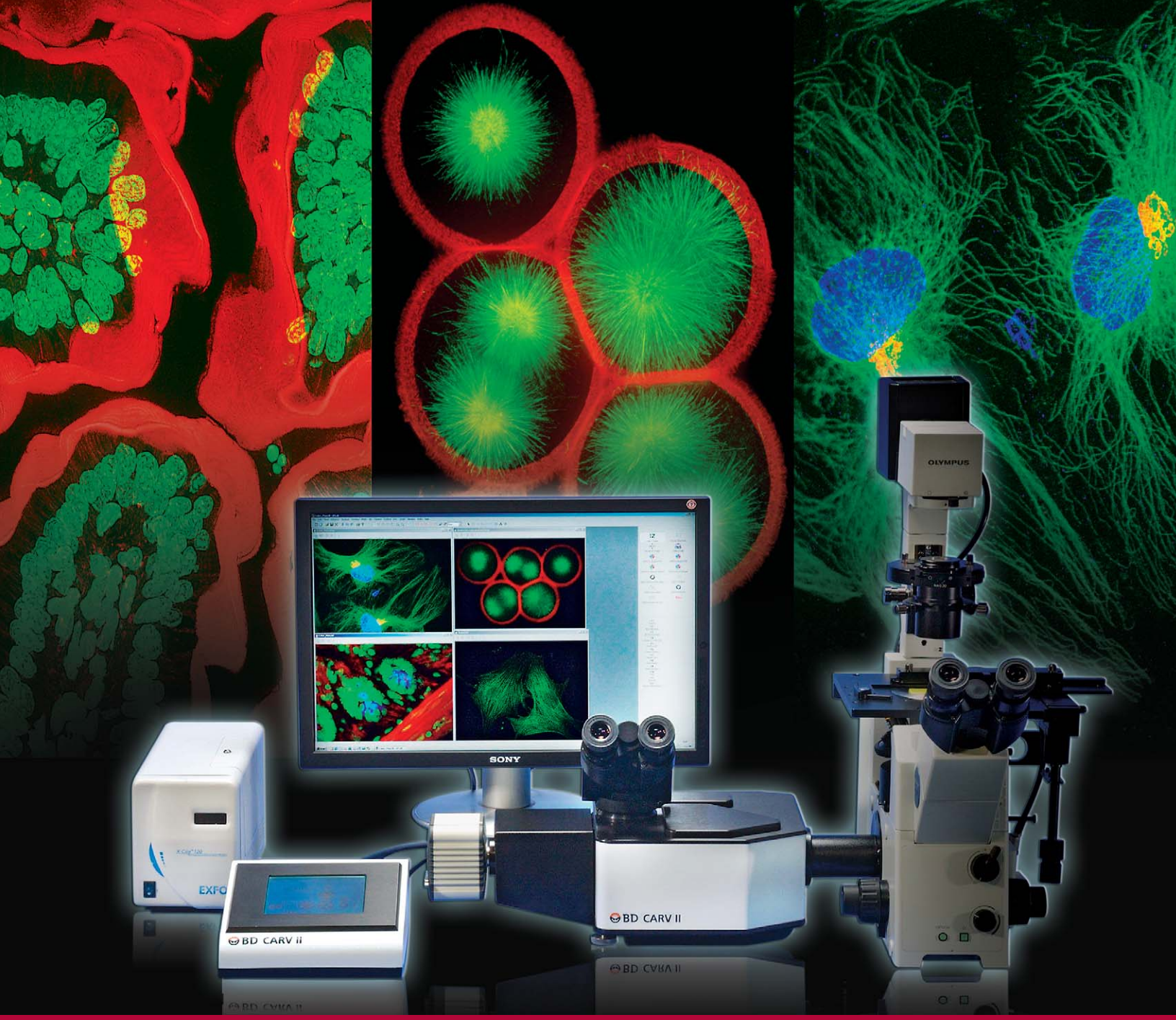


BD™ CARV II Confocal Imager

Real-time, full spectrum, personal confocal

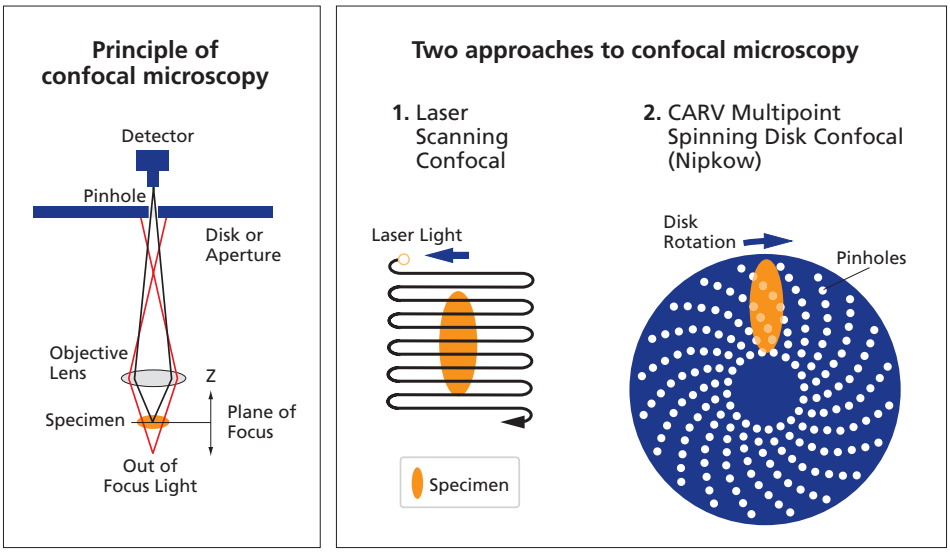
BD

BD Biosciences

Introduction to spinning disk confocal imaging

The BD™ CARV II confocal imager utilizes a Nipkow spinning disk containing multiple sets of spirally arranged pinholes placed in the image plane of the objective lens. The column of excitation light is projected through 1000 pinholes to simultaneously scan the entire field once every millisecond, thereby creating a full image of the focal plane in real-time. Emitted light is collected and imaged using a high resolution and high quantum efficiency CCD camera.

