



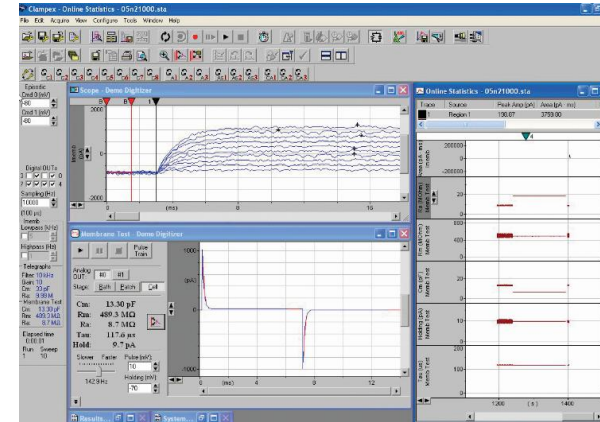
Together through life sciences.

## pCLAMP workshop

Jeffrey Tang, PhD  
2013

# Axon Conventional Electrophysiology Family

- pCLAMP software
  - Clampex-data acquisition
  - Clampfit-Data analysis
  - Axoscope-Data continuous monitoring
- Digitizer
  - Digidata 1440A-Analog-to-Digital converter
- Patch-clamp amplifiers
  - Axopatch 200B
  - MultiClamp 700B
  - AxoClamp 900A



# Flow of recording signal



Axopatch 200B



Axoclamp 900A



MultiClamp 700B

Amplifiers

Biological signals:  
current and voltage



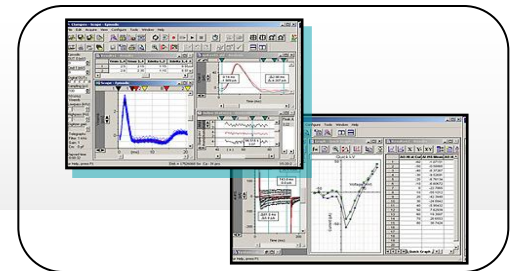
Digital  
Conversion



Digidata 1440A

Digitizer

Software



pCLAMP 10  
Software

# Electrophysiology rig



# Measurement Techniques

- **Extracellular voltage recording**
  - **Single unit and field potential recording in brain or brain slices, electrocardiograms, encephalograms and oculograms**
- **Voltage clamp**
  - **Voltage is held constant and the current passing through the cell membrane is measured**
  - **Patch-clamp recording**
- **Two electrode voltage clamp**
  - **Voltage is held constant through one electrodes and current is measured with the other electrode**
- **Current clamp**
  - **Current is held constant and the corresponding membrane voltage of the cell is measured**
- **Intracellular sharp electrode recording**
  - **Measurement of action potentials**
  - **Bridge balance for the pipette resistance**
- **Discontinuous clamp**
  - **Amplifier divides its time between passing current and recording voltage**
- **Ion-selective electrodes and electrochemistry**
  - **Measurement of small changes in ion, neurotransmitter and hormone concentrations in tissues and cells**

**MolDev Axon CEP Products**

# The position of Molecular Devices

- Market leader
  - Axon brand
    - over 25 years
  - High quality
    - ultra low noise amplifiers
    - High speed, low noise digitizers
  - Reliable
    - Lasting in a good condition for many years
  - Technical support
    - Professional assistance
- Large install base (17,000)
  - Academic research labs
  - Pharmaceutical/biotechnology drug discovery labs
  - >10K amplifiers sold
  - >10K digitizers sold
  - >10K copies of pCLAMP

# Customer support activities

- Scientific conferences
  - Society for Neuroscience
  - Biophysical Society
  - FENS
- Online webinars
  - Getting the Most Out of pCLAMP series
- pCLAMP Workshops at university
  - Duke University
- University loaner program/Support training courses
  - Cold Spring Harbor marine biology lab
  - Woods Hole marine biology lab
  - Australian Course in Advanced Neuroscience (ACAN)
  - Patch clamp workshop in Singapore
  - More...



# A Walkthrough of Protocol Editor in Clampex



# Golden Tips that help

- Study manual
- Study manual again
- Ask someone who uses pCLAMP
- Clampex tutorial
- Online HELP
- Knowledge base
- Webinar tutorials
- Technical Support

# Agenda



- Acquisition Mode
- Inputs
- Outputs
- Trigger
- Statistics
- Comments
- Math
- Waveform
- Stimulus

# Feature Highlights

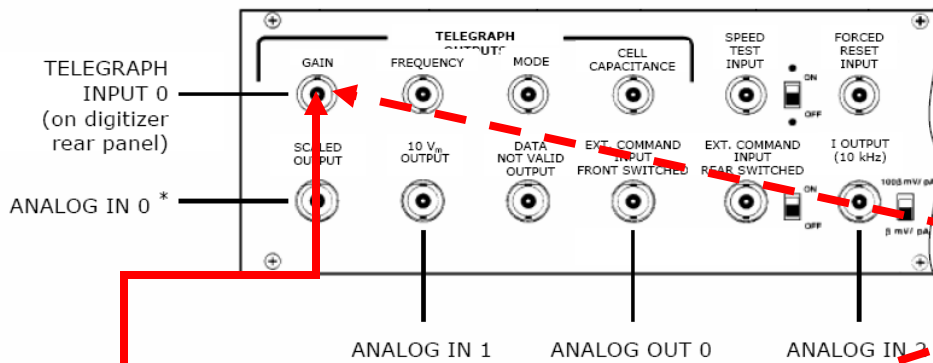
- Acquisition modes
- Scope trigger
- Holding level overrides
- Digital OUT holding pattern
- Stimulus file
- Digital bit pattern
- Pre-sweep train
- P/N leak subtraction
- User list
- Membrane test between sweeps

# Telegraphs

- **Clampex can receive and incorporate arrange of “telegraphed” amplifier settings as the variable gain, lowpass filter, whole-cell capacitance compensation.**

# Telegraph setting in AxoPatch™ 200B

## Axopatch Connections 3



Telegraphed Instrument

Input Channels

Telegraphed Instrument

Axopatch 200B

Telegraph Connections

Choose the digitizer channels to which telegraphs are connected:

Gain: Telegraph Input 0 Cm: (None)

Frequency: (None)

Amplifier Configuration

Amplifier output: Scaled output 1

Serial number: No Device Scan

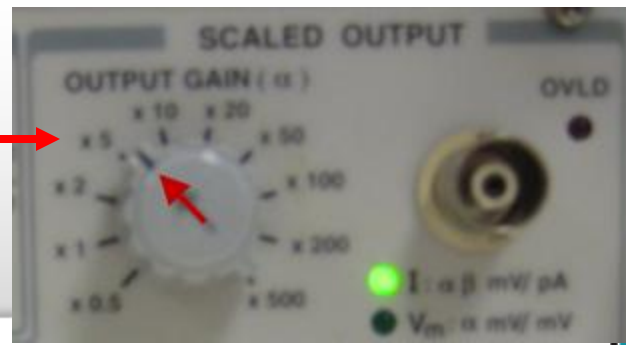
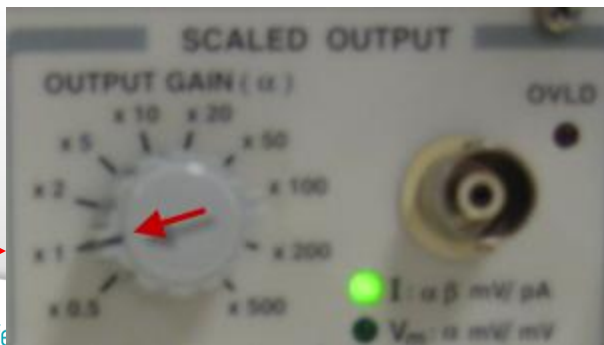
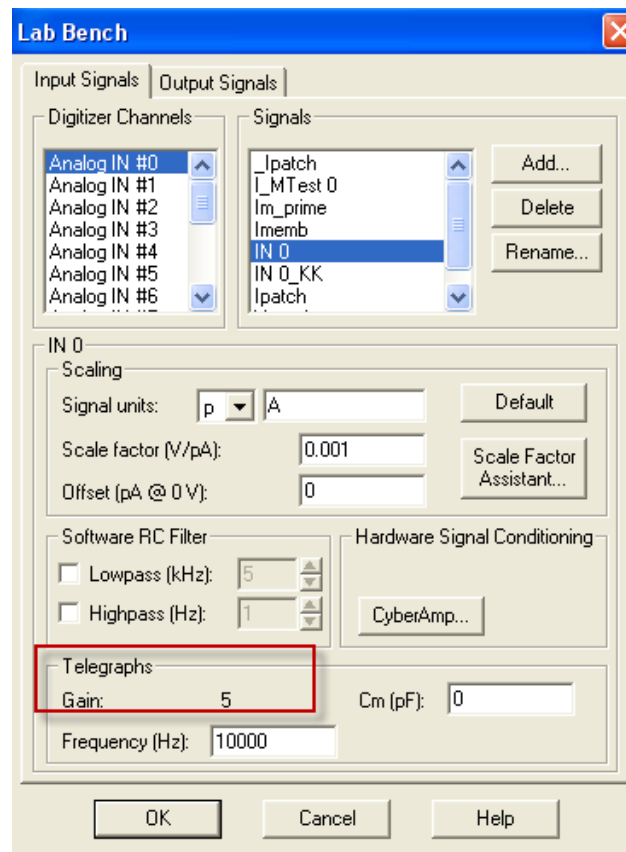
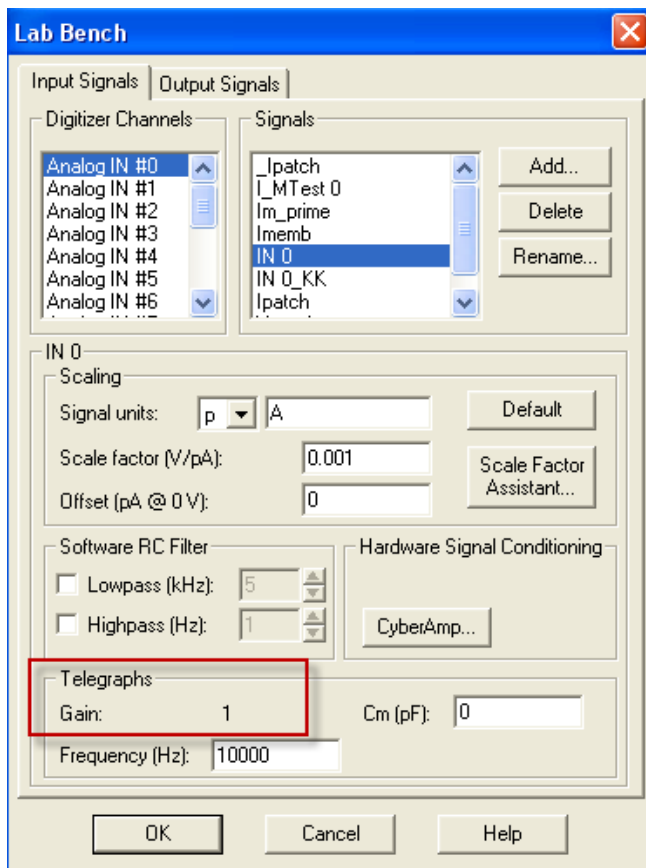
Output Channels

Choose the Analog OUT channel to which the command signal is connected:

Command: Analog OUT #0

OK Cancel Help

# Telegraph setting in AxoPatch™ 200B



# Telegraph setting in MultiClamp™ 700B

**Telegraphed Instrument**

Input Channels: Analog IN #0 to #10

Telegraphed Instrument: **Axon MultiClamp 700B**

Amplifier mode switches are automatically telegraphed.

Start new trial when mode, scale factor or units change. To simultaneously switch protocols, configure special Sequencing Keys (V-Clamp or I-Clamp IN).

Continue trial when mode, scale factor or units change.

Telegraph Connections: Choose the digitizer channels to which telegraphs are connected.

Gain: Telegraph Input 0    Cm: (None)

Frequency: Telegraph Input 1

Amplifier Configuration

Amplifier output: **Primary output 1**

Serial number: 00103420    Scan

Output Channels: Choose the Analog OUT channel to which the command signal is connected.

Command: **Analog OUT #0**

OK    Cancel    Help

**MultiClamp 700B : ( 00103420 )**

Channel 1: V (mV) -2.6    I (pA) -6.4

Channel 2: V (mV) -0.2    I (pA) 91.9

Mode: VC I=0 IC Ext

**V-Clamp 1** | I-Clamp 1 | V-Clamp 2 | I-Clamp 2

Holding: 0 mV    Seal Test: 10 mV @ 100 Hz    Pipette Offset: -3.31 mV

Cp Fast: 3.850 pF    1.73 μs    Cp Slow: 3.00 pF    370 μs    Tau x20

Whole Cell: 29.12 pF    11.22 MΩ    Rs Compensation: Bandwidth: 1.02 kHz

Correction: 0%    Prediction: 0%     Disable if oscillation detected

Primary Output: **Membrane Current ( 2.5 V/nA )**

Gain: **5**    Bessel: 10 kHz    AC: DC    Scope: Bypass

Output Zero: 0 mV    Leak Subtraction: 10.00 MΩ

Secondary Output: **Membrane Potential ( 10 mV/mV )**

Gain: 1    Lowpass Filter: 10 kHz

Pulse: 10 mV    10 ms    Zap: 500 μs    Rf: 500 MΩ

**Lab Bench**

Input Signals | Output Signals

Digitizer Channels: Analog IN #0 to #6

Signals: IN 0

IN 0

Scaling: Signal units: pA    Scale factor (V/pA): 0.001    Offset (pA @ 0 V): 0

Software RC Filter: Lowpass (kHz): 5    Highpass (Hz): 1

Hardware Signal Conditioning: CyberAmp...

Telegraphs: Gain: **5**    Cm (pF): 0.000    Frequency (Hz): 10000    Scale factor (V/nA): 0.5

OK    Cancel    Help

# Lab Bench setting in AxoPatch™ 200B

The screenshot displays the AxoPatch 200B software interface. The main window is titled "Clampex - [Scope - Demo Digitizer]". The "Configure" menu is open, showing options like "Digitizer...", "CyberAmp", "Lab Bench...", "Telegraphed Instrument...", "Overrides...", "Membrane Test Setup...", "Sequencing Keys...", "Program Options...", "Lab Book Options...", "Toolbars", "File Registration...", and "Voice Tags...". The "Lab Bench" dialog box is open, showing the "Input Signals" and "Output Signals" tabs. The "Input Signals" tab is active, showing "Digitizer Channels" (Analog IN #0 to #6) and "Signals" (Ipatch, I\_MTest 0, Im\_prime, Imemb, IN 0, Ipatch, Vmemb). The "IN 0" channel is selected, and its "Scaling" section is visible, showing "Signal units" set to "pA" and "Scale factor (V/pA)" set to "0.001". The "Output Signals" tab is also visible, showing "Digitizer Channels" (Analog OUT #0 to #3) and "Signals" (Cmd 0, OUT 0). The "Cmd 0" channel is selected, and its "Scaling" section is visible, showing "Signal units" set to "mV" and "Scale factor (mV/mV)" set to "20". The "Voltage Clamp" text is overlaid in green. Below the screenshot, two red numbered steps are listed: "1. To define signals for each digitizer channel" and "2. To set units and scale factors".

