

ZellChip

User Operation Manual

For Single Cell Bioanalyzer (SCB-402)

Manual Version: 1.3

Doc# 102-SCB-402-1.3

Table of Contents

1.	General Information	1
1.1	How to Use This Manual.....	1
1.2	Operation Safety.....	1
1.3	Product Overview	2
1.4	Product Functions.....	2
1.5	Implementation Constraints.....	2
2.	Basics of SCB-402	3
2.1	Hardware Interfaces.....	3
2.2	Module Description	4
2.3	Optical Features.....	5
2.4	Software Overview	6
3.	Experiment Tutorials	8
3.1	Automated Test Run.....	9
3.2	Microscope Scale-bar Test.....	10
3.3	Fluorescent Bead Test	10
4.	Instrumental Maintenance	11
4.1	Changing Chips	11
4.2	PMT Calibration	12
4.3	General Troubleshooting.....	14

1. General Information

1.1 How to Use This Manual

This manual aims to provide users with overall understanding of SCB-402 regarding its functions, operations and maintenance.

Section 1 contains general descriptions of the system, defining the operation requirements and capabilities for SCB-402.

Section 2 describes the overall system and various modules of SCB-402. Hardware, optics and software operations are discussed in detail.

Section 3 provides step-by-step tutorials on the basic tests that users should encounter when operating SCB-402.

Section 4 lists some common troubleshooting procedures.

1.2 Operation Safety

The points below are intended to enhance your safety awareness and to draw your attention to risks which you, the operator, can prevent. While the instrument is designed and tested to be safe, proper handling is also critical. The operators should be first trained in basic laboratory protocols and they should be familiar with the possible hazards before operating this instrument.

All instrument modifications should be performed only by qualified personnel of ZellChip. Equipment damage, personal injury or even death may result if this equipment is operated, altered or maintained by untrained personnel or in an irresponsible or improper manner.

1.3 Product Overview

SCB-402 is essentially an inverted fluorescence microscope.

Excitation wavelength of the incident light is selected by an excitation filter to irradiate the fluorescent molecules to be measured. When a laser is used as an excitation source, the excitation filter is not necessary. Emission wavelength of the fluorescent light is selected by an emission filter to measure the molecules.

1.4 Product Functions

SCB-402 provides optical bright-field vision or imaging and fluorescent measurement to be conducted simultaneously on a cell or particle. A particle such as a fluorescent polymeric bead in a microfluidic biochip (e.g SCC-001) may be optically viewed and observed while fluorescent measurement of the embedded fluorescent dye in the particle can be monitored. In the case of a biological particle such as a cancer cell, the drug concentration in the cell can be monitored.

1.5 Implementation Constraints

The fluorescent molecules are measured by a photomultiplier tube (PMT) module as signal peaks. The peak intensity depends on how long the laser illuminates the fluorescently labeled molecules. The PMT voltage decreases over time when the fluorescent molecules are in long-time laser illumination due to photo-bleaching effect.

The blue laser provides an excitation source for cells placed in the microfluidic biochip. The PMT will always pick up background noises when displaying data since light scattering by the chip material and reflection by the component surfaces is inevitable. The interior surfaces have been black-anodized to minimize the reflective effect. Background noise will generally be reduced by lowering the PMT sensitivity knob, e.g. from 9 to 8.5.

Accessories like the Chip Holder are fabricated from plastic which is less robust in mechanical strength, as compared to those made from metals.

The Azarel software is developed specifically for SCB-402 which allows users to automate the measurement, viewing and perform data management for experiments. Please refer to the Azarel Software User Manual to fully explore its capabilities.

2. Basics of SCB-402

2.1 Hardware Interfaces

SCB-402 consists of various custom-made components that support its system operation. The pictures shown below will provide the information to user to describe components within SCB-402 when communicating to ZellChip in conditions of services or upgrades.

