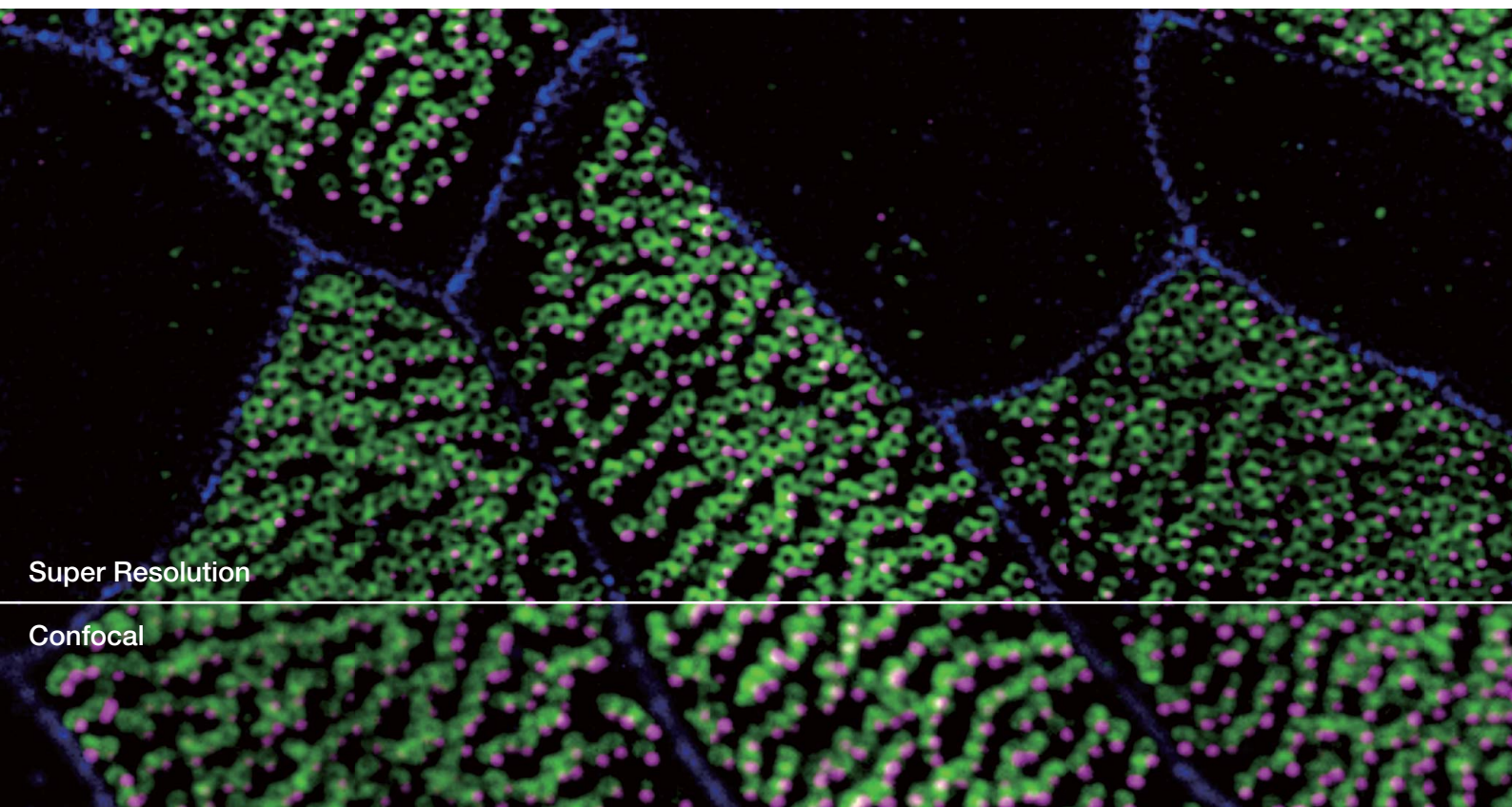


Super Resolution for All Types of Live Cell Imaging



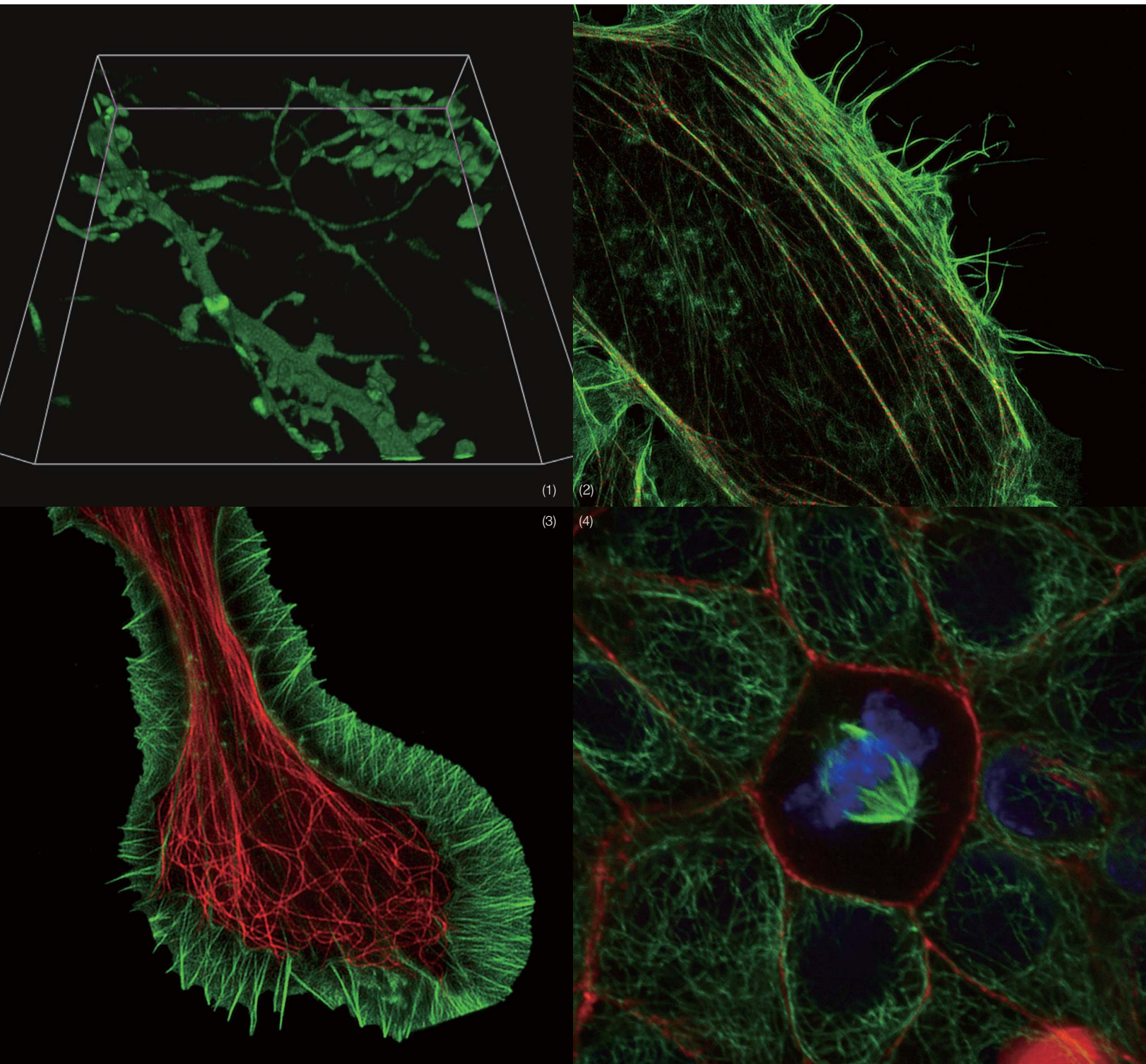
Super Resolution

Confocal





## Higher Level of Super Resolution Imaging



Designed for live cell imaging with 120-nanometer resolution, the Olympus IXplore SpinSR10 super resolution imaging system balances speed, resolution, and efficiency in a single, flexible platform. Researchers can observe the fine details and dynamics of cellular structures and processes with the ability to easily switch between super resolution, confocal, and widefield imaging. The system's advanced confocal technology enables researchers to capture super resolution images with excellent clarity.

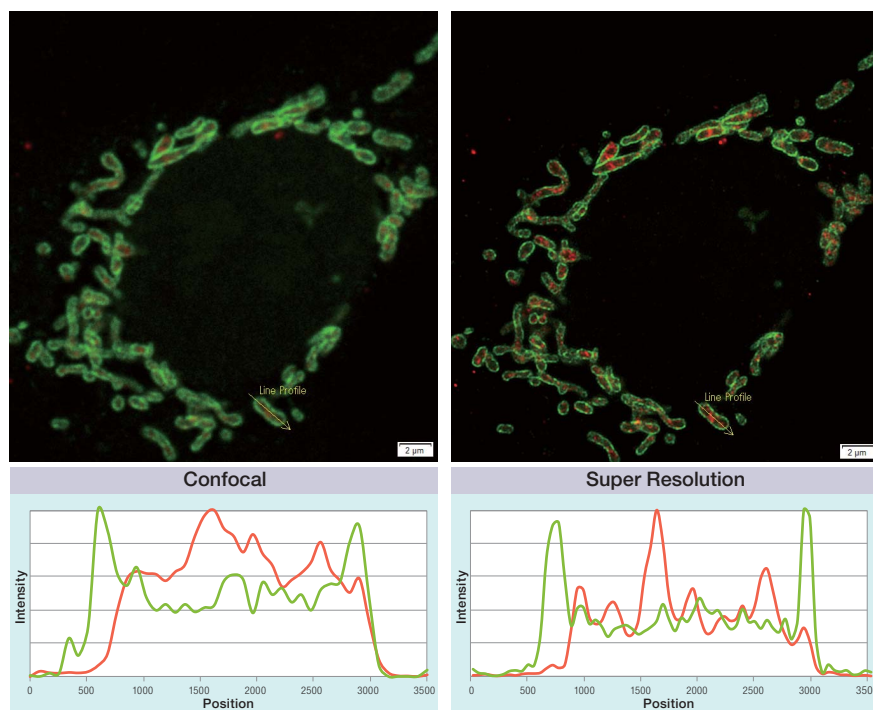
