User Manual

SpectroscoPy

SPECTROSCOPy



User Manual – Version v2.7.1
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1 General Information

1.1 Introduction

SpectroscoPy is a Python application for real-time acquisition, analysis, and visualization of single-photon count intensity as a function of time. It interfaces with the FLIM Data Acquisition Card to retrieve acquisition data and display intensity traces either in a graphical user interface (GUI) or in console mode.

1.2 Scope of the Manual

This manual covers installation, connection, basic operation, parameter configuration, data export, and error handling to support correct use of the SpectroscoPy software with compatible FLIM LABS hardware. For further information, refer to the manual of the FLIM Data Acquisition Card (https://www.flimlabs.com/products/data-acquisition-card/).



This manual describes the software **SpectroscoPy version 2.5**. Other software versions may differ in functionality and appearance. If this document does not correspond to the version of the software in use, refer to the official website at https://www.flimlabs.com/products/spectroscopy to obtain the appropriate version of the software or the corresponding manual.

1.3 User Qualification

This software is intended for qualified laboratory personnel and researchers familiar with data acquisition hardware and software. It assumes a basic understanding of photon counting and acquisition parameters. For introductory material on these topics, refer to the learning resources available on our website (www.flimlabs.com).

1.4 Developer Information

FLIM LABS makes the source code of SpectroscoPy publicly available on GitHub to support transparency and collaboration with the research community:

https://github.com/flim-labs/spectroscopy-py

You can explore the code, report issues, suggest features, and propose improvements through the repository. This collaborative approach helps adapt the software to specific research needs. For customized acquisition workflows, analysis tools, or integration with your experimental setup, contact FLIM LABS to discuss your requirements and available development support.

2 Installation

2.1 System Requirements

The following specifications are required for running SpectroscoPy on a PC:

- PC with multicore CPU
- 4 GB RAM
- USB interface for connecting the DAQ to the PC
- Windows 10 or later

Additionally, Python version 3.9 or later is required for advanced features such as console-mode acquisition and analysis scripts. For further details, see Chapter 6.

2.2 Software Installation

SpectroscoPy can be downloaded from the website: https://www.flimlabs.com/spectroscopy/.
If multiple versions are available, select the version that provides the required functionality. To install the software, proceed as follows:

1. Download the selected version. The file is provided as a Windows installer package.
2. Double-click the installer and follow the on-screen instructions.

The windows installer already includes the ZestSC3 drivers required for the correct operation of the FLIM Data Acquisition Card.

2.3 Hardware Connection

Using the software for data acquisition requires the FLIM Data Acquisition Card. Connect the device as follows:

- Connect the FLIM LABS card to the acquisition PC.
- Verify that the card is correctly recognized by the operating system.
- Connect input, trigger, and reference signals as required for your application.

For detailed instructions on hardware connections, refer to the user manual for the data acquisition card available on the website: https://www.flimlabs.com/products/data-acquisition-card/.

3 Software Overview

SpectroscoPy enables visualization and analysis of fluorescence intensity decay data as a function of time acquired with the FLIM Data Acquisition Card. The software provides a user-friendly graphical interface to access core functions and configure acquisition and analysis parameters.

3.1 User Interface

Figure 3.1 shows the graphical user interface (GUI) of the software. The GUI provides real-time visualization of photon intensity and access to all acquisition parameters and controls.

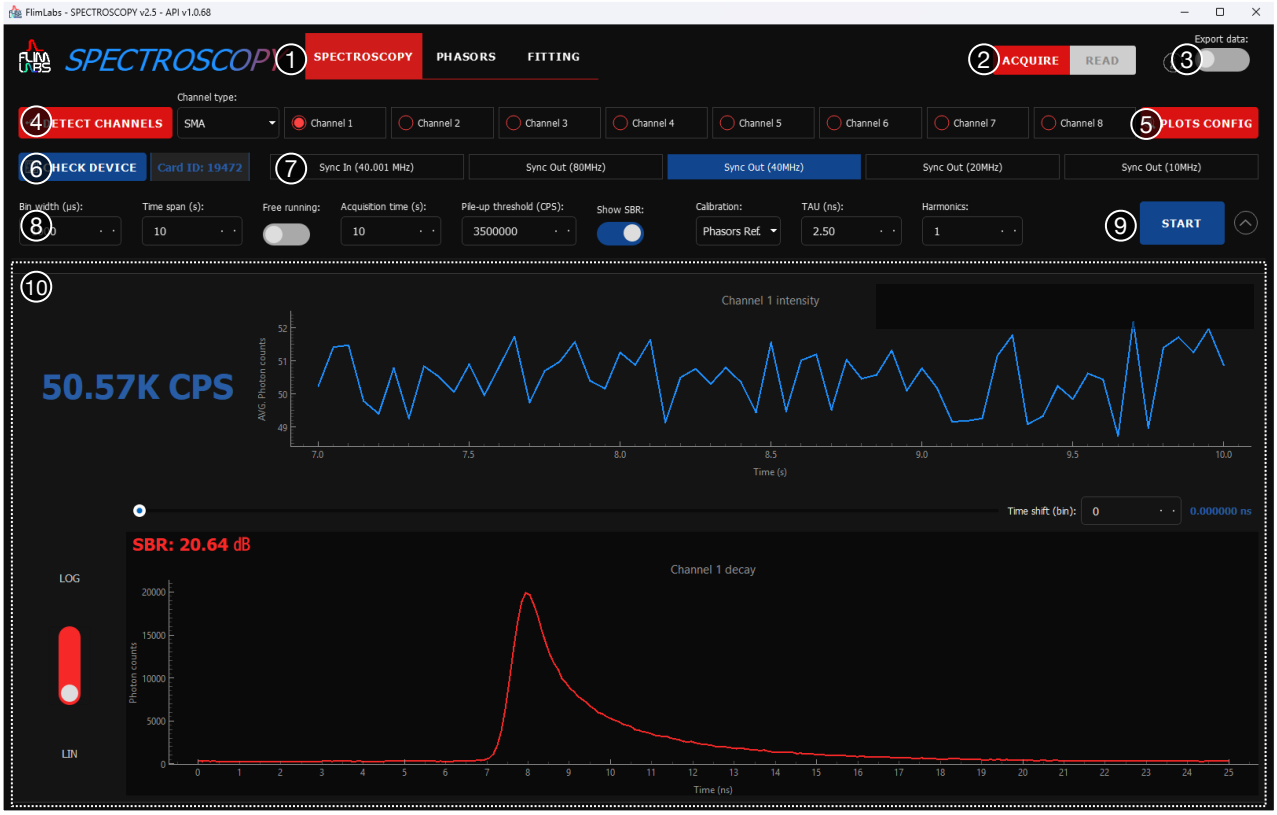


Figure 3.1: Graphical user interface of the software in *Spectroscopy Mode*.

