EnviroTech

MicroLAB Nutrient Analyzer

Operating Manual

EnviroTech LLC

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Section 1

1 Revision History

1.0   New Issue   25 February 2005   Approved

Section 2

2   Terms of Use

2.1   Limited Liability

EnviroTech LLC disclaims all product liability risks arising from the use or servicing of this equipment.

EnviroTech LLC cannot take steps to comply with laws pertaining to product liability, including laws which impose a duty to warn the user of any dangers as it has no way of controlling the use of this equipment or selecting the personnel to operate it.

Acceptance of this system by the customer shall be deemed to include a covenant by the customer to defend, indemnify, and hold EnviroTech LLC harmless from all product liability claims arising from the use or servicing of this system.

2.2   Disclaimers

All information in this manual is believed to be accurate and reliable. However, no responsibility is assumed by EnviroTech LLC for inaccuracies. Whilst every effort has been made to ensure that this document is correct, errors can occur. If you find any errors or omissions please let us know. EnviroTech LLC reserves the right to change product design, operation, specifications and documentation without notice.

2.3   Entitlements

EnviroTech LLC wishes to protect the investment it has made in producing MicroLAB. Acceptance of the product by a third party whether or not in exchange for payment or consideration implies acceptance of the following design entitlement notice:

All information contained in this manual and other documentation supplied by the Company is protected by copyright registered by EnviroTech LLC. No information relating to MicroLAB, whether or not in the public domain, may be reproduced by or for third parties except with the written consent of EnviroTech LLC.

2.4   Warranty

EnviroTech LLC warrants MicroLAB against faulty materials and workmanship for a period of <u>TWO YEARS</u> from dispatch. Faulty units will be repaired (or replaced at the Company's option) free of charge provided they are delivered to EnviroTech LLC's premises at the owners/users expense and providing all manufacturers recommendations with regard to operation, servicing and storage have been adhered to. In particular the warranty will not apply if the fault has been caused by misuse, attempted repair or modifications in a manner unauthorised by EnviroTech LLC.

Section 3

3   Health & Safety

The user should exercise care when operating or servicing this equipment. It should be used or serviced only by personnel with knowledge of and training in the use and maintenance of analytical equipment.

In the interests of safety MicroLAB users should acquire and use the following items:

- A laboratory coat
- Surgical gloves
- Safety goggles
- An eyewash station
- Chemical spill granules

Always ensure good ventilation

Never pipette by mouth

Material Safety Data Sheets (MSDS) are available from your chemical supplier. MSDS are also available from EnviroTech LLC on request.

Care should be taken when handling chemicals. If any chemicals come into contact with skin or clothing immediately wash the affected area with fresh water. If splashes enter the eye immediately use an eye-wash station or flush with copious amounts of clean fresh water and seek medical advice.

The following substances are particularly toxic and users should be familiar with their Material Safety Data Sheets:

- Cadmium

Ingestion should be avoided. If ingestion occurs immediately seek medical advice.

Section 4

4   Acknowledgments

We are indebted to our customers for their feedback and comments on the content and accuracy of our documentation. We gratefully acknowledge your contributions.

EnviroTech LLC welcomes all suggestions for new features and enhancements of our products and manuals. We are also pleased to receive any error corrections or notice of omissions.

Please e-mail any comments or suggestions to help@n-virotech.com

Section 5

5   Technical Support

Thank you for using one of our products.

We are dedicated to ensuring that you get the very best performance from our instruments throughout their lifetime. To this end we would like to make you aware of the comprehensive technical support options we provide:

On-line FAQ library

We have an extensive and searchable on-line library of frequently asked questions. This database is continuously updated. The FAQ's can be viewed by selecting the "Browse FAQs" button on our website Help Page.

On-line Help Desk

You can enter a trackable trouble-ticket at our on-line Help Desk by selecting the "Contact Help!!" button on our website Help Page. Your request will be automatically routed to the best people to help you. Help tickets entered this way will never get lost or be forgotten. Please help us to help you by using this facility.

Email Help Desk queries

As an alternative to the on-line Help Desk a new trouble-ticket can simply be emailed to our help email address.

Telephone contact

Please feel free to contact us via telephone during business hours (8:00 – 5:00 US Eastern Standard Time)

Emergency contact

For emergencies and especially out of hours assistance please use our automatic pager system by sending a short email to our emergency contact address. The maximum message length should be 130 characters and please include your name and a contact number where we can get back to you.

Website Help Page
Browse FAQ's / Enter a trouble ticket
www.n-virotech.com/help.htm

Help Desk system email
help@n-virotech.com

Phone and fax
T: 757 549 8474
F: 757 410 2382
EnviroTech LLC
Chesapeake, Virginia, USA

Hours of business 8:00 - 5:30 EST

Emergency Out of Hours Pager
In an emergency send a very short email message starting with contact details to:
emergency@n-virotech.com

Section 6

6 Introduction

MicroLAB is a submersible single-channel chemical analyzer developed and manufactured by EnviroTech LLC. It may be deployed for extended periods to automatically measure nutrient concentrations in all aquatic environments. MicroLAB uses conventional wet chemistry techniques and colorimetric or fluorimetric detection.

Each MicroLAB system is made up of five major components
1. Multi-port rotary valve and drive mechanism
2. Syringe and drive mechanism
3. The colorimeter or fluorimeter
4. Reagent housing
5. Electronic controller

MicroLAB external view with guard fitted

The rotary valve and syringe are both driven by stepper motors controlled by an electronic controller (known as the ESM). Reagents are stored in the reagent housing in plastic "IV" bags. Further details are given in the following sections.

A sample is taken by withdrawing the syringe plunger when the rotary valve is aligned to the inlet port. A chemical reaction is achieved by moving the valve from port to port and adding reagents to the sample by retracting the syringe plunger at each port. Samples and reagents can then be mixed in the syringe where the reaction develops. The sample is injected into the detector for further development or measurement. The basic flow of the sampling regime is give below.

READ BLANK >> TAKE SAMPLE >> ADD REAGENTS >> DEVELOP >> READ REACTION >> FLUSH

The detector typically consists of a narrow capillary tube with an LED light source positioned at one end and a photodiode detector at the other. An additional detector is placed adjacent to the light to measure and calibrate the LED intensity directly. The sample is placed between the source and sensor and a beam transmission measurement is taken through the sample or the fluorescence of the sample is measured.

MicroLAB performs discrete chemical analyses at a user programmable interval. The result from each analysis is stored in memory for data extraction after recovery. Between samples MicroLAB is dormant and in a low power state. MicroLAB can also be used on-line or in telemetry applications.

The analyzer utilizes a totally pressure balanced system. This avoids depth limitations and prevents problems with air in the system. Pressure balancing is achieved by the chemical circuitry being external to the instrument and at ambient pressure. Only the motors, electronics and battery are contained in sealed pressure housings.

Default analysis routines are provided. Users may also design and implement their own sample analysis procedures and sampling sequences by using of our friendly NutrientDATA 2 software. For more complex applications the Eco-Script programming language can be used. This feature makes the instrument uniquely flexible allowing analytical regimes to be developed and adapted to suit a wide variety of environments.

MicroLAB is programmed and operated via a serial communication link to a PC running NutrientDATA 2 software. A VT100 compliant terminal emulator may be used as an alternative.

Section 7

7 Getting Started

7.1 Important Notes

Power supplies

Please note that the single most frequent problem encountered with customers new to MicroLAB is the use of an inadequate or inappropriate power supply. We can supply or recommend a suitable model of power supply. Do not hesitate to ask.

Valve nozzles

The torque of the barbed adapter nozzles on the rotary valve is critical to performance. The nozzles must be tightened to the correct torque to avoid jamming (too tight) and cross-talk (too loose). See the torque settings section for full details.

O-rings

It is important that all o-rings and their sealing surfaces are in perfect condition, scratch free, absolutely clean and lightly lubricated with silicone grease before assembly. This is essential to ensure complete watertight integrity. All replacement parts including o-seals should be obtained from EnviroTech LLC.

Servicing

Inadequate servicing is the most common cause of poor data returns and catastrophic failure (such as flooding). Please pay full attention to the servicing routines.

7.2 General Advice

To get the best results out of your MicroLAB it is important that you allocate sufficient time to familiarize yourself with all the features described in the documentation package and take the time to practice with the instrument.

An on-site training course is highly recommended and these can be arranged at a convenient time and place. Please contact your supplier for further details.

The beginner should start with manual operation and individual samples. Then familiarize yourself with programming the instrument for deployment. A self-training exercise section is provided in this manual.

Once you feel comfortable with the basic operation of MicroLAB move on to the chemistry section.

Servicing and maintenance are very important aspects of operating MicroLAB and the servicing routines are described further in the Maintenance section

New users of MicroLAB are advised to perform several short or "accelerated" test deployments prior to using the instrument in the field. It is not necessary to immerse MicroLAB, it will perform logging operations in air on the laboratory bench.

Your preparation should include practising the deployment in the laboratory and at a local site if possible. If you are going to be responsible for the deployment it is strongly recommended that you practice so that you are able to use the instrumentation and software without assistance from your colleagues. Always assemble all the equipment in the deployment frame that you are going to use prior to your field deployment.

- Read the operating manual
- Don't cut familiarisation time short
- Carry out regular maintenance
- Make up chemicals and standards carefully
- Activate the cadmium column properly (nitrate only)
- Don't over tighten screws and nuts etc.
- Fit communications dummy plug prior to deployment
- Double check everything and ask if unsure
- Don't leave tube clips on for deployment

7.3   Unpacking

Carefully unpack MicroLAB and the accessories supplied. Inspect each item for shipping damage and ensure that the inventory is correct by identifying each item on the packing list. If any items are damaged or missing please contact your supplier immediately.

MicroLAB should be placed vertically on a flat surface with the reagent housing handle at the top. Do not attempt to open or operate your MicroLAB until you have read and understood at least as far as the chemistry section.

Upon receipt of the instrument it is advisable to check components and fasteners for security. Most fasteners on MicroLAB are tightened to specific torque settings immediately prior to leaving the factory however these may become loose during transit. The torque settings are detailed in the Maintenance section.

7.4   Equipment

The following equipment is required to power-up and communicate with your MicroLAB:

- A 12 volt DC _**regulated**_ power supply capable of supplying 12 volts at 2 Amps
- The communications and power deck leads
- A personal computer (PC) with a spare RS232 port
- NutrientDATA 2 software (Or a VT100 compliant Terminal Emulator)

Please note that the single most frequent problem encountered with customers new to MicroLAB is the use of an inadequate or inappropriate power supply. We can supply or recommend a suitable model of power supply. Please do not hesitate to ask.

7.5   Software Installation

NutrientDATA 2 is a Microsoft Windows application with very modest PC requirements.

To install the software:

- If you received a CD launch the NutrientDATA 2 installation from the CD autorun panel.
- If the launcher does not autorun or you downloaded the file, use Windows Explorer navigate to the NutrientDATA 2 installation file on the CD or the folder that you downloaded or saved it to as in the screen shot below.
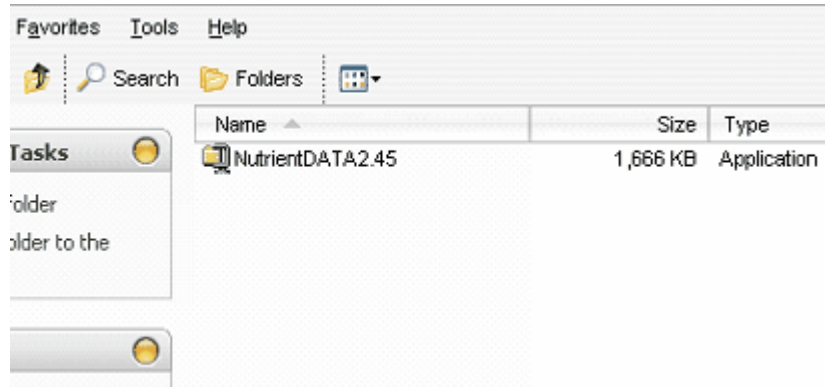
- Double-click on the application file "NutrientDATA2.xx.exe". The installation confirmation box will be displayed. Click the "OK" button.
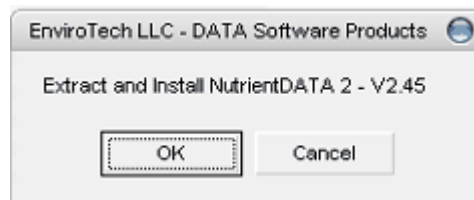
- Next click the "Setup" button and the installation will start. Follow the on-screen installation instructions to suit your requirements.
- Once the installation is complete run NutrientDATA 2 from the Windows Start menu (\Programs\EnviroTech LLC).
- A splash screen will be displayed and then the main Nutrient DATA control panel shown below.

7.6   Connecting Up

STEP 1
- Remove the dummy plugs.
- Identify the hex driver that fits the three retaining screws on the lowest row around the reagent housing.
- Unscrew these three retaining screws and carefully lower the central guard.
- Lift MicroLAB out of the guard and put the guard aside.
- Ensure that you have sufficient working space around MicroLAB analyzer module.

STEP 2
- Boot-up your PC in the normal way.
- Take the communications cable and connect the D-type connector to your spare RS232 COM port (COM1 on your PC is the default = easiest).
- Carefully plug the other end into the user serial connector of MicroLAB (4 pin).

STEP 3
- If the power supply is new to you it is highly recommended that you check the voltage output with a digital voltmeter (DVM) or multi-meter before connecting up. MicroLAB will be damaged by excessive voltage, so ensure the supply voltage is a nominal 12 volts and certainly in the range of 9 – 15 volts.
- Ensure the power supply is switched-off and connect positive/red terminal to the red jack plug on the cable and negative/black to the black jack plug.
- Carefully plug the other end into the user power connector of MicroLAB (3 pin).

- Don't switch-on yet!

STEP 4
- Make sure NutrientDATA 2 is running
- Now switch on your power supply and within 20 seconds press the "Configuration" button on the main control panel (shown above). The following configuration screen will be displayed.
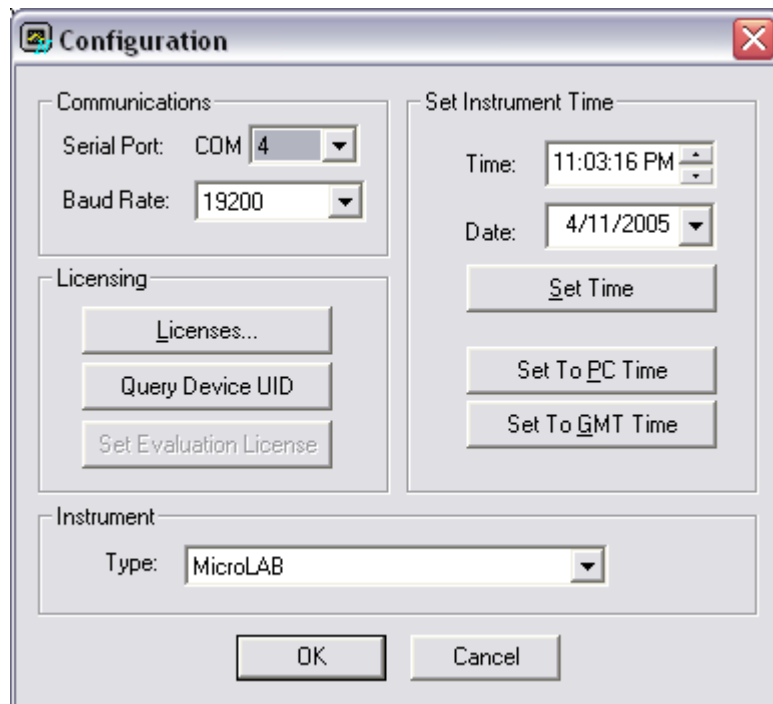
STEP 5
- Select the correct COM port on you PC (if it is not COM1)
- Press the "Set Evaluation License" button (we will come back to enter your full license later in the software section).
- Once the license is enabled return to the main control panel by pressing the "OK button".
- You are now ready to use your MicroLAB via the NutrientDATA 2 software.

Before trying to operate MicroLAB meaningfully it is essential that you familiarise yourself with the unit's modules, commands and the software interface.

Section 8

8   Operation

8.1   Main Components

To use MicroLAB effectively you must familiarise yourself with the basic anatomy of the system and some technical issues. If you are not familiar with electronic instrumentation you may find some concepts challenging at first. However, you will soon become comfortable with these aspects.

The main component parts and modules are shown in the diagram below. The reagent housing has been removed to allow the internal parts to be seen.

Analyzer Module

Reagent Housing

Upper Guard

MicroLAB with reagent housing and guard removed

MicroLAB pressure cases contain the battery back. Any other nominal 12 volt DC supply can be used as an external supply.

The photograph below shows the location of each of the major components.
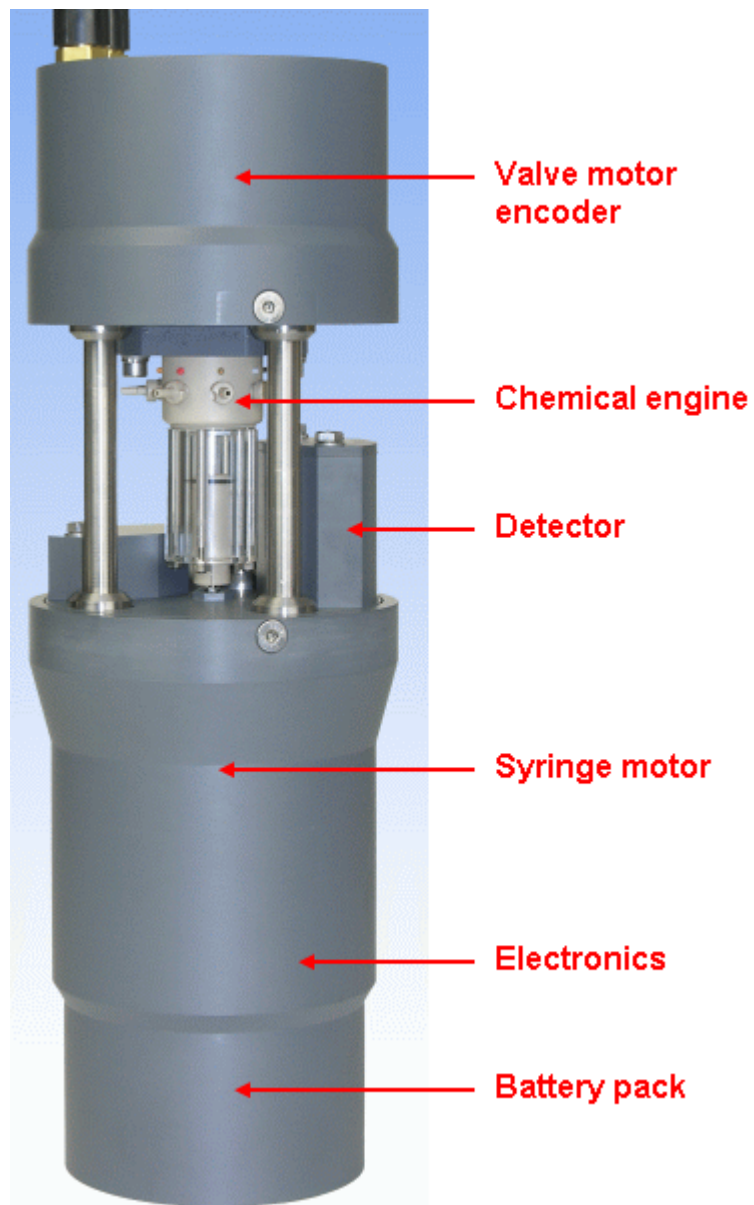
8.2 Electronic Configuration

The user communicates with MicroLAB via an RS232 link. This is a common serial communication connection and protocol found on most desktop PCs and many laptop computers. It may be necessary to install an RS232 "card" in your computer to provide a suitable COM port. If you have any queries please do not hesitate to ask.

MicroLAB contains a number of electronic sub-systems, these include the ESM controller unit, the motor drivers and the detector interface. The motor drivers and detector interfaces are individual intelligent devices that are controlled by the ESM.

Each motor and detector module has its own microprocessor built-in and is configured as a peripheral to the main controller. The peripherals are located on a "bus" and this bus

arrangement is called the Serial Peripheral System (SPS). Accordingly the motors and detectors are called SPS devices and each has an individual two-character address.

The layout of MicroLAB sub-modules is shown in the diagram below:
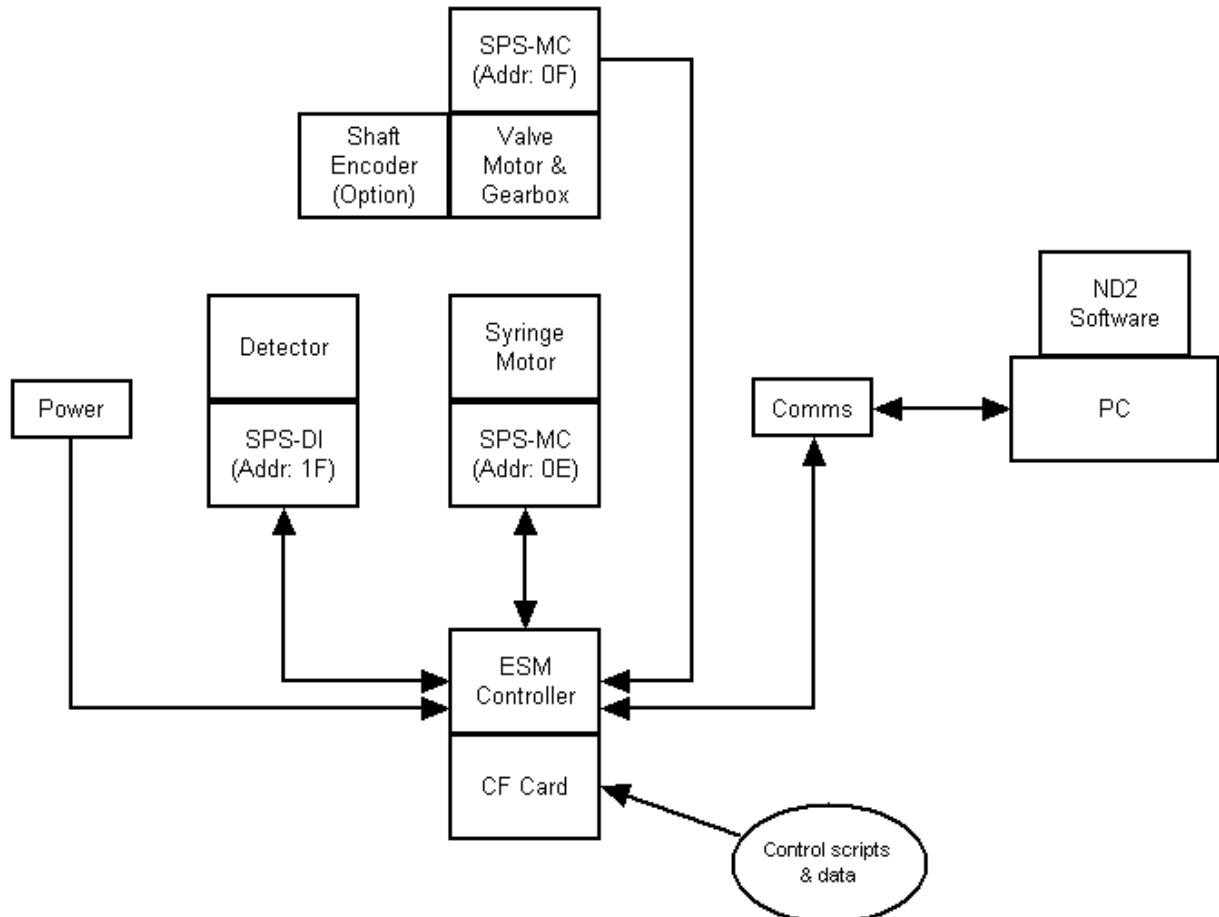
8.3   Hydraulic Configuration

MicroLAB hydraulic system has three major components and comprises a multi-port valve (normally with 8 ports), a syringe and a detector as shown in the block diagram below.