**Voltage Clamp**

1. To define signals for each digitizer channel
2. To set units and scale factors



# Lab Bench setting in AxoPatch™ 200B

**Lab Bench**

Input Signals | Output Signals

Digitizer Channels | Signals

Analog IN #0  
Analog IN #1  
Analog IN #2  
Analog IN #3  
Analog IN #4  
Analog IN #5  
Analog IN #6

\_Ipatch  
\_MTest 0  
Im\_prime  
Imemb  
IN 0  
Ipatch  
Vmemb

Vmemb

Scaling

Signal units: m V

Scale factor (V/mV): 0.001

Offset (mV @ 0 V): 0

Default

Scale Factor Assistant...

Output Signals

Digitizer Channels | Signals

Analog OUT #0  
Analog OUT #1  
Analog OUT #2  
Analog OUT #3

\_Cmd 0  
Cmd 0  
OUT 0

Cmd 0

Signal units: p A

Scale factor (pA/V): 400

Holding level (pA): 0

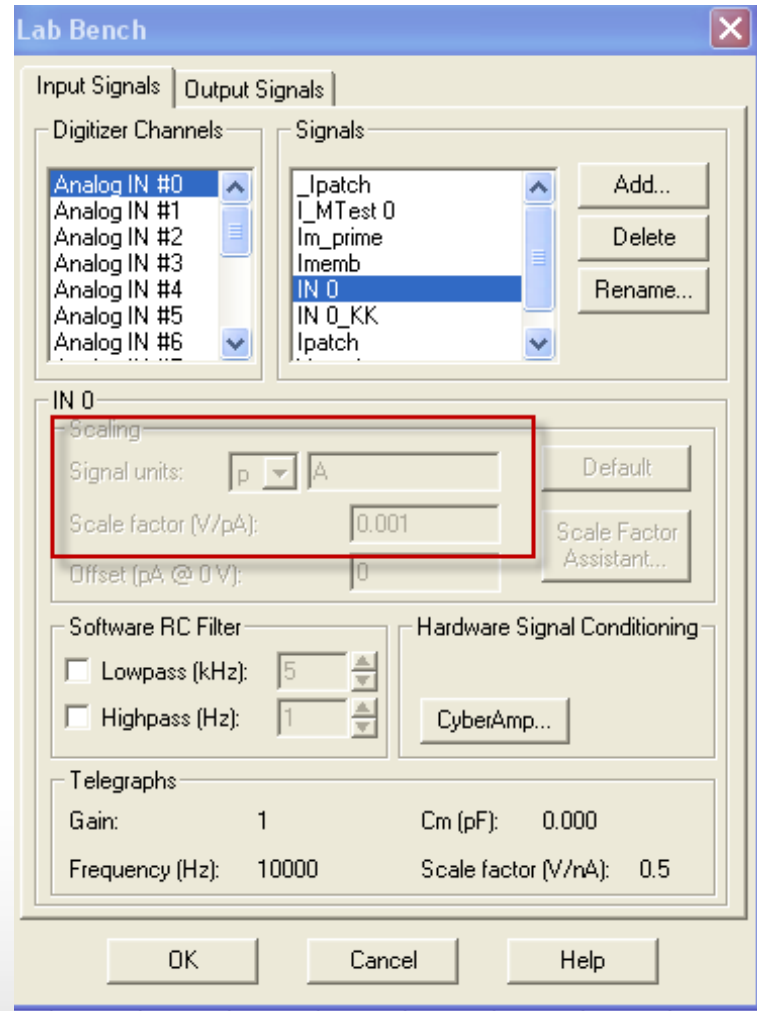
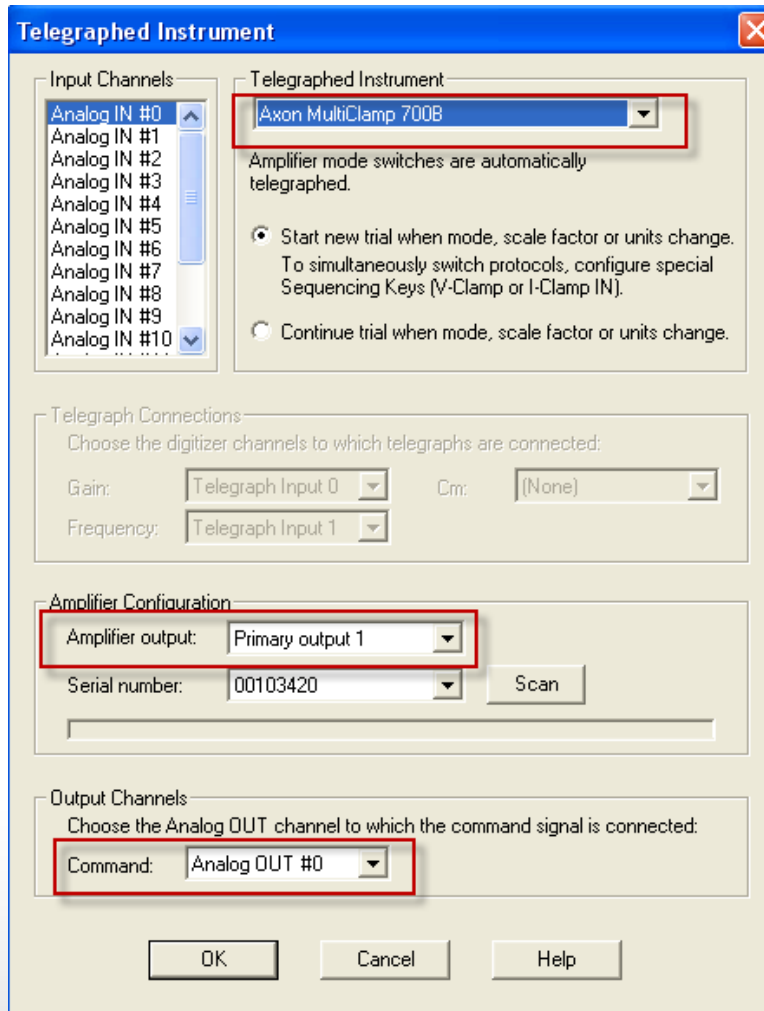
Scale Factor Assistant...

Analog holding level is disabled because of the setting in the Configure / Overrides dialog.

**Current Clamp**

1. To define signals for each digitizer channel
2. To set units and scale factors

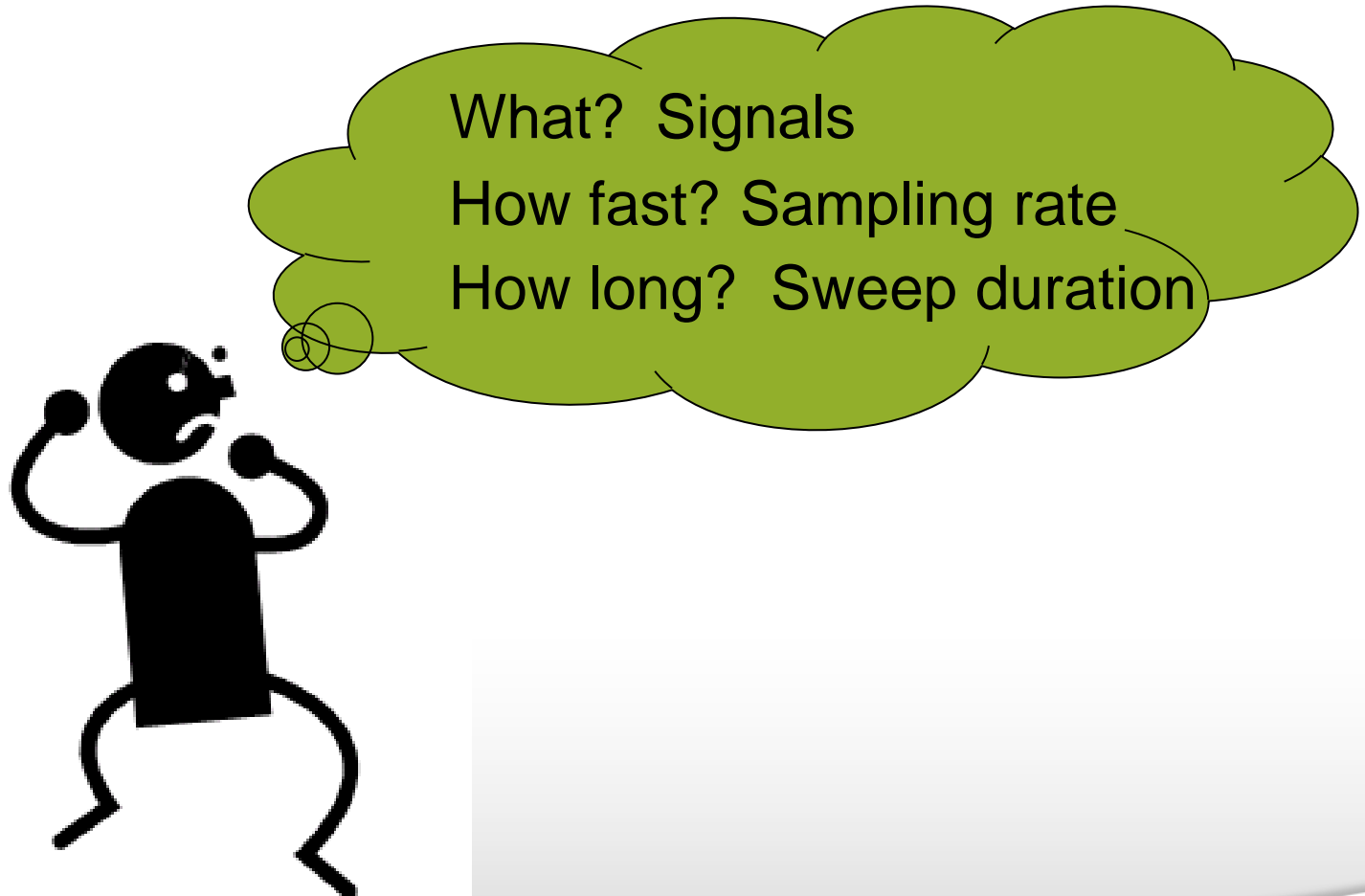
# Lab Bench setting is telegraphed in MultiClamp™ 700B



# Protocol Editor

**Protocol Editor:**  
is the "central" for protocol writing.

# Acquisition Mode?



# Mode/Rate

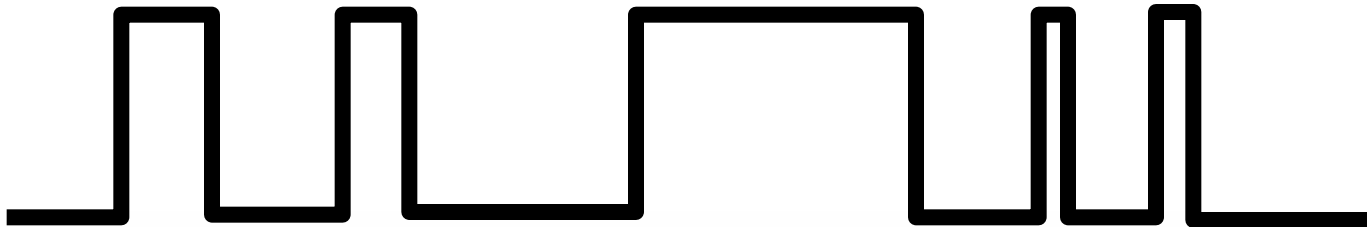


- **Passive Acquisition**
  - **Gap-free**
  - **Fixed-length**
  - **Variable-length**
  - **High-speed oscilloscope**

- **Stimulus Acquisition**
  - **Episodic stimulation**

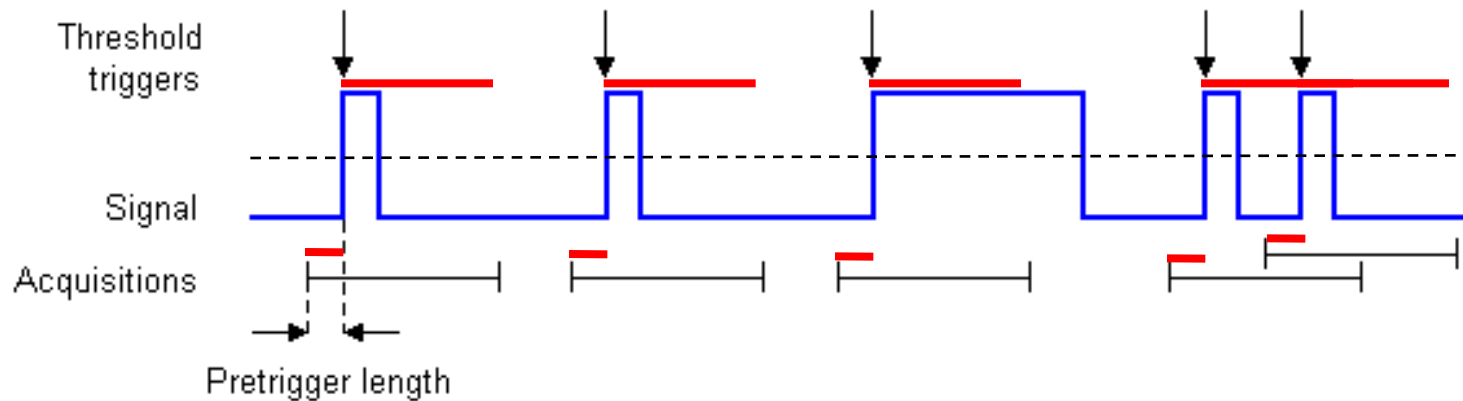
# Gap-free Mode

- Data are acquired continuously
- No gaps in data file
- single-channel or minis recordings

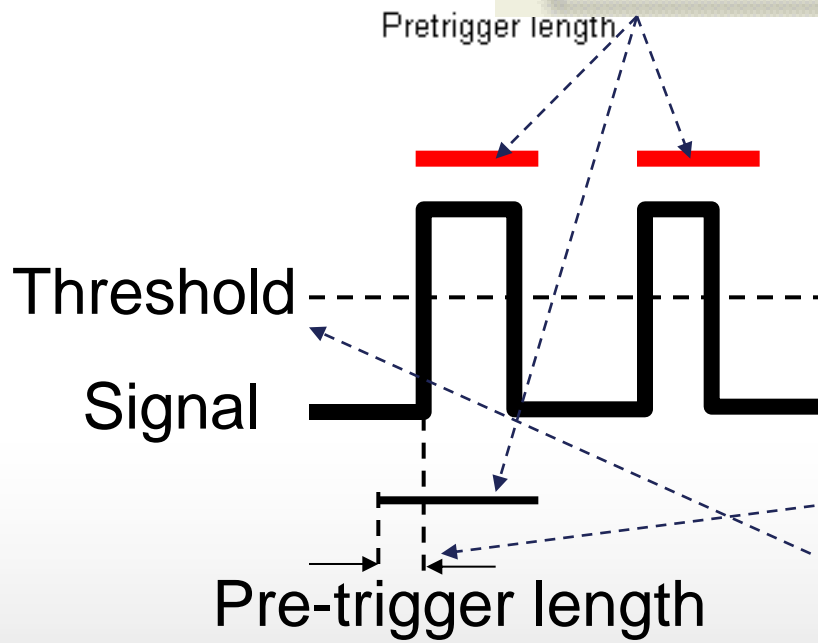
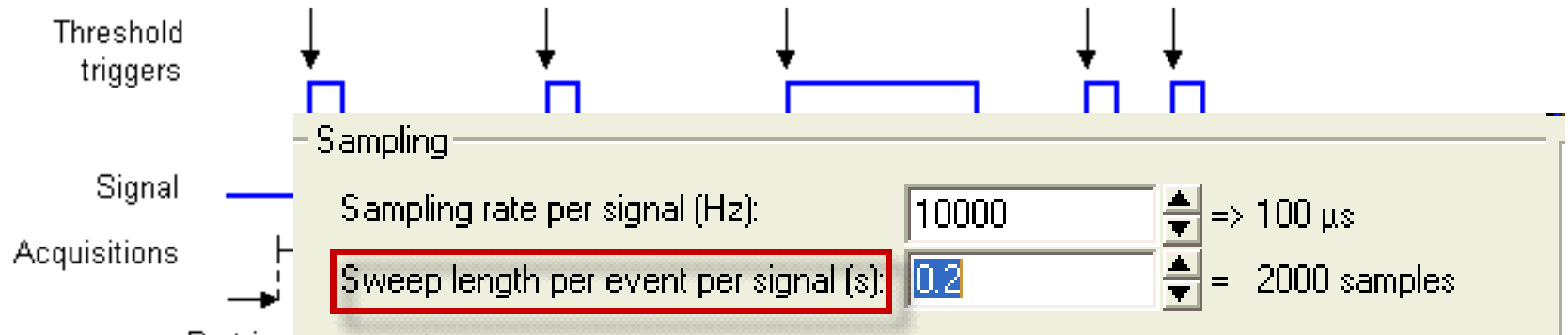


