

AppliedPhotophysics

Ultrasensitive Spectroscopy for the Life Sciences

User Service Manual SX Series Spectrometers

SX Stopped Flow Spectrometer
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Applied Photophysics Ltd,
21, Mole Business Park, Leatherhead, Surrey, KT22 7BA, UK
Tel (UK): +44 1372 386 537
Tel (USA): 1-800 543 4130
Fax: +44 1372 386 477

Applied Photophysics was established in 1971 by The Royal Institution of Great Britain

www.photophysics.com

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ESSENTIAL SAFETY INFORMATION

MAKE SURE THAT YOU HAVE READ AND UNDERSTAND ALL THE SAFETY INFORMATION CONTAINED IN THIS DOCUMENT BEFORE ATTEMPTING TO OPERATE THE SX20 SPECTROMETER. IF YOU HAVE ANY QUESTIONS REGARDING THE OPERATION OF YOUR SPECTROMETER, 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.

FOR CLARITY, MANY OF THE IMAGES IN THIS USER MANUAL SHOW THE SX20 WITHOUT THE SAFETY COVER IN PLACE. A DRIVE SHOULD NEVER BE PERFORMED UNLESS THIS COVER IS IN PLACE.



The SX20 spectrometer is powered by the mains electricity supply which can produce an electric 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.



The metal components of the spectrometer can produce an electric shock leading to serious injury or death if they are not earthed (grounded). The design of the spectrometer provides protection against electric shock by earthing appropriate metal components. This protection will be lost unless the power cable is connected to a properly earthed outlet. It is the user's responsibility to ensure that a proper earth connection can be made.



The photomultiplier tube (PMT) detector used with the SX20 operates at high voltages and can produce an electric shock leading to serious injury or death. Do not connect or disconnect the detector from the spectrometer unless the spectrometer is powered off.



The SX20 spectrometer may be equipped with a light source (150 watt xenon or mercury-xenon arc lamp) that produces intense ultraviolet radiation that can irritate the eyes and may impair eyesight. Never look directly at the light source. Do not open the lamp housing while the lamp is operating or immediately after it is powered off. Do not allow the skin to be exposed to UV radiation.



The SX20 drive rams are pneumatically driven and could trap hands or clothing, causing injury to the user. Keep hands and clothing and other items clear when performing a drive, and never perform a drive without the safety cover in place.



The interaction between ultraviolet light and oxygen leads to the formation of ozone, a very reactive gas that is damaging to health and may cause deterioration of the optical components of the instrument. If an ozone producing lamp is used, it essential that the SX20 is thoroughly purged with clean, oxygen-free nitrogen before the lamp is powered on.

NOTICE

The electronic circuitry used in the SX20 is very sensitive and must be correctly grounded to avoid electrical interference. The lamp power supply unit, the lamp housing and the mains distribution board all have earth posts that must be connected to the earth post on the optical rail via the braided earth straps provided with the system. It is the user's responsibility to ensure that a proper earth connection can be made.

NOTICE

The cables running between the power supply and the lamp housing carry high voltages during lamp ignition that could cause interference with other electronic devices and cables: keep other devices and cables at least 30 cm away.

NOTICE

The lamps used in the SX20 operate at high pressure and can explode or break without warning, causing damage to the interior of the lamp housing. This is more likely to happen with lamps with longer usage, and lamps with more than 1000 hours usage should be replaced.

NOTICE

Corrosive chemical and organic solvents can cause damage to the spectrometer. Do not allow corrosive fluids to come into contact with any part of the spectrometer except within the flow system. Do not clean the spectrometer with organic solvents. Use only a soft cloth and water or a mild detergent solution.

NOTICE

Always follow Good Laboratory Practice; wear suitable PPE and dispose of spent consumables according to local waste disposal legislations.

APPLICABLE INSTRUMENTS

This document applies to all SX Stopped Flow Spectrophotometers which run under Pro-Data SX Software. For older instruments, please contact support@photophysics.com.

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.

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INTRODUCTION

This document describes a series of tests and service procedures, which the user can perform on the SX series of Stopped Flow Spectrophotometers, in order to diagnose and/or solve issues. These procedures vary in how frequently they should be performed and dependent on how often the system is used.

There are certain procedures which are beyond the scope of this manual, for example, a full preventative maintenance service, or advanced service procedures. Procedures like these are expected to be completed by a qualified APL service engineer and no-one else.

This manual is split into four sections:

- **Routine Testing:** This section describes a list of tests which users should perform on a regular basis to assess the performance of their instrument, depending on how often the instrument is used. It also contains troubleshooting tips.
- **Advanced Testing:** This section describes some of the more advanced testing procedures. It is recommended that these tests should only be carried out where there is specific cause to do so.
- **Routine Servicing:** This section describes some of the more common service procedures which a user may need to perform on a regular basis, depending on how often the instrument is used. For any more advanced service procedures, contact support@photophysics.com.
- **Routine PC and Software Service Procedures:** This section described some of the more common procedures relating to the PC and software.

1 ROUTINE TESTING

Routine testing should be performed on a regular basis as part of a standard instrument testing procedure.

1.1 Light Throughput Check

It is advised that the light throughput check is performed each time the stopped flow spectrophotometer is used. Poor light throughput will result in a poor signal-to-noise ratio.

1.1.1 Experimental Conditions and Procedure

Turn on the instrument in the usual way, and allow 30 minutes for the instrument to equilibrate. Satisfy the experimental conditions outlined below.

Experimental Conditions for the Light Throughput Test	
Equilibration time	30 minutes
Detector	Photomultiplier tube (Absorbance detector)
Signal mode	Absorbance mode
Wavelength	350nm
Cell contents	Deionised water
Monochromator slit width	0.5 mm
Optical path length	2 mm

Tick the checkbox labelled 'Auto HV' then press the 'Reference' button.

1.1.2 Criteria

Take note of the detectors high voltage and compare it to the voltages in the table below.

High Voltage (V)	Description
200 – 240 V	Light throughput is good
240 – 450+ V	Light throughput is lower and may benefit from optimisations
1000 V	The detector is detecting no light

1.1.3 Troubleshooting

If your high voltage is much greater than 240 V, then your instrument may benefit from light throughput optimisation:

- Check that the lamp shutter is fully open.
- Check that channel 1 at the back of the electronics unit is connected to the absorbance detector and not the fluorescence detector. Check that the detectors are screwed securely into place with O-rings, and that blanking plugs are in place over any unused ports on the cellblock.
- Ensure that the lamp has been correctly aligned (Section [3.4](#)).
- Ensure that monochromator accuracy is within specification (Section [2.1](#)).
- Consider whether the slit widths are accurate. This can be tested for using a feeler gauge and the dials can be adjusted to read the correct values using a small hex key.

- Check that the cell is clean. Try cleaning the cell and flow circuit with 2 M nitric acid, or Hellmanex cleaning fluids if necessary (see Section [3.1](#)). If your cell requires a more thorough clean, see Section [3.9](#).
- Check for damage on the light guide. Hold one end of the light guide up to a ceiling light, while looking down the other end. If black spots obscure a significant portion of the observable light, you may need a new light guide.
- If the end of the light guide attached to the monochromator is not secured by two thumbscrews, does rotating this end of the light guide a few degrees improve the light throughput, i.e. does the absorbance decrease? If so, monitor the live trace display to find the point where the absorbance is least.
- If the lamp is close to 1000 hours old, consider replacing it.
- If none of the above points yield any cause of the low light throughput, the instrument may benefit from replacing mirrors in the lamp housing and/or monochromator. This can especially be the case if the instrument is old and/or an ozone-producing lamp is commonly used. Contact support@photophysics.com for more assistance.

1.2 Lamp Stability Check

The lamp stability check will determine the stability of the lamp and therefore assess its contribution to noise. The experimental conditions are similar to those in the Section [1.1](#) so it is recommended that this test follows.

1.2.1 Experimental Conditions and Procedure

Satisfy the conditions below.

Experimental Conditions for the Lamp Stability Check	
Equilibration	30 minutes
Detector	Photomultiplier tube (Absorbance detector)
Signal mode	Absorbance
Wavelength	350 nm
Cell Contents	Deionised water
Monochromator slit width	0.5 mm
Optical path length	2 mm
Sequencer	Kinetics,
Time base	0.1, 1, 10 and 100 seconds (test all in turn)
Points	1000
Trigger	Internal
Repeats	5 per time base, except 100 seconds (no repeats necessary)

Press 'Acquire'.

1.2.2 Criteria

Your acquired traces should show:

- Reproducibility by overlaying
- A peak-to-trough amplitude of < 1.25 mAU,
- No significant periodic noise, such as 50 – 60 Hz,
- No significant drift.

The traces in Figure 1.1 do not overlay well and would therefore fail the stability test.

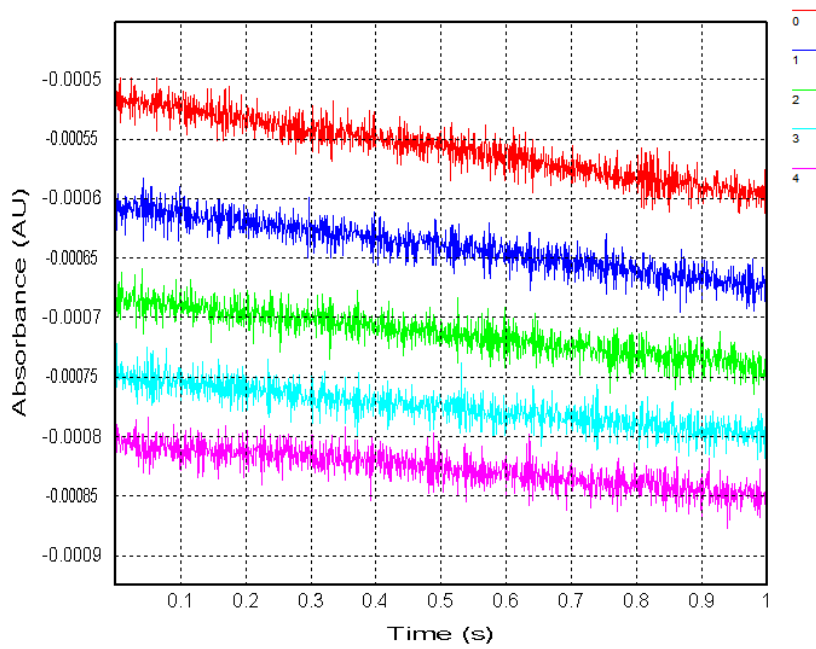


Figure 1.1 – Traces failing the stability test; poor reproducibility

The traces in Figure 1.2 fail as their peak-to-trough amplitude is > 0.00125 AU.

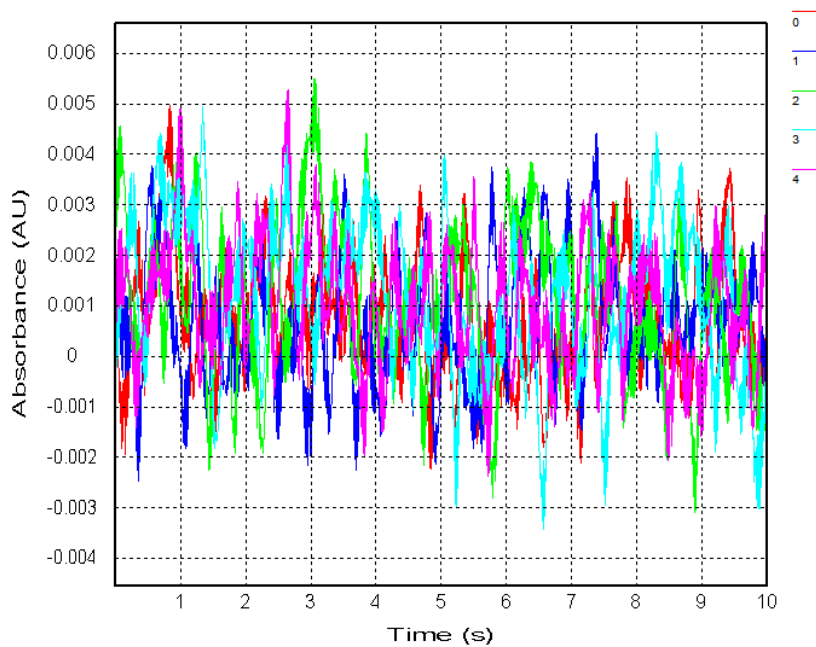


Figure 1.2 – Traces failing the stability test; peak-to-trough amplitude > 1.25 mAU

The traces in Figure 1.3 fail due to the presence of 50-60 Hz noise.

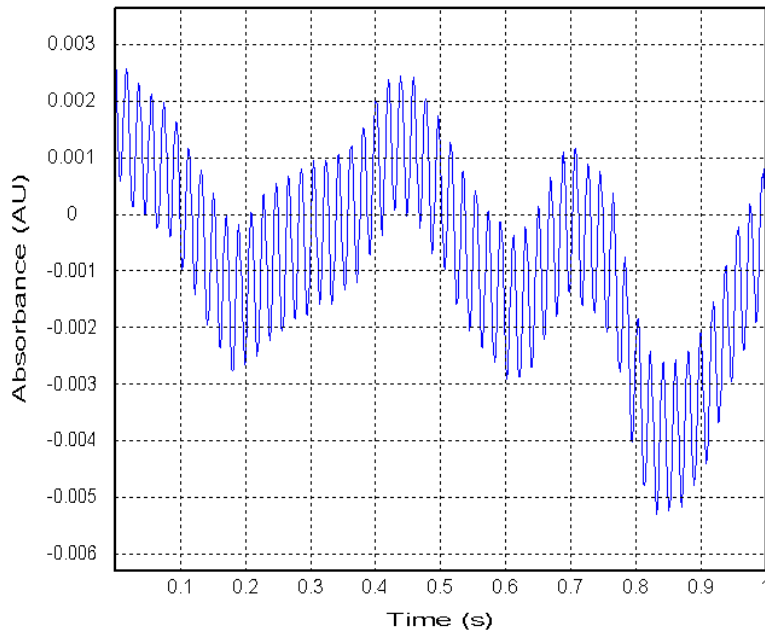


Figure 1.3 – Traces failing the stability test due to presence of periodic noise, i.e. 50-60 Hz

The traces in Figure 1.4 fail the stability test due to significant drift observed on the 100 second time base.

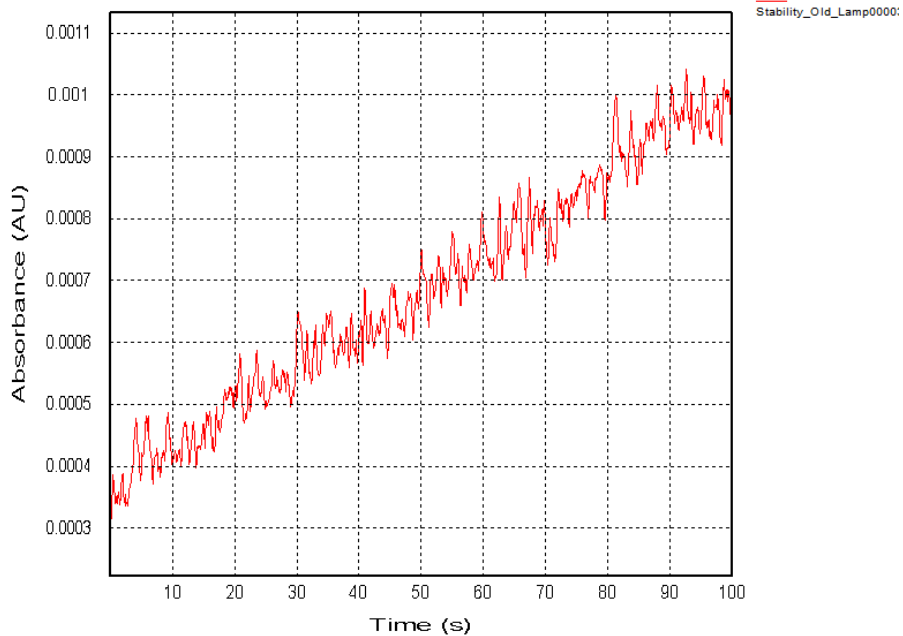


Figure 1.4 – Traces failing the stability test due to significant drift

The traces in Figure 1.5, Figure 1.6, Figure 1.7 and Figure 1.8 pass the stability tests for all respective timescales.

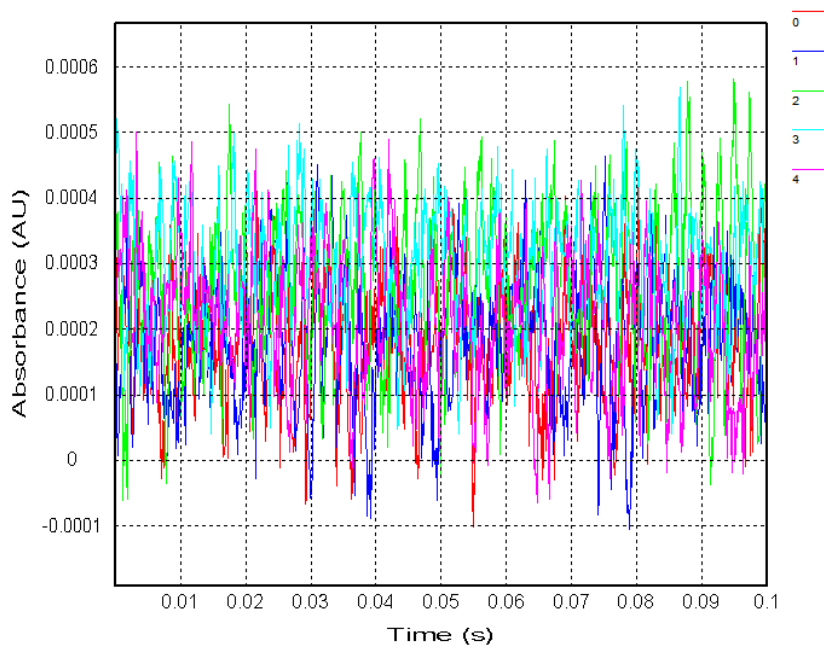


Figure 1.5 – Traces passing stability test at 0.1 seconds

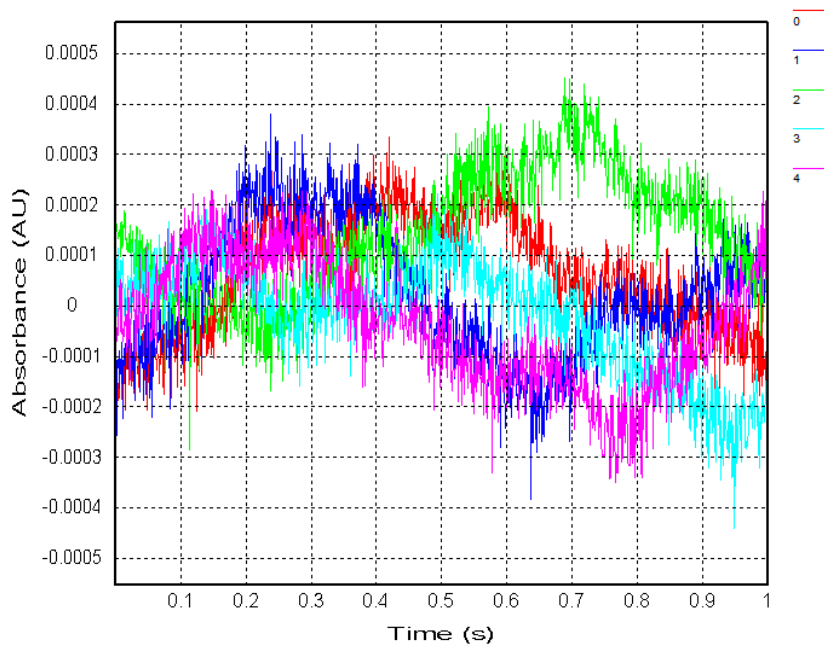


Figure 1.6 – Traces passing stability test at 1 second

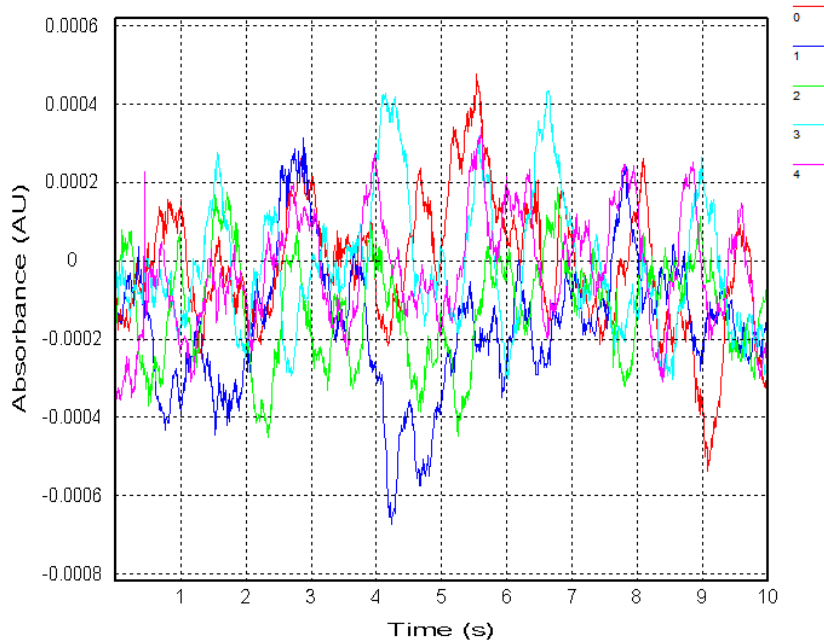


Figure 1.7 – Traces passing stability test at 10 seconds

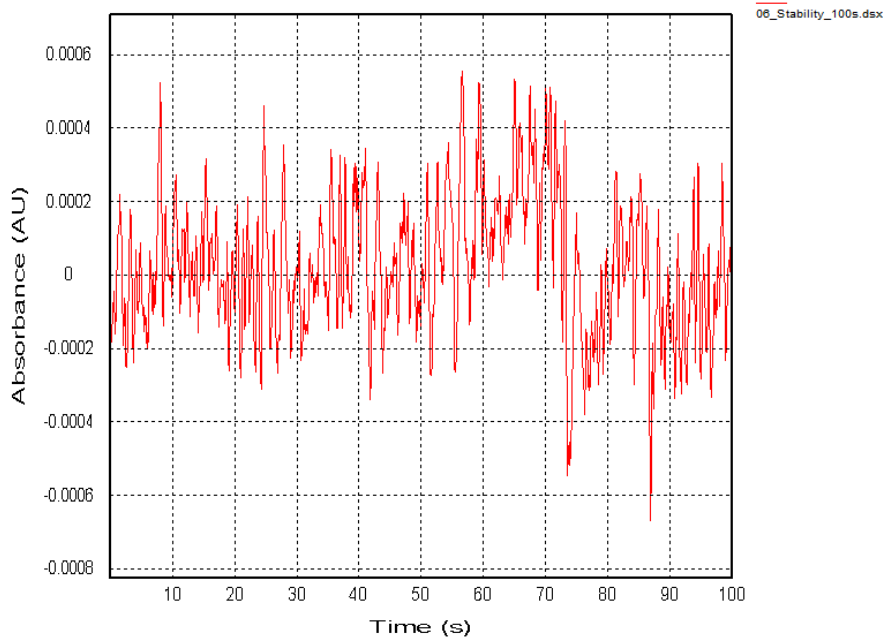


Figure 1.8 – Traces passing stability test at 100 seconds

1.2.3 Troubleshooting

If any of your traces fail to meet the criteria described above, consider the following actions to resolve the issue(s):

- Allow more time for the lamp and absorbance PMT to equilibrate. The lamp usually requires 30 minutes, the absorbance PMT should be given a few minutes with a high voltage applied to equilibrate.

