

# **Slice<sup>TM</sup> Analysis User's Guide**

**Version 1.2**  
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**and Dan Sanes**

## **0 Forward**

*Slice Analysis 2.02* is the expansion to the original version 1.0, designed by Dan Sanes and created and maintained by Fanyee Lee and Chris DiMattina in the New York University Center for Neural Science, Sanes Lab. Both versions use the same format of data from the application *Slice* and are only compatible to *Slice* data or other data file of the same format. This manual is not a continuation but a conglomerate of the old 1.0 version and the new 2.02 version of *Slice Analysis* manual.

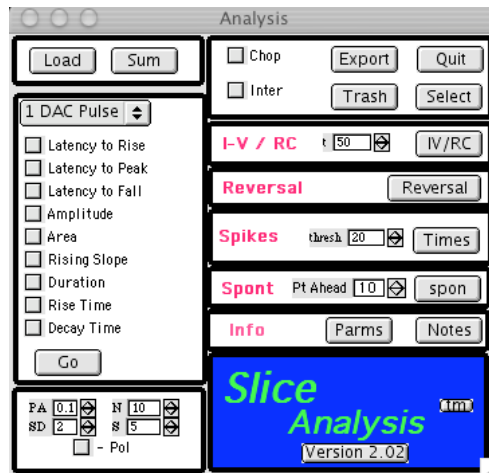
## **1 Introduction**

*Slice Analysis* is the data analysis package that accompanies the *IGOR Slice* data acquisition package. *IGOR Slice* was designed by Dan Sanes and written and supported by Fanyee Lee and Chris DiMattina for doing in vitro slice physiology; its primary design is for doing synaptic as well as intracellular studies. *Slice Analysis* uses numerical values from saved parameters in *Slice*, and extracts and analyzes these values. These values are then written to tables, which may be exported into tab-delimited text files and further analyzed with the statistics package JMP or any other package that accepts tab-delimited text. Graphing, hypothesis testing and statistical analysis are not performed in *Slice Analysis*.

## **2 Features Overview**

*Slice Analysis* is meant to support the batch analysis of selected traces from multiple files. In principle all of the analysis for an entire experiment could be performed in one session. The analyses that are supported are:

- 1) IV/RC feature to compute I-V curves and time constants of cells
- 2) Reversal feature to compute the reversal potential of a synapse
- 3) Spikes feature to generate a list of spike times for each trace
- 4) Spont feature counts the number of spontaneous events for a trace
- 5) compute 9 different parameters of synaptic events, in eight different modes of operation.

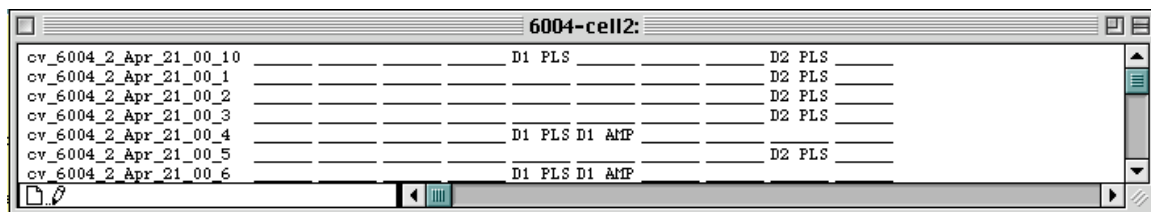


**Figure 1:** The Slice Analysis main window

Slice Analysis also allows for basic editing of the data waveforms and one may use built-in IGOR features to export data traces into a paint program such as Canvas™.

### 3 Directory Summaries

In most cases a scientist cannot recall exactly what they did simply by looking at the name of the file. In order to get an idea as to exactly what parameter sets were varied for each of the data files, we have included a feature which allows you to get a "summary sketch" of a particular directory. One may get a summary sketch of a particular directory by pressing the Sum button on the upper left hand corner of the Main window ( right next to the loading button ).



**Figure 2:** The directory summary window

The directory summary window contains the name of each file in the directory followed by ten fields which indicate the active DACS, which mode they were in and which parameters were varied. The values which each column may assume and their meanings are given in table 2.

Column	Possible Values	Meanings
1	D0 TRN,D0 PLS	D0 TRN - DAC0 in train

		mode D0 PLS - DAC0 in pulse mode
2	D0 AMP	DAC0 amplitude was varied
3	D0 LAT	DAC0 latency was varied
4	D0 INT	DAC0 interval was varied
5	D1 TRN,D1 PLS	D1 TRN - DAC1 in train mode D1 PLS - DAC1 in pulse mode
6	D1 AMP	DAC1 amplitude was varied
7	D1 LAT	DAC1 latency was varied
8	D1 INT	DAC1 interval was varied
9	D2 PLS D2 RMP D2 2PL	D2 PLS - DAC2 in pulse mode D2 RMP - DAC2 in ramp mode D2 2PL - DAC2 in 2 pulse mode
10	D3 TON	DAC3 is in tonic mode

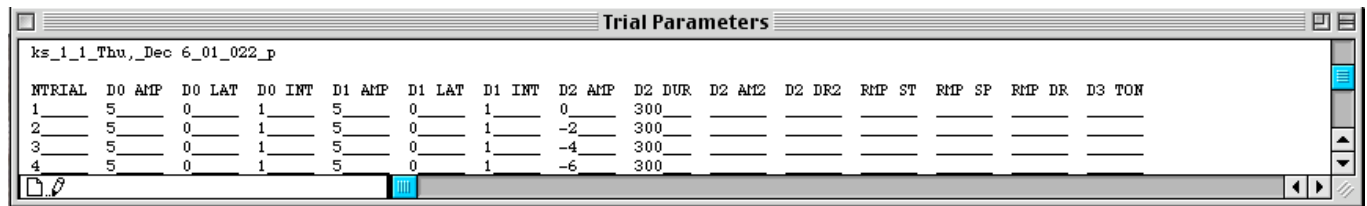
**Table 1:** Values listed in the Directory Summary window

## 4 *Loading Files*

Slice Analysis takes as its input IGOR binary files generated using Slice 1.1.1 or later. A file is loaded by pressing the "Load" button on the upper left hand corner of the Slice Analysis window. This brings up a box which may be used to select the file of interest. Please note that the associated parameters file for a given data file **MUST** be in the same directory in order for it to load properly. This is usually not a problem since the two files are automatically saved to the same directory.

