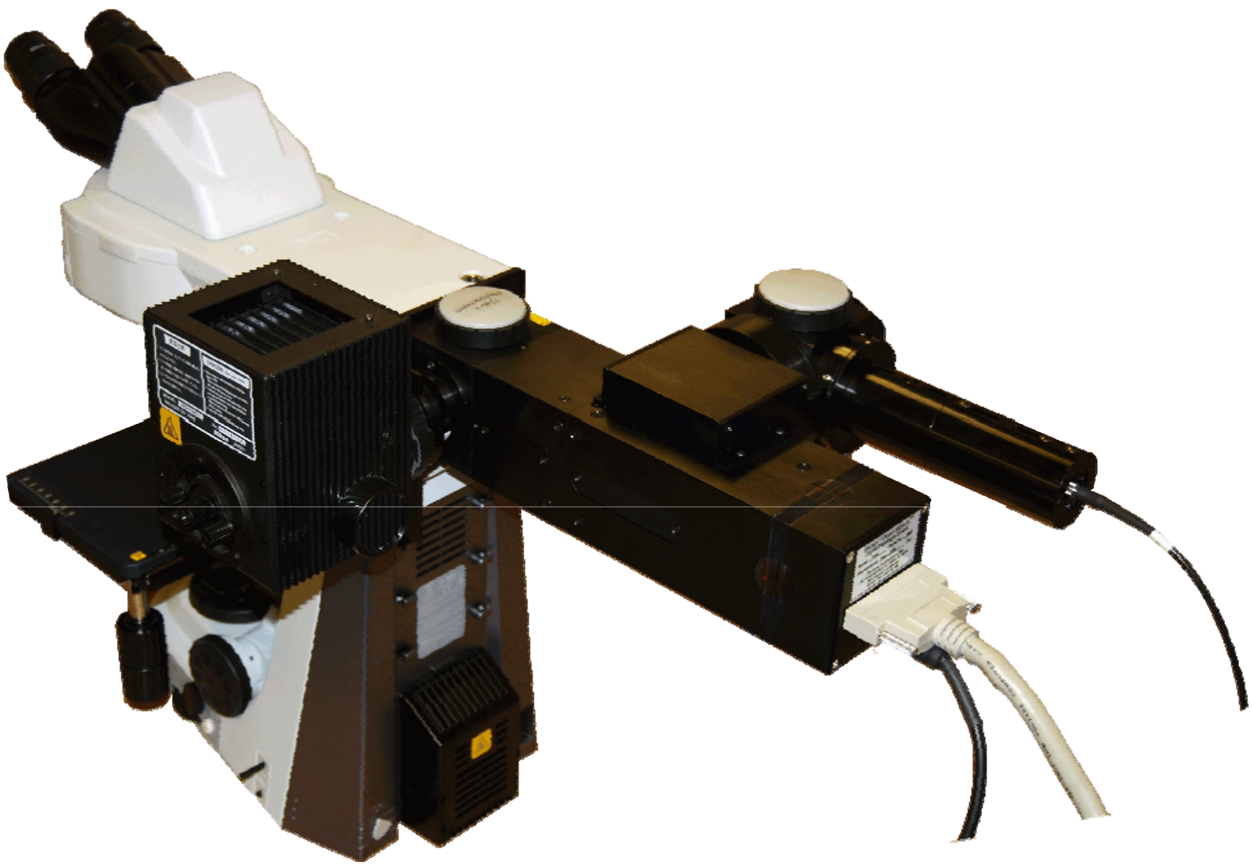
Mosaic® Digital Diaphragm System



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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 3.

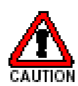
All information in this manual is subject to change without notice and does not represent a commitment on the part of Photonic Instruments, Inc. The system and various components in the system are the subject of the following US patents: 6,885,492 6,072,892 and 7,034,983 other patents pending.

Printed in the United States of America.

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 Mosaic Digital Diaphragm System

1. **Please read and understand fully this manual before attempting to operate or maintain the Mosaic 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 Mosaic Digital Diaphragm System. 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 Mosaic 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 Mosaic system. If your system is configured with a laser illumination source, it 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.

The Mosaic Digital Diaphragm 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, verify that the laser is turned off. Both the dual illuminator block and the wide field illuminator housing are "non-interlocked protective housings" per the CDRH definition and contain hazardous laser radiation.**

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 Mosaic 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 one 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 and fiber optic randomizers.
- 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.

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1 Introduction

1.1 Overview

The Mosaic Digital Diaphragm System (Mosaic) is designed to allow for spatial and temporal control over the fluorescence illumination of a research-grade optical microscope equipped for epi-fluorescence microscopy. The Mosaic system utilizes its own, highly corrected epi-illumination port in place of the microscope manufacturer's model port. The Mosaic optical head is attached to this port and an external light source is attached to the optical head. This external light source is masked (off) or reflected (on) by a Spatial Light Modulator (SLM) placed in a plane conjugate to the sample plane. An imaging computer and software control the SLM. Since the sample plane is displayed on the imaging computer's monitor, accurate spatial registration allows the user to select regions of illumination down on the sample plane. High speed deterministic timing of the SLM allows for precise temporal control. A variety of external illuminators can be used with the product, including fiber-optic sources, arc lamps, flash lamps and lasers.

1.2 Components of the System

Each Photonic Instruments Mosaic Digital Diaphragm System is specifically configured to meet the needs of the end user and the microscope with which it is to be used. In general, the system includes computer interface board(s), an optical head with SLM and optics, two integrated shuttered light paths including one for Mosaic illumination and the other for standard wide field epi-fluorescence illumination, kinematic removable beam splitter plugs and microscope / lamp house flanges.

