Covaris®

E220 & LE220 (-plus, R-plus, Rsc)
Setup & Instruction Manual

Instrument and Software Developed for High-performance
Sample Preparation using Adaptive Focused Acoustic® (AFA®) Energy

For Research Only

Model Numbers: E220-plus, E220R-plus, LE220-plus, LE220R-plus, and LE220Rsc | Software: SonoLab® 10.2

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 government 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-A3 Clinical Laboratory Safety; Approved Guideline - Third Edition, published 2012, ISBN 1-56238-797-9 (Print), ISBN 1-56238-798-7 (Electronic)
- Clinical and Laboratory Standards Institute (CLSI), M29-A4 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Fourth Edition, published 2014, ISBN 1-56238-961-0 (Print), OSBN 1-56238-962-9 (Electronic)
- 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 the Safety of Operating Personnel:

- Make sure that the equipment is properly grounded. DO NOT operate if it's not properly grounded.
- 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.
- DO NOT run an acoustic treatment with the enclosure door OPEN or without a water bath - the acoustic system will not work.
- If there is any indication that the Safety System is not functioning properly, DO NOT operate the equipment. Contact Covaris Technial Support immediately if this occurs.
- 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:

- The instruments are designed to operate in ambient laboratory conditions e.g., 19 to 25 °C (66 to 77 °F). DO NOT operate the instrument in a cold room environment. The system is designed to operate with a water bath and recirculating heater/chiller apparatus to control sample temperature.
- NEVER run a method without water in the bath since this could cause damage to the transducer. The instrument is equipped with a water level sensor to protect the transducer and degassing pump. The system will not allow the degassing pump to operate or the acoustic treatment to start unless an adequate volume of water is detected.
- Distilled or deionized water should be used to fill the water bath.
- Unless a Water Conditioning System 2.0 (WCS) is actively employed, empty the water bath and wipe it dry EVERY DAY with a lint-free cloth. DO NOT leave water in the bath for an extended time since there is no water filtration or water cleaning system within the apparatus (unless WCS is employed).
- DO NOT employ isopropyl alcohol, ammonia-based or abrasive cleaners in the system, since these chemical solutions will damage the acrylic surfaces.
- Establish a standard of operation and test the equipment periodically, as described in the maintenance section of this manual.

CAUTION: All AutoFill components have pressure ratings above Covaris' specified maximum operating pressure which have been extensively tested. However, if plant DI water is connected to the instrument, a catastrophic failure may result in substantial plant water spillage. It is the end user's responsibility to specify and implement appropriate containment/alarm schema for their facility.

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 proven 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 that the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement via 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 by misapplication from 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. if you have questions or concerns pertaining to the use of our quipment.

Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the necessary services and support necessary so end users can successfully operate their equipment. 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 – Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:
- Telephone
  - United States: Tel: +1 781.932.3959 during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Eastern Standard Time/EST (UTC−05:00)
  - Europe: Tel: 44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Greenwich Mean Time/GMT
- E-mail queries to techsupport@covaris.com or applicationsupport@covaris.com

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.

This manual includes maintenance guidelines and troubleshooting tips.

Table of Contents

1. Introduction

1.1 Overview of the User Manual

This manual contains the operation and maintenance instructions for the Covaris E220-plus, E220R-plus, LE220-plus, LE220R-plus, and LE220Rsc Focused-ultrasonicators. This document contains information that is essential to the proper use and care of your equipment. Should any unforeseen problems occur with the normal operation of the equipment, immediately contact your Covaris Technical Support representative.

The following definitions apply in this manual:

NOTE: Inconvenience if disregarded.	**WARNING**: Personal injury may occur.	**CAUTION**: Equipment damage may occur.

1.2 The E220- and LE220-plus Focused-ultrasonicator

The E220- and LE220-plus Focused-ultrasonicators are based on the Covaris Adaptive Focused Acoustics (AFA) technology. These instruments are designed to provide researchers with an integrated stand-alone tool to be used in a laboratory environment and are ideal for those who work with tissue homogenization, cell lysis, DNA or chromatin shearing, and other sample preparation steps. The E220-plus treats samples sequentially, one sample at a time. The LE220-plus treats a column of samples simultaneously.

This system is comprised of the:
- **Treatment System** which delivers the acoustic energy to the sample
- **Safety System** which protects users from inadvertently contacting the high intensity acoustic energy
- Computer and Application Software which provides an interface to control the device via the API
- **Water Management System** which consists of a cooler/heater and a UV treatment chamber with particulate filter to maintain water quality in the instrument bath

1.3 The E220R- and LE220R-plus Focused-ultrasonicator

In addition to the features of the E220- and LE220-plus described above, the E220R- and LE220R-plus are designed to be integrated with a sample handling robot in an automated work cell. The front door of the safety enclosure contains a cutout providing the robot arm access to the inside of the instrument cabinet. The instrument presents the sample tray at the opening, and the robot arm loads or unloads the sample through the opening. The instrument is controlled by custom software built using the SonoLab 10 API Toolkit.

A light curtain has been added as an additional safety interlock. The light curtain is wired into a safety rated relay controller, the output of which is connected to the Covaris controller board. If an operator reaches through the robot access opening while the system is running, the motor power and acoustics are immediately disabled.

The light curtain consists of invisible, infrared light and an infrared detector. Beams of light on 14 mm spacing are transmitted between the source and detector. Blocking one or more beams of light will trigger the safety interlock system. Acoustics and motion will remain paused until the beams are unobstructed.

1.4 The LE220Rsc Focused-ultrasonicator

The LE220Rsc is an enhanced version of the LE220R-plus. In addition to the features of the LE220-plus and LE220R-plus, the LE220Rsc contains an extended travel X axis motion stage. This longer travel stage allows treatment of 96 and 384 position well plates using the Covaris scanning treatment method. The scanning treatment method allows for continual AFA-treatment as the plate passes over the transducer, without pausing between columns. The scanning speed and number of iterations determines the total acoustic dose.

2. Installation and Setup

2.1 Assembly

The following items should be found in the crate containing the instrument:

- Main unit
- Notebook computer with AC adapter
- Cables
    - USB cable
    - Instrument power cord
    - Computer AC adapter power cord
- Accessories
    - AFA ultraCUBE, consisting of:
        ◦ Water Conditioning System 2.0 (WCS)
        ◦ CH05 Chiller
    - Chiller hose kit
    - Sample tubes & racks (varies by application)
    - Water bath (installed in main unit)
    - AutoFill accessory kit

E220R-plus Focused-ultrasonicator

Unpack the instrument, the Water Conditioning System 2.0 (WCS), and the CH05 chiller. Refer to the WCS User Manual to install the UV lamp into the WCS. The lamp is shipped separately to prevent breakage during shipment. Arrange the instrument, WCS, and chiller on a bench as shown. The chiller and WCS may be installed on either side of the system. The CH05 and WCS may also be placed on the floor or such that the hoses may reach the front of the water bath of the water bath.

> **CAUTION**: When positioning the system, please keep the fan and rear panel vents free of obstructions which can block the flow of air.
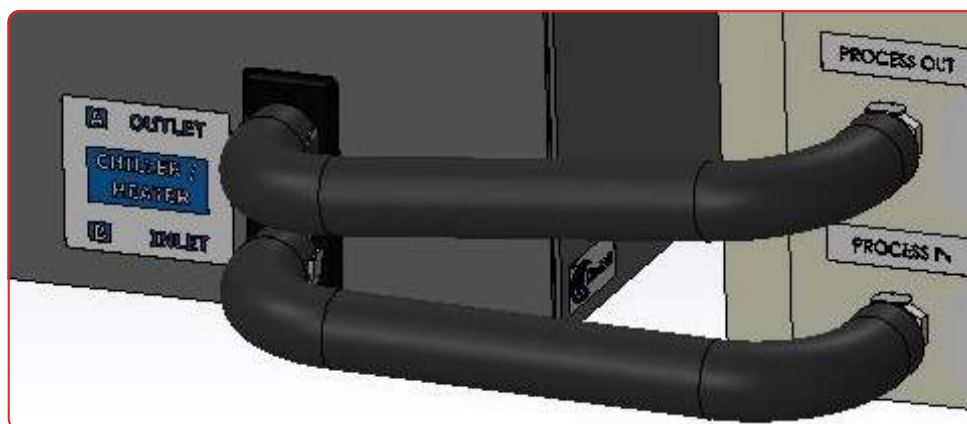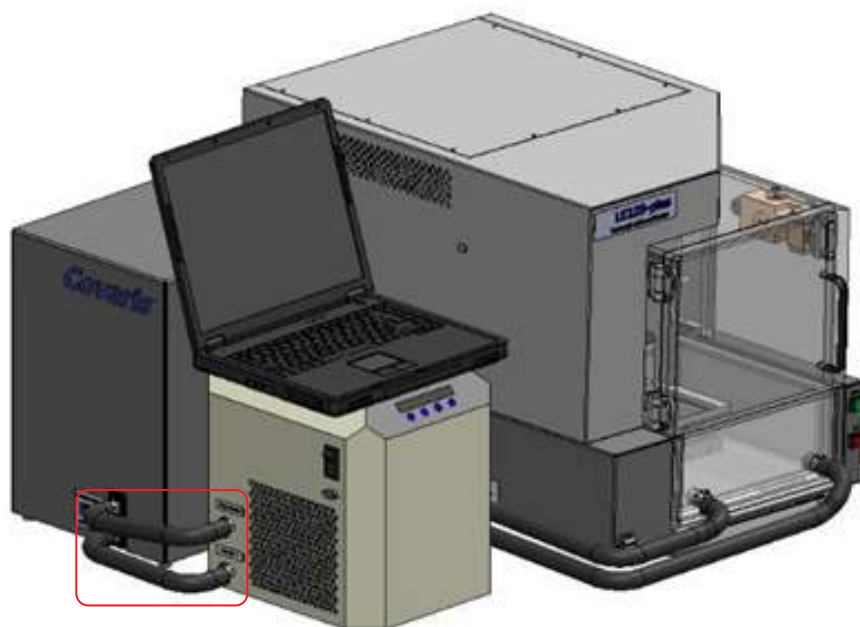
- Connect the hoses between the WCS and the CH05 as shown above.
- For the E220-, E220R-, LE220-, and LE220R-plus, connect the remaining two hoses between the two ports on your instrument water bath and the remaining two ports on the opposite side of the WCS. Connect the lower hose to the fitting on the left side of the water bath.

  For the LE220Rsc, the upper hose from the WCS 2.0 must connect to the lower fitting of the water bath. The temperature sensor for the water bath is adjacent to the two hose connections. The lower hose fitting contains a long tube to bring the cooled (heated) water from the chiller away from the temperature sensor to prevent an erroneous temperature reading.

Upper fitting from WCS must connect to lower fitting of water bath
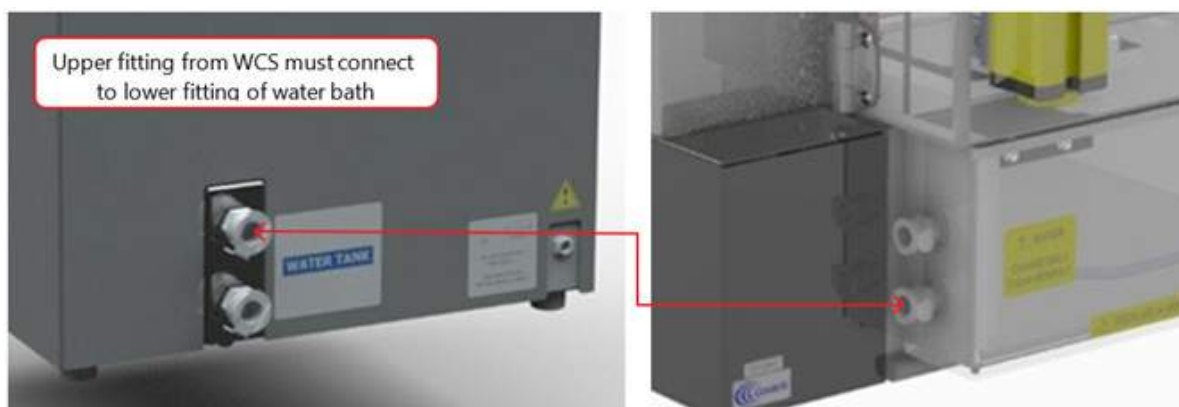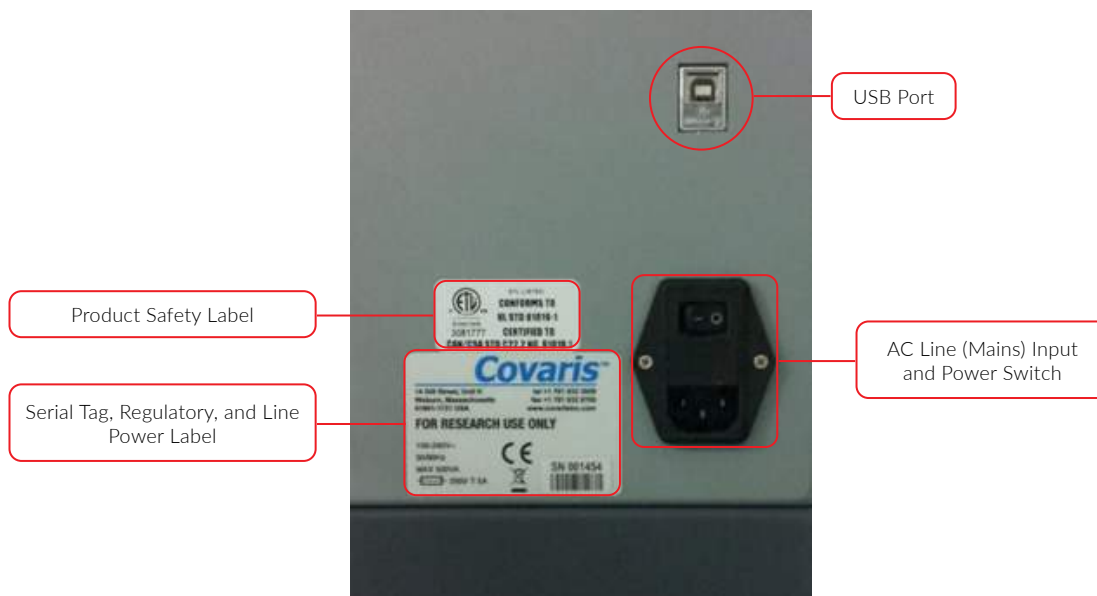
The WCS contains a particulate filter and UV lamp to prevent biological growth. This allows the water bath to be changed only once per month. Refer to the **User Maintenance Section** of this manual for additional details.

- The AutoFill option allows plant deionized water to be plumbed directly to the instrument to allow automatic filling of the water bath without any operator intervention. If the AutoFill option is going to be used, refer to the instructions included with the AutoFill accessory kit or **Appendix D** of this manual to assemble the components of the AutoFill Accessory Kit, and connect the plant water to the system.
- Plug the instrument into a power source using the enclosed line cord. The instrument is a "universal input" device and automatically employs any worldwide line voltage or frequency (100 to 240 volts AC, 50 or 60 Hz). Leave the instrument power and notebook power switched OFF during this time.
- Plug one end of the USB cable into the instrument USB Port and the other end into any USB port on your notebook computer. Connect the temperature control cable between the D-connector at the rear of the instrument and the D-connector on the CH05 chiller.

- Connect the CH05 to a power source. The CH05 is a universal input device. Do not turn on the CH05 at this time.
- Locate the WCS power supply and line cord. Connect the WCS power supply to the port provided on the WCS and connect the line cord to a power source. The power supply is a universal input device. Leave the WCS turned off at this time.
- Connect the computer to a power source. Connect the mouse or other accessories to the computer.

