

Bio-Kine32
User's Manual
Version 4.73

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1. BIO-KINE

1.1. Overview

Versions of Bio-Kine software starting from version 4.0 have been designed to control stopped-flow instruments, to perform data acquisition and analysis using different simulated devices such as a **Scanning Spectrometer**, a **Transient Recorder**, a multipurpose **Virtual Chart Recorder** or several **External Devices** controlled or not by the PCIe-6321 board.

Previous versions of Bio-Kine were only able to perform acquisition and analysis. It was necessary to use second software (MPS) to control the syringes and edit the sequences. In order to improve friendliness of instruments it was decided to link both softwares.

Bio-Kine is organized as one main window and several child windows (made active by a mouse click in it) included in this main window. Only one of these child windows is the active window. As in all Window software the menu bar corresponds to actions in the active window.

The main window is activated upon loading of the program. The menu bar of this main window is used for general installation options and for loading the child windows. These options are discussed in sections 1.2.2 and 1.3.

The ways to control the stopped-flow device, the syringe and to edit sequences are explained in section 2. All the information concerning the software is available in this section, for more precise information about the stopped-flow device please refers to its user's manual.

The Analysis windows are used to display and analyze recorded files, either Kinetic or spectral files. The use of these analysis windows are discussed in section 3. A full set of mathematical tools used to perform data analysis can be found in the software. Descriptions of all the functions available are presented in section 3.12.

Acquisition tools using MOS-200 and MOS-500 are fully described in section 5 and 6 respectively. In these sections, acquisition using a scanning spectrometer, a transient recorder and a chart recorder are explained.

Bio-Kine can also be used to drive Jasco CD Spectropolarimeter (J-810 but also J-715...) using AD/DA board or not. All the possible configurations are described in section 7.

Bio-Kine can also drive J&M Diode Array Spectrometer, in that case Bio-Kine becomes a powerful tool to follow 3D Kinetics. This configuration is described in section 8.

The multi-window organization offers the possibility to load at the same time several acquisition or analysis windows with various setups and switch from one application to another by a simple mouse click.

This manual uses some typographic conventions: words in bold indicate menus or buttons commands (e.g. **File** / **OK** button) and menu levels are separated with a comma (e.g. **File, Save As**). Shortcut keys are underlined and the full list of shortcuts available in Bio-Kine is presented in annex at the end of this manual.

1.2. Installation

For proper installation of Bio-Kine, the equipment described below is required:

Minimum PC requirements

- Pentium 200 MHz (at least 1Ghz for use with diode array detector)
- 64 Mb of RAM.
- Monitor (800 x 600) with 16 bits colors.
- Windows vista, 7 (32 or 64 bits)
- One free **PCI express** slot.
- One USB port

Analog to digital converter board model **PCIe-6321**.

Laser or ink-jet printer or plotter.

Bio-Kine installation must be done in two steps as follows:
AD/DA board and driver installation into the PC computer.
Bio-Kine software installation.

It is important to proceed in the order above; otherwise Bio-Kine may not be properly installed. Make a backup copy of your original disk set, put it into a safe place and follow the next steps.
Note : if you want to use Bio-Kine only for data analysis refer directly to section 1.2.2, in this case only the Bio-Kine software installation step is necessary.

1.2.1. Board and Driver installation

Install NI-DAQmx software

IMPORTANT: The drivers of the board have to be installed before installing the hardware itself (National instrument Board: NI PCIe 6321). Please check the Bio-Kine compatibilities in Table 1: Bio-Kine compatibilities) before the implementation of software.

Bio-Kine32 is also Windows 32-bit software designed to acquire data using the PCI-6221 or the PCI-6052E(previous boards provided with Bio-Logic instruments). With these cards, **Regular PCI slot is needed** to install the plug-and-play board.

Table 1: Bio-Kine compatibilities

<u>A/D board</u>	<u>Driver</u>	<u>OS</u>	<u>Bio-Kine version</u>
NI PCIe-6321	NI DAQ mx 9.0.2 or higher	7 (32 & 64 bits)	Bio-Kine32 V4.64 and higher
NI PCI-6221	NI DAQ mx 9.0.2 or higher	7 (64 bits)	Bio-Kine32 V4.60 and higher
	NI DAQ mx < 9.0.2	Windows 2000, XP, vista, 7 (32 bits)	Bio-Kine32 V4.45 and higher
NI PCI-6052E		Windows 2000, XP	
	NI DAQ traditional	Win95, 98, ME	Bio-Kine32 V4.44
DT-2801(A)	DT-OPEN Layer Driver	Win95, 98, ME	Bio-Kine16 V3.30

Insert the NI-DAQmx 9.x.x CD (Data Acquisition Driver Software).

If you do not see the Install NI-DAQ screen when you insert the CD, run D:\SETUP.EXE

Choose 'Install typical NI-DAQ' and follow the instructions.

When the installer completes, click restart later.

Shut down the computer from the windows start menu. Remove the power cord.

Install the PCI board into the computer.

Turn on the PC, the board should be automatically recognized and configured.

The second part of the NI-DAQmx installation should run automatically.

Check if you want to install online documents and follow the instructions to complete the installation.

Note: for any problems refer to the DAQ Quick Start Guide and the Release Notes (supplied).

1.2.2. Bio-Kine installation

The installation of Bio-Kine is described below:

Insert the CD supplied with the instrument.

Run setup.exe and follow the instructions.

By default the directory c:/bk32 is created. This will also create a new group (Bio-Kine) in the start menu.

Leave the CD in the PC to allow then the installation of the USB drivers (manual of automatic installation)

1.2.3. USB drivers installation under windows 7 only

. An automatic installation is performed to install the USB drivers under all windows OS, however for computers running under windows 7 (32 or 64 bits) it may be necessary to install the USB drivers of the MPS-70 manually.

- Connect the MPS-70 to the PC using the USB cable, and switch on the MPS-70.

- Remove the Internet connection from the PC, otherwise Windows 7 will silently connect to the Windows Update website to try to install any suitable driver it finds. No suitable driver is automatically found then the following procedure should be followed.

- Press the Windows start button to bring up the start menu and select "Control Panel".

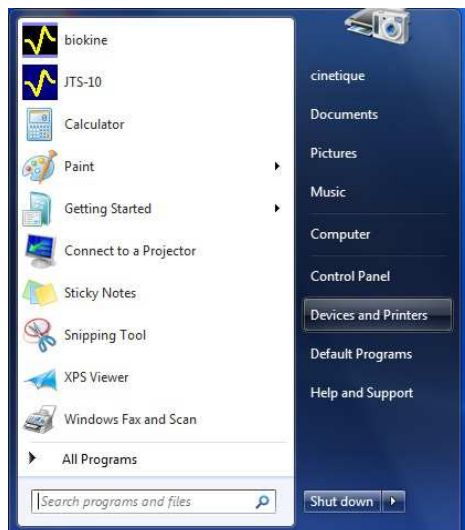


Figure 1: control panel access

- From the Control Panel window select Hardware and Sound



Figure 2: Hardware and sound selection

- At the next screen select Device Manager:



Figure 3: Devices and printers selection

In the Device Manager window there will be a **USB I/O** device under “**Other Devices**” with a yellow warning symbol to indicate a problem i.e. no driver installed. Right click on the “**Unknown device**”, and from the displayed menu select “Update Driver Software...”

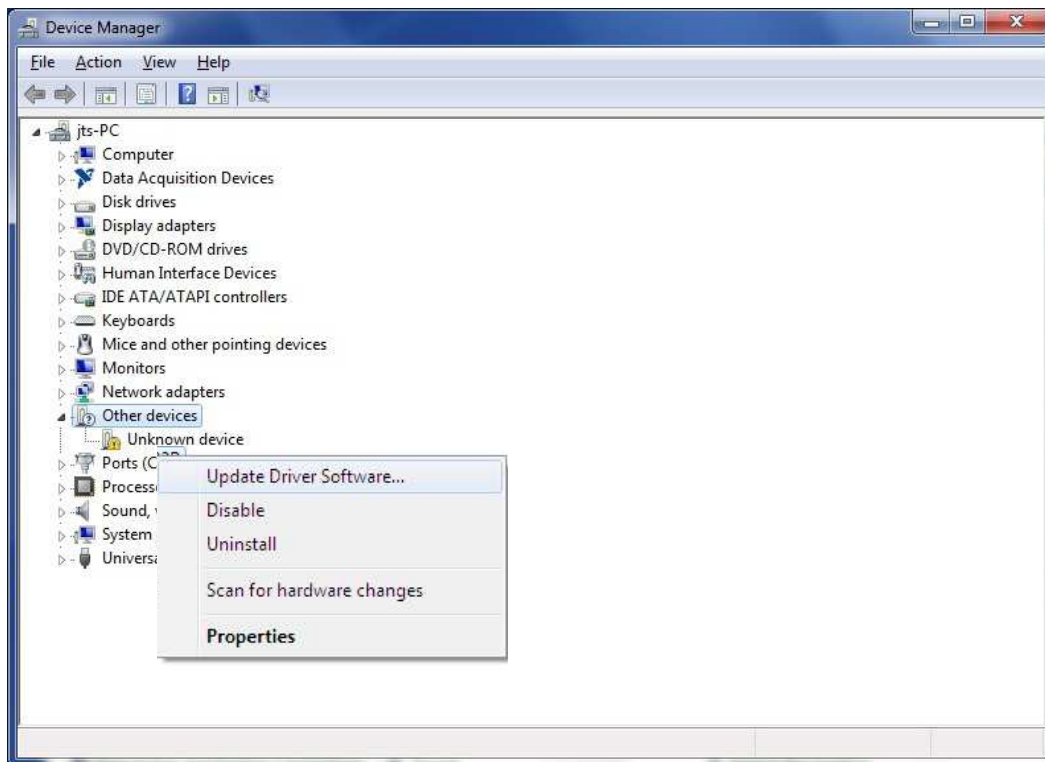


Figure 4: device installation

This then displays the option for an automatic search or a manual search (Figure 5). Select “**search automatically for updated driver software**”.

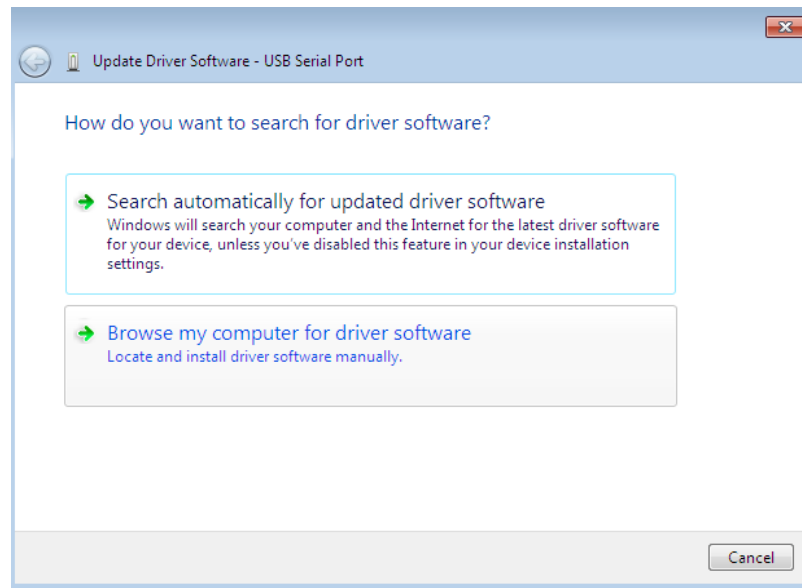


Figure 5: driver software search

The installation of the drivers is initiated,
When the installation has finished a completion screen is displayed.

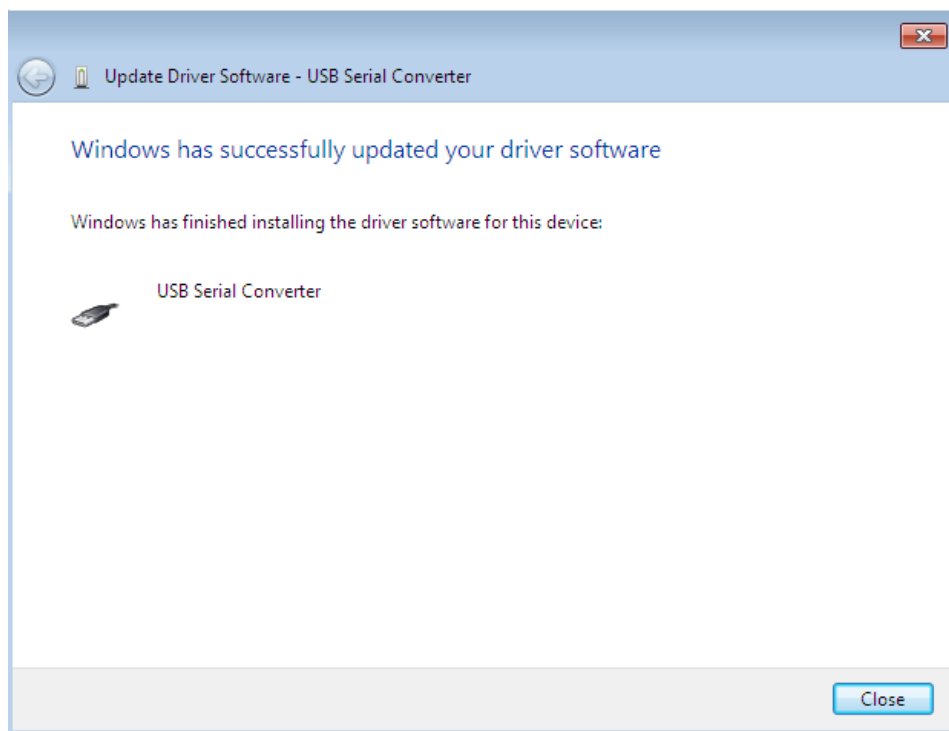


Figure 6: USB Serial converter installation

Press **Close** to close this window and go back to the Device Manager Window.

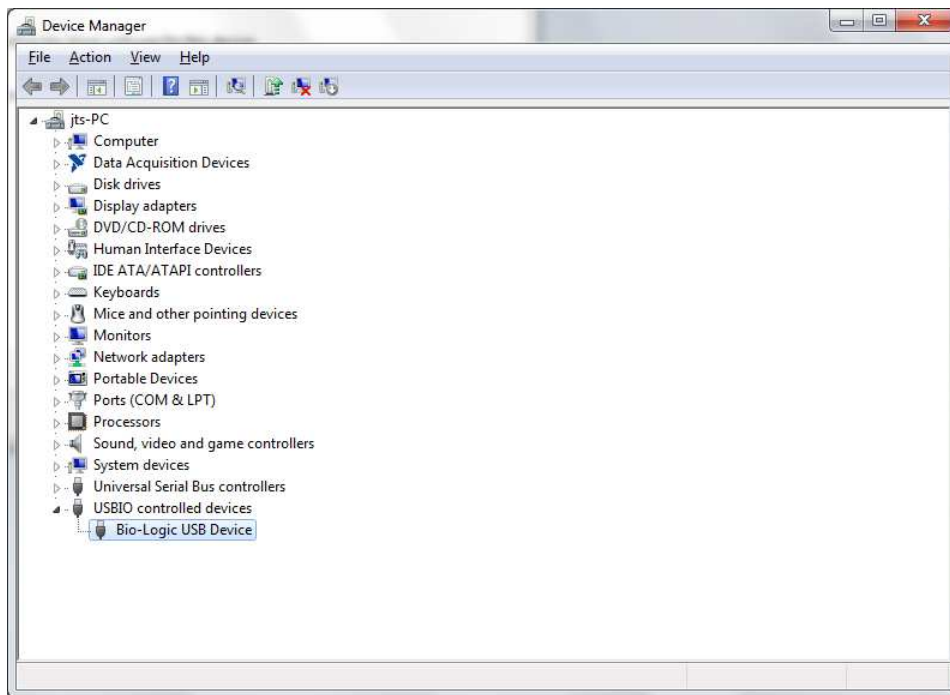


Figure 7: device manager window

The Device Manager will still show a Bio-Logic USB device. Installation is completed.

1.3. Getting started

Run Bio-Kine software from the start menu. The following screen will be displayed: Figure 8.

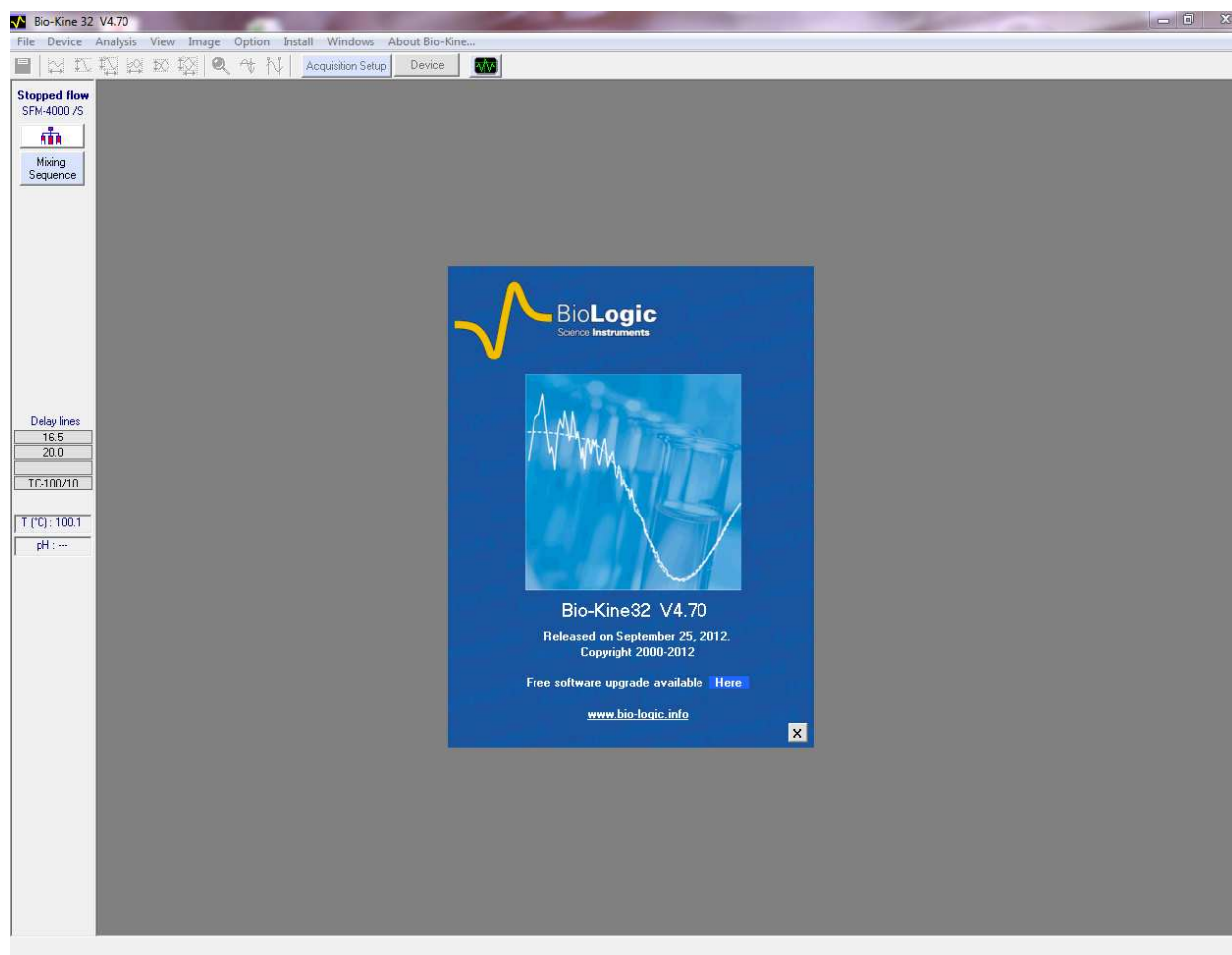


Figure 8: Starting window

As in most Windows software Bio-Kine functions are operated through the menu and the most frequent operations are obtained by actions on buttons which are menu shortcuts. Rectangular state panels (on top right corner and bottom corners of the screen) display information about the current state of the software and information on the data that is displayed on the screen.

Standard Windows actions (reduce, restore, close...) are available from this screen. It is out of the scope of this manual to describe these functions and we recommend referring to the Microsoft Windows manual for more information.

All the buttons are inactivated in the main window (Figure 9) and only four menus are available: **F**ile, **D**evice, **O**ption, **I**nstall and **A**bout Bio-Kine.



Figure 9: Bio-Kine main window menu

1.3.1. File

From the File menu Choose **Load data file...** the window shown in Figure 10 appears:

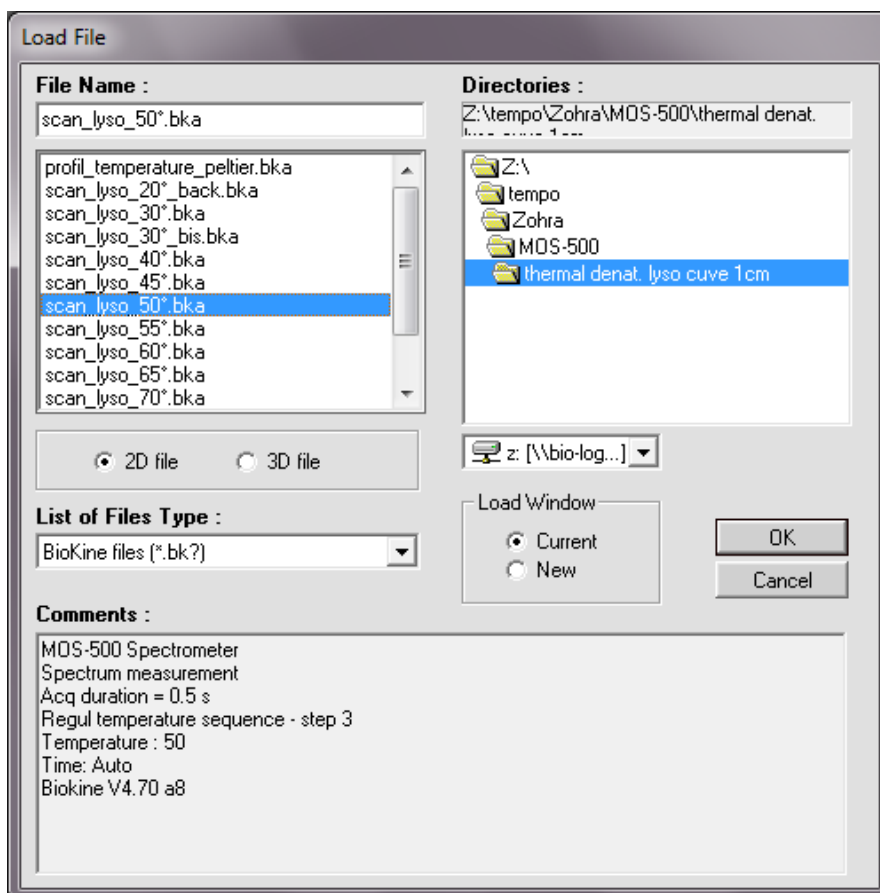


Figure 10: Load File window

Multi-file selection is allowed (using **SHIFT** or **CTRL** keys) up to a limitation of 10 files. Choose **Current** in the **Load Window** area (see Figure 10), validate the selection with **OK** button: this will close the file selection window and load an Analysis window with selected file(s) on display.

If one or more analysis window are already loaded, the **File, Load data files** command replaces all files into the active Analysis window. All the files in one Analysis window have the same X scale. Notice that It is not possible to have a spectrum and a Kinetic traces display in the same window.

If an attempt is made to load a spectrum (X unit in nanometers) into an active window, showing a Kinetic trace (X unit in seconds), the spectrum will be loaded into a new Analysis spectra window.

To open several analysis windows, select **New** in the **Load Window** (see Figure 10) before validate with the button. Then it is possible to arrange the different windows on the screen using **Window, Title** or **Window, Cascade** in the main menu.

Other menus available in the File menu will be discussed further (refer to section 3).

1.3.2. Option

From the Option menu: when loading, only the Graph and Plot area (see Figure 11) and New User's Profile window are active (see Figure 12). The New User's Profile window may be useful if different users are using Bio-Kine with different configurations.

The Graph and Plot area window permits to select the color of different plots.

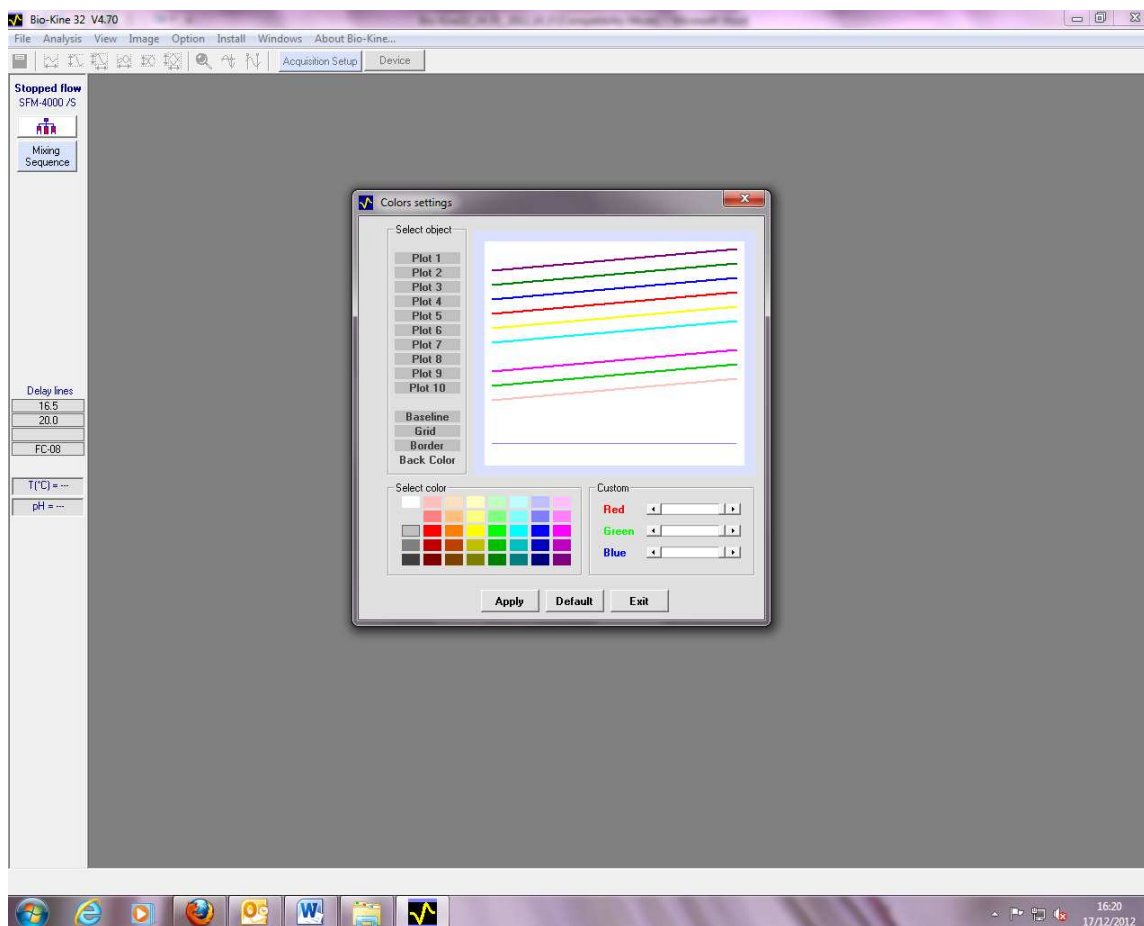


Figure 11: Graph and Plot area

To save the Bio-Kine parameters into a private configuration file (*.INI), choose **Option, New user's profile...** on the main menu. This will load a new window (Figure 12). Enter the name of the file (in the example: Bio-Kine.INI) and click on the **OK** button. Then the parameters will be recorded into the new configuration file at Bio-Kine unload.



Figure 12: User parameter window

Note: files name are limited to 8 characters plus 3 for the extension into the MS-DOS environment. This limitation was kept into Bio-Kine for the configuration files, (even if Windows 95 can support a larger numbers of characters) and the INI extension is locked.

To retrieve parameters from a given configuration file, select the appropriate configuration file name into the initialization window (see Figure 13). This window is displayed at Bio-Kine load time (if several initialization files are present on the hard disk). In the example shown below, Bio-Kine will start with USER2.INI parameters. To delete a profile select the profile in the initialization window and click on the **Delete** button.

If there is only one configuration file (BIO-KINE.INI by default) the initialization window is not displayed. Note: initialization files (*.INI) are stored into the Windows directory (usually C:\WINDOWS\BKSYS).

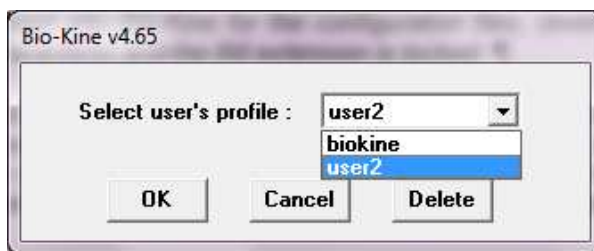


Figure 13: Initialization window.

1.3.3. ***Install***

From the **Install** menu: Install menu is composed in three parts, Device installation option, Stopped-flow configuration and MPS maintenance.

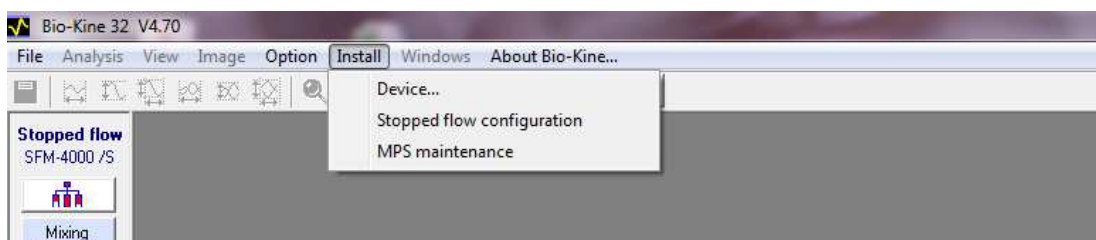


Figure 14: Install menu

1) **From the *Install menu***: choose the **Device installation** option to load the window shown in Figure 15. The stopped-flow communication is established from this window by choosing the corresponding serial port. The acquisition device and its serial port are also selected in this window, details of each configuration will be provided in further sections.

When installed the AD/DA board is automatically recognized. When AD/DA board is not necessary (for example for diode array detection) then the message '*no board detected*' is displayed. Once the system configured, click on OK to validate. Now Bio-Kine is able to drive the selected device.

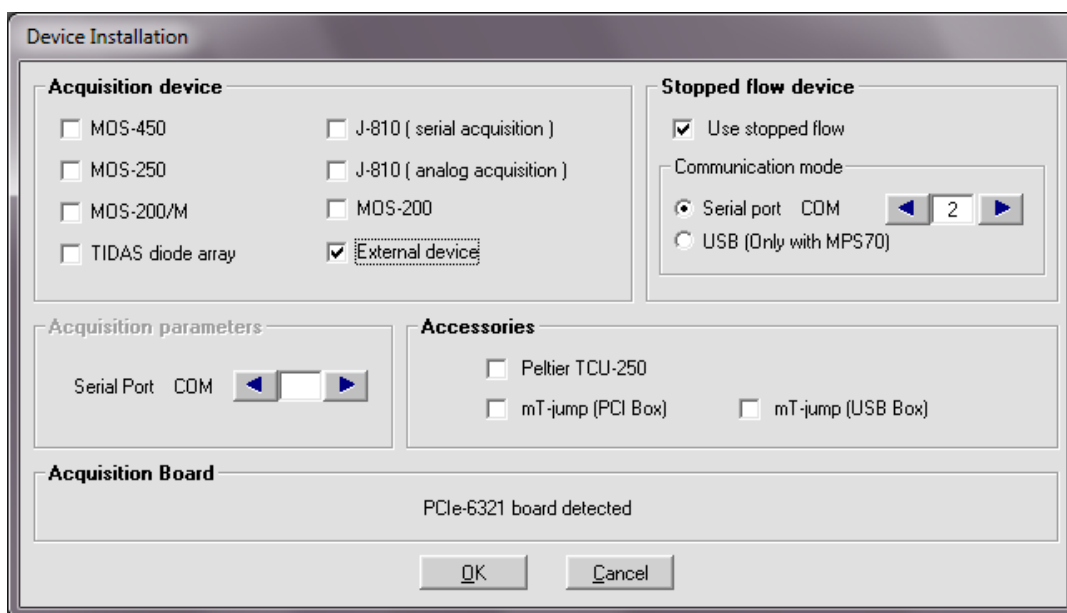


Figure 15 : Device installation

- 2) From the **Install** menu: choose the **stopped-flow configuration**: see section 2.1
- 3) From the **Install** menu: choose **MPS maintenance option**: see section 2.2

1.3.4. About Bio-Kine

From the **About Bio-Kine** menu, some information about Bio-Kine software and links to Bio-Logic's web site (possibility to download some upgraded versions) can be found.

At the following link: <http://www.bio-logic.info/rapid-kinetics/support.html>

2. INSTRUMENT OPERATION IN STOPPED-FLOW MODE

2.1. Stopped-flow configuration

Once the stopped-flow device and its serial port selected in the **device configuration** menu (refer to section 1.3), choose the **Install, Stopped-flow configuration** menu (see Figure 16).

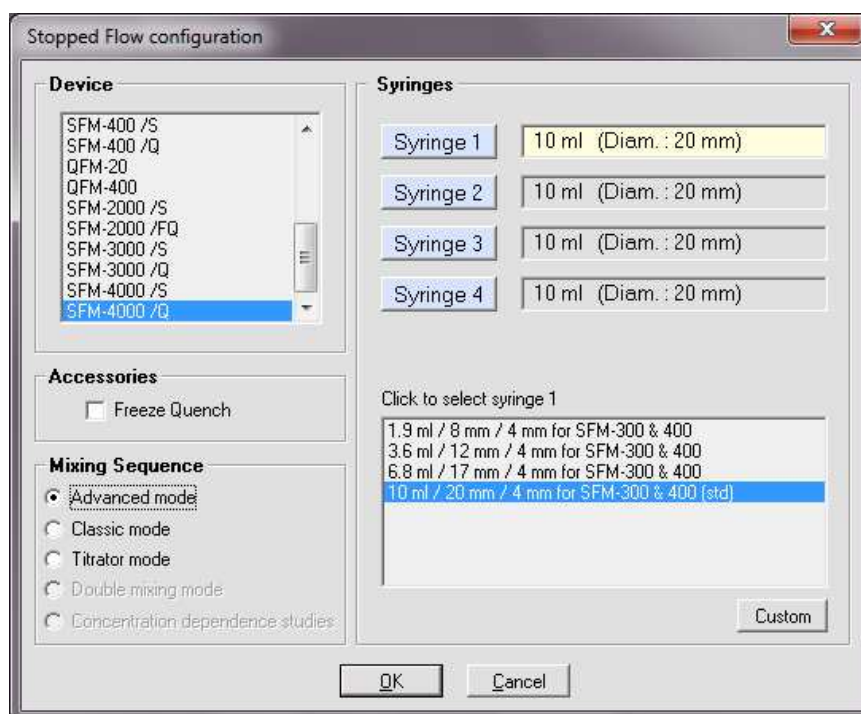


Figure 16 : Stopped-flow configuration

The device to be installed should be configured according to the instrument purchased and mode chosen for use. According to the device selected you can choose different mixing sequence:

- Advanced mode
- Classic mode
- Titrator mode
- Double mixing mode
- Concentration dependence studies

When you operate a quench experiment only classic mode can be selected. The **freeze quench** and **the titrator mode** are only available in specific configurations; please refer to their respective manual for details.

Syringe configuration is made in the same window. The active syringe is displayed in yellow; select the nature of the syringes that have been installed in each syringe position of the SFM by clicking on the right one.

The SFM comes equipped with standard 10 ml syringes and these are the default syringes installed in the software. Changes only need to be made in the software when syringes of different volumes (other

than standard) have been installed in the SFM.

Use the **Custom** button to enter syringe specifications if you have a custom syringe. In this condition the window shown in Figure 17 is displayed, it is then necessary to enter volume, piston diameter and screw pitch of the custom syringe to add it to the standard ones.




Figure 17 : Custom syringe

WARNING: Incorrect syringe configuration will cause volume and flow rate calculations to be incorrect!

2.2. MPS maintenance

From Bio-kine 4.70, the mps14. elf is automatically installed. For bio-kine version 4.50 and higher version, it is now necessary to upgrade the firmware of the MPS-70/x (mps14.elf actually). The procedure is the following:

From the Install menu choose the MPS maintenance option (Figure 18):

For upgrading the MPS firmware the MPS-70 has to be on line. To do so, switch on the MPS-70 using the button located at the rear panel of the device) and few seconds later, click on the icon , from the stopped flow window. Check at the left part of the window if the **MPS On Line** appears. It is important to check that the MPS is on line before starting the upgrade of the firmware.

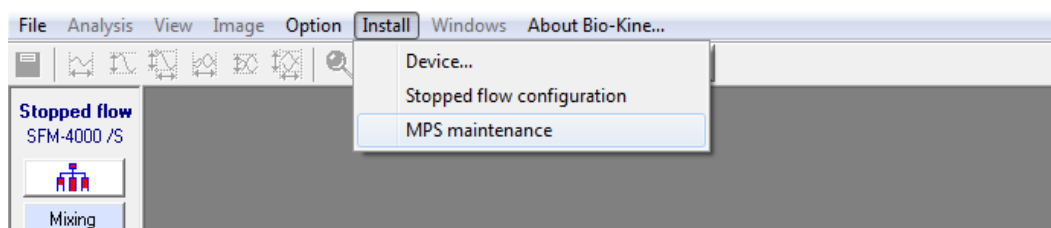


Figure 18: MPS maintenance

By clicking on MPS maintenance (Figure 18) the following window will be opened (see Figure 19).

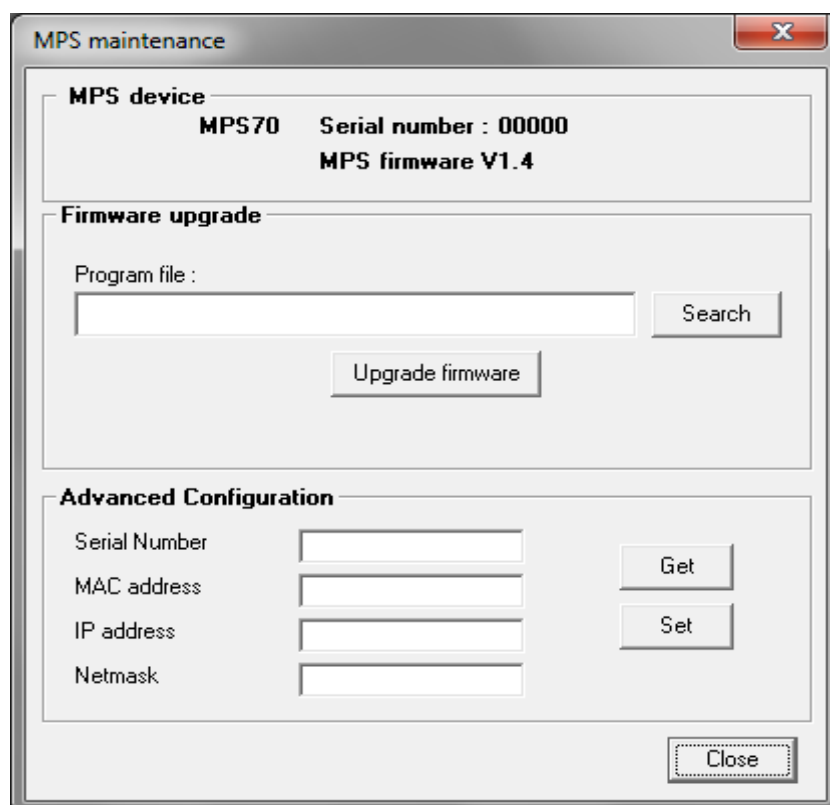


Figure 19: Firmware upgrade

Click on **Search** button, and select the proper file mps14.elf in the MPS folder of Bio-Kine software: by default, Bio-kine folders are installed in C:\bk32 (Figure 20 & Figure 21).

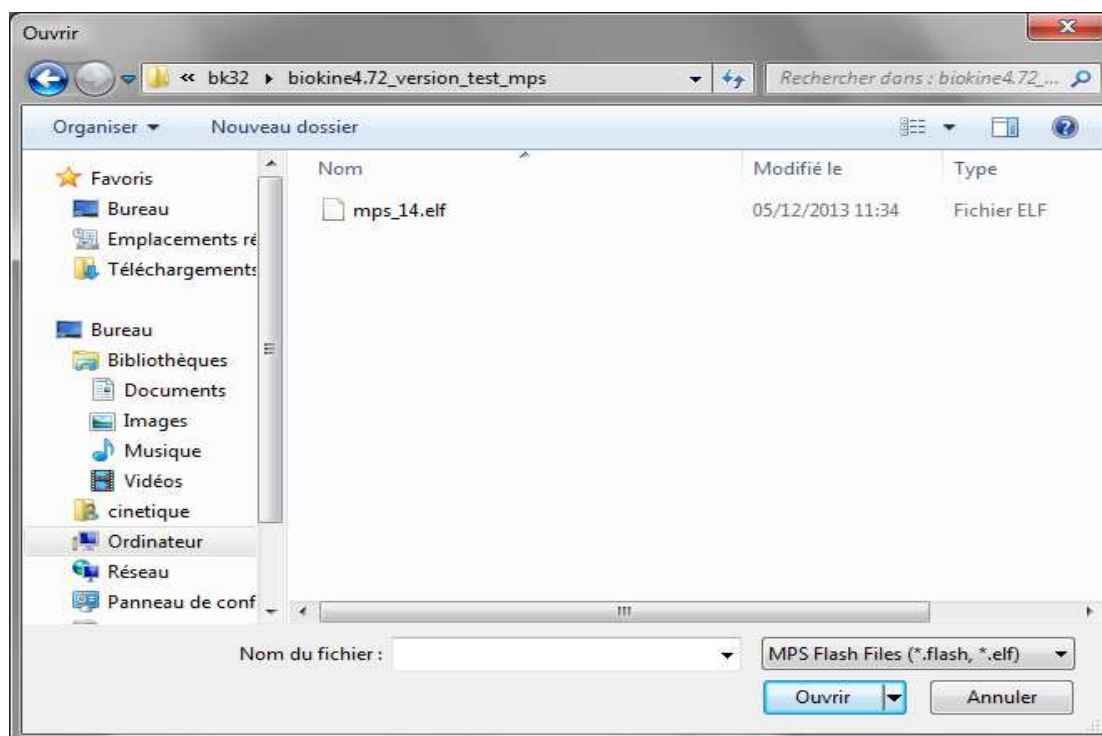


Figure 20: selection of mps14.elf

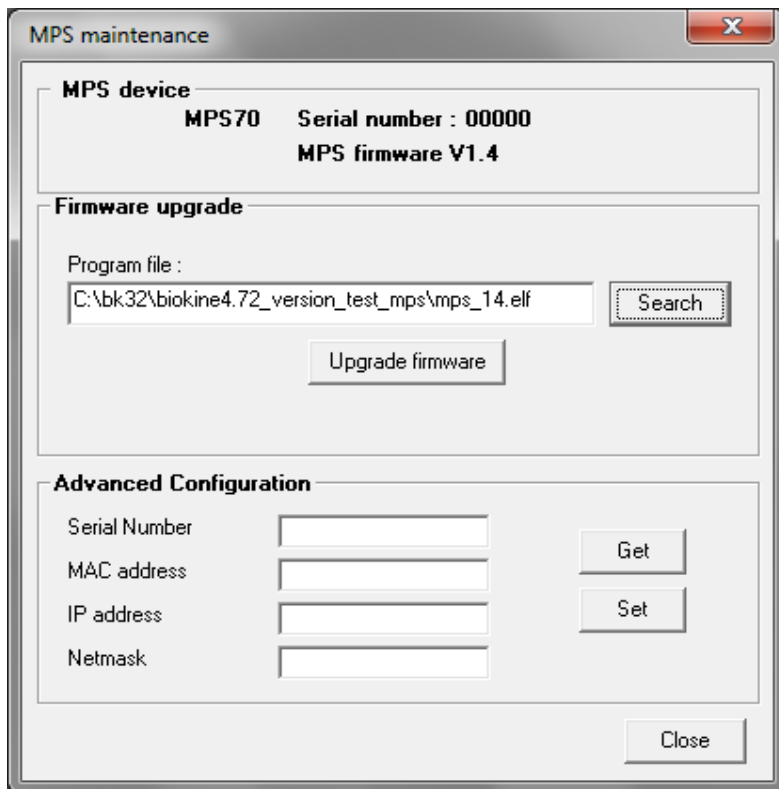


Figure 21: downloading of firmware

Start the upgrade by clicking on  button.
When the upgrade procedure is starting the following window is opened (see Figure 22)

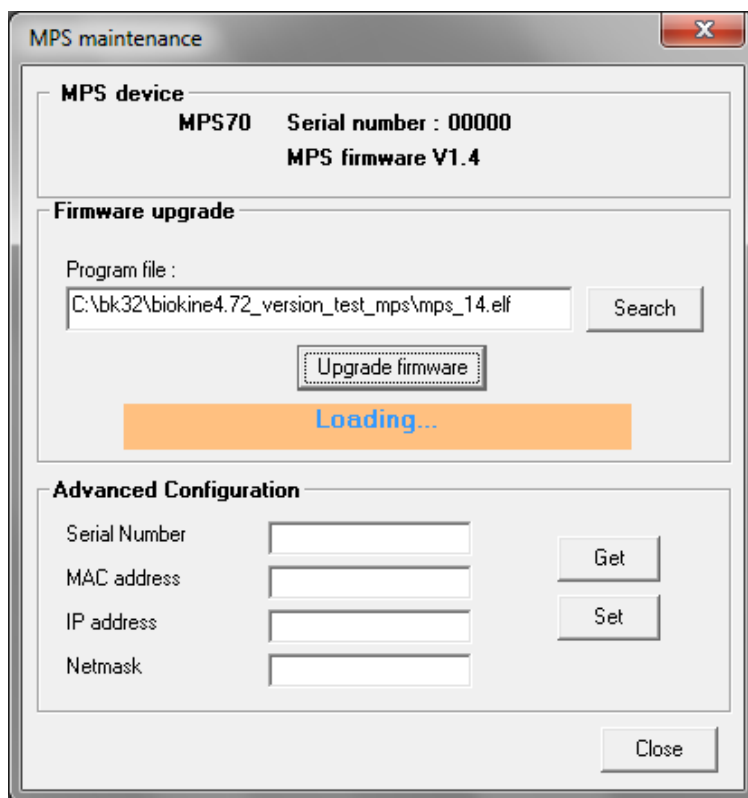


Figure 22: upgrading the MPS firmware

Finally, the procedure is completed (Figure 23).

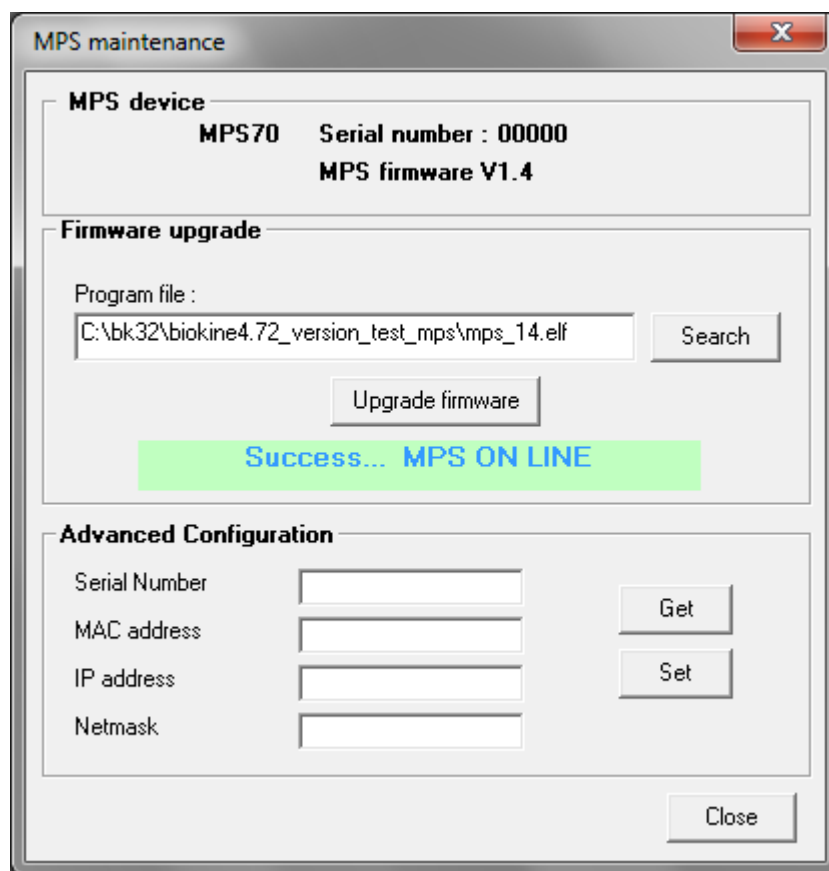



Figure 23: upgrading success

2.3. Stopped-flow status area

A vertical menu bar on the left of the screen is dedicated to the stopped-flow device (see Figure 24).

This menu bar gives access to the syringe control window using the  button (refer to section 2.4), to get the classic mode (refer to section 2.4) or advanced mode (refer to section 2.5) according to the device selected (refer 2.1) click on the mixing sequence button.

At any time, information about the configuration of the stopped-flow can be found in this bar: device, delay lines installed and cuvette type.

Once the sequence ready in the classic or advanced mode, the shot control window is displayed in the area as shown in Figure 247 (refer to section 2.6).

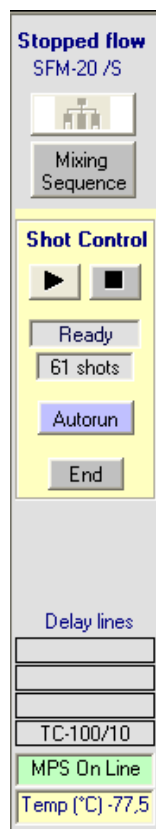













Figure 24 : Stopped-flow menu bar

2.4. Control and initialization of syringe from software

Syringe control from Bio-Kine is made through the  button in the stopped-flow status area. The window shown in Figure 25 is displayed.

The syringe to be moved is selected by clicking on the corresponding frame. The new selected syringe will be surrounded with a red rectangle.

Syringes are emptied or filled using the , ,  and  buttons. The  button move a syringe upwards by one elementary movement and the  button arrow move a syringe downwards by one elementary movement. The  button move the piston upwards by 10x elementary movements and the  button move the piston downwards by 10 x elementary movements.

The size of the elementary steps and syringe movement speed is controlled in the Manual Speed section of the window. The  and  buttons are used to change the manual speed. It could be very useful to reduce cavitation during loading of sample for example. The display shows the speed in flow rate based on the syringe installed and moved.

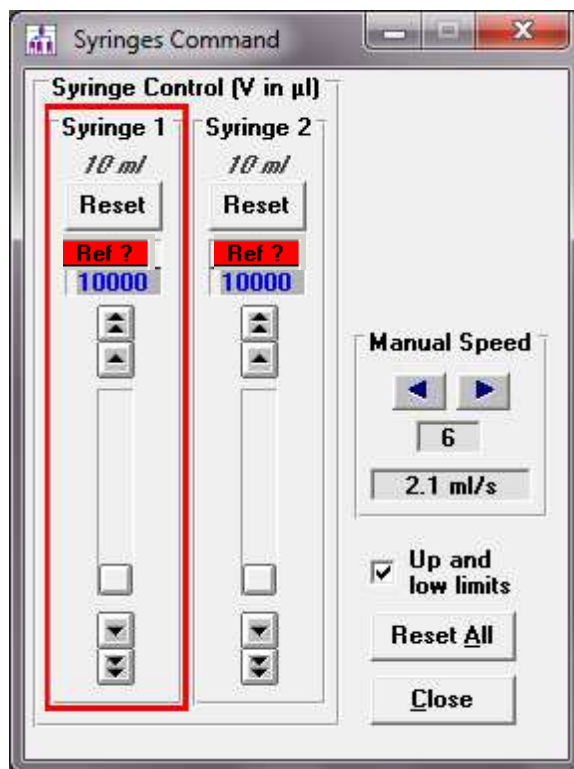


Figure 25 : Syringe control

The MPS that controls the SFM follows the movements of the syringes so that the actual residual volumes are displayed at all times (see Figure 25). When the MPS is turned on and the software started, turned on, the syringe volume counters show **Ref ?** and has to be initialized.

