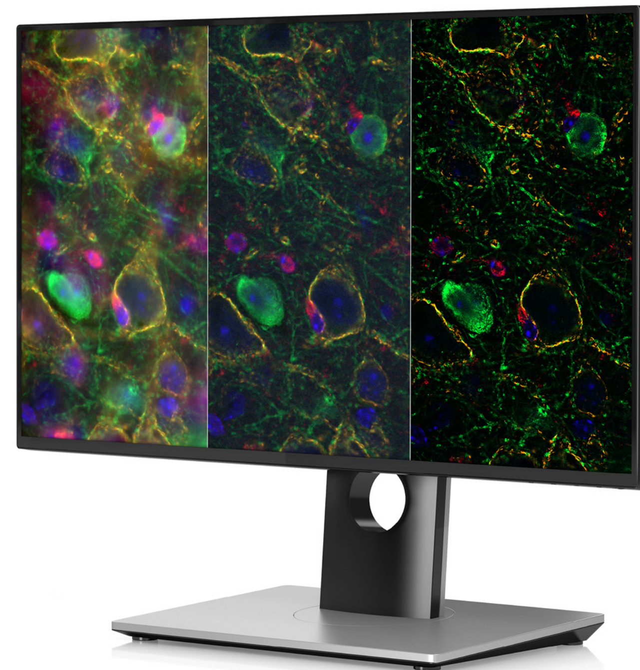
# 3D structural analysis with your widefield microscope



**ZEISS Apotome 3**

Reliable Optical Sectioning in Fluorescence Microscopy with Structured Illumination

Seeing beyond

zeiss.com/apotome

# Reliable Optical Sectioning in Fluorescence Microscopy with Structured Illumination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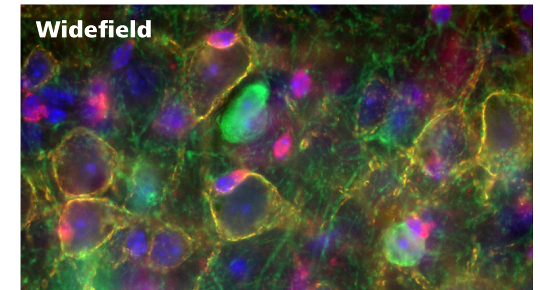
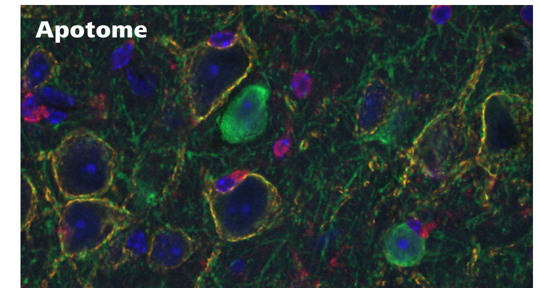
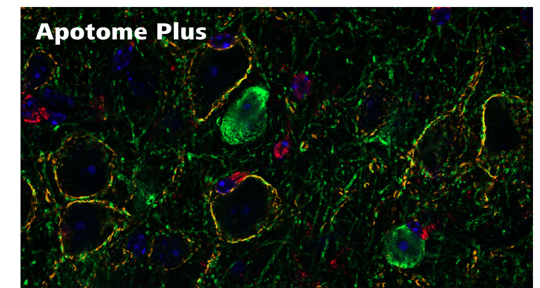
Fluorescence microscopy is one of the most important imaging techniques in the life sciences, with high-contrast images achieved from fluorescent signals emitted by the sample. However, the objective lens also collects light from outside the focal plane. Out-of-focus light which might originate from structures above or below the focal plane, must be removed to extract the in-focus information only.

Optical sectioning with ZEISS Apotome 3 allows you to efficiently minimize out-of-focus light. It's a totally reliable way to create crisp images and 3D renderings, even of thicker specimen. Yet your widefield microscope remains just as easy to operate as always. Add Apotome Plus to get even higher contrast and distinguish structures down to 180 nm – simply brilliant optical sections.




*35 µm sagittal section of adult mouse brain, imaged with ZEISS Axio Observer and ZEISS Apotome, processed with Apotome Plus. Sample courtesy of University of California, Davis / NIH NeuroMab Facility.*

# Simpler. More Intelligent. More Integrated.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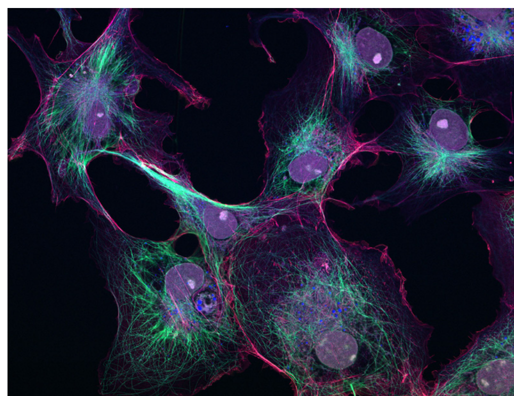
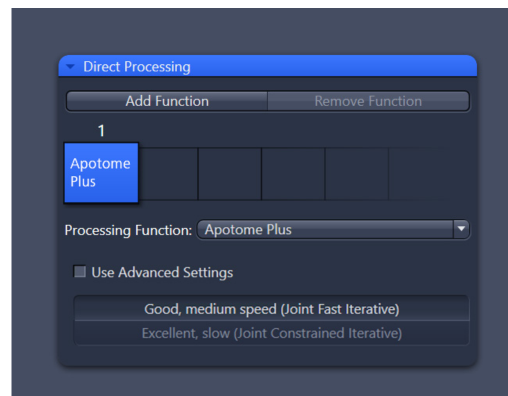
**Brilliant optical sections under varying experimental conditions**
To image structures of sizes from hundreds of micrometers to the nanometer range, you typically use objectives with different magnifications. ZEISS Apotome 3 comes with three grids of different frequencies, giving you the best resolution for each objective. You can fully focus on your experiment as ZEISS Apotome 3 adapts to your fluorophores and light source by automatically selecting the ideal grid. Enjoy high-contrast optical sections with significantly improved axial resolution compared to conventional fluorescence microscopy.

**Intuitive access to efficient workflows and smart data handling**
With just one click in the ZEN imaging software, the advantages of optical sectioning are available for your usual image acquisition. Boost the productivity of your workflows with intelligent functions like Direct Processing: combine several processing functions and process your data while acquiring the next image. The built-in lossless data compression automatically reduces the file size, thus saving storage space and making data handling easier. Whether you want to image a single plane or conduct a complex experiment, ZEISS Apotome 3 is your choice for easy and efficient optical sectioning.

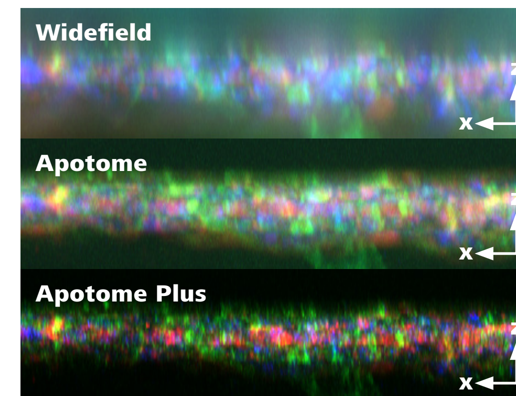
**Structural information with resolution down to 180 nm**
Compared to conventional widefield fluorescence microscopy, ZEISS Apotome 3 already significantly increases the axial resolution: you obtain brilliant optical sections that enable 3D rendering even of thick samples. With Apotome Plus, you can go one step further and achieve confocal-like image quality with your widefield microscope. The combination of structured illumination with state-of-the-art image processing substantially improves the signal-to-noise ratio. With Apotome Plus you can resolve details that were not visible before with your widefield microscope.



*Cos7 cells (nuclei stained with Hoechst, tubulin with Alexa 488 and Phalloidin with Alexa 568) imaged with Plan Apochromat 63x/1.4.*




*ZEN user interface: Adding Apotome Plus to a Direct Processing workflow*




*XZ-view of postnatal day (P) 16 mouse retina (blood vessel marker isolectin B4: Alexa Fluor 633, red; pericytic proteoglican NG2: Alexa546, green; VE-cadherin, Alexa 488, blue). Sample courtesy of E. Montañez Miralles, University of Barcelona, Spain.*

# Your Insight into the Technology Behind It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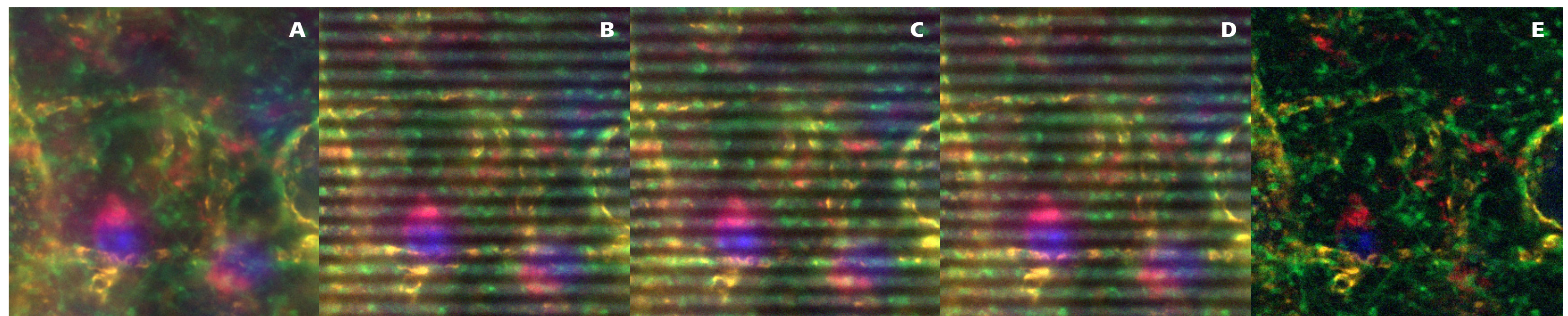
**Reliable optical sections through structured illumination**

ZEISS Apotome 3 positions an optical grid in the light path to create a pattern of intensity differences on your sample. If out-of-focus light is present at a certain region of the sample, the grid becomes invisible. After the fluorescence of a grid position is acquired, the grid moves to the next position. Each optical section is calculated from a number of images acquired with different grid positions. This proven, hardware-based approach built on well documented algorithms gives you the reliability that is expected from scientific data.

