

User's Manual



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Precautions: READ THIS FIRST

WARNING:

This instrument uses water, and under certain circumstances could present a **shock hazard**.

Use the provided GFCI-protected power cord for the control unit. If a GFCI-protected power cord is not available, connect a standard power cord ONLY to a GFCIprotected receptacle or to a circuit with a GFCI breaker. Check with your laboratory safety officer before using if you are unsure of the GFCI status.

Caution:

Read this manual before use.

Caution:

Control unit input compressed air **pressure** should not exceed 25 psi (170 kPa).

Caution:

Clean and dry out tank and water lines after use using soap and water. Leaving water in the tank or lines could result in the growth of harmful molds or pathogens.

Caution:

Do not overfill the water tank. Maximum recommended volume is 2.5 gallons (10 liters).

Caution:

Do not operate with less than 1 gallon of water remaining in the tank.

Caution:

Water tank is under pressure during use. Do **not** loosen tank lid or fittings during operation.

Caution:

A 1.5 psi relief valve is installed on the water tank. Do not tamper with or remove the relief valve.

Note:

Condensation on the relative humidity sensor can occur when using high water temperatures and humidities above 90%, and this can make measured RH values unreliable.

About Watershed™

Watershed is based on a system developed by Professor Doug Juers and his students at Whitman College, and is protected by US patent 9,789,486.

The Science Behind Watershed™

Maintaining and controlling sample hydration is a critical problem in biomolecular crystallography, as well as in cell biology, assisted reproduction, and related fields. Watershed provides a convenient, robust, low-cost solution to this problem.

When manipulating crystals contained in liquid drops on cover slips, glass slides, or in multiple well microplates, evaporation can lead to formation of precipitate and protein skins, to crystal aggregation and adhesion, and to crystal cracking and other damage.

Evaporation limits the time that a given drop can be exposed to air during crystal manipulations and harvesting before the drop and remaining crystals within it become unusable.

Evaporation during crystal soaks in solutions containing cryoprotectants, heavy atom compounds, or ligands can lead to uncertainty and to crystal-to-crystal variations in the concentrations of these solutes within the crystal.

Evaporation during these steps and during crystal harvesting onto a crystallography loop or mount can cause degradation in crystal diffraction properties and crystal-to-crystal variations in unit cell dimensions and protein structure.

Crystal dehydration during post-growth handling is the overwhelmingly dominant cause of crystal nonisomorphism in room temperature X-ray data collection, and an important cause of cryogenic temperature nonisomorphism. Typical room-temperature unit cell volume variations of 1% or more decrease to less than 0.2% when crystal hydration is carefully controlled.

On the other hand, modest controlled dehydration can, for perhaps 5-10% of protein crystals, cause improvements in overall crystal order and diffraction properties, and provides a useful salvage approach when crystal diffraction is inadequate.

Watershed[™] Applications

Watershed provides a controlled humidity environment for a wide range of common sample preparation tasks, including

- separating crystals from each other, from precipitate, and from protein skins that may form over crystallization drops;
- soaking crystals in solutions containing cryoprotectants, heavy atoms or ligands;
- harvesting crystals from drops onto X-ray sample mounts;
- blotting or wicking away excess liquid from crystals to reduce background X-ray scatter and increase sample cooling rates during plunge cooling;
- placing crystals in MiTeGen's MicroRT capillaries or glass capillaries for room temperature data collection; and
- adjusting the hydration of crystals to a desired relative humidity, which may be different from their as-grown relative humidity, to modify protein structure or optimize diffraction properties.

By using Watershed[™],

- Drop and sample **working times can be increased by a factor of 10 or more** relative to typical laboratory environments having relative humidities below 50%.
- More crystals can be harvested from each drop before it becomes unusable.
- **Crystal isomorphism is improved**, improving data quality and sometimes reducing the number of crystals required for structure determination.
- Perturbations of protein structures away from their biologically relevant forms are reduced.
- Quality and reproducibility of data are improved.

This manual provides all the technical details you need to start enjoying the benefits of Watershed's simple but powerful technology.

