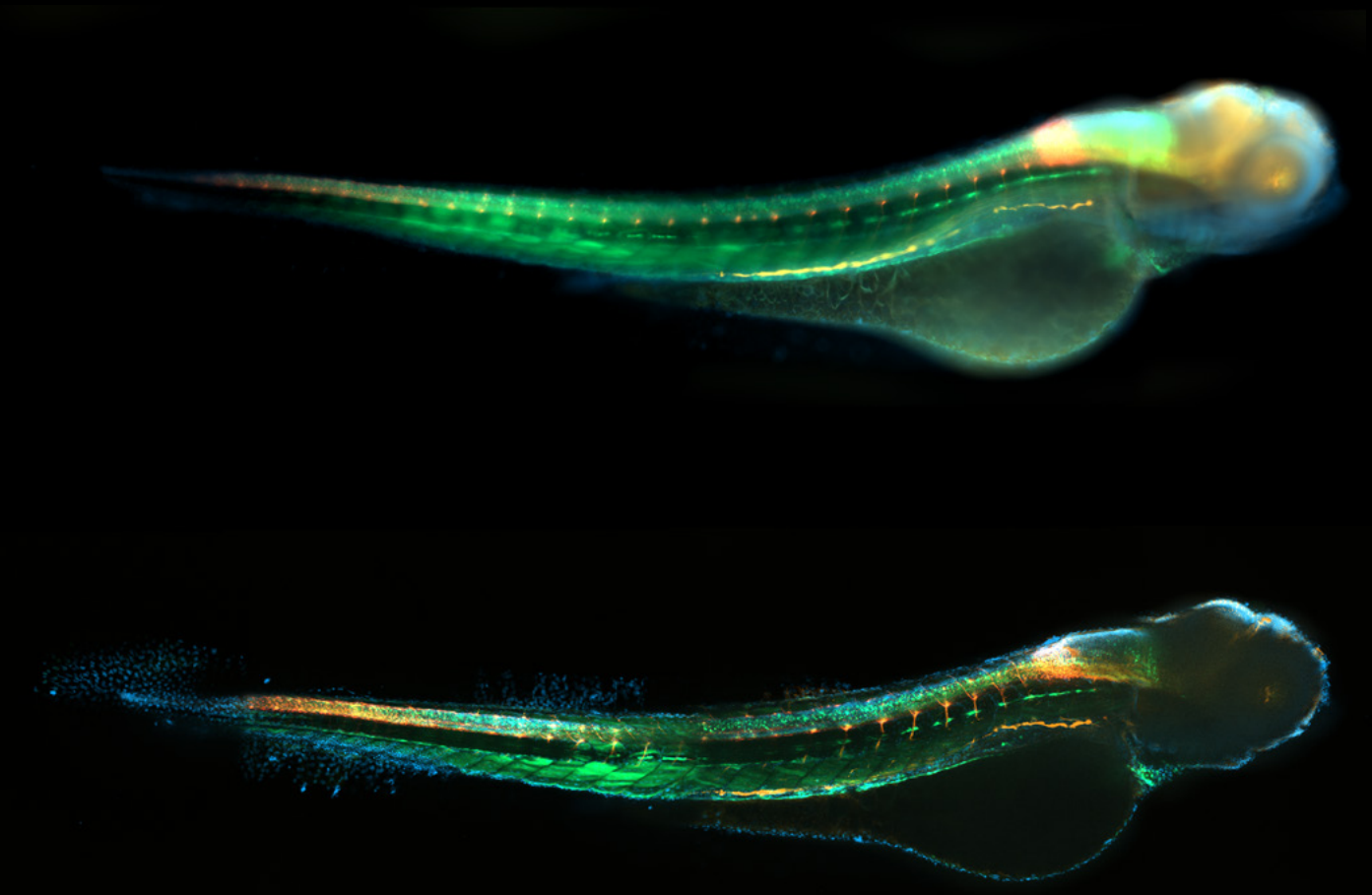
Creating reliable optical sections with structured illumination



