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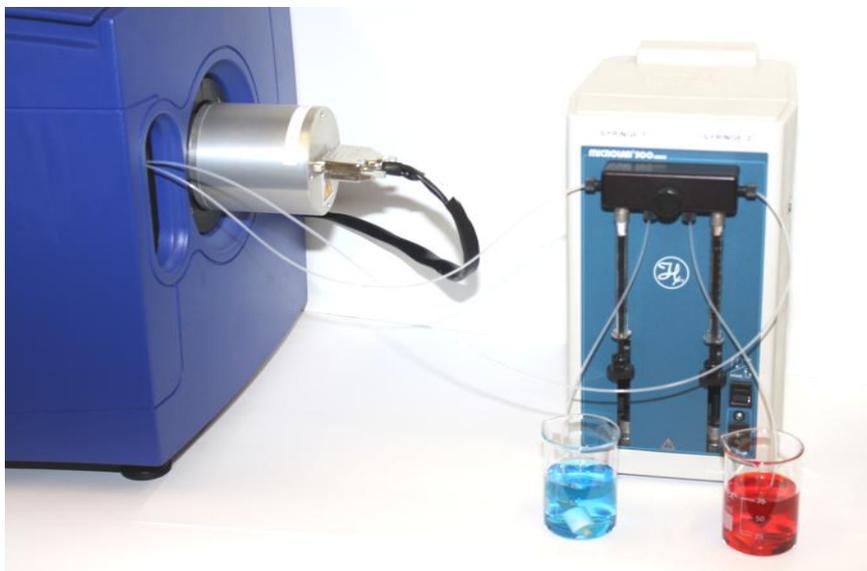
*Ultrasensitive Spectroscopy for the Life Sciences*

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## User Manual Chirscan CS/TT

**Chirscan Series Dual Syringe Automated Titration Unit**  
February 2012

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This document contains important safety information. Read this document and the Chirascan series User Manual before attempting to install or use the CS/TT accessory. Failure to do so could result in death or serious injury.

## USE OF THIS DOCUMENT

This document is intended to inform the operator of Applied Photophysics' CS/TT Dual Syringe Automated Titration unit on its design, installation and operation with either the Chirascan or Chirascan-plus spectrometer. The titrator unit is supplied by the Hamilton Company, Nevada, U.S.A, and this document should be used in conjunction with the User Manual supplied by the Hamilton Company, and that applicable to the spectrometer. It is assumed that the user of this document is familiar with the operation of the Chirascan or Chirascan-plus, and with Applied Photophysics Pro-Data software. In particular it is assumed that the user is familiar with the hazards associated with the operation of the spectrometer, and has read the hazard and other safety information contained in its User Manuals and those supplied by the Hamilton Company.

Note that the CS/TT is normally used with the CS/PCS single cell temperature control accessory, and the some of the images of the interior of the spectrometer Sample Handling Unit show this unit in place.

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The sign to the left is used to indicate a hazardous situation, which, if not avoided, could result in minor or moderate injury.

### OTHER INFORMATORY INDICATORS USED IN THIS DOCUMENT



The sign to the left is used to indicate a situation which, if not avoided, could result in damage to the instrument.

## ESSENTIAL SAFETY INFORMATION

MAKE SURE THAT YOU HAVE READ AND UNDERSTAND ALL THE SAFETY INFORMATION CONTAINED IN THIS DOCUMENT BEFORE ATTEMPTING TO INSTALL OR OPERATE THE CS/TT. IF YOU HAVE ANY QUESTIONS REGARDING THE OPERATION OF THE ACCESSORY, PLEASE CONTACT APL TECHNICAL SUPPORT SECTION AT THE ADDRESS SHOWN ON THE FIRST PAGE OF THIS DOCUMENT.

OBSERVE ALL SAFETY LABELS AND NEVER ERASE OR REMOVE SAFETY LABELS.

PERFORMANCE OF INSTALLATION, OPERATION OR MAINTENANCE PROCEDURES OTHER THAN THOSE DESCRIBED IN THIS USER MANUAL MAY RESULT IN A HAZARDOUS SITUATION AND WILL VOID THE MANUFACTURERS WARRANTY.

### **WARNING**

The Chirascan spectrometer is powered by the mains electricity supply which can produce a shock leading to serious injury or death. Do not connect or disconnect the instrument from the mains supply unless the supply is powered off at source. Ensure all communications and electrical connections are made before powering on the spectrometer. Exercise care during operation and do not operate units with their covers removed. Operate the spectrometer using only the cables provided. Never operate a spectrometer with damaged cables.

### **CAUTION**

The CS/TT has moving syringes that could trap hands, clothing or other items, causing injury to the user. Keep hands, clothing and other items clear of the syringes during operation.

### **NOTICE**

Corrosive chemical and organic solvents can cause damage to the CS/TT. Do not allow corrosive fluids to come into contact with any part of the accessory. Do not clean the accessory with organic solvents. Use only a soft cloth and water or a mild detergent solution.

## **CS/TT INSTALLATION AND OPERATIONAL REQUIREMENTS**

### Environmental and electrical requirements

The CS/TT has no environmental requirements additional to those of the Chirascan or Chirascan-plus spectrometer. Operation of the CS/TT is controlled by the Chirascan electronics, and there are no additional electrical requirements.

### Bench space

A minimum of 250 mm bench space is required to the right of the Chirascan spectrometer.

### Nitrogen purge gas

The CS/TT has no purge gas requirements additional to those of the Chirascan spectrometer.

### Servicing

Servicing of the CS/TT should only be undertaken by qualified personnel. If you are in any doubt at all please contact the Applied Photophysics Technical Support Department at the address shown on the front of this User Manual.

## GLOSSARY

The following abbreviations may be found in this User Manual

APL	Applied Photophysics Ltd.
ESHU	Equilibrium sample handling unit
SHU	Sample handling unit

## HYPERLINKS

This document contains hyperlinks between references (for example the Contents tables, or references to Sections or Figures in the text), and sources. To follow a link, place the cursor over the reference and use **CTRL+click**. Hyperlinks in the text are indicated by underlined [blue](#) font.