Each valve port can be uniquely selected by the valve motor controller and when the valve is "at" a particular port the valve nozzle is connected to the syringe chamber. For example if the valve is at the gray port, retracting the plunger will draw fluid into the syringe via that port. If the valve is then positioned at the blue port, inserting the plunger will inject the fluid into the detector. The gray port is normally designated the inlet and the blue port is connected to the detector. The other port connections are dependent on the chemistry regime used.
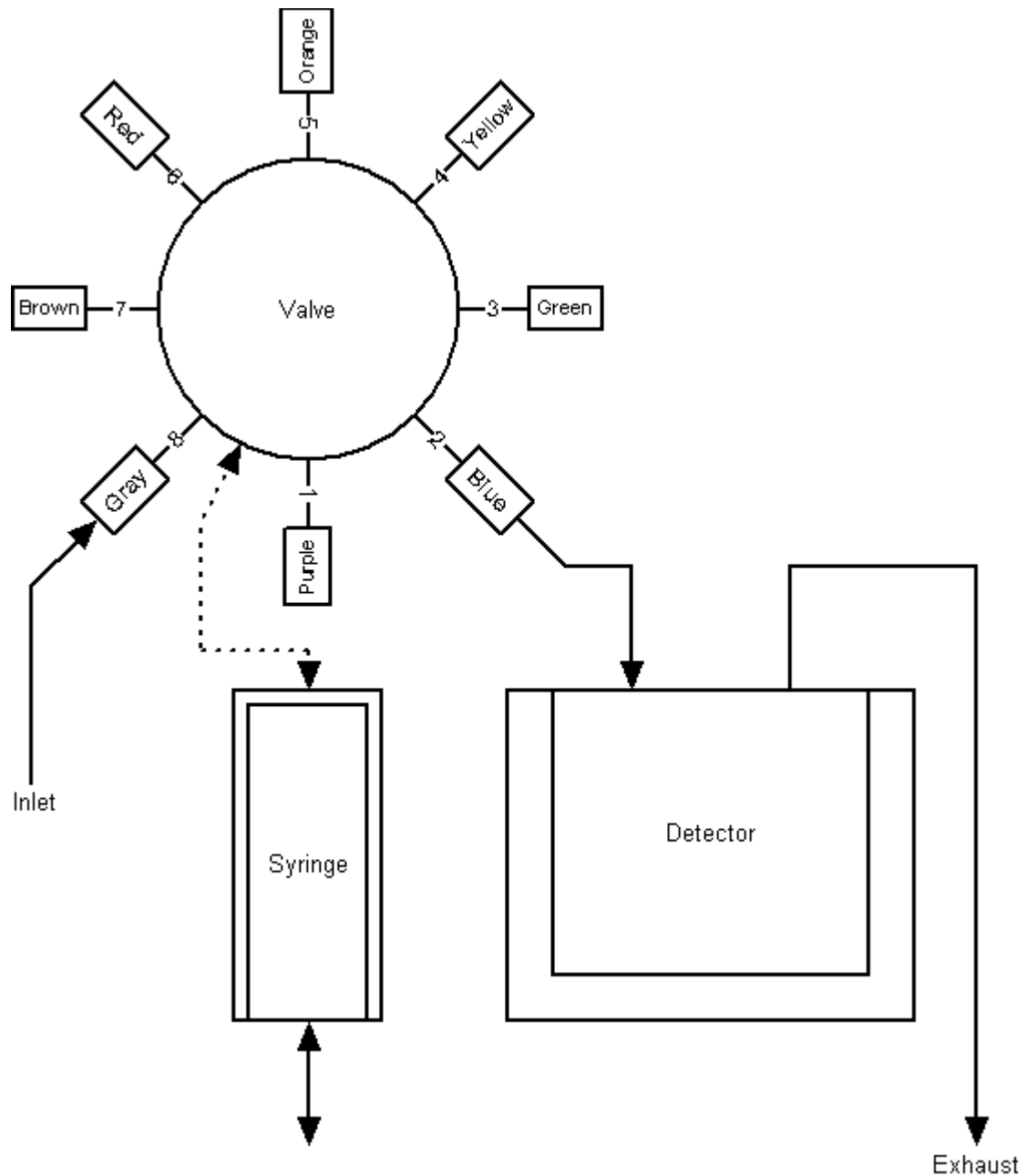
The valve is usually moved "to" a port. That is, you will either manually or programmatically move the valve to the gray or yellow (etc.) port. Unless you specify otherwise the valve SPS controller will select the shortest distance (to save time and power). Moving from the purple to the gray port is said to be moving 1 port clockwise. Then moving back to the blue port is 2 ports counter/anti-clockwise.

The valve has an alignment point that is near, but not exactly at, the gray port. This is an arbitrary reference point that provides the valve drive system with an absolute reference. The distance to the purple port is then calibrated and this is known as the valve offset. Aligning the valve only moves it to the valve reference point. It must then be moved to a port before fluid can be draw into or expelled from the syringe.

The syringe can be inserted or retracted a number of "steps". These are individual stepper motor steps and the full travel is approximately 4500 steps. This equates to approximately 3 ml volume in the syringe. More accurately:

$$1443 \text{ syringe steps} = 1.0 \text{ ml volume}$$

Motor speed may be programmed "globally" or programmatically within the scripts. Typically they are set via an initialization file known as the "init" file. See later sections for more details.

8.4   Colorimeter Design

The colorimeter design is shown in a block diagram below and in its most simple form consists of a capillary tube with an LED light source at one end and a photodiode detector at the other. The sample is injected into the pathlength between the light source (A) and the beam measurement detector (C). The intensity of the light source is monitored with a further detector (B).
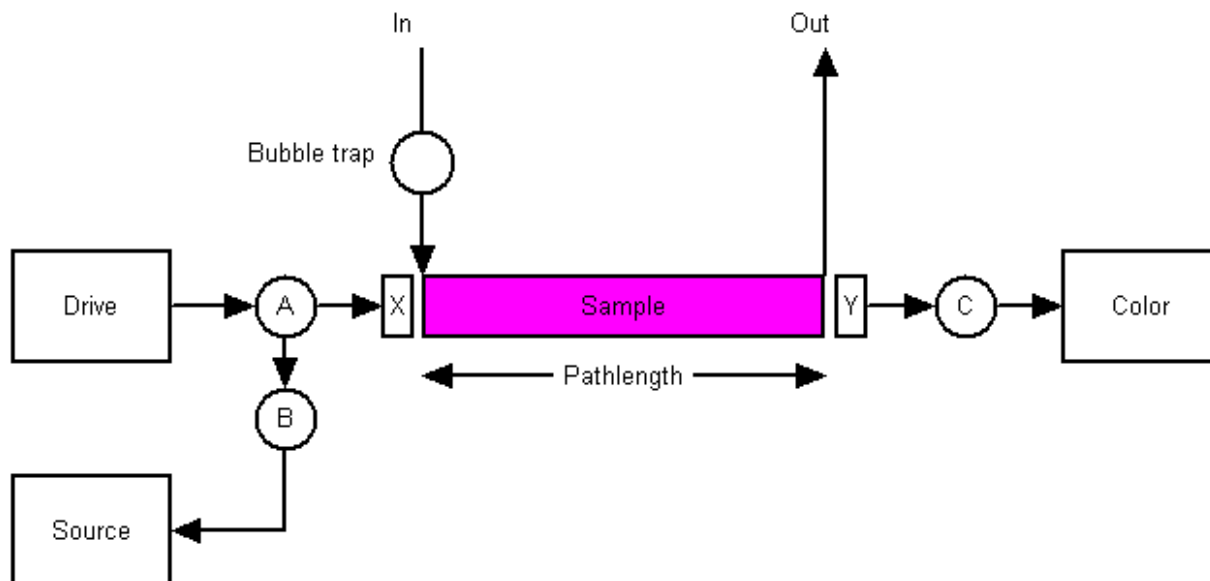
The light from (A) passes through a broadband interference filter (X) selected at the peak spectral response of the chemical method to be used. The beam then travels through the sample and when it emerges from the capillary it passes through a narrow-band interference filter (Y) also selected at the peak spectral response of the dye. Finally the light is incident on (C) the beam detector.

The light intensity is set accurately by the LED drive and a feedback mechanism that uses photodiode (B) to monitor the light launched into the sample. The light launched into the sample is know as the "source" value and the light out is called the "color" measurement. The beam intensity is normally set algorithmically using feedback or can be set directly using the digitally adjustable LED current.

The inlet has a bubble trap that is designed to prevent bubbles from building-up in the capillary. It is important that the inlet to the colorimeter is connected to the valve. Reverse connection will cause measurement noise. The inlet tube is indicated by a dot on the

detector cover.

Detectors with various pathlengths and wavelengths are available as standard and determine the analytical method and sensitivity. In accordance with the Beer-Lambert law shorter pathlengths have greater range and longer pathlengths have greater sensitivity.

Custom pathlength and wavelength detectors are available to special order.

8.5   Command Sequences

MicroLAB provides a flexible approach to configuration and control of the motors and detectors via the integrated scripting language and NutrientDATA 2 .

It is important to understand that MicroLAB operates by controlling the SPS and external devices and these are shown in the table below:

Device	Controls
Valve motor / gearbox	Rotate (clockwise & counter-clockwise), align valve
Syringe Motor	Insert and retract plunger
Detector	Set-light intensity, measure beam, heat, hold temperature.
Analog instrument	Temperature probe, chlorophyll fluorometer, etc.

To take a blank measurement MicroLAB would follow the command sequence below:

1. Align valve
2. Move valve to inlet
3. Retract plunger
4. Move valve to detector
5. Insert plunger
6. Set light
7. Measure beam

The following sections will describe these operations in more detail.

8.6   Script Basics

MicroLAB performs chemical routines by following scripts that are uploaded into memory. Your MicroLAB will be factory configured. Default or specially designed scripts (if requested) will be provided on your CD.

Normally you will simply run the default chemistry routines. However, if you wish to change the chemical routines you are able to design and upload your own scripts using the NutrientDATA 2 Script Designer.

The analysis scripts, such as the sample and standard are a sequence of commands that operate the valve, syringe and detector and ultimately perform the analysis routine.

The analysis cycle script defines the sequence of sample, standard and other analysis scripts.

The settings determine the master controls such as sample interval, deployment duration and detector light intensity.

The scripts hierarchy is shown in the block diagram below.
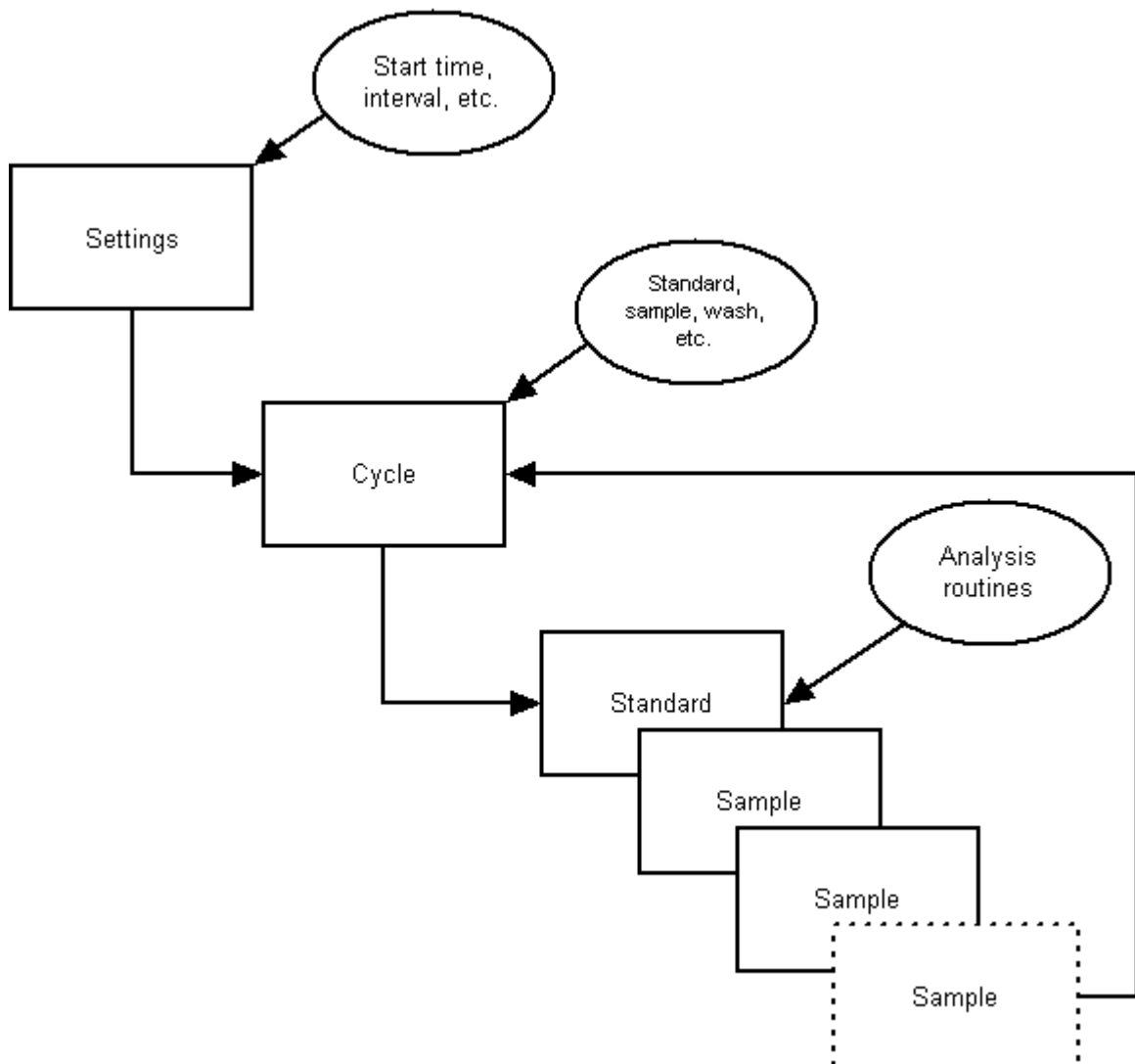
The simplest method of operation is to program the settings and use the default cycle and analysis routines.

Section 9

9 Software

9.1 Introduction

NutrientDATA 2 is an EnviroTech LLC software product that provides an easy to use interface to MicroLAB.

Features include:

- Manual controls
- Instrument configuration
- Deployment programming
- Analysis routine set-up
- Bench testing
- Data download
- Data processing & calibration

9.2 Control Panel

The NutrientDATA Control Panel is displayed after the splash screen and provides the following options:

- **Manual Control**
  Manual control of instrument functions (insert plunger, move valve, etc.)
- **Script Designer**
  Edit, upload and run scripts for automatic analysis
- **Data Processing**
  Download and process recorded data
- **Monitor Instrument**
  Check serial output from MicroLAB
- **Deploy Instrument**
  Start MicroLAB auto-sampling routine
- **Configuration**
  Configure communications, time & date and software licences
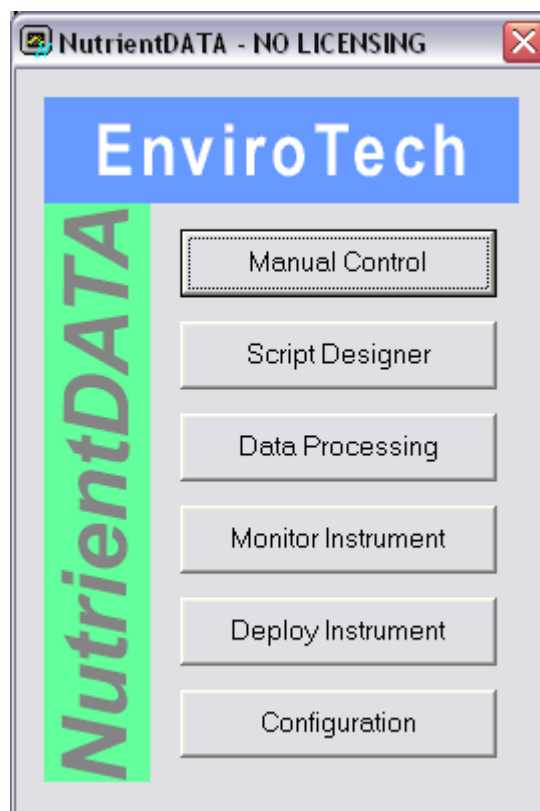
9.3 Manual Control

From the Control Panel select the Manual Control button.

You may manually control MicroLAB devices, such as the syringe motor, valve motor and detector from the Manual Control panel. This panel allows direct control of all MicroLAB functions.
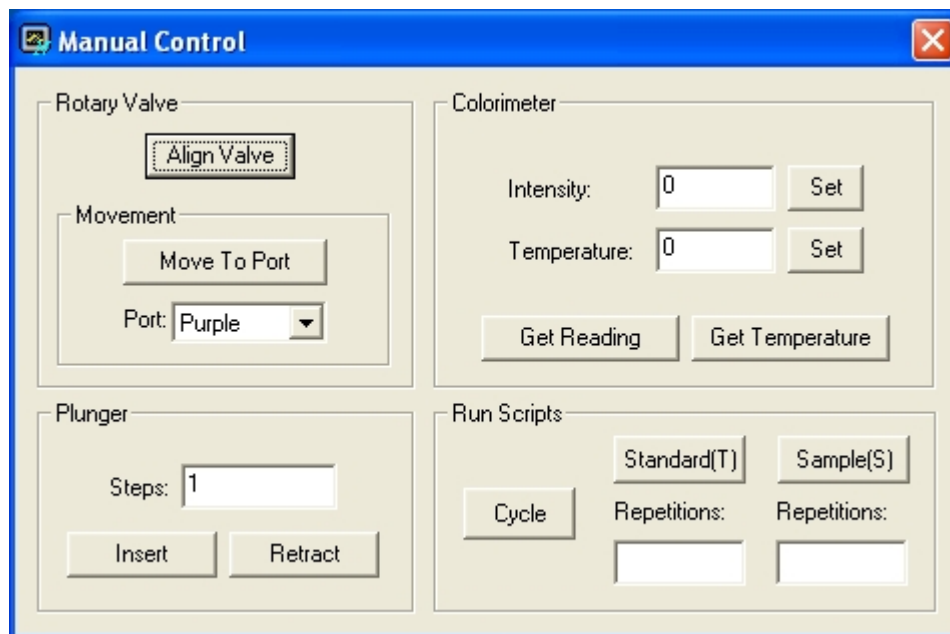
Valve rotor functions

Align - Aligns the valve rotor.

Move to port - Select a port color and click *Move to Port.* Moves rotor to a specified valve port.

Syringe plunger functions

Insert - Inserts the plunger. Enter the number of steps to move and click *Insert.* (1-1100)

Retract - Retracts the plunger. Enter the number of steps to move and click *Retract.* (1-1100)

Colorimeter functions

Get Reading - Obtains a source (supplied light intensity) and color (intensity of light transmitted through flow cell) reading from the colorimeter. (0-65535)

Get Temperature - Obtains a temperature reading from the colorimeter. (0-65535)

Set (Intensity) - Sets the source intensity of the colorimeter cell. Enter an intensity value into the *Intensity* box then click *Set.*

Set (Temperature) - Sets the heater temperature for the colorimeter cell. Enter a temperature value into the *Intensity* box then click *Set. Note: Only PO4 and NH4 employ a heater.*

Bench test routines

Sample (S) - This button and associated setting box allow the current sample routine to be run for a number or replicates. This is intended for bench testing purposes.

Standard (T) - This control operates in the same way as the same button described above.

Cycle - This button allows the user to run through the complete measurement cycle, which is also intended for bench testing.

9.4 Script Designer

A project contains all of the scripts and settings for a deployment. Projects can be loaded and saved from the File menu of the script designer. A project comprises the following components:

- **Project Description** - Description of the project
- **Deployment settings** - Collection of settable parameters.
- **Analysis Cycle** - Program cycle specifying the sequence in which analysis scripts are executed.
- **Standard (T) Script** - Script to analyze MicroLAB On-Board Standard.
- **Sample (S) Script** - Script to analyze an ambient water sample.
- **User scripts** - Any number of user defined scripts.

Projects are edited in the Main Window, which is split into two panes. The left pane shows the project component currently selected for editing. The selection can be changed by clicking one of the listed components. The right pane displays an editor for the selected component, which is one of the following:

Project Description Editor
Settings Dialog
Analysis Cycle Editor
Script Editor

9.4.1 Description Page

This is a simple text editor, allowing a text description of the project to be entered. (Tip: You can copy, cut and paste for your word processor in this panel)
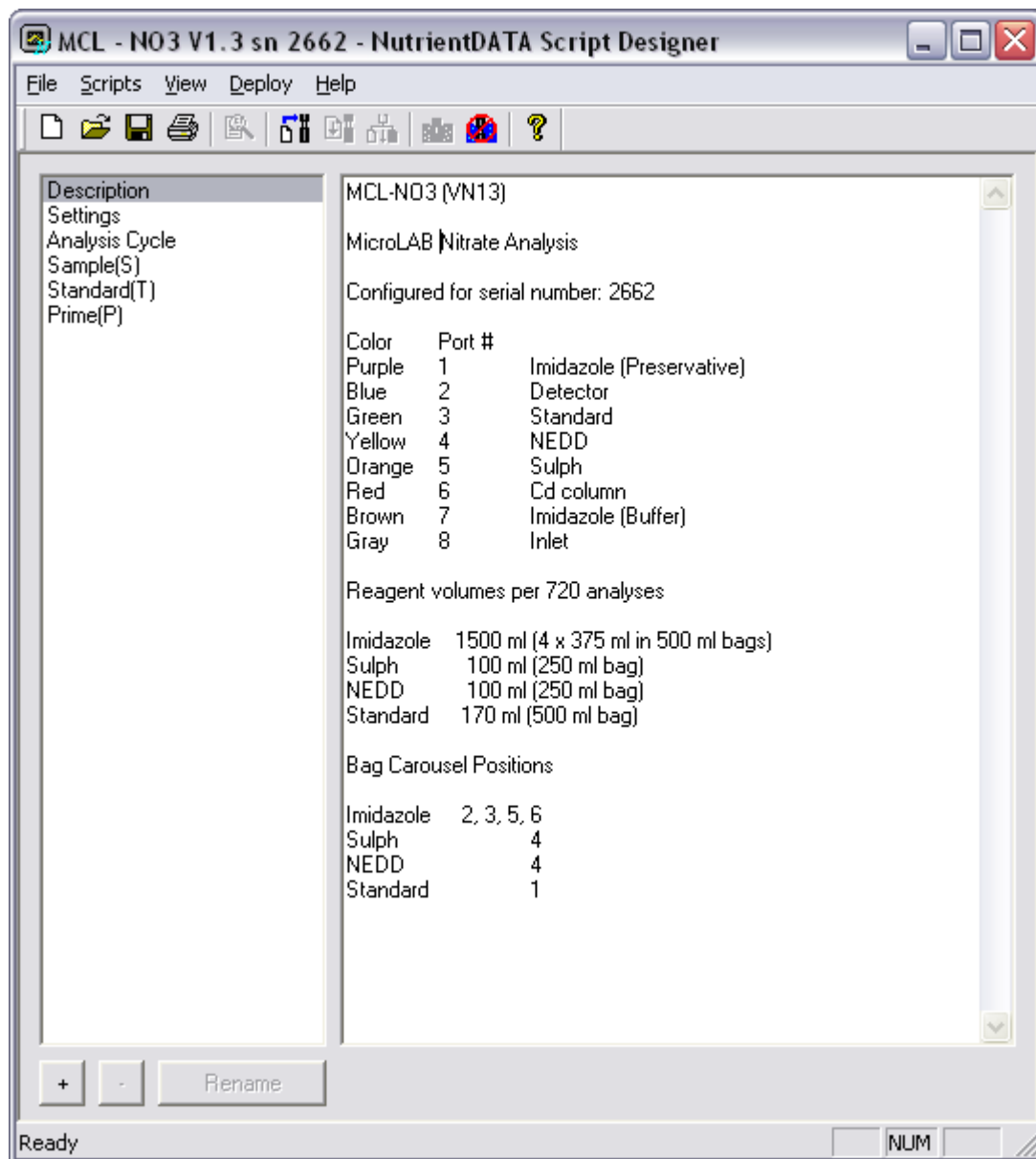
9.4.2   Settings

The deployment settings can be changed in this panel.

