LISST-200X
Particle Size Analyzer

User's Manual

Version 1.4

(September 2019)

SEQUOIA

Store Software
USB Card Here

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Welcome to the LISST-200X Particle Size Analyzer

Using this manual
This manual is divided into two sections.

I. LISST-200X contains an introduction to the LISST-200X instrument and the principles of its operation.

II. LISST-200X Operation provides a detailed set of instructions for using and caring for the instrument.

Technical assistance
For technical assistance please contact your local Distributor or Sequoia. Please be sure to include the instrument serial number with any correspondence.

IMPORTANT: Please read APPENDIX H: TECHNICAL ASSISTANCE *BEFORE* you call or email.

Sequoia Scientific, Inc. contact information:
Telephone: +1(425) 641-0944
Email: info@sequoiasci.com

PLEASE NOTE: The LISST-100X particle size analyzer will not communicate with the SOP200X software described in this manual. If you have a LISST-100X you can use SOPv5.1 to communicate with and process LISST-100X data.

Table of Contents

I. LISST-200X Introduction and Tutorial

A.   Laser Diffraction and the LISST-200X

Measurement Principle and Optics

Particle sizing by laser diffraction is currently the most widely prevalent method in research and industry. It is a multi-parameter measurement. Just as a set of 3 equations can be solved for 3 unknowns, a measurement of light scattering at multiple angles can be solved for equally as many *concentrations* in different sizes. For example, the LISST-200X measures scattering into 36 angles; consequently, one obtains concentrations in 36 size classes of particles. This is called the particle size distribution (PSD). That is the essence of this method. In order that you appreciate the importance of some steps in operation of the LISST-200X, we recommend reading the rest of this chapter.

The measurement of scattering at multiple angles is done with a collimated beam illuminating particles in water. Laser light scattering at an angle from the beam arrives, through a daylight rejection filter, at the focal plane of a receive lens at the same angle from lens axis (see figure). Thus, distance from lens axis in the lens focal plane corresponds to scattering angle. The photodetectors in the LISST series instruments are a series of silicon rings spanning 60-degree arcs. Each ring covers a small range of scattering angles. Rings are used to achieve stability of *inversion* – conversion of set of multi-angle scattering measurements to the PSD.
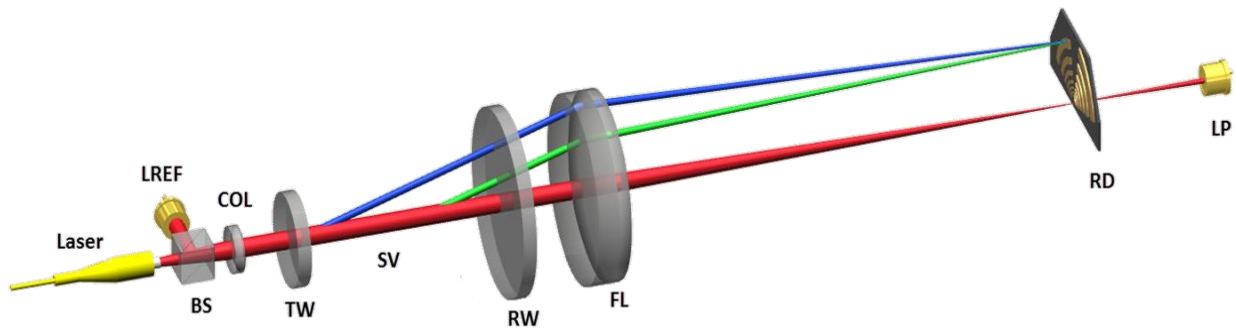
Notice that the laser beam itself is focused by the receive lens and passes through a small aperture in the ring detector, centered on the rings. This beam is sensed by a photodiode placed behind the ring detector. This is the *transmission* sensor. As light is removed from the laser beam by scattering, the beam is attenuated, i.e. the light transmitted through water is reduced in intensity. A similar attenuation also affects the light that is scattered by particles and sensed by ring detectors. Thus, the transmission sensor provides a vital measurement to de-attenuate the measured scattered light.

Clean Water Backgrounds and Background QC

Imperfections, such as micro-scratches on glass windows and lenses, also scatter laser light. This is measured and stored; it constitutes a *Background* (formerly termed *zscat,* as in scattering by zero concentration of scatterers). This background is subtracted from the total signal seen by ring detectors. It is also helpful in determining if the instrument is properly aligned, and if all optics are clean and unscratched. A *factory background* acts as a reference to compare with, to identify degradation in laser output or fouling of optical surfaces. The LISST-200X stores its factory background data in its memory, as well as the user's acquired background data.

The LISST-200X incorporates a major advance over our previous LISST-100X. This is the automatic quality control of the background data (QC). During background acquisition, after grabbing a group of scans of all detectors, their means and variances are computed. Comparing with the on-board factory background, these are used to alert the user of possibilities such as laser degradation, possible optics misalignment, contamination of data by thermal microstructure in the water used for background, bubbles or particles in the supposedly clean water, scratches on windows etc. If no warnings are generated, the background passes QC and the user has the option to save it, or repeat.

Ambient Light Rejection

The laser diffraction method requires that the light arriving on the detector be due entirely to scattering of light that originates with the laser beam. Light from ambient sunlight can distort the results. The LISST physically shields the detector from much ambient light, but not all, so daylight at shallow depths can still be significant.

The LISST-200X now can measure the ambient light independently of scattered light, and subtract it to completely remove its effects. (Note that this function requires firmware version 1.4 or later, introduced in May, 2017). See section H for more about ambient light rejection (ALR).

Quick Estimates of Particle Concentration and Mean Size

The process of inverting laser diffraction data to produce PSD requires too much computation to execute in real time within the instrument. But the LISST-200X does provide real-time estimates of the total particle volume concentration and mean particle size. These are approximate quantities, not as accurate as the fully-processed PSD, but are useful for quick characterization of water conditions. For example, if used in conjunction with a CTD package in profiling applications, the CTD software could display the concentration and mean size as a depth profile, revealing any vertical structure in the particle distribution. (Note that real-time interface to a CTD requires proper configuration of the LISST-200X's auxiliary connector; see "Configuring the LISST-200X as a Sensor for a CTD" on page 70.)

The quick estimates are based on weighted sum of the net scattered light. One set of weight factors yields the total volume concentration; the other yields the area concentration of particles. The volume/area ratio provides mean diameter, also known as the Sauter Mean Diameter (SMD). Notably, SMD can be quite different from D_{50} in broad or multi-modal size distribution situations. The SMD output is set to zero in very clear waters, i.e. when transmission is above 98%. In such clear waters, the SMD can become erratic. [*For an explanation of this method of SMD derivation, see: Shaped Focal Plane Detectors for Particle Concentration and Mean Size Observations*; Agrawal, Y.C. and O.A. Mikkelsen, (2009), Optics Express, v 17, n 25, pp 23066-23077].

Particle Shape Models – Spheres or Irregular Shape

The multi-angle scattering can be interpreted via inversion as arising from spherical particles, or from irregularly shaped particles. Provided software gives you the choice and the resulting PSD files are named differently to distinguish them. As to which particle model to use, we suggest that when working with natural waters, use the irregularly shaped model. Only in exceptional circumstances, the spherical model is appropriate.

B. LISST-200X General Description

Instrument Description

The LISST-200X is capable of autonomous operation when used with external battery packs. Windows software (compatible with Windows XP through Windows 10) is provided to program the instrument for a specific sampling schedule. The primary measurements delivered by the LISST-200X are the small-angle scattering properties of particles in water, the laser optical transmission, depth, and temperature. An Auxiliary port is available for that can be configured for recording data from an external device such as a turbidity or fluorometer or output summary data (mean size and total concentration) as analog voltages. For details of the possible configurations of the Auxiliary Port, see Appendix E: Connectors on page 106.

After recovery of the instrument, small-angle scattering data are off-loaded from the instrument and subsequently *inverted* mathematically on a computer to produce the particle size distribution. The inversion function is included in the Windows software. For MATLAB users, inversion scripts are available for download from Sequoia's website: www.SequoiaSci.com.

The LISST-200X instrument is a laser diffraction device. It consists of optics for producing a collimated laser beam, a specially constructed detector array, electronics for signal pre-amplification and processing, data storage and scheduling computer, and an external battery system.

The principal measurement—angular scattering distribution— is obtained over 36 ring-detectors whose radii increase logarithmically from 102 to 20,000 microns. The detector is placed in the focal plane of the receiving lens. The rings cover an angular range from 0.00085 to 0.34 radians. This angular range corresponds to size ranges from 1.00 to 500 µm. See Appendix B: Particle Size Bins on page 100 for more information.

The LISST-200X consists of the following parts: a solid-state diode laser operating at 670 nm wavelength and fiber-optically connected to a laser beam collimating system, a beam manipulation and orienting system, a two pressure windows, a scattered-light receiving lens, the custom designed 36-ring detector, preamplifier electronics, a ring-selecting multiplexer circuitry, and a data logger. All these components are inside the black-anodized pressure housing.

The LISST-200X does not have internal batteries and therefore should not need to be opened.

Data Storage and Interface The LISST-200X includes high-capacity data logging and storage. The logging functions are programmed via the provided software. It can be programmed with different start and stop conditions as well as different sampling rates and average durations. The data logger stores the data in non-volatile Compact Flash memory which can be later downloaded and processed into size distributions and concentration using the provided software.

The data logger will also accept commands via the RS232 interfaces. These commands can be used to program the instrument or to exchange data with another instrument.

Depth and Temperature In addition to measuring the particle size and concentration, the LISST-200X also has depth and temperature sensors. The depth sensor has a 1000 psi full-scale range[1]. The stainless steel fitting on the Connector endcap is used for testing this sensor, and keeps contamination such as salt and sediment out of the pressure sensor. The temperature is measured using a high precision thermistor imbedded into a stainless steel probe on the Connector end cap. Both values are stored automatically in the LISST-200X data file.

External I/O Port The LISST-200X is also equipped with an external Auxiliary port which is available via a 6-pin connector in the endcap. This port can be configured in different ways by the user.

The Standard Configuration has one analog input and two digital inputs. The analog voltage on this input is recorded with the size distribution data. The digital inputs can be used to start and stop the instrument.

The Analog Input Configuration has two analog inputs and switched power which can be used to power the external sensor. Both analog voltages are stored with every size distribution.

The Analog Output Configuration allows the Mean Size and Total Concentration information to be output as analog voltages. The 6-pin connector is wired to match the SeaBird CTD Auxiliary Input. This allow the LISST-200X to obtain power from the CTD and output the mean size and total concentration in real time to the CTD while the detailed size distribution data is stored internally for later downloading.

The Auxiliary port can be reconfigured by the user by moving mechanical jumpers on the main circuit board inside the instrument. Contact Sequoia Scientific for instructions on how to access the jumper on the circuit board.

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[1] A gauge pressure sensor is calibrated to measure the pressure relative to a given atmospheric pressure, as opposed to an absolute pressure sensor, which measures pressure relative to a vacuum. The pressure sensor on the LISST-200X will read 0 at atmospheric pressure.

Battery Life   The LISST-200X does not contain internal batteries (except a small battery to maintain the real-time clock), but can be powered from multiple sources including a USB computer cable, Small or Large Battery Packs (provided with the instrument) or an external instrument such as a CTD.

The standard Small Battery Pack supplied with the LISST-200X uses two NiMH rechargeable D-cell batteries and will provide about 12 hours of continuous sampling.

The Large Battery Pack contains 16 standard alkaline D-cells. For profiling applications, where the instrument is sampling continuously, the Large Battery Pack has about 200 hours of sampling time or 200 days of stand-by. Powering down the instrument between samples can greatly extend the deployment times. An Excel spreadsheet that can estimate the battery consumption is included with the software on the Ship Disk you received with your instrument. The spreadsheet can also be downloaded from www.sequoiasci.com/product/lisst-200x, click Library, then Downloads, then LISST-200X.

For laboratory or tethered usage, power can be supplied through the communications connector on the endcap. A 2-meter USB cable is provided that will power the instrument from the computers USB port. No additional power is required to operate the instrument.

Cables up to 50 meters can be provided to supply external power and communication with the instrument. This can allow real-time observation of the size distributions.

Included Accessories   The instrument is shipped pre-aligned and tested. A small chamber is provided for obtaining measurements of background scattered light from optical surfaces. This background is subtracted from actual particle scattering measurements to obtain the true particulate scattering. Additionally, small tools used to open endcaps, spare batteries, and communication cable is supplied so that a user need only provide a PC running Windows.

For extended laboratory applications various chambers are available from Sequoia.

Software is provided to communicate with the instrument, schedule an experiment, offload the data, and invert the measurements to obtain particle size distribution and volume concentration. For laboratory use or for monitoring the progress of an experiment, the software can be used for real-time processing.

C.   LISST-200X Quick Start Tutorial

This section gives step by step instructions to unpack your LISST-200X, load software, and acquire data in the lab. For more detailed instructions on specific steps, background on how the instrument works, or specific technical information such as cable pinouts please refer to the full User's manual following this Quick Start Tutorial.

Contents of Shipping Case

Let's assume that you are opening the LISST-200X shipping case for the first time. Inside you will find the following:

- User's Manual,
- USB memory card (credit card size) with the software,
- LISST-200X instrument,
- Plastic Instrument stands,
- Small Volume Test Chamber,
- USB Communications cable,
- Insulated stainless steel clamps,
- Small Battery Pack (without batteries),
- Larger Battery Pack with alkaline batteries installed,
- LISST-200X-to-Battery Pack cable,
- NiMH batteries and charger.

Step 1: Remove Instrument from Shipping Case.

Start by removing the white plastic instrument stands and set them on a flat working surface. Remove the LISST-200X from the case and set it on the stands. The LISST-200X has two distinct ends that we will refer to as the Optics endcap and the Connector endcap.

Optics Endcap

The optics endcap contains the optical windows that the laser beam passes through to make a measurement. The internal optics and laser electronics are mounted to the inside of this endcap.

Connector Endcap

The connector endcap has three underwater connectors that are used for communication, external power, and connecting to optional accessories or instruments:

3-pin connector: Used for BioBlock, an anti-biofouling accessory

5-pin connector: Used for serial communication and external power.

6-pin connector: Used for analog in/out and digital in/out signals.

See Appendix E for a full description of the wiring of the underwater connectors and mating cables.

A temperature sensor is located between the 3-pin and 6-pin connectors. In the center of the endcap is an LED that blinks when the instrument is sampling. The stainless steel fitting with small tube is the port for the depth sensor. This fitting can be removed to allow the port to be cleaned should sediment or salt deposit build up.

Also on the Connector endcap is a white plastic lever. This mechanical lever has a strong magnet embedded in the plastic. This magnet can be used to trigger a digital switch inside the instrument which can be programmed to start and stop the LISST-200X sampling.

The final item on the endcap is the zinc anode. The sacrificial zinc anode protects the instrument from corrosion during long periods of time in salt water.

Step 2: Check for Clean Windows

At this time, check the optical windows to make sure that they are clean. There are two windows: The receive window (or large window) and the transmit window (or small window). Both window need to be very clean in order to get good measurements.

The best way to check the windows is by using a flashlight. By shining light from one side and viewing from the other the surface of the windows can be easily checked for cleanliness.

If there is dirt or fingerprints on the windows clean them first by rinsing them with lukewarm water and a mild soap solution (e.g. mild hand soap, liquid dish soap) and then rinsing off all soap residue with clean, particle free water such as deionized water, distilled water or bottled drinking water. The windows can also be wiped clean with a soft cloth (e.g. a lens cloth) or glass cleaner. It is not recommended to use stronger solvents, such as acetone or toluene. Also, do not use any abrasive cleaners or wipes. Treat the windows as you would an expensive camera lens.

Step 3: Attach Communication and Power Cable

Remove the Communications cable from the plastic accessory case within the shipping case. It is the 3 meter cable with the USB connector on one end and the 5-pin underwater connector on the other. Remove the underwater cap from the Communications connector. The connectors will all look similar when the protective cap is installed. The Communication connector is the only 5-pin connector and is located next to the stainless steel pressure sensor fitting. After removing the cap install the cable making sure that proper alignment of the cable is maintained, so that the connector pins are not bent. Plug the USB cable into the computer. Please note that USB drivers may automatically install the first time the USB cable is plugged into the computer. If the driver installed correctly, you should see the green light on the USB cable blinking. For more information on establishing and troubleshooting communication with the LISST-200X see the Step-by-Step guide on page 32 of the User's Manual.

Step 4: Install the Horizontal test chamber

Remove the Small Volume Horizontal test chamber from the Accessory Case if it is not already installed on to the instrument. This one-piece chamber is designed to slip between the optical windows of the instrument such that the space between the windows can be filled with water for testing or calibration.

We can now fill the chamber with clean, particle-free water. Sequoia uses steam-distilled bottled water filtered through a 0.2 micron filter. Tap water may contain too many particles and may also contain dissolved gas that can release and form small bubbles on the optical surfaces. Pressurized filtered water can also contain dissolved gas. Often the best source of clean water is bottled drinking water.

Step 5: Install LISST-SOP200X Software

At this point the instrument is ready to go. We now need to install the software that is required for operation of the instrument. A USB memory card the size of a credit card is included with each instrument. In addition to the communication and processing program the disk also contains digital copies of this manual and other support files. Insert the memory card into a USB port on your PC to install the software. You must install the software on a computer running Windows XP or later (it is not compatible with Mac or Linux operating systems).

On the memory card you will find the 'LISST-SOP200X_Installer.exe.' The name LISST-SOP refers to the Standard Operating Procedure functionality of the program. Double click the installer executable to begin installing the software. Follow the onscreen instructions and the installer will transfer the necessary files to your computer and place a shortcut on your desktop and start menu. Do not remove the memory card from your computer until the installation is fully completed.

Please note the following with respect to the LISST-SOP200X:

- On a 64-bit Windows PC, the software will be installed in the following folder: C:\Program Files (x86)\Sequoia\LISST200X, while on 32-bit Windows machines, the install folder will be C:\Program Files\Sequoia\LISST200X
- The LISST-SOP200X **WILL NOT** work with the older LISST-100 or LISST-100X instruments. It is NOT possible to use the program to offload data from a LISST-100 or LISST-100X instrument, only a LISST-200X will work with this software.

Step 6: Start LISST-SOP200X Application

Start the LISST-SOP software by selecting the shortcut the installer placed on your desktop. See Appendix F: LISST-SOP Software Button Description on page 111 of the User's Guide for a brief description of the toolbar buttons.

Step 7: Establish Communication with the LISST-200X

The LISST-200X does not have an internal battery. Power is supplied to the instrument via a battery pack or USB cable. Please note that the LISST-200X USB cable has a USB-to-serial convertor and special power supply that converts the +5V to +11V to run the instrument built into cable. When using this cable no other power source is required.

Power can also be provided by the Small and Large External Battery Packs or another external power source such as a CTD. When using an external battery the serial communication passes through the battery pack. However, the power from the USB is not used power the instrument. Power will be drawn from the battery only.
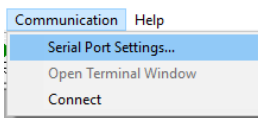
When connecting to your instrument from the software, the first step is to open a communication port with instrument. From the *Communication* menu select *Serial Port Settings*. You will see the window below:
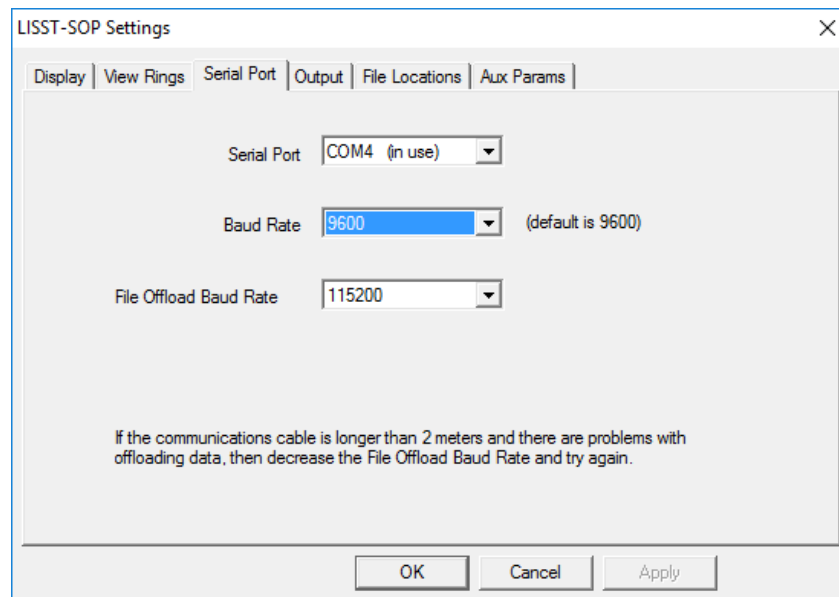
Select the port your LISST-200X is connected to from the drop down menu of next to 'Serial Port.' The other two fields can be left at their default values. Press the 'OK' button to save the changes.
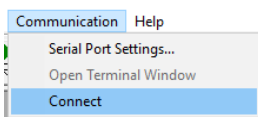
Next, open the port by selecting *Connect* from the *Communication* menu or pressing the button. After connecting, may need to wake your instrument from sleep before continuing. To wake up the instrument select *Wake Up LISST* from the *LISST* menu or press the button. A dialog box will appear counting down the maximum time required for the instrument to wake up. Upon wake up the instrument status will be displayed. If the cable connections are not correct or if
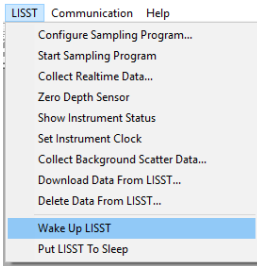
the power is not getting to the instrument a warning message will appear after the time expires. The serial port settings can be checked by selecting Serial Port *Settings* from the *Communication* menu. The default settings are COM1 and 9600 baud.

Step 8: Open Terminal window
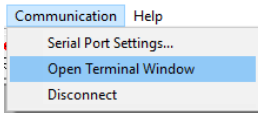
The LISST-SOP has a terminal window that allows the user to communicate directly with the instrument using a set of commands. To open the Terminal window select *Open Terminal Window* from the *Communication* window or select the ▣ button form the tool bar. A window similar to the one shown below will appear.
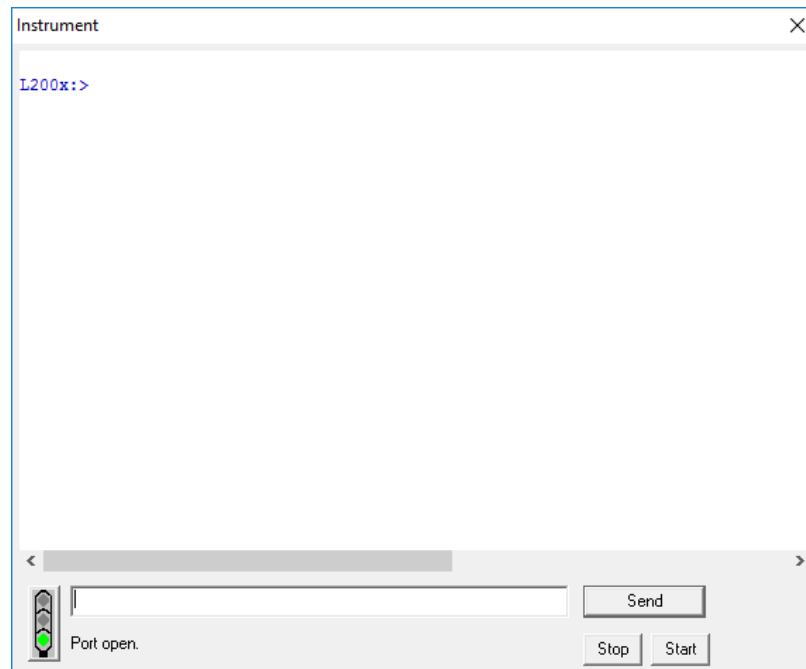
If you do not see the L200X:> prompt, click the Send button.

