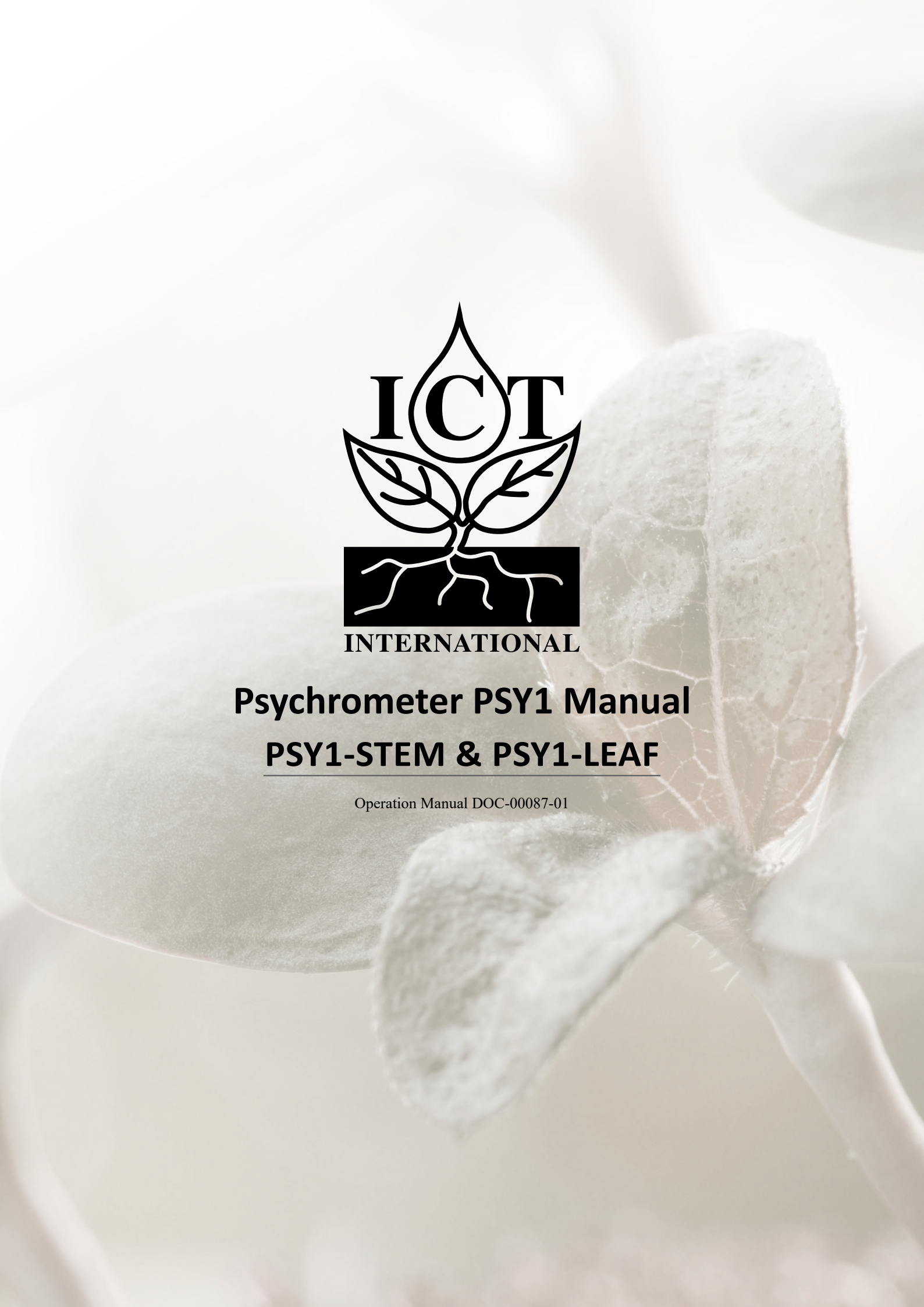


Psychrometer PSY1 Manual

PSY1-STEM & PSY1-LEAF

Operation Manual DOC-00087-01

Contents

1. Introduction

Figure 1.1. The PSY1 Psychrometer (PSY1-Stem)

The measurement of plant water potential has been traditionally carried out using Scholander pressure chambers. Although some systems are field portable and simple to use, less-destructive and the ability to continuously monitor water potential in plants in-situ is now possible with ICT International's Psychrometer.

The Psychrometer (PSY1) is a very powerful tool that integrates all the ambient environmental parameters acting upon the plant such as solar radiation, temperature, humidity, wind speed and water availability into a single continuously measurable variable.

The PSY1 is a stand-alone instrument for the measurement of stem water potential. It can continuously log changes in plant water status/ potential, which directly reflect the energy required to access water or the stress the plant is under.

The information contained in this manual allows users to install, operate and calibrate (when necessary) the instrument. This manual also includes information to operate the ICT Combined Instrument Software (CIS) and the embedded graphical user interface for PSY1.

2. Features and Specifications

Figure 1.2. PSY1 Psychrometer with Logger

The PSY1 Psychrometer (Figures 1.1., 1.2.) consists of the following items:

- Psychrometer Chamber;
- Lid/Calibration disk holder;
- Integrated, standalone data logger / meter;
- Clamps (for PSY1-Stem and PSY1-Leaf).

2. Features and Specifications

2.1. Psychrometer Chamber

The PSY1 chamber (Figure 2.1.) is made of chrome plated brass to achieve a stable thermal mass. Two welded Chromel-Constantan and one Constantan–Copper thermocouple are housed within the chamber. The psychrometer chamber well is capsule shaped that is 3.5mm wide and 8mm long.

Figure 2.1. PSY1 Psychrometer chambers

2.2. PSY1-Stem

The PSY1-Stem Psychrometer chamber has the following dimensions:

Diameter	25.5mm
Depth	20mm
Chamber volume	10.21cm³
Depth with lid	30mm

2.3. PSY1-Leaf

The PSY1-Leaf Psychrometer chamber has the following dimensions:

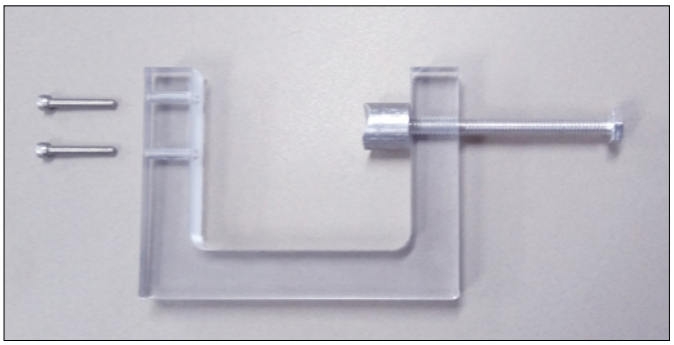
Diameter	19mm
Depth	19mm
Chamber volume	5.39cm³
Depth with lid	27mm

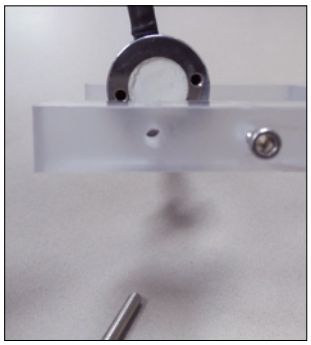
2.4. Clamps for stem installations

A clamping device is required to attach the psychrometer to a plant stem. Clamps are available in 4 sizes:

a. Small Stem Clamp (PSYS-C37) for stem sizes between 10 and 37mm in diameter
b. Medium Stem Clamp (PSYS-C55) for stems sized between 10 and 55mm in diameter
c. Large Stem Clamp (PSYS-C77) for stem sizes between 30 and 77mm in diameter
d. Extra-large Stem Clamp (PSYS-C125) for stem sizes between 78 and 125mm in diameter

Further details regarding the clamps can be found in this link. Use of the PSY1 with larger stem sizes will require a customised clamping mechanism; please contact ICT for more details.





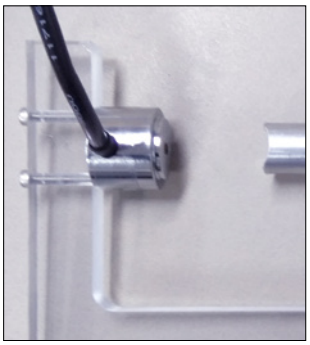


Figure 2.2 (Left) The parts of the PSY1 Stem Clamping device with hex screws including frame, moveable jaw, and jaw screw;
Figure 2.3 (Middle) Close-up on how the PSY1 connects into the frame of the PSY1 Stem Clamping device;
Figure 2.4 (Right) Showing the PSY1 attached to the frame of the PSY1 Stem Clamping Device.

2.5. Clamps for leaf installations

Leaf clamps (Figure 3.1.) come in 2 sizes with unique part numbers. To use the Stem Psychrometer chamber on leaf samples in-situ, make sure you have part number PSYS-LLC. To use the Leaf Psychrometer chamber on leaf samples in-situ, make sure you have part number PSYL-SLC.

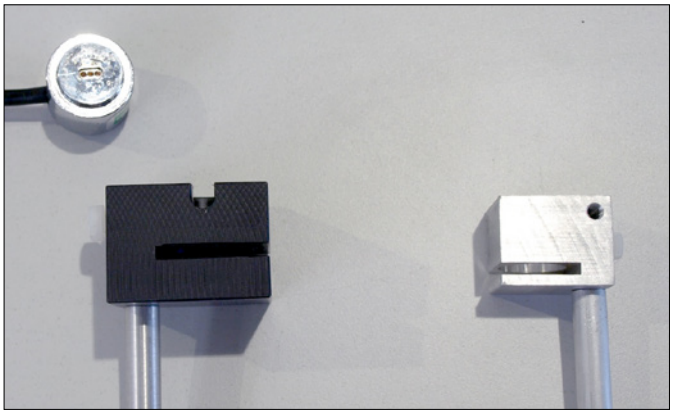


Figure 3.1. PSYS-LLC (left) and PSYL-SLC (right)

Figure 3.2. and 3.3. The PSYL-SLC shown with the PSY1

2.6. Osmotic potential

Osmotic potentials can be measured on extracted sap samples or destructively sampled leaf tissue or leaf discs. These measurements can be made in the lab or in the field. Samples are placed in the calibration lid of the PSY1 stem psychrometer.

The psychrometer chamber is housed in the Osmotic Potential Insulator (OPI; Figures 4.1., 4.2., 4.3.) to provide a thermal insulating jacket around the chamber. This eliminates introduction of thermal gradients caused by a need to handle the chamber to load samples and provides a stable insulated thermal buffer from ambient temperature gradients within the surrounding environment. This enables a very rapid equilibration time between samples. Please see Section 5.6. for more details.

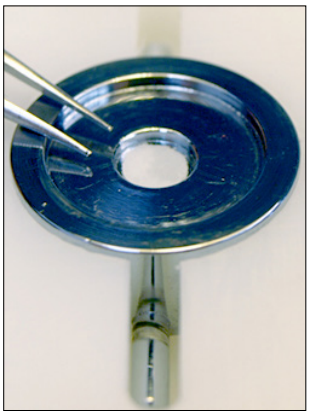


Figure 4.1., 4.2., 4.3. The PSY1 Stem Psychrometer chamber embedded in the Osmotic Potential Insulator

2. Features and Specifications

Figure 5. The PSY1 Logger

2.7. PSY1 Logger

The PSY1 logger (Figure 5.) features a highly accurate, high precision microvolt meter designed to specifically measure water potential in plants. It is an integrated stand-alone data logger consisting of a 24-bit resolution preamplifier and microprocessor with integrated Analog to Digital converter that outputs and logs processed data in calibrated engineering units (MPa).

The custom-designed enclosure of the PSY1 has an IP65 rating. Water proofing is achieved through a unique physically separated, but electrically linked dual chamber enclosure design. This ensures that the internal circuitry and battery can be electrically linked and charged from an external power supply without providing any physical pathway for water ingress. However, to ensure water proofing guarantee of the instrument, it is important not to open the enclosure without ICT International's technical assistance.

3. System Requirements

The ICT Combined Instrument Software (CIS), required to operate the Psychrometer, is compatible with the following Operating Systems:

- Windows 7, 8, 8.1, 10
- Mac OS X

The minimum hardware requirements to run CIS are:

- Intel Atom 1.66GHz and 1GB of RAM or higher.
- 11.6" or larger or a native screen resolution of 1366 x 768 or larger

4. Getting Started

4.1. Combined Instrument Software (CIS) and the PSY1 Graphical User Interface

Download and install the latest version of the Combined Instrument Software using this link: https://www.ictinternational.com/support/software/). Once installed double click on the CIS icon on your desktop or click from the Windows (Start) button to open the software (Figure 6.).

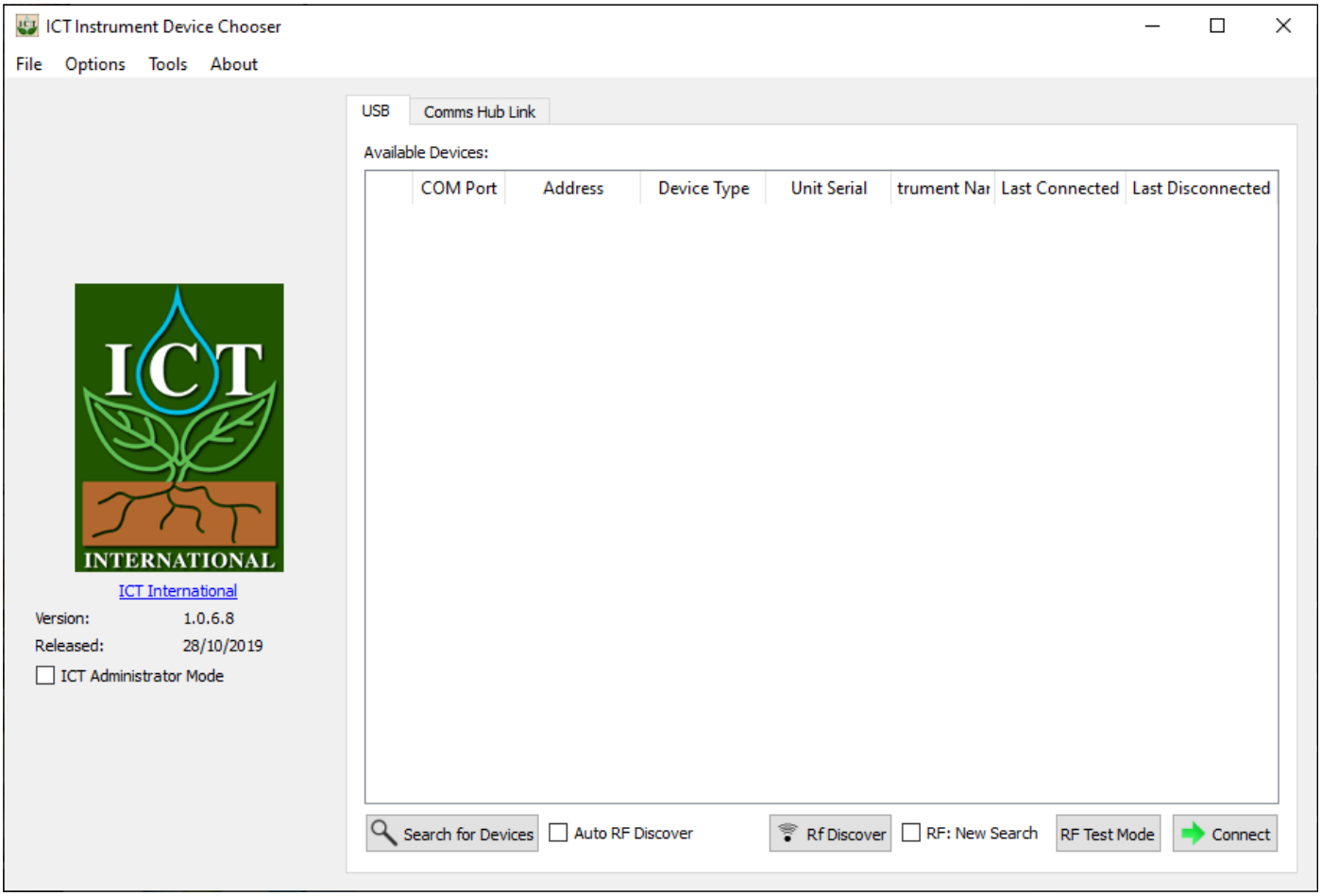


Figure 6. The ICT Combined Instrument Software

To learn how to connect to your PSY1 device using the CIS, please see 5.4.

