

8.15: Spike Sorting

Introduction

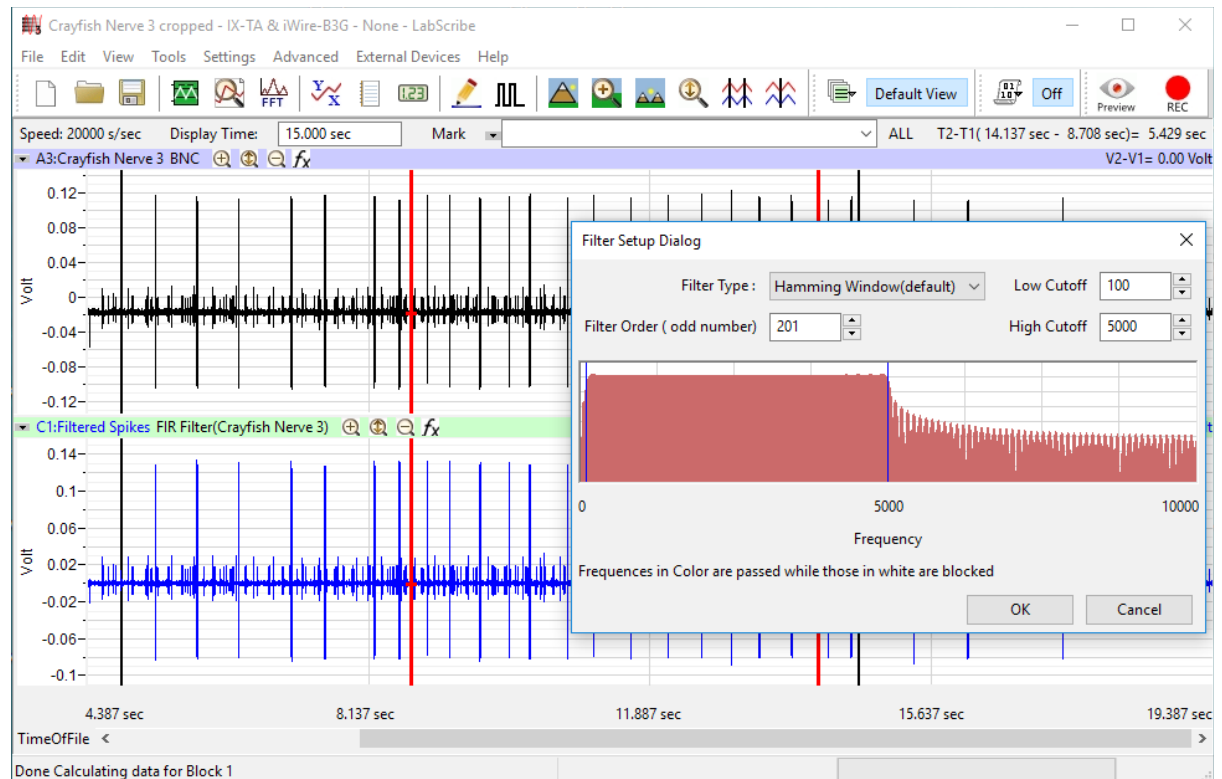
The **Spike Sorting Analysis Module** is an advanced analysis module that allows users to automatically calculate parameters from Nerve spike data.

This document includes a step by step tutorial for using the features of the LabScribe **Spike Sorting Analysis Module**. To use the step by step guide, you will need a recording of stimulus and response data file.

