

optogenix

Tapered fibers for Neuroscience

TaperScan

User Manual



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Chapter 1 Introduction

1.1. What is TaperScan?

TaperScan is a graphical user interface designed for the use of OptogeniX GalvoStation. The software allows for manual control of the GalvoStation and for the definition of stimulation protocols for both single channel and time-multiplexed dual channel measurements.

1.2. Recommended configuration

We recommend

computer systems

that meet or exceed

the following

specifications:

Processor (CPU)

Intel Core i5 (8th generation or newer)
or equivalent

Memory

8 GB RAM

Operating system

Windows 10 x64

Other

1 USB 2.0 port

1.3. Copyright and License

By downloading, installing, copying, or using TaperScan the user agrees to the terms and conditions contained in the End User License Agreement distributed with the software and available at our [website](#).

This document may not be reproduced, transmitted or translated to another language, either as a whole or in parts, without the prior written permission of OptogeniX s.r.l.

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1.4. Terms of Use and Terms of Distribution

- We shall not be responsible in any way for any kind of damages arising from the use or inoperability of TaperScan.

- We shall not be liable in any way for support of TaperScan, any kind of warranty, correction of trouble or continuation of version upgrades, etc.
- TaperScan may be subject to changes and/or updates, and we will update the content of this document regularly to reflect the current status of TaperScan. OptogeniX s.r.l. does not guarantee that any particular objective can be achieved with TaperScan.
- Provided that you consent to the aforementioned matters, you shall be permitted to use the software for nonprofit purposes.
- When "TaperScan" is mentioned, it refers not only to the executable file, but to all of the component files including Help and appended text.

1.5. Support

We call support the provision of all kinds of information including answers to questions, requests, trouble reports, and other matters, and the scope of our support shall be limited to the functions and operation of TaperScan. Problems that arise in using TaperScan cross many paths including those attributable to the personal computer, the OS, other application software, and other peripheral devices. Accordingly, we only support problems that are attributable to TaperScan. Also, TaperScan is subject to version upgrades without prior notice due to the technical advancement of hardware and software. The customer can receive and use upgraded versions of this software through a method determined by OptogeniX s.r.l. Note that version upgrades will be limited to customers who consent to the aforementioned End User License Agreement.

Chapter 2 Installation and first start

Ensure that the GalvoStation system is fully cabled as specified in its User Manual and that it is turned off. To install and configure TaperScan follow the instruction below.

- 1) Download the latest version of National Instruments NI-DAQmx drivers from [NI website](#)¹, locate the file and run the NI Package Manager setup. If you already have NI-DAQmx drivers and NI hardware installed on your computer, you can skip points 1-3.
- 2) Run the NI Package Manager.
- 3) When prompted with the choice of packages, select "NI-DAQmx Runtime with Configuration Support" only.
- 4) Connect the GalvoStation DAQ board to your PC and turn the board on.
- 5) Run NI-MAX and check that the DAQ board is recognized by the system. Take note of its name under Devices and Interface on the left menu (typically in the form «NI USB-6343 "Dev X"»).
- 6) Download the setup of the latest version of TaperScan from the [GalvoStation shop page](#)² and locate the file.
- 7) Run the TaperScan setup. Avoid to install TaperScan in directories with no writing permission for the current PC user (e.g. C:\Programs).
- 8) Start up the GalvoStation system as described in the corresponding section of the GalvoStation User Manual.
- 9) Run TaperScan. A DAQ connection error message will appear: click OK and select the GalvoStation DAQ board in the DAQ Settings dialog that opens after the error.
- 10) Leave Sample rate and Refresh view with default values.

¹URL: <https://www.ni.com/en-us/support/downloads/drivers/download.ni-daqmx.html>.

² URL: <https://www.optogenix.com/prodotto/galvostation/>.

- 11) Insert the GalvoStation Offset value in the Offset [V] field of the DAQ Settings dialog.
- 12) Press OK; a confirmation message of correctly saved DAQ parameters should appear.

Note that, particularly when antivirus softwares performing real-time scans are running, the first launch of TaperScan can take a few minutes.

Chapter 3 User Interface description

TaperScan main window is organized in panels as shown in Figure 1.

