# Cary Spectrophotometer User Guide

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https://www.agilent.com/en/products/uv-vis-uv-vis-nir/uv-vis-uv-vis-nir-systems/cary-100-uv-vis

## Introduction

The principles of spectroscopic analysis rely on Beer's law. The principle of Beer's law is that passing light of a known wavelength through a sample of known thickness and measuring how much of the light is absorbed at that wavelength will provide the concentration of the unknown, provided that the unknown is in a complex that absorbs light at the chosen wavelength.

IODP's Agilent Technologies Cary 100 double-beam UV-Vis (ultraviolet–visible) spectrophotometer is ideal for shipboard routine laboratory work. The system measures analytes in interstitial water obtained from sediment cores using standard colorimetric methodology.

The described methods are based on ODP Technical Note 15, *Chemical Methods for Interstitial Water Analysis Aboard the JOIDES Resolution*, Aug 1991; J.M. Gieskes, T. Gamo, and H. Brumsack.

## Methods

# Ammonium

Determination of ammonium concentration is of importance because this constituent is an indicator of diagenesis of organic matter in the sediments. The onset of sulfate reduction coincides with initiation of ammonium ion production. Ammonium production increases strongly in the zone of methanogenesis, presumably as a result of associated deammonification reactions. The large potential variation in ammonium concentrations, therefore, suggests that a few preliminary ammonium concentrations should be run in order to set limits to the sample dilution and range of standards to be used. Suggestions for this follow below.

The methodology is based on Solorzano (1969), originally developed to detect very small $NH_4^+$ concentrations in seawater. Although background contamination problems in seawater are enormous, the relatively high concentrations of ammonium in pore fluids (as high as 85 mM in ODP Leg 112 samples; Kastner et al., 1990) minimizes this problem when matrix blanks are run along with the samples. In areas of low sedimentation, however, very low ammonium concentrations require careful sample handling to avoid this problem.

The ammonium method is based on diazotization of phenol and subsequent oxidation of the diazo compound by Chlorox™ to yield a blue color, measured spectrophotometrically at 640 nm.

### Reagent Solutions

| | |
|---|---|
| **Ammonium Standard ( 0.10 M)**<br><br>make once an expedition | Dry ammonium chloride ($NH_4Cl$) overnight in an oven.<br><br>Dissolve 5.349 g dried $NH_4Cl$ in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• To make 25 mL - Dissolve 133.728 mg dried $NH_4Cl$ in a 25 mL volumetric flask. Bring to volume with nanopure water. |
| **Alkaline**<br><br>make once an expedition | Dissolve 7.5 g (tri)sodium citrate tribasic dihydrate ($Na_3C_6H_5O7{\cdot}2H_2O$) and 0.4 g sodium hydroxide (NaOH) in a 500 mL volumetric flask. Bring to volume with nanopure water. |
| **Oxidizing**<br><br>make fresh daily | Add 1 mL fresh sodium hypochlorite (4% available chlorine) to 50 mL **Alkaline solution.** This should be adjusted for the amount of samples to be run.<br><br>• Use sodium hypochlorite or regular strength Chlorox™ bleach. DO NOT use Chlorox that contains NaOH, fragrances, or other agents, and do not use another brand of bleach.<br>• The Clorox bleach can go bad with time. Absorbance values of the ammonium analysis will be much lower when this happens. Absorbance of the 1000 uM standard falling below 0.2 or the 100 uM standard falling below 0.01 could be indications that a new bottle of bleach is needed. |
| **Phenol**<br><br>make fresh daily | Add 1 mL liquid Phenol to 100 mL Absolute Ethanol |
| **Sodium Nitroprusside**<br><br>make fresh daily | Dissolve 75 mg sodium nitroprusside ($Na_2[Fe(CN)_5NO]$) (also known as sodium nitroferricyanide) in 100 mL nanopure water |

## Standards

Add standard to a 50 mL volumetric flask and bring to volume with nanopure water.

50 mL batches are stable for 1 month.

| concentration (µM) | volume of ammonium standard (mL) | volume of nanopure water (mL) |
|---|---|---|
| 0 | 0 | 50.000 |
| 50 | 0.025 | 47.975 |
| 100 | 0.050 | 49.950 |
| 150 | 0.075 | 49.950 |
| 200 | 0.100 | 49.900 |
| 400 | 0.200 | 49.800 |
| 600 | 0.300 | 49.700 |
| 800 | 0.400 | 49.600 |
| 1000 | 0.500 | 49.500 |
| 1500 | 0.750 | 49.250 |
| 2000 | 1.000 | 49.000 |
| 3000 | 1.500 | 48.500 |

### Procedure

Concentrations of ammonium may differ quite a bit at different sites. Typically in areas with strong evidence of organic carbon diagenesis (e.g., organic carbon–rich sediments), high concentrations of $NH_4^+$ can be expected. In that case, sample aliquots must be made appropriately small or sample dilution may be required. The range can be established by using a sample near the alkalinity maximum. Once the range has been determined, prepare standards that cover this range. In this manner, samples and standards are treated in a similar way.

**Note:** Use a smaller aliquot of sample if the result exceeds the linear range of the spectrophotometer, making up the volume with nanopure water. (For example, for a 300 µL aliquot of a sample, add 300 µL nanopure water.)

**Note**: The order of dilution (below) matters, so *do not change this order*. Shake samples after EACH addition.

| | |
|---|---|
| **1.** | Transfer 200 µL of sample/standard to a vial. |
| **2.** | Add 2 mL of nanopure water to each vial. |
| **3.** | Add 1 mL **phenol solution** to each vial and shake. |
| **4.** | Add 1 mL **sodium nitroprusside solution** to each vial and shake. |
| **5.** | Add 2 mL of **oxidizing solution** to each vial and shake. |
| **6.** | Let the color develop (in a dark place) for 6.5 hr and then determine the absorbance at 640 nm wavelength.<br><br>(From a series of measurements over 8 h, it was found that results stabilized after 6.5 hr.) |

## Phosphate

Determination of dissolved phosphate, particularly in rapidly deposited organic carbon–rich sediments, is important in the shipboard analytical program. Phosphate concentrations may vary considerably, and it is therefore advisable to obtain a preliminary idea of the concentration ranges to be expected. This can most easily be accomplished by taking samples in the region of maximum alkalinities. Typically if alkalinities are >30 mM, dissolved phosphate concentrations may be >100 µM; thus, only very small sample aliquots will be needed to establish the concentration range.

This method is, in essence, the colorimetric method from Strickland and Parsons (1968) as modified by Presley (1971) for DSDP pore fluids. Orthophosphate reacts with Mo(VI) and Sb(III) in an acidic solution to form an antimony-phosphomolybdate complex. Ascorbic acid reduces this complex to form a blue color, and absorbance is measured spectrophotometrically at 885 nm.

It is important to note that the concentrations in the final test solution cannot exceed ~10 µM. Thus, for open-ocean (low sedimentation rate, low organic carbon) sediments, one might need to do the determination on 2 mL of sample (expected range 0–10 µM), but in typical continental margin settings, where concentrations can exceed 100–200 µM, a 0.1 or 0.2 mL sample aliquot must be used. The concentration range must be established prior to running samples, and it is highly advisable to make standards that cover the range of concentrations to be expected.

**Note:** Samples with high silica concentrations may give a false increase in measured concentration of phosphate (http://dx.doi.org/10.1007/BF02071829; S. Noriki, Silicate correction in the colorimetric determination of phosphate in seawater, 1983).

## Reagent Solutions

| | |
|---|---|
| **Phosphate Standard (0.01 M)**<br><br>make once an expedition | Dry potassium phosphate monobasic ($KH_2PO_4$) in oven at 100°C for two hours; keep in a desiccator while it cools before weighing.<br><br>Dissolve 1.361 g dried $KH_2PO_4$ in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• To make 25 mL - Dissolve 34.025 mg dried $KH_2PO_4$ in a 25 mL volumetric flask. Bring to volume with nanopure water. |
| **Ammonium Molybdate**<br><br>stable indefinitely | Dissolve 2 g ammonium molybdate tetrahydrate ($[NH_4]_6Mo_7O_{24}\cdot 4H_2O$) in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• Store in polyethylene @ 4°C |
| **Sulfuric Acid**<br><br>stable indefinitely | Add 10 mL concentrated sulfuric acid ($H_2SO_4$) to ~600 mL nanopure water in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• **Caution:** Mixing sulfuric acid and water produces heat. Take appropriate precautions. |

| | |
|---|---|
| **Antimony Potassium Tartrate**<br><br>make once an expedition | Dissolve 102 mg antimony potassium tartrate trihydrate ($KSbC_4H_4O_7 \cdot 3H_2O$) in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• If using antimony potassium tartrate hemihydrate [$KSbC_4H_4O_7 \cdot ½H_2O$], dissolve 90 mg.<br>• Store in amber glass @ 4°C |
| **Ascorbic Acid**<br><br>make fresh weekly | Dissolve 3.5g ascorbic acid ($C_6H_8O_6$) in a 1000 mL volumetric flask. Bring to volume with nanopure water.<br><br>• If the reagent is discolored upon creation, the dry ascorbic acid is probably oxidized and must be replaced. |
| **Mixed Reagent**<br><br>make fresh every 6 hours | Mix the following solutions. Mix well after each addition. Can adjust the volumes to be suitable for the number of samples as long as the proportions of each reagent are maintained.<br><br>• 50 mL **Ammonium Molybdate**<br>• 125 mL **Sulfuric Acid**<br>• 25 mL **Antimony Potassium Tartrate**<br>• 50 mL **Ascorbic Acid** |