Spike Sorting Analysis: Step by Step

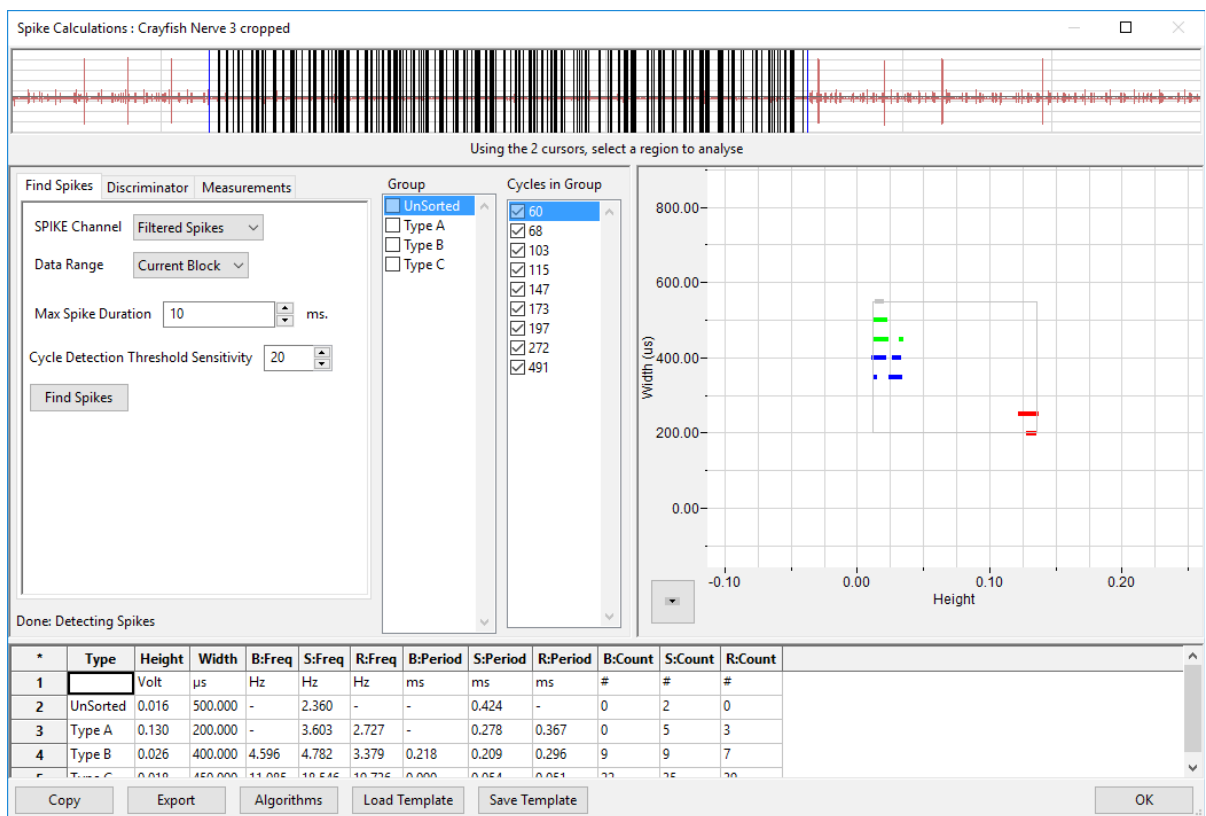
Sophisticated analysis can be done on a previously recorded data file using the **Spike Sorting Analysis Module**.

Before analyzing the spikes, it is best to filter the spike data to remove any baseline and reduce noise. This can be done in LabScribe by creating a filter channel. To reduce baseline and 60Hz mains noise, choose a Low Cutoff of 100 Hz.



The Spike Sorting Analysis dialog can be launched from the Advanced menu in LabScribe.

When the dialog is launched the first time, it will ask for the User Name and License for the Spike Sorting Analysis module. Please contact iWorx Systems for more information.

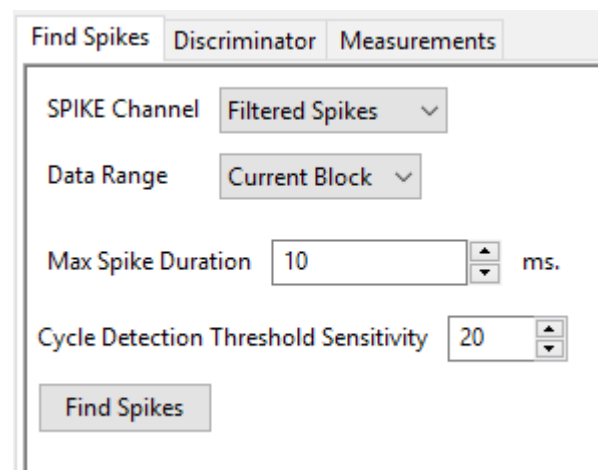


1. Familiarize yourself with the offline **Spike Sorting Analysis** dialog, pictured above.
2. Across the top of the dialog, in the channel display area, is the spike data channel from the recording.
3. The tabbed configuration dialogs are on the left below the recordings.
4. An XY graph window on the right displays the **Spike Graph, Sorting Graph or Histograms**
5. Between the configuration dialogs and the XY graph window is the **Groups and Cycles Selected** list, an editable list of the cycles that can be displayed and analyzed.
6. The **Data Table** is located on the lower part of the dialog.

