

User's Guide

PMOD Blood Sampling (PSAMPLE)

Version 3.6



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Swisstrace Blood Sampling System "Twilite"

Introduction

It has become widely recognized, that besides qualitative imaging the absolute quantification of tissue parameters is essential for the understanding of biological systems. One of the gold standard methods in quantification is PET which has sensitivity down to the picomolar range and a unique specificity due to the targeting by molecular probes. Quantitative PET requires the measurement of the arterial input function (AIF), the unchanged radiotracer in arterial plasma, and modeling of the tissue response measured in the PET image.

Swisstrace <http://www.swisstrace.com> has developed the "twilite" sampler for the online monitoring of the tracer concentration in whole blood. The design of the system was optimized for animal and human research using PET. The twilite has been proven to feature excellent sensitivity, linearity and signal-to-noise, even in the presence of significant external radiation, resulting in whole blood radioactivity curves with high temporal resolution. An experimental setup using an arteriovenous shunt allows acquiring arterial input curves without blood loss in rodents [1].

PMOD's PSAMPLE software is the acquisition software of the twilite, and supports the correction and calibration of the count data for accurately measuring the whole blood tracer activity concentration. It can be easily forwarded into PMOD's kinetic tool for the metabolite correction. The resulting AIF is subsequently be used for modeling purposes in the PKIN and PXMOD quantification tools.

Twilite Components

Device Containers

The twilite system comes in two container boxes: one box for the twilite acquisition box plus annexes, and one box for the twilite measuring head and light guides.



Measuring Head

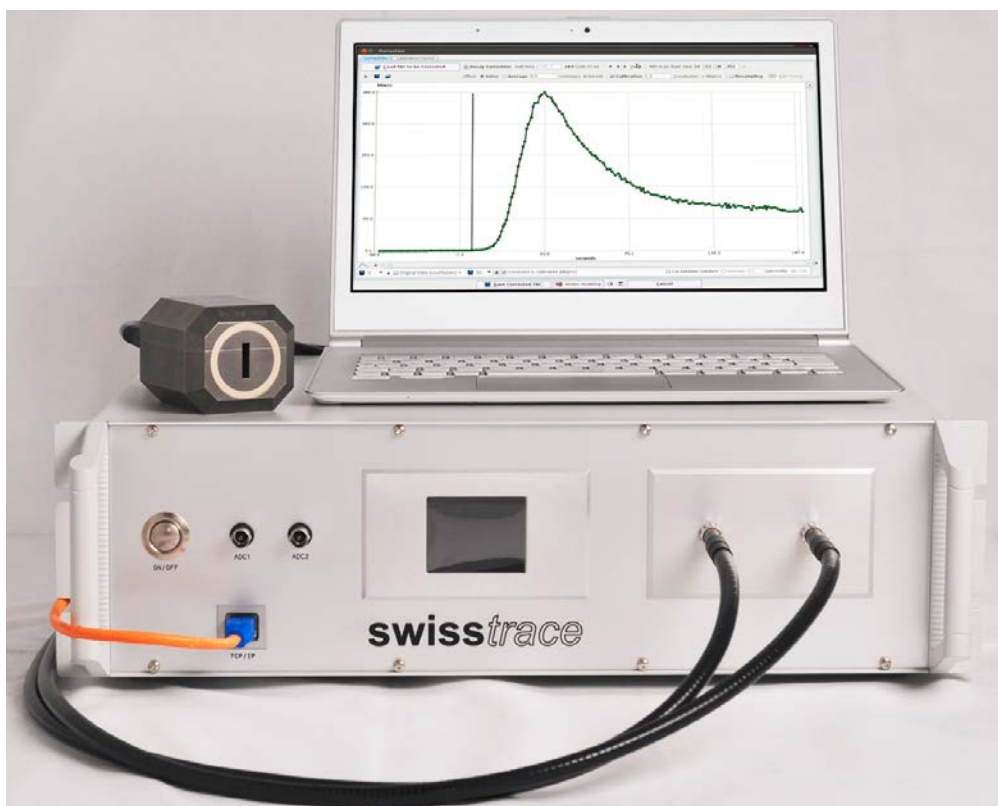
The core of the twilite system is a very compact measuring head made from two LYSO crystals enclosed by medical grade tungsten, which is fully MR compatible. The head has an opening into which the catheter templates are inserted. There are templates available for different catheter dimensions. The illustration below shows a template with a fitted human-size catheter.



The scintillations in the LYSO crystals are conveyed to the photon detection unit via two flexible high efficiency light guides. This 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.

Acquisition Box and Device Assembly

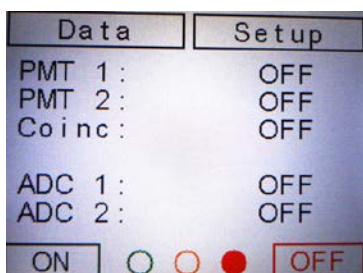
The front panel of the acquisition box has an **ON/OFF** button for powering the system. It is connected to the acquisition computer with a standard network cable. Alternatively, it can be connected to a network switch, and a remote computer used for the acquisition. To the right there are two connectors for attaching the measuring head via optical light guides.



The touch-screen on the front panel serves as the user interface and displays the current status as well as the instantaneous measurements.

Touch-Screen

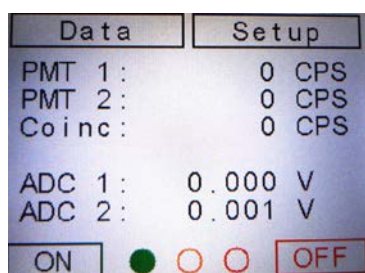
The touch screen has four main buttons: **Data**, **Setup**, **ON** and **OFF**.



The **ON** button serves for starting the device operation. It opens a confirmation window where the **Apply** button needs to be activated.



As a result, the green button next to **ON** is filled in order to indicate that the system is operational. The on-line measured values are available by activating the **Data** button when the twilite is running.



Use the **OFF** button to switch off the device. Correspondingly, the right circle is filled with red color.

Configuration Setup

Setup serves for defining the **IP** address, the **Subnet** mask and the **Coincidence** time. In addition, the current version of the hardware, the build date and the voltage reference are shown.



The twilite is provided with a default IP address that can be changed after activation of the **IP** button. The IP address consists of four numbers, each of which contains one to three digits, separated by a dot (.). Each number can be incremented or decremented using the corresponding **IP+** respectively **IP-** button. The modified numbers are shown in red color in the lower IP line. The new IP address is established by the **Accept** button, whereby the lower IP number also gets green color. Use the **Abort** button to undo the modifications.

Data		Setup	
Accept		Abort	
IP: 10. 0. 52. 25			
IP1+	IP2+	IP3+	IP4+
IP: 10. 0. 52. 25			
IP1-	IP2-	IP3-	IP4-
ON	<input type="radio"/>	<input checked="" type="radio"/>	OFF

The **Subnet** button serves for changing the network mask and has a similar user interface as the IP address interface.

Data		Setup	
Accept		Abort	
SNet: 255. 255. 0. 0			
SN1+	SN2+	SN3+	SN4+
SNet: 255. 255. 0. 0			
SN1-	SN2-	SN3-	SN4-
ON	<input type="radio"/>	<input checked="" type="radio"/>	OFF

The coincidence delay timing can be changed by activating the **Coinc** button. The delay value can be increased or decreased using the **+10**, **+1** and respectively the **-1** and **-10** buttons.