### Standards

Add standard to a 50 mL volumetric flask and bring to volume with nanopure water.

| concentration (µM) | volume of phosphate standard (mL) | volume of nanopure water (mL) |
|---|---|---|
| 0 | 0 | 50 |
| 5 | 0.025 | 49.975 |
| 10 | 0.050 | 49.950 |
| 15 | 0.075 | 49.925 |
| 20 | 0.100 | 49.900 |
| 40 | 0.200 | 49.800 |
| 60 | 0.300 | 49.700 |
| 80 | 0.400 | 49.600 |
| 100 | 0.500 | 49.500 |
| 150 | 0.750 | 49.250 |
| 200 | 1.000 | 49.000 |
| 300 | 1.500 | 48.500 |

### Procedure

**Note:** Use a smaller aliquot of sample if the result exceeds the linear range of the spectrophotometer, making up the volume with nanopure water. (For example, for a 300 µL aliquot of a sample, add 300 µL nanopure water.)

| | |
|---|---|
| **1.** | Transfer 600 µL sample/standard to a vial. |
| **2.** | Add 2 mL nanopure water to each vial. |
| **3.** | Add 4 mL **mixed reagent** to each vial and shake. |
| **4.** | After a few minutes a blue color develops, which remains stable for a few hours.<br><br>It is best to make the readings at 885 nm ~ 30 min after addition of the mixed reagent. |

## Silica

Silicon is routinely measured on the ICP, so measurement by spectroscopic analysis can be considered an alternate method.

Dissolved silica determinations are of great importance in interstitial waters. Often they represent the lithology of the sediments, and the concentrations can vary substantially, especially if highly dissolvable phases such as biogenic opal-A, volcanic ash, or smectite are present. Thus, a wide range of concentrations can be expected, typically from 50 to 1200 µM or higher (especially in hydrothermally affected sediments). The method below usually covers the range, although greater dilutions may be appropriate if sediments or sample sizes necessitate this.

This method is based on the production of a yellow silicomolybdate complex. The complex is reduced by ascorbic acid to form molybdenum blue, measured at 812 nm. The blue complex is very stable, which will enable delayed reading of the samples.

## Reagent Solutions

| | |
|---|---|
| **Silica Standard (3000 M)**<br><br>make once an expedition | Dry sodium silicofluoride in a vacuum desiccator overnight to remove excess water.<br><br>Do not heat. |
| **Sulfuric Acid**<br><br>make fresh monthly | Slowly add 250 mL concentrated sulfuric acid to ~200 mL nanopure water in a 500 mL volumetric flask. Allow to cool to room temperature, then bring flask to volume with nanopure water.<br><br>• **Caution:** Mixing sulfuric acid and water produces heat. Take appropriate precautions. |
| **Synthetic Seawater**<br><br>make fresh monthly | Dissolve 25 g sodium Chloride in ~800 mL nanopure water in a 1000 mL volumetric flask. Add and disolve 8 g magnesium sulfate heptahydrate (MgSO4·7H2O). Bring flask to volume with nanopure water |
| **Ammonium Molybdate**<br><br>make fresh monthly | Dissolve 4 g ammonium molybdate tetrahydrate ($(NH_4)_6MO_7O_{24}\cdot 4H_2O$) in ~300 mL nanopure water in a 500 mL volumetric flask. Add 12 mL concentrated hydrochloric acid (HCl). Bring flask to volume with nanopure water. |
| **Metol Sulfite**<br><br>make fresh monthly | Dissolve 6.0 g anhydrous sodium sulfite ($Na_2SO_3$) in a 500 mL volumetric flask. Add 10 g Metol (p-methylaminophenol sulfate [$(C_7H_{10}NO)_2SO_4$]). Bring flask to volume with nanopure water. When the Metol has dissolved, filter the solution through a Whatman No. 1 filter paper.<br><br>• **Note:** This solution may deteriorate quite rapidly. |
| **Oxalic Acid**<br><br>make fresh monthly | Add 50g oxalic acid dihydrate [$(C_2H_4O_2)\cdot 2H_2O$] to ~300 mL nanopure water in a 500 mL volumetric flask. Shake well, and bring flask to volume with nanopure water. Let stand overnight. Decant saturated solution of oxalic acid from crystals before use. |
| **Reducing**<br><br>make fresh daily | • mix 50 mL **Metol Sulfite solution** with 30 mL **Oxalic Acid solution**.<br>• add slowly, with mixing, 30 mL **Sulfuric Acid solution**<br>• add 40 mL nanopure water to bring total volume to 150 mL |

## Standards

Add standard to a 50 mL volumetric flask and bring to volume with nanopure water.

| concentration (µM) | volume of primary standard (mL) | volume of nanopure water (mL) |
|---|---|---|

| | | |
|---|---|---|
| 0 | 0 | 50 |
| 30 | 0.5 | 49.5 |
| 60 | 1 | 49.0 |
| 120 | 2 | 48.0 |
| 240 | 4 | 46.0 |
| 360 | 6 | 44.0 |
| 480 | 8 | 42.0 |
| 600 | 10 | 40.0 |
| 900 | 15 | 35.0 |
| 1200 | 20 | 30.0 |

## Procedure

Make sure that all reagents are prepared ahead of time. The method has a time factor built in, and therefore it is of great importance to have all necessary reagents ready to go.

Do not handle more than about thirty samples at a time in order to ensure that the 15 min time limit can be adhered to. Make sure that there are no large fluctuations in room temperature.

Do not use synthetic seawater in dilutions of the primary standard. This could cause the decrease in reactive silica in a few hours as a result of polymerization reactions.

The reason for adding 200 µL of synthetic seawater to the standards is to maintain a reasonably uniform salt content in relation to the samples, this suppressing a potential salt effect on the method.

It is important to wait at least three hours for the blue color to develop; the higher the concentration, the longer the time. The color remains stable for many hours, and reading after 4–5 hours may, in fact, be a good idea. Again, consistency in time limits is advisable.

| | |
|---|---|
| **1.** | add 4 mL of nanopure water to each vial (3.8 mL for standards). |
| **2.** | for standards, pipette 200 µL of synthetic seawater to each vial. |
| **3.** | add 200 µL sample/standard to each vial. |
| **4.** | Record time. |
| **5.** | add 2 mL **ammonium molybdate solution.** |
| **6.** | a yellow color will develop; allow to mature for exactly 15 minutes (± 15 s). |
| **7.** | add 3 mL **reducing solution.** |
| **8.** | Let color develop for at least 3 hours. Read absorbances at 812 nm. |

# Sulfide

The sulfide method is based off a method developed by Cline in 1969. This method called for very large volumes of water (50 mL). This method was modified on the BONUS Baltic Gas expedition in 2011 to work with sample volumes in the 1-5mL range.
The method is a bit tricky in that the reagent concentrations change depending on what concentration range your samples fall in:

- High range: 6-80 uM
- Low range: 1-10 uM.

All samples need to be preserved during splitting with a 1% zinc acetate solution. It can require a relatively large amount of sample in order to do this analysis. The high range method uses less sample (~500 µL), but if the sulfide level is below 6 µM, it won't be enough. It may still be worth screening samples with the high-range method in order to conserve interstitial water sample volume. The low-range method requires 4 mL (8x the amount of sample) but is sensitive down to 1 µM. The scientists will have to determine if consumption of 4 mL, possibly 4.5 mL, of sample is worth obtaining the sulfide concentration.

## Reagent Solutions

| | |
|---|---|
| **Zinc Acetate (1%)** | For Standard:<br>• Dissolve 10 g zinc acetate dehydrate into ~600 mL nanopure water in a 1000 mL volumetric flask. Add 1 mL concentrated acetic acid and bring flask to volume with nanopure water. Mix well.<br>For Splits:<br>• Dissolve 1 g zinc acetate dehydrate into ~60 mL nanopure water in a 100 mL volumetric flask. Add 100 µL concentrated acetic acid and bring flask to volume with nanopure water. Mix well. |
| **Zinc-Sulfide Standard Suspension (1 mM)** | • Before weighting sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), wash the crystals with nanopure water and dry with kimwipe to remove oxidation products.<br>• Dissolve 200 mg of $Na_2S \cdot 9H_2O$ in 1000 mL 1% **Zinc Acetate solution**.<br>• Store in plastic @ 4°C. Shake vigorously each time before using. |
| **Zinc-Sulfide Standard (100 M)** | Dilute 10 mL of **Zinc-Sulfide Standard Suspension** to 100 mL with nanopure water in a 100 mL volumetric flask. |
| **High-Range Sulfide Diamine** | For the high-range method:<br>• add 500 mL concentrated HCl to 500 mL nanopure water. Mix thoroughly and allow to cool to room temperature. Add 4 g N,N-dimethyl-p-phenylenediamine sulfate and 6.0 g iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). Mix well.<br>• Store in amber Nalgene bottle at 4°C |
| **Low-Range Sulfide Diamine** | For the low-range method:<br>• add 250 mL concentrated HCl to 250 mL nanopure water. Mix thoroughly and allow to cool to room temperature. Add 500 mg of N,N-dimethyl-p-phenylenediamine sulfate and 750 mg iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). Mix well.<br>• Store in amber Nalgene bottle at 4°C |
| **Dilution Reagent** | This reagent is used to dilute too-dark samples into the range of color covered by the standard curve, while keeping a constant concentration of the diamine reagent. Create this reagent as appropriate for the high-range or low-range method:<br>• For high-range method: add 800 L of **High-Range Sulfide Diamine** to 10 mL of nanopure water.<br>• For low-range method: add 4 mL of **Low-Range Sulfide Diamine** to 50 mL nanopure water. |

## High-Range Method

### Sample Preservation

The IW splits for sulfide should be preserved immediately with the 1% zinc acetate solution to prevent the loss of dissolved sulfide.

