

# **SpikePac User's Guide**

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SpikePac User's Guide

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# Before You Begin:

## *Installation*

Installing SpikePac enables special features in OpenEx and RPvdsEx, notably, adding an  *Insert SpikePac Macro* button to the RPvdsEx toolbars. TDT Drivers and the OpenEx Suite must be installed before installing SpikePac.

The recommended operating system for all TDT systems is 32-bit Windows XP®.

## *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.

## *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.*

## *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 detailed guide for the *SpikePac Macros* and control sets provided by SpikePac.
- **A Tutorial**  
A step-by-step tutorial for building an OpenEx Project using *SpikePac Macros*.
- **Glossary**  
A convenient listing of the common 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 R PvdsEx 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:***

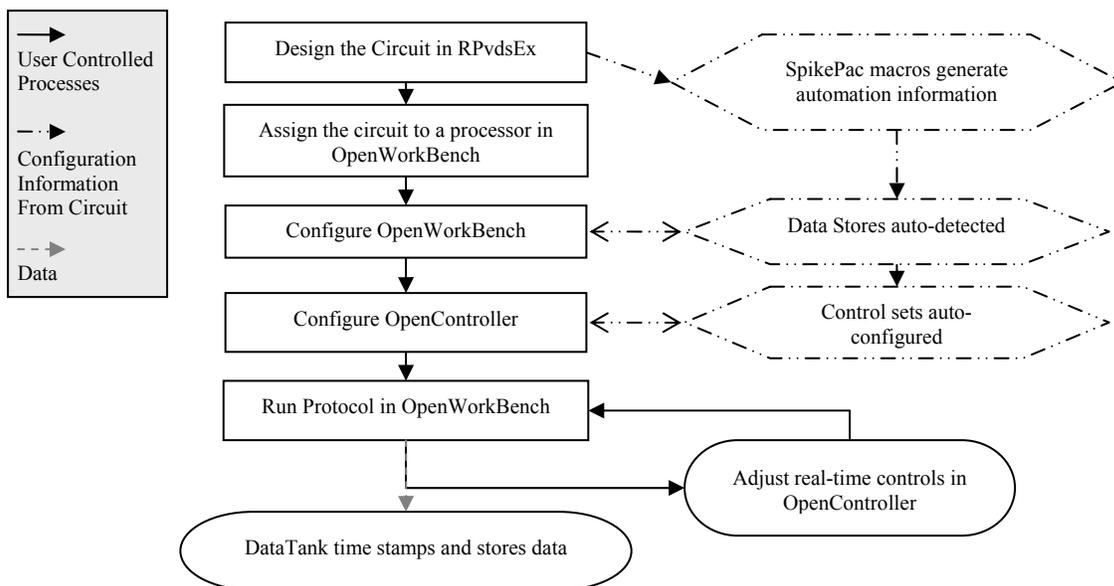
- Principal Component Feature Space Spike Sorting
- Real-Time, Time-Voltage Spike Sorting
- Filtering
- Signal Monitoring with Noise Gating
- Signal Denoising
- Electrode Site Remapping

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 R PvdsEx 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**



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# The Tools

## Overview

### SpikePac Macros

When OpenEx's Workbench 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 WindowSort 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 31, 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, channel-monitoring, or noise removal. 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.

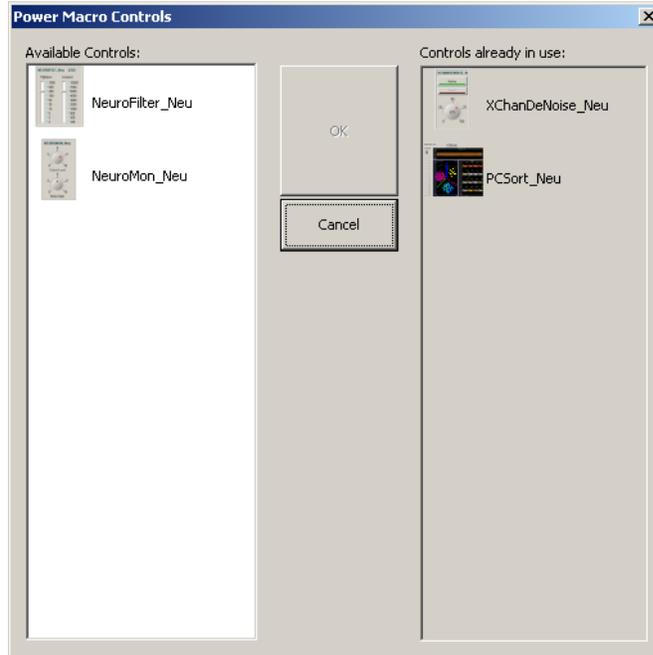
### Adding a SpikePac Control in OpenController

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. The time stamp and position of the waveform in the snippet is dependent on the time of the threshold crossing for the signal.

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, 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

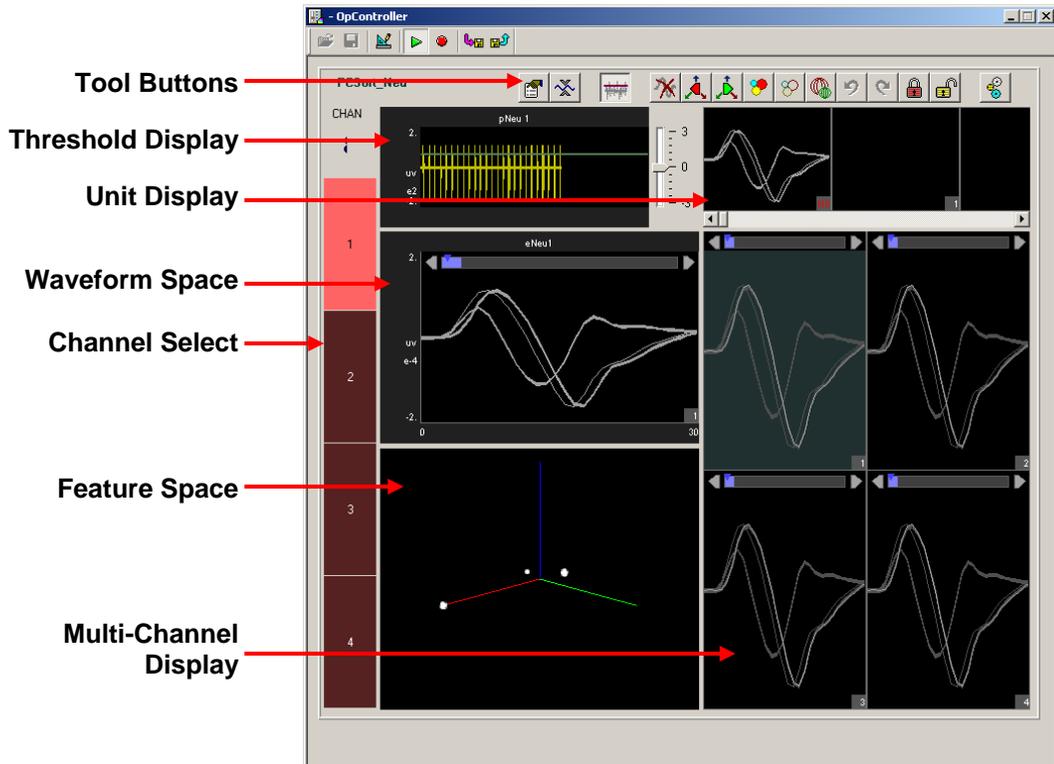
The next phase begins when clustering is initiated. Preliminary identification of units is indicated by color coding in the control's displays for visualization; however, all candidate spikes are saved to the data tank with a sort code of 0 during this phase. The default clustering method is a Bayesian 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, training termination control, sorting algorithm, and auto-clustering can all be modified by the user in the control's Setup Properties.

## The Control Set User Interface



*The Control Set window includes:*

- Channel Select** selects the active channel and displays channel status.
- Threshold Display** displays the plot decimated waveform of the currently selected channel.
- 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.
- 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.
- 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.
- Tool Buttons** provide access to tools and parameters.

**To adjust the scale of any plot:**

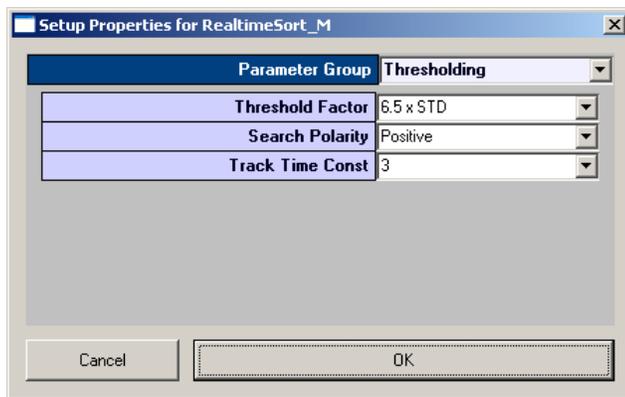
- Point to the center of the plot, press and hold down the Shift key, and drag the mouse up or down.
- Click the **Auto Scale** button  to auto scale all plots. This will not auto scale the feature space

**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 the threshold for spike detection to a value which is  $n * \text{STD}$  of the signal RMS.

**Search Polarity** – Select a positive or negative polarity for the threshold value.

**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.

**Training Control Parameter Group**

**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.

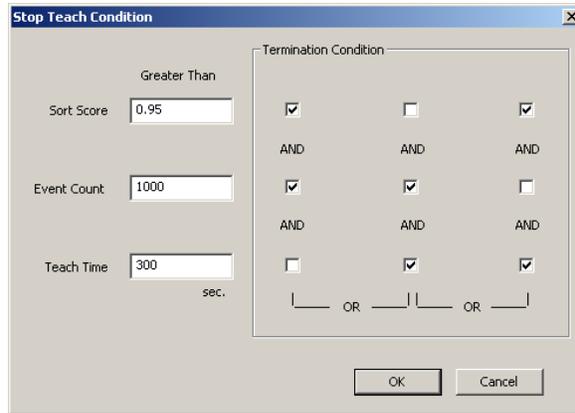
Click the  Browse button to open the Stop Teach Condition window, which allows users to create a logic statement that is used to evaluate and apply specified criteria.

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.

**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).

**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 or 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 KMean Num Clusters).

*See page 55 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 the KMeans clustering method 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.

