



## **ApoTome.2**

Optical Sections in Fluorescence Imaging

Product Information

Interactive PDF

INTERNET-LINK 

VIDEO/ANIMATION 

Release 1.0



We make it visible.

- › **In Brief**
- › The Advantages
- › The Applications
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## Simply Brilliant: Perfect Optical Section Thickness for All Magnifications

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.



# ApoTome.2: Simpler, More Intelligent, More Integrated.

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### Perfect Images – with All Magnifications

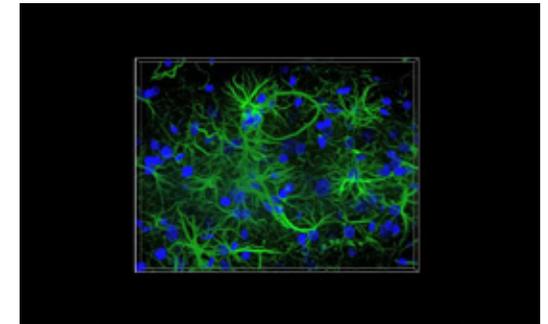
Because your applications need different objectives, you need a system that gives you the best resolution for each one. ApoTome.2 automatically uses the right grid for your objective, selecting from three grids with different frequencies. With a defined optical section thickness in the region of a Rayleigh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

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

From conventional HBO illumination to adjustment-free 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 multi-channel 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*

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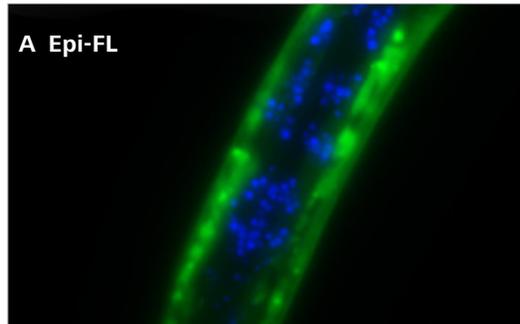
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# Three Grids Deliver Optimal Optical Section Thickness

## 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.



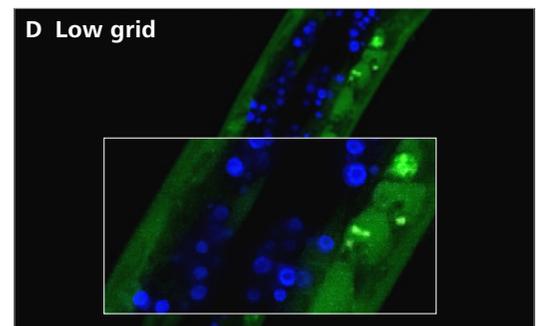
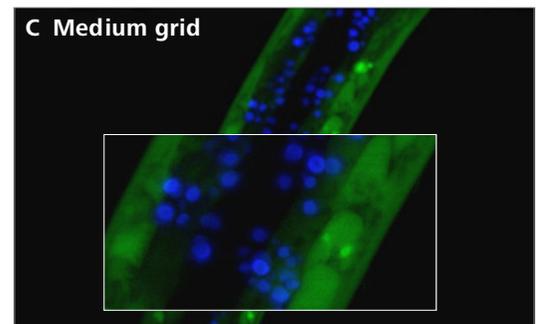
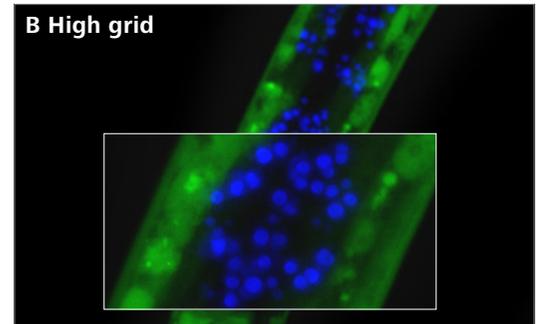
*C. elegans, whole mount, green: GFP, blue: DAPI*  
Objective: Plan-APOCHROMAT 20x/0.8  
Prof. Schnabel, TU Braunschweig, Germany