Once connected to the PSY1 device, an embedded PSY1 graphical user interface (GUI) will appear as a separate window that is defaulted to show the ***Channels*** ribbon (Figure 7.).

4. Getting Started

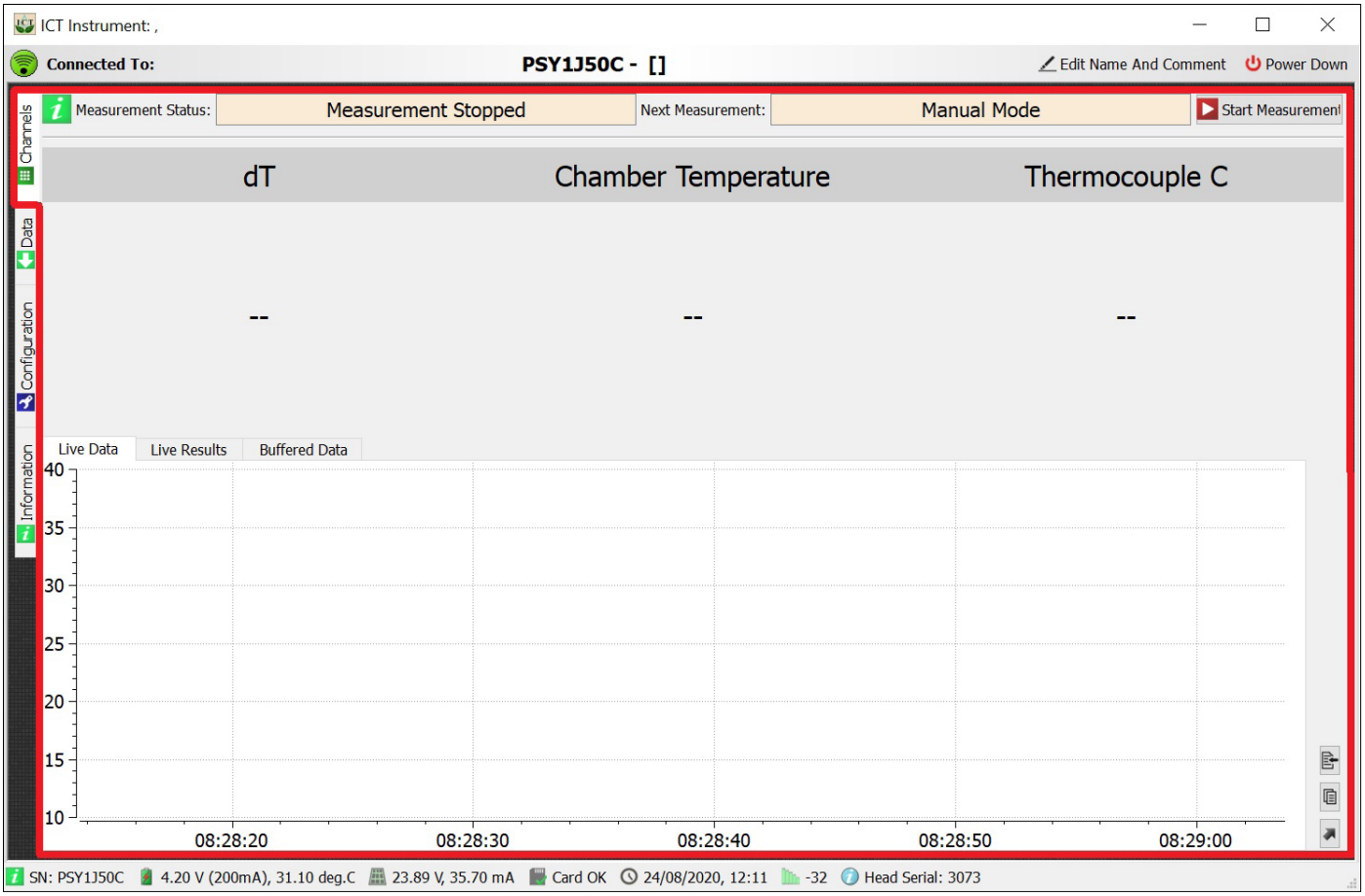


Figure 7. The PSY1 graphical user interface (GUI) showing the Channels ribbon (red area).

At this point, you can initiate a manual measurement (provided the sensor or chamber is installed properly). The ***Channels*** ribbon provides the current status of the instrument and displays 3 important parameters of the instrument (dT, Chamber Temperature, and Thermocouple C). The Channels ribbon also displays 3 tabs: ***Live Data***, ***Live Results*** and ***Buffered Data***. These tabs display current or most recent measurement outcomes for PSY1 connected to a PC and being operated on using CIS (e.g. any manual measurement done if the ***Start Measurement*** button is clicked). These tabs are explained further in Appendix 12.1.

4.1.1. dT and Chamber Temperature

Displays the difference in temperature (dT) measured in (µV) between the Thermocouple-S and Thermocouple-C. In the manual mode dT will be measured initially at the commencement of the measurement process and this value remains on display until the completion of the measurement.

Chamber Temperature is the measured temperature (°C) of the entire psychrometer chamber body. The measurement is made by a Copper-Constantan thermocouple located within the insulated base of the psychrometer chamber. It is representative of the ambient conditions under which the measurement is made. The chamber temperature measurement is made and subsequently displayed after the Peltier cooling pulse and psychrometric wet bulb measurement has been made.

4.1.2. Status bar

The PSY1 GUI displays information of the PSY1 hardware in 2 ways. The first and quicker way to view this information is through the status bar (Figure 8.). The following information is displayed conveniently at the bottom of the GUI at any point you are connected to the instrument via CIS:

- Serial Number;
- Current voltage of the internal battery;
- Current internal temperature of the PSY1 logger;
- Power status in voltage (i.e. if connected to external power such as solar panel or battery or mains power);
- microSD card status (if properly inserted or not);
- Real time clock;
- Serial number of the PSY1 sensor;
- RSSI value (if connected to the instrument via MCC-MINI).

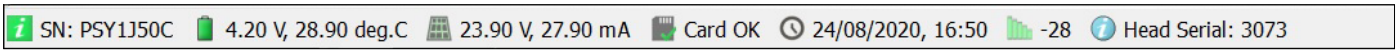


Figure 8. Status bar as displayed at the bottom of the PSY1 GUI.

New units delivered by ICT International are set to Australian Eastern time and date. If your location is different you will have to reset the real time clock of the unit. A key element of the status bar is the real time clock. Click this in the status bar and a dialogue box will appear (Figure 9.).

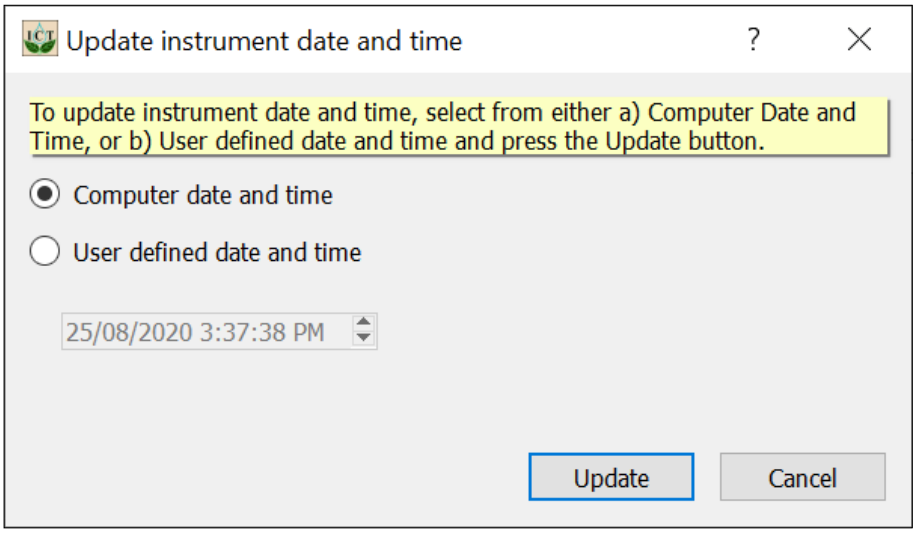


Figure 9. Update instrument date and time dialogue box

Make the necessary changes and click ***Update***.

4.1.3. Information ribbon

More information about the PSY1 logger can be accessed by clicking on the ***Information*** ribbon (Figure 10.), which, in addition to the information displayed in the status bar, displays the following:

- Application board (version and release date)
- Firmware (COM and APP version and release date)

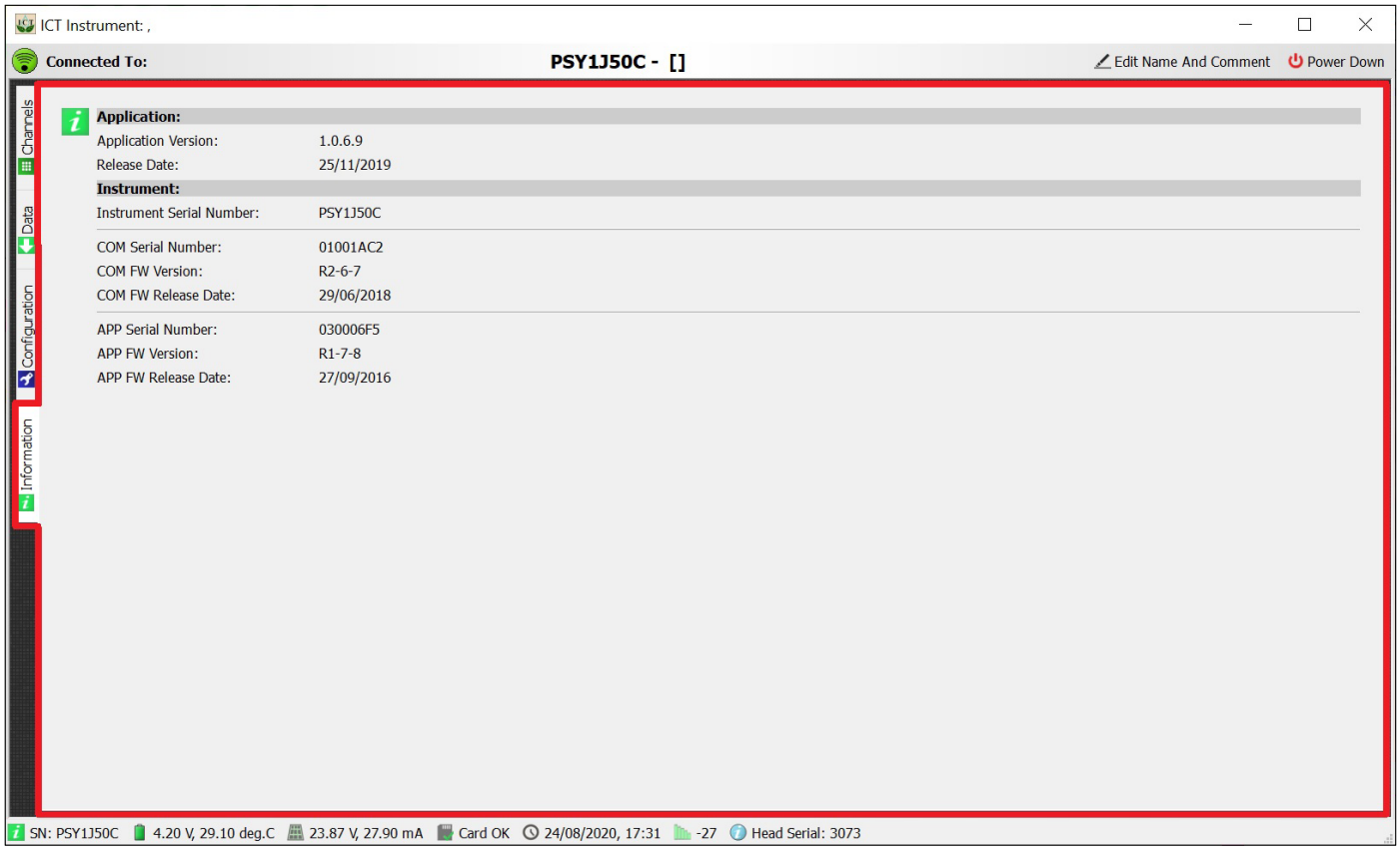


Figure 10. ***Information*** **ribbon**

4.2. Editing and annotating the PSY1 logger

The PSY1 logger can be annotated and labelled according to, for instance, a convention in an experimental design. This feature is available in the PSY1 GUI. Click ***Edit Name and Comment*** (Figure 11.).

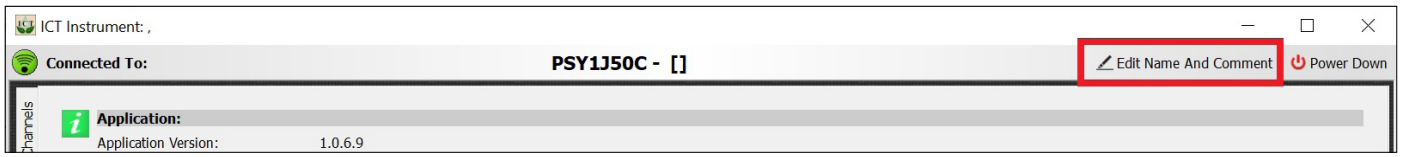


Figure 11. ***Edit Name and Comment*** **button**

A dialog box (Figure 12.) will appear which will allow for annotating the PSY1 in 2 levels: Name and Comment. Both fields can accommodate 28 alpha-numeric characters and will be printed in the header line of the data file. Be sure to click ***Update*** button in the dialogue box to save the annotations.