# Fixed-length Events Mode

- Constant data segment above the threshold and pre-trigger portion are recorded
- Action-potential spikes or other constant-width events recordings



# Fixed-length Event Mode



Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments |

Start trial with: Immediate

Threshold-based statistics source: First Acquired Signal

Scope trigger

Statistics Settings

Pretrigger length (ms): 20 = 200 samples

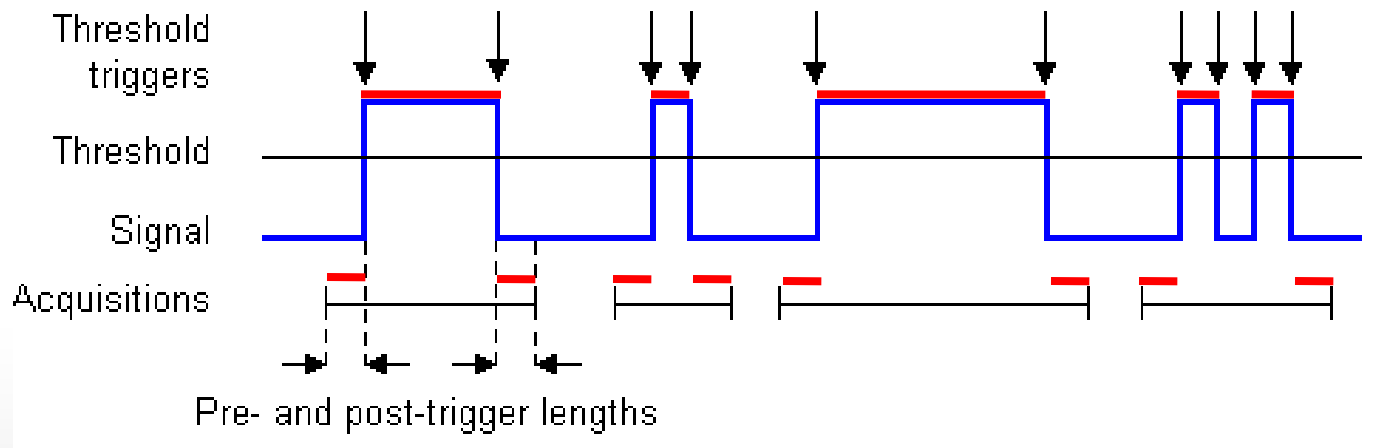
Statistics threshold (pA): 50

The screenshot shows the configuration window for the Edit Protocol. The window has tabs for Mode/Rate, Inputs, Outputs, Trigger, Statistics, and Comments. The Start trial with dropdown is set to Immediate. The Threshold-based statistics source dropdown is set to First Acquired Signal. The Scope trigger checkbox is checked. The Statistics Settings section is expanded, showing the Pretrigger length (ms) set to 20, which is equivalent to 200 samples, and the Statistics threshold (pA) set to 50.

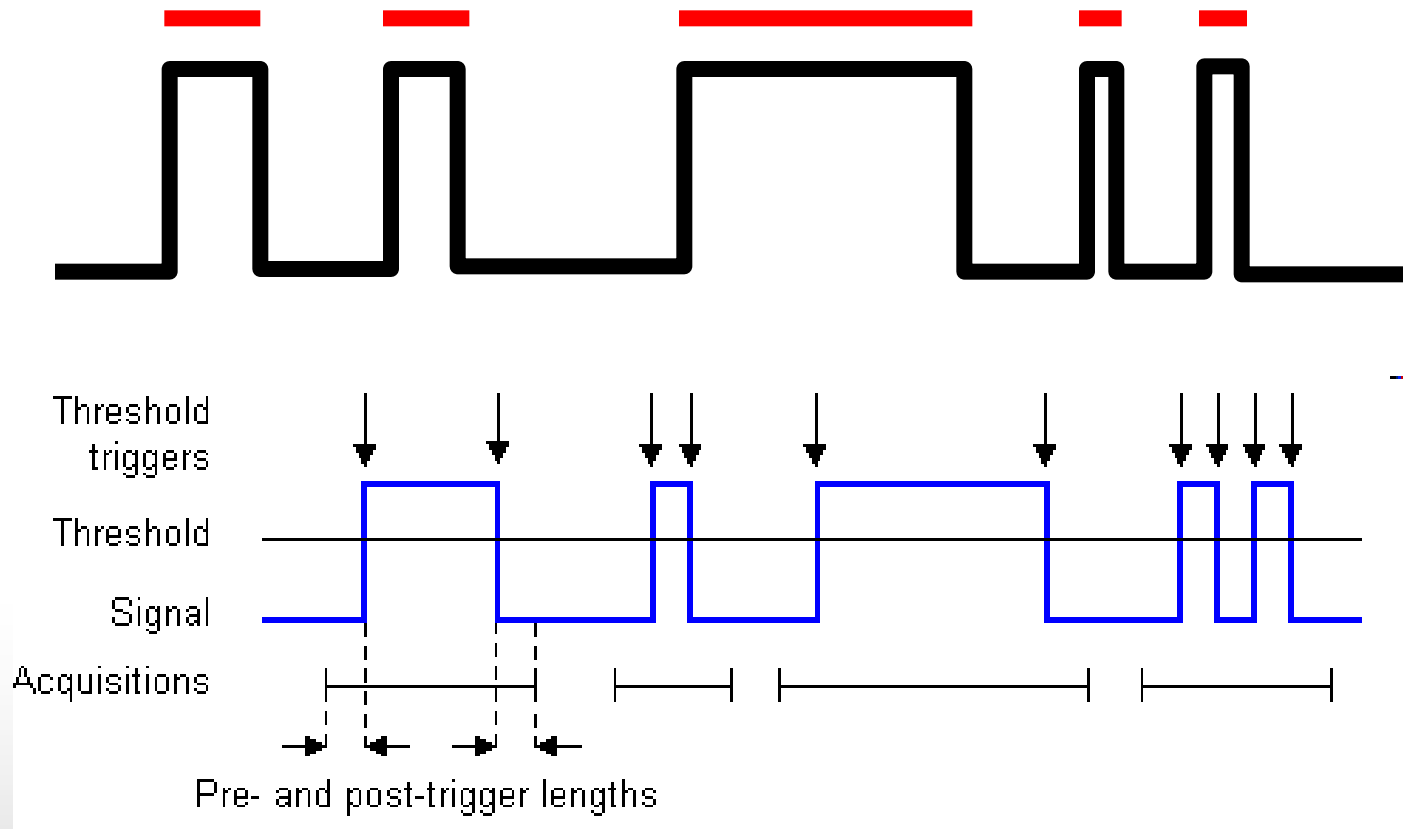


# Variable-Length Events Mode

- Data above the threshold, and for pre- and post-trigger periods are recorded.
- Variable recorded segment
- "Bursting" data recording

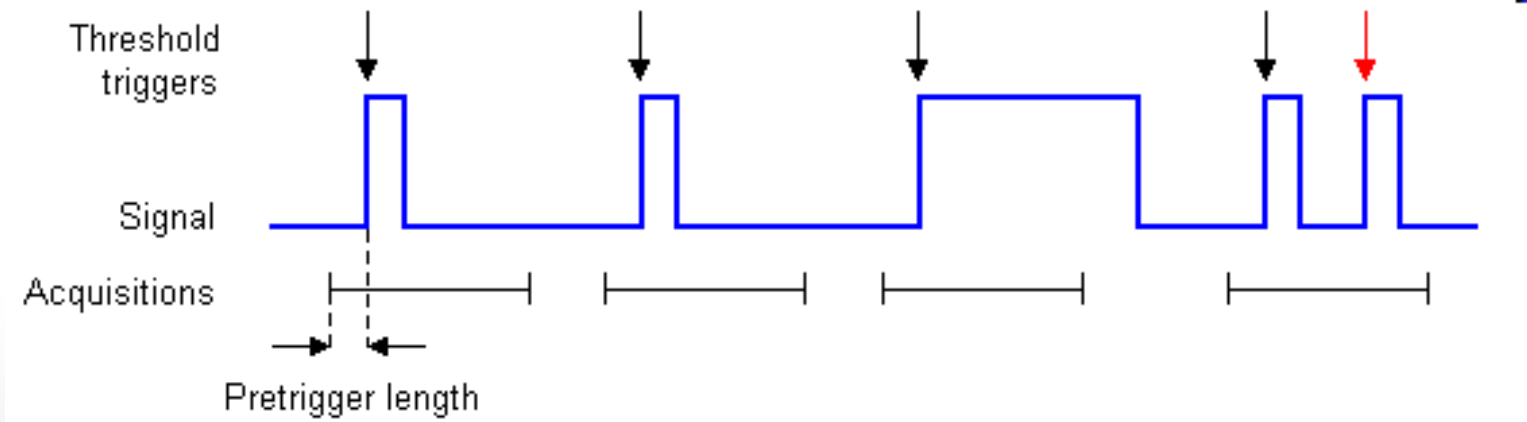


# Variable-Length Events Mode



# High-Speed Oscilloscope Mode

- Like an oscilloscope
- Data above the threshold, and for pre- and post-trigger periods are recorded.

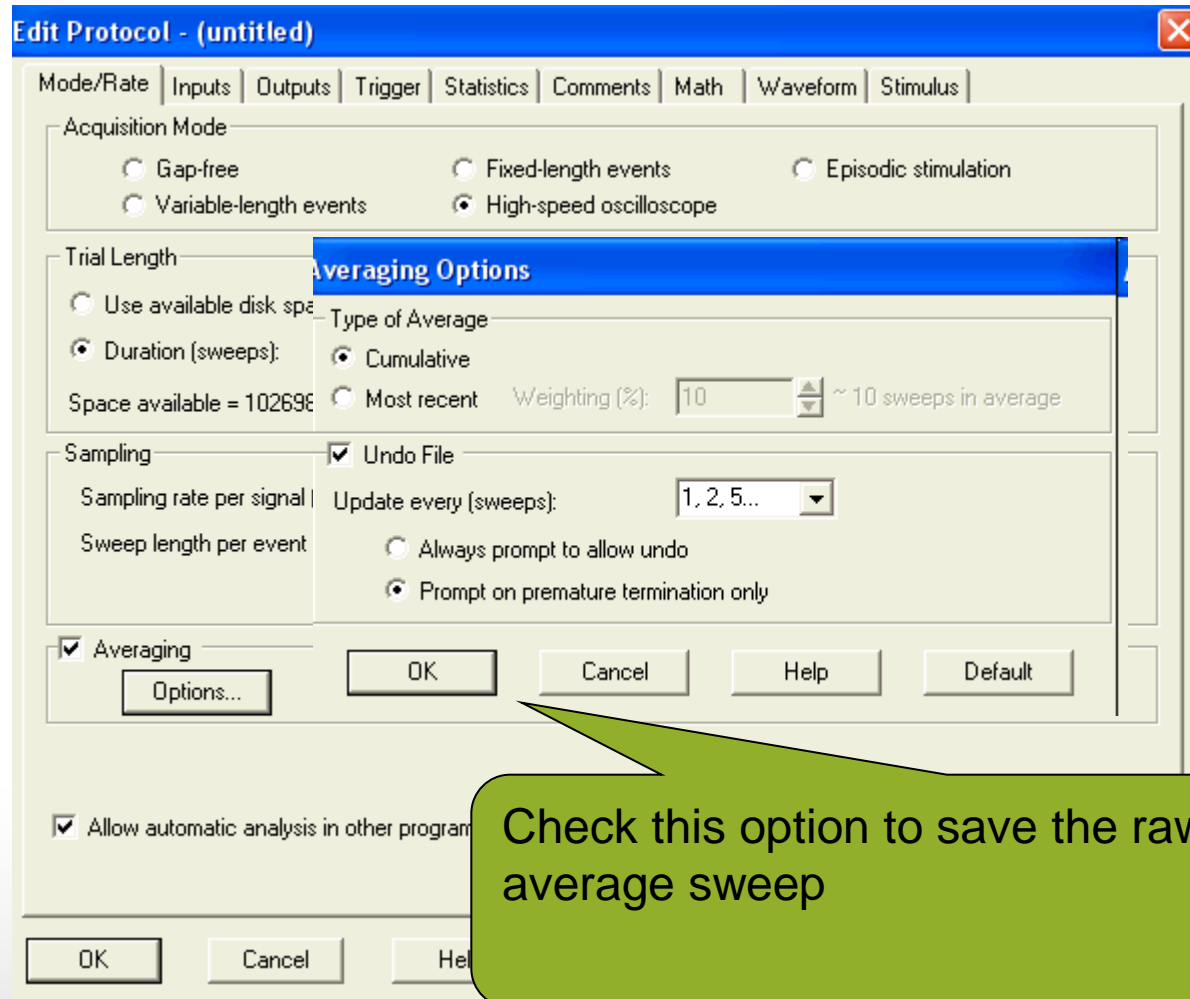


# Passive acquisition modes

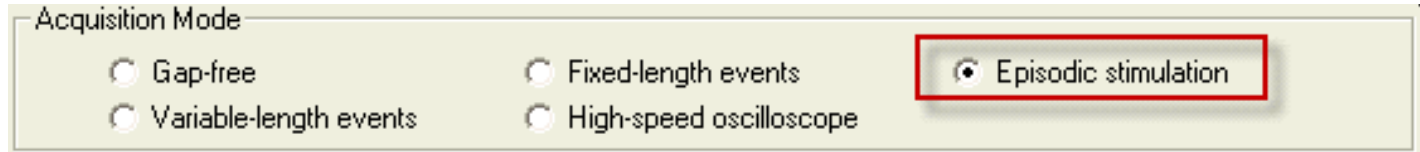
The screenshot shows a dialog box titled "dit Protocol - (untitled)" with several sections and callouts:

- Acquisition Mode:** Radio buttons for "Gap-free" (selected) and "Variable-length events". A callout points to this section: "Check this option to acquire data until the disk is full, or until acquisition is stopped manually".
- Trial Length:** Radio buttons for "Use available disk space" and "Duration (hh:mm:ss): 00:00:05 = 0.10 MB". A callout points to the duration field: "the duration of the data acquisition. in megabytes, and in hours, minutes and seconds". Below this, it says "Space available = 57:03:16 (hh:mm:ss) (28493 MB)".
- Sampling:** Input fields for "Sampling rate per signal (Hz): 10000" and "Sweep length per event per signal: 0.2". A callout points to the sampling rate field: "Specify the sampling rate of the analog-to-digital conversion".
- Checkboxes:** A checked checkbox "Allow automatic analysis in other programs". A callout points to it: "Enable Configure / Automatic Analysis in Clampfit to have new files automatically open in Clampfit."
- Buttons:** "OK", "Cancel", and "Help" buttons at the bottom.