Add 40 µL of **1% zinc acetate** to 0.5 mL sample aliquots.

### Standards

Make dilutions of the **100 µM zinc-sulfide standard** for the standards to create 0.5 mL of standard at each level.

| concentration (µM) | zinc-sulfide standard (µL) | nanopure water (µL) |
|---|---|---|
| 0 | 0 | 500 |
| 6 | 30 | 470 |
| 20 | 100 | 400 |
| 40 | 200 | 300 |
| 60 | 300 | 200 |
| 80 | 400 | 100 |

### Procedure

| | |
|---|---|
| **1.** | Shake well the zinc acetate preserved sample. |
| **2.** | add 500 µL sample/standard to each vial |
| **3.** | add 40 µL **high-range diamine reagent** to each sample/standard and shake. |
| **4.** | let color develop (in a dark place) for 30 minutes. Measure absorbance at 670 nm.<br>• If any samples are darker blue than the highest standard, dilute the sample with the **dilution reagent** until it is within the color range of the standards. |

## Low-Range Method

### Sample Preservation

The IW splits for sulfide should be preserved immediately with the 1% zinc acetate solution to prevent the loss of dissolved sulfide.

Add 800 µL of **1% zinc acetate** to 4 mL sample aliquots.

### Standards

Make dilutions of the **100 µM zinc-sulfide standard** for the standards to create 4 mL of standard at each level.

| **concentration (µM)** | **zinc-sulfide standard (µL)** | **nanopure water (mL)** |
|---|---|---|
| 0 | 0 | 4000 |
| 1 | 40 | 3.960 |
| 2.5 | 100 | 3.900 |
| 5 | 200 | 3.800 |
| 7.5 | 300 | 3.700 |
| 10 | 400 | 3.600 |

### Procedure

| | |
|---|---|
| **1.** | Shake well the zinc acetate preserved sample. |
| **2.** | add 4 mL sample/standard to each vial |
| **3.** | add 320 µL **low-range diamine reagent** to each sample/standard and shake. |
| **4.** | let color develop (in a dark place) for 30 minutes. Measure absorbance at 670 nm.<br>• If any samples are darker blue than the highest standard, dilute the sample with the **dilution reagent** until it is within the color range of the standards |

# Analyzing Samples

## Preparing the Cary Spectrophotometer

1. Turn on the unit and let it warm up for at least three hours at the wavelength in question.
2. In *Varian > Cary WinUV* start the **Advanced Read** application.
3. Click the **Setup** button and select the **Options** tab.
    - Confirm in the **Beam Mode** area that **Double Beam** is selected and **Normal** is also selected.

## SPS3 Autosampler

The Agilent Technologies SPS3 autosampler facilitates sample introduction into the Cary Spectrophotometer with minimal operator interaction.

*Figure 1 : Sample inlet tube and flow cell*

The SPS3 is connected to the PC via a RS232 cable to a Keyspan USB converter and the SPS3 is connected to the Cary unit via a RSA sample inlet tube (Figure 1).

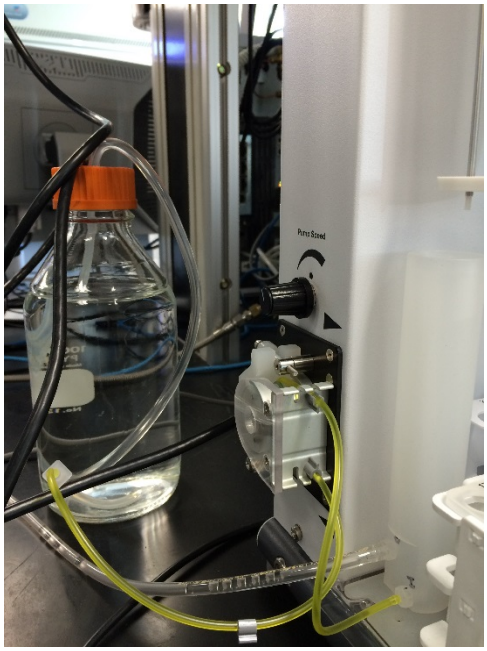


*Figure 2 : SPS3 Reservoir peristaltic pump*

The reservoir peristaltic pump (Figure 2) allows nanopure water to be pumped through the system between samples to flush lines. Pump speed is controlled by a dial above the pump. This pump fills the reservoir but does not change the flow into the Cary's flow cell; that is controlled by the spectrometer's peristaltic pump.

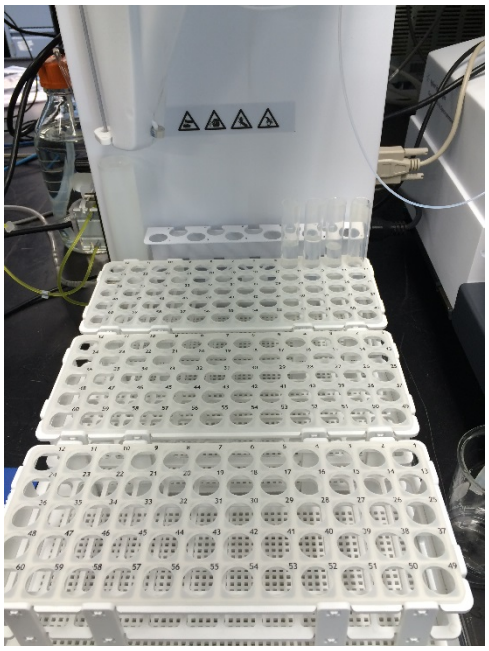


*Figure 3 : SPS3 Sample trays*

Sample trays allow up to 180 samples to be run (Figure 3). First vial is a "zero" (nanopure water).

# Software setup

## Advanced Reads setup

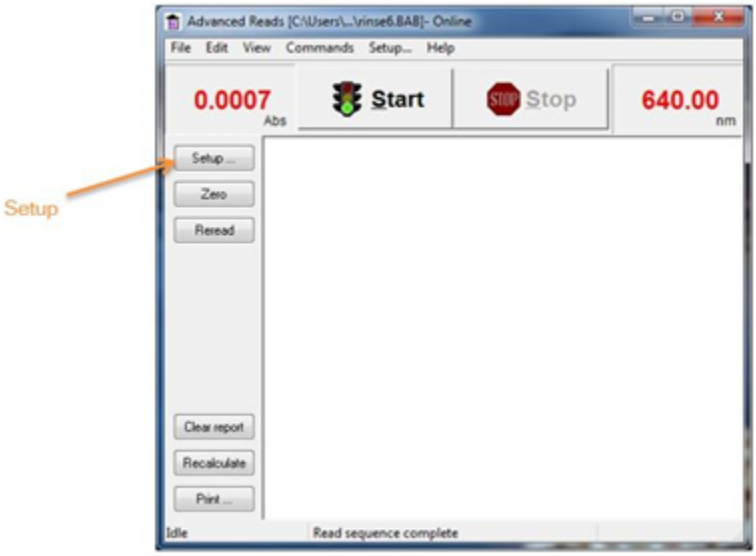



*Figure 4 : Advanced Reads main screen*

In the Advanced Reads software, click the setup button (figure4).

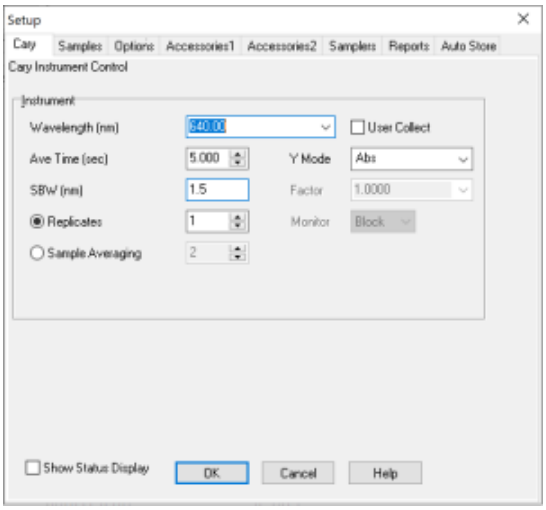



*Figure 5: Cary (Enter wavelength)*

On the Cary tab (figure 5), the wavelength can be changed to the necessary wavelength for the analysis type.

Ave Time (sec) is the length of time over which readings will be taken and averaged. The default setting is 0.1 seconds; increasing this time is beneficial for improving precision and reducing the effect of noise on the resultant measurement.

1 second is a good minimum setting for Ave Time. There are diminishing returns for precision as time increases. Setting it higher than 5 seconds provides almost no more benefit at the expense of taking more time.