A command can be typed into the box in the lower section of the window. When the Enter key or the Send button is pressed the command will be sent to the instrument. The Start button sends a GO command to the instrument to start the instrument sampling using the current settings. The STOP button sends a CTRL-C to stop the instrument from its current process. A list of the commands that can be sent to the LISST-200X can be found in the section LISST-200X Command Summary on page 75.

Step 9: Acquiring Clean Water Background Measurement
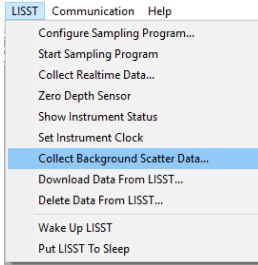
The background scattering measurement is critical to good instrument performance. It is especially critical for clean water applications where the optical transmission is greater than 90% over the 2.5cm path. The background scattering will also check the overall health of the instrument. It will verify that all of the systems are functioning and that the optics is still in alignment. The current background will be acquired and displayed relative to the factory background scattering for the instrument. The image below shows an example of this display. It is opened by either selecting *Collect Background Scatter Data* from the *LISST* menu or by pressing the button on the toolbar.
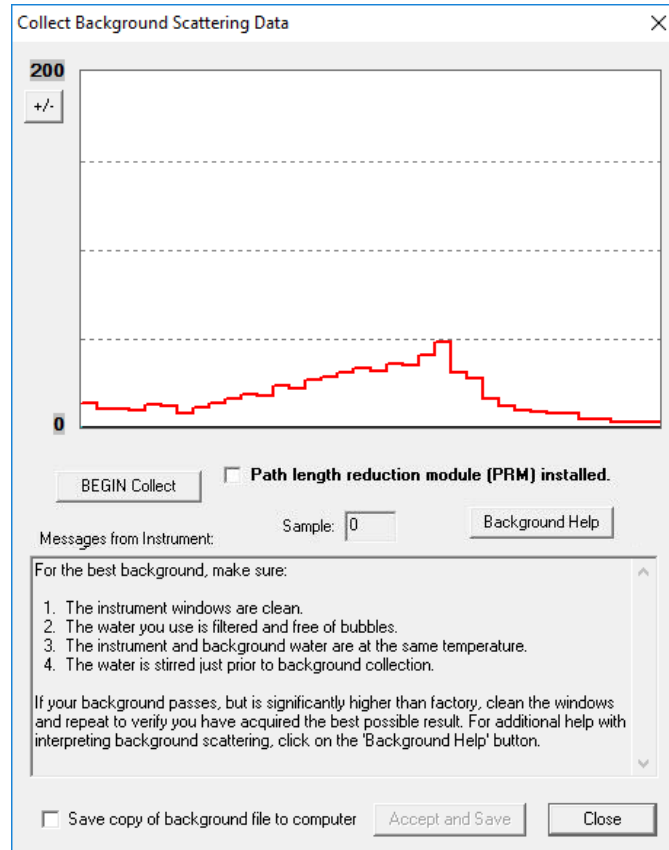
The factory background file will be automatically acquired from the instrument and displayed as a red line on the screen. Tips for collecting a background are also displayed on the screen. Pressing the 'Background Help' button will open a PDF with additional information about background measurements. When the *BEGIN Collect* button is pressed 20 samples will be displayed to the screen as they are acquired.

The graph shows the value of the 36 light scattering detectors. The red line is the factory values, the black line is the current measurement and the solid bars are the average of all 20 measurements. If the background is close to factory levels the message displayed will indicate a pass or acceptable background.

If the water or windows are not clean or if there is a problem with the instrument, error messages and suggested actions will be displayed. Dirty water or windows will generally cause higher values across the middle rings. Large bubbles or particles in the water can cause higher values on the inner rings or left hand side of the display. High values on the inner rings combined with a lower transmitted laser power value can also be an indication of optical misalignment.

If needed you can update the background, such as after cleaning the windows or replacing the water, by pressing the *BEGIN Collect* button again.

In general, the lower the background values the better the background. The goal is to get values that are at the same values as factory line. However, as the instrument is used the background may increase due to small scratches and slight alignment changes. It may not be possible to get the background down to the original factory values.

If the values are acceptable the values can be saved both onboard the instrument and to a file on your computer. The LISST-200X handles the background files differently than the LISST-100X. The background file is stored on the instrument and is saved as part of every data file that is recorded. When processing a data file the background will be automatically extracted and used during processing. Saving, tracking and selecting a background file to use for processing is no longer required. The background stored on the instrument will continue to be saved in new data files until a new background is recorded.

NOTE: If you have already saved a background file, then decide to do another one and you click Accept and Save, the background file on the instrument will be overwritten without any warning.

If you wish to store a background into file on your computer as well as on the instrument, check the 'Save copy of background to computer'. You will be prompted for location to save the file when you press the 'Accept and Save' button. It is not necessary to store the background on your computer, however, these saved backgrounds can be used during processing instead of the background saved in the data file.

Step 10: Configuring Instrument for Deployment

The Sampling Program window is used to configure the deployment parameters. To open the window, choose *Configure Sampling Program* from the *LISST* menu or press the ⬌ button on the tool bar. A window similar to the one shown will appear. The window has four tabs: Instrument Status, Operating Mode, Start Condition, and Stop Condition.
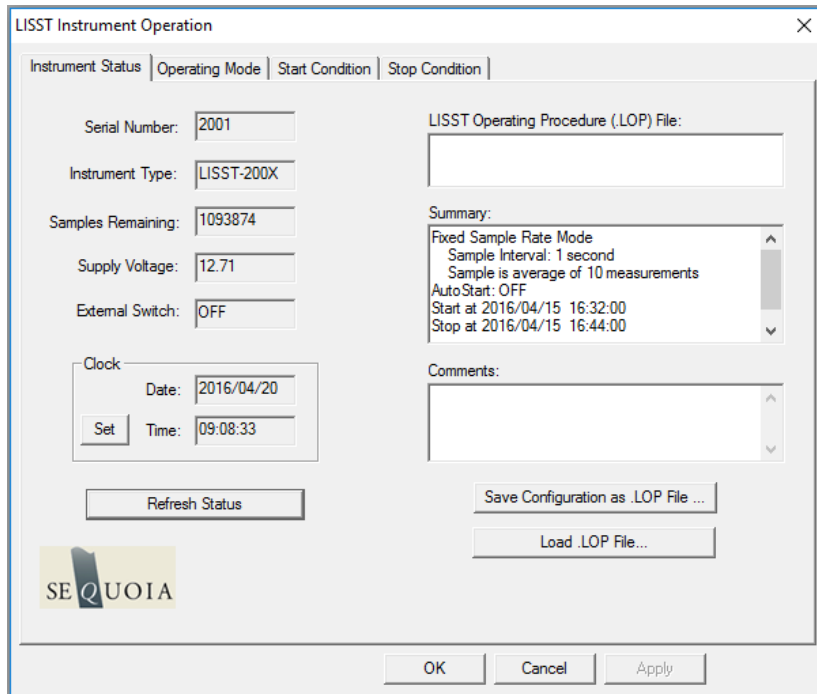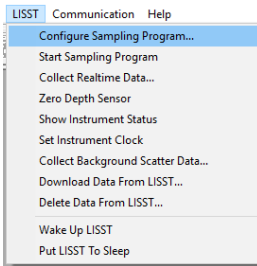
The Instrument Status page shows a summary of the instrument's current configuration. It also allows previously saved instrument configuration files (.LOP files) to be loaded on to the instrument. The ability to save the current settings to a file is also available. The. The Comments windows allows for entering data about the current configuration, for example metadata relating to the current settings.

Step 11: Setting Operating Mode
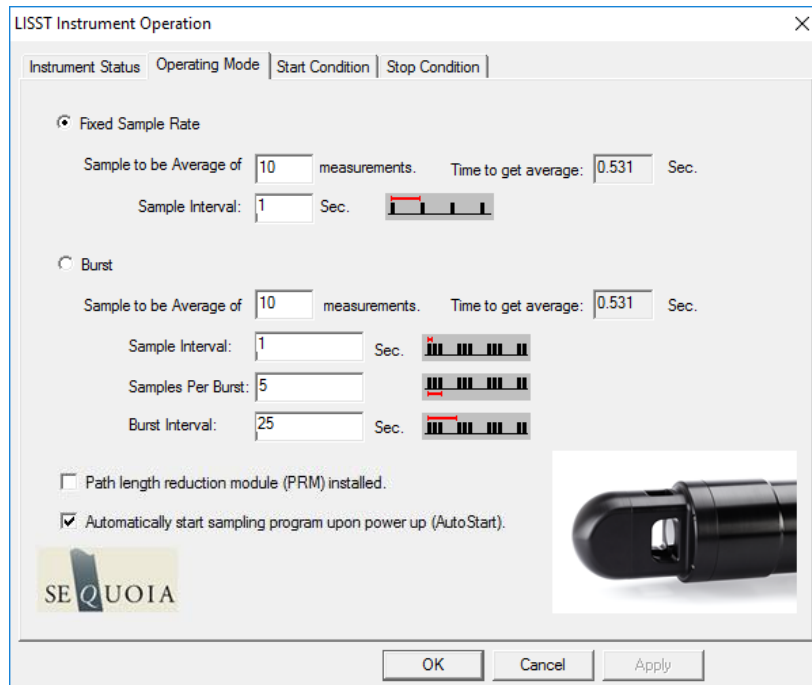
By selecting the *Operating Mode* Tab at the top of the main window the screen below appears. This screen is used to set the type of sampling; Fixed Sample Rate or Burst. You can also select the samples per average and sample rates on this screen.

Burst and Fixed Rate Modes

The Burst and Fixed Sample Rate modes are used to save data to a raw data file on board the instrument. The icons next to the various values give a better understanding of their meaning. The software automatically checks the values entered to make sure that there is no conflict. For example, when a 'Sample to be Average of' value is entered, the minimum sample interval is computed. If this value is less than the minimum permitted the value will be changed to the minimum and the text will turn red.

The LISST-200X measures internally at ~20 Hz, but the data cannot be stored to the data logger at this rate. The individual measurements are averaged into a sample, and it is this sample average that is stored at a maximum sample rate of 1 Hz. For the example shown above, the instrument is set to sample in the Fixed Sample Rate mode at a 1 Hz rate with 10 measurements per average. This average is obtained in 0.53 seconds.

AutoStart

If the AutoStart check box is selected, the LISST-200X will start the sampling program when power is applied to the instrument. If this is not selected the user must send the 'GO' command to start the sampling programing after apply power.

NOTE: AutoStart will not occur if the power is connected to LISST-200X in low power sleep mode.

Step 12: Setting Start Conditions

After selecting the Operating mode the start and stop conditions can be selected. Choose the *Start Condition* tab at the top of the window.
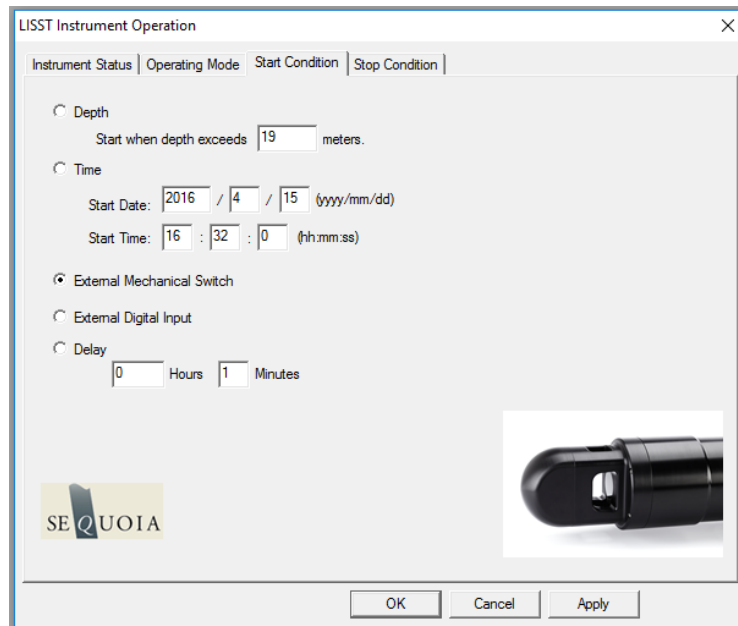
There are five options: Depth, Time, External Mechanical Switch, External Digital Input, and Time Delay. Select the mode by clicking on the button next to its label. Select the correct parameters as required. For this example let's select the External Mechanical Switch Start Condition.

Step 13: Setting Stop Conditions

Similarly the Stop conditions can also be selected. Click on the *Stop Condition* Tab to open the Stop Condition window. The available stop conditions are: Depth, Time. External Mechanical Switch, External Digital Input, Fixed number of Samples, and Memory Full (<less than 6.5 V).
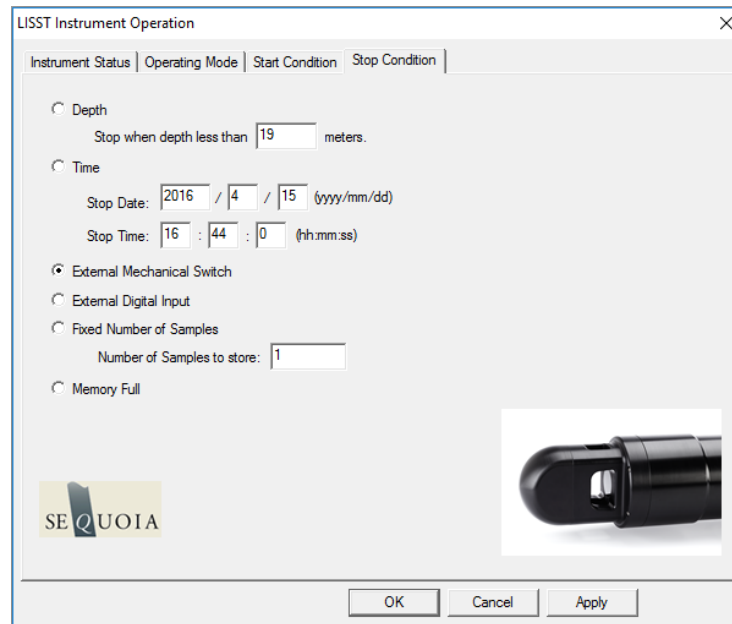
For this example, choose the External Mechanical Switch as the Stop condition.

After selecting the Stop Conditions, click the APPLY button to complete the configuration. This will transfer the settings to the instrument. Then return to the Instrument Status page by clicking on the Instrument Status Tab. It may take several seconds to configure the sampling parameters on the LISST-200X. The status bar at the bottom of the main window will indicate when a process is underway:

The status bar will return to 'Ready' when the process is completed:

Step 14: Saving Settings for Future Use	The Instrument Status Page will now display the summary of the settings that have been selected. These settings can be saved for later use by selecting the 'Save Configuration as .LOP File'. A file name will be prompted for. The file will be given an LOP extension which will identify it as a LISST Operating Procedure File. Comments can be entered before saving in the box below the summary. Previously saved LOP files can be opened using the 'Load .LOP File' button.
	Press the *OK* button to complete the configuration.
Step 15: Background File Warning	If the background file onboard the instrument is more than 6 hours old, a File Warning dialog box will appear, alerting you that final data quality could be compromised. Select from the 2 options (collect a new background or accept the risk and continue).
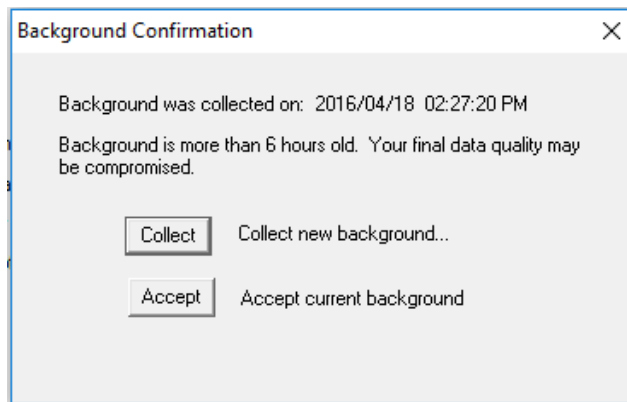
Step 16: Start Instrument	The instrument is now configured for deployment, **however, it is not yet running**.
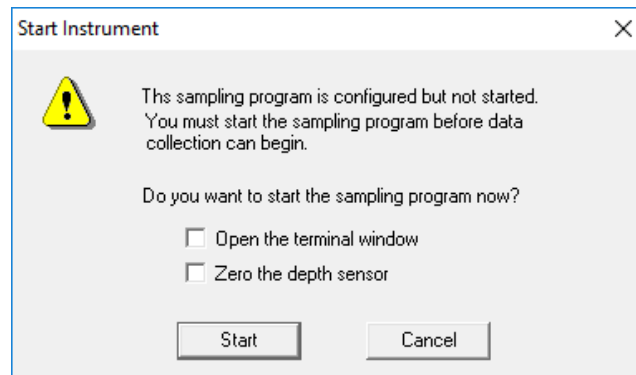
	You MUST start the instrument in order to start the samping program you just configured. If you do not do that, the LISST will never start sampling or react to the start and stop conditions.
	Note that starting the instrument is not necessarily the same as starting sampling (unless you have a delay start of 0 minutes set as your start condition). For example, if you have selected the mechanical

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switch as the start condition, the instrument will only react to the swtich after the sampling program has been started. Only then will actual sampling begin.

For convience, you can select to open the terminal window or zero the depth sensor before starting the sampling program. We recommend selecting to open the terminal window, so you can confirm the sampling program has been started.

If you select the Cancel button, the message below appears, informing you how you can start the instrument.
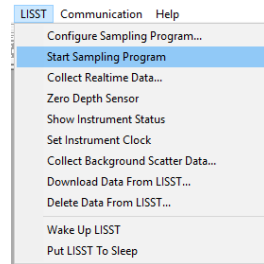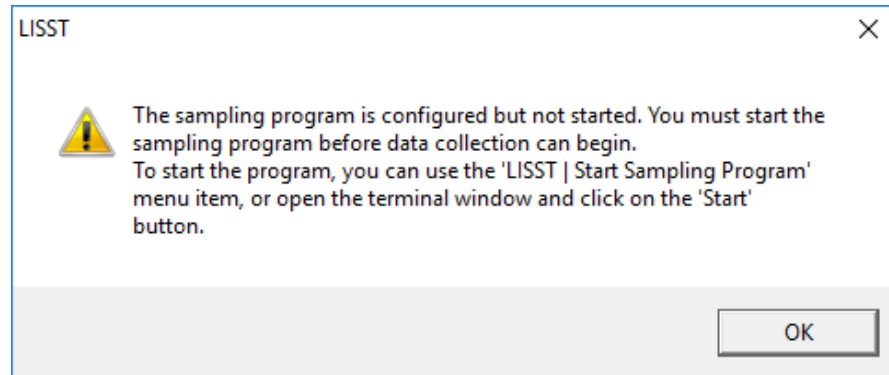
When the instrument has been started, depending upon the Start Conditions, text will be displayed approximately every 30 seconds in the terminal window. At this point the user will know that the instrument is running and is ready to be deployed. For our example it will be displaying a message that reads "Waiting for Mechanical Switch ON signal".

While waiting for the start condition the LED on the connector endcap will double blink every few seconds to alert the user that the instrument is running but not yet sampling. During sampling the LED will illuminate during the actual measurement of an averaged sample. For example if the sampling is set for 1 Hz sampling with measurements per average set to 20 then the LED will blink once per second. If settings are set for a 30 second average every 15 minutes then the LED will illuminate for 30 seconds every 15 minutes.

Step 17: Collecting Data

For our example we can move the white plastic lever on the endcap to the "1" position. Sampling will start. If the Terminal window is still open, text will be displayed as the instrument acquires data. Data

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collection will continue until the Stop Conditions are met. In our case, until the switch lever is returned to the "0" position.

If the Start and Stop Conditions are set to Depth, External Mechanical Switch, or External Digital Input the program will return to checking for the Start Condition. This will only be true if the Start and Stop conditions match. For example, Depth Start and Depth Stop. For non-matching Start and Stop Conditions, such as fixed number of samples or Time Stop, the program will terminate and the instrument will go into a low power sleep mode.

To stop a running program, or wake it from low power sleep mode, use the Stop button on the Terminal window or the [STOP] button on the tool bar.

It is good practice to always send a Stop command to the instrument before attempting any other communication with the instrument.

Step 18: Downloading Data
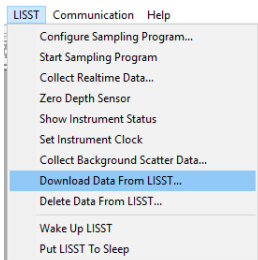
The instrument has now stored data in the on-board memory card. Pressing the Instrument Query Button **i**, will display the instrument status, including the number of samples saved. To download the data to your computer select *Download Data From LISST* from the *LISST* menu or choose the ⬇, button from the toolbar. A list of files will appear:

LISST   Communication   Help

Configure Sampling Program...
Start Sampling Program
Collect Realtime Data...
Zero Depth Sensor
Show Instrument Status
Set Instrument Clock
Collect Background Scatter Data...
Download Data From LISST...
Delete Data From LISST...
Wake Up LISST
Put LISST To Sleep

Select Files on Datalogger

Highlighting to select files on datalogger from the following list, then press OK.

Name:	Size:	Modified:
L1180735.RBN	2,400	2016-04-27 07:36:10
L1180737.RBN	7,320	2016-04-27 07:41:58
L1860154.RBN	2,160	2106-07-05 01:54:48
ZSLOG.BIN	480	2016-02-26 14:23:22

OK   Cancel

Choose the files to offload by clicking on them while holding down the CTRL key. The Shift key can also be used to select a range of files. Select OK and then choose a location to save the files to.
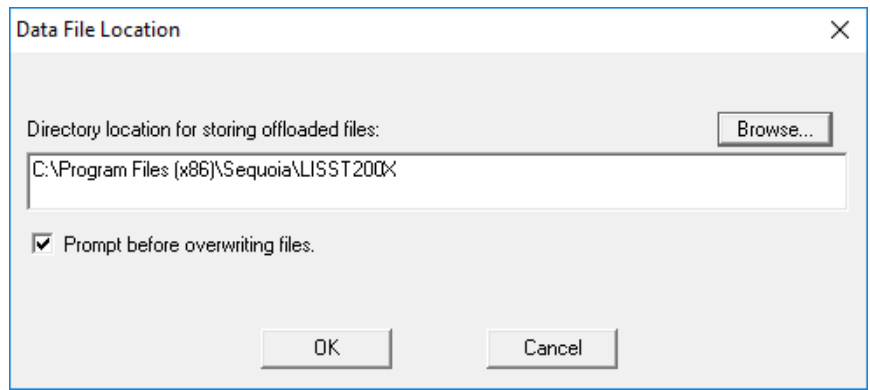
The files will be saved with names in the following format: L*dddhhmm*.RBN, where *ddd* is the day of the year, *hh* is the hour, and *mm* is the minute that the file was first written to. As the data is downloading a Transfer Status window will appear. The data is offloaded at 115K baud.
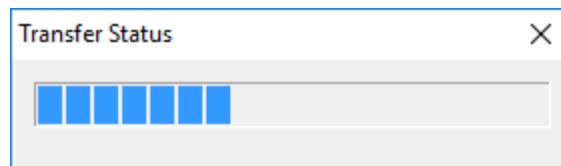
If you click the cancel button in the upper right corner of the transfer window the current file transfer (and any remaining file transfers) will be cancelled.

