Microscopy from Carl Zeiss

# Axio Imager 2

**Progress Meets Performance** 

Trend-setting technology for brilliant results in all life science research applications



We make it visible.



# Axio Imager 2. Even More Highlights.

### The optics

- IC<sup>2</sup>S beam path for high contrast
- Highest possible resolution through high-performance objectives

### The fluorescence

- Combination of DIC and fluorescence with the motorized DIC turret
- Excellent image quality due to the optimized beam path
- Triggerable LED light source
- Several light sources for uniform illumination

### The stands

- Preconfigured packages for the most common applications
- Coded and motorized components
- Modular and individually upgradable

### The imaging

- Motorized DIC turret: Combination of fluorescence and DIC for absolute artifact-free images
- Rapid image acquisition in up to 6 dimensions
- Motorized scanning stages, motorized z-focus and high-performance focus (Axio Imager.Z2) for the highest precision and positioning accuracy

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### www.zeiss.de/axioimager

## Axio Imager 2 from Carl Zeiss. Success in Series.

Always provide the best tools for the study of life – with this objective in mind Carl Zeiss introduced Axio Imager in 2004. This objective still applies. The result: the new Axio Imager product generation. With outstanding performance. With unrivaled optics. With an unmatched range of application. And with maximum ease of use.

### Axio Imager: Trailblazer in Terms of Performance

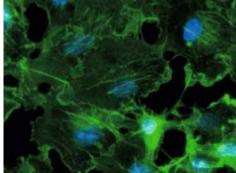
More flexibility for more performance: from simple observation and image acquisition to highly complex analyses, there are six different stands available, which allow you to adapt the system exactly to your individual application by providing many different system components. Taken together these are trend-setting performance characteristics and technical innovations for outstanding research results.

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Inhalt







Respiratory epithelium cells

COS cell culture

- Encoding: Readout of magnification, illumination or contrast settings, respectively, and transfer to the AxioVision image processing software.
- Motorization for reproducible settings and automatic procedures.
- Excellent optics and uniform illumination in transmitted light and fluorescence applications.
- Highest precision due to new high-performance focus, even in cases of constant load and heavy stages.
- Intelligent control concept for ergonomic work and multi-user operation
- Preconfigured stand configurations for a broad application spectrum
- Assured future use supported by modular system architecture



# **Optics**. **Brilliant Performance.**

Excellent optical quality: That is what the Carl Zeiss research class stands for. Axio Imager 2 boosts this performance even further. From the transmitted light beam path to the new motorized DIC turret to the high-performance objectives Axio Imager provides excellent results even with extremely weak signals.

### Visibly more information: the IC<sup>2</sup>S beam path

IC<sup>2</sup>S stands for Infinity Contrast & Color Corrected System. This patented beam path is based on the optimization of the proven Carl Zeiss ICS Infinity Optics (ICS). New: the transmitted light beam path for uniform illumination. The optics of the universal and long-distance condensers have been adapted to all applications. Even at low magnifications and large working distances considerably better resolution and contrast is achieved. Axio Imager's optical system provides you with remarkable performance: higher image contrast, perfect uniformity and unrivalled resolution in every contrast technique.

#### Simple upgrading: the freely accessible infinite space

With its freely accessible infinite space, Axio Imager allows additional components such as light sources and detectors to be added as needed. An individual system solution that is tuned to the respective application can be simply and rapidly configured.

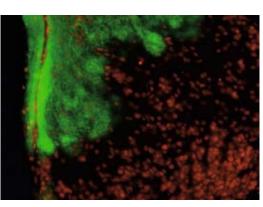
### Unrivalled in every respect: the objectives

For the new Axio Imager 2 product line Carl Zeiss has extended the high-performance objectives especially for high magnifications, for both fixed and live cell imaging applications.

- The EC Plan-NEOFLUAR universal objectives. The consistent stray light minimization results in a definite contrast enhancement, which is critical in all microscopic techniques.
- The Plan-APOCHROMAT objectives convince through their outstanding point spread function and their unparalleled planar and chromatic correction.
- The  $\alpha$  Plan-APOCHROMAT objectives 100x/1.46 Oil and 100x/1.57 HI Oil (available from Fall 2009) provide maximum resolution in fluorescence and transmitted light DIC techniques because of their high numerical apertures.
- The LCI Plan-NEOFLUAR objectives 25x/0.8 and 63x/1.3 Imm. korr. were conceived for live cell Imaging techniques and calibrated for specific temperature intervals as well as immersion media from water to glycerin.



Olfactory bulb (frog), image taken with DIC. Objective: EC Plan-NEOFLUAR 20x/0.5



Olfactory bulb (frog), multichannel fluorescence with ApoTome. The new motorized DIC turret Green: projections of olfactory sensory cells. Red: cell nuclei. Objective: EC Plan-NEOFLUAR 20x/0.5. D. Schild, Univ. Göttingen, Germany





### See more: DIC or DIC + Fluorescence

Optimized DIC for the new generation Axio Imager: uniform interference contrast at all magnifications from 5x to 100x across the entire field of view. Particularly in digital imaging the shading correction becomes obsolete. You always have a uniformly illuminated DIC image. For the first time these advantages are now also reproducible and can be adjusted via motorized control. With the new motorized DIC turret for transmitted light DIC you can now automatically shift between high-resolution and high-contrast interference contrast. The contrast settings can be separately stored for each user and for each magnification used. You can also combine DIC imaging with fluorescence excitation extremely simple and automatically. Without sample-induced artifacts.

# Constant color temperature: the LED illumination sources

The interesting alternative to conventional halogen illumination with compelling advantages: constant color temperature independent of the brightness, low heat radiation and long service life. LED illumination also has a filter mount for individual setting of the color temperature. For the first time such an illumination source is also offered with a trigger input for high frequency switching. For more simple applications there is a variant available which is attached directly beneath the condenser. In accordance with the Fixed Köhler Principle, for simple adjustment with all contrast techniques.



Motorized DIC turret for reproducible contrast adjustment



LED – the new light source for Köhler illumination



LED for Fixed Köhler illumination

## Fluorescence. Strong Components for Weak Signals.

Brilliant signals for the finest structures and extremely rapid processes – that is what Carl Zeiss fluorescence microscopy stands for. And all the components of the new generation of Axio Imager have been designed to meet this standard. With fast image acquisition in AxioVision and light sources such as Colibri. With filter sets for new dye combinations. And with high ease of operation.

#### Motorized reflector turret for rapid imaging

The investigation of rapid processes is becoming increasingly important. The motorized reflector turrets are custom tailored to this end. Six filter modules can be accommodated. Even for the use of more than six dyes simultaneously, for example, in multi-color FISH applications, the Axio Imager.Z2 provides the best possible results. The motorized 10-position reflector turret synchronized with the fast Colibri LED light source provides a wide selection of excitation wavelengths and brilliant results without pixel shift.

# Reproducible settings by means of motorized diaphragms

The intelligent, motorized aperture and luminous field diaphragm automatically controls contrast and illumination. In the reflected-light beam path as well as in the transmitted-light beam path. Objective-specific aperture adaptations can be saved and loaded again at any time for reliable reproducibility.

# Versatile as never before: the High Efficiency Filter Sets

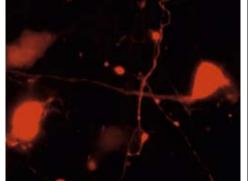
The HE Fluorescence Filters for Axio Imager provide an excellent signal-noise ratio, high transmission for excitation and emission, and for up to 50 % shorter exposure times. This protects sensitive samples to the greatest



Simply fast: the change from manual to motorized reflector turret

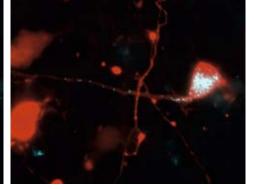
Changing to HE filter set

Motorized diaphragm sliders





Cyan: CFP-labeled peroxisomes



Multi-channel image: red and cyan channels superimposed

Red: YFP-labeled cell body Primary neurons (rat) in culture. Objective: EC Plan-NEOFLUAR 40x/0.75. Y. Okada, Dept. Cell Biol. & Anatomy, Grad.Sch.Med, Univ. Tokyo Hongo, Tokyo, Japan

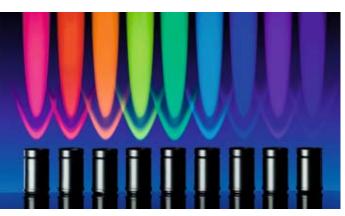
possible degree. Seven new filter sets and multi-color combinations with double and triple filter sets were developed especially for combination with new fluorescing proteins. The trend toward red dyes such as mRFP, mCherry, mPlum, mTomato was considered in optimizing the range of LED options integrated into Colibri. As a result, the energy of the available LEDs can be completely exploited.

### Light sources for every requirement

For Axio Imager you can select exactly the light source which ideally meets the specific demands of your fluorescence application.

 The self-adjusting HBO lamp has been the illumination source of choice for all standard fluorescence applications since 2004. After each lamp change and each time the device is switched on, it centers itself automatically such that uniform illumination is guaranteed.

- Metal halide lamps such as HXP 120 exhibit an emission spectrum similar to HBO lamps. Remote coupling via liquid light guide, minimizes heat transfer to the stand making it ideal for live cell imaging.
- Exact intensity control and thus ideal specimen protection, Specific wavelength selection, and flexible mixing of different wavelengths, long lifetime, and – above all – switching time in the microsecond range characterize the Colibri LED light source. It is ideal for complex applications at extremely high speeds.
- HXP 120 and Colibri can also be used in combination. In this manner dyes for which there is no LED today can be excited.



There are 11 different LEDs available for Colibri: from UV to dark red



Each LED is continuously adjustable and can be switched in the microsecond range

# Applications Infinite Diversity.

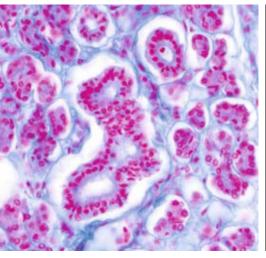
The more diverse the applications, the more flexible the imaging platform – that is what Axio Imager stands for. The modular architecture of Axio Imager 2 allows you to use a technology that optimally supports your application. And which grows with your performance requirements.

### Pathology

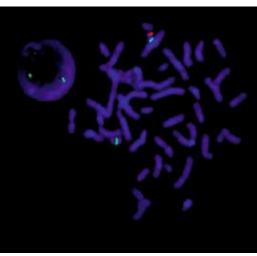
Axio Imager.A2 with LED illumination, the coded stand with Fixed Köhler illumination is ideal for pathology. In conjunction with EC Plan-NEOFLUAR or Plan-APOCHROMAT objectives it is the standard equipment for histological evaluation. The economical LED illumination has a long service life, consumes little energy, and requires no maintenance or adjustment. It provides incredible images, for instance with the typical H.-E-, DAB- or Azan staining techniques. Its constant color temperature ensures uniform light quality and brilliant image presentation over the entire intensity range.

### **Human genetics**

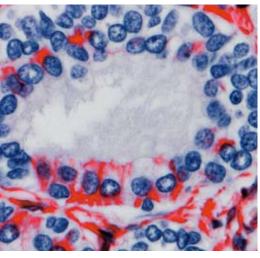
For the diagnosis of diseases which are due to a mutation in genetic material, genome analysis is a standard tool in human genetics. Karyograms are acquired and analyzed in transmitted light brightfield. The Fluorescence In Situ Hybridization (FISH) method identifies the gene loci on the chromosomes based on the DNA probes used and helps detect deviations from the healthy condition. In this context the Axio Imager provides complete support: the apochromatically corrected IC2S beam path illuminates the object field uniformly for all colors. The integrated light traps eliminate stray light in the illumination and imaging beam path. The 6-position reflector turret for Axio imager.A2 and Axio Imager.M2 as well as the 10-position reflector turret for Axio Imager.D2 and Axio Imager.Z2 allow rapid multi-channel image acquisition, the basis for FISH analyses. Control with AxioVision or MetaCyte from MetaSystems make the use of such complex applications as simple and reliable as possible.



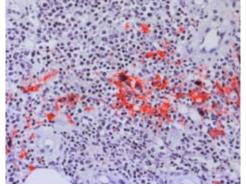
Salivary gland: azan staining; Orange: cytoplasm, Red: nuclei, Blue: collagen. Objective: Plan-APOCHROMAT 20x/0.8



Multi-color FISH preparation. Objective: Plan-APOCHROMAT 63x/1.4 Oil



Histological section – brightfield. Red: Anti-CD. Blue: nuclear counterstaining. Objective: Plan-APOCHROMAT 63x/1.4 Oil



Histological section – Red: CD61. Blue: nuclear counterstaining. Objective: EC Plan-NEOFLUAR 20x/0.5

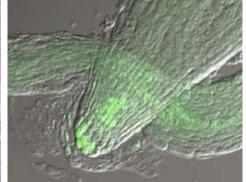


Histological section – Red: MPOX2. Blue: nuclear counter-

A. Schmitt-Gräff, Pathology, Univ. Freiburg, Germany

Objective: EC Epiplan-NEOFLUAR 10x/0.3.

staining.



Arabidopsis root thread – DIC superimposed fluorescence Green: GFP. Objective: EC Plan-NEOFLUAR 40x/0.75

### Histology

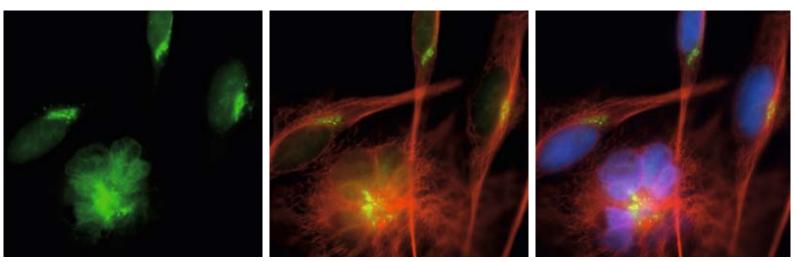
The requirements in histology and anatomy are optimum resolution in the image, perfect color presentation in the documentation of details and overviews and rapid, precise relocalization of diagnostically conclusive locations in the specimen. Ideally tailored to this: the EC Plan-NEOFLUAR and Plan-APOCHROMAT objectives in conjunction with motorized stages.

### Cell biology

The investigation of subcellular compartments such as the cell nucleus, mitochondria, vesicles or dynamic processes such as motility, mobility and cell division make special demands on the respective microscope systems. Axio Imager allows brilliant DIC, phase contrast, darkfield applications, and optical sections with ApoTome as well as fluorescence at the highest resolution. DIC and fluorescence can be combined most conveniently with the motorized Axio Imager.Z2 stand.

### Neurobiology

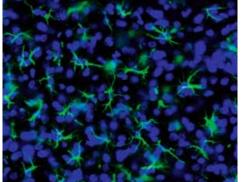
The samples are as different as the diverse range of topics in neurobiology: meaningful results must be obtained from individual cells and thin sections to thicker brain sections up to entire brains. Axio Imager is the ideal platform for this: excellent image quality in brightfield and fluorescence, high-resolution DIC for thick preparations and high-contrast DIC images for very thin sections. MosaiX provides high-resolution overview images of large specimens. The motorization of all important components and the use of the motorized DIC turret on Axio Imager.Z2 allow the storage of all important



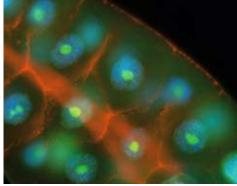
Red: alpha-tubulin

Blue: cellular nuclei (DAPI)

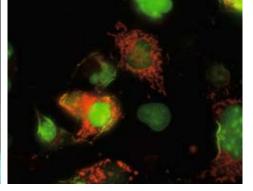
HeLa cells – multichannel image. Green: GFP Objective: Plan-APOCHROMAT 63x/1.4 Oil. L. Pelletier and T. Hyman, MPI for Molecular Cell Biology and Genetics, Dresden, Germany



Brain section (rat) – multichannel image with ApoTome. Green: GFP-labeled astrocytes. Blue: cell nuclei (DAPI). Objective: Plan-APOCHROMAT 20x/0.6. E. Fuchs, S. Bauch, DPC, Göttingen, Germany



Drosophila larval stage. Red: fibrillarin. Green: Venus-CG 8571-Transgene. Blue: cell nucleus (DAPI). Objective: EC Plan-NEOFLUAR 40x/0.75. M. Buszcak, A. Spradling, CIW-Dept. Embryology, MD, USA

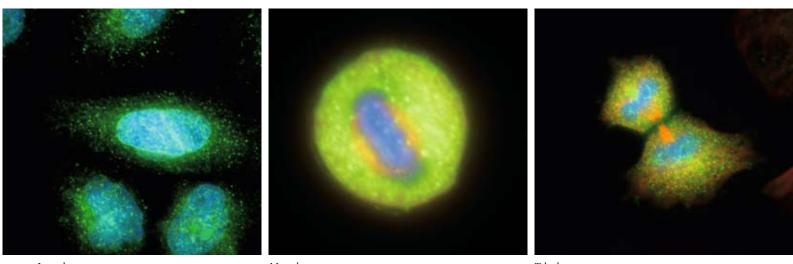


CHO cell culture. Green: GFP-histone. Red: dsRed, Objective: EC Plan-NEOFLUAR 40x/0.75. S. Haxelmans, R. Nitschke, Inst. Biologie I. Univ. Freiburg, Germany

settings for reproducible imaging and subsequent image analysis tasks.

### **Developmental biology**

The documentation and analysis of the processes which result in differentiation, regeneration or growth of cells, tissues and organisms make particularly high demands on a microscope system. Regardless of the animal model used, the highest performance of color fidelity, resolution and contrast is critical. Axio Imager provides you with the ideal uniform illumination in the common transmitted light contrast techniques, the best optical resolution, the extremely sample protecting fluorescence illumination with optimum signal-noise ratio to ensure brilliant image quality. With Axio Imager as the basis for an imaging system the processes to be investigated can be imaged at high spatial and temporal resolution and analyzed with different AxioVision Modules. The motorization of Axio Imager M- and Z-stands allows efficient and reproducible imaging. Beyond this the manipulation of the sample is clearly facilitated and the sample turnover increased with the help of the docking station and scanning stage.



InterphaseTelophaseHeLa cells – mitosis stages. Red: Alexa Fluor 594-DM1-alpha. Green: Alexa Fluor 488-Mad2. Blue: DNA (DAPI). Objective: EC Plan-NEOFLUAR 100x/1.3 OilH.Y. Li, Y. Xheng, HHMI & CIW, Dept. Embryology, MD, USA

## Imaging Systems. From Simple Observation to Analysis.

The type of task determines the system solution. Axio Imager 2 provides the appropriate system for every requirement of life science research. Sophisticated modularity and a wide spectrum of perfectly coordinated components guarantee perfect results. Quickly. At any time.

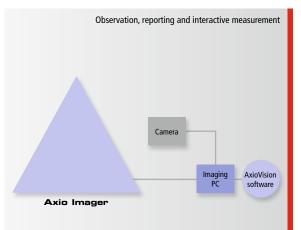
### Preconfigured and individual: the systems

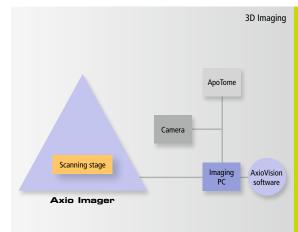
The demands on the relevant systems are as different as the nature of the tasks in life science research. The modular architecture of Axio Imager 2 allows you to make an individual configuration which is exactly tuned to your requirements. For the digital image documentation of 3 (x, y, z) to 6 dimensions (additionally t,  $\lambda$  and x,y location) Axio Imager can be expanded with highly sensitive cameras from the AxioCam family. AxioVision offers a large number of specific modules for subsequent image analysis.

#### Digital intelligence: AxioVision

AxioVision is the high-performance software for useroriented solutions in digital imaging. From image acquisition and processing to image analysis and archiving. AxioVision is practice-oriented, can be operated intuitively, and can be easily adapted to individual requirements. The modular design of the Carl Zeiss imaging software can be expanded in many ways. For example, for Zstack, multi-channel fluorescence or time-lapse images. AxioVision is the solution for growing demands.

Stand	Standard Equipment	Options	<b>Fields of Application</b>	Materials	Applications
A2 LED	<ul> <li>LED – Fixed Köhler Illumination transmitted light</li> <li>Lightmanager</li> <li>Encoded</li> </ul>	<ul> <li>Transmitted light beampath with manual filter wheel</li> <li>Reflected light beampath</li> <li>ApoTome</li> <li>Encoded stage</li> </ul>	Pathology     Histology     Cytology	<ul> <li>Histological staining</li> <li>Antibody staining</li> <li>Fluorescence In situ Hybridisation (FISH)</li> <li>Live cell staining on</li> </ul>	Evaluation     Fast routine work
A2	Universal stand transmitted light     Lightmanager     Encoded     Neutral density filter wheel	<ul> <li>Reflected light beampath</li> <li>ApoTome</li> <li>Encoded and 2-plate scanning stages</li> </ul>	<ul> <li>Biosciences research</li> <li>Medical sciences research</li> <li>Industrial research</li> <li>Bio-material-research</li> </ul>	samples of - Living cells - Fixed cells - Tissue sections - Whole-mount-samples	<ul> <li>Observation</li> <li>Image acquisition and reporting</li> <li>Interactive measurements</li> </ul>
D2	Universal stand transmitted light     Encoded     Partly motorizable:     Reflector turret	<ul> <li>Reflected light beampath</li> <li>Reflector turret 6x or 10x</li> <li>ApoTome</li> <li>Encoded and 2-plate scanning stages</li> </ul>	<ul> <li>Human Genetics</li> <li>Animal Genetics</li> <li>FISH-applications</li> </ul>		<ul> <li>Evaluation</li> <li>Image acquisition and reporting</li> <li>Semiautomatic measurements</li> </ul>
M2p	<ul> <li>LED – Fixed Köhler illumination transmitted light</li> <li>Convenience-Motorization: Parfocality, Condenser</li> <li>Encoded nosepiece</li> <li>Motorized z-drive with 25 nm step size</li> </ul>	<ul> <li>Transmitted light beampath with motorized luminous field stop</li> <li>Reflected light beampath</li> <li>TFT</li> <li>ApoTome</li> <li>LSM (entry level)</li> <li>2- and 3-Plate scanning stages</li> </ul>	<ul> <li>Pathology</li> <li>Histology</li> <li>Cytology</li> </ul>		<ul> <li>Evaluation</li> <li>Image acquisition and reporting</li> <li>Fast routine work</li> <li>Confocal Imaging (entry level)</li> </ul>
M2	<ul> <li>Universal stand transmitted light</li> <li>Motorized: Luminous field stop</li> <li>Lightmanager</li> <li>Contrastmanager</li> <li>Motorized z-drive with 25 nm step size</li> </ul>	<ul> <li>Reflected light beampath</li> <li>ACR for objectives</li> <li>ApoTome</li> <li>2- und 3-plate scanning stages</li> <li>2 TV Tube motorized</li> </ul>	<ul> <li>Biosciences research</li> <li>Medical sciences research</li> <li>Industrial research</li> <li>Bio-material-research</li> </ul>		<ul> <li>Automatic image acquisition and analysis</li> <li>3D Imaging</li> <li>Medium sample throughput</li> <li>Multi-User environment</li> </ul>
Z2	<ul> <li>High performance stand transmitted light</li> <li>Motorized: Luminous field stop</li> <li>Lightmanager</li> <li>Contrastmanager</li> <li>Motorized focus drive: <ul> <li>10 nm step size</li> <li>designed for loads up to max.</li> <li>9 kg</li> <li>designed for continuous operation</li> </ul> </li> </ul>	<ul> <li>Reflected light beampath</li> <li>ACR for objectives and filter cubes</li> <li>ApoTome</li> <li>2- and 3-plate scanning stages</li> <li>LSM</li> </ul>	<ul> <li>Biosciences research</li> <li>Medical sciences research</li> <li>Industrial research</li> <li>Bio-material-research</li> </ul>		<ul> <li>Automatic image acquisiton and analysis</li> <li>Certified image acquisition and archiving (CFR 21 part 11)</li> <li>3D imaging</li> <li>DIC-Fluorescence Imaging</li> <li>Confocal Imaging</li> <li>High sample throughput</li> <li>Multi-User environment</li> </ul>





### Proven and appreciated: the AxioCam family

Carl Zeiss offers a broad spectrum of digital cameras in different performance classes. The monochrome cameras are characterized by optimum resolution and highest sensitivity (12 or 14 bit dynamics) particularly in cases of faint fluorescent samples. The color cameras stand for the best color reproduction and highest resolution up to 12 megapixels per color channel. All the cameras have thermoelectric cooling and provide the option of rapid shutter synchronization. All AxioCam cameras are characterized by rapid live image and complete integration in the Carl Zeiss system world.