Data		Setup	
Accept		Abort	
Coincidence: 255 nS			
New Delay: 255 nS			
-10	-1	+1	+10
ON	<input type="radio"/>	<input checked="" type="radio"/>	OFF

Experimental Setup

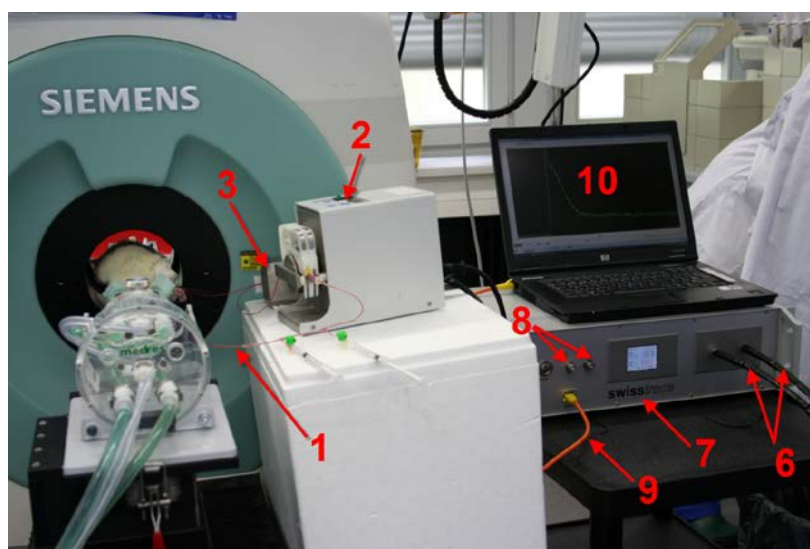
The twilite measures the radioactivity in a catheter which runs between two LYSO crystals. In animals the best solution is to install a shunt between the femoral artery and the femoral vein. This allows to continuously measure the whole blood activity without any loss of blood. Such shunts can be placed in animals as small as a mouse, using catheters differing in size.

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 appropriate catheter diameter differs according to the size of the animals. Swisstrace delivers templates for guiding the catheter through the measuring head for each catheter size, so that a well defined geometry is established. The characteristics of the shunt are described in a paper published by Weber and co-workers [1].

A typical setup of a quantitative rat PET experiment is illustrated below.



- 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 crystal 1.
- 5) LYSO crystal 2.
- 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 20 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.

The shunt can serve several additional functions, such as blood pressure monitoring, tracer injection as well as the collection of blood samples for metabolite analysis. The procedure illustrated below is recommended for collecting blood samples: a small cut is made into the catheter using a scalpel. In normal operation the catheter is bent upwards, so that the cut is

closed and blood circulates. To obtain blood samples, the shunt is shortly pressed downward for opening the cut and blood drops can be collected with practically zero dead volume.

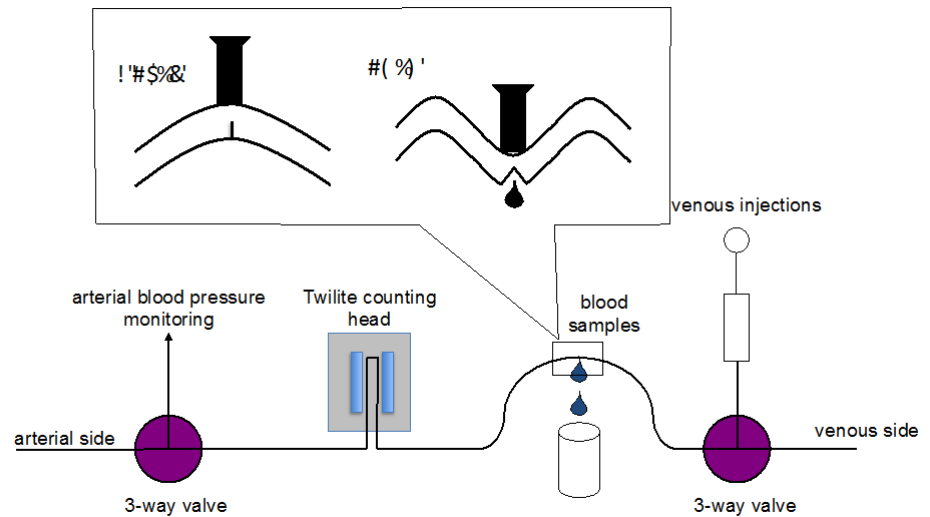


Figure 1: Shunt functions

Conventions

Please note the conventions used in this documentation:

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

PSAMPLE Configuration

The twilite is directly connected to the computer via an ethernet cable. The data acquisition is performed with the dedicated PMOD module PSAMPLE via a TCP/IP interface. Two configuration levels are required, the network configuration and the module configuration.

Network Configuration

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

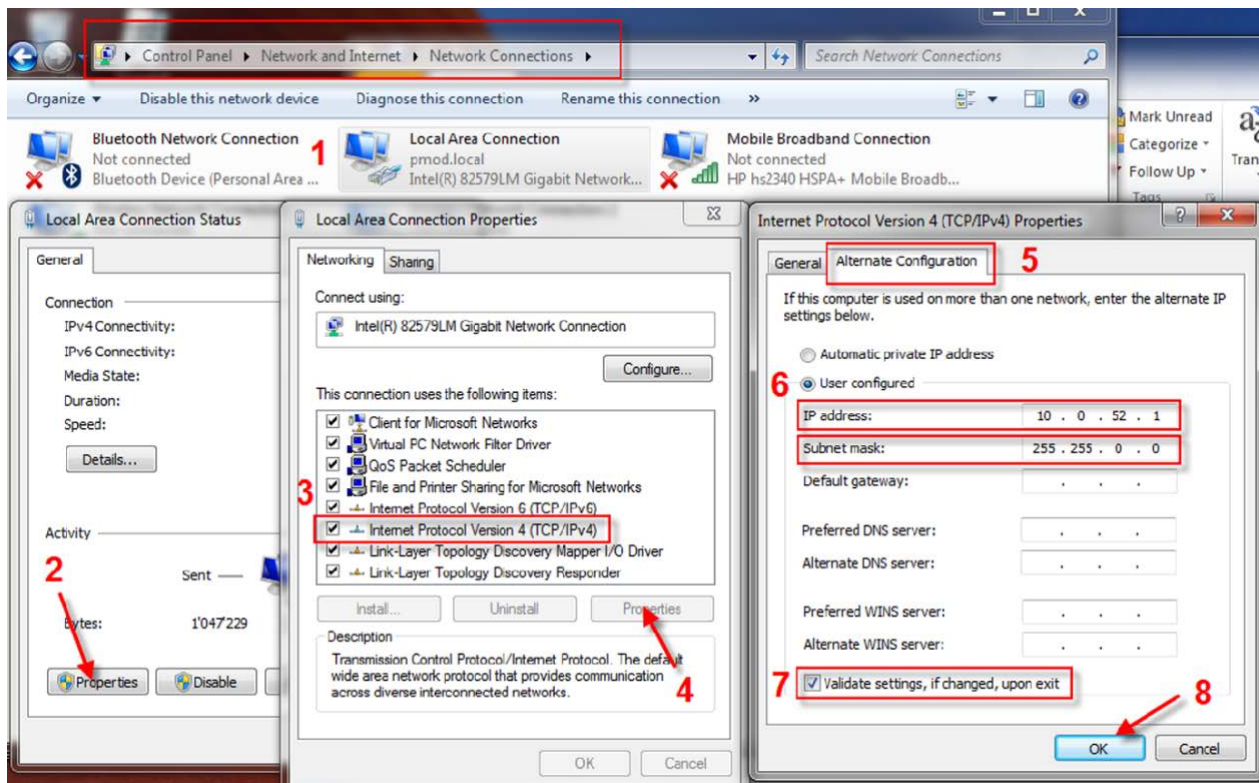
The twilite IP address is available upon the activation of the **Setup** button and then the **IP** button on the touch screen user interface.



For a twilite with IP address set to 10.0.52.25, the Windows 7 set up steps are described below:



- 1) *Control Panel/Network and Internet/Network Sharing Center.*
- 2) Select *Change Adapter Settings* and double click *Local Area Connection (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.