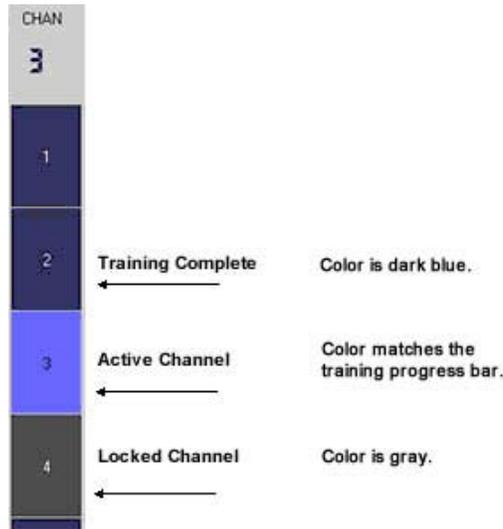
**Selecting the Active Channel**

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.

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

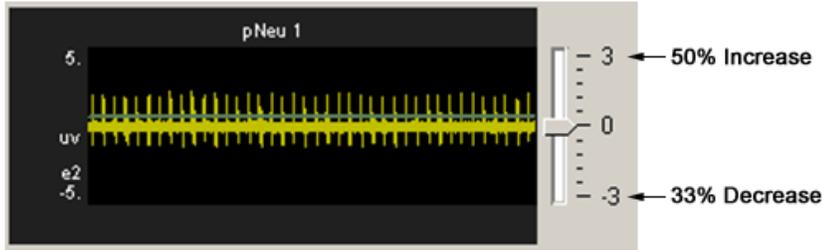


The color of the channel selector indicates the status of each channel. When training is active and the feature space is being calculated, the colors will match the training progress bar. The selector appears blue once the training is complete or if the feature space is accepted. If a channel is locked, the color on the selector will be shown as gray. *For more information about the training period, see **Calculating the Feature Space**, page 12.*

**Using the Threshold Control**

The top left plot (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).

The slider bar to the right of the plot allows the user to adjust the threshold by a +/- 3dB deviation from the base threshold value.



*To change the base threshold:*

Click the **Setup Properties**  button and select a new Threshold Factor in the Thresholding parameter group. You can also change the polarity of the Threshold in this parameter group.

### Threshold Tracking

PCSort also includes threshold tracking. If tracking is enabled, the threshold adjusts to changes in the active channel waveform's RMS. Disabling tracking will lock the threshold at a fixed voltage.

**Note:** While threshold tracking is enabled, the default time constant ( $\tau$ ) for calculating the RMS is five seconds.

*To toggle threshold tracking off or on:*

- Click the **Track Threshold**  button.

## Calculating the Feature Space

When the control is run a *training period*, during which the feature space is calculated, begins. 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 progress bar (shown below) is added to many of the plots. A pointer moves along the bar to show progress towards the maximum time for training and a colored bar.



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 side 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 are met:*

- The termination condition is satisfied (*see pg 9*).
- The feature space is manually accepted by the user.

When the training period is ended, automatic or manual clustering 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, then click **Yes** when prompted to confirm.  
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 throughout 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.

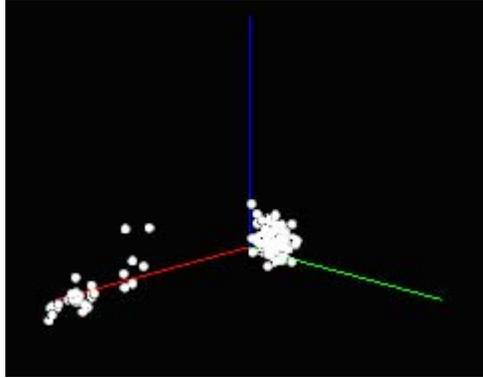
**To begin saving sort codes to the data tank:**

- 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.

## 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 mouse button and drag the feature space from the desired angle.

### *To pan the feature space:*

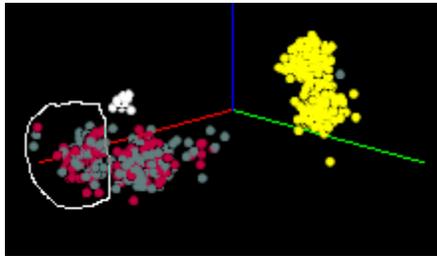
- Press and hold the **Alt** key on the keyboard and drag the mouse around the feature space.

## 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.

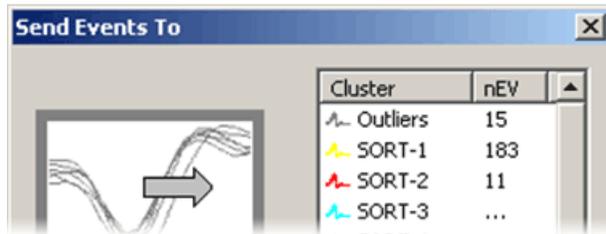
### *To assign events to clusters manually:*

- 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.

- Select the desired Sort Code.

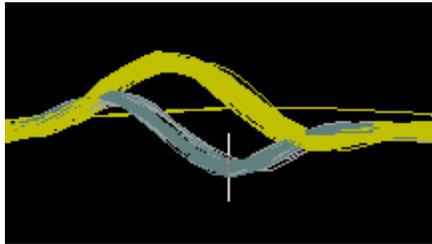


### Defining Clusters Manually in the Waveform Space

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.

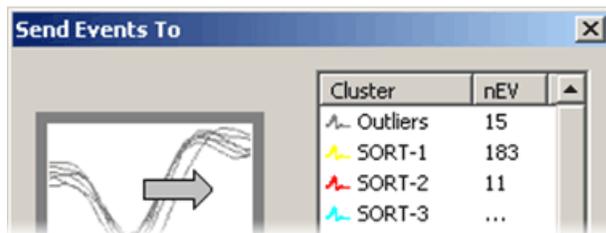
*To assign events to clusters manually:*

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



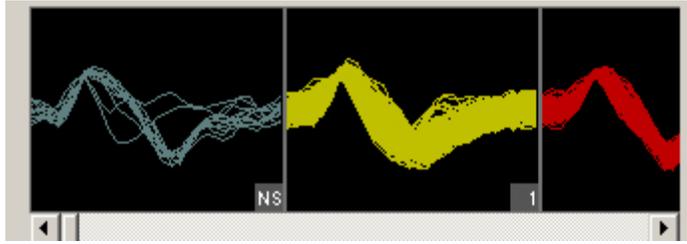
When the mouse button is released, the **Send Events To** dialog opens. The dialog box lists all possible Sort Codes as well as the number of events currently comprised by each Sort Code.

- Select the desired Sort Code.



- 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.

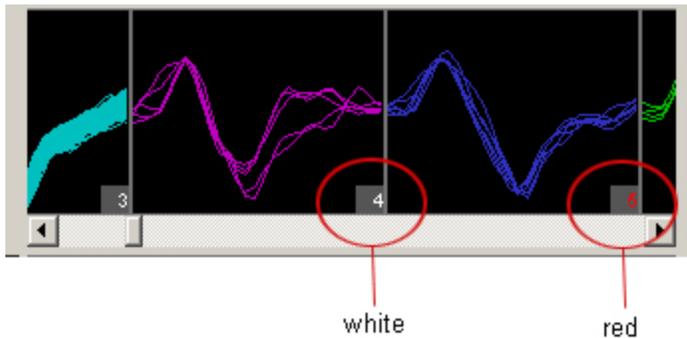
## The Unit Display



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.

However, the "maximum number of clusters" parameter in the PCSort macro determines the maximum number of sort codes (up to 5) that can be stored per channel. 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).



## Toolbar 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 all plot displays. This does not auto scale the feature space.



**Undo**

Undo previous action.



**Redo**

Redo an undone action.

	<b>Track Threshold</b>	Enables or disables auto-tracking for the auto-threshold bar.
	<b>Clear All History</b>	Clears event history for all channels.
	<b>Recalculate Feature Space</b>	Recalculate the feature space for all channels depending on the training termination conditions (recalculates the eigenvector to determine the mapping for the feature space).
	<b>Accept Feature Space</b>	Accepts the current feature space for all channels (uses the currently calculated eigenvector for all channel principle component mapping to the feature space).
	<b>Calculate Clusters</b>	Automatically stops training, accepts the feature space, and clusters the existing data for all channels.
	<b>Clears Clusters</b>	Clears clusters for all channels and removes color coding (also removes spheres).
	<b>Spheres</b>	Displays the boundaries of spheres used to define cluster shapes in the feature space.
	<b>Lock</b>	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.
	<b>Unlock</b>	Unlocks the controls mentioned above after they have been locked.
	<b>Apply Sorts</b>	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 for the active 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 **Training Control Parameter Group**, page 9, for more information.*

**Restart Space Calculation** - Restarts the space calculation for the selected channel. Only available until the training time is completed.

**Accept Space** - Accept the selected channel's feature space regardless of the training status.

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

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

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

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

# WindowSort - Time-Voltage Spike Sorting

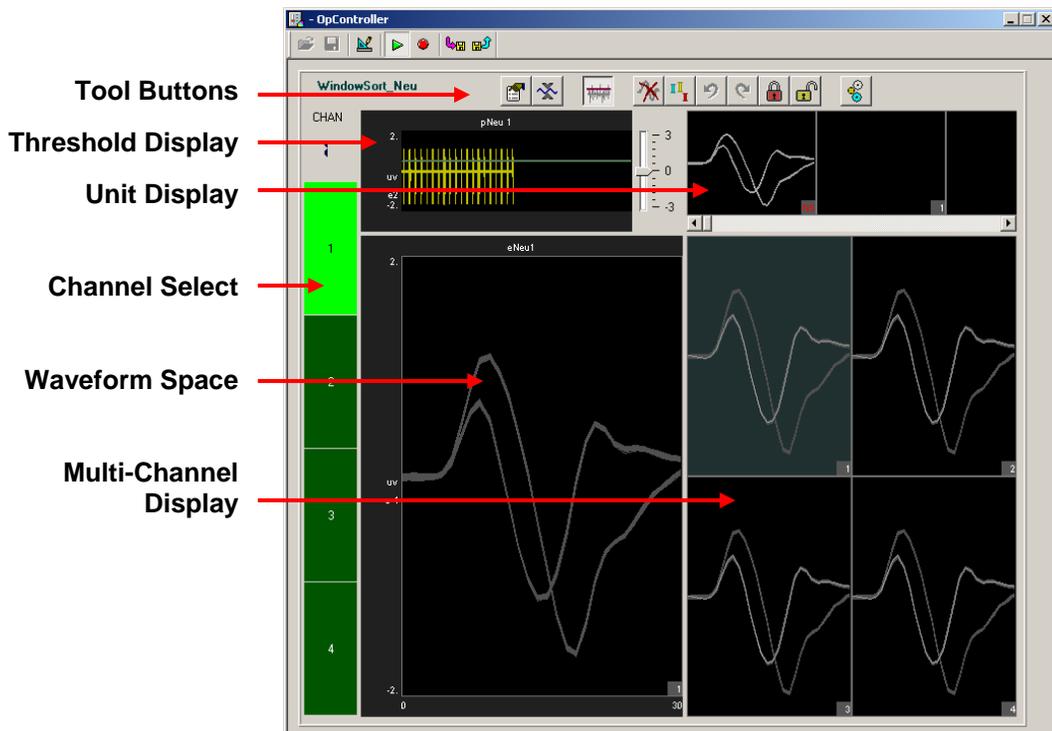


The WindowSort macro is used to time stamp and store waveform events as data snippets and support online spike sorting through the WindowSort control in OpenController. Candidate waveforms are detected when the rising edge (positive waveforms) or falling edge (negative waveforms) of the waveform crosses a threshold voltage automatically set during acquisition. The time stamp and position of the waveform in the snippet is dependent on the time of the threshold crossing.

