

Radiens AnalayticsTM Software

January 10, 2025





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Welcome to the RadiensTM Analytics Software Suite!

RadiensTM is a software solution meticulously crafted by neuroscientists specifically for neuroscientists, offering an exceptional toolset for various electrophysiological applications. Within the RadiensTM environment, you gain comprehensive laboratory management capabilities, including the creation of unique logins and event tracking for all your experiments, efficient probe inventory management, and robust data security and integrity measures. Moreover, our remote login feature allows for offsite observation during experiments, ensuring flexibility and convenience.

The RadiensTM suite comprises three seamlessly integrated applications: **Allego**, an advanced real-time signal processing software that seamlessly integrates with various data acquisition systems; **Curate**, a powerful data processing manager designed to ensure data integrity and curation of electrophysiological datasets using customizable batch processing protocols; and **Videre**, an intuitive and highly integrated software offering extensive capabilities for processing and analyzing diverse data types and formats related to electrophysiology.

Our RadiensTM suite offers highly intuitive and integrated functionalities, meticulously developed to meet your needs. The validated probe-centric approach facilitates the identification of data patterns during recording and enables swift verification of results. Furthermore, our software includes built-in 3D brain models that validate probe location, neuronal populations, and real-time neural network activity, providing valuable insights during both live experiments and file playback.

In addition, the file-based functional interface twin within Radiens[™] allows for the mapping of neural, cardiac, and muscle activity, offering a visual representation of the spatial relationship between recording sites on any NeuroNexus probe. This feature-rich software significantly accelerates neuroscience research through its exceptional performance, user-friendly interface, and forward-thinking advanced features.

For your convenience, it is crucial to ensure that you have the latest versions of our applications. Please visit our downloads page at the following link: <u>https://www.neuronexus.com/radiens-allego-download-page/</u>

If you have any inquiries or require further assistance regarding our software, please do not hesitate to reach out to us via email at support @NeuroNexus.com or by phone at +1.734.913.8858. We are dedicated to providing you with exceptional support.

Thank you for choosing RadiensTM for your neuroscientific endeavors.



Before You Begin

System Requirements

Minimum System Requirements

- 4-core CPU or better
- 16GB RAM
- USB 3 or better

For 256ch and higher, we recommend:

- 8-core, 16-thread CPU or better
- 32GB RAM

Below are the main components in the workstation NeuroNexus has available for SiNAPS and other high-channel count probe users. While GPUs are utilized, CPU performance has the biggest impact:

- Intel Xeon W3-2425 CPU
- 32 GB DDR5 RAM
- 2 TB SSD
- NVIDIA RTX A2000 12GB



Download and Install Radiens Software Application

RadiensTM is a comprehensive software suite specifically developed to seamlessly integrate with the SmartBox Pro, X-Series DAQs, and other Intan-based acquisition systems. It serves as a powerful tool for reviewing, visualizing, and curating previously recorded data. While updates are automatically installed by default, the latest versions of Radiens apps can be found on the RadiensTM download page at the following link: https://www.neuronexus.com/radiens-allego-download-page/

Please note that before you can access the software, you will be required to set up a Radiens user ID. Once the ID is created, NeuroNexus will add licenses to it, allowing access. Please refer to the next section "What is a Radiens ID?" for more detail.

For all platforms, TCP Ports 50051-50055 must be open and available for use as they are required for Radiens applications to function properly.



Launch the installer and follow the instructions.

If you receive a warning against running an unrecognized app, select "More Info", then "Run Anyway".

After the installation is complete and one of the apps is launched, Windows Defender or other security software may prompt you to authorize access for two different Radiens processes, "pybridge.exe" and "allegoserver.exe". Please allow these processes access as they are required for Radiens applications to run properly.



Download the Allego installer.

Double-click the installer to launch.

Drag the Radiens App icon into the Applications folder.

Open the Applications folder by double clicking the folder icon.



In the Applications folder, double-click the Radiens App icon while holding down the control key (holding down the control key overrides the MacOS security check, which is only needed during the initial launch).

Allego will launch after the installation is complete.

What is a Radiens ID

Your RadiensTM ID is the email address chosen to serve as your unique identifier and login for the Radiens applications. The Radiens apps can be installed on any number of computers, but only one session can be active at a time. If more than one license is on a Radiens ID, additional licenses can be delegated to other Radiens IDs. The instructions for these processes can be found in the Getting Started with Radiens section of this document.



Getting Started with Radiens

Create or Update Your Radiens ID

To create your user ID, start Allego by finding the shortcut or app on your device and launch it. Once Allego has started, it will begin the initialization process.

After initialization is complete, you will be presented with the sign-up screen. Look for the "Sign up now" option and click on it to create a new account. This will prompt you to provide the necessary information to set up your account, such as your name, email address, and a password.

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Sign in to your Account	
Email	
Password	
Forgot Password?	
SIGN IN	
Don't have an account? <u>Sign up now</u>	
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Note: The email address that you enter will be your Radiens ID, so use a frequently used address that you will remember.



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	Num #			
	Name *			
	Email Address *			
	Organization *			
	CONTINUE			
	OR			
	Already have an account? Sign In			
	© 2021 NeuroNexus Inc. All Rights Reserved.			

• On the new page, confirm password and submit.



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Confirm Password		
Password		
Confirm Password		
→ Min 8 characters long		
\rightarrow Must contain uppercase and lowercase characters		
\rightarrow Must contain a number and a special character		
SUBMIT		
Back to <u>Sign In</u>		
© 2021 NeuroNexus Inc. All Rights Reserved		

• Within a few minutes, you will receive a verification email with a 6-digit code to verify and confirm the account. The email comes from the domain "verificationemail.com" or "radients.neuronexus.com" which can sometimes get filtered as spam, so please check there as well.



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	Allego		
	Account Verification		
	Please confirm your account by entering the 6-digit code sent to		
	VERIFY		
	© 2021 NeuroNexus Inc. All Rights Reserved.		

• Once you verify your account you should be able to login with the Email ID and password that you already created.

By creating an account, you will gain access to the full functionality and features of Allego. It will allow you to leverage its advanced real-time signal processing capabilities and seamlessly integrate with a variety of data acquisition systems.

If you encounter any issues during the sign-up process or require further assistance, please don't hesitate to reach out to our support team. We are here to ensure a smooth onboarding experience for you.

Delegate Access for Additional Licenses to Other Radiens IDs

To delegate access to other Radiens IDs, login to a Radiens app and click on the "User Profile" button in the upper right corner of the UI, then in the window that spawns click "Invite User". This will spawn the "Invite a User" which will prompt for a new name and email address to be added as shown in the image below.



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spike-grid-0 × □ ⑦ ☑ ^		
 Invite a User 	×	MY SUBSCRIPTIONS Active
allego		
New User Information		
Name *		
Email Address *		
Allowed Applications Select More		
SUBMIT		
© 2023 NeuroNexus Inc. All Rights Reserved.		

Next, click on "Select More" beneath Allowed Applications to show the licenses on the primary Radiens ID. Select an available license and then click Submit. The email entered will receive the same email verification and temporary code as the primary Radiens ID did when initially set up.

Once this is complete, the new Radiens ID will now utilize the delegated license.



Checking Hardware Connection

To begin, access the module drawer on the left-hand side of the display and locate the System button. Within the System tab, navigate to the Mode dropdown and choose SmartBox Pro Hardware. When the SmartLink headstages are powered on and connected, the software will automatically detect and acknowledge their presence. If you are utilizing a different Intan-based system, click on the Mode menu and select the appropriate option accordingly.

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\vee	Signal Source Available Channel SmortBox Clossic Hordwore		
	Port A 0 Open Ephys USB3 Hardware	DESELECT ALL 3 ft V	
<u> </u>	Port B 0 Open Ephys USB2 Hardware	DESELECT ALL 3 ft V	
z	Port C 0 Intan Recording Controller 1024 Hardware	DESELECT ALL 3 ft ~	
ര	Port D 0 Intan Recording Controller 512 Hardware	DESELECT ALL 3 ft ~	
ulu	Analog In 2 Training: Simulated spike data Training: Sine waves	DESELECT ALL	
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The bottom-right corner of the UI shows a SmartBox Pro / DAQ outline which provides the ability to monitor the status of the ports connected to the DAQ. A green light will indicate which ports are currently in use. The following image demonstrates the status of a DAQ turned on with single 32-channel headstage connected to port B.



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	Signals						
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	Port C				3 ft ~		
Ó	Port D				3 ft ~		
ulu 	Analog In		AI 1 🛞 AI 2 🛞 Select More	DESELECT ALL			
#	Digital In		DI 1 🛞 DI 2 🛞 Select More	DESELECT ALL			
	Digital Out		DO 1 🛞 DO 2 🛞 Select M	DESELECT ALL			
Ŷ	Analog Out Control						
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Radiens Allego





Training and Supporting Resources

We recommend visiting <u>Radiens' web page</u> where you can access the training manuals available on the documentation center or watch instructional <u>videos</u> on our YouTube channel. These resources, including webinars, are designed to assist our users in becoming familiar with our products. If you require additional support, please feel free to reach out to our customer support team for further assistance.

To familiarize yourself with Allego prior to using it in conjunction with the SmartBox Pro, follow these steps:

- Select the System icon from the drawer on the left to open a System tab.
- From the Mode dropdown menu, choose "Training: Simulated spike data."

By selecting this mode, Allego will simulate the presence of a 32-channel headstage connected to Port A, allowing you to explore and practice using the system.

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Q	Port B			DESELECT ALL
D	Port C		Select More	DESELECT ALL
	Brd D			
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	Analog In		AI 1 3 AI 2 3 Select More	DESELECT ALL
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Additionally, you have the option to test the filtering capabilities by selecting "Training: Sine waves" mode. This mode simulates the presence of a 64-channel headstage connected to Ports



A and C. By selecting this mode, you can experiment with the filtering options available and gain hands-on experience with the system's functionalities.





Interface Overview

The Radiens platform user interfaces are designed to be easily configurable and can utilize any number of displays connected. Feature sets are grouped together in windows or tabs referred to as Modules. The module names at the top of each tab can be clicked and dragged to new position; they can be stacked together, placed next to one another, maximized, or docked to the sidebars. For more about this, please go to the "Dashboard Configurability" section below.

Module Drawer

On the left-hand side of the display, you will find the Module Drawer, which showcases various module icons. To access the Module Drawer, simply click on the arrow button located in the top left corner of the window.





The modules drawer contains all available modules. Clicking on an item in the list will either open a new tab for the selected module or bring an already opened tab into view. Moreover, it is possible to have multiple tabs of the same type saved within the workspace, all of which can be accessed from the module drawer. If you wish to extend the interface to additional monitors, creating tabs in a new window can be easily accomplished. By right-clicking on a module, a context menu will appear, offering the option to open a new tab in a new window.

Configuration

System: Offers comprehensive configuration options for signal sources, port selection, sampling frequency, and D/A (outputs).

It encompasses all the essential settings for defining the functionality of the recording system and specifying the specific recording system in use. Within the System module, you can set the Sample Rate, access information about connected headstages for each port, control analog and digital outputs, and configure triggering settings.



-0-

Electrodes: Choose the probe and headstage type connected to each port.

In the Electrodes module, you can define the headstage and probe types for each port on the recording system. This module allows you to enable or disable individual or groups of probe electrode sites, as well as arrange the voltage traces that are visualized in the Monitor tab. Furthermore, wireframe models of NeuroNexus probes are displayed within this module, providing a visual representation of the probe configuration. When working with NeuroNexus probes, it is important to select the headstage and probe type to match what is connected to the system as this can affect the probe site numbering in software.

Signal Processing: Provides the capability to create different types of filters and combine them to form Digital Signal Processing (DSP) grouping

The Signal processing module contains filtering options that will impact only visualized data, or both visualized and saved data.



Signal Collection

Monitor: Displays the signals that are currently being streamed

Within this module, you can visualize and observe the live signals from the recording system.

Z Impedance: Impedance testing

Probe impedance can be measured at 1 kHz in this module.

Visualization

HD Snapshot: capture high-resolution still pictures of the signals for closer inspection

The HD Snapshot module offers a powerful visualization tool that allows you to view waveforms of recorded signals using all captured data points. By utilizing all the data points available, the module generates a highly accurate depiction of the signal of interest. This level of detail and precision provides a comprehensive view of the waveform, enabling you to analyze and examine the signal with great accuracy and fidelity. The HD Snapshot module is a valuable resource for detailed signal analysis and interpretation.

·|||!

Signal Metrics: Configure spike detection parameters and view key performance indicators/signal quality characteristics

The Signal Metrics module allows the user to characterize activity across electrode sites using several different metrics, such as mean and standard deviation. Probe geometry is used to visualize relationships between the activity recorded in different locations on the probe.

Spike Grid: Offers a refined capability to set the spike detection threshold based on either the voltage or the standard deviation of the background noise from the mean

This module efficiently displays the detected spikes across all channels, providing a comprehensive overview of the recorded neural activity. With the Spike Grid module, you can precisely adjust the detection threshold to effectively identify and analyze spikes in your data, facilitating in-depth investigations and insightful interpretations.



Spike Sorter: Facilitates the grouping of spikes into distinct clusters

Using advanced algorithms and techniques, this module efficiently categorizes spikes based on their waveform characteristics, allowing for a more organized and structured analysis of neural activity. By grouping the spikes into clusters, researchers can gain valuable insights into the different types of neural events occurring within the recorded data. The Spike Sorter module streamlines the process of spike sorting, enhancing the efficiency and accuracy of data analysis in neuroscience research.

Raster: Provides a powerful tool for analyzing spike trains of single neurons

With this module, you can visualize and study the temporal patterns and firing activity of individual neurons. The Raster module displays spike events as vertical lines or ticks along a horizontal timeline, allowing for a clear representation of the timing and frequency of neuronal firing. This visualization aids in the identification of patterns, correlations, and other important characteristics of the neuron's activity. By examining the raster plots, researchers can gain valuable insights into the firing behavior and response properties of single neurons, contributing to a deeper understanding of neural dynamics and information processing.

3D Model: Offers an interactive brain model that presents network activity in a visually engaging manner

This module provides a three-dimensional representation of the brain, allowing users to explore and visualize the dynamics of network activity. By leveraging this interactive model, researchers can gain a comprehensive understanding of how different regions of the brain interact and contribute to overall neural activity. The 3D Model module provides a powerful tool for studying and presenting complex network dynamics, enhancing our knowledge of brain function and connectivity.

Neurons: Provides visualization of neurons and spikes detected across channels

The Neurons module provides interspike interval graphs for neurons detected by each site. The graphs show the quantity of spikes detected between intervals ranging from 2 to 20 ms.



Settings: Provides essential configuration options to activate the software, set visual themes, and define the recording file name and location.

Within this module, you can activate the software to ensure full functionality and access to all features. Additionally, you can customize the visual themes to personalize the software's appearance and create a visually appealing workspace.

Furthermore, the Settings module allows you to specify the recording file name and choose the desired location where the recorded data will be saved. This enables efficient organization and easy access to your recordings for further analysis and review.

Status Bar

At the top of the window, you will find the Status bar that includes the Stream and Record buttons. These buttons allow you to initiate and control the recording and streaming processes. Additionally, the Status bar displays the current duration of the recording and streaming activities, providing you with up-to-date information on the progress of your session. The Settings module is also accessible from this bar, allowing you to quickly customize and configure various aspects of the software.

The Status bars serve as a helpful navigation and monitoring tools, enabling efficient control over your recording and streaming tasks while providing essential information at a glance.

() Recording Dur: 0s () Streaming Dur: 0.0s 🕸 🛃 🖞 RECORD STREAM 🙇 🗸

The bottom Status bar, as depicted in the image below, shows a diagram with the current state of the system and the ports with connected headstages.



Save Workspace and User Profile

In the upper right area of the display, you will find the workspace buttons that provide convenient options for managing your workspace:

): Clicking this button saves the current configuration of your work-Save Workspace button (space. It allows you to preserve the arrangement of tabs and windows, making it easier to restore the layout at a later time.

Trashcan icon(

Ш): This button will delete the currently saved workspace and return the UI to its default state.



Restore button(): Clicking on the restore button restores the tabs to their saved locations when you launch Allego. This functionality ensures that your preferred layout is automatically applied upon starting the software.

Below is a table of features that are retained by using the Restore Workspace button:

Restore Workspace Feature Retention

System	
Mode	Yes
Sample Rate	Yes
Selected/Deselected Sites	No
Gain	Yes
AO Control	Yes
Highpass filter	Yes
AO 1 / AO 2 mapping	Yes
Digital Out Control Mode	No
Digital In Triggering State	Yes
Digital In Triggering Channel	Yes
Electrodes	
Headstage Selection	Yes
Probe Selection	Yes
Site Reordering	No
Signal Processing	
Filters	Yes
Spike Sorter	
Threshold	Yes
3d-model	
Stereotactic Coords	No
Animal Model	No
Hide Atlas Panel	No
Atlas Slice	No



Additionally, in the top right corner of the Allego window, you will find the user's profile icon(Clicking on this icon provides access to the user's profile information. Here, you can view your subscription status and the current version of Allego installed on your system.

agolabchi@neuronexus.com Admin						
MY SUBSCRIPTIONS	Active					
Radiens Analytics S	oftware Suite - Academic					
ORGANIZATION						
NeuroNexus						
ALLOWED USERS	CURRENT USERS					
1	1					
i About						
S⁺ Invite User						
\rightarrow Sign Out						

These workspace buttons and user profile information offer convenient tools for managing your workspace layout and accessing important user-related details within the Allego software.

