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Journal of Visualized Experiments
Lyndsay Troyer, Ph.D.
Science Editor
1 Alewife Center Suite 200
Cambridge MA 02140

Dear Dr. Troyer:

Further to our discussion in the fall we are excited to submit our uploaded original manuscript entitled *"Calibration Protocol and Software for Split Point Analysis and Uncertainty Quantification of Thermal-Optical Organic / Elemental Carbon Measurements"* for consideration as a research article in JoVE.

Measurements of elemental and organic carbon in aerosol samples are vitally important to many researchers, where accurate classification and quantification of carbonaceous aerosols have important impacts on climate and health. This paper first presents a detailed protocol for precisely calibrating a thermal-optical OCEC analyzer within defined uncertainty ranges. The resulting video protocol of this complicated procedure should be highly-valued by a range of researchers and labs, both as a teaching tool and as a means to better document and standardize calibration procedures.

More importantly, along with the calibration protocol we are including an open-source software tool "*OCECgo*" that enhances calibration and data analysis and enables rigorous Monte Carlo quantification of uncertainties. Notably, the software tool includes novel means to correct for instrument drift and identify and quantify the uncertainty when discriminating between OC and EC in a sample. This is a significant improvement on the uncertainty estimation in the instrument manufacturer's software, which ignores this uncertainty and otherwise uses fixed equations for relative and absolute errors (generally leading to under-estimated uncertainties and often yielding non-physical results as demonstrated in several example data sets). The demonstrated calibration protocol and new software tool enabling accurate quantification of combined uncertainties from calibration, repeatability, and OC/EC discrimination are shared with the intent of assisting other researchers in achieving better measurements of OC, EC, and total carbon mass in aerosol samples. We are excited to have this work released through JoVE.

Sincerely,

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TITLE:

Split Point Analysis and Uncertainty Quantification of Thermal-Optical Organic/Elemental Carbon Measurements

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KEYWORDS:

organic carbon, elemental carbon, thermal-optical analysis, OCECgo, split point, uncertainty analysis, Monte Carlo, aerosol, soot, OC, EC

SUMMARY:

This article presents a protocol and software tool for the quantification of uncertainties in the calibration and data analysis of a semi-continuous thermal-optical organic/elemental carbon analyzer.

ABSTRACT:

Researchers from myriad fields frequently seek to quantify and classify concentrations of carbonaceous aerosols as organic carbon (OC) or elemental carbon (EC). This is commonly accomplished using thermal-optical OC/EC analyzers (TOAs), which enable measurement via controlled thermal pyrolysis and oxidation under specific temperature protocols and within constrained atmospheres. Several commercial TOAs exist, including a semi-continuous instrument that enables on-line analyses in the field. This instrument employs an in-test calibration procedure that requires relatively frequent calibration. This article details a calibration protocol for this semi-continuous TOA and presents an open-source software tool for data analysis and rigorous Monte Carlo quantification of uncertainties. Notably, the software tool includes novel means to correct for instrument drift and identify and quantify the uncertainty in the OC/EC split point. This is a significant improvement on the uncertainty estimation in the manufacturer's software, which ignores split point uncertainty and otherwise uses fixed equations for relative and absolute errors (generally leading to under-estimated uncertainties and often yielding non-physical results as demonstrated in several example data sets). The demonstrated calibration protocol and new software tool enabling accurate quantification of combined uncertainties from calibration, repeatability, and OC/EC split point are shared with the intent of assisting other researchers in achieving better measurements of OC, EC, and total carbon mass in aerosol samples.

INTRODUCTION:

The ability to accurately measure atmospheric concentrations of carbonaceous species is exceedingly important to many researchers. Carbonaceous species in ambient particulate matter (PM, the largest environmental risk factor for early death[1]) have been suggested to be the key component of PM responsible for adverse health effects and outcomes[2–4]. Particulate carbon in the atmosphere is a critical climate pollutant, where different carbonaceous species are known to have variable, even opposite, impacts. Black carbon is potentially the second strongest direct radiative forcer in the earth's atmosphere[5–8]. When deposited on snow and ice, black carbon also reduces the reflectivity of the arctic landscape, enhancing the absorption of sunlight, and increasing the rate of melt[9–12]. Contrastingly, hygroscopic organic carbon particles act as cloud condensation nuclei, increasing the mean reflectivity of earth, and causing a cooling effect[13]. Accurate classification of sampled carbonaceous material and concurrent quantification of measurement uncertainties are thus essential aspects of particulate matter measurements.

Differentiating between organic and elemental carbon in a particulate-laden sample can be achieved using a thermal-optical analysis[14]. Commercial, laboratory-based systems for thermal-optical carbon analyses have been created[15–17] including an on-line, semi-continuous analyzer[18] that enables the execution of thermal-optical analyses in the field. The present work describes a detailed procedure for calibrating this latter OCEC instrument (see **Table of Materials**) and shares an open-source software tool for the rigorous quantification of calibration and analysis uncertainties. Although the initial release of the open-source software is designed for the output file format of the semi-continuous instrument, the software tool could readily be extended by others in future to work with outputs generated by other instruments.

The semi-continuous thermal-optical organic/elemental carbon analyzer (OCEC) quantifies organic carbon (OC) and elemental carbon (EC) in a sample volume. The analysis procedure contains four phases outlined in **Figure 1**. Firstly, a sample volume is pulled through the instrument, where particulate matter is deposited onto, and gas-phase organics are adsorbed by, a quartz filter. At the termination of sampling, the quartz filter is heated through a prescribed temperature protocol in an inert, helium (He) atmosphere. During this procedure, a portion of the carbonaceous material is thermally pyrolyzed from the quartz filter. Gaseous exhaust is forwarded to a fixed-temperature, Manganese Oxide (MnO_2) oven that converts pyrolyzed carbonaceous species into carbon dioxide (CO_2). The time-resolved concentration of released CO_2 is subsequently measured by a non-dispersive infrared (NDIR) detector. Following initial heating in the He environment, the sample is heated through a similar protocol in an oxidizing (Ox) environment. In the presence of oxygen, refractory carbonaceous species remaining on the quartz filter are oxidized and then forwarded through the MnO_2 oven and NDIR detector in the same manner. Once sampled carbonaceous species have been fully evolved from the quartz filter, a final in-test calibration procedure is performed. A fixed quantity (nominally 0.8 mL) of a 5% methane-helium mixture is introduced into the instrument, oxidized in the MnO_2 oven, converted to CO_2, and subsequently measured by the NDIR. The integrated NDIR signal during this in-test calibration phase (termed the methane [CH_4]-loop) corresponds to the known carbon mass (introduced as CH_4) and therefore quantifies the sensitivity of the NDIR, which can drift in time. This measure of NDIR sensitivity is then used to infer carbon masses from the NDIR signal during the prior He- and Ox-phases of the analysis.

[Place **Figure 1** here]

The mass of injected carbon during the CH_4-loop can be sensitive to operational conditions such that intermittent calibration is required. This calibration uses an aqueous sucrose solution of known concentration (approximately $0.99\%_{m/m}$) as an external standard. Repeated tests are performed where different known volumes of the sucrose solution are introduced in random order into the instrument and the thermal-optical analysis is performed. Each repeated test (i.e., results from each injection and subsequent analysis) yields an integrated NDIR signal during the CH_4-loop ("calibration area") and an integrated NDIR signal for total carbon (i.e., signal during the He- and Ox-phases; referred to as "total area"), which corresponds to the known mass of carbon in sucrose. Linear regression of the known carbon mass with instrument-reported "total area" provides a measure of mean NDIR sensitivity. This sensitivity is then coupled with the mean "calibration area" to yield calibrated knowledge of the carbon mass injected during the CH_4-loop. Beyond calibration, a key challenge in the interpretation of results from the OCEC instrument is the determination of the relative fractions of OC and EC in the measured sample. As OC pyrolyzes during the He-phase of the temperature protocol, a fraction chars on the filter rather than being released, oxidized in the MnO_2 oven, and detected by the NDIR. In theory, this charred refractory OC (termed pyrolyzed carbon, PC) remains on the filter until the Ox-phase, when it is oxidized alongside EC. Consequently, naively labelling all evolved carbon during the He-phase as OC and evolved carbon in the Ox-phase as EC leads to biased estimates of the true fraction of OC and EC. A common means to define the split point (i.e., the moment in time when all prior carbon evolution is considered as OC and all subsequent carbon evolution as EC) is the thermal/optical-transmittance (TOT) method[19]. Here, a laser is directed through the quartz filter during the thermal analysis and its power (optically downstream of the quartz filter) is detected by a photodetector. By assuming that OC is not optically active at the laser wavelength (i.e., OC negligibly absorbs light) and PC shares optical properties with EC, the split point can be estimated. The premise is to first measure the attenuated laser power at the start of the analysis. As OC evolves (partly into light-absorbing PC), the attenuation of the laser is enhanced such that the photodetector signal drops. As the Ox-phase is entered and EC/PC are co-evolved, attenuation is reduced, and the photodetector signal begins to increase. The split point is defined as the instance in time when the measured laser power returns to its initial value. While the logic of this approach is robust, the result relies upon the above-noted assumptions. As such, it is common to declare that reported OC and EC results are "operationally-defined" — i.e., they are specific to the technique employed to evaluate the split point[14,20,21].

Although it is true in theory that OC is evolved in the He-phase and PC/EC are evolved in the Ox-phase, it has been observed that evolution of PC/EC can indeed occur during the He-phase due to various mechanisms[22–25], such that the true split point may occur prior to the introduction of oxygen. This ambiguity in the prediction of where the split point should lie, coupled with uncertainty in the assumptions of the optical properties of OC, PC, and EC, suggests that uncertainty in the split point could be a dominant source of uncertainty in measured carbon masses. Fortunately, the methodical estimation of the split point via the TOT method enables an objective estimate of split point uncertainty. To the authors' knowledge however, there is no direct estimation (and subsequent propagation) of split point uncertainty in the manufacturer's software; reported total uncertainties are instead computed with fixed relative and absolute components[26]. As part of this work, a novel technique to estimate split point uncertainty is presented − the "attenuation decline" technique. In this technique, uncertainty in the split point is quantified as the difference between evolved carbon mass at the nominal split (via the TOT method) and evolved carbon mass at a subsequent point where laser attenuation has decreased beyond some critical quantity, a prescribed fraction of the initial attenuation. A critical attenuation decline is estimated based on the uncertainty in laser attenuation relative to its initial value; in theory, this approach captures uncertainty in the key attenuation-matching principle of the TOT method. Furthermore, to consider (at least in part) split point uncertainty due to assumed optical properties of PC and EC, the suggested critical attenuation decline is expanded by a factor of two.

This article presents a detailed protocol for calibrating the OCEC instrument alongside a software tool for rigorously quantifying calibration and analysis uncertainties. Firstly, sections 1 to 3 of the protocol outline instructions for creating the aqueous sucrose solution, preparing the instrument for calibration, and acquiring calibration data. Section 4 uses the novel, open-source, software tool (see **Table of Materials**) to analyze calibration data via the software's graphical user interface. Section 5 specifies considerations for acquiring a sample using the OCEC instrument and section 6 describes use of the above-noted software for calculating carbon masses and associated uncertainties, including contributions from the estimation of the split point. Novel techniques to improve the processing of OCEC data — including the "attenuation decline" technique introduced above − are described in the software's online documentation.