- Check that the rubber O-ring is in place between the absorbance detector and cell block.
- Check that the instrument is correctly earthed. This will reduce the interfering frequencies sometimes observed in 0.1 and 1 s traces.
- Consider replacing the lamp if it is near 1,000 hours old.
- Check lamp alignment (Sections [3.4](#))
- Consider using a lamp-stabilising magnet.
- Consider checking the stability of the lamp power supply unit. Contact support@photophysics.com for more information.
- If there is drift over 100 s and the lamp is new, but the detector PMT is old, it may be that the detector PMT is beginning to lose its vacuum and needs to be replaced. Contact support@photophysics.com for assistance.

1.3 Flow Circuit Leak Test

The flow circuit leak test is designed to test that the flow circuit is closed and free from leaks. This test should be performed if a leak is suspected, or if one has recently worked on the flow circuit, such as when changing from single to sequential mixing or back again.

1.3.1 Experimental Conditions and Procedure

Start by flushing the instrument with distilled water. Satisfy the experimental conditions below:

Experimental Conditions for the Lamp Stability Check	
Cell Contents	Distilled water
Drive Syringe F	Distilled water
Drive Syringe C	Distilled water
Equilibration	NA
Detector	NA
Signal mode	NA
Wavelength	NA
Monochromator slit width	NA
Optical path length	NA
Sequencer	Kinetics,
Time base	10 s (try 1 s first if a leak is suspected)
Points	NA
Trigger	External (check pressure hold box)
Repeats	NA

Press 'Acquire' and monitor the drive syringe plungers.

1.3.2 Criteria

If the plungers do not move upwards under the pressure held drive and 'kick back' slightly when the pressure is released, then the instrument passes this test.

If the plungers continue to move upwards during the 10 seconds of the pressure hold, you may have a leak which you will need to find and seal.

1.3.3 Troubleshooting

If you have recently worked on the flow circuit, try tightening the parts you have just worked with. If it is not immediately obvious where the leak is, empty the water bath (Section [3.2](#)), dry the system thoroughly and repeat many pressure held shots and look for the formation of droplets.

- Check all PEEK tube fittings for leaks.
- Check the drive syringes and stop syringes are not leaking themselves between the plungers and glass barrels.
- Check the pressure plate is tight enough (if applicable, SX removable cell instruments only)
- Check the stop valve does not leak by removing the waste tube from the waste receptacle and monitor it for flow.

- Check the waste tube adaptor does not leak.

If any of the above parts cannot be tightened to resolve the leak, it is possible the part(s) may need to be replaced. Contact support@photophysics.com for assistance.

1.4 Drive Reproducibility Test

The drive reproducibility test is designed to confirm that the flow circuit is free from contamination, bubbles and vibration artefacts.

1.4.1 Experimental Conditions and Procedure

Enter the experimental conditions below:

Experimental Conditions for the Drive Reproducibility Check	
Cell Contents	Distilled water
Drive Syringe F	Distilled water
Drive Syringe C	Distilled water
Equilibration	30 minutes
Detector	Absorbance PMT
Signal mode	Absorbance
Wavelength	350 nm
Monochromator slit width	0.5 mm
Optical path length	2 mm
Sequencer	Kinetics,
Time base	1 s
Points	1000
Trigger	External
Repeats	10

Be sure that there are no air bubbles within the flow circuit for this test.

Press 'acquire'.

1.4.2 Criteria

All traces must:

- Overlay,
- Display no artefacts (e.g. large noise in initial few milliseconds, or large increases or decreases in data)
- Exhibit a peak-to-trough amplitude of < 1.25 mAU

The traces in Figure 1.9 show an example of a failed drive reproducibility test. The traces show relatively large ‘jumps’ in signal during the initial stages of the traces and they don’t overlay well.

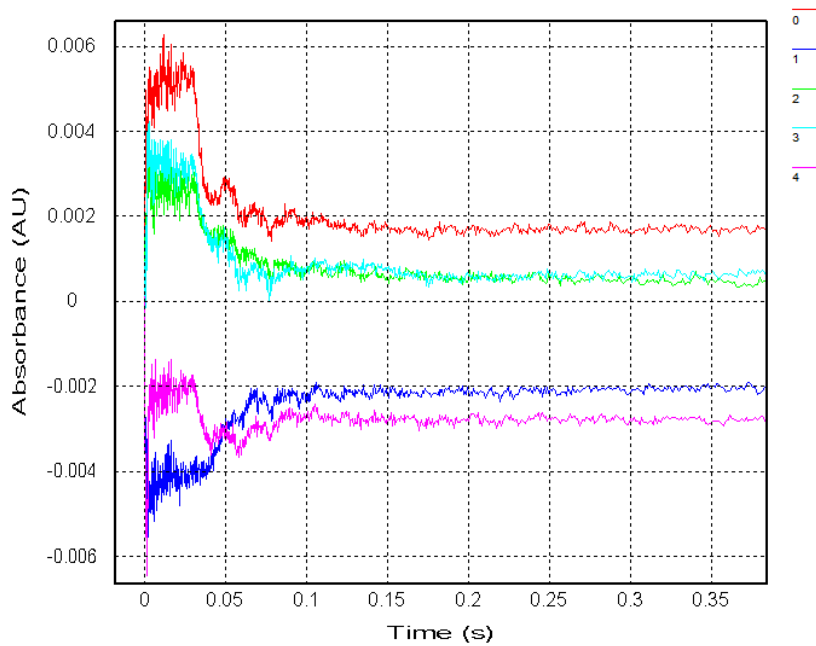


Figure 1.9 – Traces failing the drive reproducibility test, possibly due to air bubbles

The traces in Figure 1.10 also fail. They show evidence of a reaction, most likely from contamination of previous reactants.

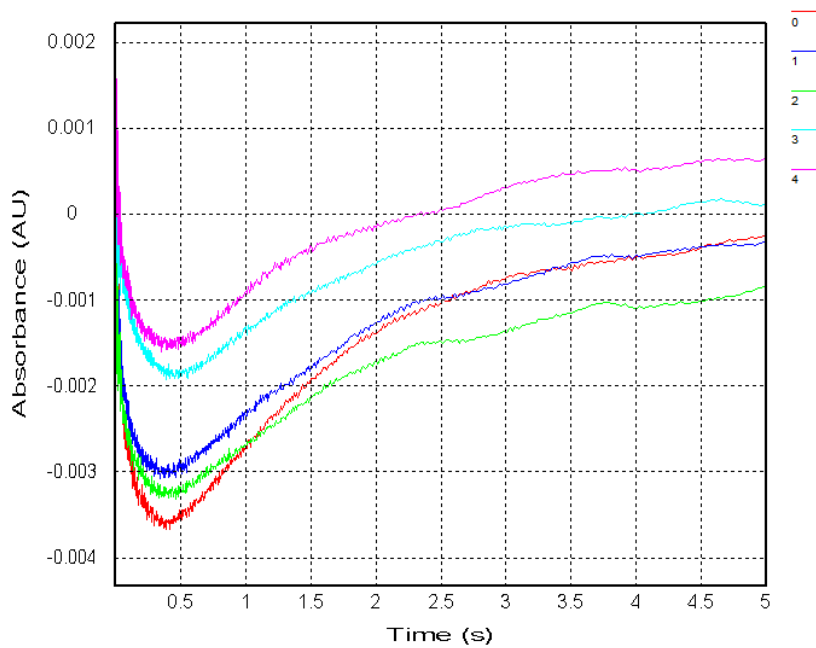


Figure 1.10 – Traces failing the water drive test, possibly due to contamination

The traces in Figure 1.11 below pass the drive reproducibility test. They overlay well and have no artefacts, vibration or otherwise.

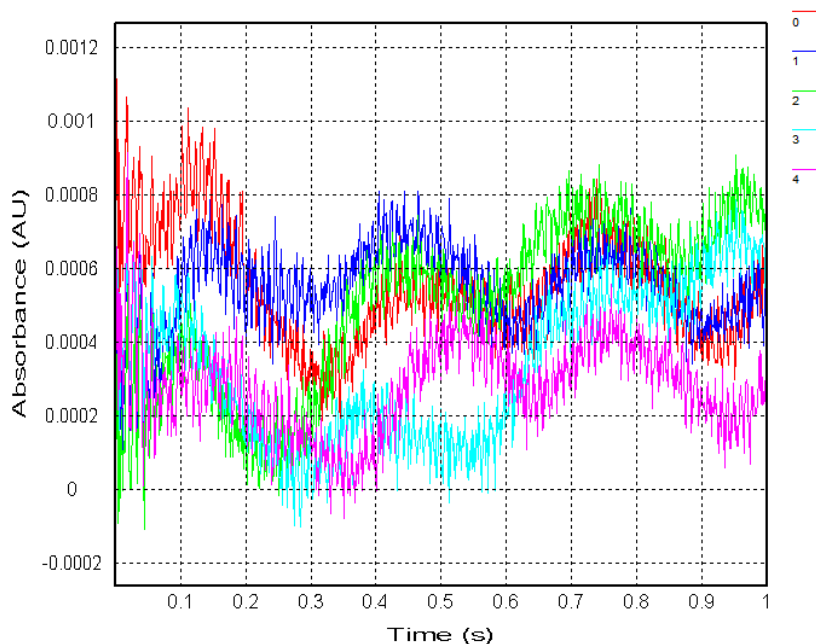


Figure 1.11 – Traces passing the drive reproducibility test

1.4.3 Troubleshooting

If your instrument fails this test:

- Flush the flow circuit with a suitable reagent to remove contamination, e.g. detergent or a mild acid (Section 3.1).
- Flush the flow circuit carefully (including the stop syringe) using ethanol or methanol to remove any air bubbles present.
- Check and eliminate possible causes of vibration artefacts (e.g. check the detectors and drive rams have O-rings in place)
- Remove the cell (Section 3.9) for a more thorough clean with concentrated nitric acid if necessary.

1.5 Absorbance Test

The absorbance detector test is designed to check that the instrument is working correctly with respect to the absorption detector. This test measures the formation of iron (III) thiocyanate.

1.5.1 Preparation of Reagents

You will require the following reagents:

1. Perchloric acid: Sigma-Aldrich product code 244252 (70% solution, approximately 11.6M)
2. Sodium thiocyanate: Sigma-Aldrich product code 251410 (MW= 81.07)
3. Iron (III) perchlorate: Sigma-Aldrich product code 326348 (MW= 354.2)
4. Distilled water

Preparation of iron perchlorate (20 mM) in Perchloric acid (80 mM)

For 500 mL, weigh out 3.54 grams of Iron perchlorate (FW= 354.21) and dissolve in approximately 400 mL of deionised water. Add to this 3.49 mL of perchloric acid (11.6M) and make up to 500mL with distilled water. Pour aliquots into labelled and sealed vials.

Preparation of sodium thiocyanate (1 mM)

For 500mL, weight out 40.54 mg of NaSCN (FW= 81.072) and dissolve in 500mL of deionised water. Pour aliquots into labelled and sealed vials.

1.5.2 Experimental Conditions and Procedure

Satisfy the following criteria.

Experimental Conditions for the Absorbance Test	
Drive Syringe F	Iron perchlorate (20 mM) in perchloric acid (80 mM)
Drive Syringe C	Sodium thiocyanate (1 mM)
Detector	Absorbance PMT
Signal mode	Absorbance
Wavelength	435 nm
Monochromator slit width	0.5 mm
Optical path length	2 mm
Sequencer	Kinetics,
Time base	1 s
Points	1000
Trigger	External
Repeats	5
Temp	25 degrees centigrade

1. Fill the cell with water and click on 'reference'.
2. Run 5 repeat kinetic traces by clicking 'acquire'.

1.5.3 Criteria

Your traces should be reproducible and look like those below:

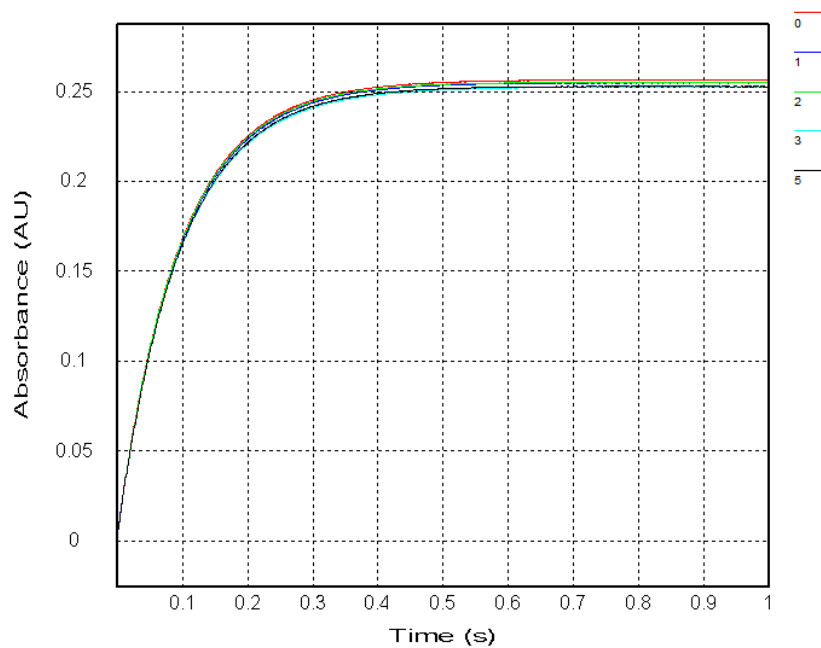


Figure 1.12 - Example of traces passing the absorbance test

The traces in Figure [1.12](#) pass the absorbance test because they are reproducible and when fitted to a single exponential curve model have the values of 'C' = ~ 0.25 and 'K' = $\sim 10 - 20$. It is worth noting that temperature variations will significantly affect this kinetic rate.

There are many factors to investigate if the absorbance test fails. Contact support@photophysics.com for assistance.

1.6 Scattered Light Test

The scattered light test is used to check that the fluorescence detector is working correctly. It is not a fluorescence test. It measures the reduction of scattered light during the formation of iron (III) thiocyanate and is suitable for testing the fluorescence channel if no cut-off filters or additional chemistry are available. For a test demonstrating fluorescence kinetics, please refer to Section [2.2](#).

1.6.1 Preparation of Reagents

The reagents required for this test are the same as described in Section [1.5.1](#).

1.6.2 Experimental Conditions and Procedure

The experimental conditions and are the same as in Section [1.5.2](#), with the exception that the fluorescence PMT is used, in place of the absorbance PMT

Experimental Conditions for the Scattered Light Test	
Drive Syringe F	Iron perchlorate (20 mM) in perchloric acid (80 mM)
Drive Syringe C	Sodium thiocyanate (1 mM)
Detector	Fluorescence PMT
Signal mode	Fluorescence
Wavelength	435 nm
Monochromator slit width	0.5 mm
Optical path length	2 mm
Sequencer	Kinetics,
Time base	1 s
Points	1000
Trigger	External
Repeats	5
Temp	25 degrees centigrade
Cut off filter (nm)	None

As the conditions and reagents used for this test are the same as for the Absorbance Test in Section [1.5](#) it is possible to perform both tests simultaneously, if dual detection is available.

1. Fill the cell with water and click on 'auto PM' for the fluorescence PMT.
2. Run 5 repeat kinetic traces by clicking 'acquire'.

1.6.3 Criteria

Your traces should be reproducible and look like those in Figure 1.13.

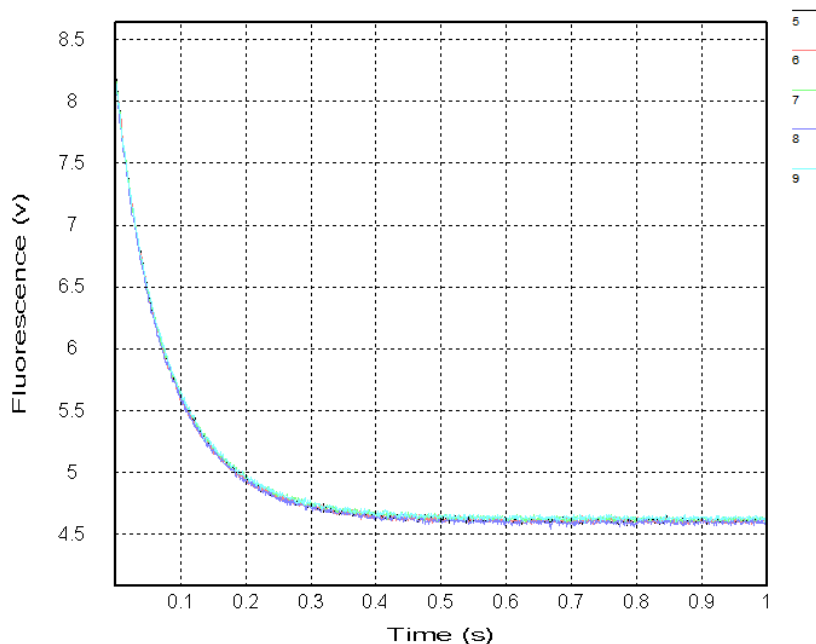


Figure 1.13 - Example of traces passing the fluorescence test

There are many factors to investigate if the absorbance test fails. Contact support@photophysics.com for assistance.

1.7 Dead Time Test

This test allows the performance of a drive to be quantified. As well as dead time, it also measures drive volumes and ram velocity, in a single shot of water. It is only possible on instruments where the sequential mixing option is available. The sequential mixing rams utilise the transducers present in the drive platforms, allowing the ram velocity to be measured and thus calculations regarding volume and time to be made.

Note: Dead time occurs before T0 on acquired traces, and should not be confused with the 'pre-stop' time interval which occurs after T0 and continues normally up to around 3 ms. During the pre-stop period, data will not be reproducible and should not be included in fitting models. It is a period of turbulence caused by compression and expansion within the flow system before a true steady-state is achieved.

1.7.1 Experimental Conditions and Procedure

Many criteria are not applicable with this test, making it very simple. Satisfy the criteria below.

Experimental Conditions for the Drive Reproducibility Check	
Sequential mixing option	Available
Drive Syringe F	Distilled water
Drive Syringe C	Distilled water
Detector	NA
Signal mode	NA
Wavelength	NA
Monochromator slit width	NA
Optical path length	NA
Sequencer	Kinetics,
Time base	NA
Points	NA
Trigger	External

1. Set up your KSHU in single mixing mode.
2. Remove the multiple shot drive ram from the left side drive platform, and install the sequential mixing flush ram (single shot ram, with a smooth circumference).
3. Click on the 'Setup' button in the KSHU window on the main Pro-Data SX program.
4. Select the sequential mixing tab to see the window in Figure [1.14](#).

