Covaris®

ML230 Focused-ultrasonicator
Setup & Instruction Manual

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

For Research Only

Model Number: ML230 | Software: SonoLab 10.1

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

Warnings

For the Safety of Operating Personnel:

- Make sure that the equipment is properly grounded. *DO NOT* operate if it is 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* attempt to operate the equipment with the safety cover in the open position or without water in the acoustic assembly; 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 °C to 25 °C (66 °F to 77 °F). *DO NOT* operate the instrument in a cold room environment; the system is designed to operate with a water bath and includes a solid state heater/chiller apparatus to control sample temperature.

- *NEVER* run a method without water in the acoustic assembly; this could damage the transducer. The instrument is equipped with a water level sensor to protect the transducer. The system will not allow the acoustic wave treatment to start unless an adequate volume of water is detected.

- *DO NOT* leave water in the water bath with instrument power turned off for an extended time as this disables the water cleaning system within the apparatus.

- AFA-grade water should be used to fill the supply water bottle.

- *DO NOT* employ isopropyl alcohol, ammonia-based or abrasive cleaners on the acoustic assembly.

- Sample plates should be stored in a dry place when not in use.

- *DO NOT* load third party hardware, software, or parts onto the system without consulting with Covaris.

Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery by an authorized representative. Any component 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), Greenwich Mean Time (GMT-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
- E-mail queries to techsupport@covaris.com or applicationsupport@covaris.com

ML230 Instrument Repair – Warranty repair of the ML230 is provided on site. All labor and expense charges are included. Failure due to accident, abuse, or improper operation is not covered. An Operator's Manual is provided with the equipment. This manual includes sections on the operating instructions, maintenance guidelines, and troubleshooting tips.

Table of Contents

1. Introduction

1.1 Overview of the User Manual

This manual contains the installation, operation, and service instructions for the ML230 Focused-ultrasonicator which are essential to the proper use and care of this equipment. Should any unforeseen problems occur with the normal operation of the equipment, contact Covaris Technical Support (techsupport@covaris.com) immediately.

The following definitions apply in this manual:

NOTE: Informational.	**WARNING:** Personal injury may occur.	**CAUTION:** Equipment damage may occur.

1.2 The ML230 and the Covaris Process

The ML230 Focused-ultrasonicator is based on Covaris Adaptive Focused Acoustics (AFA) technology. This instrument is designed to provide researchers an integrated stand-alone tool to be used in laboratory environment. The instrument is ideal for those who work with DNA shearing and sample preparation.

The system is comprised of the **Treatment System** which delivers the acoustic energy to the sample, the **Safety System** which protects users from inadvertently contacting the high intensity acoustic energy, and an embedded **Computer and Application Software** which provides users an interface to control the device.

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. Installation and Setup

2.1 Unpacking the System

Follow the included instructions for removing the ML230 instrument and accessories from their shipping materials.

2.2 Assembly

The following items should be found in the package of the Covaris ML230 instrument:

- ML230 main unit
- Accessory Box
    - Bubble Level Tool

Figure 2.1 Leveling Tool

    - Sample Tray Assembly

Figure 2.2 Sample Tray Assembly

    - 2 Plastic water bottles
    - 1 ML230 power cord
    - Sample Racks and Consumables, depending on order configuration

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CAUTION: The ML230 weighs about 80 pounds (36 kg). With the front of the instrument closest to your body, lift the ML230 by gripping from its base plate. Lifting the unit by its plastic case or doors will damage the instrument.
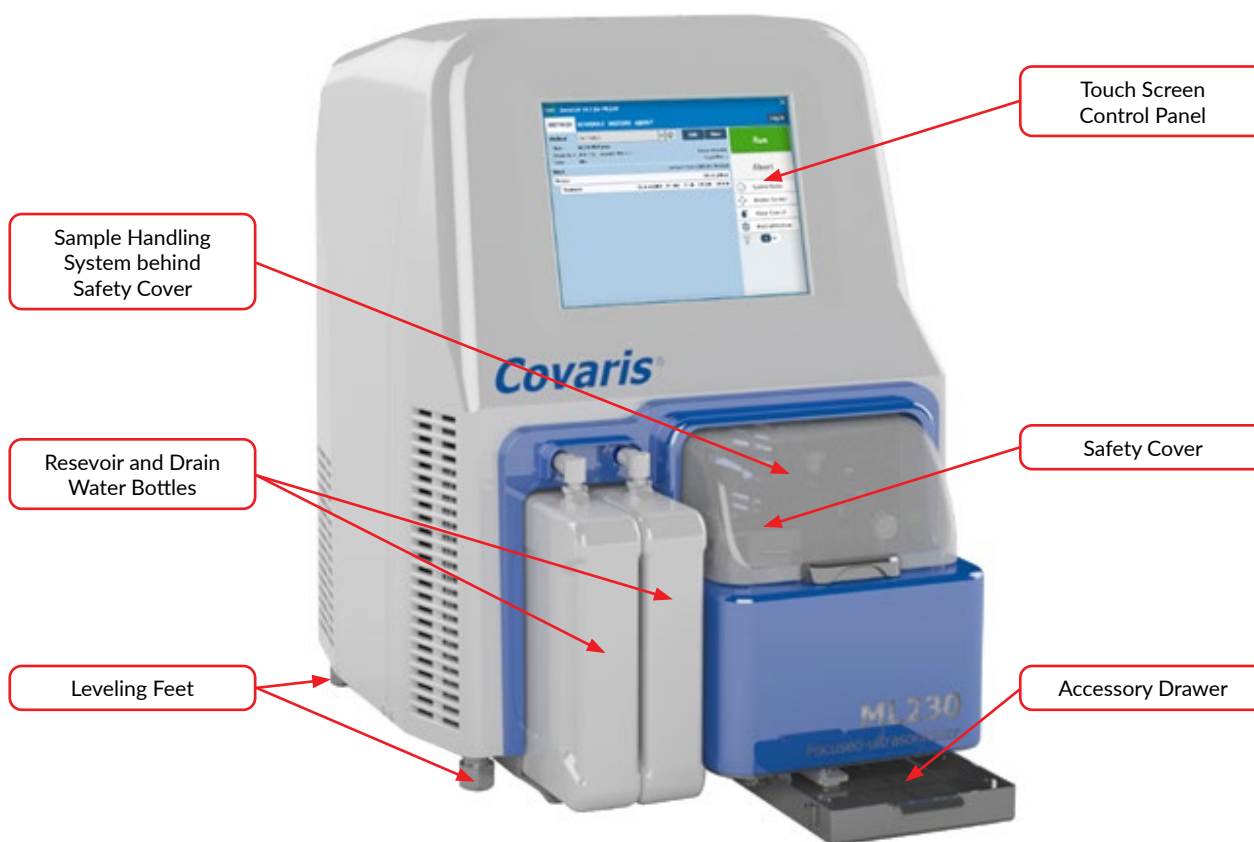

Figure 2.3 Front view of the ML230 instrument

After the instrument is unpacked, position the ML230 on a flat surface near a power outlet. Use both sides of the base to lift the instrument. *DO NOT* lift by the plastic parts at the front of the instrument.

1. Assemble the **Water Control** supply and waste bottles.

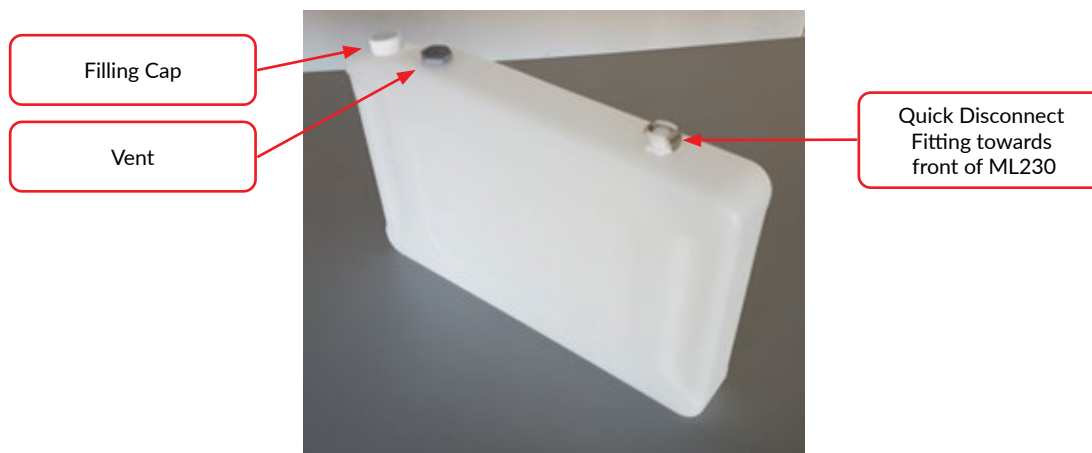   - Locate the two plastic bottles in the accessory box.

Figure 2.4 Water Control System Bottle

- Remove the cap on the 'Supply' bottle , fill the bottle with AFA-grade water, and replace the cap.

Figure 2.5 Remove cap to fill.

NOTE: The 'Waste' and 'Supply' reservoirs are not interchangeable. The 'Waste' bottle vent is marked 'Waste'.
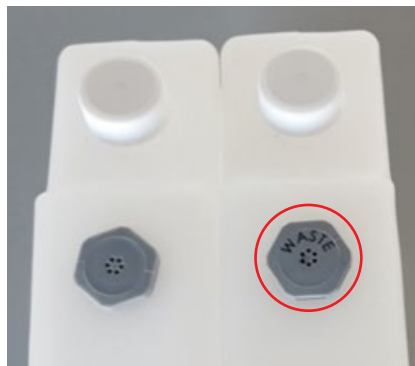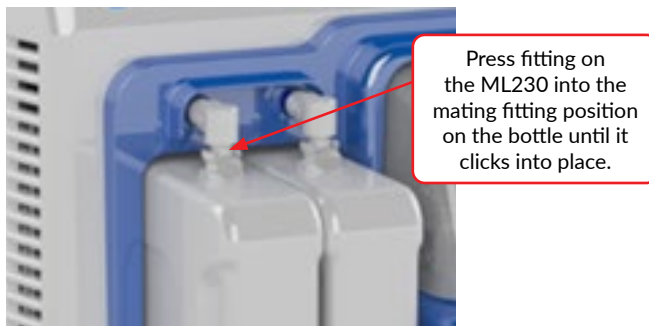

Figure 2.6 Supply and Waste Bottles with Waste Bottle Labeled.

- Holding the two quick-connect tubes up and out of the way, slide the empty Waste bottle into the left-most opening at the front of the ML230. The vent and cap are toward the rear; the Quick Disconnect port toward the front of the instrument.
- Slide the Supply bottle filled with water into the remaining opening, between the Waste Bottle and the sample handling area.


Figure 2.7 Bottles into Water Control Bay

- Press the two Quick Disconnect fittings into the Waste and Supply bottles until they click into place. See **Figure 2.4**.

> Press fitting on the ML230 into the mating fitting position on the bottle until it clicks into place.
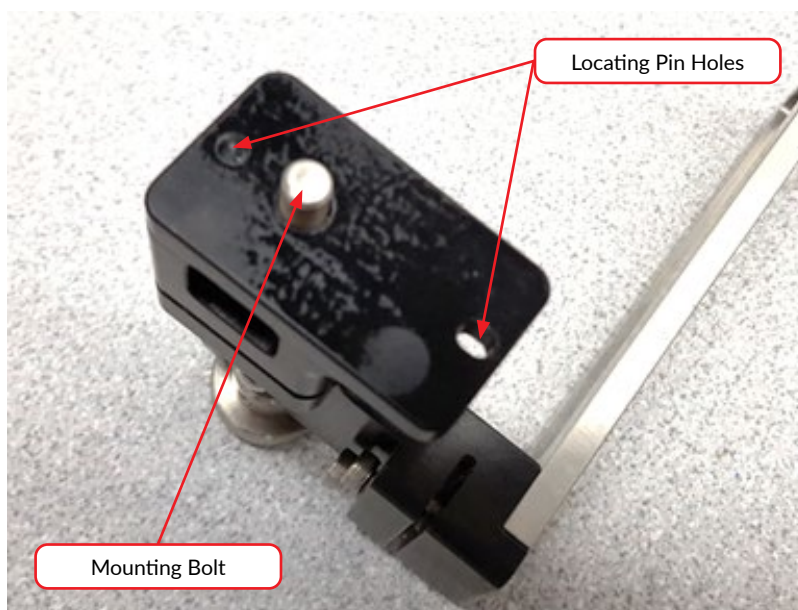
Figure 2.8 Connecting Water Control Bottle to System

