

Operator's Manual



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1 Introduction

Thank you for purchasing Decagon Devices Leaf Porometer. The Leaf Porometer measures stomatal conductance in leaves. This manual should help you understand the functions of your Leaf Porometer, make high-quality stomatal conductance measurements, and help you get the most out of your Leaf Porometer.

1.1 Customer Support

If you ever need assistance with your Leaf Porometer, have any questions, or feedback, there are several ways to contact us. Decagon has Customer Service Representatives available to speak with you Monday through Friday, between 7am and 5pm Pacific time.

Note: If you purchased your Leaf Porometer through a distributor, please contact them for assistance.

Email: support@decagon.com or sales@decagon.com

<u>Phone:</u> 509-332-5600

<u>Fax:</u> 509-332-5158

If contacting us by email or fax, please include as part of your message your Leaf Porometer serial number (found on the inside of the battery cover), your name, address, phone, fax number, and a description of your problem or question.

1.2 Warranty

The Leaf Porometer has a one-year warranty on parts and labor that begins upon receipt of the instrument.

1.3 Seller's Liability

Seller warrants new equipment of its own manufacture against defective workmanship and materials for a period of three years from the date of receipt of equipment.

Note: We do not consider the results of ordinary wear and tear, neglect, misuse, accident and excessive deterioration due to corrosion from any cause as defects.

The Seller's liability for defective parts shall in no event exceed the furnishing of replacement parts Freight On Board the factory where originally manufactured. Material and equipment covered hereby which is not manufactured by Seller shall be covered only by the warranty of its manufacturer. Seller shall not be liable to Buyer for loss, damage or injuries to persons (including death), or to property or things of whatsoever kind (including, but not without limitation, loss of anticipated profits), occasioned by or arising out of the installation, operation, use, misuse, non-use, repair, or replacement of said material and equipment, or out of the use of any method or process for which the same may be employed. The use of this equipment constitutes the buyer's acceptance of the terms set forth in this warranty. There are no understandings, representations, or warranties of any kind, express, implied, statutory or otherwise (including, but without limitation, the implied warranties of merchantability and fitness for a particular purpose), not expressly set forth herein.

2 About the Leaf Porometer

The Leaf Porometer is a battery-operated, menu-driven device that measures stomatal conductance of leaves. Stomatal conductance is a function of the density, size, and degree of opening, of stomata, which are pores in plants that open to the outside air. The Leaf Porometer measures stomatal conductance by putting the conductance of a leaf in series with two known conductance elements, and comparing the humidity measurements between them. It can display information in three selectable units:

- 1. $mmol/m^2s$ (millimoles per meter squared seconds)
- 2. $m^2 s/mol$ (meters squared seconds per mole)
- 3. s/m (seconds per meter)

2.1 Overview of the Leaf Porometer

The Leaf Porometer features an easy-to-use, six-button, menu-driven interface. There are three primary menus: the Main Menu, Data Menu, and Configuration Menu (See Section Five "Menus"). You can navigate between each of these by pressing the Menu button.

2.2 Leaf Porometer Instrument Specifications

<u>Operating Environment</u>: 5 to 40 °C; 1 to 100% relative humidity with desiccant chamber

Accuracy: 10%

Sample Chamber Aperture: 6.35 mm (0.25 in)

Measurement Range: 0 to $1,000 \text{ mmol/m}^2 \text{s}^1$

 $\frac{\text{Microcontroller Dimensions: 15.8 x 9.5 x 3.3 cm (6.2 x 3.75 x 1.3 in)}$

Data Storage: 4,095 measurements

Data Retrieval: Direct via RS-232

Keypad: Six-key, menu-driven

<u>Clock</u>: 24-hour +/- one minute per month

Interface Cable: RS-232 serial cable (included)

Power Supply: Four type "AA" batteries (included)

2.3 Leaf Porometer Components

The Leaf Porometer and its components come to you in a foampadded carrying case. You should find the following items inside when your Leaf Porometer arrives.

- <u>Leaf Porometer controller</u>
- <u>RS-232 cable</u>
- Operator's Manual and USB flash drive of Leaf Porometer Utility
- <u>Sensor Head</u>
- <u>Teflon filter disks</u>
- Replacement leaf pad/outer leaf pad
- Calibration plate/Bead Retention Screen Tool
- Bottle of USP Purified Water
- <u>Tweezers</u>
- Calibration filter paper disks Whatman grade #3 filter Paper
- <u>Bottle of Desiccant</u> DrieRite 10-20 mesh indicating desiccant
- Dispensing cap
- Spare dowel pin
- Short Dowel pin To assist with porometer head reassembly
- White Fluoropolymer agitation beads Part# 9660K13 from McMaster Carr
- <u>Bead retention screens</u>



Power: This key activates and deactivates the Leaf Porometer.

Note: The device will automatically turn off if not used for more than 10 minutes or 60 minutes if running in manual mode.



Menu: This key cycles between the three menus at the top of the screen.

Note: Pressing Menu while changing a setting will cancel any operations.



ENTER

Arrow Keys: These keys navigate within menus and sub-menus, and modify numerical values in sub-menus, as well as select different units. Holding down an arrow key allows you to scroll between different options quickly.

Escape: This key backs out of sub-menus and can be used to cancel certain actions.

Enter: This key is used to make selections within menus and sub-menus, and also begins taking measurements.

2.4 The Sensor Head

The Leaf Porometer cannot take measurements without the aid of the sensor head. This sensor head is responsible for gathering the information (vapor pressure, humidity, etc.) that the Leaf Porometer uses to calculate stomatal conductance in a leaf. Figure 1 shows the external components of the sensor head.



Figure 1: Sensor Head External Components

2.4.1 Setting the Time

You should make sure to correctly set the system time on the Porometer. This will make sure the timestamps on downloaded readings are accurate (see Section 6.2 "Organizing Saved Data"). You can do this manually in the Porometer (see "Time" in Section 5.4), or use the Leaf Porometer Utility (see "Setting The Date & Time" in Section 6.4).

2.4.2 Calibration Number

Each sensor head comes with a default seven digit calibration number that you can find on the sensor head cable tag and on the calibration certificate. Use the default calibration with desiccant. This default calibration number has been entered into the Leaf Porometer readout unit and will be applied to each measurement until a user initiates a calibration.

When you perform a user initiated calibration the sensor head generates and stores a new calibration number. This new calibration number will be applied to all subsequent measurements instead of the default calibration until the user initiates a new calibration or requests a reset to the default calibration.

3 Sensor Head Calibration

For the Leaf Porometer to make accurate readings of stomatal conductance, the two humidity sensors in the sensor head must behave in a very repeatable manner. Over the past several years, we have found that the sensors occasionally change characteristics over time. While the endpoint humidity measurement may still be accurate, the response time of the sensors can change subtly which creates inaccurate conductance measurements. The best way to ensure accurate conductance measurements is to calibrate the Porometer before taking measurements. We recommend you calibrate the Porometer every day you use it or every time you use it under a different set of environmental conditions (i.e. > 15 °C temperature change).

It is especially important to recalibrate the Porometer after you install or remove the desiccant chamber. It is also recommended that fresh desiccant be used for each day of measurement. Visit our website at www.decagon.com/porocal for an Online video tutorial of the calibration process.

3.1 Requirements

All Leaf Porometers with firmware Revision 1.30 or greater have the user Calibration feature. If your Porometer has firmware lower than Revision 1.30, you may upgrade the firmware. For instructions on how to upgrade your firmware to the latest version see "Upgrading Firmware" in Section 8.5. In addition to current firmware, you will need a Calibration Kit from Decagon to perform the calibration. If you do not have a Calibration Kit, please contact Decagon (support@decagon.com) for a kit. Please have your sensor head serial number available when you contact Decagon for the kit.

Note: The Calibration Kit contains a calibration plate, tweezers, a bottle of USP Purified Water, calibration filter paper disks, and white fluoroplymer agitation beads.

3.2 Calibration Procedure

Equilibrate to Field Conditions

It is critical that the Porometer sensor head is in thermal equilibrium with the environment where you are taking measurements. If you bring the Porometer from an air conditioned environment into hot field conditions, it can take 10 minutes or more to reach thermal equilibrium.

You can check for thermal equilibrium by putting the Porometer in "Manual" mode or using the diagnostic screen to monitor temperature readings until they are steady. It is also important that the USP Purified Water and calibration plate are also in thermal equilibrium. For example, do not keep the bottle of USP Purified Water in a pants pocket or hold the calibration block for extended periods of time.

Check Desiccant

Remove the black plastic desiccant chamber from the bottom of the leaf clip by unscrewing it (counterclockwise). Check to see that the desiccant is blue meaning that it is not exhausted. If the desiccant is purple, discard the exhausted desiccant and refill the desiccant chamber with fresh desiccant from the included desiccant storage container. It is typical to change the desiccant each day you make measurements to ensure that it is not exhausted over the course of daily measurements.

Start Calibration

To start calibration, select the "Calibrate" option on the Configuration/Calibration submenu.





Verify Sensor Head Equilibrium

You will be reminded to make sure the sensor head is in thermal equilibrium with its surroundings (see #1 above).

Press Enter to move to the next step.

Enter Serial Number

You will be prompted to enter the sensor head serial number. You can find this number on a tag attached to the sensor head. Select the number by pressing the up or down arrow keys and pressing enter after each digit.

