

R230 Focused-ultrasonicator Setup & Instruction Manual

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

For Research Only

Model Numbers: R230 | Software: SonoLab 10

R230 Setup & Instruction Manual

Part Number: 010480 Rev D | May 2021



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-A2 Clinical Laboratory Safety; Approved Guideline Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories Requirements for Safety

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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 sample cover in the OPEN position 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 and contact Covaris immediately.
- 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 damage 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 the Water Conditioning System 3 (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 tank for an extended time as 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, as these 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 WCS 3 components have pressure ratings above Covaris' specified maximum operating pressure which have been extensively tested. However, if plant DI water is connected to the WCS, a catastrophic failure may result in substantial plant water spillage. It is the 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. SHOULD YOU HAVE QUESTIONS OR CONCERNS PERTAINING TO THE USE OF OUR EQUIPMENT.

Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the necessary services and support necessary so that the customer 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.



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1. Introduction

1.1 Overview of the User Manual

This manual contains the operation and maintenance instructions for the Covaris R230 Focused-ultrasonicators. This document contains information essential to the proper use and care of the equipment.

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

The following definitions apply in this manual:

NOTE: Inconvenience if disregarded.

WARNING: Personal injury may occur.

CAUTION: Equipment damage may occur.

There is also a PDF version of this manual, typically available on the computer desktop. Open the manual by double clicking on its icon.

1.2 The R230 Instrument and the Covaris Process

The R230 Focused-ultrasonicators are based on Covaris Adaptive Focused Acoustics (AFA) technology. These instruments are designed to provide an integrated tool to be used in a high-volume production environment. This instrument is ideal for a variety of applications including DNA shearing, mammalian cell lysis, low mass sample extraction from FFPE, tissue, whole blood, compound screening, bead mixing, and pellet resuspension among other applications.

The 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 a standard user interface or 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

The Covaris Process uses adaptive focused acoustic energy to precisely control cavitation and acoustic streaming within the sample treatment vessel in a non-contact, isothermal fashion. Focused acoustic energy is generated and delivered to individual samples. The mechanical energy imparted on the sample results in a controlled series of compression and rarefaction events.

2. Unpacking and Setup

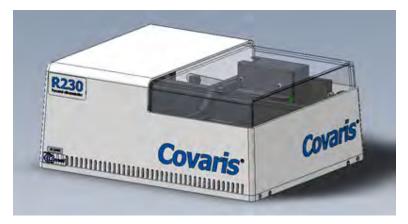
2.1 Unpack and Identify Components & Accessories

The following items are included in the packaging found with the instrument:

• Electronics Box 1.0 (eBox)



• On-Deck R230 Focused-ultrasonicator 1.0 (for placement on the liquid handler deck)



• Water Conditioning System 3.0 (WCS 3.0)





• Notebook Computer with AC Adapter. Most installations will transfer Sonolab to the dedicated computer that is connected to the Liquid Handler.

NOTE: The notebook computer should be available for IQ/OQ, PM and Service. Please DO NOT use this computer for other purposes.

- Cables
 - Computer AC adapter power cord
 - Two instrument power cords
 - Ethernet cable from eBox to Computer
 - Communications and power cables between eBox, WCS 3.0, and On-Deck AFA
- Accessories
 - The WCS Tubing Kit contains 3 hoses
 - The Reservoir Kit contains 2 carboys and 2 hoses
 - Mount adapter, sample tubes and racks (varies by application)
 - Setup kit

2.2 Install the On-Deck Module

2.2.1 Install the appropriate mounting/locating plate to the liquid handler by following the instructions provided with the mounting plate.

NOTE: A mount plate must be used for all integrations with liquid handlers.

2.2.2 Remove the shipping locks from the On-Deck module.

CAUTION: Shipping locks must be removed before powering up the instrument.

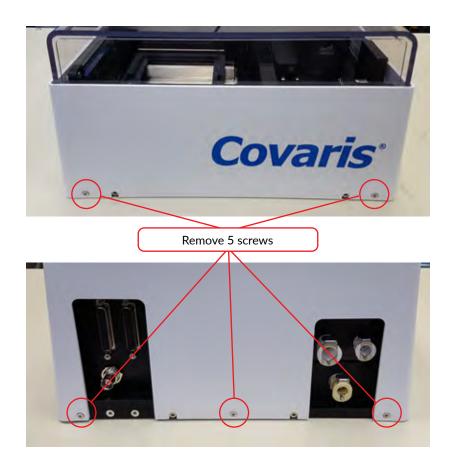
There are 2 bolts that lock the On-Deck motion system in place for shipping. The bolts are accessed from the bottom of the base plate.

- Tilt the On-Deck module up to access the bottom of the plate. In the image below, the module is resting on its back edge with the 2 shipping bolts circled. Be careful to remove only these 2 bolts.
- Lower the On-Deck module so that it is again resting flat on the bench.

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- **2.2.3** Store the shipping bolts on the On-Deck base plate.
 - Remove the 5 flat head cap screws, 2 from the right, and 3 from the left lower edge of the On-Deck cover. Do not remove the screws in the inverted "U" shaped cut-outs.

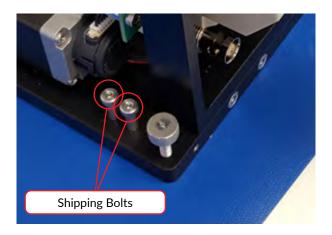


• Lift the cover off and set aside.





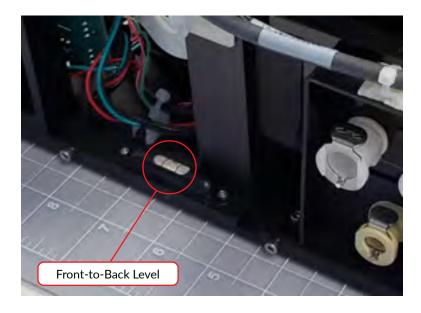
• Thread the two shipping bolts into the holes provided at the left rear of the module's base plate, near the cable connectors.



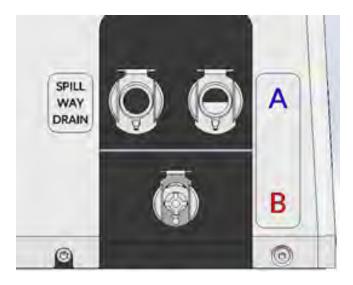
2.2.4 Level the On-Deck module in the liquid handler.