### Highly stress resistant: motorized focus and high-performance focus

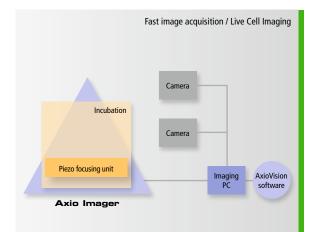
The Axio Imager offers you two different versions of the z-motor. The standard design with a step size of 25 nm at a reproducibility of  $\pm$  75 nm is always part of the M-stand configuration. And for the highest requirements such as LSM or Z-stack imaging with small intervals, a high-performance focus is available for the Axio Imager.Z2. It has a step size of only 10 nm with a reproducibility of  $\pm$  10 nm – and that at a 3-fold higher traverse rate. It was specifically developed for continuous use (24 hours / 7 days) and even with large stages guarantees absolutely precise focus movements over long periods.

### **Optical sections with ApoTome**

ApoTome has firmly established itself as the standard method in high-end research in the life sciences. For the first time it can be used with all the stands in the Axio Imager 2 family: The ApoTome slider is simply inserted in the luminous field diaphragm plane of the reflected light beam path. Via the principle of fringe projection, precise optical sections are created online. With elevated contrast and clearly increased axial resolution. The ideal solution for tissue sections and thicker, fixed samples.

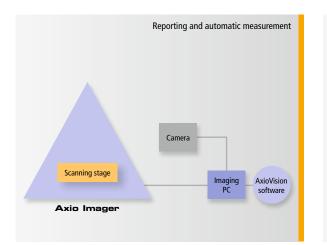
### No stray light ever again: AxioVision 3D deconvolution

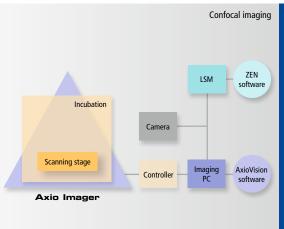
Deconvolution from Carl Zeiss calculates mathematically the stray light from outside the focal plane back to its origin. In this manner the object recorded in the 3D image stack is "unfolded". The result is a first-class image quality particularly in samples with extremely weak fluorescence where a high light yield is essential.





High-end research system with Axio Vision





# Precisely on the spot: motorized stages and z-piezo insert

They allow a precisely accurate approach to positions and the highest degree of reproducibility. Via highly sensitive piezo or step motor every desired position can be exactly set and relocated.

- Piezo stage: step size 0.2 µm, reproducibility: +/- 0.6 µm
- Mechanical stage: step size 0.1 µm, reproducibility: +/- 0.3 µm
- New stage control for stages with DC motors for direct coupling with the motorized stages (magnification-dependent traverse rate): highest reproducibility and precision in high-end applications
- z-Piezo focusing insert with 100 µm focusing range for rapid imaging with Colibri and z stack images; resolution 5 nm, reproducibility: +/- 1 nm, max. additional load 2 kg, for frame size 222 x 139 mm, available mounting frames for all common preparation shapes

The scanning stages are the prerequisite for all automated imaging techniques such as MosaiX or Mark&Find.

### New stimuli for your research: the LSM family

Confocal microscopy at the highest level: LSM 700, LSM 710 and LSM 7 MP belong to the seventh device generation of the Laser Scanning Microscope from Carl Zeiss. The use of the same first-class system components and the same software in the entire device class ensures outstanding performance and image quality without any compromises. The result: an excellent price-performance ratio. A novel beam path ensures excellent laser suppression and maximum registration of emission and results in breathtaking images. Demanding tasks such as spectral imaging, FRET, FRAP or colocalization analysis are easily managed with unprecedented image quality and high scanning speed.

LSM 710 NLO and LSM 7 MP are ideally suitable for highly sensitive deep examination of living preparations or organisms. Both systems are characterized by unrivalled sensitivity. Highly effective non-descanned detection ensures efficient depiction in deep tissue layers. These are the systems of choice for long-term developmental studies, patch-clamp- and uncaging-experiments.

#### Precision in z: the closed loop system

Axio Imager.Z2 with the focus linear sensor offers anyone who has to fulfill extremely high requirements precision of  $\pm 1$ nm in the z-direction. On the one hand, the application-independent movements of the microscope stage are detected and readjusted automatically. And, on the other hand, highly precise and reproducible Z-stacks are ensured with z-steps of equal size, which gives maximum control and reliability.



LSM 710 with Axio Imager.Z2

## Ergonomy and Ease of Operation. Efficient and Relaxed Working.

Axio Imager is intelligent technology with a trendsetting control concept. Even the most demanding experiments and long working sessions at the microscope become simple and efficient. Automated procedures allow rapid, intuitive control with either manual or motorized components, depending on the individual requirements.

### Efficient, rapid, comfortable: the Touch Screen

A good thing has been made even better: The control software of Axio Imager 2 collects all of the critical functions on one touch-sensitive TFT display. All motorized components are controlled with a touch of your finger, and their status is also displayed. The integrated light and contrast managers constantly adjust the light and contrast settings optimally.

- The Contrast Manager's control and user guidance adhere to the logic and workflow of all applications.
- Motorized components can be optionally switched to automated or manual control.
- The Favorites Page allows access to frequently used functions when switching ON the microscope.

• Individual settings can be defined for up to 10 different users.

# Ergonomically well-conceived: Control buttons and exchangeable fine drive

Ease of operation redefined: the control buttons which have been ergonomically arranged around the focus drive can be easily distinguished by their tactile surfaces. The two different fine drive buttons of the focus drive are exchangeable and can be optionally used for right or left. The motorized stand has ten freely assignable control buttons. The manual stand allows the simple setting of light intensity as well as switching of the motorized shutter in reflected and transmitted light via five preconfigured buttons.



Ergophototube for perfect convenience

 $\ Ergonomically\ distributed\ control\ buttons$ 

Ideal arrangement of the diaphragm slider and filter wheel in reflected light





 $ACR\ reflector\ module$ 

ACR objective. ACR detects objectives and reflector modules automatically

Communication connections

### Provides mobility: the control panel

Axio Imager can also be remote controlled via a free positionable control panel. Among other things, this panel has a focus drive and a brightness control. Additional arbitrary functions can be programmed. The panel provides an interface for the TFT and for the x, y-control of the motorized mechanical stage.

### Error-free control with ACR

ACR (Automatic Component Recognition) stands for the innovative concept of automatic recognition of objectives and reflector modules on the Axio Imager.Z2. When changed, the replaced components are immediately registered in the system. An important advantage for ease of operation and safety: operating errors and time-consuming programming are avoided.

### Absolutely stable: the Imaging Cell

The key elements of Axio Imager such as the nosepiece, z-guidance and the stage are decoupled from the remainder of the stand as a stable cell. The entire unit has been designed to be practically vibration-free and insensitive to thermal influences. Even in the long-term it provides the highest possible stability and absolute freedom from vibration. Ideal preconditions for imaging, particularly in long-term experiments and in time lapse imaging.



Switching of illumination on the TFT



The TFT display on the stand or in the docking station provides a transparent menu guide for control and configuration



*Control of the motorized stages via the Docking Station* 

## Stand Design. Flexibility Times 6.

Advanced technology assures that the user will select the appropriate system. The sophisticated stand design of Axio Imager 2 and well-conceived, preconfigured packages guarantee you an appropriate, configuration that meets the most demanding applications.

### Convincing technology: the stand

Progressive down to the smallest detail – even in the basic configuration, all stands have an interface to the control computer. The parameters of encoded or motorized components can be read out or controlled directly by AxioVision.

### • Axio Imager.A2 LED

Ideally appropriate for brightfield applications in transmitted light: an LED light source ensures a constant color temperature across the entire intensity range.

### • Axio Imager.A2 and M2

More flexibility: interfaces for sliders in the reflected light beam path allow convenient working with either aperture or field stop diaphragms or an attenuator in fluorescence. Optional on the Axio Imager.M2: motorized filter wheels and diaphragm sliders in reflected light (M2m) or in transmitted light (M2).



Axio Imager.A2 LED

Axio Imager.A2

Axio Imager.D2

### • Axio Imager.M2p

Automatic parfocality compensation, a light manager, the motorized condenser and manual objective changing make routine work, e. g., in pathology comfortable and efficient.

### • Axio Imager.D2

The manual high-end stand can be equipped with a 6x- or 10x-motorized reflector turret, which, above all, make fluorescence applications comfortable and fast.

### • Axio Imager.Z2

The stand has been developed to meet the most stringent requirements. A high-performance focus allows constant operation with a high sample throughput. It ensures precise focusing movements over long periods and also when using large and heavy sample stages up to 9 kg.



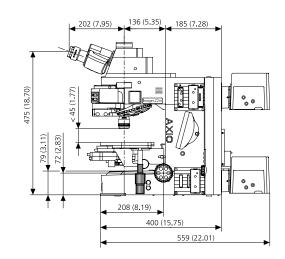
Axio Imager.M2

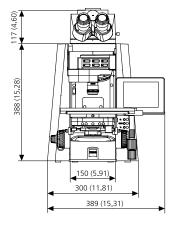
Axio Imager.M2p

Axio Imager.Z2

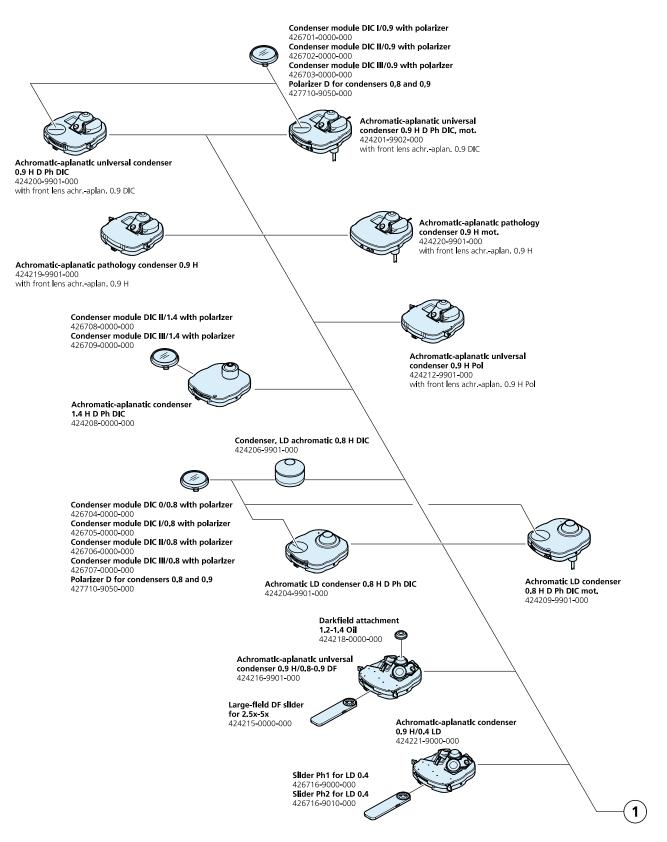
Stand         manual         +         -         0	Axio Imager 2 – Flexibility for all application an	eas										
motorized         -         +        +         +         +<	Component	Option	A2 LED	A2	M2p	M2	D2	Z2	A2m	M2m	D2m	Z2m
Fixeding         readout by computer         +         0 </th <th>Stand</th> <th></th> <th>+</th> <th>+</th> <th>-</th> <th>-</th> <th></th> <th>-</th> <th>+</th> <th>-</th> <th></th> <th>-</th>	Stand		+	+	-	-		-	+	-		-
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motorized         -         -         0         0         -         0         -         0	Encoding											
Beflector turret       6x encoded       0<	Tube lens turret			-	-	-		-				
6k motorized       -       -       0       0       0       -       +       0       0         Nosepiece       6k enoded PD       0       0       -       0					-			-		-		
6x motorized ACR*       -       -       -       0       0       -       0       0         Nosepiece       6x encoded PD/C       0 </td <td>Reflector turret</td> <td></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td></td> <td></td> <td></td> <td></td>	Reflector turret		-	-	-	-	-	-				
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Nozepiece         6x encoded HD IC         0          Modulator turret for C-DIC/TIC         matorized*****         -         -         -         +         +         +         +         +         +         0         0         0         0         0         0         0         0         0         0         0         0         0				-	-							
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7x motorized HD         -         -         0         -         0         -         0         -         0         -         0					-	-		-		-		
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motorized*****         -         -         0         -         0         -         0         -         0         -         0         -         0         -         0		7x motorized HD			-	0		-				
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Stage carrier with condenser carrier, detachable       0 mm - 25 mm Sample height       0 <td></td> <td></td> <td>-</td> <td>-</td> <td>-</td> <td>0</td> <td>-</td> <td>0</td> <td>-</td> <td>0</td> <td>-</td> <td>0</td>			-	-	-	0	-	0	-	0	-	0
Stage carrier detachable, for attachable condense carrier         0 mm - 45 mm Sample height         0          Reflected light memorized <td>Modulator turret for DL- DIC</td> <td></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>0</td> <td>-</td> <td>-</td> <td>-</td> <td>0</td>	Modulator turret for DL- DIC		-	-	-	-	-	0	-	-	-	0
Stage carrier detachable, for attachable condense carrier         0 mm - 45 mm Sample height         0          Reflected light memorized <td>Stage carrier with condenser carrier, detachable</td> <td>0 mm - 25 mm Sample height</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td>	Stage carrier with condenser carrier, detachable	0 mm - 25 mm Sample height	+	+	+	+	+	0	0	0	0	0
Transmitted light beam path       manual       -       +       -       +       -       0       0       0       0         LED transmitted light       -       +       0       +       0			0	0	0	0	0	0	0	0	0	0
motorized         -         +         +         +         -         -         -         0           Deuble filter wheel transmitted light         manual         -         +         0 <t< td=""><td>Stage carrier reflected light, detachable</td><td>0 mm - 63 mm Sample height</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></t<>	Stage carrier reflected light, detachable	0 mm - 63 mm Sample height	0	0	0	0	0	0	0	0	0	0
LED transmitted light       -       +       0       +       0	Transmitted light beam path	manual	-	+	-	-	+	-	0	0	0	0
Double filter wheel transmitted light         manual         -         +         -         0          Double filter wheel reflected l		motorized	-	-	-	+	-	+	-	-	-	0
motorized         -         -         0         -         0         0         0         0         0         0         0         0         +         -         +         -         0           Reflected light beam path         manual***         0         0         0         0         0         0         +         -         +         +         +           Luminous field stop slider reflected light         manual         0 <td< td=""><td>LED transmitted light</td><td>-</td><td>+</td><td>0</td><td>+</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></td<>	LED transmitted light	-	+	0	+	0	0	0	0	0	0	0
Reflected light beam path       manual***       0       0       0       0       +       -       0	Double filter wheel transmitted light	manual	-	+	-	0	0	0	0	0	0	0
motorized***         -         -         -         0         -         +         +         +           Luminous field stop slider reflected light         manual         0		motorized	-	-	-	0	-	0	-	-	-	0
motorized***         -         -         -         0         -         +         +         +           Luminous field stop slider reflected light         manual         0	Reflected light beam path	manual***	0	0	0	0	0	0	+	-	+	-
Luminous field stop slider reflected light       manual       0       0       0       0       0       +       0       +       0         Aperture stop slider reflected light       manual       0			-	-	-	-	-	0	-	+	-	+
motorized         -         -         -         -         0	Luminous field stop slider reflected light		0	0	0	0	0	0	+		+	
Aperture stop slider reflected light         manual         O			-			-						
motorized       -       -       -       -       0       -       0       -       0	Aperture stop slider reflected light		0	0	0	0	0		0		0	
Double filter wheel reflected light         manual         0								-				
motorized         -         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0	Double filter wheel reflected light		0	0	0	0	0	-	0	-		
Fluorescence attenuator       manual       0 <th< td=""><td></td><td></td><td></td><td>-</td><td></td><td>-</td><td></td><td>-</td><td></td><td></td><td></td><td></td></th<>				-		-		-				
motorized         motorized         -         -         -         -         -         0         0         0         0         0         0         0         0         0         0         0         0         0         0         0         0         0         1         +	Fluorescence attenuator		0	0	0		0	-				-
Lamp switch reflected light/transmitted light       manual       +       +       -       +       +       -       +       -       +       +       -       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       +       -       +       +       -       +       +       +       -       +       +       +       -       +				-		-						-
software       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +<	Lamp switch reflected light/transmitted light							-				
Mixed light with additional external power supply       manual       +       +       -       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -												
software       -       +       +       -<	Mixed light with additional external power supply											
Focus (z-Axis)       manual       +       +       -       +       +       -       +       -       +       +       -       +       -       +       +       -       +       +       -       +       +       +       +       -	Mixed light with additional external power supply											
motorized 25 nm Step size         -         +         +         -         -         +         +         -         -         +         -         -         +         -         -         +         -         -         +         -         -         +         -         -         +         -         -         +         -         -         +         -         +         -         +         -         +         -         +         -         +         -         +         -         +         -         +         -         +         +         -         +         -         +         +         -         +         -         +         +         -         +         +         -         +         +         -         +         +         -         +         +         -         +         +         -         +         +         +         -         +         +         +         +         -         + <td>Focus (z-Axis)</td> <td></td>	Focus (z-Axis)											
High Performance Focus motorized 10 nm Step size       -       -       -       +       -       -       +       +       -       +	Tocus (2-Axis)											
motorized 10 nm Step size       -       -       -       +       -       -       +       -       -       +       +       -       +<			-	-	+	+	-	-	-	+	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
TFT-Display       -       -       0       +       -       +       -       +       -       + <td< td=""><td></td><td></td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>+</td><td>-</td><td>-</td><td>-</td><td>+</td></td<>			-	-	-	-	-	+	-	-	-	+
ApoTome       -       0 </td <td>TET Display</td> <td></td> <td></td> <td></td> <td>0</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	TET Display				0							
Power supply         external         -         +         +         -												
internal       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       -       +       +       +       -       +       -       +       +       -       +       -       +       +       +       -       +       +       -       +       +       -       +       *       -       +       *       *       *       *<			-									
Mechanical stage CAN         motorized****         0         <	гомет зарру					+				+		
Scanning stages         Piezo         0	Mashanial stars CAN					-				-		
DC / Stepper motors         O					-	-	-	-				
Fast z-piezo insert         with manual stage         0	Scanning stages			~ ~		-						
with scanning stage         0	Frank and the state of the stat					-	-					
2 TV tube head motorized         -         -         0         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0         -         0 <td>Fast z-piezo insert</td> <td></td> <td></td> <td>-</td> <td></td> <td>-</td> <td></td> <td></td> <td></td> <td>-</td> <td></td> <td></td>	Fast z-piezo insert			-		-				-		
Condenser manual 0 0 0 0 0 0 0 0 0 0		0 0	-	-	-	-	-	-				
					-	-		-				
motorized 0 0 - 0 - 0 - 0	Condenser		0	0		-	0				0	
		motorized	-	-	0	0	-	0	-	0	-	0

- + = Included in stand
- O = Optionally available
- = Not available
- = Motorized (6x und 10x) reflector revolver can be used
- \*\* = ACR function not possible with "Axio Imager" D1 and D1m
- \*\*\* = A motorized shutter is included in every reflected light illumination. For fluorescence applications this can optionally be replaced by a High speed shutter
- \*\*\*\* = For the use at the "Axio Imager" A2 LED, A2, A2m, D2 and D2m USB/CAN Converter 432909 is required
- \*\*\*\*\* = Only in combination with motorized objective nosepiece
- *m* = Optimized for materials applications

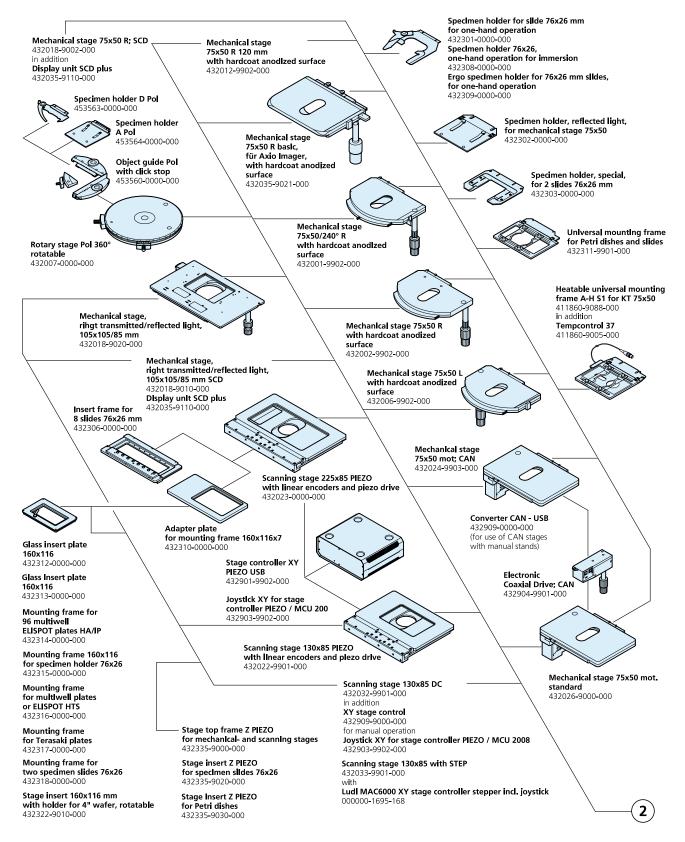




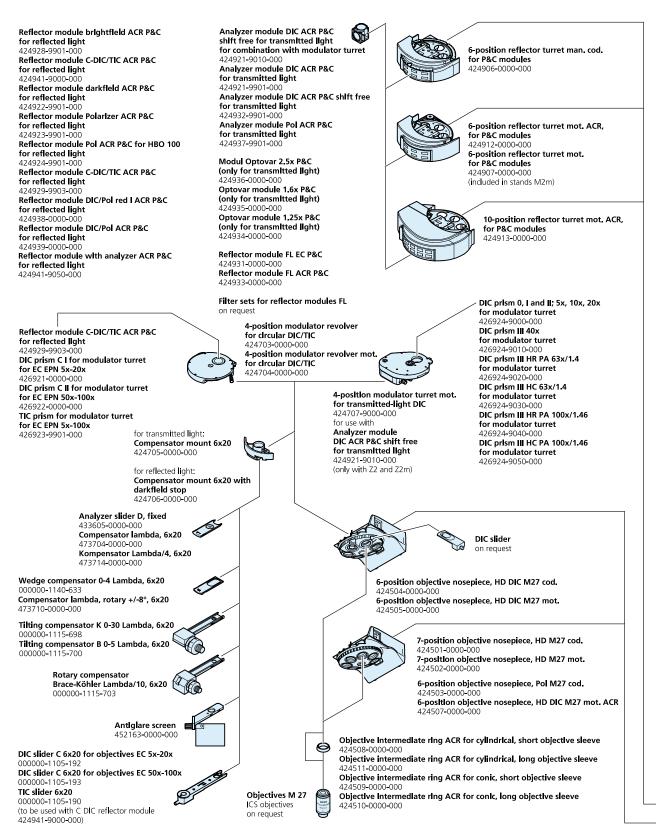
### Condensers



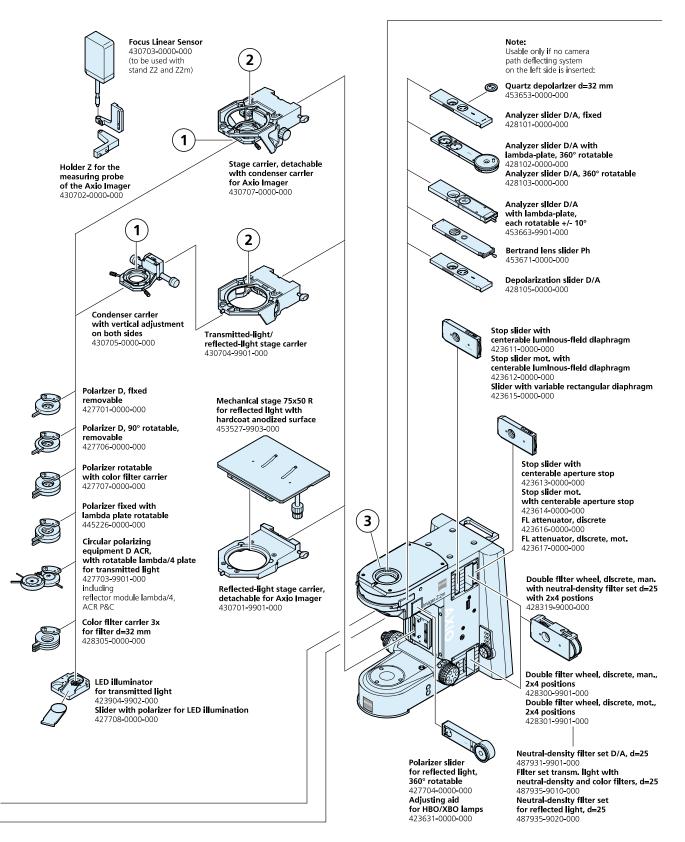
### **Microscope stages**



### Objective nosepieces, reflector turrets, filters, prisms



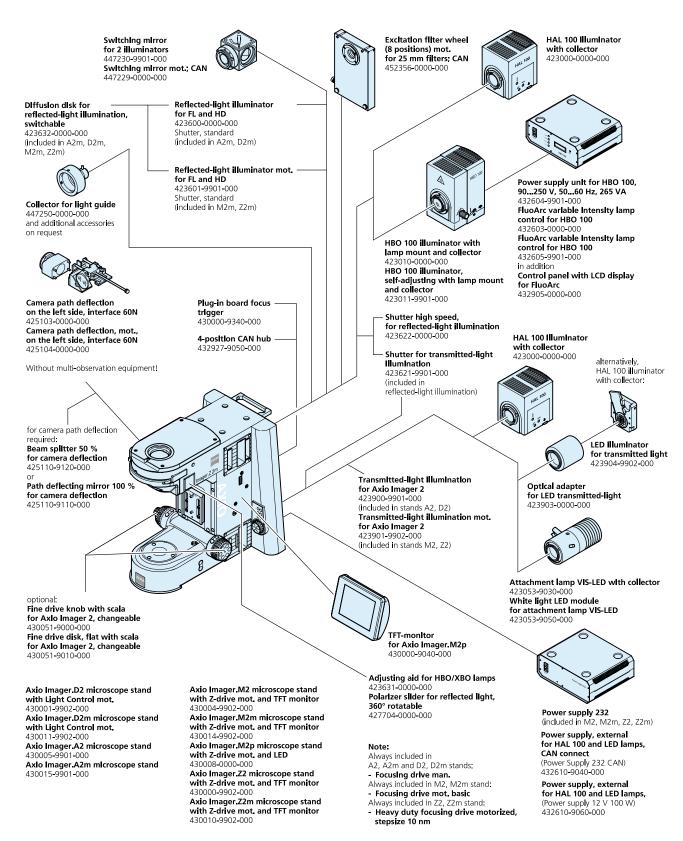
### Stand, stage carriers, polarizers, sliders