An overview of a typical Mosaic Digital Diaphragm System is presented in Figure 1-1

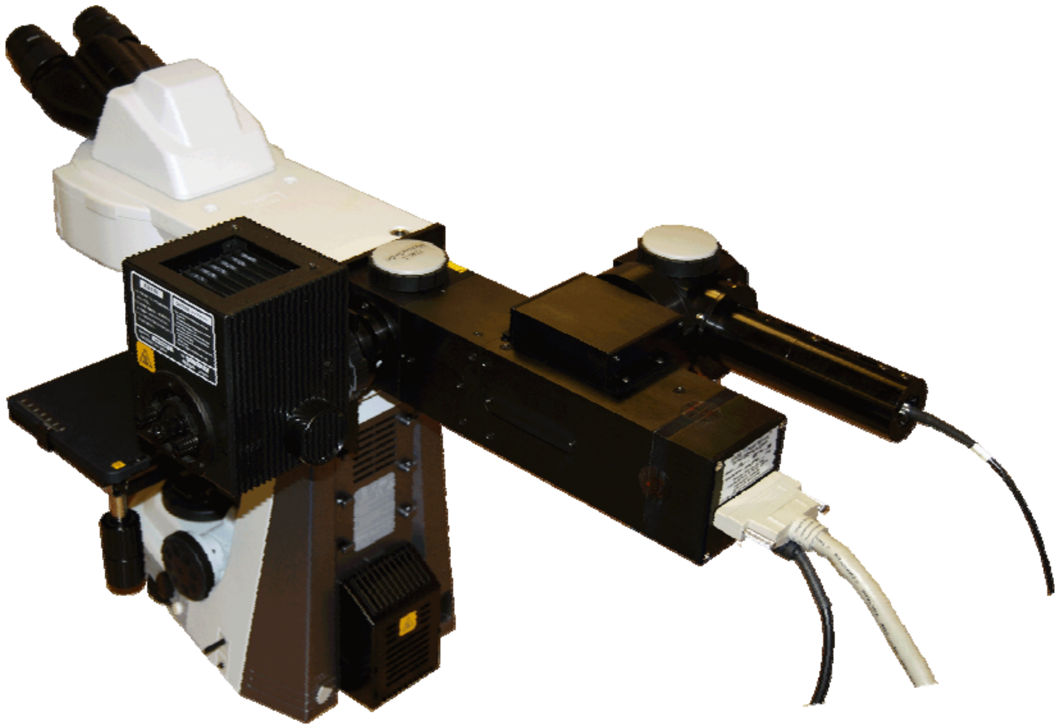


Figure 1-1: Typical Mosaic Digital Diaphragm System

1.3 Environmental, Space, Electrical and Computer Requirements

The environmental requirements (e.g. temperature, humidity range) for the Mosaic Digital Diaphragm System are similar to the environmental requirements for a research grade microscope.

The Mosaic Digital Diaphragm 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 Mosaic Digital Diaphragm System requires a horizontal distance of approximately 18" (46 cm) from the rear of the microscope.

The imaging computer must have two adjacent PCI slots (with exceptions) and a bay for BNC Trigger connectors. The cable from the Mosaic optical head to the computer is 3 m long. Application software for the Mosaic® Digital Diaphragm System is typically integrated into the imaging software provided for the microscope.

If a randomizer is employed, a fiber optic cable (4 m) is used to deliver the 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 Mosaic Digital Diaphragm System electrical requirements (excluding lamps and lasers) are:

The Mosaic Optical Head Power Supply 100-240VAC, 50/60Hz at 0.12A

The Homogenizer (if employed) 100-240 VAC, 50/60Hz at 0.6A

Note: If an Argon Ion laser is included in the system, the mains must be capable of providing 220 V.

1.4 Installation of the System

The Mosaic Digital Diaphragm System must be installed by a representative of Photonic Instruments, Inc. When the system arrives, please move it 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.
- **Routine Operation of the System** (Chapter 3) - includes information about starting the system and collecting data.

A series of appendices are included which provide specifications and other relevant information.

2 Overall Design of the System

2.1 Overview

The Mosaic Digital Diaphragm System (Mosaic) allows for spatial and temporal control over the fluorescence illumination of a sample, which is being observed by a research-grade optical microscope that is equipped for epi-fluorescence microscopy.

The system can be used in a broad range of applications, such as:

- Fluorescence Recovery After Photobleaching (FRAP) experiments
- Fluorescence Resonance Energy Transfer (FRET) experiments
- Photo Activation experiments
- Studies in the release of Caged Compounds
- Photo Switching of Compounds

The use of the Mosaic for FRAP experiments provides an excellent example of the use of the system. A FRAP experiment involves irreversibly photobleaching the fluorescent-labeled proteins in a small area of a living cell by an intense laser exposure in a short time, followed by measurement of the fluorescence recovery. The subsequent exchange of bleached for non-bleached protein is measured under normal fluorescent illumination. The high intensity and narrow wavelength of the laser combined with high temporal and spatial resolution of the Mosaic greatly benefits this technique and other studies in experimental biology.

2.2 Internal Light Pathway

A schematic representation of the Mosaic optical head [1] attached to the epi-illuminator port [2] of an upright microscope [3] is shown in Figure 2-1. A conventional light source [4] is attached to the side or rear Mosaic illumination port of the dual block illuminator [6]. The dual block illuminator may contain a beam combining element [7] (referred to here as a beam splitter plug, see 2.3 Mechanical below) to illuminate the SLM from two light sources individually or simultaneously without detachment / reattachment / realignment of those sources. Light from the light source(s) passes through a shutter [5] and illuminates the Spatial Light Modulator (SLM) [8].

The Spatial Light Modulator (SLM) contains more than 10^5 micromirrors and is positioned optically in a plane that is conjugate to the specimen plane of the microscope. With this optical arrangement, every spatial position of the array is represented by a spatial position at the specimen plane [14]. Every micromirror pixel of the SLM when angled to the ON position illuminates a corresponding area of the specimen for which we use the term “specimen pixel”. The size of a specimen pixel will vary according to objective [13] magnification, microscope model, and Mosaic model, but is usually on the order of fractions of a micron. When a micromirror is angled to the OFF position, the light reflected from the light source is directed into a light dump absorber [9], which prevents further reflection or transmission.

