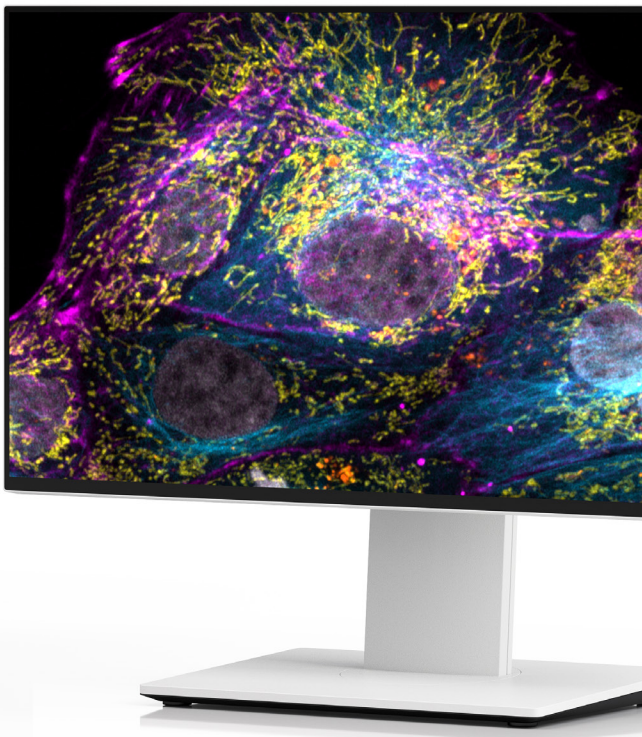
Investigating more proteins in parallel



ZEISS LSM 990 Spectral Multiplex

Advanced Spectral Imaging for In-Depth Understanding of Spatial Biology

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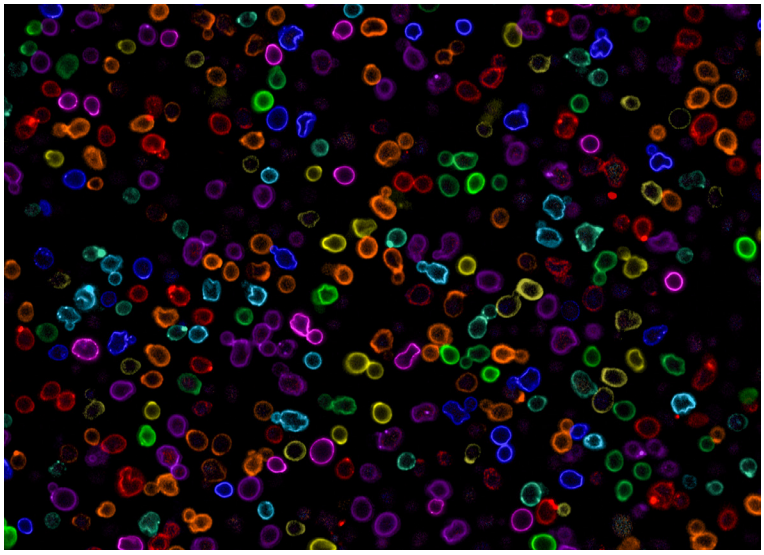
Seeing beyond

ZEISS LSM 990 Spectral Multiplex

Multi-fluorescence imaging along the entire wavelength range

LSM 990 Spectral Multiplex excels in the spectral separation of fluorescent labels. Optimize your advanced spectral multiplexing experiments with numerous protein markers and clear separation of fluorescence signals while reliably eliminating autofluorescence. Become more productive with a system that facilitates optimal imaging conditions, immediate dye identification, and streamlined workflows from acquisition to analysis.



Advanced spectral multiplexing of cell walls of budding yeast cells (Saccharomyces cerevisiae)*: 13 labels plus autofluorescence acquired in one track using 5 lasers and 36 detectors, unmixed image of the 13 labels without autofluorescence. Sample courtesy of Michal Skruzny, ZEISS Microscopy GmbH*

Efficient spectral multiplexing
Take all spectral information in a single image scan.

User-friendly experiment design
Customize your spectral experiments with ease.

Real-time spectral unmixing
Separate your fluorescent labels quickly and reliably.

Workflow automation beyond imaging
Increase your productivity by streamlining multi-faceted experiments.

