

IonOptix Corporation **HyperSwitch** Light Source System



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Table of Contents

1. Introduction	2
2. Overview	2
3. Operating Instructions	3
Power On	3
Cube Flipper – use Single or Dual excitation	3
Filter Flipper – use of ND filters	3
Changing Excitation Cubes	4
4. Electrical Connections	4
Power	4
System Interface	4
5. Maintenance	5
Beam alignment	5
Excitation filter and dichroic mirror replacement	8
Xenon Bulb Replacement	9
6. Epifluorescence adapter	13
Overview	13
Adjustment of excitation area	14
7. Technical Specification	14

1. Introduction

The heart of the IonOptix fluorescence system, whether it uses photometry or fluorescence imaging, is the light source. The light source holds the filters that provide the excitation wavelengths, the switching mechanisms that allow multiple wavelengths and the hardware to synchronize excitation with acquisition.

The IonOptix HyperSwitch™ Light Source was designed with dual-excitation fluorescence indicators in mind. It can continuously switch between a pair of excitation wavelengths at a sufficiently high rate to characterize intracellular calcium transients using the fluorescence indicator fura-2. Wavelength switching is accomplished in less than 1msec and can be continuously alternated at rates as high as 250 pairs/sec. Xenon arc light is routed through the optical system that utilizes a galvanometer driven mirror and is focused on a liquid light guide. The light source's dimensions (8 x 8 x 8") and liquid light guide coupling provide easy connection and setup with any epifluorescence microscope.

The HyperSwitch Light Source Includes

Xenon Arc Sub-system – Includes standard arc lamp housing, igniter and power supply and serves as the source of 75 watts of polychromatic light. Usable wavelength range is 300-700nm and is limited only by the availability of band pass filters

Light Guide - A liquid filled light guide delivers the excitation light to the microscope providing vibration and electrical isolation and flexible position options.

Microscope Coupling - Connects the light guide to your microscope's epifluorescence port. Couplings are available for all common microscopes or can be custom built for your particular scope.

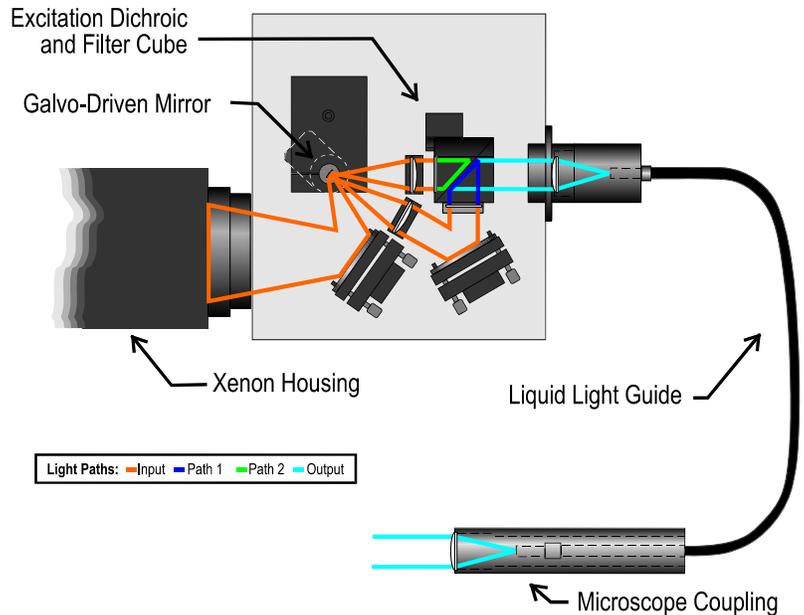
Excitation Optics - Excitation filters of your specification are included with each system and are held in a filter cube. The cube can be externally "flipped" out of light path for conventional fluorescence excitation. A manually controlled, 6-position filter wheel in the common excitation path provides a convenient means to select between neutral density filters (a 5-piece ND set is provided with HSW300).

Emission Filter / Dichroic - The appropriate emission filter and dichroic mirror is provided for your microscope.

Cables - Includes cables to connect to the Fluorescence System Interface.