The light beam reflected by the SLM passes through a series of lenses and then through an aperture in the wide field beam splitter housing [10]. A wide field illumination port [11] allows attachment of a conventional light source for wide field sample illumination. This wide field sample illumination beam is independent of SLM activity and is combined with the primary SLM beam by a partially reflecting or dichroic element in the beam splitter plug [12] installed in the housing. The combined light beam then enters the epi-illumination port [2] installed in the microscope and is relayed to the fluorescent filter cube [15], objective [13] and then to the sample plane [14] in the manner typical for fluorescence microscopy.

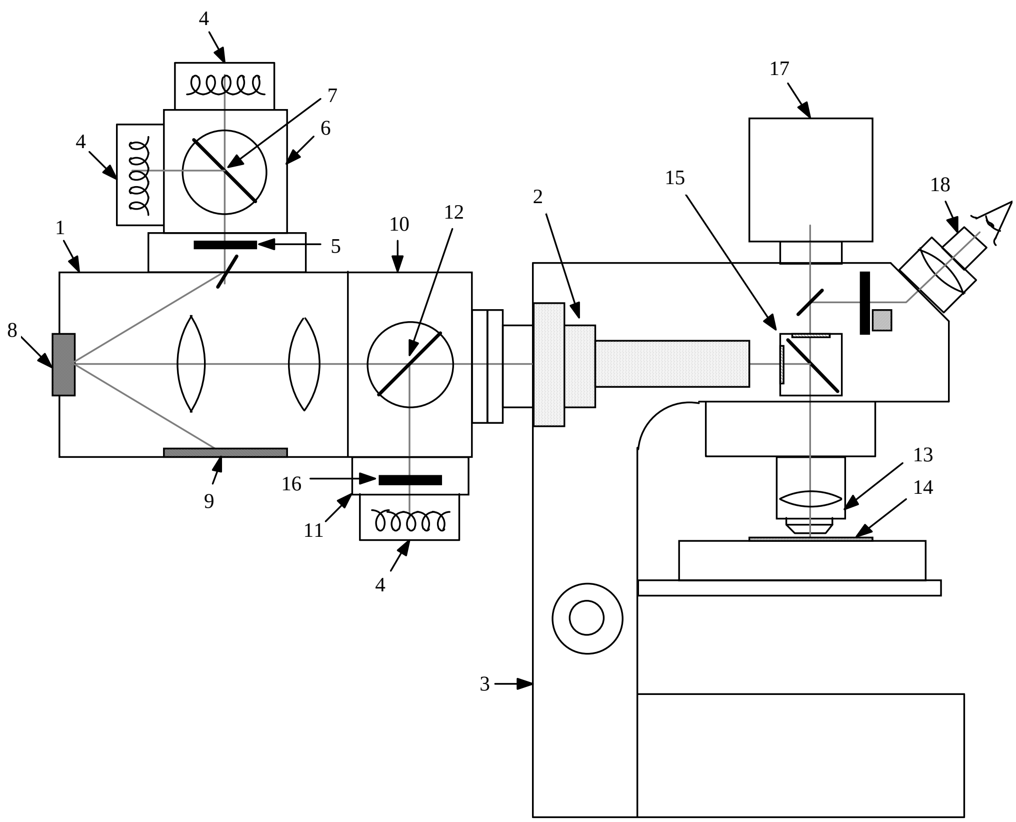


Figure 2-1: Schematic of Mosaic Digital Diaphragm System Installed on Upright Microscope

1	**Mosaic Optical Head**	**10**	**Wide Field Beam Splitter Housing**
2	**Epi-Illuminator Port**	**11**	**Wide Field Illumination Port**
3	**Upright Microscope**	**12**	**Wide Field Beam Splitter Plug**
4	**Conventional Light Source**	**13**	**Objective**
5	**Mosaic Illumination Shutter**	**14**	**Specimen Plane**
6	**Dual Block Illuminator**	**15**	**Fluorescent Filter Cube**
7	**Beam Splitter Plug**	**16**	**Wide Field Shutter**
8	**Spatial Light Modulator (SLM)**	**17**	**Camera or Imager**
9	**Light Dump Absorber**	**18**	**Eyepieces**

2.3 Mechanical

The Mosaic system is a rigid unit, deriving its strength from a heavy exoskeleton bolted to cylinders, blocks and gussets, all machined from aluminum. Unless disassembled, the Mosaic system should never go out of optical alignment.

The beam splitters [7], [12] mentioned above are each contained in an interchangeable, kinematic type mount that we call a 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, Beam Splitter Plugs are held in place in their respective beam splitter housings by magnets and kinematic mounts that assure repeatable alignment.

Removal of a beam splitter plug is performed by grasping the large gray knob on the top of the plug, giving the knob a slight twist (this helps break the magnetic field) and lifting 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.

The Mosaic illumination path and the wide field illumination port [11] each contain a manual or electronic blocking shutter [5], [16] 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, or Mosaic laser sources is done with dovetail style round flanges and clamping screws of an appropriate nature. The Mosaic optical head is also installed on to the epi-ilumination port of the microscope with a similar dovetail flange.

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.

