**Assembly Instructions and Troubleshooting Guide**

1. Overview

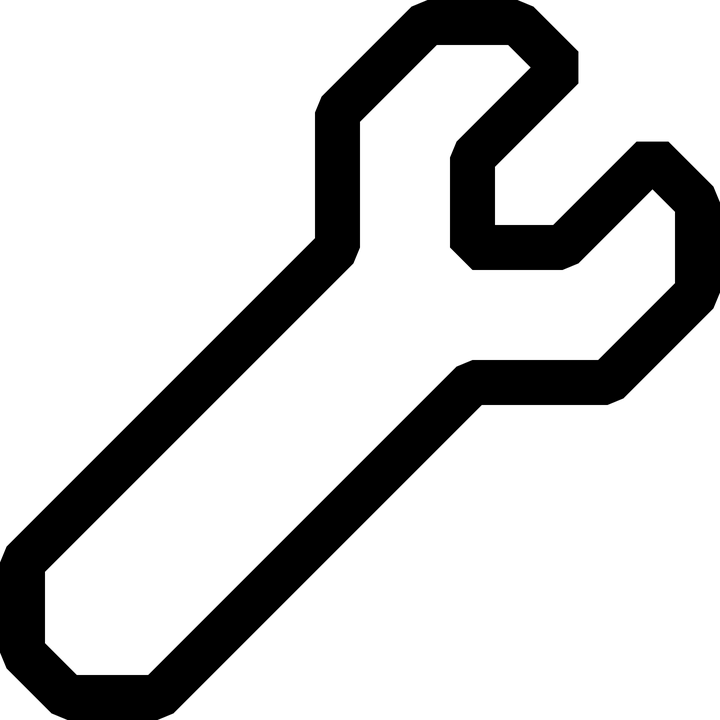
This document provides instructions on how to build the open-source CICLoPS photometry system with explicit reference to the appropriate design files provided. The subassemblies are largely independent of other subassemblies; meaning that they can be developed and tested in parallel if necessary, with a few exceptions. The entire system can be built and tested in 40 hours of work, requiring minimal experience in optical assembly or electronics.

Throughout this document, the following symbols are used:

|  |  |
| --- | --- |
| Symbol | Meaning |
| Free vector graphic: Wrench, Hardware, Tool, Workshop - Free Image ... | tools needed to complete the subassembly in a given section |
| Free vector graphic: Attention, Warning - Free Image on Pixabay ... | common problems and causes |
| File:Green check.svg - Wikimedia Commons | verify that the subassembly is working properly before moving on |

1. Subassembly 1: Optical system

The major components of the optical system are the excitation light source, the PMT detector, the optical tether, and the dichroic mirror. A light-tight cage cube system with lens tubes houses collimating, coupling, and focusing lenses while isolating the system from stray light. The time to build the system is approximately 8 hours.

 **Tools needed**

Drill press

Alan key ”

SPW602 spanner

½” spanner

Terminal block screwdriver

Current source

Optical Power Meter (PM-100 USB)

White light source

Oscilloscope

Soldering iron

Multimeter

Computer

* 1. LED Light Source

The light source is based off of a simple surface mount LED (*LXML-PB02, Lumileds*). To make this part, first fabricate the LED plate by cutting a 1”-diameter hole into perforated prototyping board, using a drill press and a 1” hole saw. Cut 2- 12” lengths of wire wrap (*R-30B-0050 (blue) and R-30W-0050 (white), Jonard Tools*), stripping ⅛-inch from the tips. Solder one wire to each of the LED pads as shown. Twist the wire wraps together. The LED may now be powered with either a continuous or modulated current source.

* 1. PMT Detector

The PMT (*H10771, Hamamatsu*) transduces light incident on its photocathode into an electrical current. It will be interfaced directly with the optomechanics to collect photons emitted from the fluorescent sample. The two coaxial cable leads are for high-voltage negative power supply and the output current. This section describes the assembly of the high voltage power supply module.