- Refer to the rear of the instrument's right-side panel to identify the following features:

Product Safety Label

Serial Tag, Regulatory, and Line Power Label

USB Port

AC Line (Mains) Input and Power Switch

The Rear Right Panel of the System

- Refer to the figure below to identify the following items:

| Safety Enclosure | Water Bath | Enclosure Door Safety Lock | Stop Button | Door Lock Release Button | LE220Rsc with extended bath and fittings on the left side of bath |

E220R-plus   LE220-plus   LE220Rsc

- Power on the instrument. The power switch is located at the right rear, near the power entry module.
- Press and hold the green 'Door' button while pulling the door handle to open the safety enclosure front door. Slide out the water bath and remove it from its plastic shipping bag. Remove and discard the desiccant pack.

  The instrument is now ready to be started.

3. Getting Started

3.1 Fill the Water Bath

The instrument's transducer must be immersed in a water bath to transmit high-frequency, focused acoustic energy to the sample tube. For best performance, employ only clean, distilled or deionized water in the bath.

Open the front door, remove the bath and the first time your system is operated, fill the bath initially about half full. The first time the WCS and chiller are operated, a substantial amount of air is forced from the WCS into the bath, which may cause the full bath to overflow. This only occurs the first time an empty WCS is used.

Once the WCS is filled with water, the bath should be filled to a level of 15. If the transducer is in a raised position, and is out of the bath, fill to 15 on the "Fill" side of the gauge. If the transducer is submerged, fill to 15 on the "Run" side of the gauge.

If Periodic Fill is enabled, after the initial filling of the bath, Periodic Fill will maintain a sufficient water level in the bath automatically. See **Section 5.2** for additional information on Periodic Fill.

3.2 Start the System

Turn the instrument power ON using the power switch located on the rear, right side of the instrument at the power entry module. When power is ON, the green door-lock release switch will illuminate and the ventilation fans in the rear panels will run.

Power on the WCS and CH05 Chiller. SonoLab software will perform its temperature control; it is not necessary to adjust the temperature set points from the CH05 control panel.

A large volume of air is purged from the chiller and WCS when they are first operated. This is normal, and only occurs the first time the chiller/WCS assembly are operated.

Start the system computer. The computer employs a Windows 10 (or later) Operating System. SonoLab is an application used to control a Covaris Focused-ultrasonicator for processing a biological or chemical sample. It is designed to create, edit, and run a processing program called a method. It has provisions to run, pause or stop a method and view execution histories which contain a record of key instrument parameter values during a run. It also contains editors for customizing system settings and performing maintenance functions.

SonoLab is installed on the computer delivered with the instrument. No further installation or configuration is required.

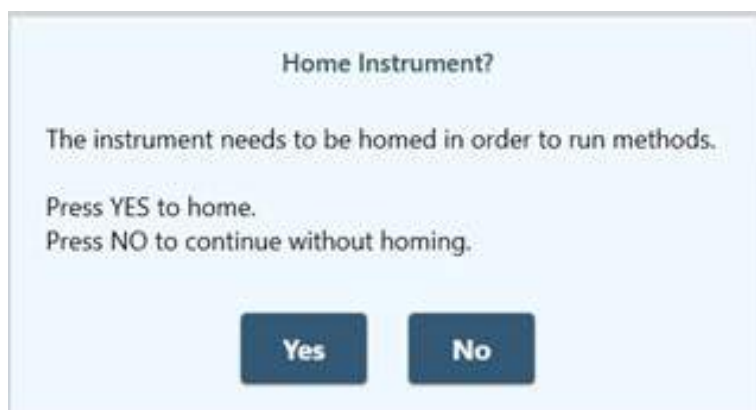Find the SonoLab icon on the desktop:

If the icon is not visible, or if SonoLab is not installed, please refer to **Appendix B** or contact the Covaris Technical Support Department for installation instructions.
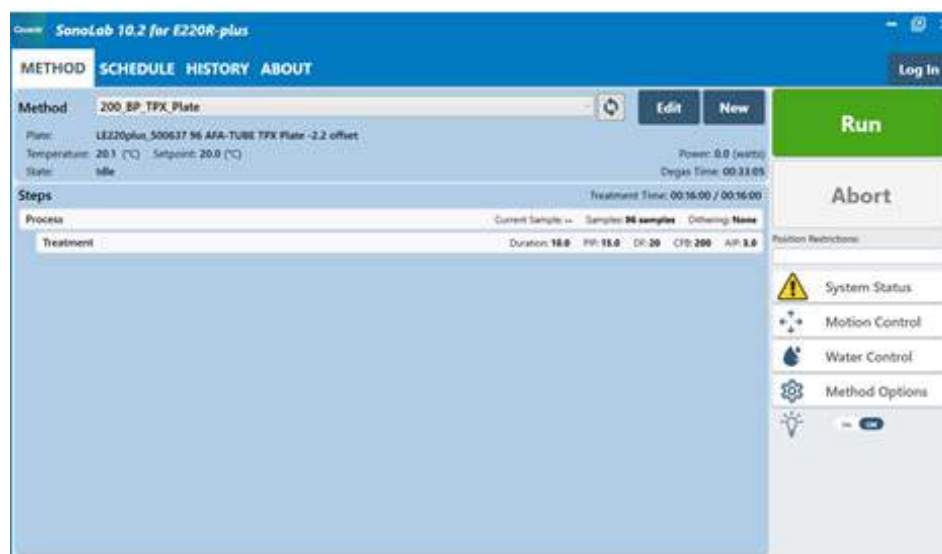
Double-click on the SonoLab icon to start SonoLab. The splash screen will appear:

Following the splash screen, the motion system homing dialog appears:

Click 'Yes', then 'OK' when prompted to empty the sample tray. The transducer and sample holder mechanical assemblies will move into position and the SonoLab METHOD Screen will display.

If SonoLab does not connect to the instrument, the following screen appears allowing SonoLab to be run in emulation mode without an instrument.

Click "Use Emulation Mode", and the normal SonoLab 'Method' screen will appear, allowing the user to operate SonoLab as if an instrument is present. This is useful for training purposes or for creating methods off-line.

3.3 Preparing your System for Use

To run an acoustic treatment, a number of conditions must be met, including:

    a. The motion system must be initialized.

    b. There must be an acoustic method selected in the Method box.

    c. Water temperature must be within acceptable Min and Max limits.

    d. The bath water must be degassed.

    e. Water should be approximately at level 15.

    f. The safety enclosure door must be closed.

> **NOTE**: The proper plate and consumables must be in place on your system's sample tray.

The "Run" button will remain gray and inactive until a method is selected, and all necessary conditions, except the temperature and degassing states are met. Your system automatically sets the temperature when a method is selected. After a specific user's initial log in, SonoLab will automatically select the last method run by this user the next time they log in. The bath water temperature will then be set according to this method. Once the method is started, SonoLab will pause if water must be added to the bath and until the bath is sufficiently degassed.

To achieve an acceptable status on the other conditions:

    a. Your system's motion system normally initializes when SonoLab starts unless the user purposely skips this step. In this case, the motion system can be homed from the Motion Control pane. Click on the Motion Control button to display the Motion Control pane.

    Click on "Home" to initialize the motion system. See additional information for the Motion Control pane in 'The Method Screen', *Section 5.1*, of this manual.

b. To select and load an acoustic method, click on the panel displaying the current loaded method, as shown below. A list of existing method names will be displayed in a drop-down list. Navigate through the list with the vertical scroll bar and select a method. If Consumable-based Method Selection is enabled, the "Method" bar is disabled. See Consumable-based Method Selection in **Appendix A** of this document for operating and configuring SonoLab for Consumable-based Method Selection.
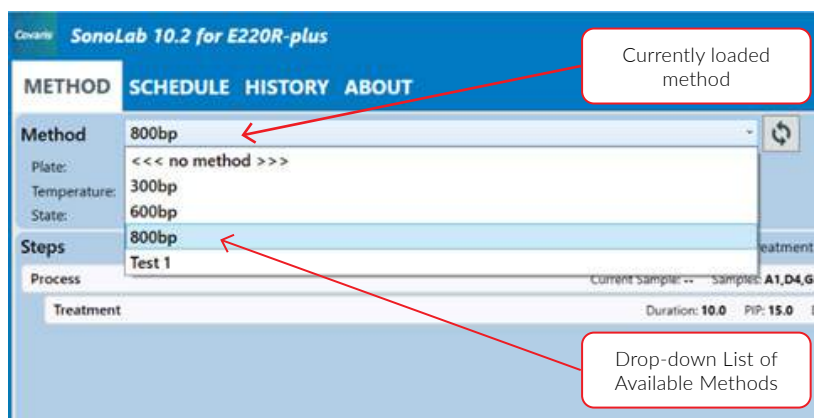
c. An important principal of the Covaris Focused-ultrasonicator is isothermal temperature control. Your system automatically adjusts the water temperature, as necessary, to match the method temperature set point once a method is selected. Water Temperature status will turn green when temperature is within the Min and Max limits (by default 3 degrees above or below the set point). The default set point (in the absence of a selected method) is 20 °C.

The bath cool down from room temperature to below 10 degrees takes about 30 minutes. Once a method starts, if the bath temperature rises beyond the user specified limit, your system finishes the current position being treated, pauses treatment, and automatically resumes when the temperature is back within limits, with no operator intervention required.

d. Properly degassed water is important for the efficient transfer of acoustic energy from the instrument's transducer to the sample being treated. If water is detected in the bath, the degassing pump is automatically turned on when SonoLab is started. The degassing time is approximately 45 seconds per millimeter of water level in the bath (about 45 minutes for a full bath). The degas pump can be manually turned on and off from the Water Works pane. See additional information below in section Other Controls on the Home Screen.

e. When the "Run" button is clicked to start the method, SonoLab checks that there is sufficient bath water for the consumables employed in the method. If the water level is incorrect, the method is paused and a message to add water is presented.

The amount of water specified will vary depending on the current water level in the instrument water bath. After the bath is filled, SonoLab will pause execution of the method to degas the bath, based on amount added. Degassing time for 1 liter added is about 10 minutes, for 2.5 liters about 30 minutes.

NOTE: If the AutoFill option connecting plant water to the E220-plus is implemented, bath water level is maintained automatically. See *Appendix D* for details of the AutoFill option.

The remaining conditions, along with their related status, are displayed in the Instrument Status panel. Click the "System Status" button to display the Instrument Status panel. An acceptable condition is indicated by a green check mark in the Status box. If a condition is not acceptable, the status will show X.

If any of the items other than "Temperature within Limits" in the Status list display X, a red X will also be displayed in the System Status button, as shown below. Water temperature will be adjusted as necessary once a specific method is started.
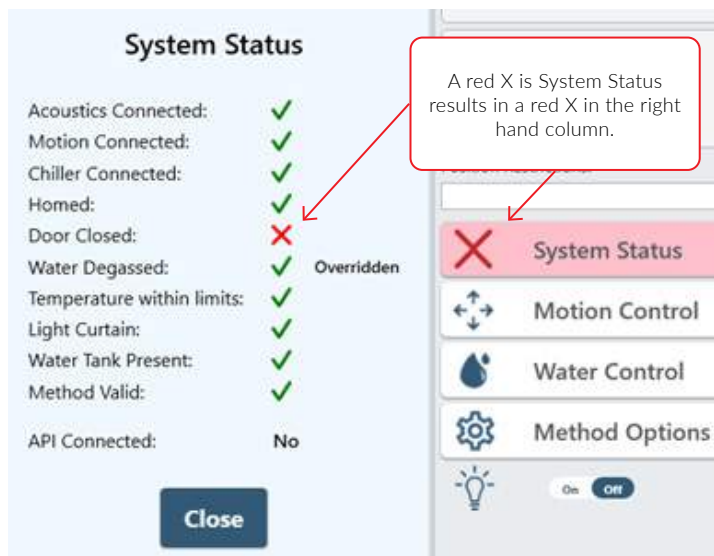
Switches are provided in the 'Method Options' pane to override the degas timer and temperature limits to allow your system to run a method without waiting for these conditions to be met. With the temperature and degas overridden, the 'System Status' display adds 'Overridden' indicators as shown.

A yellow triangle indicates that degassing time or temperature targets have not yet been met.

Once a method has been loaded and all conditions are acceptable, the 'Run' button will turn green and the selected acoustic Method can be started.
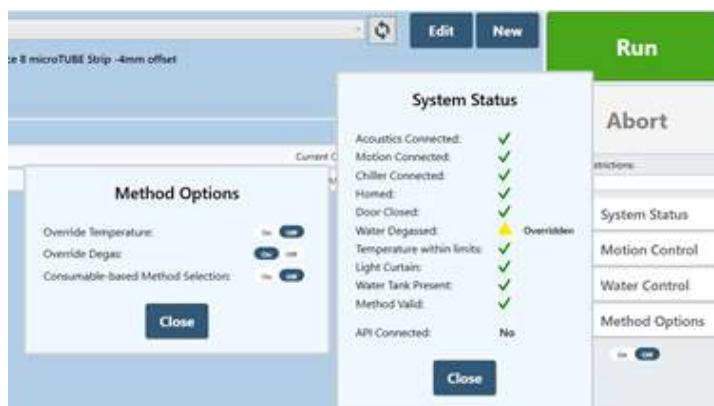
Using Position Restrictions When Running Methods

It is sometimes convenient to run a method on a plate that is only partially populated with samples. For example, consider a method that treats all 12 columns in a 96-well plate. But now you have a plate that has samples only in columns 4 to 6. Using Position Restrictions allows treating a subset of a method's specified positions. See instructions in **Appendix B** of this document, Using Position Restrictions.

To modify an existing method, refer to **Section 5**: Create and Edit Methods.

3.4 System Shutdown

If the instrument will be idle for an extended period of time (e.g. longer than two weeks), refer to the system shutdown procedure in the User Maintenance section of this manual.

When your system is finished being used for the day, leave the transducer submerged in the bath if automatic scheduling is active. At the end of the scheduled period, the scheduler will turn off the degas pump and disable cooling in the chiller, then automatically restart both as scheduled for the next day. SonoLab software must be left running and the system and CH05 chiller powered on for the scheduled events to occur.

If scheduling is not in use, click on the 'Water Control' button and then the 'Click for Standby' button. The transducer will rise out of the bath and the CH05 chiller's heater/cooler will turn off, but the chiller's pump will continue to operate, circulating bath water through the Water Conditioning System (WCS).

> **NOTE**: No scheduled events will occur when the instrument is in "Standby" mode, regardless of whether or not SonoLab is in scheduled mode.

> **CAUTION**: Do not turn off the WCS or CH05 chiller. These must remain powered on to treat the bath water against bio-growth. Leaving water in the water bath for an extended time without the WCS operating may promote algal growth in the bath and lines. This growth can interfere with the water quality and sample processing.

4. AFA Operations

AFA uses four parameters to fine tune specific acoustic treatments. These parameters are programmed into Methods as provided by Covaris. Methods can be executed manually or by the automation software, including specifying the treatment to be performed and whether a full or only part of a plate is to be treated. A brief explanation of the treatment parameters is provided below.

Peak Incident Power (PIP)

This parameter measures the peak electrical power used to drive the ultrasound transducer. In the E220- and LE220-plus instruments, the range of Peak Incident Power is between 1.5 to 500 Watts. Plate definitions selected for a treatment may further restrict maximum PIP.

Duty Factor

This parameter defines the ratio between the transducer on-time and total operation time. Duty Factor is expressed as a percentage. For a given Peak Incident Power, a larger Duty Factor results in more acoustic energy generated from the transducer. Users can define the Duty Factor from 1 to 50%.

The product of the Peak Power and Duty Factor gives the approximate Average Incident Power (AIP) generated from the transducer. For example, if Peak Incident Power is set to 10 Watts and Duty Factor is set to 15%, the Average Incident Power will be approximately 1.5 Watts. However, the real Average Incident Power from the transducer may vary due to factors such as sample tube type, water level, and water temperature.

