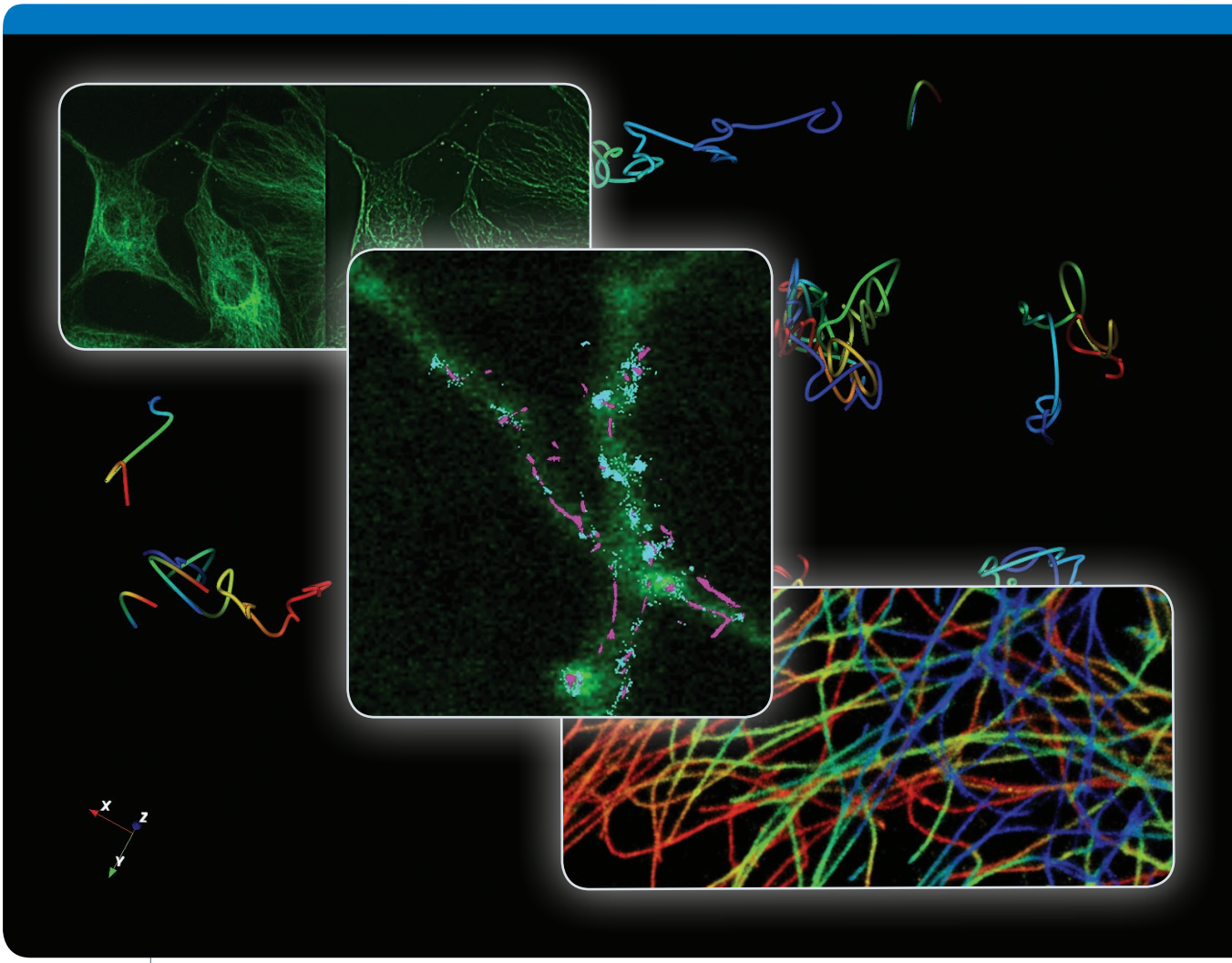


Vutara 352

- **World's Only Quantitative Super-Resolution Microscope**
  Fast and Meaningful 3D Imaging for Live Cells

Innovation with Integrity

Fluorescence Microscopy

Vutara 352

Don't Get Left Behind

Bruker's Vutara 352 is at the leading edge of super-resolution microscopy, providing the fastest super-resolution, single-molecule localization (SML) commercially available. The Vutara 352 enables 3D SML and video-rate nanoscopy through Bruker's proprietary ResEnhanced™ and Quadfield™ technologies.

