

SpikePac User's Guide



Updated: 7/22/2014

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Before You Begin

Software Requirements


TDT Drivers and the OpenEx Software Suite must be installed before installing SpikePac. The recommended operating system for all TDT systems is Windows 7[®].

Hardware Requirements

The SpikePac tools are supported by RX or RZ System 3 High Performance Processors. See the *System 3 Installation Guide* for hardware installation and set-up instructions.

Installation

Install TDT Drivers and OpenEx prior to SpikePac.

Installing SpikePac enables special features in OpenEx and RpvdsEx, notably, adding an  *Insert SpikePac Macro* button to the RpvdsEx toolbars.

OpenEx Fundamentals

SpikePac builds on existing features in the OpenEx Suite. TDT recommends completing the *Getting Started in OpenEx Tutorial* found in the *OpenEx User Guide* before working with SpikePac.

Organization of the Manual

This manual is organized in the following sections:

- **Introduction:** A brief overview of the SpikePac tools and how they work with OpenEx.
- **The Tools:** A guide for the *SpikePac Macros* and control sets provided by SpikePac.
- **Tutorial:** A step-by-step tutorial for building an OpenEx Project using *SpikePac Macros*.
- **Glossary:** A listing of the terms associated with this user guide and their definition.
- **Keyboard Shortcuts:** A useful reference to the various shortcut keys used for viewing and working with data.
- **References:** A listing of the sources that may be referenced for additional information.

Introduction

SpikePac is a powerful extension package for TDT's OpenEx software suite designed specifically for researchers doing multi-channel neural recordings. The software package includes a group of easy-to-use RPvdsEx components and paired OpenController interfaces that provide the foundation needed to build powerful recording paradigms. These building blocks handle all OpenEx integration details automatically. Most importantly, SpikePac offers this new level of integration without compromising the flexibility essential to TDT customers.

SpikePac includes:

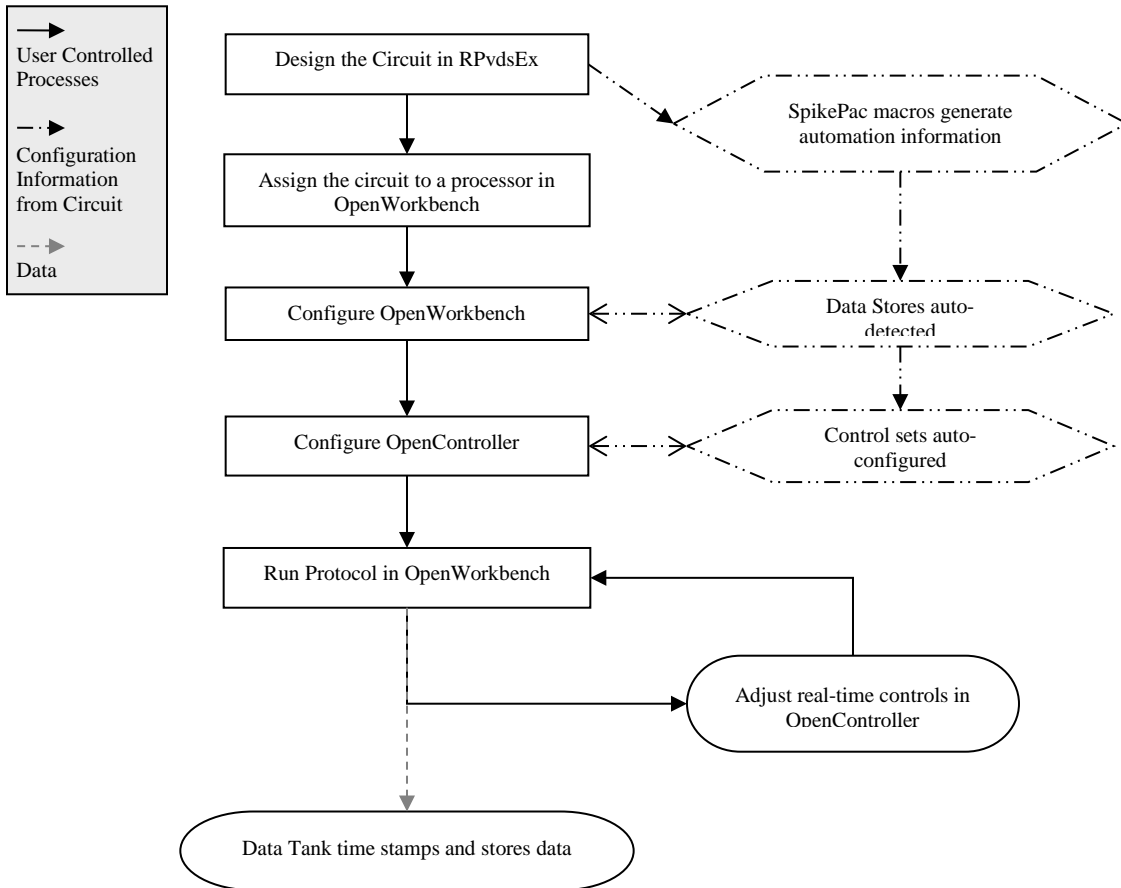
- Principal Component Feature Space Spike Sorting
- Real-Time, Time-Voltage Spike Sorting
- Tetrode Sorting
- Electrical Stimulation Generation
- Filtering
- Signal Monitoring with Noise Gating
- Electrode Site Remapping

These tools focus primarily on providing a means of detecting, storing, and classifying events (also called spikes) that are likely to be action potential waveforms that result from neural firings. The process of spike sorting attempts to separate the waveforms into distinct waveform groups associated with individual neurons (called units or clusters). Building on existing OpenEx functionality, SpikePac simplifies experiment configuration and incorporates a broader range of spike sorting techniques and performance enhancing tools.

To use SpikePac, simply add a SpikePac component to the RPvdsEx circuit for your experiment. When the circuit is loaded to a device in OpenEx any data stores are automatically inferred in OpenWorkbench and the paired user interface for that component is made available in OpenController.

The diagram below illustrates the experiment design process, highlighting the automation provided by SpikePac.

SpikePac Process Flow Chart



The Tools

Overview

SpikePac Macros

When OpenWorkbench hardware server loads a circuit containing a SpikePac macro to a processor, the OpenController client will make the corresponding control available to the user.

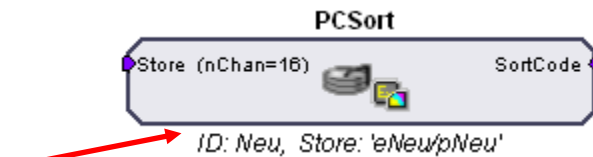
Macro Configuration

Macro configuration options are accessed through the macro setup dialog menu. Double-clicking on any macro will open the setup properties dialog menu and onboard help.

ID

IDs are used to identify the corresponding data stores and parameters that can be controlled via the runtime application. The ID is automatically appended to the end of the name of the corresponding OpenController control set and ensures that all parameter targets are found and enabled for real-time control. Users must ensure that any two instances of the same macro are assigned unique IDs. That is, if a NeuroFilter and a NeuroMon macro are added they may share the same ID. If a second NeuroFilter macro is added it must use a different ID.

Note: Store Pooled macros share the same ID and Store names. See the PCSort or BoxSort onboard macro help for more information about store pooling.



Parameter Summary

As shown above, the macro parameter summary displays the ID and other macro information, such as data Stores.

Adding a SpikePac Macro in RPvdsEx

SpikePac macros are added to circuits in much the same way as existing macros and offer the same easy-to-use parameter setup dialog box.

To quickly add a SpikePac macro:

- In RPvdsEx, click the  **Insert SpikePac Macro** button and select the desired macro then click the workspace.

See the *Tutorial: Online Spike Sorting with SpikePac*, page 75, for step-by-step practice with incorporating SpikePac Macros into your circuit design.

The Controls

The controls provided by SpikePac are pre-configured control sets for higher level tasks such as filtering, spike-sorting, or channel-monitoring. They are available in OpenController only when the compiled circuit file loaded through OpenWorkbench contains the corresponding macro. For example, adding a SpikePac filtering macro to an RpvdsEx circuit will allow a pre-configured filter control to be added in OpenController design mode. SpikePac controls save time and eliminate the need to configure controls manually.

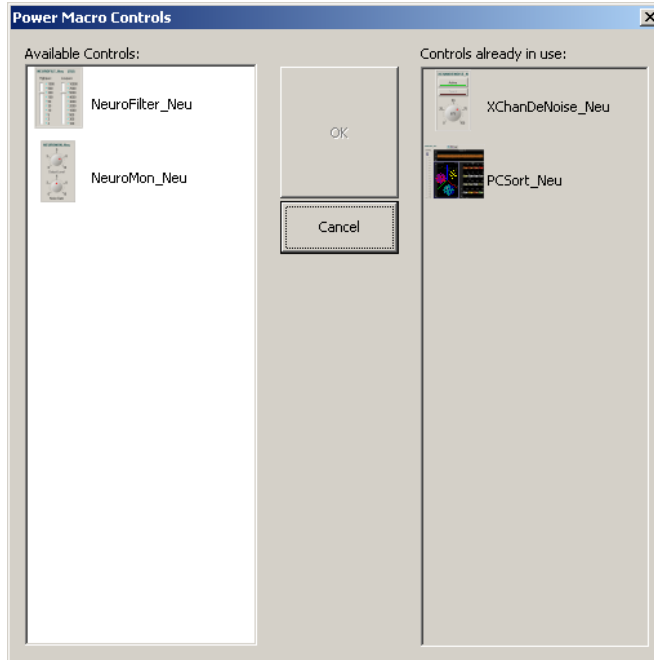
Adding a SpikePac Control in OpenController

Before attempting to add the control, ensure that the corresponding macro is used in the compiled circuit file that is assigned to a device in OpenWorkbench.

To quickly add the Control:


1. In OpenController, click the **Controls** menu, select **Power Macro**.

The Controls dialog box displays a list of controls available and a list of controls that have already been added to OpenController.



2. Select the desired control and click **OK**. The pointer changes to indicate that the control can be added.
3. Click the grid to position the upper-left corner of the control. The control is added to the grid area.

These controls are pre-configured and initialized using information in the compiled circuit file, so they can usually be used without any additional configuration. If you need to make changes to the control, double-click the control to display the setup properties

dialog box (or, if available, click the **Setup Properties**  button).

4. Before running the control, the corresponding OpenWorkbench file must be open and a protocol should be running. To run the control, click **Run!** on the menu bar.

PCSort - Principal Component Feature Space Spike Sorting



The PCSort macro is used to time stamp and store waveform events as data snippets. It supports online spike sorting through the PCSort control in OpenController. Candidate waveforms are detected based on their deviation from the noise of the system. By default, the time stamp and position of the waveform in the snippet is dependent on the time of the threshold crossing for the signal. An alternative setting allows waveform time stamp and positioning to be determined by the waveform's highest peak, aligning snippets to their respective peaks. The macro can be configured to output the sort code for each channel once sorting parameters have been loaded to the hardware through OpenController.

This macro generates two data Stores: one for sorted snippet data, designated with the prefix e, and one for the plot decimated data stream (a representation of the waveform using maximum and minimum values and used to visualize spike activity), designated with the prefix p. The ID is appended to the Store names and both are displayed in the macro's parameter summary.

Settings for configuring the number of channels, threshold mode, number of clusters and spheres, and window width for each snippet stored are defined in the macro properties dialog box. See the onboard help for more information.

The corresponding PCSort control set works in three general phases:

Training

When run, the control set implements spike detection and a training time begins. Events collected during the training time are used to calculate the feature space. By default, auto-clustering is disabled and no clustering (or sorting) takes place until initiated by the user.

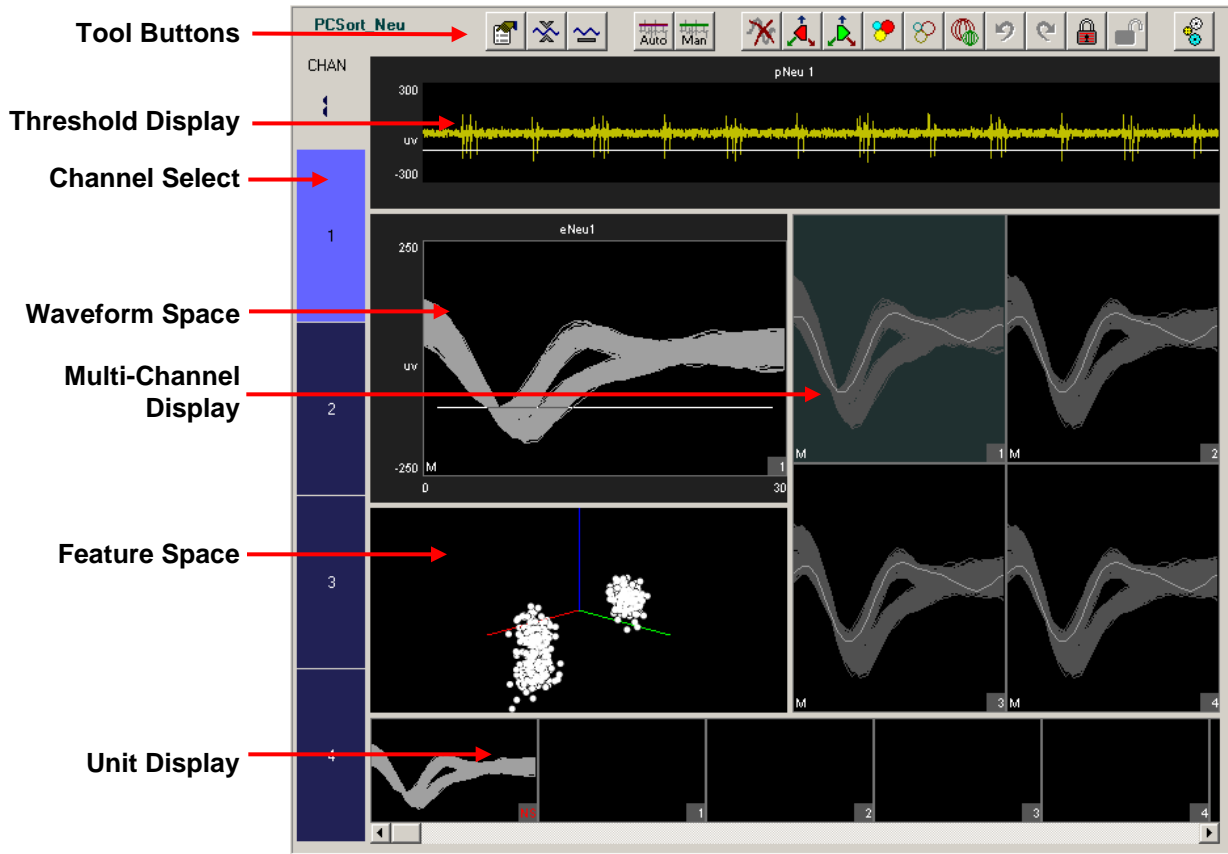
Classification

The next phase begins when clustering is initiated. Preliminary identification of units is indicated by color coding in the control's displays for visualization; however, all candidate spikes are saved to the data tank with a sort code of 0 during this phase. The default clustering method is a Bayesian algorithm, but users may choose a K-means method or edit clustering using manual cluster cutting techniques. During this phase the user can explore the data and modify sort parameters without affecting saved data.

Sorting

When satisfied with the clustering, the user can choose to *Apply Sorts*. The clustering parameters are loaded to the circuit running on the hardware and sort codes will be applied to new data as it is acquired in real-time.

The default threshold factor, peak alignment, training termination control, sorting algorithm, and auto-clustering are modified by the user in the control's Setup Properties.



The Control Set window includes:

- Tool Buttons** provide access to tools and parameters.
- Threshold Display** displays the plot decimated waveform of the currently selected channel and allows threshold adjustments under manual thresholding mode.
- Channel Select** selects the active channel and displays channel status.
- Waveform Space** displays a waveform representation of candidate spikes for the active channel in a pile plot. In this plot, users can view and manually classify waveforms by shape. A progress bar is included along the top of the plot during the training period. Indicators in the bottom left corner denote scaling and threshold tracking states.
- Multi-Channel Display** displays each channel in a separate sub-plot. The channel number is shown in the bottom right corner and waveforms are highlighted as they are added to the plot. A progress bar is included along the top of each sub-plot during the training period. Indicators in the bottom left corner denote scaling and threshold tracking states.
- Feature Space** displays the active channel of candidate spikes in terms of their principal components. This reduces the dimensionality of the data while making it visually easier to comprehend.
- Unit Display** displays a single channel of candidate waveforms by unit—each plot displays all waveforms classified with a single sort code. If there are more units than can be displayed within the width of the window, a scroll bar is displayed.

Simple Zoom

You can zoom any plot to see more or less detail. Zooming does not attempt to scale or fit the waveform shape or data in the plot, allowing you to control the view without regard to the limitations of the plot size.

Note: changing the zoom level in the Multi-Channel Display alters the zoom level for all channels in the Multi-Channel Display.

To change the zoom level of any plot:

- Point to the center of the plot, press and hold down the Shift key, and drag the mouse up or down.

To reset the view of any individual plot:

- Hold down the Shift key and double-click the mouse within the plot.

Note: this method can be used to reset the feature space to its default position.

Display Scale


When the PCSort control set is run, all channels begin at a *base scale* of 1. This type of scaling clearly shows differences in magnitude across units but can make it more difficult to see waveform shapes for some channels.

To make it easier to see waveform shapes for channels with lower magnitude, you may scale individual channels manually or normalize all channels to fit to a similar scale. If you choose to normalize the display, the PCSort control set calculates the display scale for each channel individually, attempting to fit around 80% of the signal's vertical size in each plot. In the case of the Multi-Channel Display, the waveform with the largest magnitude is used to compute the fit.

When the display has been normalized, PCSort displays either an up or down arrow in the bottom left corner of the plot or subplot to indicate whether the display has been scaled up or down.


An Auto Scale feature can be used to refresh the scale of plots either with or without normalizing the display. **Note:** changing the display scale does not alter the data being stored.

To refresh the display without normalizing plots:

1. Click the **Auto Scale**  button.
2. Click **No** when asked if you want to normalize all window groups.


Note: this will not reset the feature space display.

To normalize all channels:

1. Click the **Auto Scale**  button.
2. Click **Yes** when asked if you want to normalize all window groups.

Note: an arrow will now be displayed on the bottom left corner (next to the threshold mode indicator) which indicates positive or negative deviation from the base scale setting.

To return all channels to their base scale:

1. Click the **Base Scale**  button.
2. Click **Yes**.

Note: resetting plots to base scale does not remove any Zoom applied to a plot.

To adjust the scale of a single channel:

- Point to the desired channel in the Multi-Channel display, press and hold down the Ctrl key, and drag the mouse up or down.

Note: while adjusting the display scale, the numeric value in the lower right corner of the channel indicates the new scale value.

To return a single channel to its base scale:

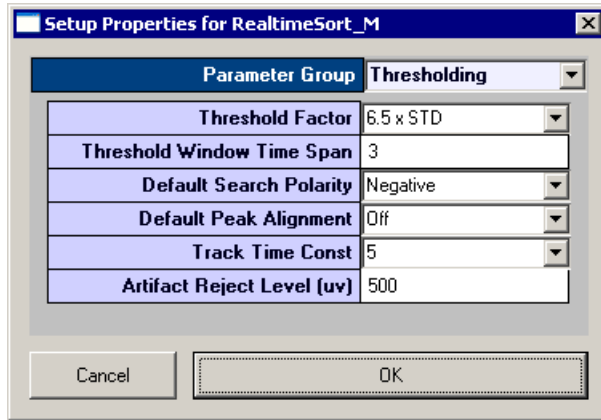
1. Right-click the desired channel.
2. Select **Reset Scaling** from the menu.

The Setup Properties

Users can configure the control, choose a Bayesian or K-mean sorting algorithm, and enable or disable auto clustering in the control set's Setup Properties.

To open the setup properties:

- Click the **Setup Properties**  button.



The Setup Properties window includes four parameter groups available from a drop down list.

Thresholding Parameter Group

Threshold Factor – Select a value to set base threshold for spike detection to a value which is $n * \text{STD}$ of the signal RMS. This setting is active only when automatic threshold tracking is enabled.

Threshold Window Time Span – Type a numeric value to set the time span of the threshold window in seconds.

Default Search Polarity – Select a positive or negative polarity for the threshold value. This setting is active only when automatic threshold tracking is enabled.

Default Peak Alignment – Sets the default alignment to Peak Align which aligns spikes according to their peak values altering the time stamp and positioning of the snippet.

Track Time Const – Sets the time constant tau to 3, 5, or 10 seconds. Shorter tau values respond more drastically to deviations in the signal RMS value. This setting is active only when automatic threshold tracking is enabled.

Artifact Rejection Level (μV) – Type a numeric value to set the artifact rejection level in micro Volts. **Note:** Artifact Rejection must be enabled in the macro setup properties in RPvdsEx.

Display Parameter Group

Clear History Counter – Enter a number to set a limit on the number of events displayed prior to a display refresh. This does not delete historical data from the data tank. It simply removes existing data displays and refreshes each plot. With the default setting of 0, plots will display accumulated historical data.

Max Channels To Display – Enter a number to limit the amount of channels that will be displayed in the Multi-Channel Display. The channel select bar will reorganize into one or more columns based on the settings applied. The default value is 64.


Channel Column Count – Enter a number to set the number of columns for the Multi-Channel Display. With the default setting of 0, the PCSort control will automatically select the number of columns to display.

Display Layout – Select either Standard or MultiChannel View modes of display. In Standard display mode the Threshold and Unit displays extend the entire width of the control set (see page 8 for an example of the *Standard Display Mode*). In MultiChannel View mode, the right side of the control set is used only for the Multi-Channel Display, extending vertically from top to bottom. This provides more room for multi-channel display.

Training Control Parameter Group

Ref Tank – Specify a reference tank that the user has previously sorted using TDT's OpenSorter. The PCSort control will load an eigenvector previously calculated for that sort and use it to determine the feature space, eliminating the need for a training time. The spike event in the selected reference tank must have the same number of samples (number of points).

Training Termination – set the conditions under which the training period for calculating the features space is complete. Three factors are considered: score (feature space stability and sort quality), number of events, and training time.

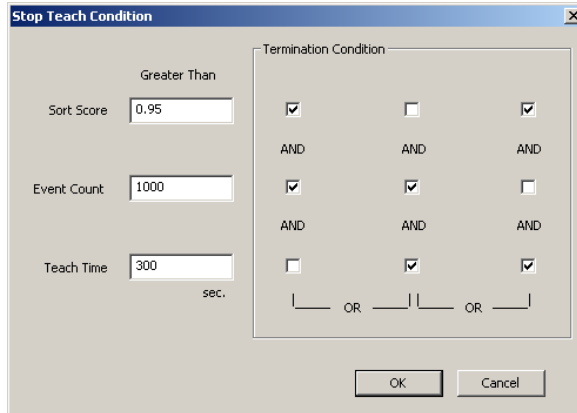
Click the  Browse button to open the Stop Teach Condition window, which allows users to create a logic statement that is used to evaluate and apply specified criteria.

The **sort score** is a value between 0 and 1 that reflects both feature space stability as well as the sort quality. The stability is measured by comparing one feature space to the next each time the feature space is recalculated during the training period. As the percentage of variability in the feature space for each successive calculation decreases, the feature space is considered more stable. The sort quality is an indication of how well the defined clusters fit the data. Quality is measured by considering the percentage of events that lie within the clusters. The overall score is a weighted average of these two factors.

The **event count** is the number of spikes detected.

The **teach time** is a time interval in seconds.

First, enter the Greater Than value for any criteria to be used. Then, select the check box to generate the logic statement. Selections in the same column are ANDed while columns are ORed. This allows for complex teach termination conditions, as shown in the example below.



Greater than:
 Score AND Event Count
 OR
 Event Count AND Teach Time
 OR
 Score AND Teach Time

If all termination conditions are omitted (unchecked), a warning will be displayed after the OK button is pressed.

Auto Clustering Parameter Group

Auto Cluster Active – Automatically clusters all channels as the feature space is being calculated. This renders the display sphere button inactive until the feature space has either been accepted or training has ended.

Clustering Method – Select between Bayesian and KMeans sorting algorithms.

Bayesian - Sorting based on expectation-maximization analysis of Bayesian probabilities.

KMeans - A binary split algorithm that attempts to find the optimum locations of the cluster centers through an iterative process and a defined number of clusters (specified by KMeans Num Clusters).

See page 97 for a list of references with more information about sorting techniques.

KMeans Num Cluster – Set the max number of clusters (1-5) for the KMeans sorting algorithm. If KMeans is selected, this number determines the maximum number of clusters the algorithm will iterate through to find the best fit for the data. If adding another cluster does not improve the efficiency of the algorithm it is not added.

Sorting Model Parameter Group

Cluster Radius xSTD – Type a value to set the radius of the sphere boundaries around each cluster. The number is a factor multiplied to the cluster standard deviation.

SortTerminateTime – Adjust the numeric value to set the termination time value in seconds during the training period. This setting adjusts the **Teach Time** in the Training Control parameter group.

SortTerminateCount – Adjust the numeric value to set the termination count value during the training period. This setting adjusts the **Event Count** in the Training Control parameter group.

SortTerminateScore – Adjust the numeric value to set the termination score value during the training period. This setting adjusts the **Sort Score** in the Training Control parameter group.

Selecting the Active Channel

The channel selector bar along the left side of the control set (shown below) determines which channel is active and displays information about the status of each channel.

To set a channel as active:

- Click the selector box for the desired channel.

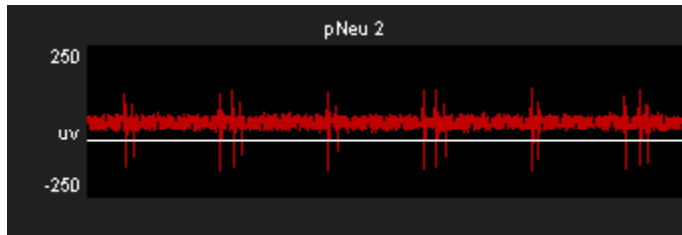
CHAN	The active channel is displayed in all single channel plots within the set and is highlighted in the multi-channel plot.		
⌵			
1			← Active Channel Color is light blue.
2			← Training Complete Color is dark blue.
3	← Locked Channel* Color is gray.		

*See *Tool Buttons* on page 20 for more information.


The color of the channel selector indicates the status of each channel. When training is active and the feature space is being calculated, the colors will match the training progress bar. The selector appears blue once the training is complete or if the feature space is accepted. If a channel is locked, the color on the selector will be shown as gray. For more information about the training period, see *Calculating the Feature Space*, page 16.

Using the Threshold Control

The threshold control displays the plot decimated waveform for the selected channel along with the threshold marker. The default value specified for the threshold factor is 6.5 x STD (standard deviations from the waveform’s RMS value).



To change the base threshold:

- Click the **Setup Properties**  button and select a new **Threshold Factor** from the **Thresholding** parameter group. You can also change the search polarity of the Threshold in this parameter group. See page 10 for more threshold parameter setting options.

Note: base threshold values only affect threshold tracking.

