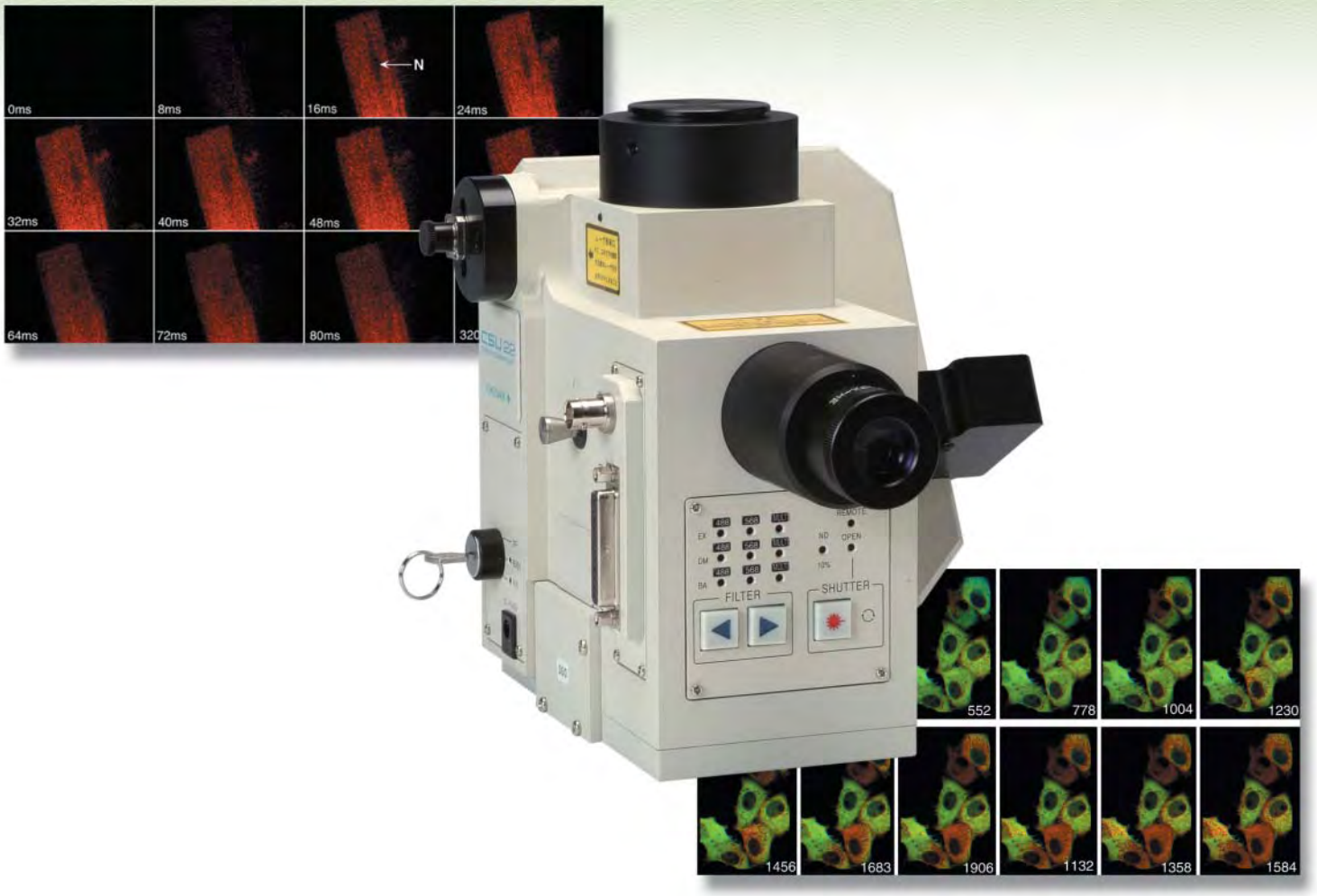
YOKOGAWA

CSU22

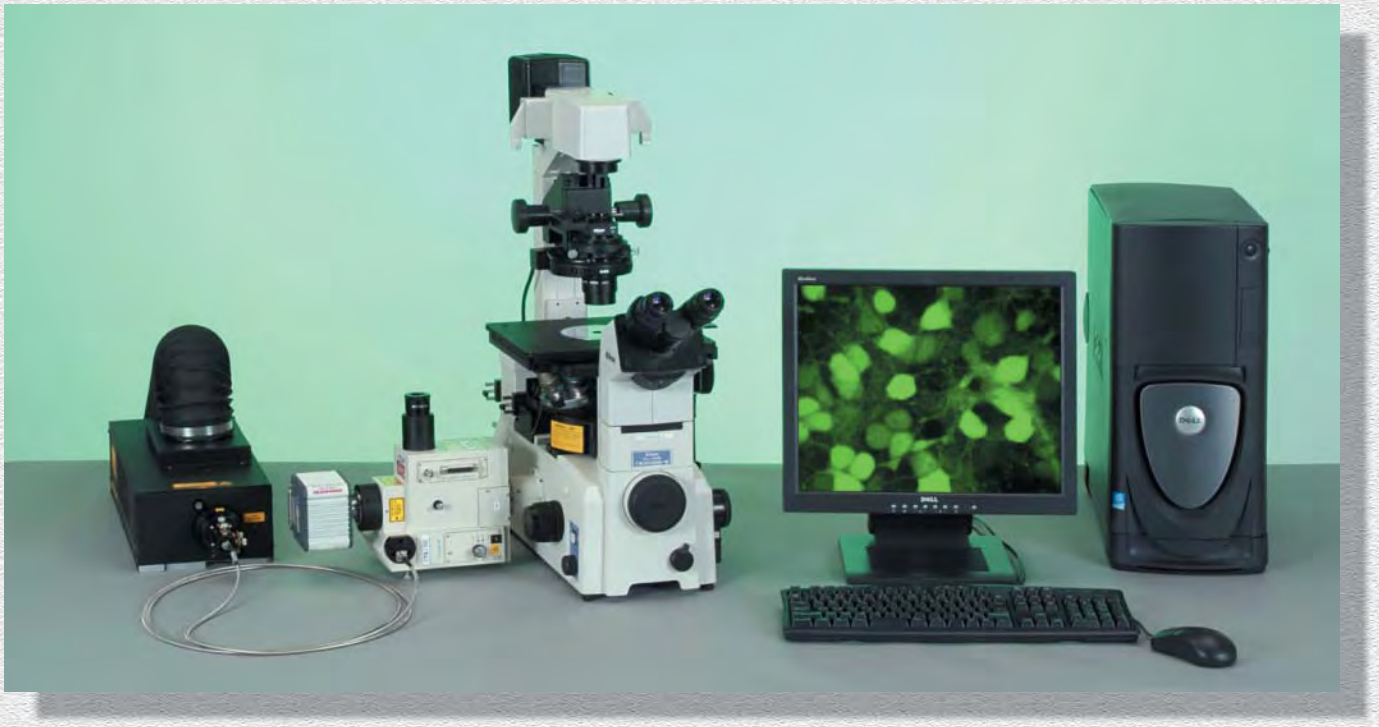
Model CSU22 Confocal Scanner Unit



Bulletin 85A07C03-01E

The most suitable tool for live cell imaging!

The mainstream of today's life science research is the observation of various life processes in living samples in real time with a high degree of sensitivity and resolution, and minimal photo bleaching and photo toxicity. Based on its innovative microlens-enhanced Nipkow disk scanning technology, Yokogawa CSU units are being used worldwide as the most powerful tool for live cell imaging.

