Understanding the fundamentals of life

ZEISS LSM 900 with Airyscan 2
Your Compact Confocal for Gentle Multiplex Imaging and Smart Analysis

zeiss.com/lsm900
What are you looking for in confocal imaging? Whatever your scientific question, you want to start with the best possible image quality and that means crisp contrast and the best resolution. You also want the highest sensitivity for gently imaging your living or fixed samples without bleaching. Your LSM 900 with Airyscan 2 has all this and more. You image with 4–8× more signal-to-noise ratio (SNR) and with a resolution down to 120 nm. You also get the highest frame rates: the Multiplex mode for Airyscan 2 adds smart detection schemes for parallel pixel acquisition. You can observe dynamic processes in living specimens gently – without sacrificing image quality. Plus, your LSM 900 has a genuinely small footprint, concentrating on the essence of a confocal and omitting needless complexity. It fits easily into your lab or imaging facility – and it’s easy to use, too.

**Your Compact Confocal for Fast and Gentle Multiplex Imaging**

See for yourself how the new Multiplex mode for Airyscan 2 gives you better data faster than ever before. Book a hands-on demonstration in one of our ZEISS Microscopy Labs now.

>> [www.zeiss.com/lsm900](http://www.zeiss.com/lsm900)

*Neurospheres, multi-color label with Dapi (blue), Tubulin-Cy2 (green), DCX-Cy5 (red). Acquired with GaAsP detectors on ZEISS LSM 900. Sample courtesy of H. Braun, LSM Bioanalytik GmbH, Magdeburg, Germany.*
Get Better Data – Faster
Combine the excellent image quality of your LSM 900 with the new Multiplex mode for Airyscan 2 to get more information in less time than ever before. You can now employ smart detection schemes to image your challenging three-dimensional samples with the highest frame rates and super-resolution. The speed and gentleness of the sensitive Airyscan area detector complement the compact point scanning confocal and allow you to image your most demanding samples with 4 – 8× more SNR.

Increase Your Productivity
Your LSM 900 with Airyscan 2 is not just compact – it’s also very easy to use. Setup is simple with ZEN microscopy software, even for complex confocal live cell imaging experiments. A wealth of software helpers lighten the load and make sure you get reproducible results in the shortest possible time. AI Sample Finder helps you in quickly finding your regions of interest, leading you to faster results. Smart Setup supports you in applying best imaging settings for your fluorescent labels. Direct Processing allows parallel acquisition and data processing. ZEN Connect keeps you on top of things at all times, both during imaging and later when sharing the whole story of your experiment. It’s easy to overlay and organize images from any source.

A Small Footprint for Greater Image Quality
Your LSM 900 is packed with innovative and clever solutions for producing the best quality in confocal live cell imaging. The elegant beam path is designed for high spectral flexibility, with each single component optimized for the highest sensitivity and contrast. Given its small footprint and reduced complexity, you’ll save valuable lab space, minimize the time needed for user-training and reduce the cost of ownership – this is especially good news for imaging facilities.

In Brief
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The System
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Service

Hela cells stained for DNA (blue, Hoechst 44332), microtubules (green, anti-tubulin, alpaca anti-mouse-alexa 488) and F-actin (red, phallaidin-Abberior STAR Red). Acquired with Multiplex mode for ZEISS Airyscan. Sample: courtesy of A. Politi, J. Jakobi and P. Lenart, MPI for Biophysical chemistry, Göttingen, Germany.

Cell division of LLC-PK1 cells, alpha-tubulin (mEmerald, magenta) and H2B (mCherry, green). With the new Multiplex mode for ZEISS Airyscan a Z-stack of 52 slices was captured every 40 seconds for a total of 40 minutes.
Your Insight into the Technology Behind It

The Airyscan Principle
Classic confocal laser scanning microscopes use point illumination to scan the sample sequentially. The microscope optics transform each point to an extended Airy disk (Airy pattern). A pinhole then spatially limits this Airy disk to block out-of-focus light from reaching the detector. Closing the pinhole gives higher resolution, but at the price of detecting fewer photons – and these photons cannot be brought back by e.g. deconvolution.

Airyscan 2 is an area detector with 32 concentrically arranged detection elements. This allows you to acquire most of the Airy disk all at once. The confocal pinhole itself remains open and does not block light, thus more photons are collected. This produces much greater light efficiency while imaging. Airyscan 2 gives you a unique combination of gentle super-resolution imaging and high sensitivity.

For further information on the Airyscan principle please refer to:
https://zeiss.ly/airyscan-principle

Comparing the field of view you can image at super-resolution in the same time using Airyscan SR (bottom) and Multiplex mode (top). COS 7 cells with labelled microtubules (alpha-tubulin 488, green) and actin (phalloidin 568, red).
Your Insight into the Technology Behind It

The New Multiplex mode for ZEISS Airyscan 2

Do you want to image large fields of view and whole sample volumes in the shortest time possible? And do you want to image with superb image quality at the same time? The LSM 9 family with Airyscan 2 from ZEISS now gives you more options to fit imaging speeds and resolution to your experimental needs. You combine an area detector with smart illumination and readout schemes, which let you choose from different parallelization options.