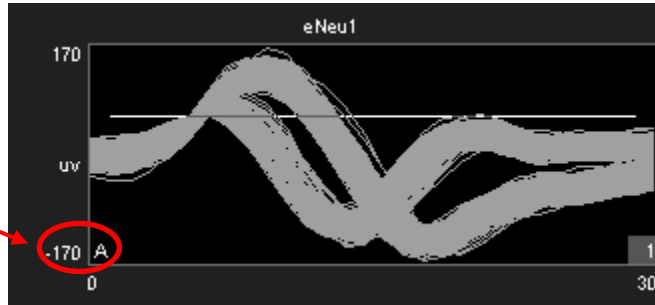
Threshold Tracking

Threshold tracking adjusts the threshold in response to changes in each channel waveform’s RMS, using a default time constant (tau) of five seconds.

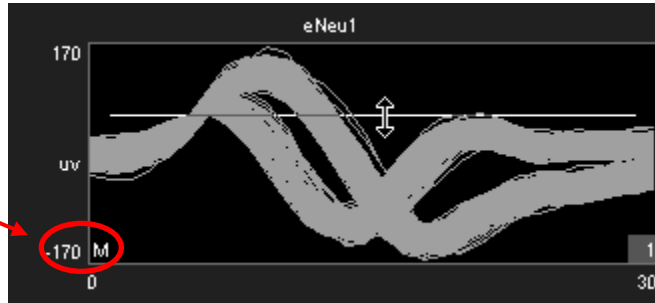
Note: tracking is performed on the DSP and is enabled by default in the PCSort macro. Automatically tracking the threshold on many channels of data can tax the processor; see *Saving Cycle Usage* below if you are recording many channels.

When threshold tracking is active the threshold bar will be locked preventing manual adjustments. An 'A' indicator is placed in the bottom left corner of the Waveform Space and Multi-Channel Display plots to denote if threshold tracking is currently active for a given channel. Otherwise, an 'M' indicator will be shown to denote that threshold tracking is disabled.

Threshold tracking is active for the current channel.




Threshold tracking is disabled for the current channel, allowing for manual threshold adjustments.



To enable threshold tracking for all channels:


Note: automatic threshold mode must be enabled in the PCSort macro setup properties.

1. Click the **Auto Threshold**  button.
2. Click **Yes** to enable threshold tracking for all channels.

To enable or disable threshold tracking for individual channels:

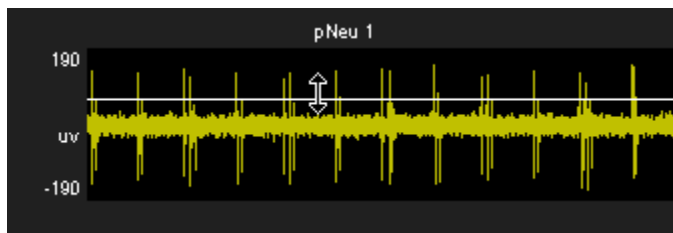
- Right-click on the desired channel and select either **Auto Threshold** or **Manual Threshold**.

To disable threshold tracking for all channels:

1. Click the **Manual Threshold**  button.
2. Click **Yes** to disable threshold tracking for all channels.

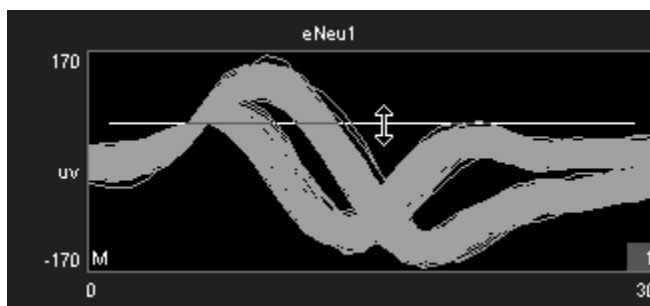
Setting the Threshold Manually

When the corresponding channel is set to manual threshold mode, the threshold bar may be adjusted manually in the threshold control window by clicking and dragging the threshold bar (shown below).



Note: hold the Ctrl key and double-click the Threshold Display to toggle threshold tracking on or off for the active channel.

Manual adjustments to the threshold can also be made in the waveform space as shown below.



To change the threshold for a single channel:

- Click and drag the threshold bar to the desired location in either the Waveform Space or Threshold Control plots.

To apply the current threshold setting for all channels:

Note: this will only set the threshold for channels in which threshold tracking is disabled.

1. Right-click the desired threshold location in the Threshold Display.
2. Select **Set Threshold Here** from the menu.
3. Click **Yes** to apply the threshold setting to all channels in manual threshold mode.


Saving Cycle Usage

Threshold tracking relies on circuit components contained within the PCSort macro. This means that the DSP is continuously tracking the threshold while it is performing other tasks such as filtering and acquisition. This process is taxing on the device cycle usage rates and may diminish the ability of PCSort to process higher channel counts. Threshold tracking can be disabled in the circuit through the PCSort macro setup properties. When disabled, only manual threshold mode is available (signified by the manual threshold button being grayed out). Manual threshold methods must be used to obtain the threshold settings but this frees up cycle usage on the DSP enabling more processing.

In addition to disabling threshold tracking, the PCSort control alters the functionality of the Automatic Threshold button. When the button is clicked, an instantaneous calculation of the data is performed by the PC to determine a reasonable threshold setting for all channels.

To instantly calculate the threshold for all channels:

Note: automatic threshold mode must be disabled in the PCSort macro setup properties.

1. Click the **Auto Threshold**  button.
2. Click **Yes** to calculate the instantaneous threshold for all channels.


To instantly calculate the threshold for a single channel:

Note: automatic threshold mode must be disabled in the PCSort macro setup properties.

1. Right-click the desired channel.
2. Select **Auto Threshold** from the menu.

Artifact Rejection

Artifact rejection is configured through the control setup properties window using the **Setup**

Properties  button. Once enabled, events that fall outside of the specified value are ignored.

Calculating the Feature Space

When the control is run, a *training period* begins, during which the feature space is calculated. As events are added to the control display they become part of the *history* or data set used for feature space calculation. The feature space is periodically recalculated using all data in the *history* at that time until the training period is complete.



During the training period, a colored progress bar (shown above) is added to many of the plots. A pointer moves along the bar to show progress towards the maximum time for training.

By default, the progress bar is colored blue. If active auto-clustering is enabled, the color of the bar indicates the current score for the feature space.

- Red = poor score
- Yellow = good score
- Green = great score

Arrows located on either end of the training progress bar can be used to restart the time variable of the training period (left arrow) or to accept the feature space (right arrow) for the active channel.

The training period continues until one of the following two conditions is met:

- The termination condition is satisfied (see page 11).
- The feature space is manually accepted by the user.

When the training period is ended, automatic or manual clustering methods can be used.

To accept the current feature space for all channels manually:

- Click the **Accept Feature Space**  button.

To recalculate the feature space for all channels:


- Click the **Recalculate Feature Space**  button.

When clicked and confirmed, this option will prompt you to either keep the existing event *history* or clear it prior to recalculating the feature space.

To clear the event history:

- Select **Yes** after selecting **Yes** to recalculate the feature space.
- or
- Right-click a plot for the desired channel, click **Clear History** on the shortcut menu.

or

- Click the **Clear All History** button  and select **Yes**.

Note: clearing the event history will not affect waveforms stored to the data tank.

Calculating Clusters Automatically

When the user is satisfied with the feature space, clusters can be calculated.

To calculate clusters for all channels:

- Click the **Calculate Clusters** button  and click **Yes** when asked to confirm.

The algorithm and options in the set-up properties are used to calculate clusters automatically.

If the training time has not yet been completed, calculating clusters will terminate training accepting the current feature space.

Once clustering begins, each sort code (or “unit”) is represented by a single color for all plots.

Applying Sorts to New Data

Sort codes are not saved to the data tank until sorts are *applied* by the user. You can re-sort or make adjustments as needed to get the best results.

To begin saving sort codes to the data tank:

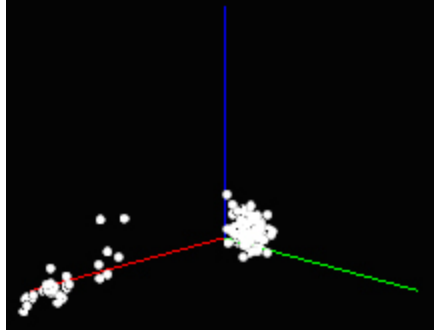
- Click the **Apply Sorts**  button.

Sort codes are applied as new data is acquired. Once sorts have been applied, any changes in sorting parameters in the display will be applied automatically.

Note: at this time, if the sort code output is enabled in the macro setup properties, the *SortCode* output of the macro will output the sort code values for each channel.

Viewing Events in the Feature Space Pane

In the feature space, waveforms can be viewed in terms of their principal components. This automatically reduces the dimensionality of the data while making it visually easier to comprehend.



To rotate the feature space:

- Click the left mouse button and drag the feature space from the desired angle.

To pan the feature space:

- Press and hold the **Alt** key on the keyboard and drag the mouse to pan the feature space.

To auto scale/reset the feature space:

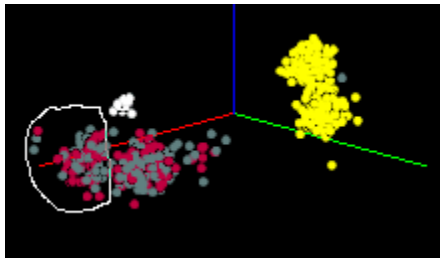
- Press and hold the **Shift** key on the keyboard and double-click the mouse anywhere in the feature space.

Manually Assigning Events to a Cluster in the Feature Space Pane

Users can manually assign waveforms to clusters using mouse based selection tools. Manual sorting can be used as the primary sorting method, however, it is more often used to edit and reassign units after automated sorting.

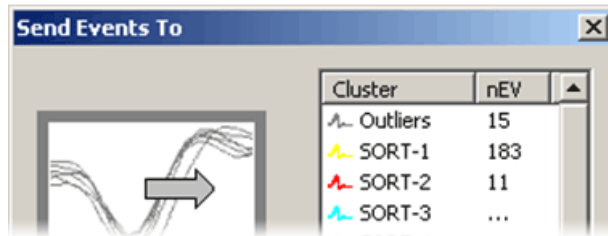
To assign events to clusters manually:

1. Press and hold the **Ctrl** key on the keyboard and use the mouse to draw an arbitrary shape around a visible cluster.



When the mouse button is released, the **Send Events To** dialog opens. The dialog box lists the available Sort Codes as well as the number of events currently sorted by each Sort Code.

2. Select the desired Sort Code from the list or click the **Next Empty Code** button.

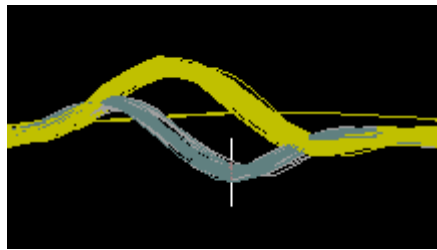


Defining Clusters Manually in the Waveform Space

The waveform space (shown below) displays waveform shapes for all events on the active channel. Users can manually assign waveforms to clusters using mouse based selection tools. Manual sorting provides the greatest degree of flexibility and control at the expense of more time and effort by the user. Manual sorting is ideal for reassigning units after automated sorting.

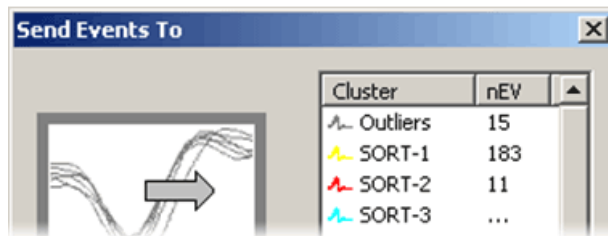
To assign events to clusters manually:

1. In the waveform space, press and hold the **Ctrl** key on the keyboard and use the mouse to draw a line across the desired waveforms.



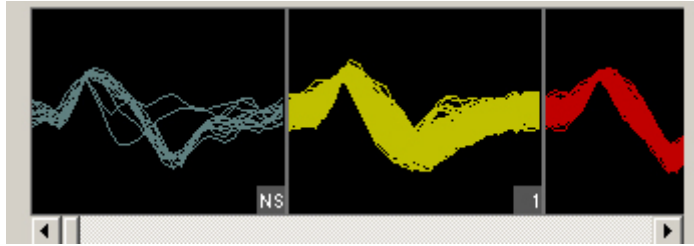
When the mouse button is released, the **Send Events To** dialog opens. The dialog box lists all possible Sort Codes as well as the number of events currently comprised by each Sort Code.

2. Select the desired Sort Code from the list or click the **Next Empty Code** button.



3. Using the selected events, a new cluster is calculated in the feature space. The selected events and any new events that fall within that cluster will be assigned the designated sort code.

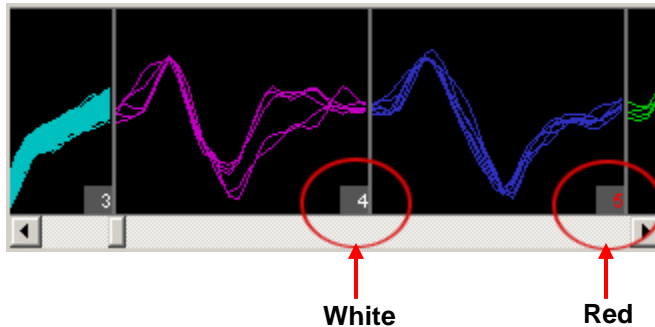
The Unit Display



In the Unit Display a single channel of candidate waveforms are grouped by unit, that is, each individual plot displays all waveforms classified with a single sort code. Units are displayed from left to right according to their numerical sort code. When present in the data set, unsorted waveforms are assigned a sort code of "0" and will be displayed to the left of all other sort codes with the label NS. If there are more units than can be displayed within the width of the tabbed window, a scroll bar is displayed.

The PCSort control will display all possible sort codes based on the sorting algorithm or user selections chosen.

The maximum number of sort codes (up to 5) that can be stored per channel to the hardware is determined by the "maximum number of clusters" parameter in the PCSort macro. Based on these limitations for data storage, the unit display uses color to distinguish between sort codes that can be written to hardware (white) and those that cannot (red). In the example below, waveforms assigned to sort code 5 in the display will be saved to the tank with a sort code of 31 (outlier).



Reassigning Units using the Unit Display

The Unit display can be used to reassign units to different sort codes or combine two or more units together into a single unit.

To reassign a sort code using the unit display:

- Click and drag the desired unit to a new or existing unit and release the left mouse button.
















Tool Buttons

The PCSort control features a set of buttons that allow you to work with all channels in the data set.



Setup Properties

Open the Setup Properties dialog box.

	Auto Scale	Auto scale and normalize all plot displays. This does not auto scale the feature space.
	Base Scale	Return all channels to the base scale of 1.
	Auto Track Threshold	Enables auto-tracking for all unlocked channels.
	Manual Track Threshold	Enables manual-tracking for all unlocked channels.
	Clear All History	Clears event history for all channels.
	Recalculate Feature Space	Recalculate the feature space for all unlocked channels depending on the training termination conditions (recalculates the eigenvector to determine mapping for the feature space).
	Accept Feature Space	Accepts the current feature space for all channels (uses the currently calculated eigenvector for all channel principle component mapping to the feature space).
	Calculate Clusters	Automatically stops training, accepts the feature space, and clusters the existing data for all unlocked channels.
	Clears Clusters	Clears clusters for all unlocked channels and removes color coding (also removes spheres).
	Spheres	Displays the boundaries of spheres used to define cluster shapes in the feature space.
	Undo	Undo previous action (disabled if the active channel is locked).
	Redo	Redo an undone action (disabled if the active channel is locked).
	Lock	Locks clustering actions for all channels. Window resizing, sphere toggle, channel selections, and acceptance of feature space are still allowed. Locking any channel(s) automatically accepts the feature space and ends training.
	Unlock	Unlocks the controls mentioned above after they have been locked for all channels.
	Apply Sorts	Applies the software sorting information for all channels to the processing chain running on the hardware.

Shortcut Menus

Right-clicking any snippet plot displays a shortcut menu that provides the following commands for the active channel:

Clear History - Clears the display for the selected channel. Data saved to the Tank remains unchanged.

Clear Clusters - Clears the currently selected channel's clusters from the display; sort codes saved to the Tank remain unchanged.

Auto Cluster - Stops training, accepts the feature space, and applies clusters to the data for the selected channel.

Recalculate Space - Recalculate the selected channel's feature space. Recalculating the current channel re-evaluates the training termination conditions. For example, if the default condition is: score > 95% OR Events > 1000 OR time > 300 secs, then the space will be accepted immediately if any of these conditions are satisfied. See, page 11, for more information.

Restart Space Calculation - Restarts the space calculation for the selected channel.
Note: only available while the training time is in progress.

Accept Space - Accept the selected channel's feature space regardless of the training status. **Note:** only available while the training time is in progress.

Lock/Unlock - Locks or unlocks the selected channel, preventing changes to clustering for that channel.

Set Threshold Here (Manual Threshold mode only) – Sets the threshold for all channels to the current mouse (pointer) position.

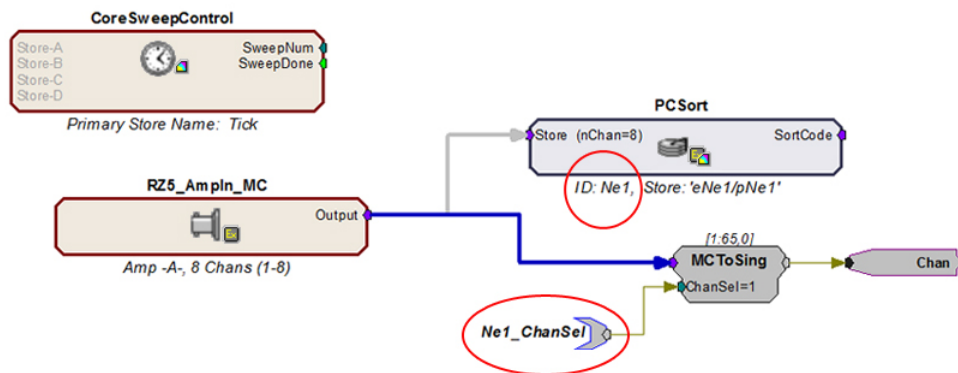
Auto/Manual Threshold – Toggles between Auto/Manual thresholding on the selected channel.

Reset Scaling – Returns the active channel to its base scale of 1.

Working with the Selected Channel

Users can access the channel that is selected (active) within the PCSort control workspace by using the <ID>_ChanSel parameter tag. This tag can be used within the RpvdsEx circuit, for example, to pick off a single channel for additional processing. The example circuit below uses the parameter tag to set the ChanSel parameter of an MCToSing component and send that channel to additional processing components. The PCSort macro updates this parameter dynamically as the active channel is changed (selected) through the PCSort control.

The tag's unique name is generated using the PCSort macro's three character Identity. In the example below, the PCSort macro Identity is "Ne1" so the parameter tag is Ne1_ChanSel.



Channel Select Circuit Modification

BoxSort - Time-Voltage Spike Sorting



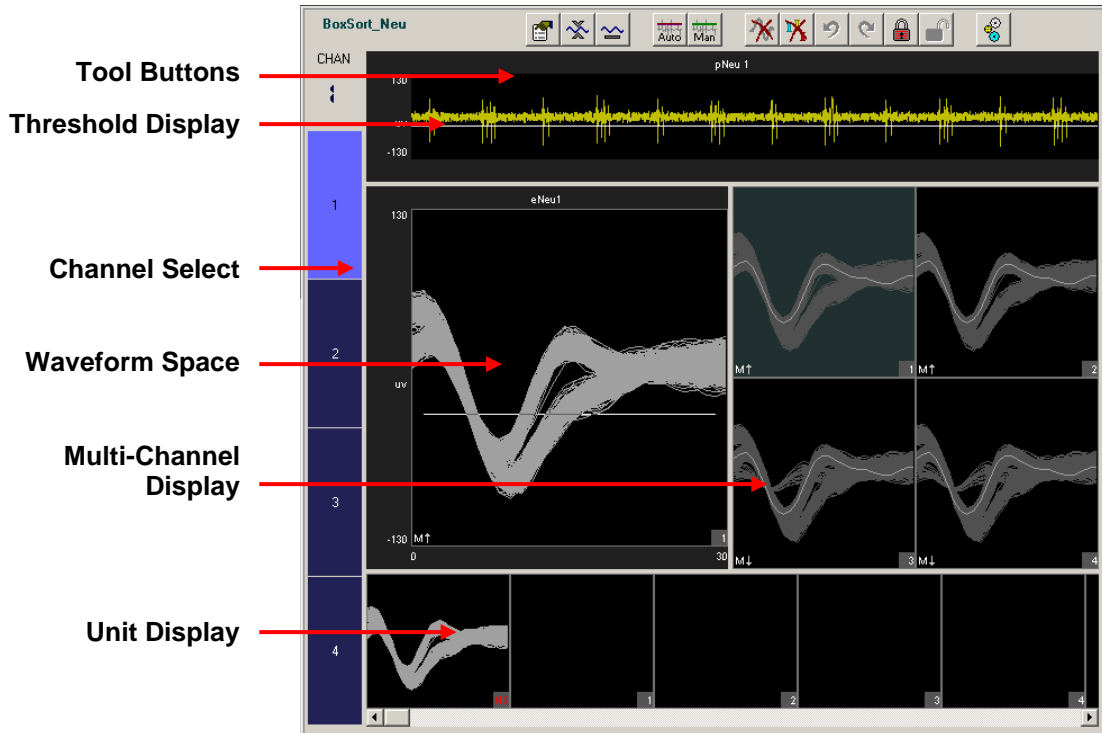
The BoxSort macro is used to time stamp and store waveform events as data snippets and support online spike sorting through the BoxSort control in OpenController. Candidate waveforms are detected based on their deviation from the noise of the system. By default, the time stamp and position of the waveform in the snippet is dependent on the time of the threshold crossing for the signal. An alternative setting allows waveform time stamp and positioning to be determined by the waveform's highest peak, aligning snippets to their respective peaks. The macro can be configured to output the sort code for each channel once sorting parameters have been loaded to the hardware through OpenController.

This macro generates two data Stores: one for sorted snippet data, designated with the prefix e, and one for the plot decimated data stream (a representation of the waveform using maximum and minimum values and used to visualize spike activity), designated with the prefix p. The ID is appended to the Store names and both are displayed in the macro's parameter summary.

Settings for configuring the number of channels, threshold mode, and window width for each snippet stored are defined in the macro properties dialog box. See the onboard macro help for more information.

The corresponding BoxSort control set offers manual sorting using time-voltage box pairs to classify potential units among candidate waveforms.

The BoxSort control set can be used to sort spikes online when the BoxSort macro is included in the compiled circuit file. Spike detection is implemented by default and the user must enable spike sorting.



The Control Set window includes:

- Tool Buttons** provide access to tools and parameters.
- Threshold Display** displays the plot decimated waveform of the currently selected channel and allows threshold adjustments under manual thresholding mode.
- Channel Select** used to select the active channel and display status information about the channels.
- Waveform Space** displays a time-voltage waveform representation of candidate spikes for the active channel in a pile plot. In this plot, users can view and manually classify waveforms by shape. Indicators in the bottom left corner denote scaling and threshold tracking states.
- Multi-Channel Display** displays each channel in a separate pile plot. The channel number is shown in the bottom right corner of each sub-plot and waveforms are highlighted as they are added to the plot. Clicking a sub-plot makes that channel the active channel for the control. Indicators in the bottom left corner denote scaling and threshold tracking states.
- Unit Display** displays a single channel of candidate waveforms by unit—each plot displays all waveforms classified with a single sort code. If there are more units than can be displayed within the width of the window, a scroll bar is displayed.

Simple Zoom

You can zoom any plot to see more or less detail. Zooming does not attempt to scale or fit the waveform shape or data in the plot, allowing you to control the view without regard to the limitations of the plot size.

Note: changing the zoom level in the Multi-Channel Display alters the zoom level for all channels in the Multi-Channel Display.

To change the zoom level of any plot:

- Point to the center of the plot, press and hold down the Shift key, and drag the mouse up or down.

To reset the view of any individual plot:

- Hold down the Shift key and double-click the mouse within the plot.

Note: this method can be used to reset the feature space to its default position.

Display Scale


When the BoxSort control set is run, all channels begin at a *base scale* of 1. This type of scaling clearly shows differences in magnitude across units but can make it more difficult to see waveform shapes for some channels.

To make it easier to see waveform shapes for channels with lower magnitude, you may scale individual channels manually or normalize all channels to fit to a similar scale. If you choose to normalize the display, the BoxSort control set calculates the display scale for each channel individually, attempting to fit around 80% of the signal's vertical size in each plot.

When the display has been normalized, BoxSort displays either an up or down arrow in the bottom left corner of the plot or subplot to indicate whether the display has been scaled up or down.


An Auto Scale feature can be used to refresh the scale of plots either with or without normalizing the display.

To refresh the display without normalizing plots:

1. Click the **Auto Scale**  button.
2. Click **No** when asked if you want to normalize all window groups.


Note: this will not reset the feature space display.

To normalize all channels:

1. Click the **Auto Scale**  button.
2. Click **Yes** when asked if you want to normalize all window groups.

Note: an arrow will now be displayed on the bottom left corner (next to the threshold mode indicator) which indicates positive or negative deviation from the base scale setting.

To return all channels to their base scale:

1. Click the **Base Scale**  button.
2. Click **Yes**.

Note: resetting plots to base scale does not remove any Zoom applied to a plot.

To adjust the scale of a single channel:

- Point to the desired channel in the Multi-Channel display, press and hold down the Ctrl key, and drag the mouse up or down.

Note: while adjusting the display scale, the numeric value in the lower right corner of the channel indicates the new scale value.

To return a single channel to its base scale:

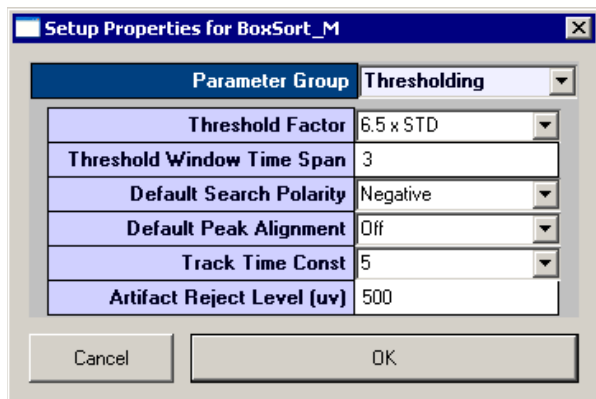
1. Right-click the desired channel.
2. Select **Reset Scaling** from the menu.

The Setup Properties

Users can configure the thresholding control and enable or disable the use of bi-directional thresholding by selecting positive or negative from the **Default Search Polarity** drop-down box.

To open the setup properties:

- Click the **Setup Properties**  button.

**Thresholding Parameter Group**

Threshold Factor – Select a value to set base threshold for spike detection to a value which is $n * \text{STD}$ of the signal RMS. This setting is active only when automatic threshold tracking is enabled.

Threshold Window Time Span – Type a numeric value to set the time span of the threshold window in seconds. **Note:** the control must be restarted to alter the time span.