The Vutara 352 is the only system of its kind, opening a new realm of research possibilities:

- Video-rate SML microscopy
- Live-cell, super-resolution protocols and workflows
- Simultaneous four-color super-resolution imaging (including a 750 nm excitation option)
- 3D super-resolution up to 15 um deep, with Z stacking capability
- All raw data is stored and available in a non-proprietary format for inspection and novel analysis
- Comprehensive online protocol and support library to speed you to publication

"The trend toward systematic and quantitative analysis of cellular systems has not been expanded to nanoscopy, possibly because high recording speeds of large FOVs have been lacking, thus impeding the use of SMSN (single-molecule switching nanoscopy) for systematic studies of complex cell biological processes."

– Huang, Bewersdorf, et al. 2013, Nature Methods

Vutara 352 Features

Patented Technology	ResEnhanced Technology; Quadfield Technology; Biplane™ Imaging
Super-Resolution Imaging	20 nm XY and 50 nm Z resolution
Optimized laser illumination	Supports up to five 1,000 mW lasers; Integrated 750 nm laser included; Proprietary top hat laser illumination edge to edge for consistent results
Superior Drift Management	Integrated thermal temperature control; Environmental isolation; Multi-modal drift correction
Custom Case	Designed for super-resolution, environmental isolation, temperature regulation, and drift minimization
Measured Point Spread Function	Actual PSFs for each color are measured during calibration to validate and optimize imaging session
Support for High-NA Objectives	Water, silicone, oil for matching refractive index to imaging medium 60x, 100x, 150x
Biology Research Design	Expert images the day of installation; Raw data stored and available in non-proprietary format for inspection and analysis; Comprehensive online protocol and support library
Options	High-speed confocal scanner; Incubation chamber for live cell imaging; External sideport; Total Internal Reflection Fluorescence (TIRF)

Video Rate Super-Resolution Nanoscopy

Rapid Imaging

For the core facility director or PI, the Vutara 352 maximizes the capacity of your facility by getting you more data, faster. Imaging sessions can last hours, but Bruker's Vutara video nanoscope accelerates the image acquisition process to seconds. With automation in mind, this system will free up your schedule and enable you to spend more time developing solid research strategies. Let the instrument do the work, while you're drafting your next publication.

Quadfield Technology

Bruker's Quadfield module is optimized for sCMOS detectors, while maintaining the benefits of the proprietary 3D Biplane. Where the Biplane was optimized for the 3rd dimension, the Quadfield module fis optomized for the 4th dimension, "time."

800 frames per second

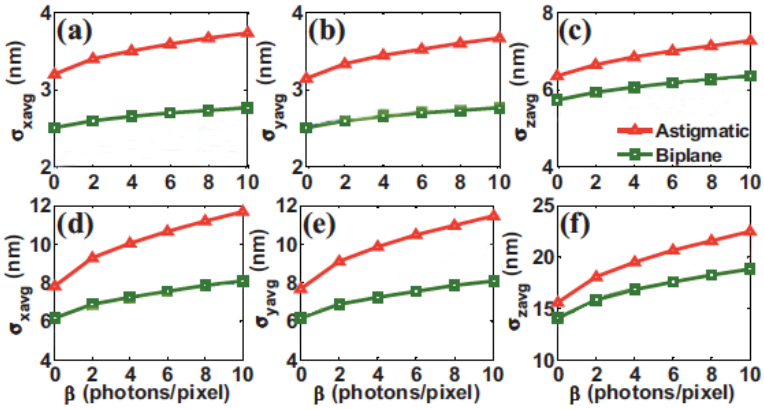
Single color BSC1 Cell labeled with AF647(XY view), 800 fps

3,000 frames per second

Single color BSC1 cell labeled with AF647(XY view), 3000 fps

Biplane vs. Astigmatism

Bruker's patented Biplane technology offers higher localization precision than astigmatism over a larger axial range, making it the preferred commercial 3D super-resolution technique. In comparison to astigmatism, the Biplane technique offers an enhanced per-pixel SNR resulting in superior localization precision. The Biplane and Quadfield modules detect the PSF from two different focal planes, and sum the total number of photons, which yields superior localization precision over a larger axial range (without the perceived loss of photons).