**Design Notes:** The design is based of the application in the C4900 datasheet. A voltage divider resistor network prohibits the controlling voltage to exceed ~3.3 V, limiting the high voltage output to a maximum of ~830 V. This is within the safe operating region for the selected PMT.

First, make the enclosure according to hvsupply\_enc.dae. We recommend a 2” length of 2”x 2” aluminum tubing (*6546K42, McMaster Carr*) and sheet aluminum to cover the open ends, using 3/8” set screws to secure the end plates.

Next, make the controllable power supply circuit for the C4900 high voltage power supply from Hamamatsu. and is provided by hvsupply\_schem.SchDoc. It is implemented on an 1.8” x 3.8” section of perforated circuit board. Leave approximately 3” of stranded wire for each of the VIN and HVOUT leads for soldering to the BNC and jack plug connectors on the enclosure. Position the rotary potentiometer near its knob’s hole in the enclosure. We recommend providing longer leads for its terminals for ease of positioning.

[insert annotated figures here of built power supply circuit and enclosure]

**Hamamatsu power supply**

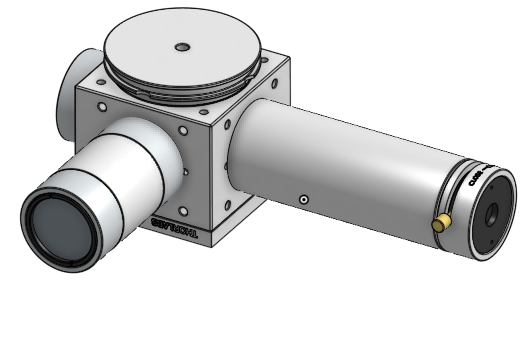
The high voltage output should vary linearly with the number of turns on the potentiometer, at a rate of ~90V per turn. Use a voltage divider circuit to read HVOUT with a conventional multimeter without surpassing its maximum operating range.

* 1. Optomechanics

The optomechanics may be thought of as having an excitation input arm, a fiber tether output arm, and a detector arm that converge on a single cage cube (*C4W, Thorlabs*). The excitation beam is launched into the system via a modulated LED and collimation lens. This beam is then reflected into the optical fiber tether output by the dichroic filter (*T495LPXRU, Chroma Technologies*). After interacting with the sample, a mix of reflected excitation and fluorescent photons travel back to the system via the optical tether, and are separated at the dichroic mirror. In the detection arm, the beam’s spectral properties are narrowed again by the emission filter (*ET470/40X, Chroma Technologies*) and then focused onto the PMT detector by the focusing lens (*AC254-040-A, Thorlabs*).

The accurate model of the optical system (optical\_system\_assembly.dae) contains exact relative positions between components in the optical system. We recommend that the builder first familiarize themselves with this model and ensure they have all parts before getting started. We recommend building outward from that cage cube one ‘arm’ at a time, as explained here. CICLoPS is an Imperial setup and is not compatible with metric optomechanical parts.

Design Notes: The use of the cage system drastically simplifies the task of aligning the beam. The use of lens tubes reduces the amount of stray light that leaks into the optical system.



***Excitation input arm***

First screw a ring retainer in 0.5” lens tube (*SM1L05, Thorlabs*) such that it is flush with the optic retention lip. Then place inside the excitation filter (ET470/40x), ensuring that it is oriented properly (the arrow on the side should point towards the retention lip, as this is the direction of the beam). Secure the filter with a second retaining rings (*SM1RR, Thorlabs*). We recommend using a spanner wrench (*SPW602, Thorlabs*) to screw the retaining rings into lens tubes, as they enable accurate distance measurements. Screw the excitation filter assembly onto any one of the four 1” tapped holes.

Next, build the collimation assembly. Screw a 0.5” retaining ring (SM05RR) into the into the 1” - 0.5” adapter (*SM1A6T, Thorlabs*), such that it is flush with the other end of the adapter. Place inside the focusing lens, such that the convex surface touches the retaining ring. Secure the lens with a second retaining ring. Next, use the 1/16” Alan Key to position the adapter within the 1” lens tube (*SM1L10, Thorlabs*), ensuring the convex surface of the lens faces the retention lip of the lens tube. The distance between the adapter and the mouth of the lens tube should be 10 mm. Use the spanner wrench to check that the adapter itself is positioned 10 mm from the mouth of the lens tube. Fasten the collimation assembly onto the excitation filter assembly.