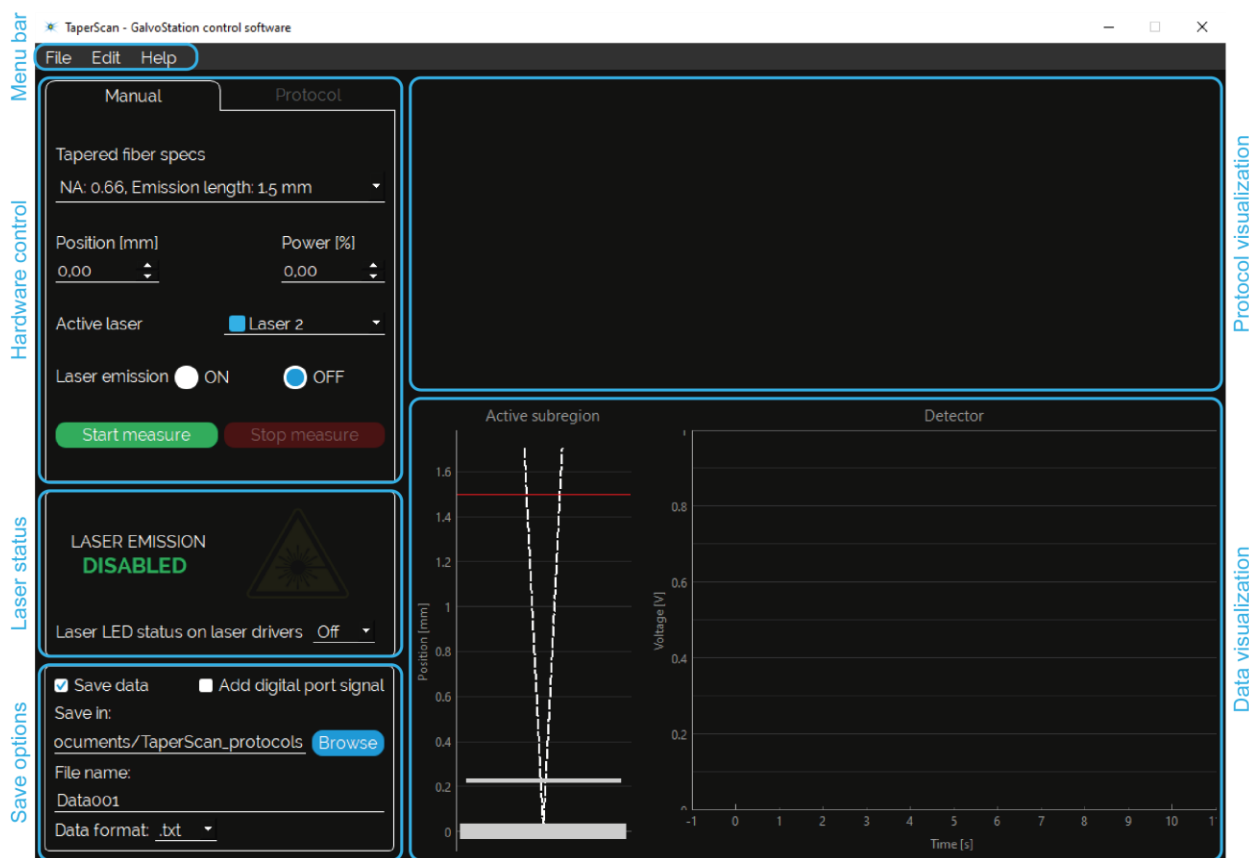


Figure 1. Panels of TaperScan main window.

3.1. Menu Bar

3.1.1 File

The File menu contains the following items:

- New site selective light delivery protocol (keyboard shortcut Ctrl+O): open the dialog to define a new protocol for site selective light delivery (single or double light source stimulation, see section 3.7 for details);
- Edit site selective light delivery protocol (keyboard shortcut Ctrl+Shift+O): open the dialog to select and edit an existing

protocol for site selective light delivery (single channel stimulation, see section 3.7 for details);

- New site selective fiber photometry protocol (keyboard shortcut Ctrl+P): open the dialog to define a new protocol for site selective fiber photometry (time-multiplexed dual channel stimulation, see section 3.8 for details);
- Edit site selective fiber photometry protocol (keyboard shortcut Ctrl+Shift+P): open the dialog to select and edit an existing protocol for site selective fiber photometry (time-multiplexed dual channel stimulation, see section 3.8 for details);
- Quit (keyboard shortcut Ctrl+Q): exit the Software.

3.1.2 Edit

The Edit menu contains the following items:

- DAQ settings (keyboard shortcut Ctrl+S): open the dialog to edit the settings of DAQ board and data visualization (see section 4.ZZ for details);
- Laser colors (keyboard shortcut Ctrl+C): open the dialog to edit the visualization color of the lasers (see section 3.10 for details).

3.1.3 Help

The Help menu contains the following items:

- User manual (keyboard shortcut Ctrl+M): open a link to this User Manual in the operating system default browser;
- About (keyboard shortcut Ctrl+A): open an information dialog about the Software.

3.2 Hardware control panel

The hardware control panel allows to control the hardware in Manual or Protocol mode.

3.2.1 Manual mode

When the Manual tab is selected, the Hardware control panel looks as in Figure 2.

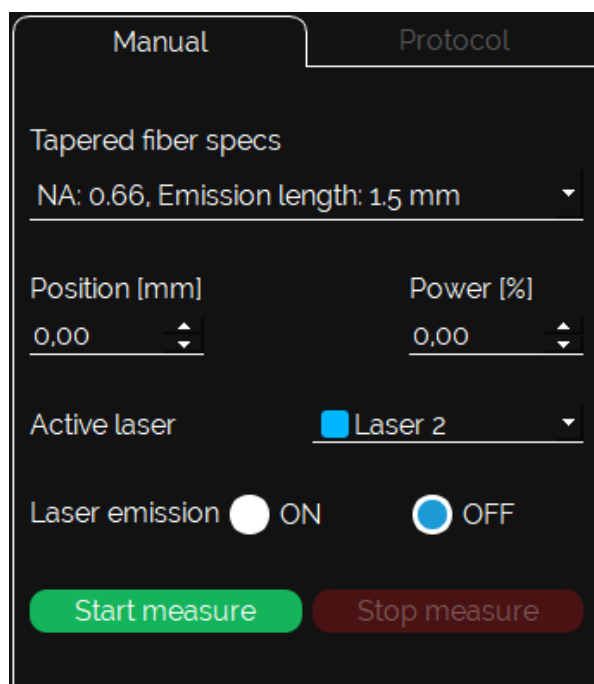


Figure 2. Hardware control panel in Manual mode.

Tapered fiber specs: this dropdown menu allow to select the specifications of the tapered fiber in use, in order to load the proper calibration of the fiber light emitting region position.

Position [mm]: by changing the value of this spin box the location of the fiber emitting region is moved across the taper; the length specify the distance between the taper tip and the emitting region.

Power [%]: by changing the value of this spin box the power of the emitted light is changed.

Active laser: this dropdown menu allow to select the laser to be used between the sources installed in the GalvoStation.

Laser emission: by using these buttons the laser emission is enabled (ON button) or disabled (OFF button), i.e. laser power is set to the specified power value or forced to zero.

Start/Stop measure: by using these buttons the acquisition of data from the detector is started/stopped.

3.2.2 Protocol mode

When the Protocol tab is selected, the Hardware control panel looks as in Figure 3.

