

User's Guide

PMOD Blood Sampling (PSAMPLE)

Version 3.9



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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]. The twilite and arteriovenous shunt have been used as gold-standard to image-derived input function in mice [2] and in quantitative imaging of high-grade glioma and radiation necrosis in rats [3]. In humans, the twilite has been used as part of a quantitative perfusion PET protocol for comparison to MRI arterial spin labeling [4].

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 used for modeling purposes in the PKIN and PXMED quantification tools.

Twilite Components

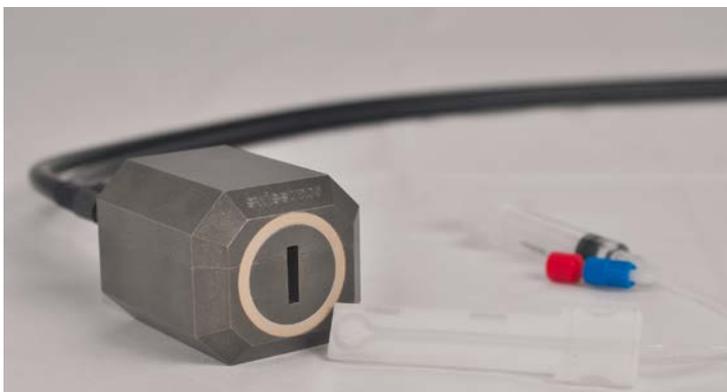
Device Containers

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 to ensure reproducible geometry. There are templates available for different catheter dimensions (currently PE10, PE50 and 2 mm OD clinical extension sets). The illustration below shows the measuring head and 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, as the detector itself does not interact with the blood or influence flow (determined by a certified infusion pump) this design minimizes any potential risks for use in human research experiments.

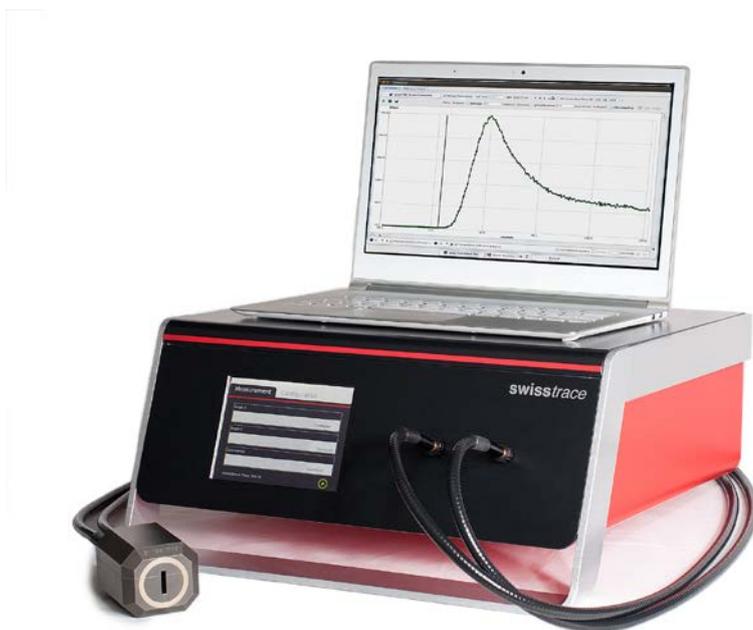
Acquisition Box and Device Assembly

The **ON/OFF** power switch can be found on the rear panel. The box 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 (configuration by DHCP is possible). On the right of the front panel there are two connectors for attaching the measuring head via optical light guides.

The light guides are connected to the acquisition box and detector head with aluminum screw fittings. Note that the screw fittings are different at each end of the light guides. The textured fitting should be connected to the acquisition box, and the smooth fitting to the detector head. Care should be taken not to over tighten the fittings, as damage to the screw threads may occur. No tools should be used to tighten the fittings.

Note: Care should be taken not to touch or contaminate the quartz end faces of the light guides.

The outer coating of the light guides should only be cleaned with a soft cloth and water.



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

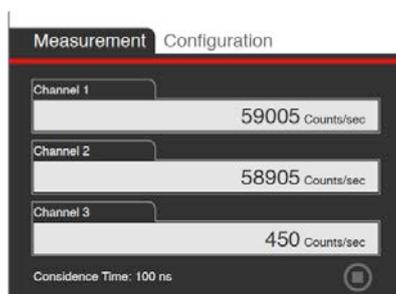
In PET/MR installations two-piece light guides are recommended, connected by an optical coupler (pictured below). The optical couplers can be mounted in a filter plate in the Faraday cage. This requires a 20 mm hole for each coupler. Alternatively the couplers can be positioned close to the Faraday cage and the light guides routed through a wave-guide tube. The light guides are connected to the optical couplers using the same screw-fitting interface as for the detector head and acquisition box. Spacers should not be added to the optical couplers as this may result in non-contact of the quartz optical surfaces and loss of sensitivity.



Touch-Screen

The touch screen has 2 modes: **Measurement** and **Configuration**.

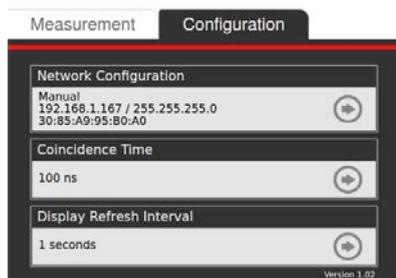
The **Measurement** mode displays the singles, the coincidences and the **Coincidence Time**. Data acquisition can be started with the green "play" button  in the lower right corner, and stopped again with the red "stop" button .



In the **Configuration** mode, several parameters are displayed and can be set:

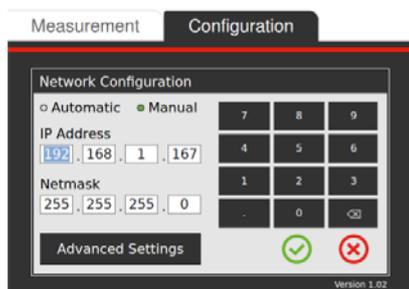
- 1) **Network Configuration**: the field displays the IP number, the subnet number and the MAC address
- 2) The field **Coincidence Time** displays the chosen coincidence time

- 3) The field **Display Refresh Interval** shows the refresh time of the singles and coincidences.



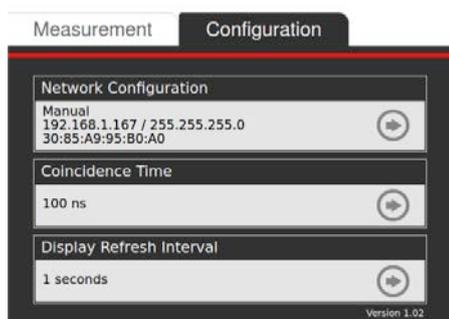
In the **Network Configuration**, the network properties are defined. The network connection can be defined manually or automatically:

- 1) **Manual**: the **IP Address** and the **Netmask** are typed in manually.
- 2) **Automatic**: **DHCP**, the **IP address** is requested from the network.



In the field **Coincidence Time** the coincidence time can be chosen. We recommend as default 100 ns.

In the field **Display Refresh Interval** the refresh interval for the singles and coincidences can be set. Longer display intervals reduce the temporal variation of the values. However, the rate at which the values are written to the acquisition file is defined in the PSAMPLE module.



Experimental Setup

The twilite measures the radioactivity in a catheter which runs between two LYSO crystals. In animals a very efficient solution is to install a shunt between the femoral artery and the femoral vein. This allows continuous measurement of the whole blood activity without any

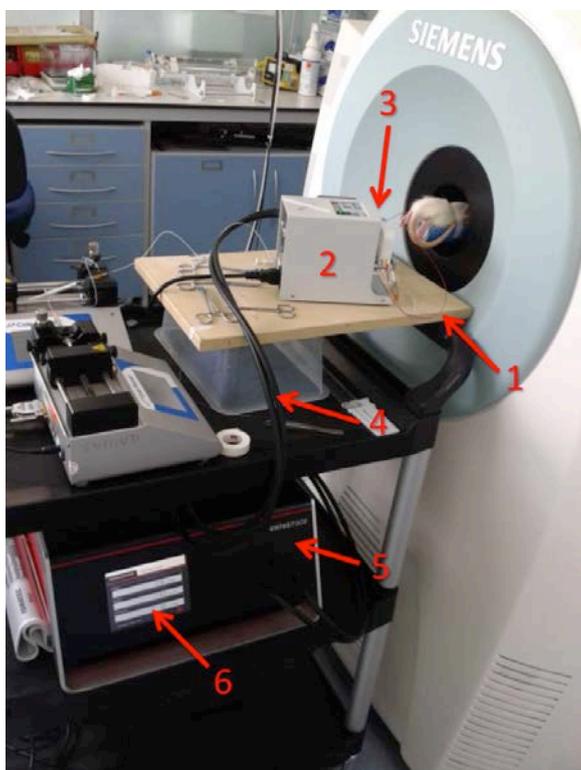
loss of blood. Such arteriovenous shunts can be placed in animals as small as a mouse, using catheters differing in size. A typical setup and schematic are shown below.

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. A schematic is shown below.

In humans the blood flow in the catheter is always controlled by a suitable certified infusion pump. In animals it is also advisable to 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 arteriovenous pressure difference.

The appropriate catheter diameter differs according to the size of the animals. Swisstrace delivers templates to guide the catheter through the measuring head for each catheter size, such that a well-defined geometry is established. The characteristics of the shunt are described in a paper published by Weber and co-workers [1], and further information on shunt and general twilite setup can be found in [2] and [3].

