Ultima Operator's Manual

Rev 1

Bruker Nano Surfaces Division
3030 Laura Lane, Suite 140
Middleton, WI 53562
Phone: +1 608-662-0022
http://www.bruker.com/nano

Table of Contents

System Overview

The Bruker Ultima is a unique laser point-scanning microscopy system that is capable of using multiple laser beams for simultaneous imaging and photostimulation experiments on *in vivo* and *in vitro* specimens. With one laser, it is possible to perform laser imaging alone and sequential imaging and photostimulation experiments. When multiple lasers are used, it is possible to perform imaging and photostimulation simultaneously. In either scenario the operator can choose to deliver electrical stimuli to the specimen as well as record electrophysiological signals from the specimen through the use of Prairie View software modules.

The Ultima scan head can be integrated with a traditional microscope base as the Ulitma In Vitro, or on a pillar system optimized for imaging larger samples as the Ultima IntraVital. The pillar system of the Ultima IntraVital can be a fixed post or can be mounted on an XY translation base.

The Ultima system is intended for research use only. The modular design of this system allows for many different configurations. The purpose of this manual is to provide an operator-level overview of the common components available for the Ultima system. Additional technical information and guidance can be obtained by contacting Bruker Nano Surfaces Fluorescence Microscopy Business Unit.

Every effort has been made to ensure that the data given in this document is accurate. The information, figures, tables, specifications, and schematics contained herein are subject to change without notice. Bruker Nano Surfaces Florescence Microscopy Business Unit makes no warranty or representation, either expressed or implied, with respect to this document. In no event will Bruker be liable for any direct, indirect, special, incidental, or consequential damages resulting from any defects in its documentation.

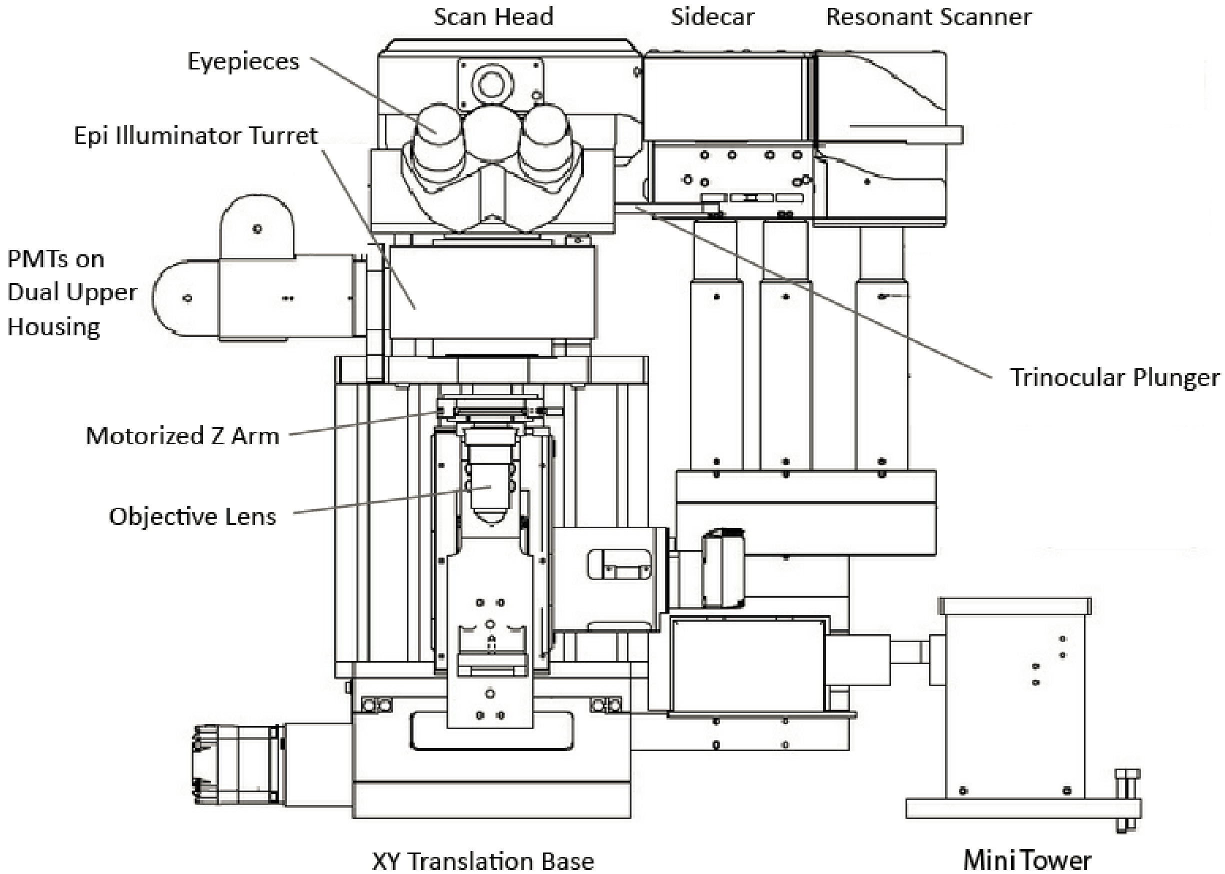
The Ultima imaging system consists of many components, including

- The microscope
    - Body, which can be either a traditional upright microscope or a pillar system
    - Ultima scan head, mounted on top of the body
    - Detectors, available in a variety of configurations
    - Z arm, to which an objective lens is attached
    - Trinocular head, which includes eyepieces
    - Epifluorescence Illuminator, with a turret for selecting a dichroic
- The electronics workstation
    - Control boxes for all components of the system
    - Computer workstation
- One or more laser light sources
- Table optics, which route light to the microscope from one or more ultrafast pulsed infrared lasers

These components are described in later sections of this manual.

Most Ultima systems are configured for imaging using an ultrafast pulsed infrared laser for multiphoton excitation of the sample. Operation of this standard system will be the focus of this manual. The Ultima system may also be configured for confocal (descanned) imaging with visible lasers, either in addition to or instead of multiphoton excitation.

Ultima IntraVital with XY Translation Base



Ratings

Electrical Rating

Voltage: 115/230 VAC ~

Frequency: 50/60Hz

Power: 700W

Weight

Electronics Rack: 350lbs (159 kg)

Microscope: 150lbs (68 kg)

Beam Cover:

Light Box:

Lifting and Carrying Instructions

Many of the protrusions from the Ultima system are not designed to support the weight of the system. Only the scan head base plate can be used for lifting, shown in the image below.

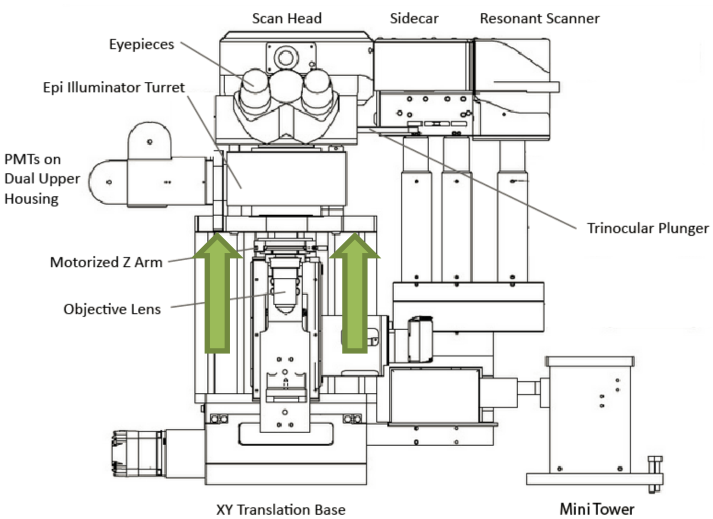


Figure 1 - Ultima lift points.

Ingress Protection rating

This system is IPX00 rated - No protection against contact and ingress of objects or liquids.

Environmental Conditions

Location: Indoor use

Altitude: up to 2 000 m

Temperature: 5 °C to 40 °C

Maximum relative humidity: 80 % @ 31 °C, 50 % @ 40 °C

Mains supply voltage fluctuations: up to ±10 % of the nominal voltage

Pollution Degree II

Vibration and Shock: Not Rated (Must use vibration damped optical table)

Accessories

Only Bruker approved accessories shall be used.

Class 4 External/Incorporated Laser

The Ultima system is coupled with an class 4 laser(s) meeting the following criteria. All class 4 lasers must be approved by an authorized Bruker representative if they do not appear in the preapproved list below.

Class 4 Laser Requirements

Wavelength(s):	650-1450nm
Maximum average radiant power:	5W
Maximum beam divergence:	1 mrad
Maximum beam diameter:	1.2 mm
Typ. Pulse repetition and duration:	80MHz, 140fsec
Maximum peak power	300kW

Preapproved Class 4 Lasers

Coherent, Inc.	
Chameleon Ultra	Mira HP
Chameleon-XR	Mira 900
Chameleon Ultra	Mira-OPO
Chameleon Ultra I	Vitara-T
Chameleon Ultra II	Vitara-T-HP

Spectra-Physics	
Mai Tai	Tsunami
Mai Tai XP	InSight DS+
Mai Tai SP	Inspire OPO
Mai Tai DeepSee	

Class 3b External/Incorporated Laser

The Ultima can be coupled to external class 3b laser(s) through fiber optic cable(s) meeting the following criteria. All class 3b external lasers must be approved by an authorized Bruker representative if they do not appear in the preapproved list below.

Class 3b Incorporated Laser Requirements	
Wavelength(s):	400-700nm
Maximum average radiant power:	500mW
Maximum beam divergence:	0.5 mrad
Maximum beam diameter at fiber aperture:	0.8 mm

Preapproved Class 3b Fiber Coupled Incorporated Lasers

Bruker	
Helios Laser Launch	

Coherent, Inc.	
Obis	

Fuse Ratings

All fuses must be replaced with 250V FST 5x20 cartridge style fuse of the appropriate rating.

Model	Description	Fuse Rating
13297	Resonant Scanner & Control Box	2A
12714	Power Supply	1A
19874	Dual PMT HV Controller	1A
507718	Dual CH GaAsP PMT & HV Box	2A
18350	Z - Piezo & Piezo Driver (new)	1A
04852	Galvo Driver	4A
15578	Helios Laser Launch & PS Box	1A

Safety Precautions

This document is provided as a reference manual for Ultima imaging system. Operators are recommended to familiarize themselves with the content, especially the safety precautions listed here.

This system is designed as a Class 1 laser product, intended for use in laser-based imaging microscopy. It is not intended for any other purpose. Use of this system is used in a manner not specified by Bruker the protection provided by the equipment maybe impaired.

Laser Safety

The Ultima Laser product is designed and built in compliance with the IEC Laser Product Safety Standard (IEC 60825-1:2014). The Ultima system is a Class 1 laser device incorporating Class 4 and Class 3B laser sources. This document is not intended to be an alternative to a laser safety training class. All operators of the Ultima system are strongly encouraged to learn about laser hazards and laser safety measures. It is also emphasized that the laser safety measures are to be followed at all times.

The parts of a person most vulnerable to laser damage are eyes and skin. Lasers are classified based on the potential hazards associated with them. Briefly,

- Class 1 lasers are not capable of any damage.
- Class 2 lasers emit in the visible wavelength range only. They can not cause any eye damage unless deliberately prolonged exposure is undertaken.
- Class 3B lasers can present eye hazards if viewed directly. Prolonged exposure can also cause skin damage.

- Class 4 lasers can cause severe eye damage even with short duration exposure to direct, specularly-reflected or diffusely-reflected beams. They can also cause severe skin damage. Flammable materials should not be exposed to this beam.

A Class 1 laser product must include a light-tight enclosure designed to encapsulate all laser emissions. Therefore, at no time should these covers be removed or modified. The interlocks on these covers are only to be defeated for maintenance procedures detailed in this manual. During these procedures, appropriate laser-safe eyewear is required.

Caution – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure. Do not attempt to open, disable, modify, or otherwise change any Ultima laser equipment.

Class 4 Laser Emission: The Ultima uses a Class 4 pulsed laser which is tunable from approximately 650nm to 1450nm and hence is mostly in the non-visible range.

Class 3B Laser Emission: The Ultima can use Class 3B fiber coupled lasers for photoactivation typically in the visible range.

Interlocked Panels for Light Box: The Light Box around the microscope completely light tight protecting the user from laser hazards. The side and back panels and the front door on the Light Box are interlocked to the Hard Shutter so that the laser path is shuttered before it enters the Light Box in case a panel has been removed and/or the front door has been slid open.

Inside the Light Box, the laser beam is enclosed inside various tubes before it enters the scan head. Thus even inside the Light Box, the laser beam is unshielded only for a few millimeters between the objective lens and the sample.

Interlock Defeats: Interlock defeats provided with the system are intended only to be used for Bruker specified maintenance procedures. Operators should not try to run the system using interlock defeats for other purposes as this might render them vulnerable to Class 4 or Class 3B laser exposure.

Beam Covers: Beam Covers enclose the Class 4 laser beam from the aperture of the laser head to the point where the beam enters the Light Box. The Beam Covers are not intended to be opened or removed by operators.

Protective Eyewear: Bruker supplies a pair of protective goggles with the Ultima system. Googles must meet the following requirements:

- (OD) 6+ for 650nm to 1450nm

The use of this eyewear is required during the installation and maintenance of the system. Operators should use this eyewear when performing maintenance procedures.

Key lock and Emission Light: The laser is equipped with a key control and also has an emission warning light on the controller.

Installation, Setup and Maintenance

This system must be installed and serviced by authorized Bruker personnel. When the Ultima is being installed or serviced, the Bruker technician should make sure that the laser is fully contained in Light Box and Beam Cover enclosures. Moreover, people without laser safety training or eyewear should not be allowed in the room until the system is fully installed and all safety interlocks have been established.

Removal of objects that can change the beam path: Unnecessary objects, especially reflective objects, should not be placed in close proximity of the laser beam path. This includes wristwatches and other jewelry worn by operators.

No disassembly of the system: No attempt should be made to disassemble the system. Disassembly of this system may result in electrical shock and other hazards, including exposure to Class 4 or Class 3B laser radiation. Do not change or replace cables, as use of an improperly rated cable may result in system malfunction or failure.

Abnormal Operation: If the system does not seem to function correctly, shutter the laser, power off the system, and contact Bruker support personnel.

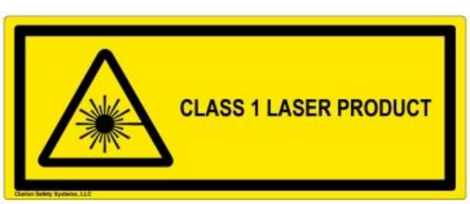
Handling: Do not subject the system to shock as that may misalign the laser beam and/or cause damage to the system. This system is designed as a precision optical instrument. Each optical and electronic component has been place with great care to assure optimal system performance. Do not pull on or bend cables or fibers. Do not handle filter cubes or dichroics except as recommended by Bruker.

Contact with Moisture: Moisture contact with any component of the system may result in a short-circuit or damage to optical components. If water gets into a system component, discontinue use of the system, turn off power, and contact Bruker support personnel.

Warning Labels

The following figures show the types and positions of safety labels on the various units, including components with apertures.

(1) IEC Explanatory Label

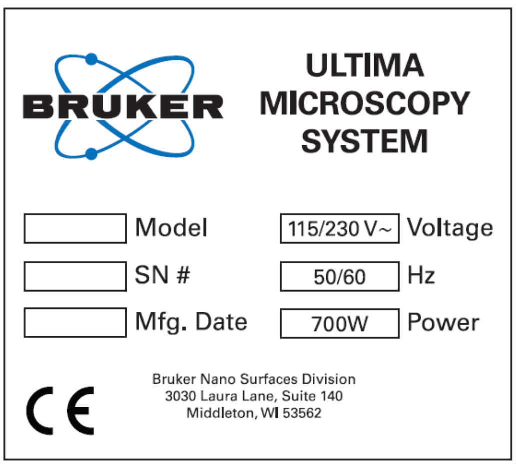


(2) IEC Interlock Defeat Label

(3) Caution

(4) Only authorized personnel may service this equipment.

(5) System Label

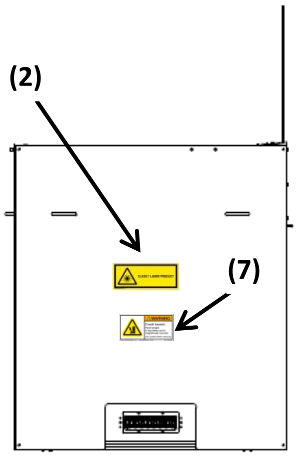


(6) IEC Laser Warning

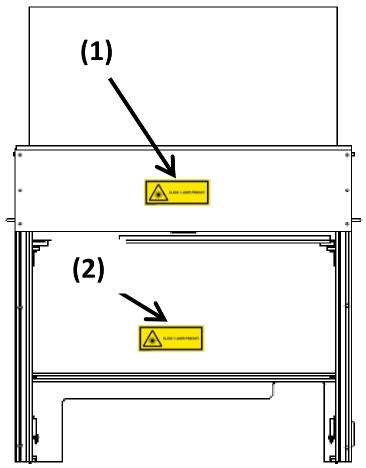
(7) Crush Hazard

Light Box

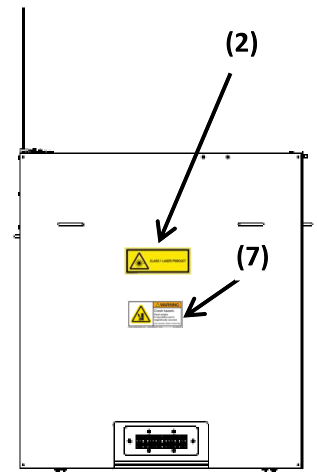
Left Side



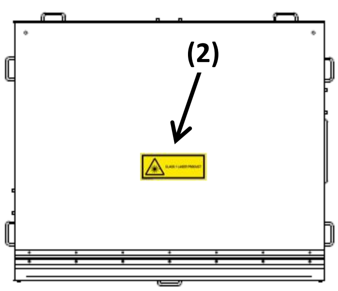
Front



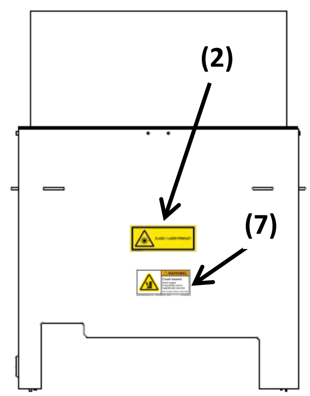
Right Side



Top

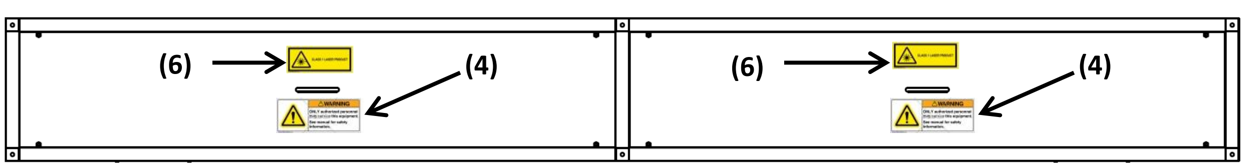


Rear



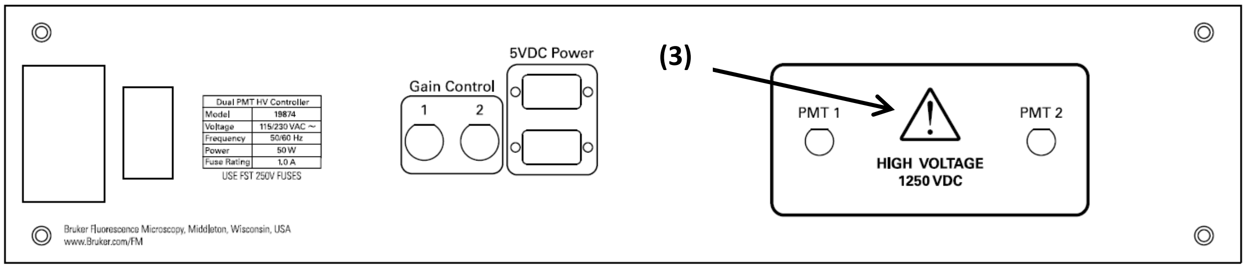
Beam Cover

Top View



Dual PMT HV Controller

Rear View



Daily Operation

Startup Sequence

1. Key on the laser(s) so it/they can ramp up
2. Turn on the main power switch(es) on the electronics rack Power Distribution Unit(s), typically located behind the monitor display
3. Press the Workstation Computer power button to start the computer
4. Launch Prairie View software via the desktop icon; a dialog box will appear and report progress as the software loads and establishes connections with various components
5. Open the cavity shutter(s) for the ultrafast pulsed infrared laser(s)
6. Turn on the XCite lamp module

Basic Imaging Guide

Additional information about selectable filters and light sources can be found in later sections of this manual. Detailed information about Prairie View software can be found in the software manual, accessed from the Help menu of Prairie View software.

1. Find the sample using transmitted or epi fluorescence light and the eyepieces
   a. Select the light path and/or optical port that directs light to the eyepieces, typically trinocular plunger pushed in
   b. Move the Epi Illuminator Turret to a position that allows the desired transmitted or epi fluorescence excitation wavelengths to reach the sample; typically an open position for transmitted white light, or a filter cube for wide field epi fluorescence
   c. Put a sample on the stage and focus through the eyepieces
2. Configure the system for laser scanning
   a. Turn off the transmitted light or close the aperture on the epi fluorescence light source
   b. Pull the plunger on the right side of the trinocular head out to the “LSM” position
   c. Move the Epi Illuminator Turret to position 1 to put the Primary Dichroic in the light path
3. Close the door of the Light Box

4. Turn off the room lights
5. Tune the laser to the desired wavelength for excitation
6. Select the objective lens currently in use from the list of calibrated objectives in Prairie View software
7. Activate the desired acquisition channels in the Image window(s) in Prairie View software
8. Click the Live Scan button in Prairie View software to start imaging
9. Adjust PMT voltage(s) to an appropriate level for the sample; reasonable starting points are around 600-700 for a Multialkali PMT and around 700-800 for a GaAsP PMT
10. Adjust the laser power slider until the image appears; the Hard Shutter will not open until the laser slider value is greater than zero
11. Continue to adjust the laser power and PMT voltage controls, along with other image controls, to get the desired image
12. Click the Stop Scan button in Prairie View to stop imaging

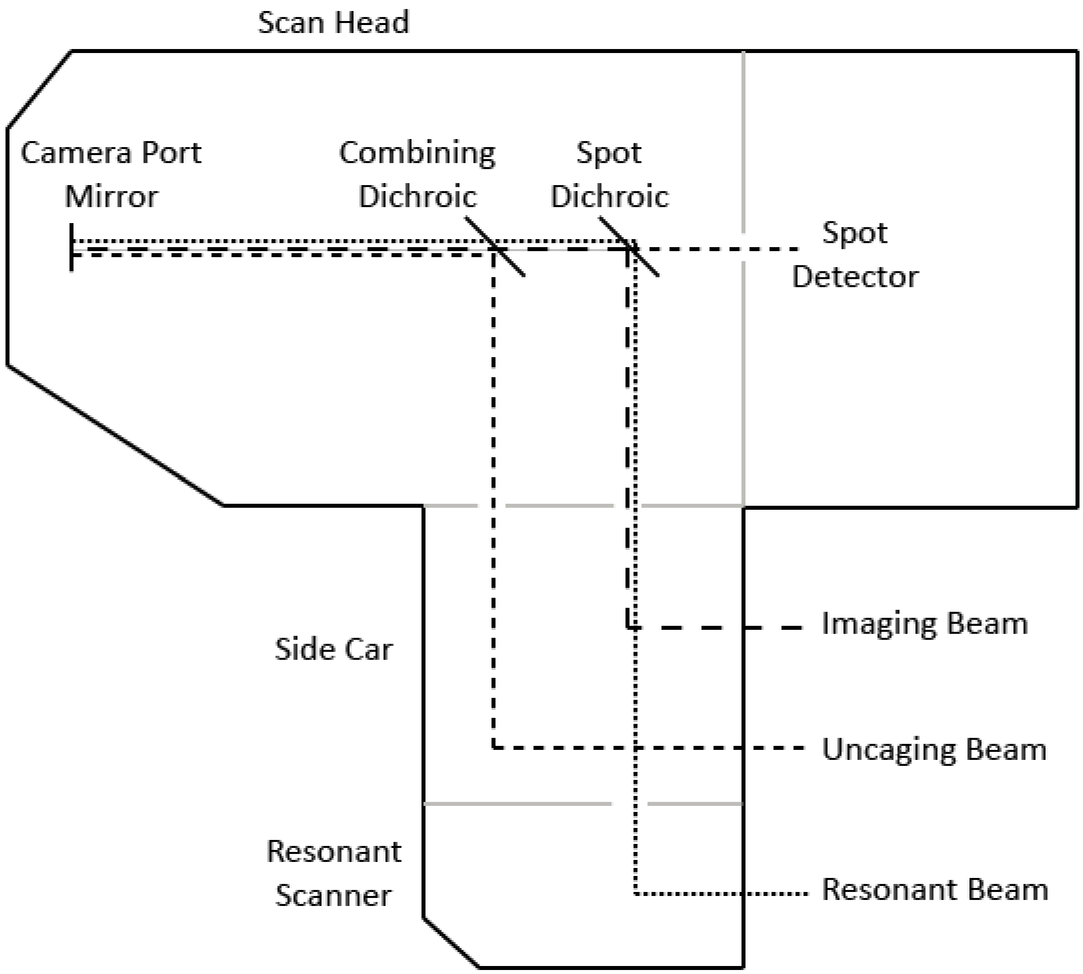
Shutdown Sequence

1. Close the cavity shutter on the ultrafast pulsed infrared laser(s)
2. Turn off the XCite lamp module
3. Exit Prairie View software
4. Shut down the computer
5. Turn off the main power switch(es) on the electronics rack Power Distribution Unit(s)
6. Key off the laser(s)

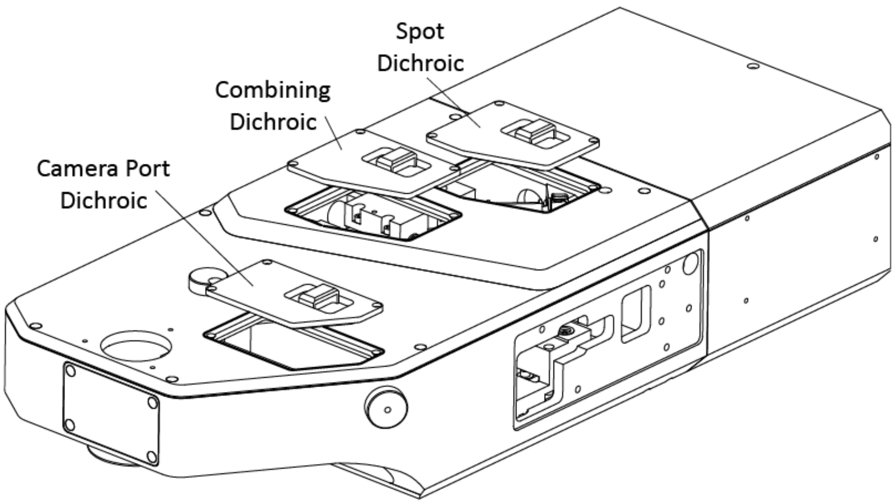
Scan Head Dichroics

Three removable hatches on the lid of the Ultima scan head allow the operator to exchange dichroic mirrors installed in the scan head. On many systems, no exchanges are necessary. However, on complex systems with many lasers, it may be necessary for the operator to exchange dichroic mirrors based on the current application or experiment. All dichroic mirrors are mounted into frames provided by Bruker. A list of the dichroic mirrors included with each system is provided to the operator at the time of system installation. Contact Bruker support personnel for information about glass for a specific system.