Confocal Microscopy

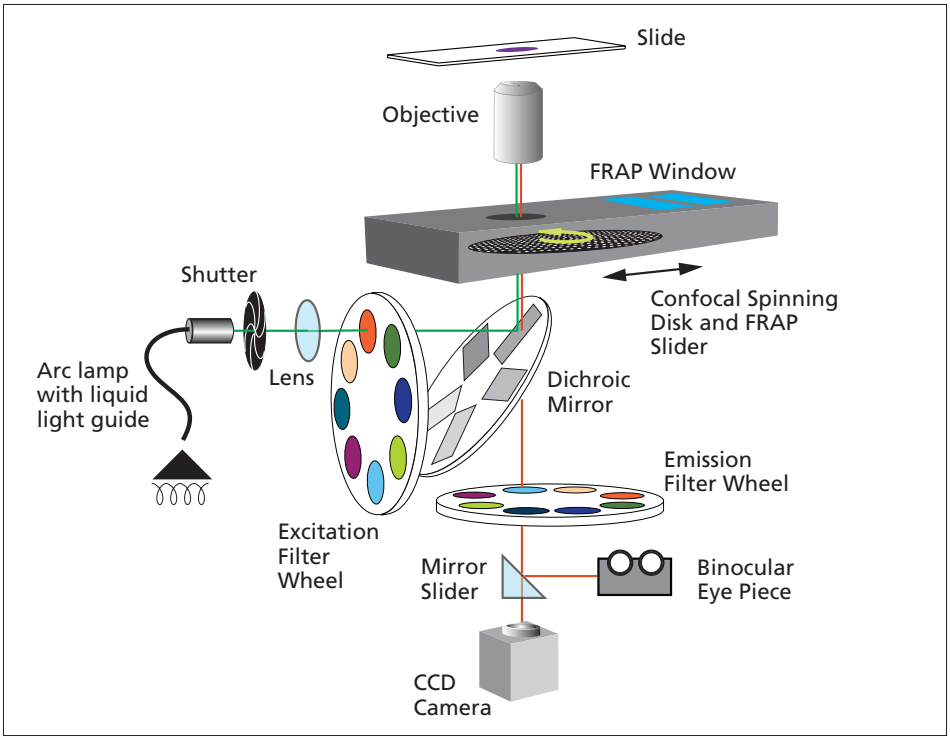


Schematic representation of confocal imaging. The left panel shows a simplified diagram of the emission path of a confocal imaging platform, indicating how out of focus light is prevented from reaching the detector. The right panel shows how the focused light is applied to the specimen by either laser raster scanning (1) or through a Nipkow disk system such as the BD CARV II. The sample is illuminated simultaneously with 1000 discrete points of light over a circular area 13 mm in diameter. A ½ inch CCD camera at 60X magnification covers an area 150 µm by 100 µm.

