Intan RHX Data Acquisition Software User Guide

11 February 2021; updated 16 April 2021

Features

♦ **Universal Intan software** works with all Intan RHD recording and RHS stimulation/recording systems.

♦ **User-selectable sampling rate** up to 30 kS/s.

♦ **Open-source**, multi-platform C++/Qt software.

♦ Multi-threaded design with GPU acceleration enables **flexible, real-time software filtering** into LFP and action potential bands, notch filtering, and threshold-based **spike detection** on all channels.

♦ **Automatically set spike detection thresholds** to a multiple of RMS levels on each channel.

♦ Flexible **multi-column display** with sweep or roll mode, oscilloscope-like triggering, custom colors, and channel grouping for tetrodes.

♦ Complete **TCP command interface** allows third-party software (e.g., MATLAB, Python) to control all functions remotely and automate tasks. Selected waveform data may be streamed into other applications via TCP.

♦ **Probe Map** window displays graphical representations of electrode sites, electrode impedance, and neural activity based on simple XML probe geometry file.

♦ **Real-time analysis tools** with easy data extraction to MATLAB, spreadsheet, or image files:

　♦ **PSTH**: Peri-stimulus time histogram

　♦ **ISI**: Inter-spike interval histogram

　♦ **Spectrogram** and spectrum analyzer

　♦ **Spike Scope** with selectable spike detection and artifact rejection thresholds.

♦ **Playback mode** to review saved data files.

♦ **Real time rewind** to review the past 30 seconds of acquired data on the screen.

♦ **Flexible referencing**: Each channel can have its own arbitrary reference, which can be a single channel or an average of multiple channels.

♦ All settings files are saved in human-readable XML format. There is an option to **auto-load a default settings file** every time the software starts.

♦ One-click *in situ* **measurement of electrode impedances** at user-selected frequencies.

♦ **Live Notes** function saves time-stamped user annotations in a text file.

♦ Selected waveforms are routed to **computer audio** or controller **audio line out** jack.

♦ **Demo mode** with synthetic data allows users to evaluate nearly all software functions with no Intan hardware present.

System Recommendations

The Intan RHX software runs on Windows 7, 8, and 10; MacOS; and Linux. A CPU with at least four cores is recommended to take advantage of the multi-threaded processing. A system with a GPU is recommended to speed up real-time software filtering and spike detection with large numbers of channels.

TOTAL NUMBER OF AMPLIFIER CHANNELS	MINIMUM SYSTEM RECOMMENDATIONS
16-32	2.4 GHz CPU with 4+ cores, 4 GB system RAM
48-512	3.0 GHz CPU with 4+ cores, with GPU, 8 GB system RAM
528-1024	3.2 GHz CPU with 4+ cores, with GPU, 16 GB system RAM

Contents

Required Intan Hardware
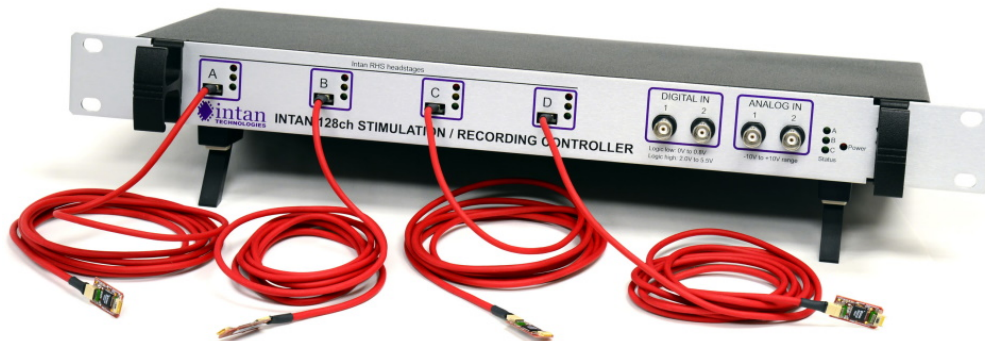
Intan RHX data acquisition software works with all Intan RHD and RHS controllers:
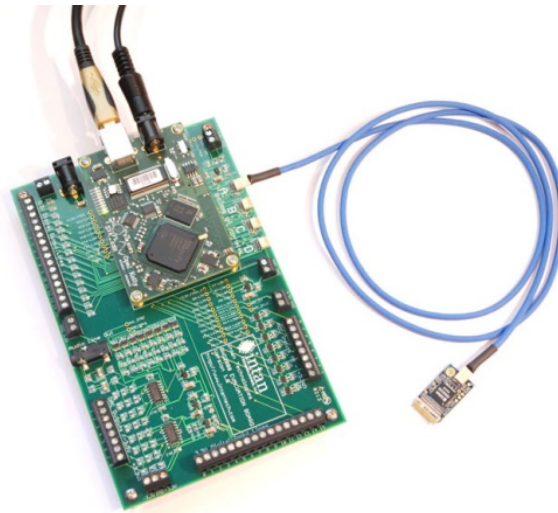
Intan RHD USB Interface Board
(up to 256 amplifier channels using RHD headstages)

Intan RHD Recording Controller (512ch or 1024ch)
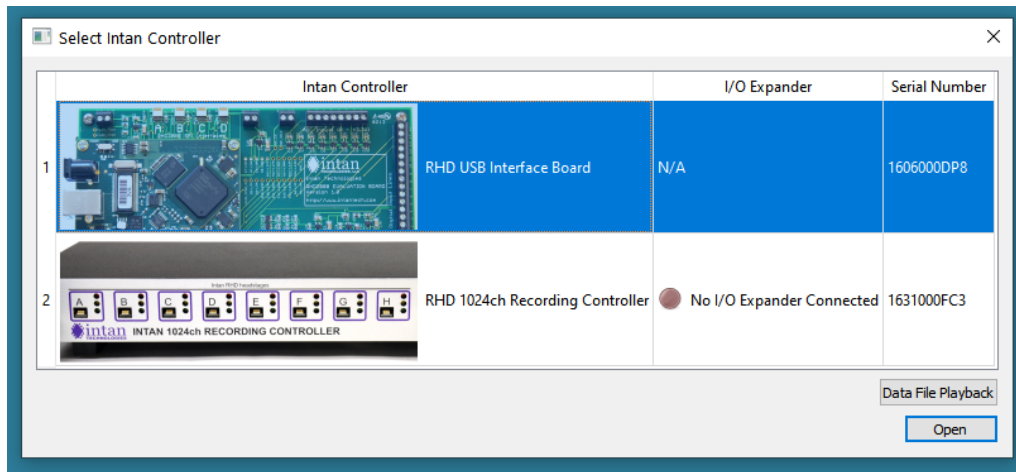(up to 1024 amplifier channels using RHD headstages)

Intan RHS Stimulation/Recording Controller
(up to 128 stim/amplifier channels using RHS headstages)

Quick Start Guide

Starting the Software

When the software starts, it scans all USB ports and displays a list of connected Intan controllers.

Select the controller you wish to use and click **Open**. Or you may select **Data File Playback** to replay a previously saved recording.

If no Intan controllers are detected, you have the option to start the software in demonstration mode or to replay a previously saved recording. **We recommend that you run the software in demonstration mode while you go through this Quick Start Guide for the first time.** The software will generate synthetic neural signals with local field potentials (LFPs) and action potentials (i.e., spikes) which will make it easy to learn the operation of the software.
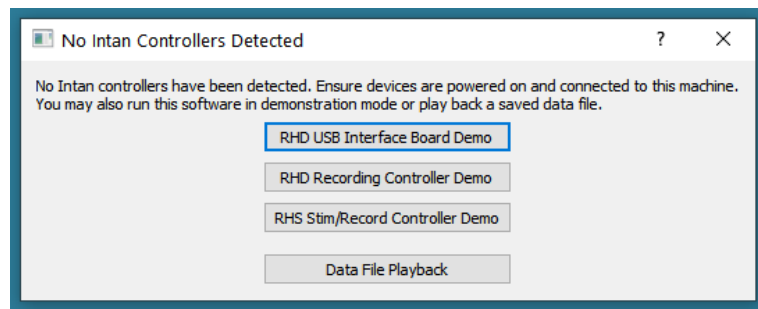
Next, the software will prompt you to select a sample rate that will be used for the acquisition session. If you selected an RHS stimulation/recording controller, you will also select the stimulation range to be used. After this point, you must restart the software to change either the sample rate or stimulation range. If you are running the software with an Intan controller present, you will have the option to set default values for these parameters for convenience.

Main Window

The main RHX window is shown below. At the top of the window is a Menu Bar and a Toolbar. Clicking the ▣ button on the left side below the Toolbar opens the Control Panel. To the right of the Control Panel is a single Display Column, but more Display Columns may be added.
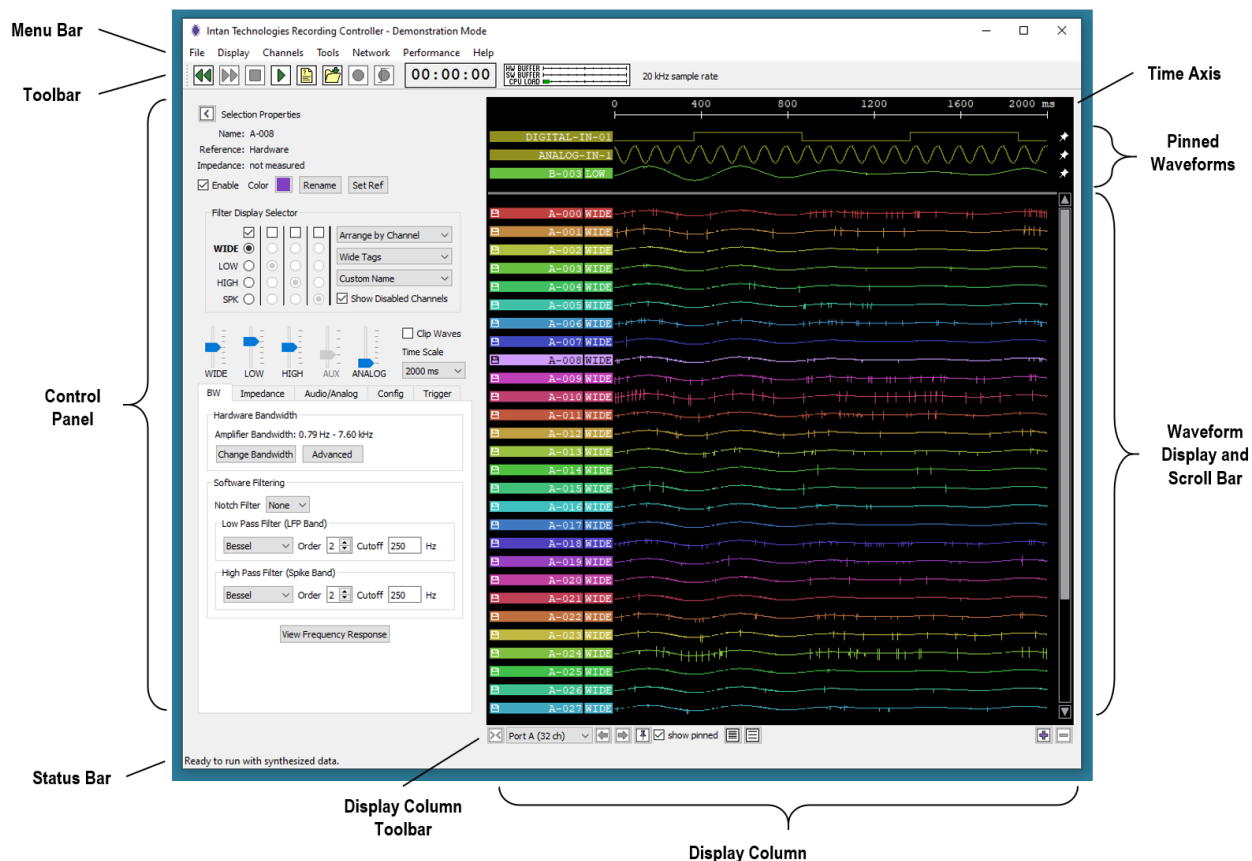
The contents of the Toolbar are shown below for normal data acquisition mode. The **Run** and **Stop** buttons start and stop data acquisition. After some data has been acquired and the system has been stopped, the **Rewind** and **Fast Forward** buttons can be used to review the last 30 seconds of data on the display, which are stored in RAM. The **Record** and **Triggered Recording** buttons start data acquisition and save the results to disk, but these buttons are disabled until the **Select Filename** button is used to select a path and base filename for the saved data files. The **Choose File Format** button is used to choose between various data formats.
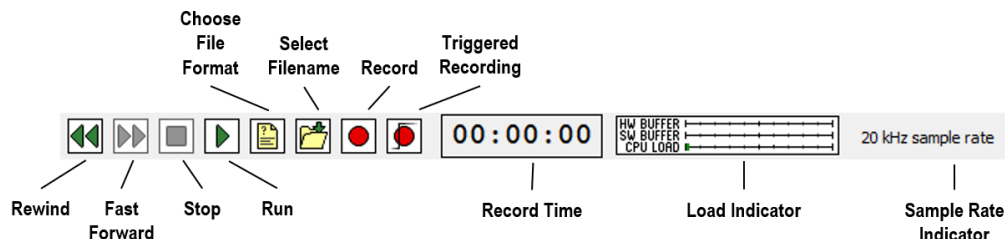
The **Record Time** indicator starts counting in hh:mm:ss format when recording begins. It also shows the negative time position when rewinding. The **Load Indicator** shows three horizontal bar graphs which provide information on how well the computer is able to keep up with reading and processing the data from the Intan controller. **HW Buffer** indicates the capacity of the FIFO in the Intan controller that feeds data to the USB bus; if the CPU cannot read the USB bus fast enough, this buffer will fill up. **SW Buffer** indicates the capacity of the main software FIFO used to store data read from the USB port while it is being processed. **CPU Load** indicates the capacity of the processor to perform all its tasks while maintaining real-time operation. Ideally these

should all be short green bars, or completely invisible. Long yellow or red bars indicate that the CPU and/or USB bus cannot keep up with the data from the Intan controller.

The **Sample Rate Indicator** shows the sample rate that was chosen when the software was started. This can only be changed by restarting the software.