Key elements of the GUI include:

1. Selection of one of three *data analysis modes* (see Section 3.2)
2. Operation mode selection: ACQUIRE / READ
3. Data export and time tagger activation: *Export data:*
4. Channel detection and activation: DETECT CHANNELS
5. Plot configuration: PLOTS CONFIG
6. Automatic device detection: CHECK DEVICE
7. Laser trigger settings
8. Acquisition and display control parameters
9. Start/stop control of the data acquisition: START
10. Data display for the selected channels

The parameters and data graphs shown in the window depend on the selected *data analysis mode*.

3.2 Data Analysis Modes

SpectroscoPy provides three display modes for data visualization and analysis. These modes can be selected using the buttons SPECTROSCOPY , PHASORS , and FITTING at the top of the GUI.
Upon startup, the software defaults to *Spectroscopy Mode*. Selecting a different mode updates the available parameters and the displayed graphs accordingly.
The following modes are available:

Spectroscopy Mode	Real-time visualization of photon intensity and decay profiles.
Phasors Mode	Visualization of fluorescence lifetime data using phasor plots.
Fitting Mode	Model-based fitting of decay curves to extract characteristic parameters.

More detailed information about each mode is provided in Chapter 5.

3.3 Operation Modes

The software can be used in two operation modes:

1. Acquisition of new data from a connected FLIM Data Acquisition Card
2. Analysis of previously acquired data loaded from a file

To switch between acquisition and data reading, use the ACQUIRE or READ buttons in the GUI.

4 Operation

4.1 Startup Procedure

Before starting the software, follow the instructions in the FLIM Data Acquisition Card manual to ensure proper device connection.
The standard workflow is listed below:

1. Connect the DAQ card to the PC
2. Connect all signal and trigger cables
3. Start the software
4. Verify that the DAQ device is recognized by the software.
5. Select active acquisition channels
6. Configure acquisition settings
7. Start acquisition

Further information about each step is described in the following sections.

4.1.1 Device Detection

The software automatically detects connected devices on startup. If the device is not detected, click CHECK DEVICE . Available devices will be listed, and the identifier of the connected device is displayed next to the button.

4.2 Configuration

4.2.1 Channel Configuration

Before starting acquisition, select the active channels and the connection type (USB or SMA). Up to 8 channels can be enabled.
Channels can be configured manually or detected automatically by clicking DETECT CHANNELS . Figure 4.1 shows the detection dialog.
Detected settings can be applied by clicking UPDATE SETTINGS , or the detection process can be repeated clicking RETRY . Close the dialog to return to the main GUI.
To assign a descriptive name to a channel, click its name to open the rename window, then enter a label such as the sample or filter name. The software remembers this name when it is restarted and includes it in the exported file, making channels easier to identify during later analysis. The channel name does not change the physical input associated with the channel; the selected channel number must still match the connected input on the DAQ card.
After completing the configuration of channels and laser synchronization, those panels can be collapsed by clicking on the up-arrow next to the start button to create more space on the display for data and acquisition parameters .



Only four channels can be displayed simultaneously. All channels are still recorded during data export. See Section 4.4 for display configuration.

4.2.2 Laser Synchronization

Laser synchronization defines the timing reference for photon detection and is essential for accurate lifetime measurements.
Two modes are available:

- **Sync In:** Uses an external synchronization signal from the laser
- **Sync Out:** Uses an internal reference signal from the acquisition system

Sync In (External Synchronization)

External synchronization is activated using the button SYNC IN . The system automatically measures the input signal frequency and reports it for confirmation.

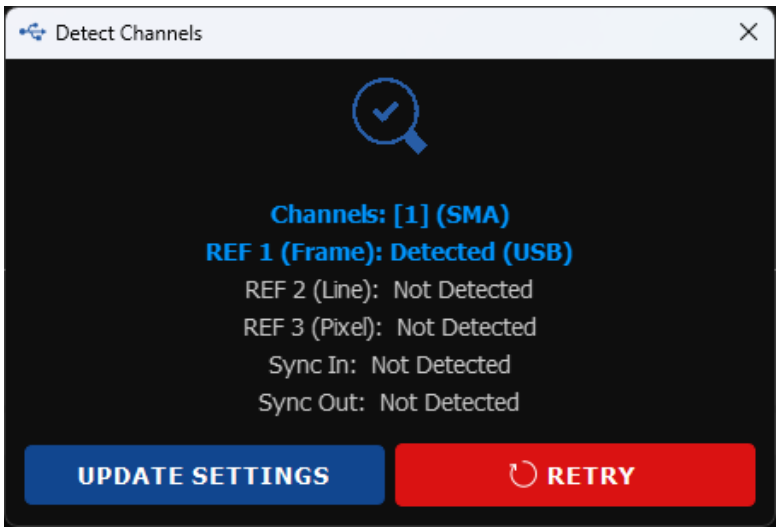


Figure 4.1: Automatic detection of connected channels.

Sync Out (Internal Synchronization)

In this mode, a fixed reference frequency is selected:

- `"sync_out_80"`
- `"sync_out_40"`
- `"sync_out_20"`
- `"sync_out_10"`

These correspond to frequencies 80 MHz, 40 MHz, 20 MHz, and 10 MHz, respectively.

Impact on Measurement

The selected synchronization frequency determines the laser period and affects temporal resolution:

- Higher frequencies result in shorter laser periods and higher temporal resolution.
- Lower frequencies result in longer laser periods and lower temporal resolution.