Dashboard Configurability

The Dashboard in Allego offers great configurability for the module tabs. You can easily customize the layout by following these steps:

- Click and hold on the tab header you want to move.
- Drag the tab to the desired location on the Dashboard.
- Release the mouse button to drop the tab in the new position.

By dragging and repositioning the tab headers, you have the flexibility to arrange them according to your preference. This allows for a personalized workspace that suits your specific needs.

Furthermore, Allego supports different viewing options for tabs. You can choose to view tabs simultaneously in a split-screen layout or stack them together, resembling tabbed web browsers. This enables efficient multitasking and easy access to multiple modules at once.



As you click and drag the tab header around the display, Allego provides a real-time preview of the layout, helping you visualize and finalize the desired arrangement before releasing the tab.

With the Dashboard's configurability, you can create a tailored workspace that maximizes your productivity and enhances your overall user experience in Allego.





The System module in Allego plays a crucial role in configuring the connected acquisition system. It allows you to define the specific system being used for your experiment. With this module, you have control over the inputs that will stream through the hardware, as well as the outputs from the recording system that interface with other components of your experiment.

By configuring the System module, you enable Allego to effectively manage and control the inputs and outputs of the recording system, facilitating seamless integration with other experimental components. This module provides the essential groundwork for accurate and synchronized data acquisition, enabling you to conduct experiments with precision and reliability.

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	Signals											
	Signal Source	Available Channels	Selected Channels			Cable Length						
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	Port B					3 ft ~						
	Port C					3 ft ~						
	Port D					3 ft ~						
	Analog In		AI 1 🗙 AI 2	Select More	DESELECT ALL							
	Digital In		DI 1 🚫 DI 2	Select More	DESELECT ALL							
	Digital Out		DO 1 🛞 DO 2	Select M	DESELECT ALL							
	Analog Out	Control										
	Gain											
										₼∎⊓⊓		EDD

Mode

The mode setting in Allego serves the purpose of configuring the software according to the type of connected recording system or for training purposes when no system is available. Allego offers multiple modes to choose from, depending on your specific requirements. Here are some important details about the available modes:



System-Specific Modes: These modes are intended for configuring Allego to work seamlessly with different types of recording systems. You should select the mode that matches the type of recording system you are using. This ensures compatibility and proper functionality between Allego and your specific hardware setup.

Training Mode with Simulated Spike Data: This mode is designed for training and practice purposes. It allows users to familiarize themselves with Allego's features and functionalities before conducting experiments with live animal data. In this mode, users can simulate spike data to practice configuring thresholds, exploring visualization options, and gaining proficiency in using Allego's tools.

Training Mode with Sine Waves: This mode is particularly useful for testing and understanding the impact of various filter stages available in the Signal Processing tab. By utilizing sine waves as simulated data, users can assess how different filter settings affect the processed signals, enhancing their understanding of the signal processing capabilities within Allego.

These different modes in Allego offer flexibility and versatility, allowing users to adapt the software to their specific needs, whether for live experiments, training purposes, or testing different scenarios.





Sample Rate

In the System tab, the next section focuses on configuring the input signals for your signal group.

Begin by selecting the appropriate sampling rate for your signal group. The default maximum value is 30,000 samples per second (30 kHz). This value is pre-selected to ensure high-quality data acquisition. However, if needed, you can adjust the sampling rate to a lower value based on your specific requirements.



By setting the sampling rate, you determine the frequency at which data points are captured and recorded. This parameter plays a crucial role in accurately representing and analyzing the signals of interest.

Allego offers a range of sampling rate options to accommodate different experimental needs. Ensure that you select a suitable sampling rate to capture the desired level of detail and fidelity in your recorded signals.

Configuring the input signals in the System tab with the appropriate sampling rate is an essential step in preparing Allego for accurate and reliable data acquisition.

625	
1000	
1250	
1500	
2000	
2500	
3000	
3333	
4000	
5000	
6250	
8000	
10000	
12500	
15000	
20000	
25000	
30000	


Signal Map

By clicking on the Signal Map button in Allego, you can access a table that provides a comprehensive overview of the end-to-end path for each detected electrode site and its corresponding headstage channel. This table presents important details such as site coordinates, amplifier channel name, connected port, and the currently selected probe and headstage files.

One key advantage of using the Signal Map is that it allows you to verify how data is being routed within Allego. While Allego's XDAT data files are sorted based on the preamplifier channels rather than the probe site numbers, the Signal Map provides a clear visualization of the data routing process.

It's important to note that the Signal Map table is updated in real-time, reflecting any configuration changes or selections made by the user within Allego. As you modify your configuration settings, the values displayed in the Signal Map will dynamically update to reflect the current state of your setup.

The Signal Map feature in Allego serves as a valuable tool for ensuring the accuracy and integrity of your data routing. By referring to this table, you can easily track the pathway of each electrode site and verify that the data is being captured and processed correctly within the software.

Signal Map								×
III COLUMNS 🖶 FILTERS 🗮 DENSITY 🕁 EXPORT								
Data Channel Index	Site Number	Site Pos X um	Site Pos Y um	Site Pos Z um	Signal type	Channel Name	Port	Headstage
0		-29.92000000000005	5.197		PRI	pri_0		hstgpassthrough_32
1		-29.92000000000005	25.19700000000003		PRI	pri_1		hstgpassthrough_32
2		-29.92000000000005	45.197		PRI	pri_2		hstgpassthrough_32
3		-29.92000000000005	65.197		PRI	pri_3		hstgpassthrough_32
4		-29.92000000000005	75.197		PRI	pri_4		hstgpassthrough_32
5		-29.92000000000005	55.197		PRI	pri_5		hstgpassthrough_32
6		-29.92000000000005	35.197		PRI	pri_6		hstgpassthrough_32
7		-29.92000000000005	15.197000000000001		PRI	pri_7		hstgpassthrough_32
8		-9.92000000000002	5.197		PRI	pri_8		hstgpassthrough_32
9	10	-9.92000000000002	25.19700000000003		PRI	pri_9		hstgpassthrough_32
10		-9.92000000000002	45.197		PRI	pri_10		hstgpassthrough_32
11		-9.92000000000002	65.197		PRI	pri_11		hstgpassthrough_32
12		-9.92000000000002	75.197		PRI	pri_12		hstgpassthrough_32
13	14	-9.92000000000002	55.197		PRI	pri_13		hstgpassthrough_32
14	15	-9.92000000000002	35.197		PRI	pri_14		hstgpassthrough_32
							Rows per page: 50	1–38 of 38 < >



Cable Length

While most system modes in Allego use an algorithm to automatically determine the best timing offset for a headstage interface cable in use, modes for some older systems like the SmartBox classic will provide a dropdown menu with a list of predetermined timing offsets. This compensates for the delay introduced depending on the length of the cable.

In the System tab of Allego, choose the cable length that most closely matches your cable.

By default, Allego assumes the use of a 3 foot cable. It is worth noting that adjusting the cable length setting can also be beneficial if you are experiencing issues with the detection of a head-stage by Allego. By altering this setting, you may improve the detection and recognition of the headstage, ensuring proper functionality within the software.

By offering built-in compensation for different cable lengths, Allego allows users to optimize their recording systems and account for any potential delays, ensuring accurate and reliable data acquisition and processing.



Signals

• **Signal Source:** In the Signals section of the System tab, you will find a comprehensive list of all the available inputs to the recording system. The image below showcases some of these inputs for reference. This section allows you to select which inputs you want to visualize during the streaming process.



In addition to the headstage ports, the SmartBox Pro offers two Analog Inputs and two Digital Inputs that can be utilized. These additional inputs provide flexibility and expand the range of signals that can be captured and processed by Allego.

Furthermore, the Signals section also allows you to select or deselect the Digital Outs for visualization. This feature enables you to choose whether you want to view the output signals in realtime during the streaming process.

By providing this level of control and customization in the Signals section, Allego empowers users to tailor their visualization settings according to their specific experimental requirements. This ensures that you can focus on the relevant inputs and precisely monitor the signals of interest during your data acquisition and analysis tasks.

lls			
Source	Available Channels	Selected Channels	
	0	Select More	
	0	Select More	
;	0	Select More	
)	0	Select More	
g In	2	AI 1 🗙 AI 2 🛞 Select More	DESELECT
l In	2	DI 1 🗴 DI 2 🗙 Select More	DESELEC
l Out	2	DO 1 🛞 DO 2 🛞 Select M	DESELECT

Available Channels: The available channels for Ports A, B, C, and D in Allego will automatically populate based on the detection of connected headstages. Allego is designed to recognize and identify the headstage connections, and as a result, the corresponding channels associated with each detected headstage will be displayed in the software.

When you connect headstages to your DAQ, Allego will detect and establish the communication with them. This detection process allows the software to dynamically populate the available



channels for each port based on the connected headstage configuration. You will be able to see the specific channels associated with each headstage in Allego's interface.

The automatic population of available channels simplifies the setup process and ensures that you have access to the correct channels for data acquisition and analysis. Allego's ability to detect and configure the available channels based on the connected headstages provides convenience and accuracy, allowing you to seamlessly work with your recording system.

Please note that the availability of channels will depend on the specific headstage models and configurations you have connected to Ports A, B, C, and D. Allego adapts to the detected hard-ware to provide you with an intuitive and streamlined user experience.

• Selected Channels: In Allego, by default, all available channels on each port are selected for visualization. This means that all channels will be displayed in the various tabs and modules throughout the software. However, Allego provides the flexibility to customize the channel selection based on your specific needs.

In the System tab, you have the option to deselect specific channels for each port. When you deselect a channel, it means that it will not be visualized in any of the other tabs or modules within Allego. However, it's important to note that even if a channel is deselected for visualization, it will still be recorded and saved as part of the data acquisition process.

This feature allows you to focus on the channels of interest and declutter the visual display in Allego. By deselecting certain channels, you can streamline your analysis and concentrate on the relevant data. It's worth mentioning that while deselected channels are not visualized in real-time, they are still recorded, ensuring that you have access to the complete dataset for post-processing and analysis.

The ability to selectively choose which channels to visualize while recording all channels in the background gives you greater flexibility and control over your data visualization and analysis workflow in Allego.

Stimulation

This section specifically deals with the selection between XDAQ One and XDAQ Core when using the XSR headstage. The choice between these options depends on your specific requirements and the stimulation configuration you wish to implement.

For more detailed information and guidance on the stimulation configuration using XDAQ One or XDAQ Core with the XSR headstage, we recommend referring to the relevant documentation or resource provided in this manual. This resource will provide comprehensive instructions and guidelines on how to configure the stimulation settings and utilize the specific features of XDAQ One or XDAQ Core in conjunction with the XSR headstage.



By consulting the provided resource, you will be able to access specific information tailored to your setup and obtain a thorough understanding of the stimulation configuration options available to you. This will ensure that you can effectively configure and utilize the stimulation capabilities of Allego in conjunction with the XSR headstage according to your experimental needs.

XDAQ One with XSR Headstage

XDAQ Core with XR Headstage

XDAQ Core with XSR Headstage

Analog Out Control

Located below the Signals panel in Allego, you will find the Analog Out Control section. This section allows you to configure the routing of live data signals to the analog outputs of the recording system or the auxiliary port on the SmartBox Pro unit. Additionally, any audio activity associated with the selected sites will be played through the computer's audio system.

Within the Analog Out Control settings, you can specify which specific signals you want to route to the analog outputs. This provides flexibility in choosing which data you want to monitor or feed into other external devices through the analog outputs.

By selecting the appropriate options in this section, you can control the routing of live data signals, enabling you to monitor and manipulate the desired signals in real-time. Moreover, any audio activity associated with the selected sites will be audible through your computer's audio system, allowing you to listen to the corresponding audio signals alongside the visual data representation.

The Analog Out Control section is an important tool for managing and monitoring live data signals in Allego, providing you with the ability to customize the output routing and audio playback according to your experimental requirements.



Analog Out Control						
Gain	Gain					
0	~					
Analog						
Out Sit	e					
Channel						
AO 1						
AO 2						
CLEAR BOTH						

- **Gain:** First set the desired amplification for both analog output channels. By setting the amplification for the analog output channels, you can control the strength and intensity of the signals being sent through these channels. This allows you to customize the output to match your specific requirements, ensuring optimal signal quality and compatibility with external devices or systems connected to the analog outputs.Please note that this only impacts the gain when listening to audio via the 3.5mm Auxiliary or Audio Out jack on the back of the DAQ and does not impact signals heard within the Radiens software.
- Analog Out Channel: The Analog Out Channel settings in Allego correspond to the AO1 and AO2 outputs located on the back of the SmartBox Pro unit. These channels allow you to route specific signals from the recording system to external devices or systems.
- Site: The Amplifier Channel selection allows you to assign a specific channel from a SmartLink headstage to the corresponding analog output. To choose the appropriate channel, follow these steps:
 - Scroll to the desired port (A, B, C, or D) in the Amplifier Channel selection section.
 - Select the channel of interest within the chosen port.
 - To easily identify the channel number based on the probe's location, refer to the probe wireframe available in the Electrodes tab.



• If you need to find the channel number based on a signal waveform, navigate to the Monitor tab, where you can correlate the waveform with the corresponding channel number.

By following these steps, you can accurately assign the desired channel from the SmartLink headstage to the analog output. This allows you to control which signals are sent to the analog output for further analysis or connection to external devices.

Utilizing the probe wireframe in the Electrodes tab or referencing the signal waveform in the Monitor tab provides convenient methods for identifying the specific channel numbers associated with your desired signals.

Please note that the channel numbers may vary depending on the configuration of your recording system and the specific probes or headstages being used.

 CLEAR ALL: To clear the settings and stop sending signals to the analog outputs on the back of the SmartBox Pro, simply click on the "CLEAR ALL" button. This button serves as the most effective way to clear any audio output selections that have been made.

By clicking on the "CLEAR ALL" button, any previously assigned channels or configurations for the analog outputs will be reset, ensuring that no signals are being sent to the analog outputs. This allows you to start with a clean slate and make new selections or adjustments as needed.

Using the "CLEAR ALL" button provides a straightforward and efficient method to clear audio output settings, allowing you to manage and control the routing of signals with ease.

Please note that clearing the settings using the "CLEAR ALL" button will not affect other configurations or settings within Allego, and it specifically targets the analog outputs on the back of the SmartBox Pro.

To configure the Analog Out Channels in Allego:

- Open the System tab in Allego.
- Locate the Analog Out Control section.
- Within this section, you will find settings for each analog output channel.
- Select the desired Analog Out Channel (AO1 or AO2) that you want to configure.



- Adjust the settings for the selected channel according to your requirements. This may include selecting the specific signals to route and adjusting the output voltage range.
- Repeat the process for the other Analog Out Channel (if applicable) to configure its settings as well.

By configuring the Analog Out Channels in Allego, you can determine which signals are sent to the corresponding AO1 and AO2 outputs on the SmartBox Pro unit. This provides you with the flexibility to connect external devices, such as oscilloscopes, data acquisition systems, or other recording devices, to capture or further process the desired signals.

Digital Out Control Modes

There are three available modes for controlling the Digital Outputs of the acquisition system in Allego:

Digital Out Control					
Mode					
● Gated ○	Manual O Events				
Channel	Indicates				
DO 1	Streaming status				
DO 2	Recording status				

- **Gated Mode:** This mode is primarily used for synchronization purposes. In Gated Mode, DO1 is used to indicate whether data is currently streaming or not streaming, while DO2 indicates if the system is recording or not. When the system is in the recording state, both DO1 and DO2 will be set to a high state (ON). This is the default mode in Allego.
- **Manual Mode:** In Manual Mode, the user has the ability to manually control the state of the Digital Outputs. You can choose to set DO1 or DO2 to either a high state (ON) or a low state (OFF). This mode is particularly useful for testing or resetting the digital output signal as needed.
- **Events mode:** Events Mode allows for communication of events between the acquisition system or Allego and other devices in the experiment. When the voltage of the signal being monitored by one of the Analog In channels crosses the voltage threshold set in this mode, a positive or negative digital output will be sent to the corresponding Digital Out channel.



These modes provide flexibility in controlling the behavior of the Digital Outputs in Allego, allowing for synchronization, manual control, or event-based communication within your experimental setup.

Digital In Triggering

By enabling the "Trigger gated recording on DIN state" checkbox, Allego is configured to monitor the input of either DIN 0 (DI1 on the back of a SmartBox Pro) or DIN 1 (DI2 on the back of a SmartBox Pro). When a high signal is detected on the selected input, Allego will automatically switch from the streaming state to the recording state. Conversely, when there is no input or a low signal is detected, the recording will be turned off.

It's important to note that this behavior can be overridden in the Monitor tab, allowing you to manually control the recording state regardless of the input signal on DIN 0 or DIN 1. This gives you flexibility and control over the recording process based on your specific experimental needs.



Stimulation

The Stimulation module, located in the System tab, is used to configure the parameters for intracortical microstimulation. This approach involves the invasive activation of neurons and is being investigated to deliver sensory percepts in cases where sensory pathways have been damaged.

Within the Stimulation module, you can define the specific parameters for the stimulation, including the amplitude, duration, and frequency of the electrical pulses. These parameters can be adjusted based on the experimental requirements and the targeted neural population.