Apotome can be used with multi-LED and white light sources. It recognizes the magnification and automatically moves the appropriate grid into the beam path to ensure optimal imaging parameters. No matter if you want to image specimen on slides, petri dishes, or multi-well plates, Apotome generates crisp optical sections with high resolution.




*Schematic illustration of the grid projection. A: Widefield image. B – D: raw images with different positions of the grid. E: resulting optical section through the sample. Out-of-focus light is efficiently removed by the structured illumination.*

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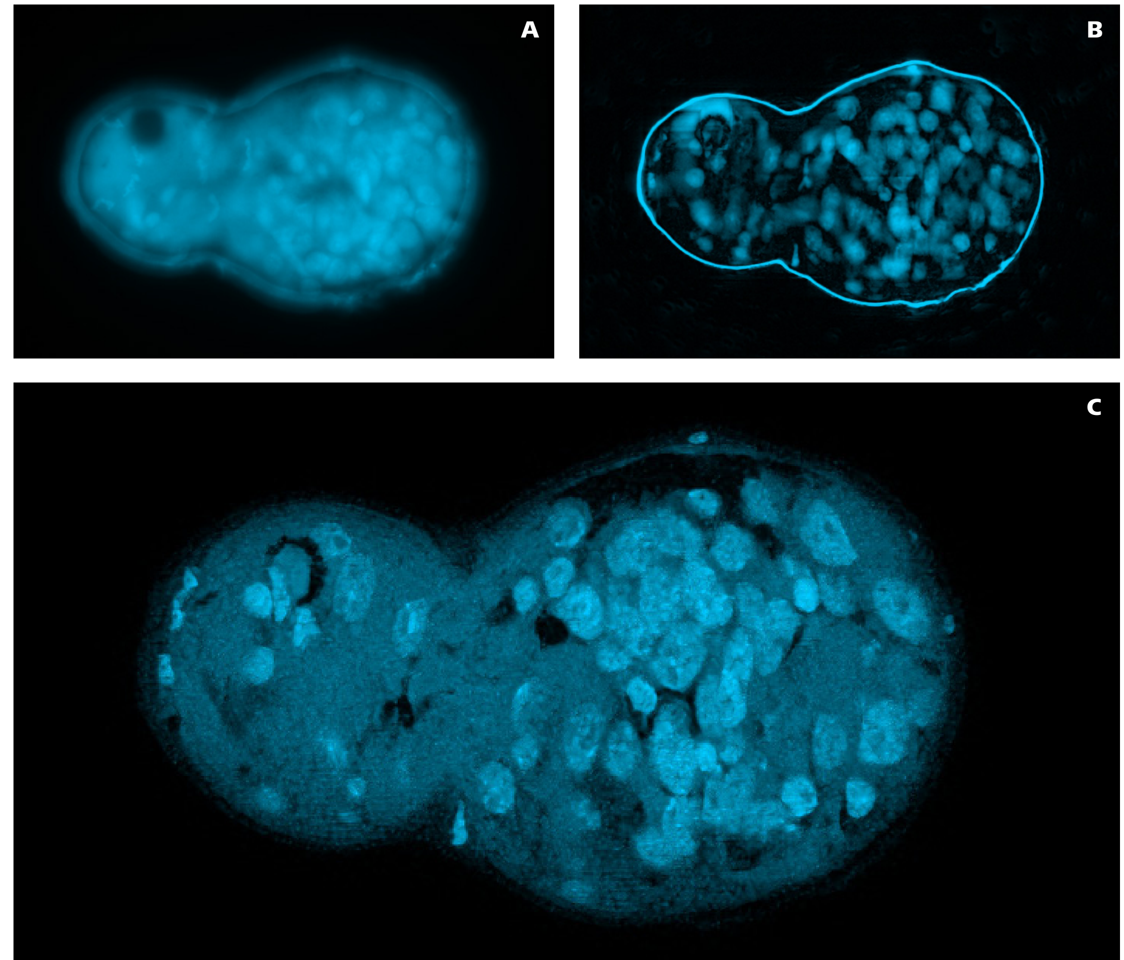
**Image data you can trust**

Compared to purely software-based approaches, Apotome uses structured illumination which has several advantages. Purely software-based methods require either prior knowledge of the sample (AI based methods) or rely on complex algorithms that have not been peer-reviewed. As software solutions can only use the acquired widefield image, users must trust that these black-box solutions exclusively produce structures that are real and do not remove structures when "enhancing" the image.

The figure shows the comparison of a widefield image, a background-subtracted image processed using a software algorithm, and an image acquired with ZEISS Apotome 3. Even though the background-subtracted image shows a high contrast that is pleasing to the eye, it falsifies information. Features are missing and structures look entirely different. Without knowing the true image, it is almost impossible to realize this. Apotome uses the information from the structured illumination combined with documented algorithms to create a crisp optical section you can trust.




*Giant live fluke stained with Hoechst 33342. The homogeneous fluorescence in the inner parts of the widefield image (A) poses a serious problem for the background correction algorithms (B). Some structures remain, but generally, there are too many black spaces between the cells. This becomes visible when comparing the results to an optical section acquired with ZEISS Apotome 3 (C). Notably, the prominent rim around the structure, as seen in the background-corrected image (B), is an artifact of interference in the widefield image, which is not seen with an optical sectioning system.*

# Expand Your Possibilities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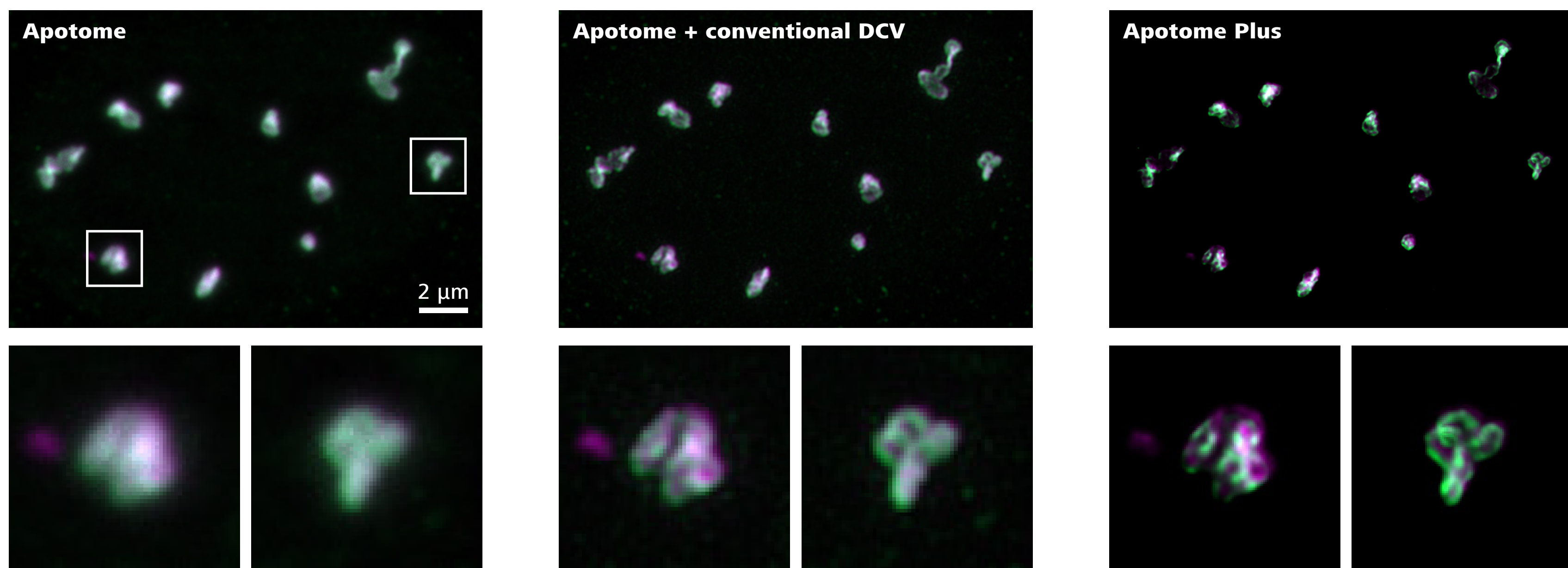
**Apotome Plus: Resolve structural information down to 180 nm with your widefield microscope.**
Compared to conventional widefield microscopy, imaging with structured illumination enables more information to be obtained for the creation of optical sections.