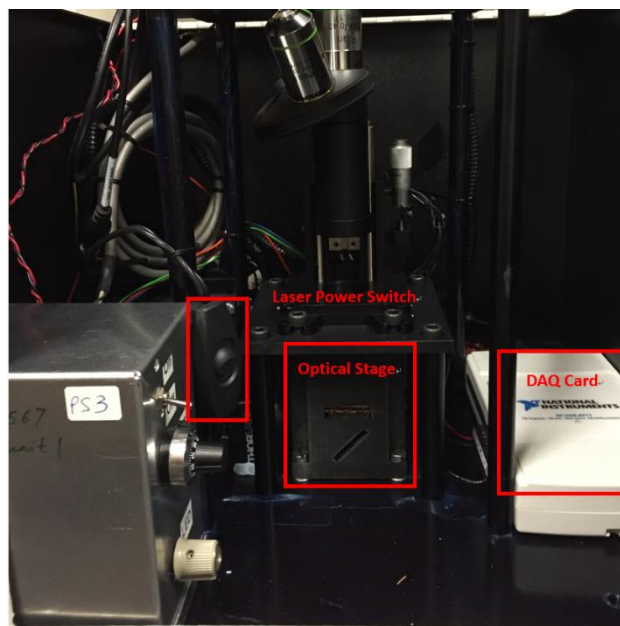


Figure 1: SCB-401 Front View

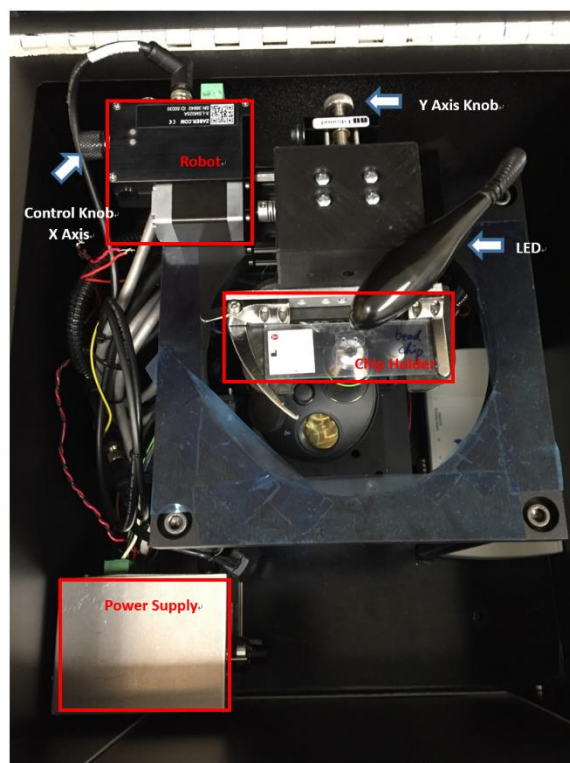


Figure 2: SCB-401 Top View

2.2 Module Description

Data Collection Module:

The intensity of light emitted by laser-excited targets will be converted to voltage signal by the PMT. Signals from PMT are detected using a National Instruments NI6211 Data Acquisition Card (DAQ). The data are displayed on user interface as Voltage vs. Time graphs.

Robot & Robot Control Board:

Driven by stepper motor, the robot travels along the left-right (X) direction. A total travel range is set to be 1" (25.4 mm) away from HOME position. Robot will automatically stop upon reaching either HOME/AWAY locations.

Robot comes with a stepper controller to ensure accurate control of robot position of up to 2 μm accuracy.

2.3 Optical Features

A systematic diagram indicating all optical features and light path is shown below:

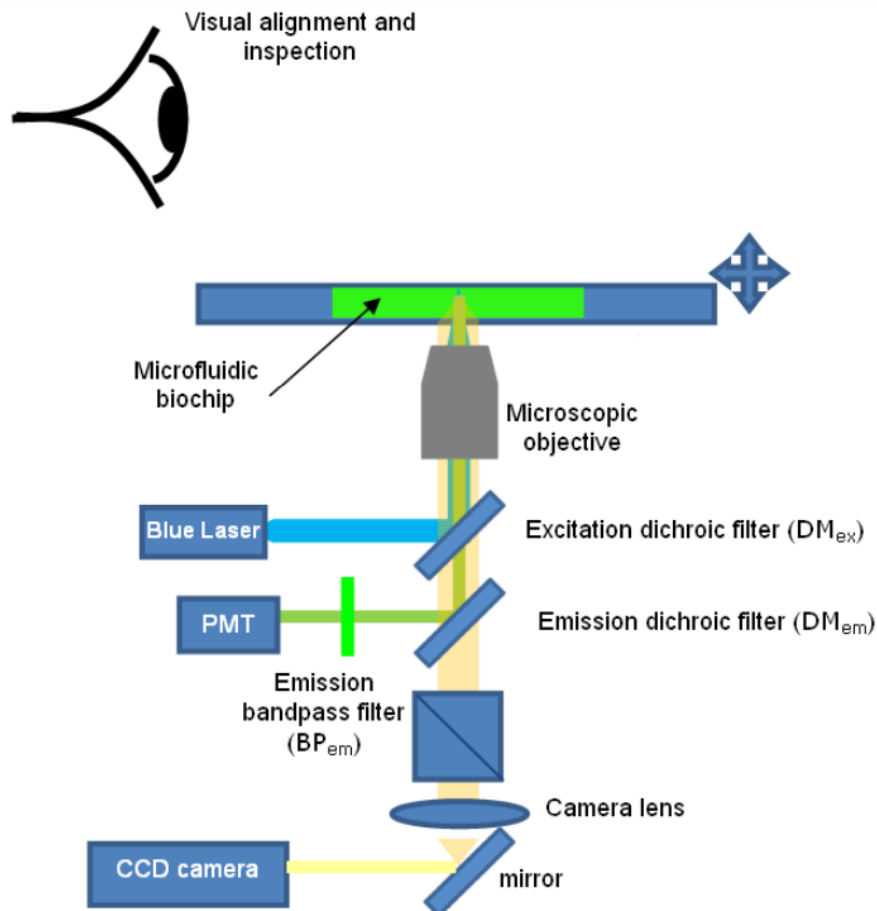


Figure 3: SCB-401 Optical Layout

The Blue Laser shines a laser beam at the excitation dichroic filter (DM_{ex}) through a neutral density filter (OD1.5). This beam will be reflected to the microfluidic biochip and excite the fluorescent molecules inside it. These molecules will emit fluorescence which will pass through DM_{ex}, but reflected by the emission dichroic filter, DM_{em} to reach the photomultiplier tube (PMT). The PMT converts the laser signal into an electrical voltage. The camera based on the charge-coupled device (CCD camera) will capture the image of the microfluidic biochip reflected on the camera mirror.

2.4 Software Overview

2.4.1 Azarel for Robot Control and Data Acquisition

Azarel software is a GUI designed specifically to operate SCB-402. It has the function of robot control and data acquisition. Users are able to define parameters in the GUI to run various experiments. Readings are automatically plotted and results can be saved after each experiment is completed. For more details on running the Azarel software, please refer to ***Azarel Software Manual***.

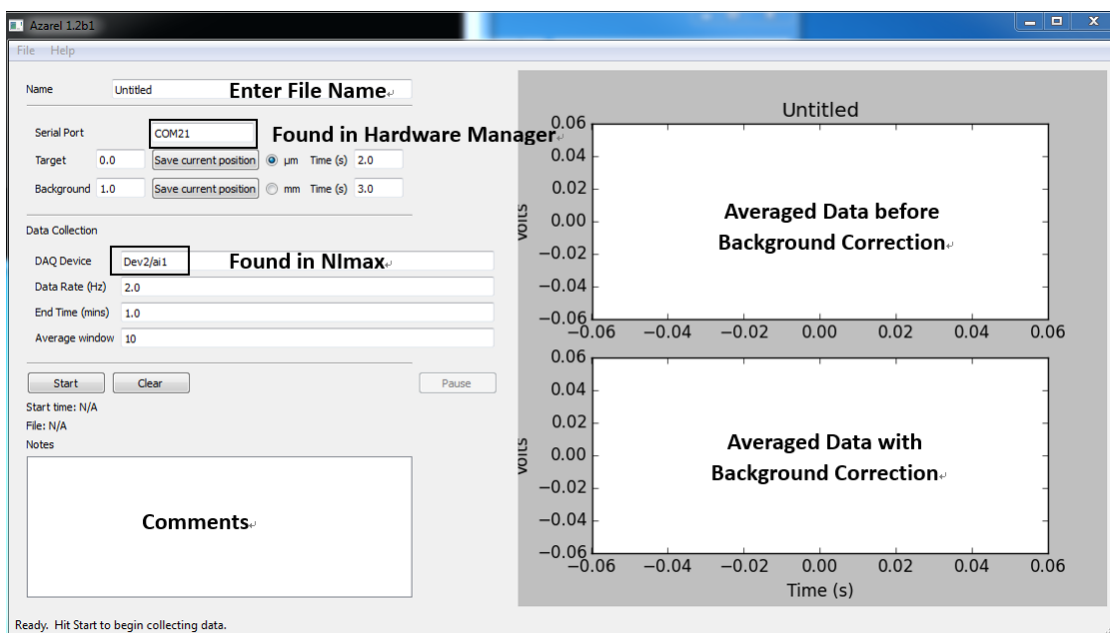


Figure 4 Azarel User Interface

Azarel has 3 features when it comes to saving data:

a) Temporary backup file

Whenever user attempts to start a new experiment, Azarel will prompt users for a saved location (default in the Azarel folder) for backup file. Data is written into the file in .csv format once it is created by users and any data acquired during the run is saved.

b) File -> Save As

After an experiment is completed, users can save the file by File -> Save in Azarel. This will contain all the information contained in backup file. This is a proper way of saving information after an experiment so it can be re-opened in Azarel in future. Aside from

experiment data, the backup file will be saved in .json format will contain every parameter the user defined in user interface such as time, date, notes, names etc.

c) File -> Export Data

If users wish to export data with 'numbers only' from an experiment, then *Export Data* should be selected to only include the numeric information in Azarel. File saves without a format but could easily be accessed from data analytical software like excel once saved as .csv format. The information will be in 4 columns going in the order of: Time (s), Raw PMT Voltage (Volts), Averaged PMT Voltage (Volts), Averaged PMT Voltage with Background Correction (Volts).

2.4.2 ThorCam for Optical Imaging

The ThorCam software allows users to visually locate test objects under the microscope in real time through the CCD camera. It also supplies features to allow user to 'draw' on screen so it would become handy to locate laser focus on screen. The software can be found and launched from the desktop.



CAUTION: The CCD Camera is directly powered by the USB cable; powering the Camera for a long time will overheat Camera.

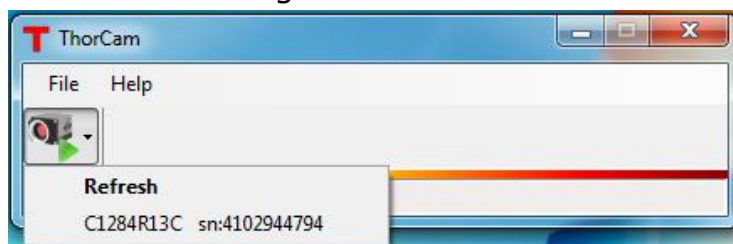


Figure 5 ThorCam Camera Scan

The software will scan for authorized Thorlab cameras located on PC. Once the camera is connected, the scan window will show the specifications of the camera selected. Select **Camera Name**.

Then, the software will proceed to the image capturing screen

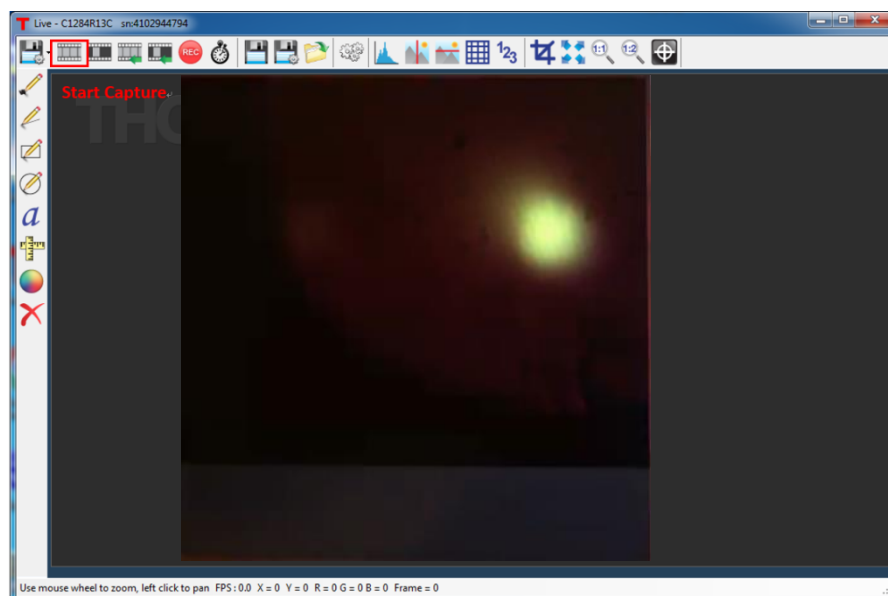


Figure 6 ThorCam User Interface with Focus Shown

Make sure to go to select '**Start Capture**' on top left corner; this will enable the camera to feed real time images into screen. Users should then be able to see the details on the microfluidic biochip by turning on LED switch once light path has been properly aligned.