2. Overview

The first figure illustrates the optical path and components of the IonOptix HyperSwitch Dual Excitation Light Source. The light output of a xenon arc bulb forms a converging beam due to the properties of a parabolic reflector in the source housing. This beam is then focused on a high-speed galvanometer controlled mirror. This mirror directs the now diverging light to one of two collecting lenses where it will be collimated. One path is sent straight into a dichroic cube where it encounters an excitation filter and then passes through the dichroic mirror towards the collecting lens at the end of the light guide. This 'straight through' path is referred to as Path 2 or Denominator path in IonOptix software. In the other path, it is steered into the dichroic cube at a 90-degree angle where it encounters a second excitation filter and is then reflected by the dichroic mirror towards the light guide collecting lens. Before exiting the box, the excitation light encounters a manually controlled, 6-position filter wheel that is typically used to hold a set of neutral density filters. The diverging light coming out of the end of the light guide is then collimated for use in any standard microscope epi-fluorescence port.



3. Operating Instructions

Power On

The power switch for the HyperSwitch is on the same side of the box as the xenon housing. It is recommended that power be turned on after the power for the Fluorescence System Interface. Conversely, power should be turned off to the HSW before switching off the controller's power. This insures that the HSW's internal servo-amplifier that drives the galvanometer has a stable control signal on both power-up and power-down.



Turn ON the HyperSwitch power AFTER turning on the FSI



Turn OFF the HyperSwitch power BEFORE turning off the FSI

Cube Flipper – use Single or Dual excitation

For experiments using single excitation fluorescence indicators, the HyperSwitch provides a mechanism to remove the Excitation Dichroic and Filter Cube from the light path. A knob on the arc-housing side of the HSW labeled Cube Flipper performs this task. The Cube Flipper has 2 positions: in one, the cube-optics are in the light path; this is when the knob-indicator points to the Cube Flipper label. This position is used for dual-excitation experiments. The second position is obtained by rotating the CF-knob counter-clockwise thereby “flipping” the cube out of the light path (so the knob-indicator points up). In this position the HyperSwitch presents the broad-band white light of the xenon arc directly to the light guide when the beam is steered to the “Path 2”.



Figure 1 - Cube Flipper Knob, Single Excitation cube position (up), Dual Excitation cube position (down)

Filter Flipper – use of ND filters

A knob labeled Filter Flipper on the light output side of the HyperSwitch box provides a manual control of a six-position filter wheel in the final output path of the excitation light. In the standard configuration of the HSW, five of the six positions contain neutral density (ND) filters. ND filters serve to attenuate the amount of excitation light by a fraction that does not depend on the wavelength. The ability to attenuate the excitation is handy in conditions where the fluorescence emission saturates the sensor (usually a photomultiplier tube).



The Filter Flipper Knob indicates the current filter position. In addition to the 6 filters, there are 3 “shutter” positions as well. The shutter positions physically block all light output from the HyperSwitch box.

The following table shows standard configuration of the ND filters and their percent transmission

Flipper Position	Neutral Density Value	% Transmission
1	No filter	100
2	ND 0.2	63
3	ND 0.3	50
4	ND 0.6	25
5	ND 1/0	10
6	ND 1.3	5

Changing Excitation Cubes

All of the excitation optics for the HyperSwitch are held in the excitation filter cube. If you frequently change excitation filter sets it is recommended that you use separate cubes for each set. To change the filter cube loosen the screw that holds the cube into the hold the rotate the cube to the up position using the Cube Flipper. Once in the up position slide the cube away from the filter wheel until it is free from its mounting rail.

When installing the cube make sure to position the cube so that the output (empty) position of the cube is facing the filter wheel. Slide the cube back onto the rail, rotate it to the down position, and then tighten the screw.

4. Electrical Connections

Two electrical connections are required for proper HyperSwitch operation.

Power

AC power entry and associated fuse holder are accessible on the back panel of the Filter Wheel Controller. The μ Step is supplied with a US-standard power cable which uses a NEMA standard power cable entry. The μ Step controller uses a universal voltage power supply and will automatically adjust to any main voltage between 110 and 240 volts AC.

System Interface

The connection to the IonOptix software is made via the 25-pin, DSub connector (labeled “System Interface” in the back-panel artwork.) Connect this to the matching 25-pin connector on an IonOptix Fluorescence System Interface.



Do not connect anything except an IonOptix System Interface to the 25-pin port!

