MicroPoint®
Laser Illumination
and
Ablation
System

www.andor.com

September 2010 Release

Notices:

This system is covered by a limited warranty. A copy of the warranty is included with this manual. The operator is required to perform routine maintenance as described herein on a periodic basis to keep the warranty in effect. For routine maintenance procedures, refer to Chapter 5.

All information in this manual is subject to change without notice and does not represent a commitment on the part of Photonic Instruments, Inc. The system and various components in the system are the subject of the following US patent: 5,933,274.

If you have any questions regarding your MicroPoint Laser, please feel free to contact Andor directly at one of the addresses shown below, via your local representative***** or supplier.

* The latest contact details for your local representative can be found on our website via the following link: http://www.andor.com/contact_us/Default.aspx

Europe	USA
Andor Technology 7 Millennium Way Springvale Business Park Belfast BT12 7AL Northern Ireland Tel. +44 (0) 28 9023 7126 Fax. +44 (0) 28 9031 0792 **http://www.andor.com/contact_us/support_request**	**Andor Technology** 425 Sullivan Avenue Suite # 3 South Windsor CT 06074 USA Tel. (860) 290-9211 Fax. (860) 290-9566 **http://www.andor.com/contact_us/support_request**
Asia-Pacific	**China**
Andor Technology (Japan) 7F Ichibancho Central Building 22-1 Ichiban-Cho Chiyoda-Ku Tokyo 102-0082 Japan Tel. +81 3 3511 0659 Fax. +81 3 35110662 **http://www.andor.com/contact_us/support_request**	**Andor Technology** Room 1116 Zhejiang Building No. 26 An Zhen Xi Li Section 3 Chaoyang District Beijing 100029 China Tel. +86-10-5129-4977 Fax. +86-10-6445-5401 **http://www.andor.com/contact_us/support_request**

Safety/Operating Symbols

The following symbols appearing in the manual are defined as follows:

The WARNING statement used throughout the manual presents dangers that could result in personal injury.

The CAUTION statement used throughout the manual presents hazards on conditions that could cause damage to the instrument or the reporting of erroneous results.

The NOTE statement used throughout the manual highlights important information about the instrument and its use.

Failure to follow these statements may invalidate the warranty.

Warnings and Safety Precautions

The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the MicroPoint Laser Illumination and Ablation System:

1. **Please read and understand fully this manual before attempting to operate or maintain the MicroPoint Laser Illumination and Ablation System. Only qualified personnel should operate or maintain the system. All service must be performed by factory authorized personnel.**

2. **The System must be plugged into a Grounded Power Line.**

   Ensure that all parts of the system are properly grounded. It is strongly recommended that all parts of the system be connected to a common ground.

   Do **not** attempt to bypass the earth ground connection. A serious shock hazard could result.

3. **Wear Protective Eyewear.**

   The laser and or lamps used in the system can damage your eyes. Always wear appropriate protective eyewear when the system is powered up.

4. **Use the System in a Proper Manner.**

   Do not use the instrument and/or its accessories in a manner not specified by the MicroPoint Laser Illumination and Ablation System manual. If you do so, the protection provided by safety equipment may be impaired.

5. **Do not Attempt to Bypass any Safety Interlocks**

   The safety interlocks are provided to comply with safety requirements of various regulatory agencies and must be employed to protect the operator.

6. **On a daily basis or before every use test and verify that the MicroPoint Laser Illumination and Ablation System laser interlock circuit is working by confirming that the laser emission indicator on the source laser turns off when the microscope binocular eyepieces are in the open position or when the articulated transmitted light arm on inverted microscopes is tilted back from the functional vertical position before using the system.**

The Laser and lamp safety is of paramount importance in the operation and maintenance of the system. The laser illumination source is certified as manufactured and installed as a U.S. Center for Devices and Radiological Health (CDRH) Class IIIb laser product. For more information on laser safety, the website **http://www.fda.gov/cdrh** is a good resource. For information on lamp safety, consult the documentation provided with your lamp source.

7. The toxicological properties of the laser dye that is used are described in the MSDS provided with the laser dye. Refer to that document for specific information about potential hazards.
8. The laser dye is dissolved in an organic solvent. Avoid open flames and sparks in the laboratory.

The MicroPoint Laser Illumination and Ablation System complies with the European Low Voltage Directive 73/23/EEC and the EMC Directive 89/336/EEC and has been tested to the following standards per the CE marking requirements.

EN 61010-1: 2001 (2nd Edition)

IEC 61010-1: 2001 (2nd Edition)

EN 60825-1: 1994 +A1: 2002 +A2: 2001

IEC 60825-1: 1993 +A1: 1997 +A2: 2001

EN 61326-1: 2006

EN 61000-6-1: 2001

WARNING **Warning: The use of controls, adjustments or performance of procedures other than those specified herein may result in hazardous laser radiation exposure. Avoid viewing the laser directly, or as reflected by a mirror or other polished surface.**

WARNING **Warning: Before changing beam splitter plugs or dye cells, verify that the laser is turned off. Before accessing any removable covers or housings on the microscope, verify that the laser is turned off.**

WARNING **Warning: The use of optical viewing instruments other than those approved at the time of MicroPoint Laser Illumination and Ablation System installation with this product may increase eye hazard.**

Warranty

Photonic Instruments, Inc. (Seller) warrants that its products will be free from defects in materials and workmanship under normal use and service in general process conditions for the effective period set out below. This warranty and its remedies are in lieu of all other warranties expressed or implied, oral or written, either in fact or by operation of law, statutory or otherwise, including warranties of merchantability and fitness for a particular purpose, which Photonic Instruments, Inc. specifically disclaims. Photonic Instruments, Inc. shall have no liability for incidental or consequential damages of any kind arising out of the sale, installation, or use of its products.

Photonic Instruments, Inc. obligation under this warranty shall not arise until Buyer notifies Photonic Instruments, Inc. of the defect. Photonic Instruments, Inc. sole responsibility under this warranty is, at its option, to replace or repair any defective component part of the product.

Except in the case of an authorized distributor or seller, authorized in writing by Seller to extend this warranty to the distributor's customers, the warranty herein applies only to Buyer as the original purchaser from Seller and may not be assigned, sold, or otherwise transferred to a third party.

No warranty is made with respect to used, reconstructed, refurbished, or previously owned Products, which will be so marked on the sales order and will be sold "As Is".

BUYER'S SOLE AND EXCLUSIVE REMEDY UNDER THIS WARRANTY IS THAT THE SELLER EITHER AGREES TO REPAIR OR REPLACE, AT SELLER'S SOLE OPTION, ANY PART OR PARTS OF SUCH PRODUCTS THAT UNDER PROPER AND NORMAL CONDITIONS OF USE, PROVE(S) TO BE DEFECTIVE WITHIN THE APPLICABLE WARRANTY PERIOD. ALTERNATELY, SELLER MAY AT ANY TIME, IN ITS SOLE DISCRETION, ELECT TO DISCHARGE ITS WARRANTY OBLIGATION HEREUNDER BY ACCEPTING THE RETURN OF ANY DEFECTIVE PRODUCT PURSUANT TO THE TERMS SET FORTH HEREIN AND REFUNDING THE PURCHASE PRICE PAID BY BUYER.

Place of Service

Seller shall use its best efforts to perform all warranty services hereunder at the Buyer's facility, as soon as reasonably practicable after notification by the Buyer of a possible defect. However, the Seller reserves the right to require the Buyer return the Product to Seller's production facility, transportation charges prepaid, when necessary, to provide proper warranty service.

Effective Date

The effective date of this warranty shall begin on the date of shipment/date of invoice, whichever is later. Products are warranted to be free from defects in materials and workmanship for parts and labor for 1 year with the exceptions indicated below:

Limitations

Products are warranted to be free from defects in materials and workmanship for parts and labor for 1 year with the following exceptions:

- Fiber Optic Elements
    - Consumable items such as lasers and lamps are excluded from this warranty. The laser is covered by a separate warranty from its manufacturer.
    - Loss, damage, or defects resulting from transportation to the Buyer's facility, improper or inadequate maintenance by Buyer, software or interfaces supplied by the buyer, unauthorized modification or operation outside the environmental specifications for the instrument, use by unauthorized or untrained personnel or improper site maintenance or preparation.
    - The sole and exclusive warranty applicable to software and firmware products provided by Seller for use with a processor internal or external to the Product will be as follows: Seller warrants that such software and firmware will conform to Seller's program manuals or other publicly available documentation made available by Seller current at the time of shipment to Buyer when properly installed on that processor, provided however that Seller does not warrant the operation of the processor or software or firmware will be uninterrupted or error-free.
    - Products that have been altered or repaired by individuals other than Photonic Instruments, Inc. personnel or its duly authorized representatives, unless the alteration or repair has been performed by an authorized factory trained service technician in accordance with written procedures supplied by Photonic Instruments, Inc.
    - Products that have been subject to misuse, neglect, accident, or improper installation.

The warranty herein applies only to Products within the country of original delivery. Products transferred outside the country of original delivery, either by the Seller at the direction of the Buyer or by Buyer's actions subsequent to delivery, may be subject to additional charges prior to warranty repair or replacement of such Products based on the actual location of such Products and Seller's warranty and/or service surcharges for such location(s).

The warranty period for data processing equipment, including data storage devices, processors, printers, terminals, communication interfaces, tape drives, and/or all similar devices, is in all cases limited to ninety (90) days from the date of shipment to Buyer.

Repaired products are warranted for 90 days with the above exceptions.

Table of Contents

1 Introduction

1.1 Overview

The MicroPoint Laser Illumination and Ablation System is designed to allow for simultaneous and precise illumination and ablation of multiple regions of interest on a sample. It allows for concurrent viewing of laser illumination or laser ablation in wide field or fluorescence through the microscope eyepiece or with the imaging system in real time. The user can obtain extremely precise illumination of the area(s) of interest, thereby protecting the remainder of the target.

The system is used in a variety of applications in the life sciences and in semiconductor processing. Typical applications include:

Semiconductor Processing	**Life Sciences**
Laser Ablation	Laser Ablation
Circuit Isolation	FRAP
Marking IC's	FRET
Marking Hard Discs & Media	Photoactivation
Flip Chip Navigation	Photobleaching
LCD Repair	Photoswitching
Micromachining	Photoconverting
Navigation	Cell Regeneration/Degeneration
Removal of Passivation	Release of Caged Compounds
Removal of Photoresist	Drug Delivery
Semiconductor Failure Analysis	Thrombosis
Probe Stations	Free Radical Release
Hard Disk & Media Processing	CALI

1.2 Overall Design of the System

The MicroPoint Laser Illumination and Ablation System (Figure 1-1) consists of a tunable fiber-optic pumped laser, coupling and beam steering optics, a microscope coupling and a selection of beam splitters and interference filters. The angular and spatial alignment of the illumination and the focus in the Z-direction can be controlled. The wavelength of the light used for illumination is selected by the user via a dye cell and an attenuator is provided to adjust energy.