- Place the On-Deck module, without its cover, into the mounting plate installed previously in the liquid handler.
- Perform any final adjustments required to the mounting plate.
- Level the On-Deck module by adjusting the 4 leveling thumbscrews, as necessary, while monitoring the 2 levels provided on the base. There is a leveling screw at each corner of the module base. Three of the screws and the left-to-right level are shown in the image of the module without the cover above. The front-to-back level is on the left side of the base plate, between the cable and hose connector panels. See image below.

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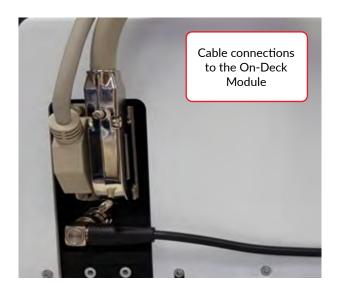


- Remove the On-Deck module from the liquid handler and reinstall the outer cover.
- **2.2.5** Complete installation of the On-Deck module. Because of limited clearance in some liquid handlers, it is easier to connect hoses and cables prior to placing the On-Deck module in the liquid handler.
 - Connect the right-angle connectors of the "A", "B", and "DRAIN" hoses to the On-Deck module. The straight end of the hoses can be connected after the On-Deck module is in place on the liquid handler. Refer to section 2.2.7 below.

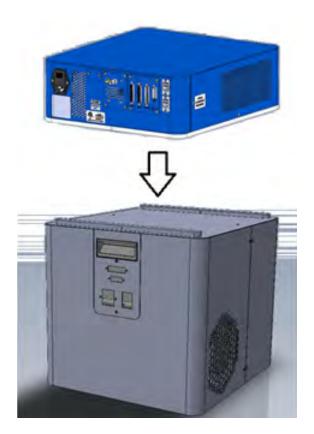


- Connect the right-angle connector of the male 25-pin d-sub cable to the female 25-pin connector on the On-Deck module.
- Connect the right-angle connector of the male 44-pin d-sub cable to the female 44-pin connector on the On-Deck module.
- Connect the right-angle connector of the coax cable to the BNC connector on the On-Deck module.





- Place the On-Deck module with cables and hoses attached into the mounting plate on the liquid handler.
- **2.2.6** Arrange the eBox and WCS 3.0 as shown. These may be stacked on the floor or an adjacent table. The eBox may be mounted on top of the WCS 3.0 as shown below. Use the two M4 x 25 button head cap screws that come in the accessories box to secure the eBox to the WCS 3.0.



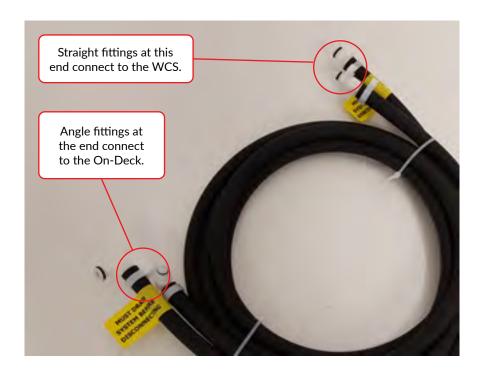
CAUTION: When positioning the system, please keep the fan and rear panel vents free of obstructions that may block the flow of air.

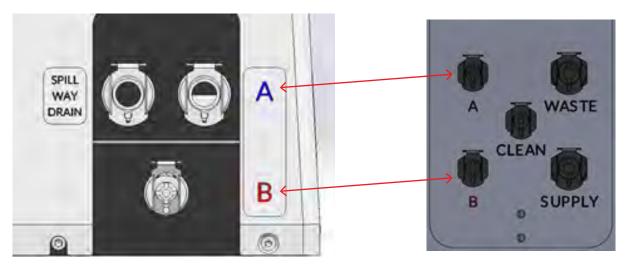


2.2.7 Complete the water connections.

There are two hose kits included with the R230. The WCS Tubing Kit is used to connect the On-Deck to the WCS and to the Waste reservoir. The Reservoir Kit includes the Waste and Supply carboys and the two hoses which connect the WCS to the carboys. From the WCS Tubing Kit:

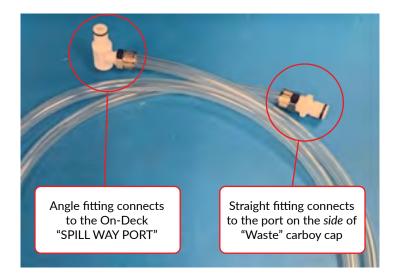
- Connect the straight end of the "A" hose to the WCS "A" port. The "A" hose has a yellow tag at each end.
- Connect the straight end of the "B" hose to the WCS "B" port. The "B" hose has smaller fittings than the "A" hose.



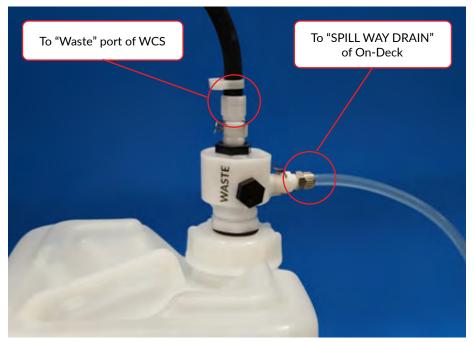


• Connect the straight end of the remaining, clear "SPILL WAY DRAIN" hose to the port on the side of the waste reservoir cap. See next three images.

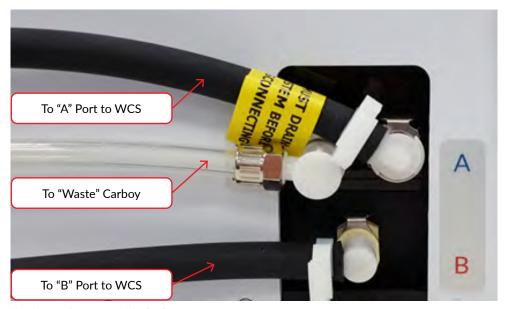
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• Connect the right angle end of the clear hose to the "SPILL WAY DRAIN" port of the On-Deck.



