

**Instruction Manual**  
**Apotome 3**

Knowledge of this manual is required for the operation of the instrument. Would you therefore please make yourself familiar with the contents of this manual and pay special attention to hints concerning the safe operation of the instrument.

The specifications are subject to change; the manual is not covered by an update service.

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3	10/2022	Implementation of UK responsible person and Swiss representative

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# 1 INTRODUCTION

## 1.1 Important notes



The Apotome 3 system is an extension to the Axio Imager, Axio Observer, and Axio Zoom.V16 microscopes. The Apotome 3 system consists of a slider, which is inserted in the plane of the luminous-field diaphragm of the incident-light beam path.

A complete Apotome 3 imaging system comprises the following components:

- microscope
- anti-vibration table or anti-vibration microscope support
- cooled digital camera with high dynamic range
- PC with at least 16 GB RAM according to Carl Zeiss specifications plus monitor, operating system Windows® 10 (64 bit version)
- Apotome 3 slider
- Imaging software ZEN Rel. 3.2 (blue edition) or higher



This Instruction Manual describes the use of the Apotome 3 hardware. For notes and instructions for the control of the Apotome 3 through software, please refer to the Instruction Manual of the imaging software ZEN (blue edition).



**Prior to the initial start-up of the Apotome 3, read the notes on instrument safety as well as the chapters Instrument Description (Chapter 0) and Startup (Chapter 3).**

## 1.2 Additional applicable operating manuals

In addition to the present Instruction manual, the following operating manuals for optional devices should also be consulted depending on the equipment of the system:

- ZEN Software Description (blue edition) (online version)
- Axio Imager, Axio Observer or Axio Zoom
- installation handbook for peripheral equipment
- control computer
- monitor
- reflected light fluorescence illuminator (HBO 100, HXP 120 V or Colibri)
- microscope stage

### 1.3 Notes on device safety

The Apotome 3 systems have been designed, produced and tested in compliance with DIN EN 61010-1 (IEC 61010-1) and IEC 61010-2-101 "Safety requirements for electrical equipment for measurement, control and laboratory use".

The device meets the requirements set forth in the European Directive IVDR (EU) 2017/746 (European Regulation on in-vitro diagnostic medical devices), the RoHS Directive 2011/65/EC, the delegated directive 2015/863, and carries the CE mark.

EMC User notice for Korean only:









#### 사용자안내문







기종별	사용자안내문
A급 기기 (업무용 방송통신기자재)	이 기기는 업무용(A급) 전자파적합기기로서 판매자 또는 사용자는이 점을 주의하시기 바라며, 가정외의 지역에서 사용하는 것을 목적으로 합니다.

This Instruction Manual contains information and warnings that must be followed by the operator.

The devices must be disposed of in compliance with the WEEE Directive 2012/19/EC.

The following warning and information symbols are used in this Instruction Manual:

Symbol	Explanation
	<b>CAUTION</b> <i>This symbol indicates a potential hazard to the user.</i>
	<b>ATTENTION</b> <i>This symbol indicates a potential hazard to the instrument or system.</i>
	<b>ATTENTION</b> <i>Read the Instruction Manual.</i>
	<b>NOTE</b> <i>This symbol indicates an instruction which requires particular attention.</i>
	CE marking (Conformité Européene)
	UKCA marking (UK Conformity Assessed)
	CSA label: product tested by CSA to meet U.S. and Canadian standards. CSA approval master number optionally given adjacent to this symbol
	Manufacturer

Symbol	Explanation
	Date of manufacture
	Swiss authorized representative
	In-vitro diagnostic medical device
	Serial number
	Catalogue number
	WEEE label: Do not discard as unsorted waste. Send to separate collection facilities for recovery and recycling.

The Apotome 3 may only be used for the techniques described in this manual.

Particular attention must be paid to the following warnings:



Any serious incident that has occurred in relation to the microscope and its components shall be reported to these institutions:

- the competent authority of the Member State in which the user is established
- the manufacturer Carl Zeiss Microscopy GmbH, Jena, Germany



The Instruction Manual of Apotome 3 must be strictly observed.



If the Apotome 3 is used in a manner not specified within these instructions for use, the protection provided by the device system may be impaired.



Please consider that the Apotome 3 is a high-precision opto-electronic instrument. Incorrect handling can easily impair the functioning of the device, or even damage it, and will render any warranty claims invalid.



Before connecting Apotome 3 via CAN-BUS cable to the microscope stand make sure, that the stand is switched off.

Do not remove the CAN-BUS cable while the microscope system is switched on.



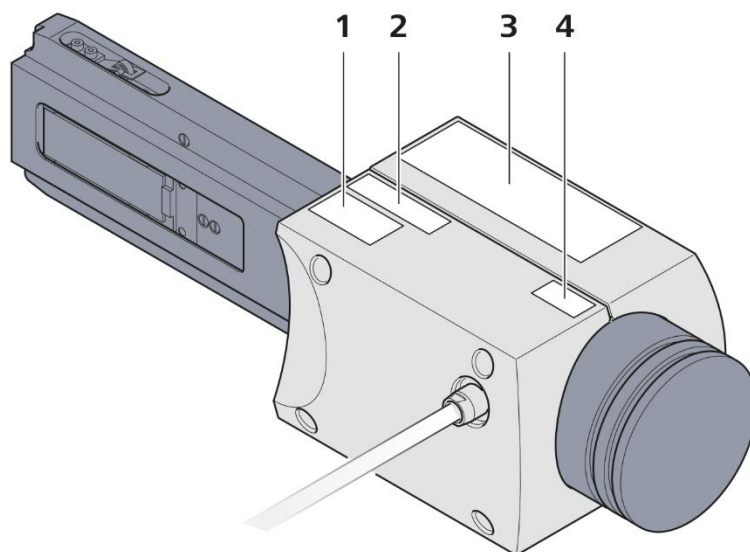
If it is determined that protective measures are no longer effective, the device must be taken out of service and secured against inadvertent operation, to avoid the risk of injury.

Contact ZEISS Service to have the device repaired.




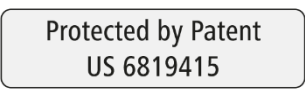


- Do not dispose of defective equipment in regular domestic waste. It should be disposed of in accordance with the prevailing national legal requirements. The manufacturer of the device is under the legal obligation to take defective devices back.
- Sample waste should be disposed of in accordance with the prevailing national legal requirements.
- Specimens should also be disposed of in compliance with the prevailing legal requirements and internal operating procedures.

### 1.4 Warning and information labels



- 1 KC label
- 2 Patent label
- 3 Type label
- 4 IVD label

**Fig. 1** Warning and information labels

No.	Label	Description
1		KC label
2		Patent label
3		Type label
4		IVD label



## 1.5 Notes on warranty

The manufacturer guarantees that the instrument is free of material or manufacturing defects upon delivery. Any defects must be reported immediately and steps taken to minimize damage. If such a defect is reported, the instrument manufacturer shall be obliged to correct the fault, either by repairing the instrument or replacing it with a new one, at the manufacturer's discretion. No warranty is given for defects caused by natural wear and tear (particularly of wearing parts) and improper use of the instrument.



