Jellyfish User Guide

Version 1.0.0

4Deep
inwater imaging

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Edition

Jellyfish User Guide - Version 2.1.0

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Safety and Information Notices

Important

An “Important” signifies helpful information in using the software/hardware. It identifies an important piece of information to guide the user in their workflow, and if not followed could result in time wasted.

Caution

A “Caution” signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in damage to the product or loss of information. Do not continue beyond a “Caution” sign until the procedure is fully understood.

Warning

A “Warning” signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in personal injury. Do not continue beyond a “Warning” sign until the procedure is fully understood.

Contents

List of Figures

1 Overview

The FluoroSea, a submersible underwater microscope is operated with the Jellyfish software.

This user guide is strictly related to the installation, use and functionality of the FluoroSea microscope and Jellyfish software.

The main function of Jellyfish is to sample algae through images and fluorescence to identify different species through these general steps:

- Identify specific species from cultured data
  - Algal species can be differentiated by the organism size and the Fluorescence ratios
  - To properly identify the species of interest, usually individual samples of algae cultures will need to be sampled by the FluoroSea to determine the Fluorescence signatures and sizes of each species
- Record data from the ocean or a pond
- Use the already classified data to classify the ocean data, in real-time
- Program Jellyfish to send an alert when the concentration of an algae species reaches a dangerous level

For example, Jellyfish can be used in:

- Harmful Algal Bloom (HAB) studies, or as a prevention device for the HAB Algae production: algae profiling
- Phytoplankton abundance
- Phytoplankton ecology
- Phytoplankton community composition

1.1 Purpose

The FluoroSea incorporates a modern imaging sensor and 3 colour fluorescence excitation for rapid detection of harmful algae species. With its new and unique method of data acquisition and analysis, this system is designed to optimize your research of the microscopic world.

Jellyfish can be operated in two modes: live sampling and offline (analysis of already captured images with the FluoroSea), which allows the user to recover all sample information.

1.2 Benefits

Compared to a traditional fluorometer, the FluoroSea system has many benefits:

- Measures fluorescence signal of each individual cell, yielding unprecedented accuracy of species detection

- Real-time, in-situ capability to detect particles
- Portable and lightweight, making it easy to deploy, yet rugged
- Low maintenance and easy to clean, as there is no pumps or tubing, with an additional copper ring around the sample space to make biofouling less of an issue
- Pressure rated from 0-50m
- Instant: live imaging of samples without any manipulation

1.3 Outline for Use

The general setup and use of the FluoroSea system are outlined below:

- Install the FluoroSea (Section 3)
- Install the Jellyfish software (Section 4)
- Use the Jellyfish and FluoroSea as one system to Collect Data (Section 5)
  - Testing the FluoroSea in a bucket with a known culture (Subsection 6.2)
- Performing Offline Analysis to identify taxa (Section 7)
- Ocean and/or Continued Sampling (Subsection 6.3)

2 Safety Information (FluoroSea)

The user should read this user guide and any other additional information supplied by 4Deep before operating the instrument.

Caution Always make sure that the proper 12 VDC power supply is used with the microscope. Failure to do so could result in critical damage to the microscope.

Caution Do not look at the LEDs in the FluoroSea for any extended time frame as it may cause damage to your eyes.

3 FluoroSea Installation

3.1 Components of the System

Important Be careful when unpacking the contents of the package. Take care not to touch the windows encasing the camera lens. For further Care and Cleaning information, see Section 8.

The following is the list of parts of the FluoroSea:

- The FluoroSea, the Submersible Fluorescence microscope, pre-assembled. For full details, see the microscope description in Section 3.3.

Figure 1: The pre-assembled FluoroSea.

- Long (10m) cable with 13 pin connector to power laser, camera, and transmit data from the camera to the host computer.

Figure 2: 10m Cable

- Power supply (12 VDC)

Figure 3: Power Supply (12 VDC)

- Carrying case

Figure 4: Carrying case

Important Please ensure all components listed above were shipped to you. If not, please contact a 4Deep representative immediately.

3.2 Recommended Extras (not included)

The following is a list of recommended extra components, suggested by 4Deep, to optimize the use of your 4Deep microscope. Note that none of these are necessary for the use of the microscope and the microscope is fully functional without.

- Molykote 44 Medium, for marrying the connectors (Subsection 3.4)
- Deployment rope/cord, to deploy the FluoroSea (Section 6)
- A female-female Ethernet connector (ex: from a computer hardware store, shown in Figure 10)
- A 9/64ths inch hex screwdriver

3.3 The Microscope and its Components

The FluoroSea consists of 5 primary components:

1. Chamber 1: houses the motor and photo diode.
2. Chamber 2: houses the camera, LEDs and control electronics.
3. Sample delivery system.
4. Sample Space: connects the two chambers, contains the sample delivery system, and determines the sample volume that will be imaged.
5. Crown.

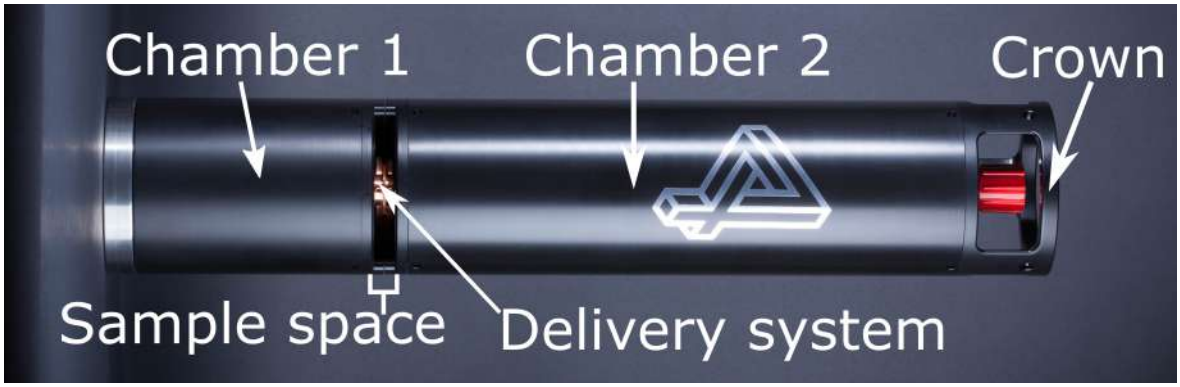


Figure 5: FluoroSea components

3.3.1 Chamber 1

This Chamber 1 contains the motor and the photo diode.