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 and window width for each snippet stored are defined in the macro properties dialog box. *See the onboard macro help for more information.*

The WindowSort control set can be used to sort spikes online when the WindowSort 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:*

- Channel Select** used to select the active channel and display status information about the channels.

<b>Threshold Display</b>	displays the plot decimated waveform of the currently selected channel.
<b>Waveform Space</b>	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.
<b>Unit Display</b>	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.
<b>Multi-Channel Display</b>	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.
<b>Tool Buttons</b>	provide access to tools and parameters.

Once sort codes are assigned, each sort code (or “unit”) is represented by a single color throughout all plots.

*To adjust the scale of any plot:*

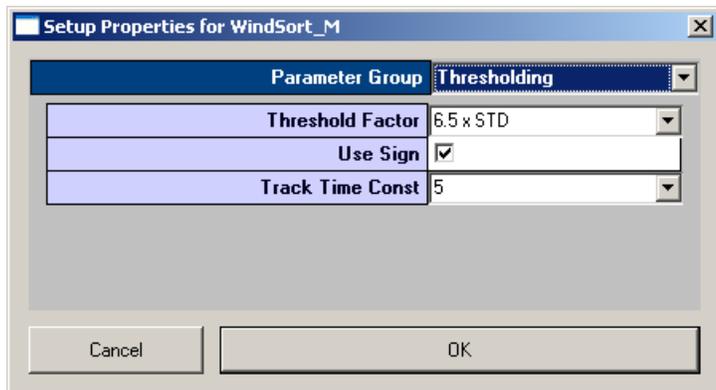
- Point to the center of the plot, press and hold down the Shift key, and drag the mouse up or down.
- Click the **Auto Scale** button  to auto scale all plots.

## The Setup Properties

Users can configure the thresholding control and enable or disable the use of bi-directional thresholding by selecting or deselecting the **Use Sign** check box.

*To open the setup properties:*

- Click the **Setup Properties**  button.



### Thresholding Parameter Group

**Threshold Factor** – Sets the auto thresholding bar to a value which is  $n * \text{STD}$  of the signal RMS.

**Use Sign** – Select whether the auto-threshold bar uses/ignores the sign of the RMS value for thresholding.

**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.

### 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.

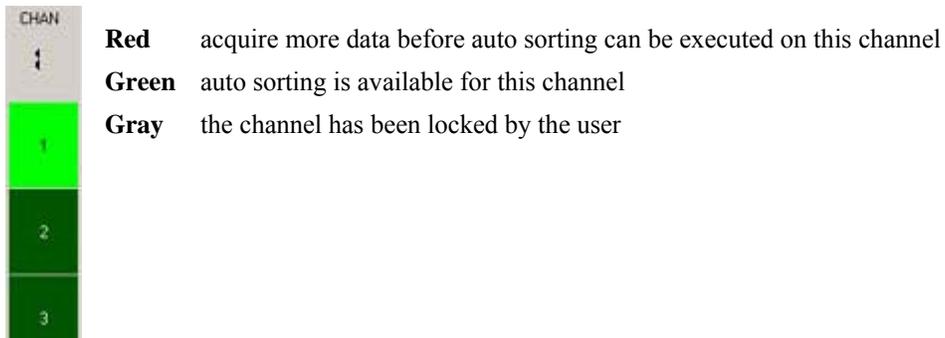
**To set a channel as active:**

- 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:**

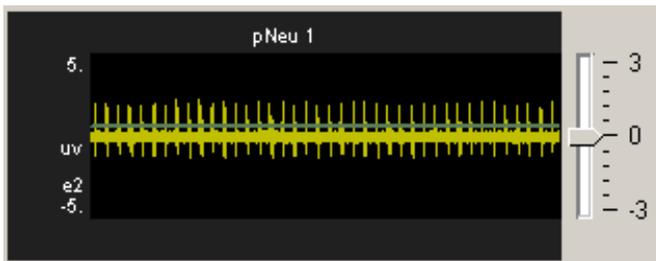
The color of the selector switch displays the status of each channel.



### Scrolling Threshold Plot

The top left plot (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).

The slider bar to the right of the plot (shown above) allows the user to adjust the threshold by a +/- 3dB deviation from the base threshold value.



**To change the base threshold:**

Click the **Setup Properties**  button and select a new Threshold Factor in the Thresholding parameter group. You can also choose to enable bi-directional thresholding in this parameter group.

### Threshold Tracking

WindowSort also includes threshold tracking. If tracking is enabled, the threshold adjusts to changes in the active channel's waveform's RMS value. Disabling tracking locks the threshold at a fixed voltage.

**Note:** While threshold tracking is enabled the default time constant ( $\tau$ ) associated with the RMS is five seconds.

*To toggle threshold tracking off or on:*

Click the **Track Threshold**  button.

### Calculating Sorts Automatically

Time-voltage windows may be automatically applied to all channels and represent a given voltage range at a specific time and are used to sort spike snippets that lie in their range. Time-voltage windows are added according to a sorting algorithm that sorts snippets from the current event history.

Clicking the Auto-Sort button will re-calculate the placement of time-voltage windows and will remove existing time-voltage windows that are no longer needed.

*To calculate Sorts for all channels:*

- Click the **Auto-Sort** button  and click **Yes** when asked to confirm.

### 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.

### 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, then click **Yes** when prompted to confirm.

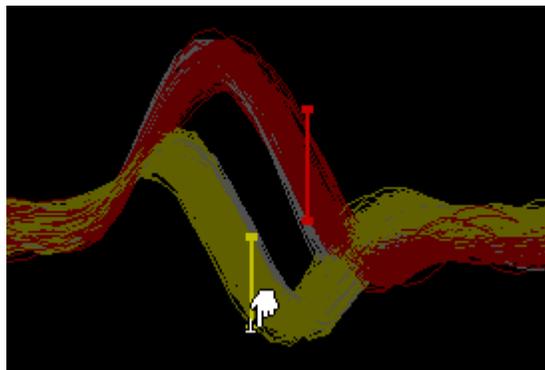
or

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

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

## Calculating Sorts Manually in the Waveform Space

The waveform space (shown below) displays all spike waveforms for 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 re-assigning units after automated sorting.



### *To manually adjust the time voltage windows:*

- Use standard click and drag techniques to adjust the markers (and thereby the time-voltage window).

### *To add a window:*

1. Hold Ctrl + drag a cut across the desired waveform.  
Upon releasing the left mouse button the **Send Events To** dialog box opens.
2. Select the desired sort code. A time-voltage window will be automatically added to the waveforms intersecting the cut.

or

- Alt + Double-click the waveform space.

Sort codes are automatically applied to the newly added time-voltage window and are auto incremented by the control.

Up to a maximum of ten sorts per channel are permitted.

### *To remove a window:*

- Drag the marker out of the plot and click **Yes** when asked to remove hand mark.

### *If a waveform passes through more than one time voltage window:*

- Sort codes are assigned based on how close a detected spike is to the center of a window marker.

## Toolbar Buttons

The buttons along the top of the control set provide the following additional commands:

	<b>Properties</b>	Open the Setup Properties dialog box.
	<b>Auto Scale</b>	Auto scale all plot displays.
	<b>Track Threshold</b>	Enables or disables auto-tracking for the auto-threshold bar.
	<b>Clear All History</b>	Clears event history for all channels.
	<b>Auto Sort</b>	Auto-sort the signal snippets based on time-voltage windows.
	<b>Undo</b>	Undo previous action.
	<b>Redo</b>	Redo an undone action.
	<b>Lock</b>	Lock sorting related actions from the user. Window resizing and channel selections are still allowed.
	<b>Unlock</b>	Unlocks the sorting related controls.
	<b>Apply Sort</b>	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:

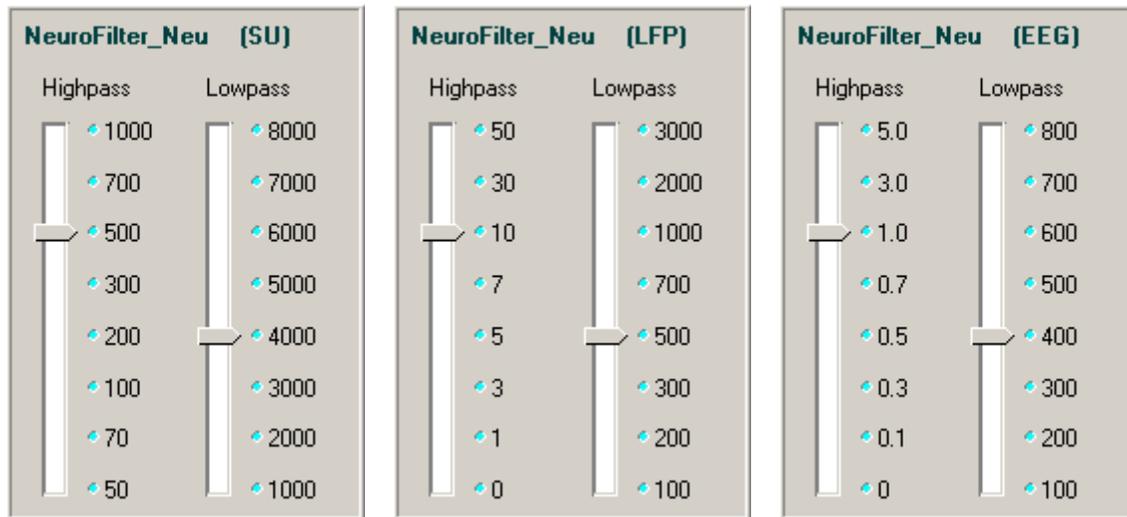
- Auto sort the current channel** – Select to sort data for the selected channel. Automatically analyzes existing data and applies time-voltage windows for display purposes. The windows will not be implemented in the processing chain running on the hardware until the Apply Sort button is selected, that is, new sort codes will not be stored to the data tank.
- Clear the current channel's sorts** – Select to clear any sorting 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.
- Clear the current channel's data 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.