Step 19: Processing Raw Data

We now have the data transferred from the instrument to the PC. To process the data file choose *Process Raw Data* from the *File* menu or press the button on the toolbar.
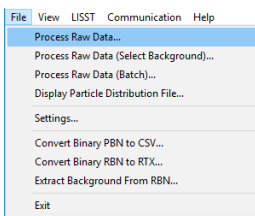
Note: You can also choose to open a raw data file using *Process Raw Data (Select Background).* Use this option to manually select a background saved on your computer to use during processing instead of the background saved in the data file. There is also an option to do batch processing of a large number of files at one time. Both of these methods of processing data are discussed in more detail in the LISST-200X User's Manual.

You will be prompted to select the raw data file to open. Raw data files have the extension .RBN, and are offloaded directly from the LISST-200X data logger. Below is list of the file types you can expect to see when using the SOP software.

Extension	Discription	Format
.RBN	Raw Data	Binary
.RTX	Raw Data	ASCII
.PBN	Processed Data	Binary
.CSV	Processed Data	ASCII
.BGT	Background File	ASCII
.LOP	Operating Procedure	ASCII

Next, specify the output file name for the processed data. Note that a .PBN file is always created. Output of other file types (.CSV or .RTX) depends on the selections in the *Settings* selection in the *File* menu.

Every LISST-200X data file contains all the necessary information to process the file. Therefore, the software will automatically determine the instrument serial number, factory background, current background and other instrument specific parameters.

A raw data file display will be generated for your selected data file.
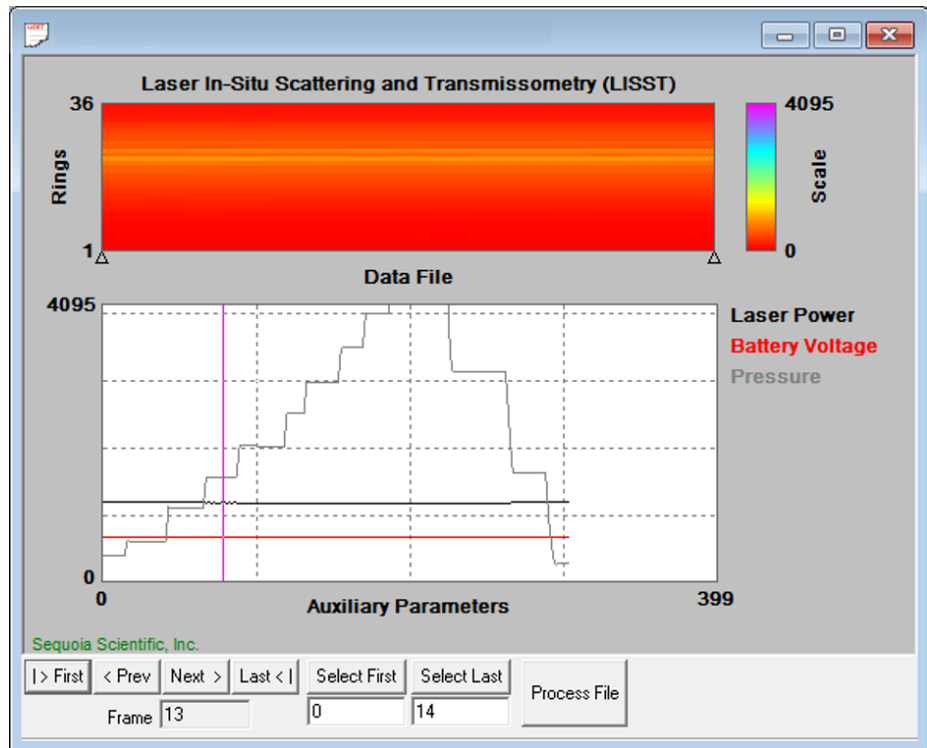
The range of samples to process can be selected by entering values in the *Select First* and *Select Last* boxes. The default selection is to process the complete file. Press the *Process File* button to convert the raw file into processed size distributions. For more details on the available options when this window is open please see the detailed

Instruction for Processing Raw data files. When the processing is complete the button label will change to read *Finished*.

Step 20: Viewing Processed Results
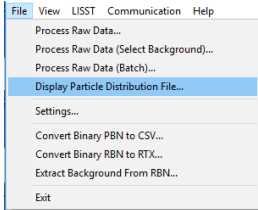
Processed data files are stored as ASCII files (.CSV) and as binary files (.PBN). The PBN files can be opened in the software and the size distribution viewed on the screen. To open a processed Particle Size Distribution file choose *Display Particle Distribution File* from the *File* menu or press the 📂 button on the toolbar. A display similar to the one below will open. The left hand figure will display a bar chart showing the volume concentration in each of the 36 log spaced size classes. The right hand plot will be the cumulative concentration. To view the samples as a movie press the *Start Display* button. The slider bar next to the button adjusts the refresh rate. The *First*, *Prev*, *Next*, and *Last* buttons allow you to step through the measurements one frame at a time. When the last frame is reached the movie display will stop. Use the *First* button to return to the first sample and press the *Stop Display* button to restart the display.

File	View	LISST	Communication	Help

Process Raw Data...
Process Raw Data (Select Background)...
Process Raw Data (Batch)...
Display Particle Distribution File...
Settings...
Convert Binary PBN to CSV...
Convert Binary RBN to RTX...
Extract Background From RBN...
Exit

ISO_Fine_CL_rs

Laser In-Situ Scattering and Transmissometry (LISST)

Particle Size Distribution   Cumulative Concentration

Mean: 8.52
Std Dev: 23.48
Total Vol Conc: 30.64

Sample Number: 5

Sequoia Scientific, Inc.

Sequoia Scientific, Inc.

Start Display |> First   < Prev   Next >   Last < | Scale +/-

0.5 Sec   0   Interval (Sec)   60

To view the value of the auxiliary parameters, such as depth and transmitted laser power, select the *Show Auxiliary Parameter* from the *View* menu.
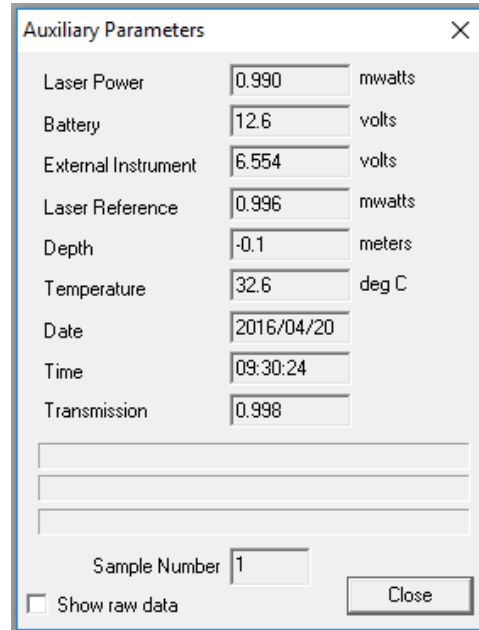
Step 21: Opening a Real Time Session

The LISST-SOP software also supports the ability to acquire, process, and display size data it in real time. The Start and Stop conditions will not be used and therefore their settings are not relevant.
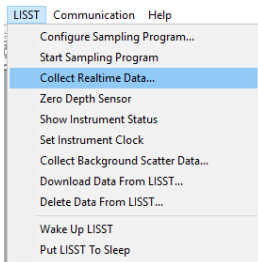
Before opening a real time session you should collect a new background and store it onboard the instrument. The real time session will use the last background stored on the instrument to process the data in real time. Therefore, you must ensure the background on the instrument is up to date before continuing.

To open the Real-Time session, choose *Collect Realtime Data* from the *LISST* menu or press the button. Choose an output PBN file. A display very similar to viewing a processed PBN file will be displayed.
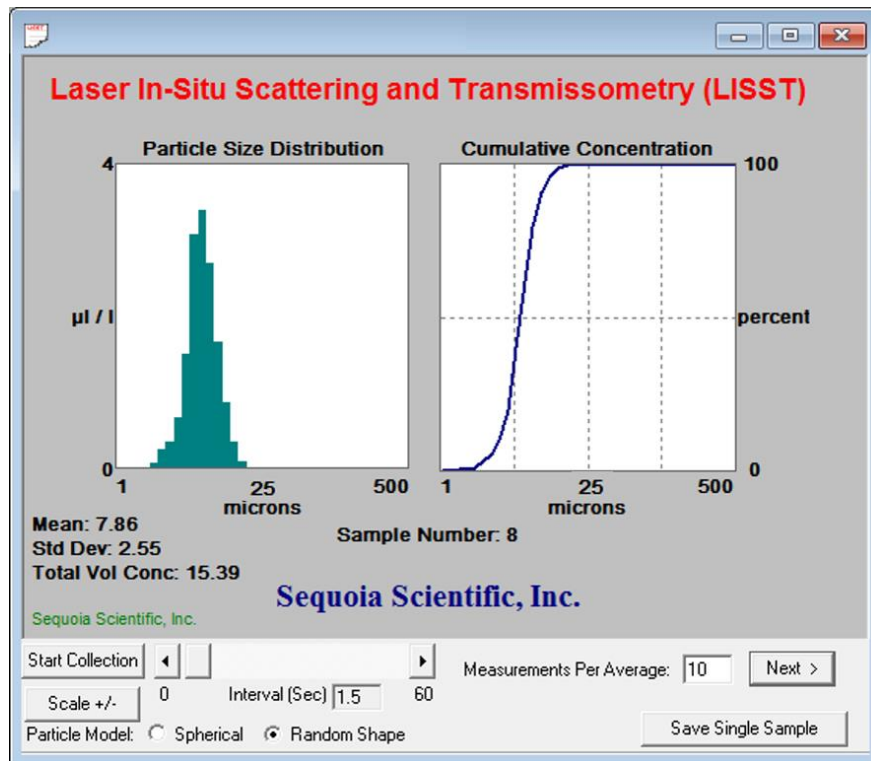
Use the Start Collection button to start and stop continuous data collection. The slider bar controls the data acquisition time.

The Measurements Per Average text box allows you to adjust the number of measurements that will be averaged together before the display is updated. The default is 10. Higher values can be used however it may cause a delay that lasts longer than your selected interval time.

The Scale +/- Button adjusts the Particle Size Distribution scale. The Save Single Sample button records a single sample to an ASCII file.

The Next button is used if the continuous data collection mode is not being used. Pressing the next button will command the LISST to make a measurement and transmit the data to the SOP for display.

The Spherical / Random Shape radio buttons can be selected in order to display the results as being processed under the assumption that the particles are spheres or randomly shaped (natural grains) particles.

The Auxiliary Parameters window can be opened during the Real-Time session so that the values of various parameters such as depth and transmission can be monitored. The raw scattering values on the detector rings can also be viewed by selecting *View Rings* from the *View* menu. A typical display is shown below.
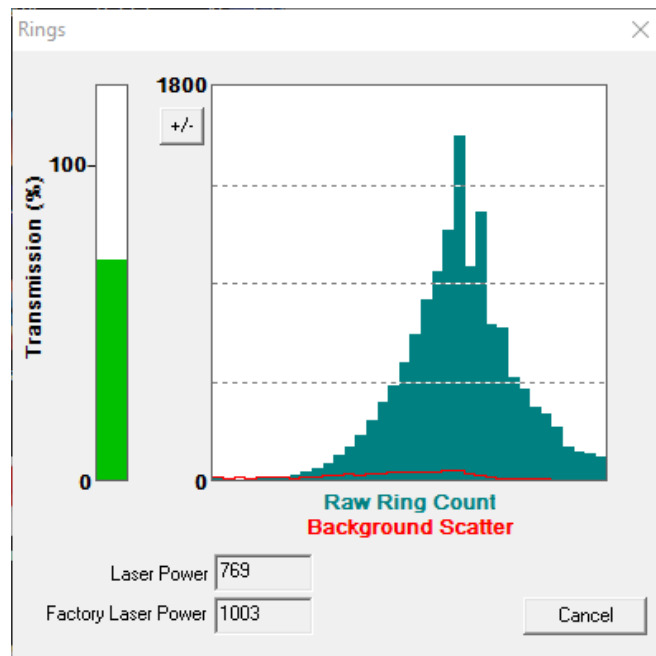
The view rings display is also available when processing a Raw Data file. However, it is not available when viewing a processed Size Distribution file.

Deploying the LISST-200X in the Field

The above steps should have given you a good understanding on the how to operate the LISST-200X. More details on all of the steps can be found in the User's Manual following this tutorial. The following steps go into deploying the instrument in the field.

Step 22: Preparing for Transport

The LISST-200X has been designed to be a robust field instrument. However, it is still a highly sensitive optical instrument and needs to be treated with care. This is especially true during shipping. The LISST-200X is shipped in a custom case with specially designed foam cushioning. Anytime the instrument is transported it should be in this case. We highly recommend using air shipping when possible to eliminate the extended vibrations that ground shipping can cause.

Because the LISST-200X does not have an internal battery there is no need to worry about putting the instrument into low power sleep mode. You can simply disconnect it from the battery pack.

The Large External Battery Pack, which has alkaline batteries, can be stored for extended period when fresh batteries are installed.

The Small External Battery Pack, which has NiMH rechargeable batteries, should be opened and the batteries removed during transport. There are not restrictions on shipping NiMH batteries. However, the batteries will have a small drain when installed in the Small Battery housing. Therefore it is recommended that they be

removed until the battery is ready to be used. The batteries should also be stored in a fully charged state.

The LISST-200X can be pre-programmed with the desired sampling program such as start and stop conditions and sampling rates. These settings will remain in non-volatile memory until power is applied. Therefore it is possible to prepare the instrument for sampling before shipping it out. It is even possible to program the instrument to automatically start upon power up. This is described in the next step.

Step 23: Configuring AutoStart

The LISST-200X can be configured to start running the sampling program upon power up. In some situations this ability can be quite helpful as you do not need to connect the instrument to the PC to start the sampling. The instrument can be preconfigured with the desired settings and the wait for the battery to be plugged in or external power from the CTD to be present before looking for the Start Condition.

To configure the LISST-200X to start its sampling program upon power up use the AS command (short for Auto Start). The AS command can be issued when the 200X> prompt is displayed in the Terminal Window. When AutoStart is enabled a message will be displayed as part of the Status command or the Query instrument results.

When the LISST-200X is powered up a message will be displayed to the Terminal window (or via the RS232 interface) that will prompt the user that the instrument will auto start in 5 seconds. Pressing the stop key or sending two CTRL-C characters will stop the instrument from starting and return to the 200X> prompt.

Step 24: Connecting to External Sensors or Instruments

The LISST-200X had the ability to connect to external sensors and instruments such as other dataloggers or CTDs through the 6-pin Auxiliary connector on the endcap. The details of using the Auxiliary connector can be found in the Step-by-Step instructions on page 69. In short, the Auxiliary connector can be configured in one of three ways: 1) Analog and Digital inputs, 2) Dual Analog Inputs, or 3) Dual Analog Outputs. When using the Analog or Digital inputs it is possible to provide power to an external sensor. By default this power output is switched off. It will need to be enabled before power will be available. You can also configure the warmup time required for the sensor after power is applied before sampling will begin.

The Analog Output configuration sends out two analog voltages, Mean Size and Total Concentration. This summary information is updated each time data for the full size distributions is recorded. When using the Analog Output configuration power can be received through the Auxiliary connector. The wiring of the connector is designed to match the SeaBird CTD Auxiliary Input connector simplifying the connection.

II. *LISST-200X Operation Details*

Section Organization

This section contains detailed instructions for performing various procedures. These are either in the form of step-by-step instructions or detailed descriptions of the various aspects of instrument operation (e.g. command list, instrument mounting and deployment).

General Precautions

- LISST-200X is a sensitive optical instrument - please handle it gently as you would handle a very expensive camera.

- Critical alignments may be disturbed if the instrument is subjected to shock or rough handling.

- Evidence of shock/rough handling will void the warranty.

- Whenever in transit, store the instrument in the provided padded shipping case.

- If placing the instrument vertically on the standoffs, be sure to do so gently as the Compact Flash Memory Card inside may otherwise come loose.
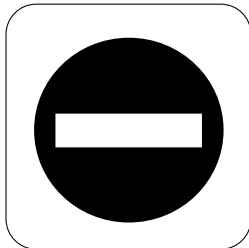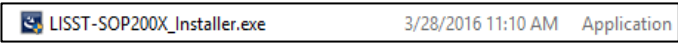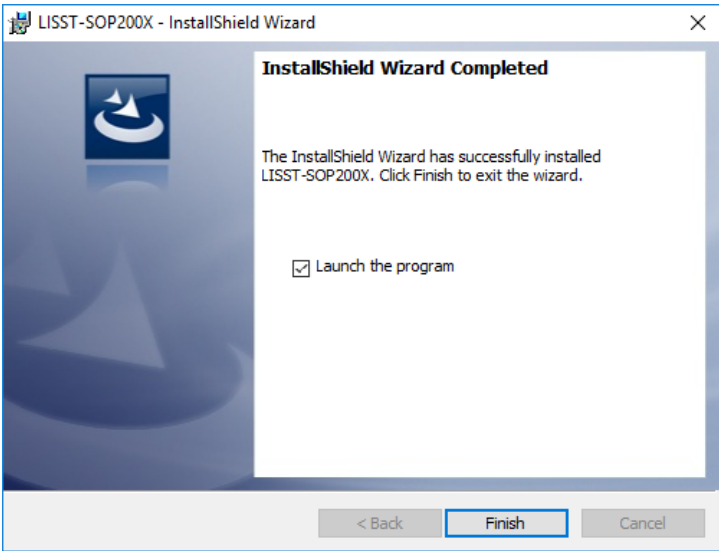
WARNING

The LISST-200X uses a laser diode emitting a maximum of 1 mW of visible (red) light at a wavelength of 670nm. The laser beam under normal circumstances is not a threat. However, if objects are placed in the path of the laser beam, the light could be reflected into the eye causing permanent damage.

D. Step by Step Procedures
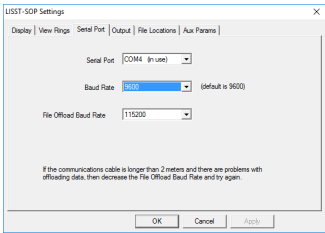
1. Installing LISST-SOP Software

Software for the PC is used to configure the LISST-200X and for downloading and processing the size distributions.

STEP	ACTION	RESULT
1	• The LISST-200X comes with a USB memory card. Plug the provided memory card into a USB port on your computer. Locate the 'LISST-SOP200X_Installer.exe' executable on the memory card. Note that this software it is not compatible with Mac or Linux operating systems. Your operating system must be Window XP or newer to run the SOP software. LISST-SOP200X_Installer.exe 3/28/2016 11:10 AM Application •	Installer was found on LISST-200X memory card
2	• Double click the 'LISST-SOP200X_Installer.exe.' Follow the onscreen instructions and the installer will transfer the necessary files to your computer and place a shortcut on your desktop and start menu. Do not remove the memory card from your computer until the installation is fully completed: LISST-SOP200X - InstallShield Wizard **InstallShield Wizard Completed** The InstallShield Wizard has successfully installed LISST-SOP200X. Click Finish to exit the wizard. ☑ Launch the program < Back Finish Cancel	Installation of SOP software in complete

2. Establishing Communication with the LISST-200X

Establish communication with LISST-200X using the supplied software and USB cable. Communicating with the LISST-200X via the LISST-SOP software should be automatic. Should there be a problem you can use these step-by-step instructions to troubleshoot the problem.

STEP	ACTION	RESULT
1	• If the SOP is open, close the program, then plug the USB communication cable to the LISST-200X and to a USB port on your computer.	LISST-200X will be powered on
2	• After plugging the USB cable into your computer, the drivers for the USB to serial converter should install automatically. You should see a notification on the task bar that drivers are being installed. If you are unsure if the drivers installed, you can install the drivers manually by running 'CDM USB Drivers.exe', located on the memory card that came with your instrument.	Computer is now set up to communicate with the LISST-200X
3	• After the driver installation is compete, open the LISST-SOP200X software.	SOP software appears onscreen
4	• Open Serial Port Settings from the Communications menu. • Select the port your LISST-200X is connected to from the dropdown menu next to 'Serial Port.' The Baud Rate and File Offload Baud Rate should stay at their default values, 9600 and 115200, respectively. Save the settings by pressing the 'OK' button. • If you have trouble finding the right port, open the Device Manger on your computer. Unplug and then re-plug the USB cable into your computer. The port that appears in the Device Manger when you plug in the USB cable is the port you need to select in the SOP software. If no port appears, go back to step 2 and reinstall the USB drivers.	SOP software will be configured to connect to the LISST-200X
5	• Open the port by selecting *Connect* from the *Communication* menu or pressing the button.	Communication port is now open

6	• After connecting, may need to wake your instrument from sleep before continuing. To wake up the instrument select *Wake Up LISST* from the *LISST* menu or press the button. A dialog box will appear counting down the maximum time required for the instrument to wake up. Upon wake up the instrument status will be displayed. If the cable connections are not correct or if the power is not getting to the instrument a warning message will appear after the time expires.	LISST-200X is awake and ready to configured or collect data

3. *Saving and Evaluating Clean Water Backgrounds*

In order to properly compute the size distribution, it is necessary to remove the light scattering from the internal optics and window surfaces so that only the light scattering from the particles of interest are used to compute the size distribution.

STEP	ACTION	RESULT
1	• Connect the instrument to the computer and establish communication in the LISST-SOP200X program. (see Step-by-Step Procedure: Establishing Communication with the LISST-200X)	LISST-SOP open and communicating with LISST-200X.
2	• Clean instrument and install Clean Water Background Test chamber. • Fill with clean filtered water and make sure no bubbles are in the water or on the windows. • The water and instrument should be at the same temperature. Using water a significantly different temperature from the water can impact the quality of the background. • Stir the water well before obtaining a background to make sure the water is well mixed. • For the best Background, make sure the instrument is clean; the water you use is filtered, and free of bubbles. Temperature fluctuations in water will seriously degrade the background. Use water at the same temperature as the instrument. Stir well.	Optics submerged in water
3	• Select *Collect Background Scatter Data* from the *LISST* menu or press the ![icon] button on the toolbar. The factory background file will be automatically acquired from the instrument and displayed on the screen. • If using the path length reduction module (PRM), you must collect a background with the PRM installed. If the PRM is installed, select the PRM checkbox in the collect background window. • When the *BEGIN Collect* button is pressed 20 samples will be displayed to the screen as they are acquired.	Background collected and displayed on the screen

	• The graph shows the value of the 36 light scattering detectors. The red line is the factory values, the black line is the current measurement and the solid bars are the average of all 20 measurements. If the background is close to factory levels the message displayed will indicate a pass or acceptable background. Even if your new background passes, but is significantly higher than factory, clean the windows and repeat to verify you have acquired the best possible result.	Acceptable Background
	• If the water or windows are not clean or if there is a problem with the instrument, error messages and suggested actions will be displayed. Dirty water or windows will generally cause higher values across the middle rings. Large bubbles or particles in the water can cause higher values on the inner rings or left hand side of the display. High values on the inner rings combined with a lower transmitted laser power value can also be an indication of optical misalignment. If needed you can update the background, such as after cleaning the windows or replacing the water, by pressing the *BEGIN Collect* button again.	Unacceptable Background

4	• In general, the lower the background values the better the background. The goal is to get values that are at the same values as factory line. However, as the instrument is used the background may increase due to small scratches and slight alignment changes. It may not be possible to get the background down to the original factory values. • Multiple cleanings or using better filtered water may be required to get the best possible background.	
	• When you have an acceptable background press the Accept and Save button to store the background on the instrument. You also have the option to save the background to a file on your computer. The LISST-200X handles the background files differently than the LISST-100X. The background file is stored on the instrument and is saved as part of every data file that is recorded. When processing a data file the background will be automatically extracted and used during processing. Saving, tracking and selecting a background file to use for processing is no longer required. The background stored on the instrument will continued to be saved in new data files until a new background is recorded. • **NOTE:** If you have already saved a background file, then decide to do another one and you click Accept and Save, the background file on the instrument will be overwritten without any warning. • If you wish to store a background into file on your computer as well as on the instrument, check the 'Save copy of background to computer'. You will be prompted for location to save the file when you press the 'Accept and Save' button. It is not necessary to store the background on your computer, however, these saved backgrounds can be used during processing instead of the background saved in the data file.	Background Accepted and saved.