A typical setup for 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) Light guides carrying the photons from the crystals to the PMT's. These guides have a standard length of 2m; they can be as long as 20m in MR compatible systems.
- 5) Data acquisition box with coincidence electronics.
- 6) Touch screen for device setup and start/stop.

- 7) Not seen here: TCP/IP connection on rear panel of data acquisition box, computer with PMOD PSAMPLE data acquisition and data analysis software.

Schematic:

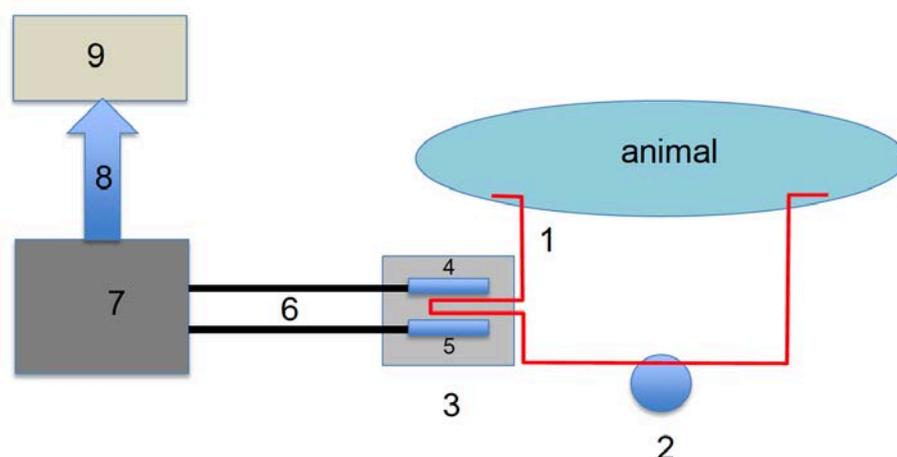
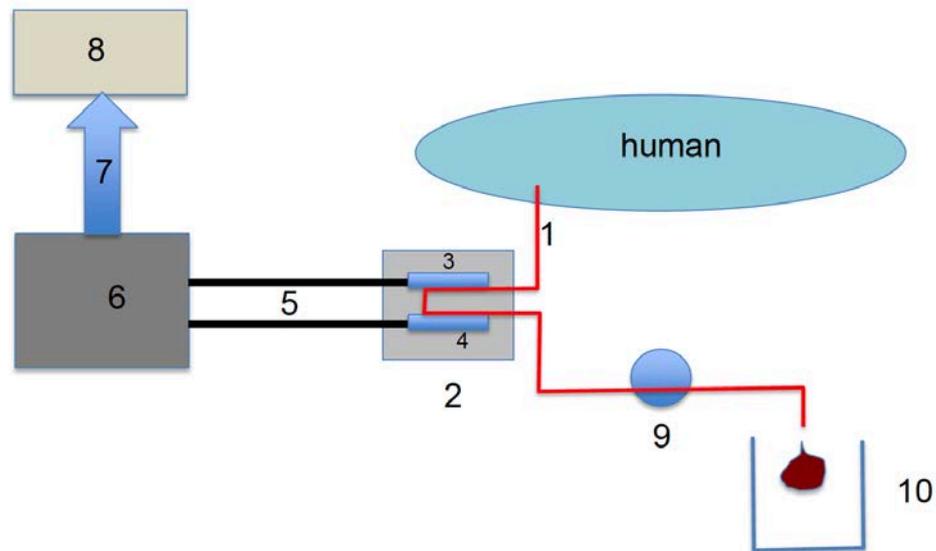


Figure 1: Shunt functions

- 1) Catheter running femoral artery to the femoral vein.
- 2) Peristaltic pump.
- 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 10 m in MR compatible systems.
- 7) Data acquisition box with coincidence electronics.
- 8) TCP/IP connection to computer with PMOD data acquisition tool PSAMPLE.
- 9) Computer with PMOD data acquisition and analysis.

The arteriovenous 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 following procedure is recommended for collection of 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 briefly pressed downwards in order to open the cut and blood drops can be collected with minimal dead volume.

In humans there is no return of arterial blood. Schematic:



- 1) Catheter running from the radial artery to the waste container.
- 2) Twilite measuring head made of tungsten.
- 3) LYSO crystal1.
- 4) LYSO crystal2.
- 5) Light guides carrying the photons from the crystals to the PMT's. These guides have a standard length of 2m, they can be as long as 20m in MR compatible systems.
- 6) Data acquisition box with coincidence electronics.
- 7) TCP/IP connection to computer with PMOD data acquisition tool PSAMPLE.
- 8) Computer with PMOD data acquisition and analysis.
- 9) Peristaltic pump.
- 10) Waste container.

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

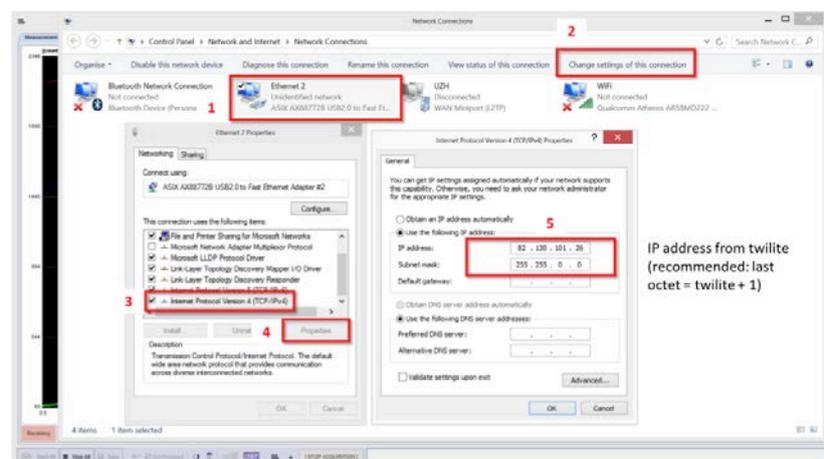
The twilite is directly connected to the computer via an ethernet cable. The data acquisition is performed with the dedicated PMOD module PSAMPLE via a TCP/IP interface. Two configuration levels are required, the network configuration and the module configuration.

Network Configuration

This procedure will normally be completed by Swisstrace upon installation of the twilite. These instructions are intended as a guide in the event that the acquisition PC must be replaced.

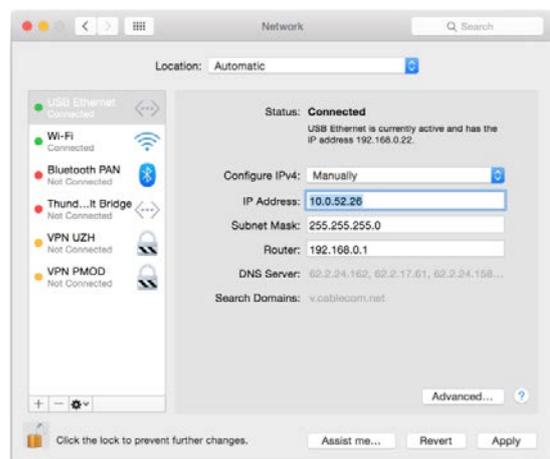
Windows (Windows 8 illustrated)

- 1) Switch on the twilite and connect Ethernet cable to PC
- 2) Check IP address and subnet of twilite on the touch screen (if DHCP is active, the IP address will be assigned by the network server)
- 3) In Windows, go to **Network Connections**
- 4) Right click the network/Wi-Fi icon on task bar and select **Open Network Connections/Network & Sharing Center** (or use: Start, Control Panel, Network & Internet, Network & Sharing Center)
- 5) Select **Change adapter settings**
- 6) Select the active Ethernet/LAN connection (= twilite) (1)
- 7) Select **Change settings of this connection** (2)
- 8) Select **Internet protocol version 4** (3)
- 9) Select **Properties** (4)
- 10) Select **Use the following IP address:** (5)
- 11) Enter an **IP address** in the same group as the twilite IP (typically the twilite IP +1 on final section) and subnet mask.
- 12) OK



Mac (OSX Yosemite on 2014 Macbook Pro illustrated)

- 1) Switch on the twilite and connect Ethernet cable to PC
- 2) Check IP address and subnet of twilite on the touch screen (if DHCP is active, the IP address will be assigned by the network server)
- 3) Go to **System Preferences, Network**
- 4) Select **USB Ethernet** and **Unlock** to allow changes
- 5) Select **Manually** from the **Configure IPv4** menu
- 6) Enter an **IP address** in the same group as the twilite IP (typically the twilite IP +1 on final section) and subnet mask
- 7) **Apply** the settings.