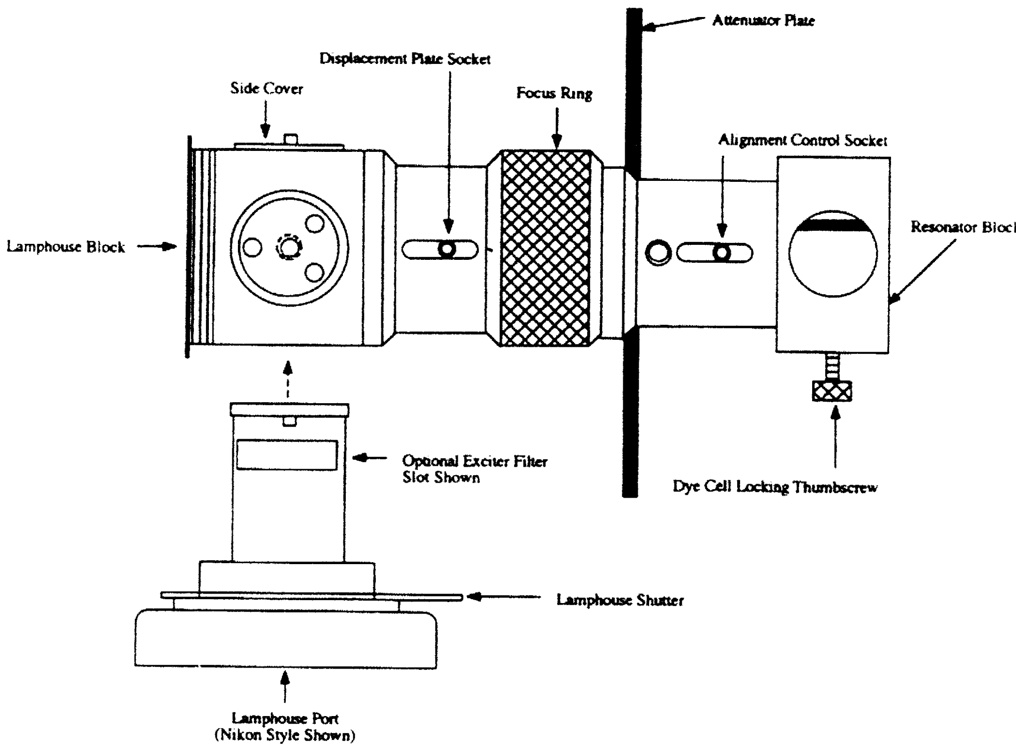


Figure 1-1: MicroPoint Laser Illumination and Ablation System (Manually Controlled System)

While each system is specifically configured to the needs of the user, there are three broad configurations of the system:

- A *manually* controlled system in which the angular and spatial alignment of the illumination is controlled via a manual joystick. The focus in the Z-direction is controlled by a knurled focus ring and the attenuator is adjusted manually.
- A *manually* controlled system in which the angular and spatial alignment of the illumination is controlled via a manual joystick. The focus in the Z-direction is controlled by a knurled focus ring and the attenuator is adjusted by an external controller.
- A *computer* controlled system in which the angular alignment of the pulsed dye laser illumination is controlled via a 2-axis joystick and the spatial alignment is computer controlled via galvanometer beam steering optics. The focus in the Z-direction is controlled by a knurled focus ring and the attenuator is adjusted by computer via an external controller. The computer controlled system is available with a USB or a Bluetooth-PDA interface.

1.3 Environmental, Space, Electrical and Computer Requirements

The environmental requirements (e.g. temperature, humidity range) for the MicroPoint Laser Illumination and Ablation System are similar to the environmental requirements for a research grade microscope.

The MicroPoint Laser Illumination and Ablation System is designed for indoor use only per IEC 60529, at any altitude suitable for human habitation, at a temperature between 10° F and 110° F, Humidity overvoltage Category 2, Pollution Degree 2, Protection Class 1.

The manual MicroPoint Laser Illumination and Ablation System requires a horizontal distance of approximately 8.5” (22 cm) from the rear of the microscope.

The MicroPoint with Galvanometer Module requires a horizontal distance of approximately 10” (25cm) from the rear of the microscope.

A fiber optic cable (1 or 2 m long) is used to deliver the laser output to the system. This cable should be configured so that all bends have a minimum radius of 4” (10.2 cm) to avoid damaging the cable.

The laser power supply that is supplied with the system is dependent on the requirements of the end user and the precise space and electrical requirements are provided in the documentation provided with the laser. A typical laser employs +24V DC 3A peak@20 Hz and has the dimensions of 11” L x 3.75” W x 3.75”H (27.9 x 9.5 x9.5 cm). Typical power requirement for the laser power supply is 120V/2A (240V/1A).

The attenuator controller has the dimensions of 11” L x 3.75” W x 3.75”H (27.9 x 9.5 x9.5 cm) and a power requirement of 120V/1A (240/1A).

The computer controlled system requires a personal computer with a PCI slot and a USB port. The Bluetooth controlled system is controlled by a Bluetooth-enabled PDA device provided by Photonic Instruments, Inc.

1.4 Installation of the System

Chapter 3 describes installation of the system. The system is normally installed by a representative of Photonic Instruments, Inc. When the system arrives, move the system to a safe area and contact Photonic Instruments, Inc. to arrange a mutually convenient time for installation. If there are any questions or issues regarding installation, please advise the technician when the installation date is arranged.

1.5 Contents of this Manual

This manual contains the following information:

- **Overall Design of the System** (Chapter 2) - presents a detailed discussion of the components of the system and explains how they are integrated.
- **Installation of the System** (Chapter 3) - describes the procedure to interface the system to a microscope and perform the necessary alignment.
- **Routine Operation of the System** (Chapter 4) - includes information about starting the system and collecting data.
- **Maintenance and Troubleshooting** (Chapter 5) - presents information about advanced alignment procedures.

A series of appendices are included which provide specific information about the computer controlled system, specifications and a list of spare/replacement parts.

2 Overall System Design

2.1 Optical Pathway

A schematic representation of the MicroPoint Laser Illumination and Ablation System [1] attached to the epi-illuminator port [2] of an upright microscope [3] is shown in Figure 2-1. Figures 2-2 through 2-4 present the top, side and front views of the system.

Figure 2-1: Schematic of a Manual MicroPoint Laser Illumination and Ablation System Installed on an Upright Microscope