The syringes are initialized by setting the syringes to their uppermost (empty) position and resetting the syringes in Bio-Kine. The syringes can be selected and moved to their uppermost positions either directly with the MPS or from the MPS software. Once a syringe has reached its uppermost position, the syringe motor will oscillate and vibrate as it becomes out of phase with the driving pulses. There is no danger to the SFM or syringe motors when this occurs, but there is no reason to unnecessarily prolong this treatment either.

The syringes can be reset individually by pushing the **Reset** button for each syringe or all at once by pushing the **Reset All** button (refer to Figure 25).

CAUTION: Measurement of residual syringe volume is made by counting the logic pulses from the controller for each syringe. If, for any reason, a syringe is blocked during a run, the pulses will not correspond to the true volume delivered and the value displayed may become erroneous (e.g. in the case of incorrect positioning of a valve). In this case, it is advisable to reinitialize the syringes.


If, by accident, a syringe is returned to its uppermost position the syringe volume counter will again show **Ref ?** and the syringe must be reinitialized. To avoid such accidents, the **Up and Low Limits** checkbox may be checked. When this box is checked, Bio-Kine will not allow the syringes to be driven beyond their upper and lower limits. This also avoids accidentally pulling the syringe plunger completely from the syringe and spilling solution onto the SFM.

WARNING: The Up and Low Limits only applies to control of the syringe from within Bio-Kine software. These limits can be bypassed by manual control of the SFM directly from the MPS.

2.5. Creating a sequence using the advanced mode

2.5.1. SFM options

An advanced menu was created to improve the friendliness of the design of the stopped-flow sequence. This mode can only be used for single mixing experiment, it is still necessary to use the classic mode for double mixing experiment or to perform pre-washing phase (refer to section 2.6 in this

case). The advanced menu can be reached clicking on the  button in the stopped-flow status area. The window shown in Figure 26 is then displayed.

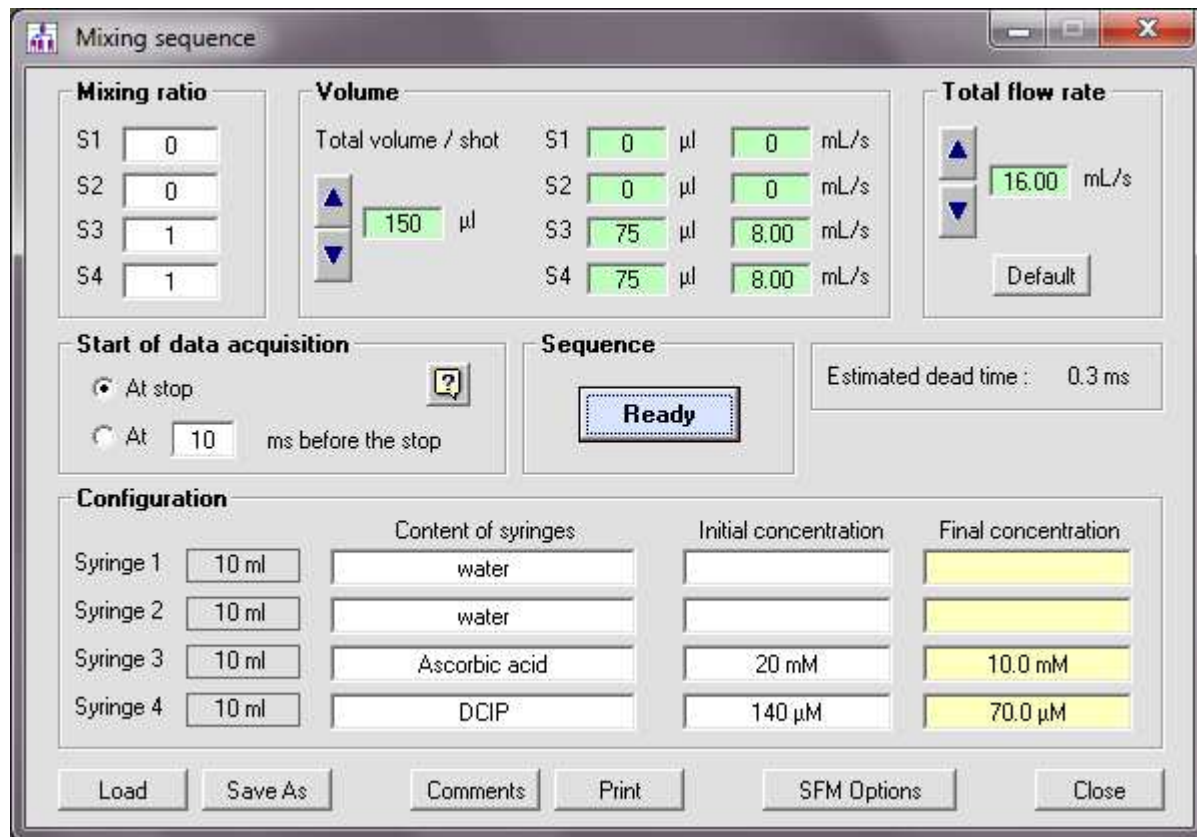
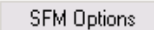


Figure 26 : Driving sequence in advanced mode

First operation should be to check the configuration of the stopped-flow, it is made by clicking on the  button (refer to Figure 27).

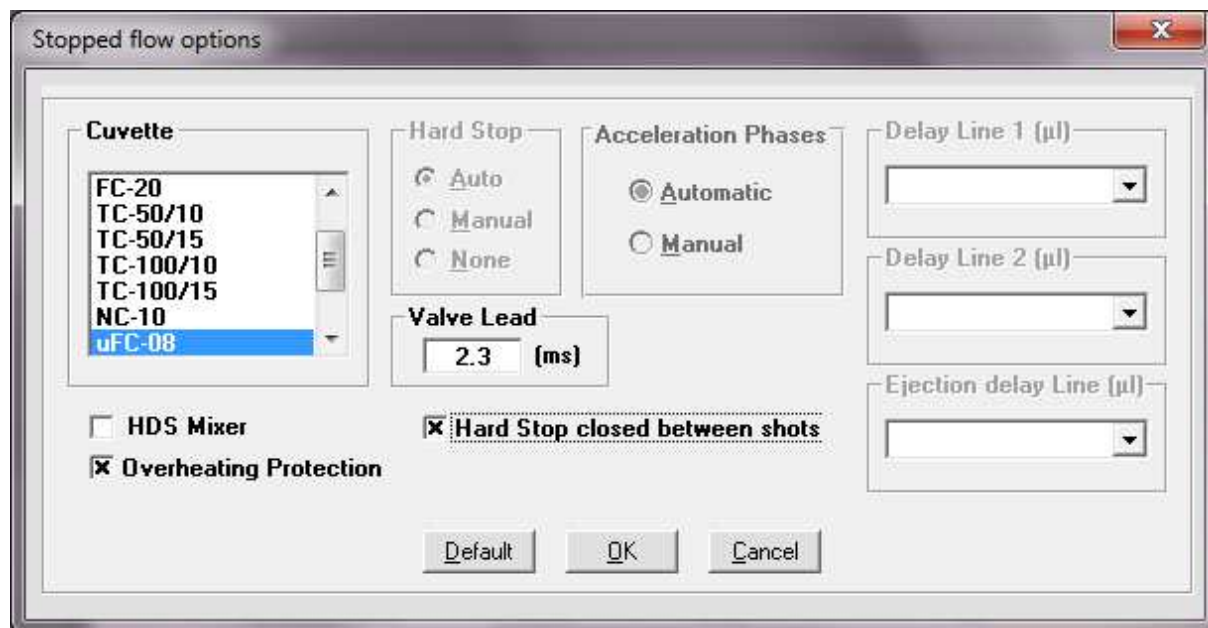


Figure 27 : SFM options in advanced mode

Select the cuvette and mixer according to the cuvette and mixer installed in the SFM (refer to the SFM user's manual for more details).

WARNING: Incorrect cuvette and mixer configuration will cause dead time calculations to be incorrect!

Valve Lead: This section of the windows allows one to enter the number of milliseconds before the flow stops that the hard stop starts closing. The default value is 2.3 ms. The lead time may be adjusted (from 0 – 5 ms) to fine-tune the quality of the stop. The precision of the setting is 0.1 ms.

Overheating Protection: Not applicable for recent MPS-60 and MPS-20. Default mode is checked. It is a protection against electronic overheating after long working day.

Keep hard-stop closed between shots: in advanced mode, the configuration of the hard-stop is automatically set: the hard-stop closes at the end of the pushing phase (or few milliseconds before if a lead time is selected) and opens at the end of the acquisition. If the user needs to keep the hard-stop closed at the end of the acquisition (to run a spectrum for example), then it is necessary to check the corresponding box.

Delay lines: In advanced mode delay lines and ejection delay lines cannot be changed boxes are not active because only single mixing experiment can be performed.

2.5.2. Design of stopped-flow sequence

The window shown in Figure 26 is separated into six areas: mixing ratio, volume, total flow rate, start of data acquisition, shots, and configuration. These different areas are respectively described below.


Mixing ratio: it is the first parameter to set, ratio of syringes which are not used must be set to 0. It is possible to enter decimal value for the ratio.

Volume: it is necessary to set the total volume of reactants to push into the cuvette using the 'up' or 'down' arrows. The volume is proportional to a micro-step volume in order to improve the reproducibility of results. Once the total volume selected the volume to be pushed for each syringe is calculated. The total volume selected should be big enough to wash the cuvette efficiently between two shots (refer to color code for the limits).

Total flow rate: total flow rate must be selected using 'up' and 'down' arrows. Once the total flow rate selected the flow rate for each syringe is automatically calculated. 1 ml/s is considered as a minimum value to get an efficient mixing. There are also limits for single syringe according to their respective volume (refer to color code).

Start of data acquisition: Using the 'stop' option only the Kinetics will be recorded, the acquisition is starting when the hard-stop is closed. To make sure the cuvette is well washed and the stationary state is reached, it is advised to start the acquisition few ms before the stop.

Configuration: In this area it is possible to find the volume of the syringe installed and the type of cuvette. The content of syringes can be entered with initial concentration, the final concentrations are calculated using the mixing ratio selected (in the example given in Figure 28 the mixing ratio used is 1/2/4/1).

Sequence: Once the sequence ready, click on the  button, the shot window is now activated on the left panel of the screen.

The estimated dead time of the reaction is given in ms, the dead time is calculated using the cuvette and mixer selected (dead volume) and the total flow rate

Configuration				
		Content of syringes	Initial concentration	Final concentration
Syringe 1	10 ml	solution 1	25 mM	3.125 mM
Syringe 2	10 ml	solution 2	150 mM	37.5 mM
Syringe 3	10 ml	solution 3	20 mM	10.0 mM
Syringe 4	10 ml	solution 4	100 µM	12.5 µM
Cuvette	TC-100/10			

Figure 28 : Example of driving sequence

A color code is used to warn the user about the choice of the parameters selected:

	Color code	message
Total volume	green	OK (depending on syringe)
	yellow	Total volume may be insufficient for washing the cuvette (<4 times the dead volume)
	red	Total volume too low for correct washing of cuvette
Volume per syringe	green	OK
	yellow	Syringe volume may be insufficient for washing of mixer
	red	Syringe volume too low
Total flow rate	green	OK
	yellow	Flow rate too low for correct mixing (< 1 ml/s)
	red	Flow rate may be difficult to achieve for this cuvette (>15ml/s)
Flow rate per syringe	green	OK
	yellow	Flow rate may be too high (will be dependant on cuvette and flow by other syringes)
	red	Flow rate out of range (too low or too high)

Standard operations can be made from the same window:

- **Load** a sequence using the button.
- **Save** a sequence using the button.
- **Print** a sequence using the button.
- **Close** a sequence using the button.

Comments: a text window is opened by clicking on the button; comments will be saved with the sequence.

2.6. Creating a Driving Sequence using classic mode

2.6.1. SFM options

Experiments are performed with the SFM through the use of a driving sequence. A driving sequence tells the SFM to automatically perform several functions such as moving the syringes, activating the hard stop, and triggering data acquisition. Driving sequences are created in the window shown in



Figure 29, this window can be reached from the button in the stopped-flow status area.

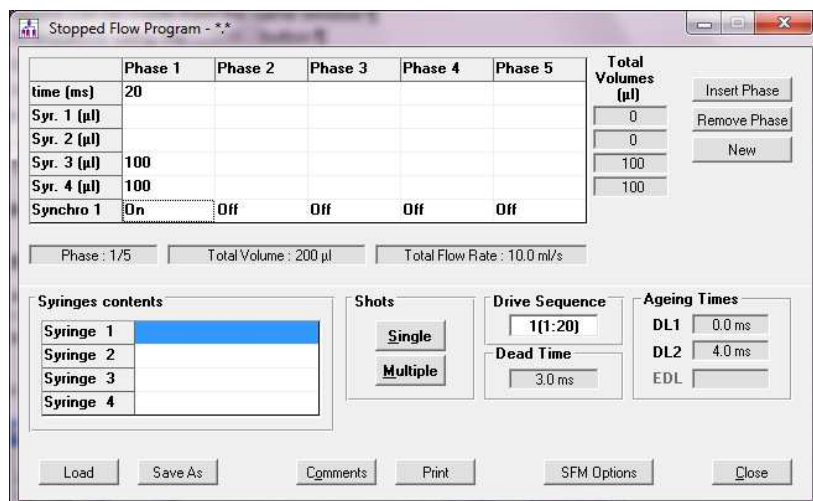


Figure 29 : Driving sequence in classic mode

First operation should be to check the configuration of the stopped-flow, it is made by clicking on the **SFM Options** button (refer to Figure 30)

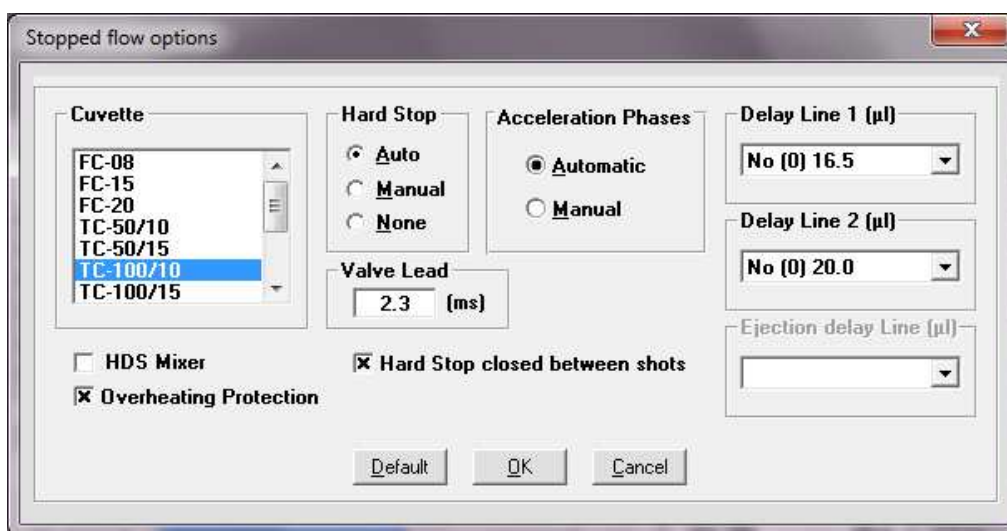


Figure 30 : SFM options

Select the **cuvette** and **mixer** according to the cuvette and mixer installed in the SFM (refer to the SFM user's manual for more details).

WARNING: Incorrect cuvette and mixer configuration will cause dead time calculations to be incorrect!

Valve Lead: This section of the windows allows one to enter the number of milliseconds before the flow stops that the hard stop starts closing. The default value is zero. The lead time may be adjusted (from 0 – 5 ms) to fine-tune the quality of the stop. The precision of the setting is 0.1 ms.

Overheating Protection: Not applicable for recent MPS-60 and MPS-20. Default mode is checked. It is a protection against electronic overheating after long working day.

Hard-stop: 'auto' is the default mode. In this position the hard stop is closed at the end of the pushing phase (or few milliseconds before depending on the lead time chosen) and remains closed until the end of the acquisition. As soon as the acquisition is over the hard-stop opens. In case the user wants to leave the hard-stop closed after acquisition (for example to perform a spectrum) it is necessary to choose the keep hard-stop closed between shots option (see Figure 31). When using the 'manual'

mode the hard-stop is programmed to open (off) and close (on) by the user (through the synchro out 2). If 'none' is selected, the hard-stop is always open.

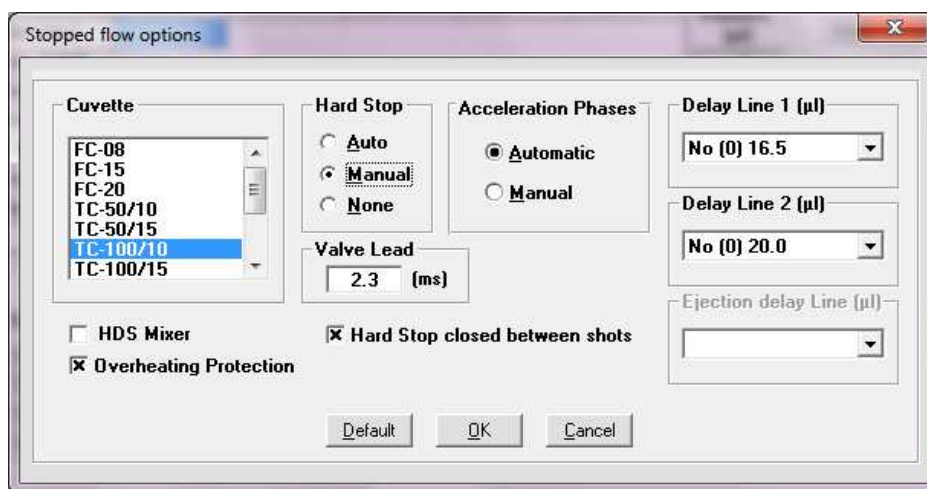


Figure 31: SFM option

By clicking on “OK”, you return back to the mixing sequence window with Sync.2/HS (Hard Stop) line see Figure 32.

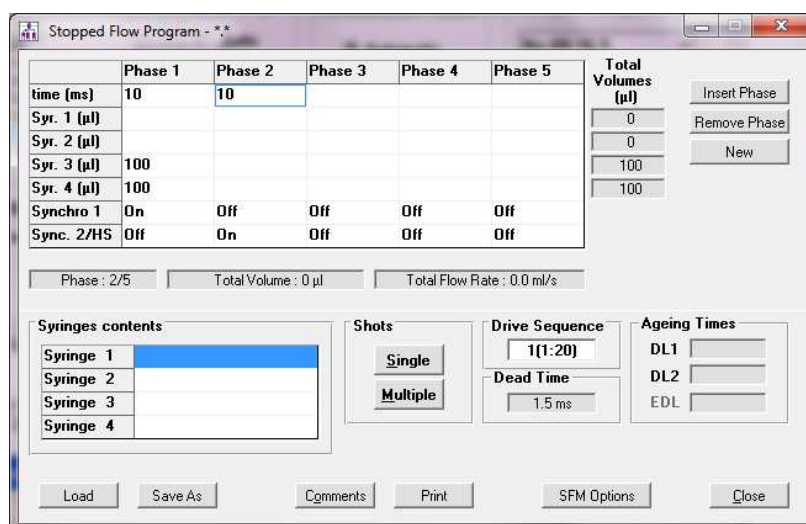


Figure 32: Sync.2/HS command

Acceleration phases: please refer to the SFM user's manual for details about the acceleration phases, default mode is automatic but one can create his own acceleration.

Delay lines: Select the delay line(s) according to the delay line(s) you have installed in the SFM. None, one or two delay lines must be configured depending on the type of device installed. Each delay line is chosen from a pull-down menu in the window shown in (Figure 30). The ejection delay line is only available in freeze quench configuration.

WARNING: Incorrect delay line configuration will cause ageing time calculations to be incorrect!

2.6.2. Design of the sequence

A driving sequence is entered in the program grid shown in Figure 29. Each column of the grid represents a driving sequence phase. Each phase contains actions for the SFM to perform. A complete driving sequence may contain from 1 to 20 phases. Although only 5 phases are shown initially, additional phases may be inserted using **Insert Phase** button or removed using the **Remove Phase**

button.

Figure 33, shows an expanded view of the program grid. The duration of a phase is entered in ms (1 – 60000 ms/phase) on the first line of the program grid. The volume in μl delivered by each of the syringes during a phase is entered on the line next to the appropriate syringe. The status of the synchronization trigger is noted on the last line of the program grid.

	Phase 1	Phase 2
time (ms)	100	
Syr. 1 (μl)	100	
Syr. 2 (μl)	100	
Syr. 3 (μl)	100	
Syr. 4 (μl)	100	
Synchro 1	On	Off

Figure 33 : Design of the sequence

To enter the phase duration and syringe volumes delivered, click on the corresponding cell or use the keyboard arrows keys to navigate between cells.

CAUTION: Blank and non-numeric values entered in the program grid are considered as zero values. Phase duration of 0ms will cause the phase to be skipped in the execution of the drive sequence.

The contents of the syringes can be entered in the **Syringe Contents** (refer to Figure 28) Each time a program grid cell value is changed, information about the current syringe, current phase and driving sequence is updated displayed below and to the right of the grid (Figure 34). This information indicates:

- 1) Current phase number and total number phases in the driving sequence.
- 2) Volume delivered by the current syringe during the current phase or current phase total volume (if an entire phase is selected).
- 3) Flow rate of the current syringe during the current phase or current phase total flow rate (if an entire phase is selected).
- 4) Total volume delivered by each syringe during the driving sequence.

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Total Volumes (μl)
time (ms)	100					100
Syr. 1 (μl)	100					100
Syr. 2 (μl)	100					100
Syr. 3 (μl)	100					100
Syr. 4 (μl)	100					100
Synchro 1	On	Off	Off	Off	Off	
Sync. 2/HS	Off	Off	Off	Off	Off	

Phase: 1/5 Total Volume: 400 μl Total Flow Rate: 4.0 ml/s

Figure 34: MPS Software: Driving Sequence Information

Indications of the Ageing Times for a driving sequence are also displayed in the window shown in Figure 29. The ageing time is calculated for the last valid phase, according to its flow rate and of the delay lines and intermixer dead volumes.

The ageing times are calculated for the current phase selected based upon the syringes flow rates, delay lines installed and intermixer volumes

The MPS software provides the possibility to repeat phases a number of times in virtually any order. This is accomplished through a macro sequence entered in the **Drive Sequence** frame of the « **Stopped Flow Program** » window (Figure 35). The macro sequence can be edited to run a single phase or many phases in a different order than present in the program grid.

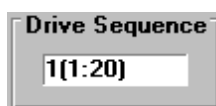


Figure 35: MPS Software, drive Sequence Macro

Once the sequence ready, click on **Single** or **Multiple** button to activate the shot window on the left panel of the screen.

Standard operations can also be made from the same window:

- Create a new sequence using the **New** button
- Load a sequence using the **Load** button.
- Save a sequence using the **Save As** button.
- Print a sequence using the **Print** button.
- Close a sequence using the **Close** button.

Comments: a text window is opened by clicking on the **Comments** button, comments will be saved with the sequence.

2.7. Titrator mode: Please refer to the titrator manual

2.8. Double mixing mode

CREATING A DOUBLE MIXING EXPERIMENT

The double mixing experiment program is used in order to mix three reactants together. This means that a SFM 3000 or SFM 4000 has to be considered. With the SFM 3000/S, all syringes will be used. With SFM 4000, the three last syringes will be used (S2, S3 and S4) and S1 is not used, however this syringe has to be loaded with a solvent (water or buffer for example).

A double mixing experiment is divided in three phases:

1/ A and B are mixed in a first mixer

2/ The solution (A+B) goes through a delay line. The solution is aged in the delay line (waiting phase)

3/ The solution from the delay line is mixed with a C solution (third reactant) in the second mixer; and the final solution goes through the cuvette for analysis.

Once the mixing sequence “Double mixing mode” is selected from the **Install, Stopped Flow**

Configuration menu, clicking on the button mixing sequence **Mixing Sequence** opens a window (Figure 36).

The double mixing window can be shared in two parts:

- Double mixing experiments, where all parameters such as content syringe, concentration, phase conditions are defined
- Global sequence: where the program sequence appears, giving information on the volume consumption for each syringe, ageing time assessed etc...

The screenshot shows the 'Double mixing experiments' software interface. It is divided into several functional areas:

- Content of syringes:** Fields for Syringe (2), (3), and (4) to enter volumes.
- Initial concentration:** Fields for A0, B0, and C0.
- Final concentration:** Empty fields for the final calculated concentrations.
- Phase 1 conditions:** Ratio A and Ratio B input fields.
- Phase 3 conditions:** A dropdown for 'Empty Delay line by using' (set to 'Syringe (B)'), 'Ratio Delay line' (1), 'Ratio C' (1), and 'Total flow rate' (10.0 ml/s, highlighted in green).
- Acquisition options:** 'Start next acquisition step' (radio buttons for 'at the end of measurement' and 'after 5 sec') and 'Start of data acquisition' (radio buttons for 'At stop' and 'Pre-trigger').
- Timing:** 'Estimated dead time' (3.0 ms) and 'Minimum ageing time' (4.0 ms).
- Global sequence:** A table for 'Structure of the sequence' with columns for Phase 1, Phase 2, and Phase 3, and rows for Time (ms), Syr. 2 (µl), Syr. 3 (µl), and Syr. 4 (µl). An 'Ageing times' table has columns for Step (1-5) and Age (ms). Below the tables are 'Repeat number' (1) and 'Total volume / Syringe' (A: 0 µl, B: 0 µl, C: 0 µl).
- Buttons:** 'Load', 'Print', 'Save As', 'SFM Options', 'Infos...', 'Edit Sequence', 'Ready', and 'Close'.

Figure 36 : Double mixing experiment, main window using SFM 3000/S

➤ **Double mixing experiments** (Figure 37):

The content of syringes and the initial concentration have to be entered. Thus the final concentration will be calculated (the final concentrations change in function of the ratio assessed, see Figure 37).

In the **Phase 1** conditions window, enter the Ratio A and ratio B,

In the **Phase 3** conditions window, select the syringe(s) used in order to empty the delay line. Product from the reaction of A+B is stored in the delay line and then mixed with the C solution. To empty the delay line of the aged solution, A or B or A+B can be used. This procedure is particularly useful to save a precious solution during this flushing phase.

Assess the ratio between the solution coming from the delay line (A+B) and C, and finally fix a total flow rate. Please notice that the flow rate has to be assessed to satisfy the turbulent conditions: in that way, a colour coded window orientates the user. The green font colour indicates adequate parameter conditions. In case of orange font colour, the total flow rate is too slow and should be increased.

Start next acquisition step: In case of a repetition of the sequence, starting the next acquisition step can be done at the end of the measurement or after a defined time.

Start of data acquisition: as in the stopped flow advanced mode, starting the acquisition can be done at the stop of the motors or few milliseconds before the stop (this time is fixed at 20 ms and cannot be changed)

Estimated dead time: An estimated dead time is calculated in function of the flow rate assessed and the volume of the cuvette (chosen from the SFM option).

Minimum ageing time: this corresponds to the time needed to fill the delay line. This minimum ageing time is equal to the delay line volume divided by the flow rate coming from syringe 1 and 2 for an SFM 3000 (A+B).



Figure 37 : editing a double mixing experiment

Clicking on SFM option **SFM Options** opens a new window (Figure 38):

Select the right **cuvette** to allow a correct estimation of the dead time (Figure 37).

Select the correct delay line. For practical reasons we advise the user to choose the delay line number 3 (90 μ L) or number 5 (190 μ L). The volume of the delay line has to be high enough to allow a correct mixing during the third phase (A+B) mixed with C.

Valve lead: this time is driving the closure of the valve of the hard stop to fine tune the quality of the stop of the kinetic.

HDS Mixer: In case a HDS mixer is used, tick this box to allow a correct calculation of the dead time

Overheating Protection: this prevents overheating of the electronic in case of intensive daily work.

Hardstop closed between shots: the hardstop closes at the end of the pushing phase and opens once the acquisition is completed. Ticking this box will left the valve closed. This could be particularly useful in order to run a spectrum at the end of a shot for example.

Use Synchro out 1 trigger: used in case an external device is connected to the MPS for synchronization.

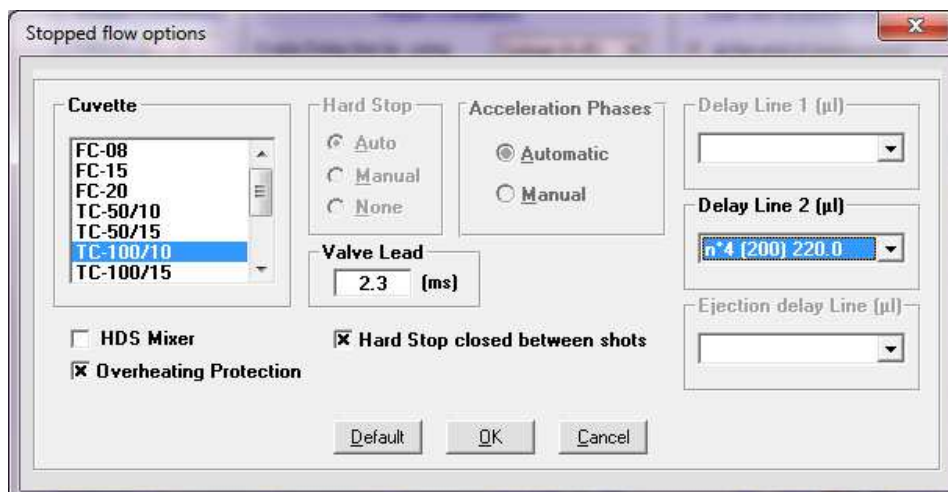


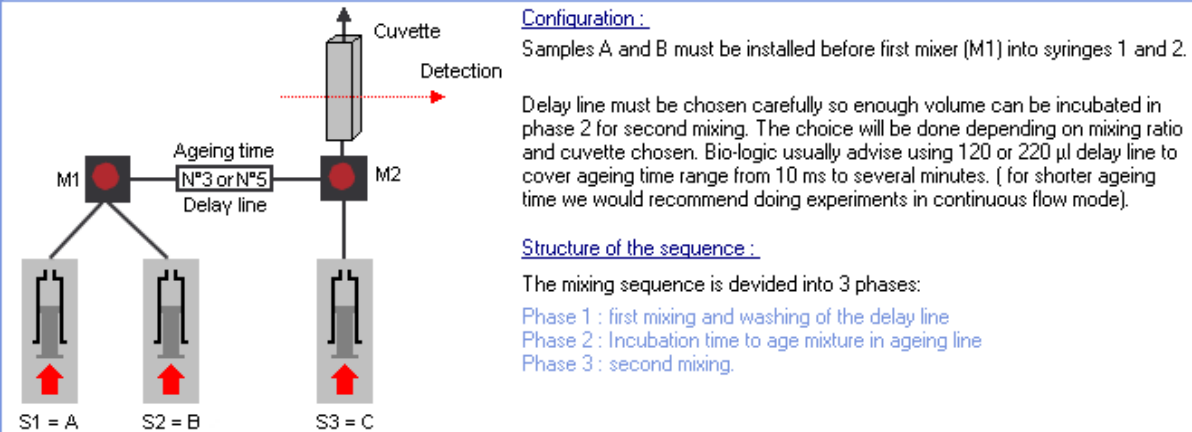
Figure 38 : stopped flow options

Once all parameters are fixed from the stopped flow option window, click on the **OK** button.

Clicking on **Infos...**, open a new window (Figure 39), giving informations on the double mixing experiments. Theory, and description of the method are explained.

A double mixing (also called double jump) experiment can be described as follows: A and B are mixed in a first mixer, after a defined incubation time in a delay line the mixture is mixed with C. The second reaction is then monitored in the cell. It is done using 3 syringes and 2 mixers according to the following diagram.

The aim of this mode is to propose automation of double mixing experiments by changing automatically the ageing time between shots, the dead time of the second reaction remains constant in each experiment.



Design of the sequence :

The user must select the mixing ratio S1/S2 to apply in phase 1 and the mixing ratio in the second mixer (phase 3). In phase 3, the delay line is emptied using S1 and/or S2. It is the user's choice (usually it would be the less valuable sample) to save expensive material.

Biokine proposes to the user a driving sequence which takes into account the volume of the cell, the type of mixer and the size of the delay line. Depending on flow rate selected, both the theoretical dead time in the second mixer and the minimum ageing time are displayed.

Theoretical dead time = Volume of the cell / Flow rate in phase 3
Minimum ageing time = Volume of delay line / Flow rate in phase 1

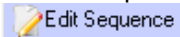
Automation :

Once the sequence is set, the user just needs to enter the ageing times he wants to reach. Biokine will automatically calculate duration of phase 2 to match desired ageing times. Once all ageing times are selected, select the number of shots you want to accumulate for each ageing step. Your sequence is now ready for automation.




Figure 39 : Help section

Once all parameters of the double mixing sequence window are defined, click on the button



This will automatically create a sequence in the global sequence window.

- **Global sequence window part** (Figure 40)

Once the user has clicked on , a structure of the sequence is automatically defined taking in account the ratios and total flow rate.

The structure of the sequence is made with four rows and three columns (phases).

The rows display times and volumes injection for Syringe 1, Syringe 2 and Syringe 3.

The columns display the three phases:

Phase 1: first mixing (S1 + S2)

Phase 2: waiting phase. Solution is stored in the delay line to be aged.

Phase 3: solution from the delay lined is emptied (using S2) and mixed with solution coming from S3. In our example in Figure 40, 47.5 μL of aged solution present in the delay line will be mixed with 47.4 μL of solution coming from S3.

Global sequence

Structure of the sequence				Ageing times	
	Phase 1	Phase 2	Phase 3	Step	Age (ms)
Time (ms)	28.4	0.5	9.5	1	10
Syr.1 (µl)	71.1		0		
Syr.2 (µl)	71.1		47.4		
Syr.3 (µl)	0		47.4		

Repeat number: 1 Total volume / Syringe : A: 71.1 µl B: 118.5 µl C: 47.4 µl



Ready  Close 

Figure 40 : Global sequence window

The volumes in the structure of the sequence can be changed manually, click on the desired box, and enter a new injection volume.


A colour code is used to warn the user about the volumes selected:

In case of orange colour, the volumes used are too small to insure an efficient washing of the cuvette or the delay line.

In case of green colour, the volumes used are adequate to run the kinetic.



The ageing times window is used in order to define an ageing time (Figure 40). The ageing time is equal to the waiting phase from phase 2 + minimum ageing time (time needed for the solution A+B to fill the delay line under a continuous flow).

When changing the ageing time, the waiting phase is automatically calculated.

It is possible to run several shots, using different ageing times for each shot. To do so, click on the button  from the Edit table. This will add a second step in the ageing time window (Figure 41).

To remove a step, click on the button .

To erase all the ageing time sequence, click on .

When several steps are created, and in case the user wants to invert two lines, the arrow  or  from the edit table can be used.

At the bottom of the Global sequence window, a function allows to repeat each step. For example in case three steps are created as in Figure 41, and a "repeat number" of 2 is introduced, the sequence will be run so that step number one is done 2 times, then, step number two is done in two times etc...

This procedure allows recording each traces and does an average.

Global sequence

Structure of the sequence				Ageing times	
	Phase 1	Phase 2	Phase 3	Step	Age (ms)
Time (ms)	38.3	47.2	14	1	40
Syr.1 (µl)	95.7		0	2	50
Syr.2 (µl)	95.7		70	3	60
Syr.3 (µl)	0		70		

Repeat number Total volume / Syringe : A B C



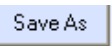
Ready  Close 

Figure 41 : step creation

SAVING/LOADING A DOUBLE MIXING EXPERIMENT

It is possible to save a sequence, by clicking on , the file is saved under a .DMX file.

2.9. Concentration dependence studies mode

2.9.1. Creating driving sequences using SFM-3000

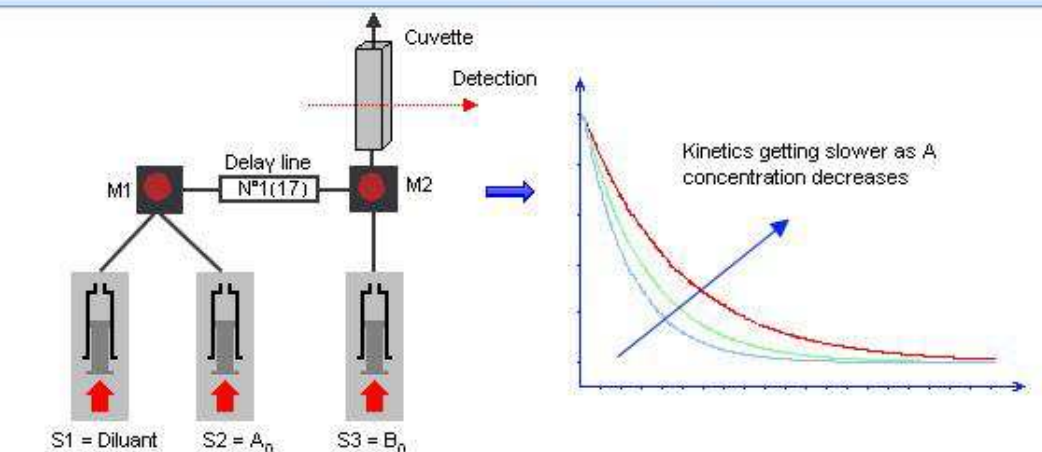
The aim of this sequence is to observe different kinetic curves by increasing automatically step by step the concentration of one reactant A against B in A + B reaction. Thus the concentration of B is maintaining constant while the concentration of A is increasing in constant steps (automatic mode) or in certain steps values (manual mode).

The first mixer M1 is used to change the concentration of reactant A by mixing reactant A with diluant while the concentration of reactant B is constant. The second mixer is used to mix solution A and solution B to start the reaction (Figure 42). The following window is obtained by clicking on the button



of the main mixing sequence window.

The aim of this mode is to follow the concentration dependency of A in the reaction $A + B$ keeping concentration B constant. It is done using 3 syringes and 2 mixers according to the following diagram:



Loading of solutions :

The first mixer M1 is used to change concentration of A, thus diluting solution is loaded in Syringe 1 and A is loaded in syringe 2. The second mixer M2 is used to start the A+B reaction, so B is loaded in syringe 3. The shortest delay line (17) must be installed between M1 and M2

Design of the sequence:

Users indicates the initial concentration A_0 and B_0 and mixing ratio to apply between (A+diluant) and B at each step in mixer M2. The same total flow rate is used for all steps so dead time remains constant.

The user builds the sequence by varying the ratio 'Diluant / A' in mixer 1 or by changing directly the final concentration of A. Once all parameters are set Biokine proposes volumes per syringe (defined according to mechanical limitations, size of cuvette, mixer...), the user has access to the volumes to customize the sequence.

Once the sequence is ready synchronization can be done with the detection part.



Figure 42: Concentration dependence setup

Loading of solutions:

Syringe 1 (S_1) is used to store the diluant solution while Syringe 2 (S_2) and Syringe 3 (S_3) are filled with reactants: respectively A and B.

The increase of the concentration of A can be achieved in two different ways: by varying the ratios between syringe 1 and syringe 2 (i.e. ratios steps) or by increasing the concentration of the reactant A (i.e. concentration steps). In both sequences, automatic mode and manual mode are available. The delay line installed between mixer M1 and Mixer M2 should be the shortest one corresponding to a dead volume of 17 μl .

Design of the sequence:

The driving sequences is created in the window shown in Figure 43, this window can be reached from



the button, in the stopped flow status area.

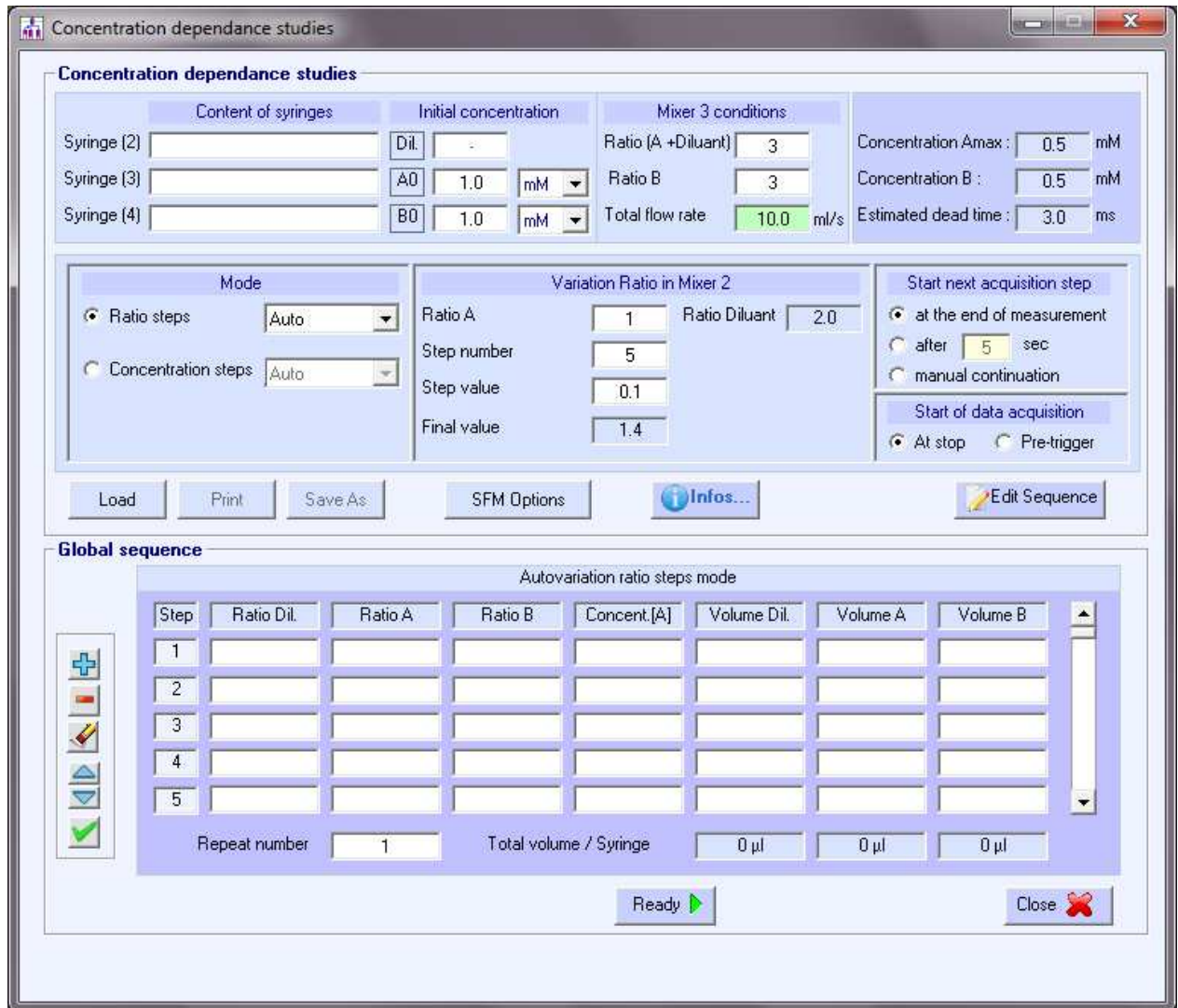


Figure 43: driving sequence for concentration dependence studies

In the sequence the user has to indicate the content of the syringes and the initial concentrations of reactant A (A_0) and reactant B (B_0).

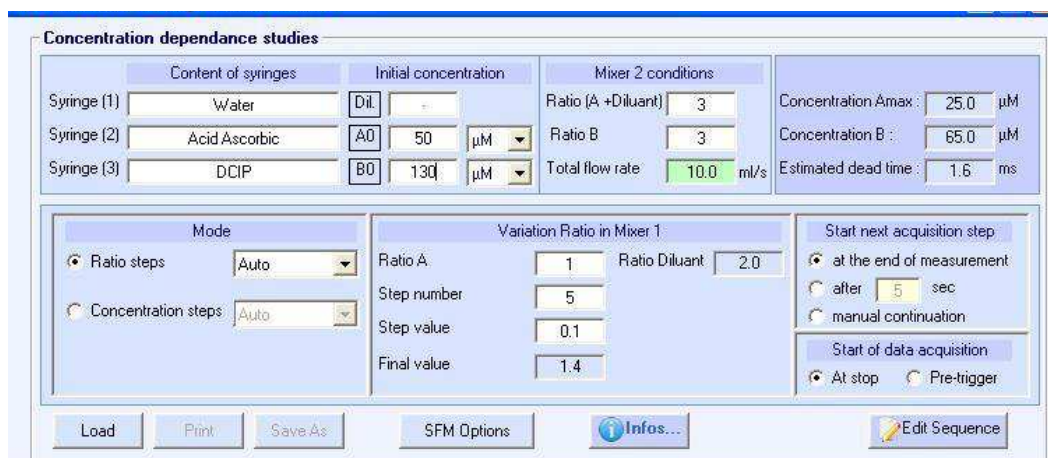


Figure 44: Mixing sequence

Then the conditions in Mixer 2 have to be entered in the following window:

Mixer 2 conditions	
Ratio (A +Diluant)	3
Ratio B	3
Total flow rate	10.0 ml/s

Figure 45: Ratio in mixer 2

These conditions correspond to a 1 to 1 mixing sequence in Mixer 2 with a flow rate of 10 ml/s. The concentration maximum of A " A_{max} " is automatically calculated while the concentration of B reactant is maintained at a constant value.

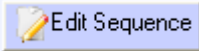
There are two ways to increase the concentration of A: the first is done by the increase of the ratio in mixer 1 step by step. The second is done by increasing the concentration of A step by step.

Ratio step Auto mode:

The variations of the ratios between Diluant and reactant A in mixer M1 have to be fixed by typing a value in Ratio A (i.e.1 in the example), choosing the steps numbers and the value of the step.

Mode		Variation Ratio in Mixer 1	
<input checked="" type="radio"/> Ratio steps	Auto	Ratio A	1
<input type="radio"/> Concentration steps	Auto	Ratio Diluant	2.0
		Step number	5
		Step value	0.1
		Final value	1.4

Figure 46: Ratio steps Mode

The sequence is edited by clicking on the button  as following:

Concentration dependence studies

Content of syringes		Initial concentration		Mixer 2 conditions			
Syringe (1)	Water	Dil.	-	Ratio (A +Diluant)	3	Concentration Amax :	25.0 μM
Syringe (2)	Acid Ascorbic	A0	50.0 μM	Ratio B	3	Concentration B :	65.0 μM
Syringe (3)	DCIP	B0	130. μM	Total flow rate	10.0 ml/s	Estimated dead time :	1.6 ms

Mode		Variation Ratio in Mixer 1		Start next acquisition step	
<input checked="" type="radio"/> Ratio steps	Auto	Ratio A	1	Ratio Diluant	2.0
<input type="radio"/> Concentration steps	Auto	Step number	5	<input type="radio"/> at the end of measurement	
		Step value	0.1	<input type="radio"/> after 5 sec	
		Final value	1.4	<input type="radio"/> manual continuation	
				Start of data acquisition	
				<input checked="" type="radio"/> At stop	<input type="radio"/> Pre-trigger

Global sequence

Autovariation ratio steps mode

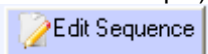
Step	Ratio Dil.	Ratio A	Ratio B	Concent. [A]	Volume Dil.	Volume A	Volume B
1	2	1	3	8.333	100	50	150
2	1.9	1.1	3	9.167	95	55	150
3	1.8	1.2	3	10	90	60	150
4	1.7	1.3	3	10.833	85	65	150
5	1.6	1.4	3	11.667	80	70	150

Repeat number: 1
Total volume / Syringe: 450 μl 300 μl 750 μl

Figure 47: Global sequence in ratio steps mode

Concentration step Auto mode:

The variations of the concentration of reactant A in mixer M1 have to be fixed by typing a value in concentration A (i.e.1 in the example), choosing the steps numbers and the value of the step. By

clicking on the button  , the following sequence is edited:

Concentration dependence studies

	Content of syringes	Initial concentration	Mixer 2 conditions	
Syringe (1)	Water	Dil. -	Ratio (A +Diluant) 3	Concentration Amax : 25.0 µM
Syringe (2)	Acid Ascorbic	A0 50.0 µM	Ratio B 3	Concentration B : 65.0 µM
Syringe (3)	DCIP	B0 130. µM	Total flow rate 10.0 ml/s	Estimated dead time : 1.6 ms

Mode	Variation Concentration in Mixer 1	Start next acquisition step
<input type="radio"/> Ratio steps Auto	Concentration A (µM) 1	<input checked="" type="radio"/> at the end of measurement
<input checked="" type="radio"/> Concentration steps Auto	Step number 5	<input type="radio"/> after 5 sec
	Step value (µM) 0.1	<input type="radio"/> manual continuation
	Final value (µM) 1.4	Start of data acquisition
		<input checked="" type="radio"/> At stop <input type="radio"/> Pre-trigger

Global sequence

Autovariation concentration steps mode

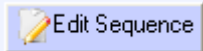
Step	Ratio Dil.	Ratio A	Ratio B	Concent.[A]	Volume Dil.	Volume A	Volume B
1	2.88	0.12	3	1	1200	50	1250
2	2.868	0.132	3	1.1	1086.4	50	1136.4
3	2.856	0.144	3	1.2	991.7	50	1041.7
4	2.844	0.156	3	1.3	911.5	50	961.5
5	2.832	0.168	3	1.4	842.9	50	892.9

Repeat number
 Total volume / Syringe

Figure 48: Global sequence in concentration steps mode

Ratio step manual mode:

By selecting Ratios steps Manual in the Mode window you have an access to the window menu then,

by clicking on the button  , the following sequence is edited or the latest sequence is automatically loaded by default.

Concentration dependance studies

Content of syringes		Initial concentration		Mixer 2 conditions			
Syringe (1)	<input type="text"/>	Dil.	<input type="text"/>	Ratio (A +Diluant)	<input type="text" value="3"/>	Concentration Amax :	<input type="text" value="0.5"/> mM
Syringe (2)	<input type="text"/>	A0	<input type="text" value="1.0"/> mM	Ratio B	<input type="text" value="3"/>	Concentration B :	<input type="text" value="0.5"/> mM
Syringe (3)	<input type="text"/>	B0	<input type="text" value="1.0"/> mM	Total flow rate	<input type="text" value="10.0"/> ml/s	Estimated dead time :	<input type="text" value="1.6"/> ms

Mode		Variation Ratio in Mixer 1			Start next acquisition step	
<input checked="" type="radio"/> Ratio steps	<input type="text" value="Manual"/>	Ratio A	<input type="text"/>	Ratio Diluant	<input type="text"/>	<input checked="" type="radio"/> at the end of measurement
<input type="radio"/> Concentration steps	<input type="text" value="Auto"/>	Step number	<input type="text"/>			<input type="radio"/> after <input type="text" value="5"/> sec
		Step value	<input type="text"/>			<input type="radio"/> manual continuation
		Final value	<input type="text"/>			

Start of data acquisition
 At stop Pre-trigger

Buttons: Load, Print, Save As, SFM Options, Infos..., Edit Sequence

Global sequence

Manual ratio mode

Step	Ratio Dil.	Ratio A	Ratio B	Concent.[A]	Volume Dil.	Volume A	Volume B
1							
2							
3							
4							
5							

Repeat number: Total volume / Syringe: µl µl µl

Buttons: Ready, Close

Figure 49: Global sequence in ratio steps manual mode

The Global sequence is created through the following table:

Click on button to create a step then type the ratio in "Ratio A" that will be used during the step and indicate the volumes. All the volumes, volume Dil., volume A and volume B have to be superior to 40µl.

- 1) Click on button to create a second step
- 2) Click on to clear all the sequence or to remove a step.

Global sequence

Manual ratio mode







Step	Ratio Dil.	Ratio A	Ratio B	Concent.[A]	Volume Dil.	Volume A	Volume B
1	2	1	3	8.333	100	50	150

Repeat number: Total volume / Syringe: µl µl µl

Buttons: Ready, Close

Figure 50: Global sequence window

The functions of the different buttons are the following:


-  is used to create or add a step
-  is used to remove a step
-  is used to remove all the sequence
-  is used to move up or down a step
-  is used to move up or down a step
-  is used to validate the sequence

Notice that a stored sequence can be automatically loaded by clicking on  button.

