</p> <p>Global or local definition by clicking or circumscribing objects, specification of thresholds using the image histogram</p> <p>Deletion of artefacts, filling of holes</p> <p>Erosion and dilatation, watersheds</p> <p>Drawing of separation lines, deletion of objects, addition of objects</p> <p>Region specific, field specific, geometric and annotation parameters</p> <p>Logical concatenation (AND/OR) of region specific parameters, definition by simple clicking on reference objects</p> <p>Rectangle, circle, freehand</p> <p>Measurement of geometric and densitometric features for single objects or the entire image</p> <p>Marking of measured objects and display of freely selectable measurement parameters in the graphics plane</p> <p>Saving of measurement data in an Excel-compatible file format (csv)</p>
Execution of measurement programs	<ul style="list-style-type: none"> – Image acquisition – Autom. assignment of scaling – Control of program execution • Program information – Display 	<p>Image acquisition via camera, all images of a folder, all loaded images</p> <p>With ZVI image format</p> <p>Activation/deactivation as well as changing of a function during the program execution</p> <p>List of executed functions with parameter settings</p>
AutoMeasure Plus	Segmentation, binary image processing, automatic measurement	
	<ul style="list-style-type: none"> • Segmentation – Thresholds – RegionGrowing – Automatic – Dynamic – Valleys – Canny – Marr • Binary processing – Bin Erode, Bin Dilate – Bin Open, Bin Close – Bin Fill, Bin Scrap – AND, OR, XOR, NOT – Bin Endidean Distance • "Skeletonizing" of binary images – Thinning – Exoskeleton • Measure 	<p>Adjustement of thresholds using histogram</p> <p>Detection of associated regions (gray values within user defined tolerance level)</p> <p>Automatic determination of thresholds using a histogram</p> <p>Threshold detection by using size information</p> <p>Detection of dark lines (valleys) in images with bright background</p> <p>Edge detection considering "steepness" of edges</p> <p>Detection of edges and associated regions</p> <p>Erosion or dilation of binary objects</p> <p>Erosion followed by dilation or dilation followed by erosion</p> <p>Filling of holes, removal of artifacts</p> <p>Bitwise logic operation on the image</p> <p>Generation of a "distance map", indicating the distance of each pixel to the object border</p> <p>Thinning of binary objects to 1 pixel wide lines (skeleton)</p> <p>Skeletonize the image background</p> <p>Automatic measurement of geometric and densitometric object features</p>

Module	Functions	Contents/Description	
Cumulus	Image cataloging and archiving	Single User	Workgroup
	Organizing catalogs, categories and key words	X	X
	Storage of assets on a server		X
	Network access for workgroup members		X
	Central management of access rights		X
	Local administration of catalogs	X	
	Data and category fields	X	X
	Voice annotation for assets	X	X
	Free definable queries to all data fields	X	X
	Short response time to complex queries	X	X
	Asset export to HTML	X	X
	On the fly e-mail assets	X	X
	TCP/IP Client/Server architecture		X
	Access to the same pool of assets – even from different operating systems		X
	User rights control for asset access		X
	ODBC compatibility	X	X
	Commander	Recording and automatic execution of steps	
	Protocol, save	Record procedures and save protocol	
	Start	Automatic run of recorded protocol	
	Edit	Edit protocol	
VBA	Programming environment		
	Visual Basic Editor	VBA environment with full access to AxioVision functionalities	