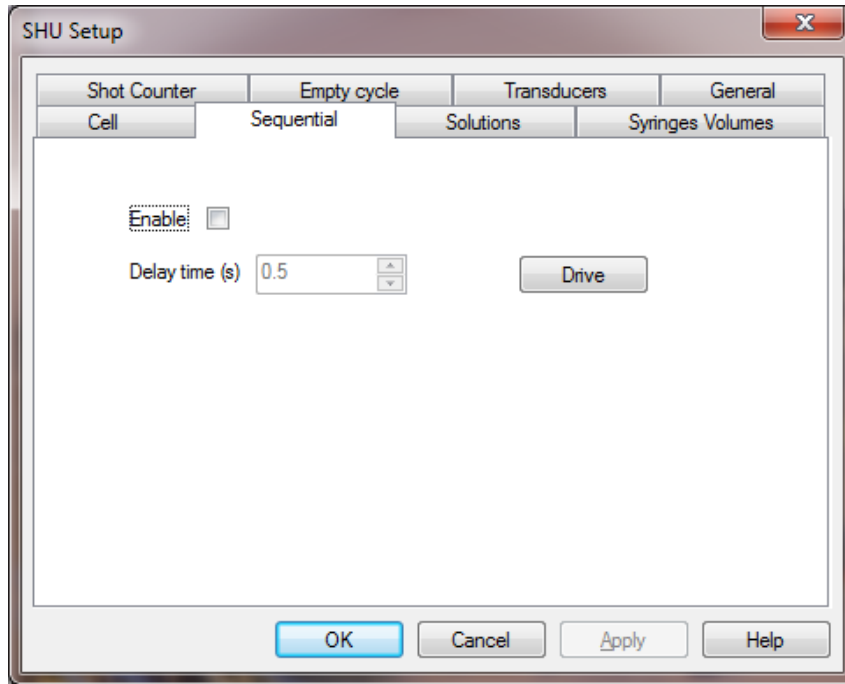


Figure 1.14 - Sequential mixing tab

5. Fill your drive syringes with distilled water and click 'Drive'.
6. You will now find that the 'profile' button on the main Pro-Data SX software has become active. Click on it to view the drive profile which will call up a window similar to that in Figure 1.15.

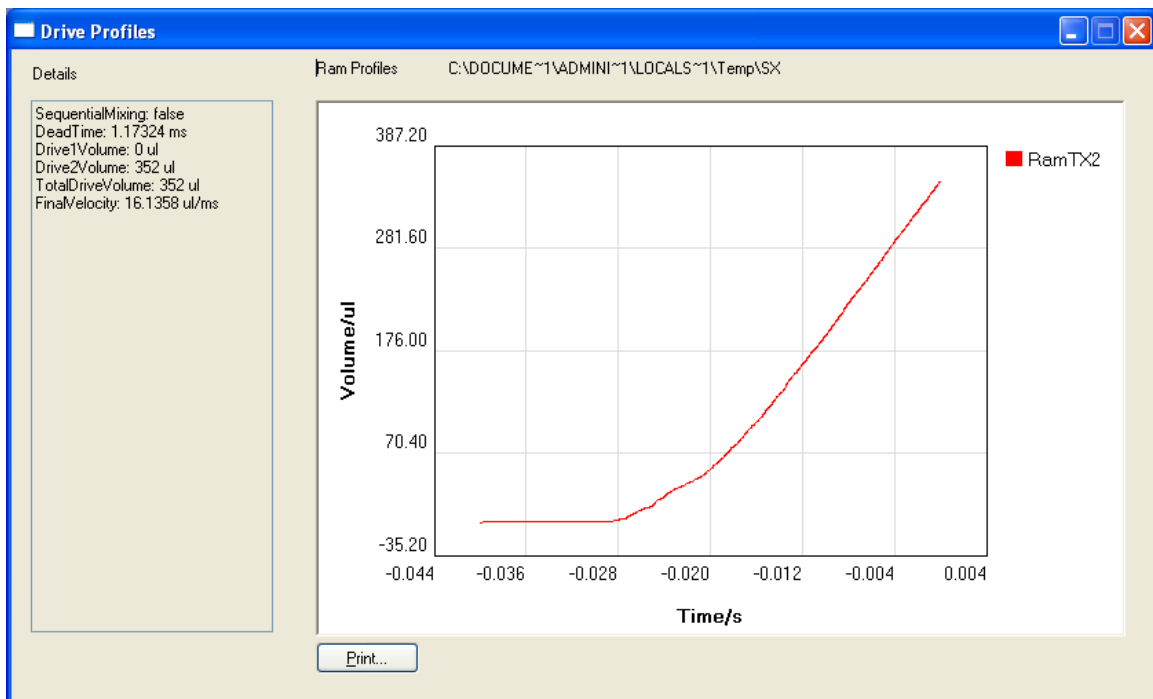


Figure 1.15 - Example of a drive profile

1.7.2 Criteria

The standard 20 μl cell will yield dead times of between 1 – 1.5 ms using a drive volume of $\sim 120 \mu\text{l}$ with a 1:1 mixing ratio. Other setups will vary. The 5 μl cell will enable dead times of 0.5 ms with 1:1 mixing.

The drive volume for 1:1 single mixing is recommended as 120 μl . Reducing this value by turning the knurled knob on the return cylinder may retain reproducibility and decrease sample throughput, but will increase dead time as a compromise. Take this opportunity to adjust your drive volume to your optimum.

Dead times will vary slightly from shot to shot.

1.7.3 Troubleshooting

If your dead time is >1.5 ms, your instrument could perform better.

Firstly, check the inlet pressure is 8 bar / 120 psi.

The next most frequent cause of poor dead times are expired syringes or stop/drive valves. Consider replacing these if they are old.

The next step to consider would be a complete maintenance visit, in which all consumables are replaced. This operation should only be attempted by a qualified Applied Photophysics engineer.

2 ADVANCED TESTING

2.1 Wavelength Accuracy Test

The wavelength accuracy test should not need to be performed regularly. Xenon lamps naturally and reproducibly exhibit peak maxima at 468 nm. This standard can be used to test for wavelength accuracy.

2.1.1 Experimental Conditions and Procedure

1. Launch the 'Pro-Data SX' software.
2. Choose to display the signal as voltage.
3. Set the monochromator to 468 nm and autoPM the absorbance PMT.
4. Acquire a spectrum running from 450 - 500 nm with a step of 1 nm and a sampling rate of one point per 0.1 s. The spectrum should look like that below (Figure 2.1).

Note: If the high voltage resets to zero, check you have deselected the box labelled 'zero HTs before spectrum scan' found under "configuration>preferences>data acquisition".

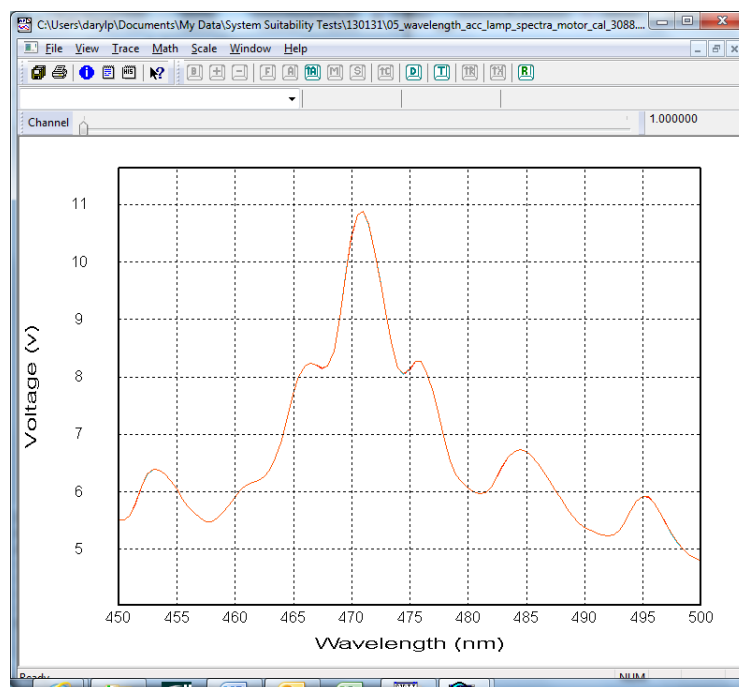


Figure 2.1 - Spectrum of a xenon lamp from 450 - 500nm

Note: If your spectrum looks very different consider running a spectrum from 200 – 700 nm to identify the lambda maximum.

2.1.2 Criteria

If the lambda max is equal to 468 ± 0.5 nm, then the instrument is operating within acceptable parameters and wavelength calibration is not required. If the lambda max is not equal to 468 ± 0.5 nm, then wavelength calibration is required.

Wavelength calibration involves changing the offset applied to the motor controlling the diffraction grating so that it is more accurate at reflecting light at specific wavelengths. This is not something which should be attempted by the user. Please contact support@photophysic.com for assistance.

2.2 Fluorescence Test

This test measures the total fluorescence signal change produced as a result of the interaction of ANS with BSA.

2.2.1 Preparation of Reagents

You will require the following reagents:

1. 8-anilino-1-naphthalene sulphonate (ANS) Sigma-Aldrich product code A1028 (MW=316.37)
2. Bovine serum albumin (BSA) Sigma-Aldrich product code A7030 (MW .66,000)
3. Deionised water
4. Phosphate buffer, 10 mM, pH 7

Preparation of ANS Working Solution (1 μ M)

Stock solution (1 mM): Weigh out 29.9 mg and dissolve in 100 mL of water.

Working solution (1 μ M): Dilute 20 μ L of stock solution to 20 mL in water.

Preparation of BSA working solution (10 μ M) in phosphate buffer (10 mM)

Stock solution BSA (100 μ M): Weigh out 33 mg and dissolve in 5 mL of pH7 phosphate buffer.

Working solution BSA (10 μ M): Dilute 1 mL stock solution in 10 mL pH7 phosphate buffer.

2.2.2 Experimental Conditions and Procedure

Start up the instrument and allow sufficient time for it to equilibrate

Experimental Conditions for the Fluorescence Test	
Drive Syringe F	1 μ M ANS solution
Drive Syringe C	10 μ M BSA solution in phosphate buffer
Detector	Fluorescence PMT (attached to default cell block face)
Signal mode	Fluorescence
Excitation wavelength	365 nm (Auto PM detector at reaction end point)
Monochromator slit width	2 mm
Optical path length	2 mm (used to excite)
Sequencer	Kinetics,
Time base	0.25 s
Points	100
Trigger	External
Repeats	5
Temp	25 degrees centigrade
Cut off filter (nm)	395 nm

1. After filling the drive syringes, run several drives to flush the cell with the reaction.

2. Auto PM the fluorescence detector on the end stage of the reaction. The reaction is quick and will require little time to reach the end point.
3. Click 'acquire' to collect the data.

2.2.3 Criteria

Your traces should be reproducible and look like those in Figure 2.2 .

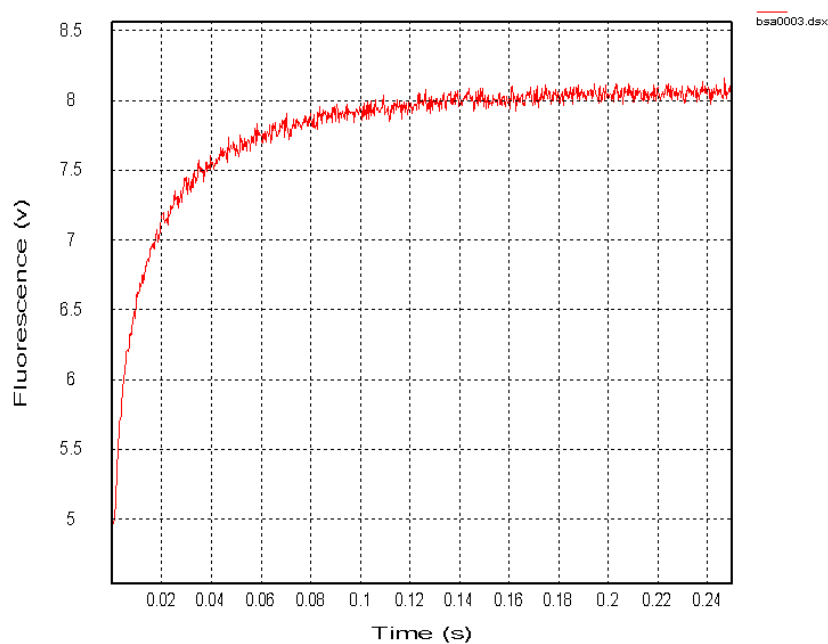


Figure 2.2 - Example of traces passing the fluorescence ANS and BSA test

There are many factors to investigate if the absorbance test fails. Contact support@photophysics.com for assistance.

2.3 PDA Test

2.3.1 Preparation of Reagents

The reagents required for this test are the same as described for the absorbance test in Section [1.5.1](#).

2.3.2 Experimental Conditions and Procedure

The following experimental conditions are required for the PDA test.

Experimental Conditions for the Fluorescence Detector Test	
Drive Syringe F	Iron perchlorate (20 mM) in perchloric acid (80 mM)
Drive Syringe C	Sodium thiocyanate (1 mM)
Detector	PDA
Monochromator slit width	Direct coupler
Optical path length	10 mm
Sequencer	Kinetics,
Time base	1 s
Points	1000
Trigger	External
Temp	25 degrees centigrade

1. Start by acquiring a baseline with distilled water in the cell. If the peak at 468 nm is not somewhere between 1.4 – 1.8 V, then consider changing the gain and offset values. A quick way to do this would be to pick another pre-set radio button (i.e. pre-set 1, 2 or 3).
2. Fill the cell with iron perchlorate (20 mM) in perchloric acid (80 mM) and click on 'baseline'. Your baseline trace should look like that in Figure [2.3](#).

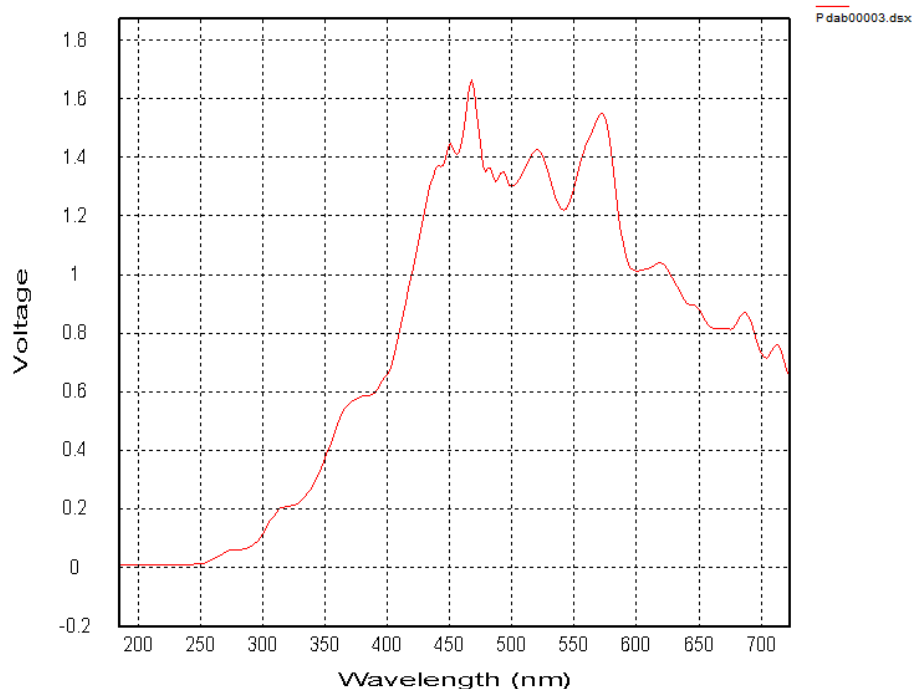


Figure 2.3 - Baseline PDA trace of iron perchlorate (20 mM) in perchloric acid (80 mM)

3. Flush the flow circuit with sample and acquire data.

4. Your data should look like that in Figure 2.4 and Figure 2.5.

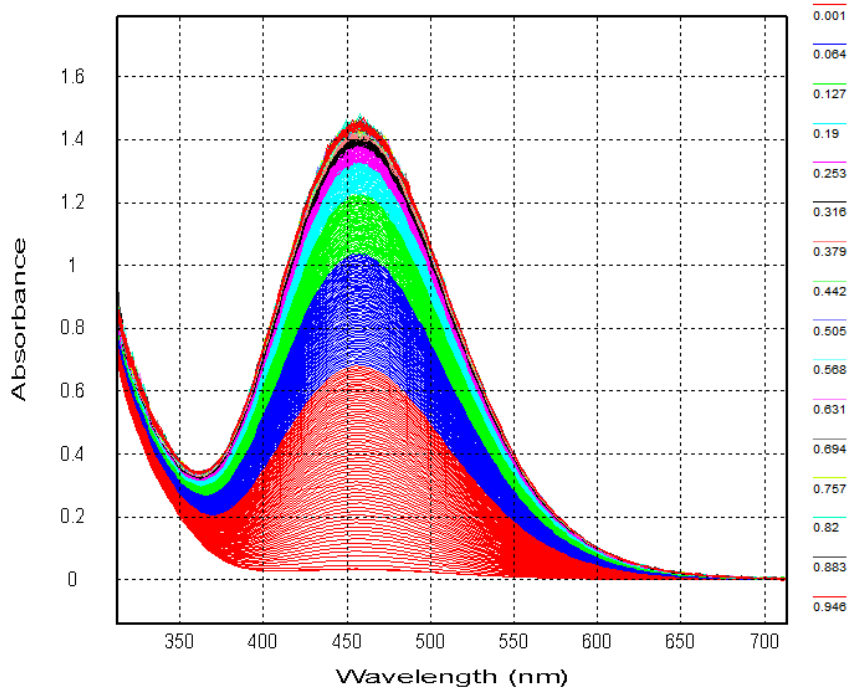


Figure 2.4 - PDA trace of iron perchlorate (20 mM) in perchloric acid (80 mM) reacted with sodium thiocyanate (1 mM) over 1 s (330 – 720 nm)

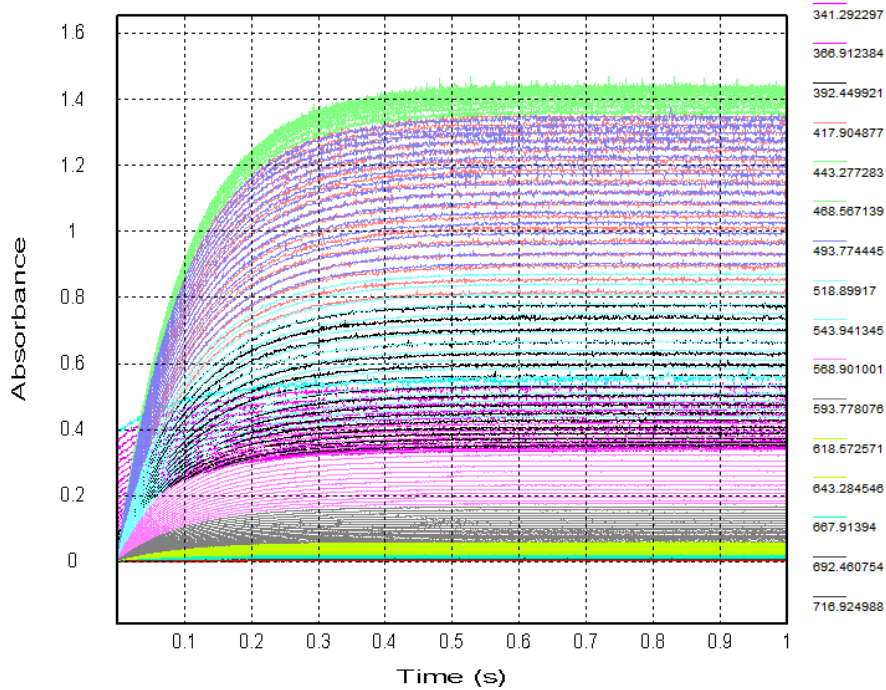


Figure 2.5 - PDA trace of iron perchlorate (20 mM) in perchloric acid (80 mM) reacted with sodium thiocyanate (1 mM) over 1 s (330 – 720 nm).

2.3.3 Criteria

Traces acquired should look comparable to those in Figure [2.4](#) and Figure [2.5](#) above. Some variation can be expected due to temperature variations and PDA set up.

The calculated kinetic rate constant should be comparable to that acquired in the absorbance test, if using the same test chemistry.

If the PDA test fails to meet the specified criteria, there may be underlying causes. Contact support@photophysics.com for assistance.

2.4 Kinetic Fluorescence Polarisation Test

This fluorescence polarization (FP) test tests the single wavelength kinetics performance of fluorescence polarization accessory in conjunction with the SX stopped flow instrument.

Phloxine B binds quite well to bovine serum albumin (BSA) and produces a good polarisation change. The optimal wavelengths when using phloxine are 535nm for excitation and 560nm for emission, however, since glass cut-off filters are used with the fluorescence polarisation accessory, 515nm has been found to be a satisfactory excitation wavelength when used with a 550nm filter.

2.4.1 Preparation of Reagents

You will require the following reagents:

1. BSA: Sigma-Aldrich product code A-4378 (MW=66000)
2. Phloxine B: Sigma-Aldrich product code P-2759 (MW= 830)
3. Buffer; pH 7 phosphate buffer (10 mM)

Be aware, phloxine may irritate the eyes. Wash hands after use.

Preparation of BSA working solution (10 μ M) in phosphate buffer (10 mM)

Stock solution BSA (100 μ M): Weigh out 33 mg and dissolve in 5 mL of pH7 phosphate buffer.

Working solution BSA (10 μ M): Dilute 1 mL stock solution in 10 mL pH7 phosphate buffer.