You may load as many files as you like, limited only by your machine's available memory. There is no unloading option however. If you do not wish to use a file in your analysis, you can simply not select any traces in that file.

When the first file is loaded, a Trial Parameters window is opened which lists information for that file.



**Figure 3:** The Trial Parameters Window

As additional files are loaded, their information is appended to this window. The file name is listed first (actually the filename of the associated parameters file) and after skipping a line there are 15 columns of data. The numbers which are listed in these columns are the values of stimulation parameters which can vary from trial to trial. If a parameter is not active at all during the particular run then a blank line is inserted in its place. The parameters which are listed in the Trial Parameters window are given in Table 1.

Column	Meaning
NTRIAL	The trial number
D0 AMP	DAC0 amplitude
D0 LAT	DAC0 latency
D0 INT	DAC0 interval
D1 AMP	DAC1 amplitude
D1 LAT	DAC1 latency
D1 INT	DAC1 interval
D2 AMP	DAC2 pulse 1 amplitude
D2 DUR	DAC2 pulse 1 duration
D2 AM2	DAC2 pulse 2 amplitude
D2 DR2	DAC2 pulse 2 duration
RMP ST	Ramp starting voltage
RMP SP	Ramp stopping voltage
RMP DR	Ramp duration
D3 TON	DAC3 tonic stimulation level

**Table 2:** Values listed in the Trial Parameters window

## 5 Chopping and Interpolation

When you load your traces, they may contain stimulus artifacts picked up by the intracellular electrode which are due to the electrodes stimulating the afferent fiber pathways are immersed in an almost perfectly conducting medium (the ionic solution!). You may want to remove these artifacts for whatever reason. This can be done as you are loading your traces by checking the "chop" box. The number of milliseconds after the pulse stimulus that will be chopped is a variable located in the lower left hand corner which is entitled **PA**, which stands for post-artifact. Instead of leaving holes in your traces, you may wish to perform a linear interpolation after the

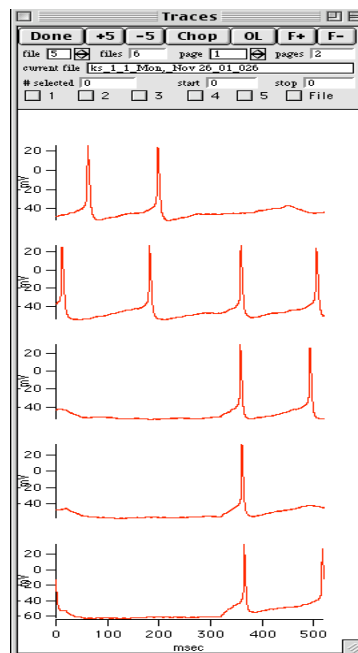
artifacts have been removed. This can be accomplished by also checking the "inter" box, situated right beneath the "chop" box.

Chopping and interpolation during the file load are not the recommended way to edit your data. A much better way is for each file, after it has been loaded to use the Chop button in the Select window so that you may see the results of your chopping. It is always best to be conservative in how much you chop out cannot re-load a file that is already loaded without exiting the program. Chopping and interpolation are discussed in more detail in later sections.

## 6 The Traces Window

Once traces have been loaded, one may open up the Traces window to see the waves collected from *Slice*. This allows you to select which traces you would like to include in your analysis. As any physiologist knows, some of the data which is collected is not suitable for analysis for one reason or another and so we provide a mechanism for paging through and selecting traces to analyze. We chose to display five traces per page since psychological studies show that the typical person can remember 7 (+/- 2) items easily in short term memory (so we chose to err on the side of caution).

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**Figure 4:** The Traces Window

There are a number of set variables, display variables, buttons and checkboxes that we've developed to help obtain a better analysis.

## **6.1 The Checkboxes**

There are six check boxes on the bottom of the control bar portion of the window. The boxes numbered from 1-5 allow you to select the corresponding trace on that page of the current file. The box labeled file allows to instantly select all of the traces in the current file. Checking and then un-checking this box is a way to de-select all of the traces in the current file and to return back down to a baseline state.

Once you have selected all of the traces in the file, the File check box automatically checks, and when you un-select a trace so that not all of the traces in the file are selected the File check box automatically un-checks.

## **6.2 The File Set Variable**

When multiple files are loaded, one may use the file set variable to select the current file. When one advances to the next file or the previous file, the Traces window displays the FIRST page of traces for that file. The filename of the current file is given in the display box labeled "current file". The total number of loaded files is indicated in the display box titled "files" which is right next to the file set variable. One may also advance to the next file by pressing the "F+" button and go to the previous file by pressing the "F-" button.

## **6.3 The Page Set Variable**

There are a maximum of five waves per Trace window, so one can flip through the pages of traces using the "Page" set variable. The number of pages of traces for the current file is displayed in the "Pages" display variable.

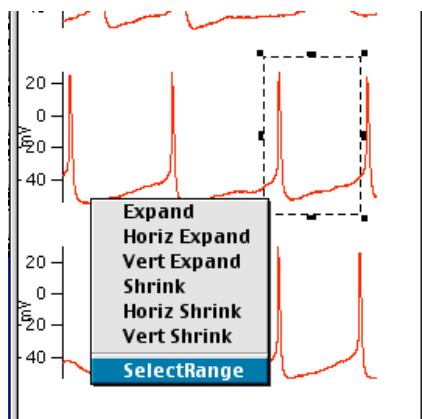
One may also advance to the next page by pressing the "+5" button and go to the previous page by pressing the "-5" button.