4. Configuring Data Collection

The LISST-SOP software can be used to configure the Start and Stop Conditions, Fixed Rate or Burst sampling and sample rates.

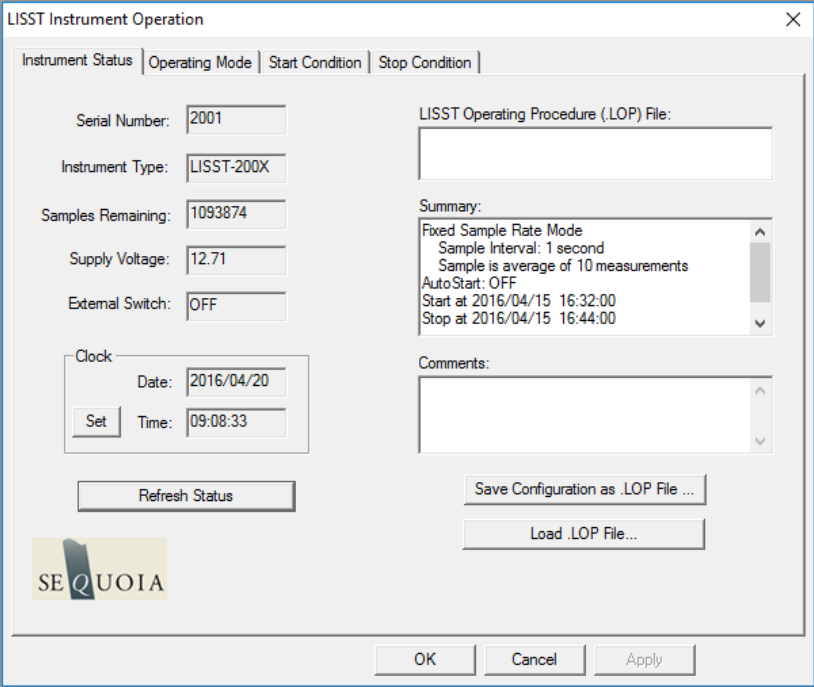| STEP	ACTION	RESULT
1	• Connect the instrument to the computer and establish communication in the LISST-SOP200X program. (see Step-by-Step Procedure: Establishing Communication with the LISST-200X)	LISST-SOP open and communicating with LISST-200X.
2	• Open the Sampling Programs window by choosing *Operating Modes* from the *LISST* menu or by pressing the ![button] button on the toolbar. ![LISST Instrument Operation dialog] • To set the LISST-200X data logger clock to the computer clock press the *Set Clock* button. Press the *Refresh Status* button to refresh the display.	Instrument Status Displayed. Clock set
3	• To load a pre-saved LISST Operating Procedure file (LOP) click the *Load .LOP File* button. • After the file is selected, the appropriate settings are loaded but not yet configure on the instrument. To apply these settings to the instrument press the *Apply* or *OK* buttons. • LISST Operating Procedure files can be saved by clicking the *Save Configuration as .LOP File* button. The setting must have first been sent to the instrument using the Apply button.	

4	• By selecting the *Operating Mode* Tab at the top of the main window the screen below appears. This screen is used to set the type of sampling: Fixed Rate or Burst. You can also select the samples per average and sample rates on this screen.	Operating Mode set
	• The Burst and Fixed sample rate modes are used to set up a sampling program that will save data onboard the instrument. The icons next to the various values give a better understanding of their meaning. The software automatically checks the values entered to make sure that there is no conflict. For example, when a Samples per Average value is entered, the minimum sample interval is computed. If this value is less than the minimum permitted the value will be changed to the minimum and the text will turn red. A similar test is done on the Burst Interval setting.	
	• If using the path length reduction module (PRM), check the PRM checkbox. This checkbox must be selected to ensuring the data are processed using the correct path length.	
	• If the AutoStart check box is selected, the LISST-200X will start the sampling program when power is applied to the instrument. If this is not selected the user must send the 'GO' command to start the sampling programing after apply power.	
	• NOTE: Auto Start will not occur if the power is connected to LISST-200X in low power sleep mode.	
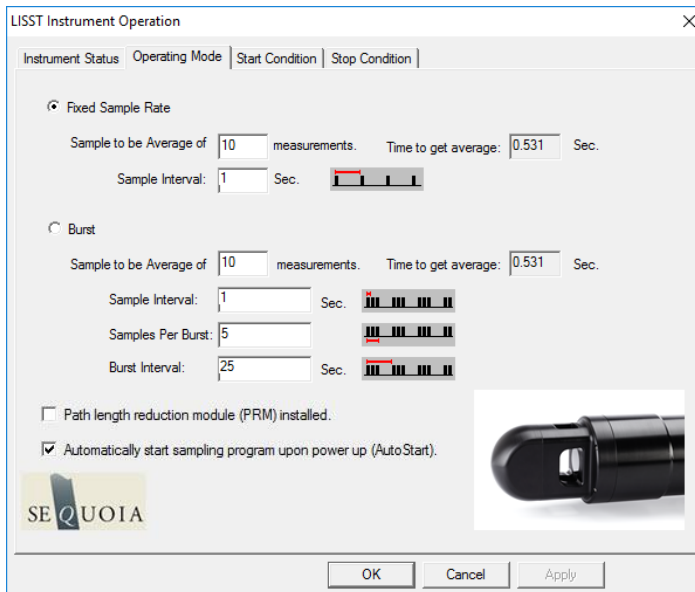
5	• Select the Start Conditions tab to configure when the instrument will begin sampling. • There are five options: Depth, Time, External Mechanical Switch, External Digital Input, and Time Delay. Select the mode by clicking on the button next to the its label. Select the correct parameters as required.	Start Condition Set

LISST Instrument Operation ✕

Instrument Status | Operating Mode | Start Condition | Stop Condition

○ Depth
    Start when depth exceeds 19 meters.
○ Time
    Start Date: 2016 / 4 / 15 (yyyy/mm/dd)
    Start Time: 16 : 32 : 0 (hh:mm:ss)
⦿ External Mechanical Switch
○ External Digital Input
○ Delay
    0 Hours 1 Minutes

SE**Q**UOIA

OK Cancel Apply
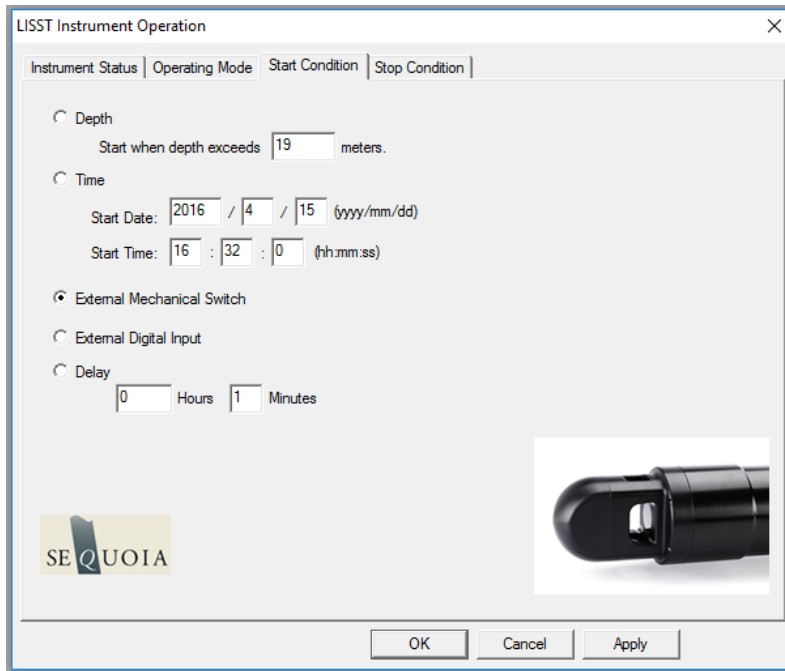
6	• Choose the Stop Conditions Tab to select the conditions when sampling should stop. • The available stop conditions are: Depth, Time. External Mechanical Switch, External Digital Input, Fixed number of samples, and Memory Full.	Stop Condition Set

LISST Instrument Operation ✕

Instrument Status | Operating Mode | Start Condition | Stop Condition

○ Depth
    Stop when depth less than 19 meters.
○ Time
    Stop Date: 2016 / 4 / 15 (yyyy/mm/dd)
    Stop Time: 16 : 44 : 0 (hh:mm:ss)
⦿ External Mechanical Switch
○ External Digital Input
○ Fixed Number of Samples
    Number of Samples to store: 1
○ Memory Full

SE**Q**UOIA

OK Cancel Apply

	• If the Stop Condition is Depth, External Mechanical Switch, or External Digital Input and the Start Condition is also one of these three options the Base Program on the instrument will return to waiting for the Start condition. For example, if the start and stop conditions were set to External Mechanical Switch then multiple sampling sessions can be obtained by flipping the switch on and off. This is useful when doing profiles. It eliminates the need to communicate with the instrument between profiles.	
7	• Select *Apply* or *OK* buttons to configure the instrument with the current settings. If the *Apply* button is pressed the program will return to the current window. Returning to the *Instrument Status* window will display a summary of the current settings.	
8	• When the OK button is pressed, an alert will apear that shows the time and date of your last background measurment. If the background is more than 6 hours old, it will alert you that final data quality could be compromised. Select from the 2 options (collect a new background or accept and continue). **Background Confirmation** ✕ Background was collected on: 2016/04/18 02:27:20 PM Background is more than 6 hours old. Your final data quality may be compromised. [Collect] Collect new background... [Accept] Accept current background • For best results, the background file should ALWAYS be taken as close as possible to the deployment. • If using the path length reduction module (PRM), this alert may also warn you if your background was collected without the PRM installed. If you are deploying the instrument with the PRM installed, the clean water background must also be collected using the PRM.	Confirm Background Measurement

9	• If the background file is accepted, the instrument is now configured for deployment, **however, the sampling program running**.	Start Sampling Program
	Start Instrument ☒	
	⚠ Ths sampling program is configured but not started. You must start the sampling program before data collection can begin.	
	Do you want to start the sampling program now?	
	☐ Open the terminal window	
	☐ Zero the depth sensor	
	[Start] [Cancel]	
	• You MUST start the sampling program in order to collect data. If you do not do that, the LISST will never start sampling or react to the start and stop conditions.	
	• Note that starting the sampling program is not necessarily the same as starting sampling (unless you have a delay start of 0 minutes set as your start condition). For example, if you have selected the mechanical switch as the start condition, the instrument will only react to the swtich after the sampling program has been started. Only then will actual sampling begin.	
	• For convience, you can select to open the terminal window or zero the depth sensor before starting the sampling program. We recommend selecting to open the terminal window, so you can confirm the sampling program has been started.	
	• If you select the Cancel button, the message below appears, informing you how you can start the instrument.	
	LISST ☒	
	⚠ The sampling program is configured but not started. You must start the sampling program before data collection can begin. To start the program, you can use the 'LISST	Start Sampling Program' menu item, or open the terminal window and click on the 'Start' button.	
	[OK]	
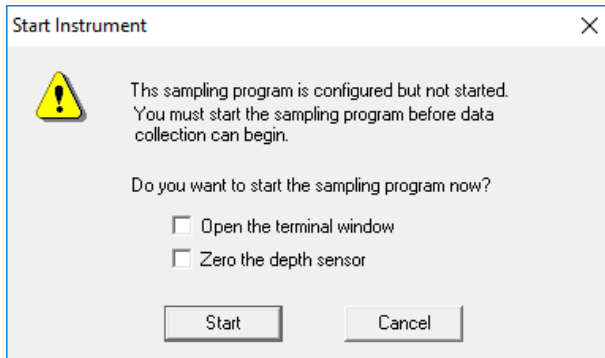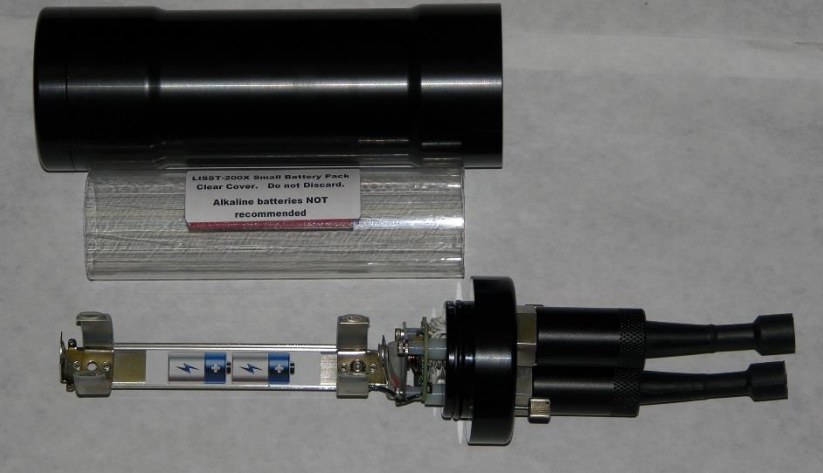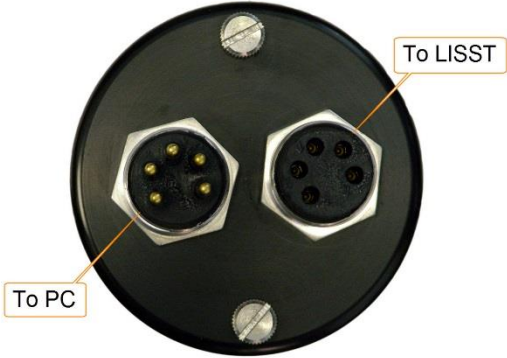
10	• Clicking the Start button instead of the Cancel button will start the sampling program immediately. In other words, the instrument will begin waiting for the start condition. If the start condition has already been met, the instrument will start sampling immediately.	
11	• The instrument will display text to the terminal window as it is running to confirm that it is running and to indicate where it is in the sampling cycle. Once the program is confirmed to be running and waiting for the correct start conditions, the LISST-SOP program can be closed and the communications cable can be disconnected. Be sure to replace the connector cap before deployment.	View Instrument Messages in Terminal Window

5. *Using the Small External Battery Pack*

The LISST-200X comes with a Small External Battery Pack that can be used for short term deployments. The instructions in this section describe how to use the Small Battery including installing and charging the NiMH rechargeable batteries.

STEP	ACTION	RESULT
1	• The LISST-200X Small External Battery Pack uses two rechargeable NiMH D-cell batteries. Four of the 1.5V 10000mAh batteries and a charger are included with the battery pack. The batteries are not installed when the instrument a new instrument is shipped. It is recommend that the batteries be removed from the battery housing when not in use. Leaving the batteries in the battery housing will cause the batteries to discharge and could cause damage to the cells. • Fully charge the batteries using the provided charger. Fully discharged batteries will take about 8 hours to charge. 	Batteries fully charged.
2	• The Small Battery housing is made of plastic and has an endcap with two underwater connectors and two captive thumbscrews on one end. • Loosen the thumbscrews. Note that the screws will not need to be fully removed. They are designed to be removed from the main housing but still remain attached to the endcap. The screws can be removed if you continue to rotate the screws through the threaded holes in the endcap. • Slide the endcap with battery holder attached out of the main housing.	Battery open and ready for batteries

3	• Slide the clear cover off of the battery holder and install the two D cell batteries. The orientation of the batteries is shown on a label on the battery holder. • Slide the clear cover over the batteries such that the rubber pad is pushing against the batteries as shown in the picture above	Batteries installed.
4	• Check that the o-ring in the endcap and the mating surface on the main housing are clean. Lightly grease with silicone grease as needed. • Carefully insert the assembly back into the main housing until the o-ring makes contact with the housing. Align the thumbscrews with the holes in the main housing and push the endcap on until it is flush with the housing. • Tighten the thumbscrews by hand **(DO NOT USE ANY TOOL). DO NOT OVERTIGHTEN.**	Battery assembled and ready for use.

5	• The battery pack can be connected to the LISST-200X using the provided 5-pin Male to 5-pin Female cable. • Connect the Male end of the cable to the Female Bulkhead connector on the Small Battery Pack. • Connect the other end of the cable to the 5-pin Male bulkhead on the LISST-200X. • Power is now going to the LISST-200X. If AutoStart is enabled the instrument may be looking for the start condition.	Instrument is powered and ready to collect data
6	• If desired connect the Communications cable to the male 5-pin bulkhead connector on the Small Battery Pack. Communications will pass through the battery to the LISST-200X. • The battery and communications cables are shown below. 	

6. Using the Large External Battery Pack

The Large External Battery Pack is used to provide power to the LISST-200X for long term deployments or extended profiling operations.

STEP	ACTION	RESULT
1	• The LISST-200X Large External Battery is shipped with 16 Alkaline D batteries pre-installed and is ready for use. The following steps describe how to open the battery and replace the batteries. Skip to Step X to connect the battery to the LISST-200X. • The new alkaline batteries can be left in the battery housing for storage. However, it is not recommended that fully discharged batteries be left in the battery housing during long term storage.	Battery ready to use when shipped.
2	• To access and replace the batteries in the Large Battery pack you will need remove the endcap with the connectors and handle. • Remove the four 6-32 X ¾ long socket head cap screws from the connector endcap using the provided 7/64" ball driver. Remove the screws uniformly or hold the cap down against the light spring force.	Endcap ready for removal

3	• Pull out the connector endcap. There are no wires or other connections. Set the endcap to the side.	Endcap removed and batteries replaced.
	• Remove the batteries making note of their orientation.	
	• Insert the batteries into the pressure case. Labels on the inside surface of the center divider will guide you on the correct orientation of the batteries for each stack.	
4	• Inspect the o-ring on the endcap and the seating surface on the pressure case to make sure there is no debris that could affect the seal. Lightly grease the surfaces as needed.	Endcap re-installed
	• Align the tab in the center of the endcap to the slot in the post in the center of the pressure case	
5	• Screw the connector endcap onto the pressure case with the four 6-32 x ¾ socket head cap screws. If necessary, apply anti-seize compound onto the threads.	Battery assembled and ready for use.

| 6 | • The battery pack can be connected to the LISST-200X using the provided 5-pin Male to 5-pin Female cable.
• Connect the Male end of the cable to the Female Bulkhead connector on the Large Battery Pack. The connector will be labeled with "TO LISST".
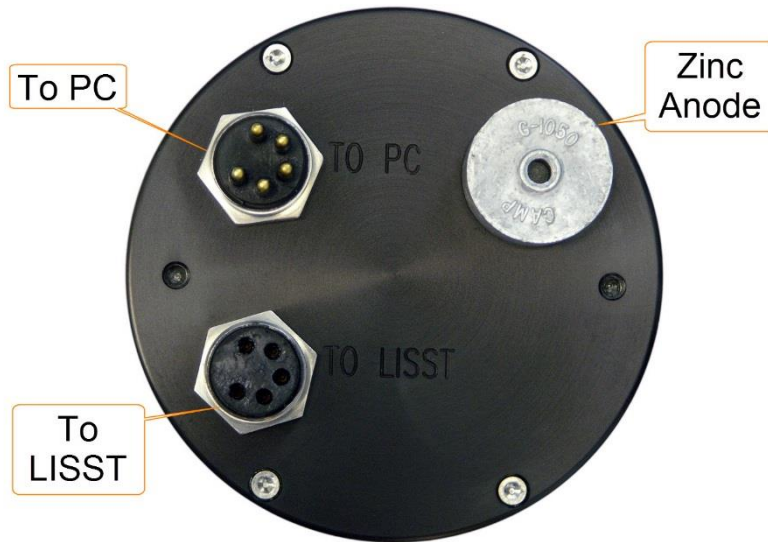
• Connect the other end of the cable to the 5-pin Male bulkhead on the LISST-200X.
• Power is now going to the LISST-200X. If AutoStart is enabled the instrument may be looking for the start condition. | Instrument is powered and ready to collect data |
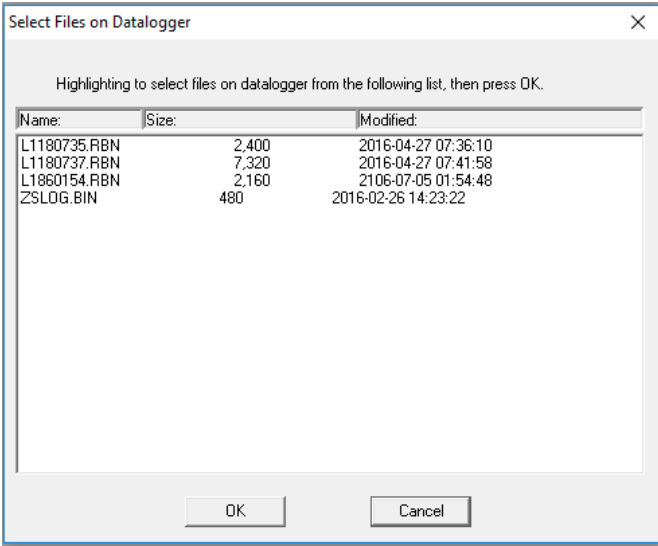
7	• If desired connect the Communications cable to the male 5-pin bulkhead connector on the Large Battery Pack which is labeled "TO PC". Communications will pass through the battery to the LISST-200X. • The battery and communications cables are shown below. 	
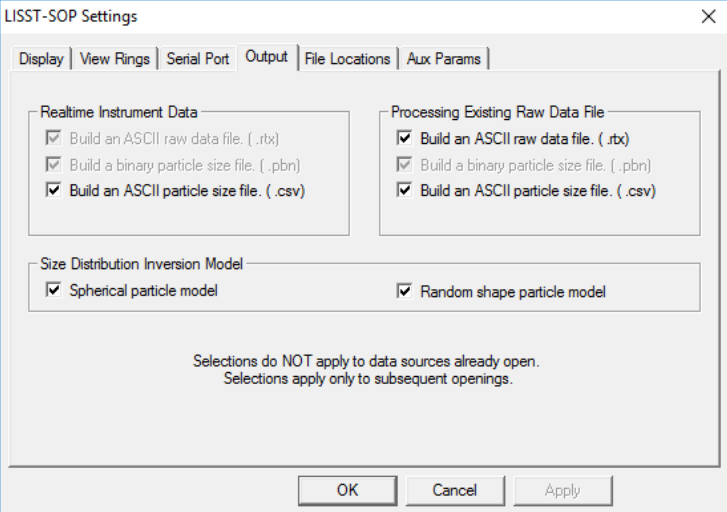
7. *Offloading and Deleting Data Files from Internal Memory*
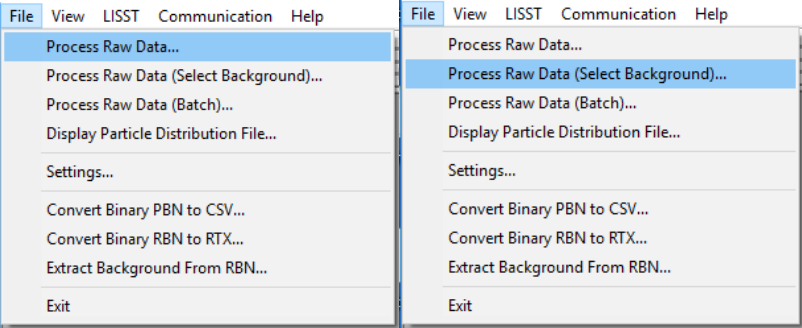
Data collected using the LISST-200X is stored on the internal data logger. This section describes how to offload and delete files from the internal data logger.