In Data File Playback mode, the Toolbar looks a bit different (see below). There are additional buttons to **Jump to Start**, **Jump Back 10 Seconds**, **Jump Back 1 Second**, and **Jump to Position** (to a specified time in the saved data). For technical implementation reasons there is no smooth rewind in Playback mode, but there is a fast forward option. The Record and Triggered Recording options are still present to allow users to re-record data in a different file format. The **Sample Rate Indicator** shows the sample rate that was used to acquire the data in the file.
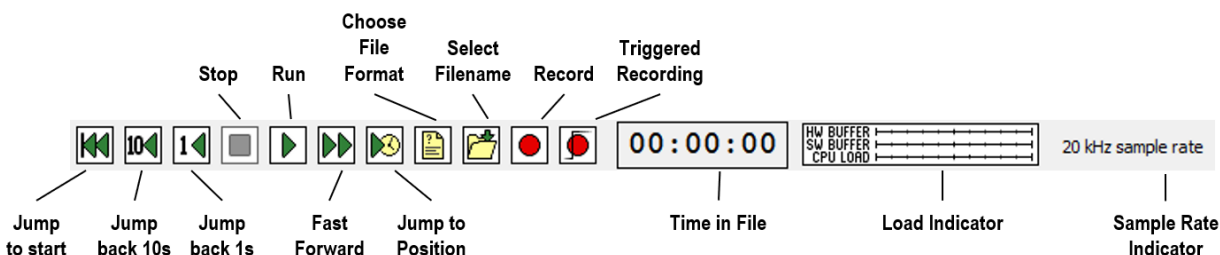

Display Columns

Each Display Column consists of the following items, moving from bottom to top: A Display Column Toolbar, the main Waveform Display with a scroll bar on the right side, optional Pinned Waveforms, and finally a Time Axis at the top. The Waveform Display plots waveforms from one selected port such as Port A, Port B, Digital Inputs, Analog Inputs, etc. The scroll bar allows you to look through long lists of waveforms. You can also use the mouse wheel, the Page Up and Page Down keys, and the Home and End keys to move up and down the list of displayed waveforms.

You can add Pinned Waveforms at the top of the Waveform Display. Pinned Waveforms have two important properties: (1) They stay fixed at the top of the display regardless of the scroll bar position; and (2) they may be signals from other ports. For example, if you are displaying Port A on a particular Display Column, you can still add channels from Port B or a Digital Input or Analog Input to the Pinned Waveforms in this column.

After a waveform has been pinned, it can be unpinned by clicking on the angled pin icon to the right of the waveform: ⬚. You can also pin and unpin waveforms by pressing Ctrl+P and Ctrl+U.

The Display Column Toolbar is shown below. The **Hide Column** button allows you to temporarily hide a display column. (You must have more than one column on the screen to hide a column. At least one column must always be showing.) The **Port Selector** is a pulldown combo box that allows you to select which port to view in the Waveform Display. The **Move Column Left** and **Move Column Right** buttons allow you to rearrange multiple columns on the screen. The **Add Pinned Waveform** button brings up a dialog that allows you to select any signal from any port to add to the Pinned Waveforms at the top of the Display Column. The **Show Pinned** check box allows you to temporarily hide all pinned waveforms. Other buttons allow you to select **Less Spacing** or **More Spacing** between waveforms in the display (you may click these multiple times to adjust the vertical spacing), and to **Add** or **Delete Columns**.

The user is encouraged to experiment with the Display Columns to set up various configurations on the screen.
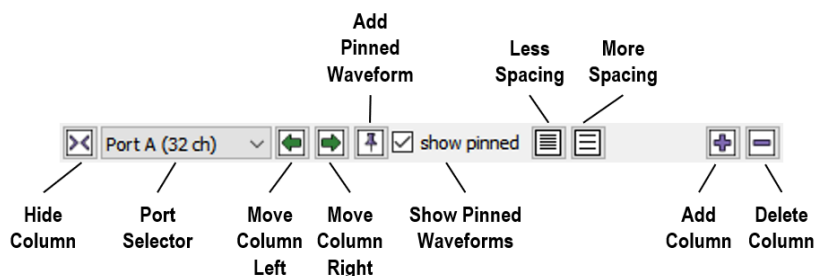
Above the Pinned Waveforms is the Time Axis. Hovering the mouse on this axis displays vertical time axes. The time scale may be adjusted with a combo box in the Control Panel.

Waveforms

All waveforms displayed on the screen have a label or "tag" on the left side of the Display Column. An example of a waveform tag is shown below. By default, the channel name is displayed (A-006 in this example; channel 6 from the Port A headstage). The Display menu or a combo box in the Control Panel allows you to change what is displayed here. For example, you can show the last measured electrode impedance instead of the channel name.
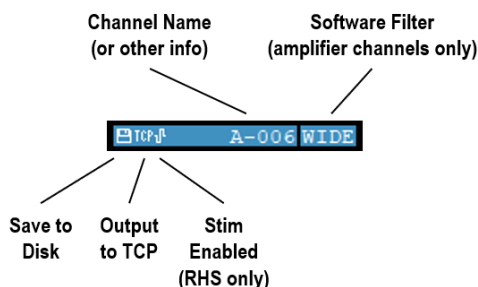

For amplifier channels, the Software Filter is shown to the right. In our example we see WIDE for wideband, indicating that this is the full-bandwidth signal from the headstage, limited only by the hardware bandwidth selected for the amplifiers, plus an optional software 50/60 Hz notch filter. Using the Filter Display Selector in the Control Panel, you can also display amplifier signals as LOW (software lowpass filtered, used to emphasize LFPs), HIGH (software highpass filtered, used to emphasize action potentials), or SPK (spike rasters, detected using thresholds applied to the highpass-filtered waveforms).

There are three icons which may appear on the left side of the waveform tag (see diagram above): The **Save to Disk** icon appears if this channel will be saved to disk during recording. The **Output to TCP** icon appears if this channel is selected for TCP Output (see Remote TCP Control section for more information). The **Stim Enabled** icon appears if stimulation has been enabled on this channel (RHS systems only).

If you wish to allocate more screen area for the waveforms themselves, you can visit the Control Panel where the default **Wide Tags** option can be changed to **Narrow Tags** or **Hide Tags**.

You can reorder the waveforms on the display by clicking and dragging a tag up or down the Waveform Display. You can select multiple waveforms by holding down Shift or Ctrl while clicking on the tags. You can hide unused channels by first disabling them (click the waveform tag to select, then press the Space Bar; or uncheck the Enable check box in the Control Panel) and then unchecking **Show Disabled Channels** in the Control Panel. Note that disabled channels will not be saved to disk.

If you hover the mouse pointer over a waveform, a vertical scale bar will appear, showing you the amplitude of the signal:
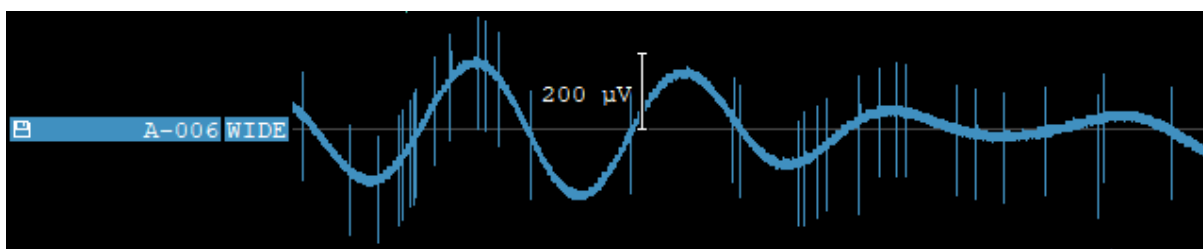
You can change the vertical scale of various waveforms using the vertical sliders in the center of the Control Panel, to the left of the Time Scale selector. There are separate sliders for the different signal types: WIDE, LOW, and HIGH are filtered amplifier waveforms; AUX waveforms are the signals from the Aux Inputs on RHD headstages (accelerometer signals if using an RHD headstage with integrated accelerometer); and ANALOG signals are analog inputs or outputs on the main Intan controller.

A check box labeled **Clip Waves** will not allow adjacent waveforms to overlap on the screen, if checked.

Filter Display Selector

The Filter Display Selector in the Control Panel (shown below) allows you to show different versions of the amplifier waveforms and to customize their arrangement. Each amplifier channel can be shown as a wideband signal (WIDE), a lowpass filtered signal (LOW), a highpass filtered signal (HIGH), or a spike raster (SPK). If an RHS system is being used, you can also see the low-gain DC amplifier signal (DC).
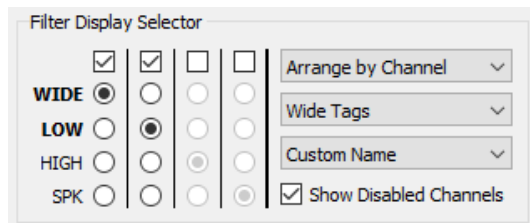
When the software starts, there is a checked box over the leftmost column, and WIDE is selected. Try changing the selection from WIDE to LOW, HIGH, or SPK while data acquisition is running, and watch the waveforms in the display change to these various filtered versions of each amplifier channel. Notice also that the right side of the waveform tags change to reflect the filter currently displayed.

Now check the box at the top of the second column from the left. If you set the first column to WIDE and the second column to LOW, you will see two waveforms for each amplifier channel in the display: one wideband and one lowpass version. Now pull down the Arrange by Channel combo box and change this to Arrange By Filter. It may look like the LOW waveforms have disappeared, but they have simply moved to the end of the waveform display list. Drag the scroll bar in the Waveform Display to the bottom and find the LOW waveforms in order after the WIDE waveforms.

By creative use of the Filter Display Selector and multiple Display Columns, a variety of useful waveform configurations may be quickly and easily set up. For example, you can display both WIDE and SPK filters and select Arrange by Filter, then add a second Display Column and show the same port on both columns. Now scroll down on one of the columns so that you view wideband waveforms in one Display Column and spike rasters in the other Display Column. You can add pinned waveforms to the top of the display columns for any "global" signals you wish to view alongside the signals from each port.

Selection Properties

The items at the top of the Control Panel show information on the currently selected waveform(s). The **Enable** check box allows you to enable/disable channels (though pressing the Space Bar is a quicker way to do this). Clicking on the square **Color** indicator allows you to change the color of selected waveforms. (You may also change the background color using an option in the Display menu.) Disabled channels have gray tags.

The **Rename** button allows you to change the custom name of each signal, and the **Set Ref** button allows you to set the reference for amplifier signals. In RHS systems, the **Set Stim** button allows you to configure stimulation settings for headstage channels, analog outputs, or digital outputs.

Basic Keyboard Shortcuts

Here are some useful keyboard shortcuts to remember. Pressing **F12** brings up a list of keyboard shortcuts.

- **+** and **-** increase or decrease the vertical scale of selected channels.
- **<** and **>** increase or decrease the time scale.
- **/** switches the display between sweep mode and roll mode.
- **Space Bar** enables or disables selected waveforms.
- **Ctrl+R** renames a channel (i.e., changes its custom name).
- **Ctrl+T** cycles the waveform tags between displaying custom channel name, native channel name, impedance magnitude, impedance phase, and reference.
- **Ctrl+Z** and **Ctrl+Y** undo and redo changes made to the ordering and coloring of channels in the display.
- **Page Up**, **Page Down**, **Home**, **End** scroll up and down a display column.
- **Cursor Up/Down** changes the selected channel to an adjacent channel above or below the current selection.

Control Panel Tabs

The bottom of the Control Panel contains several small panels accessible through tabs:

- **BW (Bandwidth)**: This tab contains tools for setting the hardware amplifier bandwidth as well as the software filtering. The **View Frequency Response** button shows users the combined frequency response of all hardware and software filters.
- **Impedance:** This tab contains tools for measuring electrode impedance and saving this data.
- **Audio/Analog:** This tab allows users to route selected amplifier signals to PC audio or hardware analog out/audio.
- **Config:** This tab contains tools for rescanning ports if headstages have been removed or added. It also contains text boxes that can be used to add custom notes to saved data files, and an option to add **Live Notes** during a recording session. This text is added to a notes.txt file in the same directory as the data files, with a time stamp for each Live Note entry.
- **Trigger:** This tab contains Display Trigger tools that function like an oscilloscope trigger and allow the display to be synced to a digital input event. Analog inputs may also act as digital inputs here.

Saving and Loading Settings

The File menu contains options for Saving and Loading settings files. These text XML files save the entire state of the software. The **Set Default Settings** menu option allows the user to select a default settings file to be loaded every time the software starts with this particular type of Intan controller. (You can always cancel the loading of a default settings file when the software starts by unchecking a box that appears on startup.)

Real-Time Analysis Tools

The Tools menu contains five options for real-time analysis and reference:

- **Spike Scope** opens an oscilloscope-like display that shows brief "snapshots" from the highpass (HIGH) filtered version of selected amplifier channels. The threshold used to detect spikes for the SPK rasters can be set independently for each channel. The user may also specify a larger Artifact Threshold that can be used to reject large artifacts (e.g., due to movement or electrical stimulation). The **Set Spike Detection Thresholds** option in the Channels menu allows users to automatically set the thresholds for all enabled channels to an absolute level or to a multiple of RMS level.

  The RMS level of the signal and the spiking rate are shown in the upper left corner of the Spike Scope display for convenience.

