User's Guide

PMOD Blood Sampling (PSAMPLE)

Version 3.3

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Contents

Swisstrace Blood Sampling System "Twilite"

Introduction

Swisstrace developed a new high sensitive blood sampler: *the twilite*. The design of the system was optimized for research using PET (small animal and human) as well as beta-probes. The experimental setup allows acquiring arterial input curves without blood loss in rodents.

The twilite's performance is outstanding: the system proved excellent sensitivity, linearity and signal-to-noise, also in the presence of high external radiation.

Conventions

Please note the conventions used in this documentation:

Mouse Operation	Type of Information
Click	The term *click* in the text means that the left mouse button is pressed down and then released. Clicking with another mouse buttons is indicated by a description such as *right click*.
Double-click	The term *double-click* means that the left mouse button is clicked twice in a fast sequence without moving the mouse.
Drag	The term *drag* means that the left mouse button is pressed and hold down while the mouse is moved.

Formatting	Type of Information
Special Bold	Items you must select in the user interface of the program, such as menu options, command buttons, or items in a list.
Emphasis	Used to emphasize the importance of a point or for variable expressions such as parameters.
CAPITALS	Names of keys on the keyboard. For example, SHIFT, CTRL, or ALT.
KEY+KEY	Key combinations for which you must press and hold down one key and then press another, for example, CTRL+P, or ALT+F4.

Please refer to the Glossary at the end of this document for information regarding specialized terms used in the documentation.

Purpose

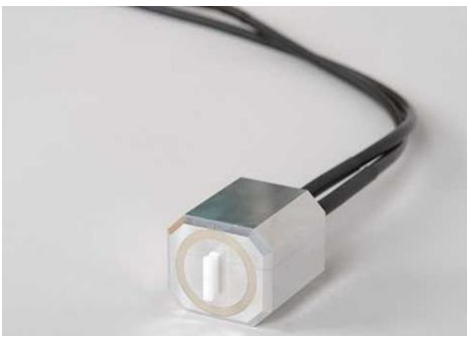
The twilite is the device created to measure the time-course of radioactivity in the whole blood with high temporal resolution. The data acquisition is performed with the dedicated PMOD module PSAMPLE. The PMOD software with its modular structure allows for comprehensive off-line analysis of radio tracer data.

The main application of the Sampler is the quantification of PET studies.

Quantification is unique and the gold standard methods require the arterial input function (AIF). The joint use of the twilite system and PSAMPLE software allows the accurate measurement and correction of the whole blood tracer activity. The corrected blood activity measurements are used to identify the plasma activity. This represents the essential information needed for quantification purposes. The PKIN module is the dedicated tool for blood data post processing and quantification. The present PKIN modeling tool offers an easy and intuitive access to the wealth of developed methods to a broader community.

Detection Principle

The core of the twilite system is a very compact measuring head machined from medical grade tungsten, which shields the LYSO crystals from outside radiation and is fully MR compatible.

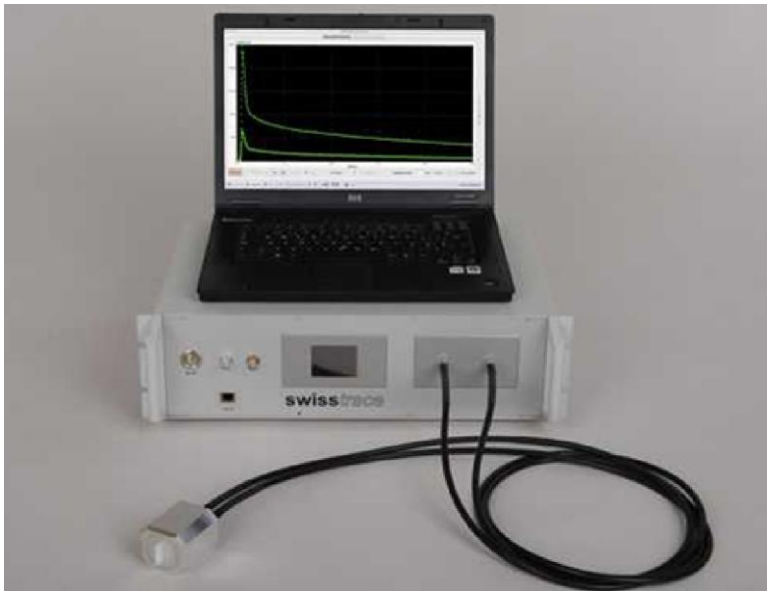


The scintillations are conveyed to the photon detection unit via two flexible high efficiency light guides. This elegant design is without any electronics in the sensor head and thus avoids any potential problems of electromagnetic interference with other devices. Furthermore, this design is minimizing any potential risks for the use in human research experiments.

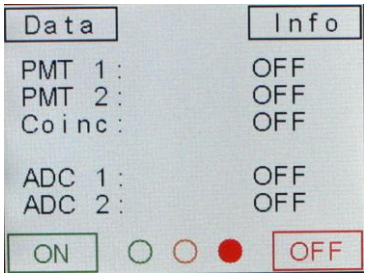
Twilite Components

The device shipment consists in two boxes: one box for the twilite acquisition box and annexes and one box for the twilite measuring head and guide lines.

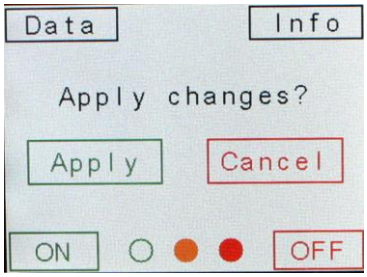
The Swiss Trace Sampler can be connected directly to a computer via a network cable, as shown below:



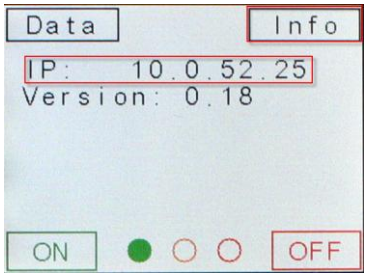
The twilite system is run as a stand-alone device. A touch-screen on the front panel serves as the user interface, and also displays the current status and measured values. The touch screen has four main dedicated buttons: **Data**, **Info**, **On** and **Off**. The last two ones describe the status of the device. The **OFF** term displayed on the touch screen interface is an indication that the device is not ready for the acquisition. In addition, the circle close to the **OFF** button is filled with red color.



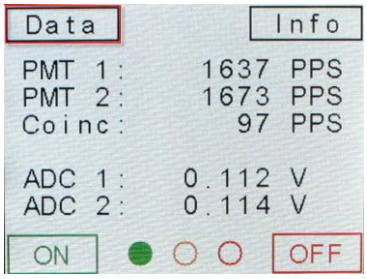
The **ON** button allows the device activation. A dialog window opens and the **Apply** button needs to be activated in order to *Apply the changes*.



The device IP information and the current version of the hardware are shown when the **Info** button is selected.



The on-line measured values are available activating the **Data** button when the Sampler is running.



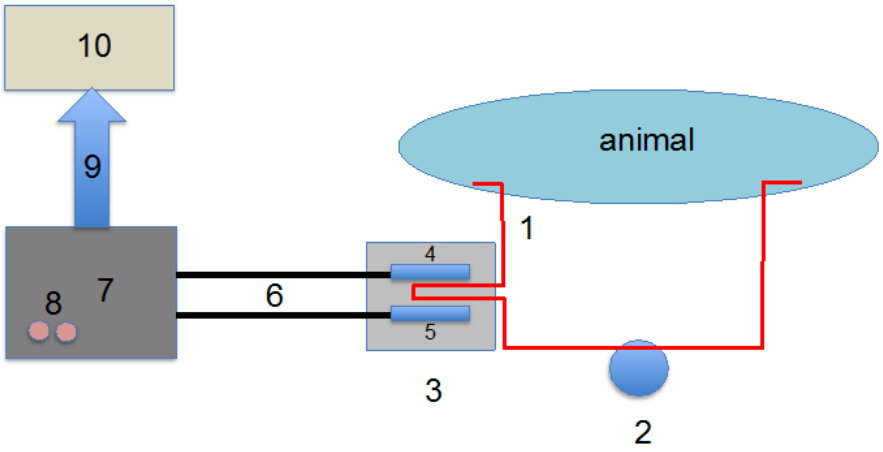
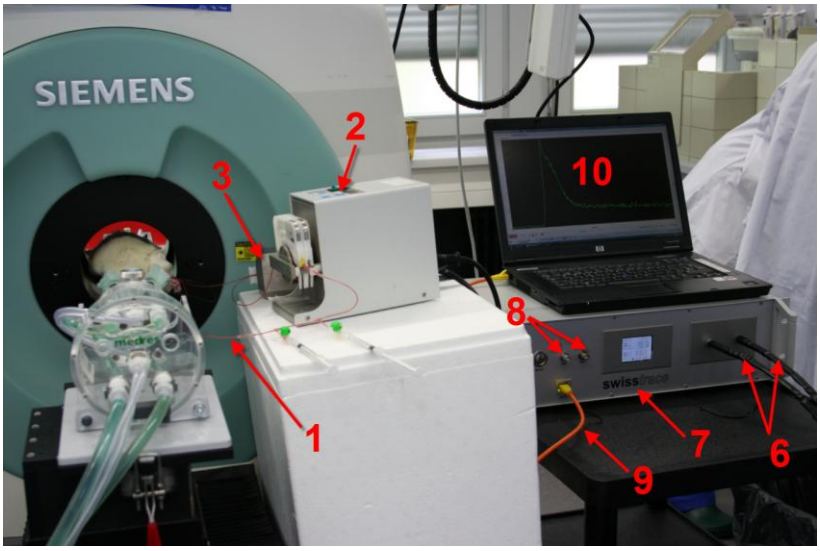


Figure 1: Twilite system



The main components of the device are illustrated in the *Figure 1* above and are as follows:

1) Shunt running from the femoral artery to the femoral vein.

2) Peristaltic pump to control blood flow in the shunt.

3) Twilite measuring head made of tungsten.

4) LYSO crystal1.

5) LYSO crystal2.

6) Light guides carrying the photons from the crystals to the PMT's. These guides have a standard length of 2 m; they can be as long as 10 m in MR compatible systems.

7) Data acquisition box with coincidence electronics.

8) Two analog input channels (for parameters like heart rate, ECG, blood pressure etc).

9) TCP/IP connection to computer with PMOD data acquisition tool PSAMPLE.

10) Computer with PMOD data acquisition and data analysis.

The twilite consists of the components 3-10. The catheters for the shunt and the peristaltic pump are not delivered by Swisstrace.

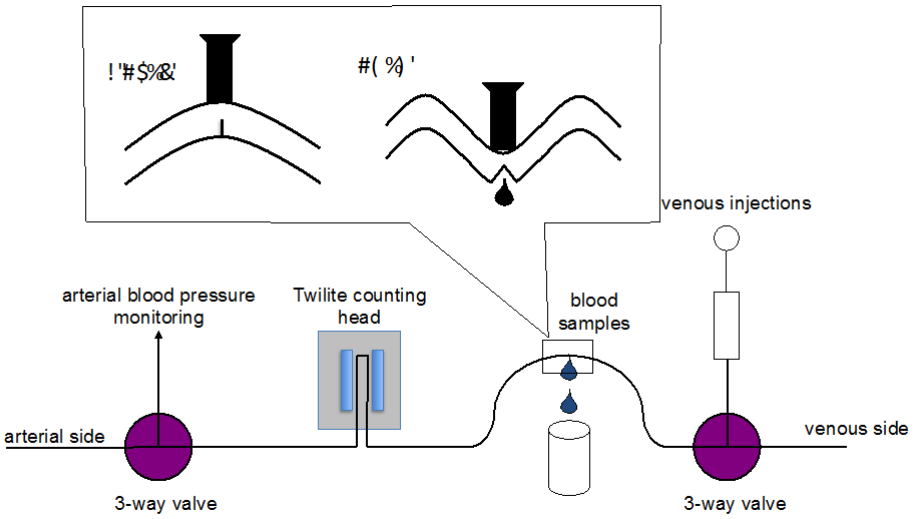


Figure 2: Shunt functions

As described in *Figure 2*, the shunt can serve several functions, such as blood pressure monitoring, tracer injection and the collection of blood samples. For the collection of blood samples, a small cut is made using a scalpel. When bent upwards, the cut is closed. To collect blood samples, the shunt is pressed downward and the cut opens. This procedure allows collecting blood samples with practically zero dead volume.

