PMOD PSAMPLE Software for twilite Blood Sampling System

USER MANUAL
Version 4.3

PMOD is a software
FOR RESEARCH USE ONLY (RUO)
and must not be used for diagnosis or treatment of patients.

π.pmod

1 Swisstrace Twilite Blood Sampling System

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 specifity 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 developed the "twilite" coincidence detector 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 features 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 arterial input curves to be acquired 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]. Version three of the twilite was released in late 2018, and features upgraded base unit ergonomics, improved touch-screen operation and a choice between direct data acquisition or internal storage and FTP-based transfer.

PMOD's PSAMPLE software is the acquisition software of the twilite, and supports the correction and calibration of the raw data (coincidence counts per second) to derive the whole blood tracer activity concentration. It can be easily forwarded into PMOD's Kinetic Modeling tool for fitting, WB-to-plasma and metabolite correction. The resulting AIF is subsequently used for modeling purposes in the PKIN and PXMOD quantification tools.

2 Twilite Components

2.1 Device Containers

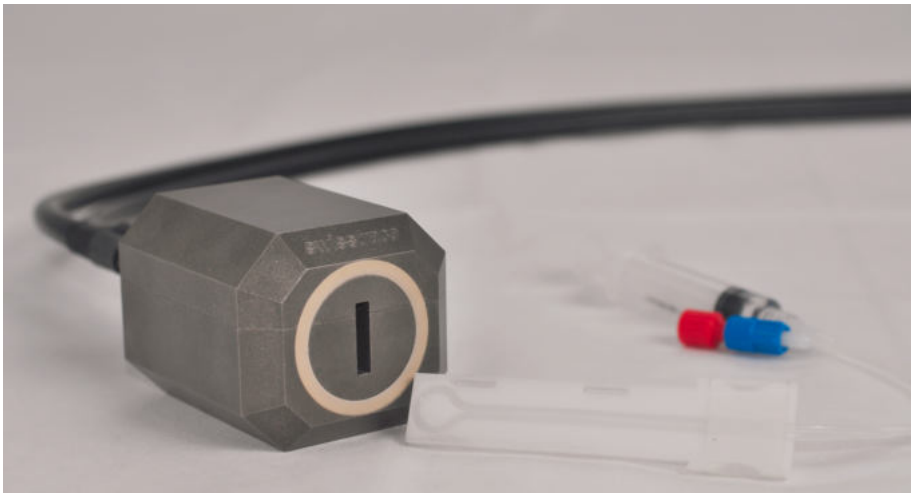
The twilite system comes in two container boxes: one box for the twilite acquisition box plus annexes, and one box for the twilite measuring head and light guides.

(please note - color may vary depending on the twilite version)

2.2 Measuring Head

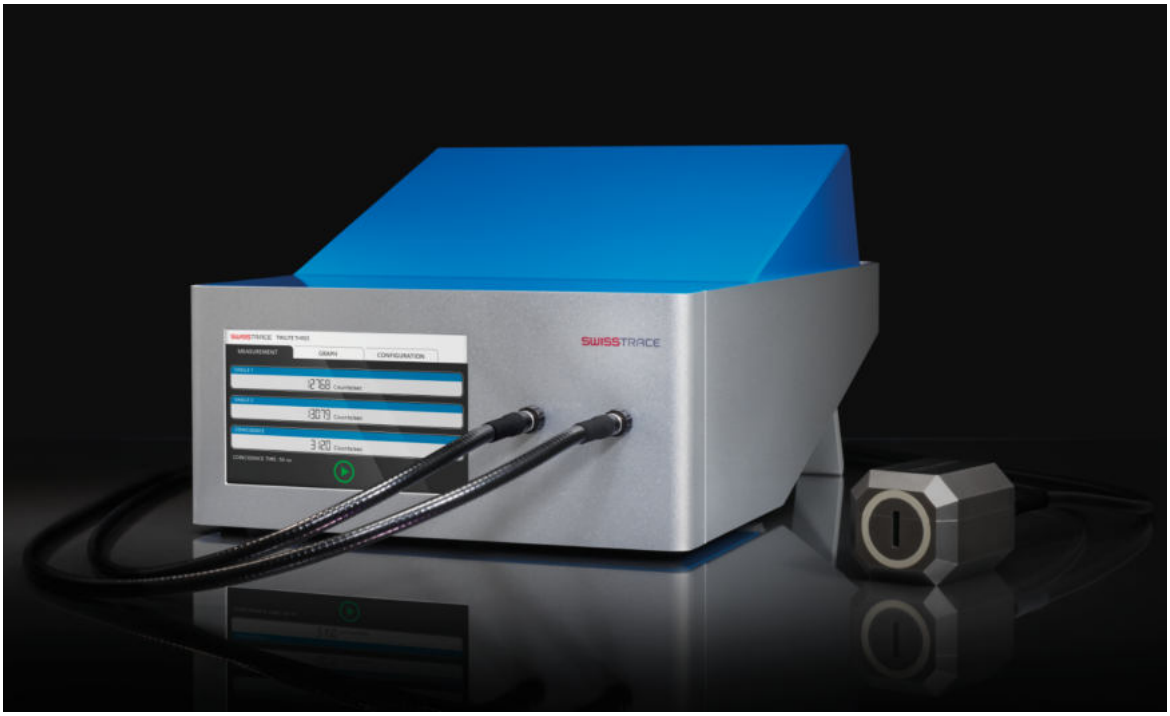
The core of the twilite system is a very compact measuring head made from two LYSO crystals enclosed by medical grade tungsten, which is fully MR compatible. The head has an opening into which the catheter templates are inserted to ensure reproducible geometry. There are templates available for different catheter dimensions (currently PE10 0.61 mm OD, PE50 0.96 mm OD, and 2 mm OD to suit common low-volume extension set tubing used in human studies). The illustration below shows the measuring head and a template with a fitted human-size catheter. Note that the

(standard) catheter enters the detector head as an unbroken loop, allowing detection of coincidences without any interaction between the twilite and the subject blood.

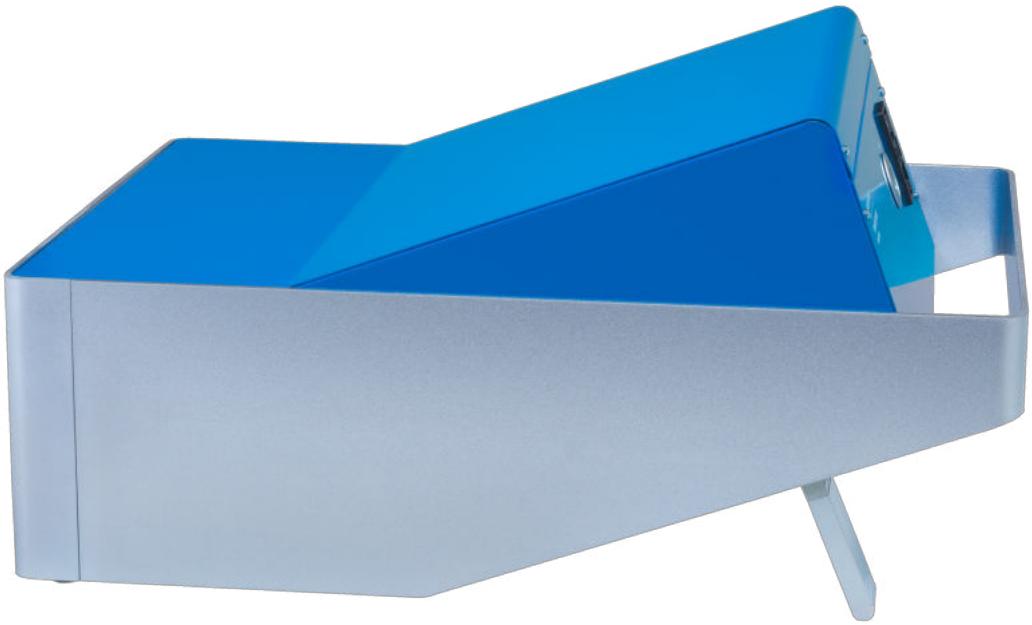


The scintillations in the LYSO crystals are conveyed to the photon detection unit via two flexible high efficiency light guides. (BGO crystals are also available for specific applications). This design is without any electronics in the sensor head and thus avoids any potential problems of electromagnetic interference with other devices, in particular MR scanners. Furthermore, as the detector itself does not interact with the blood or influence flow this design minimizes any potential risks for use in human research experiments.

2.3 Base Unit and Device Assembly



The **ON/OFF** power switch can be found on the rear panel of the base unit. The base unit can be 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). The twilite three base unit can be used with tilted front panel (e.g. when used on a bench top and viewing from above) or with vertical front panel by extending the support legs (e.g. when placed on top of a rack, facilitating viewing from below).



On the right of the front panel there are two connectors for the attachment of the light guides to the measuring head.

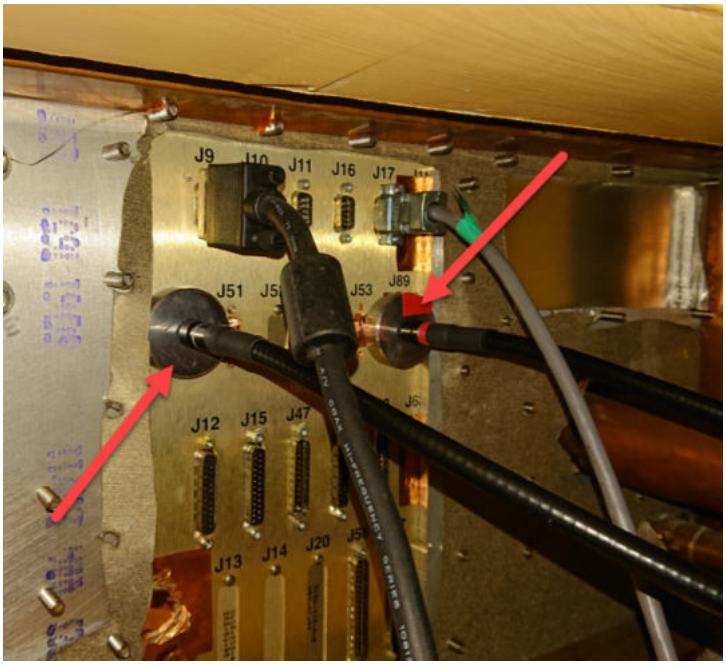
The light guides are connected to the base unit and detector head with aluminium screw fittings. Note that the screw fittings are different at each end of the light guides. The textured fitting should be connected to the base unit, and the smooth fitting to the detector head (note that the textured fittings will also not fit side-by-side at 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. Initial installation and connection of the light guides will be performed by a swisstrace engineer.

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, as illustrated below. 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.

The two parts of the optical couplers, illustrating how they can be screwed into a filter plate.



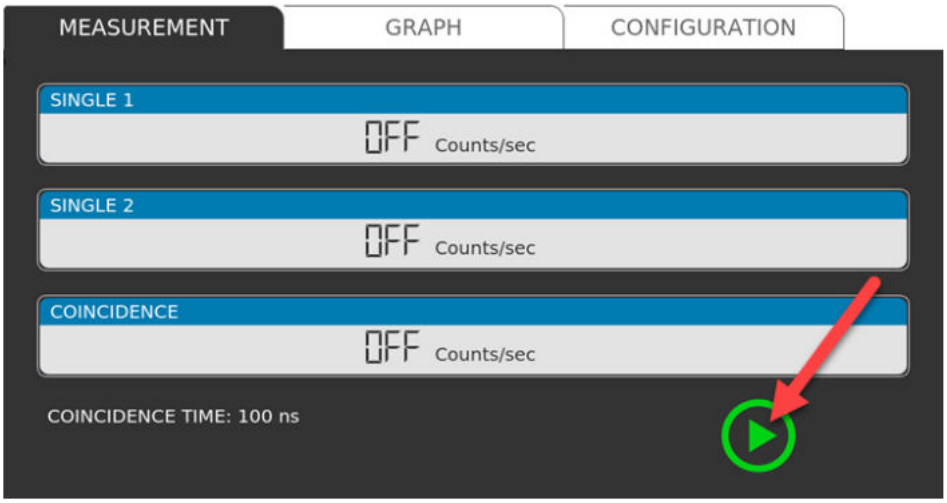
The optical couplers mounted in a filter plate (viewed from the operator room of the PET/MR).

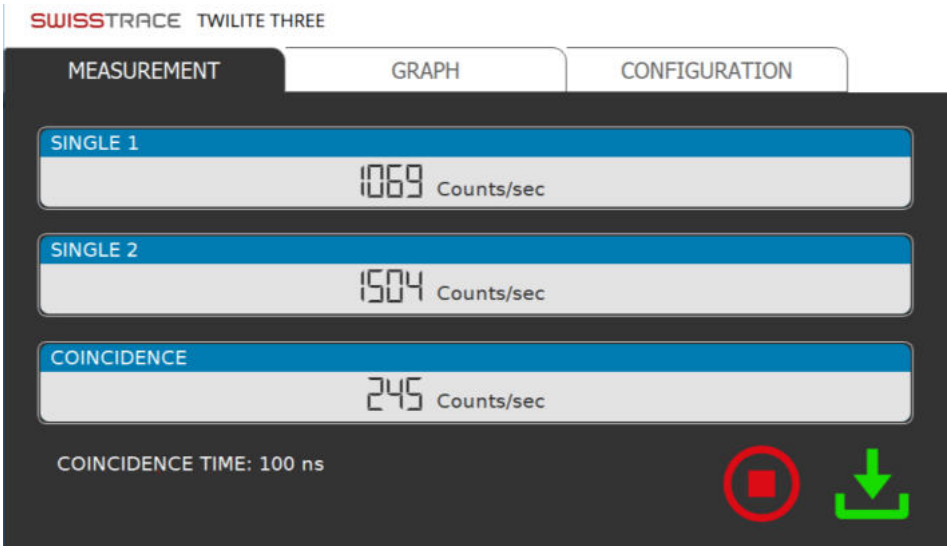
2.4 Touch-Screen

The touch screen has 3 main tabs: **Measurement, Graph** and **Configuration**.