## CONTENTS

USE OF THIS DOCUMENT.....	4
HAZARD AND OTHER INDICATORS.....	5
ESSENTIAL SAFETY INFORMATION.....	6
CS/TT INSTALLATION AND OPERATIONAL REQUIREMENTS .....	7
GLOSSARY .....	8
CONTENTS .....	9
FIGURES .....	10
1 INTRODUCTION .....	11
2 OPERATING PRINCIPLES .....	12
3 INSTALLATION .....	14
3.1 Hardware installation.....	14
3.1.1 Installing electrical connections.....	14
3.1.2 Communications settings.....	15
3.1.3 Installing and removing the valve assembly .....	16
3.1.4 Installing and removing syringes .....	16
3.1.5 Installing the blanking plate.....	16
3.1.6 Installing the flow tubing .....	17
3.1.7 Connecting to the sample cell.....	18
3.2 Software Set-up .....	20
3.2.1 The Select SHU panel .....	20
3.2.2 The Titrator - Syringe Configuration panel .....	21
4 OPERATION.....	23
4.1 Manual Operation.....	23
4.1.1 Using the step/prime switch.....	23
4.1.2 Using the control software .....	23
4.1.3 Picking up and dispensing through the same port .....	24
4.1.4 Transferring fluid from one vessel to another .....	24
4.1.5 The Status panel .....	24
4.2 Using the Protocols .....	25
4.2.1 Additional of solution 1 to specified volumes .....	26
4.2.2 Withdrawal / addition to specified concentrations .....	26
4.2.3 Withdrawal / addition by specified volumes .....	26
4.2.4 The auto correct function .....	26
4.3 Performing a Titration.....	27

## FIGURES

Figure 1.1: the CS/TT titration unit.....	11
Figure 2.1: main components of the titrator .....	12
Figure 2.2: schematic of the syringe and valve arrangement of the CS/TT. Arrows indicate the usual direction of sample flow .....	13
Figure 3.1: electrical connections to the CS/TT .....	14
Figure 3.2: the Titrator Configuration File .....	15
Figure 3.3: communications settings .....	15
Figure 3.4: CS/TT blanking plate and plug .....	16
Figure 3.5: flow tubing tightening tool .....	17
Figure 3.6: tubing between titrator and sample cell .....	17
Figure 3.7: tubing between the titrator and reservoir or waste .....	18
Figure 3.8: position of the tubing within the sample cell .....	18
Figure 3.9: connection of flow tubing to sample cell .....	19
Figure 3.10: the interior of the ESHU after connection of the CS/TT .....	19
Figure 3.11: the fully assembled CS/TT accessory .....	20
Figure 3.12: the Select SHU panel .....	20
Figure 3.13: the Titrator – Syringe Configuration dialog box .....	21
Figure 3.14: the Syringe – Advanced Setting dialog box.....	22
Figure 4.1: the control panel .....	23
Figure 4.2: the Status panel.....	24
Figure 4.3: the Titration dialog box .....	25

## 1 INTRODUCTION

The CS/TT Titration Accessory is designed to be used with the Chirascan and Chirascan-plus spectrophotometers. The CS/TT is a fully programmable, high accuracy titration system that enables automated measurement of the concentration dependent circular dichroism, fluorescence or absorbance of a sample. The titrator, shown in Figure 1.1, is a Microlab 541C supplied by Hamilton Company, Nevada, U.S.A. Fittings for use with the Chirascan are supplied as part of the accessory.

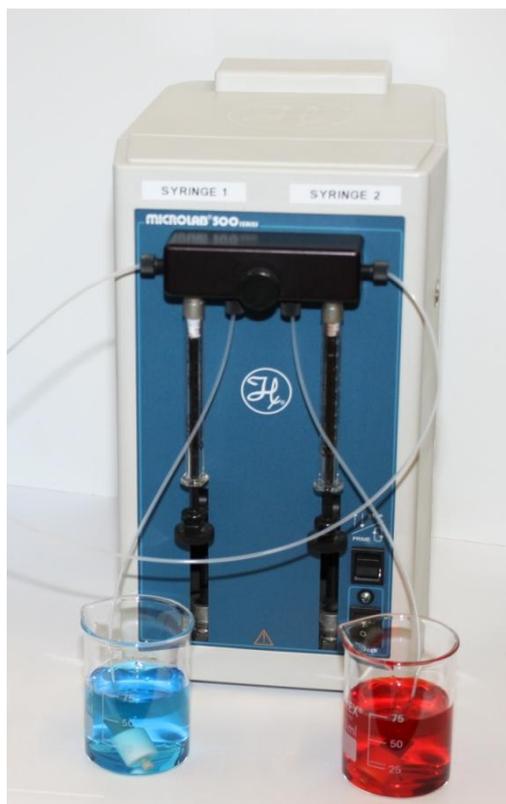


Figure 1.1: the CS/TT titration unit

The titrator is a dual syringe system capable of accurately dispensing a set volume of solution to a sample cell, or withdrawing a set volume from the cell, and can be programmed to vary the concentration of solution systematically, while maintaining a constant volume. The circular dichroism or other spectroscopic properties of the sample can be monitored following each change in concentration.

## 2 OPERATING PRINCIPLES

The main components of the titrator are labelled in Figure 2.1.