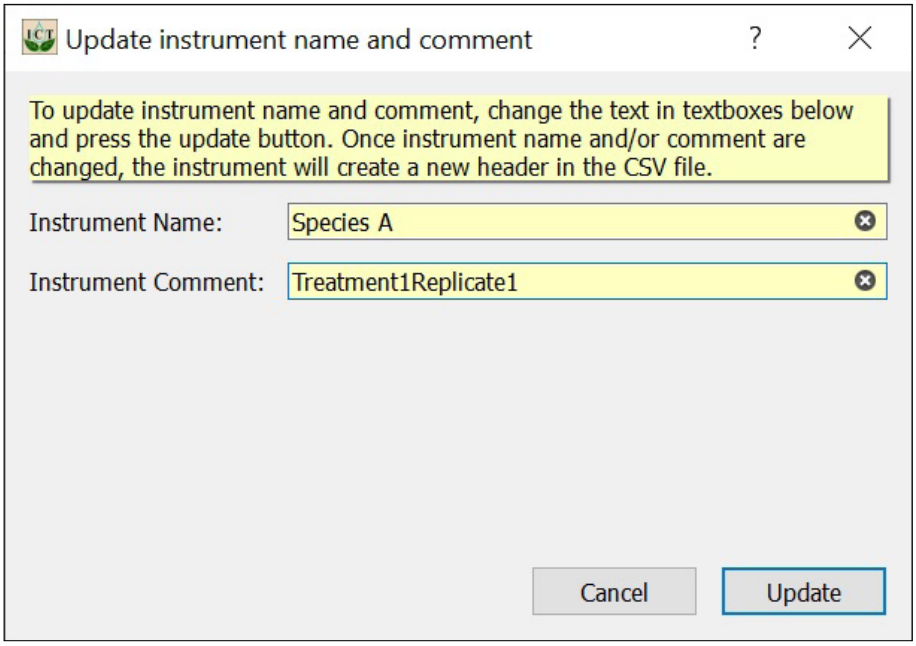


Figure 12. Edit name and comment dialogue box (with example annotations)

4.3. Charging and powering the unit

The PSY1 is a self-contained instrument that incorporates a lithium polymer battery. After receiving your new instrument, please charge the unit for at least 4 hours using a 24V charger (can be sourced from ICT International part # CH24). A fully charged internal battery is around 4.2V (indicated in the status bar on CIS).

To power the instrument for continuous measurement (at defined intervals), it is recommended to use a 20W solar panel (can be sourced from ICT International; part # SP22). The unit can also be powered using ***CH24*** provided the internal battery is cycled properly. This can be done by using the CH24 in conjunction with a timer switch. In circumstances where either a solar panel or mains power is not available, 85 Ah to 100 Ah sealed, lead gel acid, deep cycle batteries may be used in conjunction with a timer switch. Cycle the internal battery propery using a timer switch when using deep cycle batteries

The PSY1 logger is equipped with power-bus plugs to simplify electrical wiring process. Figure 13 provides wiring instructions.

An alternative powering solution (using deep cycle batteries in conjunction with solar panels) is also available. Further information is found on Appendix 12.2.

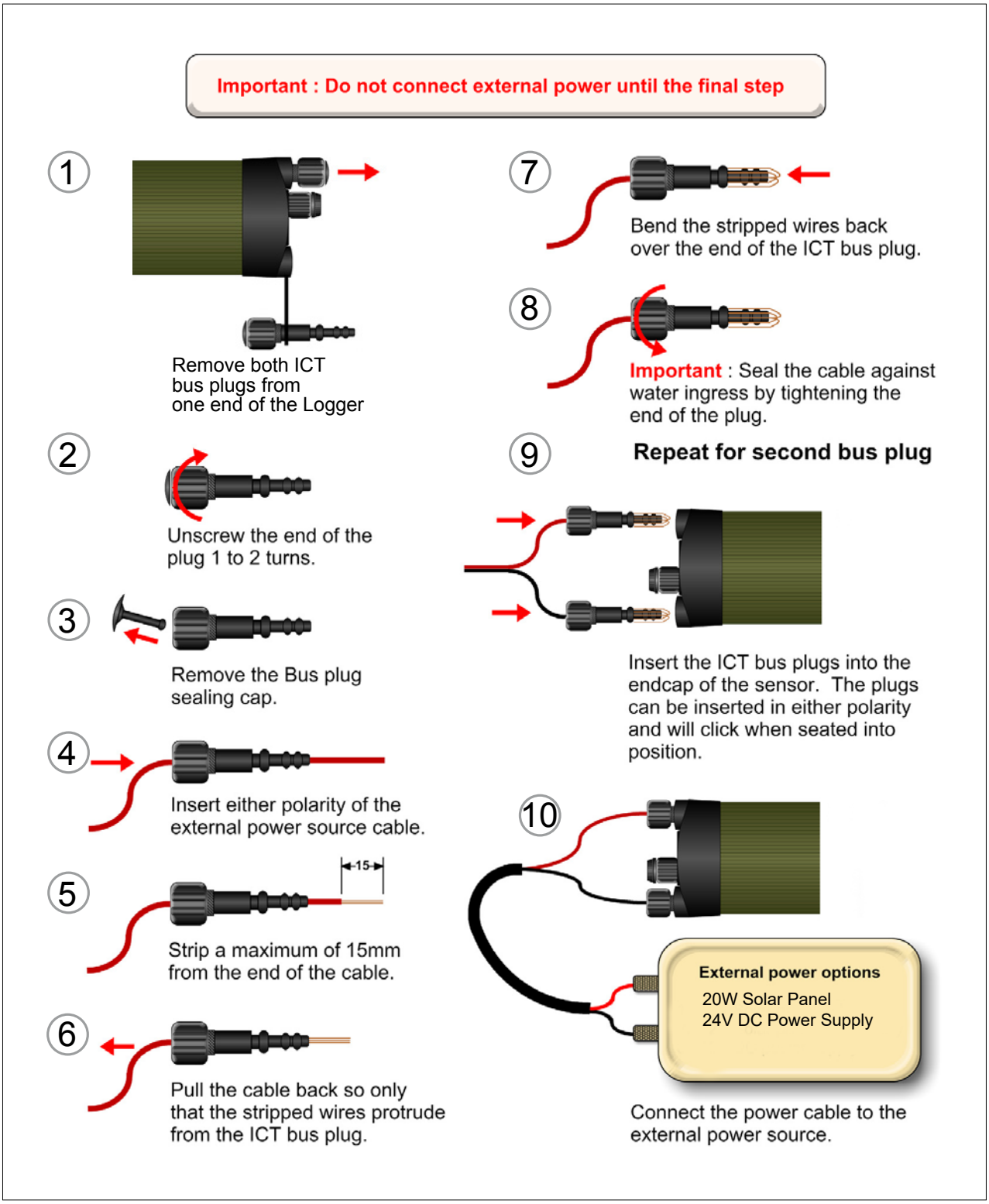


Figure 13. Wiring instruction through PSY1 bus plugs

4.4. Calibrating the psychrometer sensor (an introduction)

PSY1 are delivered with default Calibration settings using historical average slope and intercept values of a small sub-sample of all psychrometers made. It allows end-user to use the instrument to obtain relative water potential measurements (for instance to demonstrate the instrument in a class). PSY1 must be calibrated prior to first use after delivery or before reinstalling on a new or fresh installation site (within the same plant being monitored) to guarantee accurate results.

The full calibration procedure is found in Section 11. The calibration will require a controlled temperature chamber (set to 25°C) where the PSY1 sensor is placed during the entire calibration process. A 6-point calibration is recommended using varied molalities of NaCl solution. To prepare these solutions please refer to Appendix 12.3.

It is common to calibrate the psychrometer at 25°C. If one routinely uses the instrument at, say, 15°C and applies the correction, an unknown potential error could be introduced. It is recommended to calibrate the instrument at or near the temperature at which it will normally be used. A very useful exercise would be to calibrate the instrument at a variety of temperatures and assess the relationship between temperature and calibration coefficient for the individual instruments. This will enhance the reliability of the instrument.

4.5. Turning on and shutting down the instrument

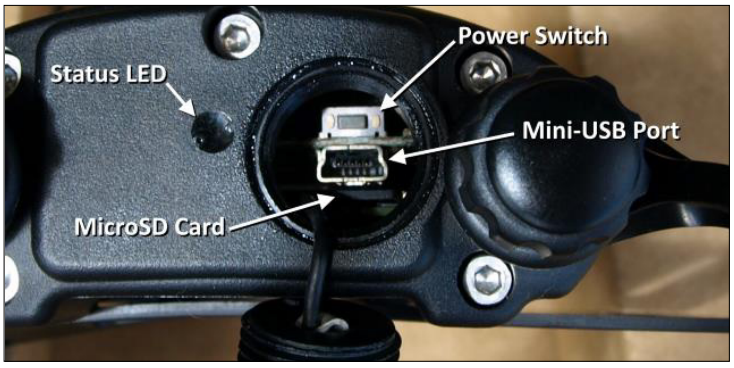


Figure 14. PSY1 access port showing parts after the knurled bung has been removed

The unit wakes up once connected to any power source described in 4.3.

To turn on the unit using internal battery, press (for 1 sec) the physical power switch inside the access port (Figure 14.). You may need a pointed tip object to do this. Once successful, the status LED will flash green for about 10 seconds. Turning off the device can be done in 2 ways. In either way, the unit has to be disconnected from any external power source to shut down the unit.

Pressing the power switch for 3 seconds will shut down the unit. A red LED will begin to flash and will slowly extinguish indicating that it has powered down successfully. Alternatively, using CIS you can shut down the unit by clicking on ***Power Down*** button and following the prompts (Figure 15.).

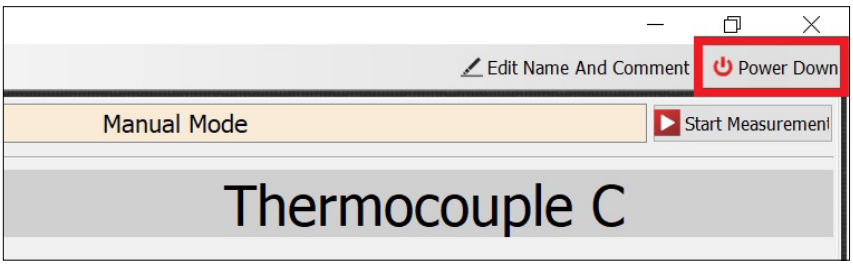


Figure 15. *Power Down* button

5. Operating Instructions

5.1. Preparing relevant accessories for installation (Installation kit)

The standard materials required for installing the psychrometer are the following:

- □ Single Edge Razor Blades;
- □ Dow Corning Vacuum grease (150 g);
- □ 10 ml Offset Syringe;
- □ Tweezers;
- □ #30 Rubber bands;
- □ Label Tape;
- □ Wash Bottle, a box of Kim Wipes;
- □ Polyester Insulation;
- □ Roll of Aluminium foil;
- □ Micro SD Card Shuttle;
- □ Screwdriver;
- □ Wire Strippers;
- □ Spare Screws for Small Clamp;
- □ Spare Screws for Large Clamp;
- □ 1.0 Molal NaCl Calibration Solution;
- □ Whatman #1 filter paper discs.

These materials come standard in the Install Kit available at ICT International (part # PSYS-IK or PSYL-IK).

Aside from these items, distilled water is required during installation. Depending on the number of installations to be performed, additional insulation materials (polyester foam, aluminium foil) may be required.

5.2. Installing the PSY1 chamber

5.2.1. Sample selection and install site preparation

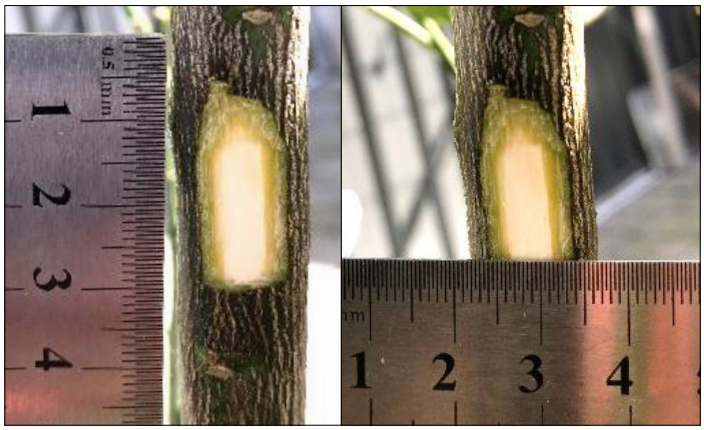


Figure 16. Suggested width and length of installation site on a small-sized stem

Samples are selected depending on measurement protocol. Select a suitable leaf or stem sample considering the dimension of the sensor and your available clamps and install gear (See 2.1-2.6).

On woody stems, expose a suitable area of the xylem tissue or sap wood (the size will vary depending on stem size; see example on Figure 16.) to ensure that the chamber well of the psychrometer will be completely covered by the sample and ensure vapour seal.

Remove the bark, phloem and cambium layers using a single edged razor blade to scrape a flat area of xylem upon which to mount the psychrometer.

Do this by using relatively short, straight passes with the razon blade in a forward and backward motion ensuring a level plane to guarantee a vapour seal when the chamber is installed (See Figure 17.).

It is very important to achieve a flat surface when exposing the xylem. The calibration lid can be used as a surrogate for the psychrometer chamber to visually check the flatness of the prepared site. Ensure that no gaps exist between the calibration lid and xylem surface by looking for flecks of light between the two surfaces. Proceed to clean the prepared site after ensuring a flat surface is achieved. Thoroughly clean the area with distilled water and wipe dry. This will remove spilled cell contents and plant tissue debris.

On a leaf, install the PSY1-Leaf chamber abaxially on leaf surface by first removing the cuticle layer to expose the lower epidermis. A polishing compound (e.g. 0.05 µm gamma alumina, Buehler, IL) can be used to remove the cuticle. A very fine grit sandpaper can also be used.

Figure 17. Razor blade angled slightly to create a level plane installation site

5.2.2. Attaching the PSY1 sensor to the install site

Once the install site is ready, prepare the PSY1 chamber for attachment. If installing on a stem, mount the chamber first to the clamp using hex screws provided. The hex screws should be tightened using finger force only (no need to use a driver). At this point the movable jaw of the clamp may have to be retreated by turning its screw.

Once mounted properly into the clamp, remove the lid / calibration disk holder and apply a thin layer of grease around the lip (close the flange) of the chamber well. Smear the grease evenly by replacing the lid and turning it gently 180° clockwise and counter- clockwise. Remove the lid. Inspect the chamber and make sure there is a thin film of grease surrounding the chamber well (but not on it).

Place the movable jaw of the clamp to the opposite side of the install site being careful that the chamber well does not touch any surface. Slowly wind the screw of the movable jaw so that the chamber gradually approaches the install site. Make sure that the chamber well is centred in the install site aligning with the exposed xylem tissue. Secure the chamber in the install site by tightening the clamp using finger pressure (Figure 18.).

At this point, do not move the PSY1 sensor. Apply grease around the installation point (junction of the sample and psychrometer chamber).

Figure 18. Installed PSY1-Stem sensor on citrus stem

5.3. Insulating the chamber and installation site

The installed instrument and portion of stem should be insulated with styrofoam or cotton wool or a suitable material. The foam insulation is not intended to prevent the diurnal temperature change. Instead, it is intended to act as a thermal buffer zone to depress the rate of temperature change during the period of measurement to determine the Psychrometric Wet Bulb Depression. Finally, wrap the foam-covered chamber and stem with aluminium foil to reflect direct radiation and prevent it from heating the whole installation (Figure 19.).

Alternatively, an insulated temperature control jacket can be fitted around the installation and connected to a bath circulating temperature controlled fluid. This is usually only possible in a laboratory situation but is particularly efficient at limiting temperature gradients and maintaining constant instrument temperature, two highly desirable factors in the reliable use of the instrument.

Figure 19. Attaching a high-density foam insulation jacket around the installation site

5.4. Connecting to the instrument

5.4.1. via USB

To connect to the PSY1 logger via USB, open CIS (see 4.1.). Make sure the unit is connected to your PC and click ***Search for Devices*** (Figure 6.). FTDI CDM driver might be required. You can download this driver from https://ictupdater.ictinternational.com/sw/CDM21226_Setup.exe. The instrument will appear in the ***Available devices*** pane. Select the instrument and click ***Connect***.