WARNING

The sensor head must be in thermal equilibrium with it's surroundings before continuing.

Sensor Offset/Stability

The sensors must give similar humidity readings to reach accurate stomatal conductance measurements. Before beginning a calibration, the Leaf Porometer checks to make sure that the two sensors in the sensor head agree well with each other. For the sensors to agree, the air in the diffusion path should be well mixed.

One of the white fluoropolymer agitation beads can be used to mix the air in the diffusion path. Sensor head versions with the metal screen should already have the bead in place. If your model does not have a screen or bead, simply open the leaf clip, insert the bead into the diffusion path in the Aluminum block, and close the leaf clip. Now the air in the diffusion path can be mixed by shaking the bead. Shaking the leaf clip briskly in a vertical orientation results in the most efficient mixing.

As the sensors come to equilibrium, you should see the indicator bar move closer to the 0% offset line. DO NOT open the clip. The water vapor in the ambient atmosphere will slow the equilibration process.



Once the sensor readings stabilize and are confirmed to be within 2% relative humidity of each other, the calibration routine automatically advances to the next screen. If the sensors do not equilibrate to similar humidity values, check for water on the sensor head. If no water is found, then the sensors may have been chemically contaminated or be otherwise malfunctioning. If this case occurs, you should recondition the sensors. Section 8.3 has more details on reconditioning the sensors.

Holding the Sensor Head

Holding the sensor head in the correct manner is very important for maintaining thermal equilibrium. Figure 2 illustrates the correct way to hold the external sensor.



Figure 2: Holding External Sensor

Preparing Calibration Apparatus

The Leaf Porometer calibration apparatus consists of a plastic calibration plate with a precisely drilled hole with known conductance of 240 mmol $m^{-2}s^{-1}$. For the conductance value of the calibration plate to be correct, a flat, moist surface must be placed across the hole on the side marked "Filter Paper." This condition is achieved with a circular disk of wet filter paper that is included in your calibration kit. Additional filter disks may be purchased if you run out.

Note: Do not touch the calibration filter paper disk with your fingers because oils in your skin will contaminate the disk and result in improper readings. To aid in handling the filter paper disk and to keep it clean, use the tweezers included in the calibration kit to hold the calibration filter paper disk.

Wetting the Filter Paper

The wetness state of the filter paper is critical for an accurate calibration. The paper must be wet enough to provide a 100% humidity surface, but must not be too wet. If the paper is too wet, free water will be pulled into the hole on the calibration plate by surface tension and will change the effective dimensions of the hole thereby changing the conductance.

The best method of ensuring the proper wetness state is to use the "wet and flick" method. Use tweezers to pick up the filter disk and then add one drop of the USP Purified Water to the filter disk to saturate it. Allow the water a couple moments to fully absorb into the filter. Then, while holding the disk with the tweezers, give the filter paper several sharp flicks of the wrist to knock off any excess water.

The resulting paper should glisten, but there should be no drop of excess water clinging to the disk. Please see the video of proper Leaf Porometer calibration technique on the Decagon website at http://www.decagon.com/education/video-sc-1-leaf-porometer-calib ration/ for visual representation of the dip and flick technique and

the proper filter paper wetness state.

After wetting the filter paper, use tweezers to lay the filter paper flat over the hole in the calibration plate on the side marked "Filter Paper." It is critical that the filter paper lay flat over the hole. Make sure that the paper is not caved into the hole or bubbled out from the hole. The filter paper also has to cover the entire hole. Turn the calibration plate over and carefully examine the hole to make sure that no meniscus of excess water is present in the hole. If excess water is observed, remove the filter paper, dry the calibration plate, and repeat the wet and flick procedure with more aggressive flicks.

If during the calibration the filter paper becomes too dry to adhere to the calibration plate, then it needs to be wet again. If this occurs during a calibration, you will need to restart the calibration procedure. The first reading after wetting the filter paper again should be artificially low.

Attach Sensor Head to Calibration Plate

Once the sensors have equilibrated, the Attach Sensor Head screen will automatically appear.



With the moist filter paper in place, slide the calibration plate into the sensor head, making sure you have oriented the side of the plate marked "Metal Block" toward the aluminum block side of the sensor head.



Figure 3: Calibration Plate Installation

Be sure to insert the calibration plate until the aluminum block seats firmly against the hard stop on the calibration plate. You should feel a seating click when the plate is placed correctly. The lip around the orifice should be fully fitted into the top of measurement hole or path.

Note: If screen is missing, simply replace screen according to replacement instructions.



Figure 4: Correct Calibration Plate Installation

This will ensure that the hole is properly aligned with the diffusion path in the sensor head. Once the sensor head is attached to the calibration plate, the Leaf Porometer will automatically sense the calibration plate and begin the first reading.

If you use the agitation bead during a calibration or regular measurement it is important to orient the sensor head with the desiccant cap facing downward. Proper orientation ensures the agitation bead sits on the Teflon filter and does not interfere with the water vapor diffusion from the leaf to the two sensors (Figure 5). The sensor can be held in this orientation by hand, or the entire sensor head and calibration plate assembly can be set on top of the Leaf Porometer Controller with the desiccant cap hanging off the edge. It is also critical that the sensor head remain still during the calibration or regular measurement if the agitation bead is in the diffusion path. The stomatal conductance measurement relies on water vapor transfer from purely molecular diffusion (no air movement) and any air mixing from agitation bead movement will cause a substantial loss of accuracy.



Figure 5: Sensor Head Diagram

Calibration Measurements

Once you attach the sensor head to the calibration plate, the Leaf Porometer will automatically sense the calibration plate and begin the first calibration measurement.



Each calibration measurement will take 30 seconds. The indicator bar at the bottom of the screen and timer at the top right of the screen indicate the status of the calibration measurement. Once the measurement is finished, you will be prompted to Equilibrate Sensors and then Attach Sensor Head. This will begin another calibration measurement.

The Leaf Porometer will continue to take calibration measurements until three consecutive measurements are all within 7.5% of each other. This ensures that the Porometer measurements have stabilized. Normally, the calibration should end after performing three to five calibration measurements.

The routine will continue for a maximum of ten measurements. If you have not achieved stable readings in ten readings, then there is a problem with the calibration and the "Too Much Drift" screen will appear.



The most common problem is that thermal equilibrium has not been reached. Let the Porometer equilibrate for a few minutes and repeat the calibration process. If you still cannot achieve a good calibration, contact Decagon for help (support@decagon.com).

Equilibrate Sensors

Once a reading is complete, you will be prompted to remove the sensor head and equilibrate the sensors.



Remove the sensor head from the calibration plate and close the clip. If you are using the agitation bead, shake the sensor head to mix the air in diffusion path to speed up the equilibration process. As the sensors come to equilibrium, you should see the indicator bar move closer to the "EQU" line. Once the sensors have equilibrated, the Attach Sensor Head screen will automatically reappear and steps 8-10 can be repeated.

High RH Reading

Once the sensors have equilibrated at similar humidity values, the Leaf Porometer checks to make sure that the air in the diffusion path is adequately desiccated. If the relative humidity is greater than 10%, the next calibration measurement does not begin, and instead a warning screen appears. In many cases, the relative humidity will continue to fall and you will be prompted to attach the sensor head to the calibration block once the relative humidity falls below 10%. However, if this process is slow or if the relative humidity never

falls below 10%, you need to change out your desiccant.

WARNING High RH Reading. Replace desiccant, recalibrate or wait until ZRH < 10Z ZRH = 12Z

Calibration Complete

Once the calibration is complete, the new calibration number is automatically saved into the handheld unit and the Calibration Complete Screen appears. At this point, you are ready to take measurements with your Leaf Porometer. The most recent saved calibration number will be applied to all subsequent measurements until a new calibration is completed or you apply the factory default calibration number.



Accuracy Verification

It is always a good idea to verify that the calibration was effective. If you wish to do this, go to the Measurement menu and make a few measurements on the calibration plate. The measured conductance should be close to 240 mmol $m^{-2}s^{-1}$.

If possible, do not wet the filter paper for the verification readings again. If wetting is necessary, disregard the first measurement taken.

3.3 Calibration without Desiccant

We recommended you always use desiccant to take readings, but if the operators wish to make non-desiccated readings, we recommend an ambient humidity of less than 30%.

For calibrating without desiccant, follow the previous steps 1 through 11 with these exceptions.

- Remove the black desiccant chamber to expose the Teflon filter to ambient conditions.
- Do not use an agitation bead in the diffusion path.
- On sensor head models with a bead retention screen, remove the bead by temporarily removing the o-ring and Teflon filter, removing the bead, and then replacing the filter and o-ring.
- There will be a three minute hydration period before taking calibration readings. During this period, the sensor head is clipped on the cal block with moist filter paper applied.
- The initial stability test (step 6) and subsequent equilibration between calibration measurements (step 9) are performed at ambient humidity. To speed equilibration at these steps, open the clip and wave the sensor head to mix air in the diffusion path.

4 Making Measurements

4.1 Before Making a Measurement

Before beginning a measurement, make sure that the Leaf Porometer is turned on and that the sensor head is clean and properly connected. It is critical operators make sure to equilibrate the sensor head to the ambient temperature. Thermal disequilibrium will cause highly inaccurate measurements.

Note: Consider if you are measuring abaxial conductance (on the top of the leaf) or abaxial (the bottom). Since the stomates of most leaves are on the bottom of the leaf, most measurements are abaxial. If the agitation bead is present in the chamber, you must take readings with the black cap facing downward so the bead is not in the diffusion path.