ZEISS Apotome 3

Hardware-based, Quantitative Optical Sectioning with Well Documented Algorithms

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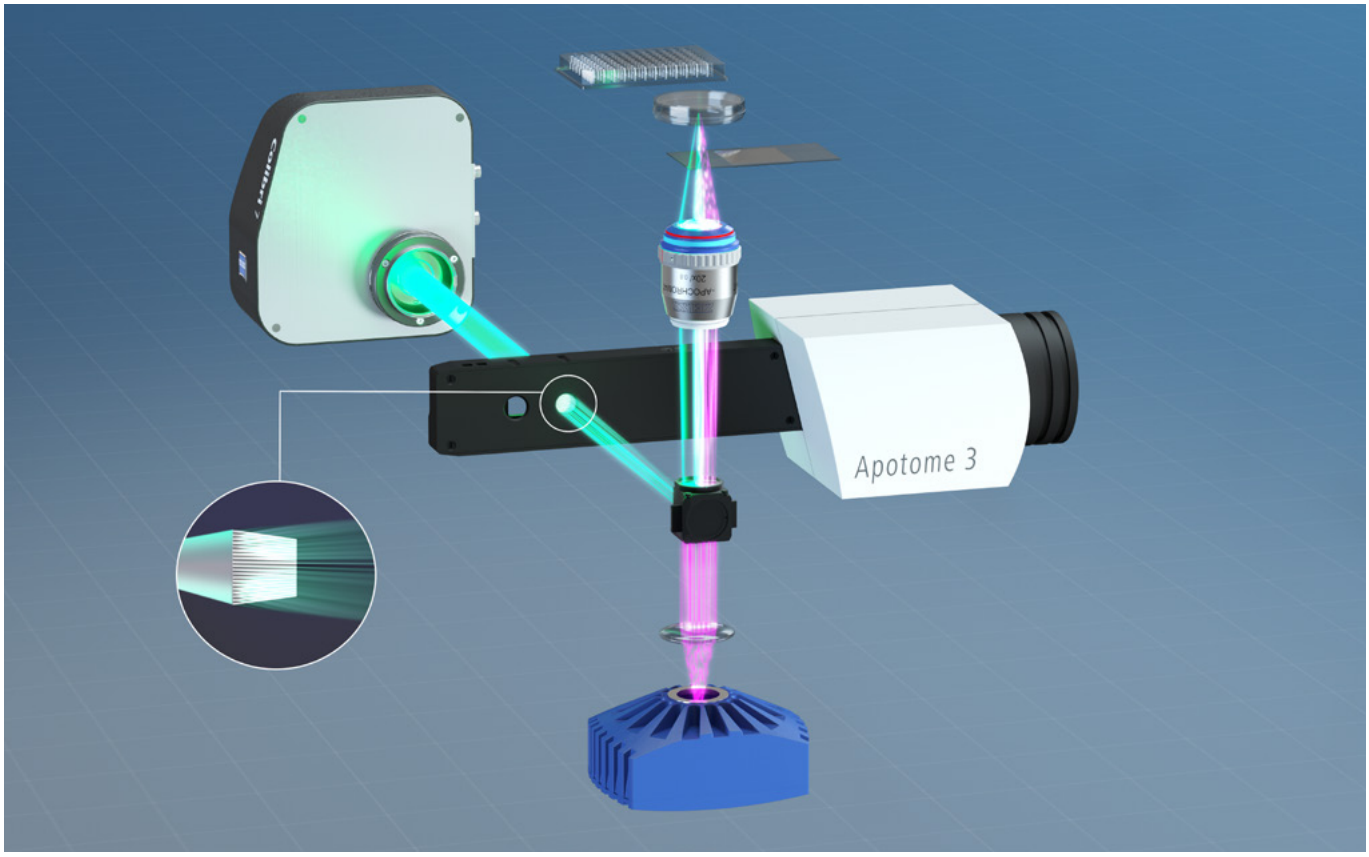
Seeing beyond

Quantitative Optical Sectioning

True Optical Sections using Structured Illumination



Fluorescence is one of the most important imaging techniques in life sciences, with high contrast images achieved when the objective lens collects fluorescent signals emitted by the sample. However, the objective lens will also collect light from outside the focal plane. Out-of-focus light, such as that which may originate from the background, needs to be removed to extract the in-focus image information. Optical sectioning allows you to efficiently minimize out-of-focus light to create crisp images and 3D renderings.

ZEISS Apotome 3 uses a grid to generate a pattern of intensity differences. 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. Using linear approaches and well documented algorithms allow you to calculate reliable optical sections from the individual images.

