

Product Information  
Version 1.0

## ZEN Correlative Array Tomography

3D Correlative Light and Electron Microscopy For Serial Sections



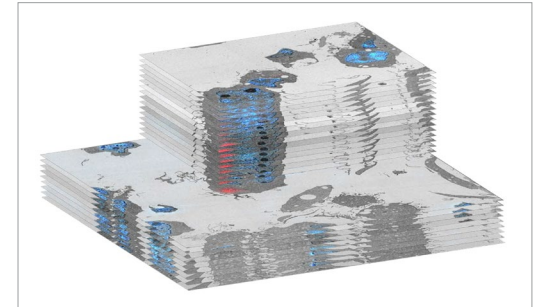
We make it visible.

# 3D Correlative Light and Electron Microscopy For Serial Sections

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With Array Tomography you use serial sections to reconstruct your sample volume. You cut your resin embedded tissue samples with an ultramicrotome into consecutive sections and image them. Precise automatic alignment of the section images allows 3D reconstruction of your sample. The section thickness determines your z-resolution.

With the unique software module ZEN Correlative Array Tomography you connect your light and electron microscope: after automated image acquisition in the light microscope (LM), you transfer the sample to your electron microscope (EM) where you find the same software tools. You automatically image hundreds of sections across length scales and combine them into one single correlative volume data set.



*Schematic representation of the information gain in 3D through combining the serial LM and SEM images resulting from a ZEN Correlative Array Tomography experiment.*



# Simpler. More Intelligent. More Integrated.

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## Perform Large Scale

### 3D Correlative Microscopy

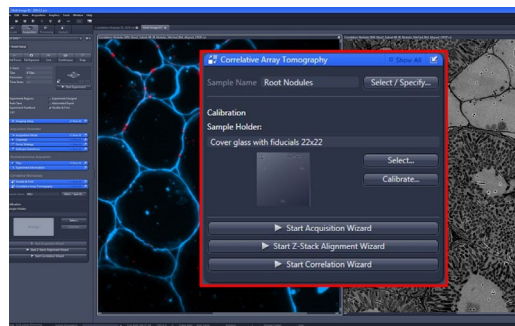
Correlative imaging of a large number of serial sections has never been easier: you simply outline one of the sections in your ribbon and ZEN Correlative Array Tomography detects and marks all other sections automatically. Then you outline the region of interest (ROI) in any of your sections. The software suggests optimal tiling set up and automatically transfers the ROI to all other sections.

## Enjoy Wizard-guided Workflows

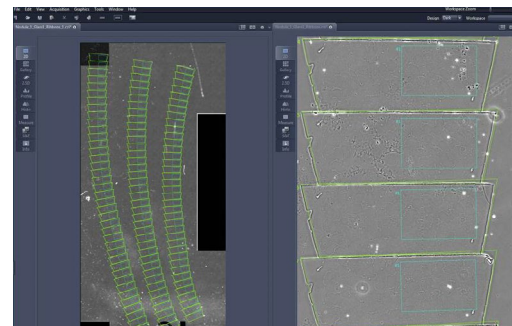
ZEN Correlative Array Tomography offers the same workflow environment at the light and electron microscope adapted to the requirements of the respective microscope. You always work in the same familiar user interface. Let ZEN software wizards guide you step-by-step through the process of imaging hundreds of serial sections on your light microscope. Then transfer your sample to your electron microscope and load the LM image. ZEN Correlative Array Tomography will provide all necessary information to seamlessly start imaging the very same regions with nano-meter resolution.

## Reconstruct Your Correlative 3D Data Set

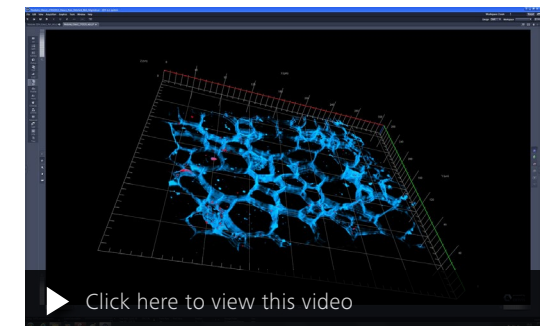
Use ZEN Correlative Array Tomography to stitch, align and reconstruct images from your light and electron microscope into 3D stacks. Then combine corresponding LM and EM data sets into one correlative 3D volume. You get an improved z-resolution based on the section thickness and not on optical constraints. Investigate the finest details in your data from light- and electron microscopy – all in one software.