The twilite measures the radioactivity in a catheter which runs between the LYSO crystals. In animals the best solution is to place a shunt between the femoral artery and the femoral vein. This allows to continuously measure the whole blood activity without any loss of blood. Such shunts can be placed in animals as small as a mouse. In humans, the withdrawn blood should not flow back into the body. One usually places a catheter into the radial artery, runs the catheter through the twilite measuring head and directs the blood into a waste container. In humans the blood flow in the catheter is always controlled by a suitable peristaltic pump. In animals it is advisable to also control the blood flow in the shunt with a peristaltic pump. However, it is also possible to let the shunt run freely driven only by the arterio-venous pressure difference.

The catheter diameter differs according to the size of the animals. Swisstrace delivers templates for each catheter size, so that each catheter has a well defined geometry in the twilite measuring head.

The characteristics of the shunt are well described in a previous paper [1] published by Weber and co-workers.

Twilite Calibration

For full quantification it is necessary to calibrate the twilite, e.g. the counts per second units of the twilite output have to be converted to the units of the PET scanner or another instrument measuring the radioactivity in the target organ. Prerequisite for such a calibration is that the geometry of the catheter loop in the twilite measuring head is exactly the same as in the experiment. To assure this, the loop inside the measuring head is guided by a precision template which Swisstrace delivers for every catheter diameter.

Experimental Setup

The procedure for calibration is as follows:

1. Measure the background activity of the twilite.
2. Fill a phantom with radioactivity, typically 200-500 kBq/cc. Shake the phantom well to homogenize the radioactivity concentration.
3. Use the same catheter as in the experiment.
4. Fill the catheter with fluid from the phantom.
5. Insert the catheter with the radioactivity into the measuring head, using the appropriate template.
6. Position the phantom in the PET scanner.
7. Start data acquisition of PET and twilite simultaneously. The same clock time have to be set for both the PET scanner and the sampler computer.
8. Reconstruct the PET image, including attenuation correction, outline a volume of interest (VOI) in the middle of the phantom and determine the average value of the radioactivity in this volume of interest. This value represents the activity to which the Sampler is going to be calibrated. The step can be easily achieved using the basic PMOD tool **PVIEW.**

Note: The average value within the VOI has to be in [**kBq/cc**].

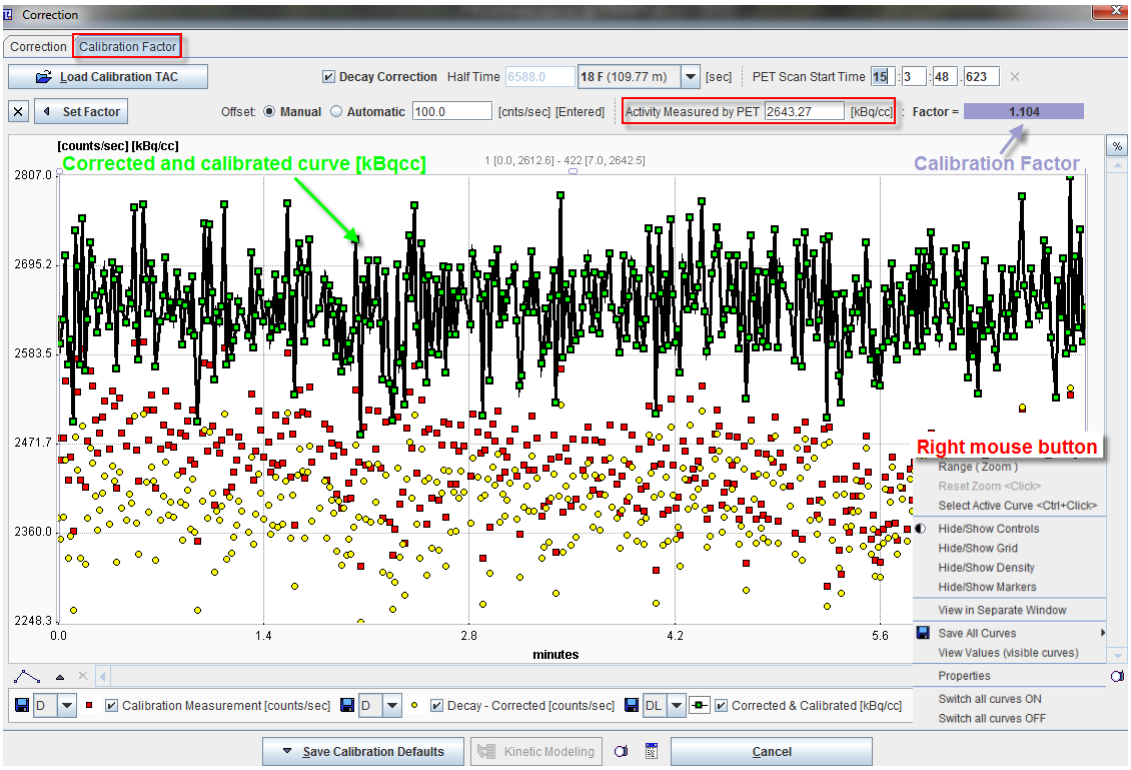
9. Use the PMOD calibration tool to calculate the calibration factor.

User Interface

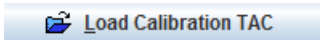
The PMOD Correction module is the suitable tool to identify the calibration factor. The correction module is started activating the **Correction** button  in the main PMOD Toolbox. The correction interface is organized in two pages: the **Correction** page and the **Calibration Factor** page.

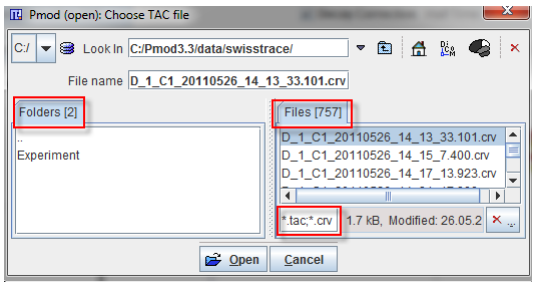
The **Calibration Factor** pane is the dedicated interface for the calculation of the calibration factor. The tool requires the twilite calibration measurement [counts/sec] and the average value of the radioactivity in the phantom image [kBq/cc].

The **Calibration Factor** window is organized in a wide curve area with the controls underneath. The setting fields and the action buttons are available above and beneath the graphic display. In the graphic area only the curves enabled for display are shown. The same curve display object is used in all PMOD tools. The detailed description is available in the PKIN documentation: the General Curve Display Functionality.

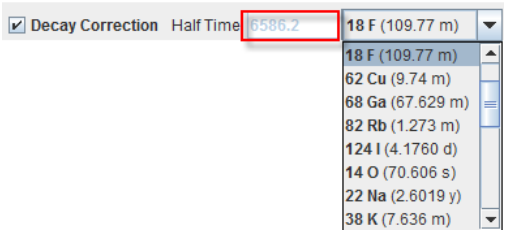
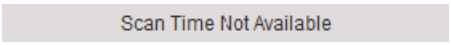


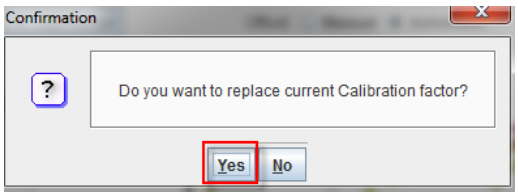
The setting fields and the action buttons are as follows:





Allows loading calibration data stored on the file system. The button activation opens a dialog window. In the upper part the current path is indicated. The program automatically points to the directory of the last successful loading operation. It has elements for changing the directory: the navigation buttons in the **Folder** section (**"."** indicating one level up). All files suitable for loading (having the right suffix, such as *.tac, *crv) in the search directory are listed in the **Files** section. The **Open** button starts loading the selected file, **Cancel** quits the operation, and **Delete** erases the file from the disk.

	The button is located under the **Load Calibration TAC** button. Its activation closes the displayed TAC. Subsequently, the graphic area becomes empty.
	Checkbox enabled: allows the selection of the radio isotope used in the experiment. Accordingly, the activity measurements are corrected for the decay. The isotope half-life is displayed in seconds in the **Half Time** field.
 	Displays the time when the PET scan was started. The text fields allow manual adjustment of the delay of several seconds that can occur during an experiment. Consequently, the "**X**" symbol becomes active and allows reseting the time to the original file start time. In case no TAC was loaded the time setting area is gray and a message is displayed: *Scan Time Not Available*
Offset  	Performs the subtraction of the background activity [counts/sec] from the measured TAC. **Manual** radio button **ON** consents the user to interactively *enter* the known value (generally around 100cts/sec). **Automatic** radio button **ON** subtracts the first measured value from the TAC when PET and twilite acquisition started simultaneously. Differently, when the *PET Scan Start Time* must be adjusted for the delay the value to be subtracted is calculated as the average value over the delay time (*[Aver]*).
	Allows entering the average activity measured within the VOI outlined on the corrected and calibrated phantom image (must be in [kBq/cc]).
	Represents the twilite calibration factor and is automatically calculated when the activity measured on the PET phantom image [kBq/cc] is available.

Set Factor 	Allows setting the calibration factor to the **Correction** page. Its activation opens a dialog window. If **Yes** button is selected the program replace the current calibration factor available in the **Correction** page with the newly identified one. Finally, the program automatically switches to the **Correction** page. The selection of **No** button preserves the current settings.
Save Calibration Defaults	The activation of the button saves and sets the calibration factor on both PSAMPLE Correction pages.
Cancel	The selection of this button closes the PSAMPLE Correction module.
	Buttons dedicated for problem reporting. The capture button creates a capture of the entire screen (not only the PMOD window) and adds it to a buffer of up to 20 captures. The console button opens the **Console** dialog window.

Workflow for Determination of the Calibration Factor

The main steps to calculate the calibration factor within the **Calibration Factor** interface are:

1) Load the calibration data.

2) Verify that the start time is correct.

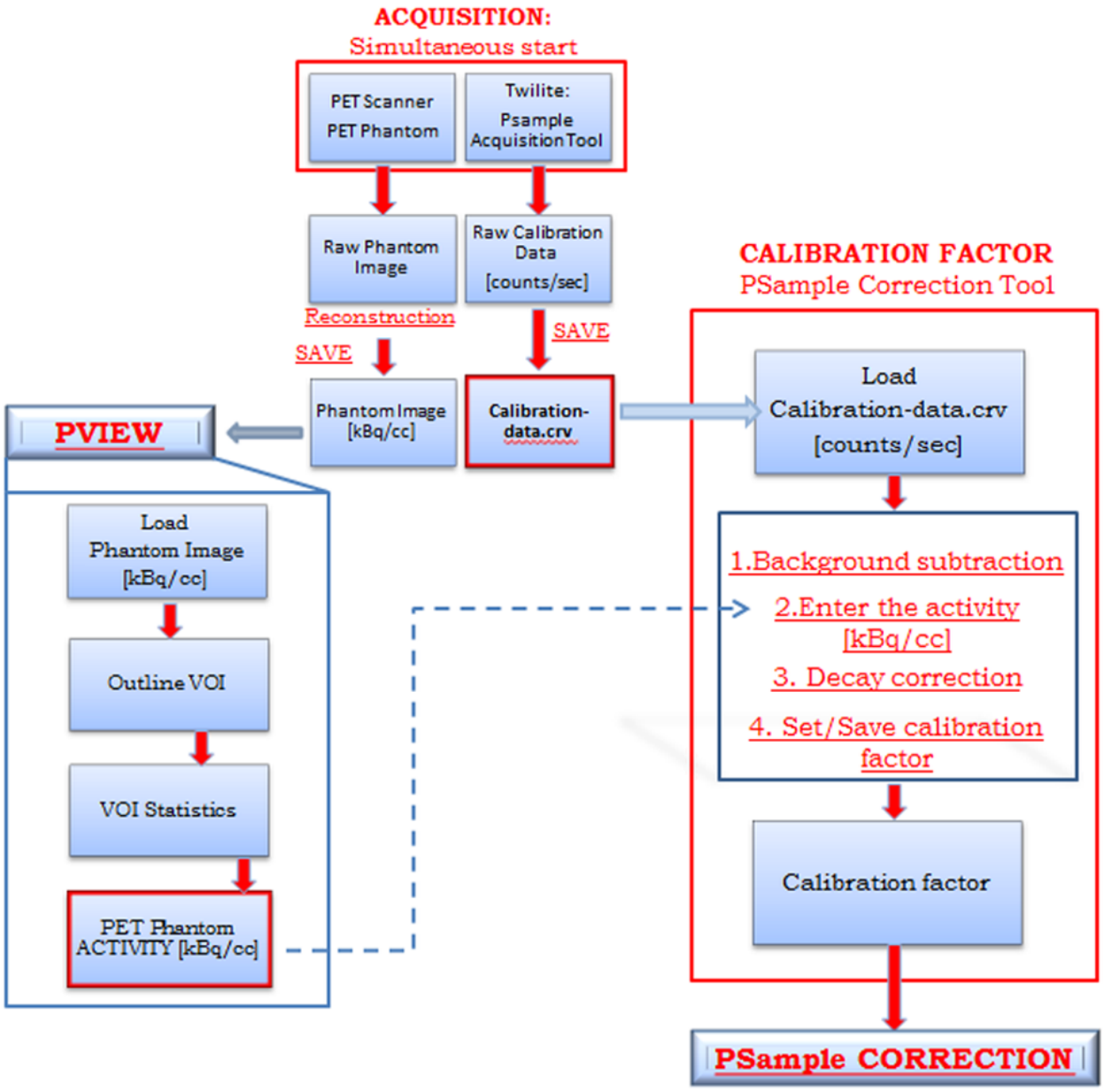
3) Enter the activity measured within the VOI outlined on the corrected and calibrated PET phantom image ([kBq/cc]).

