

# ac-9 Protocol Document

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## Acknowledgements

While virtually everyone who has sought meaningful and consistent results has contributed to this evolution of the ac-9 protocol, several scientists provided pioneering efforts, and direct input on this document. They include Scott Pegau of Oregon State University, Percy Donaghay and Mike Twardowski of the University of Rhode Island, and Alan Weidemann of the Naval Research Laboratory.



## 1. Introduction

The ac-9 was originally developed under Naval Research Laboratory sponsorship. Primary development occurred over a 6-month time span culminating in delivery in September 1993. Since initial delivery, approximately 80 more units have been used in applications ranging from tow-yos to long-term moorings. As more researchers used the meter questions as to proper usage, data integrity, and post processing requirements quickly exceeded the scope of the original user manual. This document seeks to address these issues.

The protocol for ac-9 usage breaks down into three primary sections. We discuss basic operation and deployment issues. We discuss the ac-9 laboratory and field calibration. We delineate the steps for processing and correction of the data obtained by the instrument. These three sections are prefaced by an overview of the various engineering improvements that have occurred over the past few years. A final section provides a concise summary of the data processing steps as well as a reality check table for determination of data quality.

This protocol is intended as a hands-on guide for anyone responsible for data collection or processing of data from the instrument. For more general discussions of meter applications or measurement theory you may wish to consult the references contained in the back of the document.

One should remember that ac-9 usage and data processing techniques are subject to evolution. This document attempts to summarize the state of the art techniques as they stand today. Even as the document is being written, researchers continue to explore and refine new possibilities in applications, calibration, and processing. Similarly, engineers at WET Labs continue to strive to improve instrument capabilities, reliability and ease of use. We urge researchers to stay in touch through our web site (<http://www.wetlabs.com>) or by calling us. Likewise if you have any suggestions or additions to this protocol document please let us know.

## **2. Background and Evolution**

The ac-9 has gone through several major design modifications in the last several years to improve the overall stability and reliability of the instrument. Described below are some of the more significant changes that have been successfully implemented.

### **2.1 Interference Filters**

The ac-9 uses nine band-pass filters to spectrally discriminate the light from a tungsten lamp. These nine filters are mounted on a filter wheel located in the transmitter pressure can. It was discovered that over time (6 months to 1 year) the ac-9 experienced significant drift on the order of  $1.0$  to  $5.0 \text{ m}^{-1}$ . This drift was most prevalent in the blue wavelengths. After careful examination of the interference filters, we discovered that the loss of signal over time was due to their degradation. This degradation was visible to the eye, and in some cases, severe clouding and discoloration covered the entire surface of the filter. The ac-9 now uses a more robust interference filter set that utilizes a different manufacturing process. The current filters have shown no signs of discoloration or degradation. As a result, the long-term stability of the ac-9 has been improved significantly.

### **2.2 Absorption Detector**

The absorption detectors have gone through numerous modifications in effort to improve long-term reliability, stability, and ease of manufacture.

### **2.3 Internal Optics**

Optical mounts for all the lenses and filters were improved to provide better stability and easier meter assembly.

### **2.4 Windows**

Pressure window apertures were increased to eliminate possible partial beam occlusions.

### **2.5 Flow Tubes**

The flow tubes and sleeves went through several stages of modifications. Most recently inlet and outlet nozzle diameters have been increased to provide improved flushing.

### **2.6 Improved Referencing**

The ac-9 employs a reference detector within the transmitter optics. This detector measures the output energy from the source that in turn provides a normalized output from the meter. With the original filters and optics, throughput in the blue region of the spectrum was not sufficient to allow one-to-one referencing. We thus integrated values of all three blue wavelengths and used the single value as a reference for the blue wavelengths. With the increased throughput provided by the new filters, we have returned to a one-to-one referencing scheme throughout the spectrum.



## **2.7 Electronics**

In 1995 new electronics were developed for the meter. The new board set allowed more efficient manufacturing and characterization, more flexibility in interfacing, and improved resistance to shock and vibration.

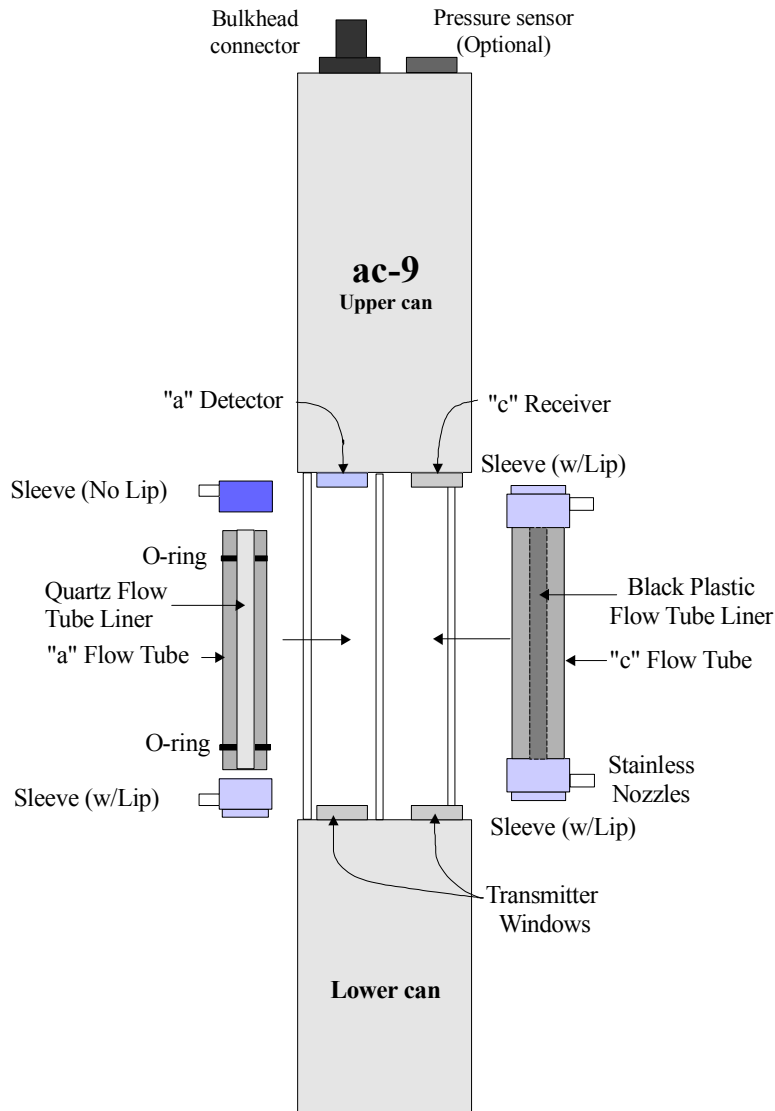
## **2.8 Mechanical**

Original ac-9 design employs a method of separating the transmitter pressure housing from the receiver pressure housing by using three independent stainless steel rods. While adequate in most cases, this design is subject to flexure and racking which would allow unwanted movement of the transmitter beams relative to the receivers. A one-piece yoke was designed to address this weakness, which we call the “unistrut.” The unistrut is manufactured from one solid piece of metal and effectively ties the ac-9 together into virtually one rigid optical assembly. This new design improves long-term stability, as well as short-term variability due to mounting stresses. Older ac-9’s can be upgraded to this new design. Please contact the factory for information.

### 3. Operation

#### 3.1 Orientation

Before testing your instrument, familiarize yourself with the ac-9 (Figure 1).



**Figure 1.** Description of ac-9 components



The instrument consists of two pressure cans separated by stainless steel spacers. The shorter of the two pressure cans houses the transmitter optics and filter wheel. The larger can contains the receiver optics and the control electronics. Two removable plastic flow assemblies reside within the area separating the two cans. These two assemblies define the flow volumes for the absorbance and transmittance measurements.

Remove the black plastic flow tubes by sliding the flow tube sleeves towards the middle of the flow tube. The flow tube will lift out, exposing the transmitter and detector windows on the lower and upper flanges respectively. Be careful not to scratch the windows. The attenuation tube is different than the absorption tube. Its flow chamber is black plastic and the two sleeves on the tube are identical. This tube installs on the “c” side of the instrument (the side with the identical looking windows). The “c” tube has no “up or down” orientation. The absorption flow assembly is lined with a quartz tube and one of the two sleeves is flat on top (the lip present on all the other sleeves is missing). This tube installs on the “a” side of the instrument that can be identified by the “a” detector that is on the upper flange and is the only window that is clearly different from the other three. The flow tube sleeve without the lip fits over the absorption detector, so in effect there is an “up and down” orientation to the “a” tube.

You may want to mark the flow assemblies and their orientation with tape or marking pen before using the instrument at sea so that there is no confusion when reinstalling the tubes after cleaning your optics. Incorrect installation of the flow tubes will result in erroneous results. Re-install the flow tubes before testing your instrument.

When re-installing the sleeves of the flow tubes, line up the white nylon set screws with the grooves in the flow tubes. This will ensure that the water flow will not be blocked by the “tabs” on the ends of the flow tubes.

### **3.2 Testing**

Before deploying the ac-9 in the field you will want to test the unit to familiarize yourself with the hardware and software, and to verify basic operation. Assuming that you are using the factory-supplied software (WETView) to perform these tests, you will require the following:

1. A clean, solid lab table or work bench;
2. The ac-9 with 10' test cable (or sea cable);
3. A 12–15 volt power supply (the ac-9 requires 10–18 VDC);
4. A Pentium® class computer running the Windows 95 or higher operating system;
5. WETView Software installed.

Installing WETView is very simple. You will need a Pentium® class computer running the Windows 95 or higher operating system with at least 16 Mb of memory and 10 Mb free hard disk space.



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Create a directory or folder to copy the necessary files needed to install WETView onto your machine. Copy the entire contents of the two disks you received with your ac-9 into this directory on your computer. You should have the following files in your directory:

- WETVIEW.001
- WETVIEW.002
- SETUP.EXE
- WETPROCE.EXE
- AC9XXXX.DEV
- AIRXXXXX.CAL

One of the files copied is SETUP.EXE; run this program and follow the online instructions to complete WETView installation.

Connect the factory supplied test cable to COM1 or COM2 of your computer. Connect the power leads to a 12–15-volt power supply. The black lead is typically the V+ lead. Before connecting the cable to the instrument, use a multi-meter to check the input power. Connect the ground probe to pin 1 on the pigtail connector (the large pin as you are looking into the connector). Connect the hot probe to pin 4 (the pin directly opposite from pin 1). You should measure 12–15 volts across these two pins. No other pins should have any voltage on them. Turn the power supply off. Connect the pigtail to the instrument. Pin one, marked on the outside of the connector by a small rubber bump, engages the large socket on the instrument’s bulkhead connector. Push the connector straight on to avoid damaging the pins. Apply power to the instrument and allow it to begin warming up.

