BD CARV II™ Confocal Imager Installation and User's Guide

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Patents

CARV II: 6,147,798 and patent pending

FCC Information

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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History

Revision	Date	Change Made
000	03/05	Initial release

Contents

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BD CARV II Hardware

The following topics are covered in this chapter:

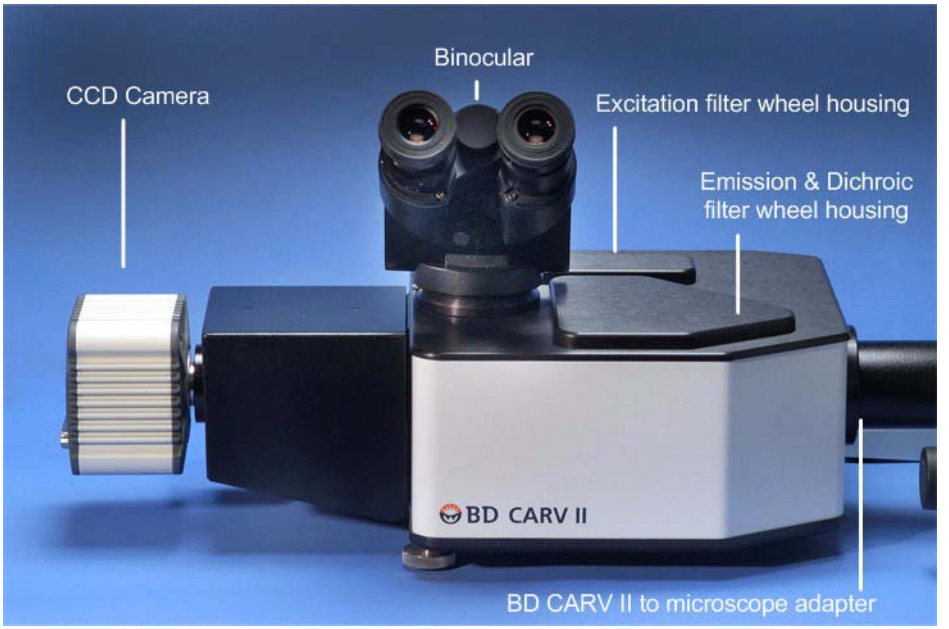
- BD CARV II Description
- BD CARV II Specifications
- BD CARV II Light Path

BD CARV II Description

The BD Confocal and Real-time Vision (CARV) II™ is a fully-automated, full spectrum spinning disk confocal with florescence recovery after photobleaching (FRAP) capabilities. It can be attached to most major inverted epi-fluorescence microscopes, and comes with its own variable intensity 120W Hg/metal halide light source, which allows for full spectrum (360nm-700nm) confocal imaging.

Synchronous automation of an eight-position excitation wheel, a five-position dichroic wheel, and an eight-position emission wheel allows for fast multi-wavelength confocal imaging. This eliminates the use of multi-band pass filter sets and provides maximum light throughput and fast sequential imaging.

BD CARV II can be used with most high-end interline or frame transfer charged couple device (CCD) cameras, as well as with such imaging software as IPLab (Scanalytics, Fairfax, VA), Metamorph (Universal Imaging Corporation, Downington, PA), and AQM (Kinetic Imaging, Nottingham, England).



In addition to the main unit, which houses all of the optics and motors, the CARV II also has a touch pad controller, which controls virtually all the input/output for the system.

The light source is an EXFO X-Cite 120W Hg/metal halide lamp which delivers light to the CARV II via a 1.5 M EXFO liquid light guide.

BD CARV II Specifications

The following table details the specifications for the BD CARV II system.

Table 1: BD CARV II Specifications

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	120W Hg-Metal Halide (1200hr)
Internal Excitation Changer	automated eight position wheel (25 mm)
Internal Dichroic Changer	automated five position wheel (25.7x36 mm)
Internal Emission Changer	automated eight position wheel (25 mm)
Filter Sets Provided	DAPI, E-GFP, TEXAS RED; (Semrock Bright line 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
Florescence Recovery After Photobleaching (FRAP)	aperture control–touch pad or RS232
Control	touch pad or RS232
Software Drivers	IPLab (Scanalytics); Metamorph (Molecular Devices/Universal Imaging Corporation) RS232 command set also available

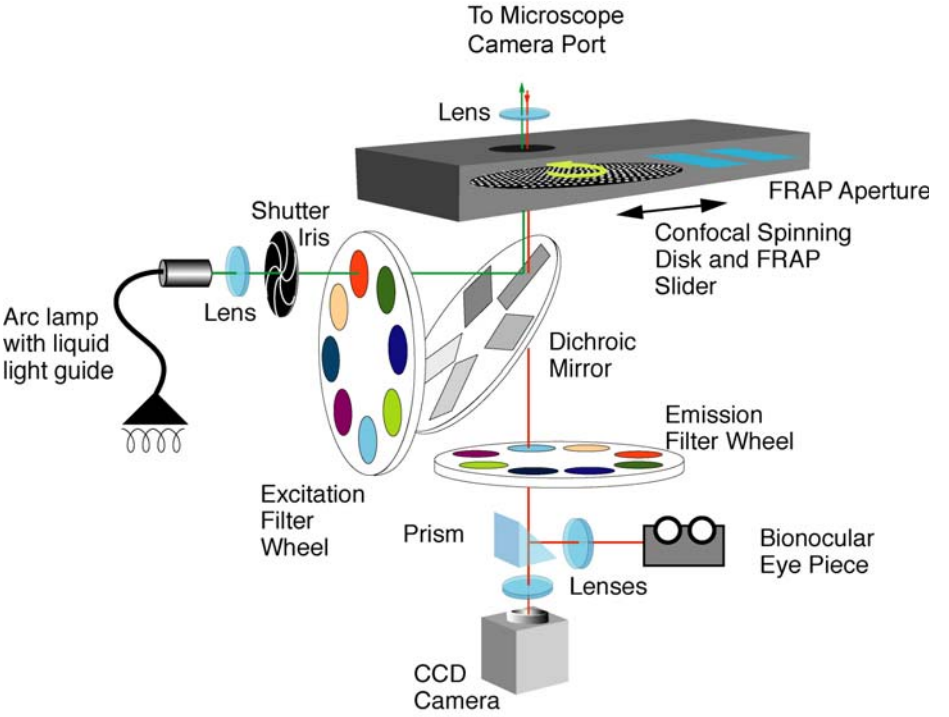
Size	11(w)x 15.5 (L) x 6 (h) inches 27.94(w) x 39.37 (L) x 15.24 cm
Weight	14.5 lbs /6.6Kg
Power	100-240 VAC, 1.2 A, 50/60 Hz

Figure 1-1 Complete System Mounted on an Olympus IX 71 Microscope with a Hamamatsu Ocra ER Camera

BD CARV II Light Path

The following diagram illustrates the light path as it travels through the BD CARV II and interacts with the various components of the system.

Figure 1-2 CARV II Confocal Imager Light Path



Arc Lamp: The light source is an EXFO X-Cite 120W Hg/metal halide lamp which delivers light to the CARV II via a 1.5 M EXFO liquid light guide. The liquid light guide delivery system produces even illumination and eliminates the need for arc lamp alignment. The lamp has typical Hg arc spectral characteristics and has a life of 1200 hours.

Shutter: The illumination shutter can be controlled via the touch pad controller or via RS232 commands (software control).

Illumination Intensity Iris: This iris allows the user to vary the illumination intensity via the touch pad controller or via RS232 commands (software control).

Excitation Filter Wheel: The quick release excitation filter wheel has eight filter positions and accepts commercially available 25 mm filters. Filter positions can be controlled via the touch pad controller or via RS232 commands (software control).

Dichroic Filter Wheel: The quick release dichroic wheel has five positions and accepts commercially available 25.7x36 mm dichroic mirrors. Dichroic positions can be controlled via the touch pad controller or via RS232 commands (software control).

Emission Filter Wheel: The quick release emission filter wheel has eight filter positions and accepts commercially available 25 mm filters. Filter positions can be controlled via the touch pad controller or via RS232 commands (software control).

Spinning Disk: The Nipkow spinning disk can be moved in and out of the light path, allowing both confocal images and wide-field images to be obtained. The spinning disk can be controlled via the touch pad controller or via RS232 commands (software control).

FRAP Slit: The adjustable FRAP slit sits on the image plane and is moved into position when the spinning disk is moved out. The FRAP disk is controlled via the touch pad controller or via RS232 commands (software control).