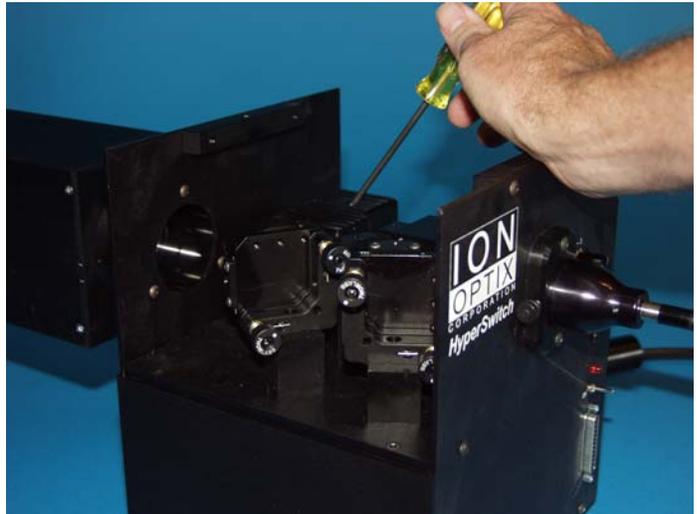
5. Maintenance

Beam alignment

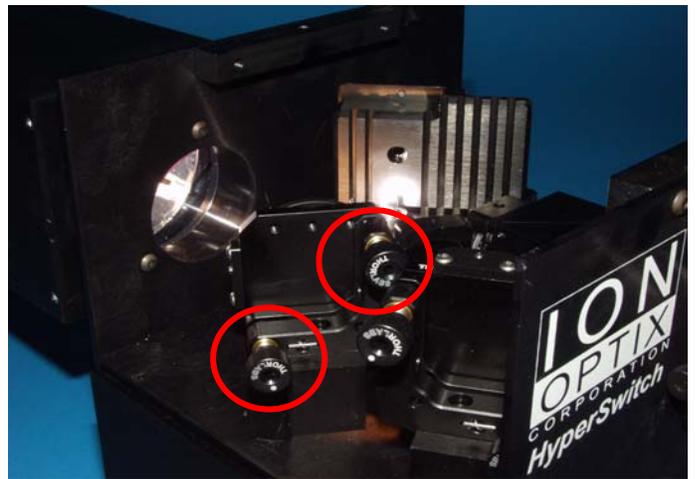
The following instructions explain the procedure required to align the beams in the HyperSwitch light source. It assumes that the lamp in the Cairn housing as been properly focused and attached to the side of the HSW.

Adjust 1st Beam Steering Mirror of HyperSwitch light source

1. Picture shows cover off and the removal of the screw that holds the cover over the galvo mirror with a 9/64" hex tool.

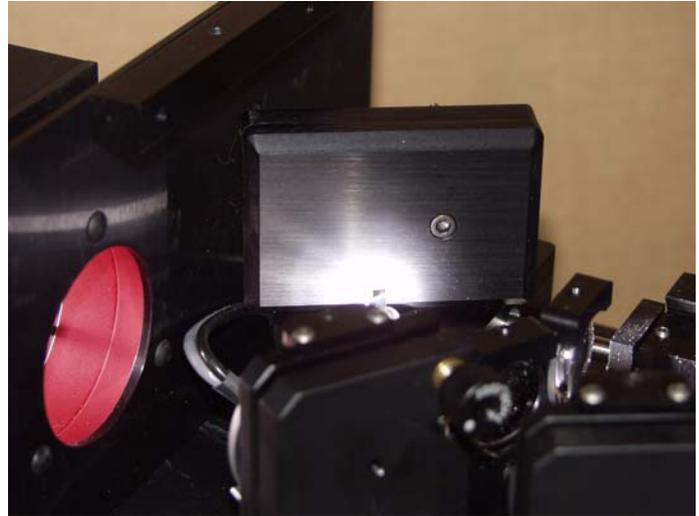


2. Thumb screws "steer" the beam in XY



3. Use the cover as a “target: for the light as it reflects off the 1st mirror in the HSW.

The idea here is to adjust the 1st mirror using the thumb screws on its holder to present the beam as centered as possible with respect to the small mirror on the galvo.



4. Though it's a little hard to see in this picture, what you want to achieve is to have the shadow cast by the galvo mirror on the cover be centered in the annulus of light that surrounds it.