4.2 Desiccant

Decagon highly recommends using the desiccant chamber to make measurements. The Leaf Porometer relies on a measurement of the humidity gradient between the leaf evaporating surface and the ambient atmosphere. As the ambient humidity increases the gradient becomes smaller, which causes small errors in the measurement of the humidity gradient to result in very large errors in the calculated conductance. The use of the desiccant chamber lowers the ambient humidity to near zero, thus ensuring a steep humidity gradient between the leaf and ambient conditions and preventing the other problems. We recommend that the desiccant chamber be used for the most consistent readings.



Figure 6: Sensor Block and Desiccant Chamber

To use the desiccant chamber, simply fill the detachable chamber with DrieRite Indicating Desiccant (included) and screw it into the bottom of the aluminum sensor block. Make sure that the chamber is screwed in far enough to compress the o-ring on the desiccant chamber and ensure a good vapor seal. If your Porometer sensor block does not have threads to accommodate the desiccant chamber, then you have an earlier version of the Leaf Porometer and need to contact Decagon to have your Porometer retrofitted with an updated sensor block.

Note: If you need to obtain a Safety Data Sheet (SDS) for DrieRite indicating desiccant, a printable version is available on our website at http://www.decagon.com/education/safety-data-sheets/.

Although several different desiccants work in the Porometer (we have had some success with silica gel and molecular sieve), we recommend DrieRite 10-20 mesh because it has the best combination of sorption rate and total water sorption properties for this application. DrieRite can be refreshed by baking at 210 $^{\circ}$ C for one hour and reused. However, from our experience, refreshed DrieRite does not absorb as much water as virgin material and therefore does not last as long in the Porometer.

When using the desiccant chamber, it is necessary to close the leaf clip in order to equilibrate the sensors in preparation for the subsequent measurement. This results in a still air environment in the diffusion path, so it achieves equilibration through molecular diffusion, which is a relatively slow process. At high temperatures, diffusion coefficients are high and the process is relatively rapid. However, at mid to low temperatures, the process can be unacceptably long (several minutes). These long equilibration times can be reduced considerably by using the white fluoropolymer agitation beads that are included with the Porometer to mix the air in the diffusion path. To speed equilibration, simply shake the leaf clip briskly in a vertical orientation. You should hear the bead bouncing between the screen and the Teflon filter.

Generally speaking, the faster you shake, the faster the porometer equilibrates. The presence of the bead does not affect the stomatal conductance measurement as long as the bead does not move during the measurement and you orient the leaf clip with the desiccant chamber down so that the bead rests on the Teflon filter (Figure 7. If you wish to measure the stomatal conductance of the abaxial (top) surface of the leaf, you must turn the leaf upside down and keep the leaf clip oriented with the black desiccant chamber down. Do not invert the leaf clip during a measurement or the bead will rest on the screen and distort the diffusion of water vapor from the leaf through the diffusion path.



Figure 7: Sensor Block and Desiccant Chamber Components

It is well known that low humidity conditions induces stomatal closure in most plant species, so prolonged exposure to the desiccated leaf clip should cause inaccurate stomatal conductance measurements. However, it is suggested in the literature that the minimum time necessary for the onset of stomatal closure in response to a change in environmental conditions is two minutes. Since the SC-1 Leaf Porometer makes an auto mode measurement in 30 seconds, the presence of the desiccated leaf clip should not cause stomatal closure unless multiple measurements are made in quick succession on the same location of the same leaf.

4.3 Manual Mode vs. Auto Mode

When the Porometer is in Manual Mode, the stomatal conductance is measured directly from the theory presented in section 7 and displayed continuously. The real time conductance measurement can be saved at any time during a Manual Mode measurement.

Note: Manual mode should only be used at low conductances (i.e. $< 100 \text{ mmol } m^{-2}s^{-1}$).

At higher conductances, the readings become inaccurate and can take an exceptionally long time to complete. For the theory in Section 7 to accurately predict the stomatal conductance using the steady state diffusion technique, true steady state conditions must exist in the diffusion path. The amount of time necessary to reach steady state conditions is proportional to the conductance. At conductances less than 20 mmol m⁻²s⁻¹, steady state conditions are generally reached in less than five minutes. At higher conductances, steady state conditions can take up to 30 minutes. Please note that leaving the sensor head on the leaf surface for more than a few minutes can cause the stomatal conductance to change in response to the presence of the sensor head clip. Some common uses for manual mode are measuring dark conductance in plants and measuring conductance of static conductance materials (e.g. fruit peels, waterproof fabric).

Auto Mode makes an accurate stomatal conductance measurement in 30 seconds. To do this, the Porometer takes the first 30 seconds of stomatal conductance data and applies a "look ahead" algorithm to predict the final stomatal conductance reading that would be achieved if unlimited time were allowed for true steady state conditions to occur. Auto Mode has several advantages over Manual Mode and other Porometers. First, the 30 second Auto Mode measurement duration ensures that stomatal conductance does not change due to the presence of the sensor head. Second, Auto Mode takes only 30 seconds per measurement, ensuring that your measurements are completed in a timely manner.

Finally, Auto Mode sets a definite endpoint for the measurement, meaning that the user does not have to pick an arbitrary endpoint, as is common with other diffusion Porometers and with Manual Mode measurements.

For the Porometer to "detect" the presence of a transpiring leaf and begin the Auto Mode measurement, the measured stomatal conductance must climb to 5 mmol $m^{-2}s^{-1}$. So, if you are making measurements on leaves with extremely low conductances, it may be necessary to make the measurements in Manual Mode.

4.4 Auto Mode Measurements

- 1. Select Auto mode from the Configuration menu.
- 2. Return to the Measurement menu. You will be prompted to press Enter to begin a reading.

3. After pressing Enter, the Measurement screen will display.



4. The reading will begin once the sensor head is placed on a leaf, and it detects a conductance value greater than 5 mmol m^{-2} s^{-1} . A progress bar will also indicate the reading is underway.

Note: If the desiccant chamber is attached, the reading will begin shortly after opening the leaf clip regardless of whether it is clipped on a leaf. It is critical to slip the leaf clip onto a leaf within five seconds of opening the clip or unacceptable errors may be introduced into the conductance measurement.



If you see the screen above, close the clip and allow the sensor to equilibrate. If an agitation bead is in place shake the sensor head briskly in a vertical orientation to more quickly equilibrate. 5. Once the sensors have equilibrated at similar humidity values, the leaf porometer checks to make sure that the air in the diffusion path is adequately desiccated. If the relative humidity is greater than 10%, the next auto mode measurement will not be initiated and the following screen will appear. In many cases, the relative humidity will continue to fall and you will be prompted place the sensor head on the leaf once the relative humidity falls below 10%. However, if this process is slow or if the relative humidity never falls below 10%, you need to change out your desiccant. Once the sensors equilibrate and the relative humidity is below 10%, you will be prompted to place the sensor head on leaf.



6. Successful measurements resemble the Measurement screen below.

Note: In Auto mode, a timer appears in the upper right hand corner of the screen that indicates how many seconds remain.



7. Once the 30 second measurement has been completed, the Porometer displays the final conductance. You may save or discard your data. The "Saving Data" portion of Section 5.2 has further instructions.

4.5 Manual Mode Measurements

- 1. Select Manual mode from the Configuration Menu. Please note that manual mode is only for special situations with conductances below 100 mmol $m^{-2}s^{-1}$. See the section on "Manual Mode vs. Auto Mode" in Section 4.3.
- 2. Return to the Main menu and press Enter.
- 3. Place the sensor on a leaf. Unlike Automatic mode, there is no time limit for taking readings, and the elapsed time is displayed directly below the system time.



4. You may save your data at any point during the reading by pressing Enter. See "Saving Data" in section 5.2 for instructions.

4.6 Measurements without Desiccant

Decagon recommends you always use desiccant, if operators wishes to make non-desiccated readings, we recommend doing so at an ambient humidity of < 30%. Note these exceptions for measurements without desiccant.

- When not using desiccant, remove the desiccant chamber from the sensor head and do not cover the hole in the bottom of the sensor head. Keep your fingers away from the diffusion path when taking measurement.
- The factory calibration is with desiccant so you need to recalibrate without desiccant as Section 3.3 describes. If you try to measure without the desiccant after having calibrated with desiccant, the Porometer prompts you to calibrate again without desiccant before continuing.
- Without desiccant, the equilibration between measurements is performed at ambient humidity.
- To speed equilibration time, open the clip and wave the sensor head to mix the air in the diffusion path. Do not insert an agitation bead into the diffusion path.
- If the desiccant chamber is not being used, the first reading in Auto mode after the sensor has been removed from a leaf for more than five minutes always reads low. Discard this reading and begin recording with the second. This is not an issue if you use the desiccant chamber.

Measuring Small Leaves & Needles

When measuring small needles or leaves, they should be inserted into the sensor as shown below. This is because single needles or small leaves (including blades of grass) may not adequately cover the aperture of the sensor.



Figure 8: Sensor Aperture with Grass

For an accurate measurement it is critical that the entire cross sectional area of the diffusion path be covered with leaf material. Sometimes it is necessary to remove the leaves/needles from the plant in order to arrange them over the opening in the diffusion path. This results in an accurate measurement as long as the measurement is completed within two minutes of leaf/needle removal, because the stomatal aperture should remain unchanged for at least that long after disturbance.