To achieve even higher resolution and better signal-to-noise ratios, Apotome Plus combines information from your Apotome hardware with advanced image processing. Compared to purely software-based techniques, Apotome Plus is less prone to artifacts because of the additional information of the structured illumination. Use your widefield microscope to resolve structures down to 180 nm laterally without sacrificing the ease of use of Apotome. With smart Direct Processing workflows, you can process your data while image acquisition is still running. What's more, you can take the opportunity to discover previously unresolved details of your samples by applying the Apotome Plus processing to existing z-stacks acquired with Apotome 3 and ApoTome.2 systems.




*Maximum intensity projection of PML nuclear bodies of U-2 OS cells transiently transfected with mRFP-SUMO-2 (magenta) and pEGFP-PML-I (green). Note that overexpression of PML and SUMO-2 leads to an increased size of the nuclear bodies. Image sizes: 2×2 µm. Sample courtesy: P. Hemmerich, Leibniz Institute on Aging – Fritz Lipmann Institute e. V. (FLI), Germany.*

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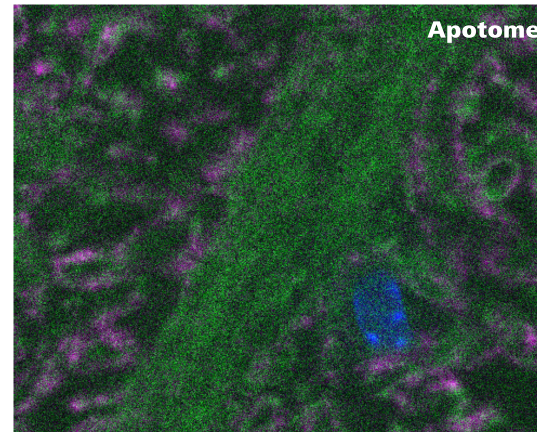
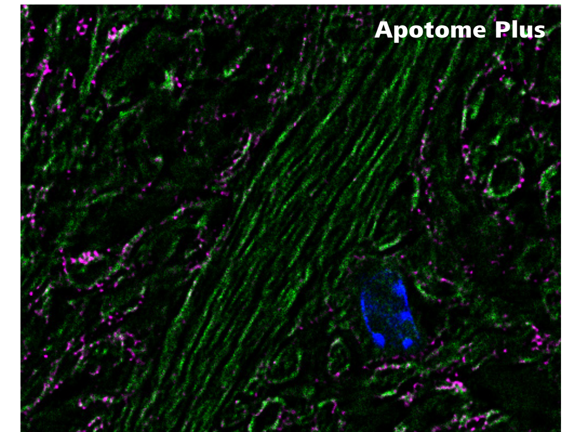
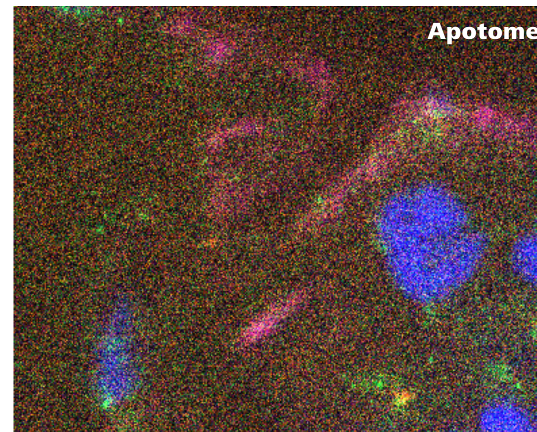
**Apotome Plus: best image quality from challenging samples**
When imaging biological samples, resolution is not always most important to answer scientific questions. Aspects like signal-to-background ratio and removal of out-of-focus information are as crucial because they directly affect image quality and subsequent data analysis.

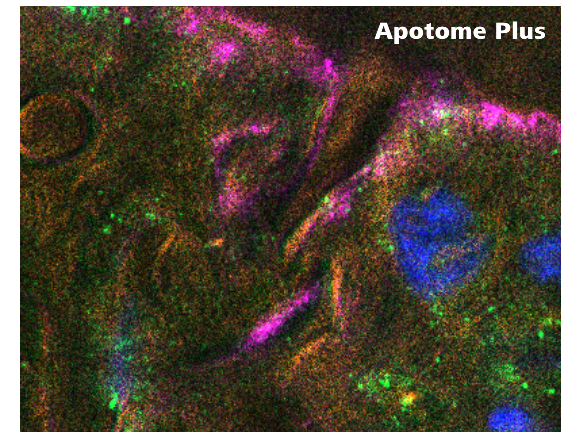
With Apotome Plus you can significantly improve the overall image quality even for dim samples with low signals. Image large volumes of tissue, cells, plants and other samples with confocal-like image quality using a camera-based microscope. No matter if you want to create well-resolved three-dimensional renderings, image thick samples with high contrast or are interested in smallest details, Apotome Plus is your choice for achieving best image quality with your widefield microscope.




*Mouse spinal cord section having nucleoli stained with DAPI (blue), myelin by Olig2 (oligodendrocyte lineage cell nuclear marker) with Alexa 555 (green) and PDGFRa (oligodendrocyte progenitor cell membrane marker) with Alexa 647 (magenta). Sample courtesy of S. C. Fernández, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Spain.*




*Low Signal-to-noise image of a marine vertebrata head (zoom-in) having nuceli stained with HOECST, neurons with Alexa 488 and the glia stained with CY3 and Alexa 647. Sample courtesy of A. Fuentes, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Spain.*

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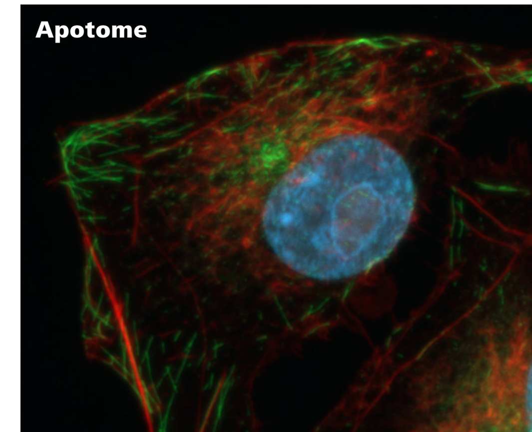
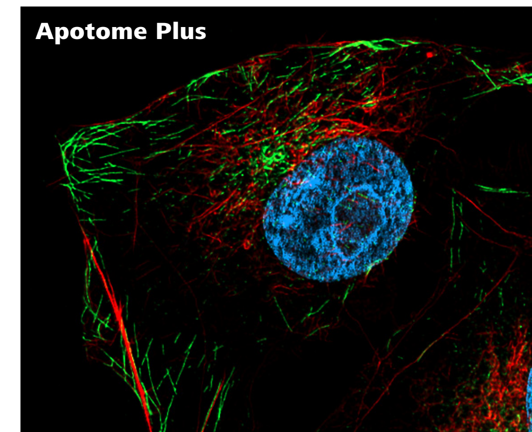
| Apotome 3 | Apotome 3 with Apotome Plus |
|---|---|
| Creates optical sections by structured illumination | |
| Established, reliable technology | |
| Activation by moving the slider in imaging position | |
| Supports white light and single LED light sources | |
| Integrated chromatic correction | |
| Automatic selection of optimal grid for each objective | |
| Supports a broad range of magnifications and numerical apertures | |
| Easy to use | |
| Full integration in ZEN | |
| Automatic, fast lossless compression | |
| GPU data processing | |
| Works with ZEISS Axio Imager, ZEISS Axio Observer and ZEISS Axio Zoom.V16 | |
| Diffraction-limited resolution | Distance discrimination down to 180 nm (xy) |
| Confocal-like optical sectioning | Optical sectioning strength down to 460 nm (50 % contrast) |
| Good signal-to-noise ratio | Superior signal-to-noise ratio |
| Good background suppression | Best background suppression |




*Maximum intensity projection of bovine pulmonal arterial endothelial (BPAE) cells having nuclei stained with DAPI (blue), F-actin with Alexa 488 phalloidin (green) and mitochondria with MitoTracker Red CMXRos (red).*

