

Opterra II

Swept Field Confocal Microscope

- Enabling 4D Live-Cell Fluorescence Imaging through Speed, Sensitivity, Viability and Simplicity

● The Most Advanced Technology for Live-Cell Research

Building on the swept field confocal (SFC) technology of Bruker's first-generation Opterra, the Opterra II system is the latest advancement in high-speed fluorescence microscopy designed specifically for live-cell studies. It uniquely combines the resolution of traditional confocal systems with the speed typically associated with wide-field imaging. With specialized input optics that produce a highly uniform field of view and a sensitive CCD camera for detection, the system produces quantitative data in all dimensions while ensuring cell viability. The user-selectable aperture sizes provide flexibility to achieve the optimal balance of speed, resolution, and fluorescence intensity in real time.



Opterra II offers scientists benefits that traditional or "next generation" spinning disk systems simply cannot match. With its short acquisition times and cell-protecting minimization of photobleaching and phototoxicity, Opterra II is ideal for advanced live-sample studies, including protein localization and trafficking, intracellular ion imaging, microtubule and vesicle dynamics, and nuclear structure and dynamics.

Only Opterra II offers market-leading...

- Flexibility in speed, resolution, and fluorescence intensity enabling optimization of experimental conditions across an array of life science research areas
- Field uniformity enabling quantitative analysis of acquired images in all dimensions
- Low phototoxicity/bleaching enabling time-lapsed volumetric studies even on the most sensitive specimens

Surpassing the Limitations of Spinning Disk Confocal

Flexibility Optimized for Every Experiment

The “iron triangle of imaging” is a concept that illustrates the balance of the three main aspects of microscopy imaging—speed, fluorescence intensity, and spatial resolution.

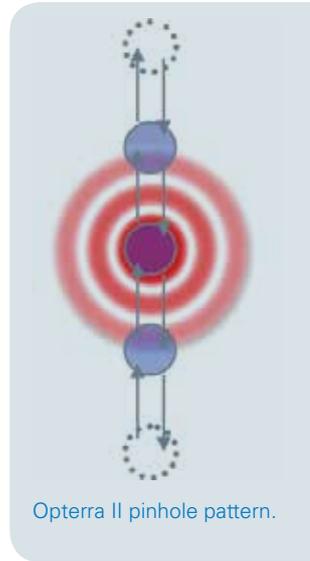
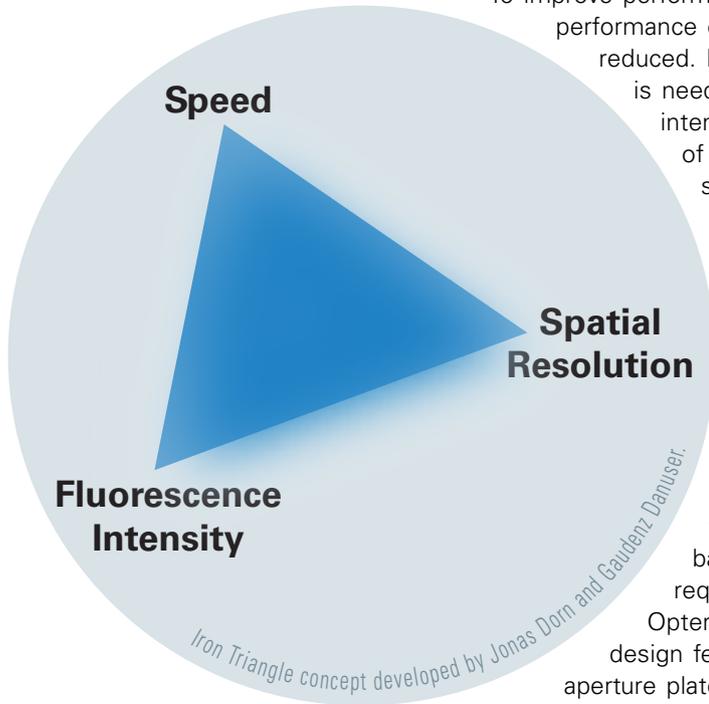
To improve performance for one facet requires that the performance of one or more of the other facets be reduced. For example, if more spatial resolution is needed, some speed and/or fluorescence intensity must be traded for it. The design of some imaging systems, such as spinning disks, is fixed so that trade-offs between these essential parameters cannot be made and the system cannot be customized to specific experimental conditions.