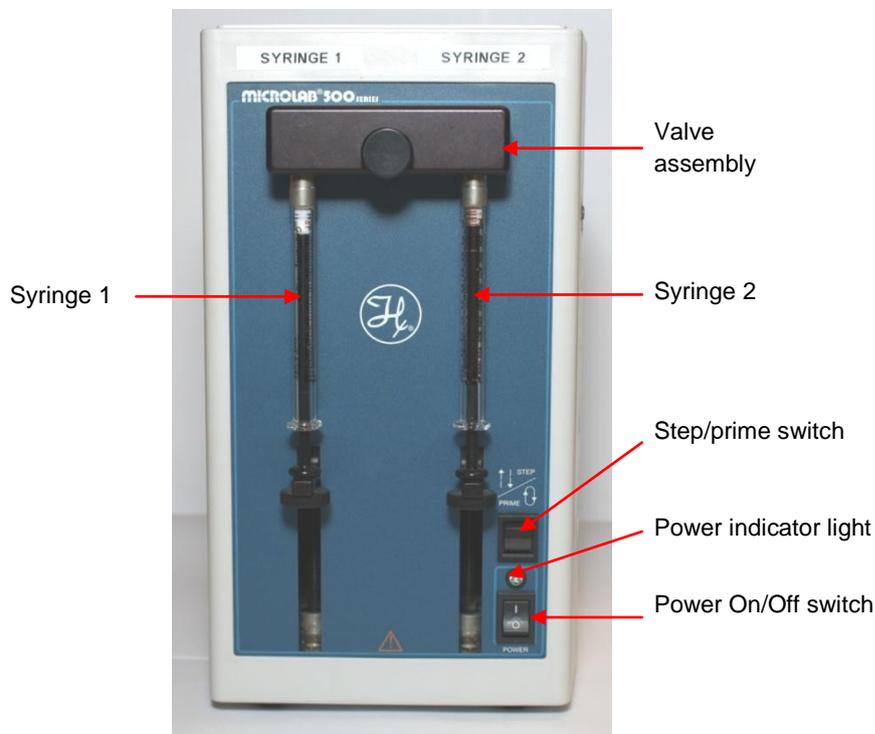


Figure 2.1: main components of the titrator

The titrator is a dual-syringe system, the syringes being driven by high-precision stepper motors. Each syringe is connected to a 3-way valve, mounted within a valve assembly. Valve settings are **In**, **Out** and **Wash**: the **In** setting connects the syringe to the input (side) port on the dispenser assembly, **Out** connects the syringe to the output (underside) port, and **Wash** connects the syringe to both ports.

Normally the input ports are connected to the Chirascan sample cuvette, the output ports to a sample reservoir or waste vessel. Although the syringe action and valve settings can be controlled manually through Pro-Data control software, it is usual to use Syringe 1 (left-hand side) to add aliquots of titrant to a solution of reagent in a cuvette; syringe 2 is used to remove reagent from the cuvette. The syringes are driven by high-precision stepper motors. A schematic of this arrangement is shown in Figure 2.2.

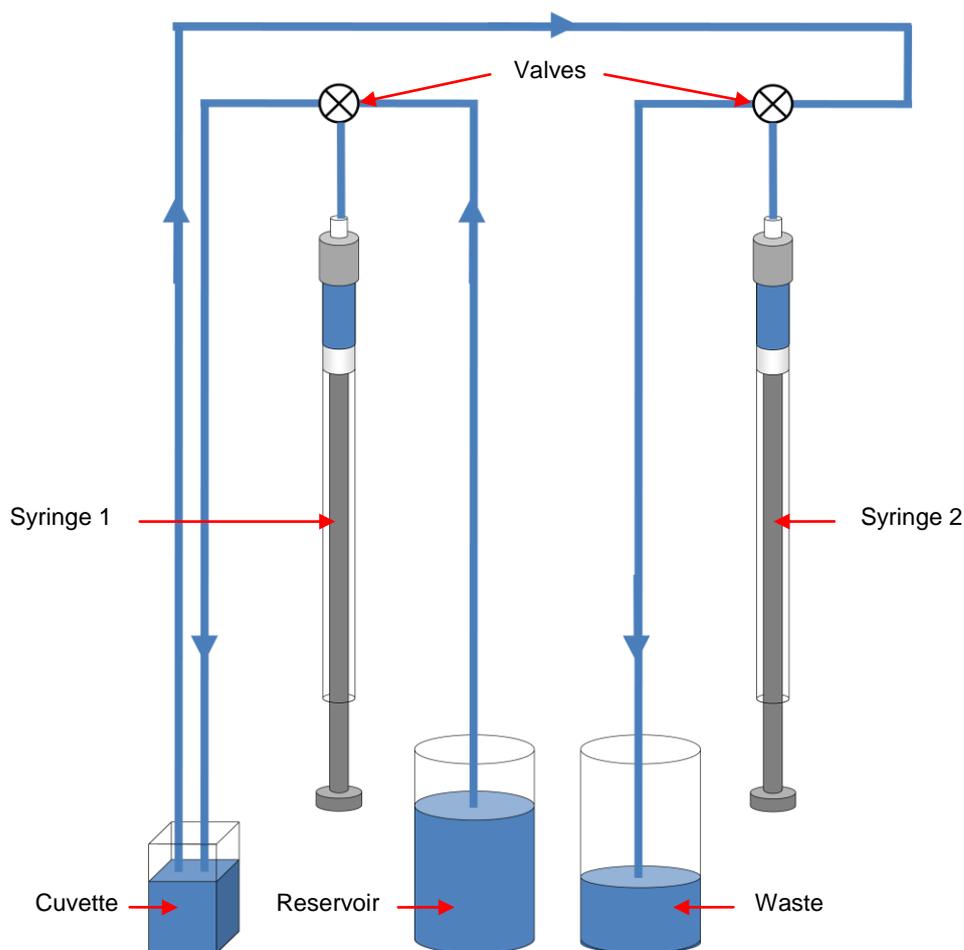


Figure 2.2: schematic of the syringe and valve arrangement of the CS/TT. Arrows indicate the usual direction of sample flow

The action of the syringes and valve positions can be controlled manually in the Pro-Data software, or through three programmable protocols.

The first protocol is a single syringe protocol, in which sample is added to the cuvette in fixed volumetric increments.

The second protocol is a dual syringe protocol, in which sample is added to or withdrawn from the cuvette to achieve concentrations increasing or decreasing in fixed increments.

The third protocol sample is a dual syringe protocol in which sample is added to or withdrawn from the cuvette in set amounts to achieve target volumes.

When these protocols are used, the left hand syringe (Syringe 1) transfers sample from the reservoir to a cuvette, the right hand syringe (Syringe 2) transfers sample from the cuvette to a waste receptacle.

### 3 INSTALLATION

This Section describes the hardware and software installation and set-up of the CS/TT. Please read through before beginning the installation.

#### 3.1 Hardware installation

##### 3.1.1 Installing electrical connections.



The CS/TT operates at mains voltages which could cause severe electric shock. Do not connect or disconnect any electrical leads without first ensuring that they are powered off at the mains socket.

Electrical connections are made to the rear of the CS/TT unit (Figure 3.1).