# High-speed Oscilloscope Mode

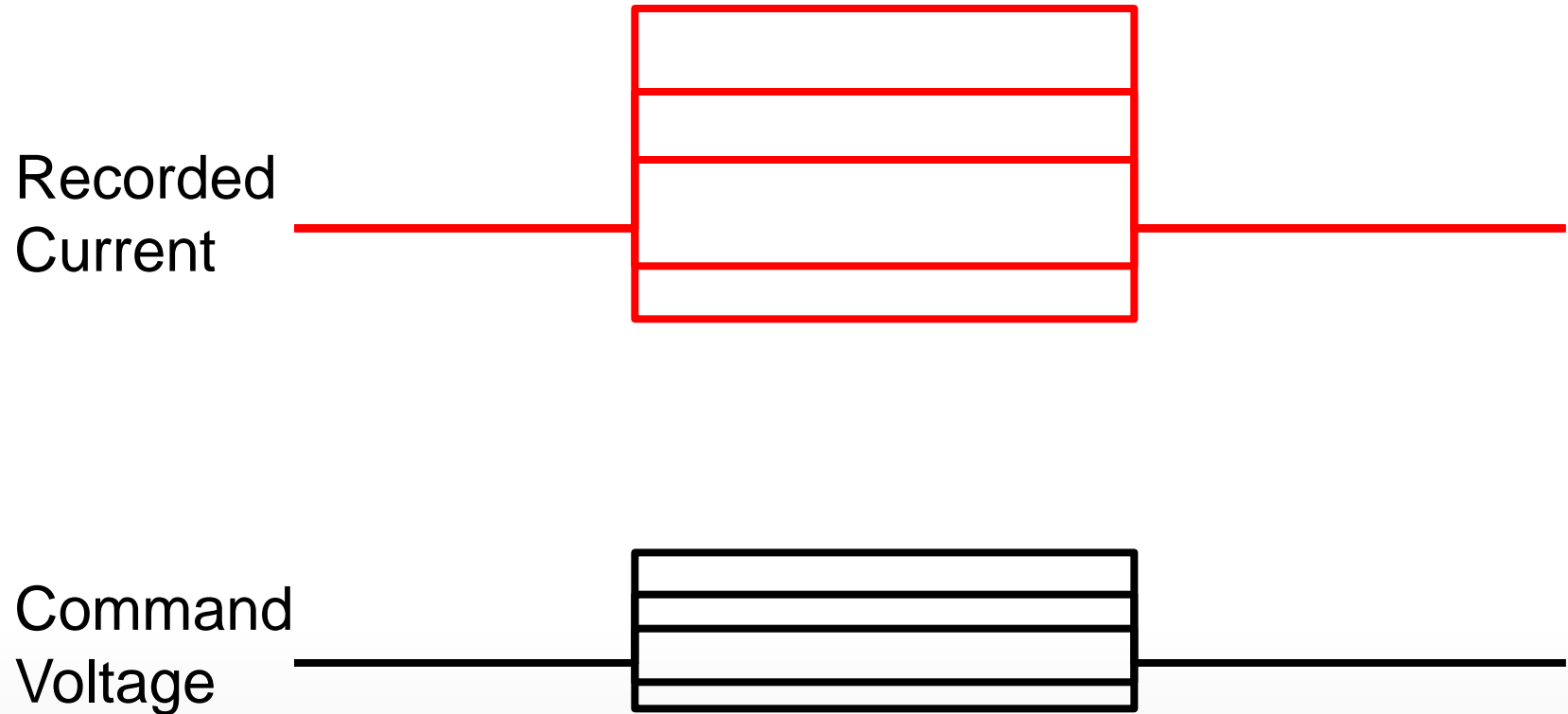


# Episodic Stimulation Mode



- Provide a command waveform and record responses, in fixed-length sweeps
- Analog waveform, holding level and/or digital pulses are outputs
- Special features include pre-sweep trains, online leak current subtraction, online event detection and statistics, and an on-line derived-math channel.

# Episodic Stimulation Mode



# Episodic Stimulation Mode

Specify a delay in seconds before the trial begins.

Specify how many runs to execute in the trial, up to 10,000. Runs are always averaged, with just the average run being saved to the data file.

Set the number of sweeps in each run, up to 10,000.

Enter the sweep duration in seconds, The maximum number of samples is 1,032,258 per sweep per channel.

Dialog box titled "Episodic Stimulation Mode" with tabs: Outputs, Trigger, Statistics, Comments, Math, Waveform, Stimulus.

Options: Gap-free, Variable-length events

Trial Hierarchy

Trial delay (s): 0

Runs/trial: 1

Sweeps/run: 10

Sweep duration (s): 0.2

First holding: 3.1 ms, 31 samples

Epochs: 193.8 ms, 31 samples

Last holding: 3.1 ms, 31 samples

Sampling Rate per Signal: Fast rate

Slow rate

Space available

Allow auto

OK Cancel Help

Acquisition mode: Episodic stimulation

Update Preview

0.04 MB = 2000 samples

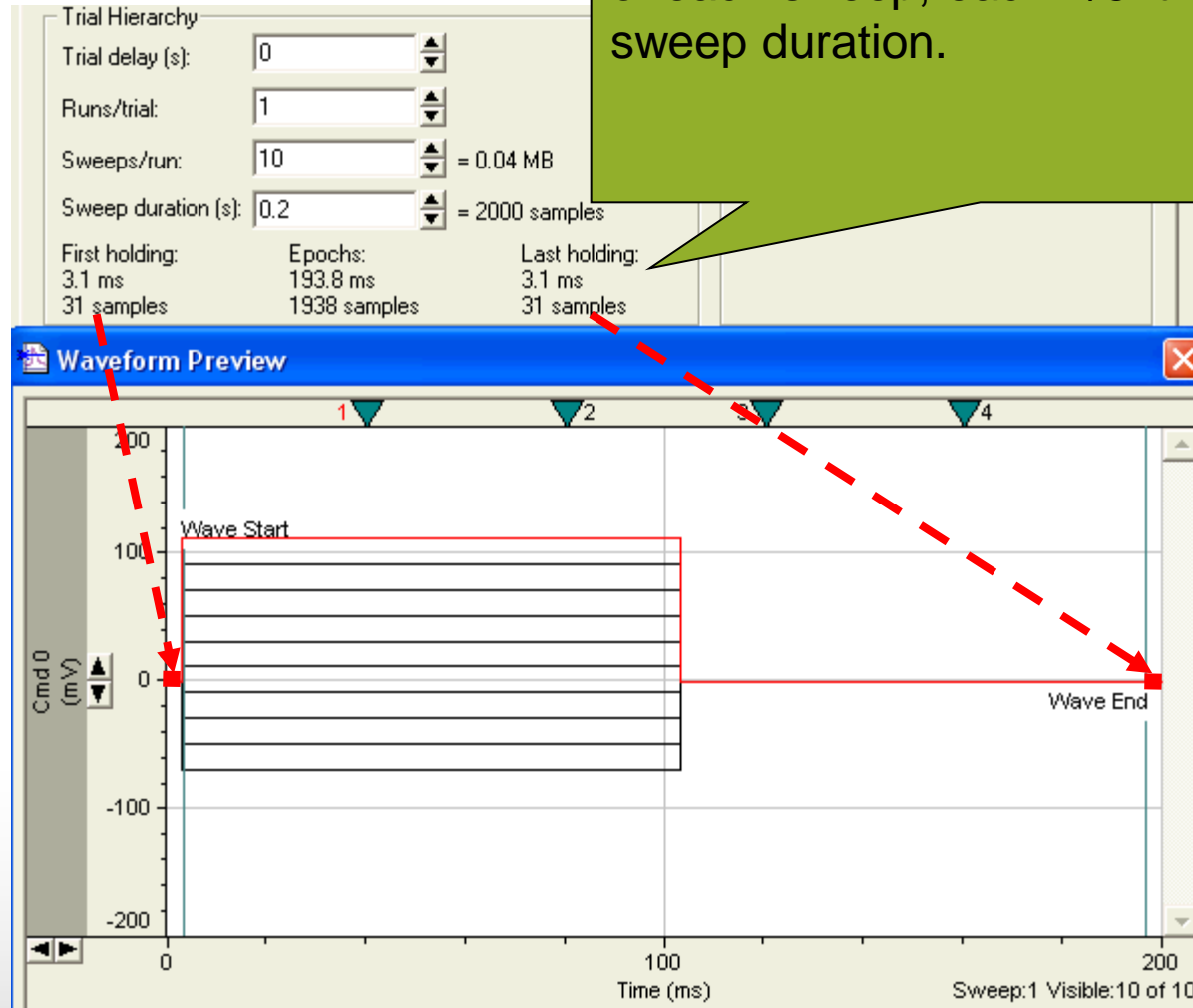
Sweep (s): Minimum

Averaging: Runs/trial = 1, no averaging



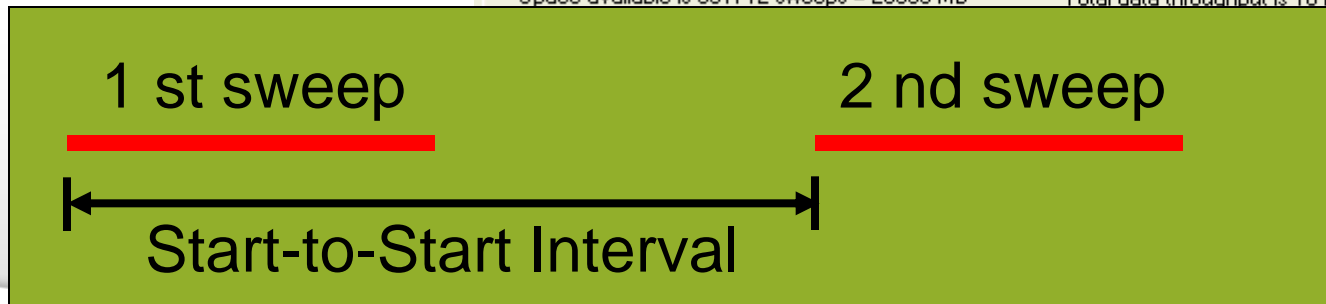
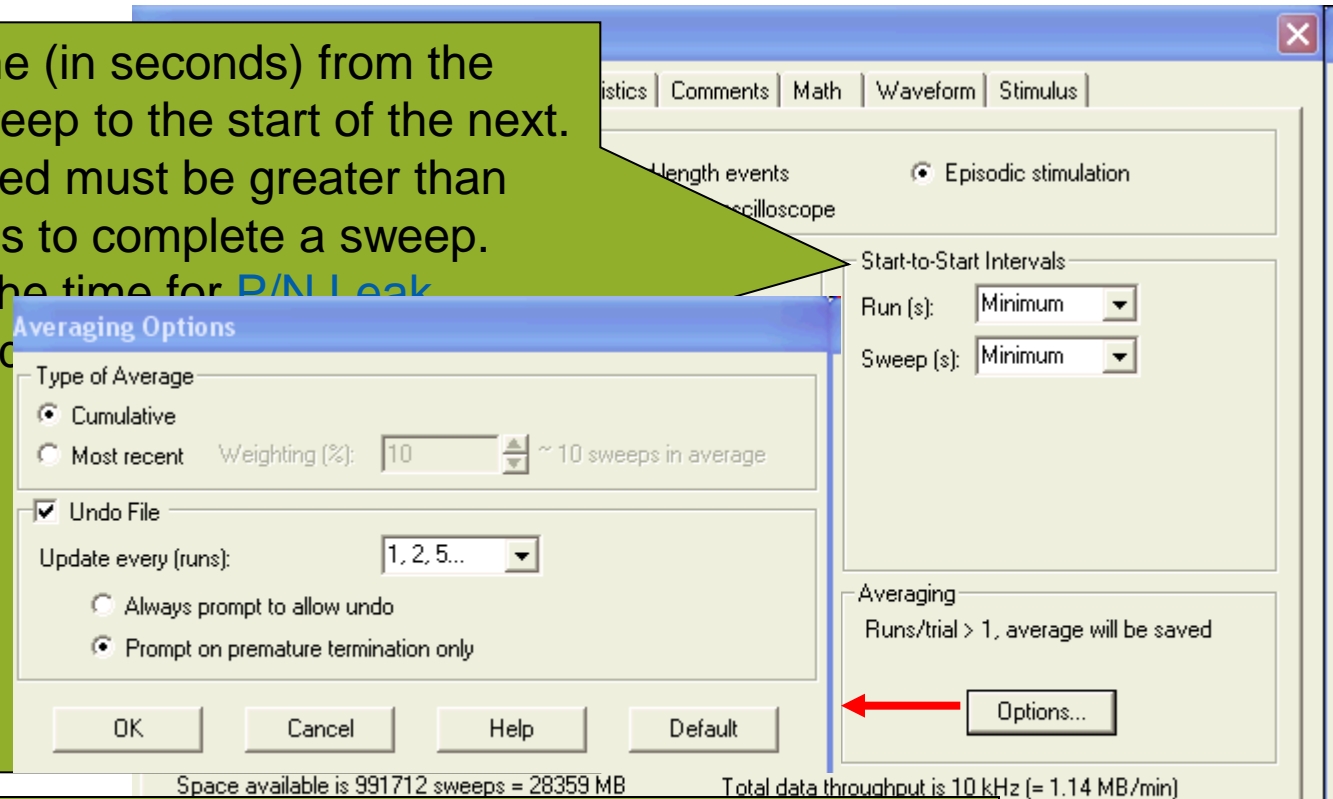
# Episodic Stimulation Mode---Holding Periods

Two "holding" periods at the start and end of each sweep, each 1/64th of the total sweep duration.

