

For research use only

E-series
USER MANUAL

an automated, batch system for
high performance sample preparation
using adaptive focused acoustic energy

Product Name: E-series

Product Number: E110, E210



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

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Covaris, Inc.
14 Gill Street, Unit H
Woburn, Massachusetts
01801-1721 USA

Printed in U.S.A.

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 without the safety door closed. When the safety door is open, the mechanical positioning system and the acoustic wave 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 instrument is 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 temperatures.
2. 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.
3. ONLY distilled or deionized water should be used to fill the water bath.
4. 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. Drain the degassing lines (see section 4.5 Shutdown Procedure).
5. Establish a standard of operation and periodically test equipment, as described in the Maintenance section of this manual.
6. Be advised that loading third party software onto the computer may interfere with the performance of the Covaris SonoLab™ software. Please consult 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. 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, 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 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.

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,
 - United States, Eastern Standard Time (EST)
 - Greenwich Mean Time (GMT-05:00)
- E-mail queries to techsupport@covarisinc.com

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

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1.0 INTRODUCTION

1.1 Overview of the Manual

This manual contains operation and maintenance instructions for the *Covaris E-series* instruments (the E110 and E210). It contains background information essential to the proper use and care of this equipment.

The following definitions apply in this manual:

NOTE: Inconvenience only if disregarded – no damage or personal injury

CAUTION: Equipment damage may occur, but not personal injury.

WARNING: Personal injury may occur – DO NOT DISREGARD.

There is also a PDF version of this manual on the system computer (if a computer was supplied with your order). Open the manual by double clicking the manual icon on the desktop.

1.2 Purpose of the Equipment

The E Series instruments are used for high-throughput, sample preparation and extraction. The technology uses controlled and highly focused nonlinear acoustic waves that are delivered to each sample. The systems are designed to work with a variety of closed or open sample vessels including vials, tubes, and multi-well plates. In general, the E110 is best for samples less than 50 mg and volumes less than 0.5 ml and the E210 is most appropriate for samples less than 500 mg and volumes less than 5 ml. These ranges may be increased or decreased depending upon buffer composition and sample/buffer ratios (contact Technical Support for more information).

CAUTION: Processes developed and optimized in the E110 system are not necessarily transferable to the E210 system. Contact Covaris Technical Support for help.

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

CAUTION: The system is designed to work primarily with closed sample holders. Open vessels may be used; however, the energy should be dramatically reduced as there is potential for leakage and spilling into the water tank. Please contact Covaris Technical Support for guidance in this area if open vessel processing is required.

1.3 Main Components

The main components of the E Series instruments include:

- acoustic transducer
- temperature sensing device
- sample positioning system
- degassing pump
- water bath
- user manual

External components include a notebook computer.

The user is expected to provide a recirculating chiller/heater. Covaris can provide specifications for recommended recirculators.



Figure 1

1.4 Principle of Operation

The Covaris Process generates acoustic shock waves to cause controlled cavitation and acoustic streaming at the focal point within the sample treatment vessel. Acoustic energy has been utilized for many years for a variety of diagnostic, therapeutic and research purposes. 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 (e.g., 15,000 cycles per second) ultrasound source into a fluid suspension of the material being treated. There are also specific clinical examples of the utilization of therapeutic ultrasound (e.g. lithotripsy) and of diagnostic ultrasound (e.g., fetal imaging).

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 may be partitioned between various states. 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 will 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 high throughput applications where treatment standardization is required.

In contrast, focused, nonlinear acoustic waves are computer generated and delivered to individually-addressable samples with either of the *E* series systems: E110 and E210. The mechanical energy imparted on the sample results in a controlled series of compression, rarefaction events. The *E-series* instruments were designed to provide the researcher with methods and systems to control the delivery of mechanical energy into a biological sample for high-throughput sample preparation applications.

2.0 INSTALLATION

2.1 Unpacking and System Setup

The Covaris system is shipped in one crate, containing the instrument, the instrument supplies, optionally purchased notebook computer, and the selected accessory kit. 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.

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.

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

WARNING: Risk of back injury. The instrument weighs approximately 110 pounds (50 kilograms).

2.2 Placement of Equipment

The area required for the instrument (see Figure 1) is approximately 24" wide by 30" deep by 19" high (61 cm x 76 cm x 48 cm). Space on, under or adjacent to the bench is required for a notebook computer. Additional space may be required for a user-supplied recirculator.

Two fans maintain a safe operating temperature in the instrument by circulating air over the internal components. Therefore, place the instrument so that there is a minimum of 1" (3cm) between the rear panel and any wall or obstruction for airflow.

2.3 Power Requirements

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

In case of a power interruption, the system will halt. The equipment will then need to be restarted. As files or data not saved prior to the power interruption will be lost, the use of an UPS (Uninterrupted Power Supply) is recommended with this system if your site has frequent power surges or loss of power. The UPS should be capable of supplying full 5 amperes at the voltage used.

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 Powering Up the System

Power up the Covaris system by moving the power switch to the "1" position. If the computer is supplied by Covaris, turn it on. Open the SonoLab software by double clicking the SonoLab icon on the desktop.

NOTE: If user is supplying a computer, see Appendix B for instructions on loading drivers.

2.4.2 Connections

Ensure that the user-supplied chiller is connected to the CHILLER IN and OUT ports on the left side of the instrument using the Covaris supplied hoses. The hoses are setup with quick disconnect fitting to be attached to the instrument and hose clamps for the user supplied chiller

Ensure there is a USB cable connected from a USB port on the computer to the USB port on the instrument (located above the power switch on the right side, near the back of the instrument).

2.4.3 Control Buttons

There are three control buttons located on the right side of the instrument as shown in Figure 2. The white DEGAS PUMP button is used to degas the water bath prior to sample treatment processes. See section 3.2.4. The red STOP button can be depressed at any time to halt operation. The green DOOR button, when lit, can be depressed to allow the operator to open the safety enclosure door (See section 3.4).



Figure 2

2.4.4 Water Bath Configuration

The water bath tank needs to be oriented properly when inserted into the system. The bath should be oriented with the label "Insert this end" at the back of the tank (see Figure 3). The "waterline" label will be displayed on the front side of the tank.