  The most recent detected spikes on each channel (up to 500 maximum) are displayed on the Spike Scope so that variation in spike shape may be observed. These most recent spikes are preserved for each channel even as the users switches between channels. For comparison of spike shapes over longer time intervals, users may use the **Take Snapshot** button to freeze the current display in the background while new spikes are displayed on top. Each channel remembers its own independent snapshot as long as the software is running, or until **Clear Snapshot** is clicked.
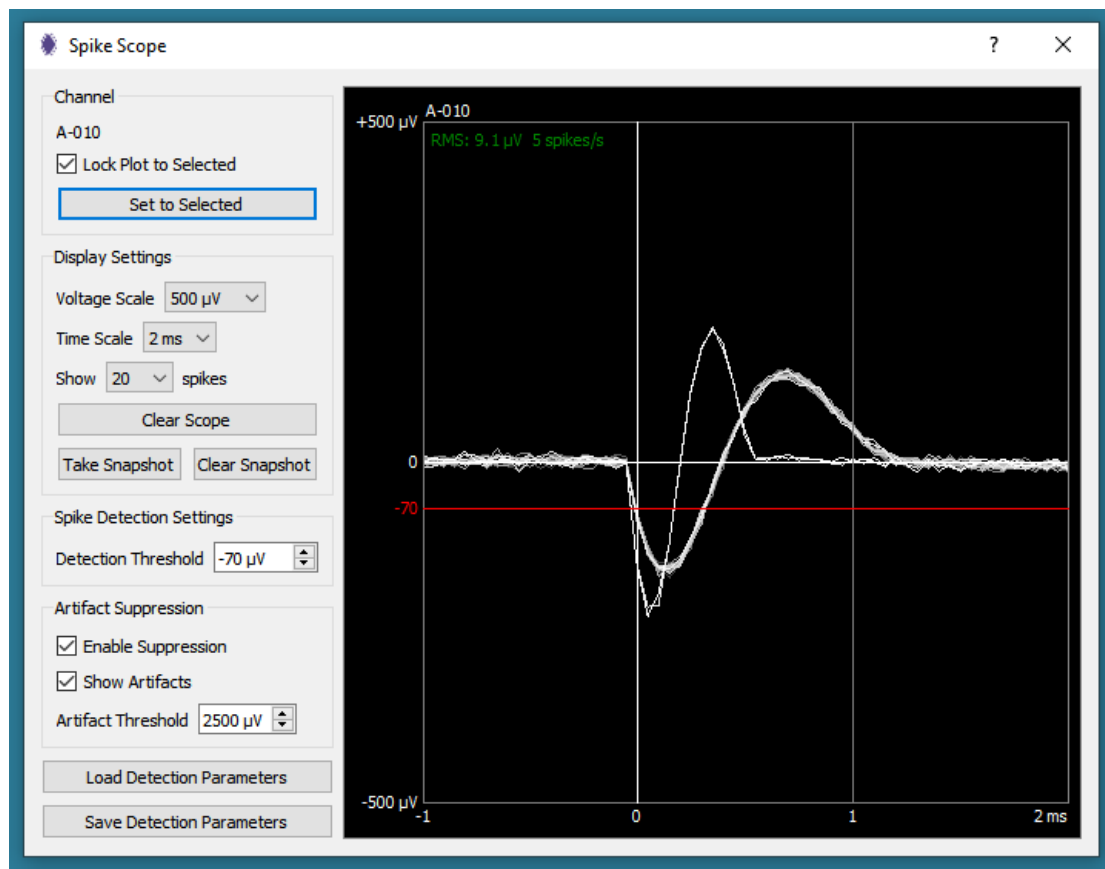
- **Inter-Spike Interval (ISI)** displays a histogram of the intervals between spikes in the spike raster (SPK filter) of the selected channel. ISI histograms can be used to look at the statistics of neuronal action potential timing or to distinguish single-unit from multi-unit activity in some cases.
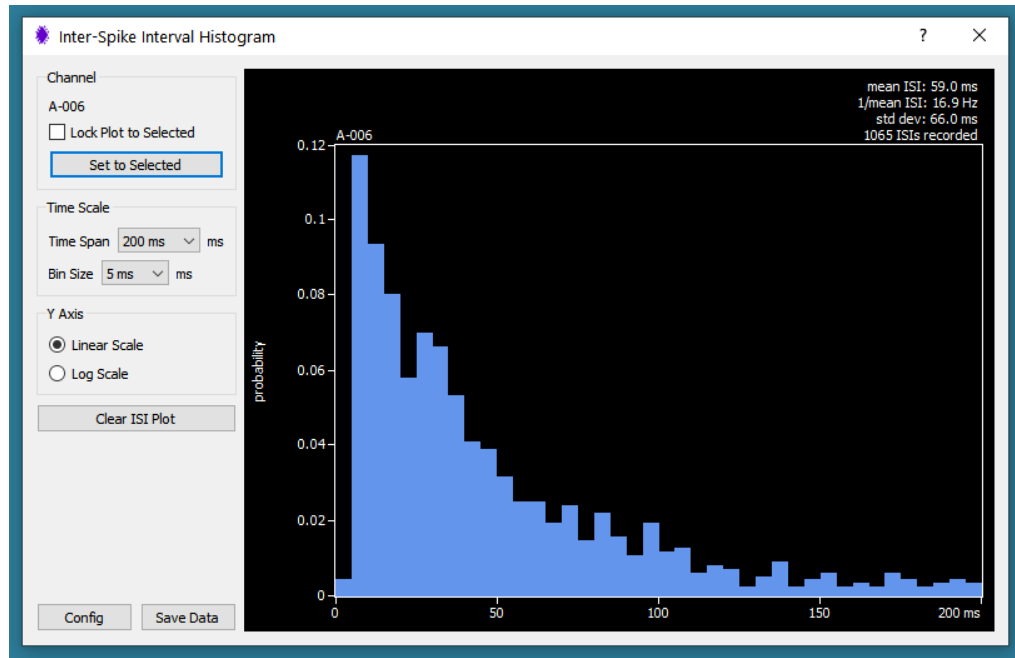
- **Peri-Stimulus Time Histogram (PSTH)** displays spike rasters in the time just before and after a trigger or stimulus marked by a digital input. (Analog inputs may also act as digital inputs here.) The digital input will typically correspond to some stimulus presented (e.g., a visual, auditory, or tactile stimulus) that elicits a neural response. For the PSTH to be measured properly, the stimulus must be repeated many times so a histogram of spikes can be created.
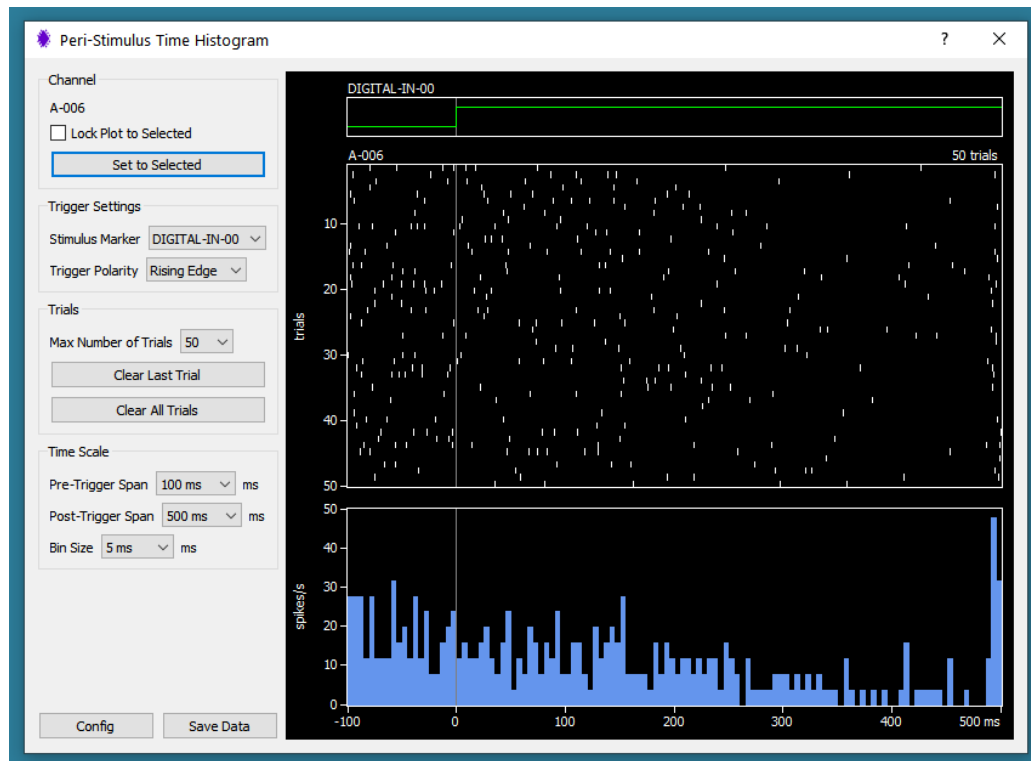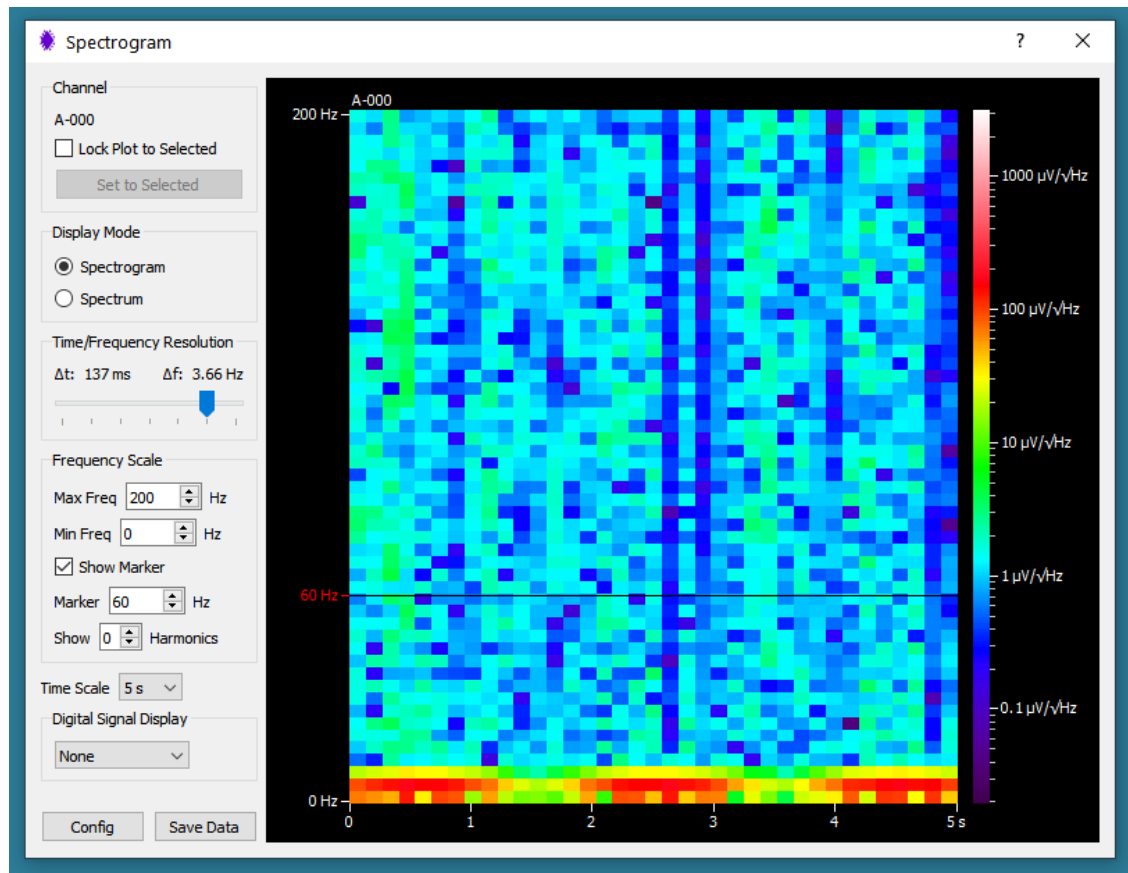
- **Spectrogram** opens a frequency analysis tool that operates on wideband (WIDE) signals, showing how the frequency content of the signal varies with time. This tool uses fast Fourier transforms (FFTs) to calculate power spectral densities over user-specified frequency and time ranges. There is a fundamental mathematical trade-off between time resolution and frequency resolution that the user can adjust with the horizontal slider.

This tool can operate either as a sliding frequency vs. time spectrogram (shown below) or an instantaneous spectrum analyzer by changing the selection under **Display Mode**. A marker can be enabled to locate frequencies of interest (e.g., 50 Hz or 60 Hz, a common source of interference). In spectrogram mode, a selected digital signal may be plotted along with the frequency vs. time display to synchronize stimulus onset with frequency response, for example.

Using the **Config** and **Save Data** buttons at the bottom, the spectrogram data may be saved as a MATLAB MAT-file, a CSV text file, or a PNG screen capture image file.

- **Probe Map** displays a graphical representation of electrode sites based on simple XML probe geometry description files. Several example probe geometry files are provided on the Intan website. Try loading AB32probe.xml when running the software in demonstration mode, then look at the XML file in a text editor to see how the probe geometry is described. In **Default View**, Probe sites that are selected in the Probe Map (see below) are also highlighted in the Waveform Display. If electrode impedances have been measured, **Impedance View** displays color-coded impedances for all electrode sites (assuming impedances have been measured). **Spike View** shows dynamic spiking activity across a probe when data acquisition is running.
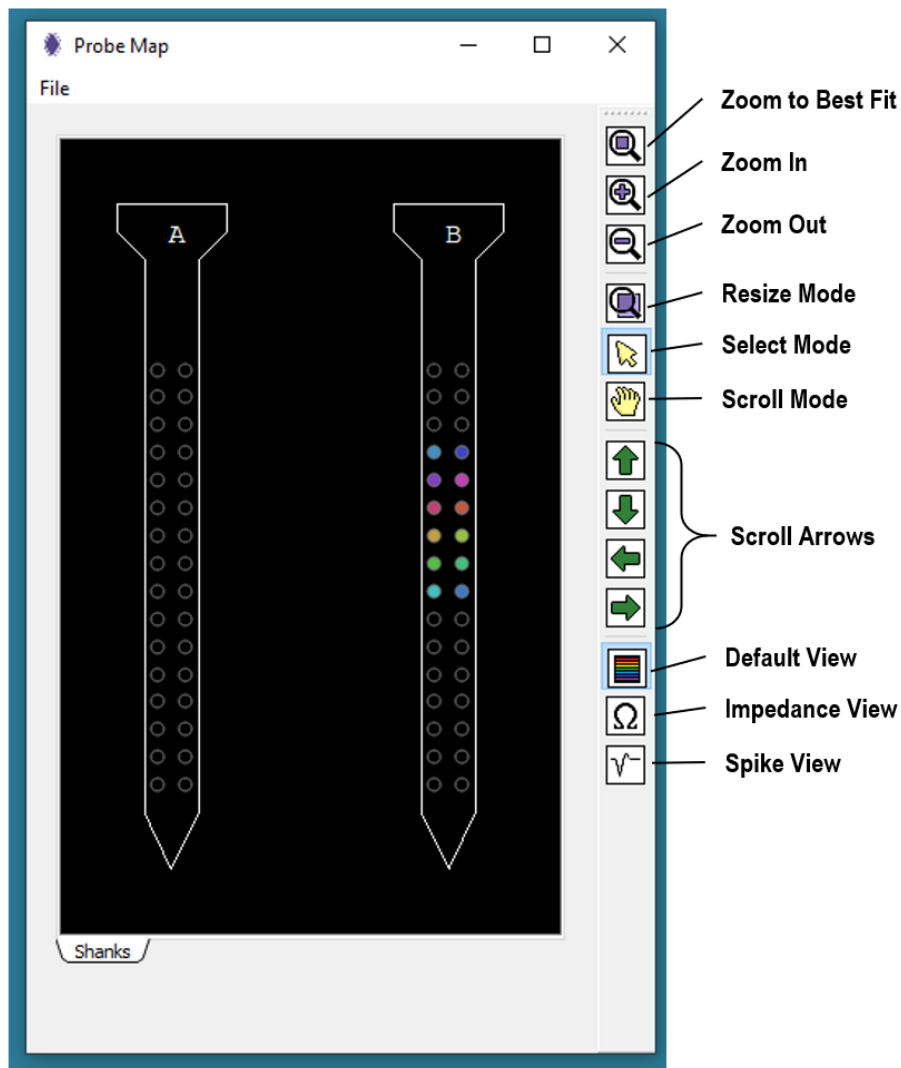
Data Viewing and Acquisition

