



Congratulations on your purchase of SiNAPS products!

The complete SiNAPS solution from NeuroNexus simplifies conducting large neuronal recordings and rapid analyses of these complex experiments.

SiNAPS active-pixel probes represent the highest number and highest quality of simultaneously recorded full-band channels currently available. The SiNAPS Interface Box hardware provides the capability to integrate SiNAPS probes into existing electrophysiology labs. Supported by Radiens software, feel confident in up to 1024 channels by viewing real-time signal metrics for every SiNAPS probe site at once, not just a subset. High-density, high-yield experiments expand the possibility of observing many neurons in one experimental trial, and many brain regions simultaneously.

A note on basic terminology: “SiNAPS box” refers to the hardware component that generates the control signal of SiNAPS probes “SmartBox Pro” refers to the latest smart data acquisition system box, i.e., the “box.” “Radiens Allego refers to the software application that interfaces with and controls the hardware box.

IMPORTANT: Please check the NeuroNexus website at

<https://www.neuronexus.com/products/electrode-arrays/activus-sinaps/> for regular updates of SiNAPS electrodes.

Note: In order to ensure you have the latest version of the applications, please visit our downloads page at the link below.

<https://www.neuronexus.com/radiens-allego-download-page/>

For questions regarding operation of the system or if you have any issues, please email us at support@NeuroNexus.com. You can also call us at +1.734.913.8858.

Hardware Components

Image below showcases our data acquisition setup, with the exception of the SiNAPS probes that are required to conduct experiments.




NeuroNexus SmartBox Pro





Note: SmartBox Pro uses 9 V AC power adapter.

Front Panel

	Power LED/Button	Indicates system status.
ABCD	Headstage Input Ports	Proprietary HDMI-style ports for connecting Smartlink headstages to Ports A-D.
RE	Reference Electrode	Banana jack for connection to Reference Electrode (potentiostat/galvanostat).
CE	Counter Electrode	Banana jack for connection to Counter Electrode (potentiostat/galvanostat).
V/I	Potentiostat/Galvanostat Port	SmartBox Pro MUX adapter to provide access to potentiostat and galvanostat functions.



Back Panel

DI/DO	Digital/Analog Inputs and Outputs	BNC connectors for connecting auxiliary signals from other instruments.
	System Ground	Banana jack for connecting the box to a low-noise earth ground point.
USB 3.0	USB 3.0 Connector	Connect to a USB 3.0 port on PC/Mac using the supplied USB 3.0 cable.
9VDC	DC Power Input	Use only the supplied AC power adapter to provide the required DC voltage input.
	Audio Output	Stereo audio Aux jack for connection to speakers to monitor the Analog Output (AO) channels.

SiNAPS interface box




Note: SiNAPS interface box uses 5 V AC power adapter

Front Panel

SMARTBOX PRO	SiNAPS system HDMI link cable	HDMI connectors for connecting SiNAPS interface box to port A of SmartBox Pro.
ACTIVUS-SINAPS	SiNAPS system HDMI link cable	HDMI connectors for connecting SiNAPS interface box to port A of SmartBox Pro.

Back Panel

5 VDC	DC Power Input	Use only the supplied AC power adapter to provide the required DC voltage input.
	System Ground	Banana jack for connecting the box to a low-noise earth ground point.

SiNAPS-SmartBox Pro HDMI cable



SiNAPS probe interface cable

SiNAPS cable attached to IST-2mm w/ bracket to hold SiNAPS probe packages. Please note that you need to connect mezzanine board to this as shown in below image.



Mezzanine adapter



- Blue USB 3.0 cable





Connect the SiNAPS–SmartBox Pro to Your Computer

- Unpack the SmartBox Pro unit and SiNAPS Kit from their packaging and place them on a secure surface with adequate ventilation.
- Plug the SmartBox Pro AC adapter into a standard (110–220 V) wall outlet and connect its DC power output pin into the 9 VDC power port of the SmartBox Pro unit.
- Plug the SiNAPS Interface Box AC adapter into a standard (110–220 V) wall outlet and connect its DC power output pin into the 5 VDC power port of the SiNAPS Interface Box
- Using SiNAPS–SmartBox Pro HDMI Cable, Connect SmartBox Pro port on the SiNAPS box front case to the Port A on SmartBox pro.



- Connect the USB cable to the back panel of SmartBox Pro and a USB 3.0 port on your computer
- With the computer on and connected to the internet, press and release the SmartBox Propower LED switch. Your computer should indicate that it has detected new hardware (Opal Kelly XEM6310-LX45). The necessary drivers should be automatically installed with no further action necessary. If the driver does not install or the Allego recording software cannot detect your SmartBox Pro unit, try downloading and installing the driver from here: <https://www.neuronexus.com/radiens-allego-download-page/>
- Connect SiNAPS Probe Interface Cable (with mezzanine adapter) to Activus–SiNAPS port on SiNAPS interface box.



- Optional - use a banana cable to attach the SmartBox Pro ground port to a low-noise earth ground. This may be beneficial for removing extrinsic noise on the signal lines depending on the laboratory environment.



Computer Requirements

The computer system requirements include:

- CPU - Intel i7-6700k 4-core or equivalent
- 16 GB RAM minimum
- Storage: SSD
- USB 3.0 or better

Note: we recommend utilizing the following specification for SiNAPS or systems planning to use more than 256 channels:

- CPU: Intel Xeon Silver 4214R, or equivalent
- 32 GB RAM

Note: Radiens platform performance is most impacted by single thread CPU performance.

Download and Install Radiens Software Application

Radiens™ 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. To ensure you have the most up-to-date version of Radiens™ Apps, please visit the dedicated Radiens™ page at the following link:

On the Radiens™ page, you can easily locate the appropriate version of the software for your operating system. Simply select the corresponding link and initiate the download process by clicking on the “Download” button. This will facilitate a smooth transfer of the software to your system, allowing you to leverage its full capabilities in analyzing data.

Thank you for choosing Radiens™ as your software solution, and we appreciate your commitment to enhancing your research endeavors.



Windows

Launch the installer.

If you receive a warning against running an unrecognized app, select “More Info”, then “Run anyway”.

Allego will launch after the installation is complete.

 **macOS**

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.

 **Linux**

On a Debian-based distro (Debian, Ubuntu, *buntu, Linux Mint, etc):

Download the .deb file

Run 'sudo apt install <path to .deb>'



What is a Radiens™ ID

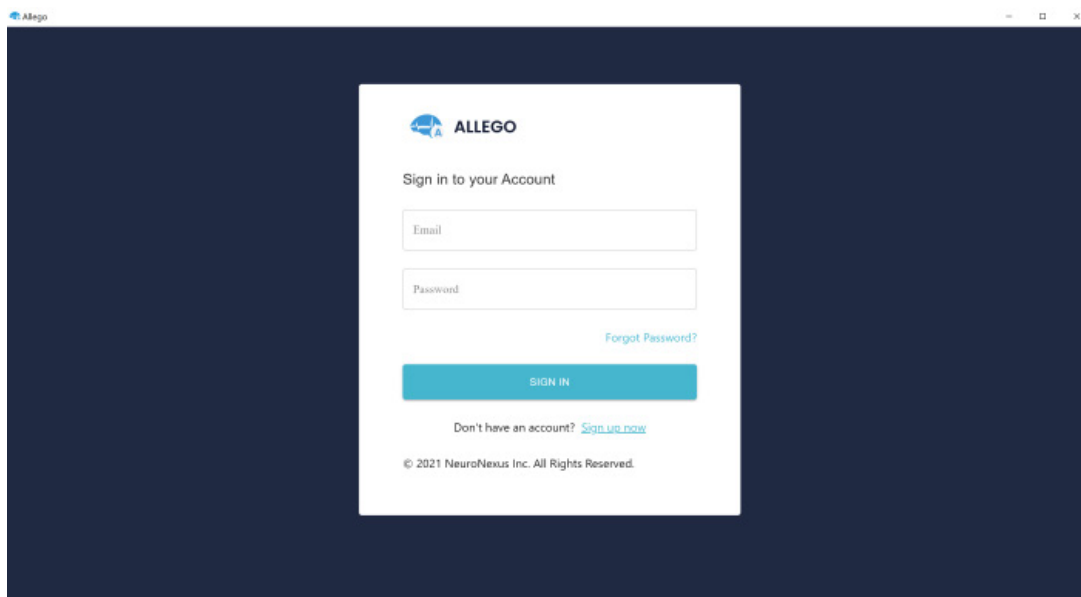
Your Radiens™ serves as your unique identifier and is linked to your email address. It is important to utilize the same Radiens™ ID across all Radiens products to ensure that they are associated with a single, consolidated account. This approach allows for a seamless and unified experience.

Having a Radiens™ ID is vital for maintaining a secure and personalized experience when using Radiens™ apps and services. It is also a prerequisite for purchasing and utilizing Radiens™ products. By using your Radiens™ ID, you can access exclusive features, personalized settings, and take advantage of the full range of benefits offered by Radiens™.

Create or Updated Your Radiens™ ID

To launch Allego, please locate the application icon on your device and click on it. Once you have launched Allego, it will begin the initialization process. Please wait for Allego to finish initializing before proceeding.

Once Allego has finished initializing, 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. Fill in the required details accurately and follow any additional instructions or prompts that may appear on the screen.



Note: The email address that you enter will be your Radiens ID, so use a frequently used address that you will remember.