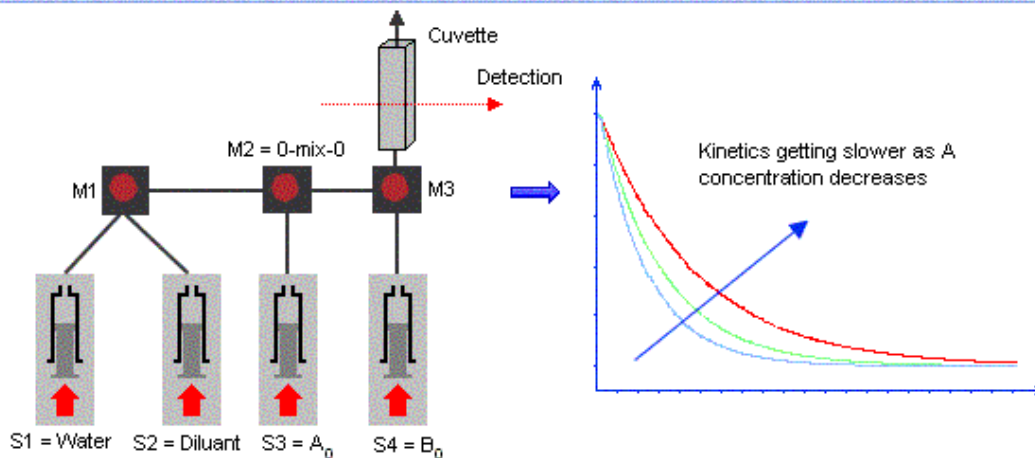
2.9.2. Creating driving sequence using SFM-4000

The aim of this sequence is to observe different kinetic curves by increasing automatically step by step the concentration of one reactant A against B in A + B reaction. Thus the concentration of B is maintaining constant while the concentration of A is increasing at constant steps (automatic mode) or at certain steps values (manual mode).

The second mixer M2 is used to change the concentration of reactant A by mixing reactant A with diluant while the concentration of reactant B is constant. The third mixer M3 is used to start the

reaction (Figure 51). The following window is obtained by clicking on the button  of the main mixing sequence window.

The aim of this mode is to follow the concentration dependency of A in the reaction A + B keeping concentration B constant. It is done using 3 syringes and 2 mixers according to the following diagram (the first syringe is not used in this configuration but must be filled with water or buffer to preserve the system from air bubbles):



Loading of solutions :

The mixer M2 is used to change concentration of A, thus diluting solution is loaded in Syringe 2 and A is loaded in syringe 3. The mixer M3 is used to start the A+B reaction, so B is loaded in syringe 4. The 0-Mix-0 mixing block (M2) must be installed between M1 and M3 to minimize sample consumption

Design of the sequence:

Users indicates the initial concentration A₀ and B₀ and mixing ratio to apply between (A+diluant) and B at each step in mixer M3. The same total flow rate is used for all steps so dead time remains constant.

The user builds the sequence by varying the ratio 'Diluant / A' in mixer 2 or by changing directly the final concentration of A. Once all parameters are set Biokine proposes volumes per syringe (defined according to mechanical limitations, size of cuvette, mixer...), the user has access to the volumes to customize the sequence.

Once the sequence is ready synchronization can be done with the detection part.



Figure 51: Automatic concentration dependence studies

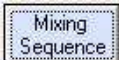
Loading solutions:

Syringe 1 (S₁) is used to store water, Syringe 2 (S₂) to store the diluant solution while Syringe 3 (S₃) and Syringe 4 (S₄) are filled with reactants respectively A and B. The reaction between reactants A and B starts in the third mixer M3.

Increasing the concentration of A can be achieved in two different ways: by varying the ratios in Mixer 2 between syringe 2 and syringe 3 (i.e. ratios steps) or by increasing the concentration of the reactant A (i.e. concentration steps). When choosing the Ratio steps or Concentration steps, an automatic and manual mode is available. The delay line installed between mixer M2 and Mixer M3 should be the shortest one, corresponding to a dead volume of 17 µl.

Design of the sequence:

The driving sequence is created in the window shown in Figure 52. This window can be reached from

the  button in the stopped-flow status area.

Concentration dependance studies

Content of syringes	Initial concentration	Mixer 3 conditions	
Syringe (2) Diluant	Dil. -	Ratio (A +Diluant) 3	Concentration Amax : 25.0 µM
Syringe (3) Ascorbic Acid	A0 50 µM	Ratio B 3	Concentration B : 65.0 µM
Syringe (4) DCIP	B0 130 µM	Total flow rate 10.0 ml/s	Estimated dead time : 1.6 ms

Mode

- Ratio steps (Auto)
- Concentration steps (Auto)

Variation Ratio in Mixer 2

Ratio A: 1, Ratio Diluant: 2.0
 Step number: 5
 Step value: 0.1
 Final value: 1.4

Start next acquisition step

- at the end of measurement
- after 5 sec
- manual continuation

Start of data acquisition

- At stop
- Pre-trigger

Buttons: Load, Print, Save As, SFM Options, Infos..., Edit Sequence

Global sequence

Autovariation ratio steps mode

Step	Ratio Dil.	Ratio A	Ratio B	Concent. [A]	Volume Dil.	Volume A	Volume B
1	2	1	3	8.333	100	50	150
2	1.9	1.1	3	9.167	95	55	150
3	1.8	1.2	3	10	90	60	150
4	1.7	1.3	3	10.833	85	65	150
5	1.6	1.4	3	11.667	80	70	150

Repeat number: 1
 Total volume / Syringe: 450 µl, 300 µl, 750 µl

Buttons: Ready, Close

Figure 52: driving sequence

In the sequence the user has to indicate the content of the syringes and the initial concentrations of reactant A (A₀) and reactant B (B₀) (see Figure 53).

Concentration dependence studies

Content of syringes		Initial concentration		Mixer 3 conditions			
Syringe (2)	Diluant	Dil.	-	Ratio (A +Diluant)	3	Concentration Amax :	25.0 μM
Syringe (3)	Ascorbic Acid	A0	50 μM	Ratio B	3	Concentration B :	65.0 μM
Syringe (4)	DCIP	B0	130 μM	Total flow rate	10.0 ml/s	Estimated dead time :	1.6 ms

Mode		Variation Ratio in Mixer 2			Start next acquisition step	
<input checked="" type="radio"/> Ratio steps	Auto	Ratio A	1	Ratio Diluant	2.0	<input checked="" type="radio"/> at the end of measurement
<input type="radio"/> Concentration steps	Auto	Step number	5			<input type="radio"/> after 5 sec
		Step value	0.1			<input type="radio"/> manual continuation
		Final value	1.4			
					Start of data acquisition	
					<input checked="" type="radio"/> At stop <input type="radio"/> Pre-trigger	

Load Print Save As SFM Options Infos... Edit Sequence

Figure 53: mixing sequence

Then the conditions in Mixer 3 have to be entering in the following window:

Mixer 3 conditions

Ratio (A +Diluant)	3
Ratio B	3
Total flow rate	10.0 ml/s

Figure 54: Ratio in mixer 3

These conditions correspond to a 1 to 1 mixing sequence in Mixer 3 with a flow rate of 10 ml/s. The concentration maximum of A " A_{max} " is automatically calculated while the concentration of B reactant is maintained as a constant value.

There are two ways to increase the concentration of A: the first is done by increasing the ratio in mixer 2 step by step. The second is done by increasing the concentration of A step by step.

Acquisition parameters:

The parameters of the acquisition have to be entered in the following window (Figure 55).

Start next acquisition step: In case of a repetition of the sequence, starting the next acquisition step can be done at the end of the measurement or after a defined time.

Start of data acquisition: as in the stopped flow advanced mode, starting the acquisition can be done at the stop of the motors or 20ms milliseconds before the stop using the pre-trigger option (this time cannot be changed)

Start next acquisition step

at the end of measurement

after 5 sec

manual continuation

Start of data acquisition

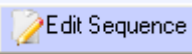
At stop Pre-trigger

Figure 55: acquisition parameters

Ratio step Auto mode:

The variations of the ratios between Diluant and reactant A in mixer M2 have to be fixed by typing a value in Ratio A (i.e.1 in the example of Figure 56), by choosing the steps numbers and the value of the step.

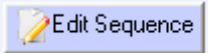
Figure 56: Ratio steps Mode

The sequence is edited by clicking on the button  as following:

Step	Ratio Dil.	Ratio A	Ratio B	Concent. [A]	Volume Dil.	Volume A	Volume B
1	2	1	3	8.333	100	50	150
2	1.9	1.1	3	9.167	95	55	150
3	1.8	1.2	3	10	90	60	150
4	1.7	1.3	3	10.833	85	65	150
5	1.6	1.4	3	11.667	80	70	150

Figure 57: Global sequence

Concentration step Auto mode:

The variations of the concentration of reactant A in mixer M1 have to be fixed by typing a value in concentration A (i.e.1 in the example), choosing the steps numbers and the value of the step. By clicking on the button , the following sequence is edited:

Concentration dependence studies

Content of syringes		Initial concentration		Mixer 2 conditions			
Syringe (1)	Water	Dil.	-	Ratio (A +Diluant)	3	Concentration Amax :	25.0 μM
Syringe (2)	Acid Ascorbic	A0	50.0 μM	Ratio B	3	Concentration B :	65.0 μM
Syringe (3)	DCIP	B0	130. μM	Total flow rate	10.0 ml/s	Estimated dead time :	1.6 ms

Mode		Variation Concentration in Mixer 1			Start next acquisition step	
<input type="radio"/> Ratio steps	Auto	Concentration A (μM)	1	<input checked="" type="radio"/> at the end of measurement		
<input checked="" type="radio"/> Concentration steps	Auto	Step number	5	<input type="radio"/> after 5 sec		
		Step value (μM)	0.1	<input type="radio"/> manual continuation		
		Final value (μM)	1.4	Start of data acquisition		
				<input checked="" type="radio"/> At stop	<input type="radio"/> Pre-trigger	

Global sequence

Autovariation concentration steps mode

Step	Ratio Dil.	Ratio A	Ratio B	Concent. [A]	Volume Dil.	Volume A	Volume B
1	2.88	0.12	3	1	1200	50	1250
2	2.868	0.132	3	1.1	1086.4	50	1136.4
3	2.856	0.144	3	1.2	991.7	50	1041.7
4	2.844	0.156	3	1.3	911.5	50	961.5
5	2.832	0.168	3	1.4	842.9	50	892.9

Repeat number: 1 Total volume / Syringe: 5032.4 μl 250 μl 5282.4 μl

Figure 58: Global sequence in concentration steps mode

Ratio step manual mode:

With this mode, the user has the possibility to create his own sequence in the global sequence window.

Click on the button .

A sequence is automatically loaded by default (Figure 59). The user has then the possibility to change different parameters.

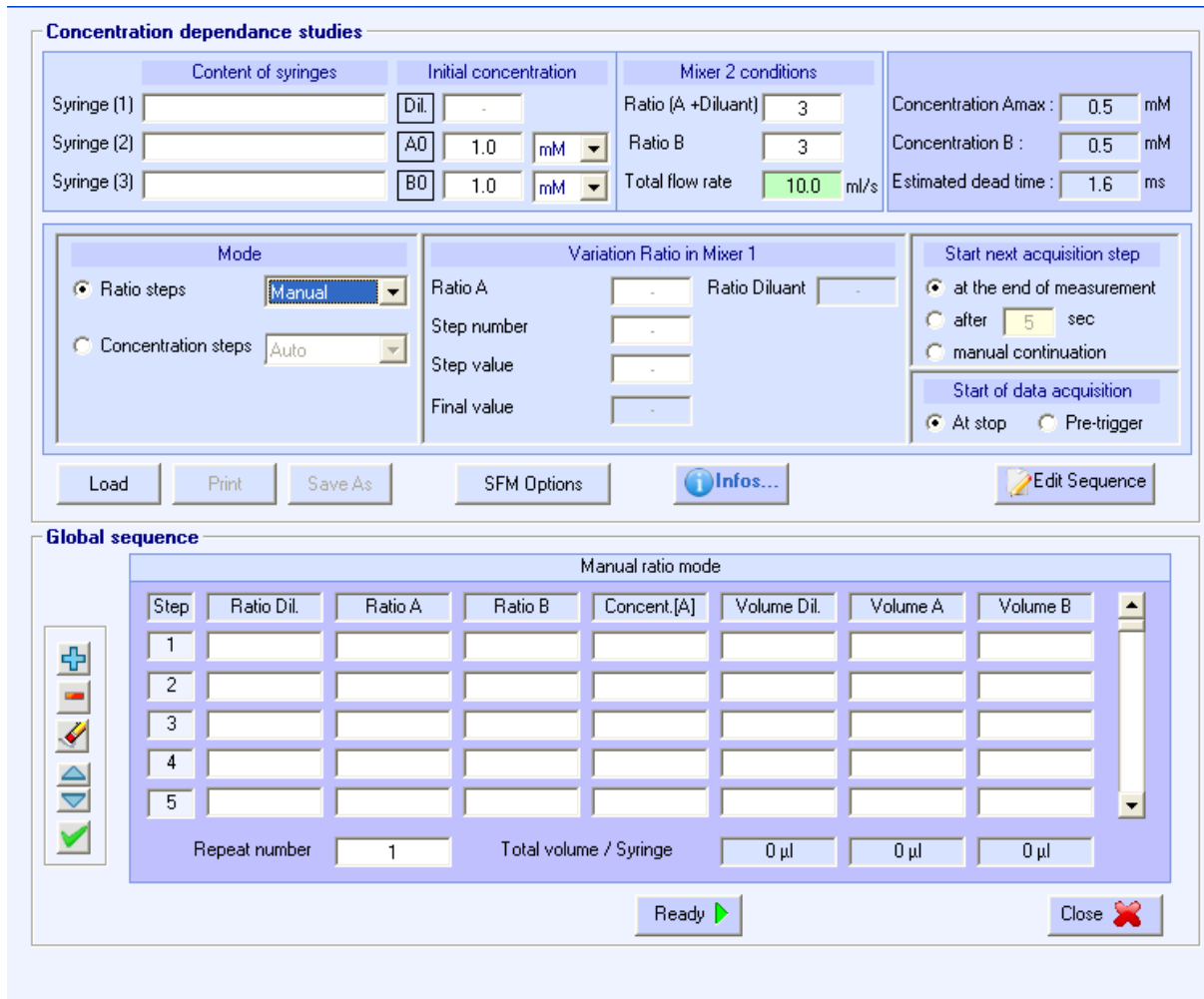


Figure 59: Global sequence in ratio steps manual mode

The Global sequence is created through the following table:

Click on button to create a step then type the ratio in “Ratio A” that will be used during the step and indicate the volumes. All the volumes, volume Dil., volume A and volume B have to be superior to 40µl.

- 3) Click on button to create a second step
- 4) Click on to clear all the sequence or to remove a step
- 5) Repeat number: is used to repeat each step.

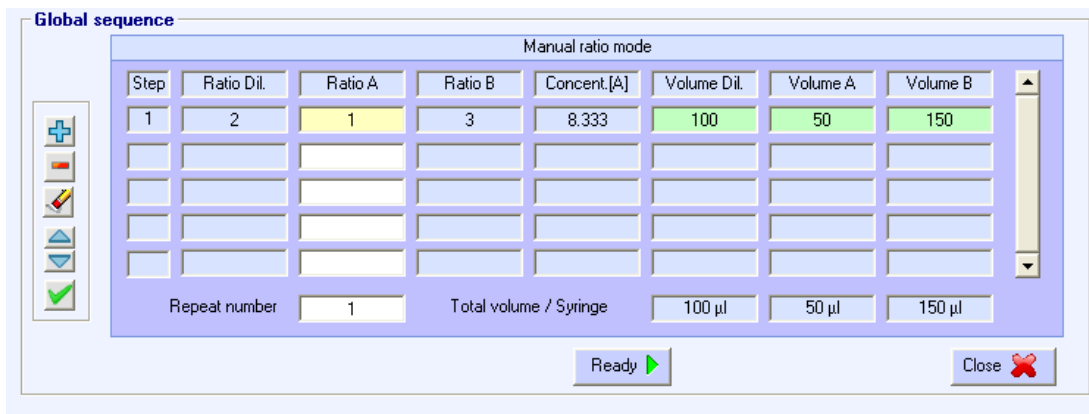


Figure 60: Global sequence

The functions of the different buttons are the following:



is used to create or add a step



is used to remove a step




is used to remove all the sequence



is used to move up or down a step





is used to validate the sequence


Notice that the latest sequence is automatically load, in this case the manual mode permits the user to change the parameters or by clicking on  to remove all the sequence.

2.10. Running a sequence

Once a driving sequence has been entered or loaded, it is transferred to the MPS by pushing the **Single** or **Multiple** button (in classic mode) or **Ready** (in advanced mode).

The MPS is now in automatic mode and the **shot control** window will be displayed in the stopped-flow status are (as shown in Figure 61).

The **Shot control** window shows the number of shots possible based the current volumes in the SFM syringes. It also indicates whether the SFM is running a driving sequence or ready for the next shot. A driving sequence is executed by pushing the  button or the **start-stop** button on the front panel of the MPS. The  button can be used to stop an experiment prematurely if necessary.

If the **Single** button was used to transfer the driving sequence to the MPS, only a single shot can be made. The  button must then be pushed to return to the driving sequence and the **Single** button must be pushed again to re-transfer the driving sequence to the MPS for a subsequent shot.



If the **Multiple** button was used to transfer the driving sequence to the MPS, the  button can be used to execute shots until the **shot** window shows that 0 shots remain. The  button is then pushed to return to the driving sequence.



Figure 61 : Shot control window

3. ANALYSIS WINDOWS

3.1. Introduction

The analysis windows are used to load recorded files. They provide a large variety of tools to perform mathematical operations on these files like: addition, subtraction, averaging, smoothing, rescaling, multi-exponential or second order filter...

The loading of spectra was explained in section 1.3. Once loaded, an analysis window as shown in Figure 62 should appear:

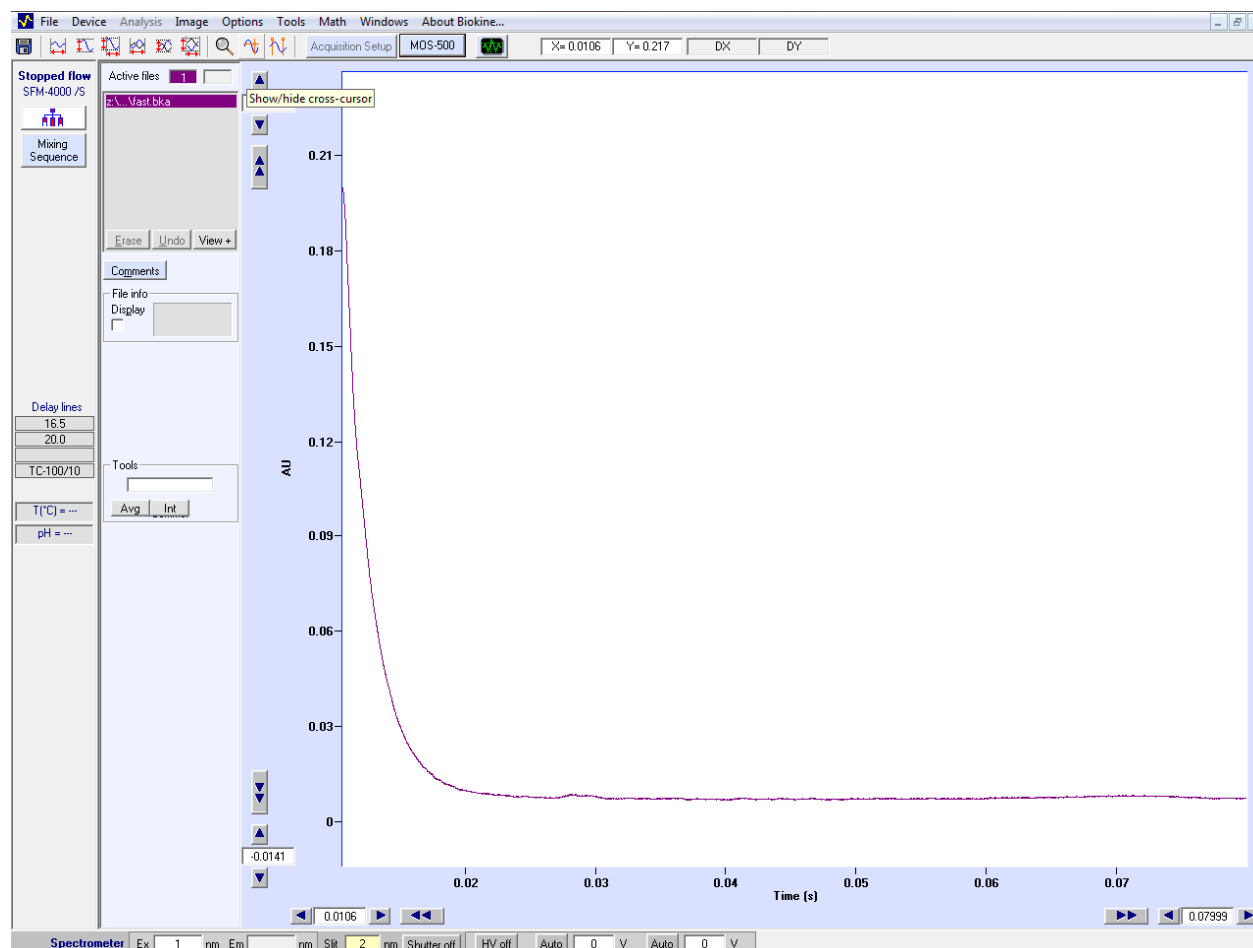


Figure 62 : Example of analysis window

To unload an analysis window, choose **Windows, Close**.

3.2. File selector window

One of the files displayed on the active window is the active file. The default active file is the last that has been loaded. To increase friendliness of software the color of the trace corresponding to the active file is indicated (see Figure 63).

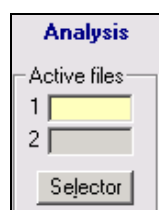


Figure 63 : Active file

To change the active file, use the up and down arrows. Selection can also be made by using the **Selector** button in the main window. The window shown in Figure 64 is displayed. Click again on the name of the file to be selected, or use the up and down arrow keys to change the selection. This will invert the file name fore color and back color. The name of the selected file is displayed into the left state panel at the top of the screen. For mathematical operations involving two files a second file has to be selected. To do this click on the name of the second file with the **SHIFT** key pressed at the same time, the name of the second file will appear into the right state panel at the top of the screen. Click on the **Close** button or again on the **Selector** button to close the file selection window.

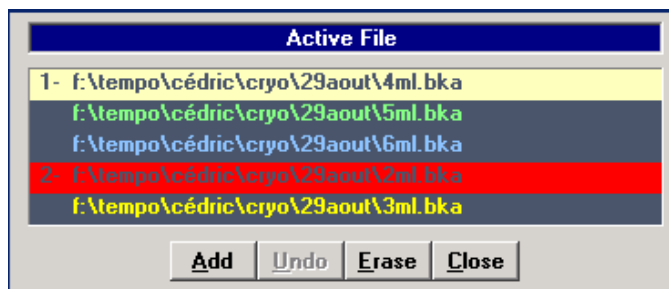


Figure 64 : File selector window

While working with the file selection window open, it is possible to erase a file by clicking on the **Erase** button. In case of mistake it is possible to restore the deleted file by clicking on the **Undo** button to cancel the operation. It is also possible to erase a file pressing the **SUPPR** key.

It is also possible to add a new file on the selection window: click on the **Add** button and choose a file or choose **File, Add** in the main menu. The new file will be added on the Analysis window only if the X units are compatible and within the limitation of a total of 10 files on the same window.

3.3. File comments

This permits to read, edit or add comments attached to the active file. Click on the **Comments** button to load the comments window (see Figure 65). To validate changes, click on the **Valid** button or change the selected file. If the current file has been acquired a Bio-Logic stopped-flow instrument, the driving parameters of the stopped-flow have been saved at the same time as the file. These parameters can be read by clicking on the **SFM info** button in the comment windows.

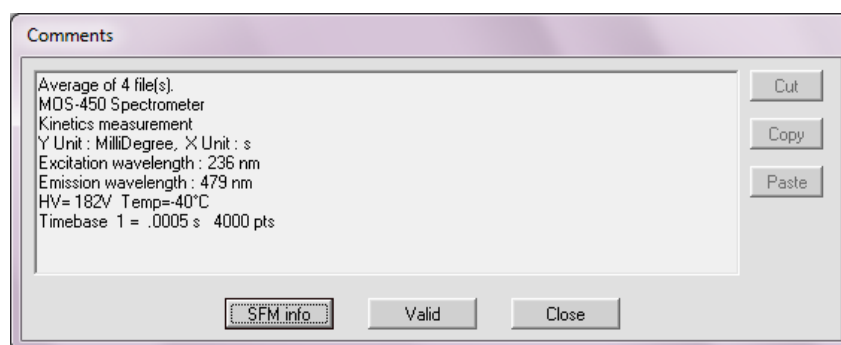


Figure 65: comments window

3.4. Print with Bio-Kine

In the main menu choose **File, Print Graph**, the window shown in Figure 66 will appear. The option **Graph and comments** prints graph and comments on the same page; the graph and its respective comments are printed in the same color (for color printers). If **Graph only** is selected on the print window, only the graph is printed.

According to the printer used it is possible to print in color or not. Its configuration can be checked by choosing **File, Printer Setup** in the main menu.

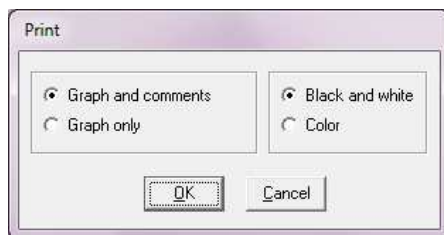


Figure 66 : Print window

3.5. File information

By checking the **display** file information box (under the comments button: see Figure 62 & 60), it is possible to display the size in bytes (if selected trace corresponds to a file on disk) and the number of data points of the selected file. At the same time a scroll bar is displayed under the window for an easy visualization of the fraction of the file displayed in the graph area. (This scroll bar may not be displayed in case the windows size is insufficient).

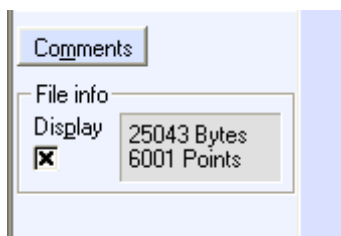


Figure 67: file information

3.6. Temporary files

Temporary files are files that Bio-Kine may create automatically (for example during large files acquisition or for new files created after mathematical operations). Choose **File, Tmp File Setup** to display the temporary file setup window shown in Figure 68. The temporary files directory is defined in the AUTOEXEC.BAT file by the command: SET TEMP=C:\WINDOWS\TEMP, and may be used by other software. Temporary files are named as follows: XXnnnn.TMP, where nnnn is replaced automatically by a temporary file number of 4 digits (nnnn) and the XX by two letters chosen by the user. Check/Uncheck **Clear on Exit** to enable clearing of the temporary files at Bio-Kine exit (at that time a message will be displayed to confirm that user wants to clear temporary files).



Figure 68 : Temporary files setup window

3.7. Scale modifications

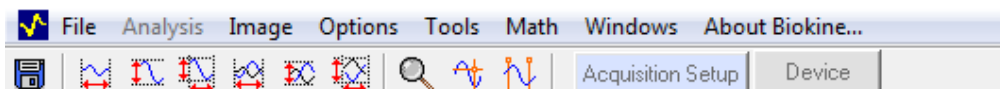
3.7.1. Manual changes

The X and Y scale limits can be entered directly in the text boxes visible at each of the axis limits.

When the window is maximized with the file information checked a green scroll bar is activated at the bottom of the graph area. This scroll bar permits an easy visualization of the fraction of the file that is displayed. Clicking on this scroll bar permits a shift along the X axis. Similar shifts are obtained using **single arrow** and **double arrows** buttons along both X and Y axis.

3.7.2. Autoscaling

Different autoscalings are available from the main menu tool bar using buttons.



Their actions are shown in the following table:

Main menu tool bar	button	action
<u>A</u> utoScale, X		autoscale X on the active file
<u>A</u> utoScale, Y		autoscale Y on the active file
<u>A</u> utoScale, XY		autoscale X-Y on the active file
<u>A</u> utoScale, All X		autoscale X on all files
<u>A</u> utoScale, All Y		autoscale Y on all files
<u>A</u> utoScale, all XY		autoscale X-Y on all files

3.7.3. Unit changes

Units can be changed on axis respectively x and y: choose **O**ption, X axis or **O**ption, Y axis in the main menu and then the desired unit (e.g. to change time in rapid kinetics mode “s” into “ms” or in scanning mode “nm” into “cm⁻¹” select **V**iew, **X** Axis, ms or cm⁻¹).

3.7.4. Logarithmic scale:

It is possible to change from linear to logarithm scale (and the reverse) with **O**ption, X Axis (or **O**ption, Y Axis), **L**inear/**L**og menu. The program will issue warnings in case negative values are present on the scale that will be turned to a logarithmic representation.

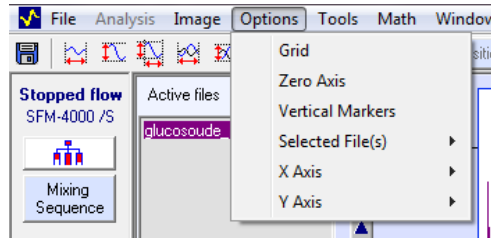
3.7.5. Zoom effects

To activate the zoom function, click on the zoom button . Then, move the mouse over the area that is going to be zoomed. Release the left button: the selected area is then loaded. Click again on the zoom button to inactivate this function.

3.8. Other graphical options and tools

3.8.1. Other Graphical options

Some additional graphical commands can be found in the **O**ptions menu from the Analyze main menu.



Options, Grid: shows or hide a grid which can help for visual interpretation.

Options, Zero axis: shows or hide the $x = 0$ and $y = 0$ lines.

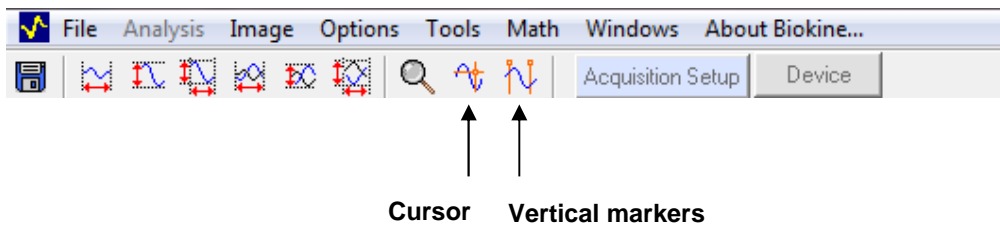
Options, selected file: By default all traces are displayed in the same style (normal style). It is possible to change the mode of display of the selected trace line style (**point** or **bold**) and the **Color**.


Option, X axis: Units can be changed on x axis.

Option, Y axis: Units can be changed on y axis.


3.8.2. Other Graphical Tools

Some additional graphical command can be activate by clicking on the buttons in main menu tool bar.



Cursor: The cursor mode can be selected by clicking on the  button. This shows a cursor on the current trace. This cursor can be moved over the data points of the active trace with the **left arrow** key and the **right arrow** key. The coordinates of the corresponding data point are displayed into the upper state panels (colored in gray) in the right corner of the main analyze window.

Measurements of ΔX and ΔY are easy in the cursor mode : click on the left button of the mouse to create a reference point, move the mouse and read the coordinates difference (between the reference point and the position of the mouse) ΔX and ΔY in the yellow state panels in the right bottom corner of the window.

Vertical markers: clicking on the following button , three vertical markers are displayed on the current graph. Select a marker by clicking on its text box (displayed in red). Moving a vertical marker is performed directly with mouse click and drag, alternatively it is made by entering the desired position from the keyboard inside the marker's text box, validate with the **ENTER** key. Click on the text box again to hide the vertical marker selected.

All vertical markers may not be necessary; they can be disabled by pushing them to the left or right origin of the graph area.

To hide the vertical markers, click again on the Vertical marker button.

3.9. Saving files Bio-Kine

Choose **File, Save** to save the current trace without changing its file name. If the current trace has not been saved before, the menu **File, Save As...** will be issued instead of **File, Save**.

Choose **File, Save As...** to save the current trace with a new file name. The window shown in Figure 69 will be displayed.

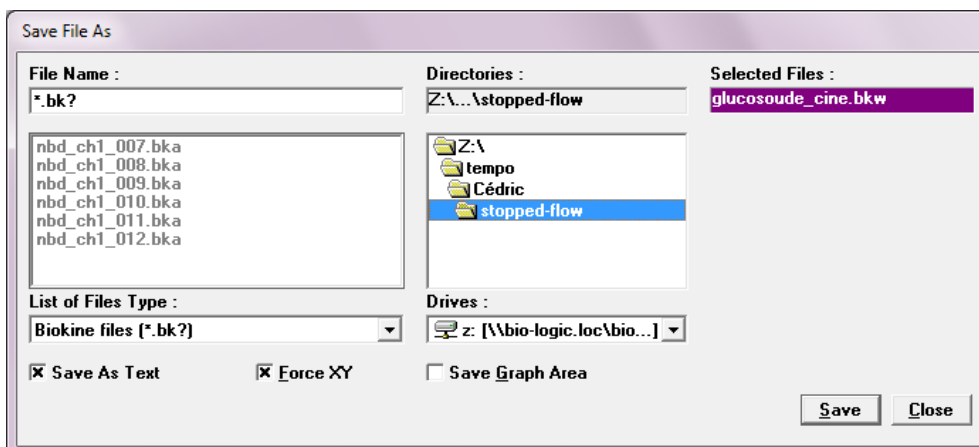



Figure 69 : 'Save file as' window

The standard files format is an ASCII format .bka but it is also possible to save files as binary files(.bkw) by checking/unchecking the **Save As Text** option (see Figure 69).

If the **Force XY** option is checked the format will be as two columns, the first for X coordinate, the second for the Y values.

It is also possible to save only the graph area by checking the **Save Graph Area** option in the **File, Save As...** window.

If several traces are displayed on the screen, it is possible to record all of them in the same files, it is just necessary to select several files using the CTRL or SHIFT key.

There is also an automatic saving function to save all the shots separately. To do this select **File, Save + Setup**, the window shown in will be displayed. The following syntax is used : <save+path>\ccccinnn.bkw, where cccc = 4 free characters, i = channel number, nnn = current ('save +') number (incremented at each 'save +' operation). Choose your parameters and then click on the OK button. Then, select **File, Save ±** or click on the  button to proceed the operation.

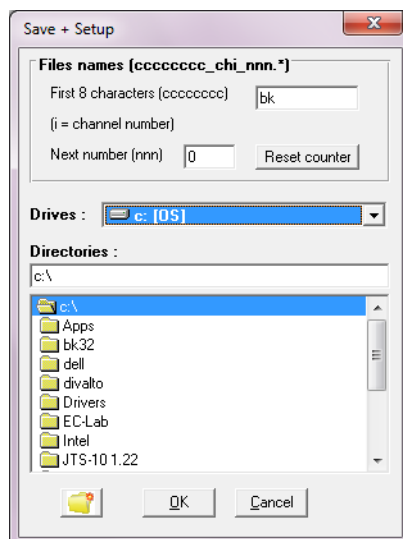


Figure 70 : Automatic renaming function

3.10. Import data

It is possible to import data from the clipboard or from an ASCII file under the condition that these data are organized into a single or a double column of data. Import is performed with the following commands

File, Import from, Text File... loads the Import From Text File windows (see Figure 71). For single Y files, First X and X Step values must be given. For X-Y files, the column separator must be selected

within {TAB, SPACE, “.”, “;”, “,”} or entered on the keyboard. For both types of files, Unit X and Y must be selected.

The imported file will be loaded in an Analysis window. The nature of the windows used will be dependent on the X unit (Analyse Kinetics window for seconds and Analyze spectra for nanometers). Each file selected is displayed into the Data grid, according to current parameters (Y or X-Y choice and columns separator). Because text files may have headers and end comments, it is possible to set markers to cut the beginning and/or the end of the file.

Clicking on the grid with the LEFT mouse button will cut data from the file beginning until the mouse current position, and clicking with the RIGHT mouse button will cut data from the current position to the end of file. In the example presented in figure 14, XYPOL.BKA header was cut down to the 6th line. To cancel the selection click on the grid zero line or past the last line, with the LEFT (or RIGHT) mouse button(s).

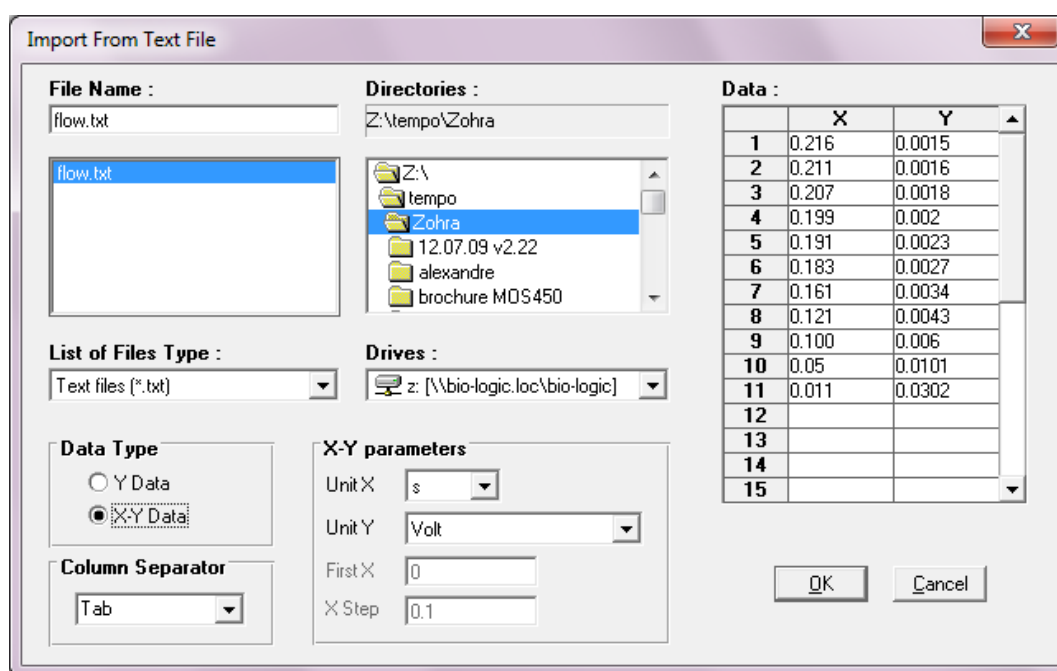


Figure 71 : Import data from text file

File, Import from, Clipboard... loads a similar window than the **File, Import from, Text File...** menu (see Figure 71), without file selection facilities. To transfer a set of X-Y data separated by the TAB character via the clipboard, use for example a spreadsheet, enter the two columns, select them and chose the copy command of this software.

Note : the imported trace will replace all traces if the import is made from an existing window.

File, Import from, Kinspec... It is possible to import files created by the KINSPEC Diode Array spectrometer. KINSPEC files (*.TRB) are stored as a two dimension matrix (see Figure 72). Wavelength scales are always linear. Time scales can be multiple (up to 4 successive time scales).

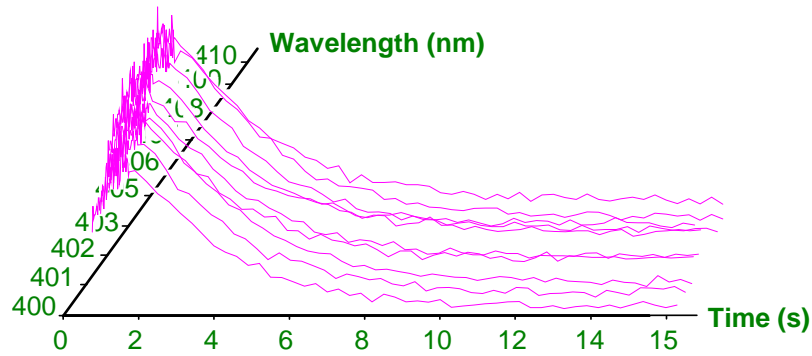


Figure 72 : Three dimension Kinspec file

The visualization of KINSPEC files by Bio-Kine can be only made partially, because Bio-Kine cannot accept directly a whole matrix. Bio-Kine can display either time or wavelength slices out of a two dimension Kinspec file.

Time slice: data are given as a function of the time and averaged over a wavelength window (see Figure 73).

Wavelength slice: data are given as a function of the wavelength and averaged over a time window (see Figure 72 and Figure 73).

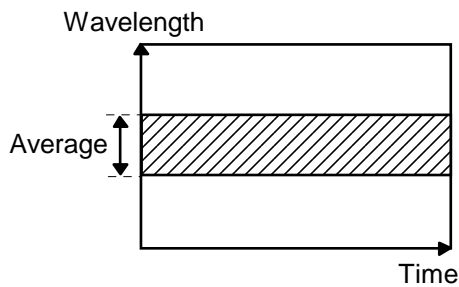


Figure 73 : time slice

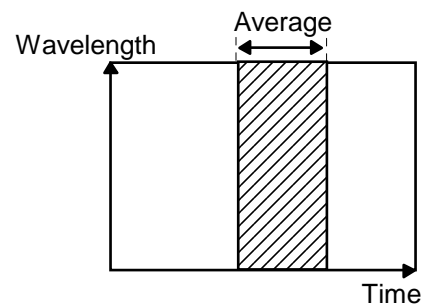


Figure 74 : wavelength slice

To import a file from KINSPEC, select **File, Import from, Kinspec...** into Bio-Kine. This will load the window shown in Figure 75. KINSPEC files informations are displayed on the left panel as files are selected. Once the slice parameters are set and the data import window selected, the import will be process by **OK** button click.

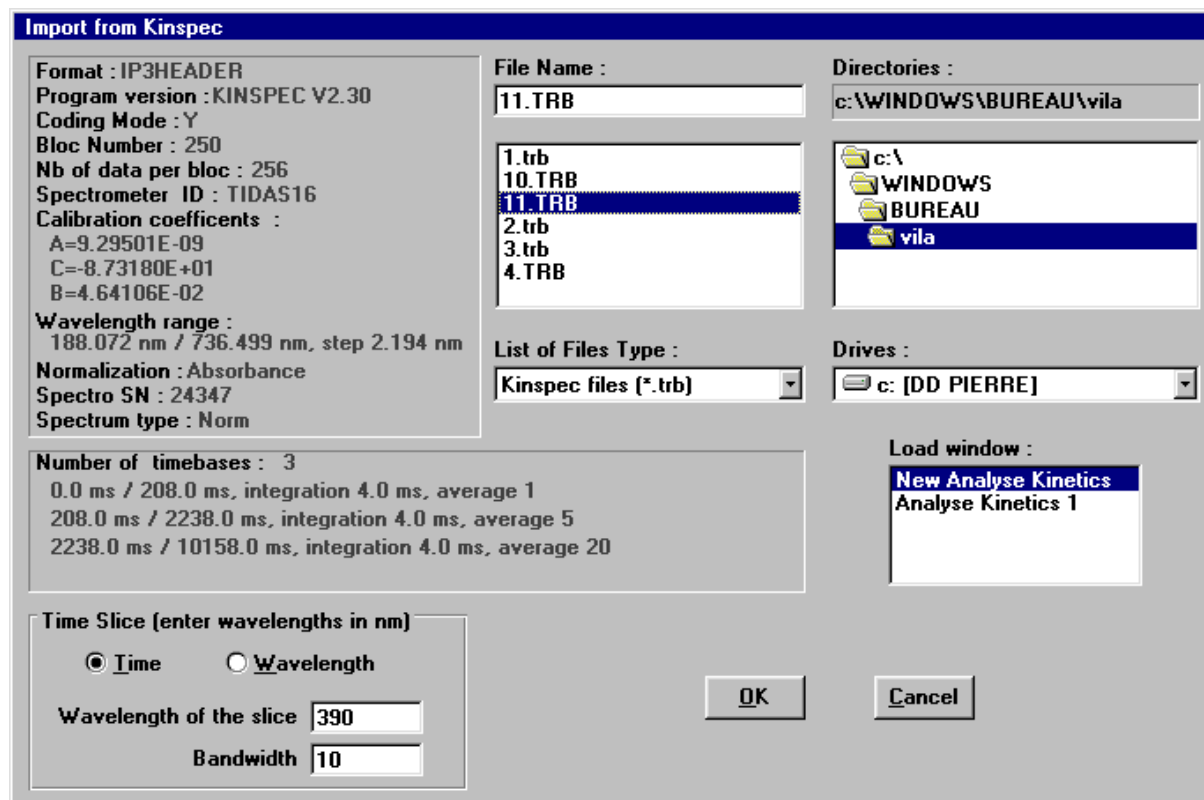


Figure 75 : Import data from Kinspec

3.11. Image copy

The menu **Image, Copy** will copy the graph into the clipboard. Then the image may be pasted (with **Edit, Paste** command generally) into several software. The Bio-Kine graphs can be directly copied into a *.wmf metafile using the command **Image, Save As Metafile...**. *.wmf format metafile can then be opened by other software.

3.12. Mathematical operations

So as to perform data analysis some mathematical operations in the **Math** menu can be used:

3.12.1. $a.(File1)+b$

Selecting **Math, a.(File1)+b** will load the window shown in Figure 76, that gives access to the **a** and **b** parameters. Once the **OK** button has been pressed a new trace will be added into the graph, the name of the new trace can be changed using the save option.



Figure 76

3.12.2. $a.(File1)+b.(File2)$

Two files must be selected to use this function (see section 3.2). Selecting **Math, a.(File1)+b.(File2)** will load the window shown in Figure 77, that gives access to the **a** and **b** parameters. Once the **OK** button has been pressed a new trace will be added into the graph, the name of the new trace can be changed using the save option.

Note : if the files have different X value the operation is canceled.

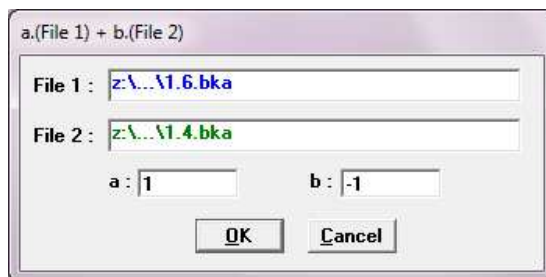


Figure 77

3.12.3. a.(File1)/(File2)

Two files must be selected to use this function (see section 3.2). Selecting **Math, a.(File1)/(File2)** will load the window shown in Figure 78, that gives access to the a parameter. Once the **OK** button pressed a new trace will be added into the graph, the name of the new trace can be changed using the save option.

Note : if the files have no common X value the operation is canceled.

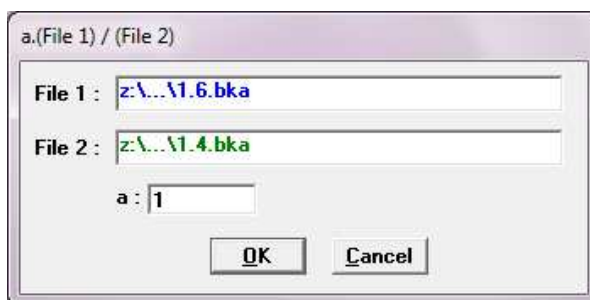


Figure 78

3.12.4. Average of files on the screen

Selecting **Math, Average** will add the average trace of the files previously displayed on the screen into the graph area, this function do not need any previous file selection.

3.12.5. Linear sampling

Select **Math, Linear Sampling** (Figure 79) and enter a **Reduction Factor** to reduce the number of points of a given trace. For example if the reduction factor is set to 10, then one single point on the reduced trace will result of the average of 10 points of the original trace. The reduced trace will have about ten time less points. Both the original trace and the reduced trace are displayed after the operation.

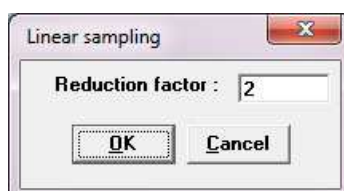


Figure 79 : Linear sampling window

3.12.6. Log sampling

Logarithm sampling: this feature of Bio-Kine permits the modification of a Kinetic trace acquired with a linear or a multiple sampling rate into a trace with a logarithm sampling. This method is very useful to reduce the number of data point of a Kinetic record. Below (see Figure 80) is shown a stopped-flow trace with an exponential Kinetics following the stop. This trace needs at least 500 data points to be correctly described over two orders of magnitude of time if a linear sampling rate is used. Only 21 points are necessary with a logarithm sampling (see Figure 81).

To apply this method, it is essential to enter an absolute time reference for the Kinetics to be studied. Before the conversion, the program will therefore ask to enter: i) the time of the stop. This time will become the new origin of the data and ii) the dead time. This will become the absolute time of the first data point.

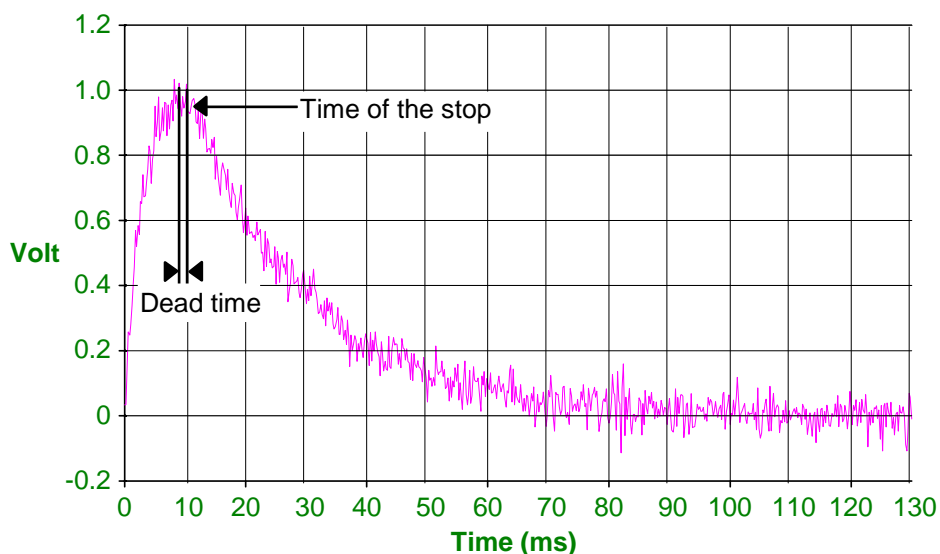


Figure 80 : Original trace (relative time, linear scale)

To perform the modification, select the trace to sample and chose **Math, Log Sampling...** (see Figure 82), enter the time of the stop, the dead time, the number of points per time decade and validate. The new data points are calculated by averaging the original data points to give the desired number of points per time decade. This has the benefit of reducing the noise as a function of time. This is equivalent of a low-pass filter whose cut-off frequency is decreasing as a function of time.

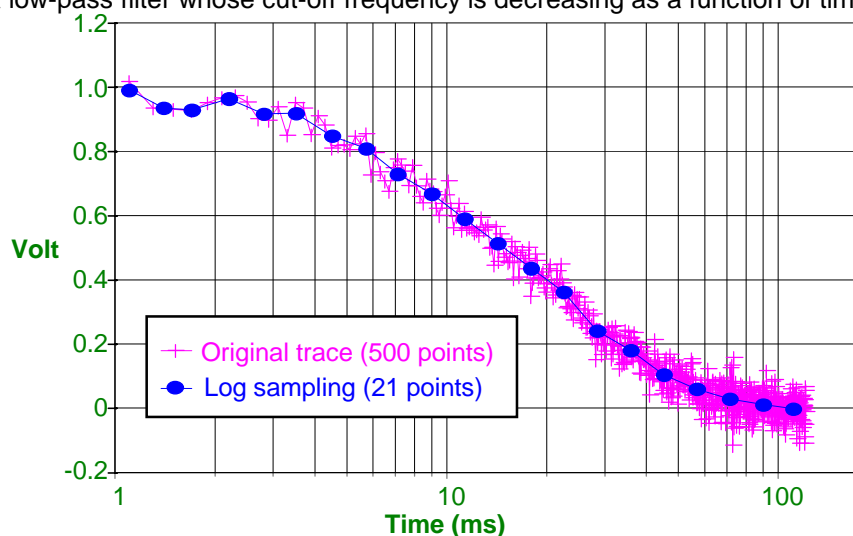


Figure 81 : Example of Log Sampling (Absolute time, logarithm scale)

From the previous example the following parameters were used:

Time of the stop = 10 ms, Dead Time = 1 ms and number of points per decade = 10. (original trace time origin was changed for log sampling trace comparison).

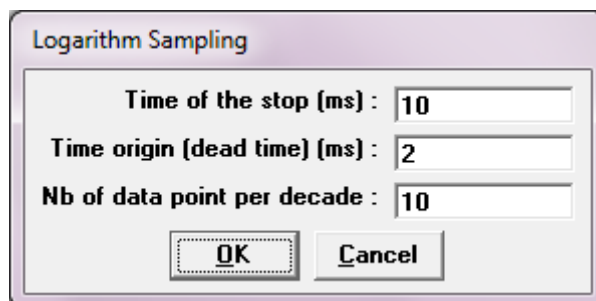


Figure 82 : Log Sampling window

3.12.7. Change of the graph time origin

Select **Math, Change Time Origin** (see Figure 83) and enter the new time origin into the appropriate field. The changes will affect all the files that are loaded into current Analysis window.