Other Headstage Waveforms

Scrolling down to the bottom of the Port A waveforms reveals extra channels: three **auxiliary analog inputs** (RHD systems only) (AUX1, AUX2, and AUX3) used to sample 3-axis accelerometers in some Intan RHD headstages. See the Intan RHD Application Note **Accelerometer Calibration** for information on how to convert these signals from voltage to acceleration. An option at the bottom of the Display menu allows these signals to be hidden if they are not needed.
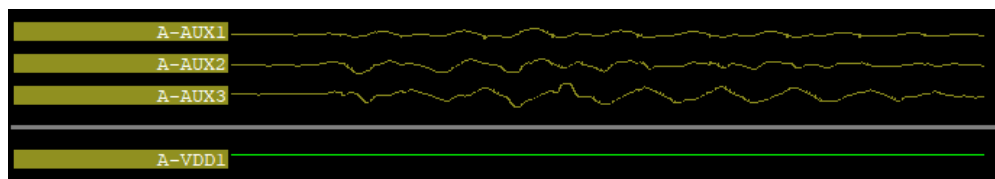
All RHD and RHS headstages also report their **supply voltage** (VDD), and this can be displayed by selecting "Show Supply Voltages" in the Display menu. A supply voltage between 3.2V and 3.6V is required for normal operation. A supply voltage between 2.9V and 3.2V can be used for derated performance (see the "3.0V Operation" section of the RHD chip datasheet for more information). Voltages below 2.9V are not recommended for proper chip operation. It is important to check the supply voltage if very long interface cables are used, as power line resistance can cause significant voltage drops. The supply voltage trace is plotted in green if normal operation is maintained. A yellow trace indicates derated (~3.0V) operation, and a red trace indicates over- or under-voltage conditions.

Reference Selection

The Control Panel contains a **Set Ref** button. This button brings up a "Select Reference" dialog that allows the user to select a particular amplifier channel (or an average of multiple channels) to use as a **software reference**.

Each channel on an Intan headstage amplifies the electrode signals with respect to a reference potential connected to the reference (REF) input. This **hardware reference** is often connected to a local ground near the recording site to reduce pickup of common-mode signals such as AC line noise and movement artifacts. In some cases, it is desirable to perform an additional digital subtraction, using a particular amplifier channel (e.g., a channel with no localized activity) to re-reference one or more amplifier signals. Any selected amplifier channel, or an average of multiple channels, may be used as a digital reference for any channel. This signal will be subtracted from the selected amplifier channel in real time.

Note: If the background noise on all channels is approximately Gaussian, this software reference subtraction will increase the background noise by 41% (a factor of the square root of two).

Amplifier Bandwidth Selection

The Bandwidth ("BW") tab in the lower-left corner of the main window contains buttons for selecting the amplifier bandwidth (see below). The "Change Bandwidth" button brings up an amplifier bandwidth selection dialog (see below) that allows users to select upper and lower cutoff frequencies for the headstages connected to the Intan controller. (Data acquisition must be stopped to access this control.) The software automatically calculates chip register values that produce actual bandwidth settings as close as possible to the desired bandwidth settings selected by the user.
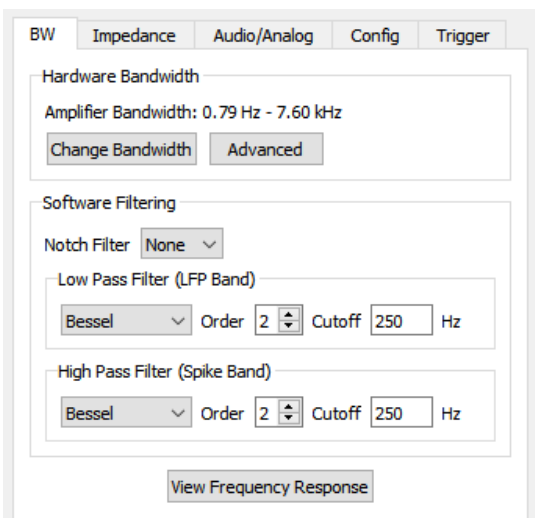
The Bandwidth ("BW") tab is used to select frequency-related parameters.
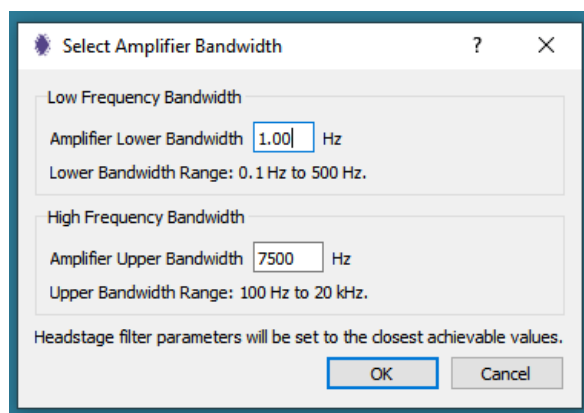
The amplifier bandwidth selection dialog allows users to set upper and lower cutoff frequencies.

It is recommended that most users use the "Change Bandwidth" button to set amplifier bandwidth. However, the "Advanced" button allows users to set amplifier analog cutoff frequencies as well as the on-chip DSP offset removal filter cutoff frequency directly. See the RHD and RHS chip datasheets for more details on the mechanisms of bandwidth selection and the operation of the DSP offset removal filter. Note that the DSP cutoff frequency has a limited frequency resolution (stepping in powers of two), so if a precise value of low-frequency cutoff is required, the amplifier lower bandwidth could be used to define this and the DSP cutoff frequency set below this point. If both the DSP cutoff frequency and the amplifier lower bandwidth are set to the same (or similar) frequencies, the actual 3-dB cutoff frequency will be higher than either frequency due to the combined effect of the two filters. The "Change Bandwidth" button performs these calculations for the user so changing the "Advanced" settings directly is not required.

The "Bandwidth" tab also contains a combo box for enabling an optional 50 Hz or 60 Hz software notch filter to help remove mains interference. Unlike older versions of Intan software, the notch filtered data is saved to disk if it is enabled.

Below the Notch Filter selection, software lowpass and highpass filters can be configured to define the frequency bands for LOW and HIGH waveforms that can be displayed for each amplifier channel. The order, type, and cutoff frequency for each filter can be set here. Software filtering is performed using GPU acceleration to maintain real-time operation. Clicking the "View Frequency Response" button brings up a graph that shows the various frequency bands graphically:
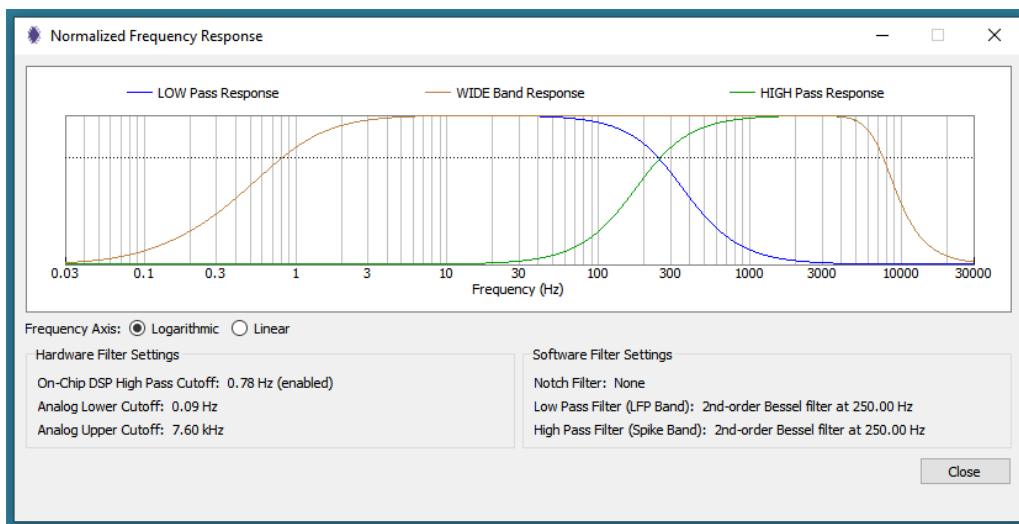
Electrode Impedance Measurement

The "Impedance" tab contains tools for measuring the impedances of all electrodes at user-specified frequencies (see below). Clicking on "Selected Impedance Test Frequency" brings up a dialog that allows users to select a measurement frequency (e.g., 1 kHz, the de facto standard for measuring neural recording electrode impedances). Data acquisition must be stopped to access this control. After executing an impedance measurement (which takes several seconds, with lower frequencies requiring more time), electrode impedances are displayed below each amplifier waveform plot (see below).
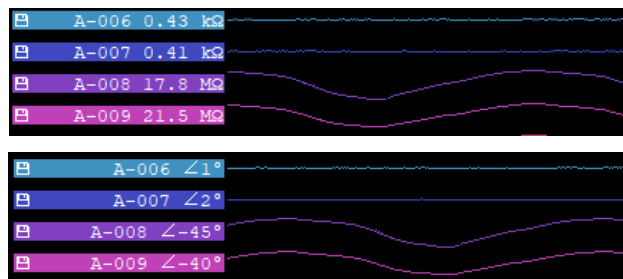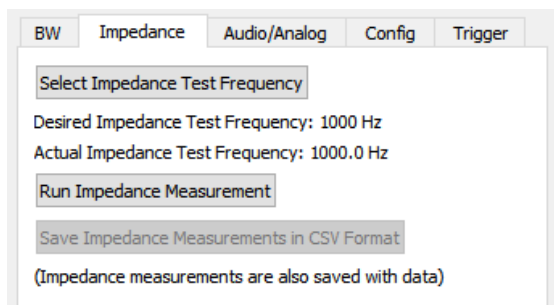
The Impedance tab is used to measure electrode impedances at specified frequencies.

Impedance magnitudes or phase angles can be displayed in each waveform tag. Press Ctrl+T to cycle the tag display.

Both the magnitude and phase angle of the complex impedance can be displayed. Ideal resistors have a phase angle of 0°; pure capacitors have a phase angle of -90°. Real electrodes have both capacitive and resistive components, and will typically have phase angles between -30° and -90°. It is important to remember that there is a fair amount of noise and uncertainty in these impedance measurements, so the values should not be considered highly precise. The best accuracy seems to be obtained at measurement frequencies no higher than 2 kHz.

Impedance measurements may be saved in CSV (Comma Separated Values) format, which is a text file that can be imported into any spreadsheet application. The most recent impedance measurement is also saved in the .rhd or .rhs header of recorded data files, and this information can be extracted in MATLAB after data acquisition is complete.

Analog Waveform Reconstruction and Audio Output

The "Analog/Audio" tab has controls for routing the select amplifier channel to the computer audio. It can sometimes be useful to scan through many channels by selecting a single channel in the Waveform Display, enabling audio, and then using the Cursor Up/Down keys to move through adjacent channels.
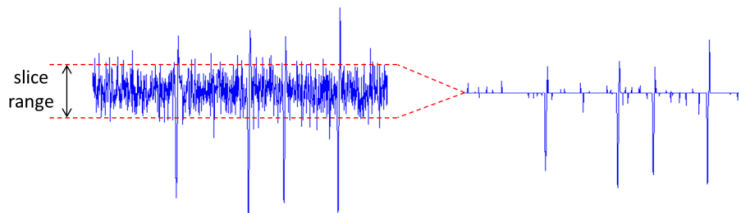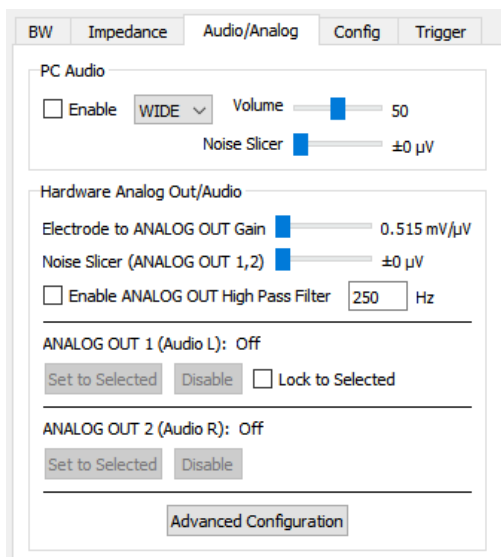
This tab also contains controls for routing selected amplifier channels directly to any of the analog outputs on the Intan controller (and optional I/O Expander) in order to reconstruct analog waveforms that may be observed on oscilloscopes or acquired using traditional data acquisition systems (e.g., National Instruments DAQ systems with analog inputs). Selected waveforms are routed directly through the Intan controller hardware to achieve latencies of less than 0.2 ms, but this means that the software 50/60 Hz notch filter is not applied to these waveforms.

ANALOG OUT 1 and 2 are also connected to the left and right channels of the Audio Line Out jack on the Intan controller. Any signals assigned to ANALOG OUT 1 and 2 will be audible if the board is connected to an audio amplifier using a standard 3.5-mm stereo cable. ANALOG OUT 1 and 2 are connected to the audio jack through DC blocking capacitors that attenuate signals below a few Hertz (far below the 20 Hz limit of human hearing), so if very low-frequency signals need to be observed as analog waveforms they should be taken directly from the analog output ports and not the audio port.

A slider at the top allows users to select the total Electrode to ANALOG OUT gain (ranging from 1.6 mV/µV to 204.8 mV/µV in powers of two). It is important to remember that the analog outputs limit at ±10.24V; large gain settings coupled with large signals from recording electrodes may lead to signal saturation.