3.3.2 Chamber 2

This Chamber contains the all of the electronics, including the camera, and LEDs.

3.3.3 Sample Delivery System

The Sample Delivery System is operated from the Jellyfish software.

When powered off, the Sample Delivery System can be moved manually. The Sample Delivery System can be removed by the user, to clean or replace the cartridge.

Figure 6: The sample delivery system, containing the white washer and copper, to reduce biofouling.

3.3.4 Sample Spacer

The Sample Space simply holds the Sample Delivery System, and determines the volume of the sample.

3.3.5 Crown

The Crown is the framing around the connector on the FluoroSea. Its main purpose is for handling and deployment.

3.4 Connect the submersible cable

Firstly, ensure the connector pins (male and female) are clean, and free of any dirt/dust before connecting.

The connector pin manufacturer suggests using Molykote 44 Medium grease before every mating (Figure 7). The manufacturer suggests greasing about 1/3 of the female socket depth (right panel of Figure 7), for a “wet mating” (ie if the connector/microscope is being submerged). The manufacturer suggests greasing about 1/10 of the female socket depth , for a “dry mating” (ie if the connector/microscope is not being submerged).

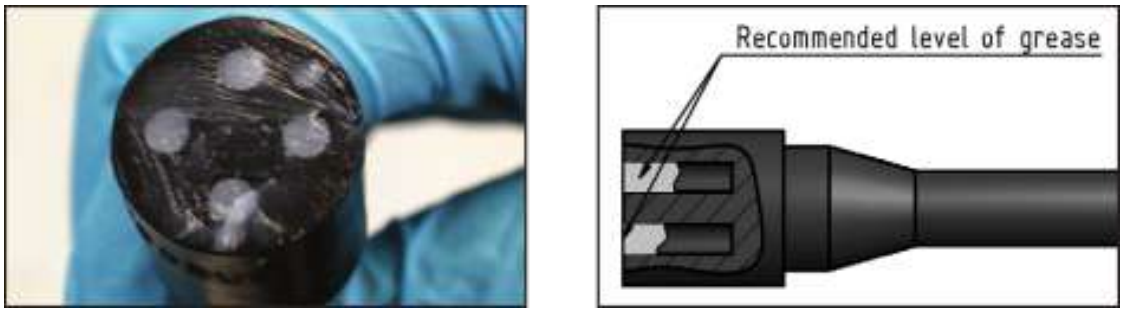


Figure 7: Mating the underwater connectors for a wet mating (Photo from MacArtney Underwater Technologies)

Caution As you push the connectors together, take care not to wiggle the cable too much, as it may cause damage the pins.

Firmly push the 13 pin connector at the end of the main underwater cable into the bulkhead connector. Push the cable connector all the way in. To verify that all of the male connectors are greased, pull the connectors apart, and check that the male pins are all coated in grease. Then reconnect the cable.

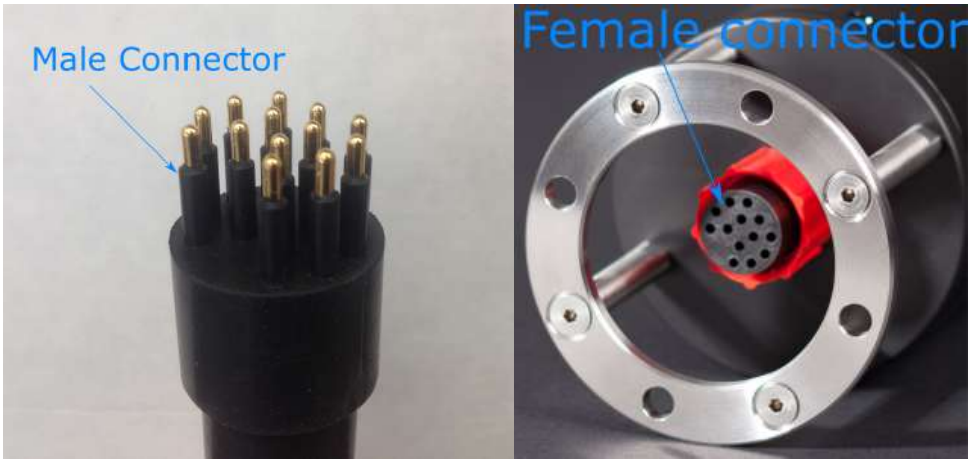


Figure 8: FluoroSea bulkhead with 13 pin connector. The male connector is in the left panel, and is attached to the underwater cable. The female is shown in the right panel and is attached to the microscope.

3.5 Screw on locking sleeve

Slide the locking sleeve over the cable connectors and screw it onto the bulkhead connector.

Figure 9: Locking sleeve on both the cable (left panel), the microscope (center panel) and connected (right panel)

3.6 Ethernet and Power

Caution It is highly recommended that you use a flexible Ethernet cable, and a female-female Ethernet connector as the underwater cable is very rigid and may damage the Ethernet port on your computer.

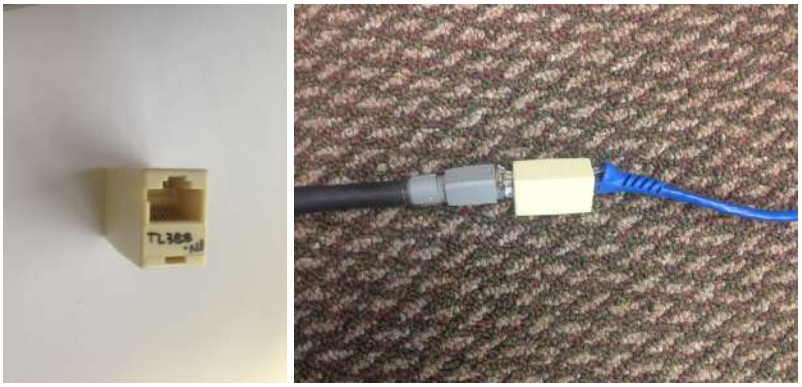


Figure 10: Use of female to female Ethernet connector. In the right panel, the black cord is the rigid Ethernet cable from the underwater cable, and the blue cable is the flexible Ethernet cable to the computer.