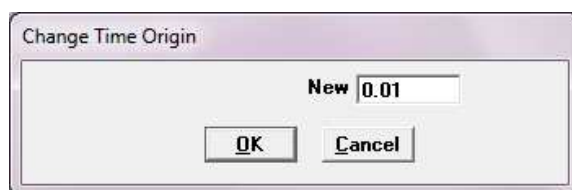


Figure 83 : Change time origin window

3.12.8. Translate trace

First select the cursor mode (see section 3.8) and then select **Math, Translate trace**. The following window will appear:

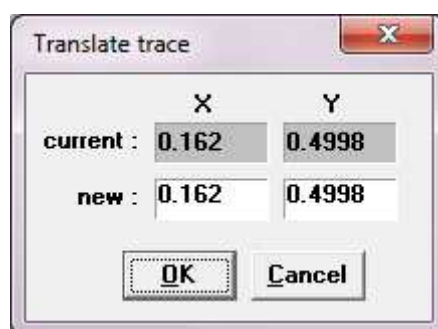


Figure 84 : Translate trace window

Choose either a X or a Y translation by filling the appropriate box, click on the **OK** button and the new trace will appear on the screen.

3.12.9. Smoothing

Smoothing using average method

Choose **Tools, Smoothing** to load the Smoothing window (Figure 85). This window allows you to enter the number of data points used for smoothing, to run and cancel the last operation with the

Proceed/Undo commands.

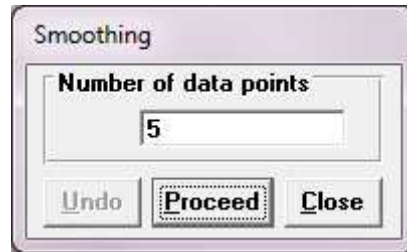


Figure 85 : Smoothing window

The smoothing operation consists in executing an average of the data with its neighbors within a sliding rectangular window. The filter width must be an odd value to center the averaging on the current data point.

Let the filter width be $2k+1$, and (x_i, y_i) $i = 1, \dots, N$, the trace to smooth, then the filtered trace will be :

$$(x_i, z_i) \quad i = 1, \dots, N, \quad \text{with} \quad z_i = \frac{1}{2k+1} \sum_{j=i-k}^{i+k} y_j$$

Smoothing using Savitzky- Golay method

The Savitzky-Golay smoothing is a filter method that performs a local polynomial regression to determine the smoothed value for each data point. This method is superior to adjacent averaging (previous smoothing method) because it tends to preserve features of the data such as peak height and width, which are usually 'washed out' by adjacent averaging.

The Savitzky-Golay method essentially performs a local **polynomial regression** (of k degree) on a distribution (of at least $k+1$ equally spaced points) to determine the **smoothed** value for each point. Methods are also provided for calculating the first up to the fifth **derivatives**.

The raw data consists of a set of n $\{x_i, y_i\}$ points ($i = 1 \dots n$), where x is an independent variable and y_i is an observed value. The approach for smoothing a series is to replace each value of the series with a new value which is obtained from a polynomial fit to $m = 2k+1$ neighboring points.

A polynomial will be fitted to a set of m (an odd number) adjacent data points separated by an interval h .

Firstly, a change of variable is made: $Z = \frac{x - \bar{X}}{h}$

Where \bar{X} is the value of the central point and z takes the values $(1-m)/2 \dots 0 \dots (m-1)/2$.

The polynomial, of degree k is defined as

$$y = a_0 + a_1 z + a_2 z^2 + a_3 z^3 + \dots + a_k z^k$$

The coefficients a_0, a_1 etc. are obtained by solving the normal equations

$$a = (J^T J)^{-1} J^T y$$

Where J is a Jacobian matrix.

Smoothing window: The filter width "**Smoothing window**" permits you to choose how many points to consider. This value must be an odd number to center the averaging on the current data point (equally spaced points). The smoothing routine will be apply to each individual point.

Polynomial Order: correspond to the degree of local polynomial regression from degree one up to seven. An improvement of fitting/differentiation routine is obtained by changing the polynomial order.

Derivative: The Smoothing dialog window provides a drop-down list for defining the derivative condition. The calculating can be done using the first derivative up to the third derivative.

You can improve the fitting/differentiation by changing the **Smoothing Window** and **Polynomial Order**

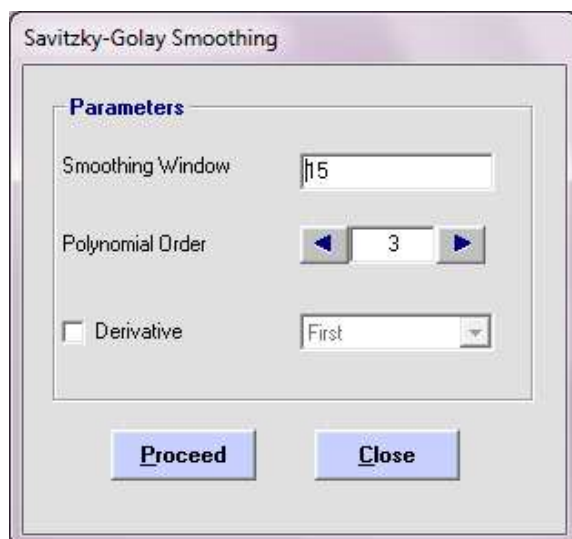


Figure 86: Savitzky-Golay smoothing window

The figure below shows, raw signal (Figure 87) and the smoothing results (Figure 88), using a second third polynomial fit with 15 data points (Figure 86).

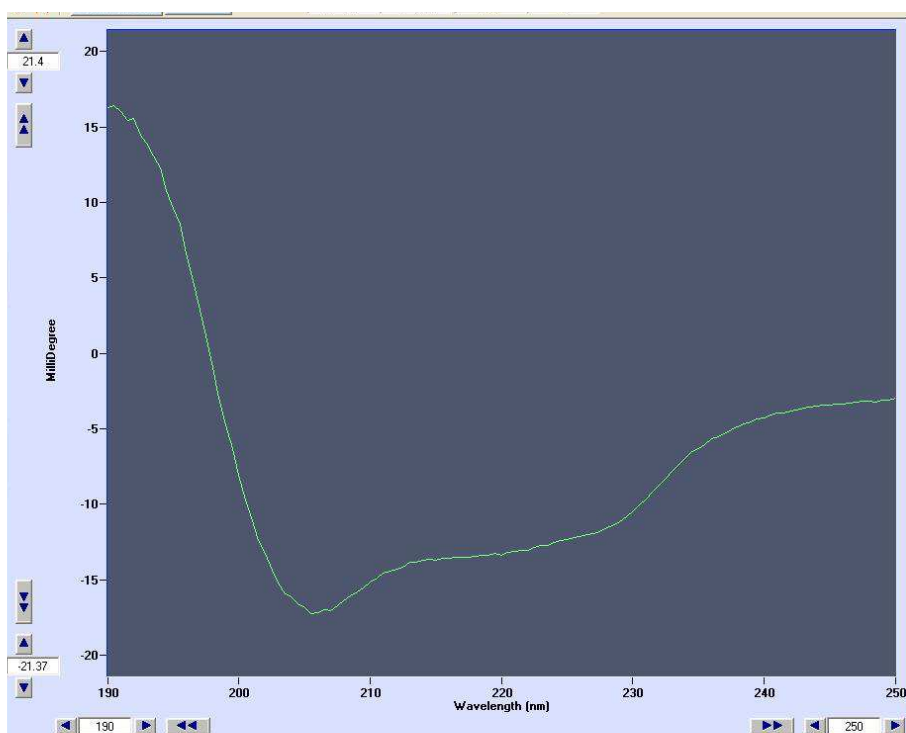


Figure 87: CD raw data

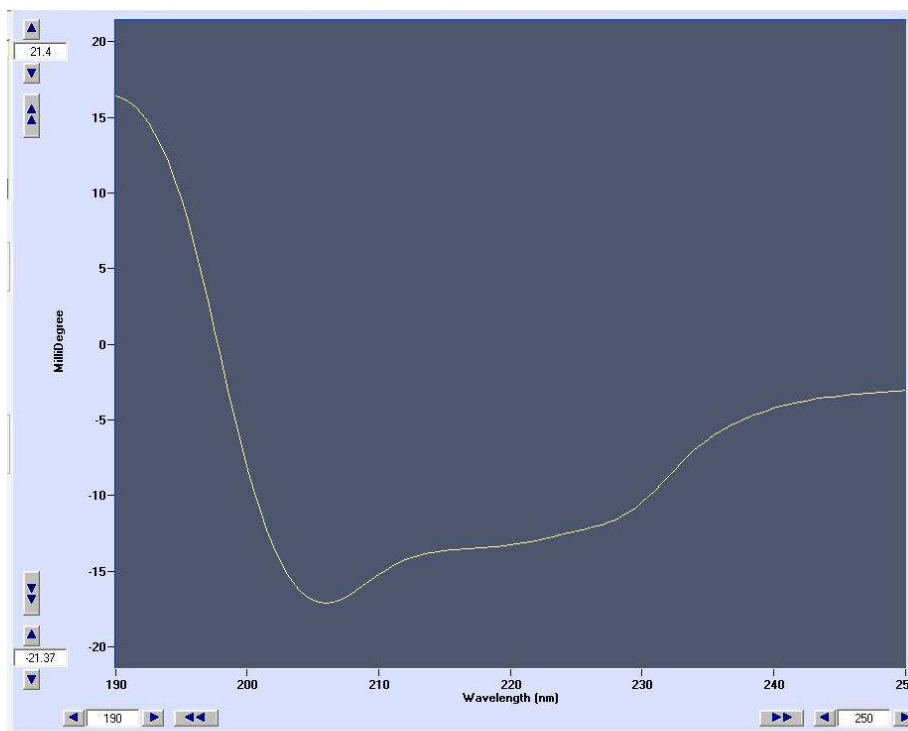


Figure 88: CD smoothed data

3.12.10. Integration

Select **T**ools, **I**ntegral to display the integral value between the markers 1 and 2 (see section 3.8), the integral value is calculated using the trapeze method. If a baseline is displayed on the graph, then the area between the current trace and the baseline is returned.

The average value between the markers 1 and 2 is the result of the integral (calculated with the trapeze method) divided by the abscissa interval length. Select **T**ools, **A**verage Value to display this value.

3.13. Baseline calculation

Choose menu **T**ools, **B**aseline, **C**ompute to load the baseline window (see Figure 89). This action displays the vertical markers at the same time (described in section 3.8).

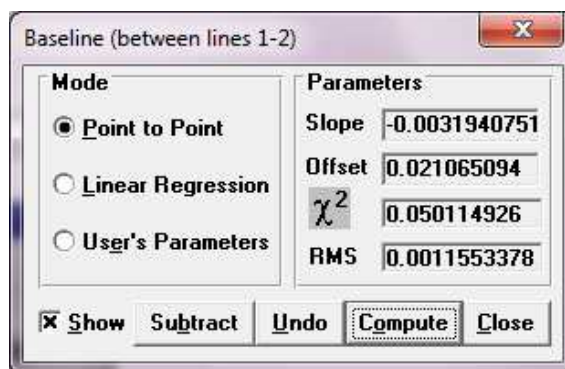


Figure 89 : Baseline calculation window

The baseline is calculated for the current file between the vertical markers 1 and 2, or between the first and the last data point of the current trace if the vertical markers are hidden.

Three modes of evaluations are available:

Point to Point: the baseline is calculated and draw directly between the intercepts of the two vertical markers and the current trace.

Linear Regression: the baseline is calculated by a linear regression taking into account all the data points between the two markers.

User's Parameters: the baseline parameters are given directly by the user.

To draw the baseline, click on the **Compute** button after having chosen the draw mode. This will display the baseline on the graph (in dashed style) and a χ^2 value. χ^2 is the quadratic error between the trace and the baseline. It is given by the following formulae:

$$\chi^2 = (1/N) \sum_{i=i_1}^{i_2} [Y(i) - (aX(i) + b)]^2$$

with:

i_1, i_2 : first and last points indexes

N : number of data points ($i_2 - i_1 + 1$)

$X(i)$: X values of the data

$Y(i)$: normalized Y values of the data (normalization is a change in the Y scale such as $\max(Y(i)) = 1$ and $\min(Y(i)) = 0$, for $i = i_1$ to i_2)

a, b : baseline slope and offset


$aX(i) + b$: base line points (with the same scale normalization)

Choose **Tools, Baseline, Show** or click on baseline window **Show** check box to display/hide baseline on the graph.

To subtract the baseline from the current trace click on the baseline **Subtract** button. The last subtraction operation can be canceled by the **Undo** button.

3.14. Difference between baselines

To perform difference between two baselines at a given abscissa, choose **Tools, Difference...** or click

on  button: this will display the difference window shown in Figure 90 and add five vertical markers to the current graph (see Figure 91).

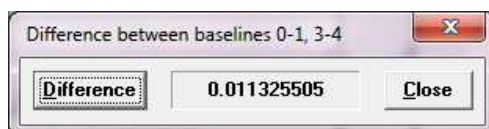


Figure 90 : Difference window

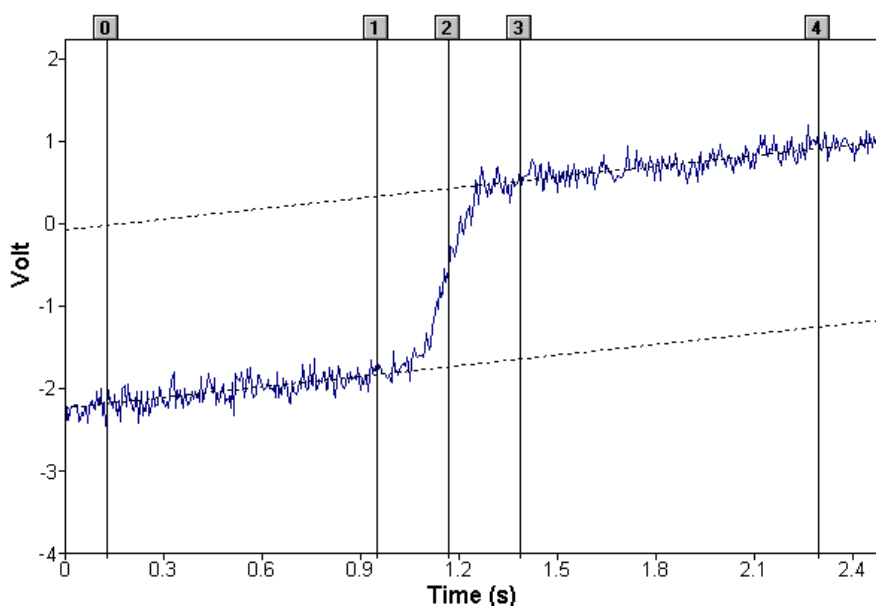


Figure 91 : Position of the markers

The two baselines are calculated between the vertical markers 0 and 1, and 3 and 4. The vertical marker 2 gives the abscissa where the distance is taken. To calculate the distance, position the vertical marker 2 (see Figure 91) and click on the **Difference** button on the difference window (see Figure 90).

3.15. Evaluation of Kinetic constants with Bio-Kine

Evaluation of Kinetics constants requires the following operations:

- determination of the starting point of the reaction (e.g. the time of stop for the stopped-flow experiments)
- determination of the baseline parameters
- determination of the relaxation Kinetics itself using first order or second order reactions.

Whatever the Kinetic model used for the determination of Kinetic constants, the region of the graph where the rate constant evaluation will be made is determined by the 3 vertical markers (refer to section 3.8). Markers N°0 and N°2 are used to determine the region of the graph where the rate constant evaluation will be performed. The marker N°0 will have to be set at the beginning of the Kinetics. Vertical markers 1 and 2 are used to mark the region where the baseline will be calculated. The values of the baseline slope and offset are given with reference to the graph coordinates. Multi-exponential parameters are given with the X axis origin set at the position of the 0 vertical markers.

3.16. First order reactions

This procedure is used in cases where the Kinetics can be analyzed as a sum of exponential functions. The fit obtained will obey the general formula:

$$Y(t) = a t + b + \sum_{i=1}^N C_i \exp(-k_i t)$$

Slope (a) and offset (b) correspond to the baseline, or in other words, to the time course of the trace at an infinite time. The C_i and k_i parameters are respectively the amplitude and rate constant of each exponential terms.

Bio-Kine provides a multi-exponential evaluation using Simplex methods for first order reactions. The Simplex method is a minimization algorithm. It minimizes the quadratic error (χ^2) between the current trace and the fit. So the performance of this algorithm is greatly depending on the starting parameters. Incorrect initialization parameters may result in a failure of convergence or in a too long search.

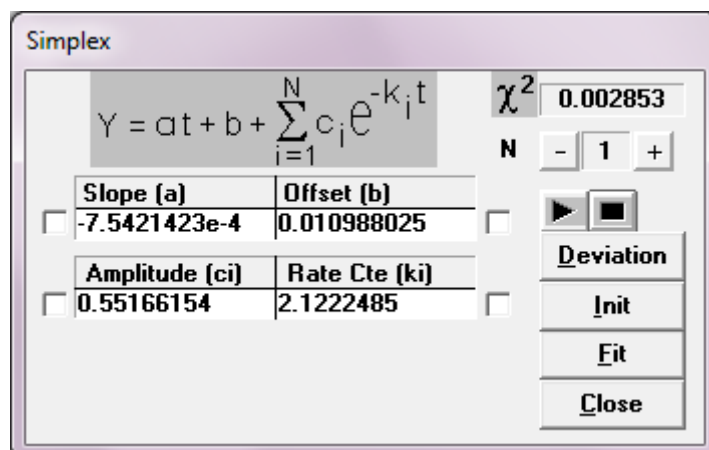



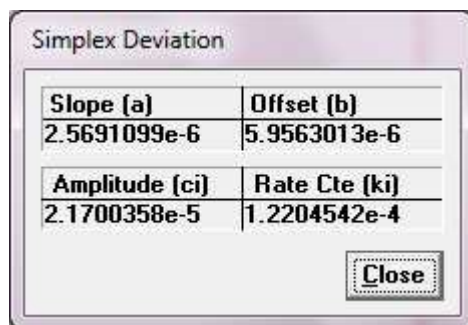
Figure 92 : Simplex method window

To run the Simplex search, choose **Tools**, **Multi-Exponential**, **Simplex**. This loads the simplex window (see Figure 92), that displays χ^2 , slope (a), offset (b), multi-exponential order (N), amplitudes (C_i) rate constants (k_i). This window has the following buttons:

Run button  : runs the Simplex search

Stop button  : stops the Simplex method

Deviation (see Figure 93): displays standard deviation for each parameters (this is a better indication of algorithm convergence than χ^2).

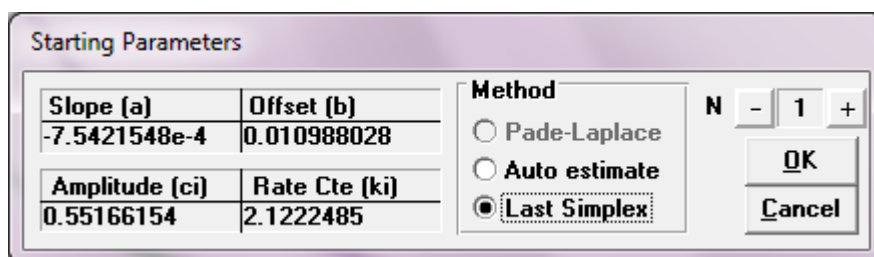


Simplex Deviation	
Slope (a)	Offset (b)
2.5691099e-6	5.9563013e-6
Amplitude (ci)	Rate Cte (ki)
2.1700358e-5	1.2204542e-4

Close

Figure 93 : Simplex deviation window

Init (Figure 94): allows to select the Simplex starting parameters between the last simplex fit. An auto-estimation of the initial parameters (used as default method by Bio-Kine).



Starting Parameters		Method	
Slope (a)	Offset (b)	<input type="radio"/> Pade-Laplace	N - 1 + OK Cancel
-7.5421548e-4	0.010988028	<input type="radio"/> Auto estimate	
Amplitude (ci)	Rate Cte (ki)	<input checked="" type="radio"/> Last Simplex	
0.55166154	2.1222485		

Figure 94 : Starting parameters window

Fit (see Figure 95): the programs will compute a simulated fit using these parameters and a graph showing the difference between the fit and the original graph is then displayed on the current window.

When the search is paused, the lastly calculated parameters (slope, offset, amplitudes and rate constants) are displayed. It is possible to lock/unlock some of the parameters with check boxes (see Figure 92). Other possible users actions are parameters editing manually and to change the number of exponential (between 0 and 6).

When the search is running (run button down and pause button up), only the value of χ^2 is displayed and updated, according to the current calculated value. It is not possible to edit or lock/unlock parameters during a search, **Deviation**, **Init** and **Fit** buttons are disabled.

Note: upon loading, the simplex method is paused (run and pause buttons down) to display the initial parameters.

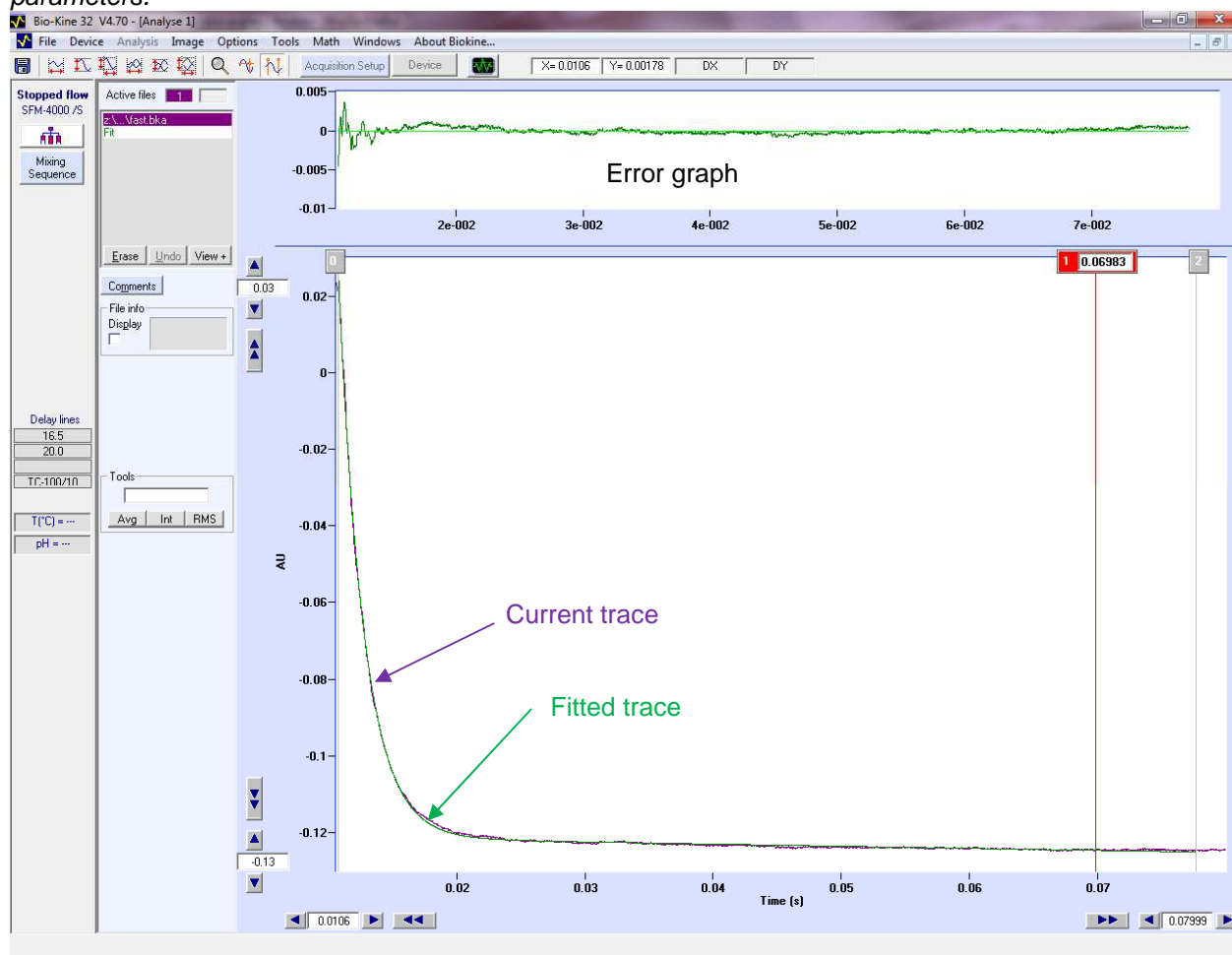



Figure 95 : Fit and error window using simplex method

The algorithm may not stop by itself during a search, it is to the user to estimate that the method has converged (according to χ^2 and standard deviations values), and to click on the stop button. THERE IS NOT WARANTEE THAT SIMPLEX ALGORITHM WILL CONVERGE TO THE RIGHT VALUES, because the simplex method may calculate the parameters that correspond to a local minimum of the χ^2 function. This is why initial parameters must be chosen carefully (see before).

*Note: The **Close** button is available only if the Simplex search has stopped.*

The Simplex search must be stopped before exiting Bio-Kine. If an attempt is made to unload Bio-Kine while the Simplex method is still running, a warning will be given to the user and the unload operation will fail.

GUIDELINE TO USE THE SIMPLEX METHOD

- Click on the vertical markers button.
- Set marker 0 to the beginning of the Kinetic.
- Set markers 1 and 2 to mark the baseline limits.
- Choose **T**ools, **M**ulti-Exponential, **S**implex in the menu.
- Choose the multi-exponential order (N).
- Run the Simplex with the  button.
- Choose **F**it to visualize the fit and the error.

3.16.1. Second order reactions

This procedure will be used in case the reaction can be analyzed as a second order reaction as in the following case:



The rate constant of this reaction (k_2) is usually expressed in $M^{-1}s^{-1}$.
The reaction will be analyzed following the equations below:

$$Y(t) = at + b + \frac{c}{1 + dt}$$

Slope a and offset b correspond to baseline parameters.
 c is the total amplitude of the observed transition.
 d is the apparent reaction rate constant (unit = s^{-1}).

$Y(t)$ is the data measured in the transition. It is a spectroscopic signal that can be proportional to the concentration of any of the compounds $[A]$, $[B]$ or $[C]$.

This apparent rate constant is the product of the total initial or final concentration of the products (as defined above) by the second order rate constant k_2 .

Choose **Tools, Second Order** to load the second order Simplex window (see Figure 96).

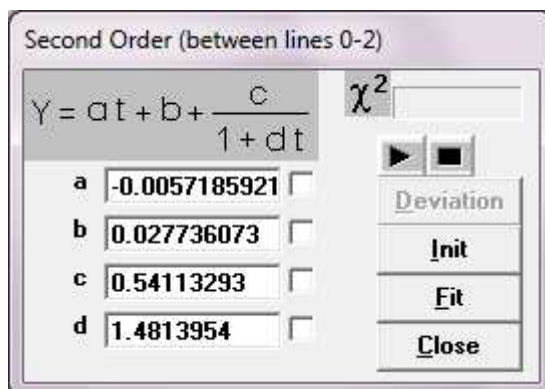



Figure 96 : Second order simplex window

The second order Simplex is the same algorithm than the multi-exponential Simplex and works exactly in the same way. Results of the search are (a), (b), (c) and (d).

Refer to section 3.16 to use the buttons presented in this window.

GUIDELINES HOW TO USE THE SECOND ORDER SIMPLEX METHOD

Click on the vertical markers button.
Set marker 0 to the beginning of the Kinetic.
Set markers 1 and 2 to mark the baseline limits.
Choose **Tools, Second order** in the menu.
Run the Simplex with the  button.
Choose **Fit** to visualize the fit and the error.

3.16.2. General simplex

The simplex algorithm can be run on any function, Bio-Kine permits to use any equations by selecting the **Tools, Simplex** in the main menu, the window shown in Figure 97 will appear.

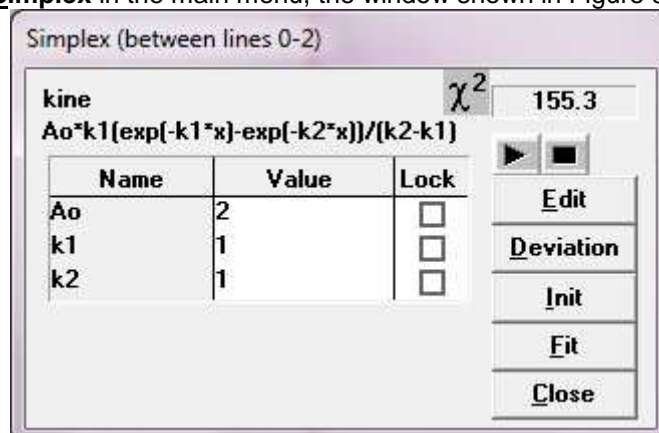


Figure 97 : General simplex window

Before any search, one must select the fitting function, in the example presented in Figure 97, the 'Kine' function is selected. To change or edit the fitting function click on the **Edit** button. The first time the general simplex window is loaded, no functions are available. So the first operation is to enter a new function.

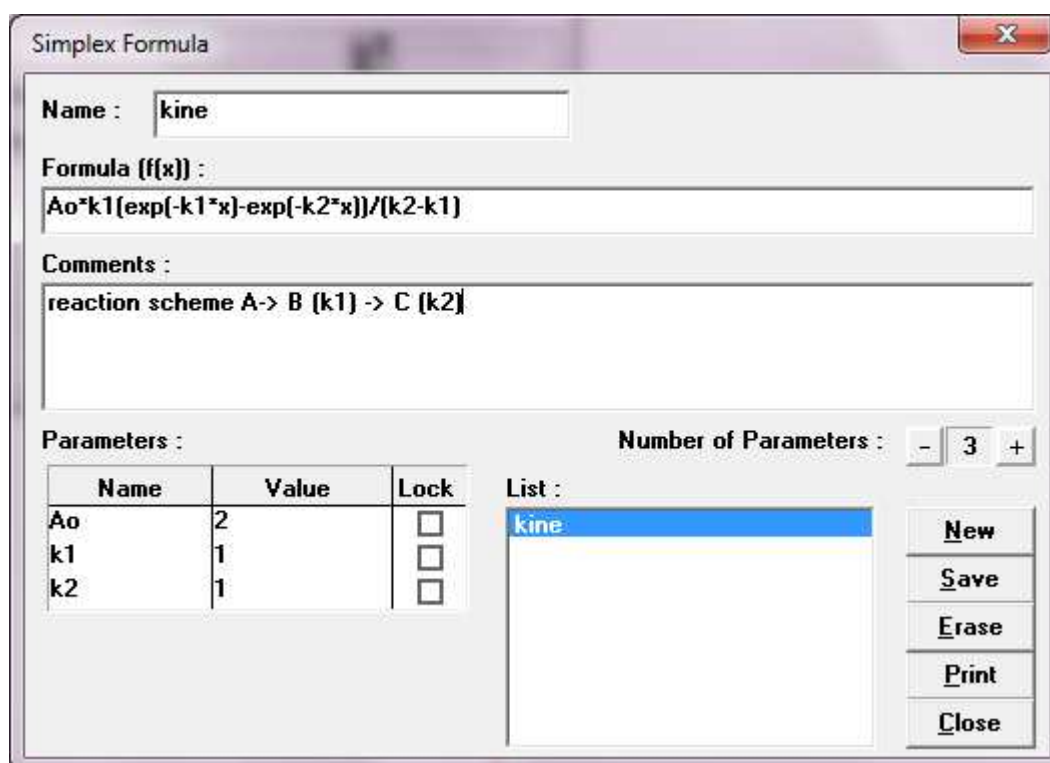


Figure 98 : Simplex edition window

The simplex edition window shown in has the following fields and buttons:
Name : enter the formula name (this field cannot be empty)

Formula: enter the formula, the operators: +, -, *, /, ^, exp, log, log10, sqr (square), sqrt (square root), sqrt3 (cubic root), the parameters name, x (for the time variable), numerical constants (ex 3.14) and the space character are allowed, Any other operator will produce a syntax error into the formula evaluation (Save button).

Comments: up to four lines of comments are allowed. It is recommended to use short parameters names into the formula and to comment their meaning instead of long parameters names.

Number of Parameters : set the number of parameters (between 1 to 10)

Parameters name: set each parameter name, this field cannot be empty.

Warning: the parameters name must be the same to the ones entered into the formula.

Parameters values: enter each parameter value (default 1). This value can be changed later into the general simplex window (see Figure 98).

Parameters: lock / unlock each parameter for the simplex algorithm. This option can be changed later into the general simplex window (see Figure 98).

List: displays the functions lists. To select a function, click on its name into the list.

New: clear the Name, Formula, Comment fields and set the number of parameters to 1 (to enter a new formula).

Save: save the new formula values if the syntax

Erase: erase the current formula

Print: print the formula

Close: close the edition window and return to the previous window.

Once the current formula is selected, the simplex search algorithm can be run through the general simplex window (see Figure 97). To operate proceed the same way as described for the Multi-exponential simplex and for the second order.

*Note: One can also re-enter the multi-exponential and the second order functions into the function edition window (see), but it is better to use the specifics menus (**Tools, Multi-Exponential, Simplex and Tools, Second Order**), the simplex algorithm will be run faster.*

3.16.3. Stopped-flow Kinetics simulation

The simulation window may be useful to exercise and for test the multi-exponential, second order and any input function evaluation algorithm. Choose **File, Simulation** in the main menu to load the simulation window (see Figure 99).

Figure 99: Stopped-flow Kinetics simulation window

In this window, enter some stopped-flow experimental parameters and click on the **OK** button to get your simulation. For example with the parameters shown in the previous figure the result presented in Figure 100 will be loaded.

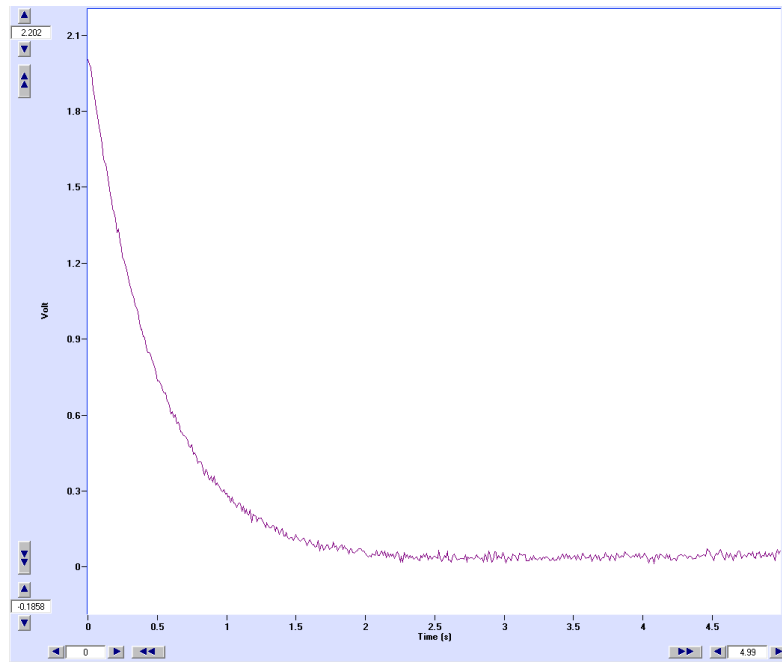


Figure 100 : Simulation window

4. MOS-200 ACQUISITION DEVICES

4.1. MOS-200 control

From the **Install, Device** menu choose external device.

1. Wavelength control

The excitation wavelength is done manually see MOS 200 user's manual

2. High voltage control

The high voltage is set manually directly on the PMS250 see MOS 200 user's manual

4.2. Transient recorder device

4.2.1. Getting started

The transient recorder device is similar to a digital oscilloscope. It is the device used to follow rapid Kinetics. The acquisition modes (fluorescence, absorbance) are controlled from this device. An acquisition is linked to a stopped-flow sequence so make sure that a stopped-flow instrument and its MPS are connected to the system. Synchronization between acquisition and driving sequence is made using Biokine software simultaneously.

In the main menu choose **Device, Transient Recorder** to load the window shown in Figure 101.

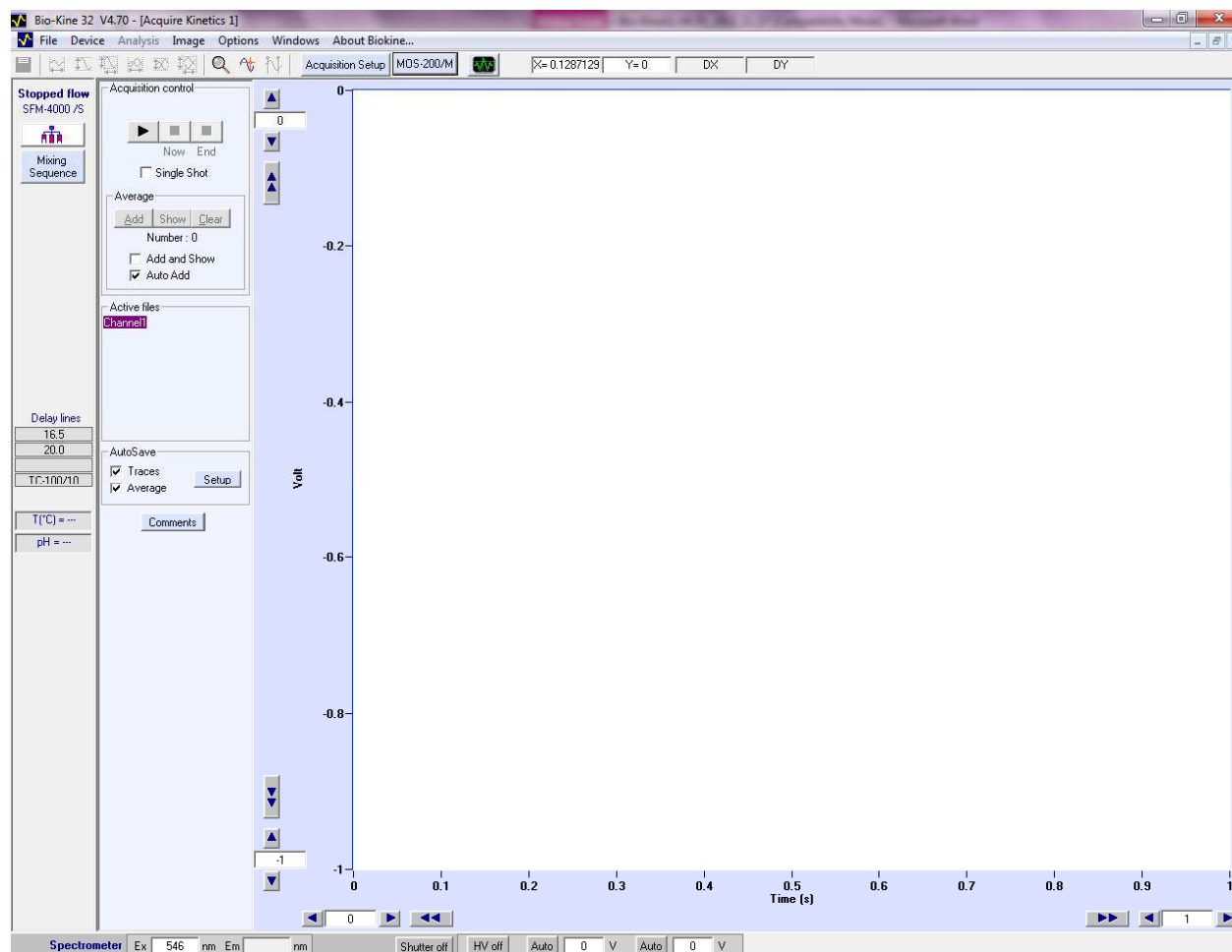


Figure 101 : Transient recorder window

Select the acquisition mode by opening the DATA Acquisition Setup window by a click on **Acquisition Setup** button. The different modes are fully described in the following section.

4.2.2. Absorbance and transmittance mode

- The data acquisition window in absorbance mode is presented in Figure 102.
- Check the **Trigger** box to control the shots from the Bio-Kine software (see Figure 102). This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments.
- For absorbance or fluorescence experiments it is necessary to record first the zero absorbance level using buffer or water. To do that, fill the cuvette with buffer (or water and click on the **Find/Stop** button ("Get reference "area). Once found click again on this button, the system is now ready for Kinetic shots.

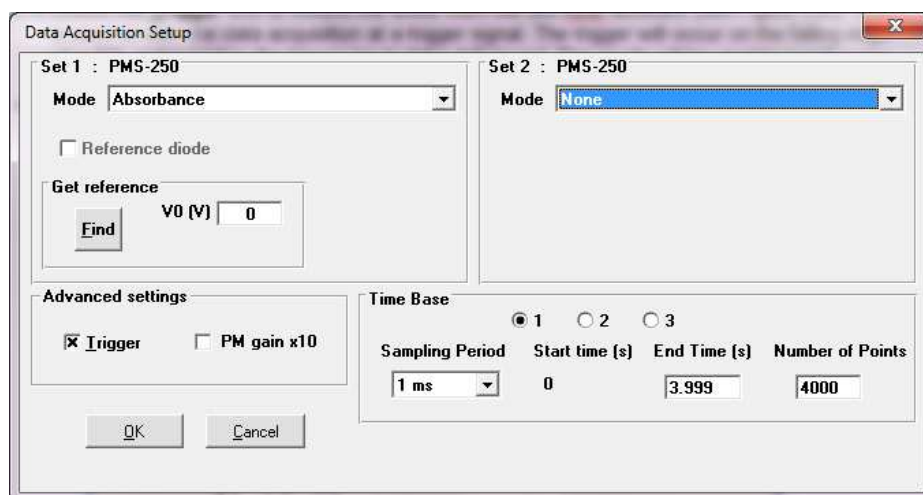


Figure 102 : Data acquisition setup window

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recordings are performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Into the 'time base area' (see Figure 103) the Number of points and End time parameters can be modified manually but notice that they are linked together. Sampling Period range is 10 μ s to 10s.

NOTE:

You need to be careful to select the output filter from the PMS250 to a value 2 times bigger than the sampling period.

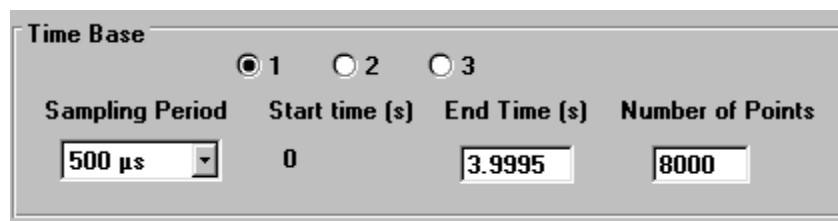


Figure 103 : "time base" area

A procedure to perform Kinetics in absorbance mode is given in section 4.3.1.

4.2.3. Fluorescence mode

- The data acquisition window in fluorescence mode is presented in Figure 104.
- Contrary to the **Scanning Spectrometer** device the **Reference Diode** is not useful. Indeed, Kinetics is followed at a single wavelength so the fluctuations of the energy of the lamp are not significant.
- Check the **Trigger** box to control the shots from the Bio-Kine program (see Figure 104). This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments.

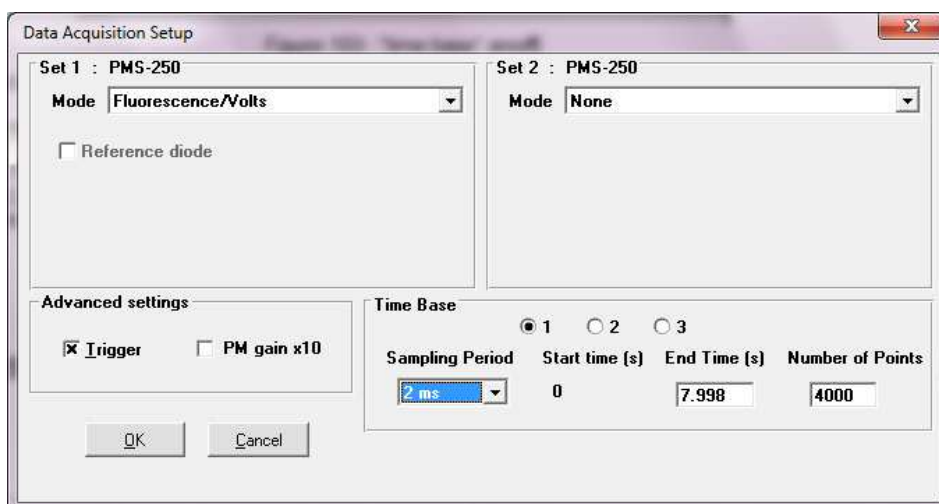


Figure 104 : Data acquisition setup window

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by over sampling on the second (and third) time bases. In other words the recording is performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is a equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Into the 'time base area' (see Figure 105) the Number of points and End time parameters can be modified manually but notice that they are linked together. Sampling Period range is 10 μ s to 10s.

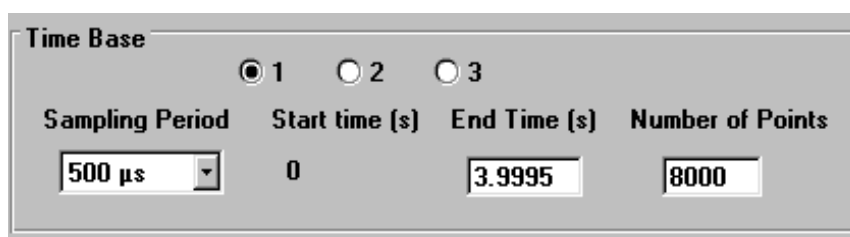


Figure 105 : "time base" area

- A procedure to perform Kinetics in fluorescence mode is given in section 4.3.2.

4.2.4. Last setting before shot

The stopped-flow control unit (MPS) generates a trigger signal to synchronize the data acquisition with the stopped-flow sequence.

Once the stopped-flow sequence ready, the shot window is activated in the stopped-flow status bar (refer to section 2.6 for more details).

Make sure that the scope is on ('waiting for trigger' must be displayed in red in the 'scope area'). The scroll option is disabled when the trigger is on (refer to).

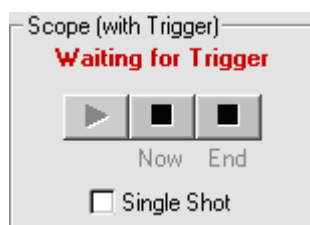


Figure 106 : Control of the scope

Click on the  button in the stopped-flow status bar to execute the shot.

More details can be found in section 4.3 where the way to run a Kinetics experiment is fully described.

4.2.5. Shot in automatic mode


The  button is used to perform automatic accumulation of shots. When clicking on this button the window shown in Figure 107. This window is available only when the **shot control** window and the **scope** are ready.



Figure 107: Auto run window

The number of accumulation is entered manually or using the arrow buttons.

The status of the acquisition is indicated in the **current** state panel during the acquisition. There is also the possibility to have a waiting time period between each shot, and then you need to check time in Restart with and enter your waiting time.

4.2.6. Graphical options

Most of the graphical tools were explained in section 2.

After each shot (Trigger with Repeat option), it is possible to perform an average of the shots, or more precisely to add the last shot to a cumulative averaging of the successive traces. Click on Add Average button to add the last trace to the average (see Figure 108). The Clear average button will erase the average.

It is not possible to show the original and the average traces at the same time. So one must click on the Show/Hide average option to display/hide the current average. An other way to display the average is to check the Add and Show box, then the current average will be displayed at the Add button click. By checking Auto Add box each curves are automatically added in the average.

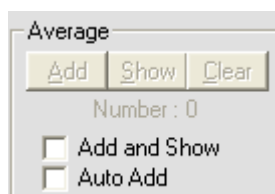


Figure 108 : Averaging window

*te: operation like **Save**, **Save As...**, **Save ±**, **Print** applies to the trace displayed on the screen. These actions are also possible while acquisition is running, during Trigger wait.*

Checking the Display measure box, instantaneous values will be displayed in a table in the left corner. Use of the Selector and comments buttons were described in section 2.

4.2.7. Go to analyses window

While working on an acquisition window, the main menu **Analysis command** will load an analysis window and set the actual data in this window. This is useful for performing an immediate analysis of the current data. Once the analysis window has been loaded, all the mathematical tools described in section 3.12 can be used so as to treat the data and Kinetic constants.

4.3. How to run Kinetics?

4.3.1. In absorbance or transmittance mode

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation..

Select external device

Select the stopped-flow and its corresponding serial port or USB port

Click OK to validate.

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.
- Push some buffer into the cuvette for further reference or High voltage adjustment.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).


Once the sequence done, click on the ready button.


The control shot window is now activated in the stopped-flow status area

Acquisition parameters

Select the Device, transient Recorder menu.

- Select an excitation wavelength, manually.

In the main window, click on the  button to adjust the High voltage automatically. Click a second time when the response is stable.

Click on the  button.

Select the absorbance acquisition mode

Do not select the reference diode..

Do not check the PM gain x 10 option.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

The cuvette is filled of water so it is possible to do a reference for further absorbance calculations.

- Click on the **Find/Stop** button in the **Get reference** area.
(*about 5V should be display in the corresponding state panel*)

Click a second time on the Find/Stop button to validate the reference.

Click **OK** to accept these parameters.

If the voltmeter check box is selected then the absorbance indicated in the corresponding channel is 0.

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

4.3.2. In fluorescence mode

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation.

External device

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the Ready button.

The control shot window is now activated in the stopped-flow status area

Click on the start button to fill the cuvette with final solution to adjust the high voltage (see further)

Acquisition parameters

Select the Device, transient Recorder menu.

- Select an excitation wavelength.
- Select an illumination wavelength (if an emission monochromator is available)
- Click Apply to validate

The wavelengths chosen are displayed in the corresponding state panel at the bottom of the screen

On the PMS 250, select a HV to get a response of 5V regarding the PM tube (make sure you are working in local mode)

Click on the  button.

Select the fluorescence acquisition mode

Do not select the reference diode.

Only check the PM gain x 10 option if the High Voltage found is higher than 900V.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

4.4. Chart recorder device**4.4.1. Data acquisition setup**

The Chart Recorder device looks very similar to the Transient Recorder, so it could be helpful to read the transient recorder part (section 4.2) for a better understanding of the chart recorder device. It is however a more specialized device that operates in the scroll mode only. It features a virtual chart recorder and one of its most interesting features is its ability to handle and to record very large files. The files created in the Chart recorder mode can have up to 100 millions points. It has to be kept in mind that a single Y file of this size represents 400 MBytes of data. Be sure to have enough hard disk space to store the data. If it is not the case, acquisition will be stopped when the disk will be full.

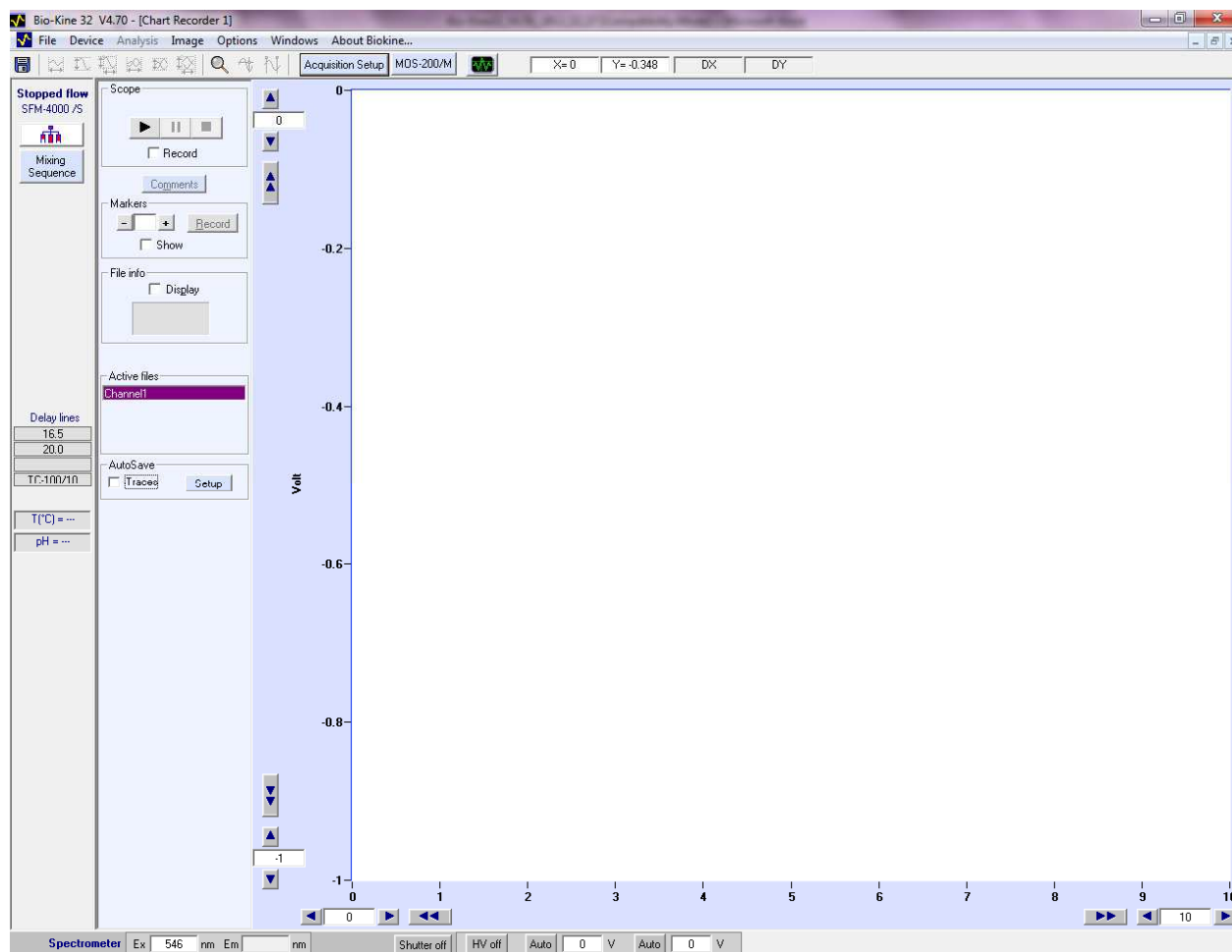


Figure 109 : Chart recorder window.