Finally, build the excitation assembly. Screw a retaining ring approximately 3 mm into the mouth of a 0.5” lens tube (*SM1L05, Thorlabs*). Place inside the LED plate and secure with a second retaining ring. Fasten the excitation assembly onto the collimation assembly.

***Fiber tether output arm***

Secure the collimation package (*F240FC-532, Thorlabs*) into its adapter (*AD12F, Thorlabs*) with the set screws and the allan key. Screw the adapter all the way into the 0.5” lens tube (*SM1L10, Thorlabs*) such that it touches the retention lip. Secure in place with a second retaining ring (*SM1RR, Thorlabs*). Fasten the fiber tether output arm onto the cage cube, 90 degrees to the left of the excitation input arm.

Next, build the dichroic mirror assembly. Pull a gap between the spring-loaded clamping mechanism of the filter mount (*FFM1, Thorlabs*) and place the dichroic mirror in between, as in Figure xx below, noting the position of the caret. Gently release the clamping mechanism. Next fasten the filter mount onto the cage cube platform (*B3C, Thorlabs*), and place into one of the 2” untapped holes of the cage cube. Rotate the platform such that the dichroic mirror is at a 45 degree angle to both of the arms, so that light from the excitation is reflected into the output arm. Fasten the FC/PC connected end of the 200 um optical fiber tether (*MFP\_200/230/900-0.48, Doric Lenses*) onto the mating end of the collimation package.

 **Collimation package keeps turning when attempting to connect FC/PC connector.**

Possible cause: Set screws on adapter for collimation package (*AD12F, Thorlabs*) are missing and/or not tight enough, OR the retaining ring (*SM1RR, Thorlabs*) was forgotten.

***Detection arm***

Place the emission filter (*ET510/20, Chroma Technologies*) all the way into the 3” lens tube (*SM1L30, Thorlabs*) to the optic retention lip and secure with a retaining ring. Using the spanner wrench, fasten another retaining ring 30.5 mm into the lens tube, and place the focusing lens (*AC254-40-A, Thorlabs*) adjacent to it, ensuring the convex surface of the lens faces the retention lip of the lens tube. Secure with another retaining ring. The distance between the retaining ring and the mouth of the lens tube should be 23 mm. Use the spanner wrench to check the position. Fasten the shutter (*SM1SH1, Thorlabs*) and then the PMT onto the detection assembly. Finally, fasten the detection assembly onto the cage cube, into the tapped hole opposite that of the output detection arm. Fasten the end cap (*SM1CP2, Thorlabs*) on the last tapped hole.

**Delivery path alignment**

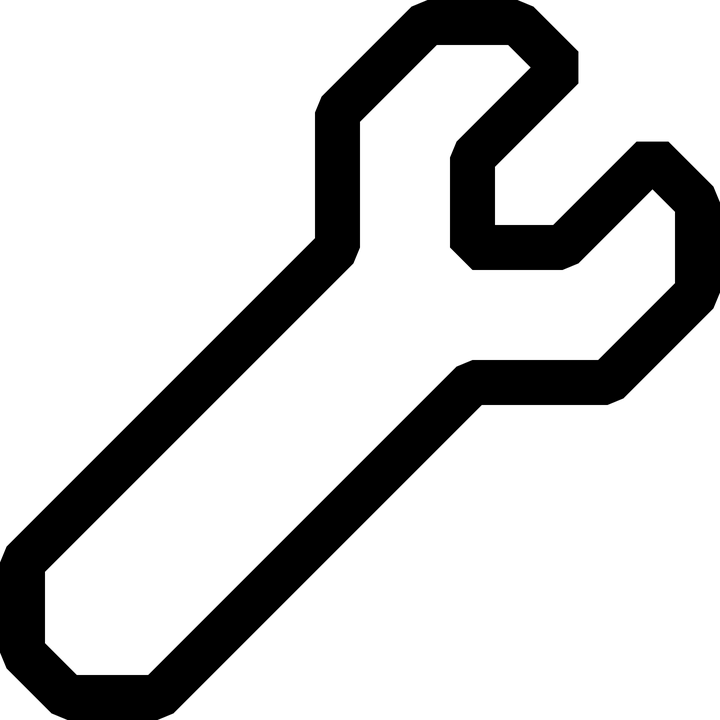
To test for alignment of the beam, supply the LED with 2 mA of continuous current from a current source. Using the optical power meter, check the excitation output of the fiber tether. The dichroic will be at the desired 45 degree angle when the the output is maximized. If the model is built as specified, the system will output xx uW when the LED is supplied with 2 mA of continuous current.