Cycles per Burst

This parameter indicates the number of electrical signals used in each acoustic "burst" of the transducer. Cycles per Burst (cycles/burst) range from 50 to 1000 in the E220- and LE220-plus instruments for different applications.

Duration

This parameter defines the amount of time for which the sample is under treatment while in indexed operation (i.e. treating one row at a time). Duration is 1 to 3600 seconds. Treatments longer than 3600 seconds (60 minutes) can be implemented using the Repeat function described in *Section 6.2*.

Treatment Records

The history of a treatment consists of three files: Measurements, Summary, and Treatments files. Refer to *Section 5.3* for details.

Measurements, a .csv file which can be opened as a spreadsheet, contains information about the methods run, including times, positions treated, acoustic power, bath water temperature, and bath water level.

The Treatments file is also a .csv file which contains treatment parameters, start and end times for the method, along with water level, and temperature data. The Treatment file contains the data sent to the LIMS.

The Summary is a .xml file containing textual information which is shown along with the graphics in the History display.

5. SonoLab Software

5.1 The Method Screen

When SonoLab is launched, the Method screen is displayed with the last successfully run treatment method.
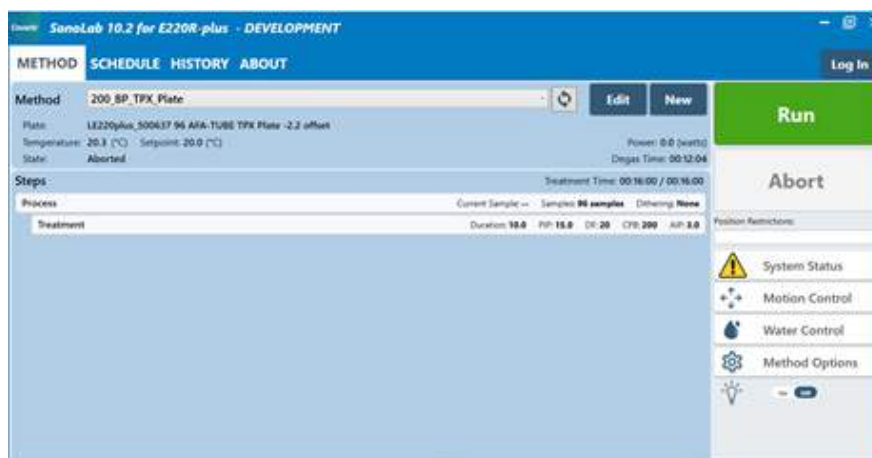
- Method displays the current selected treatment method and its status.
  - **Plate**: the name of the sample plate specified by the current selected method.
  - **Temperature**: the actual temperature of the water in the instrument bath.
  - **Setpoint**: the water bath temperature specified by the current selected method.
  - **State**: possible states, depending on what the system is doing, includes:
    - At initial startup, Idle.
    - During a treatment method, the possible states include:
      - Waiting for Water Temperature: the system waits for the water temperature to be within the range specified by the selected method.
      - Waiting for Water Degas: the system waits for sufficient time for effective degassing to occur.
      - Waiting for Water to be Added to the Bath: this occurs when the water level is low and AutoFill is not enabled.
      - Running: the system is currently performing the selected treatment method steps.
      - Paused: the method is paused by the user or the API.
    - Following a treatment:
      - Completed: the treatment terminated normally.
      - Aborted: the treatment was halted by a command from a user or the API.
      - Failed: the method run was unsuccessfully completed with the displayed reason.
      - Cancelled: in Consumable Based Method Selection (see **Appendix A**), method selection was cancelled.
  - **Run Time**: the remaining time for acoustic on, time required for motion, any delay steps or other overhead for the current method.
 - Methods saved on the system are selected from the drop-down list.

- Position Restrictions

  It is sometimes convenient to run a method on a plate that is only partially populated with samples. For example, consider a method that treats all 12 columns in a 96-well plate. But now you have a plate that has samples only in columns 4 to 6. Using Position Restrictions allows treating a subset of a method's specified positions. Refer to **Appendix B** of this document, Using SonoLab Position Restrictions.

- Additional system control functions are accessed via buttons at the right side of the screen.
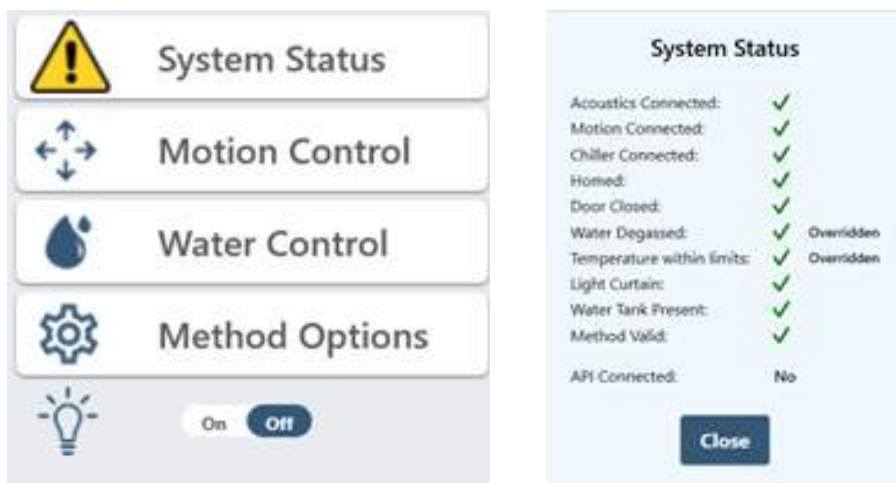
- System Status displays the System Status panel, which shows whether the instrument conditions required to run a treatment method are or are not met. A red X indicates a parameter has not been met, a green check indicates it has.
    - **Acoustics Connected**: a green check indicates the electronics have successfully connected to the software.
    - **Motion Connected**: a green check indicates the motion controller board is detected.
    - **Chiller Connected**: a green check indicates that the chiller is communicating with the instrument.
    - **Homed**: a green check indicates that the instrument's motion system has been initialized.
    - **Door Closed**: a green check indicates the safety door is closed.
    - **Water Degassed**: a green check indicates the water has been degassing for a sufficient length of time. A yellow triangle indicates that the water has not degassed for a sufficient time but may be overridden in Method Options.
    - **Temperature within limits**: a green check indicates the water is within the temperature range specified by the currently selected method. A yellow triangle indicates that the temperature has not reached the method's specified temperature range but may be overridden in Method Options.
    - **Light Curtain**: a green check indicates that the light curtain is not violated.
    - **Water Bath Present**: a green check indicates the acrylic water bath's presence is detected.
    - **Method Valid**: a green check indicates the selected method includes a valid plate definition file and treatment parameters. A red X indicates the method is not valid and cannot be run. Use the Method Editor to correct the problems.
    - **API Connected**: "No" is the default condition, indicating that the E220-plus is not under external automation control. "Yes" indicates that the instrument is under external control, but most of the functionality continues to be available, unless the external control is executing a treatment method. In this case, a method may not be started, and Motion and Water controls are locked out. A user can still pause or abort the method.

- Motion Control displays the Motion Control panel, which contains buttons for moving the sample tray to predefined positions.
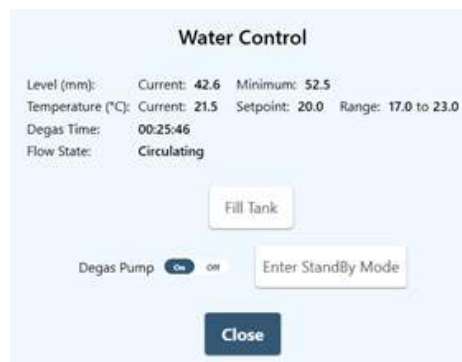

  - **Home**: initializes the motion system and moves the sample tray to its initialized position.
  - **Load**: moves the sample tray to the front of the safety enclosure to facilitate loading samples.
  - **Start**: moves the sample tray to the first treatment position as specified by the current selected method.
  - **RFID**: moves the sample tray to the position in which a plate's RFID tag is read by the RFID reader.
  - **Service**: raises the sample tray and transducer assembly allowing the water bath to be removed from the instrument.
  - **Stop**: stops all current motion.

- Water Control displays the Water Control panel, which contains both water system status indicators and user control functions.

Water Control Pane without AutoFill Enabled   Water Control Pane with AutoFill Enabled

- **Water System Indicators**
  - o Level
    - Current: indicates the current bath water level.
    - Minimum: indicates the minimum level which will allow the currently selected method to run.
  - o Temperature
    - Current: the current temperature of the water bath.
    - Setpoint: the water bath temperature specified in the currently selected method. A treatment begins when the water temperature is within the range specified by the method.
    - Range: the temperature range over which the currently selected method will run, once treatment has begun. If the bath goes outside this range while a method is running, the method is paused until the bath is again within the range.
  - o Degas Time: the remaining degassing time before a method can be run (unless the degas timer is overridden).
  - o Flow State
    - Circulating: the bath water is circulating between the bath, chiller, and WCS for temperature control and cleaning.
    - Filling: the bath is being filled automatically (AutoFill is enabled).

- **Fill**: the bath is being filled either by the optional AutoFill hardware, or SonoLab has presented a message to the user indicating water must be added to the bath.
- **Water System Controls**: the Water System Fill Bath and Stop Fill buttons will only be enabled if optional AutoFill hardware is configured.
    - o Fill Bath: clicking the Fill button causes the optional AutoFill system to fill the E220-plus water bath to a level of approximately 63 mm. The Fill button becomes the Stop Fill button while bath is filling.
    - o Stop Fill: clicking on the Stop Fill button aborts a filling process.
    - o Degas Pump: clicking this button toggles the degas pump on and off.
    - o Enter Standby Mode: clicking this button disables the chiller's temperature control but maintains water circulation through the WCS 2.0 to maintain water quality.

- Method Options displays the Method Options panel, which contains controls for overriding degassing or temperature parameters.

- Override Temp: when On, the method will run regardless of the bath water temperature; when Off, the bath water temperature must be within the temperature range specified by the method.
- Override Degas: when On, the method can be run regardless of the degas timer state; when Off, at startup, the system will degas for approximately 45 seconds per millimeter of water level in the bath (about 45 minutes for a full bath). After initial startup, if more than 1 millimeter of water are added to the bath, the degas timer is set to 45 seconds per millimeter of water added.
- Consumable-based Method Selection: limits the list of available treatment methods to those compatible with the instrument. Refer to **Appendix A** for instructions on using this feature.

- Other Controls:
    - Light is a control and indicator. Clicking on the switch toggles the cabinet light and indication of the light status on and off.

5.2 The Schedule Screen

The Schedule screen is used to configure bath Periodic Fill (if AutoFill is implemented) and scheduling daily Ready and Off states. In the Ready state, the water bath is degassed, and the water bath temperature is at the temperature specified in the current selected method. In the Off state, Periodic Fill and temperature control are turned off, but the circulation pump is on to circulate the water through the filter and UV chamber of the WCS, preserving water cleanliness. The selected method is set to none.

The operator must be logged in as Administrator to adjust the parameters on this screen.

- Fill on Startup
    - The water bath is filled when SonoLab is started if the AutoFill option is enabled. The AutoFill option consists of hardware to implement connection of plant deionized water to your system so that sufficient bath water is automatically maintained to allow instrument operation. Refer to the Appendix of this manual for instructions on making connections for AutoFill.

- Periodic Fill
    - SonoLab checks and adjusts the water bath level at the start of each treatment method. However, if the instrument will be idle for long periods of time during the day, the Periodic Fill function can be configured to periodically check the bath level while the system is idle. If necessary, the water level will be corrected, degassed, and the temperature adjusted so that the system will be ready when needed. The AutoFill option must be enabled, as described above, to use Periodic Fill. By default, the water level is checked every 15 minutes when the instrument is idle, and before each method is run. If necessary, water will be added to the bath followed by a delay to allow degassing of the added water to be executed. Degassing time is approximately 45 seconds per millimeter of water added to the bath. The 'Periodic Fill' switch on the scheduler screen is disabled if AutoFill is not implemented.
    - Click the Periodic Fill On/Off switch so that On is highlighted dark blue.
    - Enter frequency desired for checking the water level. The default value is every 15 minutes.

- Schedule
    - To configure the scheduler, first click on the Schedule On/Off switch so that On is highlighted dark blue. To configure a specific day, for instance Sunday, click in the Sunday line of the chart, then click the Edit button, or just double-click in the Sunday line of the chart, to display the Schedule Settings screen.

There are two sections of the Schedule Settings screen, READY and OFF. READY is for configuring a time for the system to be ready to run, typically ahead of a daily plant startup in the morning; OFF is for placing the system in an idle state, typically at the end of the day.

- Ready
    - Click on the on/off switch until On is highlighted in dark blue.
    - Click on the Ready Time drop down list and select the time the E220-plus will be ready to run treatment methods.
    - Click on the Method drop-down list and select the method to be run.
    - Water level will be checked at the beginning of each method run. But, if the E220-plus may be idle for long periods of time, click the Enable Periodic Fill on/off switch so that On is highlighted in dark blue. SonoLab will automatically maintain the correct water level, degassing, and temperature parameters.
- OFF
    - Click on the OFF section on/off switch so that On is highlighted dark blue.
    - Select the time for the system to switch to standby (6:00 p.m. in this example) from the drop-down list.

Click the Save button at the bottom of the screen when both Ready and OFF are configured. The updated Schedule screen is displayed.

Click Select All, and then Edit if all seven days are to use the same scheduling parameters. Click Select Weekdays, then Edit if only the five weekdays are to use the same scheduling parameters.

NOTE: If a method is running at the time the scheduler is set to switch the instrument to the OFF state, SonoLab ignores the scheduler and the instrument is not switched to standby on that day.

5.3 The History Screen

The History screen provides graphic and textual display of information for every treatment method run on the E220-plus system.

When SonoLab is started, the most recent 100 results are loaded and displayed in the scrolling list, showing the name of the method with the date and time the method ran.

The status bar's Next 100 button loads the next 100 less recent files. The Previous 100 button loads the next 100 more recent files.

The Go To control allows the operator to select files run on a specific date. The files on the specified date will be displayed at the top of the list.

Double-click on one of the listed treatments to see details of the treatment run. Click the [+ Details] button at the top left to display additional information regarding the method run above the graphics.

Start, End, Run Time, and other information

Yellow indicates the area of the plate that was treated.

Graphical representation of power and temperature during treatment. AIP should be approximately equal to PIP times Duty Factor. In this case, about 20 Watts (100 x 20%).

History files are found at C:\Users\Public\Public Documents\Covaris\SonoLab 10\History. Each treatment method's history data are contained in a folder whose name is the same as the method with the date and time of the run appended. For example, the folder for a method named "300_bp" ran on June 1, 2019 at exactly 10:30AM is named "300_bp_06_01_2019-10-30-00".

The history of a treatment consists of three files found in its folder, the Measurements, Summary, and Treatments files.

Measurements, a .csv file which can be opened as a spreadsheet, contains information about the methods run, including times, positions treated, acoustic power, bath water temperature, and bath water level.

The Treatment file is also a .csv file which contains treatment parameters, start and end times for the method, along with water level, and temperature data. The Treatment file contains the data sent to the LIMS.

The Summary is a .xml file containing textual information which is shown along with the graphics in the History display.

5.4 The About Screen

The About screen contains information about the components within the E220-plus system, such as serial numbers, software, and firmware versions. The information on this screen can be provided to your Covaris support personnel assisting with the problem resolution.

5.5 The Admin Screen

The ADMIN tab is only available if an administrative user is logged in to SonoLab. The ADMIN tab is used for managing users, their permission levels, and access to treatment methods. It is also used to turn user authentication on and off.