Eyepiece: The binocular eyepiece is used to observe both wide field and confocal images in real time.

Prism: The prism is used to divert the image either to the eyepiece or camera and is controlled via the touch pad controller or via RS232 commands (software control).

Camera: A wide range of high-end CCD cameras can be attached to the confocal unit to obtain confocal, wide-field, or dark-field images.

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BD CARV II Installation

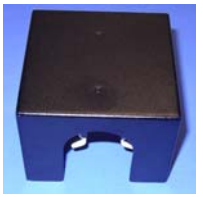
The following topics are addressed in this chapter:

- BD CARV II Components
- Filter Wheel Assembly
  - Filter Descriptions
  - Changing Filters and Dichroics
- Binocular Assembly
- Touch Pad Controller Assembly
- Light Source (EXFO X-Cite 120) Assembly
- CARV II Attachment to Microscope
- Camera Attachment
- Camera Focus Adjustment

BD CARV II Components

The following table lists all of the components of the BD CARV II:

Part	Picture	Part Description
1		Confocal head with two filter housing covers and camera tube attached.
2		Eight-position excitation filter wheel with DAPI, GFP, and TEXAS RED exciter filters in position 1, 2 and 3. (See Filter Descriptions on page 19 for more information.)
3		Eight-position emission filter wheel with DAPI, GFP, and TEXAS RED exciter filters in position 1, 2 and 3. (See Filter Descriptions on page 19 for more information.)
4		Five-position dichroic wheel with DAPI, GFP, and TEXAS RED dichroic mirrors and a mirror at positions 1, 2, 3 and 4 respectively. (See Filter Descriptions on page 19 for more information.)
5		Microscope specific adapter

Part	Picture	Part Description
6		Binocular attachment
7		Two eyepieces x10
8		Camera tube shroud
9		Touch pad
10		CARV II power supply

Part	Picture	Part Description
11		Country-specific CARV II power supply AC cord
12		Interconnect cable
13		DB 9 RS232 cable
14		BNC camera sync cable
15		EXFO X-Cite 120 power supply

Part	Picture	Part Description
16		EFXO X-Cite 120 Hg/metal halide lamp
17		1.5M liquid light guide
18		Country specific X-Cite 120 power supply AC cord
19		3mm T handle wrench
20		Hex keys (1.5 mm & 0.05 mm)

Part	Picture	Part Description
21		Filter ring spanner wrench
22		Bubble level

Filter Wheel Assembly

CARV II accommodates three filter wheels: an eight-position excitation wheel, a five-position dichroic wheel, and an eight-position emission wheel. The user is provided with three single filter sets which come mounted on the filter wheels: DAPI, E-GFP, and Texas Red (see Filter Descriptions on page 19). In addition, the fourth position on the dichroic wheel has a mounted mirror to be used in conjunction with the FRAP capability.

The topics that are discussed in the following sections are:

- Filter Descriptions
- Changing the Filter Wheels
- Changing Filters and Dichroics

Filter Descriptions

Three filters are included in the CARV II system:

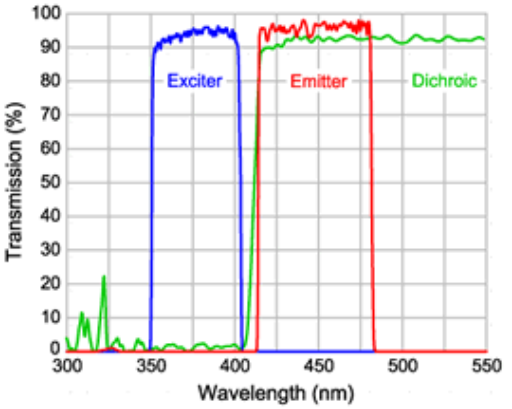
- DAPI BrightLine™ FF409 Fluorescence
- E-FFP BrightLine™ FF495 Fluorescence
- Texas Red® BrightLine™ FF593 Fluorescence

NOTICE Graphs provided by Semrock®.

DAPI BrightLine™ FF409 Fluorescence

Optimized for these fluorophores:

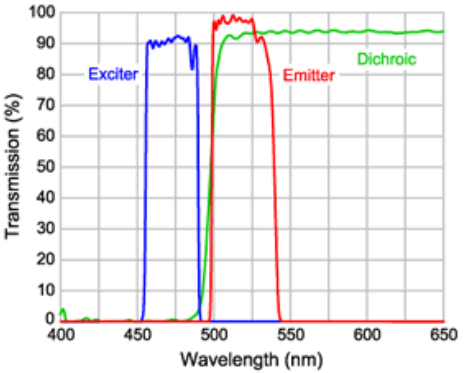
DAPI, Alexa Fluor 350, AMCA, BFP, Hoechst, LysoSensor Blue, Marina Blue, Pacific Blue, and sgBFP



E-FFP BrightLine™ FF495 Fluorescence

Optimized for these fluorophores:

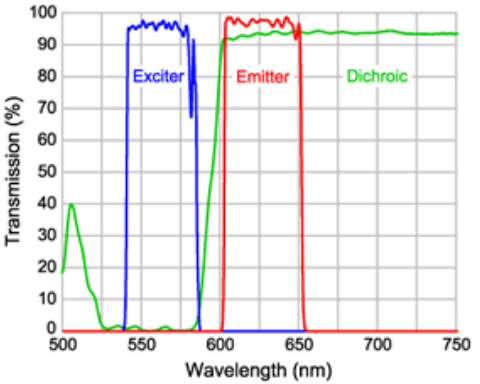
GFP, Cy2™, CyQuant, DiO, EGFP, sgGFP™, wtGFP (non-UV excitation), YOYO-1, YO-PRO-1



Texas Red® BrightLine™ FF593 Fluorescence

Optimized for these fluorophores:

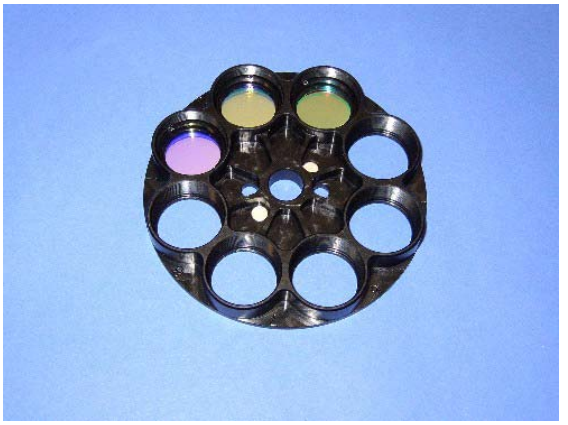
Texas Red®, 5-ROX, Alexa Fluor 568 & 594, Calcium Crimson, Cy3.5™, HcRed, and MitoTracker Red



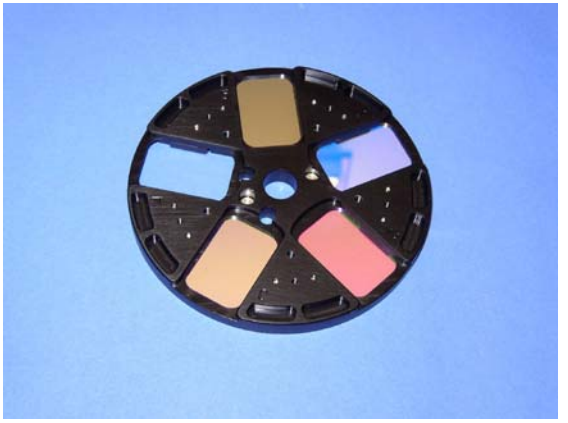
Changing the Filter Wheels

The filter wheels can be distinguished by the following features:

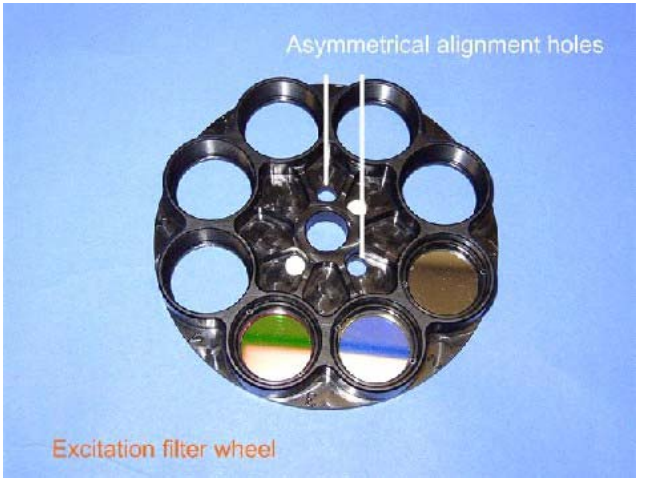
- The emission filter wheel has eight, 25mm filter positions with symmetrical alignment holes.



