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TITLE:

PARbars: Cheap, Easy to Build Ceptometers for Continuous Measurement of Light Interception in Plant Canopies

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KEYWORDS:
Canopy, ceptometer, photosynthetically active radiation, plant area index, phenotyping, transmittance

SHORT ABSTRACT:
Here, we present detailed instructions on how to build and calibrate research quality ceptometers (light sensors that integrate light intensity across many sensors arrayed linearly along a horizontal bar).

LONG ABSTRACT:
Ceptometry is a technique used to measure the transmittance of photosynthetically active radiation through a plant canopy using multiple light sensors connected in parallel on a long bar. Ceptometry is often used to infer properties of canopy structure and light interception, notably leaf area index (LAI) and effective plant area index (PAI_{eff}). Due to the high cost of commercially available ceptometers, the number of measurements that can be taken is often limited in space and time. This limits the usefulness of ceptometry for studying genetic variability in light interception, and precludes thorough analysis of, and correction for, biases that can skew measurements depending on the time of day. We developed continuously logging ceptometers (called PARbars) that can be produced for USD $75 each and yield high quality data comparable to commercially available alternatives. Here we provide detailed instruction on how to build and calibrate PARbars, how to deploy them in the field and how to estimate PAI from collected transmittance data. We provide representative results from wheat canopies and discuss further considerations that should be made when using PARbars.

INTRODUCTION:

Ceptometers (linear arrays of light sensors) are used to measure the proportion of photosynthetically active radiation (PAR) intercepted by plant canopies. Ceptometers are used widely for agricultural crop research due to the relatively straightforward nature of measurements and simplicity of data interpretation. The basic principle of ceptometry is that the transmittance of light to the base of a plant canopy (τ) is dependent on the projected area of light absorbing materials above. Measurements of PAR above and below the canopy can, therefore, be used to estimate canopy traits such as leaf area index (LAI) and effective plant area index (PAI_{eff}) (which includes stems, culms and reproductive structures in addition to leaves)[1-3]. Reliability of PAI_{eff} estimates inferred from τ is improved by modeling the effects of the beam fraction of incoming PAR (f_b), the leaf absorptance (a) and the effective canopy extinction coefficient (K); K, in turn, depends on both the solar zenith angle (θ) and the leaf angle distribution (χ)[1,4-6]. It is a common practice to correct for these effects. However, there are other biases that have not received due consideration in the past due to methodological and cost limitations.

We recently identified significant time-dependent bias in instantaneous ceptometry measurements of row crops, such as wheat and barley[7]. This bias is caused by an interaction between row planting orientation and solar zenith angle. To overcome this bias, continuously logging ceptometers can be mounted in the field to monitor diurnal cycles of canopy light interception and then daily averages of τ and PAI_{eff} can be calculated. However, continuous measurements are often infeasible due to the prohibitively high cost of commercially available ceptometers – often several thousand US dollars for a single instrument – and the requirement for measurements of many field plots. The latter is particularly evident in the -omics era where many hundreds of genotypes are required for genomic analyses, such as genome wide association studies (GWAS) and genomic selection (GS) (for review see Huang & Han, 2014[8]). We recognized that there was a need for cost-effective ceptometers that could be produced in large numbers and be used for continuous measurements across many genotypes.

As a solution, we designed easy-to-build, high-accuracy ceptometers (PARbars) at a cost of USD $75 per unit and requiring approximately one hour of labor to construct. PARbars are built using 50 photodiodes that are sensitive only in the PAR waveband (wavelengths 390 – 700 nm), with very little sensitivity outside this range, obviating the use of costly filters. The photodiodes are connected in parallel across a 1 m length to produce an integrated differential voltage signal that can be recorded with a datalogger. The circuitry is encased in epoxy for waterproofing and the sensors operate over a large temperature range (-40 to +80 °C), allowing the PARbars to be deployed in the field for extended periods of time. With the exception of the photodiodes and a low-temperature-coefficient resistor, all parts required to build a PARbar can be purchased from a hardware store. A full list of required parts and tools is provided in the **Table of Materials**. Here we present detailed instructions on how to build and use PARbars for the estimation of PAI_{eff} and present representative results from wheat canopies.

PROTOCOL:

1. Build and calibrate the PARbars

1.1) Gather all parts and tools required for the assembly in a clean workspace.

1.2) Drill a 4 mm diameter hole 20 mm from each end of a white acrylic diffuser bar (1200 mm length x 30 mm width x 4.5 mm thickness). Drill and tap threaded holes 20 mm from each end of a section of aluminum U-bar to secure diffuser. Drill and tap threaded holes to suit mounting hardware (e.g., a tripod mounting plate).

1.3) Obtain a 1.25 m length of bare copper wire (1.25 mm diameter). If the wire came on a roll, then straighten it by securing one end into a vice or clamp and the other end into the grips of a hand drill, and then turning on the drill at a low speed (100-200 rpm). Repeat with a second 1.25 m length of bare copper wire.

1.4) Mark the intended locations of the photodiodes along the edge of the diffuser using a fine-tip permanent marker, beginning with the first photodiode position at 13.5 cm from one end of the diffuser and the other positions located every 2 cm between the first diode and the far end of the diffuser.

1.4.1) Mark the position of the first copper wire on the diffuser by centering one photodiode on the diffuser bar with its electrical connection tabs pointing towards the sides of the bar, placing the wire underneath one of the tabs, and marking the wire's location.

1.4.2) Repeat the preceding step to mark the wire's position at the center and the opposite end of the bar.

1.5) Use cyanoacrylate glue to glue the first straightened copper wire to the diffuser, using the locations marked in the preceding step to align the wire.