One of the advantages of confocal imaging is the capacity to capture multiple channels simultaneously. Spectral imaging is highly relevant for various applications, particularly in cancer research through spectral multiplexing assays of cancer biomarkers and general spatial phenotyping.

LSM 990 Spectral Multiplex is the optimal choice if your experiments require the spectral separation of fluorescent labels, from simple multi-label imaging to advanced spectral multiplexing setups. Wavelength flexibility, gentle sample treatment, and efficient workflows are combined into one system. Optimize your LSM experiments with more protein markers and clear separation of fluorescence signals while reliably eliminating autofluorescence.

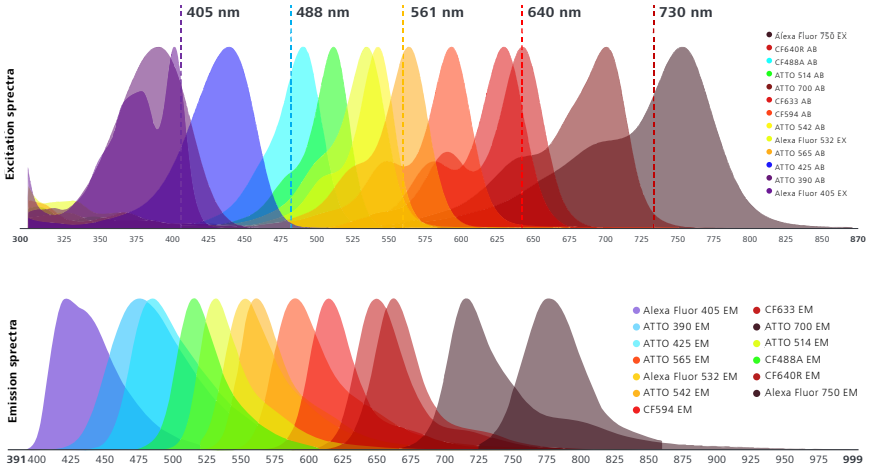
To utilize a wide array of dyes in a single experiment, freely select fluorescent labels from 380 nm into the near-infrared range at 900 nm. Employ 36 detectors to analyze more than 10 labels in a single scan. Let the system assist you in determining the optimum excitation settings and selecting the detectors with the best quantum efficiency for the desired spectral range. On-the-fly separation of all dyes for immediate identification saves data size and time, which is particularly beneficial for routine applications and screening processes.

Efficient spectral multiplexing

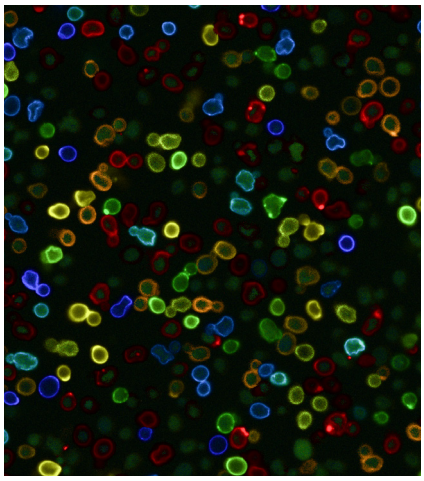
Spectral information from 380 to 900 nm taken in a single scan

LSM 990 Spectral Multiplex offers unparalleled productivity for your demanding spectral imaging experiments, covering a wavelength range from 380 to 900 nm. The intelligent design choices of the beam path eliminate compromises related to spectral separation, sensitivity, speed, signal-to-noise ratio, and resolution. With the direct Lambda track acquisition, you can simultaneously separate 10 or more individual labels. Capturing all spectra in a single scan enables instantaneous separation and real-time display of the resulting channels. This allows you to identify numerous labels in the shortest time, which is useful when enhanced spatial resolution is needed, or for imaging large volumes or live samples. Unwanted signals, such as autofluorescence, can be easily eliminated without inadvertently removing true signal from the target proteins. All Lambda information is preserved, and spectra are displayed in easy-to-read graphs to help you evaluate if you are imaging the expected fluorescent labels.