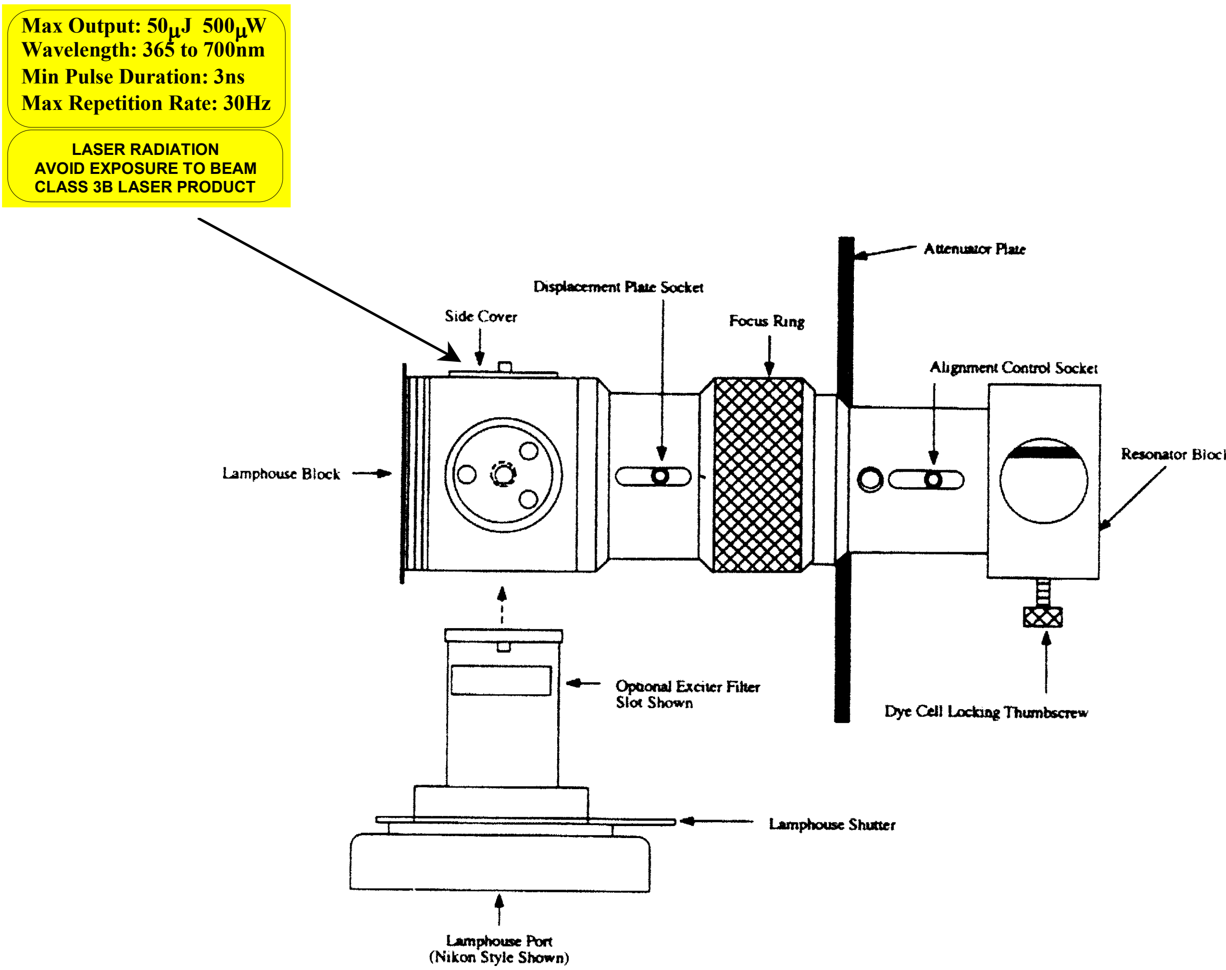


Figure 2-2: Top View - MicroPoint Laser Illumination and Ablation System

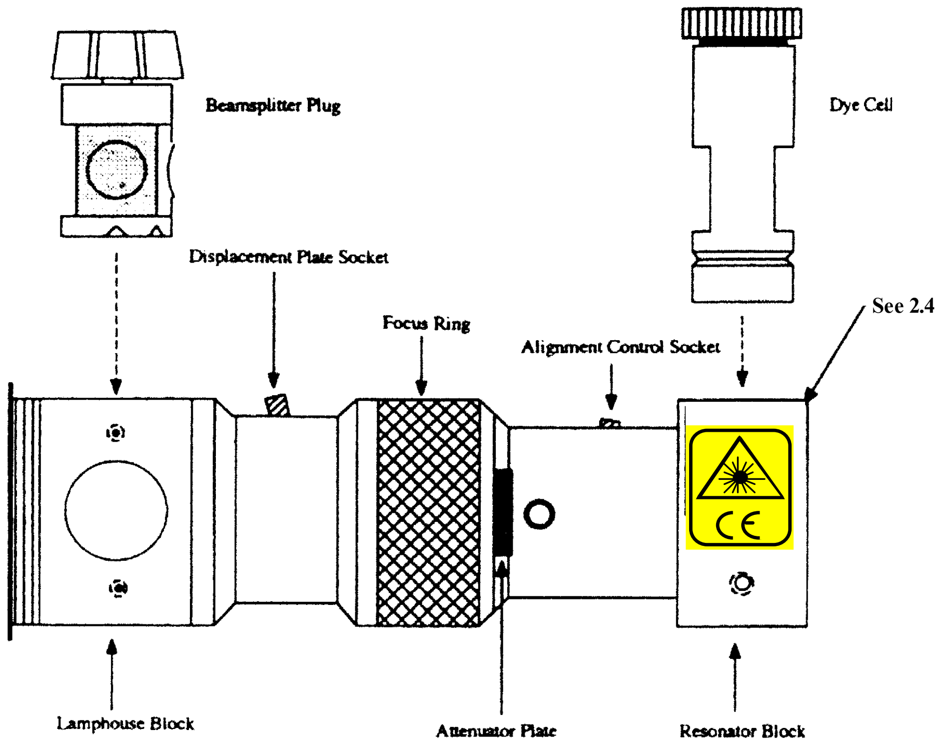


Figure 2-3: Side View - MicroPoint Laser Illumination and Ablation System

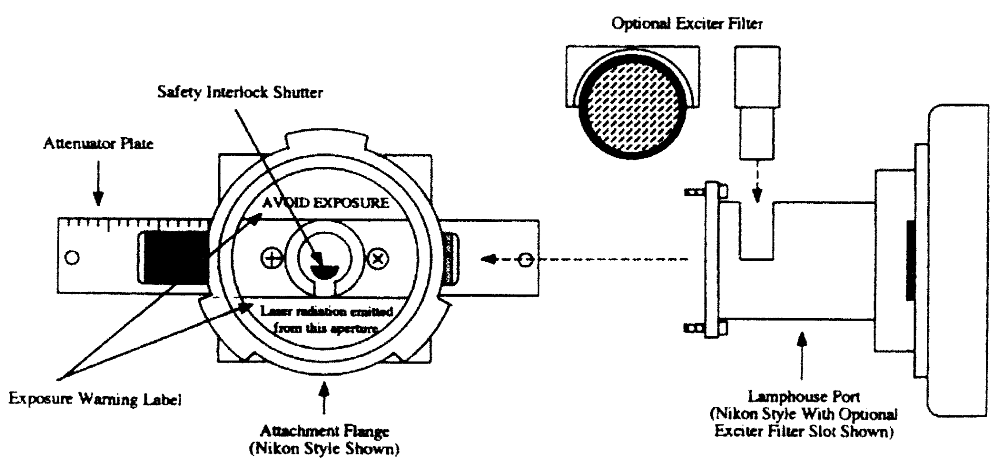


Figure 2-4: Front View - MicroPoint Laser Illumination and Ablation System

A certified class IIIb Nitrogen laser that delivers approximately 120 µ joules at 337 nm per pulse (2-6 nsec pulses). The laser transmits radiation to the resonator block via a low-loss multimode optical fiber. Coherence is lost in the fiber and is re-established in the optical head by a miniature, high performance dye laser cell, called a Dye Cell, which contains a dye solution that provides the desired wavelength.

The dye laser beam is then directed into a telescope that transfers the Gaussian beam waist from the dye laser cavity to the rear focal plane of the fluorescence port of the microscope at the solid angle required by that microscope. The beam exits the telescope through a thick-plate beam displacer that is mounted on a spherical bearing which can be positioned with a joystick (Alignment Control Socket) in order to swing the entrance angle

slightly in azimuth and elevation without changing the position of the ablation event dramatically.

System field of view alignment is adjusted by the movement of a similar displacement plate (Displacement Plate Socket or, in the case of computer controlled model, by an external controller. The alignment control socket enables the user to move the ablative spot within a 30% radius of the field of view in both the X and Y axes, while maintaining the brightness and focus of the ablative spot.

The attenuator plate is used to adjust the intensity of the ablation light and can be manually controlled or, if so equipped, controlled by an external device.

A lamp house block is provided, which can be used if additional lamps (optional) are desired for the application and a beam splitter plug is provided to direct the radiation.

The beam splitter is contained in an interchangeable, kinematic type mount (Beam Splitter plug. A beam splitter plug can contain optical elements to:

- Completely block transmission of light from the primary light source while allowing all the light from the secondary source to pass into the microscope.
- Provide both primary and secondary light to enter the microscope using a dichroic or other type of mirror.
- Allow only light from the primary source to pass through to the microscope, i.e. no optical element.

When installed, the beam splitter plug is held in place in the beam splitter housing by magnets and kinematic mounts that assure repeatable alignment.

2.2 User Controls - Manual System

Note: User controls and operation of the computer controlled system is discussed in Appendix D and user controls and operation of the Bluetooth computer controlled system is discussed in Appendix E.

The following controls are provided on the MicroPoint Laser Illumination and Ablation System:

- **Beam Splitter Selection Knob** - used to select the left or right port for the appropriate lamp house flange (can be rotated 90$^{o)}$.
- **Focus-Z Axis Alignment** - used to adjust the depth of focus of the laser's spot to achieve parfocality (all focal points in the same plane) of ablation events and the field of view (FOV).
- **Energy Density Attenuator Slide** - used to adjust the power level of the ablation laser. It has click stops and rulings to indicate the relative attenuation and allow for reproducible settings. It can be manually controlled or via an optional motorized system
- **X-Y Spot Translation** - used to locate the ablation event in the field of view. A joystick (or external device) is used to move the spot. Rotating it left or right moves the spot along one axis and moving it forward or back in the slot moves it in the other axis.
- **X-Y Alignment for Brightness** - used to control the optical axis angle of the ablation laser head and is used to compensate for differences in individual microscopes. It is activated in the same manner as the displacement plate above.

The MicroPoint Laser Illumination and Ablation System illumination path contains a manual blocking shutter that prevents any scattered radiation from being transmitted to the sample inadvertently or emitted from the open port if the light source is removed.

Attachment of conventional light sources to the MicroPoint Laser System Lamp house port is done with dovetail style flanges and clamping screws. The MicroPoint Laser Illumination and Ablation System optical head is also installed on to the epi-illumination port of the microscope with a similar dovetail flange.

CAUTION

Caution: Over-tightening of clamping screws can damage the dovetail flanges. Use appropriate torque to tighten these screws when attaching any accessories to the optical head.

2.3 Laser Source, Shutters and Interlocks

In the case where laser source illumination is employed, special hardware and special safety interlocks and labeling are required (laser safety labels with short descriptions are shown in Section 2.5). The laser source approved for pulsed nitrogen laser that has been approved by CDRH (Center for Device Radiological Health, a division of the US Food and Drug Administration). Please refer to 21CFR 1010 and 1040 for applicable regulations. The laser source has a fiber optic coupler either attached to the laser head or integral within the laser head.

The proximal end of a multi-mode quartz fiber cable is connected to this coupler. Installed safety locks prevent inadvertent removal of the fiber cable at both ends and access thereby to laser radiation.

The distal end of the fiber cable is connected to a fiber collimator device shown on the Resonator block.

A safety interlock shutter is installed inside the lamp house block to prevent any access to laser radiation when a beam splitter plug is removed.

Caution: The fiber cable should not be bent to radii smaller than 4 inches (20 cm). Breakage to the fiber may result from any sharp bend.

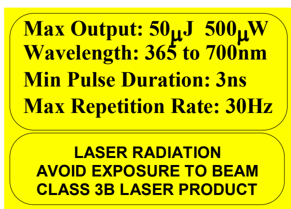
2.4 Labeling

With regard to safety labeling, the following areas are labeled in compliance with 21CFR 1010 and 1040: These labels are important in identifying potential hazards.

Sample Certification and Identification Label

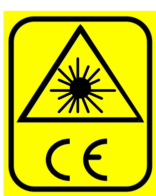
This label is permanently affixed to the rear of the MicroPoint on the resonator block.

Laser Head Label



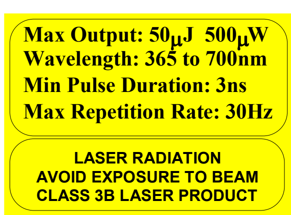
This label is permanently affixed to the MicroPoint laser head and notes the type of laser, wavelength, class IIIb, and the maximum output power.

Sample Danger Label



This label is permanently affixed to the side of the MicroPoint's Resonator block to indicate the presence of laser radiation at this junction.

Sample Microscope Label



This label is permanently affixed to an area near the epi fluorescent port of the microscope to indicate the maximum power level available to the microscope.

3 Installation of the System

3.1 Receiving and Unpacking the System

The MicroPoint Laser Illumination and Ablation System is carefully packaged and shipped with a packing list that indicates the various components included with the system. When the system arrives, please contact your local representative and confirm your installation schedule. Please do not unpack the instrument until your representative has arrived and can unpack and verify receipt of all components against the packing list together.

If there is any external damage to the shipping carton, contact the shipping agent and Photonic Instruments (or its representative) immediately.

It is recommended that you retain all shipping materials in case it is necessary to return any item to the factory.

3.2 Setting up the Nitrogen Laser

Note: A variety of lasers are provided with the MicroPoint Laser Illumination and Ablation System. For specific information about installation of the laser, please refer to the documentation provided with the laser.

3.2.1 Electrical Connections

a) Make the electrical connections shown in Figure 3-1.

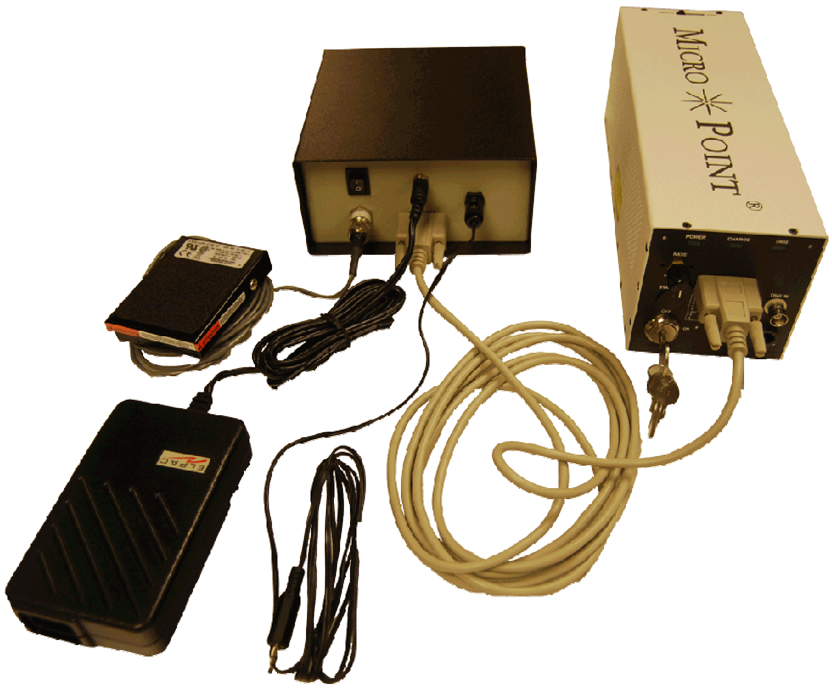


Figure 3-1: Electrical Connections for Laser

3.2.2 Laser Fiber Coupler Attachment

3.2.2.1 Standard Laser

Note: The laser power should be off for this step.

a) Attach the Fiber coupler to the faceplate of the nitrogen laser using the two screws accessible through the top plate of the coupler and that protrude through the bottom surface as indicated by the green arrows in Figure 3-2.

Note: Do not remove the fiber optic alignment screws indicated by the red arrows or and other screws accessible through the top plate.

Figure 3-2: Installation of the fiber Optic Coupler

3.2.2.2 Laser with Variable Attenuator

a) Attach the fiber coupler adapter plate to the nitrogen laser faceplate using the provided screws as shown in Figure 3-3.

b) Attach the fiber coupler adapter plate on the nitrogen laser faceplate using the two screws accessible through the top plate (green arrows) and that protrude through the bottom surface.