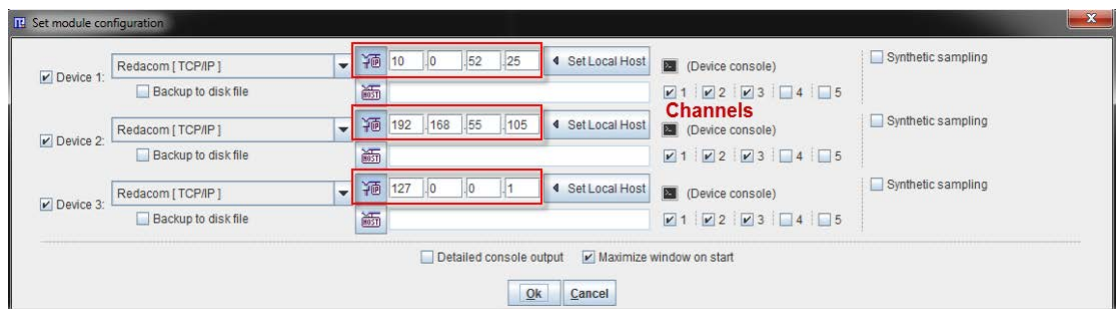
The validation is not necessary: the connection with the device is working despite the yellow triangle that appears for the local connection. Still, it might take a few minutes until the connection becomes stable.


PSAMPLE Configuration

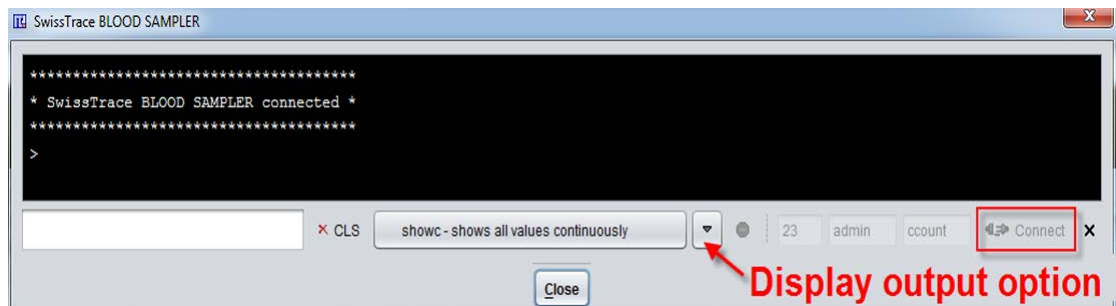
To change the PSAMPLE configuration start it with the  button in the PMOD Toolbox and activate the button  in the bottom status line. The configuration window is organized as shown below:

Connectivity

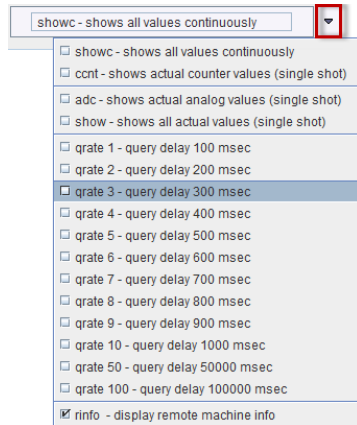
Up to three devices can be configured which can be simultaneously be used during the acquisition. Each device is characterized by an IP address that can be enter manually. For convenience, the **Set Local Host** button retrieves the computers IP address, which can then be adjusted for the twilight address. If applicable, the device can be set up within a network specifying the "host" address in the host field .



The connectivity can be tested by activating the **Device console** icon . A dialog window is opened which shows fields with the port number, the user name, and the password in the lower right. Port **23**, login **admin**, and password **ccount** are the default values of the acquisition device.



The **Connect** button tries to log into the twilite per telnet using this access information. If this succeeds, the connection elements are grayed and a confirmation is shown in the console window as illustrated above. In this state, commands can be given to the sampling device via the list menu. The responses will be shown in the telnet window.



If the connection cannot be established, please check the IP address as well as the login information.

Note: Manual connection is for testing and trouble-shooting only, but not necessary for acquiring data. PSAMPLE will perform the connection using this access information when an acquisition is started.

Channels

Up to 5 channels are available for each device. The first three are dedicated to the coincidences (channel 1) and to the singles corresponding to the two LYSO crystals (channels 2 and 3). Furthermore, the analog channels 4 and 5 can be recorded simultaneously for monitoring signals from additional instruments.

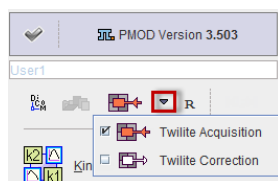
During the acquisition, the measured data is stored in a buffer. As a security option, it can additionally be written in real-time to the disk. This behavior is enabled with the **Backup to disk file** box. For each channel, a text file with the date, time and the measurements information is saved. The backup location is in the installation path: *Pmod3.6/data/swisstrace* and the file names encode the device number **D** and the channel number **C** as follows:
D_1_C_1_<date_time>.crv.

To add the samples to the log and inspect them with the PMOD console window, enable the **Detailed console output** box. This, however, may slow the acquisition down.

Data Acquisition

Start by powering the twilite system with the **ON/OFF** button. After the initialization procedure has completed, activate the **ON** field in the touch panel and confirm with **Apply**.

Start PMOD on the acquisition computer and open the acquisition window with the **Twilite Acquisition** button in the PMOD ToolBox.



It is assumed that connectivity with the twilite as been *configured* (on page 13) appropriately.

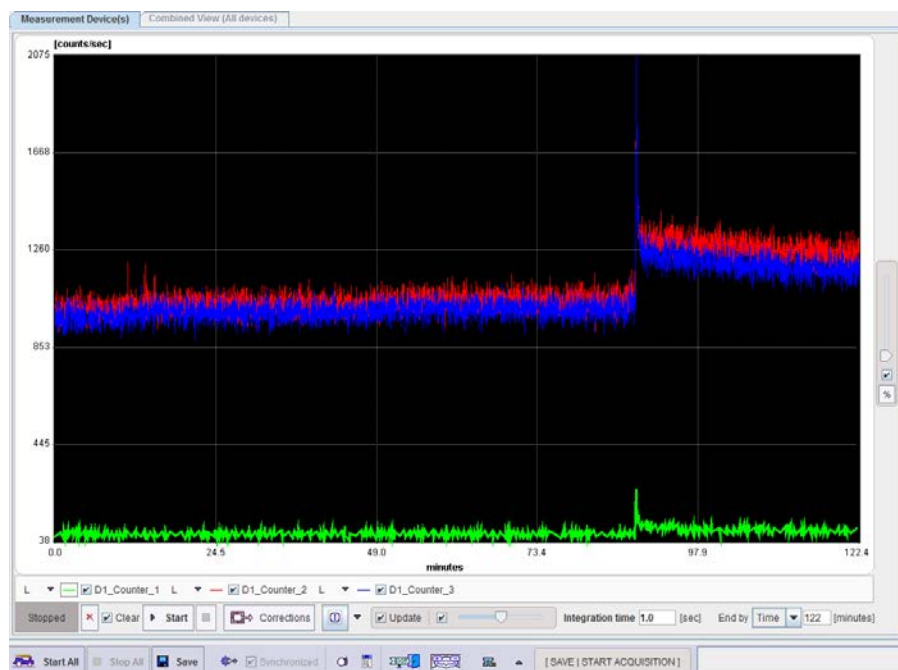
In a simultaneous experiment with a PET acquisition please make sure that the system clocks of the acquisition computer and the PET system are synchronized. Furthermore, in order to acquire enough background data, start the twilite about 20 sec before tracer injection.

Acquisition Interface

The acquisition interface is organized in two panels as shown below: the **Measurement Device(s)** page and **Combined View (All devices)** page. Not that all the configurations and settings are serialized, meaning that the configuration is stored upon program exit and restored after the next start.

Measurement View

The **Measurements Device(s)** page consists of a wide display area, with configuration and control elements below. The measurement data are displayed as time count rate curves in counts/sec as a function of acquisition time.