2. Assemble the Sample Tray Assembly to the ML230.

- Locate the Sample Tray Assembly in the accessory box

> Holes for Locating Pins Located on Rear Face of Mounting Bracket
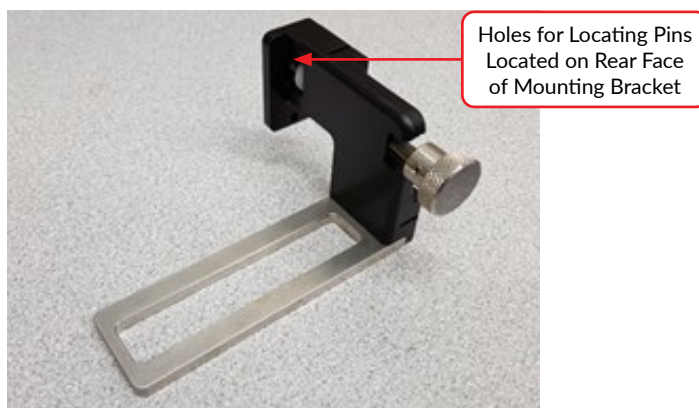
Figure 2.9 Mounting Bracket

> Locating Pin Holes

> Mounting Bolt

Figure 2.10 Mounting Bracket

- Lift the Safety Cover open.
- Slide the Sample Tray Assembly locating pin holes onto the pins on the motion system, behind the safety cover. The sample tray faces to the left. Refer to the image below.
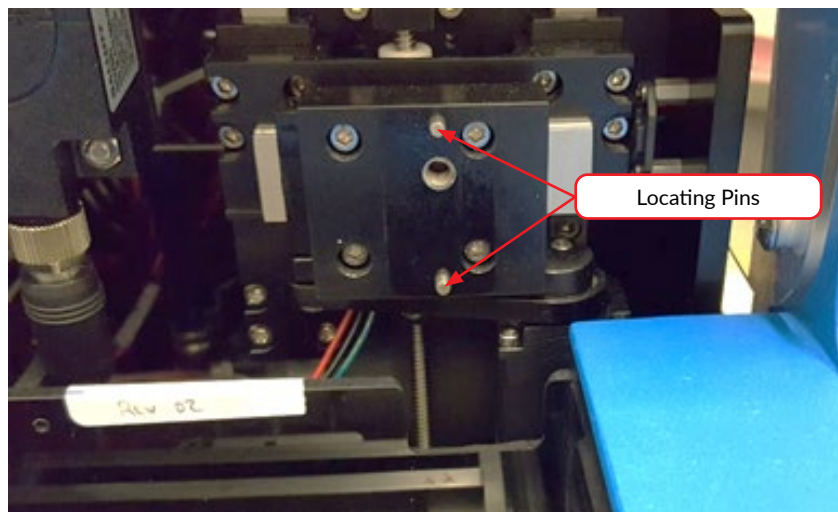

Figure 2.11 Mounting Bracket installed in ML230

- Thread the Sample Tray Assembly's thumbscrew into the motion system and snug it finger-tight.
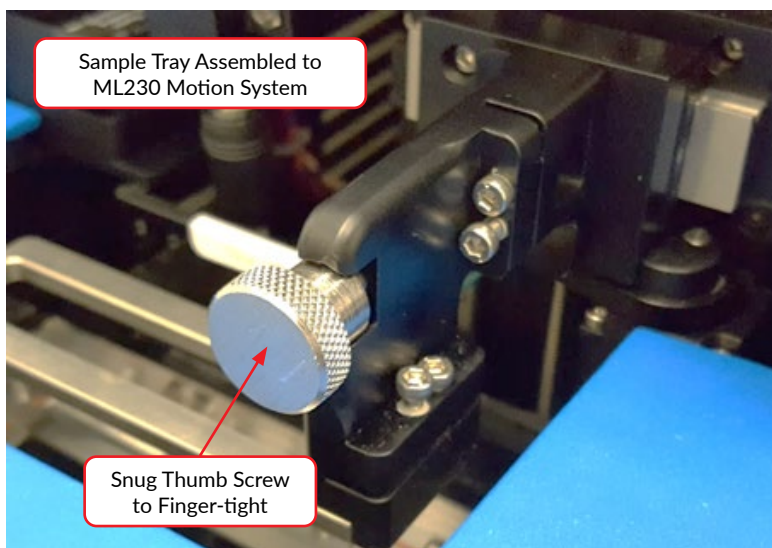

Figure 2.12 Sample Tray Assembled to ML230

Figure 2.13 The power panel of the ML230 instrument

3. As shown in **Figure 2.13**, plug the AC power cord into the power inlet located about half-way up the left side of the ML230 rear panel. Plug the AC power cord into the power outlet.

The entire instrument may be relocated in this assembled configuration by disconnecting power, lifting from the bottom, relocating, and repeating the appropriate assembly steps.

> **NOTE:** Any time the ML230 is moved to a new location, follow the instrument leveling procedure described in Section 2.3.

2.3 Leveling the ML230 Instrument

This step is very important to ensure proper filling and draining of the water bath and proper instrument operation.   Once the ML230 is installed in the location in which it will be used:

- Open the Front plastic safety cover
- Place the leveling fixture in the sample tray

Figure 2.14 Leveling Tool

- If the bubble is to the right side of the center markings of the sight glass, the right side is high, and the right side feet need to be shortened. If the bubble is to the left side, the right side is low, and the right side feet must be lengthened. Adjust both front and rear feet an equal number of turns.
- Repeat the process above to level front-to-rear, adjusting only the feet in the front. If the bubble is in the center of the fixture sight glass, no adjustment is required. If the bubble is to the front side of the center markings of the sight glass, the front side is high, and the feet need to be shortened. Alternatively, if the bubble is to the rear, the front side is low, and the legs must be lengthened. Adjust both front feet an equal number of turns. The feet can be adjusted by hand; no tools are necessary.

> **NOTE:** The feet can be adjusted by hand; no tools are necessary.

- Lengthen or shorten both legs on the right side of the instrument an equal amount, as in **Figure 2.15**, until the bubble is centered in the leveling fixture sight glass.
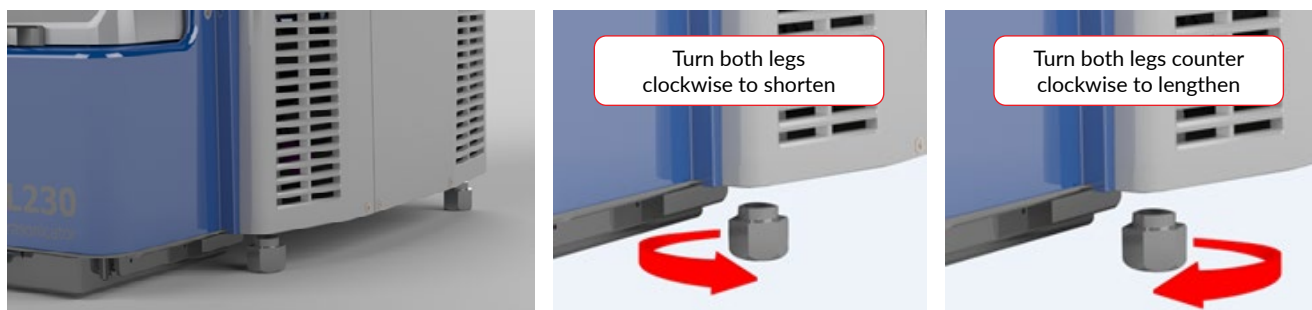

Figure 2.15 ML230 Leg Height Adjustment

2.4 ML230 Software

SonoLab 10 has been pre-installed on the ML230 instrument. No further installation or configuration is required.

SonoLab is the software used to control a Covaris Adaptive Focused Acoustics instrument for processing a sample. It is designed to create, edit, and run a sample 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.
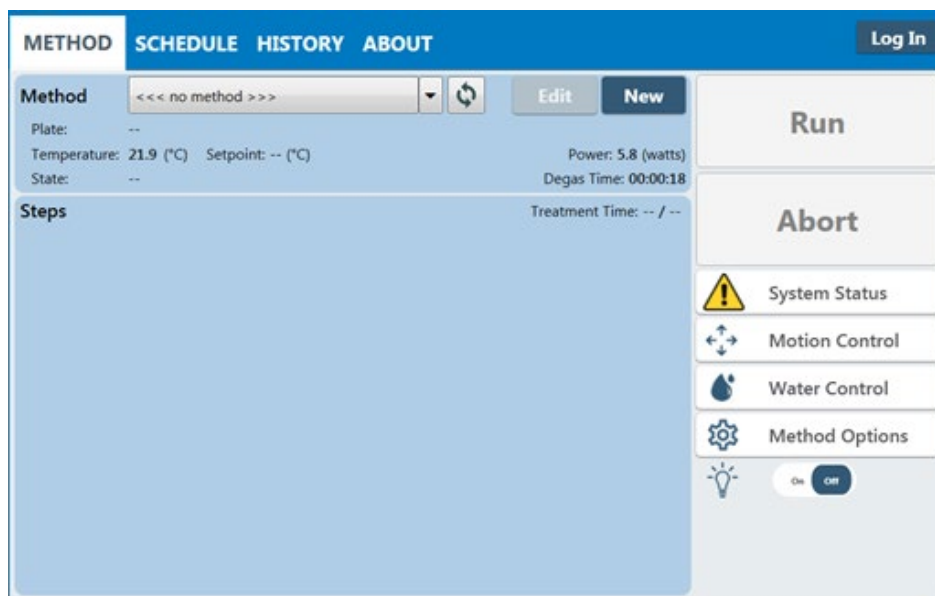
If it is necessary to install or re-install SonoLab, please contact Covaris for the latest versions, downloading, and installation instructions.

2.5 Starting the ML230

> **NOTE:** The ML230 is shipped with the sample tray removed. If the sample tray has not already been installed, follow the instructions from Section 2: Installation and Setup.

Turn on the ML230 power switch located about half-way up the left side of the rear panel.

SonoLab will automatically connect to the instrument, and a window will appear asking whether the instrument is to be homed. Touch "Yes". The motion system will perform its initialization sequence, the homing window will close, and the SonoLab Home screen will be displayed, as illustrated in **Figure 2.16**.

Figure 2.16 The SonoLab Method Screen
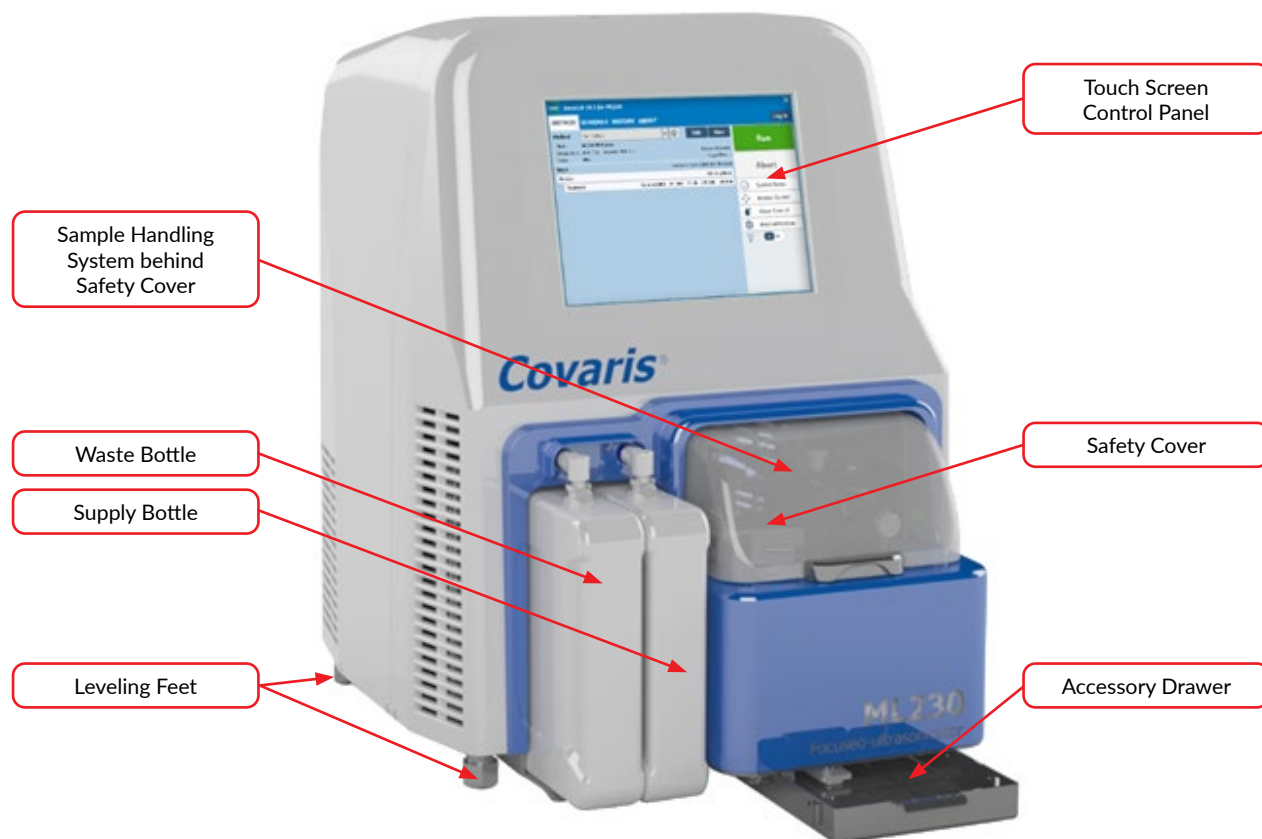
3. Operation

3.1 General Overview

Touch Screen Control Panel

Sample Handling System behind Safety Cover

Safety Cover

Waste Bottle

Supply Bottle

Leveling Feet

Accessory Drawer

Figure 3.1 Principal components of the ML230 instrument

The **Safety Cover** is used to protect users from potential acoustic hazards during the treatment. The acoustic treatment will not be started if the Safety Cover is open.