Default Search Polarity – Select a positive or negative polarity for the threshold value. This setting is active only when automatic threshold tracking is enabled.

Default Peak Alignment – Sets the default alignment to Peak Align which aligns spikes according to their peak values altering the time stamp and positioning of the snippet.

Track Time Const – Sets the time constant tau to 3, 5, or 10 seconds. Shorter tau values respond more drastically to deviations in the signal RMS value. This setting is active only when automatic threshold tracking is enabled.

Artifact Rejection Level (μV) – Type a numeric value to set the artifact rejection level in micro Volts. **Note:** Artifact Rejection must be enabled in the macro setup properties in RpvdsEx.

Display Parameter Group

Clear History Counter – Enter a number to set a limit on the number of events displayed prior to a display refresh. This does not delete historical data from the data tank. It simply removes existing data displays and refreshes each plot. With the default setting of 0, plots will display accumulated historical data.

Max Channels To Display – Enter a number to limit the amount of channels that will be displayed in the Multi-Channel Display. The channel select bar will reorganize into one or more columns based on the settings applied. The default value is 64.

Channel Column Count – Enter a number to set the number of columns for the Multi-Channel Display. With the default setting of 0, the BoxSort control will automatically select the number of columns to display.

Display Layout – Select either Standard or MultiChannel View modes of display. In Standard display mode the Threshold and Unit displays extend the entire width of the control set (see page 23 for an example of the *Standard Display Mode*). In MultiChannel View mode, the right side of the control set is used only for the Multi-Channel Display, extending vertically from top to bottom. This provides more room for multi-channel display.

Selecting the Active Channel

The channel selector bar along the left side of the control set (shown below) determines which channel is displayed in all other single channel plots within the set and highlights the active channel in the Multi-channel plot.

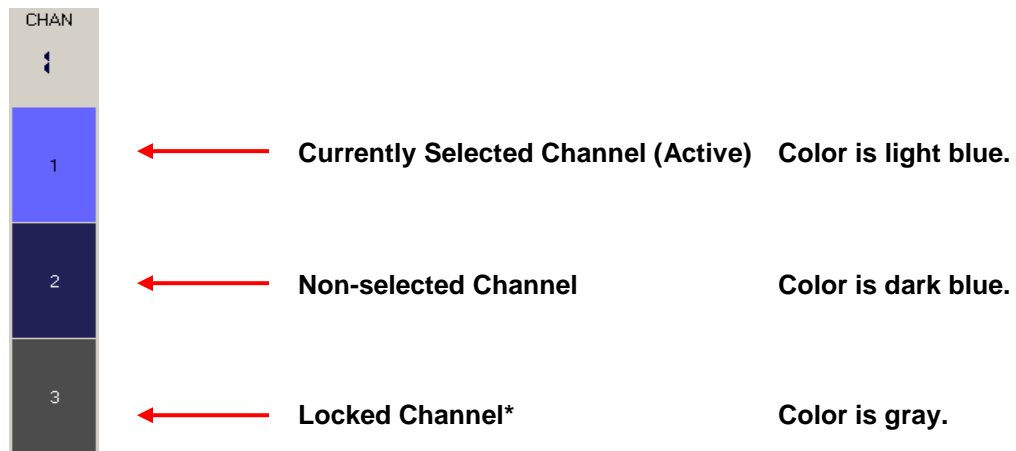
To set a channel as active:

- Click the selector box for the desired channel.

The active channel is displayed in all single channel plots within the set and is highlighted in the multi-channel plot.

Channel status:

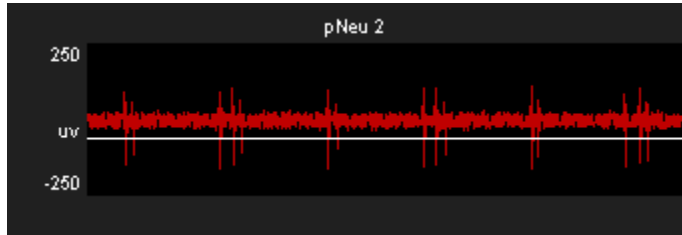
The color of the selector switch displays the status of each channel.



*See *Tool Buttons* on page 34, for more information.

Using the Threshold Control

The threshold control (shown below) displays the plot decimated waveform for the selected channel along with the threshold marker. The default value specified for the threshold factor is 6.5 x STD (standard deviations from the waveform's RMS value).



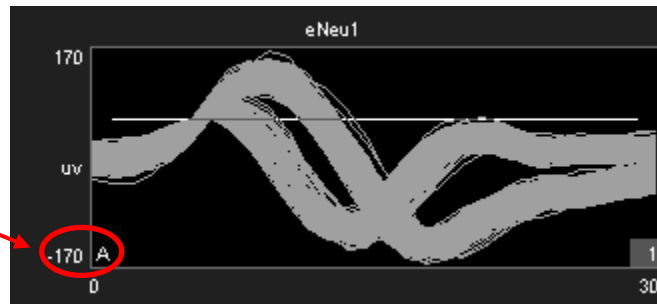
Threshold Tracking

Threshold tracking adjusts the threshold in response to changes in each channel waveform's RMS, using a default time constant (τ) of five seconds.

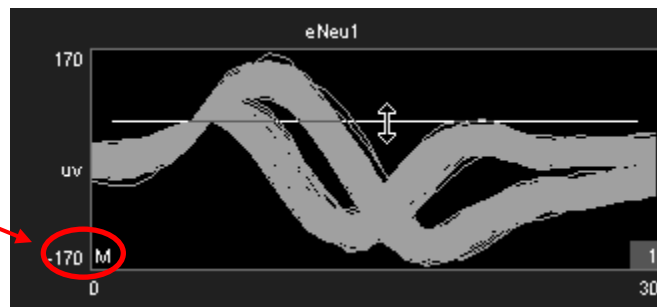
Note: tracking is performed on the DSP and is enabled by default in the BoxSort macro. Automatically tracking the threshold on many channels of data can tax the processor; see *Saving Cycle Usage* below if you are recording many channels.

When threshold tracking is active the threshold bar will be locked preventing manual adjustments. An 'A' indicator is placed in the bottom left corner of the Waveform Space and Multi-Channel Display plots to denote if threshold tracking is currently active for a given channel. Otherwise, an 'M' indicator will be shown to denote that threshold tracking is disabled.

Threshold tracking is active for the current channel.



Threshold tracking is disabled for the current channel, allowing for manual threshold adjustments.



To enable threshold tracking for all channels:

Note: automatic threshold mode must be enabled in the BoxSort macro setup properties.


1. Click the **Auto Threshold**  button.

2. Click **Yes** to enable threshold tracking for all channels.

To enable or disable threshold tracking for individual channels:

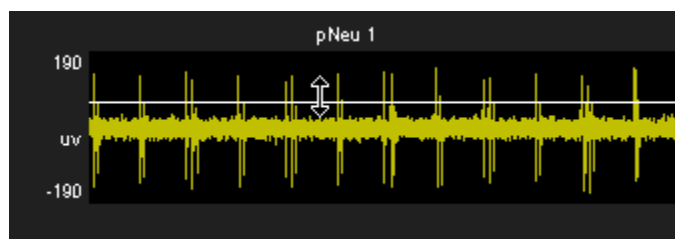
- Right-click on the desired channel and select either **Auto Threshold** or **Manual Threshold**.

To disable threshold tracking for all channels:

1. Click the **Manual Threshold**  button.
2. Click **Yes** to disable threshold tracking for all channels.

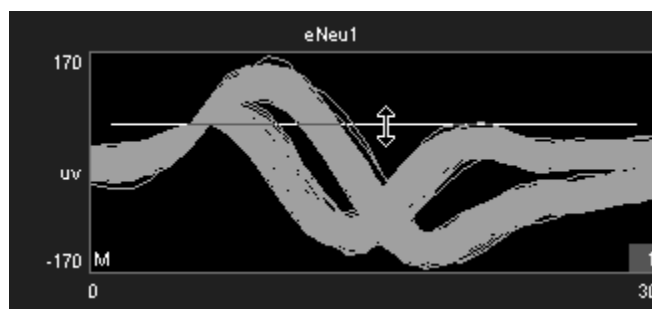
Setting the Threshold Manually

When the corresponding channel is set to manual threshold mode, the threshold bar may be adjusted manually in the threshold control window by clicking and dragging the threshold bar (shown below).



Note: hold the ctrl key and double click on the Threshold Display to toggle threshold tracking on or off for the active channel.

Manual adjustments to the threshold can also be made in the waveform space as shown below.



To change the threshold for a single channel:

- Click and drag the threshold bar to the desired location in either the Waveform Space or Threshold Control plots.

To apply the current threshold setting for all channels:

Note: this will only set the threshold for channels in which threshold tracking is disabled.

1. Right-click the desired threshold location in the Threshold Display.
2. Select **Set Threshold Here** from the menu.
3. Click **Yes** to apply the threshold setting to all channels in manual threshold mode.

Saving Cycle Usage

Threshold tracking relies on circuit components contained within the BoxSort macro. This means that the DSP is continuously tracking the threshold while it is performing other tasks such as filtering and acquisition. This process is taxing on the device cycle usage rates and may diminish the ability of BoxSort to process higher channel counts. Threshold tracking can be disabled in the circuit through the BoxSort macro setup properties. When disabled, only manual threshold mode is available (signified by the manual threshold button being grayed out). Manual threshold methods must be used to obtain the threshold settings but this frees up cycle usage on the DSP enabling more processing.

In addition to disabling threshold tracking, the BoxSort control alters the functionality of the Automatic Threshold button. When the button is clicked, an instantaneous calculation of the data is performed by the PC to determine a reasonable threshold setting for all channels.

To instantly calculate the threshold for all channels:

Note: automatic threshold mode must be disabled in the BoxSort macro setup properties.

1. Click the **Auto Threshold**  button.
2. Click **Yes** to calculate the instantaneous threshold for all channels.


To instantly calculate the threshold for a single channel:

Note: automatic threshold mode must be disabled in the PCSort macro setup properties.

1. Right-click on the desired channel.
2. Select **Auto Threshold** from the menu.

Artifact Rejection

Artifact rejection is configured through the control setup properties window using the **Setup**

Properties  button. Once enabled, events that fall outside of the specified value are ignored.

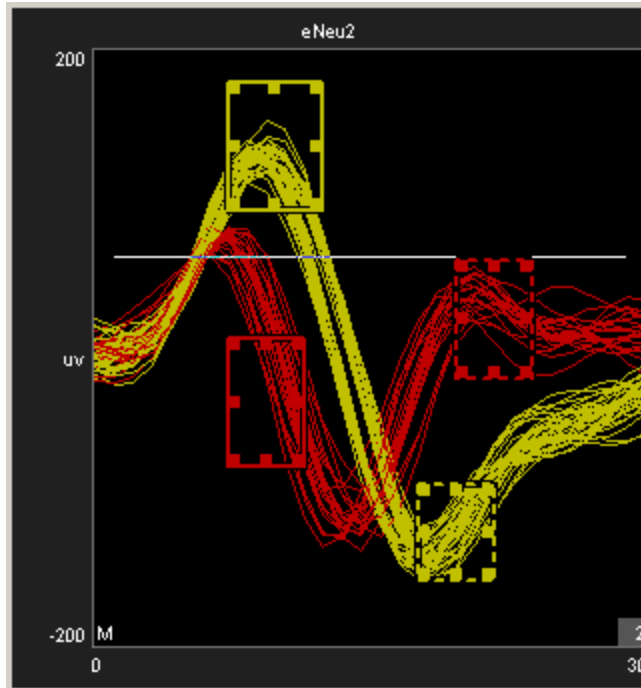
Box Sorting Using the Waveform Space

The BoxSort control set utilizes a pair of color-coded boxes (one solid and one dotted) to classify each unit. A total of eight box pairs are available for sorting candidate waveforms. Box sorting allows units to be assigned based on a set of requirements defined for each unit.

Box Sort Unit Requirements

1. Candidate waveforms must enter the solid box only one time.
2. Candidate waveforms must contain data points that pass through both boxes in the pair.
3. One digitized point of the candidate waveform must exist in each box.

The waveform space (shown below) displays all spike waveforms for the active channel. Users can identify potential units by using mouse based manipulation tools.



To manually adjust the boxes:

- Use standard click and drag techniques on any of the 8 points to adjust the boundaries of the boxes.
- Click and drag the edge of a box to move it to the desired position.

Note: the mouse icon will indicate whether the box will be adjusted or moved.

To add a box pair:

- Hold Ctrl + double click to add a new box pair to the waveform space. Sort codes are automatically assigned to the newly added box pair.

Note: the maximum number of units available for the hardware is defined within the BoxSort macro setup properties.

To remove a box pair:

- Drag either the solid or dotted box out of the plot and click **Yes** when asked to remove hand mark.

If a waveform passes through more than one box pair:

- Sort code priority is assigned based on the sort code number. This means that the lower sort code will win in the event that a waveform passes through more than one box pair.

Applying Sorts to New Data

Sort codes are not saved to the data tank until sorts are *applied* by the user. You can re-sort or make adjustments as needed to get the best results.

To begin saving sort codes for all channels to the data tank:

- Click the **Apply Sorts**  button.

Sort codes are applied as new data is acquired. Once sorts have been applied, any changes in sorting parameters in the display will be applied automatically.

Clearing the Event History

The event history of individual channels as well as all channels may be cleared at any time and will remove any displayed waveforms on the Waveform Space, Unit Display, and Multi-Channel Display plots for the desired channel(s).

To clear the event history:

- Right-click a plot for the desired channel, click **Clear History** on the shortcut menu.
- or

- Click the **Clear All History** button  and select **Yes** when asked to confirm.

Note: clearing the event history will not affect waveforms that have already been stored to the data tank.

Clearing Sort Codes

To clear Sort codes:

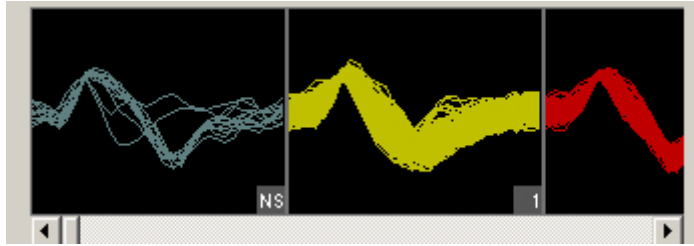
- Right-click a plot for the desired channel, click **Clear History** on the shortcut menu, then click **Yes** when prompted to confirm.

or

- Click the **Clear All Sorts** button  and click **Yes** when asked to confirm.

Note: clearing sort codes will not affect sorted waveforms that have already been stored to the data tank.

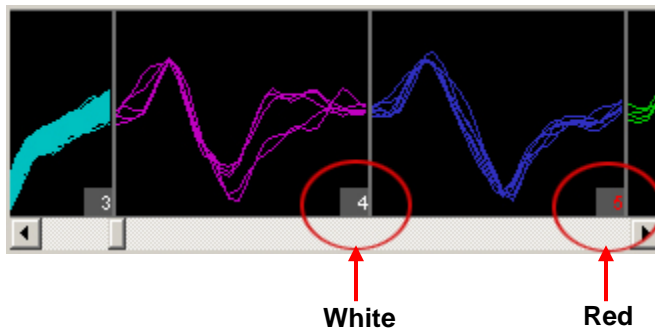
The Unit Display



In the Unit Display a single channel of candidate waveforms are grouped by unit, that is, each individual plot displays all waveforms classified with a single sort code. Units are displayed from left to right according to their numerical sort code. When present in the data set, unsorted waveforms are assigned a sort code of "0" and will be displayed to the left of all other sort codes with the label NS. If there are more units than can be displayed within the width of the tabbed window, a scroll bar is displayed.

The BoxSort control will display all possible sort codes based on the user selections chosen.

The maximum number of sort codes (up to 8) that can be stored per channel to hardware is determined by the "Max Sorts" parameter in the BoxSort macro. Based on these limitations for data storage, the unit display uses color to distinguish between sort codes that can be written to hardware (white) and those that cannot (red). In the example below, waveforms assigned to sort code 5 in the display will be saved to the tank with a sort code of 31 (outlier).



Reassigning Units using the Unit Display













The Unit display can be used to reassign units to different sort codes. Unlike PCSort, units cannot be combined.

To reassign a sort code using the unit display:

- Click and drag the desired unit to a new unit and release the left mouse button.

Tool Buttons

The BoxSort control features a set of buttons that allow you to work with all channels in the data set.

	Setup Properties	Open the Setup Properties dialog box.
	Auto Scale	Auto scale and normalize all plot displays.
	Base Scale	Return all channels to the base scale of 1.
	Auto Track Threshold	Enables auto-tracking for all unlocked channels.
	Manual Track Threshold	Enables manual-tracking for all unlocked channels.
	Clear All History	Clears event history for all channels.
	Clear All Sorts	Clears all sort codes for all unlocked channels.
	Undo	Undo previous action (disabled if the active channel is locked).
	Redo	Redo an undone action (disabled if the active channel is locked).
	Lock	Locks box sorting actions for all channels. Window resizing, and channel selections are still allowed. Locking any channel(s) automatically accepts the feature space and ends training.
	Unlock	Unlocks the controls mentioned above after they have been locked for all channels.
	Apply Sorts	Applies the software sorting information to the processing chain running on the hardware.

Shortcut Menus

Right-clicking any snippet plot displays a shortcut menu that provides the following commands:

Clear History – Select to clear all traces from the selected channel display, data saved to the Tank remains unchanged. Any existing time-voltage bars will remain.

Clear Sorts – Select to clear any sorts for the currently selected channel. This removes sort colors from the display; sort codes saved to the Tank remain unchanged.

Lock or Unlock the current channel – Select to toggle the locked or unlocked state of the selected channel.

Set Threshold Here (Manual Threshold mode only) – Sets the threshold for all channels to the current mouse (pointer) position.

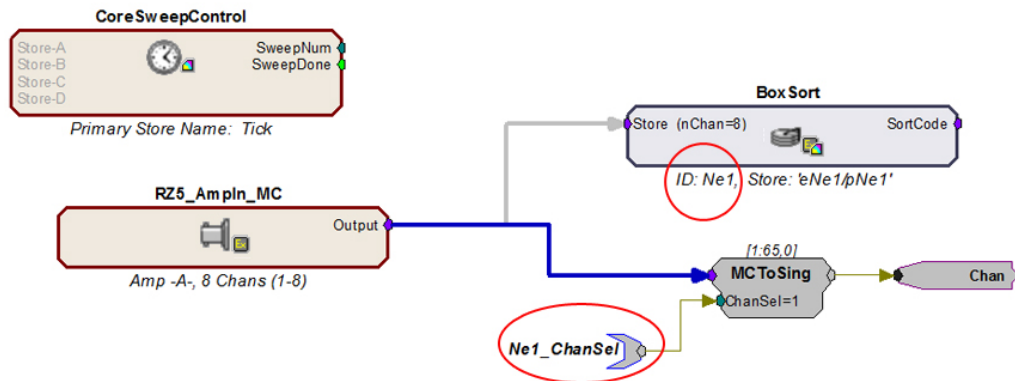
Auto/Manual Threshold – Toggles between Auto/Manual thresholding on the selected channel.

Reset Scaling – Returns the active channel to the base scale of 1.

Working with the Selected Channel

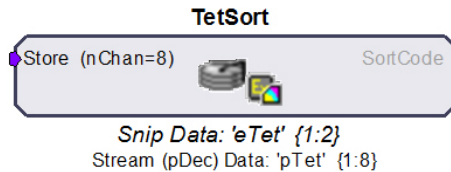
Users can access the channel that is selected (active) within the PCSort control workspace by using the <ID>_ChanSel parameter tag. This tag can be used within the RpvdsEx circuit, for example, to pick off a single channel for additional processing. The example circuit below uses the parameter tag to set the ChanSel parameter of an MCToSing component and send that channel to additional processing components. The BoxSort macro updates this parameter dynamically as the active channel is changed (selected) through the BoxSort control.

The tag's unique name is generated using the BoxSort macro's three character Identity. In the example below, the BoxSort macro Identity is "Ne1" so the parameter tag is Ne1_ChanSel.



Channel Select Circuit Modification

TetSort - Tetrode Spike Sorting



The TetSort macro timestamps and stores waveform events as data snippets and supports online spike sorting through the TetSort control in OpenController. Together the macro and control provide a flexible software interface for single-unit tetrode recording.

The macro generates two data Stores: one for snippet data, designated with the prefix 'e', and one for the plot decimated data stream for visualization, designated with the prefix 'p'. The macro ID ('Tet' by default) is appended to the Store names.

A voltage threshold is set for each channel of the tetrode, either manually in TetSort's OpenController interface or automatically based on the deviation of the waveform from its RMS. When a waveform crosses threshold on any channel in the tetrode, a snippet on all four channels in the tetrode is recorded. The channel snippets are concatenated and stored in the data tank as one large snippet, with a timestamp and a sort code.

The snippet's sort code is determined by visual spike sorting in TetSort's OpenController interface. Each channel within a tetrode is displayed in a separate waveform subplot. Channel snippets are projected onto a 2D space by first calculating user-selected metrics for one or two channels and then mapping one metric against another. Up to four 2D feature projections can be used to visualize tetrode spike clustering. Users may select from the following metrics: Peak, Valley, Height, Energy, Non-Linear Energy, Average, Area and Slope. User-defined circles in each projection plot determine each cluster's boundaries. Snippets falling inside a circle are given a sort code corresponding to that circle's color.

The TetSort control works in two modes:

Hunt Mode

In Hunt Mode the projection plots default to peak vs. peak for all six combinations of tetrode channels to provide a general overall picture of activity. Use this mode during electrode placement.

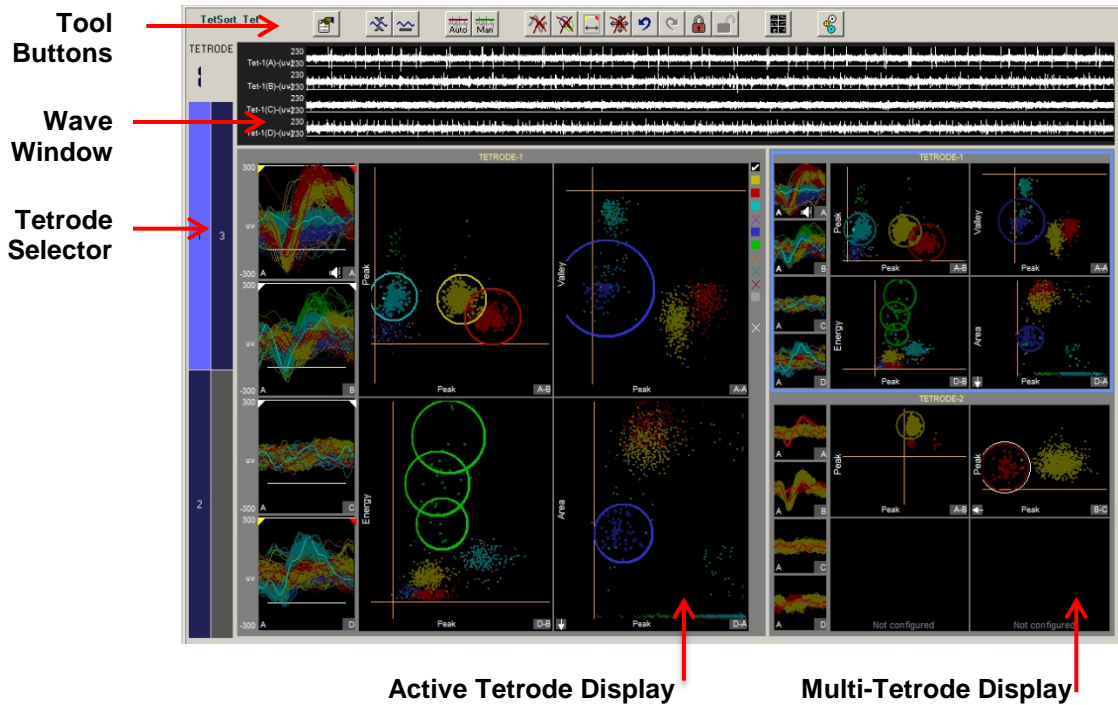
Sort Mode

After the electrode has been placed, use Sort Mode to choose new metrics for the projection plots and add sort circles.

The macro allows simultaneous recordings from multiple tetrodes. The multi-channel input stream must be arranged in groups of four; each group corresponding to one physical tetrode (a SiteMap macro may be used).

Settings for configuring the number of tetrodes, maximum number of sorting circles per projection, thresholding method and window width of the snippets, along with additional explanation of the macro functionality, can be found in the TetSort macro properties dialog. Optionally, the sort codes can be output from the macro as a stream of integers for additional real-time processing in RPvdsEx.

TetSort Control Window



The Control Window includes:


- Tool Buttons** Provides access to tools and parameters.
- Wave Window** Displays the plot decimated waveforms for the active tetrode and allows threshold adjustments under manual thresholding mode. This window can be hidden to allow a better view of the *Active Tetrode Display* during spike sorting.
- Tetrode Selector** Selects the active tetrode.
- Active Tetrode Display** Displays the active tetrode in several projection plots for easy comparison of candidate waveforms and visual sorting.
- Multi-Tetrode Display** Displays each tetrode in a smaller version of the *Active Tetrode Display* to allow the user to monitor all tetrodes while working with the active tetrode.

Selecting the Active Tetrode

The Tetrode Selector along the left side of the control set (shown to the right) determines which set of tetrode channels is active. The color of the tetrode indicator in the Tetrode Selector indicates the status of each tetrode.

To set a tetrode as active:

- Click the selector box for the desired tetrode.



The selected tetrode is displayed in Active Tetrode Display and is highlighted in the Multi-Tetrode Display.

<p>2</p> <p>1</p> <p>2</p> <p>3</p>	<p>Inactive Tetrode</p> <p>Active Tetrode</p> <p>Inactive Tetrode, All Channels Locked*</p> <p>Active Tetrode, All Channels Locked*</p>	<p>Color is dark blue</p> <p>Color is light blue.</p> <p>Color is gray</p> <p>Color is light gray.</p>
---	---	--

*See **Tool Buttons** on page 20 for more information.


Adjusting the Detection Threshold

To begin acquiring snippets, thresholds must be set for at least one channel of the tetrode. There are two methods of determining a threshold: setting the threshold manually (Manual Mode) or letting TetSort compute a threshold based on a specified multiple of the RMS level of the signal (Automatic Mode). This RMS calculation is continuously performed to adjust for fluctuations in system noise. **Note:** Automatic mode requires extra processing on the DSP and is disabled by default in the macro. It must be enabled in the macro if you want to use this feature; see *Minimizing Cycle Usage* below if you are recording many channels.

The white threshold bars for each channel are displayed in the wave window and snippet plots. They will automatically appear in Automatic Mode.

An 'A' indicator is displayed in the bottom left corner of the snippet plots if the channel is in Automatic mode. Otherwise, an 'M' indicator will be shown to denote that the channel is in Manual mode and the threshold can be adjusted manually.

Setting the Threshold in Manual Mode

If the white threshold bars are not visible in Manual Mode, and Automatic Thresholding has been disabled in the circuit macro, click the **Auto Threshold**  button and Controller will calculate a reasonable threshold for all channels.

To change the threshold for a single channel:

- In Manual threshold mode, click and drag the threshold bar to the desired location in either the scrolling waveform or pile plots.