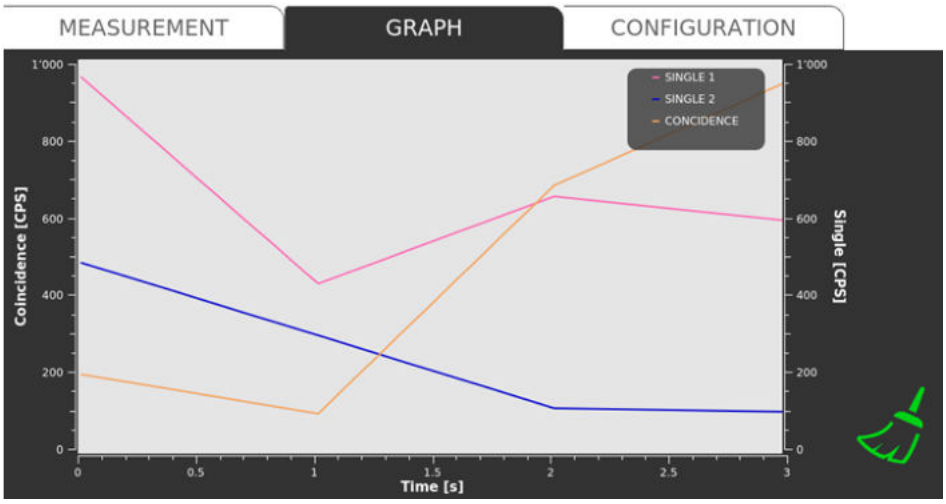
The **Measurement** tab displays the singles, the coincidences and the **Coincidence Time**. Data acquisition can be started with the green “play” button in the lower right corner, and stopped again with the red “stop” button

Internal data recording (twilite three only) is started using the "save" button , which will animate when data is being recorded (animation at sampling rate selected on Configuration tab, see below).

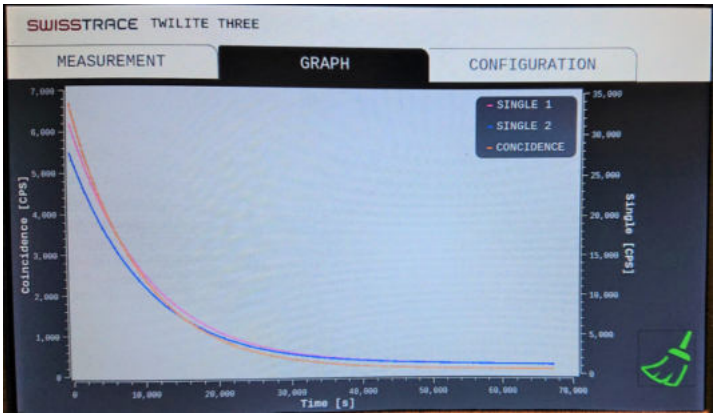




The **Graph** tab displays the counts shown on the **Measurement** tab. All data points since power up are appended to the graph, but the graph can be restarted using the "wipe clean" brush icon  .



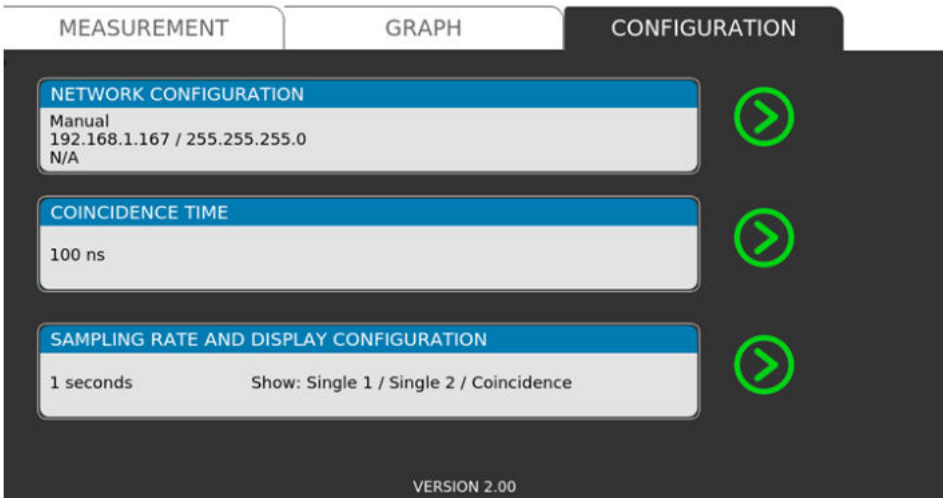
An example of data acquired during an overnight quality control measurement are shown below:



On the **Configuration** tab, several parameters are displayed and can be set:

1. **Network Configuration**: the field displays the mode (manual or automatic), IP address, subnet and the MAC address
2. **Coincidence Time** displays the chosen coincidence time

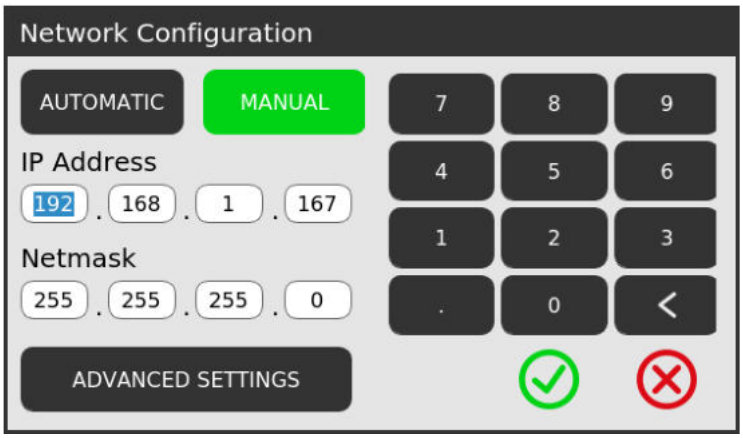
3. **Sampling Rate and Display Configuration** shows the sampling time for singles and coincidences, and which of the channels is displayed on the **Graph** tab.



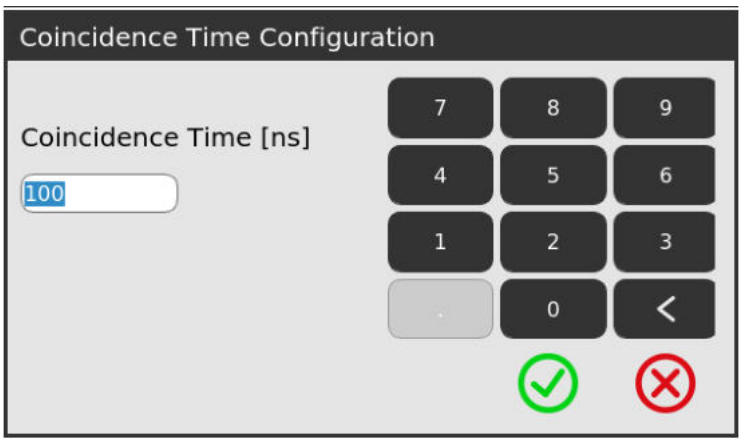
Each set of parameters can be accessed using the green "arrow" buttons to the right  .

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

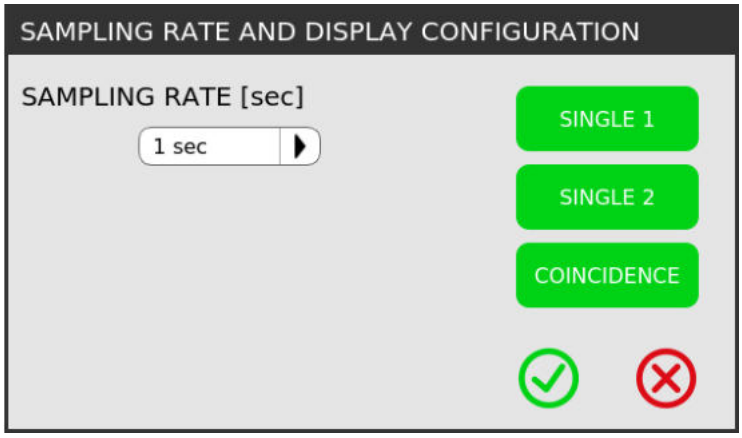
1. **Manual**: the **IP Address** and the **Netmask** are typed in manually.
2. **Automatic**: **DHCP**, the **IP address** is requested from the network.



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



In the field **Sampling Rate and Display Configuration** the sampling time can be set. Longer sampling intervals reduce noise in the resulting data, but are not recommended for studies with bolus tracer injection (the peak concentration is very transient and may be missed with sampling rates longer than the recommended 1 second). Longer sampling intervals may be useful in calibration and quality assurance measurements, described later. Sampling rates of 1, 2, 5 and 10 seconds are available. These rates are mirrored in the PSAMPLE module.



2.5 Experimental Setup

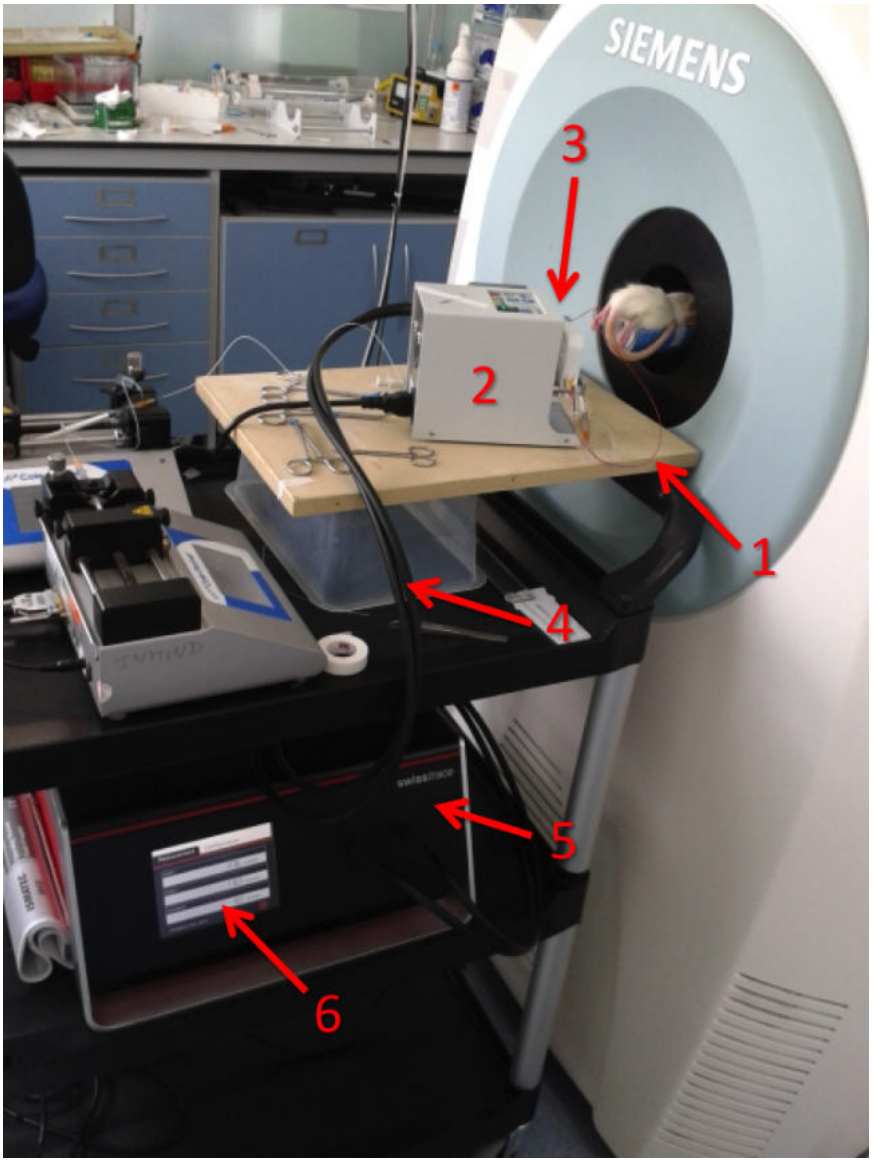
The twilite measures the radioactivity in a catheter which runs between two LYSO/BGO crystals. In animals a very efficient solution is to install a shunt between the femoral artery and the femoral vein. This allows continuous measurement of the whole blood activity without any loss of blood. Such arteriovenous shunts can be placed in animals as small as a mouse, using catheters of an appropriate size (e.g. mouse: PE10, rat: PE50). A typical setup and schematic are shown below.

In humans and larger animals, 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 all cases, swisstrace recommends that the blood flow in the catheter is controlled by a suitable pump. However, in small animals, it is also possible to let the shunt run freely driven only by the arteriovenous pressure difference.