## **6.4 The Chop Button**

One may remove artifacts in your traces using the "Chop" option. For each pulse artifact in each of your traces, the "Chop" button will remove the part of the trace from the beginning of the pulse to PA milliseconds after the end of the pulse, where PA is the value of the PA (post-artifact) set variable in the main window. This may be varied and the waves may be re-chopped repeatedly with increasingly large values of PA. Once you have removed data, you CANNOT get it back without quitting the program and re-loading. So it is advised to start with a small value of PA, and if there is still artifact left over to then increase the value until you have a good looking trace.

## 6.5 Range Selection

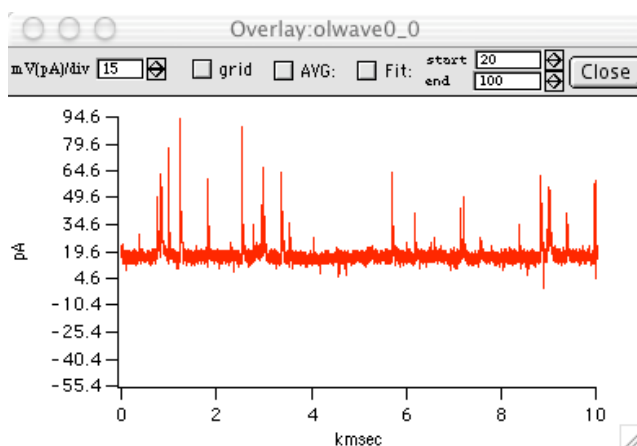
For particular analyses (for instance computing reversal potentials) you may wish to restrict your processing to a particular subinterval of the data trace. This is accomplished by drawing a marquee around the interval you are interested in and then clicking on the SelectRange option at the bottom of the marquee menu. This will set the "start" and "stop" variables displayed at the top of the screen to the time values at the left and right sides of the marquee (measured in milliseconds). The vertical extent of the marquee is not important -- the program is only concerned about the horizontal (time) range of the marquee, and will process all points of the wave which are in that range.



**Figure 5:** A Marquee used to select the time range to analyze

## 6.6 OverLay Window

Overlay window shows the explicit traces from which the users selected from the traces window.



**Figure 6: OverLay Window** with spontaneous data.

### 6.6.1 Millivolts/Picoamps per Division Variable



This variable sets the total value of units between each major tick. Adjusting this value will help the user zoom in and out of the entire trace.

### 6.6.2 Grid Button

Turns X and Y gridlines on and off.

### 6.6.3 Average Button

This button averages the selected waves and presents this wave in the FunctionedLay Window.

### 6.6.4 Fit Button with Start & End Variables

Start and End variables are used to set the range at which the single exponential is calculated for the Fit function that is activated by pressing the Fit button once. Another window labeled "FunctionedLay" will pop up for the result.

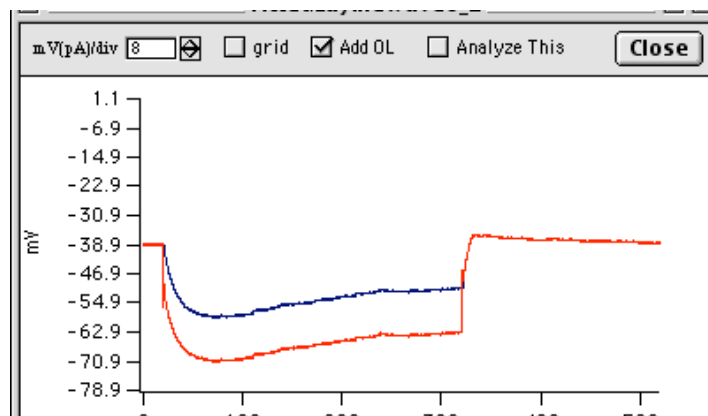
In most electrophysiology setups using micropipette stimulation the measured intracellular potential without special circuitry is comprised of the product of both the micropipette current and the micropipette resistance. The voltage drop across the micropipette is usually negated or "Bridge Balanced" ("bridge circuit and balance") to result in the intracellular potential only.

This option in Slice Analysis is geared towards users that do not have this compensation.

The algorithm for this would be a double exponential computation; where the first exponential is the supposed voltage drop and the second exponential is the actual intracellular potential. Due to certain software limitations, our algorithm is modified to calculating a single exponential to obtain the correct actual trace.

This window shows the analysis of the selected trace(s) from the OverLay window. It gives the difference of your original selection minus a single exponential constant calculated from the start and end variable ranges set in the OverLay Window. Results reflect every point of the original Overlay window traces minus a single exponential constant as called the "fitted" trace(s).

## 6.7 FunctionedLay Window



**Figure 7: FittedLay Window** with original trace in red and fitted trace in blue

#### **6.7.1 Add OverLay Button**

The "Add OL" button is used to attach the original unfitted or unaveraged wave to the FunctionedLay window in red to highlight the differences.

#### **6.7.2 Grid Button**

See section 6.7.1.

#### **6.7.3 "AnalyzeThis" Button**

Pressing this button allows for the user to analyze the fitted or averaged wave data versus using the default, original selected wave in the Traces Window.

### **6.8 Closing the Traces Window**

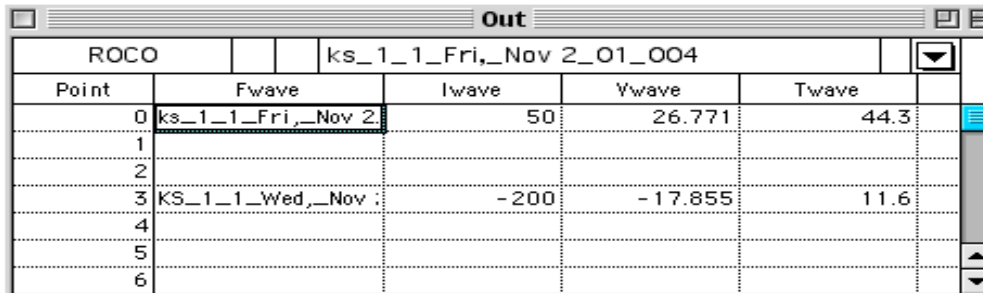
One may close the Traces window by pressing the "Done" button.

