From Eye to Insight

Leica
MICROSYSTEMS

Confocal Microscope Buyer's Guide:

Futureproof Your Imaging

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Foreword

Choosing an appropriate microscope platform for a given research project can be a daunting challenge, as competing fluorescence imaging techniques such as widefield microscopy and selective plane illumination microscopy (SPIM) [1] each have distinct advantages and disadvantages that require careful consideration.

You have decided to purchase a confocal microscope. Choosing the best option for your research is a critical decision that requires careful consideration of key features such as resolution, sensitivity, speed, and ease of use. It's also worth researching systems that support future upgrades to advanced imaging modalities, ensuring your investment can grow with your research.

With so many factors to consider, you may appreciate some insights into what matters most in the essential performance of a confocal microscope, as well as the additional capabilities of a modern platform. This guide is intended to help you choose the right confocal system for your research, both now and in the future.

Advances by M. David Egger, Mojmir Petran, and others in the late 1960s and 1970s led to improvements, such as adopting lasers as light sources [3]. Progress in computer and laser technology during the 1980s resulted in the first commercial instruments becoming available in 1987 [4]. In the late 1980s and 1990s, enhancements in optics and electronics enabled dynamic three-dimensional visualizations of living cells (Figure 1) and tissues [5,6], and access to a wider range of applications. Today, confocal microscopes are integrated systems, offering enhanced resolution, spectral flexibility, and automation for detailed molecular and cellular investigations [7].

Confocal microscopy provides life science researchers with sharp, clear images, facilitating the study of cellular and intracellular structures and organization, cell dynamics, and cellular functions in 3D and over time. The early confocal microscope was developed by Marvin Minsky in the mid-1950s [2]. It was a major step forward for the field of microscopy but, back then, lacked the necessary technology to become widely adopted.

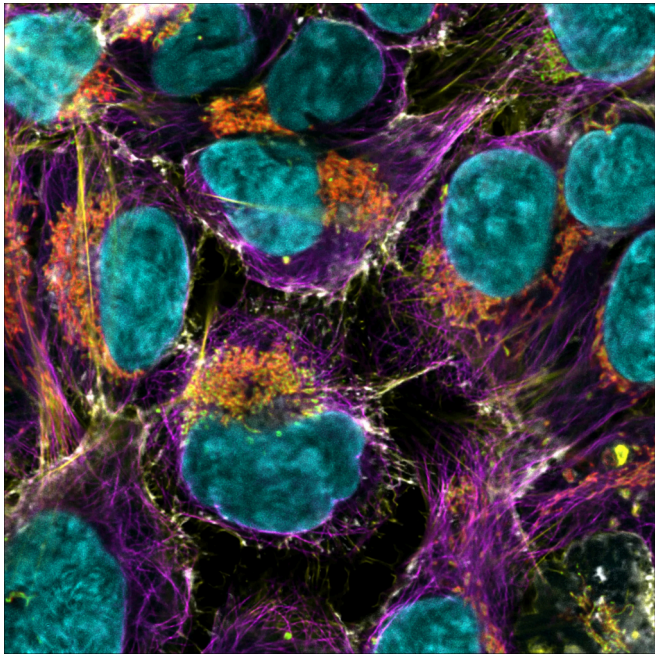
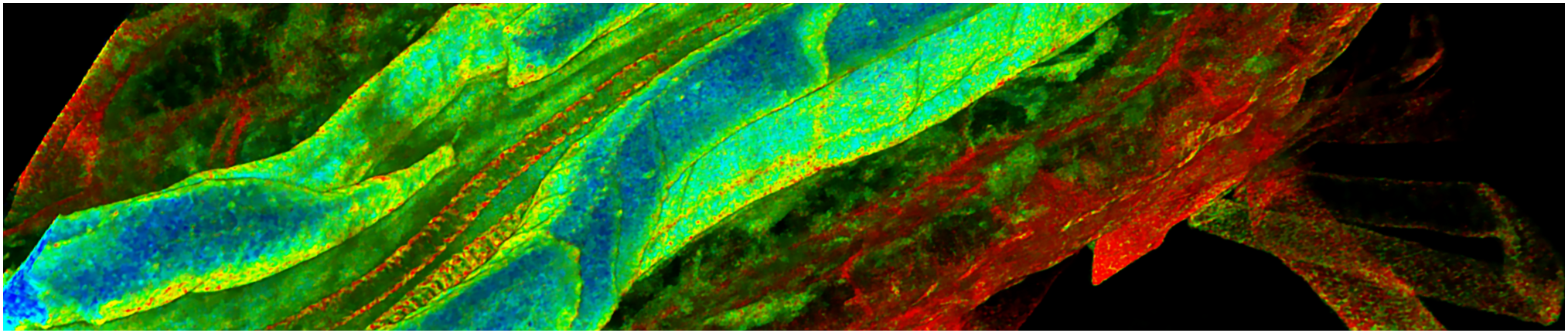


Figure 1. Mammalian cells labeled with 6 fluorescent markers: membranes (white, CF405S, WGA), nuclei (cyan, SPY505 DNA), mitochondria (green, MitoTracker Red), mitochondrial outer membrane (red, AF750), tubulin (magenta, AF555), actin filaments (yellow, SiR700).



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Chapter 1: Choosing The Best Confocal Microscope for Your Lab

When selecting an appropriate confocal microscope for your work, consider investing in a flexible system that can evolve with your research. Buying from a vendor that provides long-term technical support is also important, as it will extend the lifetime and usability of your microscope.

The next considerations should be according to your specific research requirements, including the necessary resolution, imaging depth, and the nature of the samples to be studied.

Multiplexing experiments involving multiple fluorophores require a system equipped with a tunable excitation light source to enable precise excitation wavelength selection for the specific dyes used. Integration of advanced software will help streamline your workflows and facilitate data analysis and visualization.

Step 1. Familiarize Yourself with Different Confocal Imaging

There is a variety of confocal microscopes on the market, each providing a unique set of features and designed to excel at the following imaging modes:

- Multicolor imaging
- 3D imaging of live cells
- Super-resolution imaging
- Fluorescence lifetime imaging
- Label-free imaging
- Quantitative imaging

The following information on the different imaging modes will help you decide which type of microscope is appropriate.

Multicolor Imaging

Multicolor imaging enables the simultaneous visualization of multiple targets within a single sample using different fluorescent dyes or labels [8]. This technique is essential for studying complex biological systems, such as immune cell interactions and signaling pathways in cancer research (Figure 2).

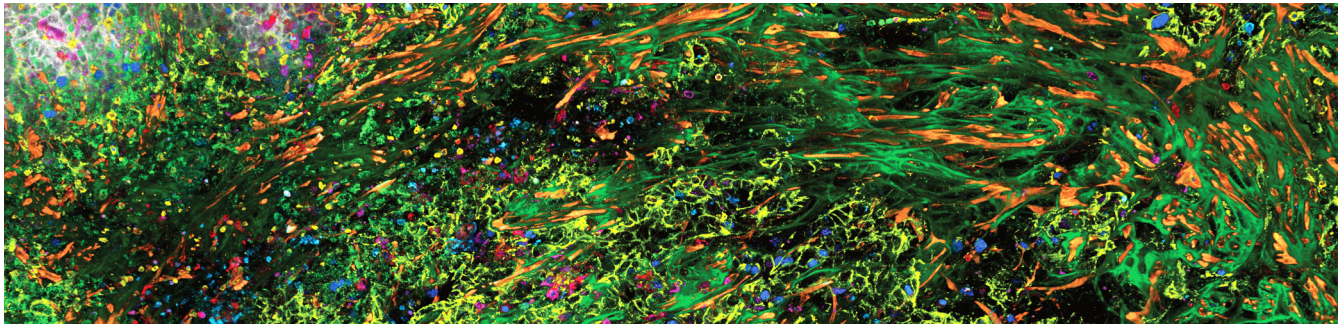


Figure 2. 3D 15-plex imaging of a mouse pancreatic tumor section using STELLARIS with SpectraPlex functionality. Image courtesy of Kunz, L., Speziale, D., et al. Nat. Methods (2024).

In multicolor imaging, spectral overlap between fluorophores can be problematic and complicate data interpretation. Careful selection of fluorophores will help avoid this. The problem can also be overcome by coupling spectra-based detectors with White Light Lasers (WLL). Spectra-based detectors allow for precise detection and separation of fluorophore signals, even in experiments with densely overlapping emission spectra.

When paired with WLLs, which provide tuneable excitation across a broad spectrum (see the section "Adaptability: What Are Your Detection Needs?" below for more information), these systems enable highly customizable precise excitation of fluorophores. This combination not only reduces spectral crosstalk but also increases the range of usable dyes, enhancing flexibility, resolution, and sensitivity in complex multicolor imaging experiments.

3D Imaging of Live Cells: High-speed Volumetric Imaging

While 3D imaging is a core feature of any confocal microscope, advanced confocal microscope configurations with high-speed volumetric imaging technology will enhance your imaging capabilities, particularly for live cell imaging (Figure 3). Capturing a series of images over time is an effective way of studying dynamic processes within living cells and tissues. Many of these processes are pertinent to important fields within biology, such as developmental biology and cancer research, including cell division, migration, and differentiation.

Loss of image resolution occurs when the speed of the biological process exceeds the acquisition speed of the microscope. Standard field-of-view scanners, based on Galvo mirror technology, offer some flexibility. However, in these setups, high-speed acquisition comes with a reduced field-of-view.

Live cell imaging typically generates huge quantities of data, and it is important to factor processing power and storage capacity into your set-up. Some confocal platforms offer integrated, dedicated analysis software to simplify the analysis of images collected at very low excitation levels and high acquisition speeds.