Average localization precision (sigma) vs background (beta) comparison for x,y,z (a,b,c) for N=6,000 (top panel) and N=1,000 photons (bottom panel). For all signal to background levels, the Biplane technique shows superior results.
(Adapted from Moerner, Badierireostami, et al. *Applied Physics*)

Uncommon Ease of Use

The Vutara 352 utilizes intuitive, step-by-step software that is specifically designed to flatten the learning curve for all users. Its open-architecture framework enables researchers to collaboratively access raw data and individually localized molecules.

Vutara 352 software provides:

- Fast real-time imaging (online)
- Advanced 3D visualization
- Integrated drift correction (three methods)
- A full palette of statistical tools
- Integrated tiling and autofocus
- Customizable speeds
- Complete control over laser and filter selection
- Variable rendering methods
- Precise localization
- Portable 3D viewing
- User-customizable features
- Variable background minimization
- Open software export options
- The capability to work with third-party algorithms
- Third-party software/module support
- Measured point spread functions

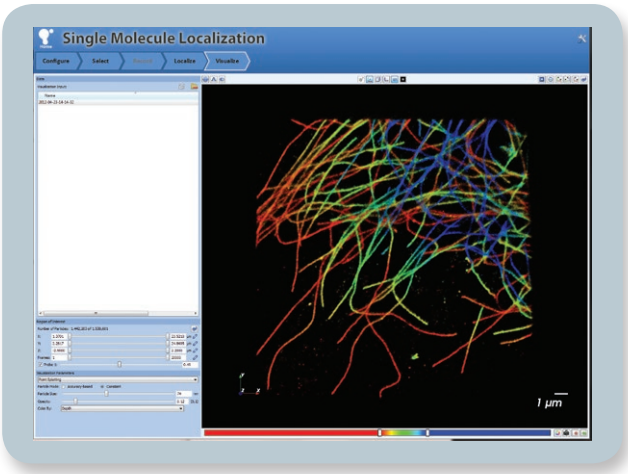
The Vutara's modular software offers simultaneous multicolor imaging, particle tracking, and offline analysis for high-use facilities. Additionally, it has an open-architecture design enabling multiple export options for maximum data analysis and visualization flexibility.

High Sensitivity

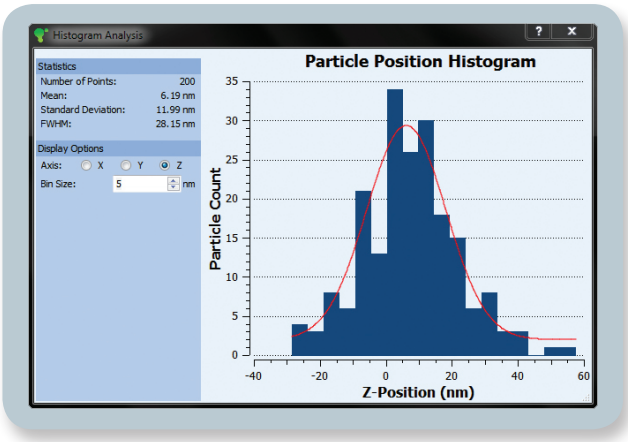
Bruker's ResEnhanced technology employs algorithms developed by the Bewersdorf lab and enhanced by Bruker, allowing extremely rapid super-resolution SML performance. ResEnhanced technology is a pixel-specific characterization and correction algorithm that uses the latest generation sCMOS imaging to enable research at frame rates previously unattainable.

Parallel Processing

Analyzing thousands of frames of data through a single CPU thread is no longer realistic, so through ResEnhanced technology and the rapid readout speed of sCMOS detectors, Bruker has harnessed the parallel architecture of CPUs and GPUs to provide next-generation processing capabilities. Using multiple threads, localization can now keep up with recording speed to acquire sub-second super-resolution images.



Real-time visualization of super-resolution image of microtubules, color-coded for depth.