## 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 AC couples (removes the DC offset) the multi-channel signal.



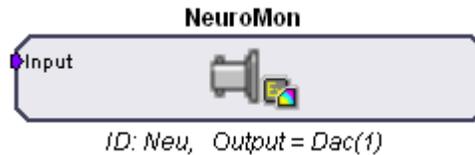
### Modifying the Corner Frequencies

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.

## Signal Monitoring with Noise Gating



### NeuroMon

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 1. However, the control is designed for use with the WindowSort 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.

## Signal Denoising



### XChanDeNoise

The XChanDeNoise macro supports the real-time implementation of a de-noising function through the XChanDeNoise control in OpenController. The macro acts as a pass through for multi-channel data until the user has enabled the de-noising function in OpenController. Once enabled, noise common to all channels is subtracted from each channel. Settings for configuring the number of channels and the teaching time are defined in the Setup tab of the macro properties dialog box. *See the onboard macro help for more information.*

The XChanDeNoise control can be used to enable and control denoising across all channels. This control is available when the XChanDeNoise macro is included in the compiled circuit file. During a user defined teach time data is sampled and the XChanDeNoise control uses a two stage multiple linear regression function to determine the weighted noise common to all channels. The weighted noise is compiled into a matrix which is used to scale the multi-channel signal, effectively subtracting the weighted noise from each channel.

### De-Noising the Signal

The control set includes two buttons and one knob control.

The **Active** button toggles the de-noising function on or off; however, the Teach function must be used before de-noising can be activated.

The **Teach** button toggles a teaching function on and off. Toggling Teach on temporarily buffers the signal allowing it to be used to predict the best representation of the common noise, which can then be used to clean each individual channel when de-noising is active.

The "teach time" is determined within the XChanDeNoise macro and the Teach function automatically toggles off when the teach time is completed. The Teach button can also be used to toggle off the teach function at any time.

When de-noising is active, the **knob** can be used to adjust the depth of the matrix (as a percentage) used to de-noise the signal.

#### **To Activate the Multi-Channel De-Noising Function:**

- Click the **Teach** button.

The control will then begin to sub-sample the multi-channel signal in order to find the noise common to all channels. Once complete, the **Active** button becomes enabled again.

- Click the **Active** Button to enable the de-noising function.
- Drag the **Knob** to the desired depth percentage.

## 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 30.*

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.

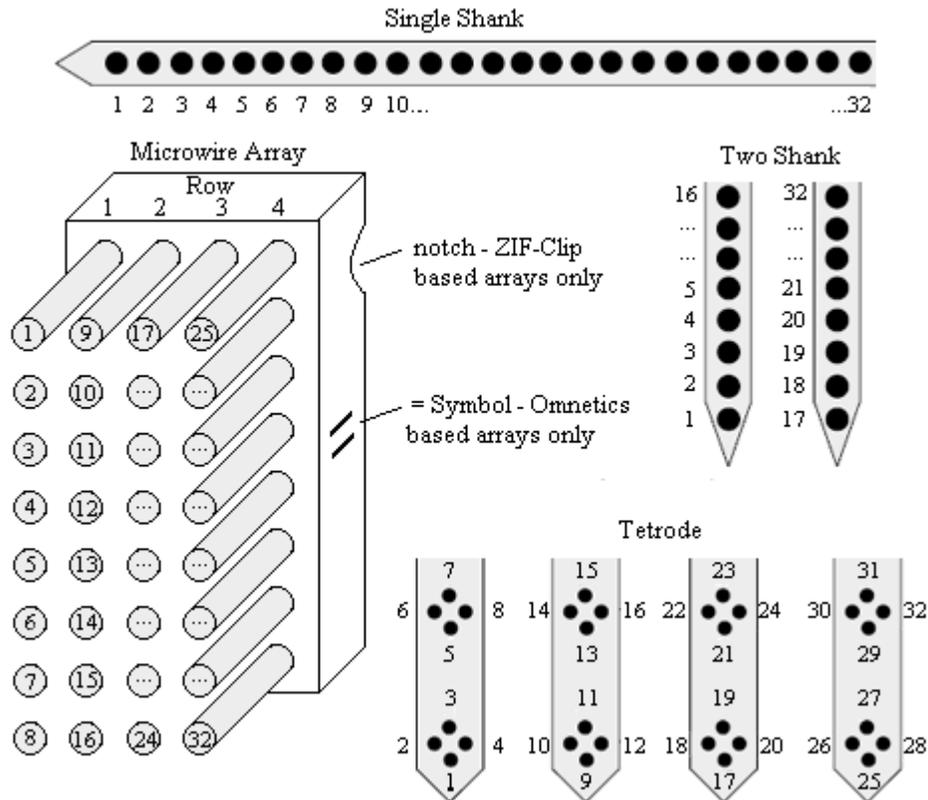
#### *To Assign a Mapping Configuration:*

- If necessary, click the **Download new specification** button to update all pin configurations.
- Click the **Browse**  button to the right of each text box. Select the desired Headstage, Adapter, and Electrode from the list then click **OK**.

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 dependant 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:***

- Acquiring plot decimated and snippet type data.
- Using macros to auto-configure real-time control sets.
- Adding real-time control with OpenController.
- Using OpenController and SpikePac control sets for online spike sorting.

## **Planning the Project**

### ***When you plan a project you need to:***

- Determine what type of data will be acquired.
- Determine what device(s) you will use.
- 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: WindowSort 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 use the WindowSort macro for real-time, time-voltage window 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 WindowSort 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). A Pentusa BioAmp System would be a good choice for data acquisition.

If you do not have a Pentusa in your system, you can still 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).

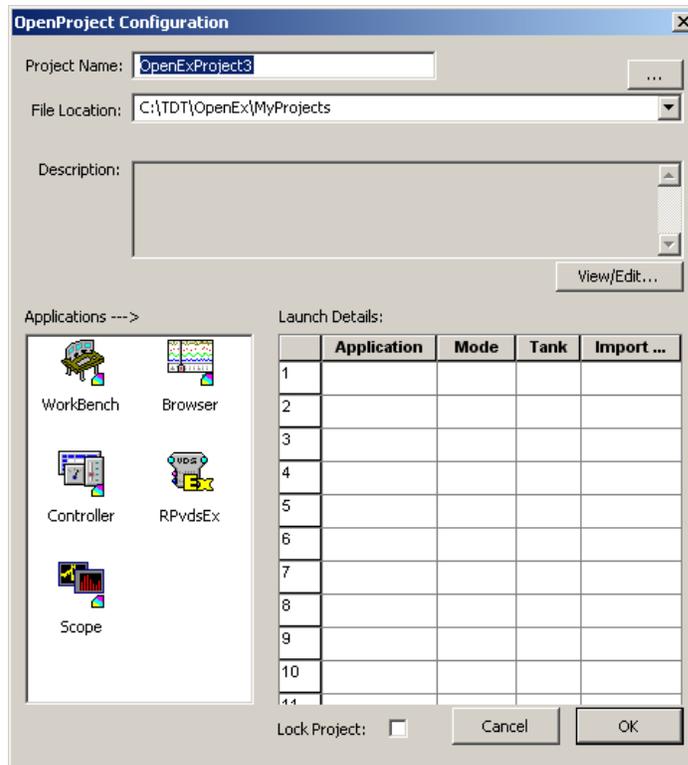
## Creating the Project

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

*To create and configure the project:*



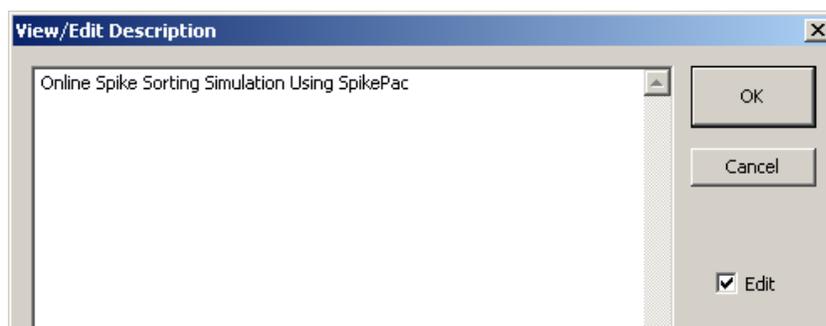
1. Double-click the **OpenProject** icon on your desktop.
2. In the OpenProject window, click the **OpenProject** menu and click **New Project**.



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.

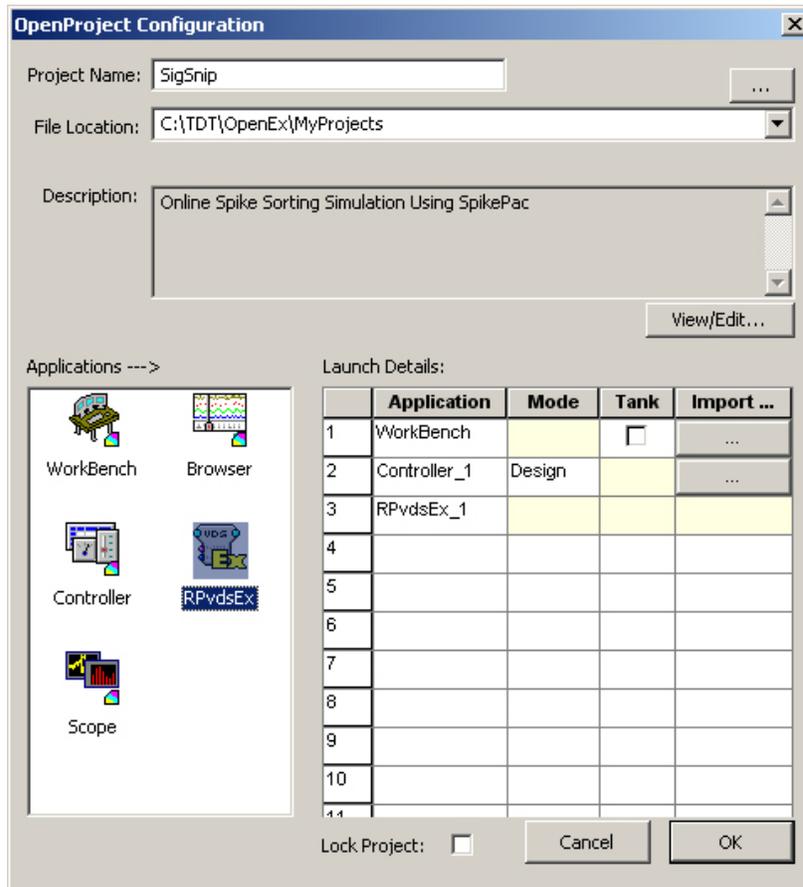


After typing a description, click **OK**.