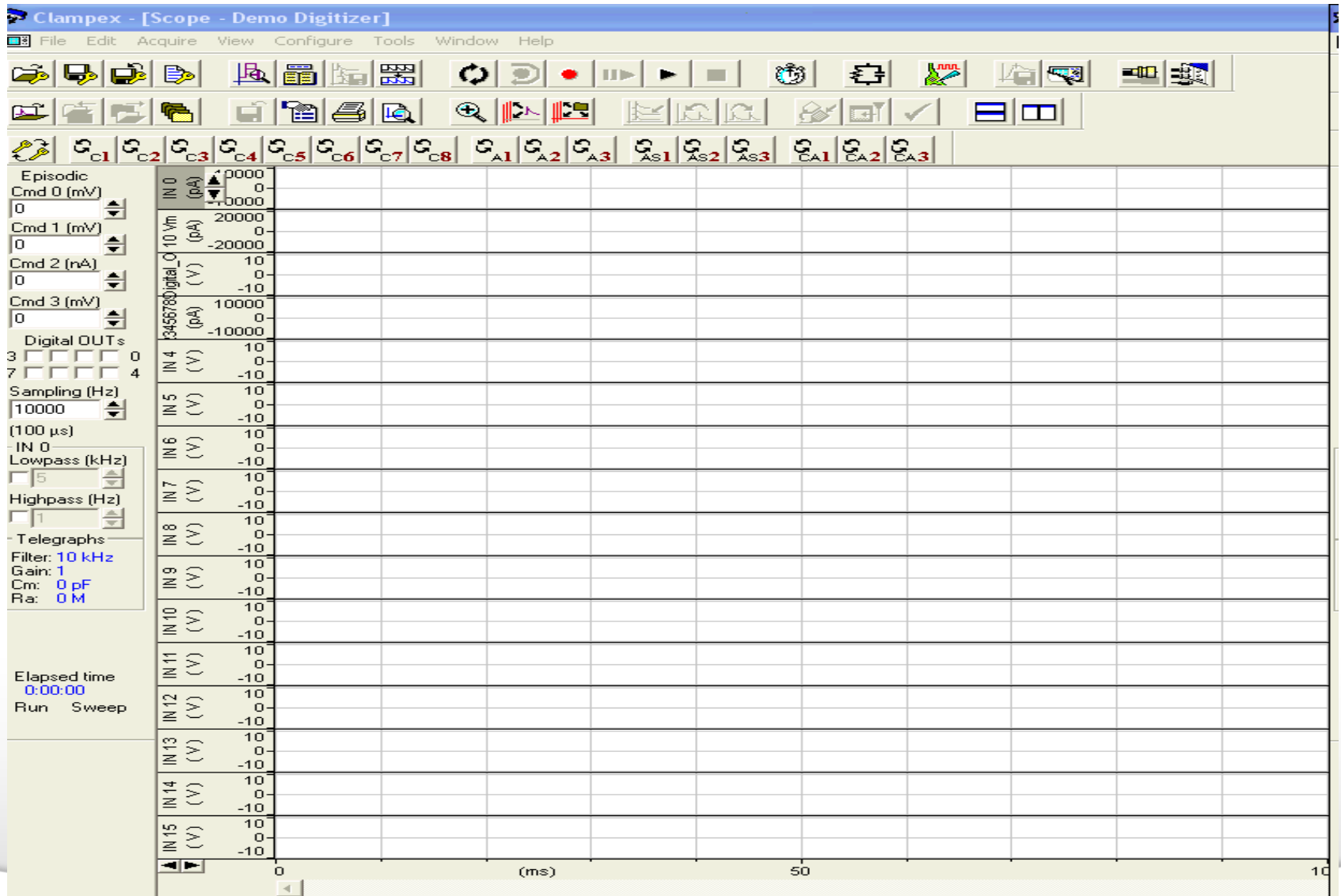


# Episodic Stimulation Mode

1. Specify the time (in seconds) from the start of one sweep to the start of the next.
2. The time entered must be greater than the time it takes to complete a sweep. This includes the time for [P/N Leak Subtraction](#) and



# Inputs



# Q & A

- Q: Why the selected input name in the Lab Bench does not show up in the scope window?
- A: You need to select the input name in the Input tab of the Edit Protocol.

# Outputs

Mode/Rate | Inputs | **Outputs** | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Analog OUT Channels

Channel #0:	Cmd 0	Range (mV): -200.00 to 199.99 at 20.0 mV/V
Channel #1:	Cmd 1	Range (mV): -200.00 to 199.99 at 20.0 mV/V
Channel #2:	Cmd 2	Range (nA): -10.00 to 10.00 at 1.0 nA/V
Channel #3:	Cmd 3	Range (mV): -200.00 to 199.99 at 20.0 mV/V

Analog OUT Holding Levels

Cmd 0 (mV):	-70
Cmd 1 (mV):	0
Cmd 2 (nA):	0
Cmd 3 (mV):	0

Digital OUT Holding Pattern

7	6	5	4	3	2	1	0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**Voltage-clamp**

**Current-clamp**

Check to specify one digital output channel to go high (and stay high) during the entire length of a trial

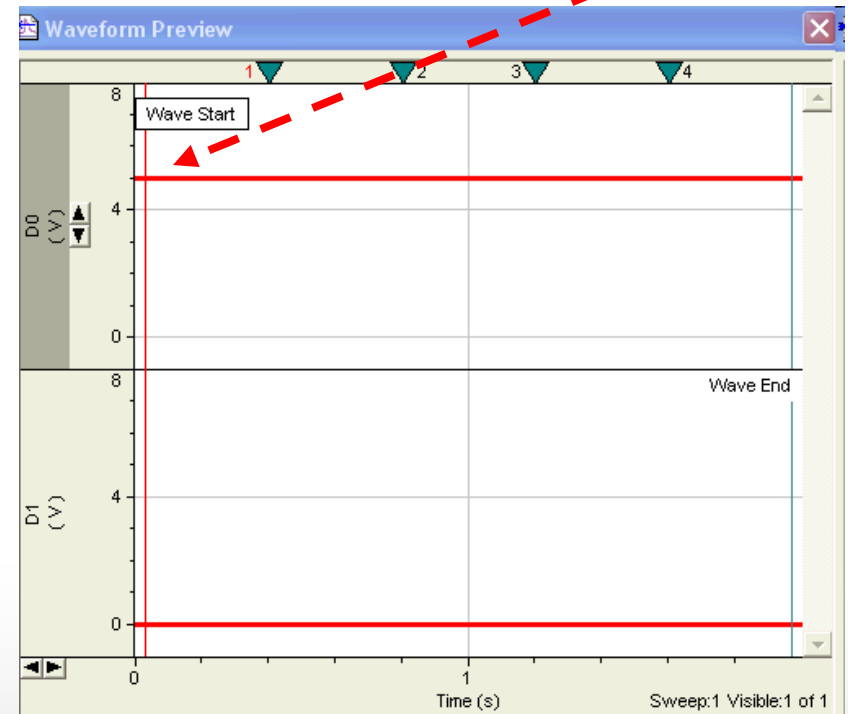
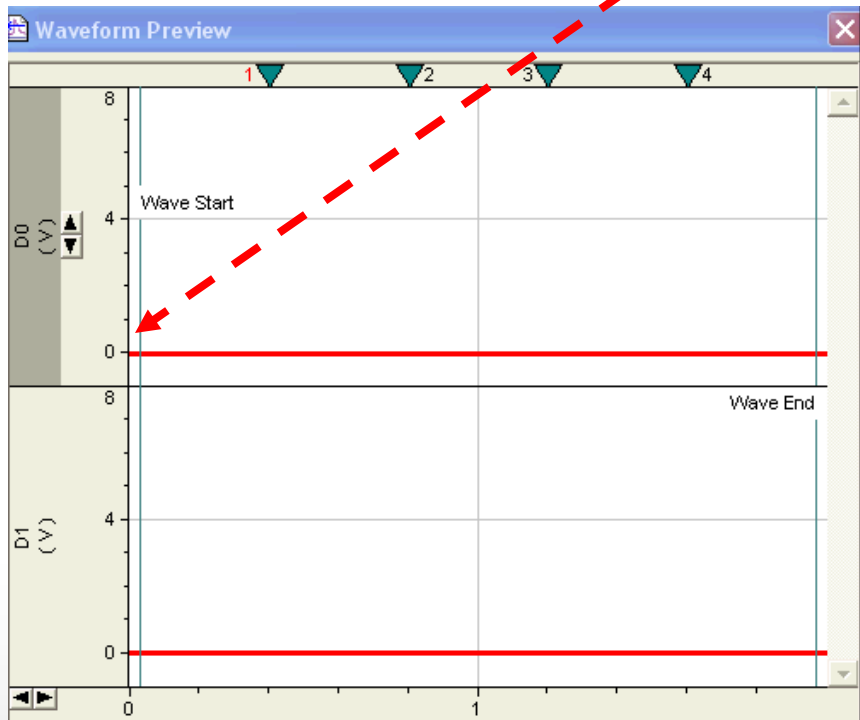
# Digital Out Holding Pattern

Digital OUT Holding Pattern

7	6	5	4	3	2	1	0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Digital OUT Holding Pattern

7	6	5	4	3	2	1	0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>



# Holding Level Overrides

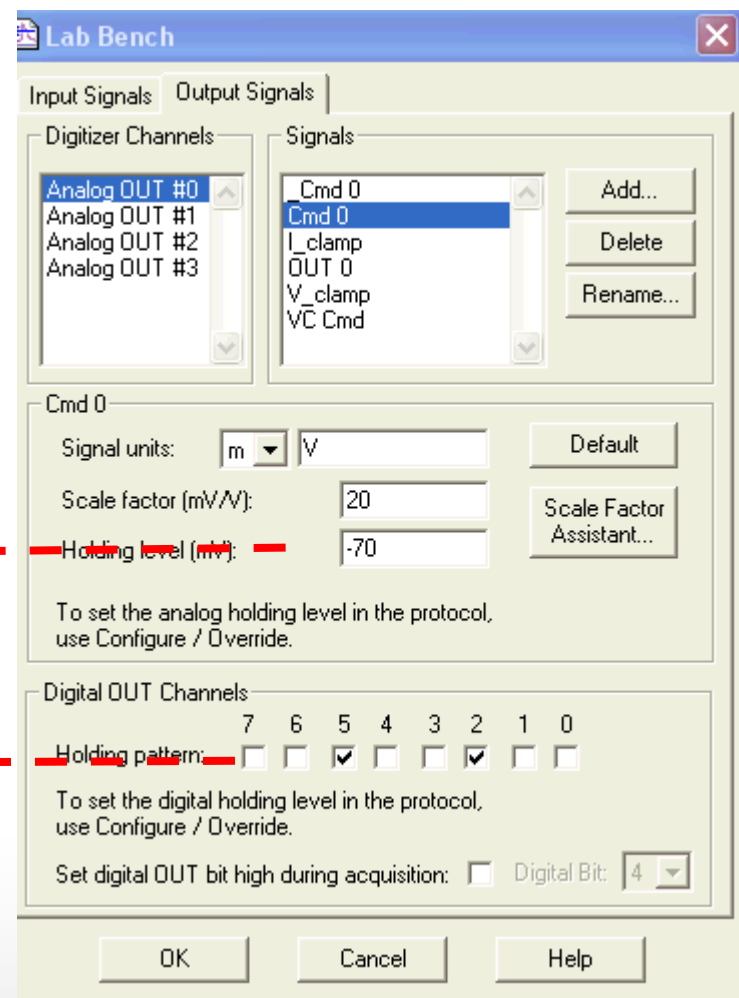
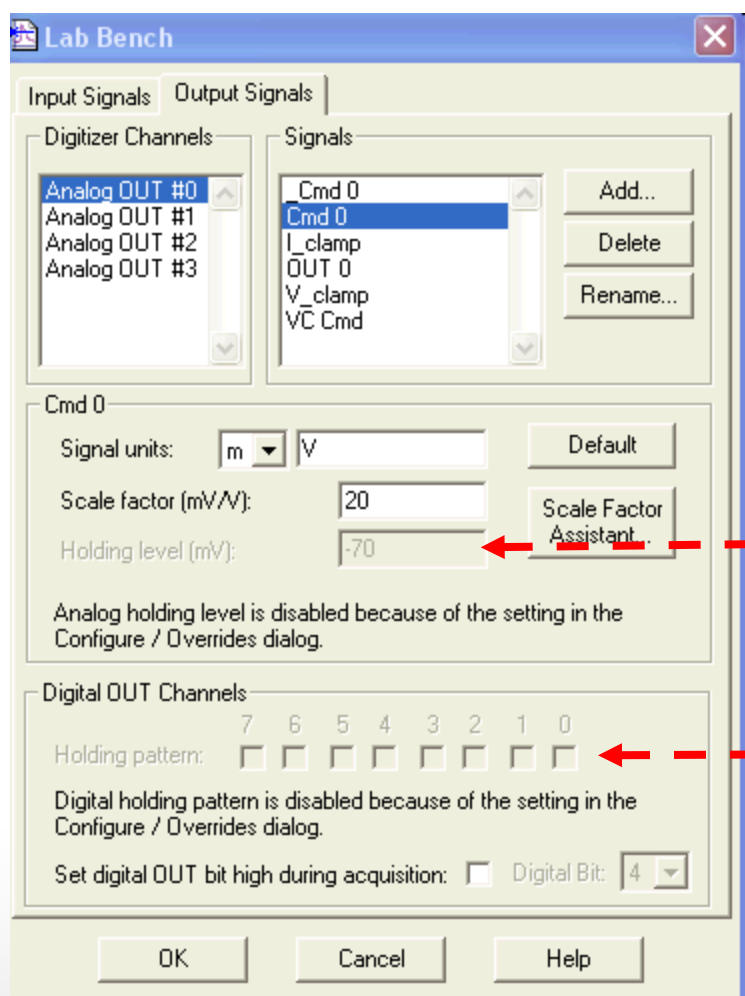
The image displays the Lab Bench software interface, specifically the 'Edit Protocol' window. The window is divided into several sections:

- Analog OUT Channels:** Lists four channels (Cmd 0 to Cmd 3) with their respective ranges and units.
- Analog OUT Holding Levels:** A section where holding levels are defined for each channel. The 'Cmd 0 (mV)' field is highlighted with a red box and contains the value '-70'. Other channels (Cmd 1, 2, 3) have a holding level of '0'.
- Digital OUT Holding Pattern:** A section with seven checkboxes labeled 7 through 1.
- Input Signals / Output Signals:** A section with two lists: 'Digitizer Channels' and 'Signals'. 'Cmd 0' is selected in both lists.
- Cmd 0 Configuration:** A detailed configuration for the selected 'Cmd 0' signal. It shows 'Signal units' as 'mV', 'Scale factor (mV/V)' as '20', and 'Holding level (mV)' as '-70'. The 'Holding level' field is also highlighted with a red box.

Red dashed arrows indicate the flow of information: one arrow points from the 'Holding level (mV)' field in the 'Cmd 0 Configuration' section to the '-70' value in the 'Analog OUT Holding Levels' section. Another arrow points from the '-70' value in the 'Analog OUT Holding Levels' section to the 'Cmd 0 (mV)' field in the 'Episodic' section of the background window. A third arrow points from the 'Episodic' section to the 'Cmd 0 (mV)' field in the 'Signals' list.