The **Acoustic Assembly**, located in the water bath below the sample tray, is used to transmit the acoustic energy from transducer to the sample through the coupling media (AFA-grade water). The assembly incorporates an Acoustic Transducer which converts electrical signals into ultrasonic acoustic signals. The assembly also incorporates Sensors for water level and temperature to ensure that these are appropriate during acoustic treatments.

The 2-axis **Motion System** is designed to automatically align the sample tube at the appropriate position and height in the acoustic field for processing. Tubes are loaded into appropriate Racks, which are then placed on the ML230 sample tray.

An **Embedded Processor** provides an interface for controlling the ML230 instrument via the touch screen at the front of the ML230.

The **Electronics Cabinet** provides the hardware for controlling the acoustic transducer, the temperature, and sample position during processing.

The **Enclosure Light** is normally lit when the instrument is turned on. The user can disable the light from the SonoLab Home screen.

The **Water Control** consists of a pump, valves, supply and waste bottles, and interconnecting plumbing. These components are controlled automatically by SonoLab software to fill, drain, and maintain the correct water level in the water bath.

When the bath is filled, the water is circulated through a UV sterilizer and particulate filter to prevent biological growth in the water control hardware.

The **Thermal Module** controls the water bath temperature. It cools or heats the acoustic assembly, maintaining the water bath at or near the setpoint programmed in the method. No external chiller is required.

> **WARNING:** During treatments, objects immersed in the focal zone above the transducer will be exposed to high acoustic intensity when the system is activated. Always close the Safety Cover prior to starting a method. Do not tamper with the door-closed sensor system.

3.2 Preparing the ML230 for Use

To run an acoustic treatment, the following conditions must be met:

a. The ML230 motion system must be initialized

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

c. The proper rack and consumables must be in place on the ML230 sample tray. See **Section 3.4**, Loading the Samples

d. Water Temperature must be within acceptable Min and Max limits

e. Water must be at the level required by the consumables specified in the selected method

f. The safety cover must be closed

The Run button will remain gray and inactive until all necessary conditions except water level and temperature are met. The ML230 automatically corrects these when "Run" is selected.

To achieve an acceptable status on the other conditions:

a. The ML230 motion system normally initializes when SonoLab starts, unless the user purposely skipped this step, in which case the motion system can be homed from the Motion Control pane.

- Touch the **Motion Control** button to display the Motion Control pane
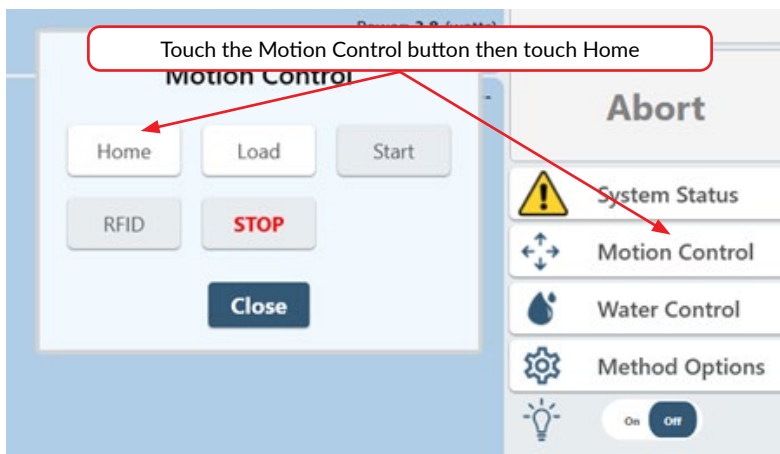- Touch "**Home**" to initialize the motion system

Figure 3.2 Motion Control Panel

b. To select and load an acoustic method, touch the "Method" bar, as shown in Figure 3.3, to display the Method Select pane. Navigate through the list with the vertical scroll bar, if necessary, and select a method.

If Consumable-based Method Selection is enabled, the "Method" bar is disabled. See Consumable-based Method Selection after **Step D** below for selecting methods with this option enabled.

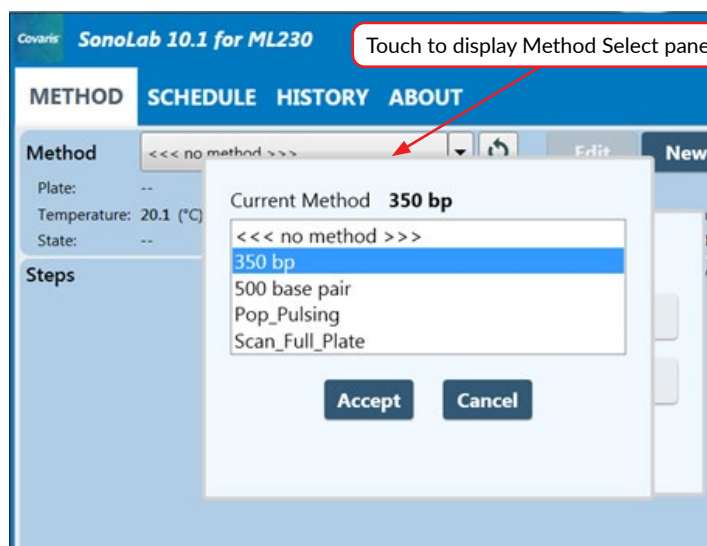> **NOTE:** Several acoustic methods will come pre-installed onto your system.

Figure 3.3 Selecting and loading an acoustic method

c. Open the safety cover and insert the samples onto the sample tray.
d. The Safety Cover Closed status will turn green as soon as the Safety Cover is completely closed.
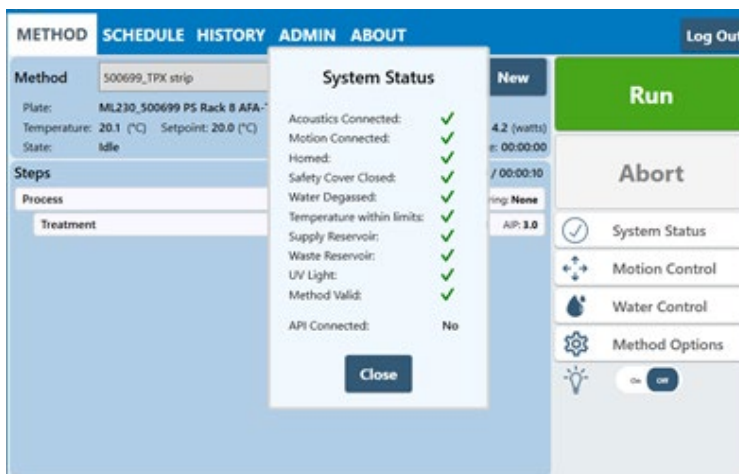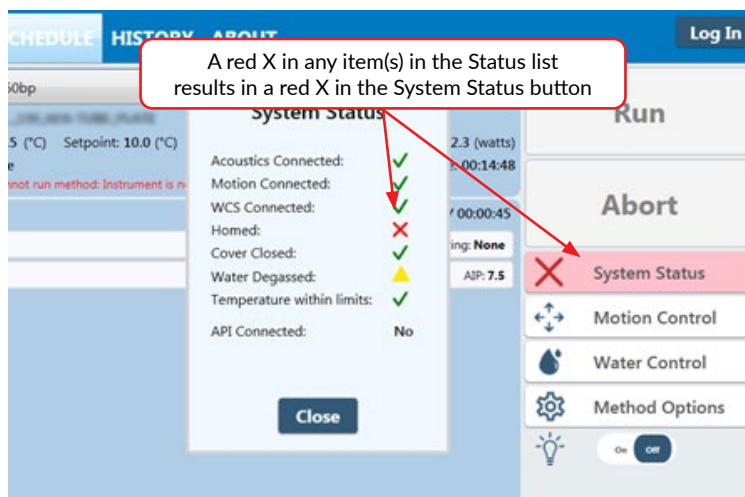

Figure 3.4 Instrument Status Panel – All OK

The remaining conditions, along with their related states, are displayed in the Instrument Status panel (see **Figure 3.4**). Touch 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 any of the items in the Status list display, a red X will also be displayed in the System Status button, as shown in **Figure 3.5**.

**Figure 3.5** Instrument Status Panel – All OK

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

Once a method has been loaded and all conditions except temperature, degas state, and water level are acceptable, the Run button will turn green and the selected acoustic method can be started.

To modify an existing method, see Create and Edit Methods in *Section 5*.

Consumable-based Method Selection

SonoLab can be configured with Consumable-based Method Selection to limit the displayed list of methods to only those compatible with the current consumable. See 'Method Options' in *Section 6.1* and 'Managing Users' in *Section 6.5.3* for details on configuring users to use Consumable-based Method Selection.
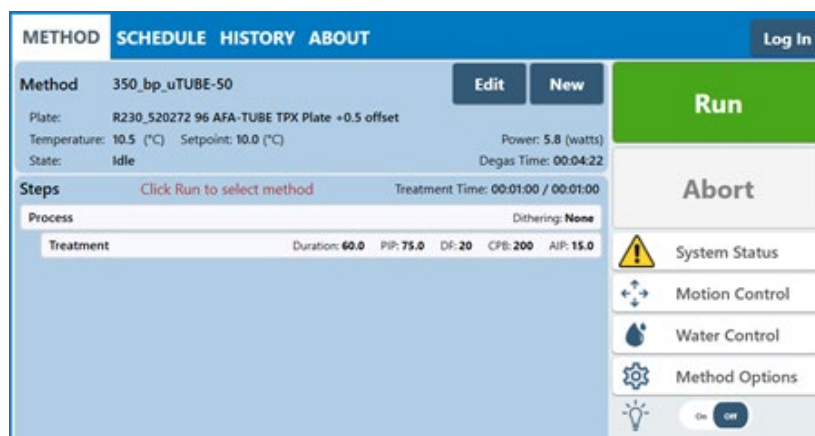
Figure 3.6 Method' Screen with Consumable-based Method Selection Enabled

To select and run a method with Consumable-based Method Selection:

1. With the consumable containing the sample on the sample tray, touch 'Run'.
2. The ML230 performs RFID validation of the inserted consumable. 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.
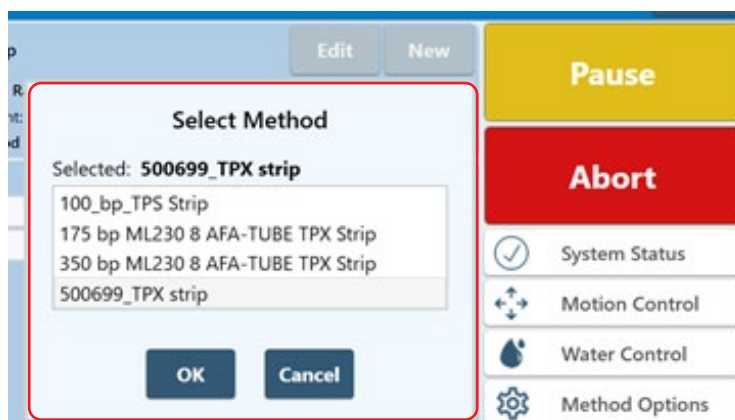

Figure 3.7 Method Selection List with Consumable-based Method Selection Enabled

4. User selects method from the list and clicks OK, or Cancel to abort.

5. System runs the method.

The method's total and remaining run time (hours, minutes, and seconds), temperature setpoint, and actual bath temperature will be displayed on the main screen as shown.
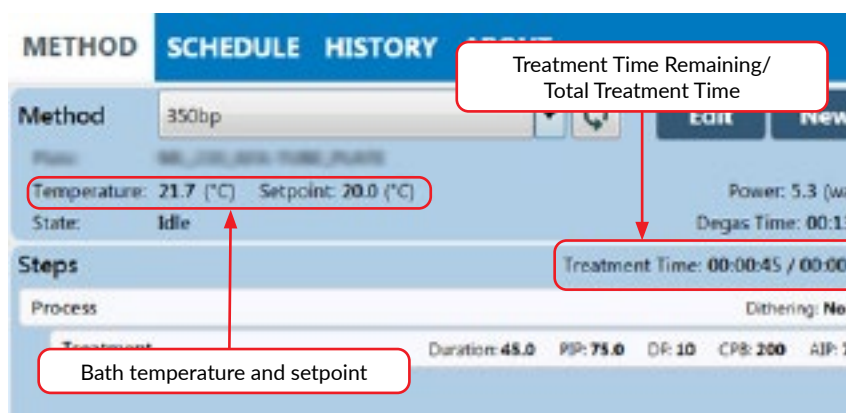

Figure 3.8 Method Run Time and Setpoint

e. The ML230 system automatically adjusts the water temperature to match the method temperature setpoint after Run is selected. Water Temperature status will turn green when temperature is within the Min and Max limits (typically 3 degrees above or below the setpoint). The default setpoint (in the absence of a selected method) is 20 °C.

f. The ML230 Water Control automatically fills the bath to the appropriate level for the tubes specified in the method when the Run button is touched to start a method. (See **Section 3.3**) The ML230 also degasses the water, as necessary.