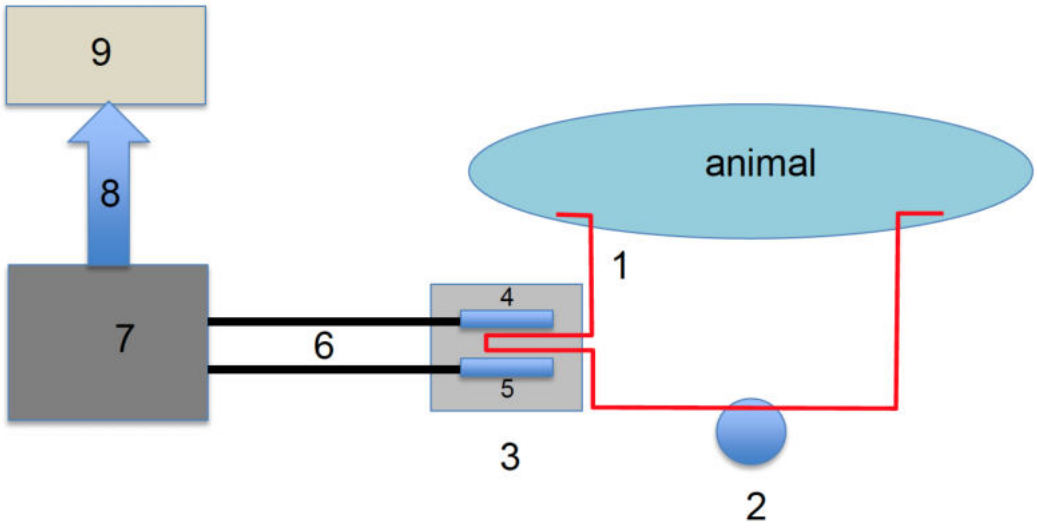
The appropriate catheter diameter differs according to the size of the animals. Swisstrace delivers templates to guide the catheter through the measuring head for each catheter size, such that a well-defined geometry is established. The characteristics of the shunt are described in a paper published 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 2 m; they can be as long as 16 m (8 + 8 m) in MR compatible systems.
5. Base unit with coincidence electronics (twilite two shown).
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 for Rats and Mice



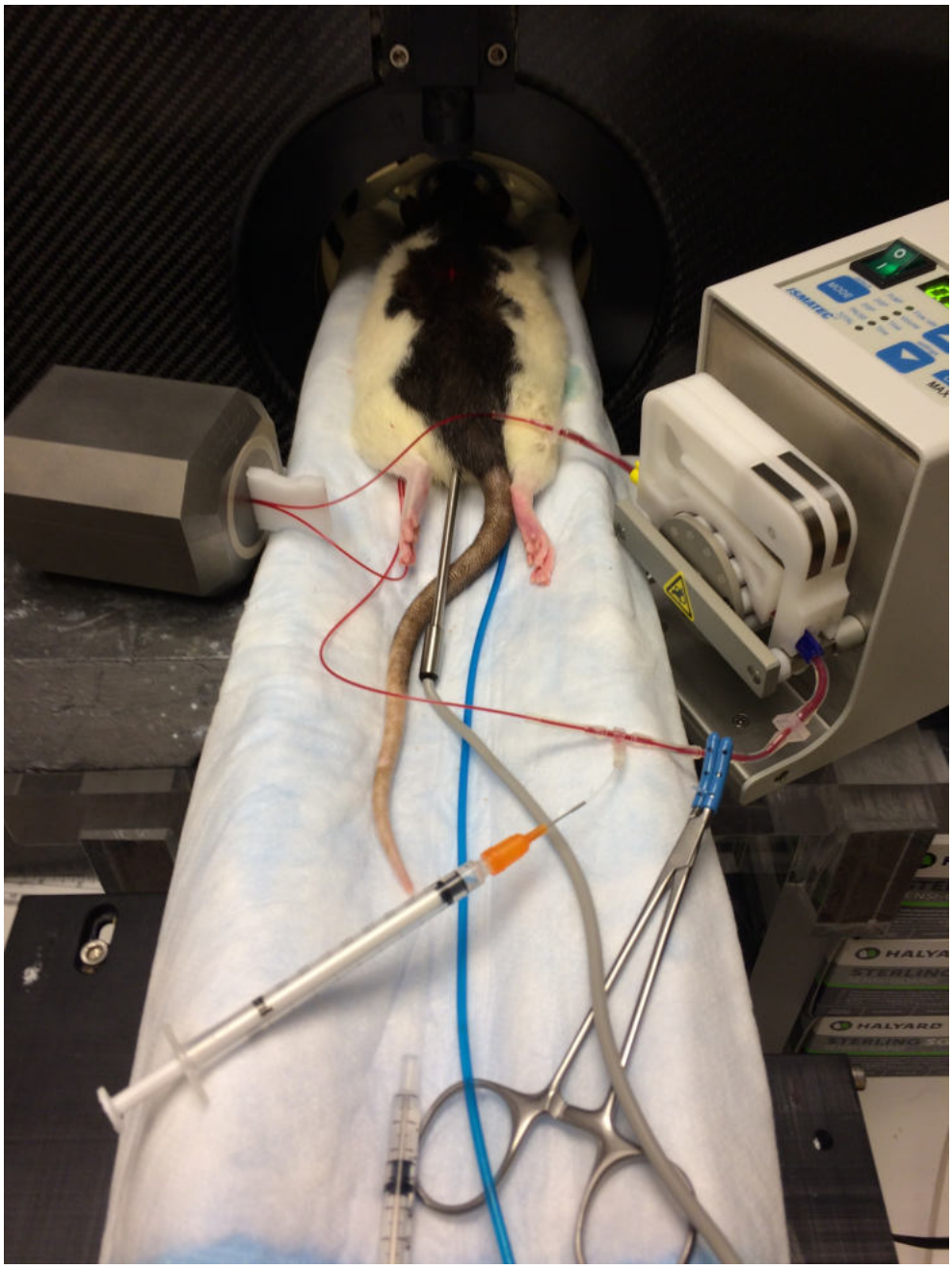
Shunt functions

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 crystal2.
6. Light guides carrying the photons from the crystals to the PMT‘s. These guides have a standard length of 2 m, they can be as long as 16 m (8 + 8 m) in MR compatible systems.
7. Base unit with coincidence electronics.
8. TCP/IP connection to computer with PMOD data acquisition tool PSAMPLE.
9. Computer with PMOD data acquisition and analysis.

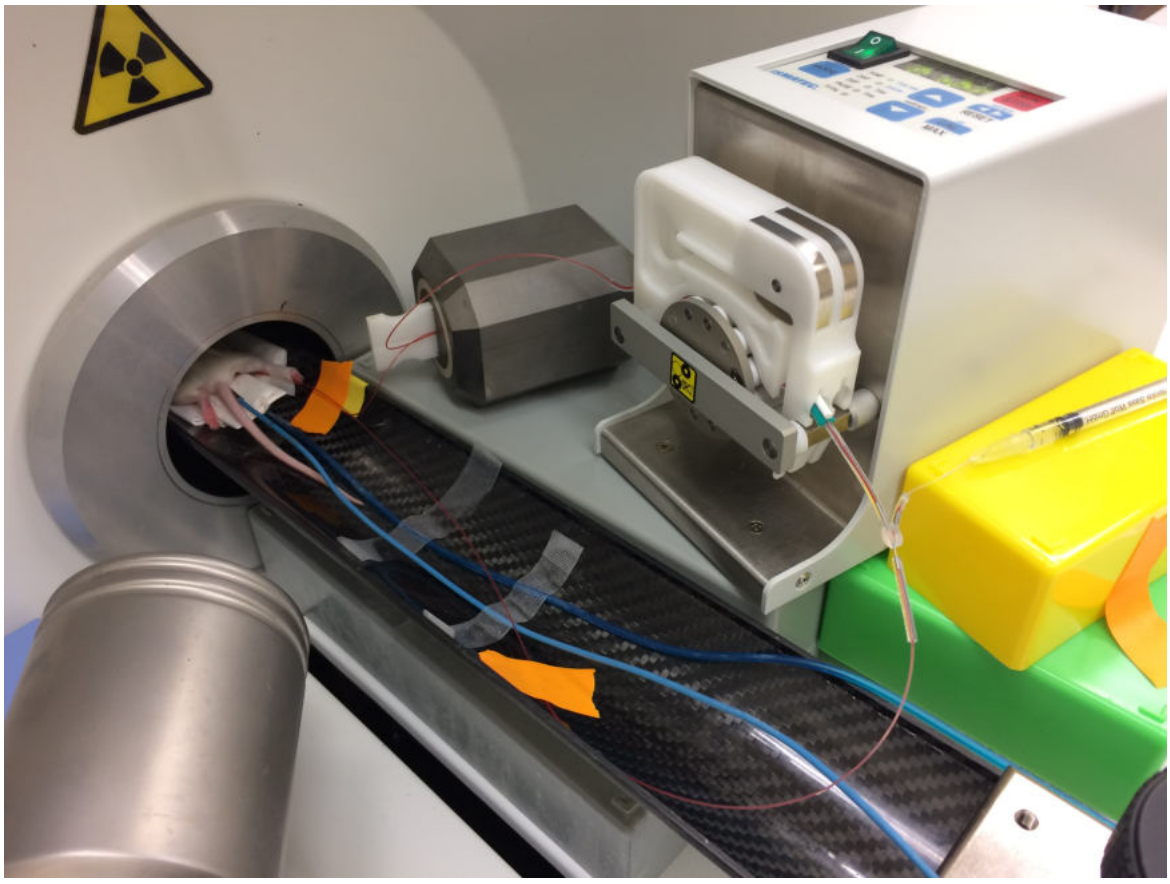
The arteriovenous shunt can serve several additional functions, such as blood pressure monitoring, tracer injection, as well as the collection of blood samples for metabolite analysis. The procedure illustrated below is recommended for collection of blood samples: a small cut is made into the catheter 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.

Additional detail of the experimental setup is seen here:

Rat

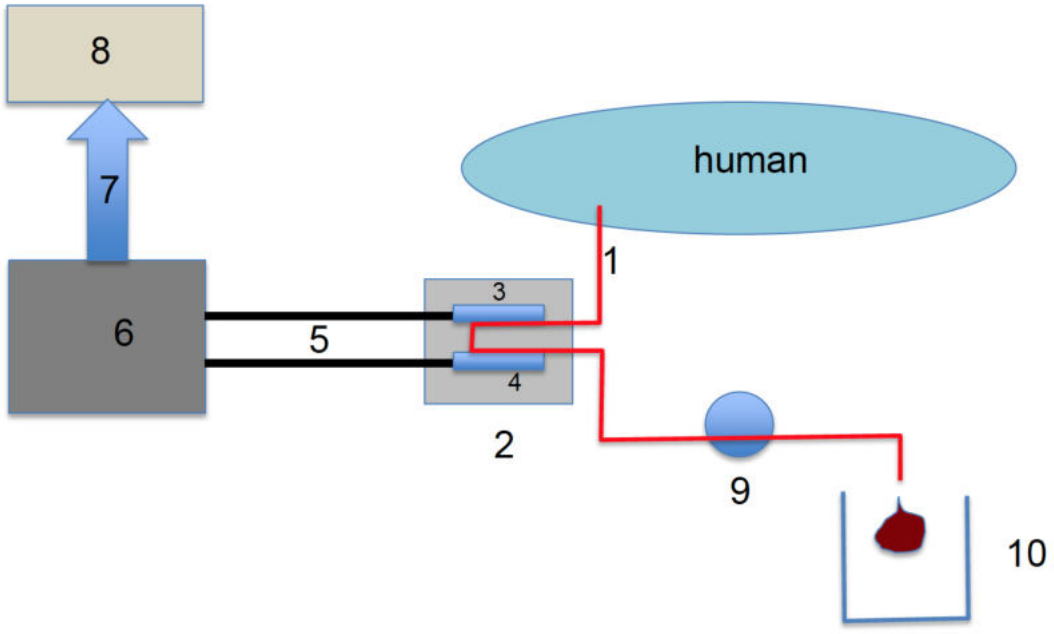


Mouse



Schematic for Humans

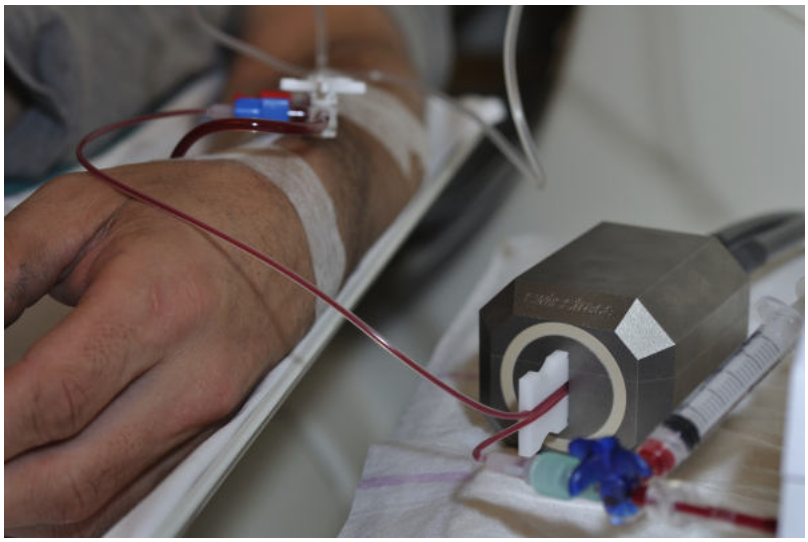
In humans there is no return of arterial blood.



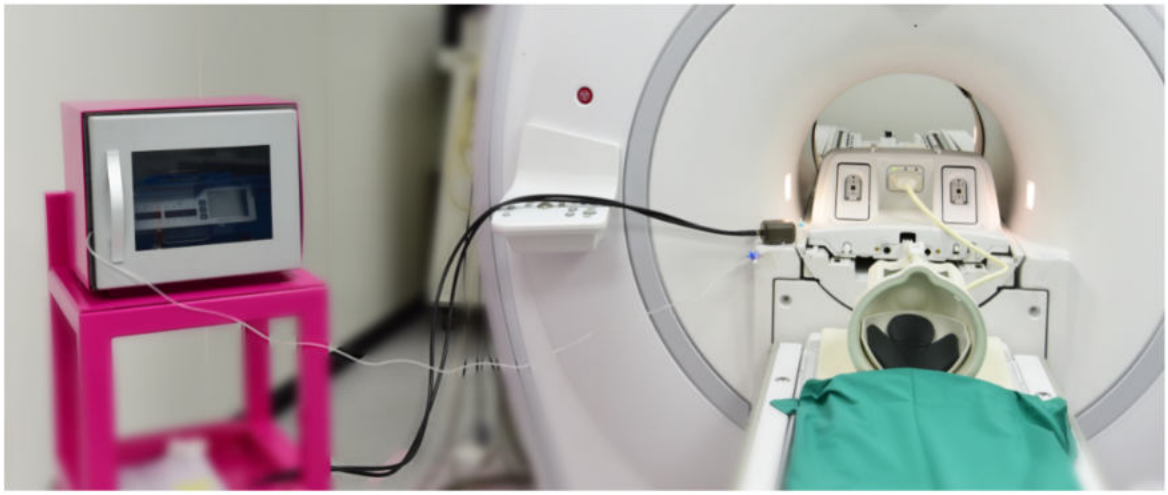
1. Catheter running from the radial artery to the waste container.
2. Twilite measuring head made of tungsten.
3. LYSO crystal 1.

4. LYSO crystal 2.
5. 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 16 m (8 + 8 m) in MR compatible systems.
6. Base unit 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.