PSAMPLE Configuration

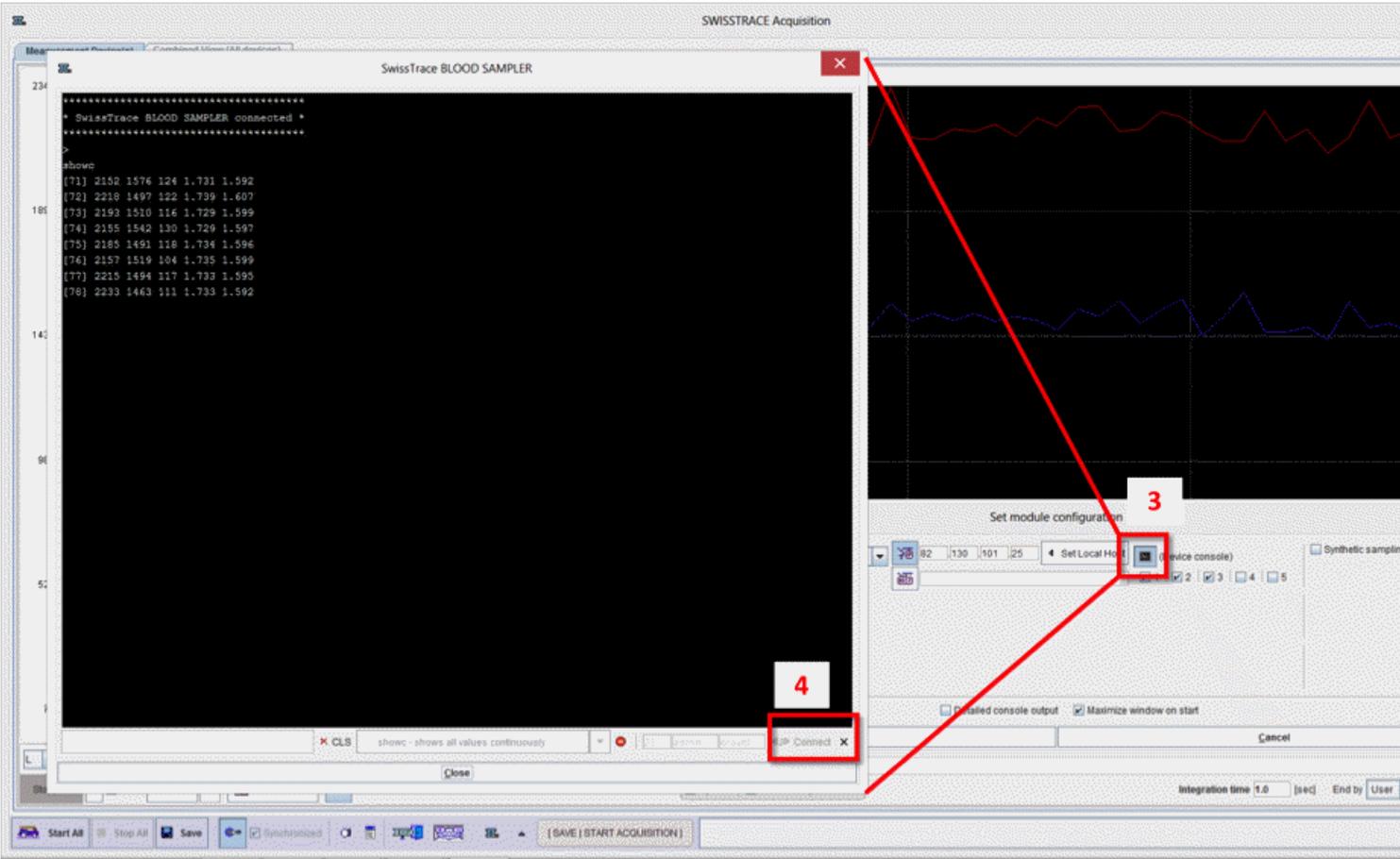
To change the PSAMPLE configuration start it with the  button in the PMOD Toolbox and activate the button  in the bottom status line. The configuration window is organized as shown below:

Connectivity

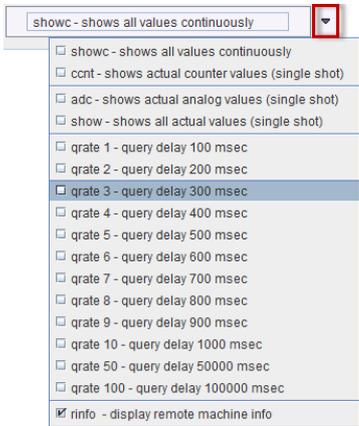
Up to three devices can be configured which can be simultaneously be used during the acquisition. Each device is characterized by an IP address that can be enter manually. For convenience, the **Set Local Host** button retrieves the computers IP address, which can then be adjusted to match the twilight address (obtained through the touch screen **Configuration** menu). 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  (3, below). 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 (4, above) tries to log into the twilite using telnet and 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). The analog channels 4 and 5 were incorporated for simultaneous monitoring of signals from additional instruments. Note that analog inputs were only available on the twilite 1, and are not available on twilite 2.

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: *Pmod3.9/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

System Calibration and Quality Assurance

Detailed console output box. However, this may slow the acquisition down.

Twilite Calibration

In experiments using the twilite and a PET system is necessary to cross-calibrate the two devices. This implies that the twilite coincidence count-rate for a given 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.

The following calibration procedure is recommended:

- 1) PET tracer is added to water such that the activity concentration is in the range of 200-500 kBq/cc. Complete mixing should be carefully ensured. Note that ideally the same isotope should be used for calibration as in the actual experiments. The reason for this is variability in the branching ratio between isotopes, namely the ratio of positron decays to other means of decay such as electron capture. Branching ratio is accounted for during the calculation of the tracer concentration by the PET system (i.e. post-processing/calibration). While the most common PET isotopes (F-18, C-11, O-15) have branching ratios above 0.95, other isotopes used in research can differ substantially, e.g. Cu-64 (0.174) and Ga-68 (0.891).

Note: Starting with PMOD v3.8 the branching ratio correction is available in the PSAMPLE Correction tool. information about the branching ratio is available from *Turku PET Center* http://www.turkupetcentre.net/petanalysis/branching_ratio.html

- 2) A catheter identical to the type used in the actual experiment is filled with the tracer solution. Extreme care is required to avoid the presence of air bubbles in the catheter. Note that tap water may contain large amounts of dissolved air that can result in air bubble formation in the catheter over time.
- 3) A suitable phantom is filled with the same (well-mixed) fluid. PET/MR users should ensure that attenuation correction can be accurately applied for the phantom used. Phantoms with thick plastic walls are known to cause problems, resulting in inaccurate tracer concentration in the reconstructed image.
- 4) After ensuring that the system clocks of the twilite acquisition computer and PET scanner are synchronized, twilite acquisition is started as *explained below* (on page 25) without the catheter guide inserted. Background counts should be acquired for at least two minutes.
- 5) Without stopping the data acquisition, the guide with filled catheter is inserted into the detector head, and counts acquired for at least two minutes. The calibration data curve thus resembles a step function.
- 6) In parallel, the activity in the phantom is measured in the PET system. Typically a protocol such as 10 minute static FDG brain is used. The data should be corrected and reconstructed in the same way as in the actual experiment, resulting in a tracer concentration in kBq/cc. It is not necessary to synchronize PET and twilite acquisitions as the **PSAMPLE Correction** tool performs decay correction to match the scan start time.
- 7) Calibration processing is performed in the **PSAMPLE Correction** tool as *outlined below* (on page 20). The activity concentration measured from the PET image of the phantom and PET scan start time are required. For example, these parameters can be measured/taken from another PMOD module. The calibration factor is finally calculated by dividing the PET concentration by the twilite count-rate:

$$F = C_{PET}/R_{twilite}$$

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 without catheter in place (or with an empty catheter in place), or by generating a step function as described above.
- ▶▶ Decay of the radioactivity must be compensated for. PET systems correct this decay to the start of the acquisition. For this reason, the twilite activity is also corrected to the PET scan start time. This is particularly relevant if there is an time offset between the start of the PET and the twilite calibration measurements.

Recommendation: Although the calibration factor is stable over time, it is recommend that calibration is performed regularly as part of a quality control procedure.

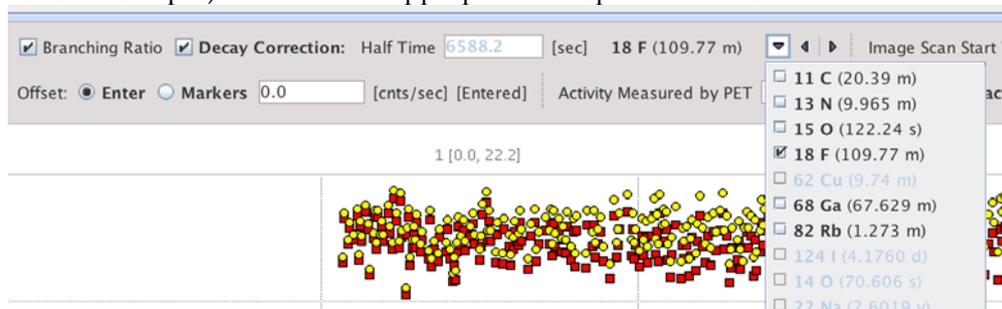
Calibration Processing

To calculate the calibration factor start the (PSAMPLE) **Twilite Correction** module from the PMOD ToolBox.