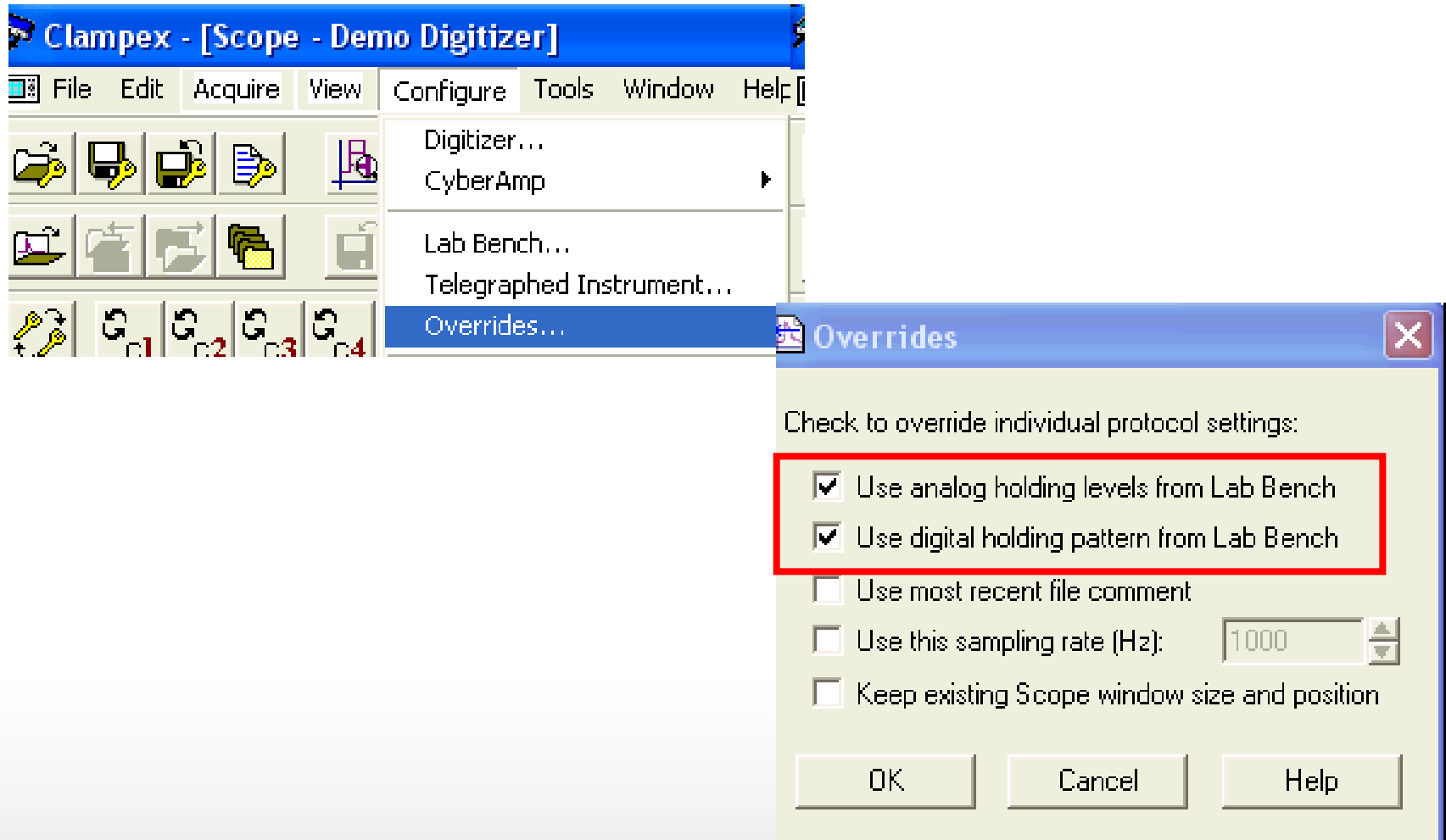
The background window shows a graph titled 'Episodic' with a y-axis ranging from 6000 to 10000. The graph displays a step function where the signal is 0 until approximately 8000, then jumps to -70.

# Holding Level Overrides



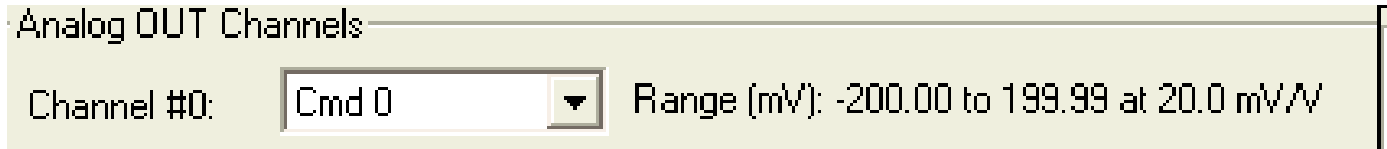


# Holding Level Overrides



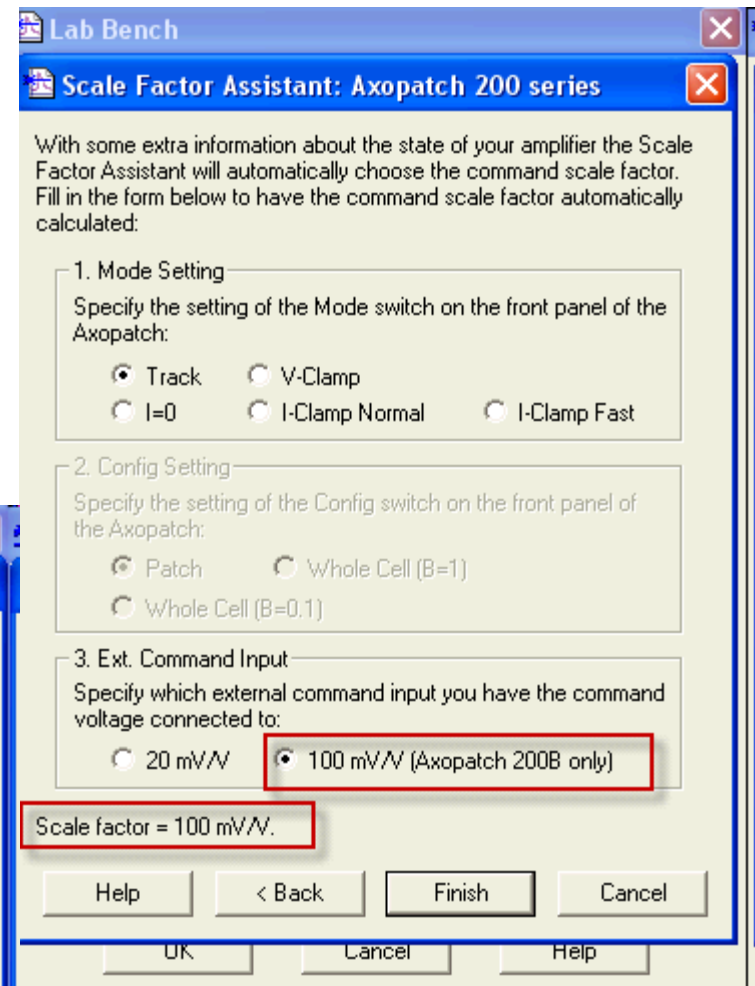
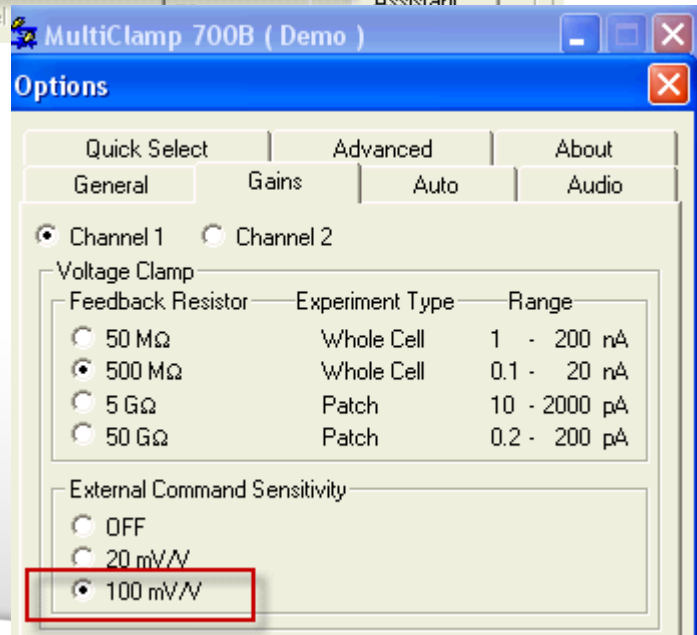
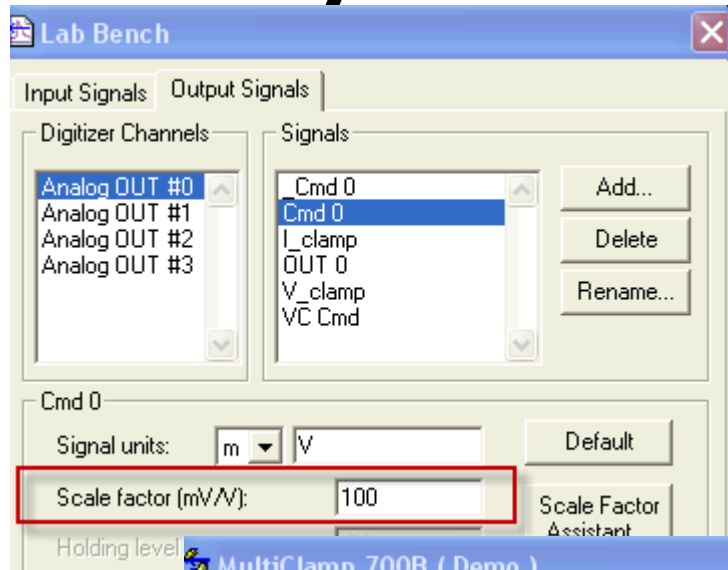
# Q & A

- Q: How do I change the command voltage for more than 200 mV



- A: You need to change the scale factor and external sensitivity of analog output signal on the Lab Bench

# Scale Factor/ External Command Sensitivity



# Scale factor/Output

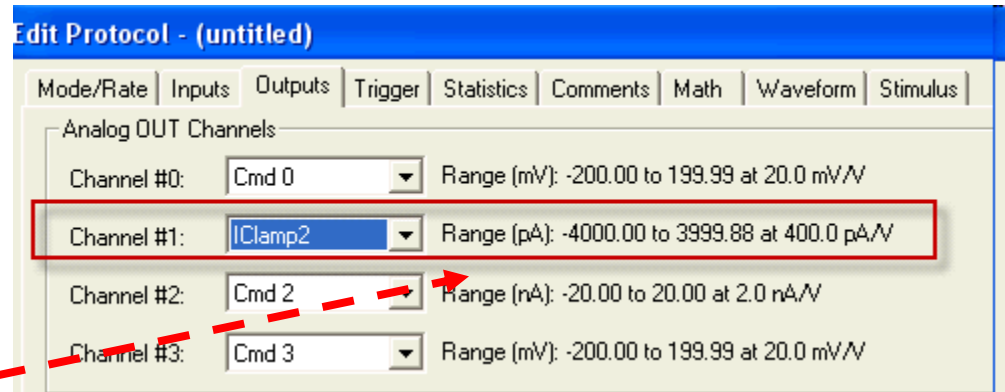
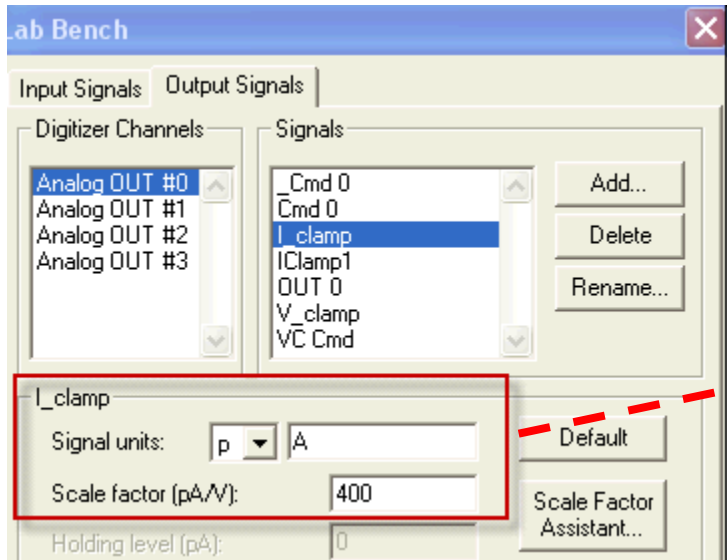
The image shows two windows from the Lab Bench software. The 'Lab Bench' window on the left is in the 'Output Signals' tab. It displays a list of 'Digitizer Channels' including 'Analog OUT #0' through '#3'. The 'Signals' list includes 'Cmd 0', 'I\_clamp', 'OUT 0', 'V\_clamp', and 'VC Cmd'. The 'Cmd 0' signal is selected. Below this, the 'Cmd 0' configuration shows 'Signal units' as 'mV' and a 'Scale factor (mV/V)' of '100', which is highlighted with a red box. The 'Edit Protocol - (untitled)' window on the right shows the 'Outputs' tab with 'Analog OUT Channels' listed. Channel #0 is 'Cmd 0' with a range of '-1000.00 to 999.97 at 100.0 mV/V', also highlighted with a red box. Other channels include 'Cmd 1' (-200.00 to 199.99 at 20.0 mV/V), 'Cmd 2' (-10.00 to 10.00 at 1.0 nA/V), and 'Cmd 3' (-200.00 to 199.99 at 20.0 mV/V).

# Q & A

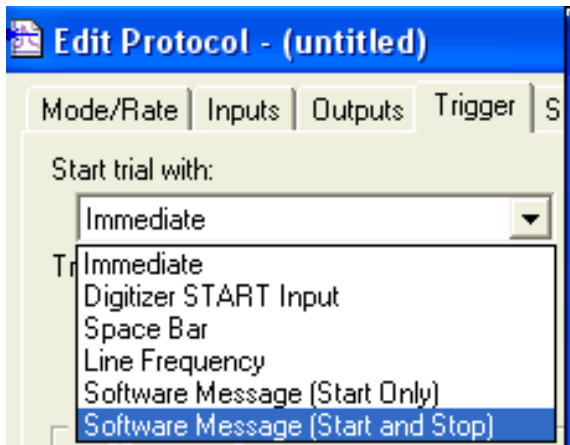
- Q: I want to perform current clamp experiment. How do I change the command voltage to current



- A: You need to change the signal unit and scale factor on the Lab Bench.