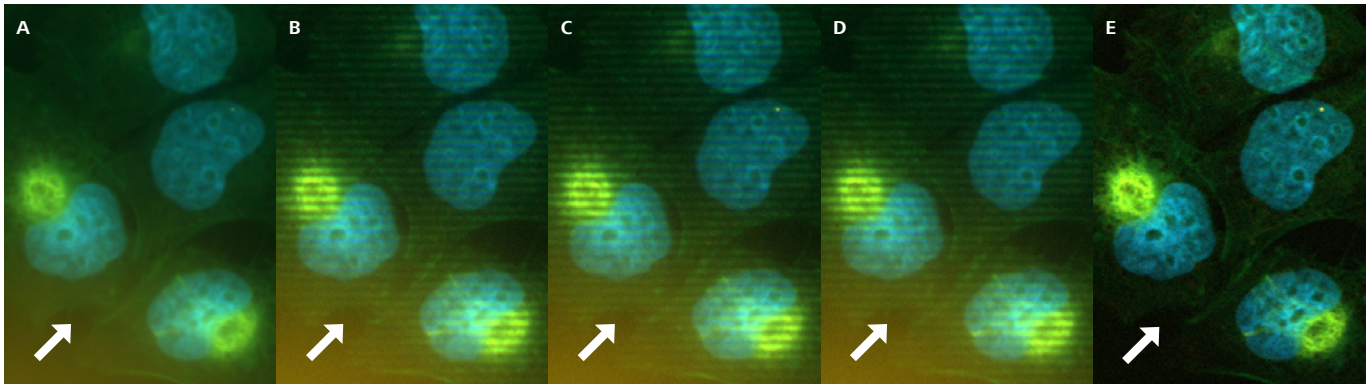


Figure 1 *Schematic illustration of the grid projection. A: Widefield image. B-D: Raw images with different positions of the grid. E: Resulting optical section through the sample. Out of focus light is efficiently removed by the structured illumination (arrow).*

Quantitative Optical Sectioning

Linear Approaches, Well Documented Algorithms

Despite hardware-based methods for creating optical sections, purely software-based solutions have emerged over the last years. These 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 only produce structures that are real and do not remove structures when "enhancing" the image.

Figures 2 and 3 show a comparison of a widefield image, a background-subtracted image processed using a software algorithm, and an image acquired with ZEISS Apotome. 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. With Apotome you can be ensured of reliable, quantitative optical sectioning that you can use for a variety of samples.

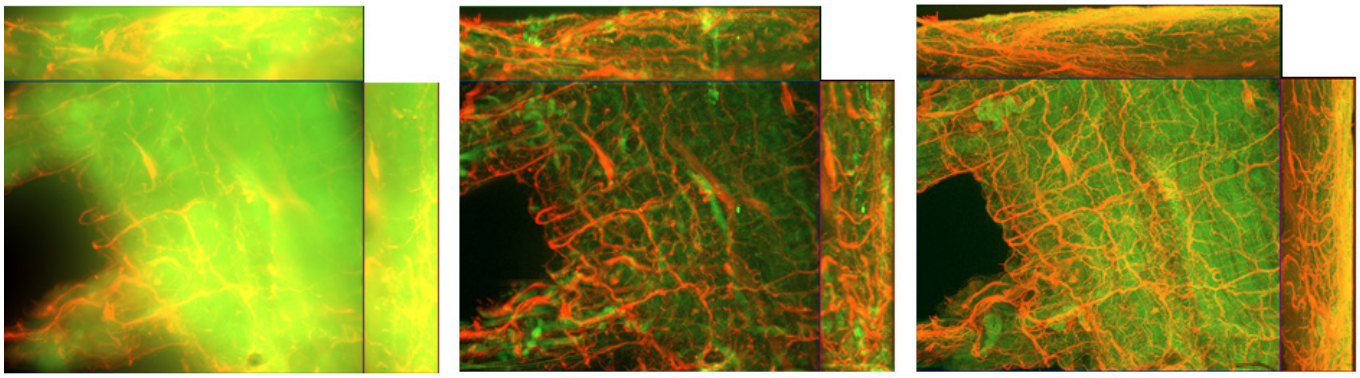


Figure 2 *Maximum-intensity (ortho) projections of a widefield image of a polychaete worm are stained in green and red (left). The image stack was processed with a rolling ball background subtraction in ZEISS ZEN lite (center). Compared to the original widefield image, more structures are visible in the background-subtracted dataset, but many details are missing when compared to the images acquired by optical sectioning with ZEISS Apotome (right). In addition, some of the compact green structure on the edges seems to have eroded. Image stack height: 160 µm, 400 planes.*