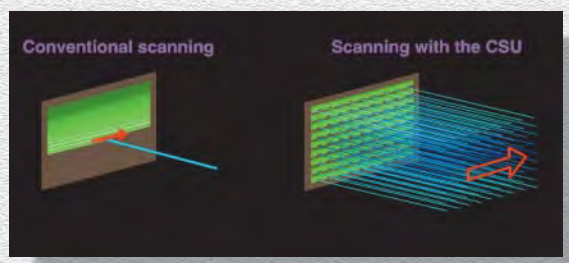


Features

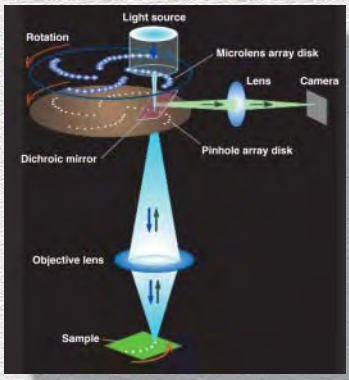
- The world's fastest scanning speed
- Minimal photo toxicity and photo bleaching
- The world's fastest 3D data acquisition (when used with optional Yokogawa RT3D system)
- Highly flexible system expandability
- Direct view of real-color confocal images
- Compact size, vibration resistant scanner

Principles of the Microlens-enhanced Nipkow Disk Scanning Technology

The most common conventional confocal microscopes use a single laser beam to scan a specimen, while the CSU scans the field of view with approximately 1,000 laser beams, by using microlens-enhanced Nipkow-disk scanning: in short, CSU can scan 1,000 times faster. Nipkow disk is a disk with spiral pattern of holes arranged to raster scan the specimen with the light illuminating through the holes; numbers of small points of lights scan the specimen when disk is spinning. Invented by Paul Nipkow in 1884, it was used for very early stage of television camera. Although capable of high-speed scanning, conventional Nipkow disks failed to provide enough amounts of light to image fluorescence from live cells. By using a disk containing microlens arrays in combination with the Nipkow disk, we have succeeded in dramatically improving the light efficiency and thus successfully made real-time confocal imaging of live cells possible.



The expanded and collimated laser beam illuminates the upper disk containing about 20,000 microlenses (microlens array disk). Each microlens focuses the laser beam onto its corresponding pinhole, thus, effectively increasing laser intensity through pinholes placed in the pinhole array disk (Nipkow disk). With the microlens, backscattering of laser light at the surface of pinhole disk can be significantly reduced, thus, dramatically increased the signal to noise ratio(S/N) of confocal images. About 1,000 laser beams passed through each pinhole fill the aperture of objective lens, and then focused on the focal plane. Fluorescence generated from the specimen is captured by the objective lens and focused back onto the pinhole disk, transmitted through each of the same pinhole to eliminate out-of-focus signals, deflected by the dichroic mirror located between miclolens array disk and the Nipkow disk to split fluorescence signal from reflected laser, passed through emission filter and then focused into the image plane in the eyepiece or camera. The microlens array disk and the Nipkow disk are physically fixed to each other and are rotated to scan the entire field of view at high speeds, thus, makes it possible to view confocal fluorescent images in real-time through the eyepiece of CSU head. As compared to conventional single point scanning, multi beam scanning by CSU requires significantly low level of light intensity per unit area, which results in significantly reduced photo bleaching and photo toxicity in live cells.



More than just a high-speed imaging tool
The CSU22 is a multifunctional easy-to-operate scanner unit with a high degree of system expandability.

● Full-frame Real-time Observation of a Variety of Samples, from Static Samples to Dynamic Samples Changing at 1-ms Intervals

The CSU22 scans a field of view as fast as 1 ms per second when its variable rotation speed is set at 5,000 rpm.
The rotation speed of the spinning disk motor can be controlled from 1500 rpm up to 5000 rpm, which enables perfect synchronization with any acquisition speed (>1ms) of a camera through TTL signal communication, in addition to NTSC/PAL synchronization.

● One-touch Automatic Selection from Up to Three Wavelengths;

· Manual Operation: With one-touch panel switch operation, selection from each three of excitation filter, dichroic mirror,emission filter, ON/OFF of ND filter, ON/OFF of laser shutter can be selected. On-off state of remote control and filter positions are shown by the LED indicators on the front panel.
· System Operation: Each filter and shutter can be remote-controlled using a PC. The CSU22 can be systematized in combination with an appropriate image processing software and peripherals, such as a camera, z-control, filter wheels, etc. (Please inquire about PC-controlled system).