By configuring the stimulation parameters in the Stimulation module, researchers can precisely control and deliver electrical stimulation to specific brain regions, allowing for the exploration of sensory perception and the potential restoration of sensory function in cases of sensory pathway damage.

It's worth noting that the Stimulation module is just one component of Allego's comprehensive system, designed to provide researchers with a wide range of tools and features for experimental control and data analysis in the field of neuroscience.



Stimulation Channel	: S 🗸 (1 en	abled) Step Size:	<u>~</u>	
Stim Diagram				^
TRIG	, D2 ↓A2 ↓D2 ↓A2 ↓Dost ↓Stort K	REFRAC	CTORY	END
Trigger	Pulse Train	Stimulation Waveform	Amp Settle	Charge Recovery
Z Enable	Pulse Repetition	Stimulation Shape	Enable Amp Settle	Enable Charge Recovery
Trigger Source	Singe Pul V	Biphasic ~	Pre Stim Amp Settle	Post Stim Charge
AI 1 ~	Post-Stim Refractory Period	Stimulation Polarity	0 µs	
Trigger Shape	1 ms		Post Stim Amp	
Edge Trigger V		100 un	Settle	Post Stim Charge Recovery Off
		μ	Ομs	Ο μs
Port Trigger Delay		Second Phase Duration (D2)		
		100 µs		
		First Phase Amplitude (A1)		
		0 μΑ		
		Second Phase Amplitude		
		0 μΑ		
SAVE ALL CANCEL	Unsaved changes (1)			

To configure the stimulation parameters for a specific headstage channel in Allego, follow these steps:

Page 47



• Click on the desired channel in the Allego interface to select it.



• Locate the expander arrow on the right side of the Stim Diagram panel to expand the display.

Stimulation Channel: S> (0 enobled) St	ap Size:	
Stim Diagram		
Trigger — Pulse Train Stimulation Waveform Amp Settle O	Charge Recovery	
SAVE ALL CANCEL		
Stim Diagram		^
TRIG		END
, DELAY ,, DI ,, D2 ,	REFRACT	rory ,
<mark>к^{рге}я</mark>	post.	
AMPLIFIER SETTLE CHARGE RECOVERY	K stor	

- This display is an illustration of the stimulation waveform (please note that the illustration is not to scale). This visualization can help you understand and visualize the stimulation waveform.
- Underneath the expanded display window, you will find the stimulation trigger settings. Enable the stimulation trigger by checking the "Enable" box.



Digital Out		DESELECT ALL	
Stimulation	- Channel: S v (0 enobled) Step Size: v		
Stim Diagram			
	LAY <u>m</u> D1 <u>m</u> D2 <u>m</u> A CURRENT AI	REFRAC	
Trigger Enable	Pulse Train Stimulation Waveform Amp Settle Charge Recovery		

• Once the stimulation trigger is enabled, a Stimulation Parameters window will appear. This window allows you to adjust the stimulation parameters for the selected channel. This visu-



alization can help you understand and visualize the stimulation waveform.

Stimulation Channel	: s v (1 en	abled) Step Size:	~	
Stim Diagram				^
TRIG DELAY DI STIM CURRENT AI AMPLIFIER SETTLE CHARGE RECOVERY	∠D2 ↓A2 ↓A2 ↓ post stort K ~	REFRA(CTORY	END
Trigger	Pulse Train	Stimulation Waveform	Amp Settle	Charge Recovery
Enable	Pulse Repetition	Stimulation Shape	Enable Amp	Enable Charge
Trigger Source	Singe Pul 🗸	Biphasic 🗸	Pre Stim Amp Settle	Post Stim Charge
× 11A	Post-Stim Refractory	Stimulation Polarity	0 μs	Recovery On
Trigger Shape		Cathodic Fi ~	Post Stim Amp	0 µs
Edge Trigger 🗸		First Phase Duration (D1)	Settle	Post Stim Charge Recovery Off
Trigger Polarity		100 µs	0 µs	0 us
Trigger on Hi 🗸		Second Phase Duration (D2)		
Post Trigger Delay		100 µs		
Ο μs		First Phase Amplitude (A1)		
SAVE ALL CANCEL	Unsaved changes (1)			

• Within the Stimulation Parameters window, you can modify the parameters such as the amplitude and duration of the stimulation pulse, and the frequency of the stimulation



waveform. Adjust these parameters according to your experimental requirements.

- When you select or interact with the stimulation parameter controls, you will notice that the corresponding arrows and labels in the waveform display(Stim Diagram) turn from light gray to red. This visual indication is provided to assist you in identifying and adjusting the specific parameters being modified.
- After configuring the stimulation parameters to your desired settings, click on the "SAVE ALL" button to apply the changes. Please note that STREAMING MUST BE OFF in order to save any parameter changes. This button will be unavailable while streaming is on.

By following these steps and adjusting the stimulation parameters for specific headstage channels, you can precisely control the electrical stimulation delivered to the target tissue. This level of control is valuable for various experimental and therapeutic applications that involve intracortical microstimulation.

Note: The exact user interface and layout may vary slightly depending on the system mode you are using, but the general steps for configuring stimulation parameters should remain similar.

Stimulation Trigger

Trigger	
Enable	
Trigger Source	
ALI	~
Trigger Shape	
Edge Triggered	~
Trigger Polarity	
Trigger on High	~
Post Trigger Delay	
0	μs



To adjust the stimulation parameters in Allego, you can select a trigger source from various options:

Trigger Source:

- Digital or Analog Inputs: Choose any available digital or analog input on the main Controller or optional I/O Expander as the trigger source. Analog inputs can function as digital inputs with a threshold of 1.65 V.
- Host Computer's Number Keys: You can use the number keys (1-8) on your computer's keyboard as triggers for the stimulation.

Trigger Shape:

Once you have selected the trigger source, you can configure the stimulation sequence type:

- Edge Triggered: Selecting this option will cause the stimulation sequence to execute once every time the trigger changes from an inactive state to an active state.
- Level Triggered: Selecting this option will cause the stimulation sequence to execute repeatedly as long as the trigger source remains in an active state.

Trigger Polarity:

You can also specify the active level for the trigger source:

- Active on High: Choose this option if the trigger source is considered active when it has a "high" logic level or when a key is pressed.
- Active on Low: Choose this option if the trigger source is considered active when it has a "low" logic level or when a key is not pressed.

Post Trigger Delay:

Additionally, you have the option to introduce a post-trigger delay:

By default, the stimulation sequence executes immediately after a trigger event. However, you can specify a post-trigger delay of up to 500 milliseconds. This feature allows you to stagger stimulation pulses across multiple channels triggered by the same source.

These settings provide flexibility in configuring the stimulation parameters based on your experimental needs. You can define the trigger source, sequence type, active level, and introduce delays to precisely control the stimulation events in Allego.



Pulse Train

Pulse Train				
Pulse Repetition				
Singe Pulse	~			
Post-Stim Refractory Period				
1	ms			

Pulse Repetition: Within Allego's stimulation module, you have the flexibility to configure both single pulses and pulse trains as stimulation sequences. The pulse repetition selector allows you to choose between these options based on your experimental requirements.

If you select a pulse train, you can define the number of pulses in the train (up to 256) and the pulse train period (up to 1 second). The corresponding frequency of the pulse train will be displayed below for your reference.

Post-Stim Refractory Period: Additionally, you have the option to set a post-stimulation refractory period. During this period, any additional trigger events will be ignored after a stimulation sequence has completed. This parameter is useful for configuring pulse trains of indefinite duration by combining single stimulation pulses with level triggering. As long as the trigger source remains high, the single stimulation pulse will repeat at a rate determined by the post-stimulation refractory period, which can be set up to one second.



Stimulation Waveform

Stimulation Wavefo	rm			
Stimulation Shape				
Biphasic	~			
Stimulation Polarity				
Cathodic First	~			
First Phase Duration (DI)				
100	μs			
Second Phase Duration (D2)			
100	μs			
First Phase Amplitude (AI)				
0	μA			
Second Phase Amplitude				
0	μA			

Stimulation Shape: Allego's stimulation system offers support for three waveform shapes: biphasic, biphasic with interphase delay, and triphasic. These waveform options provide flex-ibility in designing the stimulation patterns for your specific experimental requirements.

- The biphasic waveform consists of two phases, typically a positive pulse followed by a negative pulse. This waveform shape is commonly used in various stimulation applications.
- The **biphasic waveform with interphase delay** includes an additional delay period between the positive and negative phases. This delay can be adjusted to achieve specific timing requirements in your stimulation protocol.



• The **triphasic waveform** extends the biphasic waveform by adding a third phase, usually of opposite polarity to the initial two phases. This waveform shape allows for more intricate and versatile stimulation patterns.

By selecting the appropriate waveform shape from these options, you can tailor the stimulation to elicit desired responses and explore different aspects of neural activation and modulation.

TRIG		END
DELAY m D2 STIM CURRENT Image: Constraint of the second se	REFRACTORY	>
	Biphasic	
CHARGE RECOVERY		
TRIG	REFRACTORY	
	Biphasic with interphase delay	
CHARGE RECOVERY		
TRIG	REFRACTORY	
	Triphasic	
AMPLIFIER SETTLE CHARGE RECOVERY		

Stimulation Polarity: Regardless of the waveform shape, the stimulation polarity can be selected to deliver cathodic (negative) current first (standard practice in most stimulation experiments) or anodic (positive) current first.

Phase Duration and Amplitude: When configuring the stimulation waveform, you can adjust the pulse phase durations (D1 and D2) and current amplitudes (A1 and A2) within the limits set by the time resolution and current step size defined during the software startup.

For each phase, you can set the duration in milliseconds (up to a maximum of 5 milliseconds) and the current amplitude. The time resolution and current step size determine the precision with which you can adjust these parameters.



By fine-tuning the pulse phase durations and current amplitudes, you can precisely control the characteristics of the stimulation waveform, such as the timing and intensity of the electrical pulses delivered to the target tissue.

It's important to note that the available range for phase durations and current amplitudes may vary depending on the specific capabilities of your stimulation system and the settings configured in Allego.

The charge injected during each phase of the stimulation waveform is calculated by multiplying the duration and amplitude of that phase. The total positive and negative charges associated with the selected stimulation waveform are displayed below the amplitude selectors.

To assist with charge balance monitoring, a color-coded indicator is provided. The indicator shows whether the positive and negative charges are balanced or imbalanced. Maintaining charge balance is important, especially when using microelectrodes, as it helps reduce potential electrochemical effects at the electrode surface and can extend the lifespan of the electrode.

By monitoring the displayed charges and ensuring balance between positive and negative charges, you can optimize the stimulation parameters to achieve the desired electrical effects while minimizing potential adverse effects associated with charge imbalances.

Note: Due to inherent variations in the current sources of each stimulation channel and between the positive and negative current sources, achieving perfect charge balance may not be possible, even if the ideal balance is set in the graphical user interface.

These variations can lead to slight deviations in the actual charges delivered by the stimulation system. While efforts are made to minimize these variations during system design and calibration, it is important to be aware of the inherent limitations.

First Phase Duration (D1): Time delay in µs before the second phase of the stimulation begins

Second Phase Duration (D2): Time delay in µs before the second phase of the stimulation begins

Third Phase Duration (D3): When triphasic is selected, this is the time delay in μ s before the third phase of the stimulation begins

First Phase Amplitude (A1): Level of current in μA for the first phase of the stimulation waveform

Second Phase Amplitude (A2): Level of current in μA for the second phase of the stimulation waveform



Third Phase Amplitude (A3): Level of current in μ A for the third phase of the stimulation waveform

Amplifier Settle

Amp Settle	
Enable Amp Settle	
Pre Stim Amp Settle	
0	μs
Post Stim Amp Settle	
0	µs

Stimulation can cause large voltage transients that obscure microvolt-level neural signals (e.g., spikes) directly after a pulse. The "amplifier settle" feature is used to reduce the time it takes to return amplifier output to baseline.

This function, when enabled, can be set to activate just before stimulation (usually at zero) and remain for a duration post-stimulation, with 1 ms (1000 μ s) being a typical effective duration, but users should experiment with this value to find one optimal for their electrode. Users may also choose to maintain "amp settle" throughout a pulse train or release it after each pulse.

By utilizing the "Enable Amp Settle" option and adjusting the associated parameters, users can effectively manage the impact of stimulation artifacts on amplifier outputs, ensuring accurate and reliable recording and analysis of neural signals.



Charge Recovery



The stim chip's inherent random variations in transistors result in imperfect matching of the positive and negative current sources. This practical limitation prevents the achievement of ideal charge balance. In long-term chronic experiments, residual post-stimulation charge may have negative effects. To mitigate this, the stim chip incorporates a charge recovery feature, which involves forcing an electrode to ground or another fixed voltage.

To enable charge recovery for a specific channel, users can check the "Enable Charge Recovery" box and specify the onset and duration of charge recovery after the stimulation pulse ends. The timing of charge recovery is visually represented by the green bar in the stimulation waveform display.

It's important to note that charge recovery events can introduce recording artifacts due to the application of a large current to the electrode. This current can generate voltage transients in the recording circuitry that may be significantly larger than the desired neural signals. Therefore, careful consideration of the timing and duration of charge recovery events is necessary to minimize their impact on the recorded signals.

Furthermore, it's essential to recognize that charge recovery events may not completely eliminate residual charge on the electrode. Additional techniques such as periodic electrode polarization may be required to maintain optimal electrode performance throughout long-term chronic experiments.



By understanding the implications of charge recovery events and implementing appropriate strategies, researchers can mitigate their potential adverse effects and ensure the integrity of recorded neural signals during stimulation experiments.

Enable Charge Recovery: This option allows the user to enable or disable the post-stimulation charge recovery feature. When enabled, charge recovery is initiated a specified number of microseconds after a stimulation pulse has been sent.

In stimulation protocols, it is common to use charge-balanced pulses to prevent oxidation-reduction reactions at the electrode-tissue interface. However, achieving perfect charge balance is challenging due to inherent variations in transistor characteristics across a chip. To address this issue, recovery circuits are incorporated to dissipate residual charge after stimulation pulses.

One of these circuits is the charge recovery switch, which momentarily connects an electrode to a common stim_GND pin typically tied to ground. Additionally, each channel on the chip includes charge recovery circuits that utilize small programmable currents to pull the electrodes towards a user-defined potential.

To ensure safety and monitor unintended current, the chip also features a global fault current detector that can be inserted into a common return current path, thus detecting any unintended current flow.

By providing the option to enable charge recovery and incorporating various recovery circuits, the system aims to mitigate the effects of residual charge and maintain optimal electrode performance during stimulation experiments.





In the Electrodes tab, you can configure the headstage type, probe connector package, and probe design for each port in use. It is crucial to select the correct entries in the dropdown menus, as Allego automatically generates probe mapping based on these selections.

The tab is divided into two main sections: the signal/port configuration area at the top and the probe wireframe below. The signal/port configuration area includes dropdown menus for the Port, Headstage, and Probe. There is also an option to select whether the probe spans multiple ports, and if enabled, it will show sites from all ports.

It is important to accurately configure the signal and port settings to ensure proper probe mapping and accurate recording of signals.



Headstage

To select the appropriate headstage entry from the Headstage menu, you need to determine the connector type used by your chosen probe design. You can find this information in the last part



of the probe design code.

For example, let's say you are using a chronic Smartlink headstage with a probe design code of "A1x16-5mm-25-177-H16_21mm." In this case, the connector type is "H16," as indicated by the "H16" at the end of the code. Therefore, you should select the entry "Chronic Smartlink H16"" from the Headstage menu.

Similarly, you would choose the corresponding probe design from the Probe dropdown. In this example, the probe design to select would be "AA1x16-5mm25-177."

By correctly identifying the connector type and selecting the appropriate headstage and probe design entries, you ensure that the system is properly configured for your specific setup.



If you are using a headstage adapter to connect the probe, you can handle this within the Headstage dropdown menu as well. For example, let's say you are using the acute version of the probe with the code "A1x16-5mm-25-177-A16" and connecting it to the chronic Smartlink 16 headstage using the headstage adapter "Adpt.A16-OM16." In this case, you should select the entry "adaptor_A16_OM16" from the Headstage menu.



The headstage adapter files are designed to account for the mapping changes that occur due to the presence of an adapter, so no additional action is required from the user. The system will automatically handle the mapping accordingly.

In the Headstage dropdown menu, you will also find several entries named "Passthrough". These entries provide a generic 1:1 channel-to-site pinout, which can be used when the specific mapping based on NeuroNexus parts is not required. This can be useful if you have connected an EIB/breakout board to the headstage where the site numbers and their locations on the substrate are not necessary.

If you cannot find the necessary probe design or headstage entry in the dropdown lists, it is recommended to contact NeuroNexus support with the exact design code or part number. They will be able to assist you in adding the required entry to the system.

Probe

The Probe dropdown menu contains a list of NeuroNexus probe designs. The probe design codes are listed without a connector type because the connector information is handled within the headstage menu.

In addition to the specific NeuroNexus probe designs, there are also generic probe wireframes available in the list, indicated by "default_...". These options should be chosen when working with non-NeuroNexus probes or other nonstandard or custom devices that do not require channel remapping.

If you cannot find the necessary probe design or headstage entry in the dropdown menus, it is recommended to reach out to NeuroNexus support with the exact design code or part number. They will be able to assist you in adding the required entry to the system.





