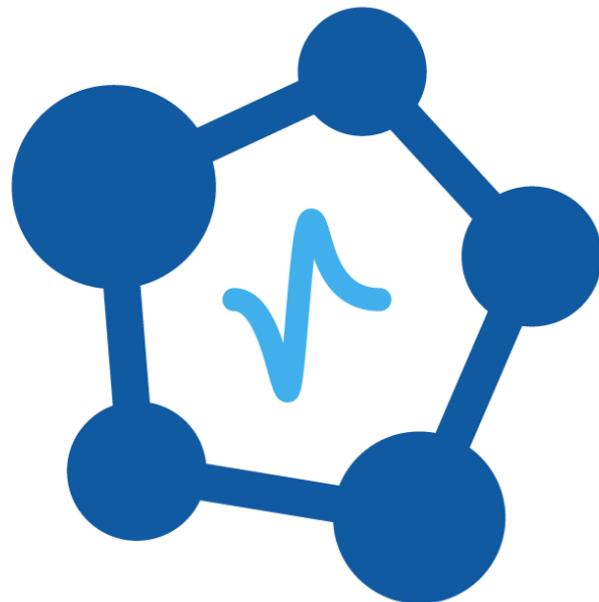




MaxLab Live Manual

MaxWell Biosystems AG



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Chapter 1. Getting Started

Welcome to the MaxLab Live Manual and thank you for using a MaxWell Biosystems product!

This chapter will guide you through the first steps of using MaxLab Live. Its sections will cover:

- How to open the software
- General graphical user interface (GUI) overview
- How to verify proper recording of signals
- How to record and replay data

1. Opening the Software

In order to open the MaxLab Live Software, look for the icons shown in Table 1.1 on the Desktop of your recording PC.

Icon	Name	Description
	Server	This icon opens the MaxLab Live Server , which provides the communication with the measurement system and reads as well as saves the data.
	Scope	This icon opens the MaxLab Live Scope , which provides a graphical user interface (GUI) for controlling the system and visualizing data.
	Stop MEA Software	This icon forces all MaxLab Live components to close and can be used to execute a clean restart of the software.

Table 1.1. MaxLab Live Software Icons

To run MaxLab Live with a measurement system, both MaxLab Live Server and MaxLab Live Scope need to be opened. While MaxLab Live Server represents the back-end tool, which communicates with the system and controls it, reads incoming data and saves it into files; MaxLab Live Scope provides the interface for the user to visualize the data and control the experiments. The reasoning behind splitting the software into these modules is to provide the highest degree of security for ongoing experiments. Even if MaxLab Live Scope is closed during an experiment, the recording of the data will be completed and the files will be saved.

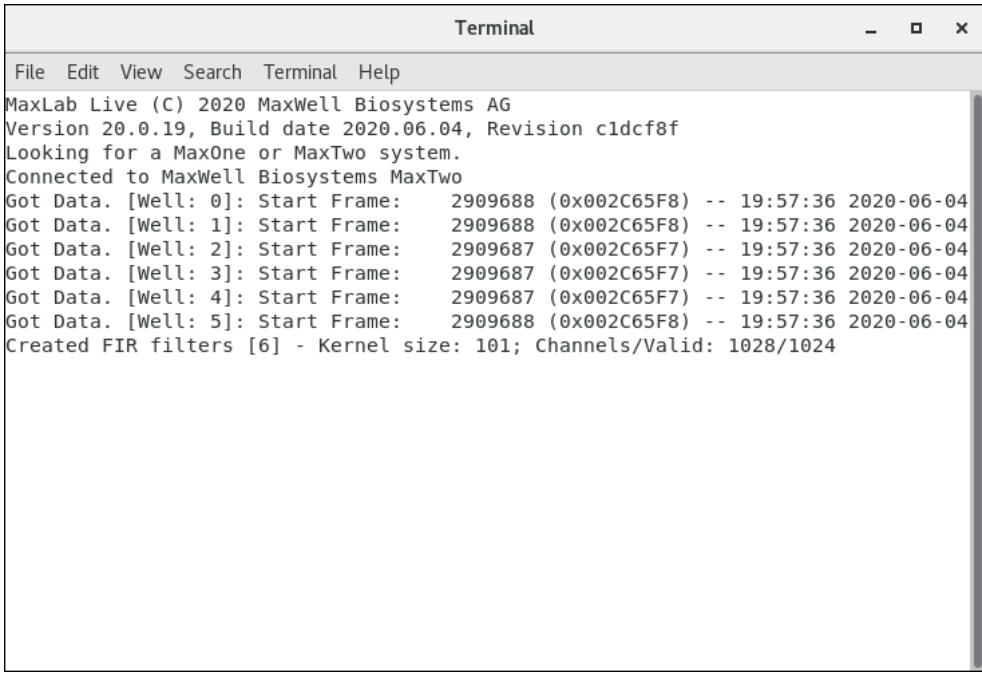
Upon opening **MaxLab Live Server**, a terminal window appears. Figure 1.1 and Figure 1.2 display the terminal window, if MaxLab Live Server is opened when connected to a MaxOne or a MaxTwo system, respectively. If the data stream from the MaxOne System is properly established, the information **Got Data** in the terminal window appears, as shown in Figure 1.1. For a MaxTwo system, the MaxLab Live Server window also indicates which wells of a wellplate are streaming data correctly (Figure 1.2).

```

Terminal
File Edit View Search Terminal Help
MaxLab Live (C) 2020 MaxWell Biosystems AG
Version 20.0.19, Build date 2020.06.04, Revision c1dcf8f
Looking for a MaxOne or MaxTwo system.
Connected to MaxWell Biosystems MaxOne
Got Data. : Start Frame: 6388332709 (0x17CC638A5) -- 20:15:19 2020-06-04
Created FIR filters [1] - Kernel size: 111; Channels/Valid: 1028/1024

```

Figure 1.1. Server Window for MaxOne System



The screenshot shows a terminal window titled "Terminal". The window has a standard OS X-style title bar with minimize, maximize, and close buttons. The menu bar includes "File", "Edit", "View", "Search", "Terminal", and "Help". The main pane displays the following text:

```
MaxLab Live (C) 2020 MaxWell Biosystems AG
Version 20.0.19, Build date 2020.06.04, Revision c1dcf8f
Looking for a MaxOne or MaxTwo system.
Connected to MaxWell Biosystems MaxTwo
Got Data. [Well: 0]: Start Frame: 2909688 (0x002C65F8) -- 19:57:36 2020-06-04
Got Data. [Well: 1]: Start Frame: 2909688 (0x002C65F8) -- 19:57:36 2020-06-04
Got Data. [Well: 2]: Start Frame: 2909687 (0x002C65F7) -- 19:57:36 2020-06-04
Got Data. [Well: 3]: Start Frame: 2909687 (0x002C65F7) -- 19:57:36 2020-06-04
Got Data. [Well: 4]: Start Frame: 2909687 (0x002C65F7) -- 19:57:36 2020-06-04
Got Data. [Well: 5]: Start Frame: 2909688 (0x002C65F8) -- 19:57:36 2020-06-04
Created FIR filters [6] - Kernel size: 101; Channels/Valid: 1028/1024
```

Figure 1.2. Server Window for MaxTwo System

Clicking on the **Scope** icon starts the user interface MaxLab Live Scope, which is explained in the next section.

Notes:

- MaxLab Live Server automatically detects whether a MaxOne or MaxTwo system is connected to the PC. In case no system is found, Server and Scope are opened for the last system type (MaxOne or MaxTwo) that was used with the PC.
- In order to inspect recorded assays (see Chapter 6) and to run analyses (see Chapter 8), MaxLab Live Scope can be used without being connected to a measurement system and without MaxLab Live Server.
- However, in order to use the **Replay Mode** (see Section 4 of this chapter), MaxLab Live Server needs to be running.

2. User Interface Overview

The main window of the MaxLab Scope graphical user interface (GUI) is organized in the following way:

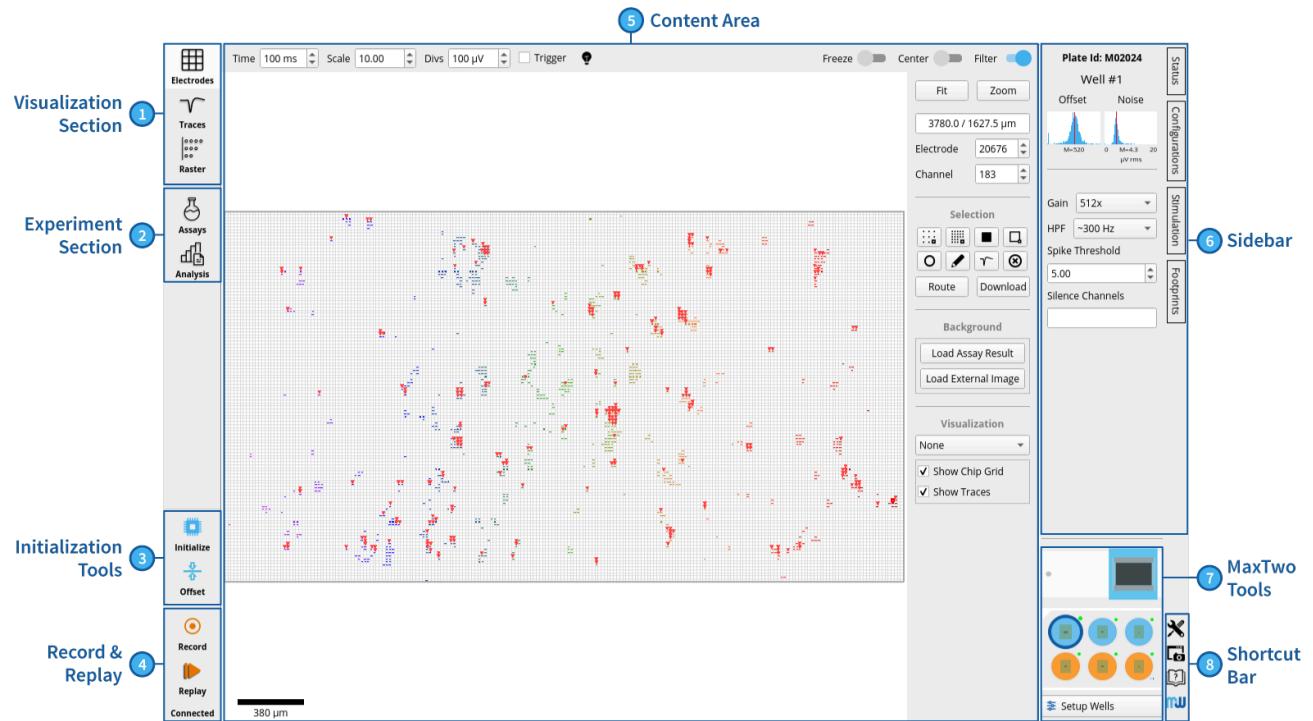


Figure 1.3. Overview of the User Interface

(1) Visualization Section

The buttons of this section allow to switch between the different modes for visualizing live data from the measurement system. The three modes are:

- Electrodes View - Displays the recorded signals at their respective position on the array
- Traces View - Displays the signal traces in individual, configurable windows
- Raster View - Displays the detected action potential (AP) events in a live raster plot mode

For more information, see Chapter 3.

(2) Experiment Section

The Experiment Section gives access to the interface for performing and analyzing automated experiments and is composed of two parts:

- Assays - Configure and execute predefined automated experiments (or assays) and visualize their results
- Analysis - Run custom analyses on recorded assays

For more information, see Chapter 6 - Chapter 9.

(3) Initialization Tools

The following tools are typically used when a new chip or wellplate is connected:

- Initialize - Initializes and resets the connection to the chip or wellplate, respectively
- Offset - Executes the on-chip automatic offset compensation routine

For more information, see Section 3.

(4) Record & Replay

Quick controls for the following actions:

- Record - Start to record streamed data
- Replay - Replay a recorded file

For more information, see Section 4.

(5) Content area

The content and interface particular to the different modes in the Visualization and Experiment Sections is displayed inside the content area.

(6) Sidebar

The Sidebar on the right side of the GUI is used to access the side tabs for basic and advanced operations:

- Status Tab - Display important information about the status and settings of the chip
- Configurations Tab - Select and display predefined and custom electrode configurations
- Stimulation Tab - Apply specified stimulation pulses at selected electrodes
- Footprints Tab - Identify individual neuronal units and their average extracellular waveform (or *footprint*) on neighboring electrodes

For more information, see Chapter 4.

(7) MaxTwo Tools

The icons in this section are only available if MaxLab Live is used with a MaxTwo system. It contains three parts:

- MaxTwo Hub Control - Controls MaxTwo hardware and indicates status
- MaxTwo Well Selection Tool - Allows to select individual wells for visualization and to display well-specific data status
- MaxTwo Wellplate Editor - Used to enter and log information about the individual wells

For more information, see Chapter 2.

(8) Shortcut Bar

The four shortcut buttons in the lower right corner of the GUI give access to:

- Settings - Open the window to control the system settings
- Screenshot - Acquire a screenshot of the current GUI window
- Documentation - Open the MaxLab Live User Manual
- About - Display the software version and check for updates

3. Operational Checkout

This section will guide you through the steps to verify that the system is set up properly and that you can record clean signals. Table 1.2 introduces functionalities that can be used when starting measurements from a newly mounted MaxOne chip or MaxTwo wellplate.

Icon	Name	Description
	Initialize	This button initializes the connection to the mounted chip or wellplate. The user is required to enter the chip-ID or wellplate-ID, which is essential for correct logging and storing of the recorded data. After initialization, the system starts to stream data from the chip/wellplate with a sparse configuration of recording electrodes.
	Offset	After initializing a new chip/wellplate or changing the configuration of recording electrodes, the recorded signals might feature DC-offsets (see below). This button starts the automatic Offset Compensation Routine that significantly reduces the DC-offset per recording channel. Note that this step is not required when using the MaxLab Live Assays (Chapter 7) for recordings, as this step is incorporated in the assay workflow.

Table 1.2. Initialization Tools

It is recommended to run the Operational Checkout while the wells being tested are filled with Phosphate Buffered Saline (PBS). This allows to inspect the signal characteristics in the absence of biological signals and to obtain an accurate estimate of the noise levels.

Before starting the Operational Checkout, please ensure that:

- The measurement system is installed and properly connected to the PC
- MaxLab Live software is updated to the latest version
- A MaxOne chip or a MaxTwo wellplate is mounted on the measurement system and the wells are filled with 1 ml of PBS
- MaxLab Live Server and Scope are running

Next, follow these steps:

1. Start measuring signals from the chip by pressing the **Initialize** button. A dialog window appears and asks you to specify the chip-ID or wellplate-ID.
2. The software now connects to the chip and starts streaming the signals, which are seen as small traces:

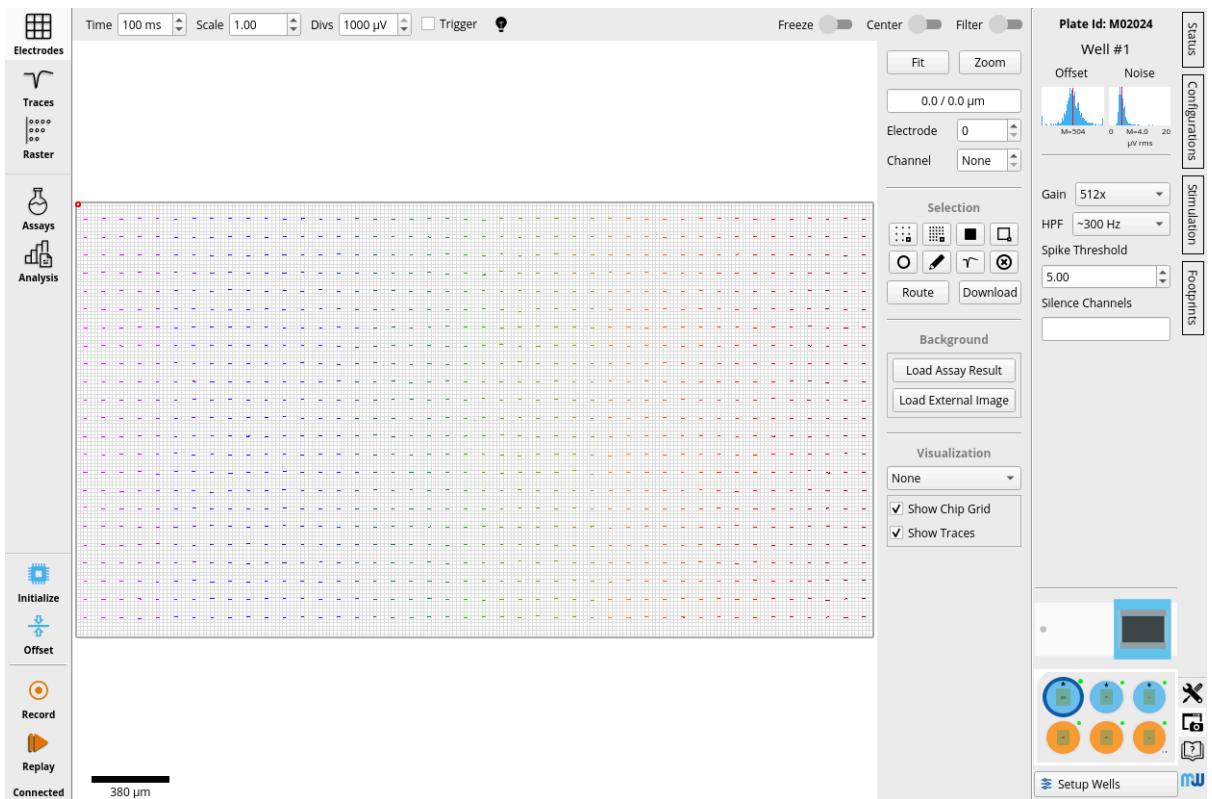


Figure 1.4. Electrodes View after Chip Initialization

If you are using a MaxTwo system, the displayed signals correspond to the marked well in the Well Selection Tool on the bottom right corner. During the subsequent steps of the Operational Checkout, you can switch anytime between selected wells to ensure proper functioning on all the wells.

3. In order to visually inspect the signals, change the view of the software by pressing the **Traces** button on the top right corner.
4. The signals are now displayed in individual rectangular windows. In order to display the data from all the channels in one single window, press the **1x1** button on the bottom left. You should now see the overlay of the traces from all the recording channels:

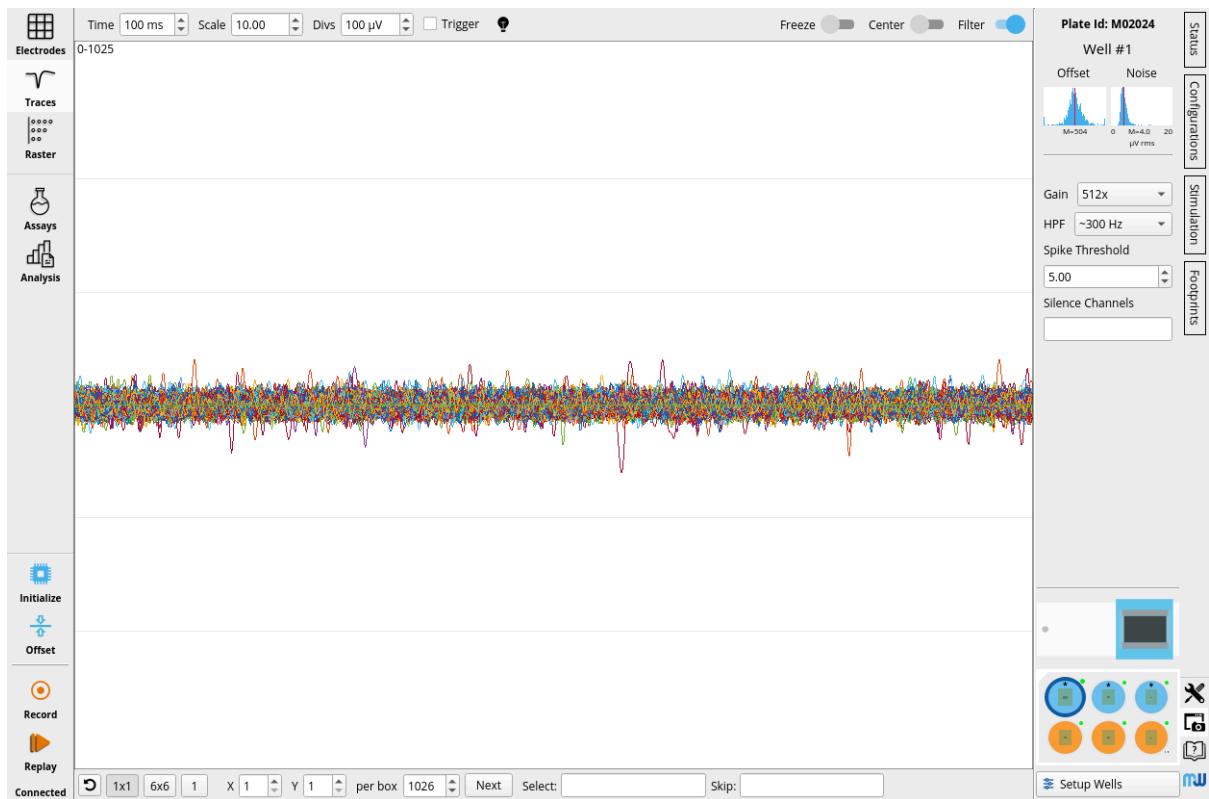


Figure 1.5. Filtered Signals from all Recording Channels in Traces View

5. The signals displayed in Figure 1.5 are band-pass-filtered by the software between 300 Hz and 3 kHz (see Chapter 5 for further information on how to modify the cutoff frequencies of the filter). Turn off the band-pass filter by clicking on the blue switch **Filter** in the top right corner. The traces will now appear vertically distributed over the signal display (Figure 1.6). If not visible yet, open the Status Tab by clicking on the **Status** button at the top right of the software window. On top of the Status Tab, two histograms display the distributions of the channel offset values and the root mean square (RMS) noise values.

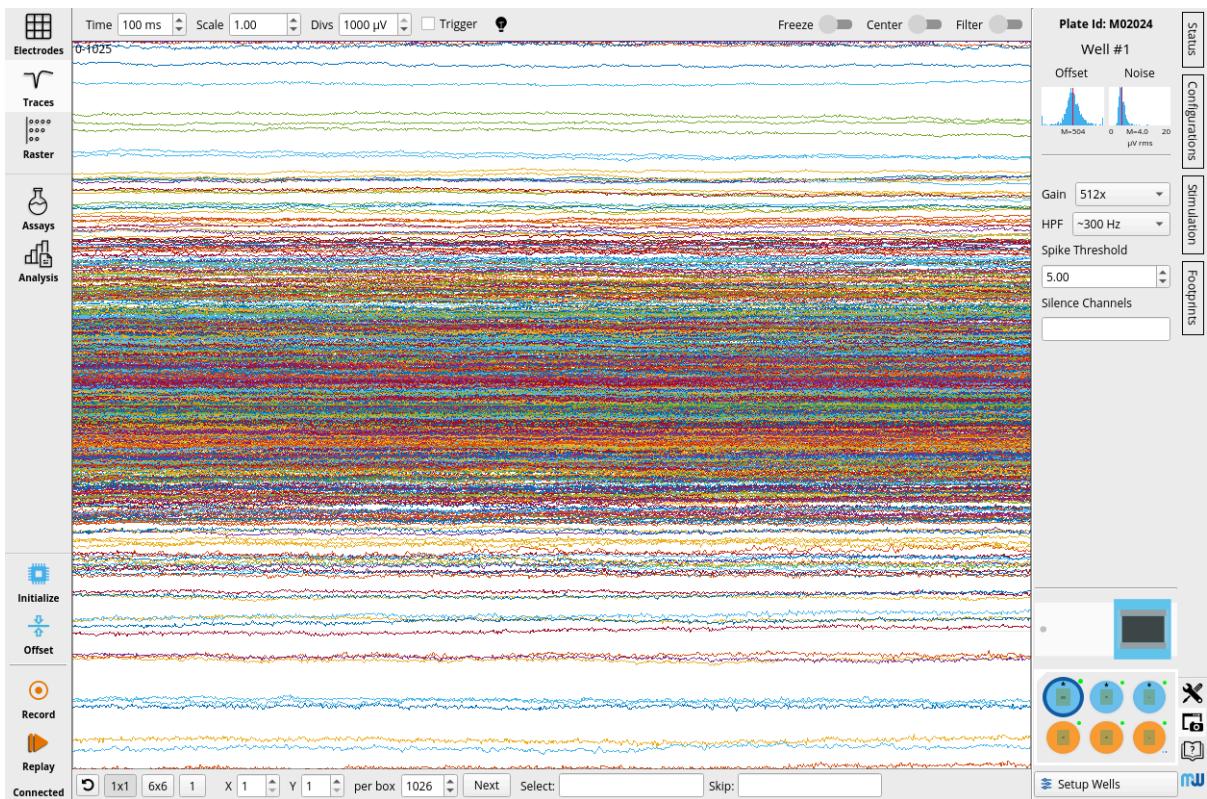


Figure 1.6. Signal Traces without Filtering

6. Figure 1.6 displays the signals as they are measured by the system. The DC offset of the individual recording channels cause a vertical spread of the signals across the measuring range (Y-axis). These offsets are also visible in the relatively broad distribution of the values in the left histogram of the Status Tab. Note that incoming light also affects channel offset and that changes in the ambient light conditions typically cause signals to move up and down. In order to ensure constant conditions, keep the MaxTwo wellplate or the MaxOne well, respectively, covered. This ensures that the signal baselines remain stable.
7. Next, press the **Offset** button on the lower left side of the window to run the automatic on-chip offset compensation routine, which significantly reduces the DC-offset per recording channel.

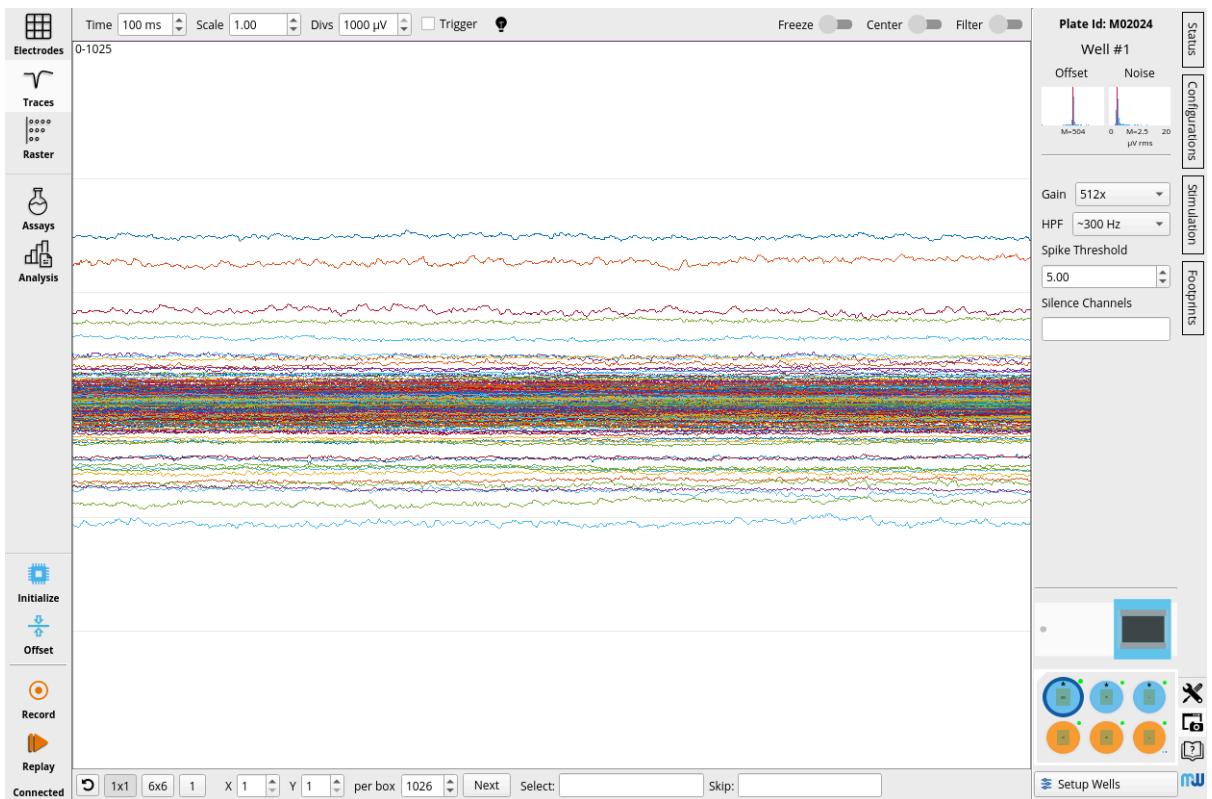


Figure 1.7. Signal Inspection after Offset Compensation

8. After a few seconds, the signal traces should become vertically arranged around the center of the traces window (Figure 1.7). Accordingly, the left histogram in the Status Tab should now show a sharp peak instead of a broad distribution.
9. Finally, verify that the signals do not oscillate and that the average noise value, which is indicated by the red line in the right histogram of the Status Tab, lies between 0 and 5 μV_{RMS} (root mean square).

4. Record and Replay

In this section, the buttons for quick data recording and replaying of recorded data at the bottom left of the software window are introduced (see Table 1.3).

Icon	Name	Description
	Record	This button starts an instantaneous recording of the data with the current electrode configuration. The recording can be stopped by clicking the same button again.
	Replay	This button can be used to replay recorded data. In the replay mode, all tools for real-time visualization, as well as the Footprint Tab functionality are available.
	Stop Replay	Stop the replay mode.

Table 1.3. Record and Replay Buttons

Chapter 6 will describe how to use **Assays** to run automatized and standardized recordings and to organize your data. As opposed to the Assays, where configuration and recording time are set before starting the recording, the **Record** button allows to start a recording instantaneously and to manually stop it. This can be especially useful when interesting data is observed and one wants to immediately start recording. This manual recording mode is also often used in combination with voltage stimulation (see Chapter 4, Section 4).

Once data has been recorded, either with the **Record** button or by using an assay, the data can be replayed in the GUI. In the Replay mode, the data is visualized in the same way as during recording. Thus, all visualization views, as well as the footprint visualization with the Footprint Tab can be used. The Replay mode is often used to inspect the data in more detail.

In some cases, one recording file can contain more than one individual recorded dataset. For example, a measurement with a MaxTwo wellplate can contain up to six datasets which were recorded simultaneously. The user will then be able to select which recording to replay (Figure 1.8). Meanwhile, an Activity Scan Assay combines datasets from sequential recording configurations into one file. When replaying such a file, the user needs to specify which dataset to replay.

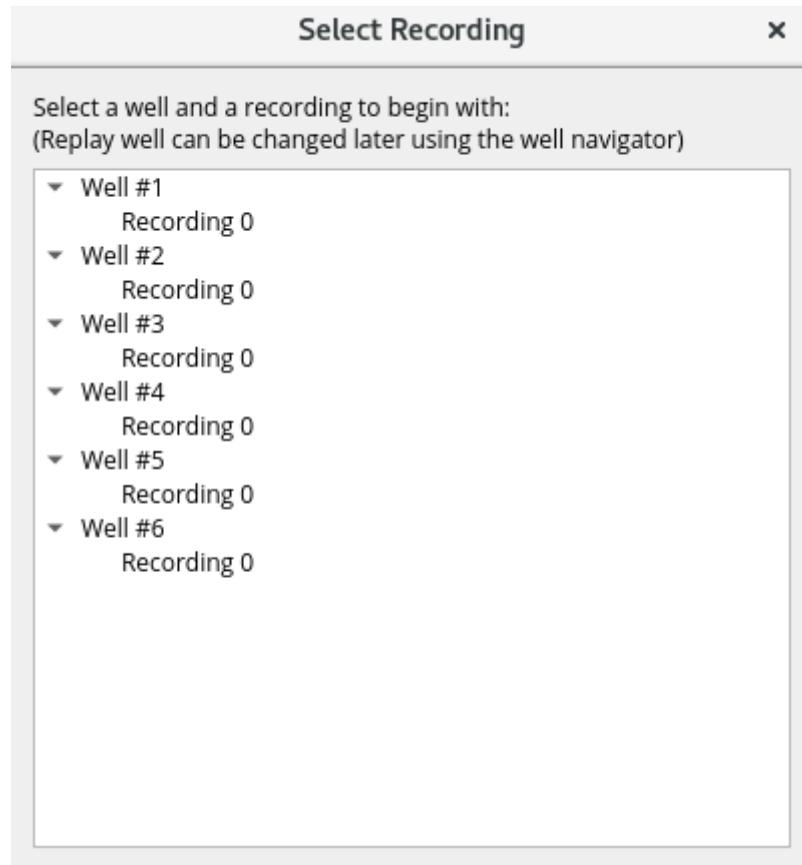


Figure 1.8. Select a Dataset for Replay

Note: in order to use the Replay mode, the Server needs to be open.

Chapter 2. MaxTwo Tools

The MaxLab Live software can be used for both the MaxOne and MaxTwo systems with the same features for visualizing signals, running experiments and analyzing data. However, when using MaxLab Live with a MaxTwo system, some particular GUI elements are available which allow to control the MaxTwo hardware and to visualize and record from multiple wells. This chapter highlights and describes these MaxTwo-specific GUI elements.