User Requirements

The following are required to set up and operate the Watershed[™] controlled humidity workstation:

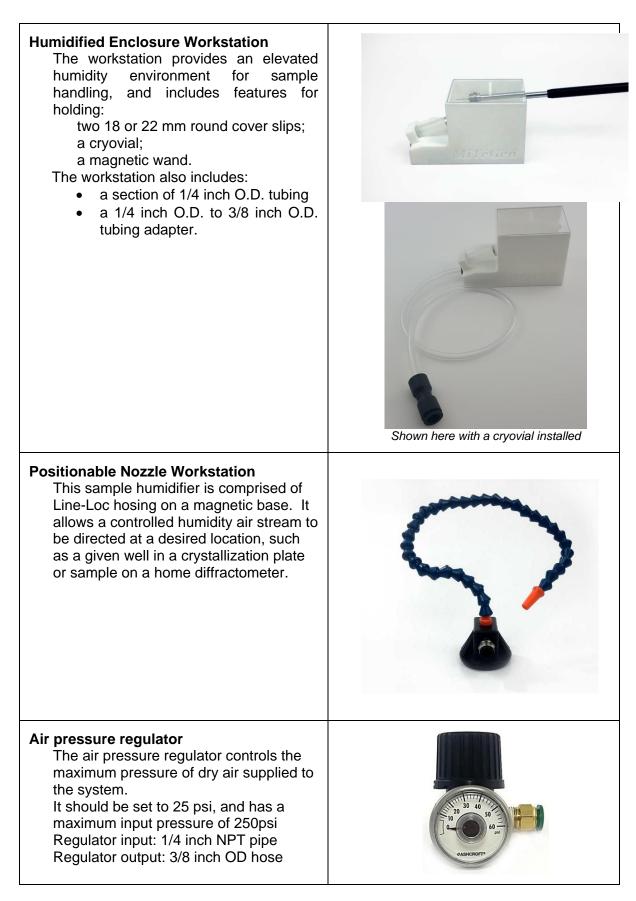
- 1. A 110-125VAC 60Hz power source with GFCI protection. GFCI protection is provided by the included GFCI power cable. GFCI wall outlets and lab wall outlets on GFCI protected circuits can also be used.
- 2. A source of dry clean air with a pressure of 25-250 psi, regulated to a pressure of 25 psi.
- 3. Wrenches and tools as required to connect to your lab's pressurized dry air supply.
- 4. De-ionized or distilled water (approximately 2 gallons / 8 liters).
- 5. Scissors or a razor blade to cut the flexible tubing for connections between the water tank and control unit.
- 6. If using the Humidified Sample Workstation, a stereo microscope with a working distance of at least 4.5 cm.

Specifications

Operational Parameters	
Minimum controllable RH 50% RH Maximum controllable RH >90% RH	
Control Unit	
Input power Power entry module fuse Height Width Depth Weight	110-125 VAC,60Hz * GFCI protection required 3 Amps 135 mm (5.3 inches) 357 mm (14.0 inches) 255 mm (10.0 inches) 5.2 kg (11.5 lbs)
Water Tank	
Capacity Pressure relief valve setting Height (to top fitting) Width Depth Weight (empty) Weight (with 2.5 gallons water) Thermocouple Heater Tubing (for controller connections)	2.5 gallons 1.5 psi 260 mm (10.2 inches) 357 mm (14.0 inches) 235 mm (9.3 inches) 2.3 kg (5.0 lbs) 11.8 kg (26 lbs) K-type 100 Watt submersible 3/8 inch O.D., 1/16 inch wall
Humidified Enclosure Workstation	
Height Width Depth Required working height for microscope Compatible cover slips Cryo-Vial holder Wand holder Tubing (from station to 3/8 inch adapter): Tubing (for controller connection)	66 mm (2.6 inches) 115 mm (4.5 inches) 55 mm (2.2 inches) 45 mm (1.8 inches) minimum round, 18 mm or 22 mm for MiTeGen Cryo-Vials compatible with Rigaku Wands (available from MiTeGen) 1/4 inch O.D., 1/8 inch ID 3/8 inch OD, 1/4 inch ID
Positionable Nozzle Workstation	
Magnetic base diameter Positionable tubing length	54 mm (2 inches) ~500 mm (~19 inches)