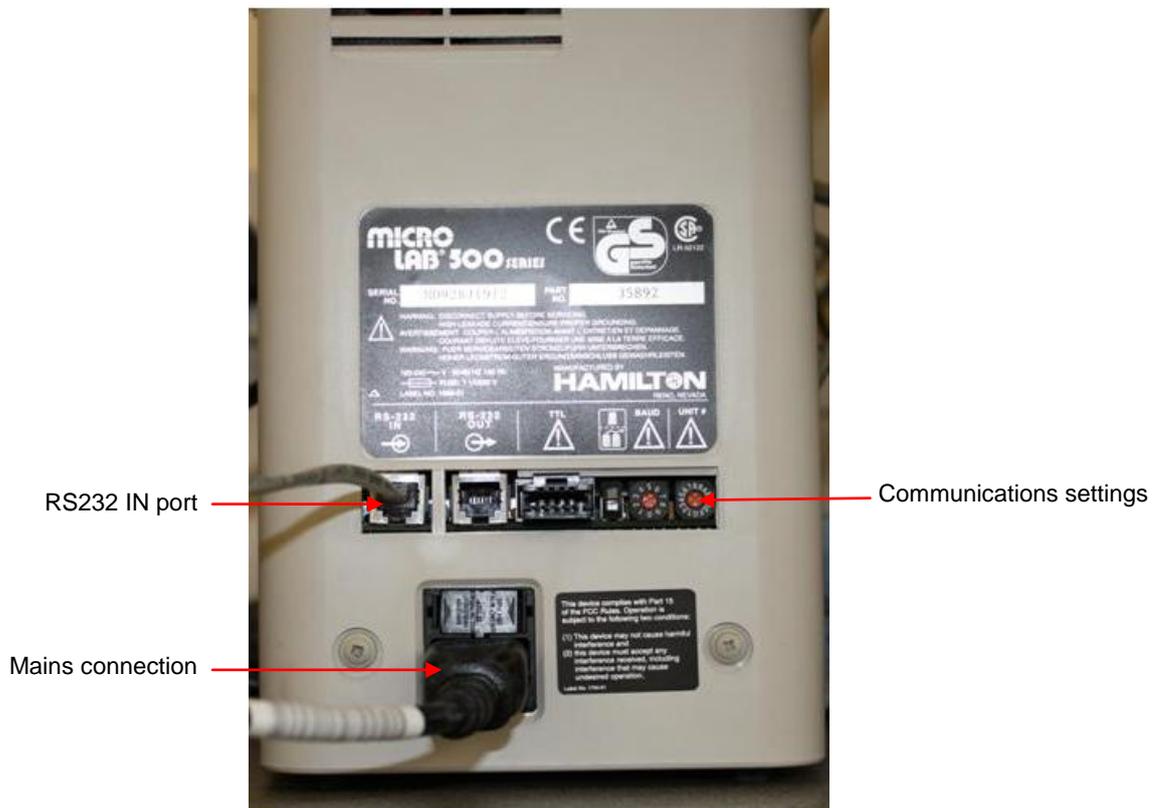


Figure 3.1: electrical connections to the CS/TT

1. Plug the mains cable into the power cable socket. The mains cable fits only one way.
2. Connect the RS232 IN port to any USB port on the computer using the RS232 cable and RS232/USB adaptor provided.
3. It is now necessary to set the USB port number in the **Titration** Configuration File. This is stored in the **Program Files\Applied Photophysics\Chirascan\Config\Devices** folder, and can be opened, for

example, with Microsoft Notepad. Open the file and set the port number where indicated, as in Figure 3.2 (the port is set as 4 in the figure), then save the file.

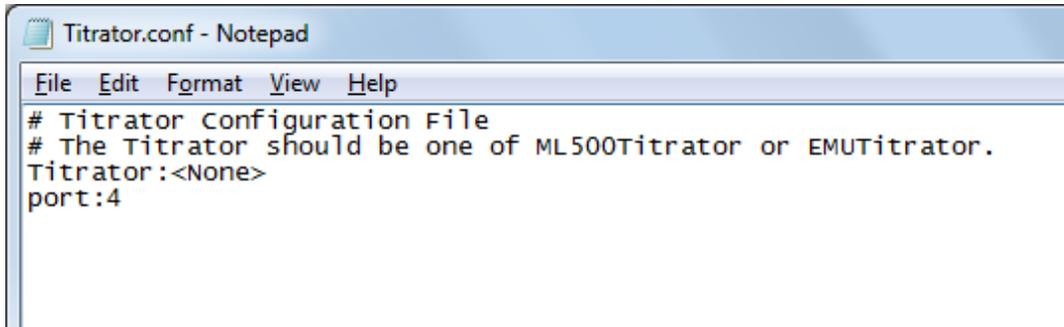


Figure 3.2: the Titrator Configuration File

4. Power on and switch on the CS/TT using the Power On/Off switch on the front of the unit.

### 3.1.2 Communications settings

The communications settings are set on the rear of the CS/TT unit (Figure 3.3).



Figure 3.3: communications settings

Set the loopback, Baud rate and Protocol switches as described below. Before setting the switches, switch off the CS/TT. To change the position of the Baud rate and protocol switches, use a small screwdriver. The position indicator on these switches is the raised white pointer.

1. Set the loopback switch to the **Up** position, which specifies a single unit.
2. Set the Baud rate switch to the **3** position, which sets the Baud rate to 9,600.
3. Set the protocol switch to **0**, which specifies a single unit.

### 3.1.3 Installing and removing the valve assembly

It is not normally necessary to remove or install the valve assembly, but the user may wish to remove it for cleaning. To install the assembly, follow these steps.

1. Ensure that the PTFE Luer fittings that attach to the syringes are in place.
2. Insert the valve assembly into the valve motor drive ports and press the valve assembly into place.
3. Tighten the thumbscrew until it is finger-tight to secure the valve assembly to the CS/TT unit.

To remove the valve assembly, perform steps 2 and 3 in reverse.

### 3.1.4 Installing and removing syringes

Syringes recommended for use with the CS/TT are the 1000 series with PTFE Luer Lock termination and PTFE tipped plunger, supplied by Hamilton Company, Reno, Nevada, U.S.A. These are available in volumes from 1 ml to 100 ml. As a general rule, in order to achieve high accuracy and precision, it is preferable to use more than 10% of the syringe's volume. If the application requires less than 10% of the total volume, a smaller syringe should be used.

To mount the syringe, use the following procedure:

1. Switch on the CS/TT unit using the Power On/Off switch.
2. Press and hold the Step/Prime switch in the Step position until the drive arm is about half-way between the Home (up) and Down positions.
3. Fasten the thumbscrew on the drive arm to the bottom of the syringe plunger by holding the plunger with one hand and screwing the thumbscrew into the threaded fitting with the other.
4. Pull the glass syringe barrel upwards, and insert the male syringe tip into the threaded female Luer fitting on the underside of the valve assembly.
5. Hold the syringe in position by rotating the metal syringe ferrule clockwise (i.e. front moves from left to right) until it is finger tight.

### 3.1.5 Installing the blanking plate

Replace the blanking plate on the Chirascan ESHU to the left of the detector with the plate shown in Figure 3.3. The plate is held in place from within the ESHU by 4 caphead screws. The plug is for use when the titration accessory is not installed.