2.4 Laser Sources, Shutters and Interlocks

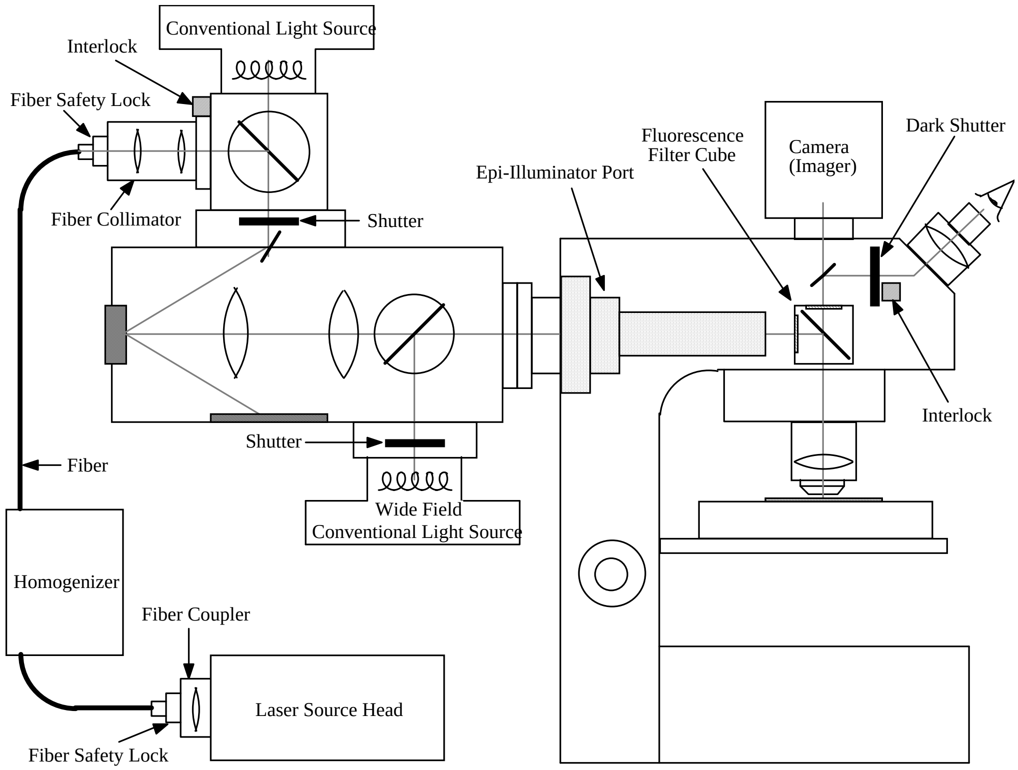


Figure 2-3: Schematic Representation of the Mosaic with a Laser Source

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). Figure 2-3 is a schematic representation of the optical paths for a laser source illuminator and associated safety interlocks and shutters.

The laser source approved for Mosaic is a continuous wave (CW) Class IIIb laser which is 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 fiber passes through a proprietary homogenizer device that randomizes the laser radiation to ensure illumination intensity flatness and homogeneity at the sample plane. In the homogenizer, laser speckle is reduced to a practical minimum and the coherence length is greatly reduced. The light exiting the homogenizer resembles a narrow wavelength band extended source more than it resembles laser radiation.

The distal end of the fiber cable is connected to a fiber collimator device shown in detail in Figure 3-2. The fiber cable has a similar safety lock at this end also. The fiber collimator has several user serviceable adjustments that will be discussed in Chapter 3. The fiber collimator mates to a dovetail style flange at the rear port of the dual illuminator block [6] Figure 2-1 with an electric interlock. Removal of the collimator disengages the interlock and shuts off the laser source.

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

The laser radiation combines with light from the conventional light source (if employed) attached to the side port of the dual illuminator beam splitter block at the beam splitter plug installed. If no conventional light source is installed, a cover for this side port is provided. After combining, the light beams propagate through the Mosaic optical head as described earlier, to the wide field combining beam splitter plug. At this point, any wide field conventional light source installed will be combined with the light beam(s) now containing the Mosaic image. Similarly to the dual illuminator block, if a conventional light source is not installed, a cover or shutter is employed to prevent unwanted radiation (light) from entering or leaving the system.

From the wide field combining beam splitter plug, the beams propagate through the epi-illuminator port installed in the microscope and on to one of the microscope's standard fluorescence filter cube assemblies. The cube(s) selected for use with Mosaic will be configured with dichroic element(s) and filter elements that are specifically selected to meet the desired wavelength characteristics of the user's application.

As is common in fluorescence microscopy, the beam(s), now considered excitation radiation, reflect off the dichroic element; pass through the selected objective and on to the sample plane. Light reflected from the sample plane, as well as emitted from the sample plane from excited fluorescence will propagate back through the objective and on to the dichroic element again. Here the reflected light is generally directed back to the Mosaic head and is absorbed in the housing. The emitted fluorescent light generally passes through the dichroic element and is directed to the video camera or other imager. Between the dichroic cube assembly and the eyepiece path is a shutter, which has an electric interlock similar to the fiber collimator. The interlock only allows the laser source to be on if the shutter is blocking the eyepieces.

Finally, in the case of inverted microscopes, a third electric interlock is installed at the pivot base of the transmitted light arm, as shown in Figure 2-4. This interlock disengages and prevents laser operation when the transmitted light arm is pivoted back to allow coaxial access to the objective.