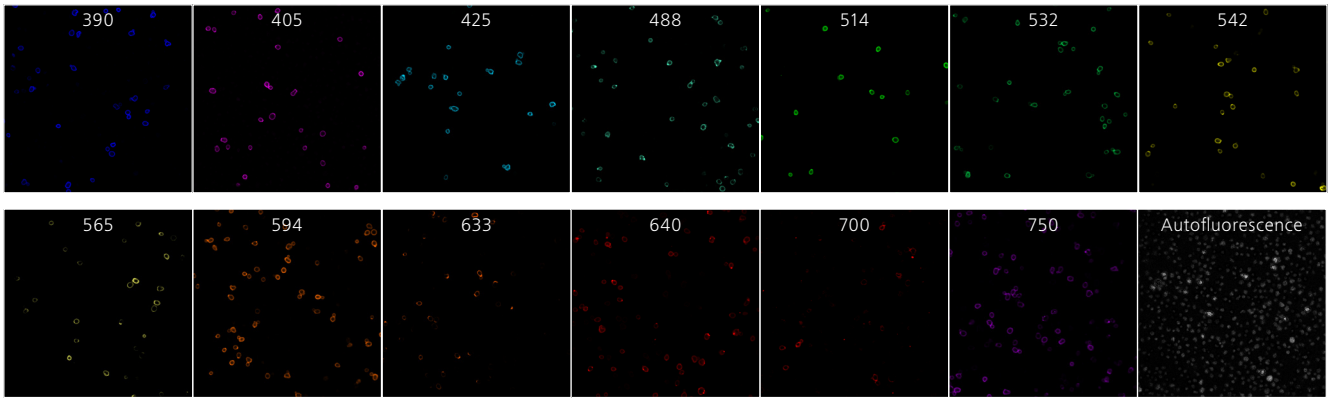
Advanced spectral multiplexing of yeast cells: 13 colors plus autofluorescence acquired at once



The 13 spectra and the autofluorescence spectrum were defined on samples labelled with 4 dyes to allow for spectral separation and clean spectra. 5 laser lines and excitation spectra (upper panel), emission spectra (lower panel)



Spectral image (Lambda stack) of 13 labels acquired simultaneously, real-color representation



Unmixed single labels and autofluorescence

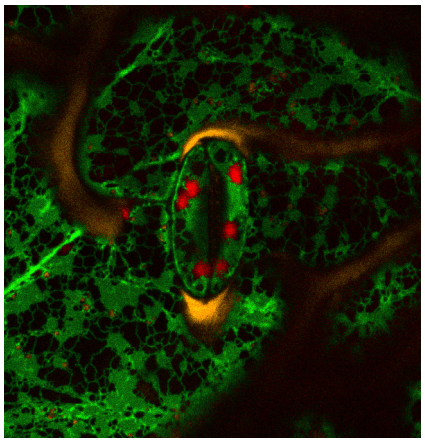
Sample courtesy of Michal Skruzny, ZEISS Microscopy GmbH

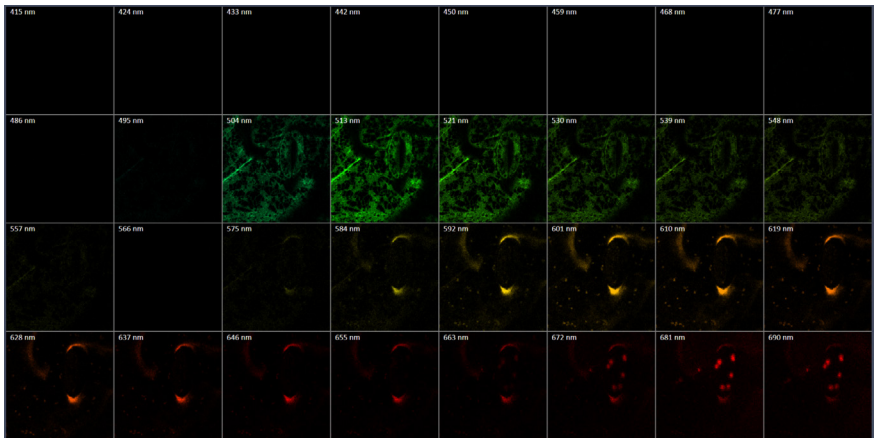
User-friendly experiment design

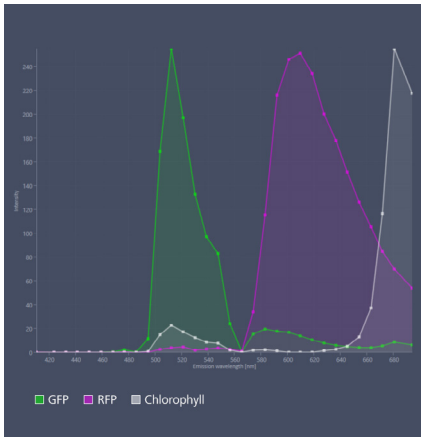
Customized spectral imaging mastered with ease