# ZEISS Apotome 3 at Work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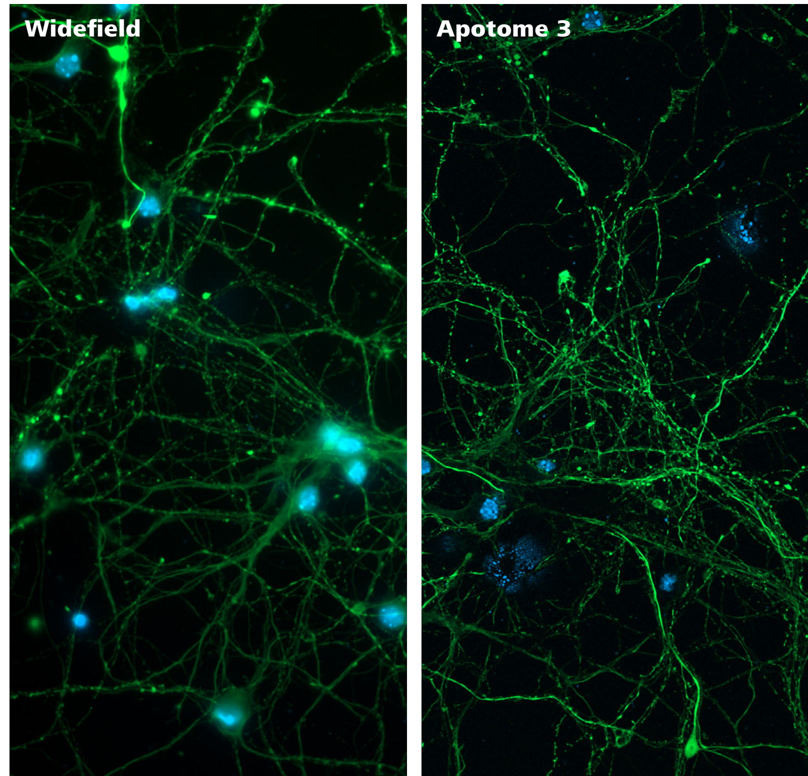
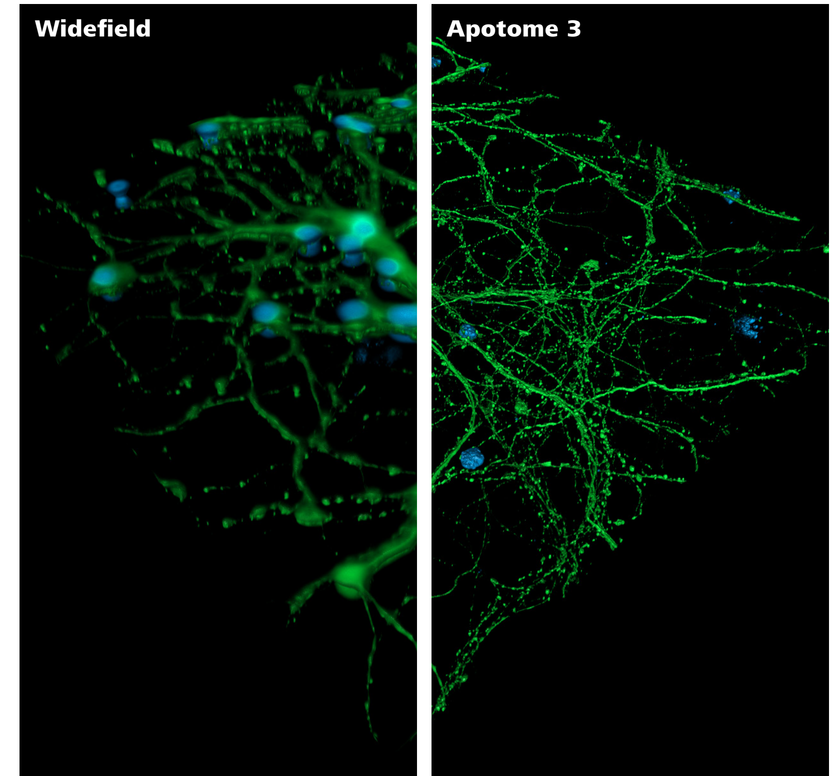



*Comparison of a widefield image of cortical neurons stained for DNA and microtubules. Courtesy of L. Behrendt, Leibniz-Institute on Aging – Fritz-Lipmann-Institut e.V. (FLI), Germany.*




*3D rendering of a section of cortical neurons stained for DNA and microtubules. The enhanced resolution improves the image quality significantly. Courtesy of L. Behrendt, Leibniz-Institute on Aging – Fritz-Lipmann-Institut e.V. (FLI), Germany.*

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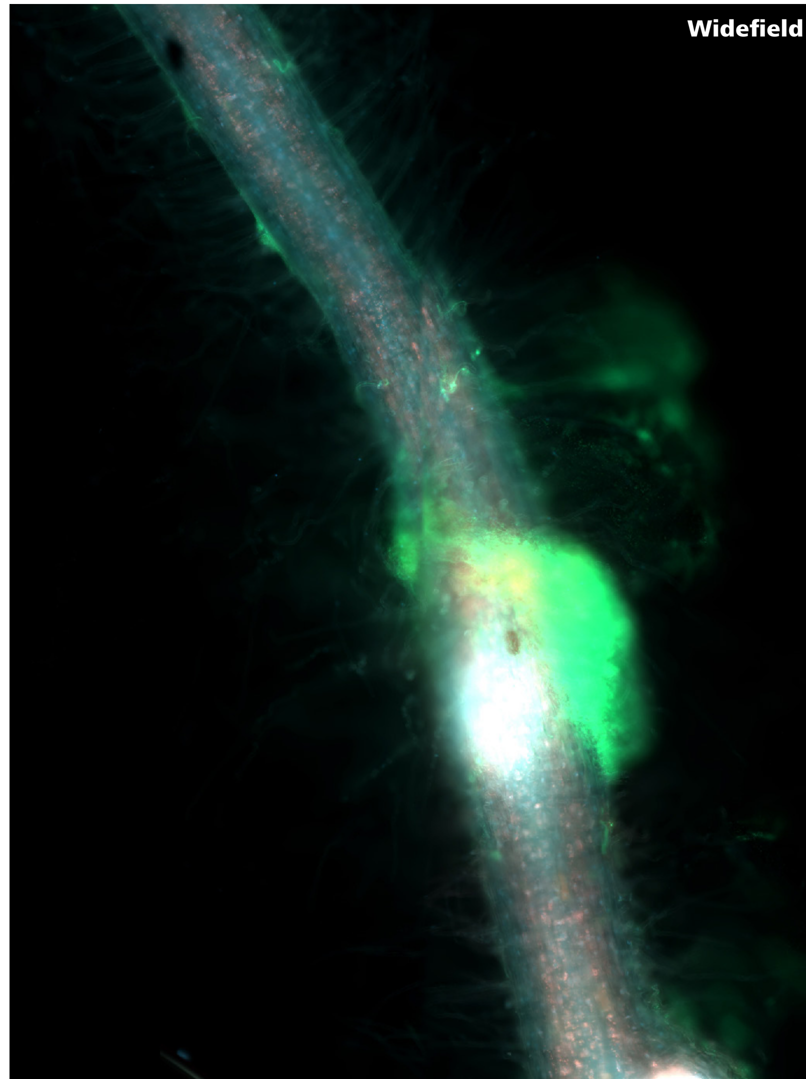


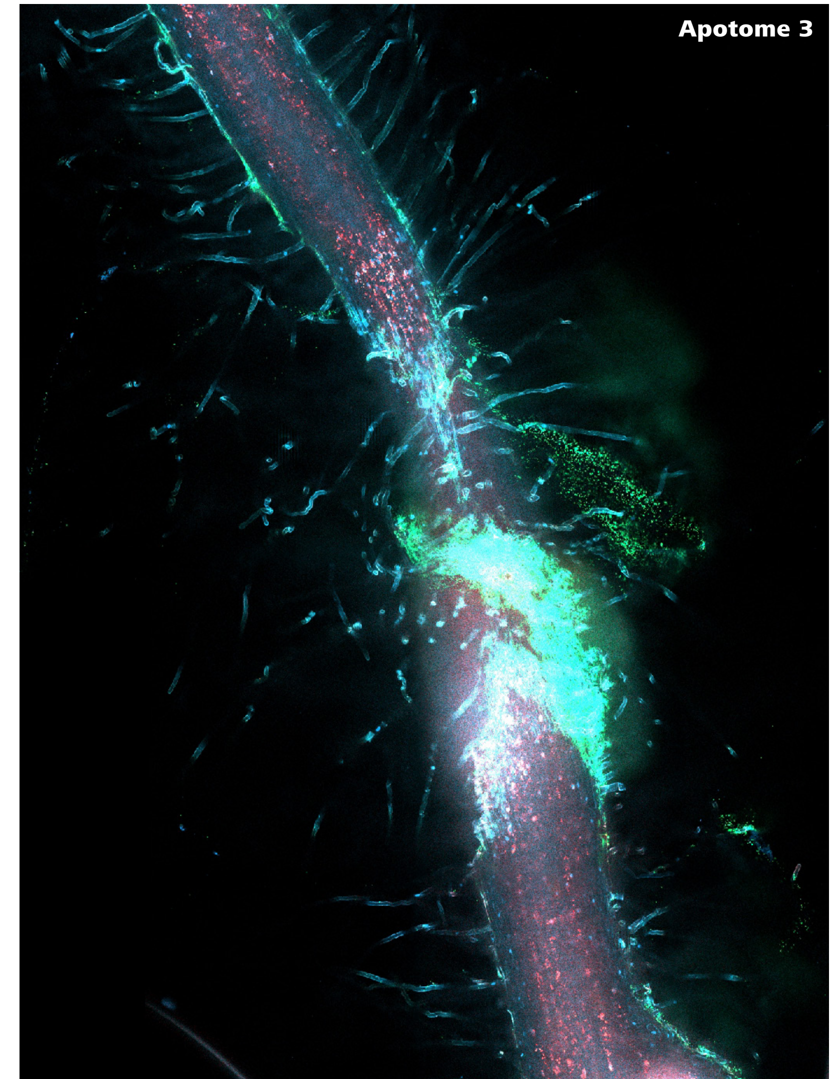


*Autofluorescence of a* Lotus Japonicus *root infected with symbiotic bacteria stained with mCherry. Courtesy of F. A. Ditengou, University of Freiburg, Germany.*