Figure 3-3: Installation of the Variable Attenuator Fiber Coupler Adapter

c) Attach the fiber coupler with variable neutral density slider to the adapter plate on nitrogen laser faceplate using the two screws accessible through the top plate (Green Arrows) and protrude through the bottom surface as shown in Figure 3-4. Do not remove the fiber optic alignment screws (red arrows) or any other screw pair accessible through the top plate.

Figure 3-4: Installation of the Optic Coupler - Laser with Variable Attenuator

d) Position the attenuator slider to the clear/full power position during fiber alignment

3.2.3 Installation of Fiber Optic Element and Fiber Lock

a) Feed the Fiber Optic Element through the small end of the fiber lock (Figure 3-5).

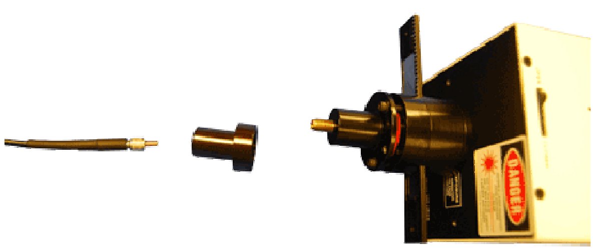


Figure 3-5: Fiber Optic Element and Fiber Lock

b) Attach Fiber to SMA connector (Figure 3-6).

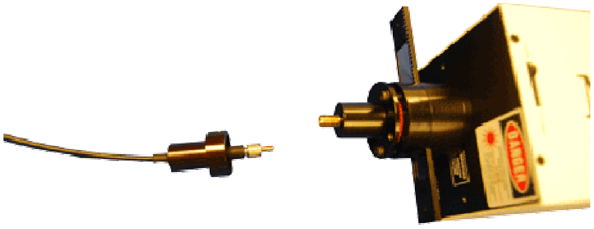


Figure 3-6: Attaching Fiber to SMA Connector

c) Install Fiber Lock over Fiber Connector (Figure 3-7).

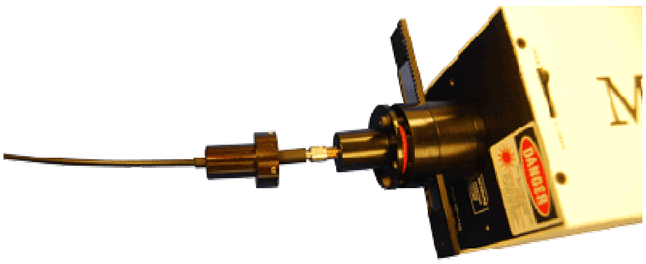


Figure 3-7: Installing Fiber Lock

d) Lock the Fiber Lock set screws (Figure 3-8).

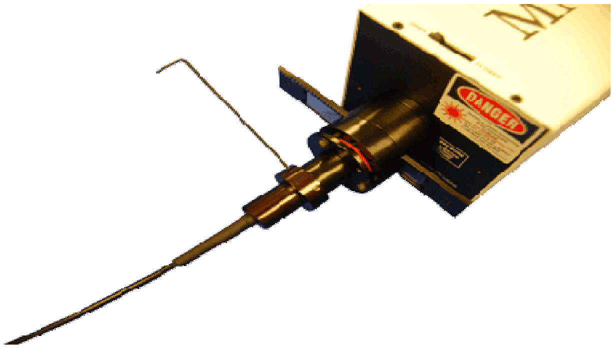


Figure 3-8: Locking Fiber Lock

3.2.4 Laser Fiber Optic Element Alignment

Caution: Do not look at the fiber tip with the laser running.

Note: It helps to do the above steps in a darkened room

a) Focus the fiber optic on an Apple Green Post-It from a distance of about 75 mm.

b) Turn on the nitrogen laser and run at about 10 Hz.

c) Adjust each of the 3 hex head screws by going in either direction using the T handle wrench (Figure 3-9). If the brightness decreases go in the other direction. The brightness will increase and then again begin to decrease. Define an arch of maximum brightness - normally about 180 degrees, and then center the screw. Make this adjustment for each of the (3) screws and then optimize by repeating the process a final time. Figure 3-10 shows the image of a properly aligned laser (a strong uniform spot with no rings).

Figure 3-9: Adjusting the Screws

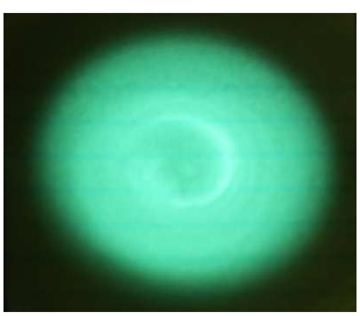


Figure 3-10: Laser Image from Properly Aligned Laser

3.2.5 Monitoring Laser Energy

The energy of a Nitrogen laser decreases over time. The normal working range for a Nitrogen laser is 150-80 µJoules (µJ). Laser voltage/output is set to 150uJ by Photonic Instruments before shipping. This corresponds to approximately 145 µJ out of the coupler and 105 µJ out of the fiber.

If desired, the laser voltage can be changed as shown in Figure 3-11.

Note: The laser lifetime is affected by voltage, increasing the voltage will shorten the lifetime of the laser.

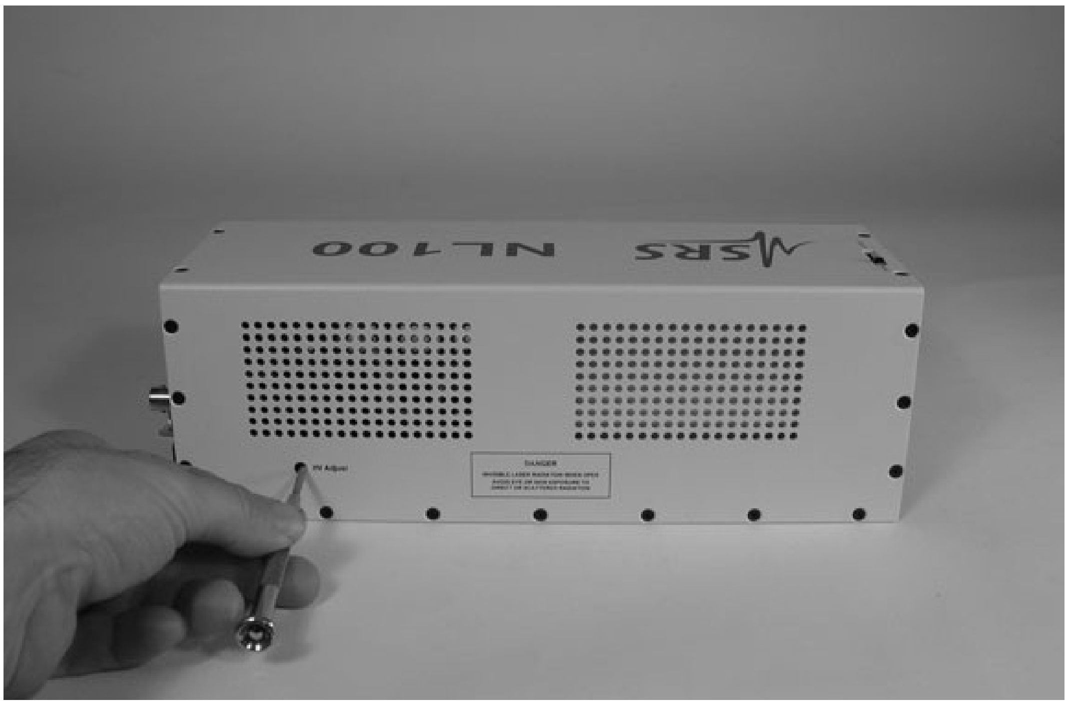


Figure 3-11: Adjusting the Laser Voltage

3.3 Attaching the MICROPOINT Laser System to the Microscope

3.3.1 Attaching the Body to the Microscope

a) Attach the **MICROPOINT** Laser System to the Microscope fluorescence lamp house port. A typical installation is shown in Figure 3-12.

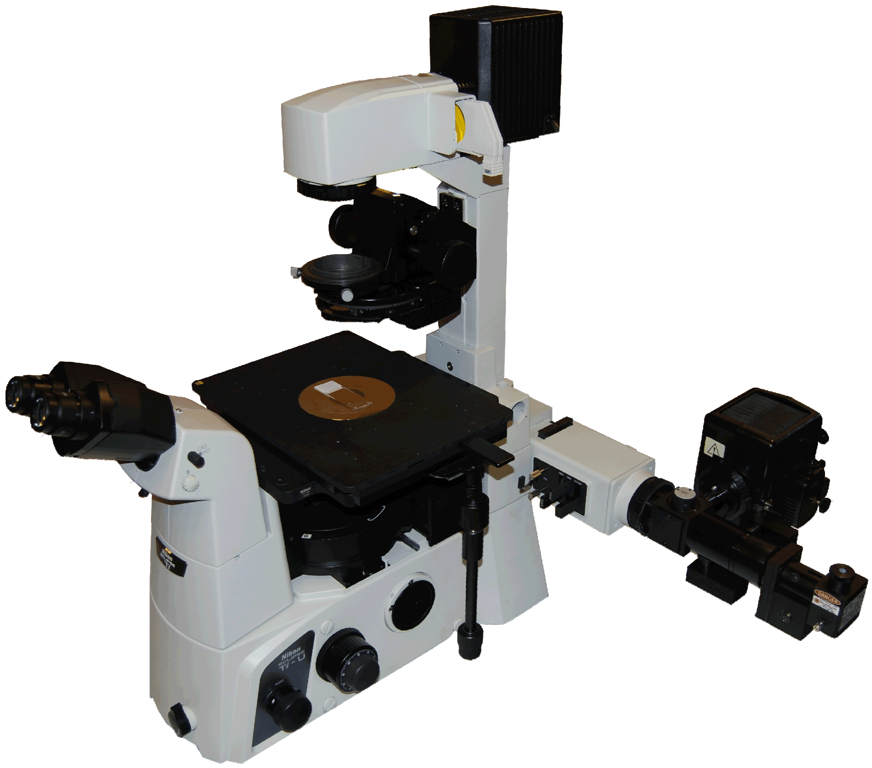


Figure 3-12: Attaching the **MICROPOINT Laser System** to the Microscope

3.3.2 Installing the Fiber Optic Cable to the Resonator Chamber

a) The fiber element and the connector are fitted into the resonator block the same way they are attached to the fiber optic coupler on the laser power supply (see Figures 3-5 and 3-6 for preparation of the fiber and connector). The assembly is fitted into the laser system as shown in Figure 3-13.

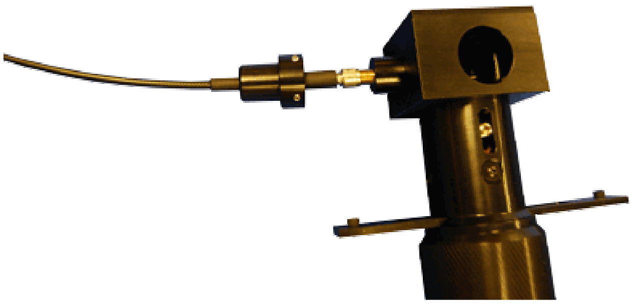


Figure 3-13: Fitting the Fiber into the Resonator Chamber

b) Tighten the set screws as shown in Figure 3-14.