1. MaxTwo Tools Overview

The elements of the user interface which are specific to controlling and handling a MaxTwo system can be found in the bottom right area of the MaxLab Live window and are shown in Figure 2.1.

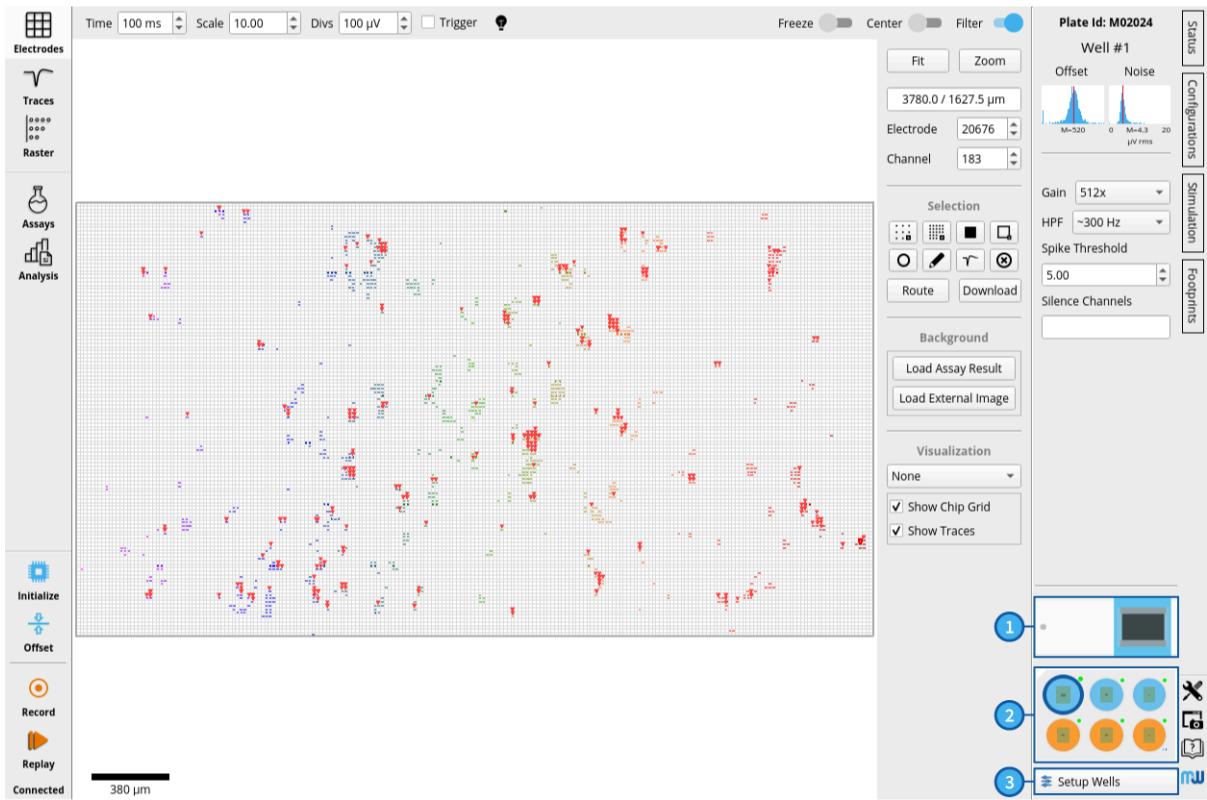


Figure 2.1. MaxTwo Tools Overview

(1) MaxTwo Hub Control

This module allows the user to operate the hub through MaxLab Live and check the hardware status of MaxTwo. Clicking on this icon opens the MaxTwo Hub Control dialog window described in Section 2 of this chapter. In the case of absence of a connection to the Hub, this icon will turn gray and display a plug disconnected from its socket.

(2) MaxTwo Well Selection and Status

Here, the user can select which well is displayed in the Electrodes, Traces and Raster Views by clicking on the desired well. The selected well is marked with a blue ring. The green dot next to the well indicates that the well is properly streaming data. The meaning of the indicator lights are further described in Section 3 of this chapter.

(3) MaxTwo Wellplate Editor

The MaxTwo Wellplate Editor allows the user to input and store information about well content and is described in Section 4.

2. MaxTwo Hub Control

The MaxTwo Hub Control allows the user to control the hub through MaxLab Live and check on the status of MaxTwo.

Summary View

Figure 2.2 shows the summary view of the MaxTwo Hub Control dialog window.

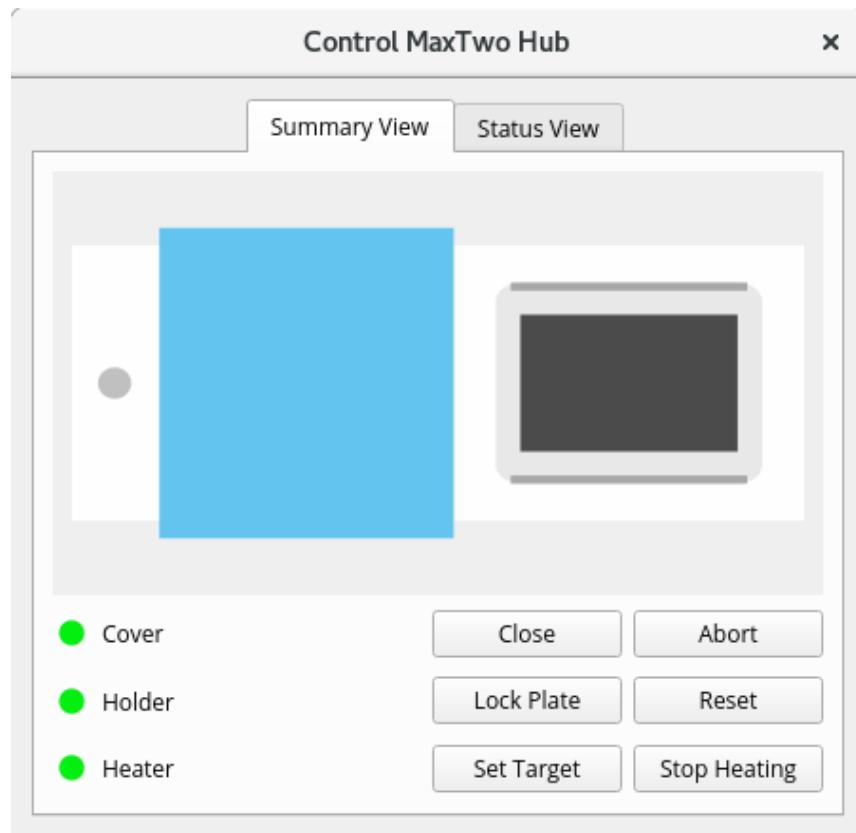


Figure 2.2. MaxTwo Hub Control - Summary View

The user interface's top section has a representation of the MaxTwo Hub as seen from above. In the **Summary View**, the blue rectangle corresponds to the cover, the dark gray rectangle to the incubation chamber and the two light gray rectangles to the lock of the wellplate holder. The bottom section allows the user to control the MaxTwo Hub **Cover**, **Holder**, and **Heater** through MaxLab Live. Indicators on the side of the three sections mark correct functioning (green), change of state (yellow), or malfunctioning (purple).

The Open/Close buttons related to **Cover** open and close the cover: when clicked, the corresponding indicator turns to yellow to indicate a change of state, reverting to green once the cover is fully opened/closed. The blue rectangle representing the cover shifts to symbolize an open or closed cover, respectively. The **Abort** button allows for emergency stopping and resetting of cover movement.

The Open/Close button related to **Holder** lock and release the wellplate in place: when clicked, the corresponding indicator turns to yellow to indicate a change of state, reverting to green once the holder is opened/locked. The light gray rectangles representing the holder become thinner or thicker to symbolize open or locked holder, respectively. The **Reset** button enables automatic calibration of the holder.

The **Set Target** button related to **Heater** allows to set the desired temperature within the incubation chamber (in °C). The **Stop Heating** button interrupts the heating.

Status View

Figure 2.3 shows the Status View in the MaxTwo Hub Control user interface.

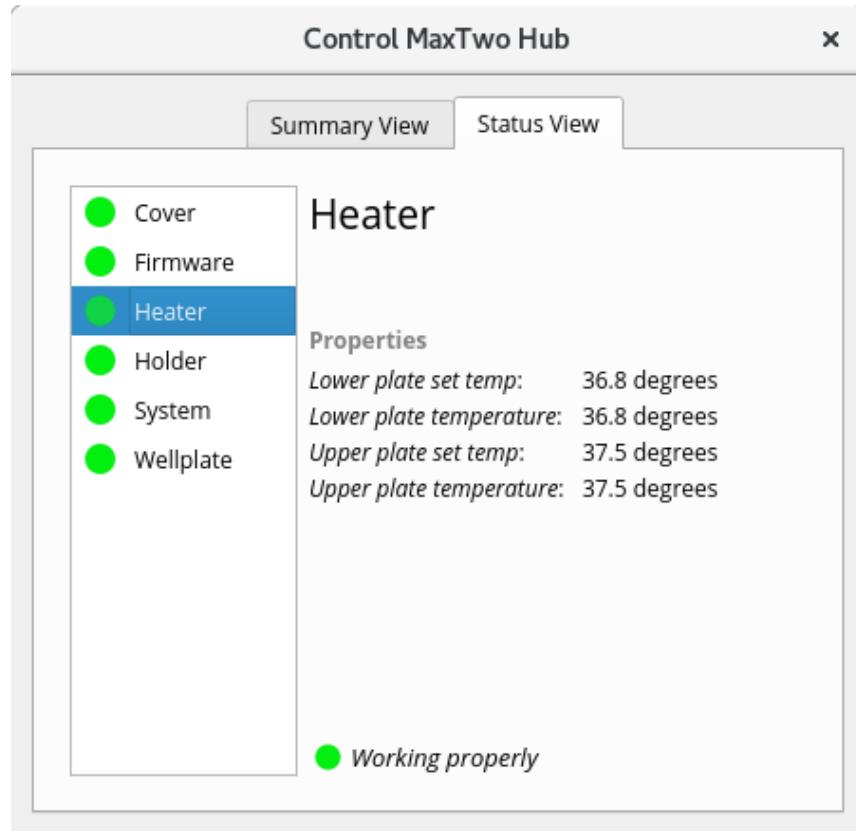


Figure 2.3. MaxTwo Hub Control - Status View

The Status View contains detailed status information of the MaxTwo Hub. It can be accessed by either clicking on the corresponding tab, or double clicking on the section of interest in the Summary View (for **Cover**, **Holder**, and **Heater**). As in the Summary View, the indicators on the side indicate correct functioning (green), change of state (yellow), or malfunctioning (purple). Each section of the left panel can be accessed by clicking on it, which displays its properties. The **Cover** section refers to the status of the cover (Open or Closed); the **Firmware** section provides information about the installed firmware version; the **Heater** section displays the set and actual temperatures of the two heating plates; the **Holder** section refers to the status of the wellplate locks (open or locked); the **System** section gives an overview of the temperature of the system and its cooling in operation; finally, the **Wellplate** section refers to both the humidity and the temperature detected at the wellplate level.

3. MaxTwo Well Selection and Status

The MaxTwo Well Selection and Status display serves two purposes. First, it conveys information about the individual wells such as their connection status, group and whether a well is defined as 'control' (see next Section). Secondly, it allows to select a particular well for live visualization (see Chapter 3).

The indicator elements of the status display are described in Table 2.1.

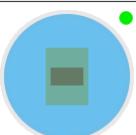
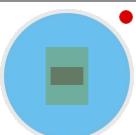
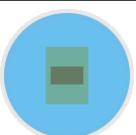
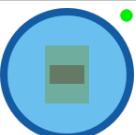
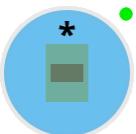
Icon	Description
	A green dot next to the well symbol indicates that the well is connected and streaming data.
	A red dot next to the well symbol indicates that there is a problem with the connection to the well.
	No dot next to the well symbol indicates that no wellplate is connected to the system.
	The blue ring around the well symbol indicates that this well is currently displayed in the Visualization Section (Electrodes, Traces and Raster views).
	A black asterisk inside the well symbol indicates that this well has been marked as control.

Table 2.1. Well Indicators

4. MaxTwo Wellplate Editor

The MaxTwo Wellplate Editor allows the user to input and store information about well content. It can be accessed by clicking the **Setup Wells** button on the bottom right of the software window.

The Wellplate Editor (Figure 2.4) shows a representation of the plate, where each well consists of a circle containing an HD-MEA chip. Wells are numbered in rows, from left to right, top to bottom.

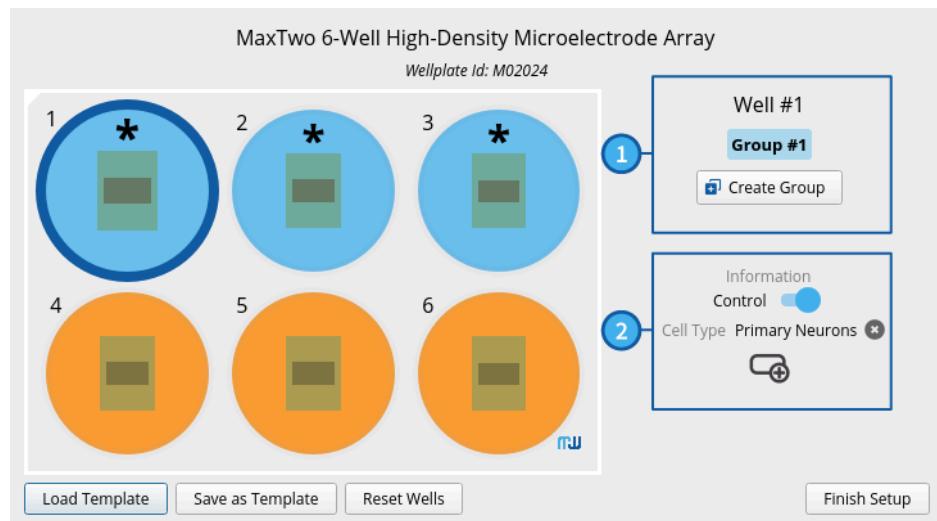


Figure 2.4. Well Plate Setup

(1) Create Group

The user can click on the **Create Group** button to form a group containing the selected wells. Colors can be attributed to the groups for easy identification. There are several ways to select wells:

- Clicking to select a single well or clicking and dragging the mouse pointer to select multiple wells
- Holding the **Ctrl** key and clicking on the desired wells
- Selecting two wells while holding the **Shift** key to include all the wells in between

(2) Information

The Information section allows the user to add and store information related to the selected wells. The **Control** switch can be toggled to identify the well as belonging to the experimental control, which will cause an asterisk (*) to be displayed at the top of the well. The **Plus** icon can be clicked to display the **Add Well Information** dialog (Figure 2.5) where the user can add extra information such as cell type or drug concentration.

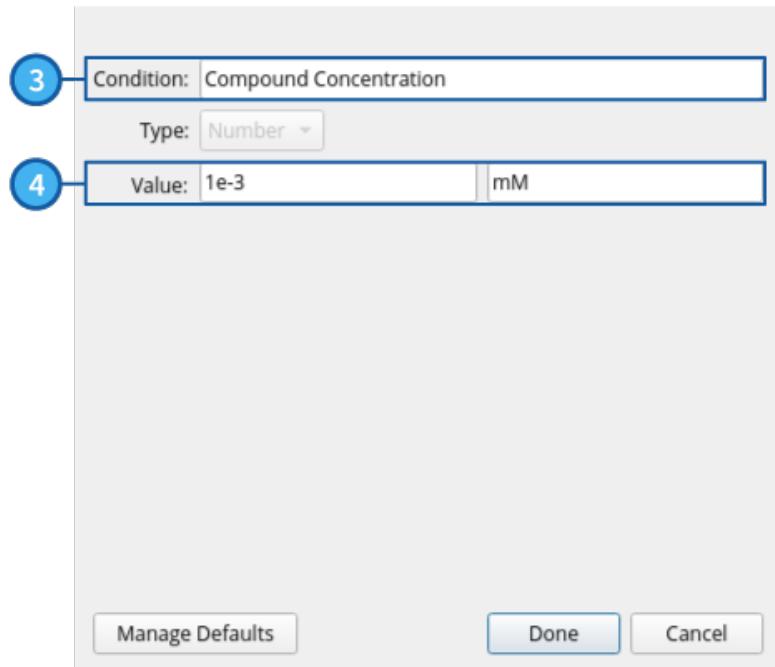


Figure 2.5. Add Well Information

(3) Condition

Start typing in the **Condition** field to see a list of possible inputs with pre-assigned input **Types** (Text, Number, or Date). Alternatively, type a new **Condition** name and assign the corresponding input **Type**. Created custom conditions will be stored by MaxLab Live.

(4) Value

Type the **Value** of the condition into this field. If the condition type is "Text", only the **Value** tab is displayed. If the type is "Number", MaxLab Live will prompt both a "Value" and a "Unit" field (scientific notation is accepted by MaxLab Live). If the type is "Date", it is possible to select the value from a calendar.

Chapter 3. Visualization

Being able to visualize and inspect cellular activity in real time is crucial to assess and judge the viability and quality of cells. For this purpose, MaxLab Live offers different ways (or *views*) for real-time visualization of the activity. Whether it is single spike waveforms, individual neuronal units or full network activity patterns, the user can select different views and alternate between them whenever required.

This chapter describes the features and functionalities associated with the real-time visualization modules.

1. Electrodes View

This view is used to visualize the activity recorded from each electrode in real-time and to prepare and inspect electrode configurations. The large rectangle in the center view depicts the electrode array ($2.1 \times 3.85 \text{ mm}^2$) and each of the 26,400 electrodes is represented by a square. The Electrodes View consists of the Signal Visualization Control Panel (1), the Array Representation Window (2) and a side tab which includes several features (3) - (8), described below. When using the software with a MaxTwo system, the Well Selection Module (8) allows to select which well is currently visualized.

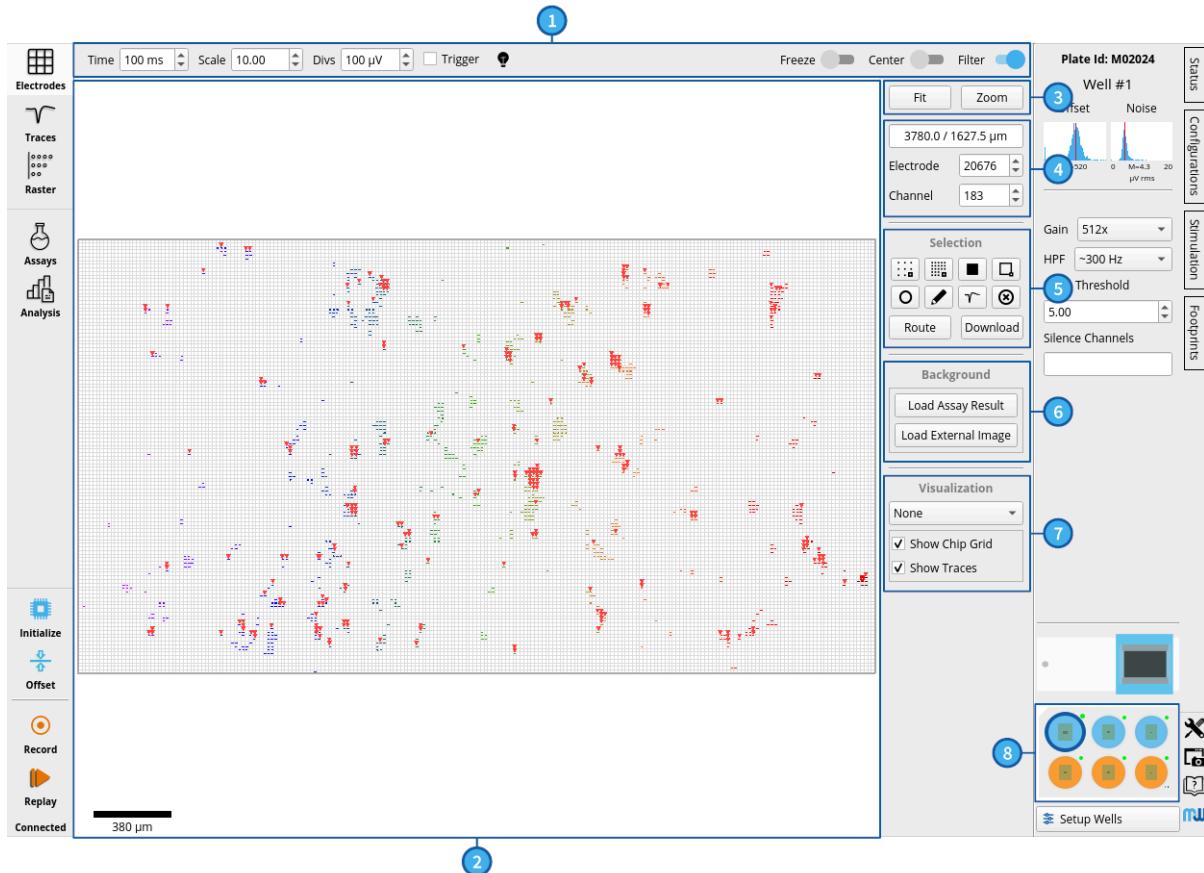


Figure 3.1. Overview of the Electrodes View

In the following, the individual elements of the Electrodes View, as displayed in Figure 3.1, are described:

(1) Signal Visualization Control Panel

This panel is used to control the signal visualization in both the Electrodes View and the Traces View, and is explained in further detail in Section 3.

(2) Array Representation Window

The main window in the center of the GUI shows the microelectrode array. Every electrode is shown as a separate square. Using the mouse and keyboard, the array can be browsed with the following commands:

- By clicking and holding the mouse, the view can be dragged around (the mouse pointer changes into a little hand).
- Using the mouse wheel, the zoom level can be changed. In the lower left corner of the screen is a scale bar that adjusts according to zoom level.

- Pressing down the shift key and using the mouse wheel moves the view horizontally.
- Pressing down the control key and using the mouse wheel moves the view vertically.
- Pressing the keyboard key 'f' will fit the whole rectangle into the window.
- Pressing the keyboard key 'z' will zoom to where the mouse point currently is.
- Clicking with the mouse pointer on one electrode will highlight this electrode with a red square.

The signal traces of the electrodes selected for recording are displayed inside the corresponding squares (colored signals in Figure 3.1). Note that the colors of the traces in the Electrode View match the colors in the Raster View (see Section 4 of this chapter).

Any detected action potentials are visualized as red triangles pointing to the corresponding electrode.

(3) Zooming Options

On the top of the right side tab, two buttons allow quick control of the scaling:

- **Fit** - the array is scaled to fit completely inside the Array Representation Window (shortcut: 'f')
- **Zoom** - the array is scaled to give a zoomed view of the area around the selected electrode (red rectangle; shortcut: 'z')

(4) Electrode and Channel Information for Selected Electrode

For the currently selected electrode, which is highlighted by a red square in the Array Representation Window (2), the information about the coordinates, electrode number and the recording channel to which it is connected is shown.

- **Coordinates**

The electrodes of the array are arranged on a XY-coordinate system, where the coordinate origin is the top left corner of the array. Every electrode coordinate value is a factor of 17.5 µm, which corresponds to the X- and Y-spacing between two neighboring electrodes.

- **Electrode Number**

Every electrode on the array has a distinct electrode number that can be used for identification. The first electrode at the top left corner of the array has the electrode number 0 and the numbering is increased by one for every electrode moving rightwards.

- **Channel**

If the selected electrode is connected to a recording channel, the channel ID will be visualized here. For electrodes which are not connected to a recording channel, 'None' will be displayed.

TIP: The Electrode and Channel fields are used to read out the values of the selected electrode, but they can also be used to find a particular electrode. Typing an electrode number or channel ID into the field and pressing 'Enter' will cause the red square marker to jump to the specified electrode.

(5) Electrode Selection Tools

MaxLab Live Scope provides a palette of electrode selection tools to design custom electrode configurations (see Table 3.1) and the corresponding section below.

(6) Background Image

This tool enables the user to display an image as background of the Electrodes View. More information can be found in the corresponding section below.

(7) Live Rendering

This module allows to render a color-coded real-time representation of selected activity features. More information can be found in the corresponding section below.

(8) Well Selection

In the well selection module, as shown in Chapter 2, the user can specify which of the available wells is currently displayed in all of the different views of the Visualization Section. The Array Representation Window shows the currently selected well.

Electrode Selection Tools

Table 3.1 provides an overview of selection tools with which the user can design custom electrode configurations.

Icon	Name	Description
	Sparse 1000	Selects 1000 electrodes sparsely distributed across the chosen area; keyboard shortcut 's'
	Sparse	Selects electrodes according to the sparsity set in the two input boxes below, labeled with X and Y; keyboard shortcut 'n'
	Block 23×23	Selects a high-density block of 23×23 electrodes; keyboard shortcut 'b'
	Rectangle	Selects a high-density rectangle; keyboard shortcut 'r'
	Circle	Selects a circular high-density block; keyboard shortcut 'c'
	Pen	Allows to freely draw electrode selections; keyboard shortcut 'o'
	Spike	Adds the currently selected electrode (highlighted by a red square) to the Footprints Tab for spike-triggered averaging (see Section 5 in Chapter 4 for more information); keyboard shortcut 't'
	Clear	Clears the current electrode selection

Table 3.1. Electrode Selection Tools

Clicking on one of the first six selection buttons activates that particular selection mode. Electrodes can then be selected inside the electrode view using the left click. The selected electrodes will be labeled yellow. Right-clicking will cancel the selection at any time.

After selecting the electrodes, the button **Route** needs to be clicked. This will send the current selection of electrodes to the server which in turn will generate a configuration and send it back to MaxLab Live. Once routed, the electrodes turn green. Clicking on the **Download** button will then activate the new configuration and the signals will now be measured by the electrodes of the new configuration.

Info: Every time a new configuration is generated by clicking on the **Route** button, a new configuration appears in the Configurations Tab of the Sidebar, which is further described in Section 3 of Chapter 4.

The following tips can help to optimize the manual electrode selection process:

- **Keyboard shortcuts**

The selection tools described above can also be accessed using the keyboard shortcuts indicated in Table 3.1. When hovering over the selection buttons in the software, the keyboard shortcuts are also indicated. Pressing the 'Esc' key will cancel the current selection mode.

- **Undo functionality**

Pressing Ctrl-Z undoes the last selection. Ctrl-Z can be pressed multiple times to go back in time through the history of electrode selections.

- **Number of selected electrodes**

While electrodes are being selected, the number of currently selected electrodes is indicated next to the mouse pointer. It is recommended to monitor this value, as no more than 1020 electrodes can be selected in a single configuration.

- **Deselect**

An electrode selection can be deselected. While selecting electrodes, holding down the 'Shift' key and clicking with the mouse pointer will remove the newly selected electrodes instead of adding them.

- **Selecting traces for visualization**

A subset of the recording electrodes can be selected for detailed inspection in the Traces View. When currently routed electrodes are selected, pressing the space key will send the selected electrode traces to the Traces View. Double clicking on an electrode trace will also send this individual electrode trace to the Traces View.

Background Image

The Background Image Tool provides the possibility to display an image in the background of the Electrodes View. The image can be either the result of a MaxOne Assay (e.g. an activity map) or an image of the biological preparation, such as a bright field or fluorescent microscopy image.

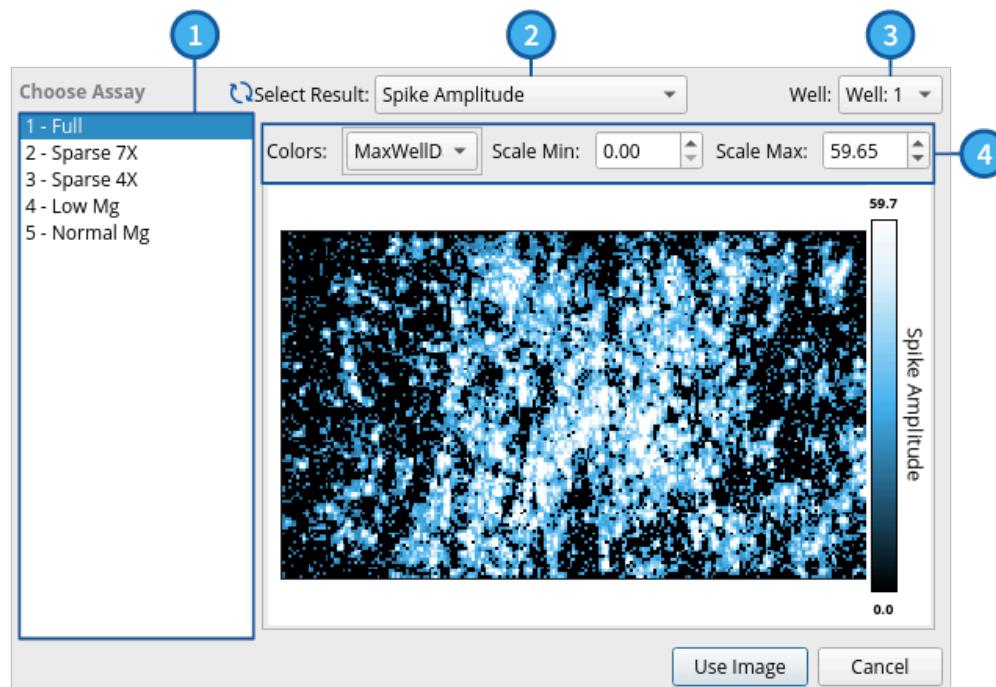


Figure 3.2. Background Assay Result Widget

Clicking on the **Load Assay Result** button (Figure 3.1 (6)) will open the Background Assay Result Widget, which is shown in Figure 3.2. In the left panel of the widget (1), which provides a list of all assays

in the current project, one assay can be selected. The visualization type, such as Spike Amplitude or Firing Rate (2) and the Well number (3) can be selected using the drop-down lists at the top of the window. Finally, the visualization parameters such as the colormap and the colorbar limits can be manually adjusted (4). Pressing the button **Use Image** will show the resulting map in the background of the Electrodes View.

Figure 3.3 (1) shows the Electrodes View after loading the Spike Amplitude map from Figure 3.2. New buttons and controls now appear in the Background Visualization tool (Figure 3.3 (2)):

- **Clear**
Clicking this button resets the background mode and goes back to the initial status.
- **Show Image**
Enables / disables visualization of the background Map.
- **Show Grid**
Enables / disables visualization of the electrode grid in the Electrodes View.
- **Transparency**
Background is shown in a semi-transparent way which facilitates the visualization of the displayed signals on top of the background image (Figure 3.3 (1)).

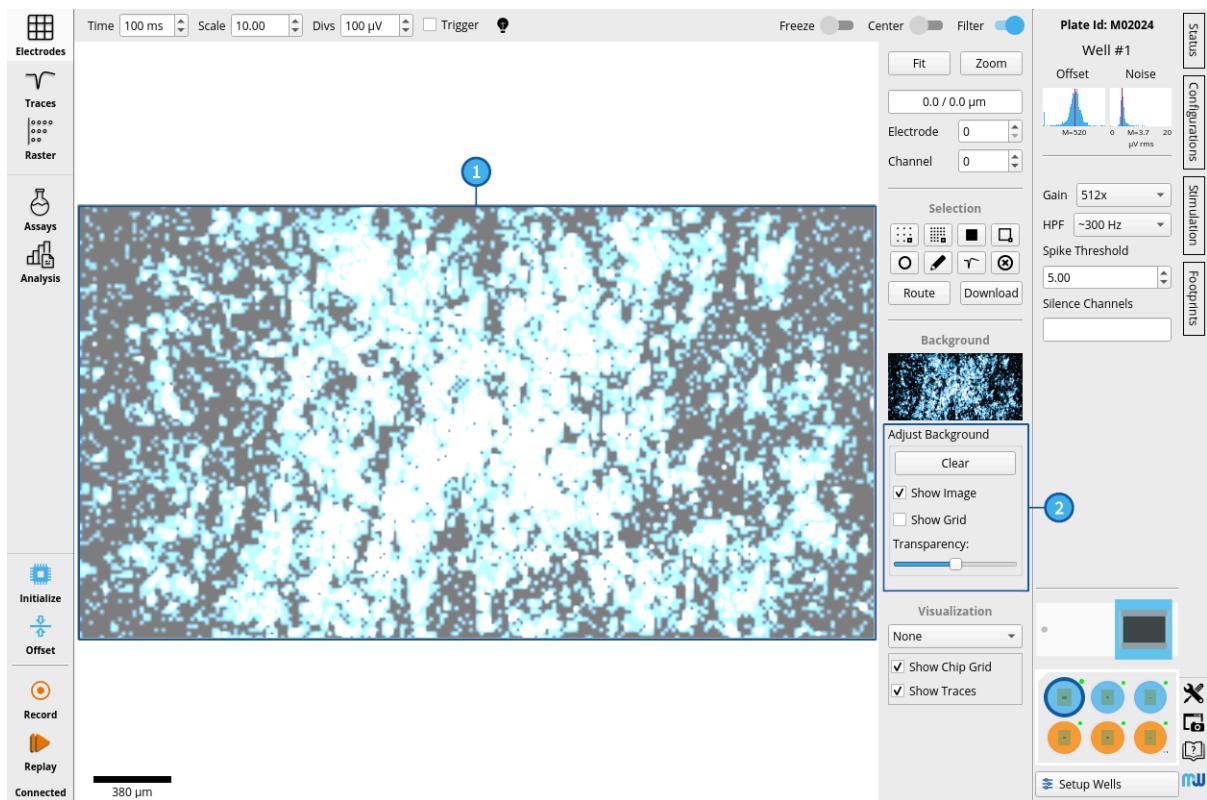


Figure 3.3. Spike Amplitude Map in the Electrodes View

The second button of the Background Visualization tool, **Load External Image** (Figure 3.1 (6)), offers the possibility to load any image and to align it to the electrode array using the MaxLab Live software. After selecting a .png, .xpm or .jpg file for background display, the user can click on the **Align** button, which will open a dialog box that explains the alignment process. The user needs to click on the four corner electrodes, starting with the top left one, and proceed clockwise. After clicking on the four corner electrodes, the software computes an affine transformation matrix which is applied to the image. A picture of a brain slice, which was aligned using this functionality, is shown in Figure 3.4. Once the alignment is finished, a new file with the aligned image and the file ending ".align" is saved to disk. This image file can then be directly loaded without the need to repeat the alignment process in the future.