- Connect the Ethernet end of the main cable to the Ethernet port on the computer.

Figure 11: Connecting to the computer

- Plug in the power adapter for the camera and plug in the laser power adapter to the connector on the same cable.
  - Note that when the FluoroSea is powered, the sample arm will move and LEDs will flash.
  - The green light on the power source should be on once the microscope is powered

Figure 12: Connecting the power. In the left panel connecting the microscope power to the included 12VDC power cord. The right panel shows a close-up of the power connector from the microscope.

4 Jellyfish Installation

The following is a list to compatibility and steps to install the Jellyfish software in your computer.

4.1 Compatibility

- The Jellyfish software requires a USB HASP key to be operated.
  - Ensure the HASP hardware protection key (dongle) was included with the shipment
- The current version of Jellyfish (2.1.0) is compatible to run on Microsoft Windows 7or later.

4.2 Installation Package

To operate the Jellyfish software, the user will need to 1) install the Jellyfish software and 2) setup the correct Ethernet settings.

1. To install Jellyfish:
   - Download the software from our website: http://4-deep.com/software-downloads/.
   - Insert the HASP key supplied and follow the onscreen instructions.
   - Install Jellyfish by running JellyfishInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.
     – Note that using Windows 10, you will also be asked to accept that “VC_redist.x64.exe” can make changes to your computer when the Jellyfish installer is around 50% complete.

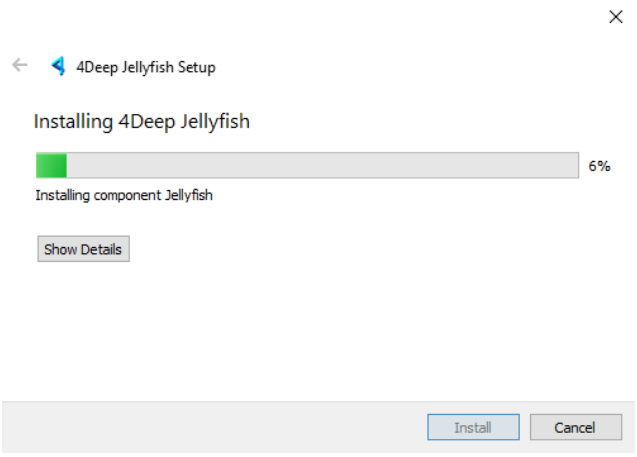


Figure 13: Jellyfish installer, selection of the installation package

2. Ethernet settings needed:

- Navigate to “Ethernet settings” -> “Change adapter settings”
  – In Windows 10, the user can search for “Ethernet” in the search bar on the bottom of the toolbar.

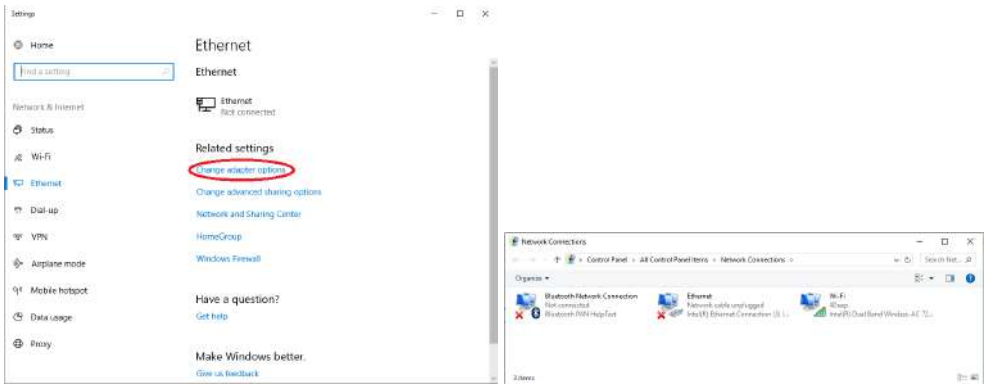


Figure 14: Change adapter options (left). Navigating to the Ethernet settings

- Right-click on the “Ethernet” option and select “Properties”. From the list, find “Internet Protocol Version 4 (TCP/IPv4)”, ensure it is enabled (check mark on) and click “Properties”.

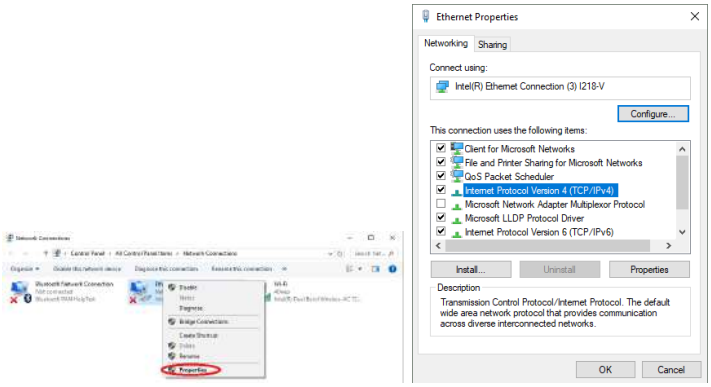


Figure 15: Changing the Ethernet settings

- Click the “Use the following IP address” and type into the “IP address:” 192 - 168 - 2 -15. In “Subnet mask”, type in 255 - 255 - 255 - 0. In “Default gateway”, type in 192-168-2-1.

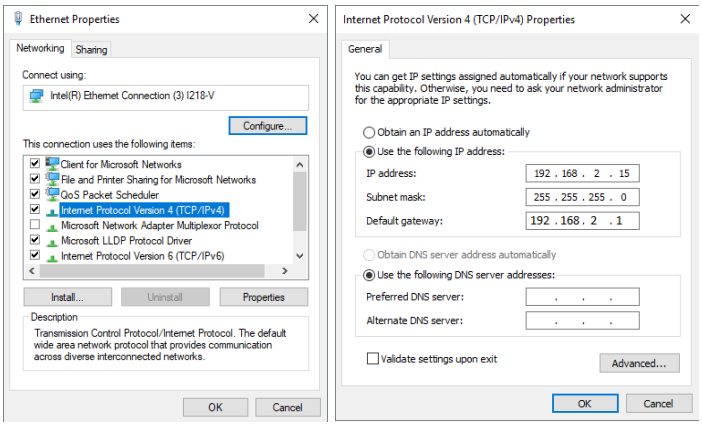


Figure 16: Setting the IP address

- Click “Ok” and go back to the Network connections page (left panel of Figure 15).
- Right-click on the“Ethernet” option and select “Properties” again. Click on “Configure...”. Note that you will need to go back to this screen to save the IP address change.
- Navigate to the “Advanced” tab. Find “Jumbo Packets” and enable them to the maximim (for example: 9014 bytes).
- Still under the “Advanced” tab, find “Speed and Duplex” from the list and set the value to “Auto negotiation”. Note that if these setting do not work, the “Speed and Duplex” can be set to “100 Mbps Full Duplex”. Note that on some Windows OS, the “Speed and Duplex” are may be located under another tab, such as “Link Speed”.