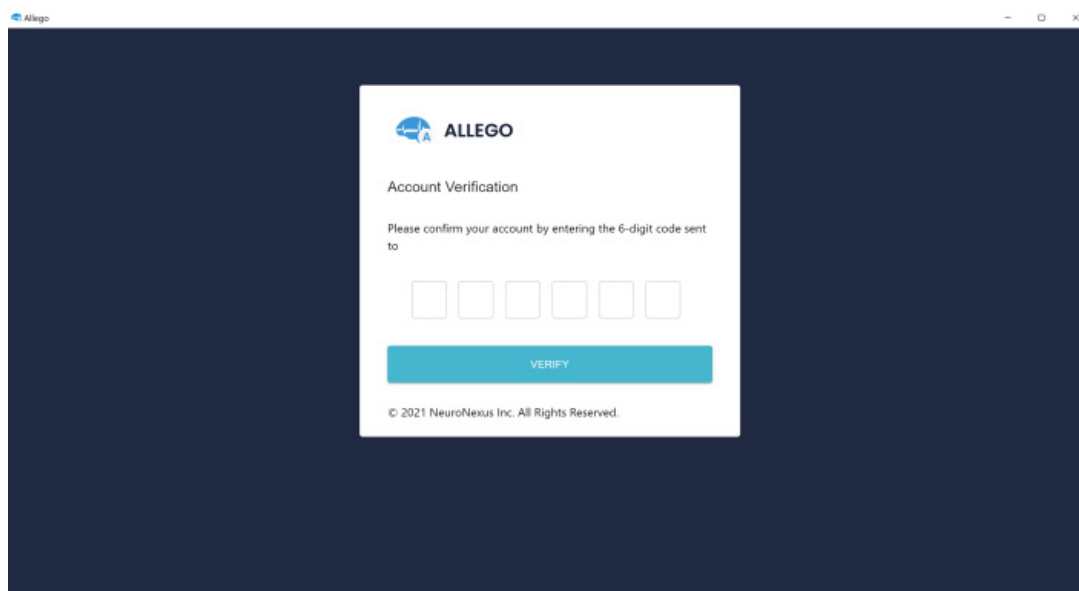


The screenshot shows a web browser window with the ALLEGRO logo at the top left. The main heading is "Create your Account". Below this, there are three input fields: "Name *", "Email Address *", and "Organization *". A blue "CONTINUE" button is positioned below the fields. Underneath the button is the text "OR" and a link "Already have an account? [Sign In](#)". At the bottom, there is a copyright notice: "© 2021 NeuroNexus Inc. All Rights Reserved."

- On the new page, confirm password and submit.

The screenshot shows a web browser window with the ALLEGRO logo at the top left. The main heading is "Confirm Password". Below this, there are two input fields: "Password" and "Confirm Password". Below the fields, there are three password requirements listed with arrows: "→ Min 8 characters long", "→ Must contain uppercase and lowercase characters", and "→ Must contain a number and a special character". A blue "SUBMIT" button is positioned below the fields. Underneath the button is a link "Back to [Sign In](#)". At the bottom, there is a copyright notice: "© 2021 NeuroNexus Inc. All Rights Reserved."

- You will receive a verification email with a 6-digit code to verify and confirm your account. You may have to wait for some time before receiving the email.



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

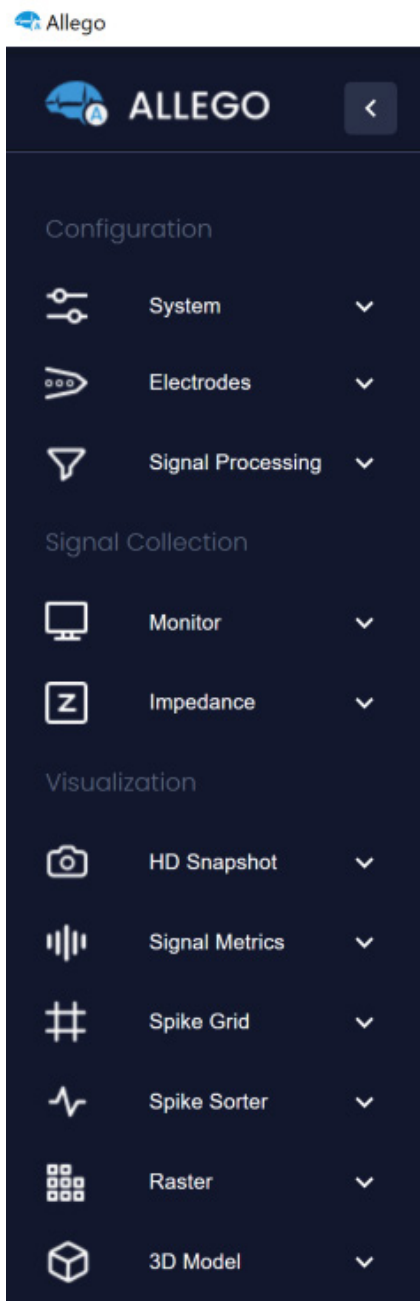
By creating an account, you will gain access to the full functionality and features of Allego. It will allow you to leverage its advanced real-time signal processing capabilities and seamlessly integrate with a variety of data acquisition systems.

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.



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 will now be presented in the form of a list. 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 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.


 **Electrodes:** Enables the selection of connected probe designs, as well as determining the specific port and SmartLink headstage to be used.

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.


 **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.

 **Impedance:** Impedance testing

Probe impedance can be measured at 1kHz 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 high 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 investigation 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.



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

The Status bars are positioned at the top and bottom of the window, providing convenient access to important features and real-time information.

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 easily accessible from this top bar, allowing you to quickly customize and configure various aspects of the software.

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



The bottom Status bar, as depicted in the image below, provides valuable information regarding the connected headstage and channel count.


Within the bottom Status bar, you will find details related to the currently connected headstage, which enables you to identify the specific hardware being used for your recordings. Additionally, the channel count information informs you of the number of channels available for data acquisition.

This information on the bottom Status bar ensures clarity and awareness of the hardware setup, allowing for accurate monitoring and interpretation of the recorded data.





Save Workspace and User's Profile


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

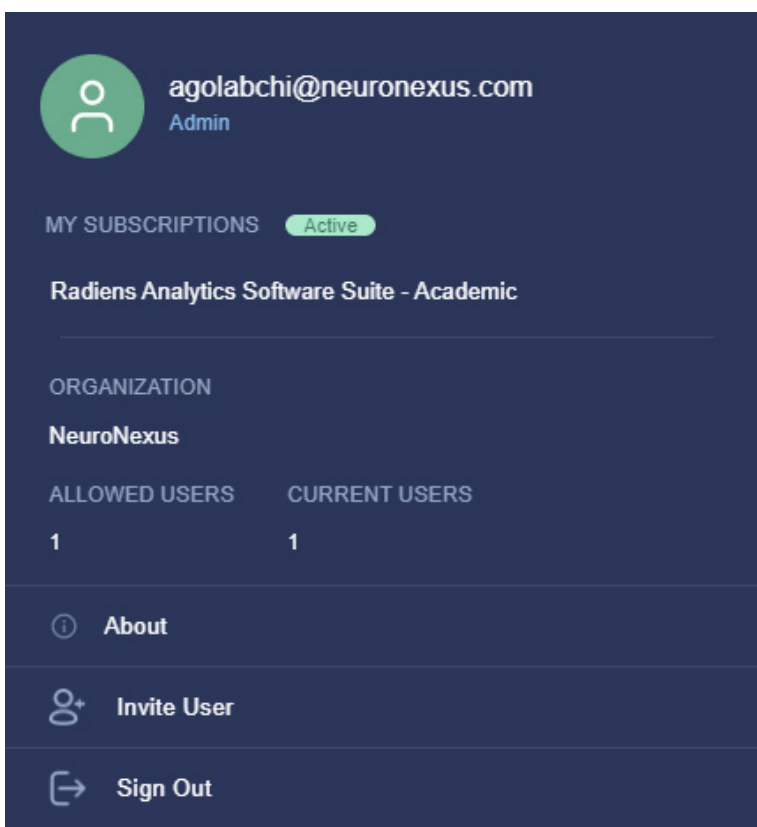
Save Workspace Button  : Clicking this button saves the current configuration of your workspace. It allows you to preserve the arrangement of tabs and windows, making it easier to restore the layout at a later time.



Trashcan icon  : Selecting a window/tab and clicking on the trashcan icon removed the selected window from the saved workspace. This feature enables you to declutter your workspace by removing unnecessary or unused windows.

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.

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.



The screenshot shows a dark blue user profile menu. At the top, there is a green circular profile icon, the email address **agolabchi@neuronexus.com**, and the role **Admin**. Below this, the text **MY SUBSCRIPTIONS** is followed by a green **Active** status indicator. The main subscription is listed as **Radiens Analytics Software Suite - Academic**. Under the heading **ORGANIZATION**, the name **NeuroNexus** is displayed. A table shows **ALLOWED USERS** and **CURRENT USERS**, both with a count of **1**. At the bottom, there are three menu items: **About** (with an information icon), **Invite User** (with a person plus icon), and **Sign Out** (with a door icon).

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.

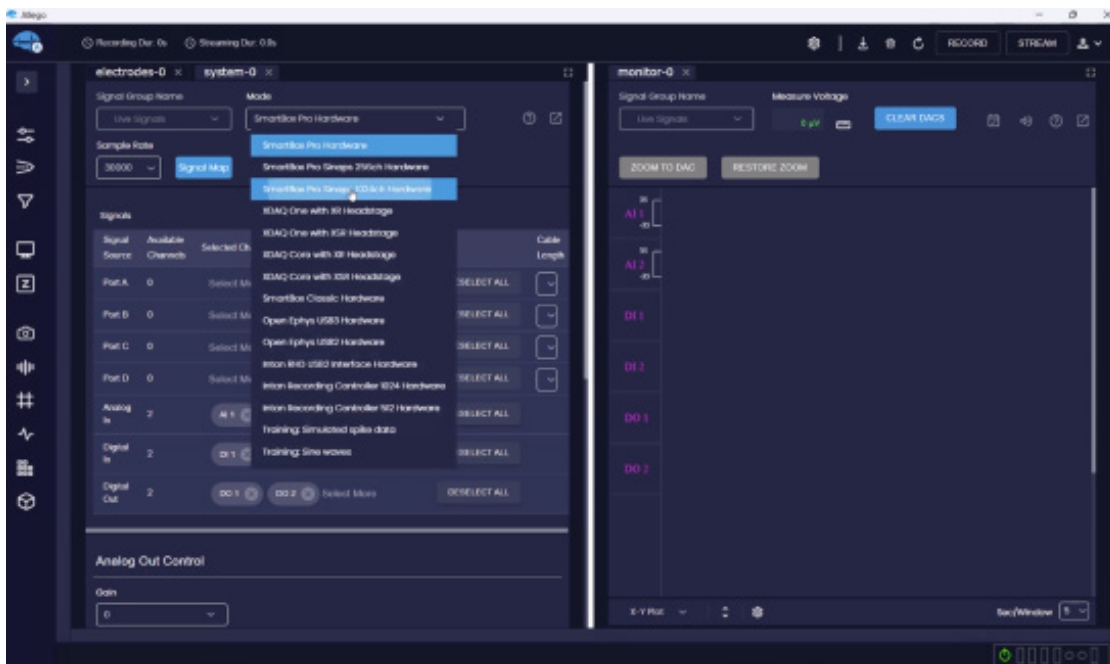
With the Dashboard's configurability, you can create a tailored workspace that maximizes your productivity and enhances your overall user experience in Allego.



