Covaris™

For research use only

S2x

USER MANUAL

a single tube system developed for
high performance sample preparation
using adaptive focused acoustic energy

Product Name: S-Series

Model Number: S2x

UNIVERSAL PRECAUTIONS

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

Warnings

For safety of operating personnel:

1. Make sure that the equipment is properly grounded. DO NOT operate if it is not properly grounded.

2. The unit is equipped with a power plug appropriate for the destination country. DO NOT, under any circumstances, remove the grounding prong from the power cord.

3. DO NOT attempt to operate the equipment with the Acoustic Assembly and sample cover in the UP position or without a water bath; the acoustic system will not run. If there is any indication that the Safety System is not functioning properly, DO NOT operate the equipment and contact Covaris immediately.

4. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

To prevent damage to the equipment:

1. The instruments are designed to operate in ambient laboratory conditions e.g., 15 to 32°C (60 to 90°F). DO NOT operate the instrument in a cold room environment; the system is designed to operate with a water bath and re-circulating heater/chiller apparatus to control sample temperature.

2. DO NOT run at high power for more than 5 minutes at one time.

3. NEVER run a process without a water bath; this could damage the transducer. The instrument is equipped with a water level sensor to protect the transducer. The system will not allow the acoustic wave treatment to start unless an adequate volume of water is present.

4. Distilled or deionized water should be used to fill the water bath.

5. Empty the water bath and wipe dry EVERY day. DO NOT leave water in the tank for extended time as there is no water filtration or water cleaning system with the apparatus.

6. Acoustic Assembly should be left in the UP position when not in use.

7. Establish a standard of operation and periodically test equipment, as described in Maintenance (see section 6.0) of this manual.

8. DO NOT load third party software onto the system without consulting with Covaris.

Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery by an authorized representative. Any component which proves defective during the stated period will be repaired free of charge or replaced at the sole discretion of Covaris, F.O.B., Woburn, Massachusetts, U.S.A. provided the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement with telephone support from Covaris personnel. If Covaris personnel are required to perform on-site repair, all travel related costs are paid by the customer. A limited warranty as specified may apply to certain components of the equipment.

Warranty Exceptions

This warranty is void if failure of the software or hardware has resulted from accidents, abuse, improper maintenance, or repair, or misapplication by the customer. It is also void if damage is caused by any unauthorized attachments or if modifications are made to the equipment. Removing or tampering with the Safety Enclosure will void the warranty, and the customer will assume all liabilities.

This warranty is limited to the original purchaser and is not transferable.

The software will perform according to the accompanying written materials and the medium on which the software is delivered is free of defects in materials under normal use and service. The warranty is void if damage has resulted from third party software not intended for use with the system.

The high power focused transducer is designed to give maximal mechanical energy output in water. Permanent damage to the transducer and electronic circuits could result if the transducer is operated without water. Operation of the system without water in the water bath voids the warranty.

CONTACT COVARIS, INC. SHOULD YOU HAVE ANY QUESTIONS CONCERNING EQUIPMENT

Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the service and support necessary so that the customer can operate the equipment successfully. Extended warranties are available at the end of the original 12 month warranty period.

Services included with the original purchase of the system are:

Technical Support – On-going assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone (+1 781 932 3959) during the hours of 9:00am to 4:00pm, Monday through Friday,
    - o United States, Eastern Standard Time (EST)
    - o Greenwich Mean Time (GMT-05:00).

Parts Replacement – Replacement of parts (excluding consumables) from normal operation of equipment are provided on a priority basis. All labor and shipping charges are included. Failure due to accident, abuse, or improper operation is not covered.

An Operator's Manual is provided with the equipment. This manual includes sections on the operating instructions, maintenance guidelines, and troubleshooting tips.

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Overview of the Manual

This manual contains operation and service instructions for the *Covaris S2x instrument.* It contains background information essential to the proper use and care of this equipment.

Should any unforeseen problems occur with the normal operation of the equipment, contact Covaris Technical Support immediately.

The following definitions apply in this manual:

NOTE: Inconvenience if disregarded.

CAUTION: Equipment damage may occur.

WARNING: Personal injury may occur.

There is also a PDF version of this manual on the system computer. Open the manual by double clicking the manual icon on the desktop.

1.2 Purpose of the Equipment

The S-series instruments are intended specifically for single sample preparation. The S2x is designed to work with the 20x125mm closed tube. In general, the S2x is best for samples larger than masses typically used in the S2. (See Appendix A) These ranges may be increased or decreased depending upon buffer composition and sample/buffer ratios.

The instruments are not intended for use in diagnostic procedures; they are designed for research use only.

CAUTION: The S-series systems are designed to work primarily with closed sample vessels.

1.3 Main Components

The main components of the S2x instrument include (see Figure 1):

Acoustic Assembly – transducer and sample holder

Temperature sensing device for water bath with cooling loop

Degassing system with water level sensor

Safety Interlock System

External components include a computer (optionally supplied by Covaris) and a recirculating chiller/heater (not shown, and to be supplied by user).