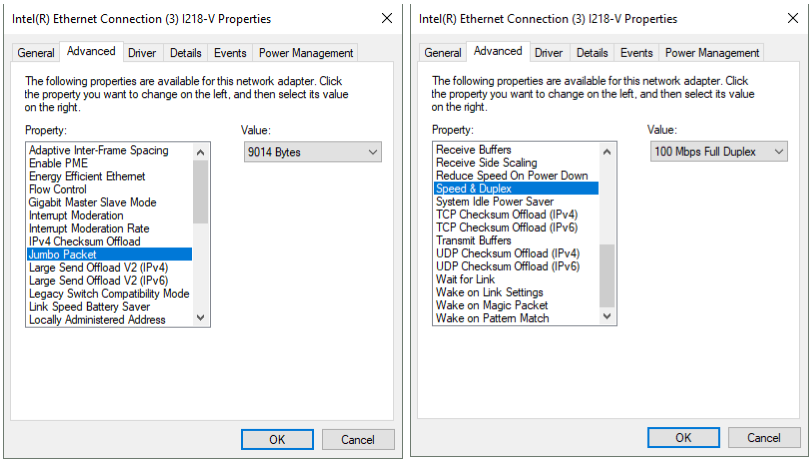


Figure 17: Enabling the Jumbo Packets (left), and Changing the Speed and Duplex (right).

- Click “Ok” and the set up is complete.

4.3 Connection of the HASP dongle and starting Jellyfish

- To operate Jellyfish, the HASP must be connected to a computer USB port. Make sure the dongle light turns on.
- Launch Jellyfish by going into **`Windows Start Menu-> 4Deep-> Jellyfish`**. The Jellyfish software will start.

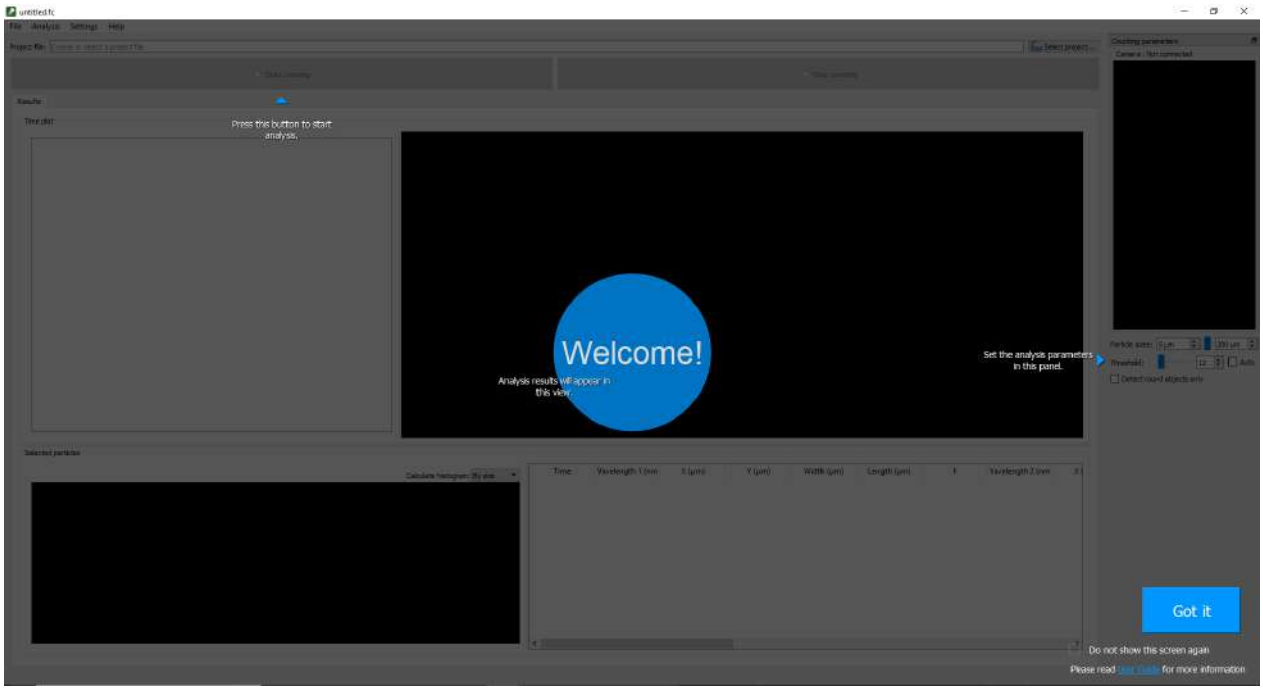


Figure 18: Jellyfish after launch of software

4.4 Main Menu

Most of the settings and functionality of Jellyfish is located in the **Main Menu** (see Figure 19). The following gives a brief explanation of each option in the Main Menu (for more details regarding these options, see the Sections referenced).

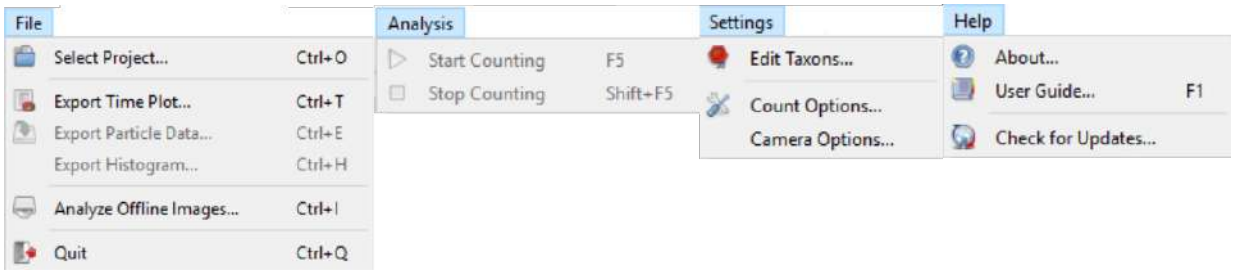


Figure 19: Main Menu, showing the options under each heading

4.4.1 File

Select Project - selects the project file. Either select a new project file, or existing project file. In case the existing project is selected, the data will be appended to the file. “Ctrl + O” also selects the project.