Schematic representation of the BD™ CARV II light path.

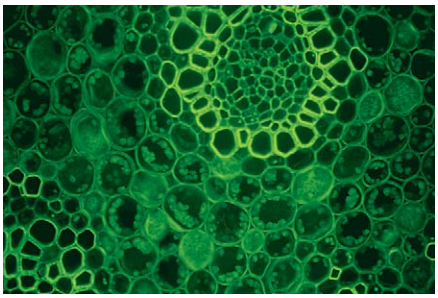
The variable intensity light from a Hg/metal halide light source passes through an excitation filter before being reflected by a dichroic mirror towards the sample. The emitted light passes through the dichroic mirror and emission filter before entering either the CCD camera or binocular eye piece. The Nipkow disk can be moved in and out of the light path to produce a confocal or a wide field fluorescence image.

A variable slit at the image plane can be used to selectively illuminate an area of the sample allowing Fluorescence Recovery After Photobleaching (FRAP) to be performed. All movable parts including the filter wheels, spinning disk shutters, and mirrors are automated and are controlled via touchpad or 3rd-party software.

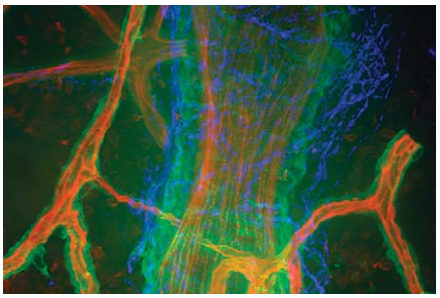


Explore the benefits of BD™ CARV II Confocal Imager

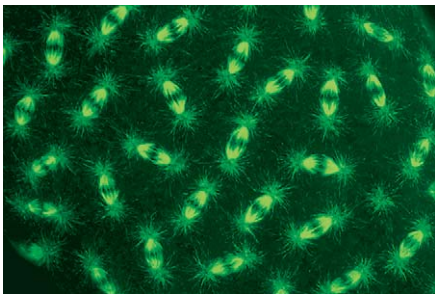
Features	Benefits
Multipoint scanning at 1000 scans/second.	Real time confocal imaging up to 100 frames per second or more. Use of CCD camera as a detector allows greater sensitivity and better signal-to-noise ratio images. Binocular viewing of confocal image allows easy set up and color viewing.
Low intensity high frequency scanning.	Minimizes photobleaching. Minimizes phototoxicity. Allows long term recording of biological events.
Mercury/metal halide light source with liquid light guide delivery.	Permits full spectrum (360 – 700 nm) confocal imaging. No light alignment required. Long lamp life (1500 hr) and low maintenance cost.
Built-in automated multi-position excitation, dichroic and emission filter wheels.	Permits fast multi-dimensional confocal imaging up to five or more fluorescent probes in the same sample. Uses commercially available filter sets.
Adapts to most major models of fluorescence microscopes.	Upgrade your fluorescence microscope to a personal confocal system.
Movable spinning disk and field aperture for selective illumination.	Permits switching between wide-field and confocal imaging. Allows Fluorescence Recovery After Photobleaching (FRAP) experimentation.
Full automation with RS232 control.	Operated by many popular imaging software packages such as Scanalytics' IPLab and Molecular Devices' Metamorph.® Allows multidimensional acquisition and analysis including, kinetics, co-localization, 1D – 5D rendering, ratio imaging, FRAP and FRET.



Plant stem
Dr. David Carter, UC Riverside, CA



Human Skin, maximum projection
CY2-Basement membrane, CY3-Neuron, CY5- Endothelial cells
Dr. William R. Kennedy and Gwen Wendelschafer-Crabb, University of Minnesota, MN



Drosophila Embryo- GFP-Tubulin
Dr. Garrett Odell, Friday Harbor, University of Washington, WA

The BD™ CARV II Confocal Imager at a glance

Image supplied by The Cooke Corporation, Auburn Hills, MI

CCD Detector

A wide range of application specific CCD cameras can be used with the confocal device including: Cooke (Sensicam QE, Sensicam EM), Roper (CoolSNAP™ ES, CoolSNAP™ HQ, Cascade™), Hamamatsu® (ORCA ER, AG), and more.

