MODEL AS-D1

DISSOLVED INORGANIC CARBON AND $\delta^{13}C$ ANALYZER

INSTRUCTION MANUAL

2023.4

Apollo SciTech, LLC

Carbon Analytical Instruments Company

18 Shea Way, Suite 108-109
Newark, DE 19713 U.S.A.
Tel: 302-861-6557
Fax: 302-861-6582
E-mail: admin@apolloscitech.com
Website : www.apolloscitech.com

CAUTION

1. **Please contact Apollo SciTech or designated representatives to schedule an online video meeting to ensure the proper setup of the analyzer and to avoid common user errors. User errors are not covered by the warranty detailed in Chapter I.**

2. **Please read this instruction manual before using the DIC-δ^{13}C Analyzer.**

3. **This product is designed for laboratory environments (land and onboard a ship).**

4. **Ensure the correct voltage is supplied to the instrument and the correct fuses are installed.**

This instrument requires a computer running Microsoft Windows to operate. We recommend that the computer is dedicated to the control of this instrument only. Connection to the Internet should be avoided. Virus protection software, Internet backdoor software, or Windows system updates may interfere with the operation of the DIC Analyzer.

Note on Safety

Apollo Scitech products have been designed to be safe when operated in the manner described in this manual. The safety of this product cannot be guaranteed if the product is used in any other way than is specified in this manual.

Markings:

WARNING	**Warnings must be followed carefully to avoid bodily injury.**
CAUTION	**Cautions must be observed to avoid damage to the equipment**
NOTE	**Notes contain important information and useful tips on the operation of the equipment**

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A. UNPACKING AND INITIAL INSPECTION

A.1. Inspect the package

The Total Dissolved Inorganic Carbon (DIC) and isotopic CO_2 Analyzer (referred to as: “the DIC-$\delta^{13}C$ Analyzer” or “the Analyzer”) was carefully packed in a sturdy carton box, with cushioning materials to withstand the shock during shipping. Inspect the exterior of the package for possible damage upon receipt. If damage to the exterior of the package is apparent, take photos and contact the shipping company immediately. The Analyzer is insured for its full price with the shipping company. Apollo SciTech LLC will not be liable for damage caused by the shipping carrier.

A.2. Unpack the package

Open the top of the package carefully and inspect for any signs of interior shipping damage. Please forward a report of any damages to Apollo SciTech LLC., in addition to contacting the shipping carrier.

When unpacking the instrument from the package, please ensure that the package contains all the items indicated on the Packing List. Please report any items on the Packing List which were not included in the shipment promptly to Apollo SciTech LLC.

B. INSTALLATION

B.1. Preparing to install your DIC-δ13C Analyzer

For DIC-δ^{13}C analysis, you need a carrier gas, an acid solution and two waste solution containers.

WARNING **Do not modify the equipment in any way, shape, or form. Any unauthorized modification may result in damage to the equipment and/or bodily injury, and will void the warranty detailed in section I.**

B.1.1. Carrier gas: CO_2-free air should be used as the carrier gas (not included with instrument). Do not use pure nitrogen or any other carrier gas; otherwise, the Picarro analyzer will not function properly. A high-quality two-stage regulator capable of delivering a stable pressure of 16 – 17 psi (1 atm = 14.5 psi) is required. Full-scale pressure for this regulator is typically 30 – 60 psi. Fluctuation of the carrier gas pressure will reduce the accuracy of the analysis.

If your compressed air contains CO_2, you can install a chemical filter to remove CO_2 and H_2O. Ascarite II is a good CO_2 scrubber and $Mg(ClO_4)_2$ can be used to remove trace amount of H_2O. You may use a 20 – 30 mL plastic syringe as a filter chamber for this purpose. Also, please install a PTFE air filter before the carrier gas enters the analyzer. Another PTFE filter is needed before the gas enters the Picarro CO_2 analyzer to protect it from being contaminated by water or particle although internally Picarro has another inline filter. The PTFE filters may be replaced annually or as needed. A hydrophobic filter (0.2 – 0.22 μm pore size) is installed on the back of the Analyzer to filter out salt aerosols in the gas line. Inspect and replace it from time to time (every 500 runs or as needed).

B.1.2. Acid solution: Use a solution with 3% H_3PO_4 and 7% NaCl (neither concentration needs to be precise) to convert all dissolved inorganic carbon to free CO_2. Use chemically resistant gloves and face shield and follow other applicable safety procedures when preparing the acid solution. Take a 2.5 L reagent bottle, add ~ 1 L of DI water to it, add 175 g of NaCl to it, and shake it, then add 89 mL 85% concentrated H_3PO_4 to it. Now add DI water to the 2.5 L line and shake it until NaCl completely dissolves and the solution is well mixed. It is possible to lower the concentration of H_3PO_4, especially if your samples have DIC values lower than that of typical seawater; please consult Apollo Scitech.

WARNING **The use of an acid solution not compatible with the above specifications or otherwise recommended by Apollo Scitech may result in damage to the equipment and/or bodily injury, and will void the warranty detailed in section I.**

B.1.3. Waste container(s): The analyzer discharges before and after each measurement. Place a container that is made of chemically resistant material and is not subject to damage by the acid solution or seawater, e.g., glass, at a position **below the analyzer** and run the waste tubing into the container. It is **IMPORTANT** that the exit end of the waste line is fixed above the liquid line in the container so that you can detect it if the waste line is clogged, pinched, or not properly installed. *Don't let the waste line go loose and spray the acid to the room*.

NOTE Check the waste container periodically and empty it as needed. Note that the waste solution is a relatively strong acid. Please discard it with sufficient dilution and follow local environmental protection regulations.

CAUTION The waste discharge valve inside the DIC analyzer is normally open. It is necessary to keep the waste discharge tubing placed inside the waste container. The connector marked “Waste” on the back panel of the DIC analyzer is fitted with an internal shut-off valve; disconnecting the plug from the connector will stop the discharge. You may do so (or keep the end of the tubing in a plastic bag) when transporting the analyzer to another location to avoid spilling acid during transport. Make sure that the waste tubing is reconnected, and waste solution flow is open before using. **If the connector is not properly connected or the waste tube is pinched, liquid will build up in the reactor, flood the gas path, and damage the Picarro analyzer. These damages are NOT covered by the warranty detailed in section I.**

B.2. Familiarizing yourself with the Analyzer

Your DIC-δ^{13}C Analyzer is composed of two main parts: the DIC analyzer (the front-end device) and the Picarro G2131-*i* Analyzer for Isotopic CO_2 (referred to as G2131-*i*; cf. Fig. 1). The CO_2 gas extraction reactor, flow control devices, and the electronic control units are inside the front-end device. On the front panel is a power switch and a temperature controller for the moisture reduction device.

Attached to the digital pump is a 12-port distribution valve with a 5- or 10-mL syringe, which is used to transfer acid and standard solution/sample for measurement. The digital pump is controlled through a computer by the original manufacturer’s program. For more information about the digital pump please refer to its user’s manual.

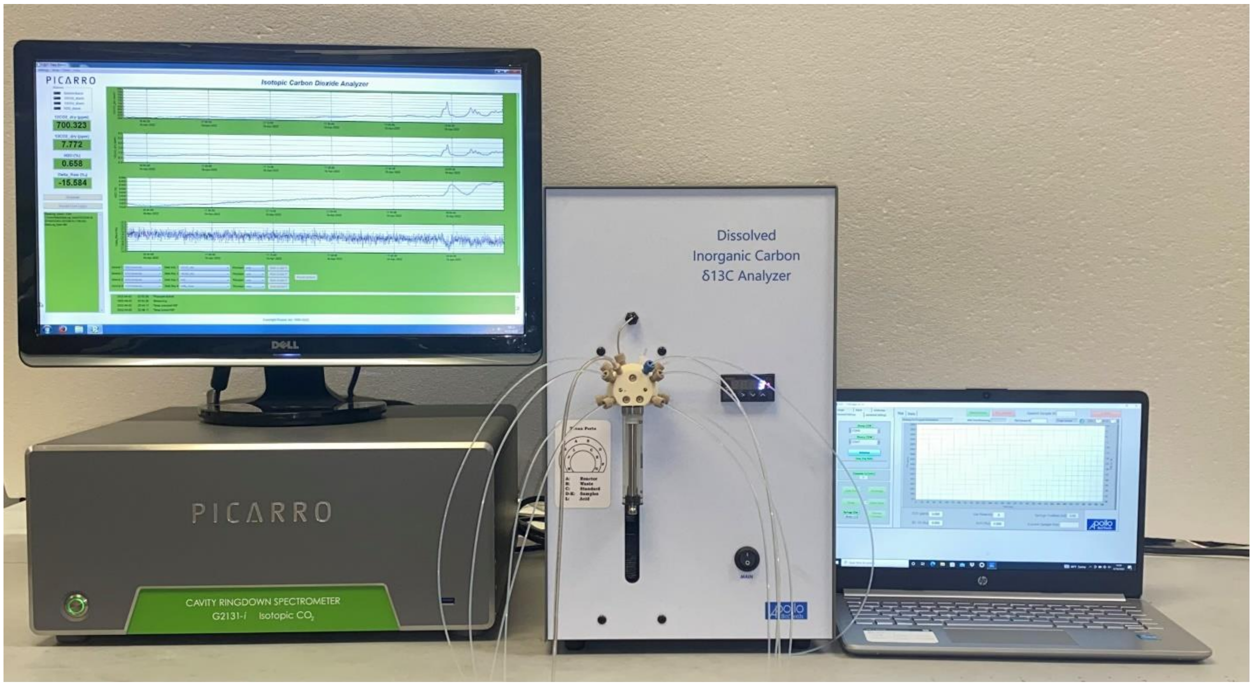


Figure 1. Front view of the DIC-δ^{13}C Analyzer model AS-D1

B.3. Carrier gas connection

Install a high-quality regulator capable of delivering 16 psi (full scale is 30 – 60 psi) to a compressed air tank. Connect a piece of ¼” Tygon® tubing (with a PTFE air filter) from the regulator to the “AIR IN” port on the back of the Analyzer. Set the regulator outlet to 16 –17 psi and check for air leakage. Fix any leakages. See B.1.1 for more information.

CAUTION Do not set the carrier gas pressure too low or too high. If the pressure is too low, it may not open the internal mass-flow-controller or deliver stable air flow at the desired flow rate. If the pressure is too high, it may burst connections inside the analyzer. A stable gas flow is critical. Inside the analyzer, a mass flow controller regulates the air flow to 60 mL/min.