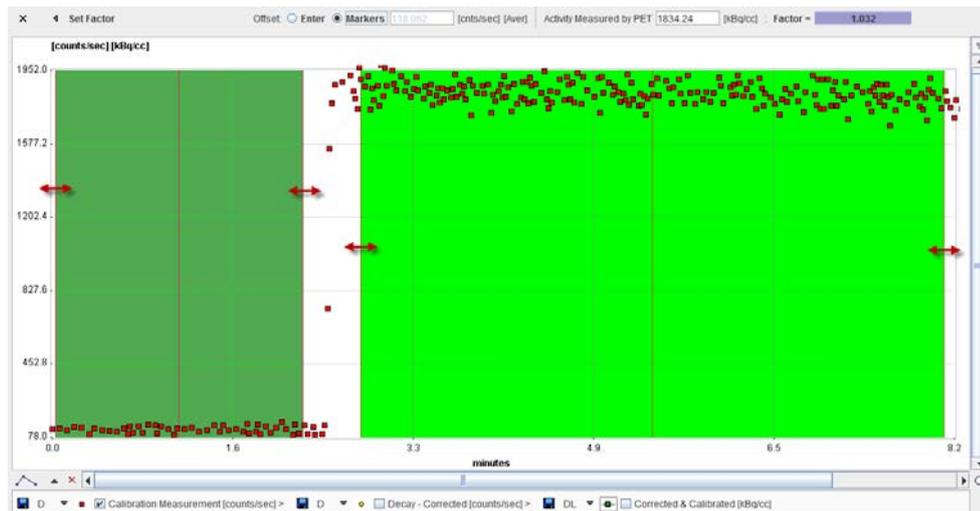


It has two pages: the **Correction** page for correcting a measured input function, and the **Calibration Factor** page for calculating the calibration factor from the calibration procedure as described above. Please proceed as follows:

- 1) Select the **Calibration Factor** page.
- 2) Load the raw data from the twilite calibration experiment using 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**, **Branching Ratio** correction (at present only available for selected isotopes) 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. **Branching Ratio** correction is a division of the counts (minus background, once defined) by the branching ratio
- 5) There are two methods provided to subtract the background activity **Offset: Enter** and **Markers**. The **Markers** operation mode is only suitable for step function 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 in order to calculate the average background rate (calculated from **Calibration Measurement [counts/sec]** with red squares), whereas the light green area defines the portion of signal from the activity to be averaged (calculated from **Decay-corrected [counts/sec]** with yellow dots, after subtraction of the averaged background counts). Therefore, the placement should be as illustrated below:



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

The **Offset: Enter** mode requires a different calibration protocol. It assumes that the entire acquisition was performed with a filled catheter inserted in the detector head, such that the signal average is calculated from the whole data range, and the background is entered manually. The background can be determined by monitoring the twilite touch screen display, or through a separate acquisition without activity and averaging of the counts.

- 6) Next, correct the **PET Scan Start Time**. Initially it is set to the time when the twilite was started. Enter the proper PET start time from the phantom PET acquisition. The differences between the two starting times will be used to decay correct the twilite data to the time of the PET scan start.

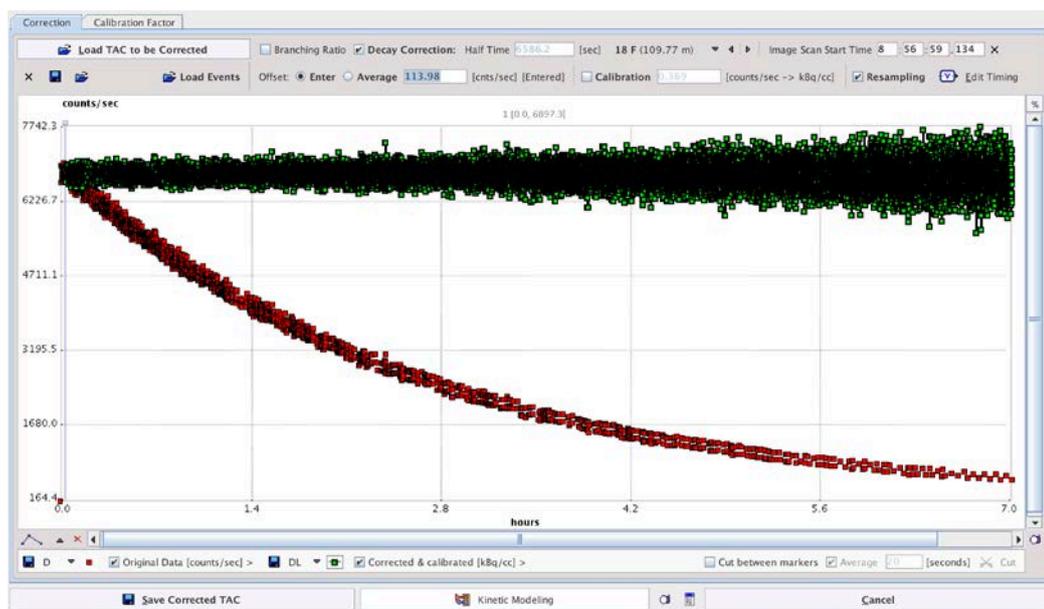


- 7) Finally the **Activity Measured by PET** is required. Enter the average activity determined with the phantom image (e.g. in a volume-of-interest, avoiding edges where spill-out may artificially reduce the average), kBq/cc.
- 8) At this time, the calibration **Factor** is determined and can be seen in the user interface. The **Corrected & Calibrated [kBq/cc]** curve in green squares illustrates the effect of the calibration factor on coincidence counts from the twilite
- 9) The calibration factor can be transferred to the **Correction** tab using the **Set Factor** button.

Quality Assurance

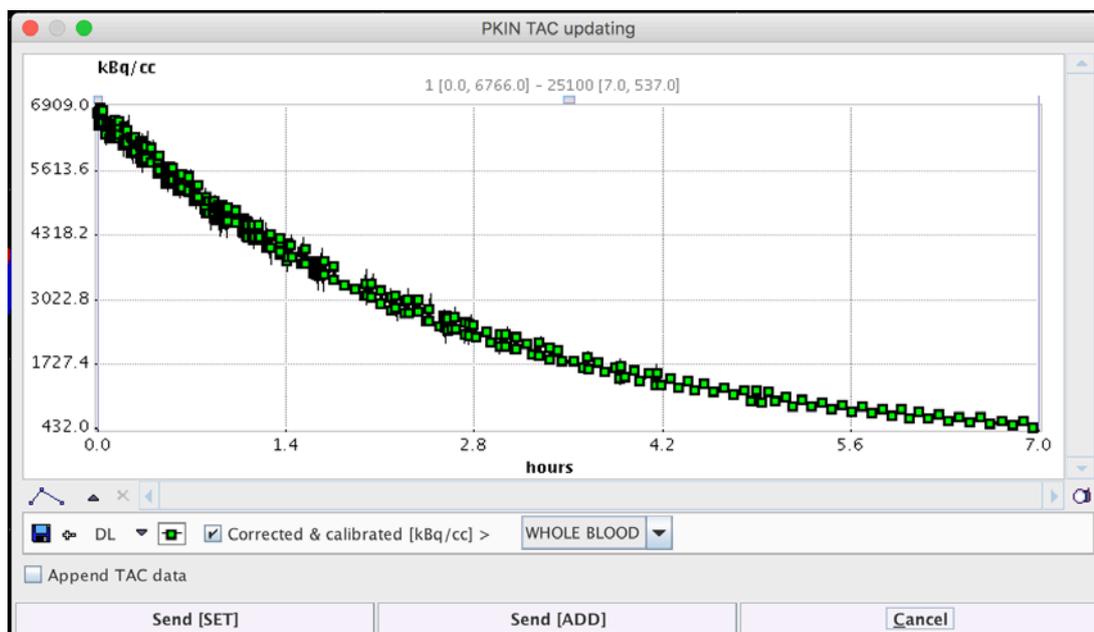
Further to calibration (measurement of sensitivity), the function of the twilite can be tested using a long (e.g. multiple of 3 to 5 times the tracer half-life) acquisition of a fixed activity solution (e.g. 5 MBq/ml for 0.58 mm ID catheter as used in rats, 2 MBq/ml for 1 mm ID as typically used in humans).

Using the **PSAMPLE Correction** tool, the data from the long acquisition can be loaded in the **Correction** tab. After subtraction of the background counts using the **Offset: Enter** method (enter the background counts calculated during calibration), apply **Decay Correction** with the appropriate isotope half time. The resulting **Corrected & Calibrated [kBq/cc]** (green squares) should be a linear, horizontal, line. Application of the **Calibration** factor and **Branching Ratio** correction is not necessary.

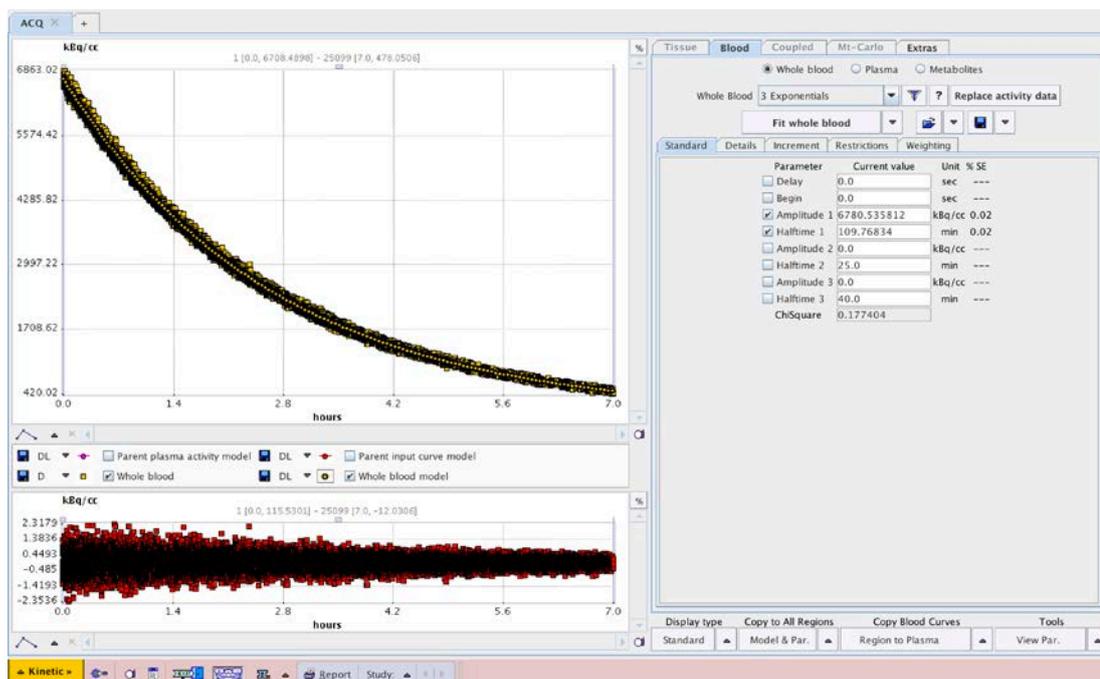


Additionally, a single exponential function can be fitted to the non-decay corrected data using the PKIN tool (if licensed). The half time of the exponential should match the isotope half-life.