Figure 1

1.4   Principle of Operation

The Covaris Process generates adaptive focused acoustic energy to cause precisely controlled cavitation and acoustic streaming at the focal point within the sample treatment vessel in a non-contact, isothermal process. Acoustic energy has been utilized for many years for a variety of diagnostic, therapeutic and research purposes. There are also specific clinical examples of the utilization of high intensity focused, therapeutic ultrasound (e.g. lithotripsy) and of low intensity, focused diagnostic ultrasound (e.g., fetal imaging).

 The physics of ultrasonic energy is well understood; however, the biophysical, chemical and mechanical effects on biological systems are often only empirically understood. Well-known uses of sonic or acoustic energy in materials processing include "sonication", an uncontrolled process of mechanical disruption involving the direct immersion of an unfocused kHz range (i.e., 15,000 cycles per second) ultrasound source into a fluid suspension of the material being treated.

Typically, when ultrasound is applied to a bulk biological sample solution (as for the extraction of intracellular constituents from tissue), the treatment causes a complex, heterogeneous, mixture of sub-events that vary during the course of a treatment dose. In other words, the ultrasonic energy effects may change between various spatial and temporal states during the course of a treatment. For example, the energy may directly treat a sample; the energy may displace the target and shift the target out of the optimal energy zone; the energy may result in interference, i.e., a "bubble shield", that reflects the acoustic energy; or the large particles may move to low energy nodes thereby leaving the smaller particles with more dwell-time in the high energy nodes. In addition, the sample viscosity, temperature, and uniformity may vary during the ultrasonic process, resulting in gradients during the process. The current low frequency, unfocused sonicator-type processes are random and non-uniform. This limits the use of ultrasound in applications where treatment standardization is required.

In contrast, with the Covaris Process, focused acoustics are computer generated and delivered to individual samples with the *S2x system*. The mechanical energy imparted on the sample results in a controlled series of compression and rarefaction events. The S-series instruments were designed to provide the researcher with methods and systems to control the delivery of mechanical energy into either a chemical or biological sample for single sample preparation applications.

2.0   UNPACKING AND INSTALLATION

2.1   Unpacking and Handling

The Covaris system is shipped in one box containing the instrument, the instrument supplies, and an optionally supplied computer. Remove all wrappings, taking care not to inadvertently discard any parts, accessories, or documents.

Normal precautions in unpacking and reasonable care in handling should be exercised to avoid possible damage to the unit. Visually inspect all external controls, indicators, and surfaces to detect any damage that may have occurred during shipment.

Included in the shipping box:

- S2x apparatus
- Two hoses with connectors for recirculating chiller/heater
- USB cable
- Power cord for S2x
- Software CD (SonoLAB and drivers) and User Manual
- Water tank
- Notebook computer (optional)

The shipping company is responsible for damage to equipment during shipment. If damage has occurred, notify the shipping company immediately to establish proper basis for a claim.

If the recirculating chiller/heater was ordered from Covaris it is shipped in a separate box.

> NOTE: In case of damage do not discard packing material until Covaris has inspected for cause of damage.

2.2   Placement of Equipment

The area required for the instrument (see Figure 1) is approximately 8" wide by 22.5" deep by 13" high (20cm x 54.6cm x 33cm). Additional space is required for a user-supplied chiller.

To accommodate the input and exhaust of an internal fan, place the instrument so that there is a minimum of 1" (3 cm) between the instrument and any wall or obstruction.

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> CAUTION: The heat sink located at the rear of the instrument gets very hot.

2.3   Power Requirements

The instrument draws a maximum of 500 VA. The Covaris S2x instrument is configured with a universal power supply. Refer to label on backside of instrument for specific power requirements.

> WARNING: **To prevent the possibility of electrical shock, always plug the system into a grounded power source.**

2.4   Set-up Procedures

2.4.1   Connections

Connect the computer to the instrument via the USB cable attached to the back of the instrument.

Ensure the user-supplied chiller is connected to the CHILLER IN and OUT ports on the back of the instrument. Slide the ends of the hoses over the inlet and outlet nozzles at the rear of the chiller, and tighten the hose clamps with a screw driver.

2.4.2   Powering Up the System

Power on the Covaris system with the switch located on the front of the instrument; it will display as Red when "ON". Power on the computer and open the SonoLAB Single™ software by double clicking the SonoLAB Single icon on the desktop.   It may take up to 20 seconds for the USB link to be established.

> NOTE: If the computer is supplied by the user, follow the software installation procedure in Appendix B.

> NOTE: Do not disconnect the USB cable or power off the S-series instrument while the SonoLab software is running. Loss of communication with the instrument may cause the software to become unresponsive, forcing the user to close the application via Windows Task Manager.

2.4.3   Acoustic Assembly

The Acoustic Assembly houses the sample holder for the tissue to be processed, the sample cover with a safety interlock that turns off the acoustic power when raised, the water bath cooling loop, and the transducer. The entire arm is pivoted up to remove the assembly from the water bath.

To access samples for loading/unloading, simply lift the sample cover up.

> **WARNING:Only the Covaris approved holder (THQ-20) for 20x125mm closed tubes is to be used with this instrument. Modification of or use of non-authorized holders will void any warranty and may present a hazard to the operation of the system.**

2.4.4   Water Bath Configuration

Fill the water bath to the level indicated on the tank. The water level will rise when the arm is lowered. Only distilled or deionized water should be used to fill the water bath. Once the transducer is submerged, the final check can be made to determine the height of the water in the water bath (see Section 3.2.3).