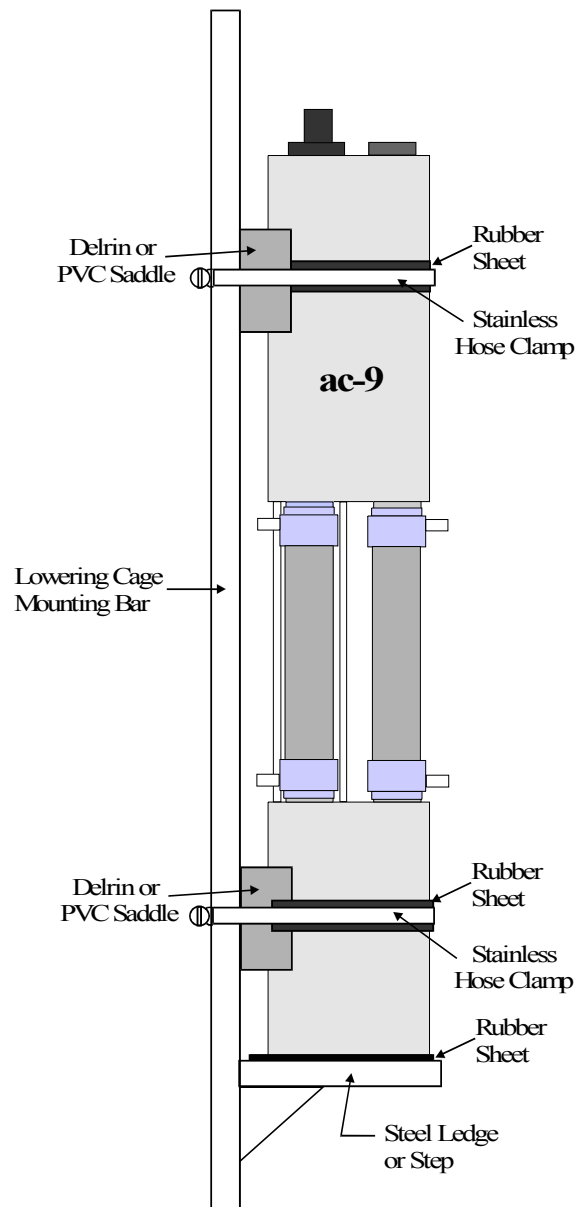
Run the WETView software by clicking on the icon in WINDOWS. When the interface is displayed, you will need to provide a device file name (DEV file). Click on the <O> button in the center top of the screen or choose “Open Device File” from the File Menu at the top left of the screen. The program will ask you to choose the proper COMM port. Select COMM1 or COMM2 as appropriate. At this point, the software will do some handshaking with the instrument and the “Start Logging” Button (or the F1 key) can be used to start data collection. After 5–10 seconds, tabular data should be displayed on the right side of the screen. A real time graph will begin to develop, using the graph parameters set at the time. Please refer to the manual for the full details of running the WETView software. After a short time, click on the F2 button. Data collection will stop and you will be prompted for a file name to apply to the data if you should want to archive it. Press ESC if you do not want to save the data. To quit the program, choose QUIT from the File menu. At this point you have successfully completed a bench test of the instrument.

Once the meter is running you may wish to verify operation by obtaining an air file, checking instrument precision and comparing results with a factory-supplied air tracking file.

We provide an air CAL file that is similar to the DEV file and can be applied in WETView in the same manner. The difference in the two files is that the DEV file provides the clean water offsets so that when measuring clean, fresh water, the instrument’s output should be very nearly 0.0 for all channels. The CAL file provides the

offsets that provide 0.0 values when the instrument is clean and dry and measuring air values. Refer to Section 4.2 for more details on using your air tracking files. Remember, when obtaining air files that it is important to block light entering the flow assemblies by covering the inlet and outlet nozzles with tape or the black plastic caps provided by the factory.

### 3.3 Mounting



Note: Tighten upper hose clamp tightly. Lower hose clamp should be just snug to avoid applying any torque to the ac-9.

**Figure 2.** Deployment cage mounting suggestion for ac-9

The ac-9 contains two optical paths that are sensitive to lateral and torsional stresses. To ensure that the unit functions properly, it is important to minimize stresses when mounting the unit to a frame. If possible the ac-9 should be mounted upright. It is preferable to rest the bottom of the ac-9 on the cage framework and attach both the upper and lower housings to the vertical framework of the cage (Figure 2). The housing attachments do not need to be any tighter than needed to hold the ac-9 to the cage as the main support is the bottom rest. If the ac-9 is to be mounted horizontally it is critical to support both housings. If the ac-9 is mounted horizontally and supported by only one housing, deformation of the optical path will occur causing the unit to provide inaccurate readings. We recommend using the air CAL file to verify that the mounting of the instrument has not altered the optical alignment of the meter (see section 4.2, “Air Tracking Procedures” for more information concerning air tracking files).

### **3.4 Plumbing and Tubing**

It is important to ensure good flow through the ac-9 flow tubes. The flow rate through the instrument should be kept above 1 liter/min. This can be achieved by maximizing the tubing size and using a pump such as the Sea-Bird Electronics SBE-5 running at a minimum 3000 rpm. The original ac-9 nozzles were designed for 5/16 inch inside diameter tubing, but 1/4 inch tubing works as well. An increase in tubing size is necessary to mate with a Sea-Bird SBE-5 pump. Sleeves with 1/2 inch nozzles are now available (Oct 1996) allowing a single size tubing for connection with a SBE-5 pump. These larger nozzles may improve the instrument performance by potentially increasing the flow rate and flushing through the flow assemblies. Regardless of the nozzle size, the tubing can be stepped down to the size necessary to fit the ac-9 near the flow tubes.

The detectors in the ac-9 are sensitive to external light so it best to minimize the external light to the unit. This is accomplished by ensuring that all tubing is dark or covered with black tape. The tubing should curve up to the pump and down for the intakes. The bend in the dark tubing reduces direct light into the flow tube.

Tygon tubing may contain plasticizers that affect the measurements. For short lengths of tubing in applications using continuous flow the type of tubing may not be a concern. When using Tygon tubing, it is best to use a thick-wall tube to prevent kinks in the tubing or collapsing the tubing once connected to the pump. Other types of tubing are available and include Teflon, Teflon-lined Tygon, and a new Tygon tubing without plasticizers. Many of the types of tubing are rigid and care must be taken to prevent kinks from forming.

The pump for the ac-9 should be placed above the upper set of nozzles of the ac-9 flow tubes. It is required that the pump pull the water through the tubes rather than push it through. It is critical that all plumbing help move bubbles out of the system. Cavitation and particles shed by the pump can create large changes in the optical properties of the water.

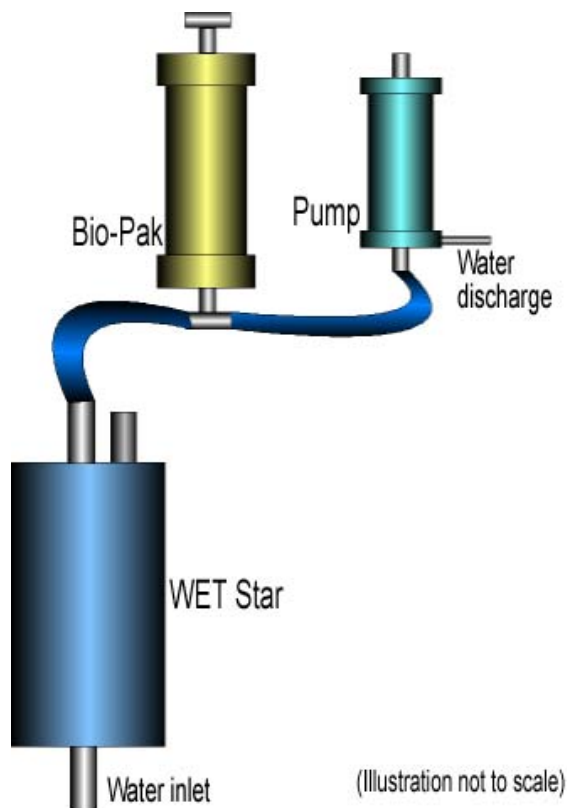
In profiling applications in which upcast and downcast information are both desired you will want to balance the inlet and outlet pressures of the flow system. This is achieved by assuring that the entrance point and exit point of the tubing are oriented horizontally with respect to the profiler package.

### 3.5 Deployment

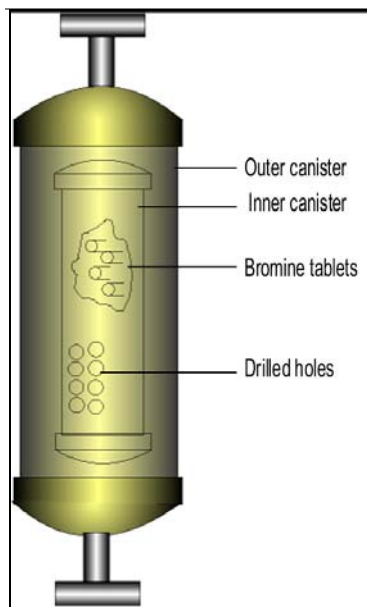
The ac-9 is used in a variety of deployment modes. While emphasis and protocol development has focused primarily upon profiling applications, the meter has also been used in moored, underway and towed applications, as well as in the laboratory. Each of these methods requires some consideration in how best to optimize results from the meter.

#### 3.5.1 Moorings

*3.5.1.1 Anti-fouling*—One of the most problematic aspects of a moored deployment of any optical device is accumulation of biological growth on the optical surfaces. The enclosed flow path of the ac-9 retards biological fouling of the meter’s windows and the reflecting tube. This retardation can be enhanced through the gradual release of bio-toxins into the sample volume between measurements. Figures 3 and 4 show schematic representations of an anti-fouling toxin delivery system.



**Figure 3.** Anti-fouling delivery system.



Construction details of a Bio-Pak designed for 6-month deployment:

- PVC for outer canister: 2-1/2 in. dia., 18–24 in. long.
- PVC for inner canister: 2 in. dia., 17–23 in. long.
- PVC caps for both canisters, secured with setscrews.
- Two plastic “T” fittings: top fitting for water inlet, bottom for anti-fouling solution outlet.
- Drilled holes are 1/8–3/16 in. diameter, 8–12 evenly spaced around inner canister.
- Tubing, 24 inches of 1/2-in. dia. (WET Star and SAFire use 3/8-in).

Note: The diffusion rate will vary depending on water temperature, number and size of holes drilled, sample rate, etc.

**Figure 4.** Details of Bio-Pak

A delivery canister, filled with time-release bromine tablets such as Bioguard (commonly used in home spas), is placed between the flow outlet of the meter and the pump. During intervals between sampling, the bromine concentration within the meter builds and diffuses throughout the rest of the flow system. During measurement periods the brominated water flushes from the flow tubes and plumbing. The time it takes to rebuild toxicity within the flow chamber depends upon the canister bromine capacity, water temperature and exposure of the canister to active water flow. Typically bromine concentration will build to toxic levels within about fifteen minutes.

Because of the wide range of environmental variables and the specific design of the delivery system it is impossible to offer an exact formula for proper incorporation of a bromine anti-fouling system. However, basic guidelines for design and implementation are provided:

1. Assume a use rate of approximately 1–2 pills per month of operation.
2. Using a test setup restrict flow between the canister and the rest of the system, so that it takes about 15 minutes to achieve minimal toxin levels within the primary flow path.



### Note

You can use a common spa tester kit to determine your concentrations. Be aware that at very high concentrations the bromine bleaches the phenyl red indicator. This rate of diffusion may require tuning depending upon sampling interval. For longer sample intervals you may want to slow down diffusion to avoid building too high concentrations within the flow chamber.