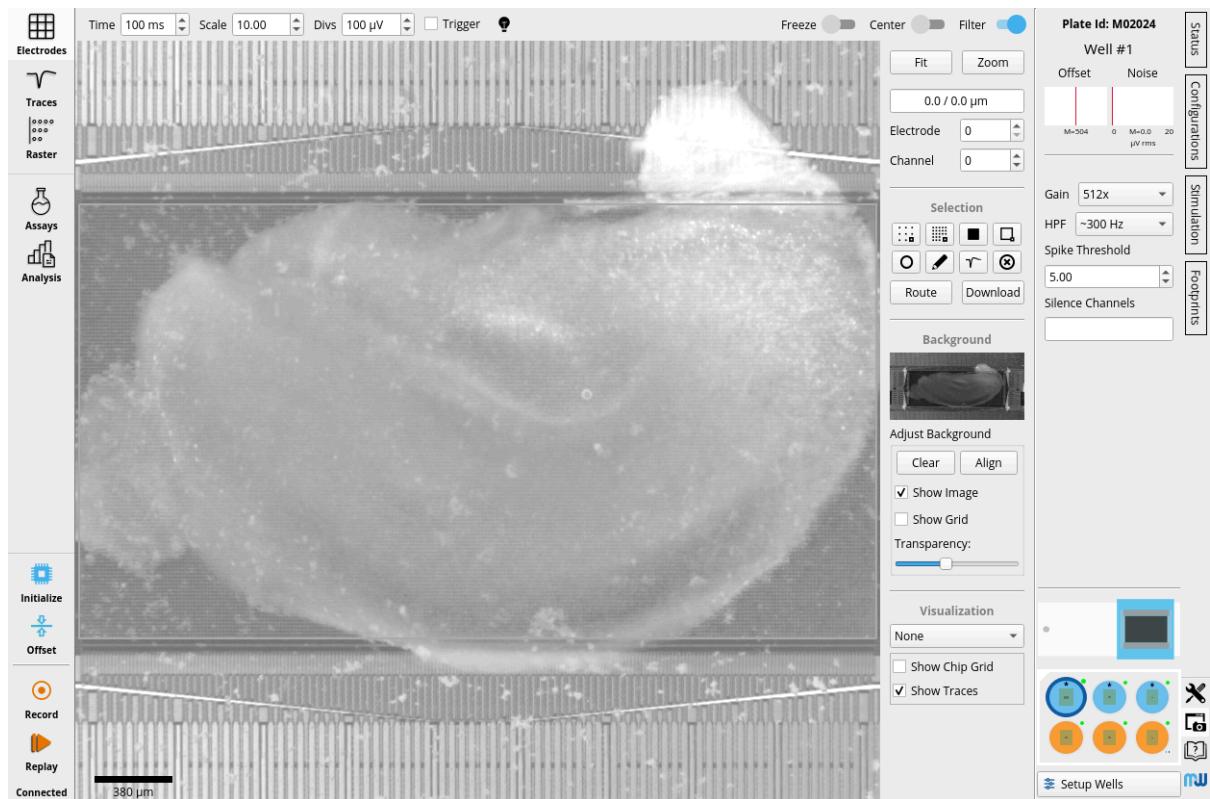


Figure 3.4. Aligned Brain Slice Background Image

TIP: To obtain a good alignment, it is important to hit the corners of the array during the image alignment as accurately as possible. The mouse scroll wheel can help to zoom into the individual corners, while the shortcut 'f' can be used to return to the entire Array Representation Window after clicking on a particular corner.

Live Rendering

This module allows to render a color-coded real-time representation of selected activity features (Figure 3.1 (7)). From the drop-down list, the following options can be chosen:

- **None**
Standard view, no rendered visualization.
- **Min Spike Amp**
For each electrode, the negative peak amplitude is color-coded.
- **Spike Count**
For each electrode, the number of detected spike events is color-coded.
- **Burst Time**
In this mode, burst events are detected. The color per electrode indicates the time delay between the burst initialization and the first detected spike event at any given electrode.

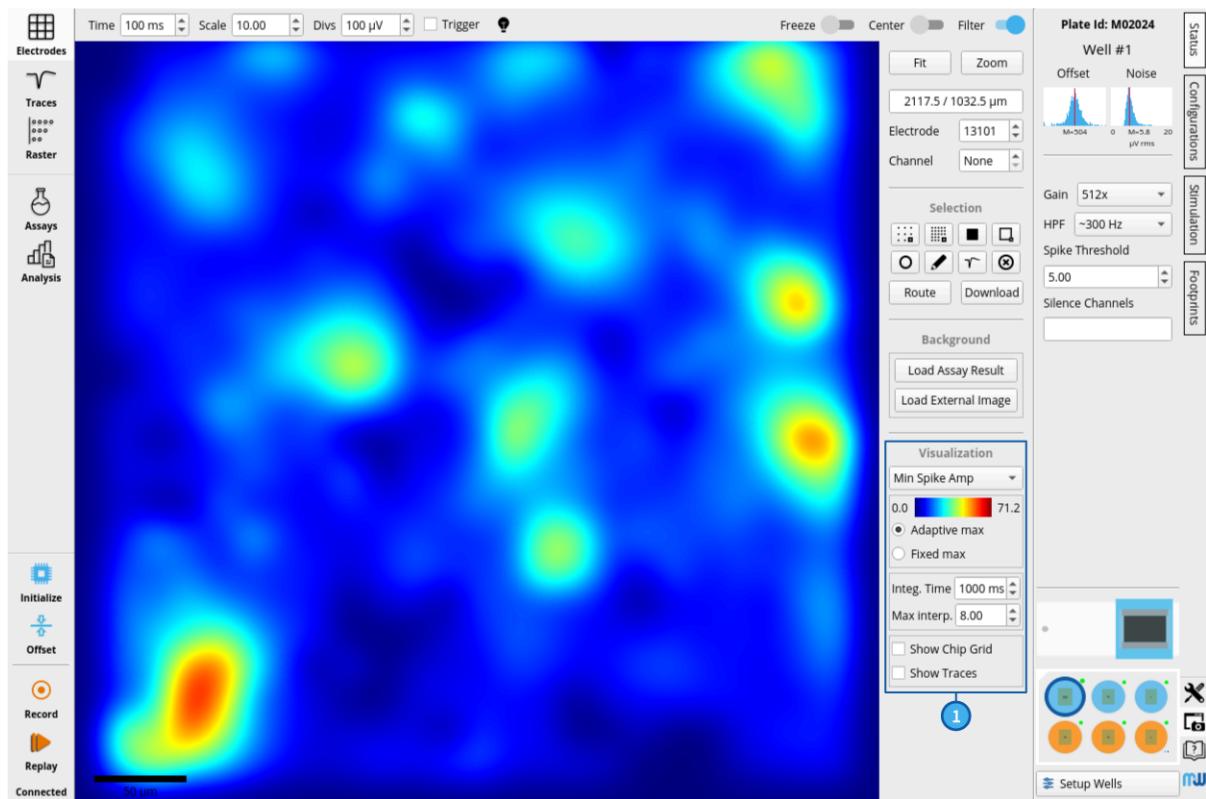


Figure 3.5. Parameters for the Visualization Module

After selecting one of the feature visualization modes, the parameters for the Visualization module appear, as shown in Figure 3.5 (1):

- **Adaptive max vs. Fixed max**
While in the "Adaptive max" mode, the limits of the colormap visualization are continuously automatically adjusted to the activity. The "Fixed max" mode allows to set the maximum displayed value manually.
- **Integ. Time**
This parameter defines the time bins in which values of the Visualization Module are computed and updated.
- **Max interp.**
When interpolating values between electrodes, the distances between those need to be considered. If the electrodes are too far apart, interpolating the in-between values might lead to sharp edges and interpolation artifacts. This parameter defines the maximum distance between electrodes (in electrode pixels), for which in-between values are interpolated. If groups of selected electrodes are spaced further apart, no interpolation will be computed and only background color will be shown between those groups.
- **Show Grid**
Enables / disables display of the electrode grid on top of the color-coded visualization.
- **Show Traces**
Enables / disables display of the signal traces on top of the color-coded visualization.

2. Traces View

The Traces View displays the voltage traces from all or a subset of the recording channels and is particularly useful to inspect the signals and the individual spike waveforms. The elements of the Traces View are illustrated in Figure 3.6.

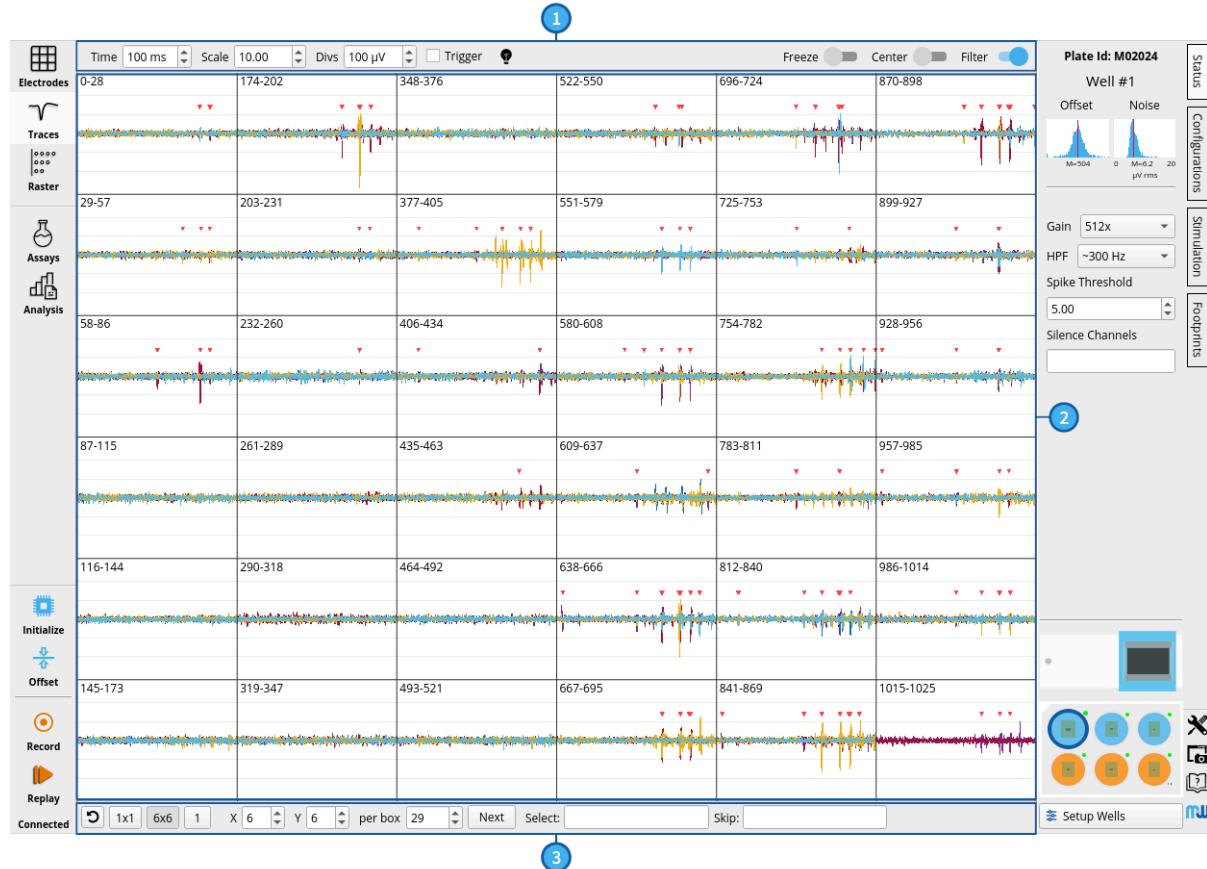


Figure 3.6. Overview of the Traces View

As for the Electrodes View, the **Signal Visualization Control Panel (1)** at the top of the window provides the controls for signal visualization and is explained in detail in Section 3 of this chapter.

The signal traces are displayed in the **Signal Visualization Area (2)**. By default, this area is divided into 6 x 6 windows, where each window displays the superimposed traces from 29 recording channels. At the top left corner of every window, a label indicates which channel numbers are overlaid.

The **Traces Windows Control Panel (3)** at the bottom can be used to change which channels are shown and how they are arranged on the display:

- **1x1, 6x6 and 1 buttons**

These buttons are used to change between three predefined window arrangement modes in the Signal Visualization Area:

1x1 - plots traces from all channels in one single window

6x6 - plots traces in 36 individual windows with 29 channels per window

1 - plots every channel inside an individual window

- **X and Y entry fields**

These inputs provide the possibility to manually specify how many windows are displayed on the X- and the Y-axis.

- **per box** entry field

Using this field, the number of channels per box can be customized.

- **Next** button
This button allows to iterate through channels. If the currently visible channels are, e.g. channel numbers 1,2 and 3; clicking on **Next** will change the visualization to channel numbers 4,5 and 6.
- **Select** entry field
If only a subset of the channels are selected for visualization, the respective channel numbers are shown here. Otherwise, this box is empty. The channels can be selected by manually entering the channel numbers separated by commas.
- **Skip** entry field
If a channel should be skipped it can be indicated here.

TIPS for using the Traces View:

- Double clicking on one of the windows “zooms” into this selection of channels. I.e. the channels in this window are shown in boxes with only one channel per box.
- Changes in channel organization can be undone by repeatedly pressing Ctrl-Z to go back through the history.
- Scrolling the mouse wheel will change the Scale in the **Signal Visualization Control Panel**, thereby moving the vertical position of the displayed signals along the Y-axis, which allows to better distinguish the signals of the individual channels.

3. Signal Visualization Control Panel



Figure 3.7. Elements of the Signal Visualization Control Panel

The control panel on top of the Electrodes View and the Traces View is used to define how the signals are displayed and provides various visualization features:

(1) Time

This parameter defines the temporal window of the visualized signal in milliseconds.

(2) Scale

The Y-axis scaling of the visualized signal can be adjusted here. The user can also change this parameter by scrolling in the Traces View.

(3) Divs

The signal windows in the Traces View provide horizontal division lines to visualize the amplitude of the acquired signals. The spacing between the horizontal lines can be adjusted using this parameter.

(4) Trigger

Checking the tick-box activates the Trigger Mode. In this mode the signal is updated every time an event is detected on a particular channel. The Trigger Mode is controlled by the two additional fields which appear next to the Trigger checkbox (see Figure 3.8) and are explained in the section below.

(5) Freeze

While data is measured, this mode can be used to stop the visualization of the streamed data and to go back in time to have a closer look at the recently measured signals. If activating the **Freeze** mode, a slider appears underneath the control panel, which can be dragged to move back and forth in time. The signal can be wound back by approximately 7 seconds.

TIP: Use the combination of 'Alt' and the left or the right arrow key to move back and forth in time instead of dragging the slider with the mouse.

(6) Center

If data is observed with the **Filter** switch disabled (see (7) in Figure 3.7), then the signal baselines are vertically distributed along the Y-axis due to the DC components of the recording channels. This makes it difficult to observe the signals and compare between the traces of different channels. Activating the Center mode will remove the offset of every channel and therefore center the signal in the middle of the displaying window.

If data is visualized with the Filter-switch enabled, the DC components of the signals are removed by the band-pass filter and the signal baseline is always 0 mV. In that case, activating the Center mode has no effect on data visualization.

(7) Filter

The **Filter** switch allows to toggle between visualizing the streamed data in the raw and in the band-pass-filtered modes. While the raw mode displays the data as it is streamed from the system, the **Filter** mode applies a software-based band-pass filter to the data. Furthermore, the online spike detection

and visualization is turned on if the **Filter** mode is enabled. Note that the spike detection threshold can be adjusted in Status Tab of the Sidebar, as explained in Section 2, Chapter 4.

Info: The characteristics of the band-pass filter can be modified in the **Settings** window (see Section 2, Chapter 5).

Using the Trigger Mode

Figure 3.8 displays the Trigger Mode which can be used to study the action potential waveform of a particular channel in more detail, or check other channels for related activity. In the example below, the signal is triggered on channel 205 (2) and will refresh every time a spike is detected in this channel.

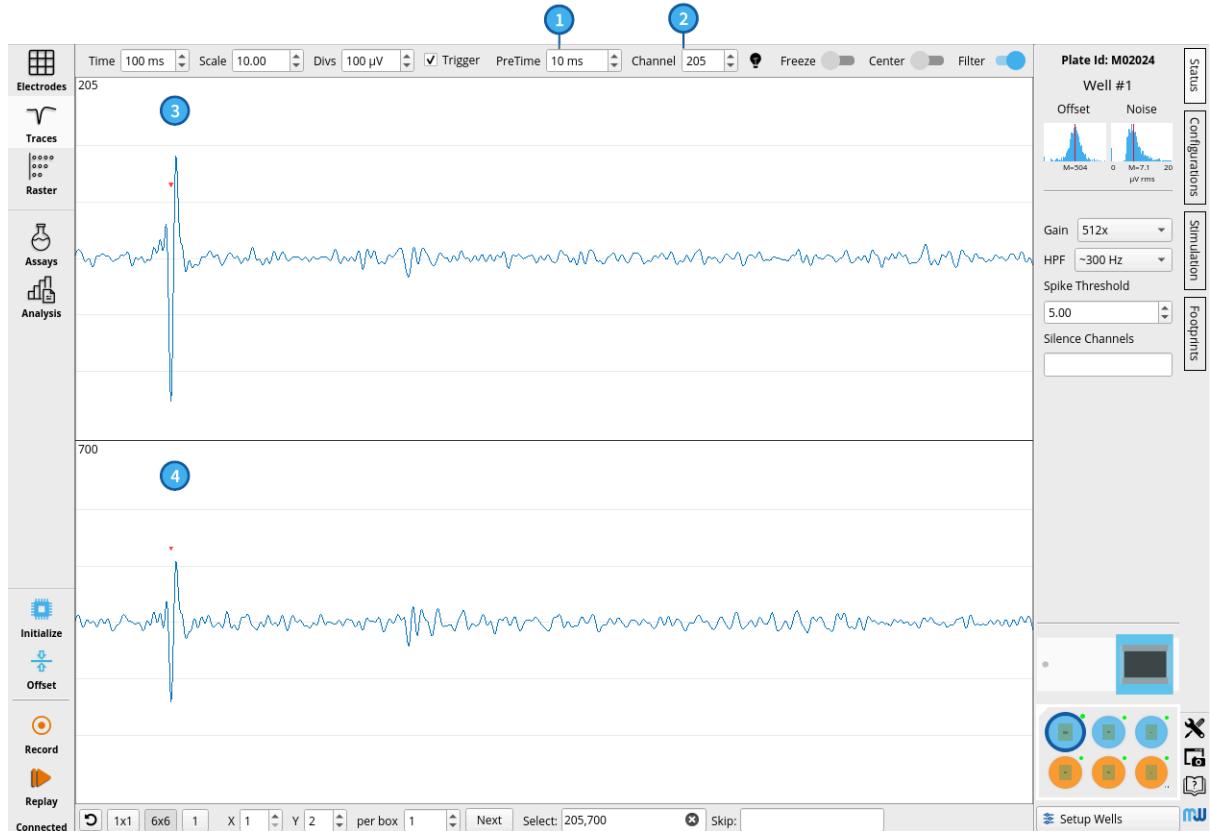


Figure 3.8. Using the Trigger Mode

Upon activating the Trigger Mode, the trigger parameters ((1) and (2) in Figure 3.8) appear on the control panel.

(1) PreTime

This time (in milliseconds) defines the extract of signal that is displayed before the detected action potential and is set to 10 ms in this example.

(2) Channel

This number specifies which channel is considered for the triggering action potentials.

(3) Triggering Action Potential

The detected event is indicated by a red marker. The trace cutout will remain visible until the next event is detected on the channel.

(4) Signal on a Neighboring Channel

Here we can see the signal on a neighboring channel that is updated every time there is an action potential in channel 205. The electrodes connected to the displayed channels are both capturing activity from the same cell, however, the triggering electrode is located closer to the axonal initial segment and thus has a higher amplitude.

4. Raster View

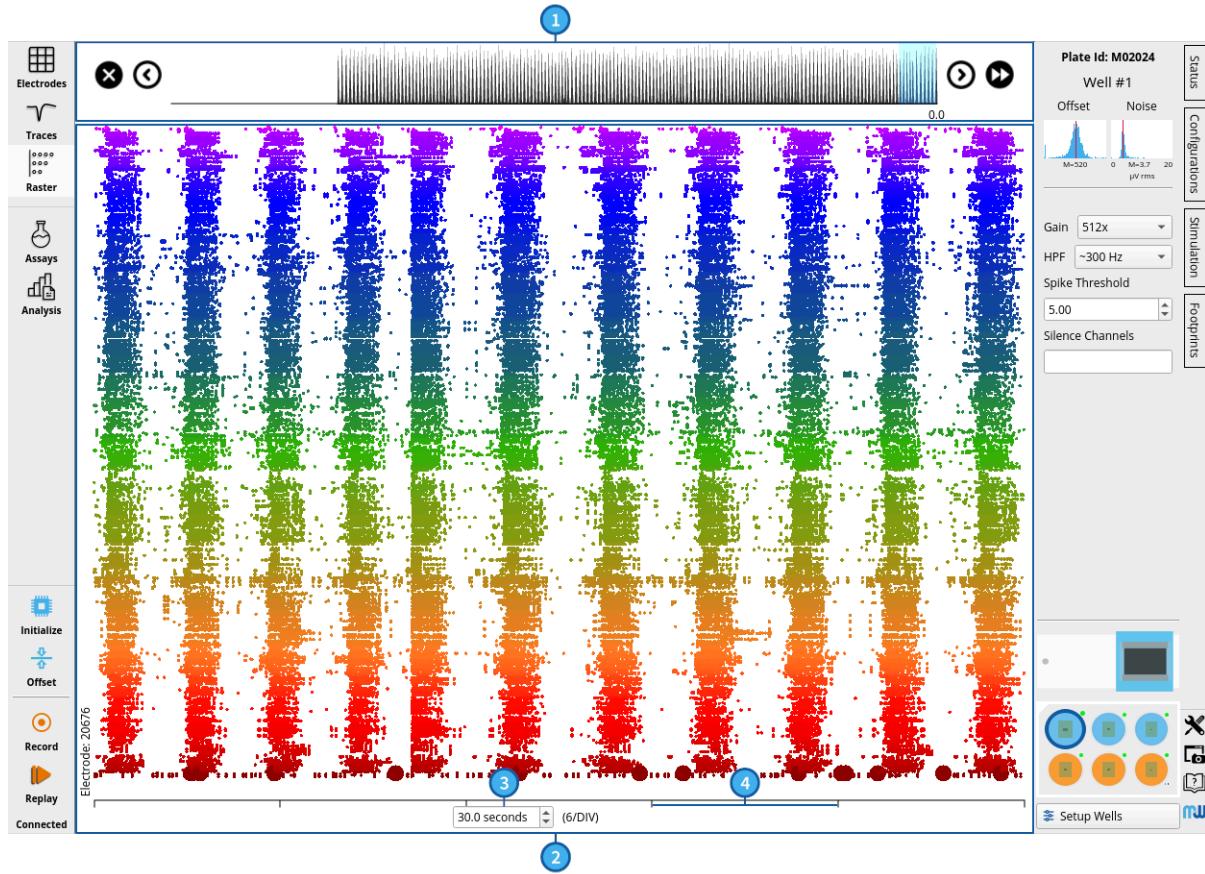


Figure 3.9. Overview of the Raster View

In contrast to the Electrodes and Traces Views, which are used to visualize the recorded signal traces, the Raster View is designed to visualize the detected spike events on all recording channels. It is therefore highly suited to gain an overview of the firing dynamics in a network of many cells and to inspect these dynamics over the time scale of tens of seconds to minutes.

Figure 3.9 provides an overview of the Raster View:

(1) Histogram Panel

The Histogram Panel at the top of the Raster View displays the history of the detected events in a histogram visualization. It contains several controls which are explained in Figure 3.10.

(2) Raster Visualization

In this part, all detected spikes are visualized in a raster plot mode. The X-axis of this view represents time while the Y-axis represents the 1024 recording channels. Every detected spike is visualized as a dot, vertically positioned according to the corresponding recording channel.

(3) Raster plot window time

This parameter defines the duration of the time window used for the Raster Visualization. The value can be manually changed using the combination 'Shift' and the scroll wheel of the mouse.

(4) Temporal divisions

The X-axis of the raster plot window is divided into five sections to better assess the temporal characteristics of the activity. As shown in Figure 3.9, the number of seconds per division (DIV) is indicated to the right of the window time (3).



Figure 3.10. Histogram Panel Overview

Besides real-time visualization of the detected spikes (live-mode), the Raster View also allows to move back in time to inspect the activity of approximately the latest 10 minutes of data (history-mode). The **Histogram Panel**, shown in Figure 3.10, provides the controls for this feature. It contains the following elements:

(1) Clear

This button resets the Raster View by clearing all the dots. After pressing the clear button, the Raster View immediately starts plotting new detected spike events.

(2) Back

The back-arrow button moves the currently displayed segment of activity back in time by the size of the currently displayed window (4). Clicking this button will change the Raster View from live-mode to history-mode. While in live-mode, the raster plot is constantly updated with newly detected events. In history-mode, the view remains frozen until another action is applied.

(3) Histogram

The histogram shows the accumulated firing activity by plotting the sum of detected events per time bin (100 ms). The histogram is filled while data is streamed and it allows to e.g. identify bursts of activity in previously acquired data.

(4) Current window

The blue window in the histogram panel shows the region of activity that is currently displayed in the raster plot. If the live-mode is used, the current window is always tied to the right edge of the histogram and the detected spiking events are visualized in real-time. The current window can be dragged with the mouse and moved back and forth in time, which will change the Raster View into history-mode. Dragging the current window to the right edge of the histogram will change the Raster View back into live-mode.

(5) Current window delay

The number underneath the histogram displays the temporal delay in seconds between the right edge of the current window and the time point of the currently-streamed data. This delay is always zero in live-mode. Once the back-arrow button is pressed or the current window is dragged and the Raster View changes to history-mode, the delay time constantly increases, indicating the difference between the actual time and the time of the current window.

(6) Forward

The forward-arrow button moves the current window forward by the current window size (4).

(7) Live-mode

This button changes the Raster View into live-mode and shifts the current window to the right edge of the histogram.

Chapter 4. Sidebar

The Sidebar, which is located on the right side of the MaxLab Live user interface, combines several important tools and functionalities for advanced users. This chapter introduces these functionalities and explains how to use them.

1. Sidebar Overview

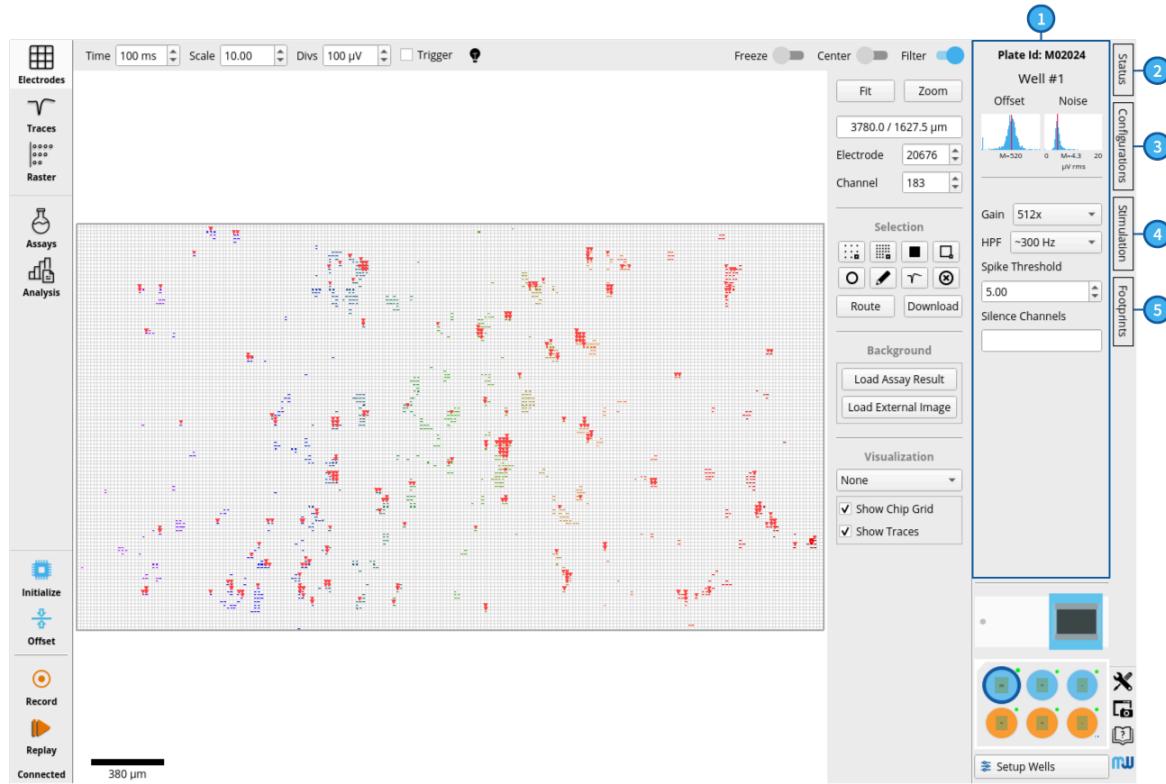


Figure 4.1. Sidebar Location

The sidebar (1) is located on the right side of the software display and contains four tabs:

(2) Status Tab

Shows information about electrode noise and offset and enables the modification of gain, filtering and action potential detection threshold.

(3) Configurations Tab

Allows to select from predefined and custom electrode configurations.

(4) Stimulation Tab

Allows to select electrodes and define waveforms for voltage stimulation.

(5) Footprints Tab

Allows to visualize spike-triggered average traces across multiple neighboring electrodes, giving a picture of a single neuron's electrical "footprint".

2. Status Tab

The status tab displays information about the chip and allows the user to modify hardware-related settings such as gain and filtering.

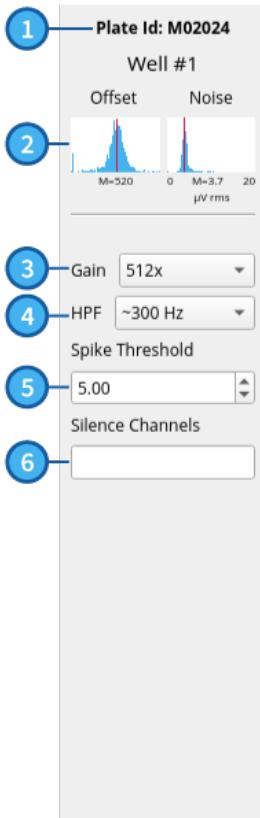


Figure 4.2. Status Tab

(1) Plate/Chip Id

Displays the Id number associated with the inserted wellplate (MaxTwo) or chip (MaxOne). The Plate/Chip Id is a convenient way of relating the experimental results to the sample and can be integrated into the data saving path (see Chapter 6, Section 2). Below the Plate/Chip Id, the currently-displayed well is indicated.

(2) Offset and Noise

These two panels convey information about the offset and noise distribution of the currently-connected electrodes.

- Offset:** The offset distribution shows a histogram of the signal offset on each electrode. After pressing the **Offset** button (see Chapter 1, Section 3) on the left side of the software window, this distribution should become much narrower. The offset values range between 0 and 1024 due to the 10 bit digitization of the signals.,
- Noise:** The histogram shows the distribution of the root mean square (RMS) values for the electrode signals and is indicated in μV_{RMS} . When measured without cells in a saline solution, the average noise of the readout circuitry in the 300 Hz – 10 kHz band is $2.4 \mu\text{V}_{\text{RMS}}$. In the presence of cellular spiking activity, however, this value is typically higher.

(3) Gain

The chip circuitry features three configurable amplification stages, allowing to control the amplifier gain. Increasing the gain improves the capability to detect small signals. This setting directly acts on the chip hardware and should not be changed during a recording.