> **CAUTION:**   Using anything besides distilled or deionized water in the water bath is a misapplication of the equipment and will void the warranty.

3.0   SYSTEM DESCRIPTION

3.1   General Overview

The *Covaris S2x* is a precision instrument for controlled single sample preparation for samples larger than masses typically used in the S2. (See Appendix A). The system is comprised of the Treatment System which delivers the energy to the sample, the Safety System which protects users from accidental contact with the acoustic energy, and the Computer and the Application Software which provide the user interface to the system.

This instrument is a very powerful, high-energy system. It is designed to disrupt and homogenize biological tissue, extract biomolecules, and accelerate chemical dissolution. Figure 2 depicts the Acoustic Assembly and the Sample Holder.

Sample Cover

Acoustic Assembly

Figure 2

3.2   Treatment System

3.2.1   Acoustic Transducer

The acoustic transducer delivers a high intensity, focused, computer-controlled acoustic shock wave to a sample as the "treatment".   One to four of these "treatments" can be combined together as a "method" that is used to process samples.

> **WARNING: Objects in the focal point of the transducer will be exposed to high energy when the system is activated.**

3.2.2   Temperature Sensors and Temperature Control

The water bath sensor, a 5 cm metal probe, monitors the temperature of the water bath. The water bath temperature limits are 5º C and 40º C. The water bath temperature is controlled by hooking up an external recirculating chiller/heater to the ports in the back of the system. The recirculator will pump a temperature-controlled fluid through a stainless steel loop immersed in the water bath. The user may set a maximum bath temperature in the user software to any value between 5º C and 40º C. If the bath temperature exceeds this limit, an indicator will flash on the software control panel and a fault notice will be posted to the user.

NOTE: Although the S2x reports the bath temperature via the Temperature Sensor, it does not control the recirculating chiller. Typically, for most applications (e.g., gene expression, proteomics, compound dissolution, and drug metabolism studies) the temperature of the recirculating chiller is set to 15 degree C with the temperature of the water bath equilibrating at 18 degree C and the software temperature alarm set to a value slightly higher (e.g. 20 degrees C).

CAUTION: A recirculating chiller is required for efficient and controlled operation of the S2x instrument.

3.2.3   Water Bath and Degassing System

The water bath holds approximately 0.5 gallons (1.6 liters) of distilled or deionized water. Fill the water bath according to the water level indicator on the front of the water bath using the "FILL" column, as shown.

The water level will be dictated by the application. If Covaris has provided an Instruction for Use (IFU) for your application, please refer to it for the recommended water level. If no water level is specified, fill the water bath to the "0" level.
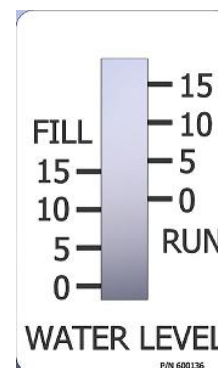
CAUTION: Do not overfill as the water may overflow during set-up operation. The water level will rise about ½ inch (1.3 cm) when the transducer is submerged.

The degassed, temperature controlled water provides an efficient energy couplant from the transducer to the sample to be processed. The water bath must be degassed for proper operation of the instrument. The degassing system is engaged by pressing the DEGAS "ON" button on the computer screen when the transducer is in the lowered position. The degassing system also circulates the water past the sample vessel being processed.

The degassing system consists of a dip tube with six small holes, a positive displacement pump, and an outlet nozzle. Dissolved gasses and vapors are reduced in the water by pulling the water through the small holes in the dip tube and pumping the resulting coalesced bubbles out the outlet nozzle before they can re-dissolve. During normal operation, there should be an intermittent stream of bubbles coming from the hole at the end of the outlet nozzle. No bubbles indicate that the six small holes in the dip tube may be plugged, typically if the water bath becomes contaminated with particulate materials. If this occurs, the dip tube may be removed and the holes cleared with a fine wire (e.g., 26 Gauge needle).

CAUTION: The pump should not be run with the acoustic assembly raised or for a prolonged period with a fault condition present in the system. The degassing pump will turn off after 10 seconds if the water tank is not filled with water to the fill line.

3.2.4   Computer

The computer uses the Microsoft™ Windows® Operating System with SonoLAB Single application software.

> NOTE: Power on the S2x instrument before starting SonoLab Single Software. Communication via the USB cable must be established with the instrument for the software to operate.

> NOTE: Any changes made to either the application software will void the warranty. Please contact Covaris Technical Support for further information.

3.3 Safety System

The safety system protects the user from hazards associated with the acoustic treatment system.

The safety system prevents generation of acoustic power if either the sample cover is open or the Acoustic Assembly pivot arm is not down. It will also ensure that the correct water level is maintained in the water tank. A "FAULT" dialog box will appear when there is a problem with the operating condition as in Figure 3. Before selecting RESUME, the "fault" condition must be resolved or the fault box will reappear. Selecting ABORT will end the method and return to the MAIN PANEL.


Figure 3

The water level sensor in the water bath detects that the appropriate water level is maintained. Incorrect water levels will cause a water level fault dialog box to appear and the acoustic power will not come on.