The instrument manufacturer shall not be liable for damage caused by misuse, negligence or any other tampering with the instrument, particularly the removal or replacement of instrument components, or the use of accessories from other manufacturers. Such actions shall invalidate any warranty claims.



With the exception of the work described in this instruction manual, no maintenance or repair work is to be carried out on the Apotome 3. Repairs may only be performed by ZEISS Service or individuals specially authorized by ZEISS Service. In the event of a problem with the instrument, please contact your local ZEISS representative.

## 2 INSTRUMENT DESCRIPTION

### 2.1 Designation, intended purpose and typical applications

#### Manufacturer's designation

The Apotome 3 system is an extension to the Axio Imager, Axio Observer and Axio Zoom.V16 microscopes.

Manufacturer's product name: **Apotome 3 for Axio Imager** (423667-9110-000)  
**Apotome 3 for Axio Observer** (423667-9010-000)  
**Apotome 3 for Axio Zoom.V16** (423667-9210-000)

#### Intended purpose

The Apotome 3 allows depth-discriminated images (= optical sections) of fluorescence specimens to be produced. Compared to conventional reflected light fluorescence methods, these optical sections feature increased contrast and enhanced optical resolution in axial direction. Apotome 3 can be used to analyze various biological samples including samples collected from humans or animals. Apotome 3 is an accessory to compatible IVD microscopes and supports their intended purpose.

#### Typical applications

Furthermore, optical sections through the specimen are the prerequisite for the three-dimensional reconstruction of structures.

Typical organizations which may use the instrument are:

- University facilities
- Pharmaceutical industry
- Biotechnology companies
- Research departments of hospitals

### 2.2 Lifetime

The Apotome 3 is an opto-electronic device. Its availability for use is significantly determined by the performed maintenance. ZEISS guarantees the ability for maintenance and repair within eight years after initial operation. This is ensured by a corresponding service and spare parts concept, thus enabling the intended purpose within this duration.

### 2.3 Instrument description and main features

The Apotome 3 hardware consists of a slider with transmission grids changer.

The slider is directly connected to the microscope. Optionally, it can be connected to the microscope by a CAN BUS cable. In this case, the Apotome 3 directly communicates with the PC via the electronic system of the microscope.

Major features of the **Apotome 3** include:

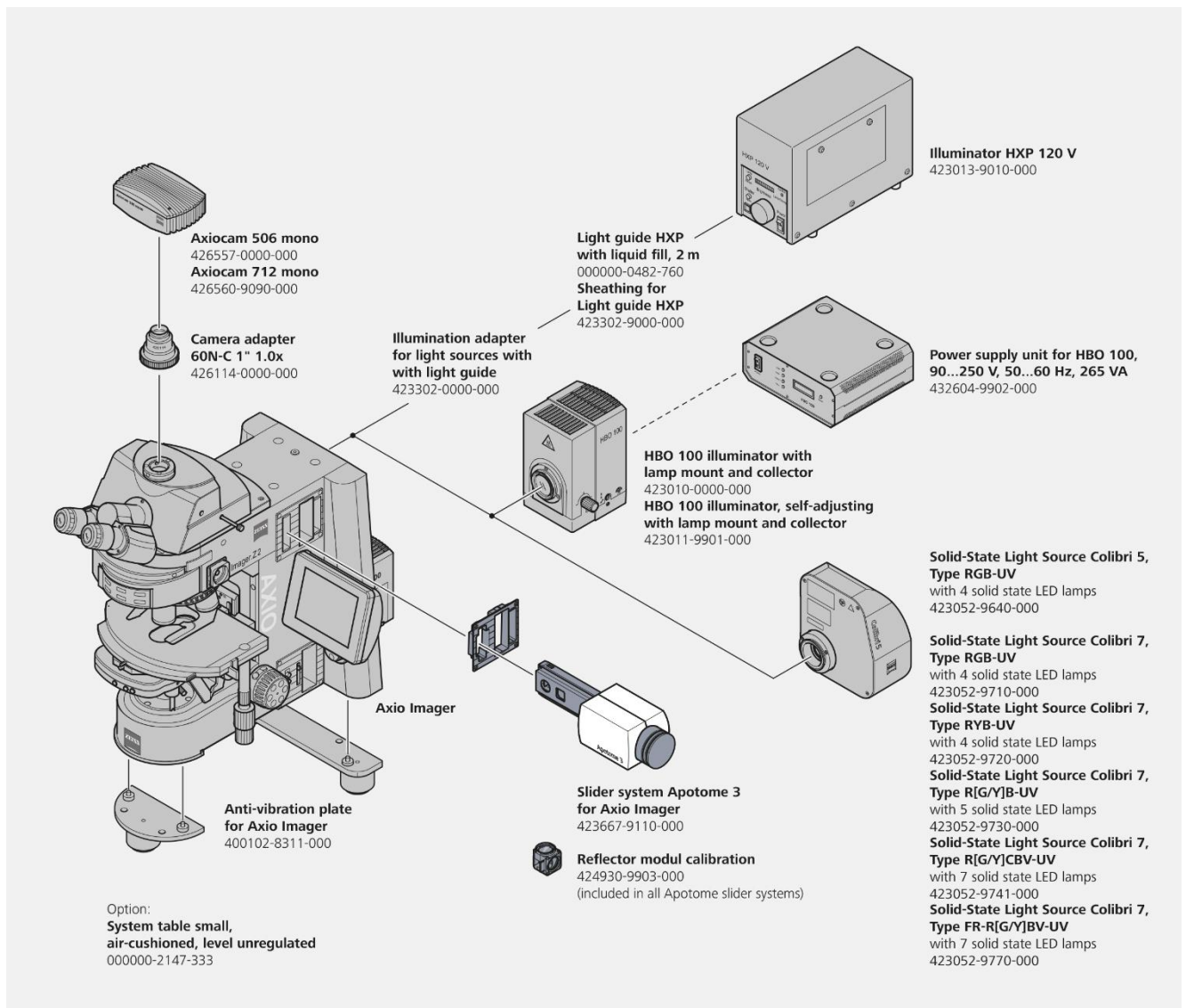
- Apotome 3 slider for the plane of the luminous-field diaphragm in the reflected light path
- Apotome 3 slider with two click-stop positions:

**Click-stop position 1:**

An open passage is provided in the reflected light path. Thus, normal fluorescence observations can be carried out in the wide field, e.g. to find interesting structures and to position the specimens.