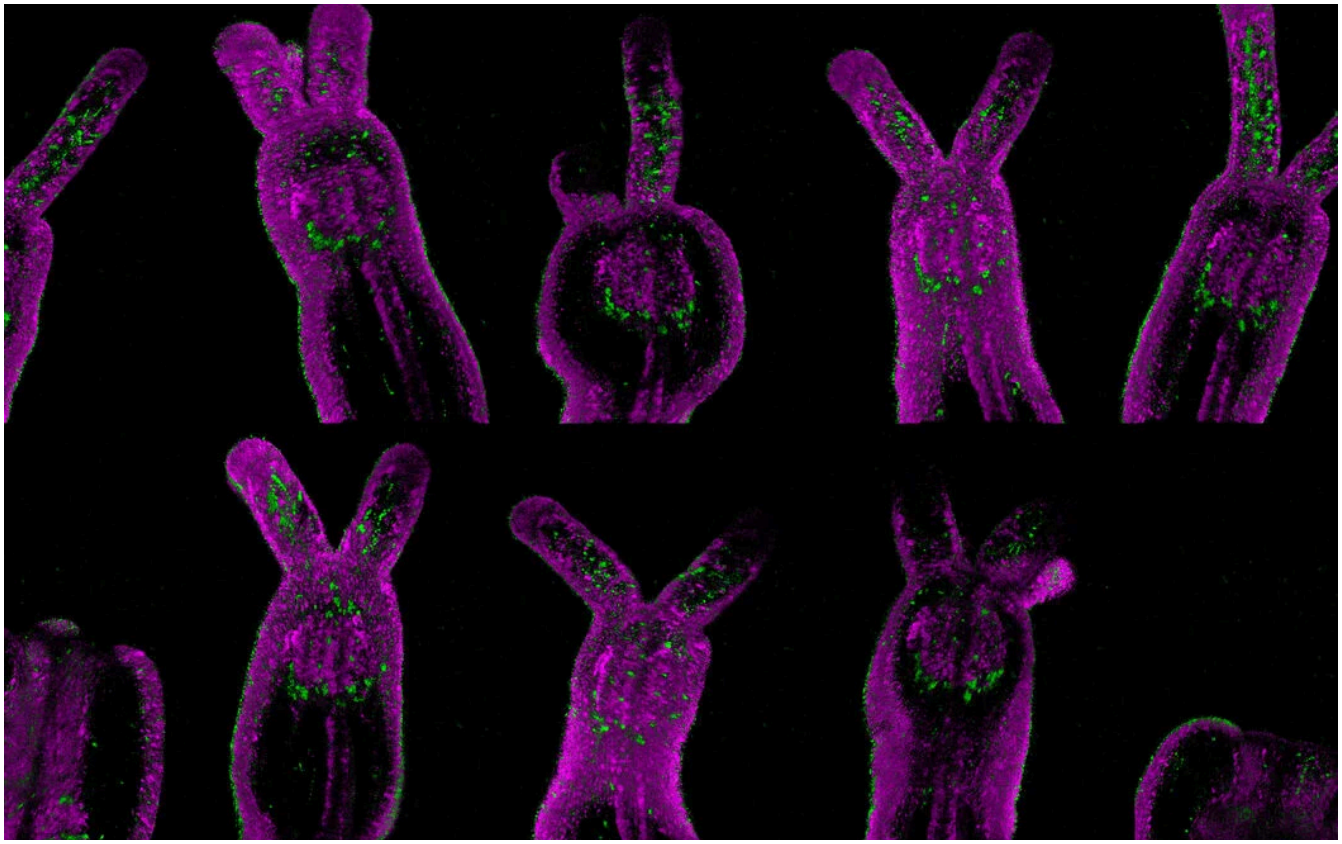


Figure 3. Fast 3D *in vivo* imaging of *Nematostella vectensis* (Cnidaria) showing endogenous nematosomes, clusters of freely-circulating cnidocytes (green), and Dextran Red fluorescence (magenta). Endogenous signals and fluorescence separated by TauGating on HyD S, 340 volumes acquired in 12 min 45 s. Sample courtesy of Anniek Stokkerman and Aissam Ikmi, EMBL Heidelberg.

Super-resolution Imaging

Super-resolution imaging surpasses the diffraction limit of conventional microscopy, allowing visualization of structures at the nanometer scale [9]. This imaging technology is crucial for detailed studies of subcellular components and molecular complexes, benefiting areas like neuroscience, cell biology, and structural biology. It requires relatively advanced microscopes, however, which can be difficult to learn how to use compared to other confocal microscopes.

When selecting a confocal microscope, consider the super-resolution techniques it supports, such as Stimulated Emission Depletion (STED) [10], Structured Illumination Microscopy (SIM) [11], Stochastic Optical Reconstruction Microscopy (STORM) [12,13], and Photoactivated Localization Microscopy (PALM) [14]. Each approach (briefly described in more detail below) has specific strengths and limitations that can influence your system's suitability for your research needs.

Super-resolution imaging can offer unprecedented insights into the subcellular organization of cells. For example, 3D STED has revealed the nanoscale organization of the hematopoietic cell-specific HS1 substrate in normal and leukemic cells [15].

Stimulated Emission Depletion (STED)

STED achieves super-resolution by selectively depleting fluorescence in the periphery of an illuminated spot, leaving a smaller central region active for imaging. A single wavelength can be used to deplete different fluorophores, facilitating multi-color imaging without complex optical adjustments (Figure 4). Fluorophore replenishment enables longer acquisition times and maintains high signal-to-noise ratios [16].

This method excels in high spatial resolution in real-time, making it suitable for live-cell imaging, and multicolor visualization allows simultaneous detection of multiple cellular components, such as actin and DNA, in a single experiment. However, STED does require high-intensity laser illumination, which may lead to photobleaching and phototoxicity in sensitive samples.

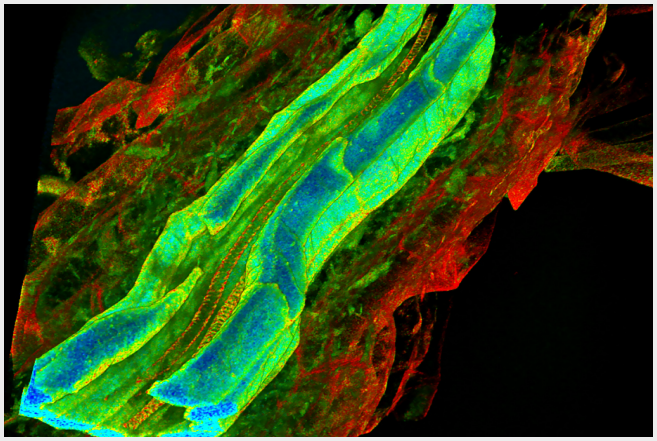


Figure 4. Endogenous fluorescence Average Arrival Time Image (TauContrast) of cleared *Arabidopsis thaliana* root samples. Courtesy: W. Busch, Salk Institute, La Jolla, CA, USA.

Structured Illumination Microscopy (SIM)

SIM enhances resolution by illuminating the sample with a patterned light source and reconstructing the image using computational algorithms. This technique doubles the resolution of conventional microscopy and is gentler on live samples due to lower light intensity requirements. While it offers versatility and compatibility with standard fluorophores, it provides less resolution improvement compared to other super-resolution methods.

Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM)

These techniques achieve nanometer-scale resolution by relying on the precise localization of individual fluorescent molecules. By activating and imaging small subsets of fluorophores at a time, STORM/PALM reconstructs a high-resolution image from the accumulated data. These methods provide exceptional resolution and are particularly effective for fixed samples but require long acquisition times and sophisticated analysis, limiting their use in dynamic or live-cell studies.

Fluorescence Lifetime Imaging

Fluorescence Lifetime Imaging (FLIM) constructs an image using the decay time of fluorescence from a sample, rather than measuring fluorescence intensity [17]. This provides insights into the local environment of cells, the changes in these environments over time, and the interactions of fluorescent molecules (Figure 5). It also prevents erroneous measurements caused by changes in sample fluorescence intensity during the experiment and autofluorescence.

FLIM is useful for studying protein-protein interactions and cellular metabolism, with applications in pharmacology and biochemistry. It can be combined with super-resolution and spatial imaging techniques to resolve molecular interactions at the nanoscale and provide detailed information about the local environment and molecular dynamics, essentially achieving high spatial resolution and temporal resolution in the same experiment.

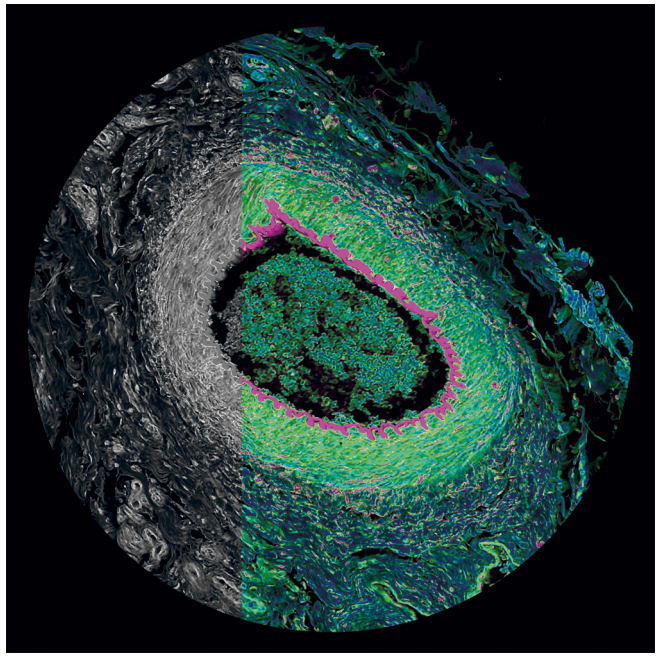


Figure 5. Histological section from a cat's eye. Simultaneous spectral (grey) and FLIM (color) confocal imaging reveals contrast by lifetime.

Label-free Imaging

Label-free imaging techniques, such as Phase Contrast Microscopy (PCM) and Differential Interference Contrast (DIC), allow the observation of cells and tissues without staining them with fluorescent labels [18].

This is advantageous for studying cells in their natural state and avoids potential artifacts introduced by staining.

For high-resolution, subcellular-level imaging, Coherent Raman Scattering (CRS) microscopy may be applicable. CRS is a powerful label-free imaging technique used for visualizing and analyzing the chemical composition of biological and material samples (Figure 6).

It uses the vibrational properties of molecules to provide highly sensitive and specific data. It provides detailed insights into the biochemical composition and metabolic processes within cells and tissues, and allows for imaging of small molecules.