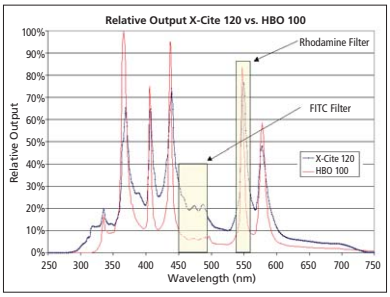


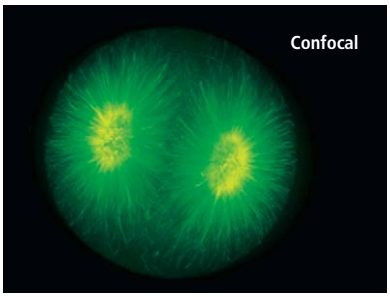
Image supplied by Exfo, Life Sciences Group, Mississauga Ontario, Canada

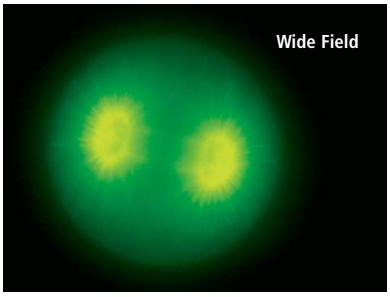
Light Source

A long life Hg/metal halide arc source, coupled to the instrument via an alignment-free light guide, allows for full spectrum (360 nm – 700 nm) confocal imaging of virtually any fluorescent probe.

Touch Pad Control

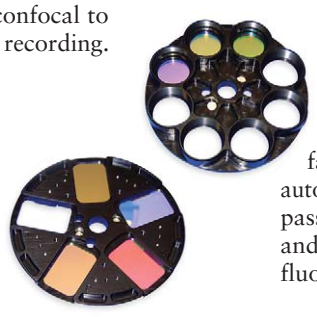
Control of and communication with the BD CARV II is accomplished via the RS-232 serial port at the back of the touch screen assembly. All automation can be controlled via the touch pad or it can be connected to a PC host that is running a standard terminal program or an application designed to integrate the BD CARV II into an imaging system.





Easily Switch Between Confocal and Widefield Imaging/Viewing Modes

The BD CARV II Confocal Imager permits direct viewing of confocal images through a binocular eyepiece and/or through the camera for fast imaging setup. It is the only pinhole spinning disk fluorescence confocal systems which allows the user to quickly switch from confocal to wide-field viewing or recording.



Automated Filter Changers

Automation of internal multi-position excitation (8 position), dichroic (5 position) and emission (8 position) filter wheels allows fast multi-dimensional confocal imaging. The automated filters reduce the reliance on multi-band pass filter sets allowing maximum light throughput and fast sequential imaging of up to five or more fluorescent probes in the same sample.

Imaging Software

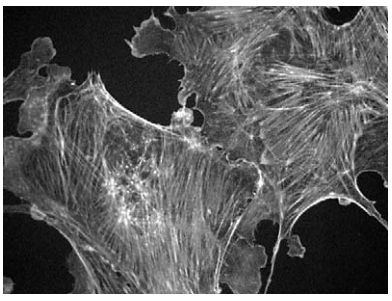
A range of state of the art 3D software packages can be used for acquisition and analysis of confocal images including IPLab (Scanalytics) and Metamorph (Molecular Devices). Command sets needed to control the BD™ CARV II are available on request.

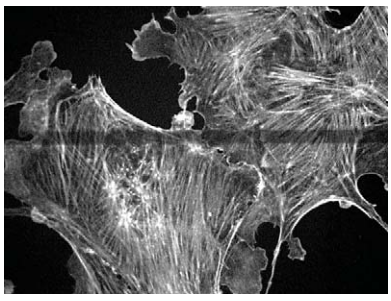
Microscope Compatibility

The BD CARV II Confocal Imager unit can be configured to most inverted and upright fluorescent microscopes converting your existing fluorescence microscope to a confocal imager.

FRAP Capabilities

A FRAP slit at the same focal plane as the confocal disk creates an adjustable rectangular aperture on the image. Passing high-intensity Hg/metal halide light through this slit enables controlled photo-bleaching of part of the sample followed by fluorescence recovery recording.