The new Multiplex mode uses knowledge about the shape of the excitation laser spot and the location of single area detector elements to extract more spatial information, even during parallel pixel readout. This allows to take bigger steps when sweeping the excitation laser over the field of view, improving your achievable acquisition speeds. In fact, the high amount of spatial information captured in the pinhole plane allows to reconstruct a final image with better resolution than the acquisition sampling. Airyscan 2 in Multiplex mode can acquire up to four super-resolution image lines with high SNR in a single sweep.

<table>
<thead>
<tr>
<th>ZEISS LSM 900 with Airyscan 2</th>
<th>Airyscan SR</th>
<th>Multiplex SR-2Y</th>
<th>Multiplex SR-4Y</th>
<th>Multiplex CO-2Y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parallelization</strong></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>120/120</td>
<td>140/140</td>
<td>140/140</td>
<td>180/180</td>
</tr>
<tr>
<td><strong>FPS at 512 × 512 pixels</strong></td>
<td>4</td>
<td>8.4</td>
<td>18.9</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>FPS at max FOV</strong></td>
<td>0.4 (Zoom 1.3)</td>
<td>0.8 (Zoom 1.3)</td>
<td>3.5 (Zoom 1.3)</td>
<td>3.5 (Zoom 1.3)</td>
</tr>
<tr>
<td><strong>Antibody labeling, fine structures</strong></td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Antibody labeling, tiling</strong></td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Live cell imaging</strong></td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

For each illumination position, Airyscan SR mode generates one superresolution image pixel. The spatial information provided by Airyscan 2 in the Multiplex modes SR-2Y/CO-2Y and SR-4Y allows to scan 2 or even 4 super-resolution image lines in a single sweep.
Your Insight into the Technology Behind It

A Streamlined Light Path with Surprising Flexibility
The compact light path with a minimum of optical elements is designed for highest efficiency. Fluorescence emission light travels through the main dichroic beam splitter with its outstanding laser suppression to deliver supreme contrast. Up to two patented variable beam splitter dichroics (VSDs) divert the spectral part of the light. You can define up to three detectors (multalkali, GaAsP or Airyscan 2).

Schematic beam path of ZEISS LSM 900.
Your Insight into the Technology Behind It

GaAsP Detectors – Your Choice for Highest Sensitivity
GaAsP PMTs – that is, gallium arsenide phosphide photomultiplier tubes – display high light collection efficiencies over a broad spectral range. Their low dark noise levels also render them the ideal tool for detecting faint signals. Enjoy outstanding image quality based on a superb signal-to-noise ratio (SNR). You might use this gain in SNR to increase productivity by achieving faster scan speeds while preserving excellent image quality. Or take advantage of the low laser powers needed in live cell imaging applications to avoid photobleaching and phototoxicity as much as possible. Or simply detect faint signals in low expressing cells. All that, and you can do it with up to three spectral channels simultaneously.

Benefit from up to Three Confocal Detectors
Investigations into localization and interaction of proteins often require multiple fluorescent labels with overlapping emission spectra. Now you can image up to four dyes, crosstalk free by multi-tracking. Or even more by performing a Lambda scan with spectral unmixing.

Typical Spectral Quantum Efficiency (QE) of multi-alkali (MA-) PMT and GaAsP-PMT detectors.

Germinal vesicle state mouse oocyte, labelled for actin (green, Phalloidin-Alexa Fluor 488), microtubules (white), Lamin A/C (magenta) and DNA (Hoechst). Sample: courtesy of K. Harasimov, MPI for Biophysical Chemistry, Goettingen, Germany.
Your Insight into the Technology Behind It

**AI Sample Finder: Automated sample identification for efficient imaging**

Microscopes are becoming increasingly automated. For sample placement, however, microscope parts such as the condenser arm often have to be moved manually. Focus adjustment and identification of the relevant areas on the sample carrier require additional manual steps.

The AI Sample Finder automates this sequence, eliminating time-consuming manual adjustments and reducing the time to image from minutes to just seconds.

You can access all sample areas directly which allows you starting your experiment faster than ever. The AI Sample Finder greatly improves productivity as you can easily image only those regions containing sample not overlooking potentially important areas.

- After you placed the sample on the loading position, the AI Sample Finder automatically moves it to the objective.
- Without the need of manual sample positioning or focusing, an overview image for fast and convenient navigation is taken within seconds. Composite darkfield illumination creates a high-contrast image even for very low-contrast samples.
- Intelligent routines automatically identify your sample carrier, regardless if you use a petri dish, a chamber slide, or a multiwell plate. Carrier properties are automatically transferred to the software, eliminating manual settings.
- Your samples are reliably identified. Deep Learning algorithms precisely detect even unusual sample regions. You can navigate and access all sample areas directly which allows you starting your experiment faster than ever.
Expand Your Possibilities

Acquire Reproducible Data with Ease

With all its various aspects and workflows, your research leaves you with no time to waste. That’s why ZEN microscopy software was created—to make your confocal imaging both efficient and enjoyable. ZEN – ZEISS Efficient Navigation - is the only user interface you will ever see on all imaging systems from ZEISS. This familiar and easy-to-learn interface will help you get reproducible results in the shortest possible time.