Caution: Avoid direct exposure to eyesight with laser in operation!

3. Experiment Tutorials

The following content will include some basic hardware validation tests that allow users to take the step-by-step guide to operate SCB-402. The automated test run tutorial is recommended for calibrating purposes.

Actual tests with cell samples are not covered in this manual. Please refer to **ZellChip Instruction for Use – Single Cell Kit** documentations for procedures.

3.1 Automated Test Run

The following procedures are highly recommended for operating SCB-402 to maximize performance during automated experiments.

Prior to any operation, check laser power switch is switched to OFF position so laser wouldn't turn on once Power Supply is powered. SCB Chip should be loaded on Chip Holder gently by opening up clamps and fitting chip into place

- a) Connect the three USB cables to PC, which includes Camera, Robot Control & DAQ Card
- b) Launch *Thorcam*, select camera and start capture
- c) Turn on Power Supply switch, wait for steady green light on robot to appear
- d) Turn Control Knob on robot to move robot to HOME position, robot will stop once HOME is reached
- e) Launch Azarel and check if robot is successfully homed by reading both background & target values. Robot is homed correctly if both brackets returns 0*
- f) Turn on Blue Laser by laser power switch, blue light should appear from microscopic lens shining on SCB Chip
- g) Turn on LED and located cell/Target on Chip on screen by turning robot knob, y-position knob & adjusting LED light
- h) Once desired target is located, select units from drop-down menu in *Target* bracket and current location should be saved in designated unit. Enter *Time* manually to indicated how long robot should stay at *Target*
- i) Move robot to background position by tuning control knob, perform steps same as (h) to set parameters for *Background*
- j) Check for steps mentioned above and close box cover
- k) Start Experiment in Azarel (Start -> Save As... -> Open)
- l) Wait for graph to be generated on the user interface
- m) Software will automatically stop after experiment ended or when user select *Stop* from interface, robot will return to HOME position for both situations
- n) Save/Discard experiment results**

* For procedure to launch software refer to section 2.4

** Check ***Installation Checklist*** for exact location of focus location on PC screen

3.2 Microscope Scale-bar Test

By mounting a scale-bar slide, we can measure the size of the field of view. The steps to this test are as simple as locating the scale bar patterns on the PC screen. Perform steps (a) to (j) from section 3.1.

Test results should look similar to this:

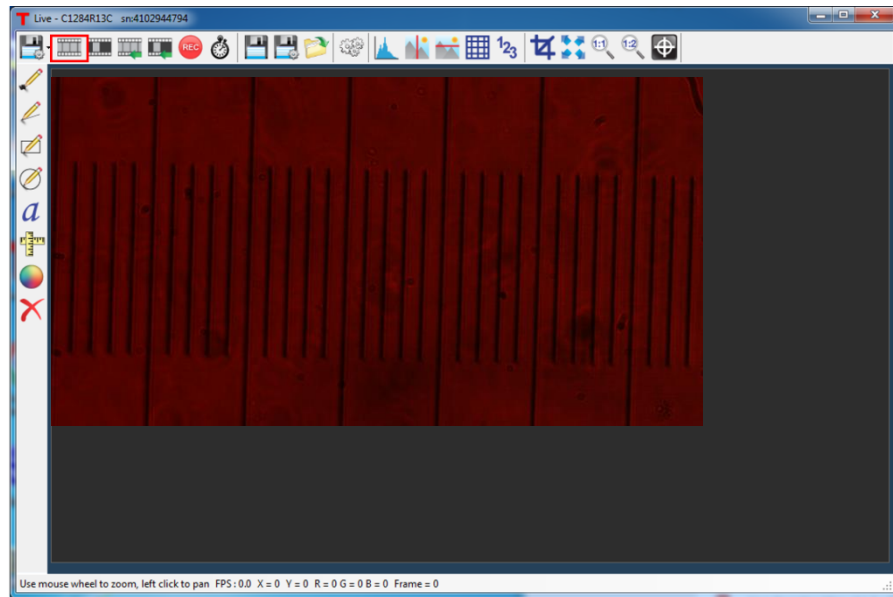


Figure 8: Microscope Scale-bar Test

3.3 Fluorescent Bead Test

Fluorescent bead test can be considered as a mocked cell test other than the test subject is done on beads. They were injected into the microfluidic biochip to glow in excitation of laser just like cells. The following steps demonstrate how this experiment is done. During preparation, users should strictly follow operation guidelines and handling requirements working inside a lab environment. Personal protective equipment like goggles with laser protection should be prepared prior to starting experiment.