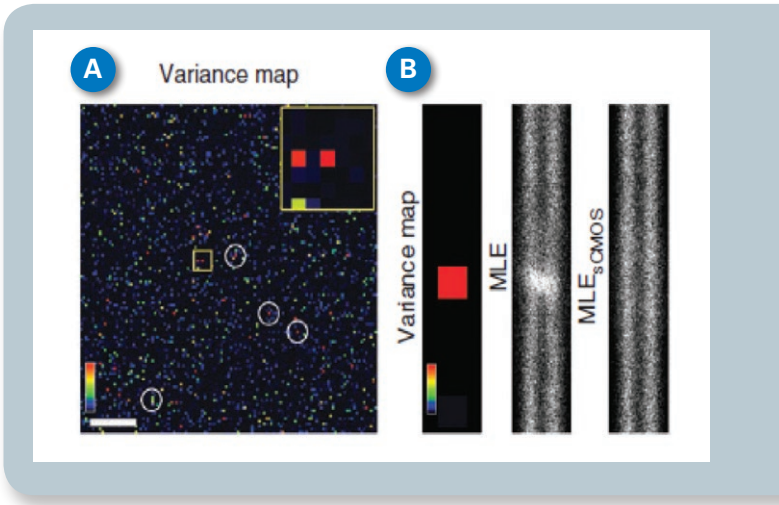
Histogram showing z data, (12 nm localization precision, 28 full-width half maximum, using TetraSpeck beads. Color co-localization x=6 nm, y= 4 nm, z=5 nm).

Software for Expanded Capabilities

Quadfield and SRX Software

Vutara's patented Quadfield and SRX software allow simultaneous two- or four-color imaging, as well as interleaved and sequential multicolor imaging.

Background signal can often be an issue with fluorescence imaging. To mitigate this issue, all Vutara systems include 1,000 mW lasers to image at high-speeds and and in four colors. Near infra-red dyes can be used to avoid autofluoresence background in samples (Tynan et al. *PLoS ONE* 2012).

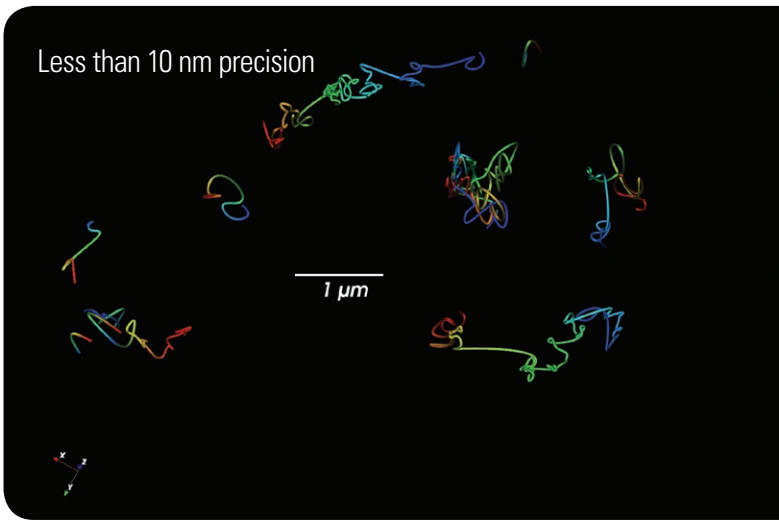


Variance map showing pixel-specific noise (A). Characterization and correction of pixel-specific noise (B). (Huang, et al. 2013, Nature Methods).

3D Particle Tracking at 3,000 FPS

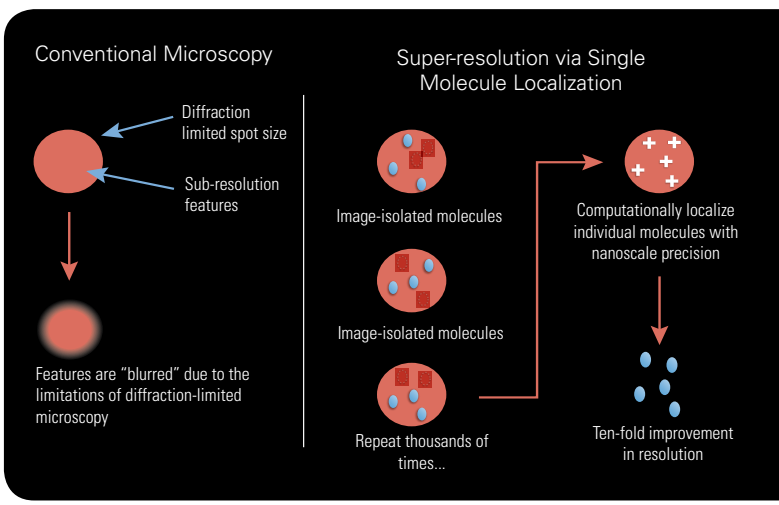
Single molecule particle tracking allows researchers to analyze areas of interest, such as the trajectory of motion or heterogeneities of motion, in regions of fast transport. For example, the trajectory of proteins or lipids in live cells can be tracked to infer the mechanisms affecting their motion.