Use Smart Setup to select your dyes and ZEN will automatically apply all necessary settings for all LSM imaging modalities. The integrated database with spectral data for more than 500 dyes helps you make an informed decision about your imaging options. You can always save imaging configurations or even whole experiments to reproduce settings quickly. The Reuse function allows you to extract and load imaging settings from the existing images. You will be amazed how easy imaging becomes when the AI Sample Finder automatically detects the sample carrier, adjusts the focus, and finds your sample regions relevant for your experiment. It takes less time to illuminate your sample and leaves you more of the precious time you’ve booked on the system for imaging. In addition, you can use the overview image to document all steps of your experiment and load it in ZEN Connect to combine with other multimodal data or aspects of your sample.

Sometimes your scientific questions will require complex acquisition strategies. Statistical analysis might call for repetitive imaging of a large number of samples with the same or even differing imaging conditions. Experiment Designer is a powerful yet easy-to-use module that images multiple regions with all imaging modalities of your LSM 900.

It gives you access to a number of hardware and software options which will always keep your sample in focus, even during the most demanding long-term time-lapse experiments. You can even view and save your valuable data during acquisition sessions to assess, analyze and react immediately.

Expand Your Possibilities

1. Repetitive manipulation experiments
2. Multiposition Z-stack acquisition with individual heights
3. Screening of multiple samples
4. Heterogeneous time lapse imaging

With the ZEN software module Experiment Designer you can set up complex imaging routines consisting of freely defined and repeatable experiment blocks with multi-position tile scans of multichannel Z-stacks.
Expand Your Possibilities

See More Details
Sometimes you need to see and assess your multi-modal images during acquisition in order to plan your next steps. ZEN gives you multiple options. You can sit at your connected computer to start the new Direct Processing function for processing your Airyscan images during acquisition.

However, confocal imaging is only one part of the big picture, and you may need data from additional imaging modalities to complement the view on your sample. ZEN Connect can bring information from all your experiments together. Keep the context of your data by collecting all images of one experiment session in a single project in which you can combine overview and detailed high-resolution images, all perfectly aligned. Once you have created a project, you can always add and align content from any other imaging source, be it ZEISS, non-ZEISS or even sketches and analysis graphs. You will stay on top of things at all times – both during your experiments and months or years later. Your ZEN Connect projects keep all associated datasets together. It’s never been easier to share results and collaborate with others as a team.

The powerful integrated 3Dxl Viewer, powered by arivis®, is optimized to render the large 3D and 4D image data you have acquired with your fast new LSM 900. You can create impressive renderings and movies for meetings and conferences. After all, a good picture can say more than a thousand words.

Section of a Thy1-YFP mouse brain. Thy-1 (green) is involved in the communication of cells in the nervous system. Overview image (A) acquired on ZEISS Axio Scan.Z1. Images B and C show enlarged ROIs imaged on ZEISS LSM with Airyscan. B) The neuronal network is clearly visible. The depth of the Z-stack is color-coded. C) shows a single neuron. Sample: courtesy of R. Hill, Yale University, New Haven, CT, USA.
Expand Your Possibilities

Get More Data from Your Sample
The real value of microscopy images is in the data they provide. The CZI file format of ZEN microscopy software makes sure that all important metadata of your experiments are safely stored and can be accessed openly for cross-platform data exchange. ZEN provides numerous analysis tools to extract all kinds of information from your images.

Building analysis workflows that adapt to specific applications is not an easy task. It requires knowledge of image processing and the ability to assemble a series of image operations. ZEN addresses this challenge with the BioApps modules for efficient image analysis. Each module is optimized for one type of application, e.g., cell counting or confluency measurement, with tailored segmentation settings and streamlined data presentation. If your applications require customized workflows, the wizard-based ZEN Image Analysis module will guide you step by step to create your unique measurements.

Within an image analysis workflow, segmentation and object classification are two of the most challenging steps. ZEN Intellesis uses the latest machine learning algorithms to make these steps easier and more accurate, also allowing you to execute training on your own data sets. You can integrate the individual models seamlessly into your ZEN image analysis workflow.

Click here to view this video

ZEN microscopy software integrates all steps from your sample to reproducible data for publication.

ZEN Intellesis: Use the power of machine learning to easily segment your images.

ZEN BioApps: From beautiful images to valuable data – analyze your images efficiently.
Expand Your Possibilities

OAD is Your Interface to ZEN Microscopy Software

- Use Python scripts to customize and automate your workflows.
- Integrate external image analysis applications into your workflows.
- Exchange image data with external programs like ImageJ, Fiji, MATLAB, KNIME or Python.
- Use feedback for smart experiments.
- Get more reliable data in less time.
  It’s your choice.

OAD enables the analysis of data acquired with ZEN microscopy software by other programs like ImageJ. Transfer your results back to ZEN for further analysis and display.

The result of overview scan using low magnification (top panel) was used to automatically detect the brain slices via image analysis. The results (XYZ position and the height/width of detected objects) were used in an automated subsequent scan using high NA objectives, where the system carried out an individual tile scan for every detected object in a complete automated fashion without any additional user interaction. Sample: courtesy of P. Grigaravicius, FLI – Leibniz Institute on Aging, Jena, Germany.
Expand Your Possibilities

As your needs grow, LSM 900 grows with you, forming the basis for a number of enhancements. Like every system from ZEISS, LSM 900 comes with open interfaces and a modular architecture to guarantee the seamless interaction of all components, now and in the future.