To load the Chart recorder, choose Device, Chart recorder in the main menu, the window shown in Figure 109 will be displayed.

Click on the **Acquisition Setup** button to load the data acquisition setup window (shown in Figure 110) Most of the tools are described in the Transient recorder device, please refer to section 4.2 for further information.

Only one time base is available in this device and it is one of the main differences with the transient recorder device. Select the sampling period for the measurements and either the number of points or the duration of the measurement (see Figure 111)

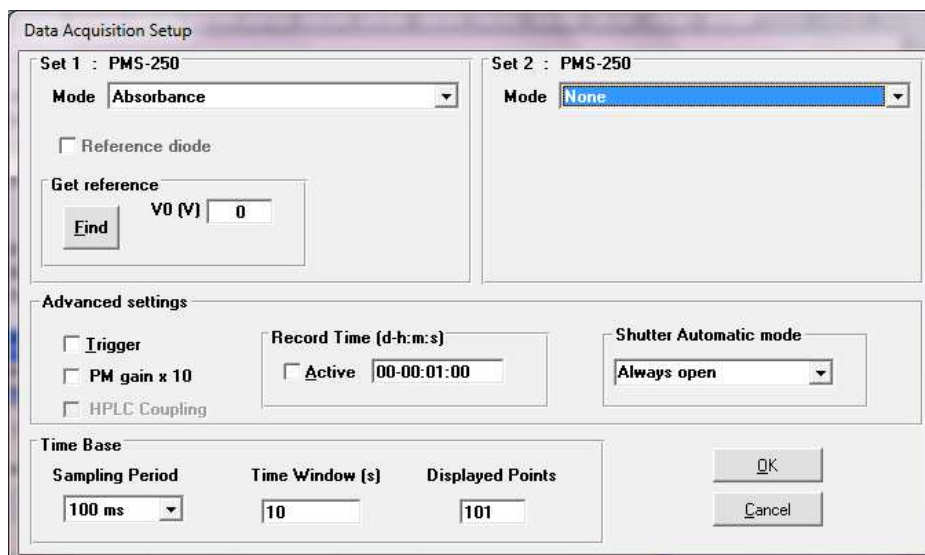


Figure 110: Data acquisition setup window

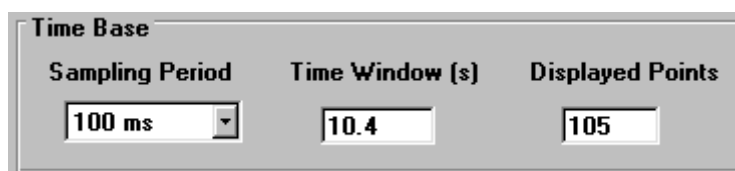


Figure 111 : Time base window

It is possible to program an acquisition time duration which will automatically stop the data acquisition at a preset time (see Figure 112).

Check Active option and enter Record Time into dd-hh:mm:ss format (for example: 00-01:30:00 for 1h 30 mn).

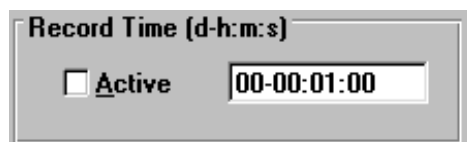


Figure 112 : Automatic stop area

4.4.2. Graphical options

Most of the graphical tools were previously described in section 3, please refer to this section for further information. Use of the Selector and comments buttons were also described in section 3.

Several tools can be found in the scope area presented in Figure 113.



Figure 113 : "scope area"

Click on run (▶) and stop (■) buttons to start and stop the acquisition. Pushing/raising the pause button (⏸) interrupt/continue the acquisition. The pause time is not added to the following acquired data.

To start recording the data on a file, click on the Record check box during the acquisition run. To stop recording, click again on the Record check box. Each time this operation is performed a new temporary file is created. Thus, several files can be recorded during the same acquisition run. If the Record box is checked before the run, recordings will start at the same time as the run button is clicked.

A display check box is inside the 'File Info area', checking this box will display the size of the file and the number of points measured during the recording.

During recordings, markers can be set at any time on the file being recorded. Clicking on the **Record** marker button will add a marker at the current position (right of the graph) into the recorded file(s).



Figure 114 : Markers area.

Checking the **Show** marker box will display the markers into the graph (see Figure 114).

Up to 50 markers are allowed on the same file. Once the acquisition has stopped, it is possible to set the file current position to be that of one given marker. Selecting one marker is made by using the left and right buttons or by entering the marker number and validate with **ENTER** key.

*Note: when a file is loaded on an Analysis window, a marker frame (without **Record** button) will be visible into the Analysis window.*

5. MOS-200M ACQUISITION DEVICE

5.1. MOS-200M control and calibration

- From the Install, Device menu choose MOS200M: see Figure 115.

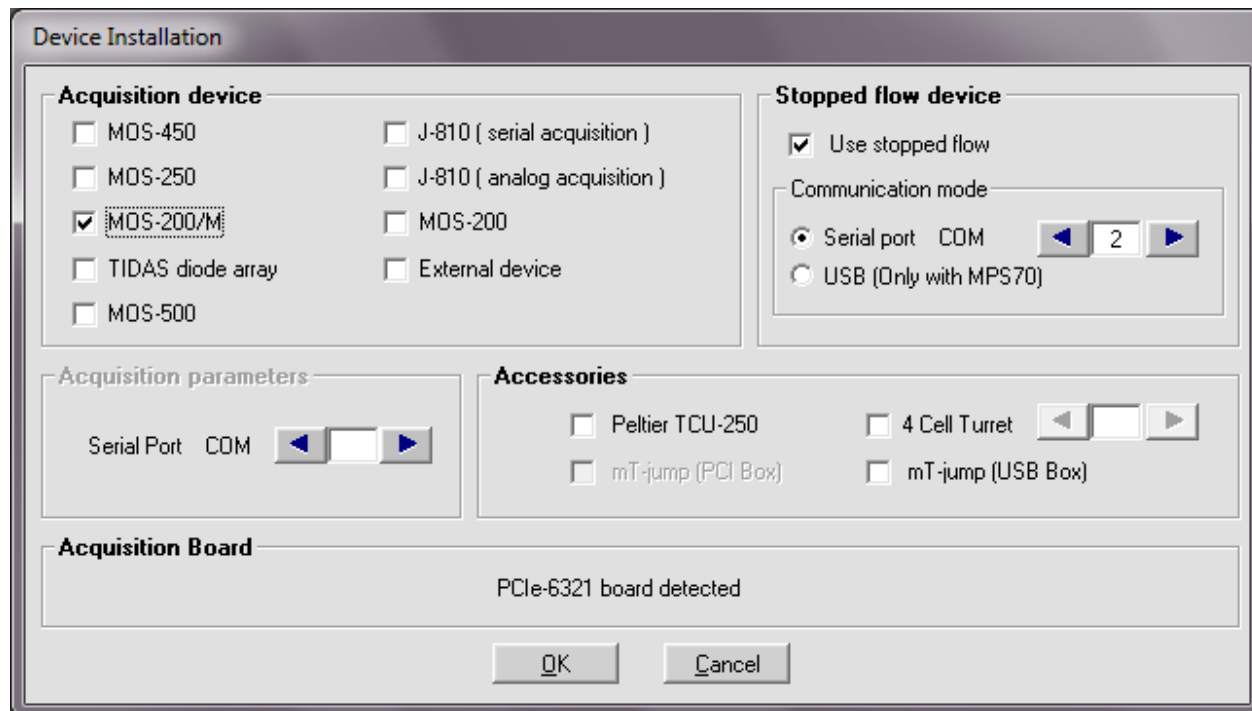


Figure 115: MOS-200/M installation

- In MOS200M, excitation monochromator is motorized and controlled through the MM450, which is the main difference with the MOS200. Therefore wavelength and high voltage setting can directly be done from Biokine software. The MOS200M was designed to follow kinetics using “wavelength tracking” which generates 3 dimension data. This is fully explained in section 5.3. Thanks to motorized monochromator, scanning mode is also available; this mode is described in section 5.4. MOS200M can also operate in transient recorder mode (single wavelength kinetics) please refer to MOS200.
- Wavelength control of the excitation monochromator can be set from his state panel at the bottom of the screen. It is also possible to control the monochromator by clicking on the “MOS200M” button and then entering the wavelength in the box as shown in
- Figure 116.

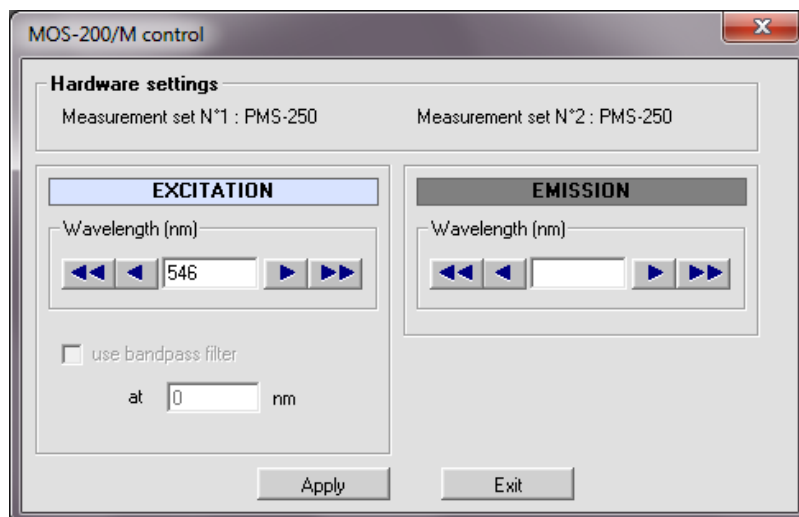


Figure 116 : Wavelength control

Choose **Install, Spectrometer advanced setting** in the main menu to load the window shown in Figure 117.

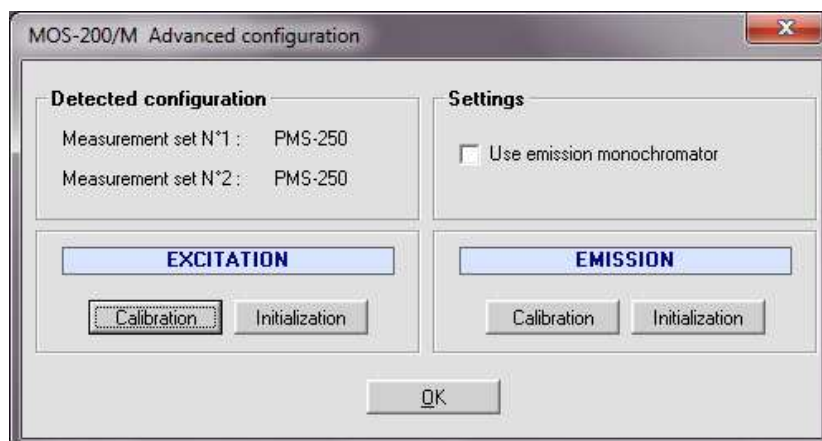


Figure 117 : MOS-200M configuration

The window displayed is separated into 2 areas:

The detected configuration is indicated on the left upper part, in this example only a PMS-250.

The excitation monochromator can be calibrated or initialized. The calibration procedure is explained in the following paragraph.

Calibration of the excitation monochromator can be accurately checked when using a Xe(Hg) lamp. Very precise peaks in intensity are available at many wavelengths to perform this calibration (see Figure 119).

Example: make a scan between 500 to 600 nm (with either the emission monochromator, set on "0"). A very clear peak of green light must be recorded at 546 nm. If this is not the case, set the monochromator at the maximum peak intensity of this green line (550 nm for example), close the Scan windows, choose **Install, Spectrometer advanced settings**, then click on the **Calibration** button to open the window shown in Figure 118. In our example 550 nm is the corrected wavelength, just enter "546" in the text box and validate. If you run a new spectrum between 500 and 600 nm, the maximum peak intensity of the green line must be observed at 546 nm.

Delta represents the difference between the mechanical wavelength and the real wavelength. Using the reset button, the real wavelength is adjusted to the mechanical wavelength.

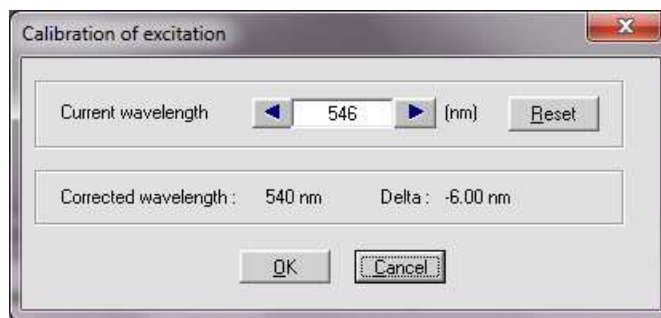


Figure 118 : Calibration window

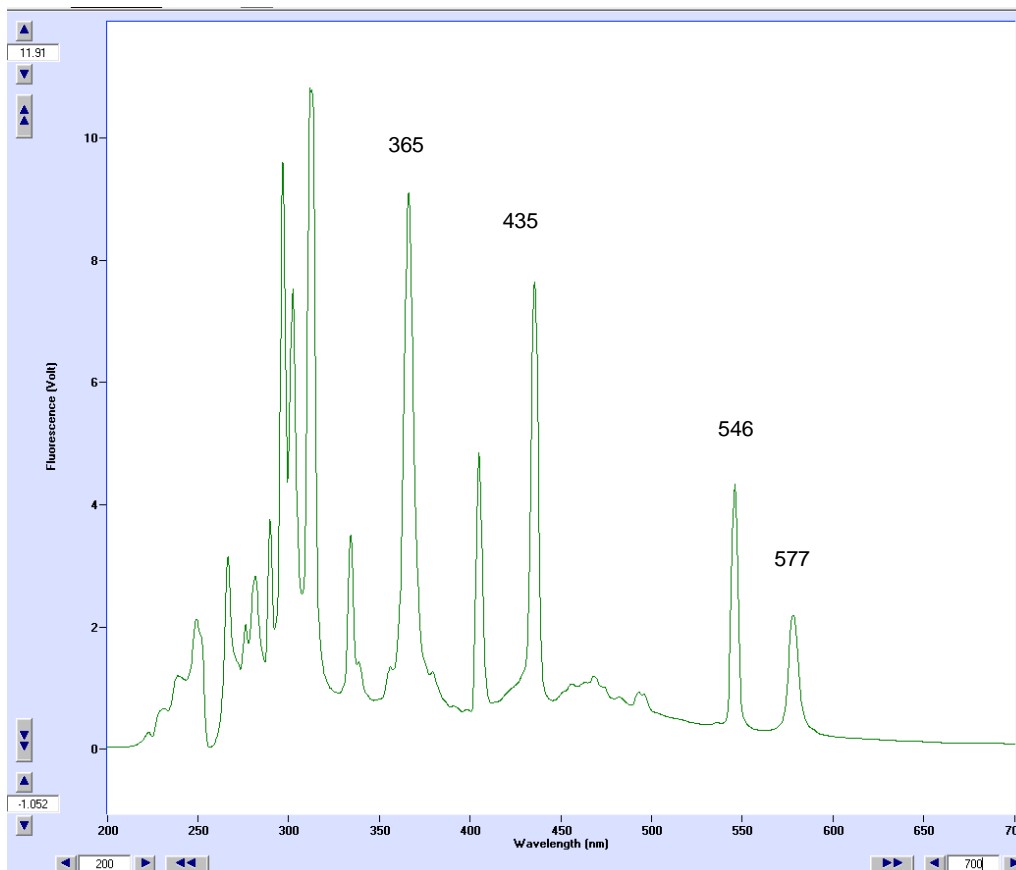


Figure 119 : Xe(Hg) lamp spectrum

Monochromator can be initialized at any time using the **Initialization** button; the aim of this function is to check the calibration of the system in three phases:


1. In a first phase the monochromator is going from the last wavelength selected to the mechanical zero of the monochromator.
2. In a second phase, the zero is adjusted considering the value of Delta found in the Calibration.
3. In a third phase, the monochromator is going from the real zero to the wavelength selected at the beginning of the first phase.

If the calibration is not done properly then the wavelength given at the end of the initialization is different from the one observed at the beginning.


These three phases of the initialization procedure are made automatically by the software when starting the system or when pressing the **Initialization** button.

5.2. High voltage control

The High voltage state panel (bottom of the main screen) enables the adjustment of the high voltage applied on the photomultiplier (PM). The Voltmeter displays the response of the PM. The number of displayed channels is modified automatically according to the number of working channels. The

voltmeter can be hidden or displayed at any time using the  button.

Two other buttons are also available to control the high voltage:

- **Auto** : this button can be used to adjust the high voltage automatically to get a 5 Volts response of the photomultiplier, once this button active its colour turns to orange.
- By clicking on the **HV on** button it is possible to turn the high voltage off, the colour of the button turns to grey () . A second click on the button will automatically set the high voltage to the last value used.

In the case the high voltage is too high and a saturation of signal is observed, the high voltage is decreased automatically to protect the PM. A warning message is displayed, it proposes to the user to reduce the value or to restore it.

If two photomultipliers are used for the detection, two **Auto** buttons are created (refer to Figure 120) their high voltage could be automatically and independently adjusted using their respective button.



Figure 120 : High voltage control

If the **Voltmeter** check box (refer to Figure 120) is checked then the voltmeter displays values in units as they are defined as in the parameters setup window.

5.3. Global Mode

5.3.1. Acquisition parameters

The global mode is use to drive the MOS-200M monochromator to perform acquisition of Kinetics data at various wavelength. The spectrometer is fully automated and synchronised with the stopped-flow mixer.

The global mode window is loaded by choosing: **Device, Global Mode**, the window shown in Figure 121 will be displayed.

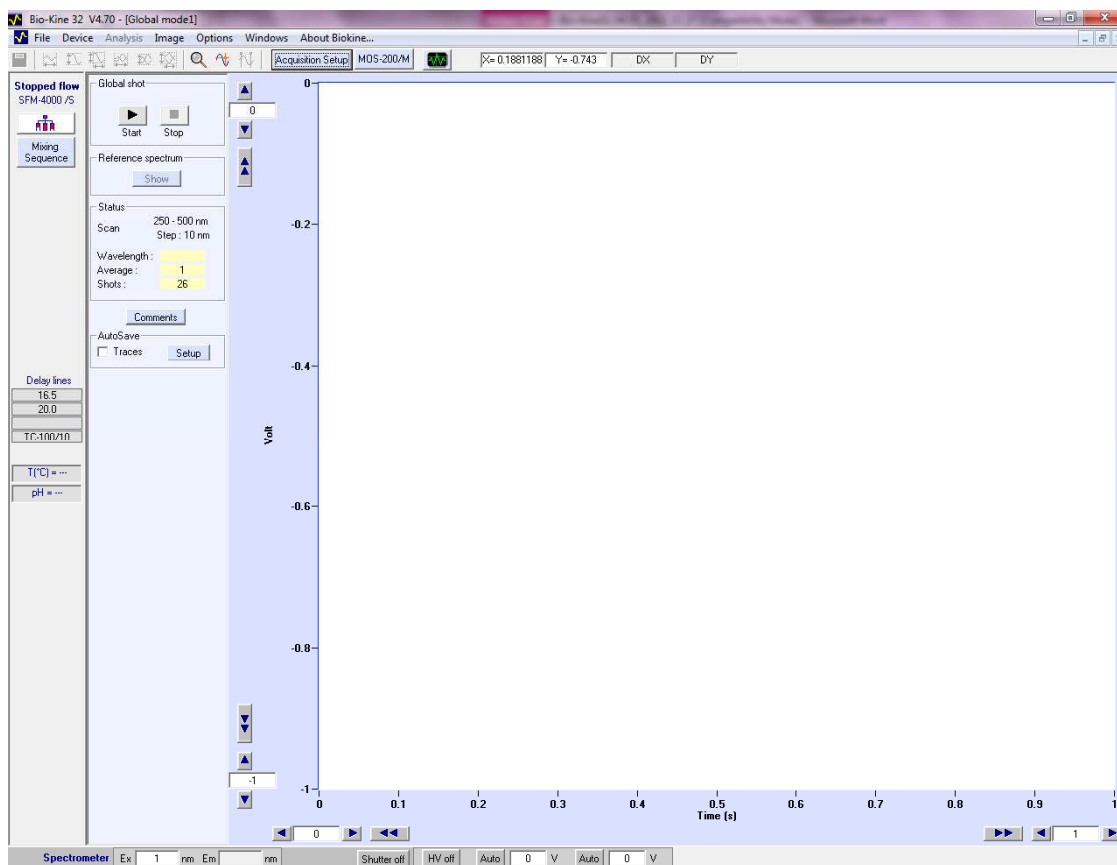


Figure 121 : Global mode window

Click on **Acquisition Setup** button to load the set-up window, the window shown in Figure 122 appears. Then choose fluorescence or absorbance acquisition mode by using Up and Down button.

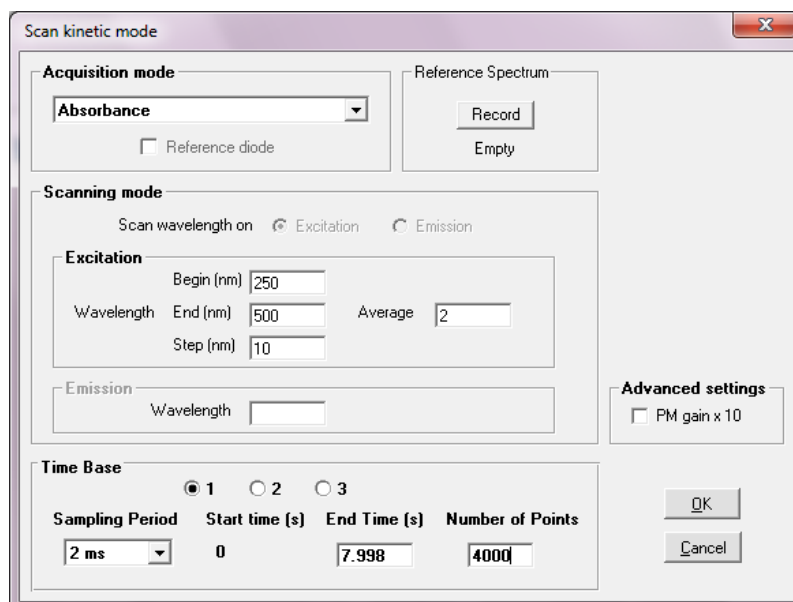


Figure 122 : Global mode window in absorbance mode

In **Scanning mode** select the scan parameters if you run some fluorescence analysis, for absorbance analysis this window is inactive.

Begin: first wavelength of the scan in nm

End: last wavelength of the scan in nm

Step: the step between each wavelength in nm

Average: number of kinetics accumulated for each wavelength

For absorbance mode a reference spectrum is required. To do that, fill the cuvette with buffer (or water and click on **Record** button. the system is now ready for Kinetic shots.

Up to three successive time bases are available. This multiple time base is performed by over sampling on the second (and third) time bases. In other words the recording is performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is a equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

The response of the photomultiplier can be amplified 10 times checking the corresponding box, it may be useful when working with very low signal and when the High Voltage is higher than 900 volts.

- When all the parameters are ready click on **OK**, the system is now ready for running the global shots.
- To run the experiment click on **Start** button in **Global Shot** (see Figure 121).

5.3.2. 3D Analysis

The file generated by Global mode is a 3 D file (Time, Absorbance, and wavelength). It is possible to transform this file using a common reference for all kinetics. This is done by using the final solution. A 3D data is obtained but it shows the change of absorbance against the final state in function of the time and not the absorbance in function of the time.

You need to choose one 2 D trace, set cursor 1 and 2 in the zone where the kinetics is finished and then go in **Tools, Global Baseline**.

5.3.3. How to run a Kinetics using Global Mode

In the main menu choose Install, Device installation.

Select MOS200M

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.
- Push some buffer into the cuvette for further reference or High voltage adjustment.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

Acquisition parameters

Select the Device, Global mode menu.

In the main window, click on the **Auto** button to adjust the High voltage automatically. Click a second time when the response is stable.

Click on the **Acquisition Setup** button.

Select the absorbance acquisition mode

Select in Excitation the 1st wavelength and the last wavelength of the scan

Do not check the PM gain x 10 option.

Choose the number of time base, your sampling period(s) and the number of points

The cuvette is filled of water so it is possible to do a reference spectrum for further absorbance calculations.

- Click on the **Record** button in the **Reference Spectrum** area.

(About 5V should be display in the corresponding state panel)

Click OK to accept these parameters.

Running the shot

Click on the start button of the scope if you want to run some shots.

Click on the start button of the global control window to run the global shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

5.4. Scanning spectrometer device

The scanning spectrometer window is used to drive the MOS-200M monochromators to perform acquisition of spectral data in various modes. Associated to the MOS-200M hardware, Bio-Kine can feature a full absorbance or fluorescence spectrometer. With the corresponding optional hardware it can also acquire fluorescence spectral data.

Interface with other hardware may also be possible, please refer to Bio-Logic for this possibility.

At its first use, the scanning spectrometer device must be installed into the main menu. Run **Install, Device installation**, to install the spectrometer option (refer to section 1.3) Once this installation is made the spectrometer window is loaded by choosing **Device, Scanning Spectrometer**, the window shown in Figure 123 will be displayed.

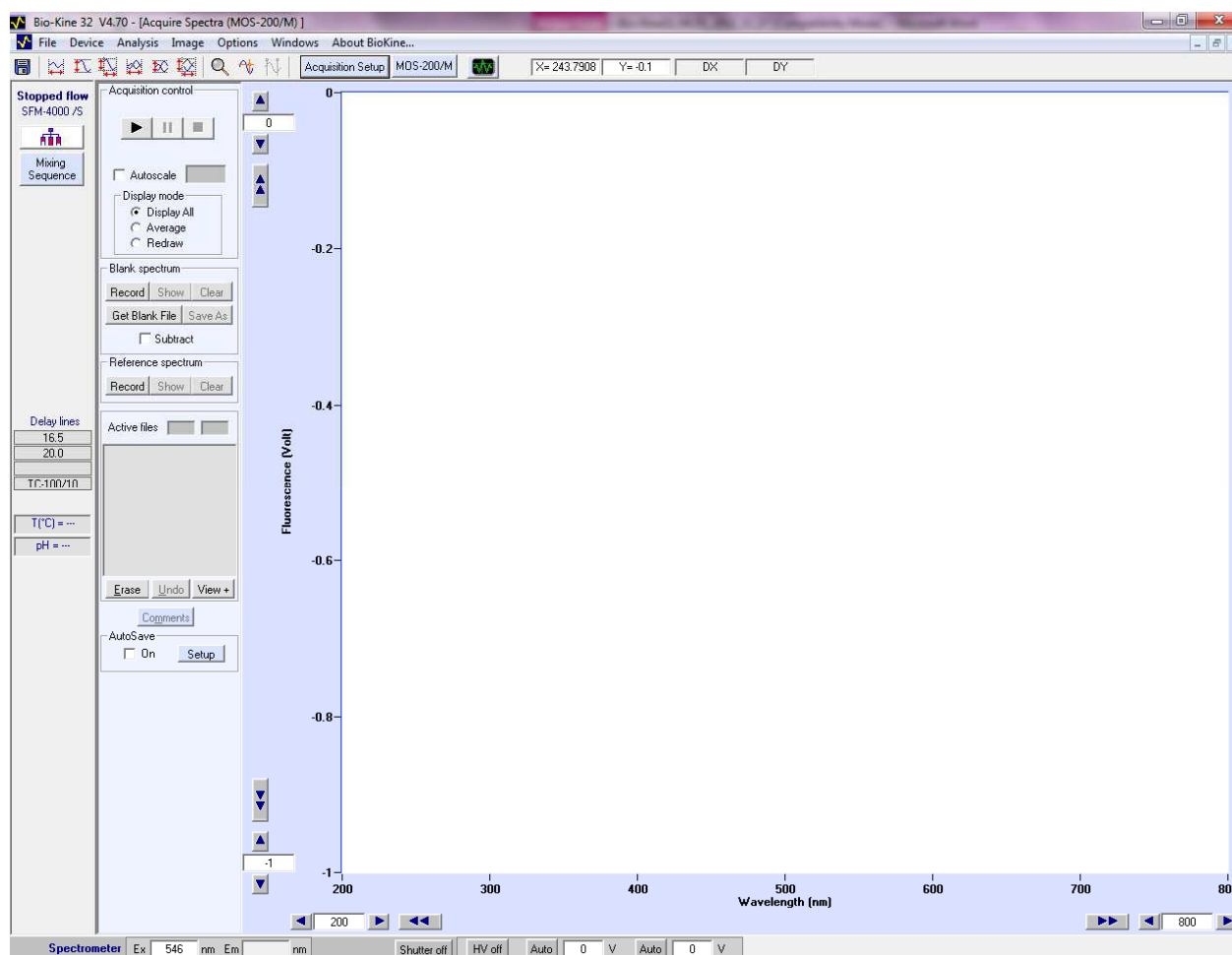


Figure 123 : Scanning spectrometer window.

5.4.1. Fluorescence scan

Click on the **Acquisition Setup** button to load the setup window. If the software was previously used in the fluorescence mode, the window shown in Figure 124 will appear. If another scan window is loaded, then choose **Fluorescence**, using the **Up and Down** button of the acquisition mode box.

Fluorescence measurements are made with the monochromator for excitation and a PM with cut-off filter for emission.

Excitation spectra are taken by moving the excitation spectrometer from one wavelength to another and by setting the emission spectrometer at a fixed value (select **mode Excitation**, with **Single Detection wavelength**). Emission spectra are taken by moving the emission monochromator at fixed excitation wavelength values. (Select **Emission mode** and **Single/Multiple Illumination wavelength**).

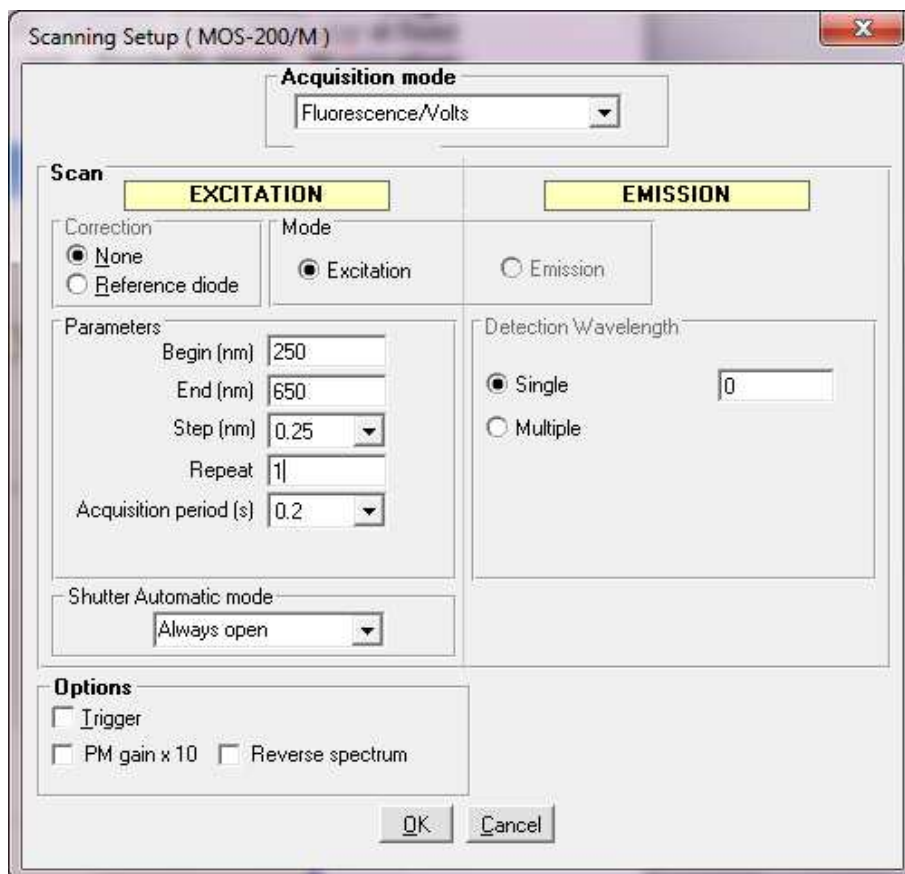


Figure 124 : Scan window in fluorescence mode.

In the parameters box, choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Acquisition duration: use the 'up and down' button to select the speed of the scan.

Scan repeat : set the desired scan number

Scan step: set the desired scan step.

For adequate fluorescence excitation spectra, corrections must be performed to compensate for the wavelength dependence of the energy of the lamp and for the wavelength dependence of the transmittance of the excitation monochromator. The correction ratio is calculated using a reference diode and the corrected spectra are performed checking the reference diode button. If not (when the none box is checked), the spectra are done without any compensations.

If a trigger is required check the trigger box in the options area.

Click on the start button of the 'scope' to run a scan.

5.4.2. Transmittance scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the transmittance mode, the window shown in Figure 125 will appear. If another scan window is loaded, then choose **Transmittance** using the **Up and Down** button of the acquisition mode box.

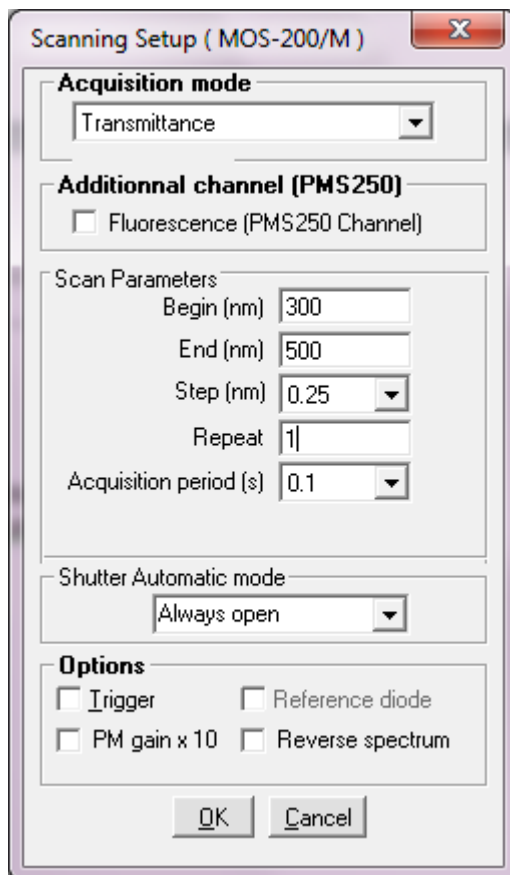


Figure 125 : Scan window in transmittance mode.

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Speed : use the 'up and down' button to select the speed of the scan.

Scan repeat : set the scan number

Scan step: set the desired scan step.

If a trigger or a diode correction are required check their respective box in the options area

In the Transmittance mode it is necessary to record a "reference spectrum" before the series of measurements. To record this spectrum open the scan setup window choose Record in the 'Reference spectrum' area so as to record it. Once this will be achieved, the Done box in the 'Reference spectrum' area will be checked. Transmittance is calculated as follows :

$$\text{Transmittance (\%)} = I(\lambda) * 100 / \text{Reference}(\lambda)$$

To display the reference trace, click on Show/Hide Ref.spectr. on the Scanning Spectrometer window (see Figure 123).

5.4.3. Absorbance scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the absorbance mode, the window shown in Figure 126 will appear. If another scan window is loaded, then choose **Absorbance** using the **Up and Down** button of the acquisition mode box.

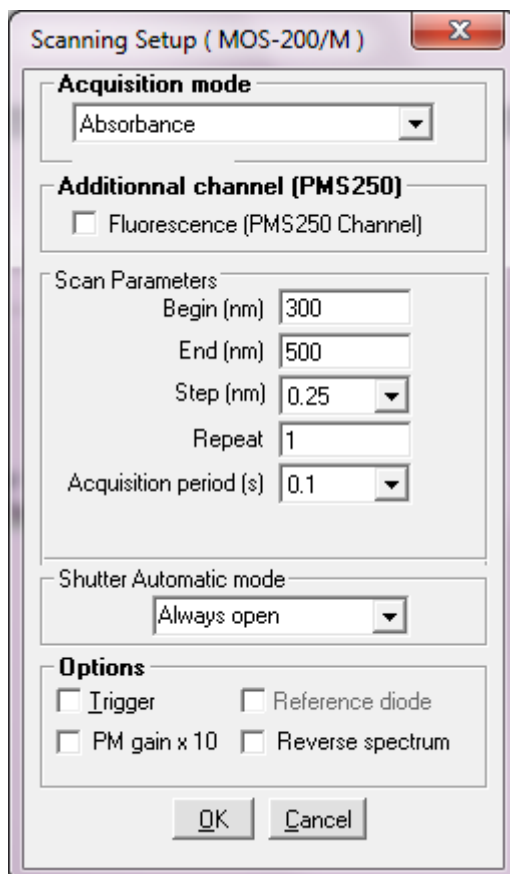


Figure 126 : Absorbance scan window.

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Speed : use the 'up and down' button to select the speed of the scan.

Scan repeat : set the scan number

Scan step: set the desired scan step.

If a trigger is required check their respective box in the options area

In the Absorbance mode it is necessary to record a "reference spectrum" before the series of measurements. To record this spectrum open the scan setup window choose Record in the 'Reference spectrum' area so as to record it. Once this will be achieved, the Done box in the 'Reference spectrum' area will be checked. absorbance is calculated as follows :

$$\text{Absorbance (A.U)} = \text{Log}_{10} (\text{Reference}(\lambda)/I(\lambda))$$

To display the reference trace, click on Show/Hide Ref.spectr. on the Scanning Spectrometer window (see Figure 123)

6. CONTROL OF MOS-500

6.1. MOS-500 control and calibration

From the Install, Device menu choose MOS-500, please refer to section 1.3 for further information. When using a MOS-500 the bandwidth of the excitation slits is set directly from the software. The wavelength of monochromators can be set from their respective state panel at the bottom of the screen. It is also possible to control these monochromators by clicking on the **MOS-500** button and then entering the wavelength in the corresponding box as shown in Figure 127.

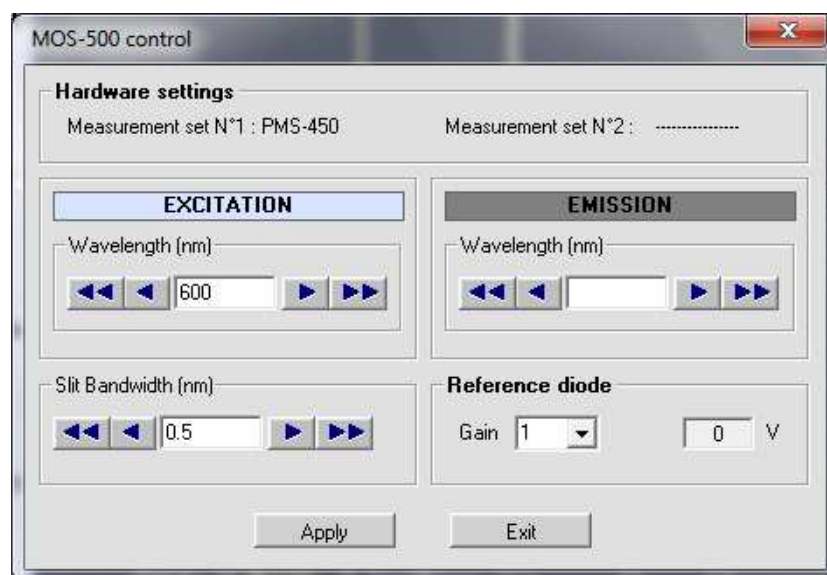


Figure 127 : Wavelength control

Choose **Install, Spectrometer advanced settings** in the main menu to load the window shown in Figure 128.

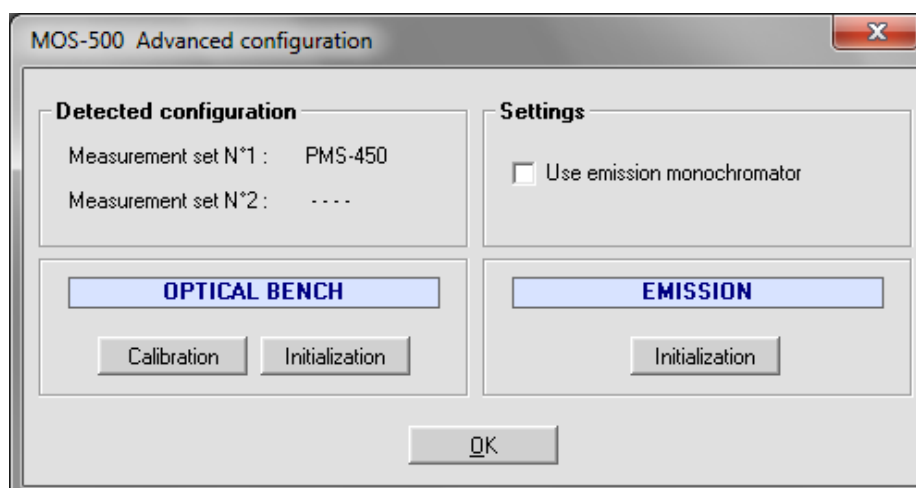


Figure 128 : MOS-500 configuration

The windows displayed are separated into 4 areas:

The detected configuration is indicated on the left upper part, in this example only a PMS-450 is detected but in some configurations a second measurement set can also be recognized.

The right upper part is useful when an emission monochromator is installed, if necessary select the **'Use emission monochromator'** check box.

Manual Calibration of the excitation monochromator is done at the factory and can be accurately checked when using a Xe(Hg) lamp. Very precise peaks in intensity are available at many wavelengths (Figure 129).

An automatic calibration of the MOS-500 is performed every time you switch on the device.

CAUTION: to allow a proper calibration of these excitation monochromator, it is compulsory to follow this order:

- 1/ Switch on the MOS-500 and the lamp .
- 2/ Launch bio-kine

For the **emission monochromator**, the manual calibration has also been done at the factory. Checking the calibration can be performed in an easy way.

Example: make a scan between 500 to 600 nm (with the excitation monochromators set on "0"). A very clear peak of green light must be recorded at 546 nm

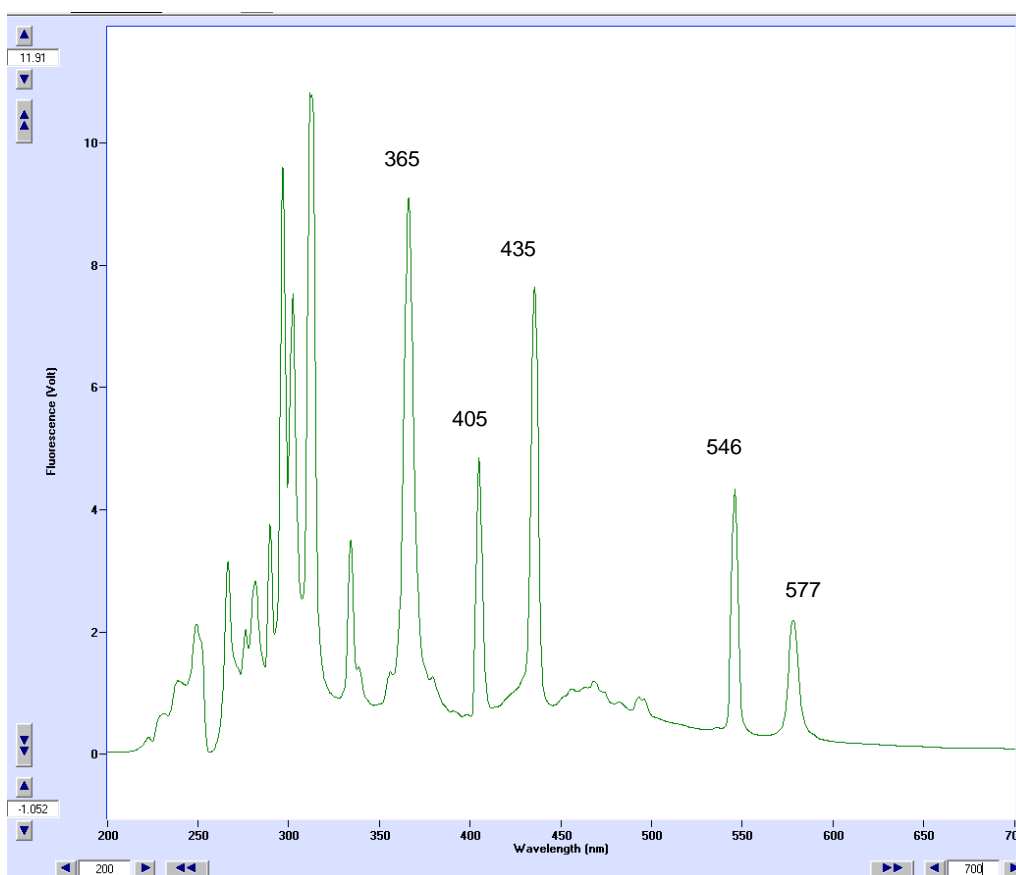


Figure 129 : Xe(Hg) lamp spectrum

• Monochromators can be initialized at any time using the **Initialization** button; the aim of this function is to check the calibration of the system in three phases:


- In a first phase the monochromator is going from the last wavelength selected to the mechanical zero of the monochromator.
- In a second phase, the zero is adjusted considering the value of Delta found in the Calibration.
- In a third phase, the monochromator is going from the real zero to the wavelength selected at the beginning of the first phase.

If the calibration is not done properly then the wavelength given at the end of the initialization is different from the one observed at the beginning.

These three phases of the initialization procedure are made automatically by the software when starting the system or when pressing the **Initialization** button.

6.2. High voltage control

The High voltage state panel (bottom of the main screen) enables the adjustment of the high voltage applied on the photomultiplier (PM). The Voltmeter displays the response of the PM. The number of displayed channels is modified automatically according to the number of working channels. The

voltmeter can be hidden or displayed at any time using the  button.

Two other buttons are also available to control the high voltage:

- **Auto** : this button can be used to adjust the high voltage automatically to get a 5 Volts response of the photomultiplier, once this button active its color turns to orange.
- By clicking on the **HV on** button it is possible to turn the high voltage off, the color of the button turns to grey (**HV off**). A second click on the button will automatically set the high voltage to the last value used.

In the case the high voltage is too high and a saturation of signal is observed, the high voltage is decreased automatically to protect the PM. A warning message is displayed, it proposes to the user to reduce the value or to restore it.

If two photomultipliers are used for the detection, two **Auto** buttons are created (refer to Figure 130) their high voltage could be automatically and independently adjusted using their respective button.



Figure 130 : High voltage control

If the **Voltmeter** check box (Figure 130) is checked then the voltmeter displays values in units as they are defined as in the in the parameters setup .

6.3. Scanning spectrometer device

The scanning spectrometer window is used to drive the MOS-500 monochromators to perform acquisition of spectral data in various modes. Associated to the MOS-500 hardware, Bio-Kine can feature a full absorbance, fluorescence or circular dichroism spectrometer. It can also acquire fluorescence anisotropy spectral data.

Once this installation is made the spectrometer window is loaded by choosing: **Device, Scanning Spectrometer**, the window shown in Figure 131 will be displayed.

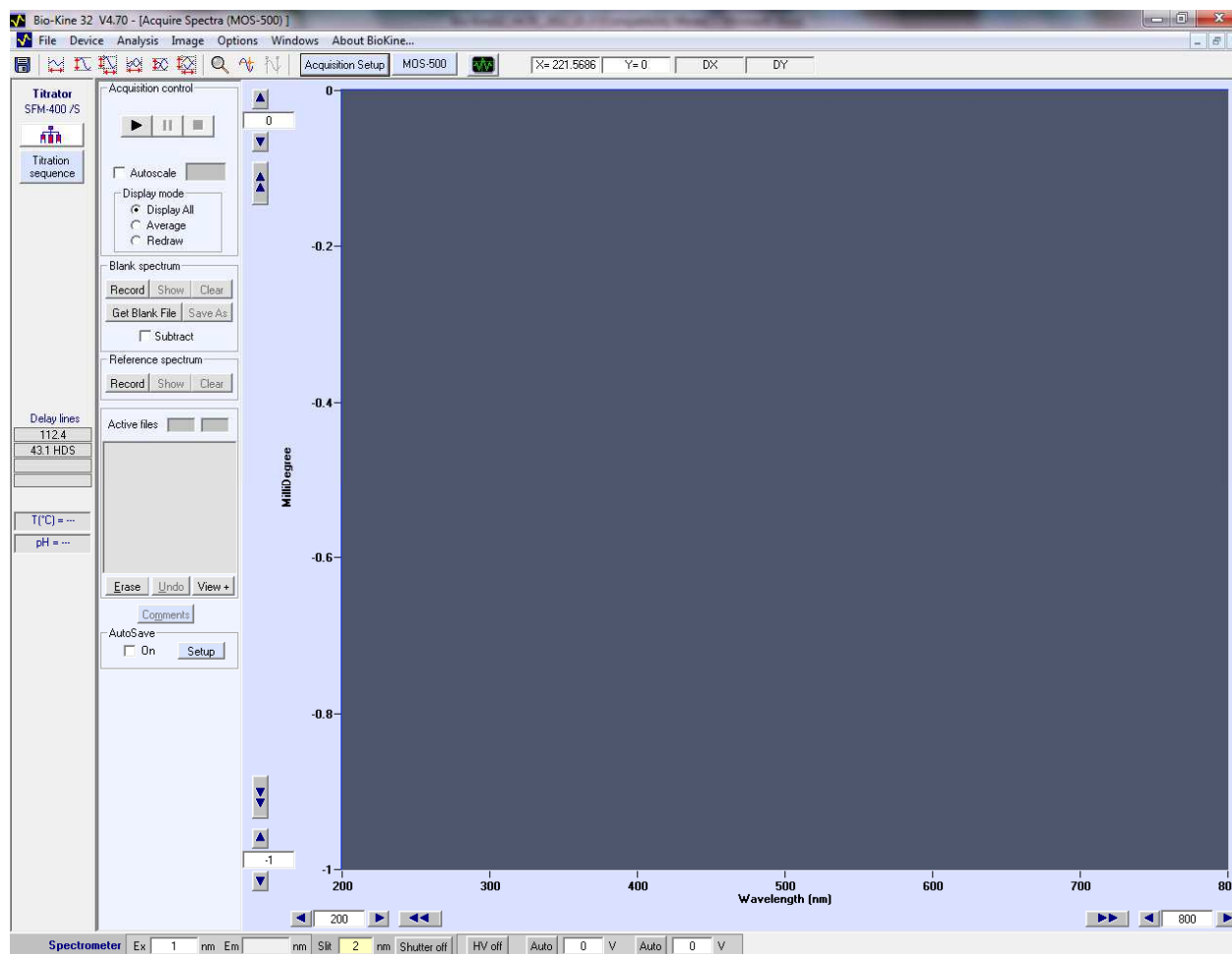


Figure 131 : Scan window using MOS-500

6.3.1. Fluorescence scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the fluorescence mode, the window shown in Figure 132 will appear. If another scan window is loaded, then choose Fluorescence using the Up and Down button of the acquisition mode box.