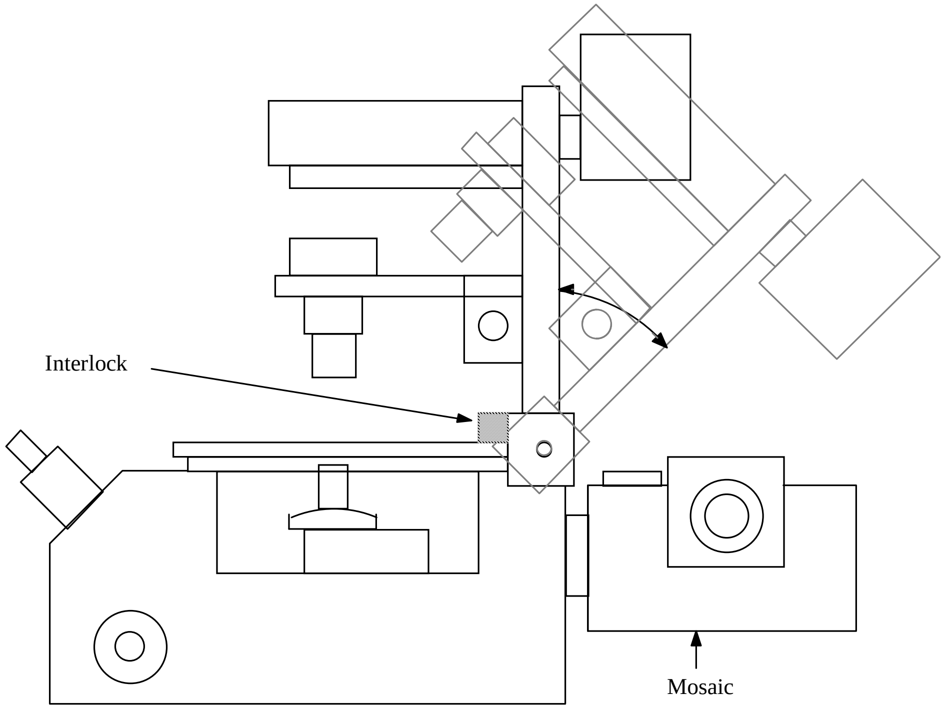


Figure 2-4: Pivot Interlock

2.5 Labeling

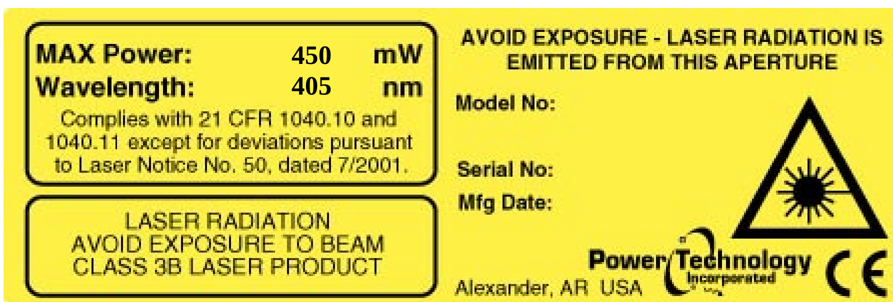
With regard to safety labeling, the following areas are labeled in compliance with 21CFR 1010 and 1040: These labels are important in identifying potential hazards.

Sample Certification and Identification Label

MICRO POINT MOSAIC™
Digital Diaphragm System
Model: 7206 **Serial No.:** 079
Manufactured: October, 2008 **For:**
Photonic Instruments, Inc.
2435 Dean St.Unit A, St. Charles, IL 60175
THIS PRODUCT COMPLIES WITH 21 CFR 1010 AND 1040
U.S. Patent 6,885,492 other patents issued and pending

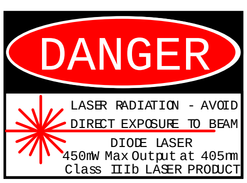
This label is permanently affixed to the rear of the device, above the data cable connection.

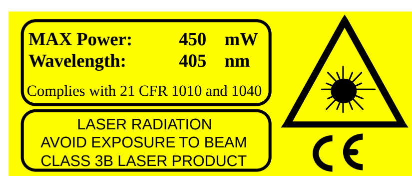
Sample Source Laser Head Label



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

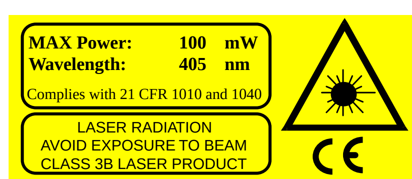
Sample Fiber Collimator Label ***Alternate Label***





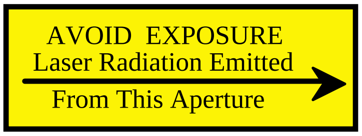
This label is permanently affixed to the fiber collimator barrel to indicate the maximum laser power at this junction.

Sample Microscope Label ***Alternate Label***

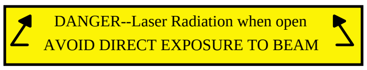


This label is permanently affixed to an area near the epi fluorescent port of the microscope to indicate the maximum power level available to the microscope.

Wide Field Beam Splitter Plug Housing Labels



This label is permanently affixed to the side of the wide field beam splitter plug housing, indicating the output aperture of the Mosaic optical head, as well as any other appropriate apertures on the installed microscope system.



This label is permanently affixed to one side of the wide field beam splitter plug housing and to one side of the dual illuminator block, as well as any other appropriate apertures on the installed microscope system.

2.6 Computer Control and User Interface

The user can control the SLM that directs illumination from the Mosaic light source into the microscope via computer hardware and software. Mosaic is usually controlled as a software toolbar in widely used imaging platforms. A video image of the specimen from a video camera or other imager attached to the microscope is displayed on the computer monitor. Using the computer mouse and standard drawing tools, the user can draw a translucent or outline overlay on to the video image, thereby selecting and area for Mosaic illumination. Prior system calibration assures accurate registration of the areas selected for illumination and the corresponding SLM pixels and thereby the corresponding specimen pixels. For information regarding the specific procedure for calibration and control, please consult the imaging platform user documentation. Other menus in the toolbar control Mosaic timing, shuttering and triggering of pre-drawn patterns and laser power.