**Click-stop position 2:**

In this position, a glass plate with evaporated grid structure is in the light path. The grid structure is laterally moved in the specimen plane by means of a scanner mechanism. By capturing three (or more) images at different grid positions and by subsequent calculation, it is possible to produce an optical section through the specimen. Three different grid frequencies selected via software are available. They allow the user to obtain different section thicknesses and to use different objectives.

**2.4 System overview****2.4.1 Apotome 3 for Axio Imager****Fig. 2 System overview Apotome 3 for Axio Imager**

2.4.2 Apotome 3 for Axio Observer

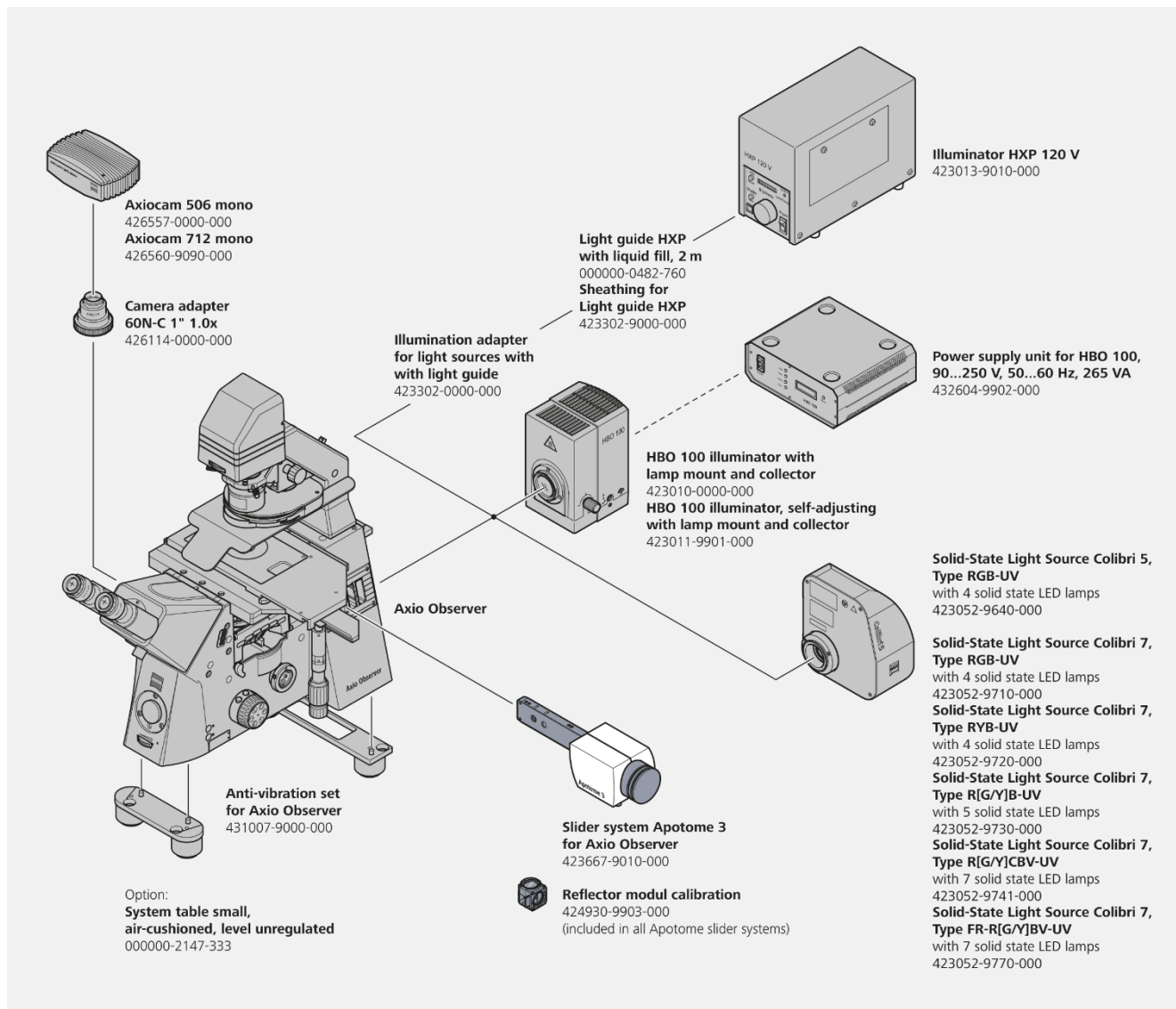


Fig. 3 System overview Apotome 3 for Axio Observer

2.4.3 Apotome 3 for Axio Zoom.V16

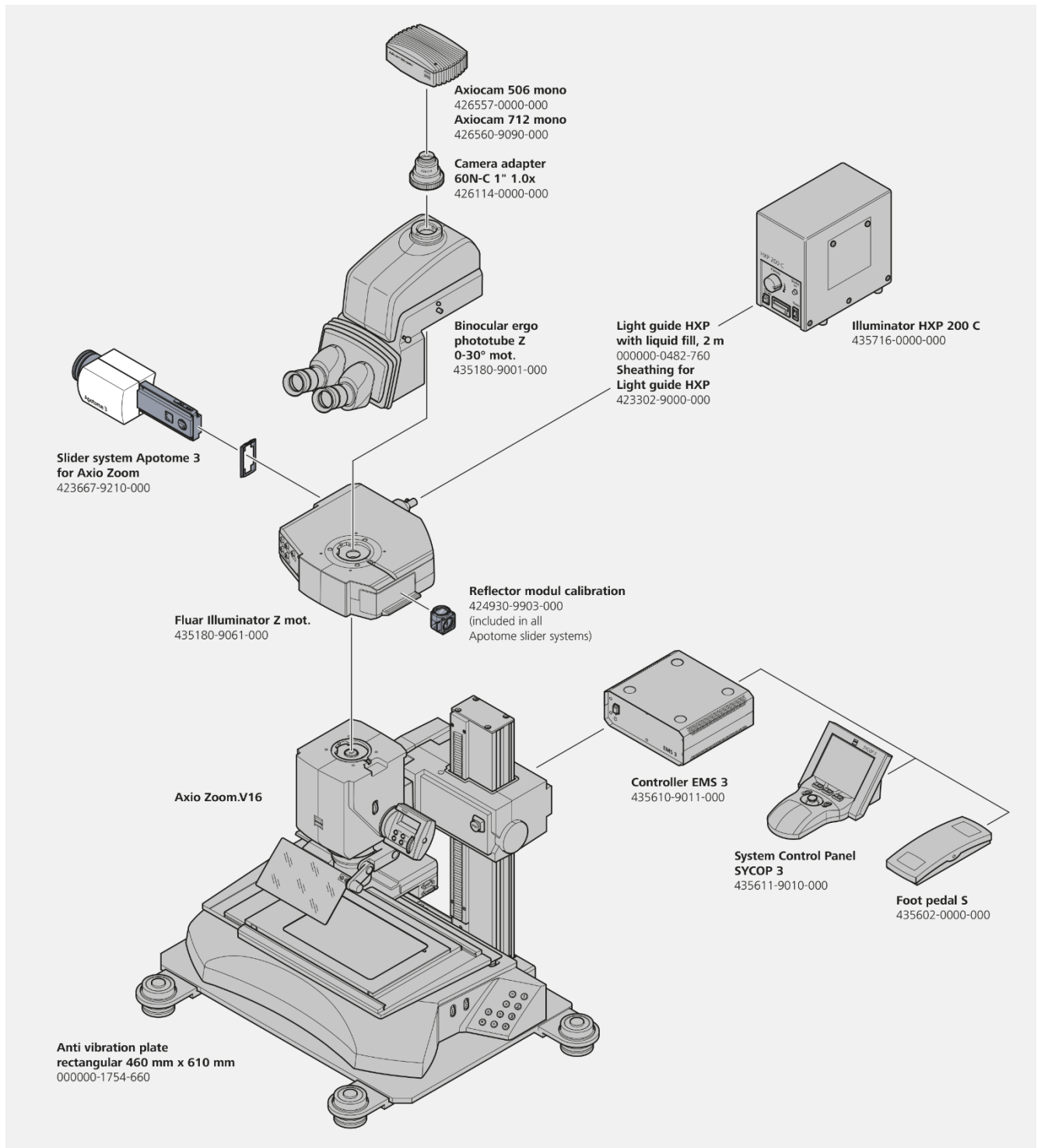
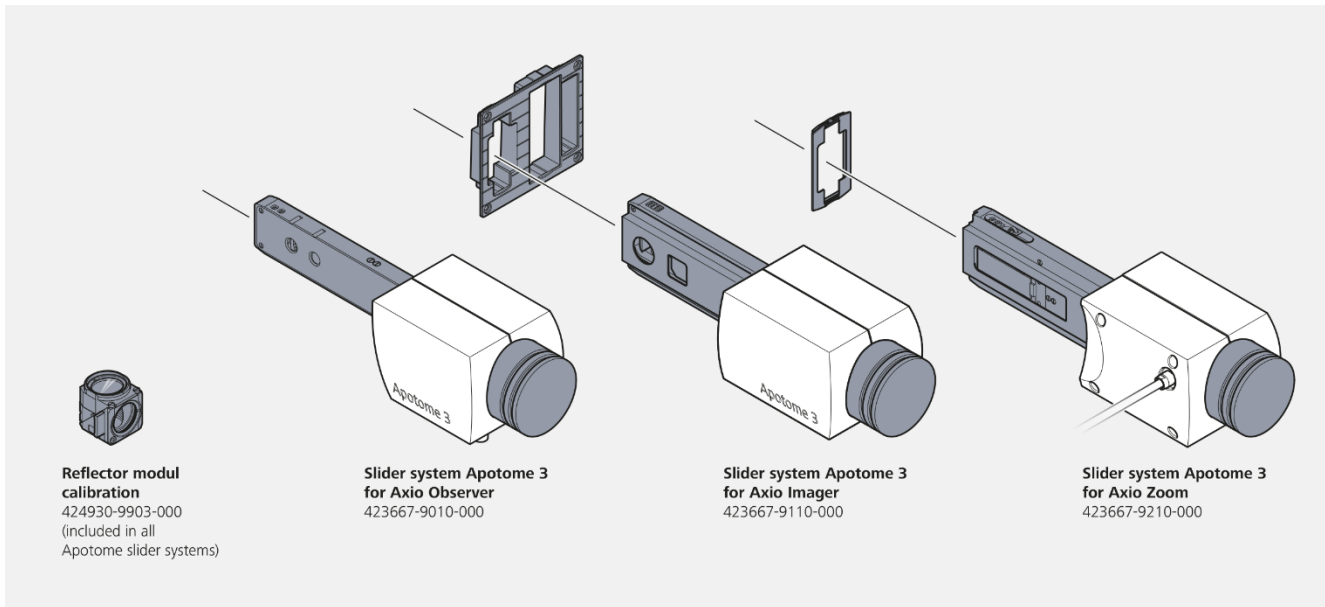


Fig. 4 System overview Apotome 3 for Axio Zoom.V16

**2.5 General view of Apotome 3**



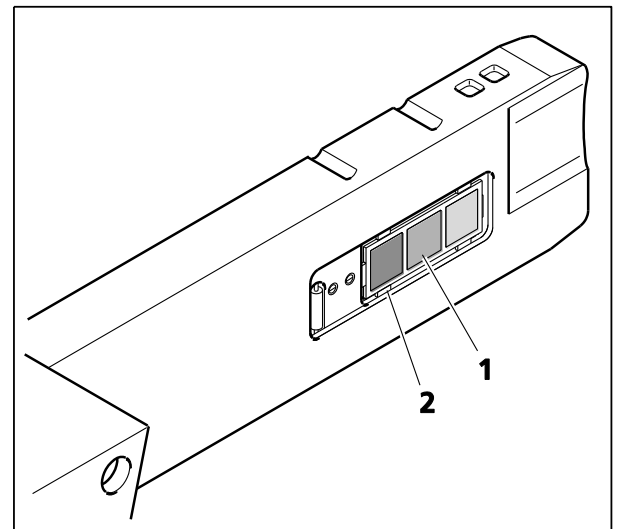
**Fig. 5 Components of Apotome 3**

**2.6 Operating principle**

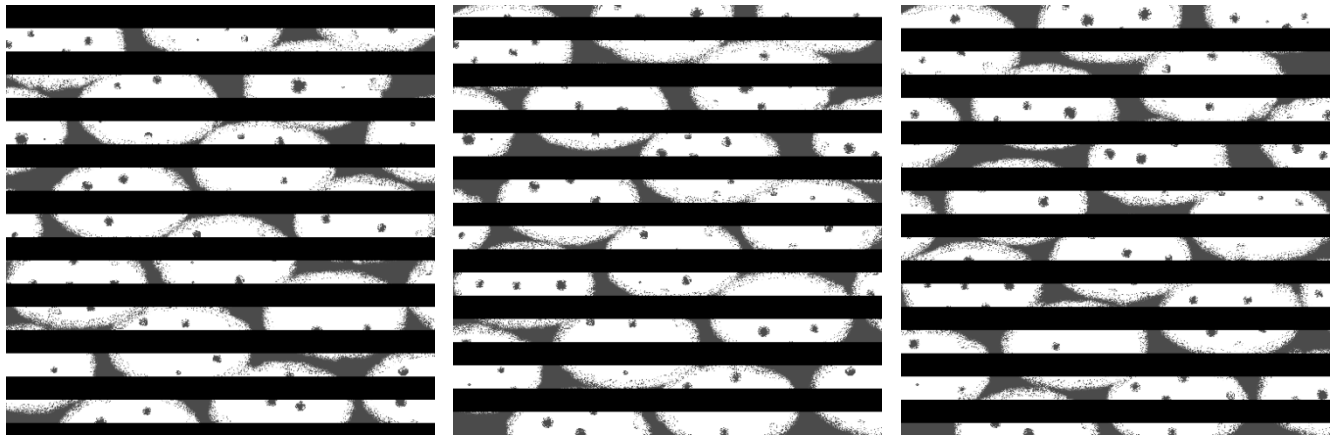
Three different transmission grids (Fig. 6/1) are installed in the slider Apotome 3 on a motorized changer (Fig. 6/2). The transmission grids are three metallic grid structures that are evaporated on a glass plate.