Export Time Plot...- exports an Excel spreadsheet with the timestamp in seconds, Date/Time and the number of each species detected. “Ctrl + T” also exports the Time Plot.

Export Particle Data... - exports an Excel spreadsheet with all the available data per particle. “Ctrl + E” also exports the Particle Data.

Export Histogram... - exports an Excel spreadsheet with particle size bins and the number of particles. “Ctrl + H” also exports the Histogram data.

Analyze Offline Images – allows for the counting of particles in previously collected images. Select the whole directory, or images from a directory to be analyzed. You must select at least 3 images. “Ctrl + I” also selects images.

Quit - closes the Jellyfish software. “Ctrl + Q” also quits.

4.4.2 Analysis

Start Counting - begins the counting process. Data will be appended to the current project file. “F5” also starts the counting

Stop Counting - stops the counting process. “Shift + F5” also stops the counting.

4.4.3 Settings

Edit Taxons - a dialog box will pop up. The user can add/remove taxon from Jellyfish

Count options - a dialog box will pop up. The user can change the counting options, such as cycle interval or shutter delays

Camera options - opens the Camera Options dialog.

4.4.4 Help

About - shows information about the software.

User Guide - opens software User Guide. “F1” also opens the User Guide.

Check for updates - Jellyfish will check for updates when prompted.

4.5 Main Window

The **Main Window** provides users with a view of the camera (smaller, right panels of Figure 20), as well as a view of the data (larger, left panel of Figure 20).

Figure 20: Main Window of Jellyfish

5 FluoroSea + Jellyfish: Collecting Data

At this point, both Jellyfish and the FluoroSea are properly set up and ready for data collection.

One full cycle of the FluoroSea + Jellyfish cycle includes:

1. the sample arm moving to the home position (if not already there)
2. pausing until the sample is steady and
3. firing each LED in the order: 540nm (green), 505nm (cyan) and 405nm (violet). Each time the LED is flashed, the camera is also activated, thus for every cycle there are three images captured.

Important If you are analyzing already collected images, you should go directly to Section 7.

To collect images, it is important to setup both the microscope and Jellyfish properly. The steps below detail how to setup and use Jellyfish *in conjunction with* the FluoroSea.

5.1 Acquisition setup for Jellyfish and the FluoroSea

1. Follow the steps in Section 3 to setup and power the microscope (Sections 4 and 3). Once the FluoroSea is powered on, the sample arm will return to the home position. If the FluoroSea does not return to home, cycle the power, as the system will not operate unless the the FluoroSea is powered.
2. During the connect stage, Jellyfish will generate calibration curves. To generate proper calibration curves, the user must connect the FluoroSea to Jellyfish in either air or distilled water. Thus, ensure the FluoroSea is connected outside the sample. Once connected to the FluoroSea, open Jellyfish, and it will automatically connect to the FluoroSea.

(a) Jellyfish should display a series of calibration messages:

i. Setting shutter to home position (sets the sample arm into the correct space in the casette)
ii. Calibrating LEDs
iii. Obtaining calibration curves (this may take a few minutes)
iv. Connecting to microscope camera

(b) It may take several seconds to connect to the camera. Often, the user will have to manually select the camera from a camera dialog, shown in Figure 21. For more help on connecting the camera, see the Troubleshooting Guide (Section 9).

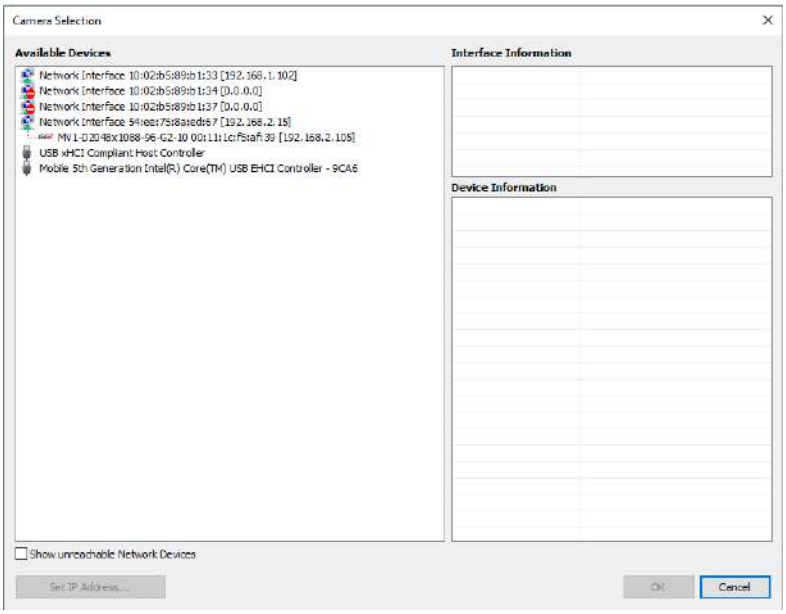


Figure 21: Connecting to microscope

3. The user will need to select and input particle settings for the software, including particle size range, threshold, and whether to detect round objects

Particle **size** - Select the maximum and minimum particle size Jellyfish should count (from 1 to 2000 micrometers).

Threshold - The minimum intensity, or pixel value, to be considered for counting (from 0 to 255). A high threshold prevents Jellyfish from counting out of focus and nondescript particles. `Auto` threshold uses Otsu algorithm to set optimal threshold for particle detection automatically.

Detect **round objects** - If only round objects are of interest, selecting this will make Jellyfish count only round objects within the volume.