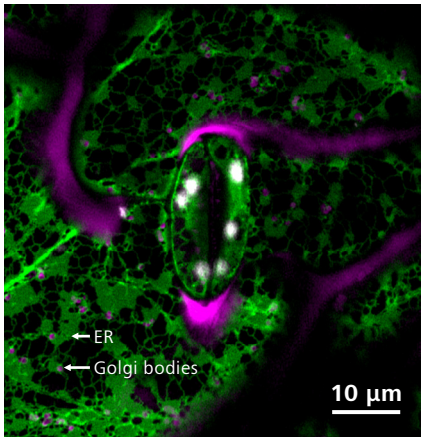
When you initiate a multi-color experiment, Smart Setup offers excitation and emission data for a wide range of fluorophores. Adjust the entire system to your requirements with a single click by choosing between configuration options for optimum spectral separation, maximum speed, or a balanced compromise. Alternatively, select the hassle-free Lambda Scan mode to capture all relevant signals of the desired spectral range in a single scan. All experimental settings can be saved and easily accessed within ZEN, facilitating quicker utilization of personal experiment configurations. Integrate the LSM Plus feature to ensure optimal signal-to-noise ratio and improved spatial resolution without slowing down your experiment.

Straightforward separation of GFP and RFP from autofluorescence in *Arabidopsis*









Arabidopsis *leaf expressing GFP-HDEL (labeling the endoplasmatic reticulum) and ST-mRFP (labeling Golgi bodies).*

Upper left: Simultaneously acquired Lambda stack of 32 spectral channels clearly shows the spectral color range of each pixel.

Upper right: Single channels of the Lambda stack from 411 to 740 nm.

Lower left: Spectra of GFP (green), mRFP (pink) and chlorophyll autofluorescence (white) as defined from Lambda stack allow to immediately distinguish different labels and autofluorescence in the image, even whilst imaging, and to unmix these.

Lower right: Unmixed and LSM plus processed image (green: GFP/ER, pink: mRFP/Golgi bodies, white: chlorophyll).

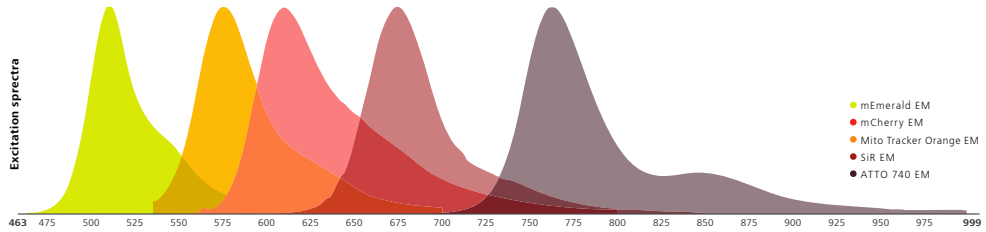
Sample courtesy of Verena Kriechbaumer, Oxford Brookes University, UK