Adjust 2nd Beam Steering Mirror

1. Remove the Light Guide and its adapter from the side of the HSW.



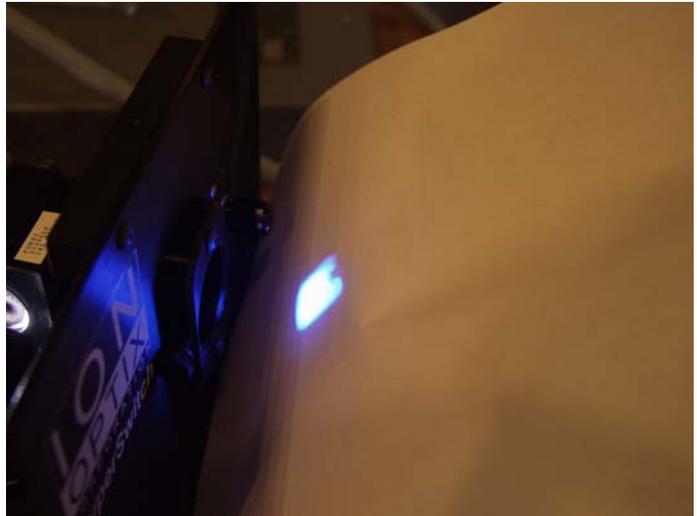
- Picture shows adjustment thumbscrews of the 2nd beam steering mirror.



- Use a piece of white paper as a target to look at the beam as it emerges from the side of the HSW.

The goal is to have the 340nm excitation beam overlap with the 380nm excitation beam. The easiest way to see this is to run an experiment where the HSW is driven switch rapidly between excitation wavelengths.

While looking at the two alternating beams, adjust the 2nd mirror to achieve a “centered” overlap.



Excitation filters and dichroic mirror replacement



Do NOT touch the center of the filters with your bare hands. Hold them by the edges or in a piece of lens paper.



Do not clean filters with glass cleaner or other solvents.

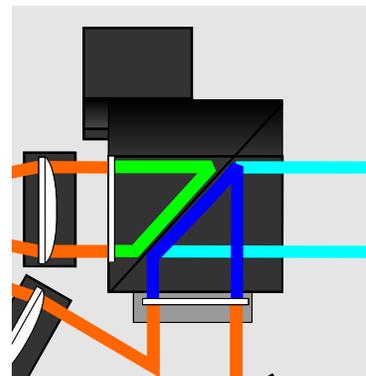


Do not use compressed air cans to clean unless they are marked “Safe for optics”.

When describing how to replace the excitation will be using position terms that are relative to the position of the cube when it is correctly installed into the HyperSwitch and the Cube Flipper is in the down position. The figure to the right is a close up of the cube in the system overview figure. This figure shows the two paths the light take through the cube in the HyperSwitch.

Path 1: white light (orange lines) enters the cube from the front of the cube (bottom of the figure) and goes through the excitation filter. The filtered light (blue lines) then hits the dichroic and is reflected out the common output path (cyan lines).

Path 2: white light (orange lines) enters the cube from the left side the cube and goes through the excitation filter. The filtered light (green lines) then passes through the cube dichroic out the common output path (cyan lines).



Once you understand the light paths through the cube, replacing the filters is pretty straight forward. On the bottom of the cube you will find a small set screw the holds the filter into the cube. Loosen this screw with a .050” (1.27mm) hex tool and the filter should drop out. When replace the filters make sure that the arrow printed on the edge of the filter is pointing “into” the cube.

To replace the dichroic mirror remove the four silver set screws that hold the two halves of the cube together. Two are located on the front edge of the left side of the cube and the other two are on the right edge of the back side of the cube (the one with the slot). The dichroic mirror is held to one of the halves with two white plastic set screws. Remove the screw that has not been notched. Lift the mirror out and replace with the new mirror.

Xenon Bulb Replacement

The following instructions explain the procedure required to replace the 75W bulb in the Cairn Housing.