To apply a threshold using point and click:


Note: This will only set the threshold for channels in Manual mode.

1. Right-click the desired threshold location in the waveform display and click **Set Threshold Here** in the shortcut menu.
2. Click **Yes** to apply the threshold setting to all channels in manual threshold mode.

Automatic Threshold Tracking (Automatic Mode)


Threshold tracking adjusts the threshold in response to changes in each channel's waveform RMS using a default time constant (τ) of five seconds. The default value specified for the threshold factor is $6.5 \times \text{STD}$ (standard deviations from the waveform's RMS value). The time constant and threshold factor can be modified in the control's setup properties.

To change the threshold factor or time constant:


- Click the Setup Properties  button and select a new Threshold Factor and Track Time Const from the Thresholding parameter group. See page 52 for more threshold parameter setting options.

When threshold tracking is active the threshold bar will be locked, preventing manual adjustments.

To enable threshold tracking for all channels:

1. Click the **Auto Threshold**  button.
2. Click **Yes** to enable threshold tracking for all channels.

To disable threshold tracking for all channels:

1. Click the **Manual Threshold**  button.
2. Click **Yes** to disable threshold tracking for all channels.

To disable threshold tracking for individual channels:

- Right-click on the snippet plot and select **Manual Threshold**.

Working with the Plots**Zoom and Pan**

Zoom and Pan are used to see more or less detail or to focus on a specific area of the plot.

To change the zoom level of any plot:

- Point to the center of the plot, press and hold down the **Shift** key, and drag the mouse up or down.

To pan in snippet plots:

- Hold down the **Alt** key, click and drag to move the snippets vertically.

To pan in projection plots:

- Click and drag to move the view.

Scale


In addition to an overall scale factor, each channel has its own individual scale factor (*base scale*) for normalizing the display of all channels in the Wave Window and Snippet Plots. All channels begin with a base scale of 1. Using the auto scale feature, you can refresh the scale of plots either with or without normalizing the display.

Note: Changing any display scale factor does not alter the data being stored.


If you choose to normalize the display, TetSort calculates the display scale for the each channel, attempting to fit around 80% of the signal's vertical size in each waveform plot. When the display has been normalized, TetSort displays either an up or down arrow in the bottom left corner of the snippet plots to indicate whether the display has been scaled up or down.

TetSort stores two sets of scale factors, one set for Sort mode and another for Hunt mode. Each set (Sort or Hunt) of scaling information includes a scale factor for the tetrode and any individual scale factors set for individual plots. This allows you to switch between modes without rescaling or losing scaling information.

To refresh the display without normalizing plots:

1. Click the **Auto Scale**  button.
2. Click **No** when asked if you want to normalize all window groups.

To normalize all channels:

1. Click the **Auto Scale**  button.
2. Click **Yes** when asked if you want to normalize all window groups.

To reset all channel and projections base scales:

- Click the **Reset Base Scale**  button.

To return all channels of a single tetrode to their base scale:

- Right-click in the **Wave Window** and select **Reset Scaling – Tetrode**.

To return a single channel to its base scale:

- Right-click in the corresponding **Snippet Plot** and select **Reset Scaling**.

Scaling the Projection Plots

In addition to being scaled when all plots/channels are scaled, the projection plots can be scaled for each tetrode or as individual plots. When a projection plot has been scaled, an arrow will appear in the bottom left corner to indicate the direction to the origin point of the plot.

To adjust the scale of all projection plots for a tetrode:

- Right-click any projection plot in the Active Tetrode Display, point to **Auto Scale**, and click **Tetrode** on the shortcut menu.

To adjust the scale of a single projection plot:

- Right-click the desired projection in the Active Tetrode Display, point to **Auto Scale**, and click **Plot** on the shortcut menu.

The projection plots also have a base scale which is computed as a reasonable estimate based on the metric combinations and typical data sets.

To return all projection plots for a tetrode to the base scale:

- Right-click any projection in the Active Tetrode Display, point to **Reset Base Scale**, and click **Tetrode** on the shortcut menu.

To return a single projection plot to its base scale:

- Right-click the desired projection, point to **Reset Base Scale**, and click **Plot** on the shortcut menu.

Because the 2D clusters don't always fit into nice circles for sorting, the projection plot axes can be independently scaled in order to skew the data set so that it does fit into a circular boundary.

To independently scale the x-axis of a projection plot:

- Hold down **Ctrl + Alt**, click the left mouse button and drag the cursor to the right or left.

To independently scale the y-axis of a projection plot:

- Hold down **Ctrl + Alt**, click the left mouse button and drag the cursor up or down.

To reset the independent scaling for a projection plot:

- Right-click the desired projection in the Active Tetrode display, point to **Reset Independent Scale**, and click **Plot** on the shortcut menu.

To reset the independent scaling for all projection plots in the current tetrode:

- Right-click the desired projection in the Active Tetrode display, point to **Reset Independent Scale**, and click **Tetrode** on the shortcut menu.

or

- Click the **Reset 2D Plot Independent Scaling**  button and select **No**.

To reset the independent scaling for all projection plots in all tetrodes:

- Click the **Reset 2D Plot Independent Scaling**  button and select **Yes**.

Highlighting Traces

By default, the most recent trace acquired is highlighted in all plots throughout the tetrode display area. Alternatively, a group of traces that are of interest can be highlighted.

To highlight a group of traces:

- Hold down the **a** key and drag the pointer across the desired traces in any pile plot in the tetrode display area.


This can be repeated to add to more selected traces. The selected pile plot traces and their corresponding dots in the projection plot(s) will be highlighted.

To remove a group of traces from the highlighted selection:

- Press and hold down the s key and drag the pointer across the desired traces in any pile plot in the tetrode display area.

To return to the default highlight mode (most recent trace highlighted as plots are updated):

- Press and hold down the s key and drag the pointer across all traces in any pile plot in the tetrode display area.

To the right of the projection plot, and below the column of filtering icons, the  highlighting icon can be clicked to clear highlighting from the projection plot and pile plots of the active tetrode.

Audio Monitoring

To select a channel for audio monitoring:

- Press and hold down the c key and click the corresponding pile plot in the tetrode display area. A speaker icon will appear in the bottom right of the pile plot. This sends the currently selected channel to a NeuroMon macro with the same ID as this TetSort macro, e.g. 'Tet'.

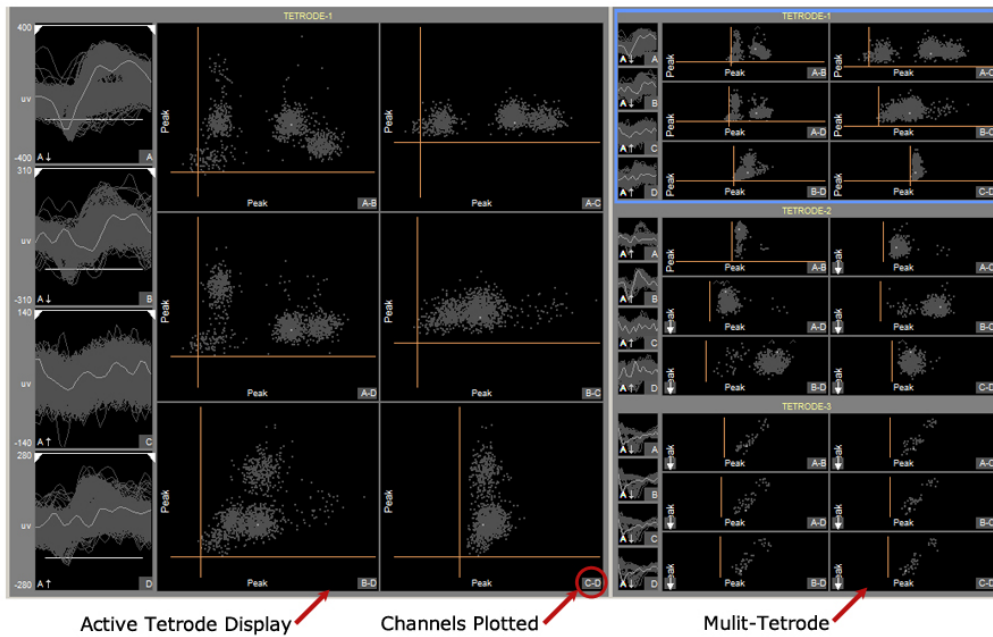
Hunt Mode

Hunt mode is designed for use during electrode placement. In this mode, the feature projections show peak vs peak for all electrode combinations to provide a general overall picture of activity. By default, the TetSort controller is in Sort Mode.

To turn on Hunt mode:

- Click the **Hunt**  button.

All sorting features are disabled in this mode, but scaling and other features are available.



The number of events that are shown in the snippet plots and projection plots can be configured in the Setup Properties.

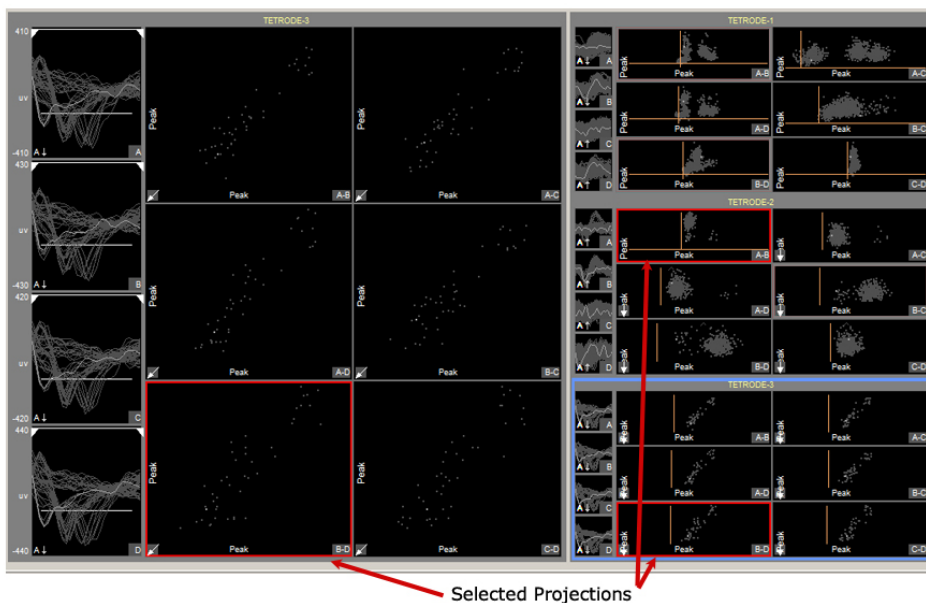
To clear all events from the display:

- Press the **Spacebar**.

You can transfer projections from Hunt mode to Sort mode to speed up projection configuration.

To select projections to transfer to Sort mode:

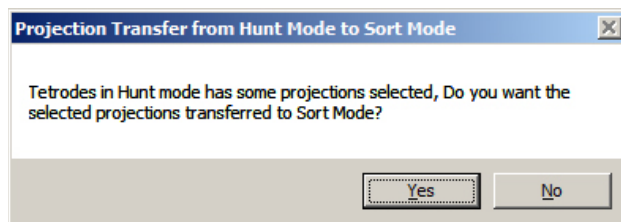
1. Press the **Ctrl** key and click the desired projection(s).



You can select multiple projections across multiple tetrodes.

2. Click the **Hunt**  button to turn Hunt mode off.

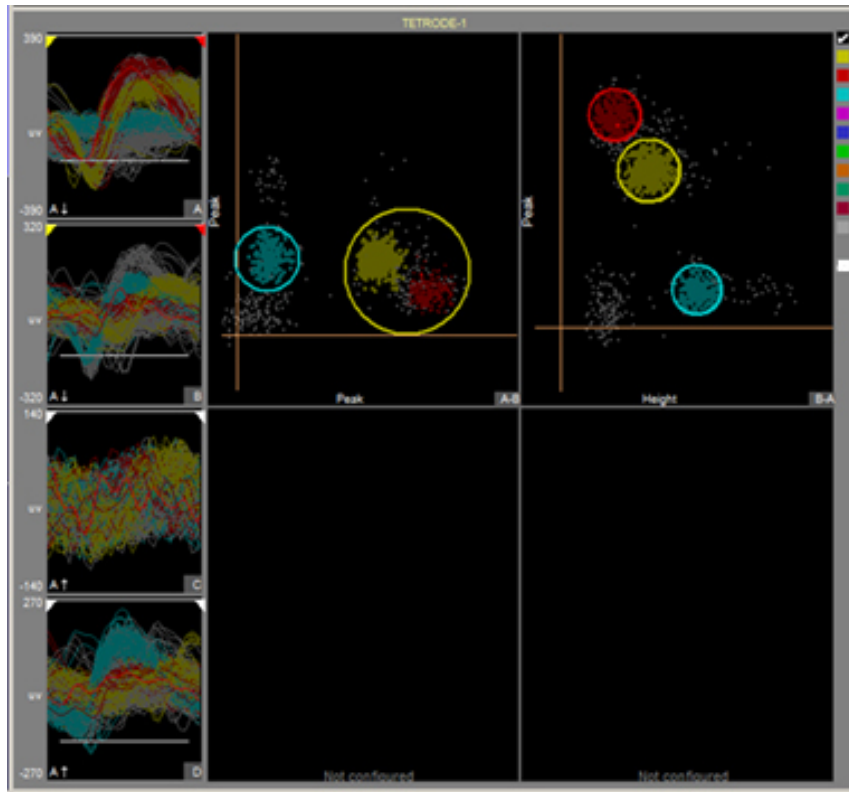
You'll be asked to confirm your selections.





3. Click **Yes**.

The display returns to Sort mode and the selections you selected are displayed.

Sorting in the Active Tetrode Plot Display



The Active Tetrode display provides an interactive space for online cluster cutting. Once reasonable thresholds are set, snippets will appear in each of the four Snippet Plots. The projection plots are created by applying metrics to the waveforms in these Snippet Plots and then plotting one metric versus another in an X-Y plane. When you are satisfied with the defined sorts, you can

begin applying the sort codes in hardware by clicking the **Apply Sorts**  button. You can lock the plots by clicking the **Lock**  button to protect them from modification.

Snippet Plots

The label in the bottom right corner of each plot corresponds to the waveform labels in the Wave Window. An arrow in the bottom left corner indicates the direction from the base scale if normalization has been applied to the channel. See *Adjusting the Detection Threshold*, page 38, for more information.

Restricting Metric Calculations to a Narrow Window

The marker at the top of the snippet plot indicates the window of snippet data that is used for metric calculations for the selected projection plot. This is called the **Min-Max Interval** and can be adjusted on the fly.



Note: This does not change the width of the window in data storage, which is defined in the TetSort macro properties.

To adjust the Min-Max Interval:

- Drag the yellow (Min) and/or red (Max) indicators to the desired position.

To reset the Min-Max Interval to the entire window width for one channel in the current projection plot:

- Right-click the snippet plot and click **Reset Min-Max Interval** on the shortcut menu.

To reset the Min-Max Interval to the entire window width for all channels for all projections of the current tetraode:

- Click the **Reset Snippet Plot Max Min Intervals**  button on the shortcut menu and select **No**.

To reset the Min-Max Interval to the entire window width for all channels for all projections for all tetrodes:

- Click the **Reset Snippet Plot Max Min Intervals**  button on the shortcut menu and select **Yes**.

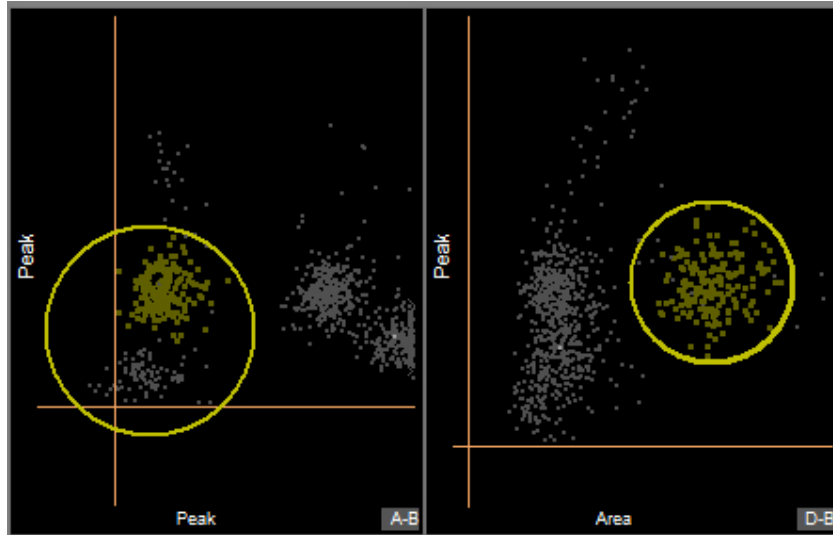
Projection Plots

The main area of the active tetraode display is divided into four 2D projection plots for dynamic visual spike sorting. Each snippet appears as a single dot in 2D space of one metric plotted versus another. By default, the top left plot will display the peak of the first channel against the peak of the second channel.

User-defined circles are drawn around dots to assign sort codes to their associated snippets. Snippets falling inside a circle are given a sort code corresponding to that circle's color. The color of the dot representing that snippet will change to the color of the circle. More than one circle of the same color can be defined in each projection. A snippet falling in any of those same-colored circles will be classified with that sort code.

Because snippets can fall into more than one circle, the actual sort code stored in the data tank is a mask of all sort codes that the candidate snippet fell inside. For example, if a snippet falls into a yellow (sort code 1) and green (sort code 6) circle, then the snippet mask will be 33 (0b100001 = 33). In this case, the color of the dot used to define the snippet in the projection plot is randomly chosen from the sort codes in the mask. A snippet that doesn't fall inside any circles is considered unsorted and has sort code 0.

If there are circles of the same color on more than one projection, a candidate snippet has to fall inside that type of circle in all projections to be given that color's sort code. The total number of circles that can be defined in any one projection plot is set in the macro properties (default is 12).



Choosing Projection Plots

Up to four 2D projections can be used to visualize tetrode spike clustering. You can preview and choose projections using the active tetrode data.

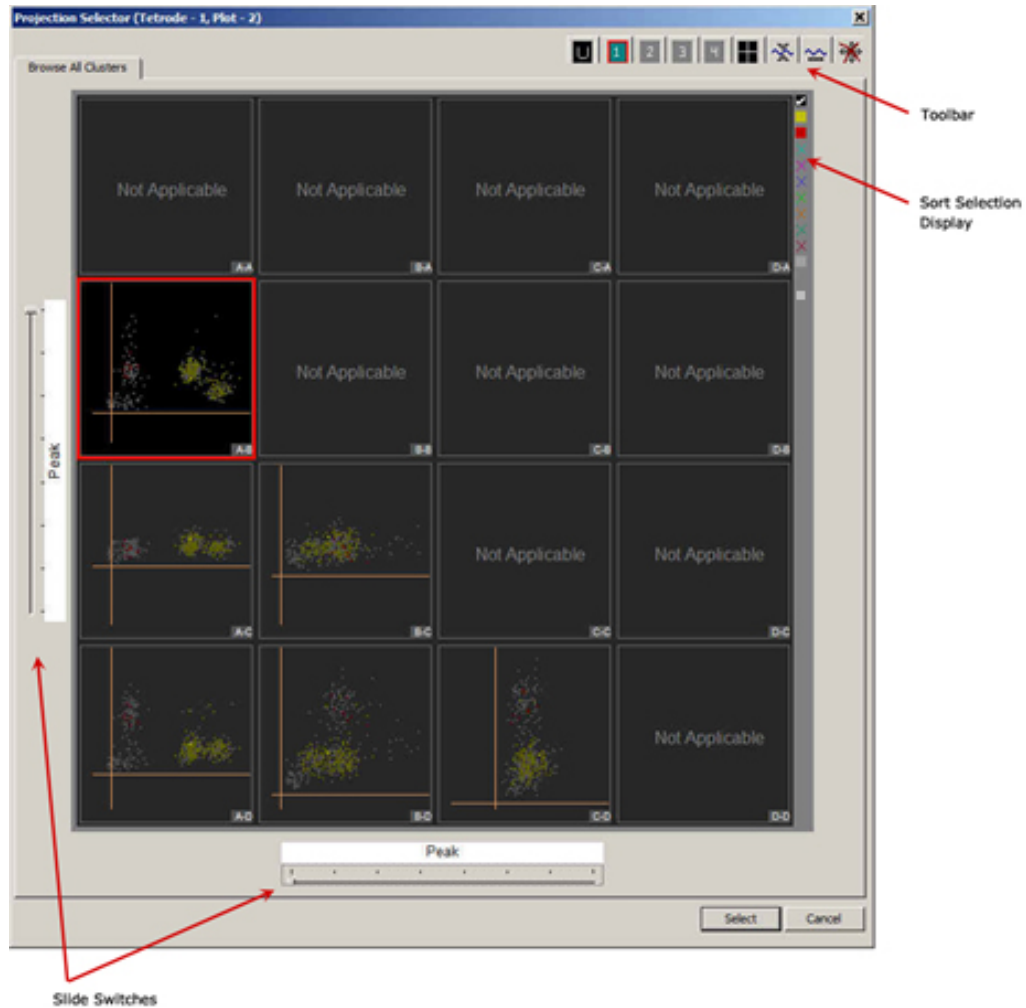
Available Metrics

Note: All metric calculations are performed on the segment of data within the min-max interval only. See *Snippet Plots*, page 44.

Peak	The highest point in the interval.
Valley	The lowest point in the interval.
Height	The difference between peak and valley in the interval.
Energy	The arithmetic mean (average) of the squares of each point in the interval.
Non-linear Energy	$\text{sum}(w(t)*w(t) - w(t-1)*w(t+1))/\text{length}(w)$, for all t in the interval, where w is the waveform.
Average	The arithmetic mean of all values in the interval.
Area	The sum of the absolute values of all points in the interval.
Slope	The height divided by the difference between the peak timestamp and valley timestamp in the interval.

To open the Projection Selector:

- Double-click a projection plot subplot. To add a projection, select a sub-plot that has not been configured.



In the Projection Selector you can choose from a variety of metrics combinations for visualization. Each new projection can help to further refine a sort or identify new sorts. All possible combinations of metrics between channels are displayed. The letters in the lower-right corner of each plot indicate the x-axis and y-axis channels for that plot. Metric combinations that are already in use in other projection plots for this tetrode will have a solid border around them.

Slide Switches on the left and bottom edges of the selector can be used to select Peak, Valley, Height, Energy, Non-Linear Energy, Average, Area, or Slope for the X and Y axes.

The **Sort Selection Display** can be used to toggle the display of individual or all sort codes and functions like the sort filter in the Active Tetrode Display. See *Filtering the Display by Sort Code*, page 51, for more information.

The **Toolbar** provides access to tools that may be used while working in the Projection Selector window.



Shows all data as unsorted events



Switch to corresponding projection (only available in plots that are already configured)



Go to selected (cluster projection preview within the selector)



Auto scale projections



Resets base scale for projections



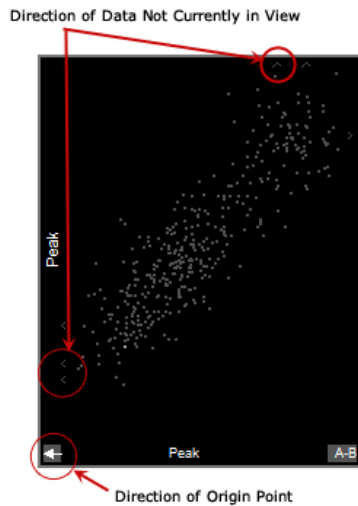
Reset independent plot scaling removes any x-axis or y-axis skew in all available projections.

To select a projection in the Projection Selector:

1. Click the desired projection preview.
2. Click **Select**.

Viewing Data in the Projection Plot

The feature projection plots can be zoomed, panned or scaled individually or by tetrode. See *Working with the Plots* page 39 for more information. When the projection plot contains data that is not in view, small carrots are positioned on the edge of the plot to indicate the direction of the data.



When the origin point of the feature projections plot is not in view, an arrow is displayed in the bottom left corner of the plot to show the direction of the origin point.

To center the origin in the plot:

- Right-click the plot and click **Center Origin** on the shortcut menu.

To clear the history:

- Right-click the plot and click **Clear Tetrode History**.

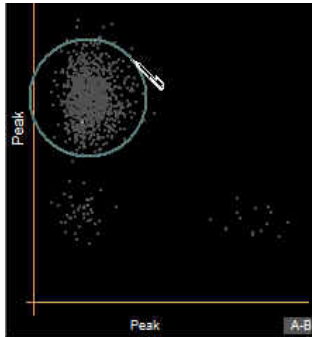
The number of points visible in the projection plot is controlled by the **Projection Plot Display History Depth** setting in the properties dialog.

Using Circles to Define Sorts

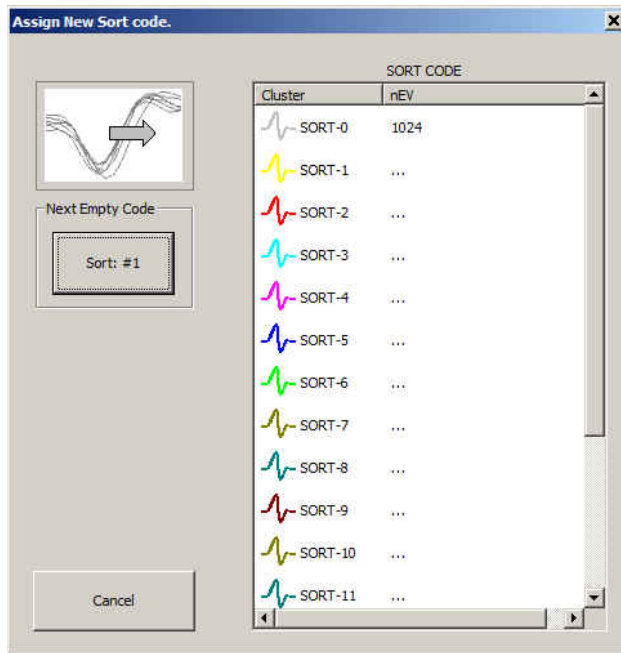
User-defined circles are drawn in each projection plot to determine a cluster's boundaries and shape. Sort codes are applied to snippets using the boundary calculated for each cluster.

To create a sort circle and assign a sort code:

1. Point to the desired projection in the Active Tetraode display, press and hold down the **Ctrl** key, click and drag the mouse up or down.



When the mouse key is released the **Assign New Sort code** dialog opens.



The Sort Code list displays the available sort codes and associated colors (Cluster) and the number of events (nEV) in each cluster. Sort-0 is unsorted events.

2. Click the sort button in the **Next Empty Code** box or click the desired code in the list.



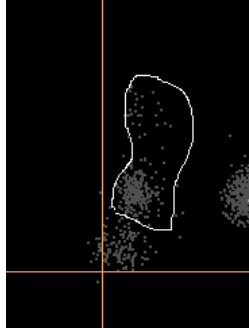
3. Repeat this process until all likely sorts have been defined.