Figure 3

CAUTION: The magnetic safety interlock will not allow the instrument to operate if the water tank is not oriented properly and fully inserted.

Fill the water bath to the water level indicated on the left side (FILL) of the water level label (see section 3.2.4). The water level will rise when the transducer is submerged. Only distilled or deionized water should be used to fill the water bath.

CAUTION: Never run a process without a water bath. This will permanently damage the transducer.

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

The system includes a water level sensor. If the water level is too low, a warning message will be issued by the software. In addition, the degas pump will not operate for more than 10 seconds if a low water level is detected. This is to protect the pump from running without water.

Prior to running a process, the water bath should be degassed for 45 minutes. Degas the system by lowering the transducer (click LOAD SAMPLE button on the MAIN PANEL of SonoLab) and then depress the DEGAS PUMP button. (This is the white button on the right side of the safety enclosure (see Figure 2)).

NOTE: The DEGAS PUMP button will not engage the degassing pump if the transducer is in the CHANGE BATH position

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

NOTE: If the degas pump switch is turned on or off while a process is running, the action is delayed until the process has ended.

3.0 SYSTEM DESCRIPTION

3.1 General Overview

The *Covaris E110 or E210*, as shown in Figure 1, is a custom instrument for high-throughput sample preparation and compound management. The system is comprised of the Treatment System which delivers the energy to the sample, the Safety System which provides intelligent interruption during the “treatment”, and the Computer and the Application Software which provide the user interface to the entire system.

This instrument is a very powerful, high-energy system. It is designed to disrupt and homogenize biological tissue, extract biochemicals, and accelerate reagent dissolution.

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. This is called the “treatment”.

WARNING: Objects at 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 5cm metal probe, monitors the temperature of the water bath. The water bath temperature limits are 5° C and 40° C. The water bath temperature is controlled by connecting 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.

NOTE: Although the E-series instrument 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.

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

3.2.3 Sample Positioning System

The major components of the system include:

- The sample positioning system (x, y, z axes)
- The transducer positioning system (z' axis)
- The sample holder plate

Figure 4 shows the sample holder plate with the A1 position identified.

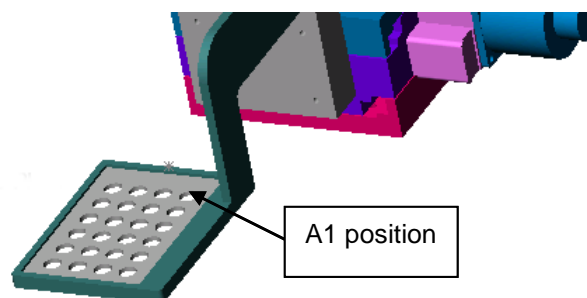


Figure 4

CAUTION: To ensure good system alignment, re-home the system daily by either restarting SonoLab or by using Process >> Initialize (Home) Motion System pull-down menu feature.

3.2.4 Water Bath and Degassing System

The water bath holds approximately 1.3 gallons (4.92 liters) of distilled or deionized water. The water level is indicated by a label on the front wall of the water bath. The left side of the label (“FILL”) indicates the water level when filling the bath. The right side of the label (“RUN”) indicates the water level when the transducer is submerged.

The water level will be dictated by the tube rack. If Covaris has provided an Instruction for Use (IFU) for your tube rack, please refer to it for the recommended water level. If no water level is specified, fill the water bath to the “0” level.

The water bath has double walls for insulation.



WARNING: The water bath is heavy when filled. Use caution when moving it.

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 depressing the DEGAS PUMP button on the right side of the instrument when the transducer is in a lowered position. The degassing system 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 outlet nozzle. If no bubbles are visible for several minutes, the six small holes in the dip tube may be plugged. If this occurs, the dip tube may be removed and the holes cleared with a fine wire (e.g., 26 Gauge needle).

3.3 Computer

The computer uses the Microsoft™ Windows® XP Professional Operating System with SonoLab™ application software.

3.4 Safety System

The safety system protects the user from hazards associated with the acoustic treatment system and the moving parts in the sample positioning system.

The safety system prevents operation of the instrument if the door is open or the water tank is not in place. The door can only be opened when the green light on the DOOR button is lit. Once lit, pressing the button will allow the door to be opened. There is also a red STOP button on the right of the safety enclosure (see Figure 2). Depressing the STOP button halts all operations and the Stop Button Dialog Box comes up which allows the user to either Resume or Abort the current operation as shown in Figure 5.

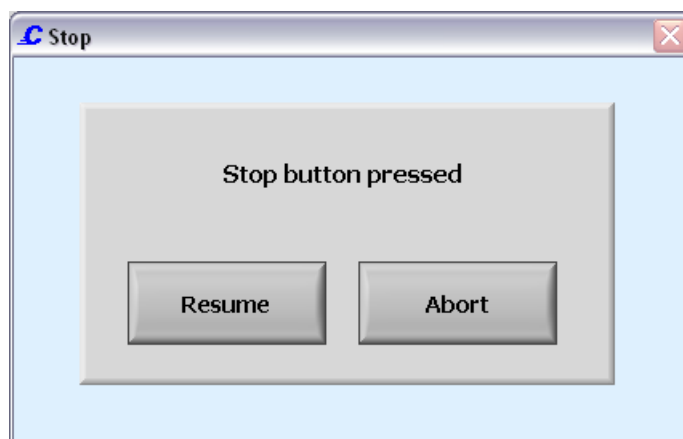


Figure 5

NOTE: The door may be opened in the event of a power failure by inserting a special tool provided with the instrument into the hole in the enclosure above the safety interlock and rotating clockwise 90 degrees. The instrument will not operate if the safety interlock is disabled. The tool is stored in a clip on the back of the instrument.

3.5 Water Bath Quality

The SonoLab Software can detect problems with the water bath that may impede the delivery of acoustic energy to the sample vessel. This feature is valid only for high power applications using round-bottom glass tubes such as 16 x 100 mm culture tubes. Water Quality Monitoring is turned off by default. It may be activated in the process configuration panel. If water quality monitoring is active and there is a problem with the water bath, the dialog box in Figure 6 will appear during the process. (See section 3.6.2).