5.4.2. via MCC-MINI (when available)

To connect to the PSY1 logger via MCC-MINI, open CIS (see 4.1.). The MCC-MINI must be connected to your PC that runs the CIS. Ensure that the ***Auto RF Discover*** is ticked then click ***Search for Devices***. CIS will then be able to detect any PSY1 device (turned on) within range (Figure 20.). Note that the first item in the list is the RF Modem connected to the PC.

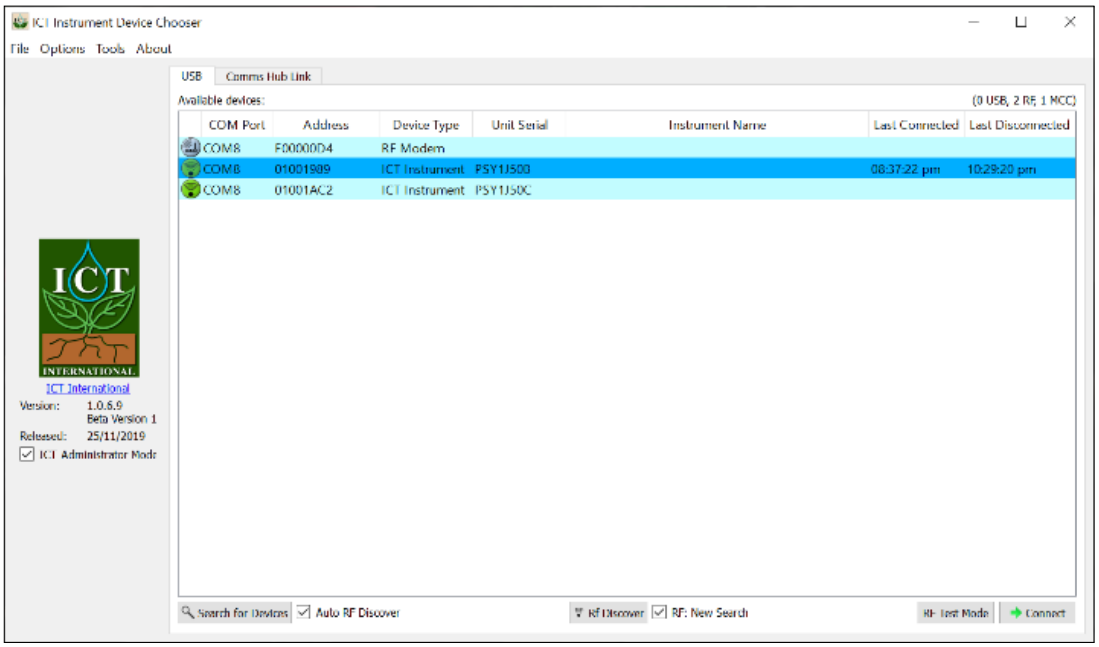


Figure 20. CIS displaying devices in the Available devices pane after clicking Search for Devices button

5.5. Setting the instrument for continuous logging

PSY1-Stem and PSY1-Leaf can be set such that water potential measurement is performed at a given interval. Click the ***Configuration*** ribbon (Figure 21.) in PSY1 GUI of the CIS. To learn more about altering measurement options, please read 6.1. to 6.4.

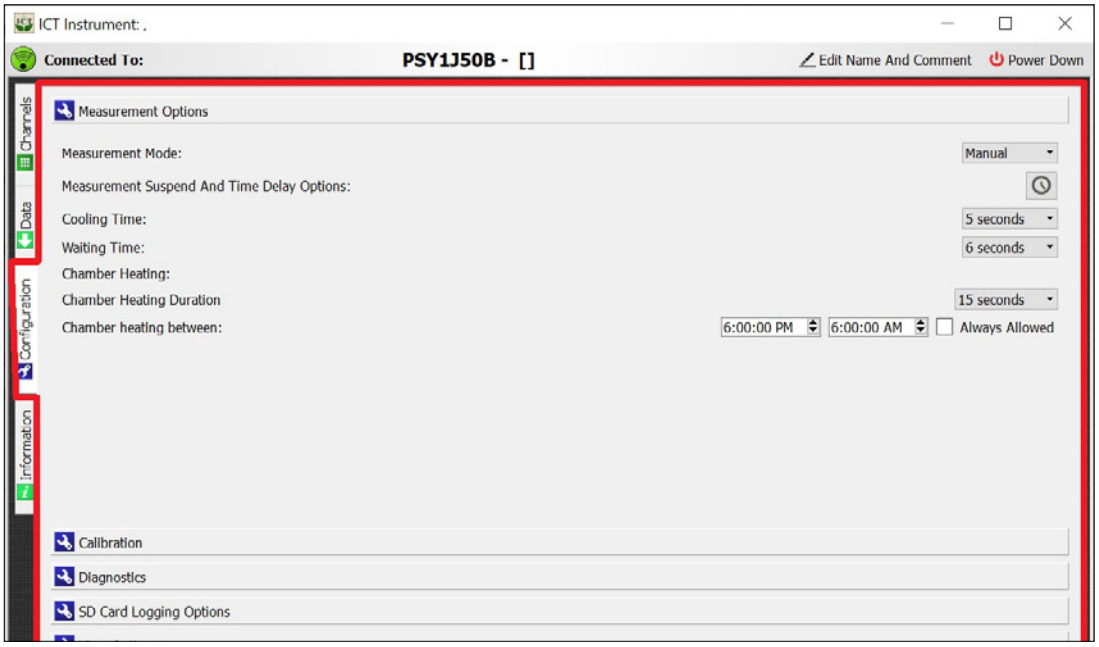


Figure 21. Configure ribbon

5. Operating Instructions

As dictated by your experimental or measurement protocol, you can select from 5 various intervals when the measurement is performed by the instrument. Click ***Measurement mode*** drop down menu to reveal these intervals. Select the appropriate interval and the click ***Update Measurement Option Changes*** and click OK. At this point you can go back to ***Channels*** ribbon to check if the instrument indicates the time of the upcoming measurement in the ***Next Measurement*** bar.

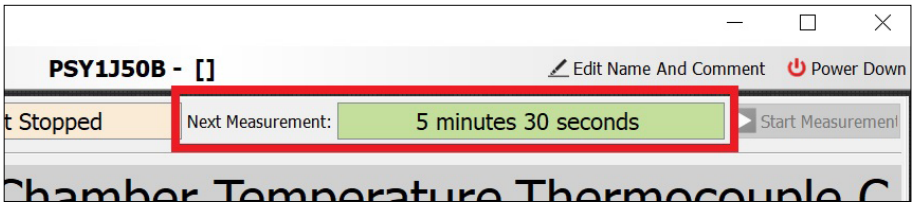


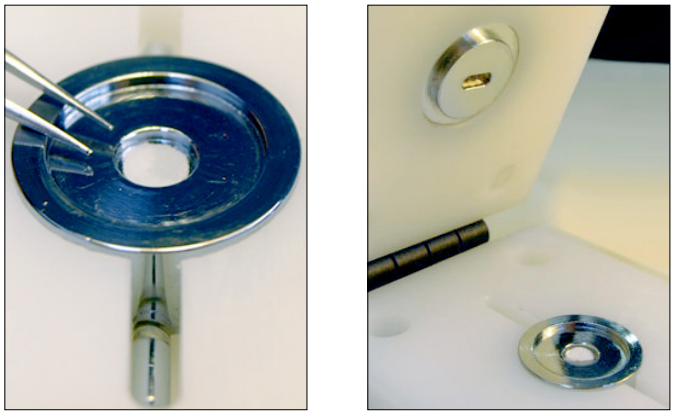
Figure 22. Next Measurement status indicator

5.6. Using PSY1-Stem for measuring osmotic potential

Osmotic potential measurements are typically performed in a manual process. Using the Graphical User Interface (GUI) the PSY1 provides the user with a "Live" mode or a Manual mode to facilitate osmotic potential measurements.

An abraded leaf disc or filter paper disc (saturated with extracted sap exudates from a suitably prepared sample using a freezing and physical disruption protocol to separate the symplastic fluid from the cells of the leaf), is placed in the calibration lid.

To prepare a sample for measuring osmotic potential, wrap the leaf in a foil envelope and include a filter paper disk which will become saturated with the expressed cell contents.



Figures 23.1., 23.2. The psychrometer chamber mounted inside the Osmotic Potential Insulator (OPI) with loaded filter paper disc soaked in an extracted sap solution to measure osmotic potential

Place in liquid Nitrogen to freeze then crush it in a vice to physically and mechanically disrupt the cell walls. Place the saturated filter paper disk in the psychrometer calibration lid and measure the osmotic potential following thermal equilibration/stabilisation of the psychrometer chamber.

6. Settings and measurement options

6.1. Suspending Measurement

The PSY1 sensor requires thermal and vapour pressure equilibrium with the sample. This takes place between 30 to 60 minutes after installation. The PSY1 can be set to commence logging after a known equilibration time. Click on the clock icon opposite ***Measurement Suspend and Time Delay Options*** (Figure 24.). A dialogue box will appear; untick ***Disable Delayed Start*** and select a time. Ensure that your real time clock is set properly (see 4.1.1.). Click ***Update Measurement Option Changes*** after making changes in this option.

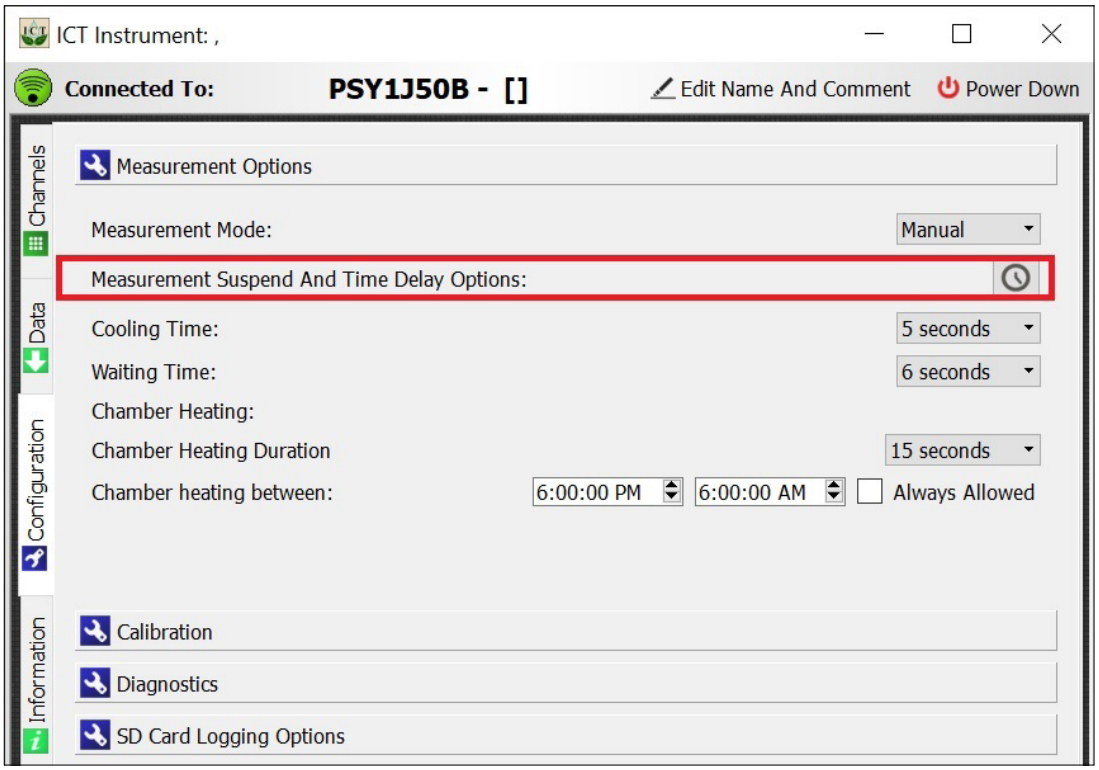


Figure 24. Suspending measurement

6.2. Cooling time

The length of the Peltier cooling pulse will determine the volume of water that is condensed onto Thermocouple-C. A short cooling time will result in a small volume of water that will quickly evaporate back into the atmosphere of the chamber. Conversely, a long cooling time will condense a large volume of water, and slowly evaporate back into the atmosphere of the chamber.

The volumes of water and the times taken to change from liquid to vapour phase will be determined by the vapour pressure within the chamber which is in equilibrium with the plant. For instance, as conditions become drier, it may be necessary to increase the cooling time to ensure a sufficient volume of water is condensed onto Thermocouple-C to generate a Psychrometric Wet Bulb Depression.

Click ***Cooling Time*** to alter length of cooling pulse and select the desired time in seconds (Figure 25.). Click ***Update Measurement Option Changes*** after making changes in this option.

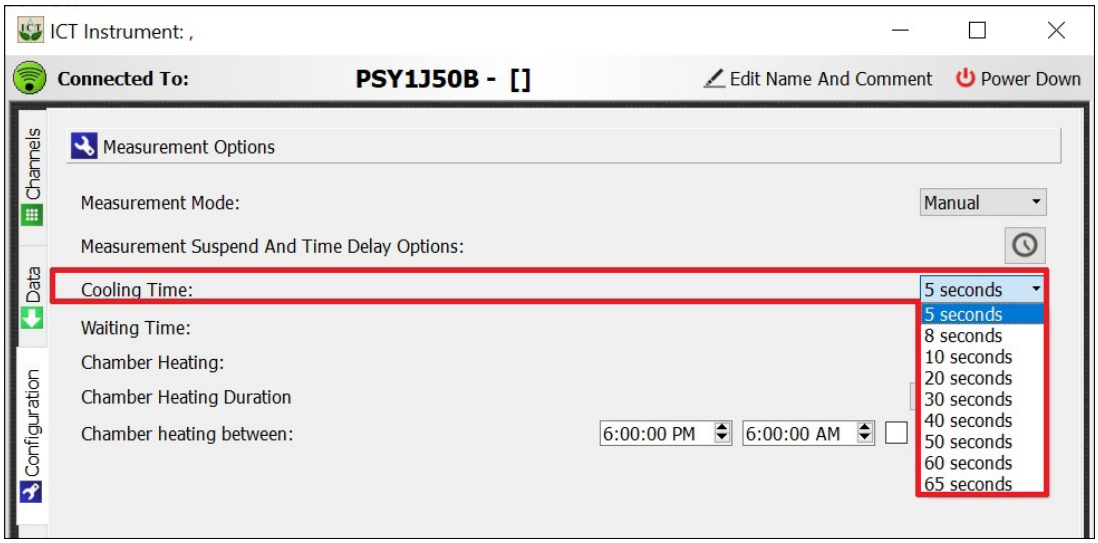


Figure 25. Setting the cooling pulse duration

6.3. Waiting time

The ***Waiting Time*** (Figure 26.) is the time after the cessation of the cooling current until a determination of wet bulb depression is made. This has been empirically determined to be 6 seconds.

