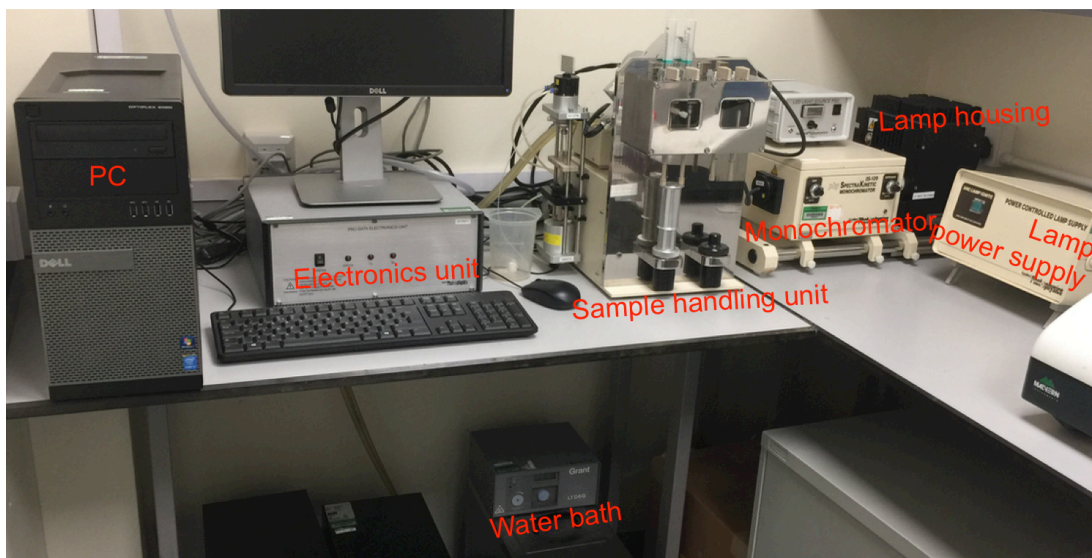# SOP for use of the SX19 Stopped-Flow Instrument

The main components of the stopped flow system are:

Always book the system before use.

Starting up and preparing the SX19 for use:

1. **Turn on the compressed air supply.**

An 8 bar gas supply powers the pneumatic rams and stop valve of the sample-handling unit. Turn the large black valve on the wall by 90° and check the pressure is 8 bar.

2. **Turn on the water bath.**

Turn on the water bath on the bench next to the instrument using the wall socket switch. Check there is sufficient water covering the heater coil, and top up with mQ water as necessary. (Let Katherine or Joe know if any air remains in the sample handling unit, which the water bath fills and controls.)

3. **Turn on the lamp.**

Power on the lamp power supply unit using the rocker switch at the rear. The green light on the "arc lamp ignite" switch should illuminate. Wait for at least 30 seconds before pressing the green button firmly to ignite the lamp. Ensure the shutter between the lamp housing and the monochromator is in the open position.

You can check the lamp is on since light comes out of the slit in the lamp housing:

If the lamp fails to ignite, then wait for two minutes before pressing the green button again.

4. **Turn on the electronics unit.**

Power on the electronics unit via the black rocker switch on the front panel. There are three light emitting diodes, one green and two red. The green diode will flash for a short time after the unit is powered on, but should then remain on constantly. Whenever the software is running the red diodes will flash continuously. It is recommended that if the instrument is to be used daily the electronics unit is left powered on.

5.   Power on the computer and load the Pro-Data software.

Power on the computer and log on. The default account is "lab" which has the password bcssxxxx (see the sticker on the side of the PC). Load Pro-Data SX using the desktop icon:


6.   Load the Pro-Data Viewer software and set the file save location and seed names.

Start the Pro-Data Viewer program using the Viewer icon on the Pro-Data Viewer toolbar:

Navigate in the Pro-Data Viewer file browser to your preferred save directory and right click on it selecting **Set Working Directory Here**. New data files will now automatically be saved in this directory:
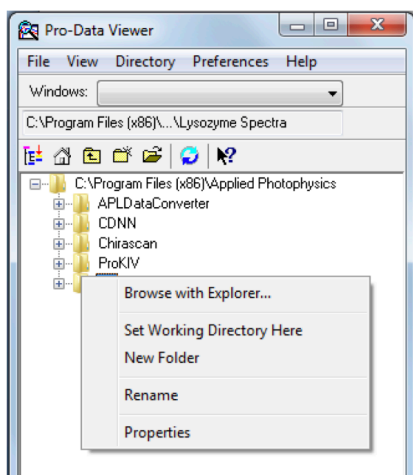
Now open the Files Names dialog box by clicking on the icon on the toolbar:

Enter a name for the new data files and baseline file (where relevant). Subsequent files of each type will be numbered starting from the values set in the window:
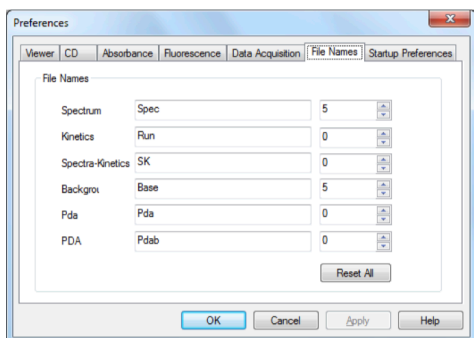
Check that the Pro-Data Viewer software is "Ready (Online)" in the lower left-hand corner. If "Offline", go to **Preferences** -> **Go online**, to connect locally.

Preparing the sample handling unit:

1. **Configure the flow lines.**

The following description applies to a single-mixing stopped flow experiment with a 1:1 mixing ratio. (See the manual for details of how to perform more complicated experiments.) The single-mixing experiment always utilises the left-hand drive ram and the reagents are always loaded in the F (left) and C (right) drive syringes:

Check that the instrument is in the configuration above before proceeding. (The drive volume is pre-set to approximately 120 µl. *Do not adjust this under any circumstances.*)

2. **Flush the system with water.**

The stopped-flow unit should have been left from previous use with mQ water/ethanol in the flow circuit and cell. In any event it is best to ensure that mQ water/buffer is in the flow circuit and cell prior to loading sample. To do this:

a) Set the beige drive valves to the "load" position:

b) Empty any contents of the drive syringes into waste reservoir syringes by pushing the drive syringe piston upwards into reservoir syringes. Always be careful when handling the drive syringe pistons as they can break.

c) Replace the reservoir syringes with ones containing clean mQ water/buffer.

d) Load each drive syringe with about 1 ml of water/buffer by pushing down on each reservoir syringe piston while at the same time pulling on the drive syringe piston. Take care not to introduce air bubbles into the flow-circuit: one of the most common causes of artifacts in stopped-flow data is the presence of air bubbles. Repeat the up and down motion a few times to develop your technique for eliminating air bubbles*.

* In general, to minimise the introduction of bubbles and to facilitate their removal, with the drive valves in the "load" position, shunt liquids back and forth gently through the drive valves whilst keeping the liquid compressed from both ends of the flow (by applying downward pressure on the piston on the reservoir syringe and upward pressure on the drive syringe piston). When loading drive syringes *avoid simply pulling the syringes down*, to suck liquid from the reservoirs, as this will encourage cavitation and bubble formation. Fill them by both pushing liquid in from the reservoirs above, while pulling the drive syringe pistons down, to keep a positive pressure in the valve and syringe cavity.

e) With the drive valves still in the "load" position, raise the drive ram until it is in contact with both drive syringe pistons.

f) Now set the beige drive valves to the "drive" position:

g) Select **Drive** on the Sample handling unit panel (using the **Drive** function rather than the **Acquire** function ensures that there will be no data collection and generation of a data file*).

* It is however more efficient to run washes using **Acquire**: use < 10 data points and < 1 s time frame, tick the panel labelled "repeats" and enter "10" as the number of repeats. The files generated can easily be deleted afterwards.

h) Do at least 5 repeat drives to ensure that the flow circuit and cell contents have been completely displaced by the clean water.