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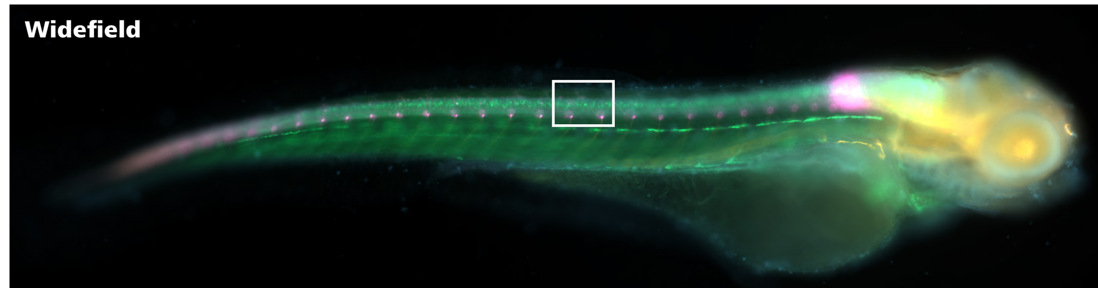


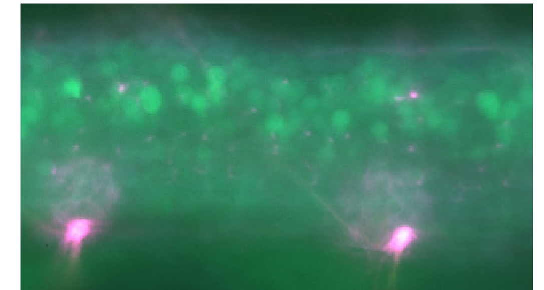

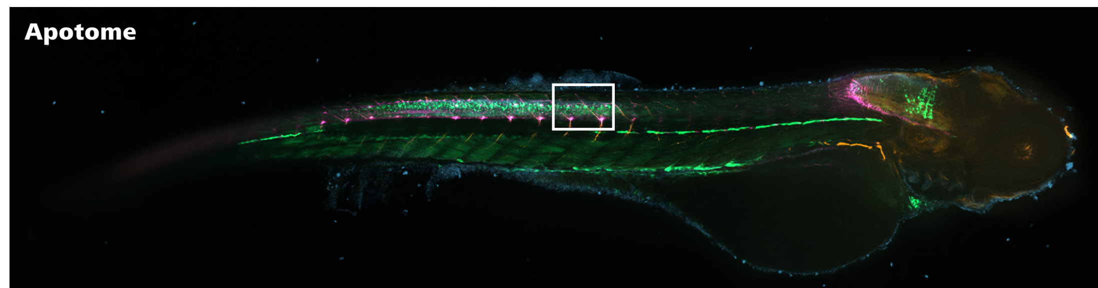


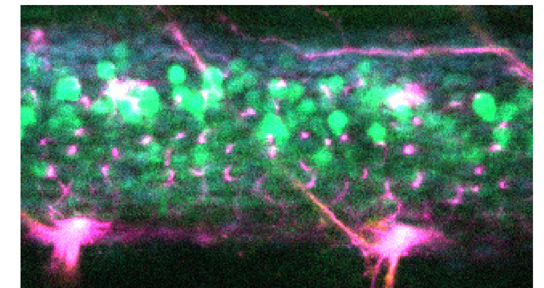

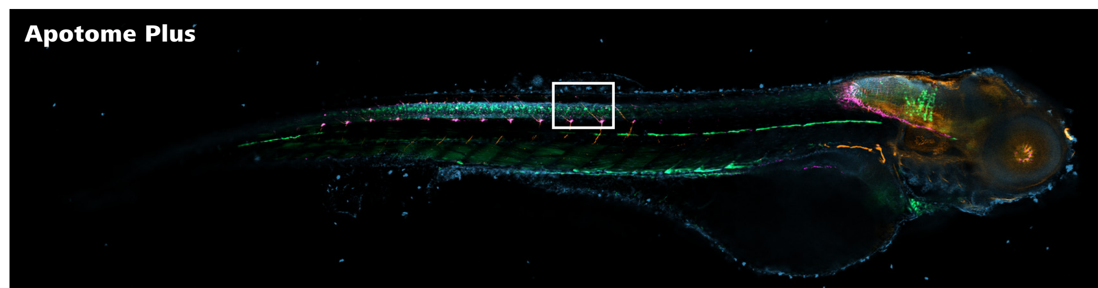


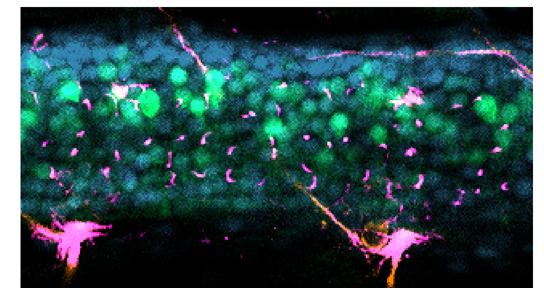

*Transgenic zebrafish larvae at 4 days post fertilization staining for: Glial fibrillary acidic protein, acetylated Tubulin, GFP and DNA. Embedded in 1.2% low melt agarose.*
*Courtesy of H. Reuter, Leibniz-Institute on Aging – Fritz-Lipmann-Institut e.V. (FLI), Germany.*

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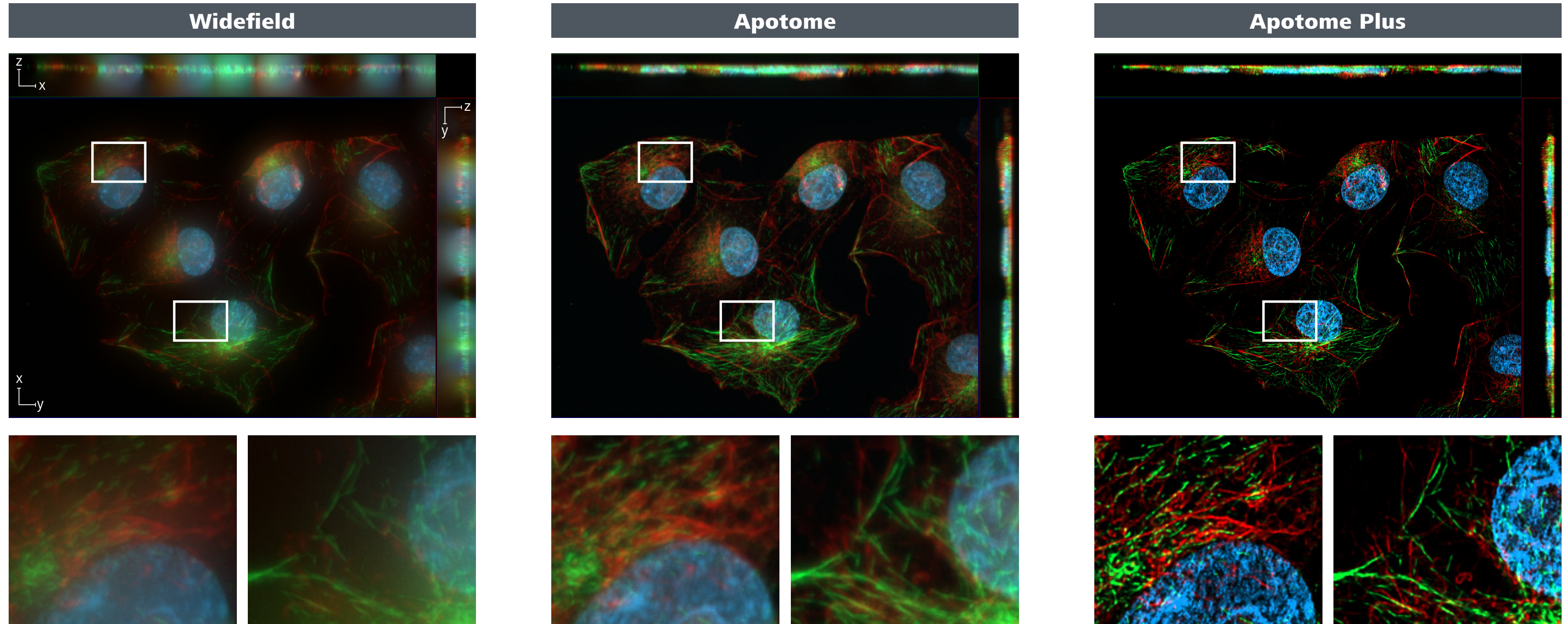


*Maximum intensity projection of bovine pulmonal arterial endothelial (BPAE) cells having nuclei stained with DAPI (blue), F-actin with Alexa 488 phalloidin (green) and mitochondria with MitoTracker Red CMXRos (red). Compared to the widefield image, Apotome removes out of focus light (see xz and yz view) creating a crisp optical section. Apotome Plus further improves image quality and resolves even finer structures.*