The various elements are described below:



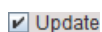
Button for opening the *configuration* (on page 10) panel.



Normalization: Scales all curves relatively with a maximum of 1.








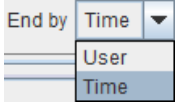
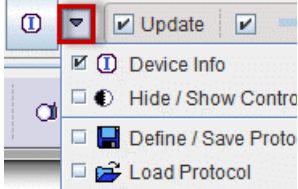


Autoscale: If enabled, the program continually adjusts the curves ranges on the display, on both the X and the Y axes.



Update box: If enabled, the display is continually updated to show all the acquired data.

Integration time

Duration, during which the counts are collected. The accumulated counts are then divided by the integration time for calculating the count rate. Default is a 1 sec integration time, minimum is 0.1 sec, and maximum 10sec. Note that rebinning to longer intervals can later be done in the correction tool.

	Button to transfer the acquired curve(s) to the PSAMPLE Correction tool.
	Button for clearing the data buffer.
	Clear box: If enabled, the data buffer is cleared when starting a new acquisition. 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.
	Creates a capture of the entire screen and adds it to a buffer of up to 20 captures.
	Opens the PMOD console window with log information.
	<p>The End By supports two settings:</p> <ul style="list-style-type: none"> » User: allows stopping the acquisition any time by activating the STOP button; » Time: the user can define the acquisition duration, in minutes, after which the measurement is automatically stopped.
	Device Info : Shows the device configuration.
	Hide/Show Controls : Allows to show or hide the curve control elements.
	Define/Save Protocol : For the definition of an <i>acquisition protocol</i> (on page 17).
	Load Protocol : For retrieving an acquisition protocol.
	Start : Starts the acquisition for the configured device. Start All starts the acquisition on all configured devices at once.
	Stop : Stops the acquisition for the configured device. Stop All stops all devices at once.

If more than one device has been configured, each if them is represented by a curve/control area as described above (maximum 3).

Protocol Configuration

Typically, twilight acquisitions are performed with a constant integration time, and subsequent re-binning for data smoothing. Alternatively, it is possible to define acquisition protocols with variable integration times using the **Define/Save Protocol** option. In the appearing dialog window the **Number of intervals** has first to be defined. When entering the number, a corresponding number of rows is set up for defining the acquisition **INTERVAL DURATION** and the **INTEGRATION TIME** to be used. The example below illustrates three acquisition phases: the first 5 min with 1 sec integration, the second 5 min with 5 sec integration, and the following 30 min with 10 sec integration.

Number of Intervals

No	INTERVAL DURATION [minutes of ACQ]	INTEGRATION TIME [seconds]
1:	<input type="text" value="5.0"/>	<input type="text" value="1.0"/>
2:	<input type="text" value="5.0"/>	<input type="text" value="5.0"/>
3:	<input type="text" value="30.0"/>	<input type="text" value="10"/>

The **Set** button establishes the protocol for the current experiment. It shows a confirmation window with summary information as illustrated below.

Number of channels: 3
 Integration Time: 1.0 [seconds]
 End Acquisition by: Time [of elapsed 7320.0 seconds (122.0 minutes)]
 Clear buffer on start: YES
 Backup to file: YES C:/Pmod/v3.5/data/pmdbase/D_1_C_<n>_<date_time>.crv

Maximal Acquisition Time: 30.0 [hours]
 Maximal Display Time: 5.0 [hours]

Display in absolute range: NO
 Update display during acquisition: YES

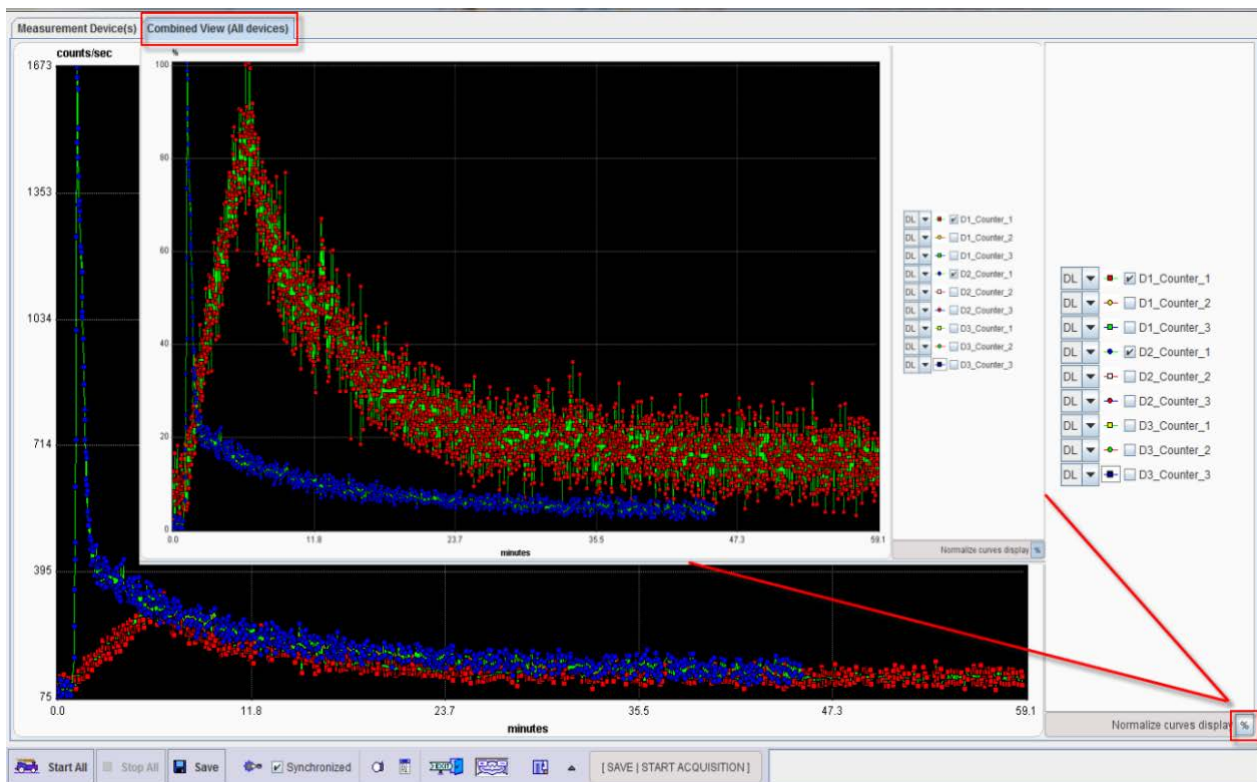
	Absolute duration [minutes]	Interval duration [minutes]	Integration time [seconds]
Number of intervals: 3 -> 1	5.0	5.0	1.0
2	10.0	5.0	5.0
3	40.0	30.0	10.0

With **Save and Set** the protocol can be additionally saved for later re-use.

Note: In the current implementation, the twilight 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, 2-3 samples are lost, independent of the sampling rate.

Combined View

The **Combined View (All devices)** page is only active, when multiple devices are configured. It shows the acquired data of all devices. The curves of interest can be enabled for display, 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 %. In the example below, the inner graphic is the normalized representation of the original one.