The software automatically adjusts the time resolution based on the selected frequency to ensure consistent sampling.
Each decay curve is represented by 256 data points, independent of the selected frequency. As a result, the temporal spacing between data points depends on the laser period.
Example:

- 80 MHz → 12.5 ns period → ≈ 48.8 ps resolution
- 20 MHz → 50 ns period → ≈ 195.3 ps resolution



When data export is enabled, file size increases with the number of active channels and temporal resolution. The estimated file size is shown in the GUI.

4.3 Data Acquisition

4.3.1 Acquisition Parameters

Figure 4.2 shows the available acquisition and display parameters.

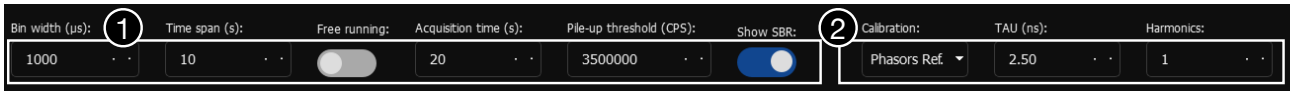


Figure 4.2: (1) Acquisition and display settings. (2) Calibration settings in *Spectroscopy Mode*.

Bin width (µs) Defines the duration of each acquisition interval (chunk). Smaller values increase temporal resolution but also data size.

Time span (s) Defines the visible time window of the intensity plot. The display continuously updates to show the most recent data within the selected interval, enabling real-time monitoring. When *Decay calc* is set to *Refreshed*, this parameter also defines the time window after which the decay curve is cleared.

Decay calc Available from version 2.5, this menu selects how the displayed decay curve is updated:

- **Cumulated** (default): Adds the photons from each acquisition interval to the previous intervals throughout the measurement. The curve becomes fuller and smoother as photons accumulate.
- **Refreshed**: Clears the decay curve at each new time window defined by *Time span* and rebuilds it using only photons acquired in that window. This option helps reveal changes in the sample over time instead of showing the accumulated decay for the entire measurement.

Free running By default the software acquires for a fixed time duration as set by the parameter *Acquisition time (s)*. In Free-running mode the acquisition continues indefinitely until stopped manually. Activate the mode by activating the switch *Free running:* . When activated the switch turns from gray to blue.

Acquisition time (s) Defines the total duration of data acquisition when free running mode is disabled. The acquisition stops automatically after the specified time has elapsed.

Pile-up Threshold Defines a count rate threshold (cps). If the measured signal exceeds this value, the system highlights the condition to indicate potential distortion due to detector saturation or pile-up effects. Insert the value expected for the detector used. For FLIM LABS SPAD detectors, refer to the specification table in the user manual.

Show SBR Enables display of the signal-to-background ratio (SBR) for each channel. This provides real-time feedback on signal quality and noise conditions during acquisition.

Calibration Selects one of four calibration options: *None*, *Phasors Ref*, *IRF Ref*, or *BIRFI Ref*. The available parameters depend on the selected option. See Section 4.5 for details.

4.3.2 Acquisition Start

Data acquisition is started using the START button on the right side of the GUI. The acquisition can be stopped at any time activating the STOP button that will appear after starting an acquisition.



Ensure the selected acquisition channel numbers match the physically connected ports on the DAQ card.

4.4 Data Display

The graphs and data displayed in the GUI varies with the selected analysis mode. In *Spectroscopy Mode*, the following are displayed:

- Counts per second (CPS)
- Intensity trace
- Signal-to-background ratio (optional)
- Decay profile

The *Decay calc* menu controls how the displayed decay curve is built. In *Cumulated* mode (the default), photons from all acquisition intervals (chunks) are added together throughout the measurement; each chunk is defined by the selected *Bin width*. In *Refreshed* mode, the curve is cleared

at each new *Time span* window and rebuilt using only photons acquired in that window. The periodic return to zero in *Refreshed* mode is expected behavior and allows changes in the sample to be monitored.

Each decay curve consists of 256 data points, independent of the laser repetition rate.

Plot interaction features

Each plot can be adjusted by zooming and rescaling using the mouse and scroll wheel based on the user needs.

Channel View Configuration

The GUI can show acquisition data from up to four channels simultaneously in the main window below the acquisition parameters. When more than four channels are selected for acquisition the data is stored but will not be displayed. The user can configure which channels to display by pressing the PLOTS CONFIG button. This will open a dialogue as shown in Figure 4.3.

Time shift

The decay curve can be shifted manually in time by inserting a bin value between 0–255 in the box next to the graph. The corresponding time shift in ns is shown for reference.

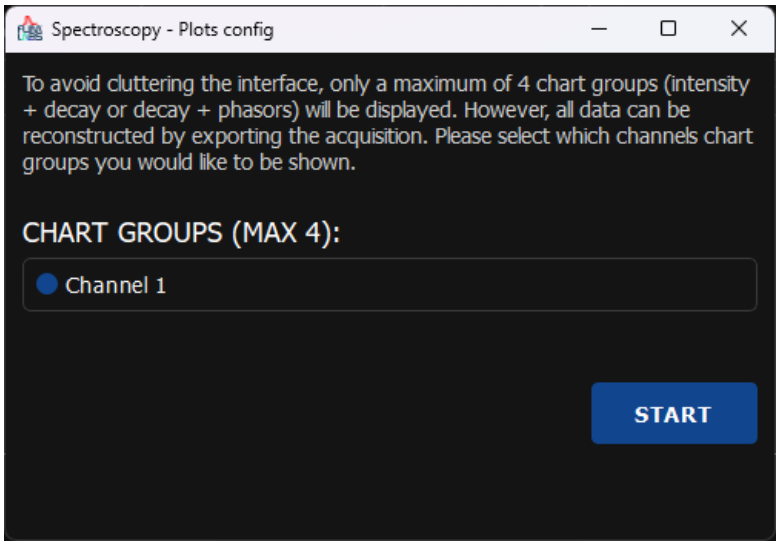


Figure 4.3: Selecting display channels.