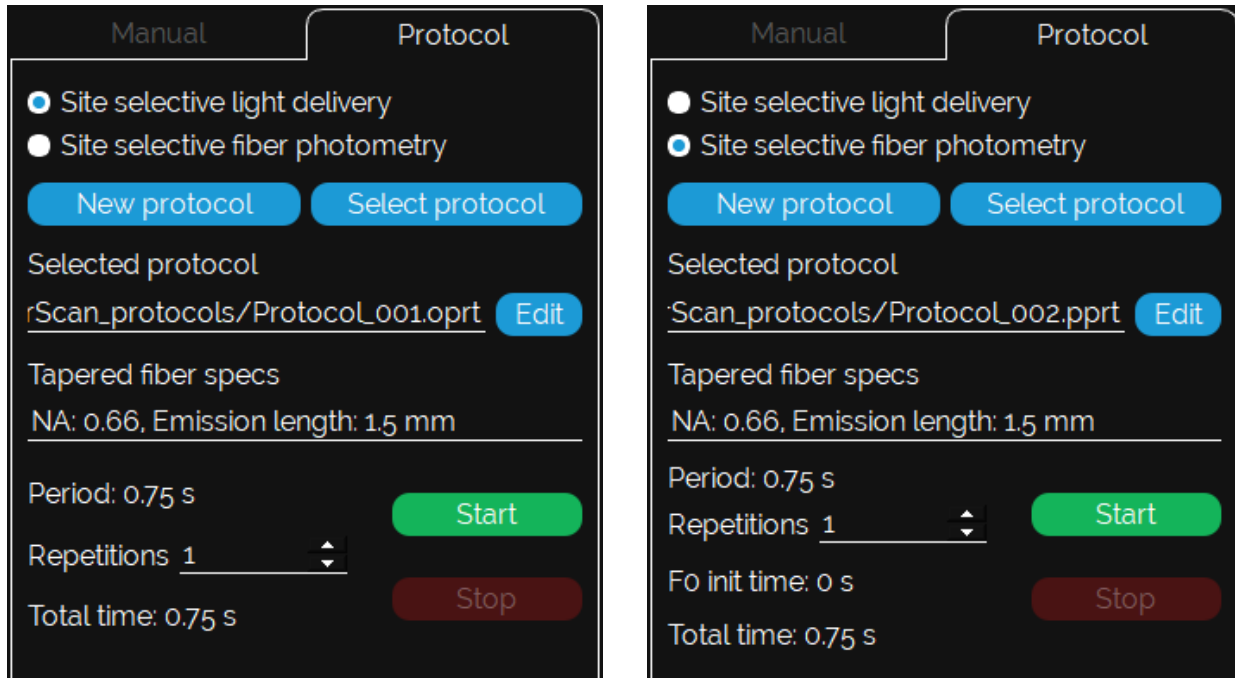


Figure 3. Hardware control panel in Site selective light delivery Protocol mode (left) and Site selective fiber photometry Protocol mode (right).

Site selective light delivery: when this radio button is selected TaperScan is configured to work with site selective light delivery protocols.

Site selective fiber photometry: when this radio button is selected TaperScan is configured to work with site selective fiber photometry protocols (time-multiplexed dual channel measurements).

New protocol: open the dialog to define a new protocol in accordance with the selected protocol type.

Select protocol: open the dialog to load a previously saved protocol in accordance with the selected protocol type.

Selected protocol: show the path of the currently selected protocol.

Edit protocol: open the dialog to edit the currently selected protocol.

Tapered fiber specs: show the specification of the tapered fiber for which the currently selected protocol is defined.

Period: show the period length in seconds of the currently selected protocol.

Repetitions: by changing the value of this spin box the number of repetition of the protocol period that will be run are defined.

F0 init time (for site selective fiber photometry protocols only): show the length in seconds of the initialization interval defined to evaluate the fluorescence reference level that can be used to compute $\Delta F/F$ values.

Total time: show the length in seconds of the total protocol execution (i.e. initialization time + repetitions * period length).

Start/Stop: by using these buttons the protocol is started/stopped.

3.3 Save options

The save data panel, shown in Figure 4, allow to define the saving options for both Manual and Protocol mode.

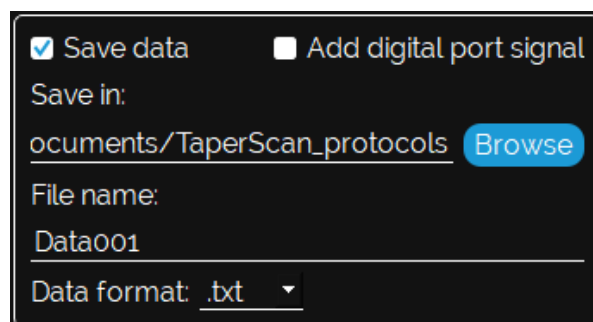


Figure 4. Save options panel

Save data: when checked enables the saving of the data collected by the system is enabled. Data will be saved when the acquisition

is manually stopped or at the end of the protocol at the specified path.

Add digital port signal: when checked the signal on the digital inputs of the DAQ board are stored in the saved data.

Save in: show the path where data will be saved, selected by using the Browse button.

File name: allow to specify the name of the file that will be saved.

Data format: this dropdown menu allow to select the format of the saved data.

3.4 Laser status

The laser status panel shows the current status of laser emission (disabled or enabled), as shown in Figure 5.



Figure 5. Laser emission panel with the laser sources disabled (left) and emitting (right).

The Laser LED status on laser drivers dropdown menu permits to the user to confirm that the laser sources are ready to emit according to the status shown on their controller.

3.5 Data visualization

The Data visualization panel shows a graphical representation of the light emitting region location and the output voltage of the fluorescence detector, both in manual and protocol mode.

3.5.1 Manual mode

When the Manual tab is selected, the Data visualization panel looks as in Figure 6. Data visualization panel in manual mode. The

active subregion is represented by three stripes superimposed on a drawing of the taper, representing the center and the boundaries of the light emitting region. The output voltage of the fluorescence detector is shown as a voltage versus time plot within a 10 seconds window. If the Add digital port signal in the Save options panel is checked, time marks are added to the Detector plot when an event on the digital ports is detected.