## Olympus Super Resolution

Olympus super resolution (OSR) technology is fast, easy to use, and can provide images from up to 100 microns deep within a cell in areas that are hard to access using other super resolution modes. Live cell super resolution images of internal cellular structures can be captured with 120 nm resolution from all kinds of samples using conventional fluorescent dyes.

Hayashi S. Resolution doubling using confocal microscopy via analogy with structured illumination microscopy. Jpn J Appl Phys. 2016;

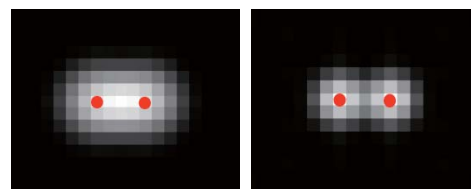


Motorized Magnification Changer

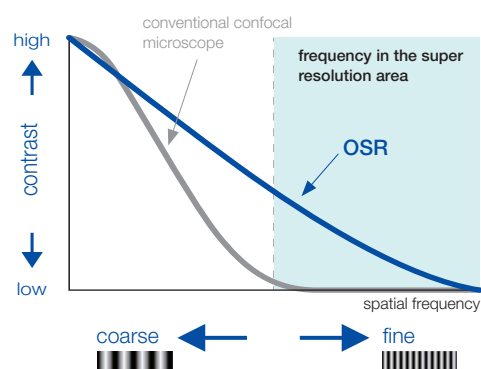


Images of adjacent 2 emission points

(• emission point)

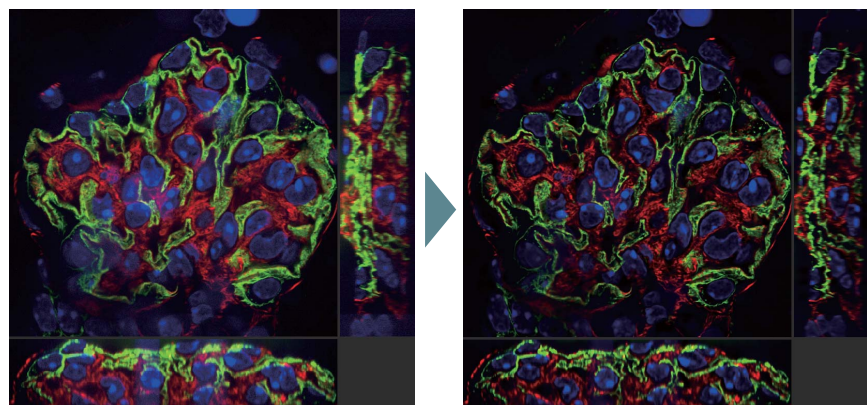


Principle of OSR



## Sharp Super Resolution Images

Olympus' dedicated deconvolution algorithm works with super resolution images to create clear, sharp 3D images.