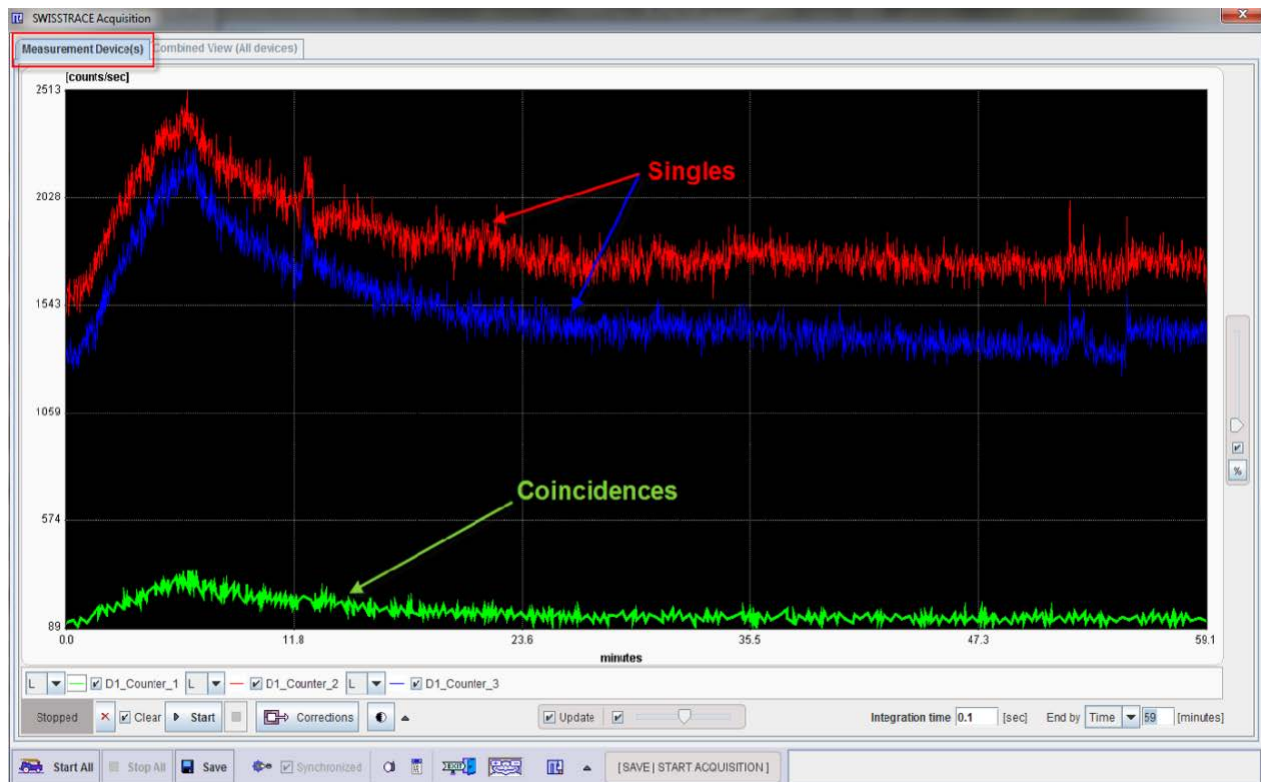


Data Acquisition

Starting the Data Acquisition

One Device Acquisition

The twilite acquisition is started with the **Start** button. With the default configuration illustrated below the display is updated each time a new sample is acquired, and the scaling adjusted in the x- and y-axes.



The curve controls

L ☒ D1_Counter_1 L ☒ D1_Counter_2 L ☒ D1_Counter_3

can be shown below the curve area or hidden by the **Hide /Show Controls** button .

Counter_1 denotes the coincidences, **Counter_2** and **Counter_3** the two singles. The check boxes next to the curve names control their display. For instance, to only see the coincidences, the checks to the left of **Counter_2** and **Counter_3** can be removed.


The acquisition will run until the end of the defined protocol, unless the stop button is activated.

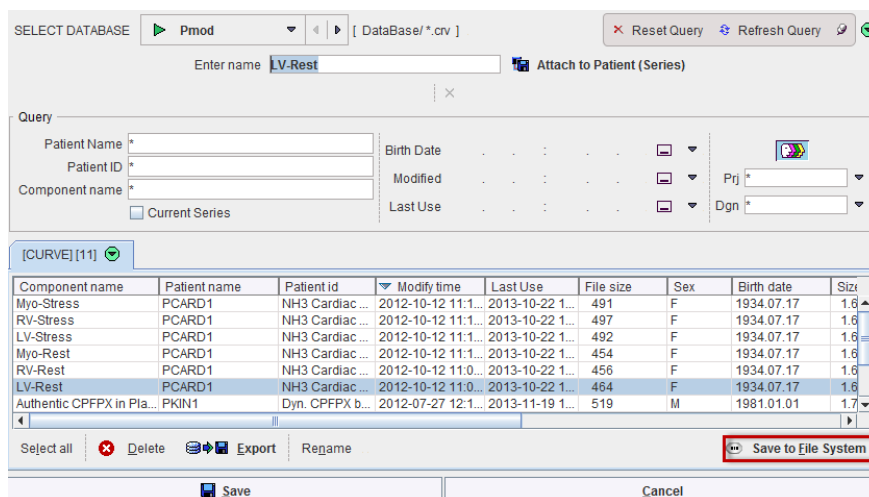
Multiple Devices Acquisition

If multiple devices are controlled by the same acquisition device, a curve display and control area is shown for each device in a row layout. The acquisition can be started individually for each device with the corresponding **START** button. Alternatively, all acquisitions can be

started at once with **Start All**. The results of a multiple device acquisition can most conveniently be inspected in the **Combined View (All devices) page**. (on page 19)

Saving the Data

Please use the  **Save** button for saving the acquisition raw data. A dialog window appears for defining the name and location of the result file. If the PMOD database functionality is enabled, a database saving dialog is shown, and a name can simply be entered.



SELECT DATABASE: Pmod [DataBase/ *.crv]

Enter name: **LV-Rest** Attach to Patient (Series)

Query:

Patient Name * Birth Date Prj *

Patient ID * Modified Dgn *

Component name * Last Use

☐ Current Series

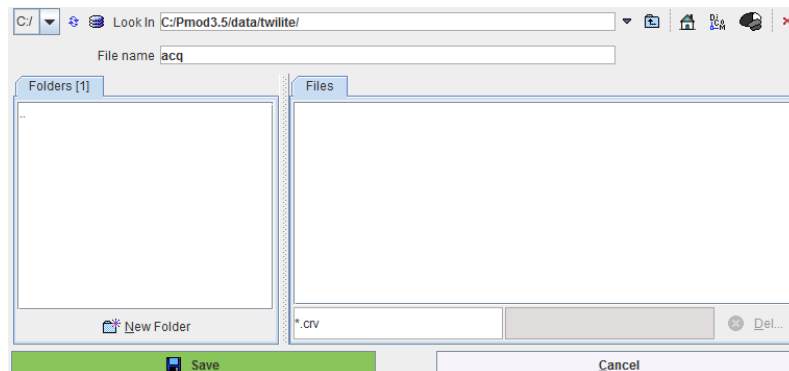
[CURVE][11] ✓

Component name	Patient name	Patient id	Modify time	Last Use	File size	Sex	Birth date	Size
Myo-Stress	PCARD1	NH3 Cardiac ...	2012-10-12 11:1...	2013-10-22 1...	491	F	1934.07.17	1.6
RV-Stress	PCARD1	NH3 Cardiac ...	2012-10-12 11:1...	2013-10-22 1...	497	F	1934.07.17	1.6
LV-Stress	PCARD1	NH3 Cardiac ...	2012-10-12 11:1...	2013-10-22 1...	492	F	1934.07.17	1.6
Myo-Rest	PCARD1	NH3 Cardiac ...	2012-10-12 11:1...	2013-10-22 1...	454	F	1934.07.17	1.6
RV-Rest	PCARD1	NH3 Cardiac ...	2012-10-12 11:0...	2013-10-22 1...	456	F	1934.07.17	1.6
LV-Rest	PCARD1	NH3 Cardiac ...	2012-10-12 11:0...	2013-10-22 1...	464	F	1934.07.17	1.6
Authentic CPFPX in Pla...	PKIN1	Dyn. CPFPX b...	2012-07-27 12:1...	2013-11-19 1...	519	M	1981.01.01	1.7

Select all Delete Export Rename **Save to File System**

Save Cancel

To save the raw data outside the database, use the **Save to File System** button. In the appearing window



Look In: C:/Pmod3.5/data/twilight/

File name: **acq**

Folders [1]

Files

New Folder

*.crv Del...