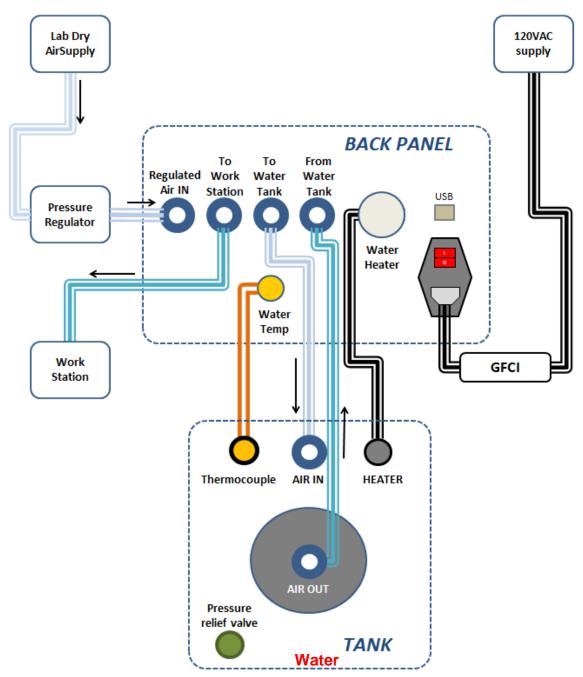
Components





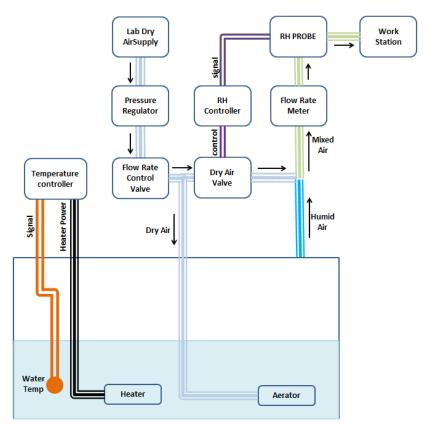
 Air pressure regulator connection accessories Two adapters can be used to connect your dry air supply to the pressure regulator: 2 inch long, 1/4 inch NPT pipe nipple 1/4 inch NPT to barbed fitting for 1/4 inch I.D. hose 	A DE LA DE L
Tubing Ample 3/8 inch and 1/4 inch OD tubing is provided for all air connections.	0
Tubing Union (for dry-out procedure) Union for 3/8" O.D. tubing	

Connection Diagram



[Office1]

Theory of Operation



- Watershed[™] generates high-humidity room-temperature air by bubbling air from a *dry air supply* through water within a *tank*. This humid air is mixed with additional dry air from the supply to create air having the desired relative humidity.
- A closed loop *RH controller* monitors the relative humidity signal from the *RH sensor* at the control unit's humid air output, and adjusts the *dry air valve* to obtain the desired humidity.
- A *flow rate meter* monitors the total humid air flow to the sample. This flow rate can be adjusted using the main *flow rate control valve*.
- With the *heater* off, the water temperature will drop due to evaporative cooling. Selecting a water *temperature controller* setpoint just above room temperature will ensure consistent water temperature and air humidification.
- The range of humidities that can be achieved at the sample depends on the moisture content of the *supply air*, the temperature of the water, and the air flow rate set by the *flow rate control valve*. The maximum achievable relative humidity can be increased by increasing the moisture content of the supply air, decreasing the air flow rate using the *flow rate control valve*, and increasing the water temperature using the *heater* by changing the setting of the *temperature controller*.