More options are available by clicking the "Advanced Configuration" button. You can also enable or disable a particular analog output by clicking the "Set to Selected" or "Disable" buttons. Disabled analog output ports are set to zero volts.

The Analog/Audio tab contains PC audio controls as well as controls for routing selected amplifier channels to analog outputs and hardware audio left/right channels.

Illustration of "noise slicing" signal processing for enhanced audibility of neural spikes in noisy waveforms. Any data points of the waveform that fall within the slice range are set to zero, and signals extending beyond this range are brought in towards zero.

The check box labeled "Lock to Selected" allows the user to lock ANALOG OUT 1 to the currently selected electrode channel. This may be useful when examining many amplifier channels while using an audio monitor.

Intan controllers include an optional signal processing feature to enhance the audibility of low-amplitude neural spikes in a noisy waveform. Both the PC audio and hardware audio have a "Noise Slicer" control. The operation of the noise slicer algorithm is illustrated above. Any data points of the waveform that fall within the slice range are set to zero, and signals extending beyond this range are brought in towards zero. The result is a dramatic improvement in the audibility of neural spikes. Users are encouraged to experiment with this feature in neural recording experiments.

It is important to note that the hardware audio noise slicing function also affects the signals on ANALOG OUT 1 and 2.

An optional single-pole high-pass filter may be enabled that applies to all ANALOG OUT waveforms. This filter may be enabled and its cutoff frequency set below the Noise Slicer slider.

Spike detection thresholds may be added to each of the signals routed to an analog output port. Intan controllers implement low-latency comparators that generate digital signals on DIGITAL OUT ports 1-8 indicating when a particular signal exceeds the selected threshold level. This feature can be used to trigger external events based on the detection of neural spikes, for example. The typical latency from electrode to comparator digital output is less than 200 µs. The "enable" box next to each DIGITAL OUT threshold must be checked to enable the threshold-based spike detector for a particular signal. If low-frequency signals (e.g., local field potentials) are present in the waveforms, the ANALOG OUT high pass filter can be used to isolate the spikes while preserving the wideband waveforms in the saved data. Using this filter in concert with the audio noise slicer function will maximize the audibility of neural spikes in the hardware audio channels.

Config Tab

The "Config" tab contains a variety of miscellaneous tools for working with headstages connected to Intan controllers. The "Rescan Ports" button causes the controller to search for connected headstages on all ports, and to account for any signal delays due to long interface cables on these ports. This function is automatically executed when the software starts, but if any boards are unplugged or reconnected, or if cable lengths are changed then this button should be clicked to update the status of all ports. If noisy, discontinuous data is observed on one of the headstage ports, this may be due to an inaccurate compensation of signal delay. The automatic signal delay estimation algorithm may be overridden by clicking the "Manual" button to bring up a dialog box allowing the delay compensation for selected interface cables to be set manually. **RHD2164 64-channel amplifier chips (used in RHD 64ch and 128ch headstages) use a double-data-rate SPI protocol and are particularly sensitive to this delay setting, so it is sometimes necessary to adjust the delay manually by ±1 step when using these chips.**

The "Auxout Pins" box (RHD systems only) contains a button that brings up a dialog box allowing the user to configure real-time control of the auxiliary digital output pin (**auxout**) on each RHD headstage connected to the recording controller. This pin is brought out to a solder point labeled **DO** on some headstages, and can drive up to 2 mA of current from the 3.3V supply. An external transistor can be added to extend the current drive or voltage range; see **RHD Application Note: Adding an LED to Headstages** for more information.
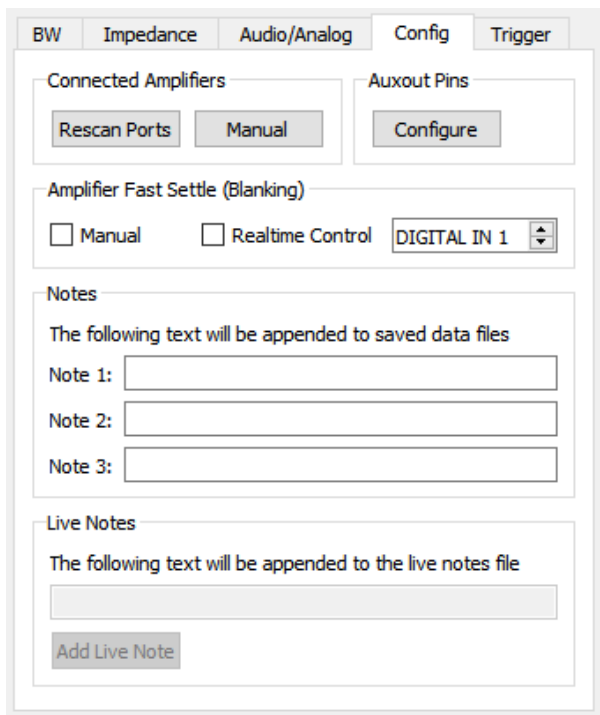
The dialog box allows users to select digital inputs on the recording controller (and optional I/O expander) to control the **auxout** pin on RHD headstages connected to particular SPI ports. There is a latency of 4-5 amplifier sampling periods (e.g., 200-250 µs at 20 kS/s) between changing the value of a digital input and seeing the change in the corresponding **auxout** pin on the chip.

The "Amplifier Fast Settle" box (RHD systems only) contains controls for the hardware "fast settle" function built into all RHD headstages that rapidly resets the analog signal path of each amplifier channel to zero to prevent (or recover from) saturation caused by large transient input signals such as those due to nearby stimulation. Recovery from amplifier saturation can be slow when the lower bandwidth is set to a low frequency (e.g., 1 Hz).
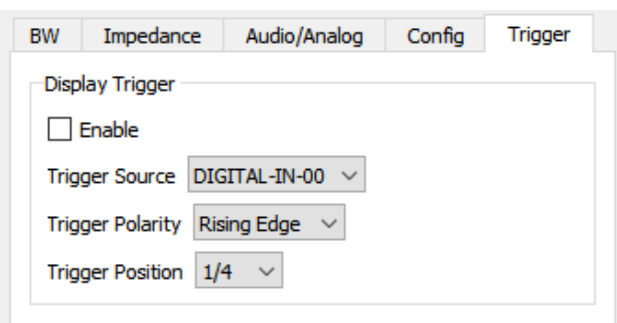
This fast settle or 'blanking' function may be enabled manually by clicking the "Manual" check box. The amplifier signals will be held at zero until the box is unchecked. Digital control of the fast settle function is enabled by checking the "Realtime Control" box and selecting a digital input that will be used to activate blanking. If this box is checked, a logic high signal on the selected digital input will enable amplifier fast settling with a latency of 4-5 amplifier sampling periods. For example, if the sampling frequency is 20 kS/s, the control latency will be 200-250 µs. By applying a digital pulse coincident with (or slightly overlapping) nearby stimulation pulses, amplifier saturation and the resulting slow amplifier recovery can be mitigated.

The "Notes" box includes three single-line text boxes in which users may add informative text that will be saved in the header of any recorded data file. This may be used to annotate various experimental parameters.

The "Live Notes" box contains a text box and "Add Live Note" button. During a recording session, the user may type notes into the text box and click "Add Live Note" at any time. A **notes.txt** file will be created in the recording subdirectory and timestamped Live Notes will be appended to this text file. This can be used to note important events during the course of an experiment. Each line in the **notes.txt** file lists the timestamp index (i.e., sample number), the timestamp in hh:mm:ss format, and the text note.

The Config tab contains miscellaneous controls as well as text fields that are appended to saved data files.

The Trigger tab includes tools for selecting digital inputs to control oscilloscope-like triggering of the waveform display.

Trigger Tab

The "Trigger" tab includes tools for oscilloscope-like triggering of the waveform display to remain synchronized with a selectable digital trigger source. Any of the digital inputs may be selected as a **trigger source**, using a rising edge or falling edge as the trigger event. The display waits for a trigger event to occur and then aligns the waveforms so that the trigger point is always aligned to a particular horizontal position on the display. This can be useful when assessing the response of neurons to various stimuli or events that coincide with a digital signal sampled by the controller (see also the **PSTH tool** in the Tools menu).
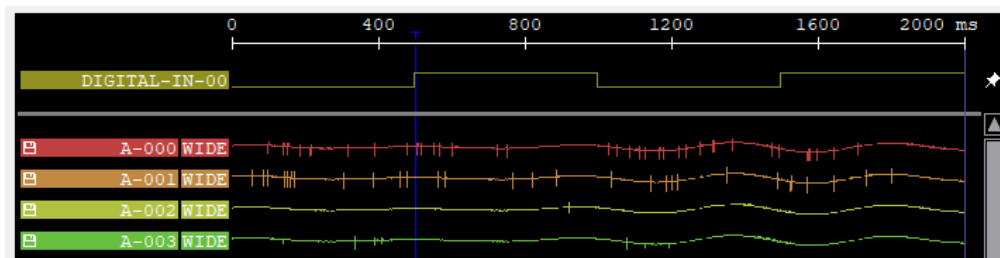
Digital trigger (shown by blue line) set to the rising edge of Digital In 0, with trigger position 1/4.

Episodic Triggered Recording Mode

After selecting a base filename, users may click the "Triggered Recording" button to pull up a triggered recording dialog window (see below). Any of the analog or digital inputs on the Intan controller or optional I/O Expander may be selected to serve as the trigger line. After trigger parameters are selected, the software will begin to display live amplifier data, but saving to disk will not commence until a high (or low, if selected) signal on the trigger line occurs. When the trigger is detected, between 1 and 30 seconds of pre-trigger data will immediately be saved to disk, and normal recording will continue until the trigger signal is de-asserted. After the trigger signal is de-asserted, between 1 and 9999 seconds of post-trigger data are saved to disk before the save file is closed. Brief sound cues indicate the onset of triggering and the end of a triggered recording. Text on the status bar at the bottom of the GUI displays the current status of triggered recording.

After the trigger signal has been de-asserted and the post-trigger data is saved, the software continues running and watching for new trigger signals. A new trigger signal will create a new data file with a unique, time-stamped name. By running in episodic trigger mode, an unlimited number of triggered events can be recorded to separate data files while running fully autonomously for hours or days.

Negative time stamps in the saved data file are used to indicate pre-trigger data; the trigger point is denoted by a time stamp of zero. By default, the digital or analog signal used for the trigger is automatically saved along with the amplifier data.
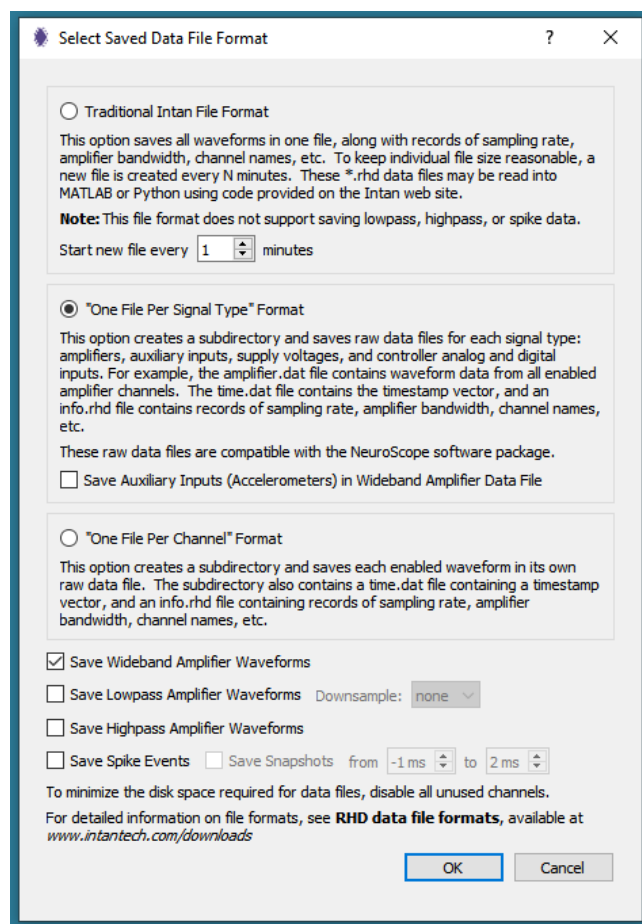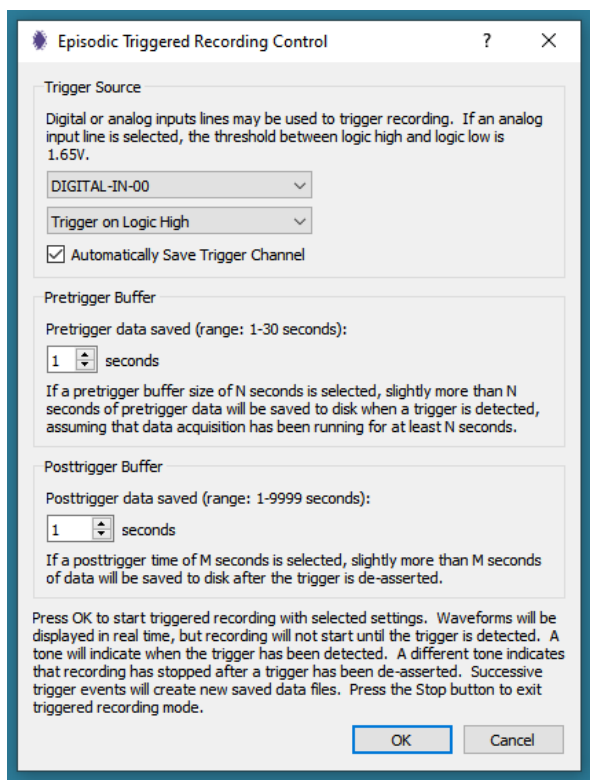
Data File Formats

The saved data file format may be selected by clicking the "Select File Format" button. This brings up the selection dialog shown in below. Details of the various file formats are described in separate documents available from the Intan website. MATLAB and Python code, also available from the website, allow users to import data stored in the default Traditional Intan file format.

Data files may grow quite large. (Watch the status bar at the bottom of the window for file size estimates in MB/minutes.) If the "Traditional Intan File Format" is selected, new data files are created at a time interval specified by the user with date and time stamps added to the base filename in year-month-day and hour-minute-second format (e.g., "mydatafile_210203_093500.rhd").

All data files are saved in a subdirectory names with the base filename and date and time stamps.