Find Spikes:

Click on the **Find Spikes** tab:

- Choose the Spike Sorting Channel.
- Set the Data Range to analyze:
 - Selection,
 - Current block
 - Complete File
- Max Spike Duration, sets the width of the spikes
- Cycle Detection Threshold Sensitivity, determines how sensitive the program is to detecting a cycle, Typically a value of 20 should work. If the program is missing some cycles,



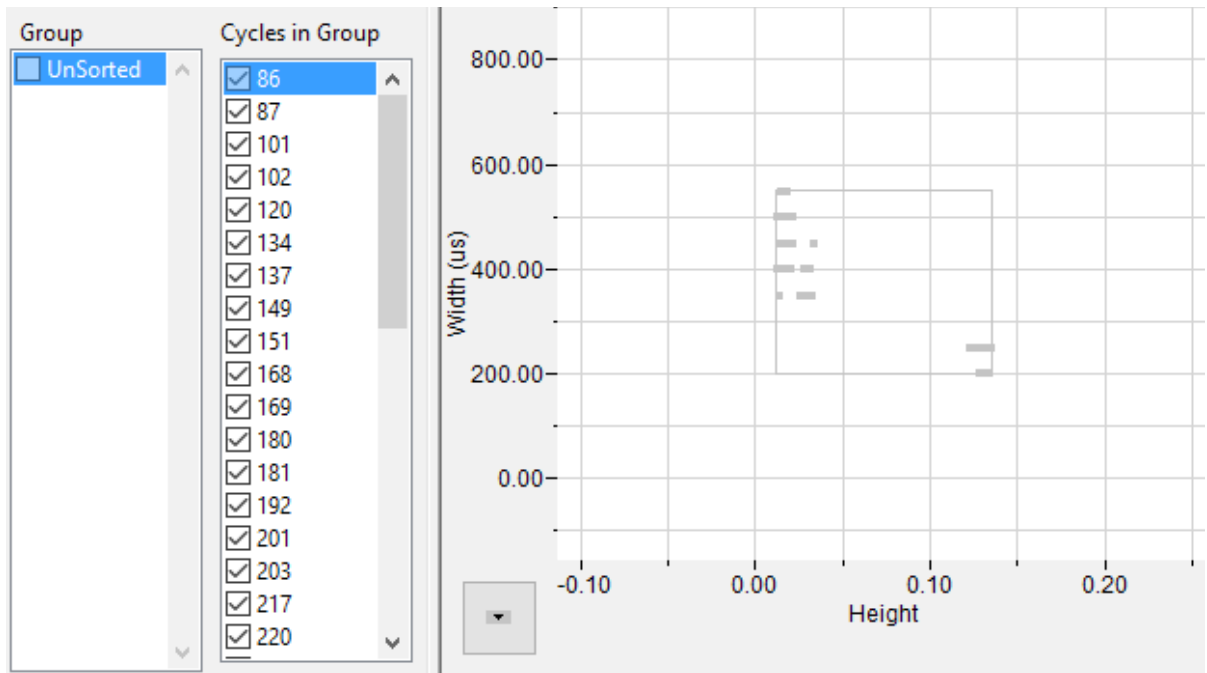
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increase the value of the sensitivity.

Select a representative area of the recording by moving the two vertical cursors in the channel display area at the top of the dialog, For a selection is the complete data set that will be analyzed

Click on the Find Spikes button: The detected spikes within the representative area will be displayed as black lines in the Channel area. The Complete list of cycles found will be listed in the Cycles Selected list box, under the unselected group.

The Spike graph will display the unsorted spikes in a sorting graph, each spike is displayed as a point, corresponding to its width and height.