Probe spans multiple ports

In the Electrodes tab, there is an option called "Probe spans multiple ports" that is always available. This option is specifically designed for configurations where a single probe is connected across multiple ports or when an adapter splits one cable and headstage across multiple ports.

When you check the "Probe spans multiple ports" checkbox, Allego allows you to select probes and headstages with a larger channel count than what was detected on the port.

This option enables you to configure Allego for setups that involve probes spanning multiple ports or adapters that split the connection across multiple ports, providing greater flexibility in channel selection and configuration.

Show sites from all ports

When you enable the "Probe spans multiple ports" option, Allego allows you to select probes and headstages with a larger channel count than what was initially detected on the port. This means that the probe may have sites that extend beyond the channels detected on a single port.



To accommodate this configuration, the "Show sites from all ports" option becomes available. By selecting this option, Allego will display the sites from all connected ports in the probe wireframe, giving you a comprehensive view of the entire probe layout and channel distribution across multiple ports.

This feature is particularly useful when working with probes that span multiple ports or configurations involving adapters that split the connection across multiple ports. It allows you to accurately visualize and configure the electrode sites across all connected ports within Allego.

Use Local Devices

Upon startup, Allego checks online for updated probe and headstage files. However, this option adjacent to the Probe dropdown menu tells Allego to disregard the files stored online and to use locally stored files only. This can be useful for users who request custom mapping from Neur-oNexus.



Probe Wireframe

The wireframe section offers a precise geometric layout of the chosen probe design. It provides comprehensive information regarding the relative positions of electrode sites, combining geometric data with recorded neural signals in Allego. This integration unlocks a multitude of analysis options that are both swift and effortless, including the utilization of the Heat Map feature found within the Monitor and Signal Metrics tabs. Moreover, the Monitor tab offers various tools for manipulating the wireframe and altering the display of sites and their corresponding voltage traces. These tools include Select, Order, and Sort By Coordinates.



Once a tool has been chosen, the application or removal of its effect on the desired electrode sites can be controlled by clicking the +/- buttons. The toolbar located in the upper right corner of the probe wireframe, as depicted in the accompanying image, provides various site selection methods and additional functions.



The box selection tool is enabled by default, while additional options such as zoom, pan, lasso selection, and the home button are also accessible to reset the view to its default setting.



Site Selection Tool

The Select tool empowers users to include or exclude specific electrode sites and their signals from being shown in the Monitor. To hide voltage traces of particular channels, make sure to select the Select tool and click the "-" buttons, then click and drag over the sites that need to be removed from visualization.



To restore electrode sites and their signals that were previously removed from visualization, simply click on the "+" button. This will allow you to add them back into the display.

Order Tool

The Order tool has an impact on the positioning and appearance of sites and traces of interest within the Monitor tab. Similar to the Selection tool, you can modify the order and remove ordering from electrode sites using the same method. Once the Order tool is applied to the desired



electrode sites, both the sites and signal traces undergo a color change, they are grouped together, and moved to the top of the display in the Monitor tab.

Subsequent applications of the Order tool will result in a different color, and selecting the "-" button will restore the sites and traces to their default color and return the traces to their original location on the Monitor display.



When a site is selected on the wireframe diagram, its size will dynamically change to indicate the selection and facilitate audio playback. This visual transformation provides a clear visual cue that the site has been chosen for audio playback.





Sorting by site coordinates offers a convenient method to swiftly organize sites based on their physical placement on the chosen probe design. This tool also incorporates color coding for both the sites and signal traces, depending on the sorting criteria. For example, sorting by X values assigns a distinct color to electrode sites on each shank. Sorting by Y values results in different colors for electrode sites at each depth. It's worth noting that the Z dimension remains unused in standard probes, but it may be utilized in specialized designs such as the 3D Matrix array or custom configurations.



V Signal Processing

The Signal Processing tab is the designated section where you can define, apply, and remove individual filters as well as filter groups. It serves as the central hub for managing various signal processing operations.

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Filter Stages

Filter groups can be applied to a signal group at three different stages:

- Hardware Stage: Filters applied at the hardware stage are implemented at the preamplifier chip level. These filters impact all recorded data and signals visualized in the Monitor tab. They are applied directly to the incoming signals before they are processed or visualized.
- **Data Stage:** Filters added to Data Stage affect both visualized and recorded data. However, instead of operating at the hardware level, these filters are applied once the data has passed through the recording system and has been received by Allego. The filters are implemented in the software and modify the signals after they have been acquired.



• Visualization Stage : Filters applied at Visualization Stage do not alter recorded data. They only impact the signals visualized in the Allego Monitor tab. If only Software Stage 2 filters are used, the data files will remain unfiltered, but the visualization within the Monitor tab will reflect the applied filters.

By understanding the distinction between these three stages, you can effectively apply filter groups to the desired level of the signal processing pipeline in Allego.



Filter Types

Allego provides five different filtering options that can be applied independently or in combination at any stage. Here is a summary of each filter type and their valid frequency ranges:

- Lowpass: This filter allows signals with frequencies below the user-defined threshold to pass through while attenuating signals with higher frequencies. The valid range for the low-pass filter is 1-300 Hz.
- **Highpass:** The highpass filter passes signals with frequencies above the user-defined threshold and attenuates signals with lower frequencies. The valid range for the highpass filter is 300-5000 Hz.
- **Bandpass:** The bandpass filter passes signals that fall within the user-defined frequency range and attenuates all other frequencies. The minimum frequency is 0.1 Hz, and the maximum frequency is 20 kHz.
- **Bandstop:** The bandstop filter attenuates signals that fall within the user-defined frequency range and allows all other frequencies to pass through. The valid range for the bandstop filter is from 1 Hz to 20 kHz.
- Notch: The notch filter attenuates signals specifically at the user-defined frequency. The valid range for the notch filter is from 50 Hz to 60 Hz.



Note: While the headstages in Allego support frequencies as low as 0.1 Hz, it is recommended to set a minimum of 1 Hz to correct for low-frequency amplifier noise.

IIR Filters	
Lowpass	O Bandstop
O Highpass	O Notch
O Bandpass	

Reference Types

Allego offers different reference types that can be used in data analysis and signal processing:

- Common Average Reference: This referencing technique eliminates noise that is common across all recording channels. It involves subtracting the average of all channels from each individual channel. By doing so, the common noise components are removed, enhancing the clarity of the signals.
- Virtual Reference: With virtual referencing, the user can choose a specific recording site to act as the reference channel. The selected reference channel is then subtracted from each channel in the data. This technique allows for more flexibility in selecting a reference and can be beneficial when there is a particular reference site of interest.
- **Paired Reference:** In paired referencing, the user selects a reference channel and a target channel. The selected reference channel is subtracted from the selected target channel. This type of referencing is useful when comparing specific channels or investigating the relationship between two channels.

These different reference types in Allego provide options for removing common noise or specifying reference channels for analysis, enabling researchers to effectively analyze and interpret their data.





Preset Filters

Preset filters in Allego offer a convenient way to apply custom filters for different types of signals quickly. You can utilize preset filters to process your live streaming data, retaining specific portions of the frequency spectrum while rejecting unwanted portions.

To apply preset filters, follow these steps:

- Select a port or channel from the dropdown menu. This specifies the target for applying the preset filters.
- Click on the button corresponding to the chosen signal type. Each button represents a different preset filter set tailored for specific signal characteristics.
- The selected filter set will be applied to the chosen port or channel. You will see the applied filters displayed in the filter group section, located near the top of the page. This provides a visual representation of the active filters and their order of application.

By using preset filters in Allego, you can streamline your signal processing workflow and efficiently manage different types of signals by quickly applying predefined filter sets to specific ports or channels.



Configure

To define and add filters in Allego, follow these steps:



- Select the desired stage (Hardware, Software Stage 1, or Software Stage 2) where you want to apply the filter.
- Choose the filter type (e.g., Bandpass) from the available options.
- Fill out the fields provided in the filter configuration box. The fields will vary depending on the filter type selected.



For Lowpass, Highpass, Bandpass, and Bandstop filters, you need to define the frequency thresholds based on your requirements.

For the Notch filter, you need to specify the notch bandwidth, which determines the range of frequencies that will be attenuated. The default value for the Notch Bandwidth field is 2, meaning that if the notch filter frequency is set to 60 Hz, the effective frequency range being attenuated will be 59-61 Hz.

Configure					
Notch Filter					
Label	Channels to filter	Frequency	Notch Bandwidth		
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Once you have configured the filter parameters, click the "Add Filter" button to apply the filter to the selected port.

In the filter group, next to each filter, you will find three buttons:

The edit button allows you to modify the properties of an existing filter in the group.

The arrow button is used to change the order of the filters within the group. Filters are applied in the order they are displayed.

To remove a filter group that has been applied to a signal group, click the "remove" button for each filter in the group.

By following these steps, you can define and add filters to the desired stage and customize their parameters in Allego's Signal Processing tab.




The Monitor tab is used to view electrical signals for connected probes, in addition to any peripheral device activity on the recording system's digital and analog inputs. Most of the space in this tab is used to show the received signals and traces, and at the top and bottom are several options to manipulate how they are displayed.

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Top Bar

At the top of this tab are the Open Event Viewer, Send GPIO channels to top, Show Monitor DSPs, Grid On/Off, Disable Cursor, Disable Volume, Help, and Float buttons.







Send GPIO channels to top

General purpose in/out channels are displayed below neural channels by default. Toggling this option will bind the GPIO channels to the top of the Monitor display, keeping them in view and on top of the neural channels.

Show Monitor DSPs

Software Stage 2 filters set in the Signal Processing tab are only applied to data being visualized while recorded data is raw. Toggling this option temporarily disables these filters in order to allow the user to view raw signals without removing the SS2 filters.

Grid On/Off

Turning this feature on will display vertical lines in Monitor, splitting the display into 10 sections.

Disable Cursor

This hides or shows the vertical green line indicating the current position where new data is being plotted.

Disable Volume

This will enable or disable audible spiking activity when a neural data channel is highlighted or bound to an analog out channel.

Configure Spike Threshold

This button will open the Spike Detection Settings window which allows you to set the positive and negative thresholds directly, and is only relevant when online spike sorting is turned on.



Melp

This button opens a link in browser to the relevant page in the Radiens online documentation.



The Float button will open the current tab in a new window.

Viz. Bandpass

The Monitor tab allows you to quickly set visualization bandpass values directly into the fields in the top-right corner of the tab. The filter set here will only impact visualized data and not the data stored to disk. Click on the trash can to remove the filter.

Viz. Bandpass 🛛 🗍 🗇

Voltage Traces

Beneath the Stream Position section is the main area of the Monitor tab where voltage traces will be displayed once data is being streamed. The port letter and probe site number are shown to the left of each trace.



In order to see how the site numbers and amplifier channels are related, please refer to the "Signal Map" section in the System tab.



Note: In the signals area of the System tab, some or all channels can be selected or deselected. All voltage traces will be enabled and displayed by default, and there are several ways to change which ones are displayed. Also, in the Electrodes tab, the Select tool can be used to achieve the same result. Individual voltage traces can be opened up for a more detailed view by double clicking on them. Thresholds and scales for that specific channel can be changed here as well.

Bottom Bar

X-Y Plot

Plot type (X-Y) Plot

The Plot type selection determines how data is displayed in Monitor. By default, X-Y Plot is selected which shows data in waveforms. The heatmap view shows vertical hashes with a color scale to indicate the strength of signals being streamed.

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Heatmap: The Heatmap is a useful option that replaces the voltage traces with a heatmap display which may make it easier to identify more active channels. This uses a 150 μ V amplitude as a default maximum value.





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Frequency scale

The Frequency scale shows two values: The selected sample frequency of the acquisition system (set in the System tab), and the plotted sample frequency in the Monitor. The plotted sample frequency is affected by the Window (s) selection in the View Settings menu. The values shown here are relevant to the View Modes options discussed below.

Units Fields Preset views (Units, Fields)

The Units preset button sets configures the Monitor in a view useful for single and multi unit recordings. Fields is intended for use with LFP, ECoG, and EEG streaming.

Spikes

View Modes

The Monitor does not plot every sample collected. There are three view mode options that impact how signals are shown:

- Raw: Signal is down sampled through simple decimation. For example, if the system is set to a sample frequency of 30 kS/s and the Monitor is set to a 5s window which has a 1 kS/s plotted sample frequency, every 30th sample is plotted while the rest are not displayed.
- Spikes: Signal is down sampled in a way that preserves high amplitude events that may be lost by decimation in Raw mode.
- Anti-Alias: Applies a lowpass filter with the upper bound defined as the Nyquist frequency (plot sample freq/2) prior to downsampling for Monitor. The signal is then resampled at the current plot sample frequency.

AOut Controls

AOut Controls

The options listed here will change the view of Monitor with respect to the analog out channels.

- Restore can be used after selecting the Zoom option and will return the Monitor to its previous configuration.
- Zoom will center the view on any neural channels bound to the analog outs.
- Clear will unbind any neural channels from analog output channels.



• Measure 0 µV Measure tool

The Measure tool is a method to quickly approximate the amplitude of a given signal in Monitor. After clicking Measure, click and drag over a signal of interest and an approximate value in μ V will be shown in the adjacent box.

Chan height (px) 20

Channel Height

The height of a streaming channel typically refers to the vertical dimension or size of the graphical representation of a recorded or streamed electrical signal on the software's display. The unit of this metric is pixel. This feature allows researchers to customize the display to focus on specific aspects of the signal or to accommodate different recording conditions.

AMP Range (μV)
 300

Amplitude Range (μV)

The amplitude range represents the voltage span covered by the channel. It is typically expressed in microvolts (e.g., $\pm 100 \ \mu V$) and indicates the minimum and maximum voltage values displayed on the y-axis.

• AIN range (V) 3

Analog IN Range (mV)

Analog IN Range (mV) specifies the minimum and maximum voltage values in millivolts (mV) that the system can accept without distortion or damage.

• Window (s) 5

Clip

Windows Duration (S)

Windows Duration represents a period of time, measured in seconds (S), during which the streaming signals are monitored.

Signal Clipping

If the recorded signal exceeds the defined amplitude range, it may result in signal clipping, where portions of the waveform are cut off or truncated. This can lead to data loss and distortion.



Show Thresholds Show Thresholds

Toggling this on will result in the positive and negative thresholds for real-time spike sorting to be shown as blue and green lines respectively on each neural channel shown in the Monitor view.

Additional Features

Neural Channel Scale

On the top neural channel, a scale is shown next to the channel label. This value changes based on the selected AMP range in the View Settings menu.

Bind Neural Data Channel to Analog Out

Clicking on a neural channel waveform will bind this channel to AO 1 and play signal activity through the left audio channel. Shift-clicking will bind the channel to AO 2 and play activity through the right audio channel.

Spike Plot

The Spike Plot pop-out is a quick way to visualize activity on a single voltage trace. The Spike Plot can be accessed by double-clicking on the trace of interest, and various options to change how spikes are determined and displayed are available. This is only show activity if real-time spike sorting has been enabled. The options in this plot are covered in





detail in the Spike Grid section of this guide.

Single Channel Scope View

CTRL+double-clicking a channel will open a new window showing the signal for a single



channel.



• Event View

New Event Viewer feature is accessible from toolbar of Monitor tab.





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The Impedance tab allows the user to measure the impedance characteristics of recording sites on connected probes. This uses the Intan RHD chip's testing method to check probe impedance values using a frequency of 1 kHz. This method is useful for verifying probe integrity and can be performed anytime the probe is connected.

In this tab, the same probe wireframe that is customized from the Electrodes tab will be shown here. To run the test, select the appropriate port from the dropdown menu in the upper left corner of the Impedance tab, then select Magnitude or Phase from the Field section as shown below and press the "Run" button.



Depending on the channel count of the probe being tested, the results may take up to 30 seconds to appear.



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Note: Currently impedance check runs on port A only.



HD Snapshot

While the real-time signals are displayed in the Monitor tab, it is downsampled data that gets used to plot the waveforms at the resolution shown. The HD Snapshot module provides the user with a way to visualize signals using all collected data points from recorded data, producing a highly accurate representation of a signal for closer inspection. Some recently streamed data that has not been saved can be visualized here as well.



There are two main areas to the HD Snapshot tab. The small section at the top of the window lets the user select the starting time stamp in the stream and the duration of the signals that will be drawn. The arrow buttons below the Start Time text box will rewind or advance the Start Time by the selected duration. The available window displays the segment of time available for this feature, with a maximum of 15 seconds.

Plotting Waveforms and Display Options

Once a Start Time and Duration have been set, hit the View Snapshot button, and the signals will be output in the large area below. By default, these traces will be each be displayed separately on a grid and only a subset of them can be seen simultaneously on the same screen. By selecting the Condensed option, the traces are stacked in close proximity and can all be seen on



the same screen. Moving the cursor over any point on these lines will display the exact time stamp and amplitude of the signal. The values available in the Duration dropdown menu are determined based on the number of channels being displayed and the sampling frequency. If the values in the Duration dropdown are not long enough, consider deselecting sites that are not of interest within the System or Electrode tabs. Since some resources are shared, this will allow the system to utilize more data points and draw longer time segments due to fewer channels that need to be generated in high resolution.



Above the waveforms are some details about the signal group, the selected ranges on the traces, and some other specifics related to the output. The Scope Frame displays how many signals were drawn, and the total number of signals available in the selected Signal Group. Also shown is the sampling frequency, the number of samples used to draw the waveform, and minimum and maximum values of the amplitude in the selected time frame.