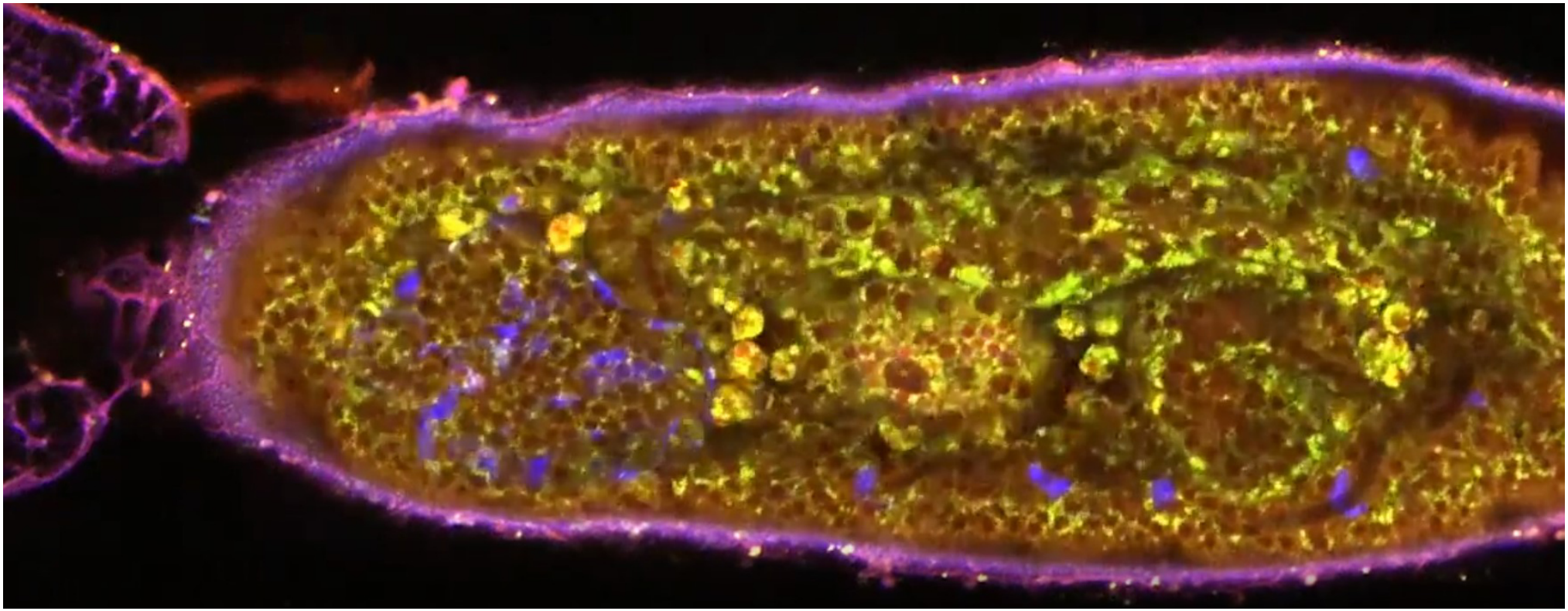


Figure 6. Fast, multiplex chemical imaging of a developing *Drosophila* embryo. Proteins (red, SRS 2940 cm^{-1}), lipids (green, SRS 2850 cm^{-1}), and a combination of second harmonic generation and endogenous 2-photon-fluorescence (blue). Still taken from a time lapse movie, 16sec / time step; run time 2h 3min (441 time steps). Sample courtesy of Dr. Matthew Benton, EMBL Heidelberg.

Quantitative Imaging

Quantitative imaging provides precise, numerical data on cellular structures and functions, facilitating rigorous analysis of biological processes such as cell division rates, intracellular molecule concentrations, and membrane potential changes [19]. It's also an important technique in drug discovery and diagnostics, where measurement accuracy is critical.

In traditional fluorescence microscopy, intensities are typically recorded as analog signals, assigned arbitrary units (a.u.) based on detector output. These values depend on factors like detector sensitivity, gain settings, and background noise, making them relative rather than absolute. This approach can introduce variability and complicated comparisons across experiments or systems.

By contrast, quantitative imaging relies on photon counting—a technique that directly quantifies the number of photons detected to provide absolute, noise-reduced data. However, as with traditional quantification methods, challenges arise when low excitation levels are used, as this causes low signals due to the low number of photons being detected.

Step 2. Decide on Your Intended Application

Depending on your research area—whether it is an established field like genetics or an emerging specialty—certain features of a confocal microscope will be more critical than others. Once you have decided on the imaging modality you want your microscope to be capable of, the following questions will further narrow down what microscope you need and any accessories.

At a glance, these questions are:

- Adaptability – What fluorophores will you use, and will your needs change over time?
- Power – How sensitive are your samples to light exposure?
- Speed – Do you need to capture fast-moving processes or large areas quickly?
- Resolution – What scale of biological structure do you need to image?
- Penetration Depth – How deep into your samples do you need to image?
- User Experience – How intuitive should the system be for your lab or core facility?
- Ease of Data Analysis – How will you process and interpret the imaging data?

Adaptability: What Are Your Excitation and Detection Needs?

Consider the types of fluorophores your lab will use and the number of imaging channels required. If you are working with a fixed set of fluorophores, fixed excitation laser lines tailored to those will be appropriate, ensuring optimized excitation while reducing unnecessary complexity.

However, if your fluorophores are likely to change over time, a WLL is a better choice. The primary advantage of a WLL is its ability to cover a broad spectrum of wavelengths, providing the flexibility needed to adapt to different fluorophores as your experiments evolve. This adaptability ensures that you can effectively handle different fluorophores without requiring significant hardware changes, making it a future-proof solution for dynamic research environments.

Power: How Sensitive Are Your Cells?

Incorporating beam splitters, such as Leica's Acoustic Optical Beam Splitter (AOBS) can help reduce sample phototoxicity. The beam splitter, along with the WLL, will allow you to excite the fluorophore at its optimum excitation wavelength, reducing the amount of excitation power your sample is exposed to, and maximizing the length of time for imaging.

In addition, technology like the AOBS is particularly beneficial for live cell imaging because it can switch wavelengths in milliseconds.

Speed: What Are the Dynamics of Your Experiments?

The speed of image acquisition is vital, especially for dynamic experiments. Will your samples be fixed or live? If working with live cells, you may need incubation equipment to maintain cell viability during experiments. Assess the data acquisition rate your research demands. High-speed imaging is essential in cell biology to capture fast cellular processes, while slower speeds might suffice for static samples.

Including a resonant scanner in your confocal setup will significantly increase imaging speed without compromising the field-of-view and maintaining image quality even at high speeds. Furthermore, configuring your microscope with a three-mirror scanner will ensure resolution is not lost during high-speed acquisitions. Unlike two-mirror systems, the three-mirror design better distributes the scanning workload. This reduced mechanical stress increases the stability of image acquisition and allows more precise control even at high speeds.

Equipping your confocal platform with chambers that maintain optimal temperature, humidity, and CO_2 levels is also essential for preserving sample viability and minimizing the risk of inaccuracies in your results caused by compromised cell health.

Resolution: What Structures Will You Be Imaging?

Consider the resolution needs based on the structures you aim to visualize (Figure 7). Are you imaging intracellular structures, intercellular interactions, or mapping larger tissues? For protein interaction studies or observing subcellular events, super-resolution technology will be necessary. Evaluate your experimental design, including the depth and size of the samples. Factors like numerical aperture and zoom capacity significantly influence resolution, so careful thought is needed to ensure the components of your intended microscope are fit for purpose.

Penetration Depth: How Deep into Your Sample Do You Need to Image?

Consider the penetration depth your studies require, particularly if your research involves working with thick samples or live cells. Multi-photon imaging enables deep tissue imaging with high spatial resolution and minimal photodamage (Figure 8). It uses near-infrared light and excels in penetrating up to 1 mm into tissues. This technique is well-suited to studies requiring high-resolution visualization of complex biological structures, such as brain tissue, tumor microenvironments, and embryos of model organisms, making it a valuable addition to advanced confocal systems.

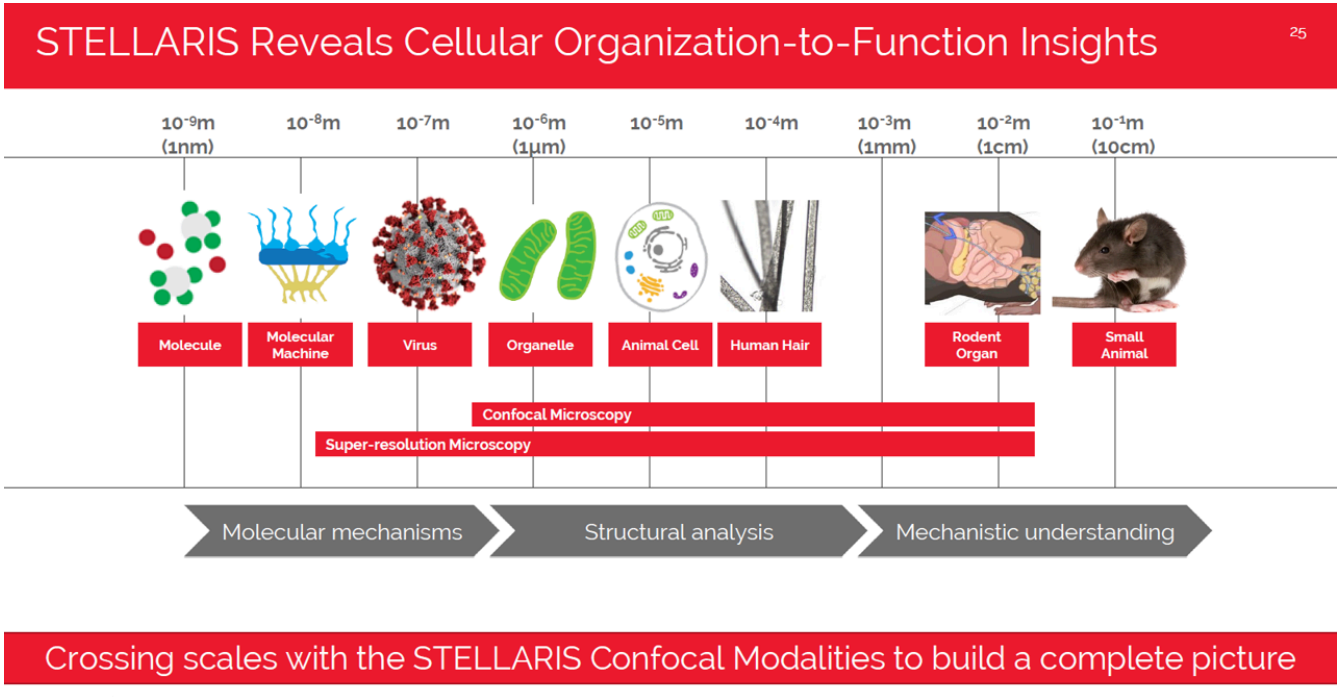


Figure 7. Confocal and super-resolution microscopy can be used to study structures ranging from molecular complexes to small animals, enabling researchers to link molecular mechanisms to larger functional insights. Leica Microsystems LSR Portfolio Introduction 2024

Resolution is influenced by several factors, including optical quality, an area in which Leica has established a longstanding reputation for excellence. Additionally, resolution is significantly impacted by the signal-to-noise ratio, which is highly dependent on the detection system and the optical design. In this context, the system's filterless optical design minimizes light loss at multiple air/glass interfaces, thereby enhancing the signal-to-noise ratio. This design approach ensures optimal performance and is tailored to meet specific research needs.