Within the presented software tool, calibration constants, measured carbon masses, and associated uncertainties are computed using a Monte Carlo (MC) method. This procedure propagates errors that are not, to the authors' knowledge, currently considered in the manufacturer's proprietary software. For calibration, these sources of error include uncertainty in the concentration of sucrose in the aqueous solution, accuracy in the volume of applied sucrose solution (instrument accuracy, inter-user reproducibility, and intra-user repeatability), and uncertainty in the linear regression. With respect to data analysis, considered sources of error include the calibration uncertainty and repeatability and, importantly, the estimated uncertainty of the split point. Ultimately, the software enables a user to accurately quantify uncertainty in the calibration of the instrument and propagate this uncertainty alongside that of split point estimation into the calculation of carbon masses. This represents a notable improvement upon the manufacturer's protocol, by directly considering key sources of error in the measurement, rather than estimating partial uncertainties using a fixed equation.

PROTOCOL:

NOTE: This instrument contains a visible, Class 1 laser. While exposure to this low-power laser is unlikely to result in harm, the instrument includes an interlock device in the form of a laser shroud that blocks the user from the optical path when the access panel is open. Removal of the laser shroud disables the laser, such that exposure to the laser throughout the presented protocol should not occur. The instrument's ovens may heat to ~900 °C during normal operation of the instrument and components manipulated throughout the presented protocol may become hot. Prior to performing work within the instrument's access panel, ensure that the instrument software reads a "Status" of "Idle" and that the "Front Oven" temperature is <90 °C. Use caution when manipulating components near the oven unit immediately following high-temperature operations.

1. Prepare aqueous sucrose solution.

1.1. On a precision balance, place a clean, sealable, glass vessel of minimum 1 L in volume. Add 10 g of high-purity sucrose and 1000 g of distilled, de-ionized (DDi) water to the vessel.

1.2. Seal the vessel and mix thoroughly by shaking until the sucrose is fully dissolved. Transfer a portion of the solution to a small, clean, glass jar (**Table of Materials**) for pipetting.

1.3. Store both solutions in a refrigerator for up to six months.

2. Prepare instrument for calibration.

2.1. Energize the instrument and allow the rear oven to come to temperature.

2.2. Run a minimum of five CH_4-loops to allow startup transients to dissipate.

2.2.1. In the **Sample ID #** field of the instrument's software, type text to indicate startup, such as **Instrument Warm-Up**. In the **PAR FILE** field, click the **…** button to browse and select the **OCECgo_WarmUp.par** protocol. In the **Output raw data file** field, click the **…** button to browse and select or create a suitable .txt file such as **yyyy-mm-dd_WarmUp.txt**.

NOTE: The parameter file **OCECgo_WarmUp.par** is available in the on-line software repository for the *OCECgo* software tool (**Table of Materials**).

2.2.2. Ensure the **Use Sample File Times** checkbox is unchecked. In the **Sample Minutes** dropdown menu, select **0**. Ensure the **Cycle** checkbox is checked. Click the **Start Analysis** button. Allow the instrument to operate for ~20 min.

NOTE: Interaction with the **Sample Minutes** dropdown menu must be by mouse. The instrument will not recognize manual text input.

2.2.3. Uncheck the **Cycle** checkbox and allow the current analysis to finish.

2.3. Replace quartz filters (**optional**).

NOTE: It is recommended by the manufacturer of the instrument[18] that filters are replaced weekly (assuming consistent use of the instrument).

2.3.1. Remove the quartz (tube) insert.

2.3.1.1. Open the access panel and remove the laser shroud (de-energizing the laser). Remove the photodetector (no tools required) by loosening the white polytetrafluoroethylene (PTFE) nut behind the photodetector, disconnecting the metal tube fitting on the left side of the photodetector, and sliding the photodetector housing off the quartz insert. Place the photodetector in the bottom of the instrument.

2.3.1.2. Remove the quartz insert by loosening the white PTFE nut holding the quartz insert in place and — while wearing non-powdered, disposable, plastic gloves — sliding the quartz insert out of the PTFE fitting.

CAUTION: The quartz insert is very fragile, place it stably on a lint-free tissue on a flat surface.

2.3.2. Using the filter removal tool, remove and dispose of the existing quartz filters. Install new quartz filters.

2.3.2.1. Place a large quartz filter on a lint-free tissue on a flat surface. Using the filter punch tool, punch out one filter.

2.3.2.2. Using clean tweezers, remove the filter from the punch and place against the PTFE housing for the quartz insert such that the textured surface of the filter is facing away from the oven. Wearing non-powdered, disposable, plastic gloves, use the quartz insert to slide the quartz filter until fully seated against the oven.

2.3.2.3. Repeat steps 2.3.2.1 and 2.3.2.2 so that two quartz filters are installed.

2.4. Install a third quartz filter (referred to as the quartz “boat”) for introduction of the sucrose standard to the instrument. Repeat step 2.3.2.1. Using clean tweezers, remove the filter from the punch and place the quartz filter “boat” into the end of the quartz insert such that the cross-sections of the filter and insert are perpendicular.

2.5. Replace components and close the instrument.

2.5.1. Wearing non-powdered, disposable, plastic gloves, re-introduce the quartz insert into the instrument. Loosely hand-tighten the white PTFE nut that secures the quartz insert in place.

2.5.2. Replace photodetector head (no tools required). Slide the photodetector housing onto the end of the quartz insert. Loosely re-connect the metal tube fitting on the left side of the photodetector to ensure proper alignment of the photodetector and quartz insert. Loosely hand-tighten the white PTFE nut securing the photodetector head onto the quartz insert. Fully hand-tighten the metal tube fitting on the left side of the photodetector.

2.5.3. Ensure all PTFE nuts are fully hand-tight and secure.

NOTE: It is critical that the instrument's oven is well-sealed from atmosphere. That is, although tools should not be used, hand-tighten all PTFE nuts as much as possible. In the authors' experience, while delicate, the quartz insert is strong in the circumferential direction — significant tightening of the PTFE nuts, which ensures good seals, is unlikely to damage the quartz insert.

2.5.4. Replace the laser shroud (re-energizing the laser) and close the access panel.

2.6. Clean newly-installed quartz filters by running a minimum of one oven clean cycle. Click the **Run** dropdown menu in the instrument software's toolbar and select **Clean Oven**, which raises the front oven temperature to above ~835 °C.

2.7. Ensure residual carbon has been removed from the filter using blank analytical cycle(s).

2.7.1. In the **PAR FILE** field of the instrument's software, click the **…** to browse and select the desired thermal protocol's .par file. Ensure the **Use Sample File Times** checkbox is unchecked. In the **Sample Minutes** dropdown menu, select **0**. Ensure the **Cycle** checkbox is unchecked. Click the **Start Analysis** button, confirm that only one analysis/cycle is desired, and allow the thermal analysis to execute.

NOTE: Interaction with the **Sample Minutes** dropdown menu must be by mouse. The instrument will not recognize manual text input.

2.8. Repeat steps 2.6 and 2.7 until the instrument-reported total carbon mass is statistically equal to zero.

3. Acquire calibration data.

3.1. Obtain one calibration point.

3.1.1. Remove the quartz insert via section 2.3.1 and following manufacturer-recommended pipetting procedures, aspirate 5 µL or 10 µL of sucrose solution. Carefully deposit the sample onto the quartz boat, as close as possible to the end of the quartz insert, ensuring a blowout procedure is executed such that the entire volume is ejected onto the quartz boat.

3.1.2. Re-introduce the quartz insert, close the instrument via section 2.5, and dry the wet filter. Click the **Run** dropdown menu in the instrument software's toolbar and select **dry wet filter**, which raises the front oven temperature to 110 °C.

3.1.3. Once the front oven has cooled, run the instrument protocol to be used in post-calibration measurements (selected in step 5.3.1).

3.1.3.1. In the **Sample ID #** field of the instrument's software, type text to indicate applied sucrose volume, such as **5 uL**. In the **PAR FILE** field, click the **…** to browse and select the desired thermal protocol's .par file. In the **Output raw data file** field, click **…** to browse and select or create a suitable .txt file such as **yyyy-mm-dd_Calibration.txt**.

3.1.3.2. Ensure the **Use Sample File Times** checkbox is unchecked. In the **Sample Minutes** dropdown menu, select **0**. Ensure the **Cycle** checkbox is unchecked. Click the **Start Analysis** button, confirm that only one analysis/cycle is desired, and allow the thermal analysis to execute.

NOTE: Interaction with the **Sample Minutes** dropdown menu must be by mouse. The instrument will not recognize manual text input.

3.2. Obtain one blank/background calibration point. Perform section 3.1 without depositing a sucrose sample onto the quartz boat.

NOTE: To obtain an accurate blank/background, ensure that the quartz boat is exposed to ambient air as though the user were depositing a sample of sucrose.

3.3. Repeat section 3.1 such that one calibration point each at 5 and 10 µL are obtained. Further repeat sections 3.1 and 3.2 as required to achieve desired calibration uncertainty as computed in section 4.

NOTE: Section 4 may be executed following each iteration of section 3.3 to support the user in determining satisfactory convergence of the calibration.

3.4. Remove the quartz boat. Remove the quartz insert via section 2.3.1. Using clean tweezers, remove the quartz boat from the quartz insert. Re-introduce the quartz insert and close the instrument via section 2.5.

3.5. To ensure the instrument is prepared for post-calibration measurements, run a minimum of one oven clean cycle as in step 2.6.

4. Compute calibration constant with uncertainty.

NOTE: The *OCECgo* software tool employs mouseover utilities to aid the user with the input of data and selection of analysis parameters. Further information, including default and permissible ranges for user-editable fields are listed in the tool's online documentation.

4.1. Load the software tool (*OCECgo*) and click to migrate to the **Calibration Tool** tab.

4.2. Input calibration data. In section (1) of the graphical user interface (GUI), input calibration data: nominal volume of the applied sucrose solution, instrument-reported integrated NDIR signal corresponding to total carbon (“total area”), instrument-reported integrated NDIR signal during the CH_4-loop (“calibration area”), and a Boolean to indicate whether specific points should be used in calibration (“1” for yes; “0” for no). Repeat for each data point, adding and deleting rows to the table, as necessary, by clicking the “+ Row” and “- Row” buttons.

NOTE: The user can alternatively click the **Import Calibration** button to upload previous calibration data and inputs to the software tool. If this option is exercised, go to step 4.4 to re-create plots in GUI section (3) or go directly to section 6 to analyze instrument data.

4.3. Define uncertainty data for use in the Monte Carlo analysis.

4.3.1. In GUI section (2)(a), input data pertaining to the aqueous sucrose solution. Input masses of sucrose and DDi water measured during step 1.1 and the absolute 2σ bias of the scale [g] used to measure DDi and sucrose masses — absolute bias is equivalent to the scale’s reported accuracy. Input the nominal minimum purity of sucrose [$\%_{m/m}$] listed on the sucrose vessel’s label and insert the range of ambient temperatures [°C] observed during the acquisition of calibration data.

NOTE: 2σ corresponds to two times the standard deviation, which in the context of a normal (Gaussian) distribution is a conservative estimate of the 95% confidence interval (CI).

4.3.2. In section (2)(b), provide data pertaining to pipette uncertainty. Input relative 2σ equipment-reported accuracy (bias error), equipment reported repeatability (precision error), precision error corresponding to intra-user repeatability, and bias error corresponding to inter-user reproducibility for aspirated volumes of 5 µL and 10 µL.

NOTE: Default pipette uncertainties correspond to the instrument listed in the **Table of Materials**. Default 2σ human-errors were estimated based on the pooled variances of studies of intra-user repeatability and inter-user reproducibility at each volume.

4.3.3. In section (2)(c), input the desired number of Monte Carlo draws for the computation of calibration metrics.

NOTE: The number of Monte Carlo draws corresponds to the number of random computations of the mass calibration constant under the Monte Carlo framework. Larger numbers yield more consistent results but take longer to process (more computational time). The default value in *OCECgo* is 10^6 while permitted values are [10^2, 10^8].