SpinSR10 image

SpinSR10 image improved using deconvolution

Mouse kidney tissue stained with Alexa Fluor 488



# Live Cell Super Resolution Imaging

The IXplore SpinSR10 system combines speed, reduced phototoxicity, and stability during time-lapse experiments to create 3D super resolution data that enables users to observe dynamic changes and phenomena within live cells.

## Live Super Resolution

The spinning disk confocal optical system acquires live super resolution images at up to 200 frames per second.

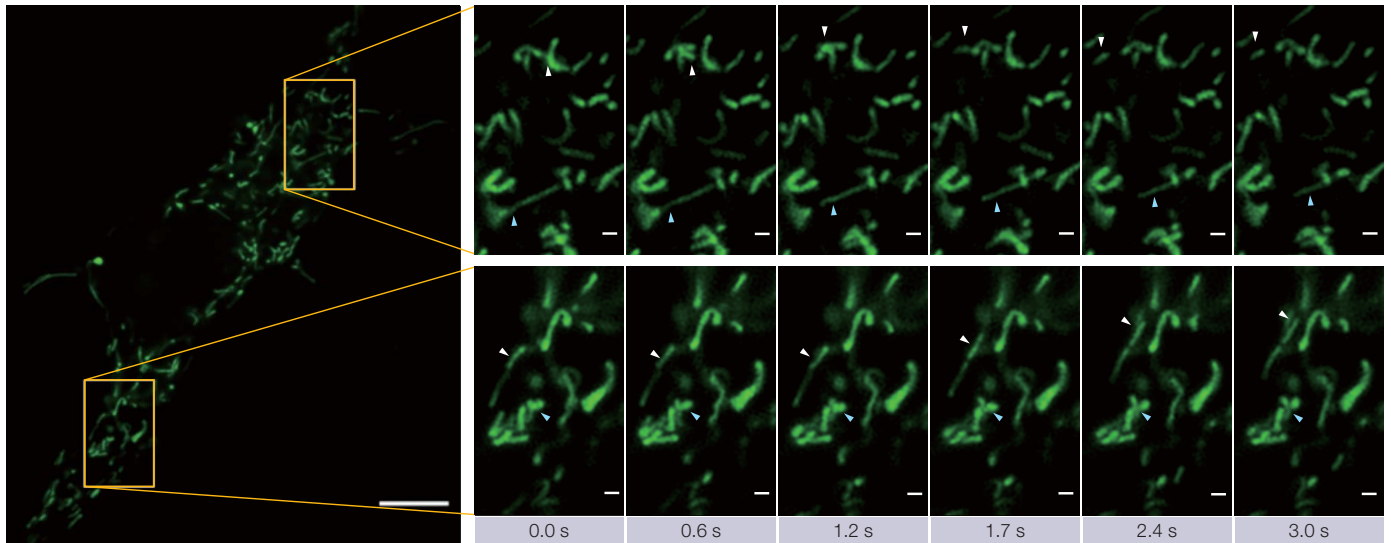
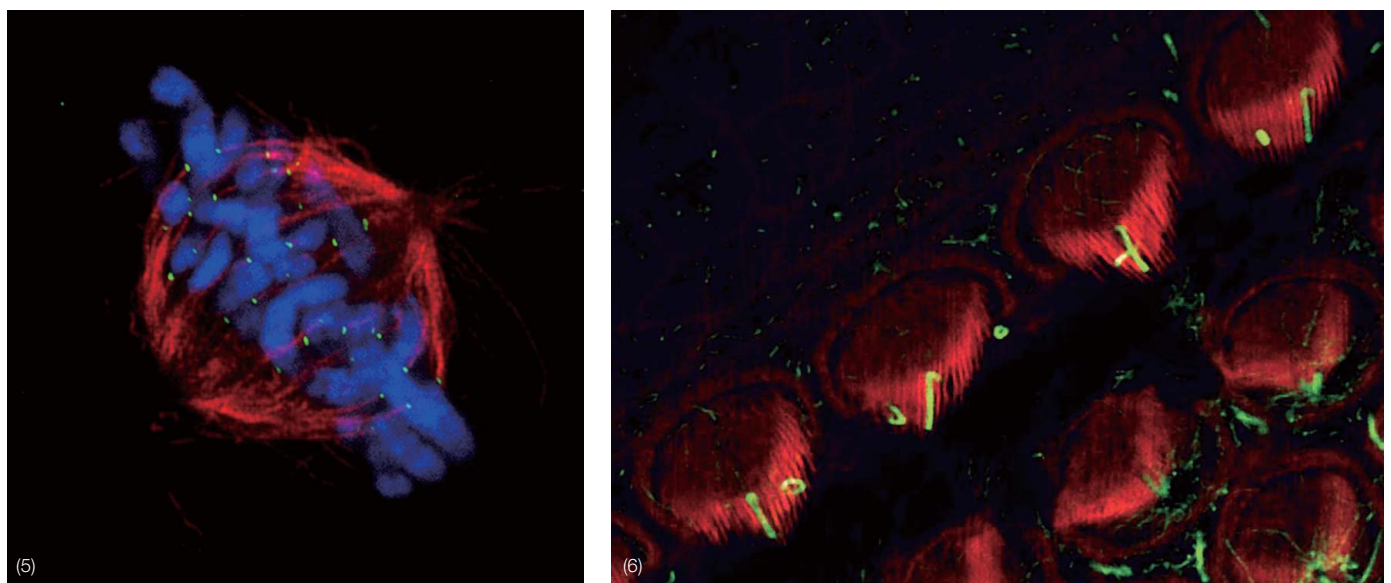


Image of mitochondria obtained at 30 fps  
Mitochondria labeled by GFP. Acquired with 30fps, able to see the individual mitochondria movements.  
Image data courtesy of: Kumiko Hayashi, Ph.D., Graduate School of Engineering, Tohoku University

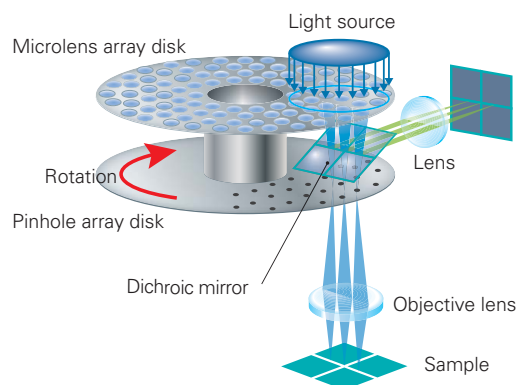
## Two-Color Simultaneous Imaging

The SpinSR10 system can use two cameras simultaneously to provide fast, two-color localization imaging.