**Excitation path alignment**

After disconnecting the PMT from the optical system, feed a white light into the output of the optical tether such that it propagates back to the system. The spot at the mouth of the remaining detection arm should be xx mm in size, as pictured below.

References

1. Subassembly 1: Lock-in Amplification Scheme

 **Tools needed**

Soldering iron

Vice

Tweezers

Solder wick

High speed photodiode (like PDA36A)

Optical Power Meter (PM-100 USB)

The model of the LIA enclosure is specified in lia\_enc.dae. For the LIA enclosure, we recommend using a 1.625” length of 1.5” x 1.5” ¼”-thick aluminum tubing, and using 1.5”-square sections of aluminum sheet as end plates. Similar to the PMT enclosure, secure the end plates with set screws.

First, populate the fabricated lock-in amplifier PCB (specified in pcb\_layout.PcbDoc) as shown in pcb\_assembly.pdf. Drill a hole in the pcb, avoiding the traces, to allow for a set screw to separate the PCB from the enclosure. Use 2” lengths of wire wrap to provide signal lines for **sig out**, **mod**, and **pmt in.** Solder these to their respective female BNC connectors (31-221-RFX, Amphenol RF Division) secured to the enclosure.

**Lock-in amplifier power supply**

**Light source modulation**

1. Subassembly 3: Software
   1. Data Acquisition

Both modulation/and demodulation and acquisition of analog data are accomplished with the data acquisition card (*USB-6351, National Instruments*), using its analog output and analog input, respectively. The daq operations are provided by C++ code developed in Microsoft Visual Studio. The v\_write solution enables acquisition of analog data, while lockin\_amplifier controls the modulation/demodulation scheme. To collect data coherently, start the lockin\_amplifier first (open Lock-in Amplifier.sln), and then begin acquiring data (V\_Write.sln).

Design notes: The modulated voltage waveform generated by lockin\_amplifier and the daq has offset of zero and amplitude of 9.9. This is the maximum amplitude given the daq’s output range. This waveform is fed directly to the op-amp LED driver. The acquisition range on the daq is set to [-1,1], which is good match to the maximum output range of the lock-in amplifier [0,0.5].

lockin\_amplifier

The solution generates a terminal window for user input. The user need only accept each of the prompts to begin generating the modulation/demodulation waveforms to the lock-in amplifier hardware.

v\_write

The solution generates a terminal window that prompts for user input on filename. After accepting the remaining prompts, the window generates a real-time display of the incoming data, with a 100 sample averaging operation applied. The user can modify this display to zoom in (z, Enter), zoom out (o, Enter), or center the trace (c, Enter) about the cumulative average of the collected samples using keyboard inputs to the terminal. The experiment can be interrupted at any time (q, Enter). The data is stored in the same folder as the solution in binary values sampled at 100 samples per second.

The acquired data from CICLoPS is written in 16-bit binary values. In any given channel, both the time and Custom Matlab script (binary\_read.m) are used to convert the data into decimal values. Example *in-vivo* data is provided to test this code.

**References**

Hamamatsu Photonics, June 2012. [Online]. Available: https://www.hamamatsu.com/resources/pdf/etd/C4900\_TACC1013E.pdf.