Understanding that no two experiments are alike, Bruker designed Opterra II to flexibly adjust speed, resolution, and fluorescence intensity based on experiment requirements. The

Opterra II's unique scanner design features a motorized aperture plate that contains pinholes of three different sizes, and slits of four

different widths. Pinholes provide maximum resolution, while slits allow for higher speed acquisitions. Aperture selection is controlled by software, so hardware changes are not required for setting up different experiment protocols, and optimally matching a selected aperture to each objective is a breeze.

Opterra II's use of one-dimensional pinhole arrays offers significant advantages over systems based on two-dimensional arrays, such as spinning disk confocal microscopes. Its array produces half, or less, of the crosstalk of a two-dimensional array, producing sharper images with superior optical sectioning and greater depth. Imaging galvanometers and piezos are precisely synchronized with a high-speed camera, filter-wheel, and piezo stage to provide unparalleled multidimensional confocal imaging of live cells and small organisms. The integrated automated bypass module provides flexibility to perform phase, DIC, brightfield, or epifluorescent imaging.



Opterra II pinhole pattern.



• Designed for Quantitative, Cell-Friendly Imaging

Uniform Sample Illumination and Emitted Light Detection

Highly uniform illumination of the field of view is a requirement for quantitative measurements of fluorescent intensities across and between images. Uneven illumination results in features with the same concentration of fluorophores erroneously displaying different and unequal intensities. Image processing algorithms cannot properly determine distributions or morphologies of objects if there is a fluorescence intensity gradient across the field of view and errors in colocalization and ratio image are produced. The effect is visually apparent in montages created by stitching multiple fields of view together.

The Opterra II has been specifically designed to evenly illuminate the field of view. When measured by a beam profiler, beyond the pinhole aperture plate, the illumination beam shows 3% to 4% deviation across the field. The fluorescence that is emitted from the specimen must also be effectively transmitted through the imaging system to the camera. Standard spinning disk microscopes have a roll-off across the field of view of 30% to 70%, preventing real quantitative measurements, and some can only guarantee performance with a single dichroic.

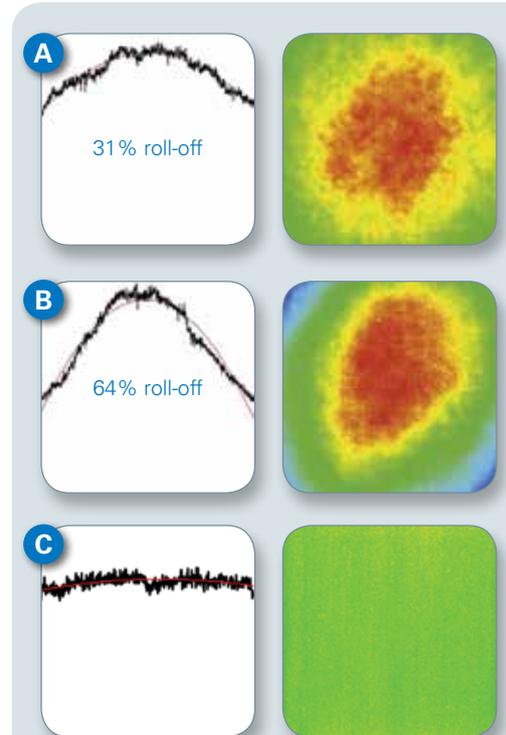
The Opterra II's excellent and guaranteed field uniformity is a standard feature, works with multiple dichroics, and provides unparalleled quantitative performance right out of the box. For example, when fluorescence emission of a concentrated dye solution is measured at the camera, the deviation across the field is guaranteed to be *less than 10%*.

Low Photobleaching and Phototoxicity

Photobleaching and phototoxicity in fluorescence live-cell imaging are largely provoked by excited fluorophores that produce reactive oxygen species. When these oxygen species react with a large variety of easily oxidizable components, such as proteins, nucleic acids, lipids and fluorophores they cause a loss of fluorescence signal called photobleaching. Phototoxicity occurs when the oxidizable components stop the cell cycle or cause cell death.

The Opterra II is designed to control the dose of excitation light by minimizing the excitation light exposure time and delivering light to specific locations only when needed. The size of the confocal aperture and the exposure time can be adjusted within the software to minimize the excitation light appropriately for each sample. The optical path also has been optimized to ensure that emitted photons are efficiently transmitted to and collected by the detector.