2.7 Description of a Typical FRAP Experiment

The mirrors of the SLM can be controlled dynamically in real time, but will be used more commonly in a “flip and hold” mode, as in a typical photo-bleaching experiment

While the SLM mirrors are held in the OFF position, and a wide field light source illuminates a sample of cells, the operator views the video image of these cells on the computer monitor. The wide field light source is one that will not damage the photo-labile compounds used in the experiment. By manipulating the virtual image of the SLM, the user “targets” which area(s) of the cell to illuminate on command with the Mosaic light source, such as a mercury arc lamp or laser. The secondary light source is turned off or blocked, eyepiece viewing is deselected on the microscope, full camera viewing is selected, and the SLM corresponding mirrors are triggered to the ON position for a period(s) of time to cause photo-bleaching of the fluorochrome labeled protein. The user can then employ the imaging software to document the recovery parameters of the fluorescent probe in the region of the cell just photobleached.

3 Routine Operation of the System

3.1 Overview

The system is designed to allow for a minimum of user interaction to function. Each system is specifically configured to meet the needs of the end user. The following information is provided to assist the user in optimizing the performance:

- General guidelines to optimize system performance (Section 3.2)
- Powering up the system (Section 3.3)
- Preparing the system for data collection (Section 3.4)
- Maintenance (Section 3.5)

Note: Since each system is configured to meet the specific needs of the end user(s), some of the components described in this chapter may not be used on a specific system. As an example, a variety of lasers and lamps have been employed on different systems to provide the appropriate wavelengths.

3.2 General Guidelines to Optimize System Performance

The following points should be implemented to optimize the overall performance of the system:

a) The computer that contains the imaging and control software should be dedicated to this use. Programs that are not relevant to the primary use of the system should not be loaded on the computer.

b) If the computer is connected to a network, make certain that the network cannot download programs or data that could be detrimental to the software. An appropriate firewall and anti-virus software should be present.

c) The configuration of the computer has been carefully selected to optimize system performance. Do not change the computer configuration or replace components without discussing the changes with Photonic Instruments Inc.

d) The communication cables between the system and the computer have been selected to ensure maximum data fidelity. If it is necessary to replace a cable, obtain a new cable from Photonic Instruments Inc.

e) Fiber optic cables are fragile and should be configured so that all bends have a minimum radius of 4” (10 cm). Arrange the fiber optics so that their position cannot be accidentally moved during data collection.

f) The computer that contains the software should be powered down if it will not be used within the next 2 hours.

g) The laser should be powered down if it is not in use.

WARNING

Before changing beam splitter plugs, verify that the laser is turned off. Both the dual illuminator block and the wide field illuminator housing are “non-interlocked protective housings” per the CDRH definition and contain hazardous laser radiation.

3.3 Powering Up the System

The following sequence should be used to power up the system:

a) Power up the microscope.

b) Power up the computer and open the imaging software.

c) Power up the homgenizer (randomizer).

d) Power up the laser.

Note: Please refer to the documentation for the microscope, laser and imaging software for any specific information about powering up these devices, including warm-up time.

3.4 Preparing the System for Data Collection

3.4.1 Angular Alignment

When a laser illumination source is installed, the microscope will have a Fluorescent Target installed in the nosepiece turret. Rotate the turret to the fluorescent target's position. In the case of an inverted microscope, remove any slide carrier or shield such that the entire window of the fluorescent target may be viewed. (Figure 3-1).

Figure 3-1: Turret, Inverted Microscope (Slide Carrier Removed)

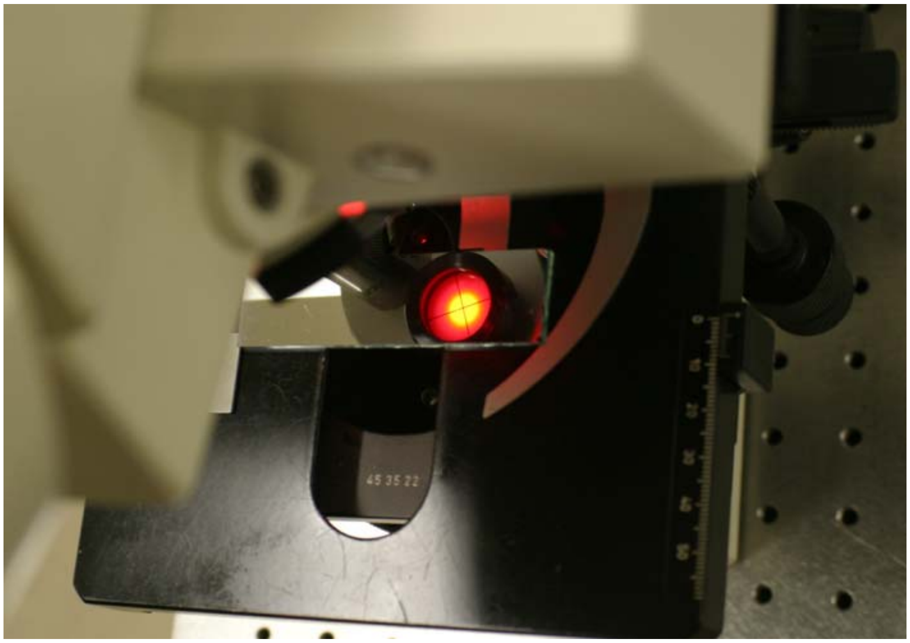


Figure 3-2: Turret, Upright Microscope

If an upright microscope is employed, place a mirror (provided) down on the stage, such that the fluorescent target window can be viewed (Figure 3-2). The fluorescent target safely shows the location of the laser illumination beam as it reaches the nosepiece plane, by converting the laser radiation entirely to non-coherent fluorescence emission.

Select the appropriate dual illuminator beam splitter plug and wide field illuminator beam splitter plug for your intended application and install these assemblies in to the Mosaic optical head. Place the provided Mosaic fluorescence filter cube into the active position.

Configure the Mosaic software or software toolbar to illuminate the entire Mosaic field of view. Set the laser to a low power setting and view the spot pattern on the target with the laser illuminator source active. An orthogonal grid of 2 to 3 spots across and down will be visible on the target and one spot will be brighter than the rest. Employ the joystick in the Angle Socket (Figure 3-4) in pitch and yaw axes to bring the bright spot to the cross line on the target. This alignment assures that the laser illumination propagates down the optical axis of the objectives. Turn off the laser.