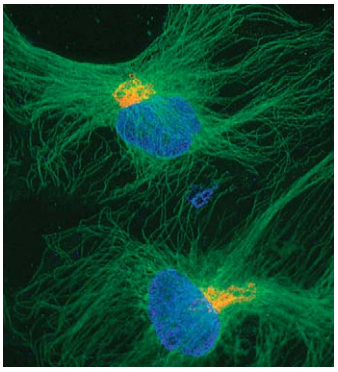




Applications

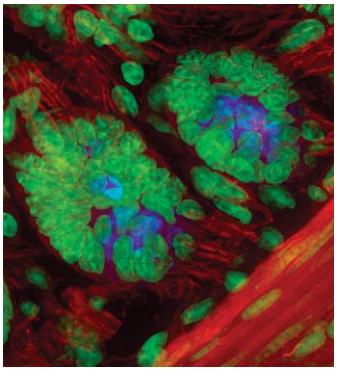
Full-spectrum, High-resolution Imaging

BD™ CARV II is suitable for both live cell and fixed cell confocal imaging using a wide variety of fluorophores.



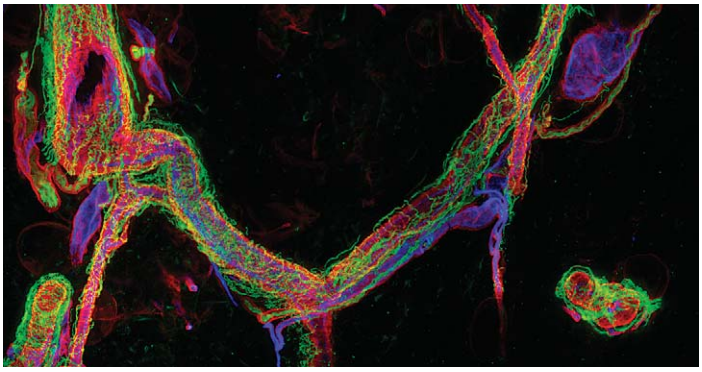
Stack projection of HeLa cells with Qdot™ Conjugates. Nucleus – Qdot™ 655; Golgi – Qdot™ 585; Microtubules – Qdot™ 525.

Quantum Dot Corporation, Hayward, CA



Stack projection of mouse intestine section labeled with Alexa Fluor® 350 WGA, Alexa Fluor® 568 phalloidin, and SYTOX® Green.

Molecular Probes/Invitrogen, Eugene, OR

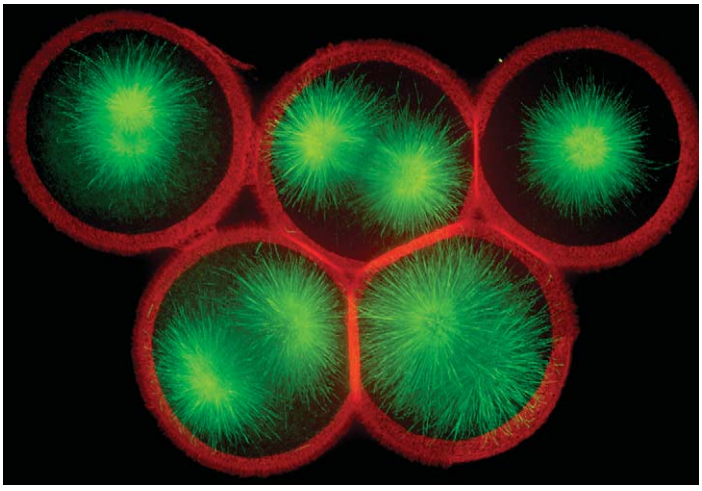


3D reconstruction of imaged skin sample labeled using pan-neuronal marker, protein gene product 9.5 localized with Cy3 and basement membrane marker, type IV collagen with CY2. Endothelial cells are stained using Cy5-Ulex europeaus agglutinin type I.

Dr. William R. Kennedy and Gwen Wendelschafer-Crabb, University of Minnesota, Minneapolis, MN

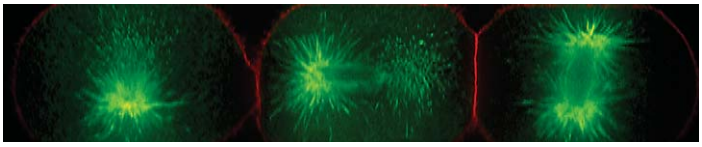
3D Reconstruction

Automation of internal multi-position excitation (8 position), dichroic (5 position) and emission (8 position) filter wheels allows multi-channel 3D and 4D images to be obtained quickly without compromising resolution. Using any of the recommended software packages, 3D and 4D reconstructions can be easily performed.