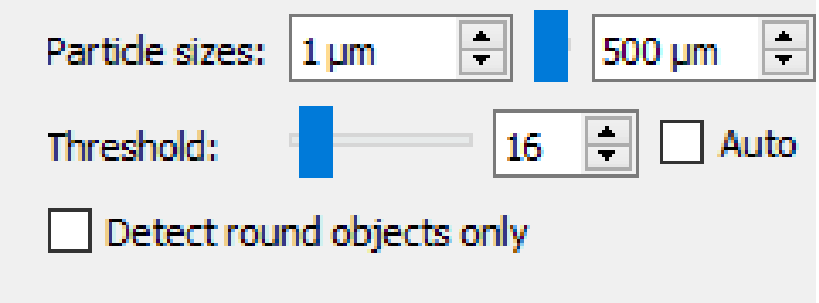


Figure 22: Particle settings for Jellyfish

4. Count options. Go to **`Settings -> Count options`**.

   - The user can change settings in Jellyfish to set the sampling parameters. These settings include:
     - **Cycle interval** - length of time, in seconds, it takes for the FluoroSea to complete one full cycle. This includes moving the sample arm to the home position, pausing for the sample to steady, and collecting images at all three LEDs. The minimum cycle time/ default time is 25 seconds.
     - **Cycles per data point** - the number of cycles to generate one datapoint. 1 cycle per datapoint means each cycle is saved as one datapoint. The user can increase the cycles per datapoint, especially in cases when the concentration of algae is low, which will reduce the number of empty or 0 datapoints.
     - **Save raw images in addition to particle data** - When this option is enabled, the raw images of the data will be saved in the same directory as the Jellyfish data file.
     - **Instrument** - there are two versions of the FluoroSea, the standard 5X, and a custom 10X. Unless otherwise directed, use the 5X option. If you are unsure, contact a 4Deep representative to verify the instrument.

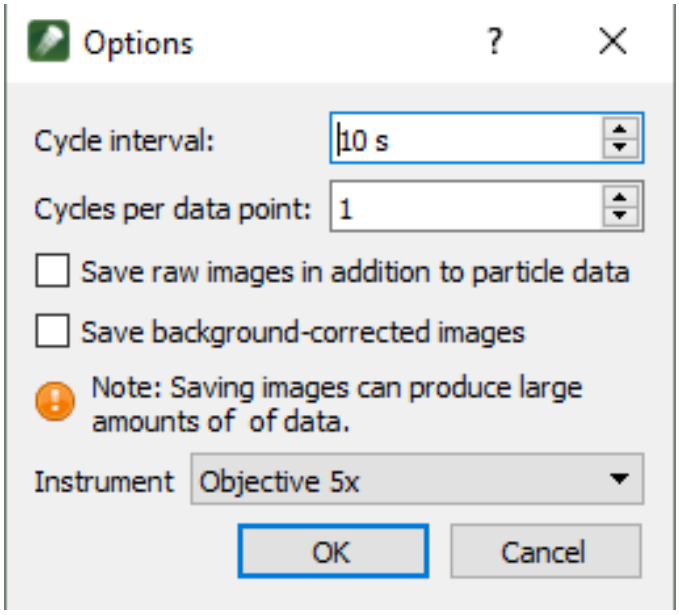


Figure 23: Count options for Jellyfish

5. Select/ Create project

   - Jellyfish will create a Jellyfish data file (*.fc)
   - Create a new project for each deployment. Save the project in a folder that is large enough to contain images, ie do not save the file to your Desktop. The file name should be the name of the species in the culture or the location the FluoroSea is deployed.
   - If the options “Save raw images in addition to particle data” and/or “Save background-corrected images” are selected, the images will also be saved in the same directory as the Jellyfish file. Thus, ensure that the directory has enough storage to save the images

6. To start collecting data, click the “Start counting” button, or go to **`Analysis -> Start counting`**.

   - As Jellyfish records data, the data can be viewed in the Main Window, as shown below.
   - Each datapoint is recorded in the top left panel. By default, one datapoint is one cycle. In the Count Options, the user can increase the number of cycles in one datapoint. Thus, if the user increases the number of cycles in a datapoint, for example, to 5 cycles, then the data will update every 5 cycles, instead of every cycle.

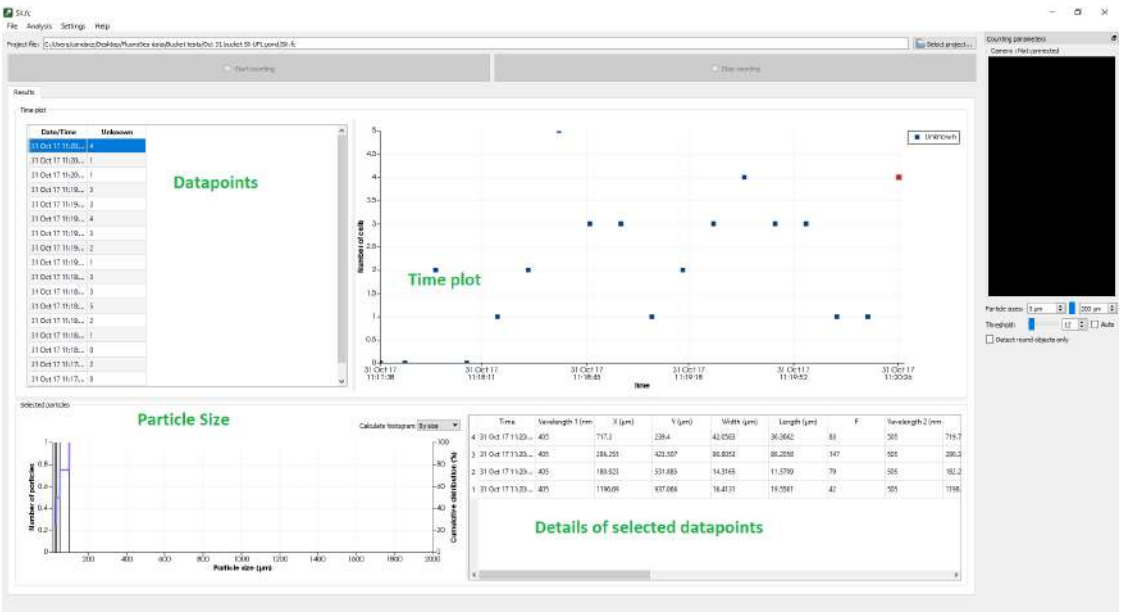


Figure 24: Results from data collection. Each datapoint is recorded on the left. The right top panel shows the datapoints over time. The bottom left plot is the particle size distribution data. The bottom right table shows the individual particle data.