5.5.1 User Authentication

SonoLab's default configuration requires no login to create, run, and edit methods. User Authentication may be enabled to limit access to SonoLab functionality. To enable User Authentication:

- Start SonoLab
- Click the Log In button at the upper right corner of the SonoLab screen
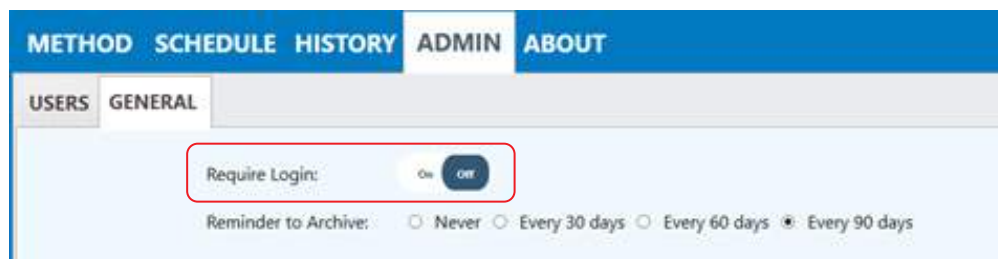

- The Login pane is displayed

- Enter the username "Administrator" and password "admin". The password is case sensitive.
- Click on Login. The ADMIN tab is displayed on the SonoLab window.
- Click on the ADMIN tab. The list of default SonoLab users, their roles, and assigned methods is displayed, along with the "USERS" and "GENERAL" tabs at the top left.

- Click on the "GENERAL" tab.

Click the On/Off switch to turn on "Require Login". If "Require Login" is not on, the Default user is active when SonoLab starts and no other user has logged in. An administrator can adjust the role of the Default user to allow this user the permissions desired. By default, the Default user has Editor permissions, access to all methods, and Optional Consumable-based Method Selection control. See **Section 5.5.2** below for details on users and their roles.

5.5.2 Users and Roles
There are three permission levels within the user authentication, Administrator, Editor, and Operator.

An Administrator:
- Can add or edit other Administrator level users.
- Can add or edit Editor level users.
- Can add or edit Operator level users.
- Can assign access to specific methods to Administrators, Editors, and Operators.
- Can run and edit any methods installed on the system.

An Editor:

- Can run and edit methods assigned to them by an administrator.
- Can create methods.
- Can review all history screens saved on the system.

An Operator:

- Can run methods assigned to them by an administrator.
- Can review all history screens saved on the system.

5.5.3 Managing Users

To create a new user:

- Log into SonoLab as Administrator and click on the ADMIN tab.
- Click the New button at the upper right side of the screen. The New User pane is displayed.

- Enter a new Username, the user's first and last names, and click on the "Assigned" button. By Default, all treatment methods on the system will be assigned to the new user.

The SonoLab default is to assign all available methods to a new user. To remove the new user's access to one or more treatments:

- Click on the method(s) to be removed in the list at the left. Multiple methods may be selected by clicking "Shift" or "Control" simultaneously.
- Click the right arrow button. The methods to be removed are moved to the box at the right.
- Consumable-based Method Selection can be set to Always On, Always Off, or Optional. Enabling Consumable-based Method Selection is done in the Method Options pane, described in **Section 5.1**. Operation is discussed in **Section 3.3**.

If Consumable-based Method Selection is set to Optional (the default), Consumable-based Method Selection can be selected by the user via the switch in the Method Options pane. Refer to **Section 5.1** for a description of the Method Pane, and **Appendix A** for selecting and running methods with Consumable-based Method Selection.

If Consumable-based Method Selection is set to Always On or Always Off for a user, this setting takes precedence, and the switch in the Method Options pane is disabled.

- Click Save to save changes and exit the New User screen. The new user now appears in the list of system users.

When a new user is created, the user must click the 'Set Password' link at the bottom of the login pane the first time the user tries to log in. The user enters their new password, and then must enter it again to verify correct spelling. Clicking "OK" returns the user to the login screen where the user may now enter their new password and log in.

6. Create and Edit Methods

The SonoLab Method Editor supports the creation of new methods and editing of existing ones.

A method consists of one or more Processes. A Process contains at least one Treatment. Most methods will contain a single process with a single treatment step.

If more complex methods are required, they can be easily constructed.

6.1 Create a New Method

1. Click the "New" button on the METHOD screen.

• The Method Editor window will appear.

Plate Definition Drop-down List

Add Process
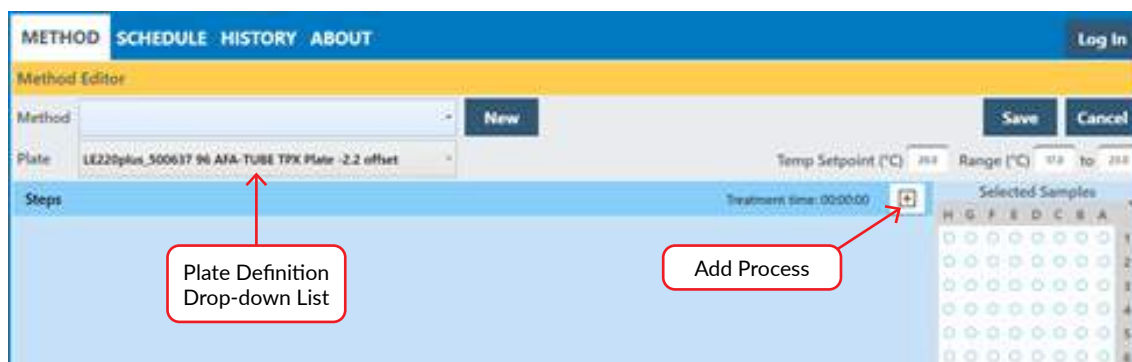
2. Select the appropriate plate for the consumables used in this method from the "Plate Definition" drop-down list.

3. Click the + New button. The "Choose a Step Type" pop-up is displayed.

4. Click "Process". The process bar is displayed.
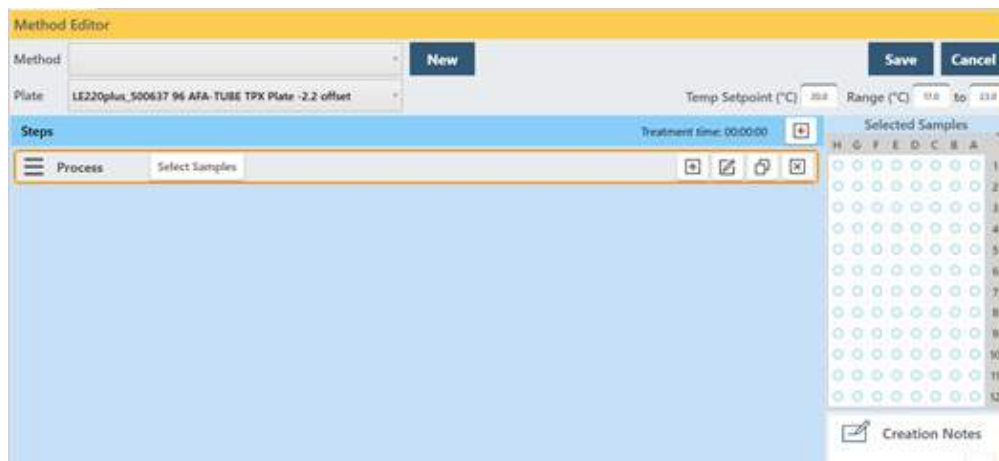
5. Click the + button inside the process bar to display the step type menu.
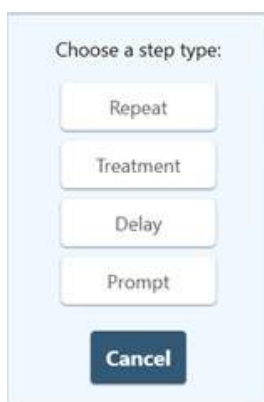
6. Choose Treatment to specify the four acoustic parameters: Duration, Peak Incident Power, Duty Factor, and Cycles per Burst.

Treatment parameters are chosen by directly editing the value boxes. The treatment parameters are:

- **Peak Power (PIP):** This parameter measures the peak electrical power used to drive the ultrasound transducer. The adjustable range of Peak Incident Power is 1.5 to 500.0 Watts.

- **Duty Factor (DF):** This parameter defines the ratio between the on-time of the transducer and total operation time and is expressed as a percentage. For a given Peak Incident Power, a larger Duty Factor results in more acoustic energy generated from the transducer. Users can specify a Duty Factor from 1 to 50%.
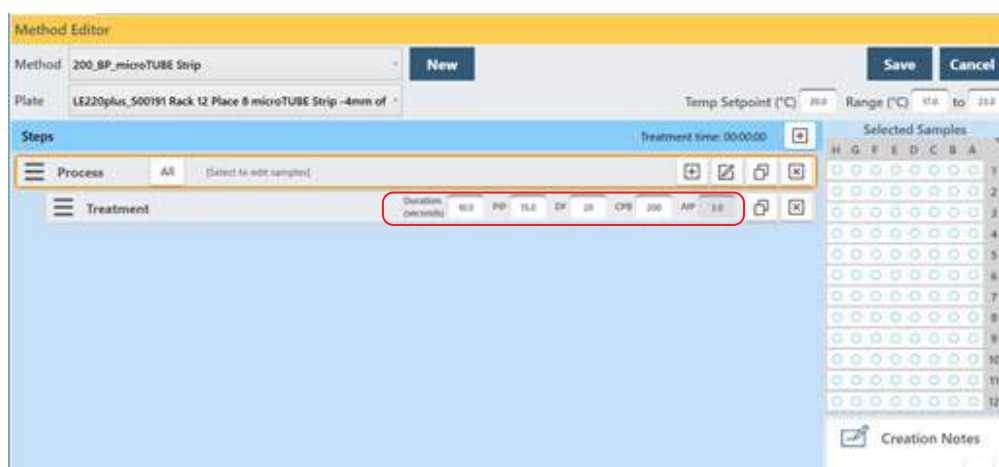
  The product of the Peak Power and Duty Factor gives the approximate Average Incident Power (AIP) generated from the transducer. For example, if Peak Power is set to 10 Watts and Duty Factor is set to 15%, the Average Incident Power will be approximately 1.5 Watts. However, the real Average Incident Power from the transducer may vary due to factors such as sample tube type, water level and water temperature.

  The maximum Average Incident Power (AIP), the product of PIP times Duty Factor (DF) is limited to 250 watts on all E220-plus and LE220-plus instruments.

- **Cycles per Burst (CPB):** This parameter indicates the number of electrical signals used in each acoustic "burst" of the transducer. The range of Cycles per Burst (CPB) is an integer from 50 to 1000 for different applications. Advanced users may use this parameter to fine tune their experiments.

- **Duration:** This parameter defines the amount of time for which the sample is under a treatment. The measurement of the Duration is in seconds. The maximum time for any treatment is 3600 seconds. If a longer treatment is necessary, "Repeat Steps" can be used to extend the treatment.

SonoLab displays the Average Incident Power, calculated from the Peak Incident Power and Duty Factor, adjacent to the treatment parameters in the treatment step bar. The maximum Average Incident Power is 250 Watts.

If a parameter exceeding the limits is entered, a red outline is displayed around the entry and a message is displayed in the "Steps" bar, as shown below.

In this example, 600 watts exceeds the 500 limit for PIP. The method Save button is disabled until the parameters are corrected.

- **Dithering**: The motion system provides dithering motion capability in the X axis (fore-&-aft), Y axis (left-to-right), and Z axis (up-and- down). If a treatment specifies dithering, click on the "Pen-&-Paper" icon on the right end of the Process bar to dislay the Process Step Parameters pane.

The X, Y, and Z +/- distance range is 0 to 5 millimeters for the X and Z axes, and 0 to 4.5 for the Y axis, unless restricted by the plate definition file. The Speed range is from 0 to 25 mm/second. The Pause Duration range is from 0 to 60 seconds.

7. Enter the desired dithering parameters, if any, and click "OK".

8. Enter the temperature set point for the protocol and the allowable range into the text entry fields. SonoLab allows a minimum range of plus/minus 2 degrees. The default range is 3 degrees.

9. Once the treatment parameters have been defined, for LE220-plus and LE220Rsc instruments, click the "All" button in the 'Process' bar to select all columns for treatment. Alternatively, click the 'Process' bar, then on individual columns to treat, or drag across columns to select multiple, contiguous columns. drag across the plate graphic to the right of the edit screen to select columns for treatment.
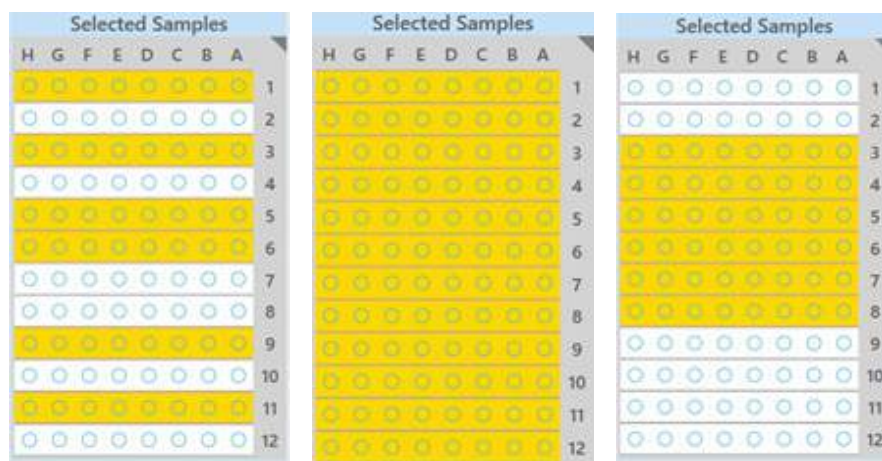
Click to Select Random Columns   All Columns Selected   Drag to select Contiguous Columns

The LE220-plus, LE220R-plus, and LE220Rsc support treatment of 384-well plates in both indexed (one column at a time) and Scanning (LE220Rsc only) modes. For treating 384-well plates, only adjacent pairs of columns may be selected for treatment. Thus, the system behaves as if it were treating a 96-well plate.

Indexed treatment steps treat two columns at a time. Scan steps treat one or more contiguous pairs of columns. When using Position Restrictions, columns are specified in a range of 1 to 12, not 1 to 24.
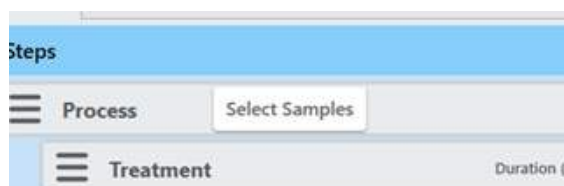
For E220-plus instruments, click the Select Sample button in the Process bar to display the Select Samples pane.
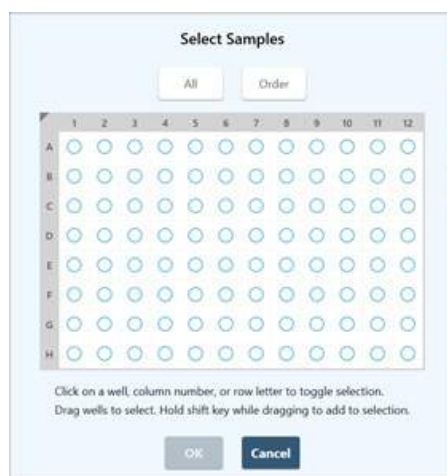
Then click in the treatment well graphic to define which plate positions will be treated.
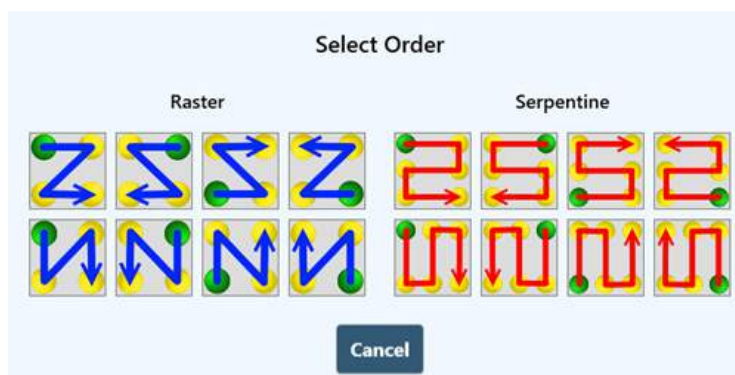
The E220-plus treats a single well at a time. Any number of wells may be selected for treatment by clicking on the wells; wells selected for treatment turn from white to yellow

Method Editor window.