## ***7 I-V curves and Time Constants***

Now that we have introduced the interface, we can learn how to extract some useful information from our traces. Suppose that we wish to use a set of traces to compute an I-V curve. After loading the files which we want to extract IV data from, and we have selected the traces from each file that we want to use, we simply set the "t" variable in the IV/RC box in the Main window to be equal to the time after the pulse onset at which we want to measure the trace voltage ( in current clamp ) or current (in voltage clamp).

This time cannot be greater than the pulse duration or else it will generate an error. You are allowed to do IV curves if DAC2 is either in pulse mode or 2-pulse mode. In two-pulse mode it will be important to set the value of t such that you are taking data from the pulse series you are interested in. For instance if you give a 100 msec pulse of constant magnitude and then step a second pulse of 100 msec then you should set your  $t = 150$  in order to capture these variations in the second pulse.

Pressing the IV button will generate a table like the following:



Point	Fwave	Iwave	Vwave	Twave
0	ks_1_1_Fri,_Nov 2_	50	26.771	44.3
1				
2				
3	KS_1_1_Wed,_Nov :	-200	-17.855	11.6
4				
5				
6				

**Figure 6:** The output table for the IV/RC analysis

This table contains four columns. The Fwave column gives the name of the file which each item of data comes from (in Fig. 6 the Fwave column is semi-collapsed for spatial efficiency). The Iwave column gives the value of the current for each selected trial. The Vwave is the corresponding voltage value. The Twave gives the time constant for each trial. In the event that a trace is not selected there will simply be a blank line where that trial would normally be.

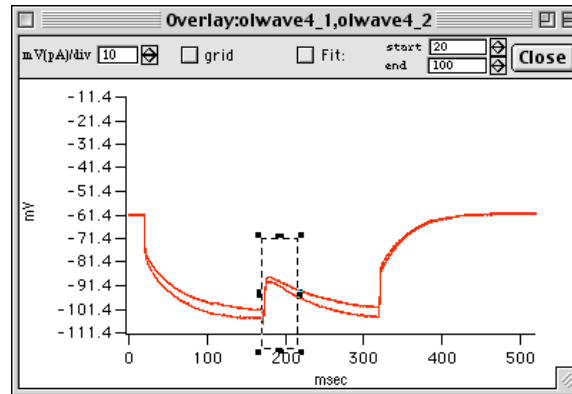
When you execute this analysis, you will notice that the Traces window closes and that you are not allowed to run any other analyses until you have decided to export or trash this data.

If you do not like the results of your analysis, you may press the "Trash" button, which deletes the waves which you have computed. If you would like to export your data to a text file, simply press "Export" and you will be prompted for the name of the file you would like to export to. Data is exported in tab-delimited text format suitable for reading with JMP.

## 8 Computing Reversal Potentials

One use which one may make of Slice is computing reversal potentials. This can be accomplished by holding the cell at a series of different voltage levels while stimulating an afferent fiber pathway at a latency, such that a synaptic current enters the cell when the cell is held at various potentials. The holding potential at which the current flow is zero is called the reversal potential. In current clamp mode, the reversal potential can be computed by plotting  $\Delta V$  versus the holding potential. The zero crossing of this plot is the reversal potential.

To compute a reversal, it is necessary to select the time range which you want to examine. Ideally you want the peak of the synaptic response to be the largest deviation from the baseline potential, which is the potential just before the pulse artifact. Then simply press the "Reversal" Button in the main panel. You may do this with chopped or unchopped data. It does not really make a difference



**Figure 7:** A range selection - the synaptic potential selected is the largest deviation from the baseline value (the value of the trace just before the chop)

The output should look something like this:

Out					
ROCO					
Point	Fwave	Bwave	Iwave	Vwave	Awave
0					
1	KS_1_1_Mon,_	-104.98	-90	15.453	100
2	KS_1_1_Mon,_	-101.75	-80	13.784	100
3					
4					
5					

**Figure 8:** The output of the Reversal Analysis

This analysis outputs a table containing the filename, the B-wave, which is the value of the voltage (in CC mode) or current (in VC mode) averaged over the millisecond preceding stimulation. The I-wave and V-wave are given as well. The A-wave gives the amplitude of the DAC0/1 stimulation, and the L-wave represents the latency of the DAC 0/1 pulse with respect to the start of the DAC2 command voltage or current.

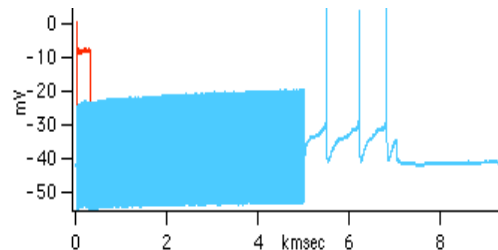
When you execute this analysis, you will notice that the Traces window closes and that you are not allowed to run any other analyses until you have decided to export or trash this data.

If you do not like the results of your analysis, you may press the "Trash" button, which deletes the waves which you have computed. If you would like to export your data to a text file, simply press "Export" and you will be prompted for the name of the file you would like to export to. Data is exported in tab-delimited text format suitable for reading with JMP.

## 9 Computing Spike Times

Slice Analysis also can extract spike times from a set of traces. To do this, go to the Spikes area of the main window, and set the criterion threshold for an event to count as a spike to the desired value. It may be useful to chop any stimulus artifacts from your trace since these will be counted as spikes if they are above the threshold level.