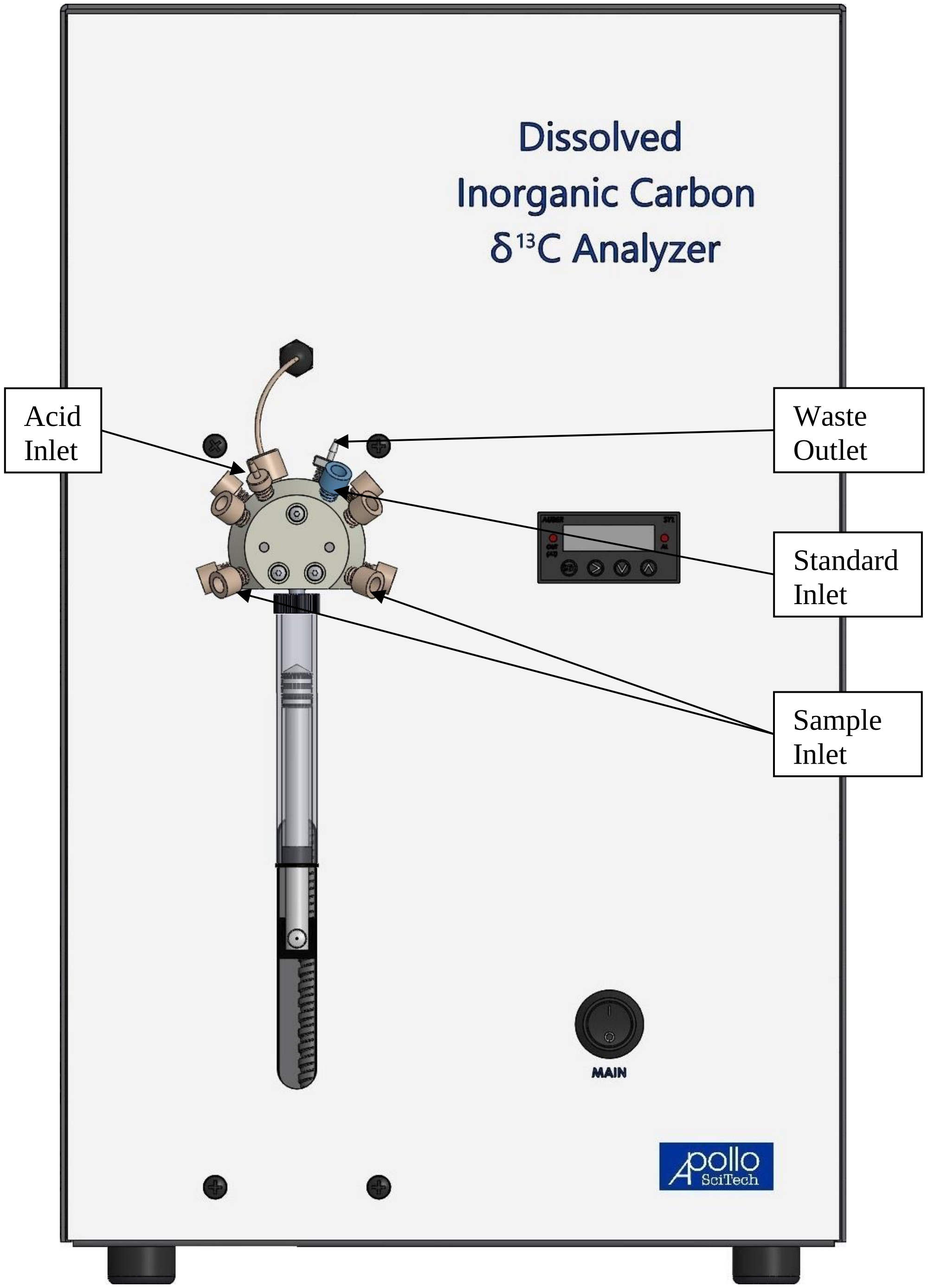


Figure 2. Front view of the AS-D1 analyzer without G2131-*i*

B.4. Acid solution and standard/sample solution supply

Place the acid solution (i.e., 3% H_3PO_4 + 7% NaCl) bottle to the left of the digital syringe. Connect a piece of 1/16" ID Tygon® tubing to port L of the digital pump, and place the tubing into the acid solution.

Connect a piece of 1/16" ID Tygon® tubing to port B of the digital pump, and place the tubing into a front waste bottle. This bottle does not need to be as large as the back waste bottle as the amount of discharge is small.

Attach 1/16" OD, 0.04" ID PTFE tubes to ports C – K of the digital syringe on the Analyzer (Fig. 1). Place the tubing into the standard (Port C) or sample (ports D – K) containers. If you choose a small sample volume (0.5 mL or less), use a 1/32" ID tubing to lower contamination by the previous sample. We recommend storing your standard or CRM in a gas-tight bag.

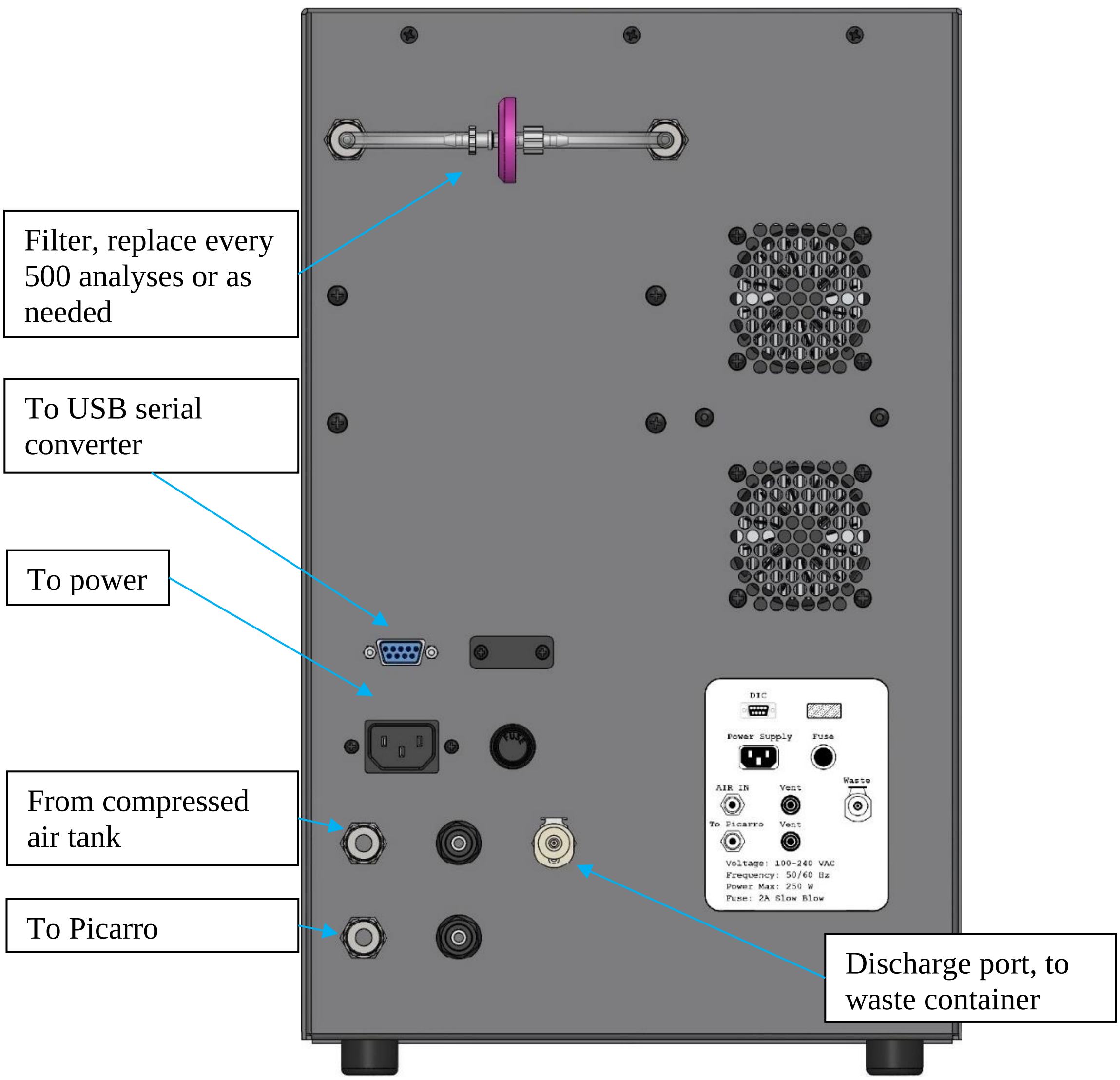


Figure 3. Back view of the AS-D1 analyzer with connection diagram

When the sample volume is very limited, such as a total of a few mL of porewater, supplying the sample directly from a syringe via a piece of short tubing is recommended. Supplying water via a syringe may also be preferred if CO_2 degassing from a limited volume sample is a concern. When plenty of the sample is available in a vial or bottle, withdrawing the sample via a tubing is the preferred method. The inlet of the tubing should be well below the liquid level to minimize the effect of CO_2 degassing to the headspace.

B.5. Computer connection

A laptop computer with the Windows® operation system is used to control the analyzer. It is connected to the Analyzer via a USB port with a USB-serial converter (from USB in the computer to two RS232 serial ports, one connected to the Analyzer, the other one to Picarro G2131-*i*). If the user chooses to supply a computer, a similar laptop or desktop computer running the Windows® operation system is required.

We recommend that the computer is dedicated to the control of this instrument only. Connection to the Internet should be avoided. Virus protection software or Internet backdoor software may interfere with the execution of the analysis.

Refer to the Picarro manual and the digital pump manual for pin assignments.

The software automatically detects two available serial ports, e.g., COM5 and COM6. Click the drop-down menu and select one for each box in sequence.

B.6. Power supply connection

Using incorrect or underrated AC power cords may result in damage to the equipment and/or bodily injury, and will void the warranty detailed in section I.

Place all components of the DIC analyzer at locations that do not block access to the AC cords.

Check that all of the power switches are in the OFF position before connecting the power cords.

Figure 4. Communication connection on the back of the Picarro G2131-*i* analyzer.

*When you turn off Picarro G2131-*i*, make sure you also turn off the power switch on the back.
** Turn the vacuum pump on BEFORE the Picarro G2131-*i* analyzer!
*** The locations of COM1 and the port for the monitor on G2131-*i* may vary and they may not be labeled.

B.7. Computer program installation

Note that the software and the USB-serial converter driver have been installed on the supplied laptop computer, thus section B.7 only applies to users who supply their own computer.

The analyzer comes with a USB Flash Drive containing an installer that installs both the DIC program and the required LabVIEW engines if needed. In case a re-install is needed, please install two runtime engines by running **NI-VISA Run-Time Engine 17.0** and **LabVIEW Run-Time Engine 2017 - (32-bit)** under the prerequisite folder or from National Instrument's website.

The installer also installs the drive for the provided USB serial converter.

The **DIC ASD1-12T.exe** program (a shortcut has been created on your desktop) is the main analytical program, which controls the instrument (refer to section E.4 for more information).

<u>Email Apollo Scitech the product code to acquire an activation code that unlocks the program.</u>

C. DIC-δ^{13}C MEASUREMENT PRINCIPLE

AS-D1 allows simultaneous analyses of both DIC and δ^{13}C precisely in water sample.

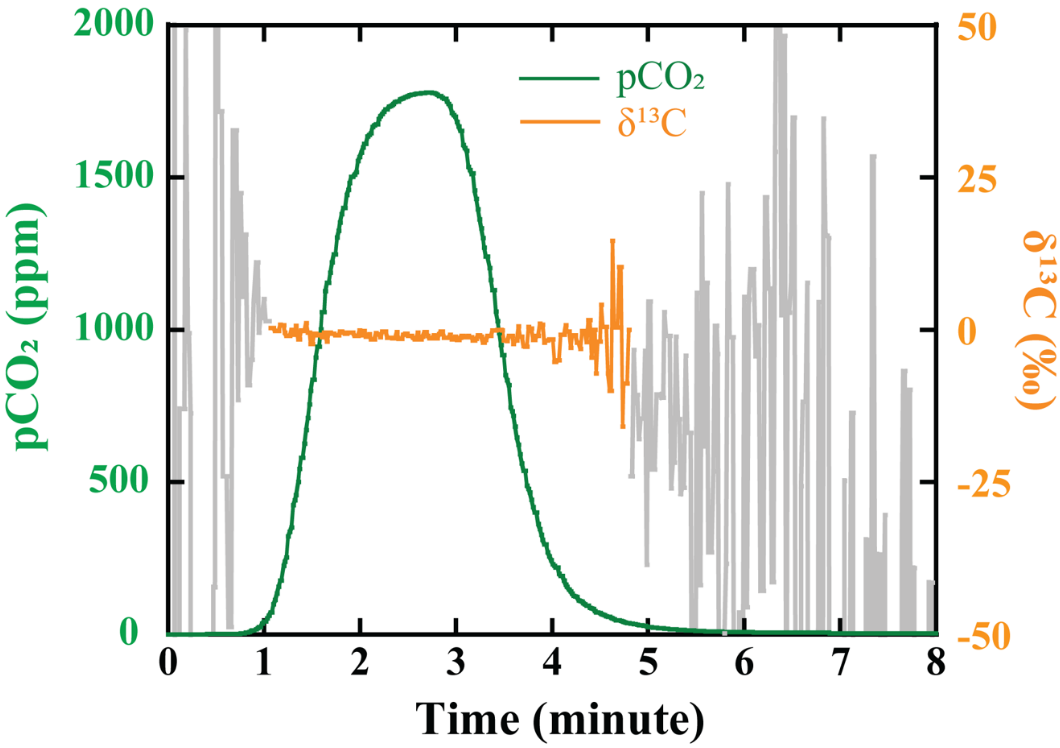


Figure 5. Illustration of the DIC and δ^{13}C signals.

The net area under the pCO₂ curve is compared to that of a standard or a Certified DIC Reference Material (CRM). The net area is calculated following Eq. (1):

$$Net\ Area = \sum \left[\left({CO_2}^{meas}{}_i - {CO_2}^{base}{}_i\right) \times \Delta t_i\right] \tag{1}$$

where ${CO_2}^{meas}{}_i$ is the measured CO_2 value from the Picarro G2131-*i* analyzer at the *i*th time interval, ${CO_2}^{base}{}_i$ is the baseline CO_2 value analyzer at the *i*th time interval, and Δt_i is the timer interval between the *i*th and the (i+1)th measurement.