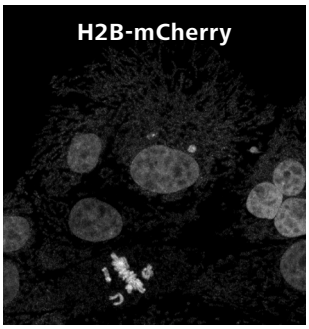
Real-time spectral unmixing

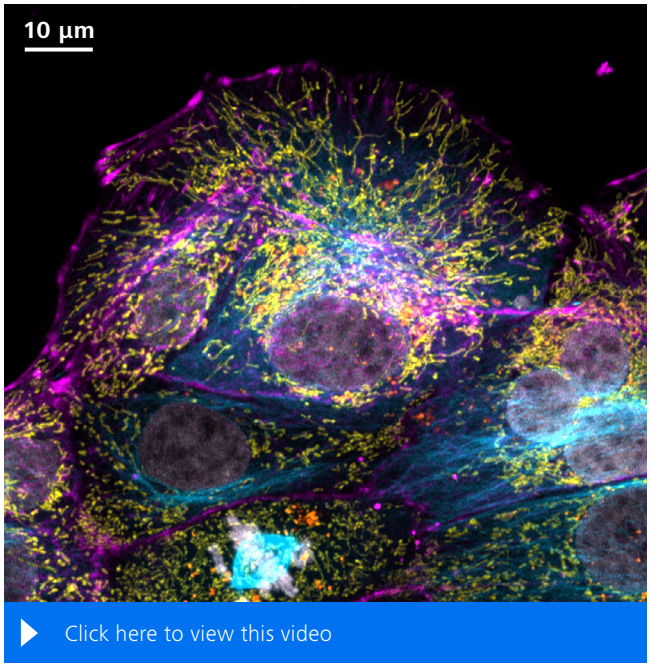
Fluorescent signals reliably separated

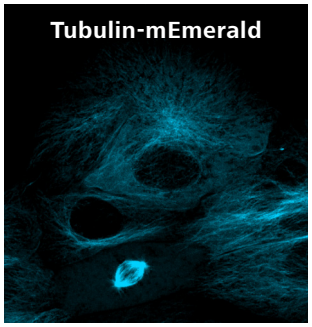
When imaging multiple channels or utilizing Lambda mode, the option for spectral unmixing is always available. Previously saved spectra can be retrieved from a local database, and alongside this information, all critical imaging settings are stored and displayed. You can manually select pixels containing spectral information from the newly acquired image, or utilize the built-in Automatic Component Extraction to identify such pixels. These sources of information can be combined in a Linear Unmixing process. Each resulting multi-channel image can undergo validation and quality control, with an optional 'residual' channel saved alongside the original data for seamless record-keeping of the experiment. Perform on-the-fly Linear Unmixing while capturing spectral information in single scans through Online Fingerprinting, leading immediately to separated signals – ideal for large volumes and screening specific combinations of fluorescent labels.

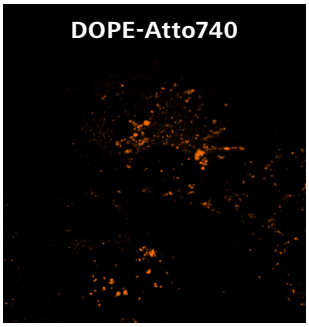
5-color live cell Online Fingerprinting of epithelial pig kidney cells

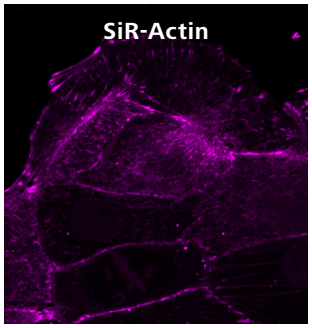


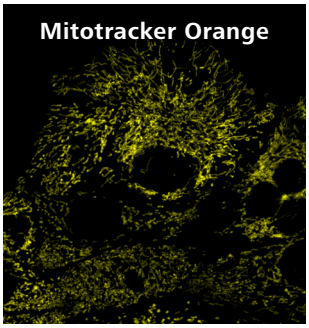












LLC-PK1 cells (pig epithelial kidney cell line) expressing Tubulin-mEmerald (Tubulin, cyan) and H2B-mCherry (Histone-bound DNA, white), additionally labeled with Mitotracker Orange (Mitochondria, *yellow), SiR-Actin (Actin, pink) and DOPE-ATTO 740 (vesicles, orange).*

Top: Excitation spectra of the 5 labels

Left: Live cell imaging (timelapse) with 5 colors imaged simultaneously and unmixed in real-time using Online Fingerprinting, processed with LSM Plus