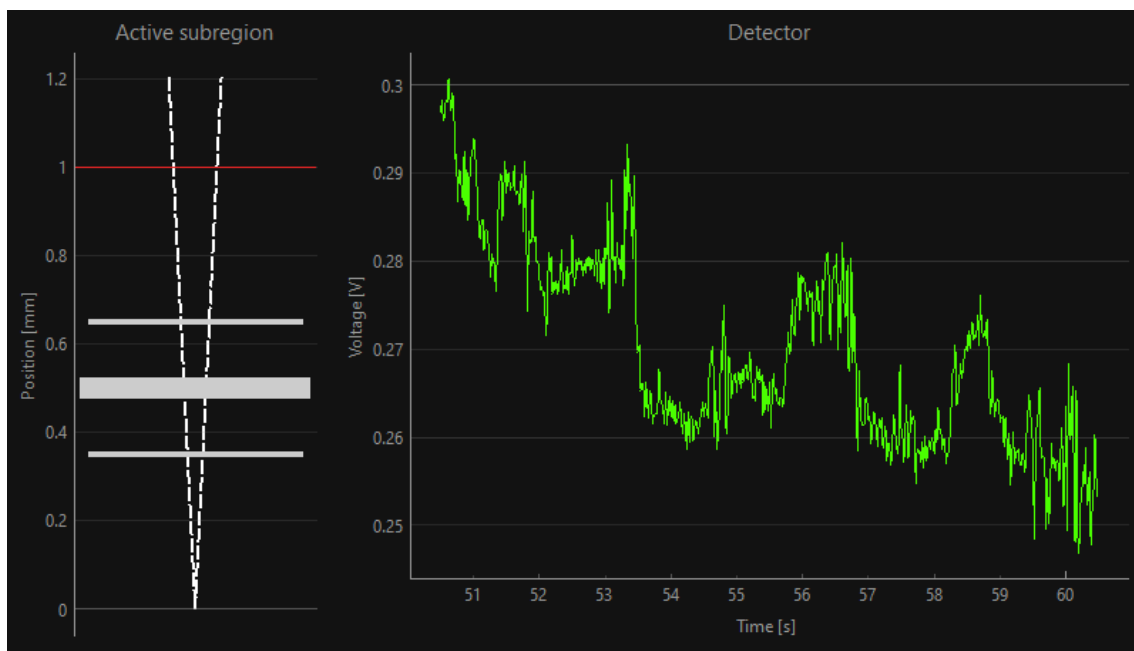


Figure 6. Data visualization panel in manual mode.

3.5.2 Protocol mode

When the Protocol tab is selected, the Data visualization panel looks as in Figure 7. Both the active subregion location and the output voltage of the fluorescence detector are shown as a plot versus time within a 10 seconds window. If the Add digital port signal in the Save options panel is checked, time marks are added to the Detector plot when an event on the digital ports is detected.

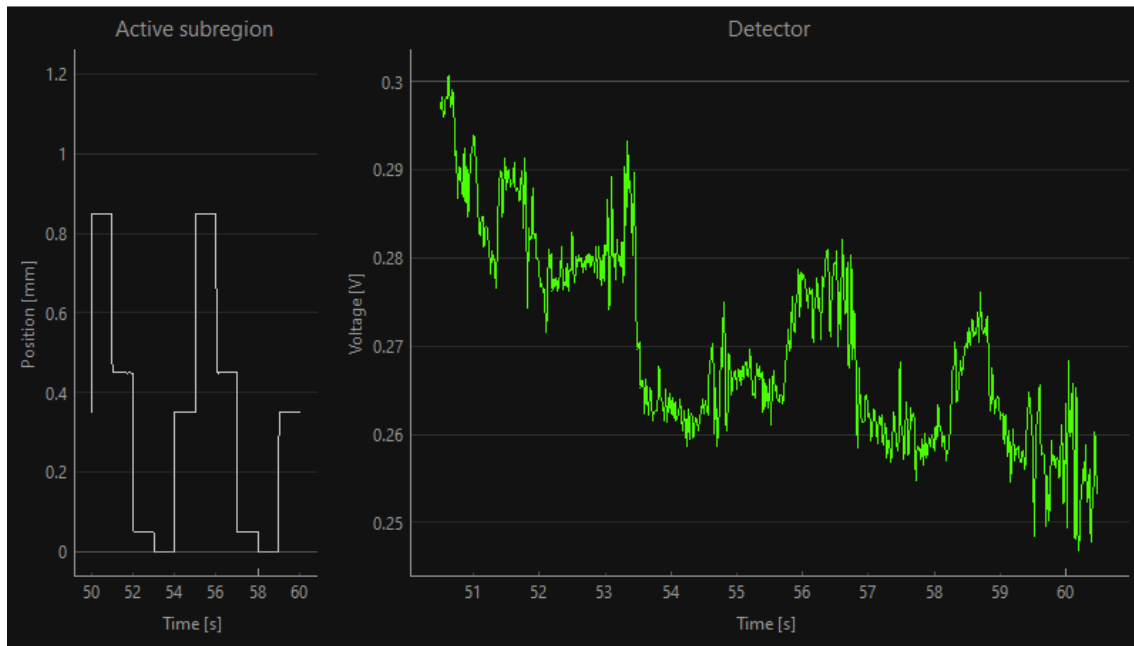


Figure 7. Data visualization panel in protocol mode.

3.6 Protocol visualization (only visible if protocol tab is selected)

The Protocol Visualization panel shows a graphic summary of the selected protocol and allow the user to navigate the plot by dragging (moving across the horizontal temporal axis) or scrolling (zooming in and out) with the mouse. A context menu with basic visualization and navigation function can be opened with a right-click on the panel.

3.7 New/Edit site selective light delivery protocol dialog

By using this dialog the user can define a new site selective light delivery protocol or edit an existing one. This dialog is organized in panels as shown in Figure 8.