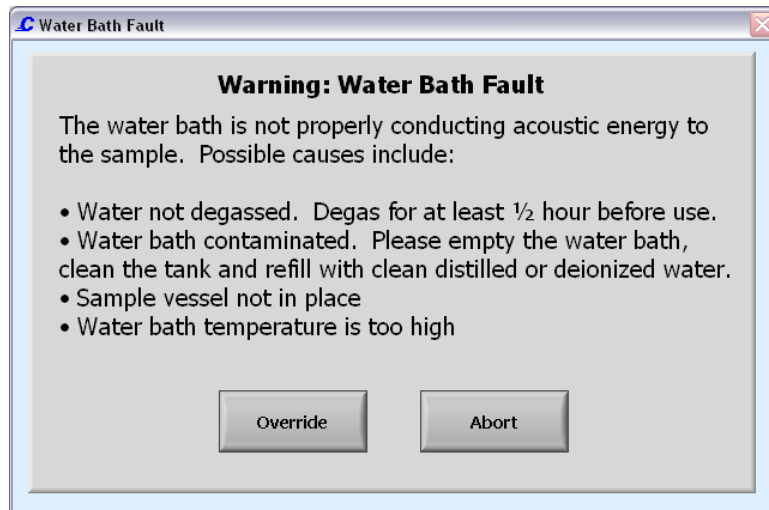


Figure 6

Resolve the problem and run the process again. The software also monitors water bath temperature. If the bath temperature is higher than a user defined limit, while a process is running, the process is halted and the following dialog box will appear.

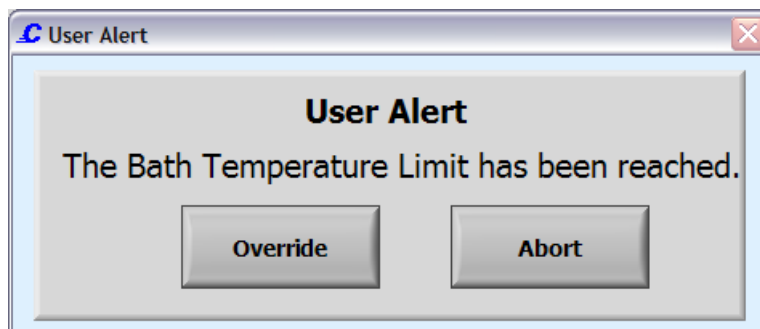
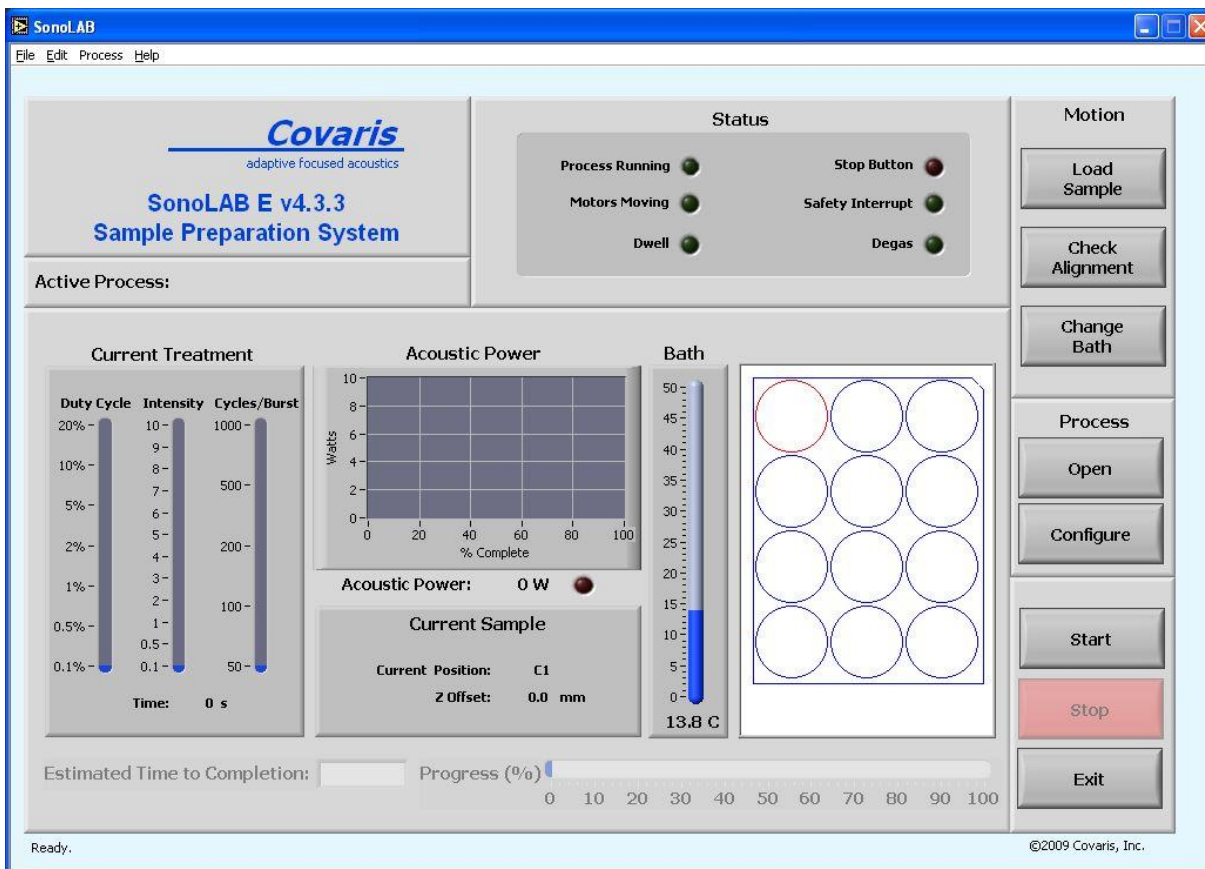


Figure 7

3.6 Application Software

3.6.1 Main Panel



Software Panel 1: MAIN PANEL

Motion Buttons

The motion buttons move the sample holder and the transducer. They operate as follows:

LOAD SAMPLE raises the sample holder out of the water bath

CHECK ALIGNMENT moves the sample holder plate to position A1

CHANGE BATH raises the transducer and sample holder out of the water bath

General Process Buttons

OPEN button loads an existing process configuration from a saved file.