Waste Carboy with Hose Connections



Hose Connections at rear of On-Deck

From the Reservoir Kit:

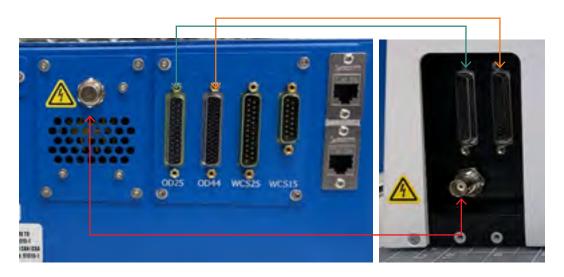
- Connect one hose from the WCS "Waste" port to the top port of the waste carboy's cap.
- Connect the remaining hose from WCS "Supply" port to the supply reservoir cap, or another source of distilled or deionized laboratory water.

The WCS contains a UV sterilizer and particulate filter to retard biological growth.

The middle connection is used only during monthly maintenance procedures (see section 8.2.2 for details on monthly maintenance). No hoses should be connected to the maintenance connector while running the R230.

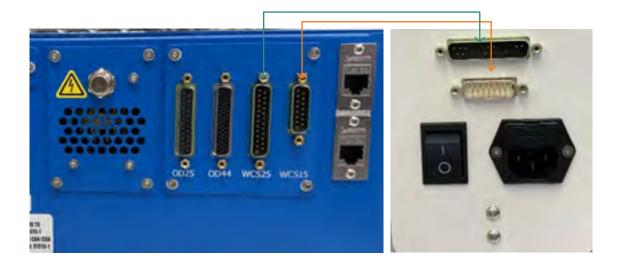
2.2.8 Complete the electrical connections from the On-Deck Module:

- Connect the eBox female 25-pin connector, labeled "OD25", to the On-Deck female 25-pin connector.
- Connect the eBox female 44-pin connector, labeled "OD44", to the On-Deck female 44-pin connector.
- Connect the eBox BNC connector to On-Deck BNC connector.





2.2.9 Complete the electrical connections from the WCS 3.0.



- Connect the eBox male 25-pin connector, labeled "WCS25", to the WCS male 25-pin connector.
- Connect the eBox male 15-pin connector, labeled "WCS15", to the WCS male 15-pin connector.
- Plug the WCS 3.0 into a power source using one of the line cords. The WCS 3.0 is a "universal input" device and automatically employs any worldwide line voltage or frequency (100 to 240 volts AC, 50 or 60 Hz). Leave power switched OFF at this time.

2.2.10 Complete electrical connections from the eBox and laptop computer.

- Connect either eBox ethernet port to the ethernet port on the laptop computer. The R230 uses 4 fixed addresses (5, 7, 10, and 22) on the 192.168.0 subnet.
- Connect the computer to a power source. Connect the mouse or other accessories to the computer.
- Plug the eBox into a power source using one of the line cords. The eBox 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 at this time.



3. Getting Started

3.1 Start the System

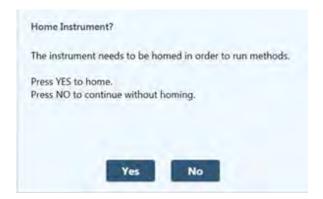
- Power on the eBox using the power switch located on the rear, right side of the eBox.
 - This provides power to the On-Deck module.
- Power on the WCS 3
 - SonoLab software will perform all temperature control.

3.2 Start SonoLab

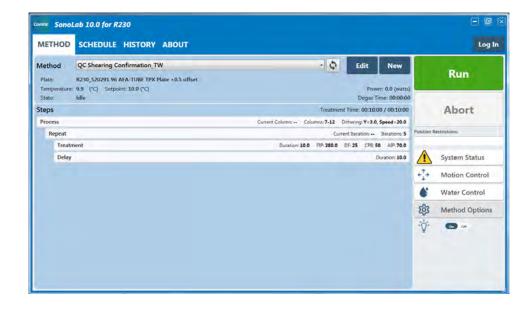
Wait 30 seconds after powering on the WCS 3, to allow the WCS 3 to initialize. Locate the Sonolab 10 icon on the laptop's Desktop.



Double click on the icon. SonoLab will load and the user is prompted to home the R230 motion system.



Click "Yes", then "OK" when prompted to empty the sample holder and the On-Deck module will go through a motion homing process. When homing is complete, SonoLab will display the *Method* screen. The *Method* screen displays the last successful treatment method run and provides access to status information and basic instrument control functions. See image below.

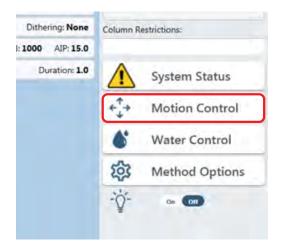




SonoLab Method Screen

The first time SonoLab is started, remove the desiccant bag from the On-Deck module and fill the On-Deck bath with deionized water:

• From the Method screen, click on the Motion Control button at the right side of the screen.



The Motion Control panel is displayed.



- Click the Open Cover button. The On-Deck safety covers will open.
- Remove or cut the plastic wrap securing the desiccant bag to the On-Deck sample tray and remove the desiccant bag.
- With the desiccant bag removed, manually pour approximately 1 liter, or a bit more, of deionized water into the water bath to prevent flow errors when the WCS 3 is powered on for the first time. It is only necessary to do this the first time the R230 is started, when the water system is completely empty.
- Click the Close Cover button in the Motion Control panel.

The R230 is now ready to use. The automation computer can connect and assume control of the R230, or treatment methods may be run directly from the Method screen.

See **Section 5** below for a more detailed description of SonoLab.

3.3 System Shutdown

If the instrument will be idle for an extended period of time, refer to the system shutdown procedure in the User Maintenance section of this manual.



4. AFA Operation

AFA uses four parameters to fine tune specific acoustic treatments. These parameters are programmed into *Methods*, 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 R230, the range of Peak Incident Power is 1.5 to 450 Watts.

Duty Factor

This parameter defines the ratio between 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 2 to 50% in the R230.

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 R230 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 column at a time. The measurement of Duration is in seconds. For scanning treatments, the sample tray is passed continuously over the transducer. In this mode, treatment time is a function of scanning speed. Scan speed ranges from 0.1 to 40 millimeters/second in the R230.