4.4. Run analysis. In GUI section (3), press GO to run the Monte Carlo analysis to process calibration data.

4.5. Update the instrument calibration file with the results presented in section (4). Open the instrument’s parameter file: **SCInstrumentParameters.txt**. Find the line of text containing the existing calibration data — this line of text includes a comment on the righthand side reading

"Calibration Constant…". Replace the numerical data with the reported "Calibrated Carbon Mass" and "Mean Calibration (CH_4-loop) Area". Save and close the parameter file and re-start the instrument's software.

4.6. Save and/or export calibration results (optional).

4.6.1. Click the **Save as Default Calibration** button to store the calibration result for default use by the software.

NOTE: Calibration results are stored in an initialization file that, upon rebooting of the software, reloads the latest calibration. The user is warned if the current date is more than 30 days from the latest calibration.

4.6.2. Click the **Export Calibration Results** button to export the calibration data.

NOTE: Numerical data are exported to a pre-formatted .xlsx file and visualization of the Monte Carlo results are exported as a .png file. This saved calibration file is useful if results are to be reanalyzed/imported at a later date using the applicable calibration.

4.7. Once calibration is complete, remove the quartz boat. Following section 2.3.1, remove the quartz insert from the instrument. Using forceps or tweezers, remove the quartz boat used for calibration. Following section 2.5, replace the quartz insert and close the instrument.

5. Obtain measurement data.

5.1. Set the desired sample flow rate. Open the instrument's valve control file: **valve_table.txt**. Set the "collect" parameter of "Valve A", which represents the target sample flow rate in liters per minute, to an integer value between 2 and 8 (inclusive).

5.2. Set the desired sampling period.

5.2.1. If an immediate sample is desired. Ensure the **Use Sample File Times** checkbox is unchecked. In the **Sample Minutes** dropdown menu, select the desired sample period in minutes.

5.2.1.1. If multiple, consecutive examples are desired, ensure the **Cycle** checkbox is checked. Otherwise, ensure the **Cycle** checkbox unchecked. Click the **Start Analysis** button, confirm that only one analysis/cycle is desired (if requested), and allow the thermal analysis to execute.

NOTE: Interaction with the **Sample Minutes** dropdown menu must be by mouse. The instrument will not recognize manual text input.

5.2.2. If it is desired to delay sample acquisition. Open the instrument's sample time control file: **SamTimePar1.txt**. Each row of this file contains a comma-separated pair of the sample time start and duration. Edit this file as desired, then save and close the file and re-start the instrument's software.

5.3. Execute the thermal analysis.

5.3.1. In the **Sample ID #** field of the instrument's software, type text to define the sample, such as **Sample_01**. In the **PAR FILE** field, click the **…** button to browse and select the desired thermal protocol's .par file. In the **Output raw data file** field, click the **…** button to browse and select or create a suitable .txt file such as **yyyy-mm-dd_Samples.txt**.

5.3.2. Click the **Start Analysis** button. If required, confirm that only one analysis/cycle is desired.

NOTE: Experience has identified, with two unique instruments, that the internal measurement of sample volume disagrees with measurements using an external high-accuracy mass flow meter, with errors exceeding 10% in some cases. Furthermore, errors in the instrument-reported sample volume have been noted to be sensitive to both sample flow rate and sample duration. It is therefore recommended to externally measure the sampled volume at the outlet of the sample pump with a high-accuracy mass flow meter such as that listed in the **Table of Materials**.

6. Compute carbon masses and uncertainties.

NOTE: The *OCECgo* software tool exploits mouseover utilities to aid the user with the input of data and selection of analysis parameters. Further information, including default and permissible ranges for user-editable fields are listed in the tool's online documentation.

6.1. Load the software tool (*OCECgo*) and click to migrate to the **Data Analysis – Inputs** tab.

6.2. Load time-resolved instrument data — GUI section (1). In sub-section (a), click the **Browse…** button and, in the file selection dialog, select the .txt results file defined in step 5.3.1. In sub-section (b), review the **Sample IDs** (as defined in step 5.3.1), and click to select the analysis of interest. In sub-section (c), review the analysis metadata, particularly the analysis' **Sample Start** timestamp.

6.3. Define data-processing options — GUI section (2).

6.3.1. In sub-section (a), select the desired laser correction procedure: a quadratic- or linear-dependence on oven temperature.

NOTE: In the experience of the authors, the laser correction procedure typically has a negligible effect — as such, the quadratic correction is recommended and is loaded as the default value.

6.3.2. In sub-section (b), select the desired NDIR correction procedure: correction via a **convex hull** to the raw NDIR data or a linear correction using the instrument-reported NDIR areas (**From results file**).

NOTE: The novel convex hull technique (briefly described in the software's online documentation) corrects the NDIR signal by fitting a convex hull as a lower-bound to the NDIR time series; this technique allows for a non-linear (piecewise) correction to the NDIR signal. In the experience of the authors, linear correction of the NDIR detector can, in some circumstances, yield non-physical results — as such, the "Convex Hull" procedure is recommended and is loaded as the default value.

6.3.3. In sub-section (c), if desired, adjust the parameters of the generalized t-distribution reported for the mass calibration constant (calculated in step 4.4) and the estimated calibration repeatability error.

NOTE: Execution of step 4.4 or import of prior calibration results (see step 4.6.2) automatically updates the generalized t-distribution parameters. Repeatability in instrument calibration ("Rep. [%]") is set to a default value of 7.90%, based on repeatability testing by the authors[27].

6.3.4. In sub-section (d), press GO → to create/update the analysis' thermogram and AVEC (laser attenuation vs. evolved carbon[21]) plots.

NOTE: If the **From results file** button is selected (step 6.3.2), in the file selection dialog, select the .xlsx results file created by the instrument.

6.4. Define the split point determination procedure — GUI section (3) and (4).

6.4.1. In sub-section (3)(a), select the desired procedure to calculate the split point and associated uncertainty: the novel "Attenuation decline" procedure described in the introduction section, a manually-defined split point and uncertainty ("Manual selection"), or the default TOT procedure of the manufacturer ("Manufacturer").

NOTE: The width of the split point range when using the manufacturer's procedure is set to zero (i.e., the manufacturer's procedure does not consider split point uncertainty).

6.4.2. In sub-section (3)(b), depending on the selected procedure to calculate the split point and uncertainty, define the nominal (mean) split point, split point uncertainty, initial laser attenuation, and/or critical attenuation decline.

NOTE: The user inputs the initial laser attenuation and a threshold of attenuation decline for the "Attenuation decline" procedure and inputs the split mean and split uncertainty for the "Manual selection" procedure. Initial laser attenuation is not used in the "Manual selection" procedure but can be tuned to support the manual selection of the split point.

6.4.3. In section (4), review the accuracy and uncertainty of the split point. Leveraging the AVEC plot, repeat steps 0 and 0 as required, until a satisfactory split point and reasonable split point uncertainty are achieved. Use the zoom in (🔍), zoom out (🔍), and pan (✋) utilities as required to manipulate the AVEC plot and support the selection of the split point and its uncertainty.

6.5. Run Monte Carlo analysis — GUI section (5).

6.5.1. In sub-section (a), insert the estimated precision of the instrument as a whole.

NOTE: Instrument precision (repeatability) in units of µg. The default value in *OCECgo* (0.031 µg) is based on estimates by the authors via replicate blank analyses.

6.5.2. In sub-section (b), insert the desired number of Monte Carlo draws for the computation of carbon masses.

NOTE: The number of Monte Carlo draws corresponds to the number of random computations of the carbon masses under the Monte Carlo framework. Larger numbers yield more accurate and consistent results, at the cost of computational time. The default value in *OCECgo* is 10^6 while permitted values are [10^2, 10^8].

6.5.3. In sub-section (c), press GO→ to run the Monte Carlo analysis to compute carbon masses and associated uncertainties.

NOTE: Following execution of the Monte Carlo analysis, the user is migrated to the **Data Analysis Tool – Results** tab.

6.6. Review results. The **Data Analysis Tool – Results** tab reports statistics of the measured OC, EC, and total carbon (TC); histograms of the Monte Carlo results; and the best-fitting posterior probability distribution of carbon masses for use in subsequent Monte Carlo procedures, chosen by the Akaike information criterion[28].

6.6.1. Press the **Export Analysis Results** button to export the Monte Carlo results.

NOTE: Numerical data are exported to a pre-formatted .xlsx file and visualization of the Monte Carlo results are exported as a .png file.

REPRESENTATIVE RESULTS:

Representative results for the calibration of the OCEC instrument are presented using example calibration data acquired by the authors, shown in **Table 1.** Here, a six-point calibration is used, obtained using the developed software and following the manufacturer's example in the instrument manual[18]. Results are shown in **Figure 2**. **Figure 2a** presents the results of linear regression on the calibration data under an MC framework. Black points correspond to the 2σ confidence interval of each of the six calibration points — i.e., carbon mass in the deposited sucrose is on the horizontal axis and instrument-reported total area (column 2 of **Table 1**) is on the vertical axis. The red shaded region represents the 2σ CI of the linear regression based on these six uncertain calibration data points − two each for 0, 5, and 10 µL of the sucrose solution (column 1 of **Table 1**). The mean carbon mass injected during the CH_4-loop is determined within the MC method. For each MC draw (2×10^7 in this example), a randomized calibration area (based on the distribution of the mean of calibration areas from column 3 of **Table 1**) is coupled with the uncertain linear model to obtain a MC-estimate of CH_4-loop carbon mass. The horizontal and vertical blue shaded regions correspond to the 2σ CIs of the mean calibration area and mass calibration constant from the MC analysis. The MC-estimates of these calibration data are shown in the scatterplot-histogram in **Figure 2b**. This example dataset yielded a calibration constant of 18.49 μg_C ± 2.78%.

[Place **Table 1** here]

[Place **Figure 2** here]

A representative data analysis using the developed software is provided for a measurement of carbonaceous emissions from a laboratory soot generator (MiniCAST 5201 Type C) burning nitrogen-diluted propane fuel[29]. Summaries of the sample data in the form of an OCEC thermogram (a plot displaying laser power, NDIR, and oven temperature during an OCEC analysis) and an AVEC[21] plot — created during protocol step 6.3.4 — are shown in **Figure 3a** and **Figure 3b**, respectively. In this example, uncertainty in the split point is estimated using the above-described "Attenuation decline" technique developed by the authors. The critical decline in attenuation was quantified as 1.342% yielding a split point uncertainty of 4.50% of TC mass. Key results of this analysis − carbon mass statistics and best-fitting posterior distributions − are summarized in **Table 2**.

[Place **Figure 3** here]

[Place **Table 2** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Thermal-optical analysis procedure. Analysis procedure of the thermal-optical OCEC instrument. Following the acquisition of a sample onto the quartz filter (step 0), three main analysis steps are performed. Two thermal protocols first in a helium atmosphere (He-phase, step 1) and then in an oxidizing atmosphere (Ox-phase, step 2) are executed, where carbonaceous components are pyrolyzed/oxidized from the quartz filter, further oxidized into CO_2 within a secondary catalyzing oven, and subsequently measured by an NDIR detector. A final in-test calibration procedure (CH_4-loop, step 3) is performed, where the oxidation of a known mass of methane provides a measure of sensitivity of the NDIR detector. Detector sensitivity is coupled with NDIR-measured CO_2 during the He- and Ox-phases to quantify carbon mass loading on the quartz filter.