All wells selected for treatment.
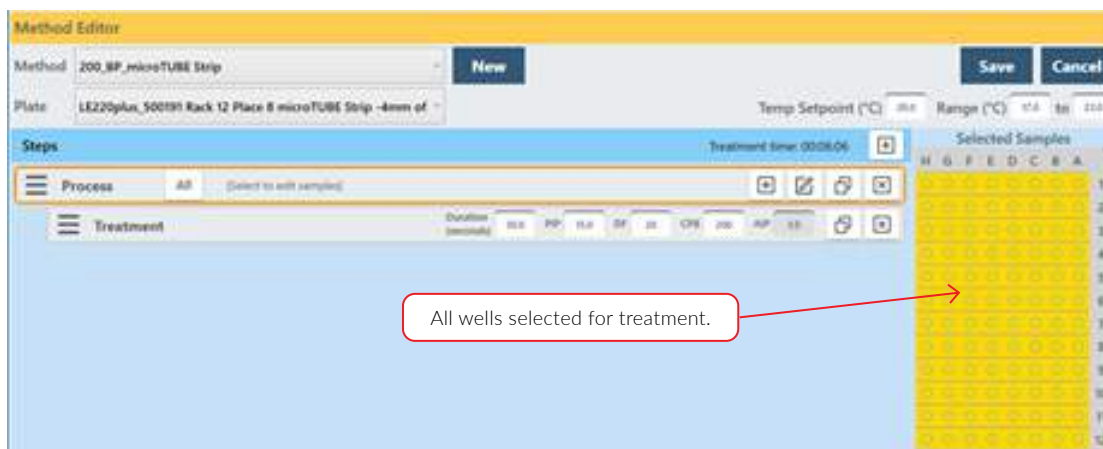
10. Add a Delay Step if desired, choose a Delay step to introduce a fixed time delay into a method to allow the sample to settle between the two different treatment steps. The delay is specified in seconds.

Enter Delay in Seconds
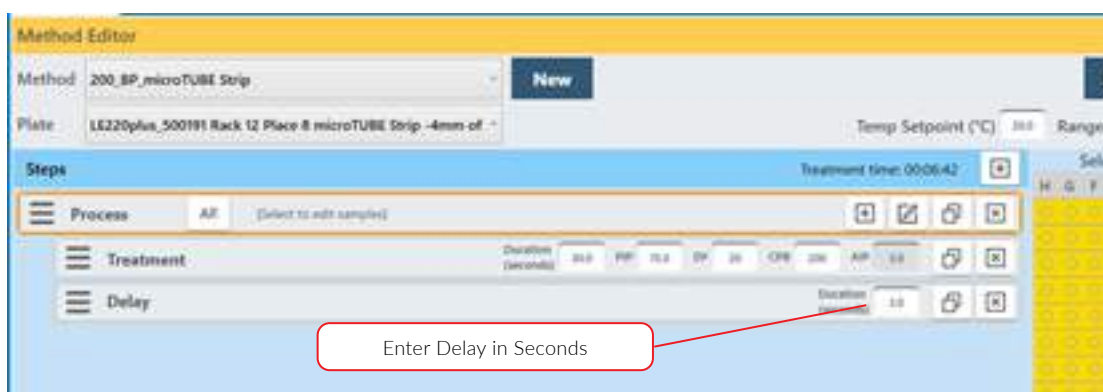
11. Add a Prompt Step if desired, choose a Prompt step to display a message on the screen and pause execution of the method until the operator clicks OK.
   a. Click the "pen & paper" button of the prompt bar to display the text entry field for the prompt.
   b. Enter the text of the prompt and click OK. See Figure below.

Click to create or edit text of the prompt
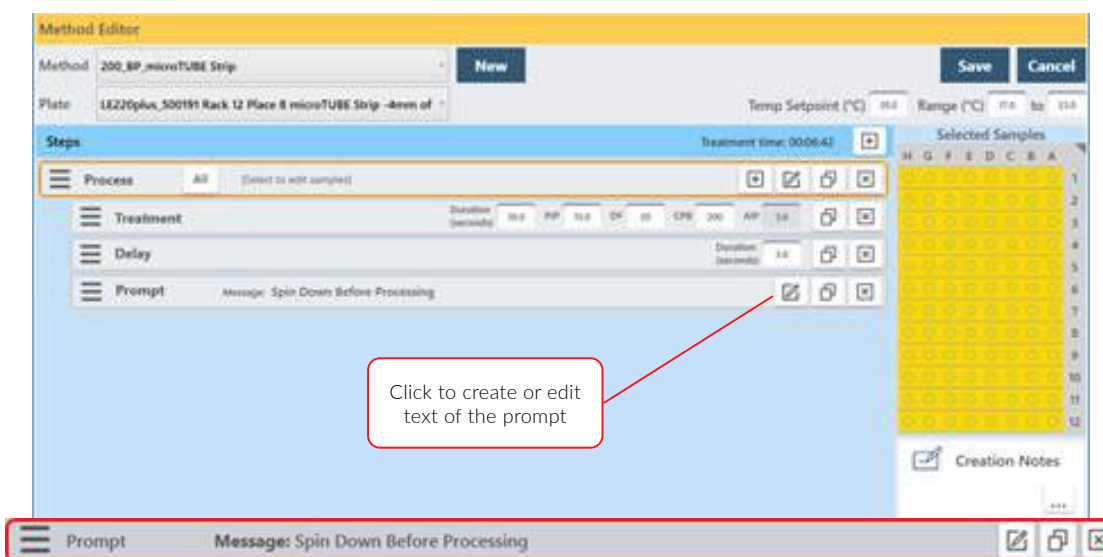
When the prompt step is reached during the treatment, the method pauses, and the prompt message will be displayed as shown.

Example of a Prompt Message Display

12. Enter descriptive text into the "Creation Notes" section at the lower right side of the Method Editor window, if desired. This field may be left blank.

13. When all method parameters and steps have been defined, click on the Save button to save changes. The user is prompted to name the method.

14. Enter a name for the treatment method and click Save.

When the method is run, the 'Current Column' ('Current Sample' for E220R-plus) Indicates the Column (Sample) under treatment.
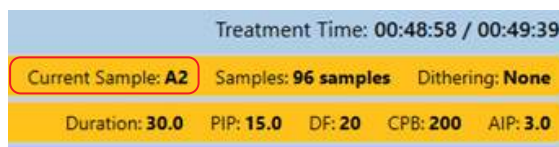
6.2 Using the Repeat Function

The Repeat Function is used when one or more treatments, delays, or prompt steps are to be applied to each column (sample in the case of the E220R-plus) of the plate multiple times. There are two scenarios for how this may need to be performed, and the required scenario determines how the repeat function is implemented.

In Scenario A, all treatment (or delay or prompt) steps are applied to the first column (sample) the number of times specified in the Repeat bar, then all steps are applied to the next column (sample). This continues until the entire plate is treated.

Scenario A is used if each column (sample) requires one or more acoustic doses, but there is no need for resting the sample between doses. Scenario A is also referred to as Pulsing.

6.2.1 Implement Scenario A:
- Create a method with a Process step, as explained previously
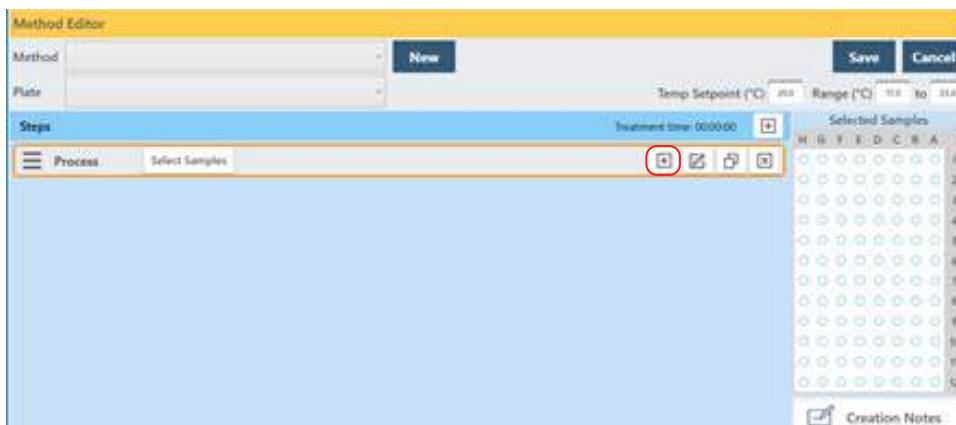
- Click the + button inside the process bar to display the step type menu.

- Click on Repeat.
- Click the + button inside the repeat bar to display the step type menu, and create two or more treatment or delay steps. See image below.

Quantity of Repeat Iterations
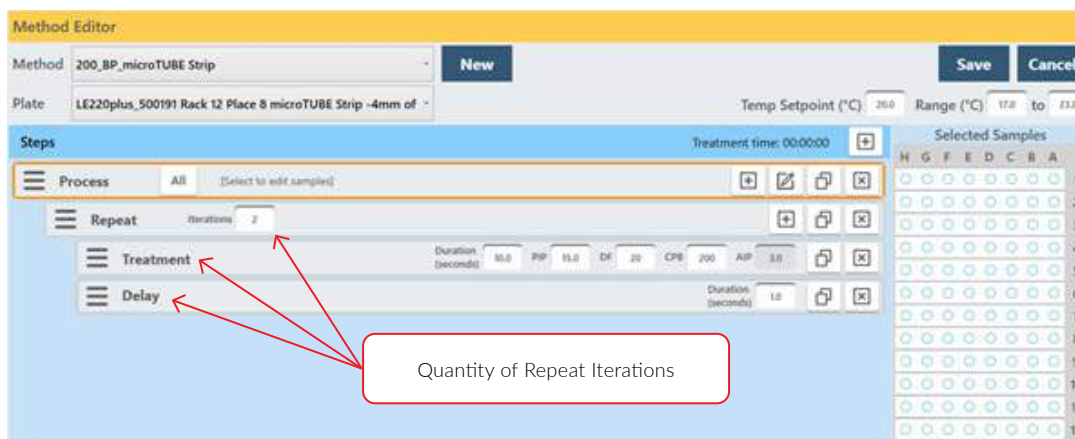
All listed treatment (or delay, etc.) steps will be applied to the first column (sample) selected for treatment based on, the number of times specified in the Repeat bar. Then all will be applied to the second column (sample), and so forth, until the entire plate is treated.

- When all method parameters and steps have been defined, click on Save to save changes.

In Scenario B, all treatment (or delay or prompt) steps are applied to all selected columns (samples) of the plate once. This repeats for the number of times specified in the Repeat bar.

Scenario B is typically used when a column (sample) is treated with an acoustic dose and is allowed to rest while the remaining positions are treated. Once the remaining positions are treated, a second acoustic dose is applied to each column (sample). Scenario B is also referred to as Cycling.

6.2.2 Implement Scenario B:
- Create a method as explained above but select "Repeat" instead of "Process" for the step type.
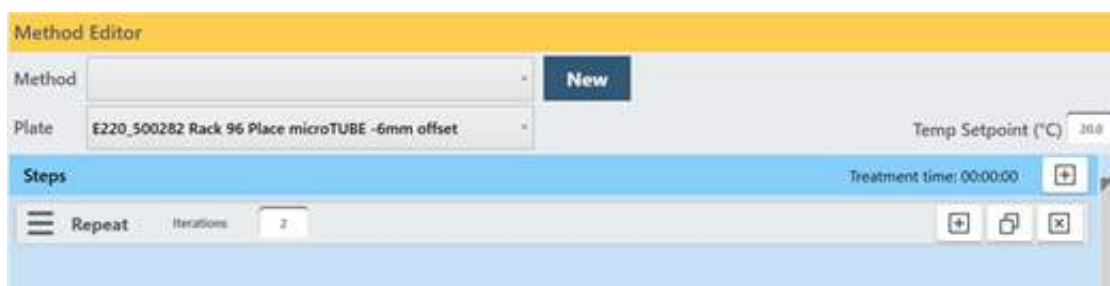
- Click the + button inside the Repeat bar and select Process.
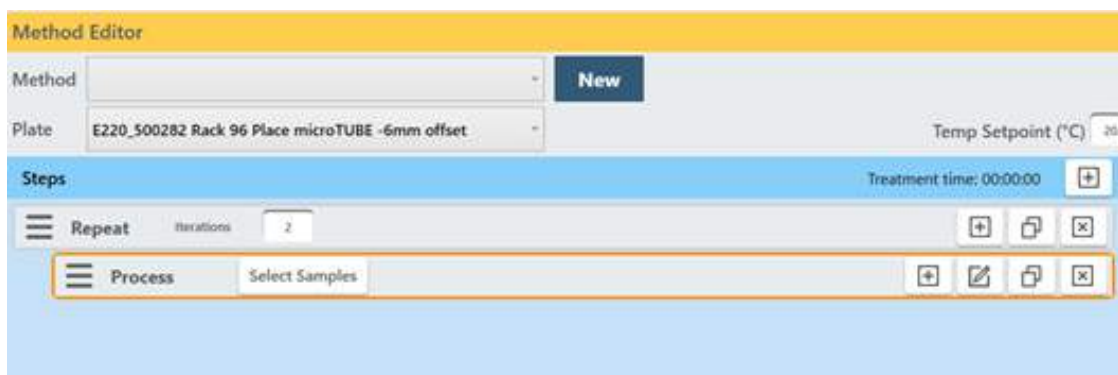
- Click the + button inside the Process bar and create one or more treatment steps. Two treatment steps are created in this example.
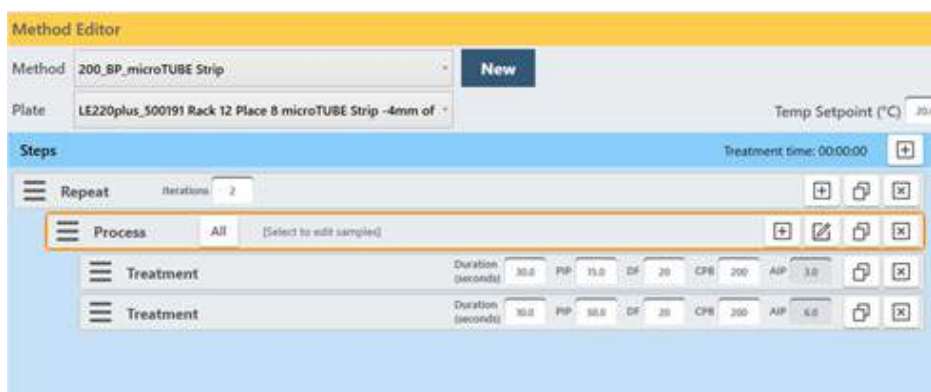
- All treatment steps will be applied to all selected columns (samples) of the plate once and then repeated for the number of times specified in the Repeat bar. That is, both programmed Treatments will be given to column 1 (sample A1), then both Treatments will be given to column 2 (sample B1), and so forth until the entire plate is treated and then the treatment steps are repeated.
- When all method parameters and steps have been defined, click on Save to save changes.

6.3 Scanning Treatments (LE220Rsc Only)

In a scanning treatment, rather than the sample tray indexing to place one column of the sample tray at a time over the transducer, the sample tray moves continuously over the transducer. In this mode, acoustic dose is a function of the speed at which the sample tray moves and the number of passes. One or more rows may be treated, but the rows must be adjacent.