3.3 ML230 Water Control

To transmit acoustic energy to the sample vessel, the bath surrounding the transducer must be filled with deionized water. Covaris recommends AFA-grade Water. You can also use highly purified water (at least ASTM Type III or ISO grade 3).

The ML230 Water Control automatically maintains the correct water level in the bath for the sample vessels specified by a method. This system either adds water from the Water Control supply bottle to raise the level of the bath or removes water from the bath to the Water Control waste bottle.

Sensors detect if the supply bottle is empty or the waste bottle is full, and these conditions are reported in the Instrument Status panel. To service the bottles, press the metal thumb latch on the Quick Disconnect Cap, as shown in **Figure 3.9** to release the Quick Disconnect Insert of the bottle being serviced, and slide the bottle out. Remove the cap at the rear of the bottle, then empty the waste bottle or fill the supply bottle, as appropriate, and reverse steps to reinstall the bottle. The Supply bottle is to the right, next to ML230 sample handling system, and the Waste bottle is to the left of the Supply bottle.

Figure 3.9 Releasing the Quick Disconnect Fittings

3.4 Loading the Samples

A variety of tubes and their associated racks are available for use with the ML230. In some cases, individual tubes are simply placed into the rack as shown.

The 8 microTUBE strip is fitted to its rack as a unit. Open the hinged rack and insert the 8 microTUBE strip. The strip is keyed to fit the rack in the correct orientation.

Figure 3.10 Assembling microTube 8-strip into Rack

Close the hinged rack cover to secure the strip. Magnets in the cover hold the cover in the closed position.

Figure 3.11 ML230 8 microTUBE Strip 50 Rack

Open the ML230 safety cover. Place tubes and rack assembly into the ML230 sample tray shown in **Figure 3.12**. The racks are keyed to load into the sample tray in the correct orientation only.
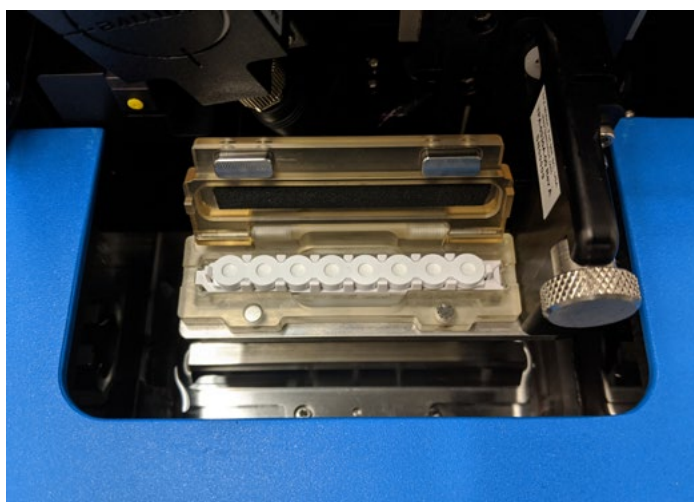

Figure 3.12 Sample Rack Loaded onto ML230 Sample Tray

Close the Safety Cover to ensure that the Door status in SonoLab turns to a green check.

3.5 Running a Method

When the "Run" button is green, the selected method may be started. Touch the "Run" button to start. The green "Run" button becomes a yellow "Pause" button, and the "Abort" button turns red. The instrument will automatically adjust the water level and/or temperature to the appropriate settings, as shown below.
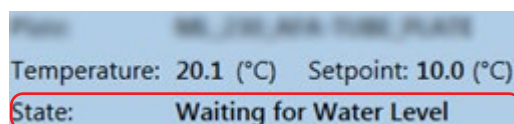
When the correct water level and bath temperature are reached, acoustic treatment begins.

As the method runs, the Process and Treatment bars turn yellow, and the actual average incident power and temperature data are displayed, as shown in **Figure 3.13**.
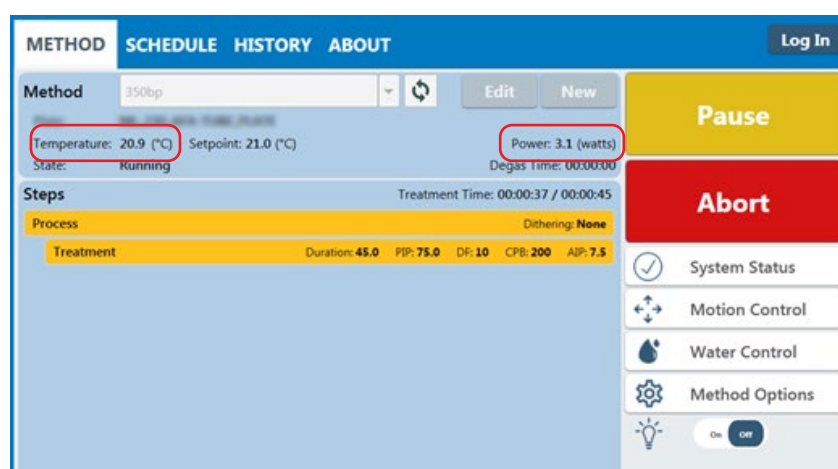
Figure 3.13 Screen Shot of a Running Method

The user can, at any time while running a method, touch "Pause" to pause a running method. The "Pause" button becomes a green "Resume" button

When the method is paused, the user may continue the process by touching the "Resume" button or terminate the process by touching the "Abort" button.

When the method has finished, the message "Method Complete" is displayed. Touch "OK", then touch the "Motion Control" button, then the "Load" button from the motion menu to raise the sample tray out of the water bath. When the sample tray stops moving, raise the safety cover and remove the treated sample rack.

3.6 Instrument Light

The instrument light is located above the acoustic assembly and inside of the Safety Cover. By default, the light will be lit once the instrument is turned on. This light may be turned off by touching the Light control on the Home screen.
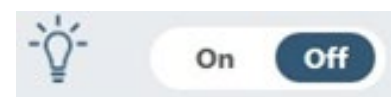
3.7 User Maintenance

The water bath should be drained when the ML230 is not in use by touching the Water Control button at the right of the main screen. The Water Control options will be displayed.
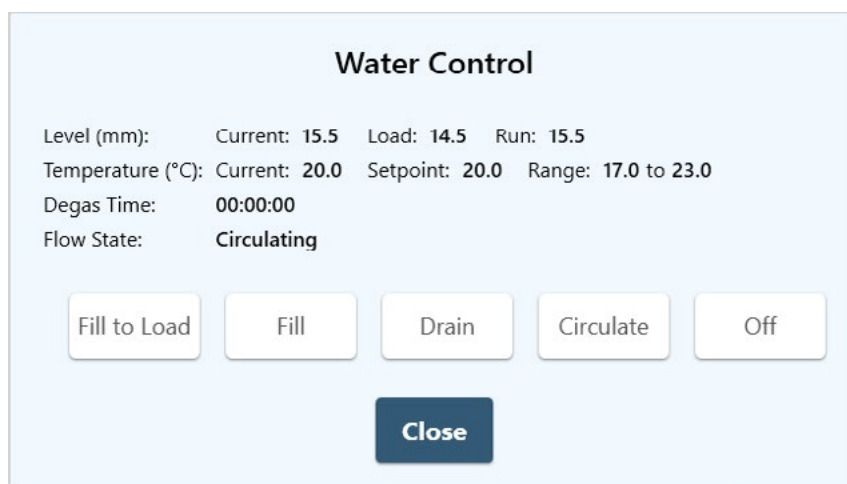
Touch "Drain". Remove the rack from the sample holder and place it on a clean, dry surface. Blot up water remaining in the bath using a soft, lint-free cloth, wipe, or swabs included with instrument. Close the Safety Cover.

Cleaning Protocol

A cleaning protocol using a solution of 10% bleach or equivalent (such as NaDCC) should be performed once a month as follows, to prevent biological growth in the water bath or plumbing of the Water Control system.

- Place a solution of 300 ml of water and 30 ml of household bleach, or equivalent, in the Water Control Supply bottle, and verify the Waste bottle is not full.

> **NOTE:** Do not use "fresh" or "pleasant smell" bleach products. Use only 70% isopropyl alcohol wipes to clean the exterior of the ML230.

- Touch the Water Control button to display the Water Control panel.
- Touch "Fill" and wait for bath to fill.
- Touch "Circulate" in the Water Control menu.
- Allow the bleach/water solution to circulate for at least 10 minutes.
- Touch "Stop", then touch "Empty" in the Water Control panel and allow the bath to drain.
- Empty and thoroughly rinse the Supply bottle, fill the Supply bottle with deionized water, and reinstall bottle to the ML230 bottle bay.
- Touch "Fill" in the Water Control menu, then "Circulate". Allow the water to circulate for 3 to 5 minutes.
- Touch "Stop", then "Empty". Allow the bath to drain.
- Repeat the above 2 steps twice more.
- Empty and thoroughly rinse the Waste bottle and reinstall the empty Waste bottle in the ML230 bottle bay.

Water Control Particulate Filter

The Water Control particulate filter element should be replaced every 12 months. Replacement filters can be obtained from Covaris Customer Service. See **Appendix C** for filter replacement instructions.

3.8 Shut-down Procedure

If the instrument will be idle for an extended time (e.g. overnight or a weekend), shut down the instrument using the following procedure: Empty the water bath and store the sample rack in a clean, dry place.


CAUTION: Leaving water in the water bath for an extended time may allow algae growth which will interfere with sample processing.

To shut-down the ML230:
- Close SonoLab by touching the white X at the upper right corner of the screen.
- When the 'Drain Bath?' pane appears, touch 'Drain Bath & Exit SonoLab' or 'Exit SonoLab'. As stated in the pane, 'Drain Bath & Exit SonoLab' is the preferred option, as leaving standing water in the bath when SonoLab is not running can result in bio-growth. The 'Exit SonoLab' option, which exits without draining the bath, may be used in the event SonoLab is to be shut down and shortly restarted.
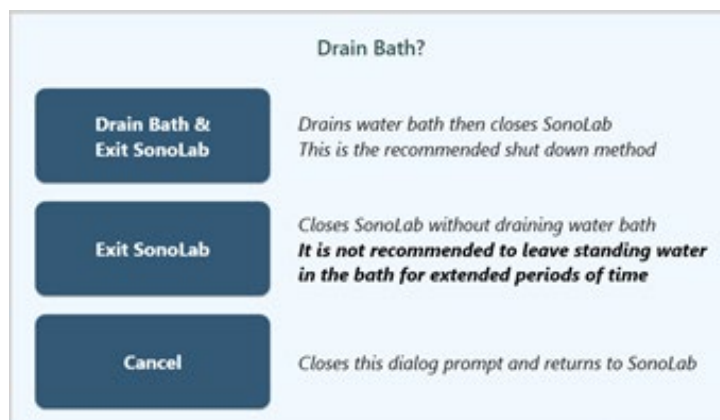
- When the bath has drained, or immediately if 'Exit SonoLab' is chosen, the 'Power Off Computer?' pane is displayed.
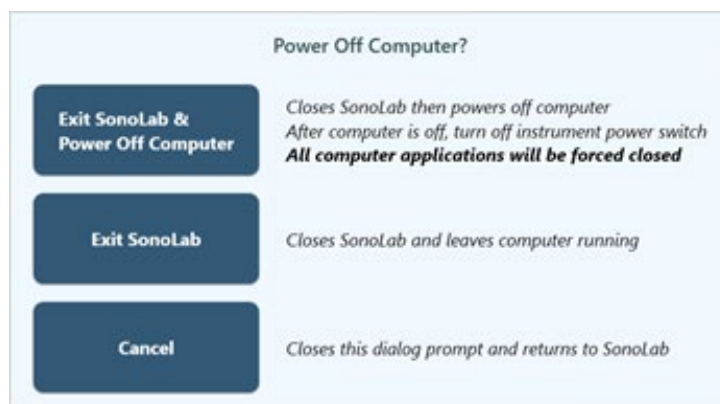
- Once the computer is shut down, move the ML230 power switch to the OFF position. The power switch is located on the rear panel of the ML230, about half-way up the left side, above the power cord.