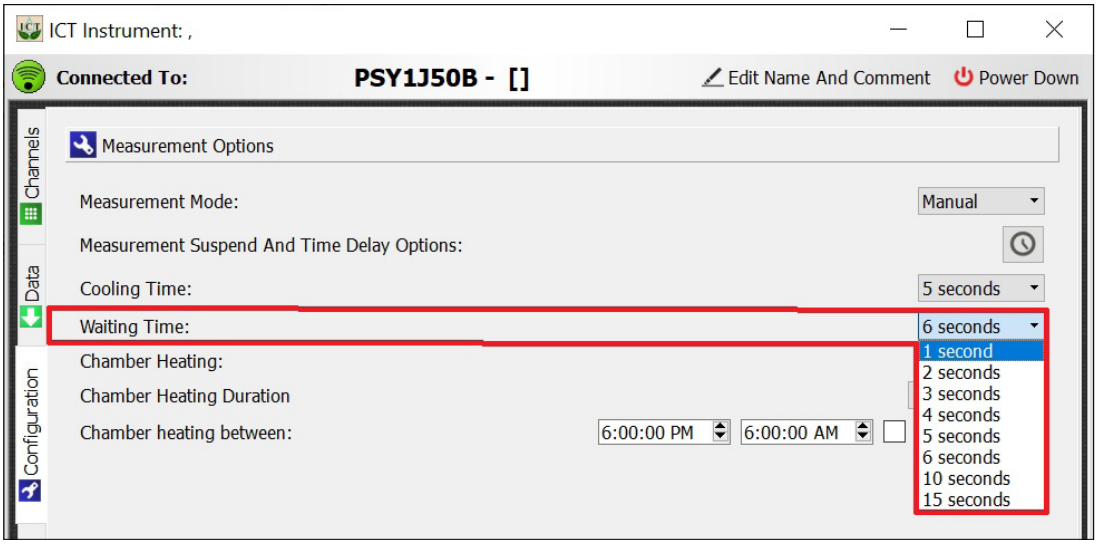


Figure 26. Setting the wait time

6.4. Chamber heating

The PSY1 chamber is equipped with heater that can be used to mitigate conditions that favour undesirable temperature gradients (i.e., the chamber is colder than the stem). The heater is a 12V (DC) resistive heater integrated into the back of the chamber and controlled by the PSY1. The chamber heater can be set to automatically turn on immediately following the completion of a measurement.

The duration of the chamber heating can be set by selecting the desired time period. The exact duration for every installation will be specific to the ambient conditions and the plant being monitored. It may be necessary to trial a range of chamber heating durations between the ranges of 15 seconds to 2 minutes until the ideal protocol for the prevailing conditions is determined. This setting can be accessed in the ***Chamber Heating*** (Figure 27.).

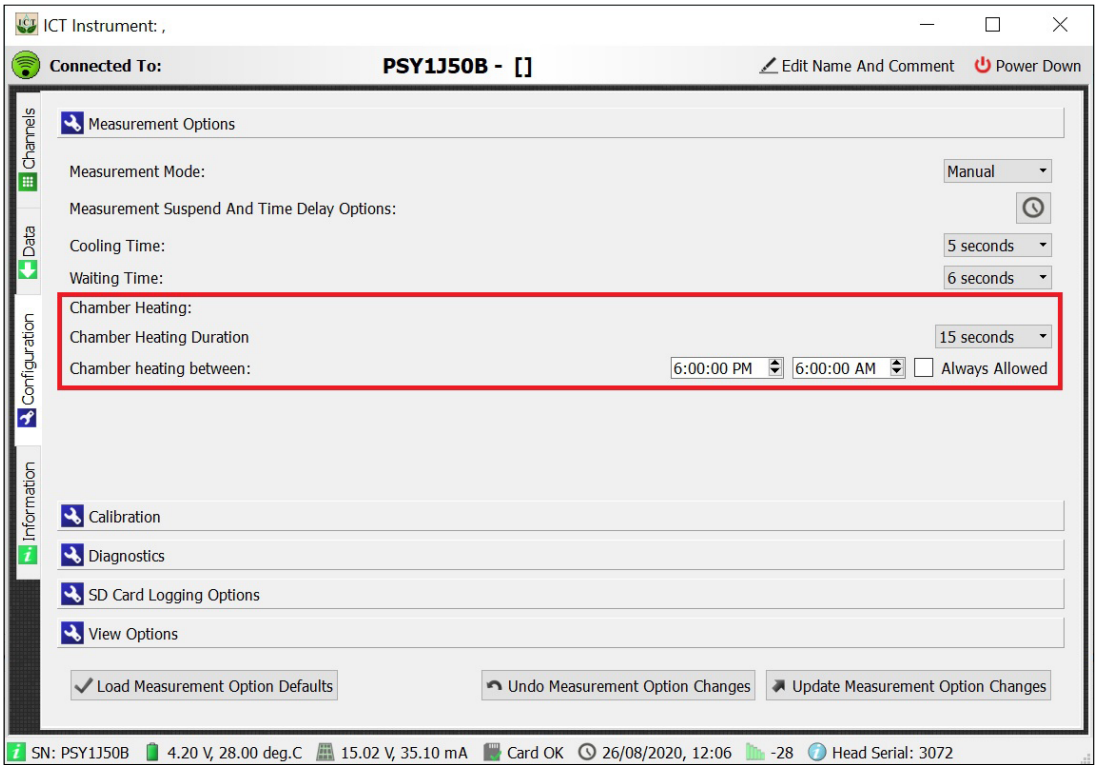


Figure 27. Chamber heating

Chamber heating can be continuously employed, but this has the potential to cause artificial drying of the stem if employed when undesirable temperature gradients are not present. As a broad guideline the most likely period for condensation to occur inside the chamber, due to the chamber being colder than the stem, is between 5 AM and 10 AM.

7. Data management

The PSY1 GUI in CIS can be used to manage instrument data in the ***Data*** ribbon (Figure 28.). The ***Download*** button will display all data stored in your device and hold it in the computer's CIS directory.

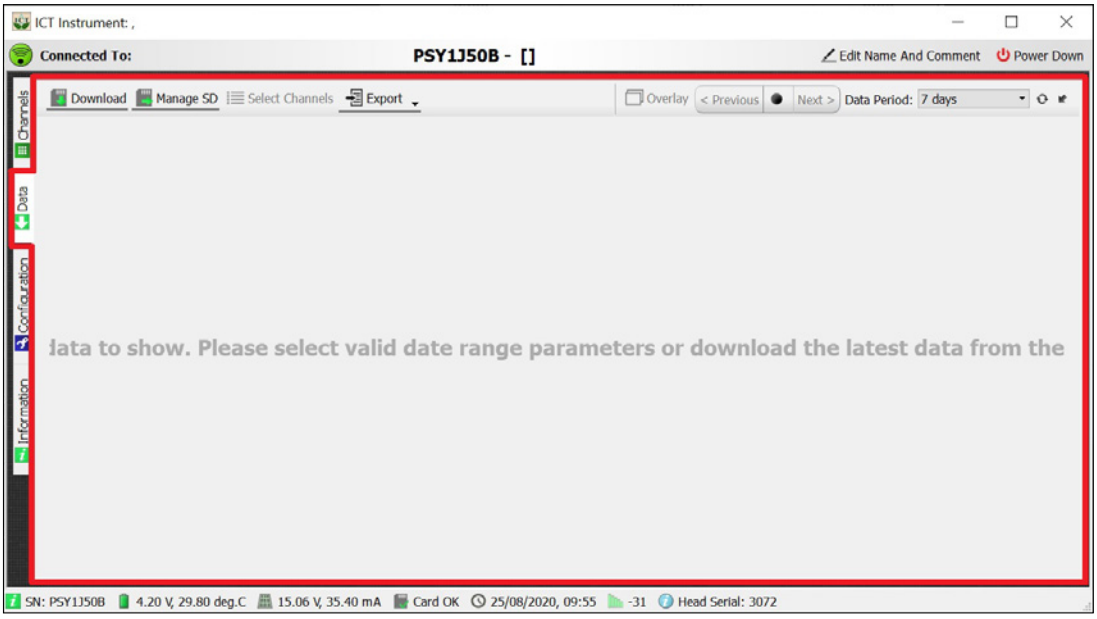


Figure 28. Data ribbon

The quickest way to check if data is stored properly in your microSD card is by clicking ***Manage SD*** button (Figure 29.). This will display any file (including file size) saved in the microSD card attached to the instrument.

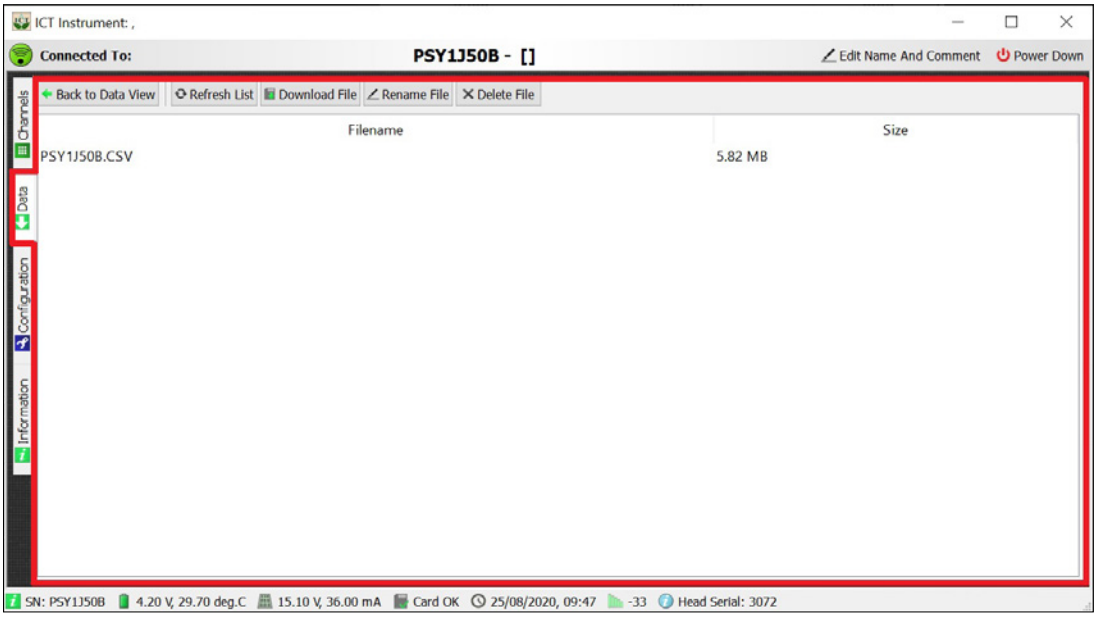


Figure 29. Data ribbon showing current file

To download data in the microSD card, remove this from the device and use the SD card reader that is provided with your instrument. See 4.5 to access the microSD card (n.b. use a tweezer or a pointed pliers). Copy the file across your computer.

7. Data management

7.1. Downloading data to view in PSY1 GUI

Clicking the ***Download*** button will display any current data that has not been previously downloaded and stored in your PC (Figure 30.). To display the measured water potential, click ***Select Channels*** and the Channels pane will appear on the right side of the GUI. Click to unselect the channels that you wish to hide and click OK. The resulting display is in Figure 31.

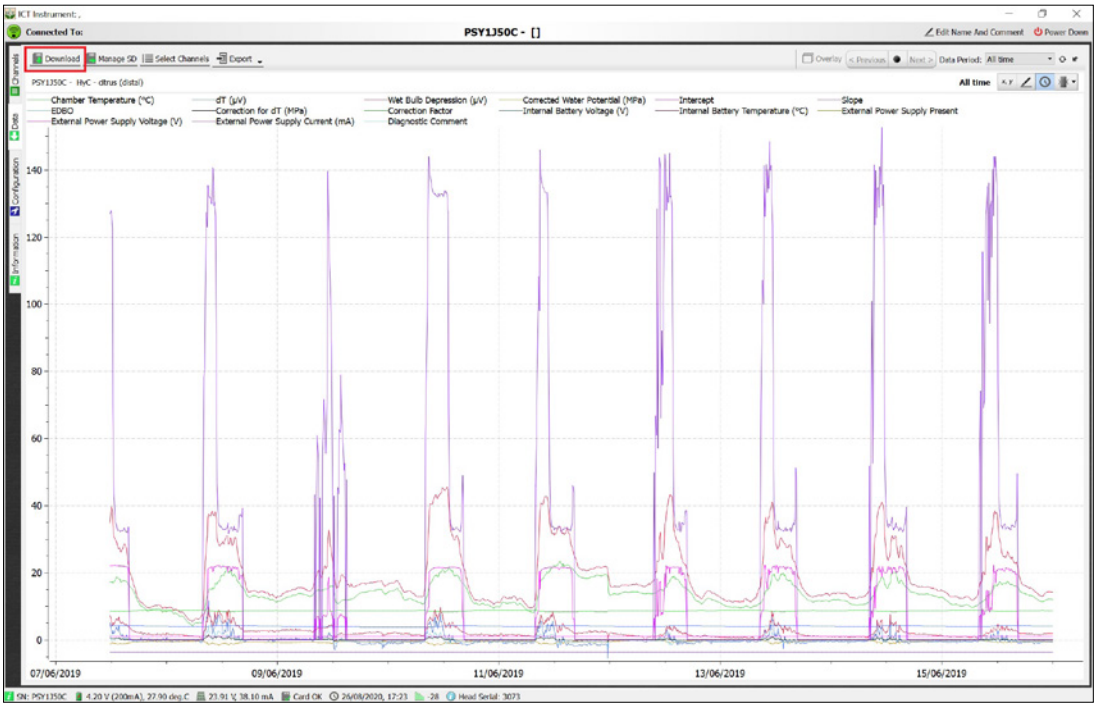


Figure 30. Data display (all channels) after *Download* button is clicked

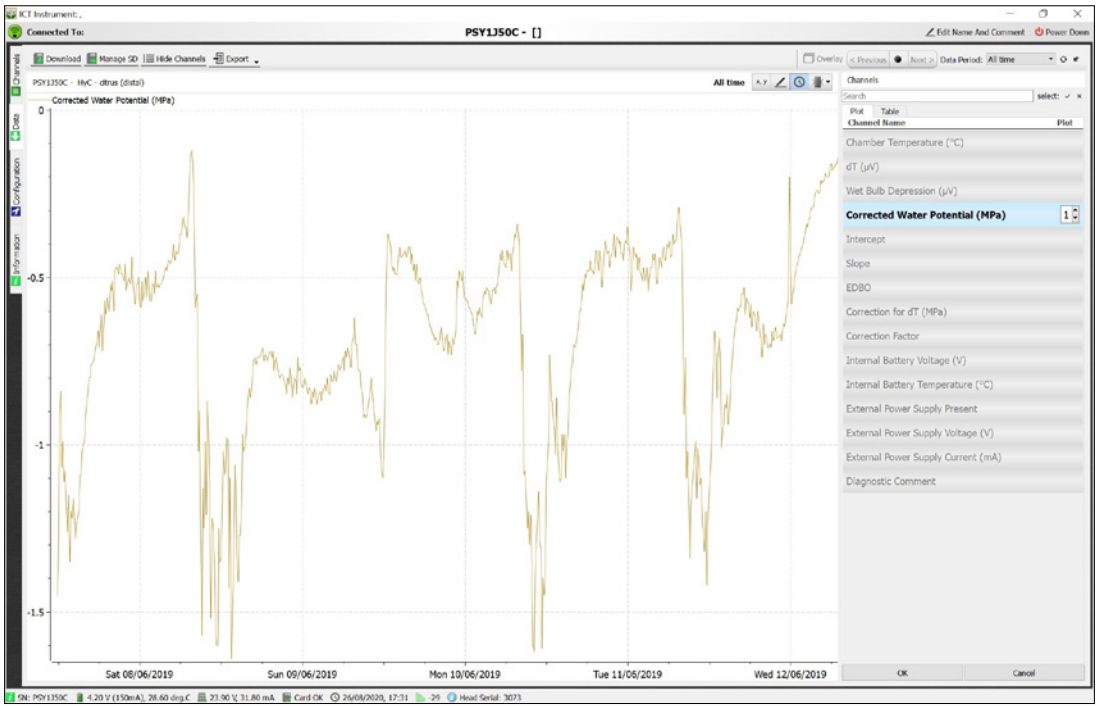


Figure 31. Channel selection for display; in this case Corrected Water Potential (MPa) is only displayed

8. Principle and Theory of Measurement

Total water potential is the measure of the plants' ability to interact with the environment. It consists of four basic components:

$$\psi = Tp - \Pi - T - g$$

Equation 1

Where: ψ = total water potential; Tp = turgor pressure; T = matric potential; g = Gravity.