6.3.1 Create a scanning treatment:
- Click the "add step" button.

- Select Repeat from the step type list.
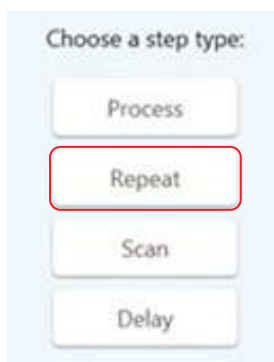
Enter the desired number of scans in the "Iterations" field. One iteration passes the sample tray over the transducer one time. If only a single scan step is desired, the 'Repeat' is not necessary and a 'Scan' step may be selected here and the next two steps below skipped.

Enter Number of Scans — Click to Add Scan Step
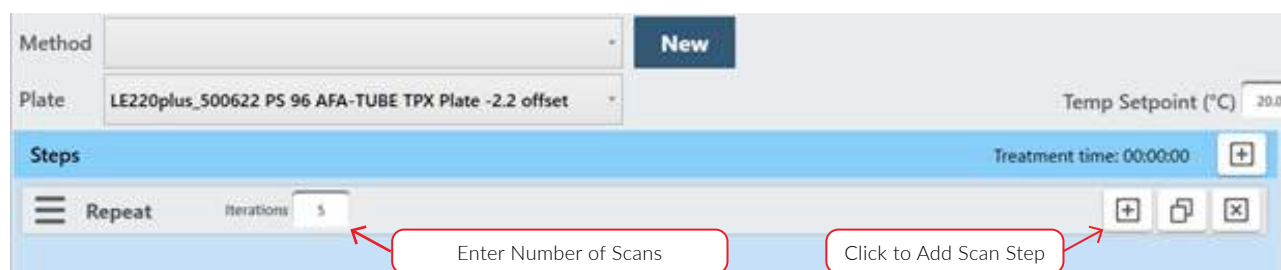
- Click the "+" button located on the 'Repeat' bar, as shown above.
- Select Scan from the Step Type list.
- Enter the desired treatment parameters as for a normal treatment step.

Note that the first parameter is "Speed", not "Second" as in non-scanning treatments. The total length of a treatment dose in a scan treatment is a function of the scan speed and the number of scans.

- With the Scan step outlined in orange, click and drag on the columns to be treated. Entire or partial plates may be treated. When treating a partial plate, the columns being scanned must be contiguous. SonoLab will not accept a non-contiguous selection. For 384-well plates only contiguous pairs wells may be selected.
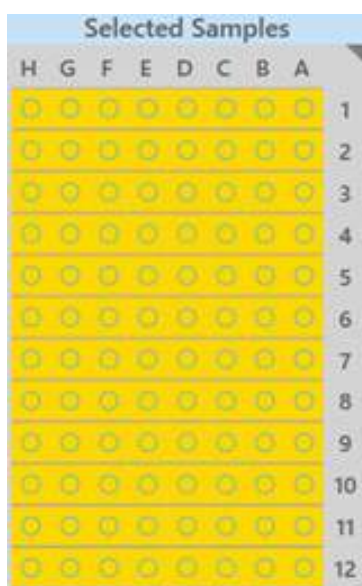
Full Plate Selected for Scanning   Partial Plate Example
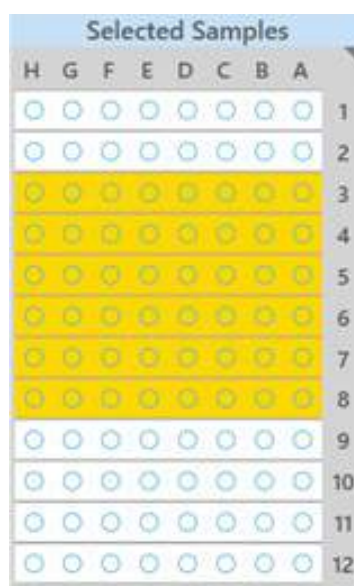
- Click the Step Edit button to display additional Scanning step options.
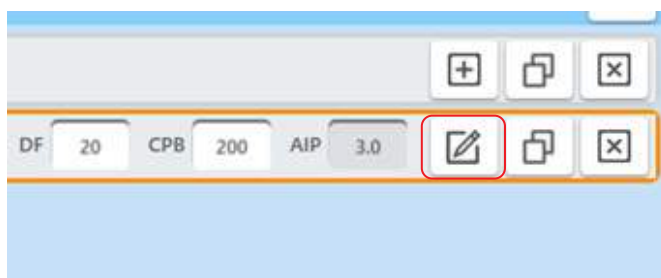
The 'Scan Step Parameters' pane provides controls to enable dithering and Bidirectional scanning.

A standard scan steps treats the sample only as the tray is moved forward over the transducer. Bidirectional scanning increases throughput by applying the acoustic dose as the sample tray moves over the transducer in both directions. Specifying 1 iteration in the Repeat step results in a single, normal scan moving toward the front of the instrument. If more than 1 iteration is specified, odd numbered iterations will move from the rear toward the front, even numbers from front to rear.

- Click Save and give the method a name as with normal treatment methods.

Contact our Covaris Applications Support Department for assistance in specifying the appropriate scanning speed and treatment parameters for your application.

6.4 Using Drag and Drop with Treatment Steps

The order of treatment steps may be rearranged, and Treatment steps may be moved in and out of Process steps and Repeat steps to optimize treatment or expedite experimenting with different orders of operations. In the example below, there are initially a single process and three treatment steps as shown.

Drag-and-Drop Handles

PIP values top to bottom:
15, 300, 100

To swap the positions of the second and third steps:
- Click and hold on the three bars at the left of the third step. A 'Move Treatment' box will hover over the mouse.
- Drag the 'Move Treatment' box over the first treatment step until a blue line shows between the first and second steps.
- Release the mouse button when the blue line appears. The former third step will be inserted below the first step ahead of the former second step.

Move Treatment Box

Blue Line between first
and second steps

PIP values now top to bottom:
15, 100, 300
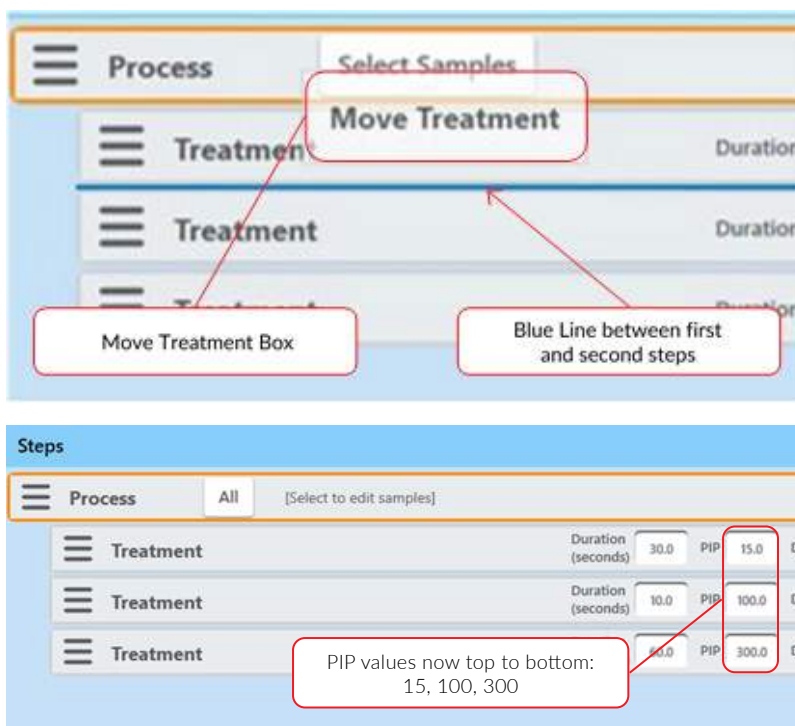
To move treatment steps into a repeat step:
- Click the '+' button in the process bar to display the step types list as explained previously.
- Select 'Repeat'. The new Repeat step displays below the three treatment steps.
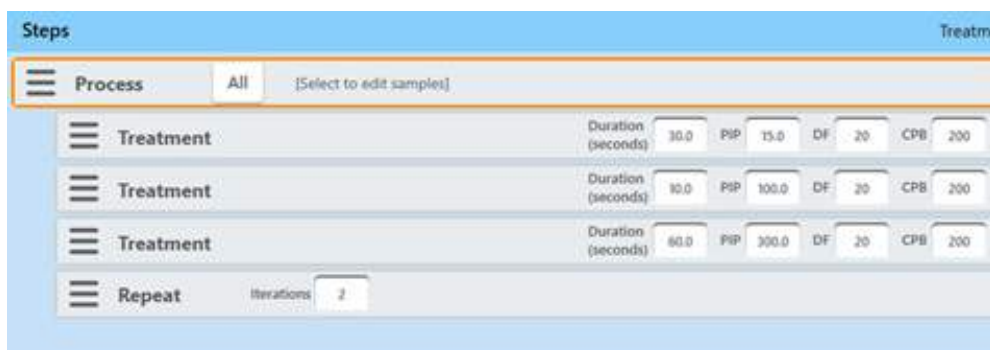
- Click on the three bars at the left of the first Treatment step.
- Drag it over the Repeat step until the blue line shows below the Repeat step.
- Release the mouse button.
- Do the same for the two remaining Treatment steps. The screen is now configured as shown below.
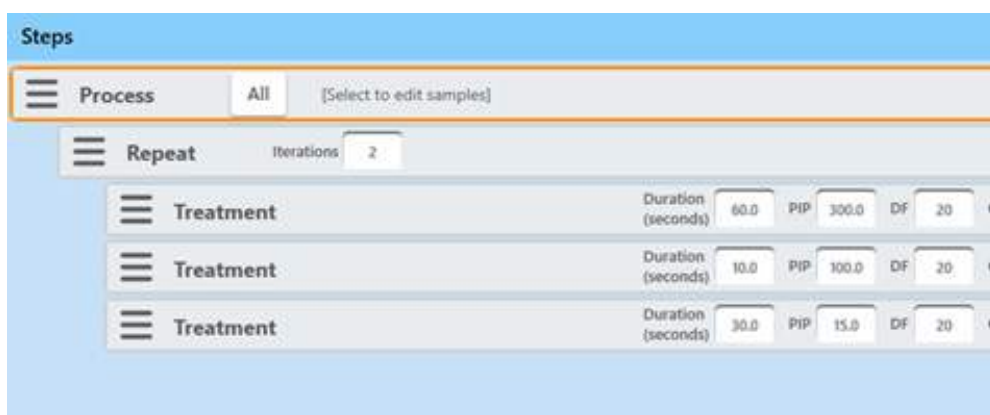
When finished, specify the samples to be treated and save the method as described in **Section 6.1** above. Behavior of Repeat steps is described in detail in **Section 6.2** above.

6.5 Modifying Existing Methods

To modify an existing method, select the method as described above, then click the "Edit" button. The method editor window will be displayed.
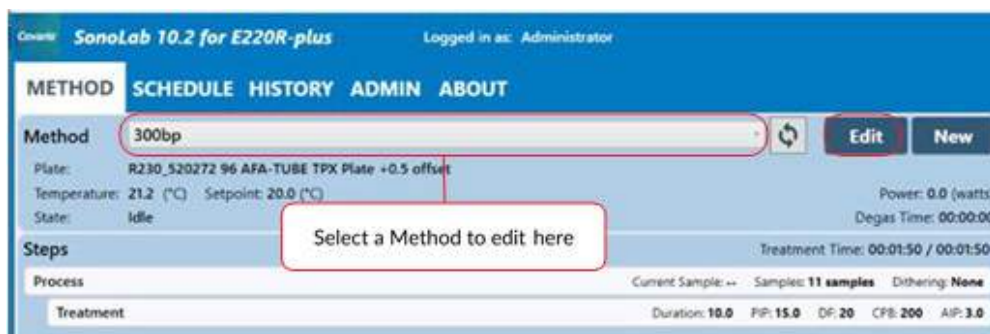
Make desired changes and click 'Save'. The user is then prompted to confirm if they wish to overwrite the existing method.

7. System Specifications

Treatment System	Bench-top; high intensity acoustic transducer, temperature monitoring device, circulation pump, water bath with safety enclosure
Treatment Power	500 Watts Peak Incident Power, 250 Watts Average Power
Dimensions	23" W x 30" D x 19" H (59 cm x 76 cm x 48 cm)
Weight	Approximately 110 lbs. (50 kg)
Power Requirements	100 to 240 VAC 500 VA maximum, 50 to 60Hz
Ambient Temperature Range	19 to 25°C (66 to 77°F)
Ambient Humidity Range	30 to 70%
Regulatory Labeling	CE, ETL Mark (for Product Safety), WEEE
Safety	Complies with Low Voltage Directive 2006/95/EC. Certified to IEC/EN/ANSI/UL 61010-1:2004 and CAN/CSA C22.2 No. 61010-1:2004, 2nd Edition "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements"
EMC	Complies with Class A Industrial/Scientific/Medical (ISM) equipment under EN 61326-1:2005, EN 61000-3-2:2004 and EN 61000-3-3:1995 for EU EMC Directive 2004/108/EC. Also FCC Part 15 Class A radio emissions requirements for the USA and ICES-003 Class A for Industry Canada.
Water Bath	Distilled or deionized water only
Computer	Notebook computer
Operating System	Microsoft Windows 10
Application Software	SonoLab 10
Data Input	Keyboard and mouse
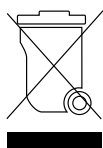
8. Maintenance

8.1 Cooling Air Intake

Unplug the unit periodically to remove any obstruction to the fans and air vents on the rear panel and to ensure that objects are not restricting the flow of air.

8.2 Water Bath Maintenance

8.2.1 Recommendations for Daily Maintenance

Only distilled or deionized water should be used to fill the water bath. To avoid algae growth, the water bath should be emptied, the degas lines purged, and the bath dried prior to longer term (more than a week) idle time. See 'Shutdown for Long Term Storage below.

8.2.2 Recommendations for Monthly Maintenance

Periodically, the water bath and degassing lines should be rinsed with a 10% solution of bleach (or equivalent, such as NaDCC).
The resultant sodium hypochlorite solution is ~ 0.5% NaOCl. Perform the following cleaning protocol:

1. Turn off the WCS and CH05 chiller and disconnect the hoses from the front of the water bath.
2. Start SonoLab and open the 'Motion Control' and 'Water Control' panes.
3. Fill the bath with approximately 450 mL of household bleach or equivalent and the rest with DI water.
4. Click the 'Home' button. The transducer will lower into the bath and the degas pump starts.
5. Operate the degas pump for approximately 5 minutes.
6. Click the degas pump 'Off' button in the 'Water Control' pane.
7. Click the 'Service' button in the 'Motion Control' pane. The transducer rises out of the bath.
8. Empty the bleach/water solution.
9. Place the empty water bath back into the instrument and click the 'Load' button. The transducer lowers into the bath.
10. Click the degas pump 'On' button in the 'Water Control' pane.
11. Wait a few seconds for the bleach/water solution to purge from the degas lines.
12. Click degas pump 'Off' button.
13. Click 'Service'. The transducer rises out of the bath.
14. Empty the bath, rinse, and fill with fresh DI water.
15. Place the water bath back into the Instrument.
16. Click 'Load'. The transducer lowers into the bath.
17. Click the degas pump 'On' button and allow the water to circulate for three or four minutes.
18. Click the degas pump 'Off' button.
19. Click 'Service' to raise transducer out of bath.
20. Repeat steps 14 through 19 two more times.
21. Fill the bath with clean DI water, reconnect and power on the WCS and CH05 chiller. Allow the water to degas for an hour prior to treating samples.

8.3 Transducer

Use 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 is damaged.