**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. In this tutorial, 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.

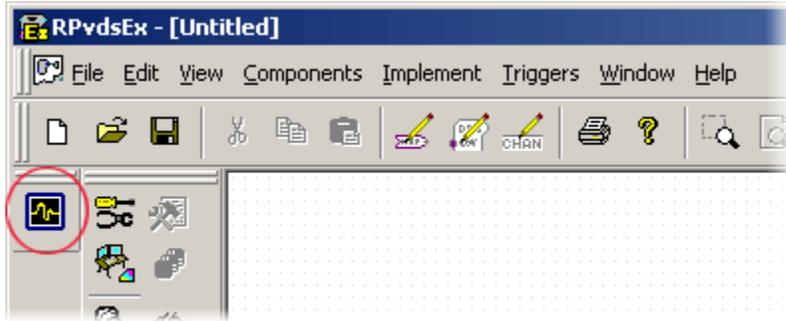
Simply add the macro based Control to the Controller workspace and you will be able to update parameters (such as spike thresholds and sorting parameters) from within OpenEx's user-friendly software environment.

**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 RIPvdsEx interface.

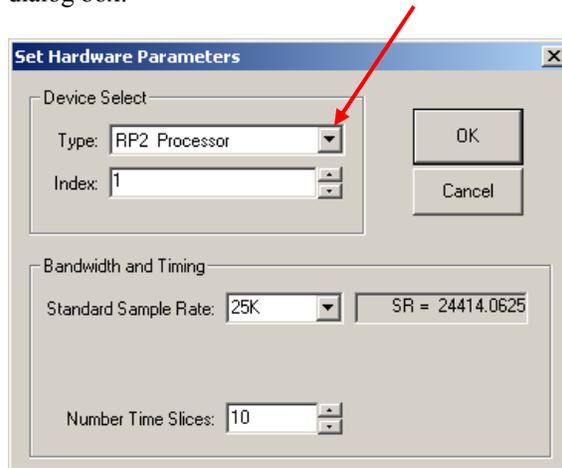


## Specifying a Device

Some of the RIPvdsEx 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.



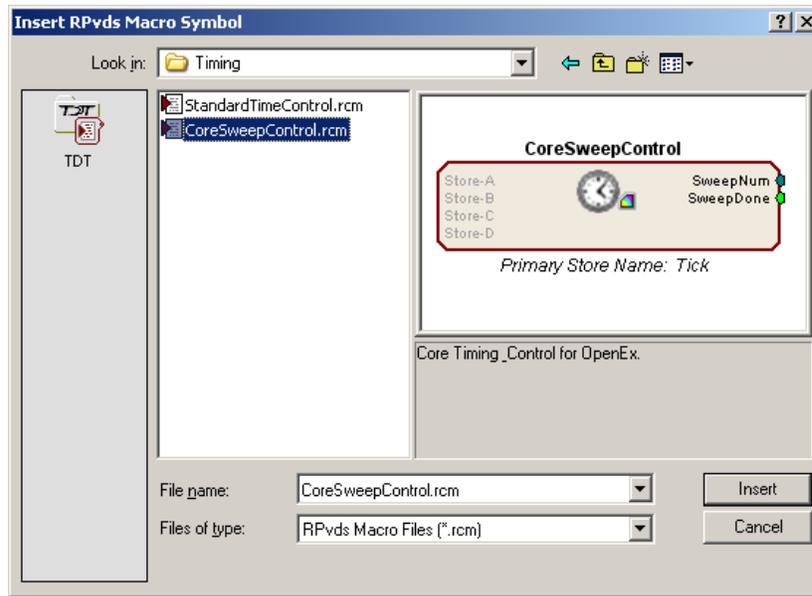
2. Select your processor from the **Type** drop-down menu in the Device Select group box.
3. Click **OK**.

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



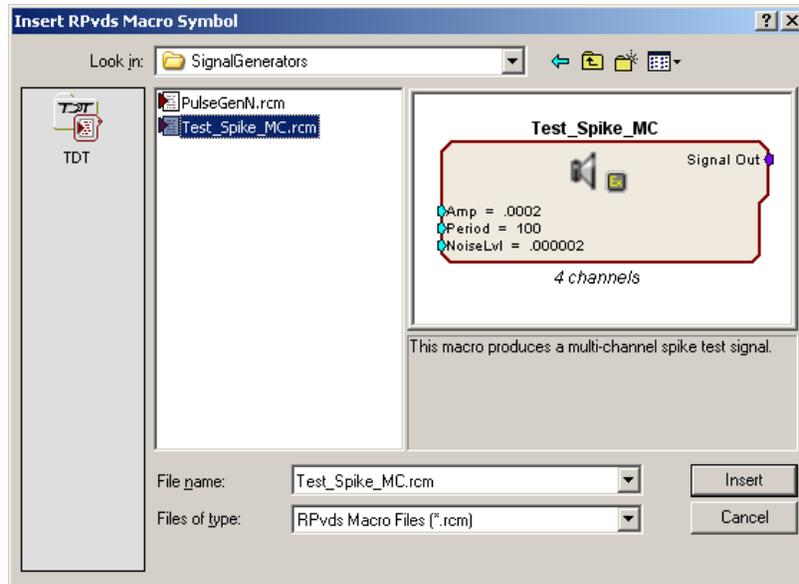
3. Click **Insert**. After the dialog box closes, click the workspace to place the component in the workspace.

### 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.

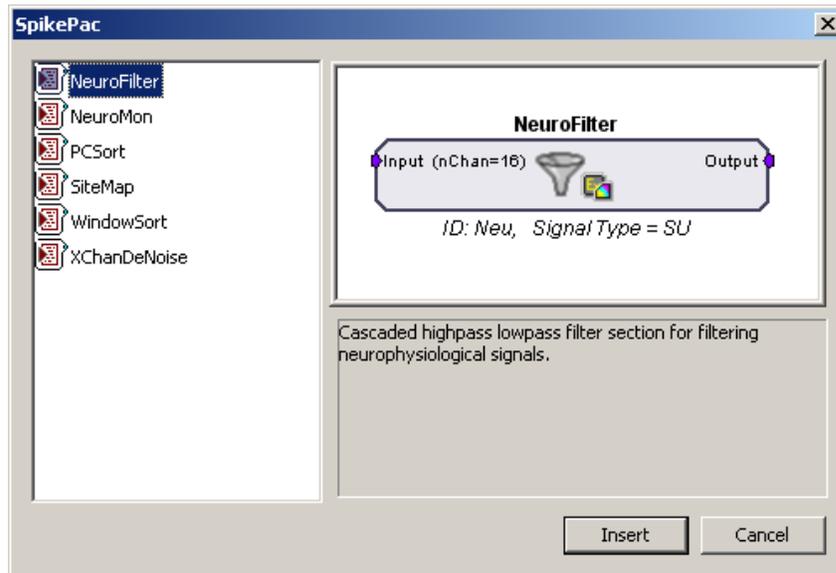


## Adding Real-Time Filtering

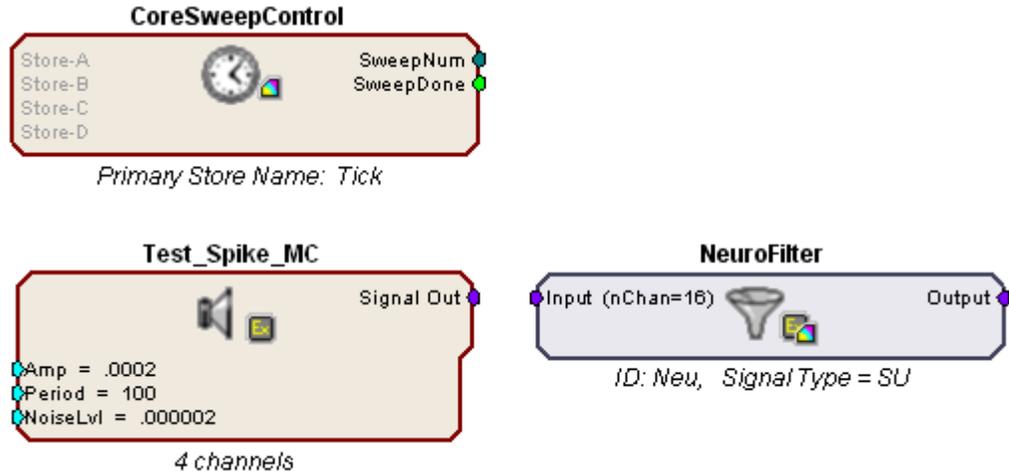
To implement real-time filtering in this project you will add the NeuroFilter, which is similar to the standard macro HP-LP\_Filter\_MC. However, when you use the macro, information is passed to OpenController and used to auto configure a control set for adjusting the filter corner frequencies.

*To add signal filtering:*

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

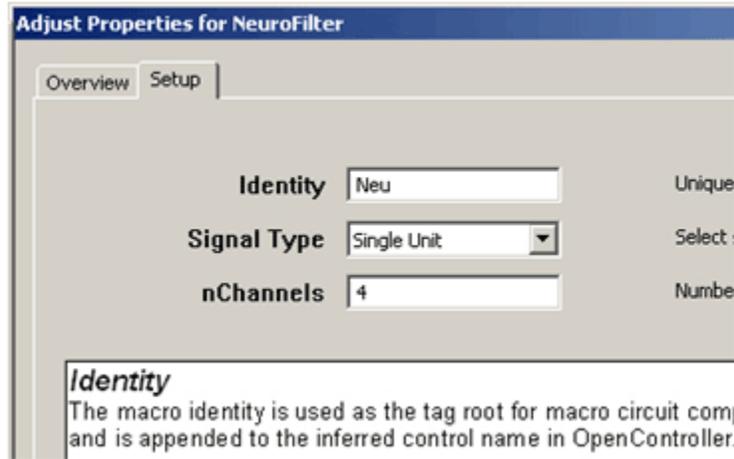


2. Click **Insert** then click the workspace to add the macro.



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.