Waveform Display Tools

The waveforms generated in this tab can also be manipulated in a number of ways using the toolbar in the top right of the waveform area. The view can always be reset to its original state by double clicking anywhere on the waveforms.



The available tools are Download Plot as a png, Zoom, Pan, Zoom in, Zoom out, Autoscale, Reset axes, Toggle spike lines. Toggle Spike Lines will turn off or on the horizontal and vertical ruler lines to assist with comparing peaks.



Ill Signal Metrics

The Signal Metrics tab is used to monitor and visualize the real-time activity of signals streaming from the connected probes. The probe wireframe selected in the Electrodes tab will also be shown here and by choosing different parameters, the neural activity of all recording sites will be monitored in real-time in heatmap style. The color of the sites is determined by the magnitude of the parameter that is selected.



Signal Metrics Configuration

At the top of this tab are the Port and Metrics dropdown menus.



The **Metric** dropdown presents different voltage parameters including Absolute, Max, Min, Mean, Std Dev, variance and signal to noise ratio.

Note: The signal to noise ratio (S/N ratio) is used to compare the level of a desired neural activity to the level of the background noise. It is important to consider that the S/N ratio values will



depend strongly on the specific spatiotemporal characteristics of neural activity and structure of background noise, which vary across brain areas and electrophysiology setups.

The **Port** dropdown allows the user to select which port is currently active in the Signal Metrics tab.

The **Live Update** option updates the probe site activity and metrics values every 2 seconds, and the **Show Table** option allows you to list all the metrics' values in the table below the probe wire-frame.

Custom scale toggle enables the custom scale range of the heatmap scale.

The **Refresh** button (^{C)}) updates all values for the selected parameter from the Metric section.

If "Show Table' toggle is selected, at the bottom of the tab, all signal metrics are available in the table, and can be sorted by column using the three-bar button () or get exported to CSV or PDF format by selecting the Export button ().

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뇨			110.6	52.9	204.2		0.0	984.0	0.0	10.3	6.9	15.8	92.6	-110.9	115.6
Z		148.7	521.8	213.8	636.7		56.0	27350.0	280.0	286.7			45.0	-74.4	80.2
â		69.4	116.7		180.0			1147.0	20.0	12.0	9.8		115.9	-128.3	132.1
		200.6	667.1	279.8	802.1	25.5	60.0	28770.0	300.0	301.6			52.9	-63.8	70.0
alte		66.4	101.1	119.6	154.9			1470.0	15.0	15.4	9.6	20.3		-148.3	154.6
#		247.5	882.5	347.8	1021.8		62.0	29543.0	310.0	309.7			59.5	-88.8	93.6
≁		440.2	1129.6	550.5	1263.2	42.4	63.0	30031.0	315.0	314.8			69.1	-98.6	107.6
		26.1	39.8	51.9	65.4		0.0	40.0	0.0	0.4	12.0	19.2	104.4	-126.5	134.5
Θ		128.2	149.5	190.9	272.8	18.9	56.0	26874.0	280.0	281.7			44.2	-48.6	53.7
		439.2	550.8	502.0	619.3	25.5	55.0	26832.0	275.0	281.3			41.4	-69.7	75.0
		135.1		228.3	412.0	26.2	61.0	29028.0	305.0	304.3			52.0	-64.2	71.2
		143.0		212.8	251.5	19.6	56.0	26745.0	280.0	280.3			41.4	-48.5	53.9
		375.8	479.7	488.2	590.0	24.7	58.0	28430.0	290.0	298.0			49.8	-62.2	68.2
		26.0		50.5	61.9		0.0	35.0	0.0	0.4		13.8	78.3	-92.3	96.7
		175.4	202.7	226.8				2747.0	35.0	28.8		14.6	72.4	-178.6	180.4
			207.8	178.0	287.7	20.1	57.0	27519.0	285.0	288.5			43.5	-52.0	56.7
														0 III	



Spike Grid

The Spike Grid is the module that allows users to visualize detected spikes across all selected channels. The user can define positive and negative spike thresholds for individual channels. Similar to other modules, the window consists of two main areas: The top bar with various settings, and the main area with pile plot boxes for each selected channel. Deselected channels will not be shown in this tab.



The top bar contains the Lookback Window, Update Period, Sorting, and Spike Grid settings.



Lookback Window: This field indicates how long spikes will remain shown in the pile plot boxes and has a default value of 5 seconds.

Update Period: The Update Period determines how frequently spikes are drawn in the pile plot boxes.

Sorting: Activate this button to sort the electrophysiological signals.



Probe layout: Toggle this button to align the grid with the arrangement of sites on the probe shank.

Spike Detection Settings:

The gear icon at the top of this tab is designed to configure spike detection settings, and it opens the window shown in the image below. The same window can also be opened from the Spike Grid tab, the next module in this manual. From here, spikes can be detected by setting the appropriate voltage threshold or a fixed multiple of the standard deviation of the voltage signal on a given recording channel in positive and negative values. Keep in mind, if the threshold is too strict, low amplitude spikes will go undetected, which may lead either to missing entire single units, or to losing a fraction of spikes from the units with mean spike amplitude relatively near the detection threshold. Conversely, if the detection threshold is too low, more noise and non-sortable spikes will cross threshold and lead to decreasing detection of larger spikes.

Spike D	etection Setting	js											
PORT	THRESHOLD TYPE	POSITIVE T	THRESHOLD	NEGATIV	E THRESHOLD	PRE	-THR	POS	ST-THR	SH	woow	Y MIN	Y MAX
۸	Voltage ~	120.00	μV 🔽	80.00	μV 🔽	1	ms	2	ms	1	ms	μV	μν
В	Voltage ~		₩ 🗆		₽V 🗆	1	ms	2	ms	0	ms	μV	μV
с	Voltage ~	120.00	μV 🔽	80.00	μV 🔽	1	ms	2	ms	1	ms	μV	γų
D	Voltage ~		₩ 🗆		۳۸ 🗖	1	ms	2	ms	0	ms	μV	μV
													SAVE

- Threshold Type This determines whether spikes are detected using voltage or standard deviations
- **Positive/Negative Threshold:** Set one or two thresholds to determine what signals qualify as a spike
- **Pre/Post-Threshold Window** The pre and post thresholds indicate how much of the signal is shown before and after the spike occurs
- Shadow: The length of time that a port will wait before another spike can be detected
- Y Min/Max: Scale of the Y axes

At the bottom of this tab, are zoom buttons to change how many pile plots can be displayed at once.



Spike Plot

Individual spike plots can each have their own thresholds set. This window is accessible by double clicking on each individual site in the Spike Grid window. The blue line indicates the current positive threshold, and the green line is for the negative threshold.







The Spike Sorter is the module that allows users to extract single-unit data from their recording. Similar to other modules, the window consists of two main areas: The top bar with various settings, and the main area with overall plots for electrode site and neuron quality.



The top bar contains a dropdown menu for the Update Period, Sort Time Range, Sorting, Rebase, Clear, and Spike Sorting settings.

l	Jpdate Per	iod	Sort Time Range					
	l sec	~	29.41, 636.80	-√r Start	5 Rebase	🗵 Clear	ϕ Initialize	View Sort Details

Update Period: The Update Period shows the dashboard refresh period.

Sort Time Range: The Sort Time Range shows the time duration of data being processed by the spike sorter.

Rebase Button: Rebase button prompts the system to calculate new spike templates for all sites.



Clustering Button/Dropdown:

- Disable Clustering: Turning this on will disable clustering and the labeling of neurons. Spikes will still be captured and labeled. By extension, this also means that when sorting is active and clustering is disabled, spike waveforms and traces in tabs like Spike Grid and Raster Plot will be in grayscale instead of colored, as a given color of these features indicate a neuron is detected on neighboring sites. This feature is disabled by default when total number of channels detected is greater than or equal to 512.
- Feature Dimension: Determines the complexity of the input used in feature extraction by specifying the dimensionality of the waveform representation used to characterize each spike.
- Position Influence: Controls the extent to which the spatial location of each spike affects its classification into distinct units.
 - Higher Values: Makes the clustering process more sensitive to differences in spike locations, leading to a higher likelihood that spikes from different positions are identified as separate units.
 - Lower Values: Allows spikes from nearby locations to be clustered together more easily, even if there are slight variations in their positions.
- Waveform Influence: Controls the extent to which the shape of each spike affects its classification into distinct units.
 - Higher Values: Emphasize waveform characteristics more strongly, making the clustering process more sensitive to differences in spike shapes. This increases the likelihood that spikes with varying waveforms are identified as separate units.
 - Lower Values: Reduce the emphasis on waveform features, allowing spikes with similar overall patterns to be clustered together more easily, even if there are subtle differences in their shapes.
- Sort: Begin a new sort with the above parameters.

Clear Button: Clear button clears all spikes from the memory cache.

Initialize Button: Resets all spike detection parameters to default and clears all spikes from memory.

View Sort Details: Neural Interface window shows the summary of spike sorting data, sites, neurons and general data about sample frequency and total dataset size in tabular format.



Neural Interface General			
III COLUMNS 👳 FILTE	rs 🗮 density 🛃 expor		
Attribute	Value		
Time Range	3.008,92.8064		
Total Spike Count	25849		
Neuron Count			
Total Available Sites			
Enabled Sites			
Sample Freq	20000		
Dataset Size	5777552		
Persistence	20		
Dataset Uid	null		
Provenance Uid			
		Rows per page:	50 1–10 of 10

Spike Detection Settings: The Spike Detection Settings window should be used to set baseline parameters for all channels on each port. This is the same window that can be configured from the Signal Metrics tab.

Spike de	tection settings												
PORT	THRESHOLD TYPE	POSITIVE	THRESHOLD	NEGATIV	E THRESHOLD	PRE-THR	WINDOW	POST-THE	WINDOW	EVENTS	HADOW	Y MIN	Y MAX
A	Voltage ~	120.00		80.00									
в	Voltage ~		μv 🗆		μν 🗆								
с	Voltage ~		μν 🗆		μν 🗆								
D	Voltage ~		^{μν} 🗆		μν 🗆								
													SAVE

- Threshold Type This determines whether spikes are detected using voltage or standard deviations
- **Positive/Negative Threshold:** Set one or two thresholds to determine what signals qualify as a spike
- **Pre/Post-Threshold Window** The pre and post thresholds indicate how much of the signal is shown before and after the spike occurs
- Event Shadow: The length of time that a port will wait before another spike can be detected
- Y Min/Max: Scale of the Y axes

In the main area there are overall plots of SNR, Noise, Neuron Yield, and Sort Efficiency located on the right of the screen.





SNR: SNR plot shows the average signal to noise ratio of all sites on each port in the current session.

Noise: Noise plot shows the average noise level of all sites on each port in the current session.

Unit Yield: Unit Yield plot shows the average number of neurons detected persite on each port in the current session.

Sort Efficiency: Sort Efficiency plot shows the average number of labeled spikes divided by the total number of detected spikes for all sites on each port in the current session.

At the left side of the main area, Elapsed Time, Units, Labeled Spikes, Active Sites, and Probe Yield are shown.

Elapsed Time:	275
Units:	61
Labeled Spikes:	40212
Total Spikes:	78452
Active Sites:	28
Probe Yield:	0.875

Elapsed Time: Elapsed Time shows sorting session duration.

Unites: The number of units sorted this session.

Labeled Spikes: The number of spike waveforms sorted this session.

Total Spikes: The number of spike waveforms processed this session.

Active Sites: Active sites shows the total number of probe sites that are detecting units.



Probe Yield: Probe yield is defined as a fraction of active electrodes that recorded neural activity during each recording session.

At the top left of the main area of the Spike Sorter tab, there are three box and whisker plots related to SNR, Noise, and Neuron Yield.

SNR whisker plot: SNR plot shows SNR statistics of all sites on each port plotted as a box-and-whisker plot.

Noise whisker plot: Noise plot shows noise statistics of all sites on each port plotted as a boxand-whisker plot.

Unit Yield whisker plot: Unit Yield plot shows Neuron Yield statistics of all sites on each port plotted as a box-and-whisker plot.



At the bottom of this tab, there is a color coded table of site Activity Panel measures as shown below.



At the bottom right of Site Activity Panel, Unit Activity Panel is presented a color coded table of Spike Rate, and SNR measures.





BBB Raster Plot

The most important information about spikes or action potentials is timing and frequency of firing. The Raster Plot is a simple way to display spiking activity of a group of neurons across all active channels over time. This module has a layout similar to the Monitor tab, but in order for activity to be displayed, spike sorting must be configured and turned on.

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The top bar in this module contains the single setting "Sorted Only" along with other common buttons like Download Image, Help and Float Tab. Toggling on the "Sorted Only" option will result in only sorted and classified hashes from being shown in the Raster tab. Turning this off will show unsorted spikes as well which are gray in color.



Like the other plotting windows, there are several functions that can be used within the Raster plot. Single click one of the traces to hear spiking activity through the left audio channel, and shift-clicking another will play the activity through the right audio channel



Double-clicking one of the channels will open an individual spike plot, where the waveform can be seen and the Y-scale and spike thresholds can be adjusted. The blue line indicates the current positive threshold, and the green line is for the negative threshold. Look back window indicates how long spikes will remain shown in the spike plot boxes and has a default value of 5 seconds. The Update Period determines how frequently spikes are drawn in the pile plot boxes.

Lastly, the length of time displayed in the window can be adjusted in the Sec/Window dropdown in the bottom right corner of the tab.







The Allego 3D Model is a powerful and unique way to visualize the recording activity of the implanted electrode in a 3D reconstruction of the brain in real time. Using this module, the probe design being used for the experiment can be positioned and the activity of the surrounding neurons can be visualized within a 3D brain of the chosen animal model.



At the top of this tab, the Port dropdown allows the user to select which port is currently active. Species dropdown presents different animal models of mouse, rat, and macaque.



Hide Atlas Panel: You can toggle the "Hide Atlas Panel" button to hide the atlas slice that is located on the right side of the window.

Listen to Pathway MPM: To integrate trajectory planning of Multi Probe Manipulator software and Radiens Allego via an HTTP server, please toggle the "Listen to Pathway MPM" button.



Note: All the current models are based on an atlas viewer from <u>Bakker et.al, 2015, Neuroin-formatics 13, 353-366</u>.

- For the mouse atlas template, adult male, 56-day old C57BL/6J Mice (Jackson Lab) are used (https://doi.org/10.1038/nature05453)
- For the rat atlas template, adult male, 80-day old, W397.6 g Sprague Dawley rats (Charles River) are used (https://doi.org/10.1016/j.neuroimage.2014.04.001; https://-doi.org/10.1016/j.neuroimage.2014.12.080; https://-doi.org/10.1016/j.jneumeth.2014.11.005)
- For the macaque atlas template, young to middle-aged male adults, 6.2 to 10.5-year old are used (https://doi.org/10.3389/fninf.2012.00027)

The 3D model tab contains two views of the brain model, with the 3D image on the left, and an atlas slice showing the regions on the right. The black dot shown in the slice indicates the current location of the tip of the probe.

Both views can be changed using controls that are common to most CAD applications. Click and hold the left mouse button to revolve around the center of the model, click and hold the right button to move the camera, and use the middle mouse button or mouse wheel to zoom in or zoom out. Also, if you hover the mouse over the brain slice region, you can find the name of the region displayed below the slice.

To the left of the brain model views, there are areas where the stereotaxic coordinates, probe angle, and other display settings are found.



Chronic SmartLink H32 - 5mm50-177	Alx32-Edge-
Probe Position from Breg	jma (mm)
ML	2
АР	0
DV	2.7
Probe Angle (degrees)	
Posterior angle (ø)	0
Axial angle (γ)	0
Lateral angle (y)	-15
Reset Probe Position	

- The top of this section shows the currently selected headstage and probe devices chosen from the Electrodes tab (Chronic SmartLink H32 and A1x32-Edge-5mm-50-177 respect-ively)
- **Probe position from bregma**: To locate the area of the implantation, you need to enter the probe's Anterior-Posterior (AP), Medial-Lateral (ML), and Dorsal-Ventral (DV) stereotaxic coordinates relative to Bregma and midline. As the probe position values are entered, the black dot in the slice view will move to that location for verification of the probe position and orientation.
- **Probe angle:** If the probe is inserted into the brain at an angle, the value can be added in the probe angle section. Enter the Lateral and Posterior angles of the probe per the stereotax and enter the Axial angle to rotate the direction the electrode sites point during insertion. Once the angle values are entered, the probe will adjust to that insertion angle.
- Site signal metrics: "Site Signal Metrics" you can select the metric of interest and by enabling the slider button during the streaming, the color of the sites on the probe model



will change from gray to a color gradient indicating the magnitude of the selected metric.

Site Signal Metrics	_
Metric	
Abs(max-min) ~	

Some of the available metrics are max (abs), abs (max-min), RMS noise, event rates, SNR and others. Upon enabling the slider button in the signal metrics section, a scale will appear between the two brain views.

Max (abs)
Cumulative Max (abs)
Abs(max-min)
Cumulative Abs(max-min)
Abs(max-min) Amplified
Cumulative Abs(max-min) Amplified
Noise
RMS
Number of Events
Cumulative Number of Events
Event Rate
Average Event Rate
Signal To Noise
Average Signal To Noise
Event Average Max
Event Average Min
Event Average Max (Abs)

Also, by activating the spike sorting slider from the spike sorting tab and defining appropriate spike threshold values in the spike detection setting window, the number of neurons detected by each site and the frequency of spikes detected from each neuron can be tracked on the 3D model.