The Correlative Array Tomography module in ZEN uses a series of wizards that guide you through calibration, automatic section detection and image acquisition of the selected regions in the LM and SEM. Afterwards you can start the image processing wizards to stitch, align and correlate your data sets.



ZEN Correlative Array Tomography user interface shows the detected serial sections (green frame) after the automatic section detection process including the defined regions of interest (blue frame).



3D reconstruction of serial sections from root nodules with the distribution of plasmodesmata. Cell wall: blue (Calcofluor white); Plasmodesmata: red (anti- $\beta$ -Glucan). The stack can be overlaid with the SEM data to investigate the relationship of bacteroid infection and distribution of plasmodesmata.

# Your Insight into the Technology Behind It

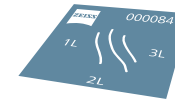
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## ZEN Correlative Array Tomography Workflow

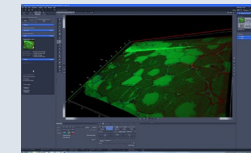
1. Cutting of serial sections with an ultramicrotome



2. Serial sections mounted on a cover glass

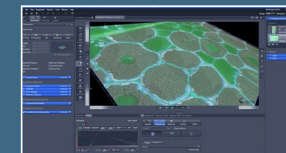
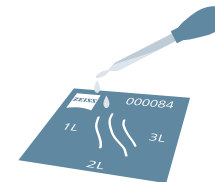


3. Image acquisition with a light microscope using the ZEN Correlative Array Tomography software



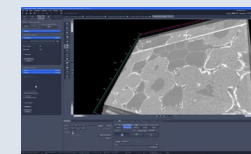
3D reconstruction of a LM z-stack

4. [Optional step]: Further sample preparation steps



Reconstruction of a correlative 3D data set

5. Image acquisition with a scanning electron microscope using the ZEN Correlative Array Tomography software



3D reconstruction of a SEM z-stack

# Tailored Precisely to Your Applications

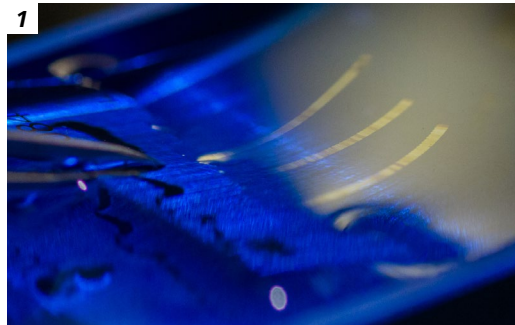
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Typical Applications, Typical Samples	Task	ZEN Correlative Array Tomography Offers
<p><b>Life Science</b></p>	<p>You prepare serial sections of resin embedded samples on glass cover slips. Perform fluorescence labelling before embedding and cutting of the sample and then preserve it during resin embedding. Or, perform fluorescent immunolabeling after cutting. Use cover glasses with fiducials from ZEISS for the highest flexibility in sample preparation for LM and EM.</p> <p>Cut your resin embedded sample with your ultramicrotome to stabilize the sample carrier during the deposition of the serial sections and mount them either on a cover glass or on a wafer.</p> <p>Data sets may include sections several mm in size, with hundreds of sections on a solid substrate such as a cover slip or wafer.</p>	<p>ZEN Correlative Array Tomography guides you through the complete workflow from light microscope to electron microscope and vice versa using several wizard systems to generate overview images, detect your sections and acquire your data.</p> <p>2D fluorescence images show the distribution of fluorescent-labeled proteins or cell components within a tissue. Images from the EM display the ultrastructural details with high resolution. Together – images from the LM and the SEM – reveal the distribution of labeled cell components in an ultrastructural context.</p> <p>With ZEN Correlative Array Tomography you reconstruct and combine both light- and electron microscopy images into one correlative 3D data set and visualize the data set with the 3D viewer module of ZEN imaging software. In addition you can export acquired images to render 3D visualizations using commercially available software and investigate the 3D ultrastructure of your tissue sample.</p> <p>The CAT workflow speeds up the image acquisition, the alignment and the correlation.</p>