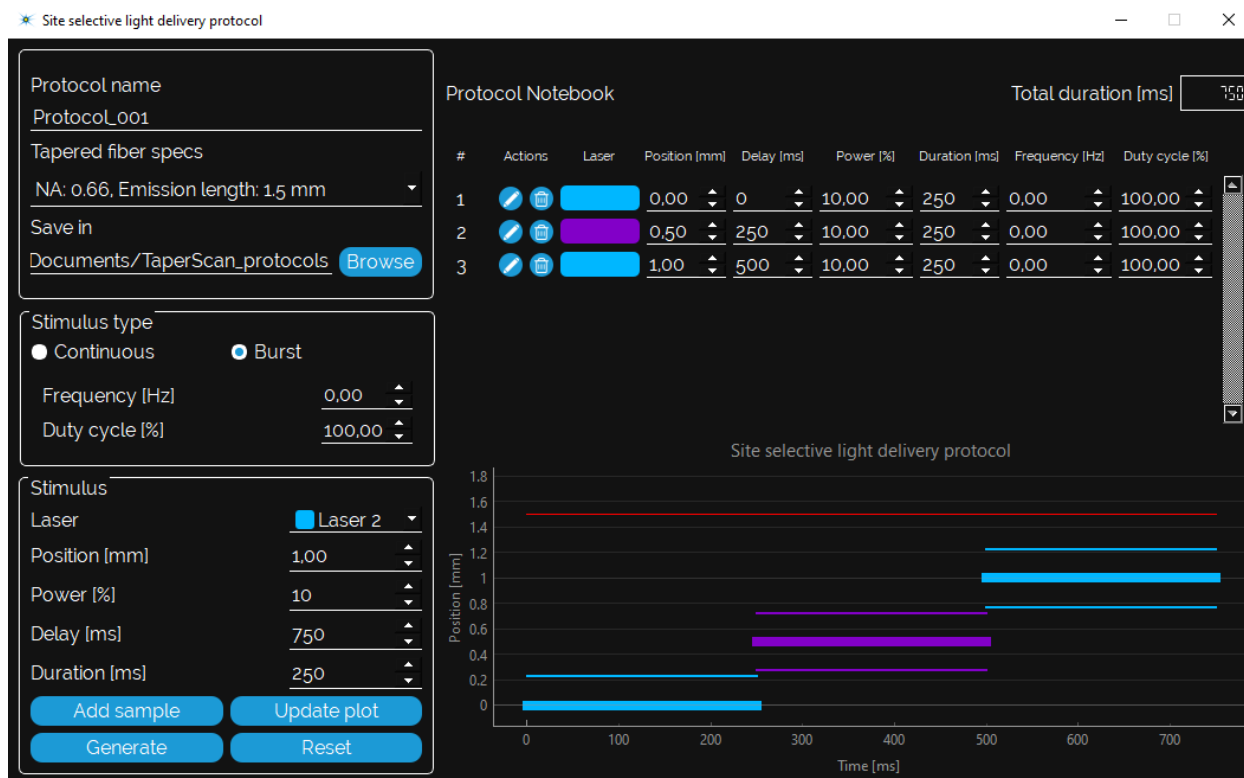


Figure 8. Panels of the Site selective light delivery protocol dialog.

3.7.1. Protocol specs

Protocol name: the name of the protocol that will be used as filename when the protocol is generated and saved.

Tapered fiber specs: this dropdown menu allow to select the specifications of the tapered fiber used in the protocol, in order to load the proper calibration of the fiber light emitting region position.

Save in: show the path where the protocol will be saved, selected by using the Browse button.

3.7.2. Stimulus type

Continuous/Burst: the selection between this two options allow to define if the stimulus in definition should be continuous or pulsed with a square wave.

Frequency [Hz]: active only if Burst is selected, defines the frequency of the pulsed stimulus.

Duty cycle [%]: active only if Burst is selected, defines the duty cycle of the pulsed stimulus.

3.7.3. Stimulus

Laser: this dropdown menu defines the laser to be used for the current stimulus.

Position [mm]: this spin box defines the location of the fiber emitting region for the current stimulus; the length specify the distance between the taper tip and the emitting region.

Power [%]: this spin box defines the power of the emitted light for the current stimulus.

Delay [ms]: this spin box defines the temporal onset of the stimulus in definition. A graphical definition of Delay is shown in Figure 9.

Duration [ms]: this spin box defines the total duration of the stimulus in definition. A graphical definition of Duration is shown in Figure 9.

Add sample: this button add the stimulus to the protocol.

Update plot: this button refresh the Protocol visualization panel.

Generate: this button generates and saves the defined protocol.

Reset: this button resets the dialog to restart the protocol definition from the beginning.



Figure 9. Definition of Delay (in orange) and Duration (in green) for two example stimuli.

3.7.4. Protocol Notebook

All the stimulus added to the protocol are summarized in the Protocol Notebook, and the Total duration [ms] of the protocol period is shown in the upper right corner. The Actions buttons allow to individually edit (pencil button) or remove (trash bin button) stimulus.

3.7.5. Protocol Visualization

The Protocol Visualization panel shows a graphic summary of the defined protocol and allow the user to navigate the plot by dragging (moving across the horizontal temporal axis) or scrolling (zooming in and out) with the mouse. A context menu with basic visualization and navigation function can be opened with a right-click on the panel.

3.8 New/Edit site selective fiber photometry protocol dialog

By using this dialog the user can define a new site selective fiber photometry protocol or edit an existing one. This dialog is organized in panels as shown in Figure 10.

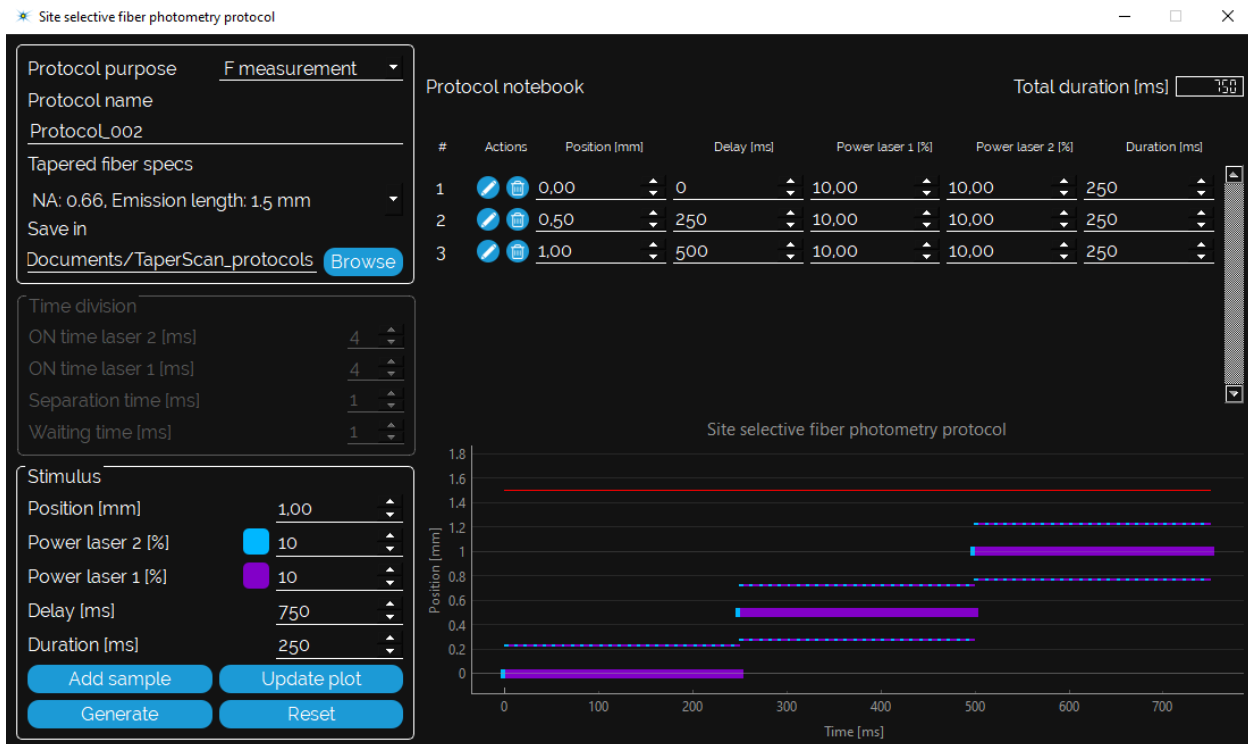


Figure 10. Panels of the Site selective fiber photometry protocol dialog.