4) Activate the **Manual Offset** radio button and enter the background activity value (generally 100 counts/sec).

5) Enable the decay correction and select the radio isotope used in the calibration experiment.

6) Set the calibration factor to the correction page activating the **Set Factor** button. Alternatively, save and set the calibration factor activating the **Save Calibration Parameters** button.

The experimental setup and the determination of the calibration factor are summarized in the workflow below:



Setting up Configurations for Different Types of Acquisitions

PMOD supports the concept of different PMOD users. Each user can maintain his own preferences such as the model selection and order, user interface font size, report layout etc, and PMOD maintains for each user independent tool configurations and loading histories.

The **USERS** tab of the configuration utility allows creating PMOD user accounts, and configuring their properties. The basic documentation includes a detailed description of the USER configuration in the section PMOD Basics/General Configuration/Users.

Note: For all changes of the settings it is important to *first select the affected user*, and then proceed with the configuration.

Dedicated PMOD users can be configured for each small animal type and each radio isotope, e.g. mouse_F18, mouse_C11, rat_F18, rat_C11, etc. The purpose of this action is to preserve simultaneously the calibration parameters for different types of acquisition.

The main steps to achieve this facility are as follows:

1) Start PMOD.

2) Select the appropriate user from the user list in the main PMOD toolbox and **Log In**.

3) Start the PSAMPLE correction tool.

4) Select **Calibration Factor** panel.

5) Determine the calibration factor as described in the *Twilite Calibration Section/Workflow for determination of the Calibration Factor*.

6) Activate the **Save Calibration Defaults** button in the bottom line to save and set the parameters.

7) Logout from the user account.

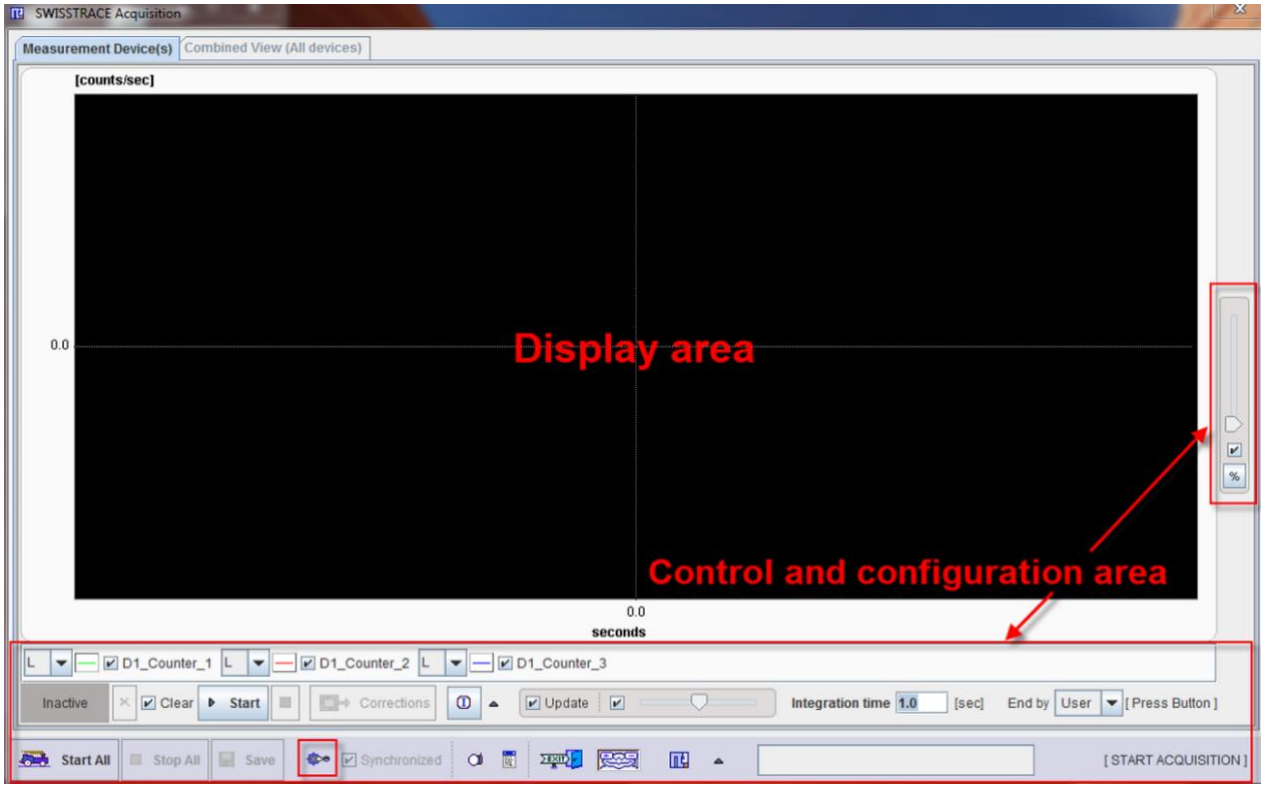
The results are therefore available only for the PMOD user account within which the determination was performed. In this way, different calibration factors are available simultaneously, but only one per user.

Data Acquisition

The data acquisition is performed with the dedicated PMOD PSAMPLE tool for acquisition. The module is started activating the corresponding button in the PMOD Toolbox.

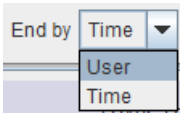
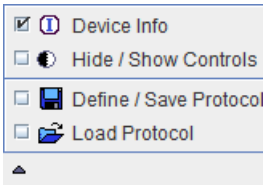
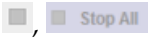
The acquisition interface is organized in two panels as shown below: the **Measurement Device(s)** page and **Combined View (All devices)** page.

The **Measurements Device(s)** page consists in a wide display area and a configuration and control area. The measurement data are displayed as time activity curves (TAC) in counts/sec over time.



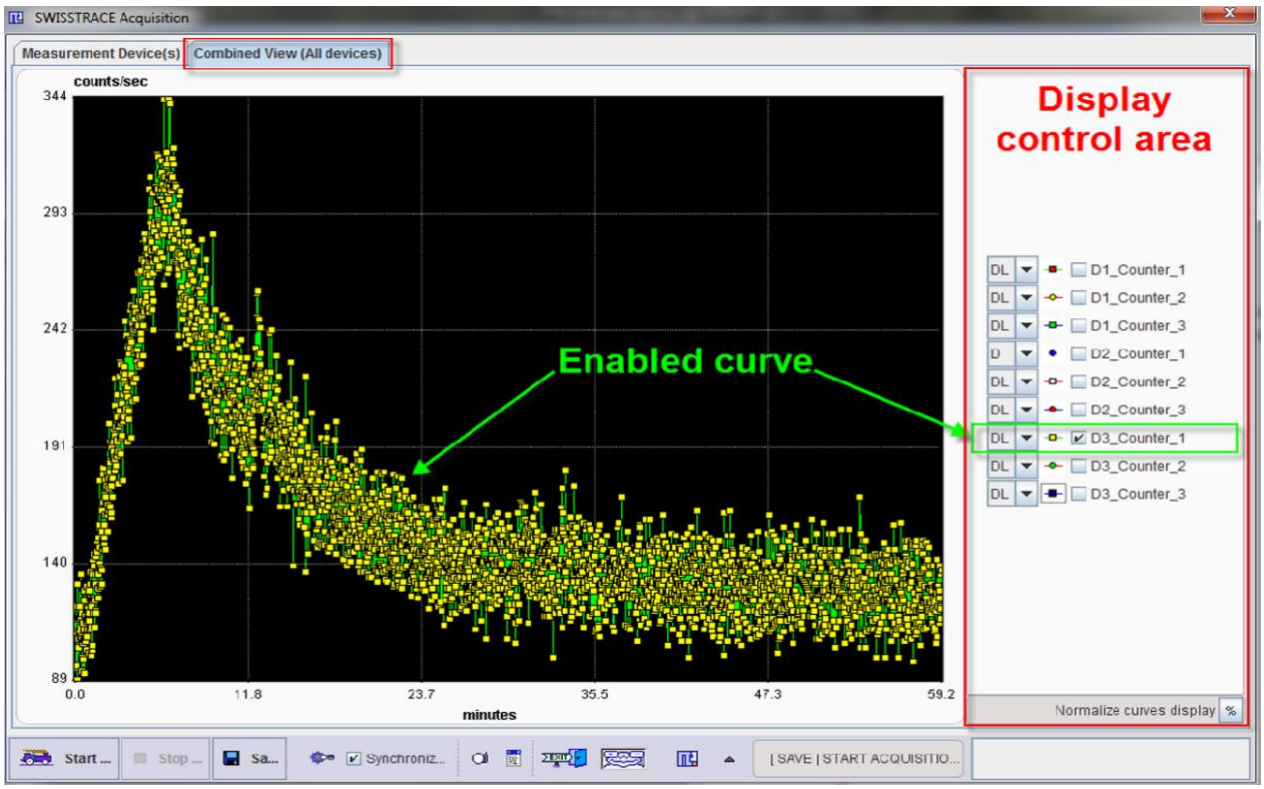
The various facilities buttons are summarized in the table below:

	Configuration button: allows the configuration of up to 3 devices.
%	**Normalization**: displays all curves normalized to 1
	Autoscale: if **ON** automatically adjusts the curves range on the display, on both X and Y axis

	**Update** checkbox: if enabled, automatically updates the display during the acquisition.
	**Corrections**: link button that allows sending directly the acquired curve(s) to the PSAMPLE Correction tool.
	**PMOD Icon**: activate the PMOD Main Toolbox from where each PMOD module can be started.
	**Clear**: is the button dedicated for the buffer cleaning.
	**Clear** checkbox: if enabled, clear the buffer on start. In particular, upon the re-start of an experiment the acquisition will overwrite the existing measurements. Contrary, if disabled, the acquisition will continue from the moment the experiment was stopped.
	**Capture**: creates a capture of the entire screen and adds it to a buffer up to 20 captures.
	**Console**: opens the console dialog window.
	The **End By** supports two setting options: ▸▸ **User**: allows stopping the acquisition any time upon activation of the **STOP** button; ▸▸ **Time**: the user can set up the time, in minutes, at which the experiment will automatically stop.
	**Device Info**: displays the set up configuration for the device; **Hide/Show Controls**: when shown allows enabling/disabling the channels signal to be displayed. **Define/Save Protocol**: is the dedicated selection for the definition of the acquisition protocol. **Load Protocol**: a protocol previously saved can be retrieved and applied to a new acquisition.
	**Start** button: upon activation starts the acquisition for the configured device. The **Start All** allows starting simultaneously the acquisition in case multiple devices are configured.
	**Stop** button: if selected the acquisition is stopped. The **Stop All** button allows ending simultaneously the acquisition for multiple devices.

The **Combined View (All devices)** page has similar layout with the first panel. This page is not accessible if only one device (e.g. Twilite) is used and configured. Differently, when multiple devices are configured the window becomes active. Thus, at the end of the acquisition, the results can be easily inspected. The shapes of different dynamic range can be

compared normalizing each curve to its own maximum. This can be easily achieved upon activation of the **Normalize curves display** button %, located on the bottom line in the display control area. In the graphic area only the curves enabled for display are shown.



PSAMPLE Configuration

The Twilite is directly connected with the computer via a network cable. The data acquisition is performed with the dedicated PMOD module PSAMPLE via a TCP/IP interface. Two configuration levels are requested:

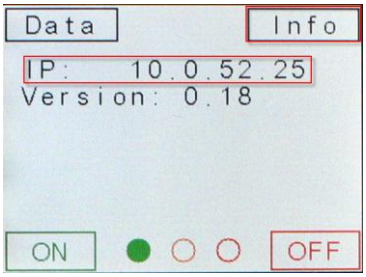
1) The network configuration.