CONFIGURE button will take the operator to the *Process Configuration Panel* which allows the user to create and/or modify a process.

START button starts running a process. After selecting the button, a warning 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 are samples in the sample holder. See the warning screen below:

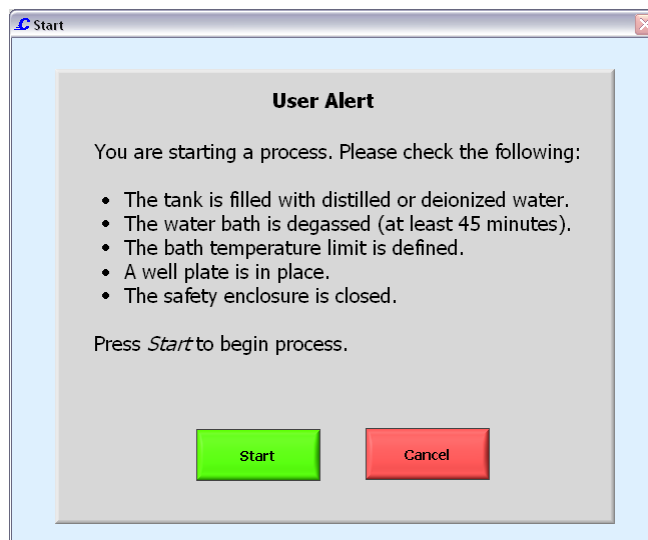


Figure 8

If the user is ready to proceed, click the START button.

STOP halts the operation of the sample positioning system.

NOTE: The STOP button on the instrument and the STOP button on the display perform the same function.

The EXIT button will exit the program.

The Status Indicator Lights

PROCESS RUNNING indicates the process has been started has not been stopped or paused and has not yet completed.

MOTORS MOVING indicate the positioning system is moving in any axis.

DWELL indicates when the stage is at the center of the well in between dithering functions.

STOP BUTTON indicates when STOP button is pressed.

SAFETY INTERRUPT indicates the software senses that the mechanical safety interlock is in the open position.

DEGAS indicates the degassing pump in engaged.

While a process is running, the following indicators display current treatment parameters:

BATH displays the temperature of the water bath.

CURRENT TREATMENT displays the parameters DUTY CYCLE, INTENSITY, and CYCLES/BURST defined for the current well being processed. (Refer to section 3.6.2)

ACOUSTIC POWER GRAPH shows the acoustic power being delivered to the sample for each treatment.

Pie Chart Well Graph

Each well position is represented as a pie chart. The pie charts fill in to indicate the progress of the treatment in each well. A well undergoing treatment is red and once completed the color changes to blue.

Progress Bar

Indicates the percent completed of the currently running process (as a percent of the total calculated time).

Pull-down Menus

Help: SHOW/HIDE HELP opens a help dialog box when the cursor passes over the button controls. A brief description is in the box.

3.6.2 Process Configuration Panel

The screenshot shows the 'Process Configuration: demo' window. The main parameter table is as follows:

Well	Duty Cycle	Intensity	Cycles/Burst	Treatment Time (s)	Radius (mm)	Rate (rpm)	Dwell (s)
A4	20%	10	1000	10.0	1.0	0.0	0.0
A4	10%	9	500	10.0	1.0	0.0	0.0
A4	5%	8	200	10.0	1.0	0.0	0.0
A4	2%	7	100	10.0	1.0	0.0	0.0
A4	1%	6	100	10.0	1.0	0.0	0.0
A4	0.5%	5	100	10.0	1.0	0.0	0.0
A4	0.1%	4	100	10.0	1.0	0.0	0.0

The data table below it contains 12 rows of configuration data:

Seq. #	Well	Duty Cycle	Intensity	Cycles/Burst	Treatment Time (s)	Radius (mm)	Rate (rpm)	Dwell (s)
1	C1	5%	6.0	100	10.0	1.0	0.0	0.0
2	B1	10%	9.0	100	20.0	1.0	0.0	0.0
3	A1	5%	10.0	100	10.0	1.0	0.0	0.0
4	C2	2%	9.0	100	30.0	1.0	0.0	0.0
5	B2	10%	6.0	100	10.0	1.0	0.0	0.0
6	A2	5%	9.0	100	20.0	1.0	0.0	0.0
7	C3	5%	6.0	100	10.0	1.0	0.0	0.0
8	B3	5%	6.0	100	45.0	1.0	0.0	0.0
9	A3	5%	6.0	100	10.0	1.0	0.0	0.0
10	C4	5%	6.0	100	60.0	1.0	0.0	0.0
11	B4	5%	6.0	100	10.0	1.0	0.0	0.0
12	A4	5%	6.0	100	10.0	1.0	0.0	0.0

Software Panel 2: PROCESS CONFIGURATION PANEL

This screen allows the user to select either a previously created process (and its associated parameters) or to create a new process. On the left side of the screen are the controls to set-up the well plate.

The SEQUENCE menu has a list of pre-defined sequences (the order of the samples being processed during a run) or the option none to allow the user to create their own sequence.

The CLICK ACTION menu uses the *replace* option to replace the last selected well position sequence with the newly selected well position. The *append* option allows the user to add the newly selected well position to the end of the list of well parameters.

The WELL PLATE menu pulls up a list of pre-defined treatment vessel configurations (multi-well plate). The diagram below it, WELL PLATE, displays the well configuration of the selected plate.

The APPLY SEQUENCE button creates a process by applying the treatment settings in the upper right panel to all the wells of the well plate definition using the ordering of the selected sequence (top left).

The PROPAGATE button creates multiple sequences per well by applying the treatments highlighted in two or more rows in the table (lower right) using the ordering of the selected sequence (top left).

Current Well Parameters

For a new or an existing process, the user may define and edit the individual well parameters or sequence in the control panel. Each sequence number correlates to a number in the WELL PLATE graphic (lower left). For multiple sequences in a WELL, the last sequence number displays. Clicking on a well in the graphic, adds the well to the sequence.