(4) Filter

This setting controls the hardware-level high pass filter (HPF) and should not be changed during a recording. To visualize action potentials (APs), an HPF of 300 Hz should be chosen. To record the signal including lower frequency components such as local field potentials (LFPs), an HPF of 1 Hz should be selected. The signal can then be post-processed to distinguish between APs and LFPs.

(5) Spike Threshold

This value determines the threshold above which a signal is classified as an AP, in units of RMS noise.

(6) Silence Channels

Channels entered in this field will be recorded as having a value of zero. This feature is useful if one channel is particularly noisy. However, this setting does not change anything at the hardware level and silenced channels reappear when the system is restarted.

3. Configurations Tab

This tab allows to select from predefined and custom electrode configurations that can be used to define which electrodes stream data.

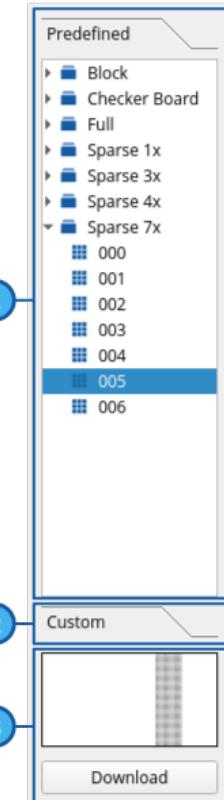


Figure 4.3. Configurations Tab

(1) Predefined Configurations

The predefined tab provides configurations that are supplied with the software. These are also the standard configurations that the user can select from when running an activity scan assay.

(2) Custom Configurations

A configuration that is created in the electrodes tab with the selection tools and routed via the **Route** button will automatically be saved. The **Custom** tab provides a view into the filesystem where custom configurations are saved. Right-clicking within this tab offers several menu entries:

- **Set directory:** This allows to define the root of the filesystem displayed in the **Custom** tab.
- **Create group:** Allows to create a new folder that can serve as a container for a set of custom configurations.
- **Rename:** Right-clicking on a folder or configuration allows to change the current name.
- **Delete:** Right-clicking on a folder or configuration allows to delete the selected file.

(3) Preview Field and Download Button

The **Preview** field contains a small visualization of the currently selected configuration. The **Download** button allows to download this configuration onto the chip, which connects the electrodes in that configuration to the readout channels.

4. Stimulation Tab

This tab provides a simple interface to deliver electrical stimulation pulses to the electrodes. There are 32 stimulation channels that can be simultaneously connected to electrodes for stimulation. The pulses are biphasic, positive-first voltage pulses.

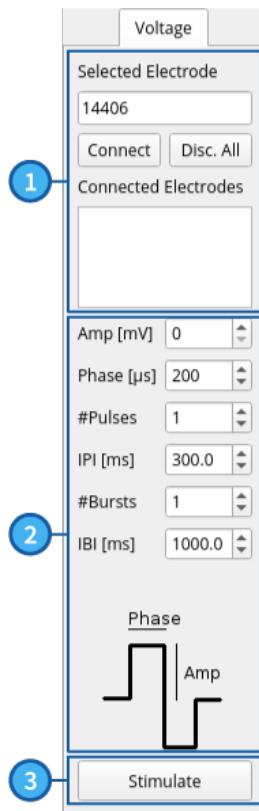


Figure 4.4. Stimulation Tab

(1) Selecting and Connecting Electrodes

As a first step, stimulation electrodes need to be selected. The user can select an electrode by entering its number into the **Selected Electrode** field, or by clicking on an electrode directly in the Electrodes View. The next step is to click **Connect** to connect the electrode to a stimulation unit. Multiple electrodes can be selected and connected in this way. Connected electrodes are marked by a lightning symbol in the Electrodes View (see Table 4.1). To disconnect all stimulation electrodes after an experiment is completed, the user can press **Disc. All**.

(2) Stimulation Settings

The electrical stimulation signal consists of a certain number of bursts that each consist of individual pulses.

Pulse Parameters:

- **Amp:** Amplitude of the voltage signal in [mV] as illustrated in the preview at the bottom
- **Phase:** Voltage signal phase in [μ s] as illustrated in the preview at the bottom
- **#Pulses:** Number of pulses that make up a single burst
- **IPI:** The inter-pulse interval, defined as the time between each stimulation pulse in [ms]

Burst Parameters:

- **#Bursts:** Number of total bursts which will compose the voltage stimulation signal
- **IBI:** The inter-burst interval, defined as the time between individual bursts in [ms]

(3)Stimulate

This button will send the voltage stimulation signal created with the parameters above to the connected electrodes. Once the stimulation experiment is completed, the user can click **Disc. All** to disconnect the stimulation electrodes.

Icon	Name	Description
	Electrode Selected for Stimulation	The lightning icon marks electrodes in the Electrodes View that are connected to a stimulation unit

Table 4.1. Electrode Selected for Stimulation

5. Footprints Tab

The Footprints Tab allows the user to visualize spike-triggered average (STA) signals of electrodes based on the 100 most recent spikes. This functionality is useful to visualize the spike waveform and spatial extent of a cell or cellular projection such as an axon. The Footprints Tab (Figure 4.5) is used together with the Electrodes View.

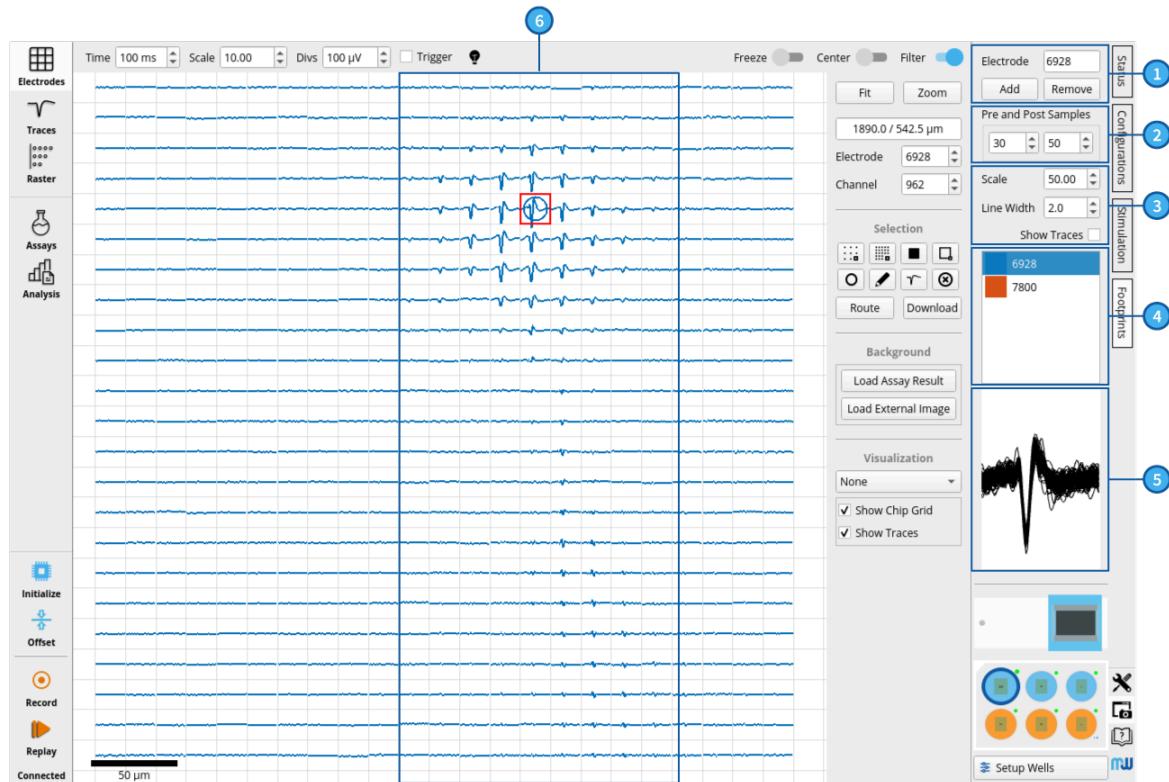


Figure 4.5. Footprints Tab

The necessary steps to visualize a footprint of a cell are detailed below. They consist of selecting the electrodes whose spikes will provide the trigger for signal averaging on all the other electrodes and adjusting the visualization settings.

(1) Selecting the Triggering Electrodes

Spikes detected on the triggering electrode will determine the time point around which signals will be averaged on all the other electrodes. To select a triggering electrode, the user has four options:

1. Clicking on an electrode in the display window and then clicking the **Add** button
2. Entering an electrode number into the **Electrode** field and then clicking the **Add** button
3. Clicking on the **Create template** button in the Electrodes View (see Table 4.2) which will allow the user to select an electrode that will automatically be added to the Footprints Tab (even if another tab is open on the sidebar)
4. Using the key “t”, which is a shortcut for the **Create template** button described above

Icon	Name	Description
	Create Template	Adds the currently selected electrode (highlighted by a red square) to the Footprints Tab for spike-triggered averaging

Table 4.2. Create Template Button**(2) Pre and Post Samples**

The pre and post sample fields determine the time window that is averaged for each electrode by defining the number of samples before and after the trigger time that will be included in the window.

(3) Scale, Linewidth, Show Traces

The scale and linewidth settings allow to modify the visualization of the footprint in the Electrodes View. For example, to visualize axonal processes, the user might want to increase the scaling to see smaller signals. The linewidth can be increased to make the traces more visible. The **Show Traces** checkbox can be unchecked to hide the live visualization of traces, so that the footprints are easier to see.

(4) Selected Electrodes

The selected triggering electrodes are displayed in this field. Clicking on an electrode will select it for visualization in the Electrodes View, while a second click will hide it. Right-clicking in this menu allows the user to **Select all** triggering electrodes for visualization or to **Deselect all** triggering electrodes to hide them.

Furthermore, right-clicking and choosing **Export selected** will export the selected electrode footprint(s) into a .csv file. Every column in the exported .csv file stands for a particular electrode. While the first and second row carry the information about the X- and Y-position of the electrode, the third to last column contain its spike-triggered average waveform.

(5) Spike-Triggered Average Preview

This preview window displays the spike-triggered average of the electrode selected in red on the Electrodes View and is composed of the 100 most recent spikes. The triggering time point is defined by the selected electrode in (4) – if a single electrode is selected – or by the most recently clicked electrode in (4) if multiple electrodes are selected.

(6) Footprint

The footprint(s) of the selected electrode(s) are displayed here. The example in Figure 4.5 shows the footprint of a cell with an axonal projection.

Chapter 5. Shortcut Bar and Settings

This chapter gives an overview of the functionalities of the Shortcut Bar, located at the bottom right corner of the software interface. Particularly, the **Settings** for customizing the software are explained.

1. Shortcut Bar Overview

The Settings Section is located on the bottom right corner of the GUI. It contains the four push buttons shown in Table 5.1.

Icon	Name	Description
	Settings Dialog	This button opens the Settings Dialog to modify visualization and filtering settings as well as the data and software paths.
	Screenshot	Acquires a screenshot of the current window.
	Documentation	The Documentation Icon opens the MaxLab Live User Manual.
	About MaxLab Live	Opens a window containing the software release version, additional information and a push button to check for software updates.

Table 5.1. Shortcut Bar Icons

2. Settings

The Settings Dialog allows the user to customize several aspects of the software. It contains four sections, which are listed on the left side of the dialog window. In the following, the individual sections are described.

Display Settings

The Display Settings, as shown in Figure 5.1, allow to adjust the colors and parameters of the software components. The description of the parameters is displayed when holding the mouse pointer on the orange light bulbs and is also provided in Table 5.2.

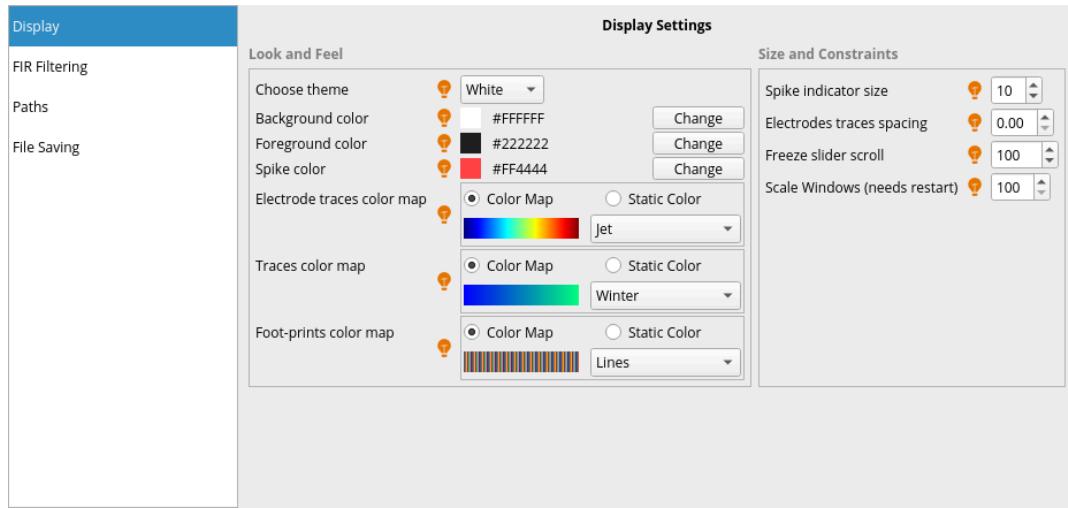


Figure 5.1. Display Settings

Parameter	Description
Look and Feel	
Choose Theme	Changes the theme of the windows including colors, look and feel
Background Color	Controls the background color of the software windows
Foreground Color	Controls the foreground color of the software windows
Spike Indicator Color	Controls the color of the spike indicator in the Electrodes and Traces View
Electrode Traces Color	Changes static color or color palette, respectively, for visualizing signal traces in the Electrodes and Raster View
Traces Color	Changes static color or color palette, respectively, for visualizing signal traces in the Traces View
Footprints Color	Changes static color or color palette, respectively, for visualizing footprints
Size and Constraints	
Spike Indicator Size	Controls the size of the spike indicator in the Electrodes and Traces View
Electrode Traces Spacing	Controls the empty space between two neighboring traces in the Electrodes View
Freeze Slider Scroll	When using the Freeze Mode in the Electrodes and Traces View, the slider can be moved back and forth using the combinations Alt-Left or Alt-Right. This parameter defines the number of samples by which the slider is moved when using these shortcuts.

Parameter	Description
Scale Windows (needs restart)	Scales the windows to the factor provided as percentage of the original size. Useful for High DPI Screens. NOTE: Needs a restart for the setting to be applied.

Table 5.2. Display Settings: Parameter Descriptions

FIR Filtering

In many applications, band-pass filtering is applied to signals in order to attenuate frequencies which lie outside the range of interest. Action potential signals, for example, are carried by signals in the range between 300 Hz and 3 kHz. It therefore makes sense to use a band-pass filter which passes signals above 300 Hz (high-pass cutoff) and below 3000 Hz (low-pass cutoff), when action potentials are detected and recorded. This will significantly reduce low-frequency oscillations, which might otherwise impede the threshold detection. At the same time, high-frequency noise from the MEA circuitry is also significantly attenuated, leading to a higher signal-to-noise ratio.

This section (see Figure 5.2) allows to customize the settings for the online bandpass filter in order to adapt it to the signals of interest.

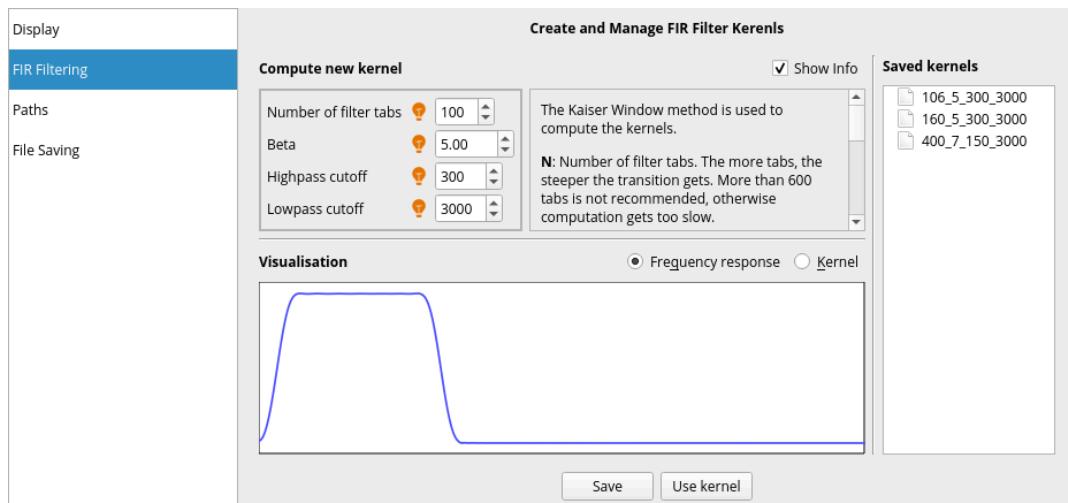


Figure 5.2. FIR Filtering

To create a new FIR filter kernel, first modify the filter parameters described in Table 5.3. After pressing **Save**, the new filter will be created and will appear on the list **Saved Kernels** at the right side of the dialog window. Finally, select the desired filter from that list and press **Use Kernel** in order to activate the filter. The filter is now selected for online filtering of the signals.

Parameter	Description
Number of Filter Taps	The number of filter taps relates to the amount of memory and calculations needed to compute the filter output. An N th-order FIR filter will require $N+1$ filter taps. FIR filters of higher orders have a steeper transition between the attenuated and passed frequency ranges. Recommended values for the number of filter taps lie within 100 and 500.
Beta Parameter	The beta parameter controls the trade-off between side-lobe level and main-lobe width. Larger beta values give lower side-lobe levels, but wider main lobe.
High-pass Cutoff	High-pass cutoff frequency in Hz - all frequencies below this value will be attenuated

Parameter	Description
Low-pass Cutoff	Low-pass cutoff frequency in Hz - all frequencies above this value will be attenuated

Table 5.3. FIR Filtering Parameters

Paths

As shown in Figure 5.3, this section contains three fields for which paths can be configured.

The field **Data Root Directory Path** allows to configure the storage location for the data of recorded assays. The fields **Python3 Executable** and **Python Path Directories** are only required for non-standard installations of Python modules. Please contact support@mxwbio.com if you require such a non-standard installation.

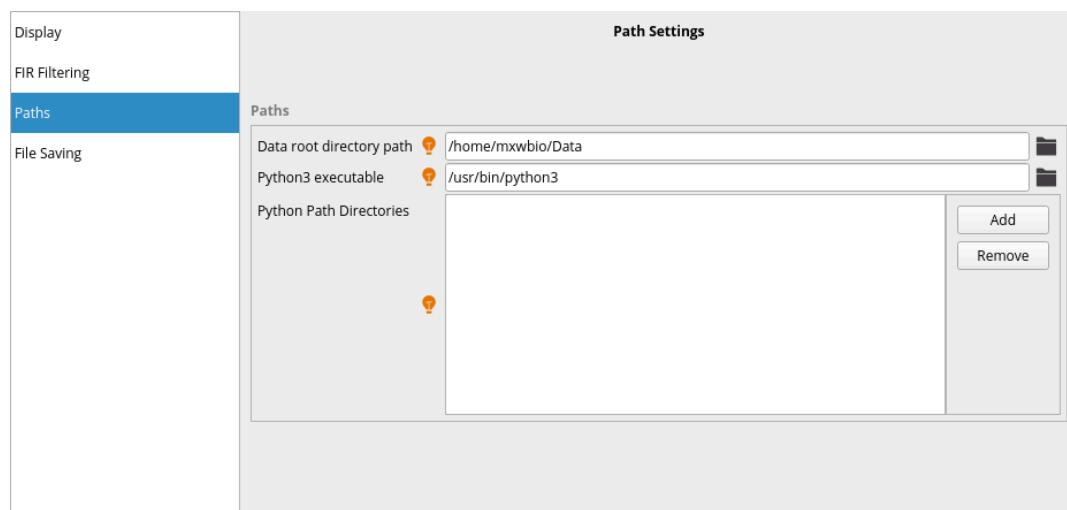


Figure 5.3. Paths

File Saving

As shown in Figure 5.4, a distinction between the file saving settings for MaxOne and MaxTwo is made. MaxOne users have the possibility to use a legacy file format, which corresponds to the standard file format before Release 20.1. This option should be selected if users have custom analysis scripts (e.g. using MATLAB) which were not yet adapted to the current file format. All MaxTwo systems automatically use the current file format.

MaxOne users which do not select the legacy file format, as well as MaxTwo users, can enable **proprietary delta compression** which significantly reduces the file size of the recorded data. We highly recommend to enable the file compression.



Figure 5.4. File Saving

3. About

The **About MaxLab Live** button opens the dialog as shown in Figure 5.5. This dialog shows important information related to the software versioning and software support.

- An overview of the most recent software features and changes is displayed when clicking on the **What's New?** button
- The current version of the MaxLab Live software is shown
- Pending software updates are indicated
- The user can see if there are new updates by using the button **Check for Updates**
- If software support is required, the logs can be exported (**Export Logs** button) into a folder. Sending the exported file to support@mxwbia.com will greatly help to identify and solve the problem.

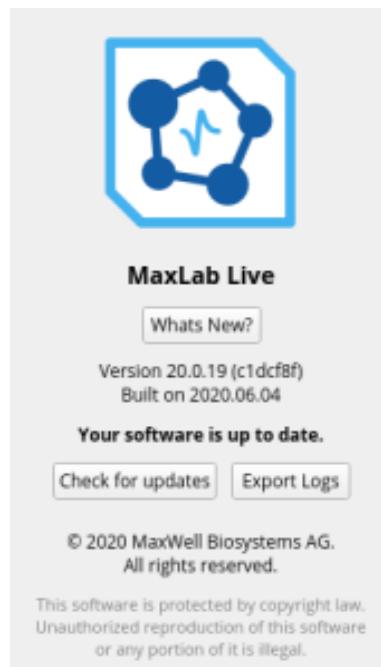


Figure 5.5. About MaxLab Live Dialog

Chapter 6. Assay Workflow

So far, the software tools and modules for controlling the system and visualizing the signals using MaxLab Live have been introduced. Those are typically used to check cell viability in the beginning of an experiment or to manually explore the cellular activity. For most research applications, however, it is crucial to have standardized and repeatable experiments. This chapter introduces the workflow and interface for running *Assays*, which are automatized experiments that involve one or multiple recording steps.

1. Assay Interface Overview

MaxLab Live Assays are automated experiments which typically involve a series of steps. The User Interface for the Assays is accessed by clicking on the **Assays** button on the left side of the GUI window.

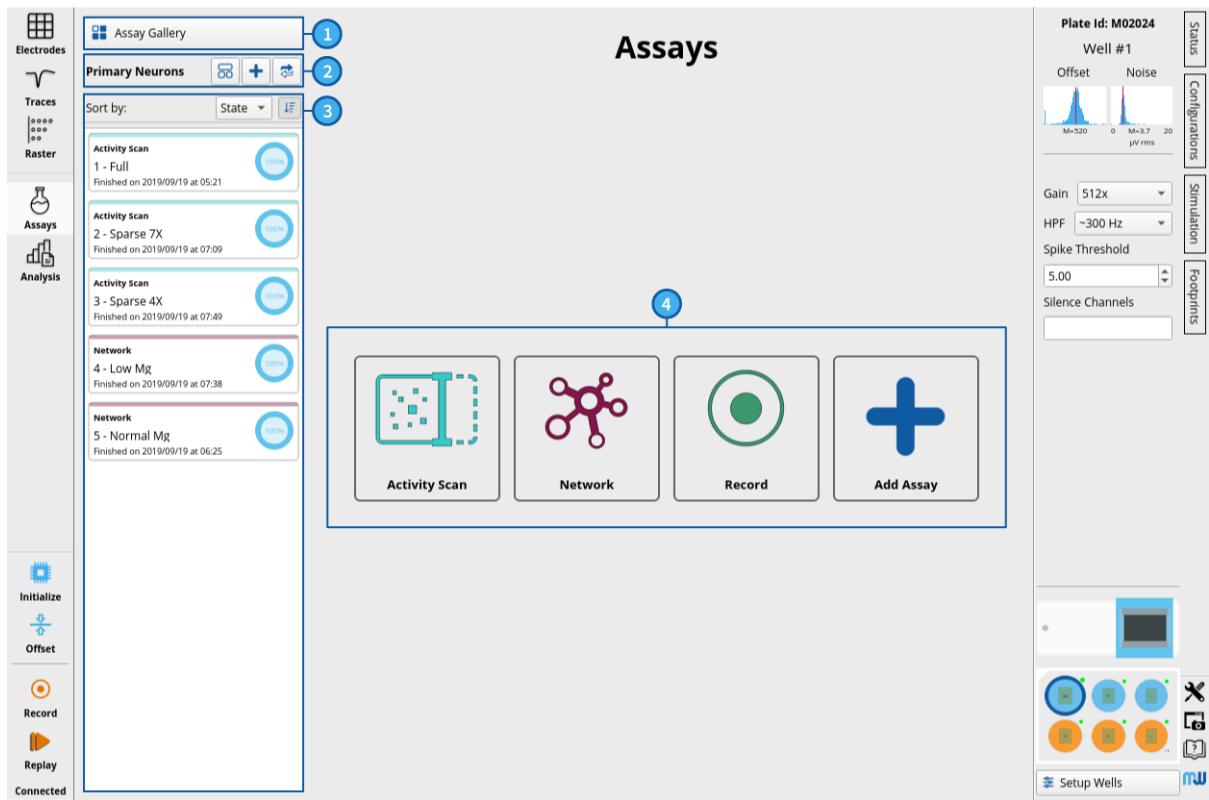


Figure 6.1. Overview of the Assays UI

Figure 6.1 shows the different components of the Assays View:

(1) Assay Gallery

Pushing this button opens the Assay Gallery (4) in order to create a new assay.

(2) Project Toolset

Projects are used to organize MaxLab Live Assays. The Project Toolset contains three buttons:

Icon	Name	Description
88	Project Overview	Opens an overview with information about the current project
+	New Project	Creates a new project
↔	Change Project	Changes the current project

Table 6.1. Project Toolset

(3) Assays List

All the executed, planned and cancelled assays are shown in the Assays List. They can be sorted in ascending or descending order according to their state, type, tag or time.

(4) MaxLab Live Assays

A new assay can be created by clicking on the corresponding icon in the **Assay Gallery**.

2. Organize Assays with Projects

A project can be seen as a container for multiple assays and helps the user to keep a better overview of the acquired data by grouping related assays. Furthermore, the project tools allow to customize the file structure to store the assay datasets. For example, a separate project can be used for every study or for every biological preparation. This feature also helps to organize data when multiple users are operating MaxLab Live.

New Project

As mentioned above, the project structure allows to customize the way in which the assay data is stored. When the button **New Project** (Figure 6.2) is clicked, the following input window appears (Figure 6.2):

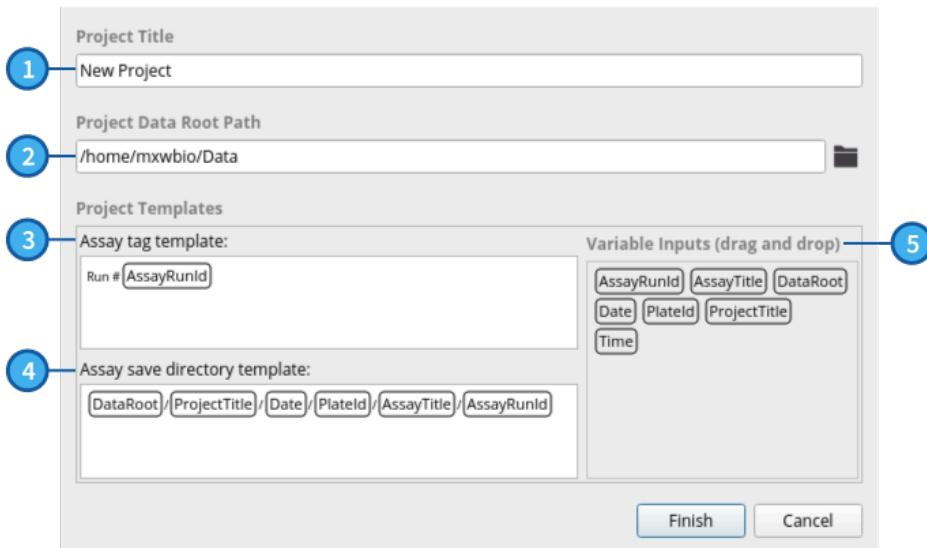


Figure 6.2. Defining Data Structure for New Project

The title of the project can be specified in **Project Title** (1) and the root path for storing the data in the **Project Data Root Path** (2). In the **Assay tag template** (3) window, the user can specify how the new assays should be named. As a default, every new assay is named "Run #" followed by the **AssayRunId**, which is a number that is increased each time a new assay is created. Finally, the folder hierarchy generated to store the data from the individual assays is shown in the **Assay save directory template** (4) window.

To customize the assay naming (3) or the assay data saving structure (4), elements from **Variable Inputs** (5) can simply be dragged and then dropped to the respective windows (3) and (4).

Project Overview

The Project Overview is accessed by clicking the corresponding button (2) in Figure 6.1 and summarizes all the relevant information of a project.

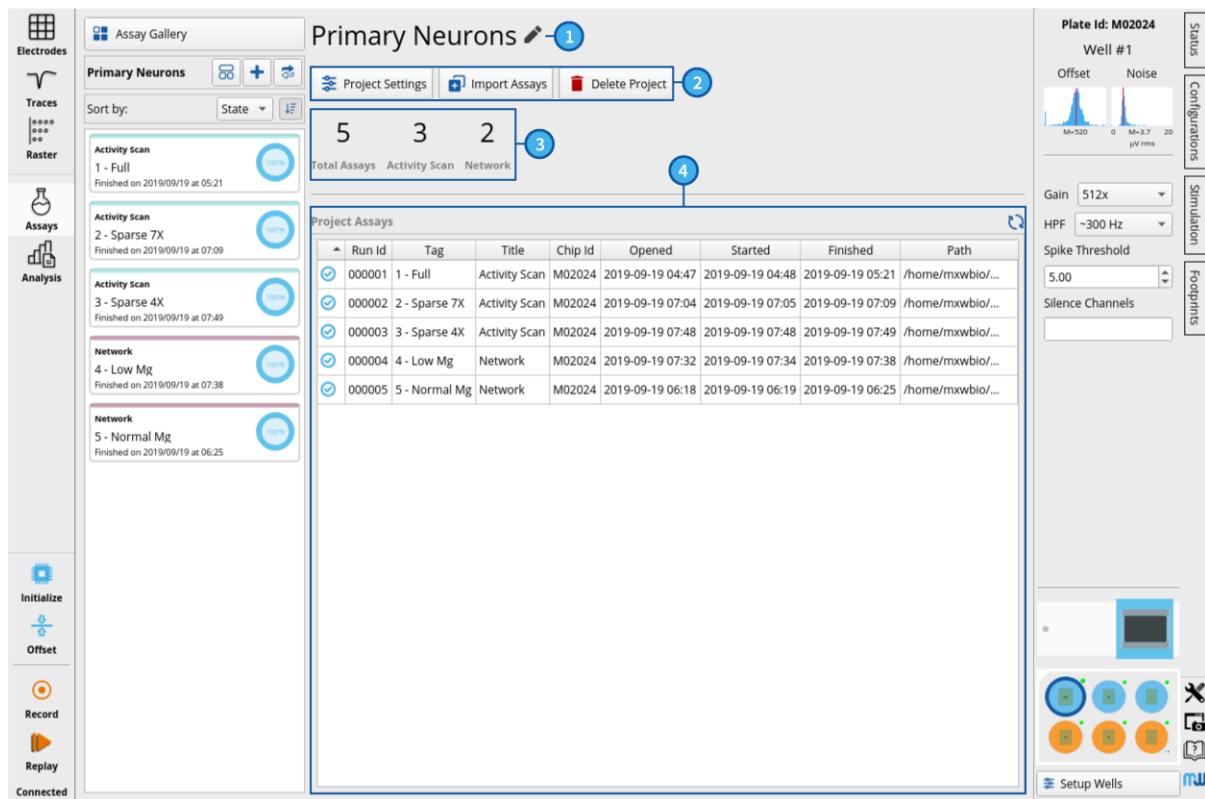


Figure 6.3. Project Overview

The individual components of this view are displayed in Figure 6.3:

(1) Project Name

The project name is shown here. Note that the project can be renamed anytime.

(2) Project Options

Here the project can be managed.

Icon	Name	Description
	Project Settings	Clicking this button will open the dialog for customizing the project settings, such as the assay data saving structure. The dialog is equivalent to Figure 6.2
	Import Assays	This functionality allows to import assays that were recorded within another project into the current project
	Delete Project	Deletes the entire project

Table 6.2. Project Options

(3) Project Summary

The total number of assays and the number of each type of assay are specified here.

(4) Detailed List of all the Assays in the Project

This list provides detailed information about every assay in the project.

3. Create New Assays

The first step to create a new assay is to open the Assay Gallery by clicking on button (1) in Figure 6.1. The available assays appear as icons in the Assay Content Area.