Arbitrary Shapes

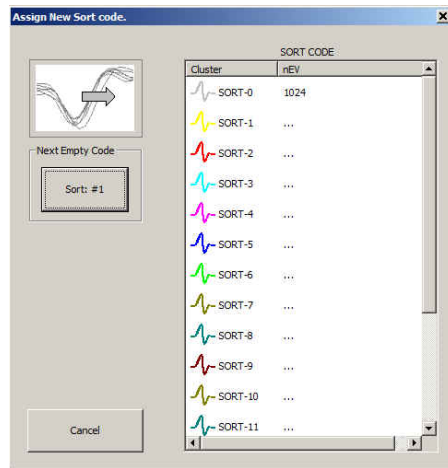
Sort circles can also be generated by drawing an arbitrary shape around traces in a projection plot. TetSort will then attempt to draw circles that will efficiently represent the selected traces. Users can control how TetSort creates the circles using the **Arbitrary Shape Uniform Distribution** check box in the Sort Display parameter group of the setup properties, see page 53 for more information.

To create sorts by drawing an arbitrary shape:

1. Press and hold the ALT key then hold down the mouse button and drag within the plot to create an arbitrary shape.



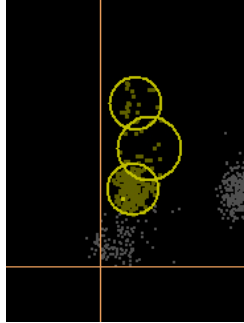
2. When the mouse key is released the **Assign New Sort code** dialog is opened.



3. Click the sort button in the **Next Empty Code** box or click the desired code in the list.



TetSort automatically creates circles to define the shape.



The number of circles it uses to define the shape depends on how accurately the shape can be approximated with circles. The Sort Display > Cluster Radius xSTD property determines how close the circle centers can be. An arbitrary shape close to a circle will only use one circle. The maximum number of circles it will use when approximating an arbitrary shape is defined in the property Sort Display > Maximum Circles per Arbitrary Shape.

If necessary, the projection plot axes can be independently scaled so that the data points fit into circular clusters. See *Scaling the Projection Plots*, page 40.

Filtering the Display by Sort Code

A column of colored squares along the right edge of the Active Tetrode display serves to filter events by sort code.

- All available sorts are displayed
- Corresponding sort is displayed
- Corresponding sort is not used
- Unsorted events are displayed


The filters can be used by:

- Clicking the black square to show all sort codes.
- Clicking individual squares to toggle display of the corresponding sort code on or off.
- Ctrl + click an individual square to show only the corresponding sort code.

Pruning Snippets from the Display

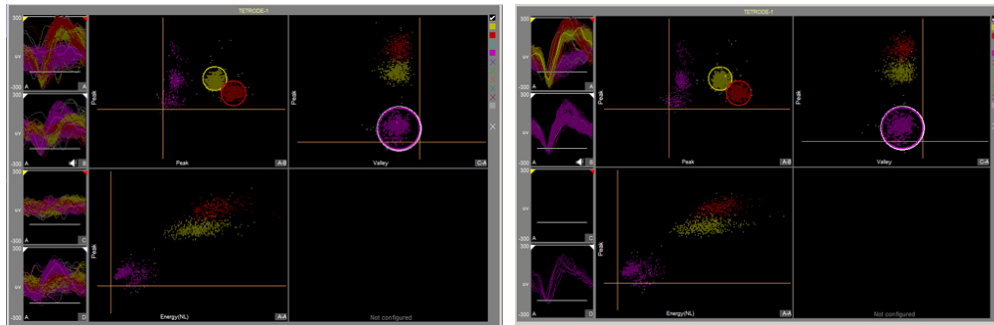
Three options to Prune Snippets from the display are provided in the Setup Properties. Similar to filtering, pruning provides more options to control the display and temporarily hide data during sorting.

To use the pruning options:

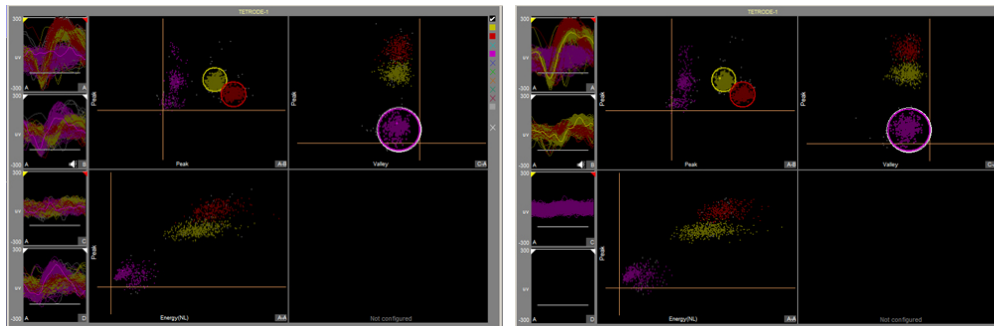
1. Click the **Setup Properties**  button and select the **Prune Snippets** parameter group.
2. Click the desired combination of check boxes.

Threshold when selected, only snippets that crossed the threshold for the channel on which they occurred are shown. The pruned snippets are hidden in all plots in the tetrode display area.

The window on the left shows the display with no pruning selected. On the right, only traces that meet the threshold for the channel in which they occurred are shown. Only the data of interest for sorting is shown.



Sort Code when selected, only show snippets if the projection plot containing a sort circle uses a metric from that channel. Hides snippets on channels not used in the projection plot metrics from the pile plots. See before and after images below.



Exclusive Shows traces only in the pile plot in which the corresponding sort code first appears. Note: this will always be the pile plot for the first channel of the tetrede, unless used in conjunction with the threshold or sort code pruning options.

The Setup Properties

TetSort control's properties are used to configure the workspace layout, thresholding parameters, history depths and other settings for both Sort Mode and Hunt Mode.

To open the setup properties:

- Click the **Setup Properties**  button.

The Setup Properties window includes four parameter groups available from a drop down list.

Thresholding Parameter Group

Threshold Factor – Select a value to set base threshold for spike detection to a value which is $x * \text{STD}$ of the signal RMS. This setting is active only when automatic threshold tracking is enabled.

Threshold Window Time Span – Type a numeric value to set the time span of the threshold window in seconds. **Note:** the control must be restarted to alter the time span.

Default Search Polarity – Select a positive or negative polarity for the threshold value. This setting is active only when automatic threshold tracking is enabled.

Default Peak Alignment – Peak Align aligns spikes according to their peak values, altering the time stamp and positioning of the snippet.

Track Time Const – Sets the time constant tau to 3, 5, or 10 seconds. Shorter tau values respond more quickly to deviations in the signal RMS value. This setting is active only when automatic threshold tracking is enabled.

Artifact Rejection Level (μV) – Type a numeric value to set the artifact rejection level in micro Volts. **Note:** Artifact Rejection must be enabled in the macro setup properties in RPvdsEx.

Common Display Parameter Group

Tetrode Column Count – Enter a number to set the number of columns for the Multi-Tetrode Display.

Max Tetrodes To Display: how many tetrodes to show in Multi-Tetrode Display.

Channel Index Type (A...D, 1...4, Absolute): how the channels inside each tetrode should be labeled. A, B, C, D... 1, 2, 3, 4... Absolute means the actual preamp channel is used.

Display Layout (Standard, MultiChannel View): Multichannel shortens the Wave Window and increases the Multi-Tetrode Display.

Sort Display Parameter Group

Snippet Plot Display History Depth: how many snippets to show in Snippet Plots in Sort Mode. 0 (default) means the 1024 most recent are shown.

Projection Plot Display History Depth: how many events to show in Projection Plots in Sort Mode. 0 (default) means the 1024 most recent are shown.

Clear on full: when checked, the Snippet Plots and Projection Plots clear when the event count reaches the display history depth specified above for the respective plots in Sort Mode. Otherwise, a circular buffer is used to display the most recent events.

Show Wave Window: when unchecked, the Wave Window is not shown in Sort Mode.

Show Snippet Plots: when unchecked, the Snippet Plots in the Multi-Tetrode Display are not shown in Sort Mode.

Show Projection Plots: when unchecked, the projection plots in the Multi-Tetrode Display are not shown.

Cluster Radius xSTD: determines how close the circle centers can be when approximating arbitrary shapes.

Maximum Circles per Arbitrary Shape: maximum number of circles to draw when approximating an arbitrary shape.

Arbitrary Shape Uniform Distribution: when unchecked, the empty spaces within the arbitrary shape are not considered significant and TetSort creates circles that tightly conform to the selected points in the projection. When checked, a bounding box is first created to contain the area within the arbitrary shape, and then circles are created to cover all areas within the bounding box. Both options use the minimum number of circles possible and no more than the user specified maximum.

Hunt Display Parameter Group

Snippet Plot Display History Depth: how many snippets to show in Snippet Plots in Hunt Mode. 0 (default) means the 1024 most recent are shown.

Projection Plot Display History Depth: how many events to show in Projection Plots in Hunt Mode. 0 (default) means the 1024 most recent are shown.
















Clear on full: When checked, the Snippet Plots and Projection Plots clear when the event count reaches the display history depth specified above for the respective plots in Hunt Mode. Otherwise, a circular buffer is used to display the most recent events.

Show Wave Window: when unchecked, the Wave Window is not shown in Hunt Mode.

Show Snippet Plots: when unchecked, the Snippet Plots in the Active Tetrode Display and the Multi-Tetrode Display are not shown in Hunt Mode.

Tool Buttons

The control features a set of buttons that allow you to work with all channels in the data set.

	Properties	Open the Setup Properties dialog box.
	Auto Scale	Auto scale and normalize all plot displays.
	Reset Base Scale	Return all displays to the base scale of 1.
	Auto Threshold	Enables auto-tracking for all unlocked displays.
	Manual Threshold	Enables manual-tracking for all unlocked tetrodes.
	Clear All Data	Clears event history for all tetrodes.
	Clear All Sort Circles	Clear all sort circles for current tetrode or for all tetrodes.
	Reset Snippet Plot Min-Max Intervals	Reset min-max intervals to endpoints of waveform for current tetrode or for all tetrodes.
	Reset 2D Plot Independent Scaling	Resets independent x-axis and y-axis scaling factors.
	Undo	Undo previous action (disabled if the active tetrode is locked).
	Redo	Redo an undone action (disabled if the active tetrode is locked).
	Lock All	Locks all tetrodes. Window resizing and tetrode selections are still allowed.
	Unlock All	Unlocks the controls mentioned above after they have been locked for all tetrodes.
	Hardware Sort	Switches to Hunt mode.
	Hardware Sort	Applies the software sorting information for all channels to the processing chain running on the hardware.

Keyboard Shortcuts

Keyboard combos:

Click + Drag

Pan Projection Plot.

Shift + Click and Drag

Projection Plot: Zoom in and out.

Snippet Plot: Y-axis zoom.

Ctrl + Alt + Click and Drag

Skew the Projection Plots up down or left right.

Alt + Click and Drag

Snippet Plot: Pan.

Projection Plot: Draw arbitrary shape.

Keyboard Projection Plot hotkeys:

~

Show all sort codes.

1-9

Toggle sort code show/hide.

Ctrl + [1-9]

Show only the selected sort code.

Minimizing Cycle Usage

There are several features of TetSort that rely on additional circuit components within the macro. These additional components raise the device cycle usage rates and may diminish the ability to process higher channel counts.

Automatic threshold tracking relies on circuit components that continuously compute the threshold while the DSP is performing other tasks such as filtering and acquisition. When automatic thresholding is disabled, only manual threshold mode is available (signified by the manual threshold button being grayed out). The TetSort control also alters the functionality of the Automatic Threshold button. When the button is clicked, an instantaneous calculation of the data is performed by the PC to determine a reasonable threshold setting for all channels.

To instantly calculate the threshold for all channels:

Note: Automatic threshold mode must be disabled in the macro setup properties.

1. Click the **Auto Threshold**  button.
2. Click **Yes** to calculate the instantaneous threshold for all channels.

To instantly calculate the threshold for a single channel:

Note: Automatic threshold mode must be disabled in the macro setup properties.

1. Right-click on the desired channel's Snippet Plot.
2. Select **Auto Threshold** from the menu.

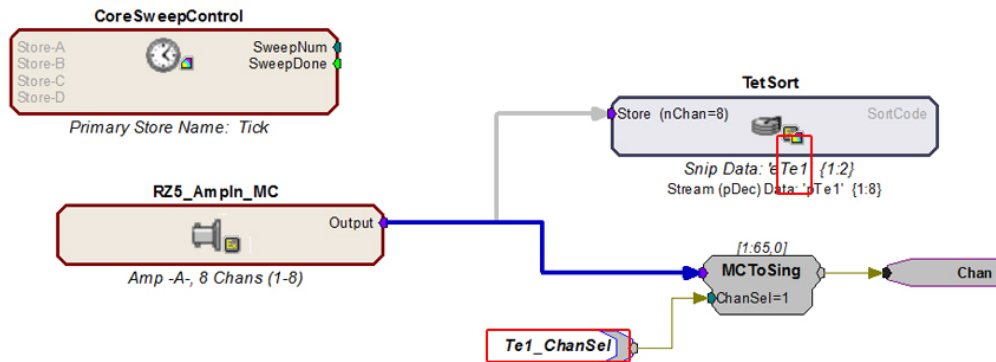
Artifact rejection also requires additional circuit components. Consider disabling artifact rejection as a means of reducing cycle usage when using high channel counts.

The sort codes can be output from the circuit macro as a multi-channel stream of integers for further processing. If this functionality is not being used, be sure to disable Sort Code Output in the circuit macro.

Working with the Selected Channel

Users can access the channel that is currently selected for audio monitoring within the TetSort control workspace by using the <ID>_ChanSel parameter tag. This tag can be used within the R PvdsEx circuit, for example, to pick off a single channel for additional processing. The example circuit below uses the parameter tag to set the ChanSel parameter of an MCToSing component and send that channel to additional processing components. The TetSort macro updates this parameter dynamically as the monitored channel is changed (by holding c and clicking on any pile plot) through the TetSort control.

The tag's unique name is generated using the TetSort macro's three character Identity. In the example below, the TetSort macro Identity is "Te1" so the parameter tag is Te1_ChanSel.



Channel Select Circuit Modification

IZ2StimGen – Stimulus Generation



ID: Stm1, Voltage Mode (16 ch), Shunt ON

The IZ2StimGen macro implements configuration and control of the IZ2 Stimulator. It supports monophasic or biphasic pulse train stimulation with up to four stimulus configurations. It features an easy-to-use interface for stimulus design, impedance checking, and real-time stimulus presentation through the IZ2 Control in OpenController. Settings for the number of channels and stimulation mode (current or voltage) are defined on the Setup tab of the macro properties dialog box. These settings control unit values and channel count for the control set.

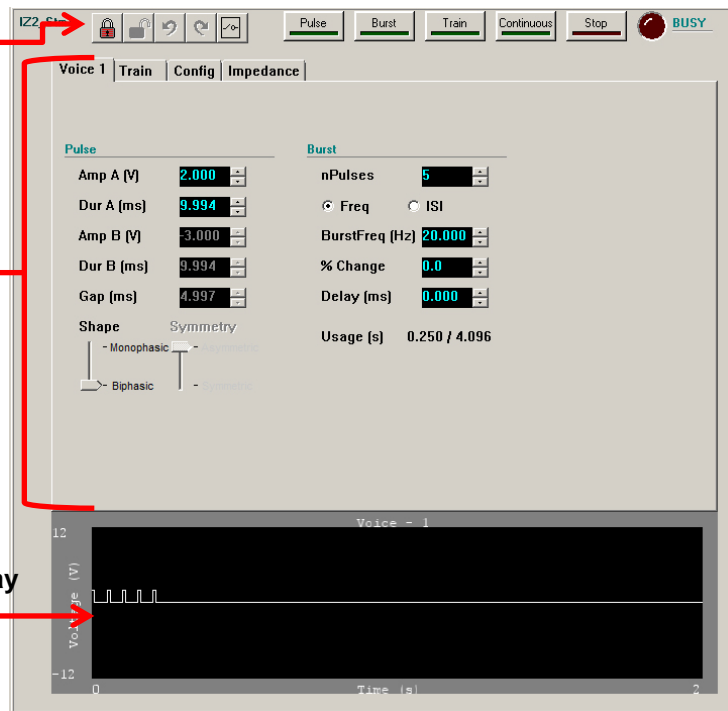
The macro also supports external triggering, data monitoring and use of the IZ2H model. More information on these options is available in the macro's internal help.

Stimulus Presentation and Control Buttons

Control Set Buttons

Tabs

Burst Display Graph



The Control

The control includes configuration tabs for signal design, channel configuration and impedance checking, as well as buttons for real-time control of stimulus presentation.

The Control Set window includes:

Buttons	Set options and control stimulus presentation.
Voice Tabs	Design up to four unique pulse and burst parameter sets.
Train Tab	Design train parameters.
Config Tab	Configure each electrode channel mode.
Impedance Tab	Check impedance of each electrode bank.

The Buttons

The IZ2 Control features two sets of buttons: one for basic control set options and a second for stimulus presentation and control.

Control Set



Lock

Lock the Voice Tabs options.



Unlock

Unlock the Voice Tabs options.



Undo

Undo last action in Voice Tab.



Redo

Redo last undone action in Voice Tabs.



Open

All channels are left Open (disconnected from the stimulator circuit) when not stimulating. When a stimulation button is pressed, all channels are set to the desired mode, 200ms later the trigger is sent. 200ms after the BUSY indicator goes dark all channels are opened again until the next stimulation button is pressed.

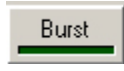
Note: this mode is not available in External Trigger mode.

Stimulus Presentation and Control



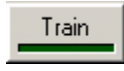
Pulse

Send one pulse.



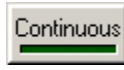
Burst

Send one burst.



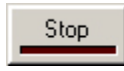
Train

Send one train.



Continuous

Run pulse trains continuously.



Stop

End stimulus presentation prematurely.



Busy

Busy LED is on when a stimulus is presented or impedance checking is active. Changes to the stimulus configuration can only be applied when Busy LED is not lit.

Note: When a signal longer than 0.5 seconds is presented, all buttons, except Stop, are disabled during stimulus presentation to prevent changes. The Voice Tabs parameters are also locked. These buttons will all be disabled if Workbench is in Idle mode, or if no channels are set to stimulate. The Train and Continuous buttons will be disabled in External Trigger mode.

Burst Display Graph

This graph displays the burst waveform for reference. Users can move or scale waveforms for better viewing. Note that this graph requires video drivers with OpenGL support. If the appropriate video drivers are not installed, a blank graph is displayed.

To move the waveform in the diagram window:

- Click and drag from side to side.

To adjust the x-axis scale of the plot:

- Press and hold the Ctrl key and use the mouse wheel to zoom in and out on the x-axis scale.

To adjust the y-axis scale of the plot:

- Use the mouse wheel to zoom in and out on the y-axis scale.

To reset the axis or auto scale the plot:

- Right-click the diagram and click the desired command on the shortcut menu.

The Shortcut Menu

Reset Scale X Reset the X-axis scale to fit the entire waveform.

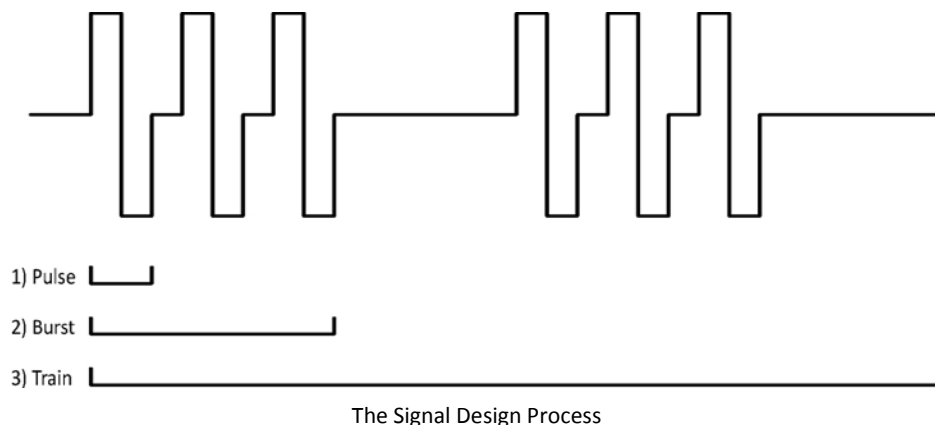
Reset Scale Y Reset the Y-axis scale to the maximum values.

Auto Scale Change the Y-axis scale to fit the waveform in 80% of the window.

Note: The Burst display is not shown in the Impedance tab.

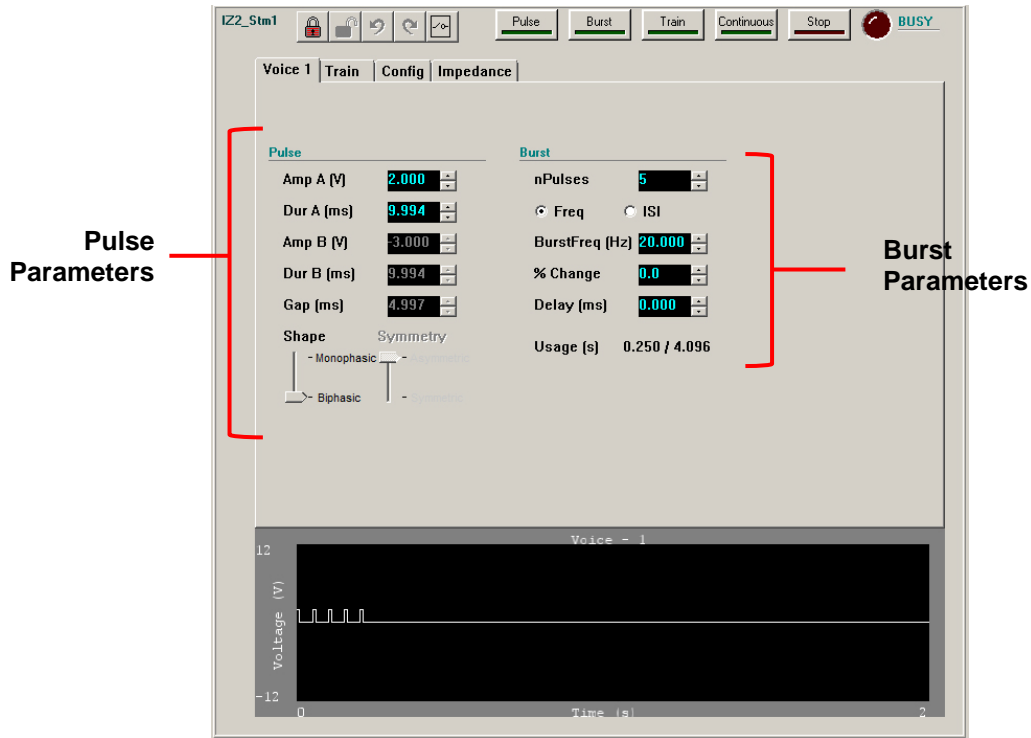
Designing the Stimulus

The IZ2 Control interface breaks down the stimulus design process into hierarchical parts. Signal design begins with the most basic component of the signal—the pulse. Once the pulse is defined, the burst and then the train can be defined with each step referencing the one before.



The IZ2 Control supports up to four unique stimuli, or *voices*. Each voice can be assigned to one or more channels for stimulation. The Train parameters (number of bursts and inter-train interval) are used globally by all voices. Each voice has its own pulse and burst parameters which are configured in the Voice Tabs.

Voice Tab – Designing the Pulse and Burst



The Control – Voice Tab

When the control is generated, the *Voice 1* tab is populated with default values for the Pulse and Burst parameters.

Configuring the Pulse

Monophasic or biphasic pulses can be configured by defining the amplitude and duration of each phase of the pulse.

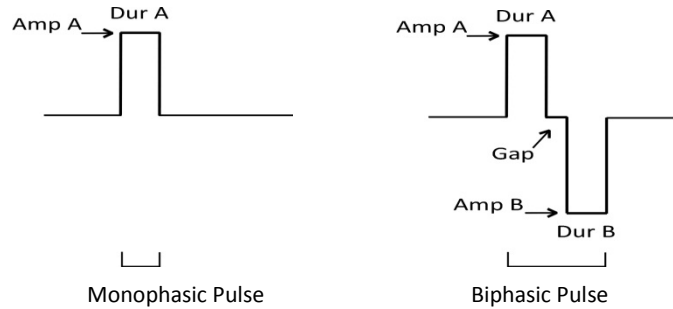
To add a second phase to the pulse:

- Drag the **Shape** slider to **Biphasic**.

By default the second phase is symmetrical with the first phase, with inverse amplitude and the same duration.

To allow configuration of the second phase amplitude and duration:

- Drag the **Symmetry** slider to **Asymmetric**.

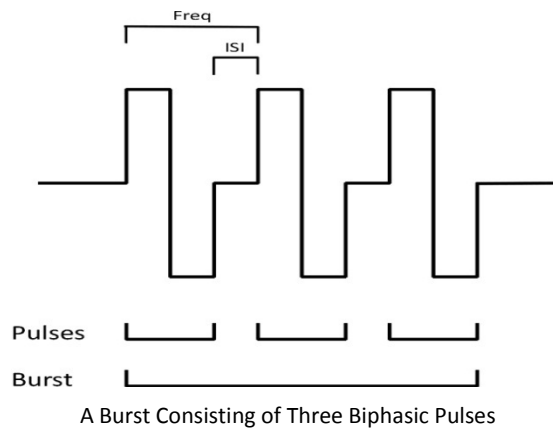


- Amp A** Set amplitude of the pulse or the amplitude of the first phase of a biphasic pulse.
- Dur A (ms)** Set duration of the pulse or the duration of the first phase of a biphasic pulse.
- Amp B** Set amplitude of the second phase of a biphasic pulse.
- Dur B (ms)** Set duration of the second phase of a biphasic pulse.
- Gap (ms)** Set the time between phases of a biphasic pulse.
- Shape** Choose Monophasic or Biphasic.
A monophasic shape consists of one phase, either positive or negative. A Biphasic shape consists of two phases.
- Symmetry** Choose Asymmetric or Symmetric.
In a symmetrical biphasic pulse, the duration of each phase is the same and the amplitude of the second phase is the inverse of the first phase. In an asymmetric biphasic pulse, amplitude and duration of phases are independent.

The unit descriptions for Amp A and Amp B in the Pulse settings are determined from the stimulation mode defined in the macro settings. In Voltage mode, the maximum voltage output is +/-12V. In current mode with an IZ2 device the maximum output is +/-300uA through a 50kOhm load. In high current mode with an IZ2H device the maximum output is +/-3mA through a 5kOhm load.

Configuring the Burst

A burst is configured by defining the number of pulses and the interval between pulses.



- nPulses** Set the number of pulses in a burst.

Freq/ISI	Define the interval between pulses using frequency or time. Enables either the BurstFreq or ISI field below.
BurstFreq (Hz)	Set frequency of pulses within a burst from onset to onset.
ISI (ms)	Set time interval from offset of one pulse to onset of the next pulse.
% Change	Defines change in pulse amplitude from beginning of burst to end of burst. If negative, the amplitude of each pulse is smaller than the last by this percentage. If positive, the amplitude of each pulse is larger than the last by this percentage.
Usage (s)	Displays the ratio of current burst duration to maximum burst duration. The maximum duration of a burst is limited to 100,000 samples. This translates to 4.096 seconds at ~25kHz sampling rate.