4.5 Data Calibration

Users can select the calibration mode from the drop-down menu next to the acquisition settings. The following options are available:

None No calibration is applied. Select this option if no reference acquisition is required.

Phasors Ref Enables the acquisition of a reference for phasor analysis. When selected, additional parameters become available:

- **TAU (ns)** Defines the fluorescence lifetime of the reference sample in nanoseconds.
- **Harmonics** Allows selection of up to four harmonics for the analysis. These parameters are used to calculate the reference values for determining phasor coordinates in Phasors Mode.

IRF Ref Records the instrument's temporal response, including the laser, cables, and detector. This reference can then be loaded in Fitting Mode for instrument response correction.

BIRFI Ref Measures a mono-exponential sample with a known lifetime. The software uses this measurement and the BIRFI algorithm to estimate the instrument response when the instrument's response pulse cannot be recorded directly.

The resulting reference can be loaded in Fitting Mode for instrument response correction.

Refer to Section 5.2 for detailed instructions on how to create and load a calibration file for phasor reference. For correction using an IRF or BIRFI reference, see Section 5.3.

4.6 Data Export and Storage

Enable data export using the switch *Export data:* in the top-right area of the GUI. A dialog will prompt for file name and location.

Data are stored in binary (`.bin`) format. See Section 9.1 for details on data formatting.

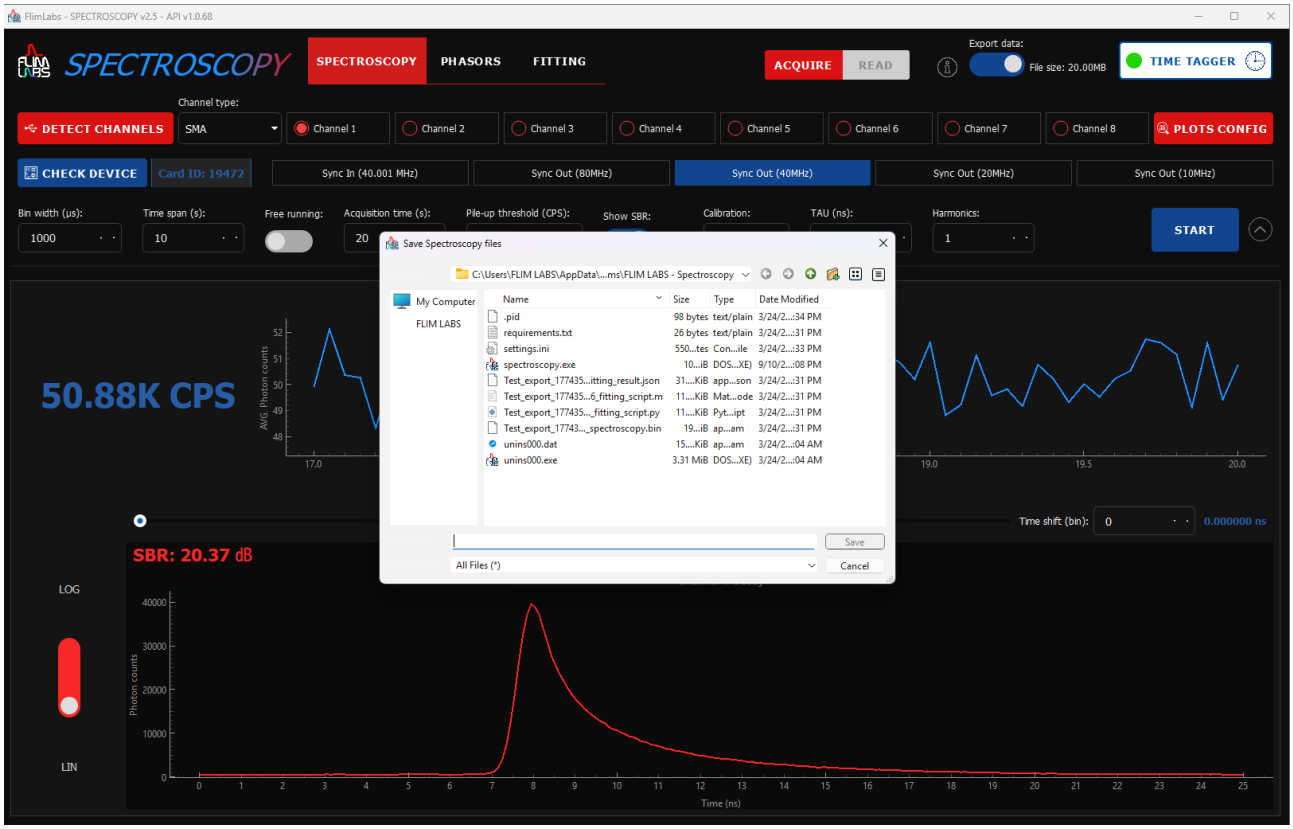


Figure 4.4: Data export dialog.

4.6.1 Time Tagger Data

Time Tagger data can be exported by activating the TIME TAGGER button that appears in the GUI on the top right when data export was activated. When Time Tagger export is enabled, additional data is stored containing event-based timing information. This includes:

- Event type (e.g., photon detection or synchronization signal)
- Micro-time (relative timing within a laser period)
- Macro-time (absolute timestamp)

This data enables detailed temporal analysis and reconstruction of photon arrival events.

5 Analysis Modes

SpectroscoPy provides three analysis modes to support different experimental and analytical workflows. The available parameters, visualizations, and analysis tools depend on the selected mode.

5.1 Spectroscopy Mode

Spectroscopy Mode is the default mode when starting the software. It enables detailed analysis of photon intensity decay as a function of time.
For each selected channel, the GUI displays:

- counts per second (CPS),
- intensity trace (time-dependent photon counts),
- decay profile (photon arrival histogram).
- SBR display: Indicates the signal-to-background ratio for noise assessment

The configuration options available in this mode were described in Section 4.3 and following.