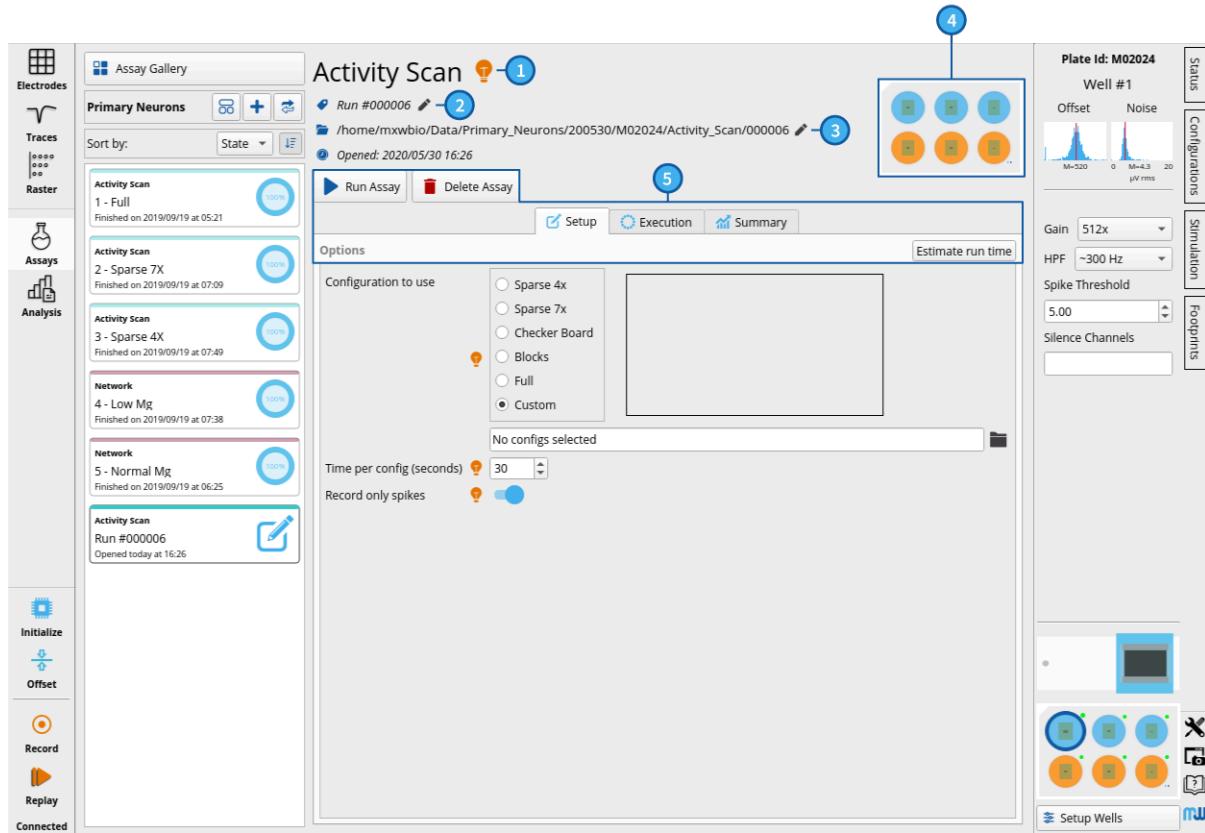


Figure 6.4. New Assay Overview

Assay-related information, push buttons and an interface providing the assay parameters are displayed in the content area, as shown in Figure 6.4:

(1) Assay type

Describes the assay type.

(2) Assay Name

Per default, the name "Run #N" is assigned to an assay, where the number N is increased by 1 for each subsequent assay. However, this name can be arbitrarily changed, e.g. to a name that describes the experiment.

(3) Data Folder

Folder where data and assay information is stored. The data saving path can be modified in the project overview.

(4) Well Selection

If using a MaxTwo system, the user can select which wells to run the assay on by clicking on the desired wells.

(5) Assay Options

The following table lists the actions available when running an assay, which are described in more detail in Section 4 of this chapter.

Icon	Name	Description
	Run Assay	Starts the assay. An assay run can be stopped at any time.
	Delete Assay	Removes the assay from the assay list in the GUI. Optionally, the corresponding data can be deleted.
	Setup	The Setup view lists all parameters that should be revised and modified before running an assay. The parameters per assay are further explained in Chapter 7.
	Execution	Displays the progress of the currently running assay
	Summary	Once the assay run is finished, the results are displayed in this tab
	Estimate run time	Estimates the total execution time of the assay before starting

Table 6.3. Assay Options

4. Run Assay and Inspect Results

Once all the assay parameters have been specified in the Setup Tab, the experiment can be started by pressing **Run Assay**. As shown in Figure 6.5, the view in the Assay Content Area changes to the **Execution** mode.

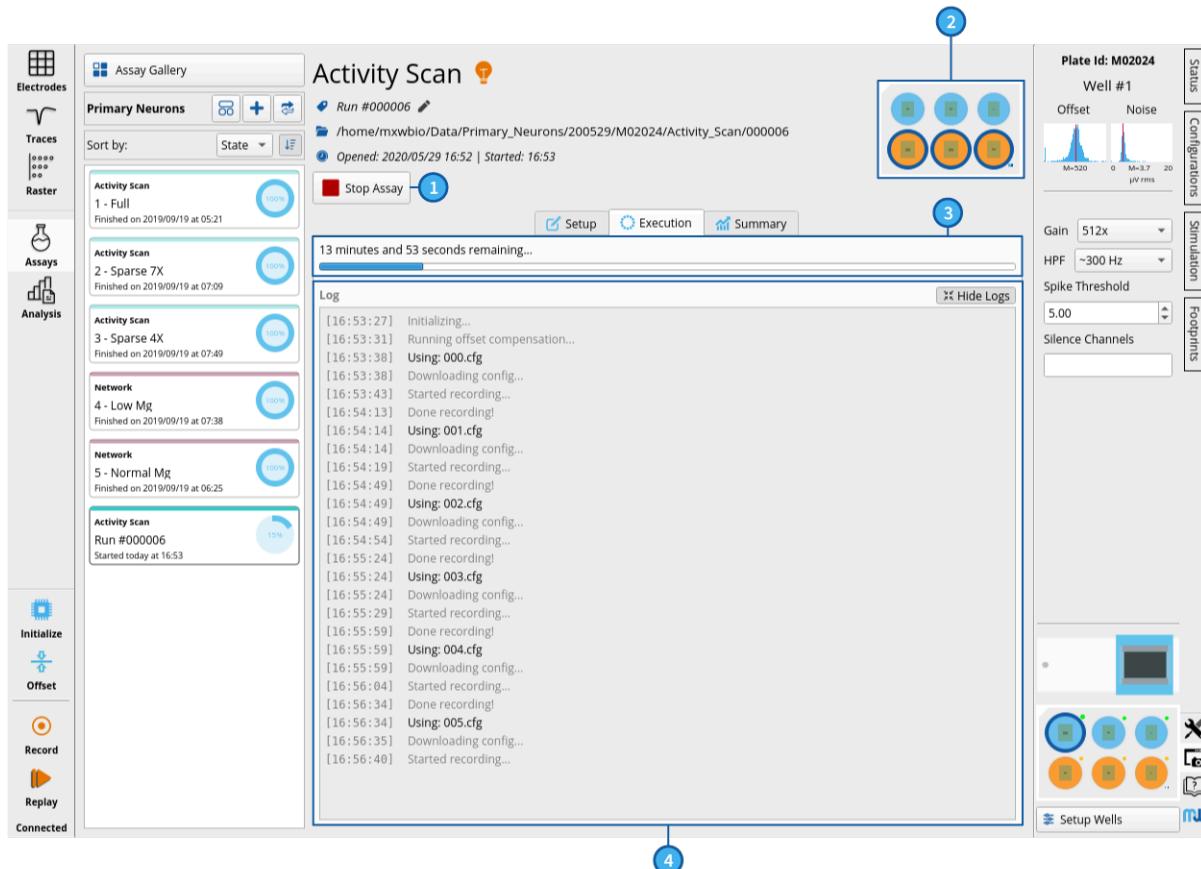


Figure 6.5. Running an Assay

- During the assay execution the only available button is **Stop Assay** (1).
- The wells which the assay is being run on are circled in blue (2); in this example, the assay is running on the bottom three wells.
- A progress bar (3) displays the remaining execution time.
- The experimental log is shown (4).

The **Summary** view is displayed once assay execution is complete. Figure 6.6 illustrates the components of the assay content area after successfully running an assay.

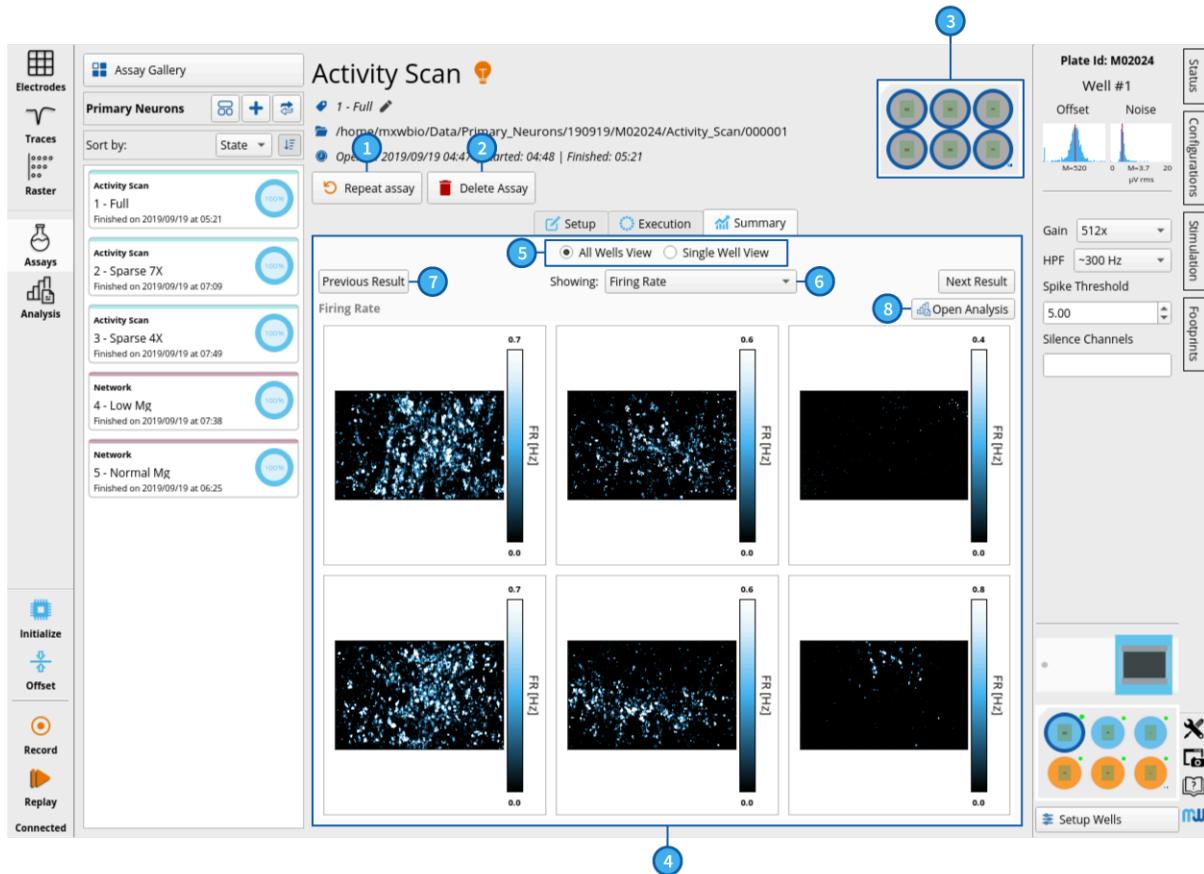


Figure 6.6. Assay Results

(1) Repeat Assay

The **Repeat assay** button creates a new assay with the same input parameters as the current one. The new assay appears at the bottom of the assay list.

(2) Delete Assay

The assay can be deleted with the **Delete Assay** button.

(3) Selected Wells

The wells which the assay was run on are indicated by blue circles; in the example in Figure 6.6, it was run on all six wells.

(4) Summary Tab

The assay-specific results are graphically displayed in the **Summary Tab**.

Note: buttons 5-7 are only available for MaxTwo recordings.

(5) Well View Selection

Here, the view can be changed between the **All Wells View**, where the results of all wells for one analyzed metric are displayed, and the **Single Well View**, where several analyzed metrics are displayed for just one well.

(6) Showing

In the **All Wells View**, the metric that is graphically displayed in the Summary Tab can be switched using the drop-down **Showing** menu.

(7) Previous/ Next Results

If the results are shown in the **Single Well View**, one can scroll through the results of the different wells using the buttons **Previous Result** and **Next Result**.

(8) Open Analysis

This button opens the current assay in the **Analysis Tab**, where further analyses and extraction of additional metrics can be carried out (see Chapter 8 and Chapter 9).

Chapter 7. MaxLab Live Assays

The **Assay Workflow**, as described in the last chapter, allows to plan and execute automatized and standardized experiments. In this chapter, the available assays in MaxLab Live are introduced and the experimental parameters, the assay functionalities as well as the assay results are explained.

1. Activity Scan

It is important to be able to assess whether cells are electrically active (i.e. firing action potentials) and to identify their position on the array. This information gives an indication of the viability and developmental status of the cells. The **Activity Scan Assay** provides a quick and automated framework to obtain this information and is typically the first step of an experiment. The assay sequentially records different configurations of electrodes, thereby scanning the entire array for action potentials. Its output can be understood as an electrical image of the cells on the array.

Setup

The Setup Tab allows to customize the different options of the Activity Scan Assay and is shown in Figure 7.1.

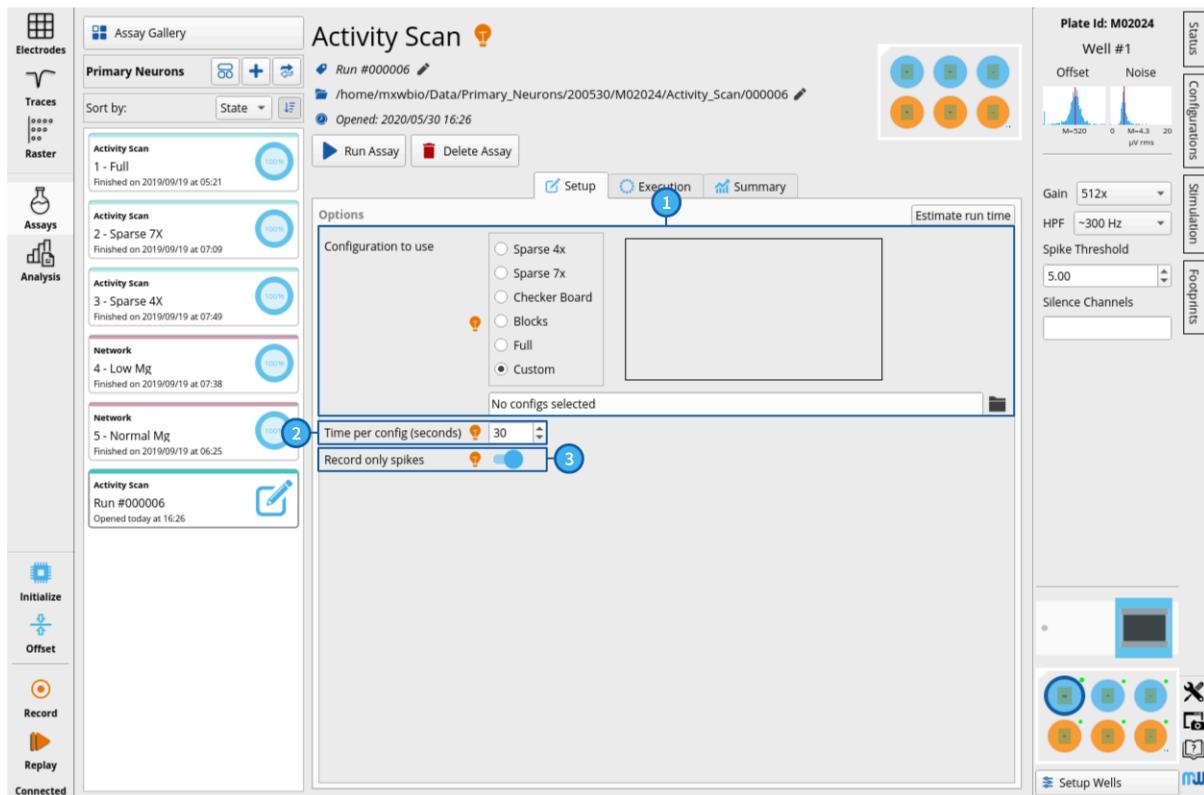


Figure 7.1. Activity Scan Assay Parameters

The main parameters of an Activity Scan experiment are (1) the configurations, which determine spatial coverage and resolution, (2) the recording time and (3) the data recording format.

(1) Configuration to use

The user can select from the following options:

- **Sparse 4x**

This option is designed to gather a quick overview of the electrical activity by scanning the electrode array in four configurations. This scan measures 12.5% of the total electrodes.

- **Sparse 7x**

The electrode array is scanned in seven configurations, where every second electrode in the x- and y-directions is sampled. This scan measures 25% of the total electrodes.

- **Checkerboard**

The array is scanned with a checkerboard pattern, thus recording from every other electrode.

- **Blocks**

This scan focuses on the center area ($1.8 \times 3.1 \text{ mm}^2$) and samples it at the highest-possible resolution. The area is scanned with 25 configurations of 20×20 electrodes (high-density blocks).

- **Full**

This scan provides the highest possible scanning resolution where the entire array is covered in 29 configurations.

- **Custom**

Here, any custom set of configurations can be selected for the Activity Scan.

The specifications of the predefined scanning options are outlined in Table 7.1. The scan duration denotes the total time required for an Activity Scan assay if every configuration is recorded for 30 seconds. This includes the time needed for offset compensation and configuration changes. Note that the Checkerboard and the Block configurations do not cover the complete array area.

	Number of configurations	Total electrodes recorded	Area coverage [mm^2]	Spatial resolution [els/ mm^2]	Scan duration [min]
Sparse 4x	4	3300	2.1×3.85	408	2.8
Sparse 7x	7	6600	2.1×3.85	816	4.9
Checkerboard	14	13133	2×3.85	1633	9.8
Blocks	25	20000	1.8×3.85	3265	18
Full	29	26400	2.1×3.85	3265	20

Table 7.1. Specifications for Activity Scan Configurations

(2) Time per config

This parameter sets the recording time in seconds for every configuration of the Activity Scan Assay.

(3) Record only spikes

The user can choose between recording the continuous voltage signals measured at the electrodes (**Record only spikes** disabled) and recording only the detected spike events. If the option **Record only spikes** is enabled, only the time and amplitude (at the negative peak) of the detected spikes are saved in the data file.

Note: When setting the experimental parameters, the trade-off between experiment duration and spatial coverage must be considered. For example, if the recording time per configuration is 30 seconds, a **Sparse 7x scan**, which includes seven sequential configurations, will last approximately 5 minutes and will provide a good overview of the cells' location and viability. The results of a **Full scan**, on the other hand, will require 20 minutes and provide a highly detailed activity image, more data for statistical analysis and a better basis for identifying isolated neuronal units.

Importantly, the recording time per configuration must be long enough to capture the dynamics of the neural activity. If a preparation exhibits strong bursting activity with interburst intervals of several seconds, the recording duration should be long enough to include a sufficient number of bursts in each configuration in order to obtain reliable firing rate values. For preparations with fast, regular spiking activity, 20 – 30 seconds per configuration is often good enough to acquire proper firing rate distributions.

Results

The Results Tab, as shown in Figure 7.2, displays the results of an Activity Scan. This window automatically appears once the scan is completed.



Figure 7.2. Activity Scan Assay Results

Both the firing rate and the spike amplitude values are displayed as an activity map (left) and as a histogram (right). The activity map representation is useful to visually assess the activity and compare different preparations or to follow the development of a neuronal culture over multiple days. The colormap of the images and the minimum and maximum values of the colorbar can be manually adjusted. On the right side, the histogram representation of the values visualizes the distribution. Here, the histogram limits and the number of bins can be modified.

(1) Firing Rate [Hz]

For every electrode recorded in the Activity Scan Assay, a firing rate value is calculated as the number of detected spikes divided by the recording time.

(2) Spike Amplitude [μ V]

For every electrode recorded in the Activity Scan Assay, a spike amplitude value is calculated, which equals the 90th percentile of the amplitude for all the detected spikes on that electrode. Note that for every spike, the amplitude of the negative peak is considered.

(3) Active Area

An electrode is considered an **Active Electrode** if it has a firing rate value > 0.1 Hz and spike amplitude value $> 20 \mu$ V. 'Active' electrodes, which likely record biological activity, are shown as active pixels (value 1) on the binary map. The percentage of active electrodes (out of the recorded electrodes of the scan) is displayed next to the colorbar and is a suitable metric to characterize the growth and development of a neuronal culture.

(4) Configs used

The individual configurations used in the Activity Scan Assay are visualized in different colors.

2. Network

While the main purpose of the Activity Scan Assay is to identify the position and activity of cells on the array through sequential scanning, the **Network Assay** allows to record activity from a large number of cells simultaneously. Based on a previously recorded Activity Scan Assay, which reveals the locations of the most active cells, subsets of electrodes can be selected and recorded. The Network Assay features several algorithms to tailor the electrode selection to the user's needs.

Setup

The Setup Tab shown in Figure 7.3 provides the controls to customize the assay.

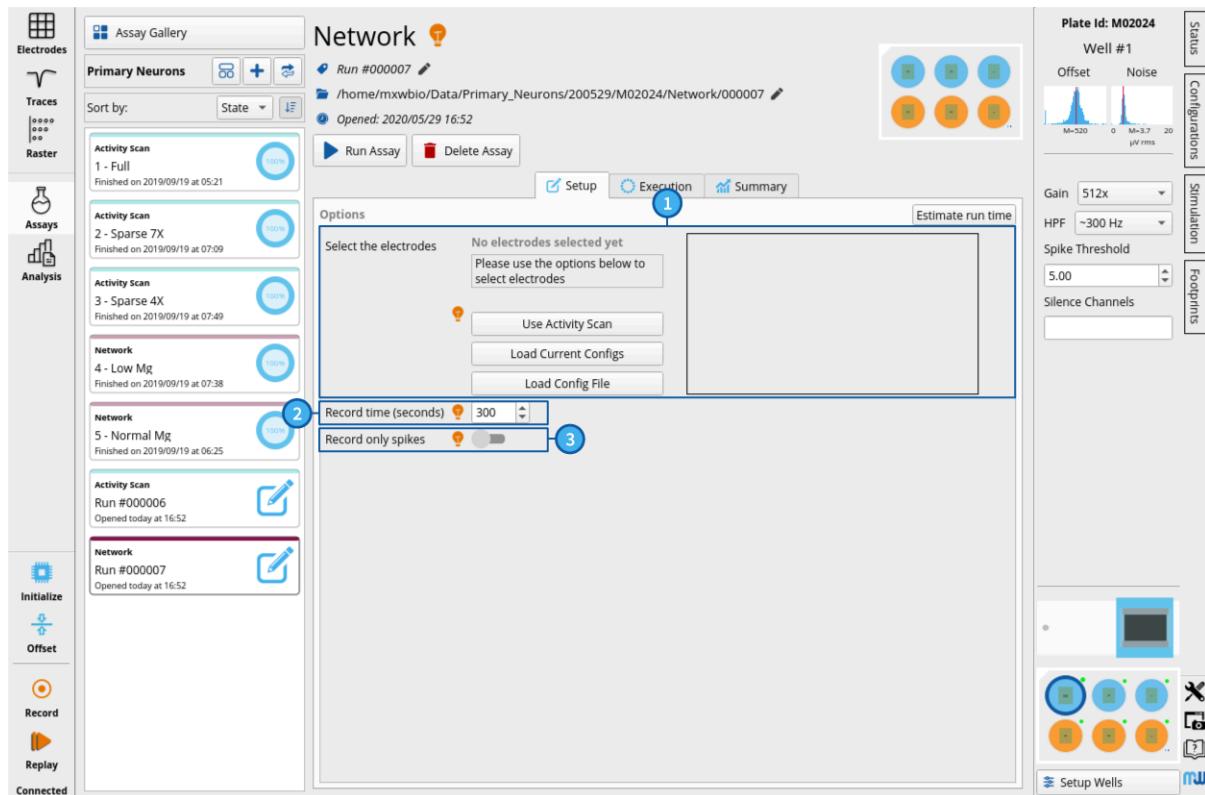


Figure 7.3. Network Assay Parameters

The Setup Tab consists of three main parts:

(1) Select the electrodes

The crucial step of the assay is selecting the electrodes for subsequent recording. Three options are available:

- **Use Activity Scan**

This button opens the window **Select Electrodes from Activity Scan** (Figure 7.4), which offers different algorithms for electrode selection to choose from. The different selection methods are outlined below.

- **Load Current Configs**

This option takes the currently active electrode selection for assay recording.

- **Load Config File**

Allows to load any existing electrode configuration for the recording.

(2) Record time

This parameter sets the time in seconds for the recording of the Network Assay.

(3) Record only spikes

The user can choose between recording the continuous voltage signals measured (**Record only spikes** disabled) or recording only the detected spike events. If the option **Record only spikes** is enabled, only the time and amplitude (at the negative peak) of the detected spikes are saved in the data file.

Select Electrodes from Activity Scan

As a prerequisite for an effective electrode selection, the location and activity of the cells on the array must have been previously identified in an Activity Scan Assay. The interface **Select Electrodes from Activity Scan**, (Figure 7.4) allows to choose between different selection algorithms and to visualize the selected electrodes.

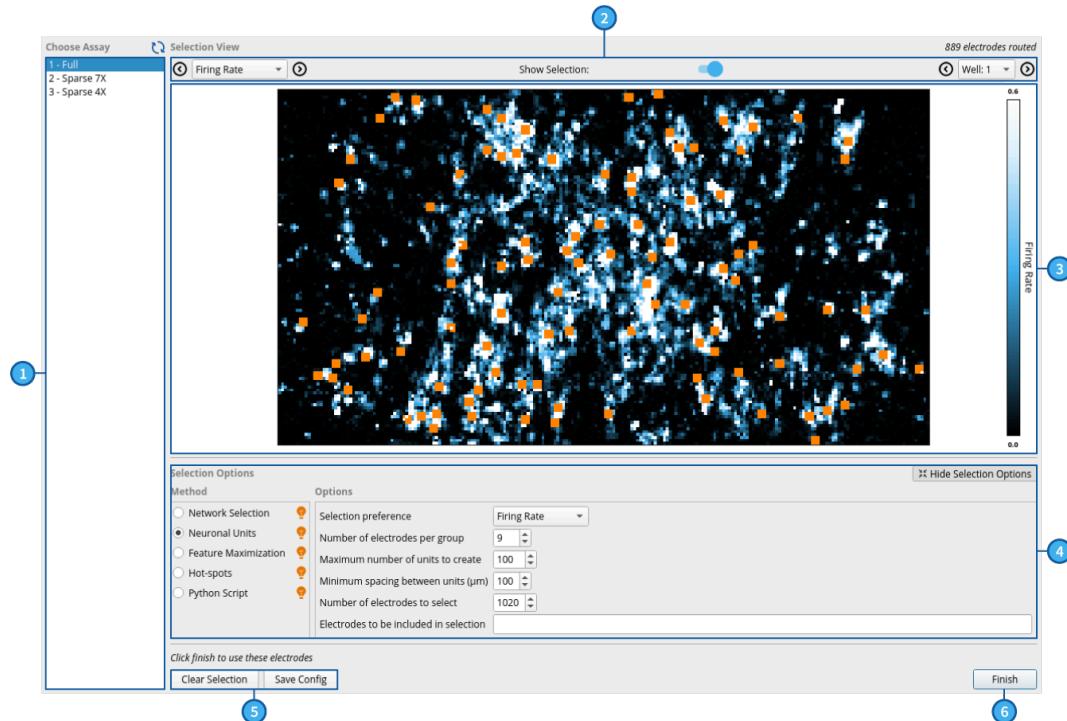


Figure 7.4. Network Selection Options

(1) Choose Assay

A list of the previously recorded Activity Scan Assays, which were run with the same chip ID as the current ID, is shown here. By default, the last recorded Activity Scan is selected.

(2) Assay View

In this section, the visualization of the selected Activity Scan Assay results can be controlled, with the options **Firing Rate**, **Spike Amplitude**, **Active Area** or **Configs used** available. If multiple wells are available (using the MaxTwo system), the well can be selected. The default is set to Well 1.

After evaluating an electrode selection (5) the switch button **Show Selection** allows to enable or disable the visualization of the selected electrodes.

(3) Visualization of Activity Map and Electrode Selection

The selected Activity Scan Assay and the electrode selection are shown here.

(4) Selection Options Section

The button **Show / Hide Selection Options** opens or hides the **Selection Options Section**. Five different **Selection Methods** can be chosen on the left side of the **Selection Options Section**. They are described in the next section.

(5) Selection Controls

The user can click **Evaluate Selection** to execute the electrode selection with the chosen parameters and display the selected electrodes in the visualization section (3). The **Evaluate Selection** button is not visible in Figure 7.4 since the selection has already been evaluated. Evaluating the selection leads to the display of the buttons **Clear Selection** (clear the current selection) and **Route Selected** (route the selected electrodes marked in (3) to recording channels). Once the electrodes have been routed, the button **Save Config** appears, which allows the user to save the selected configuration for later use. Note that the number of selected electrodes is displayed at the top of the window.

(6) Finish

To close the electrode selection dialog, click **Finish**.

Description of Selection Methods

The user can chose between different electrode selection methods which are summarized and illustrated in Table 7.2. Red dots indicate selected electrodes. The selection methods are described in more detail below.

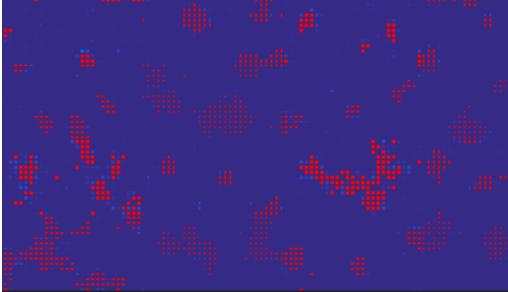
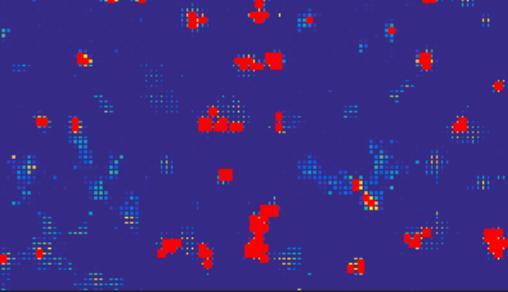
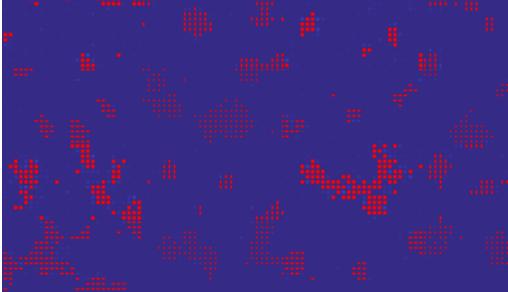
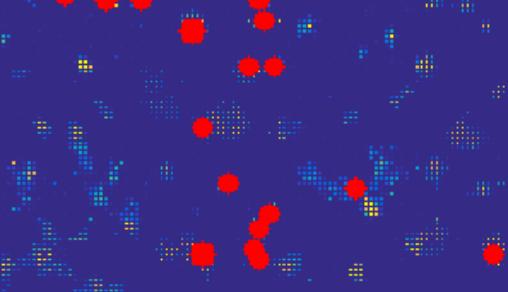
Network Selection	Neuronal Units
Number of recorded neurons is maximized	Locally confined electrodes selected with the same number of electrodes per group; ideal for subsequent spike sorting
	
Feature Maximization	Hot-spots
Electrodes with highest amplitude or firing rate selected	User-defined number of hot-spots are selected; the algorithm defines the number of electrodes per group
	

Table 7.2. Selection Methods Overview

In the following, the selection methods are explained in detail:

Network Selection

- **Summary:**

This selection method is designed to *maximize the number of cells* to be recorded with a single configuration. Out of all electrodes measuring spiking activity, a subset is defined so that the distance between the individual selected electrodes is maximized. A large distance between the selected electrodes reduces the redundancy (recording the same cells with multiple electrodes) and therefore increases the total number of measured cells.

- **Algorithm:**

Step 1 - Out of all the electrodes recorded in the selected Activity Scan Assay, those with both firing rate and spike amplitude values above the specified thresholds (see 'Parameters' below) are classified as *active electrodes*.

Step 2 - Out of all the identified *active electrodes*, the algorithm selects one electrode after the other. During this iterative process, the next selected electrode must not lie within a certain distance (distance threshold) from any other already-selected electrode. The distance threshold ensures that nearby electrodes are not selected.