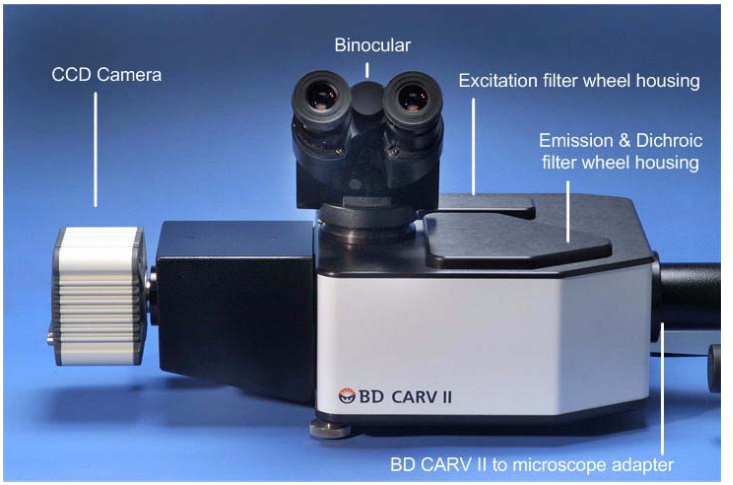
- The dichroic filter wheel is distinguished by the (25.7x36 mm) five-position dichroic mirrors.



- The excitation filter wheel has eight, 25mm filter positions with asymmetrical alignment holes.

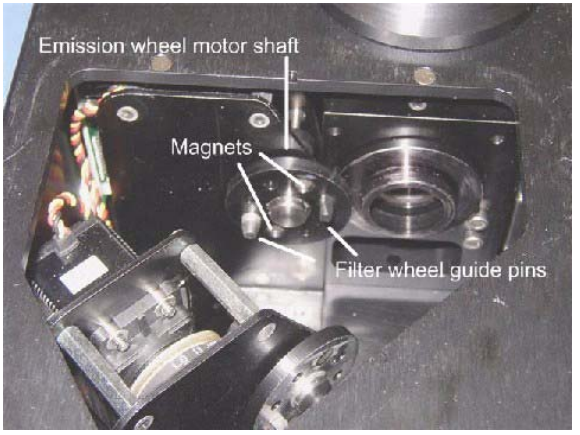


In order to access the filter wheels, remove the wheel housing covers which are held by magnets.



To change or add new filter sets, remove the filter wheel from the confocal head. Each wheel is held in place by magnets; therefore, in order to remove a wheel, grasp it with both hands using your thumb and forefinger (take care not to put your fingers on the filter surfaces) and gently pull it away from the guide pins. Once the wheel is disengaged pull it vertically clear of the housing.

To replace a wheel, use the two alignment holes to help you guide the wheel into a locked position. When attaching the wheels, lower them so that the alignment holes slide on to the two corresponding guide pins on the motor shaft.



The holes are designed so that the wheels will fit only one way. Take care not to touch the filters or mirrors when mounting the wheels. Once all three wheels are mounted, place the magnetically held covers back on the confocal head.

Changing Filters and Dichroics

Changing Excitation and Emission Filters

The 25 mm filters are held in position by a 25 mm filter ring. This ring is already screwed into each filter socket. Identify the position (1-8) where you want to place or replace the filter and unscrew the ring using the provided filter wheel ring wrench.

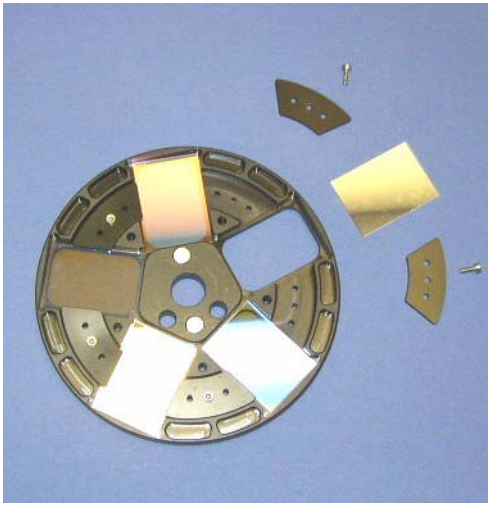
The filters need to be mounted so that the surfaces are facing in the correct direction. Both excitation and emission filters have an arrow marked on the side of the filter to indicate which direction the filters should face.

Make sure that the arrow on both the excitation and emission filters is pointing towards the sample (towards the dichroic mirror) when placed in the filter wheels.

Changing the Dichroic Mirrors

Extra precaution is necessary when mounting or removing dichroic mirrors. Lay the wheel flat on a flat surface so that the mounting screws are facing upwards.

Using the hex key provided, unscrew the holding plates on either side of the dichroic position where you wish to replace or mount the mirror. Using a clean piece of tissue paper, gently remove the holding plates.



It is important to ensure that the dichroic mirror is properly mounted on the wheel. The coated surface of the mirror should be facing downward as it sits on the flat surface. The coated surface generally has a beveled edge, or on occasion, the manufacturer will use an arrow on the edge of the mirror to point to the coated surface.

Once the mirror is placed on the filter wheel, carefully place the holding plates in position and gently tighten the screws. Make sure not to tighten the screws too much since this may cause the mirror to fracture. A good way to check for tightness is to gently shake the filter wheels after a few turns of the screws. If you hear a rattle you can tighten a bit more. Continue tightening the screw until the rattling stops.

The filter wheels are now ready for mounting on the confocal head.

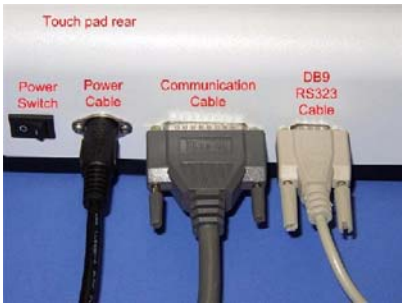
Binocular Assembly

Attach the eyepieces to the binocular assembly, then attach the binocular assembly to the confocal head using a 3mm hex key.

Touch Pad Controller Assembly

The touch pad controller and the CARV II unit share a power supply. Attach the power supply to the touch pad. Attach one end of the interconnect cable to the touch pad controller and the other end to the CARV II unit. Attach one end of

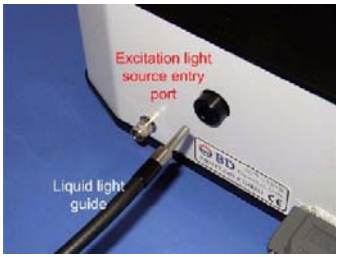
the DB9 RS232 to the touch pad controller and the other end to a com port (serial port) on the computer.



Light Source (EXFO X-Cite 120) Assembly

NOTICE Refer to the EXFO X-Cite 120 manual for information about how to assemble the light source.

Attach one end of the liquid light guide to the lamp and insert the other end into the light guide entrance on the rear panel of the CARV II confocal head. Insert the light guide as far as it will go and then tighten it with the 0.05mm hex key.



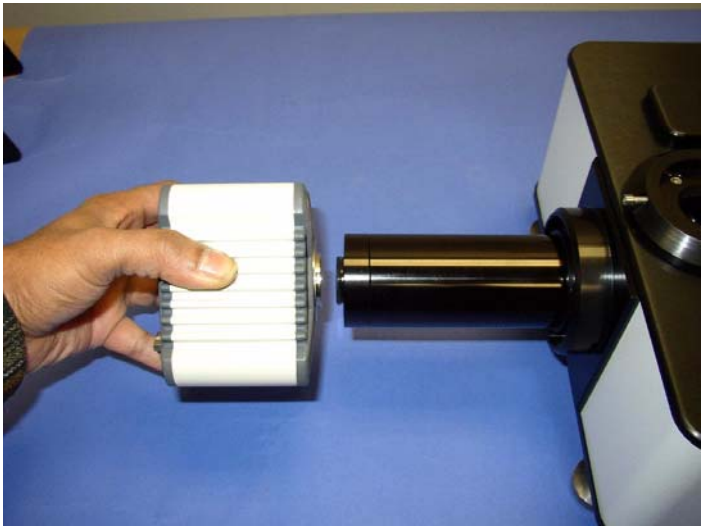
CARV II Attachment to Microscope

The CARV II comes with an adapter specific to the microscope. Slide the adapter on to the front end of the confocal head and tighten the screws to secure it (see figure; Olympus IX 70 adapter example).