STEP	ACTION	RESULT
1	• Connect the instrument to the computer and establish communication in the LISST-SOP200X program. (see Step-by-Step Procedure: Establishing Communication with the LISST-200X)	.LISST-SOP open and communicating with LISST-200X.
2	• Select *Download Data From LISST* or *Delete Data From LISST* from the *LISST* menu or click on the offload ⬇ or erase 🗑 icons in the toolbar. This will open up a window showing the data files currently stored onboard the instrument. **Select Files on Datalogger** ✕ Highlighting to select files on datalogger from the following list, then press OK. Name: Size: Modified: L1180735.RBN 2,400 2016-04-27 07:36:10 L1180737.RBN 7,320 2016-04-27 07:41:58 L1860154.RBN 2,160 2106-07-05 01:54:48 ZSLOG.BIN 480 2016-02-26 14:23:22 OK Cancel	Shows a list of files currently stored onboard the instrument
3	• Select individual or multiple files to delete or offload (by holding down either shift or ctrl). Press 'OK.'	Select files to delete or offload
4	• A dialog box with either confirm the files to be deleted or ask you to select a location on your computer to offload the files to. After pressing 'OK' the files will then be offloaded or deleted.	Files are offloaded or deleted
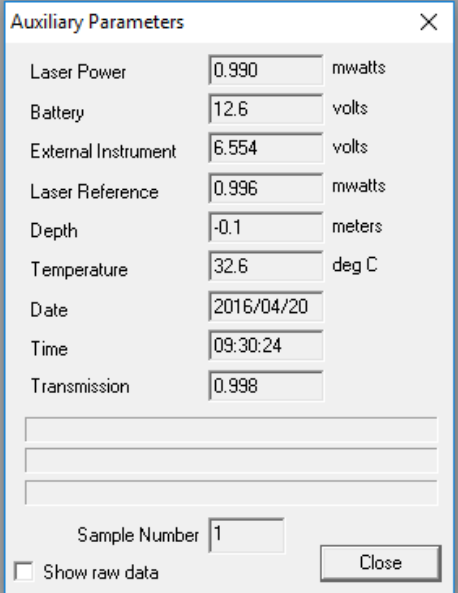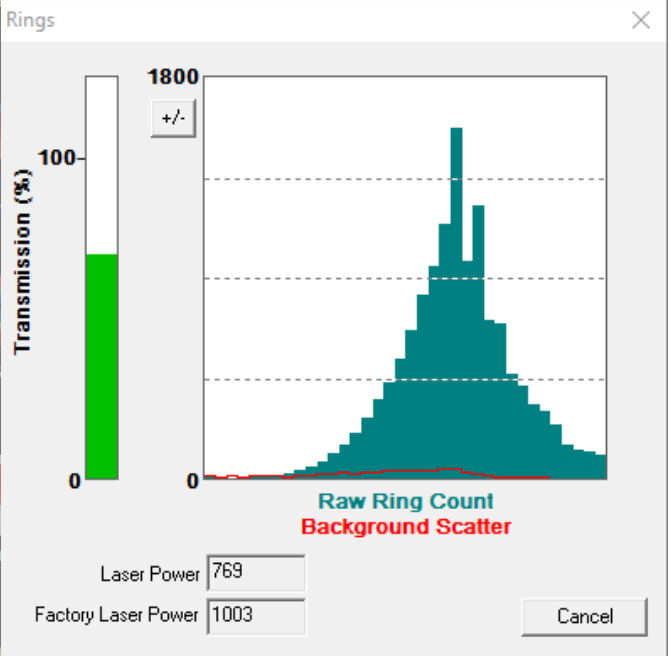
8.   *Processing a Single Raw Data File*

Data that has been downloaded from the datalogger is in a raw binary file (.RBN extension). It must be processed into particle size by the LISST-SOP program.

STEP	ACTION	RESULT
1	• Start the LISST-SOP200X program	Program started.
2	• Select *Settings* from the *File* menu and click on the Output Tab. The window below will appear prompting for the desired output to use for the future data processing. This selection must be made BEFORE starting the processing command.	Select file types to be created.
	• **NOTE:** A binary PBN file is always created when processing raw data. This is used to display the processed data to the screen using the software.	
	• The CSV file is a space delimited ASCII file containing the fully processed and calibrated data. This is useful for loading into spreadsheets such as Excel etc.	
	• The RTX file is a space delimited ASCII file containing the raw data from the LISST-200X data logger. This is useful for looking at the raw data in a spreadsheet such as Excel etc.	
3	• You must also select the inversion model to be used when processing the data: Spherical, randomly shaped or both. • For a description of the particle models, see Appendix G: Spherical and Random Shape Particle Models. • If you have selected Randomly shaped Particle Inversion Model, the processed files will have an' _rs' suffix	Select inversion method(s) to be used – spherical, randomly shaped or both.

4	• Select *Process Raw Data* or *Process Raw Data (Select Background)* from the *File* menu. Standard raw data processing will use the background file contained in the .RBN file. If you would like to use a different background file than the one in the RBN file, use the *Process Raw Data (Select Background)* option. If you use this option, you will be prompted for a .BGT file first.	Select to process a file using the background in the data file or an external .BGT file
	File View LISST Communication Help Process Raw Data... Process Raw Data (Select Background)... Process Raw Data (Batch)... Display Particle Distribution File... Settings... Convert Binary PBN to CSV... Convert Binary RBN to RTX... Extract Background From RBN... Exit File View LISST Communication Help Process Raw Data... Process Raw Data (Select Background)... Process Raw Data (Batch)... Display Particle Distribution File... Settings... Convert Binary PBN to CSV... Convert Binary RBN to RTX... Extract Background From RBN... Exit	
	• You will be asked to select the .RBN file you would like to process.	
5	• Next, select the output data file name. The default file name shown is the same as the raw file name but with a .PBN extension. You can change this name if you wish. • If other output types are selected they will automatically have the same base name but with different extensions. • **Note:** file types other than the default PBN file must have been selected before selecting *Process Raw Data* from the *File* menu (See step 2).	Name out output file.

6	• A display similar to the one shown below will appear.	Main data processing selection window displayed to the screen.
	• The top portion of the display is a graphical representation of the raw data from the 36 rings. The value of the 36 rings is represented as a vertical line, with time moving from left to right.	
	• The lower portion of the display shows the time history of some of the Auxiliary parameters. This can be useful for determining when the instrument came out of the water, when a particular profile was started, or for determining what range of the raw file to process. To change which Auxiliary parameters are displayed, choose the *Aux Params* tab from the *Settings* window.	
	• Note that only 400 samples of the Auxiliary Parameters are displayed at a time. Use the Next and Previous buttons to move through the complete data file. The triangles on the lower edge of the upper display will indicate where in the complete data file the currently displayed section is located.	
7	• Additional information is stored in the data file. Selecting the *Show Auxiliary Parameters* from the *View* menu opens a window that displays this additional information. The information displayed is for the sample located at the position of the cursor (the vertical pink line) in the lower plot of the raw data display. You can click on the lower plot to move the cursor to a new location. An example of this window is shown below.	Auxiliary parameter window displayed.

8	• The raw scattering values can be graphically displayed by selecting the *View Rings* command from the *View* menu. As with the Auxiliary Parameters, the cursor indicates which sample is being displayed. You can change what type of data is displayed in this window by going to the View Rings tab of the Settings menu 	Value of rings for current frame displayed.
9	• To select the range of raw data to process position the cursor at the desired starting sample. The display at the lower portion of the screen will show the current sample number. Pressing the *Select First* button will set the current cursor position as the starting point for processing. Similarly selecting *Select Last* will set the	Range to process selected.

		end point for processing. Use the *Next* and *Previous* buttons scroll through the complete data file. The *Select First* and *Select Last* points do not need to be on the same displayed plot.	
10		• After selecting the range to process press the *Process File* button. The display will show the processing progress and the *Process File* button text will change to *Finished* when the processing is complete. The window can now be closed.	Processing Completed.
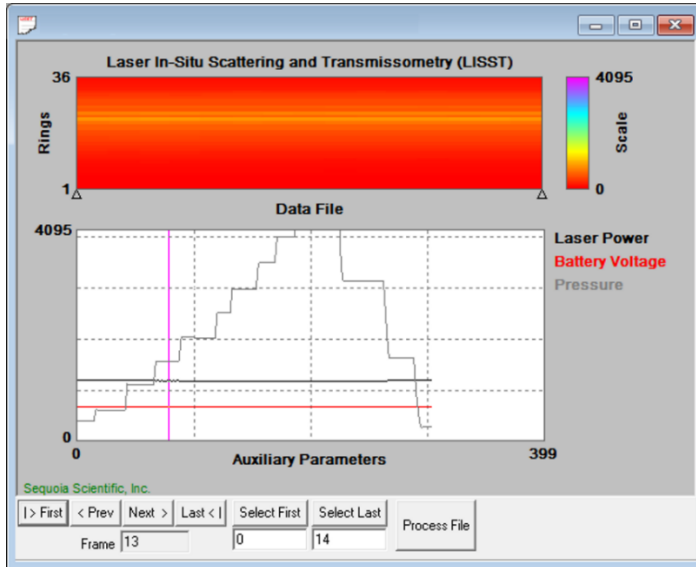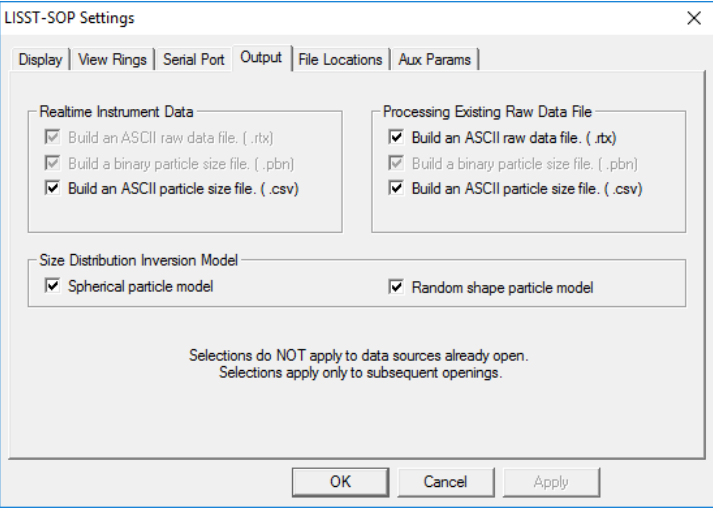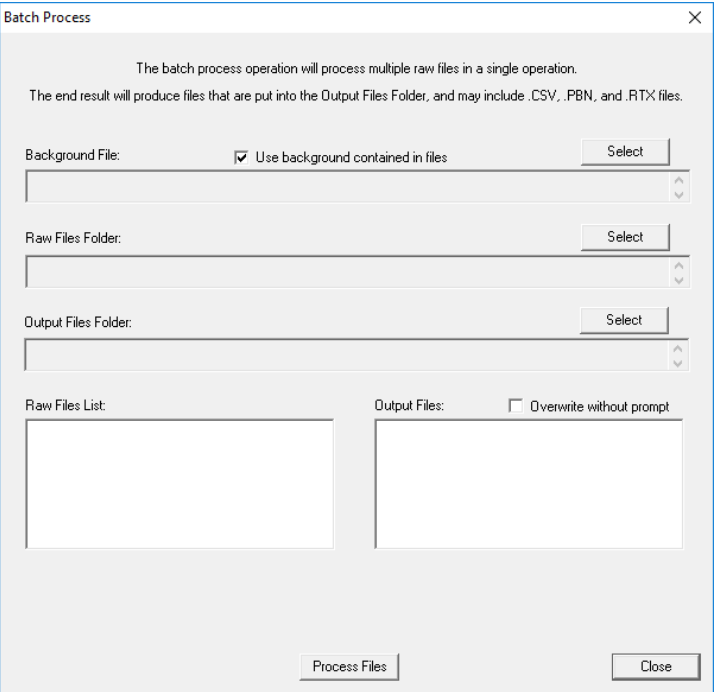
9. *Batch Processing Multiple Raw Data Files*

Data that has been downloaded from the datalogger is in a raw binary file (.RBN extension). It must be processed into particle size by the LISST-SOP program. If you have multiple raw data files to process (e.g. from a series of profiling deployments), follow these processing steps that allows you to process all files in one operation.

STEP	ACTION	RESULT
1	• Start the LISST-SOP200X program	Program started.
2	• Select *Settings* from the *File* menu and click on the Output Tab. The window below will appear prompting for the desired output to use for the future data processing. This selection must be made BEFORE starting the processing command. • **NOTE:** A binary PBN file is always created when processing raw data. This is used to display the processed data to the screen. • The CSV file is a space delimited ASCII file containing all the processed data. • The RTX file is a space delimited ASCII file containing the raw data from the LISST-200X data logger.	Select file types to be created.
3	• You must also select the inversion model to be used with the real time data processing: Spherical, randomly shaped or both. • For a description of the two particle models, see Appendix G: Spherical and Random Shape Particle Models.	Select inversion method(s) to be used – spherical, randomly shaped or both..

	• If you have selected Randomly shaped Particle Inversion Model, the processed files will have an' _rs' suffix	
4	• Select Process Raw Data (Batch) from the File menu.	
6	• By default, batch processing will use the background stored in the raw data files to process the data. If you would like to use an external .BGT file, uncheck the 'Use background contained in files' and select a .BGT file.	Select source of background files
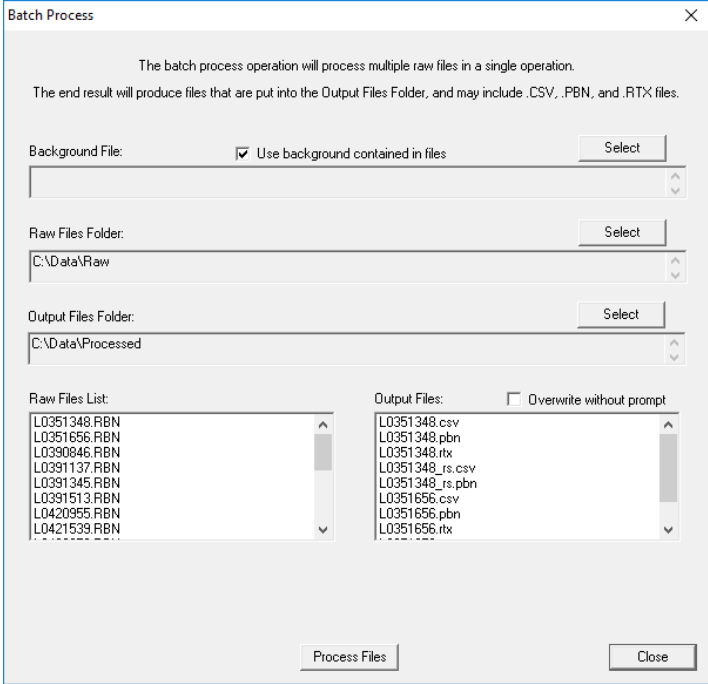
7	• Select the directory where your raw data files (.RBN) are located. Click the select button and navigate to the desired folder and select it. Please note that ALL raw data files in the folder will be processed. If you wish to only process a selection of files in a folder, these files must be copied to an empty directory, which must then be selected as the Raw Files Directory.	Raw Files Directory selected.

Batch Process

The batch process operation will process multiple raw files in a single operation.

The end result will produce files that are put into the Output Files Folder, and may include .CSV, .PBN, and .RTX files.

Background File: ☑ Use background contained in files   Select

Raw Files Folder: Select
C:\Data\Raw

Output Files Folder: Select
C:\Data\Processed

Raw Files List:
L0351348.RBN
L0351656.RBN
L0390846.RBN
L0391137.RBN
L0391345.RBN
L0391513.RBN
L0420955.RBN
L0421539.RBN

Output Files: ☐ Overwrite without prompt

Process Files   Close

• By default, the Output Files Directory will be the same as the Raw Files Directory. However, if you wish to change this to another directory, then click the select button in order to choose another directory.

• The Raw Files List box now shows all the raw data files (.RBN) that are in your Raw Files Directory, while the Output Files Box shows .CSV, .PBN, and .RTX files that may already exist in the Output Files Directory.

• You cannot change the output file names. The file name of the processed files will be the same as that of the raw data files, but with a different extension (e.g. .CSV, .PBN or .RTX). If you have selected Randomly shaped Particle Inversion Model, the processed files will have an' _rs' suffix.

• **Note:** file types other than the default PBN file must have been selected before starting the batch processing. See step 2.

| 8 | • You can now click the Process Raw Files button and all raw data files will be processed. The processed files will show up in the Output Files window. Note that the files processed using the randomly shaped inversion method have an '_rs' suffix.

Batch Process ✕

The batch process operation will process multiple raw files in a single operation.

The end result will produce files that are put into the Output Files Folder, and may include .CSV, .PBN, and .RTX files.

Background File: ☑ Use background contained in files [Select]

Raw Files Folder: [Select]
C:\Data\Raw

Output Files Folder: [Select]
C:\Data\Processed

Raw Files List: / Output Files: ☐ Overwrite without prompt
L0351348.RBN / L0351348.csv
L0351656.RBN / L0351348.pbn
L0390846.RBN / L0351348.rtx
L0391137.RBN / L0351348_rs.csv
L0391345.RBN / L0351348_rs.pbn
L0391513.RBN / L0351656.csv
L0420955.RBN / L0351656.pbn
L0421539.RBN / L0351656.rtx

[Process Files] [Close] | Batch processing.. |
| 9 | • Click the close button in the bottom right when you are done. | Close the batch processing window. |

10. View Processed Data File

After processing of the data into a Particle Size Distribution file (.PBN extension) the data can be viewed to the screen. This step-by-step procedure covers the viewing of data and optional displays.

STEP	ACTION	RESULT
1	• Start LISST-SOP200X program	Program started.
2	• Select Display Particle Distribution File from the File menu.	Open Particle Distribution File selected.
3	• Select the processed data file (.PBN) from the file selection window. Double click the file or type the file name and press Open.	Processed data file selected.
4	• After selecting the processed file, a window similar to the one shown below will appear. • The left hand display is the volume distribution in units of micro-liters/liter in each size class. The right hand display is the Cumulative Concentration.	Particle size distribution displayed on screen
5	• Only a single sample is displayed at a time. By using the buttons on the bottom of the display it is possible to manually or automatically step through the data file. The *Start Display* button will step through the data file by updating the display at a fixed rate. The rate is set by the slider bar next to the *Start Display* button.	Scroll through measurements

6	• Additional information is stored in the processed data file. The Auxiliary parameter window displays these values for the current sample. They will update when the sample number is changed. The window is opened by selecting *Show Auxiliary Parameters* from the *View* menu. An example of this window is shown below. 	View auxiliary parameters
7	• The scale of the Volume Distribution plot can be changed by using the *Scale +/-* button. After selecting the button a display similar to the one shown below will prompt you for the maximum concentration for the Y-axis of the plot. • Other options are also available for customizing the display such as changing the header and footer text or adding lines to show the mean and standard deviation. These features are located on the *Display* tab of the *Settings* window as shown below.	Display setting modified.
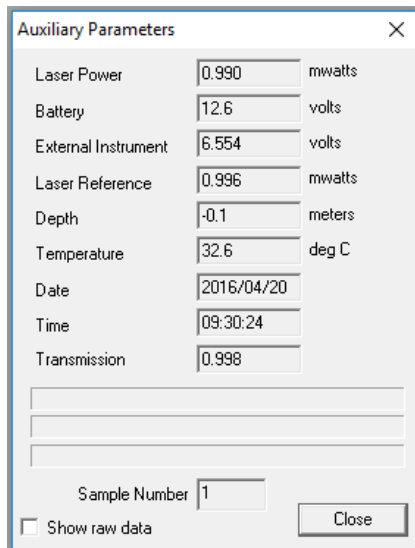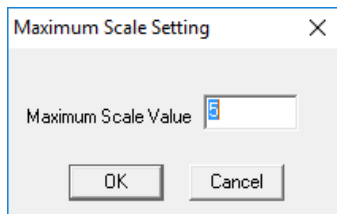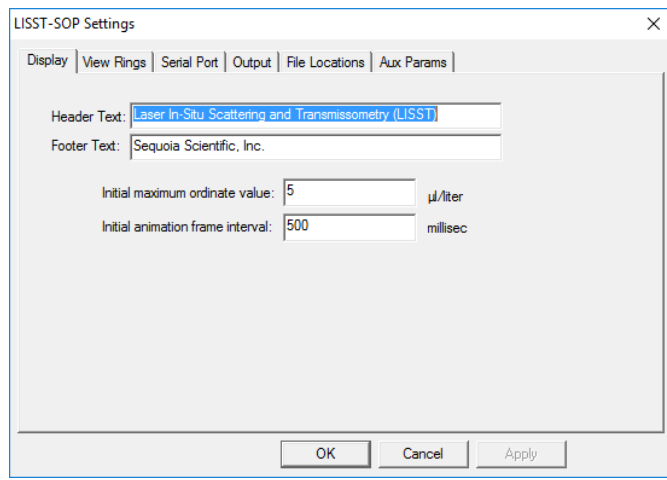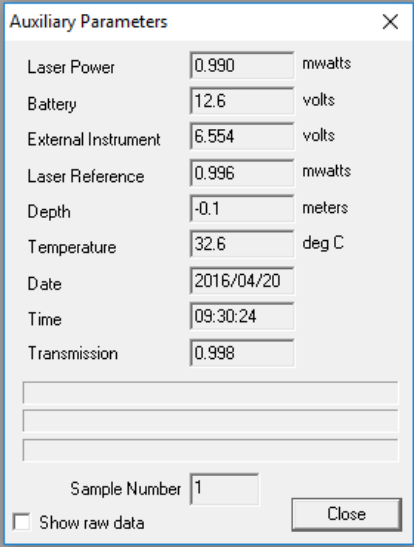
8	• The current display can be sent to the printer by choosing *Print* from the *File* menu. • *Print Preview* and *Print Setup* are also available.	Display printed.
9	• When finished viewing the processed data close the window.	Viewing of data complete.