2) The module configuration.

Network Configuration

The TCP/IP connection is automatically set up within the network when both the Sampler and the computer are **ON**. However, for Windows 7 OS an *Alternative configuration* set up is requested for proper device functionality.

The twilite IP is available upon the activation of the **Info** button on the touch screen user interface.



For a device which IP is 10.0.52.25, the set up steps are described below:

1) *Control Panel/Network/Network Connection*.

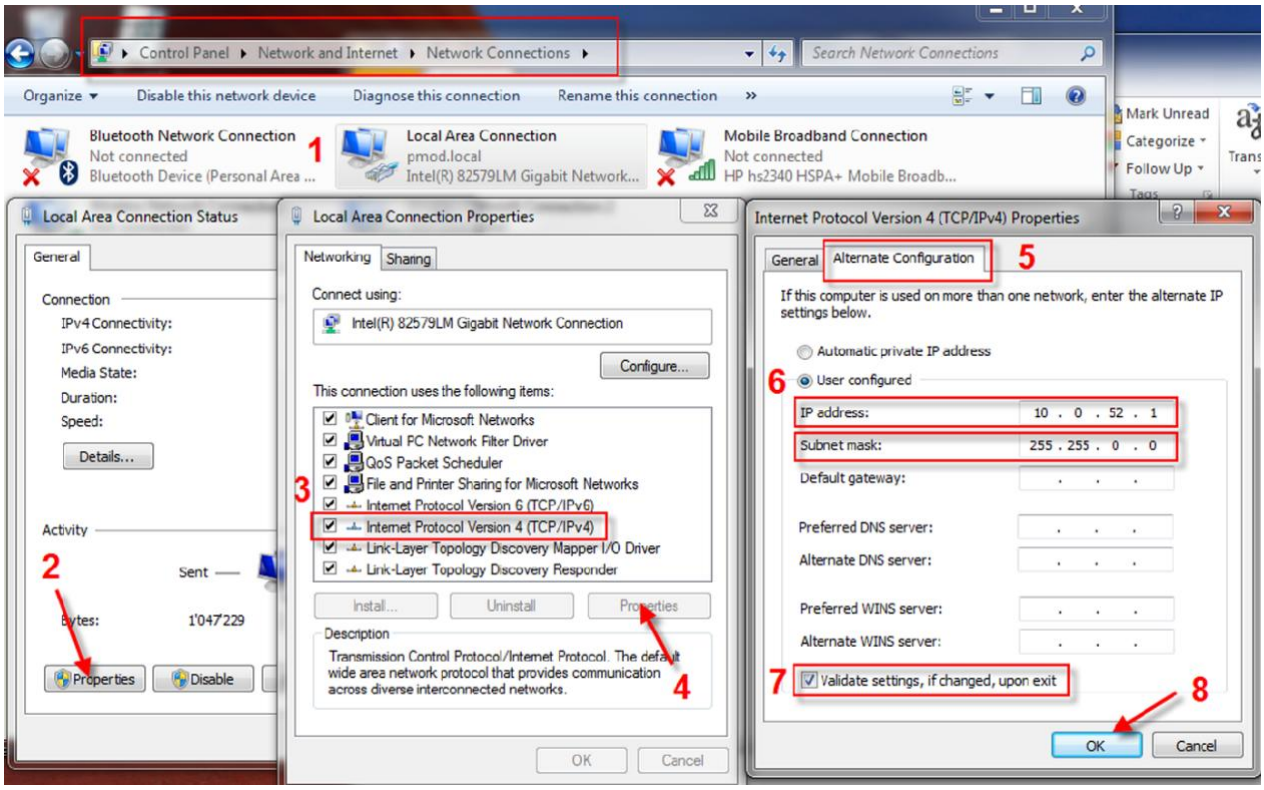
2) Select *Local Area Connection* and double click (**1**).

3) In the dialog window select *Properties* (**2**).

4) In the *Local Area Connection Properties* window select *Internet Protocol Version 4 (TCP/IPv4)* (**3**) then activate *Properties* (**4**) button.

5) In the pop up dialog window point to *Alternate Configuration* tab (**5**), activate the *User configured* radio button (**6**) and for *IP address*: type 10.0.52.1 while for *Subnet mask*: type 255.255.0.0

6) Before selecting *OK* (**8**), optionally, the *Validate settings, if changed, upon exit* checkbox (**7**) can be turned ON.



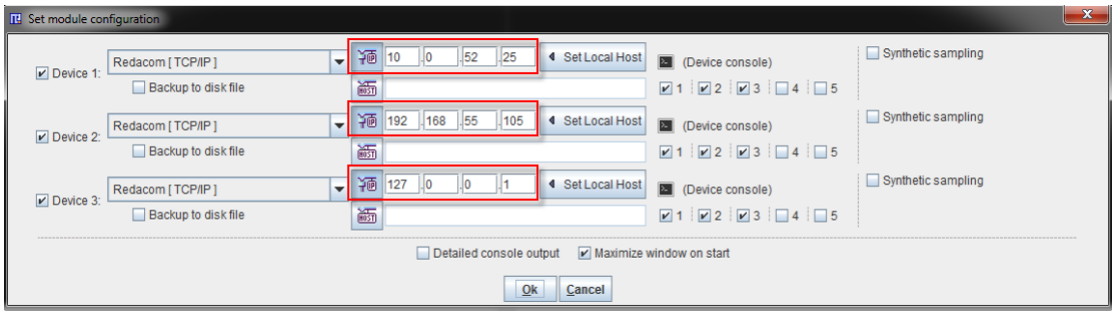
The validation is not absolutely necessary: the connection with the device is working despite the yellow triangle that appears for the local connection. Still, it might take a few minutes until the connection became stable.

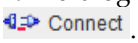
Module Configuration

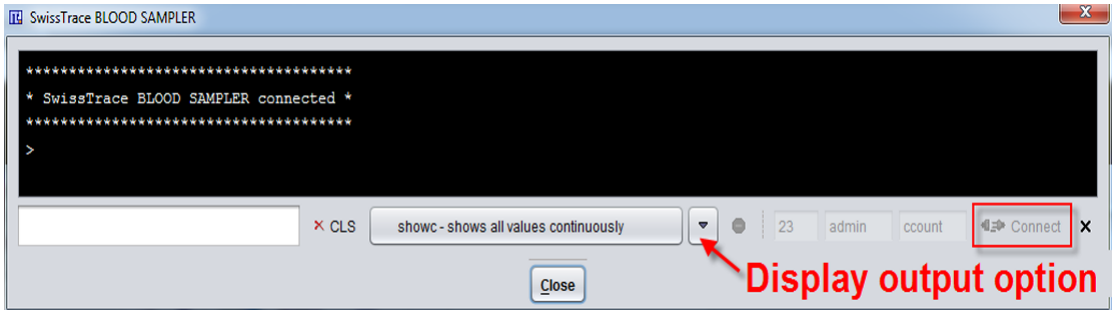
The module configuration panel can be brought up by activating the corresponding button  available on the bottom status line.

The module configuration interface is organized as shown below:

1. The area for the device(s) configuration: allows the simultaneous configuration of up to a maximum of three devices. The devices can be simultaneously used during the acquisition. Each device is characterized by an IP that can be enter manually or activating the **Set Local Host** button. Alternatively, the device can be set up within a network specifying the "host" address in the host field  .

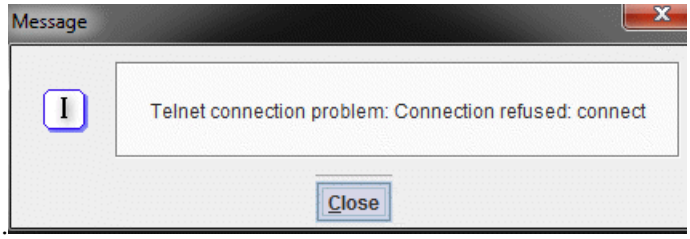


The set up can be tested activating the **Device console** icon  . A dialog window is open and the connection can be verified selecting the dedicated button  . If the connection succeeds, the connection button is disabled and the fields dedicated to the port definition, user name and password are set and become gray. Accordingly, the console returns a successful message as shown below:



The **Display type** selection allows choosing the way measurements are shown on the console.

In case the connection cannot be established a failure message is displayed in a dialog window:



Finally, the **Device console** dialog window can be closed with the **Close** button.

A number of 5 channels are available for each device. The first three are dedicated to the coincidences (number 1) and to the singles corresponding to each LYSO crystals (number 2 and 3). Furthermore, the analog channels 4 and 5 can be recorded simultaneously for monitoring signals from additional instruments. During the acquisition, the measured data can be automatically saved. This can be easily achieved enabling the **Backup to disk file** checkbox**.**. The aim of this checkbox is to avoid data lost during the experiment, in case an unexpected event occurs. For each channel a text file with the date, time and the measurements information is saved. These files obtain a .crv suffix and can easily be opened in Excel or a text editor. The files backup location, by defaults, is in the PMOD3.3 installation path: *Pmod3.3/data/swisstrace* and are encoded as follows: **D_1_C_<n>_<date_time>.crv**, where "**n**" corresponds to the channel number.

2. The synthetic sampling pane.

3. Two optional settings on the bottom area: the first consents detailed console output during the acquisition while the other one allows the window maximization upon starting. If the **Detailed console output** checkbox is enabled, the sampling data are displayed in the console.

The presence of the yellow triangles on the configuration dialog window informs that the acquisition display is slow down because of the selected settings.

The selection of the **Ok** button closes the configuration dialog window. Subsequently, a dialog confirmation window pops up.

Finally, the activation of the **Yes** button save the settings and acquisition can be started.

Data Acquisition

Once the configuration of the device(s) has(ve) been set up and confirmed, the data acquisition can be started.

Note:

1. During the data acquisition, the **Synthetic sampling** checkbox(es) must be turned **OFF.**

2. The twilite should be started around 20 seconds earlier than the PET scanner. The clock time of the PET scanner and sampler computer have to be the same.

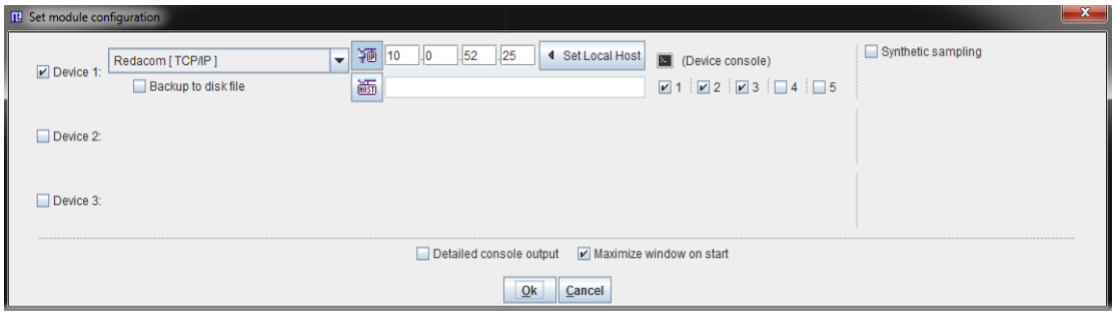
The curve display area is automatically organized in one or multiple layers (maximum 3), depending on the devices number that have been configured. Each layer corresponds to one device.

The PSAMPLE configuration interface allows the definition of one or multiphase protocol acquisition.

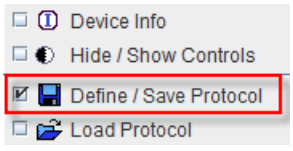
Protocol Configuration

One Device-One Phase Protocol.

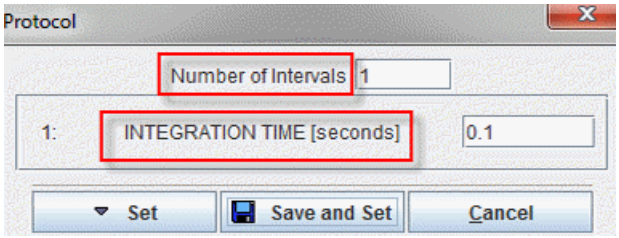
The acquisition can be performed with one device and a constant time interval sampling. The device is configured and the first three channels are enabled. The automatic backup is activated.



The integration time is defined in seconds and can be set in the main window, under the display area Integration time 1.0 [sec]. Alternatively, the time setting is available activating the **Define/Save Protocol** option in the selection list from the control taskbar.