A typical configuration for PET/CT is seen here:



And a typical configuration for PET/MR is seen here:




SIGNA
PET/MR

3 PSAMPLE Configuration

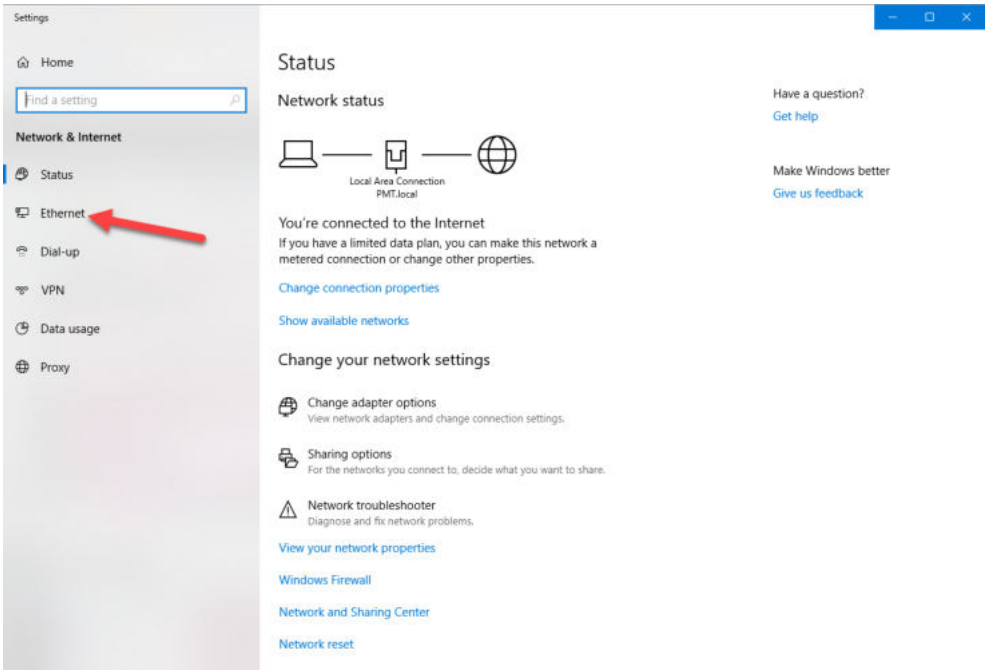
Direct data acquisition is performed with the dedicated PMOD module PSAMPLE via a TCP/IP interface (The twilite is directly connected to the computer via an ethernet cable). Two configuration levels are required, the network configuration and the module configuration.

3.1 Network Configuration

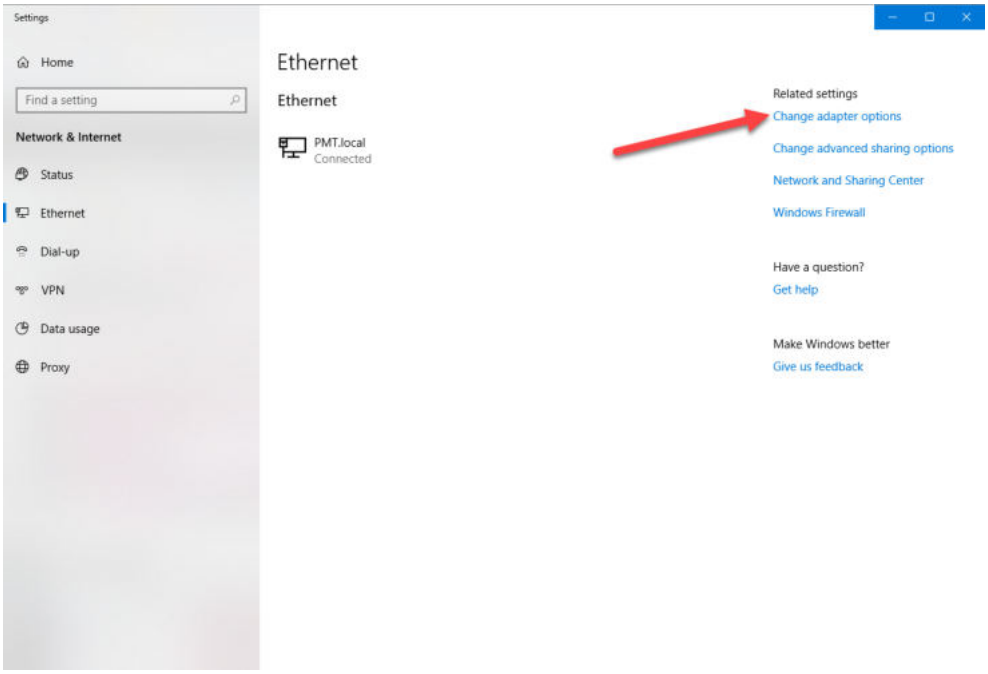
This procedure will normally be completed by swisstrace upon installation of the twilite. These instructions are intended as a guide in the event that the acquisition PC must be replaced.

3.1.1 Windows (Windows 10 illustrated)

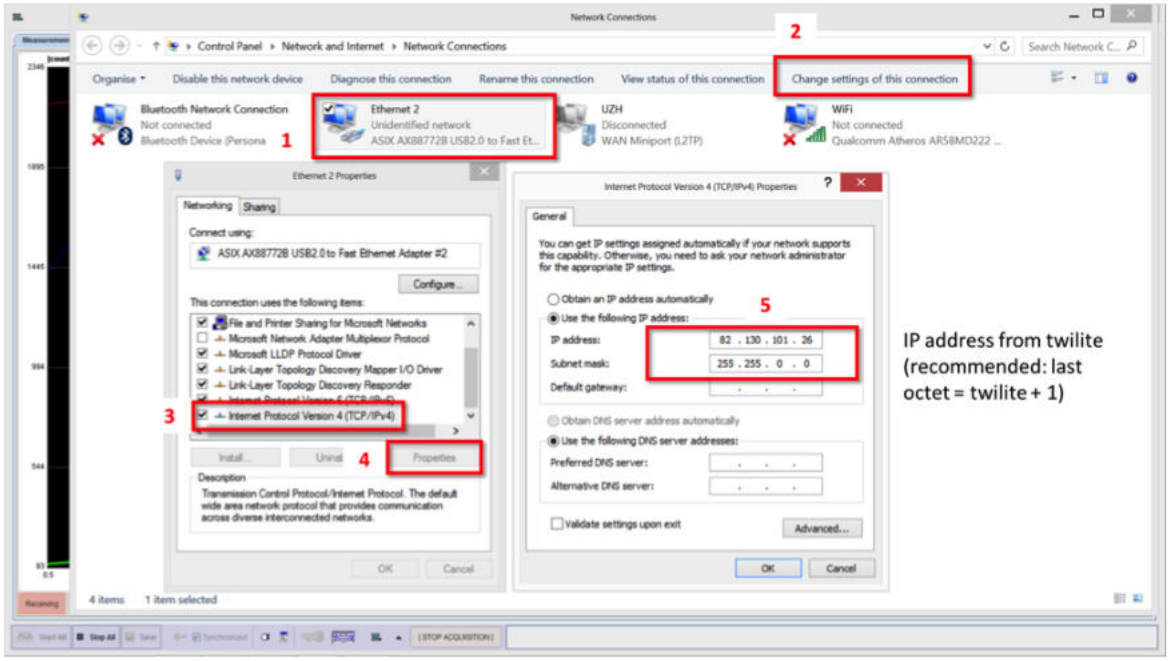
1. Switch on the twilite and connect Ethernet cable to PC
2. Check IP address and subnet of twilite on the touch screen (if DHCP is active, the IP address will be assigned by the network server
3. In Windows, go to Settings > **Network & Internet**



4. i.e. Right click the network/Wi-Fi icon on task bar and select **Open Network & Internet Settings** (or use: Start, Settings, Network & Internet)
5. Select **Ethernet**
6. Select **Change adapter options**



7. Select the active Ethernet/LAN connection (= twilite) (1)
8. Select **Change settings of this connection** (2)
9. Select **Internet protocol version 4** (3)
10. Select **Properties** (4)
11. Select **Use the following IP address:** (5)
12. Enter an **IP address** in the same group as the twilte IP (typically the twilite IP +1 on final section) and subnet mask.
13. OK



3.1.2 MacOS

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 twilte IP (typically the twilite IP +1 on final section) and subnet mask
7. **Apply** the settings.

3.2 PSAMPLE Configuration for direct acquisition

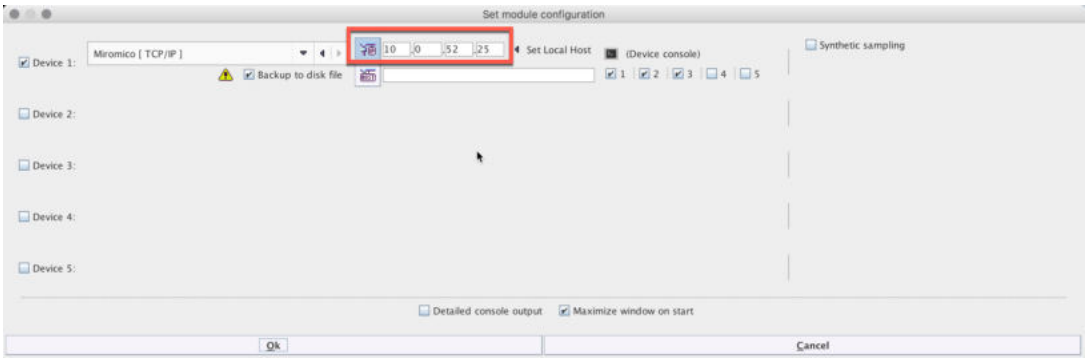
To change the PSAMPLE configuration start it with the button in the PMOD Toolbox and activate the button in the bottom status line. The configuration window is organized as shown below:

Connectivity

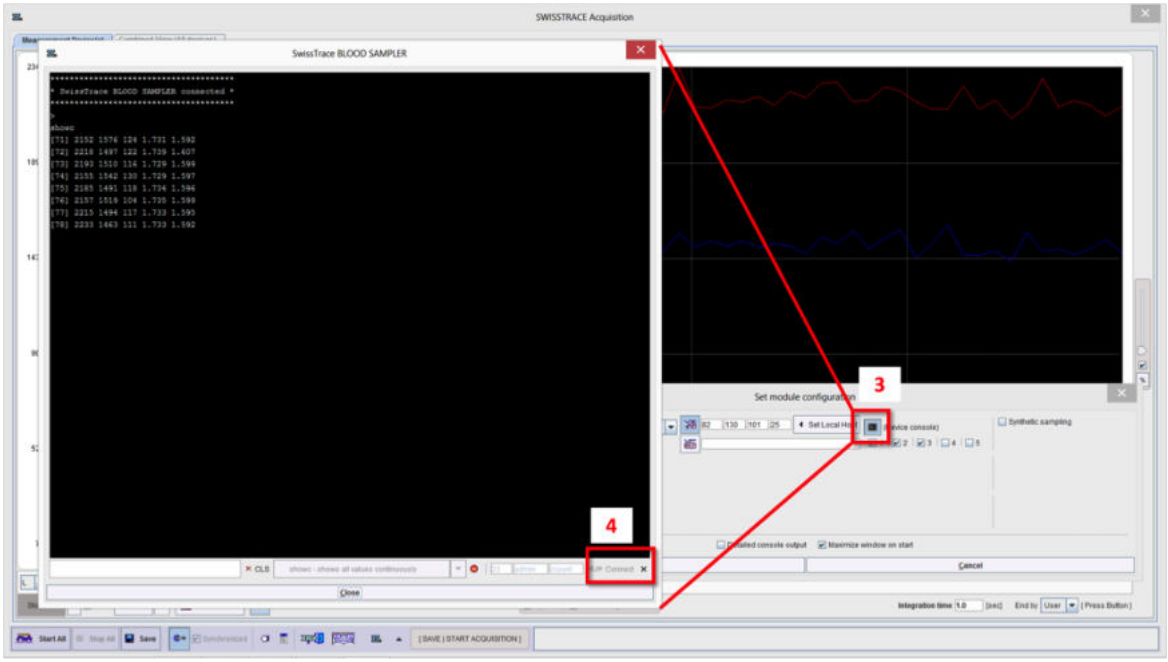
Up to five devices can be configured which can be simultaneously be used during the acquisition. Each device is characterized by an IP address that can be entered manually. For convenience, the **Set Local Host** button retrieves the computers IP address, which can then be adjusted to match the twilite 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 .

For PSAMPLE direct data acquisition with the twilite three (or for the twilite two), the **Miromico [TCP/IP]** communication protocol must be selected from the leftmost drop-down menu. *Please note that data acquisition from the twilite one is no longer supported from PMOD 4.3 onwards (the setting PerkinElmer MP-RS232 is blocked and marked as deprecated).*

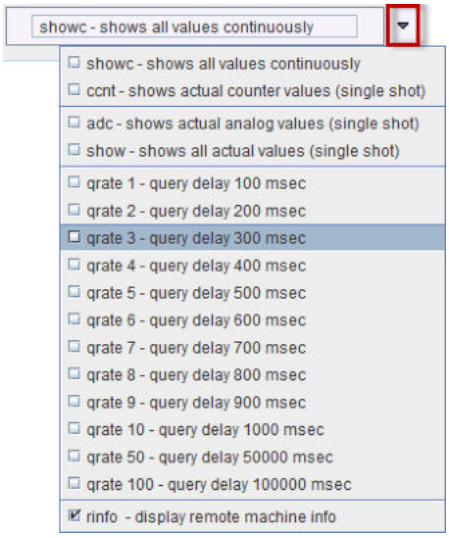
"Backup to disk file" is activated by default, and is recommended. The backup file location can be viewed by hovering the mouse cursor over the checkbox. Three back up files are generated for each twilite acquisition, for each singles channel, and for the coincidences. Data from the twilite is continuously appended to these files.



The connectivity can be tested by clicking 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 base unit.