11. Data Quality Control
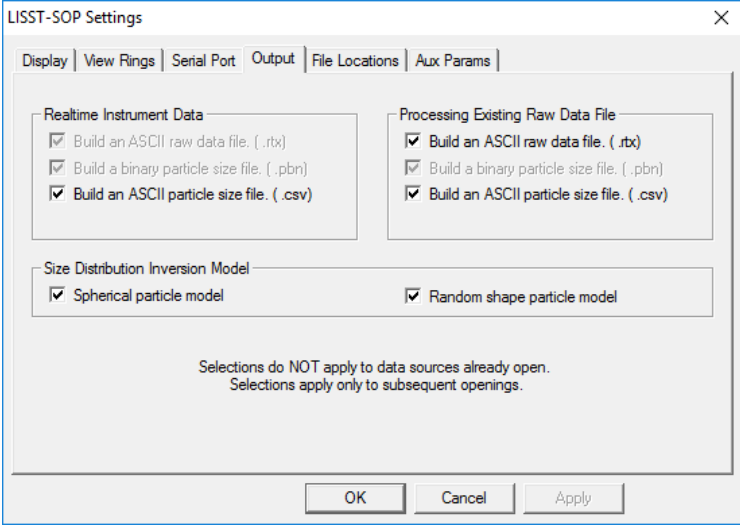
Making sure your data make sense.
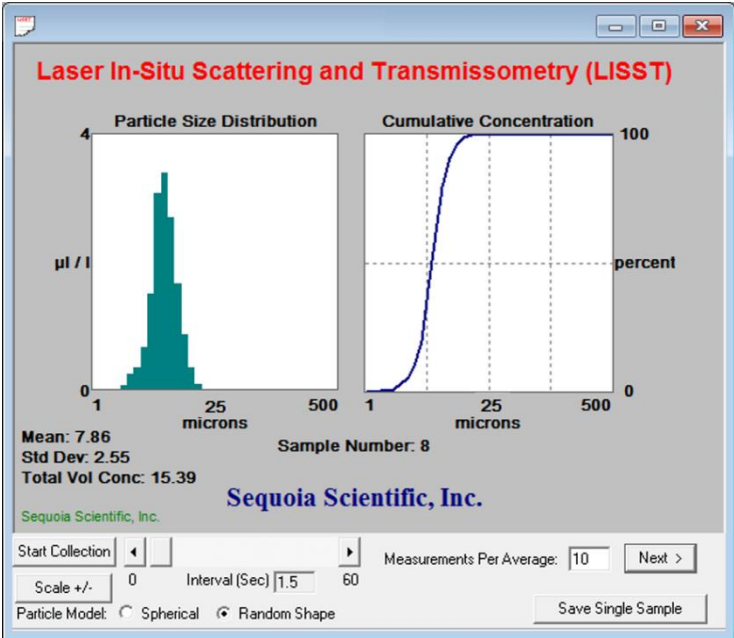
STEP	ACTION	RESULT
1	• Follow steps 1-6 in the previous section: View Processed Data File and look at the Auxiliary Parameter Frame	Data file loaded and Auxiliary Parameter frame shown.
2		
3	• The first thing to look for is to make sure that the Laser Reference box is displaying numbers different than 0. • In the example above Laser Reference is at 8.943 mW, but this number can vary from instrument to instrument, and will also vary as the instrument becomes older and, to some extent, with temperature. Values around 0.5-2 mW are the most common values. • Laser Reference should never be 0 (or very close to 0, e.g. 0.02 mW). If this is the case the laser is most likely dead. In this case only your pressure data and temperature data will be any good. The laser must be replaced. Contact Sequoia or your local distributor for instructions on what to do. • Tip: If you selected 'Create .CSV file' when processing your data, you can load the .CSV file into Excel or another spreadsheet and easily check all the Laser Reference values - the Laser Reference value is in column number 40.	Check your Laser Reference values – they *MUST* be >> than 0.
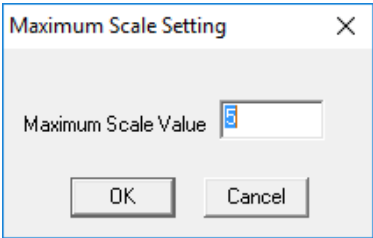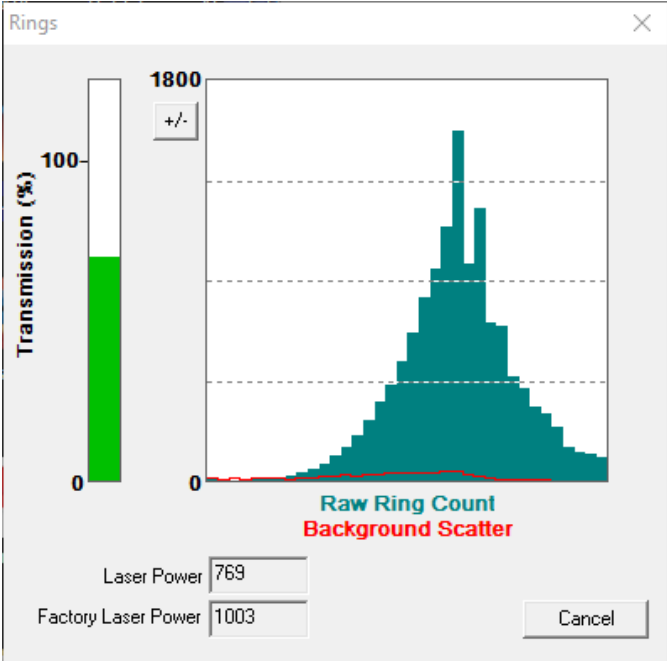
4	• The next thing to look for is to check the Transmission values. The transmission must be a number between 0 and 1. • It is physically impossible for the transmission to be negative or larger than 1 (one). • Tip: The transmission value is in column 56 of the .CSV file. • If transmission shows up as being larger than 1 (one), then your measurement is most likely taken in very clear water and/or you have a bad zscat measurement obtained with dirty water and/or dirty windows. • If your zscat measurement was obtained using dirty water, it may be possible to redo the zscat using clean water and re-process the data, then check to see if the transmission values drop below 1 (one).	It is physically impossible for the Transmission value to be less than 0 (zero) or larger than 1 (one).
5	• If your transmission values generally are in the 0.98-0.995 range, your measurements are taken in very clear water. • This means that the signal-to-noise ratio will be low, and the data may have a lot of noise in them, but can most likely still be used.	Transmission values very close to 1 yields noisy data.
6	• If your transmission values are < 0.10 (or 10%), the water is too turbid. Disregard these data.	Data with transmission values < 10% should be disregarded.
7	• If your transmission values are > 0.995 (or 99.5%), the water is too clear. Disregard these data.	Data with transmission values > 99.5% should be disregarded.
8	Summarizing • Disregard data if laser reference is 0 or very close to 0 (laser is dead, contact Sequoia). • Disregard data if transmission is > 0.995 (> 99.5%). • Disregard data if transmission is < 0.10 (< 10%). • Be wary of data collected at transmission values between 0.98 and 0.995 – low signal-to-noise ratio. • Be wary of data collected at transmission values between 0.30 and 0.10 – generally decreasing data quality as the transmission decreases below 0.30 (30%).	Quick quide for data QC

12.   *Simple Real-Time Data Processing*

This step-by-step procedure covers the acquisition, display and storage of processed data in real time.

STEP	ACTION	RESULT
1	• Connect the instrument to the computer and establish communication in the LISST-SOP200X program. (see Step-by-Step Procedure: Establishing Communication with the LISST-200X)	Communication established.
2	• Before starting a Real-Time session the output file options must be set. This must be done before selecting *Collect Realtime Data*. Select the Output tab in the *Settings* menu. The window below will appear.	Output file type selected.
	A binary PBN file must be selected before any other file type will be stored. This is used to display the processed data to the screen.	
	• The CSV type is a comma delimited file containing all the processed data.	
	• The RTX file is a comma delimited file containing the raw data from the data logger.	
	• You must also select the inversion model to be used with the real time data processing: Spherical, randomly shaped or both. For details on these selections, see Appendix G: Spherical and Random Shape Particle Models.	

3	• The background currently stored onboard the instrument will be used to process the data as it is collected in real time. • Be sure to collect a new background and store it onboard the instrument before beginning a real time session	Background is current
4	• Select *Collect Realtime Data* from the *LISST* menu or press the ⬛ button.	Open Instrument selected.
5	• A display will appear on the screen. It is similar to the main window of the View Processed Data function. • The 'Measurement Per Average' text box allows you to adjust the number of measurements that will be averaged before the display is updated. The default is 10. Higher values can be used however it may cause a delay that lasts longer than your selected interval time. • By selecting the Next button the window will be updated with the current size distribution. Note that if the water is clean the display may not show any volume distribution. • The Sample Number value displayed underneath the plots will increment each time a sample is saved. • The radio buttons for labeled 'Spherical' and 'Random Shape' allow you to display the volume distribution calculated with either the spherical or random shape particle model. The radio buttons only effect the display and have no effect on the data that is saved in the .CSV file. If you selected both particle models in part 2, data from both models will be saved to your computer regardless of the radio button selection.	

6	• The *Start Collection* button function is similar to its use in viewing processed data. The SOP will automatically acquire data at a fixed rate. The slider next to the Start Collection button controls the sample speed. Note that the interval time may be limited by the measurement average you have selected.	Start Collection button pressed and samples updating automatically.
7	• The Auxiliary parameter window can be opened to display the current values of the auxiliary parameters such as pressure and temperature. Open the window by selecting the *Show Auxiliary Parameters* from the *View* menu.	Auxiliary Parameter window opened.
8	• The scale of the Volume Distribution plot can be changed by using the *Scale +/-* button. After selecting the button a display similar to the one shown below will prompt you for the maximum concentration for the Y-axis of the plot.	Display setting modified.
9	• The raw scattering values can be graphically display by selecting *View Rings* from the *View* menu. An example of this display is shown below.	Value of rings for current frame displayed.
10	• A single sample may be saved to a separate file by pressing the *Save Single Sample* button	Averaged data acquired and stored to an ASCII file.
11	• Press the 'Stop Collection' button and close the window to end the realtime session.	Stop data collection

13. *Collecting Data from an External Analog Sensor*

The LISST-200X can supply regulated 12V power to an external sensor, and measure one or two analog voltages, depending on its hardware configuration and firmware settings. If the 12V power output is activated, it will turn on each time the 200X collects a sample or burst of samples, and turn off each time the 200X goes into a waiting state of more than a few seconds. If using the 12V output, you can specify how long it should turn on before sampling, as a warmup time for the sensor.

To provide these functions, the auxiliary connector on the LISST-200X must be in one of two configurations: Analog & Digital Input (which provides a single analog input), or Dual Analog Input configuration (which provides two).

STEP	ACTION	RESULT
1	• Determine current configuration of Auxiliary connector: in LISST-SOP, select LISST->Show Instrument Status. Look for the Endcap Configuration. If it is not one of the analog-input configurations, you will need to change it; contact Sequoia for assistance.	Auxiliary Connector configuration determined
2	• Make the appropriate connections between the 6-pin auxiliary connector of the LISST-200X and your sensor. To see the pin assignments, type the CONFIG command in the LISST-SOP terminal wipage 77). Or see Appendix E: Connectors.	External sensor connected
3	• Type SENSORPOWER into the terminal window, and respond to its prompt to select the appropriate option. • Type SENSORWARM in the terminal window to set a warmup time (in seconds). • WARNING: a USB cable may not provide enough power for external sensors. Use a battery pack or other power source to provide enough power to run external sensors.	Configured for analog collection
4	• Set the standard sampling parameters to control the sampling schedule and other options (see • Configuring Data Collection on page 38).	Ready to collect data

14. *Configuring the LISST-200X as a Sensor for a CTD*

The LISST-200X can operate as an analog-output auxiliary sensor for a CTD. For this purpose the LISST-200X's auxiliary six-pin connector must be in its "Dual Analog Output" configuration. The two analog outputs represent the total volume concentration of particles, and the Sauter mean diameter (SMD). These values are approximations for the convenience of real-time display. For highest-quality data you will still need to offload and process the detailed data from the LISST-200X after deployment.

The LISST-200X updates its analog outputs each time it collects a sample, at a maximum of 1 Hz.

STEP	ACTION	RESULT
1	• Determine current configuration of Auxiliary connector: in LISST-SOP, select LISST->Show Instrument Status. Look for the Endcap Configuration. If it is not in the Dual Analog Output configuration, you will need to change it; contact Sequoia for assistance.	Auxiliary Connector configuration determined
2	• Make the appropriate connections between the 6-pin auxiliary connector of the LISST-200X and your sensor. To see the pin assignments, type the CONFIG command in the LISST-SOP terminal window (see LISST-200X Command Details on page 77), or see Appendix E: Connectors.	Connected to CTD
3	• In LISST-SOP, set the sampling parameters as follows (see • Configuring Data Collection on page 38): o Operating mode: Fixed sample rate o Sample to be average of 20 measurements o Sample interval: 1 second o Automatically start sampling program upon power up Start condition: delay 0 minutes o Stop condition: memory full	200X will collect data at 1 Hz whenever the CTD supplies power to it

E.   Instrument Communication

Overview The cables Sequoia supplies with the LISST-200X send data through a standard USB port. However, the LISST-200X itself communicates through RS-232 serial protocol. The 5-pin male connector on the LISST-200X carries the RS-232 signals (and power). The cables incorporate adapters that convert between RS-232 and USB.

If you use a different cable that does not include the RS-232-to-USB adapter, you can use an external adapter, or connect directly to an RS-232 port if your computer has one (which is rare on any recent computer). In Windows, both RS-232 and USB connections will appear as COM ports. To establish communication with LISST-SOP or other software, you must set the software to communication with the correct COM port.

If interfacing to a data logger or controller system other than a Windows computer, you will likely also use a direct RS-232 connection.

The RS232 link communicates at 9600 baud, 8 data bits, No parity, and 1 stop bit. For offloading data files, the LISST-SOP software uses YMODEM protocol at 115K baud. The transfer rate can be changed in the settings of the LISST-SOP software. A slower speed may be required when downloading data over cables longer than a few meters.

Using the LISST-SOP Terminal Window In the LISST-SOP software, you can open a terminal window to directly view communications with the LISST-200X, and enter commands. When the LISST-200X is connected and the terminal window is the front window, the LISST-200X should respond to pressing the enter key with the L200X:> prompt. If the instrument is in the deep sleep mode, you can wake it by pressing the Stop button.

Pressing the Start button will begin the collection of data using the current sampling program (see

Configuring Data Collection).

Direct Commands In most cases, LISST-SOP software will be used to configure and operate the LISST-200X. However, some functions are available only through direct commands in the terminal window. Also, in some applications the LISST-200X may need to communicate to another datalogger or custom program. For this purpose, a large set of commands is available to operate the instrument. See the LISST-200X Command Summary on page 75 for detailed descriptions of each command.

Start Condition   The LISST-SOP software or two letter commands can configure the
                         LISST-200X with one of five Start conditions:

      1. Depth,
      2. Time,
      3. External Mechanical Switch,
      4. External Digital Input, and
      5. Time Delay.

      The details of each condition are described below.

Depth Start   The built-in depth sensor of the LISST-200X is used to check the
                         current depth to determine if the desired start depth has been
                         exceeded. The instrument is powered up and 5 measurements of the
                         depth are averaged over a two-second period. If the depth exceeds the
                         threshold the program will proceed to the data collection routine. If the
                         depth does not exceed the threshold the instrument will power down
                         and wait 28 seconds before checking the depth again. The program
                         will continue checking until the depth is exceeded or until the program
                         is stopped.

Time Start   The program will check the current time every second and compare it
                         to the Start Time. If the Start Time is equal to or earlier than the current
                         time the program will go directly to the data collection routine. It will
                         continue checking the time until the Start Time is reached or until the
                         program is stopped.

External   The LISST-200X has a white plastic lever on the endcap. This lever
Mechanical   has a magnet imbedded in it. This magnet can activate a switch inside
Switch Start   the pressure case. The base program looks at the status of this switch
                         once a second. If the switch is in the on or "1" positions the program
                         will go directly to the data collection routine. It will continue checking
                         the switch status until the switch is moved to the "0" position or until the
                         program is stopped. When in the "0" position the lever is up against the
                         zinc anode.

External Digital   If the auxiliary connector is configured for analog and digital inputs (see
Input Start   Appendix E: Connectors), pin 2 of the 6-pin connector is the digital
                         input. The program will check the status of the digital input once a
                         second. If the digital input is greater than 2 volts (relative to digital
                         ground, pin1) the program will go directly to the data collection routine.
                         It will continue checking the status of the digital input until voltage drops
                         below 0.7 volts or until the program is stopped.

Time Delay Start   The time delay start condition will cause the program to wait the specified number of seconds before continuing on to the data collection routine.

Data Collection Routine   The LISST-SOP software can configure the sampling program with one of six Stop conditions: Depth, Time. External Mechanical Switch, External Digital Input, Fixed number of samples, and Maximum Memory or Low Battery. The details of each condition are described below.

Stop Condition   The LISST-SOP software can configure the LISST-200X with one of six stop conditions:

1. Depth,
2. Time,
3. External Mechanical Switch,
4. External Digital Input,
5. Fixed number of samples, and
6. Memory full.

When storing data in the Fixed Sample Rate mode the Stop conditions are checked after each averaged sample has been saved. When storing data in the Burst mode the Stop conditions are only checked after a full Burst has been completed. The Start and Stop conditions have no effect on the Real-time sampling mode. The details of each condition are described below.

Depth Stop   The depth sensor of the LISST-200X is used to check the current depth to determine if it is less than the desired Stop depth. If the depth is less than the threshold the sampling will stop. If the Start Condition is Depth Start the program will return to checking for the start depth. For all other Start Conditions the when the current depth is less than the threshold the LISST-200X will stop and go into deep sleep.

Time Stop   The program will check the current time after each sample or burst and compare it to the Start Time. If the Start Time is equal to or later than the current time, it will stop and go into deep sleep.

External Mechanical Switch Stop

After each sample or burst the status of the Switch lever is checked. If the switch lever is in the off or "0" position sampling will stop. If the Start Condition is a Switch Start the program will return to checking the start condition. For all other Start conditions the instrument will stop and go into deep sleep.

External Digital Input Stop

The status of the digital 1 input is checked after each sample or burst. If the voltage at the input is less than 0.7 volts the sampling will stop. If the Start Condition is a Digital Input Start the program will return to checking the start condition. For all other Start conditions the instrument will stop and go into deep sleep.

Fixed Number of Samples

The program will acquire a fixed number of samples before stopping. When the number of samples to be saved has been reached the program will stop and go into deep sleep.

Memory Full

Sampling will continue until the memory is full, or the power input falls. If the memory fills, the instrument will go into deep sleep.

F.   LISST-200X Command Summary

NOTE: Command are shown in upper case for clarity, but are not case-sensitive.

Display Commands

CONFIG	Display instrument hardware configuration information
DD, DIR	Display current disk directory
DEPTH	Display current depth
DS	Display current instrument status information
DT	Display current time and date
HELP, ?	Display general help messages and command list
SW	Display mechanical switch position
TEMP	Display current temperature
VIN	Display input supply voltage

Setup Commands

MODE, OM x	Set operating mode (burst or fixed-rate)
ST x, STARTCOND x	Set start condition.
TD x, STARTDATA x	Set start condition data
SP x, STOPCOND x	Set stop condition
PD x, STOPDATA x	Set stop condition Data
SI x	Set sample interval
MA x	Set samples per average equal to x
BI x	Set burst interval
SB x	Set samples per burst

SC yyyy/mm/dd hh:mm:ss	Set clock with time and date, where *yyyy*=year, *mm*=month, *dd*=day, *hh*=hour(24 format), *mm*=minute, *ss*=seconds,
AS x	Set autostart
STOREDATA x	Suppress data storage (WARNING: can cause loss of data!)
ZD	Reset depth sensor offset
SENSORPOWER x t	Set whether power output turns on during logging
SENSORWARM t	Set external sensor power warmup time

Acquisition/Action Commands

DL filename	Delete file
GO	Start data collection using current parameters
GX	Grab sample and transmit it
XR	Transmit raw data when average saved
ZS	Acquire 20 samples and evaluate them for quality.
ZZ	Go into deep sleep mode (minimum power consumption)

G. LISST-200X Command Details

1. Display Commands

CONFIG	Display instrument hardware configuration
Syntax:	CONFIG
Description:	The current hardware configuration including Auxiliary I/O connector configuration
Example:	input: CONFIG output: Serial Number: 2001 Firmware Version: 1100 (1.100) VCC: 59371 Full Path Length: 2500 (25 mm) Eff. Path Length: 2500 (25 mm) BioBlock Installed: 0 Start Condition: Digital input (4) Start Condition Data: 0 Stop Condition: Switch (3) Stop Condition Data: 0 Measurements Per Average: 10 Sample Interval: 1 seconds Burst Mode: 0 Samples Per Burst: 5 Burst Interval: 25 minutes Transmit Raw Data: 0 Lifetime Sample Count: 529554 Lifetime Laser On Time: 1703980 seconds Endcap Configuration: 0 (Analog & digital in) Wiring of MCBH6MP connector: 1: Ground 2: Digital input 1 3: Digital input 2 4: +12V output 5: Analog input 1 6: Ground

DD, DIR	Display Disk Directory
Syntax:	DD or DIR
Description:	Display current disk directory in DOS type format. Includes total bytes used and bytes available.

Example:	input: DD
	
	output:
	       LISST-200X Disk Directory
	
	 Volume in drive C is NONAME
	 Volume Serial Number is 778B-155F
	
	 Directory of C:\
	  L040305_.BIN 4,136 03-05-04 6:30p
	  L183705.BIN 1,672 03-05-04 6:37p
	
	    2 file(s) 5,808 bytes
	    0 dir(s) 15,933,440 bytes free
	L200X:>

DEPTH	Display current depth
Syntax:	DEPTH
Description:	The current depth is displayed to the screen.
Example:	input: DEPTH output: Current depth: 567.1 meters

DS	Display current status information
Syntax:	DS
Description:	The instrument settings and status are displayed to the screen.

Example:	input: DS
	
	output:
	
	 LISST-200X Status and Settings
	  Serial number = 1004
	  Endcap Configuration: 0 (Analog & digital in)
	  Firmware Version 1.126 Apr 26 2016 18:45:52

Current Date/Time: 2016/04/27 10:27:02
Current Day of the Year: 118
Supply voltage: 12.00 V
Operating Mode: Burst Mode
AutoStart OFF
Delay Start with 0 minute delay
Measurements per Average: 10
Sample Interval: 1
Stop after 5 samples
External sensor power during logging: off
External sensor warmup time: 0 seconds
Free memory: 1023 Mbytes

input: DS 1

output:

LISST-200X Current Status and Settings
SN = 1004
OM = 0
ST = 5
TD = 0
SP = 5
PD = 0
MA = 10
SI = 1
BI = 1
SB = 1
BB = 0
AS = 0
SENSORPOWER = 0
SENSORWARM = 0
Current Time = 2016/04/27 10:32:15
Battery = 1200
Switch = 0
Memory= 1023639552 Bytes
Endcap = 0

DT	Display current time and date
Syntax:	DT
Description:	Displays current time and date to the screen.
Example:	input: DT output: Date/Time: 2001/01/05 21:05:03

HELP, ?	Display general help messages and command list
Syntax:	HELP or ?
Description:	Displays the list of command to the screen.
Example:	input: HE
	output: LISST-200X Commands (followed by 30 lines of text)

SW	Display current Mechanical Switch position
Syntax:	SW
Description:	The current position of the Mechanical Switch is displayed to the screen.
Example:	input: SW
	output: Current Switch Position: 0

TEMP	Display current temperature
Syntax:	TEMP
Description:	The current temperature is displayed to the screen.
Example:	input: TEMP
	output: Current temperature: 20.3 deg C

VIN	Display Battery Voltage
Syntax:	VIN
Description:	The current supply voltage is displayed to the screen.
Example:	input: VIN
	output: Current Input Voltage: 8.90 volts

2.   Setup Commands

OM	Set Operating Mode
Syntax:	MODE x or OM x
Description:	Sets the Operating Mode to one of the following types: 0 = Fixed rate 1 = Burst Mode OM command only without a parameter will initiate a prompt for the Operating mode.
Example:	input: OM 1 output: New Operating Mode: Burst Mode

ST, STARTCOND	Set Start Condition
Syntax:	ST x or STARTCOND x where x is the start condition code described below
Description:	The ST command sets the start condition to be used when the GO command is issued. The start condition options are: 1 = Depth Start 2 = Time/Date Start 3 = Mechanical Switch Start 4 = Digital Input Start 5 = Delay Start If no value follows command, prompts will be displayed for the value. After setting the start condition to Depth, Time or Delay, use STARTDATA to set the applicable condition value.
Example:	input: ST 5 output: New Start Condition Setting: Delay Start

TD, STARTDATA	Set Start Condition Data
Syntax:	TD x or STARTDATA x where x is the start condition data described below
Description:	The TD command sets the start condition data to be used when the GO command is issued. The start condition data is used with the Start Condition setting as follows: • If the Start Condition is Depth Start (option 1) the input will be start depth in meters. • If the start condition is set to Time/Date Start (option 2) the input for TD will be the start date and time. • If the Start Condition is Delay Start (option 5) the input will be time delay in minutes. • The TD setting is ignored for Mechanical Switch Start (option 3) or Digital Input Start(option 4). If no value follows command, prompts will be displayed for the value.