δ^{13}C is simultaneously measured by the G2131-*i* analyzer. When $pCO_2 < 100$ ppm, the δ^{13}C reading is highly unstable (gray lines in Fig. 5). When $pCO_2 > 100$ ppm, the δ^{13}C reading becomes reliable (orange line in Fig. 5). A cutoff CO_2 value serves as a filter to exclude the unreliable δ^{13}C measurements. The average δ^{13}C of the sample is calculated following Eq. (2):

$$\delta^{13}C_{Sample} = \frac{\sum_{pCO_2 > cutoff} \left[\left({CO_2}^{meas}{}_i - {CO_2}^{base}{}_i\right) \times \Delta t_i \times \delta^{13}C_i\right]}{\sum_{pCO_2 > cutoff} \left[\left({CO_2}^{meas}{}_i - {CO_2}^{base}{}_i\right) \times \Delta t_i\right]} \tag{2}$$

where $\delta^{13}C_i$ is the δ^{13}C value at the *i*th time interval.

D. OPERATION

CAUTION During every measurement cycle **the waste solution in the reactor is discharged both at the end of an analysis and at the beginning of the next analysis**. If it fails to discharge, stop running the program and check for waste discharge problems (such as blockages in the waste tubing). Do not run the analyzer until the problem is solved.

D.1. Warming up

It is preferred that the **Picarro G2131-*i* Analyzer** is powered a day before and remains on for days or weeks during the analyses of all samples. **Remember to always turn on the vacuum pump BEFORE the G2131-*i* Analyzer and turn off the pump AFTER the analyzer**. Make sure a PTFE filter is installed before the inlet of the G2131-*i* despite that there are internal filters in G2131-*i*. This will prevent water and particles from entering the G2131-*i*.

Turn the Analyzer's power switch on. Allow a warmup period of 1 hour. The temperature display on the front panel of the Analyzer should be stable at 2 – 3°C.

D.2. Settings of the G2131-*i*

SKIP this section for analyzers tested by Apollo Scitech. Otherwise, follow this section to set up new G2131-*i* analyzers or after these settings were set to default settings by Picarro's maintenance team.

1. When G2131-*i* is taking measurements, go to the desktop of G2131-*i*'s computer, run the program Stop Instrument under the folder Diagnostics. Choose "Stop software but keep driver running".
2. Run the program Setup Tool under the folder Picarro Utilities on the desktop
3. In the tab Port Manager, change Command Interface to COM1. Click "Apply" to save.
4. In the tab Command Interface, check these items only: 12CO2_dry, 13CO2_dry, H2O, and Delta_Raw. Click "Apply" to save.
5. After exit the setup tool, run the program Start Instrument on the desktop.

D.3. Digital pump HOME position setting

The HOME position of the pump is AUTOMATICALLY set by the pump when it is initialized. However, it may not correctly identify the HOME position if there is some liquid in the syringe and the outlet tube is restricted. To correct this error, make sure the liquid can flow freely out of the syringe when the plunger moves, and try initializing the pump again. Repeat the initialization multiple times, if necessary, until the syringe is free of liquid and can identify the correct HOME position.

D.4. Running the analytical program

Double-click the shortcut icon “DIC ASD1-12T” on your computer’s desktop to start. Note most of the steps below are already set in attached laptop.

Under the tab **General Settings** on the left panel. Click the downward arrow on Pump COM and Picarro COM to bring out the drop-down menu, where you will find available serial port numbers. Choose accordingly and then click initialize to establish connection between the computer and the Analyzer (you should hear the buzz from the pump). A pop-up window asks you to either select an existing .csv file to append the data to or create a new file to save the data to.

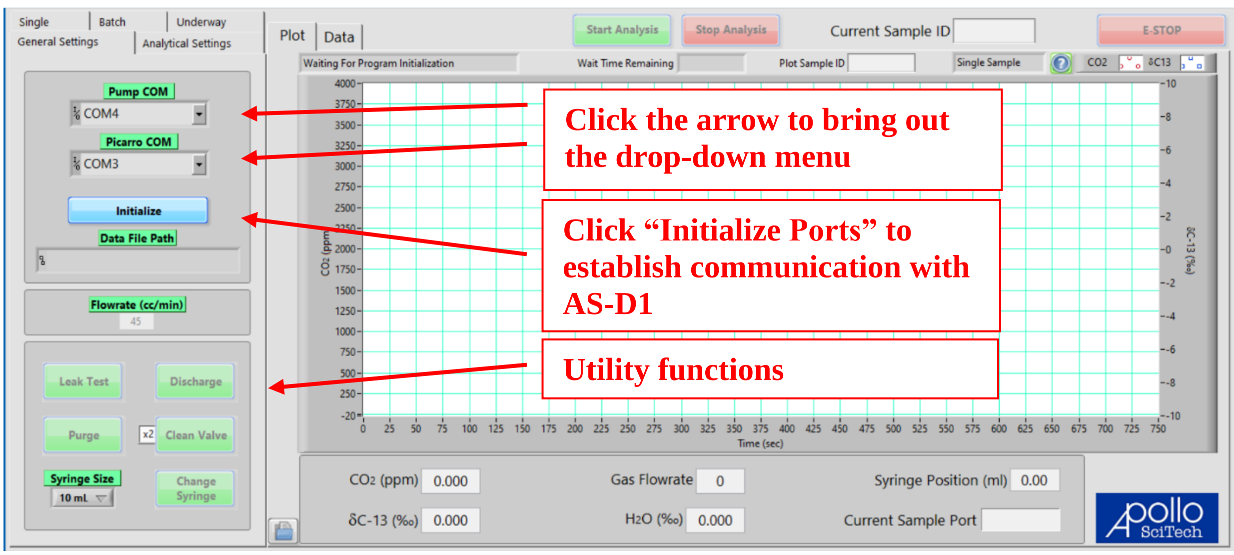


Figure 6. DIC-C13 program interface

Note that when more than one COM port number appear in the menu, some of them could be parallel ports. Use the device manager in the control panel to check which is the actual port.

After communication is established, the data file path is displayed in the box underneath the button Initialize. The utility functions that were grayed out are now available, as well as the Start Analysis button above the plot area. The status indicator should read “Ready” and the Waiting Time Remaining does not display anything. This indicates that the system is ready to take a new order. In the meantime, CO_2 (ppm), $\delta^{13}C$ (‰), H_2O (‰), and carrier gas flowrate (mL/min) are displayed underneath the plot area.

If the gas flow rate is lower than the set point for more than 10 seconds, the program will play an audible alarm, abort the current measurement, and bring the analyzer to an idle state.

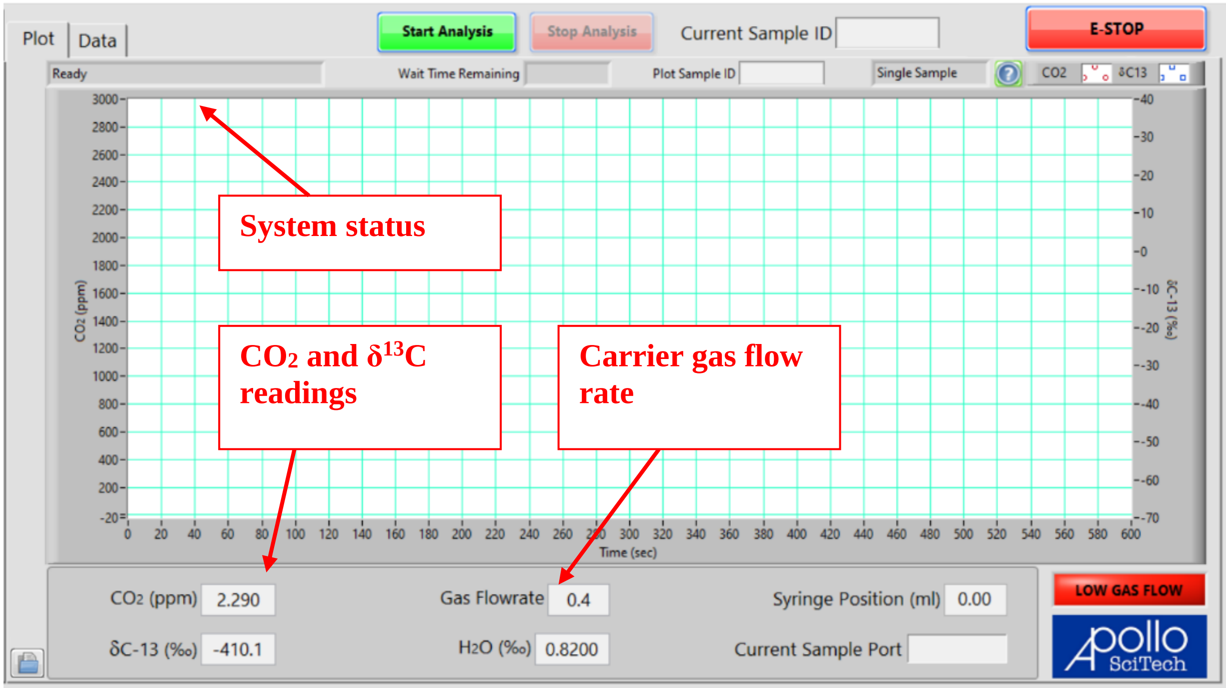


Figure 7. DIC-C13 program after initialization

Click the tab **Analytical Setting** to set up basic analytical criteria and settings.

1. **Sample Volume (ml)** determines the volume of the sample in batch and underway analyses. Single test has its own independent volume setting. The sum of the sample volume and the acid volume (see item 8 below) cannot exceed the syringe size.

2. **δC-13 Cutoff (ppm)** determines the CO_2 concentration above which the $\delta^{13}C$ values are averaged to calculate the sample's $\delta^{13}C$ value. Recommended values (RV) = 350 – 400.

3. **δC-13 Cutoff 2 (ppm)** determines another CO_2 concentration above which the $\delta^{13}C$ values are averaged. The user can adjust this number to have the program calculate the sample's $\delta^{13}C$ values with a different cutoff value.

4. **Wait Btwn Repeat (s)** determines how many seconds the program waits before starting the next analysis for the same sample. During the waiting time, the carrier gas is directed into the CO_2 analyzer, stabilizing the baseline reading for the next run.

5. **Wait Btwn Sample** (s) determines how many seconds the program waits before starting the analysis of the next sample. During the waiting time, the carrier gas is directed into the CO_2 analyzer, stabilizing the baseline reading for the next run.

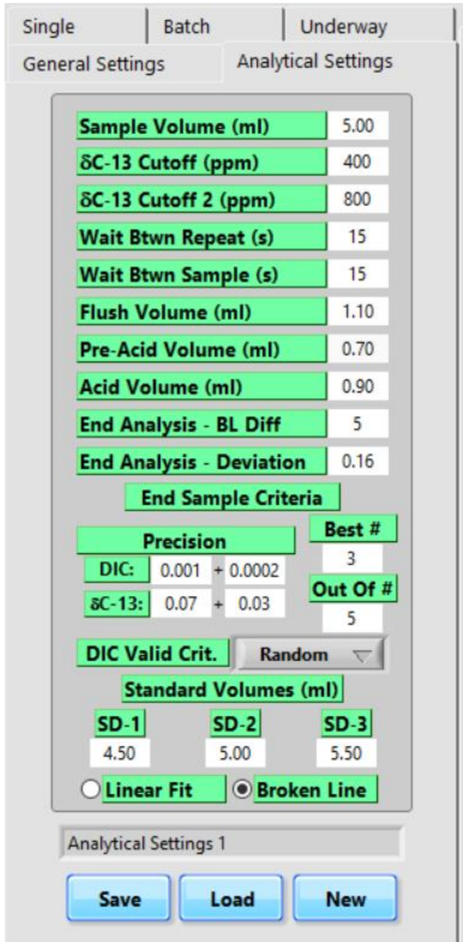


Figure 8. Analytical Settings

6. **Flush Volume (ml)**. At the beginning of each new sample, some acid and sample will be drawn to flush the sample tubing. Specifically, it draws acid for the specified "Pre- Acid Volume" one line under this item, then draws sample according to "Flush Volume", and then injects all the sample and acid to waste. The flush volume cannot exceed the difference between syringe size and acid volume.