Treatment Records

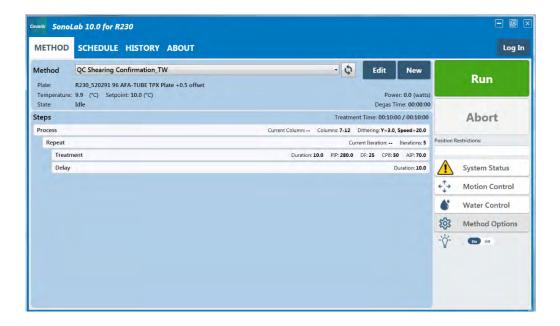
SonoLab History contains a record of parameter information for each treatment method run. An RFID scan is performed on each treated sample tray to ensure that the correct consumables are being used for the treatment and to provide information for LIMS.



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 On-Deck bath.
 - Setpoint: the On-Deck bath temperature specified by the current selected method.
 - **State:** possible states, depending on what the R230 system is doing, includes:
 - $^{\circ}$ At initial startup, Idle.
 - ° During a treatment method, the possible states include:
 - > RFID Scanning: the system is scanning and validating the RFID tag on the current treated sample plate.
 - > Waiting for Water Level: the system waits for the water to reach the level specified by the selected method.
 - > 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.
 - > Running: the system is currently performing the selected treatment method steps.
 - > Paused: the method is paused by the user or the API.

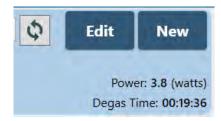


- Following a treatment:
 - > Completed: the treatment terminated normally.
 - > Aborted: the treatment was halted by a command from a user or the API.
 - > Failed: the method run was unsuccessfully completed with the displayed reason.
- *Treatment Time*: the remaining time for acoustic on plus delay time for the current method/the total time of acoustic treatment plus any delay steps for the current method.
- Methods saved on the system are selected from the drop-down list.



• The power delivered during a treatment is displayed just below the 'Edit' and 'New' buttons at the right of the method bar.

Remaining time required for degassing is displayed immediately below the power display.



• 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 or not the instrument conditions required to run a treatment method are met. A red X indicates a parameter has not been met, a green ✓ indicates it has. In the cases of Water Degassed and Temperature within limits, a yellow triangle ▲ indicates that degassing time or temperature targets have not yet been met.



- Acoustics Connected: a green check indicates the eBox has successfully connected to the software.
- Motion Connected: a green check indicates the motion controller board is detected.
- WCS Connected: a green check indicates that the chiller contained in the WCS has been recognized by the software.
- Homed: a green check indicates that the On-Deck motion system has been initialized.
- *Water Degassed:* a green check indicates the water has been degassing for a sufficient length while a yellow triangle indicates that the water has not yet degassed for a sufficient time. Overridden is displayed next to the triangle or check if the degas timer has been 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. Overridden" is displayed next to the triangle or you can check to see if the temperature has been overridden in Method Options.
- UV Light: a green check indicates that the ultraviolet water sterilizer is on and functioning normally
- **API Connected:** "No" is the default condition, indicating that the R230 is not under external automation control. "Yes" indicates that the R230 is under external control, but most 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.
- Motion Control displays the Motion Control panel, which contains buttons for opening and closing the On-Deck safety covers and moving the On-Deck 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 right end of the bath and opens the safety cover to provide access by the liquid handler.
- 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:** moves the sample tray to the right end of the bath and raises it to its maximum height to provide access for servicing the bath.
- Open Cover: opens the safety cover.
- Close Cover: closes the safety cover.
- Stop: stops all current motion. SonoLab shows the STOP button as disabled when no motion is occurring.
- Water Control displays the Water Control panel, which contains both water system status indicator and user control functions.



- Water System Indicators

Level

- > Current Level indicates the current bath water level.
- > Load Level is the level to which the method initially fills the bath with the sample tray out of the water bath.
- > Run Level is the level to which the method fills the bath with the sample tray in the bath, immediately prior to the method being run.

Temperature

- > Current Temperature is the current temperature of the water bath.
- > Setpoint Temperature is the water bath temperature specified in the currently selected method.
- Degas Time: the remaining degassing time before a method can be run (unless the degas timer is overridden).

Flow State

- > **Circulating:** the bath water is circulating between the bath and the WCS 3.0 for temperature control, degassing, and cleaning.
- > **Leveling:** filling from the supply reservoir, either to the maximum bath level, or the level specified by the currently selected method, depending on whether the filling was invoked via the Fill button, the Fill to Load button, or directly by the selected method.
- > **Draining:** the water bath is draining to the waste reservoir.



Controls

- > Off: turns off the circulation pump and control valves.
- > Fill to Load: fills the bath to the level specified by the currently selected method.
- > Fill: fills the bath to its maximum level.
- > **Drain:** drains the bath.
- > Circulate: circulates the water between the bath and the WCS 3.0.
- **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, the system will
 degas for approximately 20 minutes from startup, depending the water level specified by the current method. After
 initial startup, if more than 5 millimeters of water are added to the bath, the degas timer is set to 1 minute per
 millimeter.

· Other Controls:

- Light is a control and an indicator. Clicking on the switch toggles the light and indication of the light status on and off.





5.2. The Schedule Screen

The Schedule screen is used to configure bath Auto Fill 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, Auto Fill and the WCS temperature control are turned off, but the WCS 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, or higher permission level, to adjust the parameters on this screen.



• Fill on Startup

- SonoLab checks the level of the bath on startup and fills, or adjusts, the bath to the level specified by the currently selected method. If no method is specified, the fill is not performed.

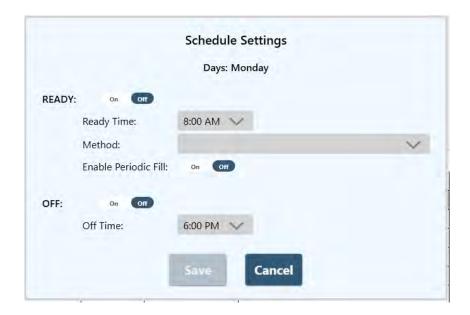
• Periodic Fill

- SonoLab checks and adjusts the R230 water bath level at the start of each treatment method. However, if the R230 will be idle for long periods of time during the day, the Periodic Fill function can be configured to check the bath level periodically 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.
- 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 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 R230 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 set at the beginning of each method run. But, if the R230 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 Schedule On/Off switch so that On is highlighted dark blue to activate the scheduler.