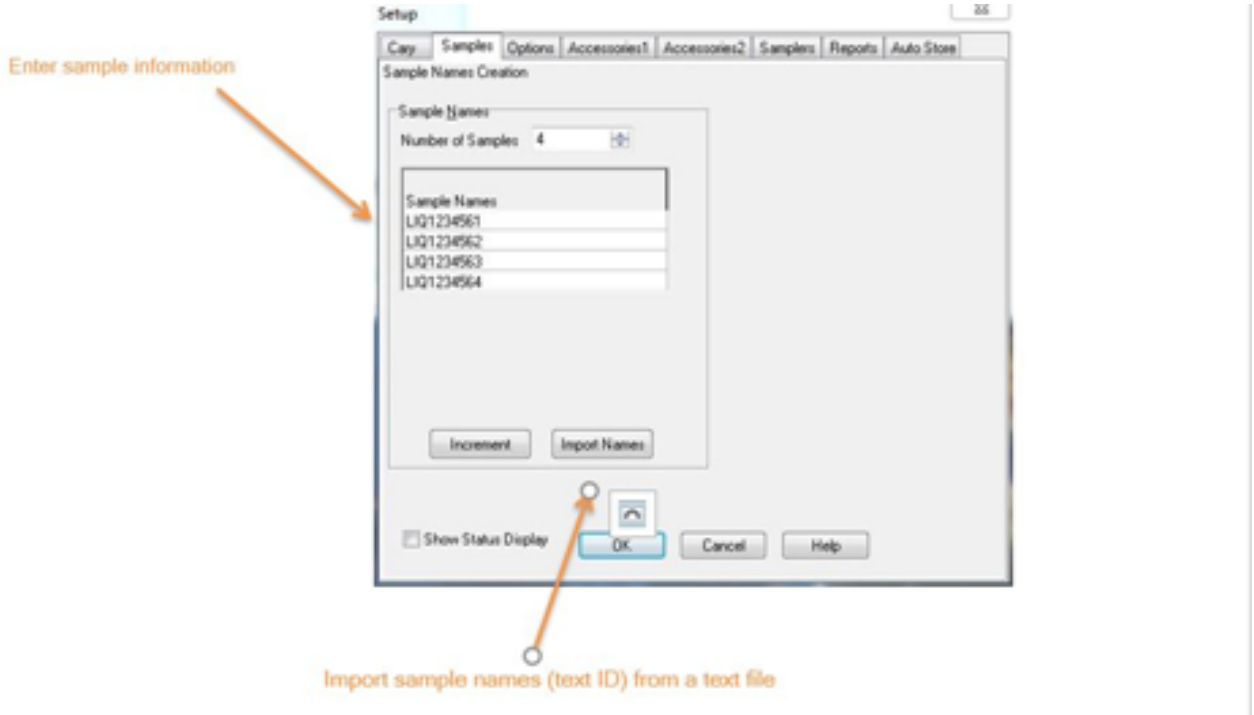



*Figure 6 : Setup (Entering sample information)*

On the samples tab (figure 6), the samples to be run can be entered.

Set the number of samples to the total combined amount of samples and standards. Enter sample names, including the standards.

An alternative is that a text file containing sample names can be imported by clicking **import names.** A text file with textID for sample names can be filled quickly with a barcode scanner (one sample per line). The file

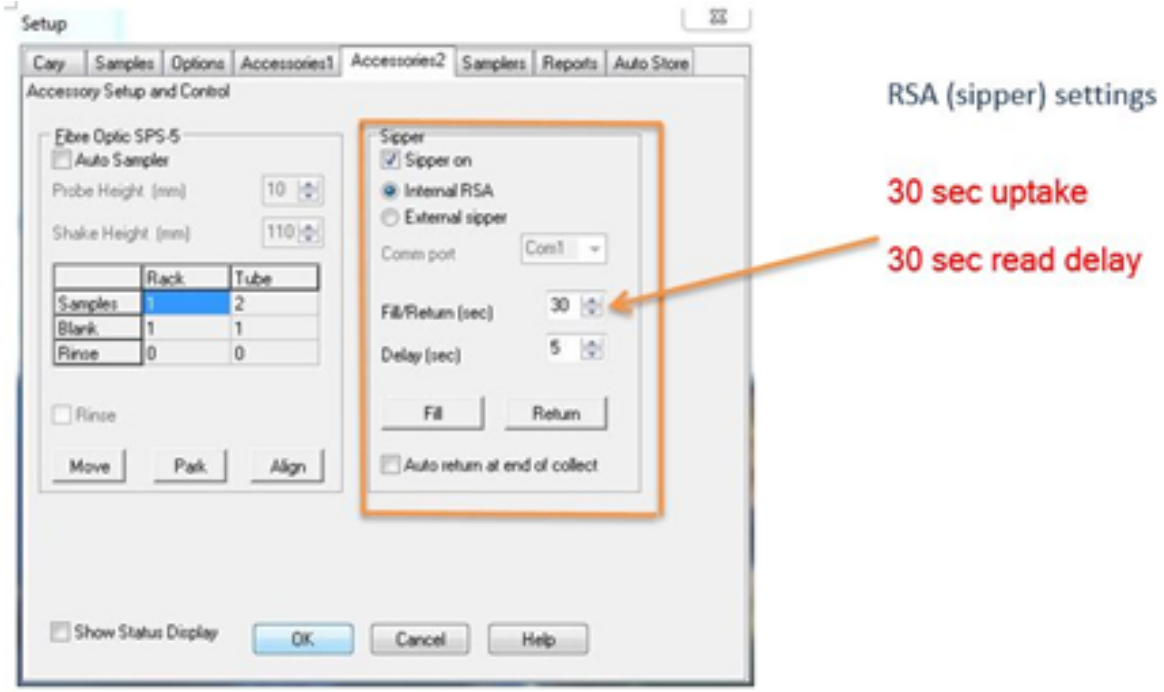
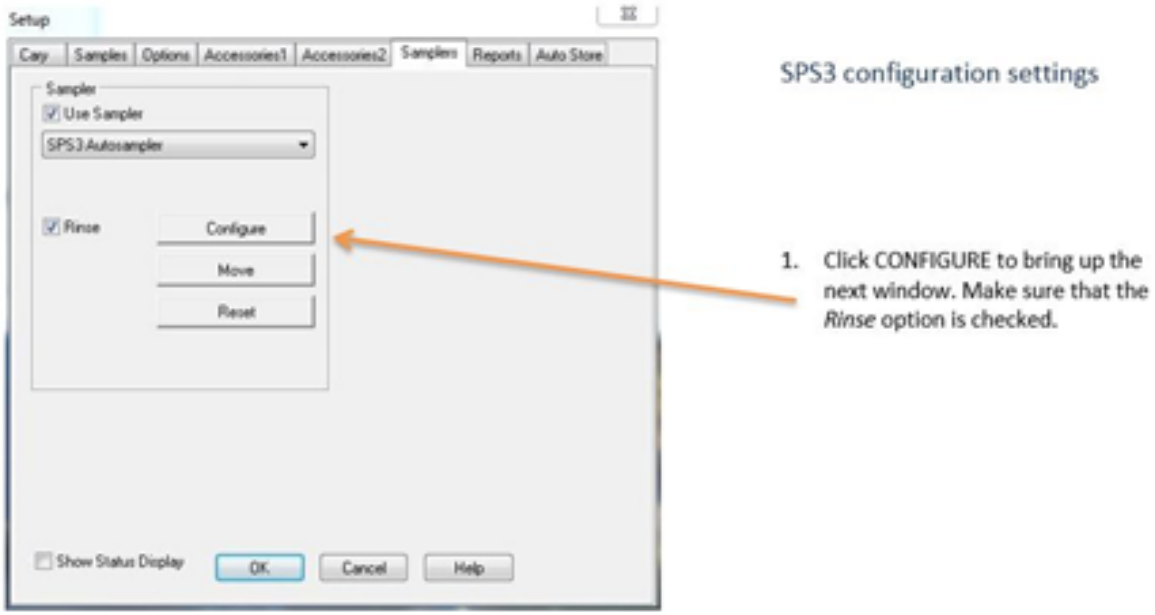



*Figure 7 : Setup (Sipper settings [Cary peristaltic pump])*

On the Acessories2 tab (figure 7), make sure **sipper on** is checked and internal RSA is selected. Set fill/return to 30 seconds and delay to 30 seconds.

*Figure 8 : Setup (Rinse/Sample trays)*

On the samplers tab (figure 8) make sure **use sampler** is checked along with **rinse**. Verify that SPS3 Autosampler is selected and displayed. Click configure for the next window.