If the transmission grid is located in the illumination beam path, the grid structure will be imaged in the specimen plane. For image capture the image of the grid structure is shifted in three defined steps ("phases") in the specimen by means of a scanner mechanism in Apotome 3. During this procedure a given point of the specimen is illuminated with different intensity depending on the phase position of the transmission grid.

The three images captured (Fig. 7) with the grid structures are calculated, so that an optical section with increased contrast and depth discrimination through the specimen will be obtained. The calculation makes the grid structures of the initial images disappear.



**Fig. 6 Changer with transmission grids**



**Fig. 7** Image capture in three defined steps (phases)

The Apotome 3 slider is provided with three different transmission grids, which cover the total magnification range, as indicated in Chapter 2.7. The software automatically recommends the optimal grid to be used. Optionally, another grid can be selected manually (e.g. to adjust the section thickness to the experimental conditions).

Please refer to Chapter 2.7 for a precise list about the correct transmission grids for the different magnifications.

## 2.7 Suitability of objectives for the use of Apotome 3

The operating principle described in Chapter 2.6 places specific demands on the correction state of the objectives used.

Especially the correction of the longitudinal color aberration must be of high quality. Shifting the grid structure along the optical axis minimizes the residual error.

The recommended objectives for the Axio Imager or Axio Observer or Axio Zoom.V16 microscopes are listed in the following tables.

### Axio Imager

Objective	M	NA	Immersion	Grid / Section thickness @ 490nm [RU/μm]			DAPI with FS 34	DAPI with FS 49
				High grid	Middle grid	Low grid		
EC Plan-Neofluar	10x	0,3	Air	2,9 / 31,9	1,7 / 18,2	<b>0,9 / 9,9</b>	✓	✓
EC Plan-Neofluar	20x	0,5	Air	2,4 / 9,2	1,4 / 5,3	<b>0,7 / 2,9</b>	✓	✓
EC Plan-Neofluar	40x	0,75	Air	1,6 / 2,8	<b>0,9 / 1,6</b>	0,5 / 0,9	✓	✓
EC Plan-Neofluar	40x	1,3	Oil	2,5 / 2,2	<b>1,4 / 1,2</b>	0,8 / 0,7	✓	✓
EC Plan-Neofluar	63x	0,95	Air	<b>1,0 / 1,1</b>	0,6 / 0,7	0,4 / 0,4	✓	✗
EC Plan-Neofluar	63x	1,25	Oil	1,6 / 1,5	<b>0,9 / 0,9</b>	0,5 / 0,5	✓	✓
EC Plan-Neofluar	100x	1,3	Oil	<b>1,0 / 0,9</b>	0,6 / 0,5	0,4 / 0,3	✓	✓
LCI Plan-Neofluar	25x	0,8	Oil, water or glycerin	2,9 / 6,6	1,7 / 3,7	<b>0,9 / 2,0</b>	✓	✓
LCI Plan-Neofluar	63x	1,3	Water or glycerin	1,5 / 1,3	<b>0,9 / 0,7</b>	0,5 / 0,4	✓	✓
Plan-Apochromat	10x	0,45	Air	4,2 / 20,4	2,4 / 11,5	<b>1,3 / 6,2</b>	✓	✓
Plan-Apochromat	20x	0,8	Air	3,2 / 4,9	1,8 / 2,8	<b>1,0 / 1,5</b>	✓	✓
Plan-Apochromat	40x	0,95	Air	1,6 / 1,7	<b>0,9 / 1,0</b>	0,5 / 0,5	✓	✓
Plan-Apochromat	40x	1,3	Oil	2,5 / 2,2	1,4 / 1,2	<b>0,8 / 0,7</b>	✓	✓
Plan-Apochromat	40x	1,4	Oil	2,4 / 1,8	<b>1,4 / 1,0</b>	0,7 / 0,6	✓	✓
Plan-Apochromat	63x	1,4	Oil	1,6 / 1,2	<b>0,9 / 0,7</b>	0,5 / 0,4	✓	✓
Plan-Apochromat	100x	1,4	Oil	<b>1,0 / 0,8</b>	0,6 / 0,5	0,4 / 0,3	✓	✓
LD LCI Plan-Apochromat	25x	0,8	Oil, water or glycerin	2,9 / 6,6	1,7 / 3,7	<b>0,9 / 2,0</b>	✓	✓
C-Apochromat	10x	0,45	Water	4,2 / 20,4	2,4 / 11,5	<b>1,3 / 6,2</b>	✓	✓
C-Apochromat	40x	1,2	Water	2,2 / 2,0	<b>1,2 / 1,1</b>	0,7 / 0,6	✓	✓
C-Apochromat	63x	1,2	Water	1,4 / 1,3	<b>0,8 / 0,7</b>	0,5 / 0,4	✓	✓
LD C-Apochromat	40x	1,1	Water	2,2 / 2,3	<b>1,2 / 1,3</b>	0,7 / 0,7	✓	✓
α Plan-Apochromat	63x	1,46	Oil	1,5 / 1,0	<b>0,9 / 0,6</b>	0,5 / 0,3	✓	✓
α Plan-Fluar	100x	1,45	Oil	<b>1,0 / 0,7</b>	0,6 / 0,4	0,3 / 0,2	✗	✗
α Plan-Apochromat	100x	1,46	Oil	<b>1,0 / 0,7</b>	0,6 / 0,4	0,3 / 0,2	✓	✗