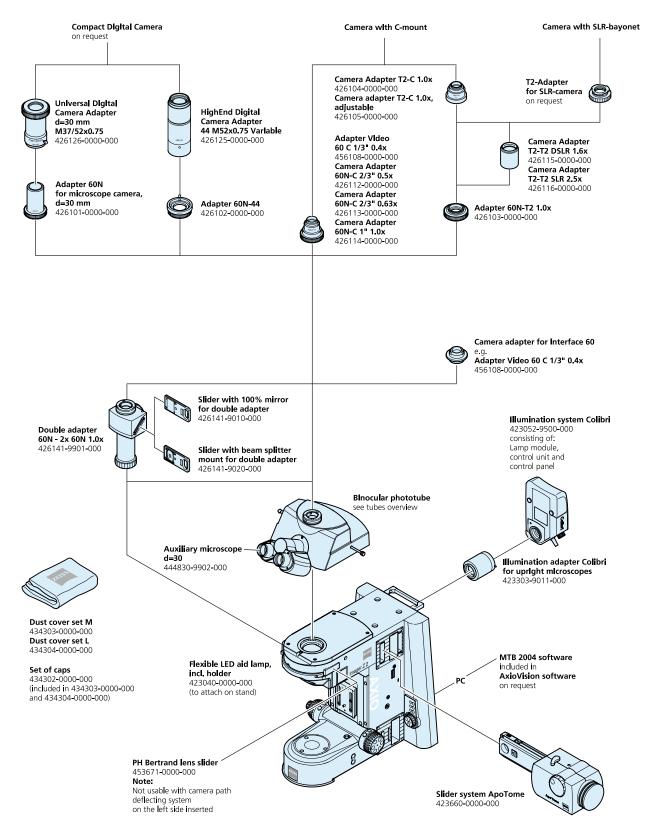
#### Binocular tube 30°/23, reversed Image, Binocular tube 30°/25, Axio Imager reversed image 425520-9060-000 425500-0000-000 **Binocular phototube** Binocular phototube 30°/25 (30vls/70doc), 30°/23 (50:50), reversed Image, Okulareinlegeplatten Axio Imager 425520-9070-000 reversed image on request 425501-0000-000 (MOH) O OO Binocular Ergotube 20°/23, Binocular phototube reversed image variable, 30°/25 (100:0/30:70/0:100), Eyepiece PL 10x/25 Br. foc. continuous vertical reversed image 444034-9000-000 Eyeplece E-PL 10x/25 Br. foc. adjustment 44 mm 425511-0000-000 425502-0000-000 444234-0000-000 Eyepiece E-PL 10x/23 Br. foc. 444235-0000-000 Binocular phototube Eyepiece PL 10x/23 Br. foc. 30°/25 mot. 444036-9000-000 **Binocular Ergophototube** (100:0/30:70/0:100), 20°/23 (100:0/0:100), with two camera ports. reversed image variable, reversed image continuous vertical 425504-0000-000 adjustment 44 mm 425512-0000-000 Binocular phototube 30°/25 (100:0/30:70/0:100), Binocular Ergophototube 20°/23 MAT (100:0/0:100), reversed Image with upright image variable, motorized eyepiece shutter, 425506-0000-000 Auxillary microscope, continuous vertical adjustment 44 mm d=30 mm 444830-9902-000 425514-0000-000 Binocular phototube Pol 15°/23 (100:0/0:100), Binocular phototube 15°/25 (100:0/0:100), upright Image Including upright image, adjustable stop 425503-9901-000 quartz depolarizer 425517-0000-000 -:~ Comfortable binocular Eraophototube 15°/23 (50:50), Ø upright image variable, continuous horizontal and vertical adjustment of the binocular component 425515-0000-000 Tube carrier multidiscussion for 2 tubes, connect linear left/right 425145-9020-000 Tube carrier multidiscussion for 1 tube, arm left deflection, connect Tube lens 1.25x Tube lens 2.5x 425145-9030-000 425305-0000-000 425303-0000-000 Tube carrier multidiscussion for 1 tube, arm right deflection, connect Tube lens 4.0x Tube lens 1.6x 425145-9040-000 Tube carrier multidiscussion for 2 tubes, end panel linear, I/r 425304-0000-000 425307-0000-000 425145-9050-000 Tube carrier for 1 Co-observer, light-intensive, end panel, left 425145-9060-000 Note: Center component for multidiscussion, The tubes 425500-0000-000 for tube carrier left and right 425502-0000-000 425141-9901-000 425503-9901-000 with: 425506-0000-000 to be equipped at most with three Filters grey 425141-8032-000 and 425515-0000-000 tube lenses: 5-position tube lens turret, cod., can be combined with a tube lens turret with Bertrand system or the center component Note: 425309-9901-000 for multidiscussion equipment. S. with tube-lens 1.0x For tube lens turrets the eyepieces 5-position tube lens turret mot., PL 10x/25 Br. foc or PL 10x/23 Br. foc have to be used. with Bertrand system 425302-9901-000 3 with tube-lens 1.0x

## Tubes, eyepieces, tube lens turrets, multidiscussion

### Stand, illumination



## Documentation





Product Information Version 1.0 **ZEISS ApoTome.2** Optical Sections in Fluorescence Imaging



# Simply Brilliant: Perfect Optical Section Thickness for All Magnifications

>	In Brief
>	The Advantages
>	The Applications
>	The System
>	Technology and Details
>	Service

Create optical sections of your fluorescent samples – free of scattered light. With structured illumination, you know that only the focal plane appears in your image: ApoTome.2 recognizes the magnification and moves the appropriate grid into the beampath. The system then calculates your optical section from three images with different grid positions without time lag. It's a totally reliable way to prevent scattered out-of-focus light, even in your thicker specimens. Yet your system remains just as easy to operate as always. You get images with high contrast in the best possible resolution – simply brilliant optical sections.





# Simpler. More Intelligent. More Integrated.

### > In Brief

#### in brief

#### > The Advantages

- > The Applications
- .....
- > The System
- > Technology and Details
- .....
- > Service

### Perfect Images –

### with All Magnifications

Because your applications need different objectives, you need a system that gives you the best resolution for each one. ApoTome.2 automatically uses the right grid for your objective, selecting from three grids with different frequencies. With a defined optical section thickness in the region of a Rayleigh unit, the image is simply brilliant.

### Optimum Results – Free Choice of Light Source and Dyes

From conventional HBO illumination to adjustmentfree metal-halide lamp HXP 120 C to Colibri.2, the LED illumination source that is gentle on your samples: with ApoTome.2 you use exactly the light you need. ApoTome.2 also gives you the choice of fluorophores. Whether you work with DAPI, FITC, Rhodamin, Cy5 or with vital dyes such as GFP or mRFP, it's your decision, not the technology's. Just change the filter and your system automatically moves the grid to the correct position. From DAPI to Cy5, you get perfect optical sections for multichannel imaging.

### Brilliant Images – Even with Thick Specimens

Your optical section thickness is close to one Rayleigh unit, a value that stands for high axial resolution with a good signal-to-noise ratio. ApoTome.2 increases the resolution in Z direction compared to conventional fluorescence microscopy: you obtain brilliant optical sections that allow 3D-rendering, even from thick specimens.







Rat, hippocampus, triple fluorescence, maximum-projection of 3D image-stack. Objective: Plan-APOCHROMAT 63x/1.4 E. Fuchs & S. Bauch, DPZ Göttingen, Germany

# **Three Grids Deliver Optimal Optical Section Thickness**

#### . . . .

### > In Brief

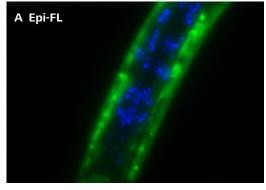
#### > The Advantages

- .....
- > The Applications
- > The System
- > Technology and Details
- -
- > Service

### Figure A:

# Acquisition with conventional epifluorescence illumination

Emission light from areas outside of the focal plane is detected. Contrast and resolution are reduced, depending on thickness of specimen.



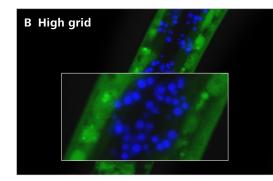
C. elegans, whole mount, green: GFP, blue: DAPI Objective: Plan-APOCHROMAT 20x/0.8 Prof. Schnabel, TU Braunschweig, Germany

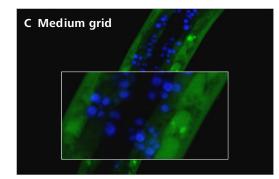
### Figures B – D:

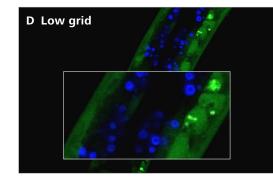
### Optical sections with different thickness

No matter which magnification you are using – ApoTome.2 automatically places the right grid in the beampath of your microscope. Reduction of unwanted background fluorescence increases with the grid frequency and the optical sections become thinner.

Structures from outside of the focal plane are suppressed (Fig. B, C and D). This improves contrast and resolution of the optical section. "Low grid" delivers the optimal section thickness in our example (Fig. D). Images of this type are particularly suitable for 3D analyses and the processing of your image data with rendering software.







# Your Insight into the Technology Behind It

### > In Brief

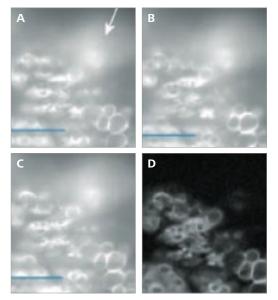
#### > The Advantages

- > The Applications
- The Content
- > The System
- > Technology and Details
- .....
- Service

### ZEISS ApoTome.2

### Brings You Structured Illumination

ApoTome.2 projects a grid structure into the focal plane of your specimen, then moves it into three positions using a scanning mechanism. At each grid position, ApoTome.2 automatically acquires a digital image. The system processes the three images into one optical section with improved contrast and increased resolution using a patented algorythm. The image that emerges is free from grid structures.



Schematic illustration of the grid projection. A – C: raw images with different positions of grid D: optical section through sample



Animation from www.zeiss.com/campus, © Mike Davidson, FSU, Tallahassee

#### ZEISS ApoTome.2 Grid in the Beampath

Fluorescence excitation light passes through two glass plates in the ApoTome.2 slider. When a grid structure is applied to the first glass plate, the grid pattern is "imprinted" in the excitation light. A scanning mechanism tilts the second glass plate and the image of the grid is laterally shifted in the focal plane of the specimen.

# **Tailored Precisely to Your Applications**

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ApoTome.2 is the cost-effective solution for creating optical sections with high contrast. Use this to your advantage in a wide range of applications from cell culture preparations via tissue sections to whole embryos.

Typical Applications / Typical Specimens	Task	ZEISS ApoTome.2 offers				
Cell Culture	2D imaging	2D single images possible				
	Fast imaging of a 2D image	Optical section available online on the monitor				
	Reliable detection of the marker even with strong background fluorescence	Automatic grid selection ensures optimum contrast with each objective				
	Combination of multiple contrast techniques	Any combination of fluorescence channels, brightfield, DIC and phase contrast. Each fluorescence channel can be individually configured as an optical section or widefield image				
Live Cell Imaging Vibratome Sections, Histological Samples	Reduction of phototoxicity	Particularly low in combination with LED illumination and EMCCD cameras				
	Time-lapse images	Depending on the exposure time, up to three images per second. Doubling of the frame rate with "burst mode"				
	3D imaging	Automatic selection of the optimum grid for each objective				
	Modification of the optical section thickness	Grid freely selectable depending on the specimen				
	Penetration depth	Depending on the optical density of the tissue				
	3D reconstruction	Rendering of the image stack via integrated software func Automatic transfer of the parameters of the individual fluorescence channels				
	Quantitative analysis	Automatic calibration of the system: reproducible size measurements				
Whole Mounts	3D imaging	Multi Channel, Z Stack and Time Lapse, Deconvolution, images in raw data mode, 3D Rendering				
	Large image areas	Automatic acquisition of large sections using Tiles & Positions				

# ZEISS ApoTome.2 at Work

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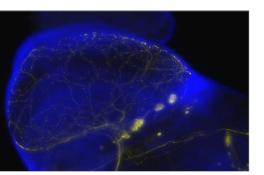


Figure A: Conventional fluorescence

Drosophila neurons, blue: DAPI, green: GFP. Objective: Plan-APOCHROMAT 20 x/0.8. Marta Koch, Molecular and Developmental Genetics, University of Leuven, Belgium

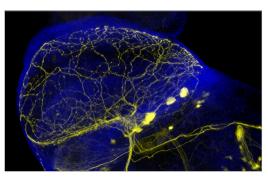


Figure B: Optical section

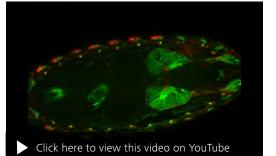


Figure C: Drosophila embryo, green: HRP, red: glia marker, 100 μm Z-stack

C. Klämbt, Institute for Neurobiology, University of Münster, Germany

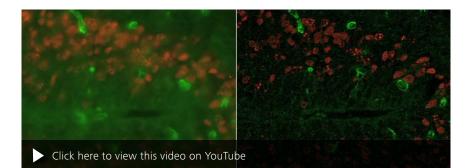


Figure D: Mouse embryo, tissue section, green: GFP, red: Cy3 Objective: Plan APOCHROMAT 40 x/1.3 Oil N. Büttner, T. Vogel, Centre for Anatomy, University of Göttingen, Germany

# **Your Flexible Choice of Components**

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### 1 Microscope

- Axio Observer, Axiovert 200 (inverted research microscope)
- Axio Imager.2, Axio Imager.Z1, Axio Imager.D1 (upright research microscope)
- Axio Zoom.V16 (Zoom microscope)
- Simple upgrading of existing systems

### 2 Objectives

Recommended objective classes with the highest level of image quality:

- C-APOCHROMAT
- Plan-APOCHROMAT
- EC Plan-NEOFLUAR

### **3 Illumination**

- Colibri (LED)
- HXP 120 C (metal halide)
- HBO (mercury vapor lamp)
- XBO (xenon)

### 4 Cameras

- Recommended cameras with high dynamic range (thick samples: at least
  - 1 : 2000; thin samples at least 1 : 1000; digitalization at least 12 bit)
- Axiocam MRm

 Alternatively, you can control these cameras: Photometrics CoolSnap HQ, Hamamatsu Orca ER2 (cameras with pixel size providing a sampling rate of < 5. This allows a pixel size of approx.</li>
 6.5 µm (Imaging in Neuroscience and Development 2005; Chapter 101; pp. 805–813)

### 5 Software

Recommended ZEN modules:

- Multi Channel, Z Stack, Time Lapse (imaging)
- Tiles & Positions (imaging with scanning table)
- 3D VisArt (rendering multidimensional image stacks)
- Image analysis modules such as Image Analysis, Colocalization

# **Expand Your Possibilities**

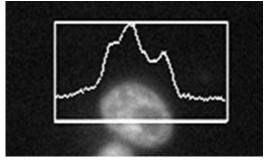
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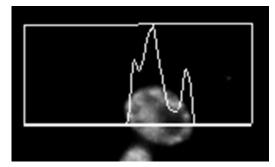
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### Deconvolution

Improve the image stacks you create with ApoTome.2 even more with deconvolution, using a patented algorithm for structured illumination:

- Acquire image stacks in raw data format individual images are saved for the different grid positions.
- Switch between conventional fluorescence and optical section after image acquisition.
- Deconvolution processes the raw data with a special algorithm for structured illumination.
- Enjoy improved image quality, contrast, axial and lateral resolution.
- The efficient suppression of any existing noise improves recognition of the object structures.





*Example image of yeast cells: (above) optical section, (below) result of deconvolution.* 

#### Literature:

L. H. Schaefer, D. Schuster & J. Schaffer, "Structured illumination microscopy: Artefact analysis and reduction utilizing a parameter optimization approach", Journal of Microscopy, Vol. 216, Pt 2 November 2004, pp. 165–174.

# **System Overview**

> In Brief

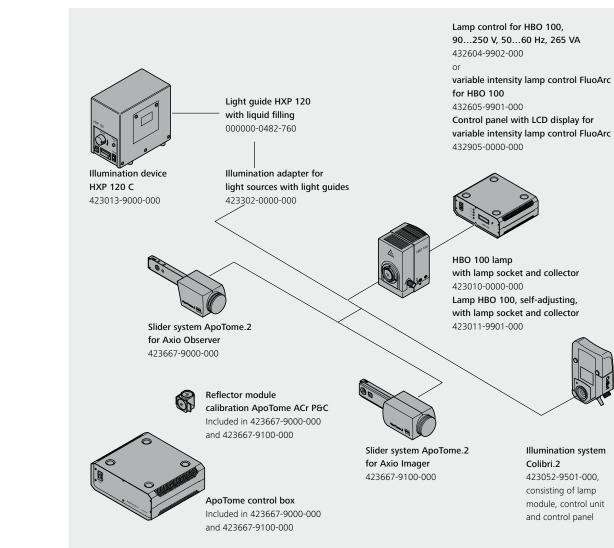
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### ApoTome.2 is Compatible with

### these Stands from ZEISS:

- Axioplan 2 imaging (serial numbers: from 35 11 000001; from 35 10 000001; from 35 02 000001)
- Axio Imager.D1 and Axio Imager.Z1, Axio Imager.A2
- Axio Imager.M2
- Axio Imager.D2 and Axio Imager.Z2
- Axiovert 200M, Axio Observer.A1
- Axio Observer.D1 and Axio Observer.Z1
- Axio Zoom.V16



# **Technical Specifications**

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Grid Table: ApoTome.2 generates optical sections of a defined thickness (in Rayleigh units, RU and microns, µm) depending on wavelength, microscope and objective used.

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Objectives for Axio Imager	v	NA	Immersion	Grid/Section	[RU/µm]	DAPI with	DAPI with	
				High grid	Medium grid	Low grid	FS34	FS49
EC Plan-NEOFLUAR	10 x	0.3	Air	2.9/31.9	1.7/18.2	0.9/9.9	Yes	Yes
EC Plan-NEOFLUAR	20 x	0.5	Air	2.4/9.2	1.4/5.3	0.7/2.9	Yes	Yes
EC Plan-NEOFLUAR	40 x	0.75	Air	1.6/2.8	0.9/1.6	0.5/0.9	Yes	Yes
EC Plan-NEOFLUAR	40 x	1.3	Oil	2.5/2.2	1.4/1.2	0.8/0.7	Yes	Yes
EC Plan-NEOFLUAR	63 x	0.95	Air	1.0/1.1	0.6/0.7	0.4/0.4	Yes	No
EC Plan-NEOFLUAR	63 x	1.25	Oil	1.6/1.5	0.9/0.9	0.5/0.5	Yes	Yes
EC Plan-NEOFLUAR	100 x	1.3	Oil	1.0/0.9	0.6/0.5	0.4/0.3	Yes	Yes
LCI Plan-NEOFLUAR	25 x	0.8	Oil, water or glycerin	2.9/6.6	1.7/3.7	0.9/2.0	Yes	Yes
LCI Plan-NEOFLUAR	63 x	1.3	Water or glycerin	1.5/1.3	0.9/0.7	0.5/0.4	Yes	Yes
Plan-APOCHROMAT	10 x	0.45	Air	4.2/20.4	2.4/11.5	1.3/6.2	Yes	Yes
Plan-APOCHROMAT	20 x	0.8	Air	3.2/4.9	1.8/2.8	1.0/1.5	Yes	Yes
Plan-APOCHROMAT	40 x	0.95	Air	1.6/1.7	0.9/1.0	0.5/0.5	Yes	Yes
Plan-APOCHROMAT	40 x	1.3	Oil	2.5/2.2	1.4/1.2	0.8/0.7	Yes	Yes
Plan-APOCHROMAT	40 x	1.4	Oil	2.4/1.8	1.4/1.0	0.7/0.6	Yes	Yes
Plan-APOCHROMAT	63 x	1.4	Oil	1.6/1.2	0.9/0.7	0.5/0.4	Yes	Yes
Plan-APOCHROMAT	100 x	1.4	Oil	1.0/0.8	0.6/0.5	0.4/0.3	Yes	Yes
LD LCI Plan-APOCHROMAT	25 x	0.8	Oil, water or glycerin	2.9/6.6	1.7/3.7	0.9/2.0	Yes	Yes
C-APOCHROMAT	10 x	0.45	Water	4.2/20.4	2.4/11.5	1.3/6.2	Yes	Yes
C-APOCHROMAT	40 x	1.2	Water	2.2/2.0	1.2/1.1	0.7/0.6	Yes	Yes
C-APOCHROMAT	63 x	1.2	Water	1.4/1.3	0.8/0.7	0.5/0.4	Yes	Yes
LD C-APOCHROMAT	40 x	1.1	Water	2.2/2.3	1.2/1.3	0.7/0.7	Yes	Yes
Plan-APOCHROMAT	63 x	1.46	Oil	1.5/1.0	0.9/0.6	0.5/0.3	Yes	Yes
Plan-FLUAR	100 x	1.45	Oil	1.0/0.7	0.6/0.4	0.3/0.2	No	No
Plan-APOCHROMAT	100 x	1.46	Oil	1.0/0.7	0.6/0.4	0.3/0.2	Yes	No

# **Technical Specifications**

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### Data for the Use of Inverted Microscopes, e.g. ZEISS Axio Observer