● Minimized Laser Damage and Fluorescence Photo bleaching

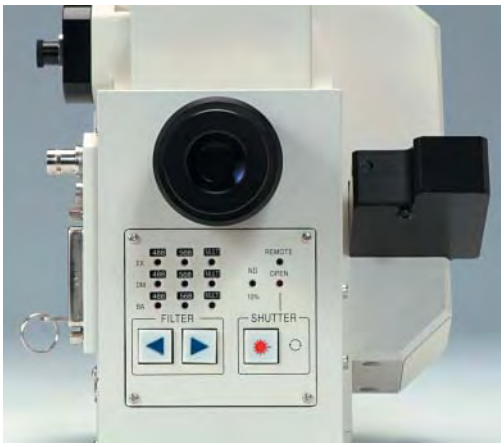
The multibeam scanning using approximately 1,000 laser beams has reduced the energy density per beam to at least one-thousandth that of conventional single-beam confocal systems. Consequently, laser damage and fluorescence photo bleaching have dramatically reduced in the case of CSU, making it possible to observe delicate life phenomena, such as cell division, for a long period.

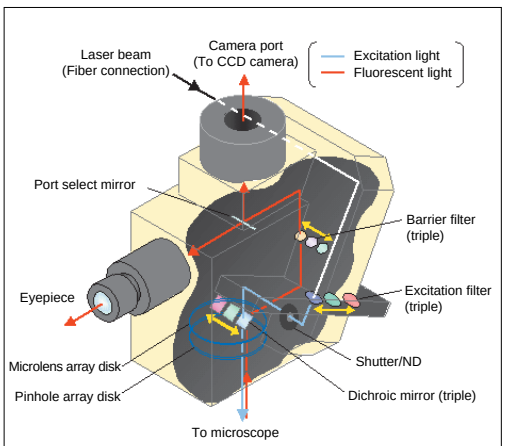
● Compact size and Vibration-resistant Design

Because of its simple common-path optical design and constant-speed rotation scanning, confocal imaging with CSU is resistant to vibration. It can be used in a regular microscopy environment without vibration isolation bench CSU22 is installed at the microscope camera port without any special optical path adjustment. This makes it easy to change the microscope to combine with the CSU22.

● A Variety of System Configurations According to Your Application Needs

The real-time, direct-view capability of the CSU22 makes it possible to leverage various cameras with different features, including high speed, high sensitivity, high resolution, and color imaging, to meet a wide range of application needs.
You can flexibly combine the CSU22 with other component units to configure a variety of systems to meet broad range of applications in the research of cellular, developmental, neurobiology, medical screening, etc. Furthermore, combining the CSU22 with fast-evolving cameras and image processing systems makes it possible to always meet your newly emerging application needs.





Optical Path Configuration in CSU22

A Wide Choice of Cameras and Laser Light Sources

1 EMCCD Cameras
ICCD video camera or II+CCD
Color CCD camera
Cooled high-resolution CCD camera
HARP cameras
High-speed camera
Film camera

2 Gas lasers
Solid-state lasers
DPSS lasers

From Analog Video Monitor to Advanced Digital Image Processing

3 Video recording/image monitoring
4 Image processing/CSU control (Mac/Windows)

3D Image Construction of Static and Dynamic Samples

5 Upright or inverted microscopes from different manufacturers
6 Piezoelectric Z-axis drive (realtime 3D observation)
7 Electromotive Z-axis drive

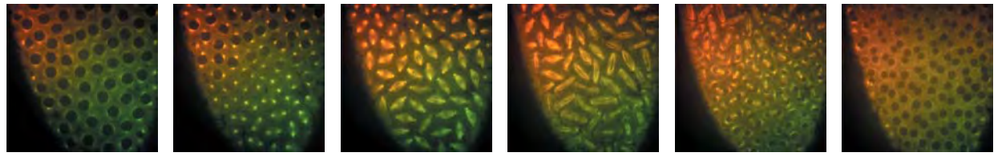
Wide Application: Imaging of Fast Biological Reactions in Milliseconds level to high resolution imaging of static specimens

Model CSU22
Confocal Scanner Unit **CSU22**

1) High-resolution Time-lapse Imaging of Living Cells

Different from conventional confocal systems , CSU uses CCD cameras to capture confocal images. Taking advantage of rapid advance in CCD camera capability, it is more and more possible to acquire high-resolution images at high speed. Especially, significantly reduced photo bleaching and photo toxicity with CSU makes it possible to image delicate changes in living cells, such as cell division or embryonic differentiation at length.