Unpacking and Inspection

The Watershed system should be inspected for shipping damage or missing parts before installation. The system consists of:

□ Instruction manual – hardcopy

- □ Watershed *control unit*
- □ *Water tank* assembly, which includes:
 - □ Water tank with pressure relief valve
 - □ Tank lid with humid air outlet
 - □ In-tank thermocouple and external lead
 - □ In-tank heater and external lead
 - □ In-tank dry-air-in diffuser

□ Main *GFCI power cord*

- □ *Humidified Enclosure Workstation*, which includes
 - □ 1/4 inch OD, 1/8 inch ID tubing
 - □ 1/4 inch to 3/8 inch OD tubing adapter
 - 22mm cover slides
 - one cryovial
- Desitionable Nozzle Workstation, which includes
 - \square 3/8 inch OD, 1/4 inch ID flexible tube
- □ Air *pressure regulator*, and the following accessories
 - □ 2 inch long, 1/4 inch NPT pipe nipple
 - □ 1/4 inch NPT to barbed fitting for 1/4 inch I.D. hose
- □ *Tubing* for connections between pressure regulator, control unit and water tank.
- □ **Tubing adapter** for dry-out procedure

Installation

- Caution: Read and follow the "Unpacking and Inspection" section of this manual prior to installation
- STEP 1. Postion the **control unit** near your microscope or other desired working area.
- STEP 2. Postion the water tank on the bench or floor near the control unit.
- STEP 3. Arrange the **power cord**, **wires** that will connect the control unit and water tank, and **tubing** that will connect the pressure regulator, control unit, and water tank so that there are **no trip**, **water spill**, **or electrical short hazards**.
- STEP 4. Connect the **3/8 inch OD gas tubing** between the **regulator** and the **control unit**, and between the **control unit** and **water tank** as indicated the connection diagram on page 9. For now, do not connect tubing to the control unit's humidified air output.
- *STEP 5.* Plug the water tank's *thermocouple connector* into the *K-type receptacle* on the back of the *control unit*.
- STEP 6. Verify that the compressed dry air supply is off. Turn the *pressure regulator* knob counter-clockwise until it stops (setting the initial setpoint pressure to 0.) Connect the *pressure regulator* to the *dry air supply*, using the included 1/4 inch NPT accessories as needed. Then connect the *3/8 inch OD tubing* between the *regulator* and the *control unit*.
- STEP 7. Connect the **control unit** to the **main power supply** using the **GFCI cable** provided.
- **Caution:** Always connect the control unit using a GFCI protected power cord, or to an electrical outlet with built-in GFCI protection. If you are unsure if your outlet is GFCI protected, contact your lab or building director.

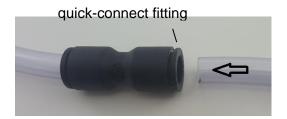
Connecting gas tubing

To **connect** tubing to the quick-connect fittings:

1. Push the tubing into the fitting

To **disconnect** tubing to quick-connect fittings:

- 1. Push and hold the collet inward toward the fitting.
- 2. While pushing and holding the collet, pull the tubing out of the fitting.





Initial Start-Up

Preparation

- STEP 1: Leave the water tank empty with the lid screwed in place.
- STEP 2: Check that the water heater cable is not connected to the control unit.
- STEP 3: On the **control unit** ensure the **air flow switch** is in the 'Air Off' position.
- STEP 4: Rotate the **pressure regulator** knob fully counter-clockwise.
- STEP 5: Turn on your compressed dry air supply.
- STEP 6: Adjust the **pressure regulator** to 25 psi, by turning the knob clockwise as required.
- STEP 7: Turn on the power to the control unit using the switch on the back of the unit.

Basic operational checks

- STEP 1: On the **control unit**, check that the **airflow gauge** is backlit.
- STEP 2: Check that the *humidity controller* screen indicates a RH between 0 and 100%.
- *STEP 3:* Check that the water *temperature controller* display indicates an appropriate set point temperature (e.g., 25 °C to start) and a reasonable measured temperature.
- STEP 4: On the control unit, set the air switch to 'ON'.
- STEP 5: Rotate the **air flow control knob** counterclockwise until the **air flow meter** indicates a flow rate of 25 l/min. * **Note:** Failure to reach 25 liters/min indicates a low feed pressure, an air source with an insufficient flow rate, or a plugged/kinked tube.
- STEP 6: Check that air is flowing out of the control unit's Humidified Air OUT output.
- STEP 7: Let air flow through the system for 5 minutes to purge the (empty) water tank.
- *STEP 8:* Note the reading on the *relative humidity display*. This is the RH of your supply air.
- STEP 9: Set the *air switch* on the front of the *control unit* to 'OFF'.
- STEP 10: Remove the water tank lid. Fill the tank with between 1 and 2.5 gallons of water.
 *Note: De-ionized or distilled water is recommended to prevent scale accumulation and bacterial growth.
- STEP 11: Screw the water tank lid on, ensuring that the tank-to-lid seal has no air leaks.
- STEP 13: Plug the water heater cable into the back of the control unit.
- STEP 14: Set the *air switch* on the front of the control unit to 'ON'.