Checking Hardware Connection

With the SmartBox Pro on and connected to SiNAPS interface box, your software recognizes connected hardware automatically.

Navigate to the System tab and select SmartBox Pro SiNAPS 256 ch/1024 ch Hardware from the Mode drop down.



Note: The sample rate on the System view is set to 20 kHz.

After selecting the appropriate mode, it is important to calibrate your probe before using it *in vivo*. To do so, follow the steps for the “Wet Test”.



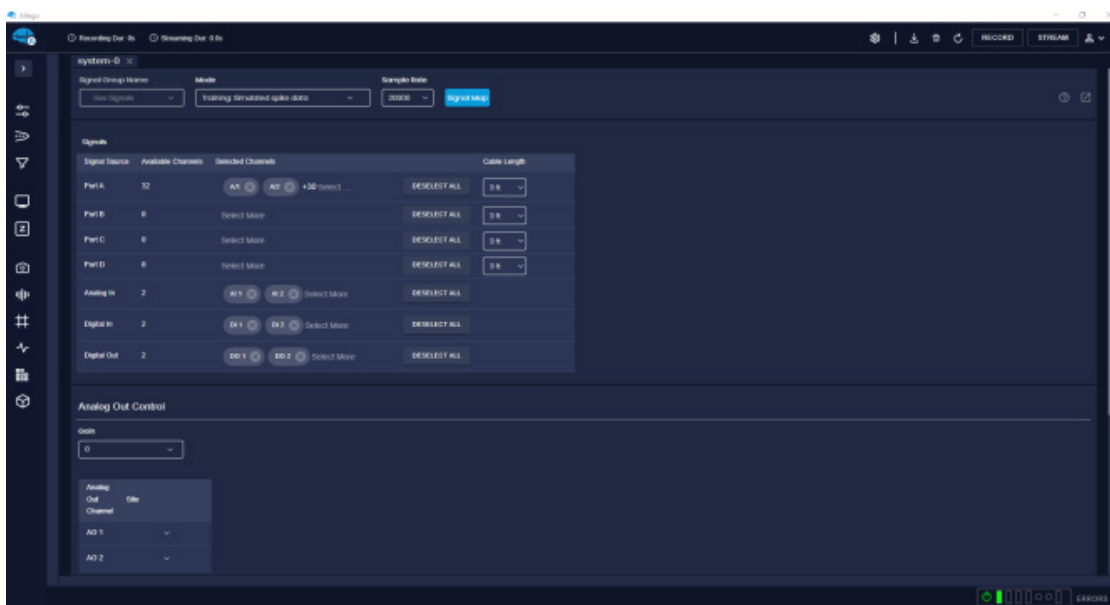
Training and Supporting Resources

We recommend visiting the [Radiens web page](#) or watch instructional videos 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.

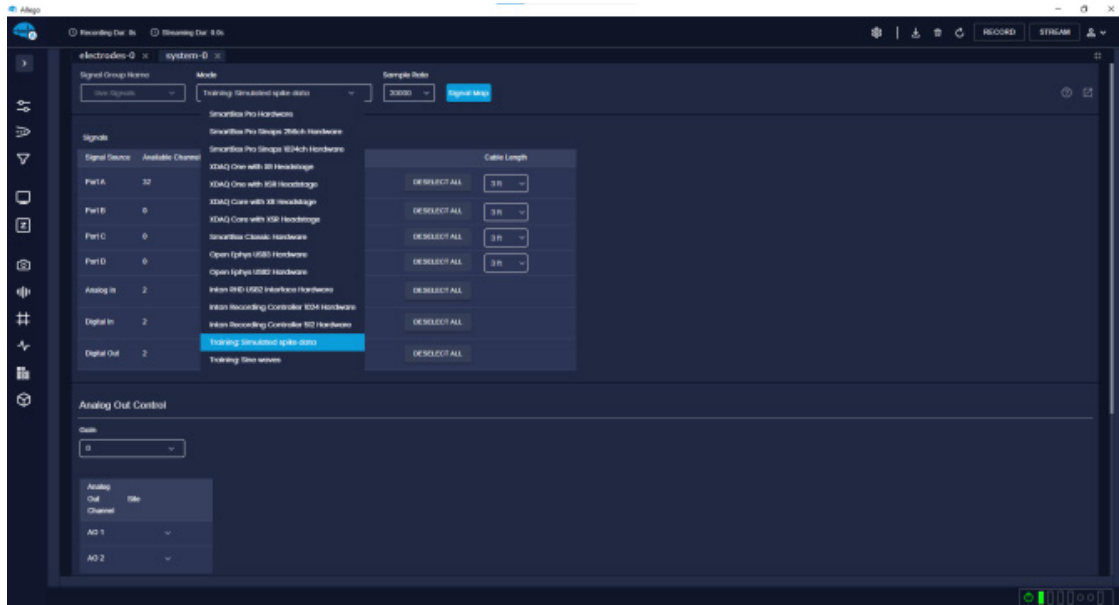
To familiarize yourself with Allego prior to using it in conjunction with the SmartBox Pro, follow these steps:

- Select the System Icon
- From the 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.



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.





Update Firmware Procedure

Updating the firmware requires two pieces of software provided by NeuroNexus:

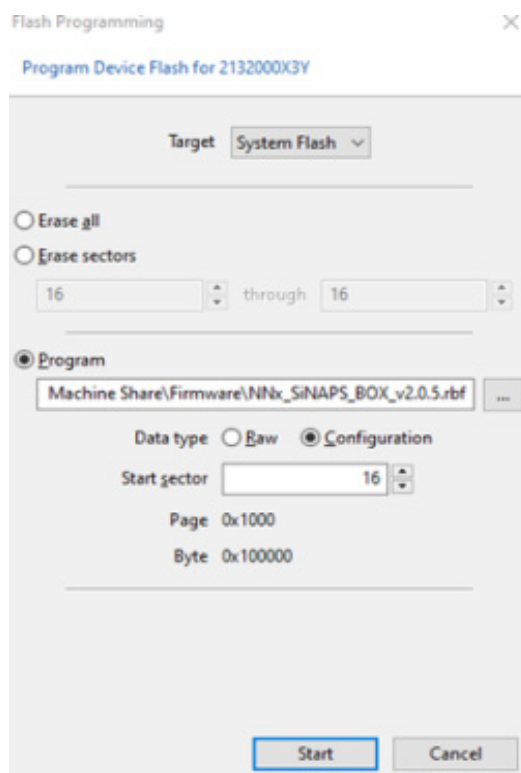
1. Firmware file

2. Opal Kelly FrontPanel tool

- Unplug the power supply from the SiNAPS interface box.
- Remove the top and back plates of the SiNAPS interface box to gain access to the small FPGA board which is connected to the large PCB inside. This will require a TR10 Torx bit or a similar sized hex key.
- Connect the blue USB cable used with the SmartBox Pro to the connector on the FPGA (figure below), and the other end to the computer. This is a USB-A to USB Micro-B cable. If you do not have this cable, please contact us.
- Reconnect the power supply to the interface box.
- Unzip and install the Opal Kelly Front Panel app from [here](#). The Model and Name of the opal Kelly board will appear on the main GUI. Run once complete.
- Click the “Program flash device”, the right-most button (figure below).



- In the new window, set the “Flash Programming” menu as follows:
 - Target: System Flash
 - Program: select the .rbf file from [here](#)
 - Data Type: Configuration
 - Start Sector: 16



- Click Start. The progress indicator on the bottom will state when the process is complete after 1-2 minutes.
- Close FrontPanel.
- Power-cycle the SiNAPS interface box to force it to load the new firmware to complete the update.

Wet Test

This test allows for checking electrical and electrodes performances of the device. We strongly advise users to perform this test in dark conditions to avoid undesired photoelectric effects, and in an electrical/electromagnetic clean environment.

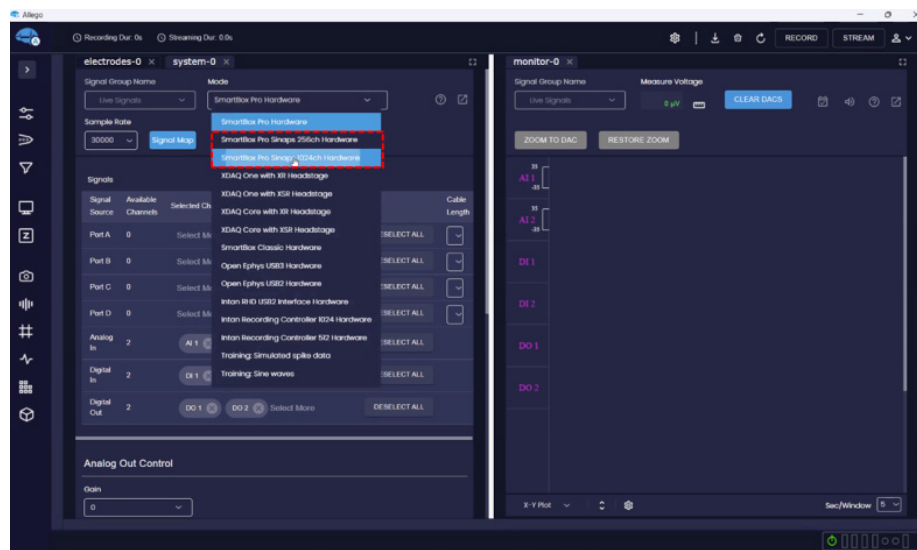
These are two exchangeable procedures, manual and automatic, to perform the wet test.



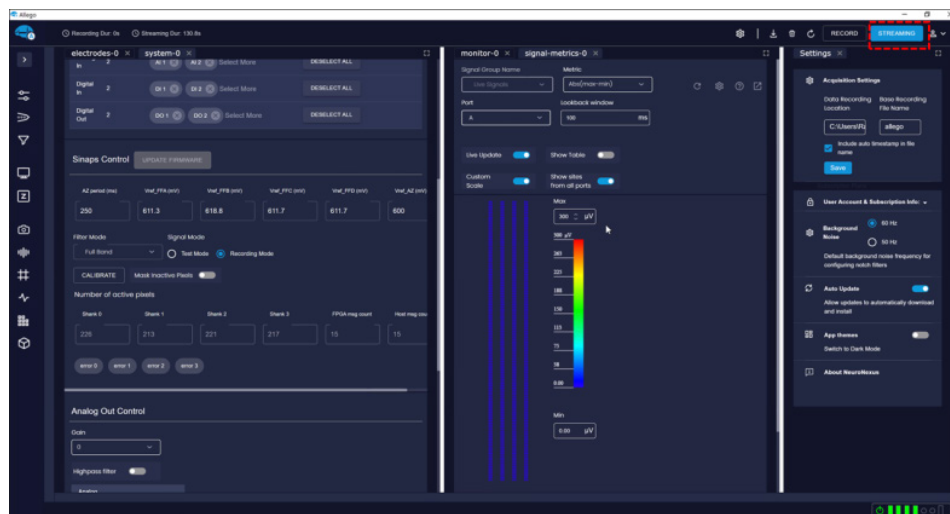
Calibration Procedure

Follow the steps on how to connect the SiNAPS-SmartBox Pro to your computer from the “Setup Hardware Connection” section. Then:

- Connect the probe to the SiNAPS probe interface cable
- Prepare a container with saline buffer (e.g. saline solution, PBS)
- Insert the probe together with a reference wire into the saline buffer
- Start Radiens Allego and login
- Turn on the SmartBox Pro
- In Allego, from the mode drop down, select the appropriate hardware mode (256 or 1024) in the System Tab, depending on the type of SiNAPS probe that is physically connected.