## Fast Super Resolution Imaging and a Wide Field of View

Instead of painstakingly scanning the entire field of view, the sensitive imaging sensor on the SpinSR10 captures snapshots of the entire sample area in one step for fast imaging, enabling researchers to observe high-speed biological phenomena. In widefield and confocal mode, the microscope's optical system has a field number (FN) of 18 to capture images with a larger field of view, while two cameras enable users to simultaneously acquire dual-color super resolution images.



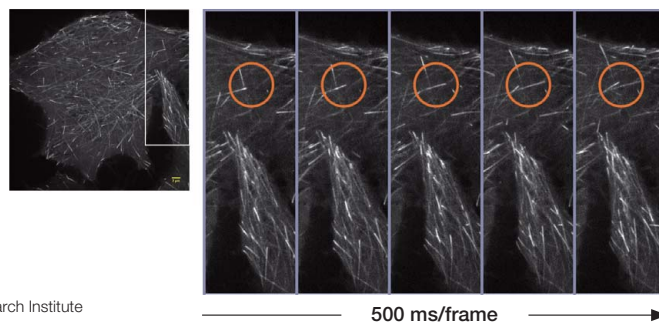
## Real-Time Super Resolution

High speed data processing algorithms enable the viewing of super resolution images in a live display window. This allows for real-time viewing of cellular activities compared to other computational super resolution techniques in live cells.

**EB3 proteins binding to the top of microtubules extending in HeLa live cells.**  
**EB3 proteins were GFP- labeled by means of transgenesis.**

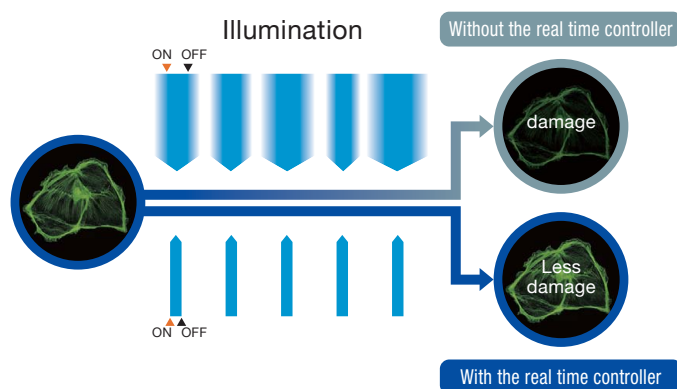
Image data courtesy of:

Kaoru Kato, PhD National Institute of Advanced Industrial Science and Technology Biomedical Research Institute



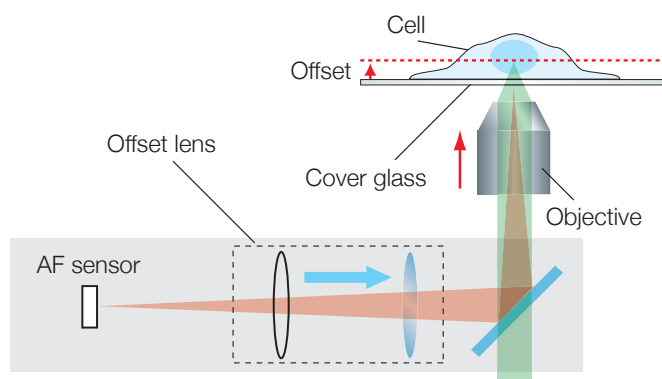
## Reduced Phototoxicity

The real time controller (U-RTCE) synchronizes the laser and camera with microsecond illumination accuracy to reduce photobleaching and phototoxicity, helping cells remain healthy during complex experiments.



## Keep Your Samples in Focus

During time-lapse imaging, minute changes in temperature, humidity, and other factors can cause your sample to go out of focus. The Z-drift compensator (IX3-ZDC2) uses a low phototoxicity infrared laser to identify the sample plane and adjust the focus for clear time-lapse images. The continuous autofocus function works with glass and even plastic vessels.



# See Inside Your Samples in Super Resolution

## Observation at Depth

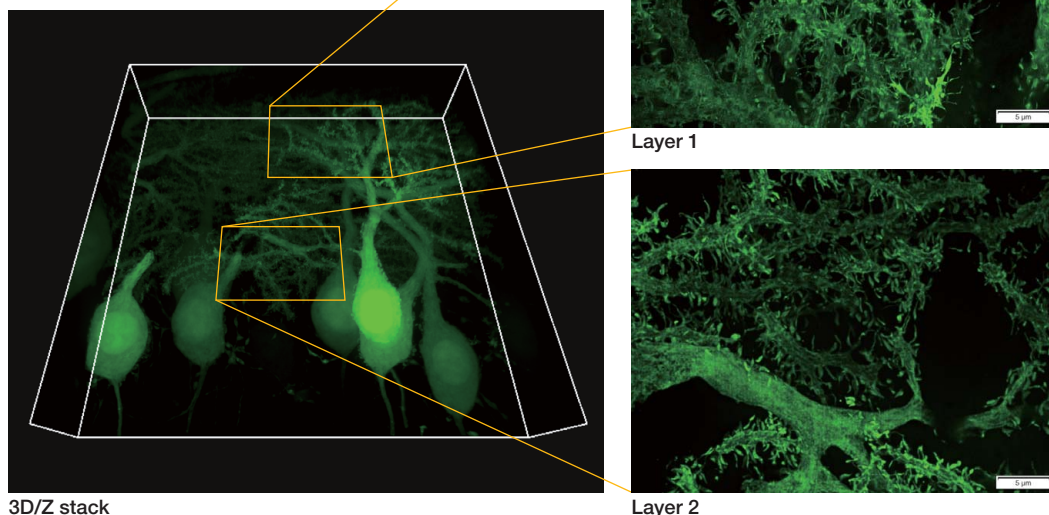
Users can clearly observe small individual spines not only on the surface of the sample, but also up to 100 microns deep within the sample.

### Purkinje cells labeled with GFP

XYZ image with confocal and super resolution image in different Z positions.