Maximum projection and orthogonal view created from confocal sections through a sea urchin embryo fixed and stained with anti-tubulin (green) and phalloidin (red).

Tubulin (green), Actin (red), (60X 1.4NA) Dr. George von Dassow, Center for Cell Dynamics, Friday Harbor Labs, University of Washington, Friday Harbor, WA



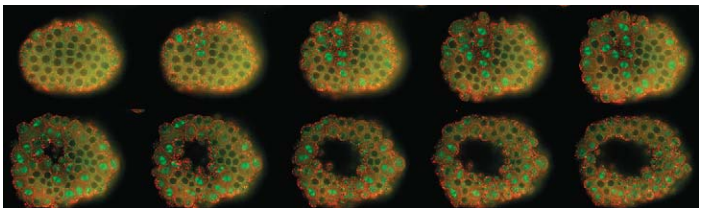
Orthogonal view (XZ)

BD CARV II Z-resolution with Olympus Objectives

Objective	Type	NA	Medium	Measured PSF (microns)
40X Water DIC	Plan Apochromat	1.15	Water	1.2
60X Water DIC	Plan Apochromat	1.2	Water	1
60X Oil DIC	Plan Apochromat	1.4	Oil	0.8
100X Oil DIC	Plan Fluorite	1.3	Oil	0.6
100X Oil DIC	Plan Apochromat	1.4	Oil	0.5

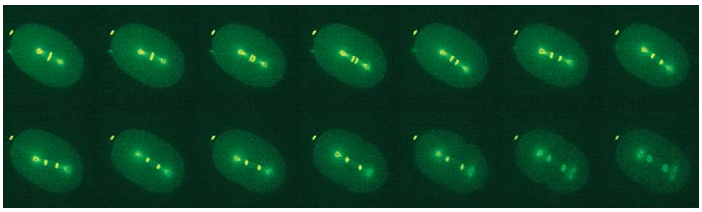
Time Lapse

Using any recommended imaging software and camera, time lapse imaging of cells or organisms can be performed for long periods of time without significant bleaching or phototoxicity. Depending on the speed of the cellular event being measured, and the installed BD™ CARV II components, time lapse at a single plane or at multiple planes (4D) can be imaged.



Time lapse images at a single confocal plane of a sand dollar embryo during mid blastula stage (rhodamine-tubulin and Alexa 488 phalloidin, Molecular Probes, Eugene, Oregon).

Dr. George von Dassow, Bill Bement, Center for Cell Dynamics, Friday Harbor Labs, University of Washington, Friday Harbor, WA

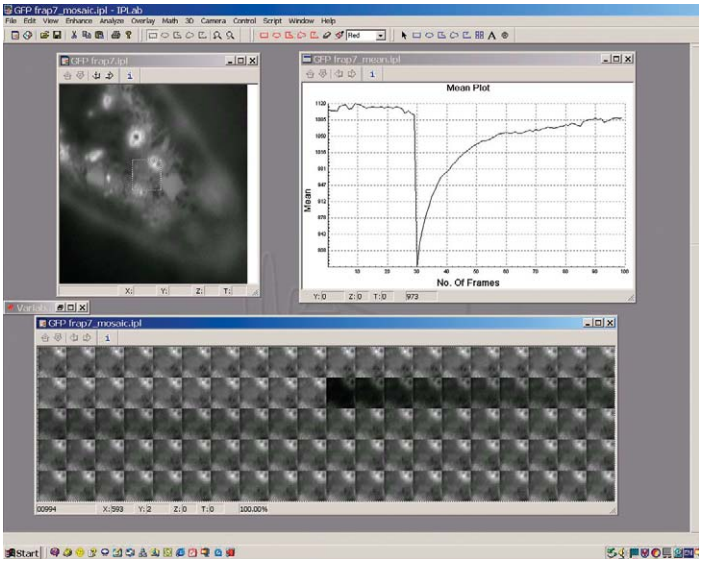


Time lapse images at a single plane of a Nematode embryo going through the first cell division stage. GFP-tubulin, GFP-histone.

Dr. Fabio Piano, NYU, New York, NY

Fluorescence Recovery After Photobleaching (FRAP)

Our FRAP capability allows software control of a variable rectangular aperture for photobleaching a selected area with full spectrum (white) light. A software protocol can then be set up to record fluorescence before and after bleaching. The recovery kinetics can be analyzed using a variety of commercially available software packages.