- Only algae that appear in all three frames will be counted as a data point and will be saved under the Results panel.

7. To stop analysis, click the “Stop counting” button, or go to **`Analysis -> Stop counting`**.

5.2 Camera Options

Caution You can change some low level camera options, if necessary. Note that the low level options may affect the image quality and reconstruction results, do not change the parameters unless you consult with 4Deep support first.

To open the Camera Options, go to **`Settings -> Camera Options`** (note that the Camera Options are only available when a camera is connected). This will open a new dialog box (shown in Figure 25; see Main Menu Figure 19 for the location in the Main Window). The Camera Options dialog provides access to the low level options of the GigE Vision camera installed in the 4Deep microscope.

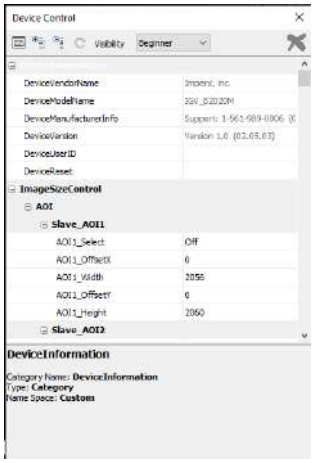


Figure 25: Camera options dialog

6 Testing and Deployment

Now that the FluoroSea and Jellyfish are connected, there are a series of tests that should be completed.

In initial testing, the user should always choose to save the raw images during capture, so as to re-analyze the data if necessary.

6.1 Dry test

The most basic test to verify the system is connected and will work consistently is a simple dry test.

Simply set up and connect the microscope in air, ie not deployed. Note that when the FluoroSea is powered on correctly, the sample arm will move to the home position, and the LEDs will flash. If this does not happen, cycle the power.

Set up the Analysis parameters, the Count Options, and Create a new project. The details are found in Section 5. For this test, ensure the “Save raw images in addition to particle data” is on, to verify that the data is being collected.

Run Jellyfish for 10-15 minutes. Check the folder that the project is saved in to verify the images are saved there. As the minimum sample time for a cycle is around 7 seconds, it will take a few minutes to generate data into the folder.

6.2 Bucket / Culture test

Once the dry test is complete, the next step is to perform a “bucket test”. Essentially, this test consists of adding a culture of algae to a bucket of water. By operating the FluoroSea in this sample of water and collecting data, Jellyfish will have a starting point with regards to data. These data will be used later to assign taxon to specific Fluorescence and size data. Recall that to generate proper calibration curves, the “Connect” stage must occur in air or distilled water.

By saving the raw images, the user can verify that the FluoroSea can detect algae. Depending on the concentration of algae in the sample, the user may need to run the FluoroSea for an extended period, such as 24 hours.

6.3 Ocean and Future Sampling

This test will occur usually after the user has completed a bucket test, and after Offline Analysis (Section 7) has been used to identify taxa. It may be beneficial to move to Section 7 before completing an Ocean test, however, it is not necessary. Recall that to generate proper calibration curves, the “Connect” stage must occur in air or distilled water.

The FluoroSea will likely be submersed into a body of water (river, ocean, etc.) off a platform (boat, wharf, etc.).

6.3.1 Deployment

The user should secure a rope or cord to the FluoroSea before deployment. To verify that the microscope powers on, the power should be connected before the FluoroSea is deployed underwater.

Caution DO NOT deploy the FluoroSea with the underwater cable alone. Attach a rope to the crown atop the FluoroSea for deployment. Submerse the FluoroSea into the body of water/fluid that you want sample. Both windows should be fully submersed in water for the accurate imaging to be performed.

Figure 26: Deployment of the FluoroSea from a stationary ship on a wharf

More complex deployment methods may also be necessary, as researchers may wish to deploy the FluoroSea in conjunction with other instruments. As this would be application-specific, we leave the details of the deployment up to the user, but give the dimensions of the system for technical purposes:

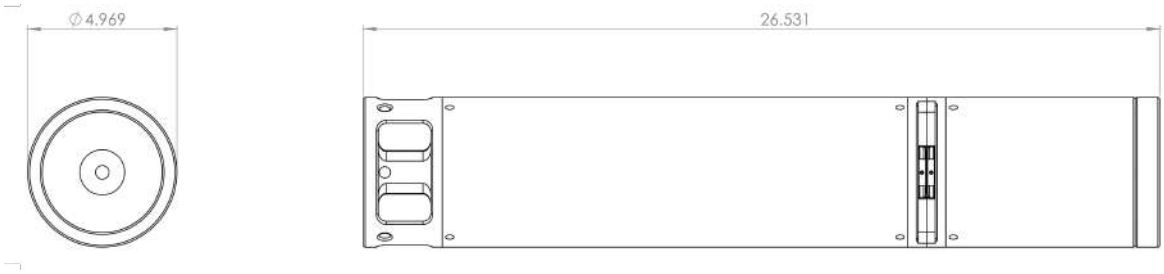
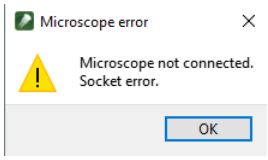


Figure 27: Dimensions of the FluoroSea, reported in inches

7 Offline Analysis

At this point, the triplet images are already recorded, and the user can analysis the images. Note that the analyze offline images, the user does NOT need to be connected to the FluoroSea.

When Jellyfish is opened without being connected to Jellyfish, an error will pop-up, as it automatically attempts to connect to the FluoroSea.



7.1 Offline Analysis

To analyze already recorded triplets:

- set the Particle Size Settings outlined in Subsection 5.1
- go to **File–> Analyze Offline Images**
- select triplets/cycles. The user can either select the entire folder that the images are saved, or select a few examples. Note that the user will need to select all 3 triplets for analysis. Note that selecting an entire folder may take some time to complete.

Once the analysis is completed, the data will be added to the Results, as in Figure 6.