Preparation of phloxine B working solution (10 μ M)

Stock solution phloxine B (10 mM): Dissolve 41.5 mg of phloxine B in 5 mL of pH 7 phosphate buffer.

Working solution phloxine B (10 μ M): Dilute 10 μ L of stock solution in 10 mL of pH 7 phosphate buffer (i.e. 1000 fold dilution)

2.4.1 Experimental Conditions and Procedure

Satisfy the following conditions:

Experimental Conditions for the Fluorescence Polarisation Test	
Drive Syringe F	BSA (10 μ M)
Drive Syringe C	Phloxine B (10 μ M)
Detector	FP accessory
Monochromator slit width	2 mm
Excitation Wavelength	515 nm
Cut off filter	550 nm
Time base	100 s
Points	1000
Trigger	External
Temp	25 degrees centigrade

1. Select Fluorescence Polarisation as the signal.

2. Under options, select 'polarisation'.
3. Click on 'Setup' and follow the onscreen prompts to setup the FP accessory. (To achieve the end point of the reaction in the cell, drive several drives of BSA and phloxine B through to the flow circuit, and then wait 5 minutes).
4. Skip the optional offset setting (steps 3 and 4).
5. Click 'Acquire'.

2.4.2 Criteria

Your data should look like that in Figure [2.6](#) below.

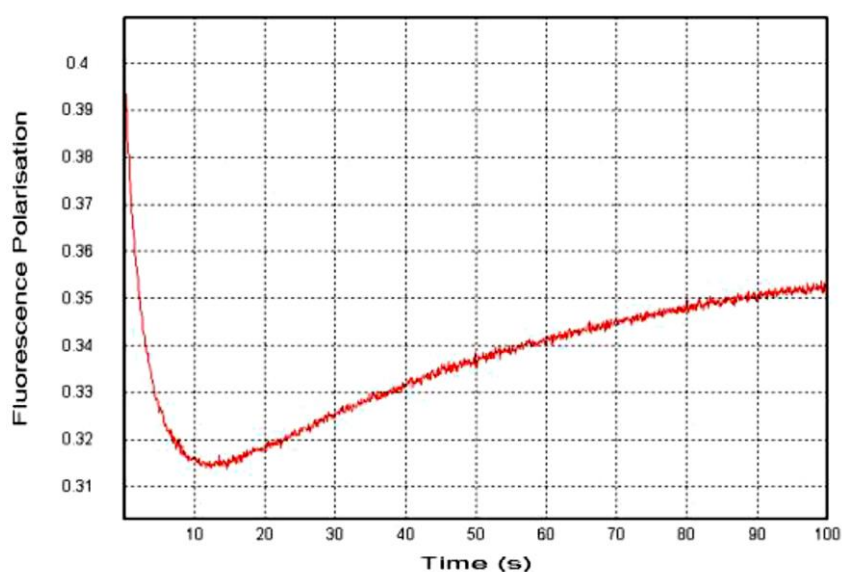


Figure 2.6 - BSA and phloxine B fluorescence polarisation kinetic trace.

If your data does not look like that in Figure [2.6](#), it could be that the setup procedure was not performed correctly. Try paying particular attention to the position of the input polariser while running through the setup once more.

2.5 Steady State Fluorescence Polarisation Test

Glycogen is a glucose polymer, which is highly soluble in water, but the large size of its molecule makes it a very efficient scatterer of visible light. Scattered light retains the entire the polarisation of the incoming polarised light and therefore has a FP signal of exactly 1.

2.5.1 Preparation of Reagents

You will require Glycogen (MW = 666.6) at 2 mg/mL in distilled water.

2.5.2 Experimental Conditions and Procedure

Satisfy the following conditions:

Experimental Conditions for the Fluorescence Polarisation Test	
Glycogen concentration in cell	2 mg/mL
Detector	FP accessory
Monochromator slit width	0.5 mm
Excitation Wavelength	525 nm
Cut off filter	No filters
Time base	1 s
Points	10
Trigger	Internal
Temp	25 degrees centigrade

1. Make sure that polarisation and not anisotropy is being collected.
2. Click on 'Setup' and follow the onscreen prompts to setup the FP accessory
3. With glycogen sample in the cell, set the Auto-PM to give a 10% value to avoid the PMTs' saturation. Check that neither of the PMTs have a HV of 1000V. Do not set the detectors' offsets and measure the G-factor with the excitation polariser in the setup position. This should be close to 1 if the accessory was setup correctly.

2.5.3 Criteria

Your data should look like that in Figure 2.7.

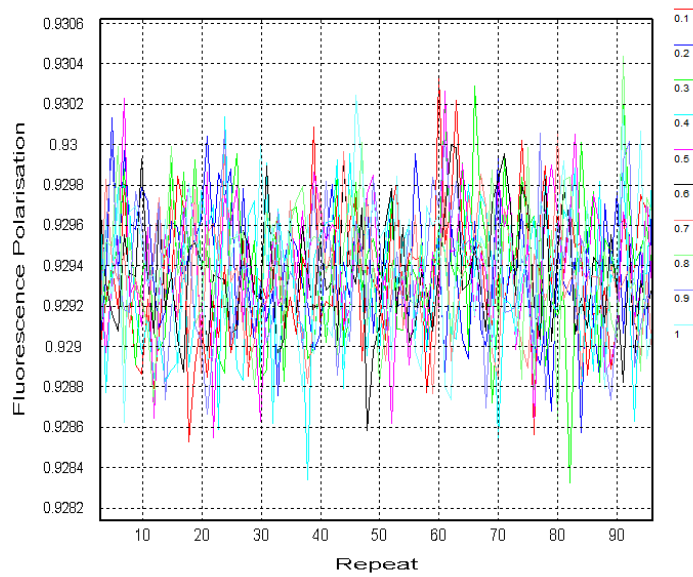


Figure 2.7 - Florescence polarisation trace of Glycogen (2 mg/mL)

The closer to 1 the FP signal value is the better performance of the accessory. The value is considered acceptable if it is over 0.9.

The lower the value of the FP, the more incorrectly polarised light is getting to one or both of the detectors. This is usually caused by inefficient (damaged) or misaligned polarisers or a stray light leakage.

3 ROUTINE SERVICE PROCEDURES

Routine service procedures should be performed periodically by the user, depending on how often the instrument is used.

3.1 *Cleaning the Flow Circuit*

To maintain the high performance of the stopped flow instrument, cleaning the observation cell and flow circuit should be included as part of the instruments routine maintenance, particularly if protein or subcellular fragment work is undertaken. A build-up of debris in the flow circuit and cell can cause serious errors in your results. How often your instrument is cleaned is determined by how often it is used, how it is used, and what chemicals it is exposed to. The following is a guideline for a suggested cleaning protocol.

3.1.1 Applicable Instruments

This procedure is applicable to all SX instruments.

3.1.2 Reagents Required

- Distilled Water
- 2 M nitric acid
- Hellmanex ® (catalogue number: 320.001)

3.1.3 Daily / Routine Cleaning

All reagents should be flushed out of the stopped flow unit following its use; in most cases, this means flushing distilled water through the instrument, but if the instrument is being used for organic work, a suitable organic solvent should be used. We also recommend the use of Hellmanex II fluid, especially if protein or sub-cellular fragment work is being undertaken. For more details on Hellmanex ® surfactant fluids, please contact Hellma.

3.1.4 Weekly / Monthly / Thorough Cleaning

If an instrument is displaying signs of excessive contamination (i.e. significant signal changes following water/water drives), then flush 2 M nitric acid through the flow circuit and leave to soak overnight. Wash the acid out with distilled water in the morning. Perform water drives to check contamination and repeat if necessary.

3.1.5 Periods of Non-use / Storage

If the instrument is being left for a single night or a long weekend before next being used, then distilled water is suitable as a storage solvent. If the instrument is being left for longer than this, then a solution of distilled water and alcohol is recommended.

Note: Overnight, solutions within the flow circuit may degas. To remove these bubbles effectively, consider flushing with an alcohol before introducing distilled water.

3.1.6 Manual Flushing

When flushing the stopped flow unit to remove old reagents and to add new ones, it is advisable to do so manually, so that the reagents in the stop syringe can be emptied more completely with each cycle. To flush reagents through manually, follow the four steps below:

1. Turn the stop valve 180 degrees clockwise, so that the narrow edge of the wedge faces away from the operator.
2. Empty the stop syringe manually by pushing the plunger upwards.
3. Return the stop valve to its normal position by turning it 180 degrees anticlockwise so the narrow edge faces the operator.
4. Fresh reagent can then be introduced into the drive syringes and flushed through the flow circuit manually (by raising the drive ram upwards against the drive syringe pistons).

This procedure can be repeated until sufficient reagents have been flushed through the flow circuit.

3.1.7 Cleaning the cell

If you have a removable cell cartridge, then this cell can be removed for a more thorough clean with 6 M nitric acid. See Section [3.9](#) for advice on how to remove the cell.

Once your cell is removed, use a needle and small syringe to fill the channels with 6 M nitric acid. Be careful not to allow the acid to come into contact with any external parts of the cell or cartridge.

3.2 Draining the Water Bath

Occasionally, the water bath will need to be drained to allow for leak tests, water circulator fluid replacement and to gain access to certain components.

3.2.1 Applicable Instruments

This procedure is applicable to all SX instruments.

3.2.2 Equipment Required

- 2.5 mm hexagon key
- Paper towels

3.2.3 Procedure

1. Remove all detectors and light guides attached to the cellblock.
2. Drain the water bath by unscrewing the bleed screw located on top of the cellblock (Figure 3.1).
3. When the water has drained away, remove the six screws on the front face of the water bath to remove the front panel (Figure 3.2). Make sure the X-ring does not fall out of the sample-handling unit as the front panel is removed.

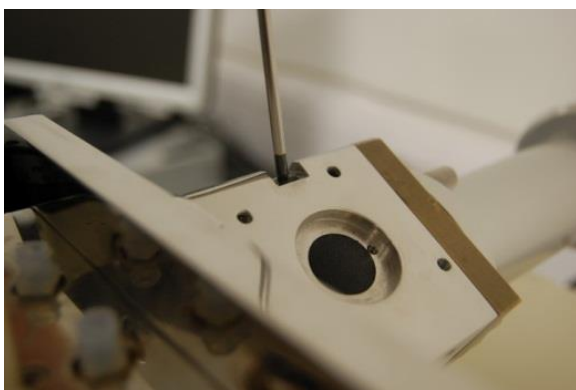


Figure 3.1 - Location of bleed screw on top of cell block

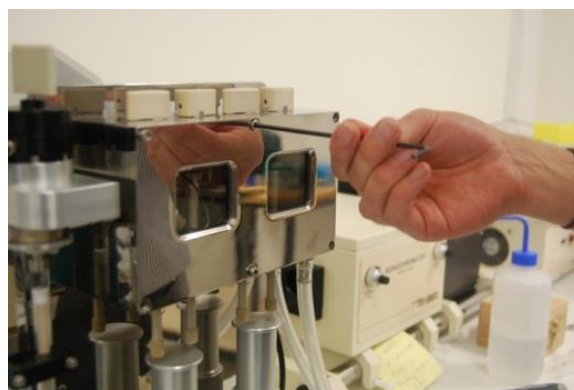


Figure 3.2 - Removing the water bath front panel

4. Remember to tighten the bleed screw when you are done, before turning the water circulator back on.

Note: If you are removing the front panel to gain access to the drive syringes, you may wish to dry the interior of the water bath with paper towels before you start the test to make any leaks more noticeable.

Note: If you are draining the water bath prior to refilling it with fresh fluids, please consult your circulator manufacturer's manual for recommended fluids and additives. A common fluid is distilled or deionised water, together with the additive ethylene glycol or an alcohol, to inhibit microbial growth.

3.3 Replacing the Lamp

Lamps are consumable and have a lifetime of approximately 1,000 hours. After this length of time, light throughput, stability and reliability can decrease significantly. The electrodes within the lamp erode with use, resulting in a deposit on the interior of the bulb and a less precise arc, which requires more and more energy to ignite.

A lamp will need replacing when:

- Ignition becomes difficult
- Output stability becomes unacceptable
- Operational lifetime is exceeded (normally 1,000 hours)

3.3.1 Applicable Instruments

This procedure is applicable to all SX instruments which feature a black lamp-housing unit. If your instrument does not feature a black lamp housing, please see Section [5.1](#).

3.3.2 Equipment Required

- 3 mm hexagon key
- 4 mm insulated hexagon key
- Sufficient PPE (i.e. a face visor)
- A new Lamp

3.3.3 Preparation

1. Switch off the lamp power supply and allow 30 minutes for the lamp to cool down.
2. Unbox your new lamp, and separate the protective case into its two halves.
3. Disconnect the red and black power cables from the rear of the lamp housing, together with the braided earth strap (Figure [3.3](#)).

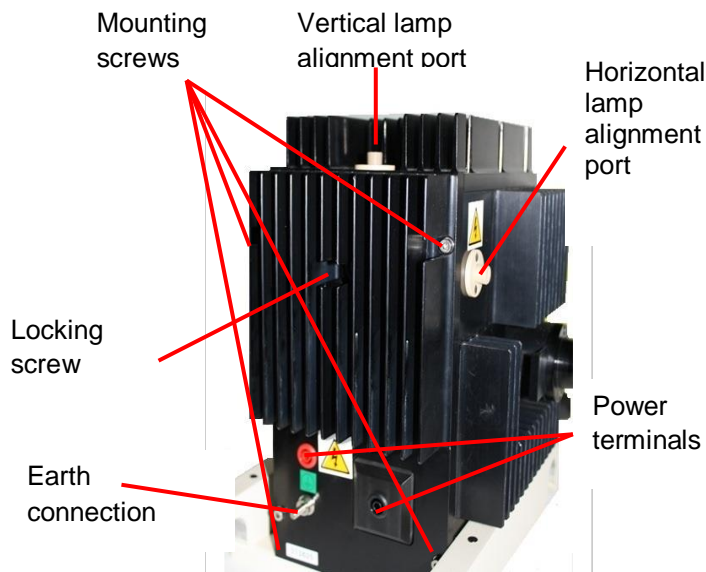


Figure 3.3 - Rear view of a black lamp housing unit

4. Loosen the four white plastic bolts, which secure the lamp housing to the optical rail.

5. Slide the lamp housing to the right then remove it from the optical rail and place on a suitable work surface.

3.3.4 Procedure

1. At the rear of the lamp housing, remove the lamp-stabilising magnet if present, loosen the lamp alignment locking screw by half a turn, and then tighten it up just $\frac{1}{4}$ of a turn so that it bites, but is not tight.
2. Unscrew the four mounting screws.
3. Carefully remove the lamp housing back panel.
4. Loosen the clamping screw on the anode-mounting block (Figure 3.4).

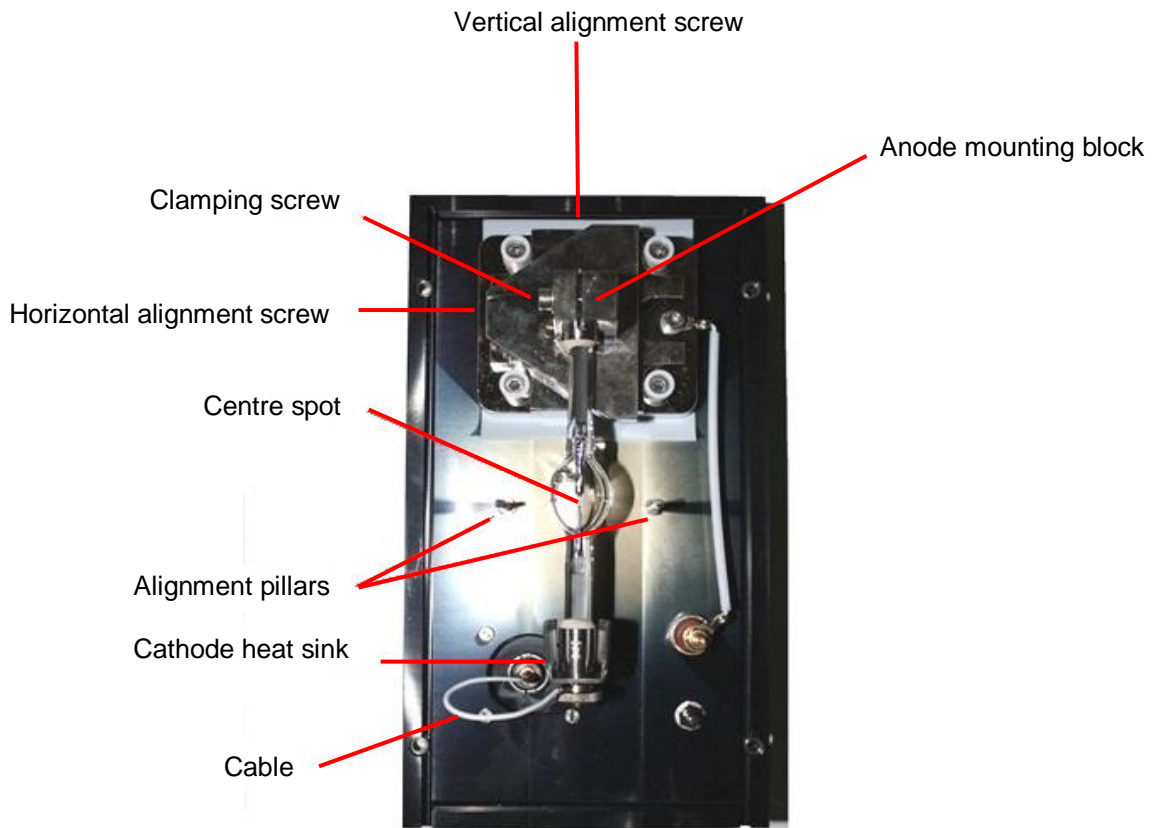


Figure 3.4 - Black lamp housing unit back panel

5. Being careful not to touch the glass, remove the lamp.
6. Gripping the cathode, unscrew the finger-tight nut and remove the cable, washer and heat sink (Figure 3.4).
7. Place the old lamp in the unused half of the new lamps protective container.

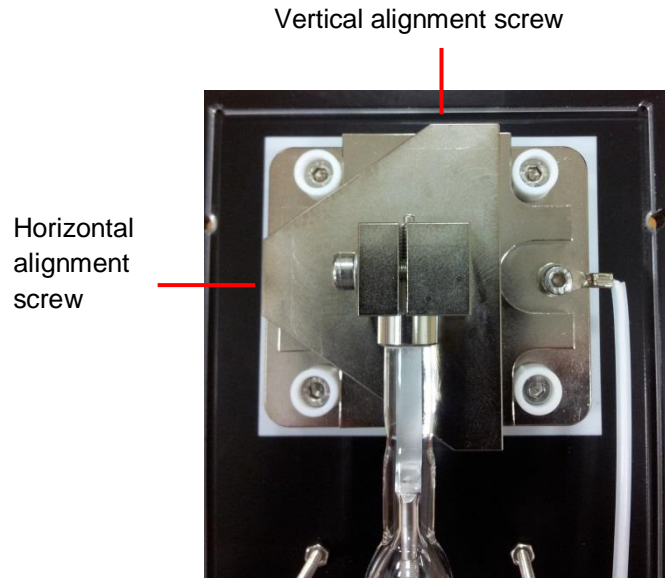


Figure 3.5 - Location of the lamp alignment screws

8. Centre the vertical position of the anode-mounting block by adjusting the vertical alignment screw (Figure 3.5).
9. Being careful not to touch the glass envelop, grip the new lamp by the cathode, and fit the cathode heat sink, washer and cable into place using the finger-tight nut.
10. Fit the anode of the new lamp into the mounting block.
11. Adjust the lamps vertical position within the mounting block, so that when viewed from the side, the gap between the two electrodes within the lamp envelope aligns with the two alignment pillars (Figure 3.6).

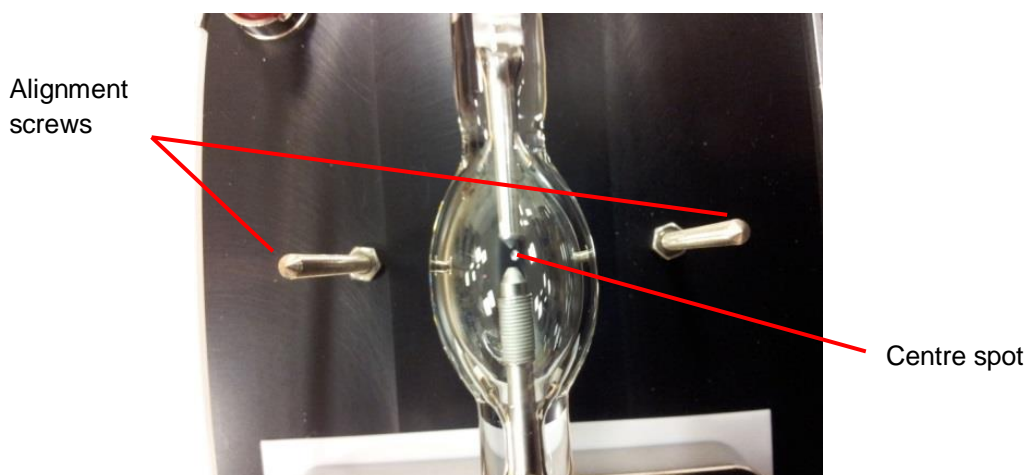


Figure 3.6 - Location of lamp alignment pillars and centre spot

12. Tighten the clamping screw (Figure [3.4](#)).
13. Adjust the lamps horizontal position using the horizontal alignment port, so that the gap between the electrodes aligns with the centre spot on the lamp housing back plate.
14. Refit the back plate to the lamp housing and secure it with the four screws.
15. Replace the lamp housing onto the optical rail and engage with the monochromator.
16. Reconnect all cables to the rear of the lamp housing unit and tighten the four white plastic bolts.
17. Switch on the lamp power supply, wait a few seconds then ignite the lamp.
18. If applicable, reset the LCD counter on the power supply.
19. Leave the lamp to equilibrate for 30 minutes. Your lamp is now ready for alignment (Section [3.4](#))

3.4 Aligning the Lamp

You may need to align your lamp if you have recently installed a new lamp, or if you need to optimise your light output, and hence improve your S/N ratio.