WELL is the current treatment position in the well plate. The set of parameters defined for the well is a treatment. When WELL is checked, multiple treatments to the same well can be achieved by selecting PASTE VALUES. The WELL number in the table goes blank indicating that the defined treatment is in the same well. When WELL is unchecked, selecting PASTE VALUES does not change the well number indicating that the treatment is the displayed well.

TREATMENT TIME is the amount of time (sec) to apply the parameters or to treat a well sample.

Acoustic energy is produced in bursts and the E-series system allows the user to modify this energy to maximize the effects on the treatment samples for any particular application. Contact Covaris Technical Support for recommendations regarding these settings.

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 DC the higher the acoustic waves per unit time is generated by the transducer. Minimum is 0.1% and Maximum is 20%.

INTENSITY: The amplitude of the pressure waves created by the acoustic transducer. 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 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. Minimum is 50 and Maximum is 1,000.

Dithering is the controlled horizontal circular movement of the treatment vessel around a center point during processing to mix the sample. For horizontal dithering, the following parameters can be set:

RADIUS is the radius (in mm) the vessel moves in a circular motion.

RATE is the speed (in rpm) of this movement of the vessel.

DWELL is the time the vessel remains at the center point between dithering operations.

To move the data to/from the current well to the control panel, use the following buttons:

COPY button copies or adds the parameter information (treatment) defined in a well to the control panel. To copy, highlight a row in the table, and select **COPY**.

PASTE VALUES copies the parameter information (treatment) defined in the control panel to a highlighted row(s)

DUPLICATE SELECTION allows the user to define multiple treatments to an individual well.

Process Wide Parameters Box

The Monitoring Water Bath Quality button is used to detect any problems that could cause the water bath not to properly conduct the acoustic energy such as contamination, high temperature, or insufficient degassing.

The Frequency Tuning Menu has three modes:

1. **FREQUENCY SWEEPING:** The acoustic frequency sweeps through a range during processing. This is the default setting for the E-series instruments. As plastic readily absorbs acoustic energy, this lower power setting is more appropriate for process development with plastic vessels. Frequency sweeping is both preferred and recommended for plastic sample vessels.
2. **POWER TRACKING:** In this mode, the instrument optimizes the operating frequency to achieve peak power in the treatment vessel for the treatment chosen. This mode delivers the most effective power and the highest level of mixing to the sample. It is also the best for most applications, especially biologically active samples (e.g., RNA extraction from tumor tissues samples or proteomic preparation) where rapid sample processing is required. This setting may also be appropriate for chemical-based samples (e.g., small molecule drug extraction from slow release tablets, compound dissolution, or metabolite extraction from plant tissue). Power Tracking is both preferred and recommended for high-power treatments in glass sample vessels.

CAUTION: When used with some high power treatments and some vessels (e.g., plastic) Power Tracking Mode may result in heating of the sample (e.g., viscous, fibrous samples).

3. **VERTICAL SWEEP** moves the sample up/down during processing. The menu is active when Vertical Sweep is selected.

VERTICAL +/- is the vertical movement in mm.

VERTICAL RATE is the frequency in cpm (cycles per minute).

CAUTION: Do not employ Vertical Sweep with non-zero Radius dither motions. If Frequency Tuning is set to Vertical Sweep, all Radius values must be set to zero.

NOTE: The ability to monitor water bath quality is not active in Vertical Sweep mode.

BATH TEMPERATURE LIMIT allows the user to select a temperature limit for the treatment. The overall operating limits of the water bath temperature are 5° C and 40° C. If the water temperature is over the specified limit, the BATH thermometer on the front panel will flash yellow and processing will be halted. It can be restarted by selecting **VERRIDE** in the temperature warning dialog box.

General Operation Buttons

NEW PROCESS button allows the user to enter a new set-up for a process. A dialog box pops up to ask for a name.

OPEN allows the user to select a process from the list of previously created processes.

SAVE allows the user to save a process set-up as a *.pcs file.

RETURN exits the screen to return to MAIN Panel

Pull-Down Menus

File: NEW PROCESS (Ctrl+N) creates a new configuration process.

OPEN PROCESS (Ctrl+O) allows opening a currently saved process

SAVE PROCESS (Ctrl+S) allows a new or opened process to be saved.

EXIT PROCESS (Ctrl+Q) exits the Process Configuration Panel.

Edit: Typical editing functions available for a currently opened process and its list of well parameters

CUT (Ctrl+ X) cut or delete selection, which can be used to paste elsewhere

COPY (Ctrl+C) copy selection

PASTE (replace) (Ctrl+V) replaces selection with last COPY

INSERT AFTER inserts the copied parameters after the sequence selected

UNDO (Ctrl+Z) undo last action

REDO (Ctrl+Y) repeats the last action.

SELECT ALL (Ctrl+A) selects all lines

DELETE SELECTION deletes selected line(s)

Help: SHOW/HIDE HELP opens a help dialog box when the cursor passes over the button controls. A brief description is in the box.

4.0 SYSTEM OPERATION**4.1 Start-Up Procedures****Chiller Connection**

The 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 left side of the instrument.

Water Bath

The water bath level needs to be checked periodically using the water level label on the tank. With the samples at the treatment position, the water level should be within +/-3mm of the appropriate waterline on the RUN side of the water level label (see section 3.2.4). Only distilled or deionized water is used in the water tank.

Special care needs to be taken when changing the water bath. First, select the CHANGE BATH button to raise the transducer. Then carefully pull out the acrylic water tank from the instrument base plate and empty it. Fill the water tank and place it back into its position, ensuring that it is oriented properly in the instrument (see section 2.4.4).

WARNING: The water bath is heavy when filled. Use caution when moving it.

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

Degassing

The Covaris system requires that the water bath be degassed to maintain repeatable acoustic energy coupling, therefore, the pump should be running whenever samples are being treated. This is especially critical for high intensity applications such as solid tissue homogenization. At room temperature, the water bath should be degassed for at least 45 minutes prior to running a treatment process. Depress the DEGAS PUMP button. Ensure the water bath is filled to the water level before turning on the pump; otherwise, damage to the pump may occur.