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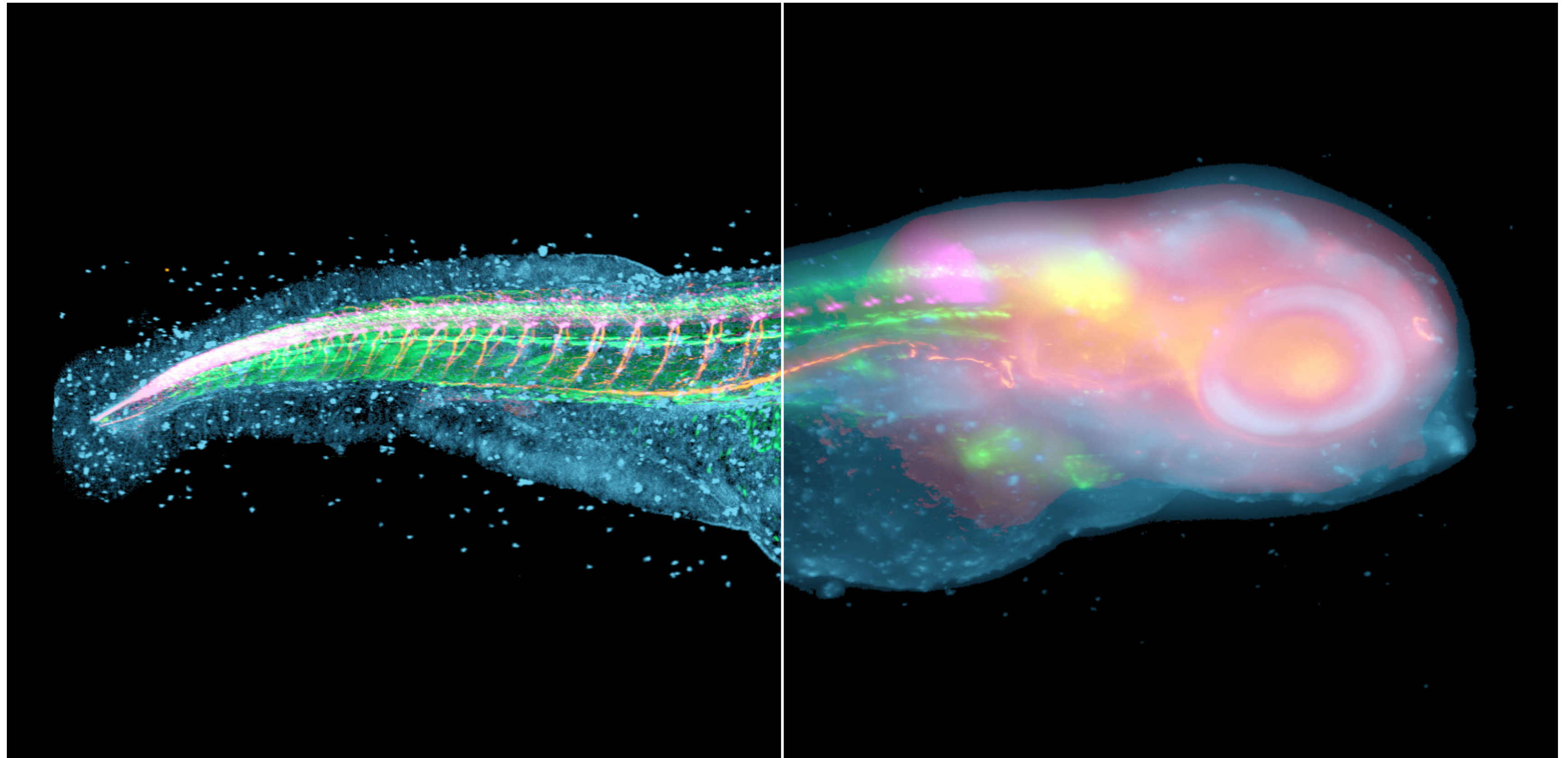
**3D reconstruction based on optical sections acquired with Apotome and Apotome Plus**



Click here to view this video

*Transgenic zebrafish larvae at 4 days post fertilization staining for: Glial fibrillary acidic protein, acetylated Tubulin, GFP and DNA. Embedded in 1.2 % low melt agarose. Courtesy of H. Reuter, Leibniz-Institute on Aging – Fritz-Lipmann-Institut e.V.(FLI), Germany.*

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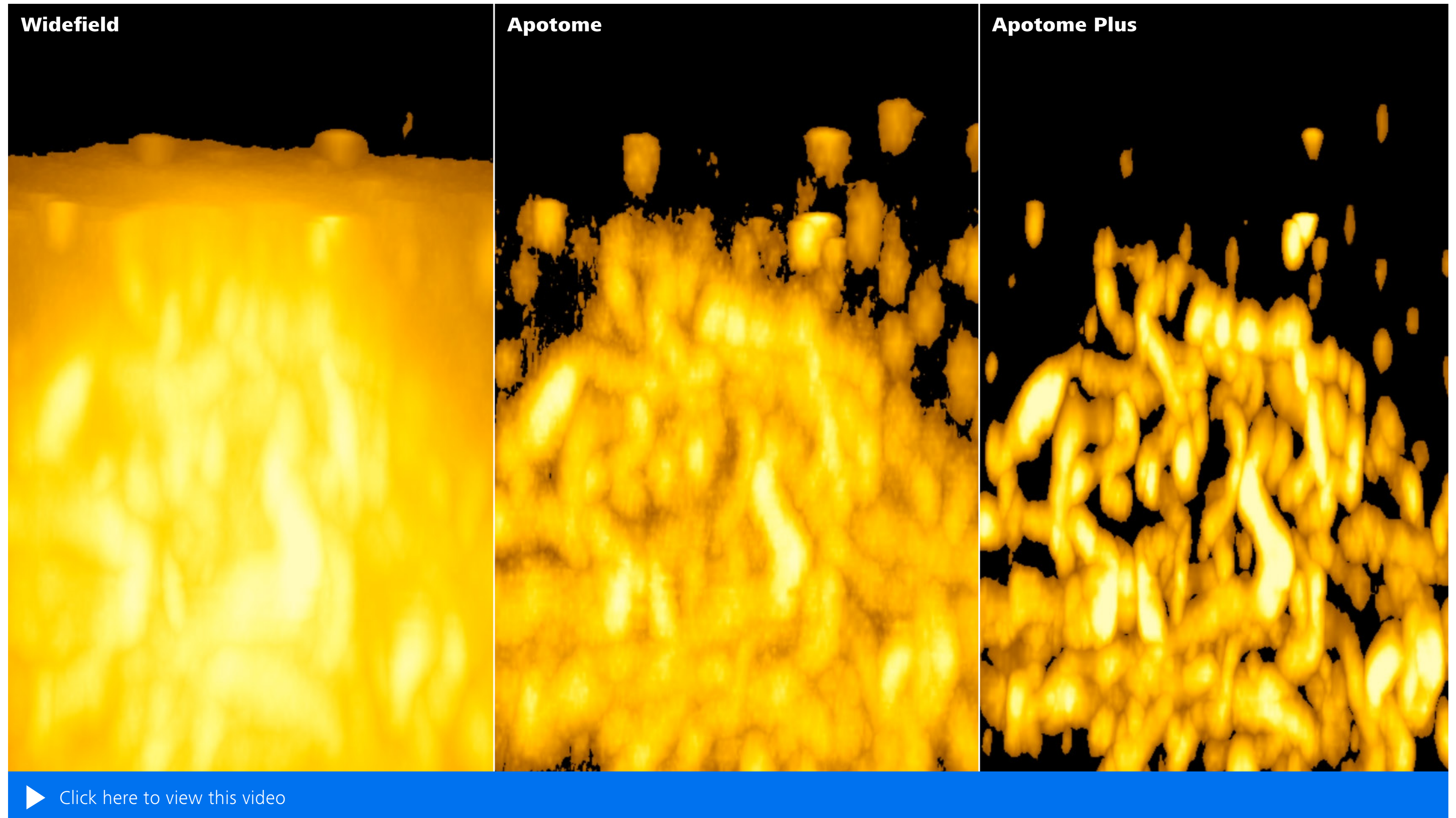


*Chromosomes of* A. Thalina *stained with REC8. Sample courtesy of S. Durand, MPI for Plant Breeding, Germany.*

# Your Flexible Choice of Components


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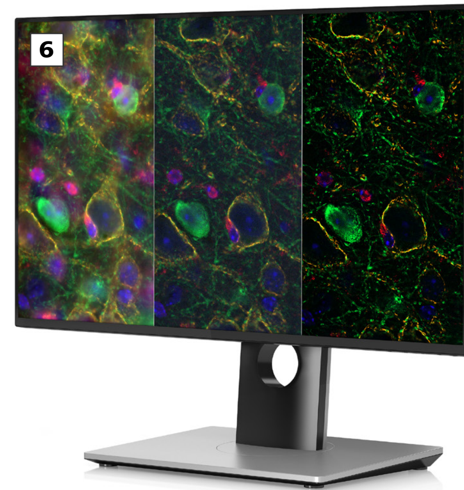


**1 Microscope**
- Axio Observer series (inverted research microscope)
- Axio Imager 2 series (upright research microscope)
- Axio Zoom.V16 (zoom microscope)
- Simple upgrading of existing systems

**2 Objectives**

Recommended objective classes with the highest level of image quality:
- C-Apochromat
- Plan-Apochromat
- EC Plan-Neofluar

**3 Illumination**
- Viluma 5, 7, 9 (LED)
- Excelitas Xylis (white light LED)
- HXP 120 C (metal halide)

**4 Cameras**
- Monochrome, low noise ZEISS Axiocam camera models
- Selected 3rd-party cameras

**5 Accessories**
- AI Sample Finder
- Definite Focus 3
- Z-Piezo
- Autoimmersion Module

**6 Software**

Recommended ZEN toolkits:
- Motorized Acquisition (z-stack, time lapse, Tiles & Position, Direct Processing)
- 3D (3D Visualization and Analysis, Advanced Processing)
- Connect (2D, 3D, third-party import)
- AI (Intellesis Segmentation, Intellesis Object Classification, Intellesis Denoising)