Excitation spectra are taken by moving the excitation monochromator from one wavelength to another and by setting the emission monochromator at a fixed value (select mode Excitation, with Single Detection wavelength). There is a possibility to repeat automatically these acquisitions for several emission wavelength by selecting Multiple Detection Wavelength: choose then the starting and ending wavelengths and also the scan step (in nanometers). If your instrument do not have an emission monochromator then it is still possible to do excitation spectrum using cut-off filter in the photomultiplier.

Emission spectra can only be made if your MOS-500 is equipped with an emission monochromator. Emission spectra are taken by moving the emission monochromator at fixed excitation wavelength values. (Select Emission mode and Single/Multiple Illumination wavelength).

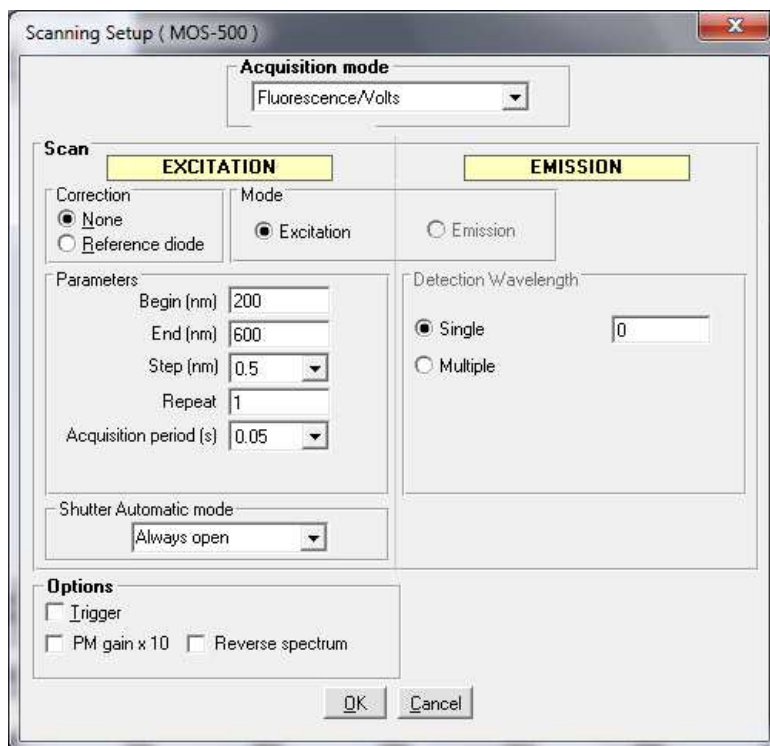


Figure 132 : Scan window in fluorescence mode

Select the excitation or emission fluorescence mode choosing the corresponding button.

In the parameters box, choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Scan step: set the desired scan step

Scan repeat : set the desired scan number.

Acquisition duration: duration of the measurement for each nm.

The acquisition duration may vary from 0.05s to 5s.

For adequate fluorescence excitation spectra, corrections must be performed to compensate for the wavelength dependence of the energy of the lamp and for the wavelength dependence of the transmittance of the excitation monochromator. The correction ratio is calculated using a reference diode and the corrected spectra are performed checking the reference diode button. If not (when the "none" box is checked), the spectra are done without any compensations.

If a trigger is required check the trigger box in the options area.

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum are also possible.

To run a scan refers to section 6.3.12.

6.3.2. Transmittance scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the transmittance mode, the window shown in Figure 133 will appear. If another scan window is loaded, then choose Transmittance using the Up and Down button of the acquisition mode box.

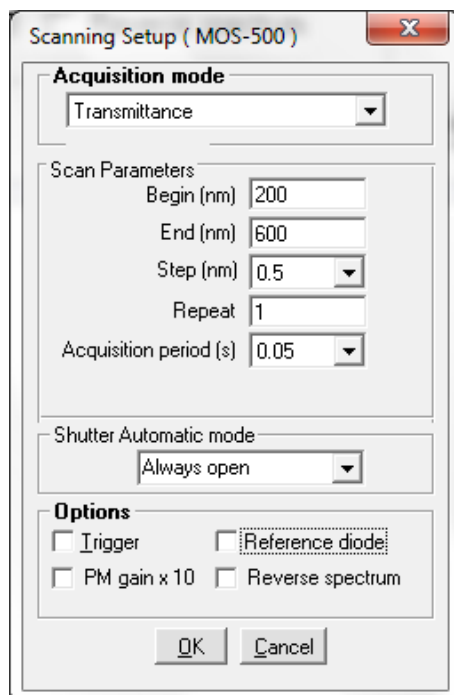


Figure 133 : Scan window in transmittance mode

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Scan step: set the scan number.

Scan repeat : set the scan number.

Acquisition duration: duration of the measurement for each nm. The acquisition duration varies from 0.05s to 5s.

If a trigger or a diode correction are required check their respective box in the options area

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum are also possible.

In the Transmittance mode it is necessary to record a "reference spectrum" before the series of measurements. To record this spectrum open the scan setup window, choose Record in the 'Reference spectrum' area so as to record it. Once this will be achieved, the Done box in the 'Reference spectrum' area will be checked. Transmittance is calculated as follows :

$$\text{Transmittance (\%)} = I(\lambda) * 100 / \text{Reference}(\lambda)$$

To display the reference trace, click on Show/Hide Ref.spectr. on the Scanning Spectrometer window (Figure 131).

To run a scan refers to section 6.3.11.

6.3.3. Absorbance scan.

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the absorbance mode, the window shown in Figure 134 will appear. If another scan window is loaded, then choose Absorbance using the Up and Down button of the acquisition mode box.

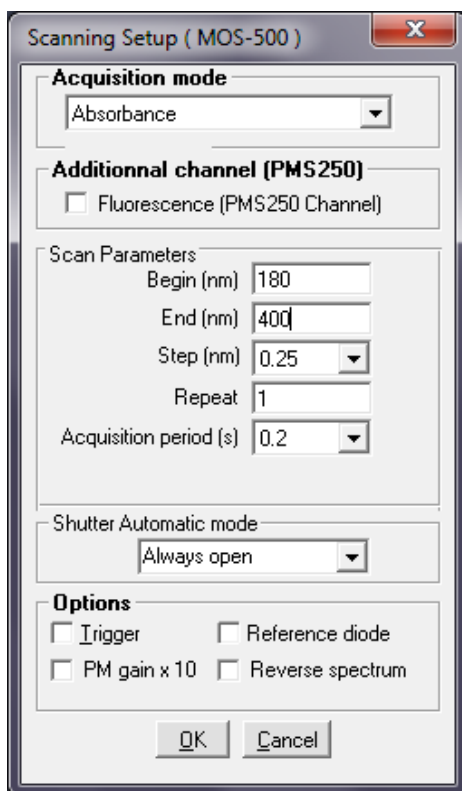


Figure 134 : Absorbance scan window

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Scan repeat : set the scan number.

Acquisition duration : duration of the measurement for each nm. The acquisition duration may vary from 0.05s to 5s.

Scan step: set the desired scan step

If a trigger or a diode correction is required check their respective box in the options area

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum are also possible.

In the Absorbance mode it is necessary to record a "reference spectrum" before the series of measurements. To record this spectrum open the scan setup window choose Record in the 'Reference spectrum' area so as to record it. Once this will be achieved, the Done box in the 'Reference spectrum' area will be checked. absorbance is calculated as follows :

$$\text{Absorbance (A.U)} = \text{Log}_{10} (\text{Reference } (\lambda)/I(\lambda))$$

To display the reference trace, click on Show/Hide Ref. spectr. On the Scanning Spectrometer window (Figure 131).

To run a scan refers to section 6.3.11

6.3.4. Anisotropy scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the anisotropy mode, the window shown in Figure 135 will appear. If another scan window is loaded, then choose Anisotropy using the Up and Down button of the acquisition mode box.

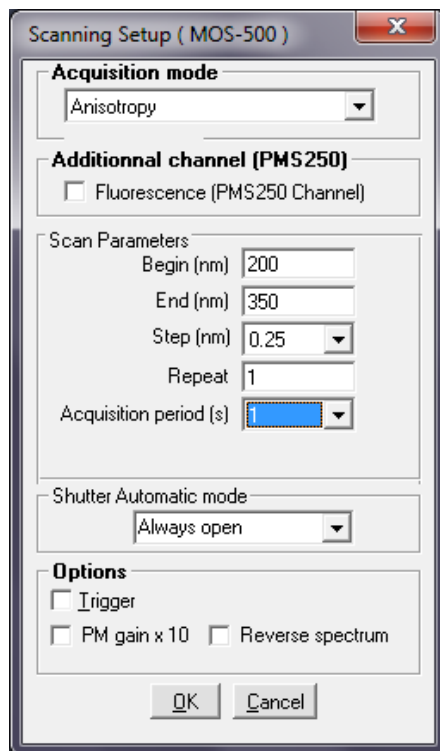


Figure 135 : Anisotropy scan window.

Choose your scan parameters :

Begin: first wavelength of the scan in nm.

End: last wavelength of the scan in nm.

Scan repeat: set the scan number.

Acquisition duration: duration of the measurement for each nm. The acquisition duration may vary from 0.05s to 5s.

Scan step: select a scan step number

The response of the photomultiplier can be amplified 10 times checking the corresponding box, it may be useful when working with very low signal and when the High Voltage is higher than 900 volts.

If a trigger is required check the appropriate box in the options area.

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum are also possible.

The PM gain x 10 option is used to amplify the response of the photomultiplier when signals are low and High Voltage is higher than 900V.

To run a scan refers to section 6.3.12.

6.3.5. Circular Dichroism scan

Click on the **Acquisition Setup** button to load the setup window.

If the software was previously used in the CD mode, the window shown in Figure 136 will appear. If another scan window is loaded, then choose CD using the Up and Down button of the acquisition mode box.

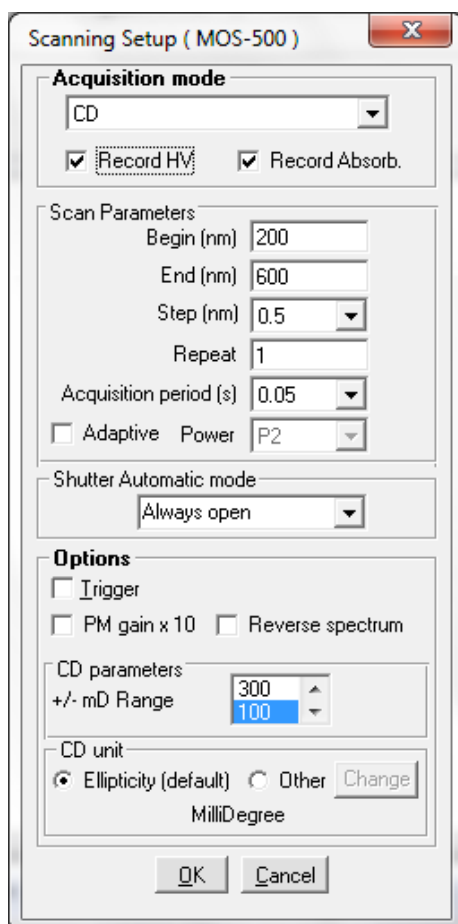


Figure 136 : Scan window in CD mode

Choose your scan parameters :

Begin: first wavelength of the scan in nm.

End: last wavelength of the scan in nm.

Scan repeat: set the scan number.

Acquisition duration: duration of the measurement for each nm (in the example above 50ms for 1nm, which corresponds to a 1200 nm/min speed for the scan). The acquisition duration may vary from 0.05s to 5s.

Scan step: select a scan step number

The response of the photomultiplier can be amplified 10 times checking the corresponding box, it may be useful when working with very low signal.

If a trigger is required check the appropriate box in the options area.

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum and Recording the High voltage (Record HV) are also available.

The CD sensitivity must be set according to the amplitude of the signal observed. Four different sensitivities are available: 1000, 300, 100 and 30 mdeg.

CD unit displayed by default is in mdeg. Other units can be selected: By clicking on “**other**”. A window opens allowing the selection of: specific ellipticity, Molar ellipticity, Mean residue ellipticity, Molar CD, Mean residue molar CD, CD (delta OD).

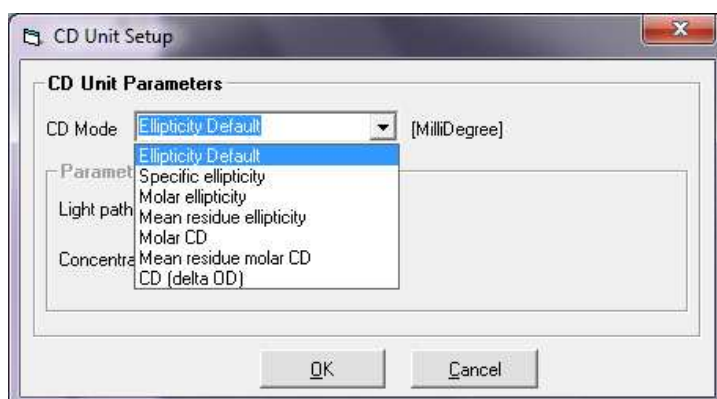


Figure 137: CD unit selection

1. Specific ellipticity: [Ψ] in (deg.cm².decagram⁻¹)

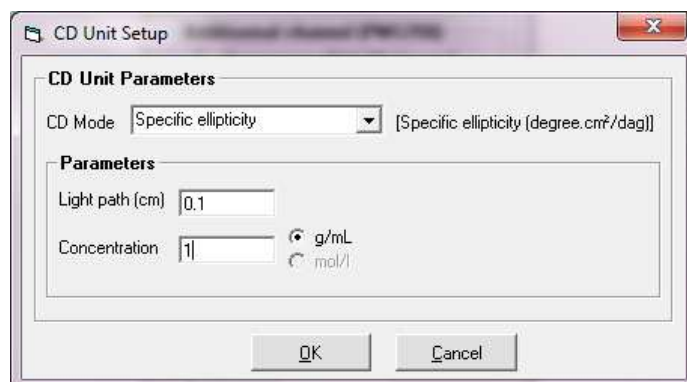


Figure 138: Specific ellipticity

The user must enter the light path of the cuvette used, in cm, and the concentration of the sample in mg.mL⁻¹, then the calculation of the specific ellipticity is performed through the equation below:

$$[\Psi] = \frac{\theta}{100 \cdot C' \cdot l}$$

θ : ellipticity (mdeg)
 C' : concentration (g/mL)
 l : Pathlength of the cell (cm)

2. Molar ellipticity: $[\theta]$ in (deg.cm².decimol⁻¹)

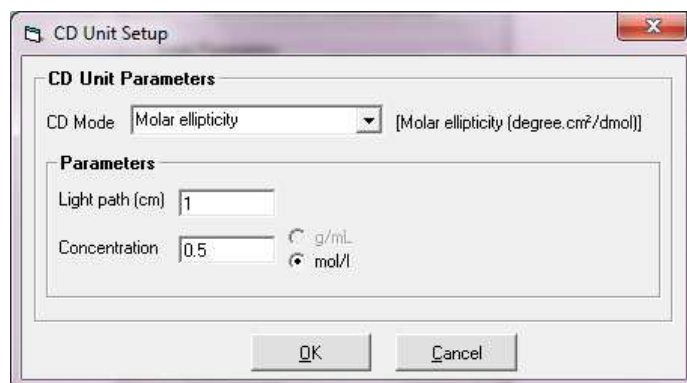


Figure 139: Molar ellipticity

The user must enter the light path of the cuvette used, in cm, and the concentration of the sample in mol.L⁻¹, then the calculation of the specific ellipticity is performed through the equation below:

$$[\theta] = \frac{\theta}{10 \cdot C \cdot l}$$

θ : ellipticity (mdeg)

C: Molar concentration (mol/L)

l: Pathlength of the cell (cm)

3. Mean residue ellipticity: $[\theta]_{MR}$ in (deg.cm².decimol⁻¹)

For macromolecules such as protein, nucleic acid etc..., the mean residue is an important unit allowing to define the molar ellipticity for individual protein residue instead of the whole protein molecules. This is a quite useful unit for comparing CD behaviors of various proteins with different molecular weight.

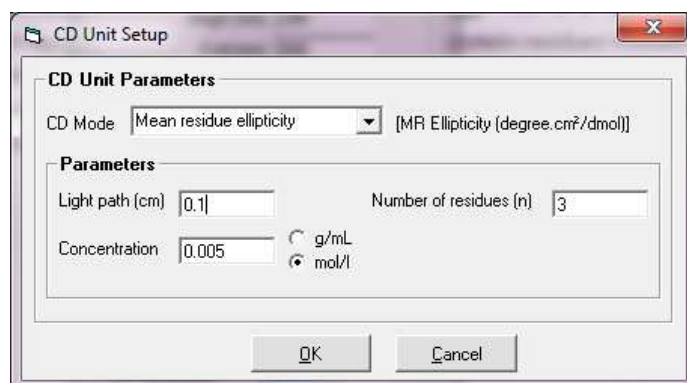


Figure 140: Mean residue ellipticity

The user must enter the light path of the cuvette used, in cm, and the concentration of the sample in mol.L⁻¹, then the calculation of the specific ellipticity is performed through the equation below:

$$[\theta]_{MR} = \frac{\theta}{n \cdot 10 \cdot C_R \cdot l}$$

θ : ellipticity (mdeg)
 C: Molar concentration (mol.L⁻¹) or g.mL⁻¹ (then Molecular Weight should be entered in the program)
 l: Pathlength of the cell (cm)
 n: number of residue

4. Molar CD or molar circular dichroic absorption: $\Delta\epsilon$ in (cm².mmol⁻¹)

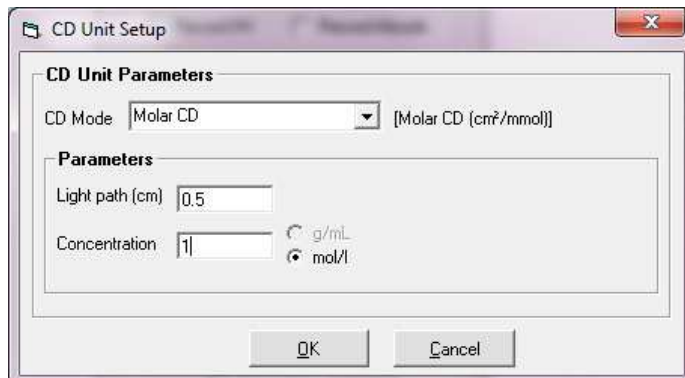


Figure 141: Molar CD units

The user must enter the light path of the cuvette used, in cm, and the concentration of the sample in mol.L⁻¹, then the calculation of the specific ellipticity is performed through the equation below:

$$\Delta\epsilon = \frac{\theta}{3298.2}$$

θ : ellipticity (mdeg)

5. Mean residue molar CD: $\Delta\epsilon$ in (cm².mmol⁻¹)

For macromolecules such as protein, nucleic acid etc..., the mean residue molar is an important unit allowing to define the molar ellipticity for individual protein residue instead of the whole protein molecules. This is a quite useful unit for comparing CD behaviors of various proteins with different molecular weight.

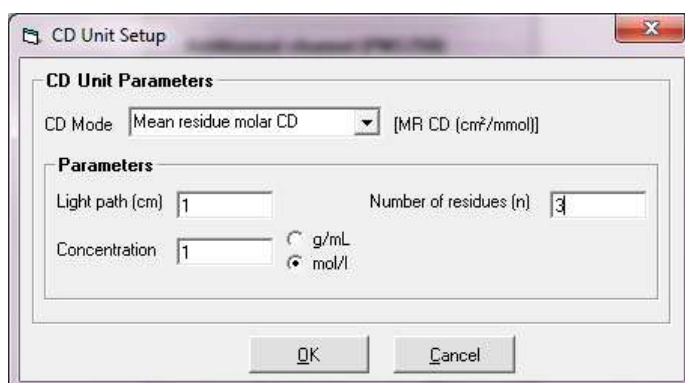


Figure 142: Mean residue molar CD

$$\Delta\epsilon = \frac{\theta}{n \cdot 3298.2}$$

θ : ellipticity (mdeg)
 n: number of residue

6. Circular dichroic absorption : CD Δ OD

Converting ellipticity to Circular dichroic absorption is done through a simple equation:

$$\Delta OD = \frac{\theta}{32980}$$

It is not necessary to integrate parameters.

In CD mode, during a scan, the high voltage is automatically set in order to get a CD/DC response constant and equal to 5.

To run a scan refers to section 6.3.13.

6.3.6. MCD Scan

MCD scan is done through the CD acquisition window. Spectra are acquired with the magnet placed on north and then south position. A computation is finally performed through the MCD analysis menu to get the MCD signal associated to the molecule studied. More information is available on the MOS-500 manual.

6.3.7. ORD scan

Click on the **Acquisition Setup** button to load the setup window, then choose ORD using the Up and Down button of the acquisition mode box.

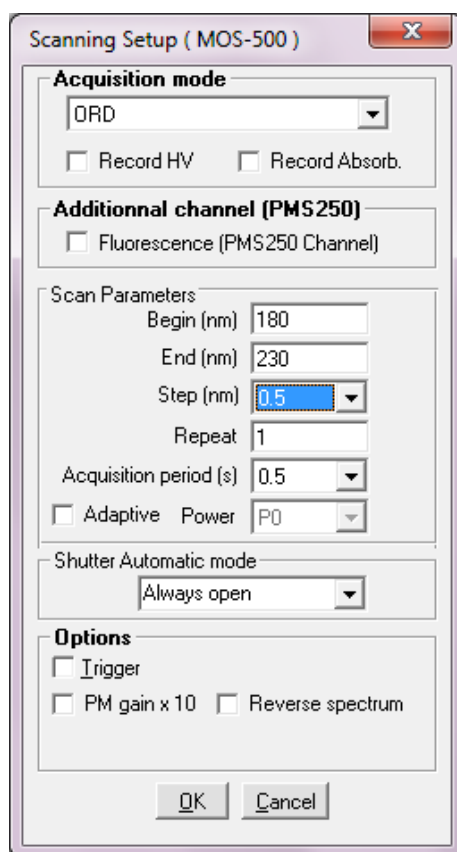


Figure 143: scan window in ORD mode

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Step: 0.25 nm to 10 nm

Scan repeat : set the scan number.

Acquisition duration : duration of the measurement for each nm. The acquisition duration may vary from 0.05s to 5s.

The response of the photomultiplier can be amplified 10 times checking the corresponding box, it may be useful when working with very low signal.

If a trigger is required check the appropriate box in the options area.

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum and Recording the High voltage (Record HV) are also available.

To run a scan refers to section 6.3.14.

6.3.8. Linear Dichroism scan

Click on the  button to load the setup window.

If the software was previously used in the LD mode, the window shown in Figure 144 will appear. If another scan window is loaded, then choose LD using the Up and Down button of the acquisition mode box.

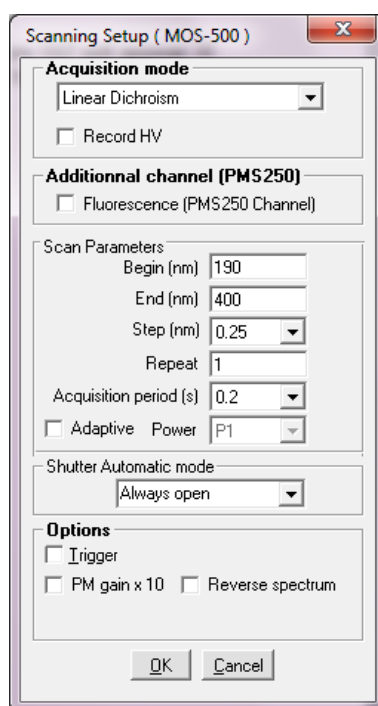


Figure 144: scan window in LD mode

Choose your scan parameters :

Begin : first wavelength of the scan in nm.

End : last wavelength of the scan in nm.

Step: 0.25 nm to 10 nm

Scan repeat : set the scan number.

Acquisition duration : duration of the measurement for each nm. The acquisition duration may vary from 0.05s to 5s.

The response of the photomultiplier can be amplified 10 times checking the corresponding box, it may be useful when working with very low signal.

If a trigger is required check the appropriate box in the options area.

If steps of 0.5nm are required please check the corresponding box. Reverse spectrum and Recording the High voltage (Record HV) are also available.

To run a scan refers to section 6.3.14.




6.3.9. *Global Mode*

The global mode is used to drive the MOS-500 monochromators to perform acquisition of Kinetics data at various wavelength. The spectrometer is fully automated and synchronized with the stopped-flow mixer. Please refer to chapter 5.3.

6.3.10. Graphical tools

For all the acquisition devices (fluorescence, transmittance, absorbance, CD or anisotropy), the graphic tools are similar to that have been described for the analysis window in section 2. Only the **Math** and **Tools** menus are specific to the analysis window. Some tools are specific to the acquisition device window (refer to Figure 131) :

To display an eventual reference trace, click on Show/Hide Ref.spectr. on the Scanning Spectrometer window.

Acquisition Run: click on Run () to start the data acquisition. the acquisition can be paused/stopped with Pause/Stop ( / ) buttons.

Display Mode: In spectral acquisition each new acquisition may be stored in a new trace (within the limit of 10 traces). There are three modes to display the new spectra :

- Display All: displays the new trace leaving the old ones on the screen.
- Average: add and average the Scan Repeat spectra acquired during the same run. If Scan Repeat is set to one than the Average mode is equivalent to the Redraw mode.
- Redraw: the new spectrum replaces the previously recorded trace.

An instantaneous auto scale may also be used checking the Auto Scale box.

A blank spectrum may also be recorded. Run the desired blank spectrum click on the Save as button in the 'Blank area' (see Figure 131) to save it. To load a previously saved blank spectrum click on the Get Blank File button. Click on the Use as Blank button to use a loaded spectrum as blank and check the Subtract box to subtract it automatically to all the spectra made later. To display the blank on the screen during spectra acquisition click on the Show button in the 'Blank area'.

6.3.11. Steady state measurement in absorbance and transmittance mode:

The method presented here is a general example to perform a steady state measurement. For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a spectrum.

Getting started

Fill a 1 cm quartz cuvette with water.

Run the Bio-Kine software.


In the main menu choose Install, Device installation.

- Select MOS-500.
- Click **OK** to validate.

Monochromators are automatically initialized.

- Select the Device, Scanning spectrometer menu.
- In the main window select a wavelength inside the limits of the scan you want to perform.
- In the main window, select a high voltage in the HV state panel in the bottom of the screen.


Scanning Setup

Click on the  Button to load the scanning set up window.

- Select the absorbance mode using the up and down button.
- Enter a scanning lower limit.
- Enter a scanning upper limit.
- Enter an acquisition time.

- Select the number of scan to repeat.
- Do not select the **trigger**, **diode reference** and **PM gain** check boxes.
- Click on the **Record** button. A reference spectrum (corresponding to the lamp spectrum) is done automatically and displayed on the screen. As soon as the reference spectrum is done the scanning setup window is displayed with the done check box (close to the Record button) checked.
- Click on **OK** to validate.

High voltage adjustment

In the Reference spectrum area on the main window click on the  button to display the reference spectrum made before.

The spectrum of the lamp is shown in dotted line. The difference of light intensity is clearly observed. To get as much light as possible on the whole spectrum, it is better to reach a maximum light intensity around 8 or 9 volts.

If the maximum light intensity obtained in your spectrum is too low, then increase the high voltage as explained before and repeat the procedure to do the reference spectrum.

Repeat until reaching 8 or 9V for the maximum.

The reference spectrum for absorbance calculation is now done


Baseline

To get a blank spectrum leave the water in the cuvette and press the  button.

A baseline is obtained; it is possible to subtract this baseline to further spectra using graphic tools.

Absorbance spectrum

Remove the water from the cuvette and fill it with sample.

Run the scan using the  Start button.

The absorbance spectrum is now displayed.

6.3.12. Steady state measurement in fluorescence and anisotropy modes :

The method presented here is a general example to perform a steady state measurement. For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a spectrum

Getting started

Fill a 1 cm quartz cuvette with water.

Run the Bio-Kine software.


In the main menu choose Install, Device: an installation window is displayed on the screen.

- Select MOS-500
- Click **OK** to validate.

Monochromators are automatically initialized

Select the Device, Scanning spectrometer menu.

Scanning Setup

Click on the  Button to load the scanning set up window.

Select the fluorescence or anisotropy mode using the up and down button.

Choose the excitation or emission mode for fluorescence measurements.

Select an excitation wavelength when working in emission mode.

Select an emission wavelength when working in excitation mode and using an emission monochromator; when working with a cut-off filter and not with an emission monochromator choose 0 nm as an emission wavelength.

Enter a scanning lower limit.

Enter a scanning upper limit.

Enter an acquisition time.


Select the number of scan to repeat.


Do not select the **trigger**.

Check the reference diode, and use the PM gain only if the high voltage used is higher than 900 volts.

Click on **OK** to validate.

High voltage adjustment


Select a set of excitation and emission wavelength inside your scan limits by clicking on the  button. Click **OK** to validate.

In the main window, Click on the  button to adjust automatically the high Voltage applied on the PM to get a 5V response.

Blank


To get a blank spectrum press the  button.

The fluorescence spectrum of water is displayed

Click on the  button to use the spectrum previously made as a blank for further measurements. Check the 'Subtract' Box to subtract automatically the blank to further measurements

Fluorescence spectrum

Remove the water from the cuvette and fill it with sample-

Run the scan using the  Start button.

The fluorescence spectrum is now displayed.

6.3.13. Steady state measurement in circular dichroism mode :

The method presented here is a general example to perform a steady state measurement. For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a spectrum.

Getting started

Fill a 1 cm quartz cuvette with water.


Run the Bio-Kine software.

In the main menu choose Install, Device: an installation window is displayed on the screen.

- Select MOS-500.
- Click **OK** to validate.

Monochromators are automatically initialized

Scanning Setup

Click on the  Button to load the scanning set up window.

Select CD mode using the up and down button.

Enter a scanning lower limit.

Enter a scanning upper limit.

Enter an acquisition time.

Select the number of scan to repeat.

Do not select the trigger and the PM gain check boxes.

Select sensitivity according to the amplitude of the signal.


Click on **OK** to validate.

Blank

To get a blank spectrum press the  button.


The high voltage applied on the photomultiplier is adjusted automatically during the scan.

The CD spectrum of water is displayed.

Click on the  button to use the spectrum previously made as a blank for further measurements. Check the 'Subtract' Box to subtract automatically the blank to further measurements

CD spectrum

Remove the water from the cuvette and fill it with sample.

Run the scan using the  Start button.

The CD spectrum is now displayed.

6.3.14. Steady state measurement in linear dichroism mode:

The method presented here is a general example to perform a steady state measurement. For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a spectrum.

NOTE: In steady state mode MOS-500 is coupled to a rotative linear dichroism cell

Getting started

Fill the LD capillary with DNA.

Run the Bio-Kine software.


In the main menu choose Install, Device: an installation window is displayed on the screen.

Select MOS-500.

Click OK to validate.

Monochromators are automatically initialized

Scanning Setup

Click on the  Button to load the scanning set up window.

Select LD mode using the up and down button.

Enter a scanning lower limit.

Enter a scanning upper limit.

Enter an acquisition time.

Select the number of scan to repeat.

Do not select the trigger and the PM gain check boxes.


Click on OK to validate.

Blank

To get a blank spectrum press the  button.


The high voltage applied on the photomultiplier is adjusted automatically during the scan.

The LD spectrum of DNA is displayed.

Click on the  button to use the spectrum previously made as a blank for further measurements. Check the 'Subtract' Box to subtract automatically the blank to further measurements

LD spectrum

Start the rotative cell with DNA inside.

Run the scan using the  Start button.

The LD spectrum is now displayed.

6.4. Transient recorder device

6.4.1. Getting started

The transient recorder device is similar to a digital oscilloscope. It is the device used to follow rapid Kinetics. All acquisition modes (fluorescence, absorbance, transmittance, circular dichroism, fluorescence anisotropy) are controlled from this device. An acquisition is linked to a stopped-flow sequence so make sure that a stopped-flow instrument and its MPS are connected to the system. Synchronization between acquisition and driving sequence is made using MPS software simultaneously.

- In the main menu choose **Device, transient Recorder** to load the window shown in Figure 145

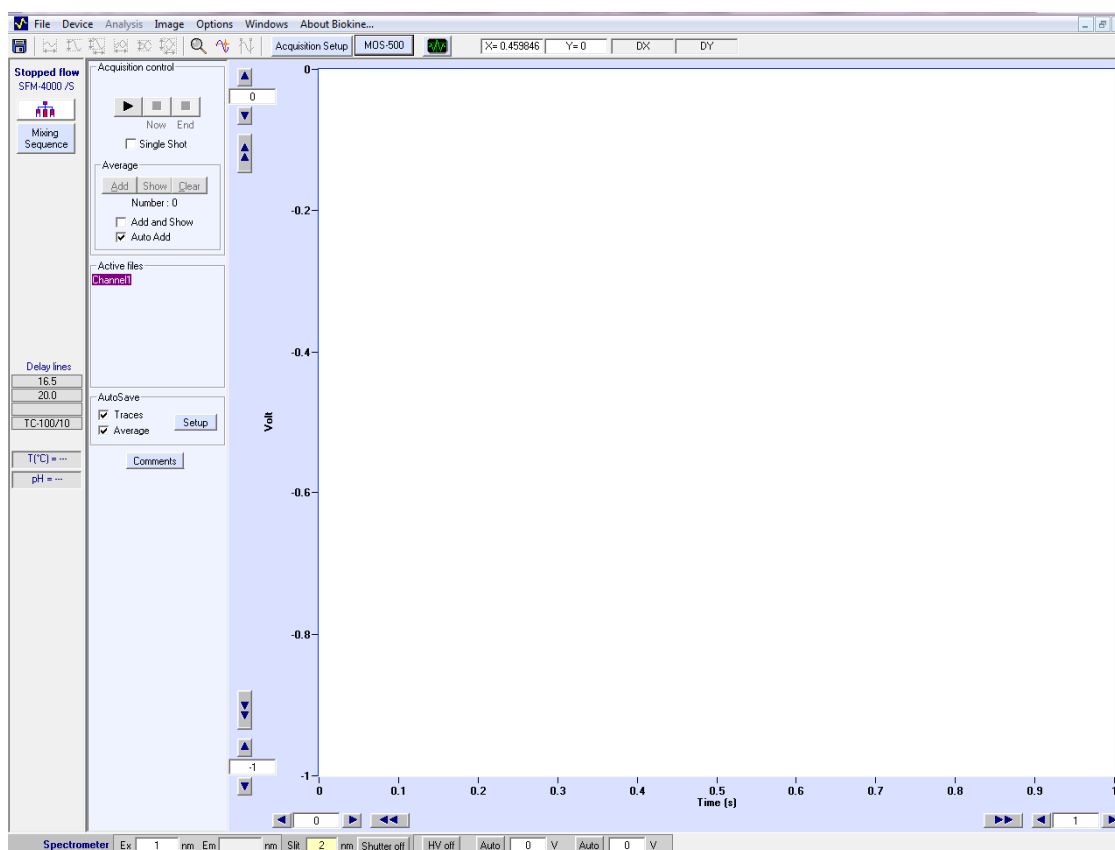



Figure 145 : Transient recorder main window

The wavelength and High Voltage control are respectively described in section 6.1 and 6.2

Select the acquisition mode by opening the Data Acquisition Setup window ( button). The different modes are fully described in the following section.

6.4.2. Absorbance and transmittance mode

- The data acquisition window in absorbance mode is presented in.
- Check the **Trigger** box to control the shots from the Bio-Kine program (see). This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments
- For absorbance experiments it is necessary to record first the zero absorbance level using buffer or water. To do that, fill the cuvette with buffer (or water and click on the **Find/Stop** button ('Get reference 'area). Once found click again on this button, the system is now ready for Kinetic shots.

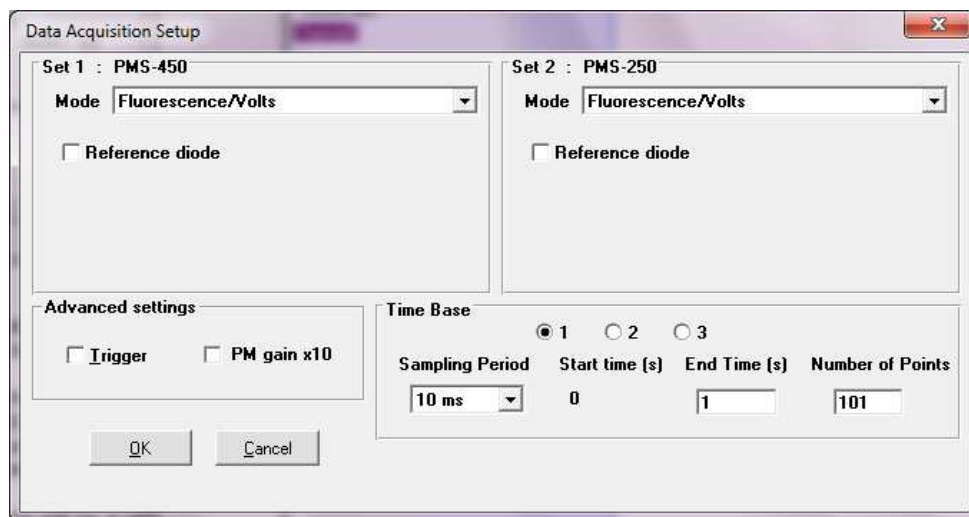


Figure 146 : Data acquisition setup window

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recordings are performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is an equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Into the 'time base area' (Figure 147) the Number of points and End time parameters can be modified manually but notice that they are linked together. Sampling Period range is 10 μ s to 10s.

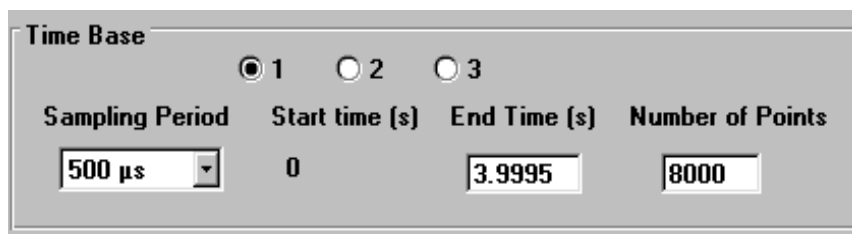


Figure 147 : "time base" area.

- A procedure to perform Kinetics in absorbance mode is given in section 6.5.1.

6.4.3. Fluorescence mode

- The data acquisition window in fluorescence mode is presented in Figure 148
- Contrary to the **Scanning Spectrometer** device the **Reference Diode** is not useful. Indeed, Kinetics is followed at a single wavelength so the fluctuations of the energy of the lamp are not significant.
- Check the **Trigger** box to control the shots from the Bio-Kine program. This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments.

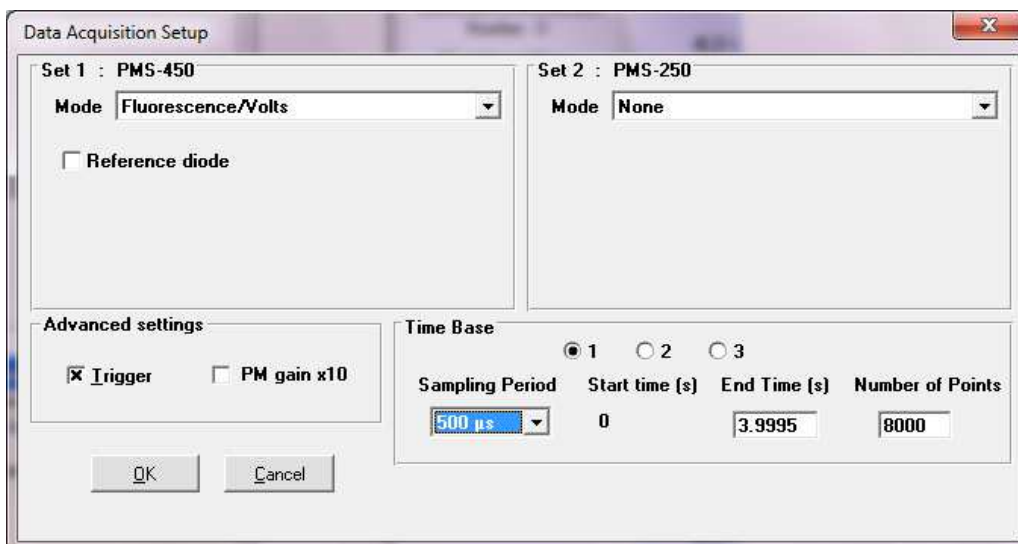


Figure 148 : Data acquisition setup window

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recording is performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is a equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

- Into the 'time base area' (Figure 149) the **Number of points** and **End time** parameters can be modified manually but notice that they are linked together. **Sampling Period** range is 10 μ s to 10

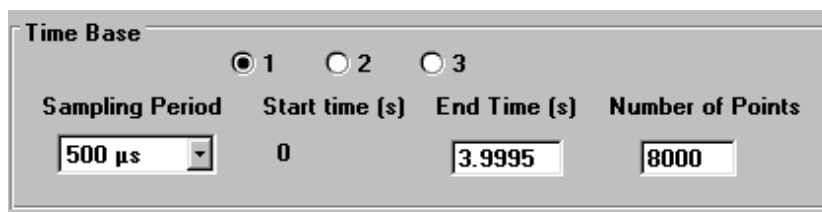


Figure 149 : "time base" area.

- A procedure to perform Kinetics in fluorescence mode is given in section 6.5.2.

6.4.4. Circular dichroism mode

- The data acquisition window in CD mode is presented in Figure 150.

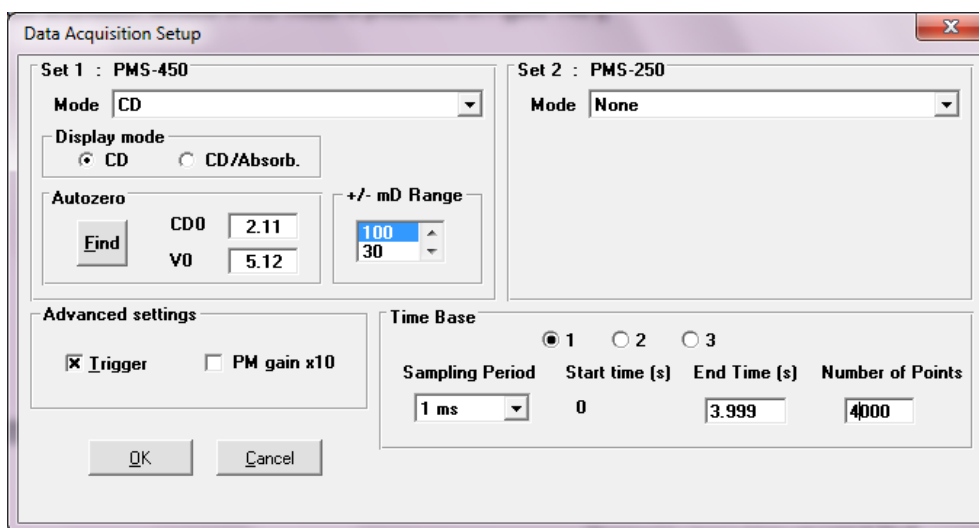


Figure 150 : Data acquisition setup window

- For CD experiments it is necessary to record first the zero CD level (**CD0**) using buffer or water. To do that, fill the cuvette with buffer (or water and click on the **Find/Stop** button ('Auto Zero' area). Once found click again on this button, the system is now ready for Kinetic shots.
- Absorbance and CD measurement can be recorded in the same time (in one shot). For this select **CD/Absorb** in "Display mode" window. For this it is necessary to record first the zero CD level (**CD0**) and zero absorbance level (**V0**) using buffer or water.
- The +/- mD range must be chosen in accordance with the amplitude of the CD signal studied, 4 amplitudes are available: 1000, 300, and 100 mdeg. For example if you estimate a CD change of 120 mdeg during your Kinetics the 300 mdeg gain is the best choice but it could also be possible to use the 1000 mdeg one.
- Check the **Trigger** box to control the shots from the Bio-Kine program (see). This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments.

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by over sampling on the second (and third) time bases. In other words the recordings are performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is an equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Into the 'time base area' (Figure 151) the Number of points and End time parameters can be modified manually but notice that they are linked together. Sampling period range is 10 μ s to 10s.

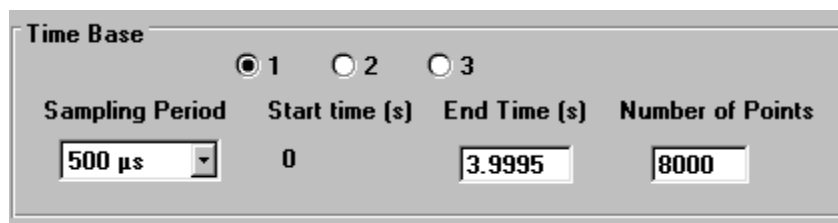


Figure 151 : "time base" area.

- A procedure to perform Kinetics in CD mode is given in section 6.5.3.

6.4.5. Linear dichroism mode

The data acquisition window in CD mode is presented in Figure 152.

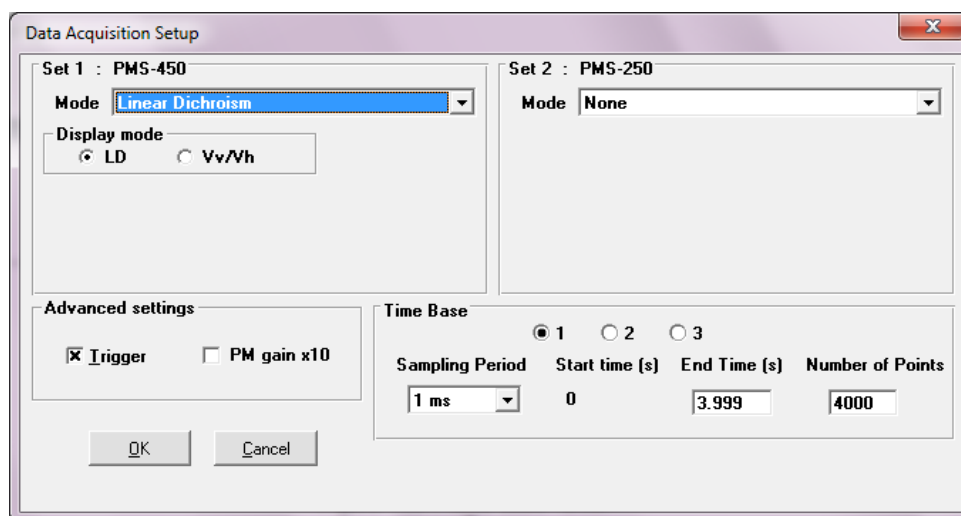


Figure 152: Data acquisition set-up window

- Check the **Trigger** box to control the shots from the Bio-Kine program. This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments.

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recordings are performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

- The **PM gain x 10** option is used to amplify the response of the photomultiplier when signals are low and High Voltage is higher than 900V.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is an equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Into the 'time base area' (Figure 153) the Number of points and End time parameters can be modified manually but notice that they are linked together. Sampling period range is 10μs to 10s.

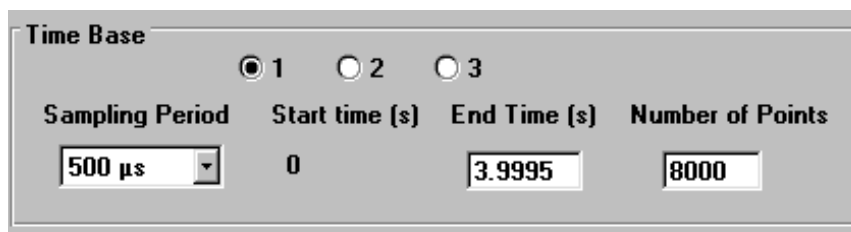


Figure 153: time base area

6.4.6. *Fluorescence anisotropy mode*

- The data acquisition window in anisotropy mode is presented in Figure 154.
- . Set n°2 is used to display the fluorescence value.

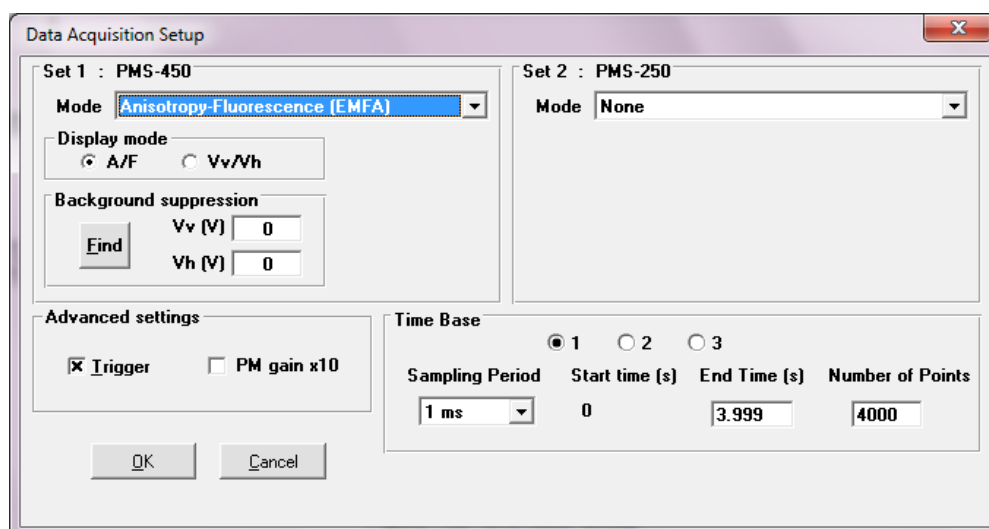


Figure 154: Data acquisition setup window

- Check the **Trigger** box to control the shots from Bio-Kine. This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with experiments
- Most of the time fluorescence and anisotropy are observed simultaneously, but it is also possible to observe directly horizontal and vertical components of the light choosing **Vv/Vh** option. In any case it is possible to switch afterward from one set of data to the other ones in the analysis menu choosing **Tools\Anisotropy\Invert display**.
- The **PM gain x 10** option is used to amplify the response of the photomultiplier when signals are low and High Voltage is higher than 900V.

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded. Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recordings are performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is an equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

Background suppression values can be used to reduce the influence of the noise. If necessary, fill a cuvette with water then press the Find/Stop button to determine the horizontal and vertical components of water, and then these values will be automatically subtracted to the signal measured during the measurement. But in most of the cases anisotropy and fluorescence measurements without using background suppression are correct.

- Into the 'time base area' (Figure 155) the **Number of points** and **End time** parameters can be modified manually but notice that they are linked together. **Sampling period** range is 10 μ s to 10s.

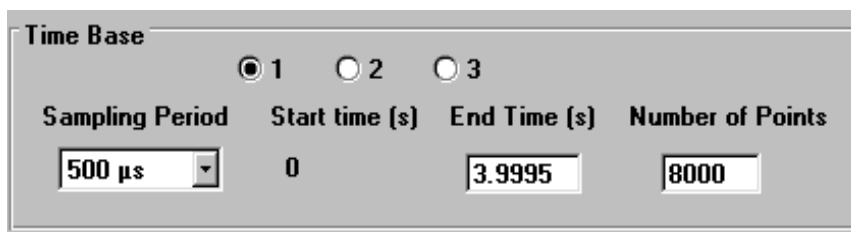


Figure 155 : "time base" area.

- A procedure to perform Kinetics in anisotropy and fluorescence is given in section 6.5.4.

6.4.7. Last setting before shot

The stopped-flow control unit (MPS) generates a trigger signal to synchronize the data acquisition with the stopped-flow sequence.

Once the stopped-flow sequence ready, the shot window is activated in the stopped-flow status bar (refer to section 2.6 for more details).

Make sure that the scope is on ('waiting for trigger' must be displayed in red in the 'scope area').



Figure 156 : Control of the scope

Click on the  button in the stopped-flow status bar to execute the shot.

More details can be found in section 6.5 where the way to run a Kinetic experiment is fully described.

6.4.8. Shot in automatic mode

The **Autorun** button is used to perform automatic accumulation of shots. When clicking on this button the window shown in Figure 157 is displayed. This window is available only when the **shot control** window and the **scope** are ready.



Figure 157: Auto run window

The number of accumulation is entered manually or using the arrows buttons. The status of the acquisition is indicated in the **current** state panel during the acquisition.

6.4.9. Graphical options

Most of the graphical tools were explained in section 2.

After each shot (Trigger with Repeat option), it is possible to perform an average of the shots, or more precisely to add the last shot to a cumulative averaging of the successive traces. Click on **Add Average** button to add the last trace to the average (Figure 158). The **Clear** average button will erase the average.

It is not possible to show the original and the average traces at the same time. Click on the **Show/Hide** average option to display/hide the current average. Another way to display the average is to check the **Add and Show** box, and then the current average will be displayed at the **Add** button click.