7. **Pre- Acid Volume (ml)** determines both the volume of acid needed in cleaning sample tubing and the volume of acid that is needed before injecting the sample to the reactor. When this portion of the acid is in the reactor, carrier gas is bubbled through it to measure CO_2 level until a steady baseline is achieved.

8. **Acid Volume (ml)** determines the volume of acid needed for the reaction with the sample, which sits on top of the plunger and underneath the sample in the syringe. The sum of the acid volume and any sample or standard volume (see item 1 above and item 12 below) cannot exceed the syringe size.

9. **End Analysis - BL Diff** determines how a measurement is deemed successfully completed by the difference. Once the average of 20 readings falls between the baseline and baseline + BL Diff, the measurement is deemed successfully finished, and result recorded. Normally, a difference of 5 ppm between the final baseline and the initial baseline is good enough. If BL Diff = 5 is selected, the next alternative value is to set a much lower value, e.g., 0.05, so that it will not be triggered.

10. **End Analysis - Deviation** determines how a measurement is deemed successfully completed by standard deviation. Once the standard deviation of 20 readings falls within the specified standard deviation value (e.g., 0.16 ppm), the measurement is deemed successfully finished, and the result is recorded. Note: when either BL Diff or Deviation is met, the measurement will be deemed finished. We recommend 0.16 as the termination condition. Once this mode is chosen, set the "**End Analysis - BL Diff**" to 1 so it will not be triggered. You may also use a higher value of 0.2 to save analytical time or a lower value of 0.12 to have a more stringent condition.

11. **End Sample Criteria** determines when a sample is considered finished.

1). If the numbers are set as shown in Fig. 8, i.e., DIC: 0.001+0.0002, $\delta^{13}C$: 0.07+0.03, best 3 out of 5, the criteria to finish a sample are as followed. The relative standard deviation (RSD) of three DIC runs needs to be within 0.001 and the standard deviation of three $\delta^{13}C$ runs needs to be within 0.07. If the first 3 runs do not yield both DIC and $\delta^{13}C$ values close to each other, it will make a fourth runs. Then it compares the 3 closest DIC runs and the 3 closed $\delta^{13}C$ runs with the required precisions. The 3 runs with closest DIC values and $\delta^{13}C$ values are not necessarily the same. It takes at most 5 runs and finds the 3 closest runs for DIC and $\delta^{13}C$. If the closest 3 DIC runs yield an RSD below 0.001, they are labeled "Valid" and the other two "Excluded"; if the closest 3 DIC runs yield an RSD between 0.001 and 0.0012, they are labeled "Within Tol. (Tolerance)" and the other two "Excluded"; if the closest 3 DIC runs yield an RSD above 0.0012, all five are labeled "Failed" and a second attempt (of up to 5 runs) will be made. The $\delta^{13}C$ values are processed similarly while the number 0.07 determines the standard deviation of the 3 closest $\delta^{13}C$ values. If it fails either criterion, the status of all runs for both DIC and $\delta^{13}C$ are "Failed", even if it meets the other criterion.

Normally we use 0.001 for the DIC precision, which is ± 0.1% or about ± 2 μmol/kg in normal seawater. In some cases, users may prefer to loosen the requirement to a larger value. For example, for river water samples with DIC < 500 μmol/kg, it may be too challenging and not necessary to get ± 0.1% (or about ± 0.5 μmol/kg), in which case, ± 0.3% may be acceptable.

2). The box "**Best #**" specifies how many measurements are needed when calculating the RSD of DIC and SD of $\delta^{13}C$. The box is limited integers between 1 and 15 and less than or equal to "Out of #". If an illegal value is input, the number closest to the attempted input that meets the criteria will be recorded.

3). The box "**Out of #**" specifies how many measurements in total are allowed in each attempt, before the program does a second attempt on the same sample or stop itself and alert the user about the condition. The box is limited integers between 1 and 15 and greater than or equal to "Best #". If an illegal value is input, the number closest to the attempted input that meets the criteria will be recorded.

4). DIC Valid Crit. determines whether the program looks for any 3 runs or 3 consecutive runs to achieve an RSD within 0.001. This does not apply to $\delta^{13}C$. The program always looks for the closest 3 runs for $\delta^{13}C$.

12. **SD-1**, **SD-2**, and **SD-3** determines a set of volumes for the CRM or your secondary standard to be used for standardization. Generally, 3 volumes (e.g., 3, 3.5, and 4 mL) are recommended to create a calibration line. A unique feature of our analyzer is to use one standard but with different volumes to create a calibration line. Note this is meant to correct the small amount of CO_2 in the acid and carried over from the previous analysis. Lower volumes may be preferred for high DIC porewater samples. It is recommended that the user estimates the DIC of the unknown sample and chooses appropriate sample and standard volumes and standard concentration so that the total carbon (and the integration area) of the sample falls within the range of the three standard volumes. The sum of the acid volume and any standard volume (see item 8 above) cannot exceed the syringe size.

The user can choose between the best-fit line of the three different volumes or the broken line using only the two adjacent standard volumes in interpolate and extrapolate.

13. You may change the value in any box in this tab and click somewhere else to finish inputting. The new values are immediately registered with the program and will be in effect the next time the program calls for that box. One does not need to save the settings for the program to execute them.

Click Save, and all settings in the **Analytical Settings** tab will be saved to the current file ("Analytical Settings 1" in Fig. 8) and reloaded each time the program is opened. If your analyzer comes with a laptop, it is recommended to use the default setting we tested for your unit, although you can make modifications and test different settings.

Click Load to reload a saved set of settings.

Click New to create a new set of settings.

D.5. Analytical procedure

There are three analytical modes available for users to choose: 1. Single analysis, 2. Batch analyses, 3. Underway analysis. Single analysis is for testing and learning purpose only. Batch analyses should be used for sample and standard analysis. Underway analysis is useful with a flowing water line (on board a ship or on a dock).

Single Analysis

While this is not the recommended mode for real sample analysis, you may this section to learn the analytical procedures and principles. In the tab Single, you may choose the sample volume for the single analysis. The sum of the single analysis sample volume and the acid volume cannot exceed the syringe size. The allowable range of the volume is from 0.5 to 4 mL (preferred range is from 2 to 3.5 mL) for a 5-mL syringe.

D.6. Standardization

This section describes how to analyze a sample and calculate its DIC against a standard sample using the linear fit approach (cf. section D.4, item 13). The software program has all of the standardization procedures automated. Use the tab Batch for real sample analysis.

Connect a standard solution of known DIC concentration to port C of the digital pump (refer to Section E.5). Then create the standard line in a spreadsheet and calculate the final sample DIC.

You may run the standard as a sample, using different volumes of a primary standard you purchased or a secondary standard you prepared. It is recommended that three different volumes be measured for the standardization. Repeat standard measurements several times for each volume. Record the volume of the standard added and the CO_2 integration area.

After the measurement of different volumes of the standard, use a linear regression software program to determine the relationship between integration area and the DIC content by converting each volume of standard solution into DIC content. For example, if the standard solution DIC = 2000.0 μM, then 1.0 mL is equivalent to 2.0000 μmol of DIC (2000 μM * 0.001 L) and 0.5 mL is equivalent to 1.0000 μmol of DIC (2000 μM * 0.0005 L). As 1.0 mL is equivalent to a DIC concentration of 2000.0 μmol and 0.5 mL is equivalent to 1000.0 μmol if they are scaled to a total volume of 1 L, you may wish to make the linear regression equation directly to this unit (μmol/L or μM). Thus, if your sample volume is 1.0 mL, a direct inverse of the regression equation will give you the concentration of your sample (in μM). If your sample volume is 0.5 mL, a factor of two should be multiplied (see Example Calculations below). This can be performed conveniently on a spreadsheet program such as MS Excel® (as illustrated in Fig. 9).

Example Calculation 1:
Sample volume: 1 mL
Measured Integration Area: 12624
Regression equation: Integration Area = 7.4207 * DIC – 143
Re-arranging the regression equation: $DIC = \frac{Integration\ Area + 143}{7.4207}$
Calculation: $DIC = \frac{12624 + 143}{7.4207}$ = 1720 (μmol/L)
Volume Correction Factor: $\frac{1720\ \mu mol/L}{\frac{1\ mL}{1\ mL}}$ = 1720 μmol/L

Example Calculation 2:
Sample volume: 0.8 mL
Measured Integration Area: 10730
Regression equation: Integration Area = 7.4207 * DIC – 143

Re-arranging the regression equation: $DIC = \frac{Integration\ Area + 143}{7.4207}$
Calculation: $DIC = \frac{10730 + 143}{7.4207}$ = 1465 (μmol/L)
Volume Correction Factor: $\frac{1465\ \mu mol/L}{\frac{0.8\ mL}{1\ mL}}$ = 1832 μmol/L

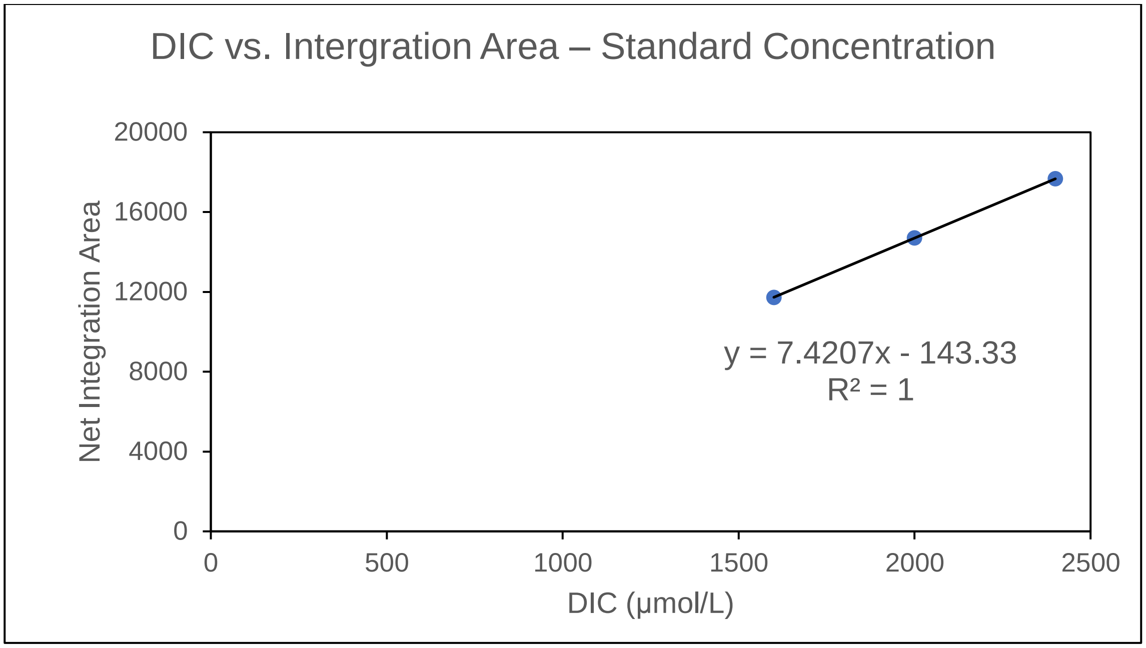


Figure 9. Example of a standard curve. Three different volumes (0.8, 1.0, and 1.2 mL) of the same standard solution were analyzed to generate this standardization curve.

For analysis of seawater samples, a standard solution of Certified Reference Material (CRM) is recommended. CRM is prepared by Dr. A. Dickson of the Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A. (http://andrew.ucsd.edu/adickson/co2qc/). This line can be extended to DIC range below or above its range. For very low DIC freshwater samples, a smaller volume of the Dickson CRM or a dilute secondary standard is used. For high DIC (> 5 mM) sediment, porewater or groundwater samples, you may prefer to prepare custom DIC standards. Such a standard may be prepared from reagent grade Na_2CO_3 after baking at 285°C for two hours. Or a $NaHCO_3$ solution can be prepared and standardized against the CRM. An aged open seawater may also be used as a standard. It is recommended that any self-prepared DIC standard solutions be standardized against the CRM.

For DIC-δ^{13}C, we suggest two types of standards.

1. Acquire a bottle of $NaHCO_3$ solid. Send a sample to a stable isotope lab with high credit to measure its δ^{13}C with isotope ratio mass spectrometry (IRMS) method. Prepare your standard with a DIC concentration similar to that of seawater, fill a few bottles, and use these bottles of artificial seawater as a standard. You may bubble DI water with compressed air to drive out dissolved CO_2 gas before using it to prepare the standard.
2. Prepare your standard with DIC concentrations similar to that of seawater and bubble this water with room air for 3 days (its δ^{13}C value will be nearly stable) before filling it in 250 mL borosilicate reagent bottles. Prepare 30 bottles. Send 3 bottles to a stable isotope lab for analysis. Use other bottles as the standard.