Bruker's exclusive Biplane technology enables the tracking of particles in 3D with 10 nm precision at rates of up to 3,000 frames per second, in multiple colors at the same time.



SML Beyond the Diffraction Limit

Vutara 352 utilizes SML to circumvent the diffraction limit of light by randomly activating and deactivating a sparse, isolated subset of fluorophores within a sample. This enables the construction of complete super-resolved images for a wide range of samples, including cells and tissues.



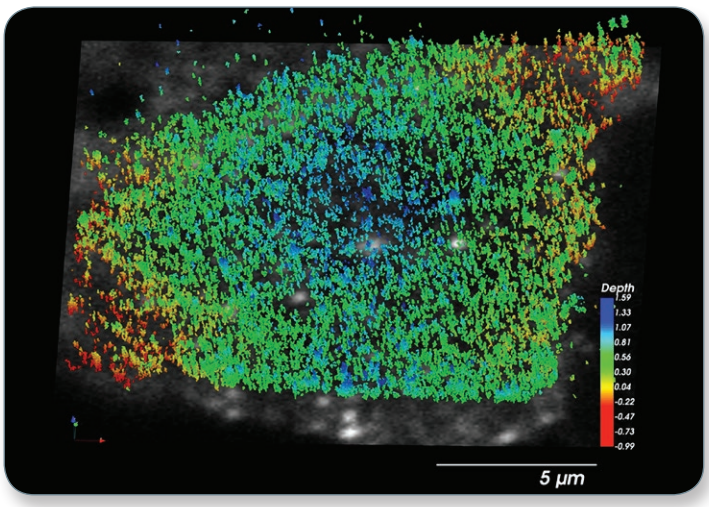
• Acquire ➔ Localize ➔ Analyze

Turn Voxels into Information

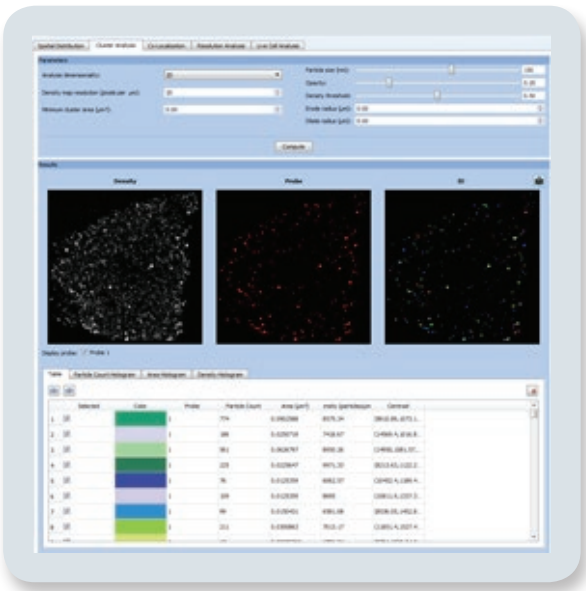
The Vutara 352 employs a wide range of statistical analysis functions to convert vibrant 3D super resolution images into meaningful quantitative data. Researchers can obtain immediate results at the instrument, or analyze data offline at an analysis workstation.

New quantitative analysis features include:

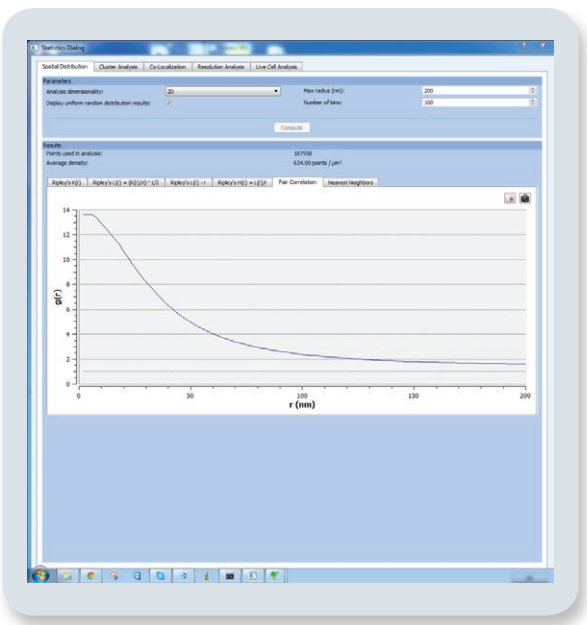
- Spatial Distribution — Provides a variety of tools for analyzing spatial distribution relationships of particles, including Ripley's K, pair correlation, and nearest neighbors
- Cluster Analysis — Counts clusters, cluster sizes, and cluster densities
- Co-Localization — Provides statistical measures on relationships between particles of two different labels
- Resolution Analysis — Quantifies resolution for images derived from localized data
- Live-Cell Analysis — Tracks clusters in live-cell experiments with mean squared displacement and angular displacement analyses



Alexafluor-633 labeled GPI-protein GFP-FR, plasma membrane protein that forms distinct clusters.



Screenshot of Cluster Analysis toolkit. Analyzes localization clusters for count, size, and area.



Pair Correlation function as a function of distance.

Add High-Speed Confocal Scanning

Modular in Design

The Vutara platform is a modular system that was designed from the ground up to grow with your research. The standard configuration includes 3D super-resolution imaging, widefield imaging, transillumination, and 3D particle tracking. Options can be added at any time to increase the capabilities of your Vutara system as you require.

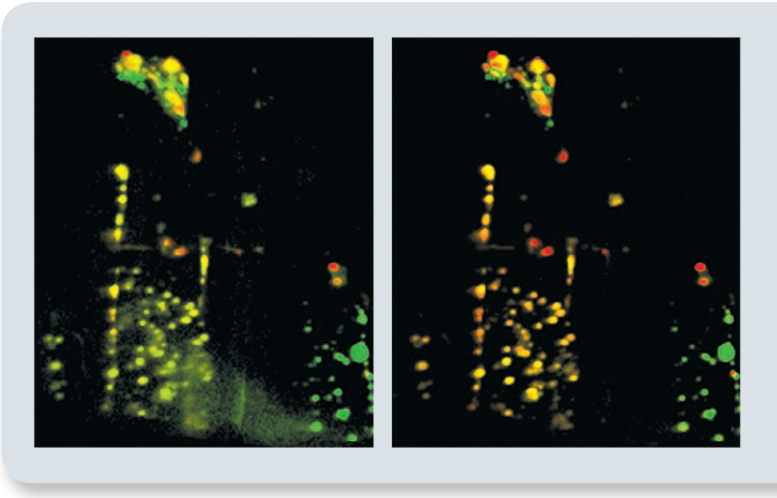
Opterra SR Confocal Scanner Option

The Opterra SR confocal scanner, which is based on Bruker's Opterra II Multipoint Scanning Confocal Microscope, adds imaging flexibility to the Vutara 352 system. The Opterra SR scanner can function as a dedicated confocal scanner, allowing confocal imaging of fixed or live-cell samples. Live-cell 4D experiments are easy to perform. Fast deconvolution built into the SRX software provides enhanced images in seconds, and interactive visualization tools allow 3D reconstructions to be viewed over time.