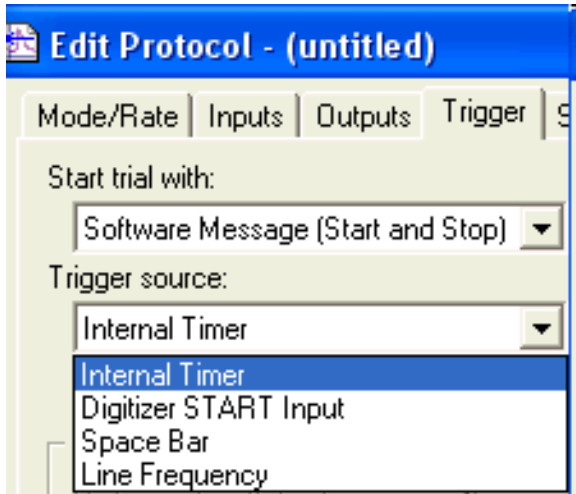


# Trigger



- **Immediate**
  - As soon as you choose **Acquire / Record** or **Acquire / View Only**, or press the equivalent toolbar button, the trial starts.
- **Digitizer START Input**
  - To trigger the start of a trial from an external device.
- **Space Bar**
  - Start the trial with a toolbar press or mouse click.
- **Line Frequency**
  - Only for series 132x Axon digitizers
  - synchronized with the mains line frequency (typically 50 or 60 Hz).
- **Software Message**
  - To trigger the trial from the other program

# Trigger



- **Internal Timer**
  - **Determines acquisition according to the length of the sweeps and Start-to-Start Intervals and any Conditioning Trains or P/N Leak Subtraction**
- **First Acquired Signal**
  - **Once a threshold in the signal from the first Analog IN Channel is crossed.**
- **Digitizer START Input**
  - **To trigger the start of a trial from an external device.**
- **Space Bar**
  - **Acquisition is started by pressing the space bar**
- **Line Frequency**
  - **Only for series 132x Axon digitizers**
  - **Synchronized with the mains line frequency (typically 50 or 60 Hz).**



# Scope Trigger

Scope trigger

Statistics Settings

Pretrigger length (ms):  = 50 samples

Statistics threshold (pA):

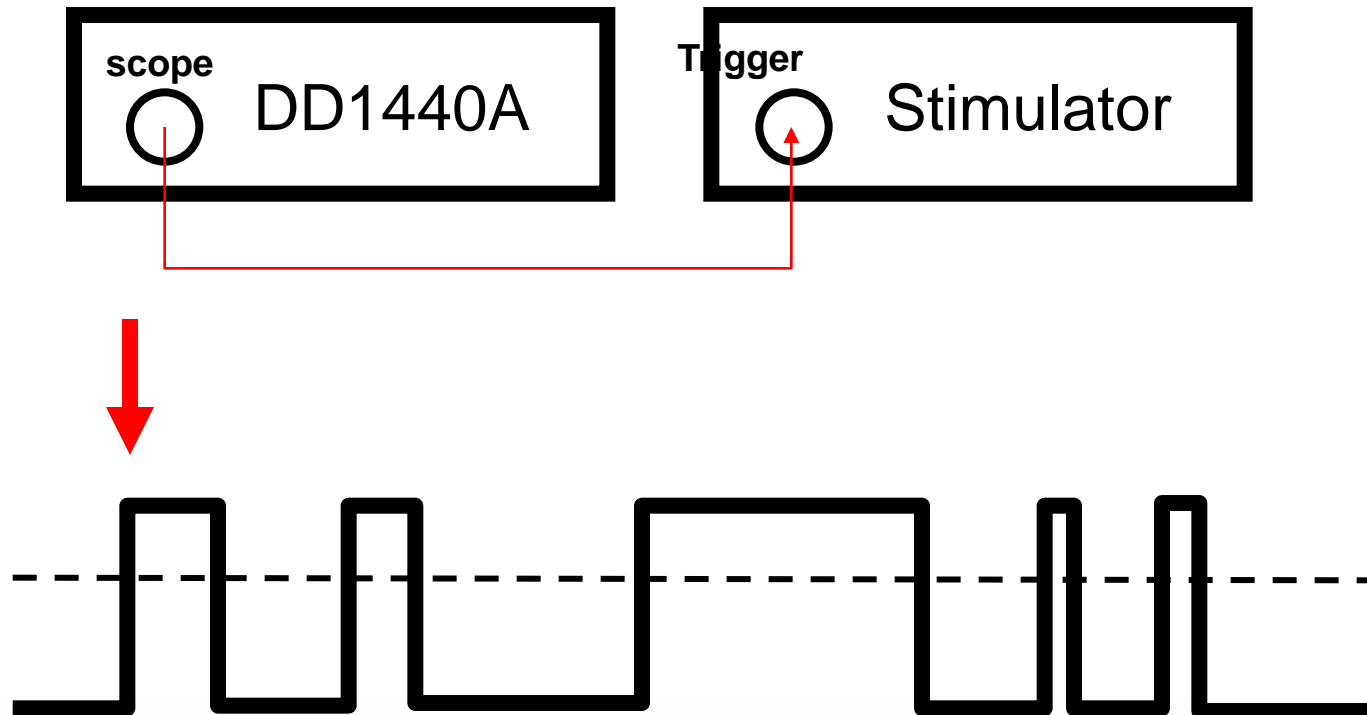
Polarity

Rising

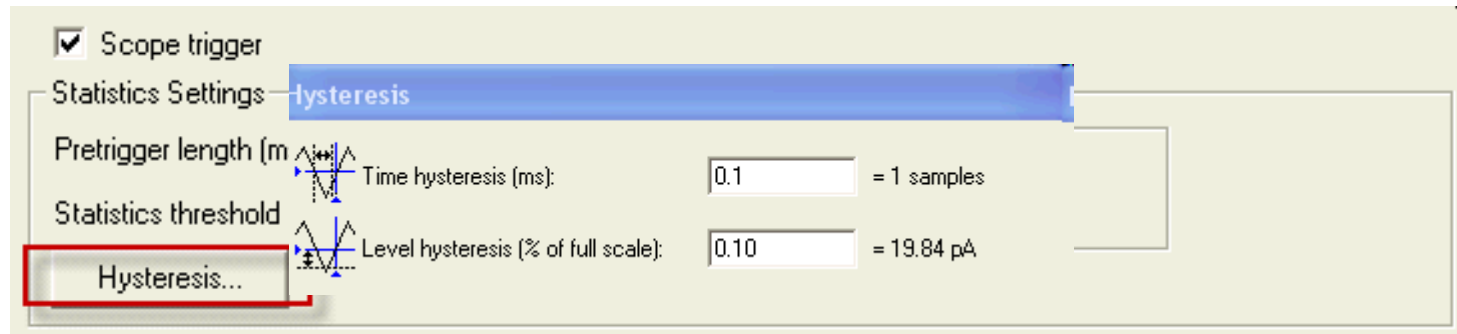
Falling

- A 5 V TTL trigger signal from a digitizer output when the signal reaches the threshold.
- The rear panel TRIGGER OUTPUT BNC (Digidata 1322)
- The front panel SCOPE BNC (Digidata 1440)

# Scope trigger



# Hysteresis



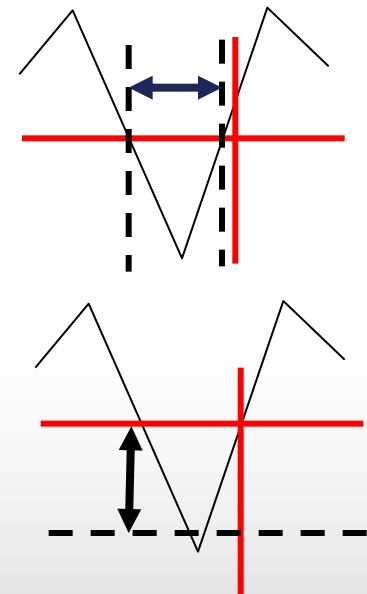
- To prevent signal noise activating false triggers, you can adjust the hysteresis settings to reduce trigger sensitivity.

- **Time hysteresis**

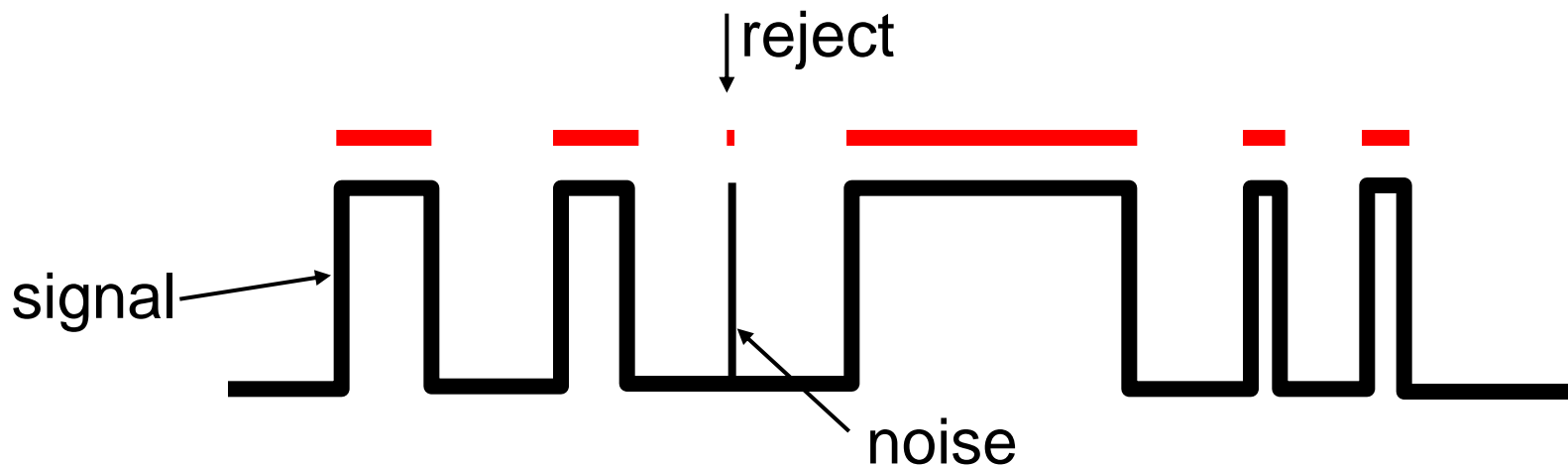
- This field adjusts the amount of time that the signal has to be under the threshold level (as determined by the Level Hysteresis setting below) to re-arm the trigger.

- **Level hysteresis**

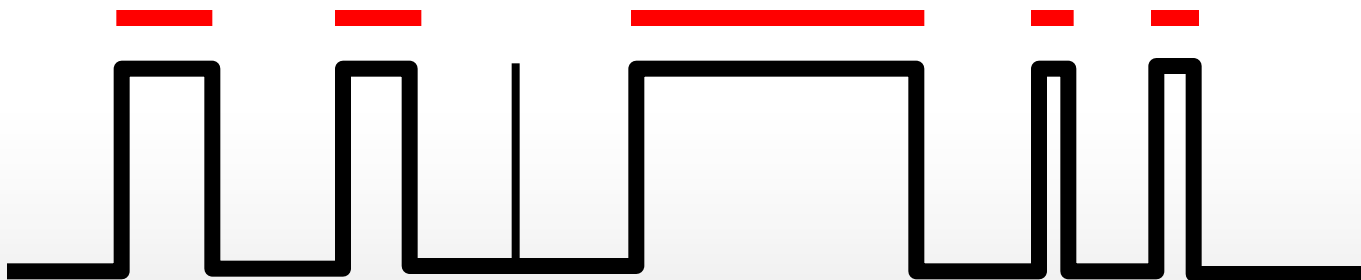
- This field adjusts the distance that the signal must be under the threshold in order to re-arm the trigger (subject to the Time Hysteresis setting above).



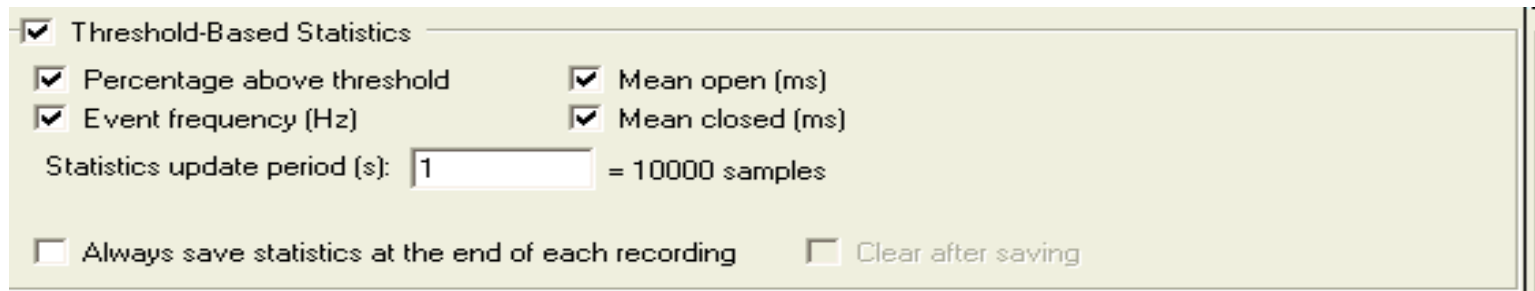
# Time hysteresis



- Adjust the time hysteresis to reduce sensitivity to avoid the false triggers.



# Threshold-Based Statistics

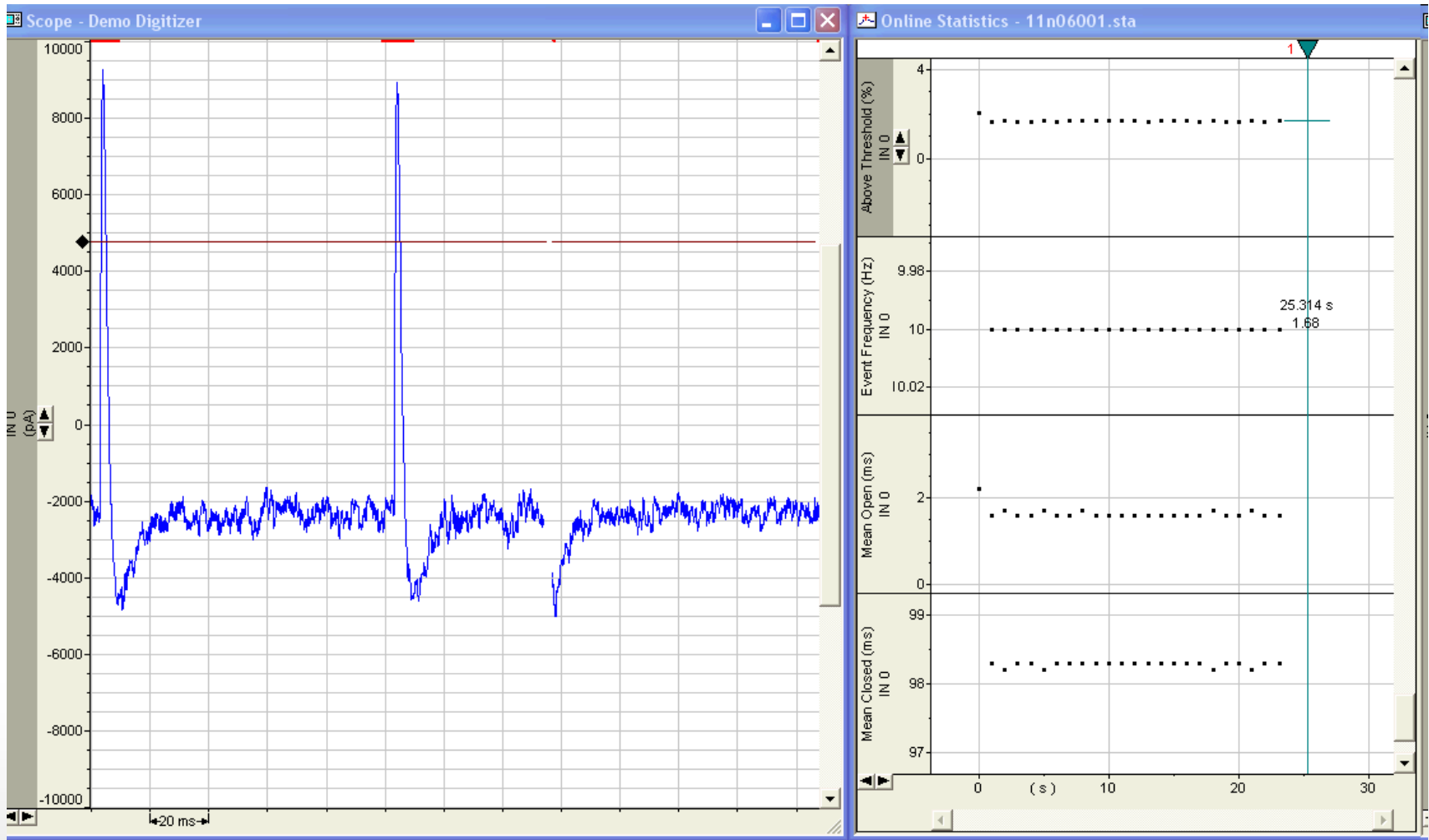


The screenshot shows a software configuration window titled "Threshold-Based Statistics". It contains several checkboxes and a text input field. The "Percentage above threshold", "Event frequency (Hz)", "Mean open (ms)", and "Mean closed (ms)" options are all checked. The "Statistics update period (s)" is set to "1", with a note "= 10000 samples". The "Always save statistics at the end of each recording" and "Clear after saving" options are unchecked.

