**Methods & Materials for building the Fluorescence Macroscope:**

***Description:***

The fluorescence macroscope is a device capable of taking large format pictures in JPEG or RAW format (@ 300 dpi) of fluorescent or non-fluorescent biological specimens. It works on the same principles as a fluorescence microscope, simply using larger parts and a less direct lighting source.

***Physical Operation:***

First the biological sample is excited with light that corresponds to its fluorophores excitation wavelength. The sample absorbs the light at that wavelength (exciting the fluorophore) which then re-emits photons at a higher wavelength (thus the system tends towards higher entropy and obeying the second law of thermodynamics). Second the higher wavelength emitted photons pass through a bandpass filter above the sample which allows only specific wavelengths of light (particularly the emission wavelength of the fluorophore) through. Finally the filtered light is captured by a detector (in this case a sensitive DSLR camera). The camera digitizes the image and sends it to the computer for storage.

***Visual representation and dimensions:***

Can be obtained from the Sheryl Rakowski or Macroscope desktop in information about macroscope folder

***Materials necessary to build:***

*Custom Box:*

3 sheets of 5x8 plywood were used to construct a wooden box of dimensions:

24”x22”x28”

The box exterior was stained and the interior was painted flat black. An 18”x23” door with hinges was installed and similarly painted.

An equal height (24” long, 1” diameter) steel plumbing pole was mounted to the top and bottom of the box with plumbing fixtures 4” from the left side of the box. The pole serves as a sturdy mounting arm for the DSLR camera and filter wheel. These items are attached to the vertical pole through 90 ° two way couplers placing the camera filter wheel perpendicular to the upright pole. A half inch hole was drilled in the side of the box to allow wiring to be run within powering lighting and transferring data to the computer.

Lights were located perpendicular to the vertical pole (on the user right side of the box) at a height of 13” from bottom. They were centered and mounted horizontally on swiveling ball sockets which can be obtained at: <http://www.amazon.com/Mini-Thread-camera-tripod-ballhead/dp/B007MZCXN8/ref=pd_sim_sbs_p_1>

*Lighting Technical Information:*

**Lights used:**

1. MR16-WW48SMD Warm White 3.2 lm/led 260 lm 2900 K 285mA

2. MR16-B48SMD Blue 800 mcd/led 38400 mcd 474 nm 265mA

3. MR16-G48SMD Green 1200 mcd/led 57600 mcd 520 nm 285mA

Raw Information on warm white light

Base GX5.3 (MR16) Beam Pattern 120 degree

Bulb Type MR16 Comparable Wattage (incandescent) 30~35 Watts

Dimmable Yes

LED Quantity: 48 LEDs LED Type: 3528 SMD

Lens Color: Clear Lumens: 260 Lumen

CE/FCC/ROHS Compliant

Total Power Consumption 3 Watt Type Bi-Pin

Operating Voltage Range 9~14.8 V AC/DC

For more information or ordering go to:

<http://www.superbrightleds.com/moreinfo/boat-rv-bi-pin/mr16-bulb-gx53-base-120-degree/467/>

Appropriate bases were attached to the lights:

<http://www.superbrightleds.com/moreinfo/empty-bases-sockets/led-product/499/>

*Wiring:*

The lighting system runs on at 12VDC, 4A (amps max) power converter. It operates at only ~300 milliamps max output to the lights (a resistor was placed in the electrical circuit), all LED’s are rated for near that amount. A 1.5 A (amp) fast blow fuse was placed in the circuit between the power converter and the distribution box (for safety of the system). A full wiring diagram can be found in the information about macroscope folder.

A 12V powered USB controlled relay (Hivitronix HR1208U4x2) was used to turn on and off lights (by grounding the parallel wired lighting, see diagram) using the manufacturer’s software.

~20 ft of 18-awg black and red wire was used to wire the system

Wires were soldered together for the connections between the power converter and distribution box and all other connections were made with Calterm butt splice joints (18-22 awg).

*Camera and Lens:*

The DSLR camera used is a Nikon D5200 body with a NIKKOR Lens AF-S Micro NIKKOR 60mm f/2.8G ED

The camera body has extremely high resolution (24.1 Megapixels) and good ability to pick up dim light. The lens is specifically designed for short range photography.

*Filterwheel and Filters:*

The starlight express filter wheel is a 5 position filter wheel with a carousel holding 50-50.8 mm unthreaded filters (small screws can be used to install new filters). It is considered a windows Human interface device (HID) and therefore can be powered and communicated with purely through the usb port connection. The device can be found:

<http://www.sxccd.com/sx-usb-filter-wheel>

*Filters:*

Were obtained from Edmund optics for the filtration of GFP and RFP emission wavelengths coming off biological samples.

<http://www.edmundoptics.com/optics/optical-filters/bandpass-filters/fluorescence-bandpass-filters/67-044>

<http://www.edmundoptics.com/optics/optical-filters/bandpass-filters/fluorescence-bandpass-filters/67049>

The GFP and RFP bandpass curves (which wavelengths of light they allow through) can be obtained from at Edmund optics website at the above links or in the information about macroscope folder on the desktop of the Macroscope user.

*Computer:*

A Dell Optiplex GX620 with Pentium D processor 3.4 GHz, 4 GB DDR2 ram and 1 TB hard drive running Windows 7 (32 bit version) were used to operate the camera and software.

*Software:*

Three forms of software operated simultaneously are required to operate the macroscope. They include:

digicamControl:

Obtainable free from <http://digicamcontrol.com/>

Hivitronix user device interface: used to turn on and off lighting through relays. Obtained from manufacturer when purchasing product.

SXFilterWheel device interface:

Obtained with device purchase or from <http://www.sxccd.com/sx-usb-filter-wheel>

Lab specific information:

Choosing more filters or lights

As you’ve probably read the filter carousel has room for 3 more filters. You may like to use those slots to detect other specific fluorescent proteins.

To choose bandpass filters and excitation lights I would recommend deciding which wavelengths to filter out and which to allow through based on the following website:

<http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cell-Analysis/Labeling-Chemistry/Fluorescence-SpectraViewer.html>

It will allow you to choose the specific fluorescence proteins you’d like to see, their excitation and emission wavelengths. You can then choose if the lights we are using will excite your specific fluorophore or if you need another wavelength of light (the lights we already have in may be sufficient if you use anything red, yellow, green or orange as a fluorescent protein). Check the excitation wavelength of your fluorophore at Invitrogen to be sure the LED emits light that will excite it. Then based on the maximum emission and what color you’d like the colonies or cells to appear choose your bandpass filter (I recommend Edmund Optics 50 mm fluorescence filters as above). Ensure you do not allow through exciting light from the LED, only allow through the emission wavelength. This will ruin your ability to distinguish between fluorescent and non-fluorescent samples! (See physical operation for more information)