Adding Voices

If more than one pulse/burst definition is desired in the same paradigm, you can add up to three additional voices. For example, this is useful for sending an inverse of Voice 1 simultaneously on other channels or sending delayed versions of Voice 1 on other channels.

To add, copy or delete a Voice tab:

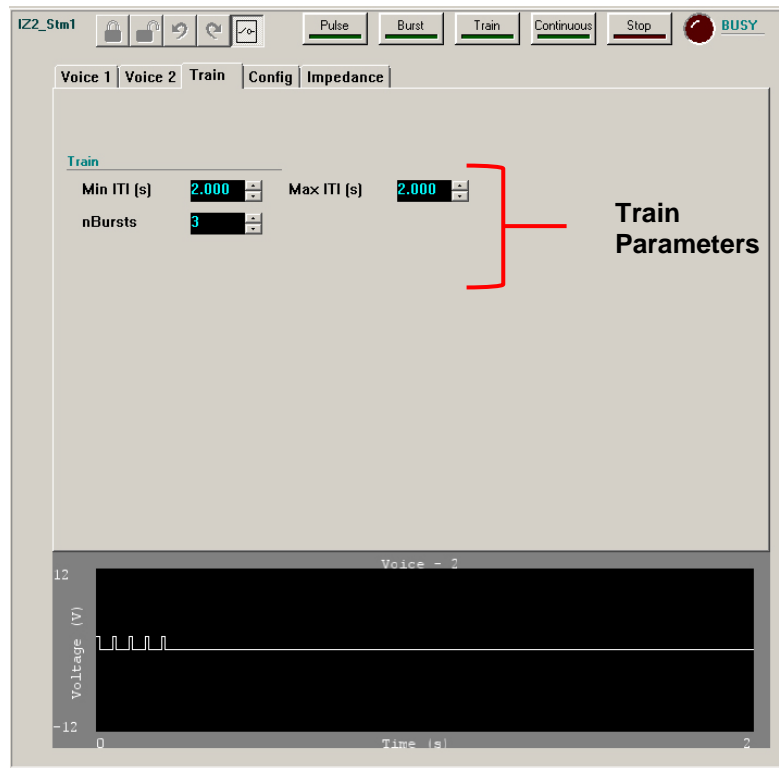
1. Right-click the current tab to display the shortcut menu.
2. Click **New Voice** then click **Add**, **Copy**, or **Delete**.

Add	Add a new Voice tab with the default values.
Copy	Add a new Voice tab with the same values as the current tab.
Delete	Delete the current voice.

Voices are assigned to channels on the Config tab.

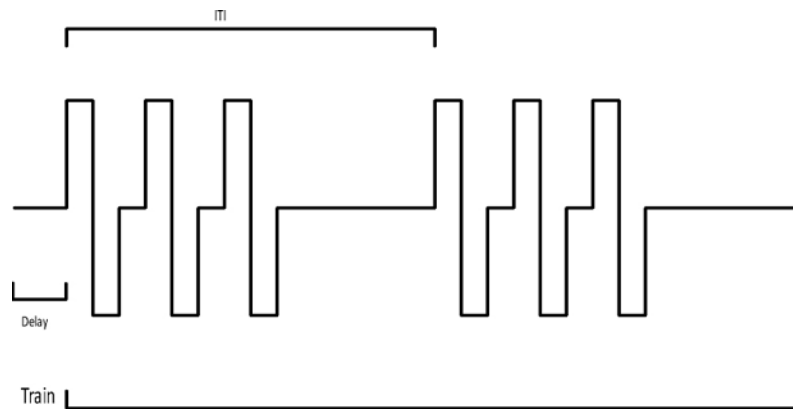
Configuring the Train

The train parameters are configured on the Train tab.



The Control – Train Tab

A train is configured by defining the number of bursts and the interval between burst onsets. A delay before the onset of the train presentation may also be set. A randomized train interval can also be set.



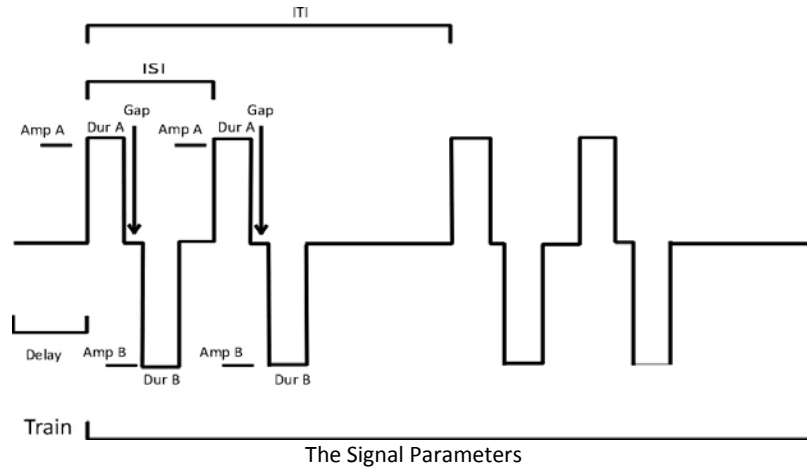
A Train Consisting of Two Bursts of Three Biphasic Pulses

- Delay (ms)** Set the time before stimulation presentation begins after the trigger occurs.
- Min ITI (s)** Set the minimum time interval between onset of one burst to onset of next burst.

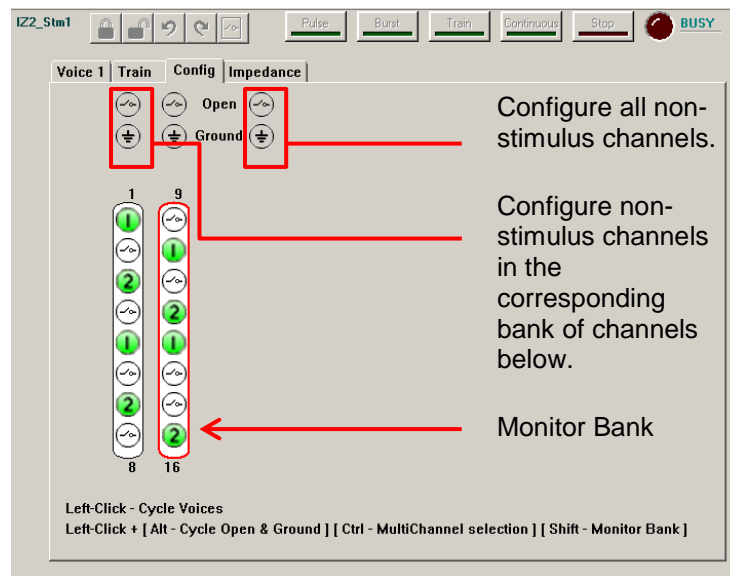
Max ITI (s) Set the maximum time interval between onset of one burst to onset of next burst.

nBursts Set the number of bursts in a pulse train.

Set Max ITI equal to Min ITI to use a uniform train interval. A randomized train interval will be used if Max ITI does not equal Min ITI.



Config Tab – Configuring Channels



The Control – Config Tab

The configuration diagram represents the stimulation channels and serves as an interface for configuring them. The channels are divided into banks of eight channels and labeled above and below the bank column in the diagram.

The selected bank (highlighted in red) is available for monitoring the actual stimulus voltage output from the IZ2StimGen macro in the circuit. The monitor output of the IZ2StimGen macro contains all of the stimulation channels, however only one bank of eight channels can be updated

at a time. The highlighted bank in the Config tab sets this bank. The rest of the banks are latched at their last monitored value.

Each channel can be configured as a stimulus channel or in one of the following three states: Open or Ground.



Stimulate Stimulus output channel (Voice 1 shown).



Open Channel left open (disconnected from stimulation circuit).



Ground Channel shorted to return ground.



Note: if the **Open** button is depressed all channels regardless of their configuration will be in the Open state when not stimulating (e.g. when the Busy LED is not lit).

To set a channel for stimulation:

1. In the diagram, left-click the corresponding circle. The circle turns green and the number 1 is displayed to indicate the first voice stimulus has been assigned to that channel.
2. To select a different voice, click the circle again until the desired voice number is displayed, or hold down the number key of the voice you would like to assign and then left-click the circle.

Note: When configuring channels, the bank that contains the most recently selected channel will automatically become the monitor bank.

To set multiple channels for stimulation:

1. Press and hold the **Ctrl** key and click the desired channels.
2. To select a different voice for a channel, continue to hold down control and click the channel again until the desired voice number is displayed, or hold down the number key of the voice you would like to assign and then left-click the circle.
3. When all desired channels are set, release the **Ctrl** key.

Note: When configuring channels, the bank that contains the most recently selected channel will automatically become the monitor bank.

To configure all non-stimulus channels in all banks:

- Click the desired option icon from the far right column of icons. This also clears all stimulus channels in all banks.

To configure all non-stimulus channels in a single bank of eight channels:

- Click the desired option icon from the column above the bank. This also clears all stimulus channels in that bank.

To configure non-stimulus channels individually:

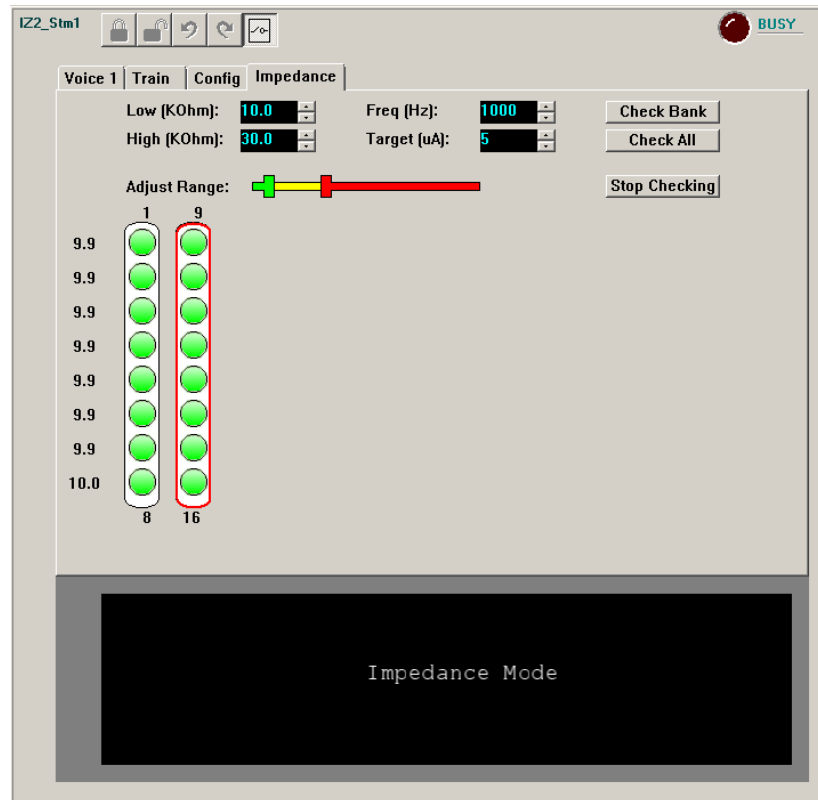
- Press and hold the **Alt** key and click the channel icon to cycle through the options, such as Open and Ground, until the desired option icon is displayed.

Note: When configuring channels, the bank that contains the most recently selected channel will automatically become the monitor bank.

To select the monitor bank without changing/setting stimulation channels:

- Press and hold the **Shift** key and click the desired bank.

Impedance Tab



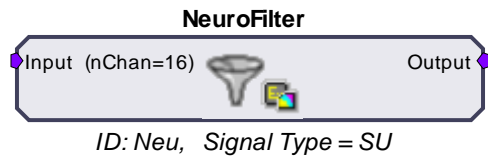
The Control – Impedance Tab

The Impedance Tab provides an easy to use interface for impedance checking. Users can set test signal parameters and define the high and low impedance thresholds for visualization. Results of the impedance check are displayed in the channel diagram.

- Low (KOhm)** Enter the low impedance threshold.
- High (KOhm)** Enter the high impedance threshold.
- Freq (Hz)** Enter the desired frequency for the test signal.
- Target (uA)** Enter the amplitude of the test signal.
- Adjust Range** Drag indicators to set minimum and maximum impedance range visually.
- Check Bank** Run an impedance check on the currently selected bank (highlighted in red). The test signal (sine wave of frequency defined by **Freq (Hz)** parameter and amplitude defined by **Target (uA)** parameter) is presented on the currently selected bank for two seconds and the impedance is measured. All other electrode channels are open circuit, regardless of the state of the Open button.

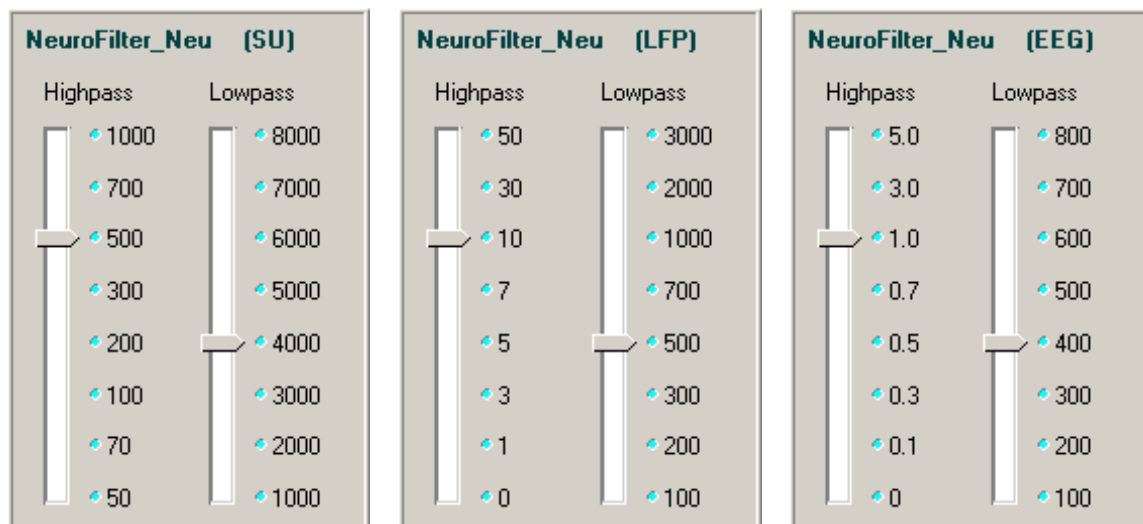
- Check All** Run an impedance check for all channels by cycling through each bank of eight channels and performing the Check Bank operation described above.
- Stop Checking** Stop impedance checking prematurely.
- Diagram** Displays a representation of all channels, divided in eight channel banks. Results of impedance check are indicated by color: below low impedance threshold (green), above high impedance threshold (red), between low and high impedance thresholds (yellow). The actual impedance values (in KOhm) for the currently selected bank are displayed in the column on the left. To change the selected bank, click the desired bank.

NeuroFilter - Filtering



The NeuroFilter macro implements filtering of a multi-channel signal and supports real-time control of corner frequencies through the NeuroFilter control in OpenController. Settings for the number of channels and signal type selection (Single Unit, LFP, or EEG) are defined in the Setup tab of the macro properties dialog box. See the onboard macro help for more information.

The NeuroFilter control is available when the NeuroFilter macro is included in the compiled circuit file. The macro's default HP-LP filter settings are initialized based on three *Signal Type* settings in the macro properties window: Single-Unit, LFP, and EEG. These settings determine the initial corner frequencies at runtime (shown below). Highpass corner frequencies for LFP or EEG settings can be set to 0. This removes the highpass filter and DC couples the multi-channel signal.



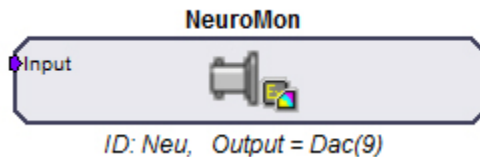
Modifying the Corner Frequencies

The control contains a slider switch control for each filter. Values on the slider switches are specified in Hz.

To Change the Filter Characteristics:

- Drag the slider pointer to the desired corner frequency for the associated filter.

NeuroMon - Signal Monitoring with Noise Gating



The NeuroMon macro outputs a single channel to a user-specified DAC output and is useful for monitoring single unit activity via a loudspeaker during an experiment. The macro supports real-time adjustment of output level and noise gating through the NeuroMon control in OpenController. The optional noise gating feature can gate the signal on and off based on the signal RMS to, in effect, play out the spike train while gating out noise.

Settings for configuring the output channel and noise gate properties are defined on the Setup tab of the macro properties dialog box. See the onboard macro help for more information. Also note, DAC output channels are device specific. Refer to your System 3 reference manual for the DAC output channel configuration for your device. In addition to the ID, the macro parameter summary displays the currently specified DAC output channel.

The NeuroMon control provides output level and noise gating control for a single channel in a multi-channel data stream. This control is available when the NeuroMon macro is included in the compiled circuit file. It is most often used when monitoring unit activity during an experiment.

The NeuroMon control modifies the monitored channel which is, by default, channel 9. However, the control is designed for use with the BoxSort and PCSort controls. When used with these controls the monitored channel is the active channel in the sort window.

The output level knob controls a factor from 0 to 10 that is multiplied by the base output gain of 1000. The base output gain value can be modified in the control's properties dialog box.

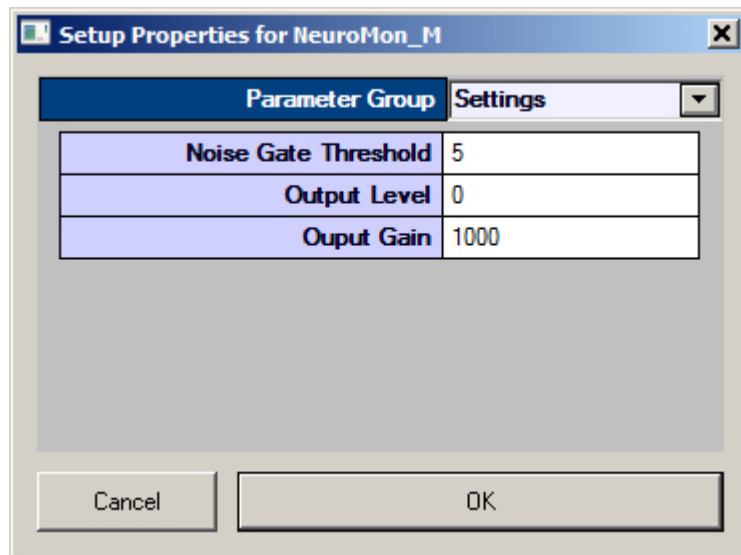
When noise gating is enabled, the noise gate knob is used to control a factor from 0 to 10 that determines the signal to noise ratio needed to pass a signal through the gate.

The Setup Properties

Double-clicking on the control opens the Settings Parameter Group dialog box. Several options can be modified.

To open the setup properties:

- Double-click the **NeuroMon** control.



Settings Parameter Group

Noise Gate Threshold – Type a numerical value to set the Noise Gate threshold factor.

Output Level – Type a numerical value to set the output level factor.

Output Gain – Type a numerical value to modify the base output gain value for the monitored signal.

Monitoring the Signal

The control includes two knobs, allowing users to adjust the output level and the noise gating threshold factors for the monitored signal by dragging the pointer on the control.

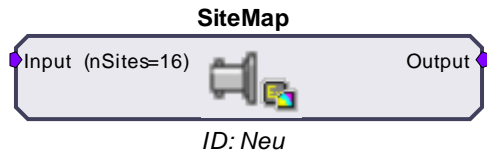
To Change the Output Level of the Signal:

- Drag the **Output Level** Knob control to the desired factor.

To Change the Noise Gate Factor:

- Drag the **Noise Gate** Knob control to the desired factor.

SiteMap - Electrode Site Remapping



The SiteMap macro is used to remap specified headstage, adapter, and electrode combinations to a multi-channel data stream. The number of sites for the electrode is specified in the macro properties dialog box. Once running in OpenController, the SiteMap control is used to select the combination of headstage, adapter, and electrode from three separate lists of available headstages, adapters, and electrodes. The control then sends the site mapping information to the macro in order to remap the multi-channel output. A **Download** button is included in the macro setup properties, which, once clicked, downloads the latest known pinouts for each Headstage, Adapter, and Electrode from the TDT website.

Mapping conventions for this macro are displayed in the macro onboard help. These channel numbers reflect the channels present at the output of the macro as they relate to the electrode sites. For a more detailed diagram representing the numbering conventions, see *Site Numbering Conventions*, page 73.

The SiteMap control can be used to remap electrode sites based on the combination of headstage, adapter, and electrode chosen.

This control is available when the SiteMap macro is included in the compiled circuit file. The SiteMap control uses a .CSV file to send the remapping list to the macro which then remaps electrode sites to a known convention making them available at the output of the macro. Manufacturers may change pinouts frequently.

Remapping the Sites

Remapping the electrode sites configures the multi-channel signal in an orientation based on a standard convention.

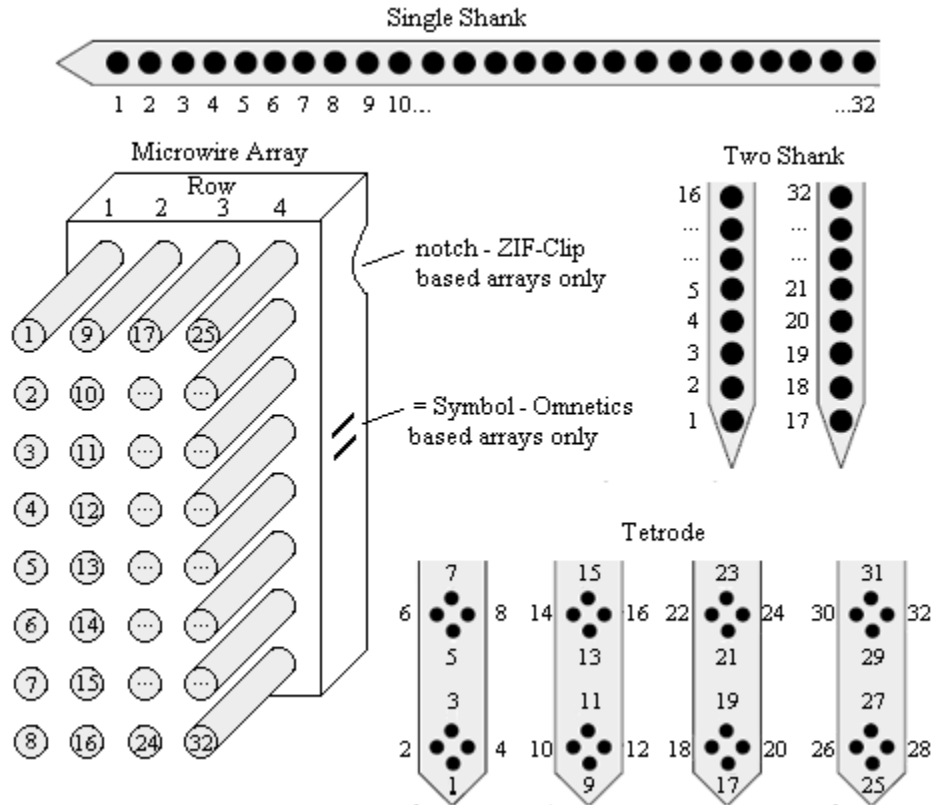
To Assign a Mapping Configuration:

- In RPsVsEx, if necessary, click the **Download** button in the SiteMap macro setup properties to update all pin configurations.
- In OpenController, click the **Browse** button to the right of each text box in the SiteMap control set. Select the desired Headstage, Adapter, and Electrode from the list then click **OK**.

Note: clicking the Show All check box enables additional selection options.

Site Numbering Conventions

Probe sites for shanks and tetrodes are arranged clockwise and in ascending order from tip-to-shank. Omnetics and ZIF-based microwire arrays are arranged in descending order top-to-bottom from left-to-right with the array symbols shown in the diagram below.



Electrode Map → **Adapter Map** → **Headstage Map** → **Amplifier Map** → **SiteMap**

Position in brain (probe positions are dependent on manufacturer).

Remapped to adapter (if necessary).

Remapped to headstage.

Remapped to amplifier.

Remapped to conventions above.

Channel numbers equal probe position in brain.



Tutorial

Online Spike Sorting with SpikePac

In this tutorial you'll build an online spike sorting project using OpenEx and the SpikePac tool set. The experiment created in this tutorial acquires four channels of simulated extracellular spike data and supports online spike detection and sorting. The project uses SpikePac macros and controls to simplify and improve experiment configuration.

This tutorial demonstrates:

1. Building and configuring a SpikePac OpenEx project.
2. Using macros to auto-configure real-time control sets.
3. Adding real-time control with OpenController.

Planning the Project

When you plan a project you need to:

1. Determine what type of data will be acquired.
2. Determine what device(s) you will use.
3. Determine which OpenEx applications you will need.

Determining What Type of Data Will Be Acquired

One of the strengths of OpenEx is that it allows you to acquire multiple types of data in one project, often from the same signal source. In this tutorial, we will learn about acquiring discrete waveforms (often called snippets or segments), a special type of continuous waveforms called plot decimated data, and a list of scalar values. Each type of data storage is configured using a specific structure in the underlying RPvdsEx circuit and is identified with a *Store* name by the TTank data server for fast efficient handling of the data. Data Stores are configured for you when you use RPvdsEx circuit macros.

The SpikePac tool set includes two data saving macros: BoxSort and PCSort. These macros primarily store spikes—discrete waveforms that are acquired as small chunks of data (<1000 samples) and occur at rates greater than once a second—and support applying and storing sort codes. In this tutorial, you will configure the PCSort macro for real-time, principle component sorting; however, experiment configuration is virtually the same using either macro.

While the raw, continuous waveform will not be acquired for data analysis in this experiment, a special type of continuous waveform, called plot decimated data, will be stored and used to view spike activity in real-time. Plot decimated data is a form of decimation where only the maximum and minimum values are stored for each chunk of data (for example 64 samples). Data in this format allows users to visualize the maximum noise floor and spike activity of an incoming signal; however, it is not a true representation of the acquired signal. It is used to get an approximation for real-time plotting without requiring the transfer of a large amount of data. A plot decimated data Store is included in both the BoxSort and PCSort macros. Finally, a pulse will be generated and acquired once per second and stored as a set of scalar values using the CoreSweepControl macro.

Determining What Device You Will Use

OpenEx supports all of the System 3 processor devices. However, because the SpikePac tool set is intended for building a neurophysiology experiment that acquires multi-channel data and

incorporates complex real-time processing, you'll need to use a multi-DSP processor (RX or RZ). An RZ2 BioAmp or Pentusa BioAmp System would be a good choice for data acquisition.

If you do not have an RZ2 in your system, you can follow through the tutorial by substituting any multi-DSP processor (RX or RZ) in the OpenWorkbench device configuration. This tutorial cannot be run on classic single processor devices such as the RP2.1 or RA16BA Medusa BioAmp.

Determine Which OpenEx Applications You Will Need

OpenEx is a suite of applications that work in a client/server environment. That means you can pick and choose the applications you need to build and run your experiment. In this tutorial you will be building the project from the ground-up. To design the processing chain that controls the processor you will use RPvdsEx, the circuit design interface. To design the experiment and to handle communication between the OpenEx environment and the hardware you'll need OpenWorkbench.