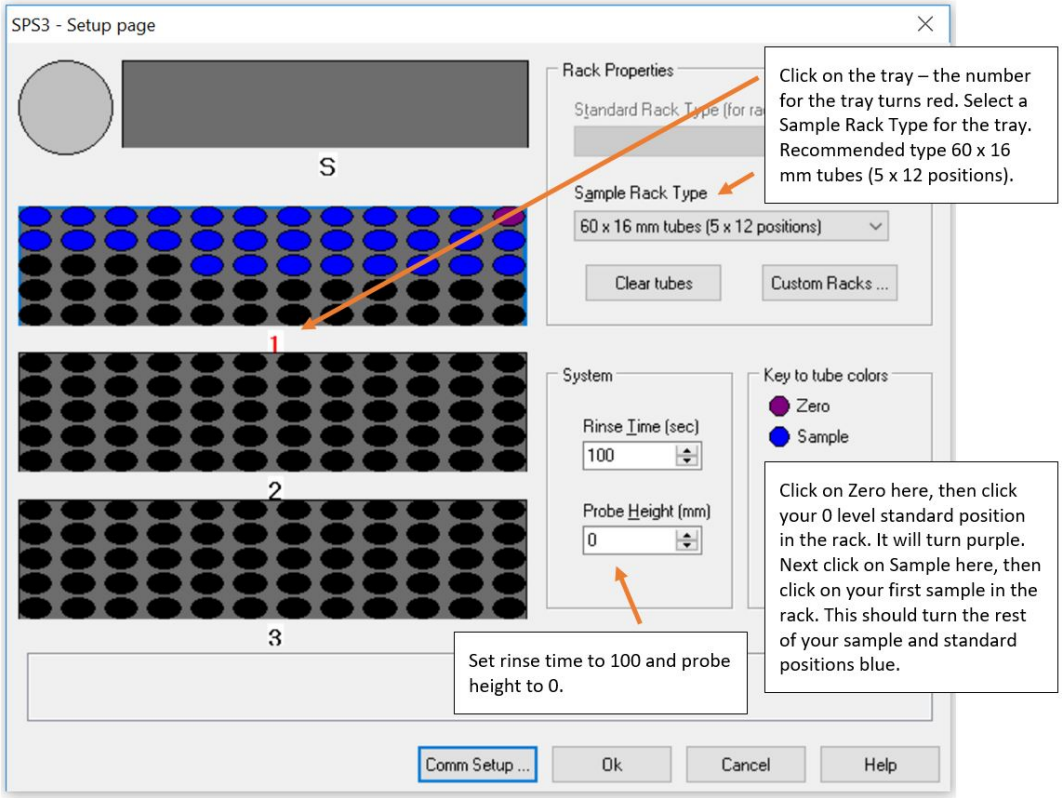




*Figure 9 : Setup (Rinse/Rack type)*

Click on a tray area and then select the 5x12 positions rack type from the **sample rack type** drop down menu.

To position your samples on the autosampler. Click on **zero**, then click on the first tube position (top right). Then click on **sample** and click on the second position. This will then populate the sample rack with the samples that were entered on the sample tab.

Verify that the display matches the loading of samples on the sample rack.

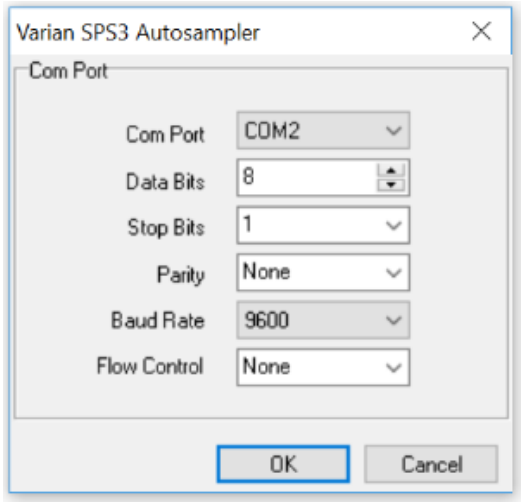



*Figure 10 : Setup (**Comm Setup**/RS232 settings)*

Com port settings (figure 10) can be reached by clicking **comm setup** (figure 9). This is just a reference for the proper com port setup in case the setup is lost or forgotten by the software.

## Running samples

- Prepare samples according to the protocol outlined in the above sections for the appropriate analyte.
- Engage the tubing on the peristaltic pump for both the Spectrophotometer and the Autosampler. Make sure that the waste lines go into a receptacle.
- Click on **START** in the main Advanced Reads window (Figure 4).
- A window will pop-up showing the samples entered in the previous steps (Figure 12). Click **OK**.
- Save the data to a file for later viewing (Figure 13).

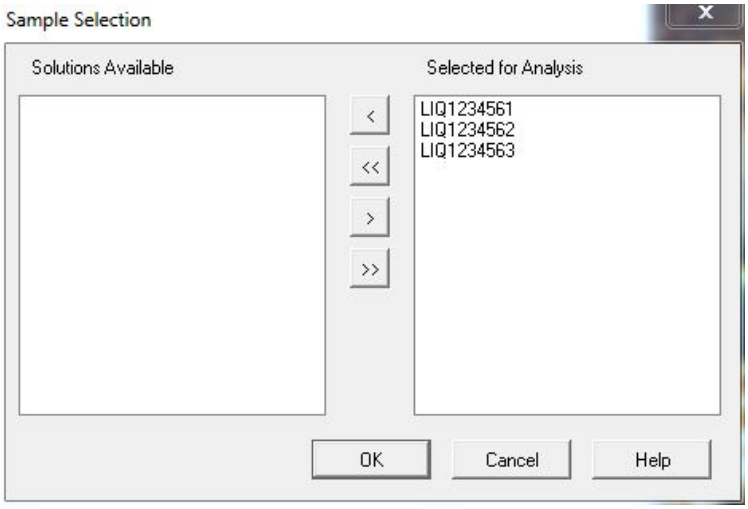



*Figure 12 : Samples ready for analyses*

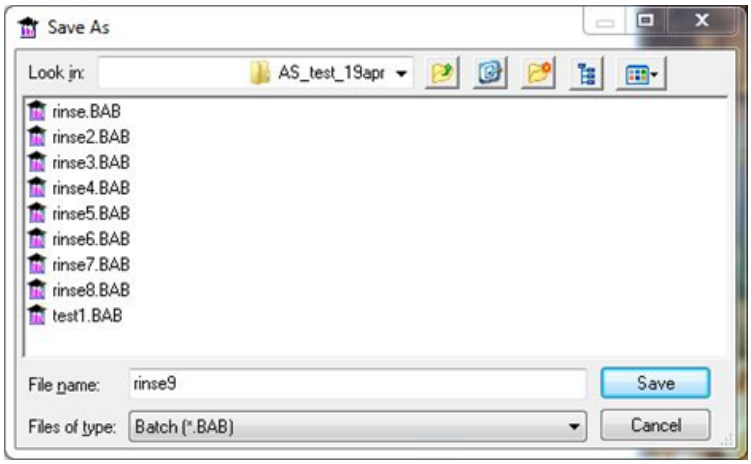



*Figure 13 : Assigning a filename for the results*

- Observe where the samples need to go (Figure 14). Click **OK**.

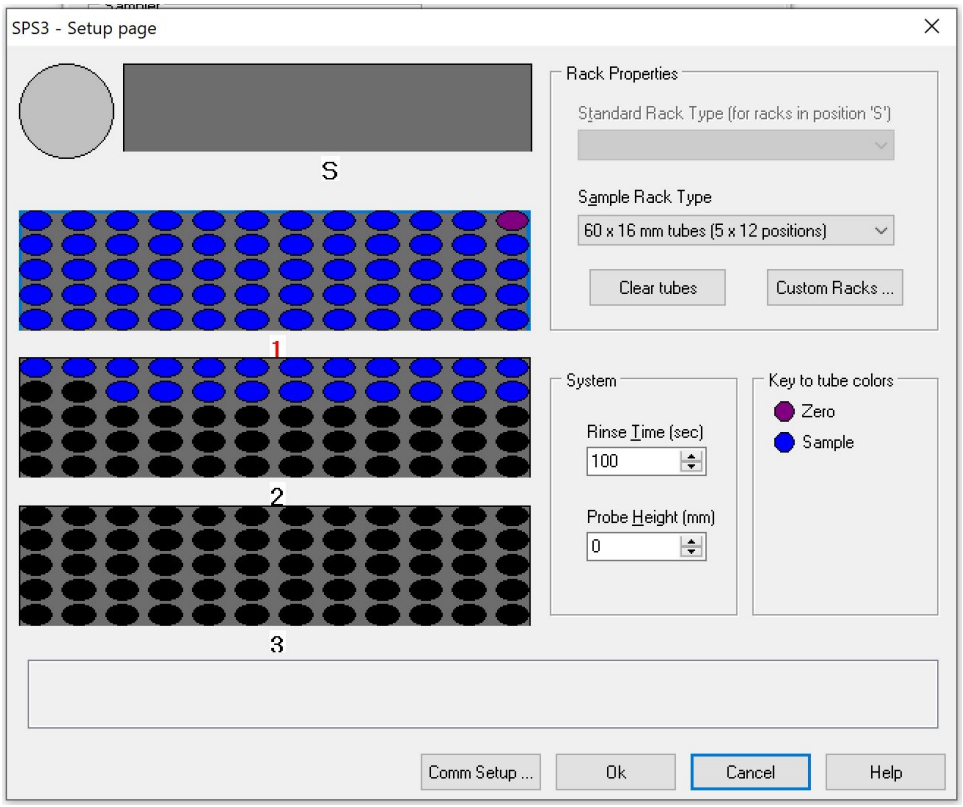



*Figure 14 : How to load the vials in the sample racks*

- View the results in real time (Figure 15).