---

4. Using a Pre-installed Method

SonoLab has protocols pre-installed on the ML230. These methods have been developed by Covaris scientists and are ready-to-use for some common applications. To select a pre-installed protocol, touch the Method field to display the list of available methods. If the "Method" bar is disabled, see Consumable-based Method Selection in **Section 3.2** above.
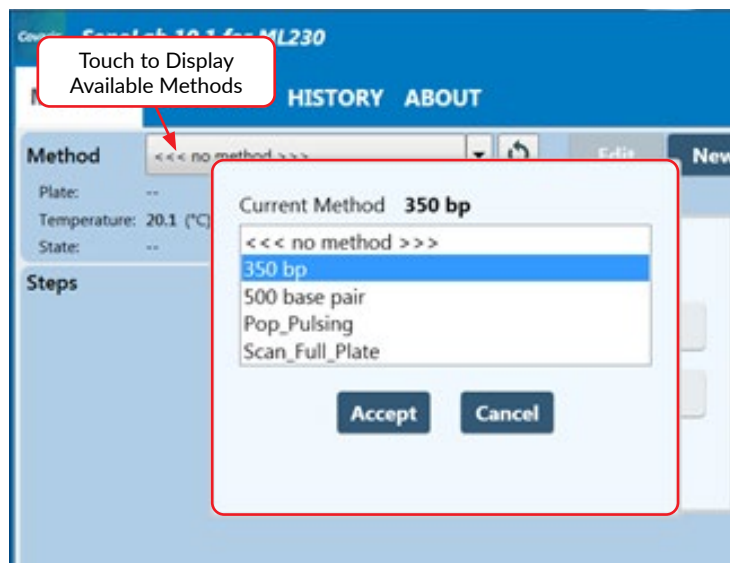

Figure 4.1 Selecting and loading an acoustic method

4.1 DNA Shearing Protocols

DNA Shearing protocols are optimized for the mean DNA fragments size, sample volume, and sample vessel used.

Naming convention is as follow:

- DNA_[size]_bp_[volume]_µl_[type of vessel]

Please contact the Covaris Applications team, applicationsupport@covaris.com, for assistance selecting parameters when creating new methods.

5. 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, containing a single treatment step. But more complex methods are easily constructed.

A Process, and its included treatment(s), may be repeated as many times as necessary. A subset of treatments within a Process may also be repeated. If all the steps of the Process are to be repeated, a global Repeat step is placed outside the process. See **Section 5.1.1** below. If only some treatment steps are to be repeated, select the Repeat step from within the Process, as explained in **Section 5.1.5** below.

5.1 Create a New Method

To create a new method, touch the "New" button on the main screen (see **Figure 5.1**).
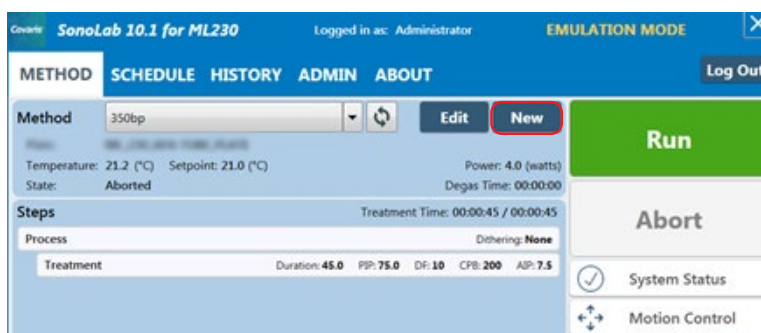

Figure 5.1 New Method Button on Main Screen

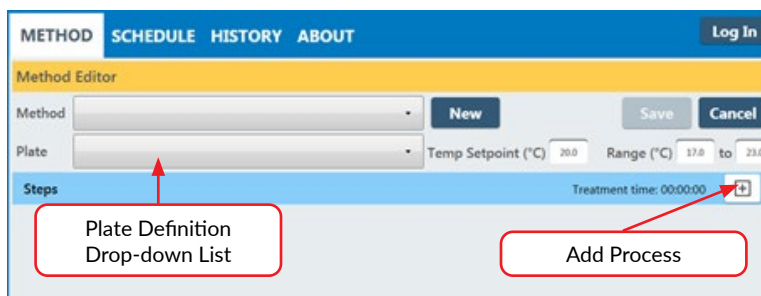The Method Editor window will appear (See **Figure 5.2**).
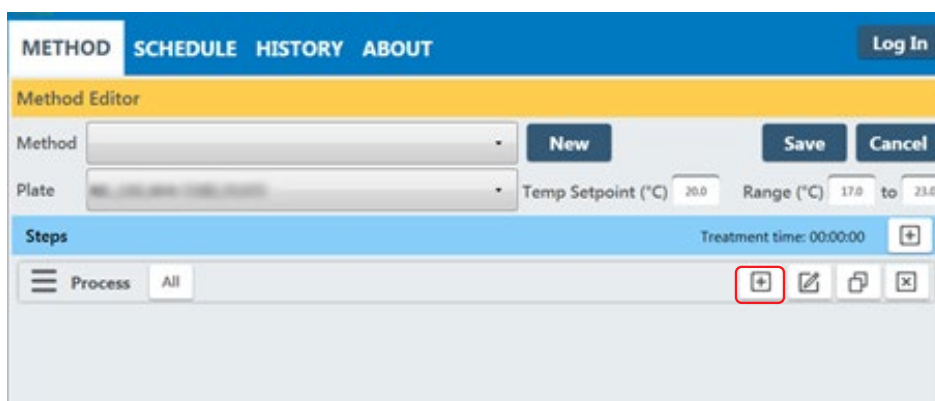

Figure 5.2 Method Editor Window

1. Select the appropriate plate for the tubes used in this method from the "Plate Definition" drop-down list.
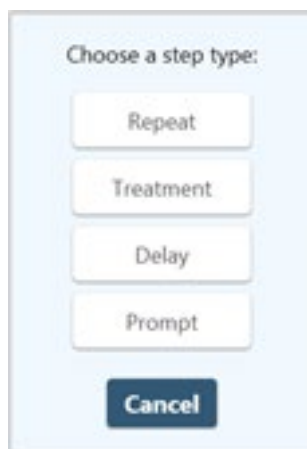
5.1.1 Add a Step in the Method

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

Touch "Process". The process bar is displayed. If steps are to be repeated, refer to the Repeat Function listed in **Section 5.1.5**.

Touch the **+** button inside the process bar to display the step type menu.

5.1.2 Choosing Treatment

Choosing Treatment allows editing of the four acoustic parameters: Duration, Peak Incident Power, Duty Factor, and Cycles per Burst.
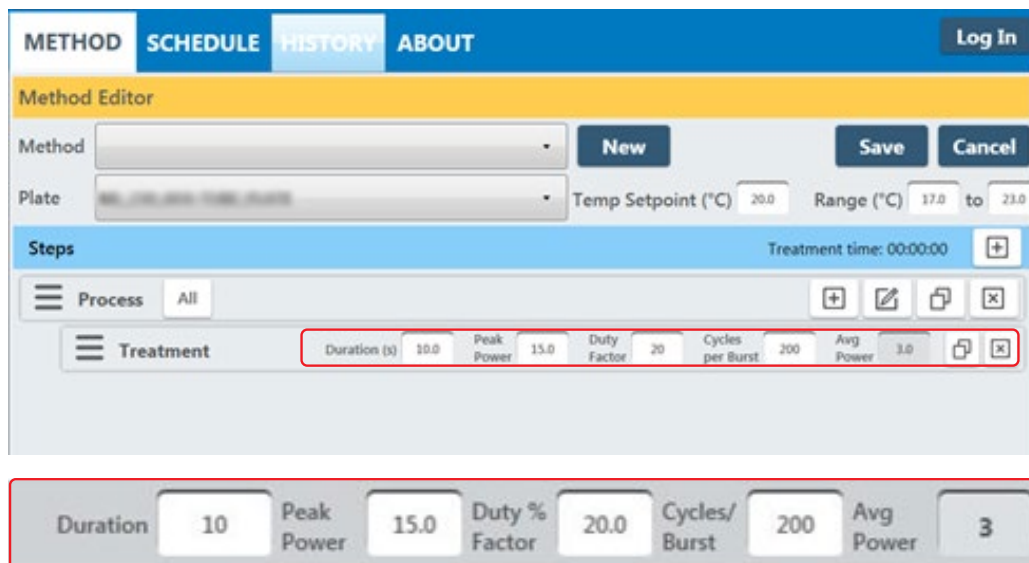

Figure 5.3 Add a Treatment

Treatment parameters are chosen by directly editing the Value box. An on-screen keyboard is displayed when the text entry fields are touched. The treatment parameters are:

Peak Incident Power (PIP): This parameter measures the peak electrical power used to drive the ultrasound transducer. In the ML230 instrument, 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 define the Duty Factor from 1 to 50% in the ML230 instrument, depending on the PIP chosen.

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.

SonoLab will not allow a combination of Duty Factor and PIP which results in an Average Incident Power less than 0.5 Watts or exceeding the ML230 limit of 100 Watts.

Cycles per Burst: This parameter indicates the number of electrical signals used in each acoustic "burst" of the transducer. One may define Cycles per Burst (cycles/burst) from 50 to 1000 in the ML230 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, "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. In the ML230 instrument, the maximum Average Incident Power is 100 Watts (for example, 450 Watts Peak Incident Power and 25% Duty Factor are selected). SonoLab does not allow the user to select a combination of Peak Incident Power and Duty Factor that results in more than 100 Watts or less than 0.5 Watts average incident power.

Dithering: The ML230 motion system provides dithering motion capability in the Y axis (left-to-right). If a treatment specifies dithering, touch the "Pen-&-Paper" icon on the right end of the Process bar to display the Dithering Parameters pane.

Figure 5.4 Dither Parameter Pane

Enter the specified dithering parameters and touch "OK".

Temperature Setpoint and Range: Enter the temperature setpoint for the protocol and the allowable range into the text entry fields. SonoLab allows a minimum range of plus/minus 2 degrees. The default value is plus/minus 3 degrees.

Figure 5.5 Entering Temperature Setpoint and Range from lower to upper limits

5.1.3 Choosing a Delay Step

Choosing a Delay step introduces a fixed time delay into a method, allowing the sample to settle between two different treatment steps, for example. The delay is specified in seconds. See **Figure 5.6**.
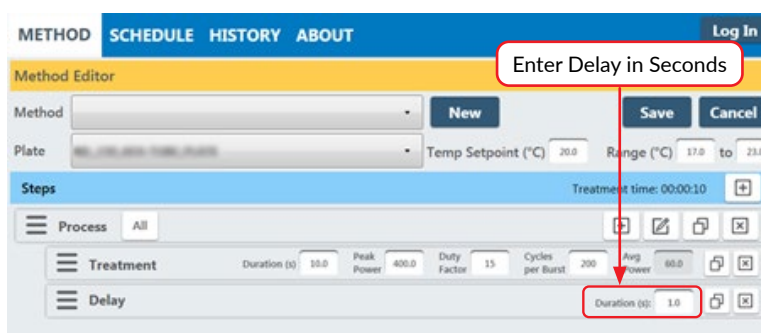
Figure 5.6 Add a Delay Step

5.1.4 Choosing a Prompt Step

Choosing a Prompt step displays a specified message on the screen and pauses execution of the method until the operator touches OK. Define the prompt message by touching the "pen & paper" button of the prompt bar to display the text entry field for the prompt. Enter the text of the prompt and touch OK. See **Figure 5.7**.
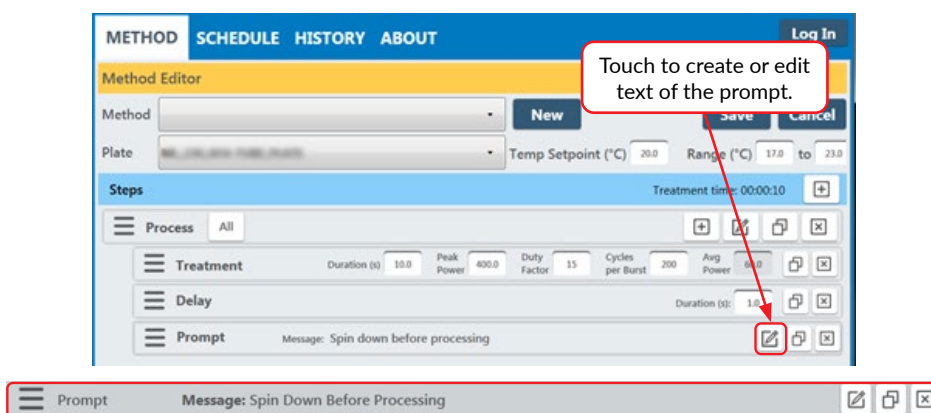