## 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

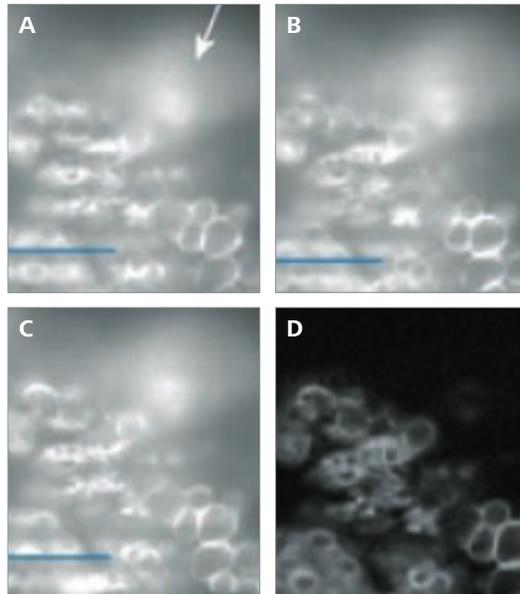
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## ApoTome.2 Brings You Structured Illumination

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 algorithm. 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](http://www.zeiss.com/campus), © Mike Davidson, FSU, Tallahassee*

## 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

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.

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Typical applications, typical specimens	Task	ApoTome.2 provides
<b>Cell culture</b>	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
<b>Live cell imaging</b>	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"
<b>Vibratome sections, histological samples</b>	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 function. Automatic transfer of the parameters of the individual fluorescence channels
	Quantitative analysis	Automatic calibration of the system: reproducible size measurements
<b>Whole mounts</b>	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

# ApoTome.2 At Work

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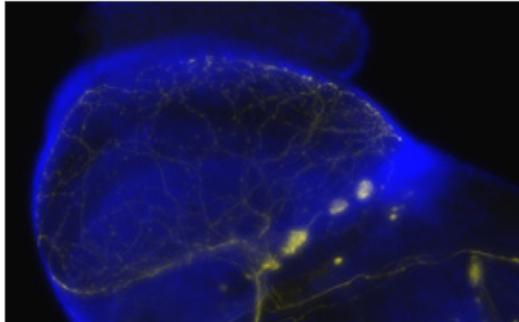


Figure A: Conventional fluorescence

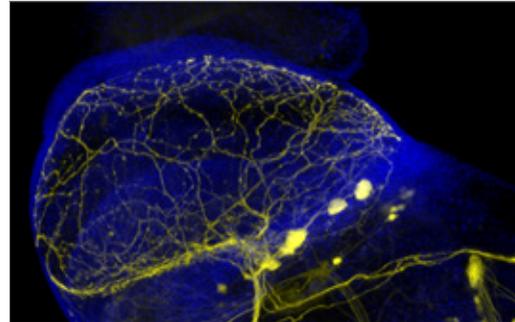


Figure B: Optical section

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

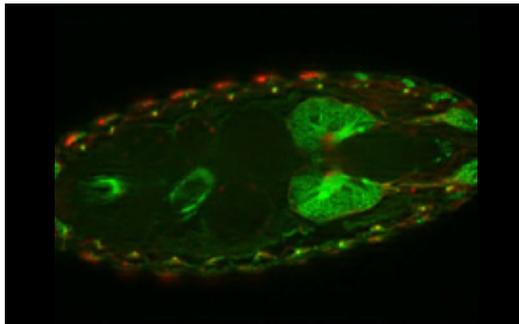


Figure C: *Drosophila* embryo, green: HRP, red: glia marker,  
100  $\mu$ m Z-stack C. Klämbt, Inst. for Neurobiology,  
University of Münster, Germany