Observation of cell division in a drosophila embryo

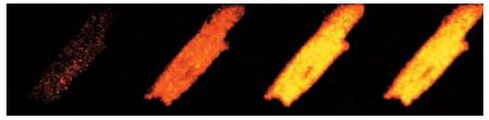


Time-lapse images of cell division: (centromere: labeled with EGFP)
By courtesy of Dr.J. Sholey, Dr. D. Sharp: University of California, Davis

2) High-speed Imaging

High-speed scanning up to 1,000 frames/sec makes it possible to record reactions at milliseconds level.

High-speed imaging using an ultrafast CCD camera with GenIV image intensifier



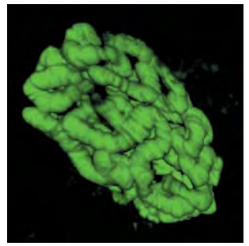
Time elapsed since electrical stimulation:
16ms 20ms 24ms 28ms

Post-electrostimulation changes in Ca^{2+} in mouse ventricular cardiac muscle cell labeled with Fluo-3, images taken at 4 ms intervals

By courtesy of Dr. Hideyuki Ishida, Dept. of Physiology, School of Medicine, Tokai University (also picture in the upper-left corner of the cover)

3) 3D Reconstruction

3D reconstruction data of living cells can be acquired at ease from stacks of sharp confocal images captured by a high-resolution CCD camera.



3D-constructed images of drosophila's salivary chromosome (labeled with PI)
(× 60 oil)

By courtesy of Dr. Yoshihiro Akimoto, Second Dept. of Anatomy, School of Medicine, Kyorin University

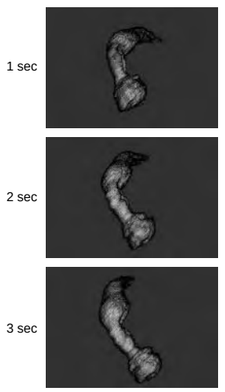
4) Real time 3D Observation

The real time 3D system (optional) enables you to perform high-speed 3D scanning of 30 cross-sectional images per second in synchronization with fast objective lens movement using piezo-actuator and a high-speed camera.

Examples of Application

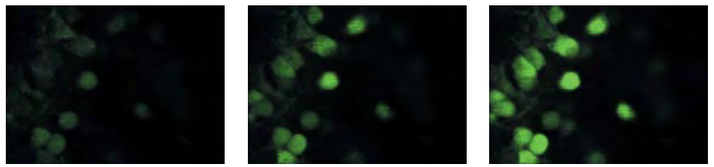
3D Reconstruction of Moving Objects

3D tracking of the behavior of C. elegans expressing EGFP-fused pharyngeal protein (3D images were reconstructed after acquiring each 30 cross section images per second)



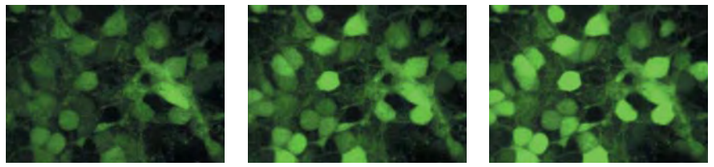
Real time 3D imaging of signal transduction

Single section confocal images



0 sec 6 sec 12 sec

Projection (overlay) of 30 cross-sectional images per second



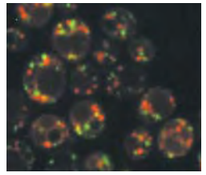
High-speed 3D scanning of glutamic acid-stimulated Ca^{2+} reactions in cerebral cortical nerve cells labeled with Fluo-3
By courtesy of Dr. Atsuo Fukuda, Dept. of Physiology, Hamamatsu University School of Medicine

5) Multicolor Dynamic Observation

CSU can generate real color confocal images thus can utilize a highly sensitive 3CCD color camera, which enables simultaneous multi-wavelength imaging as fast as at video rate.