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

Loading the Multi – Well Plate Holder

Select the LOAD SAMPLE button on the Main Panel to raise the multi-well plate holder. The system will position the stage above the water bath.

Fill the multi-well plate with the sample vessels to be run. Place the hold-down plate over the vessels. The hold-down plate ensures that the vessels stay in place for correct processing.

The plate should be loaded on the multi-well plate holder with the A-1 position in the upper right corner. See Figure 4.

NOTE: Ensure the well-plate is seated flush onto the well-plate holder to insure the samples are correctly aligned with the focal point of the transducer.

4.2 Creating and Modifying a Process in the Process Configuration Panel

To open an existing process to run or to modify, select OPEN PROCESS and choose from the list of previously saved set-ups.

To create a new process, select the NEW PROCESS button.

Select the Well Plate Configuration from the WELL PLATE menu.

Select a Sequence from the list in the SEQUENCE menu. Selecting NONE here will allow the user to customize a sequence. This is done by selecting individual wells in the well plate configuration. If some of the positions on the plate are empty, the process can be reconfigured to delete the empty positions from the process (see section 3.6.2).

Parameters may now be selected in the Process Wide Parameter box.

Select the Current Well Parameters

For each sequence number or well, a treatment can be configured using the controls at the top of the screen. See Section 3.6.2.

Save the set-up by selecting the SAVE button. The system will prompt the user to name the setup.

4.3 Running a Process in the Main Panel

Load the samples first by selecting the LOAD SAMPLE button. The motion system will move the sample holder tray to the front of the enclosure and the green DOOR button will illuminate. Press the DOOR button to open the door and position the samples on the tray.

Click the OPEN PROCESS button. Select from the list of defined processes.

Move the samples to the treatment position by selecting the CHECK ALIGNMENT button. Check the water level is at the appropriate level on the RUN side of the label.

Click the START button.

CAUTION: If the computer loses connection with the instrument during processing, the process will stop.

4.4 Manual Overrides

There is a STOP Button on the Computer screen. This will halt the current process. The process may also be stopped by pressing the red STOP button on the instrument. The green DOOR button will light once the process has stopped.

To restart, the system will prompt the user with a message to either RESUME or ABORT the process. If the door is not closed, selecting RESUME will prompt the user again to RESUME or ABORT. Selecting RESUME with the safety system closed will continue the process where it was interrupted. Selecting ABORT terminates the process.

4.5 Shut-Down Procedures

Raise the transducer out of the water bath by selecting the CHANGE BATH button. Sponge out the “bowl” of the transducer to remove water. Empty the water bath. If the instrument will be idle for an extended time, run the degassing pump with an empty water bath for 10 seconds to drain the degassing lines. The transducer must be lowered to run the pump.

CAUTION: Leaving water in the water bath or degassing lines can cause algae growth in the tank and lines which may interfere with sample processing.

Exit the SonoLab software by selecting EXIT on the main panel.

Select Shutdown from the START menu on the Windows XP screen and select the option “Shut down the computer”. The computer will power down. The instrument can be powered down by moving the power switch to the “0” position. The power switch is located at the rear of the instrument on the right side.

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

5.0 SYSTEM SPECIFICATIONS

Model	E Series
Treatment System	Bench-top: high intensity acoustic transducer, sample positioning system, temperature monitoring device, circulation pump, and water bath with safety enclosure.
Dimensions:	24" W x 30" D x 19" H (61cm x 76cm x 48cm)
Weight:	approximately 110 lbs (50 Kg)
Ambient Temp. Range:	15 to 32°C
Power Requirements	100-240 V, ~ 50-60 Hz, 500 VAC
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 computer (may be supplied by Covaris or the user)
Operating System	Microsoft Windows XP Professional 2002, SP3 or Windows 7
Application Software	Covaris SonoLab version 4
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 Water Bath

6.1.1 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 level indicator on the tank to help determine the depth required. Refer to the Instructions For Use, IFU, supplied with the tube rack to determine water depth. If no IFU is supplied or no water level is specified, fill the water bath to the "0" level.

6.1.2 Recommendations for Monthly Maintenance

Periodically, the water bath and degassing lines may be rinsed with a 10% solution of bleach (or equivalent, such as NaDCC). 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.

6.2 Transducer

When not in use, the transducer should be raised out of the water bath and the transducer "bowl" should be sponged out with a soft lint-free cloth or sponge to remove any residual water. Keeping the transducer dry between applications will help prevent corrosion and is strongly encouraged to ensure optimal performance.

Use extreme care when handling the transducer. Permanent damage could result 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.

6.3 Safety System

Test the system periodically. Make sure that the operation is halted when the STOP button is depressed.

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

6.4 Degassing the System

The degassing pump will shut down after 10 seconds without a water bath because running the pump without water will damage the pump.

Check that there are bubbles coming from the end of the degassing tube. Remove the dip tube (inlet tube) if necessary and examine it under a microscope to clear any clogged holes.

For long-term storage, all water should be removed from the degassing pump lines. This can be done by running the degassing pump for 5 to 10 seconds with an empty water bath.

6.5 Relocation of the System

Should it be necessary to move the instrument, for whatever reason, the system needs to be properly prepared. This procedure describes the steps necessary to relocate Covaris E-Series instrument. If the instrument is to be moved off site, a crate may be obtained from Covaris.

6.5.1 Prepare the System

- Raise the transducer (“Change Bath” position) and sponge out the bowl of the transducer. Empty the water tank and reinstall it in the instrument
- Lower the transducer and run the degas pump. Run the pump for 10 seconds, enough to drain the water from the pump lines.
- Raise the transducer (“Change Bath” position) and empty the residual water from the tank
- Leave the transducer in the up position (“Change Bath” position).
- Save the current process and close SonoLab software by selecting EXIT on the main panel.
- Shut down the computer from the START menu.
- Power down the instrument by moving the power switch to the “0” position. The power switch is located at the rear of the instrument on the right side.
- Disconnect the instrument power cable, the USB cable, the mouse and the computer power supply. Gather these items and keep with the instrument.
- Disconnect the Chiller from the instrument. Drain the water in the chiller into a bucket through the drain fitting. The hoses can be drained by depressing the valve in the center of each quick-connect fitting and holding over a bucket.
- Protect the chiller quick-connect fittings on the lower left side of the instrument from damage by taping foam or bubble wrap over them.