- Combine your ZEISS Axio Observer 7 with integrated incubation modules to create the perfect environment for long-term live cell imaging with stable temperature conditions.
- Add a choice of sensitive ZEISS Axiocams to your ZEISS LSM 900. It's very easy to acquire overview images for your multiposition experiments or to perform light efficient widefield imaging.
- Definite Focus 3 stabilizes the focal position of your sample compensating Z-drift. You can now perform long-term experiments that can last for multiple days.
- Z piezo stage and a leveling insert guarantee the precision needed for super-resolution applications using ZEISS Airyscan 2.
- ZEN Connect 2D and 3D Add-on is your gateway to correlative light and electron microscopy (CLEM). Combine the specificity of functional fluorescence imaging with ultrastructural information.
- Enhance your microscope with ZEISS Colibri 7. This flexible and efficient LED light source allows to screen and image your delicate fluorescent samples very gently. You profit from stable illumination and extremely long lamp life.
Expand Your Possibilities

ZEISS Predictive Service
Maximizes System Uptime

Once connected to your network and activated, this advanced technology will automatically track the health status of your instrument and collect system log files in the background to improve remote diagnosis.

Relevant technical data such as operating hours, cycle counts or voltages are periodically monitored via a secure connection to our data center. The ZEISS Predictive Service application evaluates the performance of your microscope as system data can be received and analyzed. Our support engineers will diagnose any issues by analyzing data on the Enterprise Server — remotely and without interruption to your operation.

- **Maintain highest system availability**
  Increase your uptime through close monitoring of the system’s condition as remote support can often provide immediate solutions

- **Data security**
  Ensure highest data security standards using well established technologies like PTC Thingworx and Microsoft Azure Cloud. No personal or image data is uploaded, only machine data

- **Fast and competent support**
  Use secure remote desktop sharing to easily get an expert connected

- **Optimum instrument performance**
  As the status of your system is monitored, necessary actions can be planned before they become urgent
Tailored Precisely to Your Applications

<table>
<thead>
<tr>
<th>Typical Applications, Typical Samples</th>
<th>Task</th>
<th>ZEISS LSM 900 Offers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody stained tissue slices</td>
<td>Document morphological relations of structures with a resolution of 120 nm (x/y) / 350 nm (z) at 488 nm excitation</td>
<td>Airyscan 2 with SR or Multiplex mode</td>
</tr>
<tr>
<td></td>
<td>Image large field of views and conduct tiling experiments for large specimen</td>
<td></td>
</tr>
<tr>
<td>Live cell culture</td>
<td>Study the motility of vesicles and organelles</td>
<td>Up to 8 frames per second confocal time lapse imaging</td>
</tr>
<tr>
<td></td>
<td>Screen and document cells expressing the desired fluorescent label in response to pharmacological treatment</td>
<td>Or use Airyscan 2 in Multiplex mode for up to 18 frames per second.</td>
</tr>
<tr>
<td>Live cell culture with two labels</td>
<td>Study the motility of subcellular structures</td>
<td>Airyscan 2 with GaAsP detector and Multiplex mode for time lapse imaging in 2D or 3D at up to 9 frames per second</td>
</tr>
<tr>
<td></td>
<td>Explore the interaction of two proteins exploiting the Förster Resonance Energy Transfer (FRET) effect</td>
<td>FRET analysis tool</td>
</tr>
<tr>
<td>Live cells with multiple labels</td>
<td>Image over a long time in an automated way</td>
<td>Experiment Designer software to automatically record complex multi-color experiment. Combine different acquisition modes, e.g. spectral imaging, superresolution imaging. Combine the experiment in ZEN Connect</td>
</tr>
<tr>
<td>Live or fixed cells with multiple labels and overlapping emission signals</td>
<td>Examine the interplay of multiple proteins</td>
<td>Parallel acquisition of all signals with three spectral channels and linear unmixing</td>
</tr>
<tr>
<td>Cellular structures with weak labels</td>
<td>Image subcellular structures at physiological expression levels</td>
<td>LSM 900 with GaAsP detector or Airyscan 2</td>
</tr>
<tr>
<td>Study molecular dynamics</td>
<td>Photomanipulation</td>
<td>FRAP analysis tool, classical timed bleaching or flexible interactive bleaching strategies</td>
</tr>
<tr>
<td>Plant roots</td>
<td>Follow the changes of subcellular structures over time with high resolution</td>
<td>Airyscan 2 with GaAsP detector for 140 nm super-resolution imaging beyond 40 μm deep into tissue with up to 18 frames per second in SR-4Y mode (512 x 512 pixel)</td>
</tr>
<tr>
<td>Model organisms, e.g. Zebrafish, Drosophila or C. elegans, Arabidopsis</td>
<td>See fine details of the organization and dynamics of endogeneously expressed FP proteins</td>
<td>Airyscan 2 with GaAsP detector for super resolution imaging beyond 40 μm deep into tissue with a 40x/1.0, or a 20x/1.0 water dipping objective available for confocal imaging with LSM 900 on Axio Examiner.Z1</td>
</tr>
<tr>
<td>Cleared samples</td>
<td>Image whole organs or entire organisms</td>
<td>Specialized objectives with long working distance and optimized for specific refractive indices are available for LSM 900 on Axio Examiner.Z1</td>
</tr>
</tbody>
</table>
The micrograph shows a Lilium auratum pollen grain, acquired with Airyscan 2 in Multiplex mode. Image courtesy of Jan Michels, Zoological Institute, Kiel University.
Nuclei of living HeLa Cells were labelled with 5’-610CP-Hoechst (Chem.Sci. 2019, 10, 1962–1970). The dye is added to the cell culture media in a defined concentration. The bleaching experiment (FRAP) confirms that the dye needs about 15 minutes to efficiently label DNA. The time series is recorded for 13.5 minutes with 1 frame per second, with the bleaching event in the labeled region after the first 10 frames. Sample courtesy of P. Lenart, MPI for Biophysical Chemistry, Göttingen, Germany.
Fixed starlet sea anemone (*Nematostella vectensis*) stained with Hoechst (nuclei) and Phalloidin (actin). Side view imaged with LSM 900 on Celldiscoverer 7, seamlessly combining camera based phase gradient contrast mode (top) and high sensitivity mode with Airyscan 2 (bottom). Maximum intensity projection of 19 z-planes.