Example:	input: TD 3 (if Start Condition = 1 (Depth Start))
	
	output: New Start Condition data = 3
	
	  Start Condition: Depth Start at 3 meters
	
	input: TD 2016/12/31 23:59:59 (if Start Condition = 2 (Time/Date Start))
	
	output: New Start Condition data = 2016/12/31 23:59:59
	
	  Start Condition: Time Start at 2016/12/31 23:59:59
	
	Input: TD 2 (if Start Condition = 5 (Delay Start))
	
	Output: New Start Condition data = 2
	
	  Start Condition: Delay Start with 2 minute delay

SP, STOPCOND	Set Stop Condition
Syntax:	SP x or STOPDATA x, where x is the stop condition code described below

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Description:	The SP command sets the stop condition to be used when collecting data. The stop condition options are:
	1 = Depth Stop
	2 = Time/Date Stop
	3 = Mechanical Switch Stop
	4 = Digital Input Stop
	5 = Number of Samples Stop
	6 = Maximum memory or Low Battery Stop
	If no value follows command, prompts will be displayed for the value. After setting a stop condition of Depth, Time/Date or Number of Samples, use STOPDATA to enter an appropriate parameter value.
Example:	input: SP 5
	output: New Stop Condition Setting: Fixed Number Stop

PD	Set Stop Condition Data
Syntax:	PD x or STOPDATA x where x is the stop condition data as described below
Description:	The PD command sets the stop condition data to be used when the collection data. The stop condition data is used with the Stop Condition settings as follows.
	• If the Stop Condition is Depth Stop (option 1) the input will be stop depth in meters.
	• If the stop condition is set to Time/Date Stop (option 2) the input for PD will be the stop date and time.
	• If the Stop Condition is Fixed Number Stop (option 5) the input will be the number of samples to collect before stopping.
	• The PD setting is ignored for Mechanical Switch Stop (option 3), Digital Input Stop (option 4) or Maximum memory or Low Battery Stop (option 6).
Example:	input: PD 3 (if Stop Condition =1 (Depth Stop))
	output: New Stop Condition data = 3
	Stop Condition: Depth Stop at 3 meters
	input: PD 2016/12/31 23:59:59 (if Stop Condition = 2 (Time/Date Stop))
	output: New Stop Condition data = 2016/12/31 23:59:59
	Stop Condition: Time Stop at 2016/12/31 23:59:59

input: PD 2 (if Stop Condition = 5 (Fixed Number of Samples Stop))	
output: New Stop Condition data = 2	
Stop Condition: Fixed number Stop at 2 samples	

SI	Set Sample Interval
Syntax:	SI x, where x is the number of seconds between samples, from 1 to 10,000.
Description:	In either fixed-rate or burst mode, the sample interval is the number of seconds between two consecutive samples, each composed as an average of a number of measurements (specified by the MA command).
Example:	input: SI 5 output: New Seconds between Samples: 5

MA	Set measurements per average
Syntax:	MA x The exact result depends on whether ambient light rejection (ALR) is active. Starting with firmware version 1.4 (May, 2017), ALR is on by default, and the actual number of samples averaged will be approximately x/10. That is, MA 32 will result in 3 ALR cycles per average. With or without ALR, 32 is the maximum MA value that allows 1 sample per second.
Description:	Each recorded or displayed measurement is based on an average of measurements. MA sets the number of measurements per. If no value follows command, prompts will be displayed for the value.
Example:	input: MA 32 output: New Measurements per Average: 32

BI	Set Burst Interval
Syntax:	BI x, where x is the number of seconds between the start of 2

consecutive bursts, from 1 to 10,000 seconds.	
Description:	In Burst Mode (MODE = 1), the burst interval is the number of seconds between two consecutive bursts, each burst composed of a number of samples per burst (specified by the SB command).
Example:	input: BI 900 output: New Seconds between Bursts: 900

SB	**Set Samples per Burst**
Syntax:	SB x, where x is the number of samples per burst. Each sample is taken at the sample interval (in seconds) set by the SI command. If no value follows command, prompts will be displayed for the value.
Example:	input: SB 10 output: New Samples per Burst: 10

SC	**Set Clock with time and date**
Syntax:	SC yyyy/mm/dd hh:mm:ss Where yyyy=year, mm=month, dd=day, hh=hour (24 hour format), mm=minute, ss=seconds If no values follow the "**SC**" or "**sc**" command, prompts for entering the time and date will be displayed.
Example:	input: SC 2017/01/05 21:05:03 output: Command Data in SC is: 2017/01/05 21:05:03 Current Date/Time: 2017/01/05 21:05:03

AS	**Enable Autostart Setting**
Syntax:	AS x, where x is 1 (yes) or 0 (no)

Description:	With Autostart enabled, the firmware will immediately start the sampling program when power is applied to the instrument.
Example:	input: AS 1
	output: AutoStart will occur upon power up!
Cautions:	If Autostart is enabled, the user cannot talk to the instrument when powering it up – it will immediately start sampling according to the SD defaults. In order to stop sampling and establish normal communication, the user must issue a stop command, by either pressing the STOP button or issuing a CTRL-C command in the terminal window within a few seconds of powering up the instrument.

SaveData	Save Data Setting
Syntax:	SAVEDATA x, where x is 1 or 0
Description:	The SaveData setting disables (x = 0) or enables (x = 1) data storage on the internal drive. No Ldddhhmm.DAT file will be created if Save Data mode is disabled. It is intended to be used for long-term real-time deployments, where it may not be desirable to have the data stored on the datalogger. It may be desirable to disable data storage when exporting raw data to an external data logger. See the XR command for configuring the raw data export option.
Example:	input: SAVEDATA 1
	output: Data will be stored after each sample!!!
	input: SAVEDATA 0
	output: Data will NOT be stored after each sample!!!
Cautions:	Be very careful that the correct store mode is selected before a deployment where it is the intent to store the data internally on the instrument! Issue a DS command to verify the status of the store mode setting. A warning will be displayed as part of the DS status if the store mode is disabled: WARNING: Data storage disabled. Data will not be saved!!!

ZD	Reset Depth Sensor Offset
Syntax:	ZD
Description:	The ZD (or zd) command resets the depth sensor offset so that the sensor reads a depth of 0m at zero depth (in air). You must issue the ZD command, then select 1 (yes) or 0 (no) to reset depth sensor
Example:	input: ZD output: Depth Sensor Offset Reset Procedure Started. Instrument must be at zero depth and similar temperature to field conditions. Do you wish to reset Depth Sensor offset? (1=yes,0=no): [0] ? 1 Previous offset was -14.03. New offset is -13.87. Previous Depth was -0.15 meters. New Depth using corrected offset is 0.00 meters.
Cautions:	None

3. *Acquisition/Action commands*

DL	Delete file from LISST memory
Syntax:	DL *filename*, where *filename* is the name of the file to be deleted.
Description:	DL command is used to delete file from the Compact flash module. Wildcards such as *.* can be used.
Example:	Input: DL L159*.dat Output: Are you sure (Y/N)…
Cautions:	WARNING: Make sure that the file being delete has already been offloaded before deleting the file. Once the file is delete it can not be recovered.

GO	Start Data Collection using current Settings
Syntax:	GO
Description:	Starts Fixed Rate or Burst Mode Data collection using current settings.
Example:	Input: GO Output: Waiting for start conditions…
Cautions:	To stop data acquisition before it is complete press the Stop button or **CTRL-C**.

GX	Grab sample and transmit it
Syntax:	GX
Description:	Acquires single averaged sample and displays the result to the screen.

Example:	Input: GX
	
	Output: {
	          …. 36 ring values + 24 Aux parameters
	          }

Notes:	The GX command does not store the sample to a datafile.

XR	**Transmit raw data during sampling**
Syntax:	XR x, where x = 0 through 3
Description:	This command allows data to be transmitted to an external data system during execution of the GO command, in one of several formats. The default setting is 0, with data transmission turned off. Setting 1 sends raw data in a multi-line text format; 2 sends a single line as a NMEA-compatible data sentence; 3 sends a single line of comma-separated values. Contact Sequoia Scientific for complete details of these formats.
Example:	input: XR 1 output: Raw data WILL be transmitted when data is stored
Cautions:	Format 2 requires firmware 1.3 or later. Format 3 requires firmware 1.414 or later.

ZS	**Collect and transmit background scattering data**
Syntax:	ZS
Description:	Acquires 20 averaged sample and displays the result to the screen. Evaluates the data for quality as a background measurement.

Example:	Input: ZS
	
	Output: {
	    …. 36 ring values + 24 Aux parameters
	    }
	
	repeat 20 times, then messages about the quality of the data.

Notes:	The ZS command does not store the sample to a datafile.

ZZ	**Go into deep sleep mode (minimum power consumption)**
Syntax:	ZZ

Description:	Sends LISST-200X in to low power sleep mode. Instrument will send a message every 30 seconds to indicate it is sleeping. It can be wakened at any time by sending 2 control-C characters about ½ second apart.
Example:	Input: ZZ Output: Deep Sleep… Enter zz to wake up Deep Sleep… Enter zz to wake up Deep Sleep… Enter zz to wake up

H. Performance Optimization

This section contains information on optimizing the performance of the LISST-200X.

Optical Alignment

The background measurement is the best source of information on the current health of the LISST-200X. It provides information on the current functionality of all of the major systems including laser, ring detector, data collection electronics, and optical alignment. The LISST-200X is a sensitive optical device. The laser must be aligned such that the focused spot is centered on the hole in the center of the ring detector. If this alignment in not correct the instrument will not function correctly. The background measurement can easily provide the user with information about the status of the alignment.

The first indicator of an alignment problem is a severe drop in the currently transmitted laser power when compared to the factory laser power. The laser power and laser reference values will change over time but they should track together. If the value of the laser power:laser reference ratio for your current background measurement is less than 85% of the laser power:laser reference ratio from the factory background file, misalignment may be occurring.

The second indictor of misalignment is high value on the inner rings. The inner rings are shown on the left side of the background display. The most important rings for misalignment indication are rings 1-4 in the background scatter file. As the focused laser starts to move away from the center of the ring detector it will scatter more light onto the inner rings. This will cause the inner ring values to be much higher than the factory values, and is also the reason for the decrease in transmitted laser power.

It must be noted that low laser power or high inner rings may not always indicate misalignment. Low laser power can also occur because the windows or water is dirty or if there are bubbles on the window. Large particles or bubbles can cause the inner rings to be higher than factory values. All of the other possibilities must be eliminated before it can be concluded that the instrument is misaligned.

The transmit optics on the LISST-200X do allow for the possibility of the user adjusting the alignment. A written procedure can be provided for this procedure. However, it is highly recommended that the need for re-alignment be discussed with a trained Sequoia Scientific technical service representative before attempting this procedure.

Background Measurement and its Importance

As was discussed in earlier sections, the LISST-200X uses a custom detector to measure light scattered at small angles from particles in water. In order to measure only the scattered light contributed by the particles, a measurement of the background scattering must be obtained. This background scattering can come from a number of areas. Scratches on the windows, imperfections on the optics, and other sources all contribute to the scattered light. By subtracting this background scattering from the measured data, a true measurement of

the light scattered from the particles can be obtained. The measurement of background scattering is sometimes called a "ZSCAT". The name comes from the fact that the measurement is obtained using water with zero "scatterers" or particles.

It is very important that clean and bubble free water is used. The water can be fresh or salt water. For most applications, it has been found that steam-distilled water is sufficient. Steam-distilled water is typically available in one-gallon containers. We have found that this distilled water tends to be a bit cleaner than typical bottled or packaged water. We buy steam-distilled drinking water and filter it through a 0.2µm filter.

A small volume horizontal chamber has been provided for submerging the optics while acquiring a background.   It is inserted between the windows of the optics. The instrument should be placed horizontally on the supplied white plastic supports. However, Plastic bags, Tupperware or clean containers of any kind can be used to acquire a background. The instrument's optics end can be submerged in them vertically, as long as the optics are completely submerged and there is no blockage of the windows by bubbles or other objects.

Because the area surrounding the windows is submerged, it is important to thoroughly clean and rinse this part of the instrument before acquiring a background.

Toothbrush, liquid soap and water works well for cleaning the optical end of the instrument; do not use abrasive powders, they will scratch optics and destroy instrument performance.

The low concentration limit of LISST-200X is very sensitive to the quality of the background scatter file. For this reason, when working in low concentration water, it is very important that a good background file be obtained with very clean water. As particle concentrations increase, the relative signal-to-background noise ratio also increases, thus reducing the importance of the background. However, a background should always be collected before an experiment.

Watch for Outgassing and Bubbles

Another consideration when acquiring a background is "outgassing" causing small bubbles to form on the instrument and windows. Bubbles on the windows will greatly modify the scattering pattern, rendering the background useless. Be sure to remove any bubbles from the windows before acquiring a background. Use a squirt bottle or pipette to blow the bubbles off the window.

Because the area surrounding the windows is submerged, it is important to thoroughly clean and rinse this part of the instrument before acquiring a background.

Ambient Light Rejection

Starting with firmware version 1.4 in May, 2017, the LISST-200X automatically measures and subtracts light that is not produced by scattering from its laser. This Ambient Light Rejection (ALR) prevents sunlight or other bright sources from contaminating the scattering measurements. Without ALR, as in earlier LISST instruments, high ambient light levels, if not physically blocked from the LISST's optical aperture, could falsely elevate the apparent concentration of small particles.

You can verify that ALR is active by connecting to the instrument with LISST-SOP200X, then opening the terminal window. Type DS and press enter, and look for "Ambient light subtraction: on" in the output. If it is not shown, you should upgrade the firmware. Contact Sequoia Scientific for assistance.

ALR works by measuring the signal on the LISST-200X's 36 detectors first with the laser on, then with the laser off. This on-off process is repeated 3 times per second. Because of the time required for the laser and electronics to change between the on and off states, rapidly changing ambient light may not be completely rejected. Therefore, if working in bright sunlight, near the water surface, and in conditions where the sunlight is rapidly modulated by waves, ambient light could still have an effect. ALR will reduce the average effect, but the changing ambient conditions could increase sample-to-sample noise. In those worst-case conditions it is still advisable to shade the LISST-200X optics from ambient light. The same is true if operating under intense artificial light modulated by AC power.

I.   *Instrument Mounting, Deployment Orientation, Storage and Maintenance*

This section contains information on mounting, storing and maintaining the LISST-200X.

Mount the Instrument Horizontal

The LISST-200X measures scattered light to obtain particle size distributions. Therefore it is important to keep particles from sticking to the window surface. The first line of defense it to keep the window surface vertical by mounting the instrument horizontally. This is most critical when the water motion is slow. When working in rivers or in a towed or profiling application the orientation is not as critical.

The center section of the main pressure case has a slightly smaller diameter than the ends. The smaller diameter section is the suggested mounting area. The U-shaped mounting block provided with the instrument fit this diameter and can be used for mounting the instrument. The clamps supplied with the instrument can also be used.

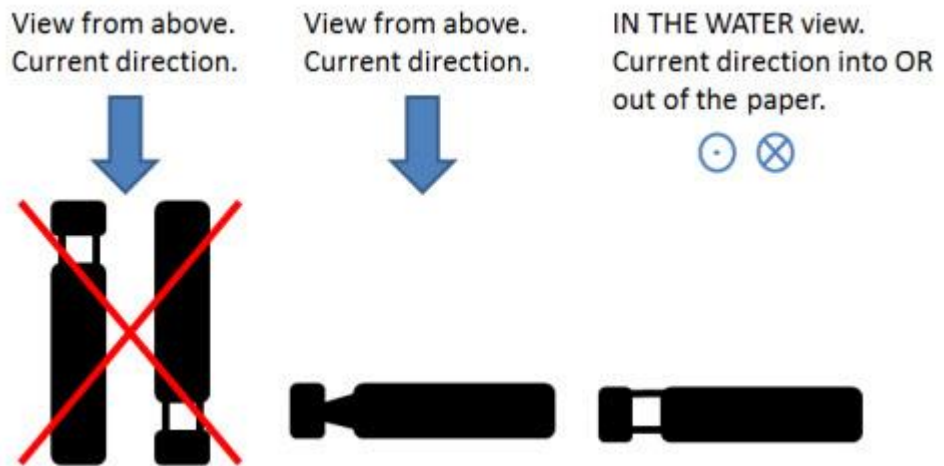Spare clamps for mounting the instrument are available from Sequoia.

Precautions for Vertical Mounting

If mounting the instrument vertical the optics endcap should always face downwards. If the connector endcap faces downwards, and the instrument is subjected to a bump or shock during deployment, the Compact Flash Memory Card inside can come loose, making it impossible to offload data.

Use the supplied U-shaped mounting blocks or the stainless steel clamps with rubberized inserts for vertical mounting.

Precautions for In-water Orientation Relative to the Current Flow

If possible, the instrument should always be deployed so that it is perpendicular to the current and the water is flowing as unobstructed as possible through the optics end of the instrument. The diagram below shows the optimum orientation with respect to the current flow.

View from above. Current direction.

View from above. Current direction.

IN THE WATER view. Current direction into OR out of the paper.

Electrical Isolation

To reduce the corrosion of the aluminum parts a zinc anode is attached to the Connector endcap. This anode must be exposed to the water for it to be effective.

When mounting the instrument be sure to electrically isolate the instrument from all other metal. Any contact with other metal can greatly increase the rate of corrosion. Isolate the instrument with rubber or plastic to keep the LISST-200X from being the sacrificial anode for the mounting hardware. Failure to properly isolate the instrument from all other metal will void the warranty.

A set of stainless steel clamps with rubberized interiors ships as part of the instrument package. Use these or the plastic clamps for mounting, and make sure that no metal is in direct contact with the pressure housing or other components of the instrument. If you lose the spare clamps, replacement clamps are available for purchase from Sequoia.

Storage Notes

Again it must be emphasized that the LISST-200X is a sensitive instrument. When not in use, the instrument should be stored in its shipping case.

Cleaning the Optical Windows

As has been noted earlier, the condition of the windows is critical to the performance of the LISST-200X. Care must be taken when cleaning the windows. The windows and the instrument should be rinsed thoroughly with fresh water after each deployment. The windows should be cleaned with a soft cloth or lens tissue. Liquid detergent/soap and water may be used. For removing grease spots, finger prints, etc, alcohol may be used. Do not use stronger solvents such as Acetone or Toluene.

Abrasive powders must never be used near the optics windows; they will scratch the windows and degrade instrument performance.

O-Rings

O-rings that seal the mating parts of the instrument must be maintained and inspected regularly. Whenever the connector end cap is removed, check the O-ring for any cuts or marks, and clean and lightly grease the O-ring before installing the end cap. Spare end cap O-rings have been provided. O-rings are inexpensive items that provide an invaluable service; replace them if in any doubt about their condition. When replacing the O-ring, be sure to clean the O-ring groove thoroughly with cotton-swabs etc. making sure that no fibers or particles of dirt are left after the cleaning.

Calibrations and Adjustments

The LISST-200X should not require any adjustment or calibrations. The pressure and temperature sensors can be re-calibrated if desired. The performance of the instrument can be checked with the use of a sample of particles of a known size distribution. Standard particles can be obtained from manufacturers of standard particles, such as Whitehouse Scientific (http://www.whitehousescientific.com/) or PTI – Powder Technology Inc. (http://www.powdertechnologyinc.com/).

Comparison of the LISST-200X's performance with that of other particle-size measuring devices that are not based on laser diffraction may result in inconsistencies because the instruments may measure different properties of the particles to interpret particle size.

Appendix A: Technical Specifications

- Parameters measured/derived:
  - Particle Size distribution
  - Total Volume Concentration
  - Optical Transmission
  - Depth
  - Temperature
  - Volume Scattering Function
- Particle size range: 1.00 - 500 µm
- Particle size resolution: 36 size classes, log-spaced
- Optical path length: 2.5 cm
- Optical transmission: 12-bit resolution
- Analog inputs (availability depends on configuration)
  - Measurement range: 0 to 6 V
  - Resolution: 0.1 mV
  - Uncertainty: 1.5% of reading + 2 mV
  - Input impedance: 500 kOhm
  - Time constant: 250 ms
- Digital input (availability depends on configuration)
  - Nominal logic levels: 0 and 3V
  - Voltage tolerance without damage: -12 to 12V
- Analog outputs (availability depends on configuration)
  - Output range: 0.1 to 3.0 V
  - Resolution: 12 bits
  - Output impedance: 1 kOhm
  - Uncertainty: 1.5% of reading + 2 mV
- Data storage memory: 1GB (~12 million measurements) standard.
- Maximum sample speed: 1 size distributions per second (standard)
- Temperature sensor
  - Range: -5 to 45 °C
  - Resolution: 0.001 °C
  - Uncertainty: approximately 1 °C
- Depth Sensor
  - Range: 0 to 600 m of sea water
  - Resolution: 1 cm
  - Uncertainty: approximately 1% of reading (if atmospheric offset zeroed)
- Input power:
  - Operating range: 9 to 24 V
  - Current during active sampling: 100 mA @ 12V
  - Current while waiting for use command: 50 mA @ 12V
  - Current during sleep: 3 mA
- Dimensions: 10.3 cm (3.95") Æ x 63.9 cm (26.18") L
- Weight in air: 5.4 kg (11.8 lbs.)
- Weight in water: 1.7 kg (3.8 lbs.)
- Depth rating: 600 m

25.18
[639,4]

16.63
[422,3]

10.16
[258,1]

4.15
[105,4]

∅ 3.95
[100,3]

∅ 3.75
[95,3]

2.25
[57,2]
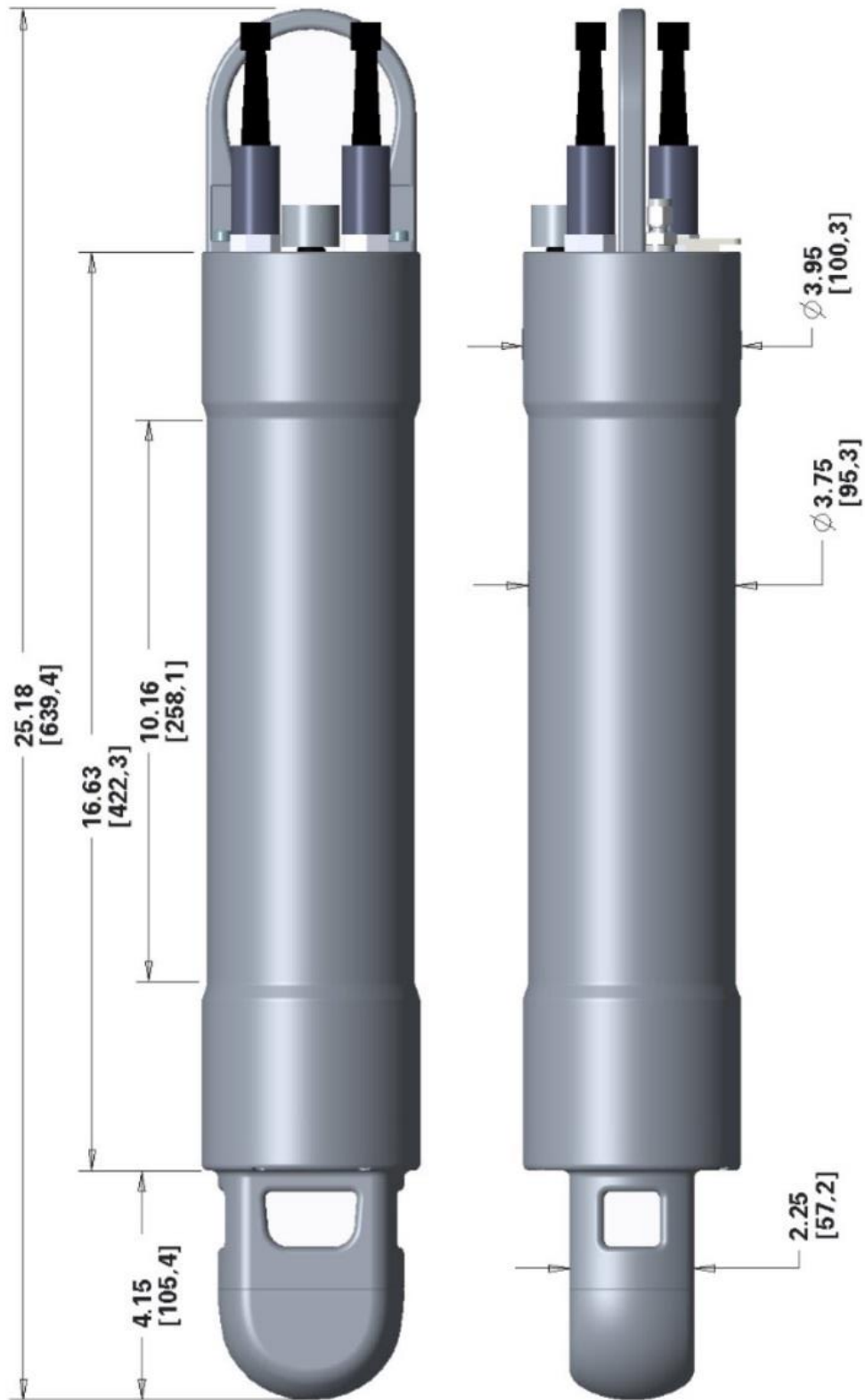
Appendix B: Particle Size Bins

There are 36 size ranges logarithmically placed from 1.00 - 500 microns in diameter. The upper size in each bin is approximately 1.18 times the lower, with the exception of bin 1. The table below shows the lower and upper limit of each size bin in microns, together with the <u>median</u> size (also in microns) for each size bin. The sizes are the same for both Spherical and Randomly Shaped inversions.