3.4.2 Centration of Laser Image

In this discussion, we assume that the eyepieces and the imaging camera are par centric and par focal.

To view the laser spot for size and centration, select the objective to be used with the Mosaic and focus on the fluorescence sample (provided).

Select the provided Mosaic fluorescence filter cube and imaging camera port for viewing. This will engage the eyepiece laser interlock and allow the laser to turn on when requested. Configure the Mosaic software or software toolbar installed in the imaging system to illuminate the entire Mosaic field of view and set the laser to a low power setting. Activate the laser illuminator source and view the spot on within imaging system field of view (Figure 3-3).

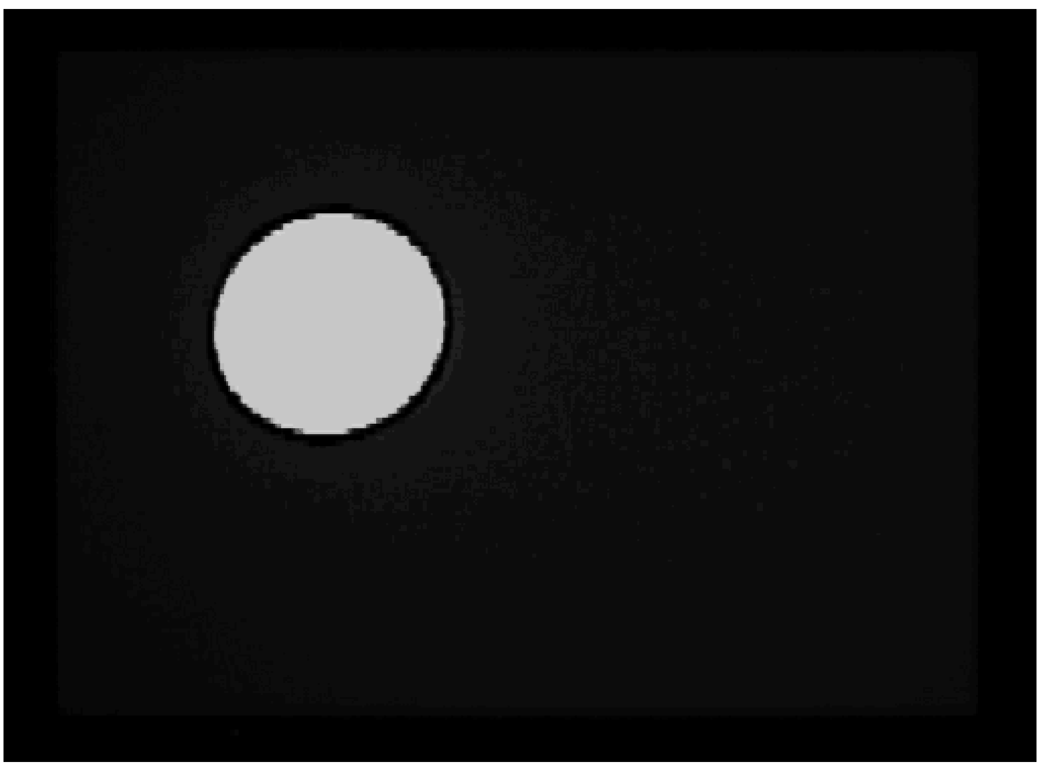


Figure 3-3: Typical (Small) Laser Spot at the Field of View at Start-up

Determine the center of the imaging system field of view using your imaging system drawing tools. Use the joystick “tee” handle (provided) in the Position Socket of the fiber collimator shown in Figure 3-4 and adjust with pitch and yaw movements to bring the laser spot to the camera center.

Figure 3-4: Fiber Collimator

Note: The angle and position socket controls both may operate as laser beam attenuators per CDRH requirement (21CFR 1040.10 (f)(6)(i)). When turned in the yaw axis between 60 and 120 degrees from nominal, each control blocks all laser radiation to the dual illuminator block and beyond. If either of these controls are employed in this manner, re-alignment per section 3.4.1 and or 3.4.2 will be required to resume operation. Scribe marks are provided inside the sockets to show nominal (open) positioning before alignment.

Because the two adjustments, position and angle, are not quite independent, it may be necessary to do a second iteration of these two adjustments for ideal alignment.

The imaging system may need Mosaic registration calibration with the objectives and fluorescence filter cubes desired for the work planned.

Note: if the system is used for a single application (i.e. the wavelengths remain constant and the zoom position is not changed) it is likely that only small changes or none at all will be required on a routine basis.

3.4.3 Determining the Desired Area for Laser Illumination

The laser illuminator subsystem (fiber collimator) is configured to illuminate a nearly circular spot on the spatial light modulator (SLM) that corresponds to the user's desired work area. For users that wish to use the highest power densities, the area should be as small as is practical.

After the spot is centered, determine the desired area for laser illumination on the imaging monitor. The diameter of the spot may be adjusted to achieve higher power densities (a smaller spot) or lower power densities (a larger spot). Adjustment is made by loosening the 2 button head screws indicated on the zoom section of the fiber collimator and sliding the screw heads (and thereby sliding the lens elements underneath) in opposite directions to obtain the desired spot size while maintaining a sharp focus at the edge of the spot.

Note: Colored dots are applied to the fiber collimator housing during installation and training to guide the user to several pre-determined positions with sharp focus.

While the focus and size are not critical, it deserves some attention. Figure 3-5 shows a picture of a poorly focused spot and Figure 3-6 shows a sharply focused large spot. .