Video: Top view of a young animal, showing mouth and four tentacle buds. Maximum intensity projection of 69 z planes imaged with Airyscan 2 Multiplex. Images were acquired using the water immersion objective with a total magnification of 25× and a numerical aperture of 1.2.

Fine image details and high signal to noise ratio can clearly be seen on the insert in the top right image, showing an enlarged view of a tentacle area.

Sample courtesy of A. Stokkermans, Ikmi Group, EMBL, Heidelberg, Germany.
Lateral line primordium migration and deposition of immature neuromasts in a Zebrafish embryo (*Danio rerio*). Animals were anesthetized and embedded using low concentrated agarose in a glass bottom petri dish.

Using Celldiscoverer 7 with integrated LSM 900 and Airyscan 2 allows to combine the best imaging modes seamlessly. Quick and easy sample navigation (top) is done by camera-based imaging of Phase Gradient Contrast and fluorescence.

Subsequent high resolution imaging with Airyscan 2 in Multiplex mode was done on individual positions identified in the widefield image (white boxes).

A) Maximum intensity projections of an immature neuromast (127 z-planes).

B) Maximum intensity projections of the lateral line primordium tip migrating through the animal (155 z-planes).

Green: LYN-eGFP (membranes); Red: tagRFP-T-UTRC (actin).

The gentle and fast image acquisition that is inherent to the Airyscan 2 Multiplex mode is very beneficial for this kind of application. The animal is unperturbed by the imaging while images with a very high signal-to-noise ratio as well as level of detail can be acquired at the same time.

Sample courtesy of J. Hartmann and D. Gilmour, EMBL, Heidelberg, Germany
ZEISS LSM 900 at Work

Human lung epithelial cell line A549 stained with MitoTracker® Orange (mitochondria) and SIR-DNA (nuclei).

With Celldiscoverer 7 and LSM 900 you seamlessly combine two imaging modes. Fluorescent channels were acquired in confocal mode using highly sensitive GaAsP detectors while the Phase Gradient Contrast was acquired with a camera.

A timelapse of 2.5 h was acquired using a 40× magnification with a numerical aperture of 0.95.
Correlative Cryo Microscopy: Image the Near-to-native State

Spindle pole bodies are difficult to localize within yeast cells. They are small and rarely occurring structures. ZEISS Correlative Cryo Workflow lets you precisely identify and image such cellular structures in the near-to-native state. The LSM with the Airyscan detector makes the identification of these structures even easier so further details can be imaged. All images — from a large overview of the entire cell to high-resolution images of these tiny structures — are organized in a ZEN Connect project, providing all data needed to re-locate these cellular structures in the FIB-SEM.

Using ZEISS Crossbeam, TEM lamella of the identified regions can be prepared for cryo electron tomography. Volume imaging is possible as well. Furthermore, the workflow solution allows you to reconnect all data after image acquisition. Images from the Crossbeam or tomograms from the TEM can be combined with the LSM data and can be rendered in three-dimensional context.

Learn more about ZEISS Correlative Cryo Workflow: www.zeiss.com/cryo

Yeast cells labeled with NUP (nuclear pore complex)-GFP and CNM67-tdTomato. Sample and tomogram courtesy of M. Pilhofer, ETH Zürich, Switzerland

a) ZEN Connect movie shows the overlay of an LM and EM dataset — from the grid overview to the region of interest identified for further TEM tomography
b) Early state of the milling process: Lamella is prepared around the marked region which was identified at the LSM
c) FIB image of the prepared lamella; lamella thickness: 230 nm
d) 3D overlay of the reconstructed and segmented tomogram with LSM dataset (Spindle pole body is false-colored in cyan); nuclear membrane and microtubules were segmented using IMOD.
e) Segmented and reconstructed tomogram
Your Flexible Choice of Components