Mount the confocal head on the side port (camera port) of the microscope. It is important that the confocal head is parallel to the side port of the microscope. This is achieved by leveling the adjustable feet on the confocal head. Place the provided bubble level on the stage of the microscope and note the position of the bubble. Next, place the bubble level on the top surface of the confocal head. Adjust the feet until the bubble position is the same for the confocal head as it was for the stage of the microscope. Now tighten the holding screws firmly to the microscope.

Camera Attachment

A range of CCD cameras can be used with the CARV II. Mount the CCD to the C-mount at the end of the camera tube on the confocal head.



Camera Focus Adjustment

Focusing the Pinholes

The spinning disk is in a plane conjugate to the image plane. As it spins, it scans the microscopic fluorescent image point by point in real-time. It is essential that the eyepieces and/or cameras be precisely focused on the image plane (the pinholes) of the spinning disk in order to obtain a sharp image.

Turn the touch pad controller on and wait for it to initialize. Turn on the bright field lamp of the microscope and send the light to the side port of the microscope. For this step, it is not necessary to have a sample on the microscope. Using the CARV II control features within the imaging software do the following:

1. Select the green florescent protein (GFP) filter set in the light path.
2. Move the spinning disk into light path (confocal mode).
3. Stop the disk from spinning.

4 Direct the light to the eyepiece.

5 Look through the eyepiece. You should be able to see the pinholes on the disk.

6 Focus the eyepiece until the pinholes are in focus.

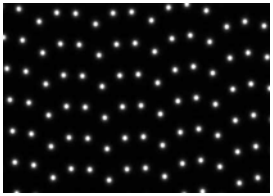
7 Next, redirect the light back to the camera.

8 Switch the camera to live mode.

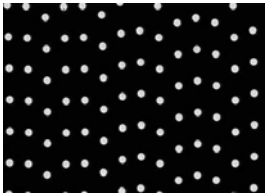
9 Adjust the exposure time to 50 ms and then adjust the bright field lamp power until an image of the pinholes can be observed.

10 The pinholes should be close to being in focus. The camera tube still needs to be adjusted until the pinholes are in focus and the image is sharp. This is necessary because, when the CARV II leaves the factory, it is focused using a CCD camera; however, the distance between the chip surface within the camera and the C-mount may vary from camera to camera.
To correct the focus, loosen the camera tube mount slightly using the three mm T-wrench provided with the system.

11 Now slide the camera tube towards or away from the microscope in very short increments until the pinholes are in focus and sharp.



Pinholes Not in Focus



Pinholes in Focus

12 Now that the pinholes are in focus, gently tighten the camera tube in place. Make sure that the camera image is squared to the screen by rotating the camera tube. Now tighten the camera tube firmly into position.

3

BD CARV II Touch Pad Controller

The CARV II consists of two major components: the main unit and the touch pad controller. The main unit, which houses all of the optics and motors, must be mounted on a microscope. The touch pad controller, which contains virtually all the input/output for the CARV II, can be placed anywhere within reach of the interconnect cable. The CARV II cannot operate unless the touch pad controller is attached to the main unit and turned on.

The following topics are covered in this chapter:

- Touch Pad Controller Connections
- Manual vs. Software Control
- Main CARV II Screen
- Secondary CARV II Screens
- Calibration
    - Calibrating the Prism
    - Calibrating the FRAP Iris
    - Calibrating the Filter Wheels

Touch Pad Controller Connections

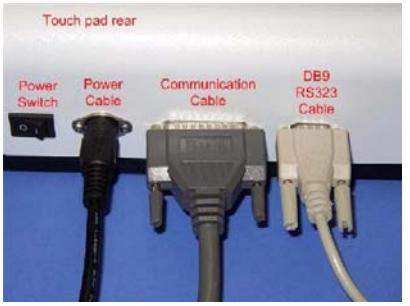
Except for the BNC connection for the optional external shutter trigger, all input/output is connected to the back of the touch pad controller.

There are three connections that need to be established:

- The DB-9 connector on the far right is the RS-232 serial connector for communications with a PC host. A standard, straight-through DB-9 extension cable is provided to establish this connection with a PC COM port.

- The next connector to the left is a DB-25 that provides all the power and signals to the main unit. The interconnect cable between the touch pad controller and the main unit is NOT a standard DB-25 extension cable. Because both power and clock signals are transmitted through the cable, it uses a larger wire gauge and more robust shielding. Replacing this cable with a standard DB-25 cable may cause the instrument to malfunction.

- The circular, five-pin DIN connector accepts the plug from the provided power supply. This power supply provides the necessary 5 and 12 V DC for the CARV II. To make sure the polarity and power capacity is correct, this supply should only be replaced with one of the same type. The power switch at the rear of the touch pad controller switches power for both the touch pad and the main unit simultaneously.



Touch Pad Controls

The CARV II can be manually controlled with the touch pad. The touch pad will respond to firm pressure from blunt objects, such as a finger or pencil eraser. Do not actuate buttons on the touch pad with the writing tip of a pin or any other pointed, hard object. The touch pad should be cleaned regularly with a lens cloth or a moist, soft cloth.

There are seven different screens available to the user for CARV II control. Computer control of all the devices in the instrument is also always available, regardless of the current screen on the touch pad. Commands sent from the computer that affect devices displayed on the touch pad current screen will update the screen.

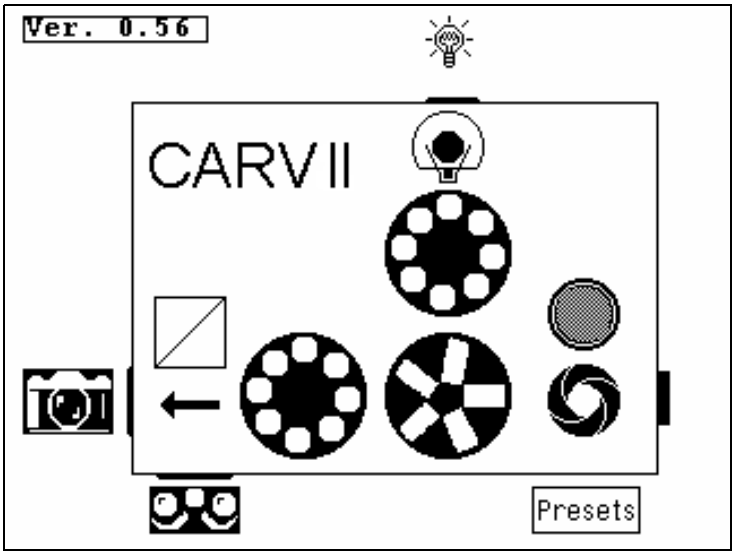
To avoid confusion and possible command collisions, avoid touching objects on the touch pad screen while simultaneously sending computer commands. Alternatively, an "M1" command can be sent to lock out the buttons on the current screen (for more information, see Command Formats on page 55). All touch pad actions will be ignored and a picture of a combination lock will be displayed in the lower left-hand corner of the current screen. The screen will still be updated with any applicable computer commands. Touch pad control can be restored with an "M0" command.

If it appears that the button action is not completely aligned with the button graphics, the touch pad may need to be recalibrated. This can be done by touching anywhere on the touch pad screen while the power switch is turned from off to on. Continue touching the screen until the calibration screen is displayed. The calibration screen has instructional text to guide you through a three-point calibration of the touch pad. Upon completion of the three-point calibration, the Main Screen is displayed. The touch pad can be calibrated at any time by power cycling and touching the screen during start up. Bear in mind that cycling power resets all devices to their default positions.

Main CARV II Screen

When the CARV II is powered on, the first screen displayed is the main screen. If, however, the touch pad is being pressed, the calibration screen is displayed. In this case, after the instructions on the screen have been followed, the main screen is displayed. An example of the main screen is shown in Figure 3-1 on page 36 (of course the version number may differ).

Figure 3-1 Main CARV II Screen



The layout of the main screen represents the light path through the main unit. The light source is the light bulb outside of the box. The illumination light passes through the shutter and excitation wheel (the eight-position circle below the shutter graphic). The light path is then reflected to the right by the five-position epidichroic wheel (which will be referred to as the dichroic wheel). In its initialized state, the CARV II now presents the FRAP iris to the light path; the iris is in its full open setting so there is no loss of intensity. The light path now leaves the CARV II and enters the microscope where it is reflected off of the sample and returns through the same opening. Since the reflected light is typically of a different wavelength, it passes through the dichroic wheel and then the emission wheel on its way to the camera.