3.4.1 Applicable Instruments

This procedure is applicable to all SX instruments which feature a black lamp-housing unit. If your instrument does not feature a black lamp housing, please contact support@photophysics.com.

3.4.2 Equipment Required

- 4 mm insulated hexagon key

3.4.3 Preparation

1. Ignite the lamp, and leave for 30 minutes to equilibrate.
2. In the meantime, flush the cell with water, and set up the 2 mm path length. Set the instrument to absorbance mode, and set the monochromator to 350 nm with a slit width of 0.5 mm.
3. Remove both the vertical and horizontal beige plastic screws from the alignment ports (Figure [3.7](#)).

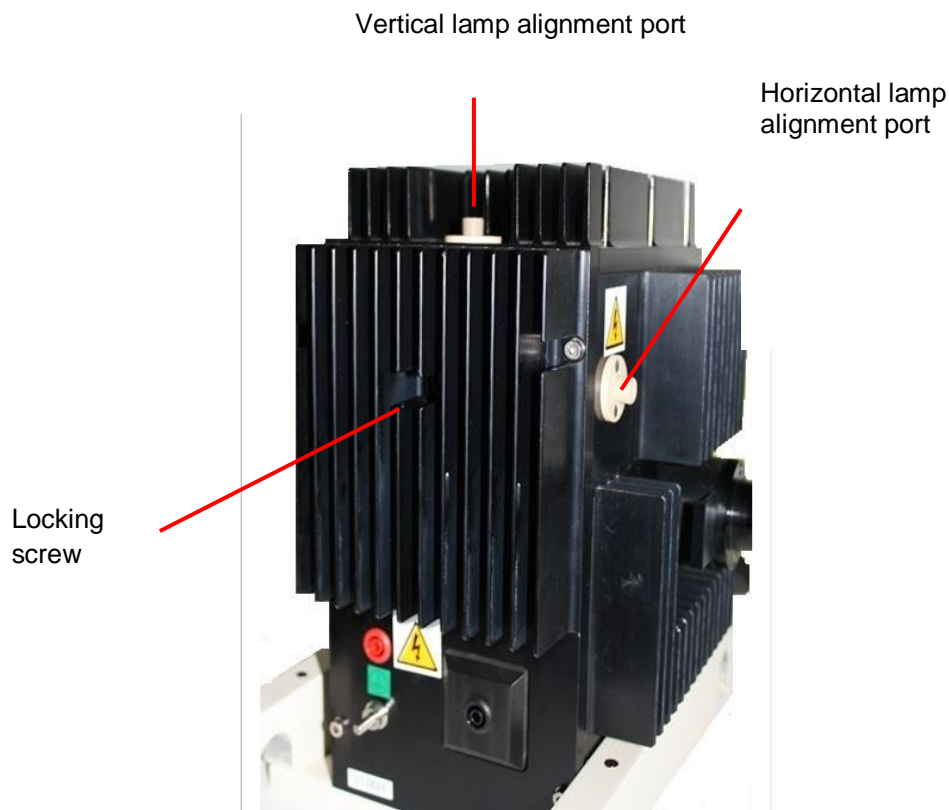


Figure 3.7 - Location of alignment ports and locking screw on the black lamp housing

4. If fitted, remove the stabilizing magnet located on the back panel of the lamp-housing unit, in between the cooling fans.
5. Loosen the locking screw on the back panel of the lamp housing. Now, tighten the locking screw back up so it bites, but not so much as to fix it in place.

- Once the lamp has equilibrated, click on the reference button to set the signal at 0 AU

3.4.4 Procedure

- Fit the insulated hex key into the vertical lamp alignment port.
- Turn the key clockwise and simultaneously monitor the live data display (Figure 3.8).

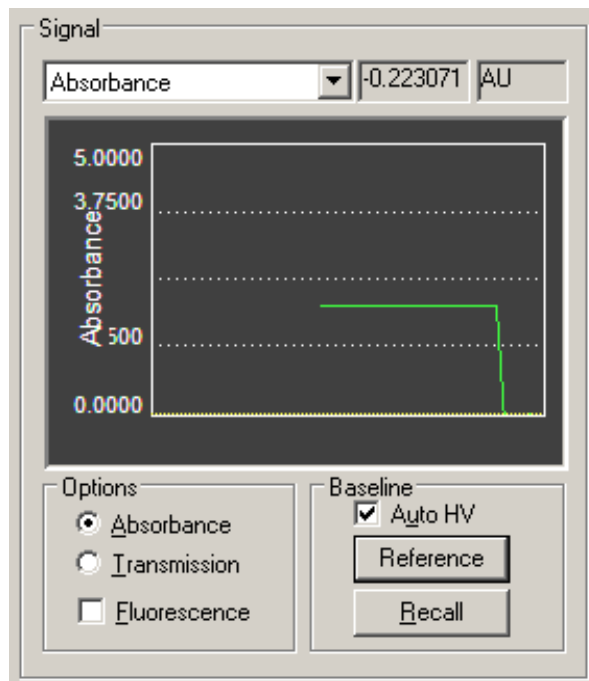


Figure 3.8 - Live trace display window

- The aim is to achieve the most negative absorbance possible (i.e. the greatest transmission possible). If the live data display decreases, indicating more light is passing through, keep adjusting in this clockwise direction until it plateaus.
- If turning the key in this direction results in an increase in absorbance, turn the key in the opposite direction. Once the signal has plateaued and absorbance cannot be reduced further, move onto the horizontal lamp alignment port and repeat this process.
- During this process, you may need to take another reference (or auto PM) if the live trace moves outside of the linear range of display.

Note: You should see the high voltage applied to your photomultiplier tube (PMT) decrease as you take a fresh reference, e.g. from 300 V to 280 V. This means that less high voltage amplification is required to get the same output from the PMT, because more light is reaching the detector.

Note: Double left click on the live data display to zoom in, double right click to zoom out.

- Once you have optimized the vertical alignment port, move onto the horizontal alignment port. After, go back and adjust the vertical port once more. Alignment is complete when adjusting the ports results in no further reduction in absorbance.

7. Auto PM your detector. New instruments can expect to see a high voltage equal to 200 – 240 V using the setup outlined in this document. Older instruments can expect high voltages of > 300 V depending on age.
8. Tighten the locking screw to secure the lamp in place, and reattach the stabilizing magnet if necessary. Screw the beige plastic screws back into place over the horizontal and vertical adjustment ports.

3.5 Replacing a Drive Syringe

Syringes are moving parts under high pressure and will naturally wear out over time. When they become worn, they may present leaks, wasting sample and introducing artefacts. To overcome these issues it may eventually become necessary to replace the drive syringes. This procedure describes the necessary steps to enable a competent user to replace the drive syringes on any SX series instrument.

3.5.1 Applicable Instruments

This procedure is applicable to all SX series stopped flow models.

3.5.2 Equipment Required

- Hexagon keys
- Paper towels

3.5.3 Preparation

Before removing drive syringe, you will first need to drain the water bath (Section [3.2](#)).

3.5.4 Procedure

1. Grip the empty drive syringe by its metal collar where it connects with the drive valve and unscrew by hand (Figure [3.9](#)).

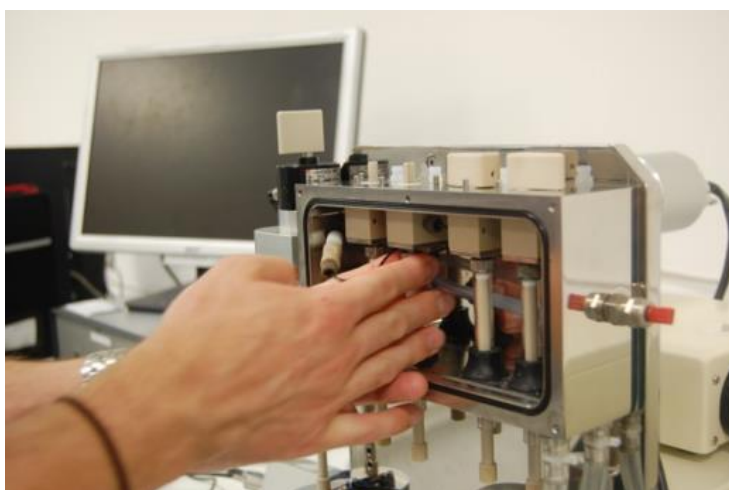


Figure 3.9 - Removing a drive syringe

Note: If the syringes are too tight to unscrew by hand, loosen the drive valve screws on top of the water bath half a turn. Remember to tighten them once the syringe has been removed.

2. Press the old syringe through its rubber sealing grommet and out of the bottom of the sample handling unit. Removing the drive ram is not essential, but may make removing the drive syringe easier.
3. With the glass barrel in the palm of your hand, grip the bottom edge of the water bath with your thumbs and push the syringe up through the grommet (Figure [3.10](#)).

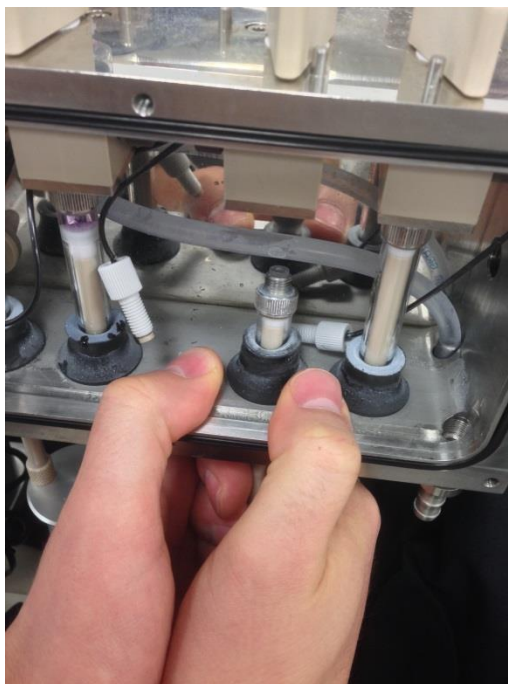


Figure 3.10 - Inserting a new drive syringe

4. Screw the syringe into the drive valve.

Note: If the syringe will not screw in easily, wet the grommet with a little water to reduce friction between the glass syringe and rubber grommet.

5. Perform a leak test (Section [1.3](#)) before reattaching the front plate and recirculating the circulator fluid into the water bath.

3.6 Replacing a Drive Valve

Drive valves are consumable items and will eventually require replacement. This procedure describes how to replace them.

3.6.1 Applicable Instruments

This procedure is applicable to all SX stopped flow models.

3.6.2 Equipment Required

- Hexagon keys
- Flow line tightening tool or needle nose pliers

3.6.3 Preparation

Before you attempt to remove a drive syringe, you should first drain the water bath (Section [3.2](#)) then remove the relevant drive syringes (Section [3.5](#))

3.6.4 Procedure

1. Half turn the drive valve knob to gain access to the grub screw located at its rear and unscrew using a 2 mm hexagon key (Figure [3.11](#)).



Figure 3.11 - Location of drive valve knob grub screws

2. Remove the drive valve knob and plastic washer found underneath.

3. Loosen the luer fitting using needle-nosed pliers and remove (Figure [3.12](#)).



Figure 3.12 - Removing the luer fittings

4. Unscrew the two 2.5 mm hexagon screws located on top of the drive valve and remove the metal holding plate from below (Figure [3.13](#)).

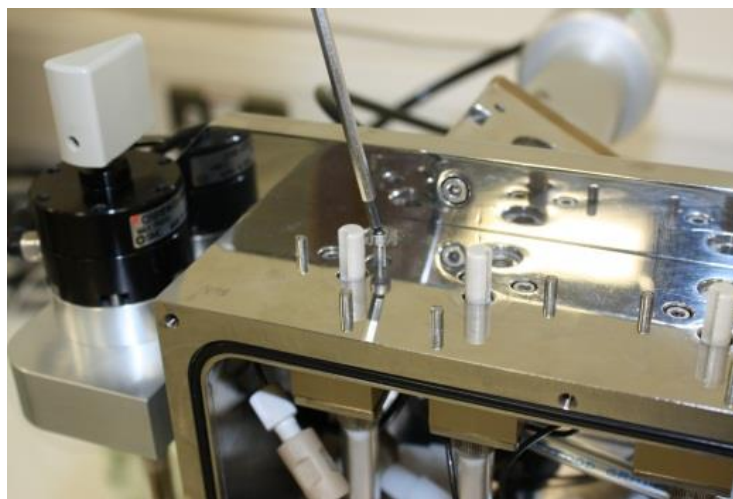


Figure 3.13 - Unscrewing the drive valve screws

- Drop the drive valve down to gain better access to the connecting flow line. Be careful not to lose the O-rings or snap the flow tubing (Figure 3.14).



Figure 3.14 - Dropping a drive valve

- Use either the flow line tool, needle nosed pliers or your fingers, loosen and detach the flow line attached to your drive valve.
- Install your new drive valve following the instructions above in reverse order, i.e. push the drive valve up through the hole in the water bath (after ensuring both O-rings are present), attach the appropriate flow line, screw the two drive screws into the holding plate, replace the washer, drive valve knob and luer fitting. Pay attention to the drive valve and holding plate orientation (Figure 3.15).

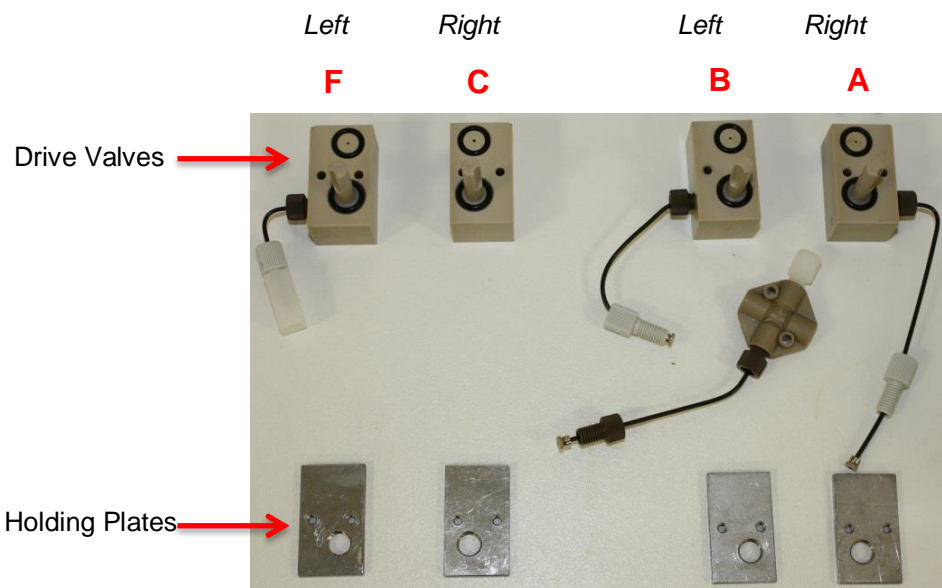


Figure 3.15 - Drive valve and holding plate orientations

8. Perform a leak test (Section [1.3](#)) before reattaching the front plate and recirculating coolant into the water bath.

Note: Drive valve holding plates can be attached in two orientations; only one will allow the drive syringe to be attached successfully. Ensure the drive syringe hole in the holding plate matches up with that on the drive valve, before attaching the drive screws.

Note: Be careful not to pinch any PEEK tubing in between the drive valve holding plates and the drive valves when tightening the drive valve screws.

Note: If the leak test fails after tightening the drive syringes by hand, loosen the drive valve screws, tighten the drive syringes once more, then tighten the drive valve screws to seal the syringes in place.

3.7 Replacing the Stop Syringe

3.7.1 Applicable Instruments

This procedure applies to all SX stopped flow instruments.

3.7.2 Equipment Required

- 2.5 mm hexagon key

3.7.3 Procedure

If you have an SX20 instrument, your autostop assembly should look like that on the left in Figure 3.16. If you have an earlier model, such as the SX18, your autostop assembly may be side-mounted and look more like that on the right. Either way, the process of changing a stop syringe remains largely the same.



Figure 3.16 - Left: SX20 autostop assembly, Right: Older SX18 and SX19 autostop assembly

1. If your instrument has a brake assembly (fitted to most sequential mixing models, not shown in Figure 3.16), remove this first using by gripping the knurled knob and rotating it anticlockwise until it comes apart. This will loosen its grip on the stop syringe plunger.
2. Next, manually empty the syringe by turning the stop valve to the waste position (narrow end pointing away from the user) and push the bottom of the plunger up into the syringe.

3. Unscrew and remove the syringe clamp if present, using a 2.5 mm hexagon key (non-side mounted models only).
4. Using your fingers, grip the metal collar at the top of the syringe, unscrew and remove the stop syringe from the stop valve.
5. If you have a brake assembly, this will fall into several pieces as the stop syringe is removed (the collar, two brake shoes and an O-ring). Leave the bracket in place so as to assist with reassembling the brake when reinstalling the stop syringe. Reassembly is the reverse of this procedure.

Note: To avoid damaging the syringes, only grip them by their metal collars. Never tighten them into place by the glass barrel.

3.8 Replacing the Stop Valve

The model shown in this service procedure is an SX20. Previous models may differ to some extent. Stop valves are a consumable item and will eventually require replacement. This procedure guides the user through the steps necessary to replace the stop valve on the SX20 model (although similar steps will apply to other models). Stop valve replacement is also included as part of the preventative maintenance service provided by Applied Photophysics.

3.8.1 Applicable Instruments

This procedure is mostly applicable to instruments which feature the front-mounted autostop mechanism, although, the principles can be applied to the older side-mounted autostop models.

3.8.2 Equipment Required

- Hexagon keys
- 10 mm wrench
- Needle nose pliers

3.8.3 Preparation

Before removing the stop valve, you will first need to drain the water bath (Section 3.2) and also removed the stop syringe (Section 3.7).

You should familiarise yourself with the components referred to in Figure 3.17.

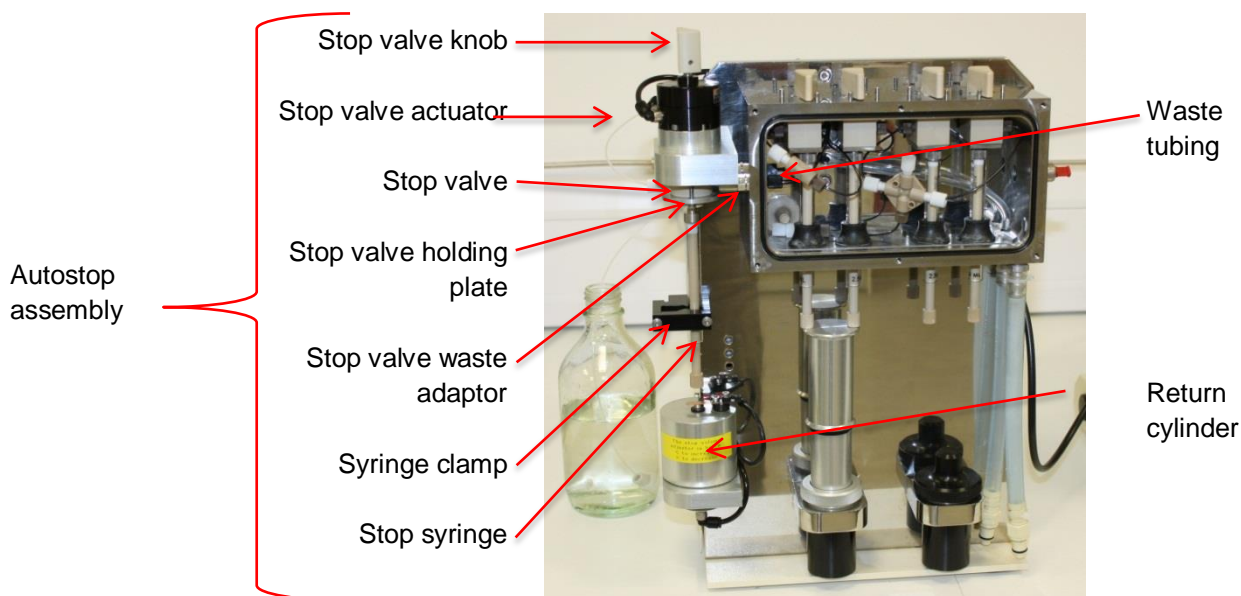


Figure 3.17 – Components involved in replacing the stop valve

3.8.4 Removing the Stop Valve

1. Disconnect the pneumatic tubing from the top of the stop valve actuator. Take note of which tube goes into which adaptor (Figure 3.18, top circle).
2. Disconnect the finger tight waste line (Figure 3.18, bottom circle).