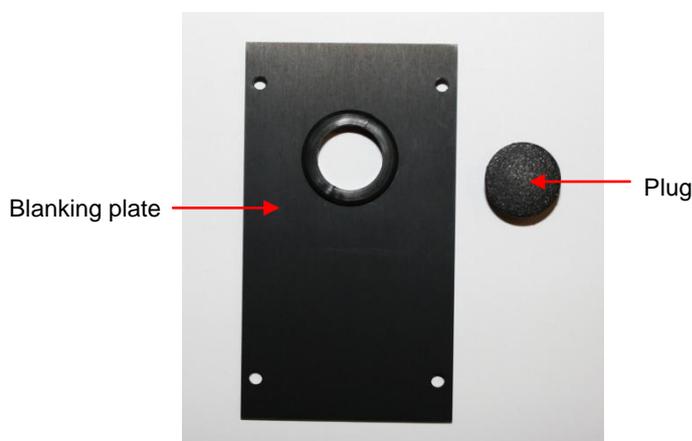


Figure 3.4: CS/TT blanking plate and plug

### 3.1.6 Installing the flow tubing

The flow tubing used is 1 mm internal diameter PTFE microtubing. The flow tubing tightening tool used with the screw connectors is shown in Figure 3.5.

**NOTICE** Overtightening the fluid tubing connectors can strip the threads and cause leakage. Use only firm finger tightness or the tool provided, do not use pliers or other tools.



Figure 3.5: flow tubing tightening tool

The tubing connecting the titrator to the sample cell is shown in Figure 3.6. Connect the longer (950 mm) length of tubing to the input (side) port of syringe 2 of the valve assembly, using the captive screw-in connector. Connect the shorter (700 mm) length to the input port of syringe 1.

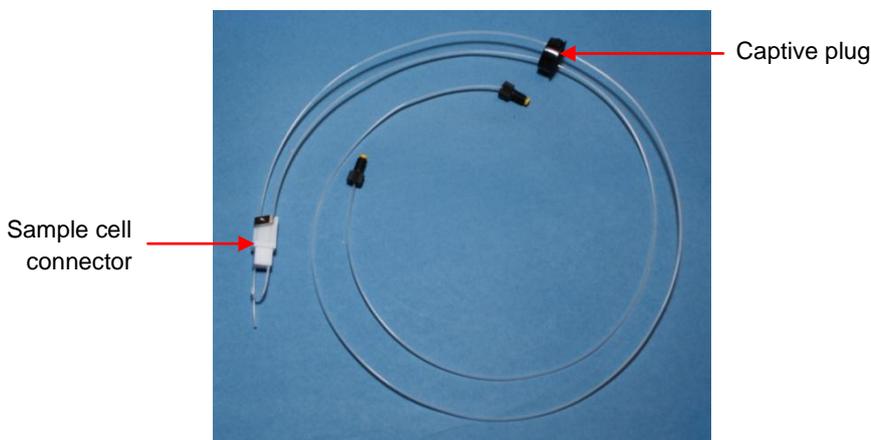


Figure 3.6: tubing between titrator and sample cell

The tubing connecting the titrator to the sample reservoir or waste receptacle is shown in Figure 3.7. The two lengths of tubing are the same, and connect to the two output (underside) ports of the valve assembly. The ends with the sinkers go to the reservoir or waste receptacle.

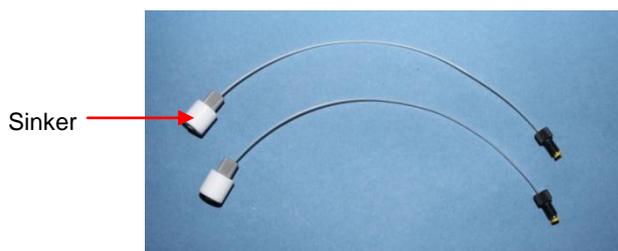


Figure 3.7: tubing between the titrator and reservoir or waste

### 3.1.7 Connecting to the sample cell

When the flow tubing connections to the titrator have been made, the connections can be made to the sample cell. A 10 mm x 10 mm fluorescence cuvette is recommended for use with the CS/TT.

Since for some titrations, the whole volume of solution in the cell needs to be withdrawn, the withdrawal tubing, connected to Syringe 2, needs to reach to the bottom of the cell. The addition tubing, connected to Syringe 1, has a hooked end to prevent the formation of drops.

Within the cell, the plain end ended tubing should reach as closely as possible to the bottom of the cell, the hooked ended tubing should reach to about 15 mm from the bottom, and the tubing should lie along the edges of the cell so that it does not obstruct the light beam, as shown in Figure 3.8. These positions can be checked in the cell before installation. To adjust the tubing, first slacken off the screw holding the stainless steel clamp; when re-tightening, the clamp should hold the tubing firmly enough to prevent it slipping or rotating, but without crushing or distorting it.



Figure 3.8: position of the tubing within the sample cell

Replace the sample cell holder with 10 mm x 10 mm cell holder with 3 mm wide aperture supplied with the CS/TT: the narrow aperture is used to prevent the tubing within the cell intruding on the light beam.

Remove the plug from the CS/TT blanking plate on the Chirascan ESHU, and carefully pass the sample cell connector through the hole in the plate.

Place the cell in cell holder, and insert the cell as shown in Figure 3.9, making sure that the tubing does not intrude on the light beam (for clarity the cell holder is shown removed from the ESHU, but it may be helpful to remove the holder from the ESHU and look through the aperture to check the position of the tubing).

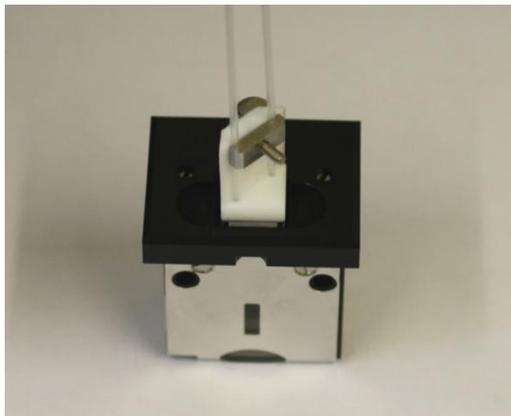


Figure 3.9: connection of flow tubing to sample cell

Insert the plug captive on the tubing into the hole on CS/TT blanking plate. The interior of the ESHU should appear as in Figure 3.10.