6.5.2 Move of the System

- If the instrument is to be moved off-site, procure a crate from Covaris. Pack the instrument in the crate using the provided foam inserts.
- Protect the system and computer with a light cloth or plastic to prevent dust contamination.
- Be sure all the components are accounted for, including tube holders and user manual.
- Transport the instrument and accessories. At least two capable people are required to lift and place the instrument. The instrument weighs approximately 88 lb (40kg). The chiller is also heavy.

6.5.3 Reinstall the System

- Instructions for reinstalling the instrument are contained in Section 2 of the User Manual. If the manual is missing, obtain a new copy from Covaris.

7.0 TROUBLESHOOTING

System Trouble Analysis Chart

SYMPTOM	PROBABLE CAUSE	POSSIBLE SOLUTION
Degassing system not generating bubbles.	<ol style="list-style-type: none"> 1. Degassing dip tube may be clogged 2. Pump not working 	<ol style="list-style-type: none"> 1. Remove and backflush 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 3. If pump does not run, contact Covaris.
Inconsistent treatment results across a plate, incorrect positioning	Home position lost, system misalignment.	Home system by selecting "initialize system" from the pull-down menu on the main panel, or by restarting SonoLab software.
Safety interrupt	<ol style="list-style-type: none"> 1. Water tank is misaligned 2. Door is open. 3. STOP button is depressed 4. Safety interlock is bypassed 	<ol style="list-style-type: none"> 1. Check tank alignment 2. Check door 3. Check safety interlock
Sample vessel hits the water jet nozzle	<ol style="list-style-type: none"> 1. System has lost the home position. 2. Incorrect nozzle position 	<ol style="list-style-type: none"> 1. Home the system 2. Reposition water jet manually
Error message that system not shut down correctly	SHUT Down command in Windows XP not used before rebooting	<ol style="list-style-type: none"> 1. Follow instructions on screen 2. Use SHUT DOWN command
Delay in powering degassing pump on/off	If a process is running, there is a delay in powering the degassing pump on/off	Wait until process completes before powering the degassing pump on/off
Values for Dithering and Vertical Sweep are different from the values that were entered	Values entered are larger than the limits for a particular Well Plate	Use lower values

APPENDIX A: Sample Preparation Requirements

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

Model E110 -	volume	100 – 1,000 µl
	mass	< 100 mg
	tube	13 x 100 mm

Model E210 -	volume	1,000 – 10,000 µl
	mass	< 1,000 mg
	tube	16 x 100 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: Installation Procedure for Covaris E-Series Software and Drivers

This procedure is for the installation of Covaris SonoLab E-Series application and supporting National Instruments software and drivers for the operation of the E-Series instruments (E110 and E210). Please follow these instructions in sequence.

1. Install the National Instruments software and drivers
 - a. Create a folder on the C drive named "NI"
 - b. From the E-DRIVERS CD, copy the Nidaq693 setup program into the NI folder and run it.
 - c. From the E-DRIVERS CD, copy the LV61Eng setup program into the NI folder and run it.
 - d. Run Setup.exe in the NI folder on the C drive.
 - i. Select LabVIEW 6.1 support.
 - ii. Select NEXT.
 - iii. Reboot when prompted.
 - e. From the E-DRIVERS CD, launch NI-VISA (NI-VISA\NIvisa26) by scrolling down, and follow the instructions. Use the (default) complete installation.
 - f. From the E-DRIVERS CD, launch VisaProvider (VISAProvider\VISA_Provider) and follow the instructions.
 - g. From the E-DRIVERS CD, launch VisaServer (VisaServer\NIVISA-Server) and follow the instructions. Select the (default) "Modify" option.
 - h. From the E-DRIVERS CD, launch IVI engine driver (IVIEngine\IVIEngineSetup) and follow the instructions.
 - i. From the E-DRIVERS CD, launch LVRunTimeEng (LVRunTimeEng).
2. Plug in USB cable to instrument and computer.
3. Turn on instrument.
4. Follow "New hardware found" wizard. It runs four times.
 - a. The message comes up, "Can Windows connect to Windows Update to search for software?" Select "No, not at this time."
 - b. Select "Install automatically".
 - c. Ignore the warning from Microsoft about unapproved drivers (continue anyway).
5. Update Measurement and Automation Explorer (MAX) (Desktop Icon)
 - a. Click View then Refresh
 - b. Look under "devices and interfaces"- "ports". Note the name of the last COM port in the list. (You will need this number for a future step.) Exit MAX.
6. On the E-DRIVERS CD, go to the GALIL directory and run the setup program (GALIL\dmcsmarterm02.exe).
 - a. Select NEXT. Ignore the customer information screen by selecting NEXT. Select NEXT again to install using the default file structure.
 - b. Select INSTALL. Reboot when prompted.
7. From the START menu, run the program: GALIL → DMC Smart Terminal.
 - a. From the menu, select Tools → Controller Registration.
 - b. A dialog box appears. Select NEW CONTROLLER.
 - c. Enter the model. Scroll to DMC-21x3/2. Use the other defaults and select NEXT.
 - d. Enter the COM port number from step 5. Enter the COM speed of 9600. Do not change Handshake options. Select FINISH. Select OK.
 - e. Close dialog box. Close program.
8. Remove the E-DRIVERS CD and load the SonoLab Software CD ver. 4.3.x.

9. Install the Covaris SonoLab software.
 - a. Create a folder C:\Covaris.
 - b. From the SonoLab Software-CD, select SonoLAB vX.X.X folder.
 - c. Copy and Paste into C:\Covaris Folder.
 - d. In the Covaris Folder, double click in the Software folder.
 - e. Right click the software application and Send to Desktop

10. Confirm the software is functioning.

NOTE: Please contact Covaris for assistance installing SonoLab software onto Windows 7 operating systems.