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3. Allow 10–20 system flushing time constants before sampling.

Bromine does have an absorptive spectral signature that can be seen by the ac-9. It also is a very strong oxidizer and in high concentrations, can attack exposed surfaces including both metal and plastics.

Ideally the bromine concentrations should reach about 6–10 ppm within 20 minutes after sampling, and should never exceed 100 ppm concentration levels. The rate of diffusion can be tuned by adjusting the flow between the meter and the canister. Alternatives to the bromine canister technique are presently being investigated. These include TPT and copper diffusion rings. You may wish to contact the factory before implementing an anti-fouling unit for your moored deployment.

*3.5.1.2 Warm-up*—The ac-9 is typically characterized and used after a 5 to 10 minute warm-up from initially powering the meter. Moored deployments typically require sampling within thirty seconds after turning the unit on. In order to assure accuracy in the field, multiple samples should be collected with a clean dry system in the lab, using the sample interval that is to be employed in the mooring. For best results the testing should occur at temperatures close to those to be found in the water. Once multiple files have been collected air values may be compared to air tracking values provided by the factory. The resultant offsets can then be applied the water values to correct for the warm-up time.

*3.5.1.3 Ground loops*—The ac-9's case operates at ground potential, effectively tying the instrument common to the seawater. Under normal circumstances this should create no problems. However, depending upon other packages tied to the mooring, inadvertent current leakage paths and ill-considered power schemes there lies potential for ground loops. In moored deployments, where packages can potentially be left unattended for months, the ground loops can drain batteries, result in noisy measurements, and damage instruments. While there is no set method for the determination and elimination of ground loops the following steps provide general guidelines:

- Create a systems grounding diagram. Consider the seawater as a ground plane.
- Note all terminations to the seawater.
- Measure voltages across these terminations to determine possible voltage potentials. Also check voltages across the instruments to the cage.

- If possible immerse the package in salt water, and repeat step three while the package is still wet.
- A 2–3 day test deployment with instruments in the water could provide important information on expected versus realized battery voltage decay.
- Mitigating a suspected ground loop is highly system specific. If you suspect a loop you may wish to consult the factory for advice.

*3.5.1.4 Plumbing*—The inability to pre-purge moored deployments in near surface waters makes it vitally important to properly plumb your system. If the tubing and meter orientation do not facilitate rapid flushing of bubbles, air could easily become entrapped within the flow assemblies. Also, in areas where the meter may be sampling large amounts of resuspended particulates, good flushing is critical to prevent sediment build-up within the meter. Pumping speeds should be set to higher speeds (4000–4500 RPM for the SBE-5) and the incorporation of the larger nozzle diameter lock sleeves is recommended.

*3.5.1.5 Post calibration*—A post calibration will allow the researcher to track any drift due to fouling or possible instrument changes. The calibration should be performed as soon as possible after removal of the mooring from the water and after stored data is uploaded. Using a field water calibration (See Section 4.3.) allows the meter to first be checked in “as-is” condition to compensate for fouling. A second calibration after the meter has been thoroughly cleaned will allow the user to track instrument specific drift. In situations where optically clean water is not available, obtaining an air tracking file after the meter has been cleaned and dried will at least provide an indication of the meter stability through the period of performance.

*3.5.1.6 Power consumption and battery life*—To assure that a viable data set is collected during the entire period of deployment one must assure that they provide enough energy capacity (batteries) to effectively operate throughout the duration. An ac-9 with pump consumes approximately one amp at twelve volts. Assuming a nominal on-time of one minute for each sample this means that the instrument will use about 1/60 of an amp-hour during each cycling. In addition one must consider the power consumed by the data logger tied to the instrument in both its “on” and “off” states. Batteries typically provide a rated capacity in amp-hours, but this can mean different things for different types of batteries. For instance a twelve volt D-cell alkaline battery pack is rated around 12–14 amp hours, but due to the near-linear decay rate of the batteries and the fact that this rating implies the amount of energy that the battery might provide until it is at 50 percent voltage, the usable capacity is only about 1/3 of the rated capacity. You must also take into account derating of the capacity due to lower water temperatures. Near zero degree Celsius temperatures could reduce usable lifetimes by 30 percent. In generally it is wise to provide ample over capacity in your power system. All things considered, the price of batteries is cheap compared to the price of lost data.

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### **3.5.2 Towed Bodies**

*3.5.2.1 Mounting*—In mounting to a towed unit you must consider both the stability of the ac-9 and the flight characteristics of the entire towed unit. While the latter consideration is out of scope for this discussion, the former topic is quite simple. The mounting should firmly secure the meter towards both ends, without torquing the unit. Neoprene lined saddle clamps are recommended for this purpose. The clamps should securely anchor to the frame. In securing the ac-9, make sure that adequate clearance is provided for plumbing and wiring. Once mounted, obtaining a good air tracking file (See Section 4.2) is imperative to ensure the meter is stable in its new orientation.

*3.5.2.2 Flow*—Flow inlets and outlets need to be placed so that the hydrostatic pressure is equivalent. This is most easily established by locating the inlet and outlet hoses at the same level.

*3.5.2.3 Pre-purge*—Typically, in towed deployments plumbing of the ac-9 is not ideally routed for purging of air at the beginning of a deployment. The simplest technique for purging the flow system in this situation is to use the water pressure of the initial descent. As the unit is initially lowered the air will naturally squeeze out. This means that data on the first descent should be considered as suspect. Likewise if the towed body breaches it will require another purge descent.

*3.5.2.4 Consider time constants*—Time constants for meter sampling should always be considered, but in the case of towed deployments, this can be especially critical, because of the potentially high descent and ascent rates or the horizontal speed of the package. Besides the six sample per second acquisition rate of the meter, one must also consider the pumping rate and plumbing for the unit. The flow path between the tubing entrance and the meter will be defined by a time constant. This will in turn result in spatial aliasing of the meter's data with respect to the rest of the package. The easiest way to determine this time constant is to directly measure it using your pump.

*3.5.2.5 Protect the meter*—If your instrument is directly exposed to water flow (mounted on an external frame as opposed to being mounted within an internal cavity) the case will be subject to erosion from the water. A simple neoprene sleeve or a plastic nose cone mounted to the meter will greatly reduce the effects of the erosion. The nose cone offers the added benefit of reducing drag upon the unit. If you require either of these items and do not have in-house resources available for their manufacture you can obtain these items from WET Labs.

### **3.5.3 Underway/Benchtop Operation**

*3.5.3.1 Keeping the meter within its specified temperature range*—Temperature characterization and compensation of the ac-9 are based upon *in situ* or underwater operation of the meter. The meter's temperature characteristics depend upon the thermal flux between the meter and its environment. Since water and air make

substantially different ambient environments, in order to assure stable operation, it's important to immerse the instrument in water for underway or benchtop operation. At very least the transmitter housing should be submerged.

*3.5.3.2 Initial purge*—Providing a bubble-free water delivery system for the unit can usually be accomplished by simply plumbing the unit so that water flows into the unit from the base and flows out from the upper side of the flow cells (i.e. the water should always flow upward.).

*3.5.3.3 Watching for bubbles*—Once you are convinced that your unit is purged, check for bubbles within the water supply by:

- Directly observing water flowing into the meter.
- Observing values obtained by the meter. If the readings rapidly increase with no apparent change in water type or if they hold a high variance, inspect your system.

### **3.5.4 Profiling**

*3.5.4.1 Mounting*—When profiling, the meter is usually mounted vertically on a cage. The pump should be located above the ac-9 flow tubes to allow it to pull water up through the flow tubes. The inlet nozzle tubing should be arranged to sample from undisturbed water below the package. (See Section 3.3 for detailed instructions on instrument mounting)

*3.5.4.2 Pre-purge*—Care must be taken to ensure that all bubbles are purged from the system before beginning to sample. Lowering the package to 20–30 meters during the initial 5-minute warm-up provides effective purging.

*3.5.4.3 Free-fall Descent*—If possible, the deployment package should be a free-fall type system, with the buoyancy set to allow a slow descent rate. By making the package a free-fall type, it becomes de-coupled from the ship's motion, allowing better vertical resolution and preventing hydrostatic surging in the flow system.

*3.5.4.4 Time Constants and Spatial Resolution*—The actual descent rate you use should depend on system capabilities and the desired vertical resolution. A descent rate of 30 cm/sec will provide 20 data points in each meter of descent allowing good vertical resolution. The flushing rate of the flow assembly also factors into this consideration. Each side of the flow assembly encapsulates a 25 ml volume. This means that for a 2 l/min flush rate the meter will completely exchange volumes 40 times. At this flow rate no significant signal degradation is seen for package descent rates on the order of 30 cm/sec. However, with significantly reduced flow rates the package descent rate needs to slow to maintain spatial resolution.

*3.5.4.5 Care of Meter Between Casts*—Requirements for cleaning the ac-9 between casts vary depending upon the interval between casts, water conditions, and signs of obvious fouling from previous casts. Basic guidelines are:

- The meter should be cleaned at least once per day. This could occur after the last cast of the day or before the first cast of the day. It can also correspond with a field calibration. (See Section 4.3 for field calibration details.)
- The meter should be cleaned if fouling is suspected.
- The meter should be cleaned if the intervals between casts are sufficiently long to allow drying within the flow assemblies.
- Profiling in very clean waters where signal changes are on the order of  $0.01 \text{ m}^{-1}$  may require more frequent cleaning.
- In addition to cleaning optical surfaces, washing down the exterior of the meter regularly with fresh water reduces possible effects of corrosion.

## **4. Calibration**

### **4.1 WET Labs Calibration Procedures**

The standard ac-9 calibration procedures at WET Labs includes a series of characterization tests to confirm the instrument's performance is within factory specifications, temperature calibration, pure water calibration, and an air calibration.

#### **4.1.1 Factory Pre-calibration Procedures**

The pre-calibration procedures at the factory confirm that the ac-9 is operating within specifications before it goes through calibration. First, a 12-hour burn-in period indicates if there are any immediate failures. The optical throughput of the ac-9 is then tested by recording the output of the signal and reference detectors. Minimum and maximum signal levels are determined to assure that the appropriate instrument precision and dynamic range can be obtained for each channel. A mechanical stability test is performed by subjecting the ac-9 to shaking/vibrations in both the horizontal and vertical positions. The ac-9 is also subject to a shock test to make certain that the output of the meter is not altered during normal shipping and handling procedures. A final performance test is performed by collecting data with the ac-9 on the bench. After a sufficient warm up period, the precision of the instrument must be less than  $0.002 \text{ m}^{-1}$ . Precision is determined by taking the standard deviation of an air data file, measured in inverse meters at a six scan integration.

#### **4.1.2 Factory Temperature Calibration**

During the temperature calibration of the ac-9, the instrument's temperature coefficients are determined. The temperature coefficients provide a correction factor for temperature for each channel of the ac-9. The temperature calibration data is also used to identify unusual instrument performance issues causing the output of the meter to change dramatically as a function of temperature.