If the "One File Per Signal Type" or "One File Per Channel" format are selected, there are also options to save lowpass amplifier waveforms (with optional downsampling to save disk space), highpass amplifier waveforms, and spike events. Spike events are saved as timestamped rasters with an option to save "snapshots" of each spike from the highpass amplifier waveform. MATLAB and Python code, available from the Intan website, allow users to import spike raster data files.

In triggered recording, a signal on a user-specified digital or analog input is used to start and stop recording to disk.

Three different data file formats may be chosen for waveforms saved to disk.

Other Menu Functions

The Channels menu contains a **Color Amplifier Channels** function that will automatically recolor all amplifier channels with a specified number of repeating colors, and optionally coloring several adjacent channels identically. This can be useful to generate uniform color patterns after disabling and/or reordering channels in the display.

The Channels menu also contains a **Group Amplifier Channels** function that will automatically group amplifier channels in groups of two, three, or four. This grouping operation has little practical effect currently but may be used to implement tetrode-oriented features in future releases of the software.

The Performance menu contains a **Performance Optimization** option. This allows the user to select the XPU (CPU or GPU) used to perform computationally-intensive operations of software filtering and spike detection. Every time the software starts it measures the speed of each processor and selects the fastest option as the default. However, this option can be changed manually here.

The Help menu contains a link to the keyboard shortcuts menu (which may also be accessed by pressing F12) as well as additional information on Intan Technologies. This menu also contains a **Generate Log File for Debug** option. If this option is checked, the software will generate a log file that can be sent to Intan Technologies for assistance in debugging the software. Under normal operation this should be left unchecked as it will cause the software to run more slowly. Note that if this option is checked, it will remain checked even after exiting from the software are restarting; it must be unchecked manually.

Stimulation Functions (RHS Systems Only)

Setting Stimulation Parameters

To set the stimulation parameters for any headstage channel, select that channel with a mouse click in the Waveform Display, and then click **Set Stim** in the Control Panel. This will bring up a Stimulation Parameters window, as shown below:
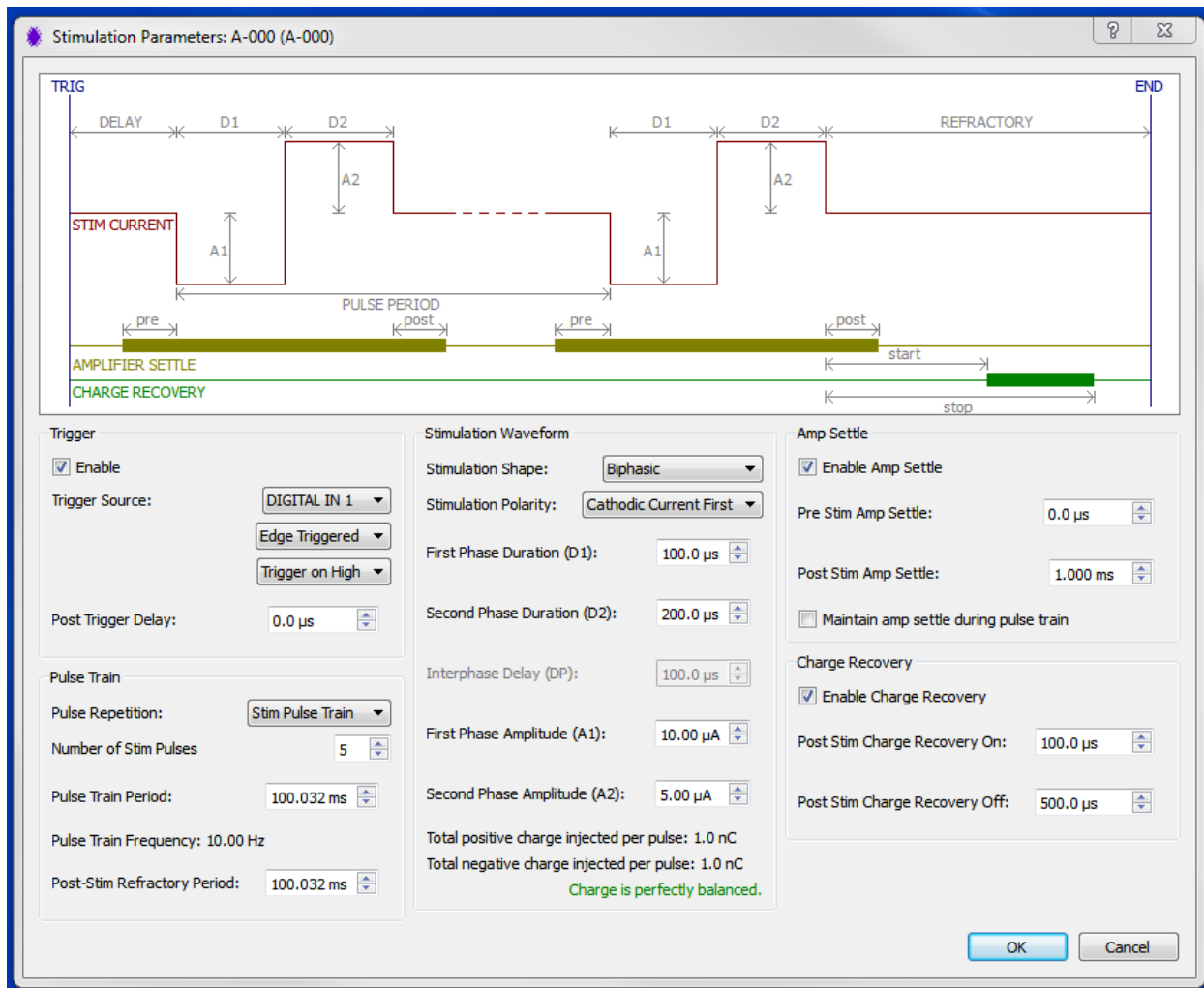
Stimulation Parameters selection window.

Stimulation Triggers

The title bar of the window lists the name of the channel that is being configured. To adjust the stimulation parameters, the stimulation trigger must first be enabled by checking the "Enable" box. Next, a **trigger source** should be selected. Any digital input or analog input on the main controller or optional I/O Expander may be used to trigger a stimulation sequence. (Analog inputs act like digital inputs for the purpose of triggering, with a threshold of 1.65V.) Additionally, keypresses on the host computer (the keys "F1" through "F8") can be used as triggers.

Stimulation sequences can be **edge triggered** or **level triggered**. Selecting "edge triggered" causes a stimulation sequence to execute once every time the trigger changes state from inactive to active. Selecting "level triggered" causes a stimulation sequence to execute and then repeat as long as a trigger source is active. A trigger source can be defined to be active on **high** (i.e., a "high" logic level or a key pressed) or active on **low** (i.e., a "low" logic level or a key not pressed).

By default, a stimulation sequence executes immediately following a trigger event, but an optional **post trigger delay** may be specified if needed. For example, this feature could be used to sequence a series of staggered stimulation pulses across several channels that are triggered by the same source. This delay may be as long as 500 milliseconds.

Note: An illustration of the stimulation waveform is displayed in the upper part of the window (not to scale). When stimulation parameter controls are selected in this window, the corresponding arrows and labels in this display turn from light gray to red as an aid to the user.

Pulse Trains

A stimulation sequence may consist of a single stimulation pulse or a train of multiple, identical pulses. The **pulse repetition** selector allows the user to choose between these two options. If a pulse train is selected, the **number of stimulation pulses** may be selected (up to 256) and a **pulse train period** can be defined (up to 1 second). The corresponding frequency is displayed underneath.

A **post-stimulation refractory period** can also be selected. After a stimulation sequence has completed, additional trigger events will be ignored during this refractory period. This parameter can be used to configure pulse trains of indefinite duration by selecting single stimulation pulses along with level triggering. As long as the trigger source remains high, the single stimulation pulse will repeat at a rate set by the post-stimulation refractory period. This period may be up to one second in length.

Stimulation Waveform

Three stimulation **waveform shapes** are supported: **biphasic**, **biphasic with interphase delay**, and **triphasic** (see below). For any waveform shape, the **stimulation polarity** can be selected to provide **cathodic (negative) current first** (this is standard practice in most stimulation experiments) or **anodic (positive) current first**.
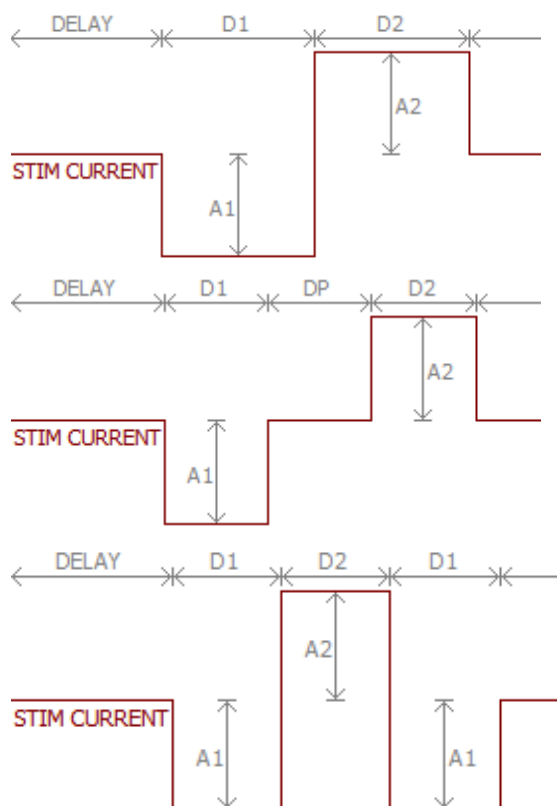
Stimulation waveform shapes: **biphasic** (top), **biphasic with interphase delay** (middle), and **triphasic** (bottom). All waveforms are shown with cathodic (negative) current first, but this may be inverted for any waveform shape.

The pulse phase durations (D1, D2, and possibly DP) and current amplitudes (A1 and A2) of the stimulation waveform may be selected, subject to the limitations of the time resolution and current step size that were originally selected when the software started up. The stimulation pulse phase durations may be set to a value up to 5 milliseconds.

The product of the duration and amplitude of each stimulation phase determine the amount of charge injected in each phase. The total positive and negative charges associated with a particular stimulation waveform are displayed below the amplitude selectors, along with a color-coded indicator that indicates if the positive and negative charges are balanced or imbalanced. When stimulating with microelectrodes, it is standard practice to maintain charge balance to reduce electrochemical effects at the electrode surface and to extend electrode life.

Note that the current sources in each RHS headstage have some intrinsic random variation from one channel to the next and between the positive and negative current sources, and will not match perfectly even if ideal charge balance is set in the GUI.
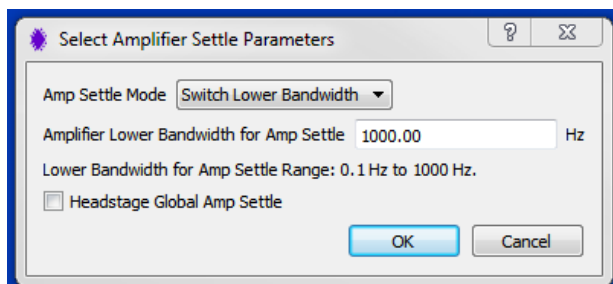
Amplifier Settle

Stimulation induces large voltage transients in microelectrodes, on the order of a volt. This creates huge artifacts that make it difficult to observe weak, microvolt-level electrophysiological signals immediately following a stimulation pulse. To mitigate these effects, Intan designed an "amplifier settle" capability into RHS headstages that can reduce the time it takes for an amplifier output to return to its baseline level after experiencing a large stimulation artifact. Checking the **Enable Amp Settle** box activates this functionality.

The user may then select the time before stimulation that the "amp settle" function should engage (typically this is set to zero) and the duration that the "amp settle" function should stay engaged after the stimulation pulse has ended. Experiments have shown that a value of 1 millisecond seems to work well here, but the optimal value for this parameter could very well change depending on the type of electrode used. The user is encouraged to experiment with this value.

If a multi-pulse train has been selected, the user can choose to maintain "amp settle" during the pulse train, or release it after each individual pulse. The dark yellow bar in the stimulation waveform display illustrates when "amp settle" is engaged based on this selection.

There are two methods for settling the amplifier after a stimulation pulse, and either of these methods may be selected in the amplifier settle configuration window (see figure below), which is accessed by selecting "Amplifier Settle Settings" under the Stimulation menu. The default mode, **Switch Lower Bandwidth**, is the recommended mode for amplifier settling. In this mode, the lower bandwidth of the amplifier is changed to a new value during the "amp settle" period. The recommended value for this parameter is 1000 Hz, but values between 100 Hz and 1000 Hz could be tried.

The second amplifier settling mode is **Traditional Fast Settle**, which is the "fast settle" technique used in the Intan RHD amplifier chips. This mode does not seem to be as effective in recovering from stimulation artifacts as the new bandwidth-switching mode.

Amplifier settle configuration window (accessible through the Stimulation menu).
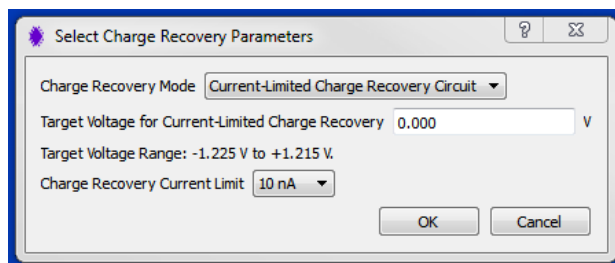
Charge recovery configuration window (accessible through the Stimulation menu).

Headstage Global Amplifier Settle

Stimulation on one electrode will typically induce artifacts on nearby electrodes due to capacitive coupling in the microelectrode array or the stim/record headstage itself. The Omnetics connector used on Intan stim/record headstages has approximately 0.15 pF of capacitance between adjacent pins, and this can lead to crosstalk between channels, especially when a volt-level stimulation pulse occurs while trying to observe a microvolt-level electrophysiological signal on a nearby channel. In such situations, it may be desirable to activate the "amp settle" function on all channels in a headstage during any stimulation pulses.

In the amplifier settle configuration window, there is a check box titled "Headstage Global Amp Settle". If this box is checked, then an "amp settle" event on any channel in a particular headstage will activate the amp settle functions in all channels on that headstage. The yellow shading in the waveform plots makes it clear when this is happening.