Every graphic on the main screen that isn't part of the box outline or text, except the “Presets” button, is a button that either actuates a device or presents a different screen to control a device or devices.

Variable Intensity Iris–Pressing this button changes the display to the Intensity Iris screen. Refer to the Intensity Iris Screen on page 42 for more details.

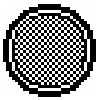
Shutter (closed)–To open the shutter, press this button. The graphic will change to the “open” icon to reflect the open status of the shutter, regardless of whether it was changed with a button press or with a computer command.

Shutter (open)–To close the shutter, press this button. The graphic will change to the “closed” icon to reflect the closed status of the shutter, regardless of whether it was changed with a button press or a computer command.

Eight-Position Filter Wheel–There are two representations of the eight-position filter wheel on the main screen, each representing a different kind of filter wheel. If the graphic below the shutter is pressed, the Excitation Wheel screen is displayed. If the graphic to the left of the dichroic wheel is pressed, the Emission Wheel screen is displayed. Refer to the Eight-Position Filter Wheel Screens on page 39 for more details.

Dichroic Filter Wheel–The Dichroic Wheel screen is displayed when this button is pressed. Refer to the Dichroic Wheel Screen on page 41 for more details.

FRAP Iris–Pressing this button displays the FRAP Iris screen. Refer to the FRAP Iris Screen on page 44 for more details. This button is not displayed when the confocal disk is in the light path.



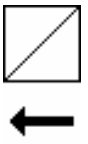
Confocal Disk (stopped)–If the confocal disk is out of the light path, pressing this button both moves the confocal disk into the light path and starts it spinning. This is represented on the touch pad when the following graphic "moves over" the FRAP iris into the light path. If the disk is in the light path, pressing this button moves the disk out of the light path. Turning the disk motor on via software will change the graphic to the following icon (that is, the spinning confocal disk) but will not change the disk position.

Confocal Disk (spinning)–This button is displayed when the confocal disk is spinning. This can be either in or out of the light path if controlled by the computer. Pressing this button with the disk in the light path stops it spinning and moves it out of the light path. The FRAP Iris button is restored. Turning the disk motor off via software will change the graphic to the previous icon but will not change the position.

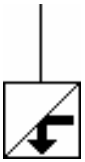
> **NOTICE** The confocal disk graphic only indicates if the disk is spinning or not. Its location indicates whether or not it is in or out of the light path. Button presses will move the disk in or out of the light path, stopping the disk if it's out, spinning the disk if it's in. Independent control of the disk is accomplished with computer commands.

Camera–Press this button to direct the light path to the camera port. The prism graphic changes as described in the **Prism (camera)** section that follows.

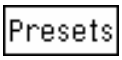
Binoculars–This button will direct the light path to the binoculars, or bincos. The prism graphic changes as described in the **Prism (binocs)** section that follows. Pressing and holding this button for about four seconds activates the prism slider calibration buttons. Refer to the calibration section (Calibrating the Prism on page 47) for more details.



Prism (camera)–When the prism is out of the light path, the light path is directed to the camera. Pressing either the binoc button, this prism button, or commanding the prism using the software, will move the prism into the light path to direct it to the binocs. The prism graphic will change to the one below.



Prism (binocs)–When the prism is in the light path, the light path is directed to the binocs. Pressing either the camera button, this prism graphic, or commanding the prism using the software will move the prism out of the light path, directing it to the camera. The prism graphic will change to the one above.



Presets–Pressing this button displays the Preset screen. Refer to the Presets Screen on page 46 for more details.

Secondary CARV II Screens

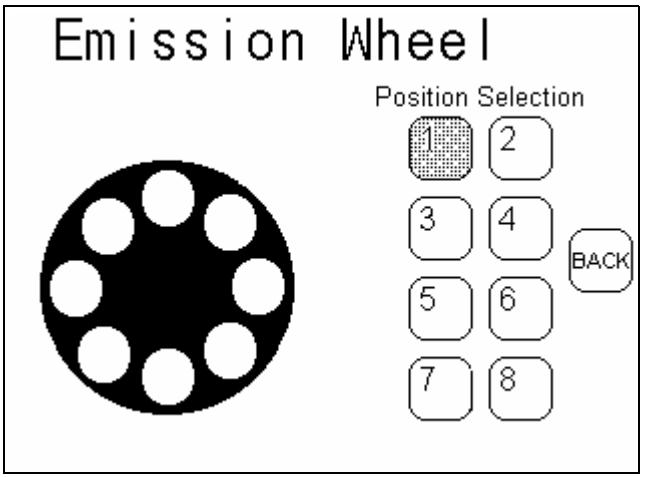
Pressing on the various icons in the CARV II main screen provides access to the following secondary screens:

- Eight-Position Filter Wheel Screens
- Dichroic Wheel Screen
- Intensity Iris Screen
- FRAP Iris Screen
- Presets Screen

Eight-Position Filter Wheel Screens

When either of the eight-position filter wheel buttons on the Main Screen are pressed, one of the eight-position filter wheel screens is displayed. The two screens look similar, as only the screen titles differ; however, the information they access is entirely different. The positions selected with one of the eight buttons and any calibration information are for either the excitation wheel or the emission wheel, depending on which screen is displayed. An example of an eight-position filter wheel screen is shown in Figure 3-2.

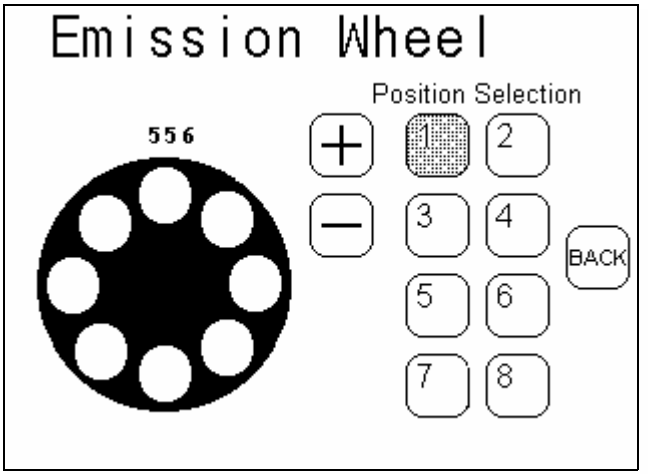
Figure 3-2 Eight-Position Filter Wheel Screen



Any position can be selected for the appropriate wheel by pressing one of the position buttons. The current position selected is indicated by a shaded button. For example, the position “1” button in Figure 3-2. To return to the Main Screen press the “Back” button.

To activate the calibration function, press the center of the large eight-position filter wheel graphic and hold it for a few seconds. The current position's calibration value is then displayed over the filter wheel graphic and a “+” and “-” button is added to the screen to adjust the calibration value, as in Figure 3-3. For more details on the calibration procedure, refer to Calibrating the Filter Wheels on page 49.

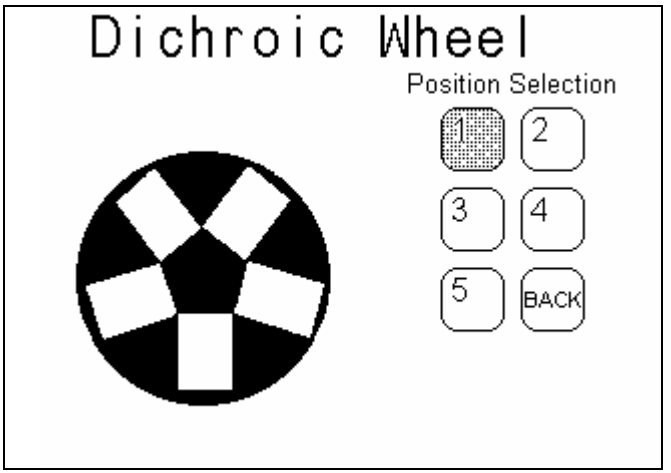
Figure 3-3 Eight-Position Filter Wheel Screen with Calibration Function Activated



Dichroic Wheel Screen

When the dichroic wheel button on the Main Screen is pressed, Dichroic Wheel screen is displayed.