Point	FNwave	TRwave	SPwave
0	KS_1_1_Wed	15	20.2
1	KS_1_1_Wed	15	23
2	ijm_3_1_Jar	1	5492.9
3	ijm_3_1_Jar	1	6212.9
4	ijm_3_1_Jar	1	6812.1
5			
6			



**Figure 9** : OverLay window with Output of the Spike Times Analysis value of -17

This analysis outputs three waves. The first column is the filename wave. The second column is the trial wave -- it allows you to group spike times with particular traces since the number of spikes will vary from trial to trial. And the SP wave gives the time of each detected spike event, relative to the onset of data collection ( NOT the time of stimulation ).

When you execute this analysis, you will notice that the Traces window closes and that you are not allowed to run any other analyses until you have decided to export or trash this data.

If you do not like the results of your analysis, you may press the "Trash" button, which deletes the waves which you have computed. If you would like to export your data to a text file, simply press "Export" and you will be prompted for the name of

the file you would like to export to. Data is exported in tab-delimited text format suitable for reading with JMP.

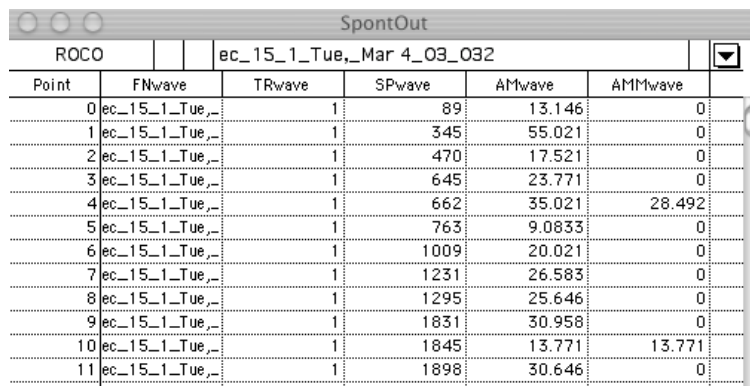
## 10 Spontaneous Analysis: Counting Activities and their relative Amplitudes

For spontaneous activity usually in the early stages of development, one requires a more refined Spike counting algorithm to pick up both subthreshold and suprathreshold activities.

### Algorithm for Analyzing Spontaneous Activity

1. Compute the mean  $\bar{W}$  of the trace during the pre-stimulus period (before the stimulus is delivered), and the standard deviation  $\sigma$  of the trace during this pre-stimulus period.
2. Walk through the trace,  $W(t)$  for  $t = 0$  to length of trace. If the trace value  $W(t_n)$  is greater than **sd** times  $\bar{W}$ , check to see if it is a local peak:
  - a. Evaluate if  $t_n$  is the local maximum of  $n(+1)$  to  $n$  plus variable **point\_ahead**
  - b. If (a) is true, then check if  $W(t_{n-(\text{point\_ahead}/2)})$  is less than  $3*\bar{W}$  and  $\bar{W}$  is not zero (for summed activity purposes).
3. After attaining all peaks with (2), check peak locations to see if there are 2 adjacent peaks with a difference less than  $2*\text{point\_ahead}$  apart. If the locations are less than  $2*\text{point\_ahead}$  apart, then the second peak is most likely has a summed amplitude – an amplitude with the first amplitude as an additional positive factor. We account for the extra amplitude of the second peak by:
  - a. Evaluate the  $\bar{W}$  of the first peak
  - b. Use the equation  $I = I_{\text{firstpeak\_amp}} * e^{-(\text{position of second peak}/\bar{W})}$  to obtain value of old peak at the position of the second peak.
  - c. Subtract old peak amplitude at the new peak position with new peak amplitude.

These results are then output into a table labeled “SpontOut” :



Point	FNwave	TRwave	SPwave	AMwave	AMMwave	
0	ec_15_1_Tue_...	1	89	13.146	0	<input type="checkbox"/>
1	ec_15_1_Tue_...	1	345	55.021	0	<input type="checkbox"/>
2	ec_15_1_Tue_...	1	470	17.521	0	<input type="checkbox"/>
3	ec_15_1_Tue_...	1	645	23.771	0	<input type="checkbox"/>
4	ec_15_1_Tue_...	1	662	35.021	28.492	<input type="checkbox"/>
5	ec_15_1_Tue_...	1	763	9.0833	0	<input type="checkbox"/>
6	ec_15_1_Tue_...	1	1009	20.021	0	<input type="checkbox"/>
7	ec_15_1_Tue_...	1	1231	26.583	0	<input type="checkbox"/>
8	ec_15_1_Tue_...	1	1295	25.646	0	<input type="checkbox"/>
9	ec_15_1_Tue_...	1	1831	30.958	0	<input type="checkbox"/>
10	ec_15_1_Tue_...	1	1845	13.771	13.771	<input type="checkbox"/>
11	ec_15_1_Tue_...	1	1898	30.646	0	<input type="checkbox"/>

**Figure 10:** SpontOut table with file name, trial number, event position, amplitude (without consideration of summed amplitude), amplitude with consideration of summed amplitude)

The amplitude column with the summed amplitude will only display an amplitude if has considered it one of the summed peaks, else the value will be zero.