The psychrometer measures the vapour pressure in the chamber attached to the water conducting tissue of the plant. It relies on the fact that the liquid phase water in the xylem sets up a vapour pressure equilibrium with the chamber. Each measurable parameter in the psychrometric equation is directly obtained by the PSY1. The PSY1 sensor is constructed of chromium plated brass to provide a large heat sink for thermal stability during the psychrometric measurement.

A psychrometric Wet Bulb Depression (WBD; defined as the temperature to which the Thermocouple-C is cooled when water condensed from the chamber air is allowed to evaporate) is measured when a Peltier cooling current condenses water from the atmosphere of the chamber which subsequently evaporates and cools the thermocouple junction. The raw Psychrometric Wet Bulb Depression is corrected for ambient temperature using an empirically derived algorithm. It is then converted to water potential with a calibration slope and intercept derived in the calibration. Finally, a correction for ΔT, or the temperature gradient between the tissue and the measuring junction is applied.

The algorithm to calculate ψ in PSY1 is:

$$\psi = \left(\frac{\left(\frac{WBD}{(C_1 * T_C) + C_2} \right) - CI}{CS} \right) + \left(\frac{\Delta T}{k} * CF_{\Delta T} \right)$$

Equation 2

Where: ψ = Corrected Water Potential; C_1 = Empirically derived temperature correction Constant; C_2 = Empirically derived temperature correction Constant; CI = Calibration Intercept; CS = Calibration Slope; WBD = (Psychrometric) Wet Bulb Depression (µV); TC = Chamber Temperature (°C); ΔT = Measured temperature difference between Thermocouple-C and Thermocouple-S (µV); k = Chromel-Constantan Thermocouple output /°C; $CF_{\Delta T}$ = Correction for ΔT - MPa/°C.

Readers are referred to read Dixon and Tyree (1984) for a detailed explanation of the measurement principle.

9. Advanced user settings and considerations

9.1. Temperature correction constants

The instrument uses default constant values for Temperature Correction (C_1 and C_2 in Equation 2.). These parameters can be modified in the ***Diagnostics*** menu in the ***Configuration*** ribbon (Figure 32.).

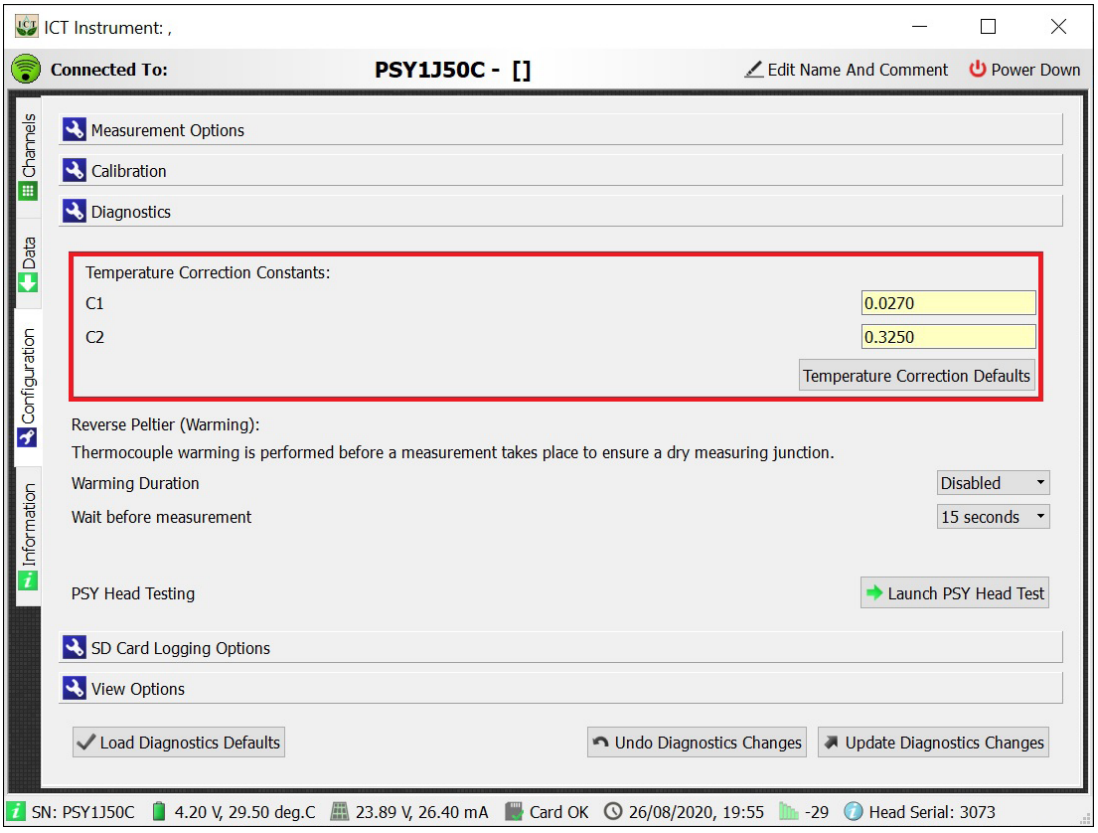


Figure 32. Modifying C_1 and C_2

9.2. Reverse Peltier

The reverse Peltier Current or "warming" is used to dry off any microscopic beads of water that may remain on the thermocouple following a measurement (Figure 33.). You can automate this to provide a user defined temporal interval for warming of Thermocouple-C prior to taking a measurement.

There is also a user adjustable wait time which prevents a measurement from being taken to allow the thermal gradients to dissipate from the chamber before the next measurement.

Reverse Peltier can be implemented under Diagnostics sub-option in the ***Configuration*** ribbon. Select appropriate lengths in the Warming Duration and Wait before measurement drop down menu, respectively.

Ensure this is saved in the device by clicking on ***Update Diagnostics Changes***.

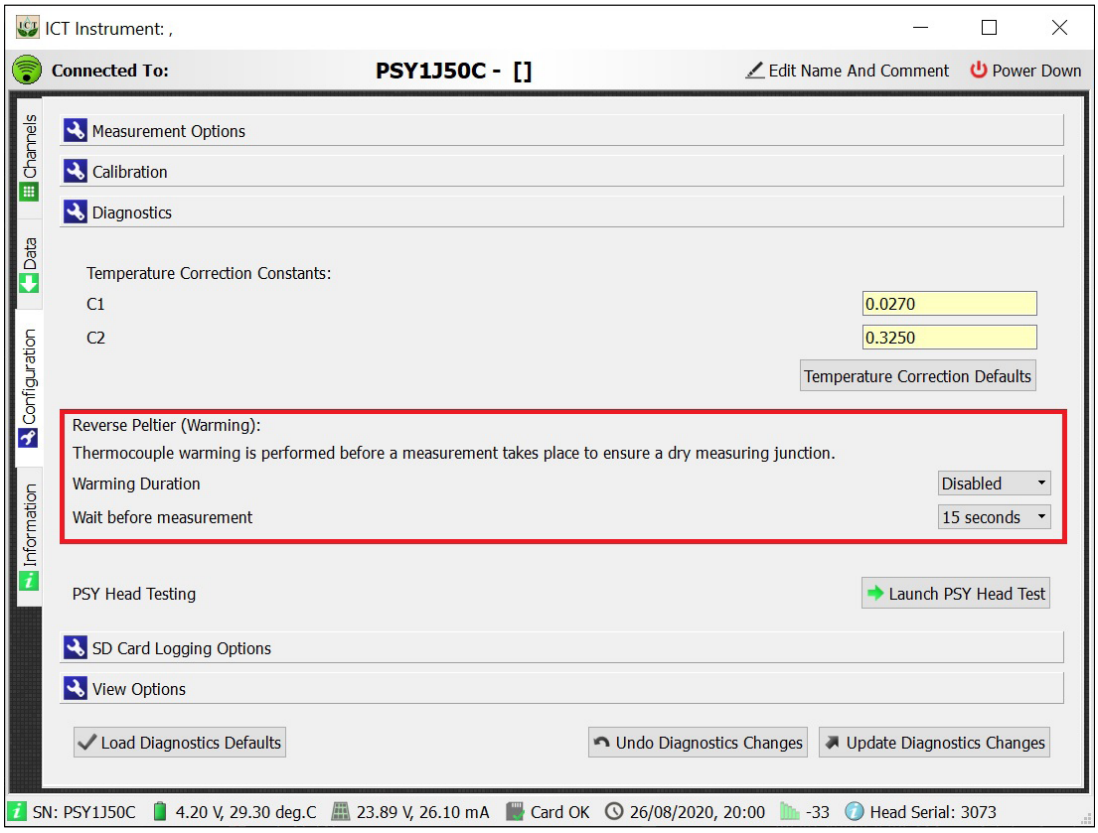


Figure 33. Applying reverse Peltier

9.3. Psychrometric error

Accuracy of the stem psychrometer at the wet end of the spectrum, around zero (0 MPa) is a limitation of the physics of the Psychrometric principle. Where there is little to no drying force, it is difficult to convert liquid water back into vapour phase, and condensation within the chamber is always imminent.

9.4. Equilibration time

The stem psychrometer exhibits rapid vapour pressure equilibration. However, chamber temperature gradients, as a result of handling the instrument or ambient fluctuations, generally require more time to dissipate. Calibration procedures require handling the instrument and usually 15 to 30 minutes are required to re-establish thermal stability under controlled temperature conditions.

Following installation on a sample, significant thermal gradients are usually apparent. Furthermore, disruption of local tissue water potential is likely to have occurred. For these reasons, some equilibration period (30 minutes to 2 hours, depending on the thermal gradients in the psychrometer chamber after installation) should be allowed between the time of installation and the first reading. Using the “Live” mode function you can “watch” in real time the thermal equilibrium occurs as the ΔT and Thermocouple-C values are displayed on screen. This data can also be logged to a .csv data file for post processing and analysis.

9. Advanced user settings and considerations

Provided adequate thermal insulation or temperature control of the installation has been employed, subsequent equilibration to even rapid tissue water potential changes will be dependent only on vapour pressure equilibration. The stem psychrometer exhibits favourable vapour pressure equilibration characteristics due to the absence of significant resistances to vapour exchange (eg. cuticular resistance) between the sample and chamber well.

9.5. Condensation in the chamber

Temperature gradients which induce condensation on the inner chamber walls should be avoided. For every bar (0.1 MPa) of measured water potential a temperature gradient of 0.012°C or more will induce condensation. If that gradient is such that the sample tissue is cooler than the chamber body, then condensation will occur on the sample and most likely be absorbed and redistributed. If, however, the reverse gradient is the case, then condensation will form on the inner chamber walls and introduce an unknown error in measurements.

Generally, this problem can be spotted before it seriously affects interpretation of measurements.

If a gradient favouring condensation on the chamber walls persists (i.e. a negative gradient from Thermocouple-C to Thermocouple-S) then measurements of apparent water potential will tend to rise and approach zero and not vary much between measurements. When it is obvious that this has occurred, remove the instrument, clean and reinstall it.

Under experimental conditions which favour undesirable temperature gradients, such as the cool early hours of the morning before sunrise through until mid-morning, the heater can be used to mitigate these problems (see 6.4.). Exact protocol must remain a subject of trial and error depending on the specific conditions experienced. However, a reasonable approach is to routinely pulse the chamber heater for periods of 15 seconds to 1 minute between measurements immediately following a measurement to allow enough time for the heat introduced to the chamber to dissipate and return to equilibrium before the next measurement. The appropriate protocol is one which maintains conditions such that condensation will not occur on the chamber walls (i.e. the chamber is warmer than the sample). Allow enough time for extraordinary gradients caused by the heater to dissipate before attempting a measurement. See Measurement Protocols for details on the setting of the chamber heating protocol.

10.1. Checking Chamber Thermocouple

Detection and diagnosis of a contaminated thermocouple is easily accomplished with the PSY1 in Manual mode. Place a known water potential sample (1.0 Molal NaCl solution) on a filter paper disk in the calibration lid of the Psychrometer. Set the PSY1 to Manual mode and perform a measurement. If the thermocouple is dirty the resulting measurement will be far from the intended outcome (-4.64 MPa for 1.0 Molal NaCl). This can be seen in ***Live Results*** upon completion of the measurement. The key factor to review also is the Wet Bulb Depression. If water had been able to be condensed on the thermocouple it would have cooled generating a µV output in the range of 16-21 µV for a 1.0 Molal solution.

10. Maintaining the instrument

10.2. Cleaning

The need for cleaning the stem psychrometer may not always be obvious from visual observation even under a 20x dissection microscope. The Stem Psychrometer consists of two very small welded thermocouples using very fine wire only 25 µm in diameter. This makes the sensor very sensitive to measuring water potential but equally as sensitive to dirt and even mild oxidation. It is recommended that before starting any measurements you clean the thermocouples. Cleaning should even be done upon receipt of new instruments from ICT or at the commencement of a field campaign, especially if they have been stored for any length of time.

Depending on the degree of chamber contamination, either cleaning using chloroform or contact cleaner or combination of both may be necessary. In case resin has accumulated in the chamber well, the use of mild acid (e.g. household vinegar or 1M nitric acid) can be used. Mild acid can also be used to remove stains and oxides that accumulate on the thermocouples and chamber well.

10.2.1. Cleaning with Chloroform

Watch this video to clean the PSY1 sensor with Chloroform.

- Invert the psychrometer chamber and flood the chamber well with the organic solvent, (chloroform). This is done using an eye dropper to deliver several drops of Chloroform directly onto the thermocouples. Let stand for between 5 to 10 seconds (longer if severely contaminated) ensuring not to allow the chloroform to evaporate;
- Using a wash bottle of distilled water, immediately rinse away the dissolved contaminants by squirting a steady stream of water into the chamber well, rinsing continuously for approx. 3-5 seconds;
- Use a Kim Wipe or other lint free tissue and place a corner of the tissue in the outer edge of the chamber well. This will wick the bulk of the water up out of the chamber well;
- Blow dry with a controlled stream of compressed air (20 to 30 psi). The drying phase is important as residual water must be removed from the chamber well. Stubborn drops may reside around the copper posts and sustained streams of compressed air may be required to remove all the water;
- A visual check of the position of Thermocouple-S using a 20x dissection microscope is required at this point to ensure correct thermocouple position.

10.2.2. Cleaning with electronic contact cleaner

Watch this video to clean the PSY1 sensor with electronic contact cleaner.