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, and are now deprecated (analog inputs were only available on the twilite one, and are not available on twilite two or twilite three). We recommend using the default settings

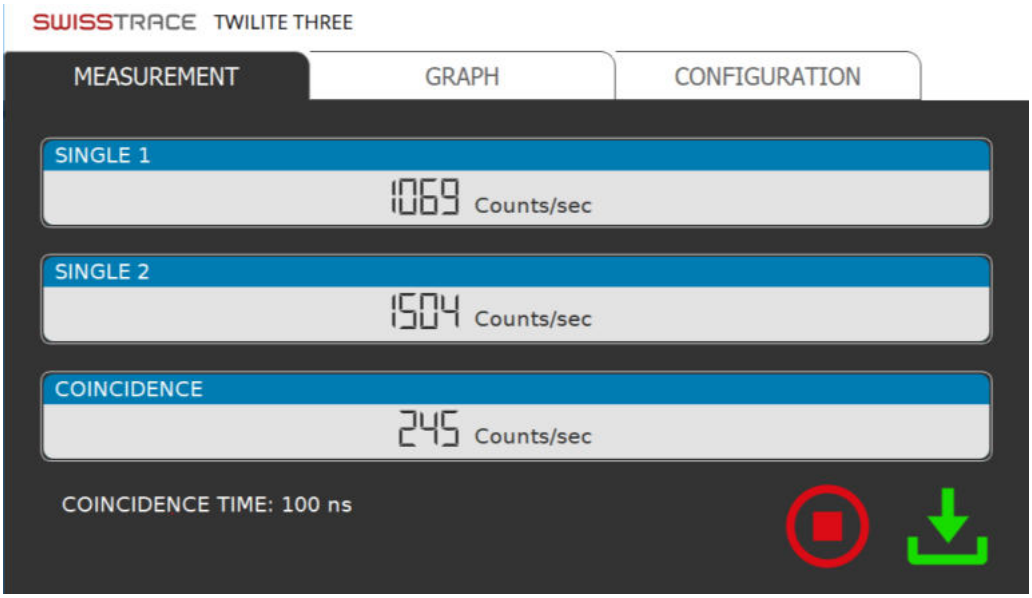
During the acquisition, the measured data is stored in a buffer. As a security option, it can additionally be written in real-time to the disk. This behavior is enabled with the **Backup to disk file** box**.** For each channel, a text file with the date, time and the measurements information is saved. The backup location is in the installation path: *Pmod4.3/data/pmdbase* 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. However, this may slow the acquisition down.

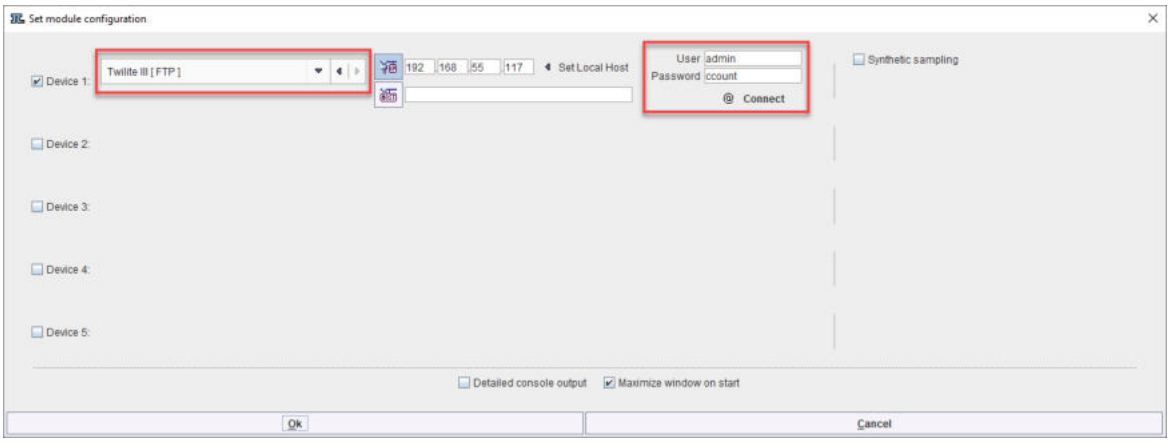
3.3 PSAMPLE FTP data retrieval

In addition to direct data acquisition to a computer running PSAMPLE, the twilite three allows internal data storage. Data stored internally is then transferred via FTP to a computer connected later (direct or via network).

Internal data collection is started/stopped using the "save" button  on the touch screen:

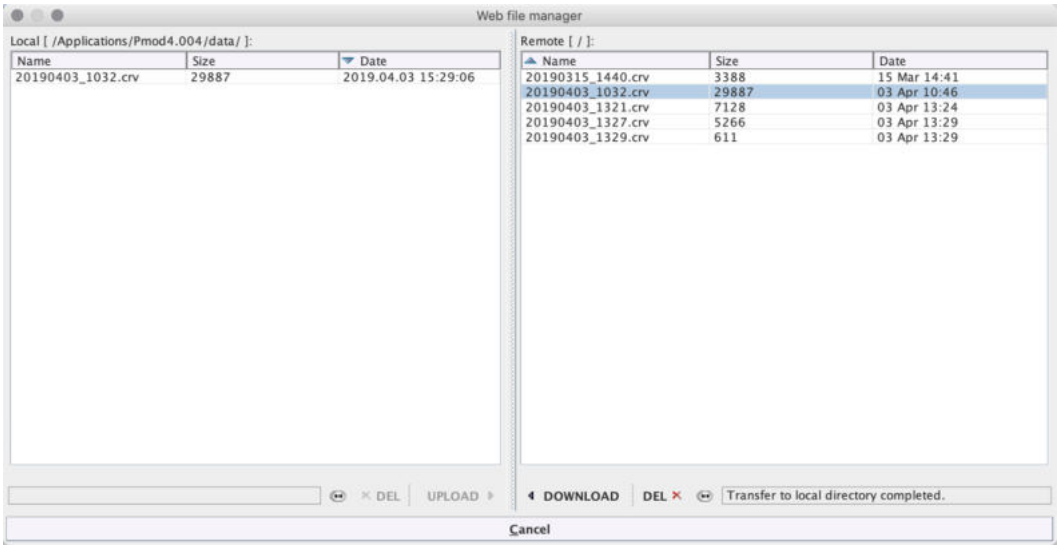


Once data has been saved internally, an FTP connection to the twilite three should be established. PSAMPLE includes an FTP interface for this dedicated purpose. It is activated in the **Configuration**:



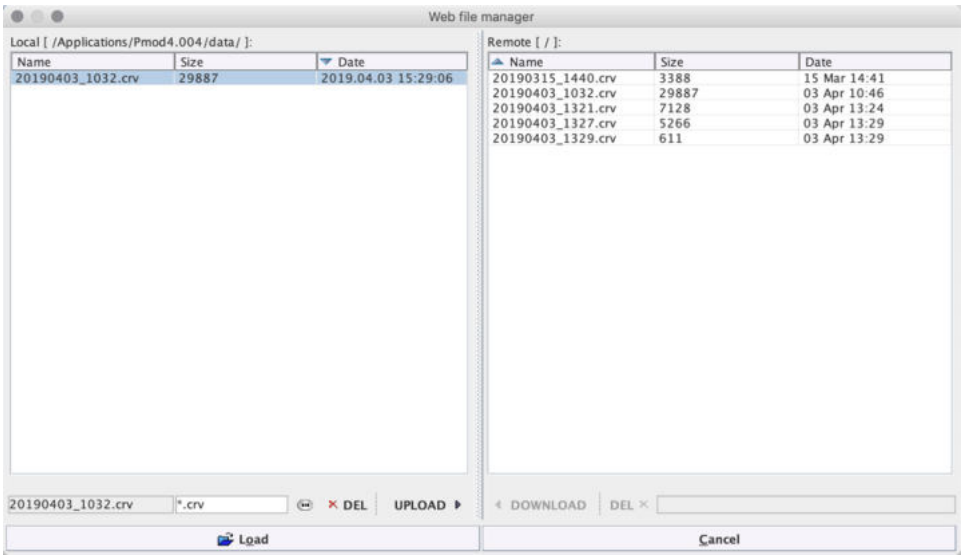
The "**Twilite III [FTP]**" mode should be selected from the Device drop-down menu. The IP address of the twilite must be entered in the IP field. Take care not to alter the User and Password fields.

The "**@ Connect**" button opens the **Web file manager** window:

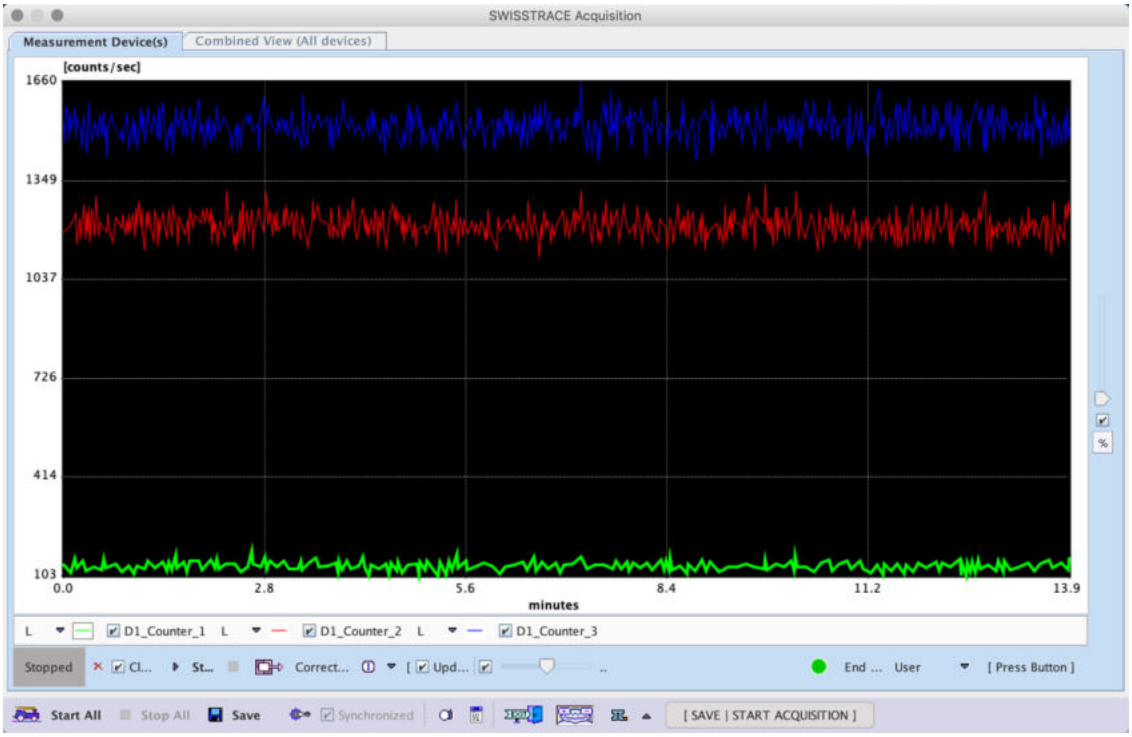


Data files on the twilite are seen on the right side (Remote). Files can be selected and copied to a local folder (PMOD data path shown in top-left) using the "**< Download**" button. These files may then be directly opened in the **PSAMPLE Correction** module, as described later in this document.

Once the "**Twilite III [FTP]**" mode has been selected in the **Configuration**, the behaviour of the **Start All** button changes. Upon *left-click*, the **Web file manager** dialog opens:



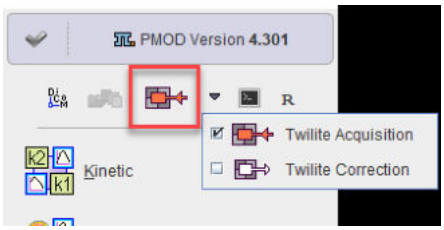
Data files can be transferred from the twilite to local data folder as described above, then a local data file should be selected and opened using the **Load** button. The data is displayed as though it had been recorded in the **PSAMPLE Acquisition** module:



4 Data Acquisition via Acquisition Computer

Start by switching on the twilite system using the **ON/OFF** switch on the rear of the base unit. 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 twilite has been configured 22 appropriately.)

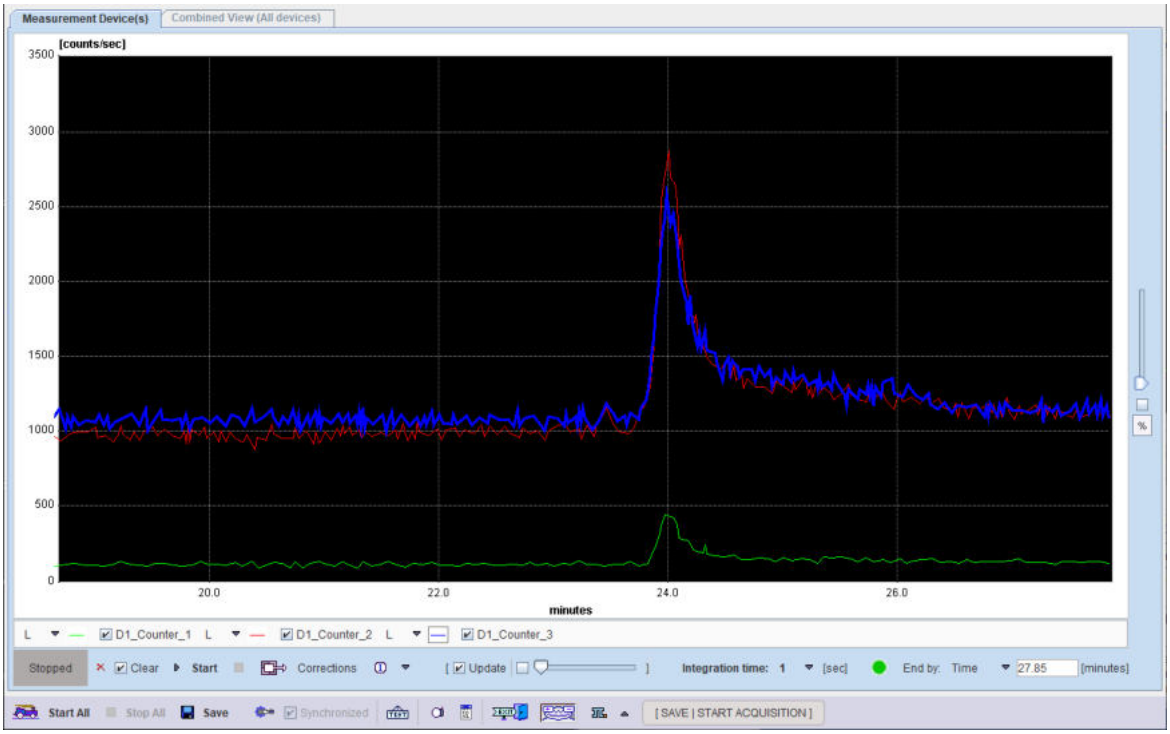
In a simultaneous experiment with a PET acquisition please make sure that the system clocks of the acquisition computer and the PET system are synchronized before starting PMOD (PSAMPLE time stamps each data point, using the system time as reference). In order to acquire background data for automatic background subtraction during AIF correction, swisstrace recommends starting the twilite at least 20 sec before tracer injection.