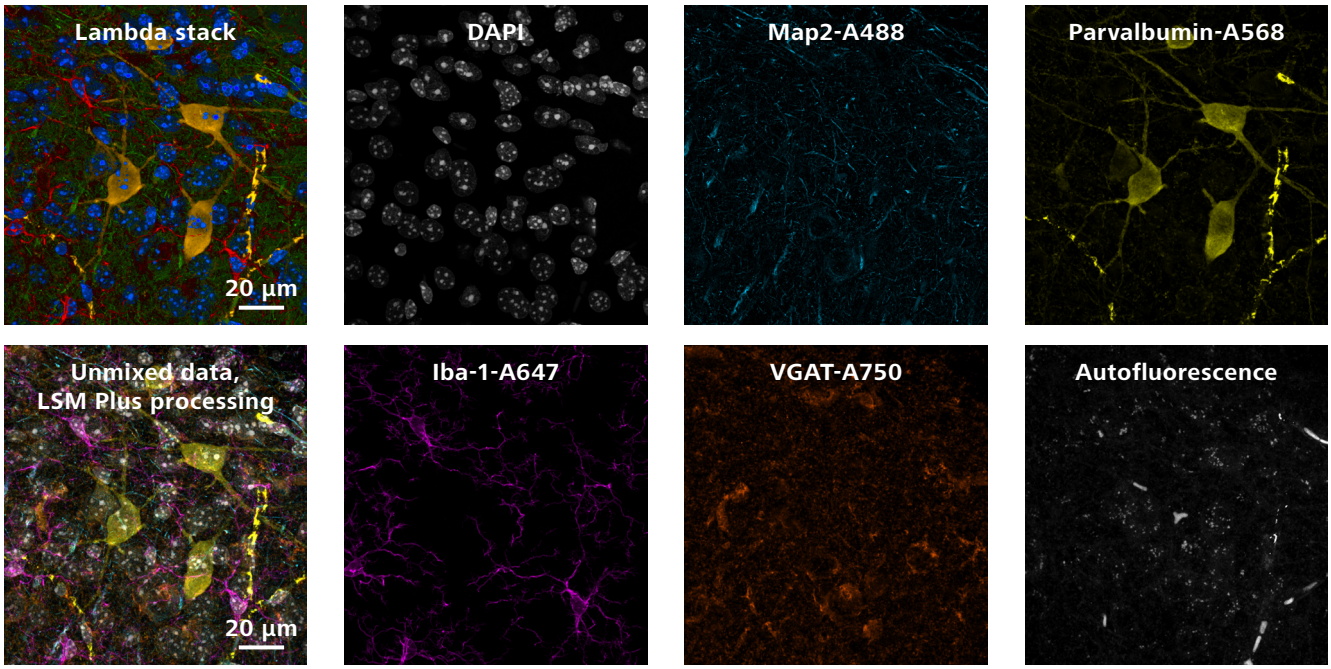
Right: Single unmixed labels

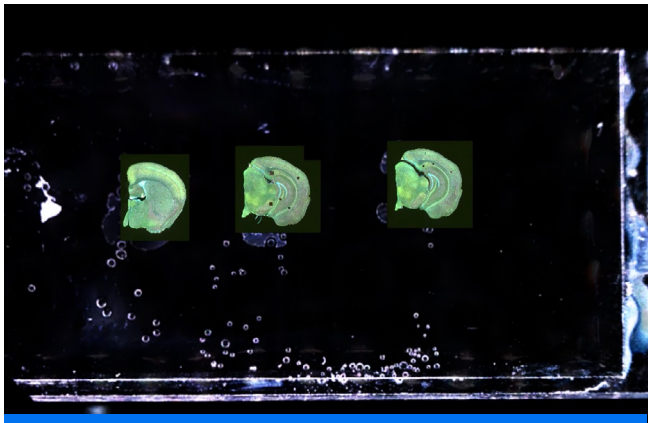
Workflow automation beyond imaging

Increased productivity through streamlined multi-faceted experiments

Combine all available spectral data acquisition methods, including Lambda Scans, Linear Unmixing, and LSM Plus for SNR enhancement, into one processing pipeline that executes all steps of multi-dimensional experiments. Automated workflows for spectral multiplexing that involve multiple rounds of staining and imaging can be simplified with automated liquid delivery systems. The individual staining, imaging, bleaching, and stripping rounds can be organized within ZEN*. Transfer the resulting data to ZEISS arivis pro for 3D registration of spectral multiplexing data, AI object segmentation, or statistical analyses, such as cell neighborhood and dimensionality reduction analyses.

Mouse brain section: Spectral multiplexing workflow from sample detection to image data processing





Click here to view this video

Fixed mouse brain, 40 µm thick sections. DAPI (nuclei), MAP2-A488 (dendrites and neuron bodies), Parvalbumin-A568 (subtype of inhibitory/GABAergic interneuron), Iba1-A647 (microglia, the resident immune cells in the brain), VGAT-750 (presynaptic terminals of inhibitory/GABAergic interneurons)

Overview was imaged with ZEISS AI Sample Finder, then overviews of the sections were added using a 10x objective, an Axiocam 705 and LED illumination. Detailed scans were acquired using the Plan-Apochromat 63×/1.4 Oil objective. A Lambda scan was set up using the 405, 488, 561, 639 and 730 nm, with 35 detectors covering the spectrum from 411 to 900 nm. The spectra for the 5 labels, plus one spectrum for autofluorescence from the tissue obtained from single stainings were then used to unmix the images. Images were processed using LSM Plus.