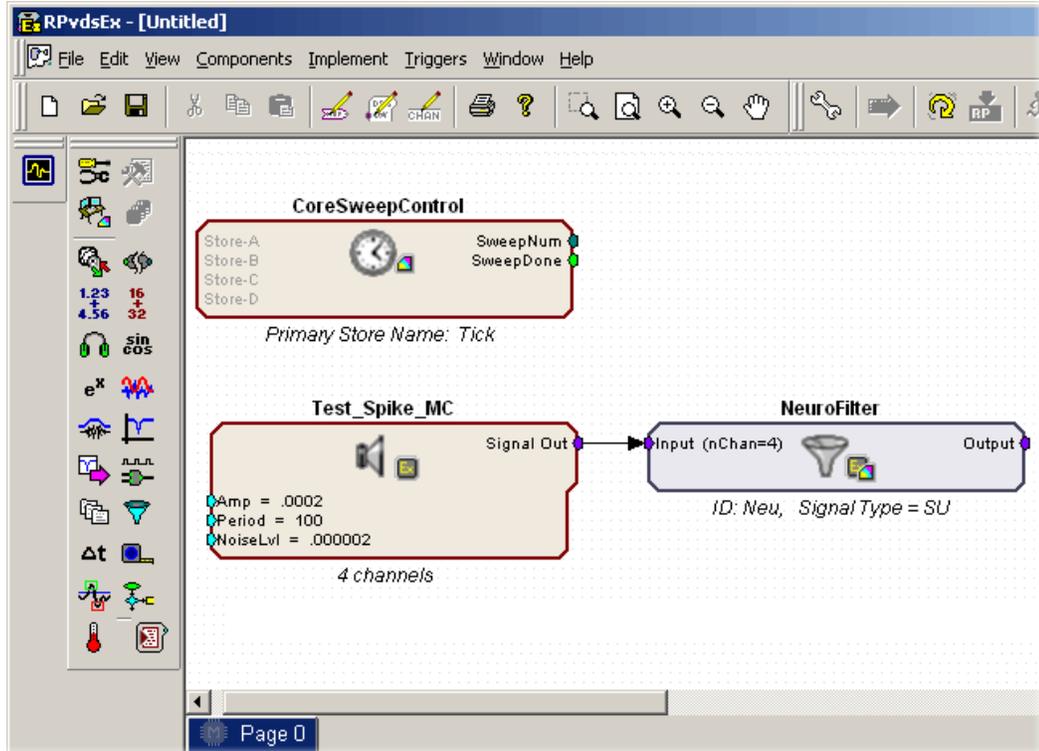


7. Finally, click **Done** at the bottom of the properties dialog.

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.

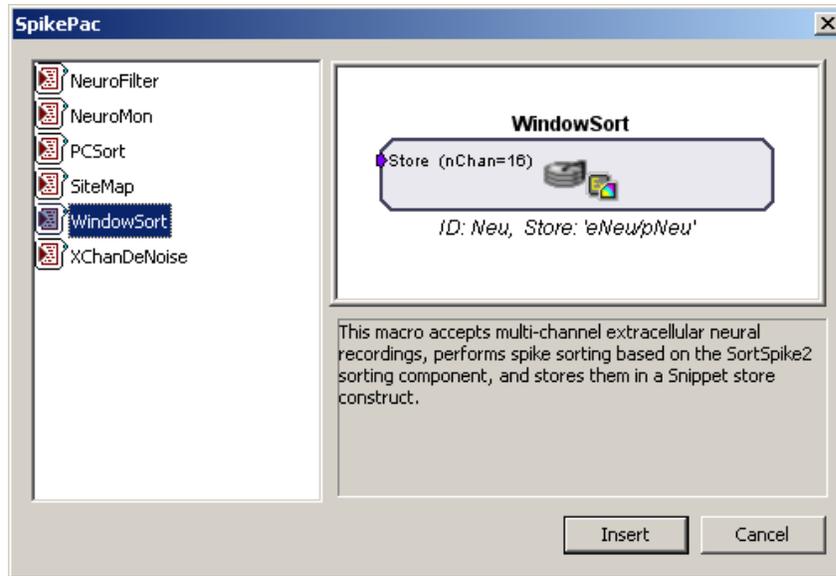
## Data Saving and Online Sorting

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

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



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

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

3. Double-click the WindowSort 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.

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

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

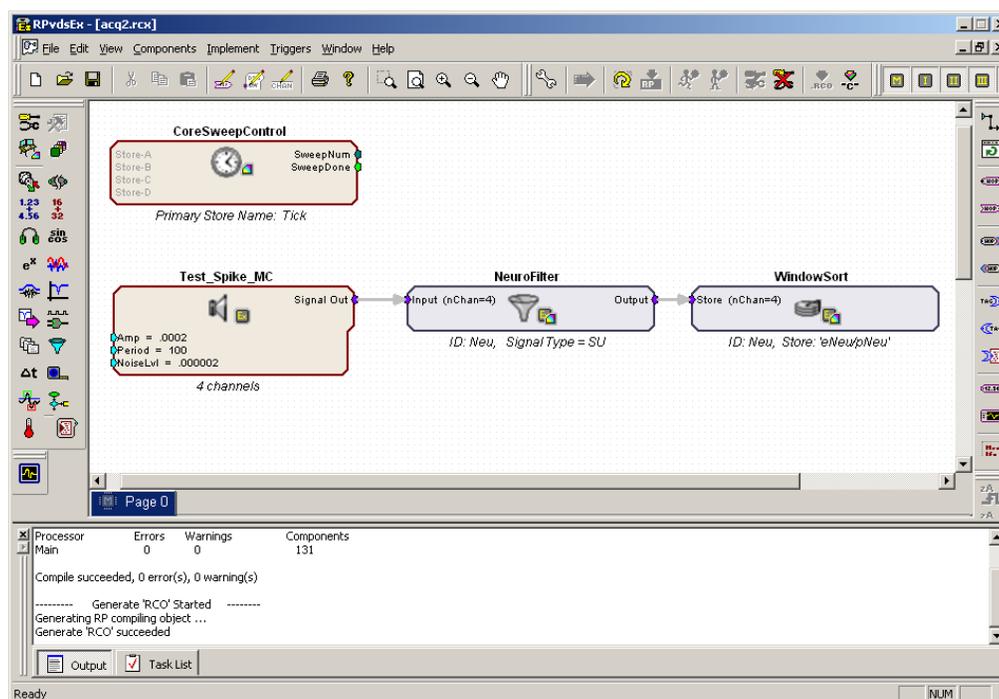
## Saving the File

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 WindowSort 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:*

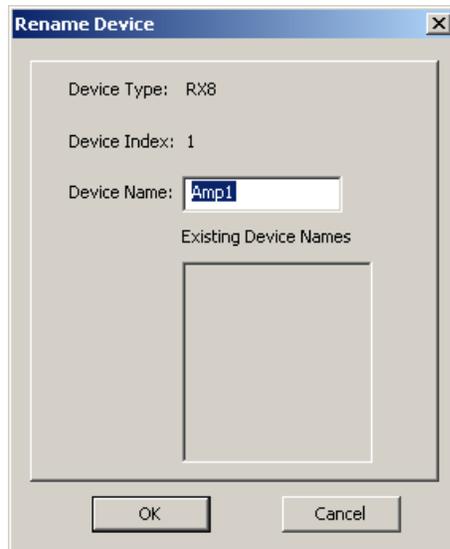
- Click the **Workbench** icon in the OpenProject window

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:**

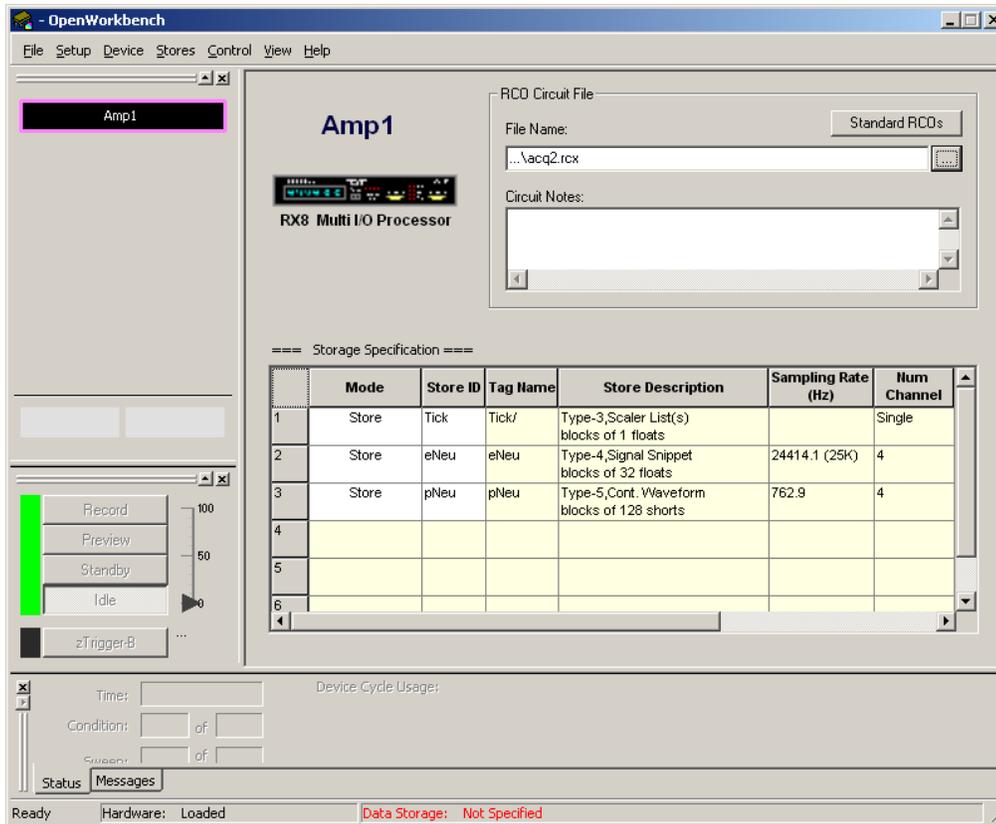
1. Select a device on which to run this example and click its gray icon in the device navigator. This will display the device configuration in the main part of the window.
2. Under RCO Circuit file, click the **Browse** button  to the right of the **File Name** box. The RCO File Select window opens to 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 WindowSort 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.