Figure 5.7 Add a Prompt Step

When the prompt step is reached during the treatment, the method pauses, and the prompt message will be displayed as shown in **Figure 5.8**.
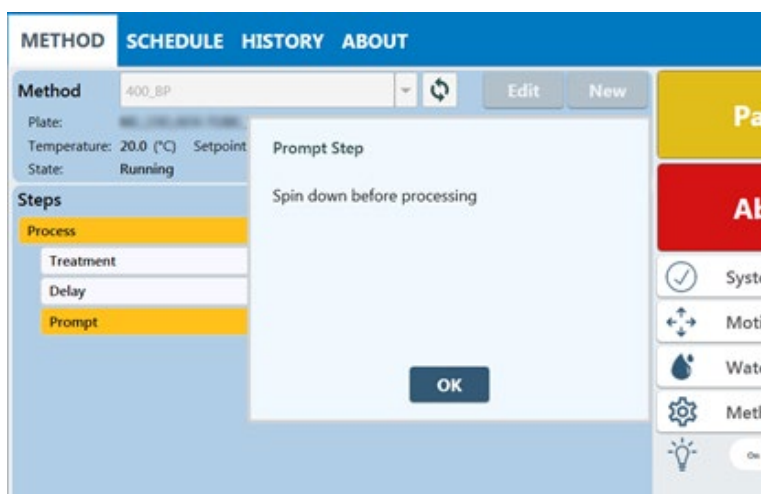
Figure 5.8 Example of a Prompt Message Display

5.1.5 Choosing a Repeat Step

Choose a Repeat Step when one or more Treatment, Delay, or Prompt step needs to be repeated one or more times. See **Figure 5.9**.
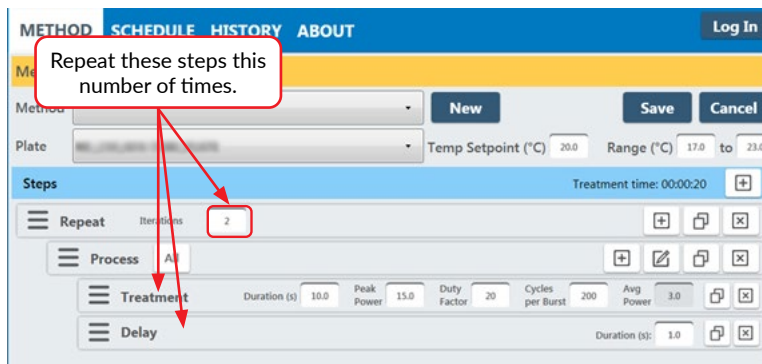

Figure 5.9 Repeat function programming for sequential sample treatment

Above: The Repeat function selected prior to the Process and Treatment Steps will provide one pulsing step and one delay step before repeating the next pulsing step and next delay step in the same manner. Please contact applicationsupport@covaris.com for inquiries regarding the repeat pulsing feature and method setup.

5.1.6 Saving Method

When all method parameters and steps have been defined, touch Save. to display the Save Method As panel. Touch the "Save" button to save the method.
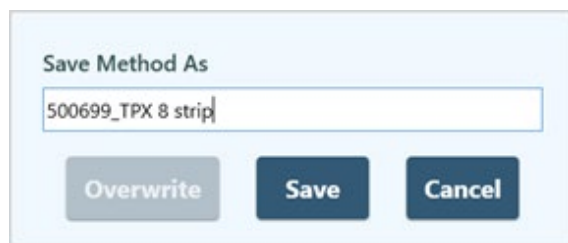

5.2 Using Drag and Drop with Treatment Steps

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.
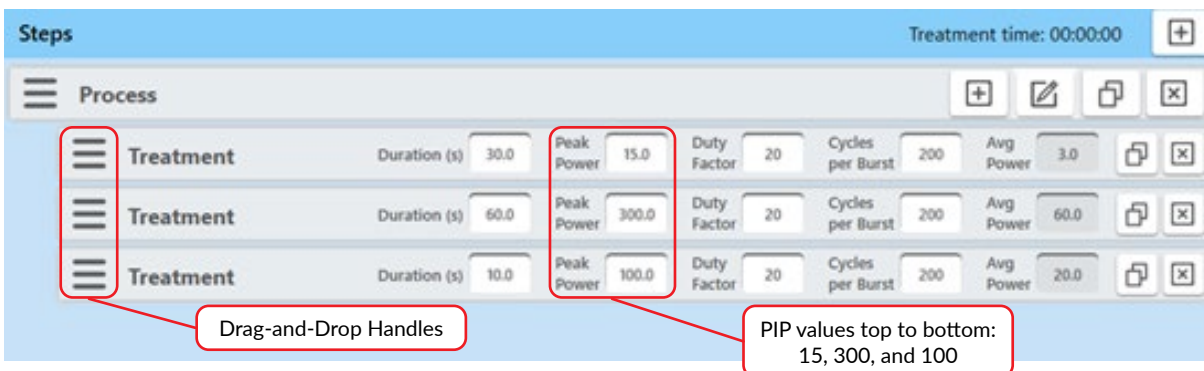
To swap the positions of the second and third steps:

- Touch and hold 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.
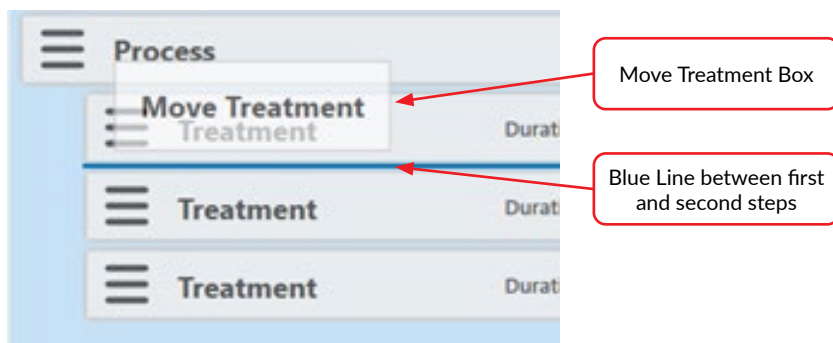
- Release the bars when the blue line appears. The former third step will be inserted below the first step ahead of the former second step.

To move treatment steps into a repeat step:

- Touch 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.
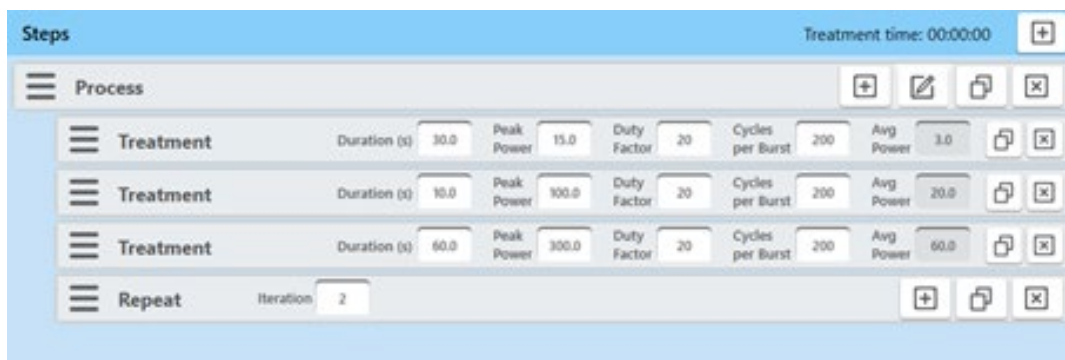
- Touch and hold the three bars at the left of the first Treatment step.
- Drag the step over the Repeat step until the blue line shows below the Repeat step.
- Release the bars.
- Do the same for the two remaining Treatment steps. The screen is now configured as shown below.

5.3 Modify Existing Method

To modify an existing method, then touch the "Edit" button to display the Method Editor screen. The touch Method bar is to display a list of methods to edit.
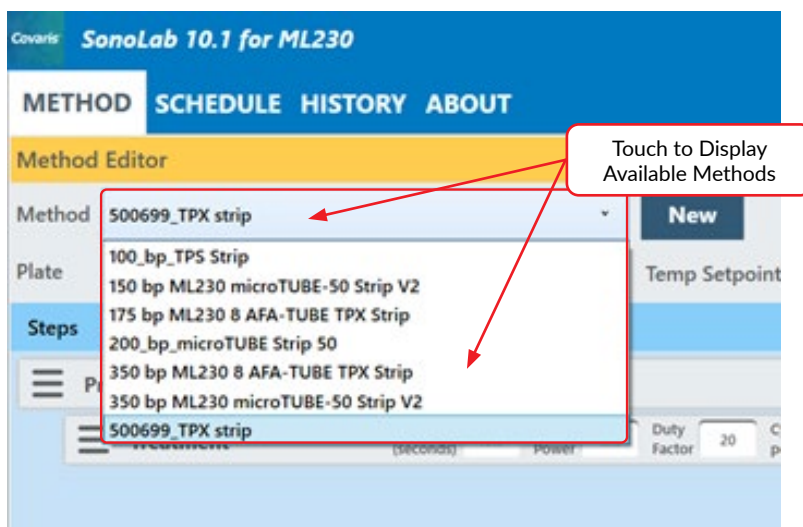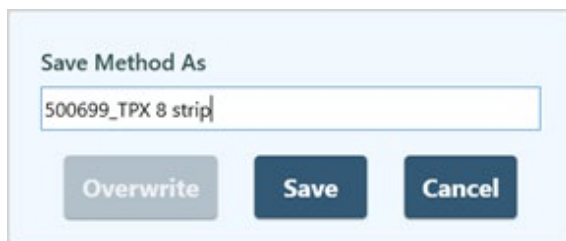

Figure 5.10 Selecting a Method to Edit

Make the desired changes to the method, touch save. The 'Save Method As' panel is displayed. Touch 'Overwrite' to update the existing method or change the name to activate the 'Save' button to save the changed method with a new name.

6. SonoLab Application Software

The main components of SonoLab software include:

- **Method** – compose or select methods to run
- **Schedule** - program times for the ML230 to automatically prepare for operation
- **History** – view system usage history
- **About** – information on system configuration

6.1 Method Screen

The Method Screen (**Figure 6.1**) is the principal user screen for the system. **Section 3** contains details on operating the instrument through the Method Screen. The right side of the Method Screen displays buttons which display additional information and controls.
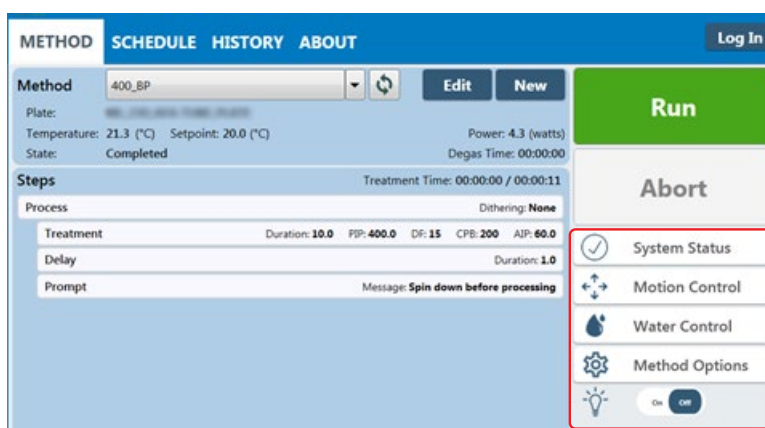

Figure 6.1 The Method Screen

Touch the 'System Status' button to display the 'System Status' pane.

The 'System Status' pane, as explained in **Section 3**, displays the states of various ML230 subsystems, indicating whether the ML230 is ready to operate.

Touch the 'Motion Control' button to display the 'Motion Control' pane.

The 'Motion Control' pane contains buttons used to move the ML230 predefined position.

- **Home**: moves the sample tray to Home position and initializes the ML230 motion system.
- **Load**: moves the sample tray to a convenient location for loading and unloading samples.
- **Start**: moves the sample tray to the initial treatment position for the currently selected treatment method.
- **RFID**: moves the sample tray to the correct location for the currently selected method's rack to be scanned by the RFID reader.
- **Stop**: stops any current motion.

Touch the 'Water Control' button to display the 'Water Control' pane.
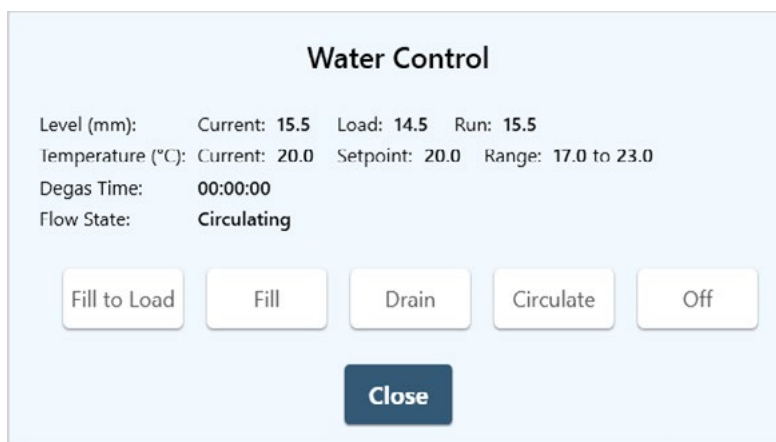
The 'Water Control' pane displays the current temperature, level, and degassing states of the ML230 water bath. The pane also contains the following buttons:

- **Fill to Load**: adjusts the bath water level to the level specified by the currently selected method.
- **Fill**: fills the water bath to its maximum fill height.
- **Drain**: drains the contents of the water bath to the waste bottle.
- **Circulate**: circulates the bath water through the UV sterilizer and particulate filter.
- **Off**: turns off both circulation and degassing pumps.