1 Microscope
- Inverted stands: Axio Observer 7, Celldiscoverer 7
- AI Sample Finder for Axio Observer
- Camera port
- Manual or motorized stages
- Incubation solutions
- Fast Z piezo inserts (for inverted stands)
- Definite Focus

2 Objectives
- C-Apochromat, C Plan-Apochromat
- Plan-Apochromat
- LD LCI Plan-Apochromat
- EC Plan-Neofluar
- W Plan-Apochromat, Clr Plan-Apochromat, Clr Plan-Neofluar

3 Illumination
- Diode lasers: 405, 488, 561 and 640 nm

4 Detection
- 2 channel Gallium Arsenide Phosphid (GaAsP) PMT or 2 channel multialkali (MA) PMT
- 1 additional GaAsP PMT, MA PMT or 40x / 63x / 100x Airyscan 2 detector with Multiplex mode
- Electronically switchable illumination and detection module (ESID) or transmitted light detector (T-PMT).

5 Software
- ZEN microscopy software, highlighted modules: Tiles & Positions, Experiment Designer, Sample Navigator, FRAP, FRET, Deconvolution, 3Dxl Viewer – powered by aris®
ZEISS LSM 900: System Overview

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Technical Specifications

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## Technical Specifications

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<th>Width (cm)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small actively and passively damped system table</td>
<td>90</td>
<td>75</td>
<td>83</td>
<td>130</td>
</tr>
<tr>
<td>Large actively damped system table (incl. corner pieces)</td>
<td>120 (129)</td>
<td>90 (99)</td>
<td>87</td>
<td>180</td>
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<tr>
<td>Vibraplate for Axio Imager (consists of three pedestals)</td>
<td>32</td>
<td>30</td>
<td>4.5</td>
<td>1.5</td>
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<td>Vibraplate for Axio Observer</td>
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<td>4.5</td>
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<tr>
<td>Scanning Module LSM 900</td>
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<td>25.5</td>
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<tr>
<td>Axio Imager.Z2; Axio Imager.M2</td>
<td>56</td>
<td>39</td>
<td>70</td>
<td>20</td>
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<tr>
<td>Axio Examiner.Z1</td>
<td>70</td>
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<td>Axio Observer 7</td>
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<td>65</td>
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<td>Component rack</td>
<td>55</td>
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<td>60</td>
<td>35</td>
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<tr>
<td>Laser module (LM)</td>
<td>40</td>
<td>25</td>
<td>14.5</td>
<td>10</td>
</tr>
<tr>
<td>Airyscan 2 (40x, 63x, 100x)</td>
<td>40</td>
<td>25</td>
<td>14.5</td>
<td>5</td>
</tr>
<tr>
<td>Power supply unit (PSU)</td>
<td>40</td>
<td>25</td>
<td>14.5</td>
<td>6</td>
</tr>
<tr>
<td>Fiber optic cable, VIS</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cables</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Microscopes

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverted: Axio Observer 7 with side port, AI Sample Finder (optional); Celldiscoverer 7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Z Drive</th>
<th>Smallest increment Axio Imager.Z2; Axio Observer 7: 10 nm; Axio Imager.M2, Axio Examiner: 25 nm; Z-Piezo stage available; Definite Focus 3 for Axio Observer 7</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>XY Stage (optional)</th>
<th>Motorized XY scanning stage, for Mark &amp; Find function (xy) as well as Tile Scan (Mosaic Scan); smallest increment of 0.25 µm (Axio Observer 7), 0.2 µm (Axio Imager.Z2), 0.25 µm (Axio Examiner.Z1)</th>
</tr>
</thead>
</table>
## Technical Specifications

### Scanning Module

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scanner</strong></td>
<td>Two independent, galvanometric scanning mirrors with ultrashort line and frame flyback.</td>
</tr>
<tr>
<td><strong>Scanning resolution</strong></td>
<td>32 × 1 to 6,144 × 6,144 pixels (Airyscan 2 max. 4,096 × 4,096 pixels), also for multiple channels, continuously adjustable (for each axis)</td>
</tr>
<tr>
<td><strong>Scanning speed</strong></td>
<td>At 512 × 512 pixels: confocal – up to 8 fps; Airyscan SR – up to 4 fps; Multiplex SR-2Y – 8.4 fps; Multiplex SR-4Y – 18.9 fps</td>
</tr>
<tr>
<td><strong>Scanning zoom</strong></td>
<td>0.45 × to 40 ×; continuously adjustable</td>
</tr>
<tr>
<td><strong>Scanning rotation</strong></td>
<td>Can be rotated freely (360°), adjustable in increments of 0.1°, freely adjustable xy offset</td>
</tr>
<tr>
<td><strong>Scanning field</strong></td>
<td>20 mm diagonal in the intermediate image plane, with full pupil illumination</td>
</tr>
<tr>
<td><strong>Pinhole</strong></td>
<td>Master pinhole with preset size and position; can be adjusted as desired for multitracking and short wavelengths (such as 405 nm); automatic alignment</td>
</tr>
<tr>
<td><strong>Beam path</strong></td>
<td>One major beam splitter for four laser lines (405, 488, 561 and 640 nm) at 10 degree with excellent laser line suppression. The 640 nm laser line can be used for internal autofocusing. Depending on the system, either one or two patented Variable Secondary Dichroics (VSD) can be used to flexibly divert the respective spectral range of light to chosen channels. Emission filters can be used to clean up the signal when imaging autofluorescent or highly scattering samples.</td>
</tr>
</tbody>
</table>