Once installed, to ensure correct operation of the software under Windows 7:

Find the SonoLAB shortcut on the desktop. Right-click and select Properties. The SonoLAB Properties window will appear. Click on the Compatibility tab. Under Compatibility Mode, check off *Run this program in compatibility mode for*. Select in the drop down menu Windows XP (Service Pack 2) Click Apply and Ok.

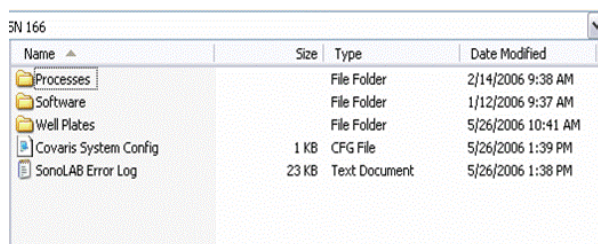
NOTE: Laptop standby and hibernate modes should be avoided while operating the SonoLAB software. To ensure this, from the start menu, open Control Panel, then Power Options. Select a Power Scheme that sets System standby and System hibernate times to Never. If standby and hibernate modes remain enabled,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

NOTE: SonoLab software has not been 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: Software Upgrade Installation Procedure for Covaris E-Series Software

This procedure is for the installation of Covaris SonoLab E-Series application software upgrade for the E-Series instruments (E-110 and E-210). Please follow these instructions in sequence.

1. Turn on the E-series instrument computer and navigate to the Covaris software C:\Covaris
2. Create a folder titled "Covaris_Old". Copy the folder "Covaris" and paste it into "Covaris_Old". This is the software backup.
3. Delete the desktop shortcut titled "SonoLab v4.x.x" and the file "E-series MANUAL 4.x.xREVx.pdf".
4. Open the original Covaris folder at C:\Covaris. Inside there is another folder titled "SonoLab v4.x.x" which contains the software, configuration files, processes, and well plate definitions needed for the E-series operation.
5. Rename this folder to the new version of software, "SonoLab v4.3.x". This folder name may also contain the system serial number.
6. Open the folder. It will contain the structure below:



Name	Size	Type	Date Modified
Processes		File Folder	2/14/2006 9:38 AM
Software		File Folder	1/12/2006 9:37 AM
Well Plates		File Folder	5/26/2006 10:41 AM
Covaris System Config	1 KB	CFG File	5/26/2006 1:39 PM
SonoLAB Error Log	23 KB	Text Document	5/26/2006 1:38 PM

7. Delete the folder titled "Software". Do not change or delete the other folders.
8. Load upgraded *Software CD E-series v4.3.x* into CD drive. Open the CD directory and copy the folder titled "Software".
9. Paste this "Software" folder into the folder titled "SonoLab v4.3.x", to create a new Software folder in place of the deleted folder.
10. Open the new "Software" folder and find the application file with the C Icon.  Create a shortcut for the desktop.
11. Copy the file "E-series MANUALrevXX.pdf" from the CD. Paste the file on the desktop.
12. Turn on the E-series instrument.
13. Using the desktop icon, launch the application.
14. Verify that the instrument and computer are communicating. Perform the homing sequence.
15. Click on OPEN PROCESS. Select any process.
16. Run the process. It will verify the system's operation.
17. If the instrument does not perform as expected, please contact Technical Support.

NOTE: Please contact Covaris for assistance installing SonoLab software onto Windows 7 operating systems.

Once installed, to ensure correct operation of the software under Windows 7:

Find the SonoLAB shortcut on the desktop. Right-click and select Properties. The SonoLAB Properties window will appear. Click on the Compatibility tab. Under Compatibility Mode, check off *Run this program in compatibility mode for*. Select in the drop down menu Windows XP (Service Pack 2). Click Apply and Ok.

APPENDIX D: Site Preparation and Equipment Installation Requirements

The Covaris E series is delivered in a crate, whose dimensions are approximately 46" wide by 25" deep by 30" high. If doorways or elevators cannot accommodate these dimensions, the instrument and accessories will have to be removed from the crate at the loading dock. If this is necessary, be certain to remove all accessories from the crate before disposal.

Placement of Equipment

The area required for the instrument (see attached drawings) is approximately 24" wide by 30" deep by 19" high (61 cm x 76 cm x 48 cm) and capable of supporting at least 150 pounds. The laptop computer may be placed on top of the E system, or on the bench to the right of the E. If the optional Water Conditioning System (WCS) was ordered, it is placed on the bench to the right of the E, and the laptop may be placed on top of the WCS.

Two fans maintain a safe operating temperature in the instrument by circulating air over the internal components. Therefore, place the instrument so that there is a minimum of 1" (3cm) between the rear panel and any wall or obstruction for airflow.

The water bath chiller is approximately 10" wide by 18" deep by 22" high (21 cm x 45 cm x 55 cm), and may be placed on the bench to the left of the E, or under the bench. The chiller hoses supplied with the E system are six feet long. If this is not sufficient to reach the chiller, the chiller accessory kit, pn 500102, consisting of six foot extension hoses should be ordered.

Allow sufficient space in front of the bench to access each component of the installation.

Power Requirements

One standard AC outlet each is required for the E system, the computer, the chiller, and the optional Water Conditioning System (WCS), if used.

The instrument draws a maximum of 500 VA. The Covaris E-Series instrument is configured with a universal power supply.

The water bath chiller can draw as much as 12 amps (at 120 VAC). If possible, provide a dedicated circuit for the chiller, separate from the E, the computer, or other electronic equipment. If a dedicated circuit is not feasible, verify that the combination of the chiller and existing loads will not overload the circuit.

The above load ratings are maximum. During typical operation, the E system and all accessories will output less than 1000 watts to the surrounding environment.

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

Water Requirements

The E system water bath requires about 6 liters of either deionized or distilled water. Since the water should be changed daily, it is useful to have a sink nearby, into which the water can be dumped. Water is added to the system by manually filling the bath; a direct connection to a tap is not necessary.

If the optional Water Conditioning System (WCS) is used, water need only be changed about once a month.

For some applications, it may be necessary to operate the chiller below 4 degrees C in order to maintain sufficient cooling of the water bath. In this case, it will be necessary to add antifreeze to the chiller reservoir.