<u>Run Settings</u>

Start Date/Time - Following deployment, MicroLAB will wait until this real date/time before commencing the Analysis Cycle.

Active Interval - Interval between running scripts from the Analysis Cycle.

Number of Cycles - Number of "loops" of the complete cycle to perform. Typically one cycle is from standard to standard.

Analog Instrument Settings

Gain Select - Gain selection applied to external analogue instruments. (If fitted).

Burst Interval - Burst interval when sampling from external analogue instruments.

Colorimeter Settings

Intensity - Source intensity. (typ.20,000 – 35,000)

Intensity Tolerance - Allowed tolerance of intensity setting. (typ. 50)

Temperature - Cell temperature. (typ. 27000)

Temperature Tolerance - Allowed tolerance of temperature setting. (typ. 500)

Temperature Timeout - Minutes allowed to achieve temperature. (typ. 6)

Burst Interval - Burst interval when sampling from colorimeter. (typ. 1000)

9.4.3 Analysis Cycle

This panel allows editing of the Analysis Cycle sequence that determines when scripts are executed during a deployment cycle. The main window is a table showing the current sequence.

These buttons are shown at the bottom of the window:

Add - Adds a script to the end of the sequence. A dialog displays a choice of available scripts to add.

Insert - Inserts a script above the highlighted grid position. A dialog displays a choice of available scripts to insert.

Edit - Displays a dialog to edit the currently selected table line.

Cut - Cuts selected lines from the table.

Copy - Copies selected lines from the table.

Paste - Inserts lines from a previous cut or copy operation into the grid below the currently selected line.
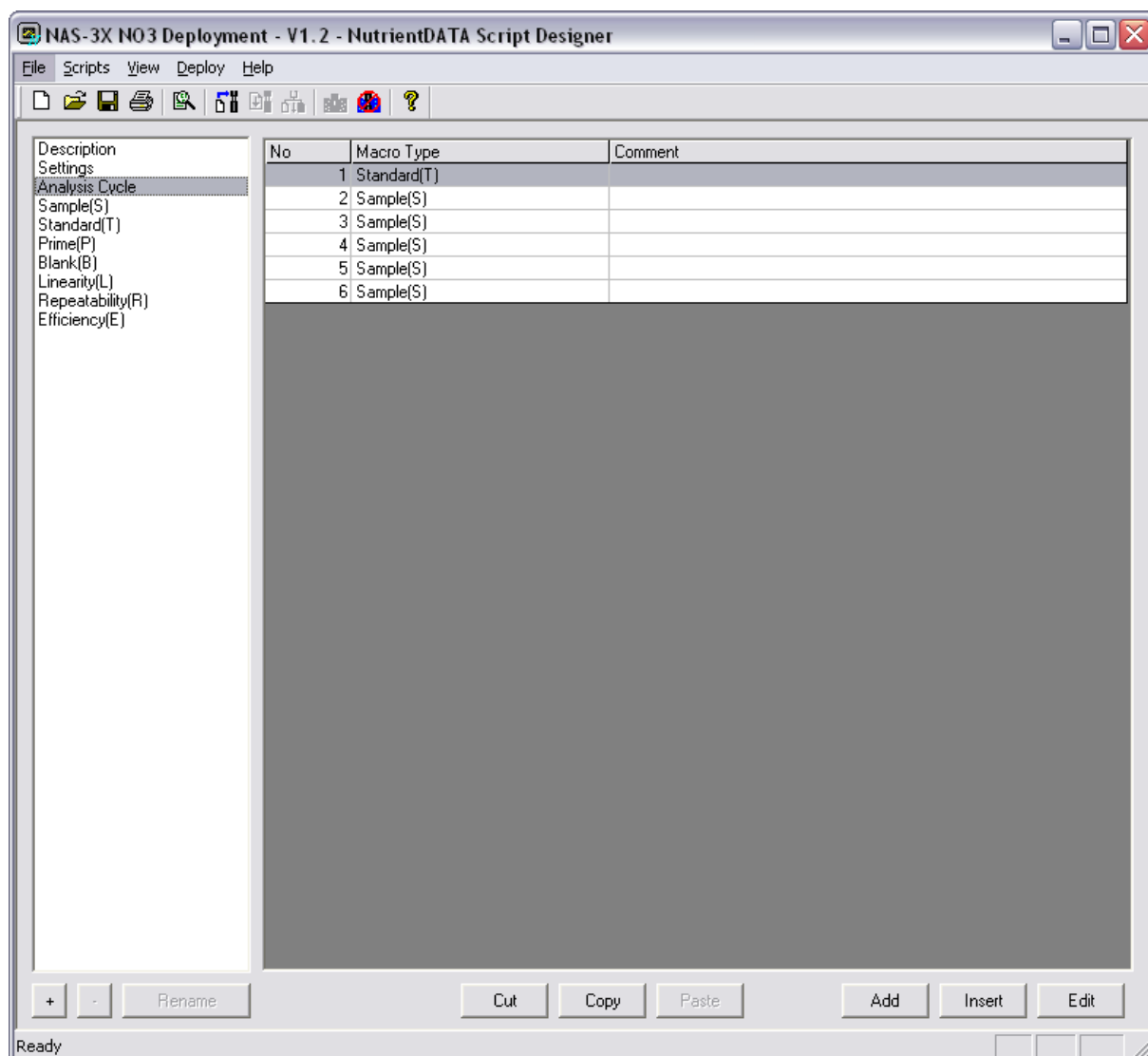
9.4.4   Script Editor

This screen allows the creation and editing of scripts using a simple graphical editor to sequence instrument operations.   A grid is displayed showing the current sequence of commands in the script.

No	Command	Port / Argument	Comment
1	Move Valve	Gray	Inlet
2	Retract Plunger	908	Volume 2.5 ml
3	Move Valve	Blue	Detector
4	Insert Plunger	908	Insert blank
5	Pause	2	Wait 2 seconds
6	Record Data Reading	5 (Blank)	Read blank
7	Retract Plunger	454	Get sample (1.25 ml)
8	Move Valve	Brown	Imidazole (Buffer)
9	Retract Plunger	454	
10	Move Valve	Red	Cd
11	Insert Plunger	200	Mix sample & imidazole
12	Pause	1	
13	Retract Plunger	200	
14	Pause	1	
15	Insert Plunger	200	
16	Pause	1	
17	Retract Plunger	200	
18	Pause	1	
19	Insert Plunger	908	
20	Pause	4	
21	Retract Plunger	200	Reduce NO3 >> NO2
22	Pause	1	Wait 1 second
23	Insert Plunger	200	
24	Pause	4	Wait 4 seconds
25	Retract Plunger	200	Reduce NO3 >> NO2
26	Pause	1	Wait 1 second
27	Insert Plunger	200	
28	Pause	4	Wait 4 seconds
29	Retract Plunger	200	Reduce NO3 >> NO2
30	Pause	1	Wait 1 second
31	Insert Plunger	200	
32	Pause	4	Wait 4 seconds
33	Retract Plunger	200	Reduce NO3 >> NO2

9.4.4.1   Scripts

The Script Editor will be displayed whenever a script is selected in the left window pane. The
script may be the Standard, Sample or a user defined script.

The buttons below the left window pane allow scripts be added, removed and renamed:

'+' - Adds a new user defined script to the project. A dialog is displayed where a
script name may be entered.

'-' - Removes the user defined script currently selected in the left window pane.

Rename - Renames the selected user defined script.

9.4.4.2   Editing

The following buttons are shown at the bottom of the right window pane:

**Add** - Adds a command to the end of the sequence. A dialog displays a choice of available commands to add.

Insert - Inserts a command above the highlighted table position. A dialog displays a choice of available commands to insert.

Edit - Displays a dialog to edit the currently selected command.

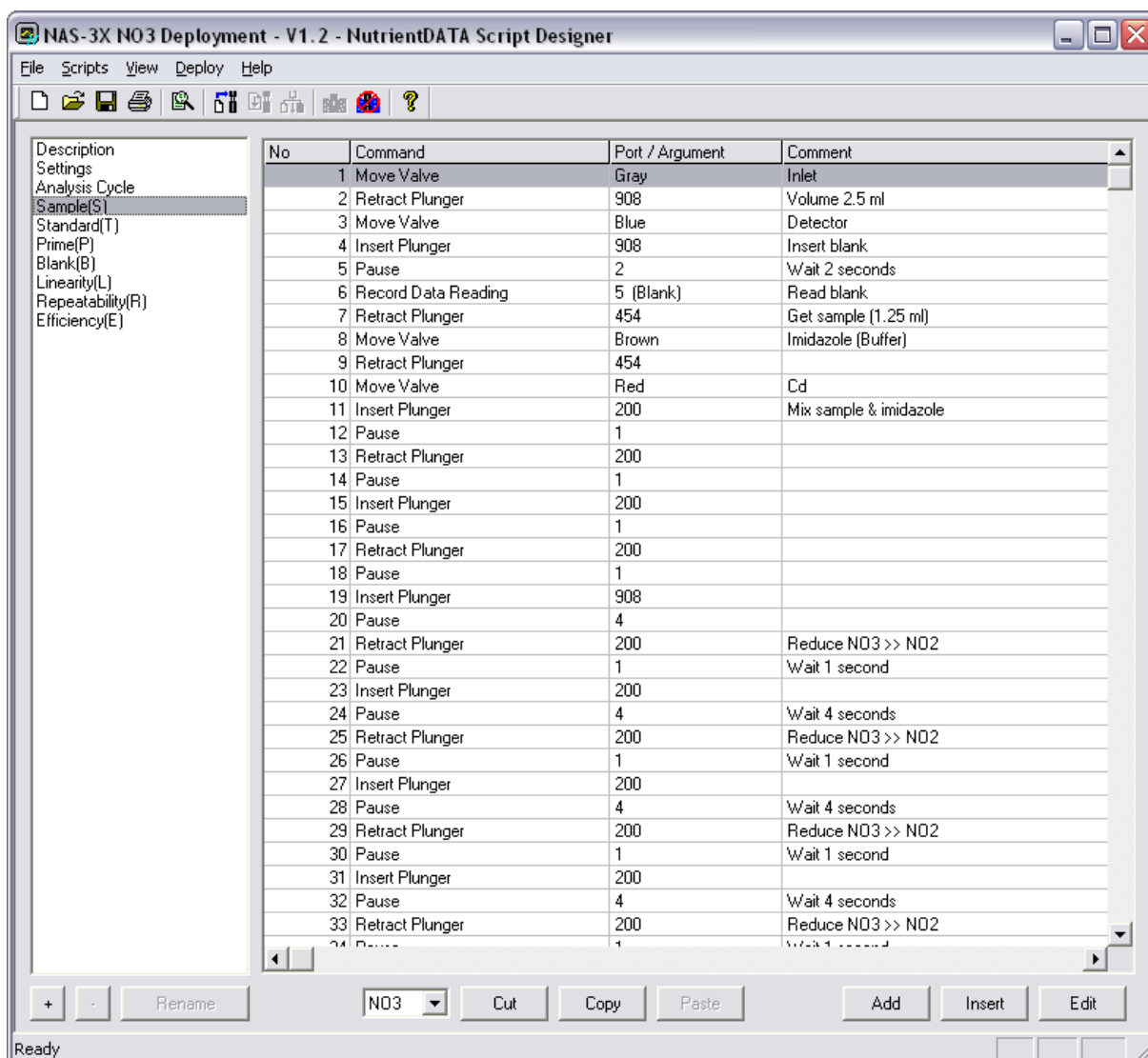**Cut** - Cuts selected commands from the table.

Copy - Copies selected commands from the table.

Paste - Inserts commands from a previous cut or copy operation into the table below the currently selected line.

9.4.4.3   Commands

Within the script editor scripting commands are to control the valve, syringe, heater, detector and external sensors.

<u>**Valve Control**</u>

Align Rotary Valve
Aligns the rotary valve to its home position. This should be done periodically to keep the rotary valve accurate.

Move Valve
Moves the rotary valve shortest distance to specified port. (color)
Port - Specifies the port.

Move Valve Counter-clockwise
Moves the rotary valve to specified port in the counter-clockwise direction.
Port - Specifies the port.

Move Valve Clockwise
Moves the rotary valve to the specified port in the clockwise direction.
Port - Specifies the port.

<u>**Syringe Controls**</u>

Insert Plunger
Inserts the plunger by a number of steps.
Argument - Number of steps.

Retract Plunger

Retracts the plunger by a number of steps.
Argument - Number of steps.

Record Data

Record Data Reading
Analyzes the light transmittance of the colorimeter flow cell and stores the data. The colorimeter is set to the intensity parameters specified in the Project Settings. The *Type* field for this command depends on whether the command appears in a Standard/Sample script or a user defined script:

Argument - The number of readings to take from the colorimeter.
Type (Standard/Sample) - Specifies whether this sample is a Blank or a Reaction sample.
Type (User Defined) - A text label which will be used to identify the data in processed data files.

Record Analogue Instrument
Takes a sample from an external analogue instrument and logs the data.
Argument - Number of readings to take.
Type - A text label which will be used to identify the data in processed data files.

Heater Controls

Heat Colorimeter
Heats the colorimeter to the temperature set in Project Settings. This command completes when the temperature has been reached or the temperature timeout has expired. The colorimeter will continue to be held at temperature until a Stop Heating Colorimeter command is issued.

Stop Heating Colorimeter
Turns the colorimeter heater off.

Pauses

Pause
Creates a delay in the script execution.
Argument - Number of seconds to delay.

9.5   Script Projects

Script projects are a set of Settings, Cycle and Analysis routines. See the Script Basics section for a description of their hierarchy.

Each project is uploaded as a complete set. That is the settings, cycle and analysis routines (sample & standard) are all uploaded together each time.

As a default each project will contain a Sample and Standard script. Further analysis routines may be added to the project as required. For example, these may be a second standard or a second diluted sample analysis.

The project can contain scripts that are not included in the main cycle script. Typically these are flushing, priming, cleaning and specific test scripts (e.g. cadmium reduction efficiency, reagent blank, etc.). The project may contain any number of such scripts.

It is recommended that you save a set of scripts for each of your instruments (e.g. MicroLAB NO3 sn 1234.ndp). Alternatively you may simply enter the unique configuration information into the Settings boxes before uploading. Typically this will be the light intensity setting and possibly the heater setting.

The unique settings for your instrument are supplied on the Equipment Configuration Record (ECR) supplied with MicroLAB. The ECR may be updated when the instrument is factory serviced. Please check for changes after the unit is returned to you.

9.5.1   Testing Projects

To test a project, it must first be uploaded to MicroLAB by clicking the *Scripts->Upload Current Project* menu item. This will transfer all of the scripts and settings to MicroLAB. Once uploaded, the project can be tested.

An individual script may be tested by selecting it in the left window pane, then clicking the *Scripts->Test Script* menu item. This will display the *Run Script* dialog, which allows the number of test runs to be entered.

An entire project may be tested by clicking the *Scripts->Test Current Project* menu item. This will follow the Analysis Cycle.

When scripts are running, an output window is displayed showing the script progress. Closing the window will terminate execution of the script.

After testing your project you may wish to download, inspect & calibrate the data. Instructions are given in the   Data Control section.

9.5.2   Deploying Projects

MicroLAB is prepared for deployment by uploading a project (*Scripts->Upload Current Project*) then placing the instrument into deploy mode. When in deploy mode, MicroLAB will automatically run the uploaded project 20 seconds after a power-on or a reset.

To set deploy mode, click *Deploy->Set Deploy Mode*. The instrument must then be switched off within 20 seconds, to prevent it from running the project. Power on again to automatically deploy the instrument.

MicroLAB may be brought out of deploy mode by clicking *Deploy->Unset Deploy Mode*.

9.6   Data Control

The following options are available from the Data Control panel:

Extract – Downloads logged data from instrument. Enter a file path into the box or use the *Browse* button to select a file location for the extracted data, then click *Extract*.

Process - Processes an extracted data file (created from the above option) through the Data Processor Dialog

Clear - Clears logged data residing on instrument.

This controls processing of extracted MicroLAB data files. The nutrient concentration is calculated and data is formatted into a Comma Separated Values (.csv) file, for easy import into other applications such as spreadsheets.
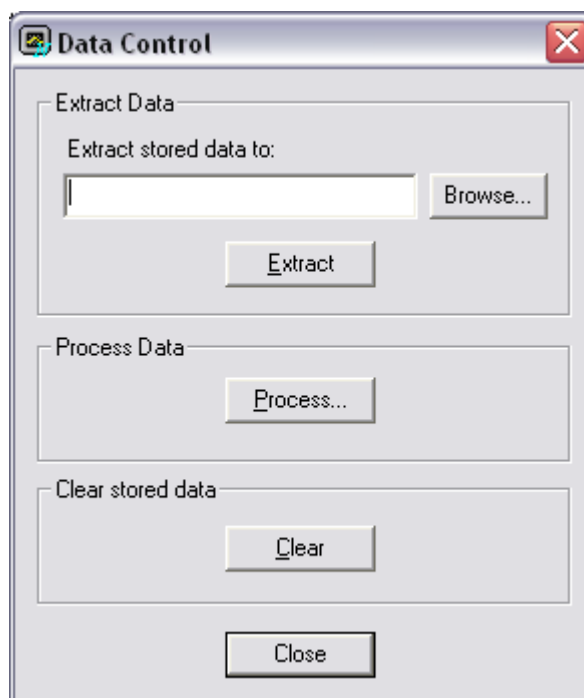
9.6.1   Extracting Data

To extract data click on the browse button and then navigate to the folder on your hard drive where you wish to store the data. Enter the file name and click the Extract button. All data in memory will be downloaded to your hard drive and the designated file.

If you have not cleared the memory since the last data extraction then all historic data plus the recently recorded data will be downloaded. i.e. Data is always appended to the main data file and does not over-write old data after downloading.

9.6.2   Processing Data

To process a data file:

1. Enter the file path of an existing (downloaded) MicroLAB data file into the *Source File* box, or use the *Browse* button to select a file.
2. Choose a filename for the processed data file in the *Destination File* box or use the *Browse* button.
3. Enter the concentration of the On Board Standard that was used by MicroLAB to create the source file.
4. Click *Process*
5. After the processing is complete a box is displayed and the CSV file will have been created in the specified location.

9.6.3 Clear Stored Data

Pressing the Clear Stored Data button will clear MicroLAB data memory. The scripts and settings are not effected. A confirmation box is displayed - select an option as appropriate. Cleared data cannot subsequently be recovered.

9.7 Monitor Instrument

The monitor window launches a screen that allows you to monitor the output from MicroLAB. This can be used to check that the instrument is running and producing data as expected. You will most commonly use it after setting an instrument into deploy mode.

The monitor is a blind terminal screen and just displays the current output of MicroLAB to the screen and is not interactive. It does not interfere at all with the operation of the instrument.

9.8 Deploy Instrument

The Deploy button launches MicroLAB main cycle in accordance with the uploaded settings.

This button start the main cycle but does NOT stop it when you close the panel, it simply reverts to monitor mode. This means that you can hit DEPLOY, see via the window that the instrument is running correctly, and then close NutrientDATA without disturbing the main cycle.

9.9 Configuration Panel

The configuration panel controls the basic set-up of the NutrientDATA 2 software.
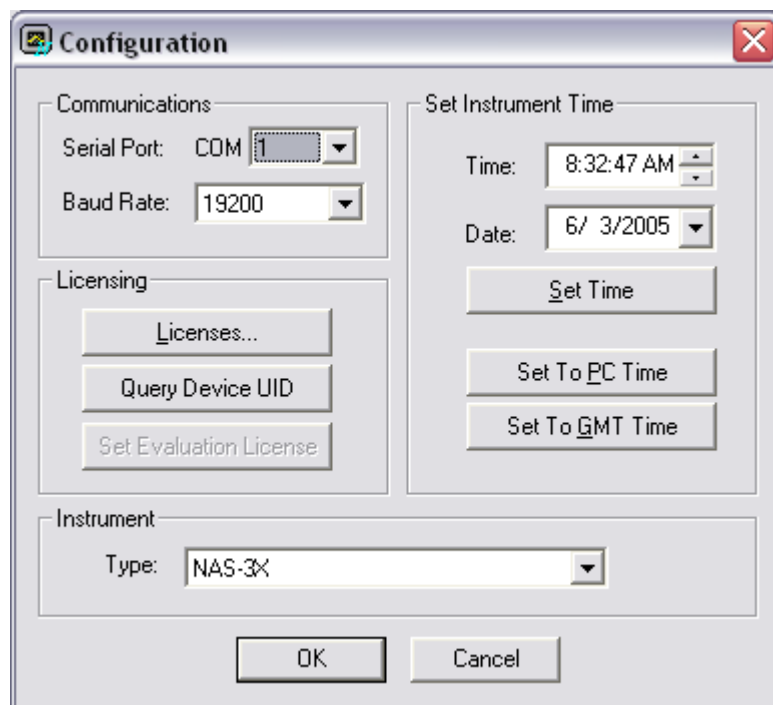
9.9.1   Instrument Type

NutrientDATA 2 works with all our single channel analyzers including AutoLAB, MicroLAB and MicroLAB. Select the appropriate instrument from the drop down box.

9.9.2   Instrument Time

Set Time - Sets the instrument clock using the *Time* and *Date* boxes

Set to PC Time - Sets the instrument clock to the current date and time of the PC

Set to GMT Time - Sets the instrument clock to GMT

9.9.3   Licensing

In order to use this software with an instrument, a license must be obtained from EnviroTech LLC for each instrument. A license entitles the use of this software with a single instrument, designated by its UID. An instrument's UID is a factory set - see instrument documentation for details.

The software will only communicate with instruments that it holds licenses for. Use the License Manager Dialog to add licenses to the software. There is no restriction on the number of licenses that may be held by the software at one time.

The License Manager allows the addition and removal of instrument licenses and displays information about licenses held. Fully licensed instruments are shown in the *Licenses Held* window.

9.9.3.1   Portable Licence Files

To eliminate the need for re-entering license keys into multiple applications, licenses can be imported/exported through Instrument License Files.

To create a license file, click *Save To File*, then select a filename. This will export all full licenses held by the software to a license file. This may then be used by another application to import all of the licenses in the file without the need to manually enter each one.

To add licenses from a file, click the *Add From File* button, then select the filename of the license file to add from.

9.9.3.2   Removing Licences

To remove an instrument license, select the instrument in the *Licenses Held* window and click the *Remove License* button.

9.9.3.3   Evaluation Mode

A 30 day evaluation period for this software exists from the time of installation. During this period, a single instrument may be used with the software without the need to obtain a full license.

Before the Evaluation License feature can be used, it must be set to an individual instrument. Note that this process "locks" the Evaluation License to that instrument and cannot subsequently be changed.

Evaluation License

The current status of the evaluation license is displayed in the bottom of the License Manager dialog:

> **Instrument UID** - Displays the instrument to which the Evaluation License is locked.
> **Days Remaining** - Displays the number of days remaining before the Evaluation License expires.

The evaluation count down clock only relates to the evaluation license which is still usable until the time expires. For example, if you have a valid license for one instrument and the evaluation license for another, you are still able to use the second instrument until the eval license expires.

If you have an evaluation license for a particular instrument and a full license is obtained for that instrument, you are automatically upgraded to a full license from the moment the software key is entered. The evaluation license will still count down but it is not active while there is a full license for the same instrument. If the license is removed, the eval license will become active again.

9.9.3.4 Adding Licences

When a license is issued, it is accompanied by a License Key. Enter this into the *License Key* box on the dialog. Then enter the UID of the instrument into the *Instrument UID* box. Click the *Add* button.

Licenses may also be added through Instrument License Files (see below).

9.9.3.5 Licence Controls

Licences - Manages instrument licenses. Displays the License Manager Dialog.

Get device Unique Identifier (UID) - Displays the UID of the connected instrument.

Set Evaluation License - Sets the Evaluation License to the currently connected instrument. This must be done in order to use the Evaluation License. Once the Evaluation License is set, this button will be disabled.