4.7 Averaging Readings

Leaf stomatal conductance depends on the amount of shade and sunlight it receives, the age of the leaf, and its position on the plant. To obtain a plant stomatal conductance average, you must take a variety of readings from different positions on the plant. The average is proportional to the type of readings taken. For example, if you know your plant receives 70% sunlight, you might consider taking seven readings from the sunny part, and three from the part in the shade. You can also obtain the average readings for a field of plants. For example, if you had a field of corn, you might decide to take a reading from the third leaf from the top on each stalk of corn on that particular plot. These readings together would give you the average for that field.

4.7.1 Measurement Precautions

General Precautions

- Before using the sensor, allow it to equilibrate to the ambient temperature. This may take 10 minutes or longer to complete if the temperature of the Porometer is very different from the sampling environment, e.g. from a cool air conditioned room to a hot outdoor environment.
- Avoid leaves that are wet or covered with dew; never take readings in the rain.
- If you blow into the sensor head, it takes a few minutes for the stomatal conductance reading to return to normal.
- Clean the sensor periodically to keep it free of dirt and pollen that can build up during use and affect readings. (Section 8.2 "Cleaning the Sensor Block")
- Avoid chemical fumes. Fumes can be extremely harmful to the sensor (i.e. alcohol, gasoline, volatile organics).
- Decayon recommends that you always use desiccant, but if operators wish to make non-desiccated readings we recommend maintaining an ambient humidity of < 30%.
- It is critical that you recess the bead retention screen in the diffusion path. If the screen extends out of the diffusion path, it will cut the leaf and cause erroneously high conductance measurements.

If the stomatal conductance reaches a value beyond the recommended range of the instrument (0 to 1000 mmol/m²s), an "approximate"

symbol appears to the left of the reading. If the stomatal conductance reaches a value greater than or equal to 6000 mmol/m^2 s, an "infinity" symbol is shown in place of the measurement. Most often, the excessively high values are caused by water on the leaf surface. If you consistently are getting high readings, check your leaf surface for the presence of water.

4.7.2 Chemical Effects

Chemical vapors can interfere with the polymer layers used to form the humidity sensors. These fumes can diffuse through the sensor and compromise sensor accuracy. It is a good idea, therefore, to never bring the sensor into contact with any sort of chemical vapor (i.e. glue, alcohol, gasoline, volatile organics). Review "Porometer Sensor Reconditioning" in Section 8.3 if you suspect your sensors have been affected by chemical contamination.

4.7.3 Environmental Effects

When making a measurement, you must account for the following effects.

Light: The amount of CO_2 required for a plant changes depending on the amount of sunlight available to it. In broad daylight, more CO_2 is required, so the stomata remain open. At dusk, the stomata close, and in darkness they are often closed completely. Readings taken on overcast days, for example, generally have lower conductances than those taken on sunny ones. Additionally, leaves in shadow or in shade usually have lower conductances that those in greater light. Therefore, make sure to consider the lighting conditions where you are take the measurements.

Temperature: Temperature also affects plant stomatal conductance. For most plants, on a very hot or a very cold day, photosynthesis slows down or stops, and the stomata narrow or do not open, to prevent internal water loss. Therefore, be sure to consider the temperature before conducting measurements. **Humidity:** A factor that causes stomatal conductance to change is the humidity at the leaf surface. As the outside humidity increases, the stomata opens. Porometers that dry the air around the stomata decrease conductance; likewise, those that allow surface humidity to increase do the opposite. Be sure not to make repeated measurements on the same spot on a single leaf especially if the desiccant chamber is used.

Carbon Dioxide (CO₂): One purpose of the stomata is to regulate the leaf usage of CO_2 , which is important to controlling the balance of humidity. An increase in CO_2 causes stomata to close, while a decrease causes them to open. Thus, it is important to consider the CO_2 levels of your measuring environment. You, the Leaf Porometer operator, can adversely affect readings by exhaling CO_2 directly on the plant you are measuring. This is particular important in growth chambers or greenhouse, where CO_2 from an operators breath can double or triple ambient levels.
5 The Menus

The Leaf Porometer features three main menus, designed for easy navigability and use.

5.1 The Measurement Menu

The Leaf Porometer displays the Measurement Menu when first powered on.



At the top of the screen is the stomatal conductance number at left, and the currently selected unit of measurement at right. Below are two columns of numbers. The left is the sensor closest to the leaf, and the right is the sensor farthest from the leaf. Each column has the temperature of that sensor, and its percent humidity. The Leaf Porometer uses these to calculate the stomatal conductance value.

5.2 Saving Data

In Automatic mode, you can save data as soon as the timer has expired; in Manual mode, you save your data without interrupting your reading. Follow steps 1 through 4 to save your data in either mode.

1. The following screen will appear (you have to press Enter in Manual mode to access it).



- 2. You have three options
- Select "Save" and press Enter to save your information as-is.
- Select "Discard" and press Enter to return to the main screen and start over with a new reading.
- Select "Annotate" and press Enter to give your data a file name. Selecting "Annotate" brings up the Annotate screen.



You will see a series of lines, with a set of arrows above and below the first. If you have annotated a previous reading, the old annotation will remain to be changed or deleted. Use the arrow keys to select a letter or number and press Enter to move to the next character.

• Repeat this until you reach the last character. If you wish to stop before the end, you still have to press Enter until you reach

the end of the row.

5.3 The Data Menu

In this menu, you can view any saved data, download data to a computer, and erase all data stored in the Porometer memory. This menu features three sub-menus.



View

Here you can view measurement data stored on your Porometer.

1. Press Enter to select the view sub-menu.



2. Each measurement shows the time acquired, stomatal conduc-

tance/resistance value (depends on which unit you have set as your default) and its annotation (if that measurement was saved with an annotation).

Download

This Download option sends the data saved in the Porometer to the terminal software running on your computer.

Note: Your Leaf Porometer comes with the software for Microsoft Windows. The Utility makes downloading data to your computer very easy. In general, you should use the Leaf Porometer Utility to download data instead of this menu option on the Porometer. Review the Leaf Porometer Utility in Section 6 for more information.

If you are unable to use the Leaf Porometer Utility, you can use terminal software to transfer the data from your Porometer to your computer. Steps 1 through 4 apply to most terminal software programs.

- 1. Configure your terminal software with the following settings:
 - 9600 baud
 - 8 data bits, 1 stop bit, no parity
 - No software/hardware flow control
 - Append line feeds to incoming line ends
 - Echo typed characters locally.
- 2. Connect your Leaf Porometer to an available serial port on your computer using the included RS-232 serial cable.
- 3. Set your terminal software to capture received data if you want to save the data.
- 4. Select "Download" from the Data Menu of the Leaf Porometer.

Erase

This will erase all your stored data records in your Leaf Porometer memory. Caution: Once this feature is activated, all data will be permanently deleted from the Porometer, and cannot be recovered!

Follow these three steps to erase data.

1. Press Enter to select the Erase sub-menu. The following screen will appear.

| Erase | | |
|-----------|--------|-------|
| Erase all | stored | data? |

- 2. Press Enter again to erase the memory, or ESC if you decide not to erase the data. "Erasing..." will be displayed as data is being removed, which usually takes about six seconds.
- 3. When this is complete, you will be returned to the Data Menu.
- 5.4 The Configuration Menu





This menu allows you to alter system settings, such as the current time, date, and operating mode, select the appropriate units for your readings, and view information about your Leaf Porometer. There is also a battery icon to the right, indicating how much battery power is currently available. This menu features seven sub-menus.

Calibration

See the "Sensor Head Calibration" procedures in Section 3.2 for a thorough explanation of the calibration menu.

Date

Follow steps 1 through 5 to change date information.

- 1. To change the current date, press Enter to select the Date display.
- 2. The current date appears in the center of the screen, in day/month/year format. A pair of arrows reside above and below the first number.



- 3. Use the arrow keys to change this number. (Holding down the up/down arrow allows you to scroll quickly between values.)
- 4. Press Enter to move to the next value, and repeat step #3. If you need to return to the previous number, press Escape.
- 5. When you have finished changing the last number, press Enter and it returns you to the Configuration Menu.

Time

Follow steps 1 through 5 to change the current time.

- 1. Press Enter to select the Time display.
- 2. The current time appears in the center of the screen, in 24-hour format. A pair of arrows are present above and below the first number.



- 3. Use the arrow keys to change this number. (Holding down the up/down arrow will allow you to scroll quickly between values.)
- 4. Press Enter to move to the next number, and repeat step #3. If you need to return to the previous number, press Escape.
- 5. When you have finished changing the last number, press Enter and you will be returned to the Configuration Menu.

Mode

The Mode Menu allows you to choose which type of measurement mode to use in the Main menu. Press Enter to select a mode. (Section 4.3) for the descriptions of Manual and Auto Modes.

Auto mode: Takes readings automatically for 30 seconds. You must press Enter before placing the sensor on the leaf for Auto mode to work properly. In this mode, you are unable to save your information mid reading. You must wait until the timer runs out after 30 seconds, then you may save your reading. Also, if initial conductance is less than 5 mmol/m²s, you will see a screen prompting you to place the sensor on a leaf. Conversely, if the initial conductance is too high, a warning screen will appear.