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Objectives for Axio Imager	V	NA	Immersion	Grid/Section	Grid/Section thickness @490nm [RU/µm]				
				High grid	Medium grid	Low grid	FS34	FS49	
EC Plan-NEOFLUAR	10 x	0.3	Air	2.9/31.5	1.7/18.5	0.9/9.8	Yes	Yes	
EC Plan-NEOFLUAR	20 x	0.5	Air	2.3/9.0	1.4/5.4	0.7/2.9	Yes	Yes	
EC Plan-NEOFLUAR	40 x	0.75	Air	1.6/2.7	0.9/1.6	0.5/0.9	Yes	No	
EC Plan-NEOFLUAR	40 x	1.3	Oil	2.4/2.1	1.4/1.3	0.8/0.7	Yes	Yes	
EC Plan-NEOFLUAR	63 x	0.95	Air	1.0/1.1	0.6/0.7	0.4/0.4	Yes	Yes	
EC Plan-NEOFLUAR	63 x	1.25	Oil	1.6/1.5	0.9/0.9	0.5/0.5	Yes	No	
EC Plan-NEOFLUAR	100 x	1.3	Oil	1.0/0.9	0.6/0.6	0.4/0.3	Yes	No	
LCI Plan-NEOFLUAR	25 x	0.8	Oil, water or glycerin	2.9/6.5	1.7/3.8	0.9/2.0	Yes	Yes	
LCI Plan-NEOFLUAR	63 x	1.3	Water or glycerin	1.5/1.3	0.9/0.8	0.5/0.4	No	No	
Plan-APOCHROMAT	10 x	0.45	Air	4.2/20.2	2.4/11.7	1.3/6.1	Yes	Yes	
Plan-APOCHROMAT	20 x	0.8	Air	3.1/4.8	1.8/2.8	1.0/1.5	Yes	Yes	
Plan-APOCHROMAT	40 x	0.95	Air	1.6/1.7	0.9/1.0	0.5/0.5	Yes	Yes	
Plan-APOCHROMAT	40 x	1.3	Oil	2.4/2.2	1.4/1.3	0.8/0.7	Yes	Yes	
Plan-APOCHROMAT	40 x	1.4	Oil	2.4/1.8	1.4/1.1	0.7/0.6	Yes	Yes	
Plan-APOCHROMAT	63 x	1.4	Oil	1.5/1.2	0.9/0.7	0.5/0.4	Yes	Yes	
Plan-APOCHROMAT	100 x	1.4	Oil	1.0/0.8	0.6/0.5	0.4/0.3	Yes	No	
LD LCI Plan-APOCHROMAT	25 x	0.8	Oil, water or glycerin	2.9/6.5	1.7/3.8	0.9/2.0	Yes	Yes	
C-APOCHROMAT	10 x	0.45	Water	4.2/20.2	2.4/11.7	1.3/6.1	Yes	Yes	
C-APOCHROMAT	40 x	1.2	Water	2.1/1.9	1.3/1.1	0.7/0.6	Yes	Yes	
C-APOCHROMAT	63 x	1.2	Water	1.4/1.3	0.8/0.7	0.5/0.4	Yes	Yes	
LD C-APOCHROMAT	40 x	1.1	Water	2.1/2.3	1.3/1.4	0.7/0.7	Yes	Yes	
Plan-APOCHROMAT	63 x	1.46	Oil	1.5/1.0	0.9/0.6	0.5/0.3	Yes	Yes	
Plan-FLUAR	100 x	1.45	Oil	1.0/0.7	0.6/0.4	0.3/0.2	No	No	
Plan-APOCHROMAT	100 x	1.46	Oil	1.0/0.7	0.6/0.4	0.3/0.2	Yes	No	

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Dimensions	(Width x Depth x Height)
ApoTome.2 slider for Axio Imager	Approx. 278 mm x 90 mm x 76 mm
ApoTome.2 slider for Axio Observer/Axiovert 200	Approx. 295 mm x 90 mm x 78 mm
Control box ApoTome.2	Approx. 255 mm x 220 mm x 96 mm
Operating Data	
Protection Class, Protection Type	I, IP 20
Electrical Safety	According to DIN EN 61010-1 (IEC 61010-1) taking account of CSA and UL regulations
Overvoltage Category	l
Interference Suppression	In accordance with EN 55011 class B
Interference Resistance	In accordance with DIN EN 61326-1
Supply Voltage	100 to 240 V $\pm$ 10%. No Adjustment of the supply voltage is required
Supply Frequency	50 to 60 Hz
Power Consumption ApoTome.2	Max. 50 VA
Fuses in Accordance with IEC 127	
Control box ApoTome.2	2 A delayed-action/H/250 V, 5 x 20 mm
Grid Frequencies	
Axio Imager slider (transmission grid high/medium/low)	5/9/17,5 lp/mm
Axio Observer/Axiovert 200 slider (transmission grid high/medium/low)	10/17.5/35 lp/mm
Installation Conditions The grid projection method used for the ApoTome.2 is sensitive	to vibration, which can have various causes (including strong draughts).

Vibrations are visible as streak artefacts in the resulting image. The microscope must therefore be set up so that it is exposed to as little vibration as possible on a vibration-damped table or on a suitable microscope base.



# Count on Service in the True Sense of the Word

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Because the ZEISS microscope system is one of your most important tools, we make sure it is always ready to perform. What's more, we'll see to it that you are employing all the options that get the best from your microscope. You can choose from a range of service products, each delivered by highly qualified ZEISS specialists who will support you long beyond the purchase of your system. Our aim is to enable you to experience those special moments that inspire your work.

#### Repair. Maintain. Optimize.

Attain maximum uptime with your microscope. A ZEISS Protect Service Agreement lets you budget for operating costs, all the while reducing costly downtime and achieving the best results through the improved performance of your system. Choose from service agreements designed to give you a range of options and control levels. We'll work with you to select the service program that addresses your system needs and usage requirements, in line with your organization's standard practices.

Our service on-demand also brings you distinct advantages. ZEISS service staff will analyze issues at hand and resolve them – whether using remote maintenance software or working on site.

#### Enhance Your Microscope System.

Your ZEISS microscope system is designed for a variety of updates: open interfaces allow you to maintain a high technological level at all times. As a result you'll work more efficiently now, while extending the productive lifetime of your microscope as new update possibilities come on stream.







Profit from the optimized performance of your microscope system with services from ZEISS – now and for years to come.

>> www.zeiss.com/microservice

# The moment your data change scientific minds. This is the moment we work for.

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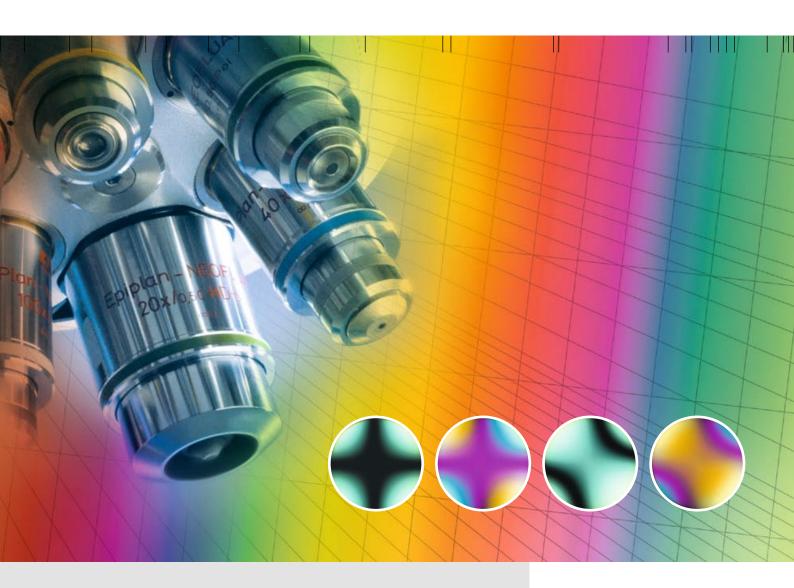


Carl Zeiss Microscopy GmbH 07745 Jena, Germany BioSciences microscopy@zeiss.com www.zeiss.com/apotome



# Michel-Lévy Color Chart

Identification of minerals in polarized light

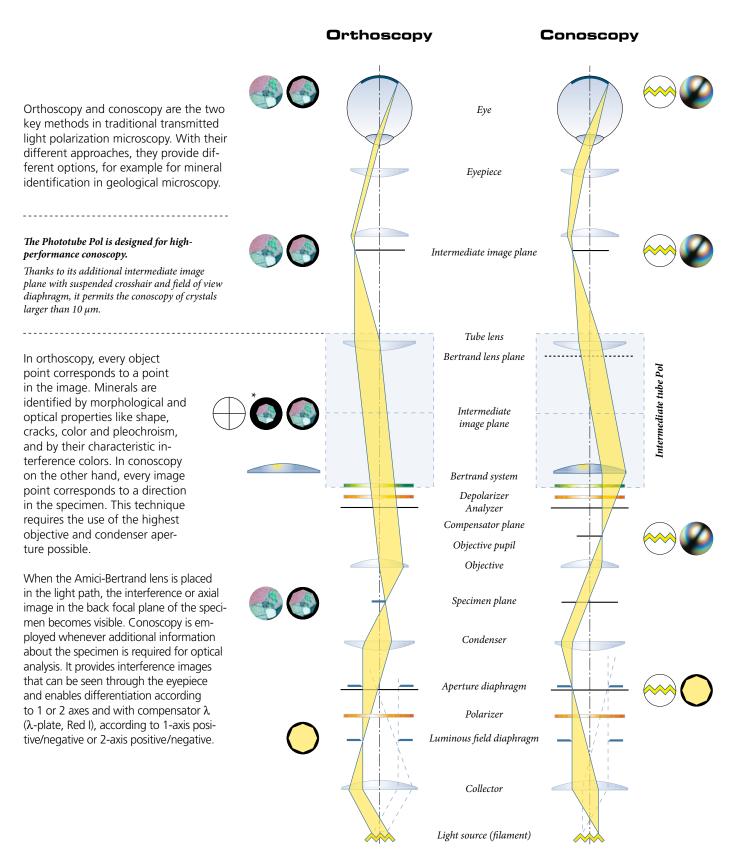


Information on Polarization Microscopy



We make it visible.

## **Polarization in transmitted light**



# Determination of birefringence by means of the Michel-Lévy Color Chart

When a ray of light enters an anisotropic medium, it is almost always split into two linearly polarized waves; the ordinary and the extraordinary ray. Both partial rays are characterized by different propagation rates due to different refraction indices. This characteristic is called birefringence. The oscillation planes of these two partial rays are perpendicular to each other.

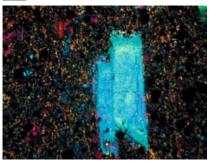
The superposition of the two partial waves (constructive or destructive) is called interference; the colors which appear under crossed (90°) polarizers are called interference colors.



Rotating the mineral into the position of extinction

Total extinction (darkest position of mineral)





# Rotating the mineral into a diagonal position

- (45° from position of extinction)
- Maximum brightness
- Identification of interference color: blue

This amounts to two distinct possibilities:

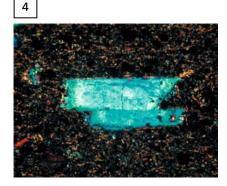
- second order blue (path difference ca 655 nm)
- third order blue (path difference ca 1150 nm)

3



Inserting the lambda compensator (Addition of a path difference of 551 nm) Assumption: second order blue (path difference ca 655 nm)

Effect: In subtraction position the mineral appears lavender- to bluegrey (655 nm - 551 nm = 104 nm)



**Rotating the mineral by a further 90°** Effect: In this position (addition position) the mineral appears greenish blue (655 nm + 551 nm = 1206 nm)

Result: The interference color has been identified as a second order blue.



### Determining the birefringence with the Michel-Lévy Color Chart

Follow the 655 nm line of the path difference across to find the intersection with the corresponding thickness line (usually  $25-30 \,\mu$ m). From this intersection, follow the "sun line" downwards towards the bottom right to pinpoint

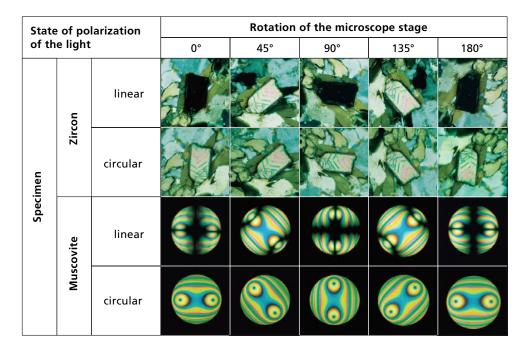
the respective birefringence magnitude on the scale on the right. In this case this leads to a birefringence value of 0.024; the mineral has been identified as an **augite**.

		Cryolite Meilite Saponite	Halloysite B-Cristobalite œ-Tricalciumphosphate	Vesuvianite Tridymite Serendibite Coesite	Orthoclase Microcline Åkermanite Kaolinite	Silicocarmotite Anorthoclase	quartz Rankinite Tricalciumsilicate Gypsum Boracite	Gehlenite Scolecite Y-Dicalciumsilicate Brushite	Petalitie Anorthite Rhodonite Trona Wollastonite	Bustamite Boehmite	B-Dicalciumsilicate Mullite Gedrite	Thomsonite	Polyhalite Amesite	Spodumene Amblygonite Brucite Gibbsite	Sillimanite Orthoferrosilite	Larnite Gadolinite Kaersutite	Borax Montmorillonite te	Cancrinite Stishovite	Glauconite	Calciumhydroxide	Sucrose	Lamprophyllite Clinoferrosilite	Stilpnomelane	Pectolite	Muscovite			hel-L or Cl		
		Analcite Leucite Apophyllite	Marialite Apatite Chabazite	Eudialyte Vanthoffite Nepheline Sanidine	Beryl Zoisite Harmotome Antigorite	Corundum Plagioclase An 20-60	Albite Celestite Struvite Bronzite Chrysoberyl	Andalusite Bytownite Natrolite Barite	Kornerupine Hypersthene Thenardite Margarite Thuringite	Jadeite Crossite	Monticellite Richterite Kyanite Na-Tramolita		Pargasite	Alunite Vermiculite Katophorite Comm. Hornbl. Glauberite	Tremolite Hastingsite	Pigeonite Omphacite Augite	Tourmaline Wavellite Hydromagnesi	Wöhlerite Fassaite Titanaugite	Phlogopite Epsomite	Paragonite Salite Hedenbergite Johannsenite	Zinnwaldite	Chondrodite Humite	Forsterite Variscite	Bischofite Olivine	Grandidierite					
		Pennine Ripidolite	Phillipsite Kämmererite	Riebeckite Chamosite Clinozoisite Arfvedsonite	T%Q ∢	오늘 형	5₽9 9₽2	Clinochlore Chloritoid Laumontite Hydronephelite			Phenakite Merwinite Syngenite	Hiortdahlite	75	Melinophan Actinolite Barkevikite Prehnite	: UE	Kainite Cookeite	Anthophyllite Glaucophane	Rosenbuschite Mizzonite Carnallite	Colemanite		Diopside	Allanite Rhönite	Prehnite	Kernite Lazulite	Catapleitte					
	Birefringence $(n_{\gamma} - n_{\alpha})$	-0.001	-0.003	-0.004 -0.005	-0.006	-0.008	-0.009 -0.010	-0.011 -0.012	-0.013 -0.014	-0.015	-0.016 -0.017	-0.018	-0.019	-0.020 -0.021	-0.022	-0.023 -0.024	-0.025	-0.026	-0.028	-0.029	0.031	-0.032	-0.033	-0.034 -0.035	-0.036					
	ն (բող																													
Thickness	50											X														 0.040	Meionite	Tilleyite Spurrite	Låvenite Nontronite	0,038 0,039
	40				AA							X													_	0.045	Datonic	Biotite Carborundum	Phengite Titanbiotite Anhydrite	0,041 0,043 0,044 0,045
																											Aegirine	Diaspore Cholesterole	Pyrophyllite Fayalite Ilvaite	0,041 0,043 0,044 0,045 0,047 0,047 0,048 0,049 0,050 0,052
	30				XA																					0.060		Silk	Piemontite	0,055
																										-0.065 -0.070 =		e Cellulose	Kieserite	0,060 0,063 0,065 0,070
	20																									-0.080 -0.090		Maltose	Stilpno mela	0,073
	20																					_			=	=		Bicalciumferrit Brownmillerite Glucose	e Cassiterite	0,090 0,096
												-	-				-					_				-0.120 	Baddeleyite Sphene	Carbamide	Xenotime	0,107 0,120
	10																						_				Brookite Columbite Aragonite Calcite	Monocalciumf	Goethite errite Whewellite	0,140 0,150 0,156 0,172
ion													_													=	Magnesite Siderite			0,180 0,195 0,241 0,270
Reading direction	0																										Pyrophanite Hematite Rutile Geikielite Lepidocrocite			0,140 0,150 0,156 0,172 0,180 0,195 0,241 0,270 0,280 0,286 0,26 0,36 0,57
Readin							I	•							•								•							0,57
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# Linearly and circularly polarized light



In contrast to linear polarization, circularly polarized light allows minerals to display their interference colors devoid of extinction. For that reason, circular polarization is the preferred method for image analytical procedures.

Behavior of optically anisotropic crystals in linearly and circularly polarized light, orthoscopy and conoscopy.

## Determination of the optical character

	Stat	te of polariza	tion of the li	ght				
uniaxial	line	ar	circular					
	without	with	without	with				
positive quartz	$\Rightarrow$							
negative calcite			0					

Determination of the optical character of uniaxial and biaxial minerals in linearly

and circularly polarized light. The reference direction ny of the  $\lambda$ -compensator is aligned in NE-SW.

			Sta	te of polariza	ation of the li	ght		
		lin	ear			circ	ular	
biaxial				comper	isator $\lambda$			
	without	with	without	with	without	with	without	with
	normal	position	diagonal	position	normal	position	diagonal	position
positive barite								
negative muskovite							P	

# **Highlights of minerals analysis**

### Auguste Michel-Lévy (1844-1911)

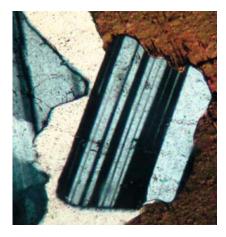
French geologist, Inspector General of Mining and director of the Geological Survey in France, made a name for himself by his research into extrusive rocks, their microscopic structure and origin.

Until this day, the interference color chart proposed by him in 1888 remains an important tool in the identification of thin sections of minerals with polarization microscopy.

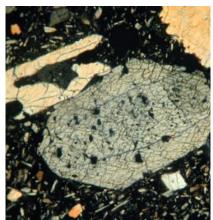
Then as now, Carl Zeiss sets benchmarks with their polarized light microscopes, in mineralogy and petrography as well as materialography and other application fields.



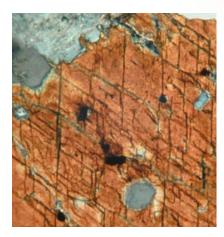
Mineralogical microscope stand of 1906.



Plagioclase (feldspar) Twin lamination



Pyroxene Cleavage angle ca. 87°



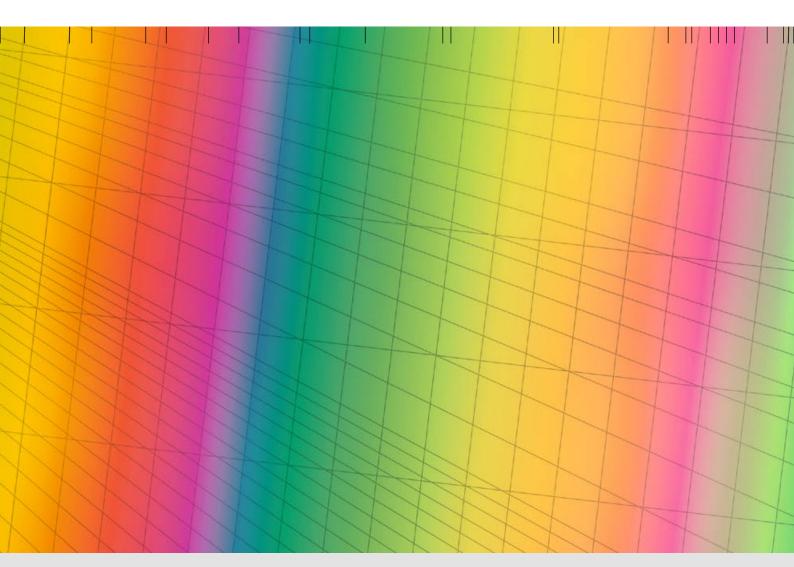
Amphibole Cleavage angle ca. 124°



Kindly supported by TU Bergakademie Freiberg

## Dr. M. Magnus

Institute of Geology and Paleontology



Carl Zeiss Microscopy GmbH 07745 Jena, Germany Materials microscopy@zeiss.com www.zeiss.com/microscopy

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# **Polarization in Focus**

Axio Scope and Axio Imager

Innovative, Economical and Strain-free: Polarization Microscopes for Education, Routine and Research.



We make it visible.

# Innovation Sets New Standards How to Get One Step Ahead in Polarization Microscopy

In the traditional fields of polarization microscopy – geology, mineralogy, metallography and the exploration of fossil fuel resources – microscopes have to meet higher standards than ever before.

Future-proof, upgradeable microscopes are an essential requirement in modern materialography as well as in the established areas of polarization microscopy. New challenges – in industries such as construction, glass, plastics, semiconductor, textile and fiber analysis as well as in forensic science – call for versatile, efficient and customized system solutions. Strain-free optics, highest optical resolution

and the availability of a wide range of contrasting and measurement techniques are a must, as is the choice of manual, motorized or encoded components. Equally important aspects are ease of use, value for money and digital analysis options, for both routine applications and research projects.





Carl Zeiss has always set the pace in polarization microscopy – with innovative, leading-edge systems that fulfill all your requirements: economical solutions for educational purposes, versatile microscopes for a wide variety of routine tasks and highly efficient instruments for each of your research endeavors. Two microscope stands form the basis for our system solutions: Axio Scope, a multi-purpose routine stand, and Axio Imager, a powerful research instrument: both embody our promise of quality. As ever, Made by Carl Zeiss means that you can meet rising demands – in the lab, research institute, university or industry – even faster, more proficiently, reliably and economically.



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# Diversity in Contrast

## What to Expect From Leading-Edge Polarization Microscopes

What to expect from Carl Zeiss polarization microscopes, no matter which contrast technique you use, is maximum performance and exceptional optical quality – time and again revealing just that little extra piece of information that can make all the difference to your results.



Dr Jutta Zipfel, Department of Meteorite Research, Senckenberg Research Museum, Frankfurt/Main, Germany

#### Contrasting: the possibilities are endless

Developed for traditional as well as current applications in polarization microscopy, Axio Scope and Axio Imager offer all relevant contrasting and measuring techniques.

#### In transmitted-light:

- Orthoscopy: linear and circular polarization
- Conoscopy
- Brightfield
- Darkfield
- Differential Interference Contrast (DIC)
- Phase Contrast
- PlasDIC

### And in reflected-light:

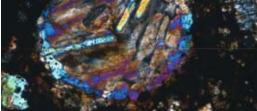
- Brightfield
- Darkfield
- Polarization
- Fluorescence
- DIC and C-DIC

## Polarizers: Classic diversity

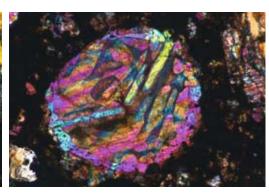
We offer a wide range of polarizers of various performance levels for both Axio Scope and Axio Imager. Each one provides a high level of polarization and color neutrality in the visible range of the spectrum, thereby ensuring exceptional images and precise measurements

Bar olivine chondrule in the Coolidge meteorite in transmitted-light Objective: EC Plan-NEOFLUAR 10x/0.30 Pol Dr Jutta Zipfel, Department of Meteorite Research, Senckenberg Research Museum, Frankfurt/Main, Germany



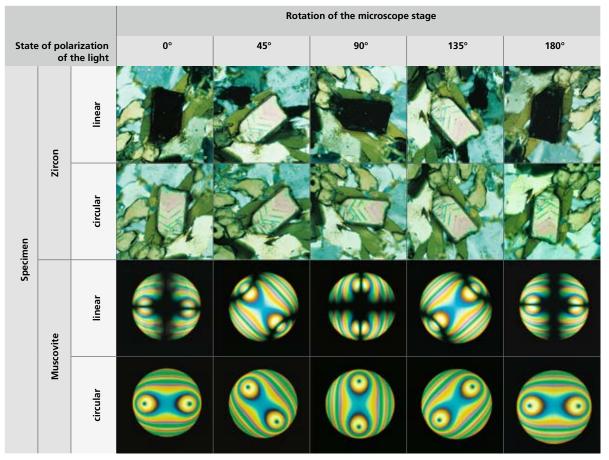


Polarization



Polarization with  $\lambda$ -plate

Brightfield



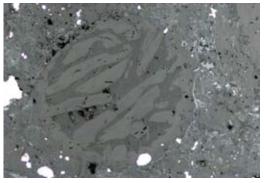
Behavior of optically anisotropic crystals in linearly and circularly polarized light, orthoscopy and conoscopy

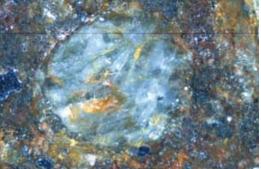
according to industrial and other standards. The range comprises fixed and rotating polarizers for transmittedand reflected-light. In addition, our portfolio includes 360° rotating quantitative analyzers with 0.1° vernier as well as combinations with a fixed or rotating lambda plate. We also offer a dedicated temperature-resistant polarizer module for the use in conjunction with the high energy arc lamp HBO103 in order to guarantee a consistent quality of polarization contrast. Circular polarization: innovation in transmitted-light

Carl Zeiss polarization microscopes offer a further leadingedge innovation focused on your everyday requirements: the circular polarization device for transmitted-light. In contrast to the linear polarization currently predominantly in use, this device enables viewing and imaging devoid of any angular-dependent extinction; all features appear in their maximum interference colors. The benefits are obvious – for the photomicrography of thin rock sections as well as for structural examinations on plastics or strain distribution in glass using digital analysis systems.