In this case, each sorted neuron is represented as a sphere projected outwards from the probe model.

The frequency of spikes from a given neuron is indicated by the size of its sphere. A sphere roughly the same size as the probe site indicates low activity, while a large sphere indicates high activity.

The last set of options on this tab are for the 3D model only, and they allow the user to show or hide the brain volume, atlas slice, and the probe model. The level of opacity can be also set for each.

Brain Volume	-
Opacity	20%
Atlas Slice	-
Opacity	100%
Probe	_
Opacity	100%





The Neurons tab is used to monitor and visualize the activity of detected neurons. To use this tab, the spike sorter must be switched on.



Analytic Dropdown: determines which spike train analytic is visualized

• Interspike Interval (ISI): The time intervals between consecutive spikes in the firing pattern of a neuron. ISIs are visualized a single histogram for each neuron, which is labeled with the channel index it's found on, and a subscript indicating the index of the neuron on that channel (in the case there are more than one neurons on a single channel).

Order: Determines how the neurons are ordered on the page (always descending). Options are:

- Channel
- Spike Rate
- SNR

Top Bar





(from left to right) -> Bin settings, clear history, decrease plot size, increase plot size:

• Bin settings menu:



- Bin number: Number of bins in each histogram
- Bin range: Start and end point for the range of histogram bins
- Note: Interpolation is performed if necessary
- Clear history: Clears spike history causing the analytic to be accumulated from this point forward
- Decrease/Inc plot size: Adjusts size of plots keeping aspect ratio the same

Pagination: Found at the bottom of the tab, this allows navigation to pages of neurons not currently visible.



Settings

The Settings tab is where data recording location and several other options are located.

Acquisition Settings

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	0) Recording Dur. 0s 🕚 Streaming Dur: 1479.4s		\$ 7 # C	RECORD	STREAMING & ~	
>	5	Settings ×					ī
		28 Acquisition Settings					peda
\$		Data Recording Location	Base Recording File Name				nge
-		C:Usersijadamsi/rada/data	aliego				X
_		Include auto timestamp in file name					
V		Record data from each port into a separate file					
		Recording like starts at timestamp 0 regardless of streaming time					
<u> </u>		Recording type Continuous data only Continuous and solar data Solars only					
Z							
		Background Noise Default background noise frequency for configuring notch filters				60 Hz () 50 Hz	
ale							
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- Data Recording Location: The directory path listed here indicates where new recordings will be saved.
- Base Recording File Name: The names of recorded files will all start with the string entered here, and a date and timestamp of when the recording was started is appended to the base name.
- Recording Type:
 - Continuous data only: Records all data streamed.
 - Continuous and spike data: Records data sets of all streamed data and files containing only spike data
 - Spikes only: Only spike data is saved and not the continuously streamed data
- **Background Noise:** Shows default background noise frequency for configuring notch filters selected in signal processing tab.



- Auto Update: Allow software application updates to automatically download and install.
- App themes: Makes changes to the Allego UI theme.



Radiens Curate




What is Curate?

Welcome to the RadiensTM Curate software!

Managing large data files, or converting and accurately saving electrophysiological data into different data file formats, can be a challenging and time-consuming task. To address these challenges, we developed "Curate", a software for reproducible data curation and manipulation with RadiensTM. Curate is the application that offers easily accessible, large-scale data management, along with an infrastructure for reproducible signal processing and batch processing through modular pipelines.

This advanced software from NeuroNexus enables extracting, structuring, and managing recorded electrophysiological data files while maintaining descriptive metadata. Together with the full RadiensTM Analytics suite, this software accelerates reproducible and transparent neuroscience research.



Curate

The Curate is a tab that can be used to extract, structure, and manage electrophysiological information recorded from data acquisition systems.

The tab is structured into two primary sections: The Data Curation Protocols area at the left, and the working space at the right.



The Data Curation Protocols area contains dropdown menus for the protocols that are indicated in the image below.





Sources and Sinks

To create a protocol for data curation, start by dragging "Source" (or "Bulk Source") in the work space as shown below.



• Then, double click at the Name bar and select the desired file from the new opened window.



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C	:\Users\AGolabchi\radix\data		Select Folder	G			
Dat	ta Files					umns \Xi Density	Export
	Base file na	Туре		∣ Date ↓	Duration	Channels	Data Size
	allego_0uid1215-16-16-29	xdat		Wed Dec 15 2021 16:16:29 G	12.1984	38	38120
	allego_0uid0928-17-04-04	xdat		Tue Sep 28 2021 17:04:04 GM	27.5584	38	86120
	sbpro_0uid0714-13-23-53	xdat		Wed Jul 14 2021 13:23:53 GM	6.6048	70	55728
	sbpro_0uid0712-14-39-31	xdat		Mon Jul 12 2021 14:39:31 GM	37.3504	134	595272
	sbpro_0uid0701-15-15-13	xdat		Thu Jul 01 2021 15:15:13 GM	9.6	38	30000
						Rows per page: 50 1-5 of 5	
Su	mmary Statistics						
		5					
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	786 786	.63 MB					

- After selecting the file, the file name will appear in the Source name section.
- Once the file is selected, drag the "Sink" (or "Bulk Sink") to the workspace. Double clicking any of the fields will open a window to select the location, file name, and output format. The available formats are xdat (.xdat), Excel (.csv), NeuroExplorer 5 (.nex), Neurodata Without Borders (.nwb), and Kilosort2 (.bin).



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	curate	2-0 ×			
	Data O	Curation Protocols			
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		Source			
		Sink	Choose		
		Bulk Source			
		Bulk Sink			
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	_	Run Protocol	Choose File		
		Remove Selected Nodes			
		Remove All Nodes	Choose Format		
				+ -	- C 🖬

• Then, select one or all of the tools below to characterize the data file and create a desired curation protocol:

Filtering

The available filtering options are High-pass, Low-pass, Band-pass, Band-stop, and Notch filters.



Drag the filtering option into the workspace, and set the frequency of the selected filter.





Referencing

To extract useful signal and minimize source of the noise, there are different selections of referencing, including common average reference, paired reference, and virtual reference.



Slicing

To extract chunk of time or channels from the previously recorded data, click on the slicing section as shown below:



Slice Time: Fast extraction of chunks of times can be done using Slice Time section. By importing the values in the "Start Time" and the "End Time" as shown below:





Slice Channels: To extract specific channels from the dataset, click on the "Slice Channels" and drag it into the working space.



Click on the Channels section to select desired recording sites. In the new opened window, using the Add and Remove buttons located at the right bottom corner of the window, select or deselect the desired recording sites.



Select Channels Port A			×
€ Rotate			Q Site Size: 2.0x Q
			⊕ ⊖ CLEAR DONE

After selecting the sites, click on 'Done" button to save the selection.

Select Channels Port A	 		×
€ Rotate			e Size: 2.0x
		⊕ ⊖	CLEAR DONE

In the slice Channels section, you should be able to see the total number of the selected sites.





Resampling

This section is designed to resample neurophysiological data.



By dragging this section into the working space, set the Sample Factor to down sample the the previously recorded data.



Run Protocol

Once all the states of data curation are set, click on run protocol to apply those to the selected data set.

Remove All Nodes

To clean the working space, click on "Remove All Nodes" button to clear all previously selected conditions.



Data Files

The Curate Data Sources tab pertains recorded data files.

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P-9	File p	ooth C:\Users\AGolabchi\rad	ix\data			Select Folder C	?	
	Data	ı Files				Columns	╤ Filters	ני Export
		Base file na	Туре	Date ↓	Duration	Channels	Sample Rate	Data Size
		allego_2uid0628-16-54-33	xdat	Tue Jun 28 2022 16:54:33 GM	209.864533333333333	38	30000	983740
		allego_2uid0628-16-53-53	xdat	Tue Jun 28 2022 16:53:53 GM	12.450133333333333		30000	58360
		allego_1uid0628-16-35-57	xdat	Tue Jun 28 2022 16:35:57 GM	1073.5701333333334	38	30000	5032360
		allego_0uid0628-16-05-54	xdat	Tue Jun 28 2022 16:05:54 GM	1561.09226666666668	38	30000	7317620
		allego_1uid0628-15-30-26	xdat	Tue Jun 28 2022 15:30:26 GM	155.6672	262	20000	3210636
		allego_0uid0628-15-14-05	xdat	Tue Jun 28 2022 15:14:05 GM	978.0288	262	20000	20171844
		allego_3uid0628-14-53-22	xdat	Tue Jun 28 2022 14:53:22 GM	0.512	262	20000	10560
		allego_1uid0628-14-33-38	xdat	Tue Jun 28 2022 14:33:38 GM	273.9648	262	20000	5650524
		allego_0uid0628-14-14-31	xdat	Tue Jun 28 2022 14:14:31 GM	1142.5216	262	20000	23564508
	Summar	a Statistics				Rows p	er page: 50 1-33 of 33	
	o anni an	- Stationes	Data Sources:33		es:98	Size:207637.0	1 MB	

File Path is the directory Curate is checking for data files.

Clicking on the top line text field will open a file manager window where the user can browse for the folder where recordings have been saved.

After navigating to the destination folder, the previously recorded files contained in that folder should populate a table under "Data Files". If they do not appear, click the refresh button at the top right of this panel to re-load the file list.

By default, data files are named with the root listed under "Base Recording File Name" in the Data Sources tab, followed by a date/time stamp as follows: uidMMDD-hh-mm-ss. Also, data files can be sorted by clicking the three vertical dot icon under Name, Type, Date, Duration, Channels, and Data Size.



Unsort
Sort by ASC
Sort by DESC
Filter
Hide
Show columns
Pin to left
Pin to right

Actions



• Copy: Copy button is designed to copy selected source files to selected folder.

Copy Files	×
allego_0uid1215-16-16-29	
Destination Folder CHOOSE Overwrite existing files?	

• Move: Move button is designed to move selected source files to selected folder.



Move Files	×
allego_0uid1215-16-16-29	
Destination Folder CHOOSE Overwrite existing files?	

Data File Table

Data File columns are:

- Name: The assigned filename for the data file.
- Type: This data file is formatted according to the type listed here.
- Date: The time listed is the timestamp showing when the recorded happened.
- Duration: The length of the data fileis shown in seconds.
- Channels: The total number of channels in the data source.
- Sample Rate: Shows sample rate that data were collected.
- Data Size: Total size of the data source in megabites.

Open File

The Open File button loads the selected data file (or files) into the Curate tab as a source (or bulk source)

Columns III Columns

The Columns button to the right of the Data Files table expands to show all available metadata categories for the listed files. These will become the columns of the data table underneath. Every column is enabled by default except Metadata Size.





Filters Filters

Filters button is used to filter which data sources are shown in the table.



The Density button is used to change the distance between the rows of the table to Compact, Standard, and Comfortable.







The Export button will prompt the user to choose a destination folder and .csv (e.g., Excel) file name.

Summary Statistics

At the bottom of Curate, there is a Summary Statistics table that reports the summary of the number of data sources, files, and the total size of the files that are shown on the Data Files table.

tistics			
	Data Sources:33	Files:98	Size:207637.01 MB



Radiens Videre





What is Videre?

RadiensTM Videre is your file-based access back into the details of an electrophysiology recording. Visualize and review data in an efficient and user-friendly way. This software from NeuroNexus includes probe geometry data, signal metrics from the experiment, spike sorting, and a twin of the functional interface visualized in 3D spatial models of the brain. Videre is capable of handling large and complex electrophysiological data sets with different formats including XDAT, CSV, NEX5, Kilosort, and NWB, and is compatible with both Windows and Mac OS.

Module Drawer

The Module Drawer in Videre is located on the left side of the display and shows module icons. To access the drawer, click on the arrow button in the top left corner of the window.



When accessed, the Module Drawer in Videre displays the modules as a list. Clicking on a module item will either open a new tab of the selected module or bring an already opened tab into focus. Multiple tabs of the same module type can be saved in the workspace, and all of them can be accessed from the Module Drawer.



The interface may be extended to other monitors by creating tabs in a new window, which is an option presented when clicking on a module.

Configuration



Data Source: Pertains recorded and converted data files.

In the Videre interface, the Data Sources tab is where all the data files are displayed along with their related information. The table view in this tab displays the data type, date of data collection, duration, number of channels, sample rate, and data size for each file. This provides an easy-to-use interface for managing and selecting data files for analysis.



Electrodes: Display connected probe designs, along with which port and headstage were used

In Videre, the Electrodes module is used to define the headstage and probe types for each port used on the recording system. Within this module, users can enable and disable individual or groups of electrode sites and arrange the voltage traces visualized in the Monitor tab. The Wire-frame models of NeuroNexus probes are also displayed in this module.

Signal Processing: Create various filter types and combine them to create DSP group-

The Signal processing module allows users to apply various filter and referencing techniques. In Videre, the Stage is restricted to "Visualization"; thus, all specified filters will not modify the original data files and are strictly for visualization purposes

Visualization

Ο

Monitor: Shows the signals being replayed from the selected File

The Monitor module is where biological data and peripheral device activity are visualized.

HD Snapshot: Takes a high resolution still picture of signals for closer inspection

The HD Snapshot module provides the ability to visualize a waveform of a recorded signal using all captured data points. This results in a very accurate depiction of a signal of



interest.

Signal Metrics: Configure spike detection parameters and view key performance indicators/signal quality characteristics

The Signal Metrics module allows the user to characterize activity across electrode sites using several different metrics, such as mean and standard deviation. Probe geometry is used to visualize relationships between the activity recorded in different locations on the probe.

Spike Grid: Set spike detection threshold using voltage or standard deviation of background noise from the mean. Displays detected spikes across all channels

The Spike Grid module allows the user to setup spike detection based on threshold types and values including amplitude threshold crossing or the standard deviation (SD) of background noise.

Spike Sorter: Scraping and Grouping spikes into clusters

The Spike Sorter module is used to perform offline sorting analysis to any data file. Spike detection and clustering parameters can be set similarly to Allego, after which, the "New Sort" button is pressed to perform the offline spike sorting. This generates a SPIKES file set, which is linked to the originally recorded data and is automatically loaded with that file set. SPIKES can be visualized in Spike Grid, Raster, 3D Model, and Neurons.

DO DO Raster: Analyze spike trains of single neurons

The raster plot is a simple way to display spiking activity of neurons across all active channels over time.

3D Model: The interactive brain model with presentation of network activity

The 3D Model module is a powerful and unique way to visualize an experiment and any detected activity in real time. The probe design being used for the experiment can be positioned and visualized within a 3D brain of the chosen animal model.



Neurons: Neurons tab visualizes detected activity.

The Neurons tab is used to monitor and visualize the activity of detected neurons. To use this tab, the spike sorter must be switched on.

Dashboard Configurability

The module tabs in videre are easily moved and repositioned by clicking on the tab header and dragging it to any other location on the Dashboard. Tabs can be viewed simultaneously via split screen or stacked together allowing them to be viewed like tabbed web browsers. Clicking and dragging the tab header around the display will provide a preview of the layout in real time.



Top Bar

The Top bar is located at the top of the window.

The Settings

The Settings module can be accessed from the top of this window.



status and videre version can be found in that section as shown below.



agolabchi@neuronexus.com Admin						
MY SUBSCRIPTIONS	Active					
Radiens Analytics So	oftware Suite - Academic					
ORGANIZATION NeuroNexus	ORGANIZATION NeuroNexus					
ALLOWED USERS	CURRENT USERS					
1	1					
i About						
S⁺ Invite User						
⊖→ Sign Out						



Stream Display Options Bar

The stream display bar is located at the bottom of the window.

5 5 × C C



Data Source

The Videre Data Sources tab pertains recorded data files.

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		Pri Mai 17 2023 12:33:24 GMT 70,009	36 20000	20200.0	
ž	allego_1_uluu222-09-25-16_53 spikes	Primar 17 2023 12:31:49 GMT 70:009	38 20000	230611.2	
0	allego_1_uid0222-09-25-16_s2 spikes	Tue Mar 14 2023 11:23:40 GM 70.009	38 20000	20286.3	
alie	allego_1uid0222-09-25-16_s1 spikes	Tue Mar 14 2023 10:01:26 GM 70.009	38 20000	20694.8	
#	allego_1uid0222-09-25-16_s0 spikes	Tue Mar 14 2023 10:00:52 GM 70.009	38 20000	20696.6	
~	Curate-Excel files csv	Wed Feb 22 2023 09:25:16 G 70.009	38 20000	225359.5	
555	Curate-Neuroexplorer5 nex5	Wed Feb 22 2023 09:25:16 G 70.009	38 20000	207852.6	
~	Curate-test bin	Wed Feb 22 2023 09:25:16 G 70.009	38 20000	87512.8	
8	allego_1_uid0222-09-25-16 xdat	Wed Feb 22 2023 09:25:16 G 70.009	38 20000	218778.1	
	allego_0_uid0222-09-25-12 xdat	Wed Feb 22 2023 09:25:12 G 1.2032	38 20000	3760.0	
	allego_0_uid0222-09-23-30 xdat	Wed Feb 22 2023 09:23:30 G 21.2096	38 20000	66280.0	
				Rows per p	age: 50 1-11 of 11 < >
	Summary Statistics				
	Data Sourc	es:m	Files:26	Size:1097.48 MB	
					allego_1uid02_ v SYNC TABS

File Path is the directory Videre is checking for data files.