Laser Beam Path through the Ultima Scan Head



Scan Head Lid With Hatches



Selecting Appropriate Dichroics

Camera Port Dichroic

The Camera Port Dichroic or Mirror is positioned at the front of the scan head to direct laser light down to the sample. Most systems have only one option for this dichroic, and it can remain installed at all times.

On most systems, there is a full mirror in this position. Specialized configurations may have a dichroic mirror instead of a full mirror, to allow some wavelengths to pass between the sample and the camera port while laser light is directed from the scan head to the sample.

The mirror position is controlled by a knob on the right side of the scan head. A labeled next to this mirror has an arrow indicating the "In for Imaging" position required for laser scanning. When the knob is rotated counterclockwise, the camera port mirror is moved into the light path, and laser light is directed down to the sample. When the knob is rotated clockwise, the mirror is moved out of the light path, and emission light from the sample can travel up toward the camera port.

With the Camera Port Dichroic moved out of the light path, it sits directly under the front hatch in the scan head lid. The operator can then open the hatch, slide the dovetail mount straight up to remove the dichroic, and slide the desired dichroic on its dovetail into position.

Combining Dichroic

The Combining Dichroic is positioned in the middle of the scan head, under the center hatch, at the position where the Imaging laser beam path and Uncaging laser beam path come together. The dichroic is mounted in the "horizontal" orientation in a rectangular frame.

After determining the wavelength(s) necessary for the current experiment, choose the Combining Dichroic which will **reflect** the **Uncaging** path laser beam toward the front of the scan head, and **transmit** the **Imaging/Resonant** path laser beam toward the front of the scan head. For example, when imaging with 820nm laser light and uncaging with 720nm laser light, choose a 760nm Long Pass (LP) dichroic.

Remove the Combining Dichroic from the scan head by lifting the hatch, pinching the top of the frame in which the dichroic is installed, tilting the top of the frame back slightly to release the magnets, and lifting the frame straight up out of the scan head. Install a Combining Dichroic by holding the top of the frame in which the dichroic is installed, lowering it into the scan head, and bringing it forward to engage the magnets. Gently tap the top corners of the frame to ensure that it is seated appropriately and not tilted at an undesired angle, as this would cause misalignment of the uncaging laser beam.

Spot Dichroic

The Spot Dichroic is positioned near the back of the scan head, under the back hatch, in the Imaging laser beam path. The dichroic is mounted in the “vertical” orientation on a rectangular tab.

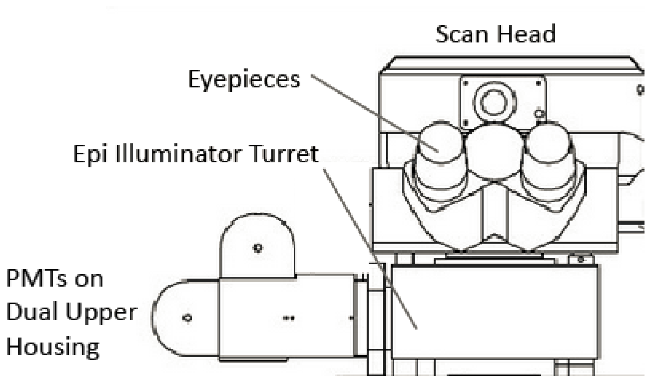
After determining the wavelength(s) necessary for the current experiment, choose the Spot Dichroic which will **reflect** the **Imaging** path laser beam toward the front of the scan head. If using an infrared laser on the Uncaging path, it can be advantageous to choose a Spot Dichroic which will also **transmit** the **Uncaging** wavelength. A small fraction of the uncaging laser beam will be reflected back through the scan head to a spot detector, which can be used when calibrating the Uncaging Galvanometers in Prairie View software. The spot detector calibration method can be used for infrared but not visible uncaging laser beams. For example, when imaging with 820nm laser light and uncaging with 720nm laser light, a 750nm Short Pass dichroic or a Mirror would perform equally well for imaging, and the 750nm Short Pass option would allow use of the spot detector.

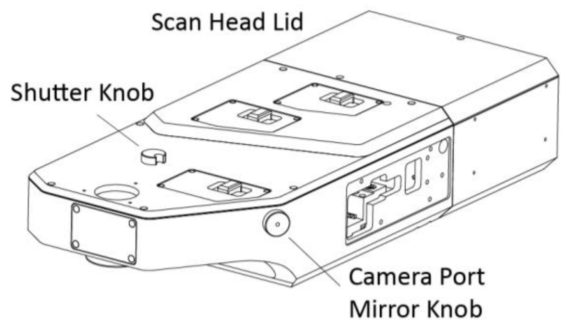
When using an Ultima system configured with a Three Channel Confocal Detector module, the Spot Dichroic must be chosen to **reflect** the **Imaging** path laser wavelength(s) toward the front of the scan head and **transmit** the **emission** wavelengths from the sample into the back compartment of the scan head. More information about the Three Channel Confocal Detector module can be found later in this manual.

Remove the Spot Dichroic from the scan head by lifting the hatch, pinching the top of the tab on which the dichroic is installed, and lifting the tab straight up out of the scan head. Install a Spot Dichroic by holding the top of the tab on which the dichroic is installed, lowering it into the scan head, and lining up the pin in the scan head with the hole in the tab. Take care not to tilt or twist the dichroic during installation, as this can cause the glass to hit other components inside the scan head, leading to system damage.

Selecting the Light Path

Several components in the Ultima system determine how light is transmitted through the system. These components are described below, followed by checklists of the position of each component for frequently used combinations.





Scan Head Internal Shutter

The scan head contains a manual shutter in the laser light path, just downstream of the galvanometer mirrors. This is primarily used by Bruker personnel, and can be kept in the Open position at all times.

The shutter is controlled by a knob on the lid of the scan head. When the knob is rotated to the right, the shutter is open and laser power can pass through the scan head. When the knob is rotated to the left, the shutter is closed and laser light is stopped midway through the scan head.

Camera Port "Turn-down" Mirror or Dichroic

At the front of the scan head, a mirror or dichroic mounted at an angle directs laser light down to the sample. On most systems, there is a full mirror in this position. Specialized configurations may have a dichroic mirror instead of a full mirror, to reduce the need for moving the component.

The mirror position is controlled by a knob on the right side of the scan head. A labeled next to this mirror has an arrow indicating the "In for Imaging" position required for laser scanning. When the knob is rotated counterclockwise, the camera port mirror is moved into the light path, and laser light is directed down to the sample. When the knob is rotated clockwise, the mirror is moved out of the light path, and emission light from the sample can travel up toward the camera port, but no laser light is reflected down to the sample.

Trinocular Head Plunger

The trinocular head, which includes the eyepieces, is controlled by a plunger on the right side. When the plunger is pushed all the way in, the light path is open between the sample and the eyepieces. When the plunger is pulled all the way out, the light path is open straight up from the sample, toward the scan head; this allows for laser scanning or imaging with a camera. If the plunger includes a discrete middle position, this engages a full block of the light path through the trinocular head.

Epi Illuminator Turret

The Epi Illuminator Turret is located below the eyepieces. The turret can be rotated to one of six positions to engage various optics.

- Position 1 contains the Primary Dichroic, which transmits laser light from the scan head to the sample and reflects emission light from the sample to the PMTs.
- Positions 2 and 6 are open, allowing transmitted white light from below the sample to travel up toward the eyepieces or camera. These positions must remain open, as filter cubes installed here would interfere with the Primary Dichroic in position 1.
- Positions 3, 4, and 5 may contain filter cubes, which each of which directs a range of excitation wavelengths from the XCite lamp to the sample, and directs a range of emission wavelengths from the sample toward the eyepieces or camera.

Epi Illuminator Shutter

At the back of the Epi Illuminator Turret is a shutter. This shutter is controlled by a black switch on the right side of the turret.

When open, the shutter moves out of the way to allow light from the XCite lamp to enter the chosen filter cube. When closed, light from the XCite cannot enter the chosen filter cube.

Though the shutter prevents light from the XCite lamp from entering the filter cube, it does not fully block this light from entering the turret. Therefore, the aperture wheel on the XCite must be rotated to the closed position before collecting laser-scanned images with the PMTs. If the aperture wheel is not closed, the stray light will flood the PMTs and introduce noise into the image.

The shutter must be closed when laser scanning to collect images with the PMTs, because the paddle of the open shutter partially obstructs the light path between the sample and the PMTs. This will cause one part of the scanned image (typically the top) to be very dim.

Transmitted Lamp

All Ultima In Vitro systems and some Ultima IntraVital systems include a transmitted white light source. This lamp is controlled by a remote connected to the light source. The remote includes an on/off button and a knob for adjusting intensity.

The light path from the transmitted light source to the sample may include a band pass filter for selecting visible or infrared illumination wavelengths.

An interlock protects the upper PMTs from direct exposure to the transmitted light by automatically turning off the light source when the Epi Illuminator Turret is in position 1.

Another interlock protects a Dodt enhanced contrast PMT (if present) by turning off the transmitted light if the detector is in the light path. This light path is controlled manually on Ultima IntraVital systems, and through Prairie View software on Ultima In Vitro systems.

Lamp Module (XCite or other)

A number of light sources are available for illumination when navigating in the sample. One common option is a mercury vapor arc lamp, such as that in the XCite 120Q, which is described in more detail later in this manual. Other options include LED light sources.

Light from the lamp module enters the Epi Illuminator Turret from the back and travels through a filter cube down to the sample.

In the case of an XCite 120Q, a power switch on the lamp module turns the lamp on and off. The lamp should not be subjected to repeated power cycles. An aperture wheel on the module controls the intensity of the light, and can be closed to block the lamp light without turning off the lamp itself.

To fully block light from the light source when it is not in use, the light source aperture wheel must be rotated to the closed position or LEDs must be turned off. The shutter mechanism in the Epi Illuminator Turret is not adequate alone, as it leaks light from the lamp house that will flood the PMTs and introduce noise into the image.

Configurations

Light Path	Scan Head Shutter	Camera Port Mirror	Trinoc Plunger	Epi Turret Position	Epi Shutter	Transmitted Lamp	XCite Aperture
Eyepiece viewing of transmitted light			In	2		On; no IR filter	
Eyepiece viewing of fluorescence			In	3,4,5	Open	Off	Open
Camera imaging of transmitted light		Out	Out	2		On; filter optional	
Camera imaging of fluorescence		Out	Out	3,4,5	Open	Off	Open
Laser Scanning with PMT imaging	Open	In	Out	1	Closed	Off	Closed

System Components

Galvanometers and Galvanometer Control Box

Pointing of the laser beam(s) in the Bruker Ultima system is accomplished by one or more pairs of galvanometer mirrors, manufactured by Cambridge Technologies, Inc., and fine-tuned by Bruker personnel. Each pair of galvanometers consists of one mirror for scanning the X dimension and one mirror for scanning the Y dimension. These mirrors rotate in response to electrical signals provided by the Galvanometer Control Box, directing the laser beam(s) to the desired points in the sample. There is one Galvanometer Control Box for each pair of galvanometers. The galvanometers are located inside the scan head of the Ultima, and are not accessible to the operator.

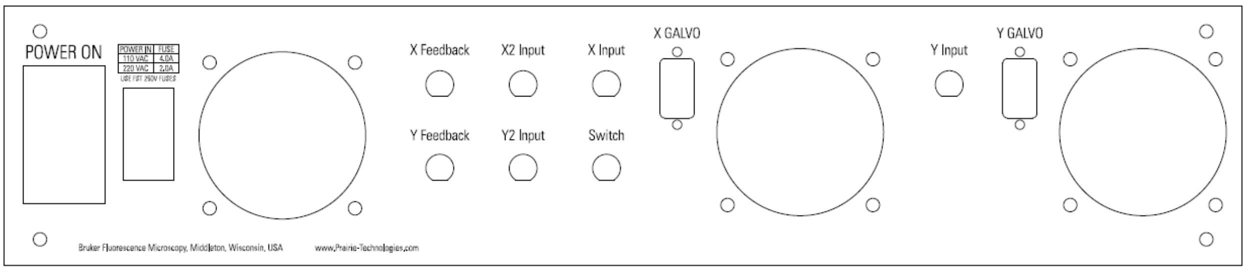
Each individual galvanometer is mated to a specifically-tuned control board, located in the Galvanometer Control Box. This means that it is not possible to operate the system with mismatched galvanometers and control boxes, or to exchange the X and Y galvanometers within a set. Doing so may cause permanent damage to the galvanometers and render the system inoperable.

Every Ultima system will have a pair of Imaging Galvanometers. Signals generated by Prairie View software drive these galvanometers in the pattern defined by the operator within the software, scanning the beam of an ultrafast pulsed infrared laser across the sample to generate an image.

Many Ultima systems contain an additional pair of galvanometers, often referred to as the Uncaging Galvanometers. The Uncaging Galvanometers operate independently of the Imaging Galvanometers, allowing a separate laser beam to be directed to discrete points or regions in the field of view for stimulation of the sample (e.g. uncaging, optogenetics, etc.) while the Imaging Galvanometers continue their scanning to generate images of the sample before, during, and after stimulation. This is referred to as simultaneous operation of imaging and photostimlulation.

If the independent set of Uncaging Galvanometers is not present, the operator can perform photostimulation functions using the Imaging Galvanometers. However, this operation will be limited in at least two ways, compared to systems with both pairs of galvanometers:

1. Photostimulation can take place between imaging events, but not simultaneously with imaging (as the galvanometers can point in two places at once); software overhead two switch signals will add additional delays between imaging and photostimulation
2. Photostimulation protocols will not be carried out as quickly, as the jumping/settling time of Imaging Galvanometers is greater than that of Uncaging Galvanometers



Operation

The operator does not interact directly with the galvanometers or the Galvanometer Control Box(es) during normal system operation. Instead, all communication is carried out by Prairie View software. The Galvanometer Control Box must be powered on and connected when the system is being used. It is recommended that the operator leave the Galvanometer Control Box power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down.

Three lights on the front panel of the Galvanometer Control Box indicate galvanometer status. The green light is on whenever the box is powered on. The left and right amber lights indicate the fault status of the X and Y galvanometers, respectively. These illuminate for 1-2 seconds on power up but should otherwise be off. If they are illuminated at any time except immediately after power up, there is a problem with the associated galvanometer. Contact Bruker support personnel for assistance with cause of the fault status.

When moving items near the Ultima system, take care not to pull, bend, or pinch the black and grey cables running from the Galvanometer Control Box into the scan head. If these cables are damaged, they can disrupt control of the galvanometers and may cause permanent damage.

Scan patterns of the Imaging Galvanometers are calculated by Prairie View software when the operator chooses the region of the sample to image and the speed at which to collect images. If present, the Uncaging Galvanometers are also controlled by Prairie View software. The position of the Uncaging Galvanometers is calculated using the point(s) or region(s) of stimulation defined by the operator, as well as the calibration file which maps locations in the image to positions of the Uncaging Galvanometers. For more information, refer to the Prairie View software manual.

When one set of galvanometers is used for both imaging and photostimulation, a switch inside the GPIO Box is typically used by Prairie View software to automatically determine which operation is carried out by the galvanometers at a given time. However, in some cases, two different signal sources may be connected to the Galvanometer Control Box at the same time, and selection may be carried out by a TTL signal sent to a switch within the Galvanometer Control Box. Contact Bruker support personnel for information about this alternate control option.

The Ultima system is aligned with the galvanometers held at their center positions. This position is achieved via Prairie View software controls, or by replacing the X Input and Y Input cables with 50Ω terminators. The galvanometers must be returned to this center position whenever the operator checks alignment of the system, as described elsewhere in this manual.

Connections

Each Galvanometer Control Box will be labeled on the back panel to indicate which galvanometers are controlled by the box. The label will indicated “Imaging” or “Uncaging” and list the serial numbers of the individual galvanometers for which the control boards inside are tuned.

Galvanometer Control Box Connector	**Cable**	**Connects to**
AC Power input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
X Feedback	BNC	Not connected; provides a scaled output of X galvanometer position used in troubleshooting
Y Feedback	BNC	Not connected; provides a scaled output of Y galvanometer position used in troubleshooting
X2 Input [1]	BNC	Not connected on most systems
Y2 Input [1]	BNC	Not connected on most systems
X Input	BNC	GPIO Box “GA X IM OUT” (imaging) or GPIO Box “GA X UN OUT” (uncaging)
Switch [1]	BNC	Not connected on most systems
Y Input	BNC	GPIO Box “GA Y IM OUT” (imaging) or GPIO Box “GA Y UN OUT” (uncaging)
X GALVO [2]	Galvanometer signal cable terminated with DB-9, provided by Cambridge Technologies, Inc.	X Galvanometer in the Ultima scan head

Y GALVO [2]	Galvanometer signal cable terminated with DB-9, provided by Cambridge Technologies, Inc.	Y Galvanometer in the Ultima scan head

[1] If there are two potential control voltages for a galvanometer axis, attach the alternate analog signal input to the X2 Input or Y2 Input. The Switch TTL input selects which source controls galvanometer position. When Switch is low (0V), X Input/Y Input controls galvanometer position. When Switch is high (5V), X2 Input/Y2 input controls galvanometer position.

[2] The cables will be labeled as X or Y and Imaging or Uncaging. Be careful not to connect incorrectly as each galvanometer is matched to its driver. Additionally, do **not** connect/disconnect the DB-9 cables when the Galvanometer Control Box power is on.

Switching cables so that the galvanometers are connected to the wrong drivers can cause damage to the galvanometers.

Troubleshooting

Symptom	**Possible Cause**	**Action**
One or both of the amber lights on the front panel of the Galvanometer Control Box is illuminated intermittently, and only while scanning	Galvanometer(s) are being driven too hard and control board is reporting a fault	Reduce scan speed in Prairie View software, contact Bruker support personnel with questions
One or both of the amber lights on the front panel of the Galvanometer Control Box is illuminated constantly	Galvanometer or DB-9 cable has been damaged and control board is reporting a fault	Contact Bruker support personnel
Green light is not illuminated on the front panel of the Galvanometer Control Box	Galvanometer Control Box is turned off	Turn on back panel switch
	Galvanometer Control Box is not receiving AC input power	Ensure that the AC power input cable is securely connected to the back panel and that it is connected to an active power source
	Improper fuse is installed or fuse has been damaged	Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation
	Circuitry controlling the green light has failed	Confirm that the system scans as expected (creates an image and uncaging/photostimulation

		points are hit as marked) in Prairie View software and ignore the green light going forward

X Feedback and Y Feedback connections on the back of the Galvanometer Control Box report the position of the respective galvanometers. These can be used during troubleshooting to determine whether a galvanometer is moving. Additionally, the Feedback signal can be compared to the Input signal coming from the GPIO Box. Note that the scale factor of the Feedback signal differs from the Input signal (0.5V/degree instead of 0.66V/degree).

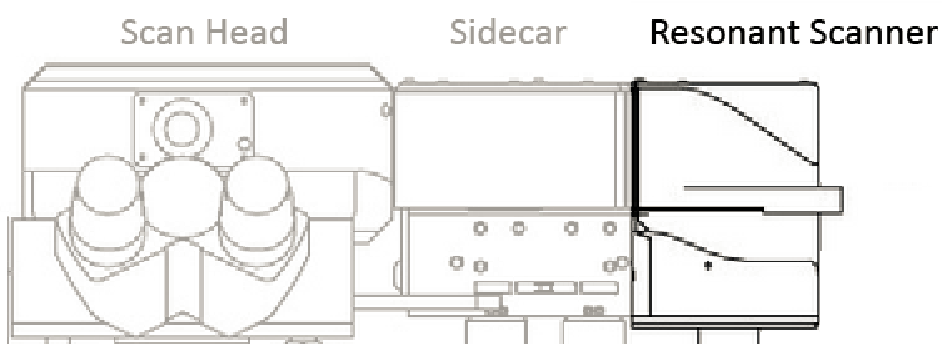
Resonant Scanner and Resonant Galvo Control Box

The Resonant Scanner is a fixed-frequency galvanometer that operates at approximately 8kHz. This scanner allows the Ultima system to scan a 512x512 pixel image at upwards of 30 frames per second. The amplitude of the scanner can be controlled via software, enabling scans at various levels of optical zoom.

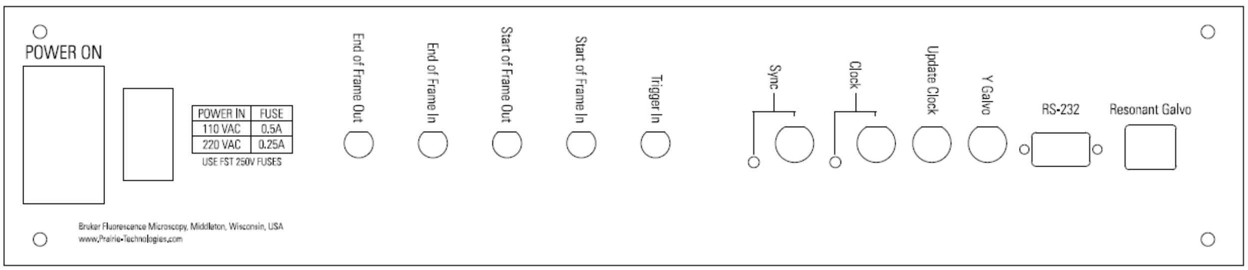
The Resonant Scanner module is installed by Bruker personnel and is attached to the right side of the basic Ultima scan head. Mirrors and lenses inside the Ultima automatically divert the ultrafast pulsed infrared laser beam from the standard galvanometer scanning path to the Resonant Scanner.

Control signals are routed through the Resonant Galvo Control Box.

Resonant Scanner



Resonant Galvo Control Box



Operation

The operator does not interact directly with the Resonant Scanner or the Resonant Galvo Control Box during normal system operation. Instead, all communication is carried out by Prairie View software. The Resonant Galvo Control Box must be powered on and connected before Prairie View software is started. It is recommended that the operator leave the Resonant Galvo Control Box power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box off when the entire electronics workstation is being powered down.

During standard galvanometer imaging, the beam from the ultrafast pulsed infrared laser does not encounter the Resonant Scanner, and the Resonant Galvo Control Box serves only as a pass-through for signals originating in the General Purpose Input Output (GPIO) Box.

When the operator chooses the Resonant imaging mode from within Prairie View software, motorized optical components inside the Ultima scan head (and inside the Beam Cover, for an Ultima IntraVital fixed post or Ultima In Vitro system) move to direct the beam from the ultrafast pulsed infrared laser to the Resonant Scanner. The laser beam path encounters the Resonant Scanner as well as the standard galvanometers, allowing the Resonant Scanner to perform high-speed scanning in the X dimension across the image, while the standard Y galvanometer provides scanning in the Y dimension and the standard X galvanometer holds the position necessary for the defined scan center; this allows the operator to select regions of interest within Prairie View software. The Resonant Galvo Control Box drives the Resonant Scanner and generates other output signals.

Because the emission path for the Three Channel Confocal Detector is descanned through the Ultima scan head, it is not possible to use the Resonant Scanner for imaging with the Three Channel Confocal Detector module.

Indicator Lights

There are three lights on the front panel of the Resonant Galvo Control Box, indicating the following

- POWER: This light is illuminated when the Resonant Galvo Control Box is turned on and receiving AC power. This light should be on any time the Ultima system is powered on.
- MODE: This light is illuminated when the Ultima system is operating in Resonant mode. The operator chooses this mode via Prairie View software.
- CRS ON: This light is illuminated when the galvanometer inside the Resonant Scanner is moving, as commanded by Prairie View software.

Connections

Resonant Scanner Connector	**Cable**	**Connects to**
Unlabeled Cat5e connector	Category 5 network cable	Resonant Galvo Control Box “Resonant Galvo”
Unlabled USB (for movement of motorized beam-routing optics)	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)

Resonant Galvo Control Box Connector	**Cable**	**Connects to**
AC Power input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
End of Frame Out	BNC	GPIO Box “PFI 8” (front panel) and Piezo Amplifier/Driver Box “Trigger 1”, if Bruker Z-piezo is present
End of Frame In	BNC	GPIO Box “PCI-6713 FTO” (front panel)
Start of Frame Out	BNC	Available for operator to poll; not connected on most Ultima systems
Start of Frame In	BNC	GPIO Box “PCI-6052E FTO” (front panel)
Trigger In	BNC	GPIO Box “RES TRIG OUT” and Piezo Amplifier/Driver Box “Trigger 2” if Bruker Z-piezo is present
Sync (output)	MMCX	General Standards “SYNC” (Workstation Computer)
Sync (input)	BNC	GPIO Box “RES SYNC”
Clock (output)	MMCX	General Standards “CLK” (Workstation Computer)

Clock (input)	BNC	GPIO Box "RES CLOCK"
Update Clock	BNC	GPIO Box "RES UP CLOCK"
Y Galvo	BNC	GPIO Box "RES Y IN"
RS-232	DB-9 serial cable	Workstation Computer (directly or via USB/Serial expander hub)
Resonant Galvo	Category 5 network cable	Ultima scan head Resonant Scanner

The Resonant Galvo Control Box must be connected to the Z-piezo to synchronize high-speed volume imaging. These connections involve T-connectors to share signals between the Resonant Galvo Control Box, Piezo Amplifier/Driver Box, and GPIO Box. The T-connectors are typically placed on the Piezo Amplifier/Driver Box. The layout of these T-connectors and lengths of the cables are determined by Bruker personnel to minimize signal offsets and reflections. Do not change the cable layout on these connections unless instructed to do so by Bruker personnel.

Troubleshooting

Symptom	Possible Cause	Action
Resonant Scanner image is not stable	Necessary scan amplitude is below lower limit of galvanometer performance	Increase the width of the scanned region in Prairie View software (decrease the optical zoom or draw a wider region of interest)
Resonant Scanner galvanometer moves (soft audible noise at 8kHz), but laser light does not reach the sample	Optics directing the laser beam to the Resonant Scanner are not in position	In Prairie View software, switch to Galvo mode and then back to Resonant mode; listening for motor movements
No green lights are illuminated on the front panel	Resonant Galvo Control Box is turned off	Turn on back panel switch and ensure that at least one green light is illuminated on the front panel
	Resonant Galvo Control Box is not receiving AC input power	Ensure that the AC power input cable is securely connected to the back panel and that it is connected to an active power source
	Improper fuse is installed or fuse has been damaged	Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation

Non-Descanned Upper Detectors

The Ultima system uses externally mounted, non-descanned photomultiplier tube (PMT) detectors to collect the fluorescence emitted from the specimen. These PMTs can be any combination of the Multialkali R3896 SEL or GaAsP H7422PA-40 SEL from Hamamatsu, described elsewhere in this manual. The standard configuration for PMTs is a two channel, top-mounted detector setup. Most Ultima systems can instead be configured for four top-mounted PMTs.