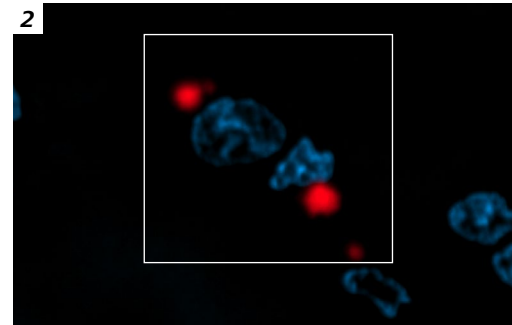
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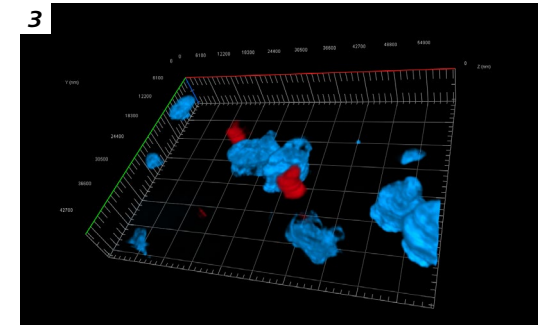
## From Serial Sections to Your Correlative 3D Data Set



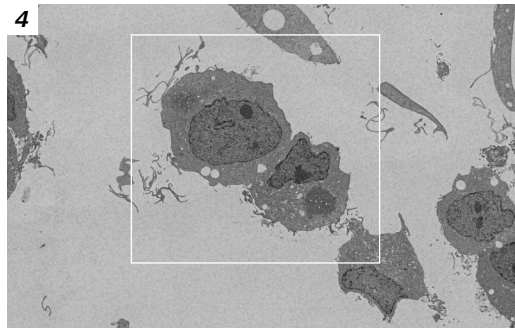
Collecting ribbons of ultrathin serial sections on a cover slip.



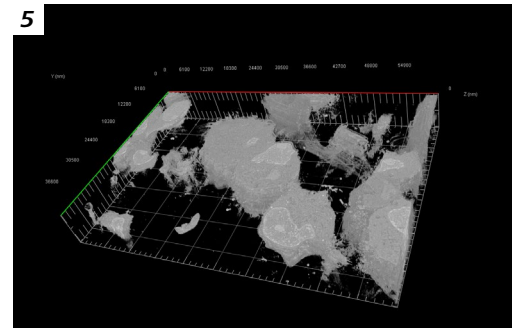
Fluorescence image of serial sections for investigation of huntingtin protein plaque formation in macrophages. DNA: blue (DAPI), huntingtin protein: red (Alexa Fluor 647).



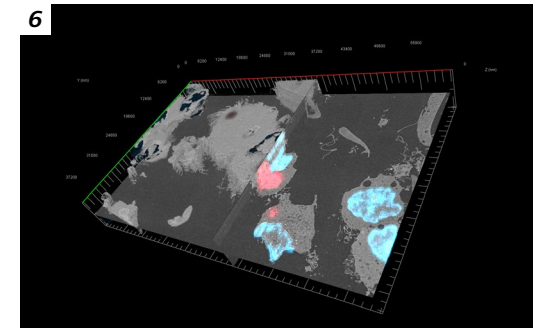
3D data set of the fluorescence images.



Corresponding SEM image showing ultrastructural information of the plaque structure and macrophage morphology.



3D data set of the SEM images.



Correlation of the LM z-stack with the SEM z-stack. The distribution of the huntingtin plaques and the location of the nucleus is clearly visible in 3D.