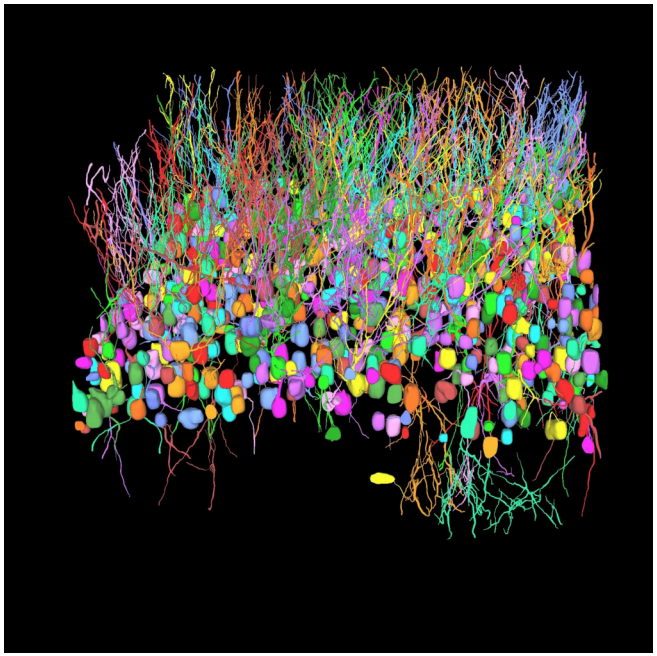


Figure 8. THY1-EGFP labeled neuron in whole mouse brain processed using the PEGASOS 2 tissue clearing method, imaged on a Leica confocal microscope. Neurons were traced using Aivia's 3D Neuron Analysis, FL recipe with Cellpose for soma detection. Image credit: Dr. Hu Zhao, Texas A&M University

User Experience: How Intuitive Does the Microscope Need to Be?

Ease of microscope use is a significant factor, especially in labs with multiple users or varying levels of expertise. A user-friendly interface will streamline workflows and reduce training time. In core imaging facilities, where researchers from diverse backgrounds must use the same system, training time is a practical reality that must be considered. A steep learning curve can hinder productivity and limit access to advanced imaging capabilities. Consider whether the control software is intuitive, how easily users can switch between different imaging modes, and how quickly personnel can be trained to use the microscope. Also look for automated features and preset configurations as these can greatly enhance user experience, enabling quicker setup and minimizing human error.

Ease of Data Analysis: How Manageable is Data Processing?

The volume and complexity of data generated by confocal microscopy can be substantial. Assess the software's capability to handle data analysis efficiently. Look for features that facilitate data processing, such as automated image stitching, 3D reconstruction, and quantitative analysis tools. Effective data management solutions, including storage and retrieval systems, are also crucial for maximizing productivity and ensuring the accuracy of your findings. Some microscopes will feature software designed to simplify data analysis and streamline workflows.

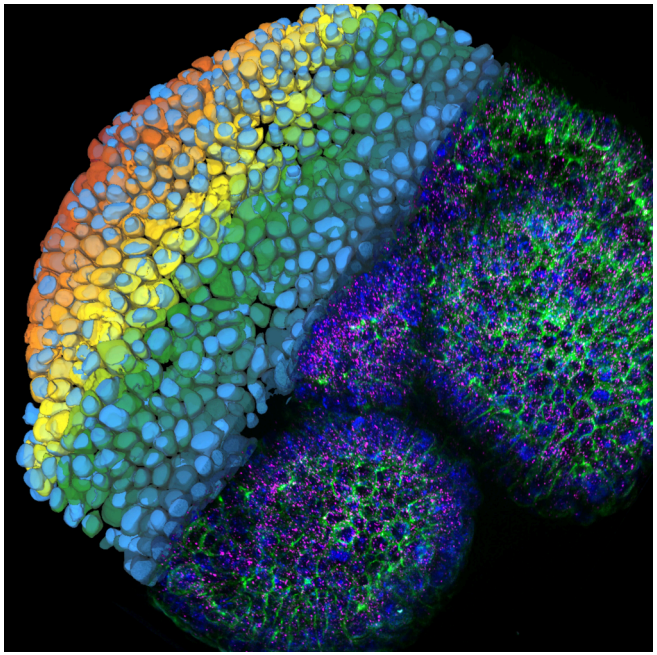


Figure 9. Intestine organoid with smFISH labeling. DNA labeling (DAPI, blue), plasma membrane labeling (green) and smFISH probe (magenta). Image courtesy Prof. Dr. Andreas Moor, Systems Physiology laboratory at the Department of Biosystems Science and Engineering at ETH Zurich.

Leica's Aivia software elevates biological image analysis with deep-learning-based cell segmentation, significantly reducing subjectivity and enhancing reproducibility. It features advanced tools that improve cell detection speeds by up to 78% and facilitate detailed exploration of complex data through interactive visualization techniques. Aivia efficiently segments and phenotypes cells in 3D multiplexed images, streamlining workflows and minimizing manual errors (Figure 9).

Inspiring and Enhancing Research Creativity with the Right Confocal Microscope

Confocal microscopes are fundamentally designed to provide excellent optical sectioning, high resolution, and high-quality image data within the traditional red, green, and blue range. While these capabilities are often taken for granted, it is important to recognize that performance can vary between different systems. For example, the STELLARIS LIAchroic system features high-sensitivity spectral detectors, a wide range of excitation lines, and an advanced optical design system, making it a powerful tool for researchers seeking these traditional capabilities (Figure 10).

However, today's research increasingly demands capabilities beyond standard 3D imaging. To meet these advanced requirements, it is crucial to consider the system's design, future-proofing, and the synergy between its components. Questions to consider include: How well has the system platform adapted to new challenges in recent years? How innovative is its design? Does the system offer scalability to meet growing research needs without significantly increasing the initial investment?

A system that meets these criteria is likely to be built on a robust platform that supports flexible solutions and maximizes the benefits for your specific research questions. Throughout this document, references are made to different applications and their respective synergy pathways. By explaining the context of the system architecture, we hope to inspire and enhance your research creativity.

Figure 10. STELLARIS LIAchroic offers spectral detection, photon-counting detectors, and confocal super-resolution. Multi-channel confocal imaging is easily achievable thanks to the smart user interface, ImageCompass, which intuitively guides researchers through experiment setup and acquisition

References

1. Huisken J and Stainier DYR. (2009). Selective plane illumination microscopy techniques in developmental biology. *Development*. 136(12):1963–1975.

2. Minsky M. (1988). Memoir on inventing the confocal scanning microscope. *Scanning*. 10:128–138.

3. Webb RH. (1996). Confocal optical microscopy. *Reports on Progress in Physics*. 59(3):427–471

4. Canette A and Briandet R. (2014). MICROSCOPY | Confocal Laser Scanning Microscopy. Encyclopedia of Food Microbiology (2nd Ed). Academic Press. 676–683.

5. White JG and Amos WB. (1987). Confocal microscopy comes of age. *Nature*. 328:183–184

6. Aslund N, Liljeborg A, Forsgren PO, and Wahlsten S. (1987). Three-dimensional microscopy using the PHOIBOS scanner. *Scanning*. 9:227

7. Nature Research Briefing. (2024). High-fidelity and fast 3D imaging of subcellular dynamics in native states. *Nature Biotechnology*.

8. Pelzer P. (2022). Multicolor Microscopy: The Importance of Multiplexing. Science Lab. URL: https://www.leica-microsystems.com/science-lab/life-science/multicolor-microscopy-the-importance-of-multiplexing/ (accessed December 12, 2024).

9. Schermelleh L, Ferrand A, Huser T, *et al*. (2019). Super-resolution microscopy demystified. *Nature Cell Biology*. 21:72–84.

10. Hell SW and Wichmann J. (1994). Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Optics Letters*. 19(11):780–782.

11. Saxena M, Eluru G and Gorthi SS. (2015). Structured illumination microscopy. *Advances in Optics and Photonics*. 7:241–275.

12. Rust MJ, Bates M, and Zhuang XW. (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods*. 3:793–795.

13. Heilemann M, van de Linde S, Schuttpelz M, Kasper R, Seefeldt B, Mukherjee A, Tinnefeld P, and Sauer M. Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. (2008). A*ngewandte Chemie International Edition*. 47:6172–6176

14. Hess ST, Girirajan TPK, and Mason MD. (2006). Ultra-High Resolution Imaging by Fluorescence Photoactivation Localization Microscopy. *Biophysical Journal*. 91:4258–4272

15. Sampietro M, Zamai M, Díaz Torres A, Labrador Cantarero V, Barbaglio F, Scarfò L, Scielzo C, and Caiolfa VR. (2021). 3D-STED Super-Resolution Microscopy Reveals Distinct Nanoscale Organization of the Hematopoietic Cell-Specific Lyn Substrate-1 (HS1) in Normal and Leukemic B Cells. *Frontiers in Cell and Developmental Biology*. 9:655773

16. Spahn C, Grimm JB, Lavis LD, Lampe M, Heilemann M. (2019). Whole-cell, 3D and multi-color STED imaging with exchangeable fluorophores. *Nano Letters*. 19(1):500–505

17. Datta R, Heaster TM, Sharick JT, Gillette AA, Skala MC. (2020). Fluorescence lifetime imaging microscopy: fundamentals and advances in instrumentation, analysis, and applications. *Journal of Biomedical Optics*. 25(7):1–43

18. Strack R. (2020). Imaging without the labels. *Nature Methods*. 19:30.

19. Jonkman J, Brown CM, Wright GD, et al. (2020). Tutorial: guidance for quantitative confocal microscopy. *Nature Protocols*. 15:1585–1611

Chapter 2: Know Before You Decide: Essentials in Your Confocal Microscope

The confocal microscope you invest in will shape your research for years to come. Your investments in essential tools today will affect the foundation of your career development. While immediate imaging needs are a priority, considering the flexibility and upgradability of your system will ensure that your microscope continues to meet your evolving requirements.