- In an open well-ventilated area (preferably outdoors), invert and hold the psychrometer chamber at 45° to the ground facing away from your body. Shake the can well (following the manufacturer's directions) and spray a steady stream of electronic contact cleaner into the chamber well for approx. 2 seconds ensuring the chamber well is fully saturated. Repeat this process at least twice leaving the chamber well fully saturated;
- Then, using a wash bottle of distilled water, immediately rinse away the dissolved contaminants by squirting a steady stream of water into the chamber well, rinsing continuously for approx. 3-5 seconds;
- Next use a Kim Wipe or other such lint free tissue and place a corner of the tissue in the outer edge of the chamber well. This will wick the bulk of the water up out of the chamber well;

10. Maintaining the instrument

- Blow dry with a controlled stream of compressed air (20 to 30 psi). The drying phase is important as residual water must be removed from the chamber well. Stubborn drops may reside around the copper posts and sustained streams of compressed air may be required to remove all the water;
- A visual check of the position of Thermocouple-S using a 20x dissection microscope is required at this point to ensure correct thermocouple position.

10.3. Adjusting thermocouples

Please watch this video on Adjusting Thermocouples.

10.4. Storing the unit

It is not always practical to clean the stem psychrometers after de-installation whilst in the field. However, this does not mean that they can be left until you next need them for a future experiment. They must be cleaned upon return to the lab and prior to storage.

Oxidation of the copper posts within the chamber of the psychrometer may affect the measured water potential. If the chamber is not cleaned and the copper posts are corroded, the psychrometer may require factory repair. Corrosion is indicated by the green colouration on the copper posts. It may be necessary to make the observation of this corrosion using a 20X microscope to be sure that the electrodes are not corroded and are suitable for use.

11. Procedure for calibrating PSY1

The calibration routine is designed to be semi-automated and implemented using the Combined Instrument Software in the Psychrometer GUI. This includes making measurements with calibration solutions on paper disk loaded to Lid/Calibration Disk Holder, to plotting the data, generating a slope and intercept and storing the calibration in the firmware of the PSY1 Instrument for real time data processing. This video provides an overview of the process. The process requires calibration solution (see Appendix 12.3.), filter paper dots and calibration chamber (see Appendix 12.4.).

To initiate the calibration, click on ***Calibration*** in the ***Configuration*** ribbon (Figure 34.).

11. Procedure for calibrating PSY1

Ensure the ***Psychrometer Head Serial Number*** is correct. Then click ***Launch PSY Calibration***.

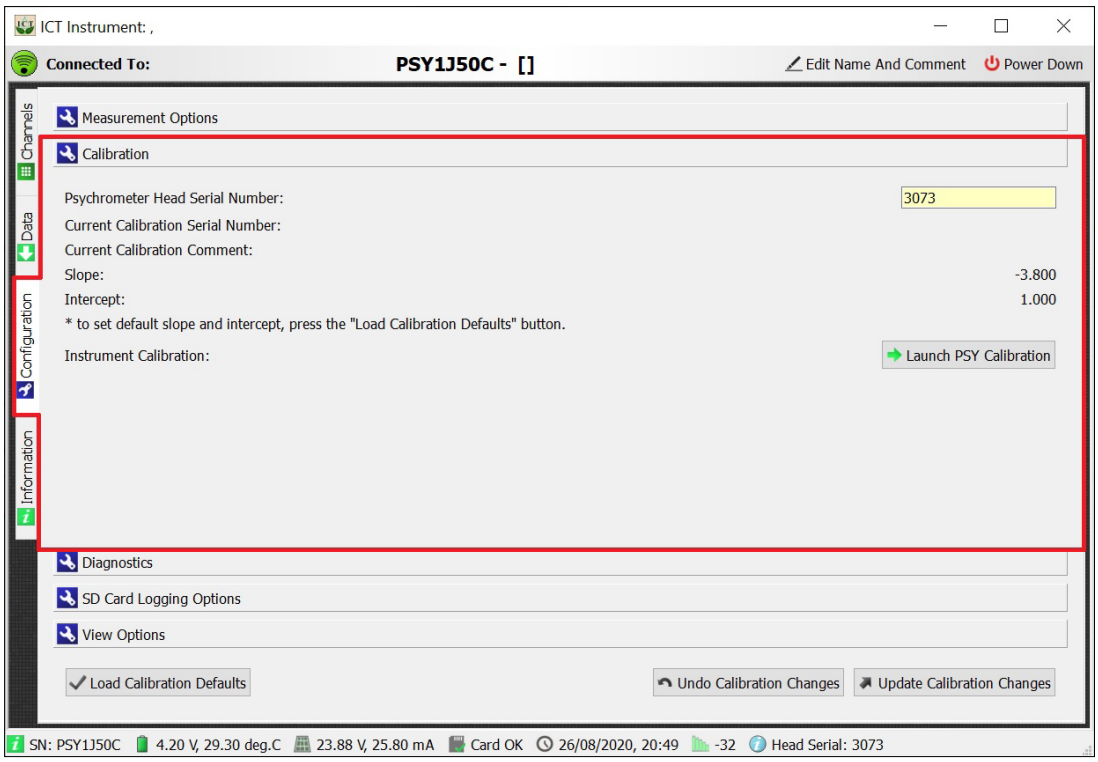


Figure 34. Initiating Calibration

The calibration screen will appear (Figure 35.) and ready to commence a new calibration.

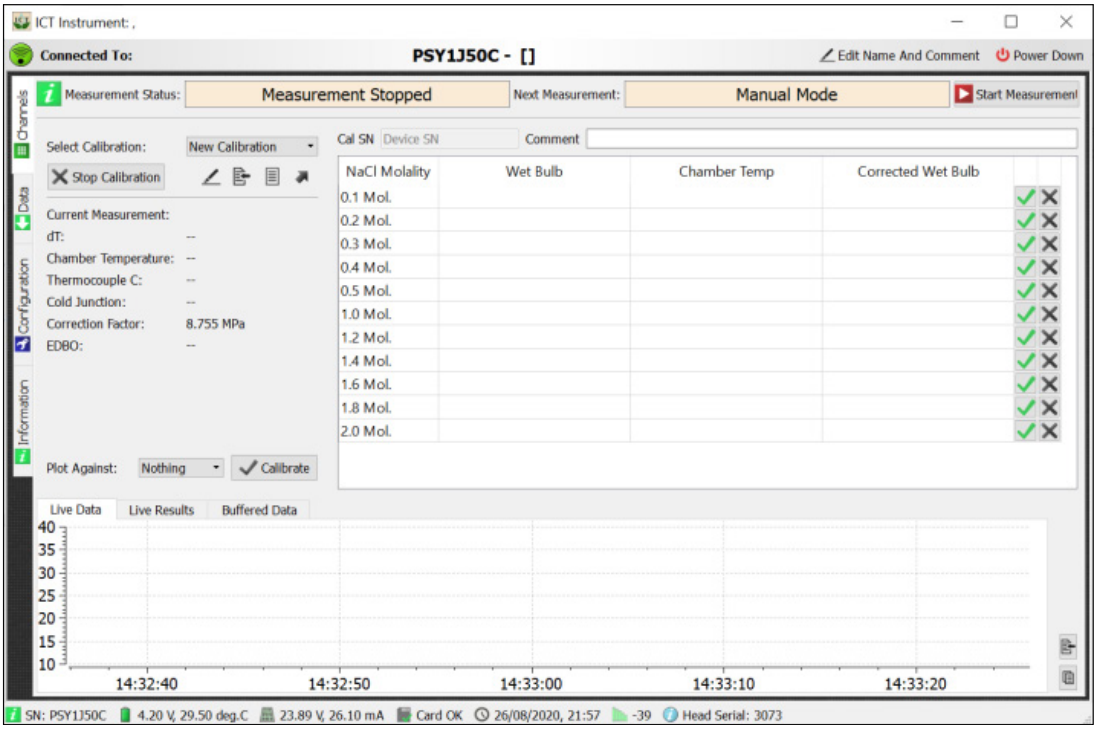


Figure 35. New calibration screen

11. Procedure for calibrating PSY1

Place a known calibration solution into the Calibration disk holder. Do this by dipping a filter paper dot into the calibration solution (starting with for instance 0.1 Molal). Apply a thin layer of vacuum grease (Dow Corning) around the chamber well. Close the chamber and twist to ensure a vapour seal. Secure with rubber band. Place the whole sensor inside the calibration chamber. If you are calibrating other sensors, repeat this process using the same NaCl solution.

Ensure a good thermal and vapour pressure equilibration (between 30-60 minutes). In PSY GUI calibration screen, select the appropriate Molality by clicking . Once clicked, the row will turn yellow. Click ***Start Measurement***. Once completed, the software will automatically populate the row with values (Figure 36.).

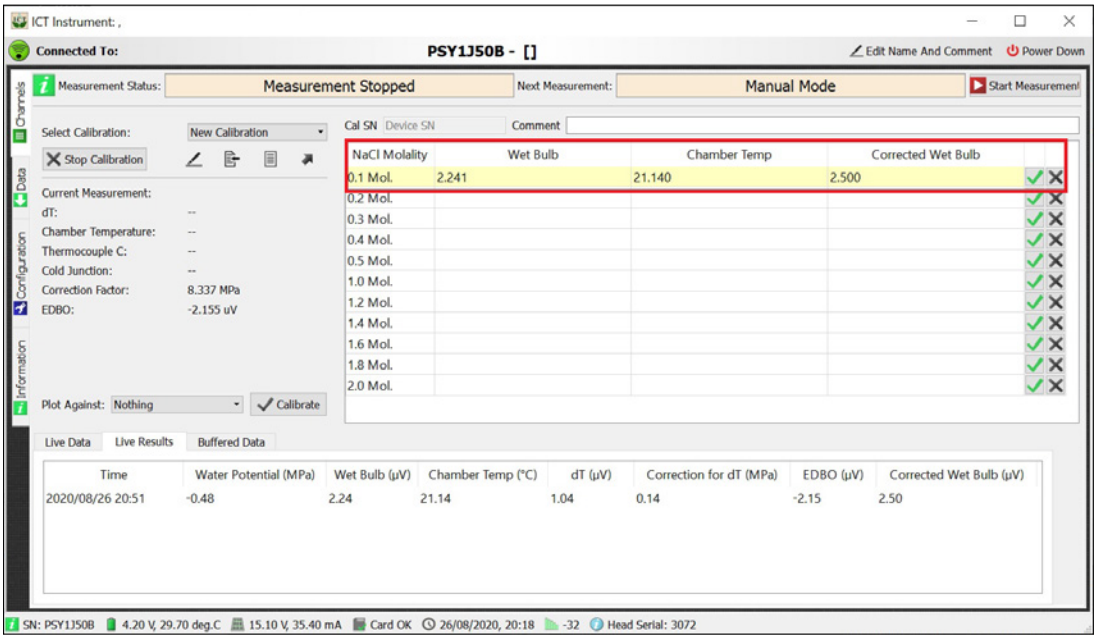


Figure 36. Calibration at 0.1 M NaCl highlighted and populated

Continue doing the above procedure until at least 6 calibration points. Ensure good thermal and vapour pressure equilibration is met at each point. Click (***Save current calibration to file***) button; follow prompt.

Once done for all points, click ***Calibrate***. A calibration curve will be generated (Figure 37.).

11. Procedure for calibrating PSY1

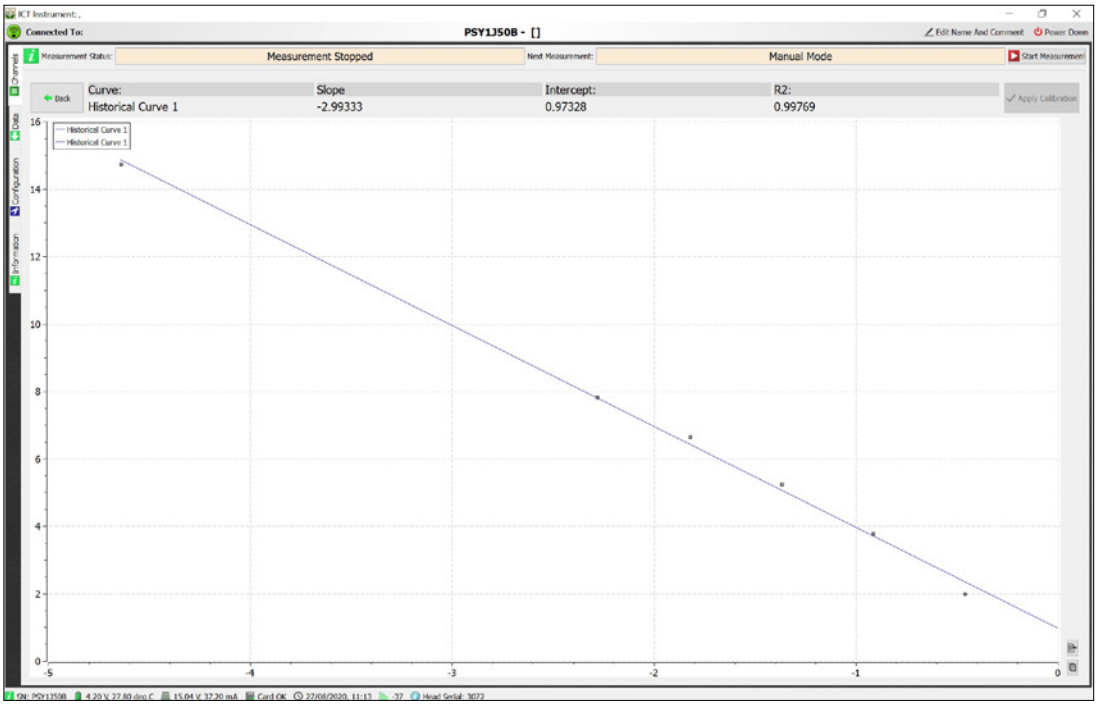


Figure 37. Generated calibration curve

Click Apply Calibration, click OK in the prompt. At this point, the ***Slope*** and ***Intercept*** are updated. Finally click ***Update Calibration Changes*** (Figure 38.).

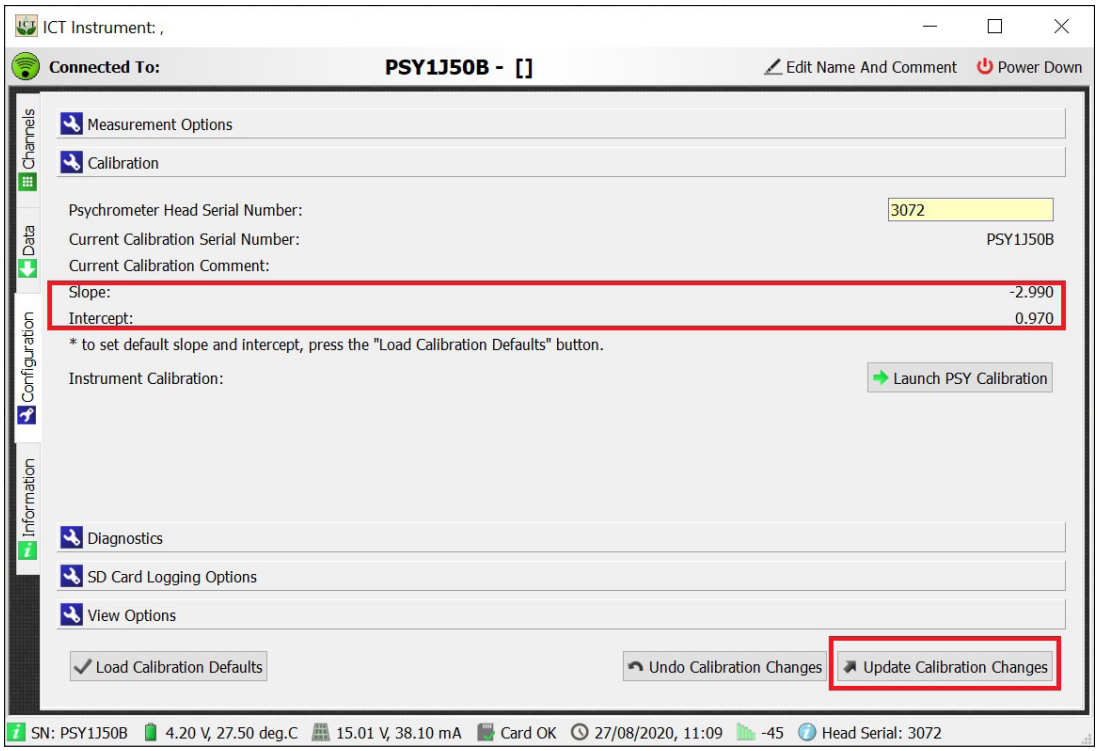


Figure 38. Updated Slope and Intercept values.