Touch the 'Method Options' button to display the 'Method Options' pane.

The 'Method Options' pane contains switches for enabling alternate behavior of treatment methods.

- **Consumable-based Method Selection**: limits the list of available treatment methods to those using a specific consumable. Refer to *Section 3.2* for instructions on using this feature.
- **Override Temperature:** allows a treatment method to run when the water bath temperature is outside the limits specified within the method.
- **Override Degas:** allows a treatment method to run without waiting for the degassing timer to complete its countdown. This can be useful if the bath water is degassed but SonoLab has been restarted. This will cause the degas timer to start over.
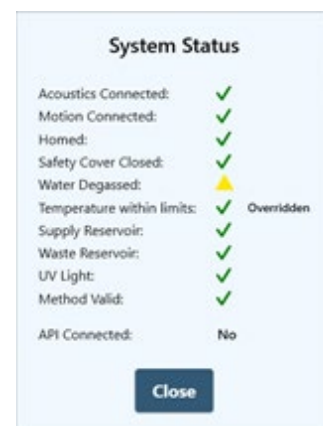
If either temperature or the degas timer is overridden, the word "Overridden" will appear next to it in the 'Status' pane as shown to the right.

6.2 The Schedule Tab

The Schedule screen is used to configure bath Periodic Fill and scheduling Ready and Off states daily. In the Ready state, the water bath is degassed, and the water bath temperature is at the temperature specified in the selected method.

In the Off state, Periodic Fill and the WCS temperature control are turned off, but the circulation pump is on to circulate the water through the filter and UV fixture, 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.
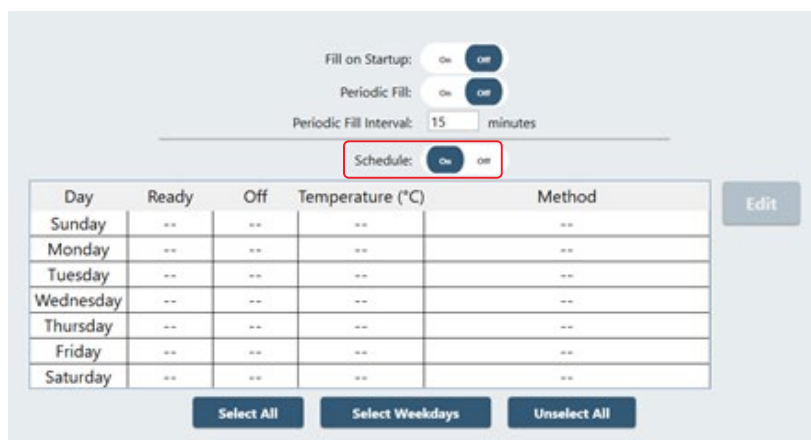
- **Periodic Fill**: SonoLab checks and adjusts the ML230 water bath level at the start of each treatment method. However, if the ML230 will be idle for long periods of time during the day, the Periodic Fill function can be configured to periodically check the bath level while the system is idle. If necessary, the water level will be corrected, degassed, and the temperature adjusted so that the system will be ready when needed.

  Touch 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 touch the Schedule On/Off switch so that On is highlighted dark blue.
    - To configure a specific day, for instance Sunday, touch the Sunday line of the chart, then touch the Edit button, or just double-tap in the Sunday line of the chart, to display the Schedule Settings screen.
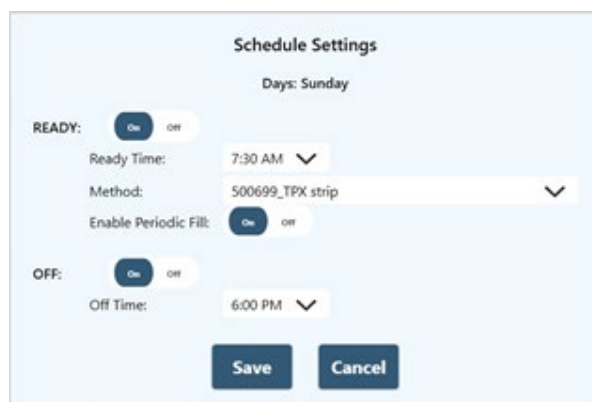
  There are two sections of the Schedule Settings screen, READY and OFF. READY is for configuring a time for the system to be ready to run, typically ahead of a daily plant startup in the morning; OFF is for placing the system in an idle state, typically at the end of the day.

  > **NOTE:** If the bath is not drained, please do not turn off SonoLab, as shutting down SonoLab means that the water will not circulate through the filter and UV light.

- **Ready**
    - Touch the on/off switch until On is highlighted in dark blue.
    - Touch the Ready Time drop down list and select the time the ML230 will be ready to run treatment methods.
    - Touch 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 ML230 may be idle for long periods of time, click the Periodic Fill on/off switch so that On is highlighted in dark blue. SonoLab will automatically maintain the correct water level, degassing state, and temperature.
- **Off**
    - Touch 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 PM in this example) from the drop-down list.

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

Touch Select All, and then Edit if all seven days are to use the same scheduling parameters. Touch 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 ML230 to the OFF state, SonoLab ignores the scheduler and the ML230 will not be switched to standby on that day.

6.3 The History Tab

The History tab (*Figure 6.2*) provides a scrolling list containing the history of every method performed on the instrument. This list can be sorted by method name, date the method was run, the operator who ran the method, or the run status (complete, aborted, failed).

Figure 6.2 The History Tab

Double-tap on a line to display its details. The left side of the screen will display textual information concerning the method. A pane on the right side contains graphs of the temperature and power levels generated for each sample treated by the method. This pane scrolls as necessary to display the graphs of all samples treated by the method. See *Figure 6.3*.
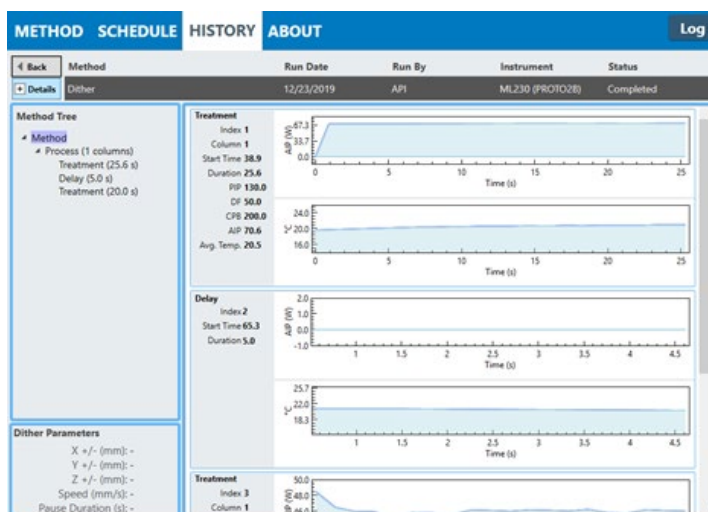
Figure 6.3 Details of a Method History

Touch the **+ Details** button at the top left. Additional information regarding the method run is displayed above the graphics.
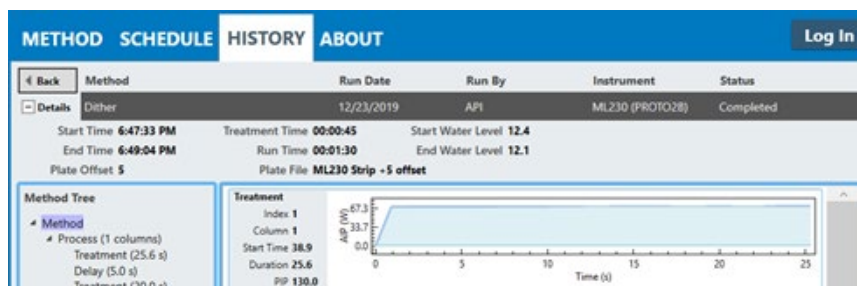See **Figure 6.4**.

Figure 6.4 Additional History Details

6.4 The About Tab

The About tab provides user basic information about the instrument such as serial number, software version, board serial numbers etc.
See **Figure 6.5**.


Figure 6.5 About Tab

6.5 The Admin Tab

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.
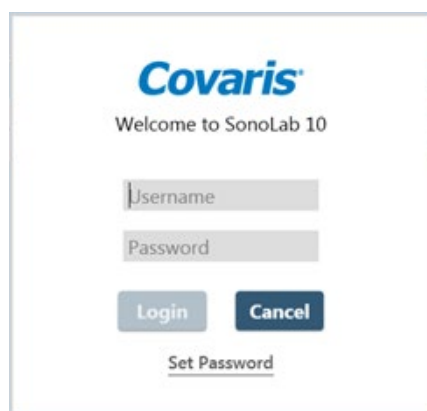
6.5.1 User Authentication

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.
- Touch the Log In button at the upper right corner of the SonoLab screen.
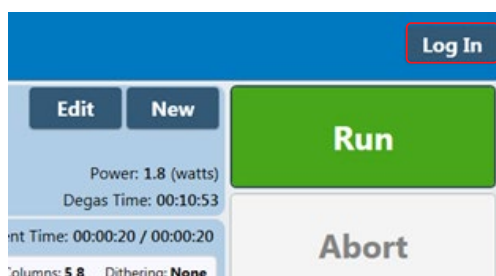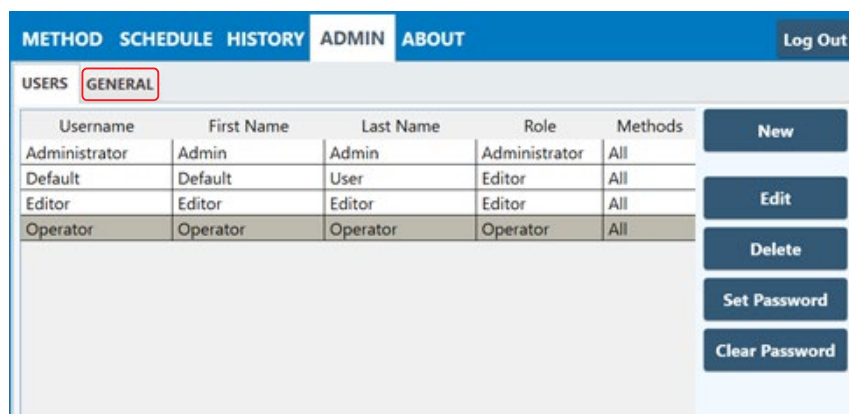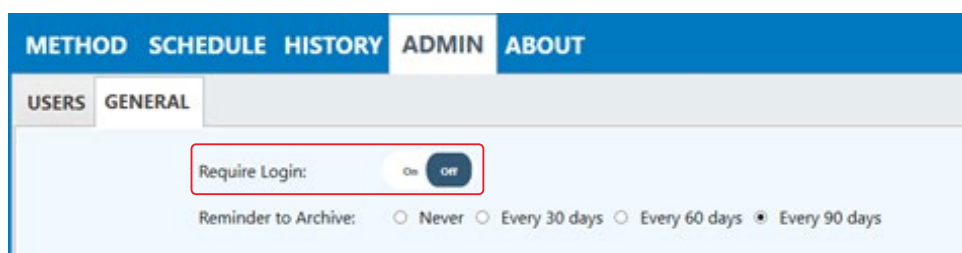

- The Log In pane is displayed.

- Enter the username "Administrator" and password "admin". The password is case sensitive.
- Touch *LOGIN* and the ADMIN tab is added to the SonoLab window.
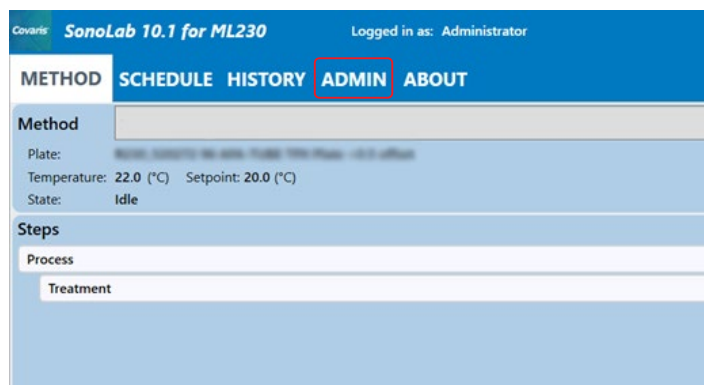
- Touch the ADMIN tab. The list of default SonoLab users, their roles, and assigned methods is displayed, along with the "USERS" and "GENERAL" tabs at the upper left.

- Touch the "GENERAL" tab.

- Touch 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 6.5.2** below for details on users and their roles.

6.5.2 User 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.
  - Can review all history screens,
- *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.
- *An Operator*:
  - Can run methods assigned to them by an Administrator.
  - Can review the history screens.