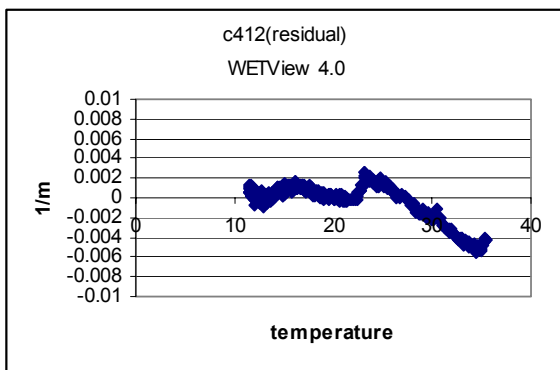
The WET Labs temperature calibration is performed by placing the ac-9 in a water bath. Initially, the ac-9's flow tubes are filled with argon and sealed off, preventing any moisture from reaching the flow path or the windows. The water bath temperature is cooled from about 35 degrees Celsius down to approximately 5 to 7 degrees Celsius over a 90-minute period. This water temperature range corresponds to an internal instrument temperature range of approximately 10–40 degrees Celsius. Data is recorded during this period. After a complete temperature cycle, the data is examined to determine if there are any unusual features in the absorption/attenuation values as a function of internal instrument temperature. If changes over temperature of the meter vary too greatly or if severe non-linearities are detected, the instrument is sent back to the production floor for examination and necessary modifications. This process may include replacement of detectors, lamps, or electronics depending on the cause of the problem. If the instrument looks good after the initial temperature cycle, the temperature coefficients are calculated and applied to the device file ultimately supplied with the meter.

Presently there are two methods of temperature correcting the ac-9 data being used. The initial compensation algorithm (WETView 4.0) applies a linear correction to the data. Since the meter's response to internal temperature is often not linear, this creates a residual error from the correction. The temperature cycle is then repeated. With the temperature coefficients applied in the processing software, the residual error must not vary by more than  $0.01 \text{ m}^{-1}$  over the entire temperature range. Once the compensated values are determined to be within specifications the correction factors are then applied to the Device File for the specific meter undergoing calibration.

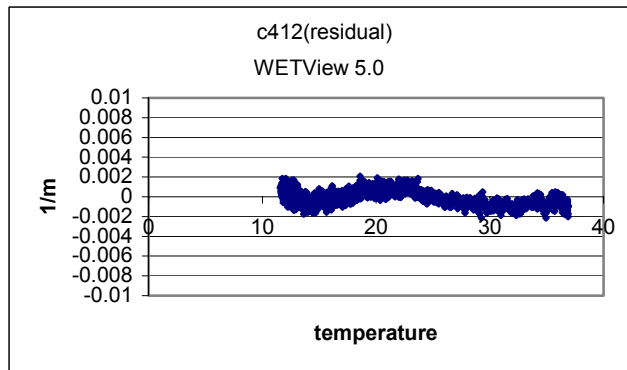
WETView 5.0, when used with the new device file type, employs a correction algorithm that uses multiple offset values,  $\Delta T_n$  values obtained by measuring output differences over small temperature increments. Instrument values are collected and averaged every one to two degrees C through the operational temperature range of the instrument. From these values we generate a table of temperature compensation offsets  $[\Delta T_n]$ . This table is contained in each instrument's device file. Using the table, WETView 5.0 then applies the algorithm,

$[a' = a_{raw} - \Delta T_n]$  for given temperatures in the table. For temperatures that fall between table values, the program applies a linear interpolation upon the data for further correction. By using this scheme, we can thus effectively compensate for non-linear changes due to temperature, in the instruments' output.

For examples and explanation of device files, refer to the Appendix.



**Figure 5A**



**Figure 5B**

Figures 5 A and B demonstrate the relative merits of both correction schemes by looking at the residual error from an instrument's c412 channel after temperature correction with both methods. The 412 nm channel was chosen because residuals are typically worst case at this wavelength. While the WETView 4.0 correction technique reduces the residual error to less than  $0.01 \text{ m}^{-1}$ , the WETView 5.0 method improves correction to within two standard deviations of the instrument's precision.

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The internal instrument temperature range, defined for each individual meter, may vary by a few degrees although typically the range extends from about 10 to 40 degrees Celsius. The exact temperature range is specified for each meter on the Calibration Sheet that is supplied for every new calibration. The ac-9 will remain within the factory temperature specifications over the internal instrument temperature range specified. Operation within this range is strongly recommended.

For an example and explanation of a Calibration Sheet, refer to the Appendix.

#### **4.1.3 Factory Water Calibration**

The purpose of the WET Labs water calibration is to determine the offset values of absorption and attenuation that result in a  $0.00 \text{ m}^{-1}$  reading with pure water in the sample volume of the flow tubes. These water offset values are listed on the Calibration Sheet and included in the device file for each meter's new calibration.

Before conducting a water calibration, the ac-9 optics are properly cleaned and the meter is allowed to warm up for at least 15 minutes (see Section 4.2.2 for detailed cleaning instructions).

WET Labs maintains a custom pure water system that includes a commercial de-ionization system and filtration system. After primary de-ionization, the water is processed by a Barnstead purification unit and stored in a 60-liter holding tank that re-circulates through an ultra-violet chamber and additional purification filters. Water for calibration is drawn through a final 0.01-micron ultra-filter at the point of delivery. The circulating holding tank allows the highly reactive de-ionized water to equilibrate with the ambient conditions and the ultra-violet chamber prevents any biological contamination from entering the reservoir. The system is continuously monitored and water quality is checked using a simple scattering detection test prior to each calibration to maintain consistent and accurate water calibrations.

During the water calibration, water from the pure water system is flushed continuously through the ac-9 at a rate of approximately 1.5 l/min. Values of absorption and attenuation are collected using WETView and the values are applied to the device file. With the offsets applied the ac-9 should then read zero on all channels. In order to confirm that the offsets are accurate, the cleaning process is repeated until the results are repeatable to within  $\pm 0.003 \text{ m}^{-1}$ . Once the offsets are collected they are applied to a Device File specific to each meter. The offsets are thereafter automatically applied when running WETView, or alternatively can be used with a user-developed program

For an example and explanation of a Device File refer to the Appendix.

Two parameters are important for the user to consider when processing data, trying to obtain water calibrations in the field, or reproducing pure water calibrations in the laboratory—the water temperature and the internal instrument temperature. The



water temperature is recorded during the calibration with typical values ranging from less than 5 to 35 degrees Celsius. When processing data it is important to account for the changes in absorption/attenuation of water that occur as a result of temperature (see Section 5, Data Processing). Knowledge of the water temperature during calibration becomes critical. Internal instrument temperature is also recorded for each water calibration. It is important that this value fall within the temperature range specified in the temperature calibration. Both the water temperature and the internal temperature of the instrument at the time the calibration is performed, are recorded on the Calibration Sheet.

#### **4.1.4 Factory Air Calibration**

The purpose of the WET Labs air calibration is to determine the offset values of absorption and attenuation that result in  $0.000 \text{ m}^{-1}$  readings with air in the sample volume of the flow tubes. These air offset values are listed on the Calibration Sheet and included in the air tracking device file for each meter's new calibration.

Before conducting an air calibration, the ac-9 optics are properly cleaned and the meter is allowed to warm up for at least 15 minutes (see Section 4.2.2 for detailed cleaning instructions). One of the most critical aspects of obtaining a good air calibration is that the exposed optics and flow assemblies of the meter must remain absolutely dry.

During the air calibration the ac-9 absorption/attenuation values are collected using WETView and the values are applied to the air tracking device file. With the offsets applied the ac-9 should read zero on all channels. To confirm the offsets are accurate, the cleaning process is repeated until the results are repeatable to within  $\pm 0.003 \text{ m}^{-1}$ .

Once the air offsets are collected they are applied to a separate device file. (This file is provided with each new meter distribution as is denoted as AIRXXYYY.DEV where the XX represents the calibration number and the YYY represents the instrument serial number. This file can subsequently be used in air tracking procedures.)

An important parameter to consider when trying to confirm or reproduce air calibrations is the internal temperature of the instrument during the air calibration. Because the output of the ac-9 is only compensated for temperature over the temperature range specified on the Calibration Sheet, any operation outside of this range may result in offsets in the data. This is most often a concern with air calibrations because the instrument is operating in air, meaning a large portion of the heat generated internally by the ac-9 is not rapidly dissipated to the surrounding environment. The temperature of the instrument during the WET Labs air calibration is recorded on the Calibration Sheet.

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## 4.2 Air Tracking Procedures

### 4.2.1 When to Use Air Tracking

Air tracking is primarily intended to be used to monitor offsets in the instrument's output due to changes in the optical system caused by shipping or mounting of the instrument to a cage or other deployment package. Air tracking can also be used to monitor instrument drift over extended periods of time. Air tracking involves the collection of data with the ac-9 in air using WETView and the air CAL file. Output collected following the Air Tracking Protocol are considered the Air Tracking Offsets. These offset values are an indication of changes in the optical throughput of the instrument since the factory air calibration and can be used in certain situations to correct data collected using the ac-9.

We recommend collecting air data immediately when you receive your ac-9 from the factory. Use the air tracking device file, AIRXXYYY.CAL, with the WETView software package. Connect the ac-9 to power and allow it to warm up for at least 15 minutes. Without removing the flow tubes or cleaning the optics, the absorption/attenuation values should be close to zero (at least within +/- 0.01). Use the Air Tracking Protocol listed below to obtain good air readings. Large offsets in the data may indicate misalignment of the optics during shipping and handling.

Air tracking data is most easily obtained in the laboratory, where the environment is consistently clean and dry. We recommend recording good air data in the laboratory before and after all ac-9 deployments. Air calibrations can be performed while in the field, however, it can be difficult to obtain good air calibrations on a ship due to the moist environment. Readings can be significantly offset by small amounts of moisture or dirt in the flow tube sample volume, resulting in considerable tracking inaccuracies.

Collecting air data has also proven extremely useful in verifying appropriate ac-9 mounting methods. Take an air data file in the laboratory prior to mounting the ac-9 to the deployment package. Repeat the test after securing the ac-9. If the air data results in significant offsets, it is a very good indication of inappropriate mounting (refer to Section 3.3 for explanation of appropriate mounting techniques).

### 4.2.2 Air Tracking Protocol

*4.2.2.1 Soap wash and rinse*—Remove flow tubes and all O-rings from the windows. Remove the collars from the flow tubes. Remove the O-rings on the flow tubes themselves. Use a mild detergent solution to gently wash all of the windows and rinse the flow tubes. Use Kimwipes or lens paper to wash the windows. Rinse off the meter completely with water to ensure no soap residue is left inside the flow tubes or on the windows.

*4.2.2.2 Dry the meter*—Place the instrument in a protected area where it can dry out completely. Using a small heater to blow warm air over the meter may help speed up

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the process. Using dry nitrogen to blow dry the meter and remove water from the small grooves around the windows will also help speed up the process. It is suggested that the instrument be left over night to dry out completely. Reassemble the meter. Carefully replace O-rings and slide the sleeves back on the flow tubes. Replace O-rings around the windows.

*4.2.2.3 Solvent Cleaning*—Use a Kimwipe or lens paper. Clean the windows with reagent grade methanol or ethanol. (When using ethanol make sure to use protective gloves.) This process should remove residual stains from oil and grease on the windows. Repeat this two to three times. Using a small flashlight to carefully examine the windows is also helpful.