Remove Xenon Housing from IonOptix light source

1. Use “red handle” hex tool (it’s 1.5mm) to loosen set screw(s) that secure the housing to the light source coupling ring



2. Usually only 1 or 2 of the 4 set screws are used to hold the housing



Open Cairn Housing and Replace the Bulb

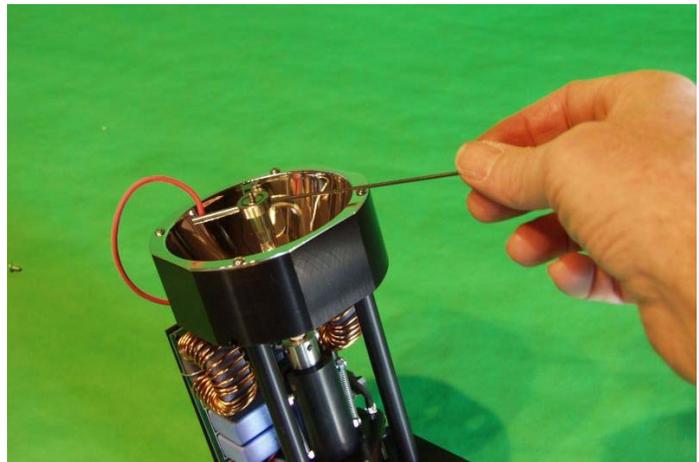
1. Remove 4 screws with Philips screw driver



2. Slide the rear plate out

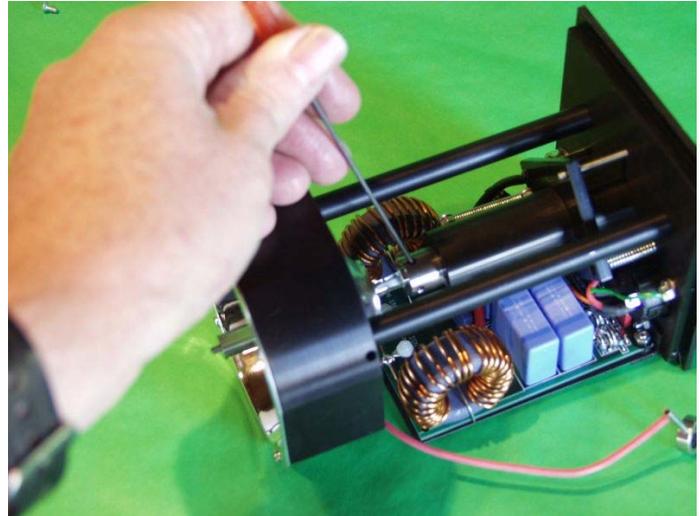


3. Loosen set screw that holds the cathode adapter



4. Loosen anode set screw and remove old bulb.

Reverse steps with new bulb



6. Point the stem of the cathode assembly towards the corner of the housing. This permits its wire to slide in more easily



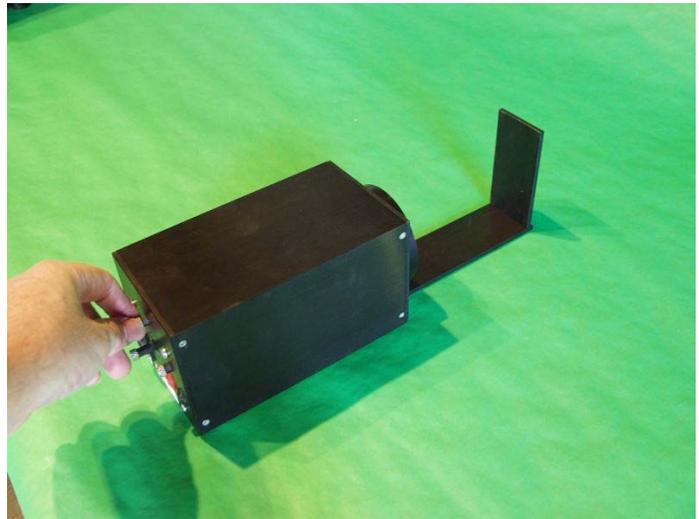
Focus new bulb

The L-Shaped, Arc Focusing Jig is provided to make initial focusing of the xenon bulb easier.

1. Put the Cairn housing on the bench. Ignite the arc and place the Arc Focusing Jig against the front of the housing



2. Adjust the 3 silver focusing knobs on the back of the housing to achieve the smallest, centered spot on the Arc Focusing Jig.



Replace the Housing on the IonOptix Light Source

Holding the housing tight against the coupling ring with one hand, use the other hand to tighten one or two of the set screws.

6. Epifluorescence adapter

Overview

The epifluorescence adapter creates the collimated beam needed by the microscope's optics from the light coming out of the liquid light guide. In addition it also provides an internal circular aperture for restricting the area of the microscope field that is illuminated by the excitation light.