1.5.1) Use cyanoacrylate glue to glue 50 photodiodes face-down along the diffuser at 20 mm intervals (as marked in the preceding step), ensuring that they are in the center of the diffuser and that all are arranged all in the same orientation such that the large tab sits on the copper wire, and the small tab sits opposite.

1.5.2) Place the second copper wire such that it sits underneath each of the smaller tabs of the photodiodes, and then glue the wire to the diffuser with cyanoacrylate glue.

1.6) Wet both tabs of one photodiode, as well as the adjacent and underlying wires, with flux using a solder flux pen. Solder each tab of the diode to the underlying copper wires using a fine tipped soldering iron at a temperature of approximately 350-400 ^{o}C. Test the solder connections by shining a light onto the photodiode from the opposite surface (through the diffuser bar) and checking for a voltage signal across the wires using a multimeter. Repeat this step for all 50 photodiodes.

NOTE: Step 1.7 is optional (if the resistor is not soldered into the PARbar, it can instead later be connected in parallel with the PARbar signal inputs on the datalogger).

1.7) Solder a 1.5 Ω low temperature coefficient precision resistor in parallel across the copper wires.

1.8) Solder the male end of a waterproof DC connector to the ends of the copper wires (the same ends to which the resistor was soldered, if you followed optional step 1.7) and then seal the connections using glue lined heat shrink tubing.

1.9) Create a continuous silicone barrier around the circuity on the diffuser to form a fluid-tight well, by applying a bead of silicone sealant to the surface of the diffuser, near the edge. Inspect the bead closely to ensure that no air gaps remain between the silicone and the diffuser bar, as gaps will permit epoxy to leak out. Once the sealant has cured, fill the well with epoxy resin.

1.10) When the epoxy resin has hardened (overnight), remove the silicone sealant using a razor blade. Bolt the diffuser to the pre-threaded aluminum U-bar using M4 bolts.

1.11) Use masking tape to secure the diffuser to the aluminum along its whole length, and then fill the void inside the ceptometer with polyurethane foam filler. Once the foam filler has set (overnight), remove the masking tape.

1.12) Solder the female end of the DC connector to a length of two-conductor cable and seal the connections with glue lined heat shrink.

1.13) To calibrate the PARbar against a quantum sensor,

1.13.1) Connect both sensors to a datalogger or voltmeter capable of measuring a differential voltage output (connect a 1.5 Ω low temperature-coefficient precision resistor in parallel with the PARbar if a resistor was not integrated into the design in step 1.7),

1.13.2) Set them outside in full sun on a level plane (level with a spirit level or spirit bubble), record the outputs of both sensors across a period during which solar radiation varies widely, such as a full diurnal cycle, and determine the calibration factor for the PARbar as the slope of a linear regression of PAR reported from the quantum sensor (as the dependent variable) vs. raw voltage output (as the independent variable).

2. Install in the field

2.1) To infer effective plant area index (PAI_{eff}), install one PARbar above the canopy (ensuring that it is not shaded by any light-absorbing elements within the canopy) and another below all light-absorbing elements whose absorptance you wish to measure (typically, below the lowest leaves), with both PARbars aligned at a 45° angle to planting rows. Ensure the upper PARbar is positioned so as not to shade the lower PARbar. Level the PARbars using a spirit level or bubble level.

2.2) Connect the PARbars to a datalogger or voltmeter using cables made in step 1.11. If a 1.5 Ω low temperature-coefficient precision resistor was not integrated into the PARbar circuit during construction (step 1.7), then connect such a resistor in parallel with each PARbar at this stage.

2.3) Convert differential voltage output to PAR using the calibration factor determined for each PARbar in step 1.13.

3. Calculate the effective plant area index (PAI_{eff})

3.1) Calculate PAI_{eff} for each pair of above- and below-canopy PAR measurements using the following equations[6]:

(1) $$\mathrm{PAI} = \frac{\left(1-{}^{1}/_{2K}\right) f_b - 1}{A(1-0.47 f_b)} \ln\tau ,$$

where $A = 0.283 + 0.0785a - 0.159a^2$ (in which a is leaf absorptance), τ is the ratio of below- to above-canopy PAR, and K and f_b are modeled by Equation 2[4] and Equation 3[9], respectively:

(2) $$K = \frac{(\chi^2 + \tan^2\theta)^{0.5}}{\chi + 1.744(\chi + 1.182)^{-0.733}} ,$$

where χ is a dimensionless parameter describing leaf angle distribution, θ is the solar zenith angle, and

(3) $$f_b = 1.395 + r\left(-14.43 + r\big(48.57 + r(-59.024 + 24.835 \cdot r)\big)\right) ,$$

where r is PAR above the canopy (PAR_{above}) as a fraction of its maximum possible value ($PAR_{above,max} = 2550 \cdot \cos\theta$); i.e. $r = PAR_{above}/PAR_{above,max}$. Consult the literature for values of a and χ appropriate to your study species (we assumed $a = 0.9$ and $\chi = 0.96$[10] for the wheat canopies used for trial measurements presented here).

NOTE: A sample R script is provided as a supplementary file to assist users in developing code for automated processing of large datasets.