Finally, you'll add the OpenController client application. Using OpenController you will control experimental parameters (such as filter settings and threshold settings for unit activity) and access acquired data and parameter variables in real-time. OpenController accesses the hardware through the OpenWorkbench Server.

Setting-Up Your Hardware

Every OpenEx experiment is run on TDT System 3 hardware. Before you begin, be sure to setup and test your hardware using the installation guide provided with your system. When the hardware is connected and turned on before you begin to build the project, OpenEx can automatically detect the devices available, speeding up project configuration.

Because this tutorial uses a simulated spike signal generated on the processor you will not actually need a preamplifier, headstage, and electrodes to "acquire" and visualize the data. You can follow along with this tutorial using any System 3 multi-DSP processor (RX or RZ).

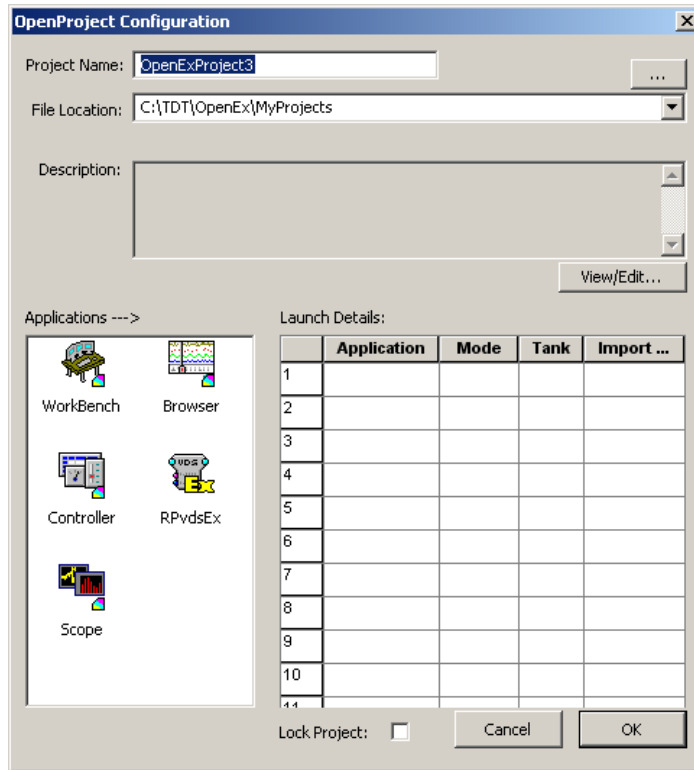
Creating the Project

Note: ensure that your TDT system is connected to the PC and turned on before creating the project.

To create and configure the project:



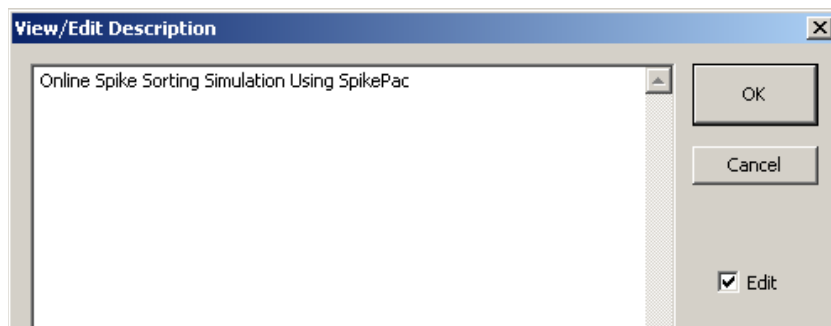
1. Double-click the **OpenProject** icon on your desktop.
2. In the OpenProject window, click the **OpenProject** menu and click **New Project**.



3. In the **Project Name** box type **SigSnip**.
4. In the **File Location** box, the default location for new project directories, the My Projects folder, is displayed. We recommend keeping all your projects under this folder, so leave the File Location information as is.

Note: if the My Projects folder is not displayed, click the down arrow to select it from the drop down list or the **...** Browse button to locate it manually.

5. If desired, click the **View/Edit** button; click the **Edit** check box to add a description.

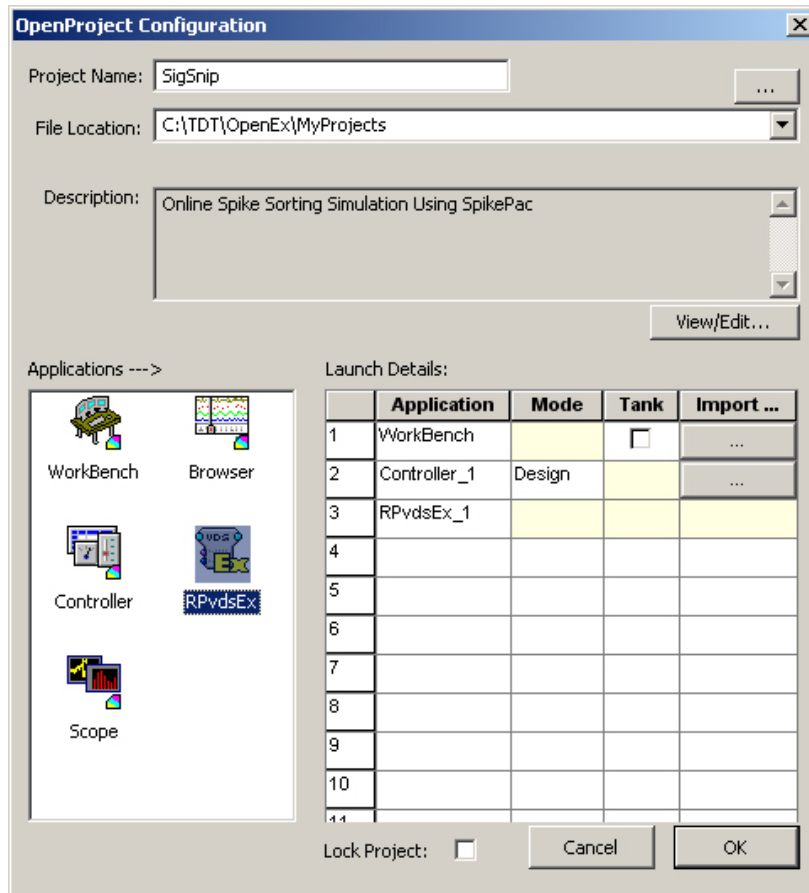


After typing a description, click **OK**.

To add applications:

1. Under Applications, double-click the **WorkBench** icon to add OpenWorkbench to the project.
2. Double-click the **Controller** icon to add one instance of OpenController.

3. Finally, double-click the **RPvdsEx** icon.



4. Click **OK** to close the OpenProject Configuration window.

As the added applications are launched, all the necessary files for the project are created and icons for the added applications are added to the OpenProject main window.

Note: because all communication with the System 3 hardware occurs through OpenWorkbench, only one instance of OpenWorkbench can be added to a project. Multiple instances of client applications, such as OpenController or OpenScope, can be added if needed.

Creating the Circuit File

In this tutorial you'll learn how to build a custom processing chain and some of the basics of designing *circuits* in RPvdsEx. You'll also learn to tap into the strength of the system—real-time control—using SpikePac macros. Circuits designed for OpenEx allow users to control timing, triggering, data storage, and modification of other parameter values. Macros greatly simplify this process, reducing the number of components and parameters that must be configured in RPvdsEx and feeding information to OpenController that allows entire control sets to be automatically configured for you.

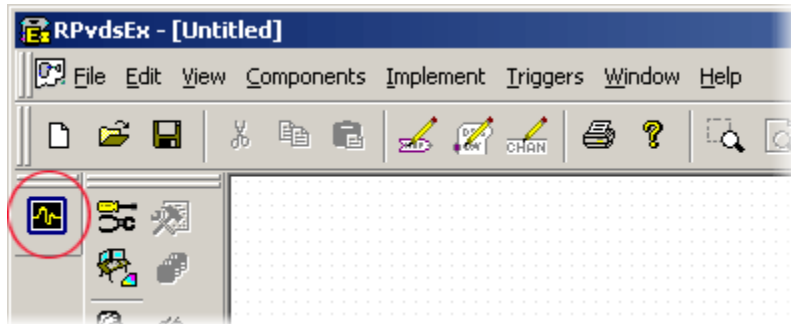
Simply add the macro based Control to the Controller workspace and you will be able to update parameters (such as spike thresholds and sorting parameters) from within OpenEx's user-friendly software environment.

To select the circuit design application and create a new file:

1. Click the **RPvdsEx** icon in the OpenProject window.

2. Click the **File** menu and click **New**.

If SpikePac is installed, a special SpikePac macro button is displayed as part of the RpvdsEx interface.

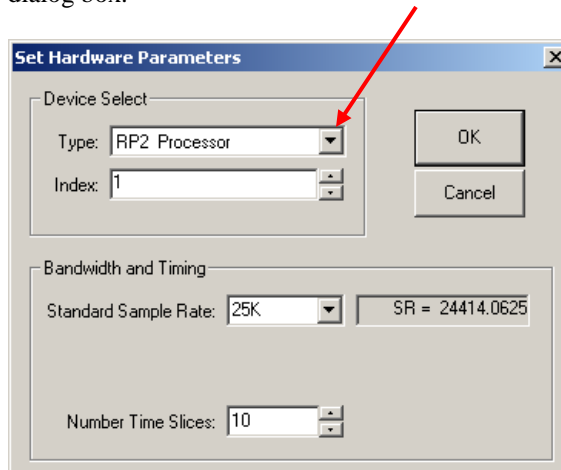


Specifying a Device

Some of the RpvdsEx features and components used in this tutorial are only supported by multi-processor devices. To ensure all the features you will need are available, you will need to specify a device type before beginning circuit design.

To set the device type:

1. Click the **Implement** menu and click **Device Setup** to open the Set Hardware Parameters dialog box.




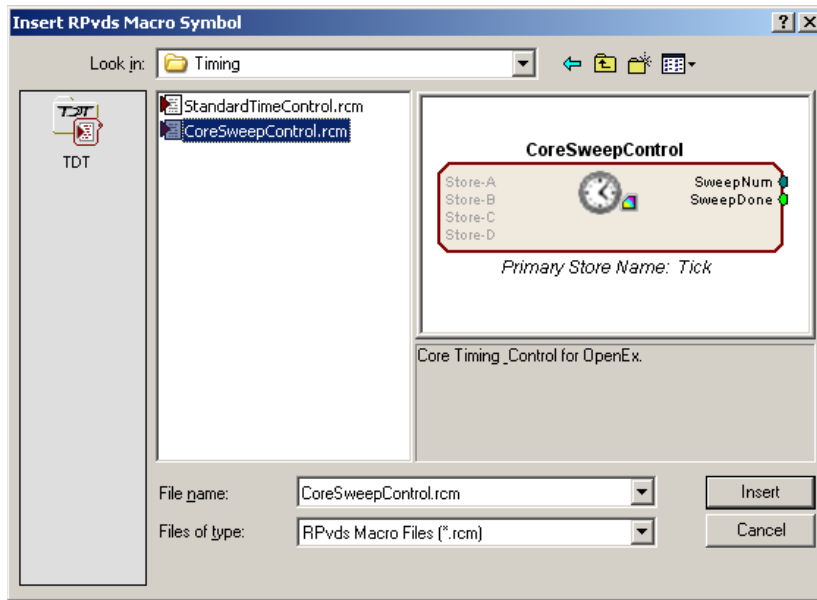
2. Select your processor from the **Type** drop-down menu in the Device Select group box.
3. Click **OK**.

Adding Timing and Synchronization

The CoreSweepControl macro or equivalent circuitry must be included in every OpenEx project to handle basic timing and synchronization for the circuit.

To add a CoreSweepControl macro:

1. In RpvdsEx, click the  **Insert Macro** button in the RpvdsEx Components toolbar.
2. In the dialog box, select the **CoreSweepControl** macro from the **Macros | Timing** folder.




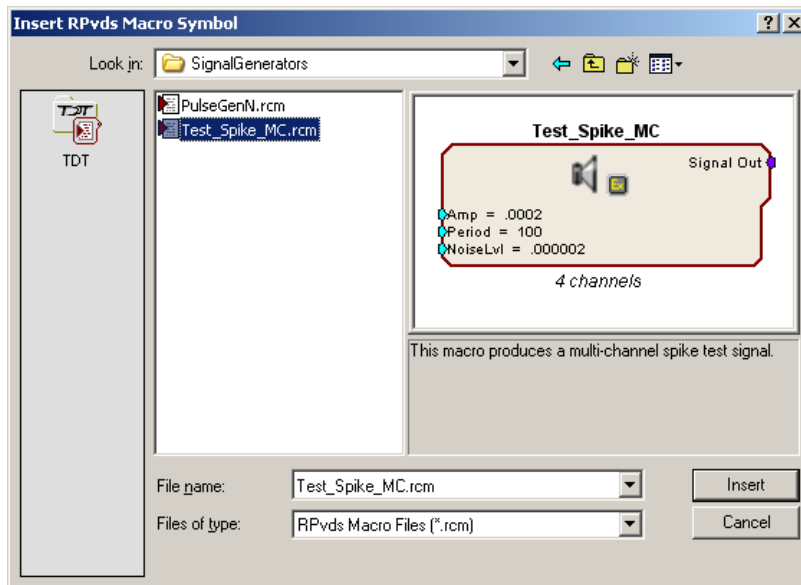
3. Click **Insert**. After the dialog box closes, click the workspace to place the component in the workspace.

Adding the Demo Signal for Demonstration Purposes

The Test_Spike_MC macro will be used to generate a fake physiological signal containing multi-channel spike data for demonstration purposes. This macro could later be replaced with A/D inputs component(s) to acquire, display, and store four channels of external analog data.

To add the demo signal:

1. Click the  **Insert Macro** button again.
2. In the dialog box, select the **Test_Spike_MC** macro from the **Macros | SignalGenerators** folder, click **Insert** then click the workspace to add the macro.

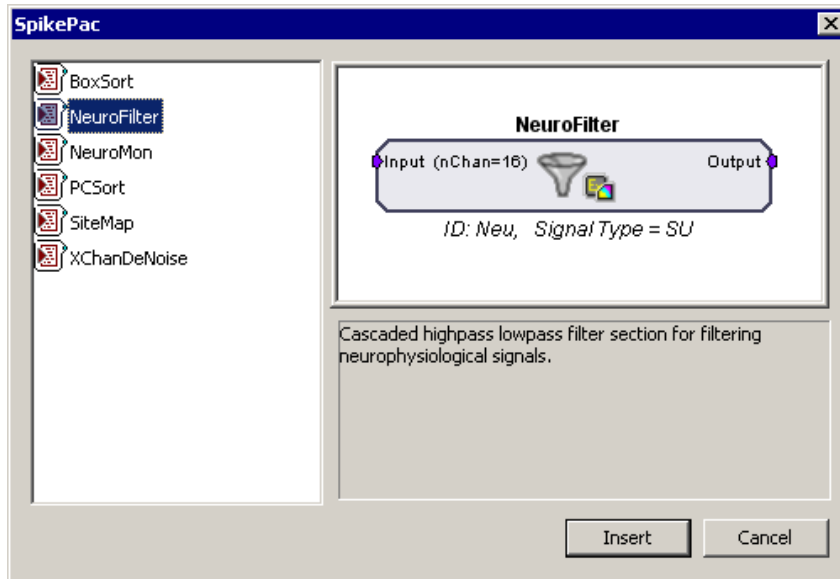


Adding Real-Time Filtering

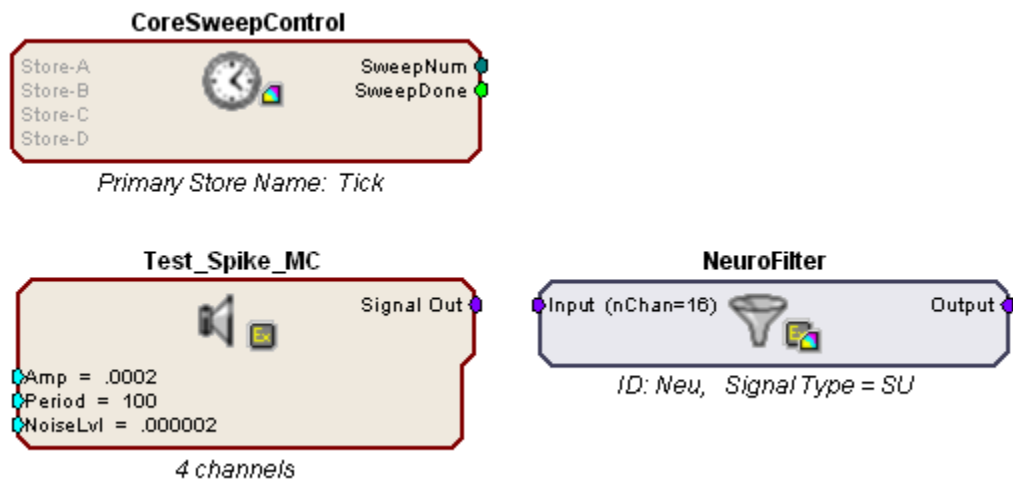
To implement real-time filtering in this project you will add the NeuroFilter, which is similar to the standard macro HP-LP_Filter_MC. However, when you use the macro, information is passed to OpenController and used to auto configure a control set for adjusting the filter corner frequencies.

To add signal filtering:

1. Click the  **Insert SpikePac Macro** button; select the **NeuroFilter** macro.



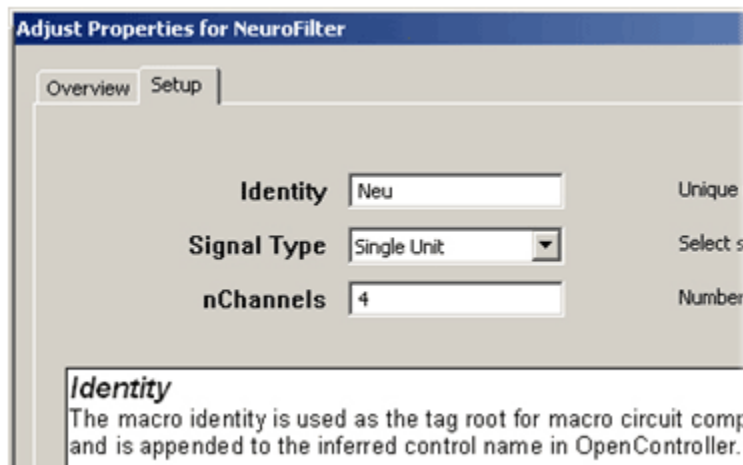
2. Click **Insert** then click the workspace to add the macro.



In your circuit, the border of the NeuroFilter macro is blue. This helps you quickly identify SpikePac macros in your circuits.

3. Double-click the **NeuroFilter** macro to open the properties window.
4. Click the **Setup** tab or click **Next**.

5. In the Setup options you can specify a Signal Type (Single Unit, LFP or EEG). This information is used to initialize the corner frequencies and configure the corresponding control with values appropriate for the type of signals you are working with. The default setting, Single Unit, is fine for this tutorial.
6. In the nChannels text box type 4 to set the number of channels to filter.

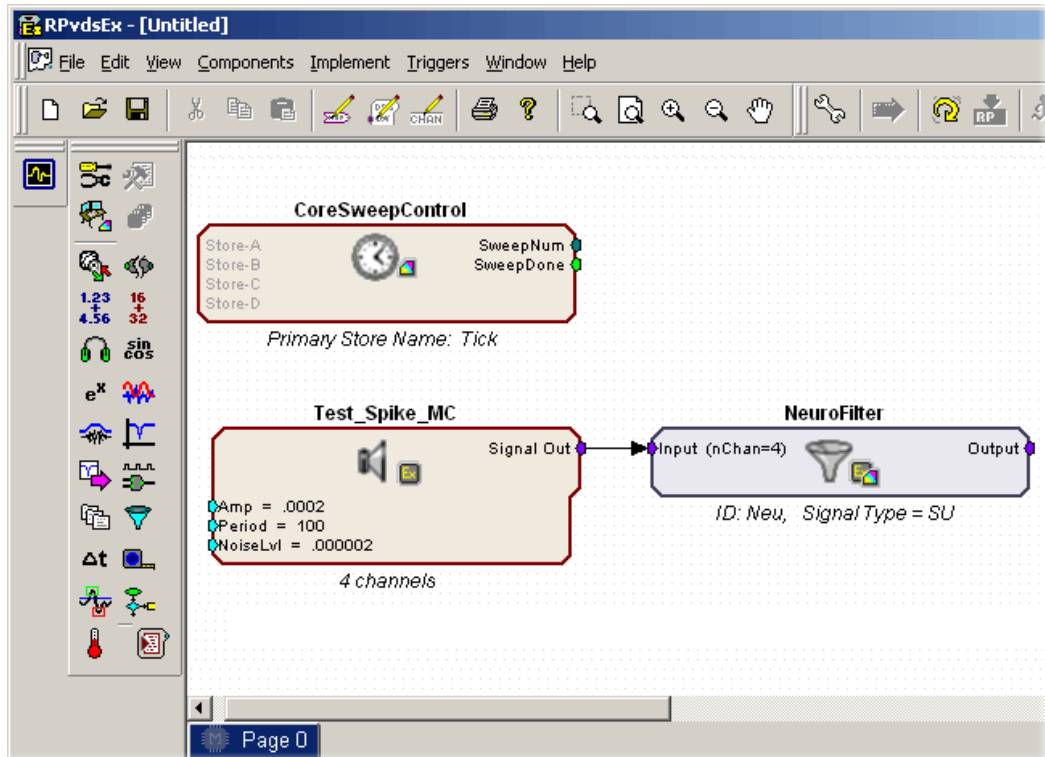


7. Finally, click **Done** at the bottom of the properties dialog.

The NeuroFilter macro should now display an input signal for 4 channel Single Unit filtering.

8. Connect the output of the **Test_Spike_MC** macro to the input of the **NeuroFilter** macro.

To link components, double-click the output of the first component and click the input of the second component.



When this circuit is loaded to OpenWorkbench control configuration information will be generated and fed to OpenController. Later in the tutorial, you will learn how to add the corresponding SpikePac Control.

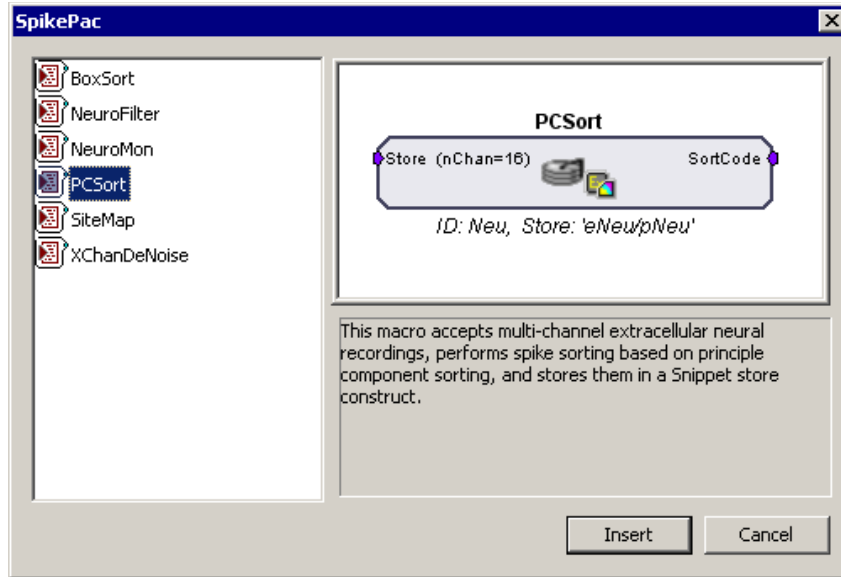
Data Saving and Online Sorting

The second real-time control element in this project is online spike sorting and is accomplished through the addition of the PCSort SpikePac macro. This macro provides support for storing, sorting, and time stamping spike snippet data streams as well as support for online principle component spike sorting in OpenController.

The macro generates two data Stores; a plot decimated streamed Store and a spike or snippet discrete waveform Store.

To add the PCSort macro:

1. Click  **Insert SpikePac Macro** button, select the **PCSort** macro.



2. Click **Insert** then click the workspace to add the macro.

By default, 16 channels are enabled when a PCSort macro is added.

3. Double-click the PCSort macro to display the set-up properties.
4. Click the **Setup** tab or click **Next**.
5. In the nChannels text box type 4 to set the number of channels, and click okay.

Note: for higher channel count applications you may need to distribute processing tasks across multiple processors. See the PCSort's **Store Pooling** tab for information about distributing data storage across processors by using multiple PCSort macros.

6. Connect the **Output** of the **NeuroFilter** macro to the input of the **PCSort** macro.

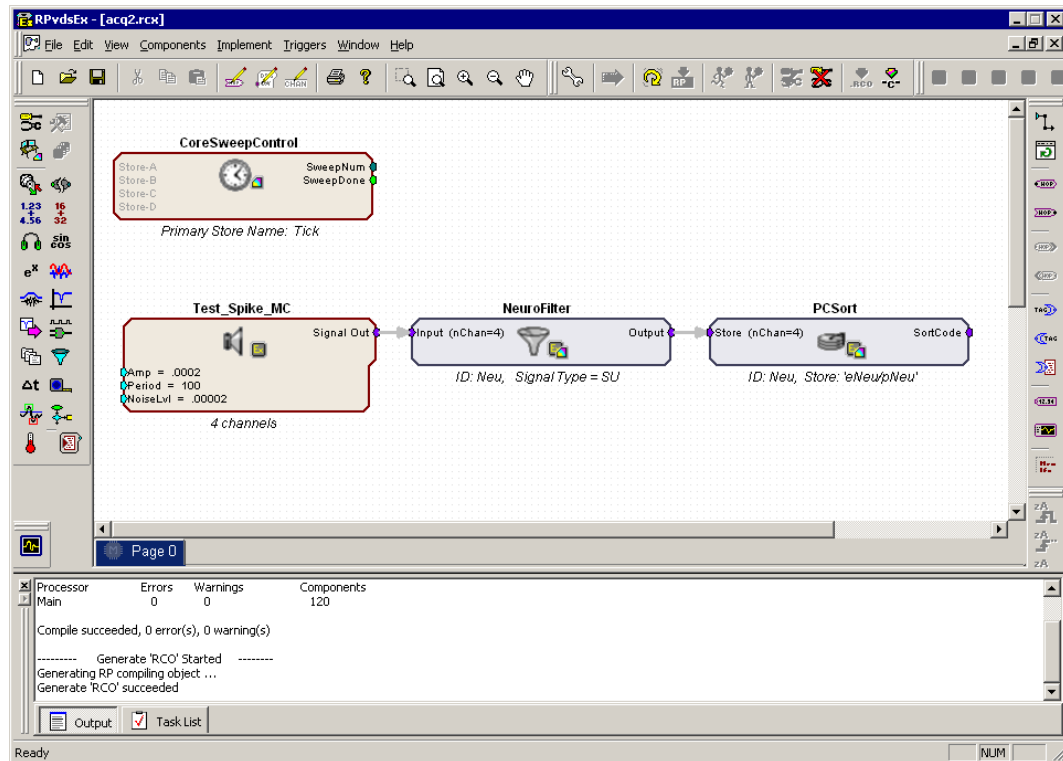
Saving the File

When the file is saved, the circuit file is compiled and any errors or warnings are displayed in the Messages window.