The adapter consists of three main parts:

1. Microscope specific adapter
2. Light guide collimator assembly
3. Liquid light guide

The microscope adapter (the outer round piece in the photo) attaches to your microscope's epifluorescence port and provides a mounting point for the Light guide collimator assembly.



The shape and attachment method of the microscope adapter will vary by microscope brand

The collimator assembly has the following parts:

1. Light guide carrier
2. Carrier tube
3. Circular aperture
4. Lens tube
5. Lens

As illustrated in the figure to the right, the light guide is held into the light guide carrier by a small set screw (top figure). The carrier is then held into the carrier tube by a 2nd small set screw in the tube (middle figure). Together these are Part B.

The lens tube holds the collimating lens on one end and a circular aperture on the other (Part A). There is a small arm that sticks out of the aperture end of the lens tube that must be aligned with a slot in the end of the carrier tube (Part B). Once Part A and Part B are connected 3 set screws in the outer edge of the carrier tube holds the two parts together. The completed assembly along with the output light path (blue) is shown in the 3rd figure. When the screws are loosened rotating Part A and Part B will open or close the aperture as shown in the 4th figure.

As show in the 3rd figure above the excitation light exits the liquid light guide and encounters a circular aperture (red). This aperture restricts the area of the microscope field that is illuminated by the excitation light. The idea here is to present the excitation light only to the area where cells can be studied. In the case of the standard IonOptix fluorescence/ contractility system, this is a circular area that is "carved out" by rotating the rectangular aperture of the Cell Framing Adapter. Restricting the area of illumination decreases the background signal and the auto fluorescence of the fluorescence objectives themselves.

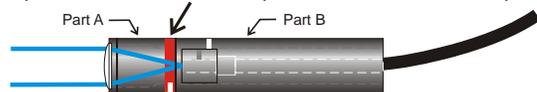
Light guide held into carrier by set screw



Carrier held into adapter by set screw



Circular aperture is connected to part A and Part B of adapter



3 set screws hold part A and part B together
loosen slightly to allow movement, tighten when done

Rotating Part A relative to Part B opens and closes aperture



Adjustment of excitation area

The excitation aperture is shipped at an “average” setting and is usually checked and, if necessary, adjusted at the time of the system installation. As discussed above the iris is adjusted by loosening the screws and rotating part A relative to Part B. This mechanism was not designed to be easily adjusted rather it is meant to be set infrequently and fixed in the ‘correct’ position.

Judging the area of illumination is most easily done with a low magnification objective (like a 10x) and a piece of white paper. The process is as follows:

1. Remove the light guide collimator assembly from the microscope adapter
2. Loosen the set screws (one on each side) holding Part A and Part B together and close the aperture to its minimum position (this is done by holding the assembly with the lens pointed towards you and then rotating the collar clockwise)
3. Lightly tighten the screws holding part A and part B together
4. Install the light guide collimator back in the microscope.
5. Use the acquisition software to project one of the excitation wavelengths from the Excitation light source
6. Using the 10x objective it should be possible visualize a small, circular area of illumination roughly centered in the microscope’s field of view.
7. Now, repeat the process after opening the aperture a small amount.
8. Repeat this process until the edge of the illumination area is just within the microscope’s field of view.

This is the largest area that ever needs to be illuminated and is a reasonable starting point. Depending on the particular type of recording done by the system, it may be possible to further restrict the area of illumination

7. Technical Specification

Standard Components

1. IonOptix HyperSwitch box
 - A. Input Power: 110-220VAC, 50-60Hz, 1.5A fuse
 - B. Active element: Galvanometer-driven optical scanner/servo amplifier sub-system
 - C. Control signal: via 25 pin DSub connector
 - D. Switching rate: 1msec between wavelengths – 250 ratios/sec
 - E. Internal optics: 1st surface, protected aluminum mirrors and fused silica lenses – optimized for UV throughput
 - F. Excitation filters: for Fura-2, narrowband filters from Chroma Technology
 - G. Excitation output: fused silica lense condenser to liquid light guide (1m in length w/ 5mm diameter active core)
2. Epifluorescence adapter: common element produces infinity projected beam via adjustable, circular field aperture. Microscope specific adapter provided
3. Cairn 75W Continuous xenon housing and power supply