REPRESENTATIVE RESULTS:

A schematic for the PARbar build is shown in **Figure 1**. A representative calibration curve for a PARbar is shown in **Figure 2**. The differential voltage output of a PARbar is linearly proportional to the PAR output from a quantum sensor, with $R^2 = 0.9998$. PARbars were deployed in wheat canopies and logged every 20 s across the development of the plants. A typical diurnal time course of the canopy light environment collected using a PARbar on a clear sunny day is shown in **Figure 3** (raw transmittance data and corrected PAI_{eff} are shown for comparison). **Figures 3b**

and 3c demonstrate the bias that could be introduced by taking instantaneous ceptometry measurements at various times of day (as per Salter *et al*. 2018[7]). The wheat plots used for the collection of this data had a row planting orientation due north-south with the transmission of light to the lower canopy peaking at 12:30 (**Figure 3b**). If an instantaneous measurement were to be taken at this point, PAI_{eff} would be underestimated, whereas if it were taken in the morning or afternoon it may be overestimated. The weatherproof PARbars can also be deployed in the field for long time periods; **Figure 4** demonstrates how PARbars could be used to monitor how canopy light environment changes as plants develop.

Figure 1. Schematics for the PARbar build. (a) Location and arrangement of the waterproof connector and the internal shunt resistor; (b) arrangement and spacing of the photodiodes; (c) drilling locations on the acrylic diffuser bar; (d) drilling locations on the aluminum U-bar; and (e) electronic circuit diagram of a PARbar.

Figure 2. Representative PARbar calibration curve. The relationship between the differential voltage output of a PARbar (mV) and the photosynthetic photon flux density or PAR (μmol m^{-2} s^{-1}) from a quantum sensor. Each point represents a single pair of measurements from the PARbar and quantum sensor, recorded once every 20 seconds over a period of 4 hours for one day.

Figure 3. Representative daily timecourse of PARbar output. Data collected on a clear day using PARbars in wheat canopies at anthesis in Canberra, Australia (-35°12'00.1008", 149°05'17.0988"). (a) PAR measured above the canopy (μmol m^{-2} s^{-1}), (b) uncorrected transmittance (the ratio of PAR_{above}/PAR_{below}) (unitless), and (c) the effective plant area index (PAI_{eff}, m^2 m^{-2}), calculated from Equation 1. Data points shown in (b) and (c) are means (n = 30), solid lines are LOESS local regressions fitted in R (α = 0.5), shaded areas are standard errors of the fit and the dashed horizontal lines represent the daily means. The shaded area between the dotted lines is the time window (1100 – 1400h) recommended for instantaneous ceptometer measurements in wheat by CIMMYT[11].

Figure 4. Representative data collected across a growing season. PARbar data collected from early tillering to anthesis in wheat canopies in Canberra, Australia (-35°12'00.1008", 149°05'17.0988"). (a) Uncorrected transmittance data (unitless), and (b) effective plant area index (PAI_{eff}, m^2 m^{-2}) calculated from Equation 1. Data points shown represent daily means for the period 1000 – 1400h (n = 30). Solid lines are LOESS local regressions fitted in R (α = 0.75), shaded areas are standard errors of the fit. Raw data was not included in further analysis if PAR_{above} was < 1500 µmol m^{-2} s^{-1} and if PAR_{below}/PAR_{above} was > 1.

DISCUSSION:

Successful implementation of the protocol outlined here for building ceptometers (PARbars) depends most sensitively on two steps: 1.5 (gluing photodiodes in place) and 1.6 (soldering photodiodes to the copper wire). Step 1.5 is prone to error by aligning the photodiodes incorrectly with respect to their intrinsic polarity. For the photodiodes that we used, and which we recommend as essential specific items, the polarity is identified by virtue of the two electrical connector tabs on the diode having clearly different sizes. Thus, before applying cyanoacrylate glue and soldering the photodiodes in place, it is strongly advised to double-check that all diodes are placed with the large connector tabs facing in one direction and the small tabs facing in the other direction. Step 1.6 is prone to failure due to poor soldering technique and formation of a cold soldered junction. This can be avoided by applying thin solder flux using a flux pen immediately before soldering and ensuring that both the wire and the photodiode tab are heated with the solder tip (at approximately 350-400 ^{o}C) before soldering itself is applied to the junction. Problems with electrical connections in a PARbar typically manifest in the form of a calibration slope distinctly different from those of other PARbars. Such problems can be caught early by testing each electrical connection during construction (as described in Step 1.6), and again after all connections have been soldered, but before they have been encased in epoxy (Step 1.9). A third potential source of error arises from the failure to use a low temperature-coefficient precision resistor, whose resistance is insensitive to temperature; using an ordinary resistor will cause the error as the resistance, and hence the voltage output per unit of light absorbed by the diodes, changes with ambient temperature. The final major source of error is not unique to PARbars, but applies to all ceptometry measurements: namely, the inference of effective plant area index or leaf area index from light capture depends on features of canopy structure (notably mean leaf absorptance and leaf angle distribution; a and χ in Eqns 1 and 2) that may vary during plant development and between genotypes.

There are two main areas in which the protocol described here could be modified or adapted. First, the PARbars that we present here were designed specifically for use in row crops, such as wheat and barley, but the design could easily be modified for other applications. For example, a shunt resistor with larger resistance could be used to enhance gain (mV output per unit PAR) at lower PAR ranges. For versatility, a low-temperature coefficient precision potentiometer (variable resistor) could be used to modify the PARbar's sensitivity range as needed or to make small adjustments to gain so that each of many PARbars have identical calibration slopes. Second, the photodiodes could also be used individually as quantum sensors, allowing the user to capture spatial as well as temporal variation within individual canopies for a much lower cost than possible using commercially available quantum sensors. This could be particularly valuable given the growing interest in dynamic photosynthesis under fluctuating light conditions[12]. Third, although we used a conventional (and expensive) datalogger for the data presented in this study, there is scope for dataloggers to instead be built using off-the-shelf componentry, enabling the creation of a combined ceptometry and datalogger system on a limited budget. The popularity of so-called maker platforms, such as Arduino and Raspberry Pi, offer great promise in this area; we suggest the open-source Arduino-based Cave Pearl project[13] as a starter for further development. Cave Pearl dataloggers were designed for environmental monitoring of cave ecosystems, so ruggedness and low power demand were key considerations in their design. Similar considerations are relevant for implementation to plant phenotyping work. Cave Pearl datalogger components are inexpensive (less than USD $50 per unit) and small, which could enable them to be directly incorporated into PARbars.