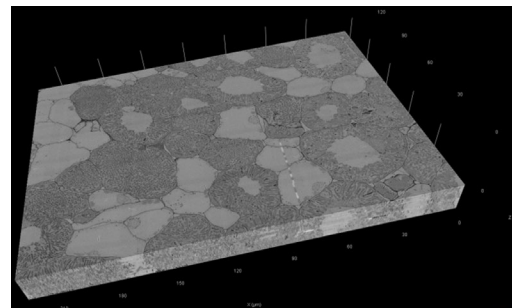
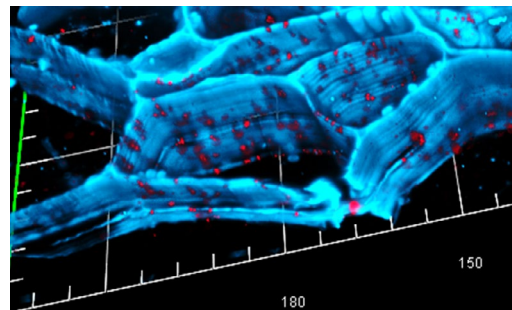
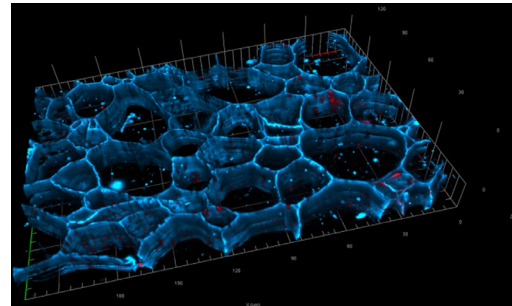


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## Investigation of the Symbiotic Relationship of Plants and Bacteria in Root Nodules

Correlative 3D images show the distribution and orientation of plasmodesmata within the cell walls of root nodules influenced by symbiotic nitrogen-fixing bacteria. The sample was embedded in Epon and cut with an ultramicrotome. The serial sections were transferred on ITO-coated cover glasses by means of a micromanipulator. Cell walls were stained with Calcofluor White (blue) and the plasmodesmata were stained against Callose with Alexa Fluor 647. The sample was post-stained for imaging in the SEM.

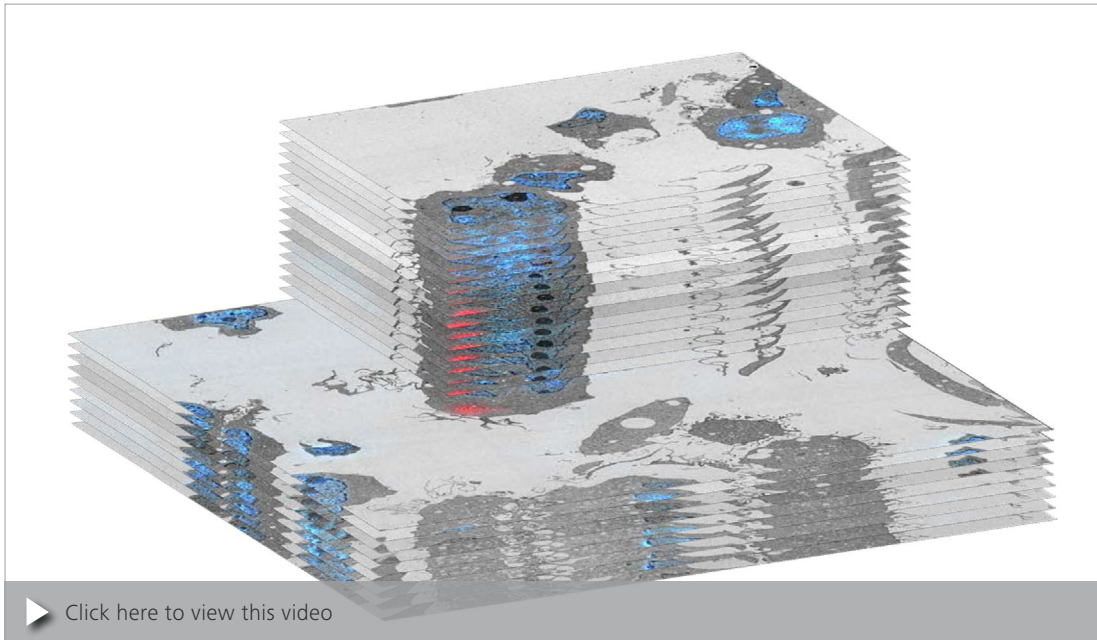


Courtesy of D. Sherrier, J. Caplan, and S. Modla,  
University of Delaware, USA.