3.4 Application Software

3.4.1 Main Panel Screen

See Software Panel 1: Main Panel.

This screen allows the user to select a previously created method and its associated parameters, go to a configuration screen to edit a method, and to run the method. The method name appears at the top of the screen.
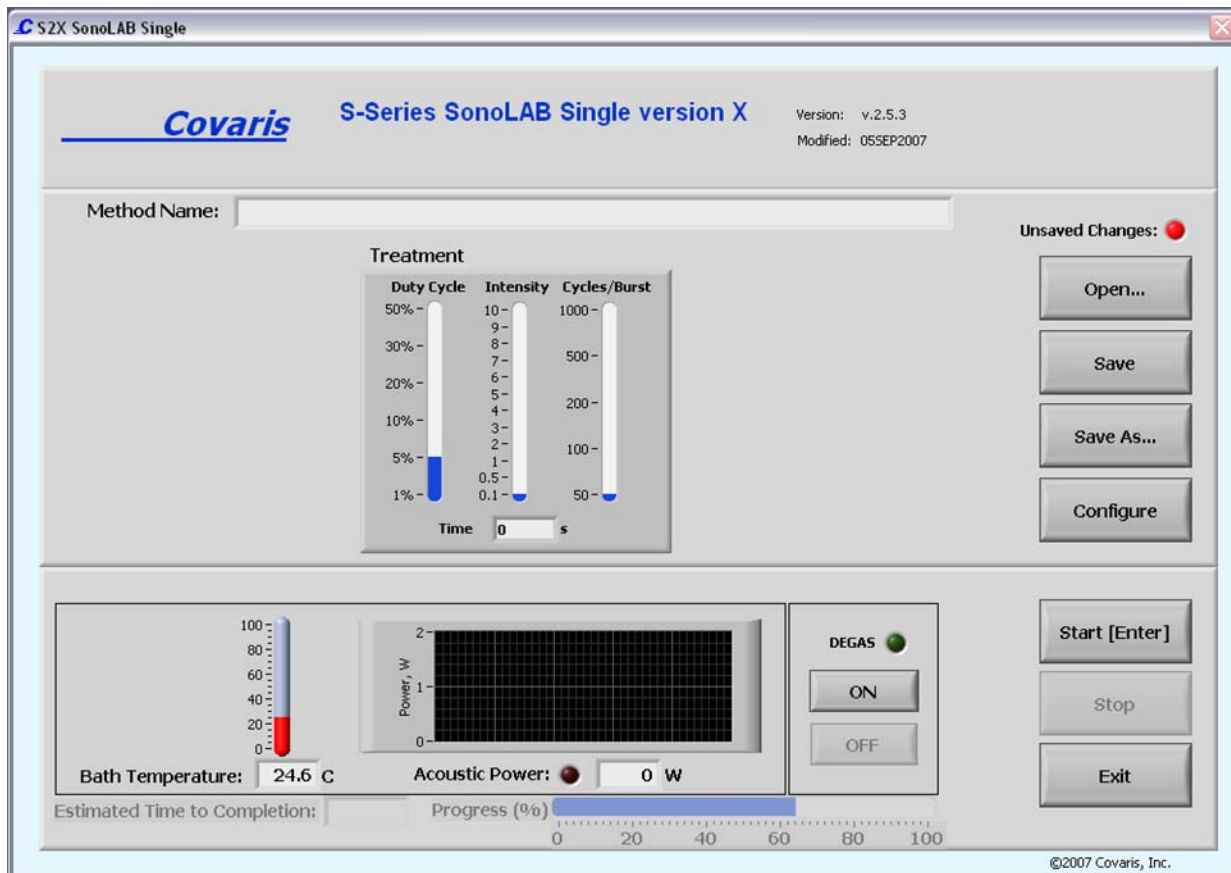
Software Panel 1: MAIN PANEL

A method consists of up to four treatments. This treatment sequence can be cycled from 1 to 999 times. Duty Cycle, Intensity, and Cycles per Burst are acoustic Treatment Parameters. See Section 3.4.2.

The Status Indicators

UNSAVED
CHANGES   changes made to the method have not been saved; the indicator will be red.

BATH
TEMPERATURE   displays the temperature of the water bath and will blink RED if the bath temperature exceeds the limit set for the method.

ACOUSTIC
POWER METER   displays the acoustic power being applied to the sample. Acoustic power reading is displayed in watts below.

PROGRESS   indicates the current method time as a percentage of the total time.

ESTIMATED TIME
TO COMPLETION   indicates the time (in sec) left to finish all treatments in a method.

DEGAS   indicator is green when the degassing pump is on. See Section 3.2.3.

General Operation Buttons

OPEN   allows the user to select a method from the list of previously created methods. The selected method will appear in the method name field.

SAVE   allows the user to save a method set-up to the current method name as a *.pro* file (in the process folder).

SAVE AS   allows the user to save the active or current method to an existing or new filename.

CONFIGURE   opens up the CONFIGURATION PANEL where the user can modify or create a new method. See Software Panel 2: Configuration Panel.

DEGAS ON   only active when the degas pump is off and allows the user to turn the degas pump on.

DEGAS OFF   only active when the degas pump is on and allows the user to turn the degas pump off.

START   initiates running a method.

STOP   is only active after a method begins and allows the user to stop the method at any time.