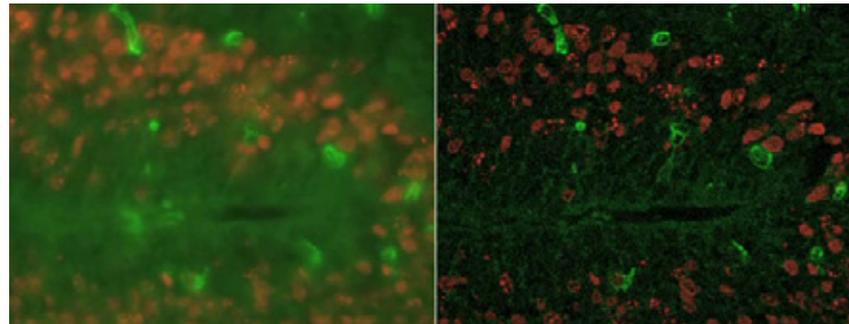


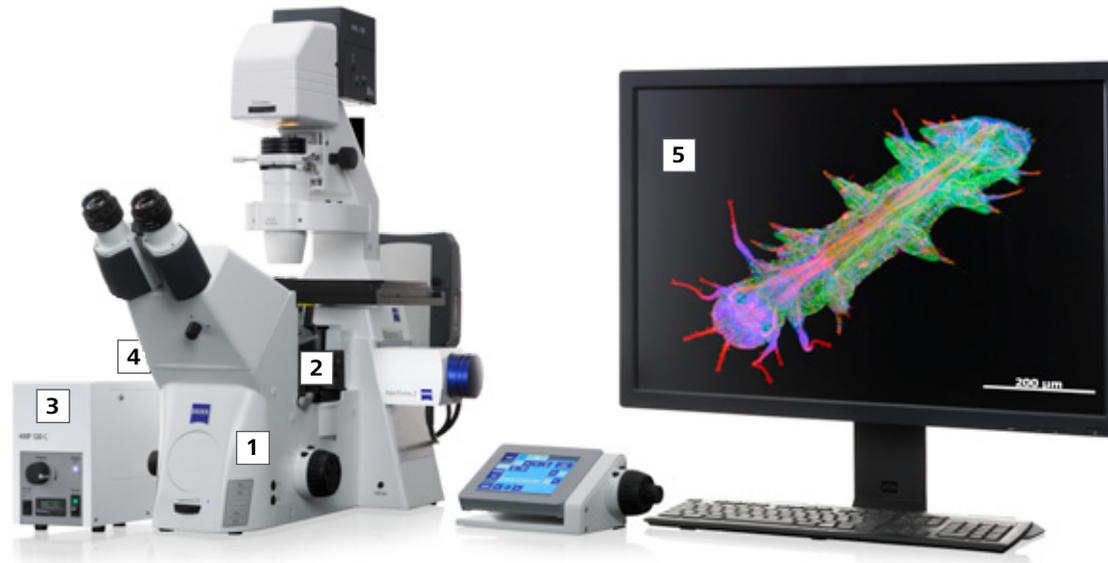
Figure C: Mouse embryo, tissue section, green: GFP, red: Cy3  
Objective: Plan APOCHROMAT 40 x/1.3 Oil  
N. Büttner, T. Vogel, Centre for Anatomy, University of Göttingen, Germany

# ApoTome.2 Imaging System: A 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 HRm
- 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.  $6.5 \mu\text{m}$  (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

## ApoTome.2

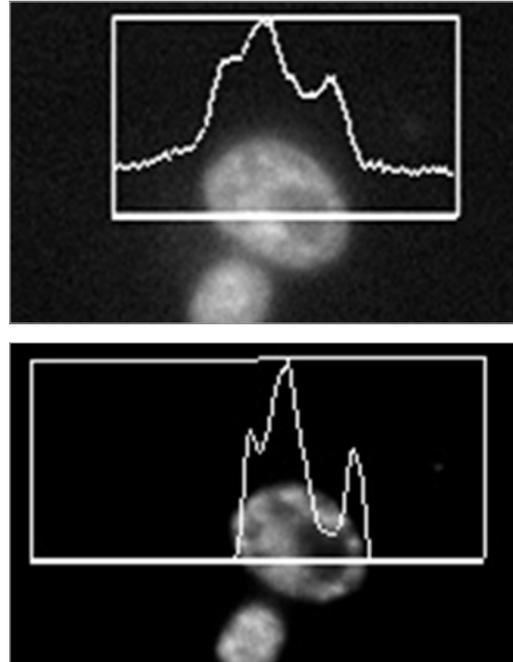
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### Deconvolution

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. SCHAEFFER, 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.