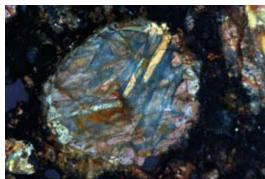
Bar olivine chondrule in the Coolidge meteorite in reflected-light Objective: EC Epiplan-NEOFLUAR 10x/0.25 Pol

Dr Jutta Zipfel, Department of Meteorite Research, Senckenberg Research Museum, Frankfurt/Main, Germany





Darkfield



Polarization



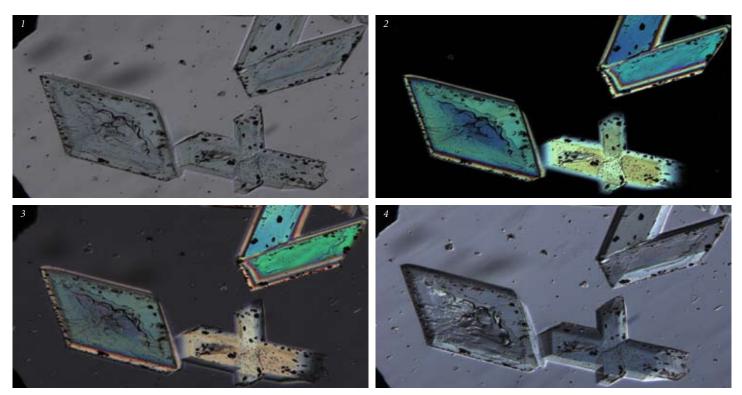
1 Brightfield, 2 C-DIC, mesosiderite in reflected-light Objective: EC Epiplan-NEOFLUAR 50x/0.8 Pol Dr Jutta Zipfel, Department of Meteorite Research, Senckenberg Research Museum, Frankfurt/Main, Germany

#### DIC or C-DIC: increased homogeneity, better contrast

The Differential Interference Contrast technique (DIC) has been further enhanced for objective magnifications from 5x to 100x, providing homogeneous illumination across the entire field of view. Circular DIC (C-DIC), an optical polarizing technique which, unlike standard DIC, uses circularly polarized light, is the perfect technique for studying oriented structures in reflected-light. Using C-DIC to contrast features with various spatial orientations has obvious advantages: Instead of having to rotate the sample in the azimuth, simply turning the DIC slider ring enables the user to view and image all features one after the other. By the way: The entire line of the general purpose objectives EC EPIPLAN requires only a single C-DIC prism for interference contrast in circularly polarized light.

#### Affordable excellence: PlasDIC

A DIC version generating polarization-optical Differential Interference Contrast in transmitted-light is available for the Axio Scope, providing excellent image quality even if the object, slide, condenser or objective lens displays anisotropic (birefringent) properties. PlasDIC is the technique of choice in the study of anisotropic samples if you would like to acquire an image with a three-dimensional, embossed character. The benefits are obvious: superior data and outstanding brilliance. In particular, compared to traditional brightfield, polarization or DIC methods, PlasDIC provides a significantly clearer distinction of specific features such as morphology or the crystal growth of anisotropic phases.



Copper sulfate crystals in transmitted-light: 1. Brightfield, 2 Polarization, 3 traditional DIC and 4 PlasDIC. PlasDIC allows this brilliant depiction of morphology for the first time.

# **Precision is Our Trademark** Quantitative Methods for Your Analysis

High performance in quantitative techniques – Carl Zeiss has a tailor-made solution for every polarization microscopy requirement

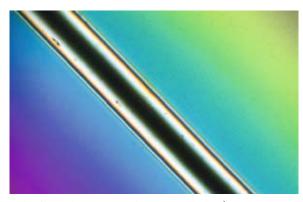
## Diversity in quantitative measurements: manual and digital

Starting with the straightforward manual measurement by a rotating, ball bearing mounted stage with 360° division and 0.1° vernier – e.g. measuring cleavage angles in minerals – all the way to determining path differences or strain measurements: Carl Zeiss polarization microscopes

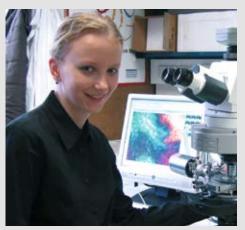


Compensators

meet just about every challenge. A wide variety of compensators for the measuring range from 0 to 30  $\lambda$  creates the basis. In addition, Axio Scope and Axio Imager offer an outstanding adaptability to a large number of other techniques. Examples include thermomicroscopy or image analysis functionalities such as grain size measurement or particle analysis with AxioVision software.



Nylon fiber, polarization, Berek compensator 0 to 5  $\lambda$ 

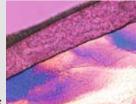


"In lyophilization microscopy we work with constant changes of magnification in order to monitor what happens to the materials in the heating chamber; which environmental conditions cause them to collapse. The motorization makes our job considerably easier. Axio Imager is extremely comfortable to work with. And we were simply thrilled with the image quality."

Dr Eva Meister Research group Dr H. Gieseler Division of Pharmaceutics, University of Erlangen-Nuremberg, Erlangen

> Above right: Poly-L-Lysine in transmitted-light Below right: Trypsinogen in transmitted-light Objective: LD Epiplan 20x/0.25 DIC, Polarizer with rotary  $\lambda$ -plate





# **Designed for Conoscopy**

## Straightforward and Confident Mastery of a Demanding Technique

Carl Zeiss polarization microscopes provide the flexibility of fast, simple and economical system extensions for conoscopic measurements to suit each of your needs.

# Economical or sophisticated: five choices for conoscopy

In many cases, the analysis of an interference image will provide even more valuable information for the classification of anisotropic material than the image of the object itself does. The polarization microscopes Axio Scope and Axio Imager from Carl Zeiss are available in a number of alternative configurations:

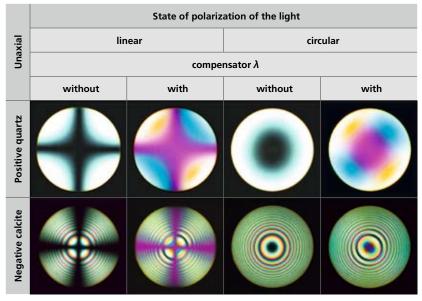
- The pin-hole diaphragm or the auxiliary microscope in the eyepiece tube The simplest and most economical version.
- 2. The conoscopy module The module is simply inserted into the reflector turret or the reflector slider, is therefore easily exchanged

without the need for tools and allows the straightforward addition of the conoscopy function for crystal analysis to the polarization microscope at any time. Using the objective N-ACHROPLAN 50x/0.8 Pol or EC Plan-NEOFLUAR 40x/0.9 Pol, this makes performing conoscopy effortless and comfortable.

3. Conoscopy with the Bertrand lens slider If the samples are uncovered or a 100x objective magnification is desired, conoscopy with the Bertrand lens slider is the solution of choice. The Bertrand lens can be focused, so that you can employ a wide range of objectives; for example EC Plan-NEOFLUAR 100x/1.30 Oil Pol or EC Epiplan-NEOFLUAR 50x/0.8 Pol.



With the conoscopy module, comprising Bertrand lens, analyzer and a high aperture objective (N-ACHROPLAN 50x/0.9 Pol or EC Plan-NEOFLUAR 40x/0.9 Pol) your microscope can be upgraded to conoscopy at any time.



Determination of the optical characteristics of 1-axis and 2-axis minerals in linearly and circularly polarized light, the reference direction  $n_v$  of compensator  $\lambda$  is aligned in NO-SW.

In addition to the economical options detailed above, two more alternatives are available specifically for Axio Imager to allow the upgrade of your polarization microscope for conoscopy.

4. 5-position tube lens turret with integrated focusable Bertrand lens

Opting for a tube lens turret in order to acquire further magnifications will allow you to perform conoscopy in addition, as the tube lens turret contains an integrated Bertrand lens. The tube lens turret is available both in an encoded and in a motorized version.

### 5. Pol phototube

The Pol phototube has been specifically designed for orthoscopy and conoscopy with Axio Imager. There is a significant advantage to this choice: Due to an additional intermediate image plane, object, cross hairs and iris diaphragm can be viewed concurrently. Thanks to the adjustable iris diaphragm this is also true for the limits of conoscopic range, down to a minimum crystal size of 10  $\mu$ m. The Bertrand optics are pre-centered and focusable and are straightforward to turn on and off with the help of a slider. As a result, the correlation of orthoscopic and conoscopic image data can be easily verified at any time. An ideal solution for fast, reliable crystal analysis.



*Small rotary stage with stage clips:* 360° *division with 0.1° vernier* 

*Pol rotary stage with adjustable* 45° *click stops and stage clips. Vernier* 0.1°, *object guide for transmitted- and reflected-light applications* (*with and without click stops*)

## Measuring Up to the Highest Standards Carl Zeiss Redefines the Limits of Optics

Optics of uncompromising quality form the basis for setting new standards in polarization microscopy. The keyword here is strain-free. This principle is embodied by the availability of a wide range of polarization objectives in various performance classes and price levels – tailor-made for your requirements.

# The six-position centering nosepiece: added convenience for polarization

The six-position Pol\* centering nosepiece offers much space for your objectives, eliminating the need for timeconsuming objective or turret changes; clearly a plus for enhanced efficiency. The rotary stage is centered in relation to the fixed turret opening which serves as the reference. Subsequently, the remaining openings are centered in the turret individually – the image position therefore remains unaffected by each change of magnification. Being equipped with M27 threads, the turret accommodates the whole range of standard contrast

\*Axio Imager: encoded; Axio Scope: manual

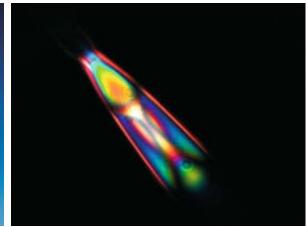
techniques in transmitted- and reflected-light, as well as reflected-light applications in darkfield. Additionally, the turret features a position to house a DIC-slider for Differential Interference Contrast.

## Polarization strain-free: the objectives

Four ranges of objectives share one ambition: Only those merit the label Pol which qualify for work in polarized light due to exceptionally low strain. Carl Zeiss offers four lines of strain-free objectives, varying in the extent of correction, price level and application area.

Pol – Polarization, DIC – Differential Interference Contrast





Glass fiber filled with liquid crystal, linearly polarized transmitted-light Objective: EC Epiplan-NEOFLUAR 50x/0.80 Pol Thomas Tanggaard Larsen, COM Research Center, Technical University of Denmark, Lyngby, Denmark; and Peter Hansen, Crystal Fibre A/S, Birkerød, Denmark

	Objective lens	Suitable up to field of view	Flatness of field	Color correction
ZEISS N-ACHROPLAN 20x/0,45 Pol #10 17	<b>N-ACHROPLAN Pol</b> The transmitted-light lenses for samples with cover glass. With further enhancement of color correction and flattening, this is the attractively-priced, entry-level line for polarizing microscopy – ideal for education and routine.	23	Very good	Very good
2EISS Plan-NEOFLU, 40x/q.9 Pol ∞/0,17	<b>EC Plan-NEOFLUAR Pol</b> The <b>E</b> nhanced <b>C</b> ontrast transmitted-light objective lenses for samples with a cover glass. With their consistent minimiza- tion of stray light and contrast enhancement, these lenses meet demanding requirements. The EC Plan-NEOFLUAR lenses feature full chromatic correction for the focal plane. With their high resolving power, they offer a crisp, high- contrast and completely flat image for observation and documentation.	25	Excellent	Excellent
ZEISS EC EPIPLAN 50X/0,7 Pol voi0	<b>EC EPIPLAN Pol</b> Transmitted- and reflected-light objective lenses for uncovered samples in routine applications. The <b>E</b> nhanced <b>C</b> ontrast series is achromatically corrected and generates a flattened field for an intermediate image size of 23 mm. The objective lenses feature blocked pupil positions and therefore allow the C-DIC contrasting technique.	23	Very good	Very good
ZEISS Epiplan-NEOFLU 50x/0,8 Pol co/0	<b>EC Epiplan-NEOFLUAR Pol</b> The transmitted- and reflected-light objective lenses for advanced applications for samples with or without a cover glass. Optimized for maximum contrast, their outstand- ing features include increased numerical apertures and therefore a higher resolving power. These lenses are suitable for transmitted-light examinations. However, due to the spherical aberration, objects with a cover glass can only be examined up to a magnification of 20x. There are no restric- tions for samples without a cover glass. Rigorous, object-side telecentricity makes these lenses particularly suitable for measuring purposes.	25	Excellent	Excellent
ZEISS piplan-NEOFL 50 x / 1,0 Oil PL ce /0	In addition, the Epiplan-NEOFLUAR Pol line offers you a selection of immersion lenses.			

You can get further information at www.zeiss.de/objectives.

## Axio Scope

## Why Twenty-Nine Stand Versions Offer a Perfect Solution for Every Application and Budget

Exceptional diversity – of stand versions and interfaces – creates exceptional flexibility and the foundation for a tailor-made configuration for your application, encompassing functionality and economic efficiency.

### A new dimension in modularity

Axio Scope is customized specifically for your applications; a microscope dedicated to your individual polarization microscopy needs. Two different polarization microscopy units are available for combination with a choice of three different bases. Your microscope configuration is tailor-made, however straightforward or complex your requirements may be; for transmitted-light, reflectedlight or both. For example, as a combined transmittedlight and reflected-light stand for geoscience training courses or as a reflected-light only stand in the exploration industry (e.g. coal mining). A significant advantage for your budget: you only invest in the components you actually need.

### **Economical upgrading**

Future upgrades to Axio Scope are both straightforward and cost-effective, thanks to the modular interface design: an attractive economical aspect, especially as many add-ons can be easily installed by the user.

#### Polarization microscopy units:

I. For transmitted-light applications in polarized light Instrument requirements: 6-position centering nosepiece Pol, including 5x H Pol, 1x H DIC; compensator mount above centering turret (for  $\lambda$ -plates, quartz wedge or quantitative compensators etc.)

II. For transmitted- and reflected-light applications in polarized light

Instrument requirements: 6-position centering nosepiece Pol, including 5x HD Pol, 1x HD DIC. 100 W halogen lamp; beam path with Koehler illumination; slots for rotary reflected-light polarizer, luminous field diaphragm, filter slider and swing-out diffusor.

#### Bases

- A. Straightforward base section, no beam path, suitable for reflected-light microscopy; can be adapted to transmitted-light with an LED (Fixed-Koehler) fixed underneath the condenser carrier
- B. For all standard transmitted-light applications
   50 W reflector lamp, beam path with Koehler illumination; luminous field diaphragm, filter slider and
   6-position filter wheel
- C. For advanced transmitted-light applications with high illumination intensity
   Beam path with Koehler illumination, luminous field diaphragm and aperture diaphragm, filter slider and 6-position filter wheel

 ${\rm H}$  – Brightfield, D – Darkfield, Pol – Polarization, DIC – Differential Interference Contrast





### From 0 to 110 mm: variable sample space

There are a number of options available for extending the sample space vertically in order to accommodate taller samples. In addition to z-travel this can be achieved by

- Lowering the stage carrier with the dovetail
- Removing the condenser carrier, for example if the stage is intended to be lowered beyond the travel range
- Inserting a 30 mm or 60 mm spacer, utilizing the customer interface between the polarization microscopy units (I, II) and the bases (A, B, C). The 30 mm and 60 mm spacer extend the maximum sample height to 80 mm and 110 mm respectively

The flexible sample space provides additional freedom of use and extends the range of applications for Axio Scope.

#### Interface for reflector modules: infinity space

The interface in the infinity space is unique in this category. Axio Scope allows you to use those reflector modules that are best suited for your applications. You have the choice of a 2-position slider, a 4-position reflector turret or a 6-position turret. No matter which alternative you prefer, all of them are easily fitted with Push&Click modules. With each option, your optics modules are held safely and dust-free.



# **Axio Imager**

## Comfort and Convenience - Provided by an Intelligent Polarization Microscope

The intelligent microscope assists in controlling your workflows, making them easier and even more reliable – with Axio Imager, Carl Zeiss has implemented a concept with regards to stand diversity, ease of use and ergonomics that will amaze you.

### The stand versions: 9 times more flexible

More economic efficiency in polarization microscopy – Axio Imager gives you the freedom to tailor your research microscope to your requirements. Nine stands are available. You can opt for the encoded, partly motorized or fully motorized version, as all key components of Axio Imager are encoded.

### The imaging cell

Stability is a major prerequisite for best results. The core elements of Axio Imager – objective turret, z-guide and stage carrier – are constructed as a compact, vibration-free unit. This stable cell is decoupled from the remaining stand, creating ideal conditions for imaging, particularly for time-lapse experiments using high magnifications.

### The touchscreen: innovation at a glance

Complex workflows made easy – the most relevant functionalities of the motorized polarization microscope are available on a touchscreen (TFT). The control of all motorized components is at your fingertips. In addition to the factory settings, complex processes can be programmed, saved and retrieved at the touch of a button on the screen.

# Light manager and contrast manager: the automatic way to optimum settings

The light manager is designed to provide reproducible illumination settings and stable imaging conditions leading to optimum illumination and contrast. This is achieved by automatically regulating the lamp voltage, ensuring



The touchscreen on the stand (left) or the docking station (right) provide clear guidance in control and configuration.



Automatic Component Recognition recognizes objective lenses and reflector modules automatically

*left:* ACR reflector module right: ACR objective lens

constant color temperature via neutral density filters and controlling the motorized luminous field diaphragm and aperture diaphragm, both in the reflected- and transmitted-light beam paths. That way, settings – e.g. diaphragm settings relating to specific objectives – can be saved to be retrieved at any time. The contrast manager on the other hand will direct you quickly and reliably to the correct setting for a given contrast technique. Simply select the desired technique on the TFT and the contrast manager will handle the complex interactions of parameters such as the position of shutter, reflector turret and/ or modulator turret.

### Control buttons: functionality you can feel

Another smart detail in the design of Axio Imager: tactile control buttons. Ergonomically arranged around the focus drive, they are easily distinguished by their position and shape.

# Operating panel: microscopy without the microscope

Designed to provide more freedom of moving around in the lab – Axio Imager can be controlled via an operating panel, which can be positioned separately from the microscope stand. The panel features a focus drive and brightness control. Other functions can be programmed by the user. The panel offers an interface for the TFT and for the x-, y-control of the motorized stage. A wellconceived design for more convenience.

# Automatic component recognition: hallmark of the research microscope

The innovative ACR – Automatic Component Recognition – system automatically identifies objectives and reflector modules in all motorized Axio Imager versions. Any exchange of components is immediately registered in the system. Confidence in the correct settings provides peace of mind. Automatically.



Two different kinds of fine focus controls are available for the focusing drive in Axio Imager: They are interchangeable and can be used either on the left or the right hand side.

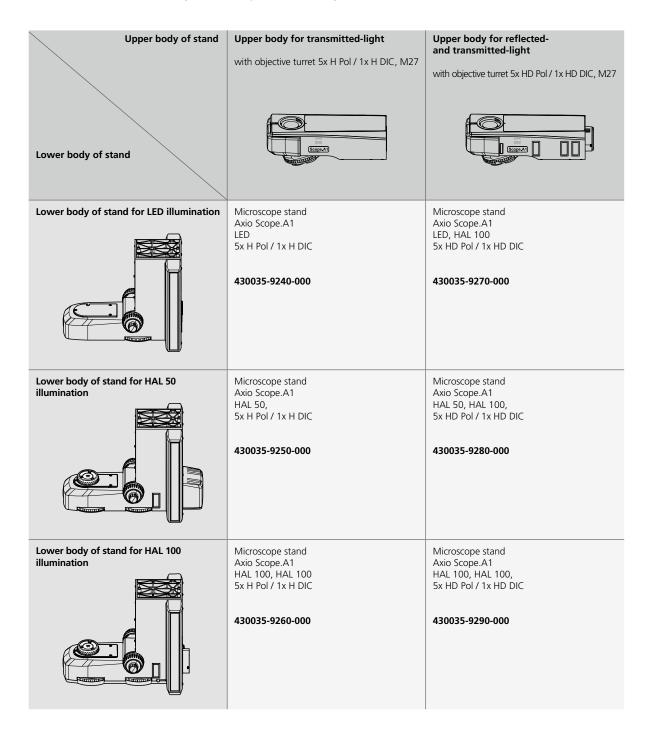


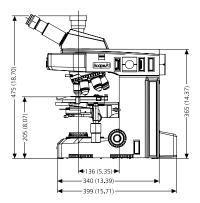
Today as ever, Carl Zeiss sets standards in polarization microscopy, providing a wide range of innovative system solutions designed to fulfill each of your requirements. Our solutions provide economy for education purposes, versatility for a wide variety of routine measurement tasks and powerful efficiency and functionality for science and research.

Two microscope stands form the basis for our system solutions: Axio Scope and Axio Imager – the former a robust multi-purpose routine stand, the latter a leadingedge microscope for research and science. Both equally signify our promise of quality in the field of polarization microscopy – from traditional areas such as mineralogy and geology to current advances in material applications, such as thin layer systems or solar cells. More than just the sum of its parts, a polarization microscope from Carl Zeiss constitutes a meticulously designed complete system solution, perfectly integrated in the Carl Zeiss system environment.