 File path
 C:\Users\asiye\radix\data

 Select Folder
 C

Clicking on the top line text field will open a file manager window where the user can browse for the folder where recordings have been saved.

After navigating to the destination folder, the previously recorded files contained in that folder should populate a table under "Data Files". If they do not appear, click the refresh button at the top right of this panel to re-load the file list.

By default, data files are named with the root listed under "Base Recording File Name" in the Data Sources tab, followed by a date/time stamp as follows: uidMMDD-hh-mm-ss. Also, data files can be sorted by clicking the three vertical dot icon under Name, Type, and Date.





Actions



• Copy: Copy button is designed to copy selected source files to selected folder.

Copy Files		×
allego_0uid1215-16-16-29		
Destination Folder	CHOOSE	

• Move: Move button is designed to move selected source files to selected folder.



Move Files	×
allego_0uid1215-16-16-29	
Destination Folder CHOOSE Overwrite existing files?	

Data Files Table

Data File columns are:

- Base file name: The assigned file name for the data file.
- **Type:** This data file is formatted according to the type listed here.
- Date: The time listed is the timestamp showing when the recorded happened.
- Duration: The length of the data fileis shown in seconds.
- Channels: The total number of channels in the data source.
- Sample Rate: Shows sample rate that data were collected.
- Data Size: Total size of the data source in kilobites.

Open Files Depen File

The "Open File(s)" button is located at the top of the dataset list. When one or more datasets is selected, the button is enabled and may be pressed to load the selected files for replay.

Data Files • Open File		I	Columns \Xi Filters	Density Export
☐ Base file name ↑	Туре	∣Date ↓	Duration	Channels
allego_2_uid1210-10-27-23	xdat	Tue Dec 10 2024 10:27:23 GN	1 79.6096	38
allego_2uid1210-10-27-23_s0				
allego_1uid1210-10-26-43	xdat	Tue Dec 10 2024 10:26:43 GN	1 35.4048	38
allego_0uid1210-10-26-04	xdat	Tue Dec 10 2024 10:26:04 GN	1 27.3984	38



		Filters Density Export
_ Base file name ↑ Type	Date ↓ Duration	Channels
✓ allego_2uid1210-10-27-23 xdat	Tue Dec 10 2024 10:27:23 GM 79.6096	38
allego_2_uid1210-10-27-23_s0 spikes		
✓ allego_1uid1210-10-26-43 xdat	Tue Dec 10 2024 10:26:43 GM 35.4048	38
allego_0_uid1210-10-26-04 xdat	Tue Dec 10 2024 10:26:04 GM 27.3984	38

Columns Columns

The Columns button to the right of the Data Files table expands to show all available metadata categories for the listed files. These will become the columns of the data table underneath. Every column is enabled by default except Metadata Size.



Filters Filters

Filters button is used to filter which data sources are shown in the table.





The Density button is used to change the distance between the rows of the table to Compact, Standard, and Comfortable.



Export	₫	Export

The Export button will prompt the user to choose a destination folder and .csv (e.g., Excel) file name.



Summary Statistics

Located at the bottom of Videre, you will find a Summary Statistics table that displays a summary of the number of data sources, files, and the total file size that are visible on the Data Files table.







In Videre, the Electrodes tab allows for configuration of probe design, probe connector package, and headstage type for each recorded data file. Videre also generates probe mapping automatically and reactively based on the selections made in each dropdown during recording.



The electrode tab is split into two primary sections:

- The signal/port configuration area
- The probe wireframe

The signal/port configuration area:

This part contains dropdown menus for the data set, Port, Headstage, and Probe.

- Port: Corresponds to the headstage ports on the front of the system (A, B, C, D) that was used during the recording.
- Headstage: Will show the connector type that was used.
- Probe: Will show the NeuroNexus probe design that was used.



Note: After selecting the dataset, the headstage, and the probe, the headstage connector and probe design used during data collection will be automatically displayed.

Probe Wireframe

The wireframe section in Videre presents an accurate geometric representation of the probe design used. The geometric data, combined with recorded neural signals in Allegro, offers numerous analysis options, such as the Heat Map located within the Monitor and Signal Metrics tabs. Additionally, several tools are available to manipulate the wireframe and alter the display of electrode sites and their corresponding voltage traces in the Monitor tab.

The available tools are Select, Order, and Sort By Coordinates.



Once a tool has been selected in Videre, clicking the +/- buttons will determine whether the tool's effect will be applied to or removed from the desired electrode sites. The toolbar located in the upper right corner of the probe wireframe provides access to various site selection methods and other functions, as shown in the following image.



By default, the box selection tool is active in Videre. However, other tools such as zoom, pan, lasso selection, and the home button to reset the view to its default setting are also available.

Site Selection Tool

Using the Select tool in Videre, users can add or remove specific electrode sites and their signals from being displayed in the monitor. To remove voltage traces of certain channels, make sure to select the Select tool and choose the "-" buttons. Then, click and drag over the sites to be removed from visualization.





Select the "+" button to add electrode sites and their signals previously removed from visualization back in.

Order Tool

The Order tool affects where and how sites and traces of interest are displayed in the Monitor tab. Ordering and removing order from electrode sites are done in the same way as described in the Selection tool. After applying the Order tool to the electrode sites of interest, the sites and signal traces are changed to a different color, grouped together and moved to the top of the display in the Monitor tab. Applying the order tool again will yield a different color, and selecting the "-" button will revert the sites and traces back to their default color and return the traces to their default location on the Monitor display.



electrodes-0 \times							\$
Signal Group Name	Port	Headstage	Probe	Probe spans mult	iple ports		
allego_1uid02_ v	A ~	Passthrough 32 v	A4x8-5mm100-200-1_ ~				0 2
¢⊋Relate	^						Q. Sim Sove 2 de Q.
Tools 🔽 🕄 Add	I/Remove 🕀 ဝ					Sorting Options	- Apply

The selected site on the wireframe diagram will change in size to reflect the selection for audio playback.



The sorting by site coordinates feature in Videre allows for quick organization of sites based on their physical location on the selected probe design. This tool also colors the sites and signal



traces based on how they are sorted. For example, sorting by X values will assign a different color to electrode sites on each shank, while sorting by Y values will result in all electrode sites at each depth being assigned a different color. The Z dimension is typically unused on standard probes, but it may be utilized in our 3D Matrix array and some custom designs.



V Signal Processing

The Signal processing tab is where individual filters and filter groups can be defined, applied and removed.

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Filter Stages

Filters can be applied to a dataset at three different stages. However, the Hardware filters and Data Filters are disabled for Videre in this tab.

• Visualization Filters: Filters applied at the Visualization Filters stage affect the signals displayed in the Videre Monitor tab.





Filter Types

Videre offers five different filtering options. They can be applied independently or in various combinations. Below is a brief description of each filter type and the valid ranges for each.

- Lowpass: Passes signals with frequencies below the user-defined threshold and attenuates signals with higher frequencies (1-300 Hz).
- **Highpass:** Passes signals with frequencies above the user-defined threshold and attenuates signals with lower frequencies (300-5000 Hz).
- **Bandpass:** Passes all signals that fall between user-defined frequencies and attenuates everything else (Min= 0.1 Hz; Max= 20 kHz).
- **Bandstop:** Attenuates all signals falling between user-defined frequencies and passes everything else (Min= 1 Hz; Max= 20 kHz).
- Notch: Attenuates all signals at the user-defined frequency (Min= 50 Hz; Max= 60 Hz).





Reference Types

- Common Average Reference: A referencing technique that removes noise common across all recording channels. The average value of all channels is subtracted from each channel.
- Virtual Reference: This allows the user to designate one of the recording sites to serve as the reference channel. The value of the selected reference channel is subtracted from each channel.
- **Paired Reference:** The selected reference channel is subtracted from the selected target channel.



Configure

To start defining and adding filters, select a stage and filter type and then fill out the fields provided in the filter configuration box as shown below for Bandpass Filter.

Configure						
Bandpass Filter						
Label	Channels to filter	Low Frequency	High Frequency			
	Port A ~	500 Hz	2000 Hz	• •	Remove all	Add Filter

Each filter can be assigned independently to specific ports on the recording system. When first opening the Signal Processing tab, the Hardware Filter Group will list a Band Pass filter for each port with the range of 1 - 7500 Hz. When adding additional filters, the Lowpass, Highpass, Bandpass, and Bandstop filters will all require frequency thresholds defined based on the user's needs.

The Notch filter requires a notch bandwidth to be defined. The notch bandwidth determines the total frequency range that is filtered out of the signal. For instance, when selecting the notch filter , the Notch Bandwidth field has a default value of 2. This means when the notch filter frequency is set to 60 Hz, the effective frequency range that is being attenuated is 59 - 61 Hz.





After the filter configuration fields are set, click the "Add Filter" button. This will apply the filter to the selected port. In the previously referenced image above, a notch filter was added to "Software Signal Group 2". Next to each filter in the filter group, there are two buttons. The arrow button is used to change the order of the filters, as each entry in the filter group is applied in the order it is displayed. The trash button removes that filter from use.

To remove a filter group that has been applied to a signal group, simply remove each filter from

the filter group in question using the button.

Preset Filters

Preset filters are custom filters applied for different type of signals, quickly. You can run your live streaming data through a specific preset filters in order to cultivate certain portions of frequency spectrum while rejecting unwanted portions of spectrum.

Select a port or channel from the dropdown menu, then click on the button for the chosen signal type. The filter set will be applied to the chosen port and will be displayed in the filter group section near the top of the page.




The Monitor tab is used to view electrical signals of the previously recorded files.

To view the recorded signal, select the data file from the data files table in the Data Sources tab. Then, click on replay button located on the left top corner of the Data Files table.

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Then, open Monitor tab in Videre. Most of the space in this tab is used to show the signals and traces, and at the top and bottom are several options to manipulate how they are displayed.





Top Bar

At the top of this tab are stream position indicators and an optional "Viz Bandpass" which can be used to apply a bandpass filter localized to this monitor for convenient visualization purposes.

- Icon Buttons:
 - Toggle GPIO channels to display on the top or bottom of the rest of the traces
 - Enable/disable monitor DSPs
 - Grid on/off
 - Cursor on/off
 - Volume on/off
 - Open threshold settings
 - Open browser to tab documentation
 - Open tab in new window
 - Collapse toolbar

Bottom Bar

On the bottom bar, you can find the following options



- **Plot Type:**The Plot Type menu is where you can change to different methods of visualizing data in the Monitor tab. The default view is an X-Y plot, and Heatmap is available.
 - X-Y Plot: each channel drawn as a trace
 - · Heatmap: each channel visualized as a heatmap



- Sample Rate:
 - fs=sample freq (plot sample freq) kHz
- Presets



- · Units: Preset visualization options optimized for units
- Fields: Preset visualization option optimized for LFP
- View Modes:



- Anti-Alias: Anti-alias filter applied to signals.
- Raw: Raw signal down sampled agnostically to signal present.
- Spikes: Signal down sampled to preserve spike structures.



Measure Voltage



Measure Voltage button is a measurement tool which allows a click-and-drag to measure the voltage of a trace.

To measure individual spike, click on "Measure voltage" button located on top of the monitor tab and draw a line over the desired spike to check the voltage value. To remove the line, click again on the draw line. Make sure that the Measure Voltage button is still active.

Videre		– o ×
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	signal-metrics-0 × spike-sorter-0 × 3d-model-0 × hd-snapshot-0 × monitor-0 ×	
	Signal Group Name Stream Position Meissure Votage	
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		Sachtfordow 5
	eost	URIOZ. V SYNC TABS

View Settings



Trace visualization options:



Chan height (px) 20
AMP Range (µV) ± 150
AIN range (V) ±15
Window (s) 5
Clip
Show Thresholds

- Chan height: Height (in pixels) of each channel on monitor display
- AMP Range: Microvolt range of amplifier channels
- AIN range: Voltage range of aux channels
- Window: Duration (in seconds) visualized in monitor display
- Clip: Clip signal from each channel to fit completely within its allocated space in the display
- Show Thresholds: Show thresholds used in spike detection





While the signals are displayed in the Monitor tab, they only utilize a fraction of the data to generate the waveforms. The HD Snapshot module provides the user with a way to visualize signals using all collected data points from recorded data, producing a highly accurate representation of a signal for closer inspection. Some recently streamed data that has not been saved can be visualized here as well.



There are two main areas to the HD Snapshot tab. The small section at the top of the window lets the user select the Signal Group Name for the data plotted, the starting time stamp in the stream and the duration of the signals that will be drawn. The arrow buttons next to the Duration dropdown either rewind or advance the Start Time by the selected duration. The available window displays the segment of time available for this feature, with a maximum of 15 seconds.

Plotting Waveforms and Display Options

Once a Start Time and Duration have been set, hit the View Snapshot button, and the signals will be output in the large area below. By default, these traces will be each be displayed separately on a grid and only several of them can be seen simultaneously on the same screen. By selecting the Condensed option, the traces are stacked in close proximity and can all be seen on



the same screen as shown in the image on the next page. Moving the cursor over any point on these lines will display the exact time stamp and amplitude of the signal.

Above the waveforms are some details about the signal group, the selected ranges on the traces, and some other specifics related to the output. The Scope Frame displays how many signals were drawn, and the total number of signals available in the selected Signal Group. Also shown is the sampling frequency, the number of samples used to draw the waveform, and minimum and maximum values of the amplitude in the selected time frame.

Scope Frame Sample Frequency 38/38 signals 20000 Hz	Time Range 299, 318	Amplitude min/max= [-686/153] μV
---	------------------------	-------------------------------------

The values available in the Duration dropdown menu are determined based on the number of channels being displayed and the sampling frequency. If the values in the Duration dropdown are not long enough, consider deselecting sites that are not of interest within the System or Electrode tabs. Since some resources are shared, this will allow the system to utilize more data points and draw longer time segments due to fewer channels that need to be generated in high resolution.

Waveform Display Tools

The waveforms generated in this tab can also be manipulated in a number of ways using the toolbar in the top right of waveform area. The view can always be reset to its original state by double clicking anywhere on the waveforms.





The available tools are Download Plot as a png, Zoom, Pan, Zoom in, Zoom out, Reset axes.

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Illi Signal Metrics

The Signal Metrics tab is used to monitor and visualize the activity of recorded neural signals. The same probe wireframe that was customized from the electrode tab during the recording, will be shown here and by choosing different parameters, the neural activity of all recording sites will be revisualized in heatmap style. The color of the sites is determined by the magnitude of the parameter that is selected.



Signal Metrics Configuration

At the top of this tab, are the Signal Group Name, Port, and Metrics dropdown menus.

Helic vot locacion wrbox <u>Aby(mov-mn) v</u> <u>x</u> <u>v</u> <u>no</u> Uvit lipidate <u>→</u> Drow Table <u>→</u> Custom Scale <u>→</u> <u>browbulget</u>

Metric dropdown presents different parameters of neural activity voltage including Absolute, Max, Min, Mean, Std Dev, variance and the signal to the noise ratio.

Note: The signal to noise ratio (S/N ratio) is used to compare the level of a desired neural activity to the level of the background noise. It is important to consider that the S/N ratio values will depend strongly on the specific spatiotemporal characteristics of neural activity and structure of background noise, which vary across brain areas and electrophysiology setups.

The **Port** dropdown shows the used port during collecting the signal.



Lookback windorefers to a specific period (ms)in the past used to analyze signal metrics. It essentially defines how far back in time you examine signal information to make evaluations.

The **Live Update** option updates the probe site activity and metrics values every 2 seconds and the **Show Table** option allows you to list all the mertrics values in the table below the probe wire-frame.

Custom scale toggle enables the custom scale range of the heatmap scale.

Recalculate will recalculate the signal metrics using the filters applied in the Signal Processing Tab.

Refreshbutton (C) let you to update all values for the selected parameter from the Metric section. Live update button periodically updates the selected metric values.

Spike Detection Settings

The **gear icon** at the top of this tab is designed to configure spike detection settings, and it opens the window shown in the image below. The same window can also be opened from the Spike Grid tab, the next module that will be covered. From here, spikes can be detected by setting the appropriate voltage threshold or a fixed multiple of the standard deviation of the voltage signal on a given recording channel in positive and negative values. Keep in mind, if the threshold is too strict, low amplitude spikes will go undetected, which may lead either to missing entire single units, or losing a fraction of spikes from the units that mean spike amplitude is relatively near detection threshold. Conversely, if detection threshold is too low, more noise and non-sortable spikes will cross threshold, and lead to decreasing detection of larger spikes.

Spike Detection Settings											
PORT	THRESHOLD TYPE	POSITIVE THRESHOLD	NEGATIVE THRESHOLD	PR	E-THR	РО	ST-THR	SH	IADOW	Y MIN	Y MAX
A	Voltage ~	μν 🗆	μν 🗆	0	ms	0	ms	0	ms	μV	μV
В	Voltage ~	۳۸ 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
С	Voltage ~	۳۸ 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
D	Voltage ~	۳۸ 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
											SAVE

- Detect Spikes: Enable or disable spike detection for this port (A, B, C, D)
- Threshold Type This determines whether spikes are detected using voltage or standard deviations



- **Positive/Negative Threshold:** Set one or two thresholds to determine what signals qualify as a spike
- **Pre/Post-Threshold Window** The pre and post thresholds indicate how much of the signal is shown before and after the spike occurs
- Shadow: The length of time that a port will wait before another spike can be detected
- Y Min/Max: Scale of the X and Y axes

At the bottom of the tab, all signal metrics are available in the table, and can be sorted by column

using the three-bar button (\blacksquare) or get exported to the CSV or PDF format by selecting the Export button (\blacksquare).