Application of the PARbars described here faces three main limitations. First, the inference of plant area index or leaf area index from measured light capture is hampered by strong time-dependent biases, particularly in row crops[7]. This can be overcome by making repeated or continuous measurements over a day. Second, inexpensive photodiodes do not have a spectral output that is exactly proportional to photon flux (the variable of greatest interest in photosynthesis research). This can cause bias when light quality changes greatly through a canopy, although previous estimates of the resulting error indicate that it is on the order of a few percent[7]. Third, PARbars cannot distinguish between the direct beam and diffuse components of incoming PAR above the canopy. As diffuse radiation penetrates deeper into the canopy than direct sunlight[14], transmittance will be increased and PAI_{eff} will be underestimated as the diffuse fraction of total irradiance increases. When all radiation is diffuse, PAI_{eff} is directly proportional to the logarithm of $1/\tau$ rather than the relationship shown in Equation 1[15]. Cruse *et al*. (2015)[16] noted that currently available commercial instruments that can measure direct and diffuse PAR are expensive and require regular maintenance, so they designed a simple and inexpensive apparatus to address this issue. Their system consists of a quantum sensor that is routinely shaded by a motorized, moving shadowband and allows for continuous measurement of total, direct and diffuse PAR. The sensor used in the Cruse *et al*.[16] system could be replaced with the same photodiode used in PARbars to further reduce cost and may be easily incorporated into the existing PARbar setup. These measurements could be integrated into the data processing pipeline and would further enhance the reliability of estimates of PAI_{eff}.

The major advantage of PARbars relative to existing commercial ceptometers is their low cost, which makes it feasible to produce them in large numbers. Recently, there has been a growing interest in novel high-throughput plant phenotyping technologies for the estimation of canopy traits (for review see Yang *et al*., 2017[17]). Whilst these methods are promising in that they produce huge amounts of data they are typically very indirect and require validation against conventional techniques. PARbars could serve as a cost-effective, ground-based validation tool for these new techniques.

The low production cost of PARbars also make them a viable option for continuous measurements in the field. This could be useful for several reasons. For example, continuous measurements can be used to characterize row-orientation biases to develop time-specific correction functions for instantaneous measurements (for more information see Salter *et al.* 2018[7]). Continuous ceptometry can also capture short fluctuations in canopy light capture over time (sunflecks and shadeflecks) caused by clouds passing overhead, movement of the canopy, etc. Photosynthesis is known to be highly sensitive to small changes in environmental conditions and 'dynamic' changes in photosynthesis are now thought to be important in driving crop yield (for review see Murchie *et al.*, 2018[12]). PARbars installed in the field with a suitably short logging interval could be used to capture these short fluctuations and provide better understanding of the dynamic nature of plant canopies.

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DISCLOSURES:

The authors confirm that they have no conflicts of interest and nothing to disclose.