D.7. Sample measurement

Port D – K are reserved for samples. In the software program, select the desired volume for the measurement. Then click the "Start Analysis" button (Fig. 10) to start sample measurement. The program will record the results and repeat until a consistent result is achieved.

Calculate the DIC content according to the linear regression function obtained during standardization. This can be performed on a spreadsheet program such as MS Excel®. Alternatively, convert the final value directly into concentration units (μM or μmol/L). Again, this is automated in the Analyzer. Note it is preferred to run samples using the batch analysis (cf. D.8.)

Record the room temperature of each measurement (sample or standard). This allows calculation of the sample density and conversion of units between μM and μmol/kg, according to this equation:

Density = (999.842594 + 0.06793952 * T – 0.00909529 * T^2 + 0.0001001685 * T^3
– 0.000001120083 * T^4 + 0.000000006536336 * T^5 + (0.824493-0.0040899 * T
+ 0.000076438 * T^2 – 0.00000082467 * T^3 + 0.0000000053875 * T^4) * S + (– 0.00572466
+ 0.00010227 * T – 0.0000016546 * T^2) * S^1.5 + 0.00048314 * S^2) / 1000 kg/L
(check: at T = 25°C and S = 35, density = 1.02334 kg/L)

(Millero and Poisson, 1981)

Millero, F. J., and Poisson, A., International one-atmosphere equation of state of seawater, *Deep Sea Res. Part A. Oceanogr. Res. Papers*, Vol. 28, Issue 6, 1981, Pages 625-629, https://doi.org/10.1016/0198-0149(81)90122-9.

Running samples with high surfactants: Some users may analyze samples with high surfactant content (such as soil pore fluid). Foams/bubbles may form during the analysis. Such bubbles may go through the gas line and into Picarro G2131-*i* and damage it. While the instrument was not designed for this type of analysis, we have recognized the need to analyze such samples and have added filters to stop the bubbles from entering Picarro G2131-*i*. Great care must be exercised in analyzing high surfactant samples. If significant amounts of foams or bubbles are noticed inside the filter or in the tube before the filter, do not repeat the measurement of the same sample. Instead, flush the contaminated tubing (and dry it with gas flow) and replace the filter.

D.8. Batch process for standard and sample measurement

We recommend users to use this function to do all of the sample and standard analyses rather than single analysis. Click the tab **Batch** to perform batch measurement.

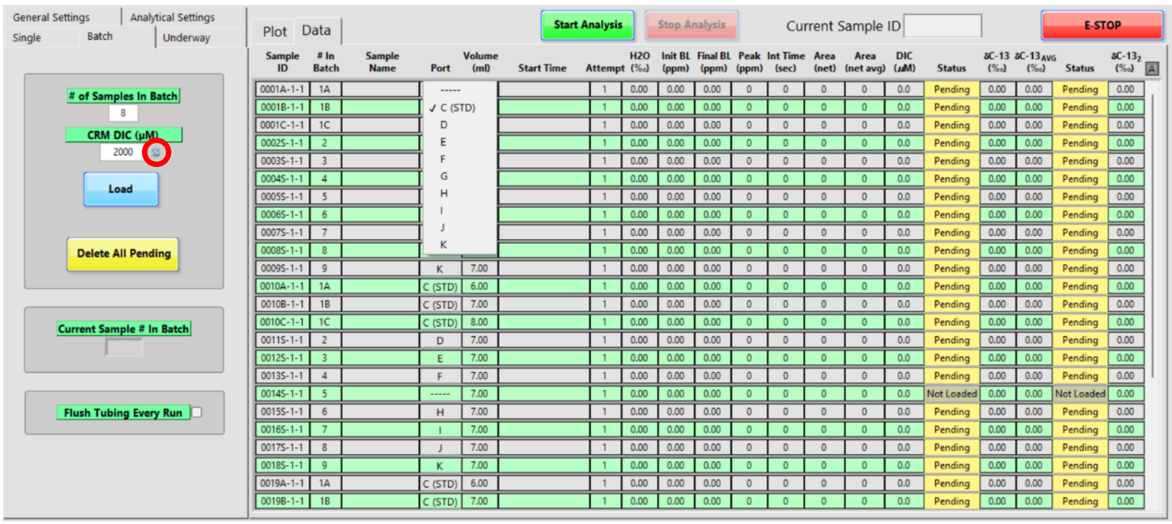
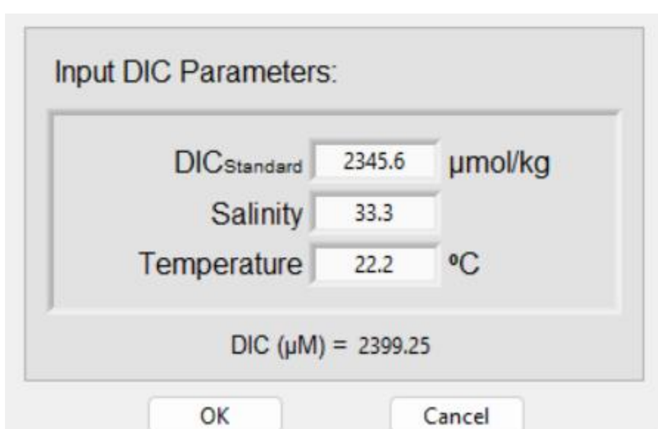


Figure 10. Batch Analyses and Data Tab

After initialization, if you choose to save data to a new file, the Data tab should be blank or if you choose to append to an existing file, the Data tab should display all the data in that file.

You can input the number of samples needed in the batch list and the DIC of the CRM.

Click the circular arrow button to the right of the number box for DIC to input the certified DIC value in µmol/kg and salinity, as well as the temperature of the standard. The DIC in µM is calculated according to section D7.



Click the button Load, then one set of standards and the specified number of samples will be added to the data tab, and the program will automatically switch to the data tab. Each volume of the standard and each regular sample takes up one line when being added to the data tab, and additional lines are inserted to the table as more measurements become available.

Manually input the sample names in the designated boxes for each sample. Put the tubes from port C to K in the corresponding standard and sample containers. Then click the green button “Start Analysis” above the data tab to start the analysis.
As the data in columns starting from Start Time become available, they will be displayed on the screen and saved in the file specified by the user.

After all samples are finished, the program will draw 0.2 mL of acid, inject it to the reactor, and discharge it every hour if the carrier gas is on. This prevents the bubbler inside the reactor from drying out when the system is in idle state for an extended period of time.

D.8.1 Data tab and the plot area

1. Sample ID is a unique sample identification in the current file. It is in the format 9999Z-9-9, where 9 stands for a number and Z stands for a letter.

    1). The first 4 digits represent the number order of a certain sample in the data file, and range between 0001 and 9999. The standard has an A, B, or C attached to the number order to represent the smallest, medium, and largest volume. A sample has an S attached to the number order, no matter what type of analysis it is from, i.e., single, batch, or underway.

    2). The number between the two hyphens represents the number of attempts. The number is limited to be 1 or 2. After the 1st attempt fails, the program will automatically make a 2nd attempt for the same sample. When the 2nd attempt fails, if the failed one is a standard, the program will stop the analysis and alert the user to check the settings and the equipment. If the failed one is a sample, the program will move onto the next pending sample in the list. If two consecutive samples fail on both attempts, the program will also stop the analysis and alert the user. Whenever there is a failed attempt, it will play an alarm continuously until the user acknowledges the pop-up message about the failed attempt, regardless whether the program continues to a 2nd attempt or the next sample, or stops the analysis.

    3). The number after the 2nd hyphen represents the number of measurements in the current attempt of the sample.

2. Sample # in Batch is the sample number within the current batch. The number can theoretically be as high as the number of samples input by the user in the left panel plus 1, because a set of standards always precedes the samples. In practice, this is usually as high as 9, as there are 8 sample ports in the valve (see item #4 below). Underway samples will have a U attached to the number in that underway sequence.

3. Sample Name is for the user to input sample names. Click on any box to input. The program will automatically fill in the sample name for the additional measurements required for the samples.

4. Port. Port assignment will cycle from D to K for the samples. The user needs to swap out sample containers at appropriate time so as not to analyze the same sample more than once. You may click the box in the Port column of a standard or a sample to unloaded, assign a port, or change the port

assignment manually. However, it is recommended to run a set of standards every 8 samples or more often, so it is recommended to input 8 or a smaller number in the box in the left panel for the number of samples.

5. Volume (ml) is the volume of standard or sample specified in the Analysis Setting tab, cf. section D4.

6. Start Time is the time stamp when the program starts injecting sample into the reactor.

7. Attempt records the number of attempt the program is currently on, cf. item #1.

8. H_2O (‰) is the H_2O reading when run is finished.

9. Init. BL (ppm) is the CO_2 baseline at the beginning of the run.

10. Final BL (ppm) is the CO_2 baseline at the end the run.

11. Peak (ppm) is the peak CO_2 concentration of the run

12. Int. Time (sec) is the duration of the integration (of CO_2 concentration with respect to time).

13. Area (net) is the net integration area of the run.

14. Area (net avg) is the average area of the 3 (or a different number specified by the user) runs close to each other. Note that the calculated average will appear with all finished runs, even when one or more are excluded from calculating the average.

15. DIC (μM) displays either the user-input DIC for the standard or the calculated DIC of the sample based on the most recent standard.

16. δC-13 (‰) is the $\delta^{13}C$ value of each run

17. δC-13_{AVG} (‰) is the average $\delta^{13}C$ value of the sample.

18. δC-13_2 (‰) is the $\delta^{13}C$ value when using δC-13 Cutoff 2, as opposed to δC-13 Cutoff, to calculate the $\delta^{13}C$ value of each run.

19. Status displays the status of sample. Both status columns are color coded (Fig. 11).

1). Pending (in yellow) means this sample is queued for measurement

2). Not loaded (in yellowish gray) means this sample not current queued for measurement.

3). Testing (in yellowish green) means data are being collected for the current measurement.

4). Analyzed (in orange) means this measurement has been completed. The status will change depending on whether the user restarts the current sample (see below for Stop Analysis) and how close the finished measurements are to each other.

5). Valid (in green) means this measurement was used for calculating the average net area.

6). Within Tol. (in light green) means these measurements yielded DIC or $\delta^{13}C$ worse than the strict requirement but within the loosened requirement.

7). Excluded (in grayish blue) means this measurement is valid on its own, but not used for calculating the average because it is very different from the rest of the measurements.

8). Halted (in coral) means the user elects to stop the analysis during this measurement, so no data are recorded for it. The program can auto abort a sample if the gas flow is abnormal.

9). Not Used (in coral, too) means this measurement was completed, but the user later elects to restart the sample, so this measurement was only recorded but not considered the one of the five (or a different number specified by the user) measurements allowed in the current attempt.

10). Failed (in red) means the current attempt has reached the maximum allowed measurements but has not yield measurements close to each other to meet the precision requirement.

18. There is a button A to the right of Status and above the scroll bar, controlling whether it auto scrolls to the newly added line when the program adds a measurement to the list. The default is on. Push that button to turn off auto-scroll.