*4.2.2.4 The flow tubes should also be cleaned*—Place a few drops of methanol on a Kimwipe and, using a wooden or plastic dowel rod, carefully slide the Kimwipe through the flow tube. Repeat this procedure with both flow tubes. Examine each flow tube when you are through, to make certain that there are no streaks or small pieces of lint left on the inside of the flow tube.

*4.2.2.5 Dry the windows*—Since small amounts of moisture can affect the air readings, it is important to ensure that the meter is completely dry. Using nitrogen to blow dry the windows immediately before replacing the flow tubes works very effectively. This will remove any water or methanol trapped in the small grooves around the window.

*4.2.2.6 Replace the flow tubes*—Carefully slide the flow tubes into place, avoiding direct contact between the window surfaces and the ends of the flow tube. Slide the collars up around the windows and over the O-rings, making certain they are firmly in place and aligned correctly. Use small black caps, or black electrical tape, over each of the nozzles on the flow tubes to provide a dark environment and to keep the meter clean and free of moisture while obtaining data.

*4.2.2.7 Allow meter to warm up*—If your meter is not yet powered, turn the meter's power on and allow the meter to warm up for at least 15 minutes. When the meter is stable you should be able to collect 10 minutes worth of data and the values should not vary more than  $0.005 \text{ m}^{-1}$  over the 10-minute time period.

*4.2.2.8 Collect data*—Use WETView and the air CAL file to record a one to two minute file and save the data. Repeat steps 4.2.2.4 through 4.2.2.6 until you can collect three data files, cleaning after each file, such that the average values for each channel vary by no more than  $0.005 \text{ m}^{-1}$ .

### **4.2.3 Applying Air Tracking Offsets**

The air tracking offsets can be applied to the users' ac-9 device file (DEV file). This process assumes that the offsets acquired in air are equal to the offsets the ac-9 would measure in water. We recommend doing this only when you are certain the air offsets are repeatable.

1. Follow the air tracking protocol to acquire data.
2. Take the average values of the acquired data for each channel. This can be done by importing the data into a spreadsheet program, such as Excel. Alternatively, by setting a relatively large binning value in the WETView software package (greater than or equal to 30) you can simply write down the values displayed upon the screen. Subtract these average values from the offsets column in the device file. Make certain to save the new device file under a new name and back up your old device file so you do not write over the original device file.

## **4.3 Field Water Calibration Procedures**

### **4.3.1 When to use Field Water Calibrations**

Maximum accuracy of measurements is obtained by performing water calibrations of the ac-9 in the field. Field water calibrations can remove the effects of small misalignments of the optical system caused by shipping or mounting of the instrument on a cage or other deployment package. Furthermore, field calibrations allow the operator to track possible instrument drift, and finally provide a superior confidence assessment of the acquired data.

When a reliable source of optically clean water is available, water calibrations are easy to perform in the field. The concept behind the water calibrations is simple. The idea is to provide the instruments with a source of clean, bubble-free water that can be used as a reference value. This is the same concept used when the factory offset values are determined.

Clean water can be produced in the lab and transported to the ship, or a portable system can be constructed for shipboard use. For shorter cruises we recommend the former of the options, however if you are inclined to produce your own clean water, we provide a general description of what is required in this document.

### **4.3.2 Field Clean Water Production System**

*4.3.2.1 Pre-Filtration Unit*—To increase lifetime of the primary filtration unit it's desirable to provide pre-filtration of the input water. A 1–5 micron commercial cartridge with possible addition of a activated charcoal filter work well for this task.

*4.3.2.2 Primary Filtration Unit*—The primary filtration unit typically consists of a commercial filtration unit such as the Barnstead E-Pure system or the Milli-Q Q-Pak treatment system. These units incorporate multiple stage filters to remove particulates, free ions, and organics. For the purposes of optically pure water

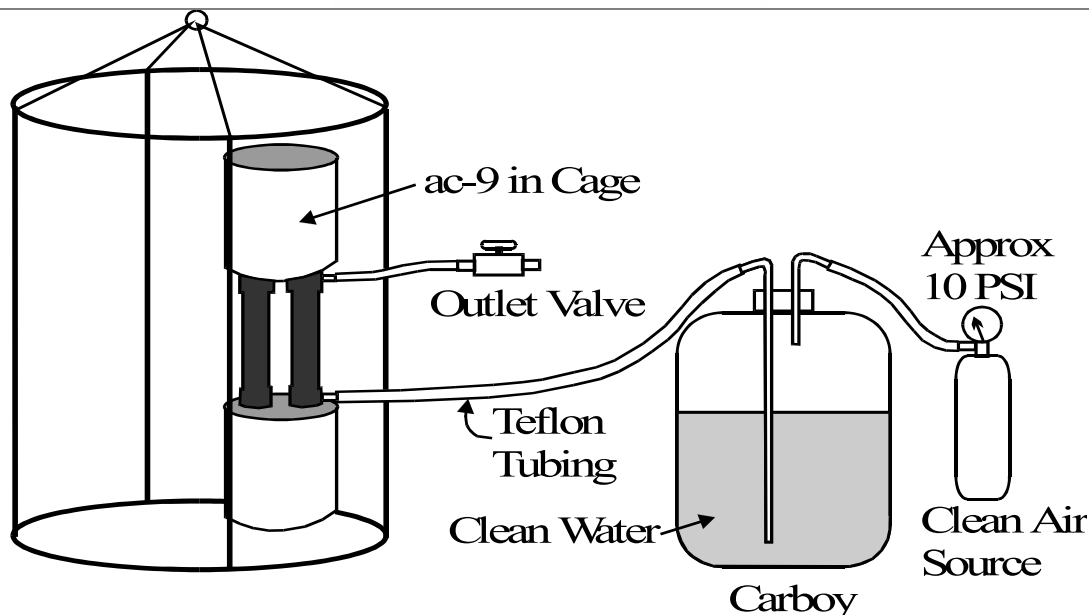
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production, organics and particulates form the dominant signals. These systems require an active AC power source so factor that in when considering use of the system.

*4.3.2.3 Water Storage Unit*—Rather than directly coupling the clean water output from the production unit to the ac-9 outlets, it's advisable to provide an intermediate storage unit. A clean 20-liter polycarbonate carboy works well for this task. The advantages of storing the water are two-fold. It allows the clean water to equilibrate with the ambient temperature, thus removing bubbles. It allows one to decouple the production unit from the delivery system. This allows one to place the production unit out of harm's way near a good water source.

*4.2.3.4 Water Delivery System*—A field water delivery system is straightforward to set-up and operate as shown in Figure 6. A polycarbonate or polypropylene carboy is recommended to store optically clean water. It is easy to clean, portable and appropriate fittings are easy to obtain.

A cap with barb fittings is used to allow for the connection of tubing to pressurize the carboy and to allow water flow to the instruments. The carboy is pressurized to approximately 10 psi using a clean air source such as an oil free air pump or a tank of nitrogen gas. The air tube inside the carboy should be short to prevent creating bubbles when pressurizing the carboy. A tube for the water should extend nearly to the bottom of the carboy. It is recommended that Teflon tubing be used to connect the carboy to the ac-9 because Tygon tubing may contain plasticizers that can add contaminants to the water. The tubing from the carboy is connected to the bottom nozzle on the ac-9 flow tube. It is best to use black tape to cover the tubing near the ac-9 to reduce the possibility of light leaks into the instrument. A short piece of tubing with a valve is connected to the top nozzle on the flow tube. The valve serves two purposes. It allows the operator to stop the water flow, conserving the calibration water. It also provides some backpressure, which helps to keep gases in solution preventing the formation of micro-bubbles. An optional 0.2-micron filter may be placed at the point of delivery.



**Figure 6.** A simple schematic of a field water-calibration delivery system.

Maintaining a viable clean water production system requires some care and common sense. Most filtration packs perform de-ionization of the water. Using a mineral rich water source may quickly foul the filter cartridge. Storage of the unit between uses may require special handling of the filters. Many systems provide a resistivity gauge that indicates the ion purity of the water. This is useful in determining whether your filters are fouled but is not a reliable indicator of optical clarity.

### 4.3.3 Field Water Calibration Protocol

#### 4.3.3.1 Obtaining a Calibration—

1. Make sure that the meter is clean (See Section 4.2.2 for cleaning techniques) and that it has warmed up for at least 5 minutes. Check the internal temperature to ensure that the unit is operating within the temperature range provided on the calibration sheet.
2. Once the delivery system is connected to either side of the flow assembly, pressurize the carboy to approximately 10 psi and open outlet valve to purge air out of the system. Typically you can directly observe the bubbles as they are pushed out of the meter.
3. Once the meter is purged, use the valve to set the flow rate to approximately 1.5 to 2 liters per minute to simulate the actual water flow during deployment. Once again check for escaping bubbles at the outlet.

4. Using WETView or your own data collection software, collect approximately 30 seconds of data into a file. Do not bin your data. This is to ensure that if a small bubble passes through the system it can be identified and removed from the data stream. Make sure that the output is stable.
5. When the acquisition is completed, turn the water off and place the tubing on the other ac-9 flow cell. Repeat steps 2–4. Calibration should not be performed on the attenuation and absorption meters simultaneously. We recommend doing the two sides separately to provide better control over the calibration procedure. Use the same carboy to calibrate both sides of the instrument.

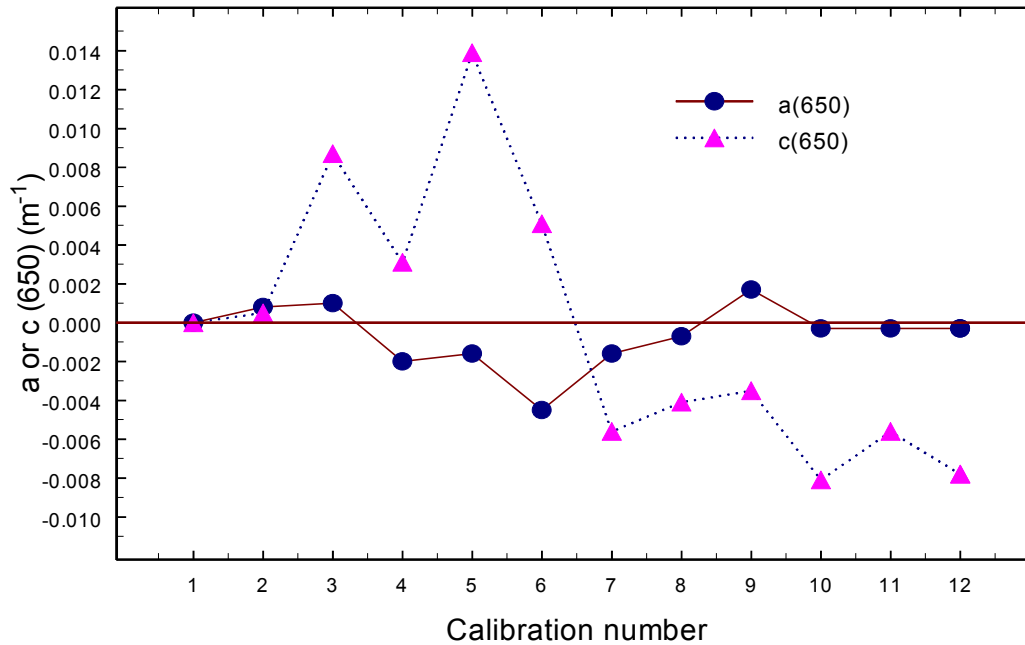
When the calibration is completed the calibration water temperature must be logged to allow water temperature corrections to be performed on the data.