EXIT   initiates shut down of SonoLAB Single.

3.4.2   Configuration Panel Screen

See Software Panel 2: Configuration

Operating Modes:

Frequency Sweeping Mode: The acoustic frequency sweeps through a range during processing. This mode delivers effective power and good mixing to the sample and is the best for most applications, especially biologically active samples (e.g., RNA extraction from tumor tissues samples or proteomic preparation). This is the default setting for the S-series instruments.

Power Tracking Mode: In this mode, the instrument optimizes the operating frequency to achieve peak power in the treatment vessel for the treatment chosen. This mode may be advantageous when intense power is needed for particular types of samples. This setting may be appropriate for chemical-based samples (e.g., small molecule drug extraction from slow release tablets or metabolite extraction from plant tissue).

CAUTION: When used with some high power treatments and some vessels (e.g., plastic) Power Tracking Mode may result in heating of the sample.
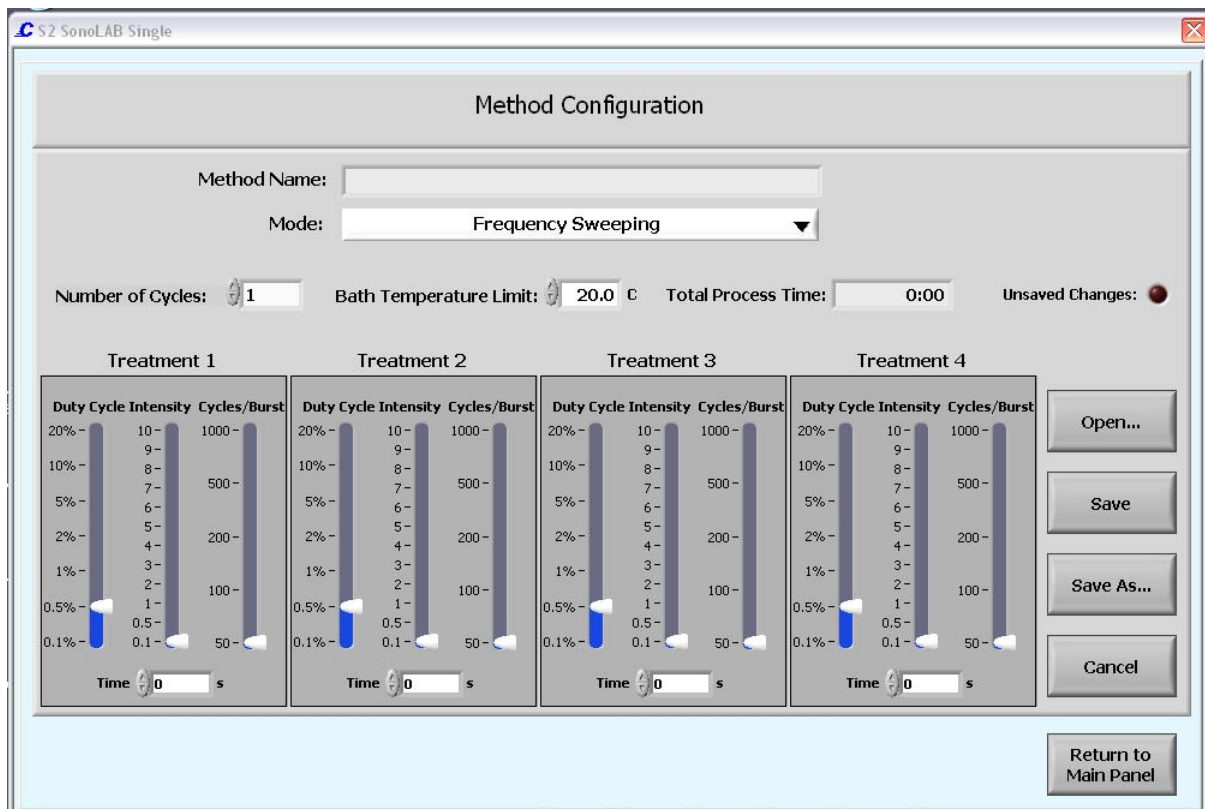
Software Panel 2: CONFIGURATION PANEL

Treatment Parameters:

NUMBER OF CYCLES allows the user to select the number of times to cycle through the treatment steps. 1 to 999 is permitted.

BATH TEMPERATURE LIMIT allows the user to select a maximum temperature limit for the treatment. See Section 3.2.2

TOTAL PROCESS TIME: this is the product of the number of cycles times the total treatment time per cycle

TREATMENT menu allows the user to configure up to four different treatments per method. Select the arrows with the left mouse key and drag to desired position.

DUTY CYCLE is defined as a percentage. For example, within a timeframe it is the percentage of the time that the transducer is creating acoustic waves; the "on" cycle. Typically, the higher the DUTY CYCLE the higher the acoustic waves per unit time is generated by the transducer. Minimum is 0.1% and Maximum is 50%.

INTENSITY: The amplitude of the pressure waves created by the acoustic transducer is proportional to the intensity. Essentially, the larger the number, the more energy each acoustic wave has. Minimum is 0.1 and maximum is 10.