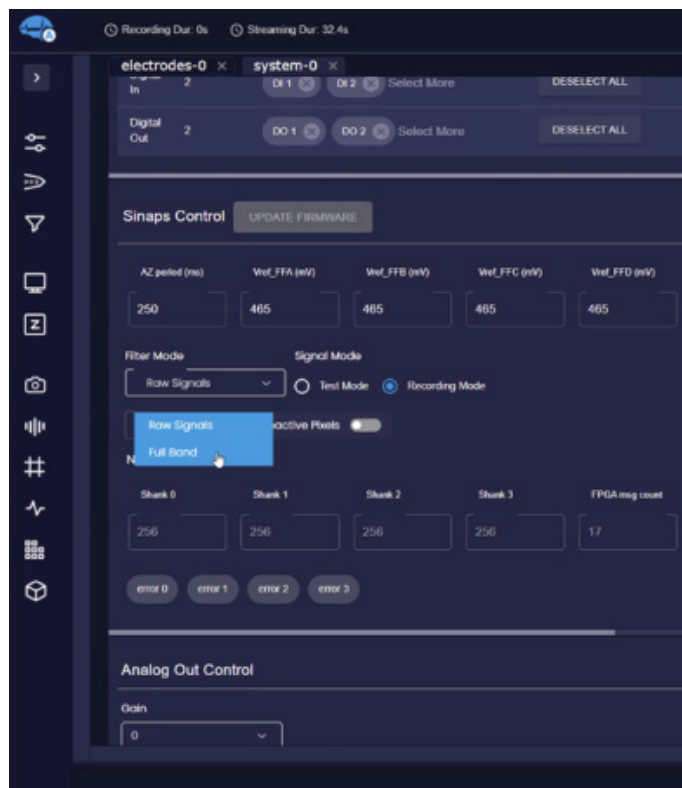
- Open Signal metrics tab and have it side-by-side with the system tab, and click on streaming button.



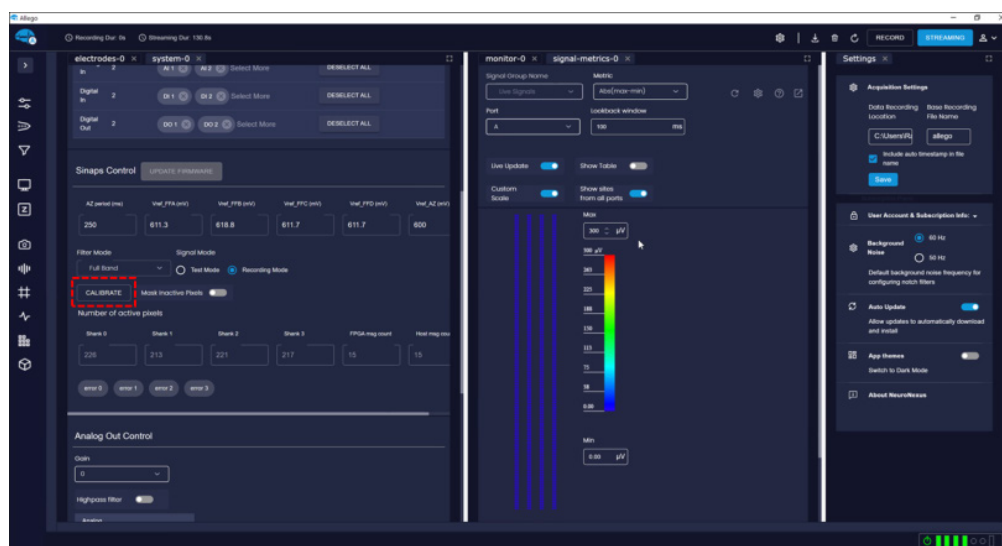


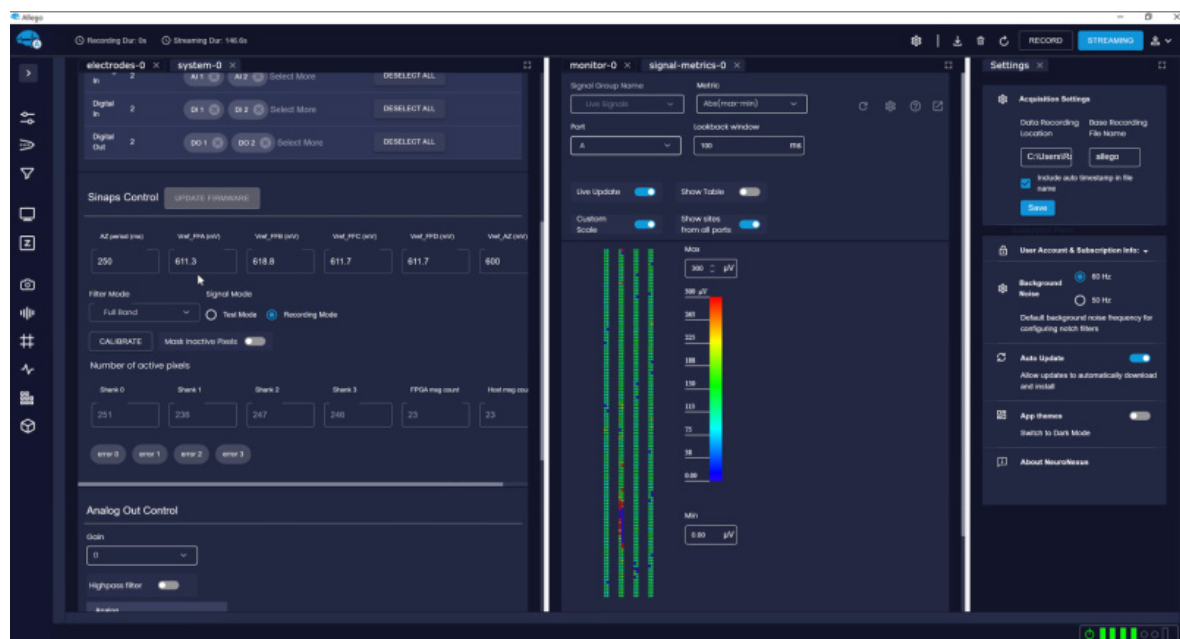
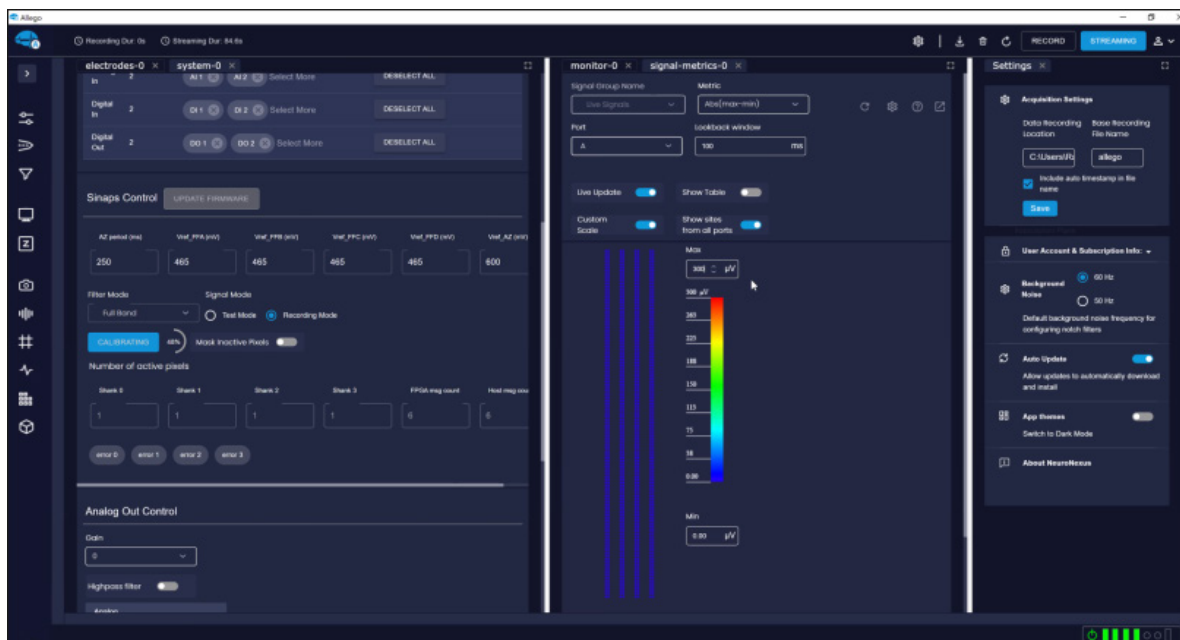
NOTE:

- Filter Mode should be set to Full band.
- Set AZ Period to 250 ms
- Set Vref_AZ to 600 mV