**Axio Observer**

Objective	M	NA	Immersion	Grid / Section thickness @ 490nm [RU/μm]			DAPI with FS 34	DAPI with FS 49
				High grid	Middle grid	Low grid		
EC Plan-Neofluar	10x	0,3	Air	2,9 / 31,5	1,7 / 18,5	<b>0,9 / 9,8</b>	✓	✓
EC Plan-Neofluar	20x	0,5	Air	2,3 / 9,0	<b>1,4 / 5,4</b>	0,7 / 2,9	✓	✓
EC Plan-Neofluar	40x	0,75	Air	1,6 / 2,7	<b>0,9 / 1,6</b>	0,5 / 0,9	✓	✗
EC Plan-Neofluar	40x	1,3	Oil	2,4 / 2,1	1,4 / 1,3	<b>0,8 / 0,7</b>	✓	✓
EC Plan-Neofluar	63x	0,95	Air	<b>1,0 / 1,1</b>	0,6 / 0,7	0,4 / 0,4	✓	✓
EC Plan-Neofluar	63x	1,25	Oil	1,6 / 1,5	<b>0,9 / 0,9</b>	0,5 / 0,5	✓	✗
EC Plan-Neofluar	100x	1,3	Oil	<b>1,0 / 0,9</b>	0,6 / 0,6	0,4 / 0,3	✓	✗
LCI Plan-Neofluar	25x	0,8	Oil, water or glycerin	2,9 / 6,5	1,7 / 3,8	<b>0,9 / 2,0</b>	✓	✓
LCI Plan-Neofluar	63x	1,3	Water or glycerin	1,5 / 1,3	<b>0,9 / 0,8</b>	0,5 / 0,4	✗	✗
Plan-Apochromat	10x	0,45	Air	4,2 / 20,2	2,4 / 11,7	<b>1,3 / 6,1</b>	✓	✓
Plan-Apochromat	20x	0,8	Air	3,1 / 4,8	1,8 / 2,8	<b>1,0 / 1,5</b>	✓	✓
Plan-Apochromat	40x	0,95	Air	1,6 / 1,7	<b>0,9 / 1,0</b>	0,5 / 0,5	✓	✓
Plan-Apochromat	40x	1,3	Oil	2,4 / 2,2	1,4 / 1,3	<b>0,8 / 0,7</b>	✓	✓
Plan-Apochromat	40x	1,4	Oil	2,4 / 1,8	<b>1,4 / 1,1</b>	0,7 / 0,6	✓	✓
Plan-Apochromat	63x	1,4	Oil	1,5 / 1,2	<b>0,9 / 0,7</b>	0,5 / 0,4	✓	✓
Plan-Apochromat	100x	1,4	Oil	<b>1,0 / 0,8</b>	0,6 / 0,5	0,4 / 0,3	✓	✗
LD LCI Plan-Apochromat	25x	0,8	Oil, water or glycerin	2,9 / 6,5	1,7 / 3,8	<b>0,9 / 2,0</b>	✓	✓
C-Apochromat	10x	0,45	Water	4,2 / 20,2	2,4 / 11,7	<b>1,3 / 6,1</b>	✓	✓
C-Apochromat	40x	1,2	Water	2,1 / 1,9	<b>1,3 / 1,1</b>	0,7 / 0,6	✓	✓
C-Apochromat	63x	1,2	Water	1,4 / 1,3	<b>0,8 / 0,7</b>	0,5 / 0,4	✓	✓
LD C-Apochromat	40x	1,1	Water	2,1 / 2,3	<b>1,3 / 1,4</b>	0,7 / 0,7	✓	✓
α Plan-Apochromat	63x	1,46	Oil	1,5 / 1,0	<b>0,9 / 0,6</b>	0,5 / 0,3	✓	✓
α Plan-Fluar	100x	1,45	Oil	<b>1,0 / 0,7</b>	0,6 / 0,4	0,3 / 0,2	✗	✗
α Plan-Apochromat	100x	1,46	Oil	<b>1,0 / 0,7</b>	0,6 / 0,4	0,3 / 0,2	✗	✗

**Axio Zoom.V16**

Objective	M	Zoom (total magnification)	Grid / Section thickness @ 490nm [RU/μm]			DAPI with FS 34	DAPI with FS 49
			High grid	Middle grid	Low grid		
Plan-Apochromat	1x	40	1,8 / 82,7	1,22 / 56,7	0,9 / 43,8	✓	✓
Plan-Apochromat	1x	80	1,5 / 25,2	1,0 / 17,4	0,8 / 13,5	✓	✓
Plan-Apochromat	1x	100	1,2 / 19,7	0,9 / 13,7	0,7 / 10,8	✓	*
Plan-Apochromat	1x	112	1,1 / 17,7	0,8 / 12,4	0,6 / 9,8	✓	*
Plan-Neofluar	1x	40	1,8 / 82,7	1,22 / 56,7	0,9 / 43,8	✓	✓
Plan-Neofluar	1x	80	1,5 / 25,2	1,0 / 17,4	0,8 / 13,5	✓	✓
Plan-Neofluar	1x	100	1,2 / 19,7	0,9 / 13,7	0,7 / 10,8	✓	✓
Plan-Neofluar	1x	112	1,1 / 17,7	0,8 / 12,4	0,6 / 9,8	✓	✓
Plan-Neofluar	2,3x	40	2,0 / 72,0	1,4 / 49,3	1,1 / 37,9	✓	✓
Plan-Neofluar	2,3x	120 <sup>1</sup>	1,7 / 9,1	1,1 / 6,3	0,9 / 4,9	✓	✓
Plan-Neofluar	2,3x	150	1,6 / 5,9	1,1 / 4,1	0,8 / 3,1	✓	✓
Plan-Neofluar	2,3x	160	1,5 / 5,3	1,0 / 3,6	0,8 / 2,8	✓	*
Plan-Neofluar	2,3x	250	1,1 / 3,2	0,7 / 2,2	0,6 / 1,8	*	*

<sup>1</sup> In ZEN (blue edition) no preset zoom positions



The following filter sets are recommended if Apotome 3 shall be used with Axio Zoom.V16: 20, 38, 43.



Axio Zoom.V16 allows the user to select also the zoom position, besides the objective. Apotome 3 may be used with Axio Zoom.V16 with a total magnification of 40x or higher.

**Further filter sets recommended for Apotome 3**

Order No.	Name	Excitation / Color splitter / Emission	Fluorophores (examples)
488001-9901-000	FS 01	BP 365/12 FT 395 LP 397	Alexa 405, BFP, DAPI
488802-9901-000	FS 02	G 365 FT 395 LP 420	Alexa 350, BFP, DAPI
488009-9901-000	FS 09	BP 450-490 FT 510 LP 515	Alexa 430, Alexa 488, FITC
488010-9901-000	FS 10	BP 450-490 FT 510 BP 515-565	Alexa 430, Alexa 488, FITC, Cy2
488016-9901-000	FS 16	BP 485/20 FT 510 LP 515	Alexa 488, FITC, MitoTracker Green
488017-9901-000	FS 17	BP 485/20 FT 510 BP 515-565	Alexa 488, FITC, MitoTracker Green, Cy2
488020-9901-000	FS 20	BP 546/12 FT 560 BP 575/640	Alexa 555, Rhodamine, dTomato