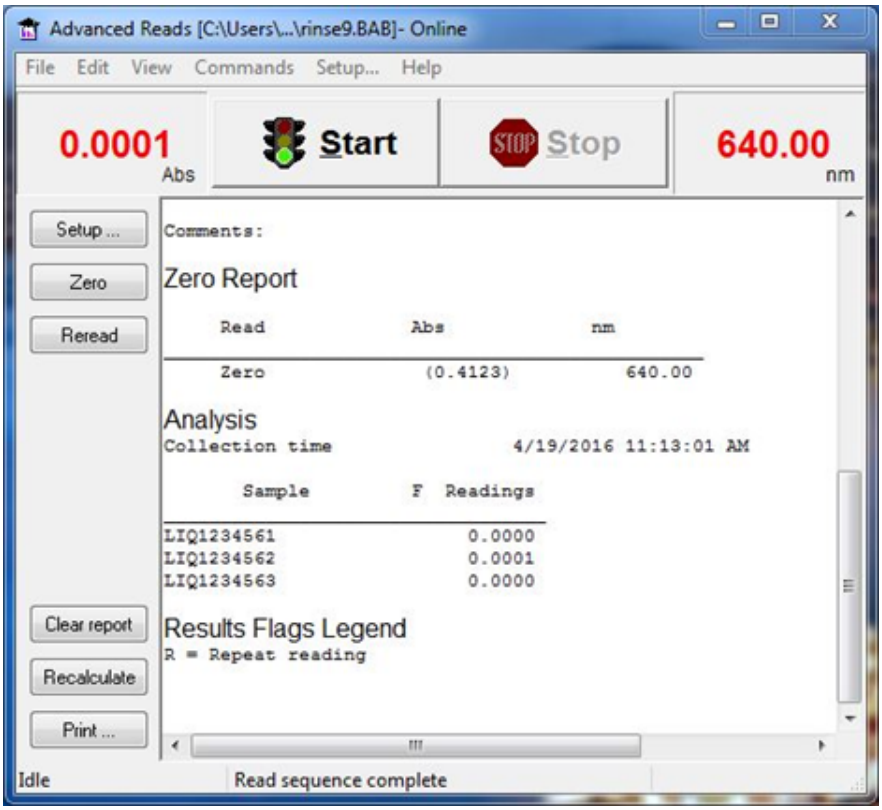



*Figure 15 : Viewing results in real-time*

- Save the results to a .csv file.
  - Select **File  Export report** (*.csv)
  - Enter the location/file name.
- Copy/paste into a calibration spreadsheet for manipulation and subsequent upload via *Spreadsheet Uploader.*
  - Using the results from the reads of the standards, create a calibration curve from the plot of the concentration vs. absorbance values. Use this equation to extrapolate the sample concentrations from their corresponding absorbance value. This can be done in the same spreadsheet as created above in the *Advanced Reads* application. This sheet can be loaded into the *LIMS Spreadsheet Loader* application as outlined in the LIMS Integration section.

## Shutting down the Instrument

Aspirate approximately eight cycles of nanopure water, release the tubing on the peristaltic pump, turn off power to the unit and exit from the software. Clean any spills that may have occurred.
Empty the waste container and rinse with tap water.

# QAQC

QA/QC for analysis consists of calibration verification using check standards, blanks and replicate samples.

## QA/QC Types

### Check Standard

A check standard for each set of analytes is run ~ every fifteen analyses depending on batch size. Check standards consist of a standard from approximately the midpoint of the calibration curve.
The check standard result is evaluated against the threshold for % variance limits for calibration verification standard against true value:

- Within ±10%: calibration is verified and sample analysis can continue.
- Outside of ±10%: check reagent solutions and rerun all samples in the corresponding analytical batch.

### Blank

A blank is run with every batch to determine if high background levels are interfering with accurate sample results.

### Replicate Samples

During each batch, a single sample should be run in duplicate and the variation of the results compared.

# Data Available in LORE

# Data Available in LORE

### Interstitial Waters Standard Report

- **Exp:** expedition number
- **Site:** site number
- **Hole:** hole number
- **Core:** core number
- **Type:** type indicates the coring tool used to recover the core (typical types are F, H, R, X).
- **Sect:** section number
- **A/W:** archive (A) or working (W) section half.
- **Top offset on section (cm):** position of the upper edge of the sample, measured relative to the top of the section.
- **Bottom offset on section (cm):** position of the lower edge of the sample, measured relative to the top of the section.
- **Top depth CSF-A (m):** position of observation expressed relative to the top of the hole.
- **Top depth [other] (m):** position of observation expressed relative to the top of the hole. The location is presented in a scale selected by the science party or the report user.
- **Sampling tool:** tool used to collect sample
- **Data columns:** header lists parameter measured and concentration units, followed by wavelength (for ICP-AES) and then analysis method.

### Expanded IC Report

- **Exp:** expedition number
- **Site:** site number
- **Hole:** hole number
- **Core:** core number
- **Type:** type indicates the coring tool used to recover the core (typical types are F, H, R, X).
- **Sect:** section number
- **A/W:** archive (A) or working (W) section half.
- **text_id:** automatically generated unique database identifier for a sample, visible on printed labels
- **sample_number:** sample number of sample. text ID with sample type prefix removed.
- **label_id:** id combining exp, site, hole, core, type, sect, A/W, parent sample name (if any), sample name
- **sample_name:** name of sample
- **x_sample_state:**
- **x_project:** expedition project the sample is uploaded under. typically the same as Exp.
- **x_capt_loc:**
- **location:** location sample was taken
- **x_sampling_tool:** tool used to collect sample
- **changed_by:** name of person who uploaded sample
- **changed_on:** date and time sample was uploaded
- **sample_type:** type of sample. typically LIQ, for liquid.
- **x_offset:** top offset of parent sample where sample was taken in m
- **x_offset_cm:** top offset of parent sample where sample was taken in cm
- **x_bottom_offset_cm:** bottom offset of parent sample where sample was taken in cm
- **x_diameter:**
- **x_idmp:**
- **x_orig_len:**
- **x_length:** length of sample in m
- **x_lengeth_cm:** length of sample in cm
- **status:**
- **old_status:**
- **original_sample:**
- **parent_sample:**
- **standard:**
- **login_by:** name of person logged into LIMS application used for this test
- **sampled_date:**
- **legacy:**
- **test changed_on:** date of last edit of analysis
- **test date_started:** date analysis was started
- **test group_name:**
- **test status:**
- **test old_status:**
- **test test_number:** unique number associated with the instrument measurement steps that produced these data

- **test date_received:** date analysis was uploaded to LIMS
- **test instrument:** instrument used to perform analysis
- **test analysis:** analysis type
- **test x_project:** project test was assigned to
- **test version:**
- **test order_number:**
- **test replicate_test:**
- **test replicate_count:**
- **rest sample_number:** sample number for sample the analysis was performed on
- **Top depth CSF-A (m):** position of observation expressed relative to the top of the hole.
- **Bottom depth CSF-A (m):** position of observation expressed relative to the top of the hole.
- **Top depth CSF-B (m):**
- **Bottom depth CSF-B (m):**
- **analyte:** the analyte measured for this test
- **concentration (uM):** concentration of analyte in uM
- **ssup_assman_id:** link to download the batch of data uploaded with spreadsheet uploader
- **ssup_filename:** filename of the batch of data uploaded with spreadsheet uploader
- **sample description:** observations recorded about the sample itself
- **test test_comment:** observations about a measurement or the measurement process; some measurement observations may be under Result comments
- **result comments:** observations about a measurement or the measurement process; some measurement observations may be under Test Comments

Data collected is transferred to the LIMS database using IODP's *Spreadsheet Loader* application.

This is best done by entering the results into an Excel spreadsheet in a format similar to the pattern below, keeping the appropriate number of columns blank, and omitting the column headers.

Then run the Spreadsheet Loader application. Go to **File** > **Load** and it should import something like below (Figure 16).

To upload into the database, go to **Lims** > **Upload** and status messages will appear in the blank window.

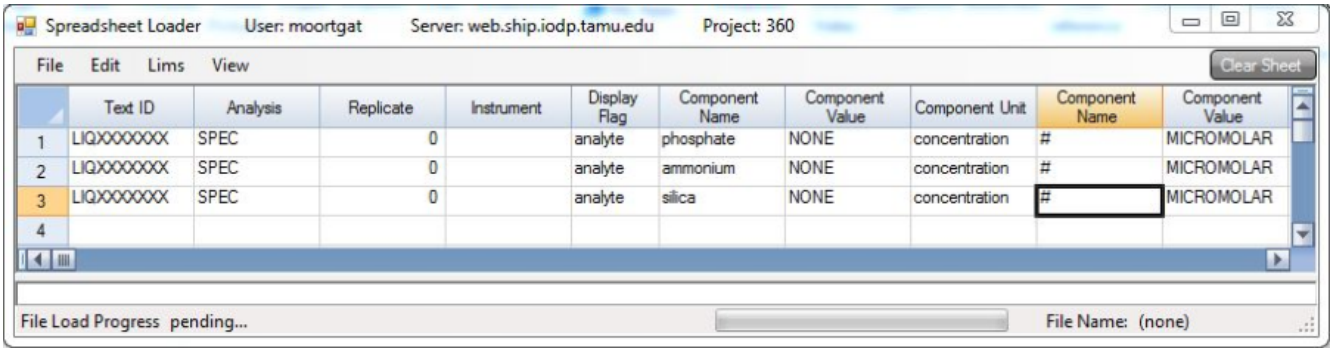



*Figure 16 : Uploading results with Spreadsheet Uploader*

# Maintenance and Troubleshooting

## Cleaning

Any spills in the sample compartment should be immediately wiped up and any deposits on the sample compartment windows should also be removed. The exterior surfaces should be cleaned with a soft cloth and, if necessary, this cloth can be dampened with water or a mild detergent.