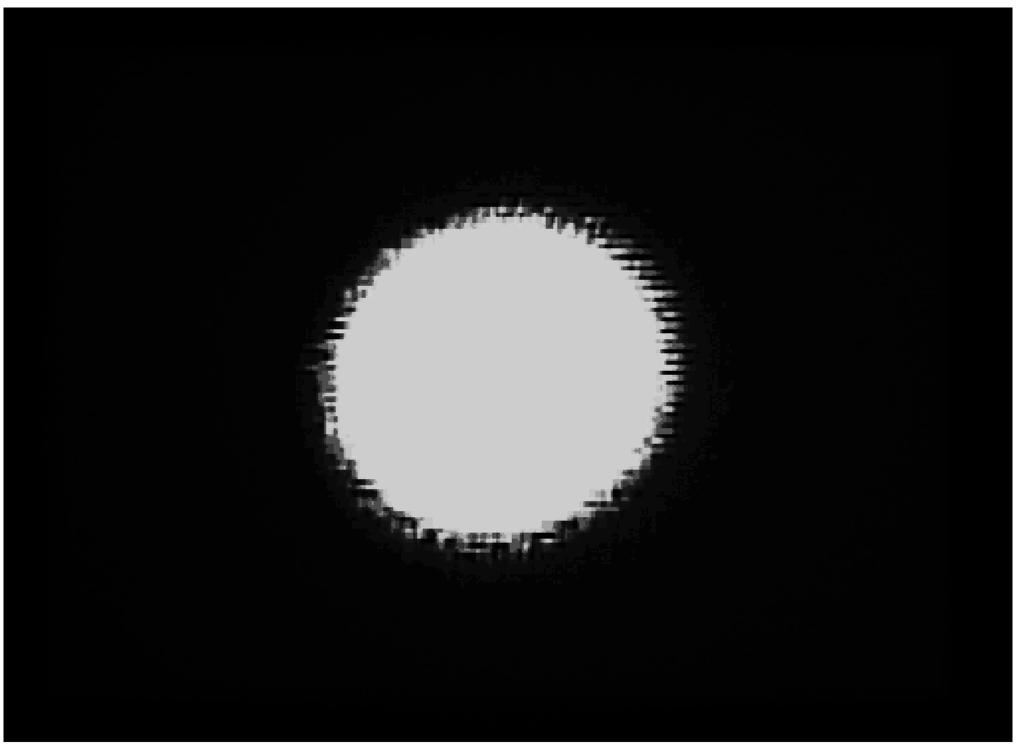


Figure 3-5: Poorly Focused Spot

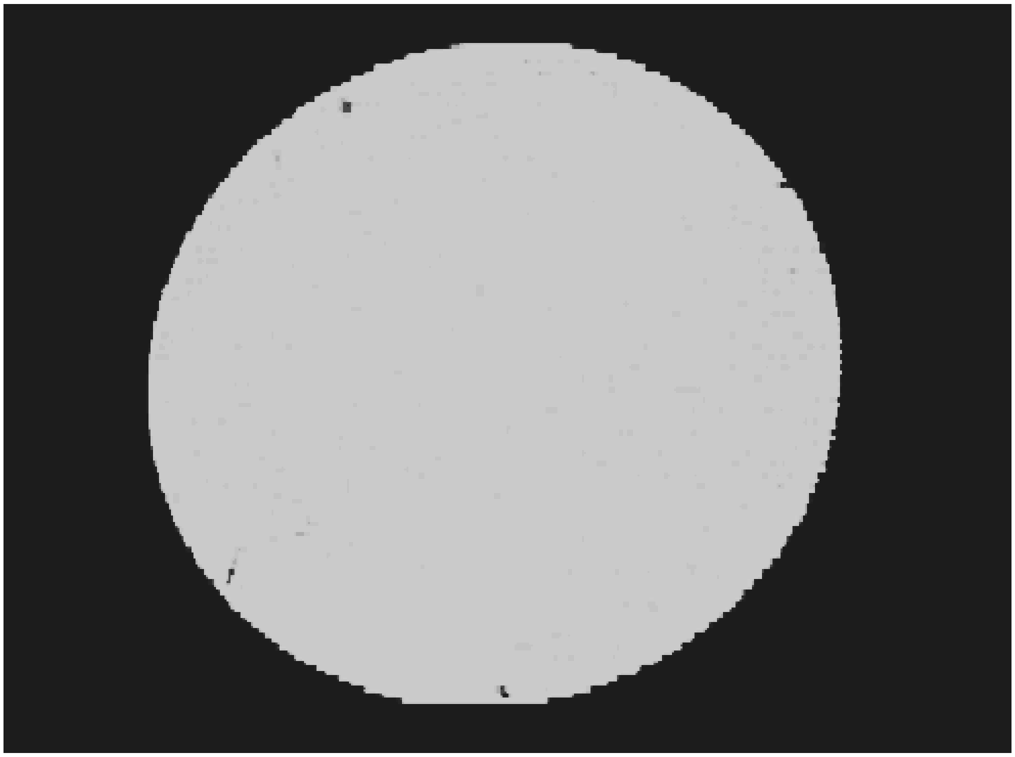


Figure 3-6: Sharply Focused Large Spot

3.5 Routine 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 source for your application is present.
- Perform any maintenance activities suggested by the microscope manufacturer
- Prepare the system for operation using the protocol described in Section 3.3.
- If configured with laser source illumination, on a daily basis or before every use test and verify that the Mosaic laser interlock circuit is working by confirming that the LED emission indicator on the laser power supply turns off when the microscope binocular eyepieces are in the open position or when the articulated transmitted light arm is tilted back from the functional vertical position on inverted microscopes before using the system. If the system fails to operate in the manner described, power down the system and call Photonic Instruments Inc. before attempting further use.

☞ **Note: If the system is used with a single set of wavelengths, only minor adjustments will normally be required on a daily basis. In a multiple user environment, the protocol described in Section 3.4 may entail iterative adjustments to optimize performance.**

On a Weekly Basis:

- Ensure that all power cables and communication cables are firmly in place.