Manual mode: Continuously measures conductance and updates the screen. This mode is only suitable for low conductances (<100 mmol $m^{-2}s^{-1}$). The user can decide when equilibrium has been reached. Pressing Enter will give you the option to save your data at any point during the reading.

\mathbf{Units}

Data in the Leaf Porometer can display in three units accessible from the Main Screen and under the Configuration Menu. Select "Units" and press Enter to select a unit When in the Configuration Menu.

Diagnostics

This menu allows you to view and save real time sensor values from your sensor head. An error message will pop up next to a reading if the value is out of range. Pressing Enter during this screen will save a Scan Record that you can view or download at a later time.



Contrast

The Contrast menu allows you to alter the screen contrast settings. To access it, use the Down arrow key to scroll to it and press Enter. The following screen will appear:



Use the arrow keys to alter the contrast level. Press Enter to save or Escape to cancel adjustments.

5.5 About Screen

This menu displays your Leaf Porometer serial number and firmware version.

About

Serial: LP1059 Firmware: LP 1.33.651 Copyright © 2005-2011 Decagon Devices, Inc.

6 Leaf Porometer Utility



Figure 9: Leaf Porometer Utility Main Screen

The Leaf Porometer Utility is a program designed specifically for interfacing with the Leaf Porometer. Use this program to download measurement data to your computer, erase the Leaf Porometer data, set the date and time, and see information about your Leaf Porometer.

System Requirements:

To use the Leaf Porometer Utility, you must meet the following minimum system requirements:

• Microsoft Windows 98 or NT 4 (SP 5) or better

- Intel Pentium Pro or better processor
- One available serial port or one available USB port
- Microsoft Excel 97 or better (for viewing data as XLS files)

You can install the Leaf Porometer Utility using the included USB flash drive(found in the inside cover of the Leaf Porometer Operators Manual). You can also download and install the latest version of the Utility from http://www.decagon.com/support/leaf-porometer-utility/.

6.1 Downloading Your Data

This transfers all saved measurement data on the Leaf Porometer to your computer. Follow steps 1 through 5 to download your data.

- 1. Make sure the RS-232 cable is connected to the Leaf Porometer, and to a serial communication (COM) port on your computer.
- 2. Open the Leaf Porometer Utility.
- 3. Choose the appropriate Comm Port from the "Use computer communication port" control on the main screen.
- 4. Click the Download button in the lower right-hand corner, or "Download Data" in the File Menu.

Note: If you encounter an error message after clicking this button, please refer to our Troubleshooting Section on page 9 for instructions.

5. The Save screen appears.

| Save in. | leaf Porom | eter Files 👻 | 🌀 🏚 📂 🛄 • | |
|---------------|---|---------------------------------|--|----------------------------------|
| (Alta) | Name | * | Date modified | Туре |
| Recent Places | 별 lune Data 열 Morch Dat 로 Porometer | a Data 7-Dec-2011 | 12/7/2011 11:07 AM 12/7/2011 11:08 AM 12/7/2011 11:07 AM | Microsof Microsof Microsof |
| | • | III | | Þ |
| Network | File name. | July Data | • | Save |
| | Save as type: | Microsoft Excel Workbook (*vis) | | Cancel |

Figure 10: Save Leaf Porometer Data Dialog

Name your data file, select where it will be saved to, and in what format. You may choose between the following formats:

- Microsoft Excel Workbook (*.xls);
- Text (Tab delimited) (*.txt);
- CSV (Comma delimited) (*.csv);
- Raw data (Comma delimited) (*.txt)
- 6. Click Save to download your data to the specified location. The progress bar shows the status of the download process.

Note: You can cancel a download in progress using the cancel button. If you cancel, the software does not save downloaded data are saved.

6.2 Organizing Saved Data

Data are organized in the same way regardless of what format it was saved in. The information will be divided into six columns:

- 1. Measurement time
- 2. Stomatal conductance/resistance
- 3. Temperature in $^{\circ}\mathrm{C}$ or $^{\circ}\mathrm{F}$

- 4. Sample ID (if saved with measurement)
- 5. Sensor head serial number
- 6. Calibration number

The utility formats measurement dates according to the Windows Local settings found in the Control Panel under "Regional and Language Options" ("Regional Settings" in Windows 98). You may override this and set the format to day/month/year by going to the Edit Menu, click Preferences, Units tab, and by clicking "Use Template" under Date/Time Format for Data Files.

Scan Records

Scan records have additional columns than other data records. They show a snapshot of the sensor values at the time of measurement. In all file types, the scan records are interleaved with the normal data records and can be identified because their annotations are labeled "Scan." There are four additional columns.

- 1. Leaf Relative Humidity in $\% \rm RH$
- 2. Leaf Temperature in $^\circ$ C or $^\circ F$
- 3. Filter Relative Humidity in %RH
- 4. Filter Temperature in °C or °F

6.3 Erasing Your Data

This will erase all data stored on your Leaf Porometer.

Caution: Once this feature is activated, all data will be permanently deleted from the Leaf Porometer, and cannot be recovered.

Follow steps 1 through 4 to erase your data.

1. Make sure that the RS-232 cable connects to the Leaf Porometer and to a Comm Port on your computer.

- 2. Choose the appropriate Comm Port from "Use computer communication port" control on the main screen.
- 3. Click "Erase," in the lower left-hand corner, or "Erase Data..." in the File Menu. If you encounter an error message after clicking this button, please refer to Section 9 "Troubleshooting" for instructions.
- 4. The progress bar shows the status of the erase process.

6.4 Setting the Date and Time

You can set the Leaf Porometer date and time by selecting "Set Porometer Date/Time" from the Actions Menu. This will automatically synchronize the Leaf Porometer date and time to your computer date and time.

6.5 Retrieving Leaf Porometer Information

Choose "View Porometer Information..." from the Actions Menu to see information about your Leaf Porometer. (Figure 11)



Figure 11: Leaf Porometer Information Screen

If you encounter an error message after clicking this button, please refer to the Troubleshooting section on page 9 for instructions. This is a good way to find the serial number, firmware version, battery status, and number of stored measurements for your Leaf Porometer.

6.6 The Menus and Their Functions

The four menus across the top of the screen allow you to change program settings, and settings on the Leaf Porometer. Below is a brief overview of the options each menu contains.

File Menu

Download Data: Saves measurement data stored in your Leaf Porometer as a data file on your computer (see instructions above.

Erase Data: Erases all data in your Leaf Porometer (see instructions above).

Exit: Quits the program.

Edit Menu

The Leaf Porometer Utility Preferences has three tabs. The Data File, Communications, and Application tabs.

1. Data File Tab

| ata File C | ommunications Application |
|------------|--------------------------------------|
| Measureme | ent Units |
| Porome | ətər 🛛 Usc Loaf Poromotor Sottings 🗢 |
| Temperat | ure 🕆 C - Celsius 🔹 👻 |
| Date/Time | Format |
| Date/Time | Format |
| | dows Regional and Language Options 📼 |
| Use Win | |

Figure 12: Preferences - Units Screen

Measurement Units: If you select "Get units from Leaf Porometer settings," this will save all downloaded measurement data in the units used on the Leaf Porometer. You can also choose to override the settings in the Leaf Porometer and save your measurement data

in one of three units:

- $\rm mmol/m^2s$ stomatal conductance (millimoles per meter squared seconds);
- $\rm m^2 s/mol$ stomatal resistance (meters squared seconds per mole); s/m stomatal resistance (seconds per meter).

Temperature Units: This allows you to choose what unit of temperature data files will be saved with °C (Celsius) or °F (Fahrenheit).

Column Headers: This option sets column headers (i.e "Date/Time," "Temperature") for saved data files. This feature is on by default. It does not apply to raw data files, which do not have column headers.

Date/Time Format: Here you can set the format with which the date and time will be displayed in saved data files. By default, the program will use Windows Regional and Language Options (accessible in the Control Panel in Windows), the system settings on your computer.

2. Communications Tab



Figure 13: Preferences - Communications Screen

Command Retries: If you encounter difficulty communicating with your Leaf Porometer, you can set the number of times the computer should automatically try sending communications commands (up to 10).

Maximum Baud Rate: You can set the maximum baud rate for talking to your Leaf Porometer. Choose a lower baud rate if you are not getting reliable communications from your device.

Force Find all Communication Ports: This will detect all Comm Ports on your computer, and should be used if your serial-to-USB adapter does not appear in the dropdown menu.

3. Application Tab



Figure 14: Leaf Porometer Utility Preferences

Automatic Internet Version Check: When selected, the utility will automatically check for utility updates for the Leaf Porometer Utility when you are connected to the Internet. You can also check for these manually by selecting "Check for Updates..." from the Help Menu.

Automatic Clock Synchronization: Automatically sets the Leaf Porometer time to your computer time, when you are connected to it. (See instructions above for setting this manually.)

Actions Menu

Set Porometer Date/Time... See the section on "Setting the Date and Time" on page 6.4.

View Porometer Information... This shows the serial number, firmware version, the number of stored readings in the Leaf Porometer, the firmware status, battery status, current date and time, and the sensor calibration number.

Help Menu

Help Accesses the Leaf Porometer Utility help file that provides detailed information on how to use the program.

Send Feedback to Decagon... Provides the user with a way to submit feedback to Decagon.

Check for Utility Updates... Goes Online and checks for utility updates.

Check for Firmware Updates... Goes Online and checks for firmware updates for the Leaf Porometer controller.