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Figure 1

Click here to access/download;Figure;Figure 1.ai

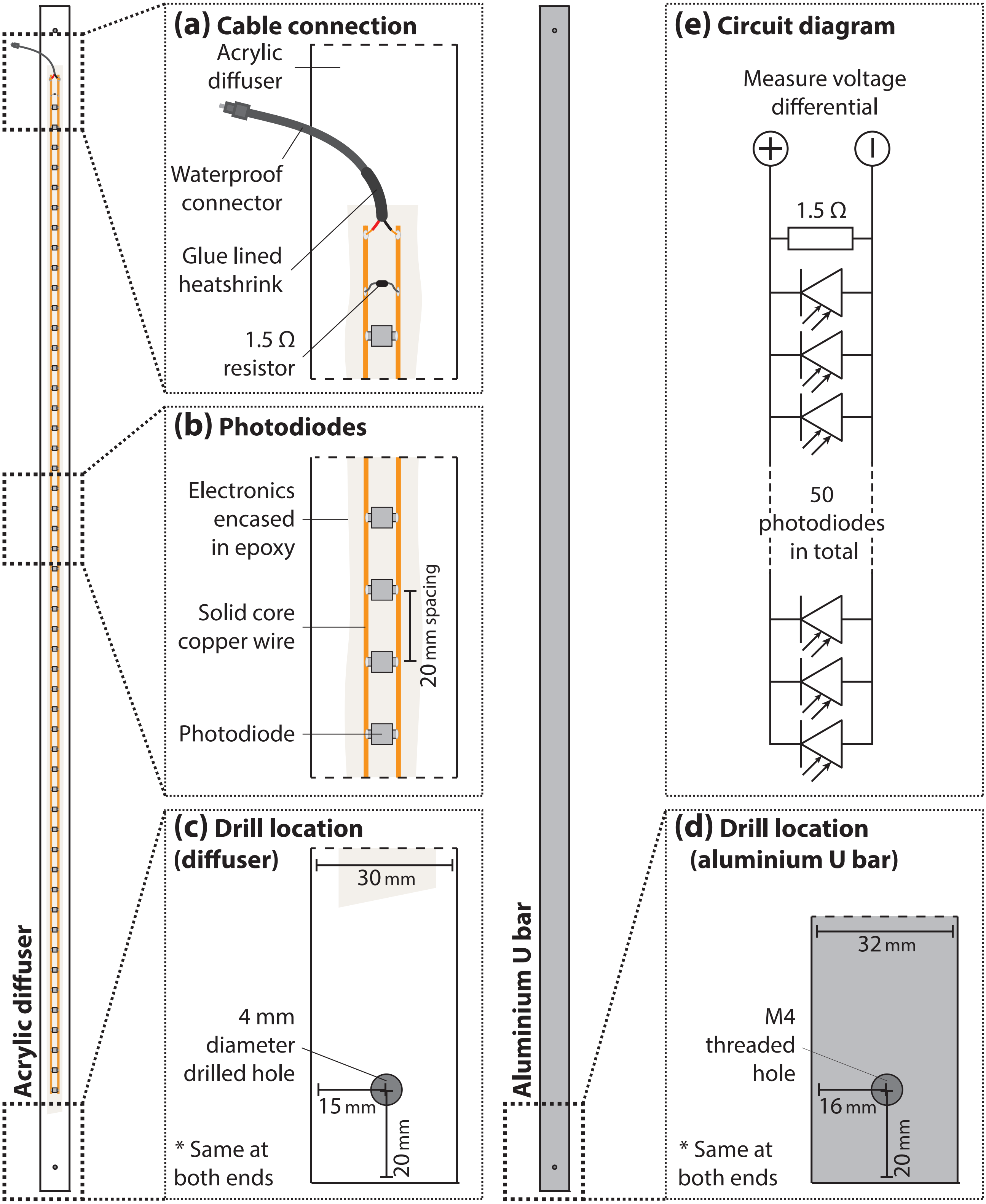

Figure 2

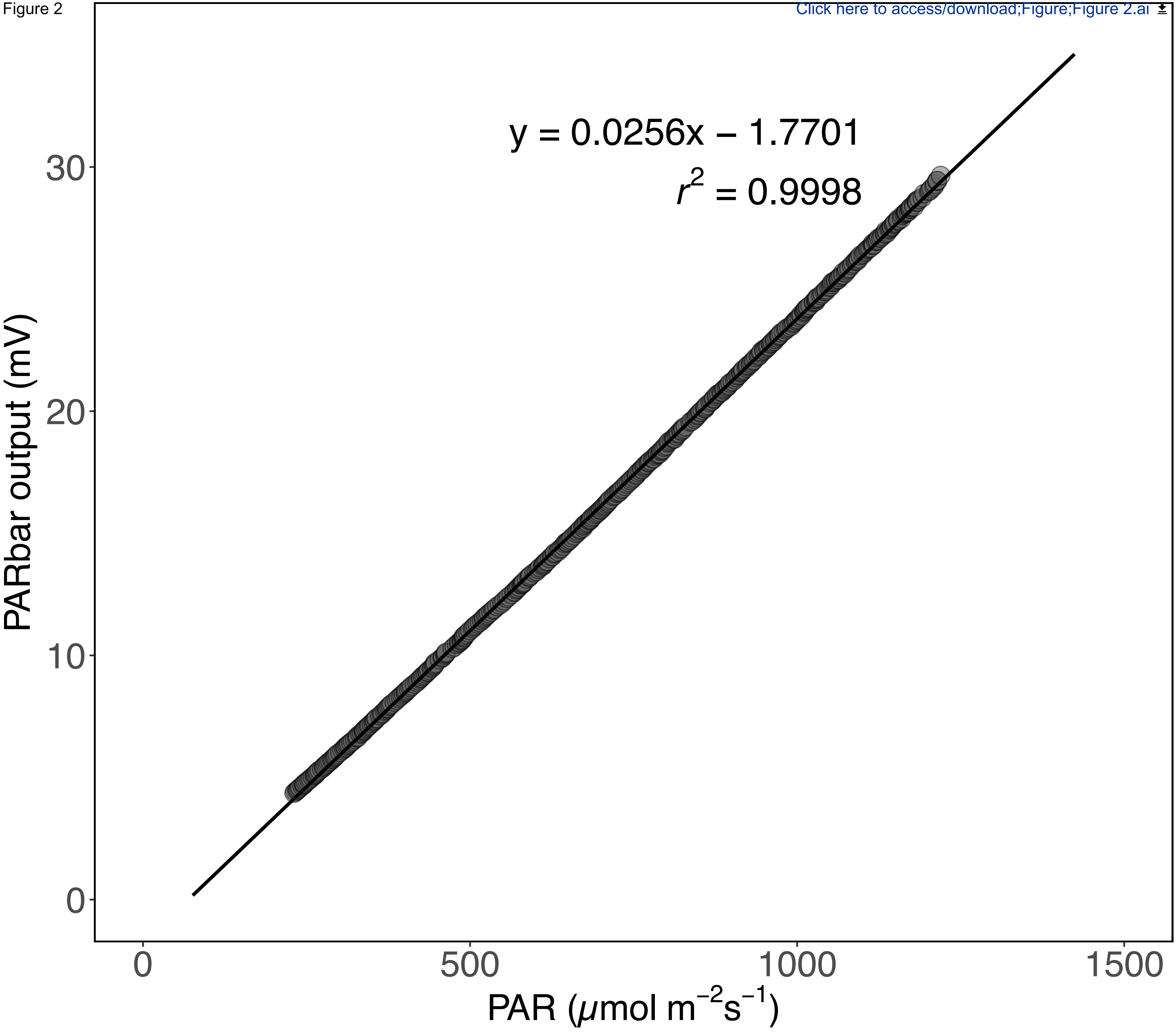
y = 0.0256x − 1.7701
r2 = 0.9998
PARbar output (mV)
0
10
20
30
0
500
1000
1500
PAR (μmol m−2s−1)