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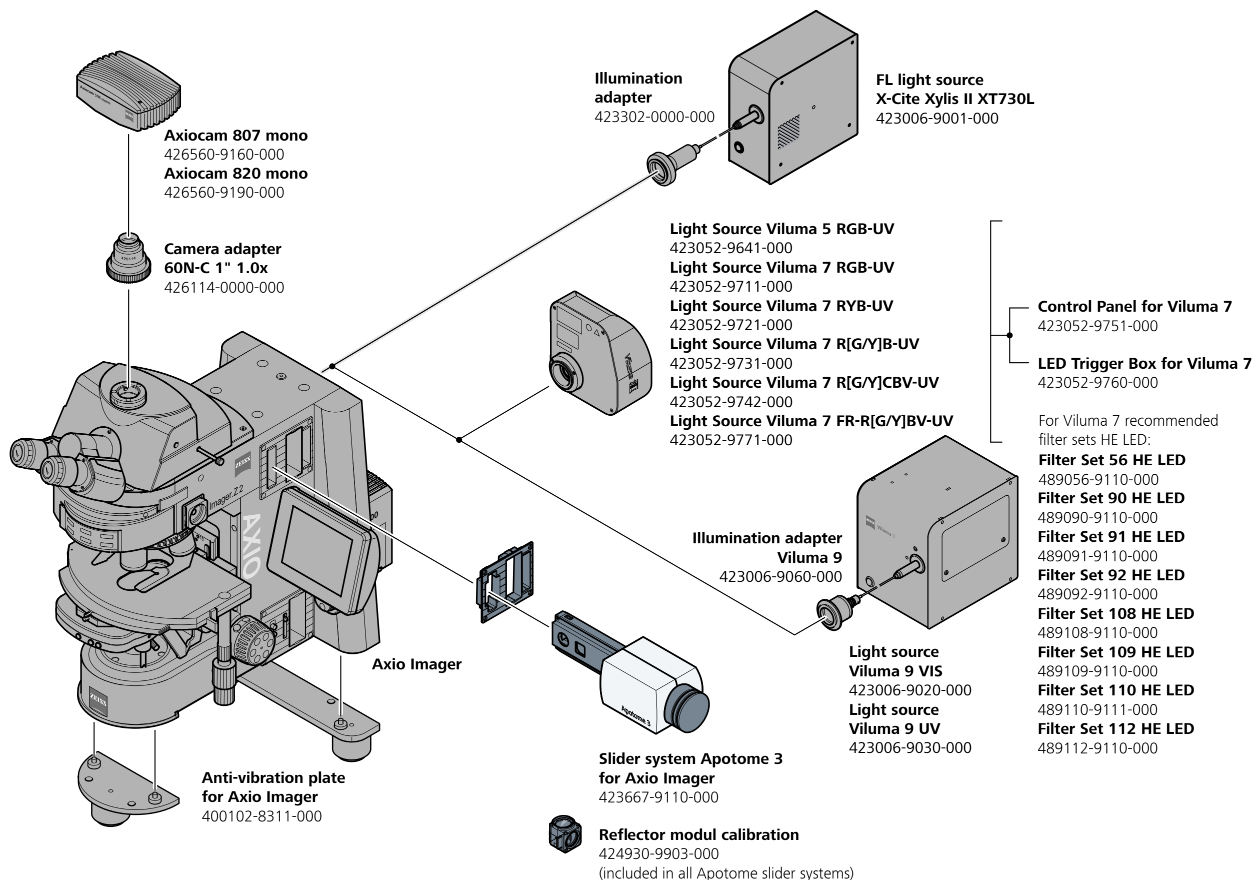

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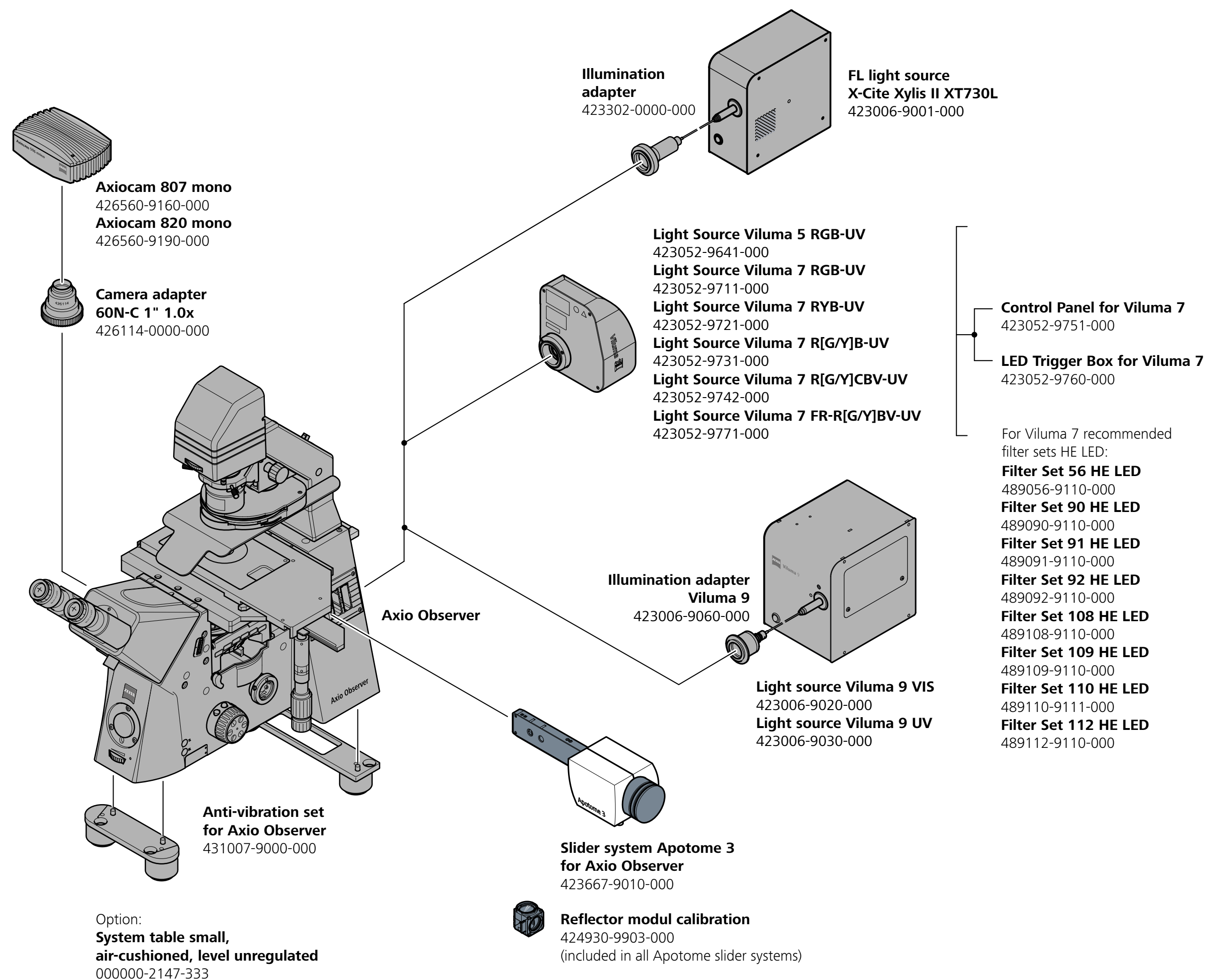

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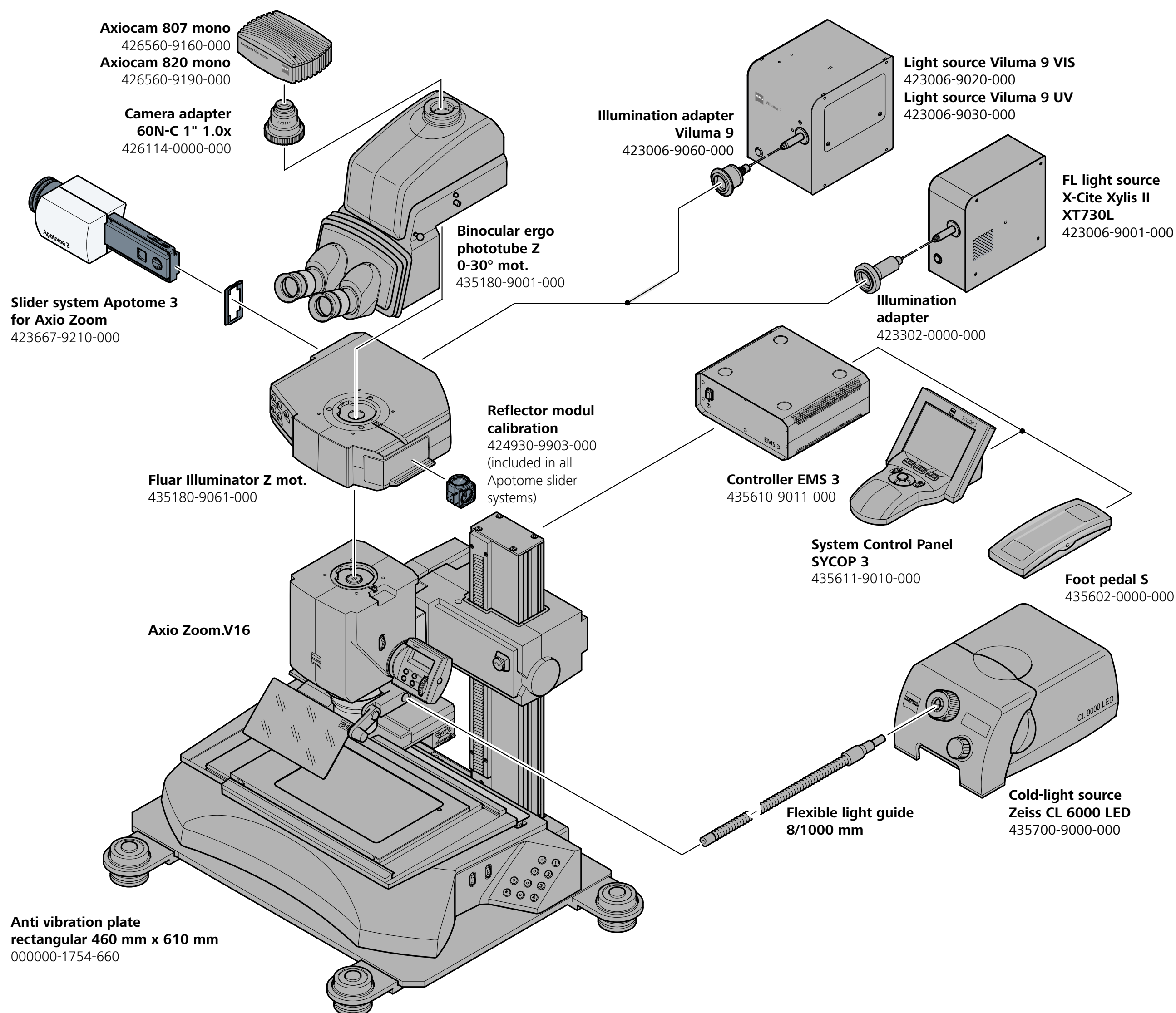