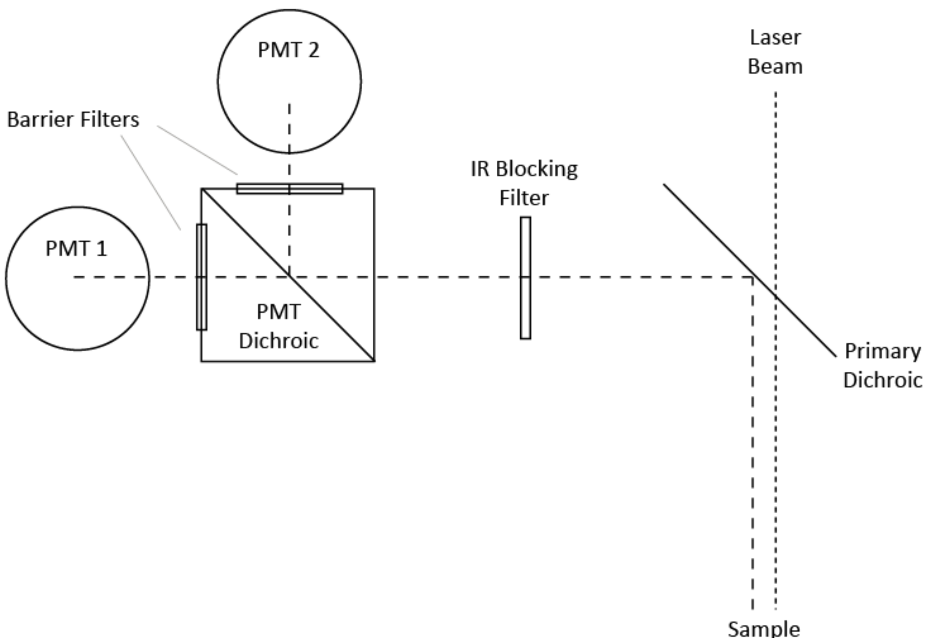
PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light and/or prevent it from reaching the PMTs, as exposure to excess light will dramatically reduce the sensitivity of the system.

Detection Light Path Overview

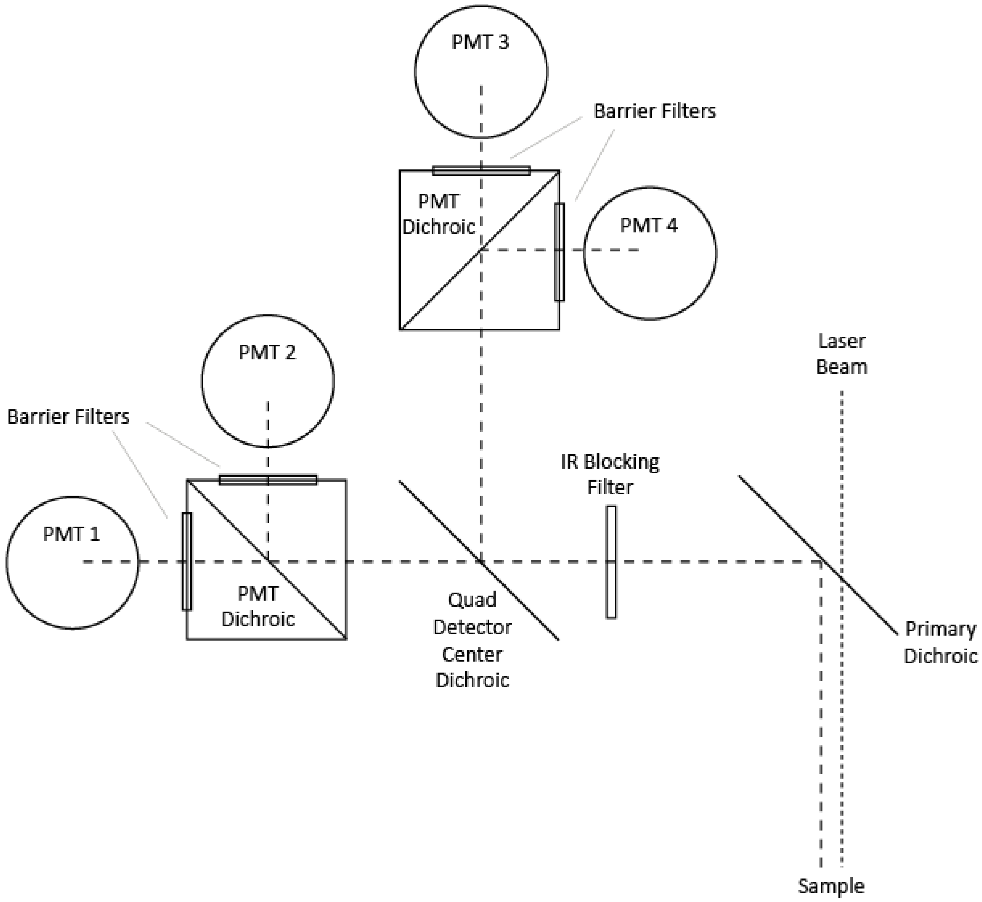
Laser light from the scan head passes down through the Primary Dichroic in the Epi Illuminator Turret and through the objective lens to the sample.

Emission light from the sample is transmitted through the objective lens, reflected off the primary dichroic, through the IR Blocking Filter, and through the various dichroics and barrier filters to the PMTs.

Dual Upper Detectors



Quad Upper Detectors



Detection Glass

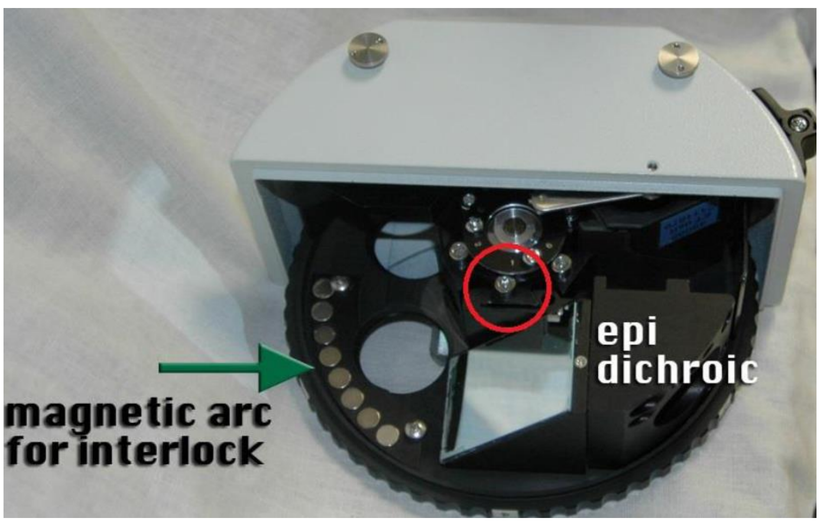
Primary Dichroic

The Primary Dichroic is located in position 1 of the Epi Illuminator Turret. Multiple Primary Dichroic options are available from Bruker. Selection is based on the laser wavelengths used for imaging and photostimulation. The selected Primary Dichroic must transmit laser light from the scan head down to the sample, and reflect all emission wavelengths from the sample to the PMTs.

Changing the Primary Dichroic

1. Use a 3mm hex driver to loosen the front-most set screw on the right side of the Epi Illuminator Turret

2. Slide the turret forward out of the Epifluorescence Illuminator
3. Turn over the turret and rotate it to expose the Primary Dichroic in position 1
4. Use the 3mm hex driver to loosen the screw on the dovetail at position 1



5. Slide the Primary Dichroic mount up off the dovetail
6. Place a different Primary Dichroic mount on the dovetail at position 1, making sure it slides all the way onto the dovetail
7. Tighten the screw to secure the mount to the dovetail
8. Put the Epi Illuminator Turret back into the Epifluorescence Illuminator
9. Tighten the screw on the right side to secure the Epi Illuminator Turret
10. Open and close the Epi Illuminator Turret Shutter switch to ensure free movement of the internal components.

In some cases, and alternate Primary Dichroic may be pre-installed in a separate Epi Illuminator Turret, making it necessary only to exchange the turrets.

The Primary Dichroic must be installed in position 1 of the Epi Illuminator Turret to preserve its relationship to the magnetic arc, which is part of the safety interlock to turn off a transmitted light source when the Primary Dichroic is moved into the light path.

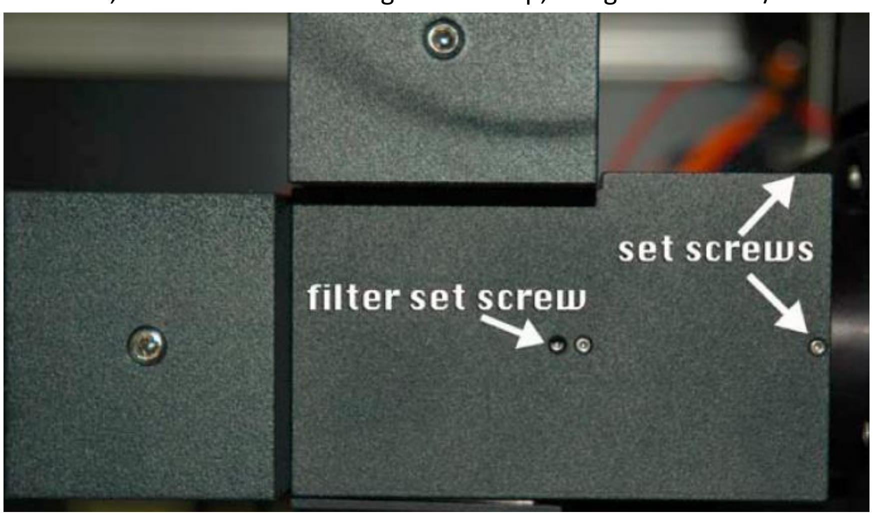
Infrared (IR) Blocking Filter

An IR Blocking Filter is mounted between the Primary Dichroic and the PMTs. It serves to prevent scattered laser light from reaching the detectors. Multiple IR Blocking Filters are available from Bruker. The IR Blocking Filter must be selected to block all laser wavelengths from reaching the PMTs, and transmit all emission wavelengths from the sample to the PMTs.

Changing the IR Blocking Filter

Dual Upper Detectors

1. Use a pencil or tape to mark the horizontal position of the detector housing along the lens tube, as it must be returned to the same position after filter exchange
2. Loosen the four set screws on the far right end of the detector housing; one small set screw on the front, one small and two large on the top, using 0.050 and 1/8 inch Imperial hex drivers



3. Slide the detector housing off the lens tube, twisting gently if the fit is tight; take care not to strain cables running to the PMTs
4. Loosen the small set screw between the PMTs on the front of the housing, using an 0.050 inch Imperial hex driver; this is holding the IR Blocking Filter in place
5. Cover the front end opening with a lens-safe cloth and tilt the housing until the filter drops into the cloth; take care not to touch the glass with bare hands or metal
6. Lower the new filter into the housing, with the directional arrows on the side of the filter facing the open end (away from the PMTs); it can be helpful to insert the wooden ends of two cotton-tipped applicators in the holes of the filter ring to use as “chopsticks” to hold the filter while guiding it into the housing

7. Ensure the filter sits flat inside the housing; adjust the ring with the handle of a cotton-tipped applicator if needed, but do not touch the applicator to the glass surface
8. Tighten the set screw holding the filter in place, using an 0.050 inch Imperial hex driver
9. Slide the detector housing back onto the lens tube, taking care to line it up to the previously marked position and orienting PMT2 on the top of the housing
10. Tighten the four set screws holding the detector housing to the lens tube, using 0.050 and 1/8 inch Imperial hex drivers

Quad Upper Detectors

1. Use a pencil or tape to mark the horizontal position of the detector housing along the lens tube and the positions of the screws/washers in the channels of the plate attaching the detector housing to the side of the microscope, as all must be returned to the same position after filter exchange
2. Remove the two screws from the slots on the plate holding the detector housing onto the side of the microscope (7/64 inch Imperial hex driver) **and** the two set screws holding the detector housing onto the lens tube (1/8 inch Imperial hex driver)
3. Slide the detector housing off the lens tube, twisting gently if the fit is tight, and set the housing aside; take care not to strain cables running to the PMTs
4. Look into the lens tube, which is still attached to the left side of the Epifluorescence Illuminator, and locate the notch in the retaining ring threaded into the tube
5. Use a fingernail or other narrow object to turn the retaining ring counterclockwise until it is out of the tube
6. Catch the filter as it drops out from inside the lens tube; with the retaining ring removed, the filter will fall on its own or can be nudged on one side with lens paper
7. Place the new filter into the lens tube, orienting the arrow on the side of the filter to point into the Epifluorescence Illuminator (away from the PMTs)
8. Re-install the retaining ring to hold the filter in place
9. Slide the detector housing back onto the lens tube, taking care to line it up to the previously marked position and orienting PMT2 on the top of the housing
10. Replace the two screws and two set screws holding the detector housing onto the microscope and lens tube (7/64 and 1/8 inch Imperial hex drivers)

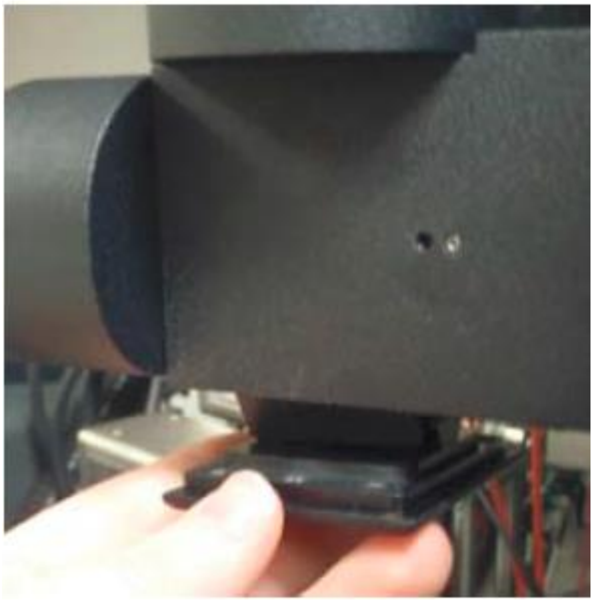
Filter Cube

A filter cube sits between each pair of PMTs to divide emission wavelengths into two channels. The cube is a BX2-style mount containing a dichroic mirror and two barrier filters. The cube can be exchanged easily to change the wavelengths detected by each PMT.

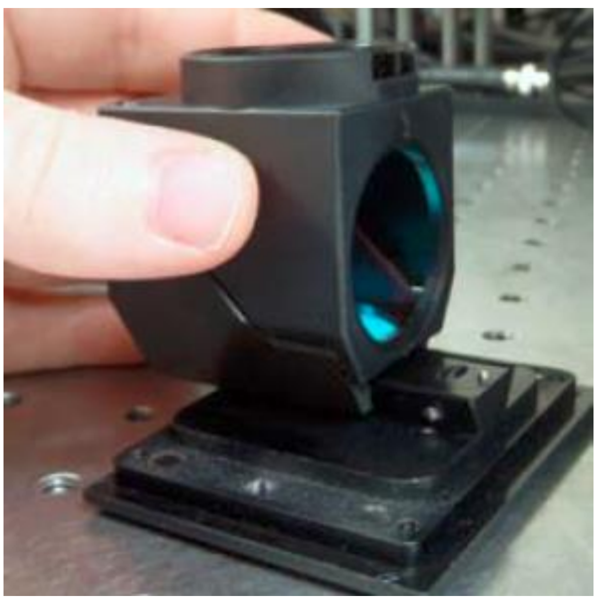
Changing the Filter Cube

1. Locate the filter cube hatch below the pair of PMTs; the hatch is held in by magnets

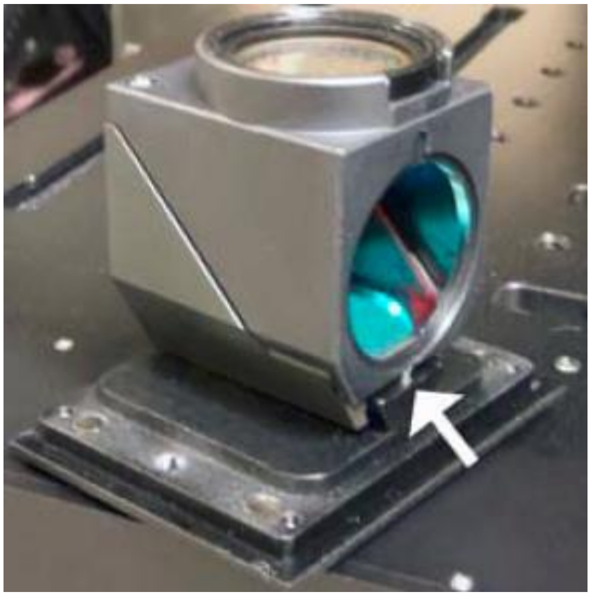
2. Pull straight down to remove the hatch and the filter cube



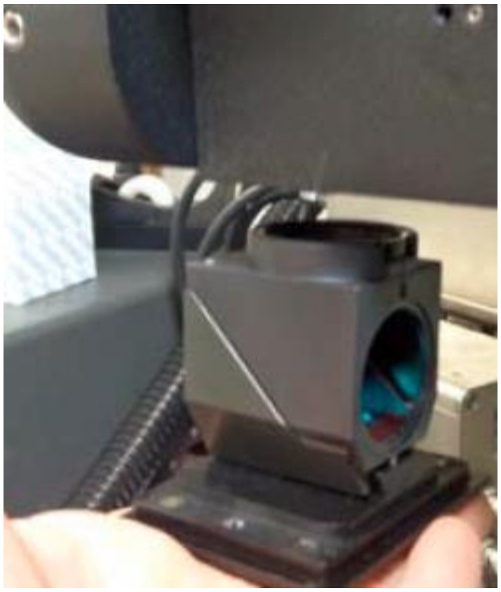
3. Slide the cube off the dovetail mount



4. Slide the new cube onto the mount, making sure to orient the open side of the cube toward the holding pin on the hatch



5. Push the cube and mount straight up into the opening below the pair of PMTs, with the open end of the cube (and the holding pin) facing away from the PMTs (toward the Epi Illuminator Turret for channels 1 and 2, or toward the front for channels 3 and 4)



Quad Detector Center Dichroic

An additional dichroic sits in the Quad Channel Detector housing to divide emission wavelengths between the two pairs of PMTs. This dichroic is selected for compatibility with the two Filter Cubes used downstream between the two pairs of PMTs.

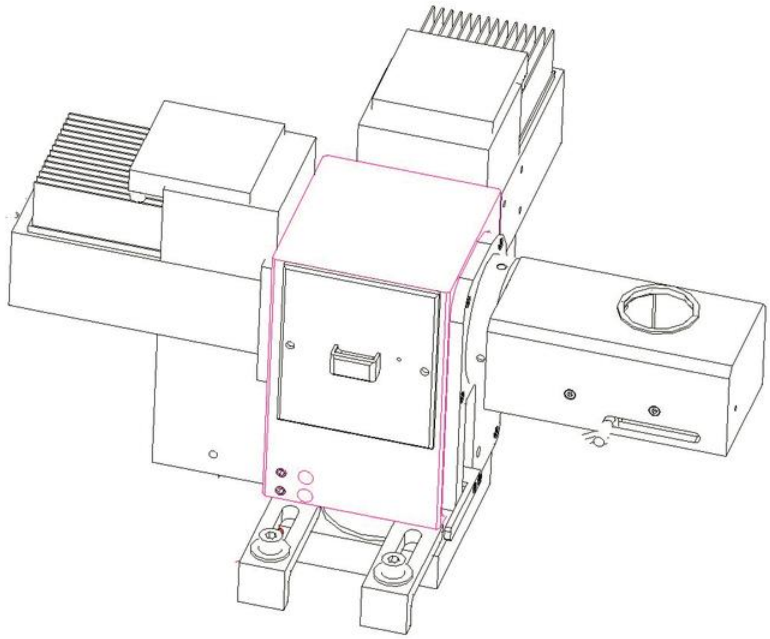
Changing the Quad Detector Center Dichroic

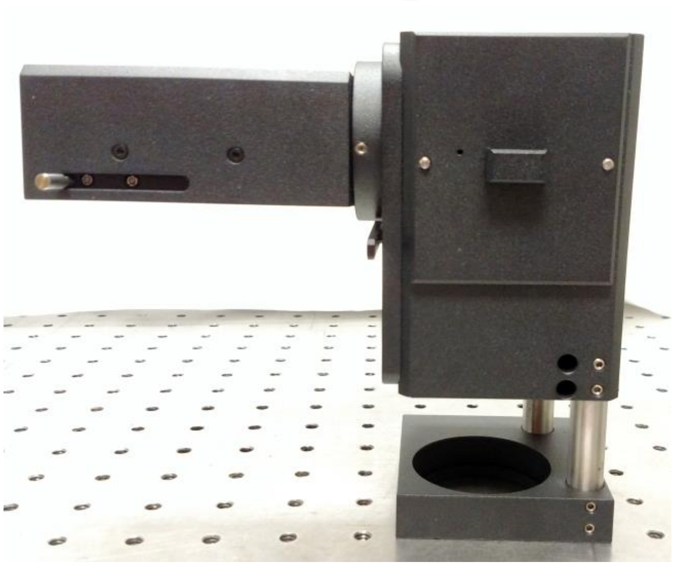
1. Locate the magnetic hatch on the front of the detector housing
2. Pull the hatch straight forward to remove the dichroic
3. Grasp the frame around the Quad Detector Center Dichroic and pull it free of the magnets on the inside of the hatch door
4. Affix the new Quad Detector Center Dichroic to the magnets on the hatch door, with the support plate on the bottom to ensure the orientation will direct light back toward the second pair of PMTs
5. Push the hatch and dichroic mount back into the detector housing

Non-Descanned Substage Detectors

In addition to the detectors described previously, many Ultima systems can be configured with two substage PMTs. These detectors are mounted below the sample and can be used to collect transmitted fluorescence and second-harmonic signal from thin samples. These PMTs can be any combination of the Multialkali R3896 SEL or GaAsP H7422PA-40 SEL from Hamamatsu, described elsewhere in this manual.

The substage detector housing is available in two versions, which are “right-hand” and “left-hand” mirror images, chosen based on the system configuration for best compatibility with other hardware present. The drawing below shows the left-hand model (with two GaAsP PMTs installed), while the photographs show the right-hand version (with no PMTs installed).

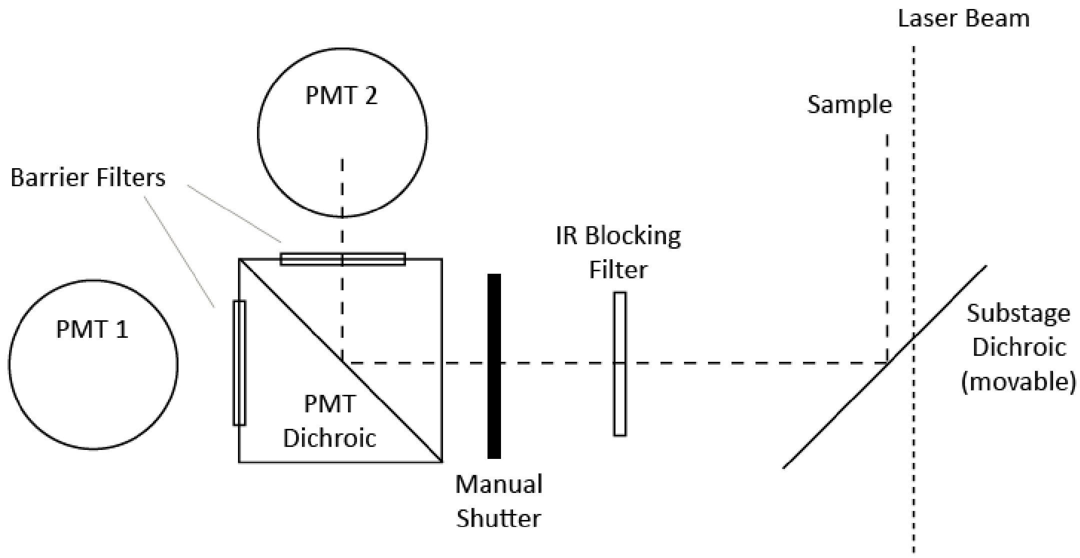




PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light and/or prevent it from reaching the PMTs, as exposure to excess light will dramatically reduce the sensitivity of the system.

Substage Detection Light Path

The emission from the sample is transmitted through the condenser lens, reflected off the Substage Dichroic, through the IR Blocking Filter, and through the various dichroics and barrier filters to the PMTs.



Detection Glass

Substage Dichroic

The Substage Dichroic is mounted in the detector housing below the condenser lens. The dichroic is mounted on a sliding track (often called a pick-up tray), allowing it to be moved out of the path when a transmitted light source is being used. Multiple Substage Dichroic options are available from Bruker. Selection is based on the laser wavelengths used for imaging and uncaging/photostimulation. The selected Substage Dichroic must reflect all emission wavelengths from the sample to the PMTs, and ideally transmit all imaging and uncaging laser light from the scan head so that laser light is not directed toward the PMTs.

The Substage Dichroic is integrated into the slider tray and cannot be removed by the operator. If it becomes necessary to exchange Substage Dichroics, the alternate dichroic must be housed in its own slider tray, and the entire tray must be exchanged on the substage housing. This can be done by loosening the two set screws on the collar near the manual shutter with a 5/64 inch Imperial hex driver.

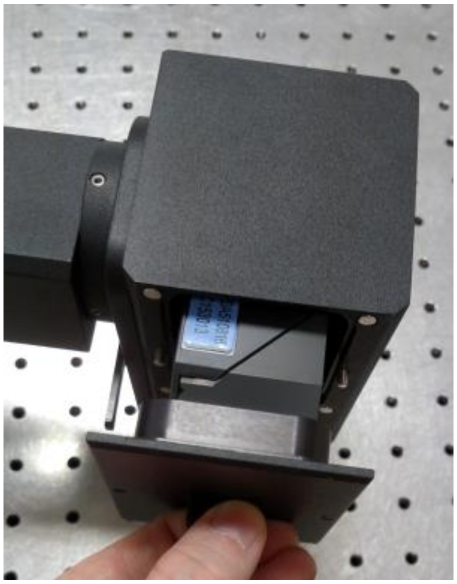
Infrared (IR) Blocking Filter

An IR Blocking Filter is mounted between the Substage Dichroic and the PMTs. It serves to prevent scattered laser light from reaching the detectors. Multiple IR Blocking Filters are available from Bruker. The IR Blocking Filter must be selected to block all laser wavelengths from reaching the PMTs, and transmit all emission wavelengths from the sample to the PMTs.