- STEP 15: Turn the **air flow control knob** on the front of the **control unit** to obtain desired flow rate, at least 15 liters/min is reccomended.
- *STEP 16:* Set the relative *humidity controller's setpoint* to the desired humidity. (See "Changing the Relative Humidity Setting" on page 17).
- STEP 17: Set the water temperature controller to obtain a temperature 1 or 2 degrees above room temperature (See "Changing the Water Tank Temperature Setting" on page Error! Bookmark not defined.).
- STEP 18: Allow the system to stabilize at the settings that were input.

Normal system start-up

- * **Note**: For best performance, run a Dry-Out procedure before or immediately after each Watershed use.
- STEP 1: Verify that there is 1 2.5 gallons of distilled or de-ionized water in the **tank**.
- STEP 2: Verify that tank lid is secure.
- *STEP 3:* Connect the 3/8 inch OD *tubing* between the *tank* and *control unit* (if disconnected during Dry-Out.)

*CAUTION: Be sure the *tubing connections* between the *water tank* and *control unit* are as shown on the *Connection Diagram* on page 9. Swapping the connections at the control unit or tank will cause water to quickly fill the tubing.

- STEP 4: Turn on your **compressed dry air supply**.
- STEP 5: Adjust the **pressure regulator** to 25 psi.
- STEP 6: Switch ON the power by means of the **power switch** on the back of the **control unit**.
- STEP 7: Set the *air switch* on the front of the *control unit* to 'ON'.
- STEP 8: Set the flow rate using the *air flow control knob* on the front of the *control unit* until the *air flow meter* is indicating a desired setting of at least 15 liters/min.
 *Note: clockwise decreases flow rate, counterclockwise increases flow rate
- STEP 9: Set the relative **humidity controller** to the desired humidity. (See the "Changing the Relative Humidity Setting" on page 17).
- STEP 10: Set the water temperature controller to the desired water temperature.
 For <u>high</u> RH settings, setting the temperature to a few degrees <u>above</u> room temperature is usually sufficient. Higher temperatures can be used, but water

temperatures significantly above room temperature will cause rapid water buildup in the unit and in the tubing.

For <u>low</u> RH settings, set the temperature at to 2 or 3 degrees <u>below</u> room temperature to prevent the heater from turning on.

(See the "Changing the Water Tank Temperature Setting" on page 16).

- STEP 11: Allow the water temperature and relative humidity to stabilize at their setpoints.
- STEP 12: Connect tubing from either the *Humidified Enclosure Workstation* or the *Positionable Nozzle Workstation* to the *Humidified Air OUT* connection on the *control unit*.

Changing the Water Tank Temperature Setting

To obtain humidified, room-temperature air, the water temperature should be close to room temperature. Bubbling air through water evaporatively cools the water, so the water must be heated to remain near room temperature. In general, a water setpoint temperature 1 or 2 °C warmer than room temperature will give stable humidity performance. Higher setpoints will give warmer air.

STEP 1: Press the up or down arrow buttons



to change the set point,

indicated by the right hand (SV or Set Value) numeric display

* **Note:** when pressing the arrow buttons, the right hand display will appear dimmer, and

the OUT1 light will be off, indicating that no power is delivered to the heater.



STEP 2: Press the enter button to confirm the new set point and return to run mode.

* Note: Once the new set point has been entered, the right hand display will appear

brighter, and The OUT1 light will be on if the set temperature is above the current actual temperature.



Changing the Relative Humidity Setting



STEP 1: Press the right arrow button

to change to "access set point 1 (SP1)" mode.