Step 3 - Once no further electrodes can be selected anymore due to the distance threshold constraint, the distance threshold is reduced and the iterative electrode selection procedure is continued. This procedure is repeated until the specified number of electrodes (see 'Parameters' below) is reached or until no further electrodes can be selected.

Note: the selection of the electrodes in steps 2 and 3 can be influenced with the **Selection preference** parameter. If e.g. the preference **Spike Amplitude** is selected, the algorithm will first select the electrode with the highest amplitude, then select the electrode with the second-highest amplitude etc., while taking into account the distance threshold as described above. If the preference **Random** is selected, the selection of the electrodes during steps 2 and 3 will be fully randomized and only limited by the distance threshold.

- **Parameters:**

- **Selection preference** - Spike Amplitude: electrodes with larger spikes are preferred; Firing Rate: electrodes with higher firing rates are preferred; Random: the selection is randomized.

- **Active electrodes amplitude threshold** - Defines the minimum spike amplitude (in μV , 90th percentile) required to consider an electrode for selection.

- **Active electrodes firing rate threshold** - Defines the minimum firing rate (in Hz) required to consider an electrode for selection.

The following parameters are identical also for the subsequent selection methods:

- **Number of electrodes to select** - Defines how many electrodes should be selected for recording.

- **Electrodes to be included in selection** - This optional field allows to manually specify a set of electrodes (comma-separated) to be included in the selection.

Neuronal Units

- **Summary:**

The selection method **Neuronal Units** should be used if subsequent spike sorting of the data is performed. The selected electrodes will be arranged as small, round groups of neighboring electrodes. Action potentials from individual units will therefore always be recorded on multiple electrodes, which greatly improves spike sorting performance.

- **Algorithm:**

The number and size of the electrode groups can be defined with the Parameters. The groups will either be centered around the electrode with the highest amplitude (Selection preference: **Spike Amplitude**) or the highest firing rate (Selection preference: **Firing Rate**).

- **Parameters:**

- Selection preference - Spike Amplitude: electrodes with larger spikes are preferred; Firing Rate: electrodes with high firing rates are preferred.

- Number of electrodes per group - Defines how many electrodes are forming a group.
- Maximum number of units to create - Defines the number of groups.

Feature Maximization

This selection method simply picks the electrodes with the highest amplitude or firing rate values, according to the chosen **Selection preference**.

Hot-spots

This method generates high-density groups of electrodes similar to the **Neuronal Units** mode. In contrast to that method, the user specifies the number of groups (parameter **Number of hot-spots**) and the number of electrodes per group is automatically set.

Python Script

Any selection method can be implemented using this option to select the electrodes with a customized python script.

Note that both the methods **Network Selection** and **Feature Maximization** only select electrodes that were recorded during the Activity Scan Assay, whereas **Neuronal Units** and **Hot-spots** can also select other electrodes. The output of the network recording for these methods will therefore also depend on the resolution of the Activity Scan.

Results

The Results Tab, as shown in Figure 7.5, displays the results and automatically appears once the Network Assay recording is finished.

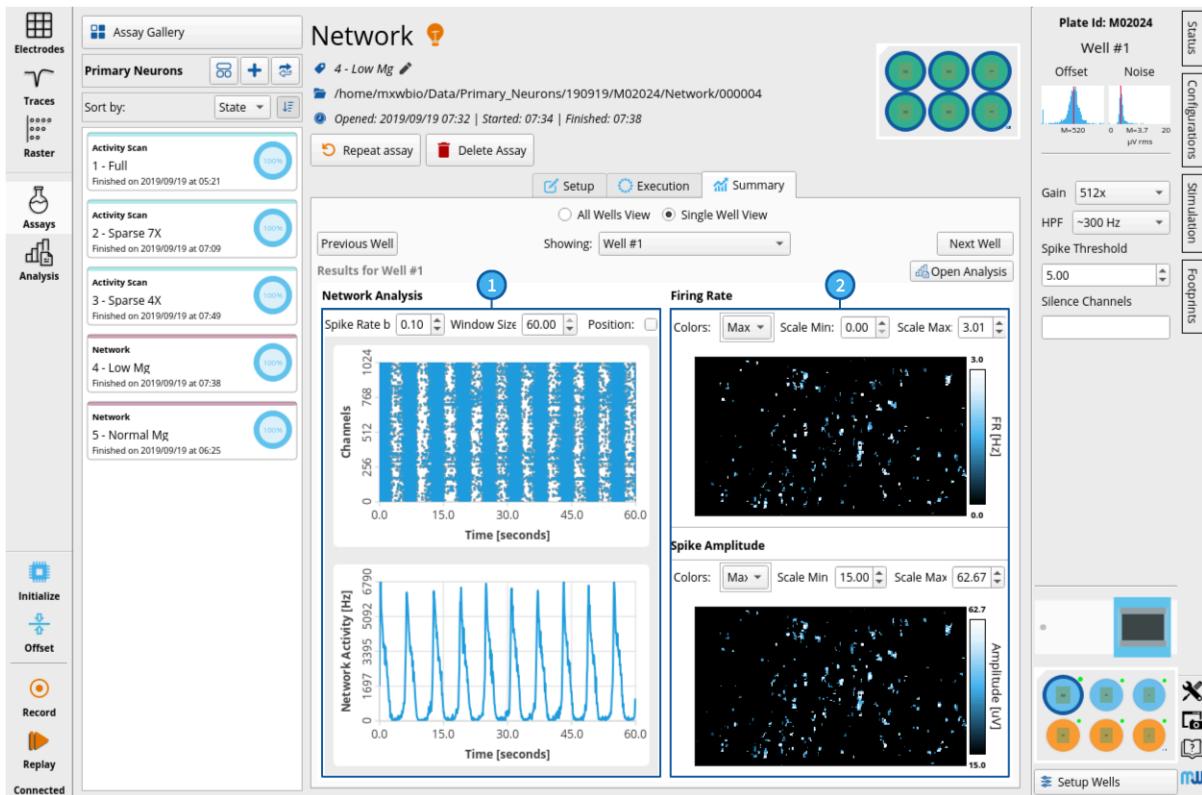


Figure 7.5. Network Assay Results

The left-hand side of the Results Tab displays the network activity, while the activity maps for firing rate and spike amplitude are shown on the right-hand side.

(1) Network Analysis

In the raster plot displayed at the top, every detected action potential is represented by a dot. The x- and y-axis represent time and individual recording channels. This visualization allows to observe whether synchronous firing (bursting) occurs.

The bottom plot represents the overall network activity binned over time. The window size, position and bin width can be modified at the top of these plots.

(2) Firing Rate and Spike Amplitude

Heat map representations of the firing rate (top) and the spike amplitude (bottom) of the recorded electrodes are shown here.

3. Record

With the Record Assay, one can load a configuration file and record for one or multiple time intervals.

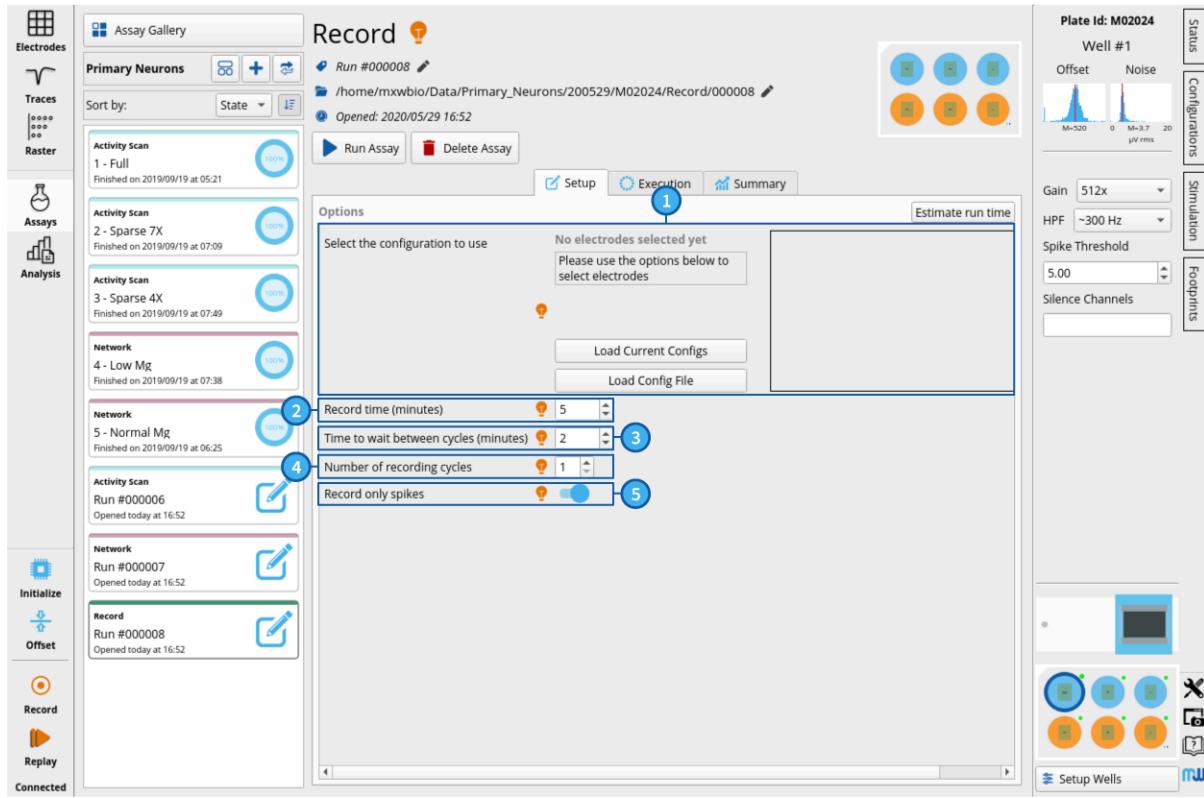


Figure 7.6. Record Assay Parameters

Setup

The Setup Tab shown in Figure 7.6 allows to set various parameters for the assay.

(1) Select the configuration to use

By clicking on **Load Current Configs**, the user can choose to use the currently active electrode selection, which can be created using the Electrode Selection Tools in the Electrodes View (Figure 3.1 (5)). Alternatively, the user can load a configuration from a saved file by clicking on **Load Config File**.

(2) Record time (minutes)

If multiple cycles are recorded, this parameter denotes the recording time per cycle. The default value is 5 min. The time can range between 1 min and 10000 min.

(3) Time to wait between cycles (minutes)

The time in between the individual recording cycles can be set here. The default value is 2 min. The time can range between 1 min and 10000 min.

(4) Number of recording cycles

The number of cycles can be set here. The default value is 1. The number of cycles can range between 1 and 50 cycles.

(5) Record only spikes

If enabled, only the time and amplitude (at the negative peak) of the detected spikes are saved in the data file. If disabled, the complete voltage signal is recorded. This option is disabled by default.

Results

After the assay recording has run to completion, the Summary Tab will open (Figure 7.7).

The firing rate and the spike amplitude are displayed as activity maps. The colormap of the images and the minimum and maximum values of the colorbar can be manually adjusted.

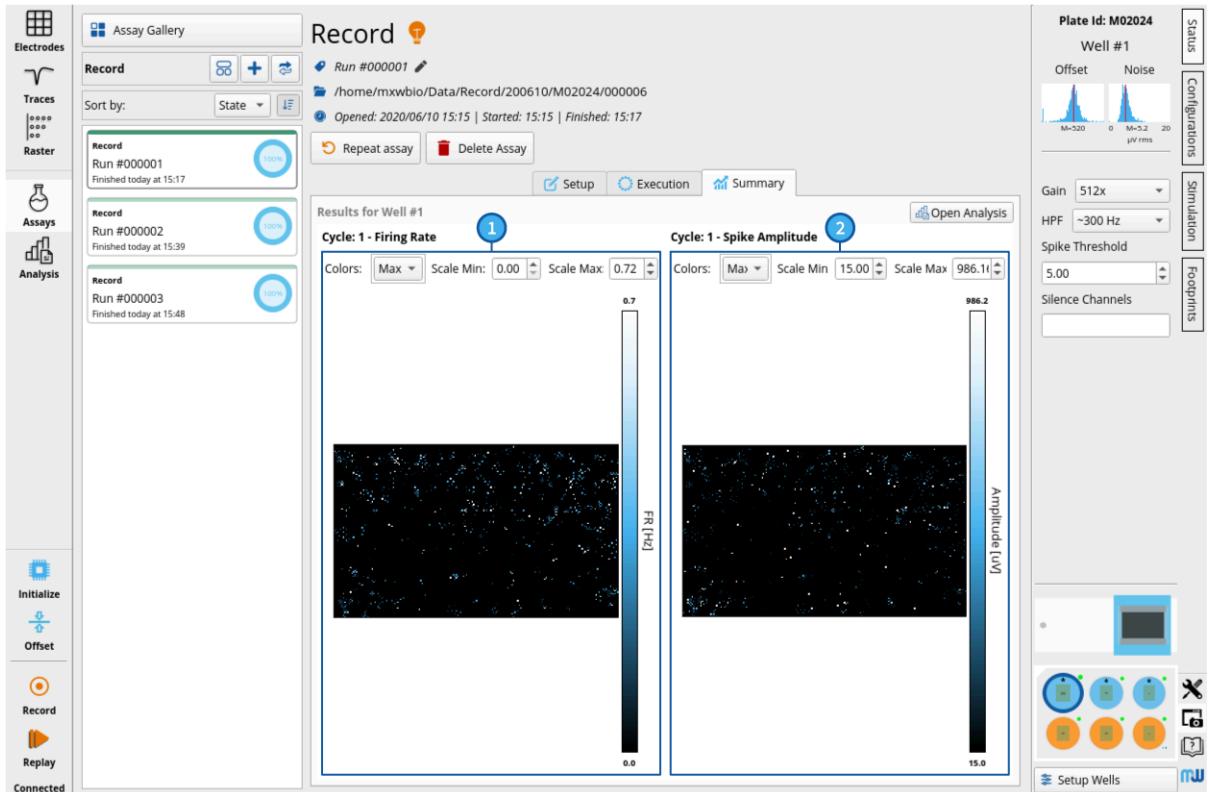


Figure 7.7. Record Assay Results

(1) Firing Rate [Hz]

For every electrode recorded in the Record Assay, a firing rate value is calculated, which equals the number of detected spikes divided by the total recording time.

(2) Spike Amplitude [μ V]

For every electrode recorded in the Record Assay, a spike amplitude value is calculated, which equals the 90th percentile of the amplitudes of all the spikes detected on that electrode. Note that for every spike, the amplitude of the negative peak is considered.

Chapter 8. Analysis Workflow

Performing an assay experiment typically produces a rich dataset. To simplify access to this data, MaxLab Live provides a set of analysis modules that can be directly applied to MaxLab Live Assay results. With the analysis modules, the user can extract meaningful features and useful metrics from the data, which can be used for subsequent statistical analysis.

This chapter describes the user interface of the Analysis Tab which includes how to run an analysis and how to export the computed results. In the next chapter, the individual analysis modules will be described.

1. Launch Analysis

Analysis of an assay can be performed with a simple sequence of steps, which include setting parameters, running the analysis, and exporting the calculated metrics. In this section, the GUI elements needed to launch an analysis are described.

Detailed descriptions of aspects specific to each individual type of analysis are provided in Chapter 9.

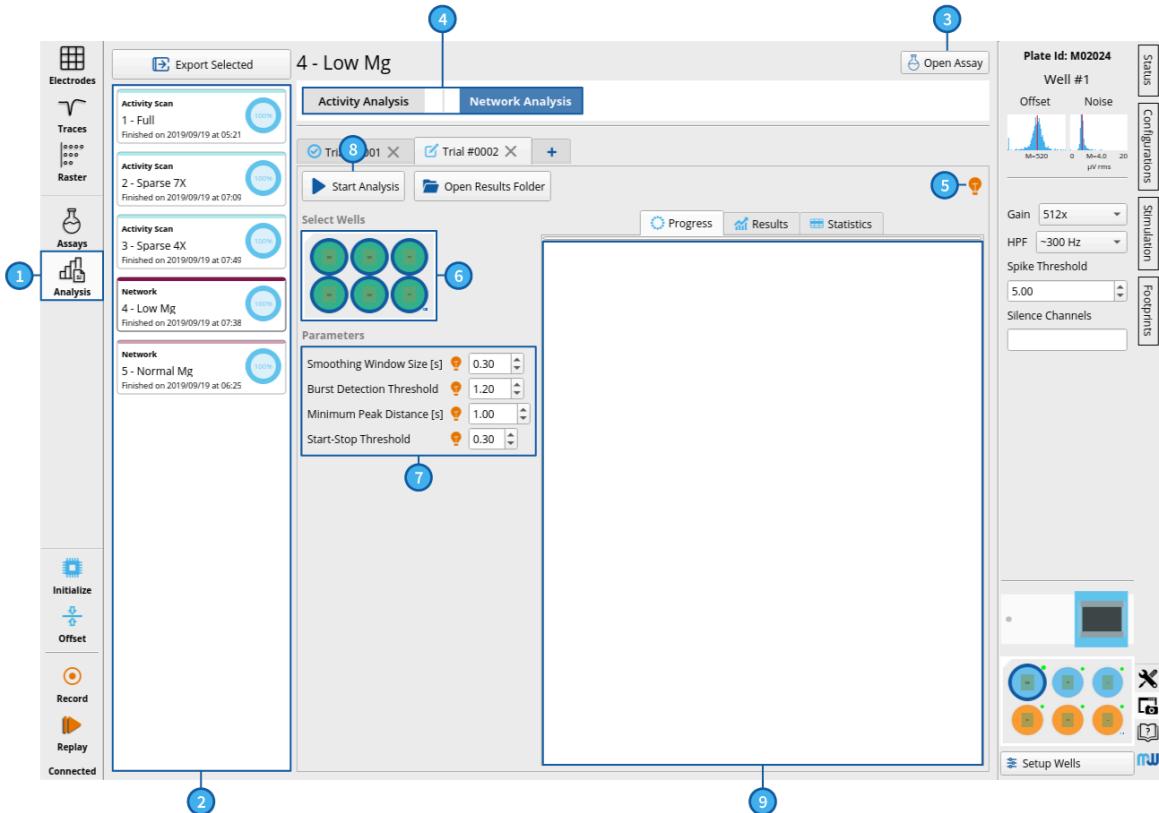


Figure 8.1. Analysis Tab Overview

(1) Open Analysis Tab

Clicking on the Analysis Tab icon opens the Analysis Interface window.

(2) Select Assay Recording in Assay List

In the left vertical panel, the recorded assays ready for analysis are listed. Select an assay by clicking on it.

(3) Open Assay

This button redirects to the corresponding Assay Summary View of the selected assay (see Figure 6.6). This allows to quickly inspect the recording outcome before analyzing it.

(4) Select Analysis from the Available Analyses

For a selected assay, the available Analysis Modules are displayed here. The most suitable analysis to the assay is selected by default, indicated by the blue coloring. A different analysis can be selected by the user.

(5) Analysis Description

Clicking on the **light bulb** button opens a description of the analysis goal and the extracted metrics.

(6) Select Wells

Wells to be analyzed can be selected/unselected by clicking on them. When selected, the well is highlighted by a blue border.

(7) Set the Parameters

Analysis parameters can be adjusted by the user. A detailed description of how to set the parameters specific to each analysis is provided in Chapter 9.

(8) Start Analysis

The analysis run is started by clicking the **Start Analysis** button.

(9) Track Analysis Progress

The display window provides information about the different stages of the analysis as it progresses. See Section 2 of this chapter for details.

2. Monitor and Inspect Analysis

Once an analysis run is started, its progress can be monitored and the results visualized.

Monitor Analysis Progress

The progress of the analysis can be monitored by three elements in the Progress Tab (see Figure 8.2).

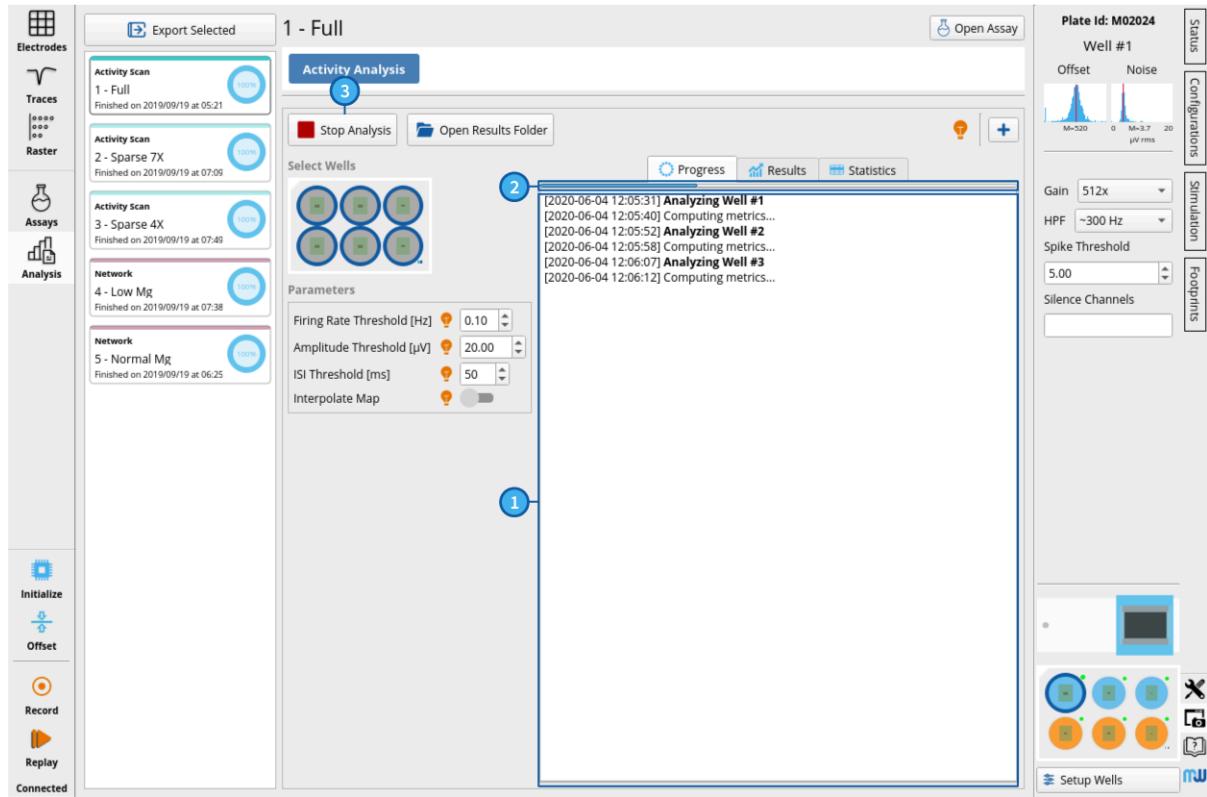


Figure 8.2. Analysis Progress Tab

(1) Log messages

Printed messages in the log window indicate the execution of the main steps. Warning or error messages to the user can also be displayed.

(2) Progress Bar

The blue loading bar indicates the analysis advancement and remaining time.

(3) Stop Analysis Execution

During execution, the user can stop the process at any time by clicking the **Stop Analysis** button.

Results Tab

Analysis results are visualized in the GUI as soon as an analysis run is complete (Figure 8.3). This allows the user to have a quick overview of the analysis outcome.

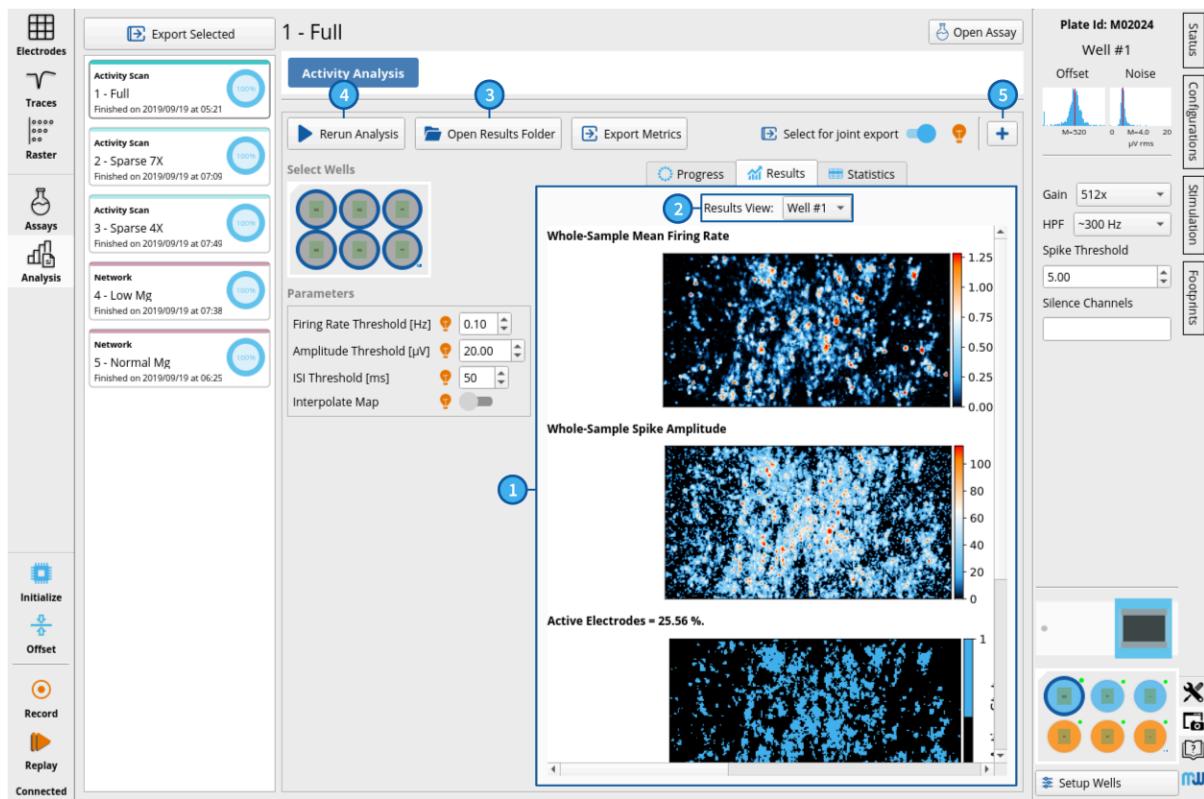


Figure 8.3. Results Tab

(1) Results Tab

Once the analysis run is done, the **Results Tab** automatically opens up on a set of figures specific to the analysis. The figures illustrate specific metrics returned by the analysis run and are detailed in Section 1, Chapter 9.

(2) Specify Displayed Well

The Results Tab shows results for individual wells. The well number for which results are displayed can be selected in the drop-down menu.

(3) Open Results Folder

All figures displayed in the Results Tab and the table shown in the Statistics Tab (Figure 8.4 (6)) are automatically saved in PNG format to the corresponding analysis folder for each well (see Figure 8.5). By clicking the button **Open Results Folder**, the folder opens up directly.

(4) Rerun Analysis

Rerun an Analysis Trial with a new set of parameters to overwrite the previous analysis results.

(5) Open a New Analysis Trial

Clicking on the **Plus** icon creates a second Analysis Trial or multiple Analysis Trials (Figure 8.4 (7)) for the same dataset. This allows the user to keep previous analysis results in order to compare them with results obtained from using a different set of parameter values. The previous analysis trial is not overwritten. As illustrated in Figure 8.5, a sub-folder is created in the analysis folder tree for every new Analysis Trial.

Statistics Tab

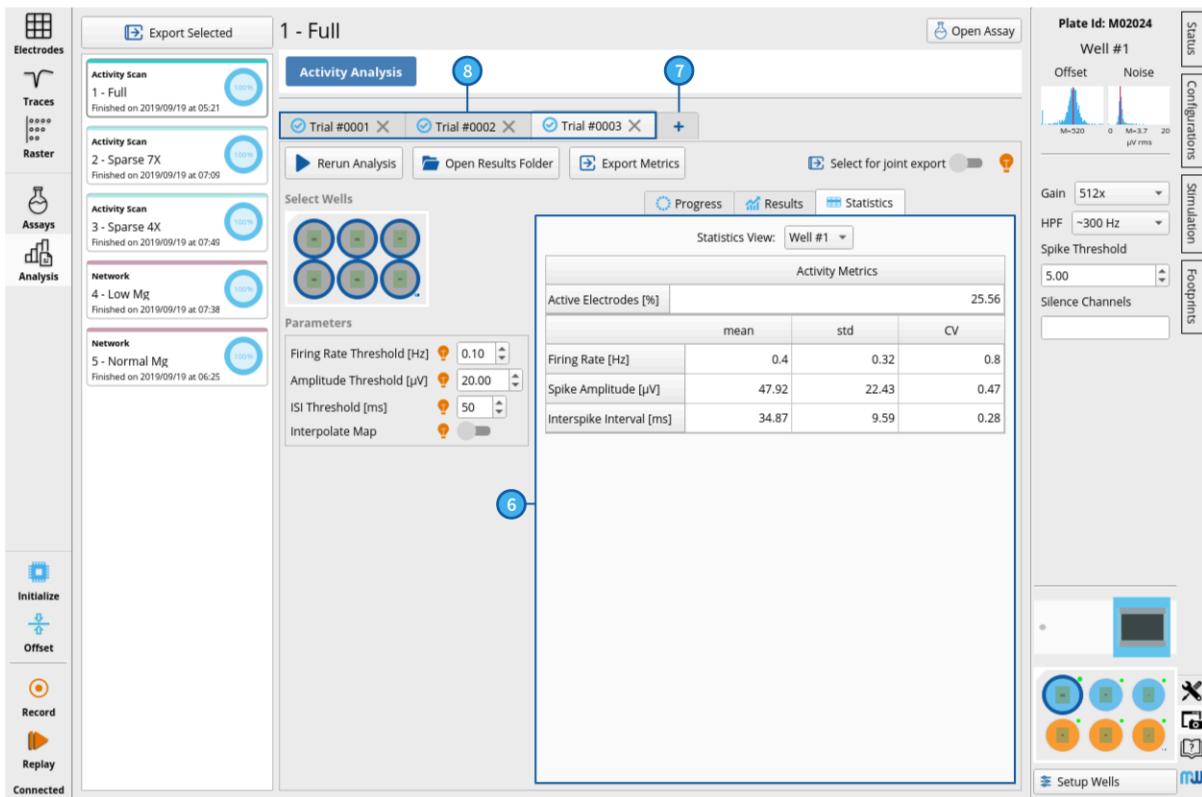


Figure 8.4. Statistics Tab

(6) Statistics Tab

The Statistics Tab provides a table summarizing key statistical quantifications of the computed metrics at the well level (mean, standard deviation, and coefficient of variation).

(7) Open a New Analysis Trial

Clicking on the Plus icon creates an additional Analysis Trial (compare to Figure 8.3 (5)).

(8) Navigating/Deleteing the Analysis Trials

The Progress, Results and Statistics Tabs of every Analysis Trial can be retrieved via the trial tabs. Additionally, an analysis trial can be deleted by clicking the cross symbol of the tab, which automatically deletes the corresponding results sub-folder.

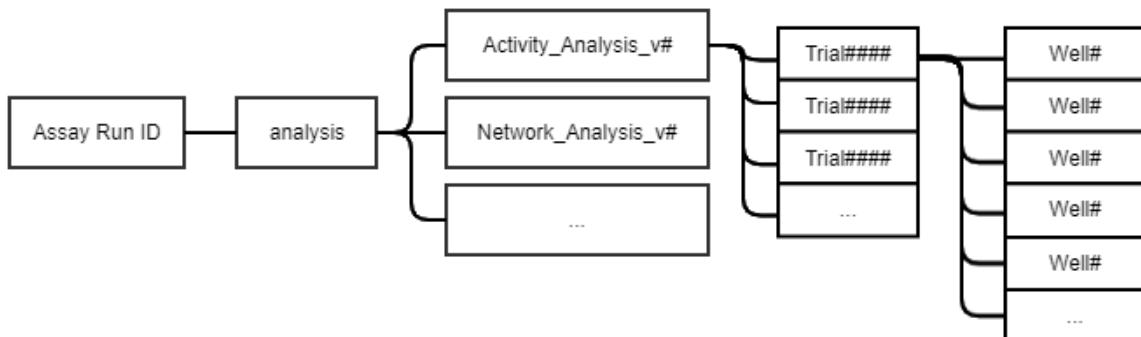


Figure 8.5. Analysis Folder Hierarchy

3. Export Analysis Results

The metrics extracted from the analyses (see Table 9.2 and Table 9.4) may be exported in CSV and Excel formats for further analysis with other statistical tools. The metrics can either be exported from a single recording's analysis (Figure 8.6) or from multiple analyses combined (Figure 8.8).

Exporting Data: Single Analysis Trial

To export data from a single analysis, navigate to the desired analysis trial and click the **Export Metrics** button (Figure 8.6 (1)).