Figure 2: Representative calibration results. (**a**) Results of the MC-regression of a six-point calibration dataset obtained by the authors. Calibration data with 2σ uncertainties are represented by black boxes and the 2σ CI of the linear regression is shown in the red shaded region. The horizontal blue bar corresponds to the 2σ CI of the mean NDIR area during the CH_4-loops ("calibration areas"), which are coupled with the uncertain linear regression to yield the 2σ CI of the mass calibration constant (represented by the vertical blue bar). (**b**) A scatterplot-histogram representing the MC-computed calibration data; mass calibration constant on the horizontal axis and mean calibration area on the vertical axis. In this example uncertainty in the

mass calibration constant was approximately 2.78%.

Figure 3: Representative analysis data. (**a**) A thermogram of OCEC-measured laser power, NDIR signal, desired (set), and measured (actual, act.) oven temperature. (**b**) The attenuation vs. evolved carbon (AVEC) plot corresponding to the presented histogram. The points correspond to instantaneous data reported at 1 Hz, with point colouring representing the instantaneous front oven (filter) temperature. The horizontal black line corresponds to the initial laser attenuation (following sample acquisition and prior to thermal analysis) used in the determination of the split point, while the horizontal red line corresponds to a 1.342% decline in initial laser attenuation used to estimate uncertainty in the split point. The grey shaded region represents the 2σ CI of the split point for this analysis, which is approximately 4.50% of TC mass.

Figure 4: Assessment of sample size on calibration uncertainty. Computed mass calibration constant for all possible combinations of calibration data listed in **Table 1** (requiring a minimum of three data, including at least one blank) normalized by the result using all six data. Relative uncertainty in the calibration constant decreases with an increase in the number of calibration data. The blue shaded area in the figure corresponds to the 2σ CI of the calibration constant computed using all calibration data. It is apparent that all nominal results are within this CI suggesting that — although uncertain — a truncated calibration procedure of just three calibration data points might be used as a "bump test" check of the instrument's calibration.

Table 1: Representative calibration data. Representative calibration data containing two blanks and two measurements each at 5 µL and 10 µL of sucrose solution, consistent with the manufacturer's example in the instrument manual[18].

Table 2: Representative carbon mass results. MC-computed OC, EC, and TC masses for the example data plotted in **Figure 3** with a 2σ uncertainty in the split point of 4.50%, corresponding to a 1.342% decline in initial laser attenuation. Listed are the mean and 2σ uncertainties of the MC data, in addition to the best-fitting posterior distributions.

Table 3: Uncertainties in mass calibration constant. Representative contribution of uncertainties in the calibration of the OCEC instrument for the example six-point calibration (see **Table 1**). Overall calibration uncertainty is dominated by bias in the NDIR detector with error due to accuracy in the pipetting of the sucrose solution, including human-errors (inter-user reproducibility and intra-user repeatability), being second-most important, followed by uncertainty in linear regression and sucrose concentration (which are both negligible).

Table 4: Uncertainties in data analysis. Contribution to uncertainty in OCEC-measured carbon masses for four example datasets from a broad range of sources and performed by different laboratories[27,29,30]. (**a**) Key numerical results from the example datasets: OC, EC, and TC masses, critical attenuation decline for the quantification of split point uncertainty, and elemental-to-total carbon ratio. (**b**) A summary of uncertainties in the calculated carbon masses. Contributing sources of uncertainty include that of the mass calibration constant, repeatability of the calibration procedure, and uncertainty in the split point (relative to TC mass) corresponding to

the critical attenuation decline listed in (a). Uncertainties in the carbon masses using the fixed equations (Eq. (1)) employed by the instrument are also shown in (b). Red and yellow highlights of the data correspond to under- and over-estimation of uncertainty, respectively, when using the fixed equations relative to the present method. In most cases, the instrument under-estimates carbon mass uncertainty however, if measured OC or EC mass is small, the instrument may over-estimate uncertainty as compared to the present software.

DISCUSSION:

Table 3 shows the contribution of the specific sources of uncertainty to the mass calibration constant for the example case described in **Table 1** and **Figure 2**. Cumulative calibration uncertainty resulting from regression of the data, bias error in the NDIR detector, bias error in the concentration of sucrose, and precision and bias error in pipetted volumes are listed. Bias error in the NDIR detector (i.e., variance in the "calibration area") tends to dominate, with bias in the pipetting procedure being second-most important (although quite small in the representative example). Proper estimation of pipetting error is thus critical to ensuring accurate quantification of overall calibration uncertainty; referring to protocol step 4.3.2, it is therefore suggested that intra-user repeatability and inter-user reproducibility is assessed for each group of users and pipette. In contrast, uncertainty due to the concentration of sucrose in the external standard and due to regression of the nominal calibration data are negligibly small. The negligible size of the latter contribution is a consequence of the good linearity of the instrument − the coefficient of determination (R^2) for a linear fit of the calibration data generally exceeds 99.95%. If calibration data are not sufficiently linear, the software automatically warns the user, who is then able to troubleshoot the dataset via the Boolean control noted in protocol step 4.2; the user can then modify their calibration dataset by acquiring replacement data as needed.

[Place **Table 3** here]

Calibration of the OCEC instrument is a time-consuming procedure, typically requiring 2−3 hours to complete depending upon the length of the thermal protocol employed. A more rapid calibration procedure is desirable. To this end, the efficacy of a modified, truncated calibration protocol was analyzed with the presented software tool. The developed MC procedure was executed using all possible subsets of the example calibration data listed in **Table 1** — limited to cases with three or more data and a minimum of one blank measurement. All resulting mass calibration constants from this analysis are plotted in **Figure 4** as a function of number of calibration data used, where calibration constants have been normalized by the full (6-point) calibration result. Unsurprisingly, uncertainty in the calibration constant increases as available calibration data are reduced. Importantly however, the means of all truncated calibrations fall within the 2σ CI of the full calibration result, which is a consequence of the above-noted linearity of the instrument. This consistency in the MC-average suggests that a modified, faster calibration consisting of few calibration data can be employed as a "bump test" check of the OCEC instrument's calibration. That is, if the MC-average of a 3-point calibration data set is within the 2σ CI of the existing calibration, it is likely that the OCEC instrument does not require re-calibration. It is also apparent in **Figure 4** that calibration uncertainty is reduced with more calibration data, but the reduction in uncertainty suffers from diminishing returns. Referring to

Table 3 and its discussion above, since calibration uncertainties are dominated by NDIR bias (quantified with the standard error of the "calibration areas"), the marginal decrease in calibration uncertainty by including the n^th^ data point can be estimated with the factor $\sqrt{(1-1/n)}$. Consequently, in the representative example, the marginal reduction in uncertainty is larger when moving from a three- to a four-point calibration than when moving from a five- to a six-point calibration. The developed software tool, which can be executed following acquisition of each calibration data point (i.e., following each repetition of protocol step 3.3), allows the user to quantify calibration uncertainty throughout the acquisition of data. Critically, this ability enables the user to not only decide upon adequate convergence of the calibration in the context of its uncertainty, but also detect the presence of spurious data – that is, an incremental decrease in calibration uncertainty that is notably different than expected highlights to the user that the most recently acquired calibration data point could be flawed.

[Place **Figure 4** here]

Calculated carbon masses and uncertainties for four example datasets are detailed in **Table 4**; these data are sourced from measurements of carbonaceous emissions from hydrocarbon flames[27,29], gas turbines[29], and fine-mode (< 2 μm) carbonaceous particles obtained from sub-ocean sediment samples[30]. Computed (MC-average) OC, EC, and TC masses are shown in **Table 4** alongside the computed critical attenuation decline and the EC/TC ratio for each dataset, showing the breadth of the example data in the context of sampled carbon composition. Summarized in **Table 4** are the sources and overall uncertainties in carbon mass using the presented software tool compared to those reported by the instrument. Within the software tool, the combination (product distribution) of calibration uncertainty and repeatability yields the overall uncertainty in MC-computed TC mass (quantified as −8.32/+8.40% in the present work), which is independent of uncertainty in the split point and therefore acts as a lower limit in the uncertainty of OC and EC masses. These representative calibration uncertainties are applied to each example dataset while maintaining the nominal mass calibration constant used in the original analyses.

[Place **Table 4** here]

For these examples, the attenuation decline technique for quantifying the split point uncertainty was employed. *OCECgo*-computed critical values of attenuation decline ranged from of 1.342% to 2.059% resulting in split point uncertainties from 0.10% to 4.50% of TC mass. While the employed values of attenuation decline are indeed somewhat subjective − particularly the employed factor to estimate uncertainty in the split point due to that of optical properties − these examples highlight the dependence of split point uncertainty upon the specific analysis data. For example, split point uncertainty is sensitive to the slope of the AVEC plot in the vicinity of the nominal split point. Consider dataset "A" corresponding to the example data in **Figure 3** and dataset "D"; despite having similar critical attenuation declines, the relatively shallow and steep slopes of their respective AVEC plots (e.g., see **Figure 3b** for dataset "A") yield the largest and smallest split point uncertainties of 4.50% and 0.10% of TC mass. Additionally, the example data show that the influence of uncertainty in the split point depends largely on its scale relative to the nominal OC and EC masses. Consider example datasets “B” and “C”, which have near-identical split point uncertainties (≈1.22% of TC mass); dataset “C” contains ≈43% OC while dataset “B” contains ≈8%; the lower relative quantity of OC in the latter results in an almost doubling of OC mass uncertainty. Critically, these results highlight the requirement to directly consider split point uncertainty in the context of the analysis’ AVEC data and overall carbon masses.

Manufacturer-reported uncertainties in carbon masses are also shown in **Table 4**. These estimates do not directly consider uncertainties in the calibration and split point, but rather are calculated using the fixed relations shown in Eq. (1)[26], where m_i represents the nominal mass of the specific carbon component.

$$\begin{aligned} U_{OC} &= 5\% + 0.2\ \mu g/m_{OC} \\ U_{EC} &= 5\% + 0.2\ \mu g/m_{EC} \\ U_{TC} &= 5\% + 0.3\ \mu g/m_{TC} \end{aligned} \qquad (1)$$

These fixed relations permit estimated uncertainties in OC and/or EC mass to be artificially less than that of TC mass − this condition occurs when OC or EC mass is less than one third of TC mass, as is the case for datasets “A”, “B”, and “D”. This result is non-physical, since relative uncertainty in OC and EC masses must be bounded below by the that of TC mass, a consequence of the propagation of split point uncertainty into the calculated OC and EC masses. Cells highlighted red and yellow in the table correspond to under- and over-estimations of carbon mass uncertainty when using Eq. (1) from the manufacturer. The fixed equations under-estimate TC mass uncertainty for all four examples, a consequence of computed TC masses being sufficiently large. In most cases, the fixed equations also under-estimate EC and OC mass uncertainty, except where OC (dataset “B”) and EC (dataset “D”) were sufficiently small to cause over-estimation via Eq. (1). This asymptotic increase in uncertainty via Eq. (1) agrees with the present software since uncertainty in OC and EC masses due to that of the split point are dependent upon their absolute magnitude; however, uncertainties via the fixed equations fail to track with those of the present software, which directly considers and propagates split point uncertainty in the context of specific analysis data.