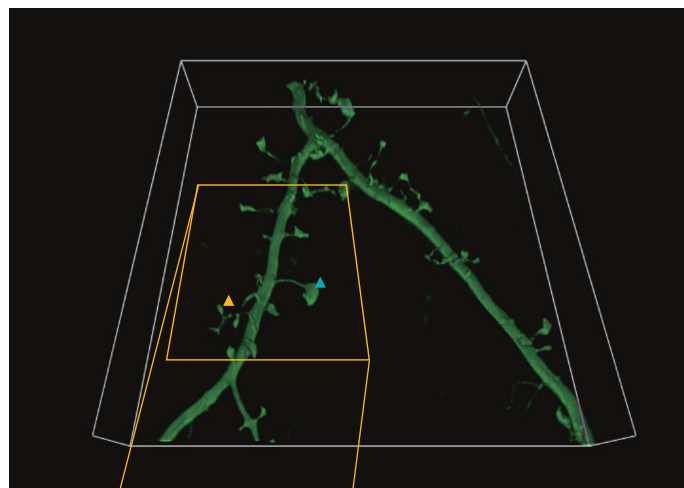
Super resolution images are projected by Z (10 slices).  
3D displayed by FV31S-DT.

Image data courtesy of: Michisuke Yuzaki, PhD. Department of Physiology, School of Medicine, Keio University



## Image Three-Dimensional Structures

Obtain detailed three-dimensional super resolution image data during time-lapse imaging.



### 3D time-lapse of neuron

Time-lapse image of mouse primary neuron labeled with EGFP after co-culture with astrocyte for 2 weeks.

Easy to see the difference between immature spine (yellow arrow) and mature spine (blue arrow), and detect the morphological change in time.  
3D was acquired with exposure time 500ms/frame, 0.15um Z step for 41 slices.

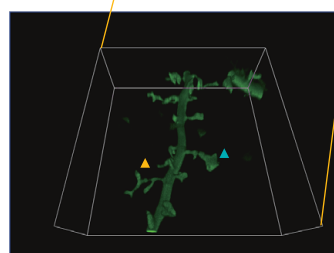
Images were acquired every 2 minutes for 1 hour.

3D displayed by FV31S-DT.

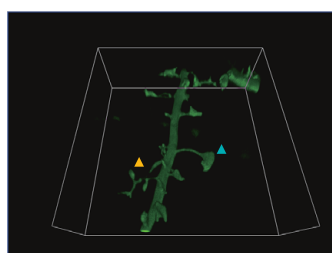
Image data courtesy of:

Yuji Ikegaya, PhD

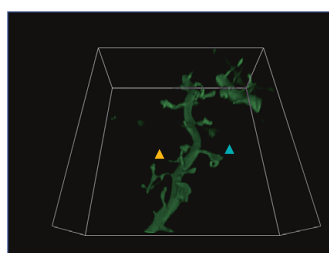
Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo



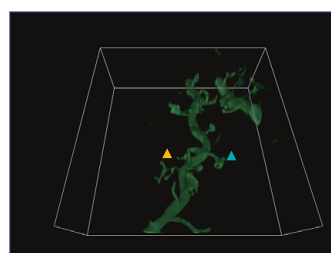
0 min



20 min



40 min



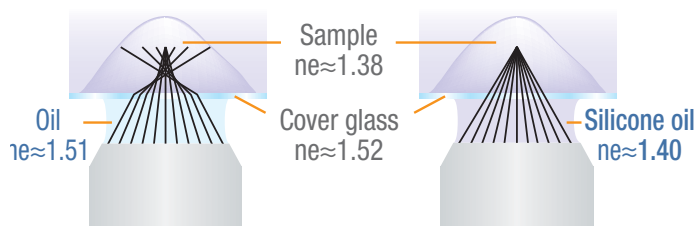
60 min

## Improved Z Resolution

Olympus silicone immersion objectives are designed for deep tissue observation. Observation depth is negatively impacted by spherical aberration caused by refractive index mismatch. The refractive index of silicone oil ( $n \approx 1.40$ ) is close to that of living cells or cultured tissue slices ( $n \approx 1.38$ ), enabling super resolution imaging of internal cellular structures at tens of micrometers in depth with minimal spherical aberration.

### The Refractive Index is Important with Deep Tissue Observation

In deep tissue observation, image quality depends on keeping the refractive index of the sample and immersion medium as close to each other as possible.



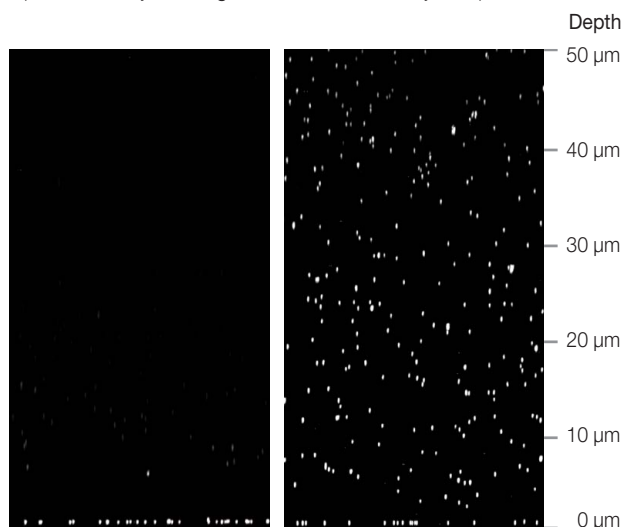
#### Oil immersion objective

When working with an oil immersion objective, the difference between the refractive index of the samples and oil results in spherical aberration in deep tissue, causing the resolution to deteriorate and fluorescence to become dim.

#### Silicone immersion objective

When working with a silicone immersion objective, the difference between the refractive index of the samples and silicone oil is minimal. This objective achieves brighter fluorescence images with higher resolution for deep tissue observation.

### Comparison of observation at depth (left: TIRFM objective, right: silicon immersion objective)



## Reduce Spherical Aberration

The remote correction collar unit is used to adjust the lens position within the objective to correct for spherical aberration caused by refractive index mismatch, resulting in dramatically improved signal, resolution, and contrast. The IX3-RCC unit works with any Olympus UIS2 objective that has a correction collar.

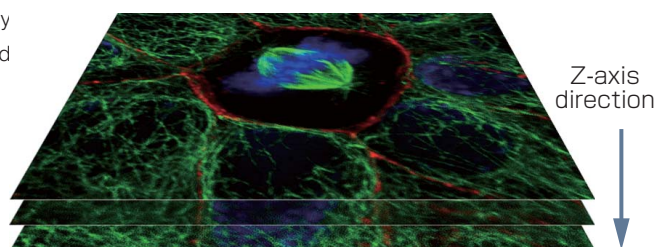


## Optical Sectioning

Based on a confocal optical system, Olympus super resolution technology enables optical sectioning to acquire clear super resolution images with reduced background.