Sample ID	# In Batch	Sample Name	Port	Volume (ml)	Start Time	Attempt	H2O (‰)	Init BL (ppm)	Final BL (ppm)	Peak (ppm)	Int Time (sec)	Area (net)	Area (net avg)	DIC (µM)	Status	δC-13 (‰)	δC-13 AVG (‰)	Status	δC-13 2 (‰)
0002S-1-1	2		D	3.50	07/28/20 12:19:25	1	7.86	1.91	8.45	2449	533	174393	174524	2001.8	Excluded	-2.37	-2.69	Excluded	-2.37
0002S-1-2	2		D	3.50	07/28/20 12:32:04	1	7.67	2.05	8.64	2442	514	174532	174524	2001.8	Within Tol.	-2.67	-2.69	Within Tol.	-2.67
0002S-1-3	2		D	3.50	07/28/20 12:44:23	1	7.74	2.05	8.76	2438	513	174516	174524	2001.8	Within Tol.	-2.60	-2.69	Within Tol.	-2.60
0004S-1-1	2		E	3.50		1	0.00	0.00	0.00	0	0	0	0	0.0	Halted	0.00	0.00	Halted	0.00
0006S-1-1	2		D	3.50	07/28/20 13:35:35	1	8.30	3.22	8.60	2454	529	175242	174663	2003.4	Excluded	-2.86	-2.86	Valid	-2.86
0006S-1-2	2		D	3.50	07/28/20 13:48:10	1	8.13	2.22	8.50	2451	523	174733	174663	2003.4	Within Tol.	-2.77	-2.86	Valid	-2.77
0006S-1-3	2		D	3.50	07/28/20 14:00:39	1	7.93	2.13	8.56	2449	516	174593	174663	2003.4	Within Tol.	-2.63	-2.86	Excluded	-2.63
0009S-1-1	3		D	3.50	07/28/20 14:16:12	1	8.06	2.02	8.60	2448	510	174361	174388	2000.2	Valid	-2.67	-2.73	Within Tol.	-2.67
0009S-1-2	3		D	3.50	07/28/20 14:28:27	1	8.17	2.15	8.77	2445	511	174414	174388	2000.2	Valid	-2.69	-2.73	Excluded	-2.69
0009S-1-3	3		D	3.50	07/28/20 14:40:43	1	7.88	2.13	8.36	2448	533	174729	174388	2000.2	Excluded	-2.69	-2.73	Within Tol.	-2.69
0012S-1-1	3		D	3.50	07/28/20 14:59:29	1	7.84	1.91	8.63	2441	504	174373	0	0.0	Failed	-2.79	0.00	Failed	-2.75
0012S-1-2	3		D	3.50	07/28/20 15:11:39	1	8.08	2.08	8.71	2443	522	174678	0	0.0	Failed	-2.79	0.00	Failed	-2.70
0012S-1-3	3		D	3.50	07/28/20 15:24:07	1	7.80	2.13	8.61	2451	520	174700	0	0.0	Failed	-2.74	0.00	Failed	-2.73
0012S-2-1	3		D	3.50	07/28/20 15:36:33	2	7.76	2.07	8.39	2441	532	174712	174613	2002.8	Valid	-2.71	-2.71	Valid	-2.63
0012S-2-2	3		D	3.50	07/28/20 15:49:12	2	7.97	2.09	8.81	2448	514	174514	174613	2002.8	Valid	-2.71	-2.71	Valid	-2.66
0014S-1-1	2		D	3.50	07/28/20 16:01:49	1	8.13	2.18	8.69	2448	530	174629	0	0.0	Not Used	-2.72	0.00	Not Used	-2.65
0014S-1-2	2		D	3.50		1	0.00	0.00	0.00	0	0	0	0	0.0	Halted	0.00	0.00	Halted	0.00
0016S-1-1	2		D	3.50	07/28/20 16:18:56	1	7.78	1.90	8.39	2446	525	174693	0	0.0	Analyzed	-2.74	0.00	Analyzed	-2.75
0016S-1-2	2		D	3.50		1	0.00	0.00	0.00	0	0	0	0	0.0	Testing	0.00	0.00	Testing	0.00
0018S-1-1	2			3.50		1	0.00	0.00	0.00	0	0	0	0	0.0	Not Loaded	0.00	0.00	Not Loaded	0.00
0019S-1-1	3		E	3.50		1	0.00	0.00	0.00	0	0	0	0	0.0	Pending	0.00	0.00	Pending	0.00
				0.00		0	0.00	0.00	0.00	0	0	0	0	0.0		0.00	0.00		0.00
				0.00		0	0.00	0.00	0.00	0	0	0	0	0.0		0.00	0.00		0.00
				0.00		0	0.00	0.00	0.00	0	0	0	0	0.0		0.00	0.00		0.00

Figure 11. Example results

After starting the analysis, the button Start Analysis is grayed out. As data become available, the program will plot them and display the curve. At the end of each measurement, the plot is not immediately cleared, to allow the user to check. At this time, the current sample ID above the plot area is different from the plot sample ID within the plot area in the inset (Fig. 12). The inset can be turned on and off by pushing the question mark underneath the button Exit.

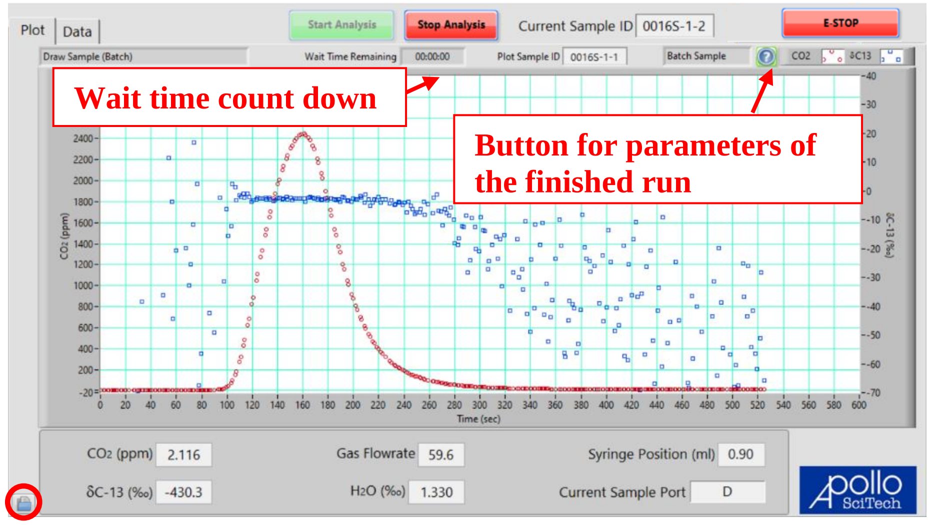
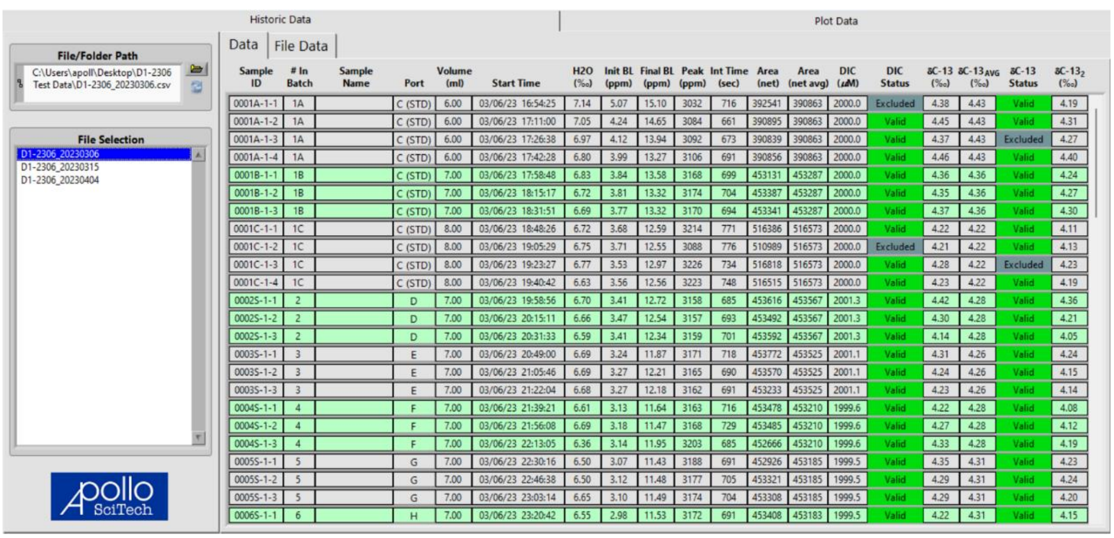


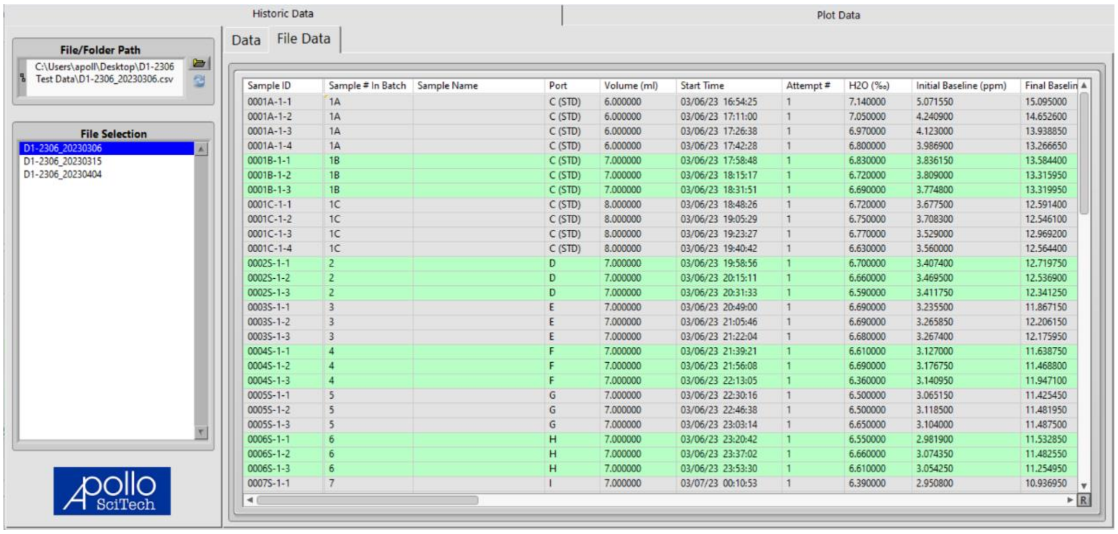
Figure 12. Plot area

Click the folder icon near the bottom left corner of the plot area to bring up the Historic Data Viewer.