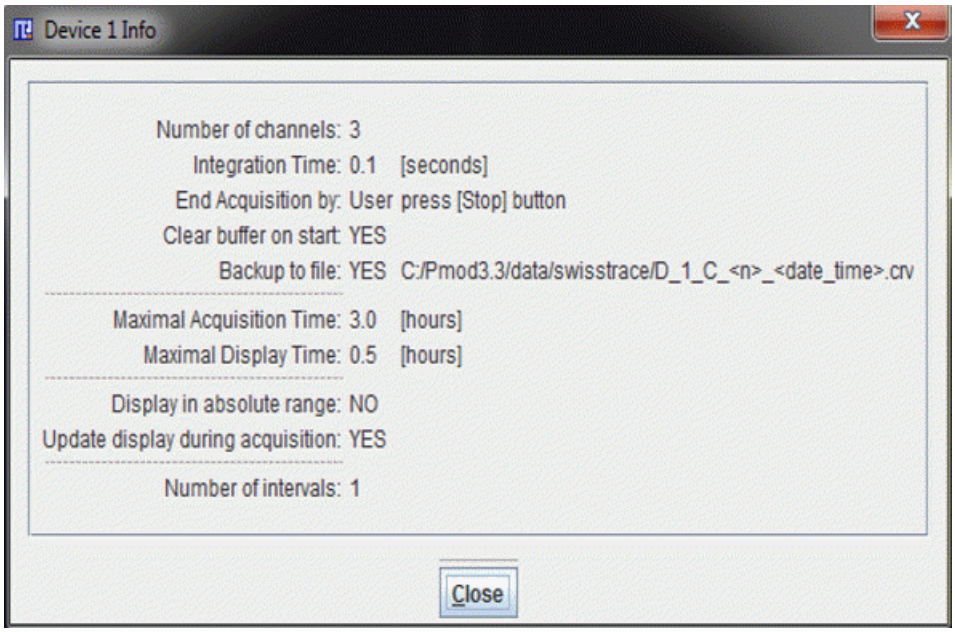
In case the alternative setting is used, a dialog window allows the definition of the *Number of Intervals* and the *INTEGRATION TIME* in seconds.



The smaller value for the integration time supported by the software is 0.1s, while the largest value is 10s.

The protocol can be **SET** for the present experiment or **Save and Set**. The last option allows, upon retrieval, the use of the same protocol in other experiments. The protocol definition can be simply aborted selecting the **Cancel** button.

In case the protocol is confirmed, a dialog window summarizes the experiment settings:



The information available is organized in four sections:

1) First section provides a summary of the device configuration (**Number of channels**, **Backup to files**) and protocol acquisition (**Integration time, Clear buffer on start** and **End Acquisition by**). The **End Acquisition by** information reflects the **End by** field setting. Two scenarios are possible: **User press [STOP] button** when the user interactively stops the acquisition and **Time [of elapsed X seconds (Y minutes)]** when the acquisition is automatically stopped after Y minutes.

2) The second section displays he **Maximal Acquisition time** and the **Maximal Display Time** for the experiment.

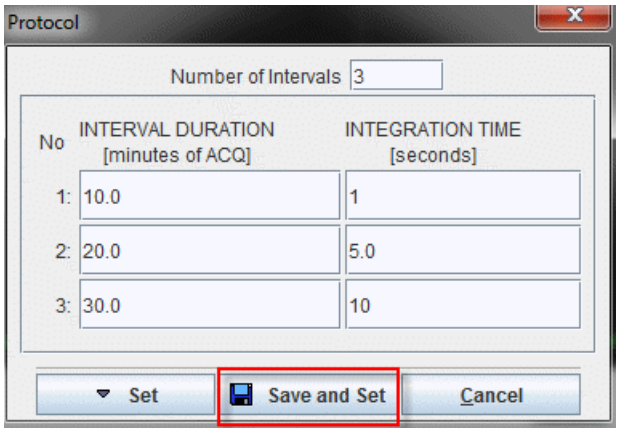
3) The third section notifies the settings for the display area

4) The last section is dedicated to the **Number of intervals**.

One Device-Multi Phase Protocol

The PSAMPLE allows the definition of multiphase protocols. The difference respect to *One Device-One Phase Protocol* consists in the time protocol set up. This can be achieved only selecting the **Define/Save Protocol** option on the control taskbar.

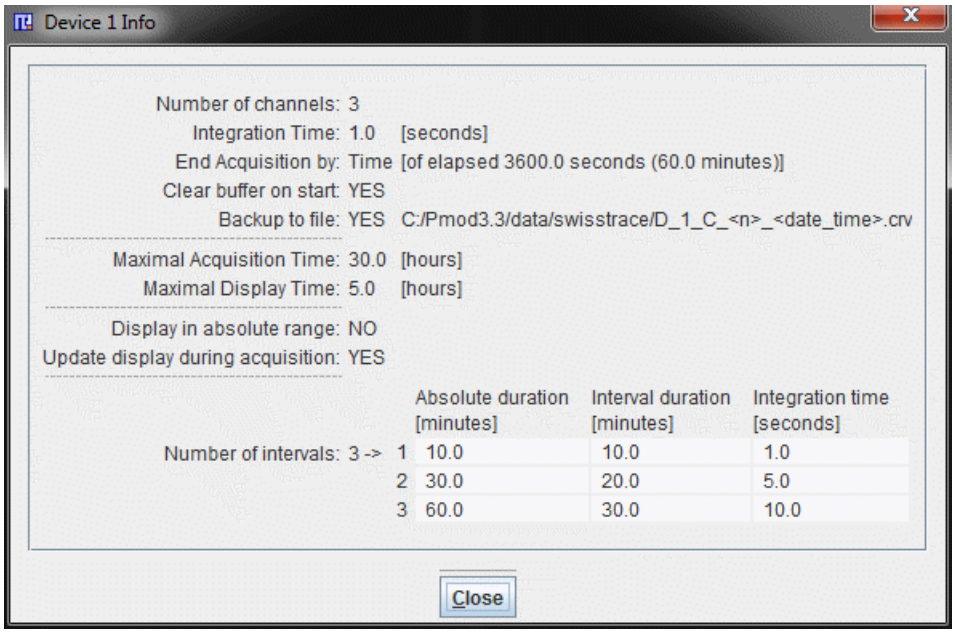
A dialog window opens and the multiphase acquisition protocol can be set. A three phase protocol set up, with duration of 60 minutes is shown below:



Initially, the **Number of Intervals** is defined. Each interval requests two additional information: the **INTERVAL DURATION** defined in terms of minutes of acquisition and the **INTEGRATION TIME** in seconds. The integration time need to be set within the 0.1 second and 10 seconds.In the example above the protocol consists of 10 minutes sampling with an integration time of 1s, followed by 20 minutes acquisition each 5s and finally 30 minutes with 10s sampling time.

The defined protocol can be **Save and Set**, such that later on it can be used in a different experiment. A saved protocol can be retrieved from the file system selecting **Load Protocol** option in the drop-down list. In alternative, the protocol can be simply **Set** to the experiment, or **Cancel** activating the dedicated buttons.

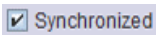
The confirmation of the acquisition protocol is a dialog window that summarizes the device settings for the experiment, as shown below:



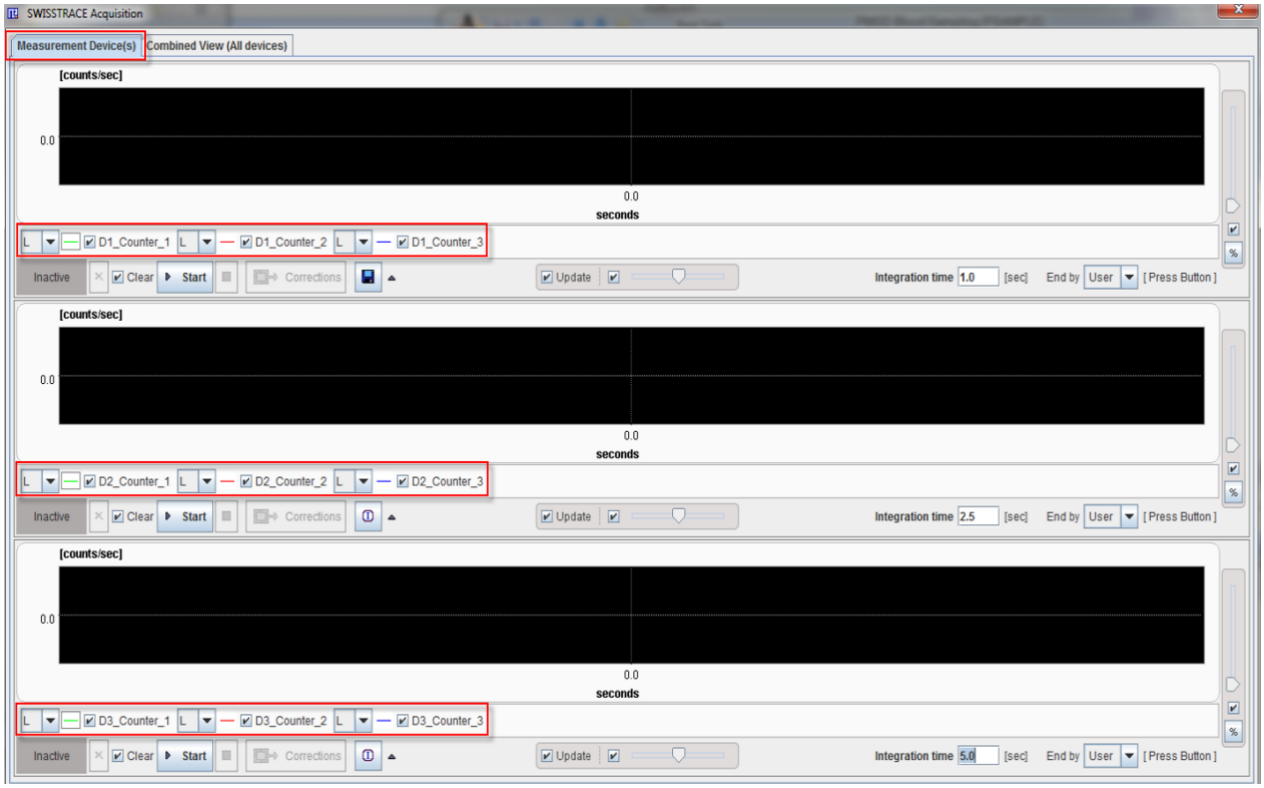
The overview layout is similar with the one available for one phase protocol definition. The difference is represented by the **Number of Intervals** section. This summarizes, in a matrix format of [Number of intervals X 3], the multi phases protocol set up. Particularly, each interval is characterized by three values: the **Absolute duration [minutes]**, the **Interval duration [minutes]** and the **Integration time [seconds]**.The bottom **Absolute duration** value in the column corresponds with the end time of the experiment.

Note: In the current implementation, the twilite multiphase acquisitions (sequences of different sampling rates) are hampered: when switching from one sample rate to another the device is automatically stopped, then the new sampling rate is set, and the sampling is re-started. During the latency time, 2-3 samples are lost, independent of the sampling rate.

Multiple Devices Protocol

PSAMPLE acquisition software allows the configuration of up to a maximum of three devices. For each device, a different number of channels can be activated. The confirmation of a similar configuration results in a display area organized in three layers, each layer corresponding to one device. During the acquisition, the signals measured with all devices can be displayed synchronized on the time axis. This can be achieved upon activation of the **Synchronized** button 

available in the bottom taskbar.



The **Combined View (All devices)** window becomes active when multiple devices are configured. This feature allows the comparison of the measurements acquired with different devices.

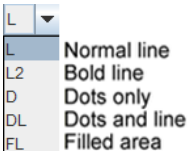
The options available for One-Device-One/Multi Phase Protocol are available for each layer. This flexibility allows the user to display one or multiple curves channels for each configured device. Further, for each device, a different time protocol can be set up or retrieved (single or multi-phase protocol).