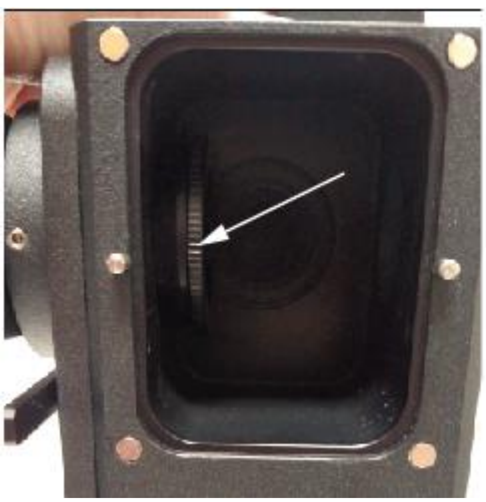
Changing the IR Blocking Filter

1. Push the manual PMT shutter lever up to shield the PMTs from ambient light
2. Locate the filter cube hatch in front of the pair of PMTs; the hatch is held in by magnets

3. Pull straight forward to remove the hatch and the filter cube; set the hatch and cube aside



4. Reach into the housing and turn the knurled IR filter holder until it is removed from the inside wall of the housing; do **not** shine light into the housing, as the manual shutter does not fully protect the PMTs while the front hatch is removed




5. Locate the notch in the retaining ring inside the filter holder and use a narrow object such as a fingernail to rotate the retaining ring and remove it from the filter holder

6. Tip the filter holder so the IR Blocking Filter drops into a piece of lens paper
7. Set a new IR Blocking Filter into the filter holder such that the arrow on the side of the filter points toward the larger knurled side of the filter holder
8. Re-install the retaining ring to hold the filter in place
9. Reach into substage housing and twist the filter holder back into the side wall; be sure to twist it in all the way to make room for the filter cube
10. Re-install the filter cube and hatch by, with the open end of the cube (and the holding pin on the hatch) facing the Substage Dichroic tray

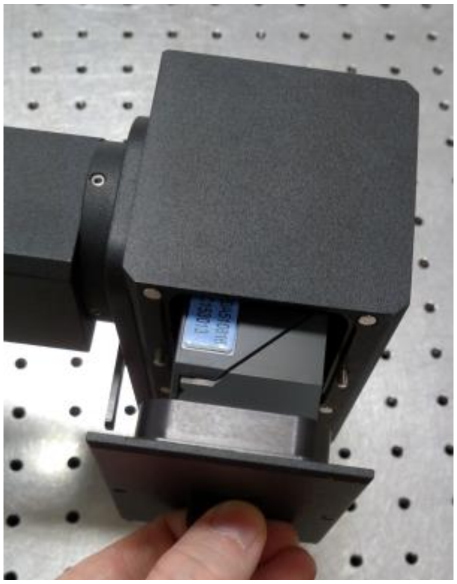
Filter Cube

A filter cube sits between each pair of PMTs to divide emission wavelengths into two channels. The cube is a BX2-style mount containing a dichroic mirror and two barrier filters. For many system configurations, the signals from the substage PMTs are combined with the signals from upper PMTs by connecting two PMTs to the same Preamplifier channel. In these situations, it is useful to use identical filter cubes in the upper and substage PMT housings to avoid summing disparate channels.

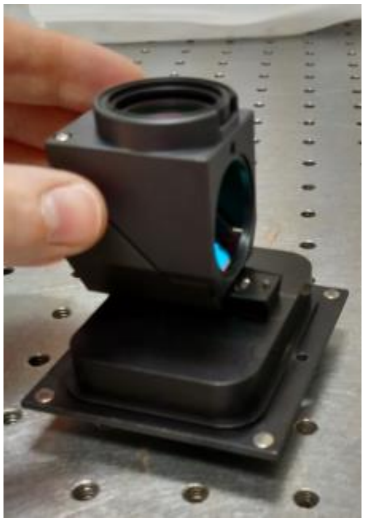
Changing the Filter Cube

1. Push the manual PMT shutter lever up to shield the PMTs from ambient light
2. Locate the filter cube hatch in front of the pair of PMTs; the hatch is held in by magnets

3. Pull straight forward to remove the hatch and the filter cube



4. Slide the cube off the dovetail mount



5. Slide the new cube onto the mount, making sure to orient the open side of the cube toward the holding pin on the hatch
6. Push the cube and mount straight back into the opening below the pair of PMTs, with the open end of the cube (and the holding pin) facing the Substage Dichroic tray

Operation

Substage PMTs can be used with thin samples. They are typically not useful for imaging in intact animals, as no emission photons reach the condenser.

To achieve maximum intensity in sub-stage detectors, the condenser aperture should be in the open position and aligned for proper Köhler illumination (focused and centered). This can be set up using the transmitted light before switching the light path for laser scanning.

The Substage Dichroic is mounted on a sliding tray inside the module. The dichroic must be moved into the light path when imaging, by sliding the handle to the position farthest away from the PMTs. The dichroic must be moved out of the light path when viewing the sample with the transmitted lamp light, by sliding the handle to the position closest to the PMTs.

A manual shutter is incorporated into the substage housing. This shutter must be opened by pushing the vertical lever down when using the PMTs. It must be closed by pushing the vertical lever up to protect the PMTs when they are not in use.

Care must be taken to protect the substage PMTs from other light sources. The manual shutter must be closed whenever the XCite lamp module is being used for epi illumination. Exposure of the substage PMTs to this light will dramatically reduce the sensitivity of the detectors.

Descanned Three Channel Confocal Detector

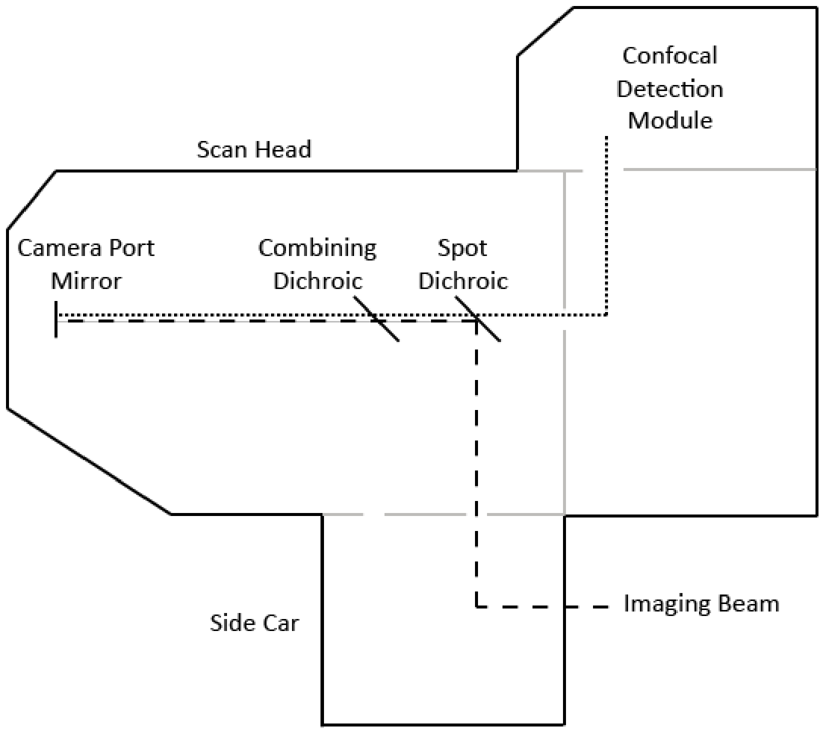
The Ultima scan head can be outfitted with additional optics to enable confocal imaging with descanned detection. The Three Channel Confocal Detector module incorporates three PMTs in any combination of Multialkali R3896 SEL or GaAsP H7422PA-40 SEL from Hamamatsu, described elsewhere in this manual.

In the confocal configuration of the Ultima, laser excitation light comes from a fiber-coupled laser launch, and enters the scan head through a Point Photoactivation Module, which is described elsewhere in this manual. The laser light is scanned across the sample by the same Imaging Galvanometers used by the ultrafast pulsed infrared laser for multiphoton excitation. However, rather than emission being reflected to the non-descanned detectors, the light is reflected back through the scan head to the Three Channel Confocal Detector module.

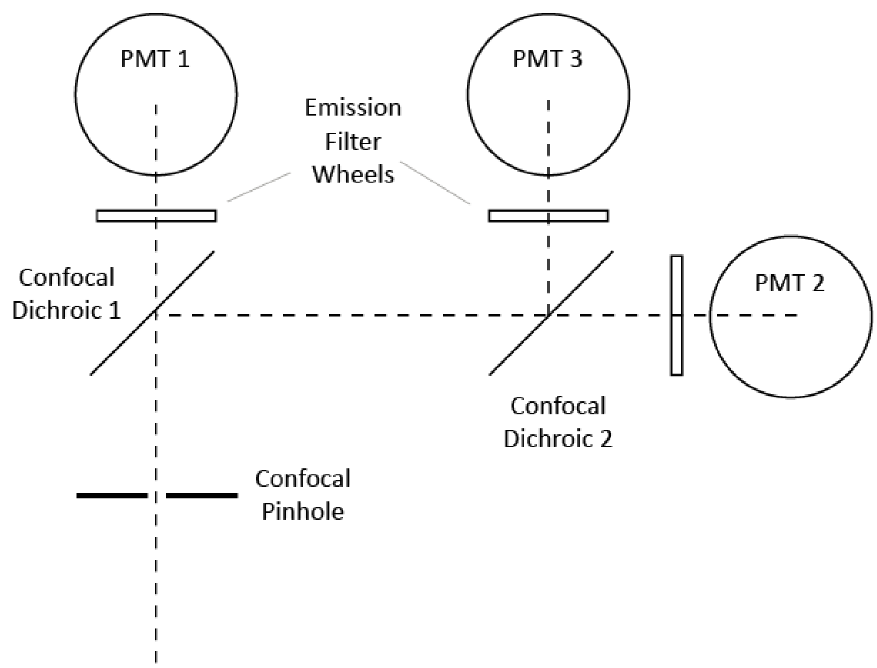
Emission light travels through a confocal pinhole in an aperture plate and then encounters a series of dichroic mirrors and band pass filters. The aperture plate, dichroics, and filters are all in motorized mounts with multiple pre-programmed positions.

Scan Head Light Path

The Three Channel Confocal Detector module is compatible with the Uncaging Galvanometers. However, for simplicity, only the Imaging laser beam path and the sample emission path are shown in this diagram.



Confocal Module Internal Light Path



Operation

The operator interacts with the Three Channel Confocal Detector module primarily through Prairie View software. Drop-down menus in the software allow the operator to choose from the available apertures, dichroics, and filters.

All of the filters and dichroics inside the Three Channel Confocal Detector module are pre-installed by Bruker personnel. However, the operator must choose and manually install appropriate glass in the scan head itself, according to the following guidelines:

- Spot Dichroic must **reflect** the **Imaging** path laser wavelength(s) toward the front of the scan head and **transmit** the **emission** wavelengths from the sample into the back compartment of the scan head; typical Spot Dichroics provided with the system will have broad transmission with narrow notches to reflect laser wavelengths
- Combining Dichroic must **reflect** any **Uncaging** path laser wavelengths and **transmit** the **Imaging** path laser wavelengths and the **emission** wavelengths from the sample
- Camera Port Dichroic must **reflect** all laser and sample emission wavelengths; a full mirror is ideal
- Primary Dichroic is not part of the confocal light path; Epi Illuminator Turret should be moved to position 2 (empty)

Because the emission path for the Three Channel Confocal Detector is descanned through the Ultima scan head, it is not possible to use the Resonant Scanner for imaging with the Three Channel Confocal Detector module.

Connections

Three Channel Confocal Detector Connector (control module on the back of the scan head)	**Cable**	**Connects to**
DC Power Input	AC adapter with integrated DC output cable	Power adapter which itself draws AC power from the Power Distribution Unit
Lower left connector on the back of the scan head	Round 4-pin connector	Channel 1 filter wheel
Upper middle connector on the back of the scan head	Round 4-pin connector	Channel 2 filter wheel
Lower right connector on the back of the scan head	Round 4-pin connector	Channel 3 filter wheel
USB	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)

Troubleshooting

Symptom	**Possible Cause**	**Action**
Image is dimmer when larger	Aperture plate is slightly mis-	Use Prairie View software

pinholes are selected	aligned	controls to nudge the pinhole aperture plate to maximize intensity on the selected pinhole
Filter wheels, dichroics, and pinholes do not respond to selections made in Prairie View software	Communication link with the Three Channel Confocal Detector module has been lost	Ensure USB and Power cable are secure Restart Prairie View software Power cycle the confocal module (disconnect and reconnect power cable) and restart Prairie View software

The motorized filter and aperture components of the Three Channel Confocal Detector module can also be operated via a stand-alone control utility for purposes of troubleshooting when working with Bruker support personnel. This utility can **not** share control of the Three Channel Confocal Detector module with Prairie View software.

Dual PMT High Voltage Controller Box with Multialkali PMT

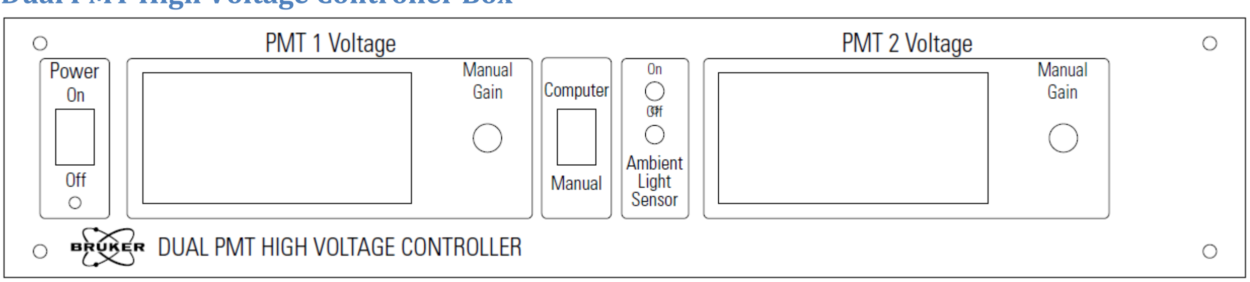
The Ultima system uses photomultiplier tubes (PMTs) to detect photons emitted from the sample. One type of PMT available for use on the Ultima is a Multialkali PMT (Hamamatsu R3896 SEL), which is both sensitive and robust.

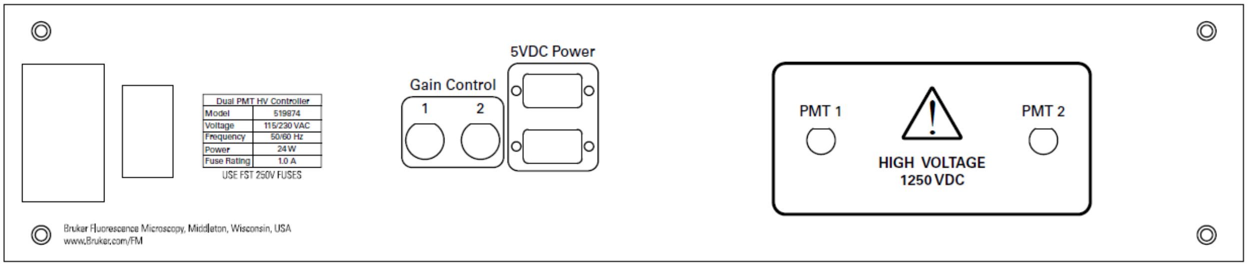
The number and position of PMTs varies by system, as described in the elsewhere in this manual.

The PMTs detect light across a wide spectrum of wavelengths. They are mounted to detector housings on the Ultima system, which include various band pass filters and dichroic mirrors to direct different emission bands to different detectors. Thus, each PMT detects a particular spectral range and feeds into a specific detection channel in the downstream electronics.

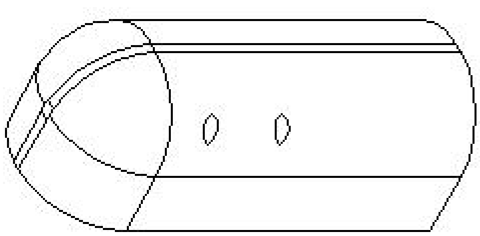
The Dual PMT High Voltage Controller Box is used to drive up to two Multialkali PMTs and to provide power for the Preamplifier.

Dual PMT High Voltage Controller Box





Multialkali PMT Housing



Operation

The primary interaction the operator has with the PMTs and Dual PMT High Voltage Controller Box will be through Prairie View software. However, there are a number of controls on the Dual PMT High Voltage Controller Box with which the operator can choose to interact directly.

When collecting images, it is recommended that the operator increase PMT voltage slowly while watching for the image to appear in the software. This gradual increase will help to prevent saturation of the detector, which can lead to a temporary increase in background noise.

There is a **Power** switch for the Dual PMT High Voltage Controller Box on the **back** panel of the box. It is recommended that the operator leave this back panel power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down.

The **Power** switch on the **front** panel of the Dual PMT High Voltage Controller Box determines whether voltage is sent from the box to the PMTs. This switch can be turned off during service or idle periods as an extra layer of protection for the PMTs. The switch must be turned on for regular system operation.

The red **LED displays** on the Dual PMT High Voltage Controller Box indicate at all times the PMT voltage in each channel. They are factory set to display the correct high voltage values for each PMT but have no effect on the voltage itself.

The LED display calibration buttons located directly below the LED displays should not be used. These controls are only used to calibrate the LED displays. They are factory set to display the correct high voltage values for each PMT but have no effect on the voltage itself. Do not change these settings, as re-calibration is difficult and time-consuming.

The **Computer/Manual** switch selects how the high voltage level is controlled for both channels. In the Computer position, the signals to the back panel Gain Control inputs control the high voltage output levels. In the Manual position, the operator can turn the **knobs** on the front panel to control the high voltage output levels instead of using software controls.

Protecting the PMTs from Ambient Light

A protection mechanism is present in the form of a light sensor on the front panel of the Dual PMT High Voltage Controller Box to detect ambient light and disable the high voltages when present. When the **Ambient Light Sensor** switch is in the On position, the photo sensor near the switch detects whether ambient light levels are high and automatically disables the high voltage to the PMTs until the ambient light level decreases. When the switch is moved to the Off position, the photo sensor is ignored and ambient light has no effect. This protection mechanism is intended to be used only to prevent accidental exposure of the PMTs to ambient light; it is not meant to be the primary mechanism for zeroing the voltage to the PMTs. Objects shielding the photo sensor will prevent this protection mechanism from functioning properly.

It is recommended that the operator move the Epi Illuminator Turret to a position other than 1 when the system is not in use. This will minimize the reflection of ambient light into the PMTs during idle periods.

PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light and/or prevent it from reaching the PMTs, as exposure to excess light will dramatically reduce the sensitivity of the system.

Connections

Dual PMT High Voltage Controller Box Connector	**Cable**	**Connects to**
AC Power input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
Gain Control 1	BNC	GPIO Box "PMT # HVCC"
Gain Control 2	BNC	GPIO Box "PMT # HVCC"
5V DC Power (2 connections)	DB-9 to 3-pin and/or DB-9 to round connector	Preamplifier power and/or GPIO Box "POWER"
PMT 1	SHV	PMT 1 voltage input (red)
PMT 2	SHV	PMT 2 voltage input (red)

Multialkali PMT Connector	**Cable**	**Connects to**

Voltage input (red)	SHV	Dual PMT High Voltage Controller Box "PMT #"
Signal output (black)	BNC	Preamplifier Channel # Input (front panel)

Troubleshooting

Symptom	**Possible Cause**	**Action**
Image doesn't respond to software selections for PMT voltage **and** Dual PMT High Voltage Controller Box red number displays read 0 or do not match software settings	Dual PMT High Voltage Controller Box high voltage output is switched off	Turn on front panel power switch (displays read 0 if power switch is off)
	Dual PMT High Voltage Controller Box control is set to Manual	Move front panel switch to "Computer" control
	Dual PMT High Voltage Controller Box light sensor detects too much ambient light	Decrease ambient light; if necessary, turn off the ambient light sensor (displays read 0 if ambient light is detected)
	Signal upstream of the Dual PMT High Voltage Controller Box has been disrupted (GPIO box or other component)	Diagnose by moving front panel switch to "Manual" control and looking for a response to the voltage knobs; contact Bruker for further diagnostics and resolution
Image doesn't respond to software selections for PMT voltage **and** Dual PMT High Voltage Controller Box red number displays match software settings	Sample is not in focus	Verify focus via eyepieces
	Light path is not transmitting emission to PMTs	Check position of the primary dichroic in the Epi Illuminator Turret (or corresponding dichroic for other detector configurations)
	Laser light is not reaching the objective lens	Diagnose by holding an IR detection card under the objective to look for laser light; check positions of optics described in the Selecting the Light Path section of this manual
Dual PMT High Voltage Controller Box red displays are not illuminated	Dual PMT High Voltage Controller Box is powered off	Ensure Dual PMT High Voltage Controller Box is powered on at the back panel

	Improper fuse is installed or fuse has been damaged	Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation

GaAsP Detector Control Box with GaAsP PMT

The Ultima system uses photomultiplier tubes (PMTs) to detect photons emitted from the sample. One type of PMT available for use on the Ultima is a GaAsP PMT (Hamamatsu H7422PA-40 SEL), which has very high sensitivity and is cooled to reduce background noise.

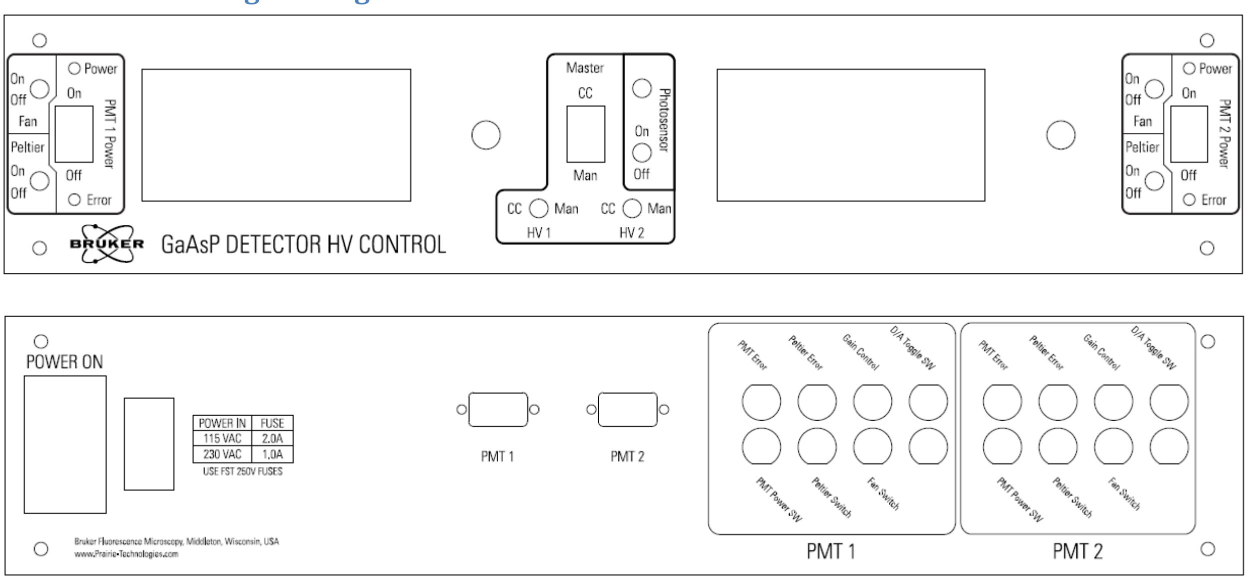
The number and position of PMTs varies by system, as described in the elsewhere in this manual.

The PMTs detect light across a wide spectrum of wavelengths. They are mounted to detector housings on the Ultima system, which include various band pass filters and dichroic mirrors to direct different emission bands to different detectors. Thus, each PMT detects a particular spectral range and feeds into a specific detection channel in the downstream electronics.

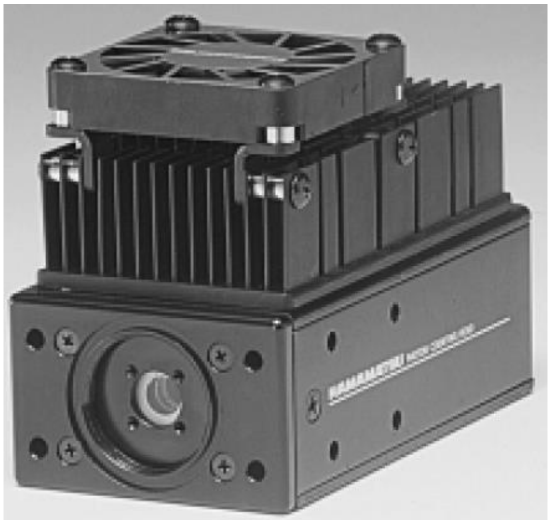
The GaAsP Detector High Voltage (HV) Control Box is used to drive up to two GaAsP PMTs.

The control box itself does not produce high voltage for the detector; the control voltage range is 0 to 0.9V, and is amplified on the detector itself. The control box also produces DC power voltage for the detectors and their cooling mechanisms.

GaAsP Detector High Voltage Control Box



GaAsP PMT



Operation

The primary interaction the operator has with the PMTs and GaAsP Detector HV Control Box will be through Prairie View software. However, there are a number of controls on the GaAsP Detector HV Control Box with which the operator can interact directly.

When collecting images, it is recommended that the operator increase PMT voltage slowly while watching for the image to appear in the software. This gradual increase will help to prevent saturation of the detector, which can cause protection circuitry to disable the detector.

There is a **Power** switch for the GaAsP Detector HV Control Box on the **back** panel of the box. It is recommended that the operator leave this back panel power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down.

Each of the two channels has a PMT **Power** switch on the **front** panel of the GaAsP Detector HV Control Box. Each switch determines whether voltage is sent from the box to the corresponding PMT. The switches can be turned off during service or idle periods as an extra layer of protection for the PMTs. The switches must be turned on for regular system operation.

The red **LED displays** on the Dual PMT High Voltage Controller Box indicate at all times the PMT voltage in each channel. They are factory set to display the correct high voltage values for each PMT but have no effect on the voltage itself.

The LED display calibration buttons located directly below the LED displays should not be used. These controls are only used to calibrate the LED displays. They are factory set to display the correct high voltage values for each PMT but have no effect on the voltage itself. Do not change these settings, as re-calibration is difficult and time-consuming.

Cooling

Additional switches on the front of the GaAsP Detector HV Control Box control the cooling devices incorporated into each GaAsP PMT.

- The **Peltier** switch controls the thermoelectric cooler inside the GaAsP PMT. The thermoelectric cooler keeps the inside of the PMT cool to reduce thermal noise. The Peltier switch should be turned on before images are collected with the GaAsP detector, as insufficient cooling will lead to noise in the images. Typically, the Peltier switch can remain on at all times, and the thermoelectric cooler can power cycle along with the rest of the system electronics from the Power Distribution Unit.
- The **Fan** switch controls a mechanical fan on the GaAsP PMT. The fan provides a second stage of cooling for the heat sink of the thermoelectric cooler, so that the Peltier can keep up with the cooling of the detector itself. Ideally, the Fan switch should be on at all times, and power cycle with the rest of the system electronics from the Power Distribution Unity. However, in some instances, the movement of the fan can introduce vibration artifacts into the images. If this occurs, the Fan switch can be turned off while imaging is in progress. The fan will automatically turn back on if the detector requires additional cooling.

Selecting Control Modes

Three **CC/Man** switches select the source of signal control for various subcomponents of the detector system. The three switches operate independently.

The **Master CC/Man** switch is used in specialized, advanced-level operation modes conjunction with the array of BNC connections on the back of the GaAsP Detector HV Control Box . These connections are not used on most systems. Contact Bruker support personnel for assistance with these controls.