6.5.3 Managing Users

To create a new user:

- Log into SonoLab as Administrator and touch the ADMIN tab.
- Touch 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 touch 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:

- Touch the method(s) to be removed in the list on the left. Multiple methods may be selected by touching "Shift" or "Control" simultaneously.
- Touch 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 6.1. Operation is discussed in **Section 3.2**. 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 6.1** for a description of the Method Pane, and **Section 3.2** 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.

- Touch **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 will be prompted to create a password either by clicking on 'Set Password' or entering their user name and then any text into the password field and clicking Login.

Once a new password is entered, it must be entered in again to verify its correct spelling. Touching 'Set Password' returns the user to the login screen where the user may now enter their new password and log in.

7. System Specifications

Treatment System	Bench-top; high intensity acoustic transducer, 1 to 8 sample batch process, temperature monitoring and controlling device, water bath with sample holder and safety enclosure
Treatment Power	1.5 to 450 Watts peak incident power, 0.5 to 110 Watts average incident power
Dimensions	18.25" W x 20.25" D x 23.00" H (46.5 cm x 51.5 cm x 58.5 cm)
Weight	Approximately 80 lbs. (36 kg)
Power Requirements	100-240 VAC 750 VA, 50/60Hz, 8 Amp Slo-Blo Fuse
Ambient Temperature Range	19 °C to 25 °C (66 °F to 77 °F)
Ambient Humidity Range	30 to 70%
Regulatory Labeling	CE, ETL Mark (for Product Safety), WEEE
Safety	Certified to IEC/EN/ANSI/UL61010-1 and CAN/CSA C22.2 No. 61010-1 "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"
EMC	Certified to EN 61326-1 "Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements". Also certified to FCC 47CFR Part 15 – Radio Frequency Devices and Part 18 – Industrial, Scientific and Medical Equipment, and ICES-003 Class A for Industry Canada
Water Bath	AFA-grade Water or Highly Purified Water, at least ASTM Type III or ISO grade 3
Method Temperature Setpoint	Programmable +10.0 °C to +40.0 °C
Computer	Integrated Panel Computer
Operating System	Microsoft Windows 10
Application Software	Covaris SonoLab
Data Input	Touch Screen
Chiller	Solid state chiller for heating and cooling (built-in)
Power	0 to 160 Watts, 24 volts
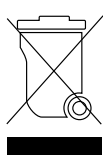
8. Troubleshooting

Problem	Corrective Actions
Unable to HOME	- Ensure the Instrument is turned on - Ensure the door is closed - You can HOME the system by going to the Motion Control button and touching HOME
Motion Errors	- The motion system should be homed by going to the Motion Control button and touching HOME if any motion related errors are encountered. - It should be standard practice to home the motion system at least once daily.
RUN button disabled	- A Method must be chosen to enable RUN. Ensure a Method has been chosen. - System status conditions have not been met. Touch the System Status button. Identify the conditions that are marked with a red X and address the indicated conditions.
Drain Taking Too Long or Water Pressure Too High message	- Ensure the Waste bottle is properly connected to the Quick Disconnect Cap (see **Section 3**). - There could be a blockage in the drain lines. Ensure the bath is clean and free of debris. This may be resolved with a bath cleaning operation (see **Section 3**). The filter may need to be changed (see **Appendix C**).
Water Level Failure Error / Method Failure Due to Water Level Adjustment Timing Out	- Rerun / Restart the method
Water in bath will not completely drain	- If the water level is below 1, as seen under the Water Control status panel, the bath will not drain further. Use the Fill button to fill the bath above level 1 then use the Empty button to start the drain operation.
Shearing not as expected or inconsistent across a rack	- Verify the ML230 is level (see **Section 2**) - Ensure the rack is properly seated into the sample arm and the weight is in place

9. Appendix

Appendix A: M-Series Site or Transport Preparation

Placement of Equipment

The area required for the instrument is approximately 18.25" W x 20.25" D x 23.00" H (46.5 cm x 51.5 cm x 58.5 cm). Allow sufficient space in front of the bench to access each component of the installation.

Power Requirements

Provide at least one standard outlet for the ML230. The ML230 draws a maximum of 750 VA. The Covaris M-series instruments are configured with a universal power supply.

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

Water Requirements

The water bath should be drained when the ML230 is not in use for more than a few days. In normal operation, water is automatically added to the bath as necessary by the Water Control system. Sensors notify the user if the Water Control supply bottle is empty or if the waste bottle is full. Covaris recommends AFA-grade Water. It is also possible to use highly purified water (Highly Purified Water at least ASTM Type III or ISO grade 3).

> **NOTE:** The ML230 Focused-ultrasonicator must not be left powered OFF with water in it. Keep the instrument power ON until the water bath is drained.

Moving or Packing the Instrument for Transport

When moving the instrument between nearby laboratories, please employ the following guidelines:

- Drain the water bath by touching the Water Control button and selecting "Empty".
- Remove the sample tray by unscrewing the captive thumb-screw securing it to the motion system and gently pulling it forward, off the locating pins. Pack the sample tray with the water bottles and other accessories.
- Turn the instrument off.
- Remove the Supply and Waste bottles, empty them, and pack separately from the ML230.
- Remove remaining water from the acoustic assembly
- Lift the unit by its base-plate (not by the plastic case or doors). Transport by cart.

When packing the unit for shipment, please follow the guidelines above. Additionally, if feasible, obtain a packing box from Covaris and employ the foam end-cap inserts to support the instrument. If this is not feasible, wrap the main instrument in ample bubble wrap or similar packing. Wrap and package accessories (laptop, safety cover, sample holder, cords) separately to avoid damage.

Please contact the Covaris Customer Support Department (customerservice@covaris.com) for guidance and assistance.

Appendix B: Updating SonoLab Software

Contact Covaris Technical Support (techsupport@covaris.com) for assistance upgrading SonoLab.

Appendix C: Replacing the ML230 Particle Filter Element (within the ML230 Filter Assembly Repair Kit, PN 500679)

The Particle Filter removes particulate that could otherwise damage the pump or negatively affect the acoustic performance of the instrument. The Particle Filter Element should be replaced annually.

1. Drain the water from the ML230 water bath.
2. Disconnect the Supply and Waste bottles by pressing the thumb latch on the Quick Disconnect Fittings and lifting them out of the bottles. See **Figure C1**.
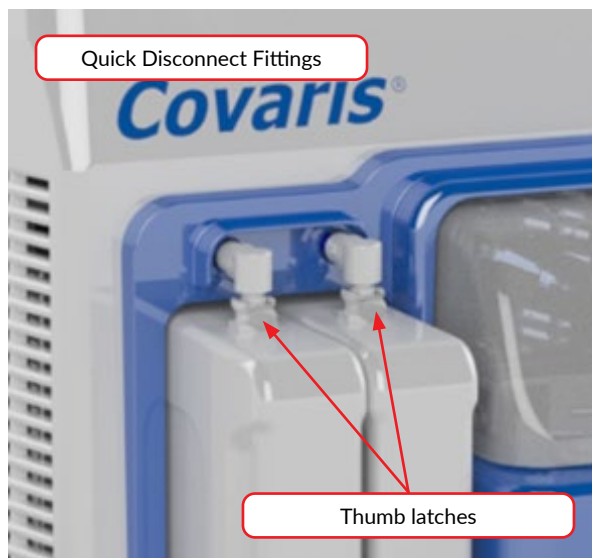

Figure C1 Quick Disconnect Components

3. Remove the Supply and Waste bottles by sliding them forward and clear of the ML230.

4. Locate the filter in a recess at the far-left side of the cabinet, inside the bottle bay.
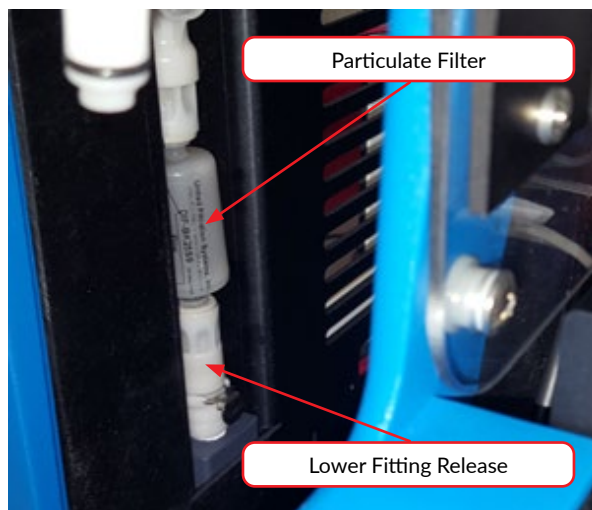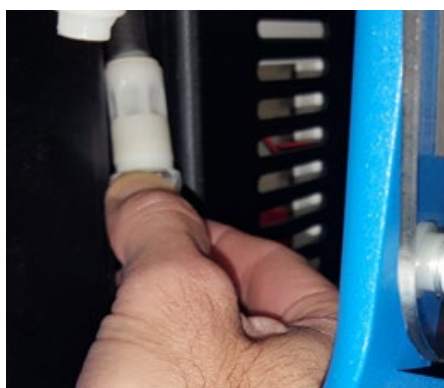

Figure C2 Particulate Filter

5. Press the release tab of the lower fitting and separate bottom of filter from the water line.

6. Press the release tab at the upper end of the filter, and remove the filter from the ML230.


7. Press the release tab at the upper end of the filter, and remove the filter from the ML230.

Figure C3 Particulate Filter Assembly

8. Orient the replacement Particle Filter Element so that the direction of flow arrow and female fitting face up. Gently, but firmly, press the upper water connector into the female fitting of the Particle Filter Element until it "clicks" into place.

9. Gently press the lower fitting of the Particulate Filter Element into the lower water fitting and stow the filter into the cabinet recess.

10. Reinstall the Supply and Waste bottles. The Waste bottle is on the left; the Supply bottle is on the right, next to the sample handling area.

11. Orient the bottle in the Water Control Bay with the label on the Quick Disconnect Cap facing out, as shown in **Figure 6**.

12. Reconnect the bottles by pressing the Quick Disconnect Inserts into the couplings on the Supply and Waste bottles until they click into place.

Appendix D: Networking Information
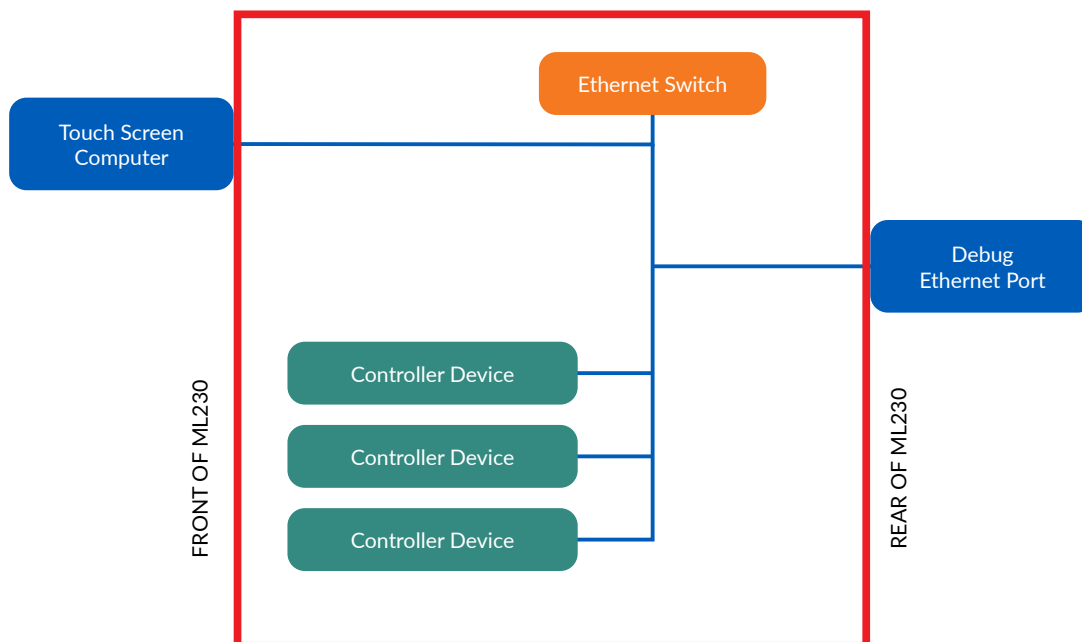
The ML230 control electronics and SonoLab software communicate via TCP/IP and utilizes static IP addresses assignments. The ethernet port at the rear of the system is only provided for Debug purposes by trained Covaris Service team members. Attempting to network the ML230 using the debug ethernet port can cause IP address conflicts and other communications errors, especially within some DCHP networks. If it is desired to network an ML230, Covaris recommends the use of a USB network interface card dongle.

ML230 Internal Network Specifications	
Assigned IP address range	192.168.0.1 to 192.168.0.50
Subnet	255.255.255.0
Gateway	192.168.0.1