Starting the Data Acquisition

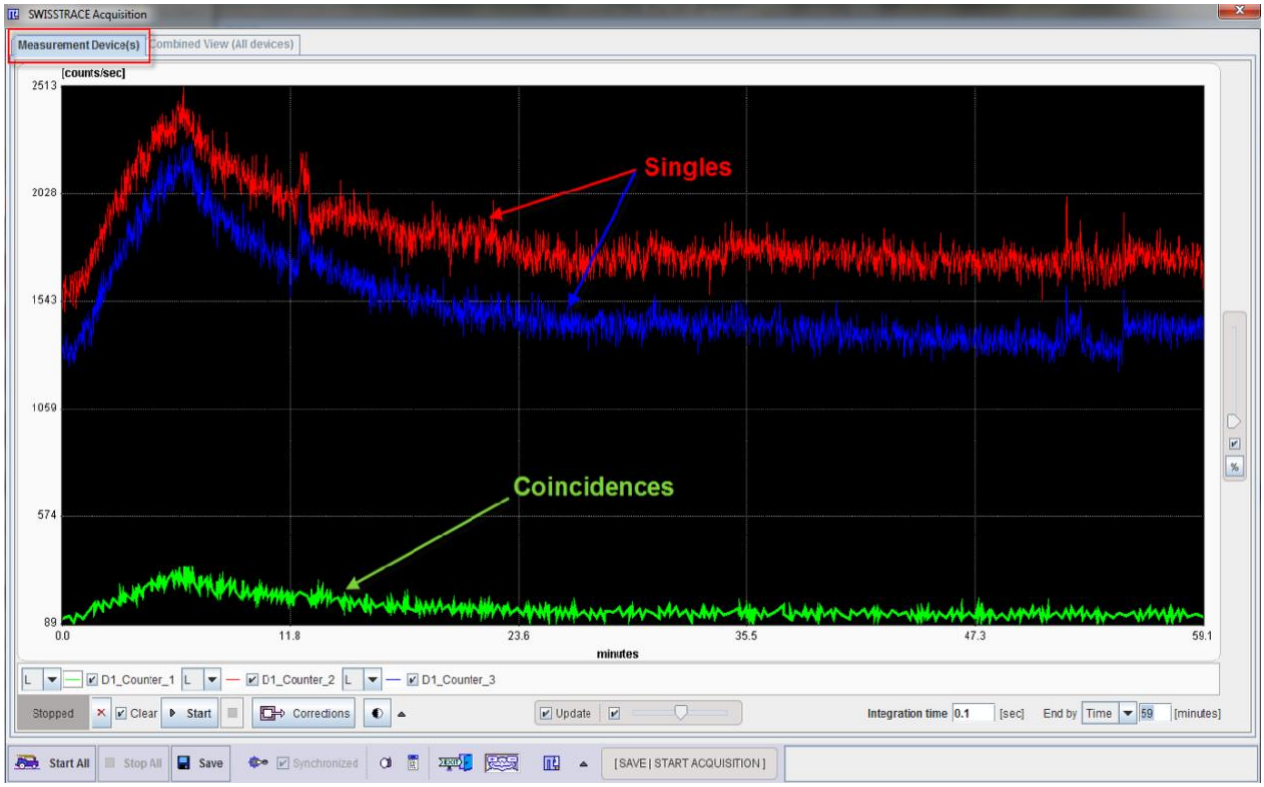
One Device Acquisition

The acquisition can be started activating the **START** button. During the acquisition, the **Hide /Show Controls** button allows the selection of the channels curves to be displayed.

The list selection can be used to change the style of the curve in the display area:



The result of a 60 minutes acquisition for one device with 3 active channels is shown below:

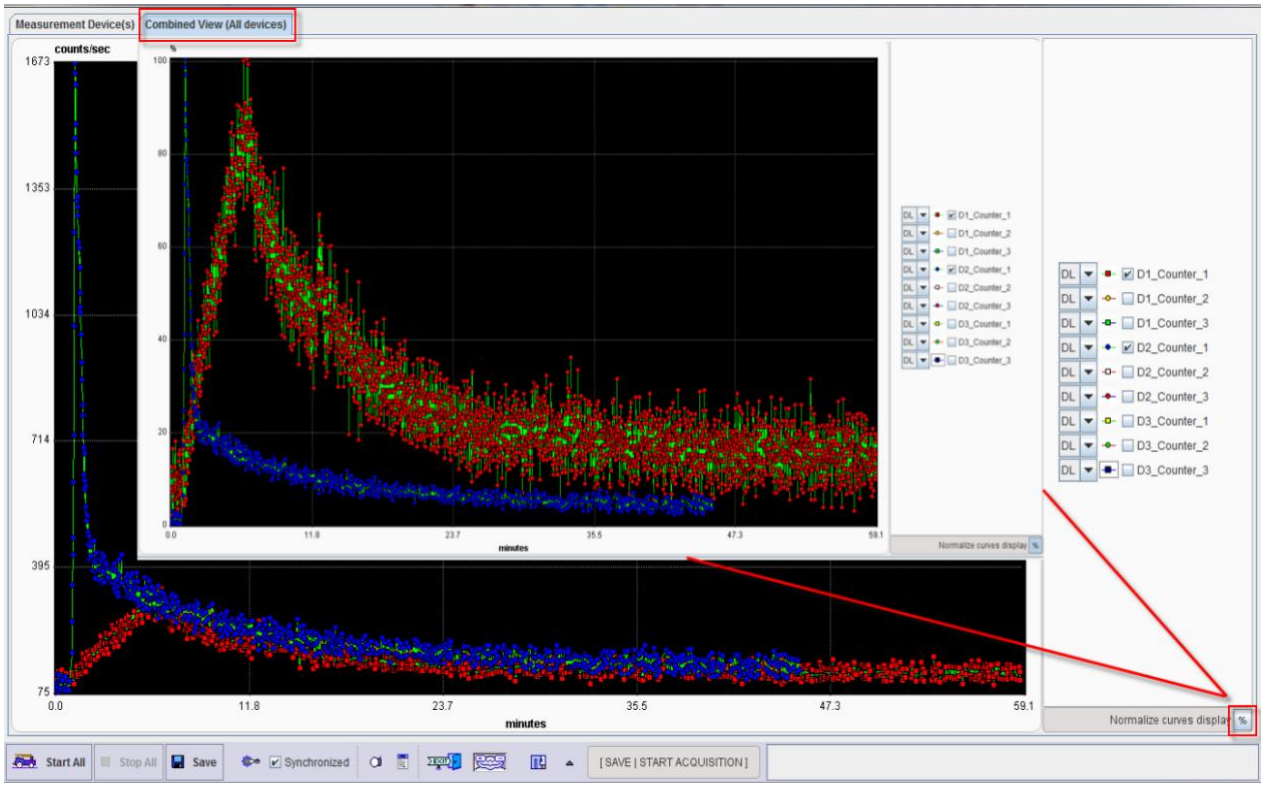


Multiple Devices Acquisition

The acquisition can be started separately for each device with the **START** button. Alternatively, the acquisition can be started simultaneously for all devices activating the **Start All** Start All button. The duration of the experiment depends on the time protocol set up. Independently of the protocols, the acquisition can be stopped simultaneously for all configured devices selecting the **Stop All** Stop All button.

The results for a multiple devices multi-channel configuration can be easily inspected in the **Combined View (All devices)** tab. The curves of interest can be enabled for display and

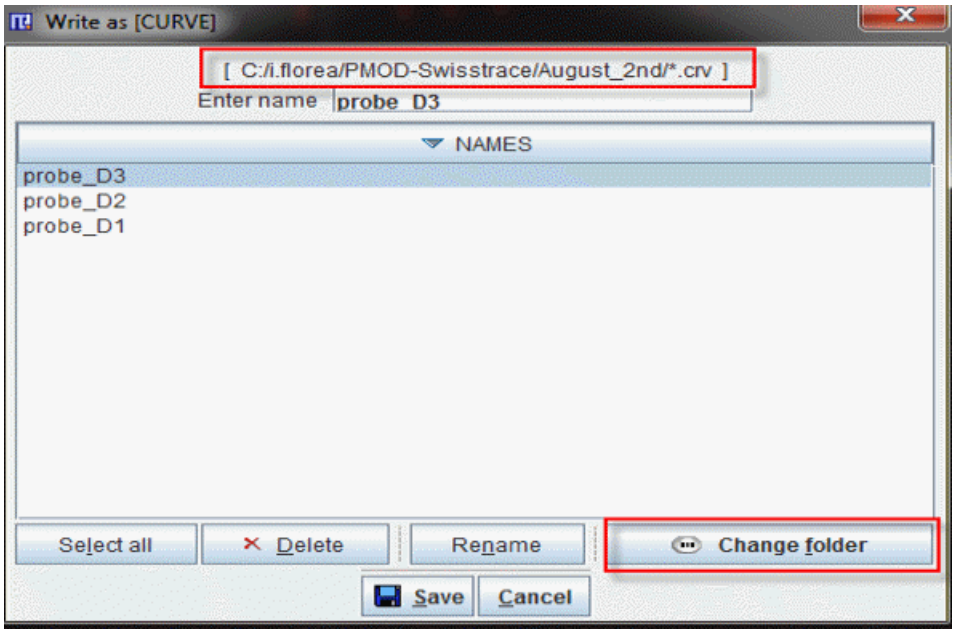
normalized to their own maximum and shown as percent values. This mode is helpful for comparing shapes when the dynamic range of the curves is very different. This display facility can be quickly achieved with the **Normalize curve display** button %, located laterally respect to the display area. In the example below, the inner graphic is the normalized representation of the original one.



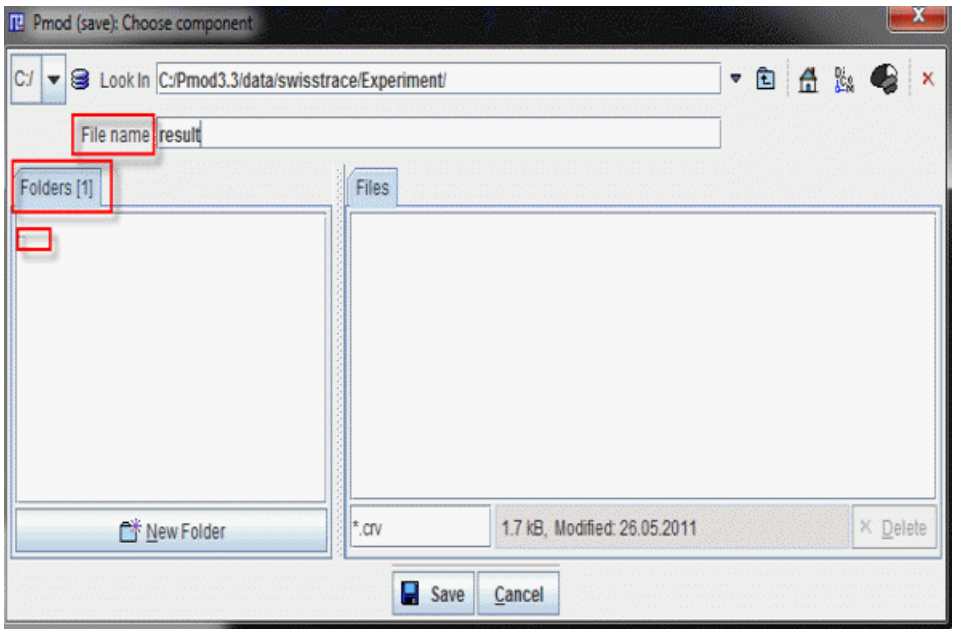
Saving the Data

Note: Upon completion of each experiment, **always** save the acquisition raw data.

The results of the acquisition can be saved in a text file activating the **Save** button. A dialog window of the following type appears during saving:



In the upper part the current save path is indicated. The program automatically points to the directory of the last successful saving operation. The new file can be saved on the same system location. **Enter name** field allows to specify the name for the file. Alternatively, the file can be saved on a different file location activating the **Change folder** button. A new dialog window appears, similar to the one below.



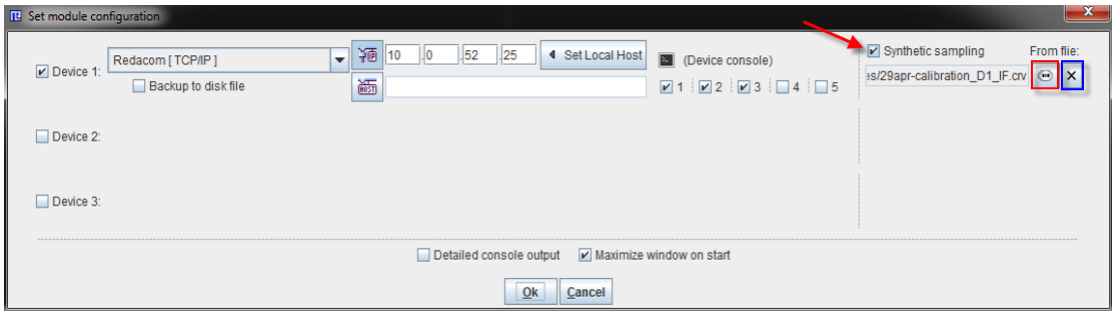
It has elements for changing the directory: the navigation buttons in the **Folder** section (**..** indicating one level up). The **File name** field allows defining the name for the new data file and the **Save** button will start saving.