Discriminator:

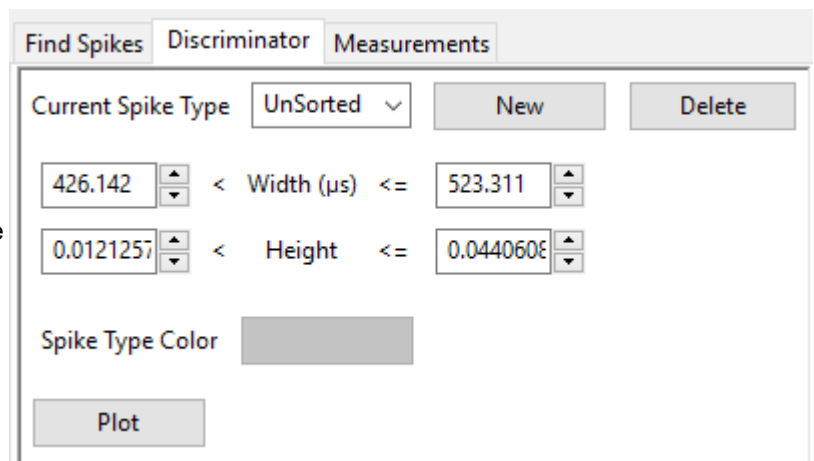
Click on the **Discriminator** tab:

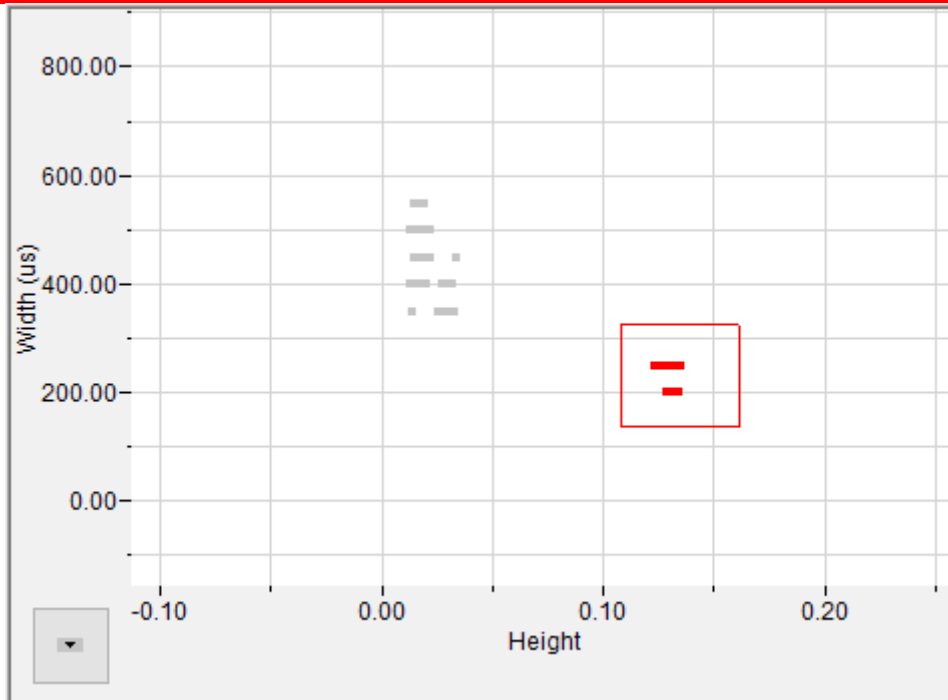
The unsorted spikes are shown in the Spike Graph on the right.

Click on **New**.

A new Spike Type **Type A** will be created, The Width and the height for the Type A spikes can be set either using the number boxes or by clicking and dragging the square in the spike graph. The color of the spike type can be changed as well.