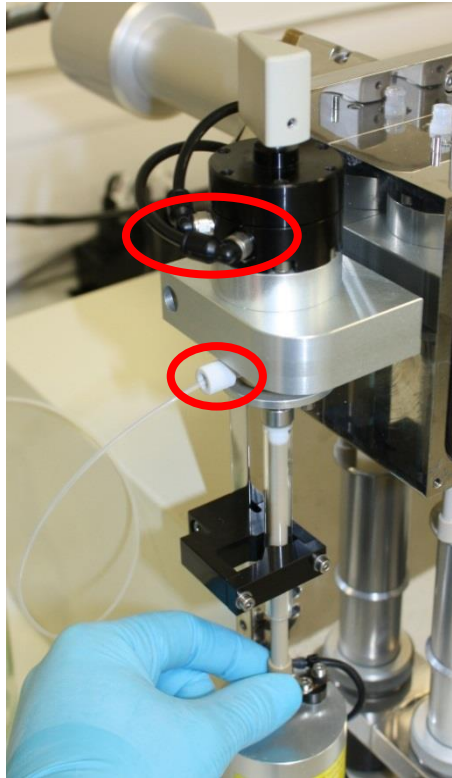


Figure 3.18 - Location of pneumatic tubing (top circle) and waste line (bottom circle)

3. Grip the stop valve waste tube adaptor using a 10 mm wrench and unscrew the waste tubing (Figure 3.19) by hand. If required, loosen the waste tubing using needle nose pliers a little before removing by hand.

Note: The metal stop valve waste tube adaptor is located between the stop valve assembly and the water bath. The stop valve adaptor is attached to the flow output tubing (Figure 3.19), which is located inside the water bath.

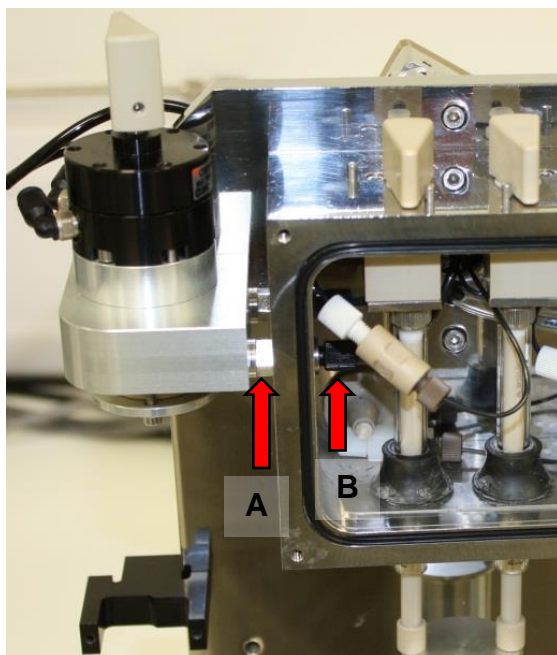


Figure 3.19 - Location of stop valve waste tube adaptor (A) and waste output tubing (B)

4. Unscrew the actuator platform screws using a 4mm hexagon key. One screw is on the side of the platform (Figure [3.20](#)); the second is accessed from the rear of the unit (Figure [3.21](#)).

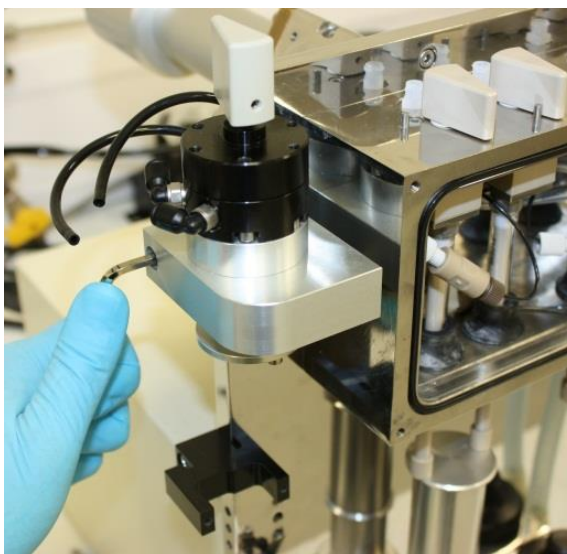


Figure 3.20 - Removing the screw on the side of the actuator platform

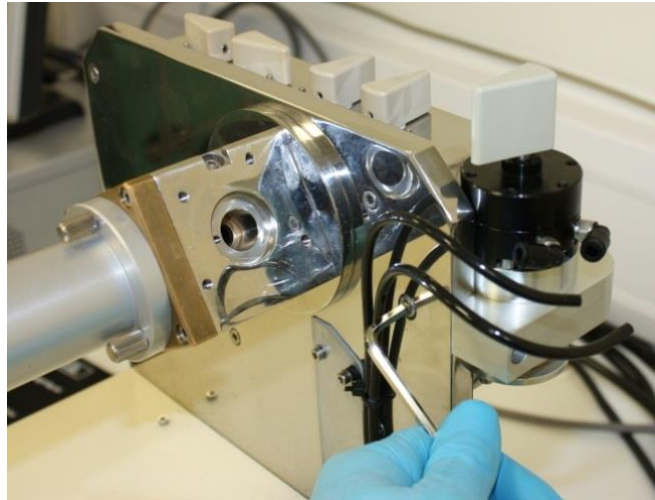


Figure 3.21 - Removing the screw on the rear of the actuator platform

5. Remove the top of the autostop assembly from the KSHU by gently pulling it to the left (Figure [3.22](#)). This may take some effort due to the sealing O-ring on the stop valve waste adaptor.

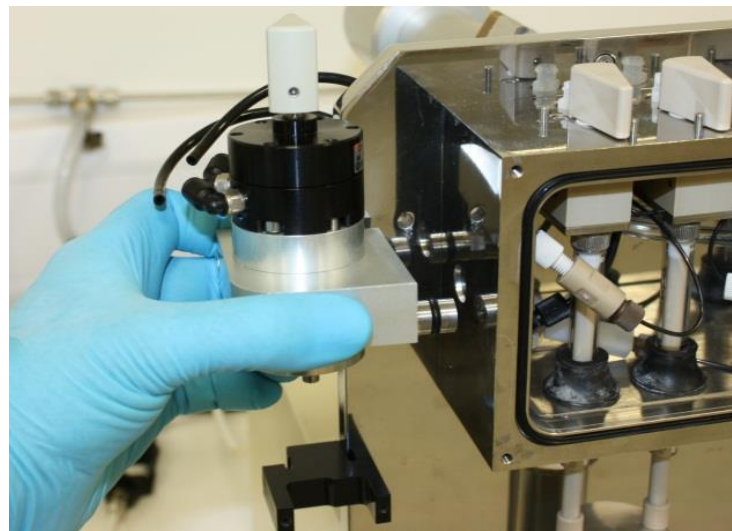


Figure 3.22 - Removing the stop valve actuator

6. Once you have removed the stop valve assembly, disassemble it into the components below (Figure [3.23](#)); remove the stop valve adaptor using a 10 mm wrench, unscrew the holding plate using a 2.5 mm hex key, then remove the stop valve from the stop valve actuator.

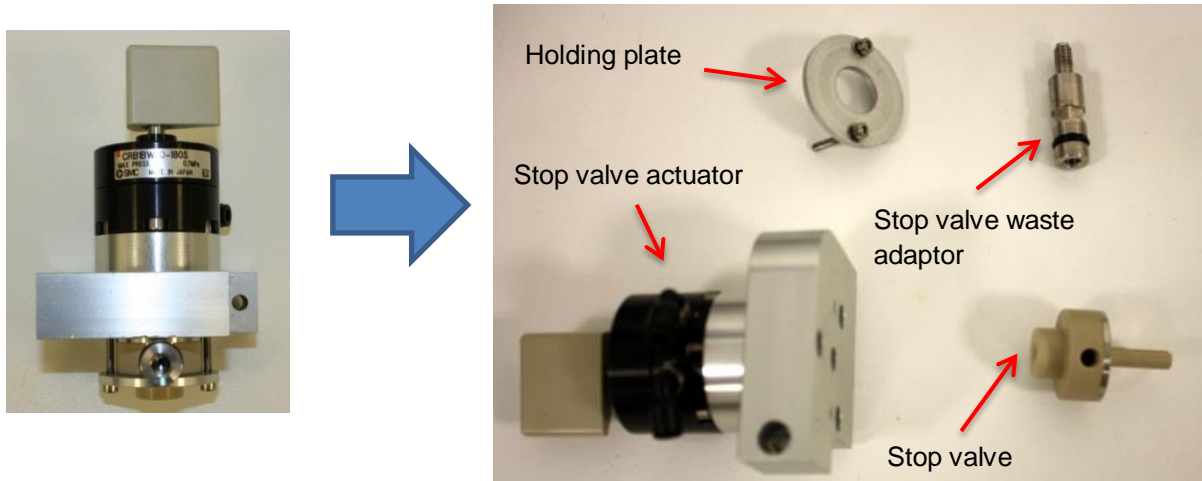


Figure 3.23 - Disassembly of the stop valve actuator assembly into its component parts

7. You can now replace the stop valve.

3.8.5 Reassembling the Stop Valve

Reassembly requires performing the directions above in reverse order, with a few considerations.

1. Screw the stop valve waste adaptor, finger-tight, into the new stop valve and insert this into the actuator (These pieces will fit together in one orientation only).
2. **Loosely** attach the holding plate using a 2.5 mm hex key. This needs to be loose to allow movement when fitting the assembly back onto the KSHU.
3. If required, grip the stop valve adaptor in one hand and the stop valve knob in the other and twist the stop valve so that the adaptor is perpendicular to the knob (i.e. the knob should point away from the user, while the adaptor should point to the right). The assembly should look as in Figure 3.24 below.



Figure 3.24 - A correctly assembled stop valve actuator

4. Attach the assembly back onto the KSHU by feeding the stop valve adaptor through the hole in the water bath and securing the two 4 mm hexagon bolts (Figure [3.21](#) and Figure [3.20](#)).

Note: If the platform does not fit, try loosening the Holding plate a little more.

5. Once attached, tighten the Holding plate (Figure [3.25](#)) and the stop valve adaptor using a 2.5 mm hexagon key and 10 mm wrench.

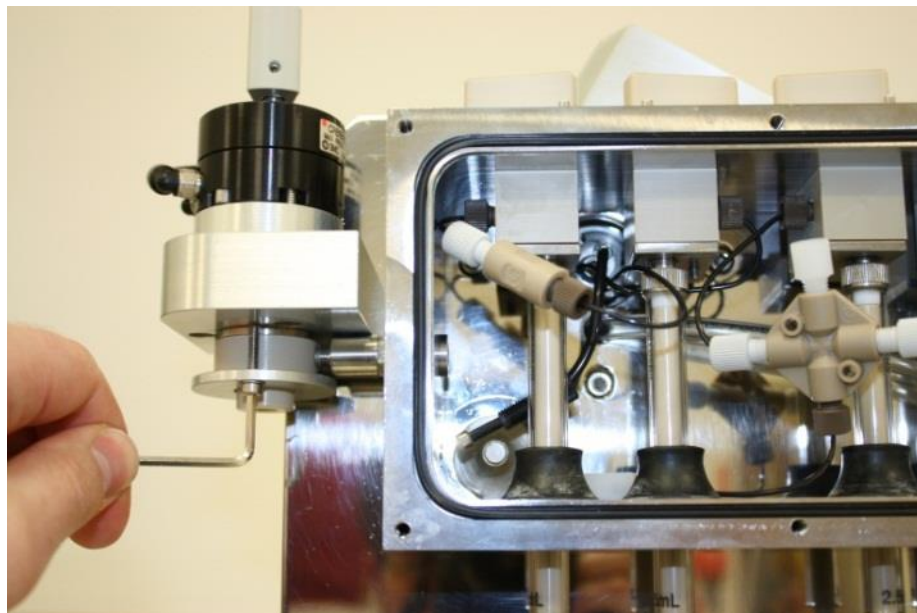


Figure 3.25 - Tightening the stop valve holding plate

Note: Both the holding plate and the waste tube adaptor need to be tight enough to prevent leaks, but not so tight as to cause difficulty in turning the stop valve knob. Test for this when tightening these components.

6. Connect the flow tubing back into the stop valve adaptor. You will need to hold the waste tube adaptor in position using a 10 mm wrench while you tighten the waste tube into it.
7. Attach the pneumatic tubes, waste line, stop syringe, clamp and if fitted, brake (Figure [3.26](#)).
8. Leak test the system (Section [1.3](#)). If necessary, use needle nose pliers to turn the flow output tubing an extra $1/8^{\text{th}}$ of a turn to achieve an effective seal. Once you are sure there are no leaks, replace the front cover, taking care to fit the X-ring correctly.



Figure 3.26 - A complete autostop assembly (no brake shown)

3.9 Removing the Removable Cell

This procedure describes how to replace a cell on the SX series of stopped flow instruments. You may wish to do this to change to the smaller volume cell, or if the existing cell is broken or requires a thorough clean.

There are two types of cell: a removable cell (RC) and a non-removable cell (non-RC). A RC can be identified by a brass or black coloured cartridge, visible on the end of the cell block, which attaches to the fluorescence detector. Similarly, a non-RC can be identified by stainless steel in the same location. This document deals with the replacement of a RC only. If you have a non-RC, please contact support@photophysics.com for assistance.

3.9.1 Applicable Instruments

This procedure is applicable to all instruments which feature a removable cell.

3.9.2 Equipment Required

- Hexagon keys

3.9.3 Preparation

Flush the instrument with deionized/distilled water and drain the water bath (Section [3.2](#)). Remove all detectors and light guides from the cell block.

3.9.4 Procedure

1. Locate the pressure plate screw, in between three PEEK tubes, inside the water bath (Figure [3.27](#), red circle). This screw holds a pressure plate in position, which in turn holds three flow lines up against the cell. Loosen this screw by 1 turn. Do **not** fully unscrew this screw.

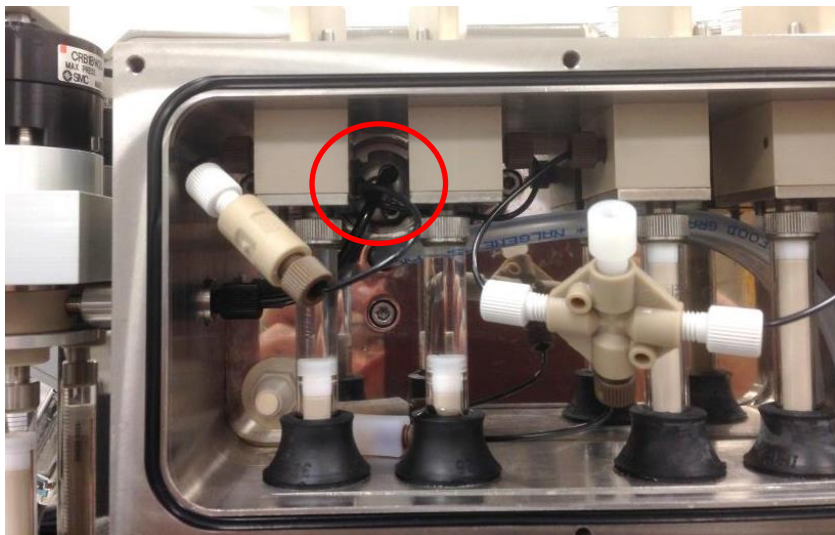


Figure 3.27 - Location of pressure plate screw

2. Loosen the four hexagon screws that hold the cell cartridge in place (your cartridge will be either black or brass) as seen in Figure [3.28](#).

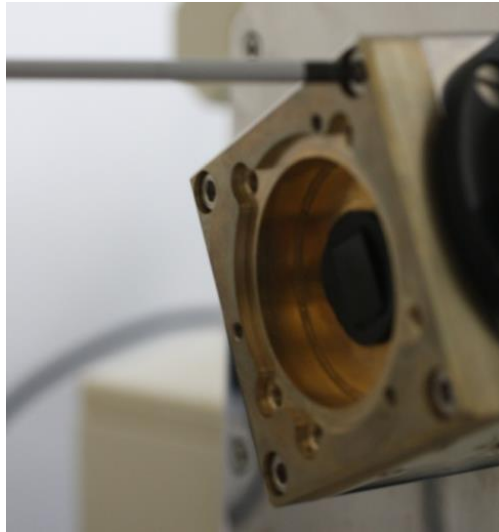


Figure 3.28 - Unscrewing the removable cell cartridge

3. Pull the cell straight out. Be careful not to cause damage to the cell (Figure [3.29](#)).

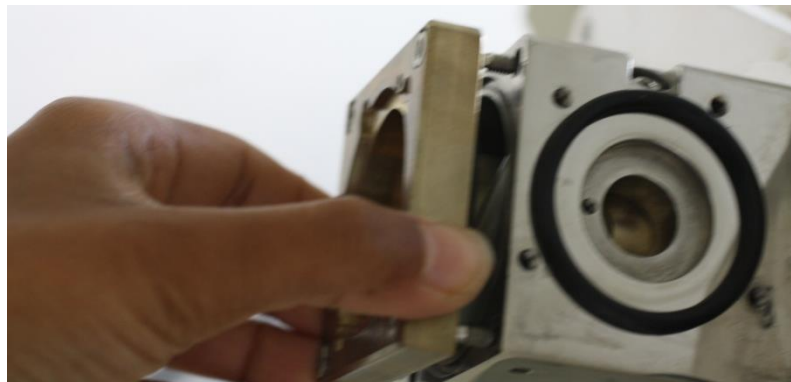


Figure 3.29 - Removing the removable cell

Note: Move the water circulator hose back into position at the rear of the cellblock if required.

Note: With the cell now removed, it can be exchanged or cleaned as desired. If it is to be cleaned, consider using concentrated nitric acid (6 M), being careful not to get the acid on the silicone sealant, which is holding the cell in the cartridge. Follow lab protocols for using such strong acids.

4. Reassembly is the reverse of the actions described above, i.e. push the cell and cartridge back into the cell block, with the bevelled corner facing upwards, screw in the four screws, and then tighten the pressure plate screw.

Note: Avoid over tightening the pressure plate screw and damaging the cell. Tighten a small amount at a time, and leak test the instrument (Section [1.3](#)).

Note: Ensure the cell and flow circuit are watertight before reattaching the front water bath panel. In addition, check the bleed screw is tight before filling the water bath once more.

3.10 Replacing Flow Tubes

This procedure describes how to replace PEEK tubing on the SX series of instruments. Some PEEK tubing is simple to replace and requires little explanation, but the 'valve-to-cell' and 'cell-to-waste' PEEK tubes require the instrument to be dismantled for correct installation. Please read this document thoroughly before attempting this procedure.

Applied Photophysics cannot accept responsibility for any damage occurring as a result of following these instructions. The user assumes all responsibility and risk.

Note: The PEEK tubing supplied by Applied Photophysics has been heat-treated, to reduce data artefacts caused by expansion and contraction. This makes the tubing more brittle than regular PEEK tubing. Handle with caution to prevent damage.

3.10.1 Applicable Instruments

This procedure applies specifically to removable cell, sequential-mixing SX stopped flow instruments. If your instrument is of the older, non-removable cell variety, or a single-mixing unit, the procedure will differ slightly. Contact support@photophysics.com for assistance.

3.10.2 Equipment Required

- Hexagon keys
- Needle-nosed pliers

3.10.3 Preparation

1. Drain the water bath (Section 3.2), remove the stop syringe (Section 3.7) and remove the stop valve actuator assembly (Section 3.8). Your instrument should look similar to that in Figure 3.30.

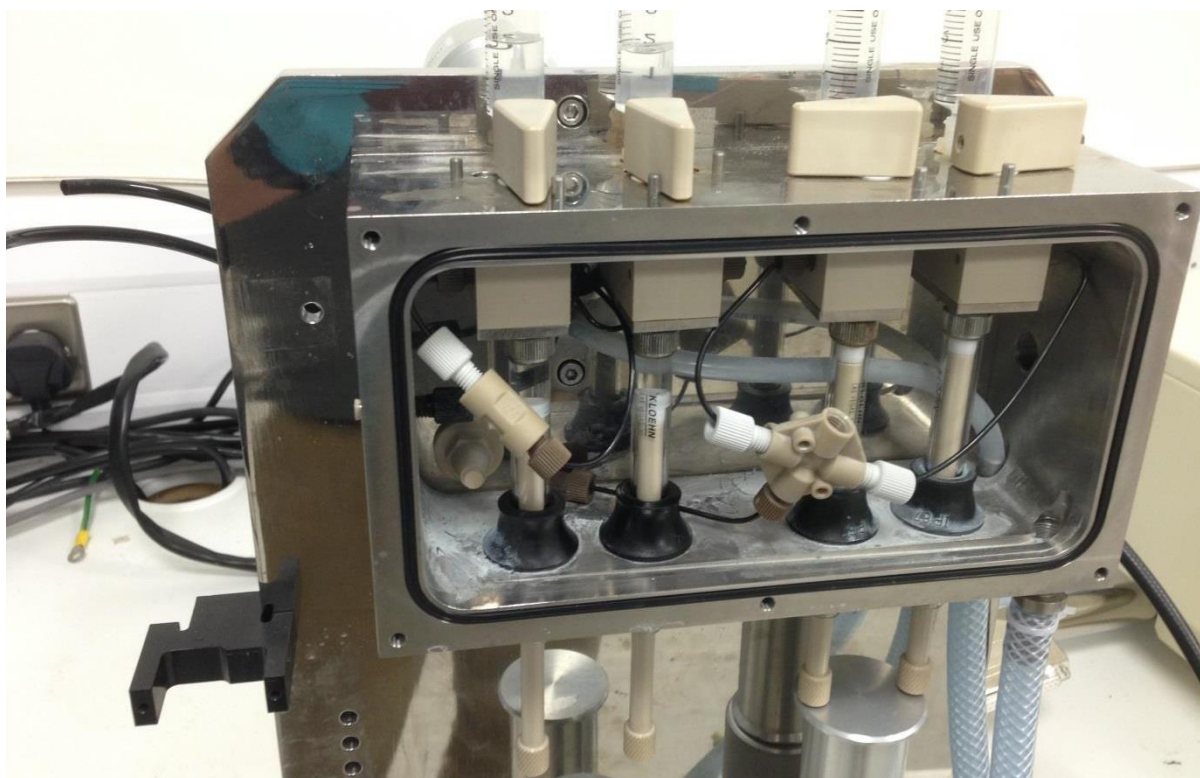


Figure 3.30 - KSHU unit with the water bath drained, and both stop valve actuator and stop syringe removed

3.10.4 Procedure

1. Disconnect the two 'cell-to-valve' flow lines (red), the 'cell-to-waste' flow line (green) and the water circulator tube (blue) shown in Figure 3.31.