Turn off **Decay Correction**, and use the Kinetic Modelling button to open the TAC export dialog. Send the curve to the PKIN tool using the **Send [SET]** button (or **Send [ADD]** if other projects are already open in PKIN).



PKIN opens on the **Blood** tab. Select the **3 Exponentials** model from the list, deactivate the second and third exponentials (uncheck **Amplitude 2/3** and **Halftime 2/3**, set **Amplitude 2/3** to zero), and enter a reasonable starting value for **Halftime 1** (e.g. 100 minutes for F-18). Ensure that **Delay** and **Begin** are set to zero. Activate **Fit whole blood** to fit the model.



Once fitted, the resulting model curve can be highlighted by clicking the **Whole blood model** button in the legend below the plot window.

Amplitude 1 represents the starting average counts, and **Halftime 1** should closely match the half-life of the isotope used in the measurements.

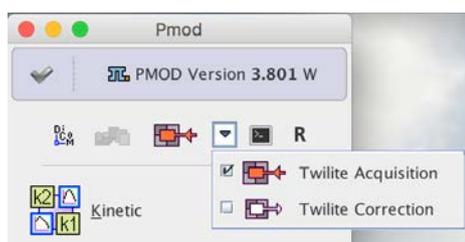
Sources of error include:

- 1) Inaccurate definition of the background in **Offset: Enter**. Determine the background counts from a calibration or simple background measurement shortly before the long decay acquisition.
- 2) Excessive starting activity (the twilite is linear, with deviation $< 1\%$, up to 6,000 counts/sec coincidences). If initial coincidences are above this threshold, stop the acquisition and restart after a suitable delay.
- 3) Continuing to acquire data after reaching the background level (flattening of the data curve tail). **Resampling** in the **PSAMPLE Correction** tool can be used to remove the tail using **Remove Sampling Segment** and an appropriate time window.

Data Acquisition

Start by switching on the twilight system with the **ON/OFF** button. After the initialization procedure has completed, press the green 'play' button on the touch screen to switch on the PMTs.

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 twilight as been *configured* (on page 15) appropriately.

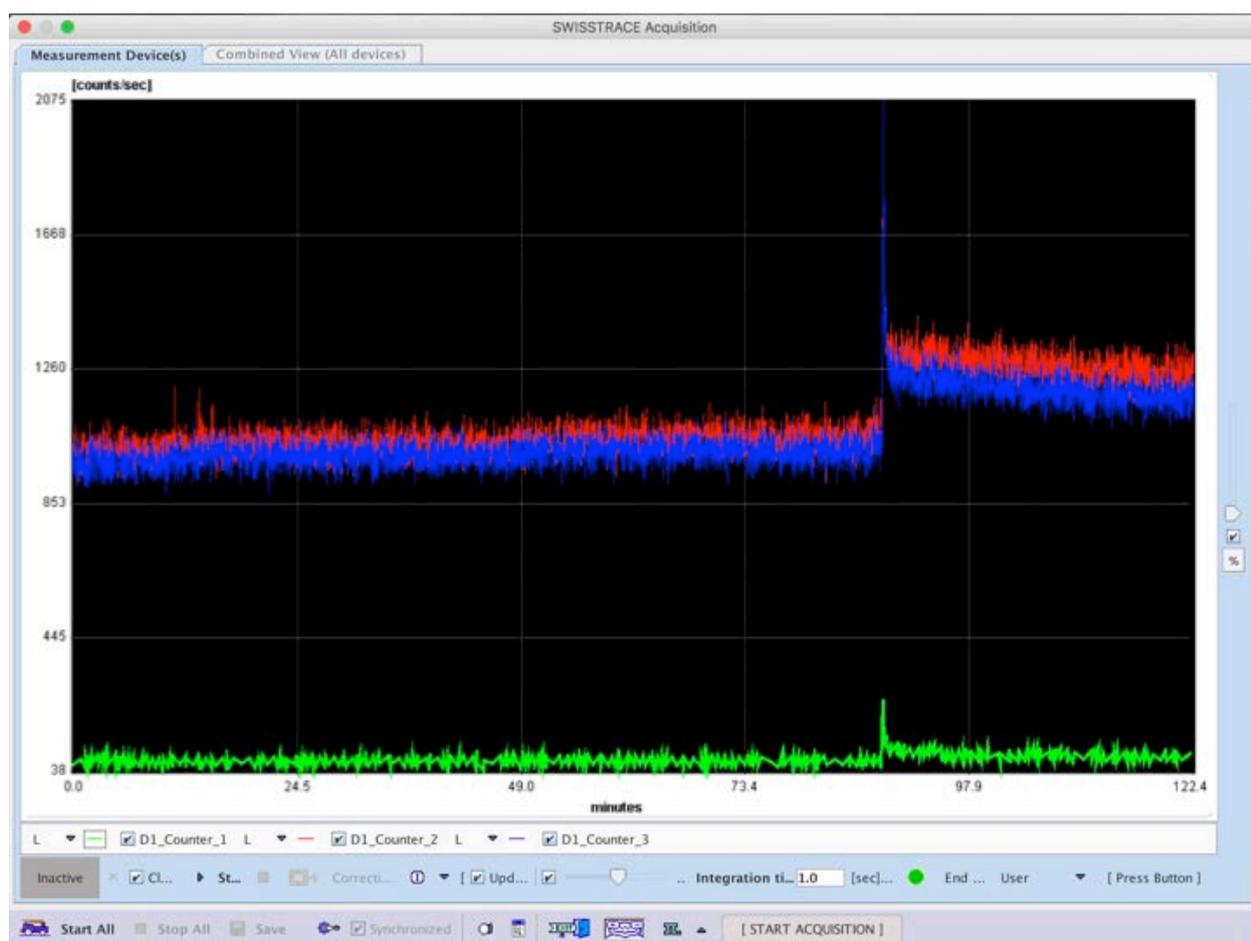
In a simultaneous experiment with a PET acquisition please make sure that the system clocks of the acquisition computer and the PET system are synchronized before starting PMOD (PSAMPLE time stamps each data point, using the system time as reference). Furthermore, in order to acquire enough background data for automatic background subtraction during AIF correction, start the twilight at least 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. Note 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 12) 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.



The **End By** supports two settings:

- ▶ **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 *acquisition protocol* (on page 28).

Load Protocol: For retrieving an acquisition protocol.



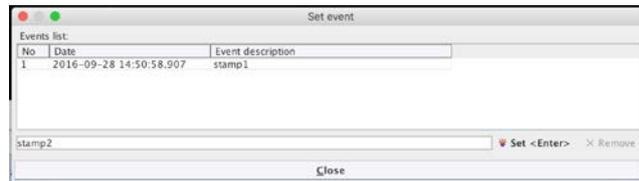
Start: Starts the acquisition for the configured device. **Start All** starts the acquisition on all configured devices at once.



Stop: Stops the acquisition for the configured device. **Stop All** stops all devices at once.



Timestamp: Opens a dialog in which the user can record events during acquisition with time stamping. Events can be entered manually and written to a file using the **Set** button.

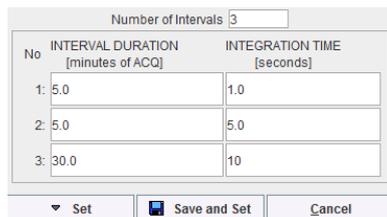


The events file will be saved along with counts data, with the suffix ***_EVTS** (format ***.crv**).

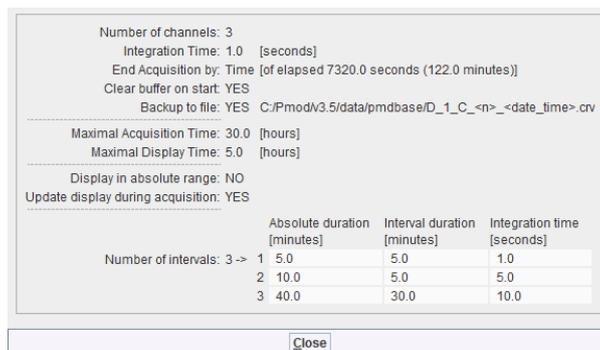
If more than one device has been configured, each if them is represented by a curve/control area as described above (maximum 3).

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 dialog window the **Number of intervals** must be defined. Once entered, a corresponding number of rows is set up for definition of 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.