The Opterra II allows researchers to perform studies on highly sensitive samples not possible with other instruments, such as standard confocal microscopes, not only because specimens are kept alive, but because cellular function is maintained as close to biological conditions as possible.



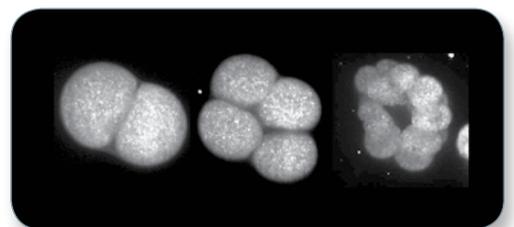
Field of view uniformity data for spinning disk imaging versus Opterra II. Graphs show captured data point values; performance curve is shown as a red line overlaying black data points.

Figures A and B reprinted from *Methods in Cell Biology*, Volume 123, J. Oreopoulos, R. Berman, and M. Browne, "Chapter 9 - Spinning-disk confocal microscopy: present technology and future trends," page 159, 2014, with permission from Elsevier.

A: Typical "good" spinning disk performance showing a 38% deviation in uniformity taken at 488 nm excitation. Field of view created through a 1.25x camera lens, captured on a 512 x 512 EMCCD.

B: Example of "poor" spinning disk performance showing a 69% deviation in uniformity taken at 642 nm excitation. Field of view created through a 1.25x camera lens, captured on a 512 x 512 EMCCD.

C: Typical imaging performance using Opterra II showing a 7% deviation in uniformity taken at 488 nm excitation. Field of view created through a 1.0x camera lens, captured on a 512 x 512 EMCCD.



Sea urchin embryo, 40x objective, 561 laser, 56 planes, 2 μm spacing, stack every 1 minute.

● Hardware for High-Performance Data Acquisition

The Opterra II tightly integrates scanner, CCD camera, illumination, filtering, and motion control devices to provide high-speed, timed volumetric, 4D imaging capabilities.

Filter Wheel

Opterra II includes a 10-position high-speed filter wheel, the fastest available, for emission wavelength selection. Switching time between positions is automatically adjusted from 45 to 110 milliseconds depending on filter position and number of filters loaded. Experimental timing is automatically adjusted and calculated based on filter selection. The filter wheel setup options provide maximum flexibility between loading and speed.

Helios Laser Launch

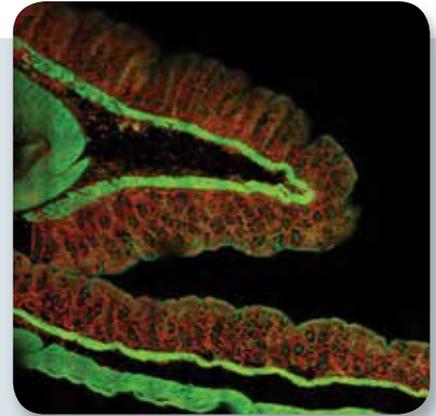
The Helios laser launch is a solid-state laser system designed specifically for laser-based microscopy applications such as confocal imaging and photoactivation. It provides ultra-stable performance for up to five laser lines (with a choice of 10+ wavelengths). Automated laser modulation and blanking ensures the laser is on only when needed and only at the fluorescence intensity needed. Timing is also auto-controlled.

LED Brightfield Lamp

Opterra II uses a high-power white light LED for brightfield illumination, and its fluorescence intensity can be modulated and blanked. Opterra II's fast switching times and synchronization capabilities enable it to create brightfield images through pinholes or slits while in confocal mode, or through the bypass module.

Stage and Focus Control

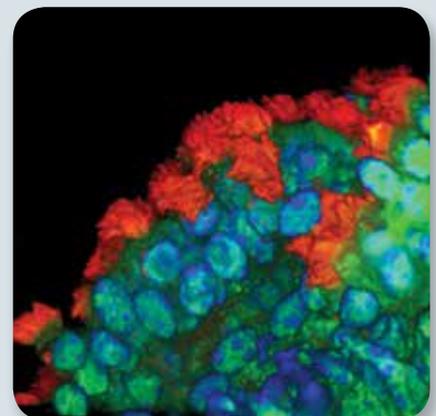
Piezo Z-focus can be combined with stage movement for efficient collection of 3D stage montages, as well as timed acquisitions at multiple stage locations in individual sample chambers or multi-well plates. The Piezo Z-focus device provides 300 microns of range with 4.5-nanometer resolution, while the stage has a 110-millimeter travel range with 0.088-micron resolution.