### Mitotic epithelial cell (Chromosome: Blue, Tubulin: Green, ZO1: Red)

Image data courtesy of:  
Hatsuho Kanoh, Tomoki Yano, Sachiko Tsukita  
Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University





# A Flexible System that Helps Simplify Your Research

Olympus cellSens image analysis software supports the complex experiments conducted with the IXplore SpinSR10 system. The software's efficient workflows enable users to effectively manage their data and perform advanced analysis that helps unlock new insights. The system integrates easily into existing protocols without necessitating major changes; labs can continue using their existing sample protocol and labeling systems.

## Easily Switch Observation Methods

The software makes it easy for you to change observation conditions. Switch between fluorescence, confocal, super resolution, and multicolor imaging modes just by clicking a button.

## Manage Complex Experiments

The process manager makes it simple to acquire multicolor, Z-stack, and time-lapse images. The programmable graphic experiment manager (GEM) enables users to design more complex automation from a visual interface to support a wide variety of experimental imaging protocols and device triggering. Customize flexible experiment protocols that can be easily changed as needed anytime during the imaging process.



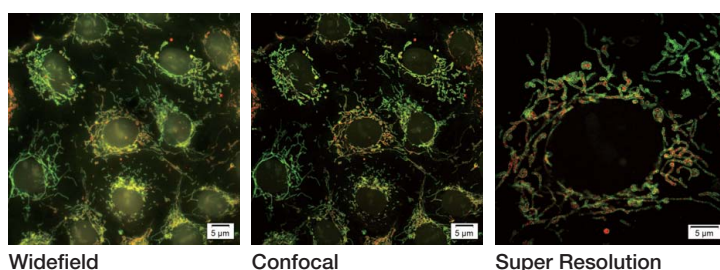
## Make Fine Adjustments

In super resolution imaging, the ability to make fine stage adjustments is critical. The highly accurate IX3-SSU ultrasonic stage is easy to use and can be controlled via software or the stage handle. The stage has a exhibits low thermal drift for reproducible multi-image acquisitions and stability during long term time-lapse experiments.



## One System, Three Imaging Modes

Researchers can use the imaging mode that most suits their sample in a single system. Users can switch between widefield, confocal, super resolution, and multicolor imaging with one click to locate areas of interest and then image fine structures.



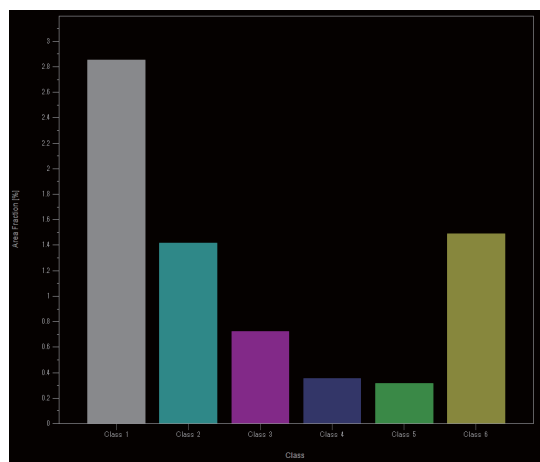
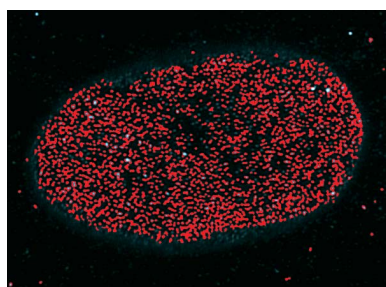
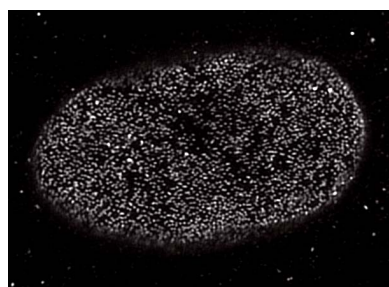


# Powerful, Intuitive Image Analysis

Olympus cellSens imaging software enables various types of numerical data to be extracted from images obtained using the software's image analysis functions. Straight line distance, boundary length, or the area of a polygon can all be measured. The following additional advanced measurements are also possible:

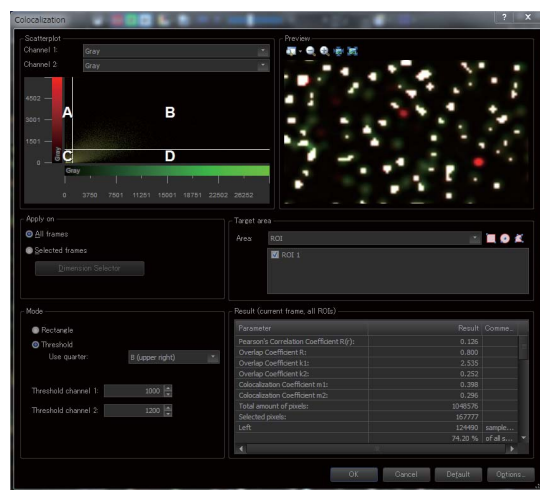
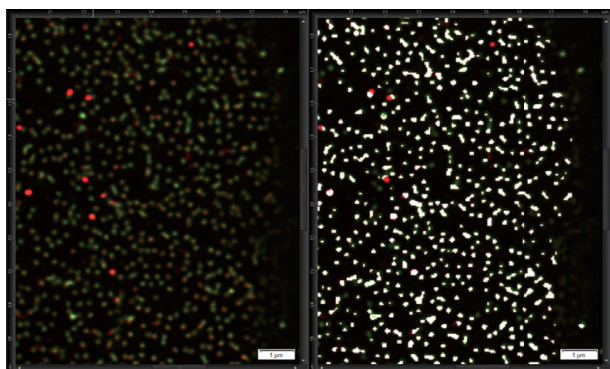
## Analyze Object Information

Analyze information about objects in your images, including the number of objects, area measurement, luminosity, and morphology.