- *STEP 2:* Press the enter button **W** to access the set point.
- STEP 3: Use the left and right arrows to select the desired RH. set point
- STEP 4: Press the enter button to input the new set point . The display will then

briefly flash **StRd**. This indicates that the new set point has been recorded.

The display will then show the actual, measured RH.

Note: If the enter button is *not* pressed after using the left and right arrows to select the RH setpoint, the display will continue to indicate that desired new setpoint, and not the actual current set point or the measured RH. If you believe the controllermay have been left in the set-point access mode simply press the enter button.

Using the Humidified Enclosure Workstation



The Humidified Sample Workstation in position on a stereomicroscope with a large working distance.

The Humidified Sample Workstation is ideal for manipulating biomolecular crystals, cells, and other dehydration-sensitive samples.

In crystallography, the workstation provides a controlled humidity environment for

- separating protein crystals from each other, from precipitate, and from protein skins that may form over crystallization drops;
- harvesting crystals from drops onto X-ray sample mounts;
- soaking crystals in solutions containing cryoprotectants, heavy atoms or ligands; and
- blotting or wicking away excess liquid from crystals to reduce background X-ray scatter.
- placing crystals in MicroRT capillaries for room temperature data collection.

Analgous manipulations are common when working with cells and tissues.

Using relative humidities approaching 90%, the Workstation can increase sample working times before dehydration becomes important by a factor of 10 to 20 relative to typical laboratory environments with relative humidities below 50%.

The workstation platform accepts samples placed on standard circular 18 mm and 22 mm cover slips (used in, e.g., 12 and 24 well crystallization plates) and on standard 25 by 75 mm microscope slides, including depression slides commonly used in serial soaking.

Samples are accessed from the open right-hand side of the Workstation, and can be viewed from above through a clear window using, e.g., a stereomicroscope with a working distance of at least 45 mm.

The workstation also incorporates a holder for a standard magnetic cryovial. A sample held in a standard goniometer base or "cap" using a standard magnetic wand can then be inserted into the humid-air-filled cryovial, the assembly transported to a dewar of liquid nitrogen or to a nitrogen gas cryostream, and the sample cooled to cryogenic temperature. This dramatically reduces the risk of crystal dehydration prior to cryocooling, especially for very small (sub-100 micrometer) crystals that can dehydrate in seconds in ambient air.

An absorbent disk placed in the cryovial can be soaked with the final sample solution (or a solution with similar RH) to further reduce the chance of crystal dehydration.

Ideally, the workstation humidity should be adjusted to be near the equilibrium RH of the solution in which the sample resides. Crystals grown with low salt concentrations and no cryoprotectants will typically require relative humidities of 90% or above, whereas crystals grown with large salt concentrations may remain in equilibrium at much lower relative humidities.

A good source for equilibrium relative humidities of common crystallization / precipitant solutions is "*Measurement of the equilibrium relative humidity for common precipitant concentrations: facilitating controlled dehydration experiments*," by M. J. Wheeler, S. Russi, M. G. Bowler, and M. W. Bowler, Acta Cryst. F **68**, 111-114, (2011), doi: doi:10.1107/S1744309111054029.

Since crystallization solutions often have multiple solutes, their precise equilibrium relative humidity may not be known. It is generally best to err on the high humidity side, since over-hydration tends to cause crystal dissolution but no crystal damage, while dehydration tends to cause crystal damage.

Using the Positionable Nozzle Workstation

The Positionable Nozzle Workstation directs a stream of controlled humidity air to a selected location.

It can be used

- to increase the humidity above opened wells in a microplate plate during sample manipulations or harvesting;
- to adjust the humidity of a sample *in situ* during X-ray or optical measurements.



The Positionable Nozzle Workstation above an open well on a crystallization plate



* Note: the magnetic base of the Positionable Nozzle Workstation can be mounted directly to the side or top of the Watershed control cabinet.

For best results, reduce or eliminate drafts and other air flows across the working area of interest.

Dry-Out Procedure

The tubing connecting the water tank, controller, and workstations should be dried out:

- if the tubing becomes filled with water drops;
- at the end of use before shutting down;
- before use if any moisture is visible.

The tubing is dried out by bypassing the water tank and running dry air through the tubing at the maximum flow rate.