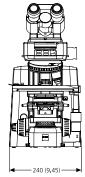
Carl Zeiss Microscopy GmbH 07745 Jena, Germany Materials microscopy@zeiss.com www.zeiss.de/polarization

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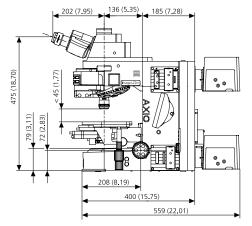
Dimensions in mm (inches)

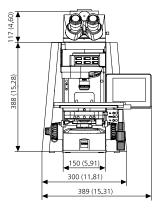


## Axio Imager 2 for polarizing microscopy – facts and figures

Axio Imager – flexibility for all fields of application										
Components	Option	A2 LED	A2	M2	D2	Z2	A2m	M2m	D2m	Z2m
Stand	manual	+	+	-	+	-	+	-	+	-
	motorized	-	-	+	0*	+	-	+	0*	+
Encoding	readable from computer	+	+	+	+	+	+	+	+	+
Tube lens turret	encoded	0	0	0	0	0	0	0	0	0
	motorized	-	-	0	-	0	-	0	-	0
Reflector turret	6x encoded	0	0	0	0	0	0	-	0	0
	6x motorized	-	-	0	0	0	-	+	0	0
	6x motorized ACR	-	-	-	-	0	-	-	-	0
	10x motorized ACR**	-	-	-	0	0	-	-	0	0
Objektiv turret	6x encoded POL	0	0	0	0	0	0	0	0	0
	6x encoded HD DIC	0	0	0	0	0	0	0	0	0
	6x motorized HD DIC	-	-	0	-	0	-	0	-	0
	6x motorized HD DIC ACR	-	-	0	-	0	-	0	-	0
	7x encoded HD	0	0	0	0	0	0	0	0	0
	7x motorized HD	-	-	0	-	0	-	0	-	0
Modulator turret for C-DIC/TIC	manual	0	0	0	0	0	0	0	0	0
	motorized****	-	-	0	-	0	-	0	-	0
Modulator turret for transmitted-light DIC	motorized****	-	-	-	-	0	-	-	-	0
Stage carrier, attachable with condenser carrier	0 mm - 25 mm	+	+	+	+	0	0	0	0	0
Stage carrier, attachable, for removable condenser carrier	0 mm - 45 mm	0	0	0	0	0	0	0	0	0
Stage carrier, reflected-light	0 mm - 63 mm	0	0	0	0	0	0	0	0	0
Transmitted-light illumination	manual	-	+	-	+	-	0	0	0	0
	motorized	-	-	+	-	+	-	-	-	0
LED transmitted-light	-	+	0	0	0	0	0	0	0	0
Double wheel filter transmitted-light	manual	-	+	0	0	0	0	0	0	0
	motorized	-	-	0	-	0	-	-	-	0
Reflected-light illumination	manual***	0	0	0	0	0	+	-	+	-
	motorized***	-	-	-	-	0	-	+	-	+
Luminous field diaphragm, reflected-light	manual	0	0	0	0	0	+	0	+	0
	motorized	-	-	-	-	0	-	0	-	0
Aperture diaphragm, reflected-light	manual	0	0	0	0	0	0	0	0	0
	motorized	-	-	-	-	0	-	0	-	0
Double wheel filter, reflected-light	manual	0	0	0	0	0	0	0	0	0
· · · · · · · · · · · · · · · · · · ·	motorized	-	-	0	-	0	-	0	-	0
FL attenuator	manual	0	0	0	0	0	0	0	0	0
	motorized	-	-	-	-	0	-	0	-	0
Light switchover reflected-/transmitted-light	manual	+	+	-	+	-	+	-	+	-
	software	-	-	+	-	+	-	+	-	+
Mixed light with additional power unit	manual	+	+	-	+	-	+	-	+	-
	software	-	-	+	-	+	-	+	-	+
Focus (z-axis)	manual	+	+	-	+	-	+	-	+	-
	motorized 25 nm	-	-	+	-	-	-	+	-	-
	High performance focus (motorized 10 nm)	-	-	-	-	+	-	-	-	+
TFT display	-	-	-	+	-	+	-	+	-	+
ApoTome	-	0	0	0	0	0	0	0	0	0
Power unit	external	-	-	+	-	+	-	+	-	+
	internal	+	+	-	+	-	+	-	+	-
Mechanical stages CAN	motorized****	0	0	0	0	0	0	0	0	0
Scanning stages	piezo	0	0	0	0	0	0	0	0	0
	DC/stepper motors	0	0	0	0	0	0	0	0	0
Fast z-piezo insert	with manual stage	0	0	0	0	0	0	0	0	0
	with scanning stage	0	0	0	0	0	0	0	0	0
2 TV tube, motorized	-	-	-	0	-	0	-	0	-	0
Condensers	manual	0	0	0	0	0	0	0	0	0

- + = Contained in the stand
- O = Optionally available
- = Not possible
- \* = Motorized (6x and 10x) reflector turret can be used \*\* = ACR function not possible with Axio Imager.D2
- and D2m \*\*\* = All reflected-light illumination devices contain a motorized shutter.
- For fluorescence applications this can be optionally replaced with a high-speed shutter \*\*\*\* = For use on Axio Imager.A2 LED, A2, A2m, D2 and
- *D2m, USB/CAN converter 432909 is required \*\*\*\*\* = Only in combination with objective turret mot.*
- m = Optimized for materials applications





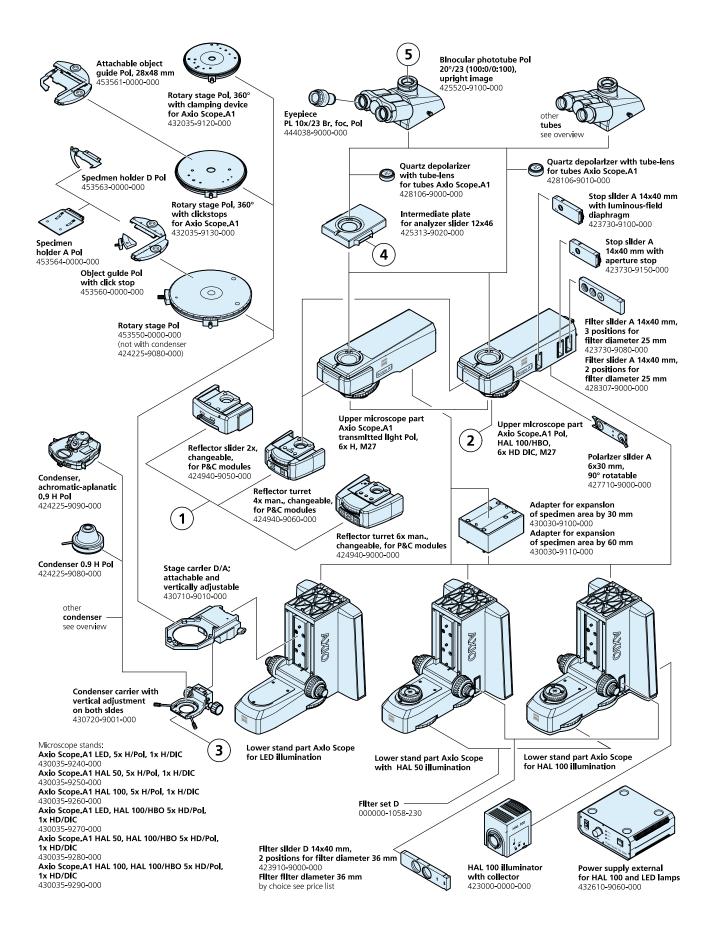
Dimensions in mm (inches)

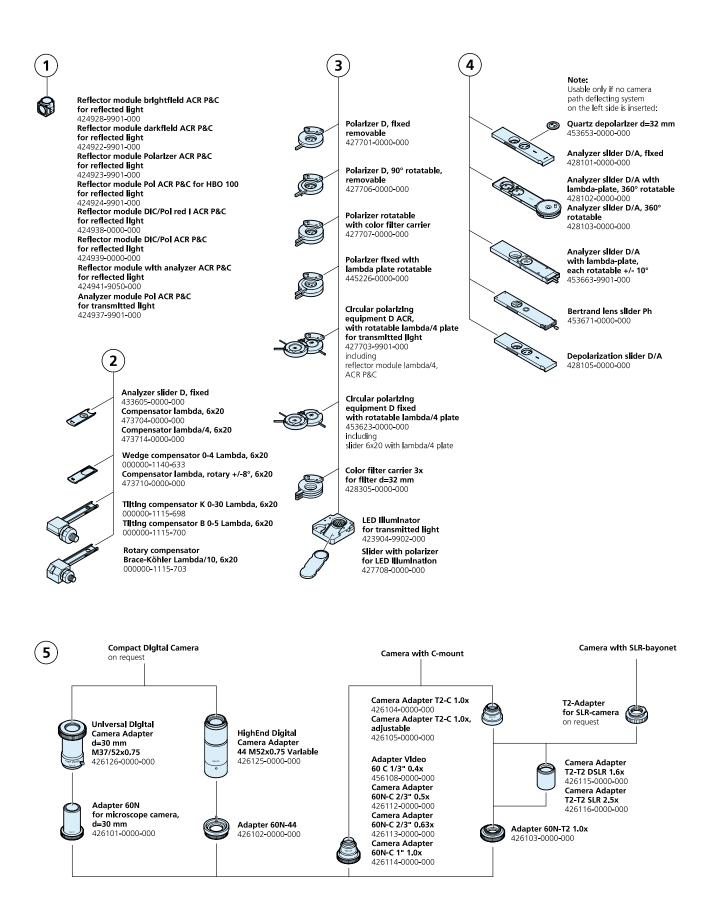
## **Concrete benefits** What details you should know for polarizing microscopy

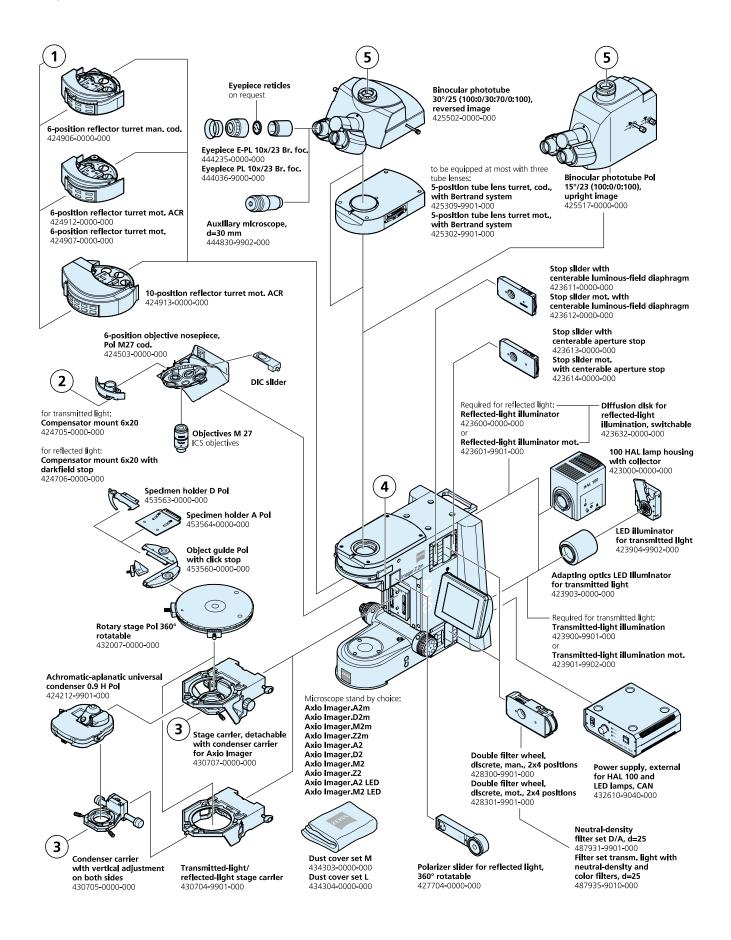
	Axio Scope.A1	Axio Imager 2							
Stands	6 manual stand models optionally available as transmitted-light, reflected-light or transmitted-/reflected-light stands	9 encoded, partly motorized or fully motorized stand versions optionally available as transmitted-light, reflected-light or transmitted-/ reflected-light stands							
Field of view number	23	23/25							
Illumination	Reflected-light: 100 W HAL, HBO Transmitted-light: 100 W HAL, 50 W HAL or LED	Reflected-light: 100 W HAL, HBO Transmitted-light: 100 W HAL, LED							
Optics	Proven ICS optics, optionally with achromatic correction lens system	Innovative IC <sup>2</sup> S infinity system for considerably more contrast in all established contrasting techniques							
	High quality and economical entry-level line of polarizing objective lense	es for transmitted-light: N-ACHROPLAN Pol							
	Enhanced Contrast, the new generations of high contrast polarizing ob Transmitted-light: EC Plan-NEOFLUAR Pol, reflected-light: EC EPIPLAN P								
Contrasting methods	Transmitted-light: qualitative and quantitative polarizing techniques, or field, Phase Contrast, Differential Interference Contrast, PlasDIC*	hoscopy, linear and circular polarization, conoscopy, brightfield, dark-							
	Reflected-light: qualitative and quantitative polarization, brightfield, day Contrast in circularly polarized light (C-DIC), fluorescence	kfield, Differential Interference Contrast (DIC), Differential Interference							
Contrast change Change of modules Push&Click without tool	Manual contrast change via Reflector slider 2x Reflector turret 4x Reflector turret 6x	Contrast change via encoded or motorized Reflector turret Reflector turret 6x encoded Reflector turret 6x mot. Reflector turret 6x mot. ACR Reflector turret 10x ACR							
Objective turret	6x centering objective turret Pol, thread M27	6x centering objective turret Pol, thread M27 encoded							
Polarizers	Transmitted-light: Polarizer (switchable), polarizer (rotary with 0° and 90° click stops), polarizer (switchable with lambda plate, rotary), circular polarizer								
	Reflected-light: Reflector module Pol, Reflector module Pol for HBO103, Polarizer rotary 0-90°	Reflector module Pol, Reflector module Pol for HBO103, Measuring polarizer, rotatable 360° with 0.1° vernier							
Analyzers	Analyzer module or analyzer slider or slider with analyzer and lambda p with 0.1° division, rotatable 360°	late, rotatable 360° or measuring analyzer							
Conoscopy	Bertrand lens module (fixed focus) Bertrand lens slider (focusable)								
		Tube lens turret with focusable Bertrand lens Pol phototube with focusable Bertrand lens Crosshairs and visual field diaphragm in additional intermediate image plane							
Ergonomy/ ease of use	Convenient intensity setting Ergo tube/ergo phototube: 20° viewing angle, vertical adjustment in range of 50 mm	Ergo phototubes Contrast manager Light manager Touchscreen Remote control							
Software	AxioVision microscope software Basic version, upgradeable with functional modules such as MosaiX, Pa	norama, particle analysis or grain size analysis							
Cameras	Open interface for each camera type (video and digital consumer came In particular AxioCam ICc 1 and AxioCam ICc 3, AxioCam HRc, AxioCa								

ICS – Infinity Color Corrected System IC<sup>2</sup>S – Infinity Contrast and Color Corrected System ACR – Automated Component Recognition

 $^{\ast}$  possible in Axio Scope only







### 21

# **Objectives from Carl Zeiss Exceeding Your Expectations**

IIII

Brilliant Imaging for Research and Routine Work in Life Sciences



We make it visible.

# When Your Research Pushes the Boundaries of What Is Visible, Only Performance Counts.

Maximum image information for the best possible result: in order to acquire meaningful images, choosing the right objective is a crucial criterion for success. To ensure reliable analysis, this applies to routine tasks as well as demanding high-end applications. The requirements of users always have one commonality - to achieve maximum resolution with extremely high contrast. Modern research demands the highest standard of optical performance from objectives – particularly in complex applications in which structural information has to be imaged with optimum quality. For over 130 years, objectives have been developed at Carl Zeiss according to scientific calculations which, consistently have set the standards in their class. Carl Zeiss has often redefined the boundaries of the technology in this area:

- First ever calculation of microscope objectives by Ernst Abbe
- Coating glass surfaces to minimize stray light
- Infinity Color Corrected System (ICS) optics
- Stray-light-minimized IC<sup>2</sup>S optics with improved contrast

At Carl Zeiss, together with you, experienced application experts define the criteria that are relevant to you. Starting from objective magnification and working distance to the selection of possible contrast techniques, numerous factors need to be taken into consideration. You will receive comprehensive support when selecting the objective that is right for your individual range of applications. In this way, you can always be sure of one thing: contrast-rich, brilliant images showing as much specimen detail as possible.

#### 1872

Introduction of the first calculated microscope objectives by Prof. Ernst Abbe

#### 1886

Development of fully colorcorrected objectives – the APOCHROMATS

1904

#### **1911** Development oj

Development of parfocal objectives which retain the focus position when the objective is changed

#### 1936

Patenting of anti-reflection coatings on lens surfaces (T-coating)

**1876** *First oil-immersion objectives* 

Discovery of fluorescence microscopy by Prof. August Köhler **1934** *First test version of objectives for phase contrast* 

#### 1938

Introduction of objectives with flatness of field – the Plan-ACHROMATS

#### Considerations when selecting an objective:

*Magnification* Image scale of the objective in the real intermediate image plane

Numerical aperture (NA) Definition of the resolving power of an objective and the light intensity

*Free working distance Distance between the front lens of an objective and the cover glass or specimen* 

*Flatness of field Correction of field curvature to avoid blurred edges* 

**Color correction** Imaging of different colors of the light spectrum in one point

**Transmission** Light transmission of an objective for certain wavelengths

#### Suitability for certain contrast techniques, e.g. Brightfield Darkfield Phase contrast (Ph)

Differential Interference Contrast (DIC) VAREL contrast PlasDIC Polarization (Pol) Fluorescence

**1950** *Introduction of objectives with specimen protection (objectives with spring system)* 

#### 1973

Introduction of infinity optics with the modular Axiomat microscope

**1982** Introduction of ICS optics

#### 1959

Development of first ULTRAFLUAR objectives with focus correction from ultraviolet through to the infrared range **1975** Development of multi-immersion objectives Plan-NEOFLUAR (Imm. Corr.) **2004** Introduction of IC<sup>2</sup>S optics

			~	Ten I	Linde Currac	or the second		OFIL	LD LCI PANADO	Comonia,	W. P. M. A. O. C.	(LD) CAPOCHO
	4.Plan	10 4 POINT	4 Ginoplay	W <sup>4CHOOL4W</sup>	Fluing	EC Plan WEDELL	1D Plan, WEDELL,	Cloanter and	<sup>10</sup> ICIP	<sup>26</sup> in 40 CHIDOL	W Plan-4P	(10) C.400
Specimen with cover glass $0.17 \text{ mm} \pm 0.01$	•	•	•	-	•	•	•	•	٠	•	-	•
Specimen with cover glass 0.14 mm to 0.20 mm	up to NA 0.7	•	up to NA 0.7	-	up to NA 0.7	up to NA 0.7	•	•	•	up to NA 0.7	-	•
Specimen without cover glass	up to NA 0.3	up to NA 0.3	up to NA 0.3	•	up to NA 0.3	up to NA 0.3	•	up to NA 0.8	up to NA 0.8	up to NA 0.3	•	-
Culture plates with glass bottom $0.17 \text{ mm} \pm 0.01$	•	•	•	-	•	•	•	•	•	•	-	•
Culture plate with glass bottom 0.14 mm to 0.20 mm		•	up to NA 0.7	-	up to NA 0.7	up to NA 0.7	•	•	•	up to NA 0.7	-	•
Culture plate with plastic bottom	-	•	-	=	-	-	•	-	-	-	-	-
Open culture plate – objective is immersed in the culture medium		_	-	•	-	-	-	O up to NA 0.8	O up to NA 0.9	-	•	-
Multiwell culture plates with glass bottom 0.17 mm $\pm$ 0.01		•	•	-	•	•	•	•	•	•	-	•
Multiwell culture plates with glass bottom 0.14 mm to 0.20 mm	up to NA 0.7	•	up to NA 0.7	-	up to NA 0.7	up to NA 0.7	•	•	•	up to NA 0.7	-	•
Multiwell culture plates with plastic bottom	-	•	_	-	-	-	•	-	-	-	-	-
Field of view	23 mm		23 mm	23 mm	23 mm	25 mm	25 mm	25 mm	25 mm	25 mm	20 mm	25 mm
Flatness	**	**	**	**	*	****	****	****	****	****	****	****
Color correction	**	**	***	***	*	****	****	****	****	****	****	****
Working distance		very long		long			very long		long		long	
High transmission in UV	**	**	****	****	****	****	***	***	***	***	***	**
Transmission in IR	****	****	****	****	****	***	***	***	***	***	***	***
Correction for 37°C for Live Cell Imaging		-	-	-	-	-	-	yes	yes	-	-	yes
Classic stains e.g. HE	•	0	•	-	0	•	0		•	•	-	•
Fluorescence	▲ VIS	▲ VIS	▲ VIS	•	•	•	•	•	•	•	•	•
3D Deconvolution	0	0	0			•		•	•	•	•	•
АроТоте	-	-	-	-	O VIS	•	0	•	•	•	0	•
Cell Observer	0	0	о	0	●UV	<b></b>	0	•	•	•	0	•
TIRF	-	-	-	-	•*	-	-	-	-	•*	-	-
Laser Scanning Microscopy	-	-	0	•	•	•	-	•	•	•	•	•
FCS	-	-	-	-	-	-	-	-	-	-	-	•*

- Particularly well suited
- ▲ Well suited
- Possible, but not recommended
  Not possible
  Variant or special version

7

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#### 1950

Introduction of objectives with specimen protection (objectives with spring system)

#### 1973

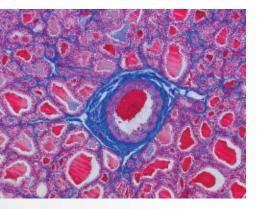
Introduction of infinity optics with the modular Axiomat microscope

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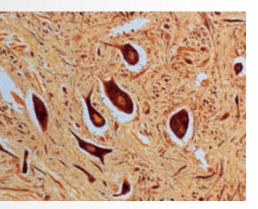
## Many Requirements Demand Many Objective Types. Each is in a Class of Its Own.



#### A-Plan - the A class

A-Plan objectives from Carl Zeiss offer sound and reasonably priced entry into the world of microscopy. They are versatile in their use and deliver good optical quality.

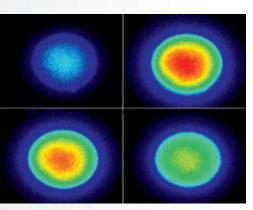




#### ACHROPLAN - the solid performers

Solid and reliable: the objectives of the ACHROPLAN class stand out through their excellent flatness of field. They are a highly recommendable solution for image documentation in pathology.





#### FLUAR - the photon collectors

The objectives of the FLUAR series are manufactured from special optical glasses. High numerical apertures, good contrast and very high transmission for the entire visible spectrum to the near UV result in great optical performance. The objectives of choice for making the weakest fluorescence signals visible.

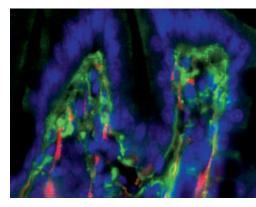


## Overview



#### EC Plan-NEOFLUAR the all-round performers

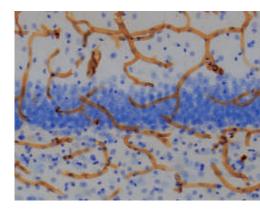
Where flexibility and multiple imaging methods are required, the EC Plan-NEOFLUAR objectives are often selected. The optimized IC<sup>2</sup>S optics makes it possible to achieve contrastrich imaging with excellent homogeneity and high resolution. From transmission to the near UV, outstanding flatness of field and achromatic correction, to high numerical apertures, the EC Plan-NEOFLUAR class meets the high demands of applications using brightfield, darkfield, phase contrast, DIC, polarization and fluorescence.





#### Plan-APOCHROMAT the precision performers

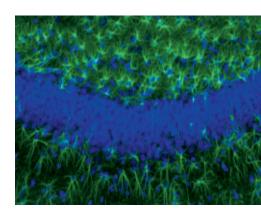
With the best color correction and highest numerical apertures, Plan-APOCHROMAT objectives deliver brilliant images in brightfield, DIC and fluorescence techniques. Their outstanding point spread function and extreme chromatic correction are particularly impressive. High resolution and excellent image sharpness make even the finest details and color nuances visible.





#### C-APOCHROMAT - the top performers

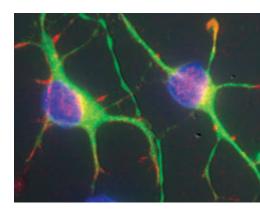
These high-performance objectives are able to compensate optically for different refractive indices and layer thicknesses of the mounting medium by means of a correction collar. They are perfectly suited to extremely demanding applications in research of living organisms and immersion specimens. For brilliant images in all applications and 3D techniques such as confocal Laser Scanning Microscopy, ApoTome and 3D Deconvolution.



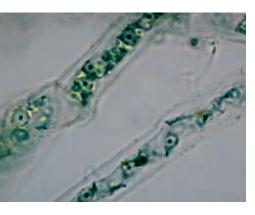


#### LCI - the immersion specialists

LCI stands for Live Cell Imaging. The high-performance objectives of this class have been specifically developed for complex applications involving living cells and tissues. They are calculated for temperature intervals of 23° C to 37° C. Spherical aberrations caused by deviating cover slip thicknesses, different temperatures or refractive indices are ideally compensated by use of correction collar. Therefore, more visible details and reliable results for your scientific analyses are possible.



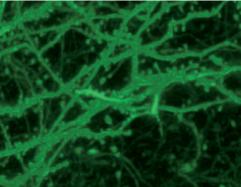
# Objectives for special applications



#### LD - the flexible performers

Special applications require special objectives. Long distance objectives are used if, for example, you need to focus deep into a thick specimen or through the plastic bottom of cell culture plates. LD objectives are as varied as the tasks they perform: with LD A-Plan and LD Plan-NEOFLUAR objectives for inverted microscopes it is possible to examine cells in plastic culture plates and specimens under a standard cover glass. LD variants of the LCI and C-APOCHROMAT series have been developed for applications involving living cells: using a correction collar, deviations in cover glass thicknesses, for example, can be compensated optically.