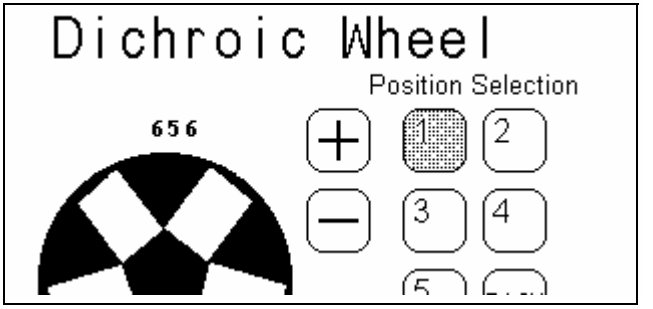
Figure 3-4 Dichroic Wheel Screen



Any position can be selected by pressing one of the position buttons. The current position selected is indicated by shaded button; for example, position “1” button in Figure 3-4. To return to the Main Screen press the “Back” button.

To activate the calibration function, press the center of the large dichroic wheel graphic and hold it for a few seconds. The current position's calibration value is then displayed over the filter wheel graphic and a “+” and “-” button is added to the screen to adjust the calibration value, as in Figure 3-5. For more details on the calibration procedure, refer to Calibrating the Filter Wheels on page 49.

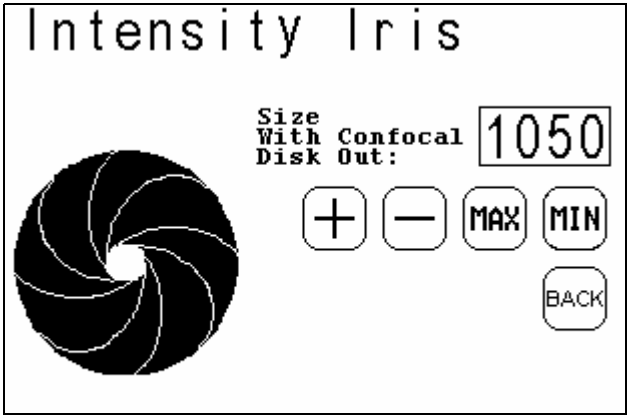
Figure 3-5 Dichroic Wheel Screen with Calibration Function Activated



Intensity Iris Screen

The Intensity Iris Screen is displayed when the light bulb graphic (Variable Intensity Iris button) on the Main Screen is pressed. This screen is used to control the variable intensity iris, which is mounted immediately after the shutter in the light path. The Intensity Iris Screen will differ depending on the location of the confocal disk. The display will show the variable intensity iris in its current setting, associated with the confocal disk either in or out of the light path, and the text will indicate that position. In the example shown in Figure 3-6, the confocal disk is out of the light path and the variable intensity iris is set to the largest allowable size, 1050. If the confocal disk were in the light path, the text would change to read “Size With Confocal Disk In” and the setting would change accordingly.

Figure 3-6 Intensity Iris Screen



The CARV II has two memory locations for the size of the variable intensity iris; one for when the confocal disk is in the light path, and one for when the disk is out. Each location is independently set and either one can be larger than the other or both can be equal. The intention of the two memorized sizes is to allow intensity compensation by the iris for the light reduction caused by the confocal disk. Therefore, the factory defaults are set to have the variable intensity iris fully open when the confocal disk is in the light path and fully closed when the disk is out. Note that the variable intensity iris does not block all the light when it is fully closed.

Whether using computer commands or the touch pad, only the size at the current position of the intensity iris can be adjusted. As a result, commands to change the setting of the variable intensity iris when the confocal disk is in the light path will only change the size of the iris in that position. The confocal disk has to be moved out of the light path to change the size of the intensity iris for that position. In this way, when the confocal disk is moved in and out of the light path, the variable intensity iris automatically changes between the two memorized sizes.

Changing the Size of the Iris

To change the size of the variable intensity iris in its current position via the touch pad, use one of the following buttons.

The plus button increments the variable intensity iris size by 10. Holding the plus button down rapidly increases the size by 10's all the way to the maximum of 1050.

The minus button decrements the variable intensity iris size by 10. Holding the minus button down rapidly decreases the size by 10's all the way to the minimum of 450.

Pressing the MAX button sets the variable intensity iris to the maximum size of 1050.

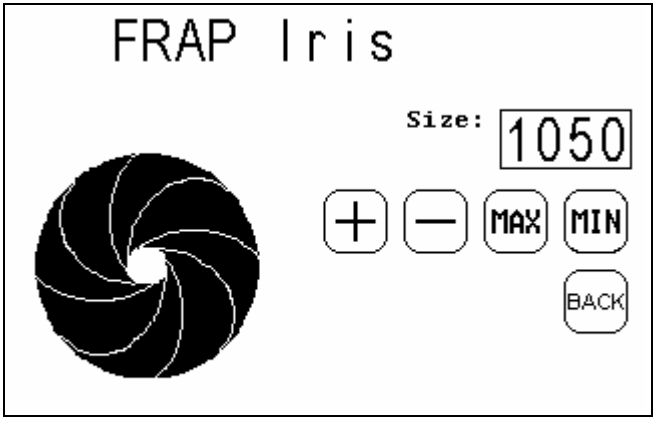
Pressing the MIN button sets the variable intensity iris to the minimum size of 450.

Press the BACK button to return to the Main Screen.

FRAP Iris Screen

The FRAP Iris Screen is displayed when the FRAP Iris button on the Main Screen is pressed. The FRAP iris is mounted on the same slider as the confocal disk such that only one is in the light path at any given time; therefore, the FRAP Iris button is not available when the confocal disk is in the light path. The FRAP Iris Screen allows manual control of the FRAP iris which creates a rectangular aperture on the image to enable controlled photo-bleaching of the sample. An example of the FRAP Iris Screen is shown in Figure 3-7.

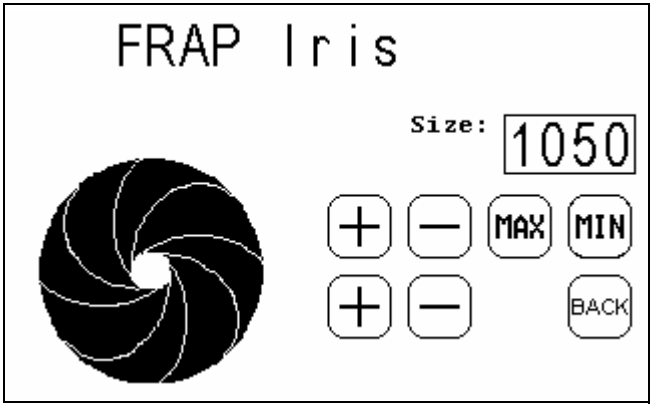
Figure 3-7 FRAP Iris Screen



The button functions of this screen are identical to that of the Intensity Iris Screen. Refer to Changing the Size of the Iris on page 44 for greater detail about the specific buttons and how they effect the FRAP iris.

As with the filter wheel screens, the calibration function can be activated by pressing and holding the center of the iris graphic for a few seconds. The calibration function is activated when a second set of "+" and "-" buttons appear directly beneath the original ones, as in Figure 3-8. The calibration function does not effect the size of the FRAP iris, but rather the position of the iris in the light path. Moving the confocal disk into the light path while the calibration function is activated changes the iris graphic to a disk graphic, and the calibration controls now adjust the position of the confocal disk in the light path. Refer to Calibrating the Confocal Disk on page 48 for more details.

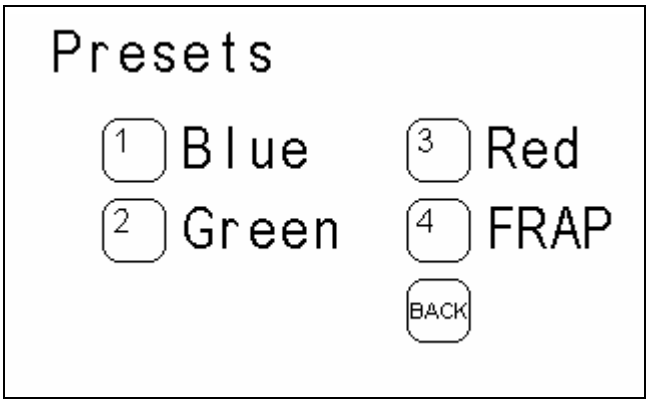
Figure 3-8 FRAP Iris Screen with Calibration Function Active



Presets Screen

Press the Presets button to display the Presets Screen. An example of the Presets Screen is shown in Figure 3-9. There are four factory-programmed presets available. They cannot be changed by the user and are designed to be used with the filter configurations shipped with the unit. Selecting preset “1 Blue” moves all three filter wheels to Position 1. Preset “2 Green” moves all three filter wheels to Position 2. Preset “3 Red” moves all three filter wheels to Position 3. Preset “4 FRAP” is unique because the excitation wheel moves to Position 5, the dichroic wheel moves to Position 5, and the emission wheel doesn't move at all. The emission wheel remains in its last position because it is not involved with the FRAP exposure. Pressing the “Back” button displays the Main Screen.