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

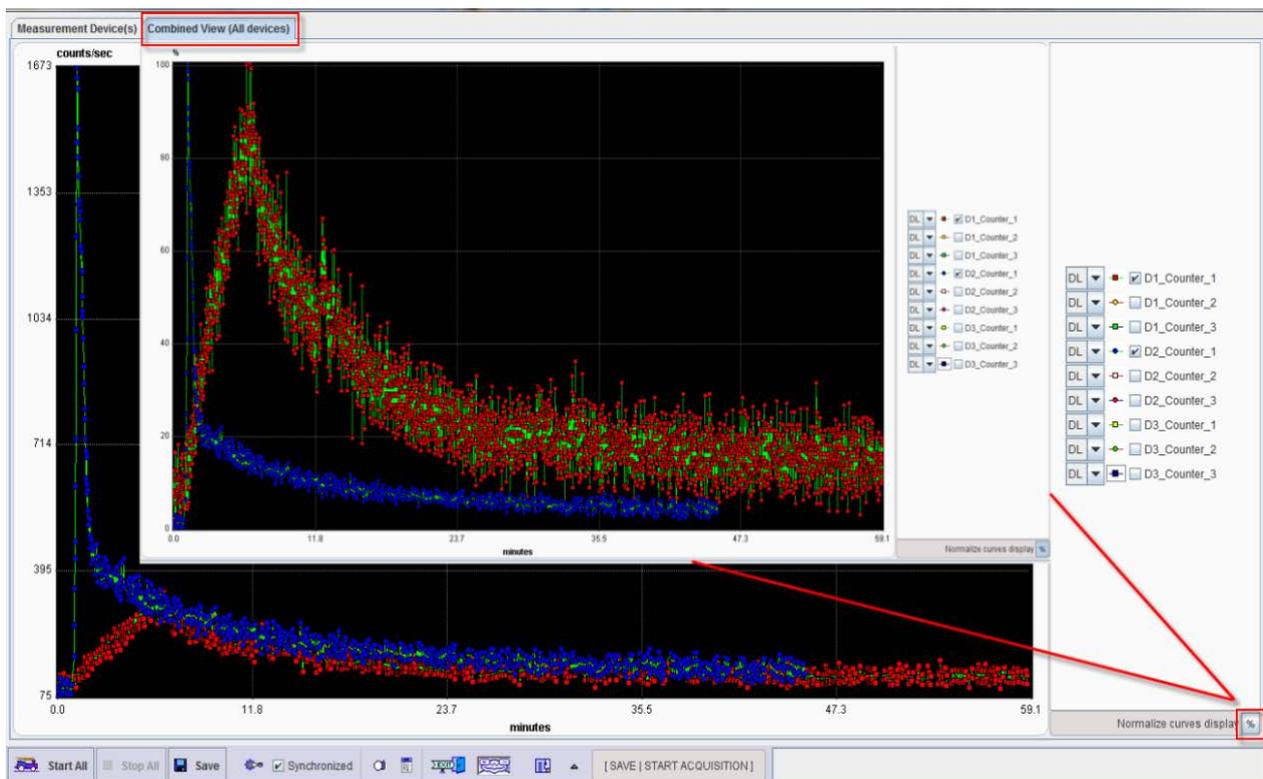


With **Save and Set** the protocol can be additionally saved for later re-use.

Note: In the current implementation, the twilight multiphase acquisitions (sequences of different sampling rates) impose data loss between intervals: 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. Resampling during postprocessing does not incur such data loss.

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.

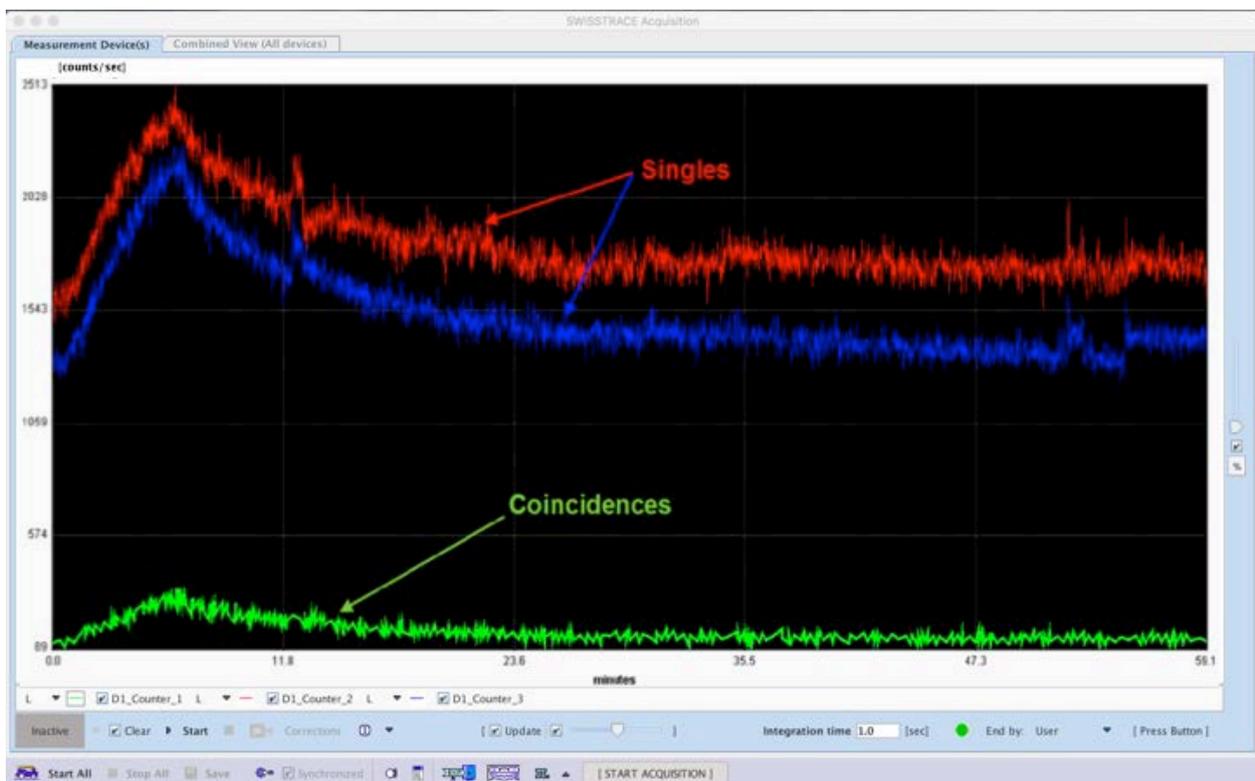


Data Acquisition

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  (in the drop-down menu below the **Info** 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.

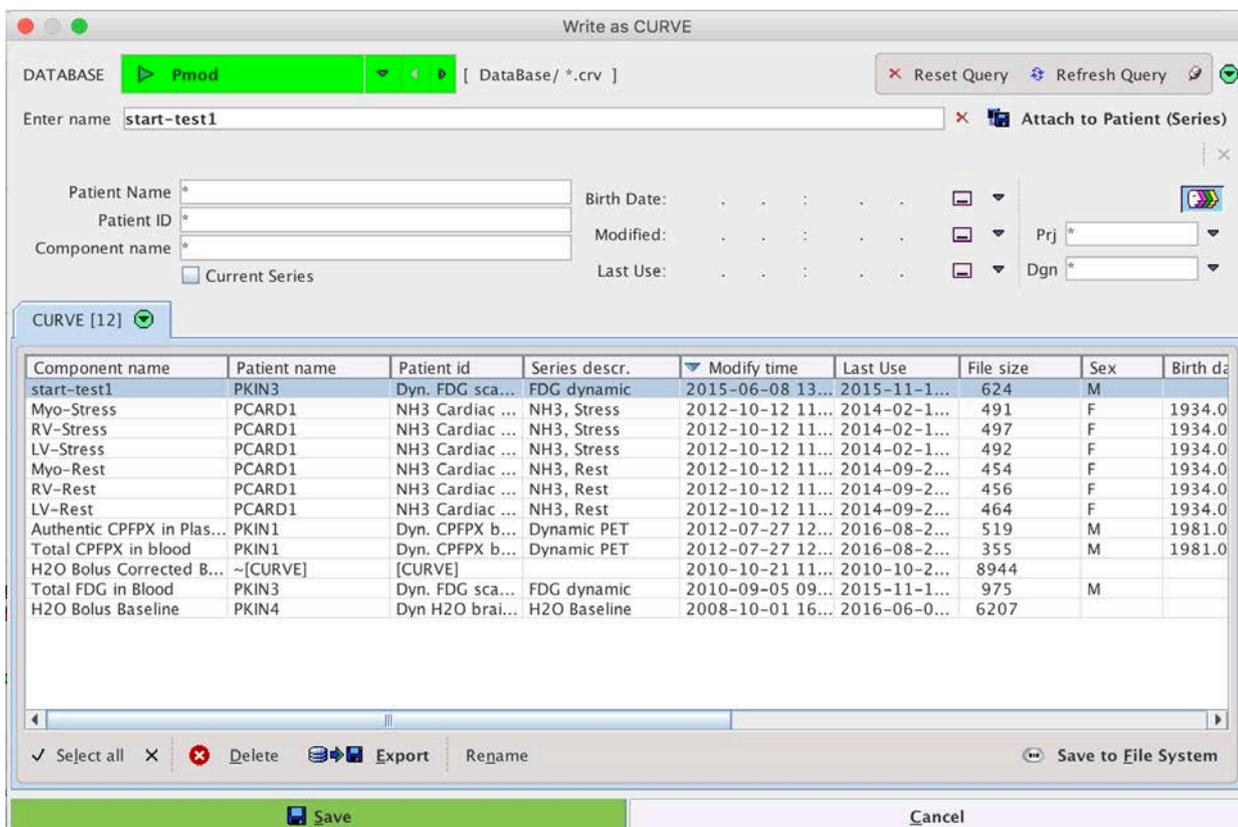
Multiple Devices Acquisition

If multiple devices are controlled by the same instance of PSAMPLE, a curve display and control area is shown for each device in a row layout. The acquisition can be started