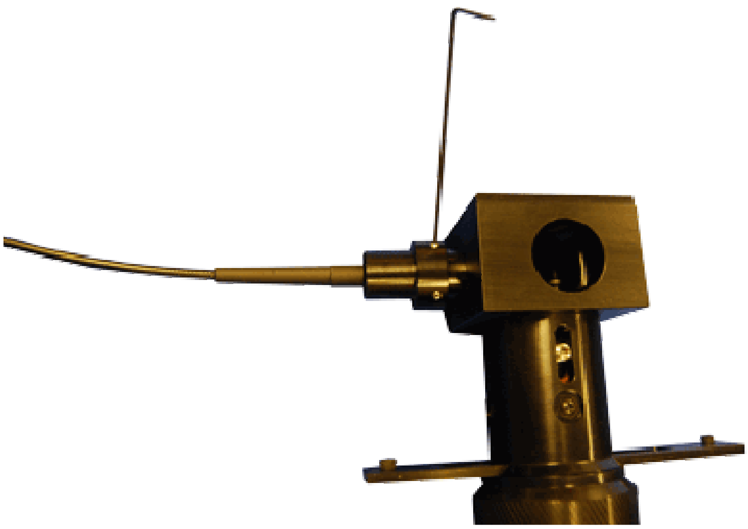


Figure 3-14: Tightening the Set Screws

3.4 Installing Ancillary Components

3.4.1 The Dye Chamber

The dye chamber is shown in Figure 3-15. A wide variety of dyes can be used to obtain the desired wavelength (a table of available dyes is presented in Appendix B). In many cases a dye providing 365 nm or providing 435 nm is employed. If the 365 nm dye is employed, a green dot is placed on top of the cell; if the 435 nm dye is employed, a blue dot is placed on the top of the cell. If an alternative dye is used, a yellow dot placed on top of the dye cell.

Figure 3-15: The Dye Chamber

To install the Dye Chamber:

a) Unscrew the cap of the dye chamber.

b) Fill the dye chamber with the dye solution mid way up to the threads.

Caution: The toxicological properties of the laser dye are described in the MSDS sheet provided with the laser dye. Refer to that document for specific information about potential hazards. The laser dye is dissolved in an organic solvent. Avoid open flames and sparks in the laboratory.

c) Place the cell in the resonator block and tighten the locking screw (Figure 3-16).

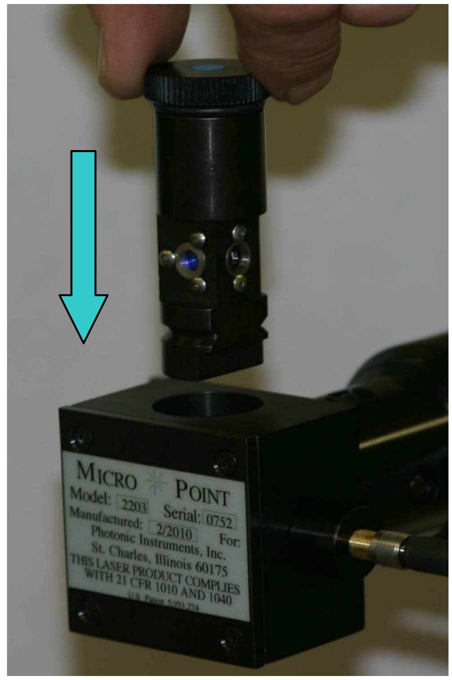


Figure 3-16: Inserting the Dye Cell into the Resonator Block.

d) Tighten the Dye Cell set screw (Figure 3-17).

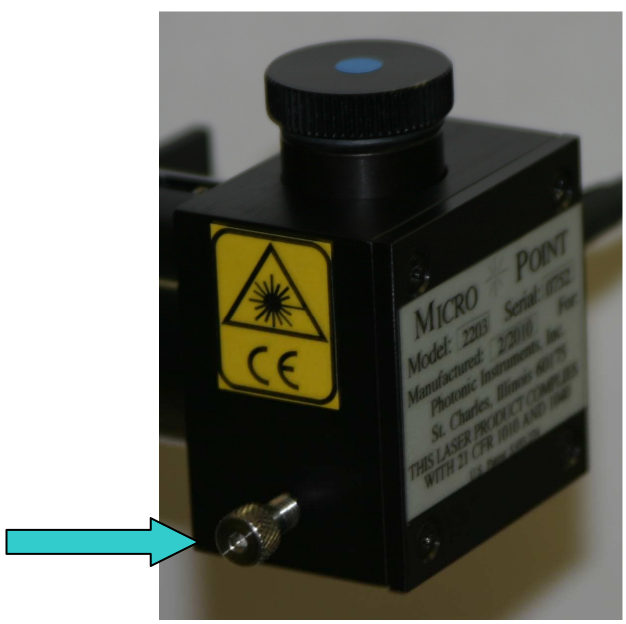


Figure 3-17: Dye Cell Locking Thumbscrew

3.4.2 Inserting the Magnetic Beamsplitter

If an external lamp is employed, a magnetic beamsplitter is included.

To install the beamsplitter, remove the plug on the top of the lamp house block and insert the beamsplitter. The beamsplitter features a magnet and kinematic mounting to ensure proper alignment.

Figure 3-18: Inserting the Beamsplitter

3.4.3 Inserting Exciter Filters

Excitation filters can be employed in the optional MicroPoint lamp house port filter slot. The filter is placed in the manner indicated in Figure 3-19. Note that the arrow is pointing away from the lamp. Filter wheels can be installed in a similar manner.

Figure 3-19: Lamp House Filter Installation

3.5 Installing an External Lamp on the MicroPoint Laser Illumination and Ablation System

If you are using an external widefield lamp house or fiber optic illuminator, simply attach the lamp house/ illuminator to the appropriate flange on the lamp house port (Figure 3-20). Lamp house port tubes can be mounted to either/both sides of the MicroPoint lamp house block.

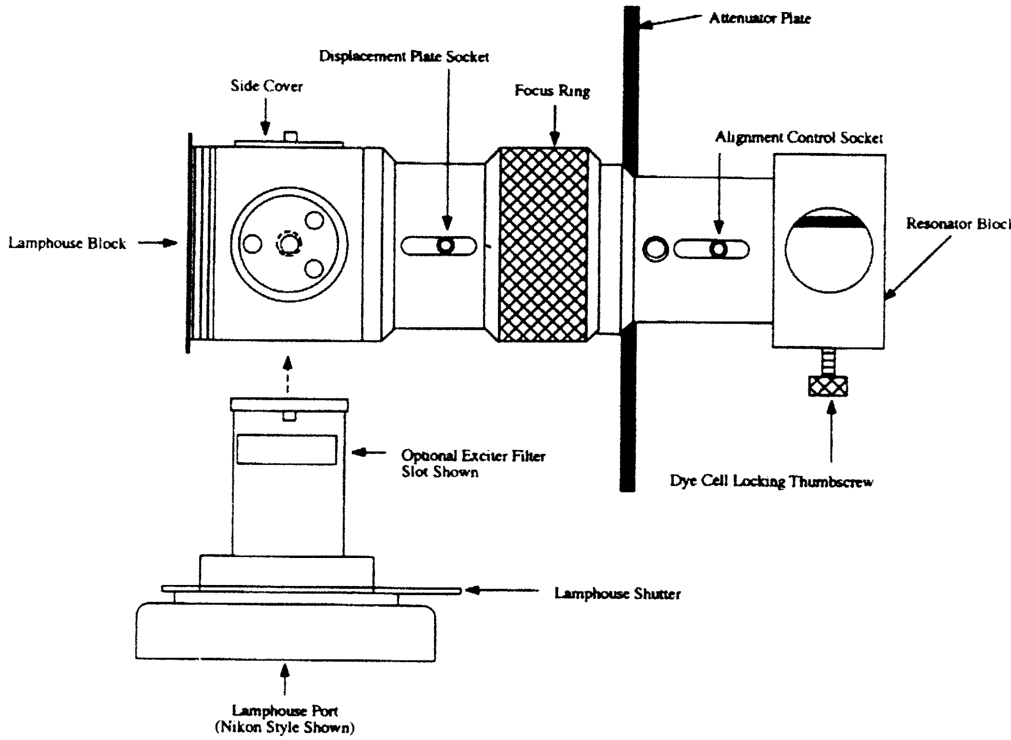


Figure 3-20: Lamp House Port

3.6 Connecting the System Interlock (Optional)

The remote interlock connector on the laser system must be connected to the magnetic reed switch on the microscope (Figure 3-21).

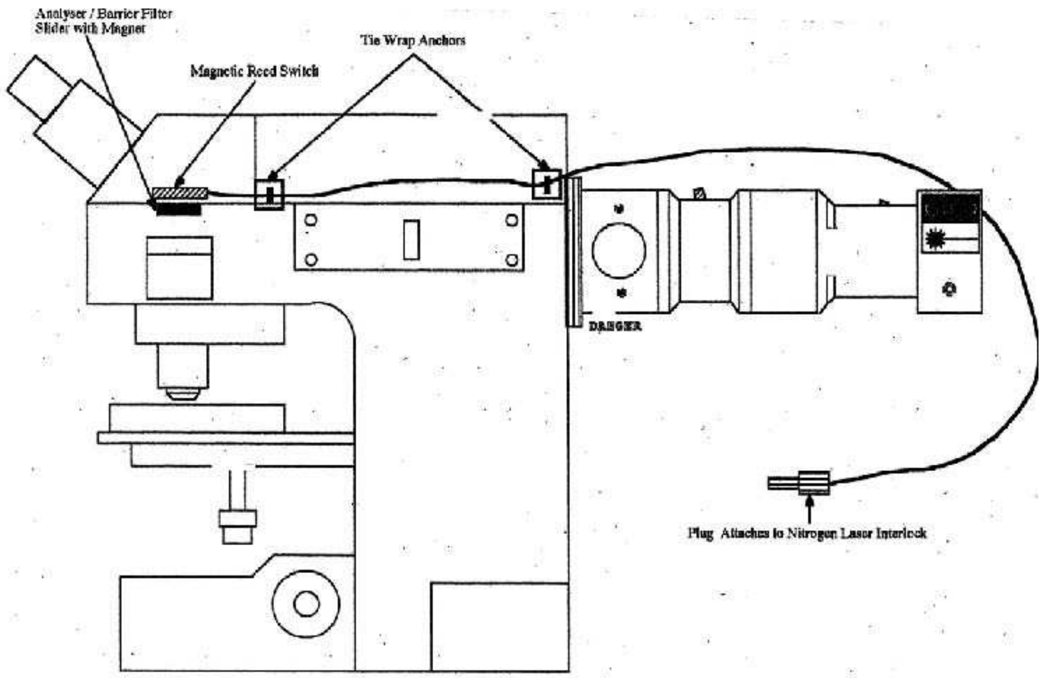


Figure 3-21: MicroPoint System Interlock

The interlock connection is made on the front panel of the laser system (9 pin D connector) or on the pulse controller, if applicable (3 Pin Kensington connector). The position of the magnetic reed switch connector varies on different microscopes. Interface information for typical microscopes is presented below, for additional information, please contact Photonic Instruments.

3.7 Alignment Procedures

Note: These instructions should be used for the Manual MicroPoint system. To align the computer controlled or Bluetooth controlled system, see Appendix D or E respectively.