Included in data added are particle data. They appear in the Selected Datapoints table and include:

- Wavelength (in nm), which is the LED wavelength
- X and Y (both in microns), which are the coordinates of the particle from the top left corner of the frame
- Width and Length (both in microns), which are the shortest and longest distance across the particle
- The image

- F - the Fluorescence signature

This is listed for all three wavelengths, thus there are 21 columns in the first portion of the table.
The last part of the table includes:

- F2/F1
- F3/F1
- F3/F2

which are the Fluorescence ratios between the Fluorescence signals listed above.

Figure 28: Section of the Selected Datapoints table

7.2 Assigning Taxon

Once there are data in the Selected Datapoints table, the user can use the Fluorescence ratios and particle size to create algae taxon:

- go to **`Settings -> Edit taxon`**. A dialog box will pop up, shown in Figure 7.2.
- use the “+” to add a taxon.
- using the data in the Selected Datapoints table, the user can name a taxon and input the Fluorescence ratios and particle size.

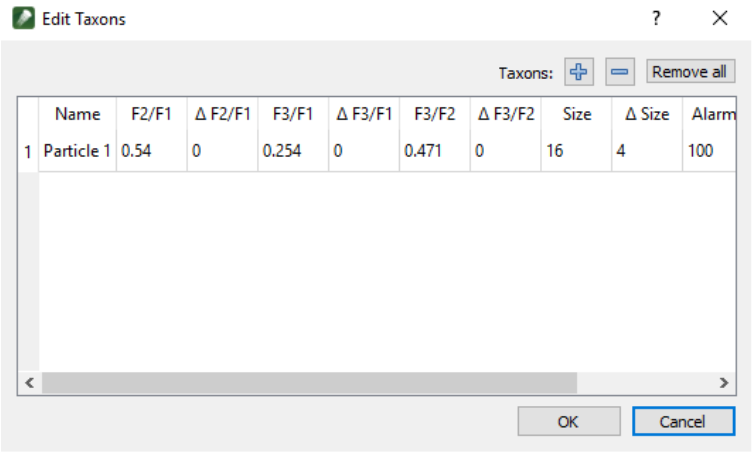
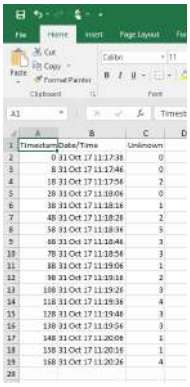


Figure 29: Edit taxon dialog

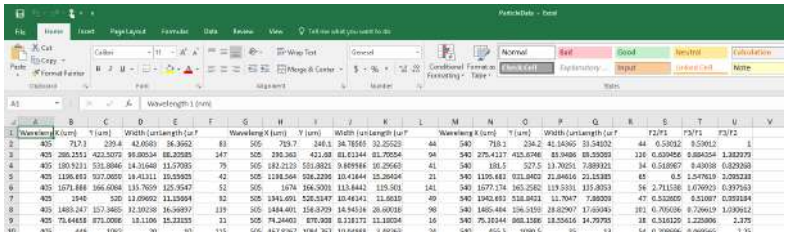
7.3 Exporting Data

Once there are data in Jellyfish, the user can export the data as Excel spreadsheets:

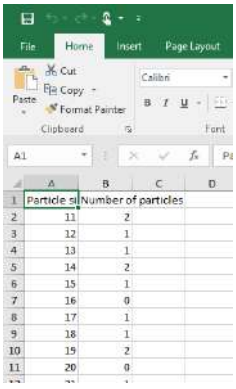
- **`File -> Export Time Plot`** exports the timestamp in seconds, Date/Time and the number of each species detected.



- **`File -> Export Particle Data`** exports all the available data per particle.



- **`File -> Export Histogram`** exports the particle size distribution data



8 Care and Maintenance

While the maintenance of your FluoroSea is minimal, some cleaning is required.

1. You microscope does NOT have to be cleaned for the first deployment.
2. Before EVERY deployment the FluoroSea connectors should be mated with grease (see Subsection 3.4)
3. After EVERY deployment the FluoroSea needs to be washed in clean fresh water to minimize the potential for corrosion.
4. After EVERY deployment the FluoroSea needs to be dried off before being replaced in the carrying case. This includes using an air duster to blow water away from crevices in the microscope.
5. After EVERY deployment (assuming the FluoroSea is used in the field), once the FluoroSea is returned to an indoor environment, it should be cleaned with a mild soap (dish soap works fine) and water. It should be thoroughly rinsed and dried before being replaced in the carrying case (see steps 3-4).
6. Eventually, the user will need to clean and/or replace the Sample Delivery System. The arm itself is part of a removable cartridge, and the user can remove it:
   (a) Disconnect the FluoroSea from its power supply
   (b) Using a 9/64ths Hex screwdriver, remove the two screws at the back of the FluoroSea that release the cartridge
   (c) Manually move the sample arm to the out position to make it easier to remove the cartridge
   (d) Clean the cartridge with water. Eventually the metal on the cartridge will corrode. Contact a 4Deep representative for a replacement.
   (e) When replacing the cleaned cartridge, ensure both the microscope sample space and the cartridge are thoroughly cleaned and dried. Do not attempt to replace the cartridge when it is still wet, as it will affect the optics, and thus the data.
7. Avoid transporting the microscope along with loose hardware (Allen keys, screws, etc) as they will scratch the sapphire window surface if contact is made.

Caution Without cleaning the microscope, there will be an accumulation of particles on the sapphire windows, which will destroy the quality of the images, making the data poor quality.

9 Troubleshooting

The most common issues, with Troubleshooting solutions listed below.

Issue	Solution
When connected to power, the FluoroSea does not perform its start up sequence (move the sample arm to the home position, flash the LEDs).	Cycle the power. If it still does not work, check the connections, and the cable for damage.
During “Connect” error message displayed “Clean calibration chamber”.	Cycle the power. If the message reappears more than 3 times, remove the sample cartridge, and clean the sample chamber thoroughly.
During “Connect” error message displayed “Failed to home sample arm”	Cycle the power. If the message reappears on the next cycle, disconnect the FluoroSea from the power supply, and move the sample arm close to the home position. Re-power the microscope. If the issue persists, disconnect the FluoroSea from the power supply and move the sample arm around and into the sample space to verify that it can easily swing into the sample space. If it cannot swing easily into the sample space, the cartridge may need to be replaced. Contact a 4Deep representative for details.

Table 1: Common troubleshooting problems and solutions