3.8.1. Protocol specs

Protocol name: the name of the protocol that will be used as filename when the protocol is generated and saved.

F0 initialization: if checked the protocol is treated as an initialization protocol to evaluate the fluorescence reference level that can be used to compute $\Delta F/F$ values.

Tapered fiber specs: this dropdown menu allow to select the specifications of the tapered fiber used in the protocol, in order to load the proper calibration of the fiber light emitting region position.

Save in: show the path where the protocol will be saved, selected by using the Browse button.

3.8.2. Time division

This panel is active only before the first stimulus is added to the protocol and allow to define the time division multiplexing parameters.

ON time Laser 2 [ms]: duration of illumination from Laser 2.

ON time Laser 1 [ms]: duration of illumination from Laser 1.

Separation time [ms]: duration of the non-illumination period between the illumination from Laser 2 and the illumination from Laser 1.

Waiting time [ms]: duration of the non-illumination period after the illumination from Laser 1.

3.8.3. Stimulus

Position [mm]: this spin box defines the location of the fiber emitting region for the current stimulus; the set value specifies the distance in millimeters between the taper tip and the emitting region.

Power laser 2 [%]: this spin box defines the power of the light emitted from Laser 2 for the current stimulus.

Power laser 1 [%]: this spin box defines the power of the light emitted from Laser 1 for the current stimulus.

Delay [ms]: this spin box defines the temporal onset of the stimulus in definition.

Duration [ms]: this spin box defines the total duration of the stimulus in definition.

Add sample: this button add the stimulus to the protocol.

Update plot: this button refresh the Protocol visualization panel.

Generate: this button generates and saves the defined protocol.

Reset: this button resets the dialog to restart the protocol definition from the beginning.

3.8.4. Protocol Notebook

All the stimulus added to the protocol are summarized in the Protocol Notebook, and the total time of the Total duration [ms] of

the protocol period is shown in the upper right corner. The Actions buttons allow to individually edit (pencil button) or remove (trash bin button) stimulus.

3.8.5. Protocol Visualization

The Protocol Visualization panel shows a graphic summary of the defined protocol and allow the user to navigate the plot by dragging (moving across the horizontal temporal axis) or scrolling (zooming in and out) with the mouse. A context menu with basic visualization and navigation function can be opened with a right-click on the panel.

3.9 DAQ settings dialog

The DAQ settings dialog, shown in Figure 11, allows the user to edit the parameters of data acquisition and visualization.

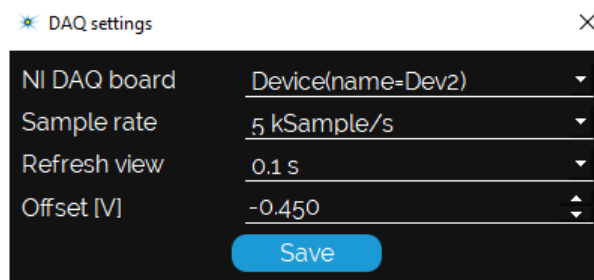


Figure 11. DAQ settings dialog.

NI DAQ board: this dropdown menu allow to select the DAQ board driving the GalvoStation.

Sample rate: this dropdown menu allow to select the data acquisition sample rate.

Refresh view: this dropdown menu allow to select the refresh rate of the data visualization plots on TaperScan main window.

Offset [V]: the offset value of the GalvoStation in use need to be input in this spin box.

3.10 Laser colors dialog

The Laser colors dialog, shown in Figure 12, allows the user to customize the visualization color of the laser sources within the software.

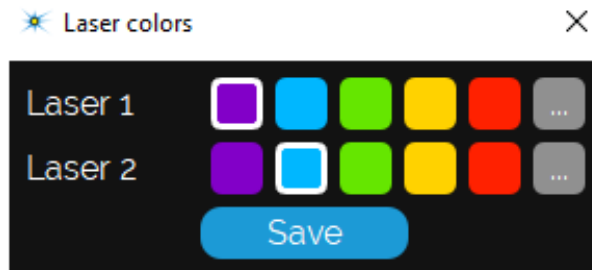


Figure 12. Laser colors dialog.

A pre-set color as well as a custom color (by using the [...] button) can be selected.

Chapter 4 Operating the GalvoStation with TaperScan

When operating the GalvoStation with TaperScan ensure that no timeout for turning off the screen or putting the system in sleep are set in the computer operating system options.

The following instructions assume that all the system components were connected as illustrated in the GalvoStation User Manual.

Please note that to run TaperScan correctly the GalvoStation system needs to be turned on.

4.1 Manual mode

When operating in manual mode, the GalvoStation is driven directly from the Manual tab in the Hardware Control panel and commands are sent immediately to the system. Note that only one laser at a time can be used in manual mode.

- 1) Start up the GalvoStation system as described in the corresponding section of the GalvoStation User Manual.
- 2) Start TaperScan.
- 3) In the Hardware control panel, select the Manual tab (see section 3.2).
- 4) In the Hardware control panel, select the appropriate fiber from the Tapered fiber specs dropdown menu.
- 5) In the Hardware control panel, select the laser to be used for light delivery from the Active laser dropdown menu. **For single laser version:** ensure that the selected laser is Laser 1.
- 6) Ensure that the Laser LED status on laser drivers dropdown menu in the Laser status panel (see section 3.4) matches the status of the green LASER indicators on the laser drivers.
- 7) The Laser emission ON/OFF buttons in the Hardware control panel act as a virtual shutter: when the OFF button is checked

the laser power is set to 0; when the ON button is checked the laser power is set to the value specified in the Power [%] spin box.

- 8) Change the value in the Power [%] spin box in the Hardware control panel to modify the laser output power: if the Laser emission is OFF, no change on the laser power happens at this moment; if the Laser emission is ON the specified output power is immediately set.
- 9) Change the value in the Position [mm] spin box in the Hardware control panel to modify the emission position along the taper. The specified position is immediately set and the active subsite visualized in the Data visualization panel is updated.
- 10) Use the Start/Stop measure buttons in the Hardware control panel to start and stop acquisition from the photodetector and save data accordingly to the values specified in the Save options panel. When the Start button is pressed the acquisition immediately start if a file with the same name does not exist, otherwise a confirmation for overwriting dialog is displayed. When the Stop button is pressed the acquisition immediately stops and the collected data are saved.