## 11 Synaptic Analysis: One DAC Pulse and Basic Definitions

### 11.1 Basic Definitions

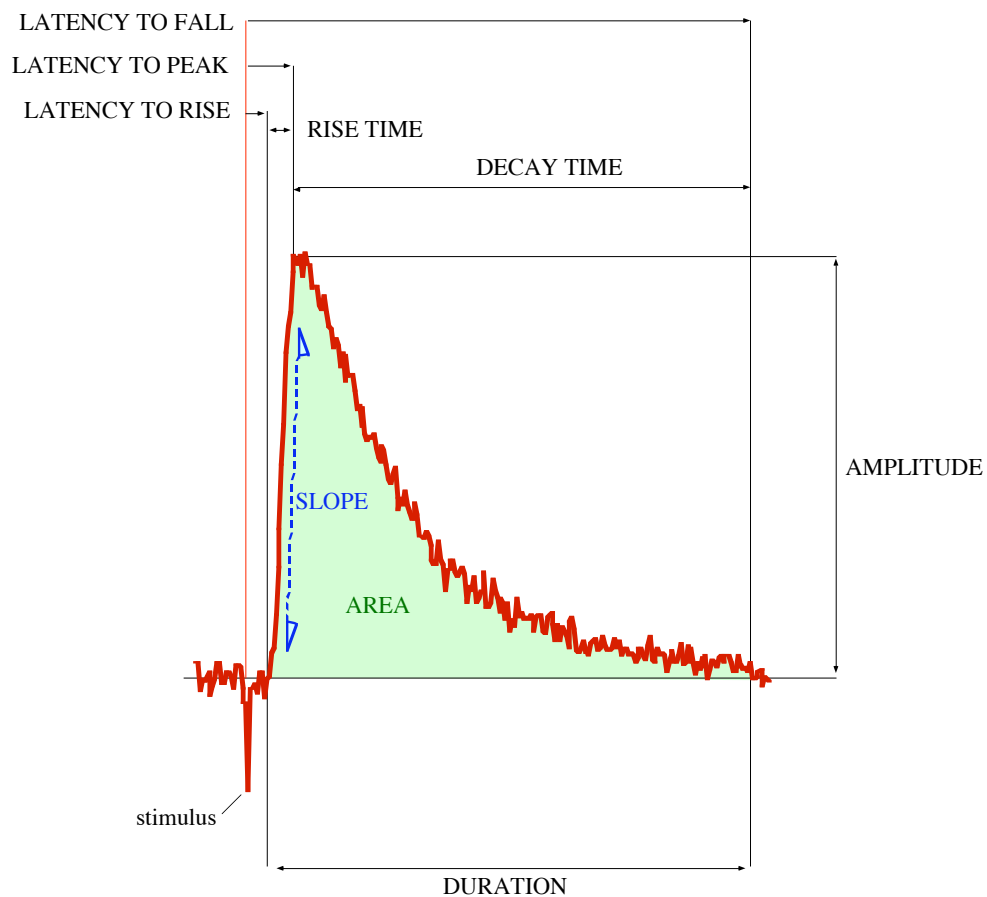
The case where a single pathway is stimulated is the simplest, and is a good starting point to discuss the analysis of synaptic data. The left portion of the analysis window contains the parameters, check boxes and popup menus relevant to the analysis of synaptic data. There are nine different analyses that can be performed, and parameters relevant to performing these analyses are in the lower left-hand corner of the

**Figure 11:** The synaptic analysis panel

main window. The 9 analyses are listed beside the checkboxes and may be performed simultaneously by checking the boxes you want and then pressing "Go". The Parameters relevant to the synaptic analyses are explained in the following table (**Table 4**):

Parameter	Definition
PA	Post-artifact: This is the number of milliseconds after the start of the pulse to begin searching for events , like the rise of the trace
N	N is the number of points in a row which

	need to be SD standard deviations above the mean value of the data collected during the pre-stimulus period in order for a rise to be detected. Details of how falls and rises are computed are given below.
SD	See above
S	S is the spacing between points used to compute the rising slope, which is the maximum slope (in absolute value) of the
-Pol	This tells us whether we are looking at traces which are positive-going or negative-going. By default we look for positive going events but checking this control will look for negative-going events. For mixed synaptic events, this allows you to specify which part you are interested in ( + or - )



**Figure 12:** A pictorial description of the different analyses of synaptic events



### 11.1.1 Latency To Peak

#### Algorithm:

1. Define a post-artifact wave for each trace consisting of all points from the original wave **PA** msec after the start of the stimulus pulse. Subtract the mean  $\bar{x}$  of the trace during the pre-stimulus period from this wave.
2.
  - a) if **-Pol** is unchecked, we find the location of the maximum value of this wave. Multiplication by sampling rate dependent factors gives us the peak in milliseconds.
  - b) if **-Pol** is checked, we find the location of the minimum value of this wave. Multiplication by sampling rate dependent factors gives us the peak in milliseconds.

### 11.1.2 Latency To Rise

#### Algorithm:

1. Compute the peak of the trace, the mean  $\bar{x}$  of the trace during the pre-stimulus period (before the stimulus is delivered), and the, standard deviation  $SD$  of the trace during this pre-stimulus period.
2. Smooth a copy of the trace (not modifying the original) and use smoothed copy for detections.
3.
  - a) if **-Pol** is unchecked, we begin our search at the peak of the trace and search backwards for **N** points in a row which are less than  $\bar{x} + SD * \bar{x}$ . The end of this run defines the rise for positive-going traces, and multiplication by sampling rate dependent factors gives this number in milliseconds.
  - b) if **-Pol** is checked, we begin our search at the peak of the trace and search backwards for **N** points in a row which are greater than  $\bar{x} - SD * \bar{x}$ . The end of this run defines the rise for positive-going traces, and multiplication by sampling rate dependent factors gives this number in milliseconds.

### 11.1.3 Latency To Fall

#### Algorithm:

1. Compute the peak of the trace, the mean  $\bar{x}$  of the trace during the pre-stimulus period (before the stimulus is delivered), and the, standard deviation  $SD$  of the trace during this pre-stimulus period.
2. Smooth a copy of the trace (not modifying the original) and use smoothed copy for detections.

3.
  - a) if **-Pol** is unchecked start from the peak and search forward to find **N** points in a row which are greater than  $\bar{x} + SD \cdot \bar{x}$ . The start of this run defines the fall for positive-going traces, and multiplication by sampling rate dependent factors gives this number in milliseconds.
  - b) if **-Pol** is unchecked start from the peak and search forward to find **N** points in a row which are lesser than  $\bar{x} - SD \cdot \bar{x}$ . The start of this run defines the fall for positive-going traces, and multiplication by sampling rate dependent factors gives this number in milliseconds.