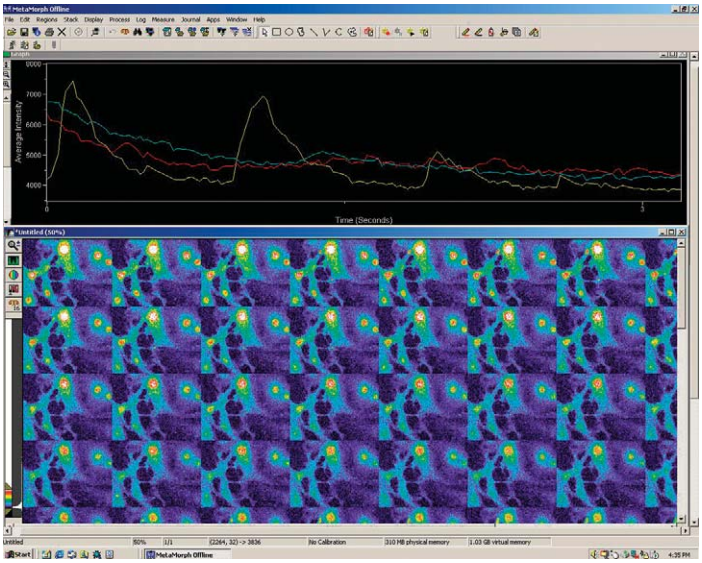


Time lapse images and FRAP kinetics of endoplasmic reticulum in a plant cell stained with the membrane dye 3,3-dihexyloxacarbocyanine iodide (DiO6(3), Molecular Probes, Eugene, Oregon).

The FRAP protocol including image capture and analysis was performed using IPLab (Scanalytics, Fairfax, VA) imaging software.

High Speed Calcium Imaging

BD CARV II, in combination with CCD cameras with on chip amplification, can be used to image fast fluorescence changes at rates ranging from 50 – 100 frames per second.



Time lapse recordings at 50 frames per second of calcium sparks in muscle cells loaded with calcium indicator dye Fluo-4, Molecular Probes, Eugene, Oregon.

The Roper Cascade 512B CCD camera in conjunction with Metamorph® (Molecular Devices, Sunnyvale, CA) imaging software was used to capture the images.

Dual Emission Imaging (FRET)

The BD CARV II, in combination with the Dual View™ adapter from Optical Insights LLC, Tucson, AZ allows you to perform dual emission imaging including CFP/YFP and GFP/RFP Fluorescence Resonance Energy Transfer (FRET).

Specifications

BD™ CARV II Confocal Imager

- Confocal scanner: Nipkow spinning disk (pinholes)
- Disk scan rate: 1000 scans per second
- Pinhole diameter: 70 µm
- Spectral transmission: 360 nm – 700 nm
- Z-resolution: 0.5 µm (PSF); 100X PlanApo 1.4NA
- Illumination source: 120 W Hg/metal halide (1200 hr)
- Internal excitation changer: Automated 8 position wheel (25 mm)
- Internal dichroic changer: Automated 5 position wheel (25.7 × 36 mm)
- Internal emission changer: Automated 8 position wheel (25 mm)
- Filter sets provided: DAPI, E-GFP, Texas Red; (Semrock Inc., Rochester, New York, BrightLine™ series)
- Operation mode: Automated confocal, wide field, bright field
- Observation: Direct confocal binocular viewing or camera port
- Detector compatibility: CCD camera – Sensicam EM, QE (Cooke); CoolSNAP™ HQ, Cascade 512B (Photometrics); ORCA ER, AG (Hamamatsu) and more
- Microscope compatibility: Most inverted fluorescence microscopes with 100% camera port
- FRAP: Aperture control – touch pad or RS232
- System control: Touch pad or Computer via RS232 interface
- Software drivers: IPLab (Scanalytics, Fairfax, VA); Metamorph® (Molecular Devices, Sunnyvale, CA) RS232 command set also available
- Size: 11(w) × 15.5(L) × 6(h) inches
  27.94(w) × 39.37(L) × 15.24(h) cm
- Weight: 14.5 lbs / 6.6 kg
- Power: 100 – 124V AC / 12V DC

Note: Specifications represented may include optional components.
Specifications subject to change. For most updated specification, please contact BD Biosciences.

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