When performing the calibrations it is important to look for bubbles in the water that will create large spikes in the data being collected, and ensure there is not a linear trend in the calibration file that may indicate cleaning of the optical surfaces with time.

The best time to perform water calibrations is upon completion of the field measurements. By performing the calibrations at this time the instrument has warmed up and the internal temperature is near the operating temperature within the water.

In most cases field calibrations only need to be performed once or twice during a cruise. More frequent calibrations will provide an indication of the instrument stability (Figure 7).

Check to see if the calibration changes after the instrument is cleaned. Apparent changes in the instrument's stability when determined prior to cleaning may well be due to environmental factors such as sediment or jellyfish pieces. Instrument stability should thus be checked with a clean instrument.



**Figure 7.** A sample of the changes in water calibrations. Calibration 1 was a field calibration after the equipment was mounted and is used as the reference for later calibrations.

*4.3.3.2 Applying Field Calibration Data*—To apply the new calibrations a new device file must be created.

Average a portion of the data collected during the calibration that does not show evidence of bubbles or linear trend.

Open the device file that was used when collecting the calibration data in a spreadsheet such as Excel.

Subtract the average values from step one from the appropriate water offset value in the device files. The offsets are given in the third column of the device file.

Make sure to save the new device file under a new name and as tab delimited text. Save all old device files for references.



## 5. Data Processing

An ac-9 acquires raw values representing light losses of a light beam propagating through a fixed path of water. In order to convert these values into meaningful units and to correct for instrument and environmental factors associated with the measurements, several processing steps are required. The following table provides an overview of these steps and subsequent sections within this chapter delineate the steps in more detail.

### 5.1 ac-9 Data Processing Steps

<b>WETView Software</b>
• Initial Parsing of Binary Data
• Ratio of Signal to Reference
• $-\ln(\text{Signal/Reference}) / \text{Pathlength}$
• Application of Temperature Coefficients
• Application of Clean Water Offsets
<b>Post Processing</b>
• Correction for Temperature and Salinity
• Correction for Scattering
• Addition of Pure Water Values

### 5.2 Basic WETVIEW Calculations

This section refers to WETView 4.0-type calculations.

WETView is the basic WET Labs software package used to collect and process data from the ac-9. WETView reads in the raw binary data from the ac-9 and then parses the data. The parsed data contains digitized signal values for signal and reference levels for each channel. WETView then applies an algorithm which:

1. Converts signal and reference values into terms of uncorrected inverse meters;
2. Applies a linear temperature correction (for the meter's internal temperature) using constants supplied in the instrument's Device File.
3. Applies clean water offsets supplied from the instrument's device file that provide a value referenced against clean water.

This formula takes the form:

$$c(\lambda) \text{ or } a(\lambda) = (c_{\text{off}} - 1/x [\ln(C_{\text{sig}} / C_{\text{ref}})]) + ((T - T_o) * K_t) \quad (\text{m}^{-1})$$

where,

$c(\lambda)$ , $a(\lambda)$	is the attenuation coefficient and absorption coefficient respectively
$c_{\text{off}}$	is the water offset value (provided on the Calibration Sheet)
$C_{\text{sig}}$	is the measured amount of light that reaches the receiver detector from the ac-9 data stream in counts
$C_{\text{ref}}$	is the amount of light measured by the reference detector from the ac-9 data stream in counts
$x$	is the sample volume path-length in meters
$T$	is the ac-9's measured internal instrument temperature from the ac-9 data stream
$T_0$	is the temperature offset provided in the DEV file (typically $T_0 = 25$ degrees C)
$K_t$	is the temperature coefficient (provided on the Calibration Sheet).

### Note

For a detailed discussion of the WETView processing of the binary ac-9 data stream, consult the ac-9 and WETView manuals.

For users writing their own code it is important to fully understand the parsing and these initial processing steps. Examples of code can be found on the WET Labs ftp site ([ftp.wetlabs.com](ftp:wetlabs.com)) or from our web site (<http://www.wetlabs.com>).

### 5.3 Temperature and Salinity Corrections

The temperature and salinity corrections discussed here are different than the temperature corrections discussed in the calibration section. These corrections relate to differences between the absorption coefficient of the optically pure water used as a reference in calibrating the ac-9 and the absorption coefficient of the water in which the measurements are being made. By referencing the ac-9 measurement to pure water, the measured absorption and attenuation coefficients represent the total absorption or attenuation minus the absorption coefficient of water.

$$a_m = a_t - a_{wr} \quad c_m = c_t - c_{wr}$$

It has been shown that the absorption coefficient of water is dependent on temperature and salinity in some portions of the spectrum. The temperature and salinity effects are largest in the near infrared and at the shoulders of the absorption spectrum in the visible. When measurements are made in these regions of the spectrum the *in-situ* measured absorption/attenuation coefficients represent the absorption/attenuation coefficients of the dissolved and particulate material plus the difference between the absorption coefficient of the water and the absorption coefficient of the reference water.

$$a_m = a_p + a_g + (a_w - a_{wr}) \quad c_m = c_p + c_g + (c_w - c_{wr})$$

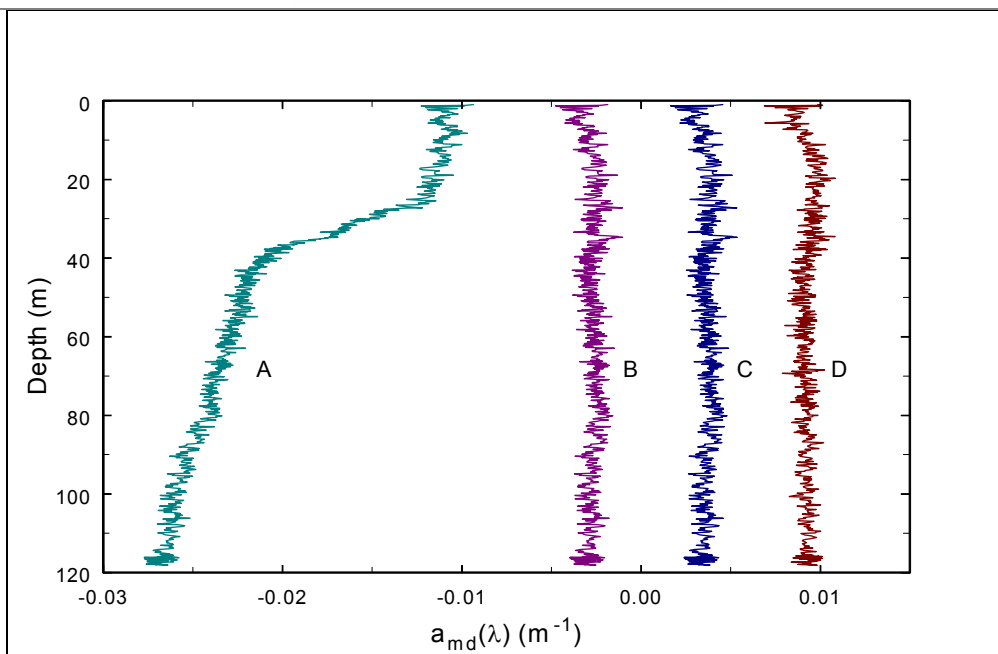
The change in the scattering coefficient with temperature or salinity is negligible except in the ultraviolet portion of the spectrum, so the change in the attenuation coefficient is driven by changes in the absorption coefficient.

To be able to do scattering corrections the water absorption/attenuation needs to be removed from the measured value to arrive at a temperature and salinity corrected measurement. Temperature and salinity effects can be removed using a simple algorithm:

$$a_{mts} = a_m - [\Psi_t * (t - t_r) + \Psi_s * (S - S_r)]$$

where the values for  $\Psi_T$  are given in Table 2, and the values for  $\Psi_S$  are in given in Table 3. The effect of salinity is small compared to temperature and it is normally sufficient to correct measurements to a mean salinity value.

Data may need to be corrected for a second salinity effect. The effect is the difference in the index of refraction between fresh and saline water that cause changes in the reflectance at the windows of the instrument. Fortunately, the change in index of refraction is linear with respect to salinity so this correction can be incorporated into the  $\Psi_s$  value. For the attenuation meter the salinity effect can be calculated in a simple manner. The effect on the absorption measurement is much more difficult to calculate since it is dependent on the optical properties of the diffuser. With slight differences in the diffusers used in some instruments there can be variability in the salinity dependence between instruments. Two methods can be used to check the salinity effect on the absorption coefficient of a particular ac-9. First, use a 0.2 $\mu$ m pore size filter at the inlet of the absorption meter in seawater where the dissolved organic load is small. The colored dissolved organic material absorption in the red may be assumed to be zero and the measured absorption coefficients at 650 and 676 nm should be approximately equal and represent the offset due to the salinity effect. Similarly, calibrations in the lab can be performed using artificial seawater and pure water to determine the change in calibrations. It is important to use laboratory grade salts and not aquarium salts as the aquarium salts tend to contain a lot of dissolved organic materials. The salt water should be passed through a 0.2 $\mu$ m pore size filter (we use a Gelman Suporcap 100) to remove suspended salts. When properly done the absorption coefficients for the salt water will be slightly higher than these of fresh water and the attenuation coefficients for the salt water will be less than or equal to that of fresh water for most visible wavelengths.



**Figure 8.** The application of temperature and salinity corrections to a gelbstoff profile.

In the example provided in Figure 8, line A is the raw  $a(715)$ . Line B has the temperature correction applied. Line C includes the temperature and salinity corrections. Line D is the absorption coefficient at 650 nm, given to provide a reference profile. Note both temperature and salinity corrections are necessary to provide a profile with positive absorption values (Pegau et al., 1997).

#### 5.4 Scattering Corrections

Attenuation measurements are limited by the acceptance angle of the instrumentation. The finite acceptance angle of the instrumentation means that the instrument collects a portion of the scattered light and thus underestimates the true attenuation coefficient. Correcting attenuation measurements for the errors caused by collection of scattered light is not recommended as it is extremely difficult and of questionable benefit (Pegau, et al., 1994). However, it is important to remember that transmissometers with different acceptance angles will provide slightly different measurements because they measure different portions of the scattering function. The differences appear to be negligible in our tests using a Sea Tech transmissometer (acceptance angles 1 degree) and the ac-9 (0.7 degrees).

Reflecting tube absorption meters and spectrophotometers do not collect all of the light scattered from the beam. The uncollected scattered light causes the instrumentation to overestimate the absorption coefficient. There are several schemes to correct absorption measurements for scattering errors. The three methods most commonly used include:

1. Subtraction of a reference wavelength where the absorption is assumed to be zero.
2. Removal of a fixed proportion of the scattering coefficient.
3. Use of a reference wavelength to determine the proportion of the scattering coefficient to be subtracted from the signal. Each of these methods require different assumptions and ancillary measurements.