9.9.4 Communications

Serial Port and **Baud Rate** configure the NutrientDATA serial port connection to the instrument. The default band rate is 19200.

Section 10

10   Chemistry

10.1   Preparation

Prior to performing analyses the user must prepare and install the various chemical reagents and the on-board standard. One or more standards of known concentrations are required for testing. For the nitrate chemistry the cadmium column must be activated and attached to MicroLAB.

This section describes how to prepare and install the reagents, standards and cadmium column (nitrate only).

The volumes given in the reagent preparation instructions are nominal and may change depending on your deployment length and sampling interval.

10.2   Chemistry Scripts

Complete standard chemistry script projects are provided either on the CD supplied with your MicroLAB or via email from EnviroTech LLC.

Scripts are currently available for:

- Nitrate / Nitrite
- Phosphate
- Silicate
- Ammonia

Please read the description page of any new script project that you receive as the latest changes and bulletins will be recorded in this area. The script project is provided as a small file with an *.ndp file extension (nutrient data project). A printout of the default scripts for your instrument are provided.

Also ensure that you have the light intensity and temperature (where applicable) settings for each instrument that you intend to use. This information is provided on the Equipment Configuration Record (ECR) supplied with MicroLAB. Copies may be requested from our technical support team.

10.3   Chemical Methods

10.3.1   Ammonia

Method

MicroLAB implements an adaptation of Kérouel and Aminot (1999) technique.

Reagents & solutions

The following list of reagents are used in this method:

Description	Supplier	Part Number

Disodium tetraborate	Merck/BDH	102674E
Isopropyl alcohol	Aldrich	44,342-5
OPA	Sigma Aldrich	P-1378
Sodium dodecyl sulphate (SDS)	Merck/BDH	442152V
Sodium sulfite	Sigma Aldrich	431621

10.3.1.1 Reagents

OPA Solution

Add 2 g of OPA o-phthaldialdehyde to 50 ml of ethanol. Use a high grade ethanol because impurities in ethanol can auto-fluoresce.

Sodium Sulfite Solution

Dissolve 1.6 g of sodium sulfite in 200 ml of de-ionized water.

Borate Buffer Solution

Dissolve 15 g of disodium tetraborate in 500 ml of de-ionized water. Stir or shake vigorously to dissolve.

Ammonia Reagent

Mix the following reagents:

- 500 ml of Borate buffer solution
- 1 ml of Sodium sulfite solution
- 10 ml of OPA solution

The reagent needs to be mixed at least 1 hour before use to allow sulfite to react with the OPA.

OPA and the ammonium reagent are light sensitive. It should be stored in the dark and should be protected from light as much as possible when in use.

10.3.1.2 Standards

Ammonia Stock Solution

Dissolve 5.35 g of ammonium chloride and transfer quantitatively to a one liter volumetric flask. Dissolve and make up to volume with distilled water. This solution contains 100 mMol/ml of NH_4Cl.

10.3.2 Nitrate

Method

EPA Method 353.4 / 4500-NO3

Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

Jia-Zhong Zhang, Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel

School of Marine and Atmospheric Science/AOML, NOAA, University of Miami, Miami, FL 33149

Peter B. Ortner and Charles J. Fischer, Ocean Chemistry Division, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, Miami, FL 33149

Reagents & solutions

The following list of reagents are used in this method:

Description	Supplier	Part Number
Ammonium chloride	Merck/BDH	100173D**
BRIJ 35	Merck/BDH	224175X
Copper sulphate	Merck/BDH	103733C
Hydrochloric acid	Merck/BDH	101254H
Imidazole	VWR	EM-5720
NEDD	Merck/BDH	104063X
Potassium nitrate	Merck/BDH	102143F
Sulfanilamide	Merck/BDH	104482G
Sulphuric acid	Merck/BDH	102765G

** Alternative for some methods

10.3.2.1 Reagents

Sulphanilamide

Dissolve 10 g of high purity sulfanilamide in 200 ml of 50% hydrochloric acid and dilute to one liter with de-ionized water. Add 2.0ml BRIJ (30%) solution.

NEDD

Dissolve 1.0 g N-1-naphthyl-ethylene diamine dihydrochloride in one liter of de-ionized water.

Imidazole

Dissolve 30.0 g imidazole in 3.0 liters of deionized water. After complete dissolution add 250 μl of 2% copper sulphate. While stirring, add drops of concentrated hydrochloric acid until the pH falls to between 7.80 and 7.85.

Hydrochloric Acid

Hydrochloric acid 50%
Add 500 ml of conc. hydrochloric acid to 500 ml of deionized water.

Hydrochloric acid 10%
Add 100 ml of hydrochloric acid to 900 ml of deionized water.

Copper Sulphate

Dissolve 2 g of copper sulphate in 100 ml of deionized water.

10.3.2.2 Standards

<u>Stock Solution</u>

<u>Nitrate</u>

Accurately weigh 1.02 g of potassium <u>nitrate</u> and transfer to a one liter volumetric flask. Dissolve and make up to volume with distilled water. This solution contains 10 mMol <u>NO3</u>-N/ml or 140 mg/l

1.0ml of this solution in 1.0 liter of deionized (DIW) or low nutrient seawater (LNS) will make a 10.0 μMol/l or 0.140 mg/l standard.

<u>Nitrite</u>

Accurately weigh 1.02 g of potassium <u>nitrite</u> and transfer to a one liter volumetric flask. Dissolve and make up to volume with distilled water. This solution contains 10 mMol <u>NO2</u>-N/ml or 140 mg/l.

1.0ml of this solution in 1.0 liter of deionized (DIW) or low nutrient seawater (LNS) will make a 10.0 μMol/l or 0.140 mg/l standard.

<u>On-board Standard</u>

The nitrate concentration of the on-board standard should be set at the mean of the expected range of values. It is usually a solution of deionized water (DIW) or low nutrient seawater (LNS) spiked to the average nitrate level expected at the area of interest.

<u>Test Standards</u>

Nitrate and nitrite test standards are prepared from deionized water or blank seawater by spiking with the stock potassium nitrate/nitrite solution.

1.0ml of this solution in 1.0 liter of deionized (DIW) or low nutrient seawater (LNS) will make a 10.0 μMol/l standard.
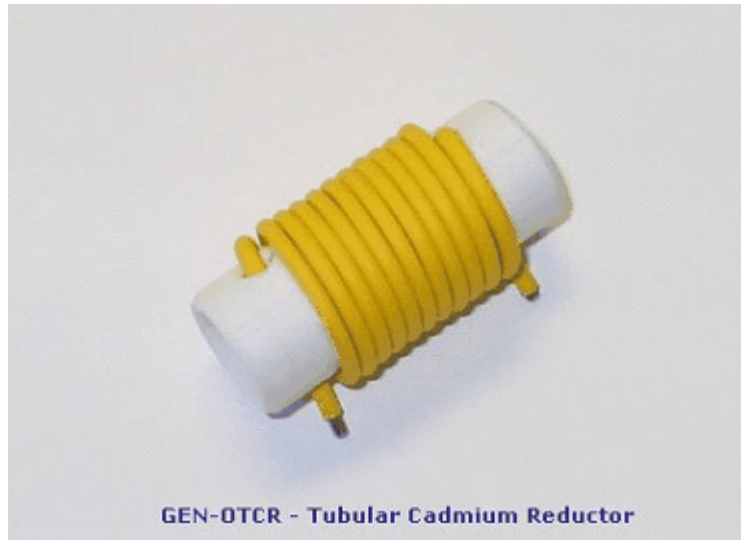
10.3.2.3 Cadmium Column

Copperized cadmium is used as a reductor for the nitrate routines. The subject of the reaction is nitrite (NO2) reduced from (NO3).

Background

The cadmium column described in this section has been introduced following a program extensive laboratory and field testing. It replaces the previous wire-spiral design. The new design is made of 36" (915 mm) narrow bore (1.3 mm) cadmium tube. The cadmium is wound in a tight spiral around a plastic former. This forces water moving through the column to take a tortuous path ensuring it comes into contact with the greatest possible area of cadmium. The characteristics of the new column are:

- Higher and more stable reduction efficiencies
- Unaffected by long periods of dormancy (if stored correctly)
- Long life with many deployments per column

- Can be easily regenerated (if stored correctly)
- Resilient to clogging and fouling

GEN-OTCR - Tubular Cadmium Reductor

Activation

To activate the cadmium column (OTCR) follow the steps below.

IMPORTANT: This procedure should be carried-out in a fume hood wearing protective gloves and safety glasses.

Column Activation Set-up

Equipment

32 cm of 2mm ID tubing
1 x Luer lock
4 x clean 60ml syringes
1 x tube pinch clip

Reagent list

- 250 ml of deionized water
- 10 ml of 2.5M nitric acid
    Measure 8.15 ml of conc. nitric acid (e.g. VWR# VW4815-6) and dilute up to 50 ml with deionized water.
- 30 ml of conc. hydrochloric acid
- 150 ml of imidazole buffer (see nitrate reagents)
- 20 ml of 2% w/v copper sulphate (see nitrate reagents)
- 40 ml of copper activation solution
    Add 20ml of 2% w/v copper sulphate to 20ml of imidazole buffer in a small beaker and mix.
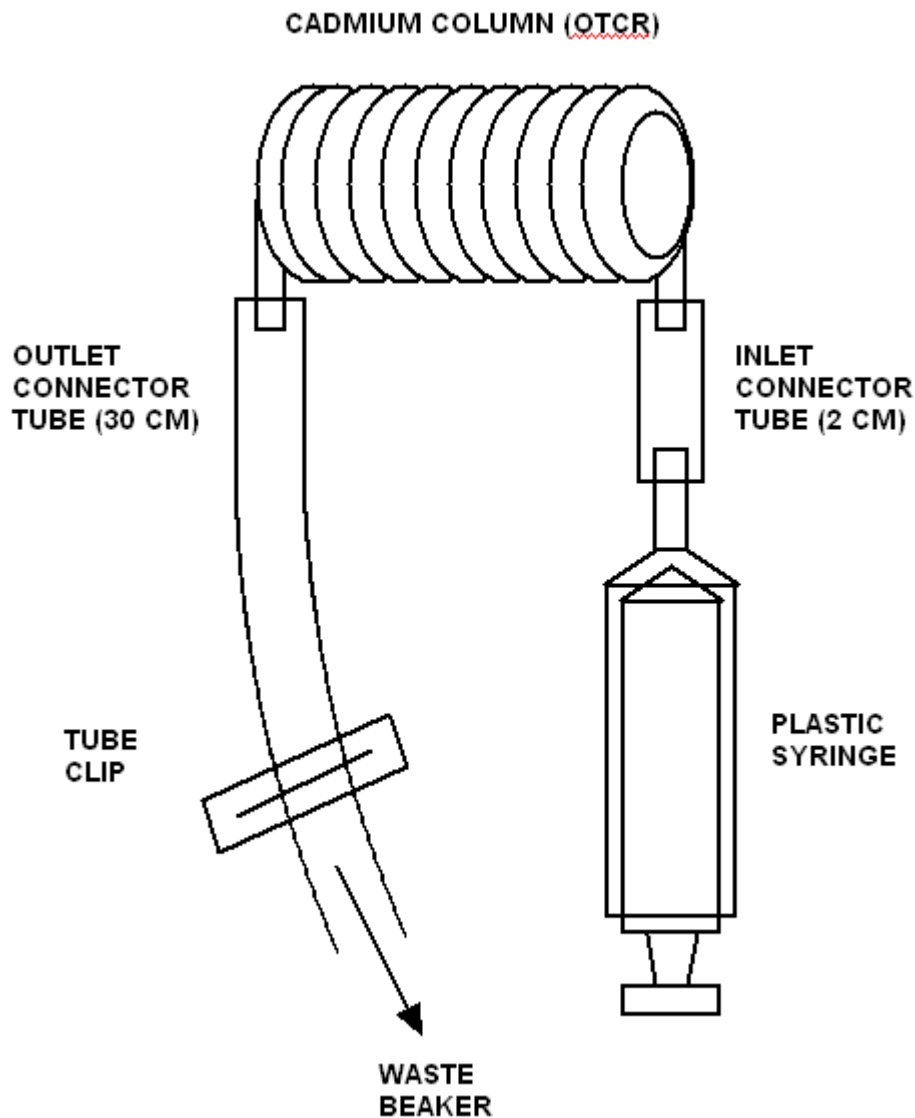
- 50 ml of nitrate conditioning solution

  The conditioning solution is 200 μM or 2.80 mg/l NO_3 diluted 50% by imidazole buffer. To make this solution add 0.5 ml of <u>stock nitrate solution</u> (see nitrate standards) in 25 ml of deionized (DIW) or low nutrient seawater (LNS) to make a 200 μMol/l or 2.80 mg/l solution. Now dilute to 50 ml with imidazole buffer (not the copper activation solution).

<u>Connections</u>

1. Attach a pinch clip to a new and unused 30 cm length of 2 mm internal diameter (ID) tubing.
2. Attach the tubing with the pinch clip to one end of the column (designated the outlet from here on).
3. Attach a Leur adaptor to one end of a 2 cm length of 2mm ID tubing.
4. Attach the other end of the 2 cm length of tubing to the vacant inlet end of the column.

The Leur adaptor will be used to safely connect and disconnect the syringe when it needs to be emptied. The pinch clip will be used to seal the tube so that liquid does not drain out of the column as the syringe is being connected/disconnected.

CADMIUM COLUMN (OTCR)

OUTLET CONNECTOR TUBE (30 CM)

INLET CONNECTOR TUBE (2 CM)

TUBE CLIP

PLASTIC SYRINGE

WASTE BEAKER

Activation Procedure

1. Using syringe #1 push 10 ml of 2.5M nitric acid through the column followed immediately by 50ml of DIW. Note: Do not be alarmed if a yellowish haze appears from the column when washed with the nitric acid.
2. Using syringe #2 push 10ml of conc. hydrochloric acid followed immediately by 20ml of DIW and repeat this step twice more. Note: Hydrochloric acid may cause pitting of the cadmium reactor interior surface if left in the column for longer than a few seconds.
3. Using syringe #3 flush the column with 50ml of imidazole buffer. Note: From this point forward air should not be allowed to contact the inside of the column.
4. Using syringe #4 push 5ml of the copper activation solution into the column and let it sit for 5 minutes. Repeat this step twice more.
5. Using syringe #3 flush the column with 25ml of imidazole buffer. If copper crumbs are flushed out during the imidazole buffer rinse continue to flush until no more appear.
6. Empty syringe #3 and using it to condition the column by pushing through 50 ml of nitrate conditioning solution.

7. Continuing with syringe #3 perform a final flush with 25ml of pure imidazole buffer
8. The column is ready for use and should be stored filled with imidazole and protected from air (e.g. submerged in a good quality glass jar filled with imidazole).

Once activated it is very important that only seawater or imidazole buffer comes into contact with the cadmium column, which is sensitive to salinity and pH.

The column should be stored in imidazole solution until required. After storage it should be vigorously rinsed with the storage medium to dislodge any loose copper particles before use. It is highly recommended that the column is reactivated after storage.

For reactivation of the column after deployments, follow the above procedure, but eliminate the nitric acid wash and substitute 50% hydrochloric acid for concentrated.

For long periods of storage, the column should be kept either dry or under nitrogen. A wash with DIW is advisable prior to prolonged storage. Each column, if properly handled, can be redeployed several times.

<u>**Attachment**</u>

The Tubular Column attaches to the appropriate valve port as per the <u>valve allocation table</u>. A short length of 2 mm inside diameter (ID) Tygon tubing is used to connect the column to the valve adapter nozzle. The tube should be pushed firmly over the end of the column and the nozzle with as little dead volume between the two as possible.

A further 2 mm ID tube is required for the exhaust end of the column. This should be 32 cm long. A waste bag or filter should be attached to the end of the exhaust tube. In the case of a waste bag this should be primed with a small amount of imidazole buffer (approx. 50 – 100 ml) to avoid air being drawn into the column.

The column should be secured to the analyzer by attaching it to the upright pillar nearest to the allocated port.
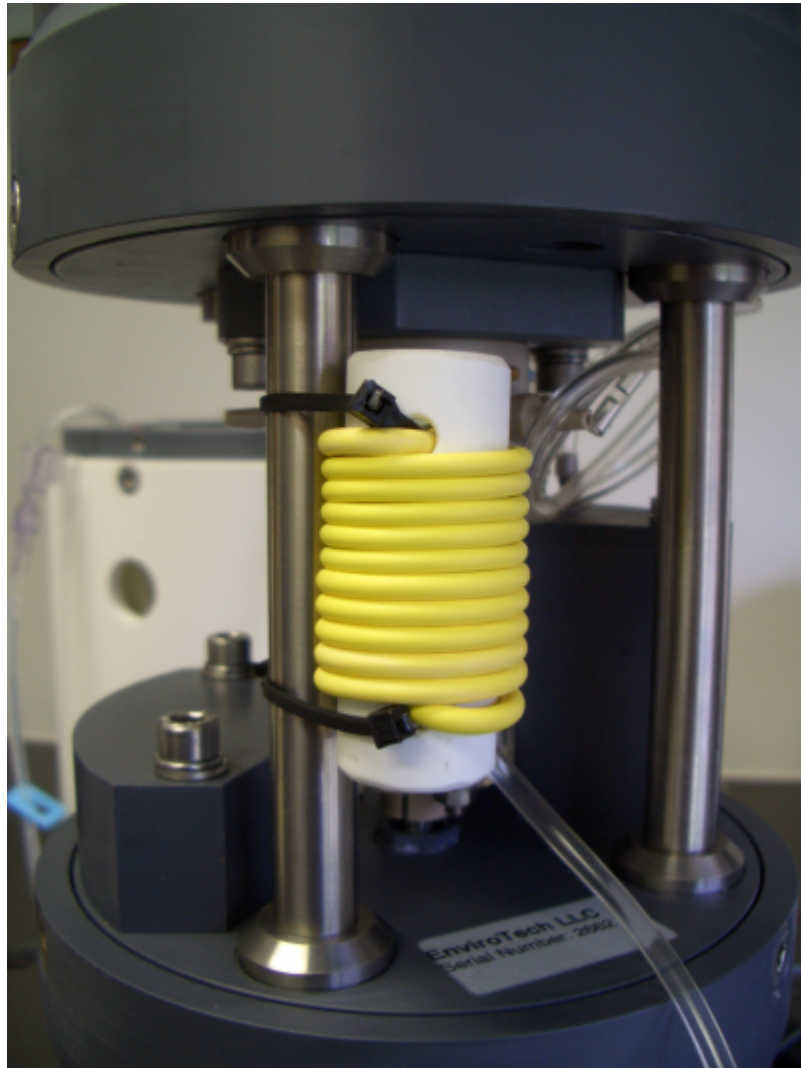
The assembly can be attached by threading small cable ties through the holes in the column holder and tightly securing it to the pillar. The exhaust tube should be coiled-up to avoid it snagging as the whole unit is assembled.

Throughout this procedure take great care not to allow air to enter the activated column. This can be easily achieved by clamping off the exhaust tube while fitting the column. Be certain to remove the clamp for the deployment.

10.3.3 Phosphate

Method

EPA Method 365.5

Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis

Carl F. Zimmermann & Carolyn W. Keefe

University of Maryland
Center for Environmental and Estuarine Studies
Chesapeake Biological Laboratory
Solomons, MD 20688-0036

Reagents & solutions

The following list of reagents are used in this method:

Description	Supplier	Part Number
Ammonium molybdate	Merck/BDH	100282H
Ascorbic Acid	Merck/BDH	103033E
Isopropyl alcohol	Aldrich	44,342-5
Potassium antimony tartrate	Sigma Aldrich	244791
Sodium dodecyl sulphate (SDS)	Merck/BDH	442152V

10.3.3.1 Reagents

Ascorbic Acid

Place 4.2 g of ascorbic acid in the bottom of a 500 ml volumetric flask.

Add 300 ml of de-ionized water and place on a magnetic stirrer until the ascorbic acid is dissolved.

Dilute the solution with de-ionized water to 500 ml

Potassium Antymonyl Tartrate

Add 5.3g of potassium antimonyl tartrate to 250 ml of DIW. Insure complete dissolution.

Ammonium Molybdate

While stirring slowly add 28ml of concentrated sulphuric acid (36N) to 220 ml of de-ionized water in a 1 liter flask.

Add 2.4 g of ammonium molybdate and continue stirring until all of the molybdate is dissolved.

While stirring, add 2.0 ml of potassium antimonyl tartrate reagent drop wise and insure that it dissolves completely.

Dilute the final solution to 500 ml and add 1ml of SDS (20g/L).

10.3.3.2 Standards

On-board Standard

Use Merck 1000 ppm as PO4 standard solution (Merck 14248 4U – 10526 uMol/l or 327 mg/l).

To make a 3 uMol/l or 0.093 mg/l on-board standard, add 1 ml of standard solution to 3.509 l of deionized water (DIW).

10.3.4 Silicate

Method

EPA Method 366.0

Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

Jia-Zhong Zhang, Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science. Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, University of Miami, Miami, FL 33149

George A. Berberian, National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Ocean Chemistry Division, Miami, FL 33149

Reagents & solutions

The following list of reagents are used in this method:

Description	Supplier	Part Number
Ammonium molybdate	Merck/BDH	100282H
Ascorbic Acid	Merck/BDH	103033E
Isopropyl alcohol	Aldrich	44,342-5
Oxalic acid	Merck/BDH	101744U
Sodium dodecyl sulphate (SDS)	Merck/BDH	442152V

10.3.4.1 Reagents

Ascorbic Acid

Dissolve 8.8 g in 500 ml de-ionized water.

Oxalic Acid

Dissolve 25 g of oxalic acid in 500 ml de-ionized water.

Sodium Dodecyl Sulphate (SDS)

Dissolve 2.5 g SDS in 50 ml de-ionized water.

Sulphuric Acid

Slowly add 140 ml of concentrated sulphuric acid to 900 ml de-ionized water.

Ammonium Molybdate

Ammonium Molybdate stock solution
Dissolve 15 g in 500 ml de-ionized water.

Ammonium Molybdate (SiO4)
Add 20 ml of dilute sulphuric acid to 160 ml of Ammonium molybdate stock solution, add 50 ml of SDS and dilute to 500 ml with de-ionized water.

10.3.4.2 Standards

<u>Silicate Stock Solution</u>

10mMol/l Silicate stock solution.
Dissolve 0.96 g sodium silica fluoride in 500 ml de-ionized water.

10.4 Installing Chemicals

10.4.1 Port Allocation

Refer to the table below for the chemical installations on the 8-port rotary valve.

Color	Port #	NO3	PO4	SiO4	NH4
Purple	1	Imidazole	Wash	Wash	Wash
Blue	2	Detector	Detector	Detector	Detector
Green	3	Standard	Standard	Standard	Standard
Yellow	4	NEDD			
Orange	5	Sulph	Ascorbic		
Red	6	Cd column	Ascorbic	Oxalic	
Brown	7	Imidazole	Molybdate	Molybdate	OPA reagent
Gray	8	Inlet	Inlet	Inlet	Inlet

10.4.2 Reagent Bags

The reagents are stored in "IV" bags during the operation and deployment of MicroLAB. The reagent bags supplied by EnviroTech LLC are (relatively) gas impermeable and this is an important feature with respect to reagent stability. Many "IV" bags are available on the market, but caution is advised as the permeability and ruggedness of different bag types varies greatly.

10.4.3 Filling Reagent Bags

The bags can be filled with reagents and standard solutions using a simple siphoning technique.

Pin the bags to the edge of a desk using two drawing pins through the bag suspension points with the tube uppermost. Place your beaker of reagent above the bag. Prime a length of tube using a syringe and then pinch it off. Keeping it pinched attach the tube to the bag neck and release your pinch. Reagent should flow into the bag. If the flow fails to start repeat the above process.

Once the bag is full or all the reagent has transferred use one of the blue pinch clips provided to close the tube about 2 inches (50 mm) from the outside end.

Once the bags have been filled with reagents (and standards) you should expel all the air. The tube clip should be released and the bag gently squeezed to expel all the air. Once the all the air has passed the tube clip it should be fastened to prevent any air returning to the bag once the squeeze pressure is released.