Use of a MC framework in the presented software tool is necessary to accurately propagate component uncertainties through the non-linear algorithms of thermal-optical OC/EC analyses. It is important to note however, through their inherently random nature, that MC methods are not deterministic and tend to yield inconsistent results if the employed number of MC draws/repetitions (see protocol steps 4.3.3 and 6.5.2) is insufficient – akin to a statistically undersized sample. Therefore, there is an inherent consistency vs. computation time trade-off to be considered when processing data using *OCECgo*. It is thus recommended to users to perform preliminary processing and troubleshooting of the data using a small number of MC draws (e.g., 10^4). Once computations yield satisfactory results, the user should then increase the number of MC draws (to 10^6–10^8) to yield a result that is less-affected by the discrete and random nature of the MC method. Beyond the necessary “operational definition” of thermal-optical OC/EC analyses, there are other limitations in the processing of these data that must also be recognized when using *OCECgo* to compute and report OC/EC data. Firstly, NDIR-based instruments (such as that listed in the **Table of Materials**) suffer from drift in the NDIR signal that must be corrected. In the present protocol (see step 6.3.2 and the *OCECgo* documentation), a novel approach to correct drift in the NDIR detector can be optionally employed by the user. While, in the authors' experience this yields improved results over the manufacturer's standard linear NDIR correction, it must be noted that uncertainty in this NDIR correction is challenging if not impossible to quantify and therefore remains an unaccounted component of uncertainty in the computation of carbon masses. In a similar vein, it is also challenging to quantify uncertainty in the necessary assumption that PC and EC share optical properties. If selected (see protocol step 0), the critical attenuation decline technique attempts to conservatively bound the effect of this assumption through a subjective expansion factor. Importantly however, this is necessarily only an estimate, and the user is suggested to assess the effect of this expansion factor (i.e., the critical attenuation decline) on their specific data. *OCECgo* is provided as an open-source tool so that it may easily be extended by the authors and other interested collaborators to not only interface with other instruments but to also include other useful, field-specific, functionalities. Overall, the developed open-source software tool coupled with the detailed calibration procedure is intended to help achieve more accurate measurements of OC, EC, and TC mass in aerosol samples while streamlining the robust calculation of measurement uncertainties.

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The authors have nothing to disclose.

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Figure 1

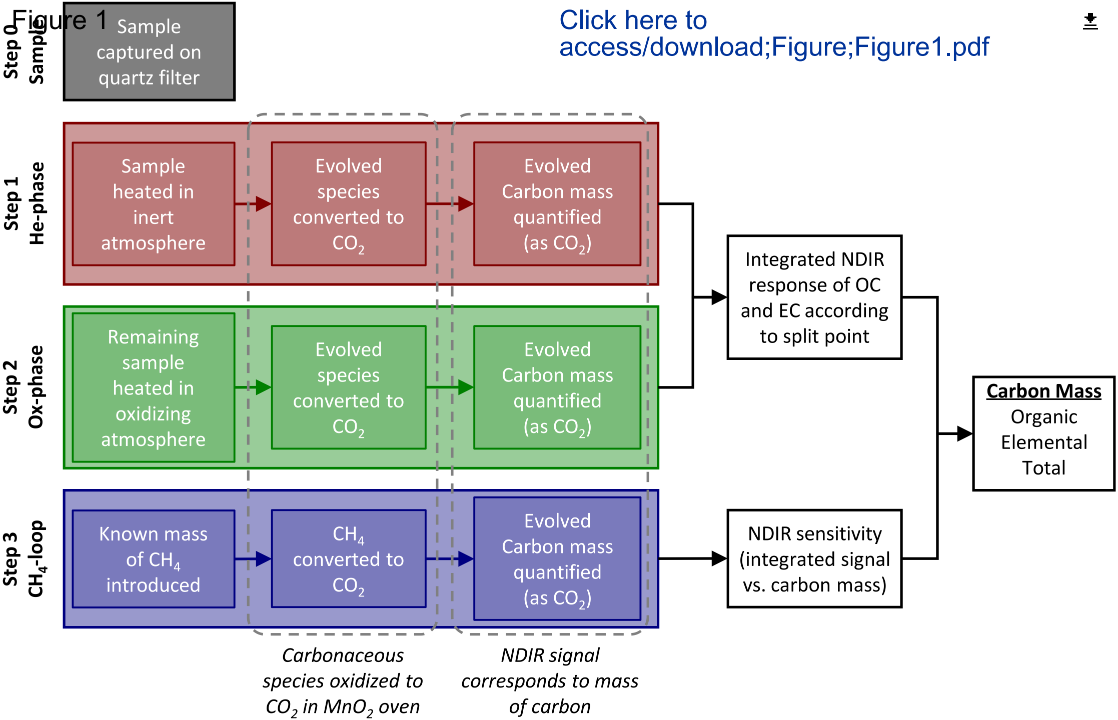

Step 0 Sample
Sample captured on quartz filter
Step 1 He-phase
Sample heated in inert atmosphere
Evolved species converted to CO_2
Evolved Carbon mass quantified (as CO_2)
Step 2 Ox-phase
Remaining sample heated in oxidizing atmosphere
Evolved species converted to CO_2
Evolved Carbon mass quantified (as CO_2)
Step 3 CH_4-loop
Known mass of CH_4 introduced
CH_4 converted to CO_2
Evolved Carbon mass quantified (as CO_2)
Integrated NDIR response of OC and EC according to split point
NDIR sensitivity (integrated signal vs. carbon mass)
Carbon Mass
Organic
Elemental
Total
Carbonaceous species oxidized to CO_2 in MnO_2 oven
NDIR signal corresponds to mass of carbon

Figure 2

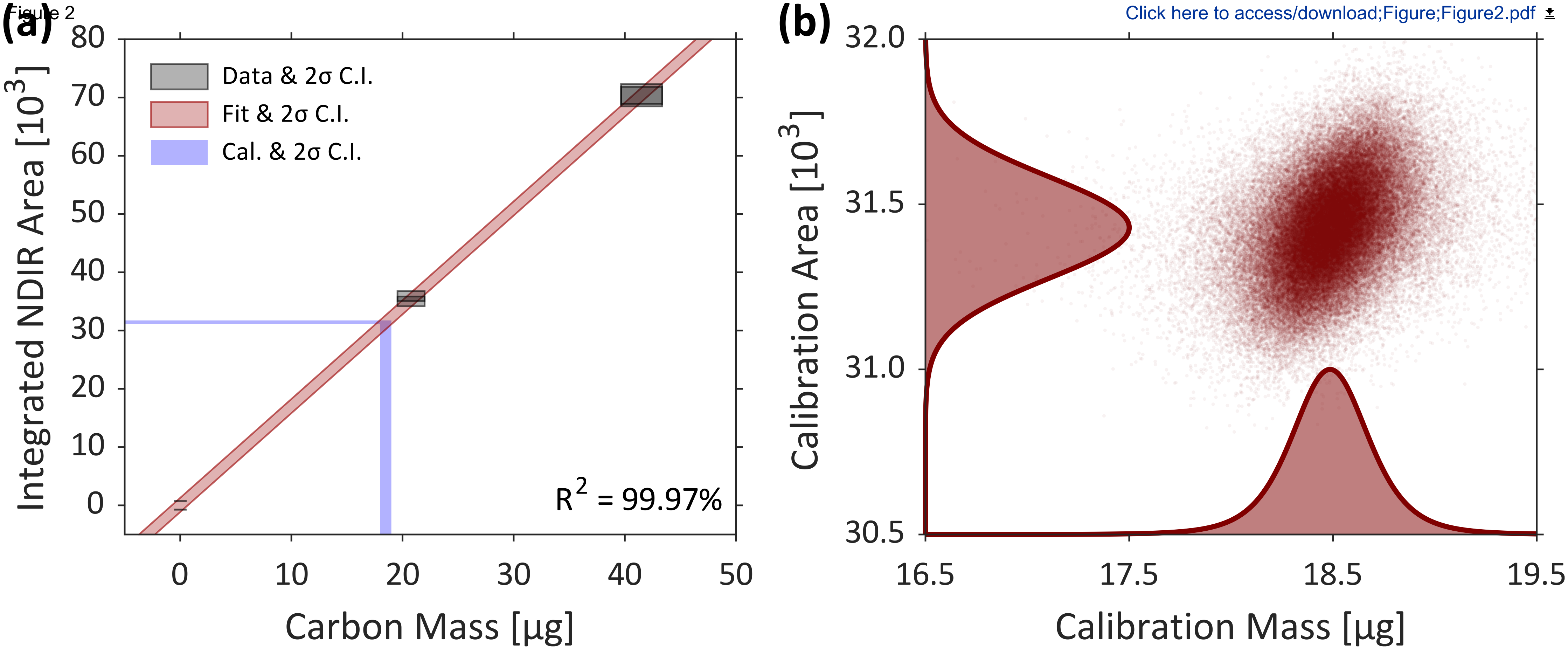

(a)
Integrated NDIR Area [10^3]
80
70
60
50
40
30
20
10
0
Data & 2σ C.I.
Fit & 2σ C.I.
Cal. & 2σ C.I.
R^2 = 99.97%
0
10
20
30
40
50
Carbon Mass [μg]
(b)
Calibration Area [10^3]
32.0
31.5
31.0
30.5
16.5
17.5
18.5
19.5
Calibration Mass [μg]

Figure 3

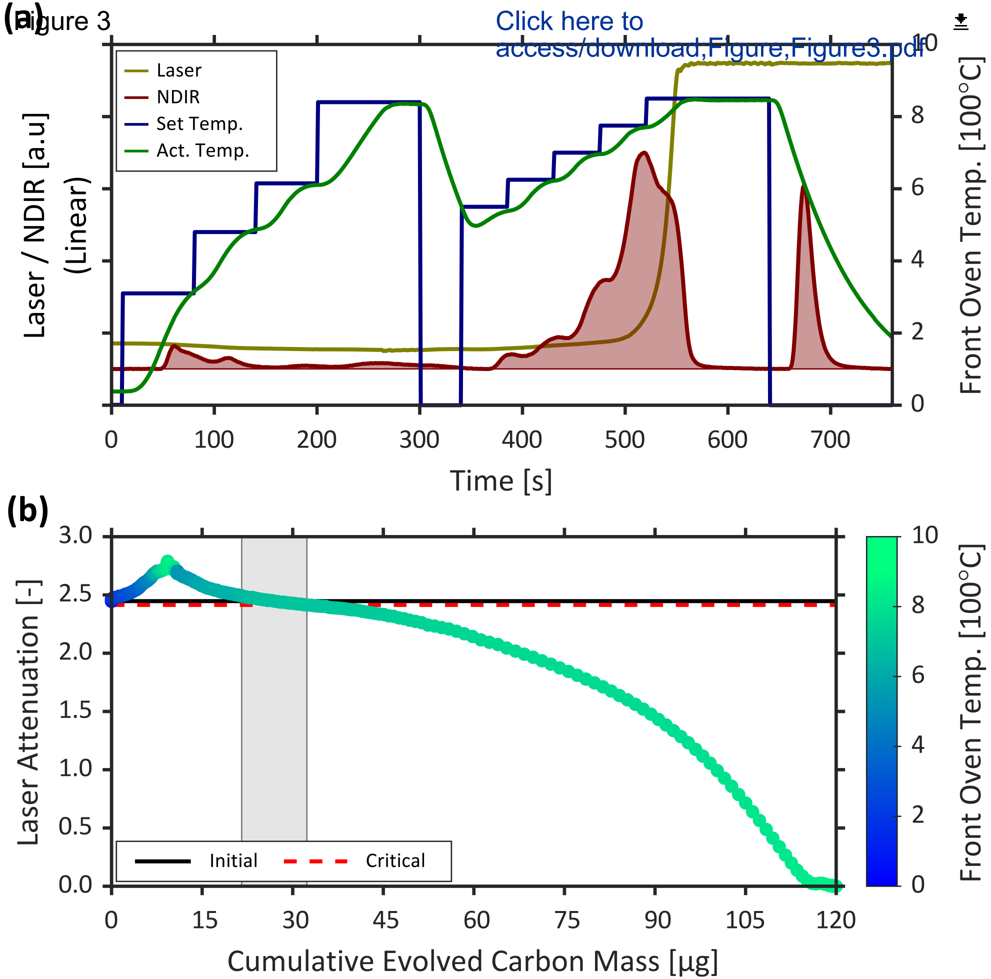
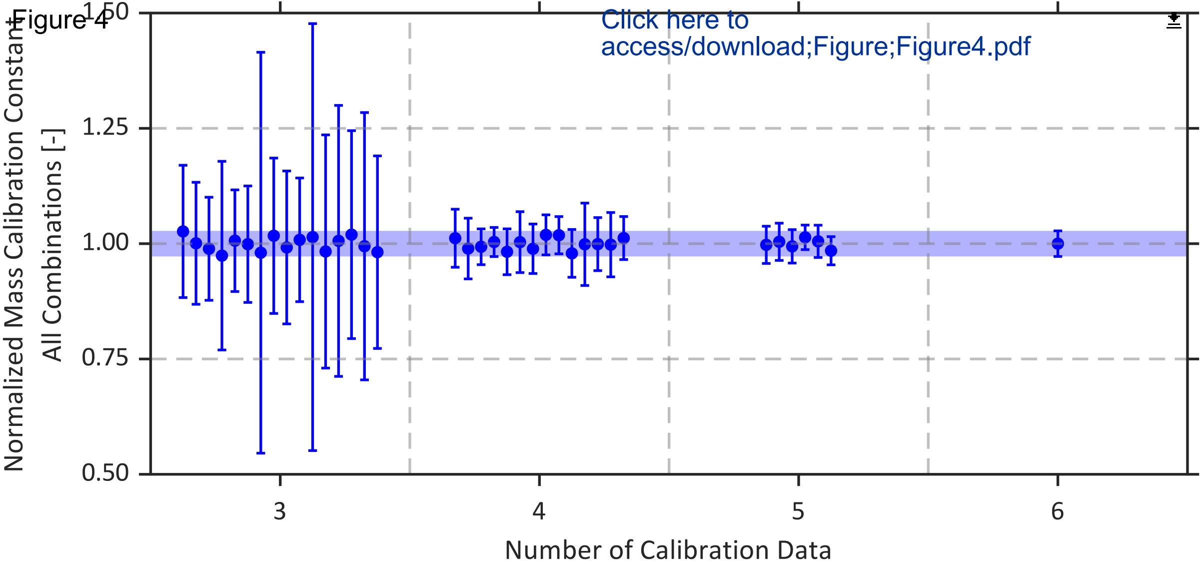