### Detection Options

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detectors</strong></td>
<td>2 spectral detection channels, GaAsP (typical QE 45 %) or multialkali (MA) PMT (typical QE 25 %)</td>
</tr>
<tr>
<td></td>
<td>1 additional GaAsP PMT, MA PMT or Airyscan 2 detector</td>
</tr>
<tr>
<td></td>
<td>Airyscan 2 for spatial detection (GaAsP) with 40×, or 63×, or 100× objectives; for superresolution (down to 120 nm) or Multiplex acquisition (down to 140 nm)</td>
</tr>
<tr>
<td></td>
<td>Transmitted light detector (ESID or T-PMT); unique transmitted fluorescence Sample Navigation with T-PMT</td>
</tr>
<tr>
<td><strong>Spectral detection</strong></td>
<td>&gt;8 sequential confocal fluorescence channels, up to three parallel confocal fluorescence channels, based on low-noise GaAsP or MA PMTs; adjustable in increments of 1 nm</td>
</tr>
<tr>
<td><strong>Data depth</strong></td>
<td>8-bit and 16-bit available</td>
</tr>
<tr>
<td><strong>Real-time electronics</strong></td>
<td>Microscope, laser, scanning module and additional accessory control; data acquisition and synchronization management through real-time electronics; oversampling read-out logic for best sensitivity; data transfer between real-time electronics and user PC via LVDS with the ability to evaluate the data online during image acquisition</td>
</tr>
</tbody>
</table>
## Technical Specifications

**ZEN Microscopy Software**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GUI configuration</strong></td>
<td>Workspace to conveniently configure all of the motorized functions of the scanning module, laser and microscope; save and restore application configurations as experiment settings or use acquired images (Reuse)</td>
</tr>
<tr>
<td><strong>Maintenance and calibration tools</strong></td>
<td>Calibration objective and software tools to calibrate the system</td>
</tr>
<tr>
<td><strong>Recording modes, Smart Setup</strong></td>
<td>Z-Stack, Lambda Stack, Time Series and all combinations (xyz, lambda, t), online calculation of signal intensities, average and summation (by line/image, adjustable), Step Scan (for higher image frame rates), quick set up of imaging conditions using Smart Setup by simply selecting the labelling dye</td>
</tr>
<tr>
<td><strong>Crop function</strong></td>
<td>Easily select scanning areas (simultaneously select zoom, offset, rotation)</td>
</tr>
<tr>
<td><strong>Real ROI Scan</strong></td>
<td>Scan of designated ROIs (regions of interest) as desired and pixel-by-pixel laser blanking</td>
</tr>
<tr>
<td><strong>ROI bleaching</strong></td>
<td>Localized bleaching in bleach ROIs for applications such as uncaging, use of different speeds for bleaching and imaging, use of different laser lines for different ROIs, flexibly define your bleaching experiments during the acquisition with Interactive Bleaching</td>
</tr>
<tr>
<td><strong>Multitracking</strong></td>
<td>Rapidly change excitation lines when recording multiple fluorescences for the purpose of minimizing signal crosstalk and increasing dynamic range</td>
</tr>
<tr>
<td><strong>Lambda scan</strong></td>
<td>Sequential acquisition of image stacks with spectral information for every pixel</td>
</tr>
<tr>
<td><strong>Linear Unmixing</strong></td>
<td>Acquisition of crosstalk-free, multiple fluorescence images using simultaneous excitation; offline unmixing; advanced unmixing logic with indication of reliability</td>
</tr>
<tr>
<td><strong>Visualization</strong></td>
<td>XY, orthogonal (XY, XZ, YZ), Cut (3D section); 2.5D for time series of line scans, projections (maximum intensity), animations; depth coding (inverse colors), brightness, gamma and contrast settings; color table selection and modification (LUT), character functions</td>
</tr>
<tr>
<td><strong>Image analysis and operations</strong></td>
<td>Co-localization and histogram analysis with individual parameters, profile measurement along user-defined lines, measurement of lengths, angles, areas, intensities and much more; operations: addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high-pass, etc., also user-definable)</td>
</tr>
<tr>
<td><strong>Image Management</strong></td>
<td>Features for managing images and the corresponding imaging parameters</td>
</tr>
<tr>
<td><strong>3DxI Viewer – powered by arivis®</strong></td>
<td>Visualization of very large data sets, fully integrated in ZEN microscopy software. Rapid 3D and 4D reconstructions and animations</td>
</tr>
</tbody>
</table>
## Technical Specifications