10.4.4 Installing Bags

Review the Port Allocation Table and carousel positions below. Now proceed to install the bags in the reagent housing, each one nearest to the appropriate port.
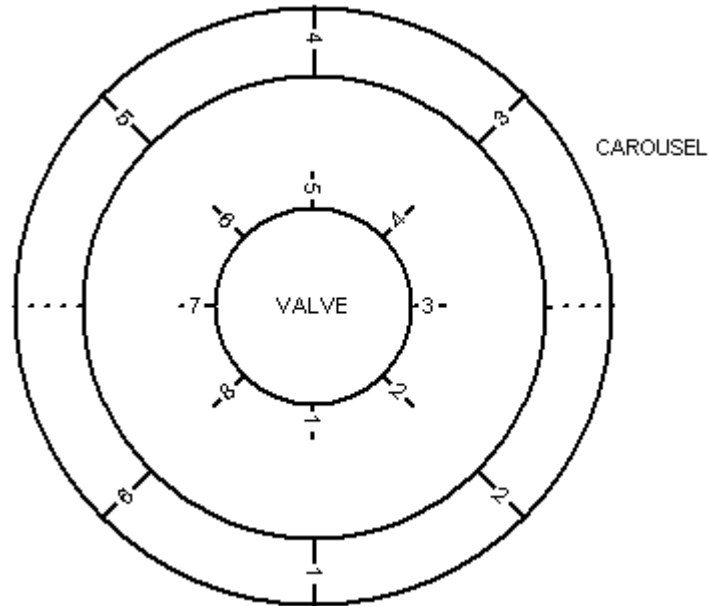

When installing the bags be VERY CAREFUL not to kink or trap a tube.

The bags are attached to the bag carousel and held in place with the semi-circular bag clamp. There are six bag positions. Each bag position may be used for one or more 250 ml or 500 ml bags. Reagents that require a supply or more than 500 ml are split between two or more bags and then inter-connected.

Remove the bag carousel and clamps and install the bags. Connect multiple bags as necessary and then check all connections. Pass the main bag tube through the feeder hole in the carousel ring and carefully clip it off.

Now install the carousel in the reagent housing and straighten the bags and tubes, checking for kinks and trapped tubes.
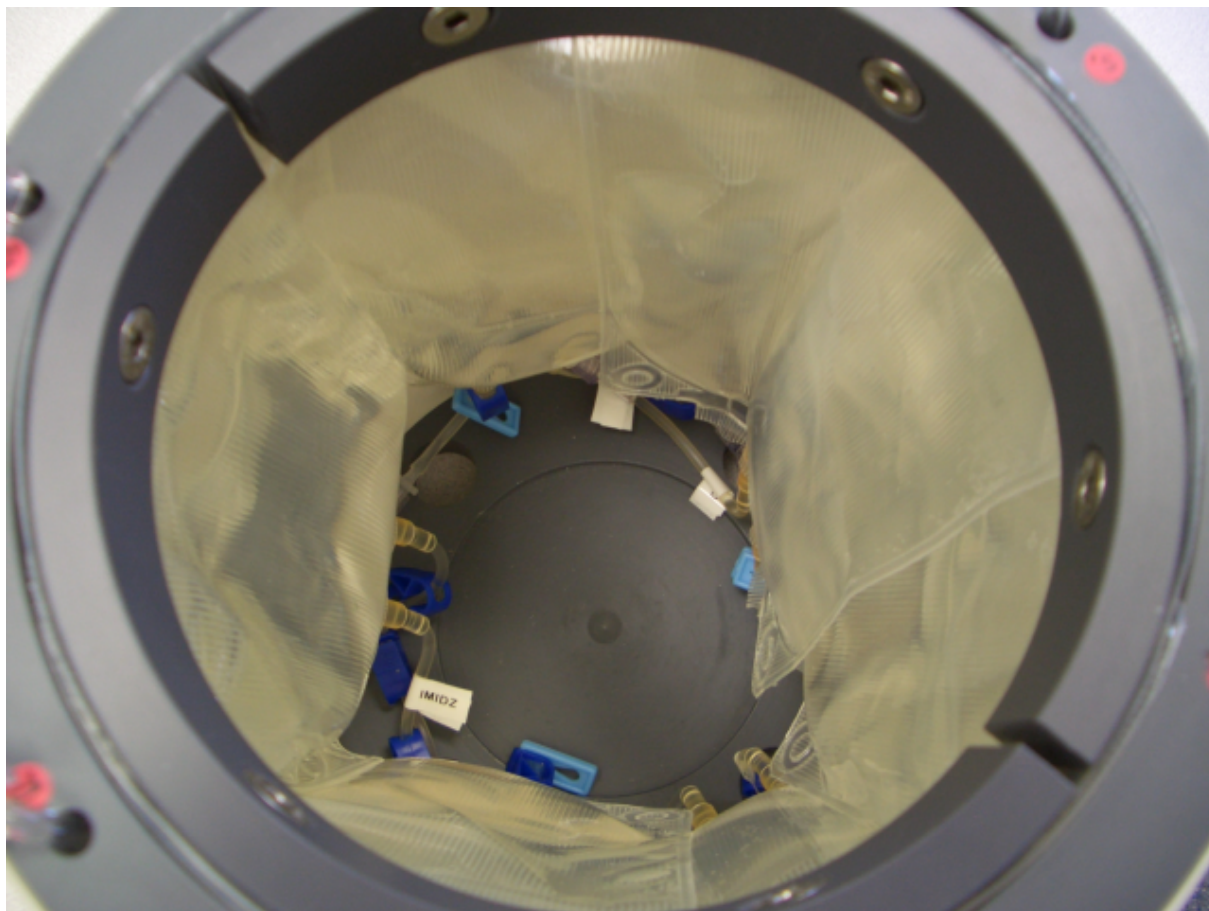
Picture of reagent bags fitted in carousel and reagent housing

Before connecting the bag tubes to the appropriate valve ports release the tube clip and bleed off any air from the tubes by running a small amount of reagent into a waste beaker.

Once connected any remaining air can be purged using the Prime(P) script to suck in a volume of each reagent and expel it via the colorimeter or inlet.

With some routines there will be spare ports on the rotary valve. These must be blanked off by attaching a short piece of tubing, sealed with a tube clip. It is essential that air is not introduced into the system.

Section 11

11 Deployments

11.1 Basics

11.1.1 On-Boards Standard Conc.

The on-board standard concentration should usually be set at the average concentration expected during the deployment.

11.1.2 Sampling Regime

The user must decide on a sampling regime before programming the instrument. The expected nutrient concentrations and deployment duration must be considered and from this the appropriate instrument set-up can be programmed.

The specific issues to consider are:
- Expected concentration
- Deployment duration
- Reagent payload
- Power budget

11.1.3 Consumables

In order to perform a successful deployment the user will require additional equipment and some standard accessories. These include an inlet filter, reagent bags, a cadmium column (nitrate only), battery pack, various cable ties, etc. Many of these items are included with MicroLAB when shipped, but replacements will be required for your second and subsequent deployments.

11.1.4 Battery Packs

MicroLAB uses either the internal battery or an external power source. An external supply may be provided by a standard BBP-10 battery pack. The battery pack are constructed of primary cells (non-rechargeable). Rechargeable and custom solutions to increase sample frequency or deployment length can be supplied to special order.

If user analysis routines are to be used it is important to calculate your power and chemical budgets well in advance of your deployment. This will determine your logging regime and the settings required.

11.1.5 Self-Training

Please be sure to run through the training exercises several times before contemplating a live deployment.

11.2 Bench Testing

11.2.1 Preparation

MicroLAB should be bench tested prior to every deployment to ensure it is functioning correctly. The pre-deployment bench test is an assessment of functionality, linearity and measurement repeatability.

Install the reagents and activate the cadmium column (if used).

Insert the cadmium column in the appropriate valve position, introducing as few air bubbles as possible. Place the open end of the column in a beaker containing approximately 200 ml of blank buffer. The outlet tube of the colorimeter should be placed in a beaker to collect waste, this should not be the same beaker as the column. It is important that during testing air is not allowed to enter MicroLAB, if air enters it will have to be purged before the test can continue.

Ensure that the rotary valve is in the correct position before priming the system, the correct starting position is at the purple port, plunger fully inserted. MicroLAB is supplied in the start position. If required the valve can be realigned by using the align command under manual control

Place the inlet tube in a beaker of blank sample (DIW or LNS). Run-off small amounts of fluid from each reagent bag using the priming script. Even if all air has been previously purged this is important if MicroLAB has been inactive for any significant time.

11.2.2 Blanks

Initiate the sample Blank(B) script. It may take a few cycles to completely flush the system and purge all bubbles. During this flushing note the blank and sample values. The blank reading should give a value of approximately 60000 counts. When repeatable values are obtained during the flushing the bench test proper may be undertaken.

If the blanks do not attain a reading of approximately 60000 then the flow-cell may need to be cleaned or the light intensity adjusted. Always begin with cleaning the flow-cell in preference to changing the light intensity setting. See the Detector Cleaning section for further details.

After the blanks have been run download and save the data. Then clear the memory.

11.2.3 Repeatability

This test assumes that reagents have been installed and the system has been primed (see the Installing Bags section) and that a blank check has been run.

The repeatability check is intended to assess the stability of the system.

This test may be run with a nitrate or nitrite standard. For nitrate the cadmium column needs to be activated / attached and the on-board standard will be spiked with potassium nitrate. For nitrite replace the cadmium column with an equivalent length of tubing and install a nitrite standard.

Ensure that the inlet tube is immersed in a beaker of DIW or LNS. Initiate the Repeatability(R) script and select 20 replicates. This will run the analysis cycle using the on-board standard and 20 discrete analyses.

Note that the analysis cycle deposits a small excess of the buffer solution back through the

column and into the reservoir beaker and so it will fill during bench testing.

After the linearity check has been run download and save the data. Then clear the memory.

11.2.4 Reduction Efficiency

The cadmium column reduction efficiency may be determined by analysing nitrite samples. The percentage efficiency can be calculated by comparing results from nitrite and nitrate samples of the same concentration. The column efficiency should be <u>at least</u> 85%.

Immerse the inlet tube in a beaker of nitrite (NO2) standard of the same concentration as the on-board standard. Initiate the Efficiency(E) script. This will run alternate analyses of the on-board standard (NO3) and the nitrite standard. The data will be used to calculate reduction efficiency.

Note that the analysis cycle deposits a small excess of the buffer solution back through the column and into the reservoir beaker and so it will fill during bench testing.

After the efficiency check has been run download and save the data. Then clear the memory.

11.2.5 Linearity

This test assumes that reagents have been installed and the system has been primed (see the Installing Bags section) and that a blank check has been run.

The linearity check is intended to provide data that can be plotted at a linear regression in order to assess the best straight line (BSL) performance of the analyzer.

This test may be run with a nitrate or nitrite standard. For nitrate the cadmium column needs to be activated / attached and the on-board standard will be spiked with potassium nitrate. For nitrite replace the cadmium column with an equivalent length of tubing and install a nitrite standard.

Ensure that the inlet tube is immersed in a beaker of DIW or LNS. Initiate the Linearity(L) script. This will run the analysis cycle using the on-board standard and process incremental dilutions over the full range of measurement with the concentration of the on-board standard being the highest measurement.

The Linearity(L) script analyzes samples at 50, 25, 90, 10, 100, 0 and 75% of the onboard standard concentration (in that order).

Note that the analysis cycle deposits a small excess of the buffer solution back through the column and into the reservoir beaker and so it will fill during bench testing.

After the linearity check has been run download and save the data. Then clear the memory.

11.2.6 After Bench Testing

After bench testing be sure to rinse all MicroLAB external surfaces with fresh water to avoid any damage due to chemical corrosion.

At this point the bench testing is complete. You should have the following data.

1. The laboratory (auto-analyzer) analysis of the on-board standard
2. The laboratory (auto-analyzer) analysis of the nitrite standard
3. A data file for blanks, repeatability, reduction efficiency and linearity

You may use the Excel template provided to import each data file and display the results.

11.3 Deployment Procedure

11.3.1 Deployment Frame

A stainless steel deployment frames are available from EnviroTech LLC.

In-line deployment frame

The frame is made from heavy gauge material with a tie bar designed to take a through load or be attached to a mooring line. The deployment frame includes clamps for MicroLAB.

MicroLAB system with the in-line deployment frame
in the foreground

Bottom deployment frame

For shallow water applications or bottom deployments a pyramidic deployment frame is available. This is made from heavy gauge stainless steel with anchor points for additional ballast, ground lines and marker floats.

MicroLAB system installed in the bottom deployment frame

Custom frames

Custom designed frames are available on request.

11.3.2 Checklist

Immediately before deployment MicroLAB should be prepared as follows:

- If filtering is to be used (highly recommended) attach the filters to the inlet and the open end of the cadmium column and if necessary secure them with a cable tie
- Set the deployment parameters
- Remove the communications cable and power lead
- Insert and secure MicroLAB to its frame taking care not to pinch any tubing
- Insert the communications dummy plug
- Place MicroLAB and battery pack in the deployment arrangement and secure all fixing plates
- Connect the battery pack lead to MicroLAB. Many users prefer to start the deployment while the instrument is in the frame.
- Deploy the assembly before the programmed delayed start elapses

There are a number of "classic" reasons for a failed deployment and human error is a significant risk. Before you deploy the instrument ensure that: the following items on your check list are complete and correct.

- The logging sequence and all variables and settings are recorded in your deployment log book
- The battery pack is connected
- MicroLAB clock is set accurately
- The instrument is verified to be in "logging mode"
- All necessary dummy plugs are inserted
- All brackets and fixings are tight (but not over-tight)
- All cables are secured by cable ties to avoid chaffing
- Sacrificial anodes are attached to the deployment frame
- All shackles are tight and locked
- Dissimilar metals are not in contact

Do not be rushed into allowing the equipment to be deployed until all the above points have been checked. If possible get a colleague to double-check.

During the actual deployment ensure that:

- Violent mechanical shocks are avoided
- The time the equipment enters the water is recorded
- The GPS position reading is recorded

A series of photographs of the equipment taken immediately before and during the deployment often prove to be useful.

11.4   Recovery

As with the deployment procedure the recovery procedure for MicroLAB is highly dependent on the mooring design and facilities available. MicroLAB should be recovered carefully and avoiding unnecessary impacts or shock.

Immediately after recovery proceed as follows:

- Connect your PC and download the data from MicroLAB. If the battery is flat you may need a further external power supply.
- Remove power from the instrument by disconnecting the battery pack.
- Remove MicroLAB from the mooring arrangement.
- The exterior of the instrument should be thoroughly washed with fresh water and dried to avoid corrosion.
- Run the Efficiency (if nitrate), Repeatability and Linearity test scripts.
- Remove the cadmium column, reagents and on-board standard from the instrument.
- Freeze a sample of the OBS for later analysis
- If post-deployment servicing is not going to be carried out immediately flush the valve, syringe and colorimeter through with deionized water or a mild solution of hydrochloric acid. Use the Flushing script.
- Service MicroLAB

Section 12

12   Data Analysis

12.1   Data Processing

NutrientDATA software makes data processing very easy. All that is required to produce a calibrated file is the downloaded data file and the on-board standard concentration. Please see the Training Exercises for a step-by-step procedure.

If you have developed your own scripts with multiple or additional readings (e.g. reagent blank) then you will need to manually process your data using a spreadsheet program or other data handling application. To do this successfully you will need to understand all the sections in this part of the manual.

12.2   Data Principles

Calculation of concentration from colorimetric beam attenuation measurements

Using the Beer-Lambert Law:

$$Absorbance = LOG\left[\frac{\text{Blank}}{\text{Reaction}}\right] = kcl \tag{1}$$

Light into and out of the flow-cell are measured for the blank and reaction of each analysis. Light transmission is the ratio of light in (source) and light out (color).

Transmission is calculated for each colorimeter reading.

$$ratio = \frac{color\,(counts)}{source\,(counts)} \tag{2}$$

To calculate concentration four readings are needed:
      Sample blank & reaction
      Standard blank & reaction

Absorbance is then calculated for each analysis

$$absorbance = LOG\left[\frac{ratio\,(blank)}{ratio\,(reaction)}\right] \tag{3}$$

Concentration is calculated by combining (1) for each of the sample and the standard.

$$A_S = kC_S l \quad\text{... and ...}\quad A_T = kC_T l \quad\text{... therefore ...}\quad kl = \frac{A_S}{C_S} = \frac{A_T}{C_T} \tag{4}$$

Where: The S suffix is sample and T is standard.

Rearrange:

$$C_S = \left(\frac{A_S}{A_T}\right) \bullet C_T$$

(5)

… or …

$$concentration = \left(\frac{absorbance\,(sample)}{absorbance\,(standard)}\right) \bullet standard\ conc.$$

(6)

12.3   Worked example

Recorded data

	Sample	Standard	
Blank source	30000	30000	Light in
Blank color	60000	60000	Light out
Reaction source	30000	30000	Light in
Reaction color	20000	30000	Light out

Onboard standard conc. = 2.50 µg/l or µMols (units not important)

Sample Analysis

$$ratio\,(blank) = \frac{color\,(counts)}{source\,(counts)} = \frac{60000}{30000} = 2.000$$

$$ratio\,(reaction) = \frac{color\,(counts)}{source\,(counts)} = \frac{20000}{30000} = 0.667$$

Standard Analysis

$$ratio\,(blank) = \frac{color\,(counts)}{source\,(counts)} = \frac{60000}{30000} = 2.000$$

$$ratio\,(reaction) = \frac{color\,(counts)}{source\,(counts)} = \frac{30000}{30000} = 1.000$$

Sample Absorbance

$$absorbance = LOG\left[\frac{ratio\,(blank)}{ratio\,(reaction)}\right] = LOG\left[\frac{2.000}{0.667}\right] = 0.477$$

Standard Absorbance

$$absorbance = LOG\left[\frac{ratio\,(blank)}{ratio\,(reaction)}\right] = LOG\left[\frac{2.000}{1.000}\right] = 0.301$$

Then

$$concentration = \left(\frac{absorbance\,(sample)}{absorbance\,(standard)}\right) \bullet standard\;conc. = \left(\frac{0.477}{0.301}\right) \bullet 2.50 = 3.96$$

12.4   Visualization

Data should be plotted as a time-series and inspected at each step during the data processing. It is especially important to visualize your raw data. You must ensure that you have a complete understanding of the sample and standard signals. In this way you will identify, and be able to correct for, any underlying issues before producing data in final concentration units.

The following graphs show steps during data processing. It is recommended that these graphs are produced and carefully examine each time data is processed.

- Standards - raw data
- Samples - raw data
- Absorbance - standard and sample
- Concentration

Review these graphs in connection with the Data Principles and Worked Example sections.

12.4.1   Standards

The graph below shows raw on-board standard data for the blank (blue) and the reaction (red). Note that the beam transmission reduces due to absorbsion by the reacted dye. Look for repeatability in the blank and reaction measurements throughout the deployment. Each pair of blank and reaction readings forms one result or analysis of the on-board standard.
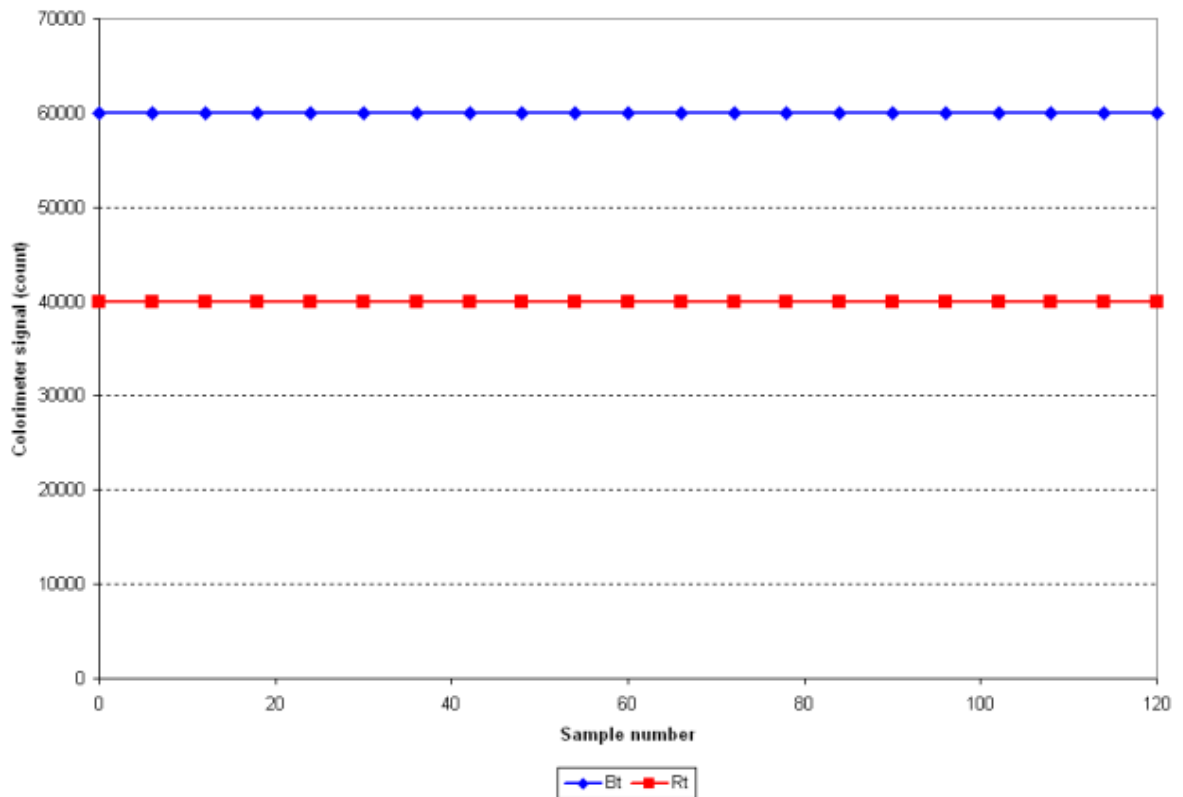
12.4.2 Samples

The following graph shows the raw on-board standard data with the sample data super-imposed. In this example the sample concentration has changed dramatically half-way through the deployment. This is shown in the sudden decrease in transmission signal in the sample reaction (gray). The sample blanks (green) are steady, but slightly lower than the on-board standard. This small difference is possibly due to background turbidity in the sample.