(a)
Laser
NDIR
Set Temp.
Act. Temp.
Laser / NDIR [a.u]
(Linear)
Front Oven Temp. [100°C]
0
2
4
6
8
10
0
100
200
300
400
500
600
700
Time [s]
(b)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Laser Attenuation [-]
Initial
Critical
0
15
30
45
60
75
90
105
120
Cumulative Evolved Carbon Mass [μg]
10
8
6
4
2
0
Front Oven Temp. [100°C]

Figure 4


Normalized Mass Calibration Constant
All Combinations [-]
1.50
1.25
1.00
0.75
0.50
3
4
5
6
Number of Calibration Data

Calibration Data		
Volume of Sucrose Solution[a] [µL]	"Total" NDIR Area [-]	"Calibration" NDIR Area [-] (CH_4-loop)
0	3041	31297
5	38229	31281
5	37321	32056
10	72472	31435
0	1589	31583
10	72914	30926

[a] Nominal concentration of 4.148 ± 0.022 g_C/L at 25 °C

Table 2

Carbon Type	Mean Mass [μg_C]	2σ Uncertainty of MC Data	Best-fitting Distribution[a]
Organic Carbon (OC)	26.94	-21.3% +22.2%	*N(26.94, 2.925)*
Elemental Carbon (EC)	93.11	-9.98% +10.4%	*Γ(385.7, 0.2414)*
Total Carbon (TC)	120.05	-8.32% +8.40%	*N(120.1, 5.014)*

[a] Normal: N(μ, σ); Gamma: Γ(a, b[scale])

Considered Uncertainties	Full (6-point) Calibration
NDIR bias	± 2.61%
+ Sucrose solution	± 2.61%
+ Pipette	± 2.78%
Nominal Result [μg_C]	18.49

a) Key Numerical Results			**Sample**	**A**	**B**	**C**	**D**
			OC Mass [μg_C]	26.94	0.80	10.18	29.72
			EC Mass [μg_C]	93.11	8.91	13.65	1.68
			TC Mass [μg_C]	120.05	9.71	23.83	31.40
			Critical Attenuation Decline	1.342%	1.756%	2.059%	1.380%
			EC/TC Ratio	77.6%	91.8%	57.3%	5.35%
b) Measurement Uncertainties[a]	**Independent Contributions**		Cal. Constant	2.78%			
			Cal. Repeatability	7.90%			
			Split Point	4.50%	1.22%	1.22%	0.10%
	Total/Combined Uncertainty	**Present Software[b]**	**OC Mass**	**21.7%**	**17.0%**	**8.83%**	**8.36%**
			EC Mass	**10.2%**	**8.47%**	**8.63%**	**8.55%**
			TC Mass	**8.36%**			
		Instrument-reported	*OC Mass*	*5.74%*	*30.0%*	*6.96%*	*5.67%*
			EC Mass	*5.21%*	*7.24%*	*6.47%*	*16.9%*
			TC Mass	*5.25%*	*8.09%*	*6.26%*	*5.96%*

[a] Root-mean-squared of negative and positive components, relative to nominal mean

[b] Total carbon mass uncertainty is not affected by the split point.

Name of Material / Equipment	Company
10% oxygen gas in helium	Local gas supplier
5% methane gas in helium	Local gas supplier
Distilled, de-ionized water	Harleco
Filter punch tool	Sunset Laboratories Inc.
Filter removal tool	Sunset Laboratories Inc.
Glass jar (4 oz.)	ULINE
Helium gas	Local gas supplier
High-accuracy thermal gas mass flow meter	Bronkhorst
High-purity sucrose	Sigma Aldrich
Lint-free tissues	Kimtech
MatLab Runtime (R2016a or newer)	MathWorks Inc.
Non-powdered, disposable, plastic gloves	VWR
OCECgo software	Carleton University, Energy and Emissions Research Lab.
Oxygen trap	Supelco
Pipette	Eppendorf
Pipette tips	Eppendorf
Precision scale / balance	AND
Quartz filters	Pall
Semi-continuous thermal-optical organic/elemental carbon analyzer	Sunset Laboratories Inc.

Catalog Number	Comments/Description
- - -	Primary or certified standard preferred
- - -	Primary or certified standard preferred
6442-85	ASTM D1193-91 Type II or Type I (preferred)
- - -	Included with carbon analyzer
- - -	Included with carbon analyzer
S-17982P-BL	Or suitable equivalent; borosillicate glass preferred
- - -	Ultra-high purity (> 99.999%) or better preferred
EL-FLOW Prestige	For accurate measurement of sample volume (see **Protocol** step 5)
S9378	Purity ≥ $99\%_{m/m}$ or higher preferred
34155	Or suitable equivalent
mathworks.com	Search "runtime compiler" and install appropriate version for the operating system
89428-752	Or suitable, properly-sized equivalent
GitHub Repository	Source and build distributions of the software are available on GitHub
22449	Or suitable GC-quality equivalent
3120000020	Model: Research® Plus 0.5 - 10 µL - Or any single-channel, adjustable volume, manual pipette
022492012	Model: epT.I.P.S.® Standard, 0.1 - 20 µL
FX-3000IWP	Precision balance with capacity > 1 kg
7202	Model: Tissuquartz 2500 QAT-UP - 47 mm
- - -	Model 4 semi-continuous analyzer

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Author(s): Bradley M. Conrad, Matthew R. Johnson

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Manuscript ID: JoVE59742
Title: Calibration Protocol and Software for Split Point Analysis and Uncertainty Quantification of Thermal-Optical Organic / Elemental Carbon Measurements
Authors: Conrad, Bradley; Johnson, Matthew

Point-by-point Response to Reviewer Comments

Reviewer 1

The reviewer has limited experience with the Sunset TOA, but does believe the OC/EC/TC measurement uncertainties estimated by the manufacture (i.e., Eq. (1)) cannot be representative. There is no reason to assume uncertainties converging at 5% for high OC, EC, and TC values while neglecting the calibration results. Therefore, the effort made by the authors should be encouraged. Information presented in the manuscript, however, are somewhat scattered and difficult to follow. The reviewer suggests moving the "Protocol" to the appendix or supplemental information and accompanying it with diagrams and/or videos to facilitate readers. The Figure and Table Captions can be moved to the end of paper, before or with the Figures and Tables.

We thank the Reviewer for recognizing the need for improved uncertainty characterization in thermal-optical instruments, especially in the context of the manufacturer's uncertainty estimates. We appreciate the suggestions with respect to the layout of the manuscript, but we believe that it is currently formatted in agreement with the JoVE guide to authors. We would gladly adjust the format as required but we suggest that this decision is left to the editor and typesetter.

Additional comments*:*

The authors should also give suggestions on the selection of "laser correction procedure" and "critical attenuation decline" in the protocol. Certainly the OC and EC results will be influenced by these selections. Are any sensitivity tests necessary to explore the selection of these parameters? How can they be consistent among users?

We agree, and in the revised **Protocol**, we now highlight that the software's mouseover utilities provide support to the user inputs while noting recommended selections.

Regarding the "laser correction procedure": our experience has shown that this has a very minor effect on the split point and, hence, OC and EC masses – the effect is often less than or on the order of the significant figures reported by the software tool. As such, we make the 3-parameter "quadratic correction" default to ensure an adequate fit to the laser-temperature relation. The mouseover utility also highlights the "quadratic correction" as recommended.

Regarding the "critical attenuation decline": as noted in the manuscript's introduction, a "critical attenuation decline" is estimated based on uncertainty in the TOT split point definition – i.e., uncertainty in laser attenuation relative to its initial value. This value is automatically updated upon plotting of the thermal analysis data (by clicking the button in section (2)(d) of the "Data Analysis Tool – Inputs" tab); the suggested value is also indicated in text directly below the appropriate input – e.g. "Suggested attenuation decline is x.xxx%".

OC and EC results can indeed be influenced by the software settings; however, other than minor variations resulting from Monte Carlo randomness, computed carbon masses are deterministic when the default software inputs are used, or any consistent inputs are used. Although sensitivity tests can indeed be useful to help capture inconsistency amongst users, we suggest that they are not required. This is based on the notion that user subjectivity is captured by the understanding that carbon masses are *operationally-defined;* furthermore, uncertainty in the parameters enabling the calculation of the *operationally-defined* carbon masses are fully propagated through the *OCECgo* software.

The procedure of Monte Carlo calculation is not described adequately in the paper. Please indicate how it is accomplished, including the equations used for error propagation. Do you use Matlab built-in toolbox? Please also indicate if the minimum detection limit is used explicitly to estimate uncertainty.

The specifics of our custom Monte Carlo (MC) method are more detailed than appropriate for the length of the JoVE manuscript. For this reason, we instead extensively detail the custom-written MC procedure in the software's documentation, which was provided with the initial submission and will be distributed with the source and build distributions of the developed software.

As elaborated in the comment below, the minimum detection limit is only useful for estimating instrument precision (via a "noise floor") and is not, in general, a reliable indicator of the total instrument uncertainty. In the present case, as detailed in the manuscript, total uncertainty is dominated by bias errors and the employed MC method rigorously captures each of these contributing sources to compute overall uncertainty in carbon mass. In response to the Reviewer's comment below, for completeness we have added the provision to propagate instrument precision error (as a final component of the total uncertainty) through our presented MC method and software tool. However, in the present case, using variance in repeated blank analyses as a surrogate of instrument precision, this contribution proves negligible.