The STELLARIS confocal microscope platform from Leica is designed with your evolving needs in mind, offering state-of-the-art core components alongside a future-proof framework that seamlessly integrates advanced imaging modalities (Figure 1).

This chapter explores the essential components of a confocal microscope system and how the STELLARIS platform incorporates these, while being compatible with advanced imaging modalities. By understanding these fundamentals, you can ensure your confocal system serves as a powerful and adaptable microscope platform that grows with your research.

Figure 1. STELLARIS is the only confocal platform with an integrated WLL, combined with the AOBS and the Power HyD detector family. STELLARIS with WLL can be combined with FAst Lifetime CONtrast (FALCON), STED, Deep In Vivo Explorer (DIVE), and Coherent Raman Scattering (CRS). This platform maximizes the potential of these modalities and gives researchers the power and potential to set new standards for research.

Essential Components of a Confocal Microscope

A confocal microscope's performance is defined by its ability to deliver high-resolution imaging with quality, sensitivity, and productivity. Your decisions on the essential parts today affect your future research. The most important parts are explained in the sections below and can be visualized in Figure 2.

To reflect the selected laser line(s) to the sample and direct the emitted fluorescence to the detectors, either dichroic mirrors or AOBS is used. The collected broadband fluorescence can then be separated into multiple channels using a prism-based spectrometer. On a STELLARIS system, emission can be simultaneously detected by up to five spectrally tunable detectors.

Figure 2. Schematic of the STELLARIS scan head: A confocal system is equipped with a mechanism to scan the sample using excitation laser light directed by mirrors, and to send the resulting fluorescence signal from samples to point detectors (for example, the STELLARIS systems use photon counting Power HyD detectors) through a variable pinhole.

Excitation: The Foundation of Fluorescence Imaging

Confocal microscopy relies on laser excitation to illuminate fluorophores in the sample. The choice of light source determines imaging flexibility, resolution, and spectral capabilities.

Fixed-wavelength lasers provide reliable and stable light sources for specific excitation wavelengths. These lasers are precise but less flexible than tunable alternatives. Researchers typically use fixed-wavelength lasers for consistent imaging protocols where the spectra are well-matched to standard fluorophores like DAPI, GFP, or Alexa 647. Fixed lasers are often more cost-effective for core facility labs, where systems must be robust and accessible to multiple users working with similar and well-defined experimental designs.

Ultraviolet (UV) lasers, which typically operate around 405 nm, are used in confocal microscopy to excite UV fluorophores such as DAPI, a common DNA stain. Their ability to highlight nuclei makes them useful for experiments involving nuclear imaging, such as cell cycle and nuclear morphology analysis. Researchers can also incorporate UV lasers into multiplexed imaging workflows to label nuclei without overlapping with visible-spectrum fluorophores.

They may also be used in molecular photo manipulation experiments, such as photoactivation or uncaging, for precise release or activation of compounds in specific regions of a sample. This spatially precise control over light-activated molecules is particularly valuable in fields like neuroscience, developmental biology, and optogenetics.

Infrared (IR) and **near-infrared (NIR)** lasers are used for deep tissue imaging due to their reduced scattering at longer wavelengths, allowing them to penetrate deeper into biological samples. Researchers typically use IR and NIR lasers to visualize complex tissue structures or thick specimens, including 3D imaging of tissue and *ex vivo* samples such as biopsies.

These lasers are particularly valuable in applications requiring the visualization of structures located deep within biological tissues, such as in neuroscience or developmental biology studies. NIR lasers are often used in combination with multiphoton microscopy, where their longer wavelengths allow for the excitation of fluorescent molecules deeper within thick specimens while minimizing photodamage.

Supercontinuum white light lasers (WLL) (Component 1 in Figure 2) simultaneously emit light over a broad range while achieving the focus of a regular laser [1]. They provide a continuous spectrum of light across a wide range of wavelengths, from blue to red, offering a more versatile solution than fixed-wavelength lasers, as filters allow selection of colors to specifically excite various fluorochromes. WLL are particularly useful in multicolor imaging, where precise control over excitation is needed, and can be especially beneficial in dynamic lab environments where new markers or dyes are frequently introduced.

The STELLARIS with WLL offers complete spectral freedom, allowing researchers to select excitation wavelengths from low in the blue spectrum up to the near infrared (440 nm up to 790 nm) [2]. This configuration incorporates Leica's Acousto-Optical Beam Splitter (AOBS) technology (Component 10 in Figure 2), which eliminates traditional dichroic filters [3]. Laser settings can be fine-tuned to match exact absorption peaks with nanometer precision, optimizing signal intensity while reducing phototoxicity [4]. The AOBS ensures a smooth and efficient workflow with ultra-fast switching between excitation wavelengths. This spectral freedom is particularly beneficial for live-cell imaging, where minimizing light exposure is essential for cell viability.

Scanners: Balancing Speed and Image Quality

Scanning mirrors direct the laser beam across the sample, defining both the speed and quality of image acquisition. **Field-of-view (FOV) scanners** (Component 15 in Figure 2) provide an optimized imaging area, allowing users to capture larger sample regions without compromising resolution or speed. By maximizing the available field, FOV scanners reduce the number of scans required, which in turn speeds up image acquisition and minimizes photobleaching, making them particularly useful for large-scale imaging studies.

However, one drawback is that field size decreases with imaging speed in classic FOV scanners. Upgrading to a scanner with a larger FOV can capture more data per image, but it also generates significantly larger files. This can lead to longer processing times and increased storage requirements.

If speed is a priority, **resonant scanners** are designed to deliver rapid image acquisition. These scanners operate at high frame rates, making them ideal for capturing fast-moving processes like cellular dynamics or the movement of live cells. Resonant scanners are often preferred in applications that require real-time imaging while minimizing phototoxicity.

Tandem scanners integrate both resonant and FOV scanners into a single system, offering flexibility and adaptability for different imaging needs. With a tandem scanner, researchers can switch between the fast acquisition of a resonant scanner and the broad field capture of an FOV scanner, depending on the experimental requirements. This combination allows for a more versatile approach, making it possible to handle a wide range of imaging tasks, from tracking rapid cellular processes to capturing detailed, large-scale tissue images.

The STELLARIS offers both resonant and traditional FOV scanning modes, giving users the flexibility to optimize imaging for different sample types. This tandem capability allows scanning of large areas with high scan-resolution by a FOV-scanner and of very fast processes by a resonant scanner, both within the same instrument [5].

Detectors: Maximizing Sensitivity and Signal Fidelity

The quality of confocal imaging depends on the efficiency and precision of its detectors. Many systems offer a combination of detection systems. **Filter-based detector**s can be adjusted to isolate a select wavelength before it reaches the detector.

Spectral-based detectors allow users to define custom spectral ranges within the emission spectrum, offering flexibility beyond fixed filters. This is particularly advantageous for adapting to new fluorophores or for differentiating between overlapping fluorophores in complex multicolor setups. Spectral detectors are a versatile option for labs anticipating the need to switch between different sample labeling strategies.

Standard Photomultiplier Tubes (PMTs) are reliable and versatile detectors that are widely used in confocal microscopy. PMTs are often used in configurations where cost efficiency and robustness are key considerations. While they may not offer advanced sensitivity, they remain a solid choice for general imaging needs.

Gallium Arsenide Phosphide (GaAsP) photocathodes are known for their high sensitivity, especially in low-light conditions. They offer a significant improvement in signal detection compared to traditional PMTs, making them suitable for imaging dim or weakly stained samples.

For researchers needing even lower noise levels and better signal-to-noise ratios, hybrid detectors that combine GaAsP with advanced amplification techniques provide even greater imaging clarity. The **Power HyD** (Hybrid) detector family provides enhanced photon detection efficiency and lower noise levels, especially in the red and NIR ranges. These detectors combine the benefits of both PMTs and GaAsP detectors, offering high sensitivity and flexible spectral detection. Power HyD detectors (Component 25 in Figure 2) are suitable for applications requiring high sensitivity and a broad spectral range, enabling more accurate and reliable data across various imaging conditions.

One of the unique advantages of the STELLARIS confocal platform is its filter-free spectral detection, enabled by the AOBS and a prism-based dispersion system. Instead of fixed filters, the system uses freely adjustable spectral windows, allowing researchers to dynamically define detection bandwidths for optimal fluorophore separation. The STELLARIS WLL configuration also has highly sensitive, photon counting spectral Power HyD detector family [6] which greatly expands the capacity of the confocal microscope for sensitive imaging.

Microscope Body: Inverted or Upright Configurations

Confocal microscopes are available in upright or inverted configurations, depending on your sample type and imaging preferences. **Inverted microscopes** are preferred for live-cell imaging and experiments using cell culture in liquid media because the objective is located below the sample. This setup is perfect for studying cells in dishes or flasks, as the flat surface of the vessel serves as the imaging base. While inverted microscopes are common in cell biology, they can accommodate most sample types with flexibility across a wide range of applications.

Upright microscopes are ideal for thick tissue sections and samples requiring immersion objectives. Upright microscopes position the objective above the sample, making them ideal for larger specimens such as whole organisms or thick tissue sections. Upright models are available with either a moving stage or a fixed stage, depending on whether you prefer to move the sample or the objective for focusing. A fixed-stage upright microscope is usually the best choice for non-disruptive imaging of large or delicate samples.

STELLARIS supports both inverted and upright configurations, ensuring flexibility across a variety of research applications.

Image Analysis: Turning Data into Insights

Confocal imaging generates vast amounts of data, requiring powerful analysis tools that enable productivity without compromising accuracy. As time-lapse imaging gets longer and multiplexing capabilities increase, so does the challenge of managing and interpreting large-scale data.

Integrating Artificial Intelligence (AI) into microscopy workflows can significantly improve both imaging efficiency and data accuracy. As you evaluate potential microscope platforms, be sure to ask vendors about their AI capabilities, particularly how these tools can be applied to streamline your specific research workflows. AI solutions are already enhancing performance across a variety of applications, including:

- Developmental biology: AI algorithms can automate the detection and tracking of rare cellular events over extended time periods. This is particularly valuable in developmental biology, where long-term imaging is required to monitor processes like cellular differentiation and tissue formation.

- Cancer research: Deep learning models can enhance image segmentation, even in complex or noisy datasets. This improves the detection of subtle morphological changes, which is critical in cancer research for identifying and tracking abnormal cellular behavior.