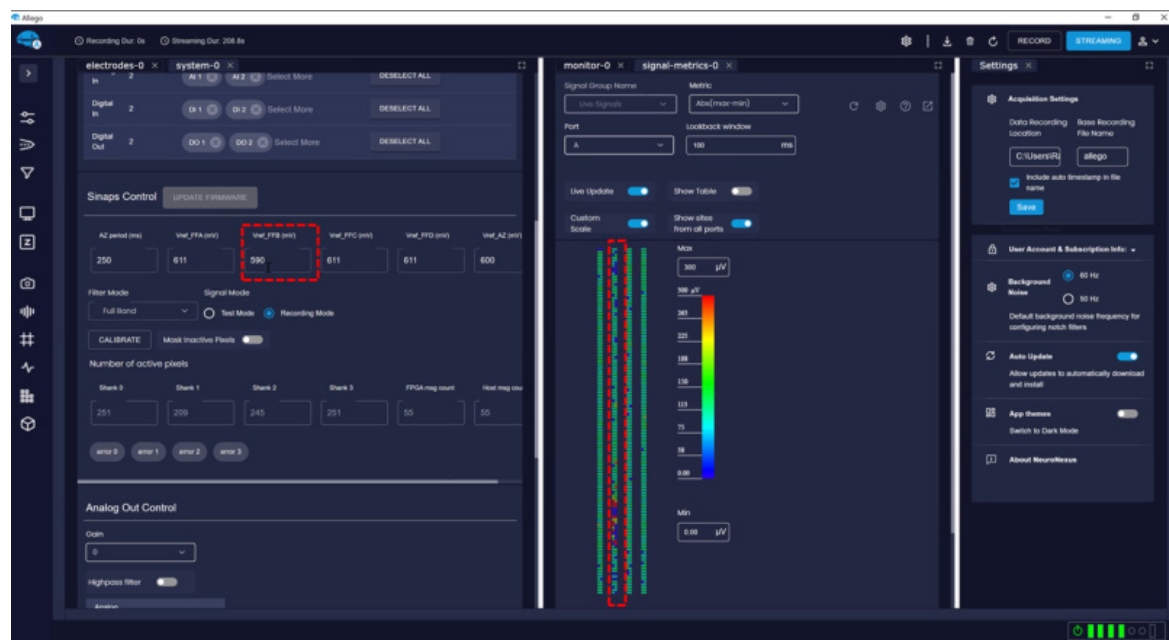
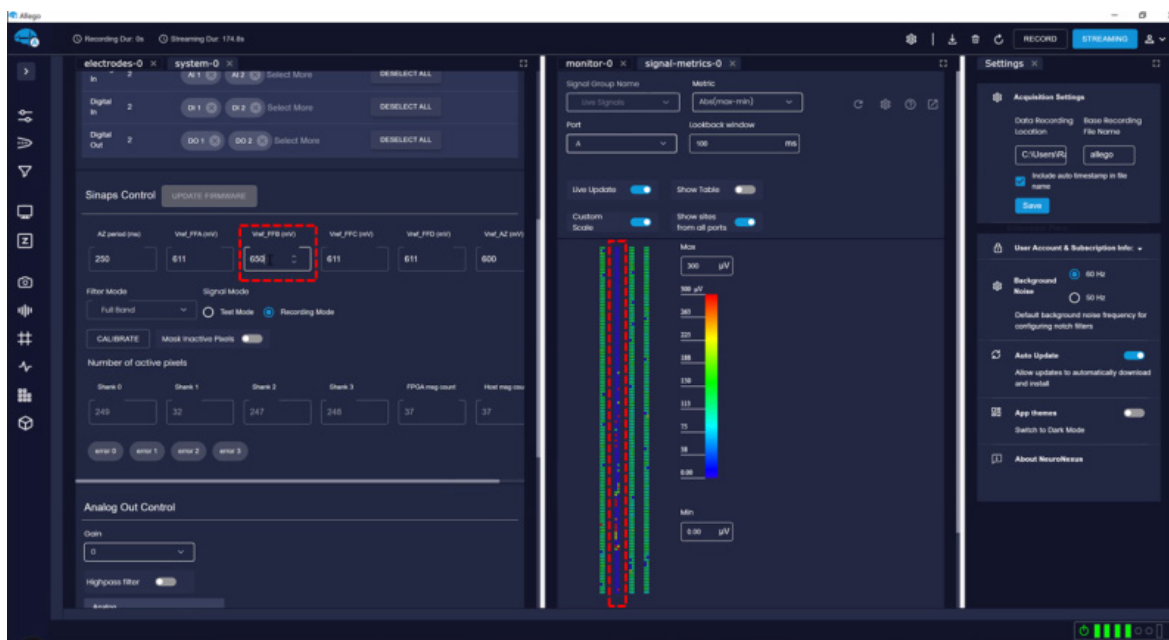


- Then, select "Recording Mode" from Signal Mode, and click on "CALIBRATE" button to start calibrating and wait until the process ends. The process can take up to 2 minutes.



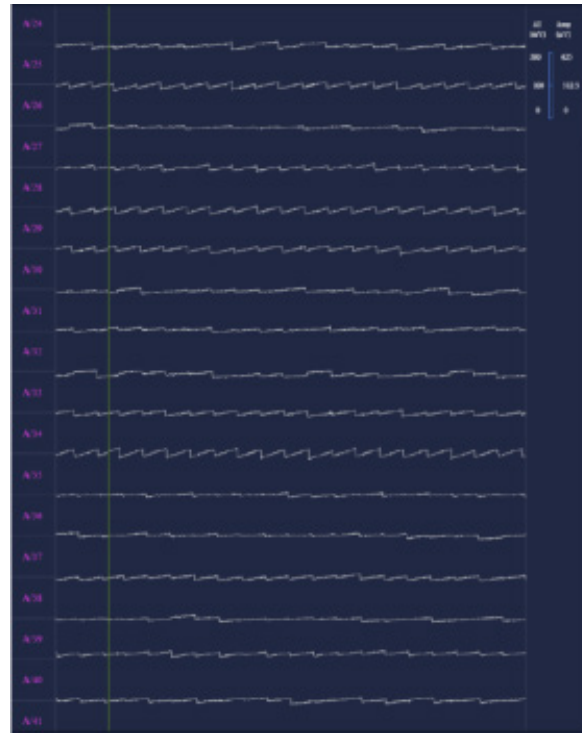


- If necessary, adjust Vref_FFA/B/C/D manually. For instance, to address dead pixels on shank 2, consider changing Vref_FF8 as shown in the following example.

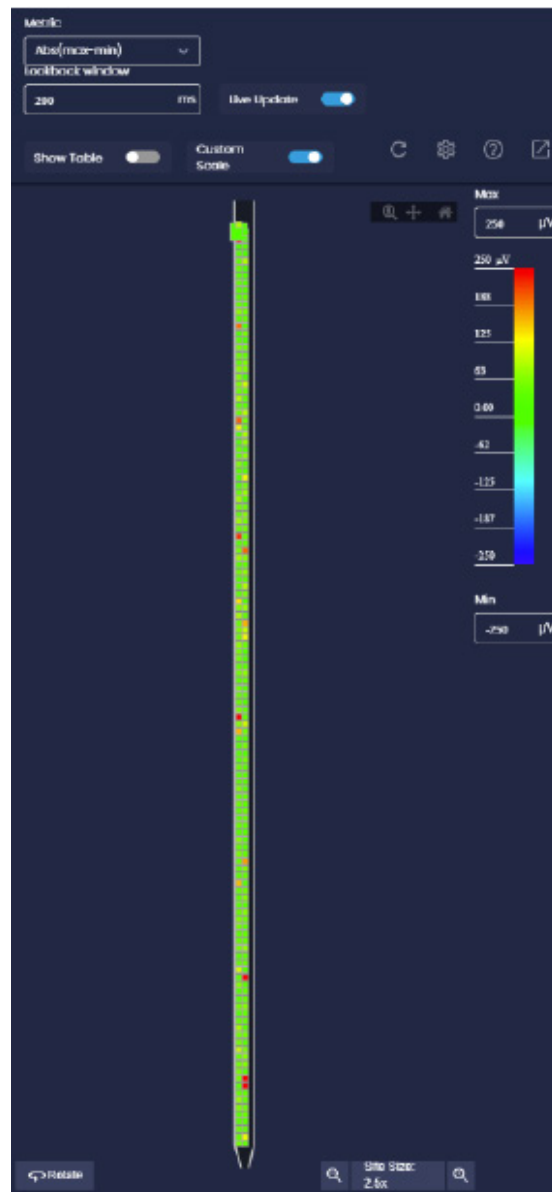


Expected Result

All the channels show a stable signal (sawtooth in a case Raw Filter mode is selected)



The probe color map has to be homogeneously colored. Few electrodes might show different dynamics with respect to the overall standard behavior.



After the probe insertion the V_{ref_FFA} has to be adjusted over time (manually or performing a new self-calibration procedure), because of the different wettability degree of the electrode interface, until it reaches its stable condition (this might require several minutes depending on the device hydrophilicity level).

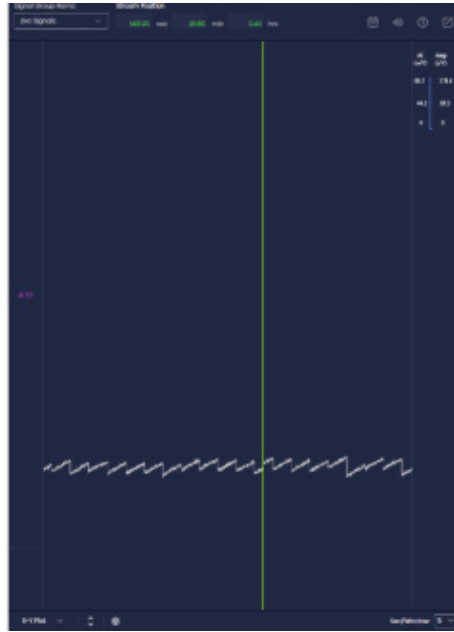
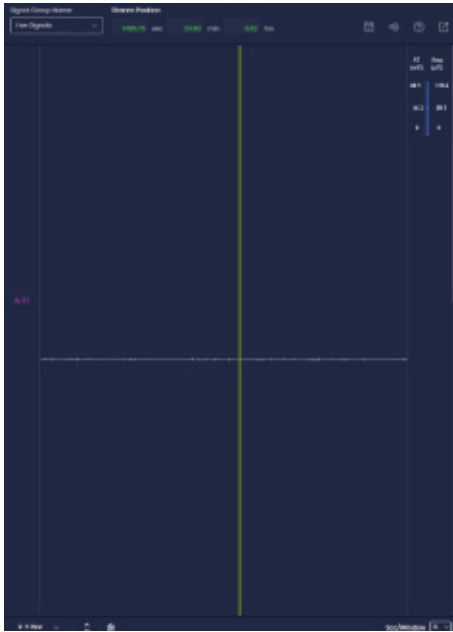


Troubleshooting

Issue	Cause	Solution
Noisy signal	<ul style="list-style-type: none"> Noisy GND/Ref electrode High hydrophobicity on electrode sites 	<ul style="list-style-type: none"> Properly ground the system Wait for the electrode surface to become less hydrophobic or do a hydrophilization treatment to the device
The probe does not work in homogeneous way (same Vref_FFA for the whole device pixels)	<ul style="list-style-type: none"> High hydrophobicity on some electrode sites Defect on metal deposition of some electrode sites 	<ul style="list-style-type: none"> Wait for the electrode surface to become less hydrophobic or do a hydrophilization treatment to the device Change device and contact NeuroNexus regarding next steps
Probe shows non-linear discharges (e.g. oscillations) over the sawtooth signals	Photoelectric artifacts	<ul style="list-style-type: none"> Perform test in darker light conditions
Signals are saturated on high ADC levels (i.e. >1600) (figure below)	Vref_FFA is too low	<ul style="list-style-type: none"> Increase Vref_FFA value
Signals are saturated on low ADC levels (i.e. <800) (figure below)	Vref_FFA is too high	<ul style="list-style-type: none"> Decrease Vref_FFA value
Digital glitches on the sawtooth traces	HDMI cable related issue	<ul style="list-style-type: none"> Shorter/higher quality HDMI cable
Probe does not respond	Unknown issue	<ul style="list-style-type: none"> Check all the wirebonding connections, check the analog voltages (VVD, Vref_FFA, Vref_AZ) on the PCB, check soldering on the PCB, check issues related with the DAQ



The effect of Vref_FFA variation on a raw trace (from Low Vref_FFA to High Vref_FFA values) are shown below. On the left and right panels, the signal is saturated. In the middle panel the amplifier is performing correctly.