To align the system:

a) Install the provided Fluorescence Centering Target on the microscope nose piece and position the fluorescence target in the active position.

b) Switch the microscope to either the laser delivery position - optional - or to the brightfield position on industrial scopes.

c) If an interlock system was provided with the system, make sure it is engaged and in the active position

d) Slide the variable attenuator all the way to one side so that the clear section is in the light path (rather than the reflective section).

e) Power up the laser and set a pulse rate of about 10 Hz.

f) Use the T-handle in the *Alignment* control socket (Figure 3-22) and center the primary beam onto the crossline on the Fluorescence Centering Target. The primary spot that is to be centered is the largest, brightest spot (Figure 3-23).

Note: The joystick responds to rotational movement, corresponding usually to X axis adjustments, and tilting forward and backward, usually corresponding to Y axis adjustments. It is never necessary to rotate any joystick further than 30 degrees from center. Doing so will vignette or block the beam.

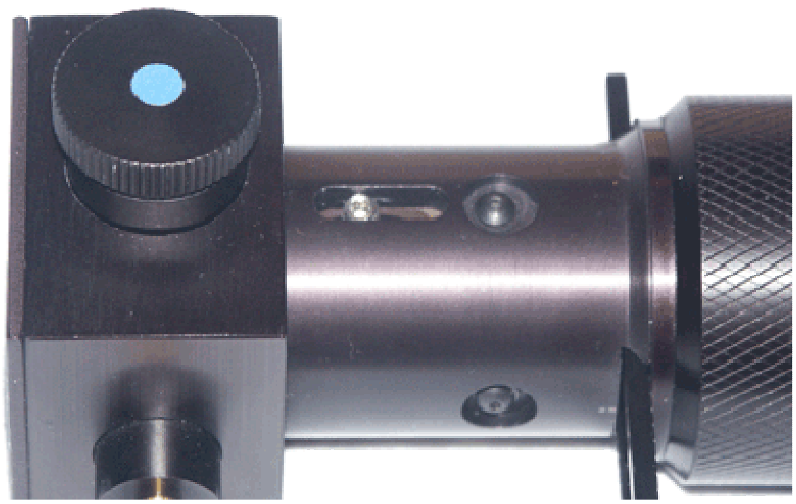


Figure 3-22: Alignment Control Socket

Note: Do not center the entire fingerprint as the entire fingerprint may include several harmonic laser beams (the smaller spots are to be ignored).

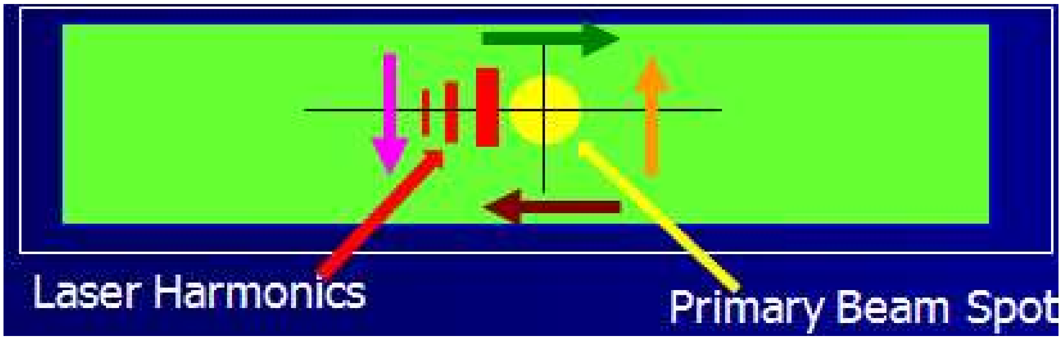


Figure 3-23: Centering the Primary Beam

If alignment is necessary, move the alignment socket as shown in Figure 3-24. The colored arrows indicate the direction which the socket must me moved to align the system, green and brown indicating CW and CCW rotation, magenta and orange indicating back and forward tilting, respectively.

Figure 3-24 shows a properly aligned system.

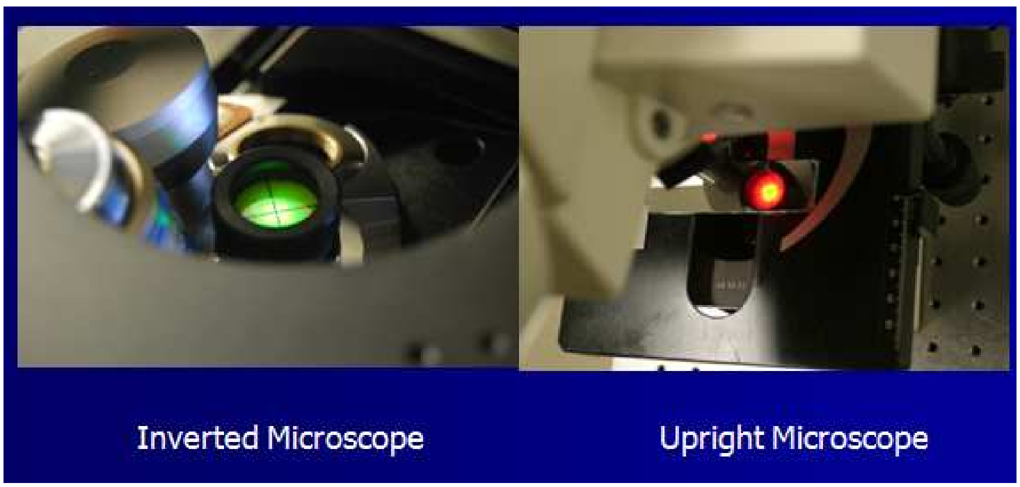


Figure 3-24: Properly Aligned System

g) Put a mirror on the microscope stage and focus on a pinhole or scratch using the transmitted light illuminator and the primary objective.

Note: Ensure that an appropriate long-wavelength pass barrier filter is installed in the viewing path.

WARNING

Warning: Using the MicroPoint grossly out of focus at high power can damage optical components!

h) Make certain the attenuator is set for minimum energy.

i) With the laser running at about 10 Hz change the position of the attenuator plate to increase laser energy until you find the cutting threshold of the mirror. The attenuator plate socket is shown in Figure 3-25. The point where more energy cuts the mirror when in focus and less energy stops cutting the mirror is the proper setting.

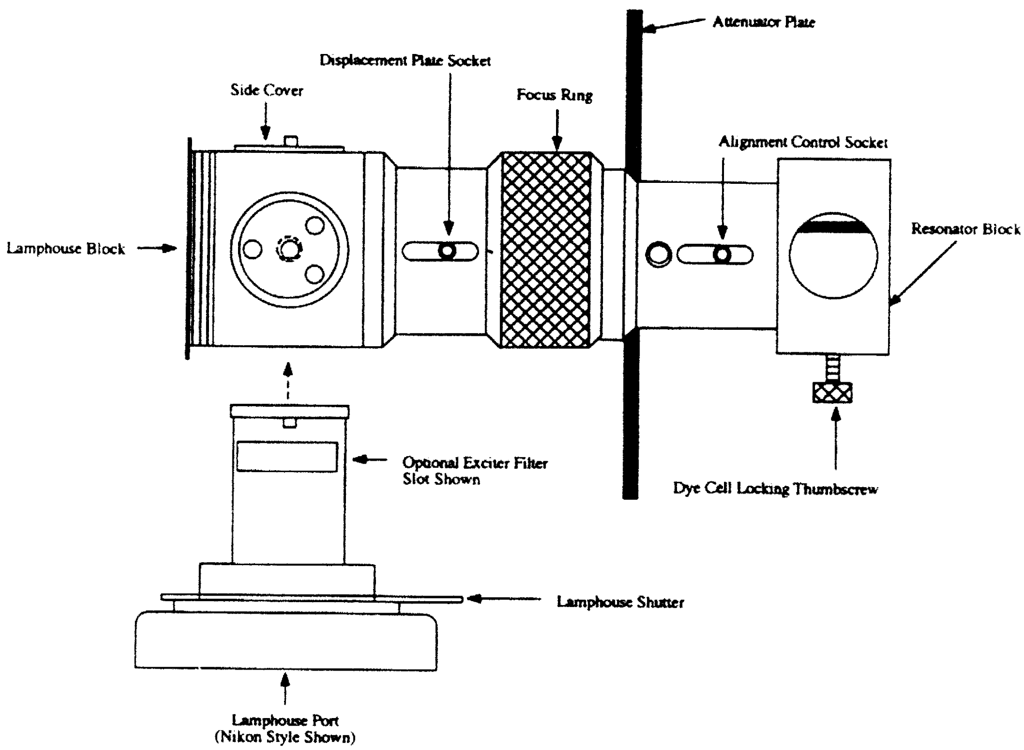


Figure 3-25: MicroPoint Laser Illumination and Ablation System

j) When you are at the cutting threshold of the mirror, slowly focus the microscope up and down relative to the mirror using the fine focus knob while continuously firing /pulsing the laser at every position /place in Z space. The laser should only be able to cut the mirror when pinholes or scratches are sharply focused.

k) If the system cuts better when out of focus; determine if the laser beam is high or low using the microscope fine focus knob. Then, return to the best microscope focus on mirror and adjust the laser focus ring on the ablation head – raise or lower the beam- until the laser and microscope optics are coincident and you can cut again. If you have enough energy to cut out of focus, you will have enough energy to cut when the laser, microscope optics/ mirror are coincident (parfocal).

l) Make sure you are perfectly coincident by repeating the above procedure while incrementally reducing laser energy until the procedure cannot be further refined.

m) Center the laser spot to your eyepiece crossline using the T-handle wrench in the Displacement Control Socket (see Figure 1-1). Move the Displacement Plate Socket until the laser holes in the mirror are superimposed onto the center of the eyepiece crossline.

n) If you have purchased an optional galvanometer beam steering system, center the laser spot to the crossline following the provided instructions in Appendix D.

o) If you are using a high power oil lens, put a standard microscope slide with cover slip on the microscope stage and focus into the coverslip just over the specimen. Make sure the attenuator is in clear or fully open position- maximum energy.

p) Fire the laser. You should observe cracks and/or a void in the coverslip where the laser was fired.

q) You are done!

3.8 Final Alignment Check

For the final alignment check:

- Verify that laser primary beam is centered on the laser Target Screen.
- Verify that the Laser and Microscope Optics are parfocal
- Verify that the laser spot is centered. Re-check alignment on the Fluorescence Centering Target.

4 Operation of the System

☞ **Note: Operation of the computer controlled system and the Bluetooth controlled system are discussed in Appendix D and E respectively.**

4.1 Overview

The MicroPoint Laser Illumination and Ablation System is a rigid unit that is designed using a heavy exoskeleton that is bolted to cylinders, blocks and gussets. All mechanical components are machined from aluminum.

☞ **Note: Do not disassemble the system, as there are no user-serviceable parts inside.**

In normal operation, the following activities may be performed:

- Advanced Alignment (Section 4.2)
- Replacing the Laser Dye (Section 4.3)
- Selecting a Lamp House Port (Section 4.4)

4.2 Alignment Procedures

Once the system is properly aligned during installation, little or no additional alignment is required. If the user desires to align (re-align) the system, the protocol described in Sections 3.7 and 3.8 should be performed.