In a real situation the sample reaction data may show spikes (rain events, flushing, discharge), oscillations (tidal cycles), constant slope (slow flushing) or a rapid reduction in signal / increased light (algal bloom).
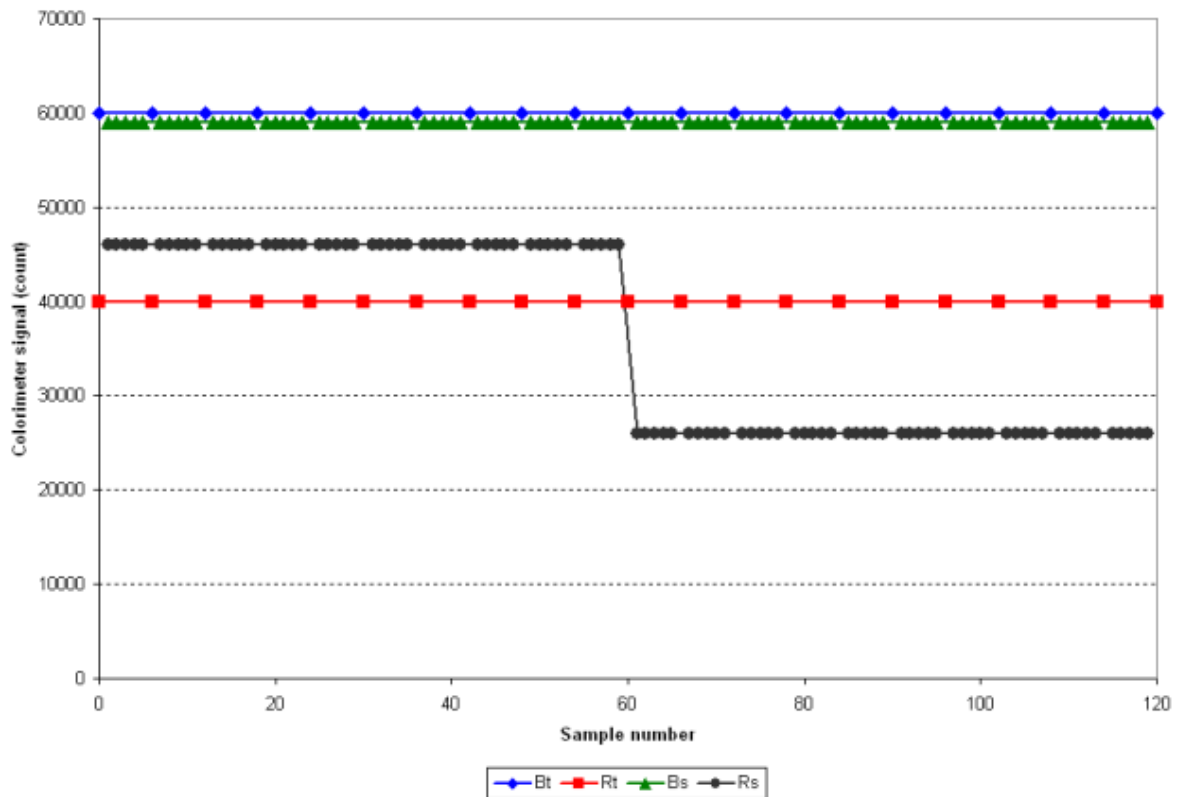
12.4.3 Absorbance

Each of the standard and sample data has been converted to absorbance in the graph below. The on-board standard (orange) should be repeatable. The sample (black) will clearly show environmental conditions at this point in the data processing.
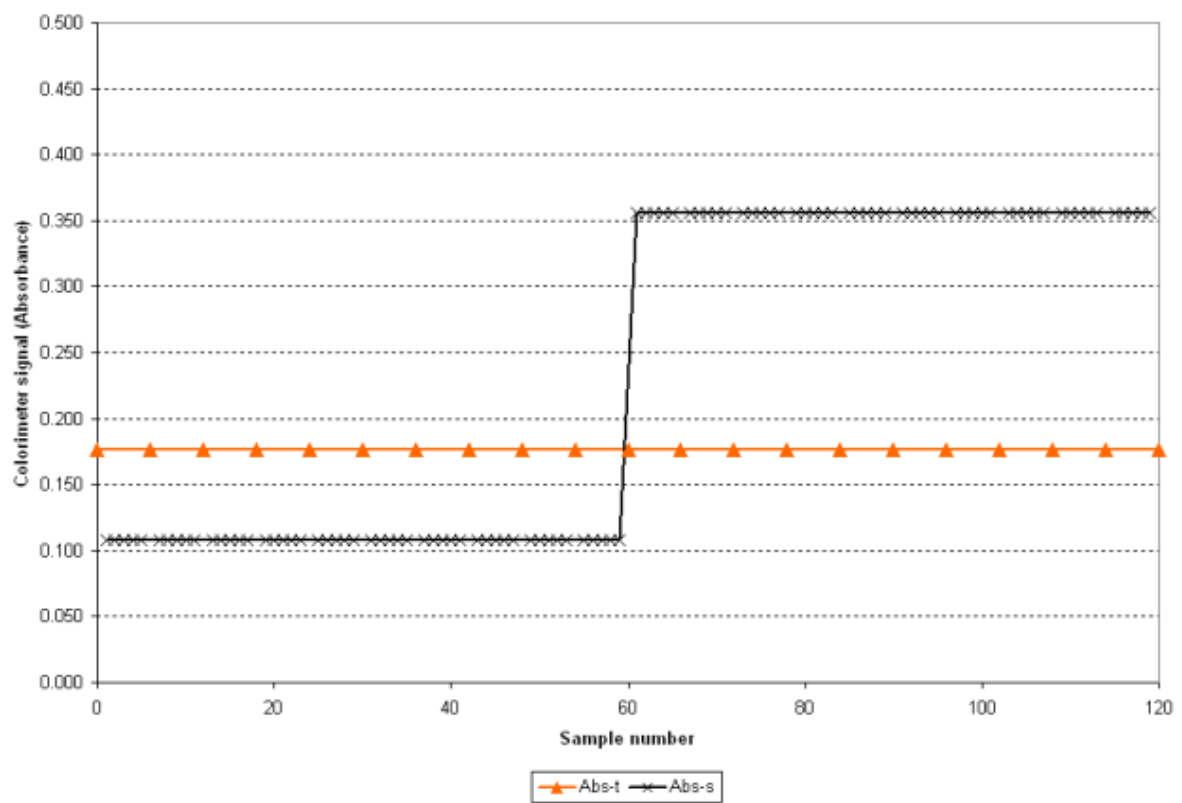
12.4.4 Concentration

The graph below shows concentration. This is the final step during data processing.

Conc
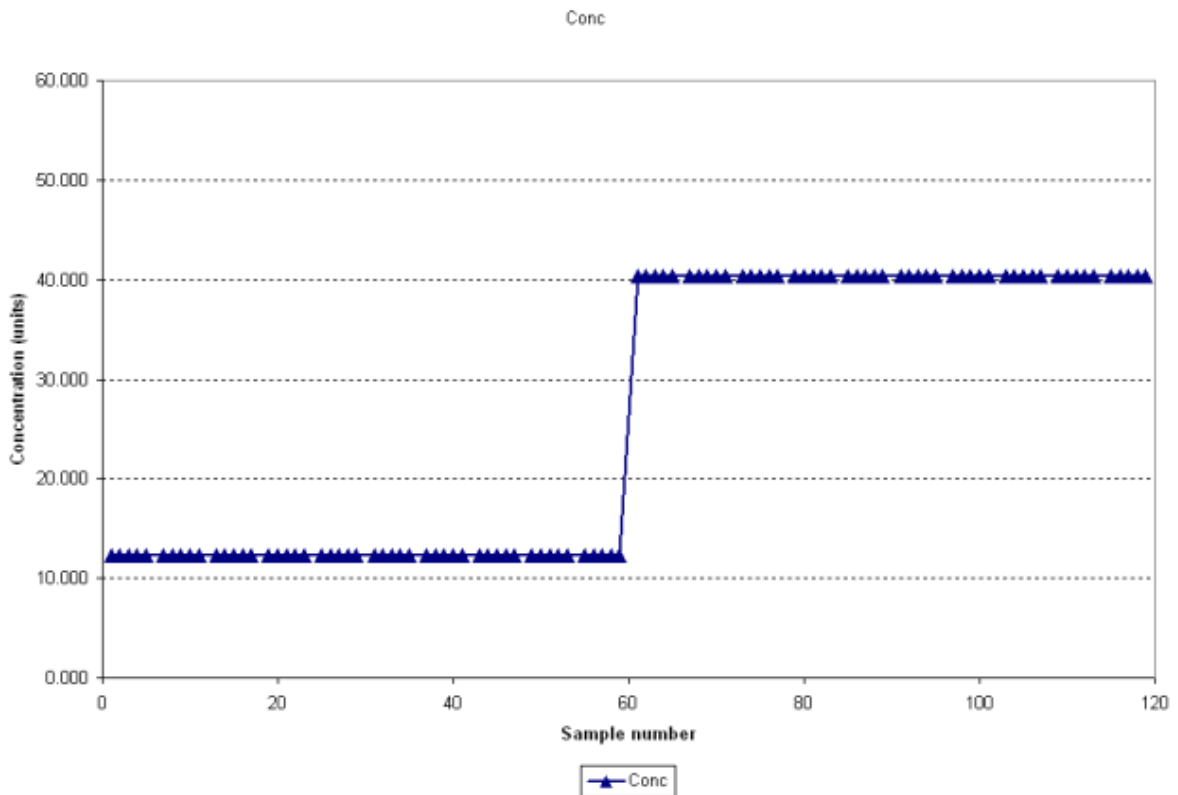
12.5 Quality Checking

Quality check the data as per the general procedure given below or your standard "in-house" procedure

1. Inspect standard blank for repeatability. Test for and discard / correct any outliers.
2. Inspect standard reaction (absorbance) for signal strength and repeatability. Test for and discard / correct any outliers Also look for and assess any drift over time.
3. Compare data from pre and post-deployment linearity checks.
4. Compare independent pre and post-deployment analysis of standard concentration. Cross reference any change in standard absorbance to the pre and post-deployment concentrations.
5. Compare pre and post-deployment reduction efficiency checks to any change in standard absorbance.
6. De-trend for changes in standard concentration, reduction efficiency and optical fouling if necessary.
7. Calculate analysis repeatability and SNR.
8. Inspect sample blank for repeatability and excessive turbidity component (compare to standard blank).
9. Calculate and subtract turbidity absorbance if necessary.
10. Inspect sample reaction data. Remove / correct any outliers.
11. Calculate concentration using (corrected) standard data.

12. Compare calculated concentrations to pre and post-deployment CTD / Rosette data and any other sample data during the deployment (e.g. from water sampler).

MicroLAB has the ability to periodically perform a multi-point linear self-calibration and nitrate reduction efficiency tests in-situ. These data are valuable for quality checking of environmental data.

Section 13

13   Consumables

Consumables for use with MicroLAB are available from EnviroTech LLC. Some are available from alternative suppliers, in particular the reagents and other chemicals. Please see the chemistry sections for reagent and chemical usage.

The following items are consumable and will require periodic replacement.

GEN-P023 - Luer Adapter

Use / clean as needed

GEN-P012 - Filter with Nozzle

Typically 1 or 2 (for NO3) per deployment

Section 14

14   Servicing

14.1   Maintenance

After each deployment MicroLAB should be thoroughly cleaned and the valve and syringe assembly serviced. The component parts must be stripped, cleaned, inspected and reassembled. Also the colorimeter should be cleaned. The servicing frequency will be dependent on the operating conditions, especially the suspended solids concentration but as a guideline it is suggested that the valve and syringe assembly is serviced after every (720 sample) deployment at the very least.

It is important that all O seals and their sealing surfaces must be in perfect condition, scratch free, absolutely clean and lightly lubricated with silicone grease before assembly. This is essential to ensure complete watertight integrity.

All replacement parts, including O seals, should be obtained from EnviroTech LLC.

Please see the service routines for specific service instructions.

14.2   Torque Settings

The table below gives torque settings for various components and fasteners.

Component	Torque setting
Barbed adapters on PEEK valve (light brown)	30 cNm (42 oz in)
M6 screws on valve plate	2 Nm (1.47 lbf ft)
M5 set screw on rotary shaft	2.5 Nm (1.84 lbf ft)
M6 screws on pressure housings	1.8 Nm (250 oz in)

14.3   INIT file

The initialization file (init) contains the main configuration set-up for each instrument. This file is not normally edited by the user unless there has been a significant change to the instrument set-up. This might be the replacement of the valve motor and/or gearbox or other major components such as the valve itself. A hard copy of the init file is supplied with the instrument. Please review the example below.

```
# init

dio F00
# Allow time for SPS turn on
wait 500

# Rotary motor setup
# --------------
transmit 0,"Valve Motor Driver\r\n"
SPS"0F","VQ"
SPS"0F","RS"
SPS"0F","XX"
```

```
SPS"0F","SH 1"          # Half step
SPS"0F","SS 38400"      # Steps per rev
SPS"0F","SE 2000"       # Steps per encoder rev
SPS"0F","SU 1000"       # Micro sec step delay
SPS"0F","SP 8"          # 8 ports per rev
SPS"0F","SO 50"         # Set initial offset
SPS"0F","SW 1"          # Encoder vertical orientation
SPS"0F","SC 0"          # Set clockwise direction
SPS"0F","ST 2"          # Set sps mode

# Linear motor setup
# --------------
transmit 0,"Syringe Motor Driver\r\n"
SPS"0E","VQ"
SPS"0E","RS"
SPS"0E","XX"
SPS"0E","SH 0"          # Full step
SPS"0E","SD 6"          # 6 ms step delay
SPS"0E","SC 0"          # Set insert/retract direction


# Detector polling
# --------------

transmit 0,"Detector\r\n"
SPS"1F","VQ"
SPS"1F","RS"
SPS"1F","XX"

dio 0000
```

Valve offset

It is likely that you will only ever have to edit the valve offset or "SO" setting during any maintenance work. SO is the offset from the single absolute reference of the encoder to Port 1 in encoder steps. This means that the absolute reference is 50 encoder steps clockwise of Port 1 (purple). See the valve alignment section for further details.

14.4 Service Routines

14.4.1 Service Schedule

A standard service schedule sheet is provided with MicroLAB and on the CD. It is recommended that you keep service records in a log book and carefully record the service history of your instrument. The service schedule is provided to assist you. Following the steps on the sheet will ensure the reliability of your equipment during deployments.

14.4.2 Valve-Syringe Assembly

Syringe

The syringe is comprised of a PEEK piston, fitted with a o-ring, inside a glass cylinder. It is important to note that this o-ring should not be twisted when fitting as this can cause the syringe to leak.

The piston and cylinder should be assembled slightly wet, preferably with a dilute BRIJ solution as the o-ring may stick if dry.

The end of the glass syringe barrel is seated on a o-ring. A clear Perspex sleeve, which is fastened to the valve by four titanium studs and nuts, retains the cylinder.

The piston shaft is connected to the linear drive shaft by a plastic coupling. This coupling is in two halves and is held together by a screw. When fitted it allows horizontal freedom to accommodate any slight misalignment of the two shafts.

The linear drive shaft is made from <u>titanium</u> and penetrates the linear drive bulkhead. The bulkhead is fitted with a seal housing which holds a special Variseal wiper/scraper seal which is pressure resistant and prevents ingress of abrasive particles to the sealing surfaces. An o-ring seal is also fitted.

The shaft is driven by a motorized linear actuator, which is controlled by the SPS driver. The syringe is designed to run "wet". If the unit is required to be run "dry" for any length of time for test or service functions, it is recommended that the clip is removed from the shafts disconnecting the syringe.

Valve

The 8-port rotary valve is a sealed unit and requires no servicing apart from cleaning after use. The valve is made from PEEK with a PTFE liner.

It is fixed to the valve plate by four <u>titanium</u> studs. The valve rotor is driven by <u>titanium</u> shaft which has an offset drive tongue. This shaft penetrates the rotary drive bulkhead via a bush with o-ring seals.

The shaft is driven by a geared rotary stepper-motor which is controlled by the on-board microcomputer.

Whenever the rotor is aligned with one of the 8 ports it is connected to the common central port and the syringe chamber.

The rotary ports are fitted with barbed adapters to suit the reagent bag tubing, the cadmium column and the colorimeter port. The ports are color coded for identification.

Service procedure

Remove the coupling, which connects the linear drive shaft to the plunger and fully retract the shaft.

Remove the two M6 hexagon head bolts from the valve mounting block, lower the mounting plate to disengage the rotary drive shaft, rotate the base until the two flats on the base align with two pillars and withdraw the complete valve and syringe assembly.

Remove the piston and the four special titanium nuts from the Perspex syringe holder. Lift off the syringe holder, the syringe cylinder and its O seal. The valve unit cannot be dismantled apart from removing the barbed adapters.
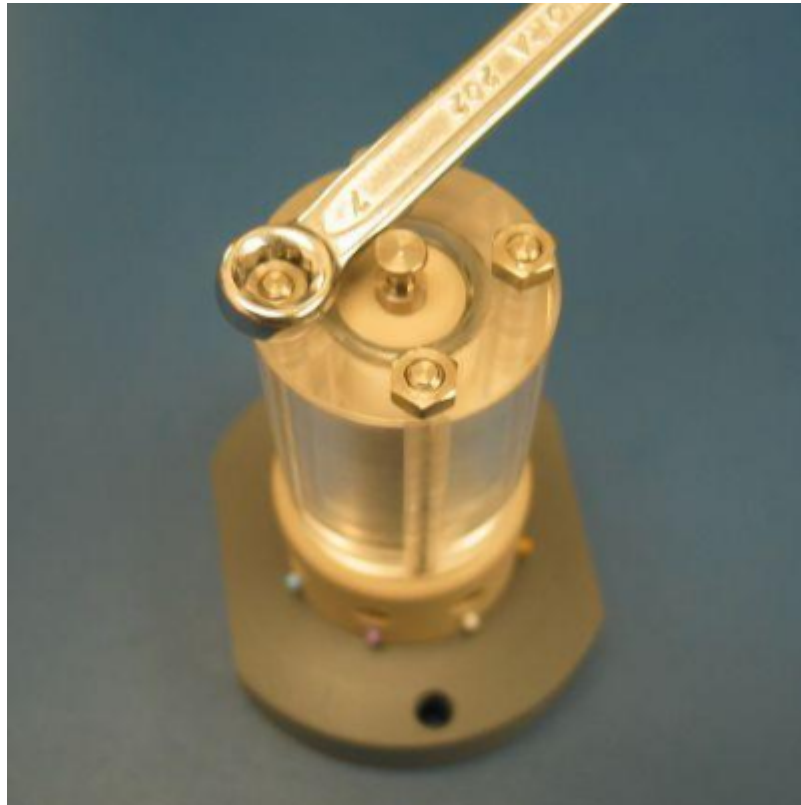
Remove four titanium nuts using the 7 mm wrench
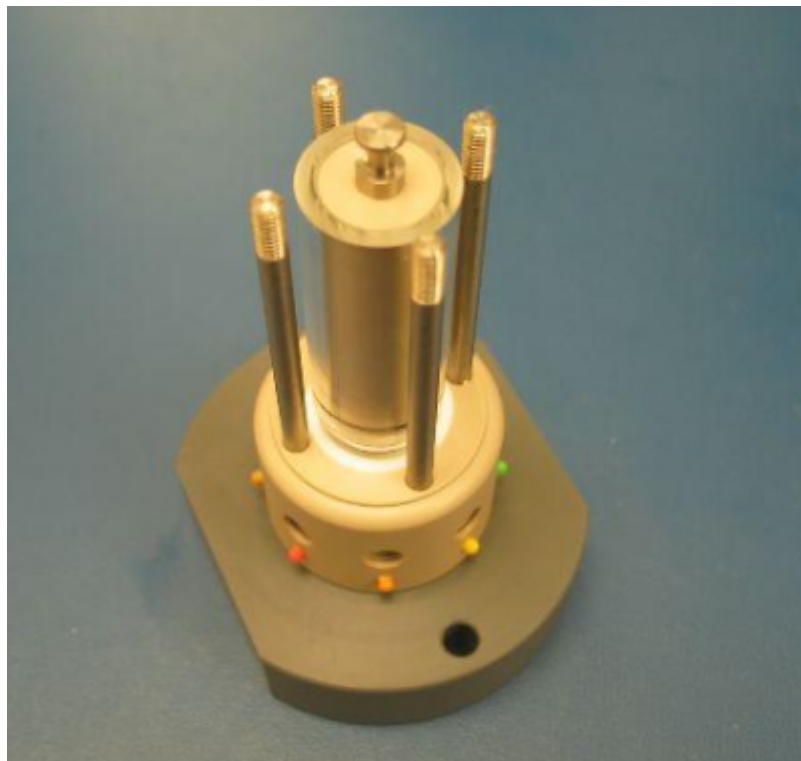
Lift of the Perspex syringe sleeve

Remove the syringe barrel and plunger. Then remove the o-ring.

Clean all parts thoroughly in clean fresh water with 2-5% of "Decon 90", "Contrad 70" or similar laboratory cleaning agent added.

Inspect all parts for wear or damage paying particular attention to the syringe barrel and piston which may require periodic replacement dependent on the abrasive content of the water. Replace the piston O ring with a genuine seal supplied by EnviroTech LLC.

Reassembly

Reassemble the valve and syringe unit in the reverse order. Lubricate the piston with water, preferably with some BRIJ solution added. Tighten the titanium nuts to a torque of 25cNm (35 oz. Inches) using a suitable torque-limiting driver. Over tightening will cause damage. The torque of the barbed adapters is critical to performance. They must be tightened to 30cNm (42 oz. Inches) using a torque limiter with the special key provided with MicroLAB. Over tightening will cause damage or seizure of the valve and under tightening will cause leakage resulting in chemistry irregularities. The tightening of these adapters should be carried out while the valve is turning.

If the linear seal housing or rotary drive shaft bushes are to be serviced do not refit the valve assembly to MicroLAB at this stage.

Take note that the rotary drive shaft tongue is off center and must be carefully aligned.

14.4.3 Syringe

Remove the syringe plunger from the barrel and inspect both parts for wear. If either is worn replace with a new set (see the spares section).

Replace the o-ring on the plunger. It is important to note that this O ring should not be twisted when fitting as this can cause the syringe to leak.

The piston and cylinder should be assembled slightly wet.

14.4.4 Valve Alignment

For this operation you will need to install and run ESM Manager 2 software (supplied on the CD or can be downloaded from our website). Please see the ESMM2 manual for full details.

To reset the valve alignment you will need to edit the init file and run some direct commands from ESMM2 software.

1. Navigate to the /eep folder and identify the init file
2. Download it to your hard drive
3. Open the init file with a text editor and set SO to 0. Save the file
4. Upload it to overwrite the version on MicroLAB in the /eep folder
5. Run the init file
6. Align the valve. The command is 0F / AL in the Direct Command panel SPS command boxes

7. Take the purple valve nozzle out and dry the hole
8. "Rotate freely" counter/anti-clockwise to the purple port (0F / RF A xx), where xx is the number of encoder steps. Start with 100
9. While rotating visually check that the hole in the rotor goes to, or past, the purple port. If not, increase xx
10. Align the valve again and repeat the RF command
11. By trial and error work out how far it is from the alignment point exactly to align with the purple
12. Set this value to be SO xx in the init file and upload it once more
13. Run init.
14. Rotate directly to the purple port using the command 0F / RP 1 in the Direct Command panel SPS command boxes
15. Check the alignment visually

14.4.5 Valve Nozzles

The torque of the barbed adapter nozzles on the rotary valve is critical to performance. The nozzles must be tightened to 30cNm.

The nozzles should be tightened using a micro torque wrench and the special nozzle adapter provided with MicroLAB (shown below). Over-tightening will cause damage or seizure of the valve and under-tightening will cause cross-talk resulting in variable results.

GEN-P015 - Torque Wrench

GEN-P002 - NAS-WMS Nozzle Adapter

14.4.6 Detector Cleaning

The colorimeter is manufactured from high-grade glass. Small-bore tubes are formed in the glass to allow sample to enter and leave the colorimeter via standard push-fit couplings. The inlet is designed to expel any trapped air in the colorimeter. The inlet is connected to the valve outlet port by a short length of plastic tubing.

If removing the tubing it should be carefully split with a very sharp scalpel. It must not be pulled off without being cut through or the tubes can be broken. If fitting tubing it should first be warmed in hot water to soften it.

Care must be taken not to replace this tubing on the wrong port of the colorimeter. The inlet port is indicated with a small mark**.**

Servicing Procedure

Attach pieces of tubing to the colorimeter ports and flush through vigorously using a syringe with a solution of 5% "DECON" or "CONTRAD" detergent or similar. Then leave to soak for 1 to 24 hours. Finally flush out and check the blank light reading with deionized water. Ensure that no bubbles are lodged in the colorimeter. The reading should be approximately 60,000 counts.

To check the light transmission through the colorimeter use the manual control panel to set the light intensity and obtain readings.

- Enter the intensity for your instrument (typically 30,000 +/- 2000) to the intensity box and press the "Set" button.