Combining the CSU22 with a high-sensitivity high-resolution 3CCD color camera makes simultaneous multicolor observation without the need for filter replacement a reality.

Observation of the process of Golgi aparatus formation in fission yeast

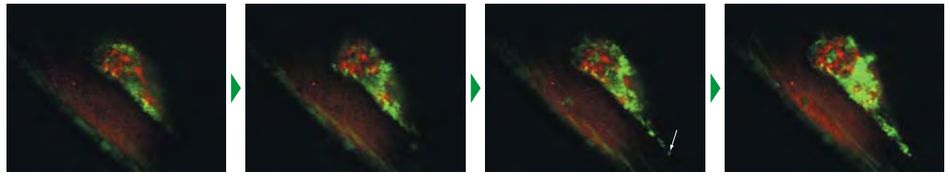


Real color imaging of formation of Golgi aparatus in budding yeast (Saccharomyces cerevisiae).
It is possible to clearly recognize cis-cisterna (EGFP) and trans-cistema (RFP) behave independently while dynamically interacting with each other.

By courtesy of Dr. Akihiko Nakano, Molecular Membrane Biology Lab., Discovery Research Institute, Riken & Prof. Graduate School of Science, University of Tokyo

By using a beam splitter in combination with a CCD camera, simultaneous dual color imaging as fast as millisecond level can be achieved.

Imaging of Microcirculation in live small animals:
Time lapse imaging of intravascular thrombus formation after laser irradiation



0 sec 2 sec 4 sec 6 sec
(Excerpts from video-rate time series)

Dynamic Movement of each individual platelet (spot indicated by an arrow in the “4 sec” image above) can be clearly recorded at video rate: platelets(green) ,fibrinogens (red).

By courtesy of Dr. Hideo Mogami, Dept. of Physiology, Hamamatsu University School of Medicine

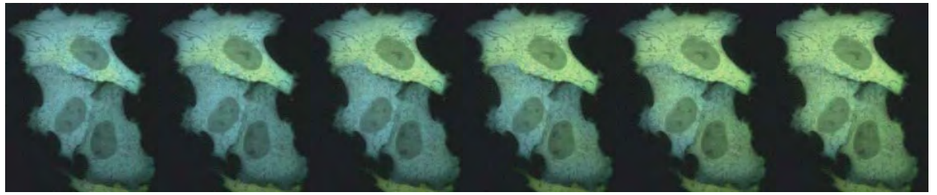
6) High-resolution, High-speed FRET Observation

Reduced fluorescence photo bleaching and high-speed imaging with CSU22 is best for confocal FRET observation in real time!

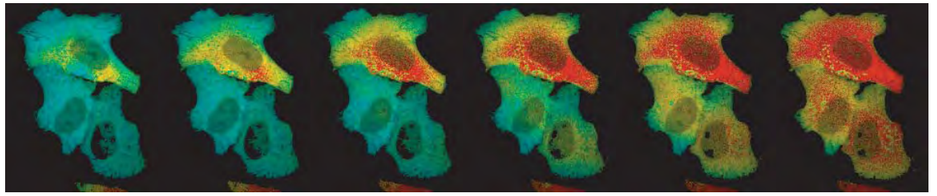
Real time, real color imaging of the initial stage of histamine-stimulated Ca^{2+} concentration in HeLa cell cytosol expressing Cameleon (YC3.60)

Video-rate FRET images: Excerpts at 264ms interval

Real-color images



Ratio images (Pseudo-colored)



By courtesy of Dr. Atsushi Miyawaki, Advanced Technology Group, Brain Science Institute, Riken and Dr. Takeharu Nagai, Lab. for Nanosystems Physiology, Research Institute for Electronic Science, Hokkaido University (pictures in the lower-right corner of the cover)

Examples of Microscope-setup of CSU22

Upright microscopes **Iinverted microscopes**

Olympus Leica Zeiss Nikon Olympus Zeiss

Note: The CSU22 can be mounted on microscopes from different manufacturers.