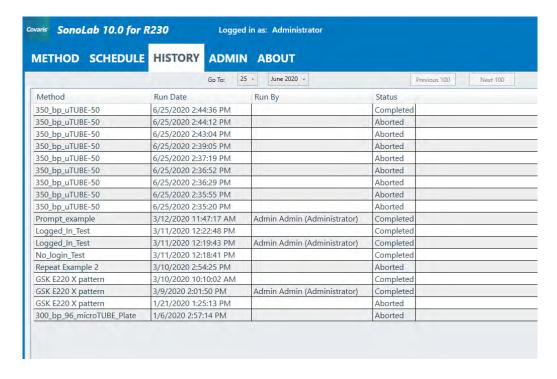
Click **Select All**, and then **Edit** if all seven days are to use the same scheduling parameters. Click **Select** Weekdays then Edit if only the five weekdays are to use the same scheduling parameters.

NOTE: If a method is running at the time the scheduler is set to switch the R230 to the OFF state, SonoLab ignores the scheduler and the R230 will not be 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 R230 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.

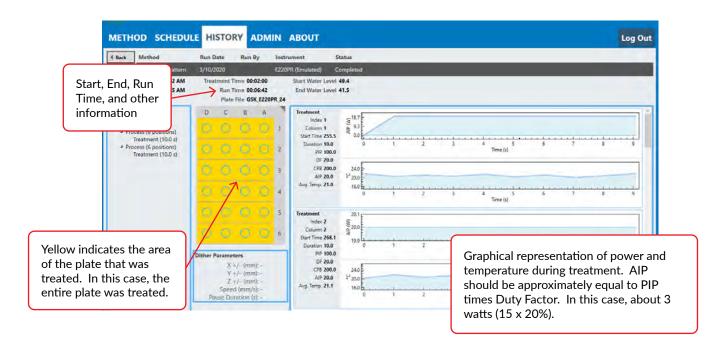
The status bar's Next 100 button loads the next 100 less recent files. The Previous 100 button loads the next 100 more recent files.

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.



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" that was run 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 is a .csv file which can be opened as a spreadsheet, containing information about the methods run, including times, columns treated, acoustic power, bath water temperature and bath water level.

The **Treatment** file is also a .csv file containing treatment parameters and start and end times for the method, along with water level and temperature data. The Treatment file contains the same data as that 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 components contained in the R230 system, such as serial numbers, software, and firmware versions. The information on this screen can be provided to Covaris support personnel assisting with problem resolution.

SonoLab 10 Version: SonoLab 10.0.0.1556 Instrument Type: R230 Serial Number: PROTO1 On-Deck Serial Number: 000010 eBox Serial Number: WCS Type: 3.1 WCS Serial Number: 000086 Controller Board Serial Number: JE4219-0068 Controller Board Version: A2 Controller Firmware Version: 10722 Transducer Serial Number: 064 Transducer Center Frequency (kHz): 497 Transducer Impedance: 47.6 Acoustic Efficiency: 1.00 Motion Firmware Version: VGEN30 API Address: IP: 10.0.0.10, Port: 8100

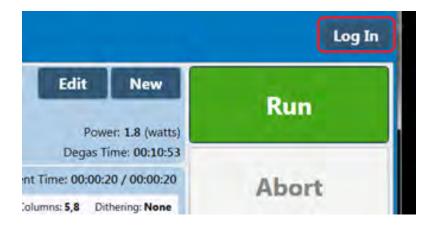
5.5 The Admin Screen

The ADMIN tab is only available if an administrative user is logged in. 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 Login and the ADMIN tab is added to the SonoLab window.

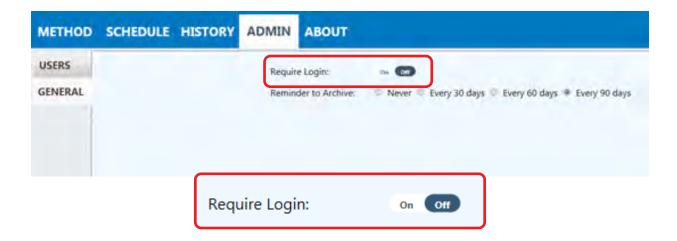


• Click on the **ADMIN** tab. The list of default SonoLab users, their roles, and assigned methods is displayed, along with the "USERS" and "GENERAL" tabs at the left side.





• Click on the "GENERAL" tab.



• Click the On/Off switch to turn on "Require Login".

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 User level users.
- Can assign access to specific methods to Administrators and Users.
- 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 edit and run methods created by this editor.
- Can review the history screens for methods run by this editor.

An Operator:

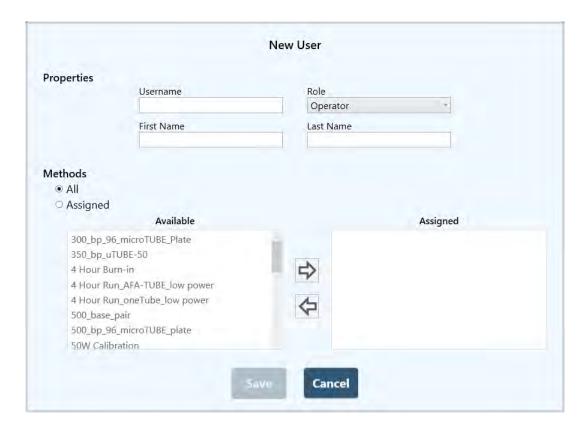
- Can run methods assigned to them by an administrator.
- Can review the history screens for methods run by this operator.



5.5.3 Managing Users

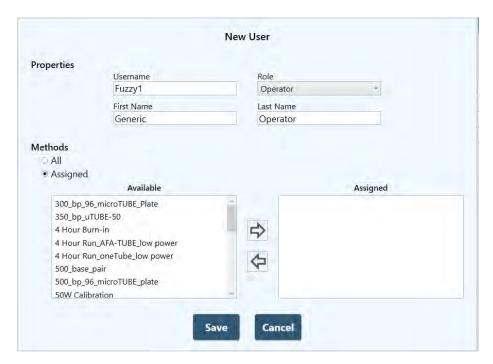
To create a new user:

- 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.