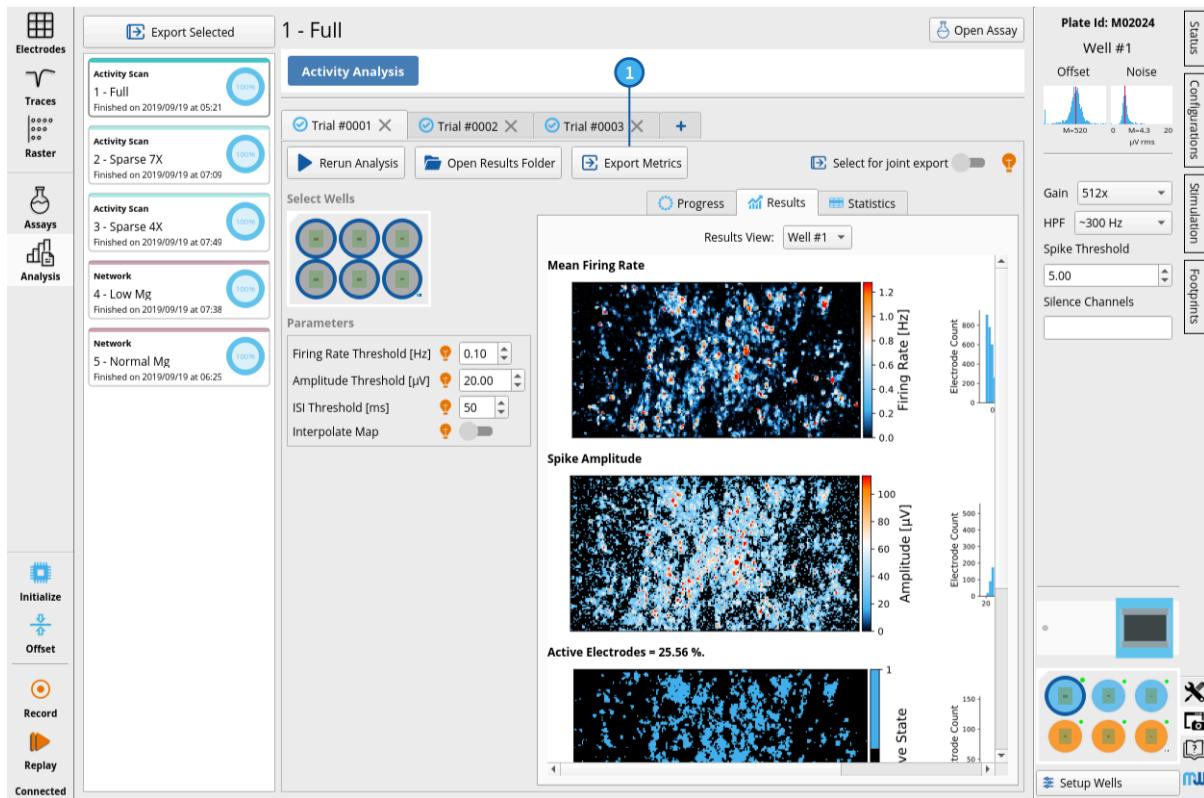


Figure 8.6. Exporting Metrics from a Single Analysis Trial

A window will appear, where the data saving path can be set (Figure 8.7 (1)) and the export format can be chosen (Figure 8.7 (2)). In addition, one can select (Figure 8.7 (3)) whether to export all metrics from an analysis (well, electrode, and spike levels for Activity Analysis and well and burst levels for Network Analysis) or just the summary metrics (well level only; shown in the Statistics Tab (Figure 8.4 (6))). Note that for Excel format, exporting all metrics from an Activity Analysis of a highly active culture (as in the example shown in the Figures) may take several minutes, since information for each detected spike will be saved. If multiple wells were selected for analysis (Figure 8.1 (6)), data from all the selected wells will be saved together.

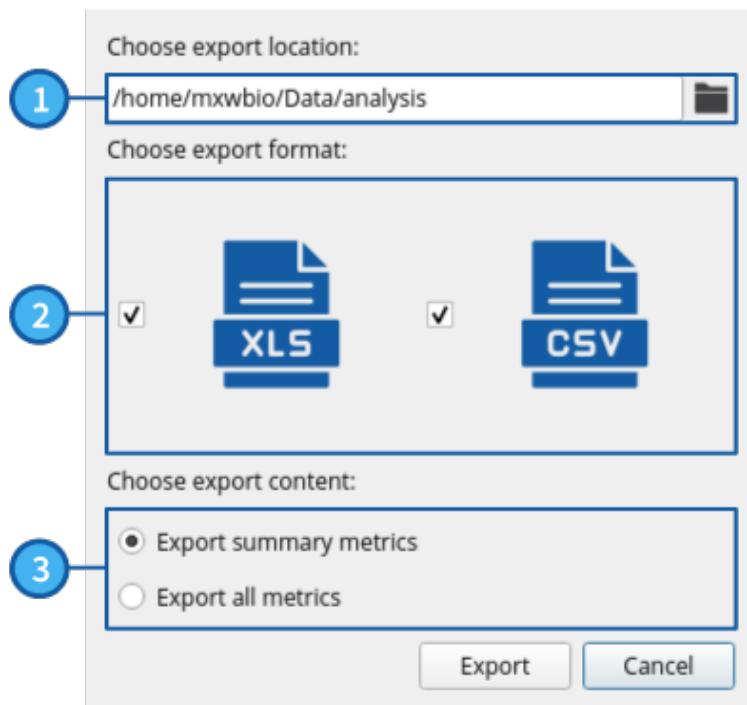


Figure 8.7. Choosing Single-Trial Export Settings

Exporting Data: Joint Export of Multiple Trials

Alternatively, data from multiple recording analyses can be exported into a single file with the following sequence of steps:

1. For every assay analysis and/or trial that is to be jointly exported, activate the **Select for joint export** switch (Figure 8.8 (1)). Trials selected for joint export can easily be recognized by the appearance of a starred shield icon next to the trial label (Figure 8.8 (2)). Note that data from multiple Analysis Trials of the same recording, as well as data from different recordings, can be selected and exported together.
2. Click on the **Export Selected** button (Figure 8.8 (3)).

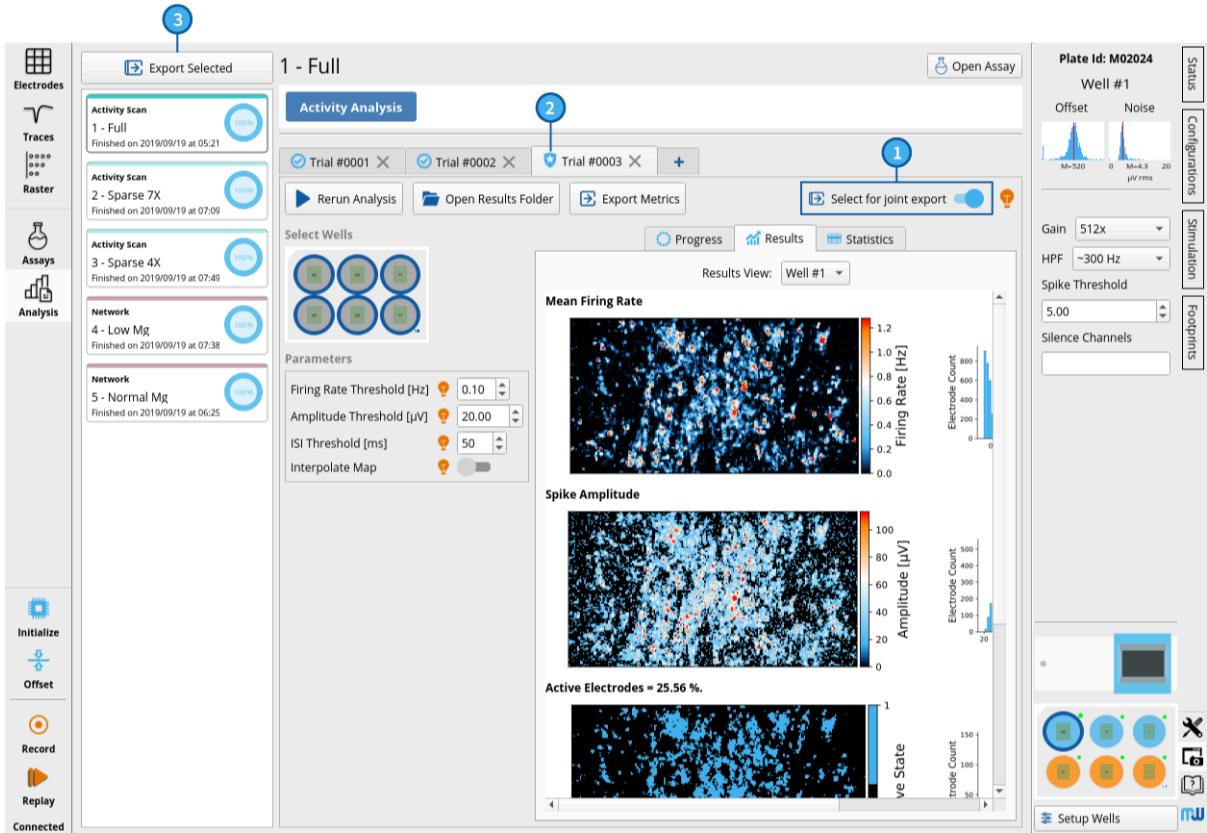


Figure 8.8. Exporting Metrics from Multiple Analyses

3. Confirm file selection. A list of selected analyses will appear (Figure 8.9). After verifying that all the desired analyses have been selected, the user may either confirm the selection to proceed to the next step or cancel the process to go back and select different analyses.

Metrics from following analysis would be exported.

Assay Tag	Analysis Type	Analysis Trial
5 - Normal Mg	Activity Analysis	Trial #0001
5 - Normal Mg	Network Analysis	Trial #0001
4 - Low Mg	Activity Analysis	Trial #0001
4 - Low Mg	Network Analysis	Trial #0001
3 - Sparse 4X	Activity Analysis	Trial #0001
2 - Sparse 7X	Activity Analysis	Trial #0001
1 - Full	Activity Analysis	Trial #0003

OK Cancel

Figure 8.9. Confirming File Selection for Joint Export

4. Set the data export path and select the data export format (Figure 8.10). Just as for single-file export, data can be exported in Excel and/or CSV formats. For multi-file export, only summary metrics (well level) can be saved, so the export content selection does not appear.

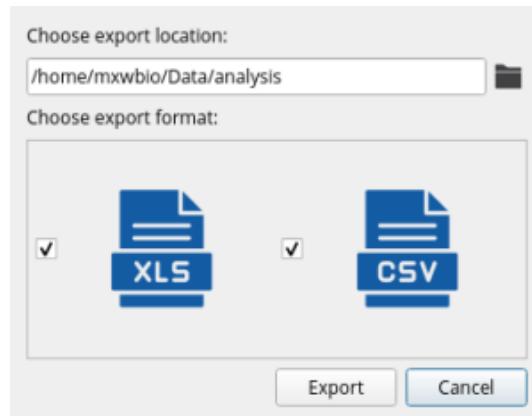


Figure 8.10. Choosing Joint Export Settings

Data Organization

The metrics data will be saved in a folder labeled with the date and time at which the export was carried out. If the data was saved in Excel format, a single file with multiple worksheets (*metrics_data.xlsx*) is saved. Alternatively, for CSV data, multiple files are saved, which correspond to the meta data table (*meta_data.csv*), the analysis parameters table (*analysis_params.csv*), and separate metrics tables for different analysis levels (well, electrode, and spike levels for Activity Analysis: *activity_summary_metrics.csv*, *electrode_metrics.csv*, and *spike_metrics.csv* and well and burst levels for Network Analysis: *network_summary_metrics.csv* and *burst_metrics.csv*). In Excel format, the different tables are saved as separate worksheets of a single file (Figure 8.11).

Instance	Well Number	Folder Path	Wellplate ID	Start Time	Stop Time	Gain [Sb (μV)]	Sampling Frequency [Hz]	HPF [Hz]	Number of Configurations	Duration per Configuration [s]	Well Group Name	Well Group Color	DIV	--	
1	1	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.062	Group #1	#e548f	10	--
2	2	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.061	Group #1	#e548f	10	--
3	3	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.061	Group #1	#e548f	10	--
4	4	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.06	Group #2	#e5e7e8	10	--
5	5	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.059	Group #2	#e5e7e8	10	--
6	6	/share/recom	M02024	2019-09-19 04:49:00	2019-09-19 05:21:19	512	6.294	10000	300	29	60.058	Group #2	#e5e7e8	10	--
7															
8															
9															
10															
11															
12															
13															
14															

Figure 8.11. Meta Data Table: Recording Information

The information exported in each data table can generally be categorized into three types:

1. Identifier Information (Figure 8.11; light blue).

- For each unique combination of Well Number + Assay Run ID + Analysis Trial (not written in the table), a unique **Instance** index is assigned. The **Instance** index can be used to link data across all exported tables: the meta recording data (Figure 8.11), the analysis parameters (Figure 8.12), and the metrics tables (Figure 8.13).

2. Wellplate Editor Information (Figure 8.11; dark grey).

- Information entered into the Wellplate Editor, such as the well group name, group color, and other user-specified values unique to each experiment (such as DIV, cell density, drug

concentration, etc.), is highly useful for data filtering, plotting, and statistical comparisons. It is saved in both the meta data table (Figure 8.11) and the metrics tables (Figure 8.13).

3. Values, Parameters, or Metrics (Figure 8.11; light grey).

- Each data table contains its unique set of values, parameters, or metrics, depending on the table's particular content. This content is described in detail below for each table type.

Three types of data tables are exported every time, regardless of whether it is a single or a multi-analyses export or whether all metrics or just the summary metrics have been exported. Below, the contents of each table type are described.

1. Meta Data Table: Recording Information (Figure 8.11)

This table contains recording information for each well in each recording assay that was analyzed and then exported together. The Meta Data table contains Identifier information, Wellplate Editor information, and recording information, the contents of which are detailed in Table 8.1 below:

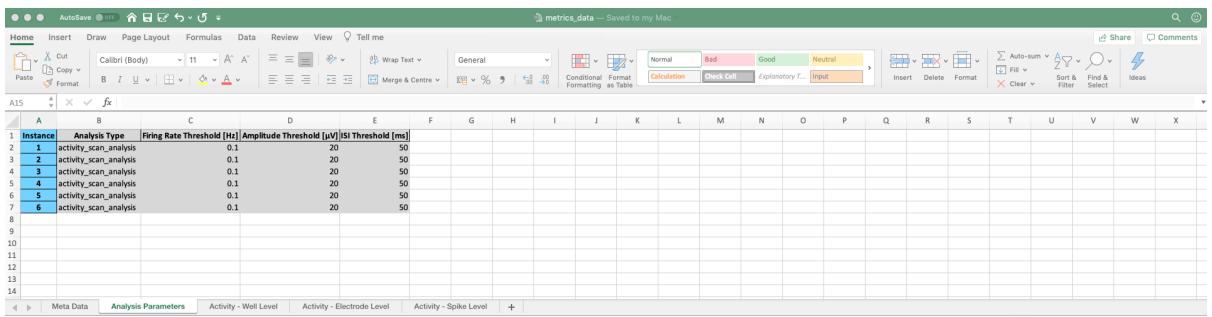
Value Name	Description
Folder Path	Path where recording file is stored.
Wellplate ID	ID number of recorded wellplate (if available; MaxTwo only).
Start Time	Start time of recording.
Stop Time	Stop time of recording.
Gain	The chip circuitry features three configurable amplification stages, allowing to control the amplifier gain. This value is set in the Status Tab of the Sidebar (see Section 2, Chapter 4).
LSB [μ V]	With a sampling resolution of 10 bits (1024 values), the least significant bit (LSB) denotes the difference between two consecutive digitization values. Increasing the gain decreases the LSB and therefore increases the digitization resolution.
Sampling Frequency [Hz]	Frequency at which recording data is sampled (10 kHz for MaxTwo or 20 kHz for MaxOne).
HPF [Hz]	High-pass filter cutoff value. Can be adjusted in the Status Tab of the Sidebar before starting the recording.
Number of Configurations	Number of configurations in a single recording file (e.g. 7 for a 7X Sparse activity scan).
Duration per Configuration [s]	Amount of time for which each configuration was recorded.

Table 8.1. Meta Data Table Contents

2. Analysis Parameters Table (Figure 8.12)

This table records the values of the parameters that were set for whichever analyses were carried out (light grey). These parameters are described further in Table 9.1 for Activity Analysis and Table 9.3 for Network Analysis. In addition, it contains Identifier information (light blue): the **Instance** index.

Analysis Workflow

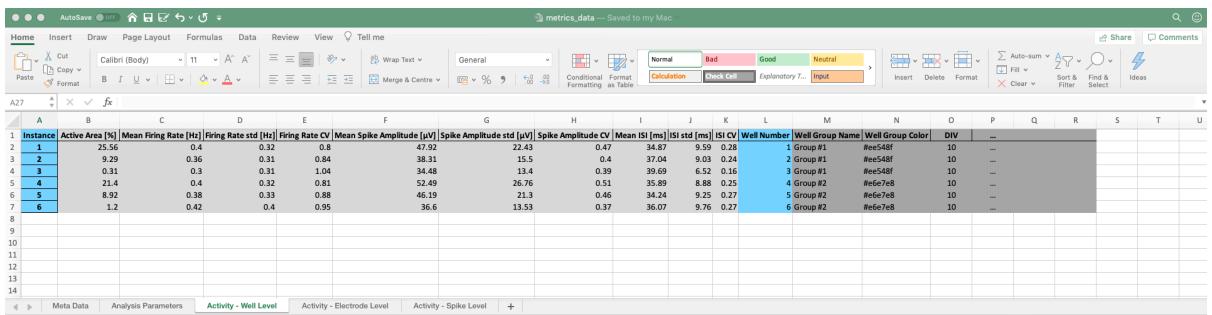


Instance	Analysis Type	Firing Rate Threshold [Hz]	Amplitude Threshold [uV]	ISI Threshold [ms]
1	activity_scan_analysis	0.1	20	50
2	activity_scan_analysis	0.1	20	50
3	activity_scan_analysis	0.1	20	50
4	activity_scan_analysis	0.1	20	50
5	activity_scan_analysis	0.1	20	50
6	activity_scan_analysis	0.1	20	50
7	activity_scan_analysis			

Figure 8.12. Analysis Parameters Table

3. Metric Tables (Figure 8.13)

The Metrics Tables contain the metrics computed in the analyses: Activity Analysis (Table 9.2) and Network Analysis (Table 9.4). When all metrics are exported from a single Activity Analysis trial, three tables are saved: Well Level, Electrode Level, and Spike Level. For a single Network Analysis trial, two tables are saved: Well Level and Burst Level. For joint export of several analyses (Figure 8.10), only the Well Level tables are saved. Besides the metrics values (light grey), each table contains Identifier information (light blue) and Wellplate Editor information (dark grey), which can be used for data selection and filtering.



Instance	Active Area [%]	Mean Firing Rate [Hz]	Firing Rate CV	Mean Spike Amplitude [uV]	Spike Amplitude std [uV]	Spike Amplitude CV	Mean ISI [ms]	ISI std [ms]	ISI CV	Well Number	Well Group Name	Well Group Color	DIV	
1	25.56	0.4	0.32	0.8	47.92	22.43	0.47	34.87	9.59	0.28	1	Group #1	#ee548f	10
2	9.29	0.36	0.31	0.84	38.31	15.5	0.4	37.04	9.05	0.24	2	Group #1	#ee548f	10
3	0.31	0.3	0.31	1.04	34.48	13.4	0.39	39.69	6.52	0.16	3	Group #1	#ee548f	10
4	21.4	0.4	0.32	0.81	32.49	26.76	0.53	39.59	8.85	0.25	4	Group #2	#e6e7e8	10
5	8.92	0.38	0.33	0.98	46.19	21.3	0.46	34.34	9.25	0.27	5	Group #2	#e6e7e8	10
6	1.2	0.42	0.4	0.95	36.6	13.53	0.37	35.07	9.76	0.27	6	Group #2	#e6e7e8	10

Figure 8.13. Metrics Tables (Well Level)

Chapter 9. MaxLab Live Analyses

The individual MaxLab Live Analysis modules can be applied to different assays and generate particular sets of metrics. In this chapter, the individual modules are explained with respect to their functionality, parameters and metrics available for export.

1. Activity Analysis

Assessing the activity of a sample is a straightforward way of evaluating the growth and development of a neuronal culture. In this framework, the purpose of the Activity Analysis is to characterize the level of activity of a biological sample by first detecting electrodes recording spiking activity and then extracting features from those 'active' electrodes such as spike amplitude and firing rate. The Activity Analysis is primarily intended to be performed on Activity Scans (see Section 1 in Chapter 7). However, this analysis can also be useful for extracting spike features from Network Assay recordings.

After setting parameter values (Figure 9.1 (1)) and running the Activity Analysis, the results can be viewed in the **Results Tab** (Figure 9.1 (2)). In addition, a table of summary metrics from the analysis is shown in the **Statistics Tab** (Figure 9.1 (3)). The results figures and the statistics table are saved in PNG format in each well's Analysis Folder (Figure 9.1 (4)), and are further described in the section called "Analysis Results and Summary Statistics".

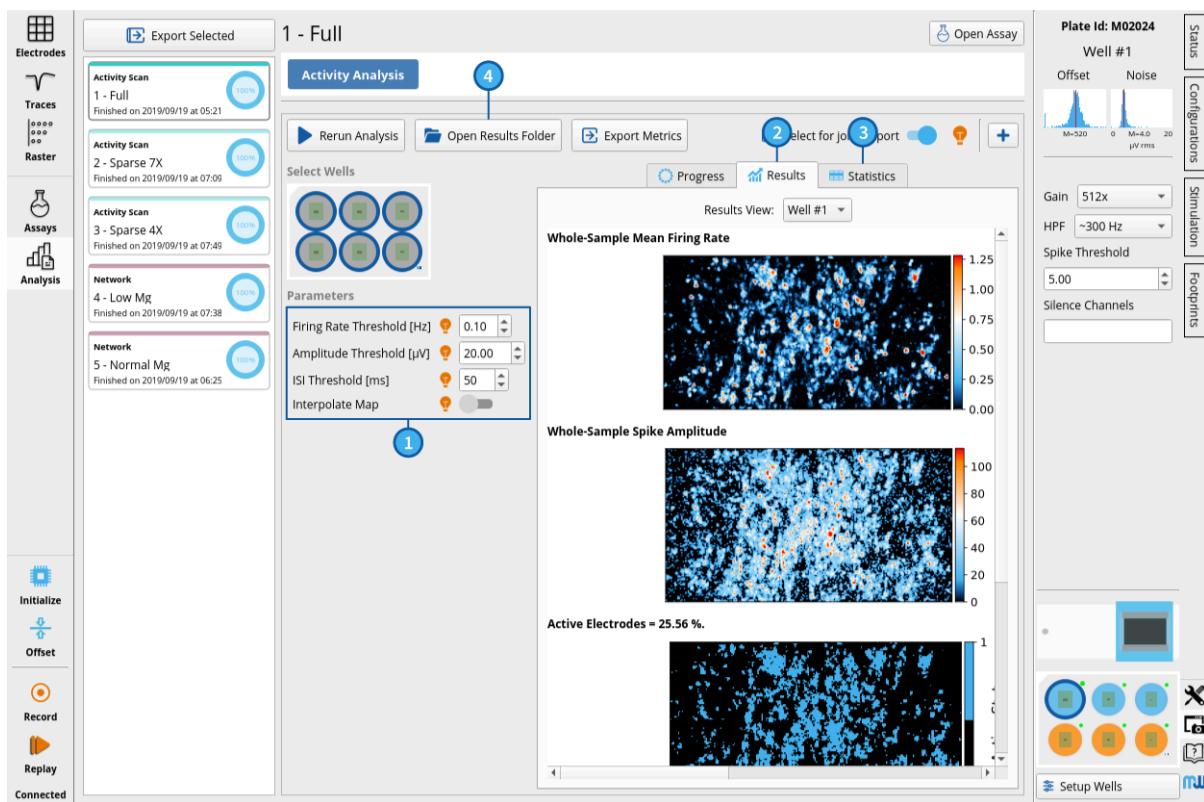


Figure 9.1. Activity Analysis Overview

Analysis Method and Parameters

Method

The key aspect of the Activity Analysis algorithm is to identify electrodes that are recording biological spiking activity rather than noise. For every electrode in an Activity Scan recording, the mean firing rate and spike amplitude values are calculated (for calculation details, see Electrode Firing Rate and Electrode Spike Amplitude descriptions in Table 9.2). If these two activity values are above the two respective thresholds set by the user in the Analysis Parameters (Table 9.1), the particular electrode is considered to be 'active' - i.e. recording meaningful biological activity from the sample. Extraction of other Activity Analysis metrics builds up on this 'active electrodes' selection step.

Parameters

To ensure optimal selection of active electrodes and subsequent extraction of activity metrics, two activity threshold parameters are available to the user ('Firing Rate Threshold' and 'Amplitude Threshold'). Two further parameters can be set to modify the interspike interval distribution ('ISI Threshold') and activity map visualization ('Interpolate Map'). These four parameters are described in detail in Table 9.1.

Parameter	Description	Adjustment Guidelines
Firing Rate Threshold [Hz]	The threshold above which the Electrode Firing Rate* must be in order for an electrode to be considered 'active'. Default Value: 0.1 [Min,Max] Values: [0, 1]	A higher Firing Rate Threshold will limit the active electrodes selection to the more strongly-firing electrodes.
Amplitude Threshold [μ V]	The threshold above which the Electrode Spike Amplitude* must be in order for an electrode to be considered 'active'. Default Value: 20 [Min,Max] Values: [0, 100]	A higher Amplitude Threshold will limit the active electrodes selection to those with high-amplitude negative peak spikes.
ISI Threshold [ms]	The threshold below which interspike interval (ISI)* values of a given electrode are included in the computation of the Mean Electrode ISI* and Electrode ISI coefficient of variation (CV)*. Default Value: 200 [Min,Max] Values: [0, 500]	The ISI threshold restricts the timescale across which the spiking dynamics are evaluated, in terms of the ISI mean and CV. For example, setting the threshold to a lower value might be used to discard undesired high ISI values arising from sparse activity patterns, allowing to capture only short-term spiking dynamics.
Interpolate Map	Estimate unrecorded electrode values using linear interpolation over the activity map (for map viewing only). Default Value: False	Most suitable for Checkerboard and 7X Sparse configurations of the Activity Scan Assay.

Table 9.1. Activity Analysis Parameters

*See Table 9.2 for calculation details on these metrics.

Metrics

The Activity Analysis extracts several key metrics for characterizing activity of the active electrodes: the firing rate, spike amplitude and ISI (interspike interval) values. Importantly, these metrics are extracted at three different levels:

- **Spike Level:** every single detected spike is characterized by one value for each of these metrics.
- **Electrode Level:** every electrode is characterized by the mean and variability measures for each of these metrics.
- **Well Level:** provides an overall activity characterization for the well. Metrics are expressed in the form of statistical quantifications, referred to as summary metrics, which are shown in the Statistics Tab. Metrics at the Well Level are derived from distributions of multiple electrode values, with three statistical quantifications provided: the mean, the standard deviation (std), and the coefficient of variation ($CV = std/mean$). The standard deviation shows the dispersion of the data: a low value indicates that the data points are close to the mean, while a higher value indicates that the data

points are spread over a wider range. The coefficient of variation normalizes this dispersion measure by the magnitude of the mean, facilitating the comparison of the variability of different datasets.

Well Level metrics can be exported in CSV (activity_summary_metrics.csv) and/or Excel formats (sheet “Activity – Well Level”) for further analysis. The Electrode Level and Spike Level metrics can also be exported (when selecting the option “Export All”; see Section 3 in Chapter 8) in CSV and/or Excel formats. Table 9.2 gives a detailed overview of all the Activity Analysis metrics that can be exported.

Metric Name	Unit	Description
Well Level		
Active Area	%	Proportion of active electrodes to the total number of recorded electrodes.
Firing Rate (mean, std, CV)	Hz	Mean, standard deviation and coefficient of variation of Electrode Firing Rate values, for active electrodes only.
Spike Amplitude (mean, std, CV)	µV	Mean, std and CV of Electrode Spike Amplitude values, for active electrodes only.
ISI (mean, std, CV)	ms	Mean, std and CV of Mean Electrode ISI values, for active electrodes only.
Electrode Level		
Electrode Number	-	The electrode number assigned on the chip array.
x Position	µm	Electrode position on the chip array in the horizontal plane.
y Position	µm	Electrode position on the chip array in the vertical plane.
Electrode Spike Count	-	Total number of detected spikes on the electrode during over the entire recording period. Note: Spike detection depends on the spike threshold set in MaxLab Live (see Figure 4.2).
Electrode Firing Rate	Hz	Electrode Spike Count divided by the total time of the recording period.
Electrode Spike Amplitude	µV	90th percentile of Spike Amplitude values for all spikes detected on the given electrode.
Mean Electrode ISI	ms	Mean of interspike interval (ISI) values for $ISI > ISI \text{ threshold}^*$, for spikes detected on the given electrode (*cf. Parameters Table)
Electrode ISI CV	-	CV of ISI values for $ISI > ISI \text{ threshold}^*$, for spikes detected on the given electrode (*cf. Parameters Table)
Spike Level		
Electrode Number	-	Number of the electrode on which the particular spike was detected.
Spike Time	ms	Spike negative peak time, relative to the start time of the recording.
Spike Amplitude	µV	Spike negative peak amplitude.
ISI	ms	Elapsed time between the given spike and the previous spike detected on the same electrode. N.B.: No ISI value for the first detected spike.
Spike Frequency	Hz	Inverse of the spike ISI. N.B.: No Spike Frequency value for the first detected spike.

Table 9.2. Activity Metrics: Well Level, Electrode Level and Spike Level.

Analysis Results and Summary Statistics

Activity Analysis metrics visualizations consist of heat maps of the chip array as well as histograms of metric values distributions (Figure 9.2). From left to right and top to bottom, the figures are:

- Firing Rate map of the chip array. Every pixel corresponds to one electrode, the color of which reflects the electrode's firing rate. The maximum value is reassigned to the 99th percentile of the distribution.
- Histogram of the Electrode Firing Rate values distribution for all **active electrodes**.
- Amplitude map of the chip array. Every pixel corresponds to one electrode, the color of which reflects the 90th percentile of spike negative peak amplitude measured at this electrode. The maximum value is reassigned to the 99th percentile of the distribution.
- Histogram of the Electrode Spike Amplitude values for all **active electrodes**.
- Binary map of the chip array. Every pixel corresponds to one electrode, the color of which corresponds to whether the electrode is considered to be **active**.
- Histogram of the mean inter-spike interval below the ISI Threshold for all **active electrodes**.

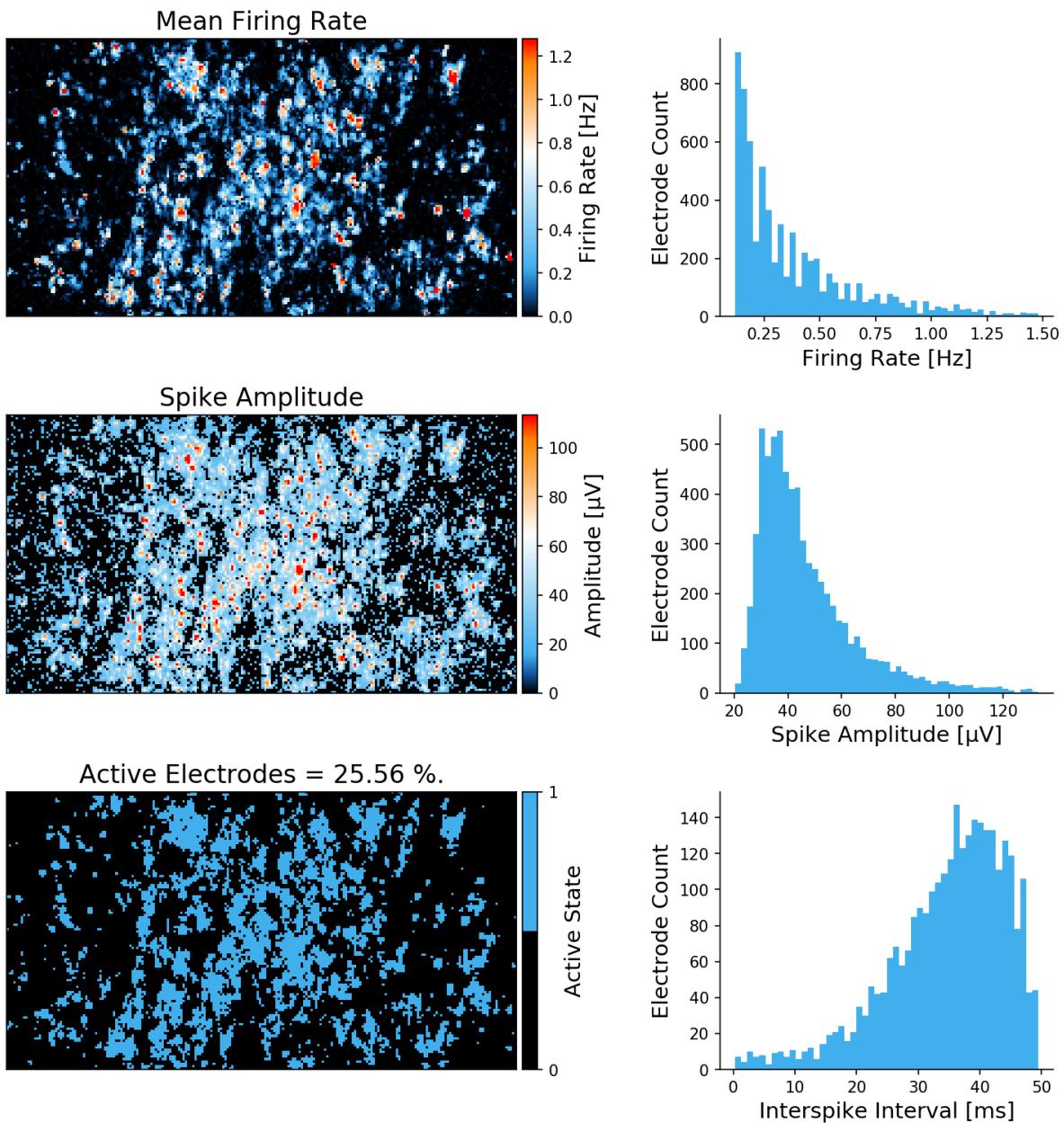


Figure 9.2. Activity Analysis Results

Well Level statistical measures describing the distributions are summarized in the Statistics Table (Figure 9.3).