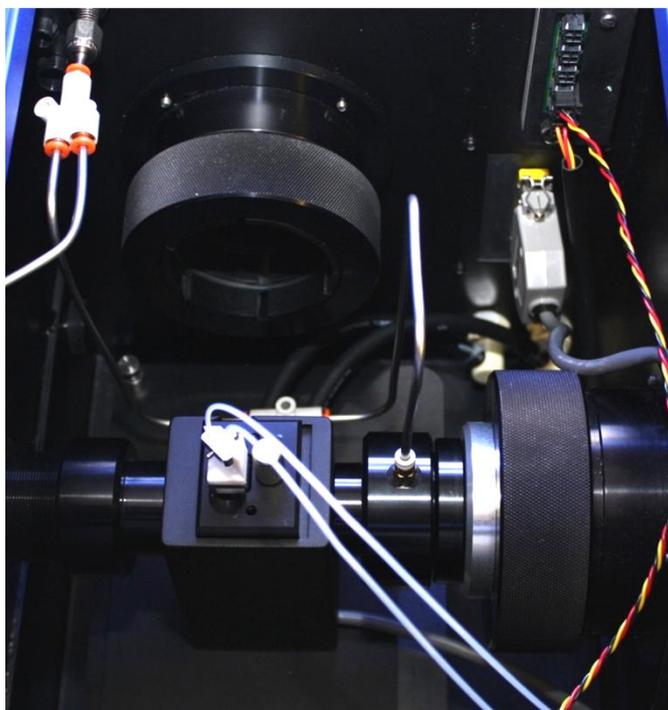


Figure 3.10: the interior of the ESHU after connection of the CS/TT

The fully assembled CS/TT accessory should appear as in Figure 3.11.



Figure 3.11: the fully assembled CS/TT accessory

## 3.2 Software Set-up

### 3.2.1 The Select SHU panel

On the **Pro-Data Chirscan** main panel, from the **Configuration** menu, click **Change Sample Handling Unit (SHU)** to display the **Select SHU** panel (Figure 3.12), and select **Equilibrium Sample Handling Unit (ESHU)**,

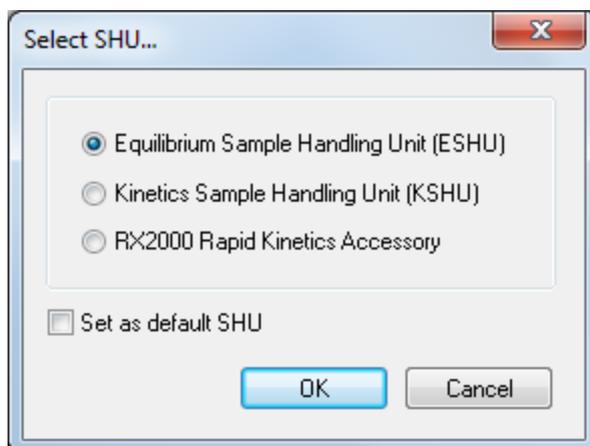


Figure 3.12: the Select SHU panel

### 3.2.2 The Titrator - Syringe Configuration panel

On the **Pro-Data Chirscan** main panel, from the **View** menu, click **Devices** and then double-click on the Titrator icon  to display the **Titrator – Syringe Configuration** panel (Figure 3.13).

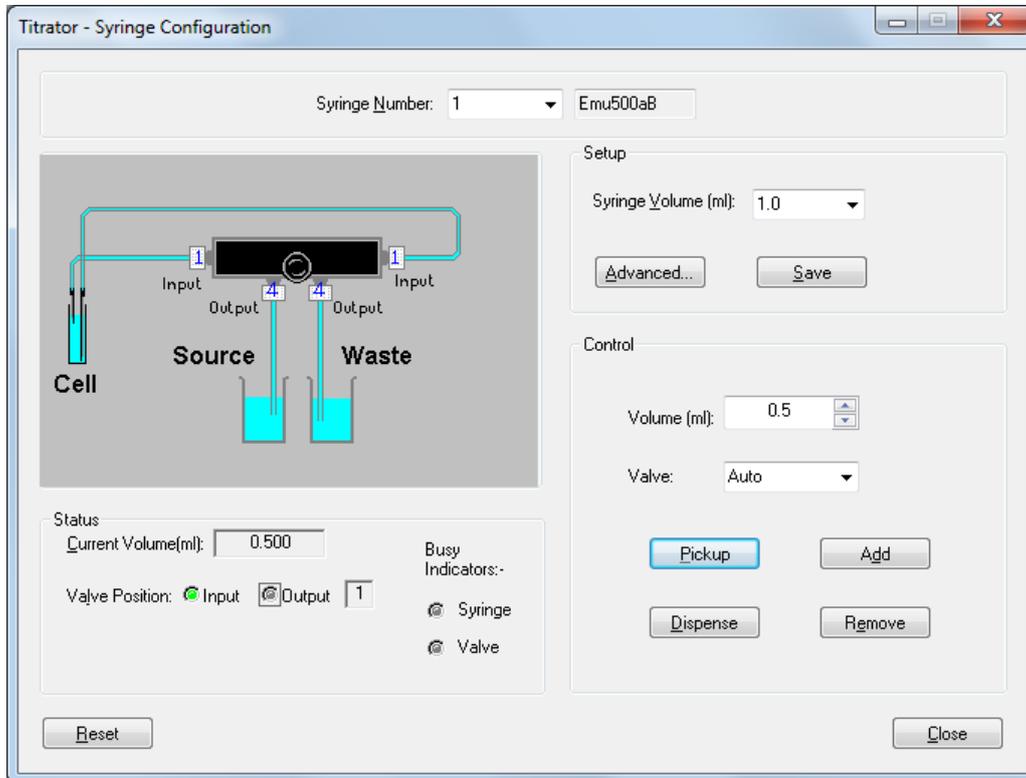


Figure 3.13: the Titrator – Syringe Configuration dialog box

The volume of each syringe is entered on this panel. Select **1** from the drop-down list of the **Syringe Number** box at the top of the panel, and enter or select from the drop down list the volume of the syringe in the **Syringe Volume** box in the **Setup** panel.

Clicking the **Advanced** button on the **Setup** panel will cause a password prompt to appear. It is unlikely that you will need to change any of the settings in the **Syringe - Advanced Settings** dialog box which is displayed when the password is entered (Figure 3.14), as these govern syringe speed, backlash correction and resolution, but if you do, please contact APL technical support for the service password and advice.