Perform steps (a) to (n) from section 3.1.

Test results should look similar to this:

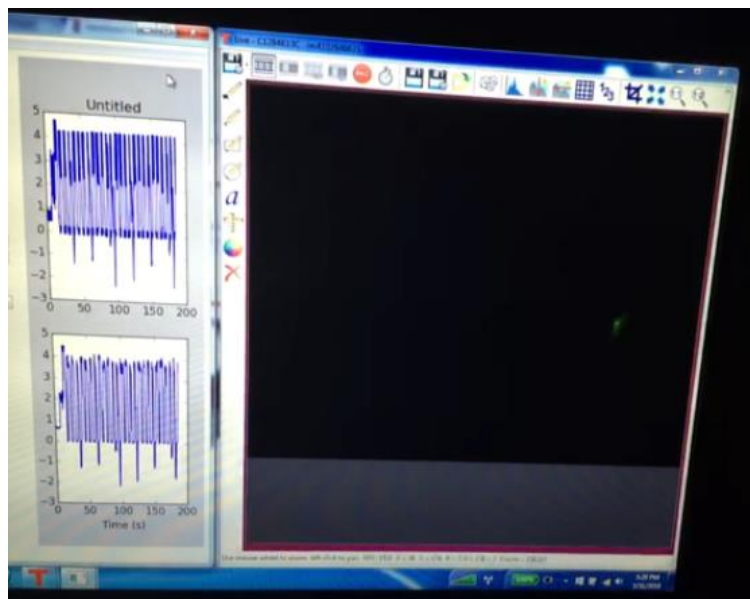


Figure 9: Bead Test

4. Instrumental Maintenance

4.1 Changing Chips

The biochip is generally mounted facing up on the Chip Holder. The slot on Chip Holder is designed to fit regular 25mm by 75 mm slides up to 2mm thickness for microscopic use. Users can load and unload SCB Chip by releasing clamp on holder. Chips should be handled in laboratory practice in order not to contaminate the surface of the chip when it is placed into Chip Holder slot. Be careful not to lose any washers underneath the 8 M3 screws holding Chip Holder on Robot, these washers were installed for tolerance considerations of Chip Holder.