#### W - for immersion

Physiological applications involving living cells and/or tissues often require water objectives which can be dipped directly into the culture medium. W objectives have a conical tip made from special inert plastic. They are often, but not only used in combination with a fixed-stage microscope. These objectives are distinguished through their outstanding optical performance with good flatness of field and high transmission for perfect results in physiology.





### **Color Coding of Objectives**

#### Labeling of the objective

Objective class, special designations are used for this, e.g. LD for Long Working Distance

#### Magnification/ numerical aperture

plus additional details on

- immersion medium (Oil/W/Glyc)
- adjustable cover glass correction (Korr.)
- contrast method

#### Tube length/cover glass thickness (mm)

ICS optics:  $\infty$ Infinity Color Corrected System

standard cover glass: 0.17 without cover glass: 0 insensitive: -

#### Mechanical correction collar for -

- cover glass thickness correction
- different immersion
- different temperature
- adjusting an iris diaphragm



#### Color of writing

Contrast method

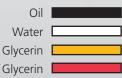
Standard Pol/DIC



#### Color coding of magnification

1.0/1.25	
2.5	
4/5	
6.3	
10	
16/20/25/32	
40/50	
63	

#### Immersion fluid



Oil/Water/Glycerin

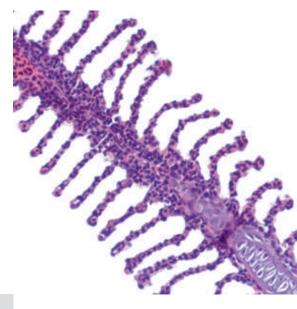
## **A-Plan**

## Every Objective Meets Just One Demand: Perfection Down to the Last Detail



## A-Plan: good entry-level product with excellent performance

From laboratory and routine microscopy through to the research class, A-Plan objectives are the right entry-level choice. They are suitable for brightfield and phase contrast and deliver good contrast. A-Plan objectives can also be used in fluorescence applications with excitation wavelengths in the visible spectral range.



- Field of view: 23 mm
- Flatness: \*
- Color correction: ★

Entry-level objectives for laboratory, routine and research microscopes



#### LD A-Plan: versatile in inverse microscopy

This entry-level line for inverted microscopy is economical, flexible and rich in contrast. These objectives have a particularly long working distance that makes it possible to carry out observations through thicker cell culture vessels. They are corrected for the use of cover glasses and vessel bottom thicknesses of up to 2 mm. From brightfield, phase contrast, VAREL to Hoffman Modulation Contrast and PlasDIC, these objectives can be used in a wide range of contrast methods for unstained cells and tissue. Suitable for excitation wavelengths within the visible spectral range, just like the A-Plan objectives these can also be used in fluorescence microscopy. A highperformance entry-level series for sophisticated microscopy.



- Field of view: 23 mm
- Flatness: \*\*
- Color correction: **\* \***

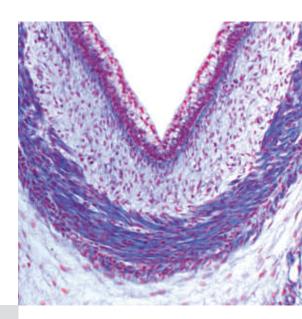
Objectives with long working distance for inverted microscopes; attachable cover glass cap for thinner cover glasses measuring 0.17 to 0.6 mm

# ACHROPLAN



#### ACHROPLAN: versatility like no other

The ACHROPLAN objectives have good flatness of field and color correction and are well-suited for microphotography. In accordance with the wide range of applications, ACHROPLAN objectives are available in various versions. The possible contrast techniques that can be used are brightfield and phase contrast in transmitted light and fluorescence with excitation in the visible range.



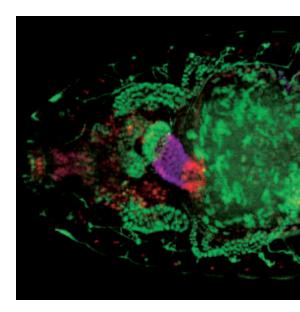
- Field of view: 23 mm
- Flatness: \*\*
- Color correction: \*\*\*

Objectives in many variations for diverse applications



#### W ACHROPLAN: dive to physiological depths

The water objectives of the ACHROPLAN series are primarily used in connection with an upright fixed-stage microscope in the area of electrophysiology. Such set-ups make it possible to dip into a medium using the immersion objective and to examine a specimen from above. Here, microscopic techniques are combined with physiological methods. Typical areas of use include the patch-clamp technique and intracellular recording in electrophysiology, intravital microscopy as well as the examination of microcirculation and of thick specimens when working with vital brain sections. Thanks to the slender tip and the long working distance, electrodes and microinjection capillaries can be brought to the specimen without any problems. Objectives belonging to the W ACHROPLAN class are impressively flexible. All contrast techniques are possible, including fluorescence and infrared DIC. The optical performance and exceedingly high transmission are particularly outstanding features for visibly more information in physiological applications.



- Field of view: 23 mm
- Flatness: \*\*
- Color correction: \*\*\*

Objectives for immersing directly into cell culture media, specifically for physiological applications

# FLUAR



#### FLUAR: detects very weak fluorescence signals

The FLUAR objectives stand for maximum light transmission and photon collection. Manufactured from special glass, these objectives have been developed specifically for qualitative and quantitative analyses of ion modifications and for demanding fluorescence applications. Good flatness of field up to 23 mm, high numerical apertures and very high transmission from a wavelength of 340 nm – making even the weakest signals clearly visible.



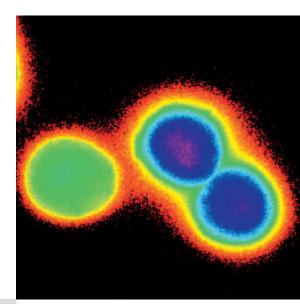
- Flatness: \*
- Color correction: \*

Objectives with high numerical apertures and extremely high transmission properties from 340 nm



#### ULTRAFLUAR: ultra-effective in UV light

ULTRAFLUAR for ultraviolet light – with ULTRAFLUAR objectives, it is possible to carry out applications using fluorescence excitation in the UV wavelength range. Only quartz glasses are used in their manufacture. These objectives demonstrate outstanding transmission from 240 nm to the infrared range. Consequently, they cover the widest spectral range and have good flatness of field up to 20 mm. With these objectives you will always obtain a reliable result, even in applications with excitation light in the UV range.



- Field of view: 20 mm
- Flatness: \*
- Color correction: \*

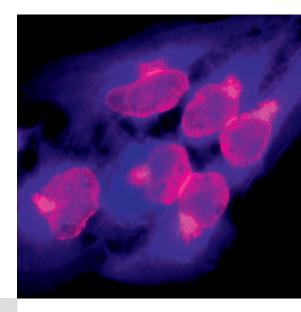
Objective for fluorescence excitation in the UV range from 240 nm

# EC Plan-NEOFLUAR



#### EC Plan-NEOFLUAR: excellent contrast

EC stands for Enhanced Contrast. In combination with the chromatic correction and high resolving power, these universal objectives deliver brilliant images that are rich in contrast, while retaining excellent flatness of field. Glass with low intrinsic fluorescence is used in their manufacture, which, in addition to their high transmission from the near UV range, virtually predestines the EC Plan-NEOFLUAR class for fluorescence applications. Special objectives in this class include EC Plan-NEOFLUAR Antiflex for reflection contrast and EC Plan-NEOFLUAR Pol for polarization.



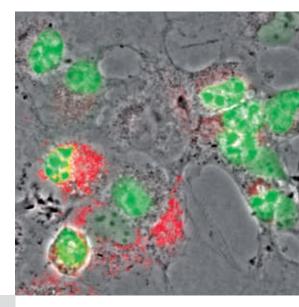
- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*

Universal objectives with excellent properties for fluorescence microscopy



#### LD Plan-NEOFLUAR: go the distance

LD Plan-NEOFLUAR objectives with an extra long working distance are objectives designed for cell culture. These objectives are used on the inverted research platforms such as Axiovert and Axio Observer. With a correction collar, the objectives can be adapted seamlessly to various optical conditions, e.g. the use of conventional cover glasses or plastic culture plates in the 0 to 1.5 mm range. Due to the outstanding fluorescence properties of all EC Plan-NEOFLUAR objectives, the LD variants are also ideally suited for fluorescence microscopy. In addition, all current contrast techniques in transmitted light, such as brightfield, phase contrast, DIC and PlasDIC, are also possible. For brilliant, high-contrast and meaningful images even at long working distances.



- Field of view: 23 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*

Objectives with long working distances for inverted research microscopy, very well-suited to fluorescence applications

# Plan-APOCHROMAT



#### Plan-APOCHROMAT: protects sensitive samples

Plan-APOCHROMAT objectives demonstrate top-class optical performance. They make it possible to see structures at the boundary of what is visible. Their outstanding performance features include: excellent correction, extremely high apertures and maximum resolution, color purity, contrast and flatness of field. All this combines to produce brilliant, needle-sharp images for observation, digital documentation and, in particular, fluorescence applications. The i Plan-APOCHROMAT of the 63x objective has been developed specifically for Live Cell Imaging – for optimal focus stability for time-lapse experiments.

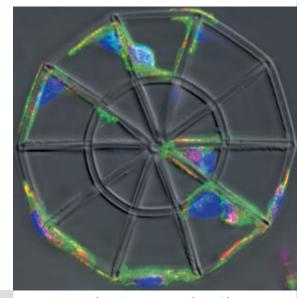


Image courtesy of Martin Bastmeyer und Franziska Klein, University of Karlsruhe, Germany

- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

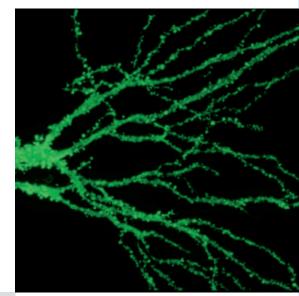


#### W Plan-APOCHROMAT: apochromatically correct

Objectives with optimum correction of flatness of

field and color; suitable for Digital Imaging

The immersion variant of the Plan-APOCHROMAT series – an addition to the water objectives of the ACHROPLAN class – has been specifically designed for electrophysiology. W Plan-APOCHROMAT objectives have apochromatic correction from visible light to the near infrared (VIS - IR) and are intended for use without a cover glass. Typical transmission values are greater than 80% from 450 nm to 1,000 nm and greater than 50% at 365 nm. These are also ideal prerequisites for use in 2-photon microscopy. The front of this slender objective is made of a special inert plastic that was originally developed for food technology.



- Field of view: 20 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

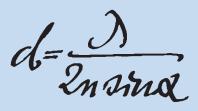
Apochromatically corrected immersion objectives for applications in physiology

## Technology

#### Resolution

The resolution of an optical system is generally defined as the smallest distance between two object structures at which these objects are still imaged separately or perceived as being separate. Due to the wave nature of light and the diffraction associated with this, the resolution of an objective is limited. This limit is theoretical, i.e. even a theoretically ideal objective without any imaging errors has a finite resolution.

Resolution can be calculated according to the famous formula introduced by Ernst Abbe and represents a measure of the image sharpness of a light microscope:



- $\lambda$  = wavelength of the light used (effective wavelength of white light: 550 nm)
- n = refractive index of the optical medium between the front lens and cover glass (air = 1;  $H_2O = 1.33$ ; immersion oil = 1.518)
- $\alpha$  = half the opening angle of the objective used

It becomes apparent from Abbe's formula that resolution is determined by the wavelength of the light used ( $\lambda$ ), as well as by the product of the refractive index (n) of the medium between the cover glass and front lens and the sine of half the opening angle ( $\alpha$ ) of the objective used. Due to the central significance of this interrelationship for imaging in microscopy, Abbe introduced the concept of numerical aperture.

#### Numerical aperture

Microscopic images are generated through the interaction (interference) of the light diffracted at the sample with the uninfluenced light that penetrates the sample. The interference of these light components leads to an intermediate image that already contains all the image information. This intermediate image is magnified in the microscope by the eyepiece. This is, therefore, also referred to as two-stage imaging process.

The larger the opening angle of an objective, the more (diffracted) light can be gathered from the sample and the higher the resolution of the resulting image.

This fundamental correlation was identified for the first time by Ernst Abbe at Carl Zeiss in 1872. He introduced the concept of the numerical aperture (NA) of an objective. This is defined as the product of the refractive index between the cover glass and front lens of the objective and the sine of half the opening angle of the objective:

 $NA = n \cdot sin(\alpha)$ 

The numerical aperture is a measure of the size of the cone of light captured by the objective, taking the immersion medium used into consideration.

Resolution values are given in the table below for a number of typical objectives with different apertures. In practice, however, these calculated resolutions are only achieved as long as the imaging system does not show any imaging errors, i.e. if the imaging is diffraction-limited.

Resolution table using green light with  $\lambda = 0.550 \ \mu m$ :

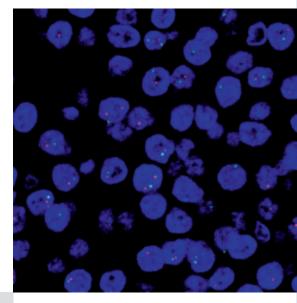
Magnification	/	NA	Resolution (µm)
10x	/	0.30	1.10
40x	/	0.75	0.45
63x	/	1.40 Oi	0.24
100x	/	1.30 Oi	0.26

# C-APOCHROMAT



#### C-APOCHROMAT: top performance in 3D

The high-quality and extremely powerful objectives of the C-APOCHROMAT class are perfect for studying living cells. They are optimally corrected in terms of flatness and color and meet even the highest requirements in 3D microscopy using confocal LSM, ApoTome Structure Illumination or 3D Deconvolution. The specimens used here are often in a watery medium that has a similar refractive index to water. With the C-APOCHROMAT objectives it is possible to compensate for spherical aberrations, which frequently occur if the refractive indices of the immersion and mounting medium are different, by means of a correction collar. Furthermore, the correction collar also allows very small deviations in the thickness of the cover glass and different temperatures to be compensated for. Optimum performance parameters provide the best possible results.



- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

apertures for 3D microscopy; optimum correction and high transmission

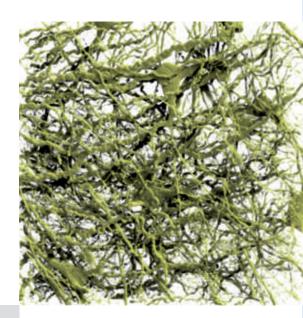
Water immersion objectives with very high numerical



#### LD C-APOCHROMAT:

#### developed for multiphoton microscopy

The LD C-APOCHROMAT objective has a long working distance while retaining a high numerical aperture of 1.1. This expert technology is particularly suited to confocal multiphoton microscopy in which extremely high penetration depths are achieved through the use of infrared excitation light. However, it is also possible to achieve excellent results with this objective using other 3D techniques such as ApoTome Structured Illumination and 3D Deconvolution.



- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

Objective with long working distance optimized for multiphoton microscopy

## Technology

#### Imaging properties

Glass lenses that are used in a light microscope fundamentally show imaging errors. These imaging errors can be reduced to a practically insignificant level by optical design measures.

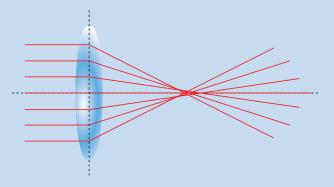
In general, imaging errors (aberrations) are understood to mean deviations from the ideal diffractionlimited imaging.

Aberrations may affect microscopic images in different ways, e.g. through reduced contrast, poor resolution or geometric distortions. Two types of aberrations well known in microscopic systems are spherical and color aberrations.

#### Spherical aberrations

Spherical aberrations, also known as aperture errors, can significantly impair the imaging quality of a microscope objective.

Spherical aberrations occur because the focal distance of an individual lens depends on the distance of the incident light beams from the center of the lens. The focal distance of the lens is shorter for light beams at the edge than for beams near to the optical axis. Consequently, there is no single focal point but a focal line along the optical axis. This leads to a reduction in imaging contrast and sharpness (see diagram). High-magnification dry objectives with high numerical apertures are particularly sensitive to this type of image error.



In principle, it is possible to correct this error in the objective. This correction can be carried out on a fixed or an adjustable basis, as in the case of objectives with a correction collar.

Spherical aberrations are not only influenced by the optical properties of an objective but also by the properties of the cover glass and mounting medium. That is why, in the case of high-magnification objectives with high apertures, the cover glass is viewed as a component of the optical system and a standard thickness of 0.17 mm is required.

In practice, there are mainly two factors that considerably intensify spherical aberration:

- 1. The difference in the refractive indices of the immersion medium and mounting medium. The more the refractive index of the immersion medium deviates from the refractive index of the mounting medium, the more marked the spherical aberration. An objective that, for example, is calculated for oil immersion (n = 1.52) therefore demonstrates a considerable spherical aberration if the specimen structures are imaged in a watery solution (n = 1.33).
- The distance between the cover glass and the specimen structure to be examined. In general, spherical aberration intensifies as the sample depth increases. Ideally, therefore, the specimen should be positioned directly under the cover glass.

In the case of objectives that do not have a correction collar, attention should therefore be paid to observing the recommended cover glass thickness and adjusting the refractive index of the mounting medium to the refractive index of the objective immersion. Otherwise, the result is an image with poor contrast and bad resolution.

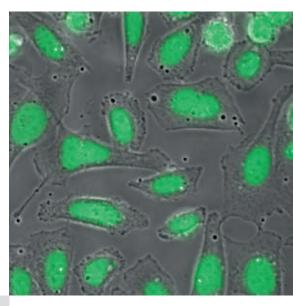
In the case of objectives that do have a correction collar it is possible to compensate for deviating values in the cover glass thickness and differences in the refractive indices of the objective immersion and mounting medium. In addition, the increasing spherical aberration that occurs when imaging deep-lying structures in thick samples can also be corrected.

# Live Cell Imaging



#### LCI Plan-NEOFLUAR: flexible with immersion

Whether it's with water, glycerin or oil – with their high numerical apertures and, therefore, optimal resolution, the LCI Plan-NEOFLUAR objectives are used for Live Cell Imaging (LCI) – with and without cover glass. The objectives of this series can always be flexibly adapted to the refractive index of the culture or mounting medium. This allows the ideal matching of the immersion fluid to the specimen's embedding media, thus eliminating spherical aberration caused by refractive index mismatch. In addition, the LCI Plan-NEOFLUAR range also allows living cells to be observed in physiological conditions of 37° C – under optimum optical conditions. With just one correction collar, immersion, cover glass thickness and temperature can all be set appropriately for brilliant insights into the dynamic processes of living organisms. The version i LCI Plan-NEOFLUAR with isolation collar is especially suited for incubation.



- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

Flexible objective for Live Cell Imaging with and

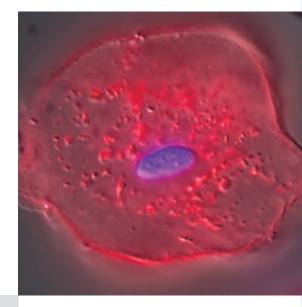
without cover glass for oil, glycerin or water



#### LD LCI Plan-APOCHROMAT: multi-tasking for Live Cell Imaging

immersion

This objective has been developed to meet the very highest requirements in Live Cell Imaging. Besides the performance features of all LCI Objectives already mentioned, LD LCI Plan-APOCHROMAT offers an extremely long working distance for this objective class of 0.57 mm. This working distance makes it possible to focus through thick specimens, e.g. through a brain section or a whole mount embryo. Color correction and flatness of field are identical to the Plan-APOCHROMAT series and represent the maximum standard of performance – despite the long working distance. Maximum quality for maximum reliability in scientific analysis.



- Field of view: 25 mm
- Flatness: \*\*\*\*
- Color correction: \*\*\*\*\*

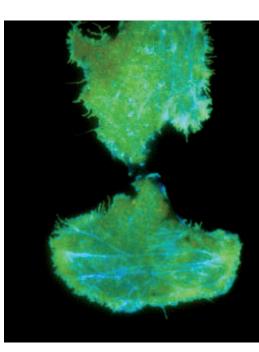
Flexible multi-immersion objective with long working distance for Live Cell Imaging

## TIRF



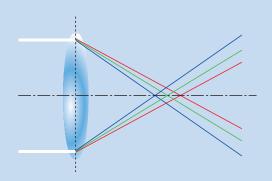
#### α Plan-FLUAR 100x and α Plan-APOCHROMAT 100x: make cell membranes visible

The special fluorescence technique TIRF (Total Internal Reflection Fluorescence) calls for objectives with particular properties. In this method fluorescence molecules are excited in a thin layer directly at the surface of the cover glass. Molecular mechanisms around the cell membrane, e.g. transport processes, are made visible – at layer thicknesses below 200 nm. In addition to high contrast and high resolution, an appropriate objective must also have a high numerical aperture of at least 1.45. Carl Zeiss has developed both  $\alpha$  Plan-FLUAR 100x with a numerical aperture of 1.45 and  $\alpha$  Plan-APO-CHROMAT 100x with a numerical aperture of 1.46 for TIRF. Both objectives have excellent transmission properties from 340 nm and, with their extremely high numerical aperture, are also ideally suited to conventional fluorescence techniques with maximum resolution.



#### Chromatic aberration

Single lenses have different focal distances for different wavelengths, i.e. different colors of light. This phenomenon is known as dispersion. Chromatic aberration shows itself in the form of narrow reddish or greenish color fringes around specimen structures.



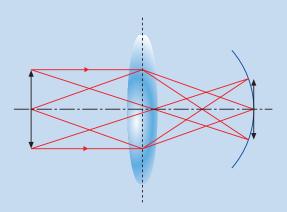
This color error can be almost completely rectified by making an appropriate choice of types of glass with various dispersion values. Chromatic aberration has practical significance because increasing the aperture of an objective improves the sharpness of the imaged object but also magnifies color errors of the optical system. High resolution microscope objectives therefore place extremely high demands on the elimination of color errors. Depending on the degree of correction, a distinction is made between, in order of increasing color error elimination, ACHROMATS, fluorite objectives and APOCHROMATS.

At Carl Zeiss, APOCHROMATS are fully color-corrected for up to 7 wavelengths from UV through to IR. APOCHROMAT objectives are virtually free of any traces of color fringes. They were calculated for the first time at Carl Zeiss by Ernst Abbe in 1886. The correction of chromatic aberration is determined through the choice of the type of objective used and can scarcely be influenced in practice. Simply using the wrong immersion medium (e.g. anisol rather than immersion oil) leads to color fringes becoming more perceptible.

## Technology

#### Field curvature

The effect of field curvature means that a flat structure is imaged on a curved surface.



This image error can be completely rectified by making a suitable choice of the lens curvatures in the objective. Objectives with a flattened field of view contain the word 'Plan' in their name. Objectives with a completely flat field of view were invented at Carl Zeiss in 1938 by Hans Boegehold. Depending on the color correction, the following Plan objectives are available: Plan-ACHROMAT, Plan-Fluorite and Plan-APOCHROMAT.

In practice, field curvature is particularly disruptive in the case of flat specimens such as blood smears or histological sections. Modern objectives of the Plan-NEOFLUAR and Plan-APOCHROMAT class are fully flattened up to a field of view of at least 25 mm.