Pancreas tumor section, 3D montage of 2 mm x 1.5 mm area at 60x.



Helios laser launch.



Mouse oviduct, maximum intensity projection, 200 Z-planes, 0.2 μm spacing, 100x objective.

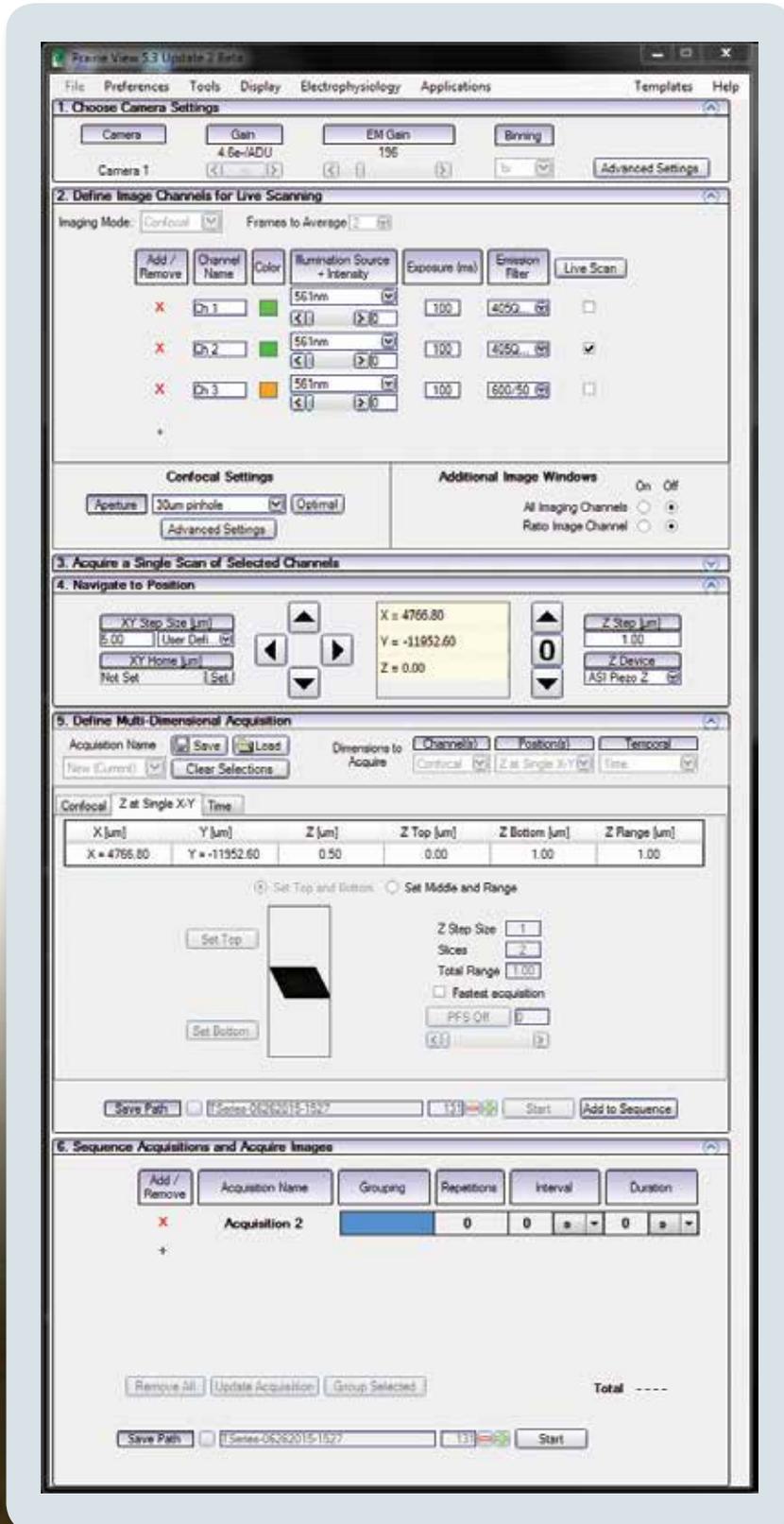
• Nimble, Feature-Rich Software That's Easy to Use

Choice of User Interfaces

Multiple user interfaces—basic, advanced, and programming—provide flexibility to meet the needs of each particular lab setting, enabling users of all abilities and individual preferences to quickly and effectively perform experiments.