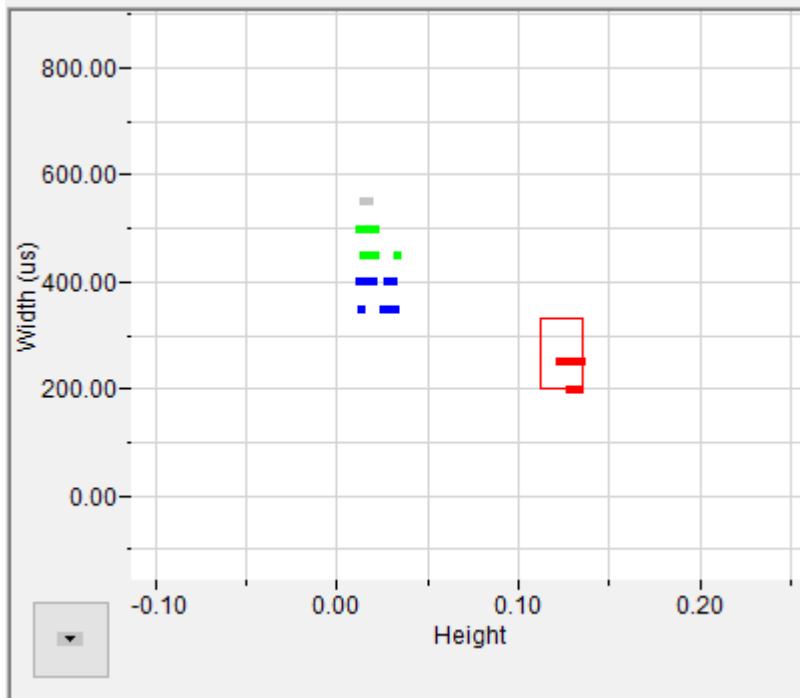
Click **Save**.



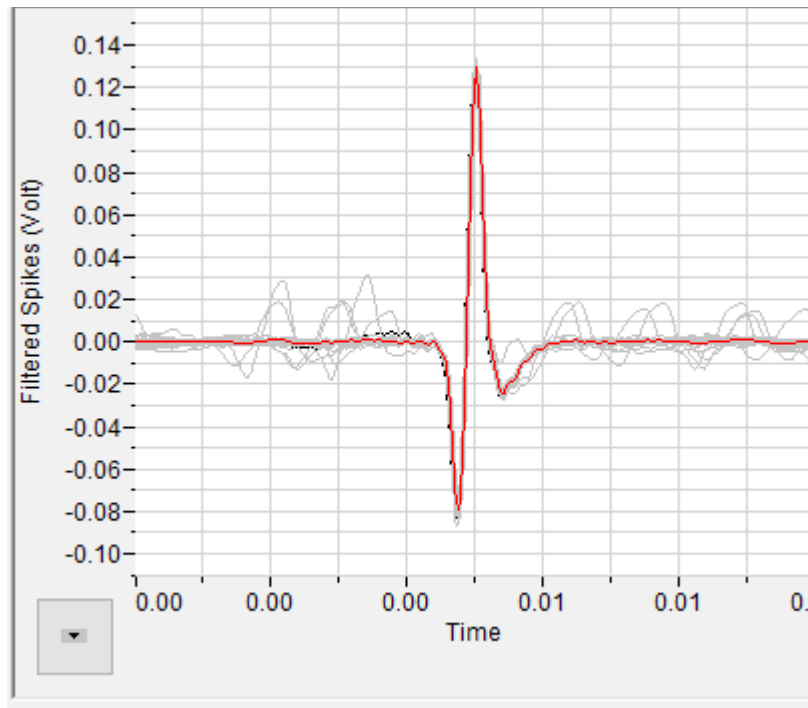


The selected spikes will be Marked in the color of the spike type.

Create other Spike Types as needed. The graph below shows Spike Types A,B,C created



In the Groups list box, click on a group to see the spikes in that group and the average spike.



Measurements:

Find Spikes	Discriminator	Measurements
Bin Size	1	sec.
(B) Baseline Mark	Baseline	
(S) Stimulus Mark	Stimulate tail hairs	
(R) Recovery Mark	Recovery	
Measure from	0	s to 2
		s after Mark
<input type="button" value="Calculate Parameters"/>		

Set the Bin Size for the histograms.

The Data set can be marked for 3 regions, Baseline, Stimulus and Recovery. Choose the marks in the record corresponding to the 3 regions. Marking the data into these regions helps in the analysis, since typically we want to see the difference from baseline when a stimulus occurs and then we want to see how fast the recovery takes place.

One can also set the time period from the beginning of each region to be analyzed.

Data Table:

The values for all the cycles are calculated and displayed in the Data Table.

The Data Table

The **Data Table** displays the chosen calculated values for each cycle.