If drying or cleaning the transducer face, use a soft lint-free cloth. If broken glass must be removed from the transducer surface, carefully pick any shards off the surface with tweezers. Do not rub them away, as this may scratch the transducer surface.

8.4 Safety System

Test the system periodically. Verify that the acoustic treatments are disabled when the 'Stop' button is pressed, or if the water level in the bath is too low. Verify that the door cannot be opened during an acoustic treatment.

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

8.5 Degassing System

Do not run the degassing pump without a water bath. Extended running without water will cause the pump head to wear out. If water is low, the pump will shut off within 10 seconds.

When the pump is running, verify that there are small bubbles coming from the end of the outlet nozzle. If you suspect that the system has become clogged, please contact Covaris for assistance. There is a small inlet tube that can be removed and cleaned.

Degassing the instrument is best performed on room temperature water, as water below 15 °C will not degas as efficiently. SonoLab prevents the chiller from coming on until the bath has been degassing for 30 minutes. If being operated manually, the operator should, likewise, begin degassing approximately 30 minutes prior to powering on the chiller. If it is necessary to have the instrument available first thing in the morning, use SonoLab's built in chiller and degas scheduler in the Water Works section.

For long-term storage, water should be removed from the degassing pump lines. Follow the System Shutdown procedure in *Section 3*.

8.6 Cleaning the System

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

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

8.7 Water Bath Storage

For Long Term Storage (more than 2 weeks).

8.7.1 Empty the water bath and purge the degas pump

- Turn off the WCS and the CH05 Chiller.
- Click on the Motion button to display the Motion Pane.
- Click on 'Service'. The degas pump will turn off and the transducer assembly will rise out of the bath.
- Empty the water bath and place it back in the instrument empty.
- Click 'Home'. The transducer will lower into the bath and the degas pump will come on, purging the lines to the degas pump.
- Click 'Service' when no additional water is coming from the lines, or when the pump shuts off. The transducer will raise out of the bath.
- Turn on the CH05 chiller, leaving it in stand-by mode. Allow it to run until no more water is pumped into the bath from the WCS.
- Empty the bath.
- Rinse it with clean DI water and dry it with a soft, lint free cloth.

If the instrument is being placed in indefinite storage, remove the particulate filter from the WCS. Refer to instructions in the WCS manual as necessary.

> **NOTE**: Since the particulate filter will remain wet internally even after draining, over a period of a   couple weeks or more, some biological growth may take place, potentially leading to filter blockage and reducing filter life.

8.7.2 Close SonoLab

Exit SonoLab by clicking on the X in the upper right corner. The following message is displayed.

Click "Cancel" to cancel this request. Click "Move" and the transducer is moved to the service position and SonoLab closes. Take no action, and the transducer is moved to the service position and SonoLab closes following a 20 second countdown. Click "Exit" to close SonoLab immediately without moving to the service position.

8.7.3 Turn System OFF

To shut down the laptop, select Shutdown from the START menu on the Windows screen and select the option SHUTDOWN. Then the computer will power down. Power down the instrument using the switch located near the power entry module of the instrument, at the rear of the right side.

> **CAUTION**: Leaving water in the water bath or degassing lines for an extended time may promote algae growth in the bath and lines, this growth can interfere with water quality and sample processing.

9. Troubleshooting

This section provides a list of symptoms and recommended actions that may be taken to correct simple problems. Please contact the Covaris Technical Support Dept. or your local representative for assistance with any issues.

- If your instrument will not connect to SonoLab:
  - Verify that the instrument is ON.
  - Verify that the USB cable is connected at both ends and undamaged.
  - Close and restart the SonoLab application.
  - Go to Windows Device Manager (right click on 'This Computer', then Properties) and verify that Windows is recognizing the instrument, and that device drivers are properly installed. Under Ports (COM & LPT), there should be four devices listed:
    - o Covaris RF Controller (COM3)
    - o Covaris RF Controller (COM4)
    - o Covaris RF Controller (Port3&4) (COM5)
    - o Covaris RF Controller (Port3&4) (COM6)

    The COM assignments may be different from those listed above (e.g. COM 7, 8, 9. 10). Under Universal Serial Bus Controllers, there should also be four devices listed:
    - o Covaris RF Controller Board
    - o Covaris RS-485 Port
    - o Covaris Serial Port 2
    - o Covaris Serial Port 3

    Each of these devices should be listed. If any appear with a yellow "!" symbol, right-click and try to re-install the device driver.
- Safety Interlock or Sensor Failures (Water Level, Water Bath Present, Door Closed, Water Temperature). These are generally failures that require the attention of a service technician, but please verify the following items before contacting Service:
  - Water Level: Verify that the water level in the bath is at or above the "0" mark on the WATER LEVEL label. Verify that the Acoustic Assembly is fully lowered into the bath.
  - Bath Present: Verify that the Acoustic Assembly is fully lowered into the bath. Verify that the bath is slid fully into the enclosure, so that the magnet embedded at the rear of the bath can be sensed. Verify that the magnet is embedded in the rear of the bath.
  - Door Closure not recognized: Verify that the door interlock is not bypassed.
  - Water Temperature limits being reached, or inaccurate temperature readings: If the water is running hot, verify that the chiller is operating and that it is circulating water through your system's water bath. Program the set point of the chiller to a lower temperature (select a method with a lower set point specified) and verify that your system's bath gets cool. Use a separate thermometer to verify the true temperature of the bath.
- Biological or chemical results (e.g. DNA shearing) not as expected:
  - Verify that the average power values reported by SonoLab are approximately correct. The typical average power is a few percent below the value "Peak Incident Power" multiplied by "Duty Factor". For example, if PIP=200 Watts and Duty Factor is 10%, then an expected power reading would be approximately 15 to 17 Watts.
  - Verify that the water temperature is correct and that the water is clear and free of algae or other particulates. Perform a cleaning protocol with a 10% bleach or equivalent solution if this has not recently been done.
  - Verify that the degassing pump is operating (observe the pump outlet on the left side of the bath – when the pump is running there should be occasional bubbles exiting the pump outlet tube). Also examine the inlet "dip tube" visible from the right side of the bath. The inlet holes (very small drilled holes in the side of the tube) should be free of obstruction. If possible, please independently verify your analysis equipment. Covaris has DNA shearing verification kits available. Please contact Covaris applications support or your local representative for assistance.

- SonoLab reported Power Level is lower or higher than expected:
  - Verify that the average power values reported by SonoLab are approximately correct. The typical average power is a few percent below the value "Peak Incident Power" multiplied by "Duty Factor". For example, if PIP=200 Watts and Duty Factor is 10%, then an expected power reading would be approximately 15 to 17 Watts.
  - Contact Covaris or your local technical representative for assistance.
- Degas Pump problems:
  - Verify that the water is clear and free of algae or other particulates. Perform a cleaning protocol with 10% bleach or equivalent solution if this has not recently been done.
  - Verify that the degassing pump is operating (observe the pump outlet on the left side of the bath – when the pump is running there should be occasional bubbles exiting the pump outlet tube). Also examine the inlet "dip tube" visible from the right side of the bath. The inlet holes (very small drilled holes in the side of the tube) should be free of obstruction.
  - If the degas pump makes a very loud noise when operating, it may need to be replaced.
- Particulates in bath water
  - Change the bath water with fresh DI water
  - Perform a cleaning protocol with a 10% bleach or equivalent solution if this has not recently been done.
- Tube Breakage
  - Verify that the acoustic parameters being employed are correct.
  - Verify that average power levels are approximately correct (see Power Level).
- RF Board Limits Exceeded Error:
  - If Peak Incident Power is set to values above 400 Watts, intense acoustic reflections may cause an overload in the electronic circuits driving the transducer. To protect the drive circuits and transducer, the system may shut down and post a message, "RF Board Limits Exceeded RF Error is Present". Should this error appear, please retry your application with the following modifications:
    - o Fill the sample tube to eliminate splashing and large bubble formation in the sample
    - o Replace the existing bath water with new, clean bath water
    - o Lower the PIP (Peak Incident Power) – this is often more effective than applying very high power.

Repeat the method to verify that overload problems have been mitigated. If not, please contact Covaris or your local representative.

Technical Support – Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:
- Telephone
  - United States: Tel: +1 781.932.3959 during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Eastern Standard Time/EST (UTC−05:00)
  - Europe: Tel: 44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Greenwich Mean Time/GMT
- E-mail queries to techsupport@covaris.com or applicationsupport@covaris.com

10. Appendix

Appendix A: Using SonoLab Consumable-based Method Selection

SonoLab can be configured with Consumable-based Method Selection to limit the displayed list of methods to only those using a specified consumable. For individual SonoLab users, Consumable-based Method Selection is set to either Always On, Always Off, or Optional, as described in **Section 5.5.3**, Managing Users. If Consumable-based Method Selection is set to Optional for a user, the user controls whether to enable it via the switch in the Method Options pane, described in **Section 5.1**.

If a user's Consumable-based Method Selection option is configured Always On or Always Off, these setting take precedence and the switch in the Method Options pane is disabled. See 'Managing Users' in **Section 5.5.3** for details on configuring users for Consumable-based Method Selection. To select and run a method with Consumable-based Method Selection:
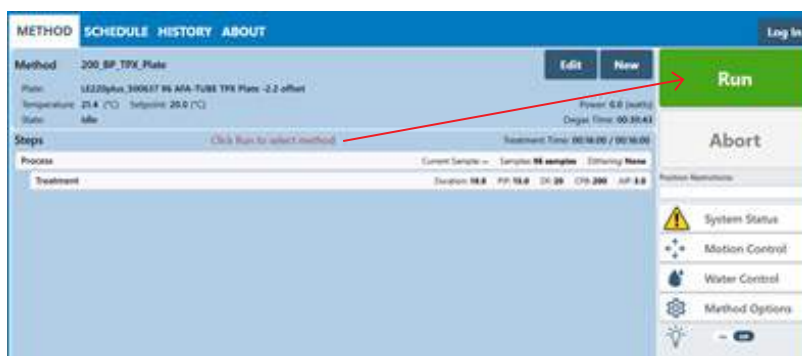
1. With the plate containing the sample on the sample tray, click the 'Run' button.
2. The instrument performs RFID validation of the inserted plate. The method run fails if the RFID check is invalid.
3. SonoLab displays a dialog listing the methods available to run. The displayed methods use the consumable part number embedded in the RFID tag. If the user is assigned specific methods, only those methods assigned to the user will be displayed in the list.
4. The user clicks on the desired method to start the treatment.
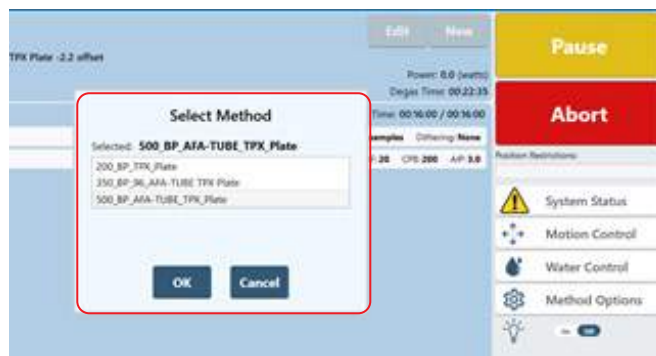
Appendix B: Using SonoLab Position Restrictions

It is sometimes convenient to run a method on a plate that is only partially populated with samples. For example, consider a method that treats all 12 columns in a 96-well plate. But now you have a plate that has samples only in columns 4 to 6. Using SonoLab Position Restrictions allows treating a subset of a method's specified positions.

Position restrictions is a list of the positions that you want to treat when a method runs. These positions are used to filter the set of positions that are treated by a method. Only positions that appear both in the method and the position restrictions list will be treated. Position restrictions do not affect the order in which positions are treated. Order of treatment is determined by the method.

The details of position restrictions depend upon whether the instrument utilizes a line or point transducer.
- Line transducers treat multiple samples (in a column) at a time. The LE220-plus, LE220R-plus, and LE220Rsc use a line transducer.
- Point transducers treat a single sample (in a well or tube) at a time. The E220-plus and E220R-plus use a point transducer.

For LE series instruments, the following types of position restrictions can be specified:
- Column number, for example. 4,5,6
- Column number range, for example. 4-6

When treating 384-well plates, columns are specified in a range of 1 to 12, not 1 to 24. Two contiguous columns are treated simultaneously.

For methods without scan steps, position restrictions can be a comma-separated list of any combination of column numbers or ranges of column numbers. For example, the following position restrictions: 1, 2, 4-6, 8-10 will treat columns 1,2,4,5,6,8,9,10.

If a method has a scan step, then position restrictions must be either a single column number or a single range of column numbers. All the numbers in position restrictions must be integers within the valid range of columns for the plate being used (1 to 12 for a 96-well plate, for example).

When specifying a range, the order of columns does not matter, 5-2 is treated the same as 2-5. Duplicate column numbers are ignored. For example, the position restriction 4-6,5,4,6 is equivalent to 4-6.

For E220 series instruments, the following types of position restrictions can be specified:

- Column number, for example. 3,5,7
- Column number range, for example. 6-9
- Row letter, for example. A,C
- Row letter range, for example. E-F
- Well position, for example. E4,F8
- Well position range is currently not supported.

All the numbers in position restrictions must be integers within the valid range of columns for the plate being used (1 to 12 for a 96-well plate, for example). All the letters in position restrictions must be within the valid range of rows for the plate being used (A to H for a 96-well plate, for example). Letters can be in upper or lower case.

When multiple position restrictions are specified, the combination of the positions are used. For example, if F,5 is specified, all wells in row F and all wells in column 5 will be treated if those positions are treated by the method. Note carefully: "F,5" is not the same as "F5".

Position restrictions are entered into the text box that is just above the "System Status" button in the "Method tab, as shown:

Appendix C: SonoLab 8.x Data Converter

The SonoLab 8.x Data Converter is a tool that will convert all SonoLab 8.x data files from the 8.x format to the format used by version 10.2. It will allow the user to continue to use their existing methods and plates as well as view their existing history files in the new 10.2 version.

The data converter is installed as part of the SonoLab 10.2 installation. The user can select to convert any or all of the following:
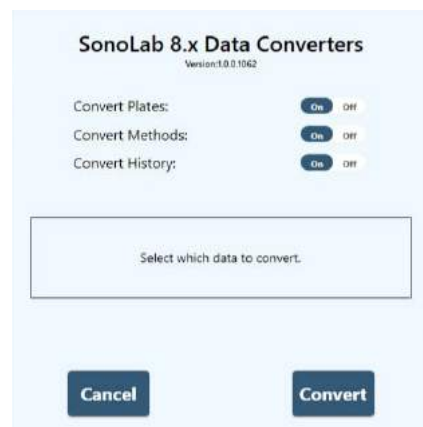- Plate Definition files
- Method files
- History files

The selected sets of data will be copied from their locations in the C:\Users\Public\Public Documents\Covaris\SonoLab 8 folder, converted to the SonoLab 10.2 format and placed in the C:\Users\Public\Public Documents\Covaris\SonoLab 10 folder. When the conversion process is finished, a report will be generated that summarizes what was converted as well as any errors in the process.

Starting the Data Converter Application

The Data Converter app is started by navigating to C:\Program Files(x86)\Covaris\ SL8xDataConverter and double-clicking on the SL8xDataConverter.exe file, selecting it from the Covaris folder in the Start menu program list, or via the search bar at the lower left of the screen. The Data Converter pane is displayed.

Select which types of files, Plate Definitions, Methods, or History, by clicking their respective buttons to "On". All three file types are "On" by default.

Converting Plate Definition, Method, and History Files

The data converter reads the plate definition, method, and history files in the SonoLab 8 folder and creates SonoLab 10 formatted plate definition, method, and history files in the SonoLab 10 folder.
All 8.x files are converted. 8.x files are not deleted, allowing the customer to revert to 8.x, if necessary. Users can delete them manually if desired.