About... Displays the current program version and Decagon contact information.

7 Leaf Porometer Theory

Stomatal Conductance

By definition, stomatal conductance is the measure of the rate of passage of carbon dioxide (CO_2) or water vapor through the stomata of a leaf. Stomata are small pores on the top and bottom of a leaf that are responsible for taking in and expelling CO_2 and moisture from and to the outside air. The Leaf Porometer measures the rate at which this happens.

7.1 How the Leaf Porometer Works

The Leaf Porometer measures the stomatal conductance of leaves by putting the conductance of the leaf in series with two known conductance elements. By measuring the humidity difference across one of the known conductance elements, the water vapor flux is known. The conductance of the leaf can be calculated from these variables.

We know the humidity at three places: inside the leaf, and at both of the humidity sensors. The Leaf Porometer effectively calculates the resistance between the inside and outside of the leaf: the stomatal conductance. It measures resistance between the leaf and the first humidity sensor and the first and second sensors. The Leaf Porometer Function diagram represents these measurement process. (Figure 15)



Figure 15: Leaf Porometer Function

The parameters listed above represent the following:

- C_{leaf} The mole fraction of vapor inside the leaf
- C_1 The mole fraction of vapor at node 1
- C_2 The mole fraction of vapor at node 2
- g_s Stomatal conductance of the leaf surface
- gd_1 Vapor conductance of the diffusion path between leaf surface and node 1
- gd_2 Vapor conductance of the diffusion path between node 1 and node 2
- d_1 Distance between the leaf surface and the first humidity sensor
- d_2 Distance between the two humidity sensors

The variable of interest is g_s or the stomatal conductance. First the vapor flux along the diffusion path will be determined using the relative humidity difference between nodes 1 and 2 as follows:

$$F_{vapor} = g_{d2}(C_1 - C_2)$$
 (1)

Where the C values are related to relative humidity by Equation 2.

$$C_i = \frac{h_r e_s(T_a)}{P_{atm}} \tag{2}$$

where h_r is the relative humidity, $e_s(T_a)$ is the saturated vapor pressure at air temperature, and P_{atm} is atmospheric pressure. Saturated vapor pressure is calculated by the Tetens formula with appropriate coefficients for water vapor:

$$e_s(T_a) = 0.611 exp\left(\frac{17.502T}{T + 240.97}\right) \tag{3}$$

Note: T must be in $^{\circ}C$

Next we must determine the value if gd_2 by the equation 4

$$g_{d2} = \frac{\rho D_{vapor}}{d_2} \tag{4}$$

where $\hat{\rho}$ is the molar density of air and D_{vapor} is the diffusivity of water vapor. Both of these quantities are temperature and pressure dependent, however when multiplied together as in equation 3 some of this dependency drops out.

$$\hat{\rho} = 44.6 \frac{P_a}{101.3} \left(\frac{273.15}{T}\right) \tag{5}$$

$$D_{vapor}(T, P_a) = D_{ref}(273.15, 101.3) \left(\frac{101.3}{P_a}\right) \left(\frac{T}{273.15}\right)^{1.75}$$
(6)

If

$$D_{ref}(273.15, 101.3) = 2.12x10^{-5} (m^2/s)$$

Then

$$\hat{\rho}D_{vapor} = (44.6)(2.12x10^{-5}) \left(\frac{T}{273.15}\right)^{0.75} \tag{7}$$

Using these C and g values we can now solve equation 1 for the flux:

$$F_{vapor} = \left[\frac{\hat{\rho}D_{vapor}}{d_2}\right] \frac{1}{P_{atm}} [h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]$$
(8)

Now that the vapor flux has been determined, you can find the stomatal conductance, g_s . This requires some assumptions. First, we assume that the relative humidity within the leaf tissue is 1.0, so by equation 9

$$C_{leaf} = \frac{e_s(T_a)}{P_{atm}} \tag{9}$$

Next, we assume that all conductance values are in series so that the flux is constant between any two nodes. We also assume that the temperature of the leaf is equal to the temperature of the first humidity sensor. (The sensor block head has been constructed from aluminum to eliminate the temperature difference.) This means that we can write equation 1 for node 1 and the leaf node and then set it equal to equation 8.

$$F_{vapor} = g_{s+d1}(C_{leaf} - C_1) \tag{10}$$

$$F_{vapor} = g_{s+d1} \left(\frac{1}{P_{atm}}\right) [e_s(T_{a1})(1 - h_{r1})]$$
(11)

$$\frac{g_{s+d1}}{P_{atm}}[e_s(T_{a1})(1-h_{r1})] = \frac{1}{P_{atm}} \left(\frac{\hat{\rho}D}{d_2}\right) [h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]$$
(12)

Solving for g_{s+d1} :

$$g_{s+d1} = \frac{\left(\frac{\hat{\rho}D}{d_2}\right) \left[h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})\right]}{e_s(T_{a1})(1 - h_{r1})}$$
(13)

We can solve for g_s using the rule for series combination of conductance.

$$\frac{1}{g_s} = \frac{1}{g_{s+d1}} - \frac{1}{g_{d1}} \tag{14}$$

Hence,

$$\frac{1}{g_s} = \frac{e_s(T_{a1})(1 - h_{r1})d_2}{\hat{\rho}D[h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]} - \frac{d_1}{\hat{\rho}D}$$
(15)

so:

$$g_s = \frac{\hat{\rho} D_{vapor}}{\frac{[e_s(T_{a1})(1-h_{r1})]d_2}{h_{r1}e_s(T_{a1})-h_{r2}e_s(T_{a2})} - d_1}$$
(16)

Therefore, g_s is a function of the distances between humidity sensors, temperature, and the two relative humidity readings.

When the conductance is small, the humidities are nearly the same, and the denominator of the denominator of equation 6 on page 6 goes to zero, causing problems. Multiplying top and bottom by the denominator gives

$$g_s = \frac{\hat{\rho} D_{vapor}[h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]}{[e_s(T_{a1})(1 - h_{r1})]d_2 - [h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]d_1}$$
(17)

Note: The resulting g_s is in units of mol/m^2s .

From the Leaf Porometer, the two distances are:

 $d_1 = 3.35 \text{ mm} \text{ and } d_2 = 11.43 \text{ mm}$

8 Care and Maintenance

8.1 Changing the Batteries

Although the Leaf Porometer has been designed to provide an excellent battery lifespan, the batteries will eventually require changing. When this happens, a low-battery indicator will appear in the upper left hand corner of the screen.

- 1. Carefully turn over the Leaf Porometer and locate the battery cover.
- 2. Place your thumb on the grooves and push upward to loosen the cover.
- 3. Remove the old batteries and insert new ones. Be sure to orient the fresh batteries properly.
- 4. Use the Leaf Porometer or the Leaf Porometer Utility to update the time and date.

8.2 Cleaning the Sensor Block

It is necessary to clean the Sensor Block periodically to remove contamination that can build up during routine use.

Follow steps 1 through 6 to clean minor contamination in the Sensor block and steps 1 through 13 for major contamination.

8.2.1 Minor Contamination (dust and pollen)

1. Remove the desiccant chamber. (Figure 16)



Figure 16: Desiccant Chamber Assembly

- 2. Use the tweezers included with the calibration kit to remove the o-ring that secures the Teflon disk.
- 3. Carefully remove the Teflon disk with the tweezers. The Teflon Disk should be handled around the edges as much as possible, and always kept free of dust and moisture.
- 4. Use a dry swab to clean out the diffusion path. Swabs are included with your kit. Do not use a damp swab, alcohol or other organic liquids to clean the diffusion path! Direct contact with water on the sensors as well as chemical vapors can cause damage! Be careful not to dislodge the bead retention screen with the swab. If you do accidentally dislodge the screen, follow the directions in the next section to remove the aluminum sensor block and install the screen.
- 5. Insert the Teflon Disk. Remember to handle the Teflon Disk around edges as much as possible. If the Teflon Disk becomes damaged discard and replace. Five replacement disks are included, contact Decagon Devices you need additional disks.
- 6. Replace the O-ring Retainer.

8.2.2 Major Contamination

When making stomatal conductance measurements on some plant types, the sensor block can become contaminated with sap and leaf exudates that can lead to inaccurate conductance measurements. This type of contamination often cannot be removed with a dry swab procedure. Instead, follow steps 1 through 13 to clean your sensor.

- 1. Remove the two machine screws from the front of the aluminum sensor block using a 3/32" hex key (Allen wrench).
- 2. Carefully pull the aluminum sensor block off of the sensor assembly. This may take some force as the sensor assembly is press-fit into the block. On some porometers, there will be a thin layer of sealant between the sensor block and sensor assembly. Make sure to place the sensor assembly on a clean surface with the sensor ports facing upwards so that debris does not get stuck in the sealant and prevent effective reassembly.
- 3. Remove the desiccant chamber from the bottom of the aluminum sensor block (if it is being used).
- 4. Use a pair of tweezers (included in calibration kit) to remove the o-ring that secures the Teflon filter inside the desiccant chamber cavity.
- 5. Carefully remove the Teflon disk with the tweezers as described in the previous section.
- 6. If necessary, use a thin implement to press the bead retention screen out of the aluminum sensor block. It is best to leave the screen in place during cleaning if at all possible.
- 7. Now the aluminum sensor block and bead retention screen should be free from all accessories and can be cleaned without damaging the sensors. Generally, a solvent like Isopropyl Alcohol is sufficient to dissolve most types of contamination, which can then easily be removed from the sensor block with a cotton swab or piece of paper towel. The bead retention screen can be cleaned in the same manner.