***In vivo* experiments**

This procedure highlights the best practice to follow before, during, and after an *in vivo* experimental session.

Surgical Procedure

Before starting your experiment, please check our suggested surgical guide for acute and chronic experiments here:

<https://www.neuronexus.com/files/technicalsupportdocuments/General-Surgery-Guidelines.pdf>

Follow the steps on how to connect the SiNAPS-SmartBox Pro to your computer from the “Up and Running” section. Then, proceed with the wet test before implanting the SiNAPS electrode *in vivo*

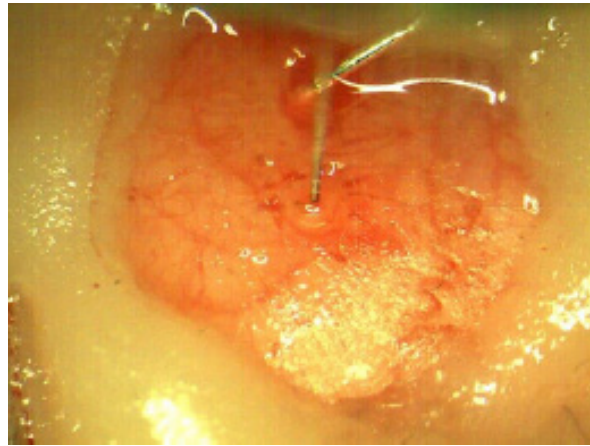
- Head fix the animal subject on the stereotaxic frame
- Perform either a big square craniotomy or two small nearby craniotomies



- Connect the probe to the system
- Insert the reference wire (already connected/soldered to the GND pin of the PCB headstage of the probe) made of silver or platinum for some millimeters inside the tissue and secure it



- Approach the probe tip to the insertion site



- Insert the device up to the desired depth



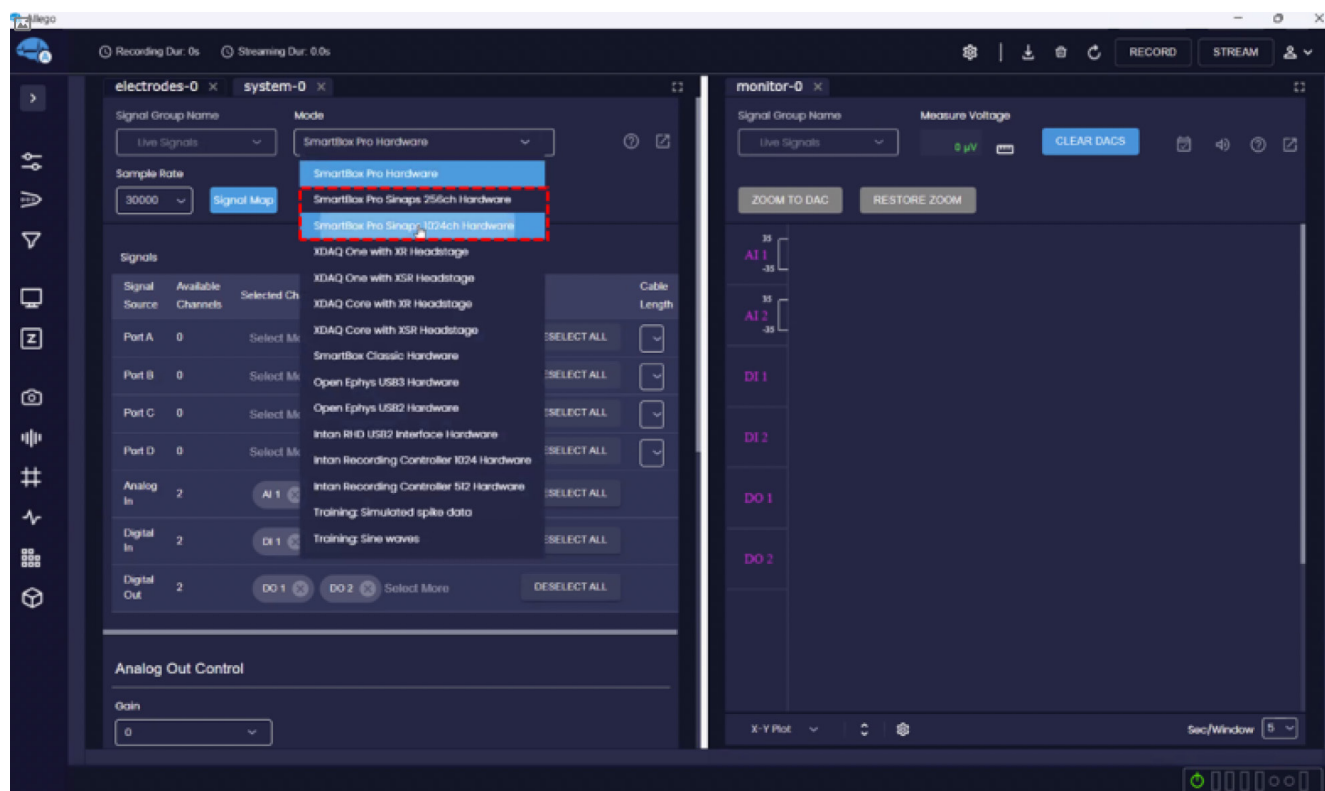
- Wait at least 15-20 minutes to let the electrode surface stabilize
- Now proceed with the calibration procedure mentioned in the Wet Test section and the *in vivo* section.



Calibration Procedure

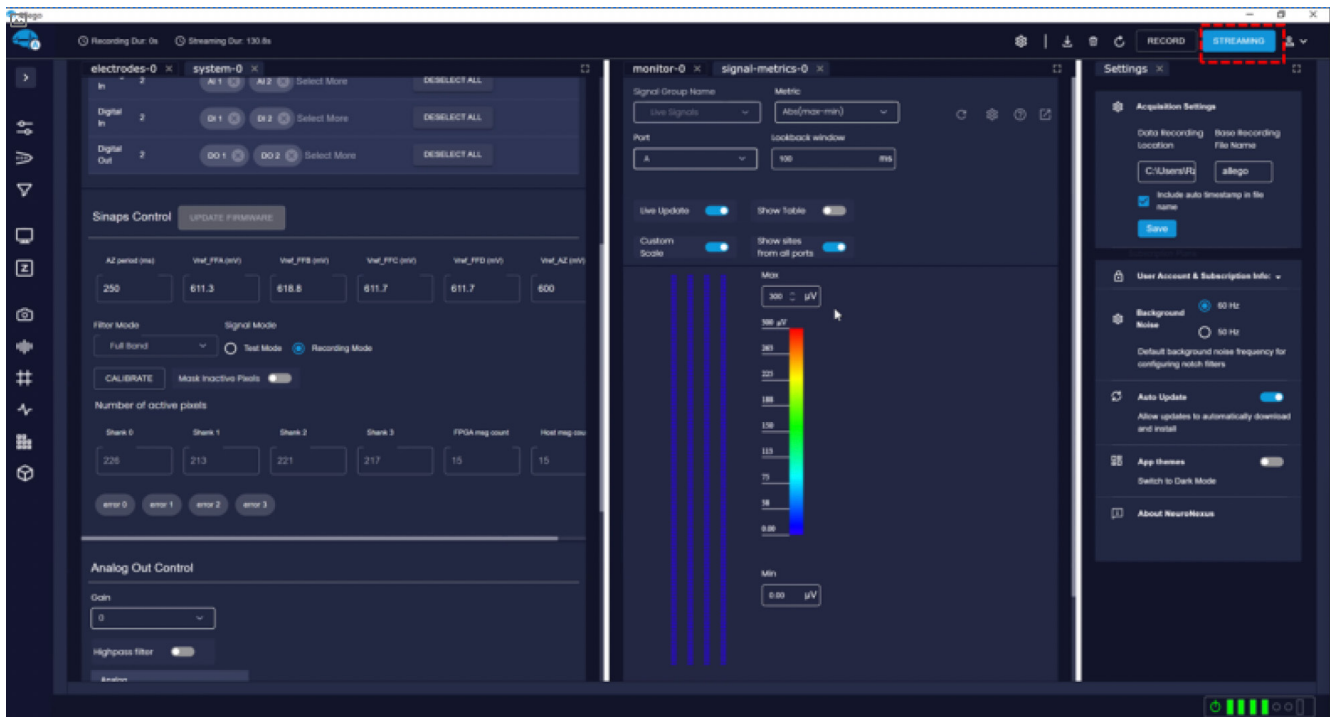
Follow the steps on how to connect the SiNAPS-SmartBox Pro to your computer from the “Setup Hardware Connection” section. Then:

- Connect the probe to the SiNAPS probe interface cable
- Prepare a container with saline buffer (e.g. saline solution, PBS)
- Insert the probe together with a reference wire into the saline buffer
- Start Radiens Allego and login
- Turn on the SmartBox Pro
- In Allego, from the mode dropdown, select the appropriate hardware mode (256 or 1024) in the System Tab, depending on the type of SiNAPS probe that is physically connected.