Save Cancel


navigate to the target directory, enter the result **File name**, and **Save**.

The saved raw data file has a **.crv** suffix and can be visualized in Excel or a text editor. It is organized in columns with the date and time of the sample, and the number of coincidences and singles during the integration interval.


Date and time	Coinc	Single1	Single2
2013 11 5 10 51 27.947	57	968	947
2013 11 5 10 51 28.947	63	992	988
2013 11 5 10 51 29.947	77	985	971
2013 11 5 10 51 30.947	73	1020	1008
2013 11 5 10 51 31.947	54	1049	969
2013 11 5 10 51 32.947	54	994	961
2013 11 5 10 51 33.947	72	1033	1004
2013 11 5 10 51 34.947	73	1063	965

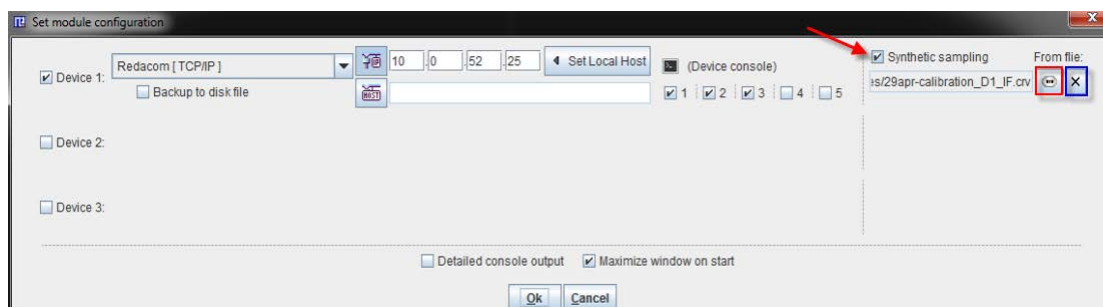
The saving operation in case of multiple devices acquisition is similar to the one described above. The activation of **Save** button will generate a text file for each device. During the saving procedure, the software appends to the file name the suffix $_D_i$, where i can be 1, 2 or 3.

Transfer to the Correction Interface

Although saving of the raw data is recommended, the acquired coincidences can also directly be transferred to the *correction* (on page 28) interface with the  **Corrections** button.

Display of Saved Raw Data

The acquisition window supports a work-around for the display of previously acquired raw data. This is achieved by enabling the **Synthetic sampling** box in the configuration window, and then selecting the raw data file by the  button.



The next time the acquisition is started with the **Start** button, the file will be read and the data displayed. To avoid confusion with a live experiment, a warning message



is displayed before the curves are shown in the interface. For returning to the normal acquisition mode, please return to the configuration and switch **Synthetic sampling** off.

Twilite Calibration

In experiments using the twilite and a PET system is necessary to cross-calibrate the two devices. This means, that the count-rate of coincidences which the twilite measures for a tracer concentration in the catheter needs to be converted into the activity concentration which the PET measures for the same tracer concentration in the field-of-view.

In principle, the following is done for the calibration:

- 1) PET tracer is added to water such that the activity concentration is in the range of 200-500 kBq/cc. Note that the same isotope should be used as in the actual live experiments. The reason is the variability of the branching factor, which is accounted for during the calculation of the tracer concentration by the PET system. While the standard PET isotopes have branching factors above 0.95, is is lower for Cu-64 (0.174) and Ga-68 (0.891).
- 2) A catheter of the type used in the live experiment is filled with the fluid.
- 3) A PET phantom is filled with the same fluid.
- 4) The catheter is measured in the twilite, producing the rate of coincidences in counts/sec. The geometry of the catheter loop in the measuring head needs to be exactly the same as in the experiment. This is achieved by inlaying the catheter in a precision templates which Swisstrace delivers for the different catheter diameters.
- 5) At the same time the phantom is measured in the PET system, the data corrected and reconstructed in the same was as in the live experiment, resulting in a tracer concentration in kBq/cc.
- 6) The calibration factor is finally calculated by diving the PET concentration by the twilite count-rate:

$$F = C_{\text{PET}}/R_{\text{twilite}}$$
 and has units (kBq/cc)/(counts/sec).

In practice, the calibration has to cope with the following challenges:

- ▶▶ The twilite has an intrinsic number of background counts due to the radioactivity in the LYSO scintillators. This background has to be subtracted from the measurement. The background can either be measured in a separate twilite acquisition with an empty catheter in place, or by a step acquisition which starts with an empty catheter, which is later filled during the acquisition by flushing the catheter.
- ▶▶ The radioactivity decays continually, which has to be compensated for. PET systems correct this decay to the start of the acquisition. Therefore, the twilite activity also needs to be corrected to the same time. This is particularly relevant if there is an offset between the PET and the twilite calibration measurements.

Recommendation: Although it was found that the calibration factor is quite stable over time, it is recommend to perform a new calibration for every live experiment.

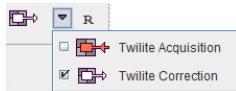
Calibration Experiment

The recommended practical calibration procedure is as follows:

- 1) Make sure the system time of the PET scanner and the twilite acquisition computer is synchronized.
- 2) Fill a PET phantom partially with water, add radioactivity, and shake to distribute the activity homogeneously. Fill the phantom entirely and shake again. The amount of activity should be such that a tracer concentration of 200-500 kBq/cc results.
- 3) Inlay the same catheter which will be used for the live experiment into the template, fill it with the phantom fluid, but don't yet insert it into the measuring head.
- 4) Position the phantom in the PET scanner.
- 5) Start data acquisition of the twilite without template inserted. A flat coincidence rate appears in the acquisition interface which corresponds to the background rate.
- 6) After 30 seconds, insert the template with the filled catheter into the tungsten head. In the acquisition interface the coincidence rate jumps up and stays level, apart from the decay which depends on the isotope used.
- 7) Start the PET scanner and record the starting time.
- 8) After 5-10 minutes stop the twilite and the PET acquisitions.
- 9) Save the twilite raw data for the calibration procedure.
- 10) Reconstruct the PET image, including all corrections, outline a volume of interest (VOI) in the middle of the phantom and calculate the average activity concentration in the VOI in kBq/cc. This represents the reference, to which the twilite is going to be calibrated.

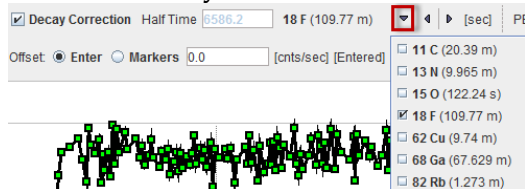
Calibration Processing

For calculating the calibration factor start the **Twilite Correction** module from the PMOD ToolBox.



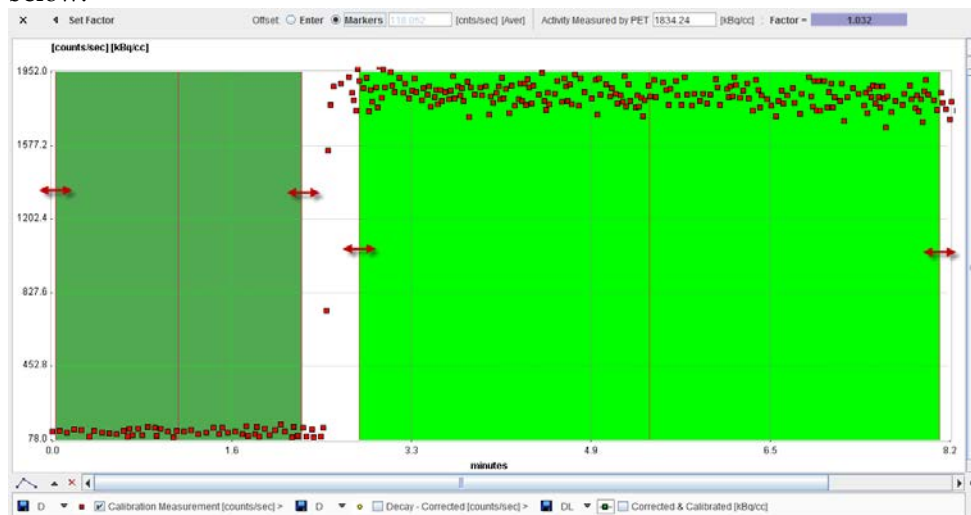
It has two pages: the **Correction** page for correcting a live experiment, and the **Calibration Factor** page for calculating the calibration factor from the calibration experiments. Please proceed as follows:

- 1) Select the **Calibration Factor** page.
- 2) Load the raw data of the twilite calibration experiment with the **Load Calibration TAC** button. The coincidence rate during the measurement is displayed in the curve area as **Calibration Measurement [counts/sec]** with red squares. It should show a level background at the beginning, and a step when the template was inserted into the head.
- 3) Enable the **Decay correction** and select the appropriate isotope from the selection list.