Note: Make sure that the humidity sensors are not exposed to

solvent vapors. Use any solvents far from the humidity sensors. Avoid scrubbing the aluminum sensor block with abrasive materials as they can remove the hydrophobic finish on the block and affect conductance measurements.

- 8. Rinse the aluminum sensor block and bead retention screen thoroughly with water and allow to dry before assembling the Porometer.
- 9. If the bead retention screen has been removed or dislodged during cleaning, carefully press it snugly into the top of the diffusion path with your finger and then place plate in place and clamp Leaf Porometer head to seat screen. Make sure that the bead retention screen is fully recessed in the diffusion path but above the first humidity sensor port. If the screen extends out of the diffusion path, it will cut the leaf and cause erroneously high conductance measurements. If it is inserted too far into the diffusion path, it will interfere with the diffusion of vapor into the upper humidity sensor port.
- 10. Install the Teflon filter and o-ring retainer. Make sure not to contaminate the Teflon filter with skin oils.
- 11. Align the aluminum sensor block correctly with the sensor assembly. There is a small oval tab on the sensor assembly that should face downwards and align with an oval cutout on the aluminum sensor block.
- 12. Press the aluminum sensor block onto the sensor assembly, firmly seating the sensor assembly in place.
- 13. Attach the sensor block assembly using the machine screws that were removed in step 1.

8.2.3 Maintaining the Seals & Teflon Disk

When you clean your sensor head, be sure to check the condition of the seals, and make sure that they are making full contact with the leaf. If the seals require replacement, a spare set has been included in your Leaf Porometer shipment. Also, the Teflon disk should be replaced if it becomes excessively dirty. If you need more replacements of either component, contact Decagon.

8.2.4 Replacing the Seal

In the event a seal falls off, you can simply replace it by peeling off the adhesive paper on the back and replacing the old seal. If the seal is very loose, it is best to peel it off and replace it in the same manner. When you replace a seal, look to see if the area you will be applying has dirt or soil particles, as these can prevent the adhesive from applying fully. You might try wiping the area with a slightly damp cloth before applying the new seal.

8.2.5 Replacing the Teflon Filter Disk

A spare set of Teflon filter disks have also been included. To replace the disk, use tweezers to remove the o-ring and then carefully remove the disk. Then, handling the disk around the edges (using tweezers is advised), insert the spare and replace the o-ring.

8.3 Porometer Sensor Reconditioning

In some situations, the humidity sensors in the Leaf Porometer can be adversely affected by exposure to chemicals.

This is most commonly encountered when the Leaf Porometer has been exposed to vapors from volatile organic compounds.

We have confirmed reports of sensors being affected by obvious sources of volatile organics such as storing the Porometer in a shed with gasoline cans. The signs of sensor "poisoning" can be manifest as a lack of agreement in humidity measurements between the two sensors, or as nonsensical humidity values from one or both sensor (e.g. >100%). If you suspect that your Leaf Porometer has been affected by chemical exposure, then the sensors can be reconditioned to return them to their natural state using the following procedure:

- 1. Remove the sensor head from the hand held readout unit as well as the desiccant chamber.
- 2. Use the metal pin from the calibration kit to hold the leaf clamp open.
- 3. Place the entire sensor head (and cable) into an oven with good temperature control.
- 4. Bake the sensor head in dry heat at 80 $^{\circ}$ C for at least 24 hours.
- 5. Remove the sensor head and let cool.
- 6. hydrate the sensor head by exposing it to 70 to 80% relative humidity at 20 to 35 °C for 12 hours. A 75% humidity atmosphere can be conveniently generated by a saturated NaCl solution. To hydrate with saturated NaCl, the Porometer head should be placed in a sealed container above a slurry of NaCl (table salt is fine) and water. There should be both liquid water and plentiful salt crystals present in the slurry.
- 7. After hydration, the sensor should be left at ambient conditions for eight hours before calibrating the sensor.

Note that reconditioning the sensors will change the sensor calibration, so the sensor must be calibrated prior to using.

8.4 General Precautions

While the Leaf Porometer has been designed for quality and durability, it should be kept clean and the batteries always fresh to ensure proper working condition. Here are a few things to remember when using this device

• Allow the sensor head to equilibrate to the ambient temperature before using.

• While this device is water-resistant, do not use it in rain, and do not use it on leaves covered with dew or water droplets.

8.5 Upgrading Firmware

From time to time Decagon will release firmware updates for the Leaf Porometer that contain bug fixes and new features to improve the performance of your instrument. You can view and download firmware updates by going to our website (www.decagon.com) and navigating to the Software

Downloads page. Once there, scroll down to the Leaf Porometer section and download the latest Firmware Updater.



Figure 17: Leaf Porometer Firmware Updater

Cautions:

• Any data records on the instrument will be erased during a firmware update so make sure to download any data you wish to save before loading new firmware.

- If upgrading to the latest firmware, you will need to verify your Porometer is equipped with a desiccant chamber.
- Do not turn off or disconnect your instrument from the communications port while the firmware is updating.

Updating Firmware:

- 1. Open the firmware updater application.
- 2. Connect your Leaf Porometer to an available communications port on your computer.
- 3. Select the same communications port in the drop down list on the firmware updater application.
- 4. Click Start to begin updating the firmware.
- 5. Wait for the firmware updater to finish before disconnecting your Leaf Porometer and closing the firmware updater application.

9 Troubleshooting

The Leaf Porometer is a high performance, low maintenance instrument, designed to have few problems if used with care. Unfortunately, sometimes even the best operators using the best instruments encounter technical difficulties.

Below is quick reference guide that will direct you to detailed solutions of some problems that may occur. If these remedies still do not resolve your problem, then please contact Decagon for help (see Customer Support in Section 1). The Troubleshooting Table lists some problems that may occur.

Note: If you purchased your Decagon instrument from one of our international distributors, please contact them for local service and support.

| If this problem occurs: | Refer to: | |
|--------------------------------------|----------------|--|
| Instrument does not turn on | Problem $\#1$ | |
| Display is difficult to see | Problem $#2$ | |
| Sensor head failure message | Problem $#3$ | |
| Low battery message | Problem $#4$ | |
| Date & time were reset message | Problem $\#5$ | |
| Invalid Cal Num message | Problem $\#6$ | |
| Missing bootstrap loader message | Problem $\#7$ | |
| Firmware corrupted message | Problem $\#8$ | |
| Communication port is already in use | Problem $\#9$ | |
| USB to Serial Port adapter not shown | Problem $\#10$ | |
| Download data to the utility failed | Problem $\#11$ | |
| No information is being displayed | Problem $\#12$ | |
| Conductance is not as expected | Problem $\#13$ | |
| Porometer will not calibrate | Problem $#14$ | |
| Equilibration time is too long | Problem $\#15$ | |
| (When using desiccant chamber) | | |
| Low RH reading | Problem $\#16$ | |
| High RH reading-replace desiccant | Problem $\#17$ | |
| Continued on next page | | |

 Table 1: Troubleshooting Quick Guide

| Table 1 – continued from previous page | | | |
|--|-------------|--|--|
| If this problem occurs: | Refer to: | | |
| High RH reading-use desiccant chamber | Problem #18 | | |
| Bead retention screen missing | Problem #19 | | |

1. PROBLEM:

Instrument does not turn on.

SOLUTION:

Make sure that the batteries are inserted correctly, and/or if they have enough power to activate the machine.

2. PROBLEM:

The display is difficult to see.

SOLUTION:

Turn off the Leaf Porometer and then turn it on again. If this does not fix the problem, the Leaf Porometer features a built-in screen for changing screen contrast. See "Contrast" in Section 5.4 for details. You may be able to fix the contrast manually, which can be done using a terminal port program. Contact Decagon for information regarding this procedure.

3. PROBLEM:

Message on screen displays the following:

| Sensor Head | Failure | |
|-----------------|----------|------------------|
| RH Leaf: | -4939.4% | ERROR |
| Temp Leaf: | 603.8°C | ERROR |
| RH Filter: | -4939.4% | ERROR |
| Temp Filter: | 603.8°C | ERROR |
| Check connectio | n! | _ t ∓ |
SOLUTION:

Verify that the sensor head is securely connected to the Leaf Porometer serial port receptacle and restart the reading. If this screen continues to reappear, it is possible that the cable or sensor is faulty and needs to be replaced. To expedite the troubleshooting process, save a Scan record by pressing Enter and then contact Decagon for further options.

4. PROBLEM:

Message on screen displays the following:



SOLUTION:

The batteries in the instrument may not be properly inserted in the battery holder or they may need to be replaced. If the batteries are not inserted properly, return the batteries to the battery holder and cycle the power on the instrument.

5. PROBLEM:

Message on screen displays the following:

WARNING

Date and time were reset!

Update the date and time.

SOLUTION:

After changing the batteries or loading new firmware onto an instrument, this message may appear. Navigate to the configuration menu and verify that the date and time are set properly.

6. PROBLEM:

Message on screen displays the following:



SOLUTION:

This screen will only be displayed when manually entering a calibration number. The best solution to this problem is to perform a user initiated calibration. Another option is to enter the default calibration from the sensors head label.