4.2 Protocol Mode

When operating in protocol mode, the GalvoStation is driven accordingly to a previously defined protocol for a chosen number of iterations.

4.2.1 Protocol definition – site selective light delivery

The site selective light delivery protocol is used when the user intends to specify the light delivery spatiotemporal pattern. To define a new protocol use the New site selective light delivery protocol item in the File menu or click on the New protocol button in the Protocol tab of the Hardware control panel (see section 3.2)

when the Site selective light delivery radio button is checked. The New site selective light delivery protocol dialog is displayed (see section 3.7).

- 1) Insert the protocol name in the Protocol name field.
- 2) Select the appropriate fiber from the Tapered fiber specs dropdown menu.
- 3) Specify the folder where the protocol should be saved in the Save in field by using the Browse button.
- 4) Use the Stimulus type and Stimulus panels to define the parameter for a sample of the protocol (see Section 3.7 for the definition of each field).
- 5) When the sample parameters are defined use the Add sample button to add the stimulus to the protocol. A new line corresponding to the sample appears in the Protocol Notebook and the Protocol Visualization is updated.
- 6) Repeat steps 4-5 until the desired protocol is completely defined.
- 7) Use the Actions buttons to individually edit (pencil button) or remove (trash bin button) already defined samples.
- 8) Use the Generate button to save the protocol accordingly to the values specified at steps 1, 3. If the protocol is saved correctly a confirmation dialog is displayed and three files with extension *.oprt*, *.oprtf*, and *.oprtw* will be saved in the specified directory.
- 9) Use the Reset button to restart the protocol definition. All specified samples will be deleted.
- 10) Use the Update plot button to force the update of the Protocol Visualization.
- 11) Close the New site selective light delivery protocol dialog.

A previously defined protocol can be edited by using the Edit site selective light delivery protocol item in the File menu or by clicking the Edit protocol button in the Protocol tab of the

Hardware control panel when the Site selective light delivery radio button is checked and the protocol is selected.

4.2.2 Protocol definition – site selective fiber photometry

The site selective fiber photometry protocol is used when the user intends to perform a time multiplexed fiber photometry experiment, e.g. to excite a fluorescent reporter of neuronal activity with temporally interleaved light pulses at two wavelengths to measure both functional fluorescence and an isosbestic control signal. To define a new protocol use the New site selective fiber photometry protocol item in the File menu or click on the New protocol button in the Protocol tab of the Hardware control panel (see section 3.2) when the Site selective fiber photometry radio button is checked. The New site selective fiber photometry protocol dialog is displayed (see section 3.8).

- 1) Select the purpose of the protocol under definition from the Protocol purpose dropdown menu. The option F0 initialization is used to specify a set of samples that will be executed only once at the beginning of the protocol to define time intervals for the evaluation of F0 reference; the option F measurement is used to specify a set of samples that will be repeated for a specified number of times to readout functional fluorescence level.
- 2) Insert the protocol name in the Protocol name field.
- 3) Select the appropriate fiber from the Tapered fiber specs dropdown menu.
- 4) Specify the folder where the protocol should be saved in the Save in field by using the Browse button.
- 5) If needed, edit the time division multiplexing parameters in the Time division panel by checking the corresponding checkbox (see Section 3.8 for the definition of each field). The default values are recommended. Note that the time division

multiplexing parameters are defined once for the whole protocol.

- 6) Use the Stimulus panel to define the parameter for a sample of the protocol (see Section 3.8 for the definition of each field).
- 7) When the sample parameters are defined use the Add sample button to add the stimulus to the protocol. A new line corresponding to the sample appears in the Protocol Notebook and the Protocol Visualization is updated.
- 8) Repeat steps 6-7 until the desired protocol is completely defined.
- 9) Use the Actions buttons to individually edit (pencil button) or remove (trash bin button) already defined samples.
- 10) Use the Generate button to save the protocol accordingly to the values specified at steps 1, 2, 4. If the protocol is saved correctly a confirmation dialog is displayed and three files with extension *.pprt*, *.pprtf*, and *.pprtw*, or three files with extension *.pprti*, *.pprtfi*, and *.pprtwi*, for F measurement and F0 initialization purpose, respectively, will be saved in the specified directory.
- 11) Use the Reset button to restart the protocol definition. All specified samples will be deleted.
- 12) Use the Update plot button to force the update of the Protocol Visualization.
- 13) Close the New site selective light delivery protocol dialog.

When a site selective fiber photometry protocol with both F0 initialization and F measurement is defined, the names specified in the Protocol name field and all time division multiplexing parameters should match.

A previously defined protocol can be edited by using the Edit site selective fiber photometry protocol item in the File menu or by clicking the Edit protocol button in the Protocol tab of the

Hardware control panel when the Site selective fiber photometry radio button is checked and the protocol is selected.

4.2.3 Running a protocol

- 1) Start up the GalvoStation system as described in the corresponding section of the GalvoStation User Manual.
- 2) Start TaperScan.
- 3) In the Hardware control panel, select the Protocol tab.
- 4) In the Hardware control panel, check the Site selective light delivery or Site selective fiber photometry radio button according to the protocol intended to be run.
- 5) Use the Select protocol button to browse for a protocol to load. The selected protocol, the fiber specs related to the protocol, and the protocol period length appear in the corresponding fields of the Hardware control Panel. The Protocol Visualization panel is updated with a graphic summary of the selected protocol.
- 6) Specify the number of time the protocol period should be repeated in the Repetitions spin box of the Hardware control panel. The total time needed for the protocol completion is updated accordingly. For site selective fiber photometry protocols the number of repetitions affects the F measurement portion only: the F₀ initialization is executed only once at the beginning of the protocol.
- 7) Ensure that the Laser LED status on laser drivers dropdown menu in the Laser status panel matches the status of the green LASER indicators on the laser drivers.
- 8) Use the Start button in the Hardware control panel to begin the control of the GalvoStation with the selected protocol. Photodetector and digital port signals will be saved accordingly to the values specified in the Save options panel. When the Start button is pressed the protocol immediately start if a file for saving the data with the same name does not exist, otherwise a confirmation for overwriting dialog is

displayed. When the protocol reach completion, a dialog is displayed.