Figure 3

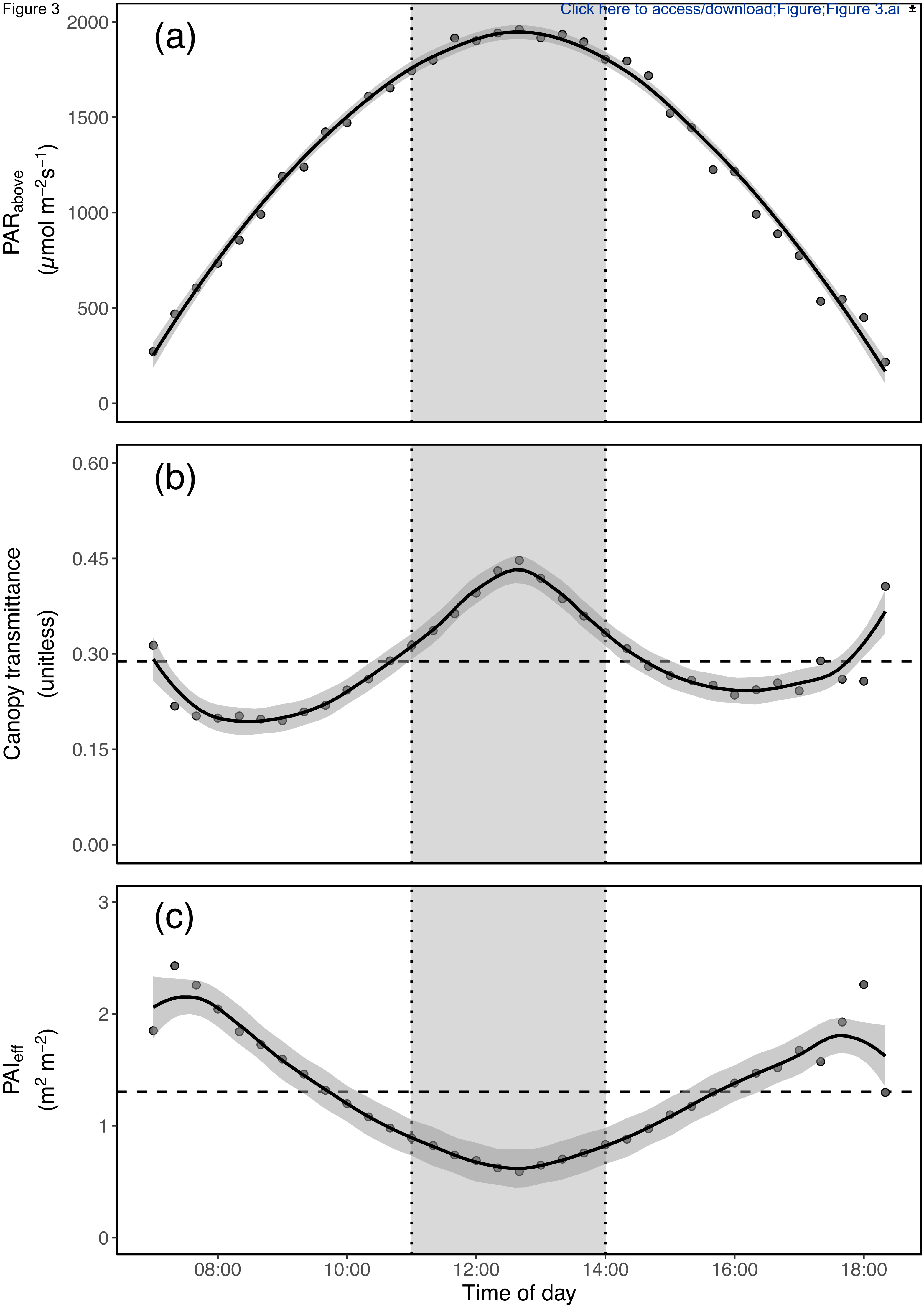
(a)
2000
1500
1000
500
0
PAR_{above}
(μmol $m^{-2}s^{-1}$)
(b)
0.60
0.45
0.30
0.15
0.00
Canopy transmittance
(unitless)
(c)
3
2
1
0
PAI_{eff}
(m^2 m^{-2})
08:00
10:00
12:00
14:00
16:00
18:00
Time of day