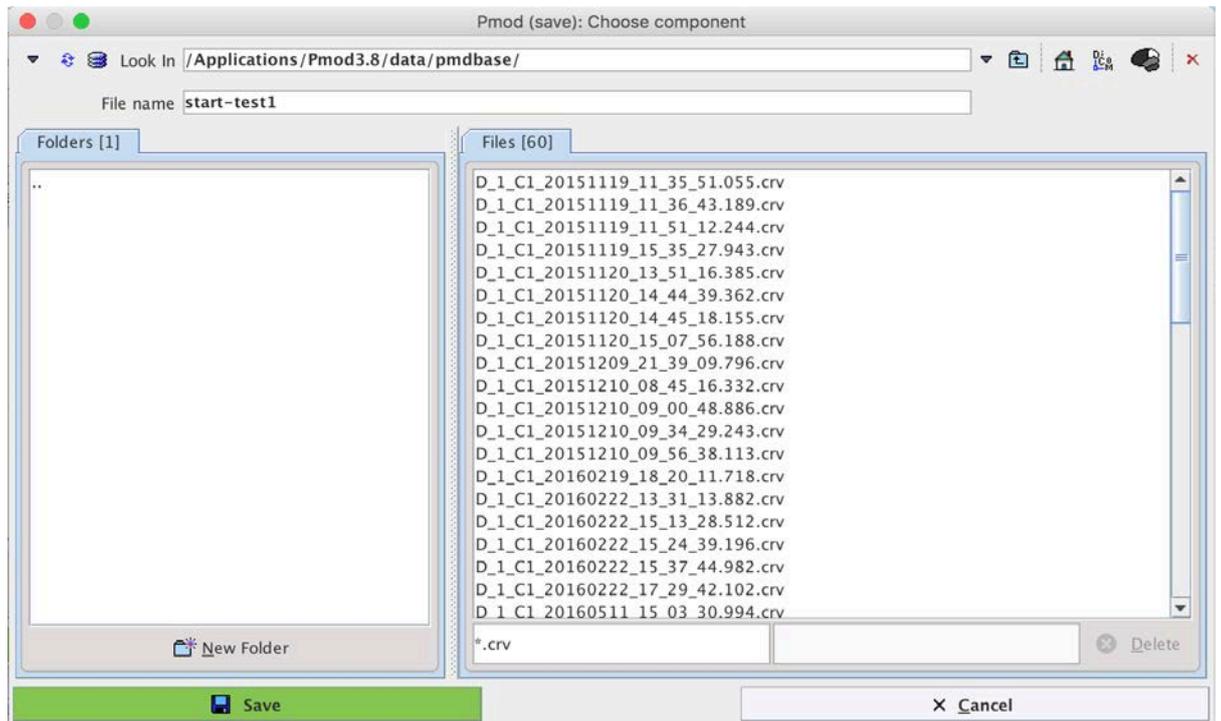
individually for each device with the corresponding **START** button. Alternatively, all acquisitions can be started at once with **Start All**. The results of a multiple device acquisition can most conveniently be inspected in the **Combined View (All devices) page**. (on page 29)

Saving the Data

Please use the  **Save** button to save the raw acquisition data. A dialog window appears for definition of 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.



To save the raw data outside the database, use the **Save to File System** button. In the dialog opened, navigate to the target directory, enter a **File name**, and **Save**.



The saved raw data file has a **.crv** suffix and can be visualized in Excel (import data from file, tab separated) or a text editor. It is organized in columns with the date and time of the sample (space separated), 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 |

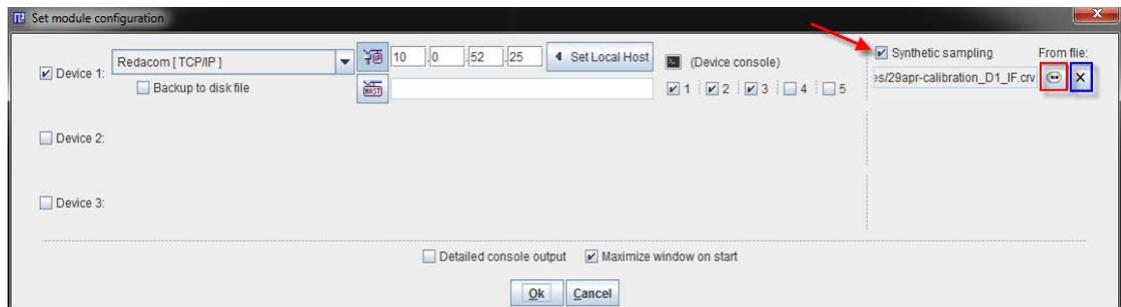
In case of multiple device acquisition, the save process is similar to that described above. The activation of **Save** button will generate a text file for each device. During the saving procedure, the software appends to the file name the suffix $_D_i$, where i can be 1, 2 or 3.

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 34) 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.

Correction and Calibration of twilight Measurements

Raw data acquired with the twilight represents a combination of background counts from the LYSO crystals, constant (with noise) at all activities, and counts (coincidences) resulting from decay of activity in the whole blood within the catheter. The number of counts from blood varies not only due to physiological kinetics, but also due to radioactive decay. Typically in quantitative PET studies we are only interested in physiological kinetics, meaning that counts in PET and blood should be corrected for decay. Thus, the following corrections have to be applied to raw twilight data in order to convert it into a whole blood arterial input function:

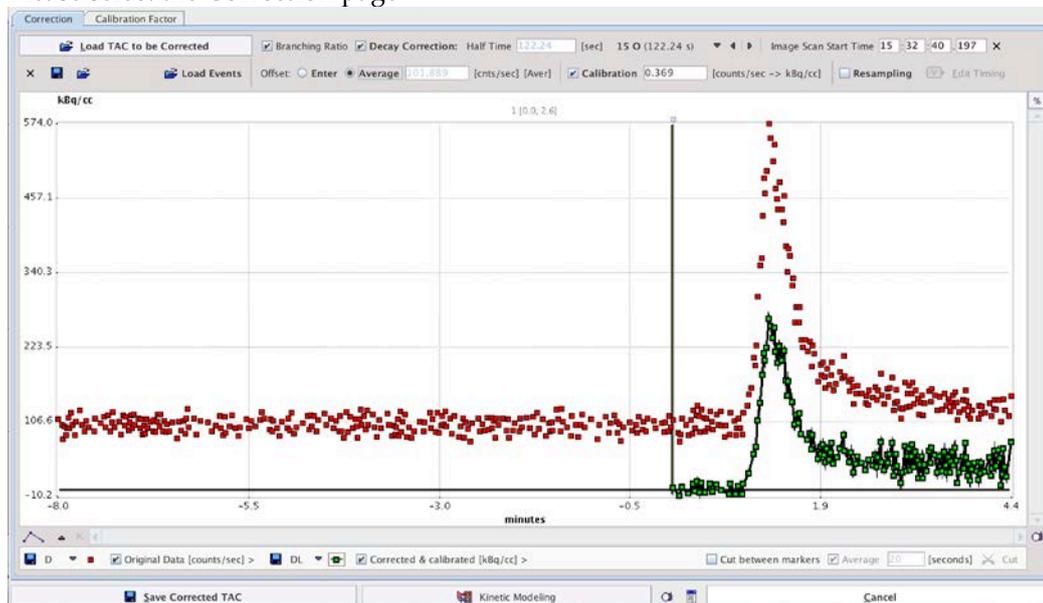
- 1) Subtraction of the intrinsic LYSO background.
- 2) Decay correction to the same time as the PET data, typically the PET scanner start time.
- 3) Correction for the isotope branching ratio.
- 4) Multiplication with the calibration factor.

Correction and Calibration Procedure

Correction and calibration of twilight raw data is performed using the PSAMPLE **Twilight Correction** 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 **Branching Ratio** and **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 corresponding to the start of dynamic imaging. Note that time 0 in the curve display is shifted to correspond to the difference between the start of twilight sampling and the true PET start time (i.e. the twilight is typically started before the PET acquisition, providing background count rate data). The decay correction will be applied relative to this time 0 to match the usual decay correction applied to PET data.
- 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, with the assumption that this is background signal only. The background average is shown in gray text and is subtracted from the **Original Data** before decay correction. If the twilight was not started before the PET acquisition, or the background was not stable before time 0 (e.g. high activity near the detector/light guides, such as ^{15}O -water generation, **Enter** should be selected

instead. The background level (e.g. that calculated during twilight calibration) should then be entered manually.