Order No.	Name	Excitation / Color splitter / Emission	Fluorophores (examples)
488026-9901-000	FS 26	BP 575-625 FT 645 BP 660-710	mPlum, Nile Red
489038-9901-000	FS 38 HE	BP 470/40 FT 495 BP 525/50	Alexa 430, Alexa 488, FITC
489043-9901-000	FS 43 HE	BP 550/25 (HE) FT 570 (HE) BP 605/70 (HE)	Alexa 546, Alexa 555, Cy3, Dil
489046-9901-000	FS 46 HE	BP 500/25 FT 515 BP 535/30	Alexa 488, FITC, eGFP, eYFP
489047-9901-000	FS 47 HE	BP 436/25 FT 455 HE BP 480/40	CFP
488049-9901-000	FS 49	G 365 FT 395 BP 445/50	DAPI
488050-9901-000	FS 50	BP 640/30 FT 660 BP 690/50	Alexa 647, Alexa 660, Cy5
489059-9901-000	FS 59 HE (Colibri)	BP 445/25 (HE) BP 510/15 (HE) DFT 460 + 520 (HE) DBP 480/22 + LP 530 (HE)	Alexa 433, Alexa 488, CFP, FITC, eYFP
489060-9901-000	FS 60 (Colibri)	BP 445/25 (HE) BP 510/15 (HE) BP 588/27 (HE) TFT 460 + 520 + 605 (HE) DBP 480/22 + 546/32 + LP 615	Alexa 430, Alexa 568, Alexa 594
489061-9901-000	FS 61 (Colibri)	BP 474/28 (HE) BP 585/35 (HE) DFT 495 + 605 (HE) DBP 527/54 + 645/60 (HE)	CFP, YFP, mRFP
489062-9901-000	FS 62 (Colibri)	BP 370/40 (HE) BP 474/28 (HE) BP 585/35 (HE) TFT 395 + 495 + 610 (HE) TBP 425 + 527 + LP615 (HE)	DAPI, CFP, YFP
489090-9100-000	FS 90 HE ms (Colibri)	EX BP 385/30 for DAPI EX BP 469/38 for FITC EX BP 555/30 for TRITC EX BP 631/33 for Cy5 QFT 405 + 493 + 575 + 653 EM QBP 425/30 + 514/30 + 592/25 + 709/100	DAPI, FITC, TRITC, Cy5
489090-9110-000	FS 90 HE LED (Colibri)	QFT 405 + 493 + 575 + 653 EM QBP 425/30 + 514/30 + 592/25 + 709/100	DAPI, FITC, TRITC, Cy5
489092-9110-000	FS 92 HE LED (Colibri)	TFT 405 + 493 + 610 EM TBP 425/30 + 524/50 + 688/145	DAPI, GFP, mCherry

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## 2.8 Technical data

### Dimensions (width x depth x height)

Apotome 3 slider for Axio Imager .....	approx. 278 mm x 90 mm x 76 mm
Apotome 3 slider for Axio Observer .....	approx. 295 mm x 90 mm x 78 mm
Apotome 3 slider for Axio Zoom.V16.....	approx. 278 mm x 90 mm x 76 mm

### Weight

Apotome 3 slider.....	approx. 1.1 kg
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### Ambient conditions

#### Transport (in packing)

Permissible ambient temperature.....	-40 to +70 °C
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#### Storage

Permissible ambient temperature.....	-10 to +55 °C
Permissible relative humidity (without condensation) .....	max. 95 % at +55 °C

#### Operation

Permissible ambient temperature.....	+5 to +30 °C
Permissible relative humidity.....	max. 80 % at +30 °C
Air pressure.....	800 hPa to 1060 hPa
Operating altitude .....	max. 2000 m
Pollution degree .....	2
Warm-up period.....	30 min

#### Functional data

Area of use.....	closed rooms
Radio interference suppression .....	as per EN 55011 Class A
Noise immunity .....	as per DIN EN 61326-1
Power consumption .....	max. 5 W
Input voltage.....	24 V DC

#### Grid frequencies

Axio Imager slider (transmission grids 1 / 2 / 3) .....	5 / 9 / 17.5 lp/mm
Axio Observer slider (transmission grids 1 / 2 / 3) .....	10 / 17.5 / 35 lp/mm
Axio Zoom.V16 slider (transmission grids 1 / 2 / 3) .....	10 / 15 / 20 lp/mm

---

## 3 STARTUP

The users can install and start up the Apotome 3 themselves.

On request, however, Zeiss Service will install or convert the Apotome 3 with costs at the customer's place.



Before installing and starting up the device carefully read the **Notes on device safety**.

### 3.1 Microscope stand requirements

The grid projection method used for the Apotome 3 is very sensitive to vibrations that may originate from different sources. Vibrations are visible in the result image as streak artifacts. Therefore, the microscope should be installed as free from vibrations as possible on an anti-vibration table or a suitable microscope support.

### 3.2 Unpacking Apotome 3

The Apotome 3 slider is delivered in a polyethylene box with outer carton package.

Besides the slider, the box contains the accessories required for the calibration of Apotome 3:

- Reflector module Calibration ACR P&C
  - Fluorescence test specimen for grid focus calibration
  - Mirror specimen with cross-line graticule for phase calibration
- Remove all units from the packaging and verify that all components listed on the delivery note are present.
  - Keep the original packaging for a possible longer storage or return of the instrument to the manufacturer or dispose of it properly.

### 3.3 Connecting Apotome 3

You can connect the Apotome 3 in the following ways:

- Connection via CAN directly to the microscope or via the CAN distributor box
- Connection directly to PC via CAN-USB converter

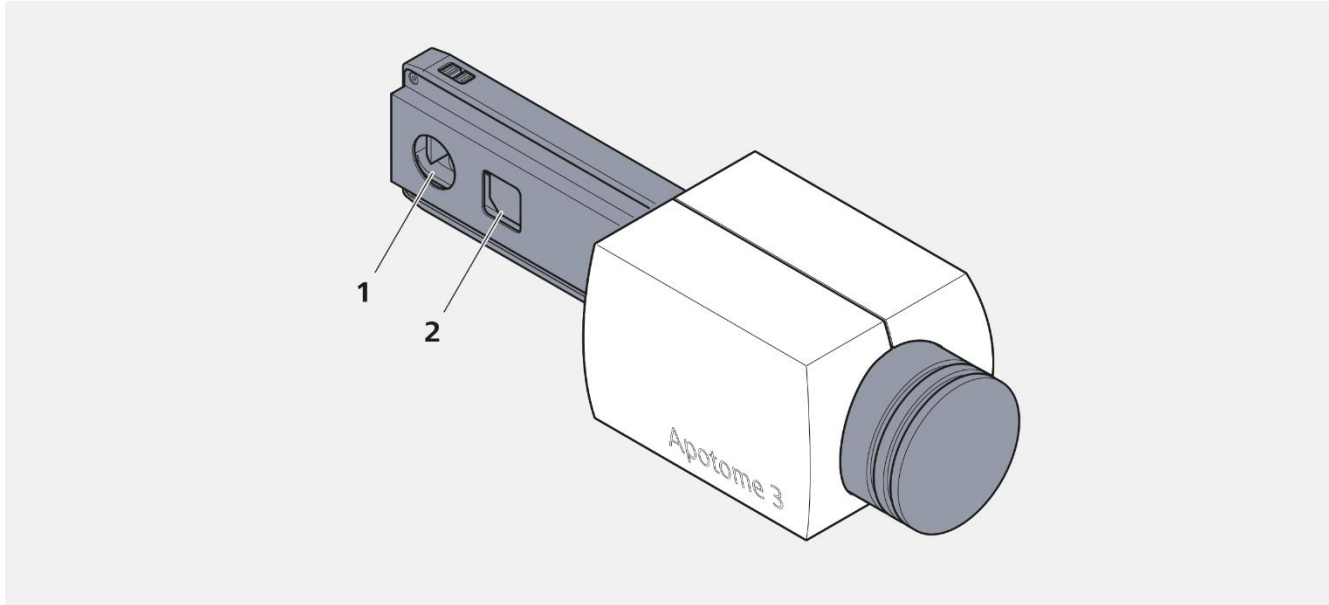


Before connecting Apotome 3 via CAN-BUS cable to the microscope stand make sure, that the stand is switched off.