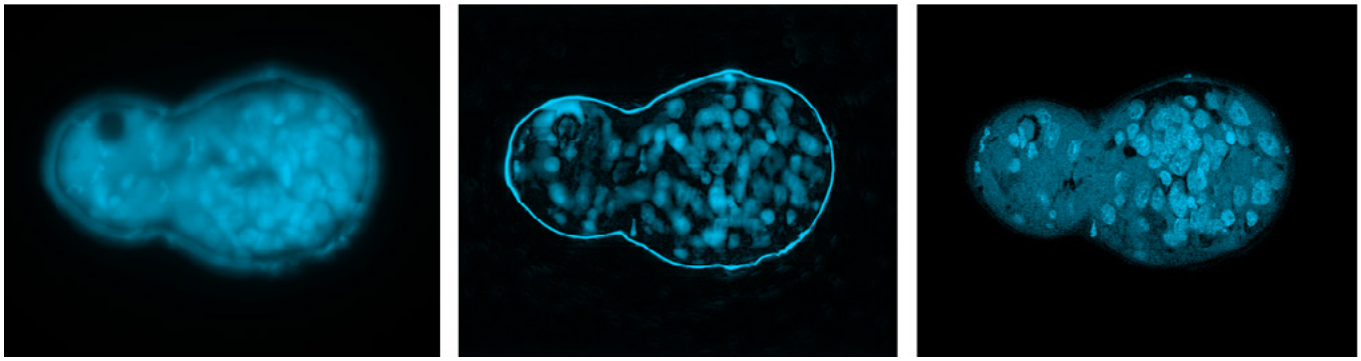
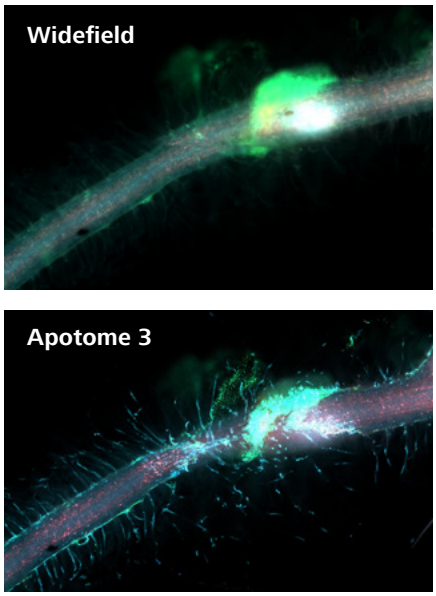


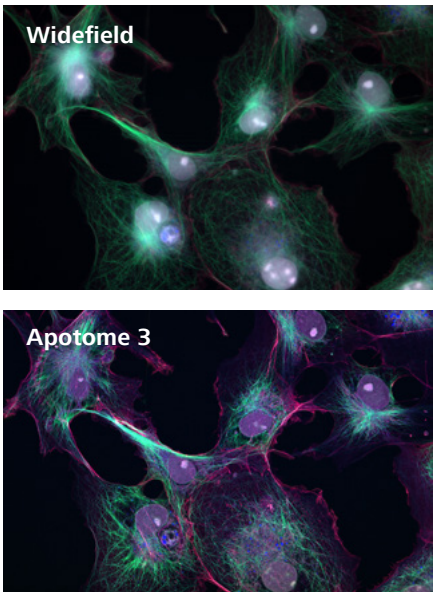
Figure 3 *Giant live fluke stained with Hoechst 33342. The homogeneous fluorescence in the inner parts of the widefield image (left) poses a serious problem for the background correction algorithms (center). Some structures remain, but generally, there are too many black spaces between the cells. This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