Figure 4

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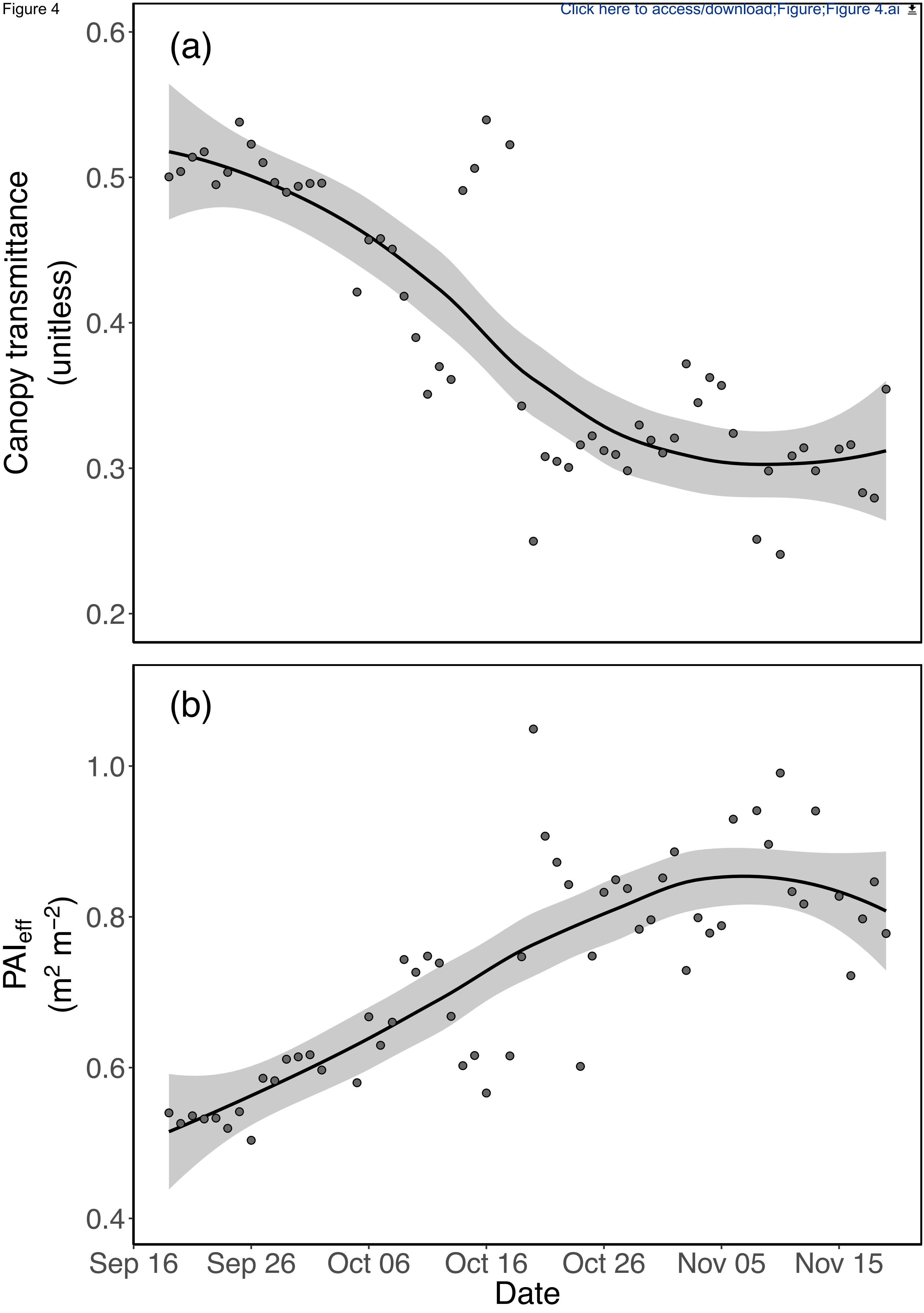

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
1.5 Ω low temperature coefficient precision resistor	TE Connectivity Ltd., Schaffhausen, Switzerland.	UPW25 series	Could be made using multiple larger resistors in parallel but they need to have low temperature coefficient (i.e. ± 3 ppm/°C).
Acrylic diffuser	Plastix Australia Pty. Ltd., Arncliffe, NSW, Australia.	445 - Opal White	1200 mm length x 30 mm width x 4.5 mm thick.
Aluminum U-bar	Capral Ltd., Bundamba, QLD, Australia.	EK9160	1220 mm length x 35 mm width x 25 mm depth.
Bare solid core copper wire	Non-specific part		1 m lengths; 1.15 mm thickness. Straightened by securing one end in a vice and the other in a drill.
Bolts	Non-specific part		30 mm M4.
Clamps	Non-specific part		
Clear epoxy potting resin	Solid Solutions, East Bentleigh, VIC, Australia.	651 - Universal Epoxy Potting Resin	Clear epoxy resin for electrical applications.
Cyanoacrylate glue	Non-specific part		
Datalogger	Campbell Scientific, Logan, Utah, USA.	CR5000	Other dataloggers that record differential voltages could be used.
Drill or drill press	Non-specific part		
Glue lined heat shrink	Non-specific part		Various sizes.
Heat gun	Non-specific part		
LED torch	Non-specific part		
Masking tape	Non-specific part		

Photodiodes (50)	Everlight Americas Inc., Carrollton, Texas, USA.	EAALSDSY6444A	It is important that this specific component is used due to spectral response.
Polyurethane foam filler	Non-specific part		
Quantum sensor	LI-COR, Lincoln, Nebraska, USA.	LI-190R	For calibration of PARbars only.
Screwdrivers	Non-specific part		
Silicone sealant	Non-specific part		
Solder	Non-specific part		
Solder flux pen	Non-specific part		
Soldering iron	Non-specific part		With fine tip.
Spirit/bubble level	Non-specific part		
Tap and die set	Non-specific part		
Two-core cable	Non-specific part		Heavy duty as the PARbars will be used outdoors.
Voltmeter	Non-specific part		
Waterproof connectors	Core Electronics, Adamstown, NSW, Australia.	ADA743	2 core waterproof connector. DC power connectors work well.

URL for commercial source

https://bit.ly/2DFuPpm

https://bit.ly/2Bq0fyc

https://bit.ly/2PPfJou

https://bit.ly/2qY0pHa

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Title of Article: PARbars: cheap, easy to build ceptometers for continuous measurement of light interception in plant canopies

Author(s): William T. Salter, Andrew M. Merchant, Matthew E. Gilbert, Thomas N. Buckley

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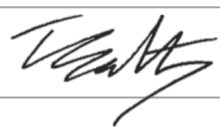
Institution: The University of Sydney

Article Title: PARbars: cheap, easy to build ceptometers for continuous measurement of light interception in plant canopies

Signature: 

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Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please use American English throughout. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Done.

2. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Done.

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Done.

4. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Done.

5. Please leave a single line space between note and the numbered step of the protocol. Please ensure that Notes should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Two notes should not follow each other.

Done.

6. The Protocol should contain only action items that direct the reader to do something.

Done.

7. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections.

Done.

8. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?

We have carefully revised the paragraph to add additional "how" details where appropriate.