Sample courtesy of Luisa Cortes, Microscopy Imaging Center of Coimbra, CNC, University of Coimbra, Portugal

** Available upon request*

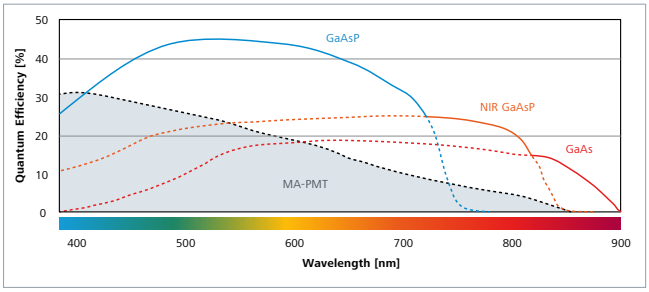
A system optimized for light efficiency

Your insights into the technology behind it

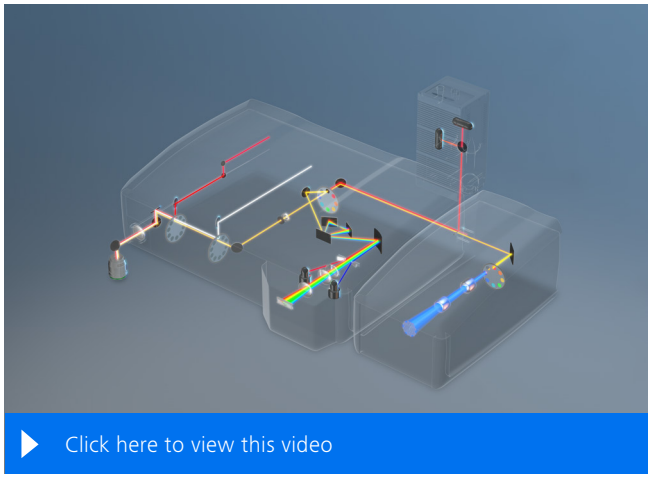
For optimal gentle imaging with multiple labels, it is crucial that all components of the imaging system work harmoniously to maximize the transmission of emission light. LSM 990 Spectral Multiplex features a detector configuration and beam path design that allow you to preserve valuable signal, and go beyond conventional multi-color imaging.

Beam path

Whether you require the flexibility to record two labels simultaneously or conduct a sophisticated spectral multiplexing experiment, the process begins with low-angle main beamsplitters (MBS) that ensure clean separation of laser excitation light from emission signals, allowing the emission light to be fully utilized without signal loss. The laser coupling of ZEISS LSM 990 is designed to accommodate a broad range of excitation wavelengths from 405 nm to 730 nm, with the additional option of multiphoton excitation through two independent wavelength-adjusted pathways and additional collimation optics. All optical elements in the emission beam path are engineered for optimal transmission of the emission spectral range from 380 nm to 900 nm, guiding the light through an apochromatic pinhole, controlled by wear-free solid-state hinges. A holographic grating ensures linear spectral separation of all emission signal. This is vital as it guarantees that all 32 channels of the detector capture the same spectral width, providing a consistent 10 nm spectral resolution for effective spectral unmixing and precise detection range definition.



Typical spectral quantum efficiency (QE) of ZEISS LSM 990 detectors



Beam path of LSM 990

Detectors

LSM 990 Spectral Multiplex features a 32-channel GaAsP detector, complemented by two side detectors and optional 2 NIR GaAs and GaAsP detectors. This unique configuration provides the highest number of detectors available in LSM systems. The detectors are strategically positioned within the scan head design to maximize quantum efficiency, ensuring optimal conversion of light into electronic signals for the relevant emission wavelengths. Each detector is linearized to guarantee quantifiable data. All detectors are calibrated relative to one another, allowing for spectral signals to be displayed in a manner consistent with spectral databases. This feature simplifies the identification of fluorophore spectra and data validation.

ZEISS LSM 990

Explore additional options for your multimodal imaging

ZEISS LSM 990

Freedom to explore

Top-class multimodal imaging combined in one confocal system

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