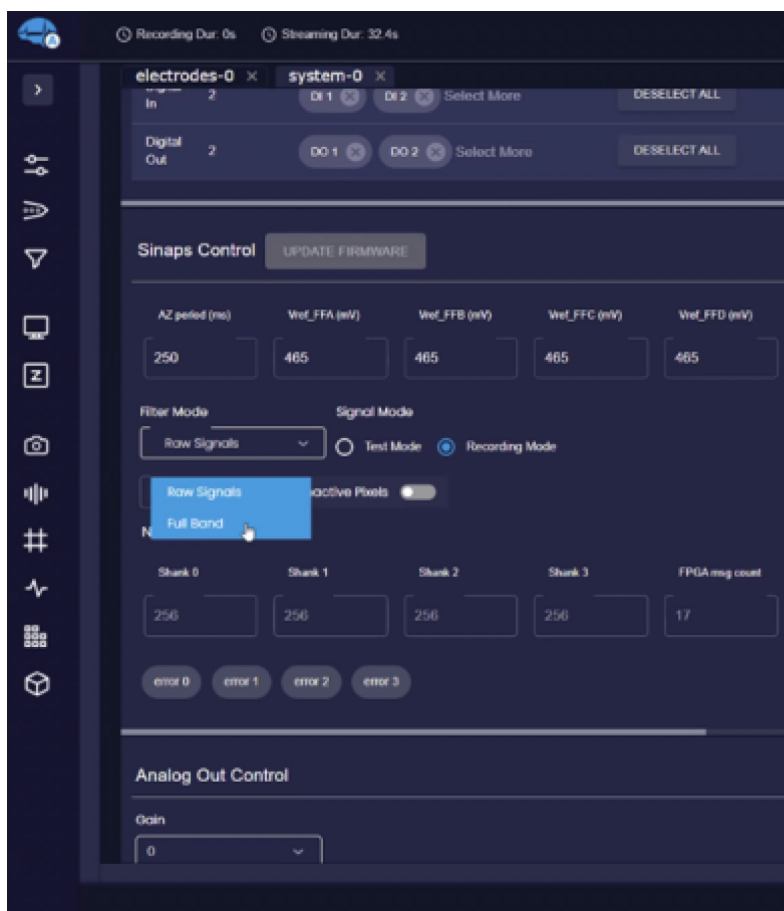


- Open the Signal metrics tab and have it side-by-side with the system tab, and click on the streaming button.

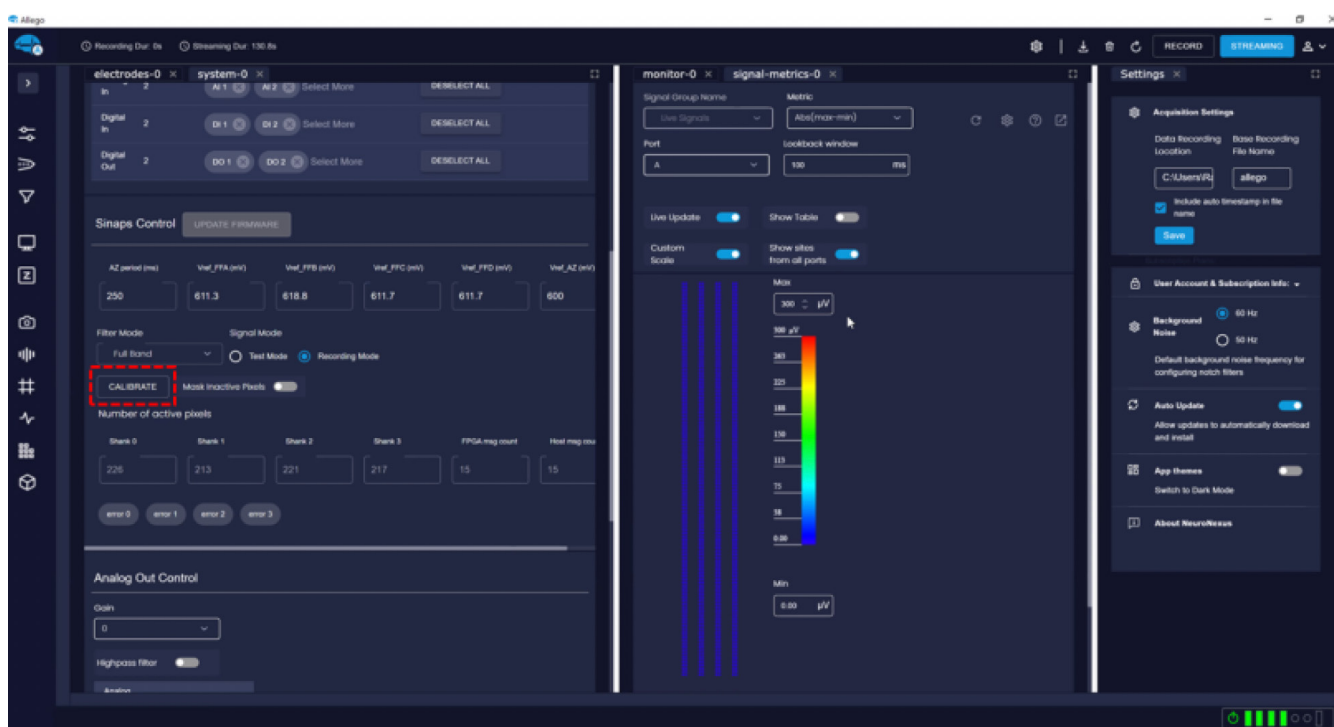


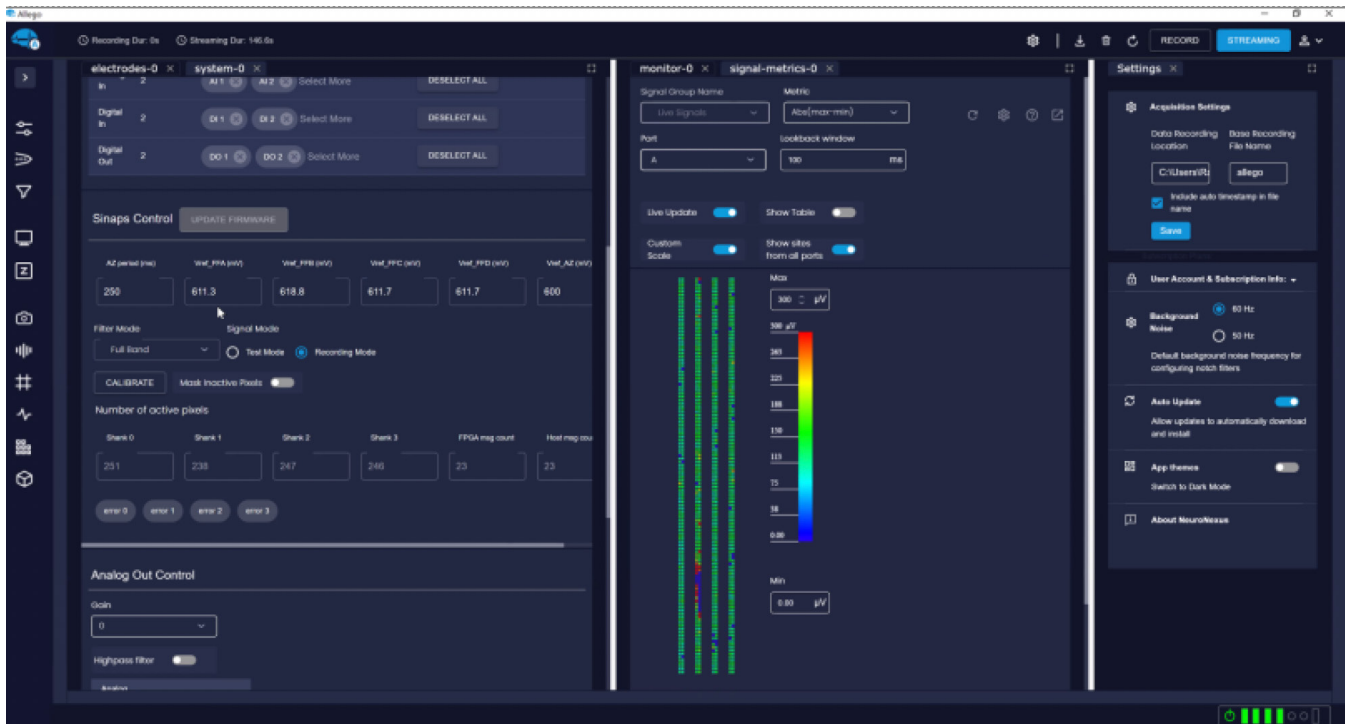
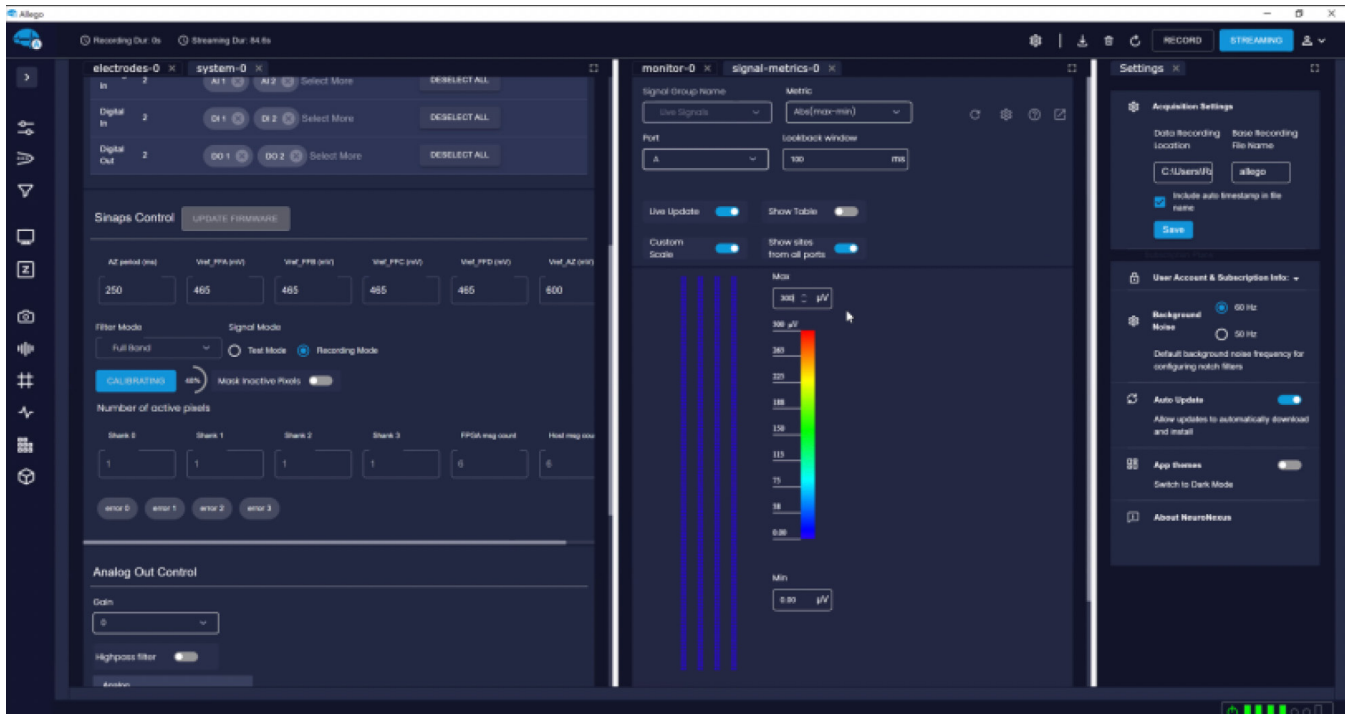
Note:

- Filter Mode should be set to Full band.
- Set AZ Period to 250 ms
- Set Vref_AZ to 600 mV



- Then, select "Recording Mode" from Signal Mode, and click on the "CALIBRATE" button to start calibrating and wait until the process ends. The process can take up to 2 minutes.