5.2 Phasors Mode

Phasors Mode enables analysis of fluorescence lifetime distributions using phasor plots. These plots provide an intuitive, model-free way to interpret fluorescence lifetime data by mapping each acquired decay onto a point in a two-dimensional phasor plot. In this representation, single-exponential decays lie on a universal semicircle, while mixtures of lifetimes appear as linear combinations inside the plot, allowing different molecular species or environmental states to be visually separated without complex fitting. This makes it particularly powerful for comparing channels, identifying heterogeneous contributions, and analyzing changes in binding or environmental conditions. For a deeper understanding of how to interpret phasor distributions and apply this method to real data, refer to the learning resources available on the FLIM LABS website.

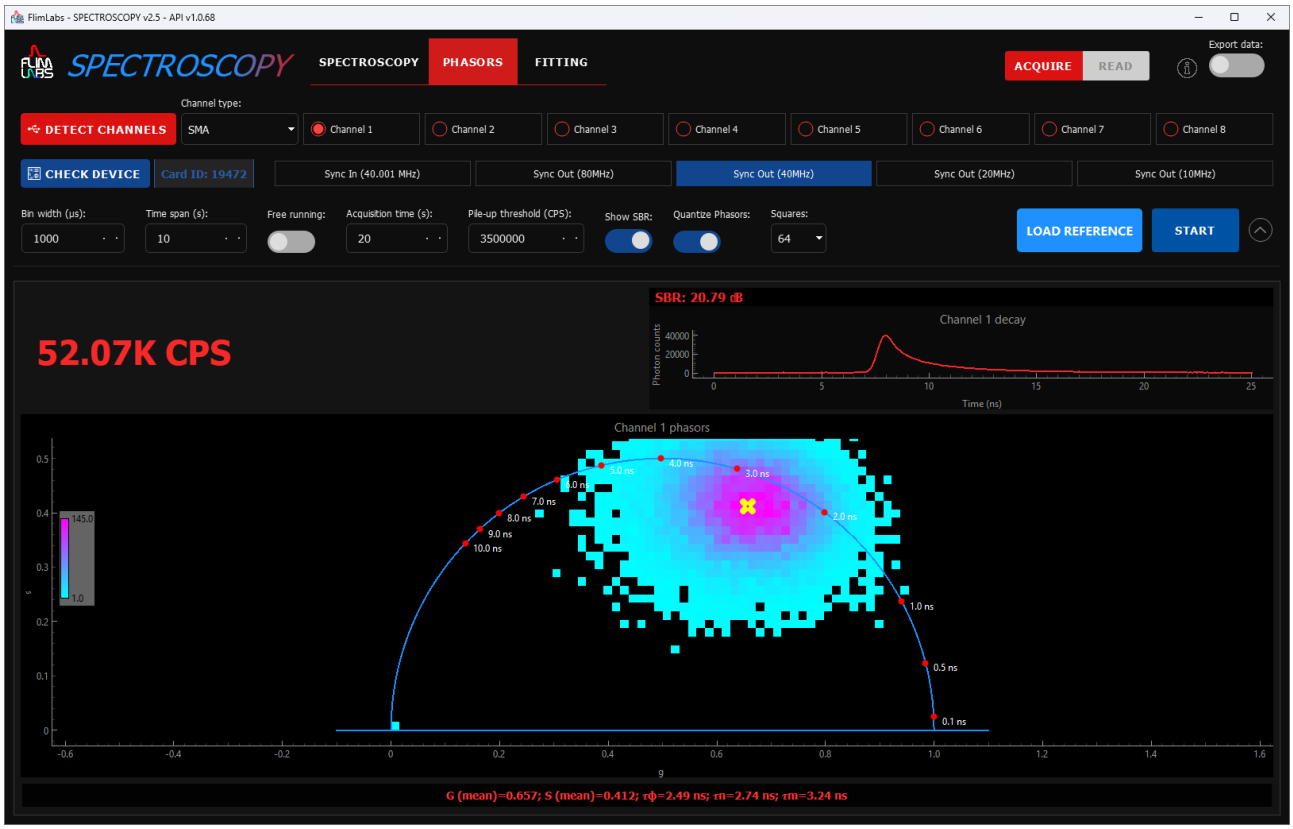


Figure 5.1: GUI display in Phasors Mode.

Figure 5.1 shows the GUI in *Phasors Mode* with one channel active. The following data is displayed for each active channel:

- counts per second (CPS),
- decay profile (photon arrival histogram).
- SBR display (optional)
- FLIM Phasor plot

Phasor points are displayed using an intensity-based colormap. A lifetime reference is included via the universal semicircle, automatically calculated from the laser repetition period. Additionally, key statistical parameters are shown below the plot, including the mean phasor coordinates (G mean, S mean) and the corresponding phase lifetime (τ_ϕ) and modulation lifetime (τ_m).

5.2.1 Configuration Options

The resolution of the phasor plot can be adjusted by grouping phasor points into a grid (quantization). The *Quantize Phasors:* switch is enabled by default.

The adjacent Squares control sets the number of squares used to group phasor points when quantization is enabled. A higher value gives a finer grid. The available values are 16, 32, 64, 128, 256, and 512; the default value at software startup is 64. This setting corresponds to `phasors_resolution` in the configuration.

5.2.2 Phasors Calibration

Phasors data can be calibrated using a reference sample with a known mono-exponential decay time τ. FLIM LABS provides dedicated calibration samples in the **FLIM-Phasors Calibration Kit**. For more information, please check the website under https://www.flimlabs.com/products/flim-phasors-calibration-kit/.

Creating a Calibration File

To generate a phasor calibration file, proceed as follows:

1. Switch to the Spectroscopy Mode tab.
2. Select `Phasors Ref` as the calibration type.
3. Insert the reference sample in your imaging setup.
4. Enter the corresponding decay constant in the field Tau (ns) in the parameter panel above the plot area.
5. Select appropriate acquisition parameters.
6. Select the desired harmonic(s).
7. Enable data export using the switch *Export data:* .
8. Start the acquisition with the START button.



The imaging and illumination parameters used during calibration should match those of the actual measurement to avoid calibration mismatch.
If the detected signal exceeds the saturation threshold of the detector, reduce the intensity using neutral density (ND) filters or by de-focusing the excitation beam.

The recorded reference data are saved as a *.json* file and can be reused for future measurements.