Before executing a drive always check that the drive valves are in the "drive" position (not the "load" position) and the drive ram is in contact with the syringe plungers. Failure to do so may result in damage to the flow circuit.
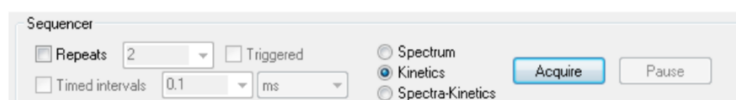
Stopped-Flow Absorbance Kinetics

1. Select Absorbance mode.

On the **Signal** panel of the Pro-Data main window select **Absorbance** from the drop down list. Ensure that **AutoHV** is ticked:
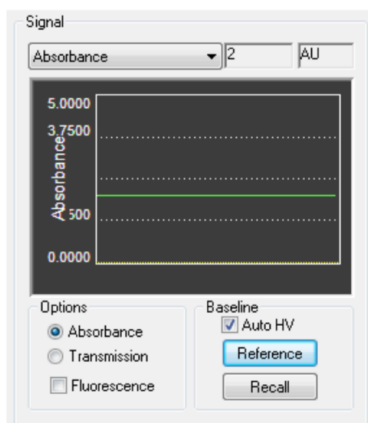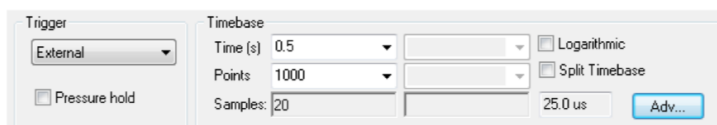

2. Select kinetics mode, set timebase and points.

Ensure that **Kinetics** mode is selected on the **Sequencer** panel:

Select **External** from the drop down list in the **Trigger** panel to activate stopped-flow operation:

On the **Timebase** panel choose an appropriate acquisition time based on the expected timescale of the reaction. The **Points** should normally be set to a value between 400 and 1000 data points.

Always tick **Pressure hold** for experiments that are less than 2 seconds long. For experiments lasting 2 – 10 seconds, only tick **Pressure hold** if you see artefacts* present in the data. **NEVER** tick **Pressure hold** for experiments over 10 seconds, it puts the instrument under too much stress and can damage components.

* The pressure hold function is to be used to prevent artefacts appearing in your data. If **Pressure hold** is *not* used, one quick burst of Nitrogen is used to drive the ram, and some samples will kick back when the burst ends and the pressure is released which can lead to artefacts. This is not seen in all experiments so for experiments up to 10 seconds, the use of Pressure hold is down to the user and dependent on the reagents. *With* pressure hold the instrument holds the sample under pressure for the entire time period of the experiment, and therefore does not allow the sample to move backwards through the flow circuit and cause the data artefact.

3. Select monochromator wavelength and slit width.

On the **Monochromator** panel enter the selected wavelength:
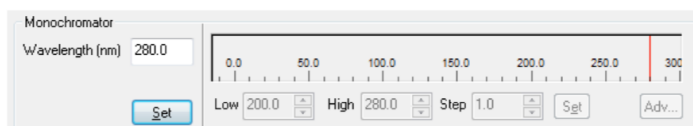
Using the dials on the front of the monochromator set the slit widths to give the required absorbance signal.

4. Zero the detector.

Fill the optical cell with a suitable reference material (e.g. water or other solvent). Click the **Reference** button in the **Signal** panel to zero the absorbance detector.

5. Load the samples.

Samples may now be loaded into the drive syringes. At least 1.3 mL is necessary for a complete series of approx. 25 drives.

Do not to push/pull the drive syringe pistons while they are in the "drive" position after loading. Equalizing the piston levels should only be done in the "load" position, otherwise the sample from one syringe (i.e. enzyme) is easily pushed into the other syringe (i.e. substrate). The reaction will then occur prematurely in the drive syringes and you will have to discard the contaminated material, and then wash and load the syringes again using fresh samples.

6. Acquire data.

(Check that the Pro-Data Viewer software is "Ready (Online)" in the lower left-hand corner. If "Offline", go to **Preferences** -> **Go online**, to connect locally.)