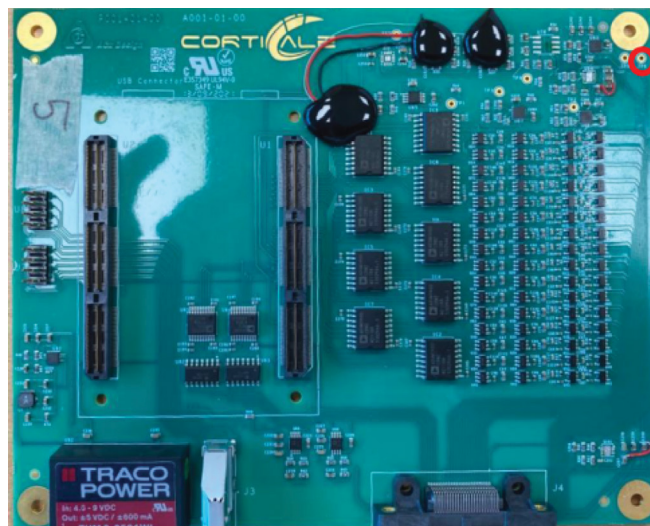


Note: If the animal is well referenced it is expected to have the device working for $V_{ref_FFA/B/C/D} > 600\text{mV}$ and $< 900\text{mV}$

- Whether necessary to remove external noise by properly grounding the animal and/or the equipment around it (for very high noise you may need to recalibrate the device after noise removal)
- In the first 15-30 minutes from the insertion continuously check the probe performances and adjust $V_{ref_FFA/B/C/D}$ whether needed, either manually or automatically by restarting the self-calibration procedure (it is expected that $V_{ref_FFA/B/C/D}$ should decrease over time because of a better electrode-tissue coupling and thanks to the increased wettability of the electrode)

Tips and Tricks

- Letting the device for some hours sit in a wet environment (e.g. saline, PBS, neurobasal, ...) prior insertion, decrease the time to $V_{ref_FFA/B/C/D}$ stabilization.
- Adding saline solutions on top of the animal brain might change the $V_{ref_FFA/B/C/D}$ needed for making the probe operative because of the different coupling between the reference and the tissue. Once the saline dries out the $V_{ref_FFA/B/C/D}$ will come back to the previous value.
- External noise might come from the stereotaxic frame. In order to avoid it we recommend to short the stereotaxic to the analog GND (TP8 pin) of the system by means of an alligator cable as shown below.



- External noise might come from the thermal pad (or other monitoring equipment connected to the animal). We strongly recommend avoiding any kind of switching electronics in contact with the animal. In the case of the thermal pad, we strongly recommend using IR thermal pads.
- After use, we recommend rinsing the device in deionized/distilled water. Check our recommended care for the SiNAPS probe [here](#).



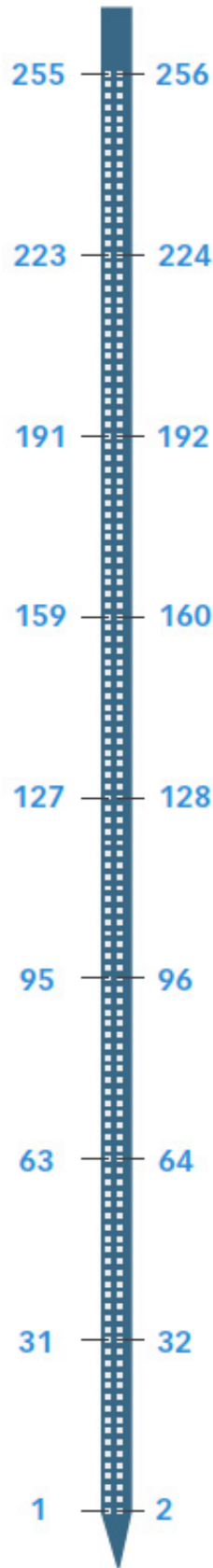
Troubleshooting

Issue	Cause	Solution
Noisy signal on all the channels	<ul style="list-style-type: none"> Noisy GND/Ref electrode High hydrophobicity on electrode sites 	<ul style="list-style-type: none"> Properly ground the system/animal Wait for the electrode surface to become less hydrophobic or do a hydrophilization treatment to the device
Noisy signal on some channels	<ul style="list-style-type: none"> High hydrophobicity on some electrode sites Defect on metal deposition of some electrode sites Damage on electrode metal layer (if an already used probe) 	<ul style="list-style-type: none"> Wait for the electrode surface to become less hydrophobic or do a hydrophilization treatment to the device Change device and contact NeuroNexus regarding next steps Change device
The probe does not work in homogeneous way (same Vref_FFA for the whole device pixels)	<ul style="list-style-type: none"> Reference not properly placed High hydrophobicity on some electrode sites Defect on metal deposition of some electrode sites Self calibration procedure fails 	<ul style="list-style-type: none"> Place again the reference, moving it closer to the insertion site and/or increase the reference contact surface and/or change reference material (whether different form Ag/Pt). Wait until the electrode surface becomes less hydrophobic or do a hydrophilization treatment Change device and contact NeuroNexus regarding next steps Restart calibration procedure or manually adjust the Vref_FFA

For all other issues please refer to troubleshooting in the “Wet Test” section

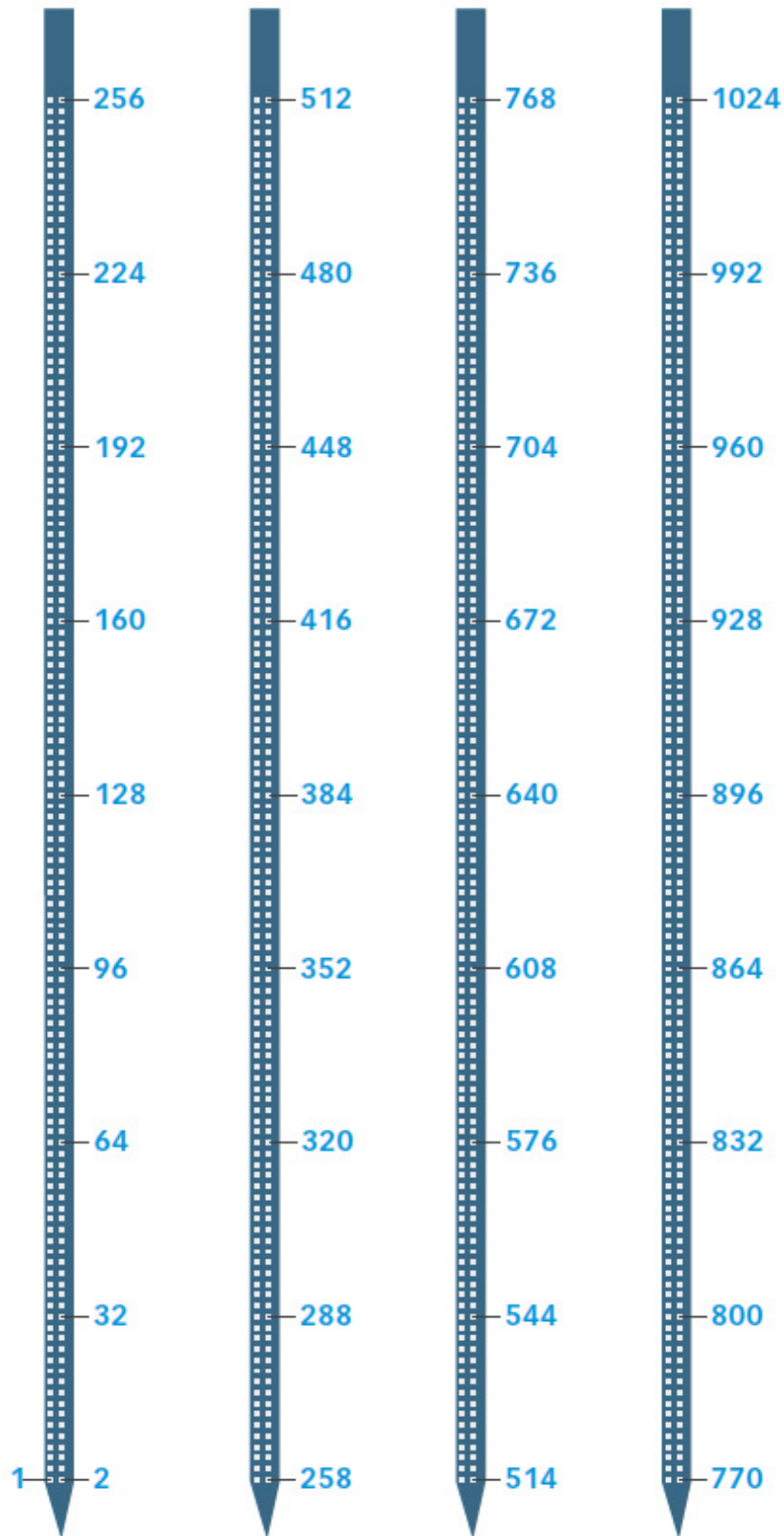


SiNAPS-1S-256





SiNAPS-4S-1024





SiNAPS-8S-1024