- g) Use the Stop button to end the execution of the protocol at any given time before completion.

4.3 Probe patch cord alignment

After the assembling of the system an alignment procedure for the Probe patch cord is required to guarantee the proper functionality of the GalvoStation. To run the alignment procedure with TaperScan follow the indications below.

- 1) Start up the GalvoStation system as described in the corresponding section of the GalvoStation User Manual.
- 2) Start TaperScan.
- 3) In the Hardware control panel, select the Manual tab.
- 4) In the Hardware control panel, select the Direct drive option from the Tapered fiber specs dropdown menu.
- 5) Select LASER 1 from the Active laser dropdown menu.
- 6) Ensure that the Laser LED status on laser drivers dropdown menu in the Laser status panel matches the status of the green LASER indicators on the laser drivers.
- 7) Change the value in the Power [%] spin box in the Hardware control panel to 20%.
- 8) Change the value in the Position [mm] spin box in the Hardware control panel to 0mm.
- 9) Remove the cap from the Probe patch cord.
- 10) Place the distal end of the Probe patch cord a few centimeter away from a piece of white paper.
- 11) Check the Laser emission ON button in the Hardware control panel: a weak laser emission should be visible on the white paper.
- 12) Slightly unscrew the Probe patch cord from the PROBE receptacle of the GalvoStation.

- 13) Gently rotate the SMA connector, without re-engaging the thread, and find the position which minimize the radius of the light disk projected on the piece of white paper.
- 14) Gently tighten the SMA connector of the Probe patch cord to the PROBE receptacle of the GalvoStation, trying to keep the fiber in the position that minimized the light disk.
- 15) Plug the cap on the Probe patch cord.
- 16) Check the Laser emission OFF button in the Hardware control panel to close the virtual shutter.

**NOTICE**

In the event that the Probe patch cord is replaced or unplugged, the alignment procedure needs to be repeated.

4.4 Calibration

Due to unavoidable tolerances of the assembling process of the GalvoStation, the patch cords, and the Lambda-plus fiber cannulae, a calibration procedure for each Lambda-plus fiber batch is needed to ensure that the light emitting region is scanned along the tapers active length with a positioning error as low as possible. To run the calibration procedure with TaperScan follow the indications below.

- 1) Place the Calibration slide provided with the current Lambda-plus fiber batch under an epifluorescence microscope equipped with a camera and with an objective giving the system a field of view large enough to frame the full extension of the Lambda-plus fiber active length, and a filter set suitable for the wavelengths in use (e.g. PBS:fluorescein solution and FITC filter for blue light).

- 2) Drop onto the Lambda-plus fiber of the calibration slide a sufficient amount of solution of fluorophore suitable for the wavelength in use.
- 3) Plug the Probe patch cord to the Lambda-plus fiber on the Calibration slide.
- 4) Start up the GalvoStation system as described in the corresponding section of the GalvoStation User Manual.
- 5) Start TaperScan.
- 6) In the Hardware control panel, select the Manual tab.
- 7) In the Hardware control panel, select the Direct drive option from the Tapered fiber specs dropdown menu.
- 8) Select LASER 1 from the Active laser dropdown menu.
- 9) Ensure that the Laser LED status on laser drivers dropdown menu in the Laser status panel matches the status of the green LASER indicators on the laser drivers.
- 10) Change the value in the Power [%] spin box in the Hardware control panel to 20%.
- 11) Change the value in the Position [mm] spin box in the Hardware control panel to 0mm.
- 12) Check the Laser emission ON button in the Hardware control panel: laser emission from the Lambda fiber should be visible.
- 13) Acquire an image with the microscope camera. Be sure to have a sharp focus on the fiber.
- 14) Repeat steps 11, 13 by changing the value in the Position [mm] spinbox in the Hardware control panel, increasing it by a constant amount (e.g. 0.5mm) up to 6.5mm.
- 15) Check the Laser emission OFF button in the Hardware control panel to close the virtual shutter.

- 16) Open the saved images as a stack in ImageJ³, and set the spatial scale in px/ μ m.
- 17) Using the *Straight* tool draw a line a few pixel away from the Lambda-plus fiber, following the taper surface, and starting at the tip position.
- 18) Run the "Lambda-plus fiber calibration" macro; you can download the macro code from [OptogeniX website](#)⁴.
- 19) Save the result table in a folder of your convenience.
- 20) Run the Lambda-plus fiber calibrator software; you can download it from [OptogeniX website](#)⁴.
- 21) Load the result table, check the TaperScan checkbox, and run the calibration analysis by clicking on the "Calibrate" button.
- 22) The Lambda-plus fiber calibrator will save a calibration file in its root directory. Rename this file in a convenient way (e.g. the serial number of the Lambda-plus fiber batch you are calibrating for) and copy it in the TaperCalibrations folder in the installation position of TaperScan.
- 23) This calibration can be used for every Lambda-plus fiber shipped together with the Calibration slide you used for the calibration procedure, and remains valid until the Probe patch cord alignment procedure is run again.

**NOTICE**

The calibration obtained through this procedure is valid for a given GalvoStation, a given Probe patch cord, and a given batch of Lambda-plus fiber.

³ ImageJ is a Public Domain software for image processing and analysis. It can be downloaded at <https://imagej.nih.gov/ij/download.html>.

⁴ URL: <https://www.optogenix.com/prodotto/galvostation/>.

4.5 Data format

To guarantee compatibility with a broad range of data analysis software and routines, TaperScan can save data in text (extension *.txt*) or comma-separated value (extension *.csv*) formats, depending on the option selected in the Save options panel. The first row is a header reporting the sample rate at which data was sampled during the acquisition. Each of the subsequent rows represents a data samples, composed by 6 columns: position of the sub-site [mm], power emitted by laser 1 [%], power emitted by laser 2 [%], output voltage of the photodetector [V], input bit at digital port 1, and input bit at digital port 2. The digital port bits are saved only if the corresponding option is checked in the Save options panel.

Chapter 5 Customization

The standard version of TaperScan is designed to allow the definition of dual light source light delivery protocol or time division multiplexed dual light source fiber photometry protocol. We are open to realize customized release of TaperScan to match specific requirements. Contact us for customization support.

Chapter 6 Contacts

For technical support or sales inquiries, please write us at or visit www.optogenix.com.