## Source Lamps

Instructions for how to change and align both the visible and UV lamps are included in the **Help** provided with the software. Care must be taken when removing lamps. Touching the glass envelope will reduce its efficiency. NEVER touch the glass surface of a new lamp. Always handle a lamp by its base, using a soft cloth.

## Fuses

To replace a fuse, disconnect the unit from the power supply, and replace the blown fuse with one of the type and rating as outlined in the hardware specifications section.

1. Disconnect the instrument from the power supply.
2. Undo the fuse cap by pressing the cap and turning it counter-clockwise.

3. Carefully pull out the cap. The fuse should be held in the fuse cap.
4. Check that the fuse is the correct type and is not damaged. If necessary, replace it.
5. Place the fuse into the cap, push the cap in and then turn the cap clockwise.
6. Reconnect the instrument to the power supply.

### Cary Win UV Help

Varian provides extensive help resources, available from the software CD. After installing the help utilities, go to *START > All Programs > Varian > Cary WinUV > Cary Help*.
Cary Help offers troubleshooting information, maintenance information like how to replace lamps, aligning lamps and mirrors, and replacing fuses and cleaning the flowcells.

### Contact Information

Varian Instruments

1 800 926 3000

customer.service@varianinc.com

# References


Gieskes, J.M., Gamo, T., and Brumsack, H., 1991. Chemical methods for interstitial water analysis aboard *JOIDES Resolution, ODP Tech. Note,* 15. doi:10.2973/odp.tn.15.1991.

Kastner, M., Elderfield, H., Martin, J.B., Suess, E., Kvenvolden, K.A., and Garrison, R.E., 1990. Diagenesis and interstitial water chemistry at the Peruvian margin—major constituents and strontium isotopes. *In* Suess, E., von Huene, R., et al., *Proc. ODP, Sci. Results,* 112: College Station, TX (Ocean Drilling Program), 413–440. doi:10.2973/odp.proc.sr.112.144.1990

Noriki, S. 1983. Silicate correction in the colorimetric determination of phosphate in seawater. *J. Oceanograph. Soc. Japan,* 39(6):324–326. doi: 10.1007/BF02071829

Presley, B.J., 1971. Techniques for analyzing interstitial water samples: Appendix Part 1: determination of selected minor and major inorganic constituents. *In* Winterer, E.L., et al., *Init. Repts. DSDP,* 7(2): Washington, DC (U.S. Govt. Printing Office), 1749–1755. doi:10.2973/dsdp.proc.7.app1. 1971

Solorzano, L., 1969. Determination of ammonia in natural waters by phenol-hypochlorite method. *Limno. Oceanogr.,* 14:799–801.

Strickland, J.D.H., and Parsons, T.R., 1968. A manual for sea water analysis. *Bull. Fish. Res. Board Can.,* 167.


# Appendix

## Hardware

The Varian Cary 100 is a double-beam, dual-chopper, monochromator UV-Vis spectrophotometer, centrally controlled by a PC. It has a high-performance R928 photomultiplier tube, tungsten halogen visible source with quartz window, and deuterium arc ultraviolet source.

| | |
|---|---|
| Name | Agilent Technologies Cary UV-Vis Spectrophotometer |
| Model | Cary-100 |
| Serial number | UV1110M021 |
| Dimensions | 26 × 26 × 13 in (unpacked) |
| Weight | 99 lb (unpacked) |
| Monochromator | Czerny-Turner 0.28 m |
| Grating | 30 × 35 mm, 1200 lines/mm, blaze angle 8.6° at 240 nm |
| Beam Splitting System | Chopper (30 Hz) |
| Detectors | R928 PMT |
| UV-Vis Limiting Resolution (nm) | 0.189 |

| | | |
|---|---|---|
| | Wavelength Range (nm) | 190–900 |
| | Wavelength Accuracy (nm) | 0.02 at 656.1 nm; 0.04 at 486.0 nm |
| | Wavelength Reproducibility (nm) | 0.008 |
| | Signal Averaging (s) | 0.033–999 |
| | Spectral Bandwidth (nm) | 0.20–4.00 nm, 0.1 nm steps, motor driven |
| | Spectral Bandwidth Accuracy (nm) | @ 0.2: 0.193; @ 2.0: 2.03 |
| | Photometric Accuracy (Abs) | @ 0.3 Abs (Double Aperture method): 0.00016 |
| | Photometric Range (Abs) | 3.7 |
| | Photometric Display | (Abs) ± 9.9999; (%T) ± 200.00 |
| | Photometric Reproducibility | (Abs; NIST 930D filters) |
| | 2 s signal averaging time @ 590 nm, 2 nm SBW<br>Maximum deviation at 1 Abs<br>Std dev for 10 measurements | <br><0.0008<br><0.00016 |
| | 2 s signal averaging time @ 546.1 nm, 2 nm SBW<br>Maximum deviation at 0.5 Abs<br>Std dev for 10 measurements | <br><0.0004<br><0.00008 |
| | **Photometric Stability** (Abs /hr)<br>500 nm, 1 s signal averaging time | 2 h warmup<br><0.0003 |
| | **Photometric Noise** (Abs, RMS)<br>500 nm, 1 s signal averaging time | 2 nm SBW<br>0.000030 at 0 Abs; 0.00014 at 3 Abs, 1.5 Abs RBA |
| | Baseline Flatness (Abs)<br>200–850 nm, smoothing = 21, baseline corrected | 0.00022 |
| | Sample Compartment Beam Separation (mm) | 110 |

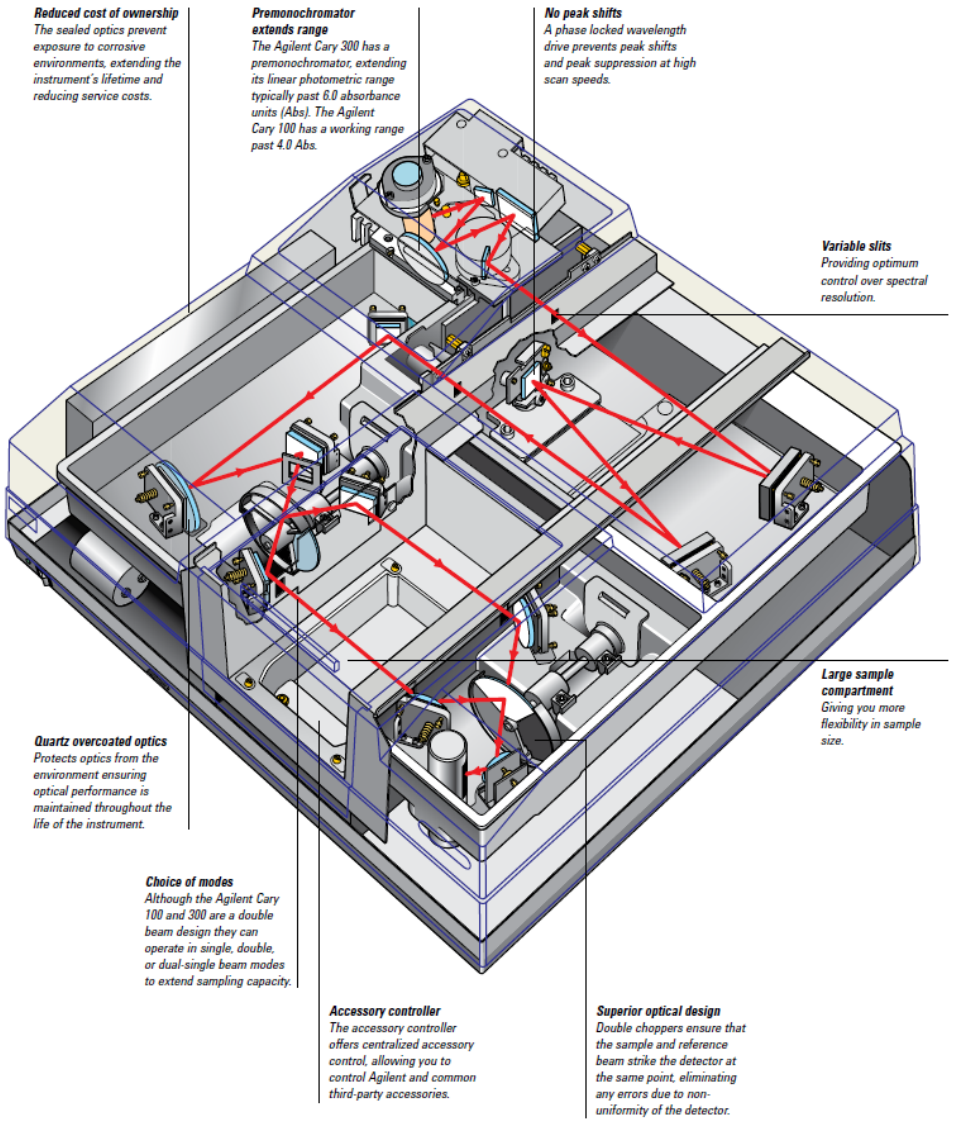



*Figure 17 : Schematic of Cary Spectrophotometer*

## Electrical

| | |
|---|---|
| Power supply (VAC) | 100, 120, 220, or 240 ± 10% |
| Frequency (Hz) | 50 or 60 ± 1 with 400 VA power consumption |
| Fuses (100–120 VAC) | T5 AH 250 V, IEC 127 sheet 5, 5 × 20 mm ceramic |
| COM port (rear) | IEEE 488 |
| PC port | USB |