If the utility detects that a file with the same name already exists in the SonoLab 10 folder, the user is notified and given 3 options:
- Replace: Replace the SonoLab 10 file with the new converted SonoLab 8 file
- Skip: Do not convert current SonoLab 8 file
- Cancel: Cancel the conversion process

If the user chooses to replace the existing SonoLab 10 file or skip converting the current 8.5 file, the user can choose to also apply this choice to all other files that already exist in the SonoLab 10 folder.

If there is an error converting an 8.5 file, the user is notified and given 3 options:
- OK: Dismisses the warning and continues to the next file
- Ignore All: Dismisses the warning and continues to the next file. Subsequent conversions will not display this warning.
- Cancel: Cancels the conversion process

All errors are logged to the application log and the summary report.

Appendix D: E220/LE220 (-plus and R-plus) Series Site or Transport Preparation

Placement of Equipment

The area required for the instrument (see drawing below) is approximately 41" wide by 30" deep by 21" high (104 cm x 76 cm x 53 cm). Leave sufficient space beside the instrument and behind the instrument for cooling fan exhaust. Do not block rear panel vents or fans. The ultraCUBE may be placed under the bench.

Power Requirements: Provide at least four standard outlets, one for the laptop computer, one for the instrument, one for the CH05, and one for the WCS 2.0. Your system draws a maximum of 12.5A. All components are configured with universal power supplies.

|	
CAUTION: To avoid derating the CH05 cooling capacity, the CH05 may be used with a step-up transformer if operated at less than 115 VAC.	**WARNING**: To prevent the possibility of electrical shock, always plug the system into a grounded power source.

Water Requirements

The instrument's water bath requires about 5.0 liters of either deionized or distilled water, which should be topped up daily. The water should be changed and cleaning protocol with 10% bleach or equivalent solution performed at least once a month.

System Relocating

When moving the instrument within a laboratory, or between nearby laboratories, please employ the following guidelines:

- Perform the System Shutdown process described earlier in this manual.
- Close SonoLab and shut down the computer.
- Disconnect power cords and USB cable. Disconnect the ultraCUBE.
- Drain the water bath and reinstall in the instrument. Please contact Covaris or your local representative for assistance.
- Upon arrival at the new location, follow the Setup and Installation instructions described earlier in this manual.
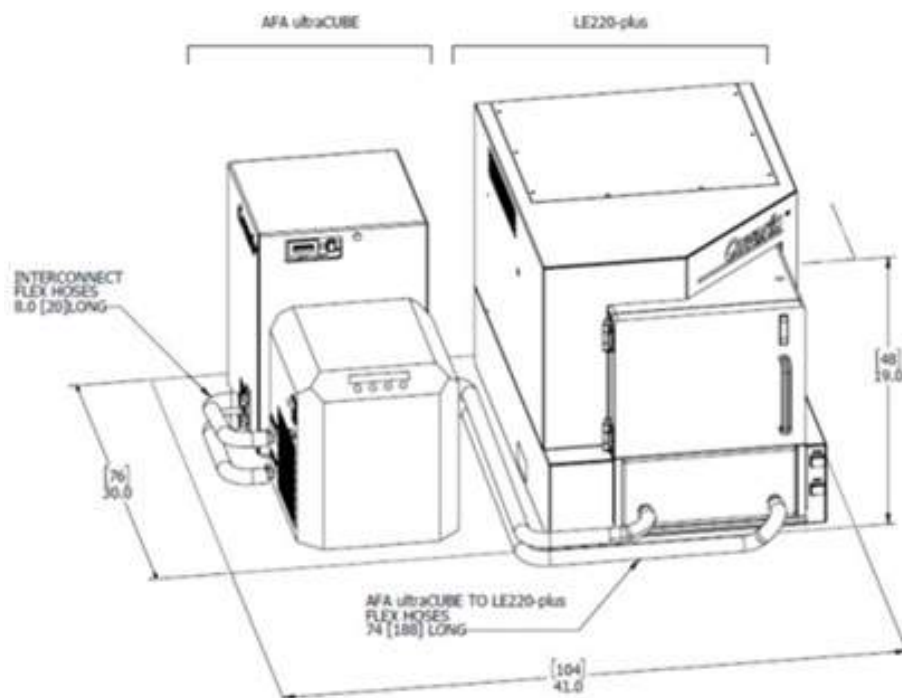
Appendix E: Installing SonoLab 10 Software

These instructions are only for installing SonoLab 10 and other required software packages on a new computer. Do not use these instructions for an incremental upgrade from one version of SonoLab 10 to a newer version. For an upgrade, simply install the new version by double-clicking its "SonoLab 10.msi" file. There is no need to uninstall the existing version prior to upgrading.

1. Install SonoLab 10: Double-click the "SonoLab 10.x.msi" file. Accept the defaults when prompted.
2. Install Baluff RFID Driver part number 210079: Copy entire 210079 folder to desktop, open and double-click the "BalluffVCPInstaller_x64.exe" file to run the installation.
3. Install Plate Installer: Copy the contents of the Plate Definition Files folder to: C:\Users\Public\PublicDocuments\ Covaris\ SonoLab 10\Plate Definitions.

Install Windows Instrument Driver

When an instrument (with power ON) is connected by USB (Universal Serial Bus) for the first time, Windows will identify the instrument by its Vendor and Product ID numbers and download from the internet and install specific device drivers (software files required for communications with a USB device).

- If an internet connection is not available at the instrument, download the USB drivers from the Covaris website, and unzip them on the new computer desktop. Then, perform the manual driver installation as described below.

Manual driver installation: If the automatic installation fails to occur, Windows will post the following message:

Click on the Windows Start button. Either by Control Panel, or right-clicking on Computer and choosing Properties, locate the Device Manager. Click on this link to open the Device Manager window. In the list of devices, locate Other devices and entries for the RF Controller Board:
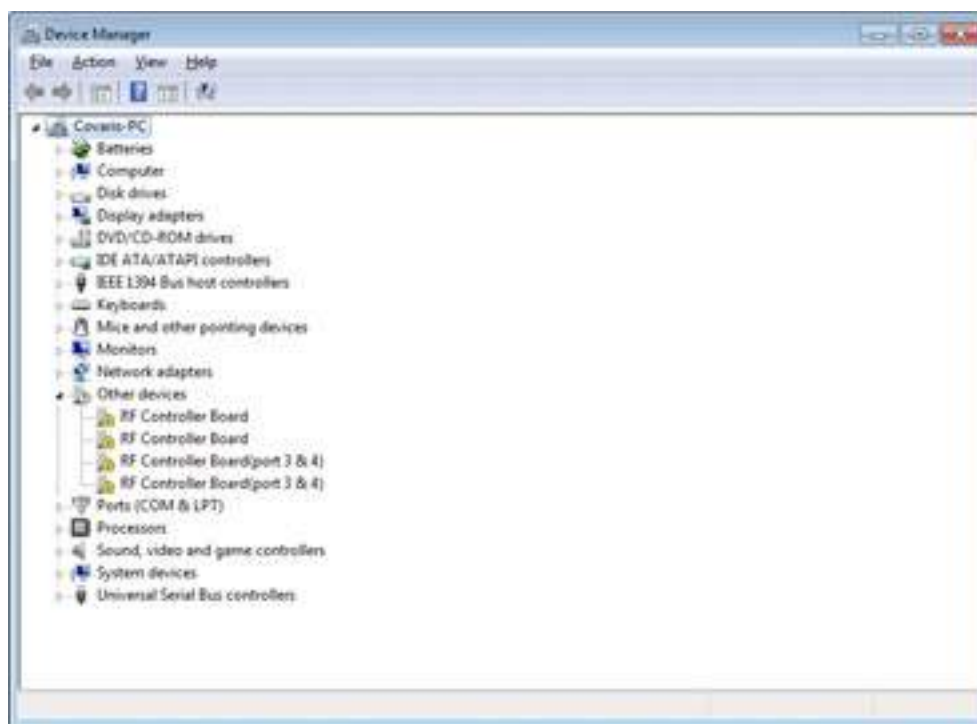
Under Other devices, right click the first device RF Controller Board and then select Update Driver Software:
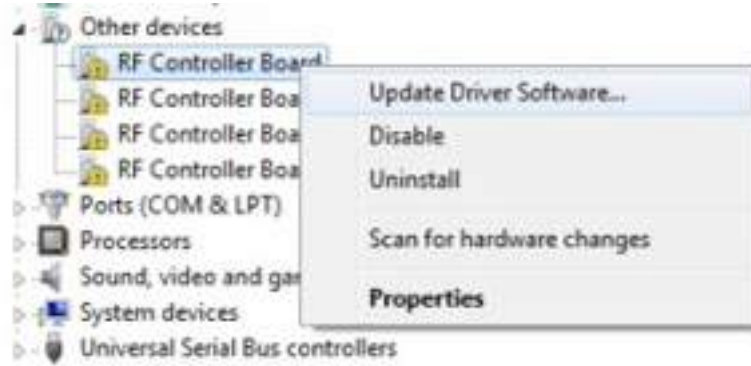
When the driver software update dialog opens, select the option to Browse my computer for driver software:
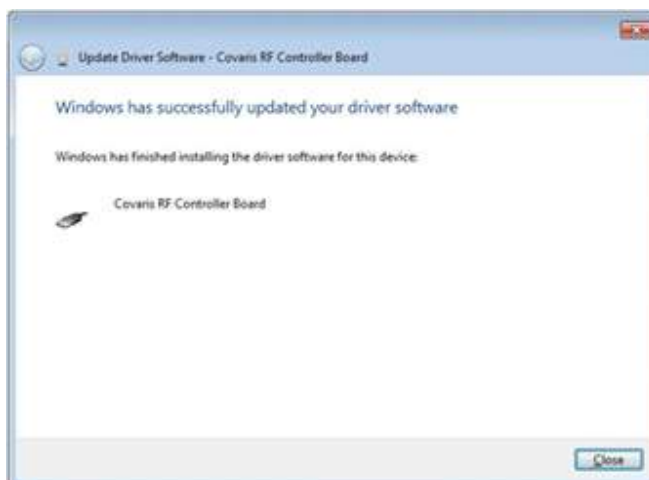

Point the file open dialog to "C:\Users\Covaris\Desktop\Signed_1152921504627718893\driver" and verify that "Include subfolders" is checked:

Windows will install the driver and post the following dialog box with a message, "Windows has successfully updated your driver software".

Click the Close button. The Device Manager screen will refresh.

Return to Other devices. Note that there is now a 'USB Serial Port' listed under the three 'RF Controller' lines. Right-click on the 'USB Serial Port' and select 'Update Driver Software...', as shown in the image below. Repeat the installation steps shown above.
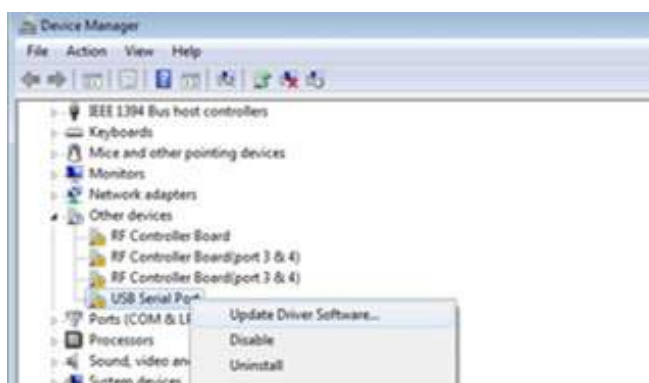
Return to Other devices and repeat this process in order (top to bottom), for the 3 remaining entries, until all Covaris device entries have been assigned a COM number under Ports (COM & LPT). The 'Device Manage' window should now show:

Note that the 'COM' numbers will vary from computer to computer but should occupy four consecutive ports.

Appendix F: Humidity and Water Management for the Covaris E220- and LE220-plus

Your Covaris instrument employs a five-liter bath of distilled or deionized water located inside the instrument housing. For the system to operate correctly, the water must be filled to an appropriate level, clean, free of algae growth, and degassed to retard bubble formation in the acoustic field outside of the sample vessel. Additionally, bath temperature is controlled by the Covaris ultraCUBE connected to your system's water bath. Managing this water and relative humidity is important for the correct operation and reliability of your Covaris system. For additional information, please refer to the following suggestions:

- When the system is powered on, the interior is vented by fans located in the rear panel to maintain an appropriate air temperature and humidity within the instrument enclosure. It is recommended, when the acoustic bath is full that the instrument be left with power on, so that these fans run and retard water condensation on interior surfaces. If it is not practical or desirable to leave these fans running when the instrument is not in use, please leave the front access door ajar so that the interior air may exchange itself with ambient air. The risk of condensate forming within the instrument is increased when operating with a Water Conditioning System 2.0 (WCS), since the WCS will add a small amount of heat to the bath water.
- The WCS 2.0 precludes the need for a daily change of bath water. It is, however, important that water which evaporates each day is made up with fresh distilled or deionized water. It is also important that a monthly cleaning protocol with bleach or an equivalent solution continue to be followed, as the WCS 2.0 will retard but cannot completely prevent algae from taking hold in hoses and interior bath surfaces. Detach the WCS water flow from system when cleaning with bleach or an equivalent.
- When the system is being operated at a low temperature, you may notice condensate water forming on the black manifold block, on hoses leading to the degas pump, and around the fittings and chassis of the WCS 2.0. Particularly in summer, when relative humidity is higher, condensate may drip onto interior surfaces and small amounts may leak onto the laboratory bench. This condensation is expected, and no harm is being done to the system. Excess condensate dripping onto a laboratory bench may be captured by placing an absorbent pad (e.g. VWR 82020-845) beneath the system and/or WCS.
- If the instrument is going to be idle for more than 2 weeks, it may be beneficial to turn all components off to prevent any damage to the instrument. Please follow the recommended sequence:

1. Turn off the chiller.
2. Move instrument to service position.
3. Turn off the ultraCUBE and disconnect hoses.
4. Empty the water bath.
5. Replace the water bath and move instrument to load position.
6. Purge the degas pump by turning on for 10 seconds. Purge the ultraCUBE lines by connecting the hoses and powering on ultraCUBE until flow subsides.
7. Turn the WCS off and disconnect hoses. Raise instrument to service position.
8. Empty the water bath and dry.
9. Gently dry the transducer and surfaces of instrument.

Appendix G: Connecting AutoFill to Plant Water

The AutoFill Accessory Kit is provided to facilitate connecting plant DI water to the AutoFill hardware of your system. The accessory kit includes a 5 micron particulate filter, an adjustable pressure regulator, 10 feet of 6 mm OD polyethylene tubing, and the fittings necessary to connect the components.

Determine the most convenient locations for the pressure regulator and particulate filter. The regulator assembly is connected directly to plant water, with the particulate filter between the regulator and the AutoFill port on the side of your system.

Based on the filter and regulator locations, cut three pieces of the provided poly tubing to appropriate lengths. One piece connects the plant water to the regulator, the second piece connects the outlet of the regulator to the inlet of the particulate filter, and the third piece connects the outlet of the particulate filter to the AutoFill inlet on the side of your system.

Assemble a male quick-disconnect fitting to one end of the tube from the filter to your system and to one end of the tube from the regulator to the filter. Refer to image below.

Install the tube from the regulator to the filter by sliding the open tube end into the push-to-connect fitting at the outlet of the regulator and clicking the other end of the tube into the inlet of the particulate filter.

Repeat the step above for the tube from the filter to the inlet port on your system.

Remove the red plug from the AutoFill inlet port on the side of your system and insert the line from the outlet of the filter.

Fully turn the regulator adjusting knob counter-clockwise and connect plant water to the AutoFill. Adjust the regulator to obtain a maximum of 30 psi water pressure. Verify that there are no leaks.