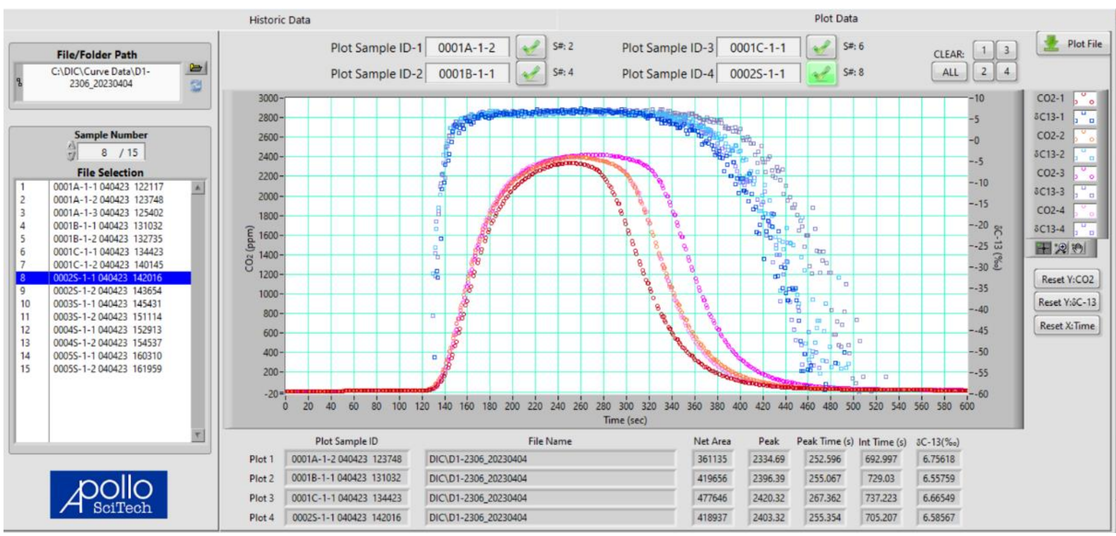


Sample ID	# In Batch	Sample Name	Port	Volume (ml)	Start Time	H2O (‰)	Init BL (ppm)	Final BL (ppm)	Peak (ppm)	Int Time (sec)	Area (net)	Area (net avg)	DIC (µM)	DIC Status	δC-13 (‰)	δC-13 AVG (‰)	δC-13 Status	δC-13 2 (‰)
0001A-1-1	1A		C (STD)	6.00	03/06/23 16:54:25	7.14	5.07	15.10	3032	716	392541	390863	2000.0	Excluded	4.38	4.43	Valid	4.19
0001A-1-2	1A		C (STD)	6.00	03/06/23 17:11:00	7.05	4.24	14.65	3084	661	390895	390863	2000.0	Valid	4.45	4.43	Valid	4.31
0001A-1-3	1A		C (STD)	6.00	03/06/23 17:26:38	6.97	4.12	13.94	3092	673	390839	390863	2000.0	Valid	4.37	4.43	Excluded	4.27
0001A-1-4	1A		C (STD)	6.00	03/06/23 17:42:28	6.80	3.99	13.27	3106	691	390856	390863	2000.0	Valid	4.46	4.43	Valid	4.40
0001B-1-1	1B		C (STD)	7.00	03/06/23 17:58:48	6.83	3.84	13.58	3168	699	453131	453287	2000.0	Valid	4.36	4.36	Valid	4.24
0001B-1-2	1B		C (STD)	7.00	03/06/23 18:15:17	6.72	3.81	13.32	3174	704	453387	453287	2000.0	Valid	4.35	4.36	Valid	4.27
0001B-1-3	1B		C (STD)	7.00	03/06/23 18:31:51	6.69	3.77	13.32	3170	694	453341	453287	2000.0	Valid	4.37	4.36	Valid	4.30
0001C-1-1	1C		C (STD)	8.00	03/06/23 18:48:26	6.72	3.68	12.59	3214	771	516386	516573	2000.0	Valid	4.22	4.22	Valid	4.11
0001C-1-2	1C		C (STD)	8.00	03/06/23 19:05:29	6.75	3.71	12.55	3088	776	510989	516573	2000.0	Excluded	4.21	4.22	Valid	4.13
0001C-1-3	1C		C (STD)	8.00	03/06/23 19:23:27	6.77	3.53	12.97	3226	734	516818	516573	2000.0	Valid	4.28	4.22	Excluded	4.23
0001C-1-4	1C		C (STD)	8.00	03/06/23 19:40:42	6.63	3.56	12.56	3223	748	516515	516573	2000.0	Valid	4.23	4.22	Valid	4.19
0002S-1-1	2		D	7.00	03/06/23 19:58:56	6.70	3.41	12.72	3158	685	453616	453567	2001.3	Valid	4.42	4.28	Valid	4.36
0002S-1-2	2		D	7.00	03/06/23 20:15:11	6.66	3.47	12.54	3157	693	453492	453567	2001.3	Valid	4.30	4.28	Valid	4.21
0002S-1-3	2		D	7.00	03/06/23 20:31:33	6.59	3.41	12.34	3159	701	453592	453567	2001.3	Valid	4.14	4.28	Valid	4.05
0003S-1-1	3		E	7.00	03/06/23 20:49:00	6.69	3.24	11.87	3171	718	453772	453525	2001.1	Valid	4.31	4.26	Valid	4.24
0003S-1-2	3		E	7.00	03/06/23 21:05:46	6.69	3.27	12.21	3165	690	453570	453525	2001.1	Valid	4.24	4.26	Valid	4.15
0003S-1-3	3		E	7.00	03/06/23 21:22:04	6.68	3.27	12.18	3162	691	453233	453525	2001.1	Valid	4.23	4.26	Valid	4.14
0004S-1-1	4		F	7.00	03/06/23 21:39:21	6.61	3.13	11.64	3163	716	453478	453210	1999.6	Valid	4.22	4.28	Valid	4.08
0004S-1-2	4		F	7.00	03/06/23 21:56:08	6.69	3.18	11.47	3168	729	453485	453210	1999.6	Valid	4.27	4.28	Valid	4.12
0004S-1-3	4		F	7.00	03/06/23 22:13:05	6.36	3.14	11.95	3203	685	452666	453210	1999.6	Valid	4.33	4.28	Valid	4.19
0005S-1-1	5		G	7.00	03/06/23 22:30:16	6.50	3.07	11.43	3188	691	452926	453185	1999.5	Valid	4.35	4.31	Valid	4.23
0005S-1-2	5		G	7.00	03/06/23 22:46:38	6.50	3.12	11.48	3177	705	453321	453185	1999.5	Valid	4.29	4.31	Valid	4.24
0005S-1-3	5		G	7.00	03/06/23 23:03:14	6.65	3.10	11.49	3174	704	453308	453185	1999.5	Valid	4.29	4.31	Valid	4.20
0006S-1-1	6		H	7.00	03/06/23 23:20:42	6.55	2.98	11.53	3172	691	453408	453183	1999.5	Valid	4.22	4.31	Valid	4.15

In the tab Historic Data, click the open folder icon to select a file. All applicable files in the same folder will be displayed in the list under File Selection. The current file is highlighted in blue. All the data normally displayed in the data tab in the main program are displayed here in the data tab.



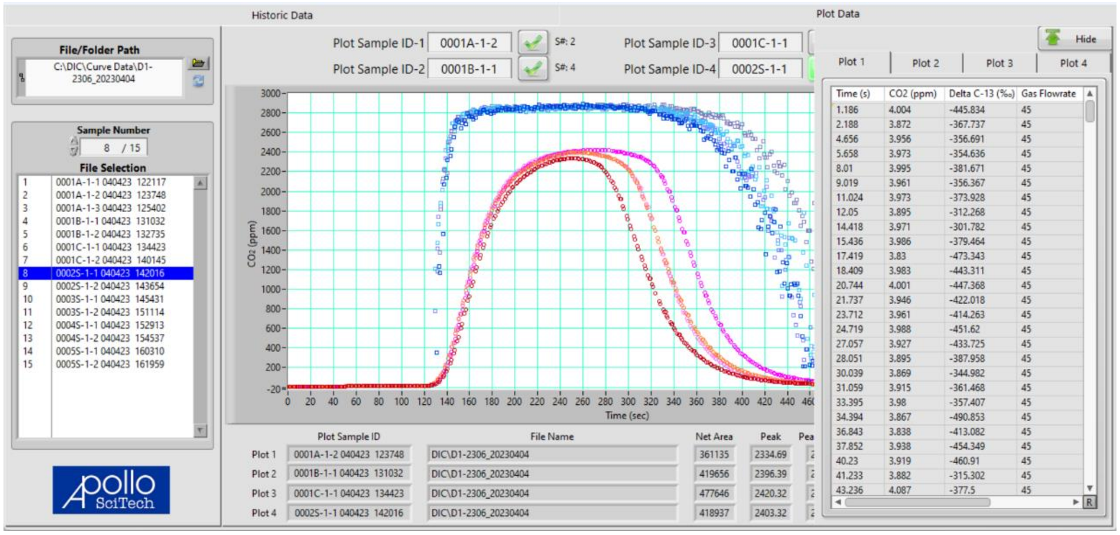
The tab File Data contains all columns saved in the .csv file. Scroll to the right to other columns. Click the dividing line between two columns and drag to adjust the column widths and click the button R near the bottom right corner to reset the column widths. All of the repeats of the same sample are highlighted in the same color.



In the tab Plot Data, click the open folder icon to select a file. In the tab Historic Data, click the open folder icon to select a file. All applicable files in the same folder will be displayed in the list under File Selection. The current file is highlighted in blue.

Click the check mark by Plot Sample ID-2 to enable it and then select a different file to compare two plots. Up to four plots can be enabled in a similar way. Only the plot with a highlighted check mark is updated when a file is selected. Clear any plot or all of them by clicking the corresponding number or ALL near the top right corner. Change the symbol and color of any plot near the bottom right corner.

At the top right corner, click Plot File to bring up the data points in the plots.



Click the dividing line between two columns and drag to adjust the column widths and click the button R near the bottom right corner to reset the column widths.

D.8.2 Stop Analysis

Click either red button, Stop Analysis or E-STOP, to stop the analysis.

If you click E-STOP, then all actions will be halted, and the program will not move the sample valve, the syringe, or the internal pinch valves. That means, if you click E-STOP during discharge, the valves may be at positions to allow gas to go through the reactor at a high speed. Re-initializing the program will reset the internal valves.

If you click Stop Analysis, and then click Stop Analysis in the pop-up window to confirm, the current sample will be halted. The system will go to the idle state. All the pending sample remain pending. You may click Cancel in the pop-up window to return to the main window without affecting the current sample.

After terminating the current sample with Stop Analysis, a Resume button will appear to the left of the Start Analysis button. Click the Resume button if you wish to continue or restart the halted sample. Two options are available (Fig. 10).

1. Restart analysis of current sample. If there are any existing measurements in the status “Analyzed”, they become “Not used” (see section D.8.1 for measurement statuses). It restarts the current sample as if all the existing measurements of this sample did not exist.

2. Restart analysis of current measurement. It continues to the next repeat of the halted sample. If there are any existing measurements in the status “Analyzed”, they remain “Analyzed” and will be potentially used for calculating the average net area.

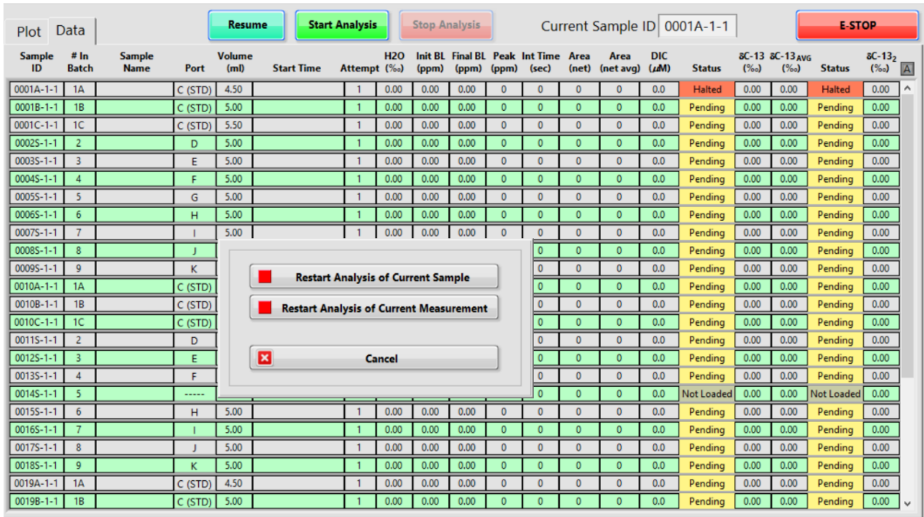


Figure 13. Restart the Halted Sample

D.8.3 Suggestions

1. The water samples need to be filtered to remove any suspended solids.

2. Before the measurements, if the standard or sample tubes are not filled with samples, put all standard and sample tubes in seawater or a solution with similar densities as your samples, use the function Clean Valve (x1) to fill the tubes with seawater. This minimizes the mixing between acid and sample when cleaning the tubes and help with the stability of the machine.

3. We recommend running aged seawater for about 1 hour (two samples with three repeats each) for the analyzer to stabilize before the analyses of the standard and the samples.

4. We recommend measuring a set of **standards** at the beginning and end of analysis (or a day), as well as every 6 to 8 samples; the current default setting for batch analysis is a set of standards for every 8 samples. Then do the calculation in a spreadsheet. This is convenient for a few reasons. As the instrument will drift slightly with time, one cannot apply the same standard curve over a day, thus adjustment is needed. Also, one will have to convert the molarity (concentration in the unit μmol/L) to molality (concentration in the unit μmol/kg) using the sample's salinity and room temperature (see the equation in p. 21), a spreadsheet is helpful.

We recommend you keep the standard in a Cali-Bond bag and connect it to port C. You can choose to run 3 volumes of the standard (3, 3.5, and 4 mL) in the beginning then run 8 samples and at the end run another 3 volumes of standards again.

5. Air bubbles may form inside the sample syringe on top of the piston or attached to the ceiling/top of the syringe). Small air bubbles of < 1 mm in size is not a problem as they stay there stable most time. If you see large bubbles (3 – 4 mm), this is often the reason of large noise in the data as bubbles change size with time and with room temperature. First check if the digital syringe is not

tight. Please tighten it by turning the syringe clockwise using two fingers. Do not overtighten it with mechanical tools. If the syringe is tight, check if the two connectors for acid and sample are tight; if not, use fingers to tighten them. Do not overtighten them with tools. One effective way to eliminate air bubbles inside a syringe is to use the Purge function (under General setting, Fig. 7). When activating Purge, lift the tube from the acid solution so that it draws air into the syringe. Once the syringe moves to about 0.5 mL, put the tube back into the liquid. The air space and the subsequent "waterfall" created this way will eliminate the air bubbles. If large air bubbles persist, leakage through the tube connectors or the 12-port valve is the reason.

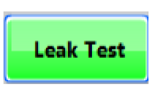
Air bubbles may also form even if there is no leakage. This problem of bubble formation occurs most during the wintertime with river/estuarine samples (cold and fresh water is rich in dissolved air) when they are brought inside a warm room. One option is to put bottles of samples and standard in cold water jackets.