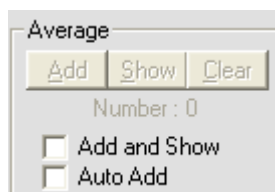


Figure 158: Averaging window.

*Note: operation like **Save**, **Save As...**, **Save ±**, **Print** applies to the trace displayed on the screen. These actions are also possible while acquisition is running, during Trigger wait.*

Use of the **comments** button is described in section 2.

6.4.10. Go to analysis window

While working on an acquisition window, the main menu **Analysis command** will load an analysis window and set the actual data in this window. This is useful for performing an immediate analysis of the current data. Once the analysis window has been loaded, all the mathematical tools described in section 3.12 can be used so as to treat the data and Kinetic constants.

6.5. How to run Kinetics?

6.5.1. In absorbance or transmittance mode

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation..
Select MOS-500.

Select the stopped-flow and its USB port.

Click OK to validate.

Monochromators are automatically initialized

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.
- Push some buffer into the cuvette for further reference or High voltage adjustment.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

Acquisition parameters

Select the Device, transient Recorder menu.

Click on the **MOS-500** button.

- Select an excitation wavelength.
- Click Apply to validate

The wavelength chosen is now displayed in the corresponding state panel at the bottom of the screen

In the main window, click on the **Auto** button to adjust the High voltage automatically. Click a second time when the response is stable.

Click on the **Acquisition Setup** button.

Select the absorbance acquisition mode

Do not select the reference diode..

Do not check the PM gain x 10 option.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

The cuvette is filled of water so it is possible to do a reference for further absorbance calculations.

- Click on the **Find/Stop** button in the **Get reference** area.
(About 5V should be display in the corresponding state panel)

Click a second time on the Find/Stop button to validate the reference.

Click OK to accept these parameters.

If the voltmeter check box is selected then the absorbance indicated in the corresponding channel is 0.

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

6.5.2. In fluorescence mode

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation.

Select MOS-500.

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Monochromators are automatically initialized

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

- Click on the **start** button to fill the cuvette with final solution to adjust the high voltage (see further)

Acquisition parameters

Select the Device, transient Recorder menu.

Click on the **MOS-500** button.

- Select an excitation wavelength.
- Select an illumination wavelength (if an emission monochromator is available)
- Click Apply to validate

The wavelengths chosen are displayed in the corresponding state panel at the bottom of the screen

In the main window, click on the **Auto** button to adjust the High voltage automatically. Click a second time when the response is stable.

Click on the **Acquisition Setup** button.

Select the fluorescence acquisition mode

Do not select the reference diode.

Only check the PM gain x 10 option if the High Voltage found is higher than 900V.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

6.5.3. *In circular dichroism mode*

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation.

Select MOS-500.

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Monochromators are automatically initialized

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.
- Push some buffer into the cuvette for further reference or High voltage adjustment.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

Acquisition parameters

Select the Device, transient Recorder menu.

Click on the **MOS-500** button.

- Select an excitation wavelength.
- Click Apply to validate

The wavelength chosen is now displayed in the corresponding state panel at the bottom of the screen

In the main window, click on the **Auto** button to adjust automatically the High voltage. Click a second time when the response is stable.

Click on the **Acquisition Setup** button.

Select the CD acquisition mode

Do not select the reference diode.

Do not check the PM gain x 10 option.

Select sensitivity according to the amplitude expected for your signal.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

CDO reference

The cuvette is filled of water so it is possible to do a reference for further CD calculations.

- Click on the **Find/Stop** button in the **Get CDO** area.

Click a second time on the Find/Stop button to validate the reference.

Click OK to accept these parameters.

High Voltage adjustment

- Click on the start button in the shot control window to fill the cuvette with final solution.
- Click on the **Auto** button to adjust the High voltage automatically according to your solution.
- Click a second time on the **Auto** button to stabilize the high voltage.

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

6.5.4. In anisotropy- fluorescence mode

For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration of the system

In the main menu choose Install, Device installation..

Select MOS-500.

Select the stopped-flow and its corresponding serial port


Click OK to validate.

Monochromators are automatically initialized

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

Click on the **start** button to fill the cuvette with final solution for background suppression (see further).

The control shot window is now activated in the stopped-flow status area

Acquisition parameters

Select the Device, transient Recorder menu.

Click on the **MOS-500** button.

- Select an excitation wavelength.
- Select an illumination wavelength (if an emission monochromator is available)
- Click Apply to validate

The wavelengths chosen are displayed in the corresponding state panel at the bottom of the screen

In the main window, click on the **Auto** button to adjust the High voltage automatically. Click a second time when the response is stable.

Click on the **Acquisition Setup** button.

Select the fluorescence anisotropy acquisition mode

Do not select the reference diode.

Only check the PM gain x 10 option if the High Voltage found is higher than 900V.

Check the trigger box.

Choose the number of time base, your sampling period(s) and the number of points

Background suppression

Close the shot control window in the stopped-flow status area.

Push some water into the cuvette.

Keep the same High Voltage as determined before.

Go into the setup menu.

Determine the background factors by clicking on the 'Find/Stop' button.

- Go back to the main menu and activate the shot control window again.

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The Kinetic trace is now displayed on the screen, all graphical tools can be used.

7. CONTROL OF JASCO J-810

7.1. Introduction

The use of Bio-Kine to control J-810 CD spectrometer offer several advantages. The most important ones are the faster acquisition process when using Bio-Kine combined to the Bio-Logic PCI board and the possibility to use up to three time base for acquisition. Analysis tools are also more developed in Bio-Kine like the average function and fit tools. It is also possible to perform data acquisition without PCI board, but in this case the communication between J-810 and Bio-Kine is done through a serial port so the acquisition parameters are the same than when working with Jasco Spectra Manager. In this case the advantages of Bio-Kine are the average function and the analysis tools. In this chapter these different configurations will be discussed.

7.2. Connection of the system

7.2.1. Using a PCI-6321e board

For the installation of the AD/DA board please refer to section 1.2. It is assumed that the J-810 has been modified to extract both CD analog signal and DC signal by adding of 2 BNC plugs. Using BNC cables connect respectively the CD and DC outputs to analog input 1 and 2 of the BNC Box. The BNC box can now be connected to the computer using the appropriate flat cable. The synchronization between the MPS and the J-810 is done connecting the trigger plug of the BNC box to Trigger out of MPS-70.

All the connections are shown in the scheme shown in Figure 159.

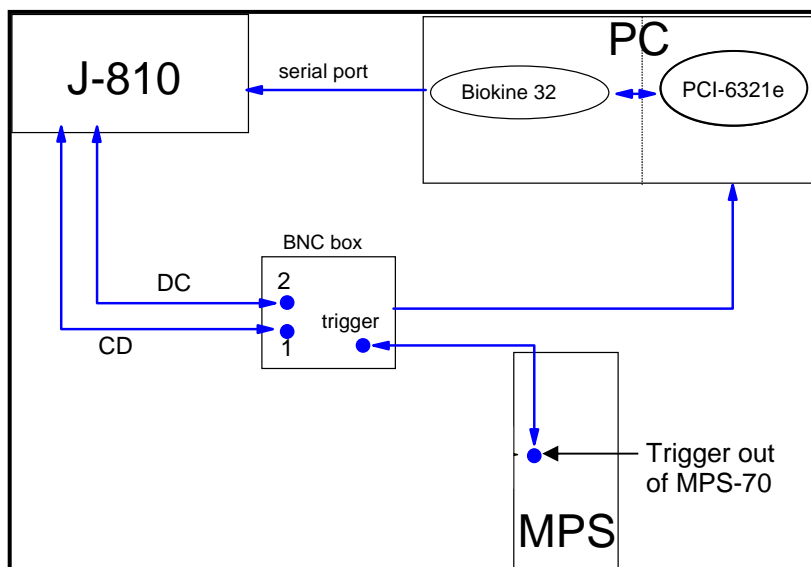


Figure 159 : Driving J-810 using Bio-Kine and PCI board.

7.2.2. Without using PCI-6321e board

It is necessary to use a special cable (BNC plug on one side, white and black connector on the other side) to link the J-810 to the MPS. Connect the BNC plug of the cable previously described to Trigger out of MPS-70. Connect the white connector to the trigger signal terminal (trig) of J-810, and the black connector to the grounding terminal (DGND). All the connections are shown in the scheme shown in Figure 160).

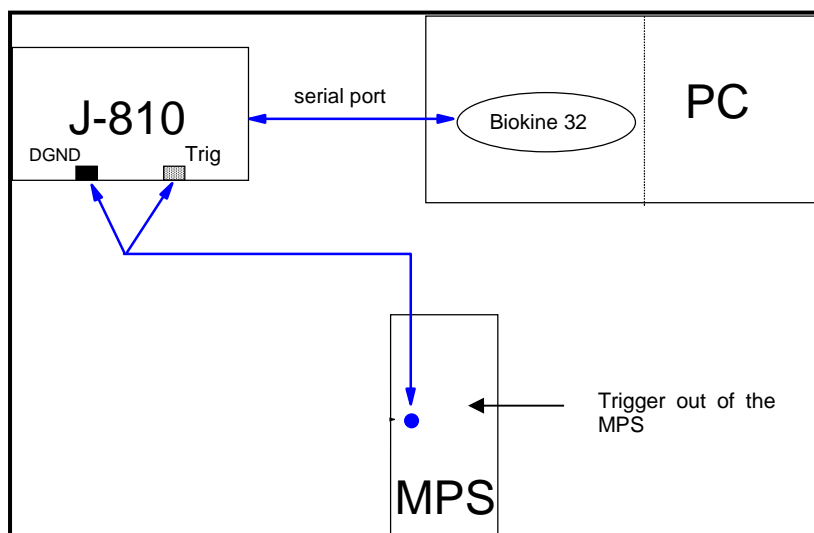


Figure 160 : Driving J-810 using Bio-Kine.

7.3. Acquisition tools

7.3.1. Installation

In the main window, select the **Install, Device installation** menu. The window shown in Figure 161 is displayed on the screen. If the acquisition is made using the PCI-6052E board (as explained in section 7.2.1) it is necessary to select **J-810 analog acquisition** and the corresponding **serial port** in the installation window.

If the acquisition is made through the serial port of the J-810 then it is necessary to choose the J-810 serial acquisition and its corresponding serial port. Click on OK to validate.

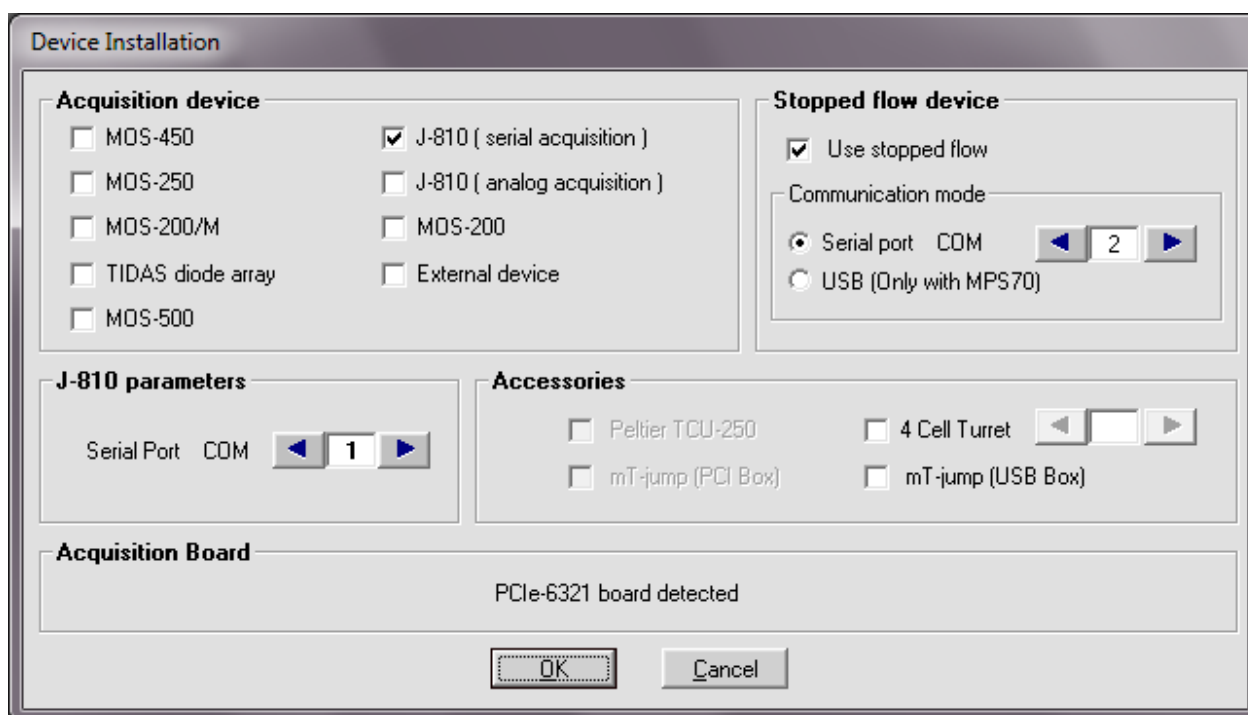


Figure 161 : Installation window.

Select the device, transient recorder menu to display the main window shown in Figure 162

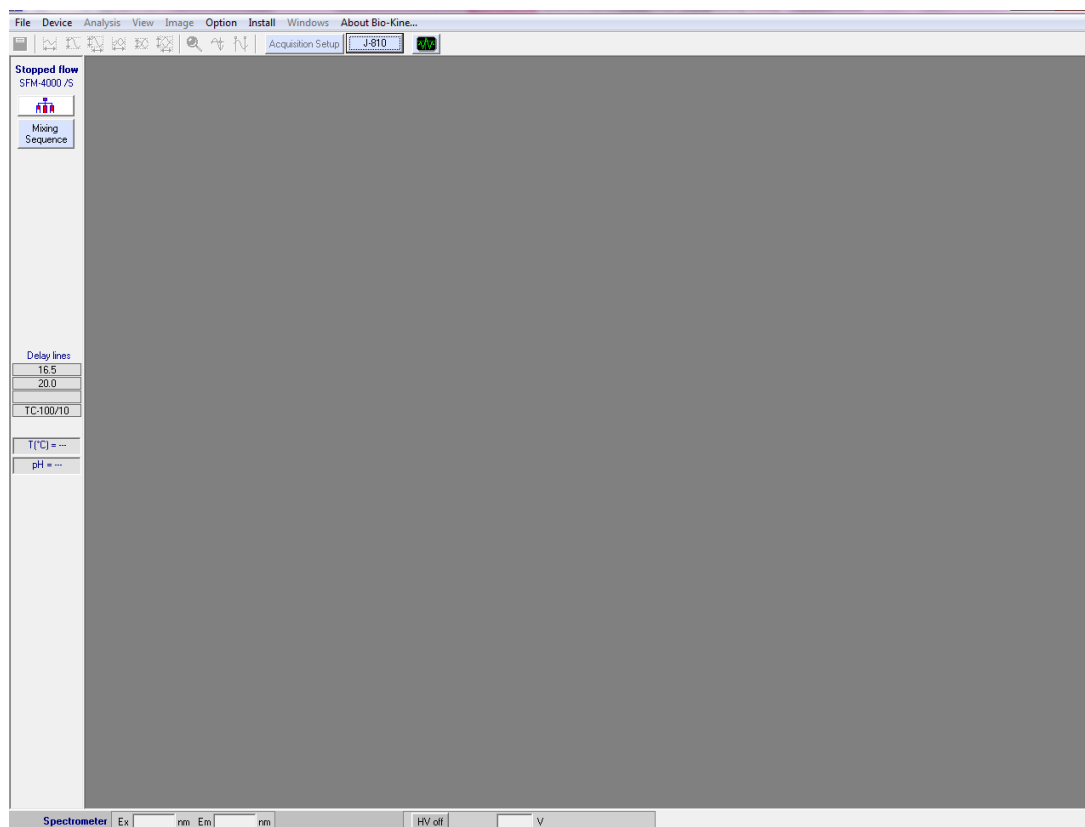
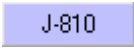


Figure 162 : Acquisition device main window

7.3.2. J-810 settings

Click on the  button to load the window shown in Figure 163

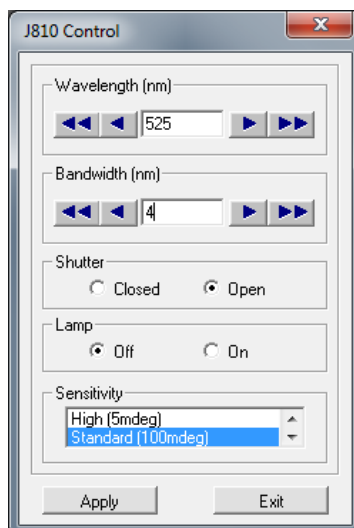


Figure 163 : J-810 settings.

From this window select the wavelength and the size of the slit (bandwidth between 1 and 20 nm). It is also possible to change the wavelength directly from the main menu using the white state panel on the left bottom of the screen (refer to Figure 162). The lamp is controlled from this window. It is important to close the shutter to avoid damaging the photomultiplier tube when opening the observation chamber or removing a light link tube between instrument and photomultiplier tube. Select one of the three sensitivities corresponding to the sensitivities used in the Jasco's Spectra Manager software.

7.3.3. High voltage control

Select the High Voltage to apply on the photomultiplier (by entering a value in the corresponding state panel at the bottom of the main window) to get a response between 0.5 and 1V. The instant value of the high voltage applied on the photomultiplier is displayed on channel 3 and the photomultiplier's response on channel 2 of the voltmeter (refer to Figure 164). The CD value is displayed on channel 1.



Figure 164: High voltage control

7.3.4. Data acquisition setup

Click on the **Acquisition Setup** button to load the window shown in Figure 165, parameters are described below.

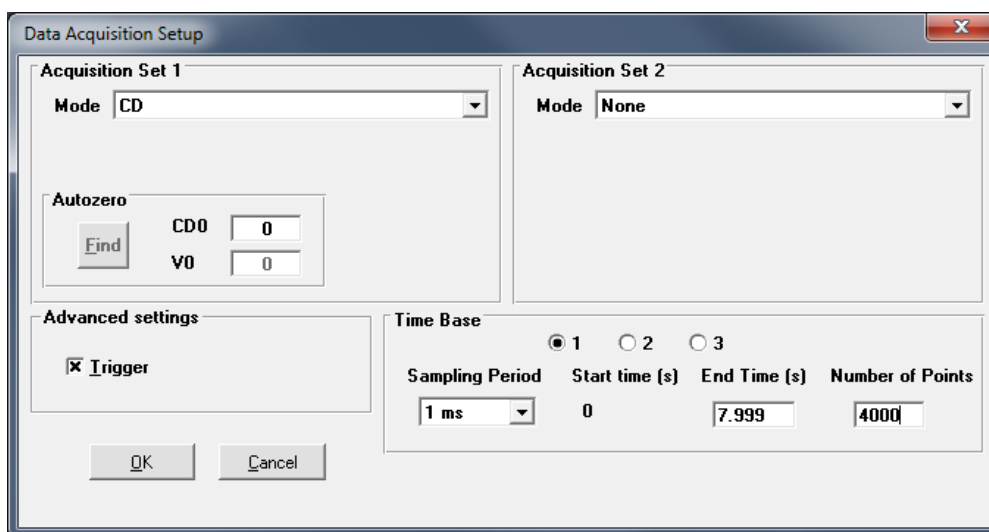


Figure 165: Setup window.

- The **set n°1** is always used to perform CD measurements, channels 1 and 2 are automatically selected (refer to Figure 165).
-

The set n°2 is used to perform fluorescence measurements, notice that it is possible to run both CD and fluorescence simultaneously with the adequate configuration. The gain is set automatically to 1, it could be modified for a specific configuration (when CD is measured with set n°2).

Scale box: enter the Y scale ratio between the two acquisition modes, the default value is 1.

Check the **Trigger** box to control the shots from e Bio-Kine (see

Figure 165). This is used to start the data acquisition at a trigger signal. The trigger will occur on the falling edge of a TTL pulse sent to the trigger input of the A/D board. This mode will be used to synchronize the data acquisition with stopped-flow driving sequence.

Bio-Kine includes a multiple time base setup that permits long time base recordings in order to limit the size of the files (within 10 to 8000 points) that are recorded.

Up to three successive time bases are available. This multiple time base is performed by oversampling on the second (and third) time bases. In other words the recording is performed on the entire duration of the record at a constant sampling rate equal to that of the first time base. The second and third time bases are obtained by averaging the data that are acquired.

The effect of this averaging will be a reduction of the noise on the second and third time bases. This is a equivalent to a digital filter whose frequency is adapted to the sampling rate. This is the best arrangement with respect to signal processing.

IMPORTANT: three time bases are only available when using the AD/DA board. If Bio-Kine is used in the configuration described in section 7.2.2 then only one time base is available.

Into the 'time base area' (see Figure 166) the **Number of points** and **End time** parameters can be modified manually but notice that they are linked together. **Sampling Period** range is 10 μ s to 10s.

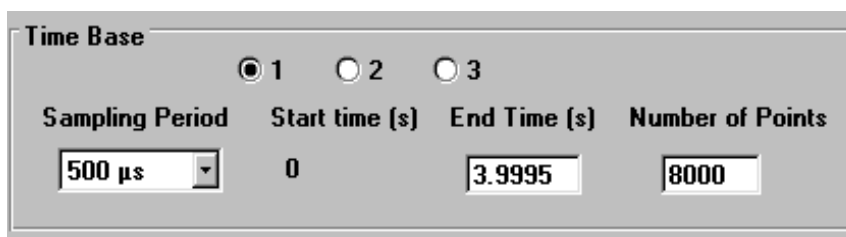


Figure 166 : Time base selection window.

7.3.5. Last setting before shot


The stopped-flow control unit (MPS) generates a trigger signal to synchronize the data acquisition with the stopped-flow sequence.

Once the stopped-flow sequence ready, the shot window is activated in the stopped-flow status bar (refer to section 2.6 for more details).

Make sure that the scope is on ('waiting for trigger' must be displayed in red in the 'scope area'). The scroll option is disabled when the trigger is on (refer to Figure 167).



Figure 167 : Control of the scope

Click on the  button in the stopped-flow status bar to execute the shot. More details can be found in section 7.6 where the way to run Kinetics is fully described.

7.3.6. Shot in automatic mode


The  button is used to perform automatic accumulation of shots. When clicking on this button the window shown in Figure 168 is displayed. This window is available only when the **shot control** window and the **scope** are ready.



Figure 168: Auto run window

The number of accumulation is entered manually or using the arrows buttons. The status of the acquisition is indicated in the **current** state panel during the acquisition.

7.3.7. Graphical options

Most of the graphical tools were explained in section 2.

After each shot (Trigger with Repeat option), it is possible to perform an average of the shots, or more precisely to add the last shot to a cumulative averaging of the successive traces. Click on **Add** Average button to add the last trace to the average (see Figure 169). The **Clear** average button will erase the average.

It is not possible to show the original and the average traces at the same time. Click on the **Show/Hide** average option to display/hide the current average. Another way to display the average is to check the **Add and Show** box, and then the current average will be displayed at the **Add** button click.

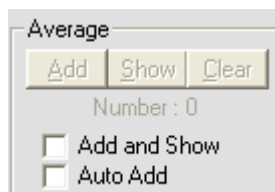


Figure 169: Averaging window.

Note : operation like **Save**, **Save As...**, **Save ±**, **Print** applies to the trace displayed on the screen. These actions are also possible while acquisition is running, during Trigger wait.

Use of the **comments** button is described in section 2.

7.3.8. Go to analysis window

While working on an acquisition window, the main menu **Analysis command** will load an analysis window and set the actual data in this window. This is useful for performing an immediate analysis of the current data. Once the analysis window has been loaded, all the mathematical tools described in section 3.12 can be used so as to treat the data and determine Kinetic constants.

7.4. Use of other Jasco spectropolarimeters (J-715...)

Other Jasco spectropolarimeters can be controlled using Bio-Kine and the PCI-6321e board. CD and DC signal must be extracted by a similar way from the spectropolarimeter but in this case it necessary to use both Spectra Manager and Bio-Kine software.

The connection of the system is the same as explained in section 7.2.1.

But in this configuration only the acquisition parameters are controlled by Bio-Kine. The wavelength, the size of the slit, the sensitivity and the High Voltage are controlled from Spectra Manager software. The spectropolarimeter must be switch to the manual high voltage mode to perform Kinetics.

7.4.1. Installation

In the main window, select the **Install** menu. The window shown in is displayed on the screen. Do not select the **J-810** check box because other Jasco instruments are not recognized the same way by Bio-Kine. It is necessary to configure the system as an external device. The **Use A/D board** check box is automatically recognized. Click on OK to validate.

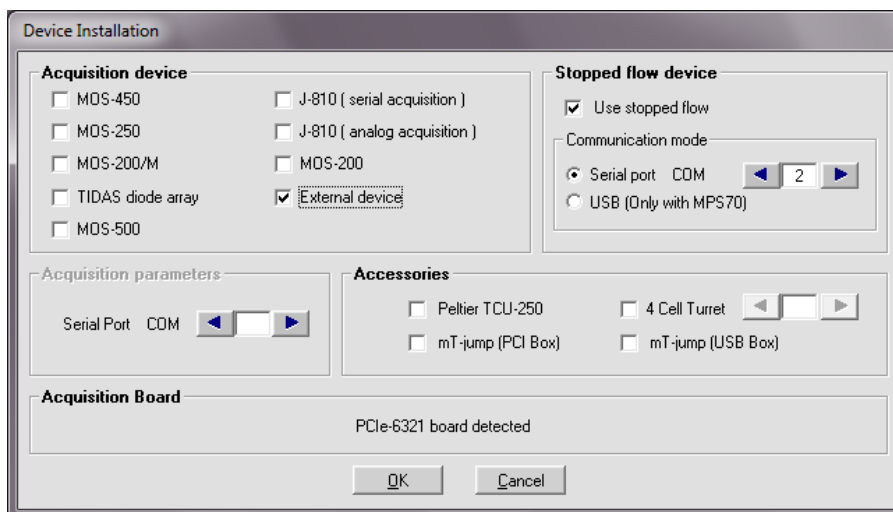


Figure 170 : Device installation

Select the device, transient recorder menu to display the main window shown in Figure 171.

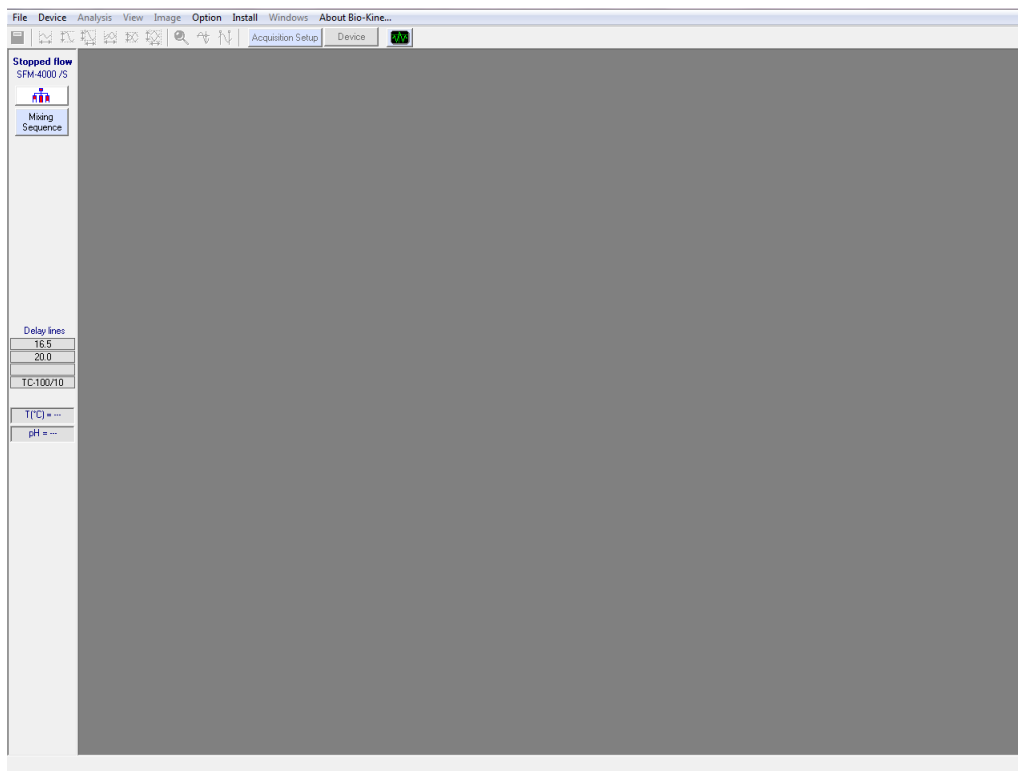


Figure 171 : Acquisition window.

7.4.2. Data acquisition setup

Click on the  button to load the window shown in Figure 172.

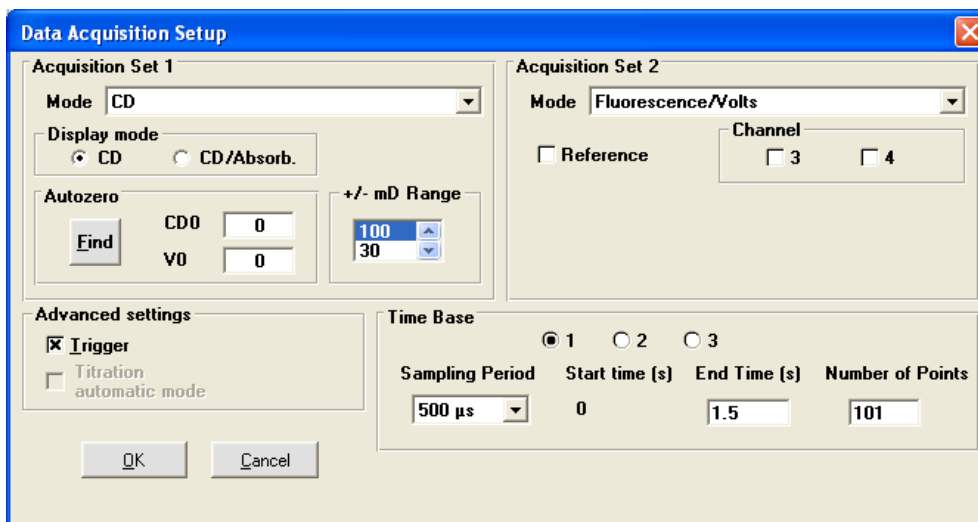


Figure 172 : Acquisition setup window.

Most of the parameters used in this window are described in section 7.3.4. The Circular Dichroism value is not calculated the same way when using the spectropolarimeter hardware or when using Bio-Kine. You need to enter a calibration factor which can be found in the following table. The calibration factor take into account the CD range value from Biokine and the sensitivity value from Spectra Manager

CD Range from Biokine in mdeg	Sensitivity from Spectra Manager		
	Low (gain 1)	Standard (gain 10)	High (gain 200)
+/- 1000 (gain 1)	3.49	0.349	0.01745
+/- 300 (gain 3)	10.47	1.047	0.05235
+/- 100 (gain 10)	34.9	3.49	0.1745
+/- 30 (gain 30)	104.7	10.47	0.5235

If the calibration is not set correctly in the data acquisition setup window, later CD values displayed will be erroneous.

To enter the calibration factor please go to acquisition window, press **Ctrl + Alt + S** together. Then appear the Gain/offset advanced set-up window. In this window enter in CD parameter window the corresponding calibration factor and click on OK to accept.

7.5. Chart recorder

The use of the **chart recorder** and **transient recorder** devices are the same, a precise description of this menu can be found in section 7.5.

7.6. How to run Kinetics

The methods presented here are general examples to perform a Kinetic experiment. For a better understanding it is necessary to have previously read the characteristics of each function above. Note all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

7.6.1. Using J-810 and PCI-6321e board

Configuration of the system

In the main menu choose Install, Device installation.

Select J-810 (analog acquisition).

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

Perform a shot to fill the cuvette with final solution and for further high voltage adjustment

Acquisition parameters

Select the Device, transient Recorder menu.

Click on the  button.

- Select the CD acquisition mode
- Check the trigger box.
- Choose the number of time base, your sampling period(s) and the number of points.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Click on the  button.

Select the wavelength.

Select the size of the slit.

Open the shutter.

Choose a sensitivity.

Click on the Apply button.

Click on the Exit button to go back to the Setup window.

High voltage adjustment

Enter a high voltage value (state panel at the bottom of the screen) to get a response between 0.5 and 1 V.

The photomultiplier's response is given in the voltmeter

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

- The Kinetic trace is now displayed on the screen, all graphical tools can be used

7.6.2. Using other Jasco spectropolarimeters (J-715...) with PCI-6321e board

Configuration of the system

In the main menu choose Install, Device installation.

Select External device, the acquisition board is automatically recognized.

Select the stopped-flow and its corresponding serial port

Click OK to validate.

Select Install, stopped-flow configuration in the main menu

- Select the device installed.
- Select the size of the syringes.
- Accept your parameters by clicking on the **OK** button and return to the main window.

Stopped-flow parameters and sequence

In the stopped-flow status area, click on the  button.

- Initialize the syringes.
- Load the solutions into the instrument.

Edit the stopped-flow sequence in either advanced or classic mode and set SFM options (cuvette, delay lines...).

Once the sequence done, click on the ready button.

The control shot window is now activated in the stopped-flow status area

- Perform a shot to fill the cuvette with final solution and for further high voltage adjustment

Settings made from Spectra Manager

Select the wavelength


Select the size of the slit.

Select a sensitivity (high, standard or low)

Choose the high voltage manual mode and enter a high voltage value to get a response between 0.5 and 1 Volt.

Acquisition parameters from Bio-Kine

Select the Device, transient Recorder menu.

Click on the  button.

- Select the CD acquisition mode
- Check the trigger box.
- Choose the number of time base, your sampling period(s) and the number of points.
- Enter the calibration factor corresponding to the sensitivity chosen
- Accept your parameters by clicking on the **OK** button and return to the main window.

7.6.3. Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

- The Kinetic trace is now displayed on the screen, all graphical tools can be used

8. CONTROL OF A DIODE ARRAY DETECTOR

8.1. Introduction

Bio-Kine can be used to control J&M Diode Array Detector. The rate of acquisition is intrinsic of the Diode Array hardware and not of the software; this rate will be discussed further in section 8.3. Communication between Diode Array Detector and computer is done through an ethernet cable. The Motor Power Supply (MPS) needs to deliver falling synchronization TTL pulses (5 to 0V) to the Diode Array detector; this is done by connecting the **trigger out** BNC plug on the rear of the MPS-70 unit to the trigger plug on the rear panel of the Diode Array Detector. Only acquisition tools are described in this section, please refer to section 2 for 2D analysis menu. (For the MPS-60 **Synchro out I** at the front panel must be connected to the trigger cable of the diode array).

8.2. Installation

Load Bio-Kine. Open the **Install, device installation** menu to display the window shown in Figure 173.

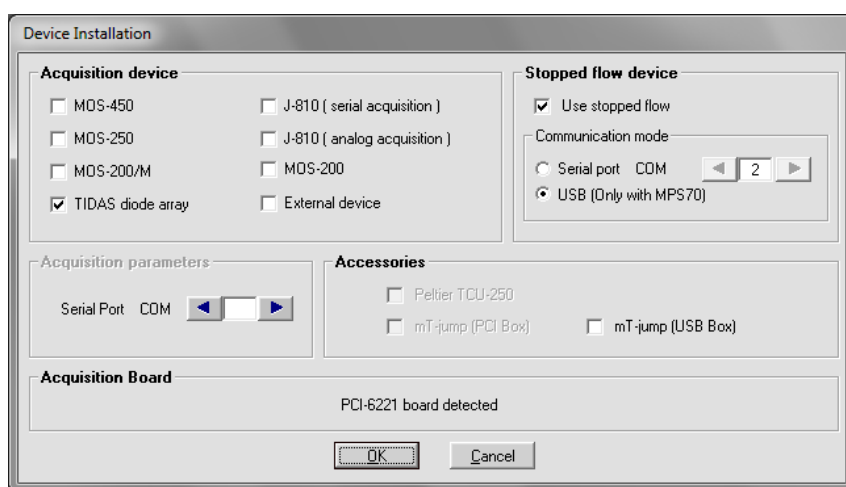



Figure 173 : Device installation window.

Select the **TIDAS Diode Array** check box.

8.3. Diode Array specifications

All the Diode Array Detector specifications can be found clicking on the  button (refer to the window shown in Figure 174).

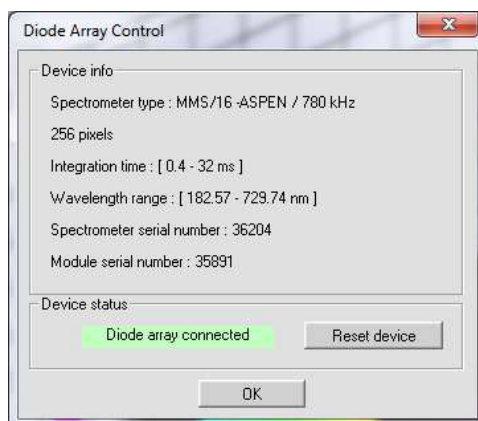


Figure 174 : Diode array specifications

The spectrometer type, the spectrometer serial number and the module serial number can be found in this window, it could be very helpful for maintenance questions or troubleshooting communications with our specialists.

The number of pixels is corresponding to the number of diodes. The whole diodes are always used for a spectrum whatever the wavelength limits chosen by the user (refer to section 8.4.2).

The integration time is the time between each spectrum; 0.4ms being the minimum time to 'scan' the 256 diodes it corresponds also to the minimum acquisition time (example referring to Figure 174).



The wavelength range is also specified. In the example described above 256 diodes are used for an 826.75nm wavelength range, so each spectrum is made of 256 points (3.2nm between two points).

Click on the **Init Device** button to initialize the system and chose **Exit** to return to the main window.

8.4. Acquisition Device

8.4.1. Presentation

Choose the Device, Diode Array Spectrometer to load the window shown in Figure 175 : the default 3D view is automatically displayed.

The 3D representation can be rotated in all directions using  buttons, and units can be modified using the corresponding state panel along the different axis. To return to the default view presented in Figure 175 click on the  button.

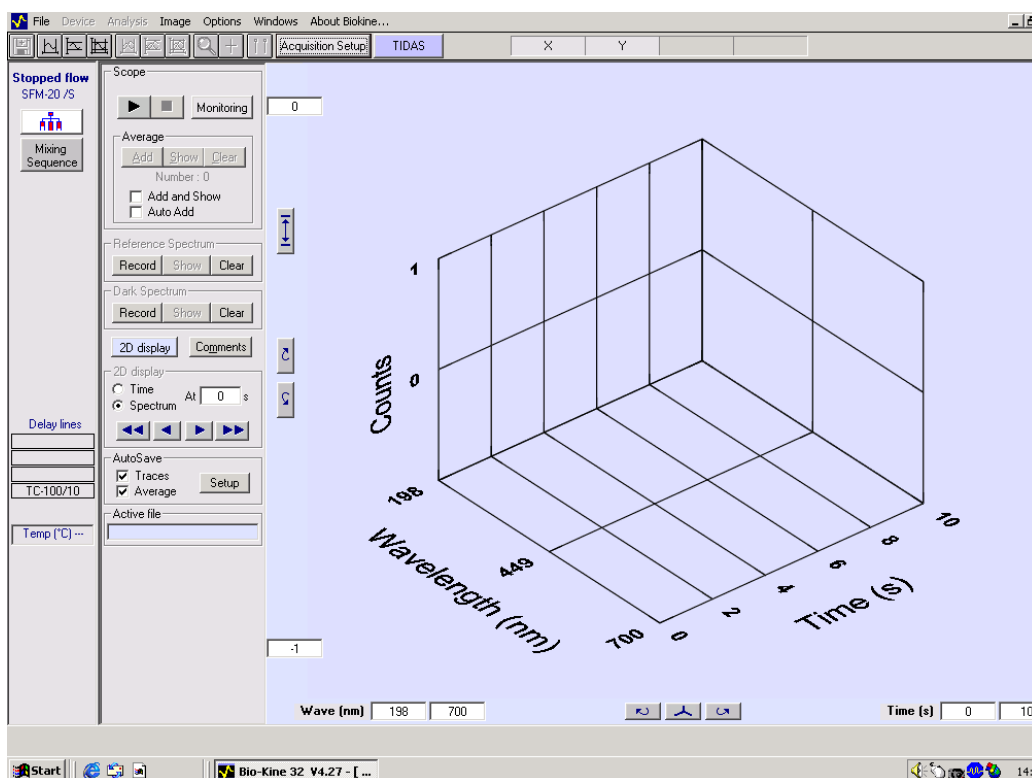


Figure 175 : Main window

8.4.2. Diode Array Setup

Click on the

Acquisition Setup

button to display the window shown in and to choose the acquisition parameters.

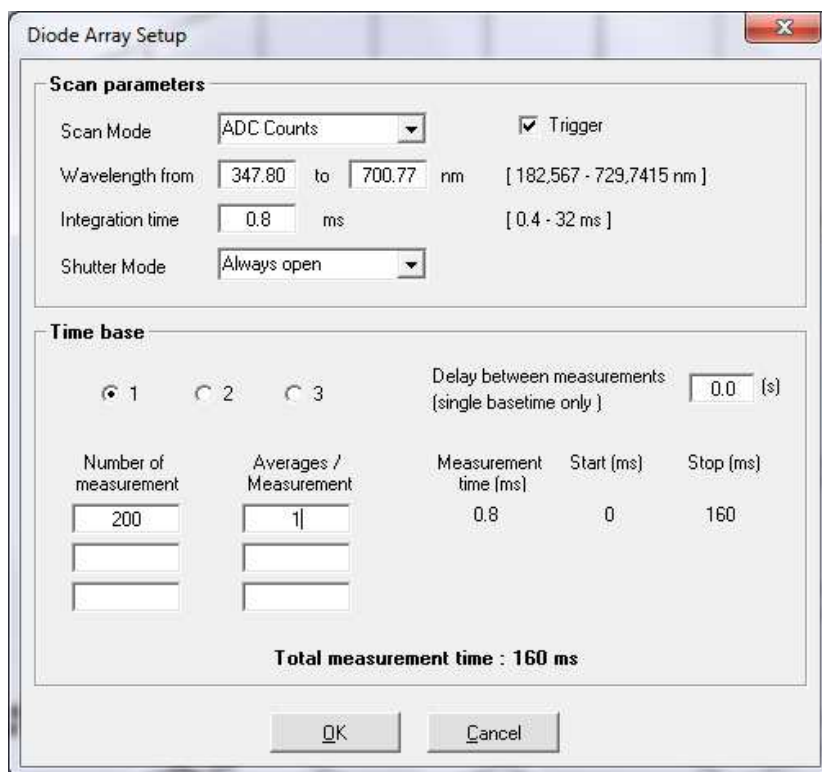


Figure 176 : Diode array setup.

3 scan modes are available: **ADC counts**, **transmittance** and **absorbance**. Select one of them using the 'up and down' button. The ADC Count mode is measuring directly the quantity of light detected by DAD without normalization (value between 0 and 65535, 65535 corresponding to a light saturation). The trigger box is checked to synchronize the stopped-flow driving sequence and the DAD.

Wavelength limits and integration time can be selected from this window; the software will adjust automatically the wavelength chosen to be in phase with the diodes.

A **delay between measurements** can be entered in the corresponding box.

Select a shutter mode: shutter always open or only open for scan.

Up to three time bases can be selected but the acquisition time remain the same for all time bases. For each time base it is necessary to enter a number of measurements and a number of averages. When the average is set to 1, the time between spectra is directly determined by the integration time. When the average value is n then the software perform n spectra, do an average of these n spectra and display the averaged spectrum.

In the example shown in Figure 176 a spectrum is done every 0.4 ms during 160 ms.

The number of spectra is limited for each kind of DAD, only 4Mbytes of memory are available so it is not possible to do more than 3000 spectra with 256 diodes and more than 750 spectra with 1024 diodes. So the use of different time bases is very important to reduce the size of files.

8.4.3. Dark current measurement

The dark current measurement is used to record the influences of the spectrometer and other equipment. The measurement is performed without exposing the diodes to light. The resulting A/D converter value is caused by remaining light, diode leaking current, thermal noise...

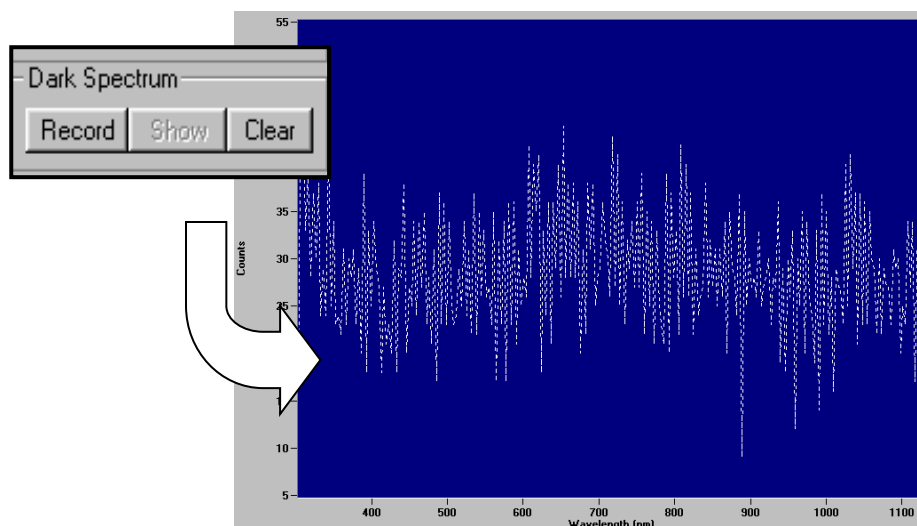


Figure 177 : Dark current measurement

In the **Dark Spectrum** graphic area, click on the **Record** button to perform a dark current measurement. After completing the measurement data are transferred to the computer and graphically displayed, a typical dark spectrum is shown in Figure 177. The dark current measurement is always done in Counts.

The dark current measurement is also done on the whole wavelength range of the instruments and not on the range specified in the **setup** menu, in that way it is possible to change experimental limits without repeating the dark current measurement.

When a dark spectrum has been made, **dark spectrum** is written in red on the screen. Click on the **Hide** button to hide the dark spectrum measurement and on the **Clear** button to get rid of it.

To perform a dark current measurement is not compulsory for Kinetics study but it is safer to include the instrument perturbations into the measurement.

8.4.4. Reference spectrum

Contrary to the dark current measurement the reference spectrum measurement is compulsory when doing absorbance or transmittance studies.

Fill the cuvette with buffer then click on the **Record** button (in the **Reference spectrum** graphic area) to perform a reference measurement. After completing the measurement data are transferred to the computer and graphically displayed, an example of reference spectrum is shown in Figure 178. If a dark spectrum was previously performed then the reference spectrum displayed is a corrected one, the reference spectrum is always given in Counts. The reference spectrum represents the lamp spectrum.

The reference spectrum measurement is also done on the whole wavelength range of the instruments and not on the range specified in the **setup** menu, in that way it is possible to change experimental limits without repeating the reference spectrum.

When a reference spectrum has been made, **reference spectrum** is written in red on the screen. Click on the **Hide** button to hide the dark spectrum measurement and on the **Clear** button to get rid of it.

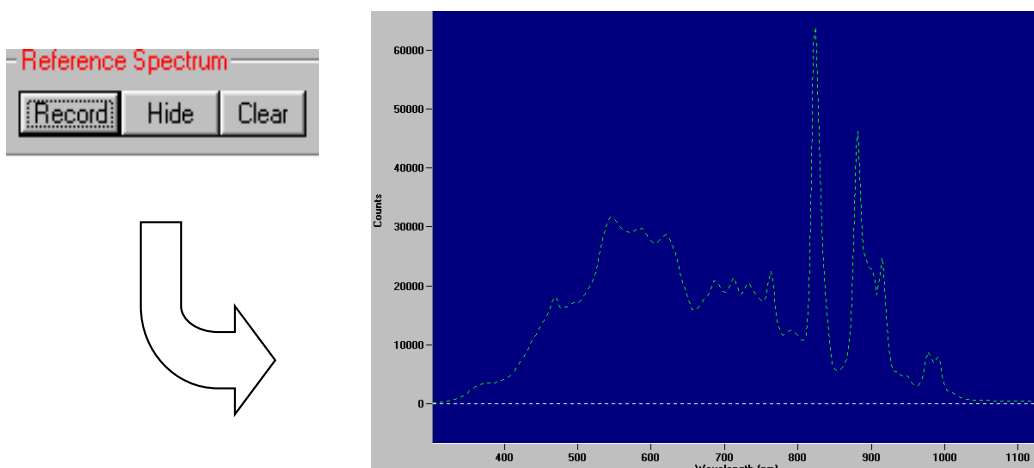


Figure 178 : Reference spectrum measurement.

It could be necessary to adjust the position of fiber optics or the intensity of the light source to avoid a saturation of the signal (65535 counts).

Hence, the spectra measured in transmission or absorbance mode are calculated using the reference spectrum (corrected with dark current or not) as explained in Figure 179.

Dark current used	Dark current not used
$\text{Transmittance (\%)} = 100 \times \frac{\text{signal} - \text{dark current}}{\text{reference} - \text{dark current}}$	$\text{Transmittance (\%)} = 100 \times \frac{\text{signal}}{\text{reference}}$
$\text{Absorbance} = -\log \frac{\text{signal} - \text{dark current}}{\text{reference} - \text{dark current}}$	$\text{Absorbance} = -\log \frac{\text{signal}}{\text{reference}}$

Figure 179 : Absorbance and transmission calculation.

8.4.5. Last setting before shot

The stopped-flow control unit (MPS) generates a trigger signal to synchronize the data acquisition with the stopped-flow sequence.

Once the stopped-flow sequence ready, the shot window is activated in the stopped-flow status bar (refer to section 2.6 for more details).

Make sure that the scope is on ('waiting for trigger' must be displayed in red in the 'scope area'). The scroll option is disabled when the trigger is on (refer to Figure 180)



Figure 180 : Control of the scope

Click on the  start button shown in the "shot control" window to execute the stopped-flow sequence.

After completing the measurement data are transferred to the computer and graphically displayed, an

example of 3D trace is given in Figure 181.

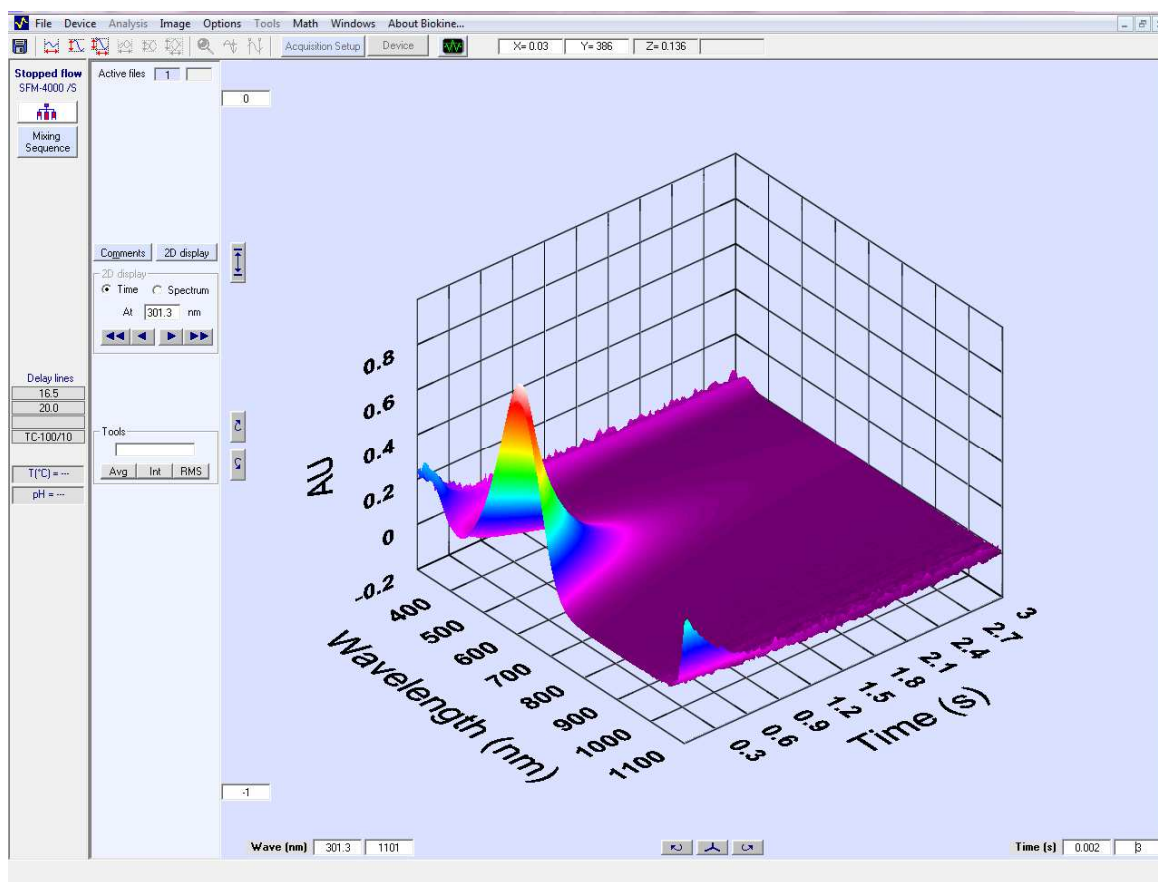


Figure 181 : Example of 3D trace.