To dry out the tubing:

STEP 1: Disconnect both pieces of 3/8" OD tubing from the water tank.

- *STEP 2:* Join the two tubing ends together using the 3/8" to 3/8" union, so that the air flow now bypasses the water tank. (See page 9.)
- STEP 3: Disconnect the tubing from the Workstation or Nozzle.
- *STEP 4:* Decrease the humidity setpoint on the humidity controller to 0%. This will ensure the dry-air mixing valve opens fully, drying the valve and associated tubing.
- *STEP 5:* Increase the airflow using the control unit's flow rate control value to the maximum level.
- *STEP 6:* Flow air through the tubing for a few minutes until the relative humidity displayed on the control unit matches the relative humidity of your supply air, indicating that the tubing is dry.
- STEP 7: When complete, either continue to the shutdown procedure or reconnect the tubing lines to begin operation again.
- * **CAUTION:** Be sure to reconnect the tubing to the correct fittings on the water tank. Switching the connections will cause water to rapidly fill the tubing.

Shut-Down Procedure

- STEP 1: Complete the **Dry-Out Procedure** on page 21 prior to proceeding with shutting down.
- STEP 2: Turn off the pressurized air supply.
- STEP 3: Once flow stops, switch OFF the air switch on the front of the control unit.
- STEP 4: Switch OFF the control unit power using the switch at the back of the unit.
- STEP 5: If you will be using Watershed within the next day or two, the water can be left in the tank. Otherwise, empty the tank and allow it to dry out.

Care and Cleaning

In normal operation, with distilled or deionized water and regular drying out after each usage, no additional care should be required.

If the tubing and water tank become contaminated, clean them using water containing a small amount of PEX, Alconox, or other standard laboratory ware cleaner.

TroubleShooting

Symptom	Possible causes and remedies
Control panel not lit	Control unit not plugged in; outlet not powered.
	GFCI tripped.
	Fuse in controller power module blown.
Water flowing in tubing lines	Tank tubing connected incorrectly. Verify tank AIR IN and AIR OUT lines are connected to the control unit as shown on page 9.
	Excessive condensation within the system. Perform a thorough dry-out procedure (page 21).
Flow meter reads zero	Compressed air supply off.
	Flow rate valve on control unit closed. Rotate it one turn counterclockwise.
	Pressure regulator set to zero. Set to 25 psi.
	Disconnected, pinched or obstructed tubing.
Flow meter oscillating and clicking.	Water pooling within the system. Perform a thorough dry-out procedure.
Indicated RH fluctuating up and down.	Flow rate may be too low. Increase flow rate to greater than 15 liters/min.
	Water may be pooling within the system. Perform a thourough dry-out procedure.
Indicated RH of <5%	Water may be pooling on part of the sensor. Perform a thorough dry-out procedure.
Indicated RH of 102% or higher	Water may be pooling on part of the sensor. Perform a thorough dry-out procedure.
Not able to maintain a low RH setting (e.g. 50-60%)	Expected RH set point may not have been entered. Verify the RH set point.
	Insufficient flow rate. Verify flow rate is at least 15 liters/min.
	Water in tank too warm. Set the temperature controller set point below room temperature to turn off heater. Add cold water to tank if needed. Perform a thorough dry-out procedure.
	Verify your compressed air supply is dry. This can be done in the dry-out procedure.
	Water may be pooling on part of the sensor. Perform a thorough dry-out procedure.

Symptom	Possible causes and remedies
Not able to maintain a high RH setting (e.g. 80-90+%)	Expected RH set point may not have been entered. Verify the RH set point.
	Insufficient flow rate. Verify flow rate is at least 15 liters/min.
	Water level too low.
	Water in tank too cold. Verify that water heater is connected to control unit. Set the temperature controller set point to a higher temperature, above room temperature. Ensure the temperature controller is in run mode, not in set point mode.
	Water may be pooling on part of the sensor. Perform a thorough dry-out procedure.
	Unit may have been left in the dry-out configuration. Remove the dry-out union and reconnect the tubing to the tank.

Technical Support

Contact support@mitegen.com or call 1-877-648-3436 for support.