Figure 3.31 - Location of tubes to disconnect prior to water bath removal

2. With one hand on the water bath, unscrew the five screws located at the rear of the KSHU unit, shown in Figure 3.32. Don't let the water bath drop! Remove the water bath, complete with drive valves and drive syringes and place it in a safe place. You may wish to remove the detectors for better access.

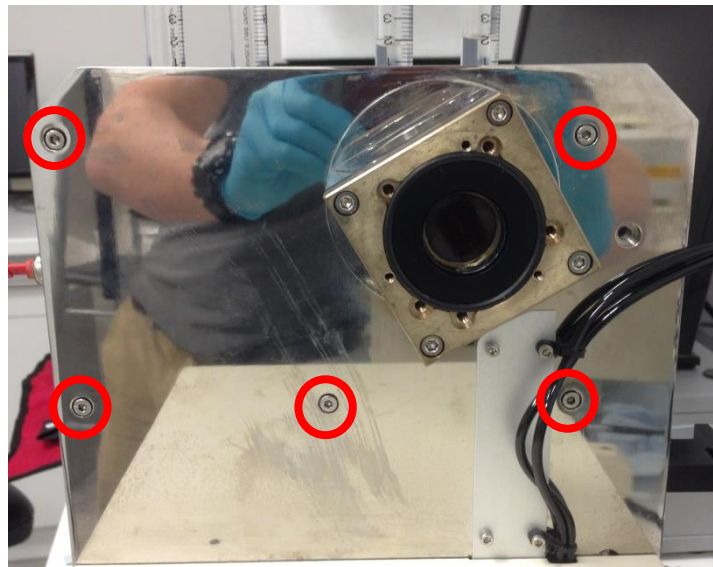


Figure 3.32 - Location of water bath screws at the rear of the KSHU unit

3. Unscrew the pressure disk screw shown in Figure 3.33 and remove the PEEK tubing assembly.



Figure 3.33 - Location of pressure disk screw

4. Disassemble this PEEK tubing assembly into the parts seen in Figure [3.34](#). Clean up any dirty parts and replace any broken tubes.

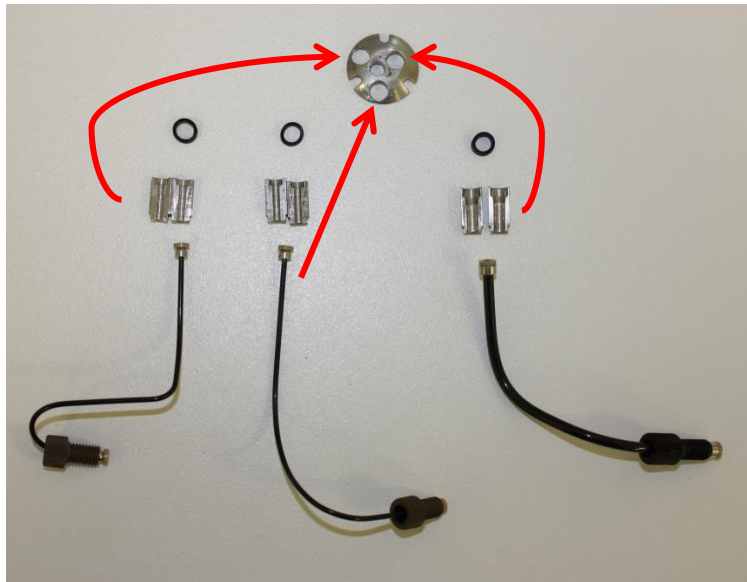


Figure 3.34 - Disassembled PEEK tubing assembly and correct location within the pressure disk

6. Reassemble the PEEK tubing assembly. The pressure disk is asymmetrical. The larger diameter, waste flow line goes through the hole which is furthest from the pressure disk's circumference (i.e. closest to the centre). The locations of the two remaining tubes are shown in Figure [3.34](#). Once the tubes have been pushed through their respective holes, their metal collars should be fitted around their ends and then fixed in place using the rubber bands (Figure [3.35](#)).



Figure 3.35 – An assembled, new and clean flow tube assembly

7. Push the flow tube assembly back into the cell block and tighten the pressure disk screw so that the tubes remain in position (Figure [3.36](#)).

Note: If you have a single mixing instrument, or an older instrument with a non-removable cell, your flow lines will differ to those seen in this procedure.

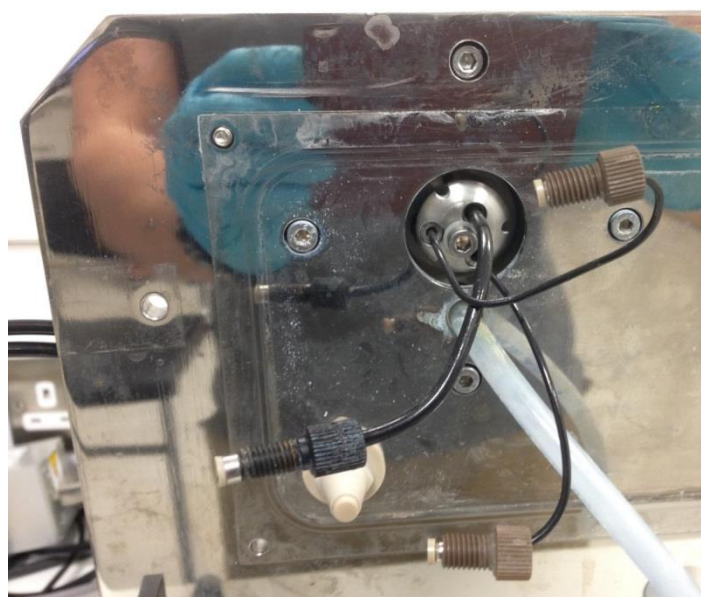


Figure 3.36 - Flow tube assembly

8. Screw the water bath back onto the front of the stainless steel plate. Be sure to check that the O-ring is not twisted, as this will lead to leaks.
9. Attach the Water circulator tubing to the circulator outlet (Figure [3.31](#), blue circle). Connect the flow lines back into their original places (Figure [3.31](#), red circles). Needle-nosed pliers may make these tasks easier. Push the waste tube through the hole in the side of the water bath (Figure [3.31](#), green circle).
10. You should now install the stop valve actuator assembly and screw the waste tubing into the waste tube adaptor (Section [3.8](#)).

11. Install the stop syringe (Section [0](#)).
12. Perform a flow circuit leak test (Section [1.3](#)) and also run the water circulator if present to check for leaks in the water bath. Common leaks are due to a loose 'cell-to-waste' tube at the waste tube adaptor and a loose pressure plate. Pay special attention to ensuring these parts are tight enough.

3.11 Installing a Small Volume Drive Syringe

This document describes how to install a small volume drive syringe (i.e. 1 mL, 500 μ L 250 μ L or 100 μ L).

The standard 2.5 mL drive syringes can simply be screwed into the metal holding plate, while the smaller volume syringes may require a different approach to achieve an effective seal.

3.11.1 Applicable Instruments

The procedure described in this document refers to all SX instruments.

Note: The photographs used in this document feature the SX20 model. Differences may exist between older models.

3.11.2 Equipment Required

- Hexagon keys (2.5 mm, 3 mm)
- Paper towels

3.11.3 Preparation

1. Before installing a small volume drive syringe, you will need to first drain the water bath (Section [3.2](#)), and remove the existing drive syringes (Section [0](#)).

3.11.4 Procedure

1. Low volume syringes are generally smaller in diameter than the standard 2.5 mL drive syringes, so will need a rubber sleeve fitted over their glass barrel to give them a water-tight seal within their grommet and prevent the water bath fluids from leaking. Small drive syringe sleeves can be ordered from Applied Photophysics. Contact support@photophysics.com for more information.
2. Loosen the two drive valve screws just one full turn each (Figure [3.37](#)). This will loosen the holding plate a little. Depending on your tools, you may need to remove the drive valve knob to gain access to the one drive valve screw. If so, remove the knob by first loosening the grub screw on its back.

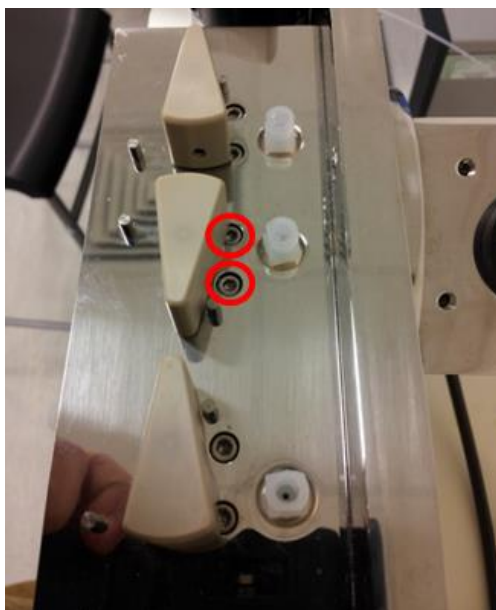


Figure 3.37 - Location of drive valve screws

3. Push the new small volume drive syringe up through its allocated grommet. Be careful not to apply uneven pressure to the glass barrel.
4. Screw your new small volume drive syringe into the drive valve holding plate (Figure [3.38](#)).

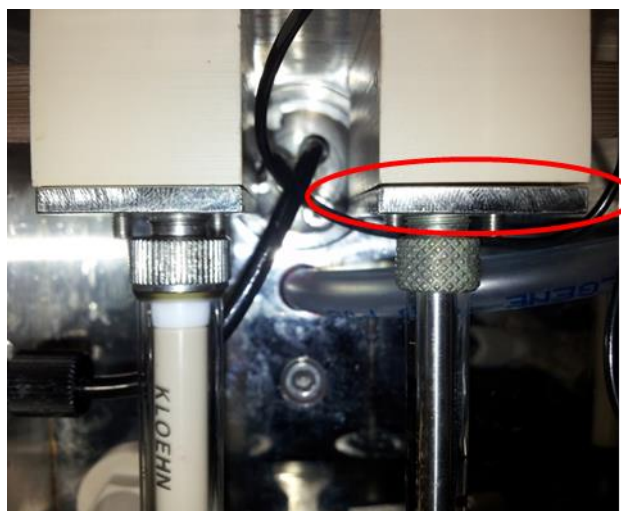


Figure 3.38 - Drive valve holding plate accepting a small volume drive syringe

Note: You will notice that there is a small gap between the holding plate and the drive valve when installing a small volume drive syringe, when compared to that of a standard 2.5 mL syringe (Figure [3.38](#)).

5. Tighten the two drive valve screws.
6. Reattach the drive ram.
7. Perform a leak test (Section [1.3](#)) before reattaching the front plate and recirculating coolant into the water bath.

Note: If you plan to perform asymmetric mixing experiments, remember to turn the external pressure regulator down to 2 bars and adjust the stop syringe volume to that required. Refer to the SX user manual for more information.

3.12 Installing a 5 μ L cell and 1 mL stop syringe

This procedure describes how to install the 5 μ L cell and 1 mL stop syringe on the SX series of stopped flow instruments. This will allow for shorter dead times and a reduced presto period when compared to the standard 20 μ L cell and 2.5 mL stop syringe.

3.12.1 Applicable Instruments

This procedure is only applicable to SX instruments featuring a removable cell (RC). Instruments with non-removable cells (non-RC) are not compatible. An RC instrument can be identified by a brass or black coloured cartridge, visible on the end of the cell block, which attaches to the fluorescence detector. Similarly, a non-RC instrument can be identified by stainless steel in the same location.

3.12.2 Procedure

1. Drain the water bath as described in Section [3.2](#).
2. Remove the existing cell as described in Section [3.9](#). Follow the rest of the procedure in this section to install the small 5 μ L cell.
3. Remove the stop syringe as described in Section [3.7](#).
4. Before installing the 1 mL stop syringe, the volume on the return cylinder should be set to about 6 turns (~240 μ L).
5. To attach the 1 mL stop syringe, perform the instructions in Section [3.7](#) in reverse order. You do not need to reduce the external pressure regulator found at the rear of the KSHU. This regulator should read 4 bars. There is no need to reduce the external regulator to 2 bars unless you are using asymmetric mixing.
6. You will need to leak test the instrument. See Section [1.3](#).
7. Once satisfied the flow circuit is sealed, expel all air bubbles from the flow circuit by flushing an alcohol through the instrument.
8. Under the 'KSHU' section of the Pro-Data SX software, select 'setup', then select the 'syringe volumes' tab. Enter your new stop syringe volume here and click on 'apply'.
9. Your instrument is now setup and ready to use.

3.13 Replacing the Trigger

3.13.1 Applicable Instruments

This procedure is applicable for all instruments featuring an autostop; side or front-mounted.

3.13.2 Equipment Required

- 2.5 mm hexagon key
- 2 M nitric acid (~50 mL)

3.13.3 Procedure

1. Remove the stop syringe as described in Section 3.7.
2. Using a 2.5mm hex-key, remove the two screws which hold the copper trigger in place and remove the trigger, exposing the third screw. Unscrew this third screw to detach the trigger cable from the return cylinder.

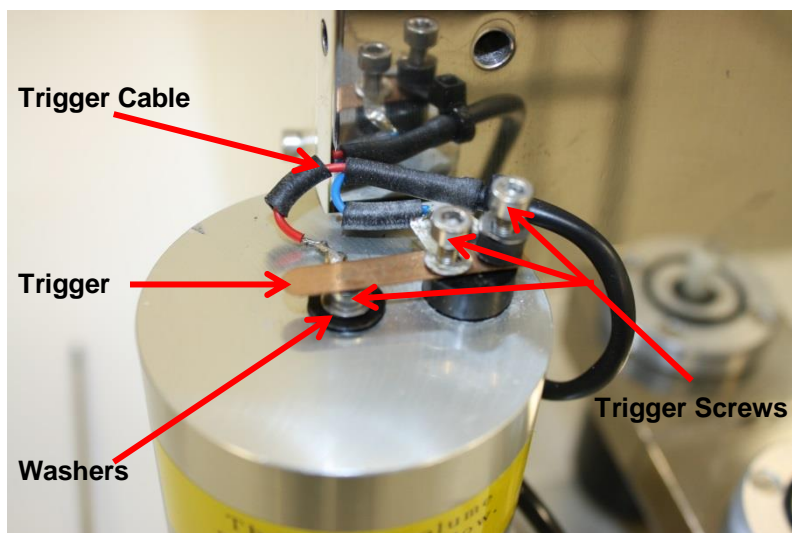


Figure 3.39 – Return cylinder trigger assembly

3. Soak the screws and the trigger in 2 M nitric acid for approximately 30 minutes to remove any build-up of contaminants which can cause triggering issues.
4. Check the integrity of the two tags on the end of the trigger cable. Give them a slight pull. If they detach, the trigger cable will need to be cut down and the tags re-soldered back on. If you do not have enough length left on the cable, contact support@photophysics.com for assistance.
5. Rebuild your trigger assembly, using as many or as few washers as required when screwing the screw underneath the trigger into place. Aim to achieve a small gap between the trigger and the screw.

4 ROUTINE PC AND SOFTWARE SERVICE PROCEDURES

4.1 Installing / Updating Pro-Data SX

Pro-Data SX is the software which controls the SX spectrometer and allows for data analysis with the Pro-Data Viewer. The latest versions of Pro-Data SX and the SX Environment are available to download from the member's area of our website at www.photophysics.com.

4.1.1 Requirements

- If you wish to install Pro-Data SX as an emulator, and/or the Pro-Data Viewer for data manipulation only, you should follow Section [4.1.4](#).
- First time installations of the full software package on a new instrument require Sections [4.1.3](#) and [4.1.4](#) only.
- If you are installing this software onto a new PC to replace an older one, you will need to follow Sections [4.1.3](#), [4.1.4](#) and [4.1.5](#).
- If you wish to upgrade your Pro-Data SX software, follow Section [4.1.4](#) only.

4.1.2 Applicable Instruments

The Environment and Pro-Data SX are suitable software for any PC controlled SX stopped flow instrument.

4.1.3 Installing the Environment

1. If you are installing the environment from a download, right click on your 'setupenv.exe' file and select "run as administrator". If you are installing the environment from a CD, insert the CD and run the installation wizard as administrator. You will find the option to install the environment under the 'Advanced' button.
2. Follow the onscreen instructions.
3. You will see a screen like the one in Figure [4.1](#)

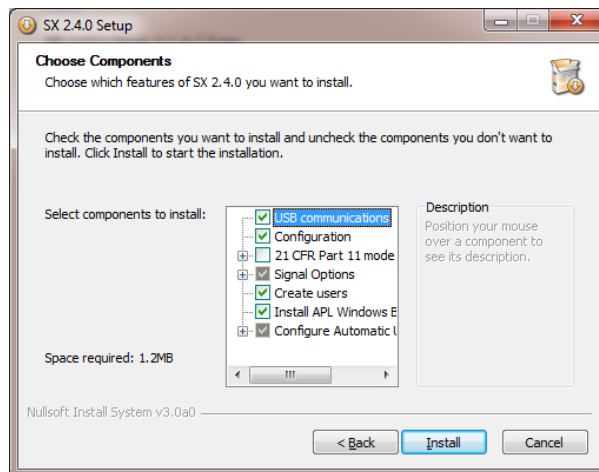


Figure 4.1 - Environment installation options

4. Select the appropriate components according to your instruments configuration. Select USB communications if you have a USB cable linking your electronics unit to your PC or select FOPCI communications if you have a fibre optic cable in place of this USB cable.
5. Ensure that 'configuration', 'create users' and 'install APL windows ...' are selected.
6. Select '21 CFR ...' if you require CFR compliance with your software.
7. Select only the signal options which are physically available with your instrument (e.g. absorbance, fluorescence etc.).
8. Disable Windows automatic updates by selecting this option. This is recommended to avoid conflicts between Windows updates and the Pro-Data software.
9. Click 'install' to complete the installation.

4.1.4 Installing / Updating Pro-Data SX

Note: If you are installing Pro-Data SX to update your current version, it is advisable to create a backup of you configuration folder before starting. Your 'config' folder will be found here

C:\Program Files (x86)\Applied Photophysics\SX

1. Run the 'setup.exe' to start the installation wizard.
2. Accept the License Agreement
3. Check the selected components are appropriate for your instrument i.e. choose the correct communications mode (FOPCI or USB) as seen in Figure 4.2. You can check which communication mode you have by identifying the cable linking your electronics unit to your PC.

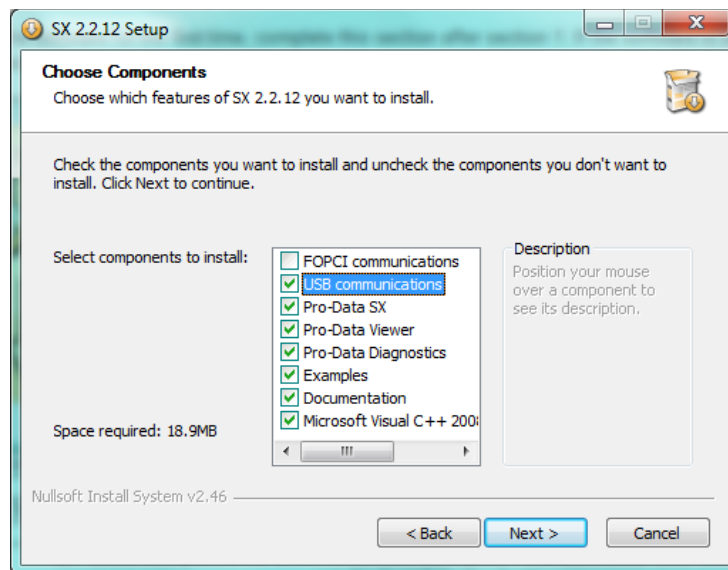


Figure 4.2 - SX Pro-Data installation components

4. Click 'next'.
5. Confirm the destination, click 'install'.
6. If the Microsoft Visual Studio isn't already installed, this installation will initiate. Accept the license terms and click 'install'. Once complete click 'finish'.
7. Click "finish" for the SX Pro-Data software installation.

4.1.5 Migrating Configuration Files

Installing SX Pro-Data will also install a configuration folder containing default settings.