The start of this run defines the rise for negative-going traces, and multiplication by sampling rate dependent factors gives this number in milliseconds.

#### 11.1.4 Amplitude

##### Algorithm:

1. Define a post-artifact wave for each trace consisting of all points from the original wave **PA** msec after the start of the stimulus pulse. Subtract the mean  $\bar{x}$  of the trace during the pre-stimulus period from this wave.
2. a) if **-Pol** is unchecked, we find the maximum value of this wave.
  - b) if **-Pol** is checked, we find the minimum value of this wave.

#### 11.1.5 Area

##### Algorithm:

1. Find rise and fall of wave as described above.
2. Define a wave which is simply the original wave in between the rise and fall minus  $\bar{x}$ .
3. Integrate this wave with respect to time

#### 11.1.6 Rising Slope

##### Algorithm:

1. Find the rise and peak location of the wave
2. As **x** varies from the start of the rise to the peak location - **S**, find the slope of the line from **x** to **x+S**, where **S** is the number of points separating the two points which define the tangent line.

### 11.1.7 Duration

#### Algorithm:

1.  $\text{Duration} = (\text{Latency to Fall}) - (\text{Latency to Rise})$

### 11.1.8 Rise Time

#### Algorithm:

1.  $\text{Rise Time} = (\text{Latency to Peak}) - (\text{Latency to Rise})$

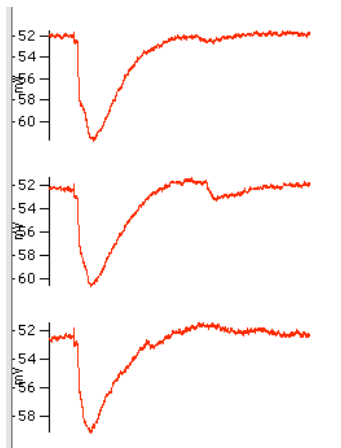
### 11.1.9 Decay Time

#### Algorithm:

1.  $\text{Rise Time} = (\text{Latency to Peak}) - (\text{Latency to Rise})$

## 11.2 Single Pulse Analysis

This is the basic synaptic analysis : to characterize properties of the waveform elicited by a single pulse delivered to the afferent fiber pathway.



**Figure 13:** Some single pulse synaptic data

[illegible]

The single pulse analysis outputs a number of waves. If one of the analysis boxes is left unchecked a blank wave is put out. Here is a list of the waves which are generated :

**Table 5:** List of waves generated by the single pulse synaptic analysis

## 12 Synaptic Analysis: Two DAC Pulse

For some experimental paradigms, one wants to stimulate two pathways which may be converging on the same cell, and do so at different latencies with respect to each other. We support this type of analysis with the Two DAC Pulse command, which performs the same analysis on both events ( the event after the first pulse and the event after the second pulse ). One restriction is that the polarity of the two events must be the same. For instance, both pathways must have an excitatory or both have an inhibitory effect.

In this case, there are two waves output in each category, one for each DAC. Also, the latencies of the two DACS are given as well.

Wave name	Information
FNwave	Filename wave -- gives the filename
LRwave0, LRwave1	Latency to Rise wave -- lists the latency to rise for selected traces
LPwave0, LPwave1	Latency to Peak wave -- lists the latency to peak for selected traces
LFwave0, LFwave1	Latency to Fall wave -- lists the latency to fall for selected traces
AMwave0,AMwave1	Amplitude wave -- gives the amplitude of the pulse
ARwave0,ARwave1	Area wave -- gives the area
RSwave0,RSwave1	Rising Slope wave
DRwave0,DRwave1	Duration wave
RTwave0,RTwave1	Rise Time wave
DTwave0,DTwave1	Decay Time wave
DAwave0,DAwave1	DAC amplitude wave -- gives the amplitude of pulses delivered from DAC 0 and DAC1
DLwave0,DLwave1	DAC latency wave -- gives the latency of the pulses delivered from DAC0 and DAC1
DDwave0,DDwave1	DAC pulse duration wave -- gives the duration of the pulses delivered from DAC 0 and DAC1

**Table 6:** List of the waves generated by the two pulse synaptic case

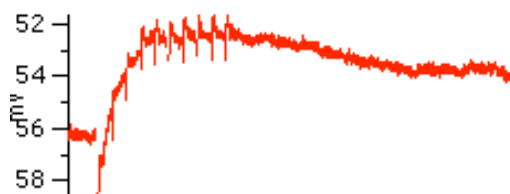
## 13 Synaptic Analysis: One DAC Train, Unitary

We support the ability to study the combined effects of a train of pulses, treating the temporally summed waveform as a single, unitary event to which we can apply the exact same measures we apply in the 1 DAC pulse case. This outputs the same waves as the One DAC Pulse case, plus two additional waves:

Wavename	Information
DIwave	DAC interval wave
DREPwave	DAC repetitions wave

**Table 7:** waves generated by the one DAC unitary train analysis

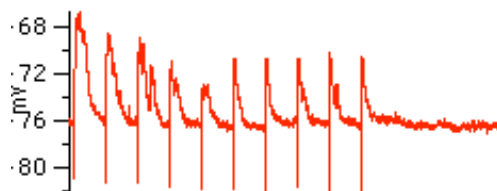
In this case it is often handy to apply the chop procedure so that you do not overestimate the pulse amplitude. For computation of area, it is recommended that you perform an interpolation once you have chopped your wave. Otherwise the area which is "under" the curve but was underneath a chopped artifact will not be counted. Alternatively, since the stimulus artifacts are usually brief and do not contribute significantly to the integral you may just compute the approximate area from the original trace.