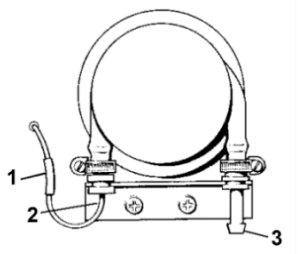
## Replacement Parts

| Item | Part number |
|---|---|

| | |
|---|---|
| Instrument fuse, 5 A time lag, ceramic, M205 | 191 000 9100 |
| Peristaltic pump tubing replacement kit | 991 005 2900 |
| Visible source lamp | 561 002 1700 |
| Deuterium lamp | 561 002 1800 |
| Dissolution cell, 715 µL, 10 mm | 661 001 5200 |
| Thumbscrew kit | 991 006 4100 |
| Spares kit: accessory locating pin, accessory fastening screws, instrument feet, instrument cover snap cap washer, snap cap, ACB cover plate, socket covers for ACB | 991 006 4300 |

### Pumps

A double-action peristaltic pump services the feed and waste.




**(1) plastic sleeve. (2) metal hook tubing. (3) waste outlet.**

# Credits

This document originated from Word documents *Cary_UG_374_draft* and *CarySPS3_QSG_374_draft* that were written by E. Moortgat (20111212). Credits for subsequent changes to this document are given in the page history.

# LIMS Component Table

| ANALYSIS | TABLE | NAME | ABOUT TEXT |
|---|---|---|---|
| SPEC | SAMPLE | Exp | Exp: expedition number |
| SPEC | SAMPLE | Site | Site: site number |
| SPEC | SAMPLE | Hole | Hole: hole number |
| SPEC | SAMPLE | Core | Core: core number |
| SPEC | SAMPLE | Type | Type: type indicates the coring tool used to recover the core (typical types are F, H, R, X). |
| SPEC | SAMPLE | Sect | Sect: section number |
| SPEC | SAMPLE | A/W | A/W: archive (A) or working (W) section half. |
| SPEC | SAMPLE | text_id | Text_ID: automatically generated database identifier for a sample, also carried on the printed labels. This identifier is guaranteed to be unique across all samples. |
| SPEC | SAMPLE | sample_number | Sample Number: automatically generated database identifier for a sample. This is the primary key of the SAMPLE table. |
| SPEC | SAMPLE | label_id | Label identifier: automatically generated, human readable name for a sample that is printed on labels. This name is not guaranteed unique across all samples. |

| | | | |
|---|---|---|---|
| SPEC | SAMPLE | sample_name | Sample name: short name that may be specified for a sample. You can use an advanced filter to narrow your search by this parameter. |
| SPEC | SAMPLE | x_sample_state | Sample state: Single-character identifier always set to "W" for samples; standards can vary. |
| SPEC | SAMPLE | x_project | Project: similar in scope to the expedition number, the difference being that the project is the current cruise, whereas expedition could refer to material/results obtained on previous cruises |
| SPEC | SAMPLE | x_capt_loc | Captured location: "captured location," this field is usually null and is unnecessary because any sample captured on the JR has a sample_number ending in 1, and GCR ending in 2 |
| SPEC | SAMPLE | location | Location: location that sample was taken; this field is usually null and is unnecessary because any sample captured on the JR has a sample_number ending in 1, and GCR ending in 2 |
| SPEC | SAMPLE | x_sampling_tool | Sampling tool: sampling tool used to take the sample (e.g., syringe, spatula) |
| SPEC | SAMPLE | changed_by | Changed by: username of account used to make a change to a sample record |
| SPEC | SAMPLE | changed_on | Changed on: date/time stamp for change made to a sample record |
| SPEC | SAMPLE | sample_type | Sample type: type of sample from a predefined list (e.g., HOLE, CORE, LIQ) |
| SPEC | SAMPLE | x_offset | Offset (m): top offset of sample from top of parent sample, expressed in meters. |
| SPEC | SAMPLE | x_offset_cm | Offset (cm): top offset of sample from top of parent sample, expressed in centimeters. This is a calculated field (offset, converted to cm) |
| SPEC | SAMPLE | x_bottom_offset_cm | Bottom offset (cm): bottom offset of sample from top of parent sample, expressed in centimeters. This is a calculated field (offset + length, converted to cm) |
| SPEC | SAMPLE | x_diameter | Diameter (cm): diameter of sample, usually applied only to CORE, SECT, SHLF, and WRND samples; however this field is null on both Exp. 390 and 393, so it is no longer populated by Sample Master |
| SPEC | SAMPLE | x_orig_len | Original length (m): field for the original length of a sample; not always (or reliably) populated |
| SPEC | SAMPLE | x_length | Length (m): field for the length of a sample [as entered upon creation] |
| SPEC | SAMPLE | x_length_cm | Length (cm): field for the length of a sample. This is a calculated field (length, converted to cm). |
| SPEC | SAMPLE | status | Status: single-character code for the current status of a sample (e.g., active, canceled) |
| SPEC | SAMPLE | old_status | Old status: single-character code for the previous status of a sample; used by the LIME program to restore a canceled sample |
| SPEC | SAMPLE | original_sample | Original sample: field tying a sample below the CORE level to its parent HOLE sample |
| SPEC | SAMPLE | parent_sample | Parent sample: the sample from which this sample was taken (e.g., for PWDR samples, this might be a SHLF or possibly another PWDR) |
| SPEC | SAMPLE | standard | Standard: T/F field to differentiate between samples (standard=F) and QAQC standards (standard=T) |
| SPEC | SAMPLE | login_by | Login by: username of account used to create the sample (can be the LIMS itself [e.g., SHLFs created when a SECT is created]) |
| SPEC | SAMPLE | login_date | Login date: creation date of the sample |
| SPEC | SAMPLE | legacy | Legacy flag: T/F indicator for when a sample is from a previous expedition and is locked/uneditable on this expedition |
| SPEC | TEST | test changed_on | TEST changed on: date/time stamp for a change to a test record. |
| SPEC | TEST | test status | TEST status: single-character code for the current status of a test (e.g., active, in process, canceled) |
| SPEC | TEST | test old_status | TEST old status: single-character code for the previous status of a test; used by the LIME program to restore a canceled test |
| SPEC | TEST | test test_number | TEST test number: automatically generated database identifier for a test record. This is the primary key of the TEST table. |
| SPEC | TEST | test date_received | TEST date received: date/time stamp for the creation of the test record. |
| SPEC | TEST | test instrument | TEST instrument [instrument group]: field that describes the instrument group (most often this applies to loggers with multiple sensors); often obscure (e.g., user_input) |
| SPEC | TEST | test analysis | TEST analysis: analysis code associated with this test (foreign key to the ANALYSIS table) |
| SPEC | TEST | test x_project | TEST project: similar in scope to the expedition number, the difference being that the project is the current cruise, whereas expedition could refer to material/results obtained on previous cruises |
| SPEC | TEST | test sample_number | TEST sample number: the sample_number of the sample to which this test record is attached; a foreign key to the SAMPLE table |
| SPEC | CALCULATED | Top depth CSF-A (m) | Top depth CSF-A (m): position of observation expressed relative to the top of the hole. |
| SPEC | CALCULATED | Bottom depth CSF-A (m) | Bottom depth CSF-A (m): position of observation expressed relative to the top of the hole. |
| SPEC | CALCULATED | Top depth CSF-B (m) | Top depth [other] (m): position of observation expressed relative to the top of the hole. The location is presented in a scale selected by the science party or the report user. |

| | | | |
|---|---|---|---|
| SPEC | CALCULATED | Bottom depth CSF-B (m) | Bottom depth [other] (m): position of observation expressed relative to the top of the hole. The location is presented in a scale selected by the science party or the report user. |
| SPEC | RESULT | analyte | RESULT analyte: analyte name of the property being measured (e.g., phosphate, ammonium) |
| SPEC | RESULT | concentration (µM) | RESULT concentration (uM): concentration of the analyte |
| SPEC | RESULT | ssup_asman_id | RESULT spreadsheet uploader ASMAN_ID: serial number for the ASMAN link for the spreadsheet uploader file |
| SPEC | RESULT | ssup_filename | RESULT spreadsheet uploader filename: file name for the spreadsheet uploader file |
| SPEC | SAMPLE | sample description | SAMPLE comment: contents of the SAMPLE.description field, usually shown on reports as "Sample comments" |
| SPEC | TEST | test test_comment | TEST comment: contents of the TEST.comment field, usually shown on reports as "Test comments" |
| SPEC | RESULT | result comments | RESULT comment: contents of a result parameter with name = "comment," usually shown on reports as "Result comments" |

# Archived Versions

- LMUG-CarySpectrophotometerUserGuide-230220-1918-164.pdf
- Cary_UG_374_draft_20181126.pdf
- Cary_UG_v1.1_20181130.pdf
- Cary_QSG_378P_20181130