The **HV1 CC/Man** and **HV2 CC/Man** switches select how the high voltage level is controlled for each PMT channel of the GaAsP Detector HV Control Box. In the CC position, the signal to the back panel Gain Control input for the channel controls the detector voltage level. In the Man position, the operator can turn the **knob** on the front panel to control the detector voltage level instead of using software controls.

Indicator Lights

Each channel (side) of the GaAsP Detector HV Control Box has both a Power and Error indicator light.

The **Power** light communicates both the on/off state of the voltage signal and the cooling state of the detector:

- Off: Front panel Power switch is off and the Peltier switch is off

- Blinking slowly (2 second intervals): Front panel Power switch is off and the Peltier is actively cooling; this is typical when the system is first powered on, as the Peltier cooler has just started operating
- Blinking quickly (0.5 second intervals): Front panel Power switch is off and Peltier has cooled the detector to its target temperature; this should happen within a few minutes of turning on the Ultima system electronics, provided the Peltier and Fan switches are also turned on
- On: Front panel Power switch is on

The **Error** light will be off during normal operation. It will be illuminated to indicate two separate fault states.

- On: Protection circuitry detects excess signal, as described below
- Blinking: A problem is detected in the cooling system

Protecting the PMTs

There are two layers of automatic protection in place to preserve the GaAsP PMTs.

Ambient Light

A protection mechanism is present in the form of a light sensor on the front panel of the GaAsP Detector HV Control Box to detect ambient light and disable the high voltages when present. When the Photosensor switch is in the On position, the photo sensor near the switch detects whether ambient light levels are high and automatically disables the high voltage to the PMTs until the ambient light level decreases. When the switch is moved to the Off position, the photo sensor is ignored and ambient light has no effect. This protection mechanism is intended to be used only to prevent accidental exposure of the PMTs to ambient light; it is not meant to be the primary mechanism for zeroing the voltage to the PMTs. Objects shielding the photo sensor will prevent this protection mechanism from functioning properly.

It is recommended that the operator move the Epi Illuminator Turret to a position other than 1 when the system is not in use. This will minimize the reflection of ambient light into the PMTs during idle periods.

Excessive Signal

The GaAsP PMT contains an additional layer of protection circuitry installed by the manufacturer. When the output signal of the PMT exceeds a certain threshold, the protection circuitry will turn off the detector voltage to prevent damage due to excessive light.

When this protection circuit detects too much signal from the sample (one or more excessively bright pixels in the image), the red LED display on the GaAsP Detector HV Control Box display a 1, the Error light will be illuminated, and an audible alarm will sound. The voltage signal to the detector will be cut off. The drive signal from Prairie View software through the GPIO Box and into the GaAsP Detector HV Control Box will remain intact, but no signal will be sent from the GaAsP Detector HV Control Box to the PMT itself.

If the protection alarm goes off, perform the following steps to resume imaging:

1. Stop the scan in Prairie View software. This will close the Hard Shutter and therefore remove the laser excitation that caused the sample to produce too much emission light. If there was no scan in progress, remove the light source that caused the alarm.
2. Lower the PMT voltage in Prairie View software.
3. Turn off the Power switch on the front of the GaAsP Detector HV Control Box for the channel in the alarm state. This will silence the alarm.
4. Turn on the Power switch on the front of the GaAsP Detector HV Control Box. This will re-activate the PMT and re-engage the voltage signaling.
5. Resume scanning with lower laser power and/or lower PMT voltage to avoid excessive signal.

Note that when the alarm goes off, voltage to the PMT is cut off, so the image in Prairie View software will go dark. No imaging can occur until the reset procedure described above is completed. This means that an alarm in the middle of an unattended time lapse imaging session will cause all subsequent images to be dark.

PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light and/or prevent it from reaching the PMTs, as exposure to excess light will dramatically reduce the sensitivity of the system.

Connections

GaAsP Detector HV Control Box Connector	Cable	Connects to
AC Power input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
PMT 1 (with serial number label to match detector)	DB-15 to 7-pin round with bundled 2-pin snap-in fan connection	GaAsP PMT 1 Power In and fan connection
PMT 2 (with serial number label to match detector)	DB-15 to 7-pin round with bundled 2-pin snap-in fan connection	GaAsP PMT 2 Power In and fan connection
PMT 1 Gain Control	BNC	GPIO Box “PMT # HVCC”
PMT 2 Gain Control	BNC	GPIO Box “PMT # HVCC”

There are several additional BNC connections on the back of the GaAsP Detector HV Control Box. These are used in advanced troubleshooting by Bruker support personnel and are not connected on most Ultima systems. Uncommon system configurations involving photon counting may use some of these connections; contact Bruker support personnel for more information on these systems.

GaAsP PMT Connector	Cable	Connects to
Power In (round) and	DB-15 to 7-pin round with bundled 2-pin snap-in fan	GaAsP Detector HV Control Box “PMT #”

Fan (snap-in)	connection	
Signal Out	BNC	Preamplifier Channel # Input (front panel)

Troubleshooting

Symptom	Possible Cause	Action
Image doesn't respond to software selections for PMT voltage **and** GaAsP Detector HV Control Box red number displays read 0 or do not match software settings	GaAsP Detector HV Control Box high voltage output is switched off (one or both channels) GaAsP Detector HV Control Box controls are set to Manual GaAsP Detector HV Control Box light sensor detects too much ambient light Signal upstream of the GaAsP Detector HV Control Box has been disrupted (GPIO box or other component)	Turn on front panel power switches (displays read 0 if power switches are off) Move front panel switches to CC (computer control) Decrease ambient light; if necessary, turn off the ambient light sensor (displays read 0 if ambient light is detected) Diagnose by moving front panel HV switches to "Man" control and looking for a response to the voltage knobs; contact Bruker for further diagnostics and resolution
Image doesn't respond to software selections for PMT voltage **and** Dual PMT High Voltage Controller Box red number displays match software settings	Sample is not in focus Light path is not transmitting emission to PMTs Laser light is not reaching the objective lens	Verify focus via eyepieces Check position of the primary dichroic in the Epi Illuminator Turret (or corresponding dichroic for other detector configurations) Diagnose by holding an IR detection card under the objective to look for laser light; check positions of optics described in the Selecting the Light Path section of this manual
Alarm is sounding and GaAsP Detector HV Control Box red displays are blinking	Protection circuitry has detected excess signal	Remove the source of the signal and follow the reset procedure described above
GaAsP Detector HV Control Box Error light is blinking	Cooling system fault is detected	Power cycle the GaAsP Detector HV Control Box to clear the error; if the problem

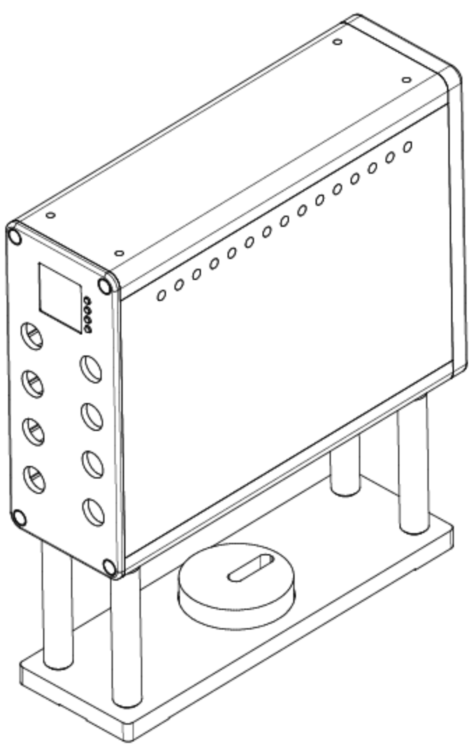
		persists, contact Bruker support personnel for assistance
GaAsP Detector HV Control Box red displays are not illuminated	GaAsP Detector HV Control Box is powered off Improper fuse is installed or fuse has been damaged	Ensure GaAsP Detector HV Control Box is powered on at the back panel Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation

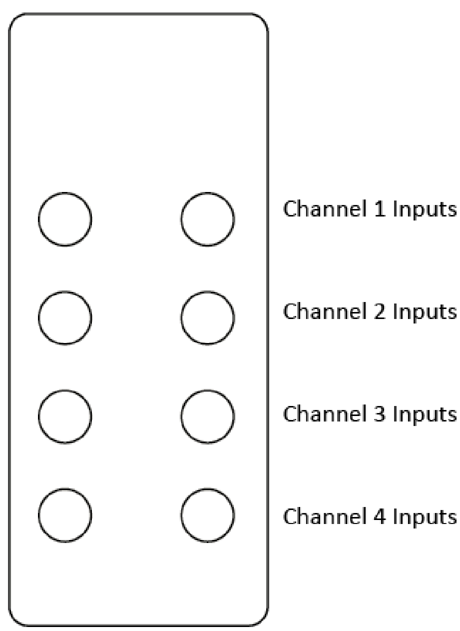
Preamplifier

The Preamplifier is one of the components involved in the process of turning photon output from the specimen into pixel intensity values to build an image. The Preamplifier uses internal circuitry, including transimpedance amplifiers, to accept current from each photomultiplier tube (PMT) and then amplify and convert those signals into voltages that can digitized by the downstream acquisition electronics.

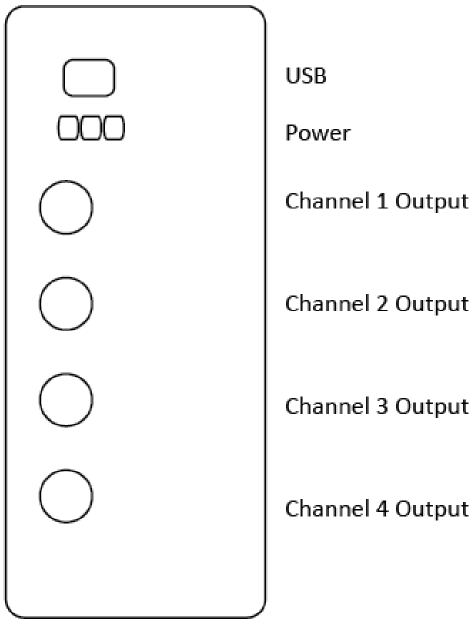
The Preamplifier is mounted to the table with a Universal Mounting Ring.

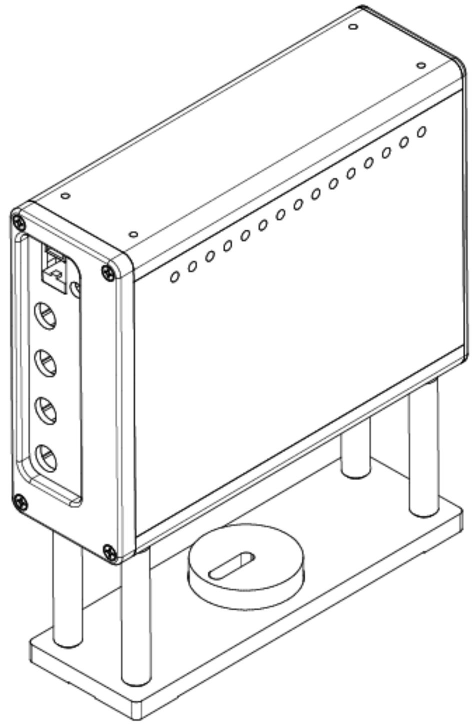
Front Panel





Back Panel





Accessories

The Preamplifier is compatible with two types of PMTs: the Multialkali R3896 SEL and the GaAsP H7422PA-40 SEL, both manufactured by Hamamatsu.

The internal components of the Preamplifier differ for Multialkali PMTs and GaAsP PMTs, because of the differences in output current from the two types of detectors. Therefore, a single Preamplifier channel cannot accept inputs from both a Multialkali PMT and a GaAsP PMT. Preamplifier channels configured for GaAsP PMTs are identified by white washers installed around the BNC connections on the front panel of the Preamplifier.

Operation

The Preamplifier can have components installed for 1 to 4 channels. Each channel is a horizontal row of BNC connections on the front of the Preamplifier and can accept signal from 1 or 2 inputs (PMTs). All inputs on a given channel will be electronically summed within the Preamplifier. The output signal for each channel is sent from the corresponding BNC on the back of the Preamplifier, out to downstream electronics for digitization of the signal. One example of a configuration in which two PMTs are connected to a single Preamplifier channel is a system having both reflected (upper) and transmitted (substage) detectors with identical optical filters. The red filtered upper and red filtered substage PMTs may be connected to the same Preamplifier channel.

The Preamplifier must be powered on before Prairie View software is launched. Prairie View software automatically initializes the Preamplifier for proper operation. Electronic filtering within the Preamplifier can be adjusted via controls within Prairie View software; refer to the software manual for further information.

Connections

Preamplifier Connector	Cable	Connects to
Channel # Input (front panel)	BNC integrated to Multialkali PMT or BNC	Multialkali signal output or GaAsP PMT Signal Out
USB	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)
Power	3-pin to DB-9	5VDC power from Dual PMT High Voltage Controller Box or Power Supply Box
Channel # Output (back panel)	BNC to MMCX (system with Resonant Galvanometer) or BNC RG68 thick cable (system without Resonant Galvanometer)	General Standards “Ch#” (Workstation Computer) or GPIO Box “PREAMP # IN”

Note: One open Channel # Input may be used to ground the Preamplifier to the surface of the table. This requires a BNC cable specially modified by Bruker personnel to connect the grounding sheath without the signal pin of the BNC cable.

Troubleshooting

Symptom	Possible Cause	Action
Image doesn’t respond to software selections for Gain, Offset, or Filter Bank of Preamplifier	Communication link with the Preamplifier has been lost	Use Prairie View software option to reset Preamplifier Ensure USB and Power cable are secure Ensure power source (Power Supply Box or Dual PMT High Voltage Controller Box) is turned on
Noise or high background in the image	Inadequate grounding of electrical components	Request a grounding cable from Bruker support personnel (specially modified for the Preamplifier)

The Preamplifier can also be operated via a stand-alone control utility for purposes of troubleshooting when working with Bruker support personnel. This utility can **not** share control of the Preamplifier with Prairie View software.

Multi Axis Motor Controller (MAMC) and Remote Interface Controller

The Multi Axis Motor Controller (MAMC) is the hub for motor movement commands for Bruker-manufactured X, Y, and Z devices. These devices include the Bruker Slim Stages, the moving platform of the Ultima IntraVital XY Translation Base, and the Z devices for Ultima IntraVital and most Ultima In Vitro systems. Third-party movement devices are not controlled by the MAMC.

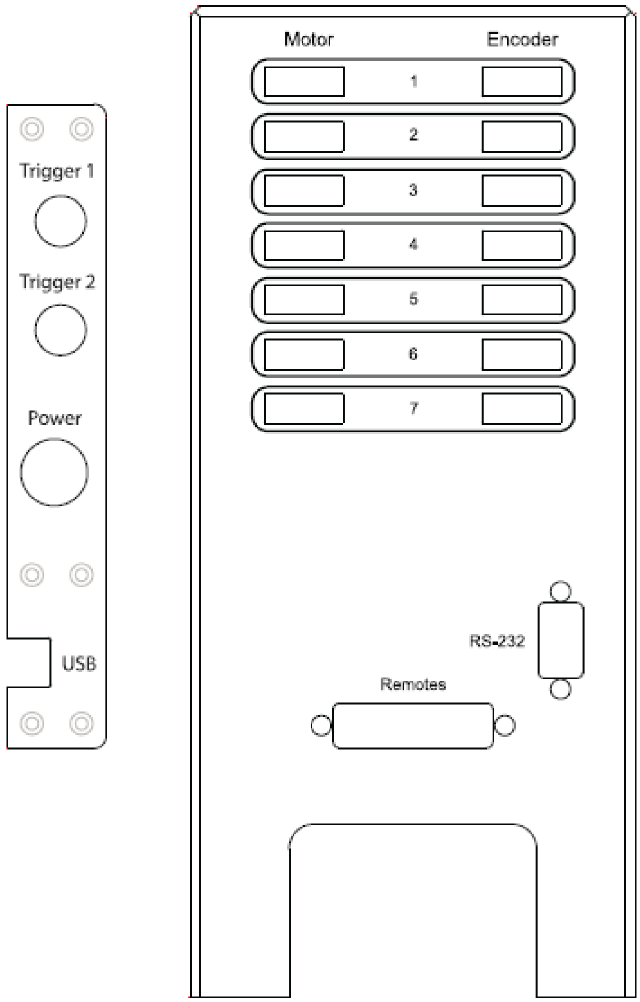
The MAMC can control up to seven motor axes.

Motor movement commands and encoder positions are communicated between Prairie View software and the movement devices through the MAMC.

The Remote Interface Controller is a knob-based unit for moving the X, Y, and/or Z motors routed through the MAMC.

The MAMC tower attaches to the table via a Universal Mounting Ring. The Remote Interface Controller can be placed on the workstation surface or the table, depending on the preference of the operator.

Multi Axis Motor Controller



MAMC Remote Interface Controller

Remote Interface Controller front view

Remote Interface Controller back view

Operation

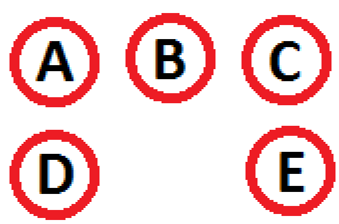
The operator interacts indirectly with the MAMC by controlling motor movements within Prairie View software or by using the Remote Interface Controller. The three knobs on this unit are:

- **X knob**: rotation of this knob drives the X axis motor of the selected device (Bruker Slim Stage or moving platform of the Ultima IntraVital with X-Y Translation Base)
- **Y knob**: rotation of this knob drives the Y axis motor of the selected device (Bruker Slim Stage or moving platform of the Ultima IntraVital with X-Y Translation Base)
- **Z knob**: rotation of this knob drives the Z axis motor of the Ultima IntraVital or Ultima In Vitro (BX51 frame)

If an Orbital Nosepiece is configured with the Ultima IntraVital system and is enabled in Prairie View software, the angle of the Orbital Nosepiece will be taken into account, and rotation of the X, Y or Z knob will move the combination of motors required to translate the objective in the specified plane. This mode of operation must be set up by Bruker personnel during installation and is dependent on proper system configuration and device firmware.

The absolute direction of motor movement (e.g. left versus right or up versus down) depends on system configuration.

There are 5 buttons on the Remote Interface Controller. Each button has two positions – up (unlit) and down (lit with a red light). For purposes of description only, these buttons are labeled A-E in the diagram below.



- **A** – Select the Primary or Secondary device for X-Y control. This is applicable to system with two X-Y translation devices, such as an Ultima IntraVital with XY Translation Base simultaneously configured with a Bruker Slim Stage. Assignment of each device as the primary versus secondary device is done in software by Bruker personnel. When the button is pressed to the down/lit state, the knobs will control the primary device. When the button is in the up/unlit position, the knobs will control the secondary device. When no secondary device is configured, the state of the button is ignored, and the knobs always control the primary device.
- **B** – Motors Continuously Engaged. When the button is pressed to the down/lit state, all motors are actively engaged even when no movement commands have been issued. This can allow operators to reduce some types of movement artifacts. When this feature is enabled, motor temperature will increase slightly; this will not affect performance. When the button is in the up/unlit state, motors are engaged only when a movement is taking place.
- **C** – Motor Backoff (Escape). This feature causes a motor to move a specified amount. Whenever the button is pressed to the down/lit position, the specified motor axis moves a distance equal to the configured backoff amount. When the button is pressed to the up/unlit position, the

motor axis returns to the absolute position it was in when the escape button was pressed. The motor axis (typically the Z axis) and amount of movement must be configured in MAMC firmware by Bruker personnel.

- **D** – Coarse versus Fine. This selection affects the absolute distance moved by the motor per revolution of the control knob. The distance moved per knob rotation in Fine mode is defined by the motor itself. The distance moved per knob rotation in Coarse mode is several times farther than in Fine mode; the ratio of Coarse to Fine control can be changed by Bruker personnel.
- **E** – Remote Interface Controller knobs enabled/disabled. When this button is down/lit, the Remote Interface Controller knobs are enabled, and rotating a knob moves the corresponding motor axis. When the button is up/unlit, the knobs are disabled, and rotating a knob has no effect on the corresponding motor axis. This can be useful for preventing unintended movements while relocating the Remote Interface Controller or when working in a dark room.

Connections

MAMC Connector	**Cable**	**Connects to**
Motor # [1] (front panel)	Mini Delta Ribbon	Motor index # (X, Y, or Z device)
Encoder # [1] (front panel)	Mini Delta Ribbon	Encoder index # (X, Y, or Z device)
RS-232 (front panel) [2]	DB-9	Workstation Computer (directly or via USB/Serial expander hub)
Remote (front panel)	DB-25	Remote interface Controller
Trigger 1 (side panel) [3]	BNC	If using Triggered Mode for faster Z Series acquisition in Prairie View software: Frame trigger signal from GPIO Box front panel "PCI-6713 FTO" or If using XZ/YZ scanning with a MAMC-controlled Z device in Prairie View software: Line trigger signal from GPIO Box front panel "PCI-611X CTR1"; Safe signal input range = 0-5V
Trigger 2 (side panel)	BNC	This connection is currently not used by the Ultima system; Safe signal input range = 0-5V
Power (side panel)	AC adapter with integrated DC output cable	Power adapter which itself draws AC power from the Power Distribution Unit
USB (side panel)[2]	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)

[1] Each motor axis has a Motor and an Encoder connection to the MAMC, made by Mini Delta Ribbon

cables. For a given motor, the Motor and Encoder connection must have the same index (i.e. be plugged into the same row on the MAMC). Axes are defined during system assembly and must match software configuration, typically in the following order:

1. Primary X axis
2. Primary Y axis
3. Z axis
4. Secondary X axis (if present)
5. Secondary Y axis (if present)

If at any time it is necessary to connect/disconnect cables from the Motor and/or Encoder connections on the MAMC, the power must first be turned off. Do not "hot plug" Motor/Encoder cables.

[2] While the connection to the Workstation Computer can be made through either a USB connection or a DB-9 RS-232(DB-9 cable) connection, Prairie View software must be configured to match the chosen method of communication. The RS-232 connection is preferred for firmware communication and updates during service/support sessions.

[3] The Trigger 1 and BNC connection is used in an alternate mode of operation, described below.

Alternate Triggered Mode of Operation (affects software control only)

Purpose

This mode allows faster Z-Series acquisitions with a MAMC by removing some of the software "handshaking" in the motor movements. Rather than waiting for a software command to move the motor between Z slices, the MAMC will move the motor every time a trigger pulse is received. A typical source of this pulse in an End of Frame trigger, so the motor will jump at the end of every frame.

Setup

1. In the Configuration Utility Z tab, enable the Fastest Acquisition checkbox
2. Connect a BNC cable from the GPIO Box 6713 FTO to the MAMC Trigger 1 input
3. In the Preferences menu, select an of End of Frame trigger

Operation

1. Define a Z-Series as usual
2. Check the Fastest Acquisition checkbox
3. Optionally, enable a Wait for Z device, and enter a number of additional milliseconds to wait between frames; this can be used to allow more time for the Z motor to finish its move before the next frame is acquired

Additional Notes

By using an End of Frame trigger to pulse the MAMC, the Z motor will start moving during the galvanometer retrace at the end of the frame. If the motor is still moving when the next frame starts, there may be some movement visible in the frame. This will be more noticeable with fast imaging (small image sizes, short dwell times). Adding a few tens of milliseconds to the Wait for Z device option on the Z-Series tab will pad some extra time into the frame retrace time to allow the motor to settle.

In this mode, there is no feedback to the software about where the motor should be during the acquisition; the feature causes the MAMC to move the motor a given distance every time a trigger is received.

The motor will still not have the speed and precision of a Z-Piezo; this mode is just faster than a regular Z-Series acquisition with the same motor.

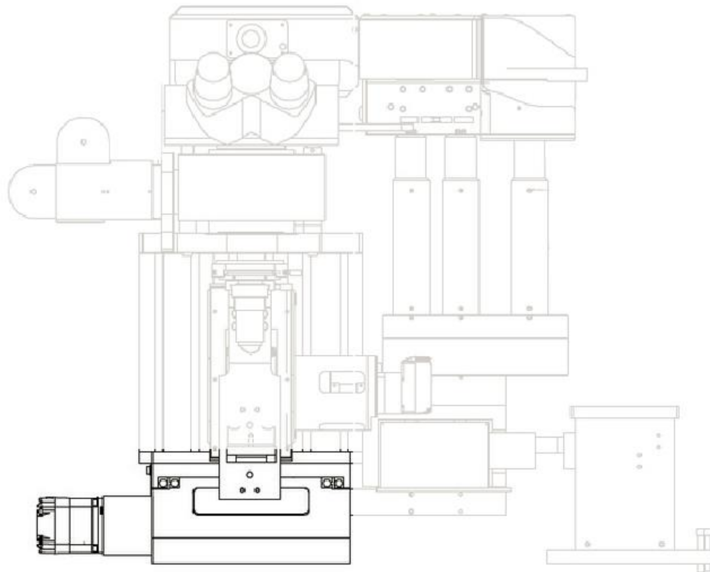
To do "regular" Z-Series acquisitions, simply un-check the Fastest Acquisition box on the Z-Series tab.

Troubleshooting

Symptom	Possible Cause	Action
Buttons do not light up when pressed down	No power is reaching the device	Make sure the power supply is connected to the side port of the MAMC and also to an AC power source
One motor fails to respond to knob rotation	Axis has reached a translation limit Command failure has disabled the axis	Move the knob in the opposite direction to stay within the allowed range of the axis Reset the MAMC via Prairie View software
All motors fail to respond to knob rotation	Remote Interface Controller knobs are disabled Command failure has disabled the axes Communication link with the MAMC has been lost	Ensure that Button E is in the down/lit position Reset the MAMC via Prairie View software Power cycle the MAMC by removing AC power from the device, waiting 30 seconds, and restoring AC power; software reset must be performed to re-establish communication with Prairie View after a power cycle

Ultima IntraVital XY Translation Base

The pillar system of the Ultima IntraVital can be mounted on an XY Translation Base. This allows the operator to move the entire microscope in X and Y while the sample remains fixed. The XY translation base has motorized X/Y control with a travel range of approximately 40mm in both the X and Y dimensions (20mm from center for each axis).



Operation

The X and Y axes of the Ultima IntraVital XY Translation Base can be moved by turning the X and Y knobs on the Remote Interface Controller of the Multi Axis Motor Controller (MAMC). Buttons on the Remote Interface Controller allow the operator to select Coarse versus Fine control and choose between devices on systems with multiple XY platforms; more information is available in the Multi Axis Motor Controller and Remote Interface Controller section of this manual.

If the XY Translation Base is configured as the primary XY device, then movement of the X and Y axes is also controlled by Prairie View software. This control is described in the Prairie View software manual.