The **Drive speed** setting controls the rate at which the syringe plunger is driven. Syringe **Return Steps** are used to compensate for the mechanical system backlash, to improve accuracy and precision. Return steps are added to all downward movements of the syringe, the same number of upwards being immediately performed. **Backoff Steps** are the number of steps away from the initialization overload position that the zero position will be set at, and is also used to improve accuracy and precision.

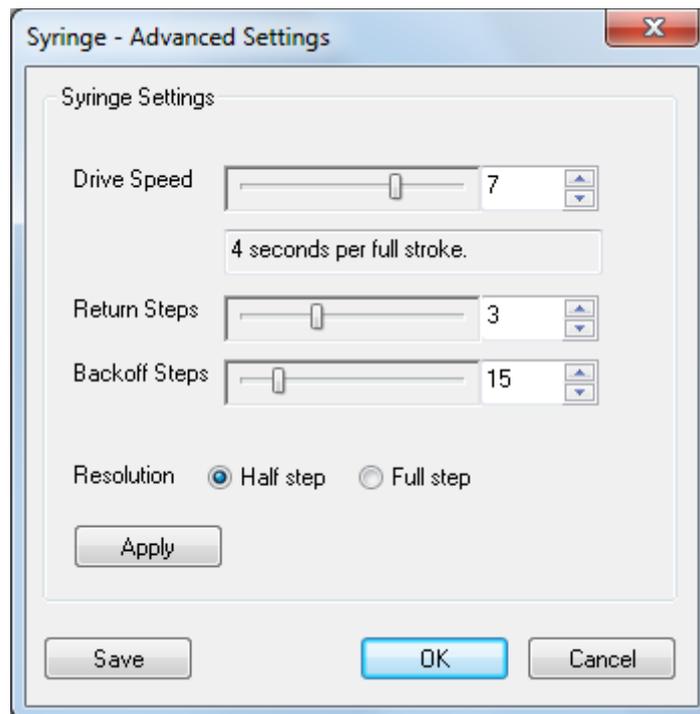


Figure 3.14: the Syringe – Advanced Setting dialog box

When the settings for Syringe 1 have been entered, click the **Save** button in the **Setup** panel to save the information. Repeat the setup procedure for Syringe 2.

## 4 OPERATION

This Chapter describes the operation of the CS/TT in manual mode or using the protocols.



The CS/TT has moving syringes that could trap hands, clothing or other items. Keep hands, clothing and other items clear of the syringes during operation.

### 4.1 Manual Operation

#### 4.1.1 Using the step/prime switch

The syringes can be operated using the Step/Prime switch, which is a 3-position rocker switch located on the CS/TT front panel directly above the power indicator light. Operation of the switch is as follows.

- When in the middle position the switch is inactive.
- When the lower part of the switch is pressed, the CS/TT goes into Prime mode. The syringe barrels are driven up and down, and the valve ports open and close so that fluid is picked up through the inlet port and dispensed through the outlet port. The syringe motion continues until the switch is set to the middle or upper positions: the syringes then move to the upper (home) position before stopping.
- When the upper part of the switch is pressed, the TT.5 goes into Step mode. The syringe motion and fluid movement is as for the Prime position, but continues only while the switch is pressed, and stops immediately it is released.

The Step/Prime switch may be used, for example, to flush the system with distilled water, to check for leaks, or to flush with reagent.

#### 4.1.2 Using the control software

The syringes can be also operated using the CS/TT control software, from the **Titrator – Syringe Configuration** dialog box (Figure 3.13). Select the syringe number from the drop down list of the **Syringe Number** box at the top of the panel. The syringe actions and valve settings for that syringe are set on the **Control** panel (Figure 4.1). The terminology used in this panel follows that used by the Hamilton Company in the Microlab<sup>®</sup> User's Manual.

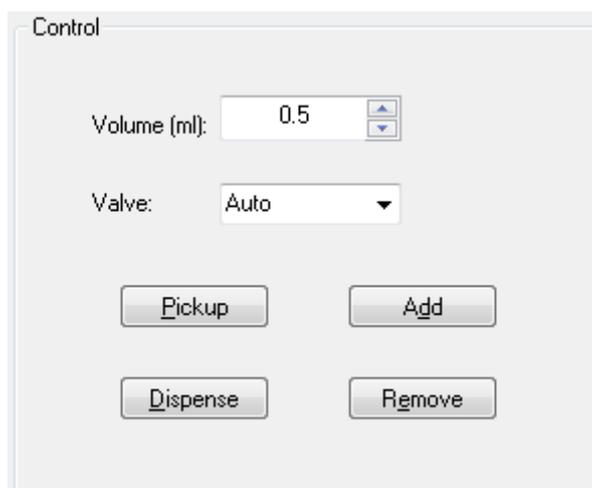


Figure 4.1: the control panel

### 4.1.3 Picking up and dispensing through the same port

Clicking the **Pickup** button will transfer a fixed volume of liquid from a vessel to the syringe. Clicking the **Dispense** button will transfer liquid from the syringe to the vessel. These buttons do not control the valve positions, which should be set from the drop-down list in the **Valve** box.

To pick up or dispense through the input (side) port, set the valve to the **In** position; to pick up or dispense through the output (underside) port set the valve to the **Out** position. If the valve is set to **Auto** the input port is used. If the valve is set to **Wash** the syringe is open to both ports.

Enter the volume to be transferred in the **Volume** box on the **Control** panel. If there is insufficient travel on the syringe to allow this, the command will not be obeyed.

### 4.1.4 Transferring fluid from one vessel to another

Solution can be transferred from one vessel to another using the **Add** and **Remove** buttons. A single syringe is used, the syringe number being entered in the **Syringe Number** box. The volume to be transferred is entered in the **Volume** box on the **Control** panel.

If the **Add** button is clicked, the solution will be picked up through the outlet port and dispensed through the inlet port, if the remove button is clicked, it will be picked up through the inlet port, and dispensed through the outlet port. There is no limit to the volume of solution that can be transferred in this way; the valves will switch automatically, the original setting in the **Valve** box being overridden.

### 4.1.5 The Status panel

The **Status** panel (Figure 4.2) shows the valve position and syringe fill condition. The Busy indicators show the operation currently being performed.

The schematic diagram in the panel above the **Control** panel is labelled following the convention used by the Hamilton Company in the in the Microlab<sup>®</sup> manual, the input valve being labelled **1** and output valve **4**.