Loading a Calibration File

To load a calibration file, click the button LOAD REFERENCE and select the desired file.

Verification of Calibration

After loading a valid calibration file:

- Phasor data should be located in the upper-right quadrant of the phasor plot (G > 0, S > 0).
- Data from a mono exponential fluorescence decay should lie on or close to the universal semicircle.

To ensure accurate calibration, keep all acquisition parameters and illumination conditions identical between the calibration measurement and subsequent experiments. Changes in system configuration may lead to calibration mismatch.

5.3 Fitting Mode

Fitting Mode allows estimation of the parameters of a mathematical model that best describes the measured decay profile.

Figure 5.2 shows the GUI layout in this mode. Clicking the button FIT on the right side of the GUI opens a separate fitting window showing the decay profile as well as the residuals of an existing fit as shown in Figure 5.3.

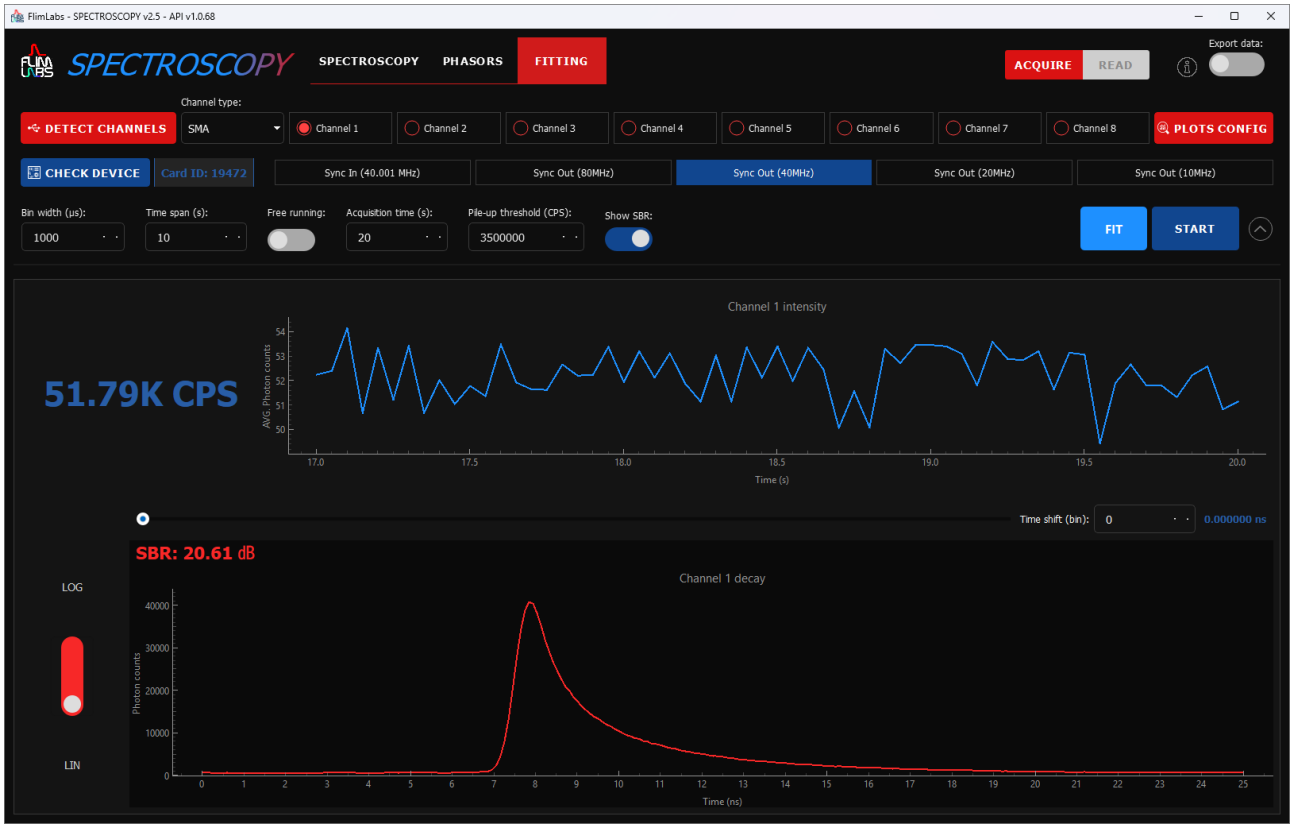


Figure 5.2: GUI display in Fitting Mode.

5.3.1 Decay Fitting

The decay profile can be fitted by pressing the button START FITTING .

The results are visualized by overlaying the fitted model curve on the measured data. The software uses the *SciPy* library to select the best-fitting model from a set of exponential decay models. A switch on the left allows modifying the y-axis scale (counts) from linear to logarithmic.

Instrument Response Correction

Load an IRF or BIRFI reference in the Fitting section and enable the *Use deconvolution* toggle to correct for the instrument response. When this option is enabled and either reference has been loaded, the measured curve is corrected for the instrument response before the lifetime calculation is performed.

An IRF reference records the instrument response directly, whereas a BIRFI reference estimates it from a mono-exponential sample with a known lifetime. See Section 4.5 for the reference acquisition options.

Residuals Analysis

The difference between measured and fitted values, labeled residuals, is shown below the decay profile. Large or systematic variations from the zero line indicate a mismatch between model and data.



Figure 5.3: Intensity decay fitting window.

Fitting Parameters

Key parameters for the fitting results are shown below the graph for decays and residuals.

- Decay constants found e.g. *T1*, *T2*,... and ratio of each decay to total intensity in %
- *B*: Constant offset of data in %
- χ^2 value. This measures the agreement between observed data points and the theoretical model by calculating the difference between residual and fit. A value close to one indicates a good fit.
- Model equation for the fit
- R^2 value (1 represents a perfect fit, <<1 a bad fit)

Region of Interest

If the fitting results are insufficient, the user can select a region of interest (ROI) by clicking and dragging the mouse in the Intensity graph. When a ROI is selected, only the selected data region is used for fitting.

Export of Fitting Data

Fitting data can be exported by clicking the EXPORT button on the top of the Fit window.