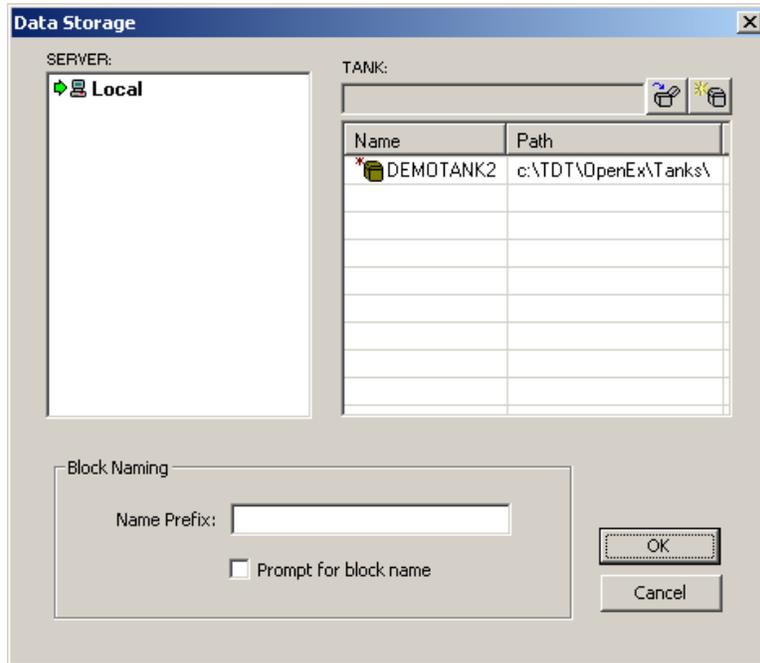
=== Storage Specification ===						
	Mode	Store ID	Tag Name	Store Description	Sampling Rate (Hz)	Num Channel
1	Store	Tick	Tick/	Type-3,Scaler List(s) blocks of 1 floats		Single
2	Store	eNeu	eNeu	Type-4,Signal Snippet blocks of 32 floats	24414.1 (25K)	4
3	Store	pNeu	pNeu	Type-5,Cont. Waveform blocks of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

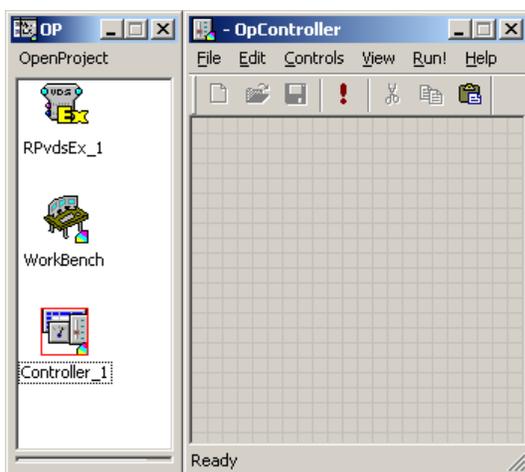
## Adding the Real-time Controls

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 set a threshold for spike acquisition, perform spike sorting, and adjust filter settings in real-time.

**To switch to OpenController:**

- Click **Controller\_1** in the OpenProject window.



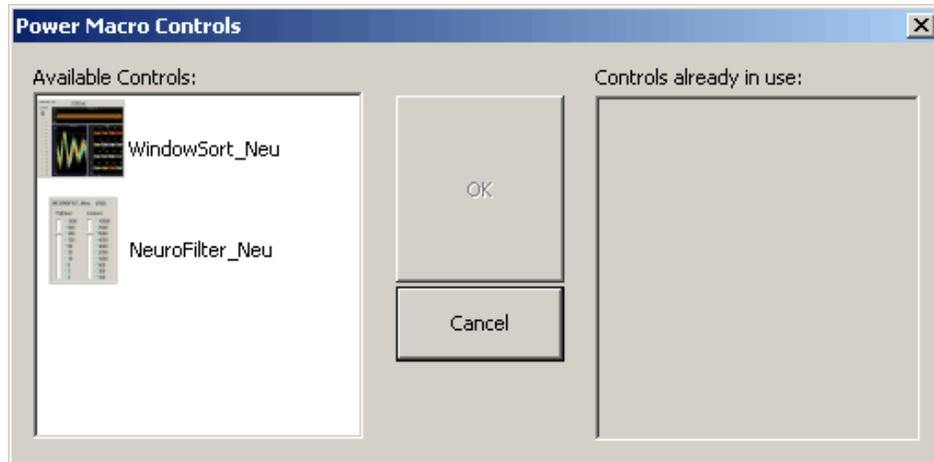
The OpenController window is displayed in Design mode. In Design mode you can add, configure, and modify controls.

## Adding the WindowSort Control for Online Spike Sorting

In this tutorial a window sort 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.

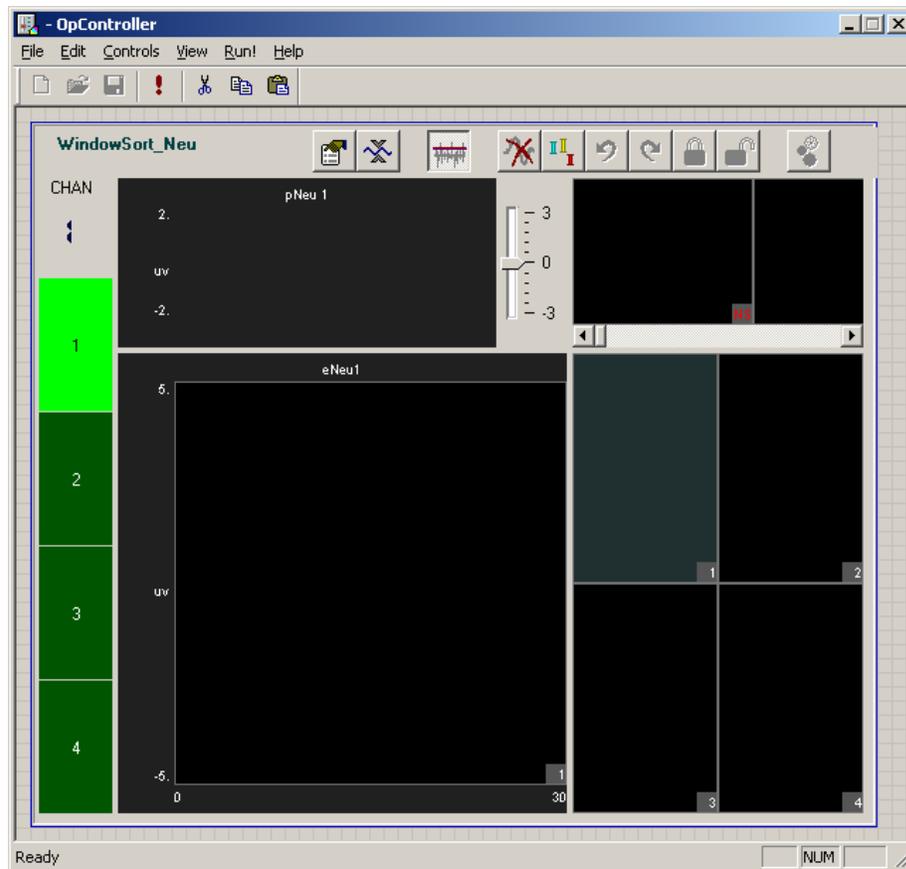
**To create the control set:**

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 **WindowSort\_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.

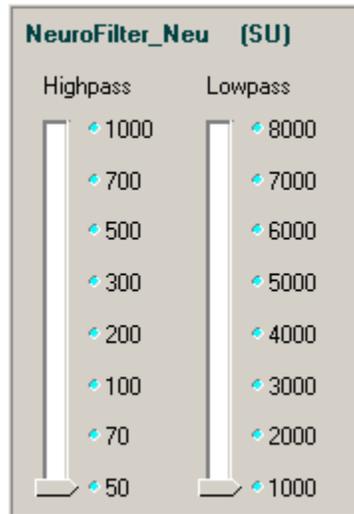


**Note:** Double-clicking any control will display additional options for the control component.

## 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

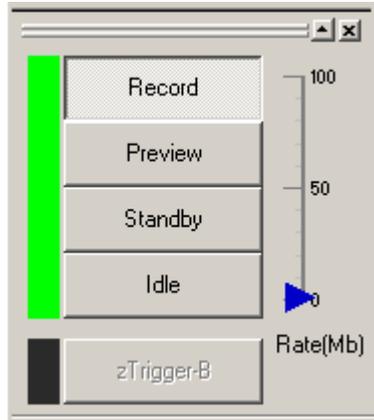
Experiments can be run from the OpenWorkbench System Control window. That means you'll need to switch between applications as you control the experimental protocol from OpenWorkbench and implement the real-time control of experimental parameters in OpenController.

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

## Beginning the Experimental Protocol

*To begin data acquisition:*

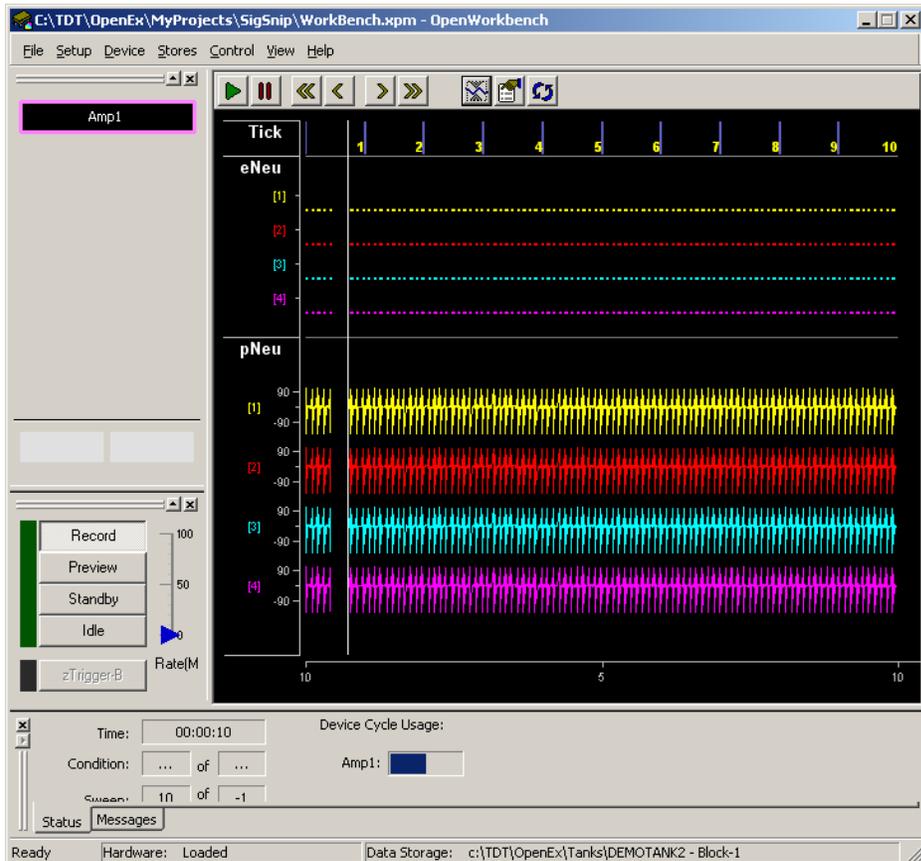
1. Switch to **WorkBench**.
2. Click the **Record** button in the System Control window. Acquired data is now being stored to the tank.