- The upper part of the table indicates the percentage of active electrodes, according to selected parameters (cf. Table 9.1).
- The lower part of the table contains some key statistical descriptions (the mean, the standard deviation (std) and the coefficient of variation (CV)) of the activity metrics at the Well Level.

	Activity Metrics		
Active Electrodes [%]	25.56		
	mean	std	CV
Firing Rate [Hz]	0.4	0.32	0.8
Spike Amplitude [μ V]	47.92	22.43	0.47
Interspike Interval [ms]	34.87	9.59	0.28

Figure 9.3. Activity Analysis: Summary Statistics Table

Tips for Parameter Adjustment

Adjusting the Firing Rate and Amplitude Thresholds

Relative to the results obtained with default parameter values (see Figure 9.1 and Table 9.1), when setting higher values for Firing Rate Threshold (e.g. 0.3 Hz) and Amplitude Threshold (e.g. 60 μ V), selection of active electrode is more restricted. A change in these two parameters generally affects:

- The sparsity of the binary color map (difference illustrated in Figure 9.4).
- The percentage of active electrodes (difference illustrated in Figure 9.4).
- The three metrics distribution histograms, and the related statistical quantification values of the distributions at the Well Level.

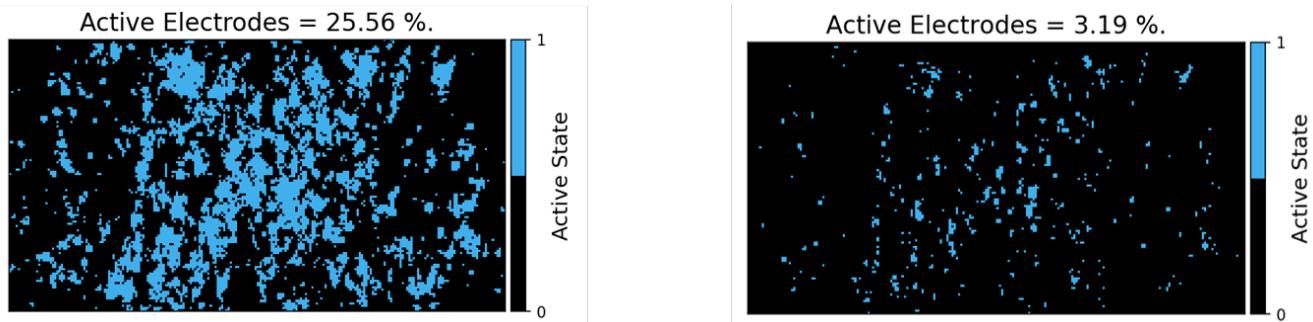


Figure 9.4. Higher Amplitude and Firing Rate Thresholds Restrict Active Electrode Selection

Using the Interpolation Option

By default, unrecorded electrodes are displayed as a black pixel (just like the zero-value electrode pixel). For a richer map viewing, it is possible to apply a 2D grid linear interpolation to estimate values at the unrecorded electrodes, as illustrated in Figure 9.5.

In general, avoid using interpolation on Network, Sparse 4x/3x/1x or very sparse custom configurations. As a rule of thumb, a configuration is too sparse for interpolation if the recording electrode coverage is < 25% of the total array surface electrodes.

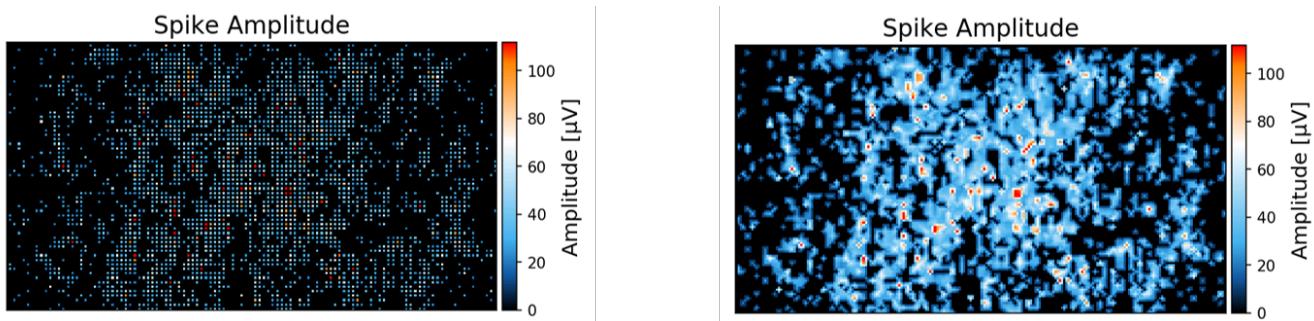


Figure 9.5. Turning On the 'Interpolate Map' Option

2. Network Analysis

The purpose of the Network Analysis is to detect and characterize synchronous activity of the recorded neurons: periods of mutual excitation followed by inhibition, which is commonly referred to as network “bursting”. The Network Analysis can be run on any Network Recording (see Section 2 in Chapter 7 and Section 1 in Chapter 8).

Once a Network Analysis has been run, the results can be viewed in the **Results Tab** (Figure 9.6 (1)). In addition, a table of summary metrics from the analysis is shown in the **Statistics Tab** (Figure 9.6 (2)). The results figure and the statistics table are saved in PNG format in each well’s Analysis Folder (Figure 9.6 (3)) and are further described in the section called “Analysis Results & Summary Statistics”.



Figure 9.6. Network Analysis Overview

Analysis Method and Parameters

Method

The burst detection algorithm involves three steps. First, spike times from all the recorded electrodes are binned into a histogram with a fixed bin size of 0.1 ms. Second, the histogram is smoothed through convolution with a Gaussian kernel (kernel width set by the parameter “Smoothing Window Size”; see Table 9.3) and normalized by the total number of recorded electrodes, giving the Network Activity plot (Figure 9.6 (4)). Third, bursts in the Network Activity are detected by comparing neighboring values in order to find all the local maxima of a curve. Only those peaks that meet criteria set by the parameters “Burst Detection Threshold” and “Minimum Peak Distance” (see Table 9.3) are selected and saved for subsequent analysis.

Parameters

Network bursting properties vary: sometimes all neurons burst synchronously and on a short time scale, leading to tight, regular bursts, while in different neuronal cultures bursts are more diffuse,

broad, and irregular. To ensure optimal burst detection, there are four parameters that can be adjusted. In addition, two visualization parameters are available to control the zoom level of the Raster and Network Activity plots (Table 9.3).

Parameter	Description	Adjustment Guidelines
Smoothing Window Size [s]	Standard deviation of Gaussian convolution kernel used for smoothing the network firing rate. Default Value: 0.3 [Min,Max] Values: [0.0001, 10.0]	Decrease to detect shorter bursts by reducing smoothing. Increase to detect longer-timescale bursts only.
Burst Detection Threshold	Threshold above which to detect burst peaks (multiple of the root mean square of the network firing rate). Default Value: 1.2 [Min,Max] Values: [0.0001, 30.0]	Decrease when bursts visible in the raster plot do not reach the threshold due to their low amplitude. Increase when there is some firing rate noise giving many smaller-amplitude peaks.
Minimum Peak Distance [s]	Minimum distance between the burst peaks detected. Default Value: 1.0 [Min,Max] Values: [0.0001, 100]	Decrease if nearby peaks above threshold are being missed. Increase to disregard smaller peaks “riding” on top of larger peaks.
Start-Stop Threshold	The fraction of the total peak height (measured from the top) at which the burst start and stop times are defined. Default Value: 0.3 [Min,Max] Values: [0.0001, 1.0]	Decrease to include fewer spikes (only spikes at the peak of the burst included) for within-burst spike statistics. Increase to include more spikes within bursts.
Plot Start Position [s]	The time point in the recording from which the Raster and Network Activity plots are displayed. Default Value: 0 [Min,Max] Values: [0, 10800]	Increase to shift the displayed activity to a later time point in the recording. This parameter does not affect burst detection. Metrics will still be extracted from the entire recording.
Plot End Position [s]	The time point in the recording until which the Raster and Network Activity plots are displayed. Default Value: 60 [Min,Max] Values: [1, 10800]	Decrease to zoom in; increase to zoom out. This parameter does not affect burst detection. Metrics will still be extracted from the entire recording.

Table 9.3. Network Analysis Parameters: Description and Adjustment Guidelines

Metrics

The Network Analysis extracts a number of metrics from the detected bursts: burst peak amplitude, inter-burst interval, burst duration, and others. Their overview is given in the **Statistics Tab** (Figure 9.6 (2) and Figure 9.12). These summary metrics can be exported in CSV (*network_summary_metrics.csv*) and/or Excel (sheet “Network – Well Level”) formats for further analysis (see Section 3 in Chapter 8).

For metrics derived from distributions of multiple burst values, three statistical quantifications are provided: the mean, the standard deviation (std), and the coefficient of variation ($CV = std/mean$). The standard deviation shows the dispersion of the data: a low value indicates that the data points are close to the mean, while a higher value indicates that the data points are spread over a wider range. The coefficient of variation normalizes this dispersion measure by the magnitude of the mean, facilitating the comparison of the variability of different datasets.

In addition, for single network recordings, individual burst metrics can also be exported (when selecting the option “Export All”; see Figure 8.7 in Chapter 8) in CSV (*burst_metrics.csv*) and/or Excel (sheet “Network – Burst Level”) formats. This data is useful for taking a closer look into the shapes of the distributions from which the network summary statistics are derived. Table 9.4 gives an overview of all the Network Analysis metrics that can be exported.

Metric Name	Unit	Description
Well Level		
Burst Frequency	Hz	Number of detected bursts divided by the total recording time.
Spikes within Bursts	%	Sum of all spikes within all detected bursts divided by the total number of spikes detected, multiplied by 100.
Spikes per Burst (mean, std, CV)	-	Number of spikes within individual bursts, for all bursts detected.
Spikes per Burst per Electrode (mean, std, CV)	-	Number of spikes within individual bursts normalized by the total number of recorded electrodes, for all bursts detected.
Burst Duration (mean, std, CV)	s	The time between the start and stop times of a given burst at a certain percentage of the peak amplitude, for all bursts detected.
Burst Peak Firing Rate (mean, std, CV)	Hz	Peak amplitude of a given burst, derived from the Network Activity plot, for all bursts detected.
IBI (mean, std, CV)	s	The time interval between the peak times of a given burst and the following one ($IBI = tpeak(n+1) - tpeak(n)$), for all bursts detected. The IBI of the last burst is assigned to 0.
ISI within Burst (mean, std, CV)	ms	The interspike intervals (ISI) for all spikes falling within a given burst, for all bursts detected.
ISI outside Burst (mean, std, CV)	ms	The interspike intervals (ISI) for all spikes falling within the interburst interval that follows each burst, for all bursts detected. The ISI outside Burst of the last burst is assigned to 0.
Burst Level		
Spikes per Burst	-	Number of spikes that fall between the start time and stop time of a given burst.
Spikes per Burst per Electrode	-	Number of spikes that fall between the start time and stop time of a given burst, normalized by the total number of recorded electrodes.
Burst Duration	s	The time between the start and stop times of a given burst at a certain percentage of the peak amplitude, which is set by the Start-Stop Threshold parameter.
Burst Peak Firing Rate	Hz	Peak amplitude of a given burst, derived from the Network Activity plot.
IBI	s	The time interval between the peak times of a given burst and the following one ($IBI = tpeak(n+1) - tpeak(n)$). The IBI of the last burst is assigned to 0.
Mean Burst ISI	ms	The mean of all the interspike intervals (ISI) for spikes that fall within a given burst.

Metric Name	Unit	Description
Burst ISI CV	-	The coefficient of variation of all the interspike intervals (ISI) for spikes that fall within a given burst. A value closer to 0 indicates spikes regularly-distributed in time, while a value closer to 1 indicates a mix of shorter and longer ISIs, as would happen when the network firing rate gradually ramps up to a peak.

Table 9.4. Network Metrics: Well Level and Burst Level

Analysis Results & Summary Statistics

Several visualizations are provided to summarize the results of a Network Analysis. Figure 9.7, which can be obtained by opening the Analysis Folder for each well that was analyzed (Figure 9.6 (3)), shows a raster plot from all the recorded electrodes and a corresponding Network Activity plot with all the bursts that have been detected. In addition, four histograms show the distributions of the number of spikes per burst, the burst duration, the burst peak firing rate, and the interburst interval.

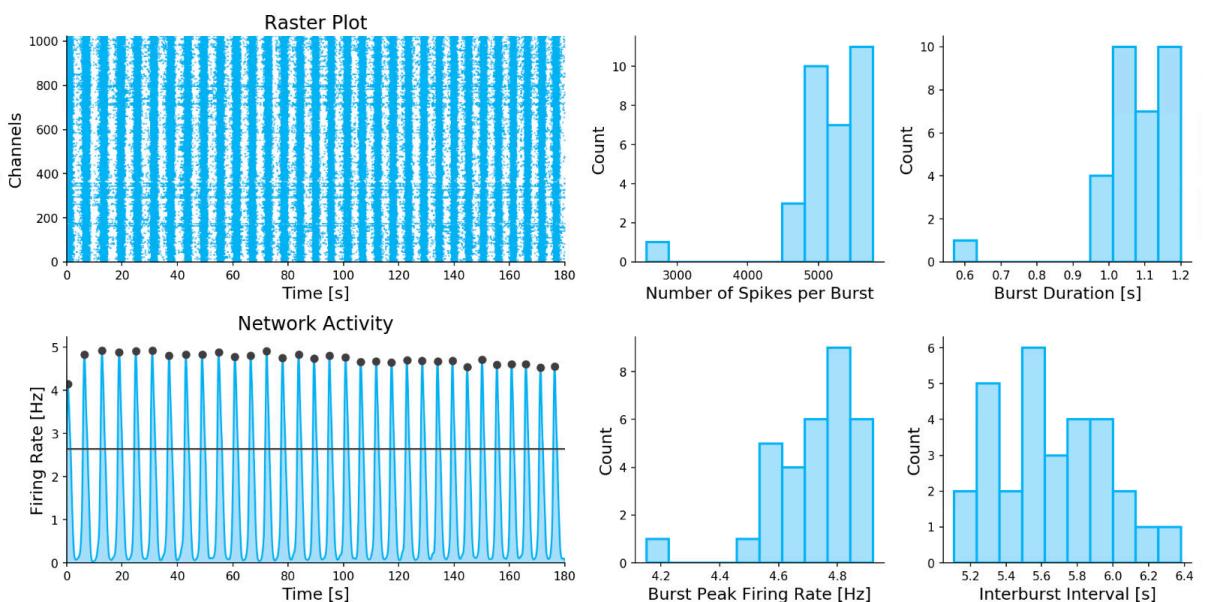


Figure 9.7. Network Analysis Results

The mean, standard deviation (std) and coefficient of variation (CV) of these distributions, along with several other metrics, can be visualized in the Summary Statistics Table (Figure 9.8), which is saved to each well's Analysis Folder. The metrics in this table are the same ones that can be exported in CSV (*network_summary_metrics.csv*) and/or Excel (sheet “Network – Well Level”) formats, as described in the section called “Metrics”. Table 9.4 – Well Level gives a detailed description of the summary metrics.

	Network Metrics		
Burst Frequency [Hz]	0.18		
Spikes within Bursts [%]	58.17		
	mean	std	CV
Number of Spikes per Burst	5129.81	569.53	0.11
Number of Spikes per Burst per Electrode	5.16	0.57	0.11
Burst Duration [s]	1.08	0.11	0.1
Burst Peak Firing Rate [Hz]	4.73	0.15	0.03
Interburst Interval [s]	5.66	0.31	0.06
ISI within Burst [ms]	125.94	109.83	0.87
ISI outside Burst [ms]	588.24	1015.04	1.73

Figure 9.8. Network Analysis Summary Statistics Table

Tips for Parameter Adjustment

As described in Section 2 of Chapter 8, a given analysis run can be repeated multiple times with different parameters, which overwrites the previous analysis results (**Rerun Analysis** button). Alternatively, different sets of results can be saved by creating multiple analysis **Trials**. The second option may be useful, for example, for analyzing network bursting properties on different timescales in cultures which show sharper bursts nested within longer bursts. In such a case, one set of parameters would be used to only detect the longer bursts, while a different set of parameters would be used to detect all the bursts on a more fine-grained level. Another use-case for creating multiple Analysis Trials would be if cultures in different wells exhibit different bursting dynamics, so that some wells would require a different set of parameters for optimal burst detection than others.

Guidelines for adjusting each burst detection parameter are given in Table 9.3. Examples of the outcome of changing each individual parameter are described and illustrated below.

Adjusting the Smoothing Window Size

The Smoothing Window Size [s] affects the Network Activity plot (see Figure 9.7), which is the basis for burst detection. The network firing rate is calculated by pooling spikes from all the simultaneously-recorded electrodes of a Network Recording, applying a Gaussian kernel to the resulting histogram, and normalizing by the total number of recorded electrodes. The Smoothing Window Size is the kernel standard deviation: the higher it is, the stronger the smoothing. For example, when the smoothing window size is increased ten times, from the default of 0.3 s to 3.0 s, the smoothing becomes so strong that the burst peaks are no longer apparent in the Network Activity plot (Figure 9.9; compare with same data in Figure 9.6, which was generated with the default parameter values). **Note:** when no bursts are detected, the histograms of the various burst parameters are not shown and the statistics table is also not available. Thus, the Smoothing Window Size should be adjusted so that the spike clusters in the raster plot match the peaks of the Network Activity plot.

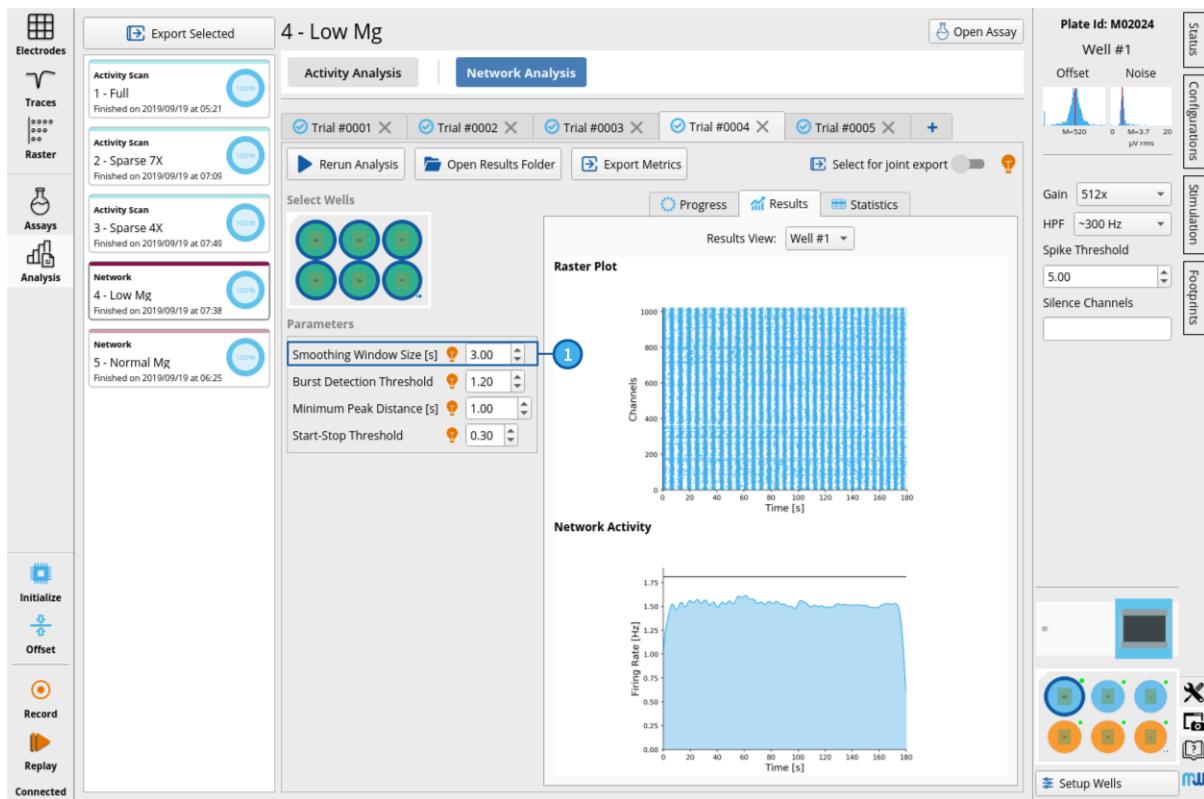


Figure 9.9. The Effects of Increasing the Smoothing Window Size

Adjusting the Burst Detection Threshold

The Burst Detection Threshold is a multiple of the root mean square of the network firing rate. The higher it is set, the fewer low-amplitude bursts will be detected. It is useful to adjust for recordings in which lower-amplitude bursts (those in which fewer neurons spike together) are interspersed with higher-amplitude bursts (those in which many neurons spike together), allowing to filter out all the weaker bursts. If the Burst Detection Threshold is set too high, however, no bursts will be detected (Figure 9.10).

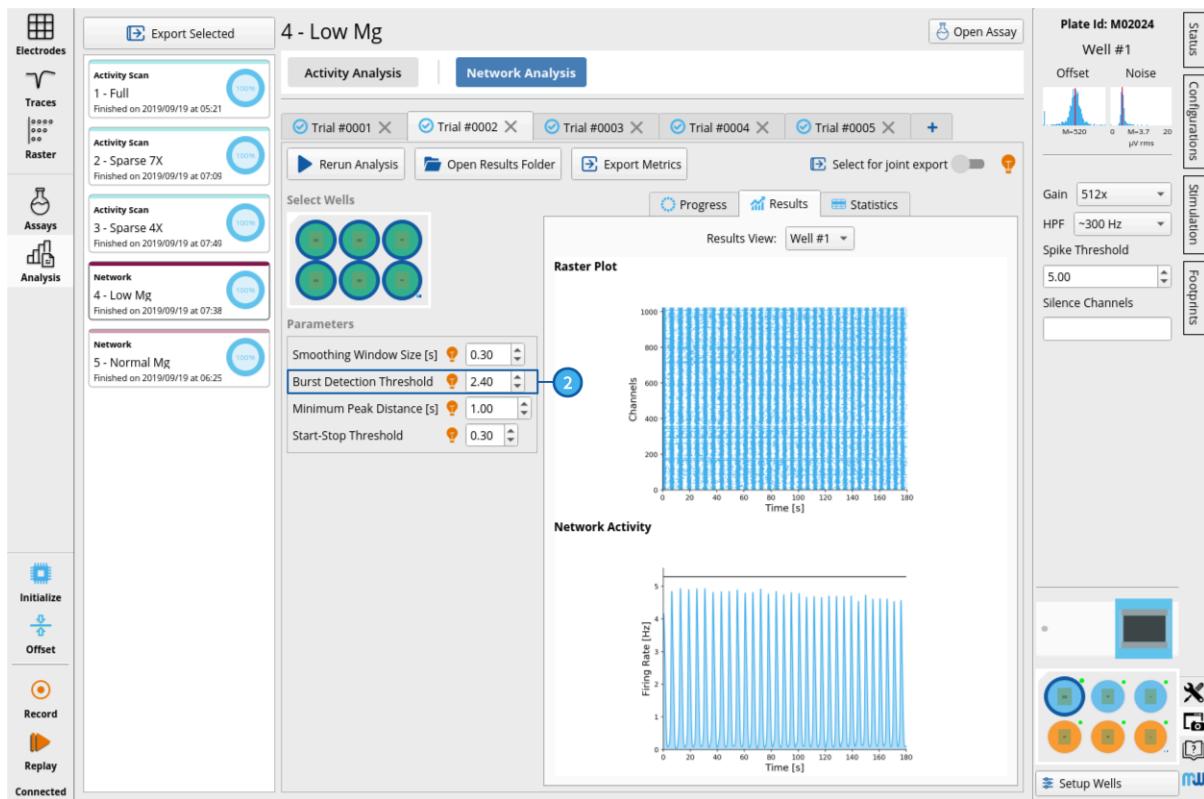


Figure 9.10. The Effects of Increasing the Burst Detection Threshold

Adjusting the Minimum Peak Distance

The Minimum Peak Distance [s] can be used to regulate whether all the adjacent peaks in the Network Activity plot are detected as bursts. The higher the Minimum Peak Distance, the more peaks after a detected peak will be skipped before another peak is selected. By setting the Minimum Peak Distance to 10 s in the following example (Figure 9.11), every other peak is skipped.



Figure 9.11. The Effects of Increasing the Minimum Peak Distance

Adjusting the Start-Stop Threshold

The Start-Stop Threshold is the upper fraction of the peak height at which the burst start and stop times are defined. The higher the value, the larger the time window around the burst peak that will be used to define the burst start and stop times, and consequently, the higher will be the number of spikes considered to occur within that burst. If it is set to 0.3, for example, then only spikes that fall within the upper 30% of the burst will be considered to occur within the burst. If it is raised to 0.6, then more spikes on the shallow parts of the slopes of the burst will be included. When comparing Figure 9.12 (Start-Stop Threshold = 0.3; default value) to Figure 9.13 (Start-Stop Threshold = 0.6), it is apparent that increasing the Start-Stop Threshold affects most burst metrics, raising the % of Spikes within Burst, Number of Spikes per Burst, Burst Duration and others.

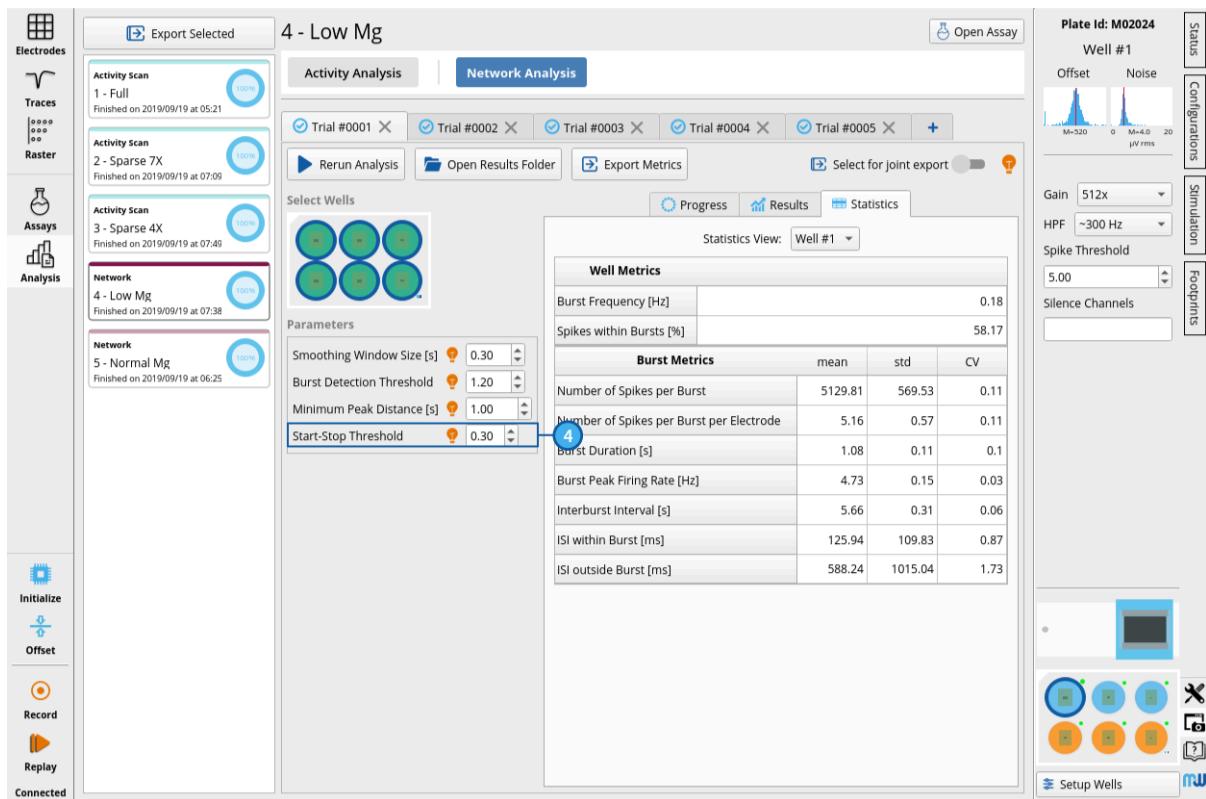


Figure 9.12. Network Analysis Statistics: Default Parameters

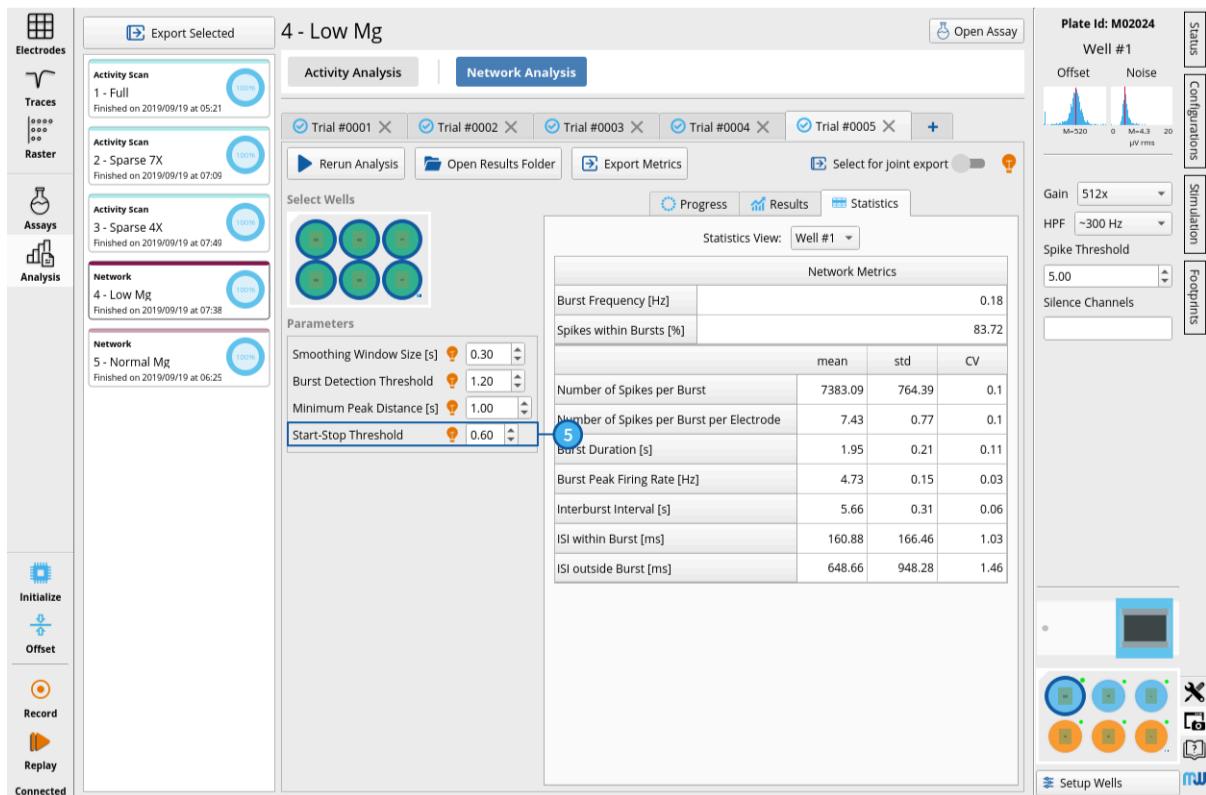


Figure 9.13. The Effects of Increasing the Start-Stop Threshold

Chapter 10. Abbreviations

AP: Action potential

CMOS: Complementary metal oxide semiconductor

CSV: Comma-separated values (file format)

CV: Coefficient of variation

DC: Direct current

GUI: Graphical user interface

HPF: High-pass filter

HD-MEA: High-density microelectrode array

IBI: Interburst Interval

ISI: Interspike Interval

LFP: Local field potential

LSB: Least significant bit

PBS: Phosphate buffered saline

RMS: Root mean square

STA: Spike-triggered average

std: Standard deviation

UI: User Interface