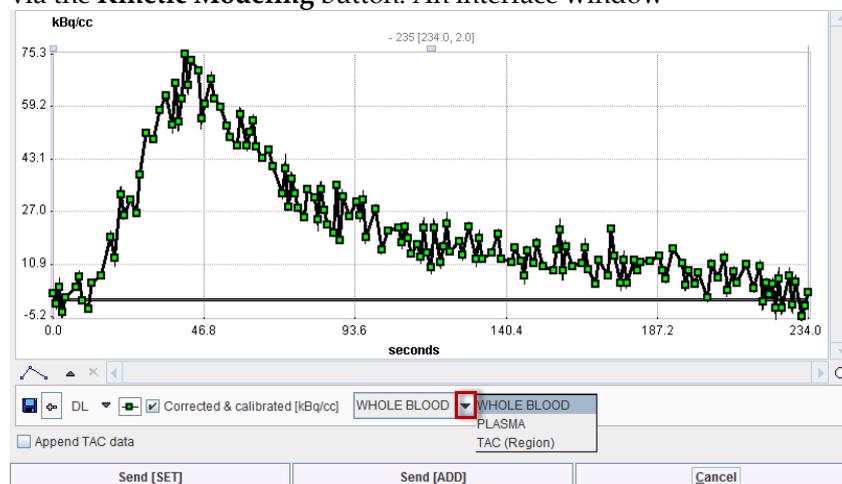
- 5) Activate the **Calibration** factor. Typically this factor has been established during the last *calibration procedure* (on page 20), but can manually be overwritten. The calibration factor is multiplied with the background subtracted, decay and branching ratio corrected data, resulting in the **Corrected & calibrated [kBq/cc]** whole blood activity concentration. Note that the curve is truncated before time 0, assuming that data prior to PET acquisition is not required.
- 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 (*.crv) Tissue (*.tac)

The **Blood** format is used when the data will be loaded as a whole blood input curve into the PKIN tool (the typical use) and has only one time column, the sample mid-time. The **Tissue** format is intended for import of 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



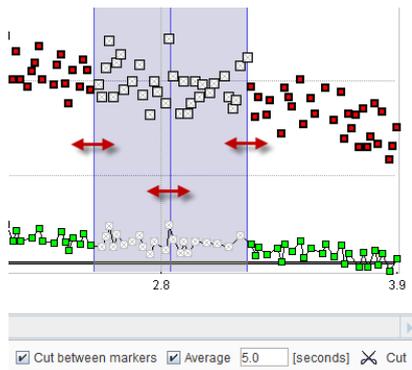
is opened which shows the curve and a drop-down menu to select how PKIN should interpret the data: as **WHOLE BLOOD**, **PLASMA** or **TAC**. Usually, **WHOLE BLOOD** should be selected. **Send [SET]** will overwrite data of the selected workspace in PKIN, whereas **Send [ADD]**, will add a new PKIN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

Cutting and Resampling

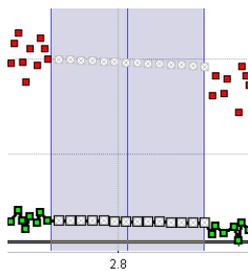
The **Correction** window offers two functions for editing of the **Corrected & calibrated** curve: removal of data segments, and data resampling.

Data Cutting

As soon as **Cut between markers** is enabled, a blue area is shown in the curve area, typically at the extreme right hand side. This cutting definition area can be placed on a curve section by dragging the edges or the center line.



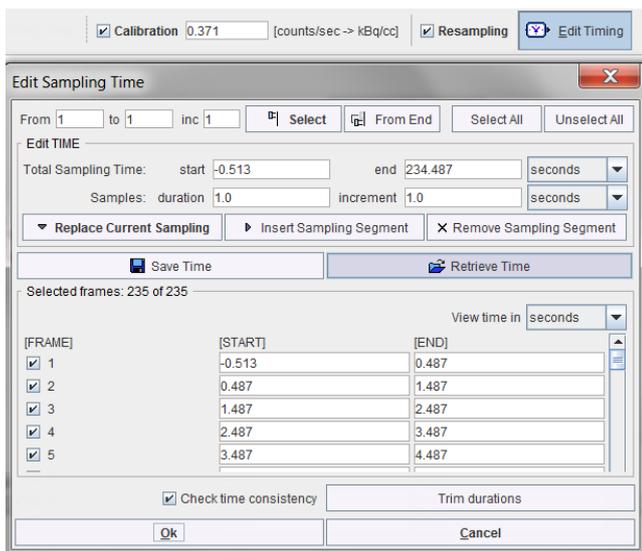
When the **Cut** button is activated, the samples covered by the cutting area are replaced by samples linearly interpolated between the left and right area boundaries as illustrated below.



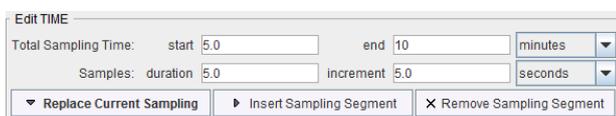
Because the boundary samples are influenced by noise, averaging of a number of samples close to the boundaries can be enabled by the **Average** flag. Samples within that duration before and after the cutting edges will be averaged to provide start and end values for the interpolated portion.

Data Resampling

If the native 1 second sampling resolution of the twilight and PSAMPLE is used, the raw data may be too dense for post-processing on older computers, slowing the calculations down. Therefore, the data can be resampled (resulting in implicit smoothing/denoising) 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.



Use the **EDIT TIME** functionality to replace the sampling interval for either the entire curve, or in sections. For instance, entering



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.

Given sufficient processing power, we recommend transfer of all data points to PKIN, where functions can be fitted to the whole blood data. This results in a noise-free input function to which whole-blood/plasma and parent correction ratios can be applied.

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.

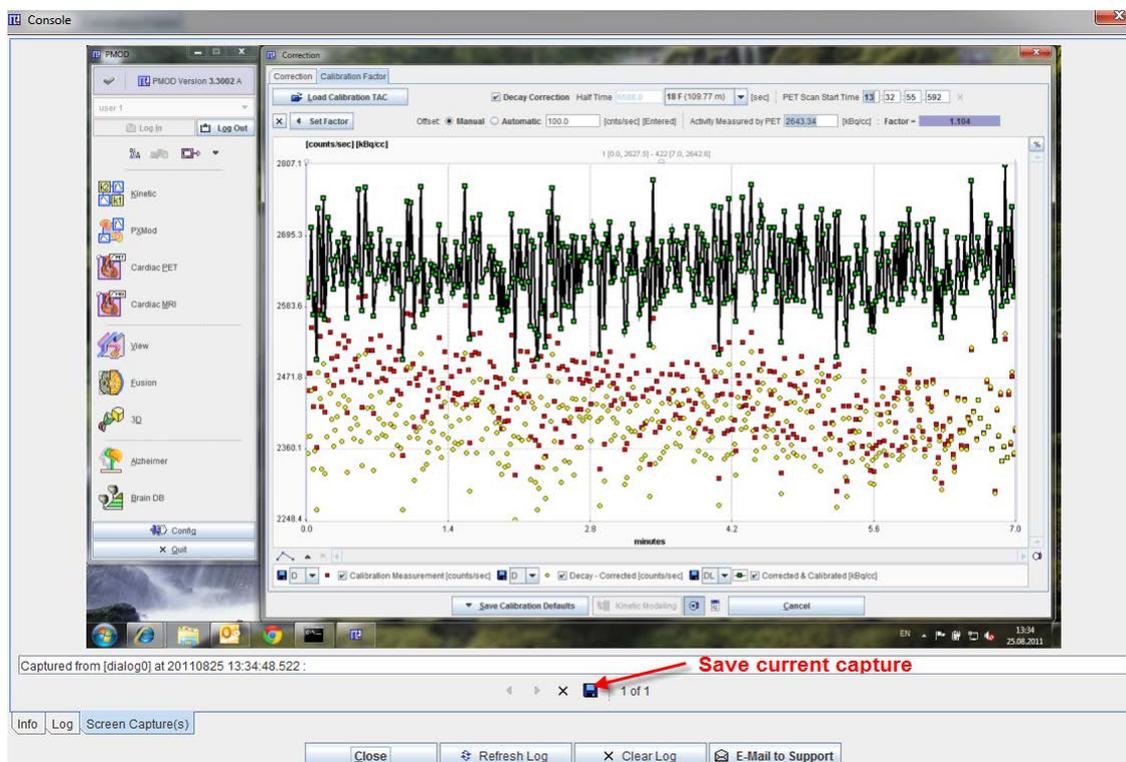
These corrections require knowledge about the plasma/whole-blood and the parent/plasma concentration ratios during the experiment, which can be acquired by taking blood aliquots for analysis (i.e. centrifugation to separate plasma, high-performance liquid chromatography or cartridge metabolite separation methods), or by applying standard ratios acquired in 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 of the techniques and the implementation in PKIN.

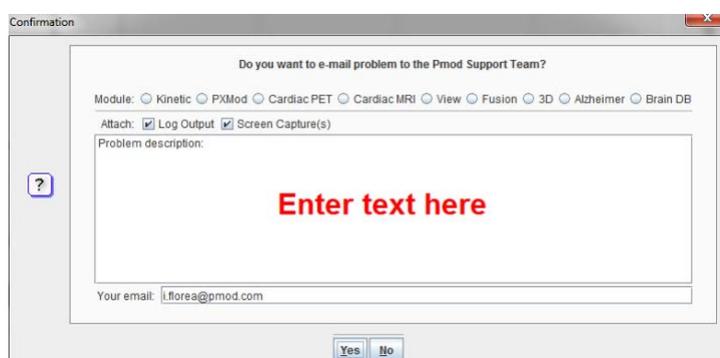
Problem Reporting

As in every PMOD module, PSAMPLE includes a functionality to directly send a problem report to the Support team (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.

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- 4) Zhang K, Herzog H, Mauler J, Filss C, Okell TW, Kops ER, Tellmann L, Fischer T, Brocke B, Sturm W, Coenen HH, Shah NJ. Comparison of cerebral blood flow acquired by simultaneous [15O]water positron emission tomography and arterial spin labeling magnetic resonance imaging. *J Cereb Blood Flow Metab*. 2014; 34(8):1373-80.

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