Do not remove the CAN-BUS cable while the microscope system is switched on.

## 4 OPERATION

### 4.1 Control and functional elements of Apotome 3



- 1 Click stop position 1 - free passage
- 2 Click-stop position 2 - transmission grid

**Fig. 8** Control and functional elements of Apotome 3

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## 4.2 Switching on safely

- Carefully insert the Apotome 3 slider in the plane of the luminous-field diaphragm of the reflected light beam path of the microscope used (see complete Instruction Manual of the microscope). On insertion, a short beep signal is generated.
- Use the on/off switch of the microscope to switch the device on.

The Apotome 3 is switched on automatically with startup of the microscope stand.

Switch on the device approximately 30 minutes before the first image capture in order to allow it to warm up to the necessary operating temperature.

The slider must be inserted in the microscope stand to warm up properly.

## 4.3 How to get the first picture



Before the Apotome 3 can be used for imaging, it has to be calibrated in the ZEN software, using Apotome Phase calibration and Apotome Focus calibration wizards.

In the first click-stop position, conventional imaging is possible (free passage). In the second click-stop position, the transmission grid is moved into the beam path.

### Click-stop position 1

In click-stop position 1 of the Apotome 3 slider, an open passage is in the reflected light beam path. In this position the microscope can be operated in the conventional way. The slider does not affect any function of the microscope stand used.

### Click-stop position 2

In click-stop position 2, the transmission grid is positioned in the beam path of rays. All functions for the adjustment and control of the instrument are operated via the software. For additional information, refer to the Instruction Manual of the software used.

## 4.4 Switching off safely

- After use, switch the device off by switching off the microscope stand.

## 5 CARE, MAINTENANCE, TROUBLESHOOTING AND SERVICE

### 5.1 Cleaning the device



Ensure that no moisture enters the device.

The care of the Apotome 3 is limited to the works described below:

- After every use, switch off the device and cover it with the protective cover (protection from dust and moisture). If necessary, store the Apotome 3 slider in the provided case.
- Do not install the device in a damp room; the maximum permissible humidity is 75 %.
- Remove dust and loose contamination from visible optical surfaces with a fine brush, rubber blower, cotton bud, optics-cleaning tissue or a dust-free cotton cloth without exerting pressure.
- Wipe off water-soluble contamination (coffee, cola, etc.) after breathing by using a dust-free cotton cloth or a moistened cloth. The water used may contain a dash of a mild detergent.
- Wipe off stronger oily or fatty contamination (immersion oils, fingerprints) with a cotton bud, lens cleaning paper or a dust-free cotton cloth using optics cleaning agent L.

This cleaning agent is made of 90 percent by volume gasoline and 10 percent by volume isopropanol (IPA). Its individual constituents are also known under the following synonyms:

gasoline: surgical spirits, petroleum ether

isopropanol: 2-propanol, dimethyl carbinol, 2-hydroxypropane

Clean the optical surfaces parallel to the alignment of the grid exerting a slight pressure onto the surface.



Clean the transmission grid with extreme care to avoid the damage of the grid structures!

For the use of the Apotome 3 in humid climatic zones, observe the following instructions:

- Store the device in a bright, dry and well ventilated room; humidity < 75 %. Store components and accessories that are especially susceptible to humidity, such as objectives and eyepieces, in drying chambers.

Precision-mechanical optical instruments are always endangered by mold attack under the following conditions:

- relative humidity of > 75% longer than three days at temperatures of +15 °C to +35 °C,
- installation in dark rooms without movement of air,
- dust deposits and fingerprints on optical surfaces.



## 5.2 Maintaining the device

- In the event of perceptible damages, switch off the instrument and safeguard it against inadvertent use. Have damages repaired by appropriately qualified staff.

## 5.3 Troubleshooting

Problem	Cause	Troubleshooting
Device does not respond to any control command	no current	Actuate power switch, connect power cable to power outlet of the microscope system.
	no connection to the PC	Check the cable connections
	device defective	Call Technical Service.
No transmission grid visible in eyepiece although the slider is in the ApoTome position (click position 2)	transmission grid not focused	To obtain an artifact-free result image, the transmission grid must be imaged with a high quality in the specimen plane. For this purpose, calibrate the system as described in the Instruction Manual of the imaging software ZEN (blue edition).

## 5.4 Wear parts and tools

Designation	Catalogue no.	Purpose
Reflector module calibration	424930-9903-000	Calibration of phase position of scanner
Mirror test specimen	000000-1182-440	Calibration of phase position of scanner
Fluorescence test specimen	000000-1213-943	Calibration of the focus position of the transmission grid for various wavelengths
Cover plate	423666-0102-000	Replacement of the original cover plate on Axio Imager

## 5.5 Requesting service

All repairs of mechanical, optical or electronic components inside the instrument and of the electrical components of the Apotome 3 may only be performed by Carl Zeiss Service staff or specially **authorized** personnel.

To ensure optimum setting and trouble-free function of your microscope over a longer period of time, we recommend that you enter into a service/maintenance agreement with Carl Zeiss.

For subsequent orders or when service is required, please get in touch with the Carl Zeiss representative responsible for your region.

For additional information, contact us at:  
[microscopy@zeiss.com](mailto:microscopy@zeiss.com)

or visit us in the Internet at:  
[www.zeiss.com/microscopy](http://www.zeiss.com/microscopy)

## 6 APPENDIX

### 6.1 List of abbreviations

CE	Conformité Européenne (Conformity with EC directives)
DIN	Deutsches Institut für Normung (German Institute for Standardization)
EC	European Community
EN	European standard
EU	European Union
HBO	Mercury vapor short-arc lamp for fluorescence
ISO	International Organization for Standardization
IVD	In-vitro diagnostic medical device
KC	Korea Certification
T	time-delay (a type of fuses)
UKCA	UK Conformity Assessed
UL	Underwriter Laboratories (USA audit authority)
UV	ultraviolet

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