More details can be found in section 8.5 where the way to run a Kinetic experiment is fully described.

8.4.6. Shot in automatic mode

The **Autorun** button is used to perform automatic accumulation of shots. When clicking on this button the window shown in Figure 182 is displayed. This window is available only when the **shot control** window and the **scope** are ready.



Figure 182: Auto run window

The number of accumulation is entered manually or using the arrows buttons. The status of the acquisition is indicated in the **current** state panel during the acquisition.

The user can save all the traces independently, the average or both (then check auto add). The choice is made by checking the right option in the window shown in.

The **Save + Setup** button can be used to implement the name of the files saved (refer to section 3.9 for more details).

The acquisition can be stopped at any moment using the **Stop** button.

The average file is displayed on the screen at the end of the acquisition.

8.4.7. Graphic options

3D/2D option

After each shot (Trigger with Repeat option), it is possible to perform an average of the shots, or more precisely to add the last shot to a cumulative averaging of the successive traces. Click on **Add** Average button to add the last trace to the average (see Figure 183). The **Clear** average button will erase the average.

It is not possible to show the original and the average traces at the same time. Click on the **Show/Hide** average option to display/hide the current average. Another way to display the average is to check the **Add and Show** box, and then the current average will be displayed at the **Add** button click.

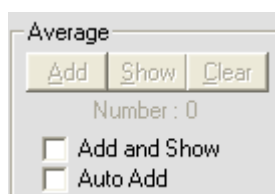


Figure 183: Averaging window.

Note : operation like **Save**, **Save As...**, **Save +**, **Print** applies to the trace displayed on the screen. 3D traces are saved as .dat files and could be open with other analysis software. At any moment it is possible to load a 3D trace by choosing the **File, Load 3D file** menu.

One of the main point of 3D analysis is the possibility to extract a 2 dimension trace: absorbance against time (Kinetics) or absorbance against wavelength (spectrum). The 2D or 3D view is selected using the **3D display** / **2D display** button.

The nature of the extracted trace is chosen through the **2D display graphic** area (shown in Figure 184)

➤ When Spectrum is chosen :

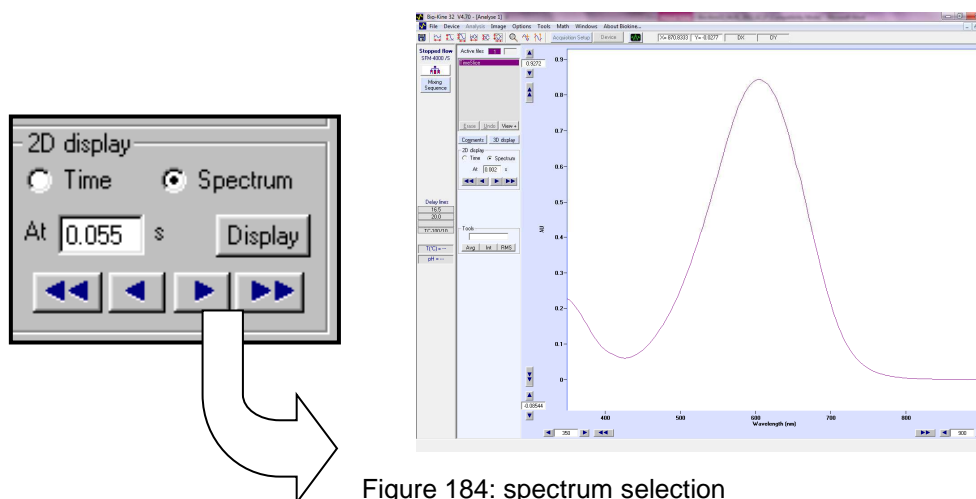


Figure 184: spectrum selection

In this configuration absorbance spectra are displayed. Enter a time in the state panel shown in Figure 184 and then validate by clicking on the display button to visualize the extracted spectrum. Using single and double arrows allows observing the next or previous spectra to the one selected just before.

When Time is chosen :

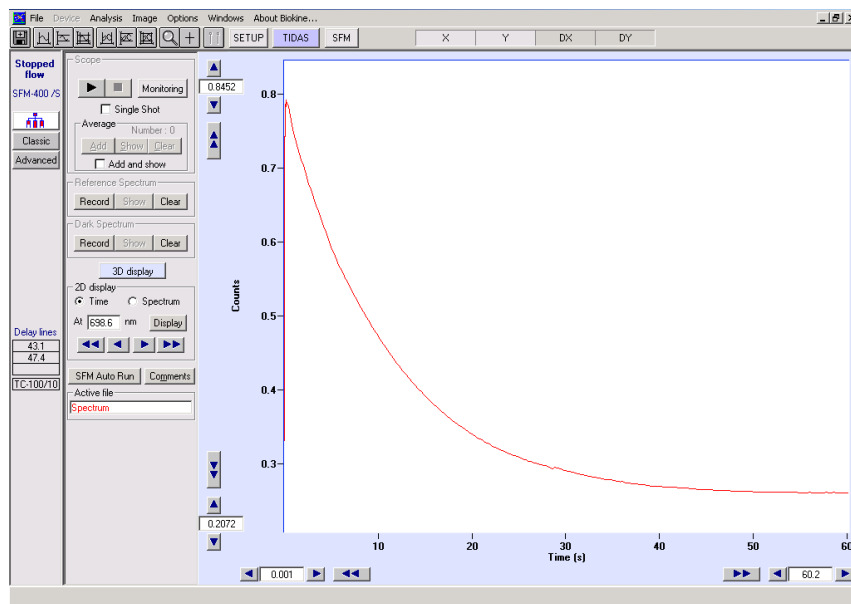


Figure 185 : Kinetics as a 2D view extracted.

In this configuration a Kinetics trace is displayed. Enter a wavelength in the state panel shown in Figure 185, and then validate by clicking on the display button to visualize the extracted time window. Using single and double arrows allows observing the next or previous spectra to the one selected just before.

- **Multi-extract option**

This option allows to extract and display, from the 3D spectrum, up to 10 spectra (at different chosen wavelength) or 10 kinetics (at different chosen time points). From the 3D spectrum, go to *Analysis*, click on *2D display*, then go to *tools* and choose *Multi-extract*.

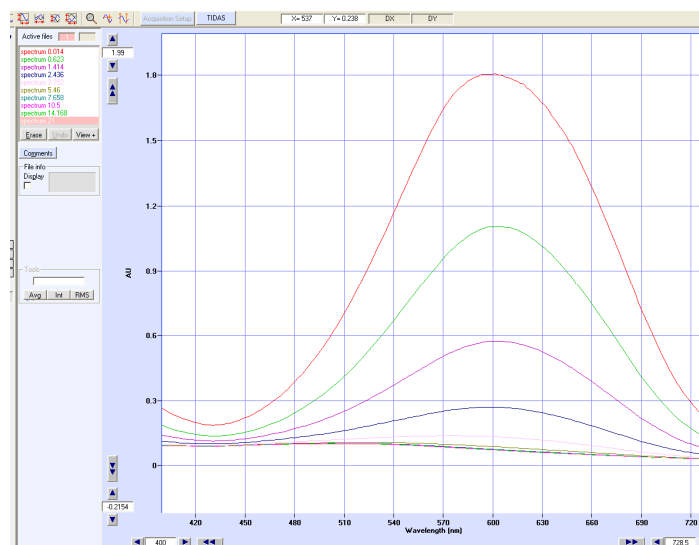


Figure 186: multi extract 2D files at different time points

Subtract plot from 3D option

This option allows to subtract one kinetic or one spectrum from the 3D spectrum.

It can be used to remove noise from the whole spectrum.

From the 3D spectrum, go to *Analysis*, click on *2D display*, from here you have to select the kinetics or the spectrum you want to subtract, then go to *tools* and choose *Subtract plot from 3D*. Then you can get back to the 3D spectrum.

8.4.8. Go to analysis window

While working on an acquisition window, the main menu **Analysis command** will load an analysis window and set the actual data in this window. This is useful for performing an immediate analysis of the current data. Once the analysis window has been loaded, all the mathematical tools described in section 3.12 can be used so as to treat the data and Kinetic constants.

8.5. How to run Kinetics

The method presented below represent a typical Kinetic experiment. For a better understanding it is necessary to have previously read the characteristics of each function above. Not all the functions are presented in this example; it is just the minimum to do to get a Kinetic trace.

Configuration

- Select Install, device installation in the main menu

Select the TIDAS diode array.

Select the stopped-flow device and USB port (for MPS-70 only)

Accept your parameters by clicking on the OK button and return to the main window.

- Select Install, stopped-flow configuration in the main menu

Select the device installed.

Select the size of the syringes.

Accept your parameters by clicking on the OK button and return to the main window.

Select Install, Tidas advanced settings, select ASPEN, and click on **Find** button, then **Refresh** to find the IP address of the diode array. Select the proper address, and then click on **OK**.

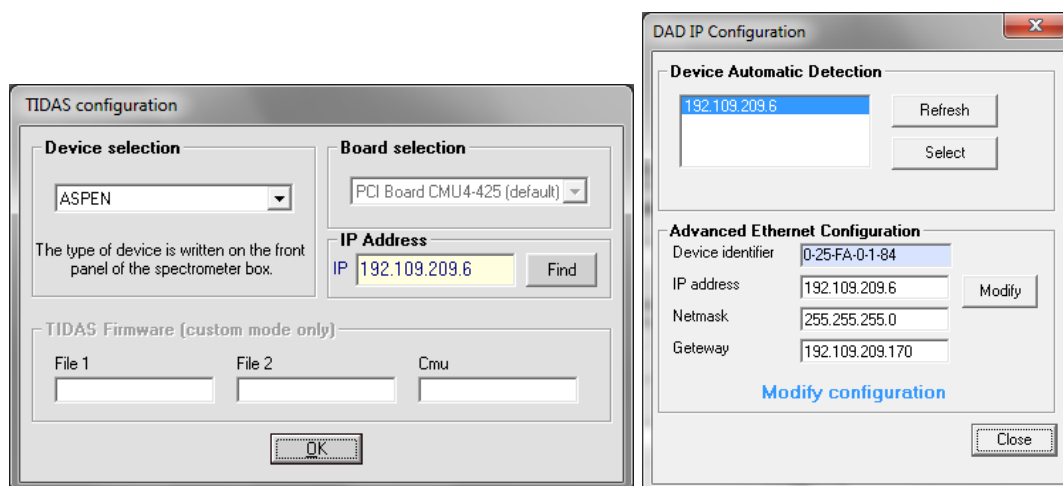



Figure 187: Tidas configuration

Note that for older tidas version a PCI board is used, and selection of the proper device has to be performed (label at the front panel of the diode array).

Stopped-flow parameters and sequence

➤ In the stopped-flow status area, click on the  button. Initialize the syringes.


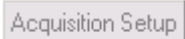
Load the solutions into the instrument.

Push some buffer into the cuvette for further reference or High voltage adjustment.

- Edit the stopped-flow sequence in either **advanced** or **classic** mode and set SFM options (cuvette, delay lines...).
- Once the sequence done, click on the **ready** button.

The control shot window is now activated in the stopped-flow status area

Acquisition parameters

- Select Device, diode array spectrometer in main menu.
 - Click on the blue  button in the main menu to check if the DAD is well recognized by the software and to initialize the system.
- Click on the  button.
 - Select the scan mode (absorbance or transmittance)
 - Check the trigger box.
 - Choose the wavelength limits.
 - Choose an integration time.
- Select a number of time bases and their parameters.
- Validate by clicking on the **OK** button.

Dark and reference spectra

Perform a dark current measurement by clicking on the corresponding Record Button.

The dark current measurement is now displayed on the screen

- Buffer being into the cuvette, perform a **reference spectrum** by clicking on the corresponding **Record** button.

The lamp spectrum is now displayed on the screen

- If light saturation is observed (over 65535 counts) then adjust the position of optic fibers and the light intensity, repeat reference spectra until disappearance of saturation.

Running the shot

Click on the start button of the scope (the message 'waiting for trigger' is displayed in red).

Click on the start button of the shot control window to run the shots.

The 3D view is now displayed on the screen.

It is now possible to do some 2D extraction from the 3D view and then use all graphical tools.

9. USING THE PELTIER ELEMENT

9.1. Description

The Peltier element is the ideal option to perform thermal denaturation or melting experiment.

The stopped-flow head is replaced by a Peltier cell cuvette holder. The Peltier element is linked to a thermostated water bath using its two input and output tubes. The Peltier element can be controlled locally from its TCU (Temperature Control Unit) or from Bio-Kine software. The wiring of the components is explained in section **Erreur ! Source du renvoi introuvable.**

When working with the peltier element, different temperatures are used, it is indeed important to understand the difference between these three temperatures (Figure 188):

T_{ref} is the temperature the user wants to reach (set by the user).

$T_{Peltier}$ is the temperature of the Peltier element, which reaches T_{ref} very fast and avoids an overshoot.

T_{bath} is the temperature inside the cuvette. The time to reach T_{ref} depends on the material of the cuvette, the amount of liquid to warm or cool.

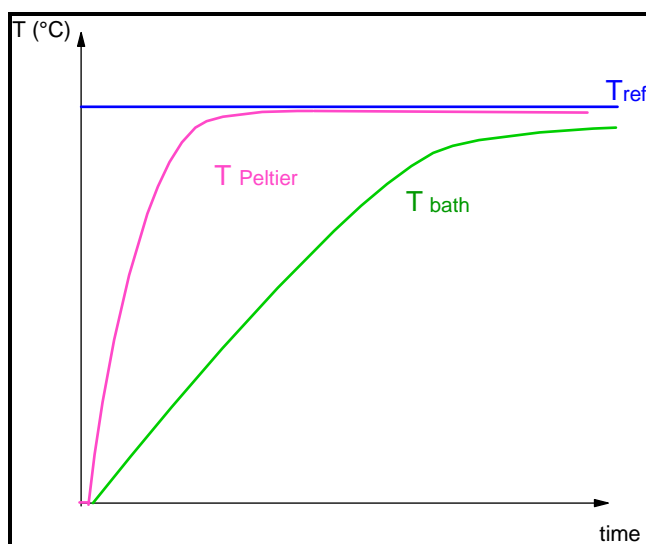


Figure 188 : T_{ref} , $T_{peltier}$ and T_{bath} .

General Specifications	
Temperature range	-40°C to 110°C
Precision of $T_{Peltier}$ ^	$\pm 0.01^\circ\text{C}$
Cooling	Water or any thermostated liquid
Cuvette	1 cm cuvette
stirring	Magnetic stirrer

9.2. Connection of TCU (Temperature control unit)

The temperature Control unit is described in Figure 189 and in the following table.

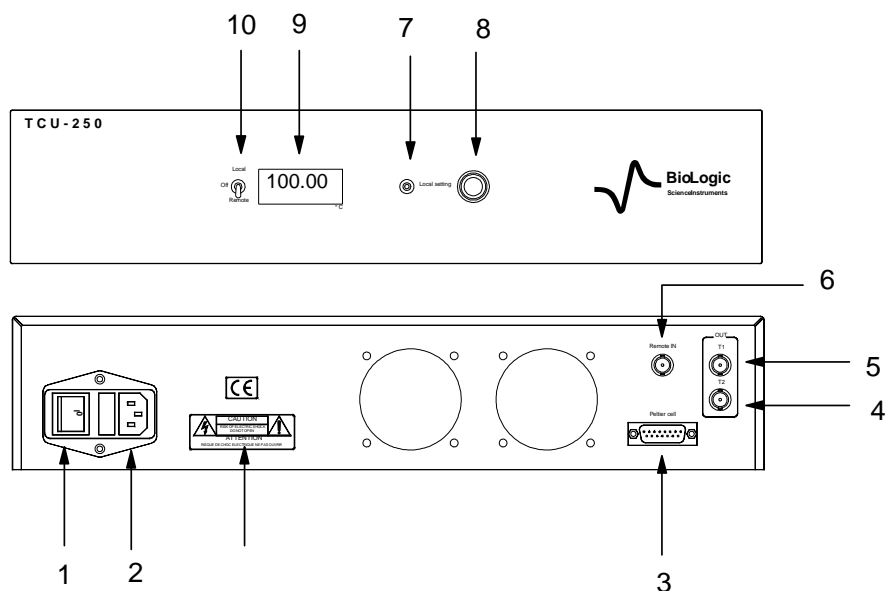


Figure 189 : description of the TCU.

	NAME	Function
1	ON/OFF switch	Turns ON/OFF the power supply
2	AC line input and fuses	Value of the fuses : T 2.5 A
3	'Peltier Cell '	Connected to the Peltier element
4	Temperature output :T2	Reads T_{bath} (temperature inside the cuvette)
5	Temperature output T1	Reads $T_{Peltier}$ (temperature of the regulation)
6	Remote In	To control the Peltier element from Bio-Kine
7	Reference setting	When pressed T_{ref} can be set (see (8))
8	Local settings	Sets T_{ref} in local mode
9	LCD (temperature)	Displays T_{bath} OR T_{Ref} accprding
10	Mode operation switch	Controls the Peltier : from TCU unit (Local) Or from Bio-Kine (Remote)

Connect the probe to the Peltier element.

CAUTION:

Before attempting any experiments with the peltier, first connect the water bath and INSURE a recirculation of the water to avoid the peltier burning.

TCU	MOS-500
Remote IN	Out1
T2 (= T_{bath})	In 2
T1(= $T_{Peltier}$)	In 1

9.3. Peltier using MOS-500

Temperature can vary from 5°C to 95°C as a standard. Cycles can be programmed and saved. In the main menu, choose: **“Device”, “Peltier controller”**. A new window appears. The yellow box displays the temperature set by the user. When entering a new value, it must be validate by pressing on **“enter”** on the keyboard, then regulation of the temperature is done on the temperature of the sample. The temperature measured by the probe plunging in the sample is displayed and is labeled **“Measure”** (Figure 190).

To display fully the peltier controller window, click on: **“full display”** (Figure 191)

The reduced window is used to have a good visibility of the acquisition window while a spectrum is plotted.

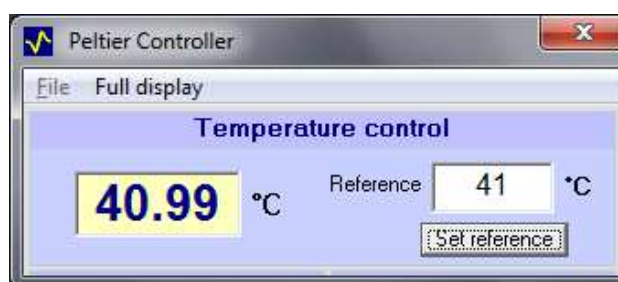


Figure 190: Peltier controller

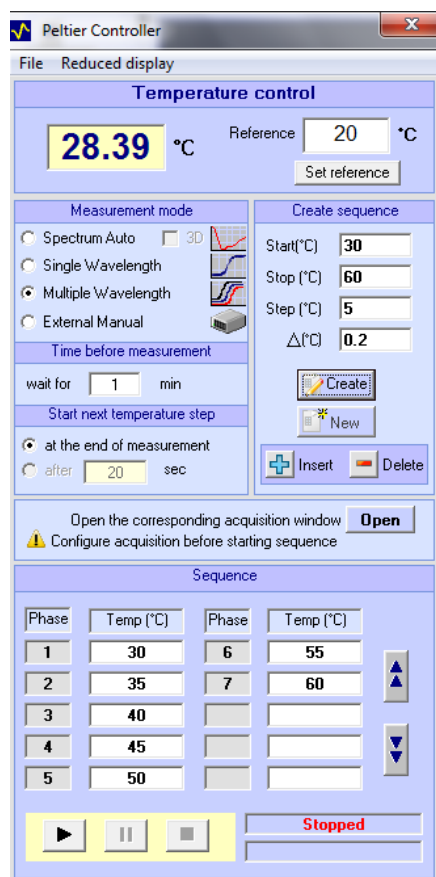


Figure 191: Peltier controller full window

This window allows assessing a temperature ramp or temperature steps.

- Temperature ramps are most of the time used for thermal denaturation. T_{initial} must be set in the reference box, when the temperature is stable, enter the final temperature (for example 95°C) in the reference box. The temperature in the cuvette increases, until reaching the T_{final} .

For such experiments, the transient recorder mode must be used (record of a signal in function of the time, and then computation to get the thermal analysis curve e.g. temperature in function of the time)

- Temperature steps can be used for spectra acquisition, single wavelength applications or multi-wavelength applications (up to 3). A program is defined in the window “**create sequence**”. Enter the starting temperature in the box “**start**”, the final temperature in the box “**stop**”, the step in the proper box. Define the Delta T acceptable for initiating the next step (we advise at least 0.2°C for good temperature accuracy). Click then on “**Create**”. The complete sequence appears then in the window “**sequence**”. Temperature steps as small as 1°C can be programmed. Before the measurement, a waiting time can be programmed from 0 min to 99 min to make sure the molecules reached a stable conformation at the temperature step before the measurement.

3D spectrum can be established while choosing the spectrum measurement mode. At the end of the measurement the software displays signal = f(wavelength, temperature).

Remark: Before running the sequence in the peltier controller, it is important to adjust acquisition parameters (blank measurement, reference, mode, acquisition time/datapoint etc..).

By default the shutter is automatically close between measurements so sample is only exposed to light during measurement.

9.3.1. Example of 3D thermal denaturation using MOS-500:

Sample: 2 mL of Lysozyme 0.01mg/mL in 1cm cuvette.

The first step is to open the scanning spectrometer menu.

The acquisition setup menu is used to adjust all parameters.

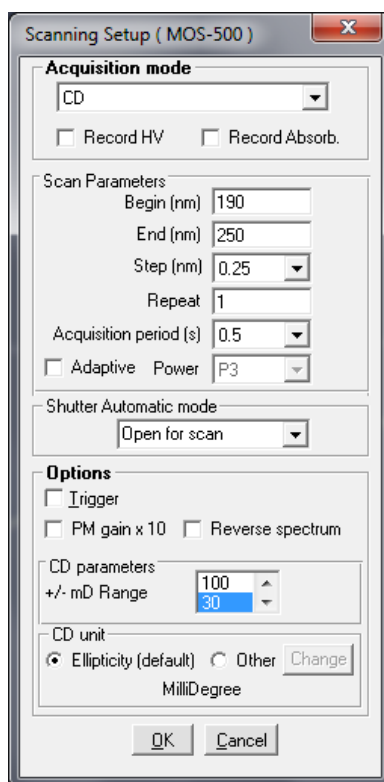


Figure 192: Acquisition setup

The 1cm cuvette is filled with water and the blank is acquired: the box subtract is checked to automatically subtract the blank to the sample's spectra.

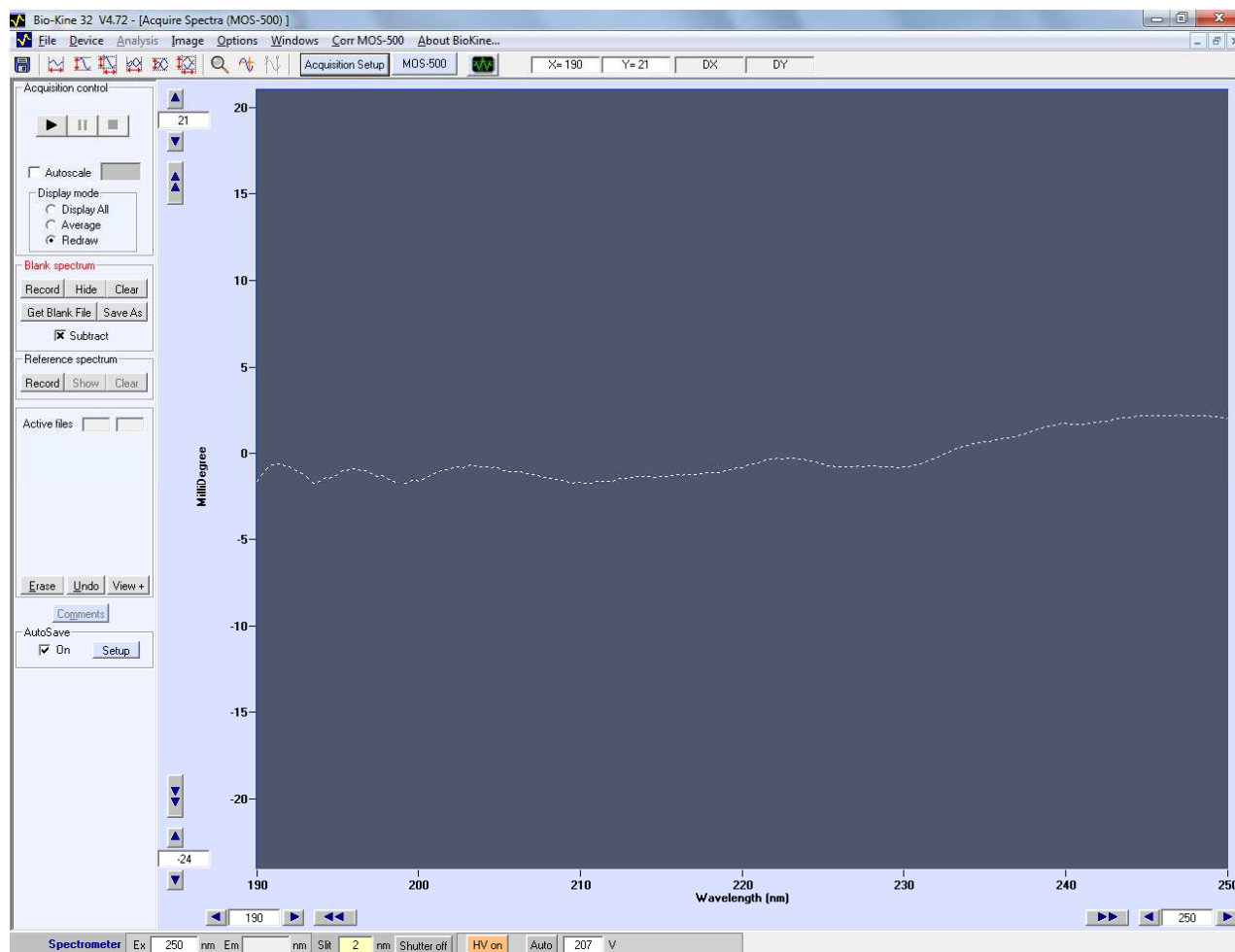
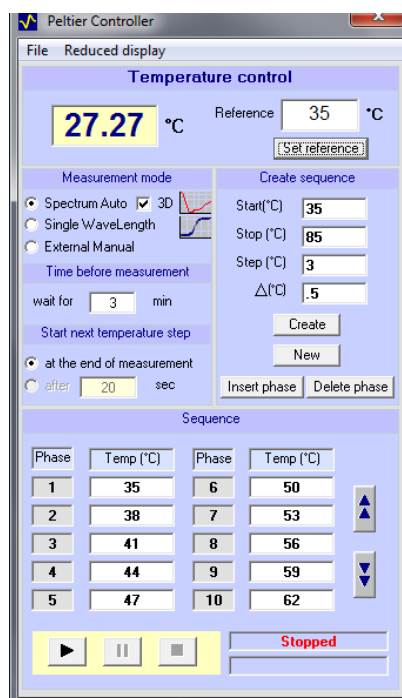


Figure 193: Blank spectrum

In the peltier controller, the program is defined so that temperature rises from 35 to 85° with 3°C steps. The $\Delta^{\circ}\text{C}$ is set to 0.5°C. **“Spectrum Auto”** is selected with the **“3D”** option. A waiting time of 3 minutes is set before the scan begins to make sure the sample reached a stable conformation. Pressing on “create” button will create the sequence. It is now possible to press on the **“run”** button to initiate the program.



All spectra are acquired. Bio-Kine displays at the end of the measurement the 3D graph. 2D extractions are possible through the “analysis” option.

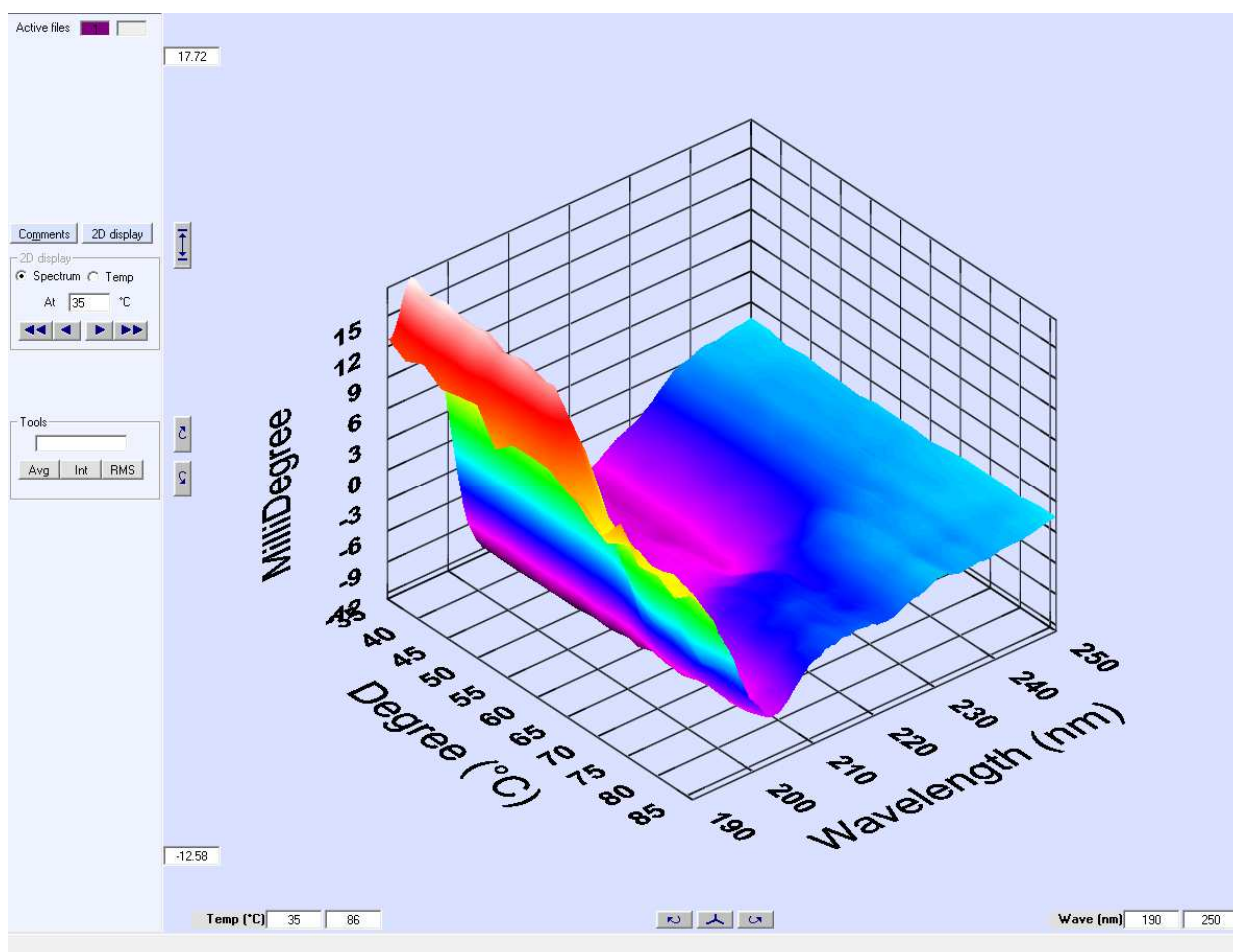


Figure 194: thermal denaturation in 3D of Lysozyme 0.01mg/mL

9.3.2. Multi-wavelength acquisition

Thermal denaturation can be followed at one, two or three wavelength simultaneously. At each temperature step signal is measured at each wavelength and denaturation curves are displayed individually.

Select Multi wavelength as shown in Figure 191 and edit your temperature sequence. Before starting the sequence you need to make sure the multi-wavelength acquisition window is open (Device, Multi-wavelength Denaturation in Biokine main menu).

Click on Acquisition Set-up button to open multi-wavelength acquisition window as shown in Figure 195.

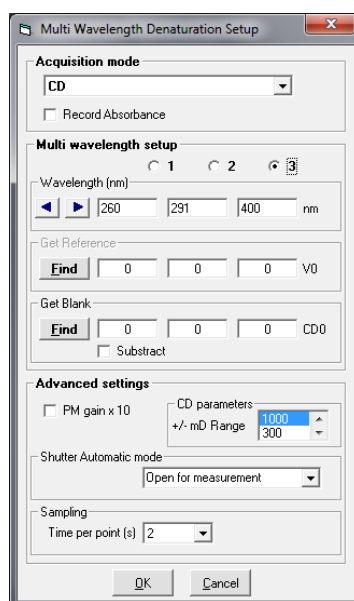


Figure 195: Multi-wavelength set-up window

Select the wavelengths of interest. Select the sampling time for each data point. By default shutter is closed between measurements. For CD measurements user may want to subtract a blank. To do so, put a cuvette with buffer in the light path and click the 'Get blank' Find button. The MOS-500 will measure CD0 at all wavelength selected. Check the 'Subtract' box so blank is automatically subtracted. If absorbance is measured simultaneously to CD repeat same operation with 'Get Reference' "Find" button.

During acquisition a temporary file name is given to each file measured so it can be easily identified on screen. For example if you are following CD and absorbance at 291 nm and 400 nm then the temporary file names would be CD291, CD400, Abs291 and Abs400. Data are saved at the end of the sequence with the name selected in the Autosave. Detection mode and wavelength are indicated automatically in the file name.

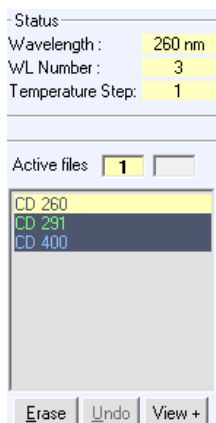


Figure 196 : Temporary files name during acquisition

9.4. Acquisition using MOS200, MOS200/M or Jasco spectrometer

With this spectrometer, a BNC connector box is used. This BNC connector box will link to the TCU following the table below:

TCU	BNC box
Remote IN	Out 2
T2 (= T _{bath})	In 4

It is compulsory to use input 4 of the BNC box to observe temperature, Inputs 1, 2 and 3 are used for signal observation.

9.5. Getting started

- Open the Bio-Kine program.
- Select **Install, Device installation** menu and check the Peltier temperature controller device as shown in Figure 197.
- Select a device and its corresponding serial port if necessary.
- Valid by clicking on the **OK** button.

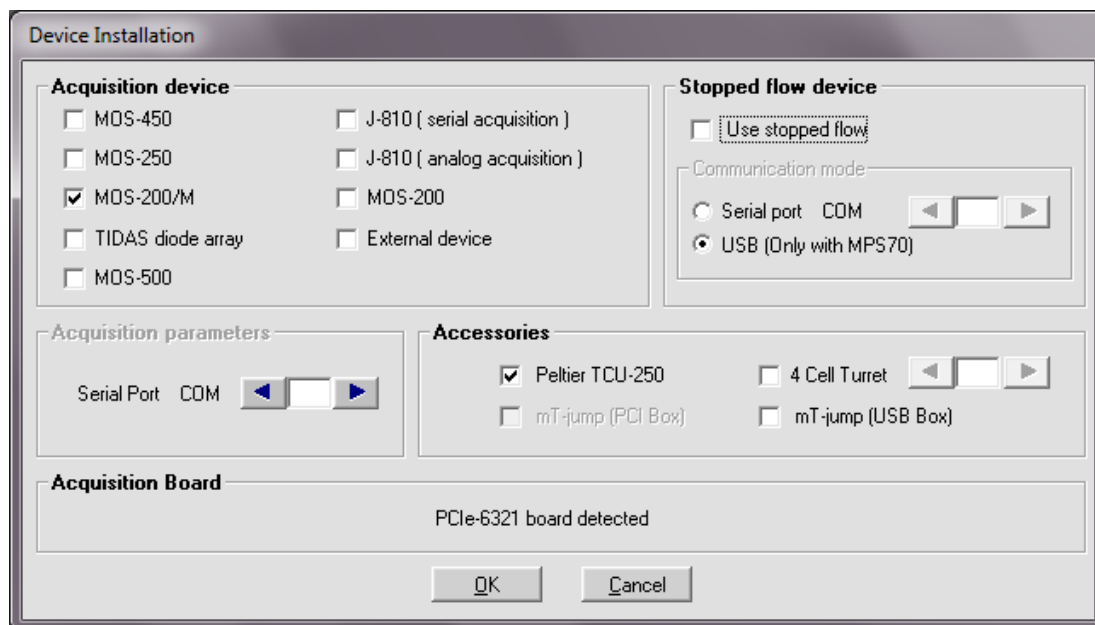


Figure 197 : Example of device installation window.

9.6. Control of the temperature

Temperature can vary from 5°C to 95°C as a standard.

In the main menu, choose: **“device”, “peltier controller”**. A new window appears. The yellow box displays the temperature set by the user. When entering a new value, it must be validate by pressing on **“enter”** on the keyboard, then regulation of the temperature is done on the temperature of the sample. The temperature measured by the probe plunging in the sample is displayed and is labeled **“Measure”** (Figure 198).

To display fully the Peltier controller window, click on **“display full”**.

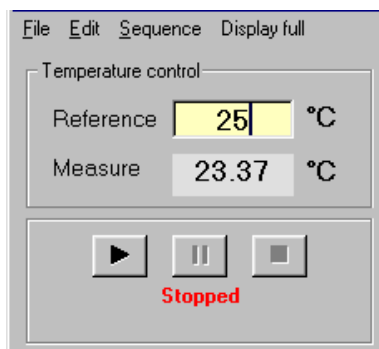


Figure 198: Peltier controller

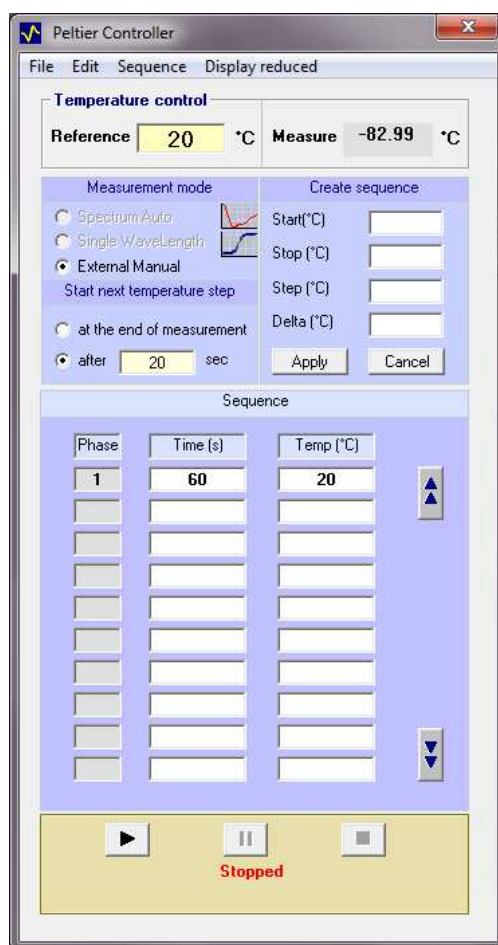


Figure 199: Peltier controller full window

This window allows assessing a temperature ramp or temperature steps.

- Temperature ramps are most of the time used for thermal denaturation. T_{initial} must be set in the reference box, when the temperature is stable, enter the final temperature (for example 95°C) in the reference box. The temperature in the cuvette increases, until reaching the T_{final} . For such experiments, the transient recorder mode must be used (record of a signal in function of the time, and then computation to get the thermal analysis curve e.g. temperature in function of the time)
- Temperature steps are most of the time used for spectra acquisition but it can also be used for single wavelength experiments. A program is defined in the window "create a sequence". Enter the starting temperature in the box "start", the final temperature in the box "stop", and

the step in the proper box. And the Delta T acceptable for initiating the next step. Click then on **"Apply"**. The complete sequence appears then in the window **"sequence"**. Temperature steps as small as 1°C can be programmed.

9.7. Denatured Protein Analysis window

Thermal denaturation data obtained using a Peltier element or the turret can be analyzed to get thermodynamic properties of protein. This analysis tool is ideal for two states denaturation and to calculate T_m , ΔH and ΔS .

First load a denaturation file (signal versus temperature) in Biokine and Select Tools, denatured Protein Analysis.

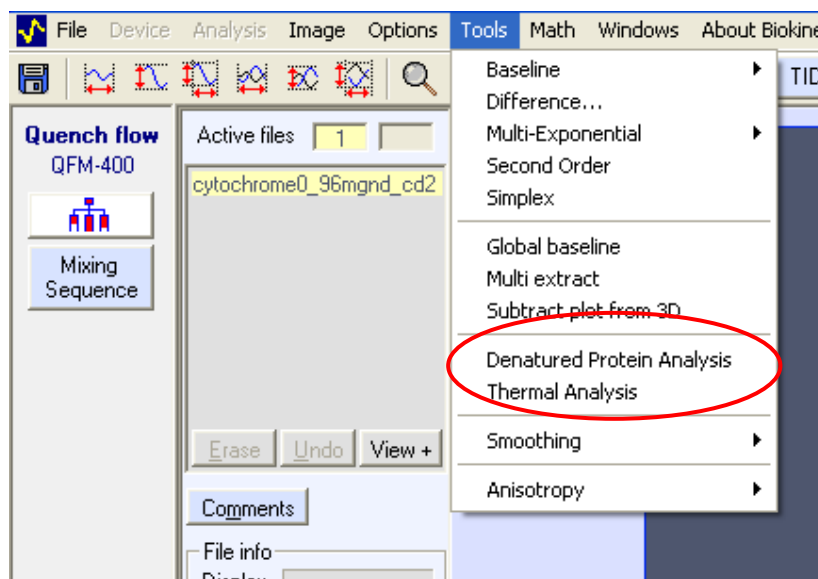



Figure 200 : denatured Protein analysis window

4 vertical markers are used. 1 and 2 are used to define the baseline of native state and 3 and 4 are used to define the baseline of denatured state. Drag the markers to define the two baselines. Once

markers are in correct position Click on 

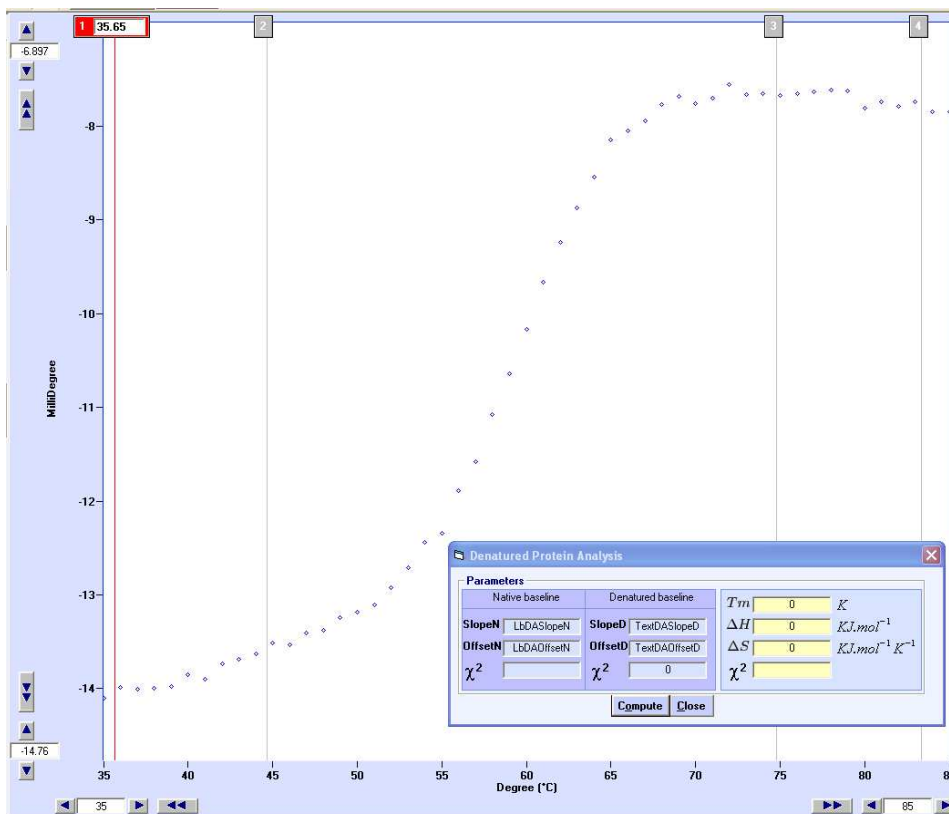


Figure 201: vertical markers

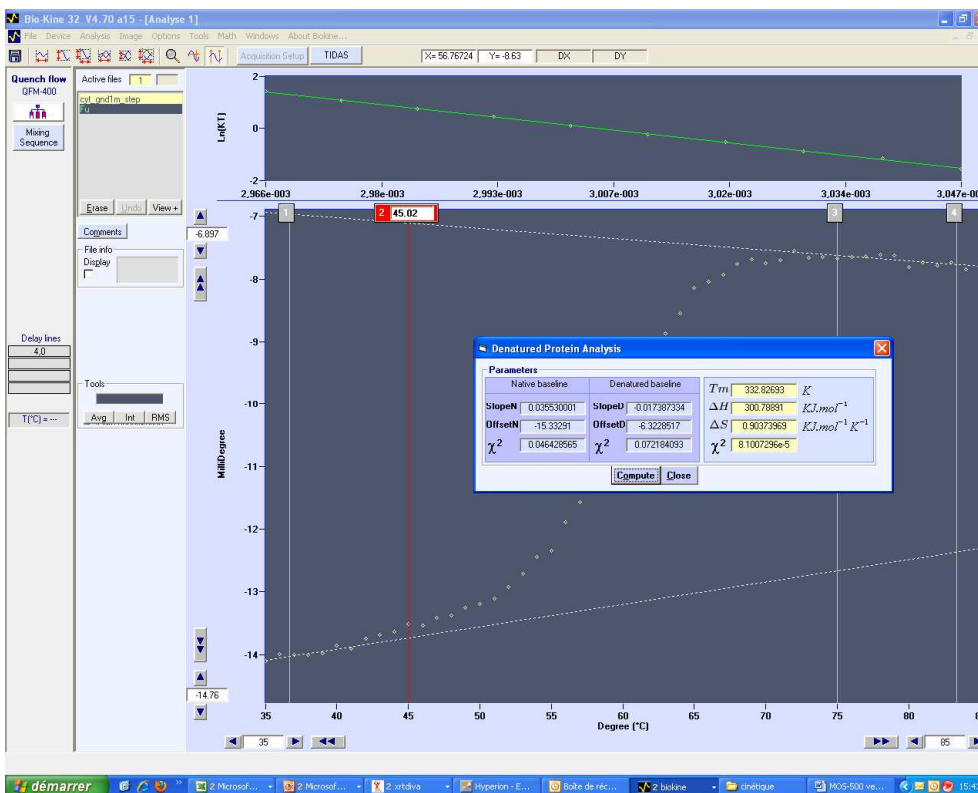


Figure 202 : analysis

The two baselines are displayed in dotted line so user can check linearity. The top view shows $\ln(K) = f(1/T)$ around the transition area. The linearity of this trace determines if the model selected was correct: T_m , ΔH and ΔS are returned in the analysis window. Fraction unfolded (F_u) is also automatically displayed.

For determination of thermodynamic parameters following equations are used:

Fraction unfolded is defined by

$$F(u) = \frac{Y_D - (Y_N + m_N T)}{(Y_U + m_U T) - (Y_N + m_N T)}$$

$F(u)$ = fraction unfolded

Y_D = signal (CD or other unit)

Y_N = native baseline

m_N = slope of native baseline

Y_U = denatured baseline

m_U = slope of denature baseline

T : temperature in Kelvin

The apparent equilibrium constant K_T is defined by

$$K_T = \frac{F(u)}{1 - F(u)}$$

And thus

$$\ln(K_T) = \frac{\Delta S}{R} - \frac{\Delta H}{R} \cdot \frac{1}{T}$$

T in Kelvin

$R=8.314 \text{ J.kmol}^{-1}$

By plotting $\ln(K_T) = f(1/T)$ and measuring slope, intercept and equilibrium user get ΔS , ΔH and T_m .

9.8. Multi-wavelength Denaturation

When a spectrum is recorded at each temperature step and data are displayed as a 3D file it is possible to determine thermodynamic properties at several wavelength simultaneously. The method used to determine T_m , ΔH and ΔS is similar to the one described in section 9.7. At the end of temperature ramp, select the 2D view and in main menu select Tool, Multi Wavelength denaturation analysis to open analysis window shown in Figure 204.

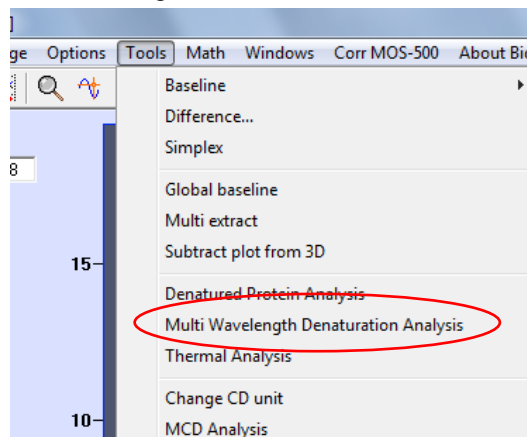


Figure 203 : Multi Wavelength denaturation analysis menu

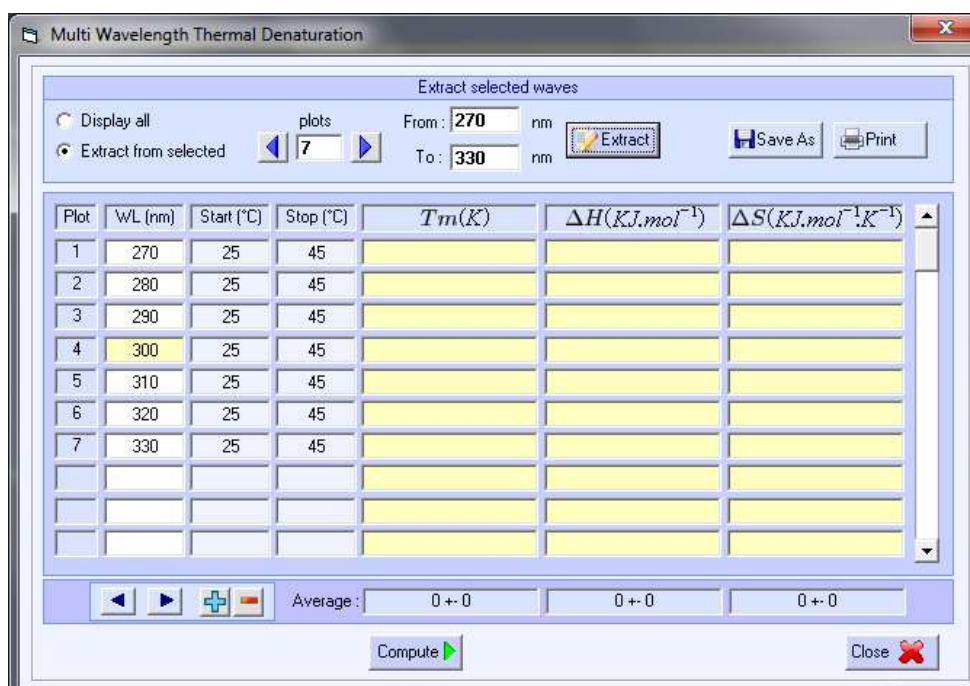

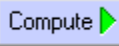


Figure 204 : Multi wavelength denaturation window.

Choose to display all recorded wavelength or to extract some wavelength of interest. In this case select number of plots you want to extract between two wavelength limits. Wavelengths are automatically extracted on the interval selected, user can manually change wavelength by entering wavelength of interest. Plots can also be added or remove manually using . Once all wavelength are selected, click  to start calculations. T_m , ΔH and ΔS are calculated for all wavelength.