### Optional Software

<table>
<thead>
<tr>
<th>Module</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Processing</td>
<td>Processing of large data during acquisition by streaming, including e.g. Airyscan, Denoising and Deblurring and storage on second PC</td>
</tr>
<tr>
<td>Deconvolution</td>
<td>3D image restoration based on calculated point-spread functions (modes: nearest neighbor, maximum likelihood, constrained iterative)</td>
</tr>
<tr>
<td>Physiology (Dynamics)</td>
<td>Comprehensive evaluation software for online and offline ratio imaging with various pre-defined formulas</td>
</tr>
<tr>
<td>FRET</td>
<td>Acquisition of FRET ( Förster resonance energy transfer) image data with subsequent evaluation; Acceptor Photobleaching and Sensitized Emission methods supported</td>
</tr>
<tr>
<td>FRAP Efficiency Analysis</td>
<td>Acquisition of FRAP (fluorescence recovery after photobleaching) experiments with subsequent evaluation of intensity kinetics</td>
</tr>
<tr>
<td>Open Application Development (OAD)</td>
<td>Python scripting interface for automation &amp; customization; experiment feedback for Smart Experiments and open interface to third party software (e.g. ImageJ)</td>
</tr>
<tr>
<td>Experiment Designer</td>
<td>Definition of advanced automated imaging</td>
</tr>
<tr>
<td>ZEN Connect and ZEN Connect 2D / 3D Add-ons</td>
<td>Exchange and alignment of image data from multiple image acquisition systems in 2D and 3D, enabling correlative workflows</td>
</tr>
<tr>
<td>ZEN Intellesis</td>
<td>Image analysis and structure detection via computational self learning technology</td>
</tr>
<tr>
<td>Sample Navigator (requires additional HW)</td>
<td>Easy to set up and perform sample overview scan with autofocus function using Axiocam or transmitted fluorescence with T-PMT</td>
</tr>
<tr>
<td>Guided Acquisition</td>
<td>Automated and targeted acquisition of objects of interest</td>
</tr>
<tr>
<td>Tiles &amp; Positions</td>
<td>Scanning of predefined sample areas (tiles) and/or position lists</td>
</tr>
<tr>
<td>3Dxl Plus</td>
<td>Combine 2D and 3D visualization in one screen</td>
</tr>
<tr>
<td>BioApps</td>
<td>Easy-to-use and modular image analysis for common assays</td>
</tr>
<tr>
<td>Airyscan RAW data</td>
<td>Optional export of complete Airyscan single channel data and the Sheppard sum for external processing, e.g. correlations, deconvolution, AI etc.</td>
</tr>
</tbody>
</table>

### Lasers

<table>
<thead>
<tr>
<th>Laser Module</th>
<th>Description</th>
</tr>
</thead>
</table>
| Laser module URGB (pigtailed; 405, 488, 561, 640 nm) | Single-mode polarization preserving fiber  
Typical total dynamic range of 10.000:1; direct modulation 500:1  
Diode laser (405 nm, 5 mW); laser class 3B  
Diode laser (488 nm, 10 mW); laser class 3B  
Diode (SHG) laser (561 nm, 10 mW); laser class 3B  
Diode laser (640 nm, 5 mW); laser class 3B |
| Laser module GB (pigtailed; 488, 561 nm)    | Single-mode polarization preserving fiber  
Typical total dynamic range of 10.000:1; direct modulation 500:1  
Diode laser (488 nm, 10 mW); laser class 3B  
Diode (SHG) laser (561 nm, 10 mW); laser class 3B |
## Technical Specifications

### Power Requirements

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line voltage</td>
<td>100 V AC ... 125 V AC (±10 %) to 220 V AC ... 240 V AC (±10 %)</td>
</tr>
<tr>
<td>Line frequency</td>
<td>50 ... 60 Hz</td>
</tr>
<tr>
<td>Max. current</td>
<td>1 phase at 9 A</td>
</tr>
<tr>
<td>Power plug</td>
<td>NEMA 5 / 15</td>
</tr>
<tr>
<td>Power consumption</td>
<td>900 VA (continuous operation; maximum)</td>
</tr>
<tr>
<td></td>
<td>260 VA (standby operation)</td>
</tr>
<tr>
<td></td>
<td>0.011 VA (off mode)</td>
</tr>
<tr>
<td>Heat Emission</td>
<td>700 W</td>
</tr>
</tbody>
</table>

### EMC Test

- according to DIN EN 61326-1
- 1. Noise emission according to CISPR 11 / DIN EN 55011
- 2. Noise immunity according to table 2 (industrial sector)

### Environmental Requirements

- For operation, the system has to be placed in a closed room.
- 1. Operation, specified performance
  - T = 22 °C ±3 °C without interruption (24 h a day independently whether system is operated or switched off)
  - It has to be ensured that the airflow of the air-conditioning is not directed at the system.
- 2. Operation, reduced performance
  - T = 15 °C to 35 °C, any conditions different from item 1. and 4.
- 3. Storage, less than 16h
  - T = –20°C to 55°C
- 4. Temperature gradient
  - ± 0.5 °C / h
- 5. Warm-up time
  - 1 h for standard imaging; ≥ 2 h for high-precision and/or long-term measurements
- 6. Relative humidity
  - < 65 % at 30 °C
- 7. Operation altitude
  - max. 2,000 m
- 8. Loss of heat
  - 700 W

LSM 900 meets the requirements according to IEC 60825-1:2014
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- 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 APEER

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

>> www.zeiss.com/microservice