The saved file receives a **.crv** suffix and can be visualized in Excel or a text editor (e.g. Notepad). It is organized in columns: the date of the experiment is stored in yyyy/mm/dd format in the first three columns; the following 3 columns are dedicated to the time of experiment hh:mm:ss. The measurement data corresponding to each activated channel is stored in order on the last columns in the file.

The saving operation in case of multiple devices acquisition is similar to the one described above. The activation of **Save** button will generate a text file for each device. During the saving procedure, the software appends to the file name the suffix $\_D_i$, where i can be 1, 2 or maximum 3.

Demo Mode

The configuration dialog window allows the display of previously acquired data available on the file system. This can easily be achieved enabling the **Synthetic sampling** checkbox. Consequently, the selection button becomes active and allows navigating to the directory where data were stored. Activate **Open** button to set the selected file for display. Upon completion of these steps, the configuration window will look similar to the one below:



Note: The **Synthetic sampling** feature was implemented for demonstration purposes only.

The maximum number of synthetic data which can be displayed simultaneously is three.

All the configurations and settings are serialized within PSAMPLE software. This means that the last configuration is stored upon exit and available when the software is started again. This feature consents the user to have an overview of the last work performed within PSAMPLE. In case of synthetic acquisition, the **Clear file or directory** button allows erasing quickly the last file location. The selection of the **OK** button confirms the new configuration.

The synthetic curve can be displayed activating the **Start** button. A dialog window shows a warning message that the synthetic acquisition is active and the TAC is going to be generated from the file and not received from the device.

The selection of **Close** button shut down the warning message. Successively, the file is read by the software. Accordingly, the curves corresponding to the activated channels are displayed.

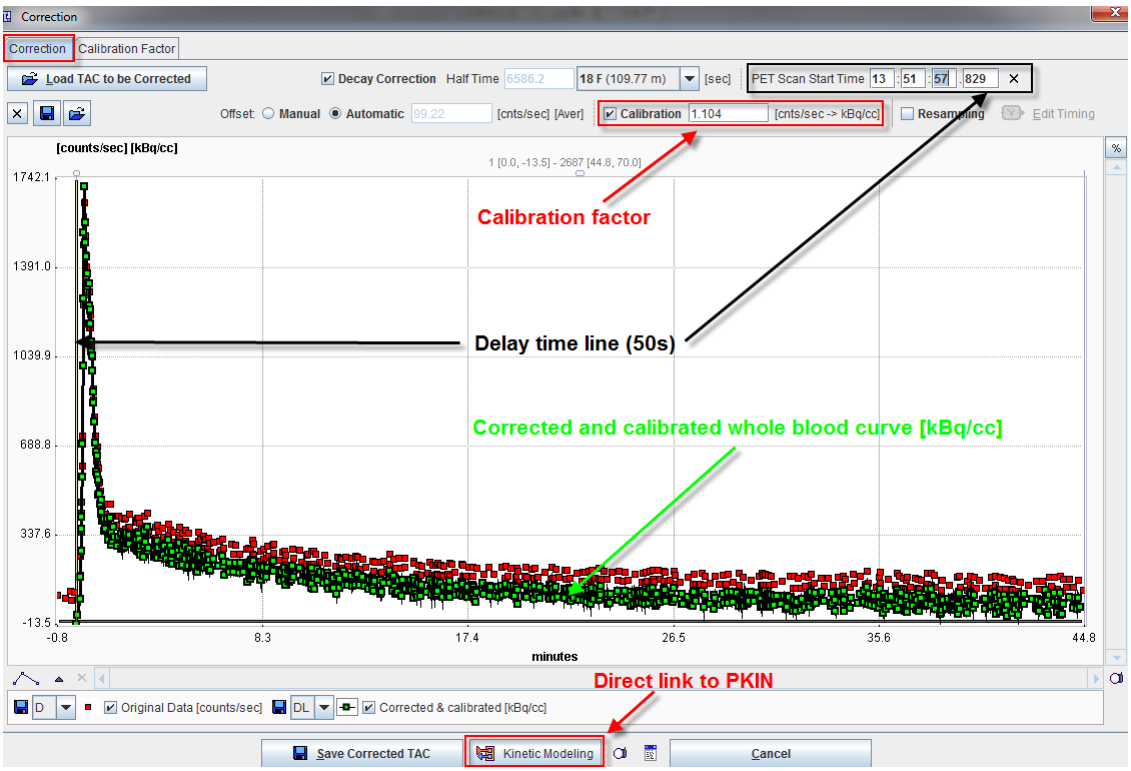
Correction and Calibration of the Measurement Data

The correction and calibration of the whole blood curved represents the essential step before performing post-processing kinetic analysis in the PKIN module. Therefore, the Sampler calibration and the identification of the calibration factor are requested. The calibration can be perform before or after the main experiment as described in the ***Twilite Calibration*** section.

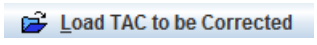
User Interface

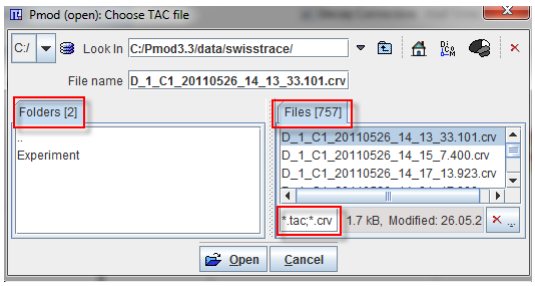
The PSAMPLE Correction module is the dedicated tool for the correction and calibration of the measurement data. The correction module can be activated selecting the **Correction** button in the main PMOD Toolbox. The interface is organized in two main pages with similar layout: the **Correction** page and the **Calibration Factor** page.

The **Correction** window allows performing all the steps necessary for the correction and calibration of the measurement data when the calibration factor is known.



The settings available on the **Correction** page are as follows:





Allows loading and correcting whole blood data located on the file system. The button activation opens a dialog window. In the upper part the current path is indicated. The program automatically points to the directory of the last successful loading operation. It has elements for changing the directory: the navigation buttons in the **Folder** section (**..** indicating one level up). All files suitable for loading (having the right suffix, such as *.tac, *crv) in the search directory are listed in the **Files** section. The **Open** button starts loading the selected file, **Cancel** quits the operation, and **Delete** erases the file from the disk.

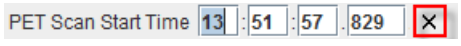
The button is located under the **Load TAC to Be Corrected** button. Its activation closes the displayed TAC. Subsequently, the graphic area becomes empty.

Allows saving the correction parameters (e.g. the calibration factor) determined in the **Calibration Factor** page. The saved file receives a **.corrPars** suffix and can be visualized in Excel or a text editor (e.g. Notepad).

Allows loading correction parameters already available on the file system. The calibration factor is read from the file and the dedicated field is updated accordingly.

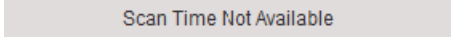
Checkbox enabled: allows the selection of the radio isotope used in the experiment. Accordingly, the activity measurements are corrected for the decay.

The isotope half-life is displayed in seconds in the **Half Time** field.



Displays the time when the PET scan was started.

The text box allows the manual adjustment of start time of the PET scan. Consequently, the "**X**" symbol becomes active, allowing to reset the time to the original file start time.

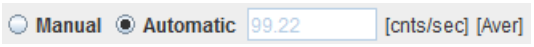


In case no TAC was loaded the time setting area is gray and a message is displayed: *Scan Time Not Available*

Offset

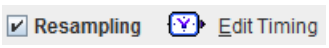
Performs the subtraction of the background activity [counts/sec] from the measured TAC.

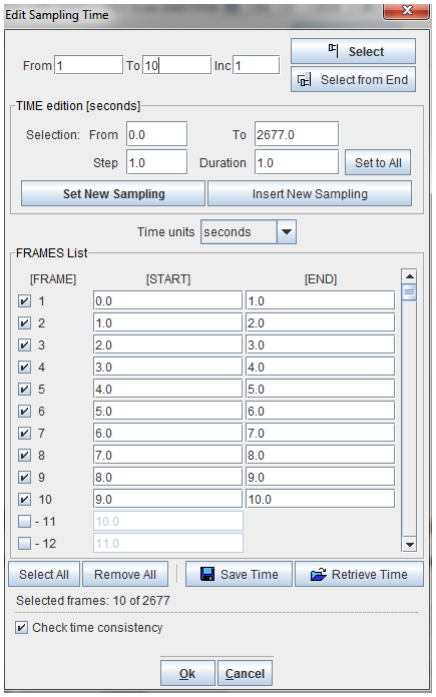
Manual radio button **ON** consents the user to interactively *enter* this value (generally around 100cts/sec).



Automatic radio button **ON** subtracts the first measured value from the TAC when PET and twilite acquisition started simultaneously. Differently, when the *PET Scan Start Time* must be adjusted the value to be subtracted is calculated as the average value over the delay time (*[Aver]*).

If the calibration checkbox is **ON** the correction for the calibration factor is applied to the curve (e.g. whole blood).





In case the **Resampling** checkbox is enabled the time can be inspected and the definition overwritten activating the **Edit Timing** button.

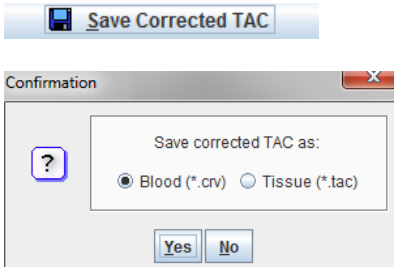
Select/Select from End: Allows selecting a subset of frames, from the first or from the last frame: **From** frame-x **To** frame-y with an increase step **Inc.**

Set to All: Allows setting the same sampling time to all existing frames. The **Step** and **Duration** must have the same value. It is a useful feature when the purpose is curve extrapolation. At the end the frame number is unchanged.

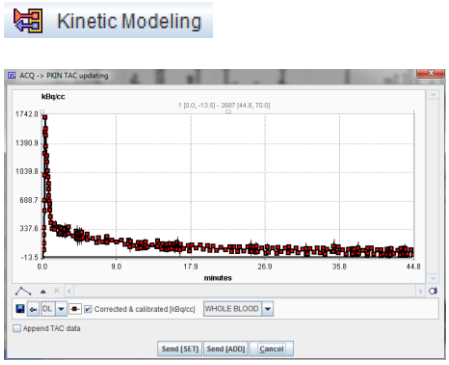
Set New Sampling: Allows defining a different ending time of the experiment in the **Time Edition** pane (**From, To** text boxes) with the same integration time (**Step, Duration**).The number of frames can differ from the initial ones.

Insert New Sampling: Allows the insertion of new frames within the existing ones or at the end of the experiment. Consequently, the frame number changes.

The **Save Time/Retrieve Time** buttons allow saving/retrieving the timing of the dynamic frames to/from a file.



Allows saving the corrected and calibrated curve. A confirmation window pops up allowing to save the corrected TAC either as a blood curve, **.*crv**, or as a tissue, **.*tac**. The saving procedure continues upon activation of the **Yes** button. Alternatively, it is possible to abort the saving selecting the **No** button.



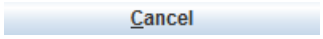
Allows sending directly the corrected and calibrated curve to the kinetic module. Upon activation, a dialog window appears which allows defining the proper type of the calculated TAC (WHOLE BLOOD = spillover curve, PLASMA = input curve, tissue TAC (Region).

The **Send[SET]** button transfers the TAC data to the currently selected tab in the PKIN tool. If the **Append TAC Data** box is checked, the curves are appended as new curves to the data existing on the PKIN tab, otherwise the data is over-written.

The **Send[ADD]** first creates a new tab in PKIN, to which the data is added. If PKIN is not running, the tool is first started and the data added.

Cancel will abort any action.

The **+-** button in the curve controls allows for simple operations such as curve scaling before sending the data to PKIN.



The selection of this button closes the PSAMPLE Correction module.

Buttons dedicated for problem reporting. The capture button creates a capture of the entire screen (not only the PMOD window) and adds it to a buffer of up to 20 captures. The console button opens the **Console** dialog window.

Note: The correction and calibration of the measurement data can also be achieved starting the correction from the acquisition panel. The difference is that the data need not be loaded: upon the activation of the **Corrections** button the data are automatically transfered to the correction module.