6. Keep the acid, standard, and sample containers in temperature-controlled water bath (or water jackets) and set the bath temperature to a constant value, 2 – 5 °C lower than the room temperature. Knowing the exact sample temperature will also help you in converting molarity (μmol/L) to molality (μmol/kg). A good AC controlled room temperature will also help achieve a highest analytical precision.

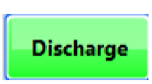
D.9 Underway Process for Sample Measurement

This function is still under development is not fully tested. The underway process is best suited for on board (or at the dockside) measurement for a continuous period of time. **Sample Interval (min)** controls the wait time between each underway sample. **Std Every #** controls how many samples are analyzed before a set of standards is measured. **Scheduled End Time** controls when the underway process should stop. **Underway Port** controls from which port the program takes underway samples.

D.10 Utility Functions

1. 

discharges the reactor, and then closes all internal gas valves for 90 seconds. If the analyzer is airtight you should notice the gas flow rate drop below 10 mL/min within 90 second.

2. 

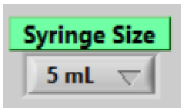
discharges all the solution in the reactor. Make sure the carrier gas is on when using this function.

3. 

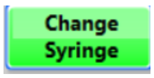
purges the valve with a full syringe of acid or DI water from port L. Make sure the carrier gas is on when using this function; otherwise the program will stop the process and inject any liquid in the syringe to the waste line (port B).

4. 

draws 0.5 mL or 1 mL (one tenth of the syringe size) of liquid from each of port C through L if the button beside it is “x1”. If it is “x2”, it will repeat this process once. Click on “x1” or “x2” to change the state.

5. 

. Click on the dropdown menu to change the syringe size between 5 mL and 10 mL to match your analyzer.

6. 

displays stepwise instructions. Click OK only after finishing all the steps in that window; otherwise, the pump may not be properly initialized.

D.11 Ending the measurement and cleaning the analyzer

> ! It is very **IMPORTANT** to follow this section to properly **CLEAN** the analyzer before shutting it down.

Put the acid inlet, standard inlet, and all 8 sample inlets into DI water. Click the button Clean Valve once at “x2”. It will draw 0.5 mL (or 1 mL with a 10 mL syringe) of DI water from each inlet tubing for port C through L and discharge from the waste tube on port B, and repeat this process. Then click the button Purge and it will draw a full syringe of DI water and inject it into the reactor and clean and discharge it.

Then remove all 10 inlet tubes from DI water and run both Clean Valve (x1) and Purge once again in air.

Close the program after cleaning the valve thoroughly.

If the external surfaces need cleaning, use only DI water to wipe down. Do not use solvents.

E. SPECIFICATIONS

Power supply:	100 – 240 VAC 50/60 Hz (universal power, auto-switch)
Fuse:	2 A slow blow
Power:	250 W
Carrier gas:	Compressed air, ~16 psi exiting pressure
DIC Repeatability:	± 0.1% at DIC level ~ 2000 μmol/L
δ^{13}C Repeatability:	± 0.05 – 0.1‰
Sample volume:	1 – 4 mL (or larger with a 10 mL syringe)
Time required:	~ 12 minutes per measurement
Computer requirement:	80486 or up
Computer operating system:	Windows 10
Communication:	USB for the main unit Cross-over RS-232 (and a USB-RS232 converter included) for the Picarro analyzer
Environment Required:	Indoor
Altitude limitations:	None
Temperature:	Room temperature (steady at 15 – 28°C) preferred. Temperature fluctuations will affect the precision of the measurement.
Humidity:	Up to 85%
Positioning:	Upright use. No angle greater than ± 15 degrees from vertical is allowed.
Dimensions:	Main unit, 16 ¾” × 10 ½” × 15” (42.5 cm × 26.7 cm × 38.1 cm, H × W × D)

F. MAINTENANCE

F.1. Picarro G2131-*i* Analyzer for Isotopic CO_2 maintenance

⚠ ***Warning:*** *Disconnect the AC power supply before opening the cover. The AC power is high voltage which is hazardous!*

Refer to its user's manual. Contact Picarro if necessary.

G. TROUBLESHOOTING

G.1. Common problems

The DIC Analyzer was completely checked during and after manufacturing and assembly operations. It was carefully packed to prevent damage during shipment. If the Analyzer is not functioning properly, check for the following common problems first:

Are all cables connected correctly?

Are there any gas and/or liquid leaks?

Does your power supply outlet match the selected value?

Were the cable connector pin-outs matched properly?

G.2. Troubleshooting guide

G.2.1. Power supply problems

1. Check the power supply cord to ensure it is connected properly.
2. Check the fuses, and replace any fuses that are burned out.
3. Verify that the power supply setting matches the supply outlet voltage.

   Note: If the voltage supplied to the instrument is greater than the selected value on the power cord switch, the Analyzer could be damaged.

Follow these steps to replace the fuse:

1. Rotate the fuse holder counterclockwise to remove it from the main unit.
2. Replace the burned-out fuse with a new slow blow 2A fuse.
3. Put the fuse holder in the main unit and rotate clockwise until it is sufficiently tightened.

G.2.2. Picarro G2131-*i* Analyzer for Isotopic CO_2 problems

Refer to its user's manual.

G.2.3. Software problems

G.2.3.1. DIC Program does not run

Check that your computer is a Windows compatible, 80486 or better system.

G.2.3.2. No communication between the computer and the Analyzer

1. Check the communication cables (RS232 cables). There are two cables required for communication between the DIC Analyzer and the computer. One is for the main DIC system, and the other is for the **Picarro G2131-*i* Analyzer for Isotopic CO_2**. Use the cables that come with the Analyzer. If the connector plugs do not fit your computer, use standard connector converters to match your computer's communication ports. Note that all the pin-outs should have good contact.
2. Check that the correct communication port numbers have been selected in the DIC program.

G.2.4. Cooling system problems

1. Make sure the ambient room temperature is not too high.
2. Check the cooling fans to see if they are blocked by foreign objects or dust.

G.2.5. Measurement stability problems

G.2.5.1. Carrier gas flow rate not stable

1. Check the air supply pressure. Confirm that the pressure is ~ 16 psi (~ 1.1 atm) or slightly higher (< 17 psi). If the pressure is too low, it will not open the mass-flow-controller's valve. If the pressure is set too high, it will burst fittings within the system.
2. Make sure the regulator valve for the compressed air is open enough to supply the flow rate of 60 mL/min.
3. The pressure of air should be stable with no fluctuation.

G.2.5.2. Solution problems

1. Check that the reagent/acid tubing is immersed into the solution and that no air bubbles are trapped inside the tubing.
2. Ensure that the sample solution is homogeneous. Stir the solution if obvious separation is visible.
3. Samples with suspended particulates should be avoided since they may block the tubing.

G.2.5.3. Syringe problems

1. If the software is communicating with the pump, you may remove the syringe from the 12-way valve by clicking the button "Change Syringe" in the program and following the instructions.
   You may also remove the 12-way valve together with the syringe.
   First, remove the lock screw on the bottom of the syringe.
   Second, use a 5/64" Hex key to unscrew the two screws that recess into the valve; firmly press the Hex key against the screw and turn counter-clockwise. Do not remove the two screws close to the bottom of the valve that are close to the surface; there is a third screw of the same type near the top of the valve; great caution should be exercised not to touch any of these three screws of the same type (see Fig. 1).
2. If the software is not communicating with the pump, you may remove the 12-way together with the syringe.

G.2.5.4. Gas path problems

1. Change the external and the two pieces of the tubes on the back of the analyzer every ~ 50 hours or as needed. If the gas flow rate decreases for no apparent reasons, change the external filter.
2. Change the internal ¼" tube immediately before the external filter following the gas path, if needed. Push the connector's movable ring evenly while pulling the tube away from the connector to disconnect.
3. Clean the inside the of the vertical stainless-steel tube inside the condensing module above the reactor.
   1). Push the elbow-shaped connector's movable ring evenly while pulling the elbow-shaped connector up to disconnect it from the stainless-steel tube.
   2). Unscrew the red-capped screw underneath the reactor. Unscrew the screw securing the reactor mount on the base board.
   3). Hold to top of the reactor (the beige-colored piece with a small connector for the tube from the front panel) and gently pull it downward to remove the reactor from the stainless-steel tube.
   4). Put a cup underneath the stainless-steel tube in the place of the reactor. Remove the tubes connected on the outside near the bottom of the reactor, if necessary, to move it out of the way.
   5). Remove the insertion inside the stainless-steel tube if there is one; otherwise, move to step #6.
   6). Use a tube brush to clean the inside of the stainless-steel tube. Collect the rinse water draining out of the tube.
   7). Reinstall the reactor, the reactor mount, the red-capped screw, and the elbow-shaped connector.
4. ALWAYS check for leakage using the software program after any component along the gas path is reconnected or changed.

H. LIST OF PART NUMBERS AND SOURCES

H.1. Air filters

1). 0.22 µm hydrophobic PTFE air filter, P/N S30PTFE022HG by Simsii,
https://simsii.net/hydrophobic-ptfe-gf-pre-filter-syringe-filters.html

2). 0.2 µm hydrophobic PTFE air filter, P/N SLFG025LS by Millipore,
https://www.sigmaaldrich.com/US/en/product/mm/slfg025ls

3). 1 µm hydrophobic PTFE air filter, P/N 9967-008 from LI-COR

H.2. Tubes

1). PEEK, 0.040" ID x 1/16" OD, P/N 1538, 1538L, or 1538XL from IDEX-HS

2). PTFE, 1/32" ID x 1/16 " OD, P/N MFLX06407-41 from VWR

3). Tygon®, 1/16" ID x 1/8" OD, P/N MFLX06407-71 from VWR

4). Bev-A-Line® IV, 1/8" ID x 1/4" OD, P/N MFLX06490-12 from VWR

5). PTFE, 1/16" ID x 1/8" OD, P/N MFLX06605-27 from VWR

6). Silicone, 1/16" ID x 1/8" OD, P/N MFLX95802-02 from VWR

7). Silicone Rubber, 1/8" ID x 1/4" OD, P/N 5236K831 from McMaster-Carr

H.3. Tube Connectors from https://www.idex-hs.com/

1). Flangeless Ferrule, ETFE, 1/4-28 Flat-Bottom, for 1/16" OD Tubing,
P/N P-200, P-200N, P-200R, P-200X, or P-200 NX

2). Flangeless Fitting Short, PEEK, 1/4-28 Flat-Bottom for 1/16" OD Tubing,
P/N P-235 or P-235X

3). Set of Ferrule P-200 and Flangeless Fitting P-235:
P/N XP-235 or XP-235X

4). ETFE Adapter, Barbed to ¼ -28 Flat-Bottom Male for 1/16" ID Tubing,
P/N P-646

5). PEEK Adapter, Barbed to ¼ -28 Flat-Bottom Male for 1/16" ID Tubing,
P/N P-668

H.4. Ferrules.

1). Nylon Front Ferrule for ¼ in. Swagelok Tube Fitting, P/N NY-403-1 from Swagelok, https://products.swagelok.com/en/c/front-ferrules/p/NY-403-1

2). Nylon Back Ferrule for ¼ in. Swagelok Tube Fitting, P/N NY-404-1 from Swagelok, https://products.swagelok.com/en/c/back-ferrules/p/NY-404-1

I. WARRANTY

Apollo SciTech offers a one year full warranty on the DIC-$\delta^{13}C$ Analyzer against defects in parts and workmanship from the date of purchase. Apollo SciTech's sole obligation under this warranty shall be to repair or replace any part of the instrument which Apollo SciTech's examination discloses to have been defective in material or workmanship without charge and only under the following conditions:

1. The defects are reported to Apollo SciTech in writing within one year after the shipping date of the instrument.
2. The instrument has not been maintained, repaired, or altered by anyone who was not approved by Apollo SciTech.
3. The instrument was used in a normal, proper, and ordinary manner and has not been abused, altered, misused, neglected, involved in an accident or damaged by act of God or other casualty.
4. The purchaser packs and ships or delivers the instrument to Apollo SciTech within 20 days after Apollo SciTech has received the written notice of the defect.
5. No-charge repair parts may be sent at Apollo SciTech's sole discretion to the purchaser for installation by purchaser.
6. Apollo SciTech's liability is limited to repair or replace any part of the instrument without charge if Apollo SciTech's examination discloses that part to have been defective in material or workmanship.

This warranty defines the obligation of Apollo SciTech and no other warranties expressed or implied are recognized.