12. Appendices

12.1. Current measurement outcomes

In this section, screen shots (i.e. example plots) of ***Live Data***, ***Live Results*** and ***Buffered Data*** are provided. The plots generated by ***Live Data*** and ***Buffered Data*** can be exported by clicking on ***Save*** to file button. Alternatively, the plot can also placed on the clipboard by clicking on ***Copy to Clipboard*** button.

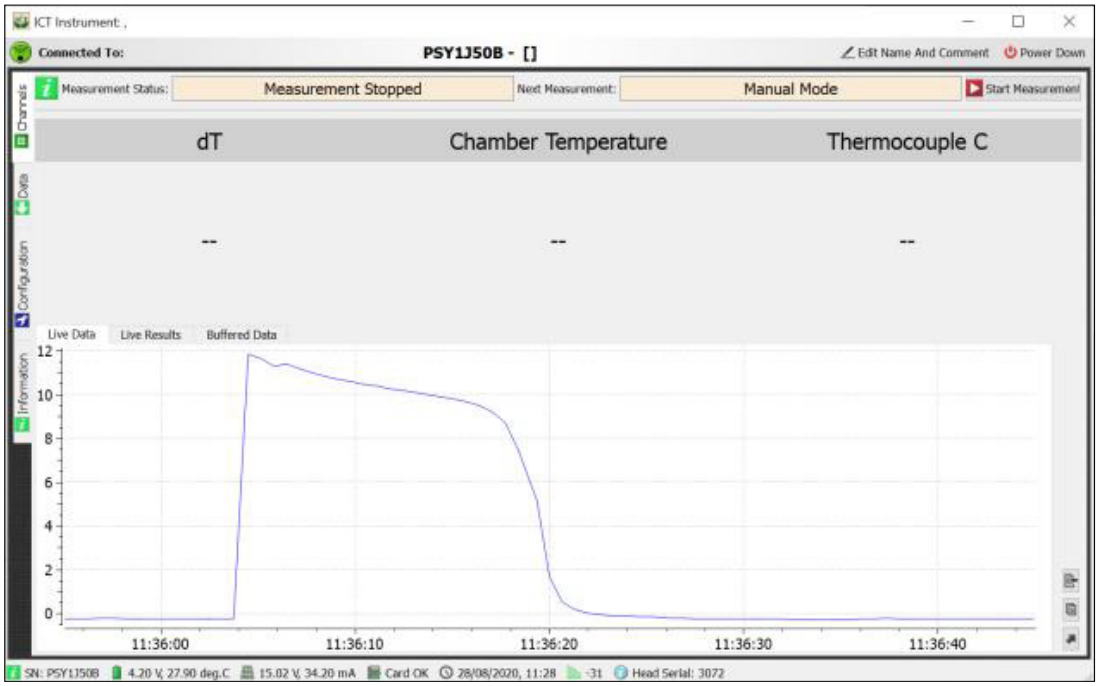


Figure 39. Live Data

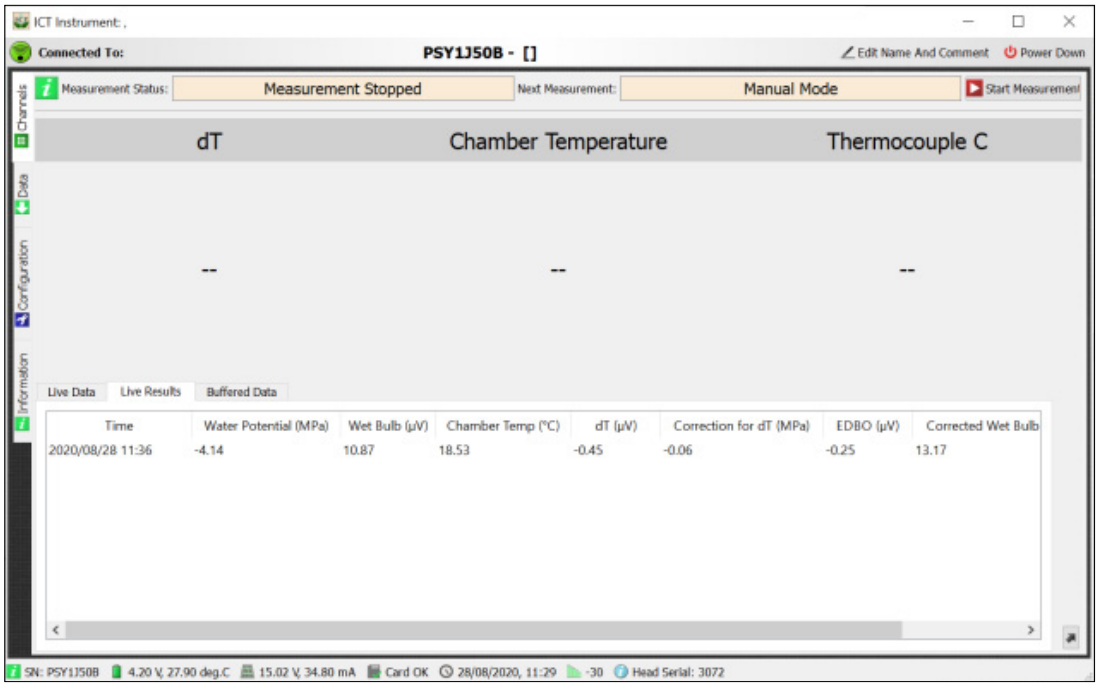


Figure 40. Live Results

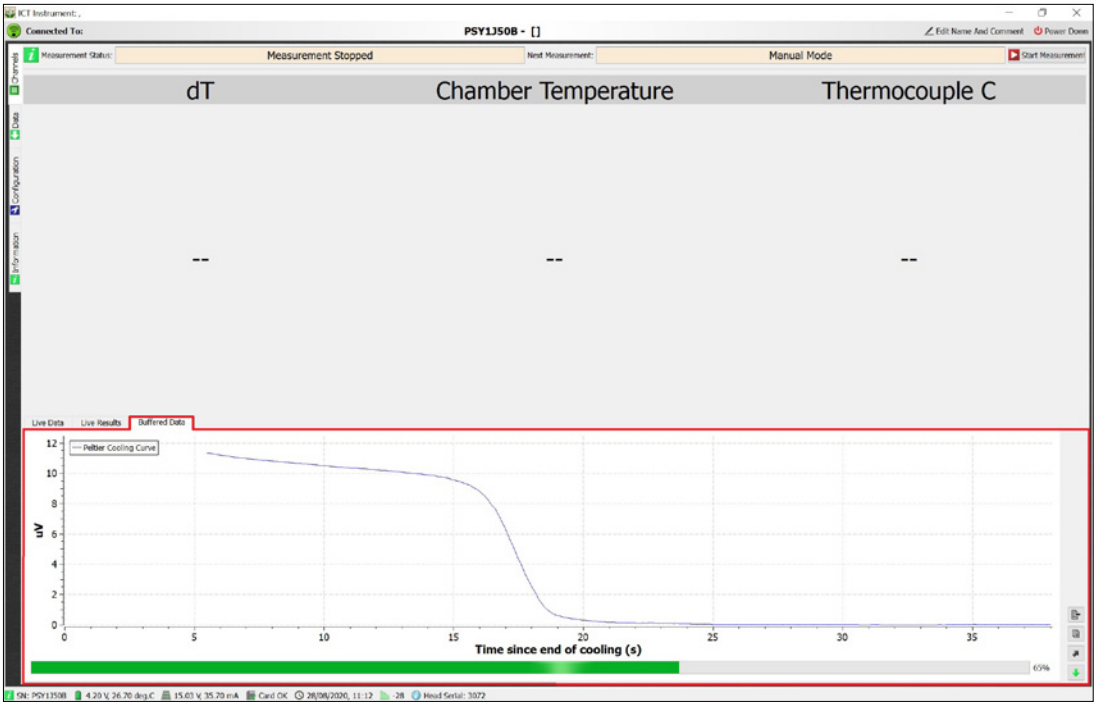


Figure 41. Buffered Data

12.2. Alternative powering solution with solar panels and deep cycle batteries

Provided access to both solar panel and external batteries are available, the use of controller is recommended. ICT International supplies part # LIGHTCHARGE. Please visit this link to learn more.

12.3. Preparing NaCl solutions for use in calibrating PSY1 sensor

Calibration over a range of water potentials is accomplished using sodium chloride (NaCl) solutions (the molecular weight of sodium chloride = 58.4428 g/mole).

Table 1. (right) represents a suitable range of molal concentrations (i.e., mass of salt per unit mass of water) of salt solutions with the corresponding water potential equivalent at 25°C.

You can make these solutions yourself using sodium chloride and distilled water by carefully measuring the NaCl and water exactly on a minimum 4 decimal balance. Alternatively, premixed calibration solutions can be purchased directly from ICT International or their distributor in your country.

Molality	Mass of NaCL (g)	Mass of Water (g)	Water Potential (MPa)
0.1	0.2992	50	- 0.462
0.2	0.5844	50	- 0.915
0.3	0.8766	50	- 1.368
0.4	1.1688	50	- 1.823
0.5	1.4610	50	- 2.281
1.0	2.9221	50	- 4.640

Table 1. Composition if g of NaCl and water at various molal concentrations and corresponding water potential.

12.4. Calibration Chamber

Here is a simple and effective means to achieve stable temperature control:

You will require a good quality circulating water bath, preferably with proportional control of water temperature. This eliminates the typical “sawtooth” temperature control of less sophisticated baths. Circulate the water through a flexible copper tube (1-2 cm diameter and 2-3 m long) which is then coiled and placed inside a box close to the inside edges. Construct the box of dense styrofoam (eg. 5 cm. thickness, Styrofoam SM) and line all inside surfaces with a reflective foil (eg. aluminium foil).

The dimensions of the box should accommodate the number of psychrometers you intend to calibrate as well as one or two small electric fans to facilitate air mixing. Internal dimensions of approximately 50x50x50 cm are appropriate. Suspend a light plastic grid in the centre of the box to serve as a platform for the psychrometers. This allows air movement around the instruments and eliminates temperature gradients from conductive surfaces. Normal equilibration times for salt solutions are quite brief but it is safest to allow between 30 to 60 minutes for each solution to equilibrate depending upon how intensively the psychrometer was handled while loading the calibration solution.

12.5. Recommended Reading

Dixon M.A., & Tyree M.T. 1984. A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. Plant Cell & Environment 7: 693-697.

Dixon M.A., & Johnson R.W. 1993. Interpretation of the dynamics of plant water potential. In: Water Transport in Plants under Climatic Stress. Edited by M. Borghetti,

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Shackle, K. 1984. Theoretical and experimental errors for in-situ measurements of plant water potential. Journal of Plant Physiology 75: 766-772.

Tyree, Melvin T, Fiscus, Edwin L, Wullschleger S.D & Dixon M.A 1986. Detection of Xylem Cavitation in Corn under Field Conditions. Plant Physiology 82:597-599

Johnson, R.W, Dixon, M.A, and Lee, D.R. 1992. Water relations of the Tomato during fruit growth. Plant Cell & Environment 15: 947-953.

Edwards D. R, and Dixon, M.A. 1995. Mechanisms of drought response in *Thuja occidentalis* L. I. Water stress conditioning and osmotic adjustment. Tree Physiology 15:121-127

Edwards D. R, and Dixon, M.A. 1995. Mechanisms of drought response in *Thuja occidentalis* L. II. Post-conditioning water stress and stress relief. Tree Physiology 15: 129-133

Chamberlain, C.P, Stasiak M.A. and Dixon M.A. 2003. Response of Plant Water Status to Reduced Atmospheric Pressure. SAE International 2003-01-2677

Robinson, S, Dixon M. A, and Zheng Y. 2007. Vascular blockage in cut roses in a suspension of Pseudomonas Fluorescens. Journal of Horticultural Science & Biotechnology 82 (5) 808–814

12. Appendices

FISHER, Rosie, A., WILLIAMS, Matthew, LOBO DO VALE, Raquel, LOLA DA COSTA, Antonio & MEIR, Patrick 2006. Evidence from Amazonian forests is consistent with isohydric control of leaf water potential. Plant Cell & Environment 29:151-165

Lang, A.R.G, Osmotic Coefficients and Water Potentials of Sodium Chloride Solutions from 0 to 40°C 1967. Australian Journal of Chemistry, 20, 2017-23.

Warranty & Service Terms

What is Covered

All products manufactured by ICT International are warranted to be free from defects in materials and craftsmanship for a period of one (1) years from the date of shipment from our factory. To be considered for warranty coverage an item must be evaluated either at our factory or by an authorized distributor.

What is Not Covered

The customer is responsible for all costs associated with the removal, re-installation, and shipping of suspected warranty items to our factory. The warranty does not cover equipment that has been damaged due to the following conditions:

1. Improper use or abuse.
2. Operation of the instrument outside of its specified operating range.
3. Natural occurrences such as lightning, fire etc.
4. Unauthorized modification.
5. Improper or unauthorized repair.

Who is Covered

This warranty covers the original purchaser of the product or other party who may own it during the warranty period.

What We Will Do

At no charge we will:

1. Either repair or replace (at our discretion) the item under warranty.
2. Ship the item back to the customer by the carrier of our choice. Different or expedited shipping methods will be at the customer's expense.

How To Return An Item

1. Please do not send any products back to ICT International until you have filled out an online RMA (Return Merchandise Authorization) and have been advised to return the item by our service team. The form can be found at http://www.ictinternational.com/support/rma-form/. We will use your RMA number for tracking of the service item.

2. Send all RMA sensors and meters back in the following condition: Clean the instruments exterior. Do not modify the sensors or wires, including splicing, cutting wire leads etc.

3. Please write the RMA number on the outside of the shipping container.

4. Return the item with freight pre-paid and fully insured to our factory address shown below. We are not responsible for any costs associated with the transportation of products across international borders.

5. Upon receipt, ICT International will determine the cause of failure. If the product is found to be defective in terms of operation to the published specifications due to a failure of product materials or craftsmanship, ICT International will repair or replace the items free of charge.

Repairs / Replacement

If it is determined that your product is not covered under warranty, you will be informed and given an estimated repair/replacement cost. The available remedy of defects under this warranty is for the repair or replacement of the original product, and ICT International is not responsible for any direct, indirect, incidental, or consequential damages, including but not limited to loss of income, loss of revenue, loss of profit, loss of wages, loss of time, loss of sales, accruement of debts or expenses, injury to personal property, or injury to any person or any other type of damage or loss.

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