Figure 3-9 Presets Screen



Calibration

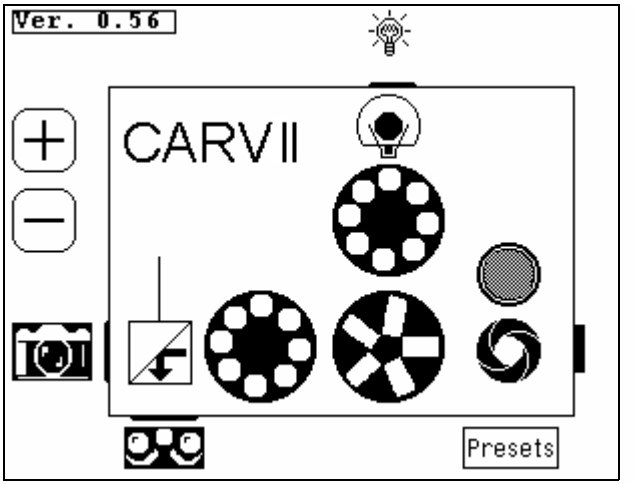
Each CARV II instrument is individually calibrated at the factory before shipping. Each position of each device is aligned to the light path. Most of the factory calibration involves internal hardware that is not accessible in the finished instrument. However, each position on the filter wheels, the position of the prism in the light path, and the position of the confocal disk in the light path can be calibrated using the touch pad. Because calibrating the devices within the CARV II can adversely affect the optical performance, only qualified personnel should perform these procedures.

Calibrating the Prism

NOTICE Once the calibration for the prism has been changed, there is no way to restore the factory setting without optically aligning the instrument; therefore, use caution when adjusting.

Press and hold the binoc button for a few seconds to activate the calibration function and adjust the position of the prism in the light path. Once the calibration function is activated, the Main Screen will appear as in Figure 3-10.

Figure 3-10 Main Screen with the Calibration Function Activated



Press the "+" and "-" buttons to move the prism further in and out of the light path to align the image out of the binoc port. The position selected becomes the position the prism will acquire when it is placed in the light path. The instrument functions normally even if the calibration buttons are displayed. Once a different screen is displayed, the calibration buttons will be hidden when the Main Screen is displayed again. Pressing and holding the binoc button again will start the calibration function. To hide the calibration buttons without leaving the Main Screen, press the camera button.

Calibrating the Confocal Disk

NOTICE Users should never attempt to calibrate the confocal disk; these instructions are for trained technicians only.

The confocal disk position can be adjusted in the light path in much the same way as the prism, above. However, several steps must be performed before the calibration controls are available on the touch screen. First, calibration controls for the confocal disk are activated through the FRAP Iris Screen. Instructions for activating the calibration function for this screen and a screen shot with the calibration function activated can be found in the FRAP Iris Screen on page 44. The calibration buttons will be hidden if the BACK button is pressed and then

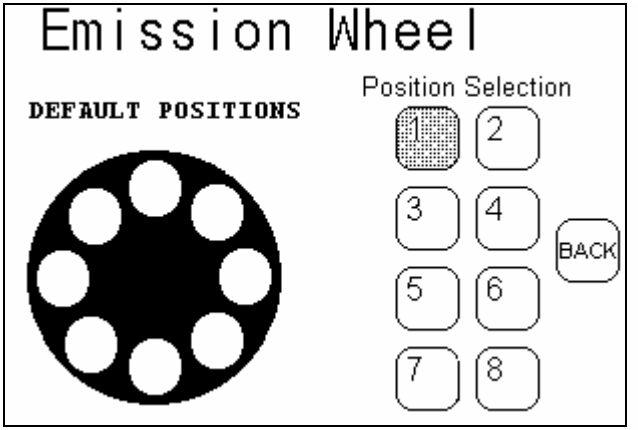
the screen is re-displayed. The calibration functions that are first displayed effect the position of the FRAP iris and do not effect the confocal disk. The size of the FRAP iris is not effected either. This positional adjustment is used for factory calibration and has no optical effect. Calibration adjustments of the FRAP iris can cause noise from the servo motor and therefore should not be changed.

In order to calibrate the position of the confocal disk, move it into the light path with a serial command while the FRAP Iris Screen has the calibration function activated. The iris graphic changes to a disk graphic and the calibration buttons set the position of the confocal disk in the light path. When the confocal disk is moved into the light path the calibrated confocal disk position is used. Calibration adjustments to the confocal disk can also cause noise from the servo motor and must be done carefully.

Calibrating the Filter Wheels

Because the positioning of the filters is so critical to an optimized light path, the calibration of the filter wheels is done in a different manner. Like the other calibration-equipped devices in the CARV II, each position of each filter wheel has its calibration value stored in memory and each one can be adjusted independently. Every CARV II is loaded with the same default values designed to place each position in roughly the correct place. When each instrument is calibrated during production, these positions may be modified to optimize alignment. These new positions are stored in non-volatile memory with a checksum. Each time the instrument is turned on, the memorized positions are checked against the checksum. If this check should fail, the default values for all positions of the failed filter wheel will be loaded and the appropriate wheel screen will indicate that the default positions are implemented, as in Figure 3-11.

Figure 3-11 Emission Wheel Screen Default Filter Positions



When in the calibration mode, the checksum for the wheel is calculated and saved when the BACK button is pressed. If the power is disconnected before the BACK button is pressed, the checksum will fail upon the next startup and the default positions will be used. If this happens, the cause is probably a minor failure of the non-volatile memory and the positions on the filter wheel will have to be recalibrated. Memory failure is a rare occurrence and does not indicate any permanent flaws in the instrument. If there is any memory failure at all it most likely will be with only one of the wheels. Therefore, only one wheel screen will display the "DEFAULT POSITIONS" text and only this wheel will need to be re-calibrated.

Instructions for activating the calibration function for the filter wheels and a screen shot for each screen can be found in the Eight-Position Filter Wheel Screens on page 39 or the Dichroic Wheel Screen on page 41. Notice that the calibrated value for the active position on the wheel is displayed when the calibration function is activated. These values can be read for all positions on all wheels with one command. All positions on a filter wheel can be written with another command. If any position is recalibrated outside of the factory, all the values should be read and stored, either copied by hand during calibration or read via software and saved as a file. If these new values then need to be restored, they can either be entered by hand by calibrating each position one at a time, or written to with a software command. Refer to the software manual for the imaging software you are using for the syntax and a description of each command needed to do this.

4

BD CARV II Command Sets

The following topics are covered in this chapter:

- Software Communication
- Devices
- Command Conventions
- Command Formats

Software Communications

Software control of and communication with the CARV II is accomplished via the RS-232 serial port at the back of the touch screen assembly. This DB-9 connector accepts a standard, straight through DB-9 extension cable to connect a PC host that is either running a standard terminal program or an application designed to integrate the CARV II into an imaging system. Communication in this manner is directed toward the touch screen itself; therefore, the touch screen must be turned on and displaying the CARV II graphics in order to establish communication. The cable that joins the touch screen assembly and the main CARV II unit need not be present in order for the software to communicate with the touch screen–relevant commands will have a reaction on the touch screen display; however, no actual movement occurs in the main unit without the cable. Regardless of the controlling application, the settings for the COM port used to connect to the CARV II are as follows:

- 9600 baud
- 8 data bits
- 1 stop bit
- no parity
- no flow control

Devices

The CARV II has nine individual devices that can be actuated to affect the light path. Each device has a single capital letter name (Device ID), which is used to address the individual device with the commands that are described later in this manual. The number of allowable positions differs for different types of devices and they are all listed in the following chart.