Quantitative Optical Sectioning
in 2D and 3D for a Variety of Samples

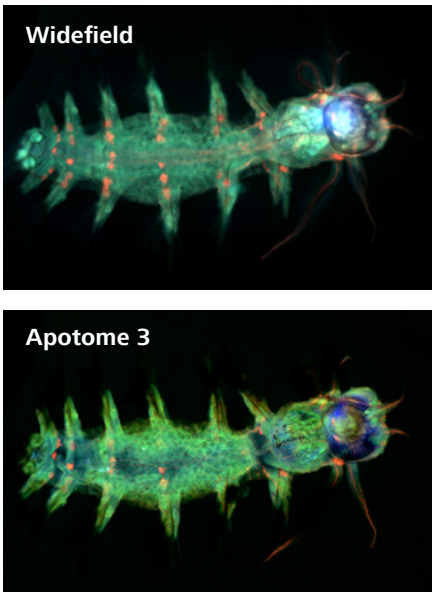
2D images



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

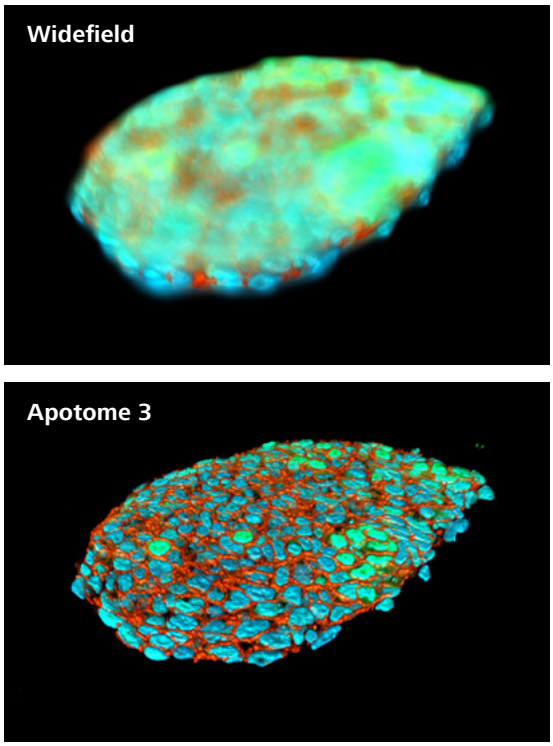


Cos7 cells having nuclei stained with Hoechst, tubulin with Alexa 488 and Phalloidin with Alexa 568.

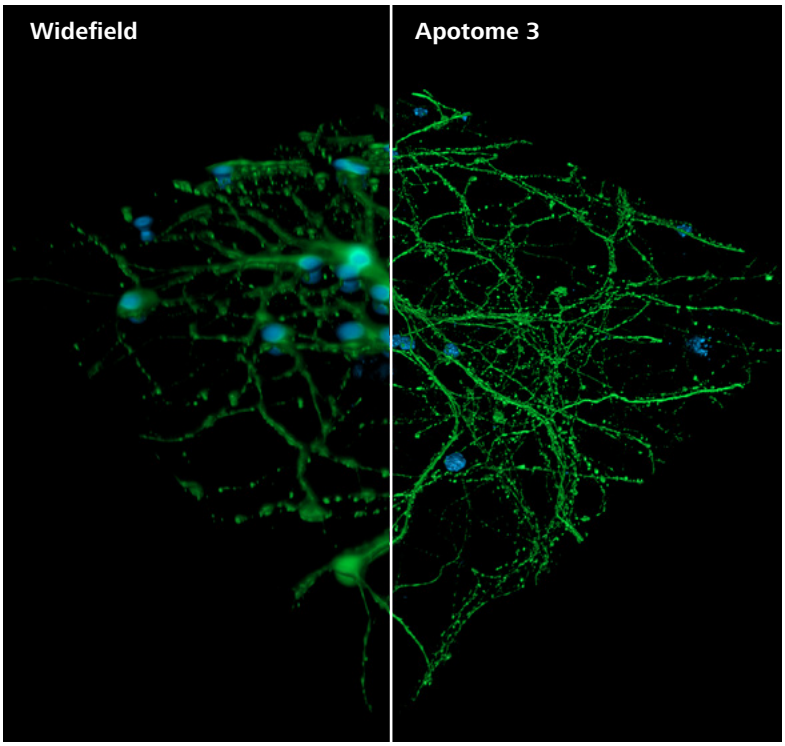


Streptosyllis websteri *worm having nuclei stained with DAPI, tubulin with Alexa 488 and Phalloidin with Alexa 568.*

3D renderings



Murine gastruloid 5µm-thick sections. ECM protein stained with Alexa 647, membrane protein with Alexa 568, transcription factor with Alexa 488, and nucleus with DAPI.
Courtesy of Prisca Liberali and Simon Suppinger, FMI.



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.

ZEISS Apotome 3

Quantitative Optical Sectioning using Structured Illumination

Flexible Choice of Stands:

Microscope

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

Objectives

Recommended objective classes with the highest level of image quality:

- C-Apochromat
- Plan-Apochromat
- EC Plan-Neofluar

Illumination

- Colibri 5 and 7 (LED)
- Xylis LED (white light LED)
- HBO (mercury vapor lamp)
- HXP 120 C (metal halide)

Cameras

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

Software

Recommended ZEN modules:

- Multi Channel, Z Stack
- Tiles & Positions (imaging with scanning table)
- Deconvolution (image processing)
- Direct Processing
- 3Dxl (rendering multidimensional image stacks)
- Image analysis modules such as Image analysis + Intellesis, BioApps and APEER

Suitable Applications

- Cell culture
- Live cell imaging
- Vibratome sections, histological samples, cleared tissue imaging
- Whole mounts

Reference

White Paper: How to get better images from Widefield microscopes.

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