The Opterra II features three different user interfaces: a basic user-friendly mode for novice users, an advanced mode for complex cases, and an externally programmable interface. Users may also seamlessly switch between the different user interface modes while setting up an experiment based upon their application needs and comfort level. Prairie View software enables users to integrate external devices into their protocols by providing a rich environment for creating triggers and analog signals, as well as recording analog signals from other devices. Time-lapse, Z-series, stage montage and multidimensional acquisitions can be defined in a matter of seconds.

PrairieLink allows communication between Prairie View and external programs to create a closed-loop system. For example Prairie View can be configured to acquire data and pass it to a third-party program for real-time analysis. The third-party program can then command Prairie View to run certain acquisition tasks based on the analysis performed.



Multiple Modalities

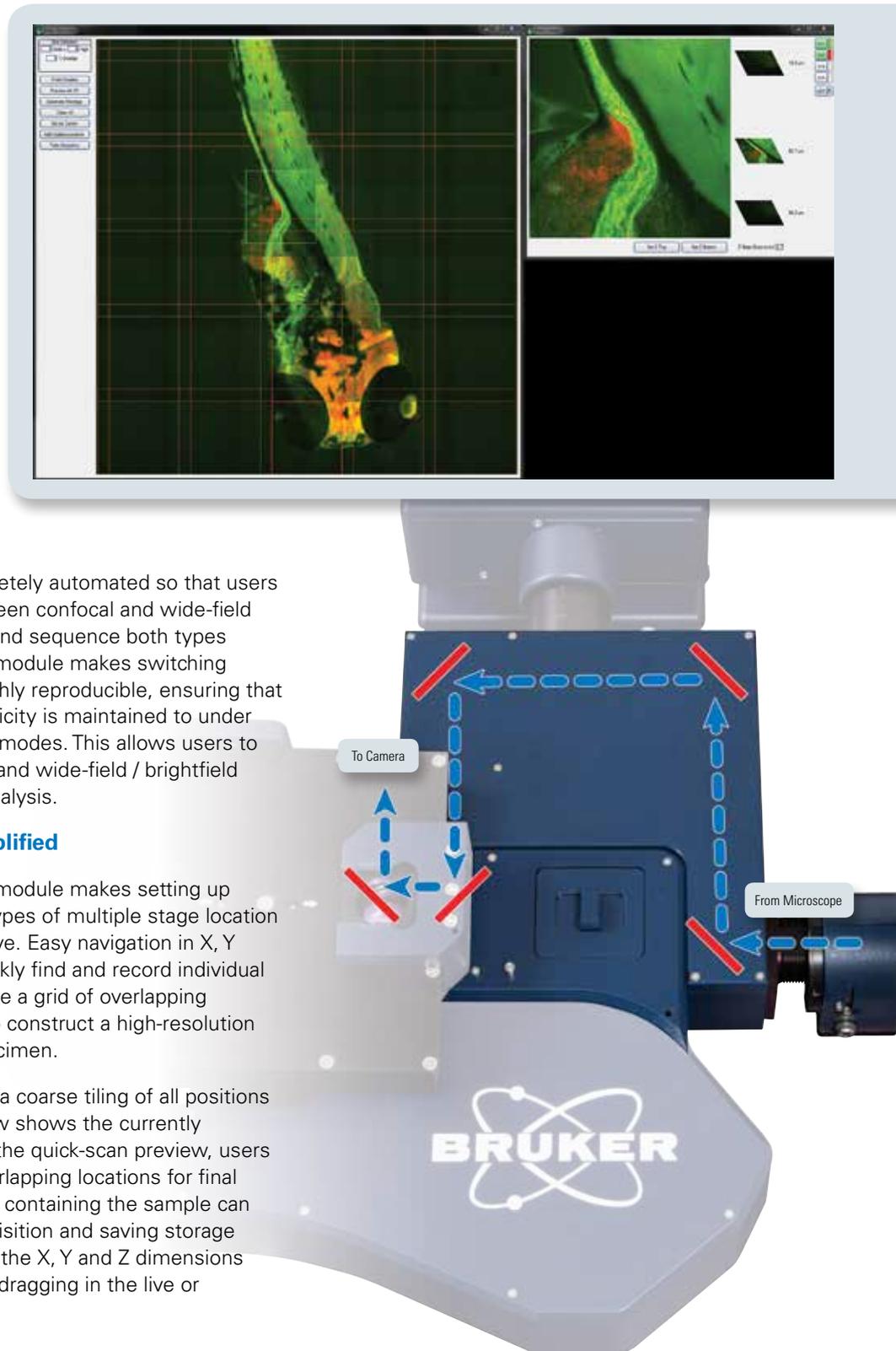
The Opterra II base system is equipped with a bypass module that uses a series of mirrors to alter the light path so that it bypasses the confocal scan head, passing light directly to the camera. This provides researchers with the flexibility to perform wide-field fluorescence imaging or brightfield imaging on the same system, using the same camera.