# Technical Specifications


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Grid Table: Apotome 3 generates optical sections of a defined thickness (in Rayleigh units, RU and microns, µm) depending on wavelength, microscope and objective used.

**Data for the Use of Upright Microscopes, e.g. ZEISS Axio Imager**

| Objectives for Axio Imager | V | NA | Immersion | Grid / Section thickness @490 nm [RU / µm] | | | DAPI with FS34 | DAPI with FS49 |
|---|---|---|---|---|---|---|---|---|
| | | | | High grid | Medium grid | Low grid | | |
| EC Plan-Neofluar | 10× | 0.3 | Air | 2.9 / 31.9 | 1.7 / 18.2 | 0.9 / 9.9 | Yes | Yes |
| EC Plan-Neofluar | 20× | 0.5 | Air | 2.4 / 9.2 | 1.4 / 5.3 | 0.7 / 2.9 | Yes | Yes |
| EC Plan-Neofluar | 40× | 0.75 | Air | 1.6 / 2.8 | 0.9 / 1.6 | 0.5 / 0.9 | Yes | Yes |
| EC Plan-Neofluar | 40× | 1.3 | Oil | 2.5 / 2.2 | 1.4 / 1.2 | 0.8 / 0.7 | Yes | Yes |
| EC Plan-Neofluar | 63× | 0.95 | Air | 1.0 / 1.1 | 0.6 / 0.7 | 0.4 / 0.4 | Yes | No |
| EC Plan-Neofluar | 63× | 1.25 | Oil | 1.6 / 1.5 | 0.9 / 0.9 | 0.5 / 0.5 | Yes | Yes |
| EC Plan-Neofluar | 100× | 1.3 | Oil | 1.0 / 0.9 | 0.6 / 0.5 | 0.4 / 0.3 | Yes | Yes |
| LCI Plan-Neofluar | 25× | 0.8 | Oil, water or glycerin | 2.9 / 6.6 | 1.7 / 3.7 | 0.9 / 2.0 | Yes | Yes |
| LCI Plan-Neofluar | 63× | 1.3 | Water or glycerin | 1.5 / 1.3 | 0.9 / 0.7 | 0.5 / 0.4 | Yes | Yes |
| Plan-Apochromat | 10× | 0.45 | Air | 4.2 / 20.4 | 2.4 / 11.5 | 1.3 / 6.2 | Yes | Yes |
| Plan-Apochromat | 20× | 0.8 | Air | 3.2 / 4.9 | 1.8 / 2.8 | 1.0 / 1.5 | Yes | Yes |
| Plan-Apochromat | 40× | 0.95 | Air | 1.6 / 1.7 | 0.9 / 1.0 | 0.5 / 0.5 | Yes | Yes |
| Plan-Apochromat | 40× | 1.3 | Oil | 2.5 / 2.2 | 1.4 / 1.2 | 0.8 / 0.7 | Yes | Yes |
| Plan-Apochromat | 40× | 1.4 | Oil | 2.4 / 1.8 | 1.4 / 1.0 | 0.7 / 0.6 | Yes | Yes |
| Plan-Apochromat | 63× | 1.4 | Oil | 1.6 / 1.2 | 0.9 / 0.7 | 0.5 / 0.4 | Yes | Yes |
| Plan-Apochromat | 100× | 1.4 | Oil | 1.0 / 0.8 | 0.6 / 0.5 | 0.4 / 0.3 | Yes | Yes |
| LD LCI Plan-Apochromat | 25× | 0.8 | Oil, water or glycerin | 2.9 / 6.6 | 1.7 / 3.7 | 0.9 / 2.0 | Yes | Yes |
| C-Apochromat | 10× | 0.45 | Water | 4.2 / 20.4 | 2.4 / 11.5 | 1.3 / 6.2 | Yes | Yes |
| C-Apochromat | 40× | 1.2 | Water | 2.2 / 2.0 | 1.2 / 1.1 | 0.7 / 0.6 | Yes | Yes |
| C-Apochromat | 63× | 1.2 | Water | 1.4 / 1.3 | 0.8 / 0.7 | 0.5 / 0.4 | Yes | Yes |
| LD C-Apochromat | 40× | 1.1 | Water | 2.2 / 2.3 | 1.2 / 1.3 | 0.7 / 0.7 | Yes | Yes |
| Plan-Apochromat | 63× | 1.46 | Oil | 1.5 / 1.0 | 0.9 / 0.6 | 0.5 / 0.3 | Yes | Yes |
| Plan-Fluar | 100× | 1.45 | Oil | 1.0 / 0.7 | 0.6 / 0.4 | 0.3 / 0.2 | No | No |
| Plan-Apochromat | 100× | 1.46 | Oil | 1.0 / 0.7 | 0.6 / 0.4 | 0.3 / 0.2 | Yes | No |

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# Technical Specifications

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

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

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# Technical Specifications

| Apotome Plus | |
|---|---|
| Resolution | Lateral resolution (x/y): After processing, distances down to 180 nm can be discriminated (measurements performed using DNA Nanorulers, excited at 475 nm, imaged with Plan-Apochromat 63× / 1.4 Oil DIC and Axiocam 820 mono; resolution is sample and SNR dependent, optical resolution is diffraction limited). |
| Optical sectioning strength | Optical sectioning strength (z) down to 460 nm at 50 % contrast (measured using Argolight SIM slide V2 excited at 475 nm, with Plan-Apochromat 63× / 1.4 Oil DIC and Axiocam 820 mono; resolution is sample and SNR dependent) |

| Dimensions (width × depth × height) | |
|---|---|
| Apotome 3 slider for Axio Imager | Approx. 278 mm × 90 mm × 76 mm |
| Apotome 3 slider for Axio Observer | Approx. 295 mm × 90 mm × 78 mm |
| Apotome 3 slider for Axio Zoom.V16 | Approx. 278 mm × 90 mm × 76 mm |

| Weight | |
|---|---|
| Apotome 3 slider | Approx. 1.1 kg |

| Functional Data | |
|---|---|
| Area of use | Closed rooms |
| Radio interference supression | As per EN 55011 Class A |
| Noise immunity | As per DIN EN 61326-1 |

| Operating Data | |
|---|---|
| Interference Suppression | In accordance with EN 55011 class A |
| Interference Resistance | In accordance with DIN EN 61326-1 |
| Supply Voltage | 24 V DC |
| Power Consumption Apotome 3 | Max. 5 W |

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| Ambient Conditions for 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 |

| Grid Frequencies (transmission grid high / medium / low) | |
|---|---|
| Axio Imager slider | 5 / 9 / 17.5 lp / mm |
| Axio Observer slider | 10 / 17.5 / 35 lp / mm |
| Axio Zoom.V16 slider | 10 / 15 / 20 lp / mm |

**Installation Conditions**

The grid projection method used for the Apotome 3 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.

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# ZEISS Service – Your Partner at All Times

Your microscope system from ZEISS is one of your most important tools. For over 175 years, the ZEISS brand and our experience have stood for reliable equipment with a long life in the field of microscopy. You can count on superior service and support - before and after installation. Our skilled ZEISS service team makes sure that your microscope is always ready for use.

## Procurement

- Lab Planning & Construction Site Management
- Site Inspection & Environmental Analysis
- GMP-Qualification IQ/OQ
- Installation & Handover
- IT Integration Support
- Startup Training

## Operation

- Predictive Service Remote Monitoring
- Inspection & Preventive Maintenance
- Software Maintenance Agreements
- Operation & Application Training
- Expert Phone & Remote Support
- Protect Service Agreements
- Metrological Calibration
- Instrument Relocation
- Consumables
- Repairs

## New Investment

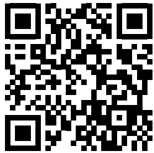
- Decommissioning
- Trade In

## Retrofit

- Customized Engineering
- Upgrades & Modernization
- Customized Workflows via ZEISS arivis Cloud

Please note: Availability of services depends on product line and location

www.zeiss.com/microscopy/service



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