- Now press the "Get Reading" button. The result should be 60,000 +/- 2000.
- If not, first check the flow-cell is clear from bubbles.
- Then increase or reduce the intensity setting to achieve a reading of 60,000 counts. The colorimeter intensity (source) and reading (color) are proportional.
- The intensity setting is your new setting for the instrument. Carefully make a record of it.
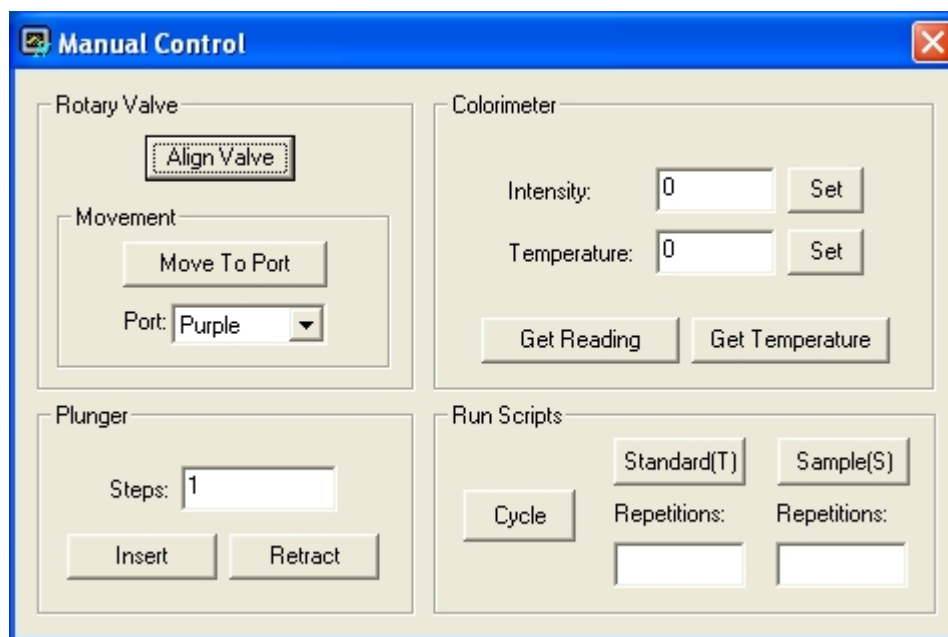
If the reading is drastically lower this suggests that foreign matter is lodged in the light path. If further flushing does not remove it, it may be necessary to fill the colorimeter with a 25% hydrochloric acid solution and leave to soak before flushing and re-testing. Do not allow the acid to contact external surfaces.

14.4.7 Syringe Motor

To access the syringe motor remove the lower pressure housing.

Lubrication

The linear syringe requires occasional servicing. It is lubricated prior to despatch. A small amount of this lubricant (black graphite grease) is included in the spares kit supplied. The lubrication of the lead screw should be checked periodically and grease re-applied as necessary. Retract the plunger fully and apply a pea-sized amount of grease to the lead screw immediately underneath the motor. Now run the motor in and out through its full travel five times.

Retaining bar

The horizontal "T-Bar" known as the Retaining Bar should be checked periodically for tightness and security. You should also ensure that the there is a 1 mm clearance between

the top of the Retaining Bar and the rear of the syringe motor <u>when the plunger is driven fully home</u>.

14.4.8 Linear Shaft

To access the syringe motor and lead-screw remove the lower pressure housing. Retract the plunger, remove the syringe coupling screw and take off both halves of the syringe coupling.
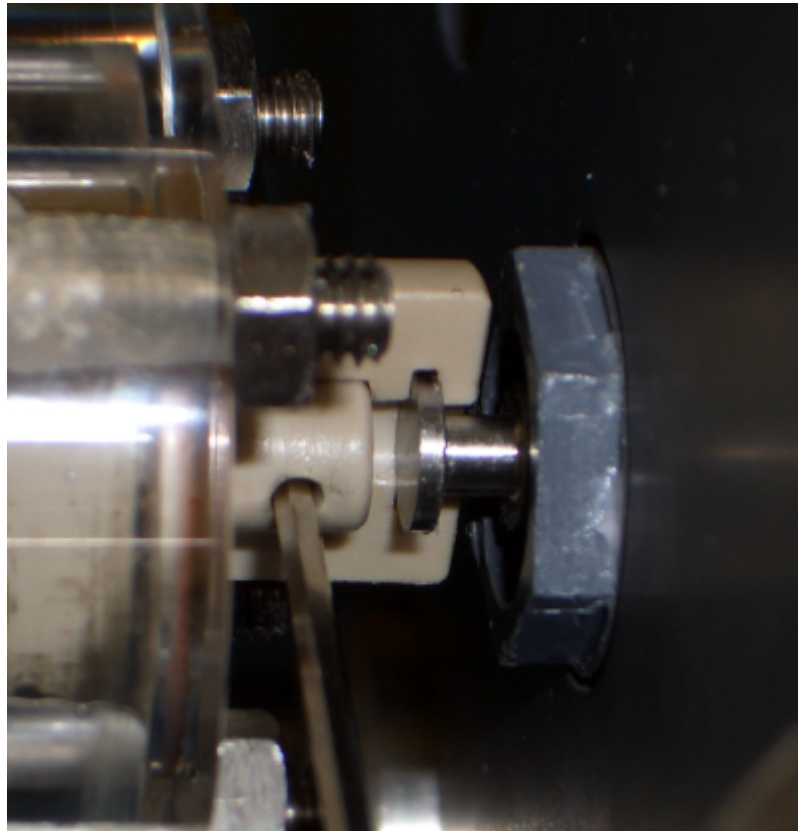
Remove the
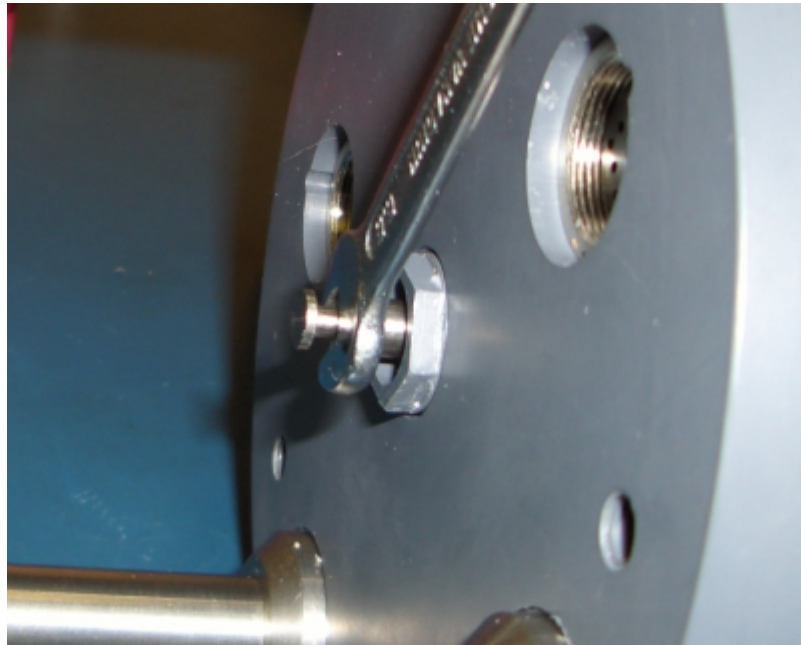
MicroLAB Linear Shaft

Fit the new seal housing which has the top seal pre-fitted and ensure that the back o-ring is in place.

MicroLAB Linear Seal Housing

Inspect the shaft for wear or damage, especially longitudinal scratches and replace it if damaged. Use your finger to feel for any wear.

Inject a small amount of silicone grease into the seal housing and insert the shaft from the tapered end. Never insert the shaft from the end with the flange and hexagon as this may damage the seals.

Screw the shaft fully onto the motor shaft and tighten securely.

14.4.9 Rotary Shaft

Remove the electronics housing and the rotary valve housing

Remove the rotary gearbox mounting screws and withdraw the geared motor complete with the shaft.
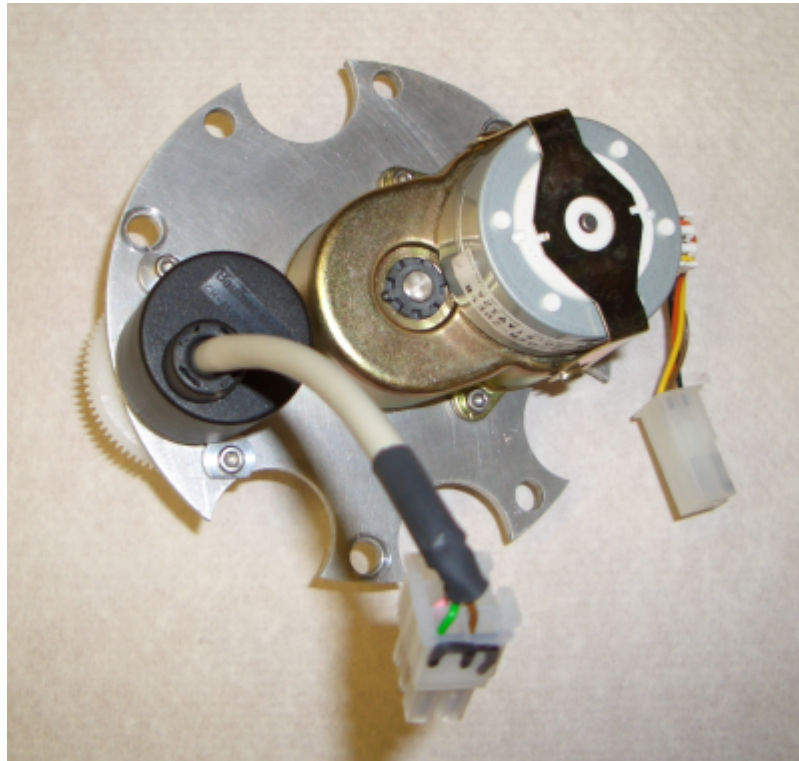
Valve motor drive mechanism

The shaft is locked to the motor using high strength adhesive, do not attempt to remove it. Push out the mounting bush.
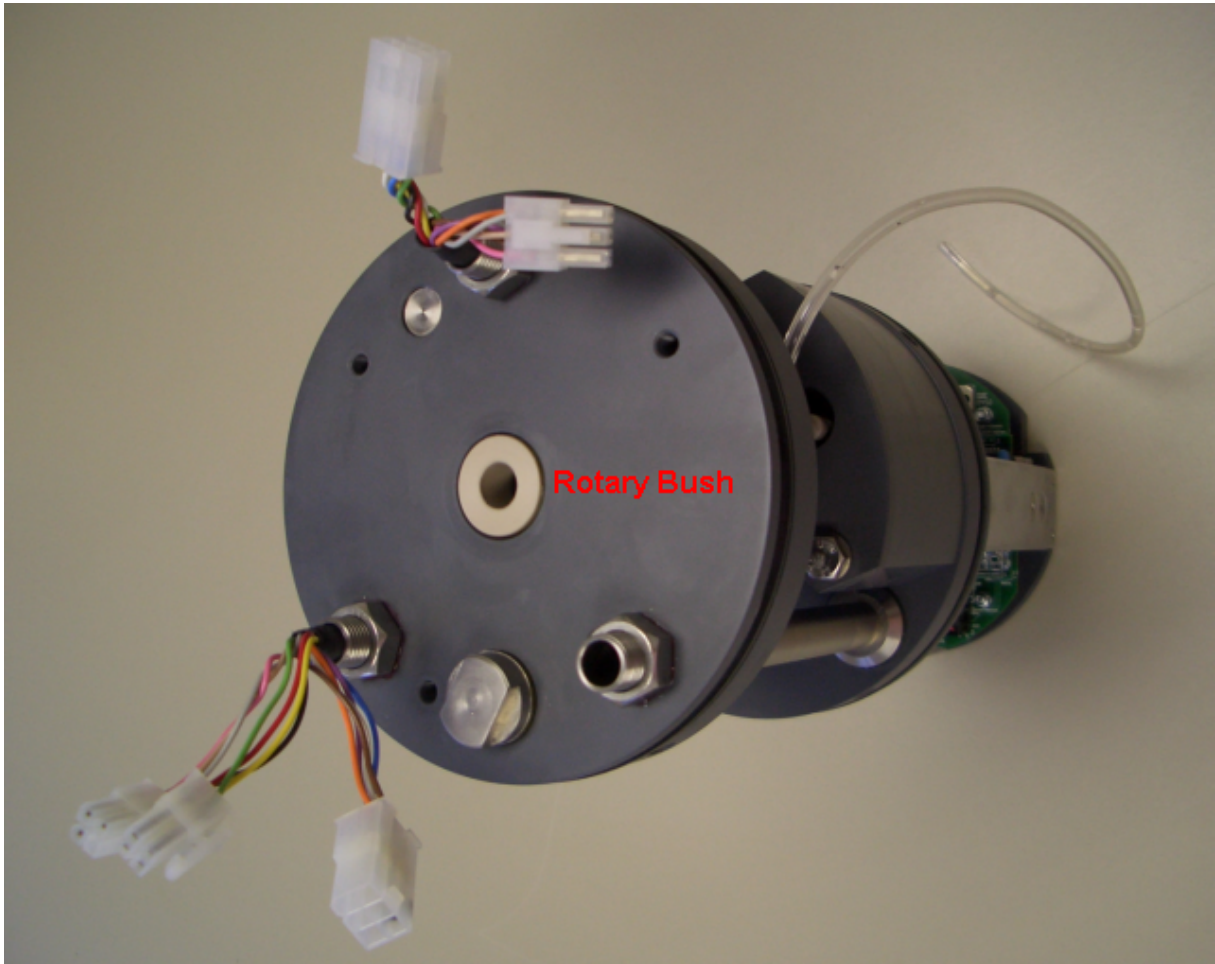
Picture showing rotary bush after removal of the valve drive mechanism

Remove and discard the O rings from the shaft. Remove the O ring from the bush. Clean all parts and inspect for wear or damage. Pay particular attention to the bore of the bush. This should have a good finish, free from any longitudinal scratches and must be replaced if damaged

MicroLAB Rotary Shaft Assembly

Fit new O rings to the shaft and re-fit the O ring to the bush, lubricating them with clean silicone grease and re-fit the bush and the motor/shaft assembly.

14.5 Spares

MicroLAB design incorporates moving parts. As with all moving parts they will eventually wear out. It is also recommended that some parts are periodically replaced in accordance with the servicing instructions (see the standard service schedule provided).

Please note that EnviroTech LLC offers a complete range of spare parts and a full instrument overhaul service. All spare parts and service are available via appointed distributors.

Inspect and replace as needed. Main pressure case seals should be cleaned and/or changed every major service (700 - 3000 samples).

Linear Shaft - Regularly inspect for wear.

GEN-P004 - Standard Syringe Plunger Set

Inspect and replace this assembly as necessary due to wear from suspended load. If leaking remove o-ring, clean groove and replace with new seal.

GEN-P003 - NAS Valve Nozzles

Required for spares purposes only. Check torque regularly.

Lead Screw - Inspect for corrosion at top and regularly lubricate with graphite grease (black).

Linear Seal Housing

Inspect and replace typically regularly (supplied assembled).
Replace rear o-ring and clean sealing surfaces every inspection.

Rotary Shaft Assembly
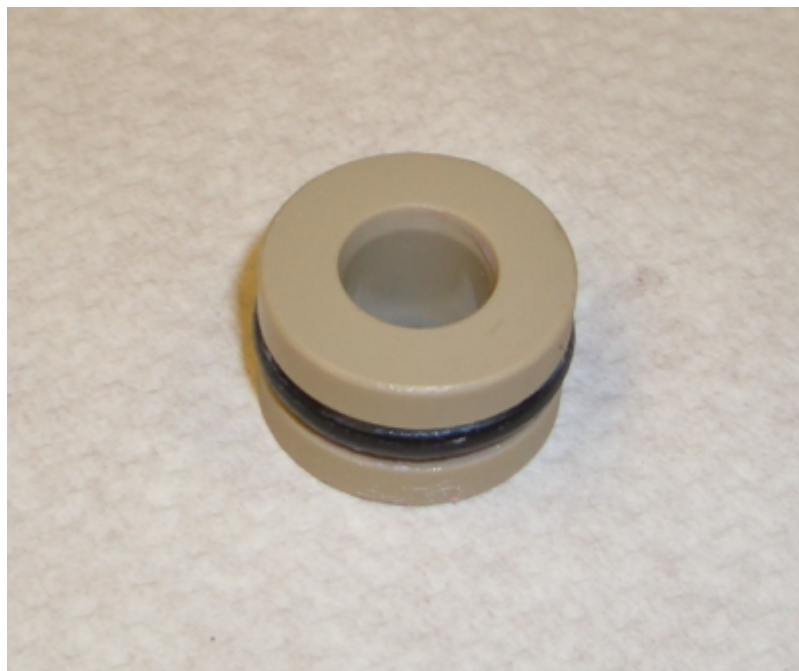
Rotary Bush - Inspect during service and replace as needed.

GEN-P005 - 8-Port Valve

Replace if worn and exhibits excessive cross-talk.

Syringe Coupling - Spares only.
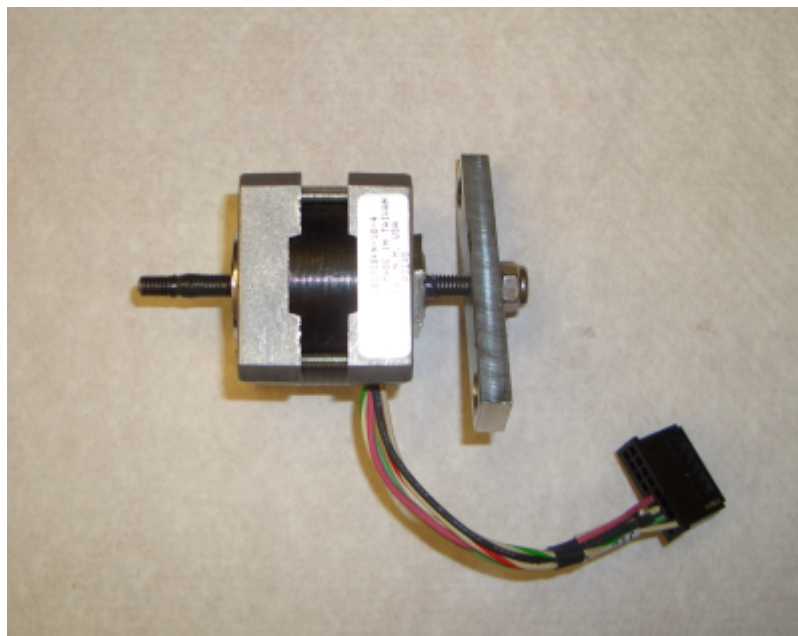
Syringe Motor and Retaining Bar - Spares only.

Valve and Syringe Assembly - Spares only.
Useful spare for quick turn-around between deployments.

14.6　Firmware Upgrades

From time to time you may wish to upgrade the firmware in your MicroLAB. New versions of firmware are posted for download on our website. If you are known to us as a user you will also received email bulletins each time a new version is posted.

Firmware is a program that is inserted into programmable read-only memory and becomes a permanent part of a computing device. The kernel is the corner-stone of the firmware. The firmware contains the basic command structure and the programming language, Eco-Script.
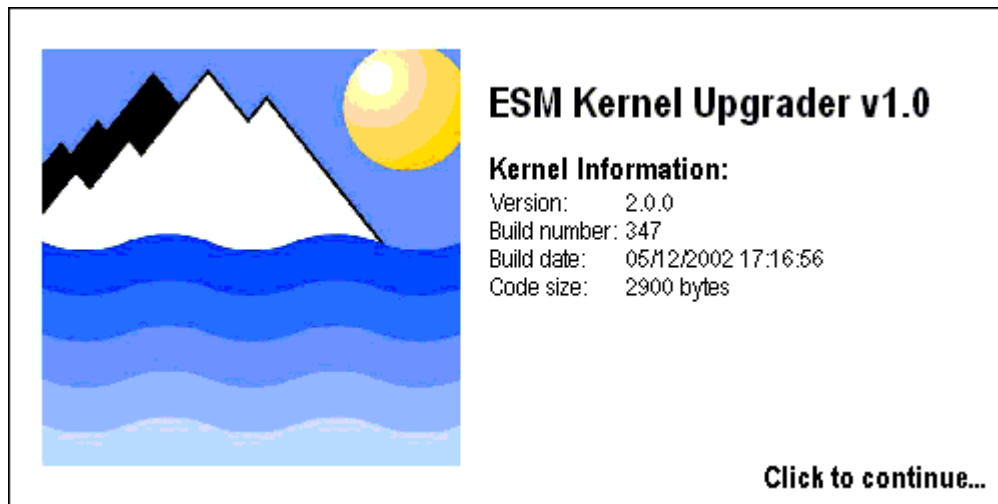
STEP 1
Upgrading the Kernel
- All versions of ESM 2.1.7 and earlier will first need the kernel upgrading before loading 2.1.8 and newer. If you have 2.1.8 or higher please go on to Step 2.
- Establish communications with your device using a terminal program (default baud 19200, N,8,1)
- Type firmwareupgrade[ENTER]
- Now close your terminal program completely
- Run (double-click on) the "ESM Kernel Upgrader" program, and click Load Kernel.
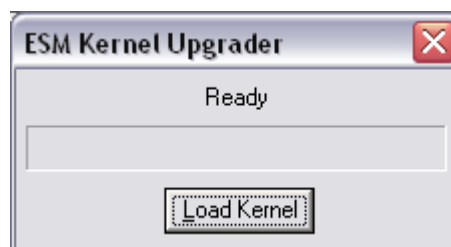
- After completion, turn off the ESM for 10 seconds.
- The "ESM Kernel Upgrader" program will not be needed in the future

There are two versions of firmware and firmware upgrader program. MicroLAB requires the firmware file ESMNV_2_x_xx.rec and the 7 MHz Firmware Upgrader Program.
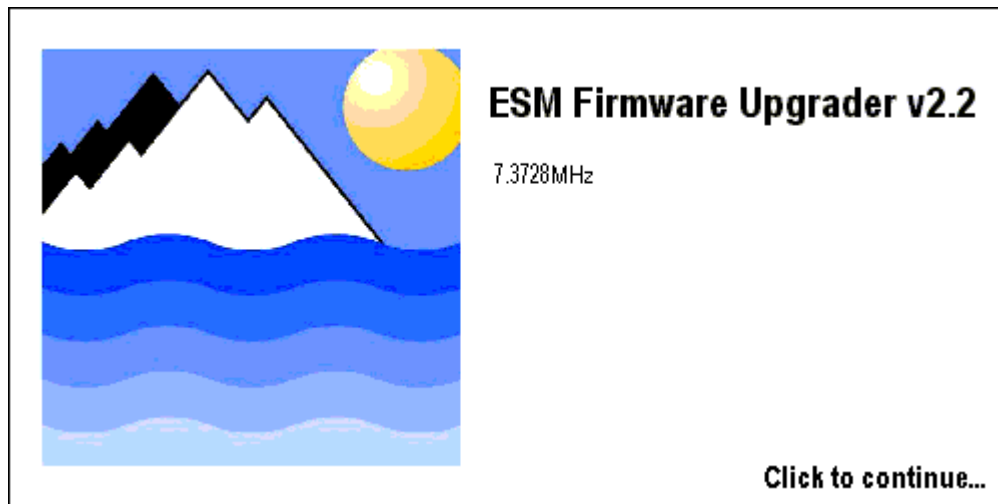
STEP 2
Upgrading the Firmware
There are four firmware files. Please ensure you have the correct type before proceeding.
1. Establish communications with your device using a terminal program (default baud 19200, N,8,1)
2. Type firmwareupgrade[ENTER]
3. Close your terminal program completely
4. Run the "ESM Firmware Upgrader" program.
5. Click Load Image, and select the appropriate *.rec file

7. Click "Flash".
8. Select the 57600 as the transfer baud rate (this doesn't need to be the same as the ESM is normally configured - they will auto negotiate)
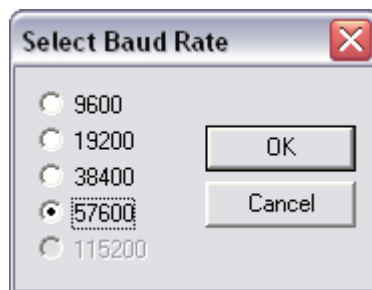
1. Once the upload is complete close the "ESM Firmware Upgrader" program
2. The "ESM Firmware Upgrader" program is no longer needed
3. Establish communications with your instrument in the normal way

You have successfully upgraded your ESM firmware

Troubleshooting

1. Make sure you are trying to install the correct version of firmware.
2. If the ESM Firmware Upgrader program fails after the Kernel has been upgraded connect to your ESM at 9600 baud and check that the message ROM FAILED is output. If is the case then the Kernel has been successfully upgraded. If ROM FAILED is not shown connect at your normal baud rate (19,200 baud by default) and type firmwareupgrade[ENTER]. Now proceed from step 2 a.
3. USB to RS232 converted cables are a known problem for the firmware upgrade application. Because these cables are not a true RS232 COM port and only an emulation, the interpretation of the UART specification for the COM port made by the USB converter manufacturer can prevent the upgrade from working. Use a built-in or PCMCIA card COM port to avoid this problem.