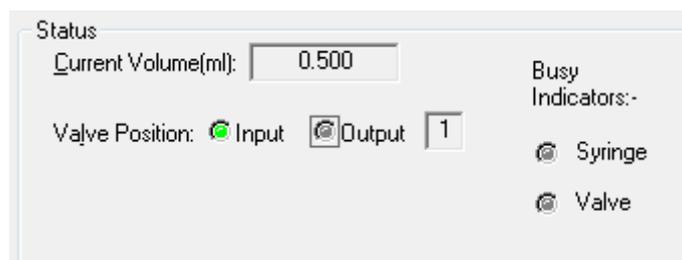


Figure 4.2: the Status panel

## 4.2 Using the Protocols

The protocols are setup up to allow the user to add or withdraw solution from the cuvette in increments to achieve fixed volumes or concentrations. After each addition or withdrawal, a spectrum will be run on the Chirascan at the conditions set up in the Pro-Data software in the usual way (see the main Chirascan manuals for further information on setting the spectrum conditions).

To enable the CS/TT to be operated using the Protocols, click the **Titration** button on the **Sample Handling Unit (ESHU)** panel of the Pro-Data control panel. The **Titration** dialog box will be displayed (Figure 4.3).

The screenshot shows the 'Titration' dialog box with the following settings:

- Enable Titration
- Protocol:**
  - Single Syringe - Addition of solution 1 to specified volumes.
  - Dual Syringe - Withdrawal / Addition of solution 1 to specified concentrations.
  - Dual Syringe - Withdrawal / Addition of solution 1 by specified volumes.
- Cell / Cuvette Parameters:**
  - Cuvette capacity (ml): 2.5
  - Pathlength (mm): 10.0
  - Initial volume of solution in cuvette (ml): 2.0
  - Initial concentration of titrant in cuvette: 0.000000 M
- Syringe1 (Solution 1):**
  - Solution 1 description: Reagent B
  - Volume of Syringe 1 (ml): 1
  - Concentration of solution in syringe 1: 0.5
- Target Volumes/Concentrations:**
  - Auto correct for titrator errors.
  - From: 0.000000
  - To: 0.5
  - Step: 0.100000
  - Generate list >>
  - Total volume of titrant required: 4.566505

Volumes (ml):	Concentrations: (M)
0.000000	0.000000
0.400000	0.100000
0.500000	0.200000
0.666500	0.299975
1.000005	0.399988
2.000000	0.500000

Figure 4.3: the Titration dialog box

Activate the dialog box by ticking the **Enable Titration** box at the top.

Select the required protocol on the **Protocol** panel.

#### 4.2.1 Additional of solution 1 to specified volumes

The first option in the **Protocol** panel uses Syringe 1 to transfer reagent from the reservoir to the cuvette in fixed amounts; Syringe 2 is not used.

On the **Target Volumes / Concentrations** panel enter the required final volume in the **To** box and the volume to be added at each step in the **Step** box. Click the **Generate list** button: the volumes of reagent in the cuvette at which a which a spectrum will be run will appear in the **Volumes** box, and the total volume of reagent added will appear in the **Total Volume of titrant required** box.

#### 4.2.2 Withdrawal / addition to specified concentrations

The second option in the **Protocol** panel uses Syringe 1 to add reagent to the cuvette, and Syringe 2 to withdraw reagent, to achieve step changes in concentration: the overall volume in the cuvette remains constant.

On the **Target Volumes / Concentrations** panel enter the required final concentration in the **To** box and the concentration change for each step in the **Step** box. Click the **Generate list** button: the concentrations of reagent in the cuvette at which a which a spectrum will be run will appear in the **Concentrations** box.

The volume replaced at each step can be viewed in the **Volumes** box by clicking the  button.

The calculation is performed as in Equation 4.1:

$$V_{rep} = V_{cuv} * C_{step} / (C_{syr} - C_{init}) \quad (4.1)$$

where  $V_{rep}$  is the replaced volume,  $V_{cuv}$  is the volume in the cuvette,  $C_{step}$  is the step change in concentration,  $C_{syr}$  is the concentration in the syringe, and  $C_{init}$  is the concentration before replacement. The total volume of reagent added will appear in the **Total Volume of titrant required** box.

#### 4.2.3 Withdrawal / addition by specified volumes

The third option in the **Protocol** panel uses Syringe 2 to withdraw a specified volume of reagent from the cuvette, which is replaced by the same volume from Syringe 1. The first action is for Syringe 2 to withdraw the specified volume from the cuvette, the second is for Syringe 1 to add the same volume to the cuvette. The overall volume in the cuvette therefore remains constant.

Enter each volume to be replaced in the **Volumes** box. The concentration after each replacement can be viewed **Concentrations** box by clicking the  button.

The concentration is calculated as in Equation 4.2:

$$C_{final} = (C_{init} \times V_{cuv} - C_{init} \times V_{rep} + C_{syr} \times V_{rep}) / V_{cuv} \quad (4.2)$$

where  $C_{final}$  is the concentration after replacement,  $C_{init}$  is the concentration before replacement,  $C_{syr}$  is the concentration in the syringe,  $V_{cuv}$  is the volume in the cuvette, and  $V_{rep}$  is the replaced volume. The total volume of reagent added will appear in the **Total Volume of titrant required** box.

#### 4.2.4 The auto correct function

If the **Auto correct for titrator errors** box is ticked, the values in the Volumes or Concentrations lists may differ from the expected values. This is because the stepper motor that drives the syringes moves in fixed steps, and may not be able to go to exactly the position calculated or requested. In that case the motor will move to the

closest position possible. If the Auto correct box is ticked, the software will allow for this in when calculating the volumes and concentrations.

### 4.3 *Performing a Titration*

Set up the experimental details on the ProData control page as required.

Open the **Titration** page and select the required protocol. Ensure that the **Enable titration** box is ticked, and fill in the writable fields in the **Cell / Cuvette parameters** and **Syringe 1 (Solution 1)** panels. Fill in the **Target Volumes/Concentration** panel as required, and click **OK** to close the dialog box and return to the ProData control page.

Use the titrator in manual operation mode to flush the system thoroughly with distilled water, and then with titrant.

Load a clean dry cell with enough sample to cover the light beam. For a standard cell this would be to a depth of at least 20 mm, i.e. a volume of 2.0 ml for a 10 mm × 10 mm cell. Place the cell in the cell holder in the ESHU, and insert the titration tubing. Close the ESHU lid.

Click the **Acquire** button to perform the titration.