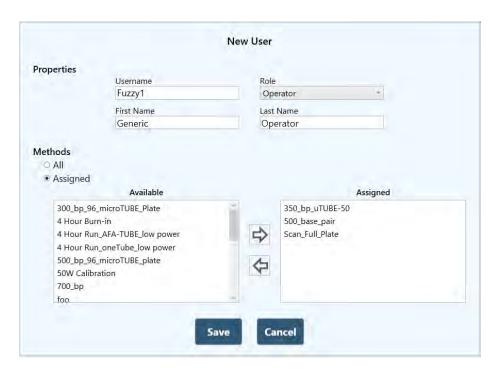
• Enter a new Username, the user's first and last names, and click on the "Assigned" button. By default, all treatment methods on the system will be assigned to the new user.





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 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.





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

METHOD SCHEDULE HISTORY			ADMIN ABOUT	ABOUT		
USERS	GENERAL					
Username		First Name	Last Name	Role	Methods	
admin		Bruce	P	Administrator	All	
Admini	istrator	Admin	Admin	Administrator	All	
Editor		Editor	Editor	Editor	All	
Fuzzy1		Generic	Operator	Operator	3	
Operat	or	Operator	Operator	Operator	All	

The first time a new user is logs in, the user will be must click on "Set Password" to display the Set Pa password pane.





The user enters their Username, 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. Creating & Editing Treatment Methods

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

A method consists of one or more *Process or Scan Steps*. A Process contains at least one Treatment. Most methods will contain a single process, containing a single treatment step.

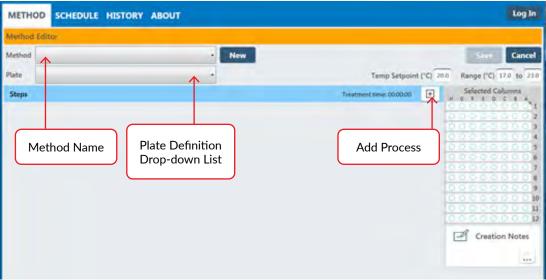
If more complex methods are required, they can be easily constructed.

6.1 Create a New Method

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



• The Method Editor window will appear.



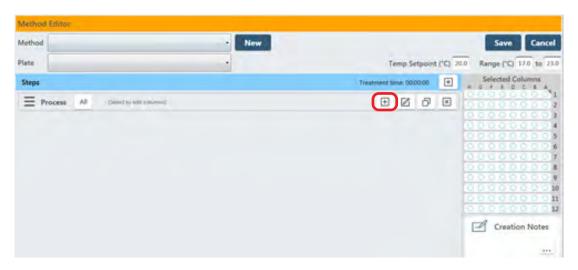
Method Editor Window

- 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.



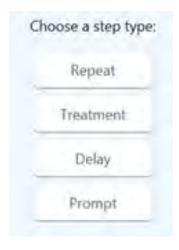


4) Click "Process". The process bar is displayed.



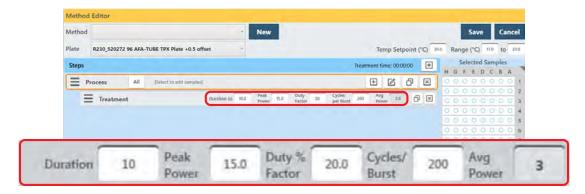
For descriptions of *Delay and Prompt steps*, see step 11 of this section below. *Repeat* steps are discussed in *Section 6.2* of this document. *Scanning* is discussed in *Section 6.3*.

5) Click the + button inside the process bar to display the step type menu.

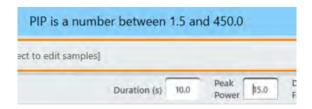


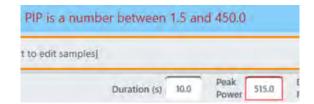
6) Choose Treatment to specify the four acoustic parameters: Duration, Peak Incident Power, Duty Factor, and Cycles per Burst.





Treatment parameters are chosen by directly editing the value boxes. When a parameter's value box is selected, the valid parameter range is displayed in the blue bar above. If an invalid parameter is entered, the value box is outlined in red and the valid parameter range is displayed in red.





Peak Incident 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 450 Watts.

Duty Factor

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 2% to 50%.

The product of the Peak Power and Duty Factor gives the approximate Average Incident Power (AIP) generated from 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.

The range of Cycles per Burst (cycles/burst) is from 50 to 1000 for different applications. Advanced users may use this parameter to fine tune their experiments.

Duration

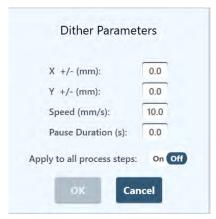
This parameter defines the amount of time for which the sample is under a treatment. The measurement of the Duration is in seconds. The maximum time for any treatment is 3600 seconds. Should a longer treatment be necessary, "Duplicate Steps" can be used to extend the treatment.



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 allowed Average Incident Power is 110 Watts.

Dithering

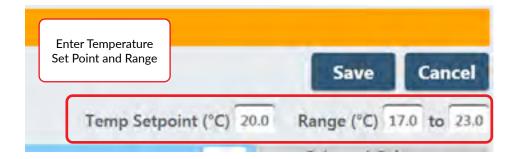
The motion system provides dithering motion capability in the X axis (fore-&-aft), Y axis (left-to-right), and Z axis (up-and-down). If a treatment specifies dithering, click on the "Pen-&-Paper" icon on the right end of the Process bar to display the Dithering Parameters pane.



Dither Parameter Pane

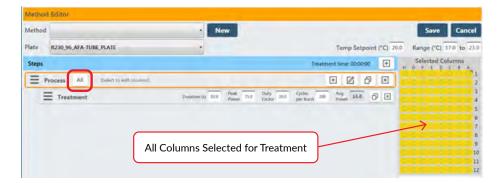
The X, Y, and Z +/- distance range is 0 to 5 millimeters. The Speed range is from 0 to 25 mm/second. The Pause Duration range is from 0 to 60 seconds.

- 7) Enter the desired dithering parameters, if any, and click "OK". If dithering is to be applied to all treatment steps of the method, click the 'Apply to all process steps' switch to toggle it on. 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 3 degrees.