Charge Recovery

As mentioned above, the positive and negative current sources in each RHS headstage are not perfectly matched due to random variations in transistors. This means that ideal charge balance cannot be obtained in practice. For acute experiments this may not have any practical consequence, but for long-term chronic experiments, residual post-stimulation charge may have deleterious effects. To mitigate the effects of residual charge, RHS headstages have the capability to perform **charge recovery** by forcing an electrode to ground, or to another fixed voltage. For any channel, charge recovery may be enabled by checking **Enable Charge Recovery**, and the onset and duration of charge recovery after the end of a stimulation pulse may be specified. The green bar in the stimulation waveform display illustrates (not to scale) the timing of charge recovery.

There are two types of charge recovery that may be selected globally for all channels in all headstages. This selection is accessed in the charge recovery configuration window (above), which is accessed by selecting "Charge Recovery Settings" under the Stimulation menu. The default is **Current Limited Charge Recovery Circuit**, which pulls the electrode toward a fixed voltage (user selectable, in the range of -1.225V to +1.215V) with a driver that has a limited current drive capability, selectable between 1 nA and 1 µA. The other option is the **Charge Recovery Switch**, which activates a transistor-based switch that connects the electrode to ground. This switch has an 'on' resistance on the order of 1 kΩ.

Please refer to the RHS2116 chip datasheet for more details on the charge recovery circuitry.

Note that charge recovery events typically induce recording artifacts.

**Custom Pulses on Analog and Digital Output Ports**

In many experiments, it may be desirable to generate analog and/or digital voltage pulses in response to particular trigger events. For example, these pulses could be used to trigger optogenetic light sources or other experimental apparatuses. Any Analog Out or Digital Out ports on the stimulation/recording controller or optional I/O Expander may be selected, and their stimulation parameters may be set by clicking "Select Stimulation Parameters".

Figures below show the parameter selection windows for Analog Out and Digital Out ports. The stimulation parameters are a subset of the parameters used for electrode current stimulation described above. The Analog Out stimulation parameters also include the ability to select a Monophasic stimulation shape, and the ability to select a non-zero baseline voltage. Pulses may have phase durations up to 5 milliseconds in length. The post trigger delay can be as long as 500 milliseconds. The pulse train period and post-stim refractory period can be as long as one second.
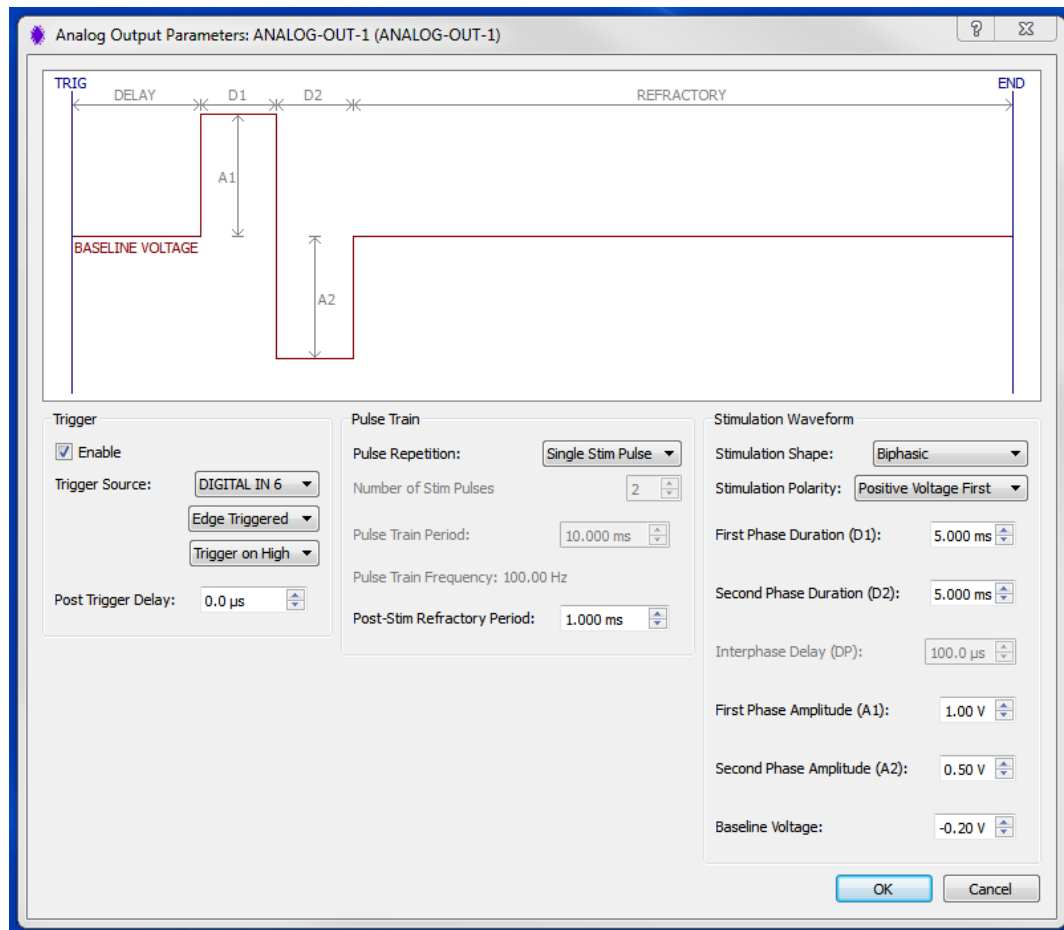
Analog Output custom pulse parameters selection window.
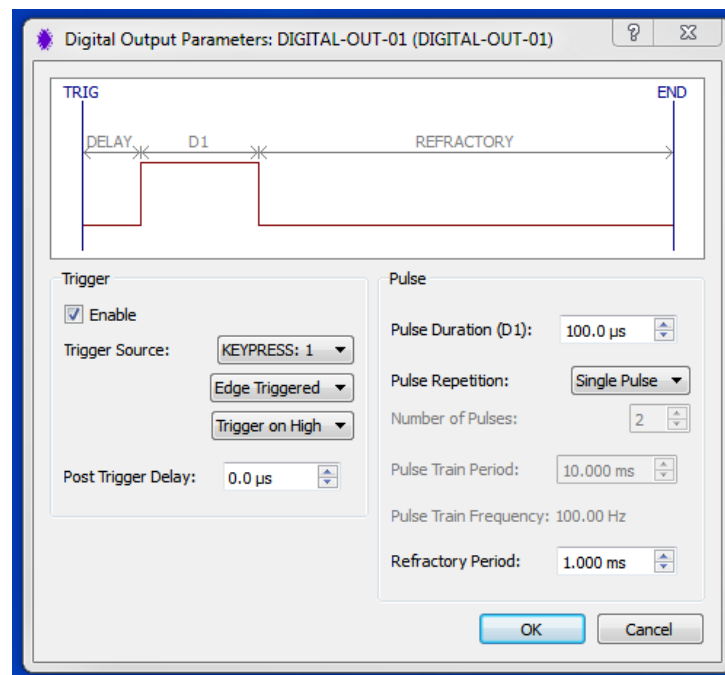
Digital Output custom pulse parameters selection window.

Copying, Pasting, Saving, and Loading Stimulation Parameters

Once stimulation parameters have been configured for one channel, they can be copied and pasted to other channels using the Stimulation menu or the standard keyboard shortcuts for copy and paste.

Stimulation settings for all channels can be saved (in XML format) and loaded using functions in the Stimulation menu.

Stimulating Between Electrodes

When a stimulation current is produced on a single electrode this current will return through the ground electrode. In some experiments it may be desirable to direct current from one electrode to another electrode instead of to ground. This can be achieved by setting up the stimulation parameters on the first channel, then copying these stimulation parameters to a second channel and inverting the Stimulation Polarity from Cathodic Current First to Anodic Current First. If the stimulation pulses on both channels have the same trigger source, amplitude, and duration, and differ only in polarity then current will be directed between the electrodes.

It should be noted that current sources do not match perfectly across channels, so there will likely be some residual current that must still return to ground.

Display of Stimulation Events in Waveform Plots

When a stimulation trigger is enabled on a particular channel, the trigger source is displayed in red text near the corresponding waveform plot (see below). When a stimulation pulse occurs, the background of the waveform plot is highlighted in light red for the duration of the current pulse. (No graphical distinction is made between anodic and cathodic current.) When the amp settle function is engaged, the background is highlighted in light yellow, as shown below.
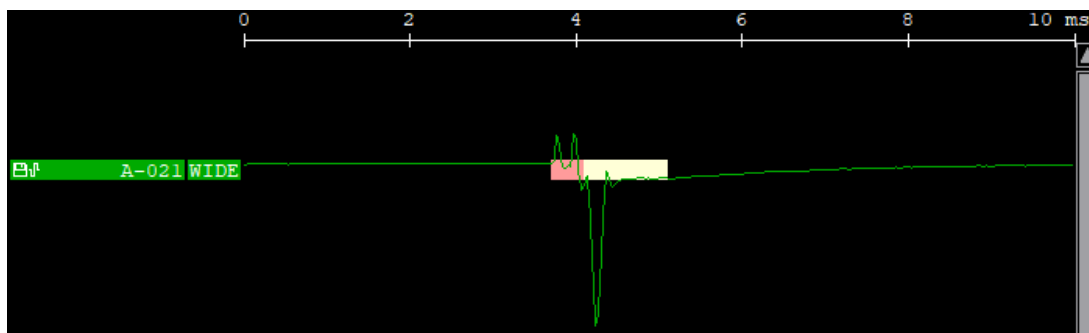
Shading indicating active **stimulation** (light red) and **amp settling** (light yellow). The red label "DIN1" on the left indicates that this stimulation event is triggered by the Digital In 1 port.

If the charge recovery option is selected in the Stimulation Parameters window, the duration of charge recovery will be highlighted in light green:
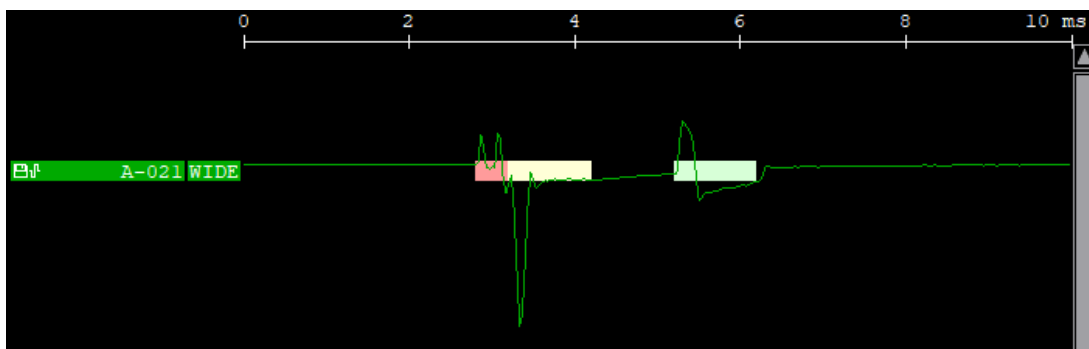
Light green shading indicates active **charge recovery** following stimulation.

Each channel in an RHS headstage contains an integrated **compliance monitor**. This circuit signals a **compliance limit** warning if the voltage on the electrode has moved so close to the positive or negative stimulation power supplies (±9V) that the current sources are unable to deliver the specified current. Compliance limit warnings are shown in dark red on the waveform plot (see below). If a compliance limit is reported, the possible solutions are to reduce the total amount of charge injected into the electrode (by reducing the amplitude and/or duration of stimulation current in each phase) or to use electrodes with lower impedances. Compliance limit warnings can also indicate broken or disconnected electrodes.
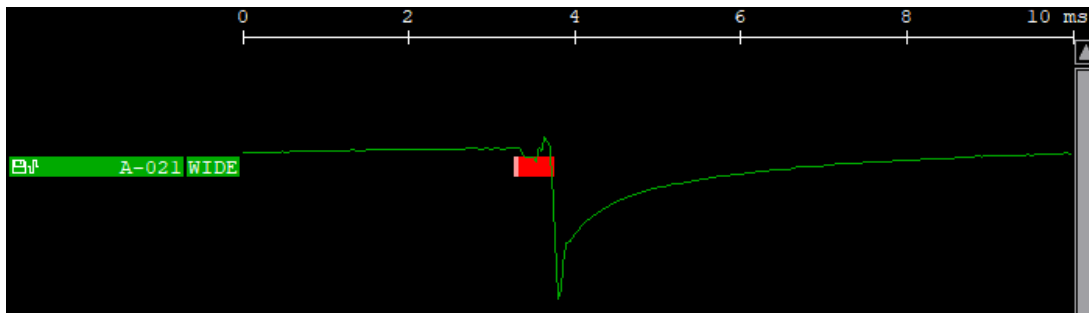

Dark red shading indicates a **compliance limit**, meaning the voltage on the electrode has moved so close to the positive or negative stimulation power supplies (±9V) that the current sources are unable to deliver the specified current.

Monitoring the DC Voltage on an Electrode

Each channel in an RHS headstage contains two types of amplifiers: a high-gain AC-coupled amplifier used for monitoring weak electrophysiological signals, and a low-gain DC-coupled amplifier that may be used to monitor larger voltage excursions induced by stimulation. To view the DC amplifiers, select "DC" from the **Amp** selector to the right of the **Voltage Scale** selector in the main window. The figure below shows an example of a DC amplifier plot when a biphasic current pulse of ±10 µA is being driven into a 500 kΩ resistor, yielding a ±5V waveform as predicted by Ohm's law. Real electrodes have complex impedances that are nonlinear with voltage and do not yield such a simple current-to-voltage relationship.

Also visible below is a brief transient current blip or "overshoot" at the onset of stimulation. This is an imperfection caused by the current source circuitry on the RHS headstage, and is most visible for current amplitudes less than 10 µA. For larger currents, this onset transient is a minor departure from the ideal current waveform. For current amplitudes less than 1 µA, it may lead to a significant deviation from the desired amount of charge injected during the first phase. This behavior is not well characterized and seems to depend strongly on electrode impedance.
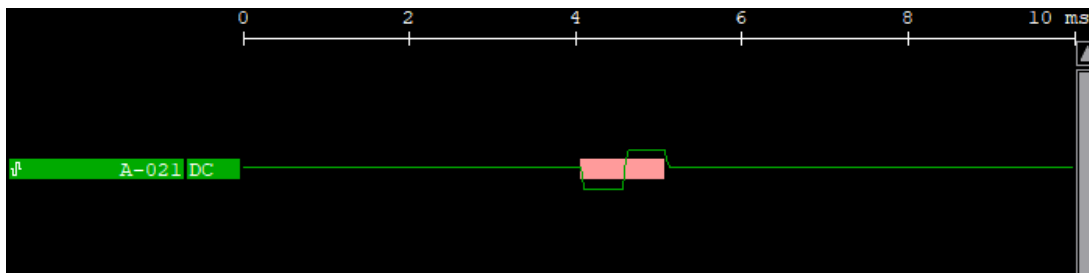
Viewing the electrode voltage using the low-gain DC-coupled amplifier. Note the label indicating a voltage scale of ±10V on the vertical axis. In this example a biphasic current of ±10 µA is being driven into a 500 kΩ resistor.