- Drug discovery: Pre-trained AI models accelerate the analysis of large, high-throughput datasets with minimal setup. This makes them ideal for drug screening workflows, where fast, accurate image interpretation is essential for identifying potential therapeutic candidates.

User-friendly Software

Proprietary software often varies between confocal systems, so it is important to choose one that is intuitive and aligns with your workflow. A well-designed interface should be easy for both new users and experienced researchers to navigate, reducing the learning curve across your team. Investing in user-friendly software not only streamlines day-to-day use but also saves valuable time on training, enabling your lab to stay focused on research rather than troubleshooting.

Advanced Imaging Modalities Compatible with Confocal Imaging

A confocal microscope should not only meet present needs but also offer pathways for expansion. STELLARIS is a true multi-modality platform, designed to integrate seamlessly with advanced imaging techniques, such as Fluorescence Lifetime Imaging (FLIM), Stimulated emission depletion (STED), multiphoton imaging, and Label-Free Chemical Imaging.

Fluorescence Lifetime Imaging (FLIM)

Fluorescence Lifetime Imaging (FLIM) measures the time a fluorophore takes to return to its ground state after being excited. This fluorescence lifetime is a characteristic parameter of each fluorescent dye and it can change according to its nanoscopic environment or conformational state. Thus, FLIM provides information on the spatial distribution of a fluorescent molecule together with its nano-environment [7].

Leica's Aivia image analysis software is a complete analysis workflow from accurate deep learning-based cell segmentation to automatic phenotyping and data exploration for 3D multiplexed images. This AI-driven solution reduces human subjectivity in data analysis and improves consistency and reproducibility across experiments. Researchers at all levels can rapidly extract meaningful insights from complex imaging datasets, improving workflow efficiency and bridging the gap between data complexity and research productivity.

FURTHER INFORMATION

Aivia AI Image Analysis Software

Find Out More

The STELLARIS with WLL is ideally suited to be upgraded to FLIM capabilities, forming the STELLARIS FALCON. This configuration is built on fast electronics and sensitive spectral hybrid detectors for measuring fluorescence lifetimes. The system has ultra-short dead time and powerful built-in algorithms for data acquisition and analysis [8]. TauSense technology is used to process FLIM data, providing access to additional information [9]. TauSense tools are found in STELLARIS platform and include:

- **TauContrast, which provides immediate access to functional information**
- **TauGating, which improves the quality of your images by removing unwanted fluorescence contributions**
- **TauSeparation, which helps you expand the combination of fluorescent signals in your experiment beyond the spectral options**
- **TauInteraction, which provides straightforward detection and quantification of molecular interactions (e.g., protein-protein interactions)**

Super-Resolution Imaging

Stimulated emission depletion (STED) is one of the super-resolution techniques that has significantly improved our ability to understand the spatial distribution and relationships of cellular components and processes. It uses precise laser control to deplete fluorescence around a central focal point. The gentle illumination with STED allows for extended imaging of delicate specimens (more frames) or of larger volumes (more planes) without sacrificing resolution. This is particularly suited for sensitive fluorophores and for studies of cellular dynamics that require high imaging speeds [10].

Multiphoton Imaging

In contrast to fluorescence microscopy, multiphoton microscopy uses excitation wavelengths in the infrared, taking advantage of the reduced scattering of longer wavelengths. This makes it perfect for deep tissue imaging in thick sections and living animals. Applications range from the visualization of whole brain architecture to the study of tumor development and metastasis. While multiphoton imaging enables deep tissue visualization, it typically requires higher laser power, which can increase photodamage or photobleaching in live samples. The potential imaging depth is correlated to the peak power of the multiphoton laser and how well this peak power can be maintained in the imaging system [12].

For deep tissue imaging, STELLARIS DIVE™ (Deep In Vivo Explorer) multiphoton microscope extends imaging depth beyond 1 mm [13]. This configuration leverages:

- Near-infrared excitation for reduced scattering and photodamage
- 4Tune, a spectrally tunable non-descanned detector, giving you the flexibility to adapt to the combination of fluorophores you need
- The combination of DIVE™ with STELLARIS also enables the use of fluorescence lifetime-based information intrinsic to fluorophores

Label-Free Chemical Imaging

Label-free chemical imaging techniques such as Coherent Anti-Stokes Raman Scattering (CARS) and Coherent Raman Scattering (CRS) microscopy provide molecular contrast without the need for dyes or fluorescent labels. These methods rely on the specific vibrational properties of chemical bonds in response to laser light. Because nearly all molecules exhibit vibrations that serve as a molecular signature, these techniques allow researchers to generate detailed chemical maps of biological samples in a completely label-free manner [14].

Label-free imaging is especially useful for studying cells, tissues, or model organisms that are difficult to stain or prepare using conventional fluorescence methods. CRS imaging offers high-resolution, dynamic insights into the biochemical composition of living systems, supporting applications in neurodegenerative disease research, cancer diagnostics, stem cell and developmental biology, pharmacology, and 3D tissue imaging. It is also capable of detecting small molecules in real time without perturbing their natural function [15].

To push resolution beyond the diffraction limit, STELLARIS STED combines lifetime-based information with spatially resolved super-resolution imaging. This combination allows researchers to extend live-cell imaging sessions while maintaining nanoscale resolution [11]. With TauSTED Xtend, these capabilities are taken even further. Researchers can use standard fluorophores and follow their preferred protocols, simplifying experiment design and expanding accessibility. TauSTED Xtend also enables expanded multicolor imaging even when using a single STED laser line. It also supports gentle live-cell imaging at remarkable nanoscale by significantly lowering both excitation and STED light doses. This makes it possible to study dynamic biological processes at the nanoscale over extended periods.

The STELLARIS CRS configuration enables researchers to visualize the chemical composition of complex biological samples, such as tissues, organoids, or whole-mount model organisms, in 3D and at subcellular resolution, using only the sample's inherent chemical properties [16]. By combining CRS with the STELLARIS platform's high-resolution optical design and modularity, users can perform live, label-free imaging that complements traditional fluorescence approaches.

Multiplexing Imaging

Understanding complex biological systems requires imaging multiple markers simultaneously. STELLARIS SpectraPlex enables 3D high-multiplexing with up to 15+ colors, providing flexibility in experiment design. This configuration allows you to design experiments in advance with integrated functionalities, and manage data intelligently, with flexible advanced control.

The Right Platform Grows with Your Research

Selecting a confocal microscope is not just about meeting your current needs—it is about future-proofing your research. STELLARIS offers a comprehensive combination of essential components including WLL, AOBS, spectral-based ultra-sensitive detectors, and sophisticatedly designed electronics. With its modular architecture, STELLARIS evolves alongside your research with inbuilt compatibility for advanced imaging modalities.

References

1. Knight JC, Birks TA, Russell TS, *et al.* (1996). All-silica single-mode optical fiber with photonic crystal cladding. *Optical Letters.* 21(19):1547–1549.

2. Leica Microsystems. (2020). STELLARIS White Light Lasers. URL: https://www.leica-microsystems.com/science-lab/life-science/stellaris-white-light-lasers/ (accessed February 13, 2025).

3. Birk H, Engelhardt J, Storz R, *et al.* (2002). Programmable beam splitter for confocal laser scanning microscopy. Internation Symposium on Biomedical Optics, San Jose, CA, United States. (15 May 2002) *Proceedings of the Society of Photo-Optical Instrumentation Engineers.* 4621.

4. Borlinghaus R, Kuschel L, and DeRose J. (2012). The Principles of White Light Laser Confocal Microscopy. URL: https://www.leica-microsystems.com/science-lab/life-science/the-principles-of-white-light-laser-confocal-microscopy/ (accessed February 13, 2025).

5. Borlinghaus R. (2019). What is a Tandem Scanner? URL: https://www.leica-microsystems.com/science-lab/life-science/what-is-a-tandem-scanner/ (accessed February 13, 2025).

6. Schweikhard V, *et al.* (2020). Application Note. The Power HyD family of detectors for confocal microscopy. Advertisement Feature. Nature Methods. 17(10).

7. Kuschel L and DeRose J. (2022). What is FLIM – Fluorescence Lifetime Imaging Microscopy? Leica Microsystems. URL: https://www.leica-microsystems.com/science-lab/life-science/flim/what-is-flim-fluorescence-lifetime-imaging-microscopy/ (accessed May 26, 2025).

8. Alvarez L, Ossato G, Kuschel L, *et al.* (2019). A novel concept in fluorescence lifetime imaging enabling video-rate confocal FLIM. URL: https://www.leica-microsystems.com/science-lab/life-science/flim/a-novel-concept-in-fluorescence- lifetime-imaging-enabling-video-rate-confocal-flim/ (accessed February 13, 2025).

9. Leica Microsystems. (2020). TauSense Technology Imaging Tools. URL: https://www.leica-microsystems.com/science-lab/life-science/tausense-technology-imaging-tools/ (accessed February 13, 2025).

10. Leica Microsystems. (2021). TauSTED: Pushing STED Beyond Its Limits with Lifetime. Nature Methods, Article Advertising Feature. URL: https://media.nature.com/original/magazine-assets/d42473-021-00241-0/d42473-021-00241-0.pdf (accessed May 26, 2025).

11. Disapro A. (2021). Benefits of Combining STED and Lifetime. Leica Microsystems. URL: https://www.leica-microsystems.com/science-lab/life-science/benefits-of-combining-sted-and-lifetime/ (accessed February 13, 2025).

12. Mülter A. (2019). Principles of Multiphoton Microscopy for Deep Tissue Imaging. Leica Microsystems. URL: https://www.leica-microsystems.com/science-lab/life-science/principles-of-multiphoton-microscopy-for-deep-tissue-imaging/ (accessed May 26, 2025)

13. Leica Microsystems. STELLARIS DIVE™ Multiphoton Microscope. URL: https://www.leica-microsystems.com/products/confocal-microscopes/p/stellaris-8-dive/ (accessed February 13, 2025)

14. Potma EO. (2012). CARS Microscopy – Imaging Characteristic Vibrational Contrast of Molecules. Leica Microsystems. URL: https://www.leica-microsystems.com/science-lab/life-science/cars-microscopy-imaging-characteristic-vibrational-contrast-of-molecules/ (accessed May 26, 2025)

15. Schweikhard V. (2022). The Potential of Coherent Raman Scattering Microscopy at a Glance. Leica Microsystems. URL: https://www.leica-microsystems.com/science-lab/life-science/the-potential-of-coherent-raman-scattering-microscopy-at-a-glance/ (accessed May 26, 2025)

16. Leica Microsystems. STELLARIS CRS Coherent Raman Scattering Microscope. URL: https://www.leica-microsystems.com/products/confocal-microscopes/p/stellaris-8-crs/ (accessed February 13, 2025).