Size bin #	Lower	Upper	Median
1	1.00	1.48	1.21
2	1.48	1.74	1.60
3	1.74	2.05	1.89
4	2.05	2.42	2.23
5	2.42	2.86	2.63
6	2.86	3.38	3.11
7	3.38	3.98	3.67
8	3.98	4.70	4.33
9	4.70	5.55	5.11
10	5.55	6.55	6.03
11	6.55	7.72	7.11
12	7.72	9.12	8.39
13	9.12	10.8	9.90
14	10.8	12.7	11.7
15	12.7	15.0	13.8
16	15.0	17.7	16.3
17	17.7	20.9	19.2
18	20.9	24.6	22.7
19	24.6	29.1	26.7
20	29.1	34.3	31.6
21	34.3	40.5	37.2
22	40.5	47.7	43.9
23	47.7	56.3	51.9
24	56.3	66.5	61.2
25	66.5	78.4	72.2
26	78.4	92.6	85.2
27	92.6	109	101
28	109	129	119
29	129	152	140
30	152	180	165
31	180	212	195
32	212	250	230
33	250	297	273
34	297	354	324
35	354	420	386
36	420	500	459

Appendix C: Data File Formats

File Types and Extensions Used by the LISST-SOP200X Software

Extension	Discription	Format	Comments
.RBN	Raw Data	Binary	Raw data file produced by the LISST-200X.
.RTX	Raw Data	ASCII	Comma delimited ASCII representation of .RBN file (59 columns, 1 measurement per row)
.PBN	Processed Data	Binary	Contains size distribution data that can be viewed in the LISST-SOP200X software
.CSV	Processed Data	ASCII	Comma delimited file containing size distributions and meta data (61 columns, 1 measurement per row)
.BGT	Background File	ASCII	Comma delimited file containing the raw ring values from a single background measurement (1 column, 59 rows)
.LOP	Operating Procedure	ASCII	File containing sampling parameters that can loaded onto an instrument in the LISST-SOP200X software

The values in the processed data file (.CSV extension) are stored in the order shown below. Each sample is stored in one row.

Column #	Parameter
1:36	Volume concentration for size class 1 through 36 [µL/L]
37	Laser transmission Sensor [mW]
38	Supply voltage in [V]
39	External analog input 1 [V]
40	Laser Reference sensor [mW]
41	Depth in [m of sea water]
42	Temperature [C]
43	Year
44	Month
45	Day
46	Hour
47	Minute
48	Second
49	External analog input 2 [V]
50	Mean Diameter [µm]
51	Total Volume Concentration [PPM]
52	Relative Humidity [%]
53	Accelerometer X [not presently calibrated or used]
54	Accelerometer Y [not presently calibrated or used]
55	Accelerometer Z [not presently calibrated or used]
56	Raw pressure [most significant bit]
57	Raw pressure [least significant 16 bits]
58	Ambient Light [counts – not calibrated]
59	Not used (set to zero)
60	Computed optical transmission over path [dimensionless]
61	Beam-attenuation (c) [m^{-1}].

ASCII Raw Data Format

The values in the raw ASCII data file (.RTX extension) are stored in the order shown below. Each sample is stored in one row.

Column #	Parameter
1:36	Raw ring values [counts]
37	Laser transmission Sensor [counts]
38	Supply voltage [0.01 V/count]
39	External Analog input 1 [10^{-4} V/count]
40	Laser Reference [counts]
41	Depth [0.01 m/count + 10 m]
42	Temperature [1 m°C/count + 5 °C]
43	Year
44	Month
45	Day of month
46	Hour
47	Minute
48	Second
49	External Analog input 2 [10^{-4} V/count]
50	Sauter Mean Diameter [0.1 µm/count]
51	Total Volume Concentration [0.1 PPM/count]
52	Relative Humidity [%]
53	Accelerometer X [counts] [not presently used]
54	Accelerometer Y [counts] [not presently used]
55	Accelerometer Z [counts] [not presently used]
56	Raw pressure [most significant bit]
57	Raw pressure [least significant 16 bits]
58	Ambient light [counts]
59	Not used

Appendix D: Processing Data Files Using MATLAB

Two Matlab functions for processing data are provided on the instrument USB card. They are also available from the 'Software and Downloads' tab on the LISST-200X webpage. The processing is split into two steps: (1) reading in and applying corrections to raw data files (.RBN) and (2) inverting the corrected scattering to a particle size distribution.

The same results can be achieved by processing data files using the LISST-SOP200X software. However, the following functions allow Matlab users to write their own processing and plotting code.

The first function is used to read in raw data from an RBN file. The function will import the data, apply the necessary corrections, and return the corrected data in a structure. The syntax is as follows:

```
RBNdata = getscat_L200X(datafile)
```

'Datafile' is the path to a binary .RBN file downloaded from a LISST-200X.

Optionally, you may specify a different clean water background file (.BGT) as a second argument. The data will then be corrected using the specified background file instead of the background contained in the RBN file.

```
RBNdata = getscat_L200X(datafile,backgroundFile)
```

'RBNdata' is a structure with the following fields:

Field	Description
cscat	Corrected scattering
date	Timestamp in Matlab datenum
transmission	Optical transmission
depth	Depth in meters
temperature	Temperature in degrees Celsius
estMeanDiameter	Estimated Sauter mean diameter (um)
estTotalConc	Estimated total concentration (uL/L)
Lp	Transmitted laser power (mW)
Lref	Laser power reference (mW)
analog1	Analog input 1

analog2	Analog input 2
supplyVolts	Supply voltage (V)
humidity	Internal instrument relative humidity (%)
accelXYZ	Accelerometer X, Y, and Z (not presently calibrated or used)
raw	Raw data as it appears in the RBN file
factory_bkgrd	The factory background (corrections applied to aux data)
bkgrd	User collected background (corrections applied to aux data)
ambientLight	Counts of ambient light removed from ring values
config	Structure containing various instrument information
dcal	Ring area coefficients
Ta	Vector to convert cscat to estimated total area concentration
Tv	Vector to convert cscat to estimated total volume concentration

The second function inverts the corrected scattering to a particle size distribution. The syntax is as follows:

```
[vd, dias] = invert_L200X(Cscat,Random,Sharpen,ShowProgressBar)
```

The first argument is the corrected scattering (**cscat**) from the structure returned from 'getscat_L200X.' The following three argument are set to zero or one.

Random – If set to 1, the randomly shaped particle matrix is used to invert the data. If set to zero, the spherical particle matrix is used. See Appendix G: Spherical and Random Shape Particle Models for more information.

Sharpen – If set to 1, the function checks the width of the size distribution and increases the number iterations if the size distribution is wide (recommended).

ShowProgressBar – If set to 1, a progress bar will display the processing status.

The function will return the volume distribution in uL/L (**vd**) and the midpoint of each size bin in microns (**dias**).

Appendix E: Connectors

IMPORTANT NOTE

In September 2019, starting with serial number 2116, LISST-200X instruments and cables use connectors manufactured by SubConn. Previous instruments have connectors from Teledyne Impulse. Connectors from the two manufacturers are very similar, but are not guaranteed to be compatible.

For reliable connections, only mate SubConn cables with SubConn bulkhead connectors. Sequoia's warranty does not cover any problems arising from mismatched connectors.

To Identify the connector type, note:

- SubConn bulkhead connectors have **brass**, golden-colored bodies. They may develop a green tint after exposure to sea water.
- SubConn cables and dummy plugs have **red** locking sleeves.
- Impulse bulkhead connectors have **stainless steel**, grey-colored bodies.
- Impulse cables and plugs have **black** locking sleeves.
- The name of the manufacturer is embossed on the rubber body of each connector.

<table>
<tr><td align="center">SubConn
(as of September 2019)</td><td align="center">Impulse
(older instruments)</td></tr>
</table>

The LISST-200X has 3 separate underwater connectors: A 5-pin, a 6-pin, and a 3-pin connector. The photograph shows the placement of each connector. The following text describes the detailed wiring for each connector. Note that in new instruments starting in September 2019, these are SubConn connectors with brass bodies.
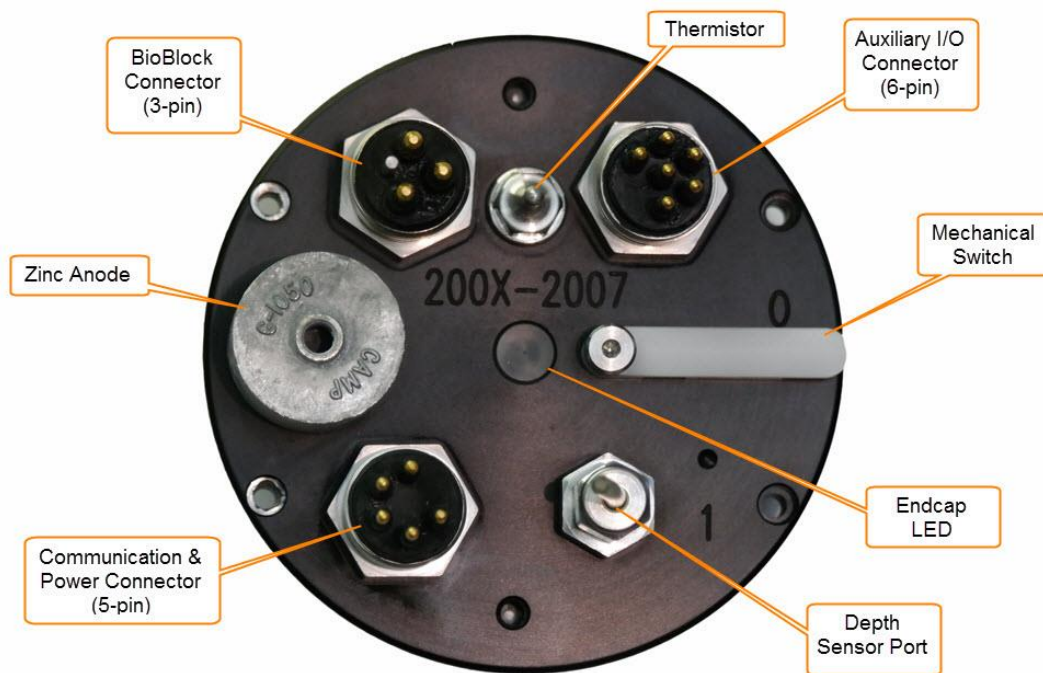
LISST-200X Connector Endcap
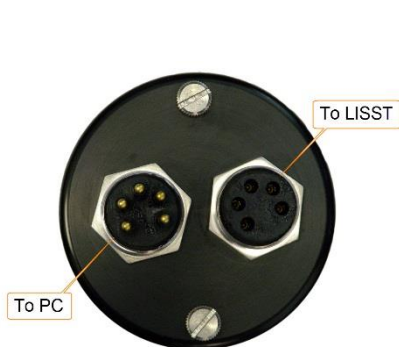
Small External Battery Pack
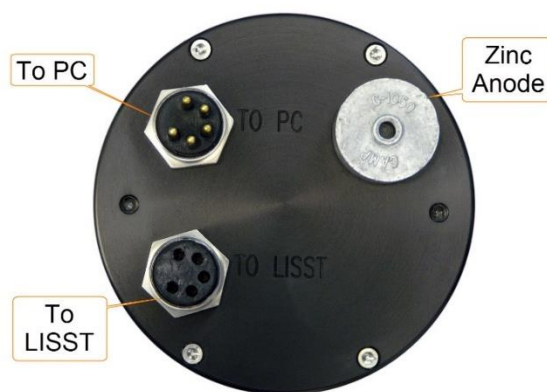
Large External Battery Pack

Communications and Power Connector (5 pin connector)

Bulkhead connector: SubConn MCBH5M
Mating cable connector: SubConn MCIL5F

Bulkhead Endview

Cable Endview

Connector Pin #	Use
1	Power/Serial Ground
2	External Power In (+9V nom. 6-15V)
3	Power/Serial Ground
4	Serial Out (to DB-9 Pin 2)
5	Serial In (to DB-9 Pin 3)

BioBlock Connector (3 pin connector)

Bulkhead connector: SubConn MCBH3M
Mating Cable Part Number: SubConn MCIL3F

Bulkhead Endview

Cable Endview

Connector Pin #	Use
1	Signal Ground
2	Signal (Open/Closed)
3	Battery Sense

Bulkhead connector: SubConn MCBH6M
Mating Cable Part Number: SubConn MCIL6F

Bulkhead Endview

Cable Endview

Analog and Digital Configuration

Connector Pin #	Use
1	Ground
2	Digital In #1
3	Digital In #2
4	+12V out
5	Analog In (0 to 5V max)
6	Ground

Analog Input Configuration

Connector Pin #	Use
1	Ground
2	No connection
3	Ground
4	+12V out
5	Analog In #1 (0 to 5V max)
6	Analog In #2 (0 to 5V max)

Analog Output Configuration

Connector Pin #	Use
1	Ground (Power)
2	Analog Output: Mean Size
3	Ground (signal)
4	Analog Output: Total Concentration
5	Ground (signal)
6	Power In (8 to 24VDC)

The current configuration of the Auxiliary I/O connector can be found by issuing a DS command at the command prompt.

Large and Small External Battery Connectors (5 pin connectors)

Bulkhead connector: SubConn MCBH5F
Mating Cable Part Number: SubConn MCIL5M

Bulkhead Endview

Cable Endview

To LISST Connector (5-pin Female Bulkhead)

Connector Pin #	Use
1	Ground
2	Battery Power Out (+9V nom. 6-15V)
3	Ground
4	Serial Out (to DB-9 Pin 2)
5	Serial In (to DB-9 Pin 3)

Bulkhead Endview
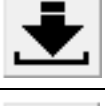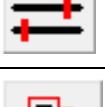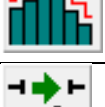
Cable Endview

To PC Connector (5-pin Male Bulkhead)

Connector Pin #	Use
1	No Connection
2	No Connection
3	Ground
4	Serial Out (to DB-9 Pin 2)
5	Serial In (to DB-9 Pin 3)

Appendix F: LISST-SOP Software Button Description

Button	Label	Description
	Open Real-Time Session	Runs the instrument for Real-Time operation.
	Open	Open raw or processed file. Depending on extension of file requested to open, the appropriate procedure is started.
	Query Instrument	Queries the instrument for its current configuration and displays the information to the screen.
	Zero Depth Sensor	Adjusts the pressure offset so the current pressure is registered as zero depth.
	Offload	Offloads stored data to a file for later processing.
	Erase	Erases user selected files from the data logger.
	Stop	Sends a CTRL-C to the instrument to stop a running program.
	Set Instrument Clock	Sets the internal instrument clock to match the currently connected computer clock
	Operating Modes	Opens the Instrument Sampling Configuration window where operating mode and sampling schemes can be configured.
	Collect Background	Opens the real-time background collection windows where background-scattering files can be collected in real-time.
	Connect/Disconnect	When the instrument communication has not been initialized the Connect/Disconnect button will be a red light. Pressing the button will toggle the communications status.
	Open Terminal	Pressing the Open Terminal button will bring up the terminal window for communication with the instrument including Start and Stop buttons.

	Program Settings	The Program Settings button bring up the general settings windows for setting display parameters and other software settings, such as File Output, Com Port settings, and Plotting preferences.
	Print	When viewing processed data the Print button will be activated. Pressing the button will send the current display to the printer.
	Put LISST to sleep	Attempts to wake up instrument from low-power sleep mode. This may take up to 40 seconds.
	Wake up LISST	Puts instrument into low-power sleep mode.

Appendix G: Spherical and Random Particle Shape Models

We briefly comment on shape effects here. Particles of different shapes scatter light differently. Two models are offered by Sequoia – the spherical model, and a random shape model. The resulting inversion of data will differ slightly for the two models.

The spherical particle model performs the mathematical inversion of scattering data under the assumption that the particles that scattered light are all spheres. Light scattering by spheres of any size and refractive index is modeled by Mie theory. According to Mie theory, the angular scattering depends on the size of the spherical particle, and its refractive index relative to water. Sequoia employs the full Mie scattering model, i.e. no simplifications, for inversion of LISST measurement as a distribution of spheres. The Mie solution is a generalized solution to the scattering of light from spheres and is commonly used as the standard model by all laboratory laser diffraction instrument manufacturers.

Sequoia provides an alternate model to Mie theory to invert the measured scattering pattern with the assumption that the scattering particles are randomly (or irregularly) shaped. This is an empirically determined model, since theoretical development of such models is not complete. The exact details of how this scattering model was established are described in a paper by Agrawal et al. [Light scattering by random shaped particles and consequences on measuring suspended sediments by laser diffraction. Journal of Geophysical Research, Vol. 113, C04023, doi:10.10-29/2007JC004403.], which can be downloaded from the library section on Sequoia's website (www.SequoiaSci.com). The direct URL is http://www.sequoiasci.com/library/technical-papers/. The paper is also included on your ship disk. A brief version of the method is described in this Article on Sequoia's website: http://www.sequoiasci.com/article/random-shaped-particles-lissts/. It is noteworthy that no other instrument manufacturer has a scattering model for irregular particles. Instead, they often use a Mie model with large imaginary refractive index, in effect, assuming the particles to be highly absorbing (i.e. black). This is an obvious fudge with consequences!

Key differences in results of the spherical vs random shape model are: the random shaped model interprets particles to be smaller than the spherical model, and when scattering from irregular particles is inverted with the spherical model, the resulting particle size distribution *invents* fine spherical particles.

So a LISST user is likely to ask which model she/he should use. If the plan is to compare LISST results with those from another laser particle sizer, one should choose the spherical particle model. If comparing with sieved particle size data, the irregular shape model is appropriate. When using the spherical particle model, the PSD measured by LISST instruments should generally match those measured by other instruments. Subtle differences may arise due to different refractive indices built into the instrument software.

Generally, it is recommended that you keep both boxes checked during inversion (this is also the default option), so that you won't have to reprocess your data because you need to see what the data look like when processed as randomly shaped particles.

Appendix H: Technical Assistance & Troubleshooting

Q: I am getting errors offloading data from my LISST-200X.

A: In some cases the offload baud rate may need to lowered in order avoid offload errors. The offload baud rate can be reduced in the SOP software by going to File -> Settings. Then select the 'Serial Port' tab:
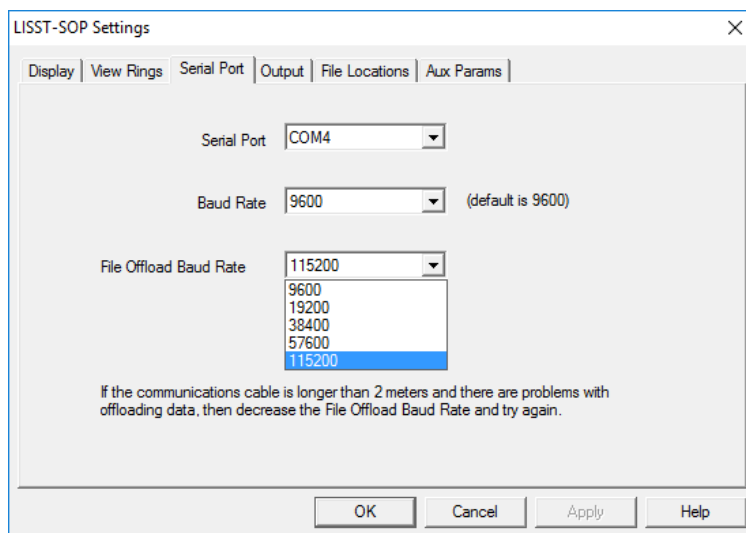
Select a lower File Offload Baud Rate, then select OK. Repeat the file offload. If the errors persist, even at the lowest baud rate (9600), contact Sequoia Scientific.

Q: I think that I have a problem with my data and/or my data processing and would like you to have a look at them - can you do that? What data do I need to send to you?

A: We'll be happy to look at your data and help you figure out what is going on. In order to troubleshoot the problem, we need the .RBN file(s) that contain the raw data.

Email the data to your local Sequoia distributor, together with a detailed explanation of what you were doing and how the data were collected. If you purchased the instrument directly from Sequoia, email the data to info@SequoiaSci.com.

PLEASE NOTE: We _cannot_ use the .PBN or .RTX files produced by the LISST-SOP for troubleshooting. We can only help you if you supply .RBN files.

Q: When I try to query the instrument I get this error:
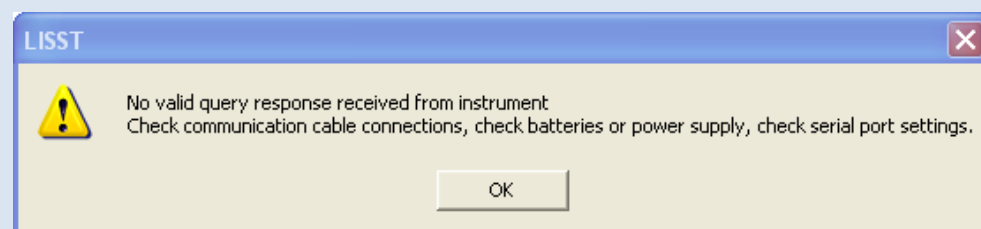
A: In addition to checking the cable, power and serial port settings, make sure that the instrument is not logging data or sleeping. Open up the terminal window and confirm that no program is running. If a program is running, stop it by clikcing the [STOP] button, close the terminal window and try to query the instrument again.

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circumstance beyond SEQUOIA's reasonable control.

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monitoring situations. If Customer chooses to purchase a Product or Products for use in
applications that could result in damages in excess of the price of the Product if the Product does
not operate properly or otherwise fails, Customer acknowledges and agrees that it is Customer's
responsibility to provide for redundancy and/or other safety or back-up measures sufficient to
assure that failure of a Product(s) will not cause such damages. Customer agrees that it will defend
and hold SEQUOIA harmless from any and all claims and costs (including but not limited to
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Revision History

Version 1.4   Show change from Impulse to SubConn connectors.

Version 1.3B   Note that accelerometer data are not presently calibrated or used (they are reserved for possible future use)

Version 1.3A   Edit XR command description. Remove incorrect warning about maximum voltage on digital input, and clarify its logic sense (p. 72). Correct units of data fields in Appendix C: Data File Formats.

Version 1.3   Add Ambient Light Rejection.

Version 1.2   Added support for path length reduction module (PRM) in LISST-SOP200X version 1.2. Extra fields added to .csv file, including ambient light. Added Appendix D: Processing Data Files Using MATLAB.

Version 1.1   First released version.