7. PROBLEM:

Message on screen displays the following:

ERROR

Missing bootstrap loader!

See User's Manual for details.

SOLUTION:

The instrument cannot download new firmware updates. To download new firmware to the Leaf Porometer, or to stop this message from displaying, the instrument must be serviced by Decagon.

8. PROBLEM:

Message on screen displays the following:



SOLUTION:

This screen means that your firmware was not loaded properly, and that an update is needed. (The firmware for the Leaf Porometer is user-upgradeable.) To obtain this, visit Decagon's website to see if new updates are available; if not, contact Decagon for the newest firmware version.

Note: You will lose any saved data records when you install the new firmware. Therefore, download any data you wish to save before reloading the new firmware. If this is not an option, contact Decagon for further help.

see the section on "Updating Firmware" on page 8.5 for more information on obtaining new firmware for your Leaf Porometer.

9. PROBLEM:

The Leaf Porometer Utility tells me the communication port I want to use is in use by another application, but I do not think any other programs are running.

SOLUTION:

Some PDA synchronization software monitor serial communication ports. Disable Microsoft's ActiveSync or Palm HotSync system software while using the serial port with the Leaf Porometer Utility.

10. PROBLEM:

My USB to Serial adapter is not showing in the communication port picker.

SOLUTION:

Enable "Force find all Communication Ports" in the Preferences Menu by going to the Edit Menu, clicking Preferences, then the Communication tab, and enabling the check box at the bottom of the screen.

Enabling this option may find other serial ports that are not available for use by the Leaf Porometer Utility (for example, modems installed in your computer).

11. PROBLEM:

Downloading data stops in the middle with an error message saying the utility lost connection with the Leaf Porometer.

SOLUTION:

A noisy serial connection can disrupt the connection between the Utility and the Leaf Porometer. If this error happens regularly, you can try setting your baud rate lower or increasing the number of times a command is sent to the Leaf Porometer. Choose the "Communications" tab in Preferences to alter this.

12. PROBLEM:

No information is being displayed.

SOLUTION:

Any conductance that is lower that 5 mmol/m²s¹ will not be displayed in Automatic mode. Check to see what mode you are in before beginning. It is advisable to use Manual mode if you know that your leaf has a conductivity that is lower than the minimum threshold for Auto mode. Also, remember that you must press Enter in Automatic mode before you begin taking a reading. The Leaf Porometer Operator could have gotten moisture inside the sensor assembly or Teflon disk by touching it with your finger.

This will distort the sensor abilities to take accurate readings.

13. PROBLEM:

You are not getting the conductance reading you expected.

SOLUTION:

Try recalibrating the sensor head. Navigate to the Calibration option in the configuration menu to begin a calibration. If you are still having problems, the inside of the sensor assembly may be dirty. Remove the Teflon disk and clean it thoroughly (Section 8.2"Cleaning the Sensor Block"). The Teflon disk also may either be wet or dirty and may need to be dried out or replaced. Additionally, if your sensor was exposed to any out gassing materials, it may require repairs or reconditioning. Section 8.3 "Porometer Sensor Reconditioning" provides additional information).

14. PROBLEM:

Your Porometer will not calibrate, even after multiple attempts.

SOLUTION:

A Porometer that will not calibrate using the user initiated calibration sequence is generally a symptom of one of two things. The first is thermal non-equilibrium. The Porometer leaf clip temperature must remain somewhat stable during the calibration sequence for the calibration to succeed.

Another problem that can prevent calibration is contaminated sensors. If the sensors are exposed to volatile organic compounds, their accuracy and dynamic response can be profoundly affected, which can cause the two sensors to read quite differently from each other during a measurement, and can cause the calibration to fail. If you find that your sensors are not well matched, we recommend that you recondition the sensors as described in section 8.3.

15. PROBLEM:

When using the desiccant chamber, the time required for the sensors to equilibrate again between measurements is excessively long.

SOLUTION:

Insert one of the white fluoropolymer agitation beads, included with your Porometer, into the diffusion path and close the clip. Shake the leaf clip and the bead will mix the air in the diffusion path more quickly and speed the equilibration time significantly. The presence of the bead will not affect the stomatal conductance measurement as long as the bead does not move during the measurement and the leaf clip is oriented with the desiccant chamber down so that the bead rests on the Teflon filter.

16. PROBLEM:

Message on screen displays the following:

WARNING

Low RH Reading. Please recalibrate with desiccant before continuing.

SOLUTION:

This displays only if the calibration was conducted using no desiccant and the internal sensors have equilibrated at a humidity less than 10%. If you wish to use desiccant for future readings, please recalibrate.

17. PROBLEM:

Message on screen displays the Warning "Low RH Reading"



SOLUTION:

This displays only if the calibration was conducted using desiccant and the internal sensors have equilibrated at a humidity greater than 10%. Please ensure that desiccant is in place and replace with a fresh batch.

18. PROBLEM:

Message on screen displays the following:

WARNING Desiccant exhausted or not present. Replace or add desiccant to avoid unstable measurements. See User's Manual for details.

SOLUTION:

This displays when the internal sensors have equilibrated at greater than 10% humidity to remind you that measurements with desiccant are generally more accurate and stable. We recommend that the desiccant chamber is always used, but most importantly when ambient humidity is greater than 30%. See the section 4.2 on "Desiccant." You can press enter after seeing this display to proceed with non-desiccant measurements. **19. PROBLEM:** Bead Retention Screen Missing

SOLUTION:

Replace bead retention screen with the calibration plate that serves as a bead retention screen insertion tool. Place screen in hole, push snug with finger, and then use the calibration plate to seat the screen firmly in the porometer head. If screen continues to fall out, contact Decagon Devices for further instructions.

9.1 Repair Instructions

If your Leaf Porometer should ever require a repair, call Decagon at 509-332-5600. We will ask you for your address, phone number, your Leaf Porometer serial number, and your current firmware version. For non-warranty repairs, we will also ask for a method of payment.

Before shipping your instrument to Decagon, please contact Decagon to obtain a Request Maintenance Authorization Number (RMA). This will allow Decagons repair staff to keep track of your Porometer. Once you have acquired an RMA, send your Leaf Porometer to Decagon. Please include a document listing the complete shipping address, name, and department of the person responsible for the instrument, as well as a description of the problem. This will better help our technicians and shipping department to expedite repair on your Leaf Porometer, and ship it back to you.

Please pack your Leaf Porometer carefully. Ship it back in the carrying case, preferably inside a cardboard box. Ship to:

> Decagon Devices Inc. 2365 NE Hopkins Court Pullman, WA 99163

Repair Costs

Manufacturer defects and instruments under warranty will be repaired at no cost. For non-warranty repairs, costs for parts, labor, and shipping will be billed to you.

10 Further Reading

For more information on porometry, stomatal conductance, and how Porometers work, consider reading:

Campbell, G. S and Norman, J.M., (1998). An Introduction to Environmental Biophysics. Springer-Verlag: New York, Heidelberg, Berlin. Second Ed.: pp. 90-92.

Jones, H.G., (1983). *Plants and Microclimate*. Cambridge University Press: Cambridge.

Marshall, B. and Woodward, F.I., (1985). Instrumentation for Environmental Psychology. Cambridge University Press: Cambridge.

Pearcy, R.W. et al., (1989) *Plant Physiological Ecology: Field Methods and Instrumentation*. Chapman and Hall: New York, London: pp. 137-158.

11 Declaration of Conformity

| Application of Council Directive: | 2004/108/EC and $2011/65/EU$ |
|--|--|
| Standards to which conformity is declared: | EN 61326-1:2013 and EN 50581:2012 |
| Manufacturer's Name: | Decagon Devices, Inc 2365 NE Hopkins Ct. Pullman, WA 99163 USA |
| Type of Equipment: | Leaf Porometer |
| Model Number: | SC-1 |
| Year of First Manufacture: | 2005 |

This is to certify that the Leaf Porometer, manufactured by Decagon Devices, Inc., a corporation based in Pullman, Washington, USA, meets or exceeds the standards for CE compliance as per the Council Directives noted above. All instruments are built at the factory at Decagon and pertinent testing documentation is freely available for verification.

12 Appendix A - Send Feedback

Decagon Software makes it easy to send feedback, bug reports, and feature requests to Decagon or your Decagon Distributor. Choose "Send Feedback to Decagon..." from the Help menu. This opens the Feedback window.

| Name (required) | Company Name |
|------------------------------------|-------------------------------|
| User Name | User Company |
| Email Address | Telephone (include area code) |
| user@example.com | 555-555-5555 |
| Type of feedback | Please respond via |
| General Feedback | ▼ Email ▼ |
| | |
| Send the following file (file size | e can be up to 8 MB): |
| Serd the following fie (file size | e can be up to 8 MB): |

Figure 18: Feedback Window

Enter your name, and other contact information. Tell us what type of feedback you are sending (General Feedback, Feature Suggestions, Bug Report, or Other). Indicate how you want us to respond to your feedback (E-mail or telephone).

Use the description area to give details for your feedback. If you are reporting a bug, it is very helpful for you to tell us what steps you took for the bug to happen and any error message you saw. By default, bug reports also include the software error files.

You can send Decagon a file using this form too. This is useful for sending data files that you have questions about. If you work directly with a Decagon representative, put their E-mail address in the field at the bottom of the form. This sends the contents of the form to them. Your Decagon representative can follow-up with you directly.

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