# ApoTome.2: System Overview

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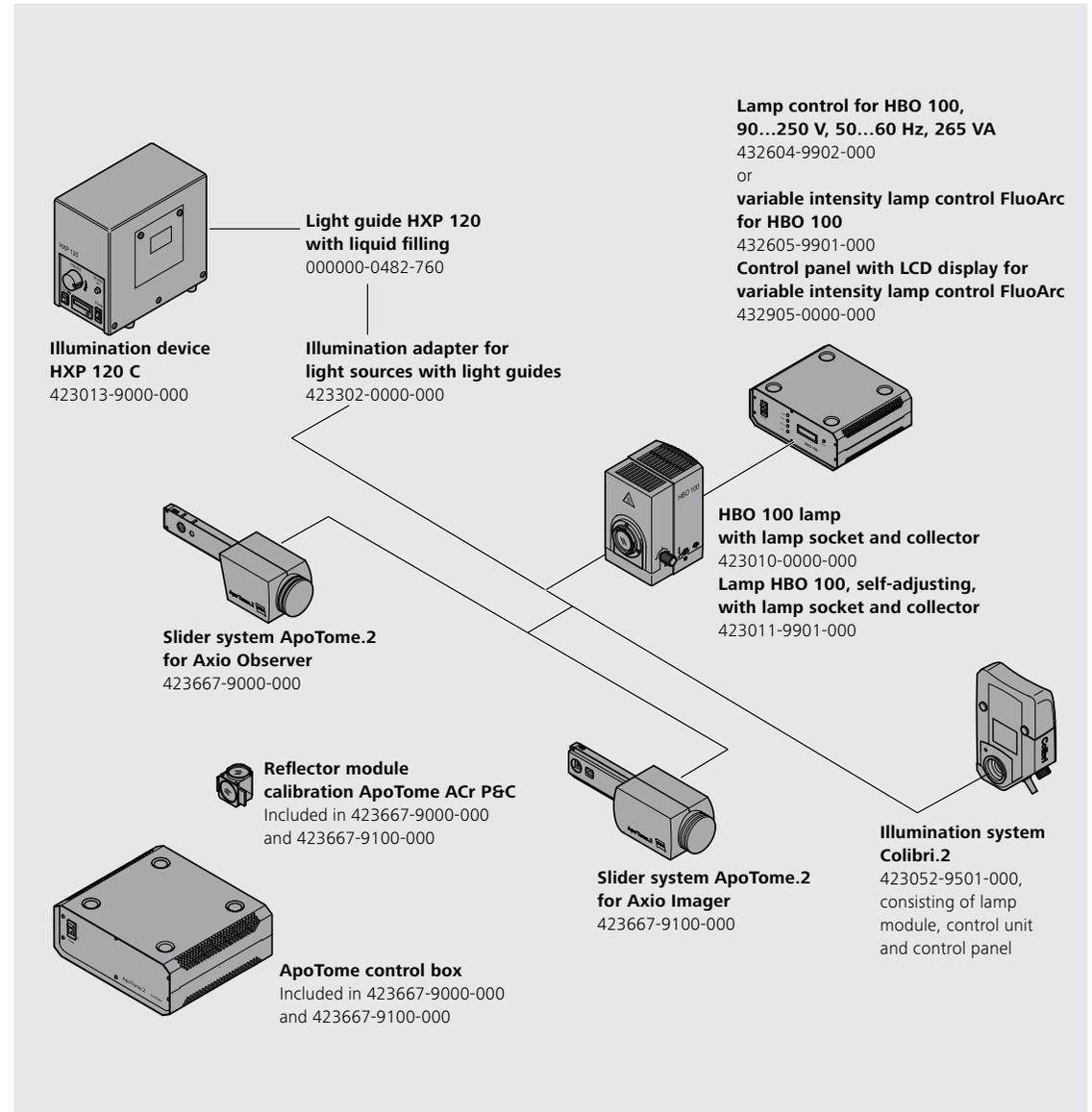
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## ApoTome.2 Is Compatible with These Stands from Carl Zeiss:

- 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,  $\mu\text{m}$ ) depending on wavelength, microscope and objective used.

Data for the use of upright microscopes, e.g. Axio Imager

Objectives for Axio Imager	V	NA	Immersion	Grid/Section thickness @490nm [RU/ $\mu\text{m}$ ]			DAPI with FS34	DAPI with FS49
				High grid	Medium grid	Low grid		
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

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Plan-APOCHROMAT	40 x	0.95	Air	1.6/1.7	0.9/1.0	0.5/0.5	Yes	Yes
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# Technical Specifications

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	II
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
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## 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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Your results really matter to us: we want you to get the best you expect from your microscope. Depend on Carl Zeiss for everything you need: technology, software, advice and service. We stay with you long after installation of your microscope on site. Carl Zeiss specialists will continue to maintain your systems, repair them, supply spare parts and much more. Just call us: we are always here for you.

### **Total Protection with Your Carl Zeiss Service Contract**

It's the safe and practical way to preserve the efficiency of your microscope system. Our service contract protects you against expensive downtime.

### **Preventive Maintenance Plus Optimizes Performance**

Our specialists will maintain and tune your system at regular intervals. You get valuable advice and comprehensive answers to any and all questions. We will also keep you right up to date on developments in your field of application.

### **The Standard Contract Also Includes Repairs and Support**

In addition to all the services of Preventive Maintenance Plus, the standard contract covers all repair and support services. The only costs you will ever pay are for replacement components. Another important aspect of the standard contract is installation of software updates – your system will always be running the latest software version.

### **The Premium Contract Covers Spare Parts Too**

Opt for the premium contract and you will have all services of the standard contract, plus free spare parts. This means you can predict your running costs precisely – and budget for them.



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We make it visible.