Ensure that the drive valves are in the "drive" position and click the **Acquire** button to perform a drive and collect the data. The first few drives will consist of buffer and then diluted sample. By the fourth or fifth drive clean sample should be mixed with each drive and results should be consistent.

Absorbance measurements will now be in units of absolute absorbance since water was used when measuring the reference. It is possible to measure the reference with something other than water in the cell, in which case the measurements will now be relative absorbance. This may be convenient when measuring small absorbance changes on significant backgrounds. Collecting a reference when only one reagent (often colourless) is pushed into the cell is one typical option as is reference collection to the chemical end point of the chemical reaction under investigation.

When you have finished your experiments:

1. Quit the software and save your data to a **clean** USB drive.
2. Power off the computer.
3. Power off the lamp.
4. Power off the electronics unit.
5. Turn off the water bath.

## Cleaning the observation cell and flow circuit after use:

1. Flush the drive syringes.

With the beige valves in the "load" position, push up the drive syringes to empty them as far as possible (taking care as they are fragile). Replace the reservoir syringes with ones containing mQ water and flush the drive syringes with water. Replace the reservoir syringes with ones containing 2% Hellmanex (provided next to the instrument; a supply of neat Hellmanex is under the fume hood and mQ water is in the plastic container by the sink in 0.14 if this solution runs out). Flush the drive syringes with at approx. 5 mL of this cleaning solution.

2. Wash the cell and stop syringe.

The cleaning solution can be pushed through the cell, stop syringe and associated circuitry using repeated drives as described on pages 3-4 (put the valves in the "load" position and the drive ram in contact with both drive syringe pistons, then put the valves in the "drive" position and perform repeated single **Drives**, or **Acquire** with repeats). However, this is unlikely to fully replace the liquid in the stop syringe. To flush the stop syringe, cell and associated circuitry completely it is necessary to perform manual drives. To do this, put the drive valves in the "load" position and move the drive ram so it is in contact with the syringe plungers, then put the valves in the "drive" position.

Next, turn off the pressure using the large valve handle on the wall, and then release residual pressure by depressing the in-line red button behind the sample-handling unit:
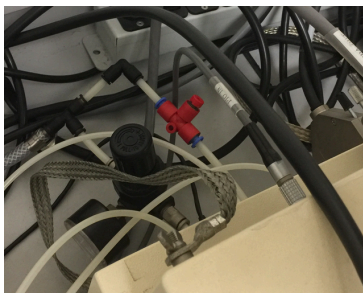
Identify the stop valve, which will be in the "inject" position (facing forwards):
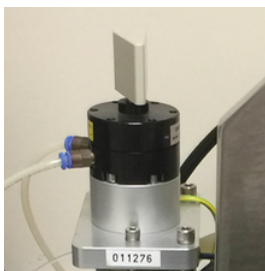
Turn it by hand 180° clockwise ("waste" position; it will only turn if there is no pressure left in the system) and empty the stop syringe manually by pushing it upwards before returning the stop valve to its normal position. **While doing this, take great care not to (a) move the stop assembly, which is only weakly fixed to the sample handling unit, or (b) touch the copper trigger plate.** Fresh mQ water can then be introduced into the drive syringes ("load" position) and flushed through the flow circuit manually (by raising the drive ram upwards against the drive syringe pistons in the "drive" position). The stop syringe will fill, and can be emptied again by turning the stop valve to "waste". This procedure can be repeated (e.g. x5) until the user is confident that the circuit is clean and also free from air bubbles.

Wash the stop syringe using the manual drive procedure described above. Repeat approx. 5 times until you are confident that the circuit is clean. Leave it filled with 80:20 water:ethanol when not in use.

3. **Final wash.**

Finish by replacing the reservoir syringes with ones containing clean purified water and 20% ethanol. Flush the drive syringes with this solution and leave them filled with 80:20 water:ethanol when not in use.

4. **Turn off the air supply.**

Turn off the compressed air supply on the wall, and release the residual pressure by depressing the red in-line button behind the sample-handling unit.

5. **Tidy up!**

Please keep the area around the instrument clean and clear. Re-fill empty bottles and wipe up any spills immediately.

Katherine Stott and Joe Maman, January 2019. *Updated February 2021.*