Workflow for Data Correction and Calibration

The correction and calibration of the measurement data consists in the following procedure:

1) Load the data to be corrected.

2) Verify that the start time is correct. Usually a delay of 30 to 60 seconds is recorded between the start of the acquisition and the tracer delivery in the animal circulation. In the **PET Scan Start Time** field the delay can be adjusted interactively.

3) Turn **ON** the **Automatic Offset** radio button to correct the measurement data for the background activity.

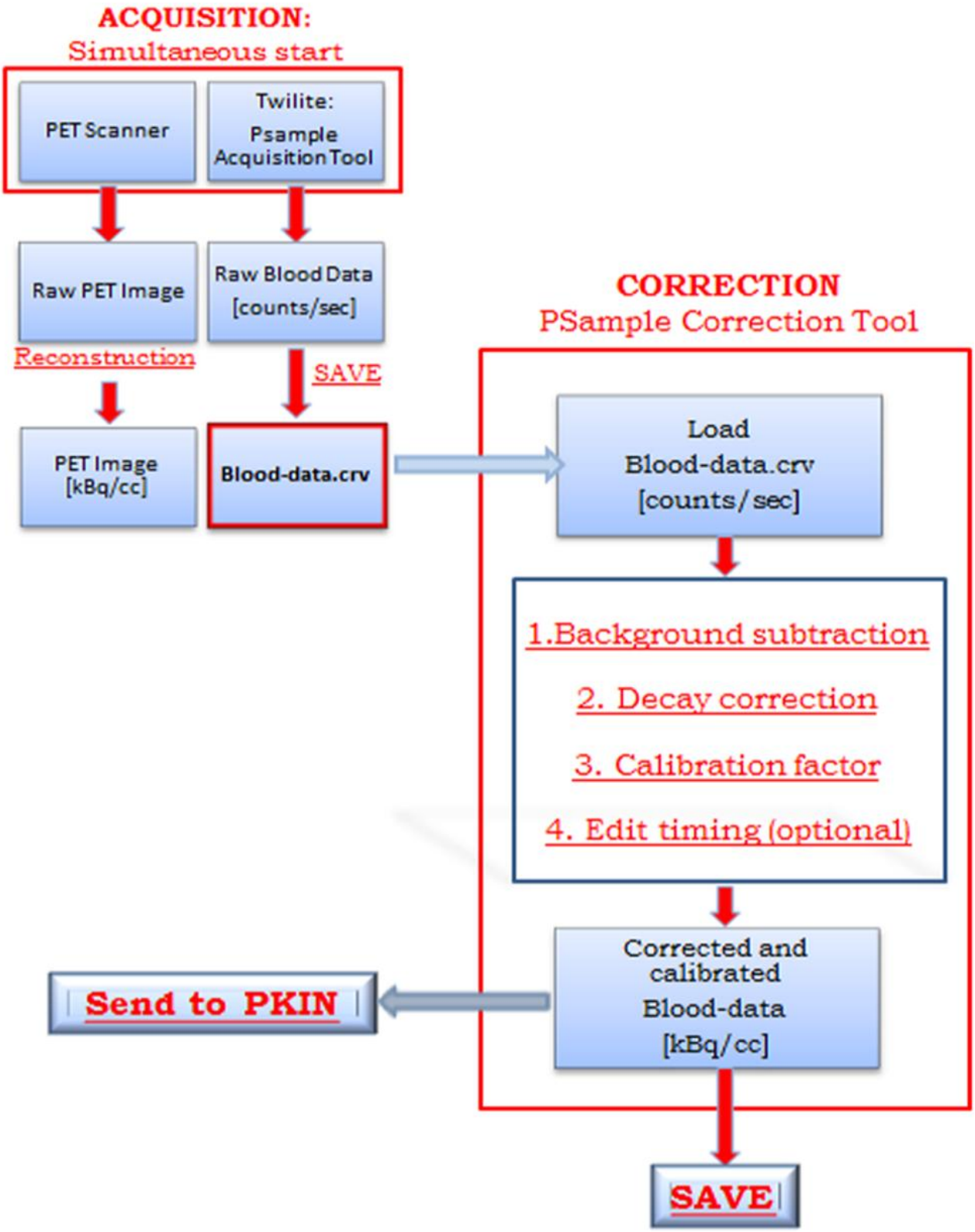
4) Enable the decay correction and select the radio isotope used in the experiment.

5) Apply the correction for the calibration factor activating the **Calibration** checkbox.

6) Optionally, the time definition can be inspected and overwritten. This can be easily achieved enabling the **Resampling** and then activating the **Edit Timing** button.

7) Finally, corrected and calibrated data can be saved activating the **Save Corrected TAC** button. Alternatively, the data can be sent directly to the PKIN tool selecting the **Kinetic Modeling** button.

The twilite acquisition experiment, the save of the raw measured data and data correction and calibration are schematically summarized below:


ACQUISITION:
Simultaneous start
PET Scanner
Twilite:
Psample
Acquisition Tool
Raw PET Image
Raw Blood Data
[counts/sec]
Reconstruction
SAVE
PET Image
[kBq/cc]
Blood-data.crv
CORRECTION
PSample Correction Tool
Load
Blood-data.crv
[counts/sec]
1.Background subtraction
2. Decay correction
3. Calibration factor
4. Edit timing (optional)
Corrected and
calibrated
Blood-data
[kBq/cc]
Send to PKIN
SAVE

Post Processing: Kinetic Analysis

In principle, compartmental modeling requires the knowledge of the time-course of authentic tracer in arterial plasma. The whole blood input function is also needed to account for the activity in the vascular compartment.

Methods for Obtaining the Arterial Input Function (AIF)

The identification of the arterial input function (AIF) can be achieved in two ways:

- Direct measurement of the AIF.
- Indirect derivation from the whole blood curve measured with the twilite.

Direct measurement of the arterial plasma input function

This method requires taking actual blood samples at various time-points, which have to be centrifuged. Then the total activity in plasma is measured in a defined aliquot. In addition, the total plasma activity has to be separated into true tracer and metabolites. If the metabolites do not enter the target organ, the input function needed for compartmental modeling is the time-course of true tracer in plasma. This procedure is cumbersome. It can be used in human studies, where enough blood can be drawn for the analysis. However in small animals like rats and mice, one has to resort to an indirect method to derive the plasma input function, which is also more convenient.

Indirect derivation from the whole blood curve measured with the twilite

This method consists in several steps to be performed as follows:

1. The time course of the activity in whole blood (C_{WB}) and total plasma activity (C_{TP}) is measured in a series of animals, using actual blood samples. The time-course of the ratio (total plasma/whole blood) is then approximated by a mathematical function, often one or a sum of exponentials.

$$\frac{C_{TP}}{C_{WB}} = f_1 \qquad (1)$$

2. The time-course of the ratio true tracer (C_{FP}) to total plasma activity C_{TP} is determined by metabolite analysis and is fitted by a mathematical function, which is often another single exponential or sum of exponentials

$$\frac{C_{FP}}{C_{TP}} = f_2 \qquad (2)$$

3. Once f1(t) and f2(t) are established, the true tracer concentration in arterial plasma can be derived from the whole blood input function measured with the twilite

$$C_{FP} = C_{WB} * f_1 * f_2 \quad (3)$$

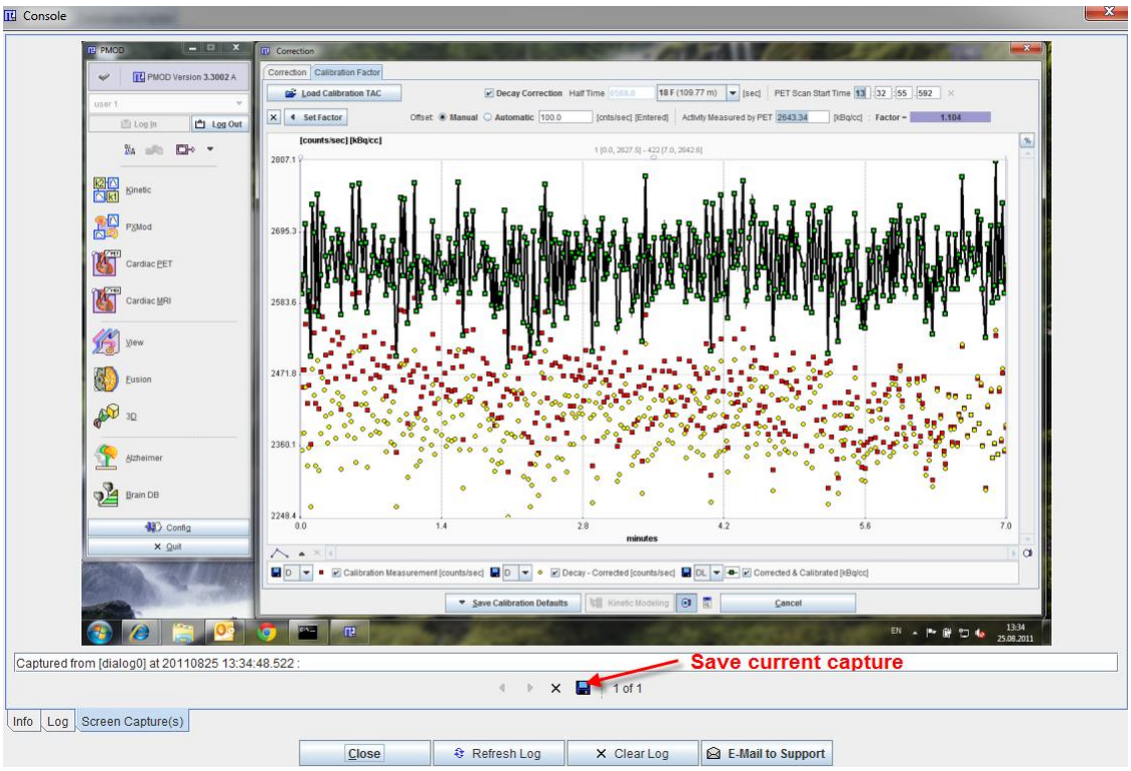
PMOD allows calculating C_{FP} from the whole blood input function according to equation (3).

Problem Reporting

PSAMPLE includes a functionality to directly send a problem report to the support staff of PMOD Technologies. This report can include the log output, screen captures and a problem description entered by the user.

Every PSAMPLE module contains in the bottom line the functions for creating the report  .

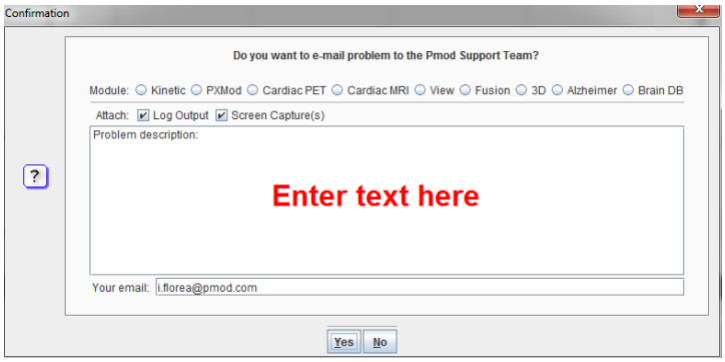
The capture button  creates a capture of the entire screen (not only the PMOD window) and adds it to a buffer of up to 20 captures. The console button  opens the **Console** dialog window illustrated below.



The captures can be inspected on the **Screen Capture(s)** pane with the left/right arrow buttons, and the current one saved in JPEG or one of the available formats. The **Log** pane

contains the log messages, if the terminal output has been configured to be saved in a file on the **On Start** tab of the **Users Configuration**. Its contents can be updated by the **Refresh Log** button. Once in a while it is recommended to use **Clear Log**, to avoid an excessive length of the log file. The **Info** contains some more general information.

To submit a problem description please activate the E-mail to Support button. It opens a dialog window,



wherein the user can select the affected **Module**, and confirm whether the **Log Output** and the **Screen Capture(s)** should be included. The problem description should be typed into the text field, and the user's email address into the **Your email** field. Note that multiple addresses can be specified, separated by the colon character (;). use the **Yes** button to submit the report, or **No** to cancel

Note: Although the standard mailing port is used, corporate firewalls may prevent PMOD from submitting the e-mail. In this case a notification will be shown, and the user needs to report the problem either through his support login, or by standard e-mail.

References

[1]. Weber B, Burger C, Biro P, Buck A. A femoral arteriovenous shunt facilitates arterial whole blood sampling in animals. Eur J Nucl Med Mol Imaging. 2002 Mar; 29(3):319-23.

report them to us in writing. PMOD Technologies Ltd does not warrant that this document is error-free.

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