Once the treatment parameters have been defined, click in the process bar to highlight it, then click in the treatment well graphic to define which plate positions will be treated. The R230 treats an entire column at a time. Therefore, clicking anywhere in a column causes the row to be selected. Any number of columns may be selected for treatment; columns selected for treatment turn from white to yellow. Clicking the All button (circled below) selects all rows for treatment. Clicking on a column (or *All* button) a second time removes it from the list to be treated. Multiple columns may be selected by clicking on one column and dragging to adjacent columns. Holding down the shift key while doing so allows multiple groups of columns to be selected.

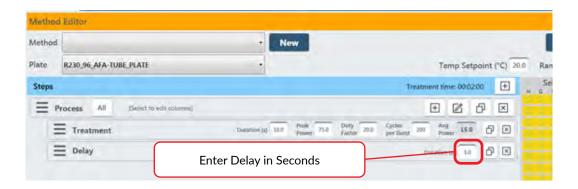




When the method is run, the column under treatment is displayed in the 'Process' bar.

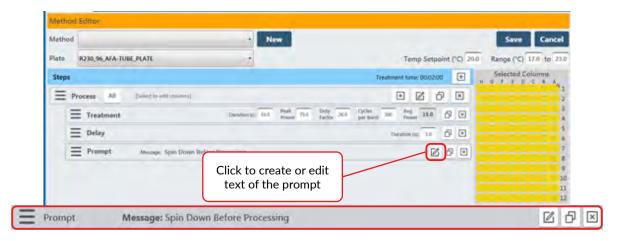


9) As an example and if desired, choose a Delay step to introduce a fixed time delay into a method to allow the sample to settle between the two different treatment steps. The delay is specified in seconds.



Add a Delay Step

- 10) If desired, choose a Prompt step to display a message on the screen and pause execution of the method until the operator clicks OK.
 - Clicking the "pen & paper" button of the prompt bar to display the text entry field for the prompt.
 - Enter the text of the prompt and click OK. See Figure below.





Add a Prompt Step

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

- 11) 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.
- 12) 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.



13) Enter a name for the treatment method and click Save. If the method name is the same as an existing method, the 'Save button is disabled and the 'Overwrite' button is enabled. Clicking the 'Overwrite' button will result in the previous version being replaced.



6.2 Using the Repeat Function

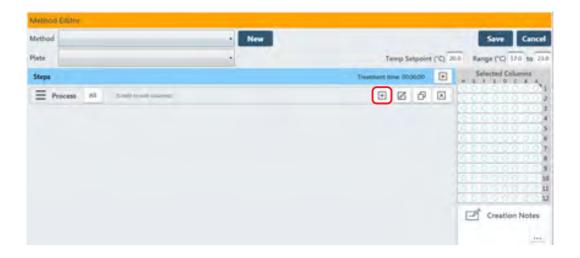
The Repeat function is used when one or more treatment, delay, or prompt steps are to be applied to each column of the plate multiple times. There are two scenarios for how this may need to be performed, and the required scenario determines how the repeat function is implemented.

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

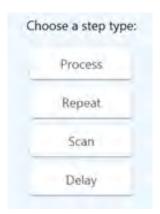
Scenario A is used if each column requires one or more acoustic doses, but there is no need for resting the sample between doses.

6.2.1 Implement Scenario A:

• Create a method with a Process step, as explained previously.

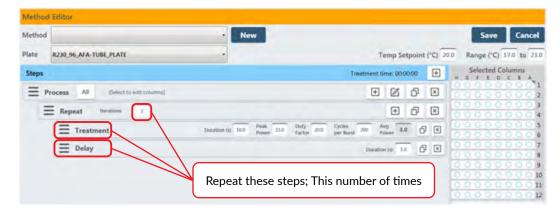


• Click the + button inside the process bar to display the step type menu.



- 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.





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

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

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

Scenario B is typically used in "pulsing". With pulsing, a column is treated with an acoustic dose, and is then allowed to rest while the remaining columns are treated. Once the remaining columns are treated, a second acoustic dose is applied to each column.

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 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 A, then both Treatments will be given to column B, and so on 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

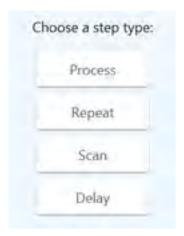
In a scanning treatment, rather than the sample tray indexing to place one column of the sample tray at a time over the transducer, the sample tray moves continuously from under the sample tray. In this mode, treatment time is a function of the speed at which the sample tray moves. One or more columns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

6.3.1 Create a Scanning Treatment:

• Click the + Process button.

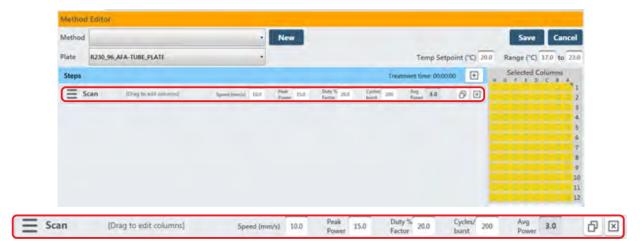


• Select Repeat from the step type list.



- Enter the desired number of scans. One scan passes the sample tray over the transducer one time.
- Click the add process button again.
- Select Scan from the Step Type list.
- Enter the desired treatment parameters as for a normal treatment step.



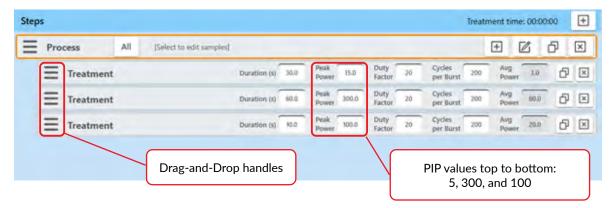


• Click **Save** and give the method a name as with normal treatment methods.

Contact Covaris Applications Support for assistance in specifying an appropriate scanning speed and treatment parameters.

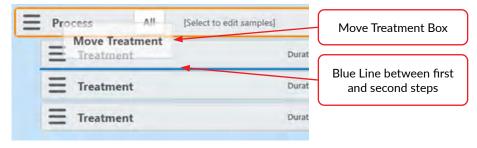
6.4 Using Drag and Drop with Treatment Steps

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.