CYCLES PER BURST: The number of pressure waves generated by the transducer in a burst. For example, 100cpb at 10% duty cycle is a wavetrain of 100 "on" energy

cycles followed by 900 "off" energy cycles. This may occur during tissue sample homogenization. Minimum is 50 and Maximum is 1,000.

TIME is the amount of time (in sec) to treat a sample. Each treatment is limited by the control software to 60 seconds.

> NOTE: Although an individual treatment is limited to 60 seconds, the total time may be readily increased by having multiple cycles

The Status Indicators

UNSAVED
CHANGES   changes made to the method have not been saved; the indicator will be red.

General Operation Buttons

OPEN   allows the user to select a method from the list of previously created methods. The selected method will appear in the method name field.

SAVE   allows the user to save a method set-up to the current method name as a *.pro* file (in the process folder).

SAVE AS   allows the user to save the active or current method to an existing or new filename.

CANCEL   removes any unsaved changes.

RETURN TO
MAIN PANEL   closes the method configuration panel and returns to the main panel.

4.0   SYSTEM OPERATION

4.1   Start-Up Procedures

Chiller Connection

A recirculating chiller (required) should be connected to the treatment system using the hoses supplied. The chiller inlet is connected to the fitting labeled "IN" and the chiller outlet is connected to the fitting labeled "OUT" located on the back of the instrument.

Water Bath

The system will determine if there is adequate water in the tank prior to starting a process. When the tank is properly filled, and the transducer is slowly lowered, the water level will be approximately ½ inch (1.3cm) below the rim of the tank. Only distilled or deionized water is to be used in the tank.

Special care needs to be taken when changing the water bath. First, verify the degassing pump has been turned "off". Then grasp the front handle and slowly lift the acoustic assembly arm to raise the transducer. Then carefully pull out the acrylic water tank from the instrument base plate, empty, and wipe dry. Fill the water tank to the fill line on the rear of the tank and place it back into its position, ensuring that it is oriented properly in the instrument.

> NOTE: Raise and/or lower the Acoustic Assembly slowly to avoid

disturbing the water in the bath.

Degassing

Prior to running a process, degas the water bath for ½ hour (30 minutes). Degas the system by selecting the degas button on the computer screen. An indicator on the screen will appear in green to denote the pump is degassing the system.

NOTE: The degassing pump will turn off after 10 seconds if the water tank is not filled with water to the fill line.

Loading the Sample Holder

Raise the sample holder cover. Only one sample is loaded. This cover ensures that the vessel stays in place for correct processing.

NOTE**:**   Ensure sample is seated flush onto the holder so that it is correctly aligned with the focal point of the transducer.

Processing the Sample

Press the START button on the computer screen.

After selecting the START button, a User Alert dialog box opens to ensure the system is ready to proceed. The warning reminds the user to check that there is water in the water bath, that the system has been degassed, and that there is a sample in the sample holder. See the warning screen in Figure 4.
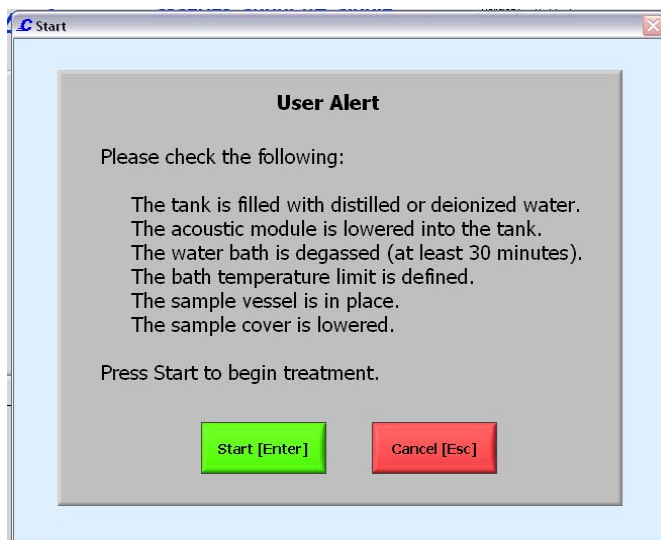

Figure 4

CAUTION: Never run a method without a water bath; this will permanently damage the transducer.

After selecting START, the following User Alert dialog box will appear if the degassing pump has not run for 30 minutes.
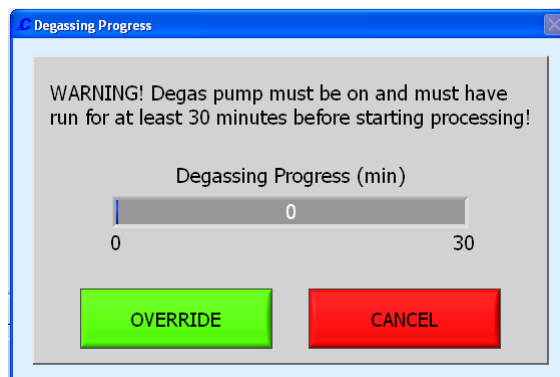

Figure 5

Either CANCEL and run the pump for additional time or OVERRIDE the warning and continue processing. The method will run until completion unless the STOP button is pressed.

NOTE**:**   Do not lift sample cover to stop the method with multiple treatments. To keep the treatment times accurate, use the STOP button instead.