4.1 Acquisition Interface

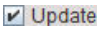
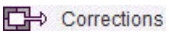
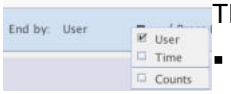
The acquisition interface is organized in two panels as shown below: the **Measurement Device(s)** page and **Combined View (All devices)** page. Note that the configuration is stored upon program exit and restored after the next start.

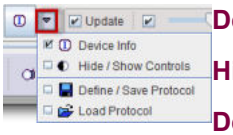
4.1.1 Measurement View

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 to access the configuration 19 panel.
	Normalization: Scales all curves relatively with a maximum of 1.
	Autoscale: If enabled, the program continually adjusts the curve display ranges (both 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 to calculate the count rate. Default is a 1 sec integration time, the minimum. The maximum Integration time is 10sec. Note that rebinning to longer intervals can be performed later 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. I.e. upon the re-start of an experiment the acquisition will overwrite the existing measurements. 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. ▪ **Counts**: the user can define a threshold after which the measurement is automatically stopped.



Device Info: Shows the device configuration.

Hide/Show Controls: Allows the curve control elements to be shown or hidden.

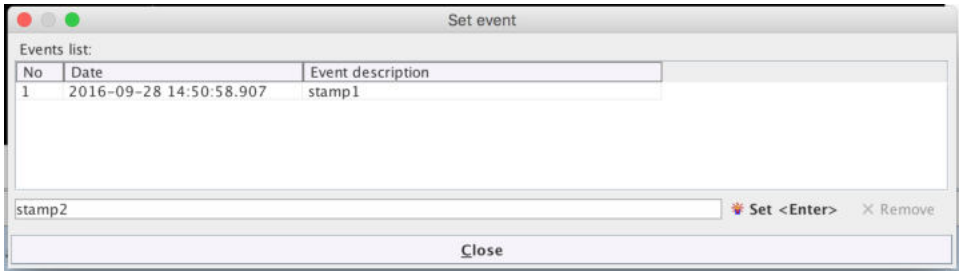
Define/Save Protocol: For the definition of an acquisition protocol.

Load Protocol: To retrieve an acquisition protocol.

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

Launches the Acceptance Test. This tests connectivity with the twilite.

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 5).

4.1.2 Combined View

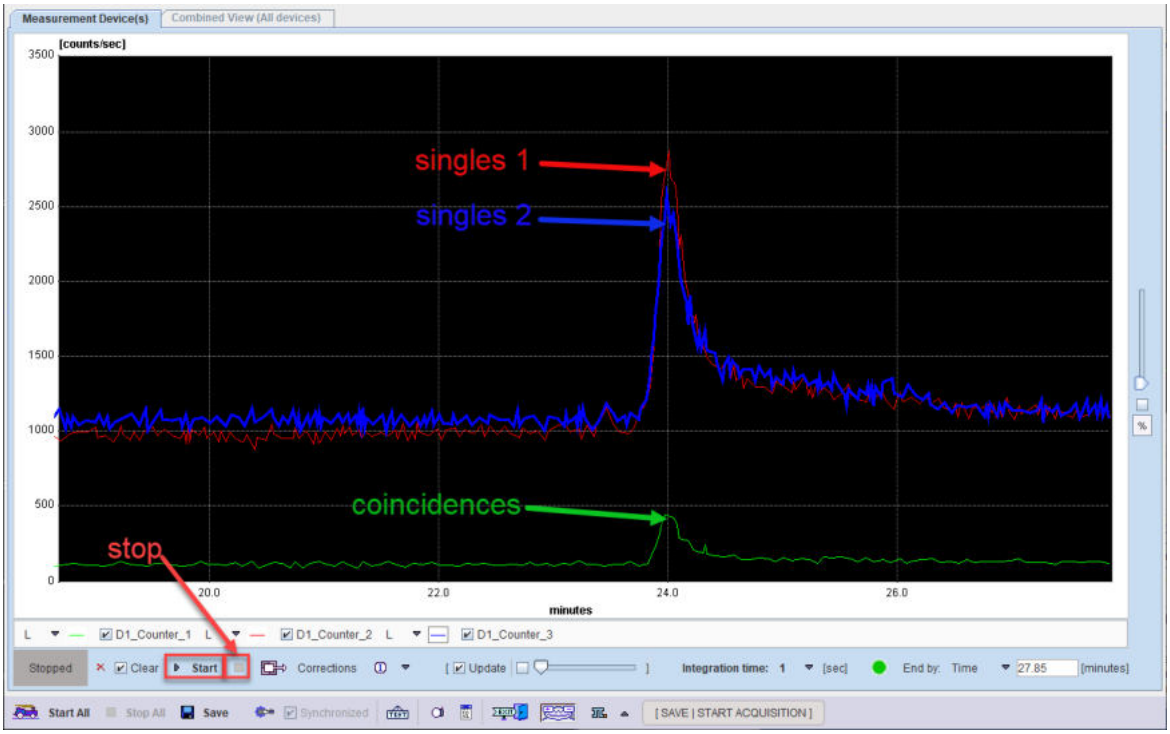
The **Combined View (All devices)** page is only active, when multiple devices are configured. It shows the acquired data of all devices. The curves of interest can be enabled for display, normalized to their own maximum and shown as percent values. This mode is helpful for comparing shapes when the 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.

4.2 Data Acquisition

4.2.1 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

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 channels. 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.

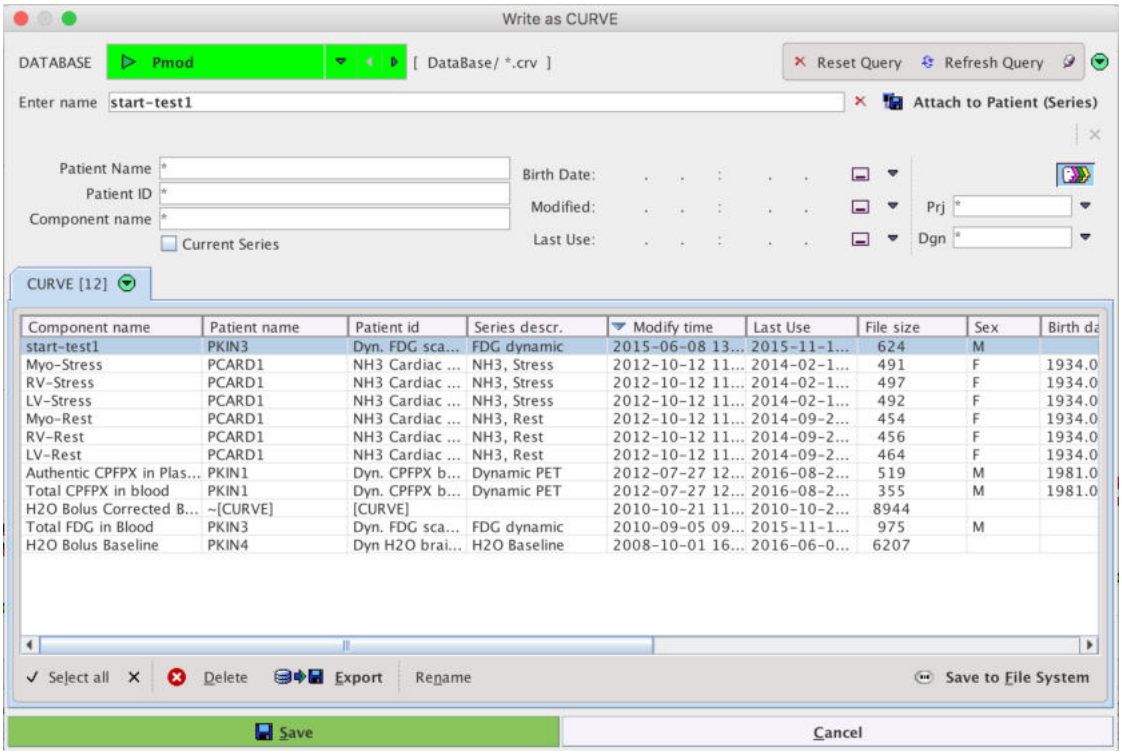
The acquisition will run until the end of the defined protocol, unless the stop button is activated. Note that **Start All** and **Stop All** can also be used for single device acquisition.

Multiple Devices Acquisition

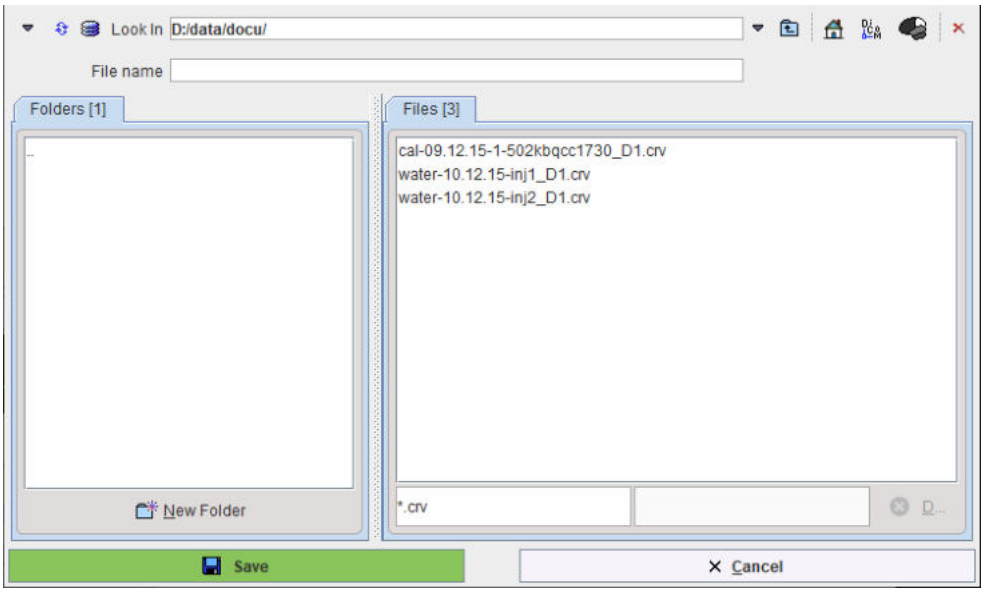
If multiple devices are controlled by the same instance of PSAMPLE, a curve display and control area is shown for each device in a row layout. The acquisition can be started individually for each device with the corresponding **START** button. Alternatively, all acquisitions can be started at once with **Start All**. The results of a multiple device acquisition can most conveniently be inspected in the **Combined View (All devices)** page. 29

4.2.2 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

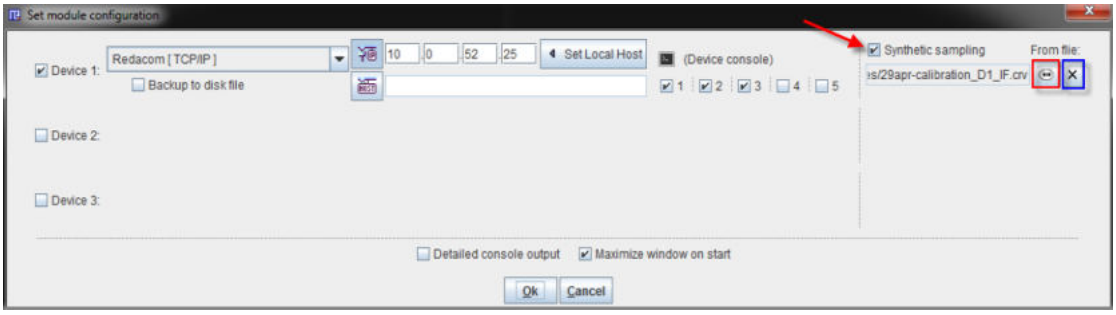
In case of multiple device acquisition, the save process is similar to that described above. The activation of **Save** button will generate a text file for each device. During the saving procedure, the software appends to the file name the suffix $\_D_i$, where *i* can be 1, 2, 3, 4 or 5.

4.2.3 Transfer to the Correction Interface

Although saving of the raw data is recommended, the acquired coincidences can also directly be transferred to the correction 36 interface with the Corrections button.

4.3 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 using the file browser.



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. To return to the normal acquisition mode, please return to the configuration and switch **Synthetic sampling** off.

5 Twilite Calibration

In experiments using the twilite and a PET system it is necessary to cross-calibrate the two devices. This implies the derivation of a calibration factor for the twilite that converts the coincidence count rate for a solution of known activity concentration to the kBq/cc activity concentration measured for the same solution by the PET. Note that this additionally implies that the PET has been calibrated to return kBq/cc voxel values, typically using the manufacturer's recommended procedures and corrections (e.g. normalization of detector efficiency, system dead time correction, correction for random coincidences, scatter and attenuation correction).