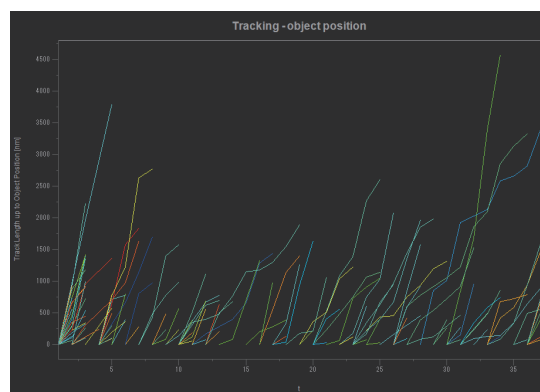
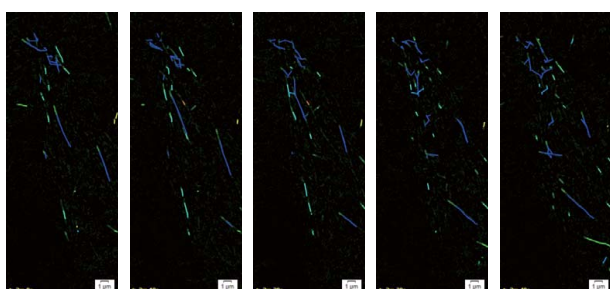
## Discriminate Spectrum Overlaps

The colocalization function analyzes the fluorescent spectrum and discriminates between overlapping spectra.



## Track Time-Lapse Imaging Data

During time-lapse imaging, the tracking function enables users to measure and analyze cell migration and division as well as luminosity.



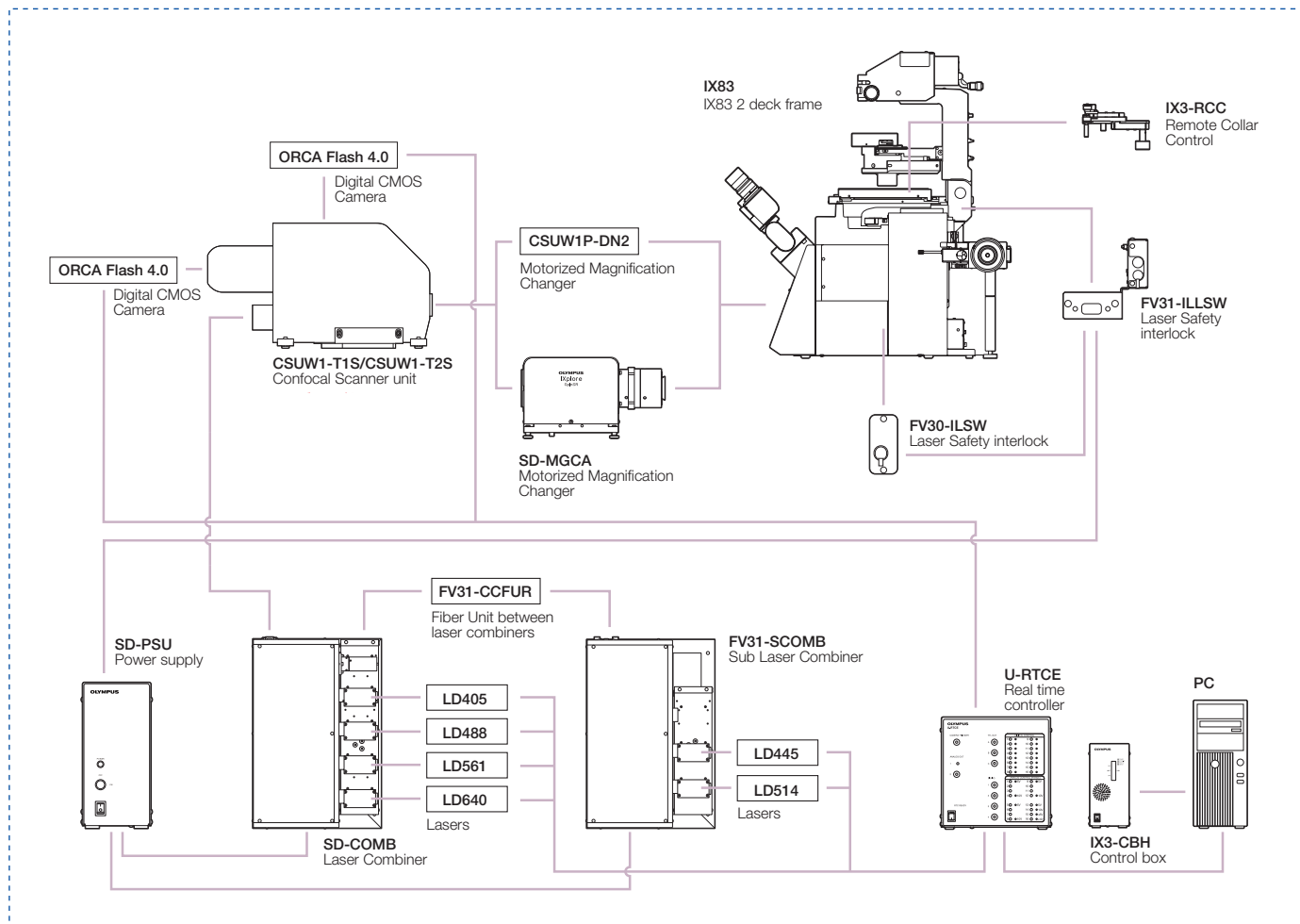
# System Description

## SpinSR10

The IXplore SpinSR10 system is capable of performing widefield, confocal, and super resolution image observation quickly and easily.