Chapter 3: Considerations Beyond Specifications and Features

When investing in a confocal microscope, it's important to consider not just the platform's specifications and features but also the reputation and support offered by the microscope vendor. Consider whether each vendor has a vision for supporting your research as it grows, with proven innovation, complete workflow solutions, and the service they can offer in the days, weeks, months, and years after purchase. This chapter will take you through the most important considerations to make before you invest.

Vendor Vision

To future-proof your research, look for a vendor that's building a complete imaging solution—not just selling you a microscope. A vendor with a clear roadmap for supporting the full imaging workflow, from sample preparation to data analysis, can offer more than just an instrument. A quality vendor can become a research partner, capable of adapting to scientific progress and user needs over time. Choosing a vendor that actively invests in innovation and integrates tools across the imaging pipeline ensures your microscope system remains relevant and scalable as your research evolves.

Leica Microsystems is committed to providing a complete imaging solution. Through strategic acquisitions and continued product development, Leica has expanded its offerings beyond hardware to support every stage of the imaging journey. The acquisition of ATTO-TEC, a specialist in high-quality fluorescent dyes and reagents, has strengthened Leica's ability to support optimized sample preparation. In addition, the integration of the AI-powered 3D image analysis platform, Aivia, has enabled researchers to extract deeper insights from complex datasets using machine learning tools. These additions reflect Leica's vision of delivering end-to-end solutions that enhance the quality, efficiency, and impact of scientific imaging. The essential components of a confocal microscope system and how the STELLARIS platform incorporates these, while being compatible with advanced imaging modalities. By understanding these fundamentals, you can ensure your confocal system serves as a powerful and adaptable microscope platform that grows with your research.

Vendor Reputation and Support

Your vendor's reputation, its established community of users, and its responsive service support will have a huge effect on your career-long experience with your confocal platform. Make sure to engage with scientific communities that share your specialism to learn about others' experiences with different brands. When considering long-term support, it's essential to invest in a company that not only excels in technology but also backs it with comprehensive support services to limit downtime and to help integrate new techniques as your research aims grow.

Specific Questions to Consider Before Buying

To ensure you're selecting a vendor that will be a long-term partner, consider the following questions:

How will the vendor help me use the full potential of the product?
You want to do business with a company that will help you get up and running, not just drop off an instrument at your lab. Look for a well-defined, personalized onboarding process, as well as ongoing follow-up, to make sure that everything is working as planned. Ask the sales representative whether the company offers extra training or webinars to help keep your knowledge fresh.

Are resources, such as training and access to experts, available to improve my skills?
Imaging core facilities are often staffed by experts who can help with new user training, troubleshooting, and routine maintenance. If your lab lacks this internal support, it's especially important to have a reliable tech support system to call for even simple questions. Explore the level of scientific expertise the vendor offers and ask how accessible these experts will be if you have a question. Also consider whether they can access your instrument remotely to solve any urgent problems.

Are local sales and imaging specialists available?
Does the company have field representatives who will make a visit to your lab if you need help resolving an equipment problem? What kind of lead time is typical for a visit? Microscope downtime causes delays to research, so it's important to estimate in advance what the impact on your lab could be.

Innovation and Collaboration at Leica Microsystems

Leica Microsystems has a strong culture of innovation and has historically fostered close collaboration with the scientific, medical, and industrial communities. Guided by the philosophy, "with the user, for the user", Leica collaborates with partners in microscopy and Centres of Excellence. This allows product innovation to be driven by the needs of the customer to solve real-world issues. For example, our advancements in techniques like STED have enabled cutting-edge nanoscale imaging capabilities critical for fields such as neuroscience and molecular biology.

Like our users, we are scientists devoting ourselves to making leading-edge scientific research possible. Leica Microsystems is part of Danaher Corporation, a global science and technology innovator with over 20 operating companies committed to improving quality of life around the world. Danaher's Life Sciences companies, including Leica, make leading-edge scientific research possible at clinical research facilities, academic institutions, and government agencies. Our shared purpose is helping realize life's potential.

Industry-leading Reputation and Support at Leica Microsystems

Leica Microsystems is widely recognized for optical precision and is one of the market leaders in microscopy and scientific instrumentation. In fact, Leica and its users have been shaping the future for more than 175 years, having been founded in the 19th century in Wetzlar, the "City of Optics", Mannheim, and Heerbrugg. These decades of innovation and industry-leading support ensure that your investment in Leica is not just for today, but for the future of your research.

Leica's mission is to help you unlock the full potential of your confocal platform with continuous expert support. With a diverse team of scientifically trained specialists, we are here to assist you in enhancing your imaging, from acquisition to analysis. Discover how you can benefit from our services below:

Local application support: Local sales and Advanced Workflow Specialist (AWS) teams are available to provide information and resources to help you. They can connect you with additional experts to address complex applications.

Application training at your instrument: Learn how to operate your instrument and how to set up experiments alongside one of our AWS team members on-site.

Remote and hands-on application training: Get trained on selected topics or refresh your knowledge with an AWS team member remotely connected to your imaging system.

Application helpdesk: Get support for your application by contacting our AWS team via the tailored portal, ensuring you receive timely and relevant assistance.

Digital material for self-learning: Train yourself whenever needed on various microscopy technologies and AI-based image analysis topics using our digital training tutorials and published articles.

Learn & Share knowledge portal: Explore the knowledge portal of Leica Microsystems, which offers scientific research and teaching material on microscopy at https://www.leica-microsystems.com/science-lab/life-science/.

Live and on-demand webinars: Stay informed about the latest developments and applications in microscopy presented in collaboration with leading researchers. Watch on demand at https://www.leica-microsystems.com/science-lab/life-science/#/adv/y,4/p,1 or subscribe to receive invitations for upcoming webinars.

Workshops: Hands-on workshops and specialized training events provide researchers with opportunities to apply cutting-edge imaging techniques to their specific applications, building deeper understanding and practical skills.

Conclusion

The vendor you choose will shape your experience with your confocal microscope far beyond the initial investment. Long-term success in microscopy isn't just about the hardware, it's about partnering with a reputable company that prioritizes innovation and collaboration and that has the ability to support your evolving research goals for years to come.

Researchers working with Leica benefit not just from world-class instruments but from a vendor built on a tradition of innovation that actively supports and collaborates with its users to drive scientific discovery. With Leica Microsystems, researchers gain access to a global support network, cutting-edge training resources, and tailored imaging expertise to maximize productivity and scientific outcomes.

Chapter 4: Glossary of Terms

As you and your team explore and compare confocal microscopy options, it's helpful for everyone to be familiar with the common terminology associated with the technology. This glossary provides clear definitions of key terms, helping to align your understanding and support informed decision-making.

Microscopy Techniques, Platforms, and Terms

Confocal Microscopy

A microscopy technique that uses a point light source and spatial filtering to produce high-resolution images by removing out-of-focus light. It is particularly useful for creating sharp, detailed images of cells and tissues, offering superior clarity compared to widefield microscopy.

Diffraction Limit

The fundamental resolution limit imposed by the wavelength of light. In standard microscopy, objects closer than ~200 nm apart cannot be distinguished. Super-resolution techniques overcome the diffraction limit to visualize structures at the nanoscale level..

Fluorescence Lifetime Imaging (FLIM)

A technique that measures the time it takes for a fluorophore to emit fluorescence after excitation, providing insights into the molecular microenvironment and interactions in a sample. It is particularly useful for studying protein-protein interactions and cellular metabolism.

Fluorophore

A molecule that absorbs light at a specific wavelength and emits light at longer wavelengths, producing fluorescence. Fluorophores are used to label specific biological structures or molecules for visualization under fluorescence microscopy. The term **fluorochrome** is sometimes used interchangeably, particularly when referring to fluorescent dyes.

Live Cell Imaging

A technique used to observe living cells over time, allowing researchers to study cellular dynamics such as movement, division, and responses to stimuli. Key concerns include phototoxicity and photobleaching, where too much light can damage cells.

Localization Microscopy

Techniques like PALM and STORM that rely on widefield imaging to build super-resolution reconstructions, offering high lateral resolution for fixed samples.

Multiphoton Imaging

An advanced imaging technique that uses near-infrared lasers to visualize deeper tissue layers with minimal photodamage, ideal for thick specimen imaging in fields like neuroscience and developmental biology.

Structured Illumination Microscopy (SIM)

A super-resolution technique that uses structured light patterns to produce high-resolution images. SIM is relatively easy to implement and compatible with conventional fluorophores, making it a user-friendly option for enhancing imaging resolution.

Super-resolution Microscopy

Advanced imaging techniques that provide higher resolution than traditional confocal microscopy, down to the nanoscale. Common techniques include STED, PALM. STORM, and SIM, each offering unique strengths for specific research applications.

Uncaging

A technique that uses light to release biological molecules from a light-sensitive precursor (a "caged" compound) at precise locations and times. Uncaging allows researchers to control when and where specific signaling molecules become available within a sample, making it a valuable tool for studying dynamic cellular processes, signaling pathways, and functional responses.

Widefield Microscopy

A conventional type of fluorescence microscopy where the entire specimen is illuminated, producing images with lower resolution than confocal microscopy but allowing for faster acquisition of images.

Microscopy Terms

Beam-splitting Device

A device used to separate different wavelengths of light, typically reflecting excitation light and transmitting emitted fluorescence. Common beam-splitting devices include dichroic mirrors and Acousto-Optical Beam Splitters (AOBS).

Modularity

The ability to upgrade or adapt a microscope over time by adding new components or features, such as additional imaging modes or laser lines. This ensures the microscope can evolve with changing research needs.

Numerical Aperture (NA)

A key optical parameter that defines the light-gathering ability of a lens. Higher NA values correlate with better resolution and the ability to capture fine details in microscopy.

Resolution

The ability of a microscope to distinguish between two closely spaced objects. Higher resolution provides more detailed images and is essential for studies requiring precise structural details.

Z-stacking

The process of capturing multiple images at different focal planes (depths) to create a three-dimensional representation of a sample. Z-stacking is vital for studying thick specimens and obtaining detailed spatial information.