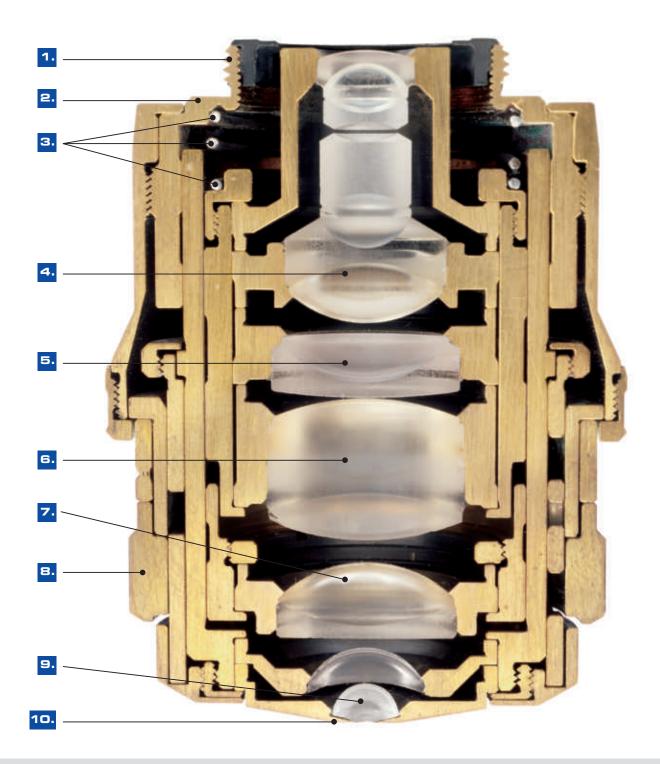
#### Cover glasses and mounting media

Cover glasses have a decisive influence on the imaging quality of a microscope as they form an optical component of the objective. Many objectives are calculated for a cover glass thickness of 0.17 mm exactly. For special purposes, e.g. smears, there are also objectives for uncovered objects (cover glass thickness = 0). If, however, the thickness of the cover glasses used deviates from the calculated value, the result is a clearly perceptible deterioration of the image caused by spherical aberration. It should also be taken into consideration that the thickness of the mounting medium also has an impact on the effective cover glass thickness. In practice, a deviating effective cover glass thickness becomes noticeable above a numerical aperture of 0.35. Above a numerical aperture of 0.7, even extremely small deviations (+/- 0.01 mm) from the specified cover glass thickness have a significant effect on the image. For this reason, many high-aperture objectives are equipped with a correction collar.

In practice, please set the correction collar as follows:

- Set the correction collar on the objective to 0.17 mm/to a marking that corresponds with this value.
- Use a position on the specimen with small structures and as high contrast as possible. Focus this using the fine focusing control.
- Carefully turn the correction collar in one direction and observe the change in imaging quality – pay particular attention to the contrast of the image. As a rule, the image sharpness is lost during this process. This should be readjusted through continuous refocusing using the fine focusing control.

If the imaging becomes worse, turn the correction collar back slightly in the opposite direction and optimize the image until the structures are imaged sharply with exceptional contrast.



#### Cross section of an objective

- 1 Objective thread
- 2 Stop face of the objective
- 3. Spring system for the specimen-protection mechanism
- 4 7. Lens groups for the correction of image errors8. Correction collar for adapting to deviating cover glass thicknesses or temperatures
  - 9. Front lens system
  - 10. Front lens holder

### Where to Find More Detailed Information

88.54

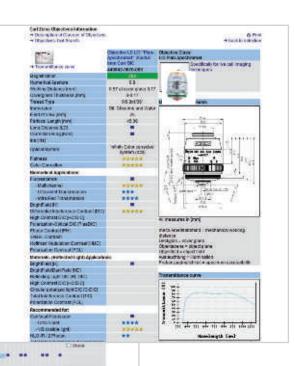
-

-

Choosing the right objective depends on a number of different factors and users may find themselves faced with unexpected issues. You will find everything that you want to know about each individual objective – down to the last detail – in the comprehensive Carl Zeiss objective database. From field of view, flatness of field, color correction and transmission properties to technical details and dimensions – it's all here. Naturally, you can also make selections on the basis of search criteria such as magnification, numerical aperture, contrast technique, etc.

In the objective class database you will find the most up-todate information and the ideal solution for you: the best Carl Zeiss objective for your application.





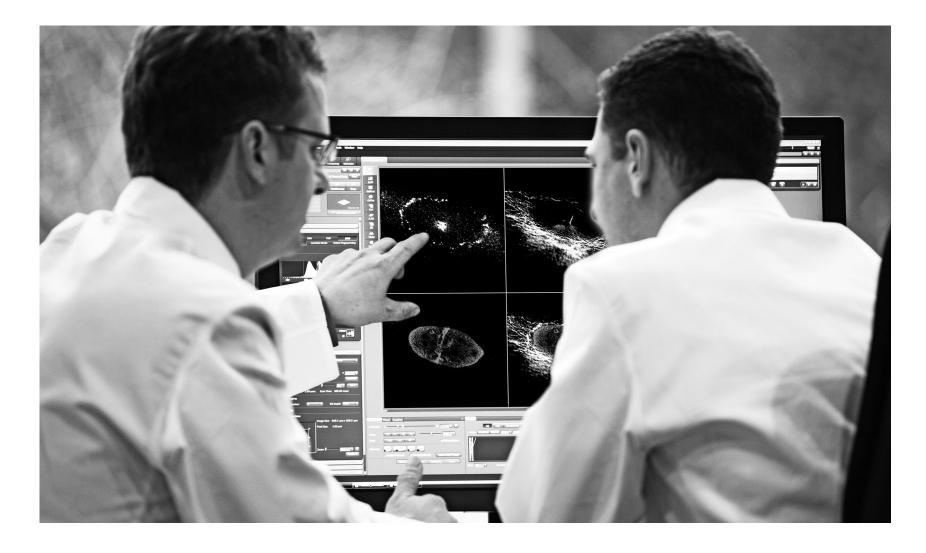
#### www.zeiss.de/objectives

In addition to the right objective, clean microscope optics are prerequisite for perfect images. For more information see the website below.

www.zeiss.com/cleanmicroscope

Carl Zeiss Microscopy GmbH 07745 Jena, Germany microscopy@zeiss.com www.zeiss.com/microscopy





Product Information Version 2.0

## **ZEN Imaging Software**

Faster. Easier to Use. More Universal. The Software for All Systems.

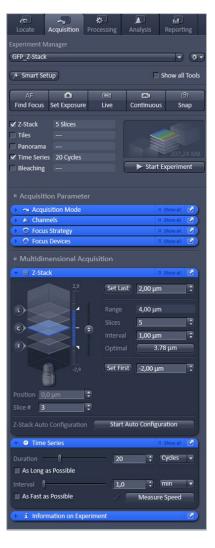


### **ZEN Shortens the Path to Your Goal**

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- > The Applications
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- > The System
- > Technology and Details
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- > Service

ZEN – ZEISS Efficient Navigation – is the single user interface you will see on all imaging systems from ZEISS. ZEN software leads you simply and quickly to the result. At all times you see which options the system is making available to you and which step is appropriate to take next. ZEN makes it easy to operate every imaging system from ZEISS correctly and intuitively. As a result you save time, reduce training and support costs, and get faster answers to your questions.





### ZEN: Simpler. More Intelligent. More Integrated.

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#### ZEN: The Essentials Count – Focus on What You Need

ZEN controls all imaging systems from ZEISS, letting you operate all of your devices with the same convenient interface. ZEN arranges operating elements in a way that follows your workflow. Functions you use only rarely are hidden away, out of sight – but always there with a single click.

#### Smart Setup: Select Fluorophore. Acquire. Done.

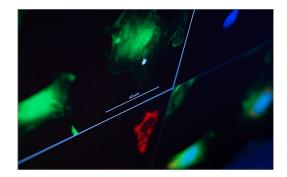
Smart Setup is the core of ZEN – your intelligent control centre. Select the dye for your sample from the database with more than 500 dyes and ZEN automatically applies all necessary settings for your imaging system. The innovative "Motifs" feature helps you to further optimize your imaging with a single click.

#### A Secure Format for Important Data

The security of your data gets top priority as ZEN stores each of your experiments with all its metadata. Using the data format .czi from ZEISS you can even process the huge amount of data you acquire with our fast 3D imaging systems. Alternatively, store your images as OME-TIFF, the image format specification of the Open Microscopy Environment including metadata, to facilitate crossplattform image data exchange.



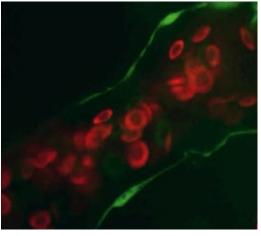




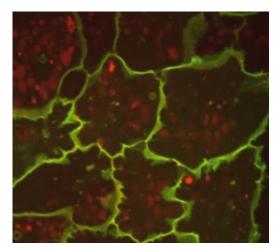
### ZEN at Work

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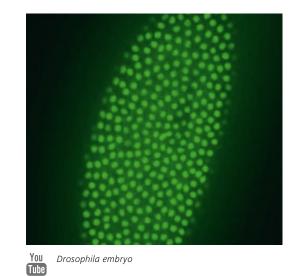
- Image subcellular trafficking in 3D over time with maximum acquisition speed
- Visualize cytoskeletal dynamics with highest sensitivity
- Carry out photobleaching experiments
- Perform functional imaging of cellular signal transduction with high temporal resolution
- Perform confocal live cell imaging with highest sensitivity

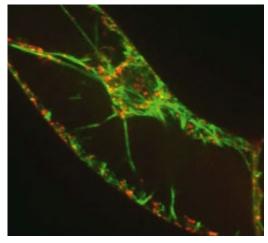


You Zebrafish blood



You Tube Xenopus explant







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ZEN features the following	module packages:
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ZEN lite	Is the free basic version of the high-performance microscopy software ZEN. You do not need a license for ZEN lite, unless you want to extend this version with specific modules for your applications
ZEN desk	Supports you in your offline analyses. Extend this version with modules for image processing and analysis
ZEN pro	Controls all imaging systems except laser-based 3D systems
ZEN system	Is the software package for all imaging systems, including laser-based 3D imaging systems (LSM 780, LSM 710, Cell Observer SD, Laser TIRF 3)

Basic functionality	ZEN desk	ZEN pro	ZEN system						
User Interface and files	Graphical user interface switchable light or dark design to adapt to ambient brightness*								
	User interface with stepless scaling and zooming								
	All functional elements can	be displayed either in basic or advanced mode							
	Configuration options for the graphical user interface: creation of menu bars and customized buttons, saving of workspace configurations, definition of properties of standard graphic elements and application of functions to TFT soft keys								
	Image import (LSM, ZVI, BMP, TIF, JPG, GIF, PNG) and function to convert images (TIF, JPG, BMP) into CZI format								
	Export to OME-TIFF - image format specification of the Open Microscopy Environment, including metadata, to facilitate cross-platform image data exchange								
	Export into ZVI, BMP, GIF, JPG, PNG, TIFF, HDP image and AVI and WMF video formats								
	Batch Export of images and	videos							
Hardware Control		Full integration of ZEISS microsco	pes, cameras and accessories						
	Interactive and automatic control of the motorized microscope components								
	Transfer of information from encoded components into the software								
		Reproducible acquisition with mil	lisecond precision – digital I/O card) for hardware control						

\* ZEN (blue edition) only

> In Brief

ZEN features the following module packages:

The Advantages	Basic functionality	ZEN desk	ZEN pro	ZEN system
The Applications	Image Acquisition			utomatic creation of experiments to acquire multichannel fluorescence and transmitted ligh e to further optimize acquisition experiments for quality or speed
The System			Acquisition experiments can be acquisition parameters	configured, saved and reloaded. Re-Use function from images automatically restores
- 1 1			Movie Recorder enables fast and	d simple acquisition of movie clips through use of Start and Stop
echnology and Details			Sequence of acquisition dimens	ions can be selected (depending on active dimensions)
ervice			Interactive graphical representat	tion of the microscope light path
			Fully automatic assignment of g accuracy	eometric scalings for image acquisition. Manually created scaling supported for even high
				tion history as metadata in CZI image format. This format has been developed to be as ecification, Copyright 2002-2012 OME (Open Microscopy Environment)
			Automatic saving of acquired in	nages in CZI (including metadata) to prevent image data loss
	Analysis, Processing and Views	Navigator window		
		Interactive measurement, Sc	ale Bars and Text Annotations	
		Management, visualization a	and printing of metadata and images	
			tandard operations for image optimization: a, colors, smoothing, sharpening, geometric corre	ctions
		Image file browser		
		Up to three independent ima	age containers, image comparison view	
		Gallery view		
		View for histogram measure	ement	
		View for profile measurement	nt	
		2.5D (pseudo-3D) view		
		Info view for metadata, part	tially editable	
		Functions for working with a	data tables: filtering and sorting of tables	
		Diagram view to display date	a in the form of histograms, line plots, bar and pi	e charts or x/v scatter plots

ZEN features the following module packages:

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Service

Task	Module	ZEN provides:
Basic	ZEN	Detector Control, AxioCams and PMTs from ZEISS included, third party devices optionally available
		Laser-/Lasermodule-Control
		Microscope Control
	Macro Recorder and Editor	Use a programming language to generate macros ZEN (black edition): VBA, ZEN 2012 (blue edition): Python
	Visual Macro Editor, VME	Use symbols to generate macros for complex acquisition strategies
	Visual Basic Macro, VBA	Use a macro recorder or freely program VBA code
Acquisition	Multi Channel	Record different fluorescence and transmitted light images in independent channels
	Time Lapse	Record images over time
	Z Stack	Record Z-stacks with the help of a motorized focus drive
	Manual Extended Focus	Acquire images manually and calculate a 2D image out of a Z-stack
	Autofocus	Determine the focus position of your specimen
	Tiles & Positions	Record exact, highly resolved images by automatically scanning pre-defined specimen areas Produce images with the help of position lists. Configure tile regions and individual positions
	Panorama	Manually acquire highly resolved overview images from individual 2D images
	Experiment Designer	Configure non-homogeneous imaging experiments
	ROI-HDR	Acquire and display HDR image data with extended dynamic range, incl. illumination blanking

Module

Extended Focus

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- Analysis

Task

Processing

Select your modules according to your requirements

Deconvolution	
Deconvolution	Improve Z-stacks with 3D deconvolution algorithms
3D VisArt	Visualize and render 3D image stacks
Spectral Unmixing	Perform spectral unmixing of lambda stacks or multichannel images, via reference spectra or component analysis
Colocalisation	Analyze colocalisation between two fluorescence channels quantitatively
Measurement	Use extended interactive measurement tools
Image Analysis	Use an assistant to create an automatic measurement program
Advanced Processing & Analysis	Add Acquisition-feedback capability and hierarchical measurements to your Image Analysis
FRAP Efficiency Analysis	Analyze acquired FRAP/FLAP or similar time series with bleach events, including mean ROI measurements
FRET plus	Analyze FRET data with either sensitized emission or acceptor photobleaching method, including mean ROI measurement
3D Analysis	Evaluate and display 3D image data stacks with various measuring tools
ASSAYbuilder	Carry out "high content" analyses (HCA) of .zvi images
Physiology	Analyze physiological time series data
FCS for GaAsP and APD	Analyze single molecules with GaAsP and APD detectors, FCS, spectral FCS and FCCS with LSM 780, LSM BiG and ConfoCor 3
Enhanced FCS	Perform interactive and global fitting using extended and self defined fit models
Photon Counting Histogram	Histogram of the photon counting populations for all FCS systems
Image Correl. Spectro. RICS	Analyze single molecules with Raster Image Correlation Spectroscopy for LSM 710 with PMT or GaAsP detectors
Торо	Analyze surface data and visualize measurement results
	Spectral Unmixing         Colocalisation         Measurement         Image Analysis         Advanced Processing & Analysis         FRAP Efficiency Analysis         FRET plus         3D Analysis         ASSAYbuilder         Physiology         FCS for GaAsP and APD         Enhanced FCS         Photon Counting Histogram         Image Correl. Spectro. RICS

ZEN provides:

Calculate a 2D image out of a 3D Z-stack

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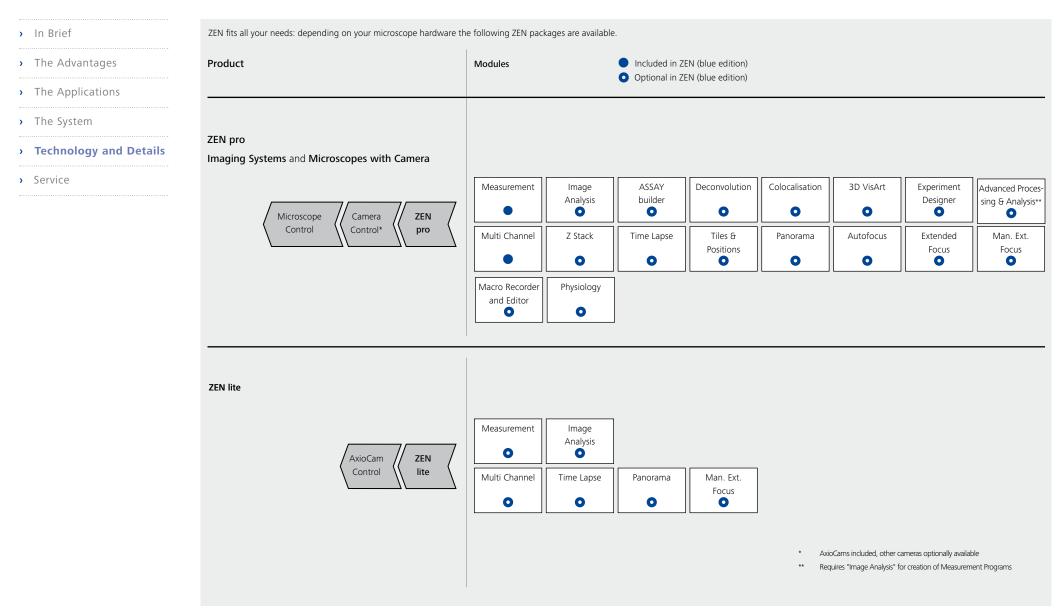
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ZEN fits all your needs: depending on your microscope hardware the following ZEN packages are available.

Product	Modules		<ul> <li>Included in ZEI</li> <li>Optional in ZEI</li> </ul>	, ,		<ul><li>Included in ZEN (black edition)</li><li>Optional in ZEN (black edition)</li></ul>			
ZEN system Laser based 3D Imaging Systems	Measurement	Image Analysis	3D Analysis	ASSAY builder	Advanced Proces- sing & Analysis	Shuttle & Find	Visual Basic Macro		
Laser Microscope Detector ZEN	Deconvolution	Colocalisation	3D VisArt	Торо	Spectral Unmixing	Physiology	FRAP Efficiency Analysis	FRET plus	
Control Control* System	Experiment Designer O O	Visual Macro Editor, VME	Macro Recorder and Editor	ROI-HDR	Enhanced FCS	FCS for GaAsP and APD	Photon Count. Histogram	Image Correl Spectro. RICS	
	Multi Channel	Z Stack	Time Lapse	Tiles & Positions	Panorama	Autofocus	Extended Focus	Man. Ext. Focus	

ZEN desk Offline tasks only	Measurement	Image Analysis	3D Analysis	ASSAY builder	· · ·	Advanced Proces- sing & Analysis***		
ZEN	Deconvolution	Colocalisation	3D VisArt	Spectral Unmixing	Physiology  • • • • • • • • • • • • • • • • • •	FRAP Efficiency Analysis	FRET plus	
desk	Visual Macro Editor, VME	Macro Recorder and Editor	ROI-HDR	Enhanced FCS	FCS for GaAsP and APD	Photon Count. Histogram	Image Correl. Spectro. RICS	
	Tiles & Positions**	Extended Focus	Visual Basic Macro	** Only stitching a	nd processing of existing	etc.) included, 3rd party o data ion of Measurement Pro	. ,	available



## **Technical Specifications**

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		Included in ZEN	(blue edition)			<ul> <li>Included in ZEN (black edition)</li> </ul>	
		Optional in ZEN	• Optional in ZEN (black edition)				
Product/Option		ZEN lite	ZEN desk	ZEN pro	ZEN system	ZEN desk	ZEN system
Basis	ZEN	•	•	٠	•	•	•
Tools	Control of AxioCams	•		٠	•		•
	PMT Control						•
	Other Cameras			0	0		
	Laser- / Lasermodule-Control				•		•
	Microscope Control			•	•		•
	Visual Macro Editor, VME					0	0
	Macro Recorder and Editor		0	0	0	0	0
Acquisition	Multi Channel	0		•	•		•
	Time Lapse	0		0	•		•
	Z Stack			0	•		•
	Manual Extended Focus	0		0	0		
	Autofocus			0	•		•
	Tiles & Positions			0	0	<b>O</b> *	0
	Panorama	0		0	0		
	Experiment Designer			0	0	0	0
	ROI-HDR					0	0
	Shuttle & Find			0	0		0
Processing	Extended Focus		•	0	•	•	•
	Deconvolution		0	0	0		
	3D VisArt		0	0	0	0	0
	Spectral Unmixing					•	•
	Colocalisation		0	0	•		

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		Included in ZEN (	blue edition)			Included in ZEN (black edition)	
		• Optional in ZEN (		• Optional in ZEN (black edition)			
Product/Optio	on	ZEN lite	ZEN desk	ZEN pro	ZEN system	ZEN desk	ZEN system
Analysis	Measurement	0	•	•	•	•	•
	Image Analysis	0	•	0	•	•	•
	Advanced Processing & Analysis		0	0	0		
	FRAP Efficiency Analysis					0	0
	FRET plus					0	0
	3D Analysis					0	0
	ASSAYbuilder		0	0	0		
	Physiology		0	0	0	0	0
	Enhanced FCS					0	0
	FCS for GaAsP and APD					0	0
	Photon Counting Histogram					0	0
	Image Correl. Spectro. RICS					0	0
	Торо					0	0

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System requirements				
ZEN lite	ZEN pro / ZEN desk	ZEN system		
Intel <sup>®</sup> Core 2 Duo E8400 3.0 GHz	Intel <sup>®</sup> Core 2 Duo E8400 3.0 GHz	Intel <sup>®</sup> Xeon X5650 6-Core 2.66 GHz		
Intel <sup>®</sup> iQ45 chipset	Intel® iQ45 chipset	Intel <sup>®</sup> 5520 (Dual) chipset		
4 GB DDR3-RAM	4 GB DDR3-RAM	6 GB DDR3-RAM		
	Graphics interface PCIe x16	Graphics interface PCIe x16		
	Graphics adapter 1920 x 1200 resolution, 32-bit true color, 128 MB RAM, DirectX 8.0 or higher	Graphics adapter ATI FirePro 2560 x 1600 resolution, 32-bit true color, 512 MB RAM, DirectX 8.0 or higher		
	Monitor 20" TFT 1600 x 1200	Monitor 20" TFT 1600 x 1200		
	Hard disk 160 GB SATA2, DVD-ROM drive	Hard disk 1x 250 GB SATA2 (configured as 250 GB hard drive) and 4x 1 TB SATA2 (configured as 2 TB RAID 10 hard drive), DVD-ROM drive		
	1x free PCI slot 5 V, 32-bit (PCI specification 2.1) non shared interrupt, to insert camera interfaces	1x free PCI slot 5 V, 32-bit (PCI specification 2.1) non shared interrupt, to insert camera interfaces		
		1x free PCI Express Generation 2.0 x16 full height slot		
		Trigger board and Signal Distribution Box		
1x FireWire IEEE 1394a interface	2x FireWire IEEE 1394a interface	2x Firewire IEEE 1394a interface		
	2x serial interfaces (COM1 and COM2)	4x serial interfaces (COM1 – COM4)		
2x USB interfaces	2x USB interfaces	4x USB interfaces		
Microsoft® Windows® 7 64-bit Ultimate (Multilanguage), no special customer adapted versions	Microsoft® Windows® 7 64-bit Ultimate (Multilanguage), no special customer adapted versions	Microsoft® Windows® 7 64-bit Ultimate (Multilanguage), no special customer adapted versions		

### Count on Service in the True Sense of the Word

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Because the ZEISS microscope system is one of your most important tools, we make sure it is always ready to perform. What's more, we'll see to it that you are employing all the options that get the best from your microscope. You can choose from a range of service products, each delivered by highly qualified ZEISS specialists who will support you long beyond the purchase of your system. Our aim is to enable you to experience those special moments that inspire your work.

#### Repair. Maintain. Optimize.

Attain maximum uptime with your microscope. A ZEISS Protect Service Agreement lets you budget for operating costs, all the while reducing costly downtime and achieving the best results through the improved performance of your system. Choose from service agreements designed to give you a range of options and control levels. We'll work with you to select the service program that addresses your system needs and usage requirements, in line with your organization's standard practices.

Our service on-demand also brings you distinct advantages. ZEISS service staff will analyze issues at hand and resolve it – whether using remote maintenance software or working on site.

#### Enhance Your Microscope System.

Your ZEISS microscope system is designed for a variety of updates: open interfaces allow you to maintain a high technological level at all times. As a result you'll work more efficiently now, while extending the productive lifetime of your microscope as new update possibilities come on stream.

Please note that our service products are always being adjusted to meet market needs and maybe be subject to change.







Profit from the optimized performance of your microscopesystem with services from ZEISS – now and for years to come.

>> www.zeiss.com/microservice

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