9. 1.4: What are the intended locations? How do you determine the same?

We have expanded the description in this step to clarify the exact positions.

10. 1.6: How much solder flux is applied? How do you solder? Any specifics to be taken care of?

We have added additional information to clarify this step.

11. 1.9: How do you create the barrier?

We have added additional information to clarify this step.

13. 2.1: What does PAIeff refer to?

Effective plant area index. We have added this information.

12. 1.1.3: how do you connect the sensor and datalogger?
14. 2.2: Please provide discrete experimental steps. How do you commence logging? Do you perform any button clicks in the software? Do you use graphical user interface, scripts if any, etc?

RE comments 12 and 14: Datalogger connections and datalogger procedures are generic and not unique to this protocol. They depend entirely on the particular datalogger and manufacturer. In fact, a datalogger is not strictly required; any device capable of measuring a differential voltage, including a digital multimeter, will suffice. We have reworded this step to clarify this: "*2.2) Connect the PARbars to a datalogger or voltmeter capable of measuring a differential voltage output, using cables made in step 1.11. Remember to connect each PARbar in parallel with a 1.5 Ω low temperature coefficient precision shunt resistor if a resistor was not integrated into the PARbar during construction.*"

15. Table 1: Please remove the part number and Link from the table and move these details to the table of materials. Else this table can be combined with the table of materials.

We have eliminated this table from the main text and merged its contents into the Table of Materials.

16. Figure 1: What does color coding represent in the figure.

There is no color coding as such. The colors are indicative of the actual colors of the items; the only parts with notable color are the copper wires, which appear orange in Figure 1.

17. Figure 2: Please clarify what are the datapoints and how many datapoints are there per experiment.

Each datapoint is a single pair of measurements recorded by the datalogger from the PARbar and quantum sensor. There are a total of 642 measurements. We have clarified this in the figure legend.

18. Figure 3: Please clarify what are the datapoints and how many datapoints are there per experiment. What does the gray area represent?

All of this information was provided in the Figure legend.

19. For formatting the units please use m2/m2 and not m2 m-2. Please leave a single space between the number and the units.

This is contrary to SI guidance and mathematically incorrect for the units of PAR ("μmol m^{-2} s^{-1}" is not the same as "μmol/m^2s" [the latter is equal to μmol s m^{-2} by rules of operator precedence], and "μmol/m^2/s" is impermissible due to the use of successive divisors).

20. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable).

We have modified figure legends accordingly.

21. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

The original manuscript did explicitly over most of these items; the only omission was (a). We have removed text that did not apply to any of these descriptions, and have reordered the rest to match the sequence described above.

22. Please sort the table of materials in alphabetical order.

Done.

Reviewers' comments:

Reviewer #1:
In this paper Salter et al. report a detailed step-by-step protocol describing how to build cost-effective and highly accurate continuous logging ceptometers, and provide evidence on the need for continuous

measurements of light transmission in row crop canopies to monitor changes in canopy light environment and to avoid bias in PAI estimation.

The protocol is well-written and easy to follow, and the technical diagrams and figures are representative. Some lacking details on the protocol should be provided and few minor points addressed:

1. Please provide more details on the field installation setup: size of the plots and number of rows, height and position at which PARbars are placed above and below the canopy.

The size of plots and numbers of rows are arbitrary and variable between research facilities and different crops; they do not affect the protocol described here. The height of PARbars above the canopy does not matter (only that they are not shaded by any canopy material); the position below the canopy is determined by the height of the lowest light-absorbing elements that the investigator wishes to account for. We have modified the text to explain these last two points.

2. Were the measurements performed in different wheat genotypes and at which development stages? Perhaps the authors could briefly comment on how changes in canopy architecture given by plant development and/or genotypic variation may affect measurements and PAI estimation.

The wheat developmental stage for the sample measurements was noted in the legends (for Figure 3, it was at anthesis; for Figure 4, it ranged from tillering to anthesis), in a single genotype. We have added a brief description to the first paragraph in the Discussion regarding how differences in canopy structure may influence the inference of PAI from light capture measurements.

3. Besides the price of the components ($75), a rough estimate on the time needed to build a PARbar could be of interest.

We have added this information to the last paragraph in the Introduction (1 hour).

4. R script: most readers would appreciate having access to a small dataset example (PARbardata.csv) for running the provided R script.

This is a good idea; we have added a sample dataset.

Reviewer #2:
Manuscript Summary:

The study itself appeared well described, and appears to have thoroughly addressed the specific objectives stated. Some minor comments are listed below that the authors might consider:

L55 - Please specify the "commercial models tested".

We are not permitted to name commercial products in the ms (as noted in item 3 under the Editorial comments above).

L156 - Was the quantum sensor LI-190R used to calibrate the PARbars described? (If yes, please specify in this section)

L157 - Was the datalogger CR5000 the one used in the present study? (If yes, please specify in this section).

Reference to this specific quantum sensor and datalogger has been removed, following instruction in the Editorial comments.

General: Avoid using "We" through out the writing part of the manuscript.

We disagree. First person is acceptable in the modern scientific literature, and it improves readability by reducing the use of passive voice.

Major Concerns:

My only concern about the manuscript it is not including a cheap datalogger. In the case, using a PARbar will require an expensive datalogger. Are the research team planning to develop /adapt/test a cheap datalogger suitable for using with the cheap PARbars described?

We have clarified, both in the Protocol and in the Discussion, that use of PARbars does not in fact require an expensive datalogger. Any device capable of measuring a differential voltage will suffice.

PAI calculation R script

Click here to access/download
Supplemental Coding Files
PAI calculation_revised.R

Sample dataset

Click here to access/download
Supplemental Coding Files
PARbardata.csv