4.3 Dye Cell Evaluation

A broad range of liquid laser dyes can be used to meet the needs of the user; the only requirement for the laser dye is that it optically pumps at 337 nm (the output of the nitrogen laser). A number of dyes are available from Photonic Instruments, Inc. (Appendix B). Laser dyes have a finite lifetime and should be replaced on a periodic basis as described in Section 4.4

The laser dye is located in a cylindrical, removable assembly called a Dye Cell. In use, the Dye Cell is housed in the resonator block and the dye should be replaced either every 30,000 pulses or minimally of every 2 months.

WARNING

Warning: The toxicological properties of the laser dye that is used is described in the MSDS provided with the dye. Refer to that document for specific information about potential hazards.

To determine if the dye cell is contaminated or out of tune:

a) Put an apple green Post-it on a microscope slide and position it under the microscope nosepiece.

b) Swing the microscope nosepiece to an open position.

c) Move the attenuator plate to the clear or fully open position.

d) Switch the microscope to the laser delivery filter set.

e) Turn on the laser and run it at approximately 10 Hz. Ensure that the laser beam is projected down onto the Post-it.

f) The laser image should be as shown in Figure 4-1.You should observe a large primary laser beam with several harmonics to one side (either image is acceptable, depending on your microscope illuminator).

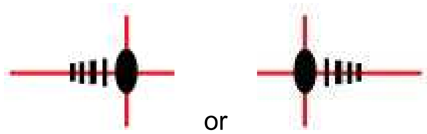


Figure 4-1: Laser Image

g) If you see a large fuzzy beam without defined harmonics, the dye cell is not clean or is out of alignment.

4.4 Replacement of the Laser Dye

To replace the dye:

Warning: The laser dye is dissolved in methanol and the solvent is used to rinse the cell. Avoid open flames and sparks in the laboratory.

a) Remove the dye cell from the resonator block and unscrew the cap.

b) Discard the old dye in a manner that is consistent with local environmental regulations.

c) Fill the dye cell with a laboratory cleaner such as Alconox, Sparkle window cleaner or an optical glass cleaner.

d) Obtain a cotton swab and remove any loose cotton fibers from it. Then compact the cotton tip.

e) Insert the cotton tip into the dye resonator cavity and plunge the cotton swab 1-2 times. Be careful not to overuse/ over plunge the cotton swap so as to not contaminate the cavity with loose cotton fibers as they may obstruct the beam.

f) Empty the dye cell and rinse the dye cell several times with water until you are sure that you have removed/diluted out all of the cleaning solution

g) Rinse the cell with spectral grade methanol and let the cell dry out.

h) If possible, inspect the resonator windows under a dissection microscope to make sure that they are clear and contamination free.

i) Refill the cell with dye using the supplied transfer pipette. The level of the dye solution should be up to the middle of the screw threads, replace the cover and tighten gently. Invert the cell to ensure that it does not leak (if a leak is observed, tighten the cover but do not over-tighten). Do not under fill the dye cell.

j) Insert the dye cell in the resonator block. Orient the cell so that it drops freely in the bore (do not force the cell in the resonator block). When the cell is properly installed, the bottom of the cell should be flush with the bottom of the resonator block. When the cell is in place, gently tighten the stainless steel thumb screw located on the side of the resonator block.

Note: If you change to a different dye, it is recommended that you use a new cell, as traces of the old dye in the new dye solution may have deleterious effects.

4.5 Selecting a Lamp House Port

You can select the left or right lamp house port by rotating the beam splitter selection knob so that one mark points to the microscope and the other points to the desired lamp house port. Magnets and kinematic mounting on the beam splitter plug align and lock the unit in position.

If it is necessary to remove the beam splitter plug, grasp the large gray knob on the top of the plug, give the knob a slight twist (this helps break the magnetic field) and lift the assembly from the housing or block, as the case may be. Replacement of a beam splitter plug is performed by placing the selected plug into the block or housing desired and rotating the plug after it contacts the bottom until a strong (magnetic) detent is felt.

Note: Removing the beam splitter plug will make the system inoperable. A safety shutter in the unit blocks all emission from the laser until the beam splitter is properly reinstalled.

5 Maintenance and Troubleshooting

5.1 Maintenance

Maintenance describes a series of activities that should be performed on a routine basis to optimize the performance of the system and minimize down time. The system is designed to require a minimum amount of maintenance.

Note: The suggested frequency of the operations described below is dependent on the amount of use of the system and the number of operators. As the user gains experience with the system, it will be found that the frequency of some activities should be done more frequently and others can be done less frequently.

The most critical aspect of maintenance is to ensure that the system is a clean environment that is suitable for sensitive electro-optical equipment. The laboratory should be free of dust, fumes and other materials that could affect the system.

On a Daily Basis

- Visually inspect the system.
- In a multi-application environment, check that the appropriate filter and dye for your application is present.
- Perform any maintenance activities suggested by the microscope manufacturer.

5.2 Troubleshooting

Ensure that the laser is functioning properly. Refer to the documentation provided with the laser for additional information.

If the laser is functioning properly and no ablation events occur, place a piece of white paper on to the specimen plane and determine if faint pulses of light (the color of the laser dye used) are visible on the paper when the system is pulsing.

- If no pulses are seen, refer to Section 5.2.1.
- If faint pulses are seen, refer to Section 5.2.2.

Note: If the procedures described below do not remedy the problem, contact Photonics Instruments, Inc. or your sales engineer. Do not attempt to dissemble the unit.

5.2.1 If No Pulses are Seen

a) Check power cable and fuse.

b) Verify that the exciter filter has been removed from the microscope's dichroic cube and the cube is properly inserted into the microscope.

c) Verify that the variable attenuator is at minimum attenuation (clear section inside unit).

d) Verify that the laser is pulsing properly and the shutter on the laser is not closed or partially closed.

e) Verify that there is light output at the end of the fiber (do not look at the fiber).

f) Make sure that there is laser dye in the cell and the dye has not been photobleached. If necessary, change the dye as described in Section 4.3.

g) Verify that there is a clear light path from the fluorescence port to the sample. To do this, turn off the laser, remove the ablation unit from the microscope port and with the sample in bright transmission, verify that light exits the fluorescence port (coming thru the sample). The light should contain the color of the laser dye to be used. If light is not observed, the filter, shutter and or diaphragms in the microscope are the indicated problem.

h) Verify light output from the laser ablation unit. Point the unit at a sheet of white paper from a distance of 4-8" and activate (pulse) the laser. A spot should appear on the paper. Turn off the laser.

5.2.2 If Dim Pulses are Seen

If dim pulses are observed, it is likely that:

- Check that you have selected the correct beamsplitter
- Make sure the objective/fluorescence cube you are using transmits/reflects at the wavelength you are using.
- The dye should be replaced as described in Section 4.3.
- The system should be realigned as described in Section 3.3.

Note: Paraffin samples ablate at low power levels. It may be worthwhile to use this material during alignment.

5.3 If Difficulties are Observed when Focusing

If you cannot properly focus the MicroPoint system after using the directions in Section 3.7, the following are suggested:

- Carefully recheck laser centering using the Target Screen.
- Carefully focus on the mirror slide in transmitted light.
- Rotate the MicroPoint Laser System focus ring and observe if the beam increases or decreases in size. Continue in the direction that focuses the beam diameter to the smallest possible diameter…a tiny point.
- Follow the parfocalization sequence again. (see **3.7**)
- If you cannot attain perfect parfocality between the MicroPoint and the Microscope, please consult Photonic Instruments.

Warning: Using the MicroPoint grossly out of focus at high power can damage optical components!

Appendix A Specifications – Manual System

The MicroPoint system is available optimized for a factory set range of focus. The angular and spatial alignment of the pulsed dye laser illumination is controlled via 2-axis joy stick. The focus in the z-direction is controlled with a knurled focus ring.

Model	2200	2203	2204	2205
Range of focus (mm)	-300 to -150	-38 to +500	+38 to +∞	-30 to +400

A.1 Tunable Fiber Optic Pumped Dye Source

Average power	750microWatts @ 15Hz
Peak power	12kW
Pulse energy	50microJoules
Stability	+/- 3%
Pulsewidth	3 to 5nsec
Pulse repetition rate	0 to 15Hz
CDRH Class	IIIb

A.2 Optical

Transmission	365nm to 700nm
Spectral bandwidth	4nm FWHM (typical)
Attenuation options	Manual, 28 steps, 1.0%-100% transmission logarithmic
	Motorized, 90 steps, 0.1%-100% transmission
Resolvable spot size	Near diffraction limited

A.3 Mechanical / Electrical

Illumination port clear aperture	Ø22mm or Ø34mm
Illumination port filter size	Ø25mm or Ø38mm
Dimensions	8.5" (l) x 6.0" (w) x 2.75" (h)
	10.0" (l) x 6.0" (w) x 3.75" (h)
Weight (laser head)	3 pounds
Lifetime	20,000,000 laser pulses
	30,000 laser pulses per refillable dye cell

A.4 Illumination Port Options

Dichroic beamspliter	Single pass, specify wavelength
	Multi pass, specify wavelength
Beamsplitter	380nm to 800nm, R = 100, 70, 50 or 30%
Excitation filter	360nm / 40nm (DAPPI)
	480nm / 20nm (GFP)
	470nm / 40nm (FITC)
	535nm / 40nm (Rhodamine)

Additional illumination port options on request.

Appendix B Laser Dyes

DYE (part number)	Peak Wavelength	Efficiency (relative to 440 nm)
27-365	364 nm	66%
27-388	388 nm	46%
27-390	390 nm	60%
27-404	404 nm	38%
27-419	419 nm	40%
27-422	422 nm	60%
27-435	435 nm	[100%]
27-471	471 nm	70%
27-481	481 nm	64%
27-514	514 nm	50%
27-521	521 nm	76%
27-539	539 nm	70%
27-543	543 nm	30%
27-551	551 nm	84%
27-576	576 nm	54%
27-582	582 nm	69%
27-590	590 nm	73%
27-593	593 nm	52%
27-613	613 nm	42%
27-622	622 nm	56%
27-626	626 nm	71%
27-651	651 nm	52%
27-656	656 nm	52%

Appendix C Spare Parts List

- 2250-0 Dye Cell For 435nm
- 2250-1 Dye Cell (all wavelengths except 435&365nm)
- 2250-2 Dye Cell For 365nm
- 2225 Fiber Optic Element 1-Meter
- 2226 Fiber Optic Element 2-Meter
- 337-USAS-PC Plasma Cartridge For Nitrogen Laser

See Appendix B for Laser Dyes.

Appendix D Computer Controlled System

D.1 Overview

The computer controlled Micropoint® Laser Illumination and Ablation System, when used in conjunction with the application program supplied by the microscope manufacturer can be used to control the attenuator and/or the galvanometers. While most systems include both, they are described separately as their operation is independent of each other.

Galvanometers - Two galvanometers (one for the x-axis and one for the y-axis) are located in the computer controlled Micropoint® system and replace the Displacement Plate Socket described in Section 2.1. The application program provided by the microscope manufacturer includes the controls for the galvanometers and the user should refer to the documentation provided with the microscope for information about controlling the galvanometers.

Automated Attenuator - The automated attenuator replaces the manual attenuator described in Section 3.7. The application program provided by the microscope manufacturer includes the controls for the attenuator and the user should refer to the documentation provided with the microscope for information about controlling of the device.

An interface box is used to provide communication between the computer and the computer controlled Micropoint® system (Section D.2).