Features and Hardware

Acousto-Optical Beam Splitter (AOBS)

A device that controls how light is directed by using acoustic waves to change a crystal's refractive index, which diffracts the light in different directions depending on its wavelength. This allows the system to quickly and precisely select the right wavelengths for imaging, making it possible to switch between different dyes or fluorophores almost instantly, without physically changing filters.

Detectors

Devices that capture emitted light from a fluorescent sample and convert it into an image. Common detectors in confocal systems include photomultiplier tubes (PMTs) and hybrid detectors (HyDs), each offering varying levels of sensitivity and noise reduction.

Excitation Sources

Components responsible for illuminating the sample with specific wavelengths of light, exciting the fluorophores for imaging. Examples include fixed-wavelength lasers, white light lasers, and UV lasers.

Field of View (FOV) Scanners

Scanners optimized for capturing large imaging areas without sacrificing speed or resolution, making them suitable for large-scale imaging studies with minimal photobleaching.

GaAsP Photocathodes

Gallium Arsenide Phosphide (GaAsP) detectors that offer superior sensitivity in low-light conditions, improving signal detection in weakly stained samples.

Non-descanned Detectors (NDDs)

Detectors located close to the sample in multiphoton microscopy setups to capture faint signals from deep within thick biological tissues. NDDs are ideal for deep tissue and multicolor experiments.

Photomultiplier Tubes (PMTs)

Standard detectors used in confocal microscopy, providing cost-effective and reliable signal detection. Though less sensitive than advanced detectors, PMTs remain suitable for general imaging needs.

Power HyD Detectors

Hybrid detectors offering high sensitivity and low noise, particularly in the red and near-infrared ranges. These detectors are used in applications that require both broad spectral detection and enhanced sensitivity.

Tunable Lasers

Lasers capable of emitting light across a range of wavelengths, providing flexibility for various applications in microscopy. Tunable lasers allow researchers to choose the optimal wavelength for specific fluorophores, enhancing imaging capabilities.

Ultraviolet (UV) Lasers

Lasers emitting ultraviolet light, typically around 405 nm, used for imaging specific nuclear stains like DAPI or for applications involving photoactivation and uncaging.

White Light Laser (WLL)

A laser that emits a continuous spectrum of light, exciting multiple fluorophores in a sample. It provides flexibility in multicolor imaging by reducing spectral overlap between dyes.

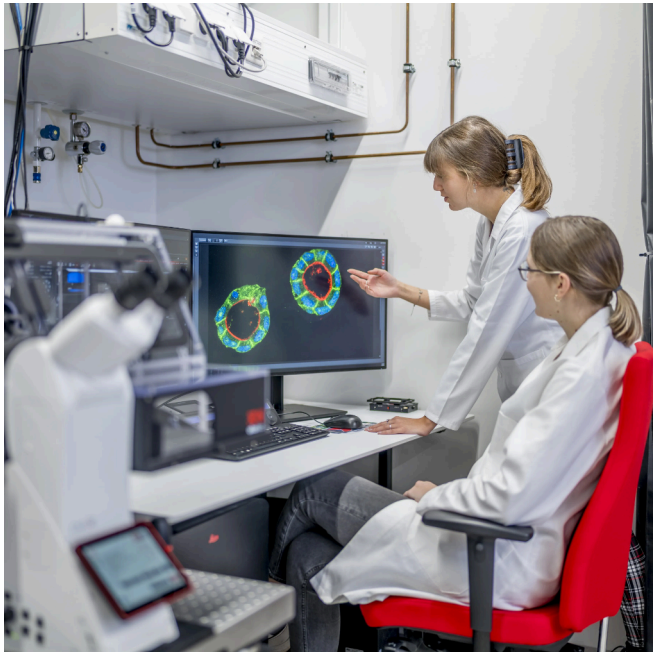
Vendor and Support Services

Advanced Workflow Specialists (AWS)

Experts provided by the vendor to help labs optimize their imaging workflows. AWS teams offer tailored advice for complex microscopy applications, ensuring that researchers can achieve the best results with their equipment.

Field Support

On-site assistance provided by a vendor to troubleshoot equipment issues. Vendors with field support teams can quickly resolve technical problems, minimizing downtime and ensuring the continuous operation of confocal microscopes.



Global Service Teams

A network of technical and application experts deployed by the vendor to provide both remote and on-site support. These teams are crucial for maintaining the functionality of confocal microscopes and minimizing operational interruptions.

In-house Experts

Qualified professionals employed by the vendor to provide specialized technical support. These experts assist in user training, addressing complex queries, and guiding researchers through advanced microscopy techniques.

Onboarding Process

The structured program provided by vendors to help new customers become familiar with their confocal microscopes. It often includes personalized training, follow-up sessions, and educational resources to ensure users can operate their systems effectively from the start.

On-demand Educational Resources

Continuously updated materials provided by vendors, including webinars, training modules, and learning tools, to ensure users remain informed about new developments in microscopy technology and techniques.

User Community

A network of researchers and professionals who use the same microscopy systems, offering peer support and knowledge sharing. Established communities provide insights into best practices, troubleshooting tips, and practical advice for optimizing research workflows.

Vendor Reputation

The standing of a microscope vendor in the scientific community, often determined by their history of innovation, product reliability, and customer satisfaction. A vendor with a strong reputation typically offers cutting-edge technologies validated by extensive research.

Data Management and Analysis

AI-facilitated Image Acquisition

AI-driven algorithms that automate image acquisition in microscopy, enabling the detection and capture of rare or time-sensitive cellular events with greater efficiency and precision.

AI-powered Image Segmentation

Artificial intelligence tools that improve the accuracy and reproducibility of segmenting images, particularly in complex datasets, offering significant advantages in cancer research and developmental biology.

Deep Learning

A subset of artificial intelligence where neural networks are trained to analyze and interpret large datasets. In microscopy, deep learning models enhance image analysis and data segmentation, improving research workflows.

Data Management

Refers to the tools and software used to store, analyze, and manage the large amounts of data generated by confocal microscopes. Efficient data management is essential for maintaining productivity and ensuring data accessibility for future research.

Machine Learning

A subset of artificial intelligence that focuses on the development of algorithms that enable computers to learn from and make predictions or decisions based on data. In microscopy, machine learning techniques can enhance image analysis, automate segmentation, and improve data interpretation, leading to more accurate results and streamlined workflows.

Segmentation

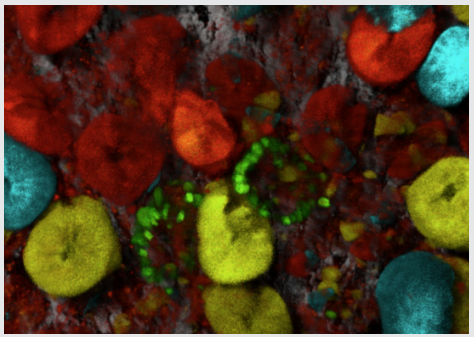
The process of dividing an image into distinct regions or objects, such as separating individual cells or structures from the background. Segmentation allows researchers to analyze specific features within an image, including size, shape, intensity, and location. Modern confocal systems often use AI-powered segmentation to improve accuracy and reduce manual processing.

Choosing the Right STELLARIS Configuration for Your Research

The STELLARIS platform is a modular system, built to grow with your research. Every configuration starts with the same STELLARIS core platform, and additional technologies can be added as your scientific questions evolve.

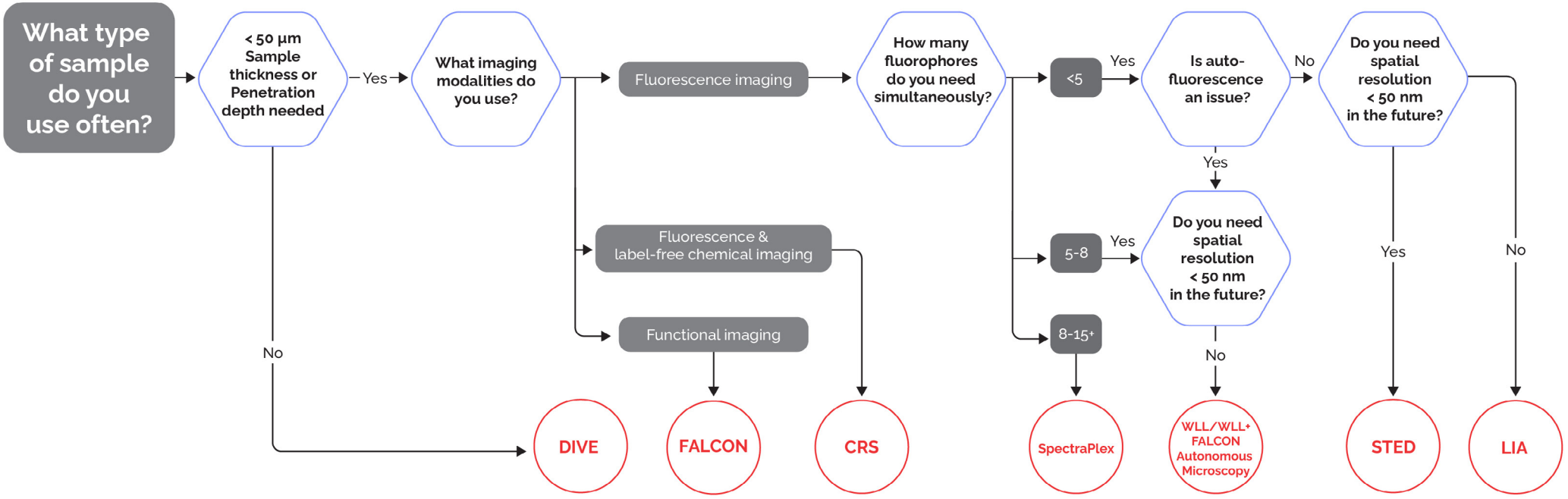
This decision tree will help you see which technologies fit your current needs, and which add-ons you can choose in the future.

DIVE X



STELLARIS DIVE multiphoton microscope provides you with flexible multicolor imaging beyond 1 mm in depth. Furthermore, you can expand the potential of your experiments with additional label-free imaging capabilities.

Figure: Confetti mouse small intestines: Collagen1 is shown in gray (label free SHG) and lineage traced stem cells are shown in cyan, green, yellow, and red. Sample courtesy of Jacco van Rheenen, Netherlands Cancer Institute, NL.



Reset Buttons

Click a module to find out more

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Front cover image: STELLARIS confocal microscopes can be combined with all Leica modalities, including FLIM, STED, MP, DLS, and CRS. The next generation STELLARIS confocal platform is your fast track to rewarding research.

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