SpinSR10 System diagram



## SpinSR10 Specifications

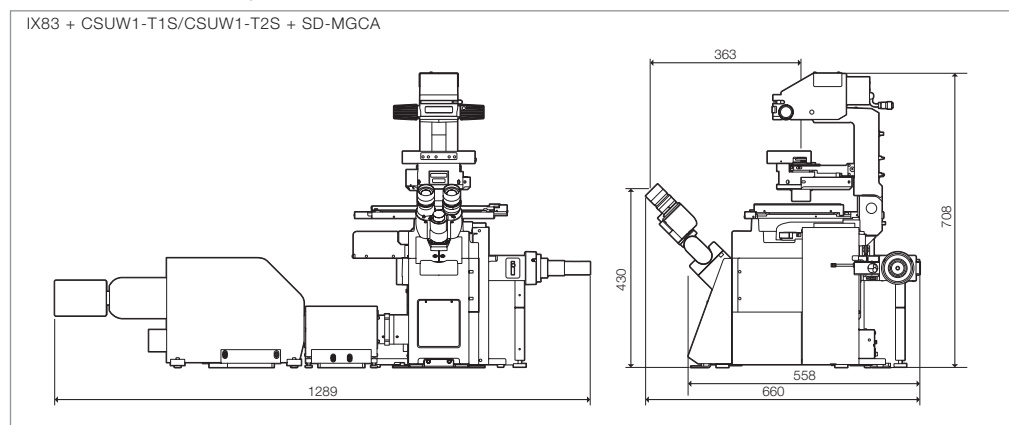
		Super Resolution/Confocal Configuration	Confocal Configuration*
Laser lines		405 nm: 50 mW, 445 nm: 75mW, 488 nm: 100 mW, 514 nm: 40mW, 561 nm: 100 mW, 640 nm: 100 mW	
Laser Combiner		Main combiner: 405 nm, 488 nm, 561 nm, 640 nm + 1 line (445 nm or 514 nm) Sub combiner: 445 nm, 514 nm 2x Interlock shutter available	
Laser Light Control		Direct Modulation by U-RTCE, ultra-fast ON/OFF control and intensity modulation with individual laser lines, continuously variable (0 % - 100 %, 1 % increments)	
Scanner	Yokogawa CSU-W1		Single 50 µm pinhole disk, 1 or 2 camera model
	Super Resolution Imaging	Acquisition Speed (max)	5 ms/f
		Optical Zoom	3.2 X
		Optical Resolution	120 nm**
		Field Number	5.9
	Regular Confocal Imaging	Acquisition Speed (max)	5 ms/f
		Optical Zoom	1 X
		Field Number	18.8
	Dichromatic Mirror		3 position (motorized slider)
Filter Wheel (emission)		10 position (motorized wheel)	
Imaging Sensor		HAMAMATSU ORCA Flash 4.0 V3 (CameraLink)	
Microscope	Motorized Microscope		Inverted IX83
	Motorized Stage		IX3-SSU
	Objectives for Super Resolution		UPLSAPO60XS2, UPLSAPO100XS, PLAPON60XOSC2, APON60XOTIRF, UAPON100XOTIRF
	Super Resolution Adapter		Confocal/Super Resolution Lightpath Changer (Motorized)
Workstation	PC		OS: Windows10 Pro 64 bit
Imaging Software	cellSens Dimension		Multi-Dimensional Acquisition and analysis
			Super Resolution Imaging Module

\* Confocal configuration is the system w/o super resolution function, able to upgrade to super resolution/confocal configuration

\*\* Typical experimental FWHM values with UPLSAPO100XS, 100nm diameter beads at 488nm excitation

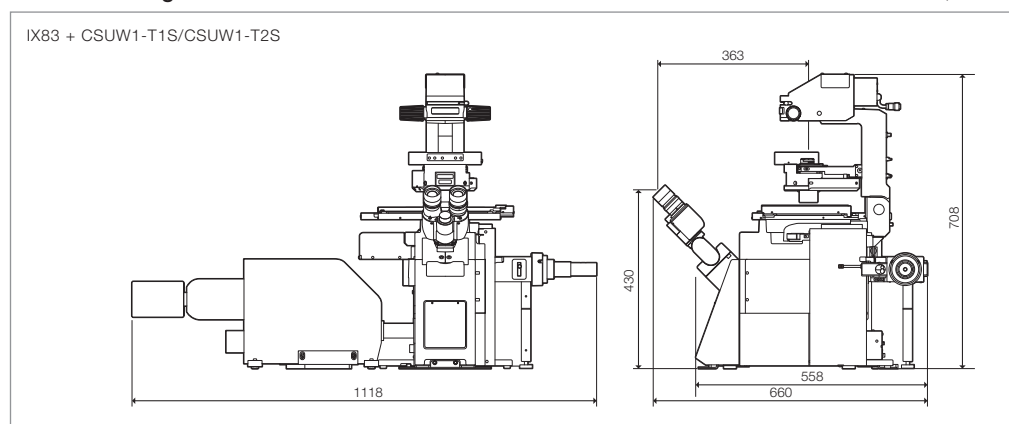
## Super Resolution Configuration Dimensions

(unit: mm)



## Confocal Configuration Dimensions

(unit: mm)





**Image data courtesy of:**

(cover) Trachea multi-ciliated epithelial cells (Culture):

Immunofluorescence microscopy: Odf2 staining (Alexa Fluor 488, green), Centriolin staining (Alexa Fluor 568, magenta), ZO-1 staining (Alexa Fluor 647, blue) Staining for Odf2 encircled the base of cilia at the upper part of the basal body (green). Staining for Odf2 revealed the basal foot at one side of basal body (magenta). Staining for ZO-1 revealed the tight junctions (blue).

Hatsuho Kanoh, Elisa Herawati Sachiko Tsukita, Ph.D.

Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University.

(1) 3D time-lapse of neuron:

Time-lapse image of mouse primary neuron labeled by EGFP after co-culture with astrocyte for 3 weeks. 3D was acquired with exposure time 500ms/frame, 0.2µm Z step for 26 slices.

Yuji Ikegaya, PhD

Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo

(2) Stress fibers of HeLa cell:

Antibody staining with Alexa Fluor 488 (green) for actin, Alexa Fluor 568 (red) for myosin heavy chain. Image courtesy of: Keiju Kamijo, Ph.D. Division of Anatomy and Cell Biology, Faculty of Medicine, TOHOKU Medical and Pharmaceutical University

(3) Fluorescent staining of microtubules (red: Alexa Fluor 594) and actin (green: Alexa Fluor 488) in growth cone of NG108 cells. Image courtesy of:

Dr. Kaoru Katoh, Biomedical Research Institute, National Institute of Advanced Industrial Sciences and Technology (AIST)

(4) Mitotic cultured epithelial cell. (Chromosome: Blue, Tubulin: Green, ZO1: Red):

Hatsuho Kanoh, Tomoki Yano, Sachiko Tsukita

Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University

(5) Mitotic spindle at metaphase cell. HeLa cells derived from human cervical cancer

were fixed and stained for α-tubulin (microtubules, red) and Hec1 (kinetochores, green), respectively. DNA was stained with DAPI (chromosomes, blue). Chromosomes interact with microtubules constituting mitotic spindle via kinetochores assembled on centromere region of chromosomes. Masanori Ikeda and Kozo Tanaka, Department of molecular oncology, Institute of Development, Aging and Cancer

(6) Stereocilia and kinocilia of inner hair cells in the organ of Corti. (Actin: Orange, Tubulin: Green):

Hatsuho Kanoh<sup>1</sup>, Toru Kamitani<sup>1,2</sup>, Hirofumi Sakaguchi<sup>2</sup>, Sachiko Tsukita<sup>1</sup>

<sup>1</sup>Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University

<sup>2</sup>Department of Otolaryngology-Head and Neck Surgery, Kyoto Prefectural University of Medicine



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