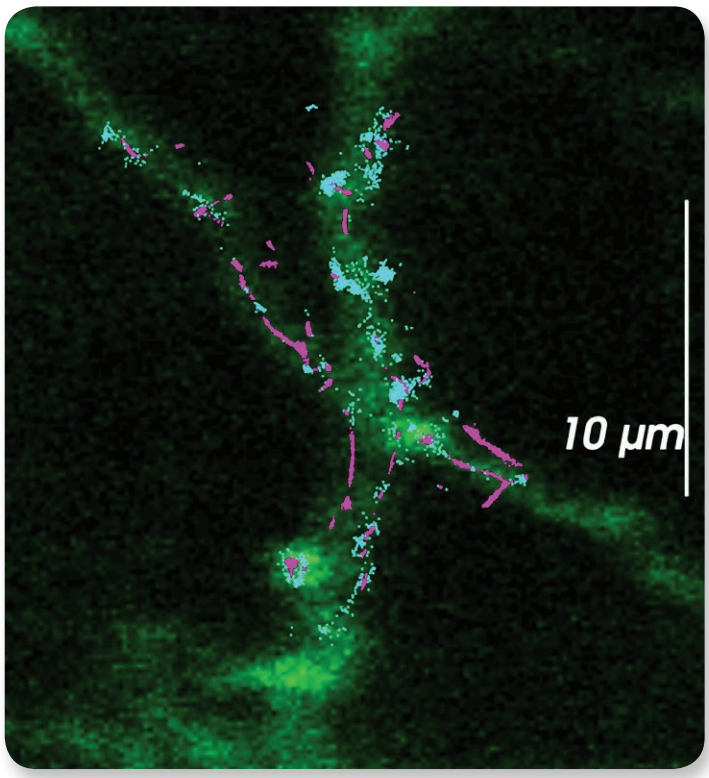
The Opterra SR option also allows correlative imaging. The confocal scanner can be used to navigate and investigate samples to find areas of interest for super-resolution imaging. Confocal images can be saved as reference images, and post acquisition can be overlaid by super resolution image results. The contextual information provided by the confocal image aids in interpretation of the SML data. Combined with quantitative analysis, the high-resolution confocal images provide a guide for creating measurement ROIs for statistical comparisons based on micro-anatomical detail.

The Opterra SR confocal scanner provides:

- Correlative imaging between high resolution (confocal) and super resolution
- Capability to perform standard confocal imaging applications with live cells for fixed preparations
- Instant deconvolution of confocal images



Maximum intensity projections of Z stack acquired from *C. elegans* expressing synaptotagmin-GFP. Image on the left is raw, image on the right is after deconvolution.



Cultured nerons expressing GFP, imaged by confocal microscopy overlaid with single molecule localization showing HaloTag®.

Vutara 352 Specifications

Overview	3D video-rate, super-resolution, single-molecule localization imaging
Lateral Resolution	20 nm*
Axial Resolution	40 nm to 70 nm*
Acquisition Speed	Up to 3,000 fps
Cameras (two included)	sCMOS camera (4 MP, 6.5 µm x 6.5 µm pixel size for super-resolution imaging); CCD camera (1392 x 1040 for widefield imaging)
Imaging Depth	Up to 20 µm* via high-precision piezo Z stage (100 µm option with select samples)
Stage	Fully motorized XY scanning stage
Piezo	Objective-mounted piezo for rapid Z focus (100 µm or 400 µm travel option)
Imaging Modes	Simultaneous two-color; Interleaved multi-color; Sequential multi-color; Ultra-fast particle tracking; Wide-field imaging; Transillumination
Field of View	Wide-field: 200 µm x 160 µm; Super-resolution: 20 µm x 20 µm, optional 40 x 40 µm; Single molecule particle tracking: 20 µm x 20 µm x 1µm, optional 40 µm x 40 µm; Confocal: 82.2 µm x 82.2 µm
Imaging	Simultaneous two- or four-channel imaging; Standard Quadfield™ module, simultaneous orange/red or green/orange; Custom Quadfield module, four-color simultaneous (2D)
3D Particle Tracking	<10 nm precision*; 20 µm x 10 µm x 1µm volume at up to 3,000 fps
Laser Options	100 mW: 405 nm; 1000 mW: 488 nm, 561 nm, 640 nm, 750 nm, optional 532 nm
Objectives	Water: 60x /1.2; Silicone: 60x /1.3; Oil: 60x /1.42, 60x /1.49, 100x /1.49, 150x /1.49
Software Features	Simple step-by-step "wizard" acquisition; Multi-threaded real-time localization; Full 3D rendering; Multimodal drift correction including fiduciary drift correction; Vutara Viewer; Non-proprietary export
Z Stacks	Acquisitions up to 15 µm of Z stacks via high-precision Z stage
Custom Stand	Drift-minimizing inverted microscope stand; Integrated thermal control for sample temperature stability (20°C to 37°C); Environmental control
Filter Wheels	2 filter wheels included
Modular Options	Modular options available upon request (e.g., Opterra SR confocal scanner, TIRF, industry-standard side port)

*Performance is sample and room dependent, typical resolutions can vary based upon preparation. Lateral resolution 15 nm to 40 nm, Axial resolution 50 nm to 80 nm. Some dyes require switching media. Some features available upon request (e.g., 750 nm laser).

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