Table 4-1 Allowable Positions for Device Types

DEVICE	DEVICE ID	Valid Positions	Homes To
Excitation Filter Wheel	A	1, 2, 3, 4, 5, 6, 7, 8	Position 1
Emission Filter Wheel	B	1, 2, 3, 4, 5, 6, 7, 8	Position 1
Epifluorescence Dichroic Wheel	C	1, 2, 3, 4, 5	Position 1
Confocal Disk Slider	D	0,1	Disk Out of Light Path
Shutter	S	0,1	Closed
Confocal Disk Motor	N	0,1	Disk Off (not spinning)
Prism Slider	P	0,1	Light Path to Camera
FRAP Iris	I	450 – 1050	Full Open
Variable Intensity Iris	V	450 – 1050	Full Open

When the CARV II is first turned on, all the devices are initialized to their "Home Position" listed in the chart above. The "H"(Home) command can also be used to return all the devices to these positions.

Command Conventions

Regardless of their origin, all commands must adhere to the following conventions:

1. All commands or series of commands must be terminated with a carriage return <CR>. The CARV II will not even attempt to recognize any characters sent on the serial port until it receives a <CR>.

2. For simultaneous motion from more than one device, commands can be chained together with only one <CR> at the end. For example, the excitation wheel can be moved to Position 2 and the shutter opened with A2S1<CR> or A2 S1<CR>.

The command buffer in the CARV II is 50 characters long. Compound commands must be 49 characters or less, including spaces and any requisite punctuation, to allow for the <CR> terminator. Overflowing the input buffer could result in either the loss of the beginning of the compound command as new commands overwrite the earlier commands or worse, completely errant behavior.

Execution of all lower case commands (commands that read information from the CARV II) and the "H" (Home) command will cause any commands that follow to be ignored and the response string will look as if only the last command of this type was sent. For example, the compound command D1rCrAP0<CR> will move the confocal disk into the light path and read the position of the excitation wheel, but the prism will not be commanded to direct the light path to the camera. The return string will look like this–rA3<CR>–or whatever position the excitation wheel is in.

Be aware that, although sending compound commands is the fastest way to execute several commands in quick succession, the action of the devices may not actually be simultaneous. The processor handles the individual commands sequentially and sets each device in motion in that order. Faster devices or devices that make a shorter move may complete motion before a slower device even starts motion. For the most time efficient coordination of moves, slower devices or ones making a longer move should appear in the compound command first.

3 When the entire command string (all strings preceding the <CR>) has been processed the entire command string is echoed back to the PC host. The exceptions are the lower case commands. Lower case commands signify the command requests information from the CARV II. The responses to these commands append the requested information to the original command. Responses to motion commands are executed immediately after commanding the motion to start and they do not signify that motion has been completed or even if it is successful. This occurs, for example, if the device has encountered an obstruction preventing it from achieving the commanded position.

4 Erroneous commands, either invalid Device ID or invalid parameters, are ignored by the system but are echoed back to the host as they were sent. Any valid command/parameter combinations in the command will be executed. For example, AA6C8B 3D1<CR> will be echoed back exactly as

it appears and the excitation wheel will move to Position 6 and the confocal disk will move into the light path. The other filter wheel commands will be ignored because “8” is not a valid parameter for the dichroic wheel and in “B 3" the space before the 3 invalidates the emission wheel command.

WARNING: If the touch screen is operated to actuate devices manually while characters are sent to the serial port, the touch screen could crash catastrophically. To prevent this, the touch screen can be put into a computer control only mode (refer to Command Formats on page 55). The touch screen will display a combination lock graphic in the lower left corner and ignore all touch actions but the display will update as commands are sent from the host.

Command Formats

In the following command descriptions the Format describes how the command should look as an individual command. Individual commands can be appended with other commands to make a compound command as outlined in the command conventions above. Parameters describe any characters that must accompany the command or are returned with the echo of the command. The Return Value describes how the echo of the individual command will look. The Example illustrates the sending of the command and its subsequent echo using actual parameters.

M – Lockout Touch Screen for Computer Control

Format	M<state><CR>	
Parameters	<state>	0 = touch screen or computer controlled
		1 = computer controlled only
Return Value	M<state><CR>	
Examples	M0<CR> →M0<CR>	
	Touch screen now responds to touch and serial commands.	

R – Response Activation: Turn on/off responses to Host

Format	R<active><CR>	
Parameters	<active>	0 = no response sent to host
		1 = responses sent to host
Return Value	If <active> = 0, no response	
	If <active> = 1, R1<CR>	
Examples	R0<CR> →	
	R1<CR> → R1<CR>	
	All commands generate a response to the host.	

NOTICE All lower case commands the "read" information from the CARV II will always respond regardless of the state set by this command.

H – "Rehome" All Devices

Format	H<CR>
Parameters	none
Return Value	HA1B1C1D0P0N0S0I1050V450<CR>
Examples	H<CR> → HA1B1C1D0P0N0S0I1050V450<CR>
	All devices are in their "Home Positions" as reported.
	See Table 4-1 for more information.

DEVICE ID – Move Device

Format	<device ID><position><CR>	
Parameters	<device ID>	ID assigned to device (see Table 4-1)
	<position>	Desired position or state
Return Value	<device ID><position><CR>	

Examples	P1<CR> → P1<CR>	
	The prism is now in the light path (light path directed to the binocs).	

S – Set Source of Shutter Trigger

Format	S<source><CR>	
Parameters	< source >	X = External trigger source (BNC camera sync cable)
		I = Internal trigger source
Return Value	S<source><CR>	
Examples	SX<CR>→SX<CR>	
	The shutter trigger source is now set as external.	

NOTICE External shutter triggering means that a 5VDC signal on the 50-ohm BNC connector on the main CARV II unit opens the shutter. 0VDC closes the shutter. Internal triggering comes from the touch screen, either manually or serially.

F – Set Calibration Values of Filter Wheel

Format	F<device ID>:<position><value><CR>	
Parameters	<device ID>	ID assigned to a filter wheel (see Table 4-1)
	<position>	Position of device that receives the <value>. For example, A=1, H=8
	<value>	Calibrated value for <position>
Return Value	F<device ID>:<position><value><CR>	
Examples	FA:A450D825<CR> → FA:A450D825<CR>	
	Excitation filter position 1 is calibrated to 450 and position 4 is calibrated to 825.	

NOTICE Allowable values range from 450 to 1050. Any or all positions of one device can be calibrated in one command. Calibration is used to align each filter position to the light path. **Improper calibration will negatively affect the optical performance of the CARV II.**

f – Read Calibration Values of All Filter Wheels

Format	f<CR>	
Parameters	<device ID>	ID assigned to device (see Table 4-1)
	<position>	Position of device that is calibrated to <value>. For example, A=1, H=8
	<value>	Calibrated value for <position>
Return Value	F<device ID>:<position><value><position><value> . . .<CR>	
	<device ID>:<position><value><position><value> . . .<CR>	
	<device ID>:<position><value><position><value> . . .<CR>	
Examples	f<CR> → fA:A450B575C708D825E893F956G1050<CR>	
	B:A450B582C715D808E895F966G1050<CR>	
	C:A450B596C702D823E876<CR>	

NOTICE The calibration values for each filter position of each filter wheel are reported on separate lines.

q – Query State of All Devices

Format	q<CR>	
Parameters	<A>	Device ID and position of Excitation Filter Wheel
	<B>	Device ID and position of Emission Filter Wheel
	<C>	Device ID and position of Dichroic Filter Wheel
	<D>	Device ID and position of Confocal Disk Slider
	<P>	Device ID and position of Prism Slider

	<R>	Device ID and position of Confocal Disk Motor
	<S>	Device ID and state of Shutter
	<I>	Device ID and position of FRAP Iris
	<V>	Device ID and position of Variable Intensity Iris
Return Value	q<A> <B> <C> <D> <P> <R> <S> <I> <CR>	
Examples	q<CR> → qA8 B8 C5 D1 P0 N1 S0 I910<CR>	

NOTICE There is no positive feedback between the motor and the processor. Therefore, the "current" position is the last commanded position–not necessarily where the device actually is. Incorrect reporting is probably a sign of hardware malfunction.

r – Read Current Position of Individual Device

Format	r<deviceID><CR>	
Parameters	<device ID>	ID assigned to a device (see Table 4-1)
	<position>	Current position or state
Return Value	r<device ID><position><CR>	
Examples	rC<CR> →rC1<CR>	
	The dichroic filter wheel is in position 1.	

NOTICE There is no positive feedback between the motor and the processor. Therefore, the "current" position is the last commanded position–not necessarily where the device actually is. Incorrect reporting is probably a sign of hardware malfunction.

v – Read Version of Firmware

Format	v<CR>	
Parameters	<version>	Version of touch screen firmware
Return Value	v<version><CR>	

Examples v<CR> → vVer. 1.02<CR>

The touch screen firmware version is reported as Ver. 1.02.

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