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  Autoscale button located at the top of the plot window.



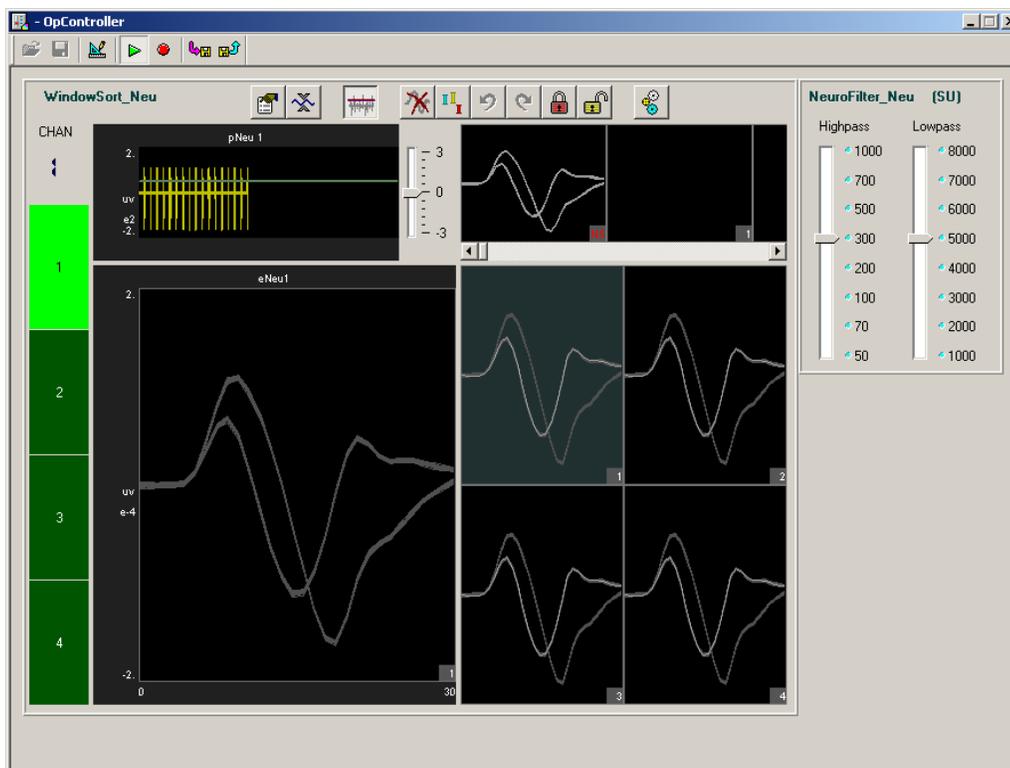
Your project should now appear similar to the illustration above, with the timing and sweep number of the Tick onset epoch shown across the top of the plot, and the four channels eNeu[1 - 4] displaying a histogram of the candidate spikes, and pNeu[1 - 4] displaying the plot decimated spike waveforms.

## 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.

The NeuroFilter\_Neu slide switches can be used to adjust the highpass and lowpass filtering for the incoming signal. Drag the slide pointer bars to change the corner frequency for the filters.

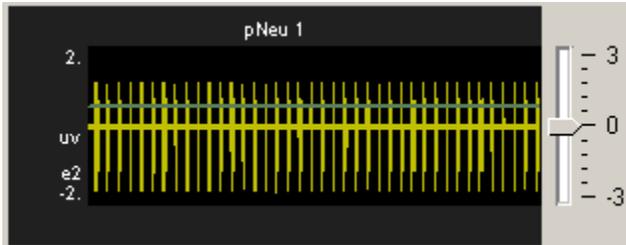
The Window Sort Control is used to sort spikes using a time-voltage window method while viewing the spike activity. The automated features of Window Sort Controls allow you to quickly assess unit activity across multiple channels without resorting to a tedious process of manually setting the threshold and then sorting spikes for each channel on a high channel count system.

### To adjust the scale of any plot:

- Point to the center of the plot, press and hold down the **Shift** key, and **drag** the mouse up or down.
- Double-click the plot while holding the **Shift** key to auto scale back to normal.
- Click the **Auto Scale** button  to auto scale all plots.

## Adjusting the Threshold

The top left plot (shown below) is a threshold plot that displays the plot decimated waveform for the selected channel along with the threshold marker. It features a slider bar that allows you to adjust the threshold by a +/- 3dB deviation from the auto-threshold value.



## Channel Selector and Clustering Ready Indicator

The channel selector bar along the left side of the control set (shown below) determines which channel is displayed in all single channel plots within the set. The color of the selector switch displays the status of each channel. If a channel on the selector switch is red, the channel cannot be auto sorted yet. A green colored selector switch indicates auto sorting for a particular channel is available.



## Auto Sorting

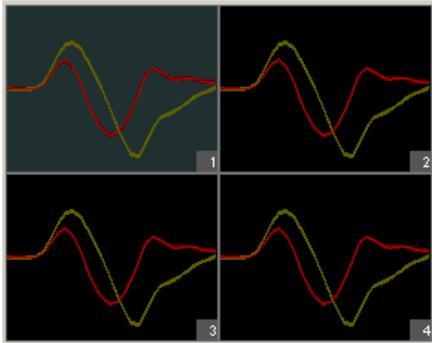
*To sort spikes automatically for all channels:*

- Click the **Auto-Sort** button  and click **Yes** when asked to confirm.

Once sort codes are assigned, each sort code (or “unit”) is represented by a single color throughout all plots.

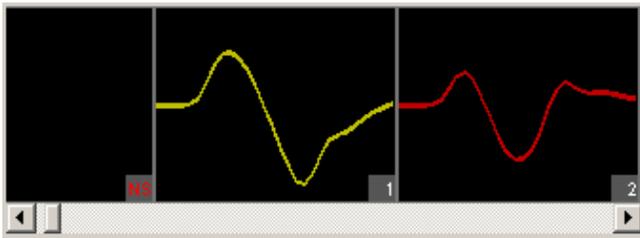
## The Multi-Channel Plot

The bottom right plot multi-plot (shown below) displays each channel in a separate pile plot. The channel number is shown in the bottom right corner of each sub-plot.



## The Unit Display

The top right plot (shown below) is a unit display. It provides a convenient means of viewing and comparing waveform shapes across different units, or sort codes. A row of individual waveform plots display the sorted waveforms by unit—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.



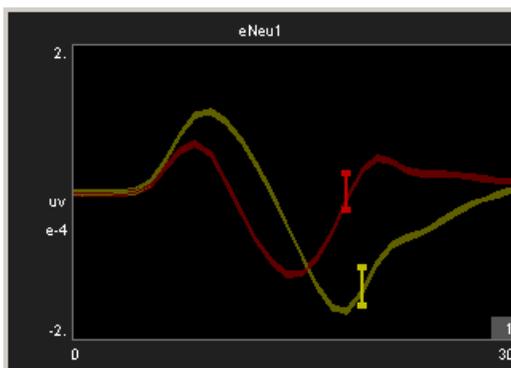
## Manually Adjusting Sort Windows

The bottom left plot (shown below) displays the waveform and the time-voltage window markers (also called sort bars or hand marks).

- You can use standard click and drag techniques to adjust the markers (and thereby the time-voltage window).

You can also add or remove markers or sort spike manually in this control.

- Double-click the control to add sorts or drag the marker out of the plot to remove them.



## Saving Sort Codes to the Data Tank

The WindowSort and PCSort controls perform spike sorting in software and send only the detected spikes to the TTank server. By default, all the snippets received by the TTank server from these controls are unsorted and assigned a sort code of '0'.

*To save software generated sort codes to the data tank:*

- Click the **Apply Sorts**  button.

Once pressed, new snippets that are assigned sort codes will be sent to TTank and subsequently saved to the data tank with the associated sort code. Any existing snippets that were sorted prior to pressing the Apply Sorts button will have a sort code of '0'.

When you are ready, you can stop the controls and the experiment.

## 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 55.

## **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 55.

## **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.

<b>Arrow Keys</b>	Adjust switches that have current focus
<b>Alt + Click and Drag</b>	Pan Plots
<b>Shift + Click and Drag</b>	Zoom Plots
<b>Shift + Double Click</b>	Reset Plot Position (resets pan and zoom changes)
<b>Alt + Double Click</b>	Manually Sort Waveform Space (WindowSort)
<b>Ctrl + Click and Drag</b>	Manually Sort Waveform Space or Feature Space (WindowSort and PCSort)
<b>Ctrl + Spacebar</b>	Toggle Design/Control mode for OpenController

# References

1. Pelleg D., Moore A., "*X-means: Extending K-means with efficient estimation of the number of clusters,*" in ICML 2000.
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3. Lewicki M.S., "*A review of methods for spike sorting: the detection and classification of neural action potentials.*" *Network: Computation in Neural Systems*, 9 (4): 53-78, 1998.
4. Devore J., Peck R., *Statistics: The exploration and analysis of data.*
5. Schmitzer-Torbert N., Jackson J., Henze D., Harris K.D., Redish A.D. (2005) "*Quantitative measures of cluster quality for use in extracellular recordings.*" *Neuroscience*, 131:1-11
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.