- 4) The decay correction is applied relative to the start of the twilite acquisition and the corrected data shown as **Decay-corrected [counts/sec]** with yellow dots.

- 5) There are two ways to subtract the background activity **Offset: Enter** and **Markers**. The **Markers** operation mode is the one to use with the step calibration data. It activates the display of two shaded areas, one in dark green to the left, and another in light green to the right. The dark green area should be placed over the background area for calculating the average background rate, whereas the light green area serves for averaging the signal from the activity (plus background). Therefore, the placement should be as illustrated below:



The area can be placed by dragging the edges, or the center line.

- 6) Next, correct the **PET Scan Start Time**. Initially it is set to the time when the twilite was started, and to which the twilite data is decay corrected. Enter the proper PET start time. The differences between the two starting times will be used to decay correct the twilite data to the time of the PET scan start.
- 7) The last information needed is the **Activity Measured by PET**. Enter the VOI average determined with the phantom image kBq/cc.
- 8) At this time, the calibration **Factor** is determined and can be seen in the user interface. It incorporates the following calculations: (1) The average in the background window is subtracted from the **Calibration Measurement** (red). (2) The resulting signal is decay corrected to the PET scan start (**Decay-Corrected**, yellow). (3) The **Activity Measured by PET** is divided by the average of **Decay Corrected** in the light green signal area.
- 9) Establish the calibration factor of the correction of the live experiment by the **Set Factor** button.

The **Enter Offset** mode requires a different calibration experiment. It assumes that the entire acquisition was done with a filled catheter so that the signal average is calculated from the whole data range, and the background is entered numerically. The background can be determined by monitoring the twilite display, or by a separate acquisition without activity.

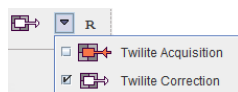
Correction and Calibration of twilight Measurements

Data acquired in a live twilight experiment represents the count-rate of activity in whole blood. The following corrections have to be applied for converting it into a whole blood tracer activity concentration:

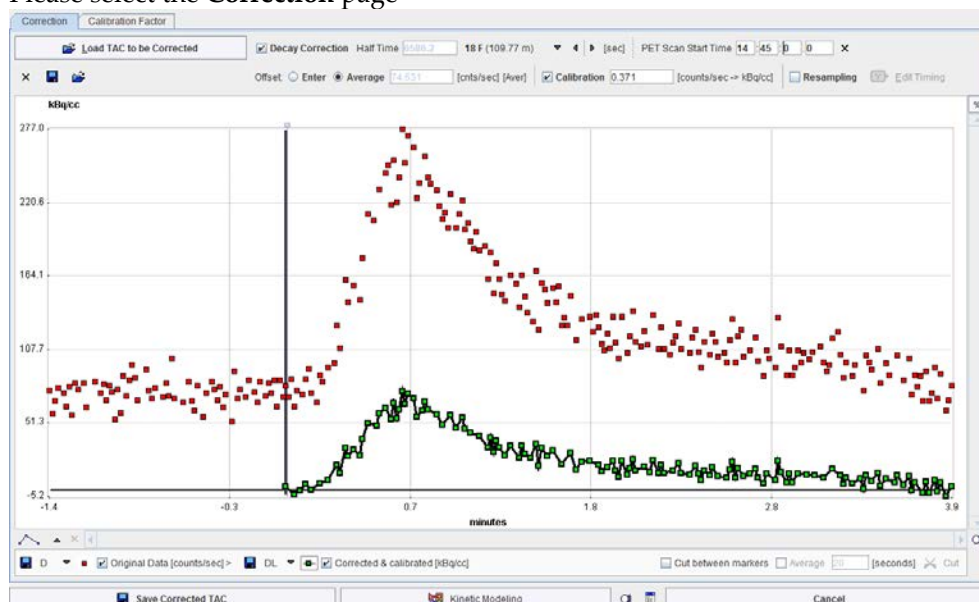
- 1) Subtraction of the intrinsic background.
- 2) Decay correction to the same time as the PET data, namely the PET scanner start time.
- 3) Multiplication with the calibration factor.

Correction and Calibration Procedure

For correcting twilight raw data and applying the current calibration factor start the **Twilight Acquisition** module from the PMOD ToolBox.



Please select the **Correction** page



and proceed as follows:

- 1) Load the raw data of the twilight experiment with the **Load TAC to be Corrected** button. The coincidence rate during the measurement is displayed in the curve area as **Original Data [counts/sec]** with red squares. It should show a level background at the beginning before the activity was injected, followed by a peak when the activity in the blood reaches the detector head.
- 2) Enable the **Decay correction** and select the appropriate isotope from the list.
- 3) Correct the **PET Scan Start Time**. Initially it is set to the time of the first twilight sample. Enter the proper PET start time. Note that time 0 in the curve display moves to the sample when the PET started. The decay correction will be applied relative to this time 0.
- 4) Select the **Average** option for background subtraction.

Offset: ☐ Enter ☒ Average 74.631 [cnts/sec] [Aver]

With this setting, all samples before time 0 are averaged, assuming this is background signal only. The background average is shown as gray text and is subtracted from the **Original Data** before decay correction. If **Enter** is selected instead, the background level has to be entered manually into the number field.

- 5) Activate the **Calibration** factor. It has been established by the last *calibration procedure* (on page 26), but can manually be overwritten. The calibration factor is multiplied with the background subtracted, decay corrected data, resulting in the **Corrected & calibrated**

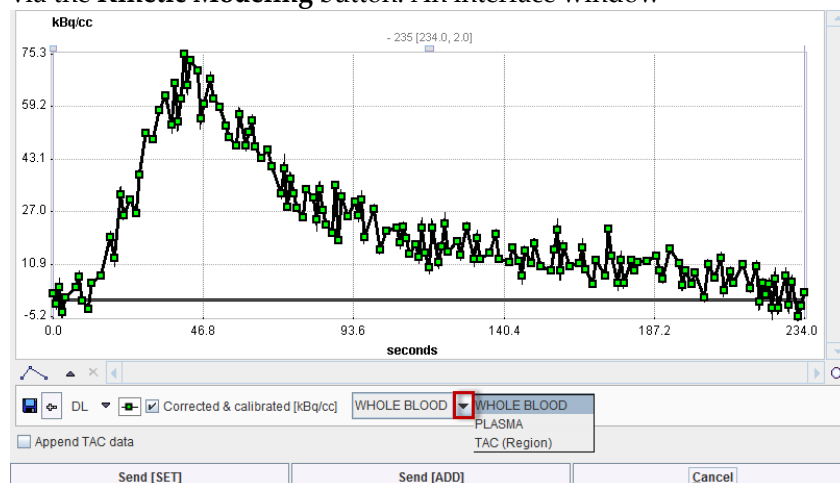
[kBq/cc] whole blood activity concentration. Note that the curve is truncated before time 0, assuming there was not activity.