**Figure 15:** A chopped summed EPSP resulting from delivering a train of pulses

## 14 Synaptic Analysis: One DAC Train, by Event

For some analyses, such as paired pulse facilitation or depression, you want to give a train of pulses to a synapse and analyze each event separately.



**Figure 16:** A train of pulses which we want to analyze on an event by event basis

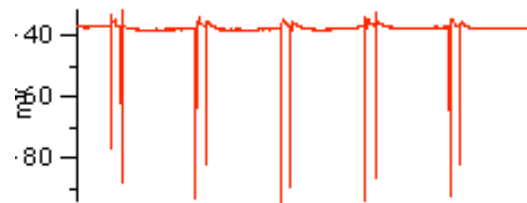
This analysis outputs the same waves as the unitary train analysis, with one addition. In the second column, an event wave (EVwave) is put out. This tells which pulse event the data in each row is associated with.

Out												
ROCO	cd_999_2_May_30_00_1											
Point	FNwave	EVwave	LRwave	LPwave	LFwave	AMwave	ARwave	RSwave	DRwave	RTwave	DTwave	
0	cd_999_2_May	1	4.6	26.1	134.3	9.277	532.07	3.125	129.7	21.5	106.5	
1	cd_999_2_May	2	3.3	9.1	100.7	7.5583	324.87	3.4375	97.4	5.8	91.4	
2	cd_999_2_May	3	4.9	8	111.4	7.0583	331.13	3.0625	106.5	3.1	103.4	
3	cd_999_2_May	4	5.2	7.9	76.4	5.0583	161.47	1.9375	71.2	2.7	66.4	
4	cd_999_2_May	5	5.6	28.2	72.1	3.1833	110.99	1.125	66.5	22.6	43.4	
5	cd_999_2_May	6	5.2	7.7	48.7	5.402	101.23	1.8125	43.5	2.5	43.4	
6	cd_999_2_May	7	5.3	9	71.2	5.3395	101.09	1.8125	65.9	3.7	62.4	
7	cd_999_2_May	8	5	8.5	65.8	5.3708	132.88	1.8125	60.8	3.5	51.4	
8	cd_999_2_May	9	5.2	8.4	67.5	5.8395	139.13	2.3125	62.3	3.2	59.4	
9	cd_999_2_May	10	5.4	8.8	76.1	5.527	125.55	1.875	70.7	3.4	67.4	
10												

**Figure 17:** The output of the train by event analysis

## 15 Synaptic Analysis: Two DAC Train, by Event

Often one wants to study the summation of currents injected from two fiber pathways. For instance in coincidence detection circuitry, one is often interested on the effect of relative stimulus latency on the post-synaptic potential.



**Figure 18:** Two DAC trains

This analysis assumes that the relative latencies of the two DAC pulses are small with respect to the inter-pulse interval, and analyzes the time from the end of the second of the two pulses to the first of the next pulse. In this analysis, it is required that the inter-pulse interval and the pulse duration be the same for both DACs.

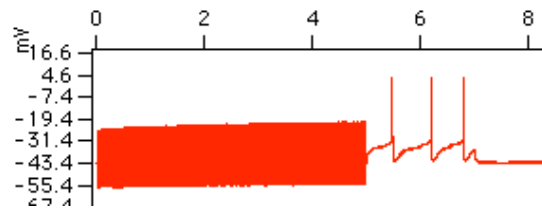
This analysis outputs the same waves as the One DAC by Event analysis, plus the following additional waves:

Wavename	Information
DAwave0	Amplitude of DAC0 channel
DAwave1	Amplitude of DAC1 channel
DLwave0	Latency of DAC0 channel
DLwave1	Latency of DAC1 channel

**Table 8:** Waves generated by the 2 DAC train analysis

## 16 Synaptic Analysis: *SynTrain&IntCell*

This option was made to analyze synaptic stimulation in train mode (DAC 0/1) and intracellular (DAC 2/3) stimulation experiments. Scientists can use this option to observe and analyze synaptic response, intracellular response, and the combination of both responses on each other. A practical application for this option is observing intracellular response after synaptic stimulation of tetanus.



**Figure 19:** synaptic tetanus with integrated intracellular response

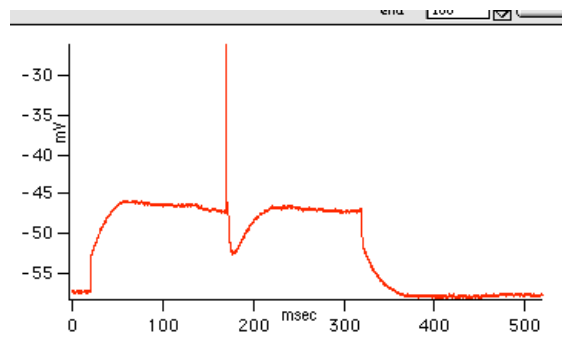
In observing synaptic responses during a long tetanus stimulation, it is advised to check off the interpolate and the chop and set the PA (post-artifact) to an appropriate time and then loading the file to save time during the chop.

## 17 Synaptic Analysis: *SynPulse&IntCell*

This feature is similar to the previous analysis, in that it analyzes data collected from both synaptic and intracellular stimulation. The only difference being that this one has a pulse form synaptic stimulation.

Waves generated from this analysis include FNwave, LRwave, LPwave, LFwave, AMwave, ARwave, RSwave, DRwave, DTwave, Lwave, DAWave, DDwave, and Iwave. The Iwave is included to indicate the intracellular stimulation used per trial.





**Figure 20:** FunctionedLay window showing both the fitted curves (in blue) and the original pipette recording (in red) after checking off the "Add OL" and grid button.

## Epilogue

We did not design this software to do every conceivable analysis. There are probably many of experiments that you want to try which are not covered by our simple little package. This means that you will need to write your own analysis software. A tutorial is currently under development that explains the principles employed in the analysis package, which allows you not only to develop new analysis programs but also to modify the existing program to keep current with any modifications you may make to the **Slice** Software.