Application kits	Functions	Contents/Description
AxioVision FRET	Measurement of molecule interaction	
	• User modes	User mode for routine measurements
	• Evaluation agent	Adjustment of system parameters for the acquisition: exposure time, method (acceptor bleaching or correction method), time lapse parameter Acquisition of multichannel images according to definition Background measurement in all channels Signal measurement in all channels Definition of ROIs by threshold, circles or freehand Choice of evaluation method Youvan, Gordon, Xia, Acceptor Ratio, Siegel Presentation of raw data as table Storage of raw data
	• Display	Display of all raw data as histograms: – channel by channel (FRET-Donor, FRET-Acceptor, Donor-Acceptor) – all FRET-methods against time Generation of false color images for FRET signals for each method (Youvan, Gordon, Xia, Akzeptor Ratio, Siegel)
KS ELISPOT	Exact immune response measurement	
	• User modes	Administrator mode – to set up and tune the system User mode – for routine measurements
	– Direct evaluation of wells	Definition of the well area on the motor stage for evaluation Selection of a configuration file for evaluation Start of image acquisition including measurements Storage of raw data
	– Evaluation of stored images	Definition of the image folder Definition of the well area in the plate field Selection of a configuration file for evaluation Start of evaluation Storage of raw data
	• Display	Result presentation in internal rtf format
	• Spot Teaching	Training the system using the unique "Teach mode"
	• Report	Creating a report as a Word document

Entry-level program	IM: Interactive Measurement	IM incl. measurement assistant	AutoMeasure
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Region specific parameters				
			• Geometric	
		X	AcpX, AcpY	x- and y-coordinates of the top object point
X	X	X	Area	Area of the region
		X	AreaConvex, AreaFilled	Area of the convex shell of the region and of the filled region
X	X	X	CenterX, CenterY	x- and y-coordinates of the geometric center of gravity of the region
		X	EllipseMajor, EllipseMinor	Length of the main axis and the secondary axis of the ellipse with the same geometric moment of inertia as the region
		X	Ellipse Angle	Angle of the main axis of the ellipse with the same moment of inertia
X	X	X	Perimeter	Perimeter of the region
		X	Perimeter Convex	Perimeter of the convex shell of the region
		X	Perimeter Filled	Perimeter of the filled region
		X	Perimeter Crofton, Perimeter Crofton Filled	Perimeter of the region and perimeter of the filled region according to Crofton
		X	Perimeter X, Perimeter Y	x- and y-projection of the perimeter
		X	Perimeter XF, Perimeter YF	x- and y-projection of the perimeter of the filled region
		X	Perimeter XY, Perimeter XYF	Diagonal projection of the perimeter and the perimeter of the filled region
		X	BoundTop, BoundBottom, BoundLeft, BoundRight	x- and y-coordinates of the bounding box
X	X	X	BoundWidth, BoundHeight	Width and height of the bounding box
		X	AreaFrame	Area of the measurement frame
		X	FeretMinimum, FeretMaximum	Minimum and maximum feret of the region
		X	FeretMinimumAngle, FeretMaximumAngle	Angle of the minimum and the maximum feret of the region
		X	FeretRatio	Ratio of the ferets ($\frac{FeretMin}{FeretMax}$)
X	X	X	Diameter, radius	Diameter, radius of the circle with equivalent area
		X	FormCircle	Circular shape factor of the region
		X	FibLength	Length of a fiber-like thin region
		X	ID	Explicit characteristic of the region, of the squares
X	X	X	Distance measurement	Distance between 2 points
X	X	X	Angle measurement	Angle in °
			• Densitometric	
X	X	X	Mean	Densitometric mean value of the region (gray and color values)
X	X	X	Standard deviation	Standard deviation of the densitometric values of the region (gray and color values)
		X	Minimum, Maximum	Minimum and maximum densitometric value (gray and color values)
		X	Sum, Sum Square	Sum of the densitometric values of the region, sum of the squares (gray and color values)
Field specific parameters				
			• Geometric	
		X	FldArea	Area of all regions
		X	FldAreaPer	Percentage area of all regions in the measurement frame
		X	FldCount	Number of the measured regions
		X	FldPerim	Sum of all region perimeters
			• Densitometric	
		X	FldDensMean	Densitometric value of all regions (gray and color values)
		X	FldDensStd	Densitometric value standard deviation in all regions (gray and color values)
		X	FldDensMin, FieldDensMax	Minimum and maximum densitometric value in all regions (gray and color values)
Further parameters				
X	X		Counting events	
	X	X	Marker	

More information:

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Subject to change