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 typically 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). Information about the branching ratio is available from Turku PET Center.
   Note*: Branching ratio correction is available in the PSAMPLE Correction tool.*
2. A catheter identical to the type used in the actual experiment is filled with the tracer solution from the phantom. *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. 3) A suitable phantom is filled with the same (well-mixed) fluid. Complete mixing should be carefully ensured. For PET/CT a suitable phantom is a 250 or 500 ml plastic drinks bottle. This is readily mixed and can be well sealed. Large air bubbles should be avoided. For small animal PET scanners a 20 ml syringe, 20 or 50 ml Falcon tube may alternatively be used. For PET/MR it may be necessary to use a larger phantom for which the mu-map for attenuation correction is available. In this case the activity concentration may be reduced (lower limit 50 kBq/cc), and care should be taken that the total activity in the scanner field-of-view does not exceed the limit for noise-equivalent count rate (NECR). In all cases it is necessary to confirm that the tracer does not stick to the plastic walls of the phantom, as this will lead to incorrect determination of the activity concentration in solution.
4. After ensuring that the system clocks of the twilite acquisition computer and PET scanner are synchronized, twilite acquisition is started 27 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/decay correction to match the scan start time.
7. Calibration processing is performed in the **PSAMPLE Correction** tool as outlined below 34. The activity concentration measured from the PET image of the phantom and PET scan start time (DICOM Acquisition Time) are required. For example, these parameters can be measured/taken from another PMOD module. The calibration factor is finally calculated by diving 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 (with LYSO detector head) has an intrinsic number of background counts due to the radioactivity in the LYSO scintillators. This background has to be subtracted from all measurements. 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.
- Continual 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 offset between the PET and the twilite calibration measurements.

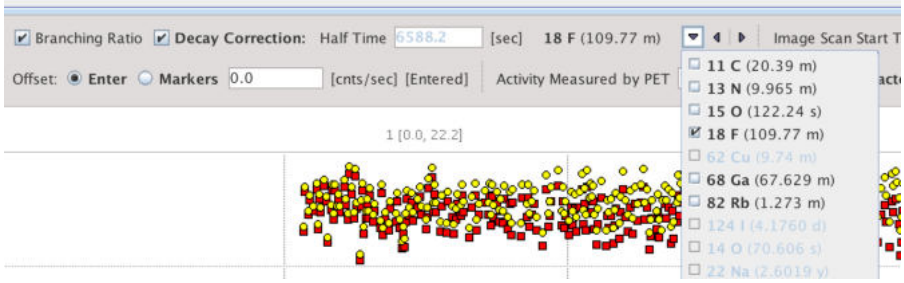
Recommendation: Although the calibration factor is stable over time, it is recommended that calibration be performed regularly as part of a quality control procedure.

5.1 Calibration Processing

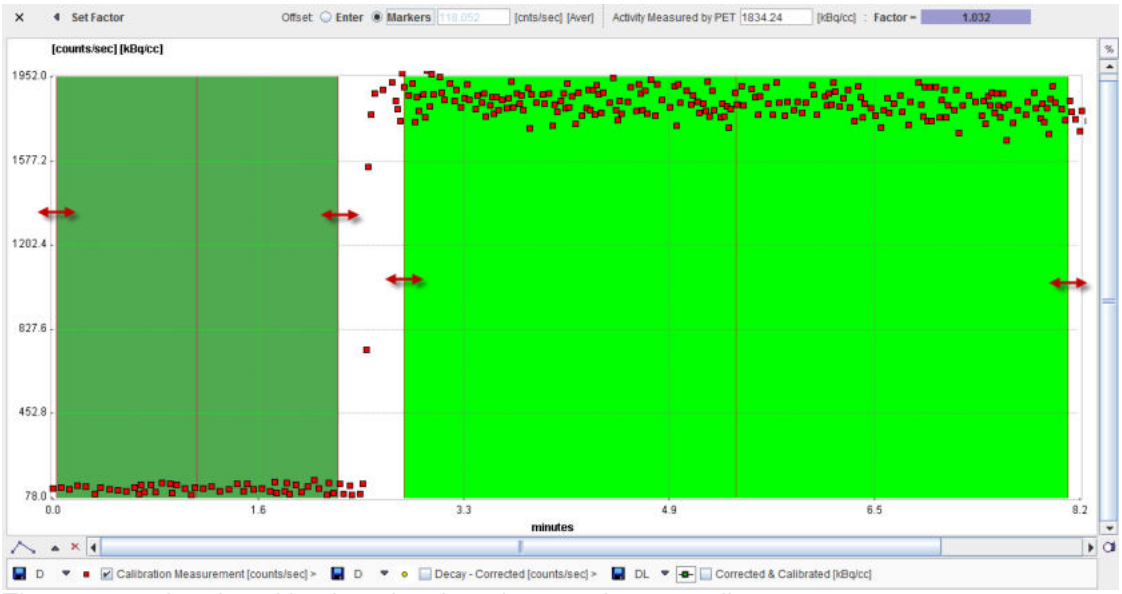
To calculate the calibration factor start the (PSAMPLE) **Twilite Correction** module from the PMOD ToolBox.

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** and **Branching Ratio** correction (at present only available for selected isotopes) and select the appropriate isotope from the selection list.



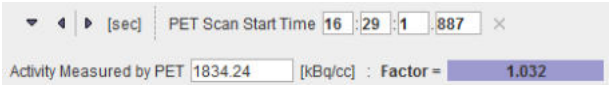
4. The decay correction is applied relative to the start of the twilite acquisition and the corrected data shown as **Decay-corrected [counds/sec]** with yellow dots. **Branching Ratio** correction is a division of the counts (minus background, once defined) by the branching ratio.
5. There are two methods 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]**; red squares), whereas the light green area defines the portion of signal from the activity to be averaged (calculated from **Decay-corrected [counts/sec]**; 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, and the calibration configuration saved using the **Save Calibration Defaults** button.

6 Correction/Calibration of twilite Measurements

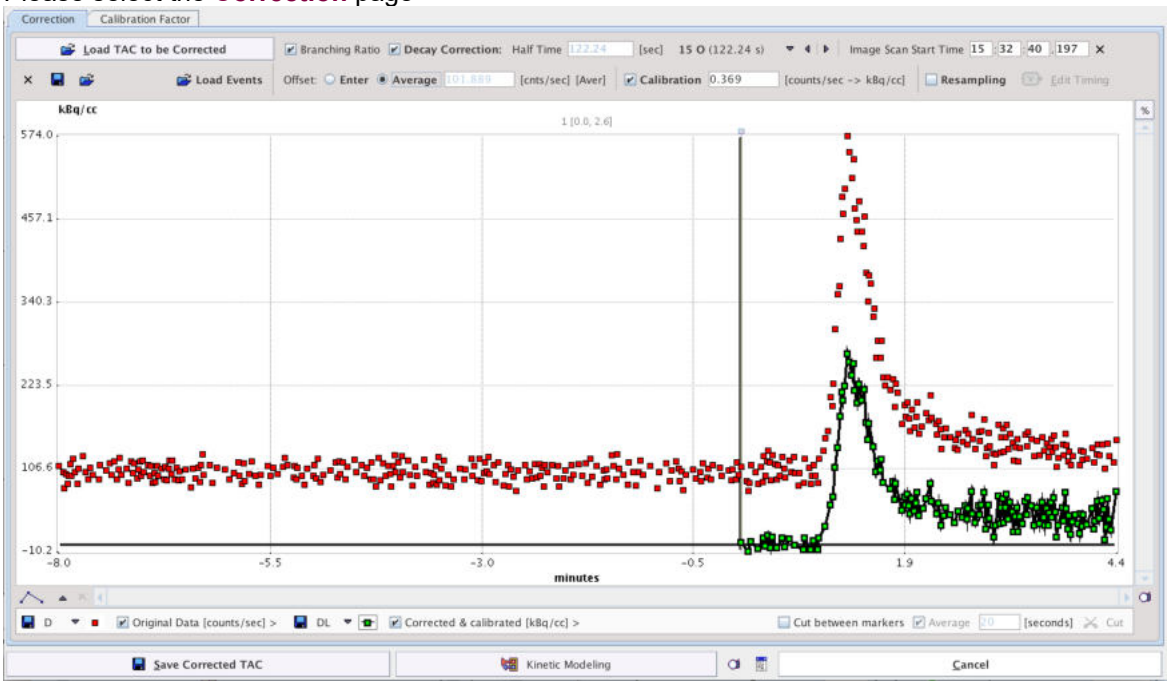
Raw data acquired with the twilite (and LYSO detector head) represents a combination of background counts from the LYSO crystals, constant (with noise) at all activities, and counts (coincidences) resulting from decay of activity in the whole blood within the catheter. The number of counts from blood 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 twilite 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 (`DICOM Acquisition Time`).
3. Correction for the isotope branching ratio.
4. Multiplication with the calibration factor.

6.1 Correction and Calibration Procedure

Correction and calibration of twilite raw data is performed using the PSAMPLE **TwiliteCorrection** module from the PMOD ToolBox.

Please select the **Correction** page



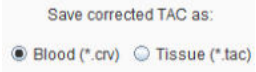
and proceed as follows:

1. Load the raw data of the twilite 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 twilite 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 twilite sampling and the true PET start time (i.e. the twilite 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.

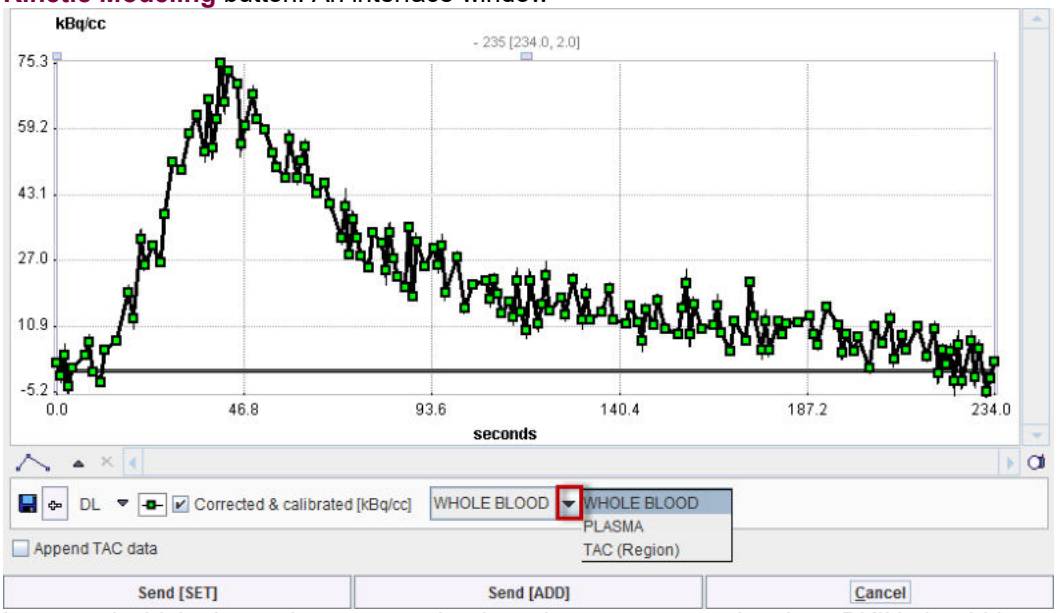
With this setting, all samples before time 0 are averaged, with the assumption that this is background signal only. The background average is shown in gray text and is subtracted from the **Original Data** before decay correction. If the twilite was not started before the PET acquisition, or the background 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 twilite calibration) should then be entered manually.

5. Activate the **Calibration** factor. Typically this factor has been established during the last calibration procedure 34, 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.



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



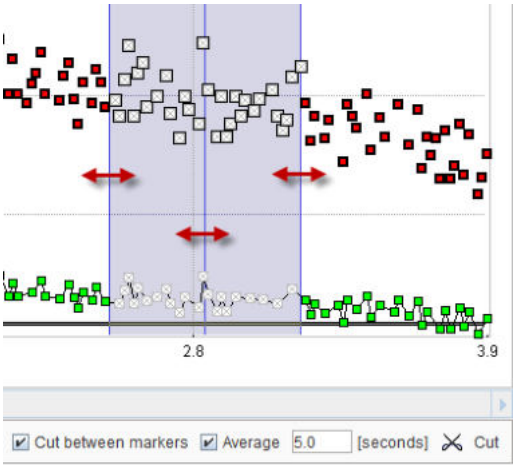
is opened which shows the curve and a drop-down menu to select how PKIN should interpret the data: as **WHOLE BLOOD**, **PLASMA** or **TAC**. Usually, **WHOLE BLOOD** should be selected. **Send [SET]** will overwrite data of the selected workspace in PKIN, whereas **Send [ADD]**, will add a new PKIN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

6.1.1 Cutting and Resampling

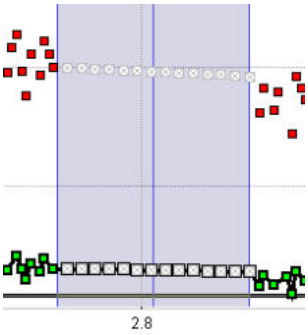
The **Correction** window offers two functions for editing of the **Corrected & calibrated** curve: removal of data segments, and data resampling.

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.



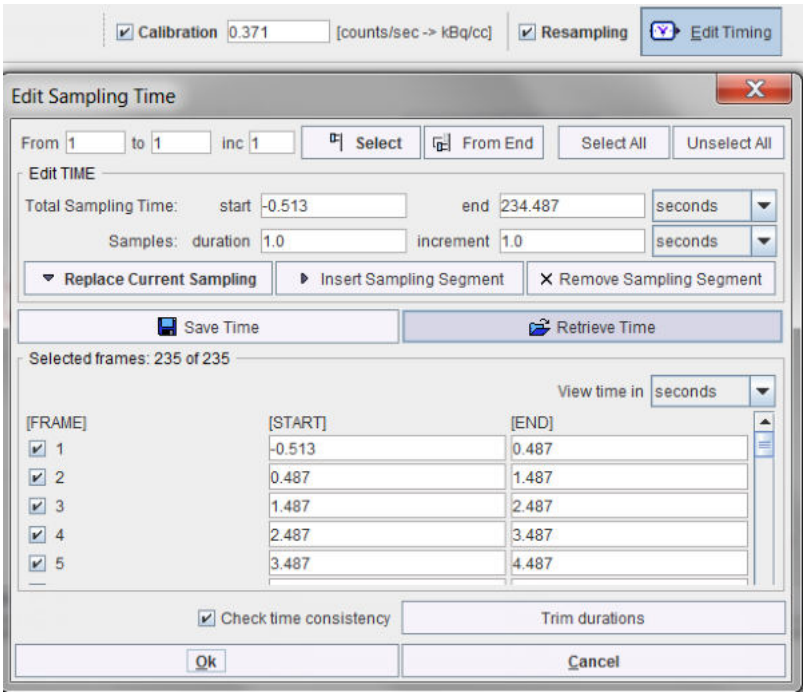
When the **Cut** button is activated, the samples covered by the cutting area are replaced by samples linearly interpolated between the left and right area boundaries as illustrated below.