4.2   Shutdown Procedure

To shut down the instrument, turn off degassing and raise the acoustic assembly to lift the transducer out of the water.

CAUTION: Failure to raise the transducer from the water bath when not in use may result in permanent damage to the system.

If the instrument will be idle for an extended time, the degassing lines should be purged. Empty the water tank. Replace the water tank and lower the arm. Press the Degas button on the computer screen, run the pump for 5-10 seconds, and then shut it off. Then empty the water bath again and wipe the tank dry with a lint-free cloth.

CAUTION: Leaving water in the water bath or degassing lines may allow biological (e.g., algae) growth in the tank and lines which will interfere with sample processing.

Exit the SonoLAB Single software by closing all screens and selecting EXIT on the Main Panel. See Figure 6. Power off the instrument after the SonoLAB Single software has closed. Power off the instrument with the switch located at the front of the instrument.

To shut down the laptop, select Shutdown from the START menu on the Windows XP screen and select the option SHUT DOWN THE COMPUTER. Then computer will power down.

Figure 6

5.0   **SYSTEM SPECIFICATIONS**

Model	S2x
Treatment System	Bench-top: high intensity acoustic transducer, temperature monitoring device, circulation pump, and water bath with safety enclosure.
Dimensions:	8" W x 22.5" D x 13" H (20cm x 54.6cm x 33cm)
Weight:	Weight: approximately 30 lbs (13.6 Kg)
Power Requirements:	100-240 VAC 500 VA, 50-60Hz, 5A fuse
Ambient Temp. Range:	15 to 32°C
Regulatory Labeling:	CE and ETL Mark
Safety:	Meets Low Voltage Directive 2006/95/EC.
	Tested to EN/UL/CSA 61010-1:2004 "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements"
EMC:	Meets EMC Directive 2004/108/EC. Tested to EMI emissions requirements for Industrial Scientific or Medical (ISM) equipment EN55011:1998 + A2:2002 Class A Group1 and EMC requirements - Electrical Equipment for Measurement, Control, and Laboratory Use EN 61326-1:2005 Table 2, EN 61000-3-2:2004 and EN 61000-3-3:1995 +A1:2001.
Water Bath:	Distilled or deionized water only
Water Temp. Alarm Limit:	Can be set at +5.0°C to +40.0°C
Computer:	Notebook or desktop computer. Optionally supplied by Covaris
Operating System:	Microsoft Windows XP
Application Software:	Covaris SonoLAB Single
Data Input:	Keyboard, mouse
Chiller:	Chiller/heater re-circulating system - not included
	Connect with the 3/8 inch I.D. hoses and quick connect fittings supplied

6.0   MAINTENANCE

6.1   Air intake

Periodically, unplug the unit and clean the air intake to ensure that dust or dirt is not restricting the flow of air for component cooling. Otherwise, maintenance of the instrument should be performed under the direction of Covaris personnel.

6.2   Water Bath

6.2.1   Recommendations for Daily Maintenance

Only distilled or deionized water should be used to fill the water bath. The water bath should be emptied every day to avoid algae growth. Do not use an algaecide.

The water level is dependent upon the sample vessel type. There is a water line in the tank to help determine the depth required. Refer to the technical notes appropriate to the sample vessel to determine water depth.

6.2.2   Recommendations for Monthly Maintenance

Periodically, the water bath and degassing lines may be rinsed with a 10% solution of Clorox bleach. The resultant sodium hypochlorite solution is ~ 0.5% NaOCl. With this solution in the water bath, lower the transducer and run the degassing pump for a few minutes. Repeat with fresh water in the water bath.

6.3   Transducer

When not in use, the transducer and acoustic assembly should be raised out of the water bath. Keeping the transducer dry between applications will help prevent metal corrosion and is strongly encouraged to ensure optimal performance.

Use care when handling the transducer. Permanent damage could result if the transducer is dropped, if anything is dropped onto the face of the transducer, or if the face of the transducer strikes something.

Permanent damage could also result if the transducer is overheated, i.e. running the transducer without a water bath.

> **WARNING: Do not attempt to remove the transducer as irreparable damage may occur.**

6.4   Safety System

Test the system periodically. Make sure that the acoustic power does not go on when the sample cover or acoustic assembly arm is raised, or if the water level in the tank is too low.

> **WARNING: Any failure of the Safety System must be reported immediately to Covaris. DO NOT attempt operation if the Safety System is malfunctioning.**

6.5   Pump, Hoses, and Cables

The degassing pump should not be run without a water bath. This will cause the pump head to wear out.

Check that there are bubbles coming from the end of the outlet nozzle. If there are no or few bubbles present, remove the dip tube, by pulling downward, and examine it under a microscope to clear any clogged holes (e.g., small pin or purge with compressed gas). Reinstall it by pushing up into fitting. Be sure the tube is fully seated in the fitting.

For long-term storage, water should be removed from the degassing pump lines. Follow shutdown procedure in Section 4.2.

6.6   Relocation of the System

Should it be necessary to move the instrument, for whatever reason, the system needs to be properly prepared. Prior to this movement, make sure all steps on the following checklist are completed.

- Follow the shutdown procedure in Section 4.2.

- Lower the Acoustic Assembly to the DOWN position.