14.7   Factory Services

A range of factory services are available. These include:

- **Service**
  Ad hoc and routine / scheduled maintenance
- **Repair**
  Refurbish and upgrade
- **Test**
  Functional and pressure tests
- **Calibration**
  Linearity & repeatability
- **Deployment preparation**
  Chemical set-up and checks
- **Training**
  Standard and tailored courses
- **System engineering**
  Integration of sensors and hook-up with telemetry systems
- **Custom design**
  Pathlength, wavelength, chemistry, depth capability, etc.

Please contact us for further information on any of these services.

Section 15

15 Returning Equipment

Before returning equipment for inspection, repair or service you must request a Return Material Authorization (RMA) number. Please email help@n-virotech.com with details of your equipment and the return request.

Include the following information:

1. Your contact details
2. product/model
3. serial number
4. reason for return (e.g. repair)
5. description of the damage, defect or issue that requires attention

You will then be issues with an RMA sheet. Please put a copy of this sheet in the box when returning it.

We are committed to providing you with the best possible service. On receipt your equipment will be cataloged and inspected. Initially you will be sent a copy of our Equipment Receiving Record, followed later by an Inspection Report.

Unfortunately we are unable to accept items that are coated in anti-fouling paint for health and safety reasons.

Where possible and appropriate please include your deck-leads with the equipment so that we are able to perform a full system test.

You may request a status or progress report at any time using the ticket created by your original RMA request to help@n-virotech.com.

Section 16

16   Training Exercises

16.1   Manual Control

1. Start NutrientDATA 2 and switch on your power supply
2. Within 20 seconds press the "Manual Control" button on the main control panel. The Manual Control panel will be shown.
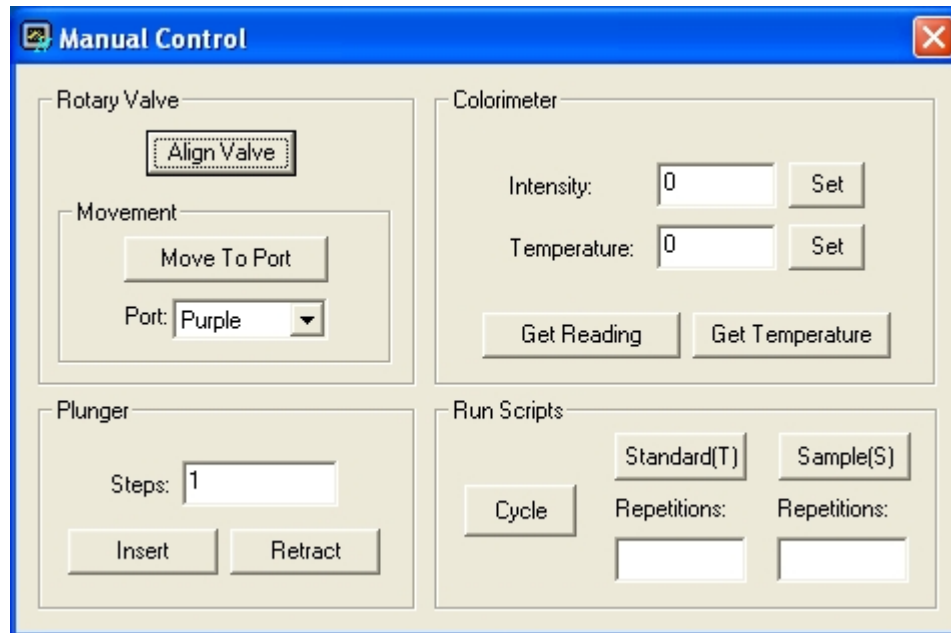
Valve and syringe movements

1. Ensure the detector inlet tube is connected to the blue port
2. Connect the inlet tube to the gray port
3. Immerse the inlet tube in a beaker of DIW or LNS
4. Put the detector outlet tube in a waste beaker
5. Press the Align Valve button (wait until this action is completed)
6. Select the Gray port from the drop down box
7. Press the "Move To Port" button
8. Enter 1000 in the Plunger Steps box
9. Press the "Retract" button (water will flow into the syringe)
10. Select the Blue port from the drop down box
11. Press the "Move To Port" button
12. Make sure 1000 is in the Plunger Steps box
13. Press the "Insert" button (water will flow into the detector)
14. Repeat from step 6.

Read colorimeter

1. Enter the correct setting for the connected MicroLAB into the colorimeter intensity box
2. Press the "Set" button (wait until this action is completed)
3. Press the Get Reading button

4.   Note the results

16.2   Reading Blanks

1.   Ensure the detector inlet tube is connected to the blue port
2.   Connect the inlet tube to the gray port
3.   Immerse the inlet tube in a beaker of DIW or LNS
4.   Put the detector outlet tube in a waste beaker
5.   Initiate the Prime(P) script as shown below.
6.   Enter the number of replicates required (typically 3 - 10)
7.   Monitor the results in the display box
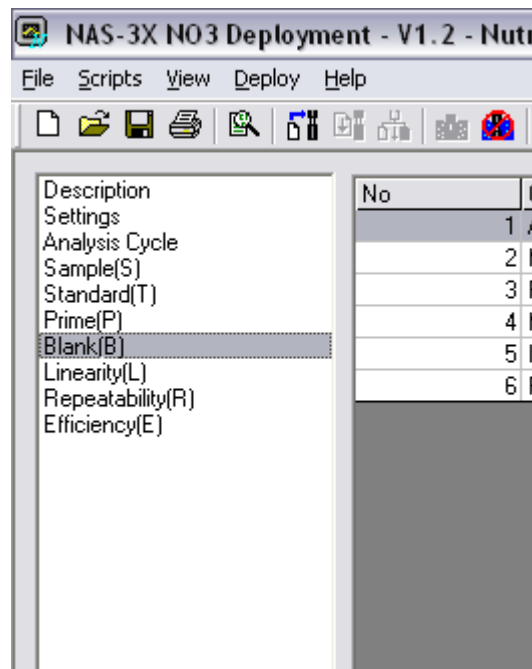
16.3   Downloading Data

1.   Connect-up the instrument
2.   Select "Data Processing" button from the Main Panel
3.   Click "Browse" and select a target folder for downloading the data to
4.   Enter a filename
5.   Click the "Extract" button
6.   The data will download
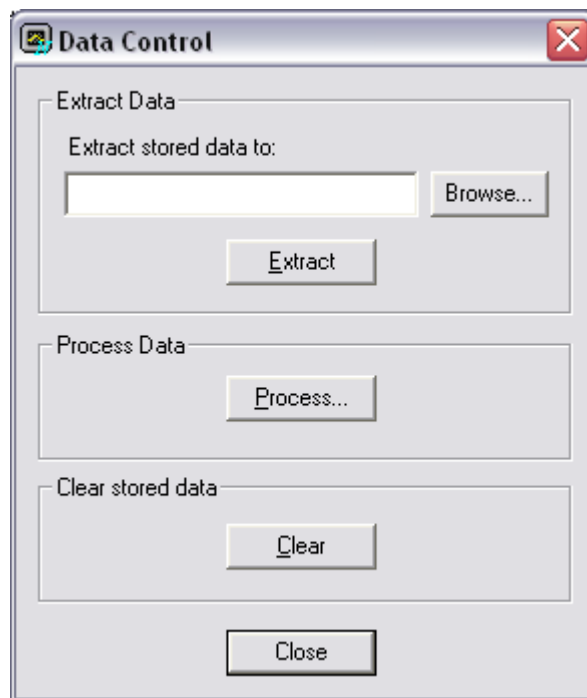
16.4 Priming Routine

1. Connect all reagent tubes
2. Place the inlet in a beaker of DIW
3. Put the colorimeter outlet in a waste beaker
4. If used the cadmium column tube should have the outside end in a beaker of imidazole (an empty plastic tube can be used in place of a cadmium column if desired).
5. Run the Prime(P) script as shown below.

16.5 Analyze Standards

1. Run through the Priming Routine exercise before running standards
2. If running nitrate either: A) activate and connect the cadmium column or B) use a nitrite standard and a length of tubing as a dummy column
3. Place the inlet in a beaker of DIW or LNS
4. Put the colorimeter outlet in a waste beaker
5. Initiate the Standard(T) script as shown below.
6. Enter the number of replicates required (typically 3 - 10)
7. Monitor the results in the display box
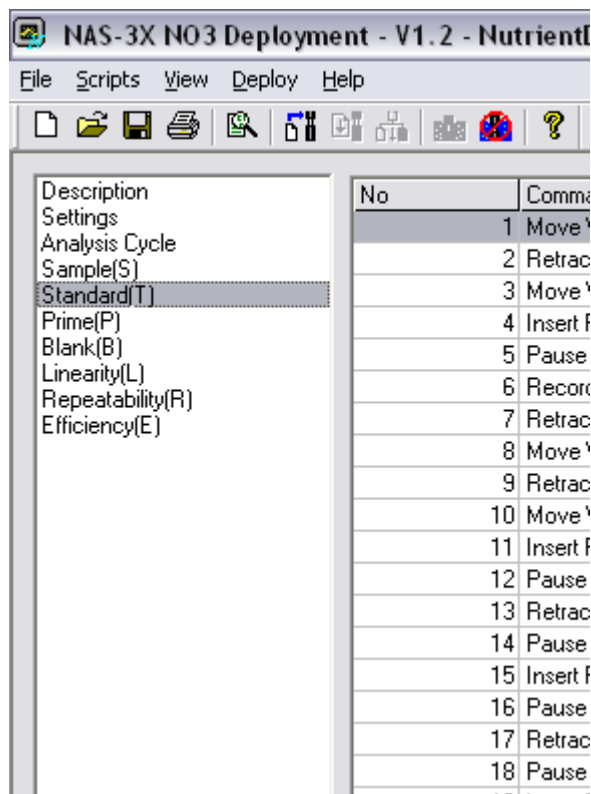
16.6 Analyze Samples

1. Run through the Analyze Standards exercise before running samples
2. If running nitrate either: A) activate and connect the cadmium column or B) use a nitrite standard and a length of tubing as a dummy column
3. Place the inlet in a known concentration test standard of DIW or LNS
4. Put the colorimeter outlet in a waste beaker
5. Initiate the Sample(S) script as shown below.
6. Enter the number of replicates required (typically 3 - 10)
7. Monitor the results in the display box
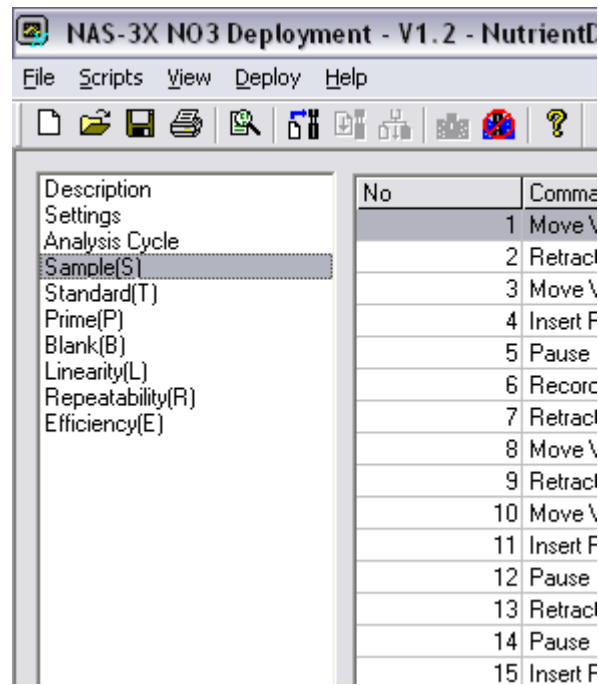
16.7 Settings

1. From the Script Designer select the Settings option.
2. Enter the settings as below, with an appropriate data and time and the correct Colorimeter Intensity for the instrument.
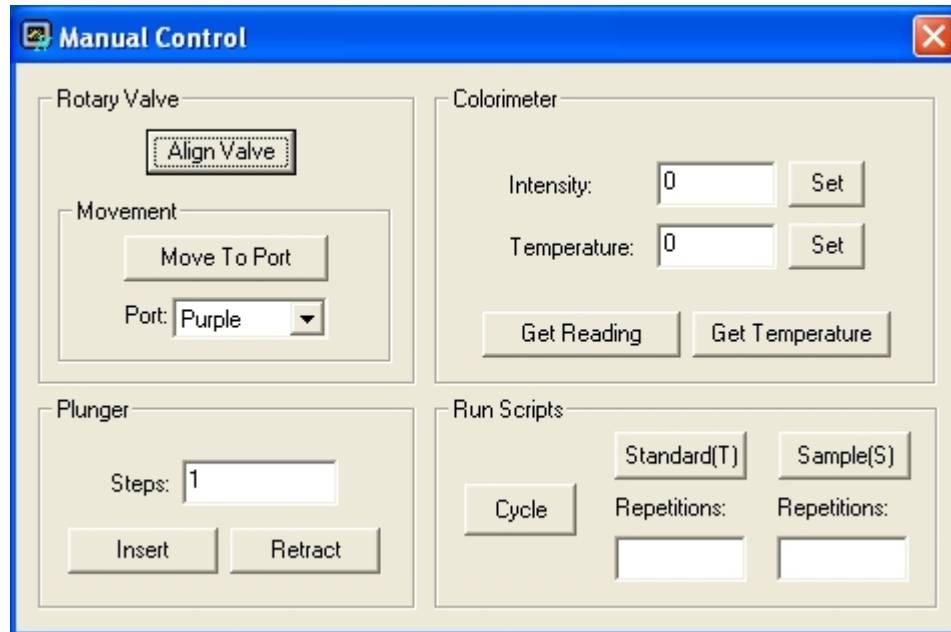3. Press OK.
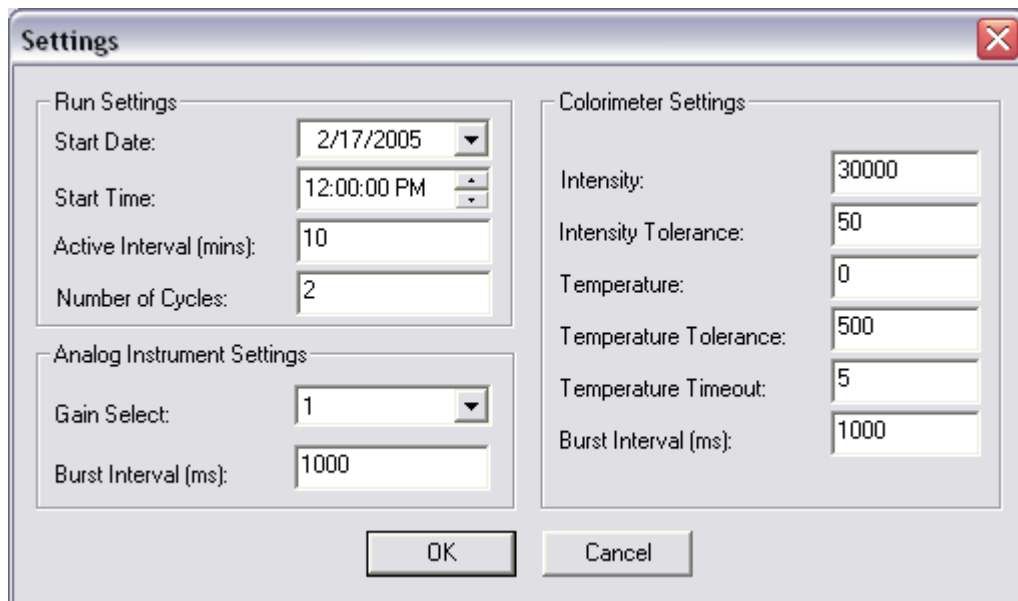
16.8 Run Cycle

This exercise will take 2 hours to complete
1. Complete the Standard, Samples and Settings exercises before Running the cycle

2. Select the Manual Control button from the Main Panel
3. Press the Cycle button
4. Monitor the data on the screen

16.9   Process Data

1. Work through the Downloading Data exercise immediately before processing data.
2. From the Data Control panel select the "Process" button.
3. Browse / enter a source file. This is the file you downloaded.
4. Browse / enter a destination file. This will be your calibrated file.
5. Enter the on-board standard concentration in the units you require (typically ug/l or uMol)
6. Press the "Process" button
7. Once complete, navigate to the Destination file with Windows Explorer and double-click on it. If you have Excel installed it will open in a spreadsheet. Otherwise open the file with a text editor.
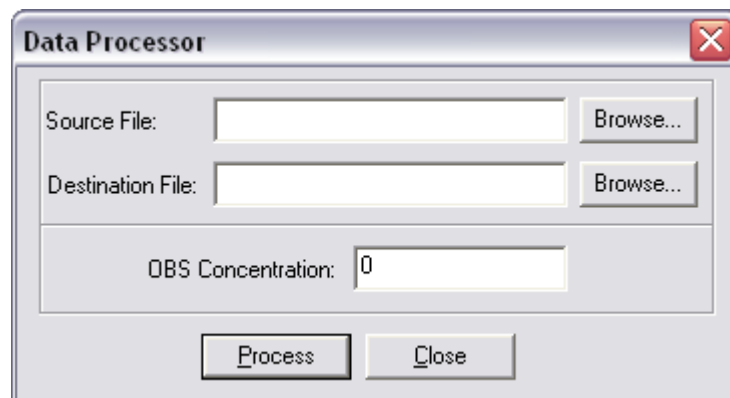
16.10 Flush Routine

1. Attach a length of tube to each of the valve nozzles and place the outside end of each one except the inlet tube in the waste beaker.
2. Place the inlet tube in a beaker of DIW or dilute (5%) DECON/CONTRAD solution if your system is dirty
3. Initiate the Flush(F) sequence.
4. Enter a number of replicates (Typically 5 - 10)
5. Once complete if you used DECON/CONTRAD repeat the process above with DIW.
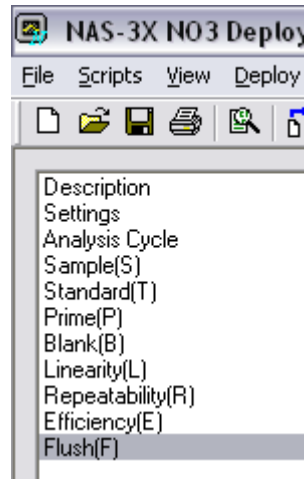
Section 17

17   Interfacing

MicroLAB can be interfaced to a host controller or telemetry system in one of four modes:

1. Constant power / asynchronous data
2. Switched power / asynchronous data
3. Constant power / polled data
4. Switched power / polled data

For modes 1 & 2 the master controller / datalogger will just "listen" for data. For modes 3 & 4 the host controller "asks" for data.

Mode 1 - Constant power / asynchronous data

```
At the start of the deployment MicroLAB is either simply powered-up and
allowed to "drop" into its automatic cycle or the command

       main.eco<CR>

is issued. Once started MicroLAB will perform the pre-configured chemical
analysis and stream data for each individual reading.

At the end of each analysis cycle the analyzer will "sleep" until the next
pre-programmed wake-up time or interval. During sleep the standby current
is 150 uA. In many respects this is the simplest interfacing method.
```

Mode 2 - Switched power / asynchronous data

```
In this mode each time an analysis is required the host controller must
initiate the sequence. The controller will first switch-on power to the
instrument. The instrument will then sign-on with a welcome message.

At this point the host can instruct the analyzer to perform one of many
functions. Typically this will be either to run a sample or standard
analysis sequence. The respective commands are:

       sample-s.eco<CR>

       standard.eco<CR>

During the analysis data will be streamed as each reading is taken.

At the end of the analysis the main prompt will be displayed and MicroLAB
can then switch off power to the unit.
```

Mode 3 - Constant power / polled data

```
At the start of the deployment MicroLAB is either simply powered-up and
allowed to "drop" into its automatic cycle or the command

       main.eco<CR>

is issued. Once started the unit will perform the pre-configured chemical
analysis and stream data for each individual reading.

At the end of each analysis cycle the download prompt will be displayed. At
this point the host controller will download either all or new data. There
```

are a variety for formats.

To poll the data the "extract" command is used. For example to retrieve a synopsis of all "new" data the following command is used:

        extract /store/flash/data,syn,new

This will download only new data since the last download from the /data folder, in synopsis format. This is a common option. The "graph" format is also often used. In this case the command is:

        extract /store/flash/data,graph,new

The graph format is compact and can be used if the host or on-shore "system" is aware of the exact data format and the current sequence (i.e. sample or standard).

After the download is complete the unit will return to the download prompt. At this point the host controller will close the download mode and allow the unit to continue with its automatic cycle. The command is:

        off<CR>

Mode 4 - Switched power / polled data

In this mode each time an analysis is required the host controller must initiate the sequence. The controller will switch power to the instrument. The instrument will then sign-on with a welcome message.

At this point the host can instruct the analyzer to perform one of many functions. Typically this will be to run a sample or standard analysis sequence. The respective commands are:

        sample-s.eco<CR>

        standard.eco<CR>

At the end of the analysis the main prompt will be displayed. The host may download either all or just the new data in a variety for formats.

To poll the data the "extract" command is used. For example to retrieve a synopsis of all new data the following command is used:

        extract /store/flash/data,syn,new<CR>

This will download only new data since the last download from the /data folder, in synopsis format. This is a common option. The "graph" format is also often used. In this case the command is:

        extract /store/flash/data,graph,new<CR>

The graph format is compact and can be used if the host or "system" is aware of the exact data format.

After the download is complete the host can then switch off power to MicroLAB.

Communications

All data and commands are ASCII and the baud rate is programmable from 300

```
to 1115.2k baud. The format is 8 bits, no parity, 1 stop bit.
```

```
The default communication configuration is 19200,N,8,1.
```

Prompts

```
Sign-on
```

```
        eESM-1
        Firmware Version: 2.1.27
```

```
Command mode prompt (for Mode 2 & 4)
```

```
        Admin:/store>
```

```
Download mode prompt (for Mode 3 & 4)
```

```
        Download time left: 280
        Download:/store>
```

Data formats

```
Streamed data (for Mode 1 & 2)
```

```
        @<date/time>,<sample
number>,<tag>,<chanw>,<chanx>,<chany>,<chanz><cr>
```

```
        example:
```

```
        @2004012016034267,1,[Bs-NO3],+12.7,31742,60684,12.1
```

```
Downloaded data (for Mode 3 & 4)
```

```
Synopsis
```

```
        21/10/2003 20:14:23 – Bs NO3
        Samples: 1, Interval: 1000ms
        Included Devices: "Source C0":SPS14, "Colour C0":SPS14
          31742,60684,12.1
```

```
Synopsis – Graph format
```

```
        21/10/2003 20:14:23,[Bs-NO3],+12.7,31742,60684,12.1
```

Section 18

18   ESM Manager 2

ESM Manager 2 (ESMM2) software is a Windows application that provides an easy to use interface for all our current products. Features include the ability to upload, view and run scripts as well as extract data, manage folders and files, directly control peripherals and set the integral real-time clock. ESMM2 eliminates the need to used third party terminal emulators and learn the system command syntax.

To operate any of our products you will also have to use or be familiar with the appropriate Operating Manual. If you intend to modify or develop your own scripts then you will need to refer to the Eco-Script Manual for details of the scripting keywords, syntax and conventions.

Quick Reference

You will quickly become familiar with the ESMM2 interface and the toolbar buttons. For those that prefer a very quick start the buttons are described below. Each button is also replicated as a menu command if menus are preferred. The interface is designed to me intuitive and has a minimal learning curve. Each button also shows a "tool tip" when you "mouse over".

Connect | Disconnect | Show Files | Delete | Create Folder | View File | Upload | Download | Extract | Run | Direct Commands | Stop

ESMM2 is free software for use with our products and is provided with a full manual on the CD supplied with your equipment. It is supported and will be updated from time to time. Please see our website for updates.

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