Connections

XY Translation Base Connector	Cable	Connects to
X motor (left side of base, inner connector)	7-pin to Mini Delta Ribbon	MAMC Motor 1 (or Motor 4, if base is the secondary XY device)
X encoder (left side of base, outer connector)	10-pin to Mini Delta Ribbon	MAMC Encoder 1 (or Encoder 4, if base is the secondary XY device)
X limit switch (left side of base, 3-pin pigtail)	3-pin branch from encoder cable	
Y motor (back side of base, inner connector)	7-pin to Mini Delta Ribbon	MAMC Motor 2 (or Motor 5, if base is the secondary XY device)
Y encoder (back side of base, outer connector)	10-pin to Mini Delta Ribbon	MAMC Encoder 2 (or Encoder 5, if base is the secondary XY device)
X limit switch (left side of base, 3-pin pigtail)	3-pin branch from encoder cable	

Troubleshooting

Symptom	Possible Cause	Action
One motor fails to respond to movement commands (via knob rotation on the MAMC Remote Interface Controller and/or Prairie View software commands)	Axis has reached a translation limit Command failure has disabled the axis	Move the knob in the opposite direction to stay within the allowed range of the axis Reset the MAMC via Prairie View software
Both the X and Y motors fail to respond to knob rotation on the MAMC Remote Interface Controller	Remote Interface Controller knobs are disabled Remote Interface Controller is moving a different XY device (for systems with multiple XY devices) Command failure has disabled the axes Communication link with the MAMC has been lost	Ensure that MAMC Remote Interface Controller Button E is in the down/lit position Change the current state of the MAMC Remote Interface Controller Button A Reset the MAMC via Prairie View software Power cycle the MAMC by removing AC power from the device, waiting 30 seconds, and restoring AC power; software reset must be performed to re-establish communication with Prairie View software after a power cycle
Both the X and Y motors fail to respond to movement commands from Prairie View software	Command failure has disabled the axes Communication link with the MAMC has been lost A different XY device is configured in Prairie View software	Reset the MAMC via Prairie View software Power cycle the MAMC by removing AC power from the device, waiting 30 seconds, and restoring AC power; software reset must be performed to re-establish communication with Prairie View software after a power cycle Only the primary XY device can be controlled by Prairie View software; move the primary XY device or contact Bruker support personnel for assistance changing the primary

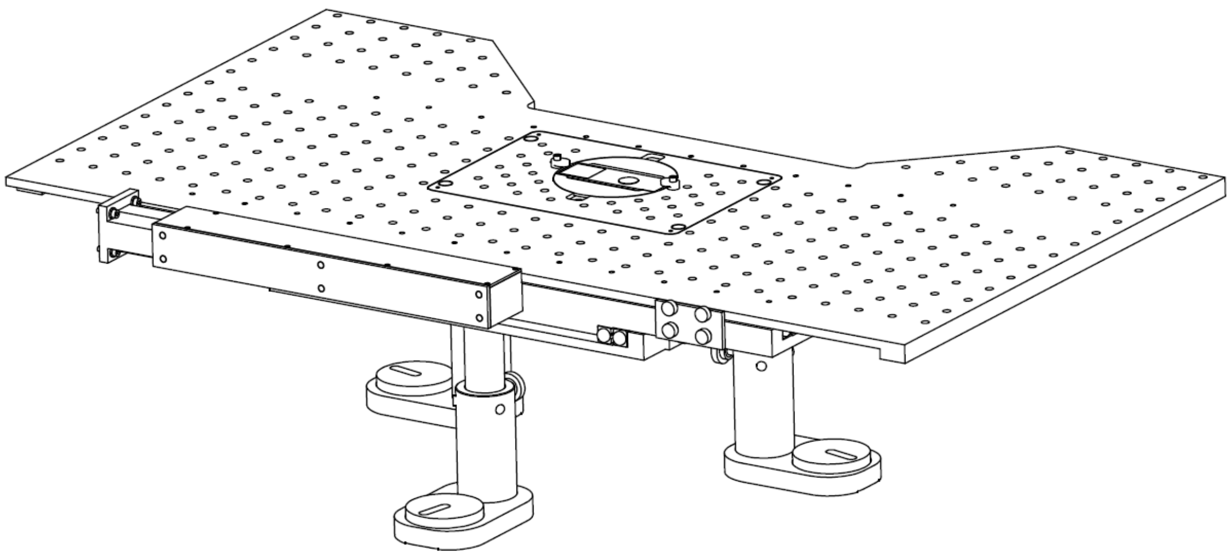
		and secondary designations

Slim Stage with XY Translation

The Slim Stage is a large-platform specimen stage which mounts to the table in front of the Ultima microscope body to position a sample under the objective lens. The large top plate provides room to mount additional equipment near the sample. The stage has motorized X/Y control with a travel range of approximately 150mm in the X dimension and 75mm in the Y dimension.

The stage is available in a variety of configurations, purchased based on the type(s) of sample(s).

- Legs can be an adjustable rack-and-pinion style or a fixed low-profile style
- Top plate shape is determined by the type of microscope body (Ultima IntraVital versus Ultima In Vitro)
- Various rectangular inserts are designed for different types of specimen holders (slides, plates, chambers, etc.)



Operation

Mounting a Sample onto the Stage
Various inserts are available for the central rectangular portion of the stage. Each is designed to accommodate a particular style of sample holder, such as a 50mm culture dish, a slide, or a perfusion chamber. Brackets or clips on the insert must be adjusted to hold the sample securely.

Before mounting a sample onto the stage, ensure that the Z mechanism has been adjusted upward to allow enough clearance between the objective and the sample.

Moving the X and Y Axes
The X and Y axes of the Slim Stage can be moved by turning the X and Y knobs on the Remote Interface Controller of the Multi Axis Motor Controller (MAMC). Buttons on the Remote Interface Controller allow the operator to select Coarse versus Fine control and choose between devices on systems with multiple XY platforms; more information is available in the Multi Axis Motor Controller and Remote Interface Controller section of this manual.

If the Slim Stage is configured as the primary XY device, then movement of the X and Y axes is also controlled by Prairie View software. This control is described in the Prairie View software manual.

Mounting Accessories on the Stage
A variety of accessories can be mounted to the surface of the specimen stage, by means of the grid of threaded holes for ¼-20 (Imperial) screws. Ensure that the screws do not extend below the top plate of the stage assembly. Screws that are too long will put pressure on the lower plates of the stage and prevent the stage from moving properly; this can damage the motor on the stage if tension is too great.

If the Bridge Attachment is present and includes the optional magnetic top surface, magnets can be used to mount components onto the Bridge Attachment.

Changing the Height of the Sample
When samples of different types (e.g. live animals and fixed slides) are used on the system, there may not be enough travel on the Z mechanism to focus the objective on all samples in one stage setting. Sample height should be adjusted such that the objective lens can be focused on the sample, with enough “escape” room to pull the objective up when mounting the sample on the stage. Depending on the stage configuration present, there are a variety of ways to adjust the height of the sample.

Spacers for the Stage Insert
One way to change the height of the sample is to add/remove spacers between the main platform of the Slim Stage and the central rectangular insert. These spacers are available in a variety of thicknesses, and can be stacked together for additional flexibility.

The stage insert is attached to the main platform of the Slim Stage by four screws, which can be removed with a Phillips-head (+) screwdriver. It may be necessary to use different screws when changing spacers. **It is essential that the screws are the correct length for the spacer(s) in use**. They must be long enough to reach into the main platform, but not so long that they extend below the top plate. Screws that are too long will put pressure on the lower plates of the stage and prevent the stage from moving properly.

Bridge Attachment

If the Slim Stage is configured with a Bridge Attachment, the central portion of the stage can be raised and lowered quickly at discrete intervals.

1. Loosen the two knobs on the front of the Bridge Attachment legs
2. Grip and squeeze the two triggers on the legs
3. Slid the Bridge Attachment up or down to the desired height
4. Release the two triggers on the legs
5. Tighten the two knobs on the front of the Bridge Attachment legs

Rack and Pinion Legs

If the Slim Stage is configured with rack-and-pinion legs, the entire stage can be raised and lowered over a defined range. Bruker personnel will consult with the operators during installation to set the height of the stage for the expected samples. Record the height of the stage before making any adjustments, as the height at installation is the "home" position to which the optical alignment has been optimized.

Do no adjust the legs of a Slim Stage on an Ultima In Vitro system before consulting Bruker support personnel. Because of the design of the microscope body, there is a very small range of appropriate heights for the In Vitro architecture.

If it becomes necessary for the operator to adjust the height of the stage on an Ultima IntraVital system, it is suggested that two people work together on the process, as the platform is heavy.

1. Clear the area of fragile items such as objectives and samples
2. While supporting the platform from underneath, loosen the two hex-head set screws on each of the three legs
3. While continuing to support the platform, loosen the small knob set screw on one leg
4. Turn the large knob on that leg to raise or lower the post
5. Tighten the small knob set screw on that leg
6. Repeat steps 3 through 6 for the other two legs
7. Put a bubble level on the stage platform and make additional adjustments as needed to level the platform
8. Tighten the hex-head set screws on each of the three legs
9. Loosen (but do **not** remove) the three hex-head screws holding the stage legs to the surface of the table, then tighten them again; this relieves any tension on the legs introduced during height adjustment

Connections

Slim Stage Connector	Cable	Connects to

Xm	Integrated Mini Delta Ribbon	MAMC Motor 1 (or Motor 4, if Slim Stage is the secondary XY device)
Xe	Integrated Mini Delta Ribbon	MAMC Encoder 1 (or Encoder 4, if Slim Stage is the secondary XY device)
Ym	Integrated Mini Delta Ribbon	MAMC Motor 2 (or Motor 5, if Slim Stage is the secondary XY device)
Ye	Integrated Mini Delta Ribbon	MAMC Encoder 2 (or Encoder 5, if Slim Stage is the secondary XY device)

Troubleshooting

Symptom	Possible Cause	Action
One motor fails to respond to movement commands (via knob rotation on the MAMC Remote Interface Controller and/or Prairie View software commands)	Axis has reached a translation limit Command failure has disabled the axis	Move the knob in the opposite direction to stay within the allowed range of the axis Reset the MAMC via Prairie View software
Both the X and Y motors fail to respond to knob rotation on the MAMC Remote Interface Controller	Remote Interface Controller knobs are disabled Remote Interface Controller is moving a different XY device (for systems with multiple XY devices) Command failure has disabled the axes Communication link with the MAMC has been lost	Ensure that MAMC Remote Interface Controller Button E is in the down/lit position Change the current state of the MAMC Remote Interface Controller Button A Reset the MAMC via Prairie View software Power cycle the MAMC by removing AC power from the device, waiting 30 seconds, and restoring AC power; software reset must be performed to re-establish communication with Prairie View software after a power cycle
Both the X and Y motors fail to respond to movement commands from Prairie View software	Command failure has disabled the axes Communication link with the MAMC has been lost	Reset the MAMC via Prairie View software Power cycle the MAMC by removing AC power from the

		device, waiting 30 seconds, and restoring AC power; software reset must be performed to re-establish communication with Prairie View software after a power cycle
	A different XY device is configured in Prairie View software	Only the primary XY device can be controlled by Prairie View software; move the primary XY device or contact Bruker support personnel for assistance changing the primary and secondary designations

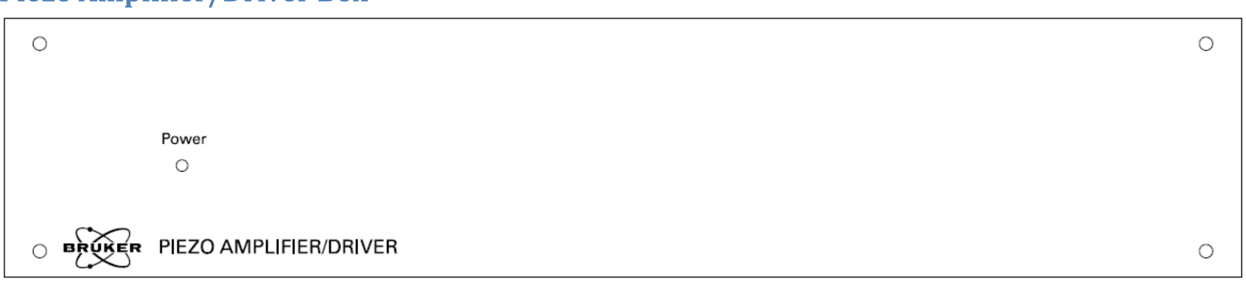
Z-Piezo and Piezo Amplifier/Driver Box

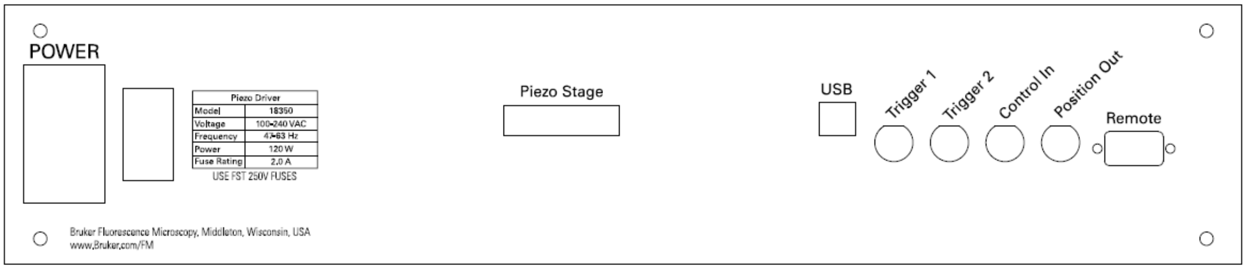
The Z-Piezo is a fast, precise positioning device for the objective lens. It allows the Ultima to perform high-speed volume imaging, especially when used in conjunction with a Resonant Scanner. The movement of the Z-Piezo is carried out by a proprietary capacitance feedback algorithm calibrated through Prairie View software. Operation is the same for all models of the Z-Piezo (250um, 400um, and 1000um travel ranges).

The Z-Piezo module is installed on the nosepiece between the Z arm and the objective lens. Control signals are routed through the Piezo Amplifier/Driver Box.

Z-Piezo

Piezo Amplifier/Driver Box





Operation

To avoid damage to the Z-Piezo, avoid excessive shearing forces on the component. Take care not to drop the Z-Piezo.

The operator does not interact directly with the Piezo Amplifier/Driver Box during normal system operation. Instead, all communication is carried out by Prairie View software. The Piezo Amplifier/Driver Box must be powered on and connected before Prairie View software is started. It is recommended that the operator leave the Piezo Amplifier/Driver Box power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down.

During Z-Series acquisition in Prairie View software, the Z-Piezo can be controlled in multiple modes. In Step mode, the Z-Piezo moves a discrete distance between consecutive images. In Calibrated mode, the Z-Piezo is driven in a continuous, smooth motion during the acquisition. Mode selections are made by the operator within Prairie View software.

Mounting an Objective Lens on the Piezo and Ultima

In all steps of this procedure, avoid over-tightening. Only gentle pressure is needed to make good connections. Over-tightening will damage the threads on each component, and can also damage the Z-Piezo mechanism.

1. Thread the objective to the appropriate spacer/adapter provided by Bruker personnel during installation; see additional information about spacers/adapters below
2. Thread the spacer/adapter with the objective into the Z-Piezo
3. Thread the Piezo Dovetail Adapter and its threading adapter into the Microscope Arm Dovetail
4. Slide the Z-Piezo onto the Piezo Dovetail Adapter
5. Tighten the thumbscrew on the Z-Piezo to hold the Z-Piezo onto the Piezo Dovetail Adapter
6. Slide the Microscope Arm Dovetail onto the Ultima Z arm, orienting the Z-Piezo cable to reduce tension and bending; when using the Z-Piezo in conjunction with an Orbital Nosepiece, it is recommended that the body of the piezo be oriented facing the operator, away from the pillar of the microscope body
7. Tighten the thumbscrew on the Ultima Z arm

Many objective lenses will require threaded spacers and/or adapters for proper mounting on the Z-Piezo. These spacers/adapters account for physical differences in the diameter and length of various objectives. During manufacturing, Bruker personnel determine the correct spacers/adapters for objective lenses purchased with the Ultima system. Contact Bruker support personnel for guidance on choosing spacers/adapters for any objective lenses added to the system after initial installation.

Positioning

Movement of the Z-Piezo is controlled through Prairie View software; the operator does not position the device directly.

Because the Z-Piezo is mounted onto the Z arm of the Ultima, gross positioning can be done by moving the Ultima Z motor, either via the MAMC's Remote Interface Controller, the manual knob on the side of the Ultima body, or through Prairie View software.

Suggested operation is as follows:

1. Install the Z-Piezo and desired objective lens onto the Ultima Z arm (see instructions above)
2. Using Prairie View software, move the Z-Piezo to the middle of its travel range
3. Adjust the Ultima Z arm to position the objective onto the sample
4. Close the Light Box door
5. While collecting images, navigate the Ultima Z arm to the area of interest in the sample
6. Define the desired stack to collecting using the Z-Piezo; be aware of the Z-Piezo travel limits and re-adjust the Ultima Z arm before defining the Z stack, if needed

Connections

Piezo Amplifier/Driver Box Connector	Cable	Connects to
AC Power input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
Piezo Stage	15-pin cable integrated into Z-Piezo mechanism	
USB	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)
Trigger 1	BNC	If a Resonant Scanner is present: T-connector to Resonant Galvo Control Box “End of Frame Out” and GPIO Box “PFI 8” Optionally, a switch box may be incorporated to change between End of Frame signals and the GPIO “PCI-611X CTR1” signal for XZ/YZ scans If a Resonant Scanner is **not** present: GPIO Box “PCI-6713 FTO” (front panel)

Trigger 2	BNC	If a Resonant Scanner is present: T-connector to Resonant Galvo Control Box "Trigger In" and GPIO Box "RES TRIG OUT" If a Resonant Scanner is **not** present: GPIO Box "PCI-611X PFI0" (front panel)
Control In	BNC	Used for driving the Z-Piezo with an external analog signal, 0-10V mapped to the travel range of the Z-Piezo; Not connected on most Ultima systems
Position Out	BNC	Used for diagnostics; Not connected to other Ultima components on most systems
Remote	DB-9	This connection is currently not used by the Ultima system

The Piezo Amplifier/Driver Box must be connected to the Resonant Galvo Control Box to synchronize high-speed volume imaging. These connections involve T-connectors to share signals between the Resonant Galvo Control Box, Piezo Amplifier/Driver Box, and GPIO Box. The T-connectors are typically placed on the Piezo Amplifier/Driver Box. The layout of these T-connectors and lengths of the cables are determined by Bruker personnel to minimize signal offsets and reflections. Do not change the cable layout on these connections unless instructed to do so by Bruker personnel.

Troubleshooting

Symptom	Possible Cause	Action
Z-Piezo does not move as commanded in Prairie View software	Travel range limit has been reached	Move the Z-Piezo in the opposite direction, and re-position the Ultima Z arm to bring the center of the Z-Piezo travel closer to the area of interest
Image appears to move laterally when changing the position of the Z-Piezo	Piezo Dovetail Adapter and/or Microscope Arm Dovetail are not seated properly	Loosen the thumbscrew(s), use gentle pressure to push the dovetail(s) back toward the Ultima body, and re-tighten the thumbscrew(s) while holding gentle pressure back on the dovetail(s)
Green light on the front panel is not illuminated	Piezo Amplifier/Driver Box is turned off	Turn on back panel switch

	Piezo Amplifier/Driver Box is not receiving AC input power	Ensure that the AC power input cable is securely connected to the back panel and that it is connected to an active power source
	Improper fuse is installed or fuse has been damaged	Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation

Workstation Computer, Magma Box, and Accessories

The Workstation Computer is assembled by Bruker personnel to meet the demands of the Ultima system. All drivers and software needed to operate the system are pre-installed. Multiple cards will be pre-installed as well, according to the specific system configuration.

The Magma Box is an expansion box in which PCI cards are installed. This box serves as an extension of the Workstation Computer.

Additional components associated with the Workstation Computer include the following:

- Monitor display
- Keyboard
- Mouse
- 4 port USB-to-Serial expander hub(s)
- USB expander hub

Each of these components has been specifically chosen and tested for compatibility with the Ultima system. The information provided by the manufacturers will be included with the Ultima system.

Operation

After turning on the Power Distribution Unit for the electronics workstation, confirm that the Magma Box power light is on, and turn on the Workstation Computer by pressing the round start/power button on the front panel.

The Magma Box must be powered on any time the Workstation Computer is running. The Magma Box must be turned on before the Workstation Computer, and not turned off until after the Workstation Computer is turned off. It is recommended that the operator leave the Magma Box power button on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down. The operator does not directly interact with the Magma Box during typical system operation.

It is recommended that the AC power switch on the back of the Workstation Computer be left on at all times, and that the monitor display power be left on as well. The main power switch on the Power Distribution Unit will control AC power for these components, and the monitor will automatically start up when the Workstation Computer is powered on. The remaining accessories of the Workstation Computer draw power from their USB connections, and will therefore turn on when the Workstation Computer is powered on.

When shutting down the Ultima system, power down the Workstation Computer via the Start menu. Wait for the Workstation Computer to shut down completely before turning off the Power Distribution Unit.

It is recommended that the operator shut down the Workstation Computer when it is not in use.

Connections

Workstation Computer	**Cable**	**Connects to**
Magma host card	PCI	Magma Box interface
Power	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
USB (multiple)	USB-A to USB-B	Preamplifier, GPIO Box, Resonant Scanner, Piezo Amplifier/Driver Box, MAMC, Helios Laser Launch, Keyboard, Mouse, USB expander hub, 4-port USB-to-Serial expander hub(s)
Graphics card	HDMI or DVI	Monitor Display

If there are more USB devices than available USB ports on the Workstation Computer, a USB expander hub may be installed to provide the additional USB ports.

The 4-port USB-to-Serial expander hub will be connected to up to 4 DB-9 cables, running to other components such as the MAMC, Resonant Galvo Control Box, ultrafast pulsed infrared laser power supply, etc. The port identification numbers associated with these components are configured in Prairie View software, so the cables must not be exchanged.

Magma Connector	**Cable**	**Connects to**
Magma Interface	PCI	Magma host card, inside Workstation Computer (pre-installed by Bruker personnel)
Power	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
National Instruments PCI cards	SH68-68-EP	GPIO Box PCI connections with corresponding card names

The slots inside the Magma Box are numbered 4 through 10, and typical card positions are:

4. Empty
5. Empty
6. PCI 6110
7. Option PCI 6714
8. PCI 6052E
9. Base PCI 6713
10. Empty

Additional connections may be present on the Workstation Computer, as additional cards are installed in the Workstation Computer according to system configuration for cameras, various signal output devices, etc. The most common additional card is a General Standards card used in systems with the Resonant Scanner. Connections for this card are listed in the table below.

General Standards Card (inside the Workstation Computer)	**Cable**	**Connects to**
Ch#	MMCX to BNC	Preamplifier Channel # Output (back panel)
Sync	MMCX	Resonant Galvo Control Box "Sync" (output)
Clock	MMCX	Resonant Galvo Control Box "Clock" (output)

Troubleshooting

Symptom	Possible Cause	Action
Workstation Computer does not boot up or Workstation Computer does not recognize National Instruments cards upon startup	Magma Box is not on	Confirm that the green light on the front of the Magma Box is illuminated Confirm that the power button on the front of the Magma Box is pressed in Confirm that the AC power jumper from the Power Distribution Unit is connected to the back of the Magma Box Shut down the Workstation Computer and power cycle the Power Distribution Unit
Monitor Display is blank while Workstation Computer is on	Monitor Display is looking for input from the wrong connection	Press the input selection button on the monitor to cycle through available connections

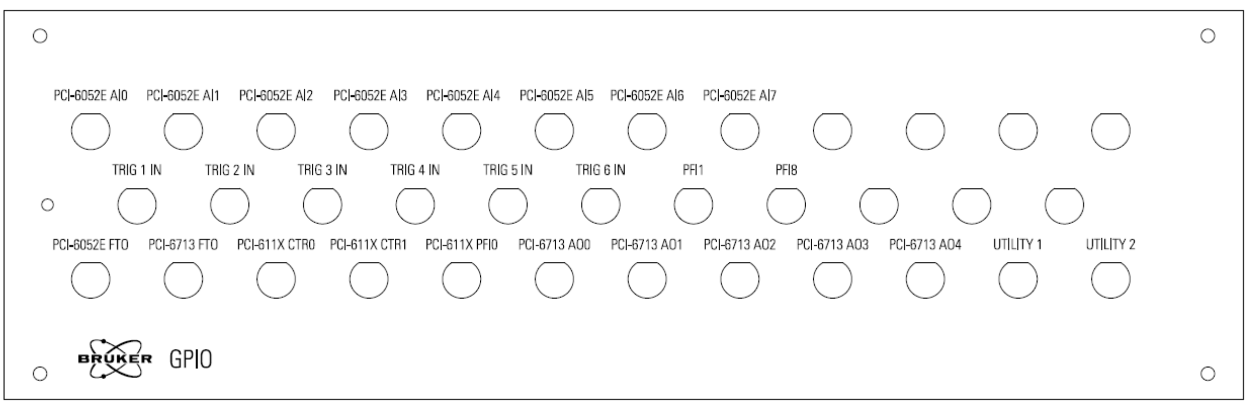
If it becomes necessary to add or remove cards from inside the Magma Box, the box must be powered off before removing any covers. This should only be done under the instruction of a Bruker service technician.

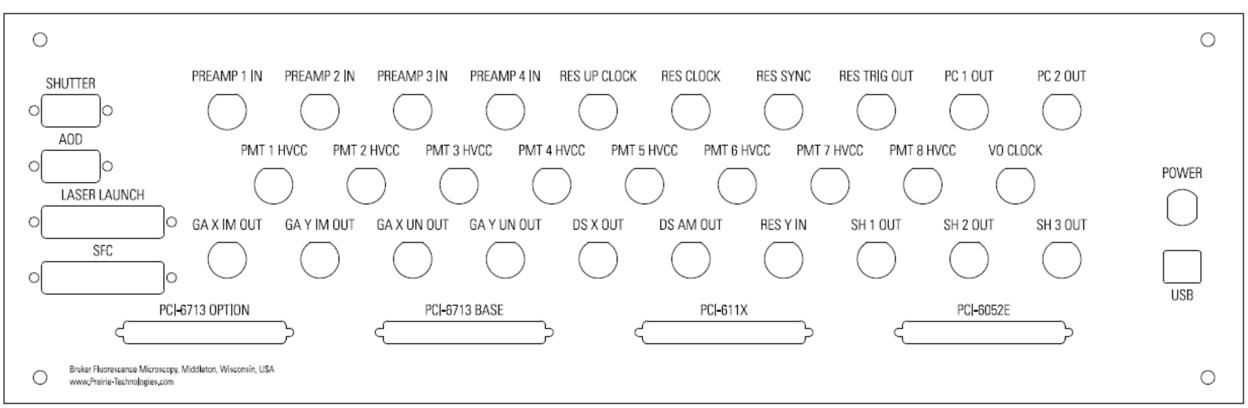
Additional information about troubleshooting issues with accessories can be found in materials from the manufacturers of those accessories.

General Purpose Input Output (GPIO) Box

The General Purpose Input Output (GPIO) Box is the main hub of connectivity for analog and digital signals in the Bruker Ultima system. There are many connections that are made on the front and back panels of the box, and even more that are internal to the box. All internal and external connections required to operate the Ultima system will be made by Bruker personnel during manufacturing and installation.

Additionally, several external connections are available to the operator. Several outputs can be monitored to collect feedback on the state of various components of the system, and can be used to synchronize third-party accessories with the Ultima system. Multiple inputs are available to allow the Ultima to perform operations upon receipt of input triggers from third-party devices or to record analog inputs from third-party sensors. These outputs and inputs are described below, and additional information is available in the Prairie View software manual.