6 Advanced Operation

6.1 Console Mode

In addition to the graphical user interface, SpectroscoPy can be operated in console mode. In this mode, data are streamed and displayed directly in the terminal without a graphical interface.

The console output provides the following information:

- acquisition channel number,
- elapsed acquisition time (s),
- decay curve values represented by 256 data points.

6.1.1 Requirements

Operation in Console Mode requires the installation of Python Version 3.9 or later. Please refer to Python.org for additional information.

6.1.2 Configurable Parameters

Table 6.1 lists the parameters available for console operation.

Table 6.1: Console Acquisition Parameters

Parameter	Data Type	Description
`selected_sync`	string	Defines the synchronization source. Supported values are `"sync_in"` (default), `"sync_out_10"`, `"sync_out_20"`, `"sync_out_40"`, and `"sync_out_80"`.
`sync_in_frequency_mhz`	float	Defines the laser synchronization frequency (MHz) when `selected_sync = "sync_in"`. The value can be set manually or determined automatically.
`enabled_channels`	list of integers	Defines the active acquisition channels. Up to 8 channels can be enabled. Example: `[0,1,2,3,4,5,6,7]`.
`bin_width_micros`	float	Defines the time bin width (µs) used for photon count accumulation.
`acquisition_time_millis`	float or None	Defines the total acquisition duration (ms). A numerical value sets a fixed duration, while `None` enables continuous acquisition.

Sync-In Frequency

The synchronization frequency can be set manually or determined automatically.

- **Manual configuration:**

```
sync_in_frequency_mhz = 40.0
```

- **Automatic detection:**

```
sync_in_frequency_mhz = detect_laser_sync_in_frequency()
```

Starting Data Acquisition

After configuring the parameters, start the acquisition from the project directory:

1. Create a virtual environment:

```
python -3.9 -m venv venv
```

2. Activate the virtual environment:

```
venv/scripts/activate
```

3. Install dependencies:
```
pip install -r requirements.txt
```
4. Start the console application:
```
python console.py
```

6.2 Data Processing Scripts

When exporting data, SpectroscoPy automatically generates scripts for *Python* and *Matlab*. These scripts are stored together with the acquisition file and enable efficient data processing of the `.bin` files.

The scripts allow users to:

- parse binary data into structured arrays,
- reconstruct intensity traces and decay curves,
- perform custom analysis workflows.

Python

To visualize the data using Python:

1. Open a terminal and navigate to the export directory:
```
cd YOUR_DOWNLOADED_DATA_ROOT_FOLDER
```
2. Create a virtual environment:
```
python -m venv venv
```
3. Activate the virtual environment:
```
venv\Scripts\activate
```
4. Install dependencies:
```
pip install -r requirements.txt
```
5. Run the script:
```
python YOUR_SCRIPT_NAME.py
```

Matlab

To visualize the data using Matlab:

1. Open the MATLAB command window.
2. Navigate to the export directory:
```
cd YOUR_DOWNLOADED_DATA_ROOT_FOLDER
```
3. Run the script:
```
YOUR_SCRIPT_NAME
```

7 Troubleshooting

7.1 Common Issues

Table 7.1: Common issues, causes, and solutions

Issue	Cause	Solution

8 Safety, License and Support

8.1 Safety and Operational Limitations

The software does not perform hardware safety checks. Users are responsible for correct configuration and operation.

For detailed safety instructions, refer to the user manual of the FLIM Data Acquisition Card: https://www.flimlabs.com/products/data-acquisition-card/

8.2 License

This software is distributed under the MIT License. It permits free use, modification, and redistribution, including for commercial applications, provided that the original copyright notice and license disclaimer are included.

8.3 Contact and Support

For additional information, visit: https://flim-labs.github.io/spectroscopy-py/

For technical support, contact FLIM LABS at: `info@flimlabs.com`

9 Technical Reference

9.1 File Format

The exported `.bin` file contains time-resolved photon counting data acquired during the measurement. The file structure is optimized for efficient storage and high-speed data processing.
Each file includes:

- photon count data for all active channels,
- time-resolved decay information,
- acquisition parameters and metadata.

The file is organized into three main parts:

1. a fixed file header for format validation,
2. a metadata block describing the acquisition settings,
3. a sequence of data records containing the measured data.

The following sections describe the structure in detail.

Spectroscopy File Format

The spectroscopy binary file stores time-resolved decay data for one or more active channels. The structure enables reconstruction of the photon decay curves over time.
The overall layout of the file is shown in Table 9.1. The file begins with a header and metadata section, followed by a sequence of data records.

1. **Header and Metadata**
   The header and metadata appear once at the beginning of the file and apply to all subsequent data records.
2. **Data Records**
   After the metadata section, the file contains a continuous sequence of data records. Each record corresponds to a single acquisition interval (chunk) and contains:
   - a timestamp indicating the acquisition time,
   - a full decay histogram (256 bins) for each active channel.

The number of channels and acquisition parameters required to interpret the data are defined in the metadata section.

Table 9.1: Byte-level structure of the spectroscopy binary file format.

Field	Size [bytes]	Data Type	Description
Header	4	ASCII	Fixed file identifier: `SP01`. Used to validate the file type.
JSON length	4	Unsigned integer	Length of the JSON metadata block in bytes.
JSON metadata	Variable	UTF-8 / ASCII string	Metadata in JSON format containing acquisition settings such as enabled channels, bin width, acquisition time, laser period, and lifetime values (τ in ns).
Timestamp	8	Double	Acquisition time in seconds for the current data record.
Channel 1 curve	1024	256 × uint32	Cumulated decay curve for active channel 1. Each curve contains 256 values, each stored as a 4-byte unsigned integer.

Field	Size [bytes]	Data Type	Description
Channel 2 curve	1024	256 × uint32	Cumulated decay curve for active channel 2, if enabled.
Channel … curve	1024 each	256 × uint32	Additional channel curves for each further active channel.

Each data record has a fixed size depending on the number of active channels:

$$\text{Record Size} = 8 + 1024 \times N_{\text{channels}} \text{ bytes}$$

where N_{channels} is defined in the metadata.
The data records are stored sequentially without separators and must be parsed continuously until the end of the file is reached.