H Spike Grid

The Spike Grid is the module that allows users to define positive and negative spike thresholds for individual channels. Similar to other modules, the window consists of two main areas: The top bar with various settings, and the main area with pile plot boxes for each selected channel. Deselected channels will not be shown in this tab.



The top bar contains a dropdown menu for selected signal groups. Continuing from left to right, this is followed by the Lookback Window, Update Period, Spike Grid settings, and zoom buttons to change how many pile plots can be displayed at once.

to spikes available 5 sec 0.5 sec Probe knyout

Lookback Window: This field indicates how long spikes will remain shown in the pile plot boxes and has a default value of 5 seconds.

Update Period: The Update Period determines how frequently spikes are drawn in the pile plot boxes.

Probe layout: Toggle this button to align the grid with the arrangement of sites on the probe shank.



Spike Detection Settings: The Spike Detection Settings window should be used to set baseline filters for all channels on each port. This is the same window that can be configured from the Signal Metrics tab.

Spike Detection Settings											
PORT	THRESHOLD TYPE	POSITIVE THRESHOLD	NEGATIVE THRESHOLD	PRE	e-THR	PO	ST-THR	SI	HADOW	Y MIN	Y MAX
А	Voltage ~	μν 🛛	μν 🗖	0	ms	0	ms	0	ms	μV	μV
В	Voltage ~	μν 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
с	Voltage ~	μv 🗆	μν 🗖	0	ms	0	ms	0	ms	μV	μV
D	Voltage ~	μν 🗖	μV 🗖	0	ms	0	ms	0	ms	μV	μV
											SAVE

- Detect Spikes: Enable or disable spike detection for this port (A, B, C, D)
- Threshold Type This determines whether spikes are detected using voltage or standard deviations
- **Positive/Negative Threshold:** Set one or two thresholds to determine what signals qualify as a spike
- **Pre/Post-Threshold Window** The pre and post thresholds indicate how much of the signal is shown before and after the spike occurs
- Shadow: The length of time that a port will wait before another spike can be detected
- Y Min/Max: Scale of the X and Y axes





The Spike Sorter is the module that allows users to analyze electrophysiological data. Similar to other modules, the window consists of two main areas: The top bar with various settings, and the main area with overall plots for the site and neuron quality.



When "New Sort" is pressed, the progress status will be shown to the right.





Sort Parameters



Feature Dimension: Determines the complexity of the input used in feature extraction by specifying the dimensionality of the waveform representation used to characterize each spike.

Position Influence: Controls the extent to which the spatial location of each spike affects its classification into distinct units.

- Higher Values: Makes the clustering process more sensitive to differences in spike locations, leading to a higher likelihood that spikes from different positions are identified as separate units.
- Lower Values: Allows spikes from nearby locations to be clustered together more easily, even if there are slight variations in their positions.

Waveform Influence: Controls the extent to which the shape of each spike affects its classification into distinct units.

- Higher Values: Emphasize waveform characteristics more strongly, making the clustering process more sensitive to differences in spike shapes. This increases the likelihood that spikes with varying waveforms are identified as separate units.
- Lower Values: Reduce the emphasis on waveform features, allowing spikes with similar overall patterns to be clustered together more easily, even if there are subtle differences in their shapes

Sort: Begin a new sort with the above parameters



View Sort Details:

Neural Interface General window shows the summary of spike sorting data, sites, neurons and general data about sample frequency and total dataset size in tabular format.

Neural Interface			
General			
III COLUMNS 🛨 FILTE	ERS 🗮 DENSITY 🛃 EXPORT		
Attribute	Value		
Time Range	3.008,92.8064		
Total Spike Count	25849		
Neuron Count			
Total Available Sites			
Enabled Sites			
Sample Freq	20000		
Dataset Size	5777552		
Persistence	20		
Dataset Uid	null		
Provenance Uid			
		Rows per page: 50	1–10 of

Spike Detection Settings: The Spike Detection Settings window should be used to set baseline filters for all channels on each port. This is the same window that can be configured from the Signal Metrics tab.

Spike Detection Settings											
PORT	THRESHOLD TYPE	POSITIVE THRESHOLD	NEGATIVE THRESHOLD	PRE-	THR	POST	-THR	SH	ADOW	Y MIN	Y MAX
A	Voltage ~	μν 🛛	μν 🗖	0	ms	0	ms	0	ms	μV	μV
В	Voltage V	μν 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
С	Voltage ~	μν 🗖	μν 🗖	0	ms	0	ms	0	ms	μV	μV
D	Voltage ~	μν 🛛	μν 🗖	0	ms	0	ms	0	ms	μV	μV
											SAVE

- Detect Spikes: Enable or disable spike detection for this port (A, B, C, D)
- Threshold Type This determines whether spikes are detected using voltage or standard deviations



- **Positive/Negative Threshold:** Set one or two thresholds to determine what signals qualify as a spike
- **Pre/Post-Threshold Window** The pre and post thresholds indicate how much of the signal is shown before and after the spike occurs
- Event Shadow: The length of time that a port will wait before another spike can be detected
- Y Min/Max: Scale of the X and Y axes

In the main area there are overall plots of SNR, Noise, Neuron Yield, and Sort Deficiency located on the right of the screen.



SNR: SNR plot shows the average SNR of all sites on each port in the current session.

Noise: Noise plot shows the average noise level of all sites on each port in the current session.

Neuron Yield: Neuron Yield plot shows the average number of neurons detected/site on each port in the current session.

Sort Efficiency: Sort Efficiency plot shows the average number of labeled spikes divided by all detected spikes of all sites on each port in the current session.

At the top of this area, the number of neurons, spikes, Elapsed time, and processed data are shown.



Time Range	[2.99, 73.00]
Units:	65
Labeled Spikes:	18121
Total Spikes:	30253
Active Sites:	28
Probe Yield:	0.875

Time Range shows sorting session duration.

Unites: The number of units sorted this session.

Labeled Spikes: The number of spike waveforms sorted this session.

Total Spikes: The number of spike waveforms processed this session.

Active Sites: Active sites shows the total number of probe sites that are detecting units.

Probe Yield: Probe yield is defined as a fraction of active electrodes that recorded neural activity during each recording session.

Located at the top center of the main section of the Spike Sorter tab, you will find three box and whisker plots that display information about SNR, Noise, and Neuron Yield.

SNR whisker plot: SNR plot shows SNR statistics of all sites on each port plotted as a box-and-whisker plot.

Noise whisker plot: Noise plot shows noise statistics of all sites on each port plotted as a boxand-whisker plot.

Unit Yield whisker plot: Unit Yield plot shows Neuron Yield statistics of all sites on each port plotted as a box-and-whisker plot.





At the bottom of this tab, there is a color coded table of site Activity Panel measures as shown below.



At the bottom right of Site Activity Panel, Unit Activity Panel is presented .





Baster Plot

The most important information of spikes or action potentials is timing and the frequency of the firing and the raster Plot is a simple way to display spiking activity of a group of neurons across all active channels over time. This module has a layout similar to the Monitor tab, but in order for activity to be displayed, spike sorting must be configured and turned on.



The top bar in this module contains the Signal Group Name and stream position in seconds, minutes and hours.

Like the other plotting windows, there are several functions that can be used within the Raster plot. Single click one of the traces to hear spiking activity through the left audio channel, and holding shift while clicking one will play the activity through the right channel.

Lastly, the length of time displayed in the window can be adjusted in the Sec/Window dropdown in the bottom right corner of the tab.









The Videre 3D Model is a powerful and unique way to visualize the activity of the previously recorded data in the 3D reconstruction of the brain. Using this module, the probe design that was used for the experiment can be positioned and the activity of the surrounding neurons can be visualized within a 3D brain of the chosen animal model.



The Port dropdown shows the port that was active during recording. Species dropdown presents different animal models of mouse, rat, and macaque.

Gliogo.2__uidi210-10-27-23_50 v A Mouse v Hide Alias Panel Distinto Pathway MPM

Hide Atlas Panel: You can toggle the "Hide Atlas Panel" button to hide the atlas slice that is located on the right side of the window.

Listen to Pathway MPM: To integrate trajectory planning of MPM software and Radiens Allego via an HTTP server, please toggle the "Listen to Pathway MPM" button.



Note: All the current models are based on an atlas viewer from <u>Bakker et.al, 2015, Neuroin-formatics 13, 353-366</u>.

- For the mouse atlas template, adult male, 56-day old C57BL/6J Mice (Jackson Lab) are used(https://doi.org/10.1038/nature05453)
- For the rat atlas template, adult male, 80-day old, W397.6 g Sprague Dawley rats (Charles River) are used (https://doi.org/10.1016/j.neuroimage.2014.04.001; https://-doi.org/10.1016/j.neuroimage.2014.12.080; https://-doi.org/10.1016/j.jneumeth.2014.11.005)
- For the macaque atlas template, young to middle-aged male adults, 6.2 to 10.5-year old are used (https://doi.org/10.3389/fninf.2012.00027)

3D model tab contains two views of the brain model, with the 3D image on the left, and an atlas slice showing the regions on the right. The black dot shown in the slice indicates the current location of the probe.

Both views can be changed using controls that are common to most CAD applications. Hold the left mouse button to revolve around the center of the model, click and hold the right button to move the camera, and the middle mouse button or mouse wheel to zoom in or zoom out. Also, if you hover the mouse over the brain slice region, you can find the name of the region below the slice.

Below the brain model views, there are areas where the stereotaxic coordinates, probe angle, and other display settings are found.



Probe Position from Bregma (mm)				
ML	0			
AP	0			
DV	0			
Probe Angle (degrees)				
φ (ring angle)	0			
γ (axial angle)	0			
ψ (arc angle)	0			
Reset Probe Positio	n			

- Probe position from bregma: To locate the area of the implantation, you need to enter the spatial relationship of the stereotaxic coordinates of Anterior-Posterior (AP), Medial-Lateral (ML), and Dorsal-Ventral DV by considering Bregma as the common landmark. As the probe position values are entered, the black dot in the slice view will move to that location for verification of the probe position and orientation.
- **Probe angle:** If the probe is inserted into the brain at angle, the value can be added in the probe angle section. Once the angle value is entered, the probe will adjust to that insertion angle.
- Site signal metrics: In the "Site Signal Metrics" you can select the metric of the interest and by enabling the slider button during the streaming, the color of the sites on the probe wireframe will change from gray to a color gradient to indicate the magnitude of the selected metric.





The available metrics are absolute max, max, min, mean, noise, variance, and signal to noise ratio. Upon enabling the slider button in signal metrics section, a scale will appear between the two brain views.

Max (abs)
Cumulative Max (abs)
Abs(max-min)
Average Abs(max-min)
Noise
Number of Events
Cumulative Number of Events
Event Rate
Average Event Rate
Signal To Noise
Average Signal To Noise
Event Average Max
Event Average Min
Event Average Max (Abs)

Also, by activating the spike sorting slider from the spike sorting tab and defining appropriate spike threshold values in spike detection setting window, the number of neurons detected by each site and the frequency of spike detected from each neuron can be tracked.

In this case, the quantity of neurons detected is shown as one or several spheres projected outwards from the probe wireframe and each sphere represents a neuron.

Also, The frequency of spikes from a given neuron is indicated by the size of the sphere. A sphere roughly the same size as the probe site indicates low activity, while large ones indicate high activity.



The last set of options are for the 3D model only, and they allow the user to show or hide the brain volume, atlas slice, and the probe model. The level of opacity can be also set for each.

Brain Volume	
Opacity	20%
-0	
Atlas Slice	
Opacity	100%
	0
Probe	
Opacity	100%
	O





The Neurons tab is used to monitor and visualize the activity of detected neurons. To use this tab, the spike sorter must be switched on.



Analytic Dropdown: Determines which spike train analytic is visualized.



• Interspike Interval (ISI): the time intervals between consecutive spikes in the firing pattern of a neuron. ISIs are visualized a single histogram for each neuron, which is labeled with the channel index it's found on, and a subscript indicating the index of the neuron on that channel (in the case there are more than one neurons on a single channel).



• Average Waveform: average waveform over specified time range.

Order: Determines how the neurons are ordered on the page (always descending). Options are:

- Channel
- Spike Rate
- SNR



Time Range (+Plot): Calculates the selected analytic for a specific time range.



Top Bar



(from left to right) -> Bin settings, decrease plot size, increase plot size, export graph, help, float tab, collapse toolbar

Bin settings menu:

Bin Number
- o
Bin Range
••



- Bin Number: Number of bins in each histogram
- Bin Range: Start and end point for the range of histogram bins (interpolation is performed if necessary)

Decrease/Inc plot size: Adjusts size of plots keeping aspect ratio the same



Export graph: Export to png

Help: Open documentation for this tab

Float tab: Open tab in new window

Collapse toolbar: Collapse toolbar for larger viewing window

Pagination: Found at the bottom of the tab, this allows navigation to pages of neurons not currently visible.



Settings

The Settings tab is where Background Noise and several other options are located.

Videre			- ø ×
-		\$\$ Ł @	ి జి ~
>	Settings ×		
8	Background Noise Default background noise trequency for configuring notch filters	● 60 Hz C) 50 Hz
1 Å	C Auto Update Allow updates to automatically download and initial		-
Ģ	Bi App Bennes Switch to Dark Mode		-
_ ©	[] About NeuroMeaus		
alie			
#			
∿			
Θ			
		allego_1uid02_ ~	SYNC TABS

- **Background Noise:** Shows default background noise frequency for configuring notch filters selected in signal processing tab.
- Auto Update: Allow software application updates to automatically download and install.
- App themes: Change the UI theme mode



Python & Matlab API

The *radiens* Python package provides programmatic access to the Radiens Analytics Software Suite, enabling you to integrate its powerful neuroscience data acquisition, processing, and analysis capabilities into your Python workflows. By using radiens, you can harness the capabilities of a neuroscience data engine to automate tasks, design custom workflows, and execute real-time or offline protocols—all from within Python.

MATLAB users can also leverage its built-in Python compatibility to access the full functionality of the radiens API. This allows you to seamlessly integrate Radiens capabilities into MATLAB scripts, providing programmatic control and workflow automation without requiring direct Python coding.

Key features of the radiens:

- Seamless Integration: Leverage Radiens' advanced data processing capabilities within your Python (or MATLAB) scripts.
- Flexible Use Cases: Perform anything from single API calls to full, closed-loop experimental protocols.
- Enhanced Efficiency: Extend Radiens' functionality to fit your specific research needs, all while maintaining the reliability and performance of its underlying system.

Whether you're running real-time experiments or processing large datasets offline, radiens offers a clean, intuitive API, so that you can focus on solving complex research challenges rather than managing cumbersome code.

The radiens package is available on PyPI (https://pypi.org/project/radiens/) and requires an active Radiens license to connect to a running system. For detailed documentation, including function references and examples, visit our online guide: https://neuronexus.github.io



Frequently Asked Questions

Here is a brief list of frequently asked questions about Radiens. If you are not able to find the answers to your questions please <u>contact us</u>.

Once we receive your inquiry, it will be routed to the appropriate support engineer at NeuroNexus. Please expect to get a personal follow up message via email within one business day.

How do I set up my experiment using Allego?

• You can find detailed instructions in the Allego manual documentation, but briefly, choose your electrode and headstage based on your experiment, then open the monitor tab and click on streaming button.

Is Allego compatible with other data acquisition systems?

• Yes, currently besides SmartBox Pro and SmartBox classic, the OpenEphys and Intan recording systems are supported. For other data acquisition systems please <u>contact us</u>.

What is the allowable range of sample rates?

• Allego allows users to select 17 sample rates ranging between 1,000 and 30,000 samples/sec. This range is sufficient for nearly all electrophysiological applications.

How do I track behavioral files using Allego?

• Time stamps will be part of the recorded data. Please find detailed instructions in the manual documentation.

I could not find my probe map in Allego. What should I do?

• Our Allego library is equipped with the mapping of the majority of the standard NeuroNexus probes. If you could not find it in our current library please <u>contact us</u>. We will either help you in finding it or built you the new one if your electrode is customized.

What should I do if my electrode is not made by NeuroNexus?

• We have default maps for different channel counts. If you'd like to have the exact design and mapping of your electrode provider, <u>contact us</u>. We support all available electrodes in the market.



Is Allego available for Windows, and Mac computers?

• Yes, Allego is fully compatible with Windows, and Mac systems and runs natively on each system. Allego runs with roughly comparable performance across each type of system. We do not recommend one type of system over another; it is up to the user and the lab.

How I can view the recorded data using Radiens?

• Videre is the software that is designed for offline visualization of recorded data. Open data sources tab in Videre and look for the data that you are interested to view. Then click on

the replay button, **button**, to create a replay file. Once the replay file is created you should be able to view that from the monitor tab. Remember to change the Signal Group name at the top of your monitor window.

How do I setup the thresholds of my data streaming?

• The Threshold can be manually selected in Spike Grids or Signal Metrics tabs.

What are the file formats that I can save my recordings?

• We save raw data alongside meta-data in popular, non-proprietary file formats for straightforward integration into existing data analysis workflows with NeuroExplorer and kilosort2. Matlab, Python, and R friendly.