Operation

All internal and external connections required to operate the Ultima system will be made by Bruker personnel during manufacturing and installation. The operator must not disconnect cables installed by Bruker personnel, as this may disrupt system functionality.

The GPIO Box must be powered on and connected before Prairie View software is started. As the GPIO box receives power from either a Power Supply Box or a Dual PMT High Voltage Controller Box, these boxes must be powered on before Prairie View software is started. It is recommended that the operator leave all boxes in the electronics workstation switched on at all times, and use the main switch on the Power Distribution Unit to turn off the boxes only when the entire electronics workstation is being powered down. The green light on the front of the GPIO Box will be illuminated when the box is receiving power.

All inputs and outputs designed to be used directly by the operator are BNC connections located on the front panel of the GPIO Box. Some of these connections may be in use already by other components of the Ultima; do not remove connections made by Bruker personnel.

The table below lists connections available for the operator. Additional information on the use of these connections can be found in the Prairie View software manual.

GPIO Connection	Row	Flow	Maximum Range	Purpose and Notes
PCI-6052E AI0 through PCI-6052E AI7	Top	Inputs	±10V	Signal recording by the Voltage Recording module of Prairie View software
TRIG 1 IN through TRIG 6 IN	Middle	Inputs	TTL (0-5V)	Input for externally triggered start of various operations in Prairie View software; send a TTL pulse from third-party hardware
PFI1	Middle	Input	TTL (0-5V)	Input for externally triggered start of various operations in Prairie View software; send a TTL pulse from third-party hardware • Used for non-imaging operations • Hardware trigger with shorter latency than TRIG 1 IN through TRIG 6 IN
PFI8	Middle	Input	TTL (0-5V)	Input for externally routed frame triggers used by Prairie View software
PCI-6052E FTO	Bottom	Output	TTL (0-5V)	Frame trigger signal; Depending on Prairie View software selections • If no frame trigger is selected, or only one frame trigger is selected, no signal is generated • If both Start of Frame and End of Frame triggers are selected, the Start of Frame signal is generated Output is not relevant in Resonant, Camera, or

				SFC acquisition modes
PCI-6713 FTO	Bottom	Output	TTL (0-5V)	Frame trigger signal; Depending on Prairie View software selections • If no frame trigger is selected, no signal is generated • If only one frame trigger is selected, that signal is generated (either Start of Frame or End of Frame) • If both Start of Frame and End of Frame triggers are selected, the End of Frame signal is generated Output is not relevant in Resonant, Camera, or SFC acquisition modes
PCI-611X CTR0	Bottom	Output	TTL (0-5V)	Sample clock signal • In Galvo mode, there is one pulse every .4us • In Resonant mode, the signal is meaningless, but may be produce anyway • In AOD mode, there is one pulse every 0.1us • In FLIM mode, this signal is the pixel clock rather than the sample clock (one pulse per pixel) • In PhLIM mode, this signal is the pixel repetition clock (one pulse per repetition of each pixel) Polarity of the signal depends on system configuration
PCI-611X CTR1	Bottom	Output	TTL (0-5V)	Blanking signal which determines when data is being acquired when imaging in standard Galvo mode • May also be used to turn on/off lasers in some control modes (not common on current systems) • Polarity of the signal depends on system configuration • May be used to trigger stepping of supported Z devices for XZ/YZ scanning
PCI-611X PFI0	Bottom	Output	TTL (0-5V)	Start of acquisition signal; marks the start of every imaging acquisition (Z-Series, T-Series, etc.) • Polarity of the signal depends on system configuration • Signal flips briefly when Prairie View

				software is preparing for a scan, then flips again at the start of the scan and remains constant for the duration of the acquisition
PCI-6713 AO0 through PCI-6713 AO4	Bottom	Output	±10V	Analog outputs programmed in the Voltage Output module of Prairie View software
Utility 1 and Utility 2	Bottom	Output	TTL (0-5V)	Digital output which changes state (0V or 5V) when the operator clicks a corresponding button in Prairie View software; available to drive the state of an external switch or other device; must be configured to be active in Prairie View software

Connections

Back Panel

Connections on the back panel are made during manufacturing/installation by Bruker personnel and are not intended to be changed by the operator. Actual connections depend on system configuration; not all connections will be made on all systems.

GPIO Box Connector	**Cable**	**Connects to**
SHUTTER	DB-9	External shutter switch box
AOD	DB-9	AOD plunger selection sensor
LASER LAUNCH	DB-25	Helios Laser Launch “Laser Control”
SFC	DB-25	SFC Scan Control Box “AOTF”
PCI-6713 OPTION	SH68-68-EP	Magma Box National Instruments card “6713 OPTION”
PCI-6713 BASE	SH68-68-EP	Magma Box National Instruments card “6713 BASE”
PCI-611X	SH68-68-EP	Magma Box National Instruments card “6110” OR “6115”
PCI-6052E	SH68-68-EP	Magma Box National Instruments card “6052E”
POWER	Round connector to DB-9	Power Supply Box “POWER SUPPLY” (typically #1) or Dual PMT High Voltage Controller Box “5VDC Power”
USB	USB-B to USB-A	Workstation Computer (directly or via USB expander hub)
PREAMP 1 IN through PREAMP 4 IN	BNC RG68 thick cable	Preamplifier “Channel # Outputs” (back panel); Connected only if no Resonant

		Scanner is present
RES UP CLOCK	BNC	Resonant Galvo Control Box "Update Clock"
RES CLOCK	BNC	Resonant Galvo Control Box "Clock"
RES SYNC	BNC	Resonant Galvo Control Box "Sync"
RES TRIG OUT	BNC	Resonant Galvo Control Box "Trigger In"
PC 1 OUT	BNC	Primary laser path Conoptics Box "J3"
PC 2 OUT	BNC	Secondary laser path Conoptics Box "J3"
PMT 1 HVCC through PMT 8 HVCC	BNC	Dual Channel Multi-alkali PMT High Voltage Box "Gain Control #" or GaAsP Detector HV Control Box "Gain Control"; Connections will vary based on the type(s) and number of detectors present on the Ultima system
VO CLOCK	BNC	NI 2110 Box "USER 1"
GA X IM OUT	BNC	Galvanometer Control Box – Imaging "X Input"
GA Y IM OUT	BNC	Galvanometer Control Box – Imaging "Y Input"
GA X UN OUT	BNC	Galvanometer Control Box – Uncaging "X Input"
GA Y UN OUT	BNC	Galvanometer Control Box – Uncaging "Y Input"
DS X OUT	BNC	Dual Scan Control Box "6115 DAC IN"
DS AM OUT	BNC	Dual Scan Control Box "VCO/PA AM IN"
RES Y IN	BNC	Resonant Galvo Control Box "Y Galvo"
SH 1 OUT through SH 3 OUT	BNC	Hard Shutter control or Helios Laser Launch "Shutter"

Front Panel

Connections on the front panel are intended to be accessed by the operator. These are described in the table above in the Operation section. In many system configurations, other components of the Ultima system must also use these connections. Do not remove connections made by Bruker personnel. Common Bruker-installed connections are listed below; others may also be present.

GPIO Connection	Cable	Connects to
PFI8	BNC	Resonant Galvo Control Box "End of Frame Out" or a trigger output signal from a camera
PCI-6052E FTO	BNC	Resonant Galvo Control Box "Start of Frame In"
PCI-6713 FTO	BNC	Resonant Galvo Control Box "End of Frame In" or Piezo Amplifier/Driver Box "Trigger 1"
PCI-611X CTR0	BNC	FLIM card breakout cable
PCI-611X CTR1	BNC	MAMC "Trigger 1" and/or Piezo "Trigger 1"; May be routed through a switch box
PCI-611X PFI0	BNC	Piezo Amplifier/Driver Box "Trigger 2"
PCI-6713 AO0 through PCI-6713 AO4	BNC to DB-25	Helios Laser Launch "Laser Control" breakout cable
Utility 1 and Utility 2	BNC	External switch box "Switch"

Troubleshooting

As the main hub of connectivity for signals in the Bruker Ultima system, the GPIO Box is a complex component. Troubleshooting may involve one or several connections or sub-components of this hub. Detailed diagnostic and troubleshooting support should be obtained from Bruker support personnel; only global issues are addressed here.

Symptom	Possible Cause	Action
Green light on the front panel is not illuminated or is only dimly illuminated	GPIO Box is not receiving input power	Ensure power source (Power Supply Box or Dual PMT High Voltage Controller Box) is turned on
Prairie View software does not load, or does not respond to desired changes to laser and PMT controls	Communication link with the GPIO Box has been lost	Ensure USB and Power cable are secure Ensure power source (Power Supply Box or Dual PMT High

		Voltage Controller Box) is turned on Manually reset the GPIO Box (steps described below)
	Connection to one of the NI cards in the Magma Box is not secure	Ensure that all SH68-68-EP cables between the Magma Box and GPIO Box are secure and are not bent at extreme angles

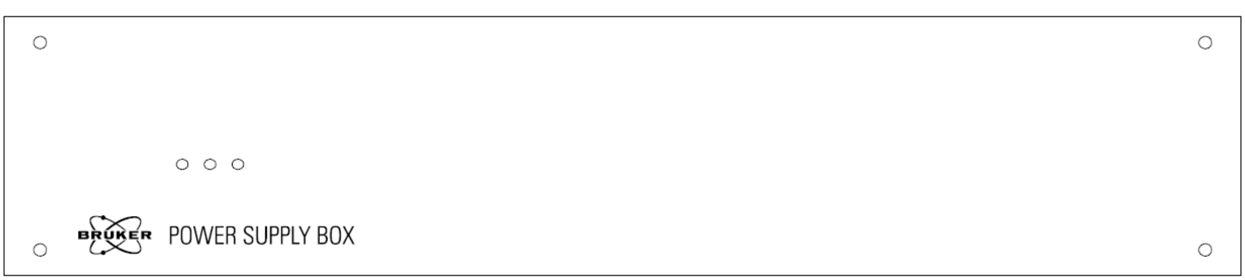
If the connection between Prairie View software and the GPIO Box is interrupted, the GPIO Box must be manually reset using the following sequence of steps:

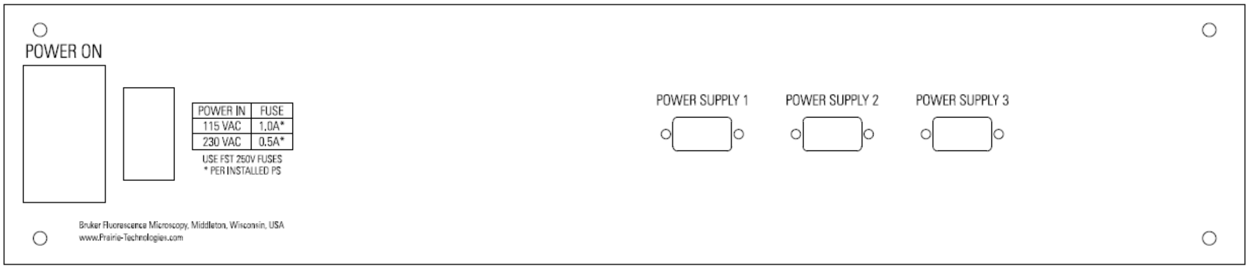
1. Exit Prairie View software
2. Unplug the USB cable from the GPIO Box
3. Turn off the power source for the GPIO Box (Power Supply Box or Dual PMT High Voltage Controller Box); ensure that the correct box has been turned off by checking that green light on the front of the GPIO Box goes dark
4. Wait 30 seconds
5. Restore power to the GPIO Box by turning on the power source (Power Supply Box or Dual PMT High Voltage Controller Box); ensure that the green light on the front of the GPIO Box is illuminated
6. Reattach the USB cable to the back of the GPIO Box, and ensure the other end is firmly seated in the Workstation Computer
7. Launch Prairie View software

The GPIO Box can also be operated via a stand-alone control utility for purposes of troubleshooting when working with Bruker support personnel. This utility can **not** share control of the GPIO Box with Prairie View software.

Power Supply Box

The Power Supply Box contains 1, 2, or 3 internal power supplies. These supplies provide 5V DC power for various components of the Bruker Ultima system, including the Preamplifier and the General Purpose Input Output (GPIO) Box.





Accessories

The Power Supply Box is intended to be used only with Bruker electronics, including the Preamplifier and the General Purpose Input Output (GPIO) Box.

Operation

When the Power Supply Box is turned on, one or more of the green lights on the front panel will be illuminated. The number of lights illuminated indicates the number of internal power supply units installed.

These components are built as needed for each Ultima system with one, two, or three separate internal power supplies. Each internal power supply unit corresponds to a connector on the back panel of the Power Supply Box. Every Power Supply Box will have at least one internal power supply unit, connected to “POWER SUPPLY 1” on the back panel. If a second internal supply unit is present, it will be connected to “POWER SUPPLY 2” on the back panel. If a third internal power supply is present, it will be connected “POWER SUPPLY 3” on the back panel.

It is recommended that the operator leave the Power Supply Box power switch on at all times, and use the main switch on the Power Distribution Unit to turn off this box only when the entire electronics workstation is being powered down. In this case, the operator will not directly interact with the Power Supply Box during normal operation of the Ultima system.

If it becomes necessary during troubleshooting to power cycle components connected to the Power Supply Box, the operator can use the power switch on the back of this box to turn off the Power Supply Box and any connected accessories.

Connections

Power Supply Box Connector	**Cable**	**Connects to**
AC Power Input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
POWER SUPPLY 1 POWER SUPPLY 2 POWER SUPPLY 3	DB-9 to round connector or DB-9 to flat connector	GPIO Box “POWER” or Preamplifier “POWER”

Troubleshooting

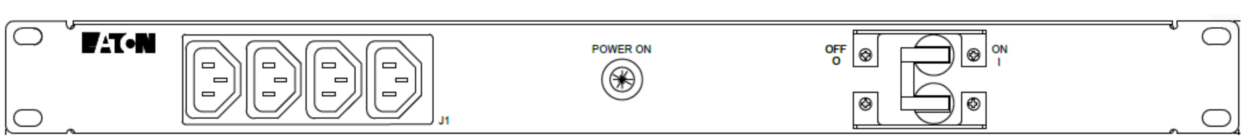
Symptom	**Possible Cause**	**Action**

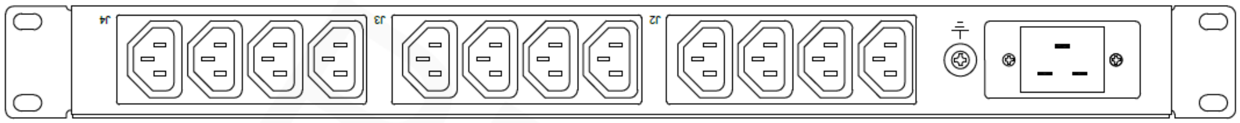
Accessory component does not power on	Power Supply Box is turned off Accessory is connected to an output with no internal supply	Turn on back panel switch and ensure that at least one green light is illuminated on the front panel Ensure that the number of lights illuminated on the front panel of the Power Supply Box matches the number of POWER SUPPLY output connectors in use on the back panel. For Power Supply Boxes populated with fewer than 3 internal units, the outputs must be used in numerical order, starting with POWER SUPPLY 1.
No green lights are illuminated on the front panel	Power Supply Box is turned off Power Supply Box is not receiving AC input power Improper fuse is installed or fuse has been damaged	Turn on back panel switch and ensure that at least one green light is illuminated on the front panel Ensure that the AC power input cable is securely connected to the back panel and that it is connected to an active power source Refer to fuse rating information on the back of the component; contact Bruker support personnel for guidance on fuse selection and installation

Power Distribution Unit

The Power Distribution Unit (PDU), manufactured by Eaton, provides a common source of AC power for most components of the Ultima system. A single PDU contains 16 IEC-C13 outlets; Ultima systems requiring more than 16 outlets will be equipped with an additional PDU.

The PDU(s) will typically be mounted in the electronics workstation behind the Workstation Computer monitor.





Operation

The switch on the Power Distribution Unit serves as the main power switch for the Ultima system. Power switches on the individual components of the Ultima should be left in the "on" position at all times, and power should be controlled by the PDU.

An initial step in turning on the Ultima system is to move the large white switch on the PDU into the "ON" position. The green "POWER ON" light will be illuminated when the PDU is on. Wait a few seconds after turning on the PDU for electronics to initialize, before starting up the Workstation Computer.

When shutting down the Ultima system, the very last step is to move the large white switch on the PDU to the "OFF" position. Be sure the Workstation Computer has been properly shut down **before** turning off the PDU.

Connections

Power Distribution Unit Connector	Cable	Connects to
IEC-C13 Outlets (16)	AC Power Jumper with IEC-C13 and IEC-C14 ends	Control Boxes and other electronics in the Ultima system
IEC-C20 Inlet	Main power cable with one IEC-C20 end and one end specific to the standard plug of the country/region where the Ultima is installed	Wall outlet (dedicated circuit preferred); Power may also run through and uninterruptable power supply and/or line conditioner
Ground	Braided ground strap	Table surface

Troubleshooting

Refer to the manufacturer's materials for specific information on troubleshooting issues related to the Power Distribution Unit.

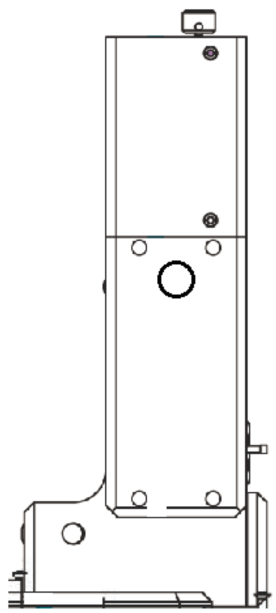
If the PDU power switch is in the "ON" position but the green "POWER ON" light is not illuminated, ensure that the main power cable is securely plugged in to the PDU and the power source.

Point Photoactivtion Module (Fiber Input)

The Point Photoactivation Module sits on top of the Side Car compartment of the Ultima Scan Head. It is the input module for fiber-coupled lasers and laser launches with FC connectors, including the Helios Laser Launch. Optics inside the module enable the operator to adjust the size and shape of the laser beam after it exits the fiber.

The module is typically installed on the Uncaging laser beam path for visible lasers used for photostimulation during imaging.

Alternatively, the module can be installed on the Imaging laser beam path for visible lasers used for imaging with the Three Channel Confocal Detector module. In this configuration, the module is rotated 180 degrees.



Operation

Beam Shape and Spot Size

The operator can adjust the size and shape of the laser spot at the sample by manually adjusting optics inside the Point Photoactivation Module.

The small metal plunger on the right side of the module moves a lens in and out of the light path. There is also an iris inside the module, which can be opened and closed by turning a black knob on the front of the module. Note that if the fiber input module is on the imaging path (instead of the uncaging path), the plunger is on the left and the knob is on the back.

The standard configuration is to have the plunger pulled out, so that the lens is out of the beam path, and the iris all the way open. The beam forms an hourglass shape as it exits the objective, with a diffraction-limited spot at the sample. Spot size at the focal plane is determined by the wavelength of the laser, the NA of the objective, and the diameter of the beam (if it does not fill the back aperture of the objective).

With the metal plunger pushed in, the lens is pushed into the beam path. This produces a pencil of light coming out of the objective. The spot size at the focal plane is larger than with an hourglass beam shape, while the cross-section of the beam at out-of-focus planes is smaller than would be found with an hourglass shape.

Spot size for the pencil beam can be adjusted by the operator. With the plunger pushed in, turn the knob on the front of the module to open/close the internal iris to adjust the spot size. Closing the iris reduces the spot size.

The laser spot size in the focal plate is affected by a number of other factors (e.g. internal design of the objective, where in the range of z-travel the objective is located, etc.), so combined with the various ways to define the "edge" of a Gaussian beam profile, reported beam size is an approximation. However, an approximation of the size for comparison is that with a 40x/0.8NA objective, the diffraction-limited spot (lens plunger out, iris open) would be less than 1um, while the pencil beam would vary from about 12um down to about 2um in diameter, as the iris is closed down. Spot sizes are smaller for a 60x/1.0NA objective, and bigger for a 20x/0.5NA objective.

Confocal Imaging Excitation Beam Shape

A diffraction-limit laser spot is needed for descanned confocal imaging. Therefore, when using a Point Photoactivation Module installed on the Imaging path in conjunction with a Three Channel Confocal Detector module, the plunger should be pulled out and the iris opened completely.

Alignment

Two mirrors inside the Point Photoactivation Module are used to align the laser beam to targets in the Ultima system. These mirrors are aligned by Bruker personnel during system installation, and are unlikely to need any adjustment by the operator. More information about alignment can be found elsewhere in this manual.

Focusing Knob

A knob on the top of the Point Photoactivation Module is used during manufacturing to adjust the focal position of the internal lens. This is set by Bruker personnel during manufacturing for proper shaping of the pencil beam. The focusing knob should not be adjusted by the operator.

Ultrafast Pulsed Infrared Laser

The primary light source used with the Ultima system is an ultrafast pulsed infrared laser. The Ti:Sapphire laser is modelocked to provide short pulses of photons, and can be tuned over a range of wavelengths. This laser provides the excitation power used for imaging and/or photostimulation of samples for the Ultima system. Multiple lasers can be configured on the same Ultima system. Additionally, a laser beam can be split and shared by two Ultima systems.

The ultrafast pulsed infrared laser consists of a laser head, which is mounted to the table, and a power supply, which typically sits below the table. Additional components include a chiller to cool the laser and an air recirculator to control the environment inside the laser cavity.

Original product inserts are provided with the laser, and additional information can be obtained from the manufacturer(s).

Operation

The information in this section applies to the use of an ultrafast pulsed infrared laser in the context of operating the Bruker Ultima system. Refer to the manufacturer's materials for specific information on the use and maintenance of the laser.

Follow institutional guidelines for laser safety procedures, including posting signs on doors to the work area.

The laser must be plugged into a dedicated power circuit and **not** the Power Distribution Unit used for the rest of the electronics in the Ultima system.

Ensure that air can circulate around the power supply and chiller components to prevent them from overheating.

Prairie View software can be configured to control many of the available lasers. This is not necessary for basic operation, but does enable the operator to read laser status and change the wavelength from within the software, rather than using the buttons on the laser power supply or stand-alone software provided by the laser manufacturer.

Starting the laser

1. Insert the key into the laser power supply and turn it 90 degrees to the "On" position
2. Allow time for the laser to warm up and reach modelocked status; status can be read from the display on the front of the laser power supply or in software
3. Open the laser cavity shutter, using the Shutter button on the laser power supply, or controls in Prairie View software, or the manufacturer's laser control software
4. Tune the laser to the desired wavelength, using either manual controls on the laser power supply, or the software controls

It is recommended that the operator allow at least 20 minutes between these steps and the start of imaging, as the transmission of laser power through the optical system will take some time to stabilize.

Shutting down the laser

When finished with the Ultima system for the day, close the cavity shutter, and use software controls and/or the key to put the laser in its standby mode.

Connections

Refer to the manufacturer's information for connections between the laser head, power supply, chiller, and air recirculator, as well as AC power connections. The laser must be plugged into a dedicated power circuit and **not** the Power Distribution Unit used for the rest of the electronics in the Ultima system.

If the laser is to be configured for control in Prairie View software, a DB-9 cable must connect the power supply unit to the Workstation Computer, via a Serial expander hub.

Additional connections may be necessary in some cases, such as systems configured for FLIM or PhLIM imaging. Contact Bruker support personnel for information about these configurations.

Troubleshooting

Refer to the manufacturer's materials for specific information on troubleshooting issues related to the laser.

Table Optics

Bruker provides the required table optics for the direction and modulation of an ultrafast pulsed infrared laser beam into the Ultima scan head. These components have been carefully installed and precisely aligned by Bruker personnel and should not be adjusted by the operator.

In some situations, such as addition, removal, or replacement of a laser or an optical component, it may be necessary to have the table optics re-aligned. This re-alignment should be performed only by a Bruker technician.

All mirrors are specially coated to withstand high-powered IR light. Still, infrared laser light will damage these mirrors over time. It is recommended that mirrors be inspected and/or replaced and table optics re-aligned every two years by a qualified Bruker technician.

After exiting the laser cavity, the beam typically passes through a zero-order half-wave plate. This, used in conjunction with a beam splitting cube, allows the laser light to be shared between two Ultima systems. For single Ultima installations, the second beam path is sent into a beam dump.

Each ultrafast pulsed infrared laser beam is sent through a power modulator, described later in this section.

Downstream of the modulator, a pick-off mirror is placed in the path of the attenuated beam. This mirror sends approximately 2-5% of the beam power to a power meter for monitoring purposes.

An interlocked electronic safety Hard Shutter is included in the beam path to prevent laser light from entering the scan head or light box when not imaging.

The last optical component on the table is a periscope assembly which directs the laser beam(s) up into the Ultima scan head. For Ultima In Vitro and Ultima IntraVital fixed post systems, the periscope assembly includes the Dual Beam Riser. For Ultima IntraVital systems on XY translation platforms, there is a horizontal periscope assembly including the Mini Tower, followed by a vertical periscope integrated into the XY translation portion of the system.

Power Modulator

The ultrafast pulsed infrared laser emits a particular amount of power, based on the laser itself and the wavelength to which the laser is tuned. The amount of laser power used for imaging/photostimulation is controlled via a power modulator in the form of a Pockels Cell and its driver control box from Conoptics, Inc. Detailed information about the Pockels Cell and driver are available from the manufacturer.

The Pockels Cell is an electro-optic device which uses a voltage signal to change the polarization of laser light exiting the internal crystal. The change in polarization causes a change in laser intensity downstream of the modulator.

Operation

The voltage signal controlling laser power is provided from Prairie View software, routed through the Conoptics driver box. The “zero” point and subsequent signal changes made in Prairie View software are relative to the bias setting on the driver box. The bias setting for each Pockels Cell is determined by Bruker personnel during installation. The bias is set such that the Prairie View software setting for zero laser power corresponds to the minimum laser power output achievable for the Pockels Cell. This “zero” output is not an absolute, but instead is a minimum laser power achievable, based on the total laser power and the extinction ratio of the Pockels Cell.

To check the bias setting of the Conoptics driver for the Pockels Cell:

1. Start up the Ultima system and tune the laser to the desired wavelength

2. Allow at least 20 minutes for the laser beam to enter the Pockels Cell and reach a thermally stable point; adjusting the bias setting before the Pockels Cell is thermally stable will result in subsequent drift
3. Align a bright sample in the field of view and configure the Ultima for multiphoton imaging
4. Set Pockels Cell (laser) power to 0 in Prairie View software
5. Use the Prairie View software Maintenance Dialog (Tools menu) to open the Hard Shutter
6. Begin a Live Scan of the sample
7. Set the Image Window channel color to Range Check
8. Slowly increase PMT voltage until a dim image of the sample can be discerned
9. Adjust the black knob on the front of the Conoptics box to minimize the brightness of the sample
10. Increase the PMT voltage and fine-tune the bias setting
11. Note the number displayed on the Conoptics box LED for the current laser wavelength

Bias settings may vary slightly based on the laser wavelength in use.

Some modes of laser power control in Prairie View software are dependent on a particular bias setting. If the laser power control in Prairie View software has been calibrated (either for Attenuation Mode or full Calibrated Mode of operation), do **not** adjust the bias setting.