Replicate analysis (i.e., analyze the same samples multiple times) is a widely used alternative to quantify measurement uncertainties in TC, OC, and EC. This may be a legitimate method to confirm your uncertainty estimate based on error propagation. As part of the paper, the reviewer would recommend to compare uncertainties from the two approaches.

We agree that replicate analysis is a useful tool to quantify the component of the total uncertainty attributable to instrument precision (unstructured/random/repeatability error); however, replicate analysis does not yield information on instrument bias (structured error). One key contribution of our presented software is the estimation of instrument bias through the consideration of fundamental contributing uncertainties, as detailed in the **Introduction** of the manuscript. Instrument precision (repeatability) error, we suggest, will be instrument-, user-, and application-dependent and could indeed be computed by a user via replicate analysis. This measure of precision could be combined with the bias uncertainties computed with *OCECgo*. Based on the reviewer's comment, we have included an additional field in the software tool (Data Analysis Tool – Inputs; Section (4)(a)) where a user could optionally provide appropriate replicate analysis results to propagate instrument precision into the uncertainties of computed carbon masses.

Reviewer 2

Manuscript Summary:

The paper describes a calibration protocol for any existing/commercial TOA instrument. They provided an open-source Mat-Lab based software tool for quantifying the uncertainties in the measurement of OC and EC. The software tool goes over and above that given by the manufacture which uses fixed equations for the relative and absolute errors. The title appropriately describes the method presented. The abstract has a nice length and provides a nice overview of the paper. The introduction describes the presented method and explains why the presented method can be useful. The protocols give detailed steps for obtaining improved measurements of OC and EC using the Sunset TOA and their improved software tool for error estimates.

We thank the reviewer for their positive feedback and recognition of the utility of the developed protocol and software.

Major Concerns:

None

Minor Concerns:

There are some suggestions how to further improve this paper, below:

Introduction:
Provide motivation why the Sunset Model 4 is preferred over other TOA instruments. How does the Sunset measurements or indeed your improved measurements with the Sunset instrument compare with the more recognizable NIOSH method 5040?

We do not mean to suggest that the Sunset Model 4 (semi-continuous) is the preferred instrument for thermal-optical organic/elemental carbon analyses. Indeed, the Magee Scientific (Desert Research Institute) instrument and the Sunset laboratory-based instrument (with flame ionization detector) are viable alternatives. Our software however, has been developed to integrate with the results file output by the Sunset Model 4 (with non-dispersive infrared detector) but, as we note in lines 65-66 of the original manuscript, "…the software tool could readily be extended … to work with outputs generated by other instruments."

The presented software and the studied instrument are both agnostic to the specific thermal protocol − example data generated by our cited references were created using the standard NIOSH method 5040 and the European EUSAAR2 method.

Protocol:
The following safety issues must be highlighted either in the protocols or in the discussion

1. *Laser, mention the necessary precautions needed when handling the laser system.*
2. *The high temperatures involved (870 ℃) must be highlighted either in the protocols or in the discussion*

We agree that these safety concerns are important for safe operation of the instrument and have added text to this effect as step 0 revised **Protocol**.

Table of materials:
I think the following items described in the protocols should also be included in the materials table

Helium (preferably UHP
Helium/Oxygen mixture
Helium/Methane mixture
Filter punch tool
Filter removal tool

We have added the suggested materials to the revised **Table of Materials**.

Reviewer 3

Manuscript Summary:

The authors describe a protocol for determining the uncertainty in quantification of organic and elemental carbon using thermal-optical analysis. A MatLab based program was designed to assist in the calibration and quantification of sources of uncertainty with the TOA assessment.

Major Concerns:

None

Minor Concerns:

A discussion of how the user should evaluate the uncertainty results and ways to decrease uncertainty would be helpful for the readers, particularly for use with unknown compounds. Please see below for comments on individual sections.

In agreement with the Editor's comment regarding content of the **Discussion**, we have added significant text noting the critical steps of the protocol, evaluation of results, and means to reduce uncertainty.

Introduction:

The introduction is well written, providing a background on carbonaceous compounds, TOA measurement techniques, and the issues with current calibration processes. Details on how system overcomes described issues? Comparison.

We thank the reviewer for the positive feedback. General issues with thermal-optical analysis approach and the underlying "operationally-defined" distinction between OC and EC that is intrinsic to thermal analyses cannot be overcome because they are inherent to the measurement technique. However, as detailed in the manuscript the developed protocol and software enables rigorous, quantitative calculation of uncertainties associated with both calibration and operation of the instrument, particularly the determination of the split point between OC and EC. This is a significant advance that will improve accuracy and robustness of measurements as noted by all reviewers. Table 4 and associated discussion compare uncertainties quantified with this new approach versus the simplified uncertainty estimates output by the manufacturer's software for a variety of samples.

Line 61 - does the calibration have to be performed manually? Could the program be used with other instruments?

As will be shown in the protocol visualization through the video, the calibration procedure (specifically the steps in **Protocol** sections 2 and 3) requires significant manual manipulation of the instrument − i.e., for each calibration data point, the instrument must be opened, and the sucrose solution must be manually applied.

At present, the *OCECgo* software tool is designed to integrate with the Sunset Model 4 instrument; however, as noted in lines 65-66 of the original manuscript, "…the software tool could readily be extended … to work with outputs generated by other instruments."

Protocol:
The protocol is well written with sufficient detail for the reader to perform.

Step 3.3 - In the author's experience, what is an appropriate number of initial calibration points to perform prior to moving to Step 4?

The appropriate quantity of calibration data is dependent upon the user's desired uncertainty in the mass calibration constant. However, one very useful feature of the *OCECgo* tool (specifically, step 4 of the protocol) is that it can be used in parallel with the acquisition of calibration data (i.e. as step 3 is repeated) to calculate calibration uncertainties in real-time, enabling the user to decide when sufficient repeats have been performed and uncertainty in the mass calibration constant is acceptable. As discussed in the **Representative Results** section of the original manuscript, the standard quantity of calibration data according to the instrument's manual[1] is six; however (referring to the **Discussion** of the original manuscript), satisfactory calibration may be achieved with fewer data and the acquisition of further data comes with diminishing returns. Additionally, we note in the manuscript that a brief three-point calibration set can be used as a "bump-test check" of the instrument to assess whether a full calibration is required.

Step 4.3.2.1 - Please define 2σ.

As noted in the revised manuscript, 2σ is conventional notation for two times the standard deviation (σ). Assuming a Gaussian/Normal distribution, 2σ uncertainty is a conservative estimation of the 95% confidence interval of an uncertain metric. In the context of step 4.3.2 of the original protocol, this corresponds to the pipette's manufacturer-reported statistics.

Step 4.5 - Do the authors have suggestions to review the Monte Carlo results?

Review of the Monte Carlo results is necessary to assess the convergence of the calibration procedure. This is best performed through the analysis of statistics resulting from the Monte Carlo analysis and the subjective definition of "satisfactory" calibration uncertainties.

Step 4.5 has been rewritten in the revised manuscript. When the Monte Carlo analysis is completed, OCECgo automatically graphs the fitted calibration data with confidence limits and shows the uncertainty distribution of the calibrated mass results. The final calibration data with 95% confidence intervals (shown as a % uncertainty) and parameters describing the shape of the distributions best representing these results are shown in Section 4 of the GUI. As noted in the revised manuscript (lines 570−573), if the calibration data are not sufficiently linear, the software automatically warns the user, who is then able to troubleshoot the dataset via the Boolean control noted in step 4.2 of the **Protocol**; the user can then modify their calibration dataset by acquiring replacement data as needed.

Results:
Please provide the details of the sucrose solution used for the representative results.

The sucrose solution used in the **Representative Results** corresponds to that developed using **Protocol** section 1. As now noted in line 89 of the revised manuscript, this solution is approximately 0.99% sucrose by mass. At typical room temperature of 25°C, the carbon mass concentration of the solution employed in our specific analysis was 4.148 ± 0.022 g_C/L (2σ), which has been added as a footnote to Table 1 of the manuscript.

Table 1 - Is the order of the volume of sucrose solutions due to the example given by the OCEC instrument? A comment on this would be helpful.

The order that the volumes of sucrose solution are provided to the instrument for calibration is at the user's discretion. It is recommended however, that the order is randomly permuted from calibration to calibration to avoid any unknown hysteresis effects. To note this, we have updated text at lines 89−91 of the revised manuscript:

> *"Repeated tests are performed where different known volumes of the sucrose solution are introduced **in random order** into the instrument and the thermal-optical analysis is performed."*

What is the original uncertainty/calibration provided by the OCEC instrument?

We present the instrument's uncertainty calculation in Equation (1), where there is a relative and absolute component for each carbon mass (OC, EC, and TC).

The original calibration of the instrument corresponds to the first implementation of the presented calibration protocol once the instrument is received, and is therefore not a standard value. Nominally however, during the CH_4-loop, 0.8 mL of the 5% CH_4-He gas mixture is injected into the instrument. Assuming standard volumetric conditions of 25°C and 1 atm, this corresponds to a carbon mass in methane of ~19.6 μg_C.

Discussion section:

What are the critical steps in the protocol to ensure success?

In response to this comment and the Editor's comment to the same effect, we have revised our **Discussion** section to include this important discussion.

Are there additional ways other than more calibration points to decrease uncertainty?

Referring to Table 3 of the original manuscript, bias in the instrument's NDIR is the most dominant contributor to overall uncertainty. This NDIR bias is estimated by *OCECgo* as the relative 2σ error of an imposed normal distribution representing the calibration areas (step 4.2.1 of the original **Protocol**) reported by the instrument. Unfortunately, this largest source of uncertainty is outside of the user's influence. Marginal reductions in uncertainty might be obtainable by reducing those of the pipette, however (as suggested by the data in Table 3) this would yield a very minor effect.

Reviewer 4:

Manuscript Summary:

The manuscript entitled "Calibration Protocol and Software for Split Point Analysis and Uncertainty Quantification of Thermal-Optical Organic / Elemental Carbon Measurements" by Conrad and Jhonson have tried to develop a protocol for calibrating the OC/EC instrument alongside a software tool for accurate and precise quantification of calibration errors and uncertainties during OC/EC measurements. The manuscript is well written with descriptive methodology and precise introduction to the topic. Results are well presented and discussed.

Overall the manuscript does fulfill the standard of the Journal and I suggest the editor accept the manuscript after incorporating minor suggestions.

We appreciate the positive feedback of the reviewer and thank them for their effort in reviewing our work.

Major Concerns:

Why the statistical part was only validated through MATLAB, why not with other open source programs such as R.

There indeed exist myriad software tools that could have been used to perform this work. Using MatLab allowed us to develop the graphical user interface of the *OCECgo* software and was well-suited to efficiently performing all calculations and Monte Carlo analyses. Although the full MatLab development package is not open-source, the runtime required to execute *OCECgo* is open-source and will be freely distributed with the open-source software upon publication.

OC/EC estimation is largely influenced by different fractions of interfering substances, although authors have tested with four different samples from different sources still it remains a question that how to reduce the interference which also influences the accuracy of measurements. What authors suggest for removing those bias.

The Reviewer is correct that OC/EC estimation is indeed sensitive to not only interfering substances in the sample, but also the specific organic constituents and other unexpected inorganic substances. This fact supports the standard notion that thermal-optical analysis-quantified carbon masses are *operationally-defined*, in that the specific thermal protocol and definition of the split point influences the resulting carbon masses. This concern however, is left to the specific research and researcher and is therefore out of the scope of the present study, which instead presents a software tool that enables *consistent calculation of operationally-defined carbon masses* and *rigorous quantification of associated uncertainties*, despite the specific operational-definition employed by the user.

How many replicates were run for each sample?