*	Sel	Time	Period	Inst.Freq	Sys. Dur	Dias. Dur	Cycle Dur.	Amp	dFdT max	dFdT min	Amp*Inst.Freq	Sys Area	Dias. Area
units		hh:mm:ss	sec	#/min	sec	sec	sec	mN	mN/sec	mN/sec	mN/min	mN.sec	mN.sec
1	Yes	29.715	12.945	4.635	353.000	2236.000	12.945	2.513	3.591	-1.485	11.648	855.839	3253.007
2	Yes	42.660	11.455	5.238	338.000	1953.000	11.455	2.952	3.533	-1.473	15.461	873.221	2979.405
3	Yes	54.115	12.690	4.728	350.000	2188.000	12.690	2.544	3.591	-1.511	12.029	895.177	3152.478
4	Yes	66.805	11.850	5.063	382.000	1988.000	11.850	2.868	3.365	-1.484	14.520	896.500	3001.600
5	Yes	78.655	11.800	5.085	365.000	1995.000	11.800	2.588	3.535	-1.439	13.161	906.208	2977.816
6	Yes	90.455	11.620	5.164	395.000	1929.000	11.620	2.558	3.425	-1.425	13.209	860.521	2983.804

Copy Export Algorithms Table Options Load Template Save Template

Clicking the asterisk at the upper left of the **Data Table** displays two options: **Autosize** and **Copy Selection**. **Autosize** will optimize the size of the **Data Table** boxes, and **Copy Selection** copies any selected **Data Table** cells to the clipboard.

The **Data Table** displays the chosen calculated values for each of the cycles checked in the **Cycles Selected** window, as well as the mean, standard deviation, and range of each of the chosen parameters averaged over all the selected cycles.

Clicking **OK** in the **Offline Calculations Options Dialog** saves the settings for future analyses.

There are four buttons across the bottom of the Dialog: **Copy**, **Export**, **Algorithms**, **Table Options**, **Load Template**, and **Save Template**.

- All the calculated data in the **Data Table** can be copied to the clipboard by clicking the **Copy** button, or exported by clicking the **Export** button. The data are exported in a tab (*.txt) or comma (*.csv) separated text file, and the XY graph can be exported as a Portable Network Graphics (*.png) or JPEG (*.jpg) image.
- Clicking **Algorithms** opens an information window describing the mathematical equations used to compute a number of the offline parameters.
- *LabScribe* is able to calculate a large number of lymphatic contraction calculations for each cycle. By clicking **Table Options** at the bottom of the **Spike Sorting Calculations Dialog**, the **Offline Calculations Options Dialog** opens, and calculations to be displayed in the dialog data table can be chosen from the list of all possible calculations. Marks indicating temperature and activity can also be added to the file, and those values can be chosen from the **Table Options** list and included in the **Data Table**.
- Clicking **Load Template** or **Save Template** displays a dialog allowing you to name and save a specific configuration for future use or to load a previously saved template.

Calculations:

- **Time:** Time (in seconds) from the start of the selection, or the time of day of the recording, depending upon which option is chosen.
- **Period:** Time interval between contractions
- **Inst Frequency:** Instantaneous contractions per min, calculated from the period of each cycle
- **Amplitude:** Amplitude of the Contraction (Maximum of the cycle - baseline)
- **Baseline Period :** Time Duration before the systolic start to calculate baseline
- **EDV:** Voltage before the upstroke during the cardiac cycle, where the derivative crosses zero.
- **DFmax :** The smoothed derivative of the channel is calculated, using 2 points on either side.
- **DFmin :** The smoothed derivative of the channel is calculated, using 2 points on either side.
- **AmplInstFreq:** $\text{Amp} * \text{InstantaneousFreq}$
- **Amp*AvgFreq:** $\text{Amp} * \text{Average Freq}$
- **Sys. Area:** Area under the curve from systolic start to Max, with respect to the baseline
- **Diast. Area:** Area under the curve from Max to diastolic end, with respect to the baseline
- **Sys. Dur:** The time, seconds, from Systolic Start to Max.
- **Dias. Dur:** The time, seconds, Max to Systolic Start of next cycle
- **Cycle Dur:** Systolic Duration + Diastolic Duration.