The first method to correct for scattering errors is to subtract the absorption measurement at a reference wavelength.

$$a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - a_{mts}(\lambda_{ref})$$

It is assumed that at the reference wavelength the absorption by particulate and dissolved materials is zero so that the measured absorption coefficient is caused strictly by scattering. It is further assumed that the shape and magnitude of the volume scattering function is independent of wavelength. This technique does allow the scattering correction to vary with changes in the materials contained within the sample. Commonly the reference wavelength selected is in the near infrared portion (715 nm on an ac-9) of the spectrum and will require temperature and salinity corrections to be applied before the scattering corrections are computed (See Section 5.2).

The second method assumes that the scattering correction is a fixed proportion of the scattering coefficient  $b(\lambda)$  such that  $b(\lambda) = c(\lambda) - a(\lambda)$ .

$$a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - \varepsilon * [c_{mts}(\lambda) - a_{mts}(\lambda)]$$

Where  $\varepsilon$  is the proportion of the scattering coefficient not detected by the sensor and has a value of  $\sim 0.14$  for waters where biological particles dominate scattering. The value of  $\varepsilon$  increases to  $\sim 0.18$  when sediments dominate the scattering. These values of  $\varepsilon$  are empirically derived from field data and the third scattering correction technique as well as being modeled by Kirk (1993). We use the measured absorption and attenuation coefficients to estimate the scattering correction. Since the measured absorption has an error the estimated scattering coefficient is not equal to the true scattering coefficient, so  $\varepsilon$  is slightly larger than models would predict. This method assumes that the shape of the volume scattering function is independent of wavelength and type of material. Restated, the assumption is that the proportion of the scattering error (i.e. the proportion of the total scattering coefficient represented by the scattering error.) is independent of wavelength. The magnitude of the scattering correction is, however, allowed to vary with wavelength. Since a reference wavelength is not used there is no requirement that the absorption coefficient equal zero at the reference wavelength and for most visible wavelengths no temperature correction needs to be applied.

The third method to scattering correct the absorption measurements is covered in detail in the ac-9 manual. This technique is a combination of the first two techniques. It is assumed that there exists a reference wavelength at which the absorption coefficient of particulate and dissolved materials is zero. It is further assumed that the shape of the volume scattering function is independent of wavelength. The correction technique is written as,

$$a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - \frac{a_{mts}(\lambda_{ref})}{[c_{mts}(\lambda_{ref}) - a_{mts}(\lambda_{ref})]} * [c_{mts}(\lambda) - a_{mts}(\lambda)]$$

This technique allows for automatic changes in the scattering correction magnitude with wavelength and changes in types of materials present. It requires the largest number of ancillary measurements (two attenuation values, two absorption values, temperature, salinity, and calibration water temperature and salinity) which makes this technique the most difficult to do correctly. However, if the assumptions are correct, it is the most accurate of the techniques.

Although there are three main scattering correction schemes in many cases only one will be of practical use. Table 1 provides a matrix of possible corrections to use based on these considerations

**Table 1.** Choosing the right scatter correction

Available Data	Method 1. Base Sub.	Method 2. (c-a)%	Method 3. Zaneveld
<b>a</b> Only	Yes	No	No
<b>a</b> and <b>c</b> Only	Yes	Yes	No
<b>a</b> , <b>c</b> and Temperature	Yes	Yes	Yes

Method 1. Baseline Subtraction method

Method 2. Removal of a fixed % of scattering coefficient

Method 3. Use of reference  $\lambda$  to determine proportion of scattering coefficient (after Zaneveld, Oregon State University)

If one obtains a negative value for the absorption coefficient (which may happen in very clean waters), this is probably due to the calibration water not being absolutely pure optically. If “a” is within  $0.005 \text{ m}^{-1}$  of zero, the instrument is performing within specification and “a” can be set to zero.

## 5.5 Addition of Pure Water Values

Since the ac-9 measurements are referenced to optically pure water, it is necessary to add the optical properties of the reference water to arrive at the total absorption or attenuation coefficient. Many sources are available for the absorption coefficient of pure water (Table 2); however, large differences in the absorption coefficient of water exist between the various authors.

**Table 2:** Volume absorption and scattering coefficients for pure water,  $a_w(\lambda)$  and  $c_w(\lambda)$ , respectively. Values for  $a_w(\lambda)$  are those of Pope and Fry (1997) [400–700 nm], and are computed from the complex refractive index measurements of Kou *et al.* (1993) [705–750 nm]. Values of  $b_w(\lambda)$  used to compute  $c_w(\lambda)$  were obtained from Buiteveld, *et al.* 1994. The linear temperature dependence of pure water absorption,  $\Psi_T$ , are from Pegau and Zaneveld (1993) and Pegau *et al.* (1997).

$\lambda$ nm	$a_w$ $m^{-1}$	$c_w$ $m^{-1}$	$\Psi_T$ $m^{-1} \text{ } ^\circ\text{C}$	$\lambda$ nm	$a_w$ $m^{-1}$	$c_w$ $m^{-1}$	$\Psi_T$ $m^{-1} \text{ } ^\circ\text{C}$	$\lambda$ nm	$a_w$ $m^{-1}$	$c_w$ $m^{-1}$	$\Psi_T$ $m^{-1} \text{ } ^\circ\text{C}$
400	0.0066	0.0119	0.0000	520	0.0409	0.0427	0.0002	640	0.3108	0.3116	-0.0001
405	0.0053	0.0103	0.0000	525	0.0417	0.0434	0.0002	645	0.325	0.3257	0.0000
410	0.0047	0.0095	0.0000	530	0.0434	0.0451	0.0001	650	0.340	0.3407	0.0000
415	0.0044	0.0089	0.0000	535	0.0452	0.0468	0.0001	655	0.371	0.3717	0.0001
420	0.0045	0.0088	0.0000	540	0.0474	0.0489	0.0001	660	0.410	0.4107	0.0002
425	0.0048	0.0089	0.0000	545	0.0511	0.0526	0.0001	665	0.429	0.4296	0.0002
430	0.0050	0.0089	0.0000	550	0.0565	0.0579	0.0001	670	0.439	0.4396	0.0002
435	0.0053	0.0090	0.0000	555	0.0596	0.0610	0.0001	675	0.448	0.4486	0.0001
440	0.0064	0.0101	0.0000	560	0.0619	0.0632	0.0001	680	0.465	0.4656	0.0000
445	0.0075	0.0109	0.0000	565	0.0642	0.0655	0.0002	685	0.486	0.4866	-0.0001
450	0.0092	0.0125	0.0000	570	0.0695	0.0707	0.0002	690	0.516	0.5166	-0.0002
455	0.0096	0.0127	0.0000	575	0.0772	0.0784	0.0002	695	0.559	0.5595	-0.0001
460	0.0098	0.0128	0.0000	580	0.0896	0.0907	0.0003	700	0.624	0.6245	0.0002
465	0.0101	0.0129	0.0000	585	0.1100	0.1111	0.0004	705	0.704	0.7045	0.0007
470	0.0106	0.0133	0.0000	590	0.1351	0.1362	0.0006	710	0.827	0.8275	0.0016
475	0.0114	0.0140	0.0000	595	0.1672	0.1682	0.0008	715	1.007	1.0075	0.0029
480	0.0127	0.0152	0.0000	600	0.2224	0.2234	0.0010	720	1.255	1.2555	0.0045
485	0.0136	0.0160	0.0000	605	0.2577	0.2587	0.0011	725	1.539	1.5394	0.0065
490	0.0150	0.0173	0.0000	610	0.2644	0.2653	0.0011	730	1.983	1.9834	0.0087
495	0.0173	0.0195	0.0001	615	0.2678	0.2687	0.0010	735	2.495	2.4954	0.0108
500	0.0204	0.0225	0.0001	620	0.2755	0.2764	0.0008	740	2.787	2.7874	0.0122
505	0.0256	0.0276	0.0001	625	0.2834	0.2842	0.0005	745	2.836	2.8364	0.0120
510	0.0325	0.0344	0.0002	630	0.2916	0.2924	0.0002	750	2.857	2.8574	0.0106
515	0.0396	0.0414	0.0002	635	0.3012	0.3020	0.0000				

The value of  $\Psi_S$  for the absorption and attenuation measurements were obtained using standard ac-9 wavelengths (Table 3). The value of  $\Psi_S$  of absorption includes multiple reflections between the diffuser and water-glass interface. The reflections, and hence the value of  $\Psi_S$  may be very slightly instrument-dependent.

**Table 3.** Value of  $\Psi_S$  for absorption and attenuation

Wavelength	$\Psi_S$ absorption	standard deviation absorption	$\Psi_S$ attenuation	standard deviation attenuation
412	0.00018	0.00005	0.00007	0.00005
440	0.00008	0.00002	-0.00007	0.00002
488	0.00008	0.00002	-0.00007	0.00002
510	0.00009	0.00003	-0.00007	0.00003
532	0.00004	0.00006	-0.00008	0.00006
555	0.00008	0.00003	-0.00008	0.00003
650	0.00011	0.00003	-0.00005	0.00003
676	0.00008	0.00002	-0.00007	0.00002
715	-0.00018	0.00006	-0.00032	0.00006
750	0.00070	0.00001	0.00059	0.00003
850	-0.00034	0.00001		
900	-0.00264	0.00003		
975	0.00221	0.00065		



## 5.6 Reality Checks

For one unfamiliar with ac-9 data it can be difficult to determine whether or not acquired and processed data is within the realm of reality. While this is a matter of experience and exposure, the following table outlines some of the boundary conditions and possible causes of problems that may be encountered.

Symptoms	Possible Causes	Solutions
Air readings are very noisy	1) Moisture in flow cells	Disassemble flow cells; allow to dry completely.
	2) Ambient light leakage	Cover stainless nozzles with tape or plastic caps
	3) Internal meter malfunction	Contact company
Air readings show significant offset from air tracking file	1) Meter operating outside of compensated temperature zone	Allow meter to cool. Keep instrument temp within cal range. Immerse transmitter housing into water while * calibrating.
	2) Instrumental drift	Compensate with air cal values or re-calibrate
Water - short term variance of signal exceeds 10% of mean	1) Bubbles/air entrapment	Tilt meter, purge all bubbles from the flow path
	2) Particles or debris caught in flow cell	Clean instrument, check water supply for sediment
a > c uncorrected	1) Bad calibration file	Use correct cal file, re-calibrate instrument
	2) Instrumental drift	Apply air cal corrections, re-calibrate instrument
	3) Bubbles/air entrapment	Tilt meter, purge all bubbles from the flow path
	4) Caught particle	Clean instrument, check water supply for sediment
a or c < -0.005*	1) Instrumental drift	Apply air cal corrections, re-calibrate instrument
	2) Bad calibration file	Use correct cal file, re-calibrate instrument
	3) Bubbles/air entrapment	Tilt meter, purge all bubbles from the flow path
	4) Meter out of specified temperature zone	Allow meter to cool. Keep instrument temp within cal range
	5) Over corrected scattering error	Check scattering error correction calculations

*\*If a or c are < 0 but > -0.005, the instrument is operating property and a or c may be set to zero.*

## 6. References

The following is a partial list of references pertaining to the ac-9 and its use.

Buiteveld, H., J. H. H. Hakvoort, and M. Donze, The optical properties of pure water, *Ocean Optics XIII*, SPIE 2258, 174–183, (1994).

Kou, L., D. Labrie, and P. Chylek, Refractive indices of water and ice in the 0.65 to 2.5mm spectral range, *Applied Optics*, 32:3531–3540, 1993.