Contact Bruker support personnel if frequent and/or large changes in bias settings are required, as this is an indication of problems with the system.


The power modulator is a delicate, consumable electro-optical device. The incorrect alignment of this device will cause it to fail. Do not adjust the power modulator or the optical components directing the laser beam into the power modulator.

Connections

Conoptics Driver Box Connector	**Cable**	**Connects to**
AC Power Input	AC Power Jumper with IEC-C13 and IEC-C14 ends	Power Distribution Unit
Signal Input J3	BNC	GPIO Box “PC 1 OUT” or “PC 2 OUT”
Amplifier Output J1	Specialized BNC provided by Conoptics	Pockels Cell J1m
Amplifier Output J2	Specialized BNC provided by Conoptics	Pockels Cell J1m

The switches on the back of the Conoptics Driver Box should be set as follows:

- Bias control to “Front Panel” (up)

- Signal Input to “1K” (down)
- Format to “Unipolar” (down)
- Unipolar to “rising edge” (left)

Troubleshooting

Refer to the manufacturer’s materials for specific information on troubleshooting issues related to the Pockels Cell and Conoptics Driver Box.

Beam Expander

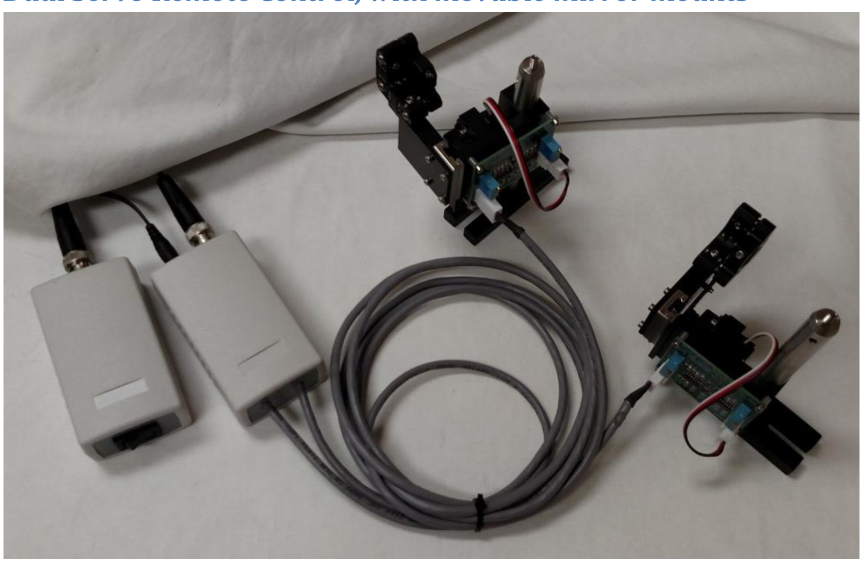
The Beam Expander is used to change the diameter and collimation of the ultrafast pulsed infrared laser beam. The optical components of the Ultima imaging system perform optimally when the laser beam is collimated prior to entering the scan head. Proper collimation also ensures that in systems with two or more ultrafast pulsed infrared lasers, all lasers focus to the same depth in the sample. Additionally, in many applications, it is desirable to increase the diameter of the raw laser beam to optimize image resolution when using objectives with high numerical aperture (NA).

In some cases, however, a narrower laser beam can provide more power and better performance at greater depths into the sample. For this reason, additional optics may be installed to allow the operator to choose between the raw laser beam and the expanded/collimated laser beam. This is accomplished by installing additional mirrors in the beam path. Some of these mirrors are on motorized mounts, allowing them to be moved in and out of the light path via a remote control. These additional mirrors change the path of the laser beam to one of two options. One path goes through the Beam Expander, while the other bypasses the Beam Expander. The operator chooses the path and moves the mirrors via a remote switch called the Dual Servo Remote Control.

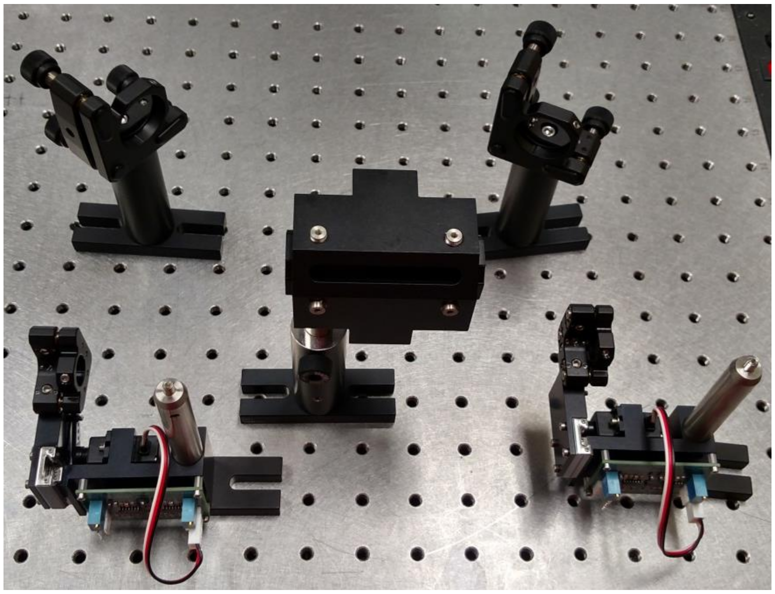
The Dual Servo Remote Control consists of two small grey boxes. One grey box contains a black rocker switch by which the operator selects the beam path. The other grey box uses the position of the rocker switch to drive the movable mirrors.

Beam Expander Mount

Dual Servo Remote Control, with movable mirror mounts



Optical components for Beam Expander and Bypass, with movable mirror mounts



Operation

The Beam Expander and associated mirrors will be installed and aligned by Bruker personnel. The operator does not need to adjust these components.

If a Dual Servo Remote Control is present, the operator can select the beam path (Raw versus Expanded) by moving the black rocker switch on the remote control unit. The Dual Servo Remote Control contains a relay which moves mirror mounts in and out of the laser beam path, determining whether the laser beam travels through or around the Beam Expander.

Connections

Dual Servo Remote Control	Cable	Connects to
Remote	BNC	Rocker switch for position selection
Power	AC adapter with integrated DC output cable	Power Distribution Unit
Integrated servo connector cables		3-pin connection on each movable mirror control board

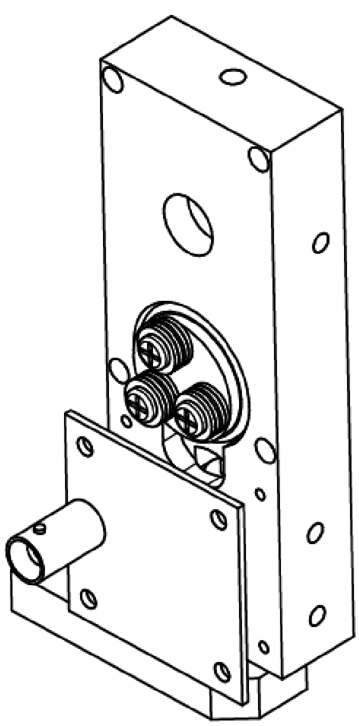
Troubleshooting

Symptom	Possible Cause	Action
All mirror mounts fail to move when rocker switch position is changed	No power is reaching the Dual Servo Remote Control	Make sure the power supply is connected to the side port of the Dual Servo Remote Control and also to an AC power source

	Connection between the rocker switch and the integrated servo connector cables has been lost	Make sure the BNC cable is connected between the two grey boxes of the Dual Servo Remote Control
One mirror mount fails to move when rocker switch position is changed	Integrated servo connector cable between the Dual Servo Remote Control and one mirror mount has become disconnected Control board or motor on one mirror mount has failed	Make sure the integrated cable is connected securely to the control board on the mirror mount Contact Bruker personnel for service or replacement
Movable mirror mount produce an audible noise or "twitches" in either position	Movable mirror mount has traveled past its usable range	Contact Bruker personnel for service or instructions on adjusting a potentiometer on the mirror mount control board

Hard Shutter

The Hard Shutter is a table-mounted shutter that blocks the laser beam from a table-mounted laser (typically an ultrafast pulsed infrared laser) when Prairie View software is not using that laser beam. The shutter is also interlocked to the Light Box to prevent the operator from being exposed to laser hazards. Each ultrafast pulsed infrared laser beam path will have its own shutter.



Operation

The Hard Shutter is automatically opened by Prairie View software when the laser associated with that shutter is used by the software. For example, a Hard Shutter on the imaging laser beam path will be opened automatically when that laser is used for a scan. The operator can also use Prairie view software controls to open the Hard Shutter when not scanning to perform operations such as troubleshooting or checking laser beam alignment.

The shutter will be closed automatically in any of the following instances:

- Prairie View software is not running
- System power is shut off
- Sensors detect that the Light Box is open (door or side/back panels)

The state of the shutter is controlled by a 5Vcontrol signal.

The 2-pin interlock sensor connection on the Hard Shutter plugs into the interlock sensor connector of the Light Box. When the Light Box is closed (or defeats are in place), the connection is made to allow the shutter to open when the GPIO Box signal is present. If the Light Box is open (door open or side/back panel removed), the shutter will not open, no matter the state of the GPIO Box signal. If the operator needs to access the inside of the Light Box while the Hard Shutter is open, interlock defeats must be placed on the Light Box to override the safety sensors. This should be performed only as instructed by Bruker personnel. Precautions must be taken to protect the operator from the laser beam, including the use of laser safety goggles.

Connections

Hard Shutter	Cable	Connects to
Control Signal (5V)	BNC	GPIO Box “SH # OUT”
Power	AC adapter with integrated DC output cable	Power adapter which itself draws AC power from the Power Distribution Unit
Interlock Sensor	2-pin Interlock	Light Box sensor [1]

[1] If an interlocked Light Box is not present, the Hard Shutter Interlock Sensor will be connected to an electronic defeat loop. In this case, the state of the Hard Shutter is controlled only by Prairie View software commands, with no safety override in place.

Troubleshooting

Symptom	Possible Cause	Action
Shutter will not open	Power is not connected	Ensure the AC adapter cable is securely connected to the Hard Shutter and that the AC adapter itself is plugged in to a power source
	BNC cable is not connected	Ensure the BNC cable is

		properly connected to the "SH # OUT" port on the back of the GPIO Box
	Light Box is open	Confirm that the side and back panels on the Light Box are in place, with the magnets lined up to the sensors on the top of the frame Press the Light Box door down and in such that the bumpers behind the door contact the door switches on the frame

Light Box (Sliding Door)

The Light Box is a large metal enclosure that surrounds the microscope body and the work area around the microscope. The Light Box performs three functions:

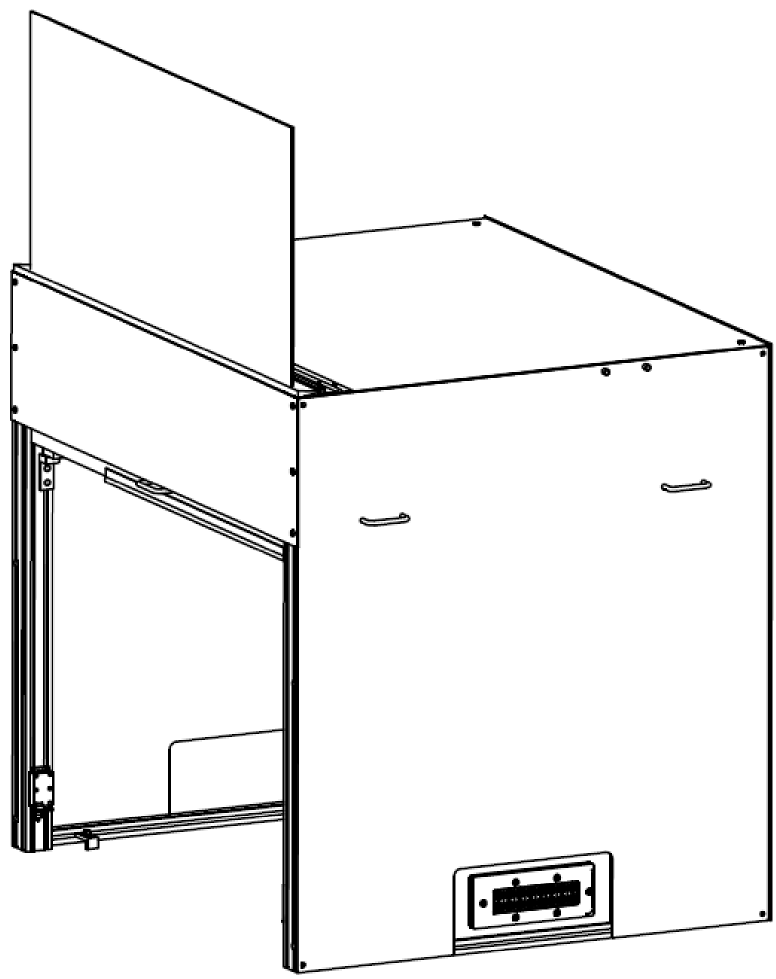
- The Light Box reduces the amount of ambient light contaminating the images produced by the Ultima, though it is still recommended that room lights be turned off for optimal system performance.
- The Light Box serves as a Faraday cage, reducing electrical noise that may otherwise contaminate both images and associated electrophysiological recordings.
- The Light Box contains safety interlocks designed to protect the operator from laser exposure by closing the table-mounted Hard Shutter when the Light Box door is opened or side/back panels are removed. When used in conjunction with the Hard Shutter and Beam Covers, these safety interlocks reduce the laser hazard of the ultrafast pulsed infrared laser from Class 4 to Class 1.

The Light Box consists of many subcomponents.

- The metal frame forms the edges of a box around the microscope body and work area. The frame is secured to the surface of the table by multiple clamps and screws, and is intended to remain in a fixed position after installation.
- The left and right side panels rest on the surface of the table and are held onto the frame by strong magnets. Cut-outs in the lower portions of these panels allow cables to pass from the work area to external control boxes.
- The back panel rests on the surface of the table and is held onto the frame by strong magnets. A large cut-out in the lower portion of this panel allows the tube(s) shielding the laser beam(s) to pass from the Beam Covers into the rest of the Ultima system.
- The top panel is affixed to the frame by screws.

- The door of the Light Box slides up to allow the operator to access the microscope body and surrounding work area.
- Two types of safety interlock sensors detect whether the Light Box is open or closed. Sensors along the top of the frame align with magnets in the side and back panels to detect whether a panel has been removed. Additional sensors on the front of the frame detect whether the door is open or closed. The sensors connect to the safety interlock of the Hard Shutter.
- Grounding wires connect each of the panels and the door to the surface of the table.

When closed, the standard Light Box is 43.25 inches wide, 36 inches deep, and 40 inches tall. A clearance of 60 inches above the surface of the table is needed to allow the door to open fully. Height may be increased by installing booster rails below the standard frame.



Ultima Laser Safe Light Box

Operation

Sliding Door

During typical operation of the Ultima system, the operator must raise the sliding door to access the work area around the microscope body. Pull up on the handle on the front of the Light Box door to slide the door up. The door is held up by three coiled springs inside the Light Box. Additionally, a catch assembly on each side of the door frame can be rotated out to provide support for the open door.

When the operator is finished positioning the sample and other items in the work area, the Light Box door must be closed prior to imaging. If the catch assemblies have been used, they must be rotated back into the Light Box before the door can be lowered. Pull down on the handle in the center of the door and push in slightly as the door reaches the fully closed position. Pushing in slightly ensures that the bumpers on the inside of the door make contact with the safety interlock sensors on the door frame.

Side and Back Panels

Occasionally, the operator may need additional access to the work area for positioning equipment or troubleshooting with Bruker support personnel. In such cases, a side panel of the Light Box can be removed.

1. Disconnect any grounding wires connecting the panel to other surfaces. These connections are white 2-pin fittings that can be pulled apart by hand.
2. Grab the panel by the two handles near the top.
3. Tip the top of the panel down and away from the frame to overcome the force of the magnets holding the panel to the frame, and set the panel aside.

When replacing the panel, be sure not to pinch any cables under the panel or between the panel and the frame. Line up the two black sensors on the top of the panel with the corresponding sensors in the top rail of the frame. Reconnect the grounding wires.

Warning: Use caution when removing side and back panels. Panels weight 15lbs (6.8kg) and weight is easily misjudged during removal.

Safety Interlocks

The safety interlock sensors in the Light Box connect to the Hard Shutter mounted in the laser beam path on the table. If a side or back panel is removed or not lined up properly, or if the door is open, the Hard Shutter will not open and no laser illumination will be available for imaging. These interlocks protect the operator from accidental exposure to the laser beam.

Emission Warning Light

The emission warning light located next to the sliding door will illuminate when laser emission is present. This light is intended to warn a Burker service technician laser emission maybe present when an interlock is defeated.

Connections

Light Box Connector	Cable	Connects to
Door safety sensor (front frame, one each lower left and right edge)	Traveler and sensor wires to 2-pin fitting	2-pin fitting for side panel traveler and sensor wires in the upper front corners of the frame
Left side panel safety sensor	2-pin fitting for left side panel traveler and sensor wires in the top of the frame on the left side	2-pin fittings for door safety sensor (upper front left corner of the frame) and back panel safety sensor (upper back left corner of the frame)
Right side panel safety sensor	2-pin fitting for right side panel traveler and sensor wires in the top of the frame on the right side	2-pin fitting for door safety sensor (upper front right corner of the frame) and Shutter safety sensor (upper back right corner of the frame)
Back panel safety sensor	2-pin fitting for back panel traveler and sensor wires in the top of the frame on the back	2-pin fitting for left side panel traveler and sensor wires (upper back left corner of the frame) and Shutter safety sensor (upper back right corner of the frame)
Shutter safety sensor connector	Three 2-pin fittings in a Y-shaped configuration, running down the back right leg of the Light Box frame	Top: 2-pin fittings for right side panel in the back and back panel on the right side Bottom: Hard Shutter or Shutter breakout cable
A series of **grounding wires**, typically green, connect the panels of the Light Box to the surface of the table. Each wire in this series has a ¼ inch ring terminal on one end, to attach to a handle or screw on a panel or to the surface of the table. The other end of each wire has a white 2-pin connector to allow easy separation when removing panels. Starting at the front these grounding wires connect the door, right side, back, and left side panels. The back panel also connects to the top panel and to the surface of the table.		

Troubleshooting

Symptom	Possible Cause	Action
Door will not close all the way	Door sensor interlock defeats are in place	Remove the magnetic interlock defeats from the door sensors on the left and right sides of the door frame
Side panels will not fit after removal	Left and right panels have been exchanged	Swap the left side and right side panels so that cable cut-outs accommodate cable positions and magnetic sensors line up with sensors in the top of the frame
Door is closed but does not contact	Door is slightly bowed out so	Open the door and close it

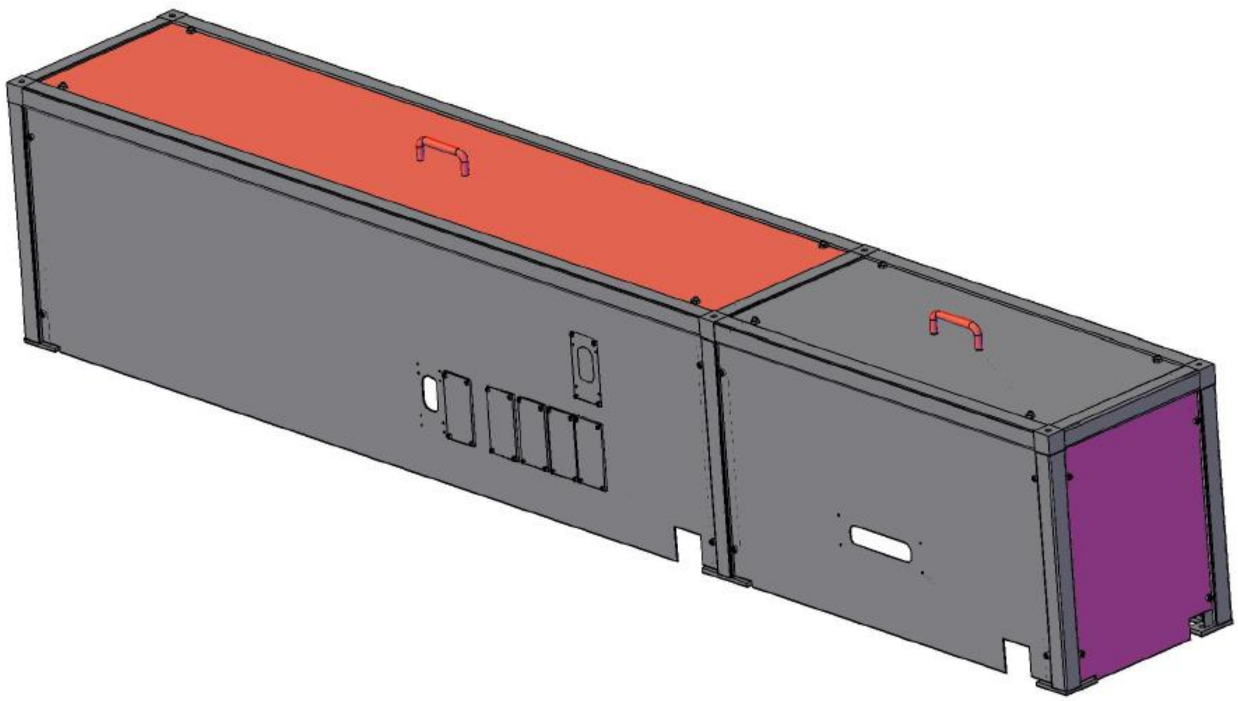
door safety interlock sensors	that bumpers sit in front of rather than on sensors	again while pushing in slightly on the handle

Beam Cover

The Beam Cover is a low metal enclosure surrounding the table-mounted optical components that direct and shape the beam of the ultrafast pulsed infrared laser into the microscope itself. The primary purpose of this enclosure is to protect the operator from scattered laser light. Additionally, it protects the optics from dust, air currents, and accidental contact by passersby.

An opening on the side panel of the beam cover allows light from the laser head to enter the beam cover. One or more smaller plates are removed by Bruker personnel during system installation to allow the laser beam(s), inside a beam-shielding tube, to pass from the Hard Shutter(s), through a cutout in the back panel of the Light Box, into the input port(s) on the Ultima.

The Beam Cover consists of a frame and panels. The metal frame is attached to the surface of the table by clamps and bolts. Side and top panels are held onto the frame with small bolts.



Operation

The operator will not interact directly with the Beam Covers during typical operation. The Beam Covers will be installed by Bruker personnel and are not intended to be moved by the operator.

Do not place heavy objects on the Beam Cover, as it is not designed to be load-bearing.

Connections and Troubleshooting
There are no electrical connections on the Beam Cover, and therefore no troubleshooting guidelines.

Xcite 120Q
The XCite 120Q, manufactured by Lumen Dynamics, is a light source which incorporates a mercury vapor arc lamp. This light source can be used on the Ultima system to view the sample in widefield epifluorecence mode when using the eyepieces or a CCD camera detector to navigate within the sample. The XCite is **not** used for illumination when scanning with the Ultima to collect confocal or multiphoton images.

The XCite 120Q consists of a lamp module which delivers light to the Ultima via a liquid light guide. The liquid light guide enters the Ultima through an adaptive coupler on the back of the system. The lamp module typically sits on the Ultima electronics workstation.

Original product inserts are provided with XCite, and additional information can be obtained from the manufacturer (Lumen Dynamics/Excelitas Technologies).

Lamp Module


Liquid Light Guide


Operation

The information in this section is specific to the user of the XCite 120Q in the context of operating the Bruker Ultima system. Refer to the manufacturer's materials for specific information on the use and maintenance of the XCite.

The power switch on the front of the XCite lamp module turns on the lamp itself.

The aperture wheel allows the operator to choose the amount of light transmitted through the fiber. The lowest position closes the aperture, and four additional positions allow increasing amounts of light to be transmitted.

A lamp will be installed in the XCite lamp module by Bruker personnel during system installation. Refer to the manufacturer's materials for information about replacing the lamp.

The XCite may be plugged into a dedicated power circuit rather than the Power Distribution Unit used for the rest of the electronics in the Ultima system, to allow power cycling of the other electronics without affecting the lamp inside the XCite.

Ensure that air can circulate around the XCite lamp module to prevent it from overheating.

Avoid pulling or bending the liquid light guide at extreme angles, as this can cause damage and decrease transmission of light.

Viewing the sample with illumination from the XCite 120Q

1. Turn on the power switch on the front of the XCite lamp module
2. Wait for the lamp to warm up, as indicated by the constant (not flashing) bulb icon on the display
3. Push in the plunger on the side of the Ultima trinocular head
4. Rotate the Epi Illuminator Turret to engage a filter cube
5. If using a camera for detection of the fluorescence, move the camera port mirror out of the light path by rotating the small black knob on the front/right of the scan head clockwise
6. Rotate the aperture wheel on the XCite lamp module to open the internal iris (up)
7. Move the shutter switch on the Epi Illuminator Turret to the Open position
8. Adjust the XCite aperture wheel to choose the required level of illumination

Returning the Ultima system to the laser scanning configuration

1. Move the shutter switch on the Epi Illuminator Turret to the Open position
2. Rotate the aperture wheel on the XCite lamp module to the closed (bottom) position
3. If it was previously moved out of the way, return the camera port mirror to the light path by rotating the small black knob on the front/right of the scan head counterclockwise
4. Rotate the Epi Illuminator Turret to engage the primary scanning dichroic (typically position 1)
5. Pull out the plunger on the side of the Ultima trinocular head

Though they may seem redundant, it is essential that the Epi Illuminator Shutter **and** the XCite aperture are closed before collecting laser-scanned images with the PMTs on the Ultima.

Turn off the XCite lamp module at the end of the day to avoid accumulating unnecessary hours on the lamp. However, do not turn off the lamp module if it will be used again within 5 hours, as re-ignition shortens the lifetime of the bulb.

Connections

XCite Connector	Cable	Connects to
Light Guide port	Liquid Light Guide	Coupling adapter on the back of the Ultima Epifluorescence Illuminator
Power	3-pin to DB-9	5V power from Dual PMT High Voltage Controller Box or Power Supply Box

Troubleshooting

Refer to the manufacturer's materials for specific information on troubleshooting issues related to the XCite.

If the XCite lamp module is functioning properly but no light reaches the objective lens, review the steps above for viewing the sample with illumination from the XCite.

Preventative Maintenance

Component	Description	Frequency
Workstation Computer	From the back of the Workstation Computer, slide the filter tray out from between the bottom feet; blow away any dust with compressed air; reinstall the filter tray, making sure it slides into the bottom of the Workstation Computer and doesn't just sit on the shelf below	Annual
Galvanometer Control Box	Clean fan filters on rear panel	Annual
Table Mirror Replacement	Bruker personnel inspect table mirrors and replace any showing signs of damage from infrared light	2 years
Table Mirror Alignment	Bruker personnel re-align alignment of table optics to optimize system performance	2 years
Magma Box	Remove the four tiny black Phillips-head screws on the front of the box	Annual

	and remove the front cover; remove the mesh filter and blow away any dust with compressed air; reinstall the filter and the cover, lining up the cutout for the power button, and reinstall the four black screws	
Pulsed Laser	Refer to lasers user manual	2mo to 2 years