As the immediate boundary samples are influenced by noise, averaging of a number of samples close to the boundaries can be enabled by the he **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 twilite 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 authentic parent correction ratios can be applied.

6.2 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 function (AIF) in kinetic modeling. In principle, the AIF 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 (authentic parent) is determined by correcting plasma activity for radiolabeled metabolites.

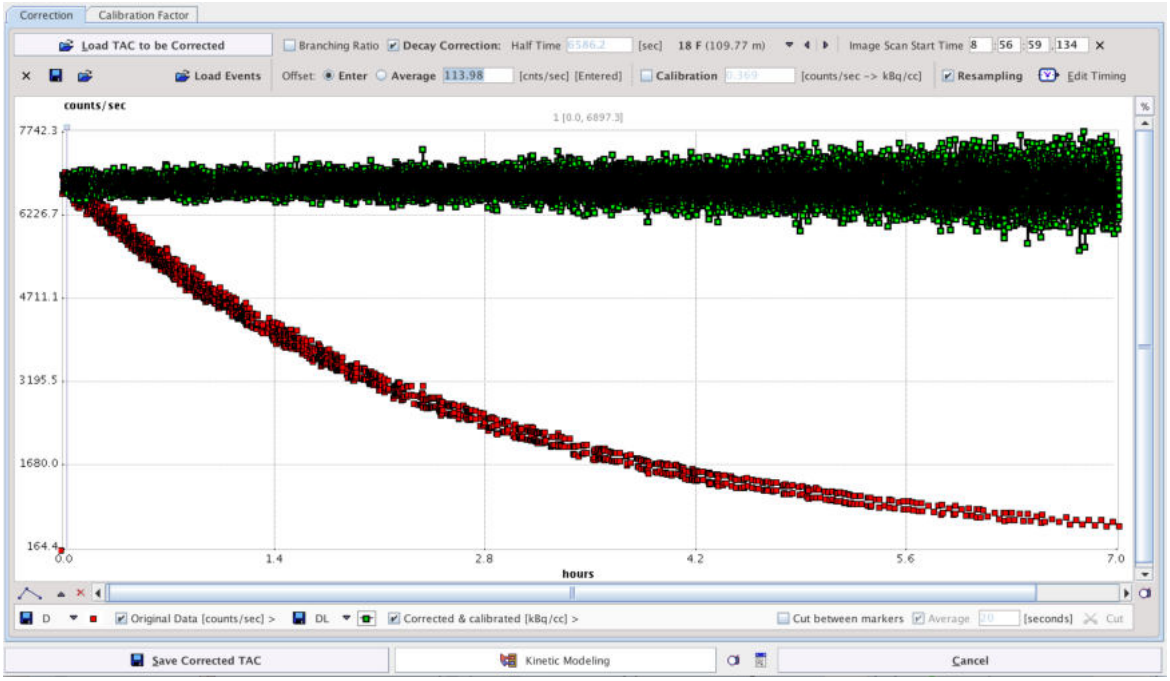
These corrections require knowledge of the plasma/whole-blood and the parent/metabolite concentration ratios during the experiment, which can be acquired by taking blood aliquots for analysis (i.e. centrifugation to separate plasma, high-performance liquid chromatography or cartridge metabolite separation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

7 Quality Assurance

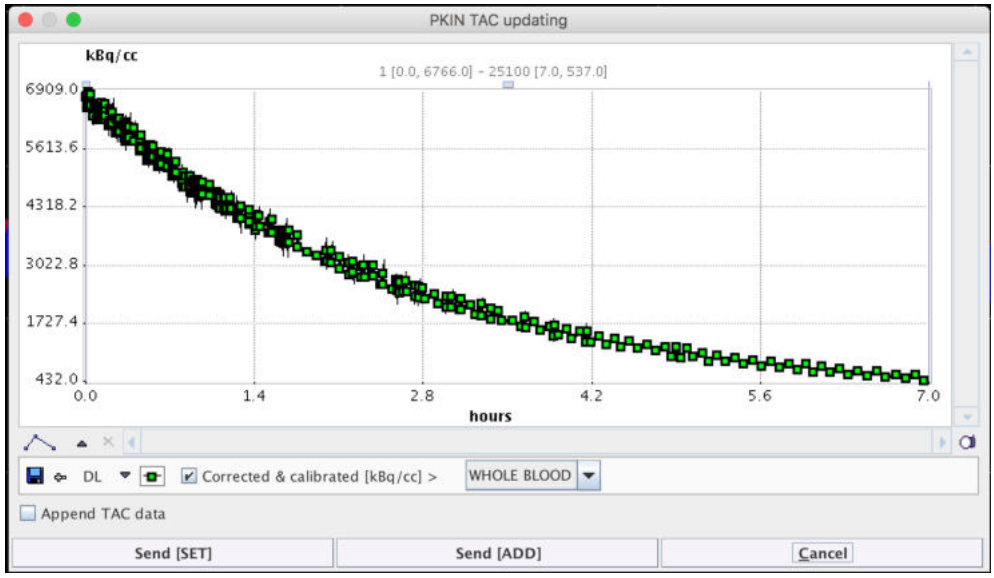
Further to calibration (measurement of sensitivity), the function of the twilite can be tested using a long (e.g. multiple of 3 to 5 times the tracer half-life) acquisition of a fixed activity solution (e.g. 5 MBq/ml for 0.58 mm ID catheter as used in rats, 2 MBq/ml for 1 mm ID as typically used in humans).

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.

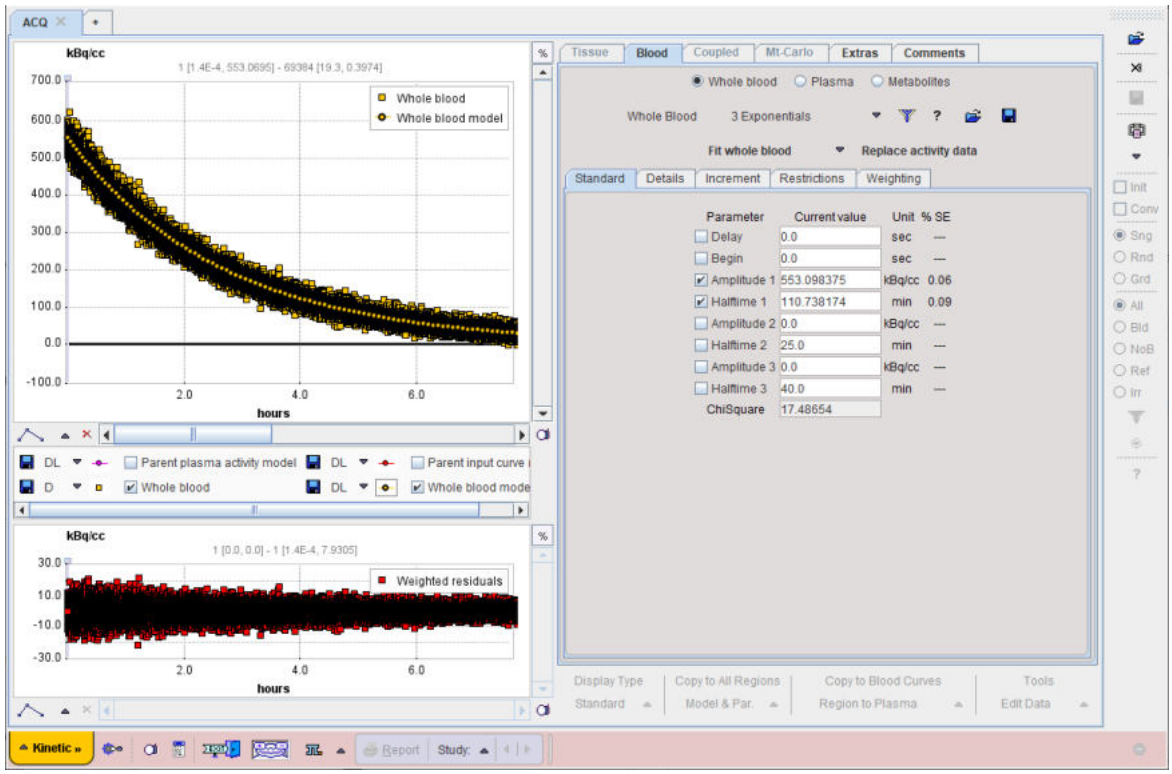


Additionally, a single exponential function can be fitted to the non-decay corrected data using the PKIN tool (if licensed). The half time of the exponential should match the isotope half-life (in minutes).

Turn off **Decay Correction**, and use the **Kinetic Modeling** 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:

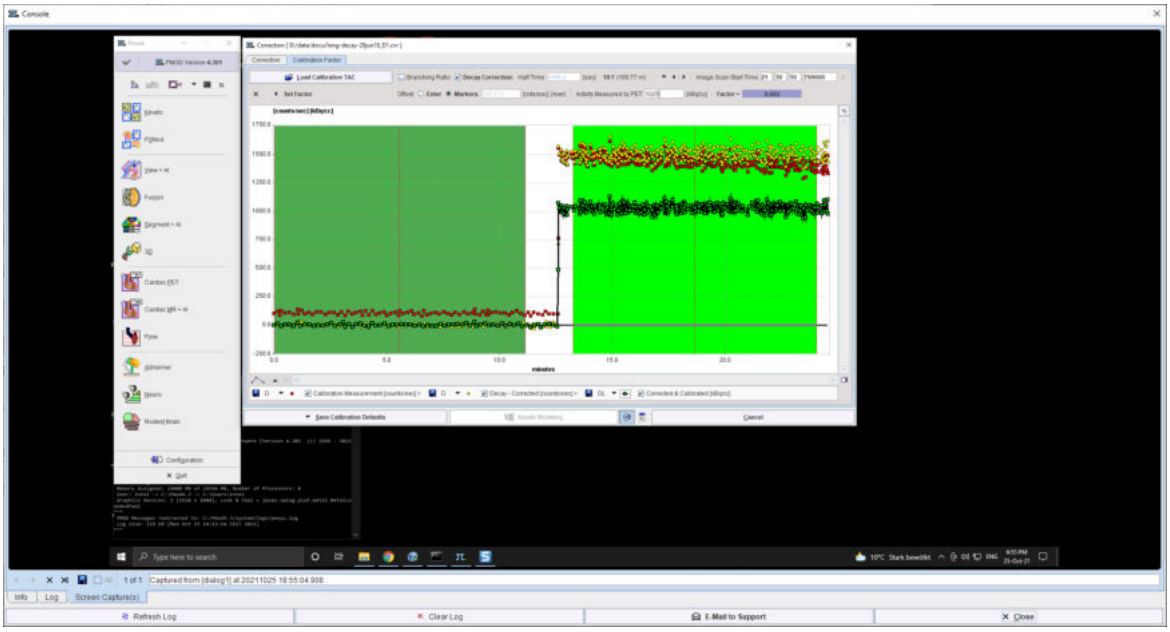
1. Inaccurate definition of the background in **Offset: Enter**. Determine the background counts from a calibration or simple background measurement shortly before the long decay acquisition.

2. Excessive starting activity (the twilite has deviation measured vs. expected counts < 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.

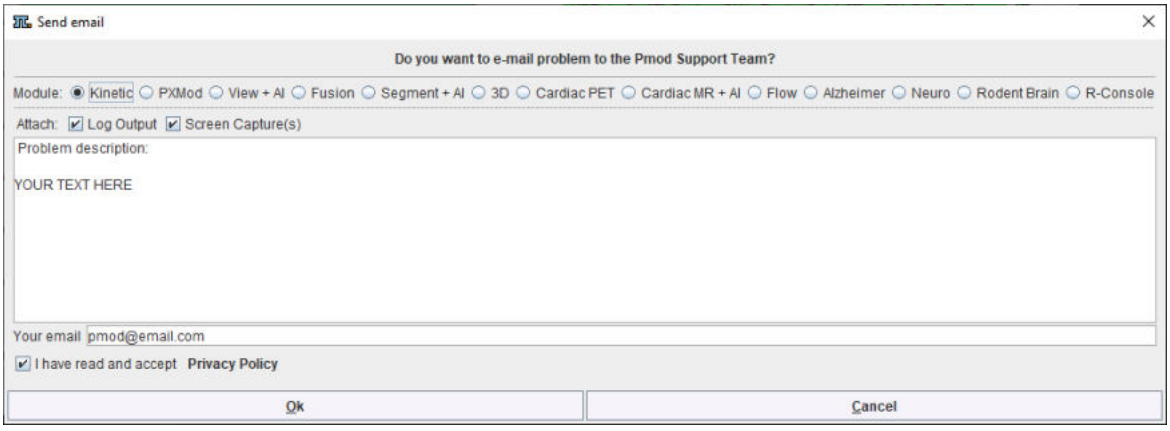
8 Problem Reporting

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,



in which the user can select the **Module** affected, 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/institutional 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.

9 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.
2. Alf MF, Wyss MT, Buck A, Weber B, Schibli R, Krämer SD. Quantification of brain glucose metabolism by 18F-FDG PET with real-time arterial and image-derived input function in mice. J Nucl Med. 2013; 54(1):132-8.
3. Bolcaen J, Lybaert K, Moerman L, Descamps B, Deblaere K, Boterberg T, Kalala JP, Van den Broecke C, De Vos F, Vanhove C, Goethals I. Kinetic Modeling and Graphical Analysis of 18F-Fluoromethylcholine (FCho), 18F-Fluoroethyltyrosine (FET) and 18FFluorodeoxyglucose (FDG) PET for the Fiscrimination between High-Grade Glioma and Radiation Necrosis in Rats. PLoS One. 2016 Aug 25;11(8):e0161845.
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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