To save the circuit diagram and compile and store the processing chain in the circuit file:

1. Click the **File** menu and click **Save As**.
2. Enter **acq2** in the file name box and click **Save**.

Your circuit should appear similar to the illustration below.



Build the OpenWorkbench Experiment

OpenEx experiments are designed and run with the OpenWorkbench application. An OpenWorkbench configuration file can contain information about timing control and triggering, device properties, and data storage. When OpenWorkbench is run a new OpenWorkbench file is created automatically. The new file includes default settings for timing and triggering. By default, the zTrgA trigger, a zBus hardware trigger, is used to begin acquisition. The timing and control settings are available from the Setup menu.

In this experiment, data will be stored asynchronously. That is, snippets are stored whenever a spike is detected, while the plot decimated waveform is stored continuously. The CoreSweepControl macro handles all of the necessary timing signals for the PCSort stores. A sweep loop is used only to control the tick pulse (generated by the CoreSweepControl macro) that will be acquired and used as a reference epoch. The sweep count is set to -1, allowing the sweeps to continue indefinitely.

Next, you must configure the hardware device and data tank before data can be acquired.

Configuring the Processor

The next step in configuring the project is defining which hardware devices (RX5, RX6, etc...) are controlled from OpenWorkbench and what circuit file will be used to control the device.


To switch to OpenWorkbench:

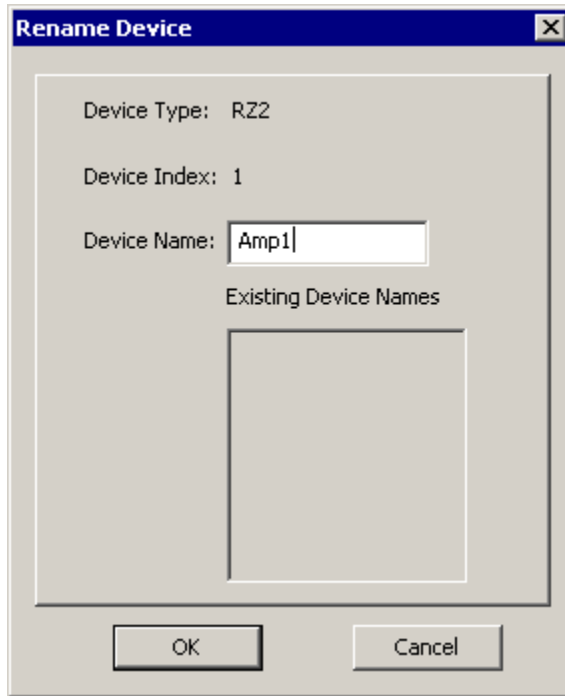
- Click the **Workbench** icon in the OpenProject window

In the OpenWorkbench window, the Device Navigator sub window displays all DSP modules turned on and connected to your PC.

Note: if you do not see your hardware devices in the Device Navigator, ensure they are turned on and connected then select **Reset Hardware** from the **Control** menu.

To configure the device:

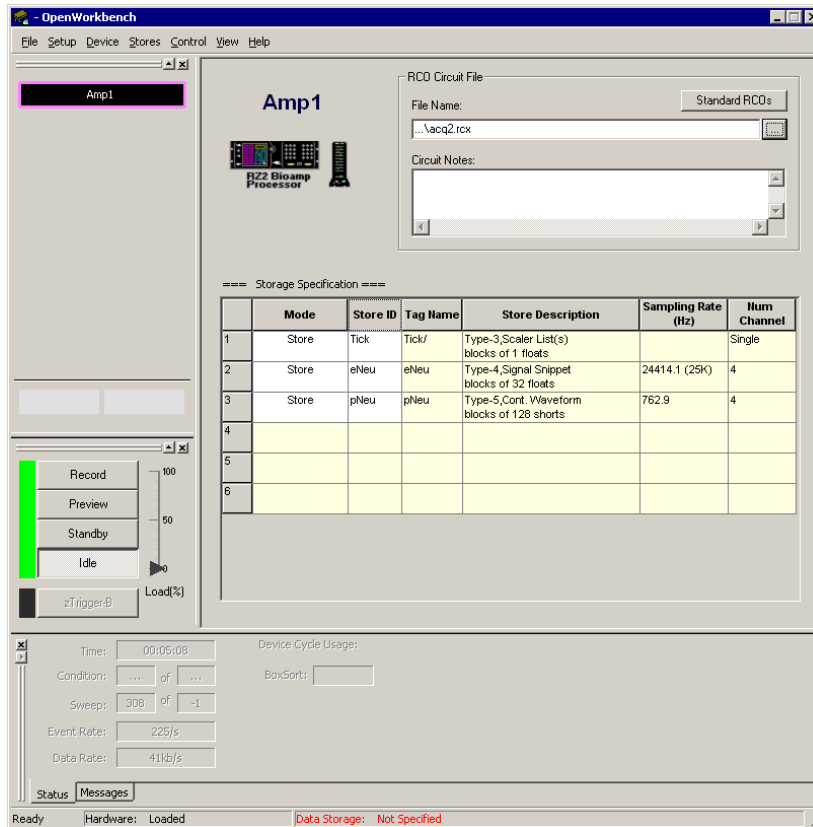
1. Select a device on which to run this example and click its gray icon in the device navigator. This will display the device configuration in the main part of the window.
2. Under RCO Circuit file, click the **Browse** button  to the right of the **File Name** box. The RCO File Select window opens to the RCOcircuits folder for the current project. Select the acq2.rcx you created.
3. Click **Open**.
4. You will be prompted to rename the Device. In the Device Name box, type **Amp1**.



5. Click **OK**.

By default, the OpenProject preferences are set to make a local copy of any compiled circuit files used by the project. That means that the file will be copied and stored with this project. This allows you to modify a local copy without altering the original file.

At this point, OpenWorkbench automatically loads the compiled circuit file, finds any data Stores included in the file, configures data storage parameters, and populates an editable Storage Specification table.



In the Storage Specification table, you can review information about the type of data that can be stored with the selected circuit file and enable or disable individual Stores.

The Storage Specification Table

In the Storage Specification table shown below, row 2 indicates that the compiled circuit file selected will store spike waveform data acquired from four channels at a ~25 kHz sampling rate. The tag name, eNeu, is set within the circuit file and is automatically generated by the PCSort macro. The Store shown in row 3 with the tag name pNeu stores the plot decimated waveform of our test signal. Although both types of data are recorded from the same source the lower sampling rate for pNeu reflects the decimation used with this type of data. The final store Tick, is added by the CoreSweepControl macro. This store will mark the sweep period defined in the Workbench by recording a pulse.

==== Storage Specification ====

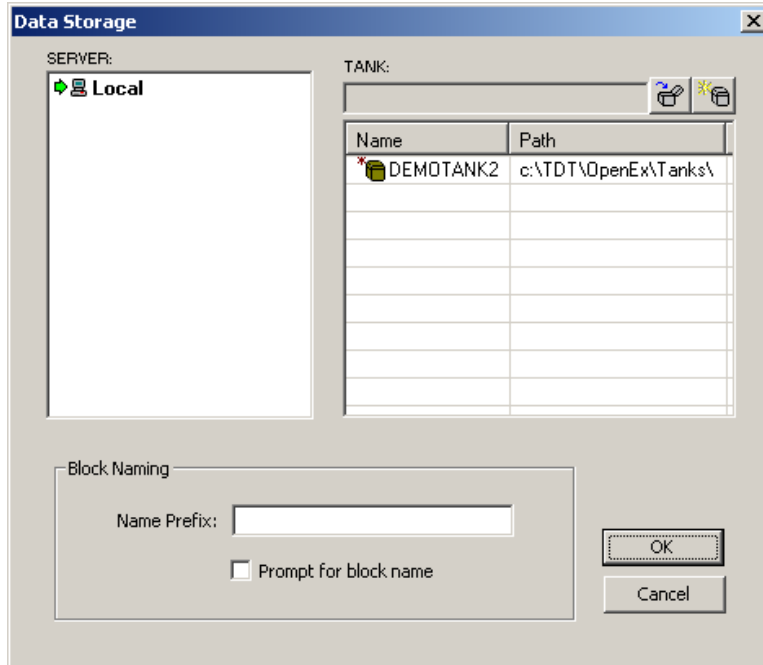
	Mode	Store ID	Tag Name	Store Description	Sampling Rate (Hz)	Num Channel
1	Store	Tick	Tick/	Type-3,Scaler List(s) blocks of 1 floats		Single
2	Store	eNeu	eNeu	Type-4,Signal Snippet blocks of 32 floats	24414.1 (25K)	4
3	Store	pNeu	pNeu	Type-5,Cont. Waveform blocks of 128 shorts	762.9	4
4						
5						

Selecting a Tank

Before data collection can begin, you must specify a data tank for data storage.

To specify a Tank:

1. Click the **File** menu, and click **Data Tank**. The Data Storage dialog box is displayed and any tanks that are registered on the selected server will appear in the list.



2. To add new data to the next block in the demo tank supplied by TDT, click DEMOTANK2 in the list and click OK.

Setup is complete and the Workbench controls are enabled.

Saving the Changes

The project file and applications files were created when the project was configured, however, you'll need to save the changes you've made.

To save the Project file and all application files:

- Click the **OpenProject** menu, and click **Save Project**.

When you are working in OpenProject, the save functions of the individual applications (such as OpenWorkbench and OpenController) are not available. Saving the project saves all of the project's application files.

Be sure to save frequently while you are working in the tutorial or when you begin to design your own experiments.

What's next?

So far you have learned to build a processing chain from the ground up and you've designed the experiment in OpenWorkbench. Now you are ready to build the real-time controls for this project. OpenWorkbench will remain open in the background while you switch to the OpenController application.

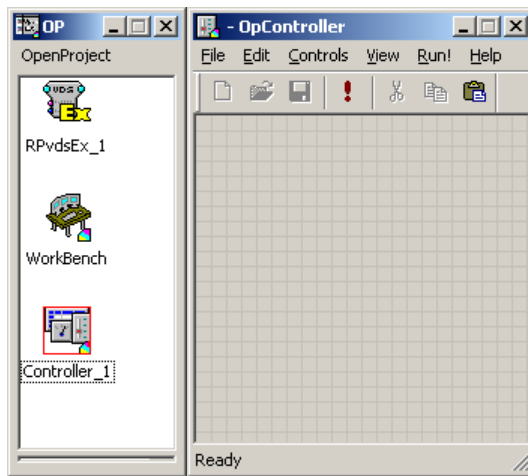
Adding the Real-time Controls

OpenController is a visual interface for implementing real-time control and data visualization for OpenWorkbench experiments. OpenWorkbench generates a map (in memory) of all the data Stores and accessible parameters (or parameter tags, often contained in macros and transparent to the user). As a client of OpenWorkbench, OpenController accesses this map to modify parameter variables and to read data for visualization. Since this map is updated in real-time (several times a second) changes to parameters occur in real-time and data displayed in OpenController is also displayed in real-time.

SpikePac controls are available when *SpikePac Macros* are contained in the compiled circuit file. In this tutorial, you will utilize SpikePac controls to setup a real-time control for thresholding, spike acquisition, and filter adjustments.

To switch to OpenController:

- Click **Controller_1** in the OpenProject window.



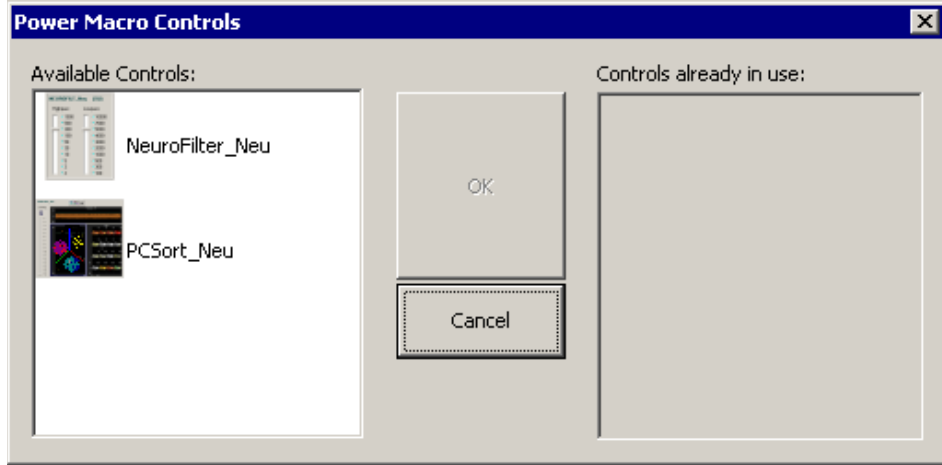
The OpenController window is displayed in Design mode. In Design mode you can add, configure, and modify controls.

Adding the PCSort Control for Online Spike Sorting

In this tutorial a PCSort control set is used to visualize the plot decimated and snippet waveforms as well as to adjust the spike threshold and assign sort codes to spikes.

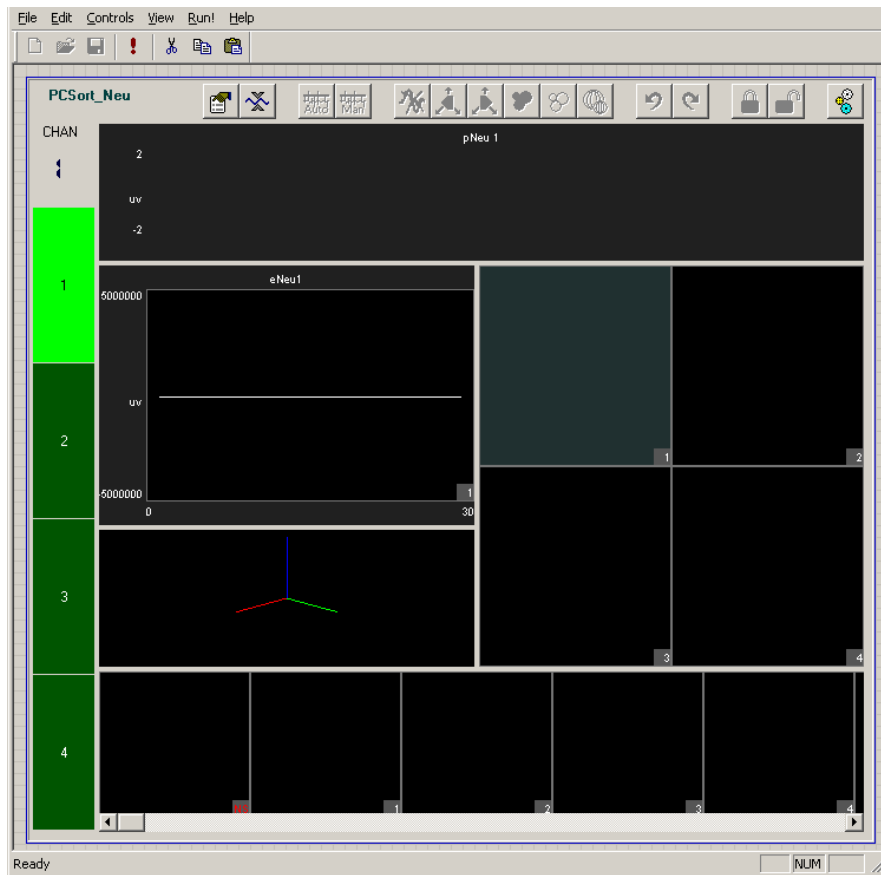
To create the control set:

1. Click the **Controls** menu and click **Power Macro**.



When SpikePac macros are used in the circuit a control for each macro will be displayed in the Available Controls list.

2. Click **PCSort_Neu** in the list and click **OK**.
3. Click the workspace to place the control set. Standard click and drag techniques can be used to resize or move the controls.

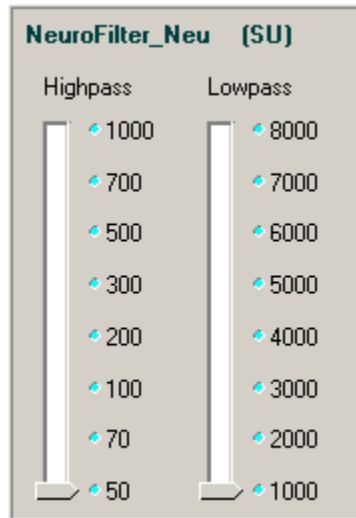


Note: double-clicking any control will display additional options for the control component if available.

Adding the NeuroFilter Control for Signal Filtering

To place the filter control:

1. Click the **Controls** menu item and click **Power Macro**.
2. Click **NeuroFilter_Neu** in the **Available Controls** list and click **OK**.
3. Click the grid to place the component on the work space.



Running the Experiment

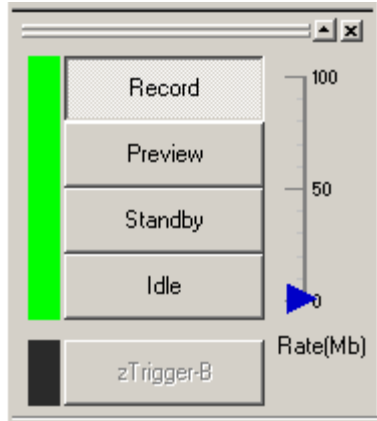
Experiments can be run from the OpenWorkbench System Control window. That means you'll need to switch between applications as you control the experimental protocol from OpenWorkbench and implement the real-time control of experimental parameters in OpenController.

You can switch between applications by clicking the application's icon in the OpenProject window.

Beginning the Experimental Protocol


To begin data acquisition:

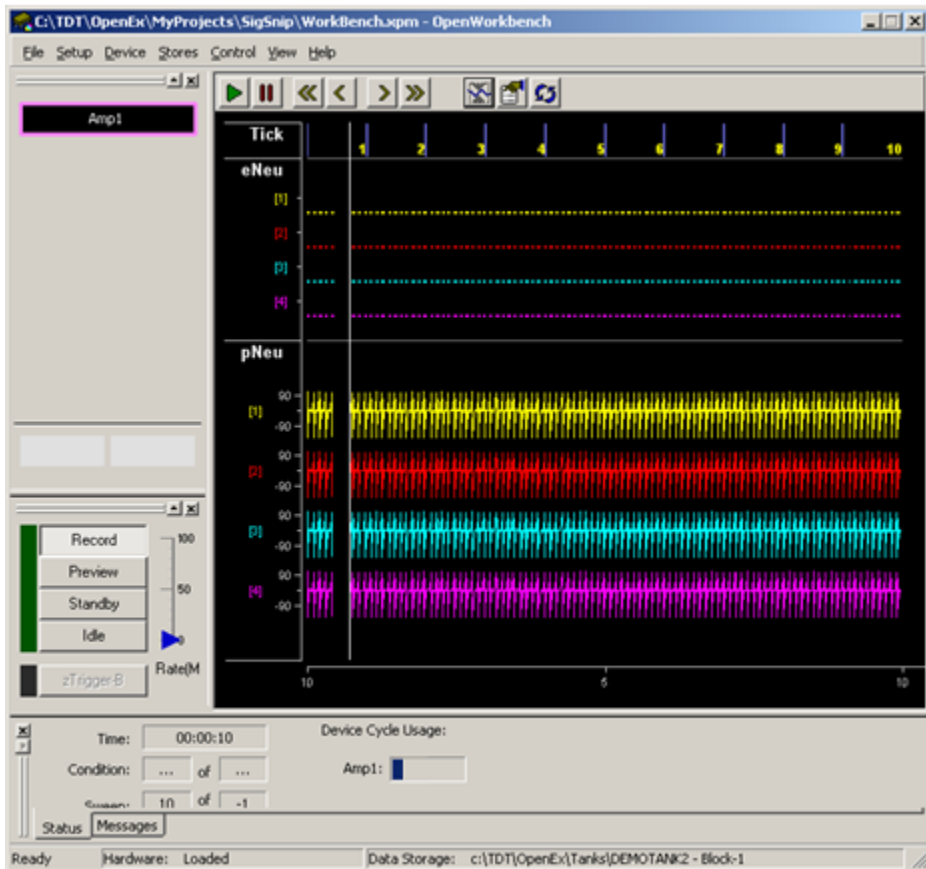
1. Switch to **WorkBench**.
2. Click the **Record** button in the System Control window. Acquired data is now being stored to the tank.



The arrow to the right of the control buttons indicates the rate of events being stored. It changes color from blue, to amber, then red to alert you when the event rate is high.

The bar to the left of the buttons will flash red when errors occur.

- To scale and view the data in the plot window, click the  **Auto Scale** button located at the top of the plot window.




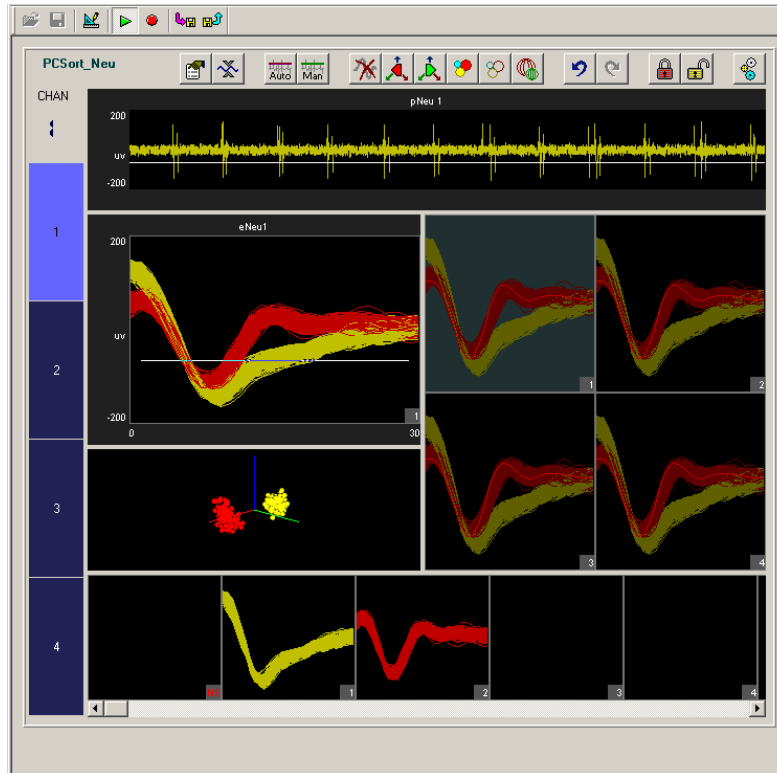
Your project should now appear similar to the illustration above, with the timing and sweep number of the Tick onset epoch shown across the top of the plot, and the four channels eNeu[1 - 4] displaying a histogram of the candidate spikes, and pNeu[1 - 4] displaying the plot decimated spike waveforms.

Running the OpenController Control Set

Upon entering Preview or Record mode in Workbench, the controls will initialize to the correct settings defined in your circuit file, but you'll need to switch to OpenController to run the controls.

To run the real-time controls:

1. Click **Controller_1** in the OpenProject window to switch to OpenController.
2. Click the **Toggle Mode**  button on the OpenController toolbar. OpenController switches to Run! mode and the controls are automatically run.




With the project running you have an opportunity to test your system and practice spike sorting. Normally this might be done in Preview mode so that the signals acquired would not be permanently stored as data. In this tutorial, however, you are recording the fake data for demonstration purposes.

See the *PCSort* section on page 7, for more information on using the PCSort control set.

Stopping the Experiment

In this project stopping the experiment involves two steps: stopping the controls and stopping the protocol.

To stop the controls:

- Click the **Stop**  button on the OpenController toolbar.

To stop the experiment:

- Switch to OpenWorkbench and click the **Idle** button in the System Control Window

Wrapping Things Up

The project file and application files were created when the project was configured, however, you'll need to save the changes you've made.

To save the Project file and all application files:

- Click the **OpenProject** menu, and click **Save Project**.

Once an experiment has been designed and saved as a project, you'll be ready to run the experiment at any time simply by opening the project file.

What's Next?

In this tutorial you created a project from the ground up using RPvdsEx macros and the SpikePac tool set. The OpenEx help file includes reference books for each of the OpenEx applications. The reference books provide more information about the workspace and settings in each application as well as step-by-step instructions for performing many tasks.

The RPvdsEx User Guide is also a good resource for circuit design. Be sure to explore the Multi-Channel Circuit Design and Multi-Processor Circuit Design sections of the manual.

Glossary

Bayesian

The Bayesian algorithm provides fully automated sorting. With this algorithm, OpenController evaluates the specified sorting Feature Space of the data set and automatically computes the number of units present in the data and the waveforms that comprise those units. Initially, the entire data set is treated as one parent cluster. This is split to form two child clusters in an iterative process that continues as long as the Bayesian Information Criterion (or BIC score) of the split data set is better as a result of the split and the distortion statistics calculated for the children are not scaled chi-squared distributions. More information regarding the Bayesian algorithm can be found in the *References* listed on page 97.

Convolution

A mathematical operation that produces a moving average of two functions by computing the integral of the product of the two functions after one is reversed and shifted.

Eigenvector

A special vector that is used in linear transformations to map a scalar to a span called the eigenspace.

Feature Space

An abstract space where each event is represented as a point in n-dimensional space. Each measurement ("feature") about the event gives the coordinate of the point along one axis of the space. The dimensionality of the feature space is equal to the number of features used to describe the event.

KMeans

The K-means algorithm is a semi-automated sorting method. The only input required from the user is the desired number of clusters that the data set is to be divided into. A binary split algorithm uses this number as an input and attempts to find the optimum locations of the cluster centers using an iterative process. Data points are then assigned to those clusters based on either their distances away from the cluster center (smallest value) or their probabilities of being allocated to each of the clusters (largest value). More information regarding the KMeans algorithm can be found in the *References* listed on page 97.

Linear Regression

A statistical method which attempts to relate two variables, such as the relative noise of a channel and its signal by fitting a linear equation between the two.

Principal Component

A multi-dimensional representation of the data set where the first principal component is that parameter that represents the maximum variance in the spike shapes; the second principal component represents the next highest variance in the spike data and so on. Transforming spike data in terms of its principal components allows representing the data in parameters that best describe the differences within the data set while also reducing its dimensionality. The number of principal components that can be obtained for a data set equals the number of sample points in each waveform. However, for data sets with well-defined spike shapes, the first two-three principal components represent a substantial majority of the variance in the data set.

Keyboard Shortcuts

The keyboard shortcuts below allow quick keyboard access to the mouse tools for viewing and working with data.

Arrow Keys	Adjust switches that have current focus.
Alt + Click and Drag	Pan Plots.
Shift + Click and Drag	Zoom Plots.
Shift + Double Click	Reset Plot Position (resets pan and zoom changes).
Ctrl + Double Click (on Waveform Space)	Add a new box pair in the waveform space (BoxSort).
Ctrl + Double Click (on Threshold Display)	Toggles threshold tracking (BoxSort and PCSort).
Ctrl + Click and Drag	Manually Sort Waveform Space or Feature Space (PCSort).
Ctrl + Spacebar	Toggle Design/Control mode for OpenController.

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6. Bolshakova N., Azuaje F.: "*Cluster validation techniques for genome expression data.*" *Signal Processing* (2003) 825-833.
7. Wheeler B.C., "*Automatic Discrimination of Single Units in Methods for Neural Ensemble Recordings,*" ed. By Nicolelis, M., CRC Press, Boca Raton, 1999.