- Save all files and close the SonoLAB Single software by closing all screens and selecting EXIT on the main panel. Select SHUTDOWN in the dialog box (see figure 6).

- Power down the instrument by moving the power switch to the OFF position. The power switch is located at the front of the instrument, on the top, on the right side.

- Select Shutdown from the START menu and make sure the option to SHUTDOWN is selected. The computer will power down. Turn off the monitor.

- Disconnect the cable from the computer to the instrument.

- Remove the water tank. Secure the acoustic assembly by wrapping tape around the base and over the sample holder.

- Cover the system and computer with a light cloth or plastic to prevent dust contamination.

6.7   Cleaning the System

Clean the external surfaces of the equipment as necessary with a damp lint-free cloth. Rinse the water tank with clean water and wipe dry with a lint-free cloth.

7.0   TROUBLESHOOTING

System Trouble Analysis Chart

SYMPTOM	PROBABLE CAUSE	POSSIBLE SOLUTION
1. Degassing system not generating bubbles. 2. Samples take longer to process 3. Process is noticeably noisier 4. Repeated "water bath fault" messages	1. Degassing dip tube may be clogged 2. Pump not working	1. Remove and back flush under running water. 2. Examine the six holes (e.g., using a stereo microscope) to see if clogged and use a pin to unclog them and/or purge with compressed gas. 3. If pump does not run, contact Covaris
Safety interrupt panel is displayed	1. Water tank is misaligned or in backwards 2. Cover is not closed completely 3. Water level is too low	1. Check tank alignment, fill lines should be at front of tank when installed correctly 2. Fill water tank to the fill line
Instrument unable to communicate with hardware	1. Computer was turned on without instrument on or connected 2. Cable is loose or damaged	1. Turn on instrument and select retry button 2. Check the cable
Software Application is unresponsive	1. The USB becomes disconnected while the Application is running 2. The S-series is turned off while the Application is running 3. The S-series is turned on when the laptop goes into standby or hibernate modes	1. Reboot the computer 2. Terminate the Application; may need to use Windows to terminate 3. Turn off S-series instrument when the SonoLAB software is closed or See appendix B for standby and hibernate mode settings
Water sensor comes on when water tank is correctly filled	Acoustic Assembly not fully lowered	Verify Acoustic Assembly is down
Error message that system not shut down correctly	SHUT Down command in Windows XP not used before rebooting	1. Follow instructions on screen 2. Use SHUT DOWN command

APPENDIX A

Sample Preparation Requirements

Although sample preparation requirements are diverse, for most samples the following ranges apply:

Model S1 -   volume   100 – 1,000 µl
                 mass   < 100 mg
                 tube   13 x 100 mm

Model S2 -   volume   1,000 – 10,000 µl
                 mass   < 1,000 mg
                 tube   16 x 100 mm

Model S2x -   volume/mass: typically larger than those used with the regular S2, however, exact size depends on the tissue and buffer composition
                 tube   20 x 125 mm

The system may also be configured for other tubes and vials, temperatures, and robotic systems. Please contact Covaris for any additional questions or comments regarding sample processing.

APPENDIX B

Procedure for a *new* install USB drivers and Covaris SonoLab Single™ software on a Windows XP computer

NOTE:	Follow the instructions in sequence.

1. Begin with the USB cable disconnected and the instrument powered off.

2. Load the INSTALL CD onto the Laptop's CD-Drive. The SonoLAB installer will start in about 30 seconds.

3. A standard Windows install wizard will run. Click Next > to proceed. The SonoLAB and NI-VISA software will install automatically. Click Finish to complete.

4. When installation is complete, leave the CD in the CD-Drive. Turn on the instrument and plug the USB cable into a USB port on the computer.

5. The Windows hardware Install Wizard will start. Choose "No, not this time" when queried about connecting to Windows Update, and then choose Automatic installation. If Hardware Installation warnings appear, click "Continue Anyway" to proceed with the installation.

NOTE:	The hardware install Wizard will run twice.

6. When the driver installation is complete, the system should be ready to use.

7. For convenient usage, find the executable file under c:\Covaris\S-Series v2.xx\Program and create a shortcut to the desktop (Do not drag executable file to the desktop, just "create shortcut".

8. Double click on the shortcut to start SonoLab Single™.

> **NOTE:** If the software is being installed on a computer already running a previous version of SonoLAB Single™, only the SonoLAB installation is required. The NI-VISA and driver installations will not be necessary. Details can be found in the ReadMe file of the install CD. Contact Covaris for assistance.

> **NOTE:** Laptop standby and hibernate modes should be avoided while operating the SonoLAB Single software. To ensure this, from the start menu, open Control Panel, then Power Options. Select a Power Scheme that sets System standby and System hibernate times to Never. If standby and hibernate modes remain enabled, then the SonoLAB Single software should be closed and the system powered down whenever the system is not in use.

> **NOTE:** SonoLab Single software was not tested with other application software and therefore we recommend that no additional application software is loaded. Wireless Network Connections on the Laptop should be disabled to avoid loading of any unwanted software. Go to Network Connections in the Control Panel. Highlight the Wireless Network Connection and select "disable this network device".

APPENDIX C

References

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety
- Botwell, D. and Sambrook, J. (2003) DNA Microarrays, A Molecular Cloning Manual, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, NY.