The DC amplifiers have a very low resolution and cannot be used to observe any known electrophysiological effects. They are only intended to provide a gross indication of electrode voltage. Note that the **Voltage Scale** selector changes to a new set of ranges (from ±1V to ±10V) when DC amplifiers are selected.

By default, the DC amplifier waveforms are not saved. To save these waveforms to disk during a recording session, click on the "Select File Format" button on the Toolbar to bring up the Data File Format window, and then check the box titled "Save DC Amplifier Waveforms".

Using the Software/DAC High-Pass Filter during Stimulation

Stimulation in microelectrodes induces large voltage transients at the electrode-tissue interface, and these transients can take tens or hundreds of milliseconds to return to baseline. These low-frequency artifacts can make it difficult to observe small signals such as neural action potentials, even if the amplifiers are not saturated, due to the fact that the spikes may be riding on very large, low frequency transients. The Software/DAC High-Pass Filter, discussed previously, can be very useful in these cases since it filters out low frequencies on the display, but it does not affect saved data. Setting the Software/DAC High-Pass Filter to values between 200 Hz and 800 Hz can leave spikes visible immediately following a stimulation pulse while rejecting the large deflections from baseline.

Remote TCP Control

The Network menu contains the **Remote TCP Control** tool. This allows other software applications on the same computer or on a networked computer to communicate with the RHX software, both to send commands and to receive data. Example applications for this are provided in MATLAB, Python, and C++, but are not limited to these languages – as long as TCP connections can be made, written to, and read from, the programming language these applications are made with is of little importance. TCP example code as well as a complete TCP command glossary are provided on the Intan website.

Whether TCP is used to command the software or receive data, the RHX software acts as a server and the separate application acts as a client. The first step is to establish a TCP connection by choosing the desired host address and port for the communication and clicking **Connect** to open the RHX software server so that it listens for a client application's attempt to connect. While the status is "Pending", the client application should attempt to connect at the same location, and if the connection is successful, the status will read "Connected".

Command Mode

For command mode, the client application can send commands as ASCII text to control various parameters of the RHX software. For example, "Set RunMode Run" will begin data acquisition, the same as if **Run** were clicked on the main window toolbar. The current state of these parameters can also be queried and read by the client application. For example, "Get RunMode" will prompt the RHX software to send the client application an answer, which can then be read by the client application; typical values would be "Stop" or "Run". Refer to the **Intan RHX TCP Guide** for a comprehensive list of commands.
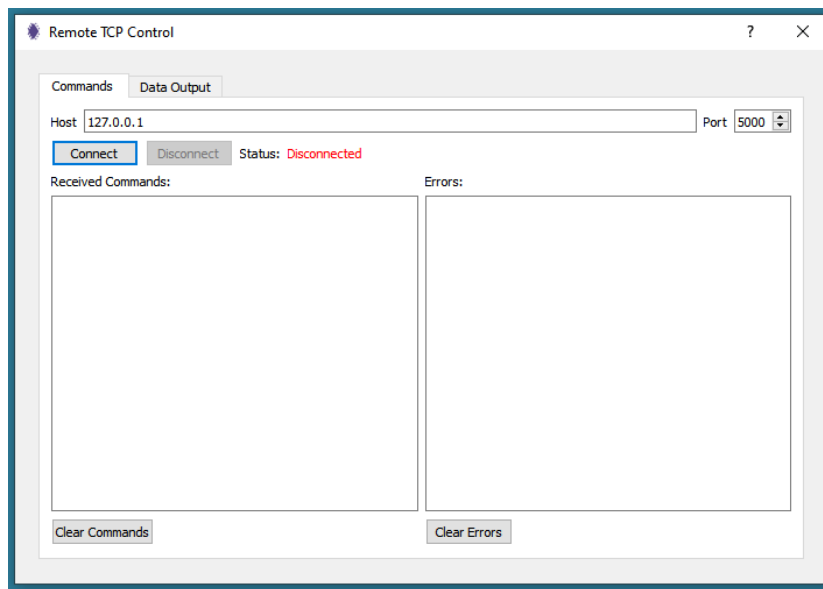
Data Output Mode

For data output mode, the RHX software streams the data that might normally be saved to disk over the connected TCP socket(s). The channels and filter bands to be output can be configured by adding them to **Channels To Stream**. For all waveforms excluding spike rasters, this data will be output through the Waveform Output socket. Spike raster event data will instead be output through the Spike Output socket. It is important that the client application reads the data output quickly, otherwise the memory allocated for data output will fill up and halt data acquisition. This can be somewhat alleviated by reducing the sample rate and limiting the number of channels having their data output via TCP, but it is still vital that the client application not allow the TCP data output sockets to back up.
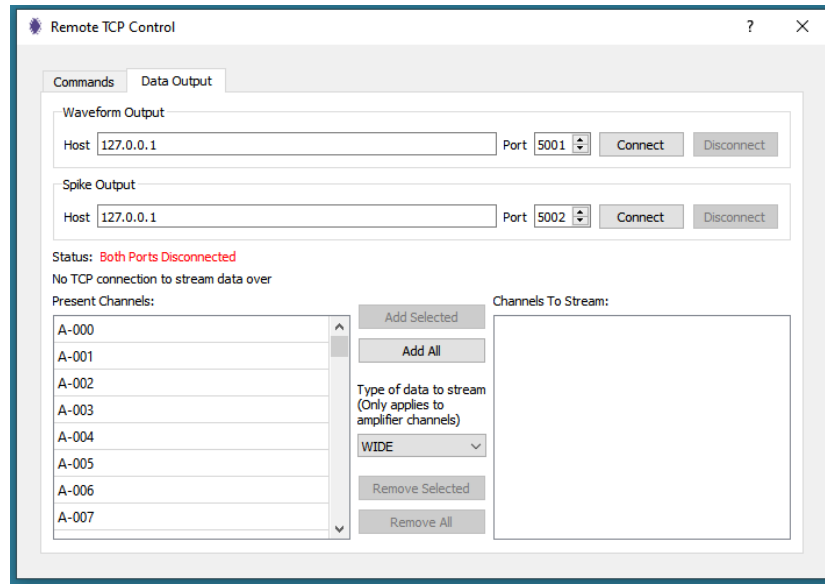
Uses

Remote TCP Control opens up many possibilities for experiment automation. Configuration of recording parameters (e.g., bandwidth, software filters, data file format, save location) and channel-specific stimulation parameters for RHS (e.g., stimulation triggers, shape, magnitude, duration) can be written into the client application to automate the setup steps that may take several minutes to complete manually, similar to loading a settings file. However, even starting and stopping recording can be automated in this way, so that an entire recording session can be controlled through the client application. See the provided MATLAB, Python, and C++ demo applications that demonstrate how each of these languages can be used to control the RHX software.

Because TCP is a network protocol, another possibility is for the client application to run on a computer separate from the host application. As long as the host computer (which runs the RHX software) is connected to the Internet and attempting to connect at a certain address and port, a connection can be made from any other Internet-connected computer to that host application. This allows for experiments to be controlled remotely, as long as the RHX software can be manually started and have its TCP functionality opened.

Aside from the ability to control recordings via TCP, the Data Output capability allows for users to develop near real-time analysis tools (with latency sources being USB to the host computer, and TCP to the client). As long as a client application is able to receive and process data over TCP quickly enough, that client application could be written to perform any number of analyses on data while the recording is still underway. For example, if a user wanted to implement a spike-sorting algorithm, they would want to receive a chunk of high-pass data from the TCP socket, parse and analyze it with their custom algorithm, and prepare to receive the next chunk. Care must be taken in order to avoid data backup on the TCP socket, so limiting the sample rate or number of channels with TCP output may be necessary. Look to the "Realtime" example MATLAB script as an example of how data can be parsed and plotted in real-time while data acquisition is underway.

Importing Recorded Data into MATLAB

Intan Technologies provides open-source m-files (**read_Intan_RHD2000_file.m** and **read_Intan_RHS2000_file.m**) for importing data recorded from the RHX software into MATLAB. Make sure you have the latest version of this m-file (**version 3.0 or later**) to ensure compatibility with the new version of the software. Running this m-file brings up a file selector dialog with which the user locates and selects the desired .rhd or .rhs data file. The m-file then loads and parses the data file and creates several variables in the base MATLAB workspace containing all voltage waveforms, time vectors, bandwidth information, and amplifier channel settings (e.g., name, channel number, last measured impedance). Since the m-file creates variables in the base workspace, it is recommended that all other variables be deleted by using the "clear" command before running this function.

Following is a transcript of a typical MATLAB session loading a recorded data file and looking at several data structures:

```
>> clear
>> read_Intan_RHS2000_file

Reading Intan Technologies RHS2000 Data File, Version 3.0

Found 32 amplifier channels.
Found 32 DC amplifier channels.
Found 0 board ADC channels.
Found 8 board DAC channels.
Found 0 board digital input channels.
Found 0 board digital output channels.

File contains 15.428 seconds of data.  Amplifiers were sampled at 30.00 kS/s.

Allocating memory for data...
Reading data from file...
10% done...
20% done...
30% done...
40% done...
50% done...
60% done...
70% done...
80% done...
90% done...
100% done...
Parsing data...
No missing timestamps in data.
Done!  Elapsed time: 1.3 seconds
Extracted data are now available in the MATLAB workspace.
Type 'whos' to see variables.

>> whos
  Name                      Size                    Bytes  Class      Attributes

  amp_settle_data           32x462848           118489088  double
  amplifier_channels        1x32                    43008  struct
  amplifier_data            32x462848           118489088  double
  board_dac_channels        1x8                     11808  struct
  board_dac_data            8x462848             29622272  double
  charge_recovery_data      32x462848           118489088  double
  compliance_limit_data     32x462848           118489088  double
  dc_amplifier_data         32x462848           118489088  double
  frequency_parameters      1x1                      3128  struct
  notes                     1x1                       528  struct
  spike_triggers            1x32                    15616  struct
  stim_data                 32x462848           118489088  double
  stim_parameters           1x1                       920  struct
  t                         1x462848              3702784  double

>> frequency_parameters

frequency_parameters =

            amplifier_sample_rate: 30000
            board_adc_sample_rate: 30000
         board_dig_in_sample_rate: 30000
```

```
        desired_dsp_cutoff_frequency: 5
         actual_dsp_cutoff_frequency: 4.6650
                         dsp_enabled: 1
              desired_lower_bandwidth: 5
       desired_lower_settle_bandwidth: 1000
               actual_lower_bandwidth: 5.0044
        actual_lower_settle_bandwidth: 1.2059e+03
              desired_upper_bandwidth: 7500
               actual_upper_bandwidth: 7.6038e+03
               notch_filter_frequency: 0
      desired_impedance_test_frequency: 1000
       actual_impedance_test_frequency: 1000
                      amp_settle_mode: 0
                 charge_recovery_mode: 0

>> amplifier_channels(1)

ans =

              native_channel_name: 'A-000'
              custom_channel_name: 'A-000'
                     native_order: 0
                     custom_order: 0
                     board_stream: 0
                     chip_channel: 0
                        port_name: 'Port A'
                      port_prefix: 'A'
                      port_number: 1
       electrode_impedance_magnitude: 0
          electrode_impedance_phase: 0

>> plot(t, amplifier_data(1,:))
>>
```

The time vector is contained in the variable `t`. Corresponding waveform data are stored in the array `amplifier_data`, with units of microvolts. If DC amplifier data was selected for saving, it is stored in the array `dc_amplifier_data` with units of volts. Stimulation current waveforms for each channel are stored in `stim_data` with units of microamps. Other stimulation-related events are saved in `compliance_limit_data`, `amp_settle_data`, and `charge_recovery_data`; each of these three arrays have values of 0 or 1 at each time to indicate a compliance limit detection, the activation of amp settle circuitry, or the activation of charge recovery circuitry, respectively.

The Analog Out port waveforms are saved as `board_dac_data` with units of volts.

The data structure `amplifier_channels` contains information on each amplifier channel whose data is contained in `amplifier_data`. The `spike_trigger` data structure contains threshold levels set in the Spike Scope. (This is provided for informational purposes only; the spike triggers do not influence how waveform data is saved. However, spike trigger information could potentially be used in a later post-processing step to compress waveform data.)

The `frequency_parameters` structure contains information on amplifier bandwidth and sampling rates. The `stim_parameters` structure contains information on stimulation settings. The `notes` structure contains text notes entered in the Configure tab.

In this example, no analog or digital input channels were enabled when the data file was saved. If those waveforms had been present, additional MATLAB variables would have been created containing this data as well.

Note that this MATLAB m-file supports the "Traditional Intan File Format", as well as the informational header files for the other two saved data formats. Information on reading waveform data from these other file formats may be found in the documents **RHD Application note: Data file formats** and **RHS Application note: Data file formats**, available from the Intan Technologies website.

Upsampling Waveform Data

Intan Technologies also provides an m-file for upsampling waveform data by a factor of two: **upsample2x.m**. Following is an example of upsampling amplifier data from the previous file from from 20 kS/s to 40 kS/s:

```
>> [t_2x, amplifier_data_2x] = upsample2x(t, amplifier_data);

Upsampling waveforms by 2X...
10% done...
20% done...
30% done...
40% done...
50% done...
60% done...
70% done...
80% done...
90% done...
100% done...
Done!  Elapsed time: 11.7 seconds

>> plot(t_2x, amplifier_data_2x(1,:))
>>
```

Users may type `help upsample2x` at the MATLAB prompt for more information on this function.

To save disk space, users may wish to sample amplifiers at a lower rate and then upsample data to achieve a higher time resolution for spike sorting algorithms, for example. This m-file uses cubic spline interpolation to perform the upsampling.

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