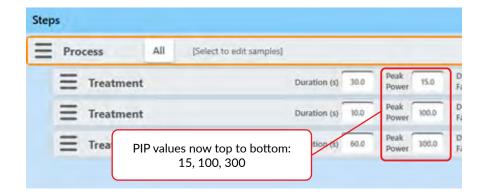
To swap the positions of the second and third steps:

- 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.





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 wells 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.



7. System Specifications

Treatment System	High intensity acoustic transducer, temperature monitoring device, circulation pump, water bath with safety enclosure
Maximum Treatment Power	450 Watts Peak Incident Power, 110 Watts Average Power
Dimensions Electronics	410.5 mm x 372.4 mm x 119.3 mm (16.2" x 14.7" x 4.7")
Weight Electronic Unit	5.8 kg (12.8 lbs.)
Dimensions Acoustic Unit	362.3 mm W x 286.8 mm D x 151.2 mm H (14.25" x 11.3" x 6.0")
Weight Acoustic Unit	8.7 kg (19.2 lbs.)
Dimensions Chiller WCS	406.4 mm x 368.3 mm x 368.3 mm (15.9" x 14.5" x 14.5")
Weight Chiller WCS	25.9 kg (57 lbs.)
Power Requirements	100-240 VAC 500 VA maximum, 50-60Hz, Electronic unit
Power Requirements	100-240 VAC, 50-60Hz, 9-4A, Chiller WCS unit
Ambient Temperature Range	19°C to 25°C (66°F to 77°C)
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
Temperature Alarms:	Can be programmed from +6.0 °C to +40.0 °C
Computer	Notebook computer
Operating System	Microsoft Windows 10
Application Software	Covaris SonoLab 10
Data Input:	Keyboard, mouse







8. Maintenance

8.1 Cooling Air Intake

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

8.2 Water Bath

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 tank dried prior to longer term (more than a week) idle time.

8.2.2 Recommendations for Monthly Maintenance

The R230 Bleaching Protocol should be performed monthly as follows:

Start SonoLab and open the 'Water Control' panel by clicking the 'Water Control' button on the right of the 'Method' screen.



Use the 'Fill', 'Drain' and 'Circulate' buttons on the 'Water Control' pane as instructed below.

- 1. Empty 'Waste' reservoir and fill 'Supply' reservoir with 600 mL of household bleach and 5.4 liters of deionized water to create a 10% bleach solution (approx. 0.6% sodium hypochlorite).
- 2. Drain the R230, if not already empty.
- 3. Fill the R230.
- 4. Circulate the bleach/water solution for 5 minutes.
- 5. Drain the R230.
- 6. Disconnect and empty the 'Supply' reservoir and 'Waste' reservoir. Rinse out the Supply reservoir thoroughly with deionized water. Fill 9.5L of deionized water into the 'Supply' reservoir. Reconnect 'Supply' and 'Waste' reservoir.
- 7. Fill the R230.
- 8. Circulate the water for 3 minutes.
- 9. Drain the R230.
- 10. Repeat steps 7 through 9 twice more.
- 11. Empty 'Waste' reservoir and Refill 'Supply' reservoir with 4.0 L deionized water.



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 WCS 3.0

The particulate filter should be changed annually. The filter part number is 500175 and may be purchased from Covaris Customer Service. Instructions for changing the filter are in Appendix A of this document.

8.5 Safety System

Contact the Covaris Technical Support Department for more information.

WARNING: Report any failure of the Safety System immediately to Covaris. DO NOT attempt to operate if the Safety System is malfunctioning.

8.6 Degassing System

The degassing system is incorporated into the WCS 3.0 and operates automatically.

8.7 Cleaning the System

Clean the external surfaces of the equipment as necessary with a damp lint-free cloth.

CAUTION: Do not employ isopropyl alcohol, ammonia-based, or abrasive cleaners on the water tank, as these will damage the acrylic surfaces.

Leaving water in the water bath or degassing lines for an extended time may promote algal growth in the tank and lines. This growth can interfere with water quality and sample processing.



9. Troubleshooting

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

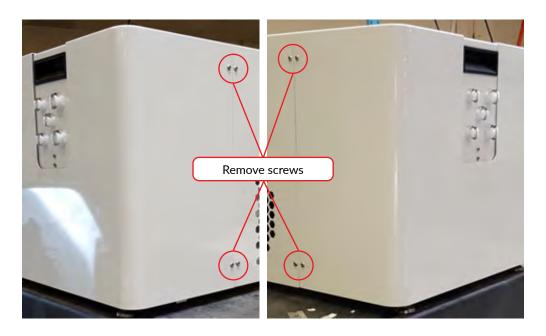


Appendix A: Replacing the WCS 3.0 Particulate Filter

The WCS particulate filter is the only user serviceable component and should be changed annually at minimum. The part number of the particulate filter is 500175 and can be purchased from Covaris Customer Service.

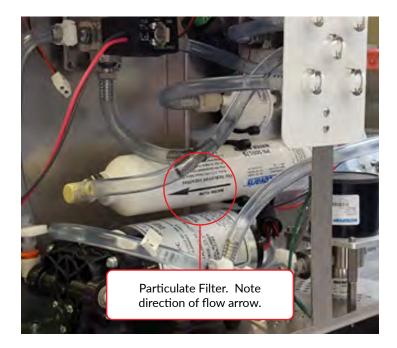
Changing the Filter

- Power off the WCS 3.0 and disconnect the power cord from the power source.
- Note which hoses are connected to which ports on the WCS and remove the hoses.
- Remove the cover at the hose side of the WCS by removing the 4 button head cap screws, 2 on each side, and slide the cover off. Removing the hoses is not necessary but gets them out of the way when the cover is removed.



• Locate the Particulate Filter and note the direction of flow arrow.

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• Disconnect the hoses at each end of the filter by depressing the release clip on the quick-disconnect fittings.



• With both fittings removed, grasp filter and gently pull it from single retaining clip near the center of the filter.





Reverse the steps to install the replacement filter.

- Note the direction of flow arrow, and place filter into retaining clip. Be careful not to pinch fingers behind filter as the filter snaps firmLy into place.
- Press the hose connections back into each end of the replacement filter until they click into place.
- Reconnect the hoses and power source. Power on the WCS and check for leaks.
- Slide the side panel on (it is not necessary to remove the hoses to reinstall the side cover) and secure with the four button head screws.