General Specifications

Model CSU22 Confocal Scanner Unit	
Confocal scanning method:	Microlens-enhanced Nipkow disk scanning
Video synchronization:	Scan-speed synchronization through NTSC or PAL composite signals
External synchronization:	Scan-speed synchronization through pulse signals Input: TTL level, 25 to 83.33 Hz Output: TTL level, 25 to 83.33 Hz (Corresponding to a Nipkow disk spinning speed from 1,500 to 5,000 rpm)
Excitation wavelength:	Standard (CH1): 488 nm Optional (CH2): 532 or 568 nm Optional (CH3): Multiple wavelengths (488, 568 and 647 nm) Contact Yokogawa for wavelength bands other than the standard wavelength
Excitation light ND filter:	0% (no light), 10%, and 100% (through)
Laser beam input:	Single-mode fiber connected with FC-connector
Fluorescence wavelengths to be observed:	Standard (CH1): 520 nm or longer Optional (CH2): 570 nm or longer, or 600 nm or longer Optional (CH3): Multi-wavelength (520 to 540 nm, 590 to 620 nm, and 680 to 710 nm) Note: For channels without filters, laser shield panel will be fixed instead of filters for laser safety.
Light path switching:	Manual switching between eyepiece for direct view and C-mount camera port using the port switching knob
Operation panel:	Switch for opening/closing the excitation light shutter Switch for selecting an ND filter Five wavelength filter combinations can be selected from three groups each consisting of three options of excitation filter (EX), dichroic mirror (DM), and barrier filter (BA).
External control:	RS-232C interface Requires the use of an optional external connection interlock key and cable (specify either Windows or Macintosh).
Microscope mount:	Add-on by using a direct C-mount adapter of the microscope
Operating temperature range:	15 to 30°C
Operating humidity range:	10 to 75% RH
Power consumption (main unit):	12 V DC, 1 A
Power consumption (AC adapter):	Input: 100 to 240 V AC ±10%, 50 or 60 Hz, 74 VA max. Output: 12 V DC, 2.5 A max.
External dimensions (outermost):	183 (W) × 205 (L) × 245 (H) (mm)
Weight:	5 kg (main unit)

Note: Use of Infinity corrected microscope with high NA objective lenses (i.e., Plan Apo) are recommended.

General specifications are subject to change without prior notice. The standard model does not include any peripherals such as a microscope, a laser unit, optical fiber, camera, image monitor, or image processing unit. For more information, contact the office indicated below.

Safety Precautions

* Read the user's manual carefully in order to use the instrument correctly and safely.
* If used in combination with a laser light source, this product falls under the category of class 3B laser products. Do not look directly into the beam and avoid touching it or any other direct exposure to it.

YOKOGAWA

Bio Centre , Life Science Business Headquarters
YOKOGAWA ELECTRIC CORPORATION

Kanazawa 2-3 Hokuyodai, Kanazawa-shi, Ishikawa, 920-0177 Japan
Phone: (81)-76-258-7028, Fax: (81)-76-258-7029

Tokyo 2-9-32 Nakacho, Musashino-shi, Tokyo, 180-8750 Japan
Phone: (81)-422-52-5550, Fax: (81)-422-52-7300

E-mail: CSU_livecell_imaging@ml.jp.yokogawa.com
URL: http://www.yokogawa.co.jp/SCANNER/english/e-index.html

Distributed By: Advanced Imaging Concepts, Inc.
301 North Harrison St., Building B, Suite 266
Princeton, NJ 08540 USA
Phone: (609) 921-3629, Fax: (609) 924-3010

info@aic-imagecentral.com www.AIC-ImageCentral.com