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## Understanding Huntington's Disease and Inflammatory Processes



*Assembly of a correlative 3D data set. The data set shows a z-stack of macrophages expressing the protein huntingtin. Macrophages are used as a model system for the investigation of the Huntington's disease. An overexpression of huntingtin results in an aggregation of the protein clearly visible within the z-stack. An antibody was used against GFP-huntingtin to locate plaques of huntingtin within the cells (Alexa Fluor 647, red). The nucleus is shown in blue (Hoechst). Courtesy of J. Caplan, E. Kmiec and S. Modla, University of Delaware, USA.*



# Your Flexible Choice of Components

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## 1 Microscopes

### Light Microscopes

- Axio Imager.Z2
- Axio Observer.Z1

### Electron Microscopes

- GeminiSEM 300/500
- Sigma 300/500
- Merlin
- Merlin Compact
- Auriga
- Supra
- Ultra

## 2 Software

### Light Microscopes

- ZEN 2.1 (blue edition)
- ZEN module Correlative Array Tomography
- ZEN 3Dxl Viewer – powered by arivis®

### Electron Microscopes

- SmartSEM 5.09
- ZEN 2.1 SEM (Basis software)
- ZEN SEM Module Correlative Array Tomography

## 3 Accessories

- Specimen holder CorrMic Life Sciences for cover glasses 22 x 22 mm
- SEM Adapter for Specimen holder CorrMic Life Sciences
- ZEISS Cover Glasses with Fiducials

# Technical Specifications

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Feature	Specification
<b>Aligned Correlative Workflow Solution for LM and SEM</b>	<p>One software user interface</p> <p>Perfectly aligned ZEN software module for the light microscope and the scanning electron microscope allowing the identical operation on both systems; the software module is adapted to requirements of the system</p> <p>Intelligent Workflow Solution; both modules recognize the existing image data and the software reacts accordingly</p> <p>Wizard-based software solution consists of 4 wizards:           <ul style="list-style-type: none"> <li>■ Calibration Wizard</li> <li>■ Acquisition Wizard</li> <li>■ Alignment Wizard</li> <li>■ Correlation Wizard</li> </ul> </p>
<b>Image Characteristics</b>	Save image data as czi file, export functions available on both systems
<b>Repositioning Accuracy</b>	<p>&lt;25 µm (initial accuracy, depending on stage specification)</p> <p>&lt;5 µm (using software option for fine calibration)</p>
<b>Calibration</b>	<p>Manual or semi-automatic calibration based on three reference markers</p> <p>Definition of user-defined sample holders</p>
<b>Image Acquisition</b>	<p>Wizard-guided hierarchical image acquisition starting with an overview image up to detailed image acquisition for the defined regions of interest</p> <p>Automated section detection</p> <p>Automated transfer of a user-defined region of interest to all identified sections</p> <p>Designed for automated image acquisition and tiles imaging</p> <p>Re-acquisition of single tiles and regions of interest</p> <p>Definition of multiple regions of interest per section</p>
<b>Focus</b>	Optimized Focus Strategy for ultrathin sections
<b>Experiment Settings</b>	<p>Experiment Settings are saved within the image on the LM and SEM</p> <p>LM: Experiment Settings are saved and can be re-used</p>

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Feature	Specification
<b>Autosave</b>	Images are automatically saved
<b>Data Management</b>	Sample information Logical name allocation
<b>Alignment of z-stacks</b>	Image Review and substitution of single images Automatic alignment of LM and EM 2D images Manual Correction possible
<b>Correlation of z-stacks</b>	Automatic Correlation by means of a 4-Point Correlation Manual Correction possible
<b>Image Processing</b>	Stitching functionality, Brightness and Contrast adjustment All processing functions delivered by ZEN
<b>Accessories</b>	Specimen holder CorrMic Life Sciences for cover glasses 22 x 22 mm SEM Adapter for Specimen holder CorrMic Life Sciences ZEISS Cover Glasses with Fiducials ZEN 3Dxl Viewer – powered by arivis®

# 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.

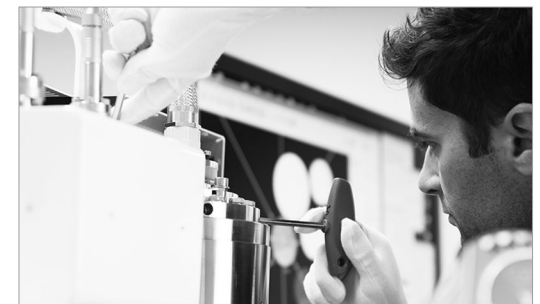
## **Repair. Maintain. Optimize.**

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.*

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