D.2 The Interface Box

The interface box is used with the computer controlled Micropoint® system. The box has a green LED on the front panel which is illuminated when it is powered up and a variety of sockets/cables on the rear panel (Figure D-1). There are no controls on the interface box and all settings are made via the personal computer.

Figure D-1: Interface Box and Connections

The USB cable should be connected to a USB socket on the personal computer, the laser trigger cable should be connected to the laser power supply and the attenuator cable should be connected to the DIN socket on the automated attenuator.

Note: If the system includes automated galvanometers, do not connect the interface box to the mains until Section D.3 is completed. If the system does not include automated galvanometers, simply plug in the Power supply (120/240V, 50/60Hz, autoswitching) and power up the interface box by the switch.

D.3 Installation of Automated Galvanometers

To install Automated Galvanometers:

Note: The installation procedure for the two galvanometers is the same.

a) Unscrew the clamping screw that holds the galvanometer assembly in place and remove it from the system. To remove the galvanometer at the bottom of the system, rotate the head by 90^{o} to allow for easier access.

CAUTION

Caution: When the galvanometer is removed from the system, hold it by the metal clamp and take care that you do not touch the glass element. If fingerprints or foreign matter are deposited on the galvanometer, clean it with a lint free tissue saturated with spectroscopy grade methanol.

b) Remove the four screws that attach the rectangular plate and remove the plate to expose the connector (Figure D-2 and D-3).

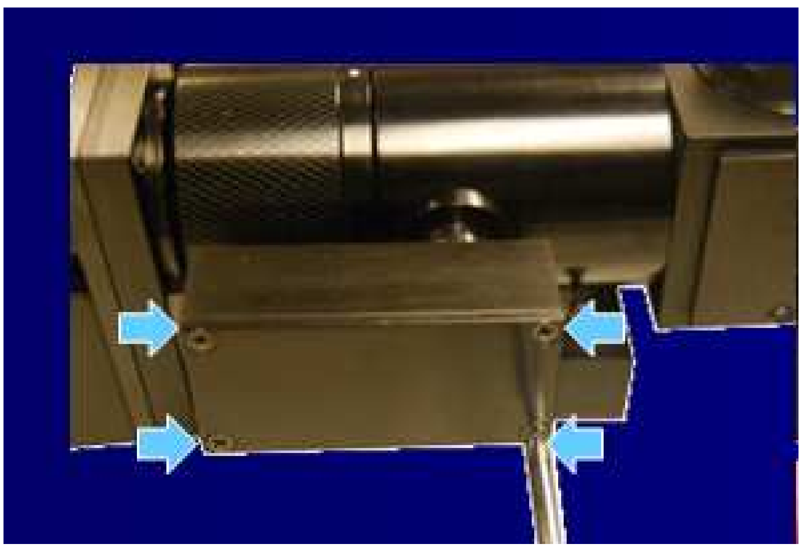


Figure D-2: Screw Locations

Figure D-3: Removal of Cover Plate

c) Remove galvanometer assembly (Figure D-4).

Figure D-4: Removing the Galvanometer System

d) Open the cable connector lever (Figure D-5).

Figure D-5: Opening Cable Connector Lever

Note: The cable for the Y axis has a red dot on it and the connector for the Y axis also has a red dot. The corresponding components for the X axis have blue dots. When you are installing a cable, make certain that the red cable is used with the red connector and the blue cable is used with the blue connector.

e) Insert cable from the interface box with the appropriate dot in the socket (Figure D-6).

Figure D-6: Insert the Cable into the Socket.

f) Close the lever, replace the galvanometer in the assembly, replace the cover and screws and replace the assembly in the Micropoint® system. Tighten the clamping screw.

Note: There is a horizontal slit in the metal base of the galvanometer, which should be in the axis of the system.

g) Plug in the Power supply (120/240 V 50/60 HZ, autoswitching) and power up the interface box by the switch on the rear panel of the interface box.

D.4 Troubleshooting

There is a fuse inside the interface box which can be replaced by the user, if necessary.

To replace the fuse:

a) Remove the four screws attaching the upper cover of the interface box.

b) Remove the fuse (Figure D-7).

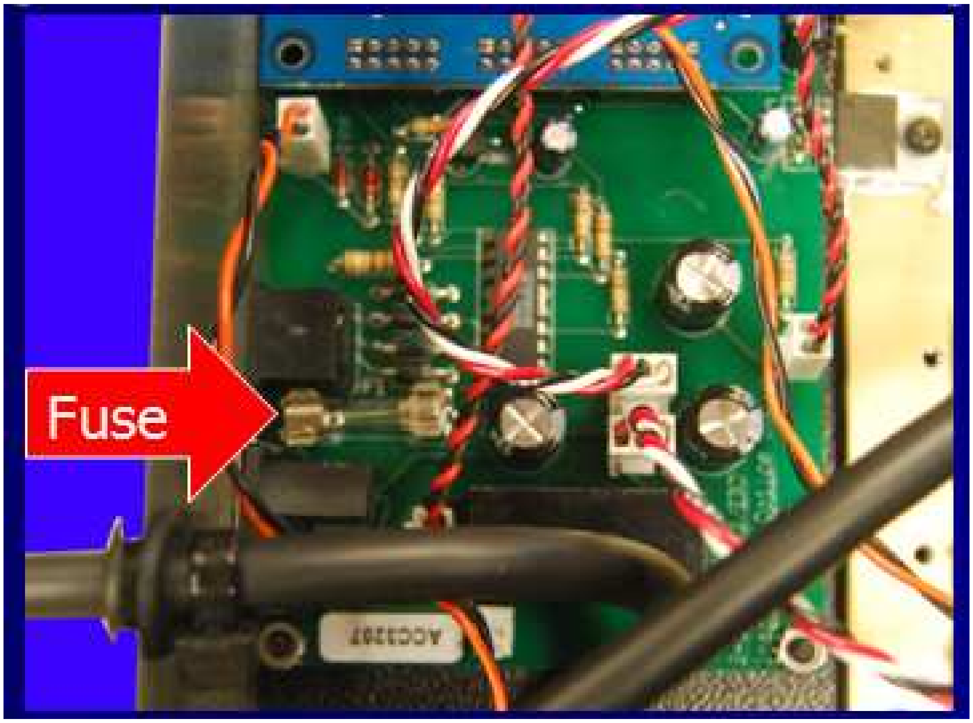


Figure D-7: Location of Fuse

If the replacement fuse blows, contact Photonic Instruments for assistance.

D.5 Uneven Power Distribution of Observed When Calibrating

Make sure you are using a flatfield objective.

After centering the galvanometers to absolute 0,0 using your imaging system, carefully recheck laser centering on the Fluorescence Target Screen

Appendix E Bluetooth Controlled System

E.1 Introduction

The Bluetooth controlled system uses a Personal Digital Assistant (PDA) which is supplied by Photonic Instruments and an antenna is installed on the lower right corner of the rear panel of the interface box to receive signals from the PDA. This system is commonly used to select regions of the sample to be ablated.

E.2 Installation of the Bluetooth Controlled System

The Bluetooth controlled system employs an interface box that is similar to that described in Section D.2. The box has a green LED on the front panel which is illuminated when it is powered up and the relevant sockets on the rear panel. The rear panel of the interface box for a Bluetooth controlled system is shown in Figure E-1.

Figure E-1: Rear Panel of the Interface Box for Bluetooth Controlled Systems

☞ **Note if the system includes automated galvanometers and/or an automated attenuator, the cabling/sockets, described in Section E.2, are provided.**

The USB cable should be connected to a USB socket on the personal computer and the laser trigger cable should be connected to the laser power.

There are no controls on the interface box and all settings are made via the personal computer and/or the PDA.

- If the system includes automated galvanometers and or the automated attenuator, refer to section E.2 for installation of these devices and do **not** connect the interface box to the mains until Section E.3 is completed.
- If the system does not include automated galvanometers, simply plug in the Power supply (120/240V, 50/60Hz, autoswitching) and power up the interface box by the switch.

E.3 Initialization of the Bluetooth Controlled System

The recommended order of power-up activities is as follows:

a) Power up the PDA and wait approximately 30 seconds

b) Turn the interface box on

c) Turn on the microscope

d) Turn on the laser.

When the PDA is powered up, the main screen is presented (Figure E-2). The *Micropoint EBD.exe* entry should be selected to present the **Calibration** page of the application program (Figure E-3).

Figure E-2: Main PDA Screen

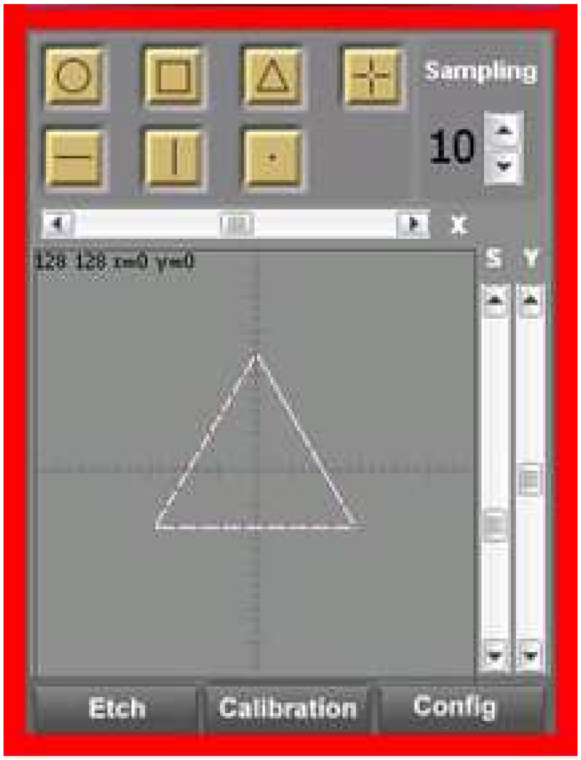


Figure E-3: Calibration Screen

There are three screens in the Bluetooth program, the *Calibration* screen, the *Configuration* screen and the *Etch* screen, which are accessed by the tabs on the bottom of the PDA.

E.4 Using the Bluetooth Screens

E.4.1 The Calibration Screen

The *Calibration* screen is used to generate an etch pattern, location and size to the region to be etched, which is then stored for use via the *Etch* screen (Section 4.2).

The central screen presents the selected etch pattern. The size of the etch pattern is selected by the S slider and the location of the etch pattern is controlled by the X and Y sliders.

☞ **Note: If the pattern in not totally on the screen, the laser will not perform the desired cut.**

The number of points for each element of the selected pattern is indicated by the up and down arrow in the upper right corner. In the above figure, ten points are used on each of the three legs of the triangle (the points at the apices are used on the two legs in common).

The *Calibration* page of the application program (Figure E-3) is accessed by pressing the **Calib** button at the bottom of the screen. It is used to set the laser power and select the format to be used for calibration.

E.4.2 The Etch Screen

The *Etch* screen (Figure E-4) is used to etch the sample.

Figure E-4: The Etch Screen

The pattern to be used for the etching is selected by pressing one of the seven buttons in the center of the screen and the power used for etching is selected by the Pwr ↑and Pwr ↓ buttons.

The green indicator at the right bottom of the display indicates the status of the battery and is not relevant in the general operation of the program, and the **Home** button returns the *Main* PDA screen.

E.4.3 The Configuration Screen

The *Configuration* screen (Figure E-5) is used to set the Laser Rep Rate. Other fields are Bluetooth related and are not used in the normal operation of the MicroPoint system.

Figure E-5: The Configuration Screen

Index