Moore, C., In-situ, biochemical, oceanic, optical meters, *Sea Technology*, 35(2):10–16, 1994.

Moore, C., J.R.V. Zaneveld, and J.C. Kitchen, Preliminary results from an *in-situ* spectral absorption meter, *Ocean Optics XI*, Proc. Soc. Photo-Optical Instrum. Eng. (SPIE), 1750:330–337, 1992.

Mueller, J.L., Ocean optics protocols for satellite ocean color sensor validation, NASA/TM 2003-211621/Rev 4-Vol IV (Erratum 1), 2003.

Pegau, W.S., D. Gray, and J.R.V. Zaneveld, Absorption of visible and near-infrared light in water: the dependence on temperature and salinity, *Applied Optics* 36(24):6035–6046, 1997.

Pegau, W.S., and J.R.V. Zaneveld, Temperature dependence of the absorption coefficient of pure water in the visible portion of the spectrum, *Ocean Optics XII*, Proc. Soc. Photo-Optical Instrum. Eng. (SPIE), 1994.

Pope, R.M., Optical absorption of pure water and seawater using the integrating cavity absorption meter, Ph.D. Thesis. (Texas A & M, College Station, TX, 1993).

Pope, R.M., and E.S. Fry, Absorption spectrum (380–700 nm) of pure water, II. Integrating cavity measurements, *Applied Optics*, 36:8710–8723.

Zaneveld, J.R.V., J.C. Kitchen, A. Bricaud, C. Moore. Analysis of in-situ spectral absorption meter data, *Ocean Optics XI*, Proc. Soc. Photo-Optical Instrum. Eng. (SPIE), 1750, 187–200, 1992.

## Appendix 1. Device Files

### Caution

ac-9 DEVICE files are not interchangeable. They provide critical calibration information to the WETView processing software. Always ensure that you are using the proper device file when running your ac-9 in real time or processing logged data from an M-Pak or MODAPS. Using an out of date device file or one created for another instrument will cause WETView to process the data incorrectly.

The ac-9 device file provides WETView with the instrument specific information it needs to carry out primary processing of the binary data. It is an ASCII file that can be edited with any standard text editor however, the file must be saved as tab delimited or it will not be read in by WETView. It contains the instrument serial number, pressure sensor coefficients if applicable, path length and the filter set installed in the ac-9. Note: the serial number contained in the DEV file allows WETView to compare the number to the one burned in to the instrument's NVRAM. Additionally, the format of the serial number tells WETView whether to use the previous blue reference-averaging algorithm or to use the 1-1 reference averaging which all instruments calibrated with WETView 4.0 need to use.

Examples of ac-9 DEVICE files for both WETView 4.0 and WETView 5.0 are provided below. Italicized annotation marked by brackets; [*comment ...*] are provided for amplification. These remarks are not found in a real DEV file.

### Note

WETView 5.0 is backward compatible with the WETView 4.0 device file type.

#### Device file formats

WETView 5.0 device file listing below;

#### AC9 Absorption and Attenuation Meter

```
00000121      ; serial number
2             ; structure version number
Reserved     ; reserved for future
5.3 0.3      ; depth calibration
19200        ; baud rate
0.25         ; path length (meters)
15           ; number of temperature bins
5.5233 8.4553 11.4712 ... ; temperature bins
a650 Blue 7.6242 0.1411 0.1028 0.0389 ...
a676 Green 7.6819 0.1403 0.1041 0.0393 ...
```

a715 Brown 7.6963 0.1369 0.1034 0.0409 ...  
 c510 Red 6.8377 0.1351 0.1045 0.0427 ...  
 c532 Magenta 6.9157 0.1299 0.1034 0.0423 ...  
 c555 Black 7.0137 0.1203 0.0987 0.0423 ...  
 a412 LtBlue 7.1391 0.1169 0.0980 0.0427 ...  
 a440 LtGreen 7.2244 0.1115 0.0959 0.0436 ...  
 a488 Yellow 7.2360 0.1029 0.0893 0.0411 ...  
 c650 Blue 7.1407 0.1001 0.0901 0.0435 ...  
 c676 Green 6.8605 0.0979 0.0861 0.0429 ...  
 c715 Brown 7.2408 0.0949 0.0851 0.0428 ...  
 a510 Red 7.3416 0.0935 0.0839 0.0417 ...  
 a532 Magenta 7.3578 0.0924 0.0827 0.0431 ...  
 a555 Black 7.3922 0.0911 0.0824 0.0436 ...  
 c412 LtBlue 6.0476 0.0903 0.0815 0.0442 ...  
 c440 LtGreen 6.4225 0.0903 0.0805 0.0431 ...  
 c488 Yellow 6.5098 0.0876 0.0793 0.0405 ...  
 0.0035 0.004 0.015 0.02 ... ; reserved info  
 0 ; auxiliary capabilities

WETView 4.0 device file listing below;

AC9 Absorption and Attenuation Meter	[Instrument type]
181 ; Serial Number	[Instrument Serial Number]
Reserved	[Reserved for future use]
Reserved	[Reserved for future use]
0.000000 0.000000 ; Depth Cal	[Pressure sensor calibration information]
19200 ; Baud Rate	[Communication Baud Rate]
0.250000 ; Path Length	[Path length, used in processing calculations]

NOTE 2.

a650	Yellow	7.621854	25.000000	0.000329	[ac-9 Wavelengths ]
a676	LtGreen	7.571334	25.000000	0.000398	
a715	Green	6.995948	25.000000	0.000419	
c510	Brown	7.485022	25.000000	0.000442	
c532	Red	7.518616	25.000000	0.000370	
c555	LtRed	7.575243	25.000000	0.000494	
a412	Cyan	7.799076	25.000000	-0.000982	
a440	LtBlue	7.752078	25.000000	-0.000107	
a488	Blue	7.743129	25.000000	0.000242	
c650	Yellow	7.363869	25.000000	0.000504	
c676	LtGreen	7.188561	25.000000	0.000444	
c715	Green	6.717293	25.000000	0.000988	
a510	Brown	7.745366	25.000000	0.000439	
a532	Red	7.753649	25.000000	0.000371	



a555	LtRed	7.759310	25.000000	0.000718
c412	Cyan	6.843933	25.000000	0.000268
c440	LtBlue	7.092329	25.000000	0.000112
c488	Blue	7.426465	25.000000	0.000271

===== End of DEV file example =====

Note 1. Old serial numbers starting with a 'C' indicate that ac-9 was calibrated using WETView 3.22 or earlier, implying that the blue reference averaging will be applied. Instruments serial numbers which do not start with 'C' like the example above must be used with version 4.0 of WETView or later so that the 1-1 reference averaging will be applied. WETView will choose the correct algorithm based on the serial number format. It is highly recommended to use version 4.0 of WETView for all data processing since it will work with instruments calibrated with either algorithm.

Note 2. The first column in this section describes the ORDER that the data will be presented in the WETView output files. In the example above, the output data file will present the data in columns starting with three absorption wavelengths (650, 676 and 715 nm) followed by 3 attenuation wavelengths (510, 532 and 555 nm) etc. The remaining columns in each line includes information such as line color (the color in which WETView will display the wavelength in the graphic window), water offset value, temperature offset ( $T_o$ ) and temperature coefficient ( $K_t$ ).

a650 Yellow 7.621854 25.000000 0.000329

In the example line above, the 650 nm absorption will be displayed as a yellow trace. Its clean water offset value is 7.621854. This is the instrumental offset required to provide a 0.0 value in optically pure water. The temperature offset ( $T_o$ ) of 25.00000 is the value used in the instrument temperature correction algorithm about which the temperature correction pivots. The temperature coefficient of 0.000329 is the slope of the linear temperature correction.



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## Calibration Sheet

The following is an example Calibration Sheet supplied with each meter with each calibration performed.

WET Labs, Inc. Philomath, OR 97370 541-929-5650

CALIBRATION RESULTS AND SCALING FACTORS      Cal: 003

Model #: 9502002              Date: 04-24-1996

Serial #: AC90152            Job: 003

### DIGITAL CALIBRATION

(Kt)

Chan. Air Val. Water Off. Temp. Co. Prec. Hex Water Hex Air

-----  
a650 -0.8950 6.0761 -0.00021 0.0002 4FA5 6437  
a676 -0.9613 6.1511 -0.00025 0.0002 6479 8076  
a715 -1.4817 5.7630 -0.00041 0.0003 3A13 54CF  
c510 0.2612 7.5054 -0.00083 0.0002 7645 6E8D  
c532 0.2956 7.4744 -0.00070 0.0003 2A4E 273B  
c560 0.2085 7.6300 -0.00063 0.0003 3D67 3A39  
a412 -0.7238 4.6620 -0.00171 0.0012 0803 099D  
a440 -0.7611 5.0389 0.00023 0.0006 136E 178B  
a488 -0.7366 5.1652 -0.00050 0.0003 4356 5145  
c650 -0.0458 7.5292 -0.00052 0.0002 7227 737C  
c676 -0.1431 7.4983 -0.00048 0.0001 8C57 9193  
c715 -0.7146 6.9088 -0.00073 0.0002 4DC1 5D79  
a510 -0.7425 5.3572 -0.00024 0.0002 4A96 5A0C  
a532 -0.7188 5.5060 -0.00035 0.0005 1B5A 20E2  
a560 -0.7363 5.6730 -0.00037 0.0004 273A 2F64  
c412 0.4077 6.7811 -0.00104 0.0014 0A26 0923  
c440 0.3038 7.2107 -0.00037 0.0005 1B2E 1921  
c488 0.2691 7.3607 -0.00141 0.0002 69B2 629E

TCal: 25.2 degrees Celsius

ICal: 30.1 degrees Celsius

Temperature Range: 12.9 to 41.2 degrees Celsius

-----  
TCal: Water temperature in flow tubes at time of water calibration.

ICal: Instrument internal temperature at time of water calibration.

Temperature Range: Internal instrument temperature range, over which the temperature coefficients are calculated.

Air Values: Air reading, in inverse meters, once the temperature corrections are applied.

Water Offset: The measured value of pure water, in inverse meters, at TCal with temperature corrections applied.

Temperature Coefficients (Kt): Temperature correction scaling factor.

Precision: The standard deviation in air, measured in inverse meters, at a six scan integration.

Hex Water: Raw water values in hexadecimal.

Hex Air: Raw air values in hexadecimal.

Flow rate of 1.5 liters per minute.





### Revision History

Revision	Date	Revision Description	Originator
A	01/11/00	Begin revision tracking	H. Van Zee
B	02/10/00	Correct table 3, absorption at 715 nm (DCR 16)	D. Hankins
C	07/11/00	Correct temperature correction equation (DCR 45)	C. deLespinasse
D	12/06/00	Replace anti-fouling graphic (DCR 74)	H. Van Zee
E	01/29/02	Correct references to tables (DCR 191)	H. Van Zee
F	07/08/02	Correct scattering correction equation (DCR 229)	D. Hankins
G	03/26/03	Correct table 2 c value at 510 nm (DCR 289)	D. Hankins
H	09/09/03	Correct table 2 after Mueller erratum (DCR 333)	A. Barnard
I	02/05/04	Correct two scattering correction equations, Section 5.4 (DCR 366)	A. Barnard