The bypass module is completely automated so that users can seamlessly switch between confocal and wide-field modes within the software and sequence both types of experiments. The bypass module makes switching between imaging modes highly reproducible, ensuring that both parfocality and parcentricity is maintained to under 3 microns between imaging modes. This allows users to confidently overlay confocal and wide-field / brightfield images as a part of image analysis.

Multi-Field Imaging Simplified

Prairie View's Atlas Imaging module makes setting up stage montages and other types of multiple stage location paradigms simple and intuitive. Easy navigation in X, Y and Z allows the user to quickly find and record individual locations of interest, or define a grid of overlapping locations that can be used to construct a high-resolution image of up to an entire specimen.

The preview window shows a coarse tiling of all positions scanned, and the live window shows the currently selected field of view. From the quick-scan preview, users can define a montage of overlapping locations for final acquisition. Tiles in areas not containing the sample can be turned off, speeding acquisition and saving storage space. Intuitive navigation in the X, Y and Z dimensions is performed by clicking and dragging in the live or preview windows.



● Swept Field vs. Spinning Disk Confocal

Opterra II is the clear winner in side-by-side comparisons with spinning disk systems. It is a true next-generation, high-speed confocal system specifically designed for the myriad experimental variations required for quantitative imaging of live-cell specimens. Opterra II offers scientists market-leading features and benefits that even the most advanced spinning disk systems simply cannot match.

Features	Opterra II	Spinning Disk
High-speed operation	● Standard	● Standard
Highly uniform field of view enabling quantitative imaging	● Standard	● Option available on some systems (at additional cost)
Reduction in bleaching and phototoxicity compared to point-scanning confocals	● Significant	● Some
Availability of slits for acquisitions of up to 1,000 frames per second	● Standard	● None
Ability to automatically select different pinhole sizes to match objectives to obtain highest resolution possible with no hardware changes	● Standard	● Limited option (at additional cost) available on some systems and requires hardware change
Improved axial resolution and improved imaging depth with linear pinhole configuration	● Standard	● 2D cross talk between pinholes
Flexibility to optimize speed, resolution, and sensitivity based on experimental conditions	● Standard	● None
Automated bypass module enables wide-field imaging (Phase, DIC, brightfield, and epifluorescent)	● Standard	● Option available on some systems (at additional cost)

Optional Components	Opterra II	Spinning Disk
Simultaneous photomanipulation and imaging	● Standard	● Option for some systems
Simultaneous photomanipulation and imaging with no emission signal loss	● Standard	● Not possible
Spectral imaging at 4 frames per second with 15 spectral channels per frame with 512x512 pixels per image	● Significant	● Not possible
Choice of spectral unmixing algorithms including real-time blind unmixing	● Standard	● Not possible
Simultaneous multi-wavelength	● Standard	● Option for some systems

● Bruker Nano Surfaces Division

Middleton, WI • USA
 Phone +1.608.662.0022
productinfo@bruker.com

www.bruker.com/opterra

Cover images: Top left: Xenopus embryo – measuring calcium change.
 Center: *C. elegans* – GFP and mCherry.
 Top right: Mouse oviduct – maximum intensity projection.
 Bottom left: Sea urchin embryo – phototoxicity.
 Bottom right: Xenopus embryo – photoconversion and photoactivation.