The sample data presented in Table 2, 4, and Figure 3 and discussed within the manuscript were provided to the authors by others for use as proof of the utility of the *OCECgo* software tool. As such, these data are from singular measurements performed by other researchers using the same type of instrument.

Why experiment was performed with a single instrument? Why not validate in other instruments? Instrument age is also a major factor.

The sample data obtained from other researchers as noted above were acquired on different instruments. The Reviewer is correct to note that instrument age may be an important element in the operation and calibration of the thermal-optical instruments, and this is a key factor contributing to the need for continual calibration of the instrument. For example, the efficiency of the MnO_2 oven in oxidizing evolved carbon into CO_2 is likely to change (reduce) over time, but this effect would be appropriately captured in regular calibrations assisted by the OCECgo software. Indeed, we have used the *OCECgo* tool to calibrate our instrument as it has aged and these calibrations have been performed satisfactorily with newly-installed, moderately-aged, and highly-degraded oven sub-units. It may be interesting for someone to use the presented *OCECgo* software tool to quantify variations in operation of a single instrument through longitudinal study of calibration results.

Why only 2 concentrations of sucrose were used, the minimum should be 3.

Following the manufacturer's manual[1], the *six-point* calibration we discuss in the manuscript includes two measurements at each of three different *volumes* (0, 5, and 10 μL) of the aqueous sucrose solution. The Reviewer is correct to suggest that three data points are needed to perform the linear regression necessary to compute the mass calibration constant (since regression degrees of freedom = number of data – $2 \geq 1$); however, the mathematics of the calibration are agnostic to the specific data and quantity of data. That is, if insufficient calibration data are acquired, regression uncertainties − which ultimately yield calibration uncertainties − will be unsatisfactorily large. In our experience, which appears to be mirrored by the manufacturer's manual, the presented six-point procedure is satisfactory and, as noted in the manuscript, further calibration data yield diminishing returns.

Data on Table 4 shows that whenever the mass of one component is very low there is a higher uncertainty in instrument reported as well as in present work as estimated by software. How the authors justify this.

As shown in Table 4 of the manuscript, OC and EC uncertainties are negatively correlated with the reported carbon mass. This is a consequence of the split point uncertainty. For a given *absolute* split point uncertainty in mass units, the resulting uncertainty in OC and EC mass depends on the nominal mass of OC and EC. By inspection, when EC/TC > 50% (such that EC > OC), uncertainty in OC is larger (examples A−C). In contrast, when EC/TC < 50% (such that OC > EC), uncertainty in EC is larger (example D). This same effect is also observed when using the fixed equations (Eq. (1) in the original manuscript) for OC and EC as employed by the manufacturer. In this case, there is a fixed component (5%) of uncertainty in addition to an inversely-mass-dependent component, such that the relative uncertainties in OC and EC masses (in %) increase as the measured carbon masses decrease.

Minor Concerns:

What is the actual precision of the instruments?

In light of this Reviewer comment and those of Reviewer #1, we have estimated the precision of our instrument by performing successive blank analyses using the NIOSH 5040 thermal protocol. The estimated 2σ precision/repeatability error was quite low at ~0.031 μg_C, and negligible in comparison to the total uncertainty of the results. Nevertheless, we have updated the Monte Carlo procedure of the *OCECgo* to allow a used to propagate instrument precision into the final carbon mass calculations. Because this is an instrument-dependent property, we have included this as a user-defined input into the *OCECgo* software, with the default value equivalent to our observation.

What is the detection limit of the mass balance used in this study?

The detection limit (reported as “minimum weighing value”) of the mass balance listed in the **Table of Materials** (AND model FX-3000iWP) is 0.01 g, equivalent to 0.1% and 0.001% of the desired sucrose and water masses, respectively.

What can be done to reduce the pipetting error?

Pipetting error is a function of both manufacturing tolerances of the chosen pipette and user error. The suggested pipette listed in the **Table of Materials** is typical of adjustable-volume manual pipettes in terms of accuracy and cost. User error can of course be minimized through the use of manufacturer-recommended pipetting procedures. Nonetheless, refering to Table 3 in the manuscript, pipetting error has only a marginal effect on overall calibration uncertainty, which is dominated by bias in the instrument’s NDIR detector. Therefore, we suggest that any single-channel, adjustable volume, manual pipette used in accordance with recommended procedures is appropriate for this calibration of this instrument.

Table 2. Why uncertainty was more for OC?

Referring to the fourth-previous response to the Reviewer, uncertainty in OC was larger than that of EC in this example measurement due to the relative effect of split point uncertainty − i.e., OC mass was less than EC mass and, hence, affected more by split point uncertainty.

Table 1. Why total NDIR area was almost double at 0 concentration of sucrose at first run compared to fifth run?

As presented in the note following original **Protocol** step 3.2.1, these data correspond to blank/background (zero-volume) analyses. These analyses are necessary to correct for background gas-phase organic carbon that can be adsorbed during deposition of the sucrose solution onto the quartz “boat” and would subsequently bias the mass calibration constant high. Deviation between the two analyses presented in Table 1 of the original manuscript is a consequence of:

1) Precision in acquisition of the blank − i.e., adsorbed ambient OC during these zero-volume analyses is variable through fluctuations in exposure time and ambient OC concentration, and/or
2) Bias of the NDIR detector − i.e., natural drift of the NDIR detector

Despite the above, we do feel that it is important to note that, regardless of the source of variability in blank/background analyses, the effect of this variability is captured in the Monte Carlo method employed to calculate the mass calibration constant and its uncertainty.

In Table 4. TC mass for sample C should be 23.9, not 23.8, please correct it.

We have updated the table to fix the noted rounding errors.

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

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

We have thoroughly proofread the revised manuscript to ensure no spelling or grammar issues are present.

2. Keywords: Please remove commercial language (Sunset Labs).

We have removed this language.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by "(Table of Materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Sunset Labs, MatLab, etc.

We have removed commercial language from the manuscript and now instead forward the reader to the **Table of Materials**.

4. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "Sunset" within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

In accordance with Editor Comment #3, we no longer refer to the manufacturer within the manuscript; however, since the software is designed to integrate with their specific instrument, we maintain the commercial language in the **Table of Materials** and refer the reader to the **Table of Materials** as required.

5. Please note that the highlighted protocol text will be used to generate the script for the video and must contain everything that you would like shown in the video. Software must have a GUI (graphical user interface) and software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g. button clicks or menu selections for software actions, numerical values for settings, etc.). There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:

We have revised the below examples as noted and have added more detail to the protocol steps.

6. 1.4: How to mix? On a stirrer?

We have updated the text for clarification.

7. 1.5: Please specify the portion of the solution transferred?

We have updated this step (step 1.2 in the revised **Protocol**) to refer to the **Table of Materials** where we now list a suitable borosilicate glass jar (4 oz in volume).

8. 2.3: Please describe how to replace quartz filters.

Detailed steps for replacing the quartz filters are included as sub-steps 2.3.1 and 2.3.2 in the revised manuscript.

9. 3.1.5: How to run the instrument's "dry wet filter" protocol? By pressing a button or selecting a menu from a program?

This has been updated to instruct the user to select the protocol from a dropdown menu in the instrument software's tool bar.

10. 4.2.1: Please specify the calibration data used in this protocol.

We had originally detailed the required calibration data within sub-steps. In the revised protocol, this procedure and specification of the calibration data have been amalgamated into a single step (now step 4.2).

11. 4.3.1.2: What is the absolute 2σ bias? How is it calculated?

Absolute 2σ bias is equivalent to the reported accuracy of the scale in units of grams. We have added text to clarify this.

12. 4.3.1.3: How to obtain the minimum purity?

The minimum purity of the sucrose is provided on the label of its vessel − e.g. ">99.5%". We have added text to clarify this.

13. 4.3.2 substeps: Please specify how to obtain these values.

Note: these substeps have been combined into step 4.3.2 of the revised protocol, in accordance with Editor comment #18. These values were calculated using the pooled variances of studies of intra-user repeatability and inter-user reproducibility at each volume. Text to this effect has been added as a note to this protocol step.

14. 4.3.3: What is the descried number here? Please provide some guidance.

This step corresponds to the user's input of the "desired number of Monte Carlo draws". We have added text to clarify the influence of this number on results, the default value, and the range of values permitted by the *OCECgo* software.

15. 4.5.1: Please describe how this is done.

We have removed original protocol step 4.5.1 as this step is not necessary for the calibration procedure. We have, however, added a more-detailed description to original protocol step 4.5.2 (step 4.5 in the revised protocol) instructing the user to update the instrument's calibration file with the new calibration results.

16. 5.1.2: What is the descried volume?

We have revised this step (now described in steps 5.1 to 5.2 of the revised manuscript) to describe in detail how the user controls the desired sample volume via control of the sampling flow rate and sampling period.

17. 5.2: This is too brief. Please provide more details.

We have revised section 5 of the original manuscript to provided further detail on key steps such as that highlighted by the Editor. In accordance with Editor comment #5, this revised section provides detailed instructions to the user including the necessary interactions (e.g. mouse clicks, text input, etc.) with *OCECgo* and the manufacturer's software.

18. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

We have combined shorter protocol steps as requested.

19. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

20. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Notes cannot usually be filmed and should be excluded from the highlighting.

21. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

In accordance with Editor Comments #19-21, we have highlighted ~2.75 pages of protocol text to identify the most critical steps for visualization of the protocol. We believe our highlights within the revised manuscript are now acceptable in preparation of protocol visualization.

22. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

In our revised **Discussion** section, we have added mention of steps that we deem most critical to the successful implementation of the presented protocol. These and other critical steps are further supported by the inclusion of notes, warnings, and cautions within our original and revised **Protocol**.

b) Any modifications and troubleshooting of the technique

In the revised manuscript, we note a number of modification/troubleshooting techniques available to the user to ensure appropriate and ideal use of the *OCECgo* software tool. These include:

- A modified (truncated) calibration procedure as a "bump test" check of the instrument's existing calibration.
- Means to detect for the presence of spurious calibration data manually, through the monitoring of incremental changes in calibration uncertainty, and automatically, through a newly-added quantitative test of instrument linearity.
- Recommended troubleshooting of the necessary assumption that PC and EC optical properties are equivalent, through a cursory test of the effect of the critical attenuation decline.

We would also like to highlight the fact that, to support troubleshooting, the *OCECgo* contains numerous warning and error dialog boxes should the user input invalid data.

c) Any limitations of the technique

We have updated the revised **Discussion** section (see the final paragraph) to include mention of key limitations of our presented technique.

d) The significance with respect to existing methods

In the **Discussion** section, we perform a critical comparison of our presented method with the existing method of the manufacturer. We show through the data in Table 4b that the manufacturer's method tends to yield non-physical estimates of carbon mass uncertainty and, in majority, tends to under-estimate uncertainty in measured carbon masses. As recognized by Reviewer 1, these are significant findings. Moreover, this important result is specifically supported by four unique data performed by various researchers using different instruments in different research contexts.

e) Any future applications of the technique

In the final paragraph of the revised **Discussion** section, we have echoed our statement in the **Introduction** that this protocol and software tool could be extended in future to work with outputs generated by other instruments and that this open-source software tool is designed to be collaborative in the sense that other useful (often field-specific) functionalities could be easily included in the software tool.

23. Table 3: Please insert a space after the "±" symbol.

We have updated Table 3 as requested.

24. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the items in alphabetical order according to the name of material/equipment.

We have updated the **Table of Materials** as requested.

References

1. Sunset Laboratory Inc. *Organic Carbon and Elemental Carbon Field Instrument: Model 4 User's Manual*. Hillsborough, NC.

OCECgo software and associated files

Supplemental Coding Files

ConradJohnson_JoVE_OCECgo-SoftwarePackage.zip