- 6) Save the calibrated whole blood activity concentration measurement using **Save Corrected TAC**. There are two formats available, from which the user has to choose in a dialog window.

Save corrected TAC as:
☒ Blood (*.cnv) ☐ Tissue (*.tac)

The **Blood** format is used for loading the result as a whole blood curve into the PKIN tool and has only one time column, the sample mid-time. The **Tissue** format is intended for importing the data as a tissue TAC into PKIN, and therefore has a sample start and end time column.

- 7) As an alternative, the **Corrected & calibrated** curve can directly be transferred to PKIN via the **Kinetic Modeling** button. An interface window



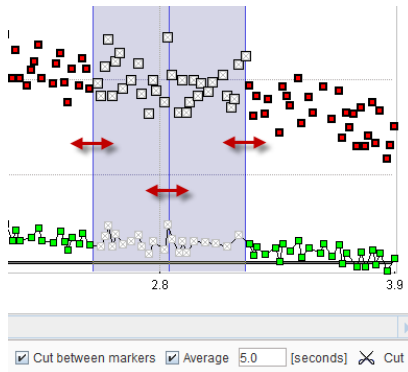
is opened which shows the curve and which has as selection, how PKIN interprets the data: as **WHOLE BLOOD**, **PLASMA** or **TAC**. Usually, **WHOLE BLOOD** should be selected. **Send [SET]** will overwrite data of the selected workspace in PKIN, whereas **Send [ADD]**, will add a new PKIN workspace for the transferred data.

Cutting and Resampling

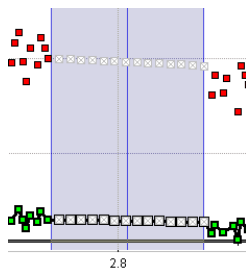
The **Correction** window offers two functions for editing the acquisition, removal of data segments, and data resampling.

Data Cutting

As soon as **Cut between markers** is enabled, an area is shown in the curve area, which can be placed on a curve section by dragging the edges or the center line.



When activating the **Cut** button, the samples covered by the cutting area are replaced by samples linearly interpolated between the left and right area boundaries as illustrated below.



Because the boundary samples are noisy, averaging of a number of samples close to the boundaries can be enabled by the **Average** flag.

Data Resampling

The acquired raw data samples may be too dense for the post-processing, slowing the calculations down. Therefore, the data can be resampled (and smoothed) as follows: Enable **Resampling**, and activate **Edit Timing**. A dialog window is shown which lists each sample with a **START** and an **END** time relative to the PET scan start.

☒ Calibration 0.371 [counts/sec -> kBq/cc]
 ☒ Resampling

Edit Sampling Time [X]

From 1 to 1 inc 1

Edit TIME

Total Sampling Time: start -0.513 end 234.487 seconds

Samples: duration 1.0 increment 1.0 seconds

Selected frames: 235 of 235

View time in seconds

[FRAME]	[START]	[END]
<input checked="" type="checkbox"/> 1	-0.513	0.487
<input checked="" type="checkbox"/> 2	0.487	1.487
<input checked="" type="checkbox"/> 3	1.487	2.487
<input checked="" type="checkbox"/> 4	2.487	3.487
<input checked="" type="checkbox"/> 5	3.487	4.487

☒ Check time consistency

Use the **EDIT TIME** functionality for replacing the sampling. For instance, entering

Edit TIME

Total Sampling Time: start 5.0 end 10 minutes

Samples: duration 5.0 increment 5.0 seconds

and then activating **Insert Sampling Segment** will replace the 1 sec samples between 5 and 10 minutes by 5 sec samples, whereby 5 raw samples are averaged. **Insert Sampling Segment** can be applied several times to create a sampling scheme with variable intervals.

After confirming the timing definition with **Ok**, the **Corrected & calibrated** curve is shown with the new sampling and can be saved for post processing.

Use in PKIN

The result of the PSAMPLE correction and calibration procedure is a time vector of total radioactivity concentration in whole blood, which forms the basis for the arterial input curve (AIC) in kinetic modeling. In principle, the AIC is derived in two steps from the whole blood activity concentration:



- 1) The activity concentration in the plasma fraction of whole blood is determined by accounting for concentration differences in whole blood and the plasma, which actually exchanges with tissue.
- 2) The concentration of unchanged tracer in the plasma (parent) is determined by correcting plasma activity for radiolabeled metabolites.

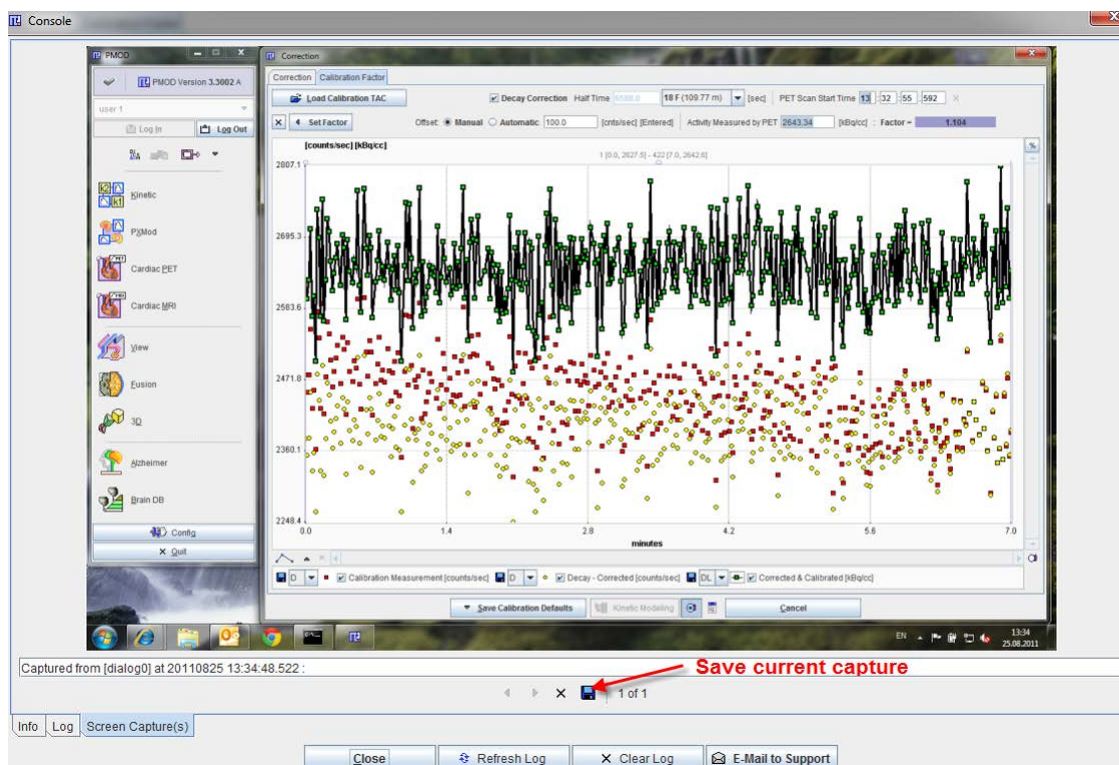
These corrections require knowledge about the plasma/whole-blood and the parent/plasma concentration ratios during the experiment, which can be acquired by taking blood aliquots and their proper analysis, or by applying standard ratios acquired for a population.

All the related processing steps are implemented in the PKIN tool. Please refer to the *PMOD Kinetic Modeling Tool Users Guide* for detailed information about the background and the implementation.

Problem Reporting

As every PMOD module, PSAMPLE includes a functionality to directly send a problem report to the support@pmod.com. This report can include the log output, screen captures and a problem description entered by the user.

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 one of the available formats. The **Log** pane contains the log messages, if the output has been configured to be saved in a file on the **On Start** tab of the **Users Configuration**. 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 define, 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 email, 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.

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