1. On your new PC, locate the configuration folder (config), found at this address:

C:\Program Files (x86)\Applied Photophysics\SX

2. Replace this configuration folder with the more appropriate one (e.g. the one obtained from your old PC or from Applied Photophysics).

4.2 Calibrating a PDA

This document describes how to optimise the photodiode array (PDA) settings on the SX series of stopped flow instruments, to get the best detectability out of your PDA detector.

Before attempting to implement this procedure, ensure that you have contacted support@photophysics.com so that precautions can be taken to protect your instrument configurations. You will not be able to proceed without the APL login and service passwords. Failure to heed this advice may seriously affect the performance of your instrument.

4.2.1 Applicable Instruments

The procedure described in this document refers to all SX instruments which include a PDA module and run under Pro-Data SX.

4.2.2 Preparation

1. Turn on the lamp and allow 30 minutes for it to equilibrate. While waiting, turn the electronics unit on and fill the cell with water.
2. Ensure the PDA is attached, using your desired pathlength (make a note of the pathlength). Connect the light guide either directly to the lamp housing, or attach it to the monochromator and set the slit width to 4 mm.

Note: Make a note of this setup, as subsequent optimisation of gain and offset values will be dependent on them remaining constant.

4.2.3 Checking Calibration Values

1. Turn on the computer and log in as the 'APLservice' user. The password is protected. Please contact support@photophysics.com for assistance.
2. Launch the 'Pro-Data SX' software as administrator (right click>run as administrator).
3. Set the signal to 'Photodiode Array'.
4. Select 'view' from the main control panel, then 'devices'. Double click on the PDA icon which appears in the next window (Figure 4.3). This area of the software is protected by a service password. Please contact support@photophysics.com for assistance.

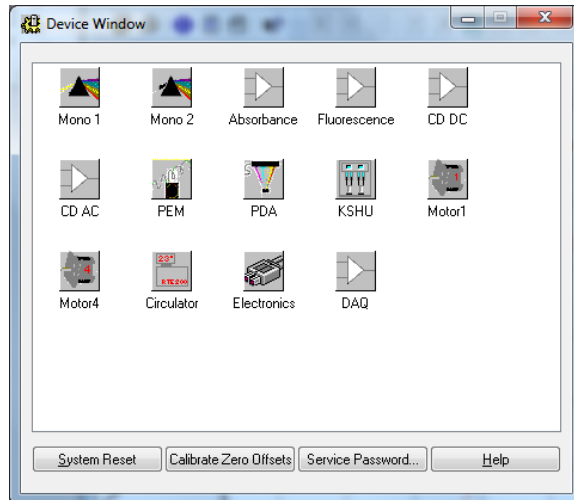


Figure 4.3 - Devices window

5. You will see a window such as in Figure 4.4.

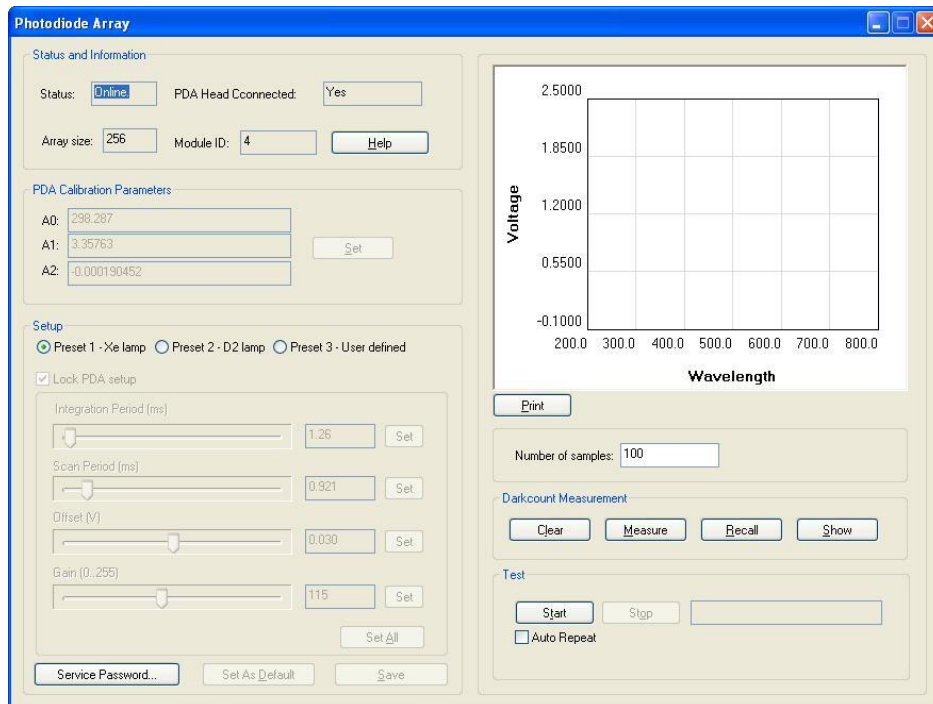


Figure 4.4 - PDA devices window

6. Click on the 'Service Password' button. This area of the software is protected by a service password. Please contact support@photophysics.com for assistance.

7. Un-tick the 'Lock PDA setup' tick-box.

Check that the PDA Calibration Parameters denoted 'A0', 'A1' and 'A2' all match up with what is physically written on the underside of your PDA. If they are not for whatever reason, make a note of them before changing them to match. Click set, then click save.

4.2.4 Setting the Darkcount Measurement

1. Choose either Pre-set 1, 2 or 3 using the radio buttons.
2. 'Integration Period' and 'Scan Period' should not be altered. 1.00 ms and 0.921 ms respectively are good starting values.
3. Close your lamp shutter and press 'Start' to take a measurement. You should see a trace appear, as in Figure 4.5.

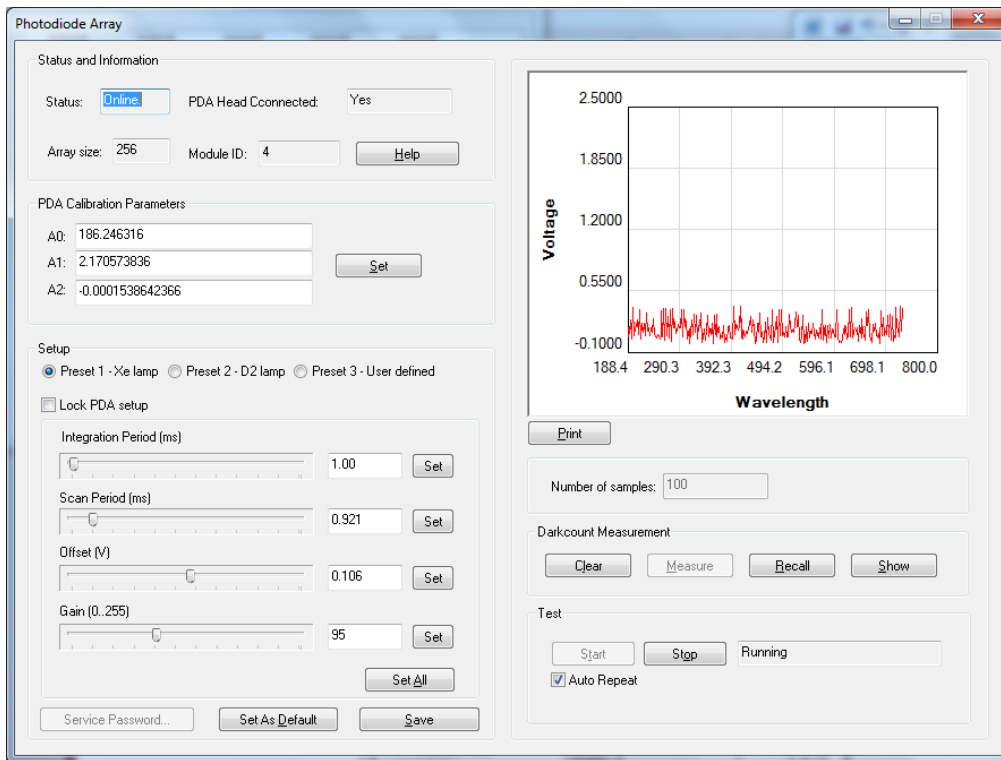


Figure 4.5 - PDA darkcount measurement

4. You will now need to set the darkcount measurement. Click on 'Measure' in the Darkcount Measurement section. Double check when prompted that the lamp shutter is closed. Your darkcount measurement is now set.

4.2.5 Setting the Gain and Offset Values

Once you have measured the darkcount, the gain and offset values need to be optimised. Gain is artificial and enhances the amplitude of both signal and noise. Therefore, if possible, it is more beneficial to increase the light throughput than it is to increase the gain.

1. Open the lamp shutter.
2. Click 'Start' to take another measurement.
3. You should see a profile of the lamp with a peak maximum at around 468 nm. This peak maximum should be between 1.5 - 1.8 V. If it is too low, increase the gain, click set, then take another measurement by clicking on start. If it is too high, decrease the gain, click set, and then click start. The trace shown in Figure 4.6 requires a decrease in gain.

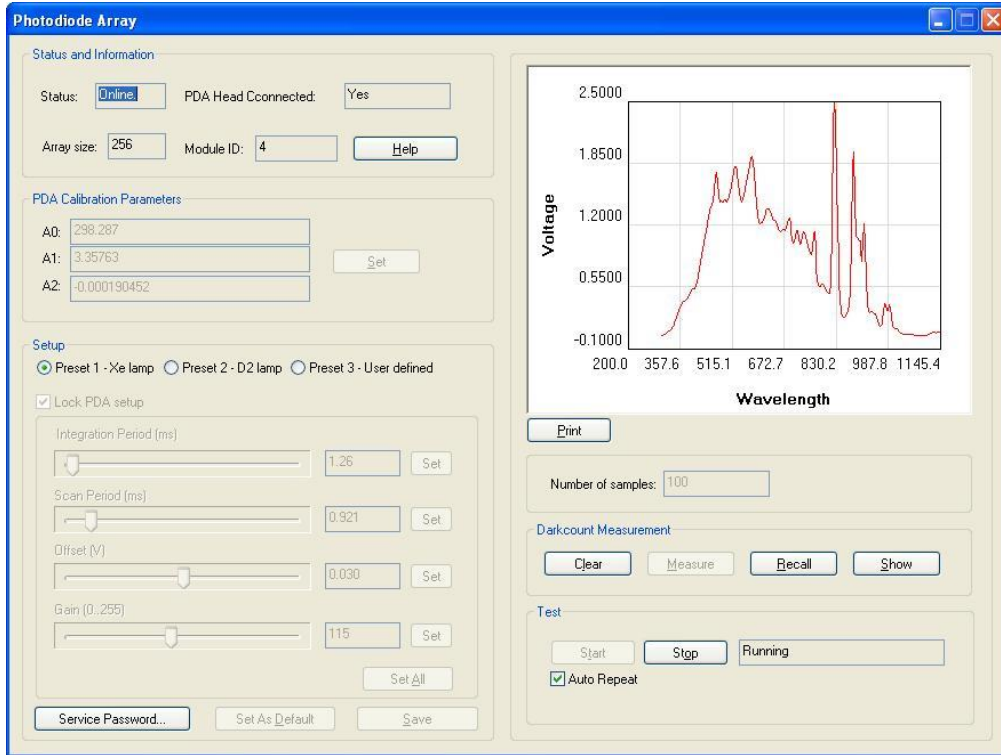


Figure 4.6 - PDA gain and offset settings

4. Once the gain has been set, save the settings
5. Now make a note of the 'Offset' value. Close the lamp shutter and alter this value until the live trace is around 80 mV. (If your data display does not update, close the devices window and reopen it). Click on the live-trace window to zoom if this helps. Save your settings.

Notes: The setup of the instrument (i.e. slit width or direct couple and pathlength) will determine what the gain and offset need to be. If you change these conditions, remember to reset the PDA gain and offset accordingly.

5 APPENDICES

5.1 Appendix A: Installing and Aligning a Lamp within a Cream Lamp Housing

During the evolution of the SX Stopped Flow Spectrometer, the lamp housing was upgraded to include passive cooling fins, to aid heat transfer. The newer models are painted black, the older models were painted cream. Instruction for replacing and aligning a lamp differs for each model. The following is a procedure applicable to the older cream lamp housing.

5.1.1 Applicable Instruments

This procedure is applicable to all SX instruments which feature a cream lamp-housing unit.

5.1.2 Equipment Required

- Sufficient PPE (i.e. a face visor)
- 4 mm insulated hexagon key
- Flat head screw driver

5.1.3 Lamp Installation

Note: Ensure you read and follow all safety instructions which are included with your new xenon lamp. Wear PPE, which should include a visor. **Xenon lamps may explode**, causing damage to the instrument and user. Always exercise caution when handling xenon arc lamps.

1. Switch off the lamp power supply and allow 30 minutes for the lamp to cool down. Remove it to a suitable worktop.
2. Referring to Figure [5.1](#), unplug the two power terminals at the rear of the lamp housing (red and black), and disconnect the earth strap (green).

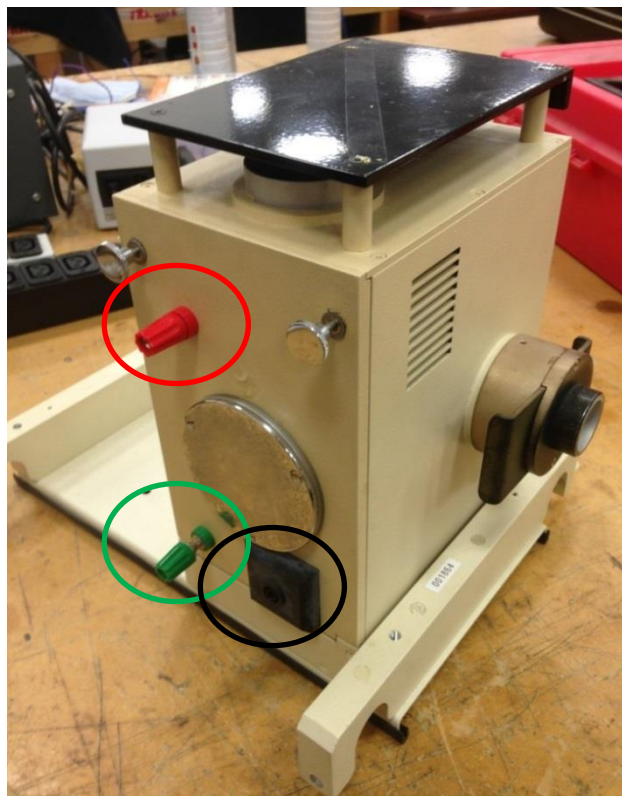


Figure 5.1 - Location of power terminals (red and black) and earth terminal (green) on the rear of cream lamp housing

3. Unbox your new lamp, and separate the protective case into its two halves.
4. Unscrew the two finger tight screws at the top of the rear of the lamp housing (Figure [5.1](#)).
5. Drop the back panel of the lamp housing down (Figure [5.2](#)).



Figure 5.2 - Opening the cream lamp housing

6. Loosen the finger tight screw (A) shown in Figure 5.3 to release the lamp from the anode mounting block.

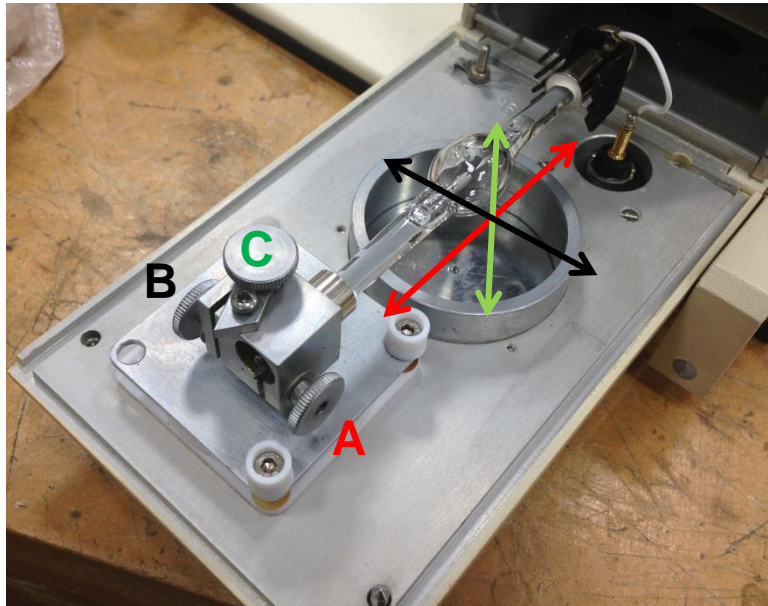


Figure 5.3 - Location of interior lamp adjustment screws

7. Detach the finger tight screw from the cathode end of the old lamp and remove the wire / washers / heat sink. Take note of their order.
8. Attach these parts back onto the cathode of the new lamp and tighten the finger tight screw into place.

9. Insert the new lamp into the anode mounting block, and adjust its position along its three axes by using the three knurled finger tight knobs (A, B and C) seen in Figure [5.6](#).

Note: There exists an alignment mirror, which makes lamp installation more effective. It is a circular concave mirror, which can sit behind the bulb of the lamp to aid alignment. If you have this mirror, you can install it by removing the cover plate at the rear of the lamp housing unit (Figure [5.](#)) and pushing it through before fixing the plate back into position.



Figure 5.4 - Location of cover plate for lamp alignment mirror.

Note: With the alignment mirror installed, adjust the position of the lamp along its axis using the knobs A, B and C (Figure [5.3](#)) until the reflection of the two electrodes overlap with one another (as perfectly as possible). This will indicate that the lamp is in the optimum position to begin alignment.

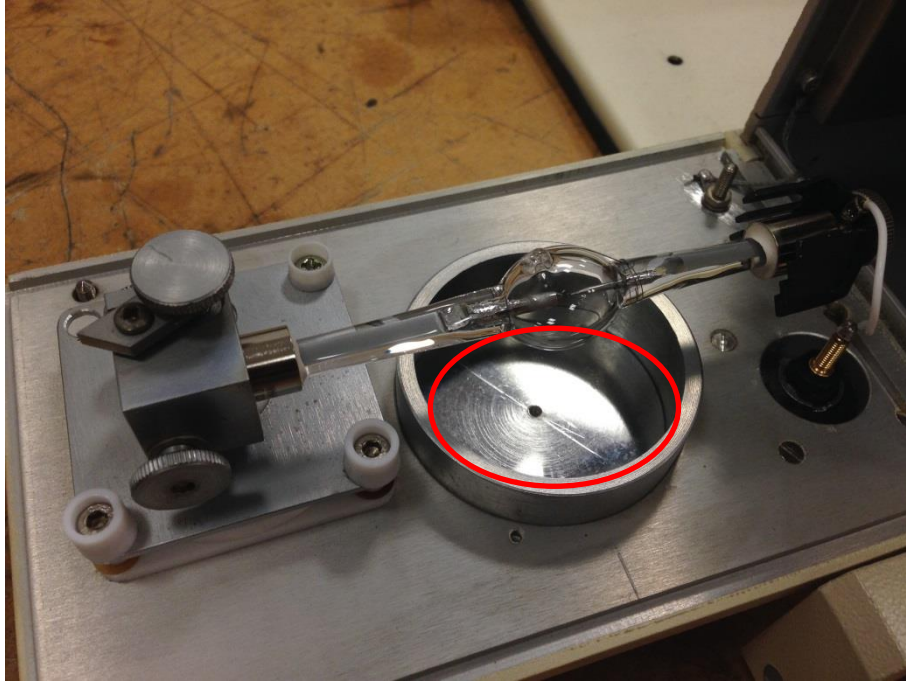


Figure 5.5 - Location of lamp alignment mirror / circular back plate in a cream lamp housing (alignment mirror not shown)

10. If you do not have the alignment mirror, you will have to simply position the lamp as central as possible in relation to the circular back plate which you will find in place of the mirror (Figure [5.5](#)).
11. Once the lamp has been repositioned, screw the back plate into position, reattach the cables and position the lamp housing unit back with the rest of the instrument. Your lamp is now ready for realignment.

5.1.4 Lamp Alignment

Satisfy the conditions below:

Conditions for Aligning a Lamp in a Cream Lamp Housing	
Absorption pathlength	2 mm
Detector	Absorbance PMT
Monochromator slit width	0.5 mm
Wavelength	350 nm
Cell Contents	Distilled Water
Lamp Shutter	Open

1. Click on 'reference', or 'autoPM'. Take note of the high voltage, which should be around 200-400 V.
2. Identify the mirror adjuster on the right hand side of the lamp housing, just above the lamp igniter (Figure 5.6). It is a silver knurled knob. Rotating this adjuster will move a mirror, inside the lamp housing, which, in turn, will move the light beam from side to side.



Figure 5.6 - Location of mirror adjuster on cream lamp housing

3. Double left click on the live trace data display in the Pro-Data software to zoom into the live trace.
4. Monitor this trace as you twist the adjuster knob (it doesn't matter which way). Your absorbance signal will go up or down. You want it to go down as much as possible (i.e. greater transmission).

5. Your signal will probably go below 0 AU. This is expected. If the signal drops out of the bottom of the live display, stop adjusting the knob and reset the PM volts by clicking on "AutoPM" or "reference". This will return the signal to 0 AU. Double left click on the trace once more to zoom in.

6. Continue adjusting the knob until no improvement in signal is observed. The optical system is now aligned. The system must always be aligned after the Xenon lamp has been replaced.

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