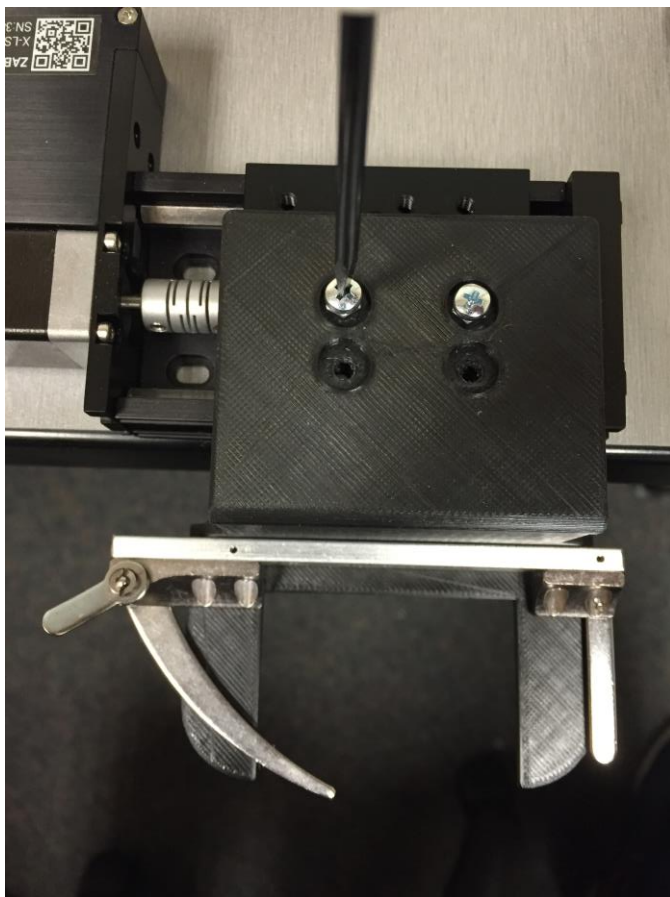


Figure 10: Unscrew Chip Holder by loosening four M3 screws

4.2 PMT Calibration

The dial located on the upper left corner of the power supply can be used to adjust the PMT driver voltage. By turning the knob in the clockwise/counterclockwise direction, users can adjust the sensitivity of PMT before data collection. Reading ranged from 1 to 10, the PMT sensitivity increases as the number goes up. The optimal sensitivity value for SCB-402 by experiment is from 7.5 to 9. With a higher sensitivity, more background noise is captured by the PMT and peak saturation is easily obtained if the signal level is high. See below for sample experiments at different PMT settings.

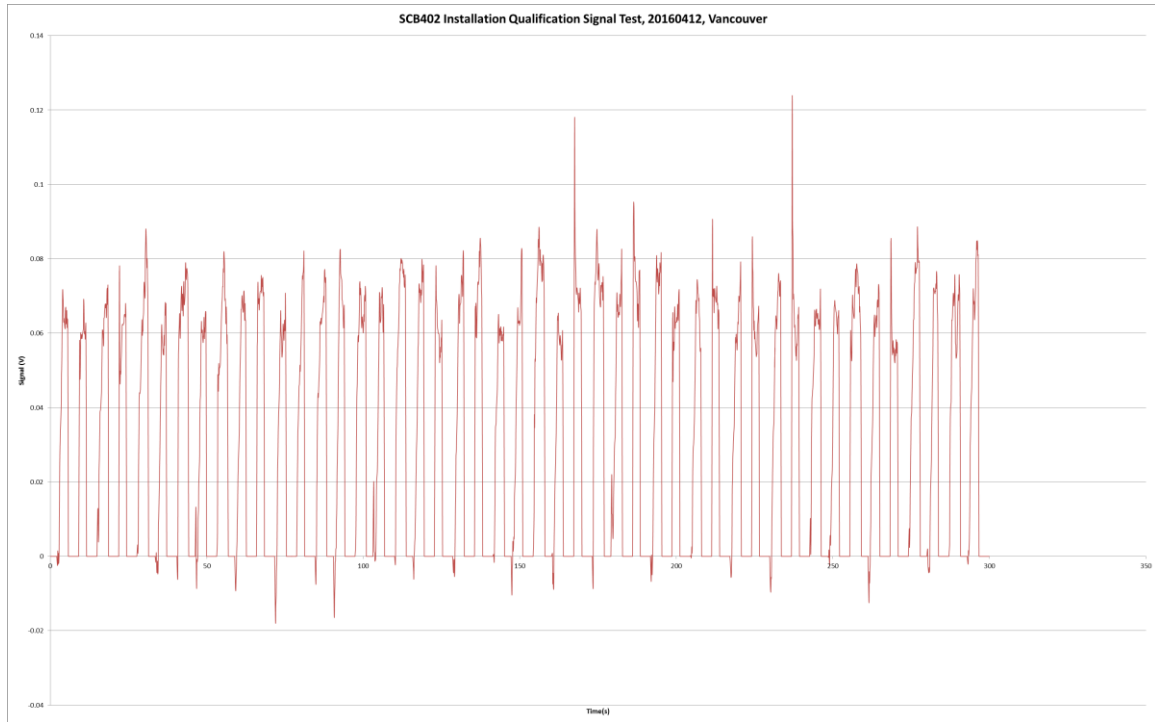


Figure 11 : Bead Data when PMT is set to 5.2 During Qualification Testing

4.3 General Troubleshooting

Common Problems	Action
Power Supply wouldn't power on when the switch is ON	Check if the power cable is connected to the correct voltage source
All other modules are powered other than module A(LED, Laser, PMT & Robot)	Check cable connection on Power Supply for loose wires, reconnect wires according to wiring diagram; Contact ZellChip for advice
Robot not going back to target/background location upon starting experiment	Power off device and reboot. Ensure robot is moved back to HOME position by control knob. Re-launch software. Contact ZellChip if problem escalates