Phasors File Format

The phasors binary file stores frequency-domain lifetime information in the form of phasor coordinates. Each record represents a phasor measurement at a given time, for a specific channel and harmonic.
The overall structure is shown in Table 9.2. As with the spectroscopy format, the file consists of a header, metadata, and a sequence of data records.

1. **Header and Metadata**
   The header and metadata are located at the beginning of the file and define all acquisition parameters required to interpret the phasor data.
2. **Data Records**
   Each data record contains the following fields, in this order:
   - a timestamp in nanoseconds (8 bytes),
   - a channel index (4 bytes),
   - a harmonic index, stored as an unsigned integer from 1 to 4 (4 bytes),
   - the phasor coordinate g (8 bytes),
   - the phasor coordinate s (8 bytes).

The phasor components g and s represent the real and imaginary parts, respectively, of the normalized Fourier transform of the fluorescence decay at the specified harmonic.

Table 9.2: Byte-level structure of the phasors binary file format.

Field	Size [bytes]	Data Type	Description
Header	4	ASCII	Fixed file identifier: SPF1. Used to validate the phasors file type.
JSON length	4	Unsigned integer	Length of the JSON metadata block in bytes.
JSON metadata	Variable	UTF-8 / ASCII string	Metadata in JSON format containing acquisition settings such as enabled channels, bin width, acquisition time, laser period, harmonics, and lifetime values (τ in ns).
Timestamp	8	Unsigned integer	Timestamp of the phasor record in nanoseconds.
Channel index	4	4-byte identifier	Channel identifier associated with the phasor record.

Field	Size [bytes]	Data Type	Description
Harmonic index	4	Unsigned integer	Harmonic number of this phasor point (1 to 4).
g	8	Double	Real part of the phasor value (X-axis).
s	8	Double	Imaginary part of the phasor value (Y-axis).

Each data record is 32 bytes long $(8+4+4+8+8)$ and records are stored one after another, without separators, until the end of the file.

9.2 Acquisition and Processing Parameters

This section provides an overview of all configurable acquisition and processing parameters available in the software. These parameters control data acquisition, visualization, phasor analysis, and data export.

Table 9.3 summarizes all available parameters, including their data type, default values, and functionality.

Table 9.3: Summary of configurable parameters.

Parameter	Data Type	Configuration	Default	Description
`enabled_channels`	number[]	List of channel indices (0–7)		Defines which channels are active for photon data acquisition.
`connection_type`	number	0 = USB, 1 = SMA	1	Selects the acquisition interface. The corresponding firmware is automatically applied.
`bin_width`	number	1000–1,000,000	1000	Integration time in µs for photon counting per bin.
`free_running`	boolean	True / False	False	Enables continuous acquisition. If False, acquisition time must be defined.
`time_span`	number	1–300	10	Time window [s] displayed on the real-time intensity plot. Also defines the decay-curve clearing interval when Decay calc is set to Refreshed.
`acquisition_time`	number / None	1–1800	None	Total acquisition duration [s] when not in free-running mode.
`cps_threshold`	number	≥ 0	0	Threshold for counts-per-second (CPS) highlighting per channel.
`show_sbr`	boolean	True / False	False	Enables visualization of the signal-to-background ratio (SBR).
`sync`	string	`sync_in` / `sync_out` (+ frequency)	`sync_in`	Configures laser synchronization mode.

Parameter	Data Type	Configuration	Default	Description
`calibration`	number	0 = None, 1 = Phasors Ref; see description	0	Menu options: None, Phasors Ref, IRF Ref, and BIRFI Ref. Supports reference acquisition for phasor analysis or instrument response correction.
`tau_ns`	number / None	Positive value	None	Lifetime value [ns] used for phasor reference calculations.
`harmonics`	number	1–4	1	Number of harmonics used in phasor analysis.
`quantize_phasors`	boolean	True / False	True	Enables binning of phasor points for visualization.
`phasors_resolution`	int	16, 32, 64, 128, 256, 512	64	Number of squares used to group phasor points when Quantize Phasors is enabled. A higher value gives a finer grid. The control is shown in the GUI as Squares.
`plots_to_show`	number[]	Up to 4 plot indices		Defines which plots are displayed during acquisition.
`time_shifts`	dict	{channel: shift}	{}	Time offsets applied to decay curves per channel.
`lin_log_mode`	dict	{channel: LIN/LOG}	{}	Sets linear or logarithmic visualization mode per channel.
`write_data`	boolean	True / False	True	Enables export of raw data and associated reader scripts.
`time_tagger`	boolean	True / False	False	Enables export of Time Tagger data.
`pico_mode`	boolean	True / False	False	Enables high-resolution acquisition mode (100 ps firmware).

9.3 GUI Parameter Saving

The software automatically stores all GUI configuration parameters. Any change made through the graphical user interface is immediately written to an internal configuration file (`settings.ini`).

The `settings.ini` file is updated dynamically during operation. Upon application restart, the previously saved configuration is automatically loaded. If the configuration file is not present, or if specific parameters have not yet been defined, default values are applied as described in Section 9.2.

File Structure The configuration file uses a standard INI format with key–value pairs grouped into sections. The `[General]` section contains the main acquisition and processing parameters.

Example Configuration

An example of the `settings.ini` structure is shown below:

```
[General]
app_version=2.5
free_running=false
tau_ns=1
calibration=1
connection_type=0
```

```
plots_to_show="[2, 3]"
channel_0=false
channel_1=false
channel_2=true
channel_3=true
channel_4=false
channel_5=false
channel_6=false
channel_7=false
sync=sync_out_40
time_shift="{\"2\": 64, \"3\": 0}"
acquire_read_mode=acquire
harmonic=4
write_data=true
acquisition_time=10
phasors_resolution=64
quantize_phasors=true
lin_log_mode="{\"2\": \"LOG\", \"3\": \"LIN\"}"
pico_mode=false
```

Notes

- Boolean values are stored as `true` / `false`.
- Lists and dictionaries are serialized as strings and must be parsed accordingly.
- Channel activation is stored as individual flags (e.g., `channel_0`, `channel_1`, etc.).