<input checked="" type="checkbox"/> Threshold-Based Statistics	
<input checked="" type="checkbox"/> Percentage above threshold	<input checked="" type="checkbox"/> Mean open (ms)
<input checked="" type="checkbox"/> Event frequency (Hz)	<input checked="" type="checkbox"/> Mean closed (ms)
Statistics update period (s): <input type="text" value="1"/> = 10000 samples	
<input type="checkbox"/> Always save statistics at the end of each recording	<input type="checkbox"/> Clear after saving

- **Percentage above threshold**
  - Display the percentage of time that the signal is above the threshold value
- **Event frequency (Hz)**
  - Display the frequency of threshold-crossing
- **Mean open (ms)**
  - Display the average time that the signal is above threshold.
- **Mean closed (ms)**
  - Display the average time that the signal is below threshold.

# Threshold-Based Statistics



# Statistics

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | **Statistics** | Comments | Math | Waveform | Stimulus

Shape Statistics

Analog IN Signal IN 0  
 Positive-going Selected signals: IN 0  
 Negative-going  
 Absolute

Baseline Region: Cursor Region  
From (ms): 0 => sample 1  
To (ms): 11.1 => sample 112

Search Region 1  
Range: Cursor Region  
From (ms): 22.2 => sample 223  
To (ms): 33.3 => sample 334

Smoothing window (samples): 1

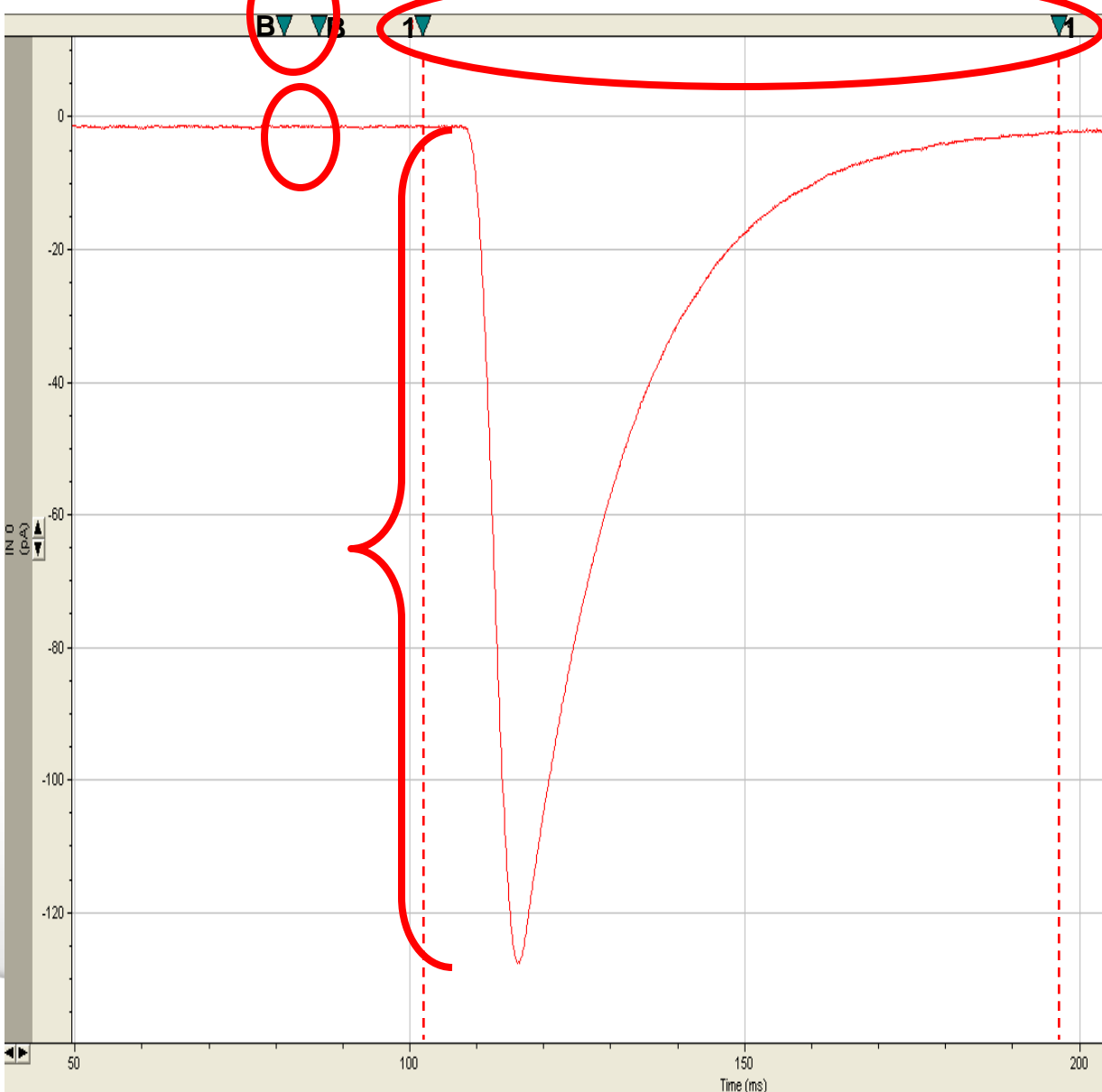
Always save statistics at the end of each recording  Clear after saving

Measurements

- Peak amplitude (pA)
- Time of peak (ms)
- Antipeak amplitude (pA)
- Time of antipeak (ms)
- Mean (pA)
- Standard deviation (pA)
- Area (pA · ms)
- Half-width (ms)
- Maximum rise slope (pA/ms)
- Time of maximum rise slope (ms)
- Maximum decay slope (pA/ms)
- Time of maximum decay slope (ms)
- Slope (pA/ms)
- Baseline (pA)
- Rise slope (pA/ms) } from  % to  %
- Rise time (ms)
- Decay slope (pA/ms) } from  % to  %
- Decay time (ms)

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview

# Statistics



Shape Statistics

Analog IN Signal IN 0

Positive-going Selected signals: IN 0

Negative-going

Absolute

Baseline Region Cursor Region

From (ms):  => sample 1

To (ms):  => sample 112

Search Region 1

Range: Cursor Region

From (ms):  => sample 223

To (ms):  => sample 334

Smoothing window (samples):

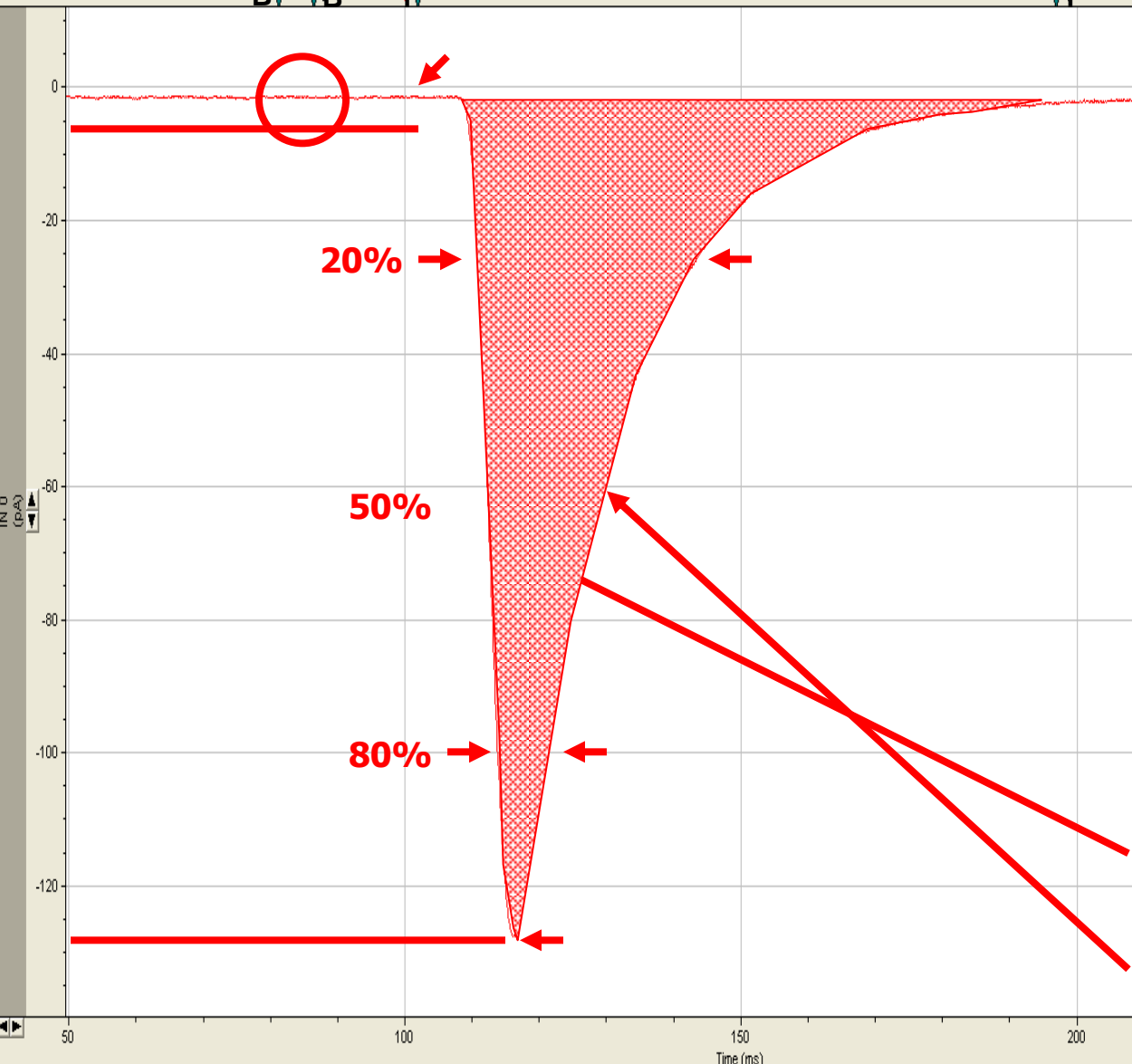
Always save statistics at the end of each recording



# Statistics

B ▾ ▾ B 1 ▾

1 ▾



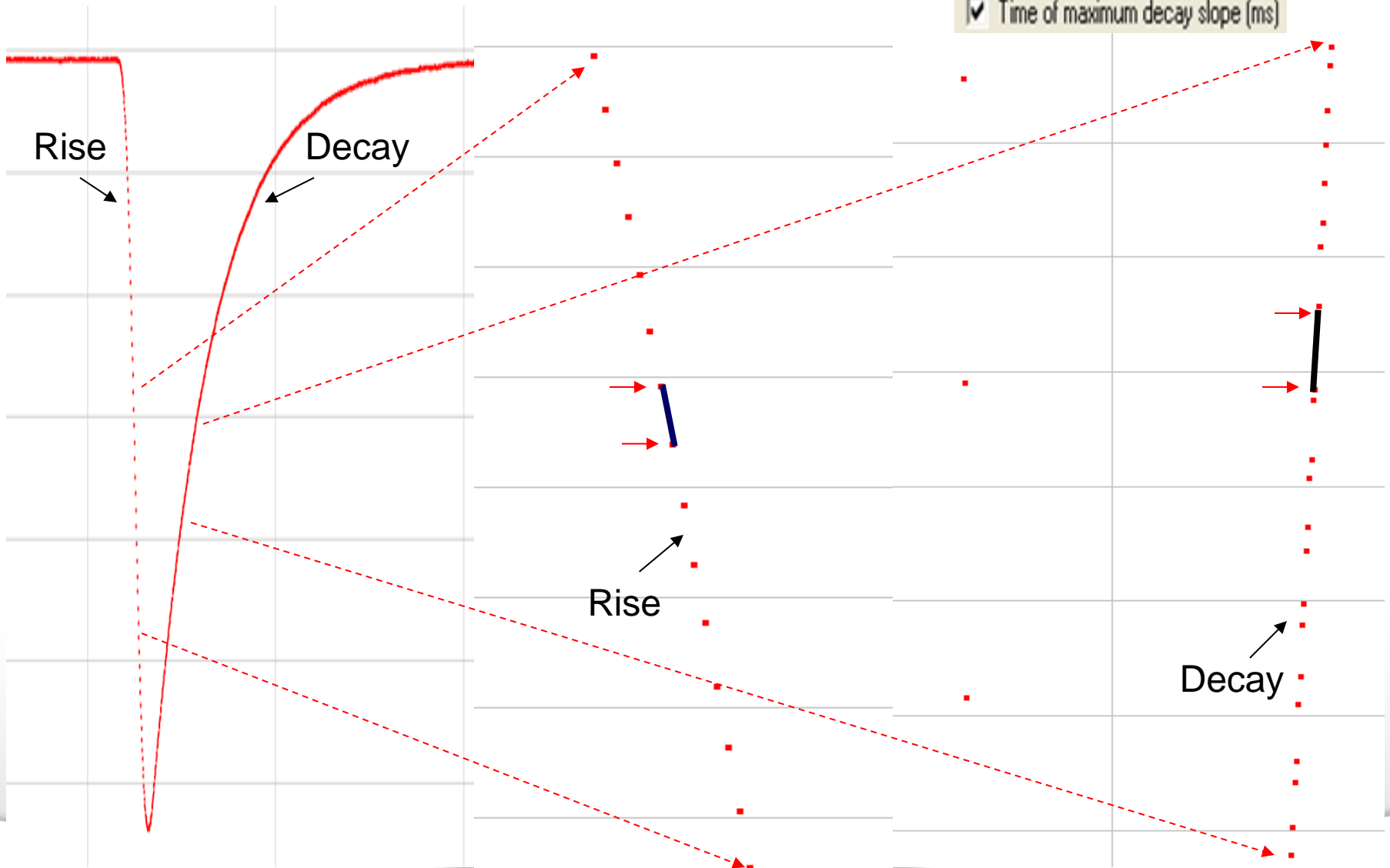
Measurements

- Peak amplitude (pA)
- Time of peak (ms)
- Antipeak amplitude (pA)
- Time of antipeak (ms)
- Mean (pA)
- Standard deviation (pA)
- Area (pA · ms)
- Half-width (ms)
- Maximum rise slope (pA/ms)
- Time of maximum rise slope (ms)
- Maximum decay slope (pA/ms)
- Time of maximum decay slope (ms)
- Slope (pA/ms)
- Baseline (pA)
- Rise slope (pA/ms)
- Rise time (ms)
- Decay slope (pA/ms)
- Decay time (ms)

From  % To  %

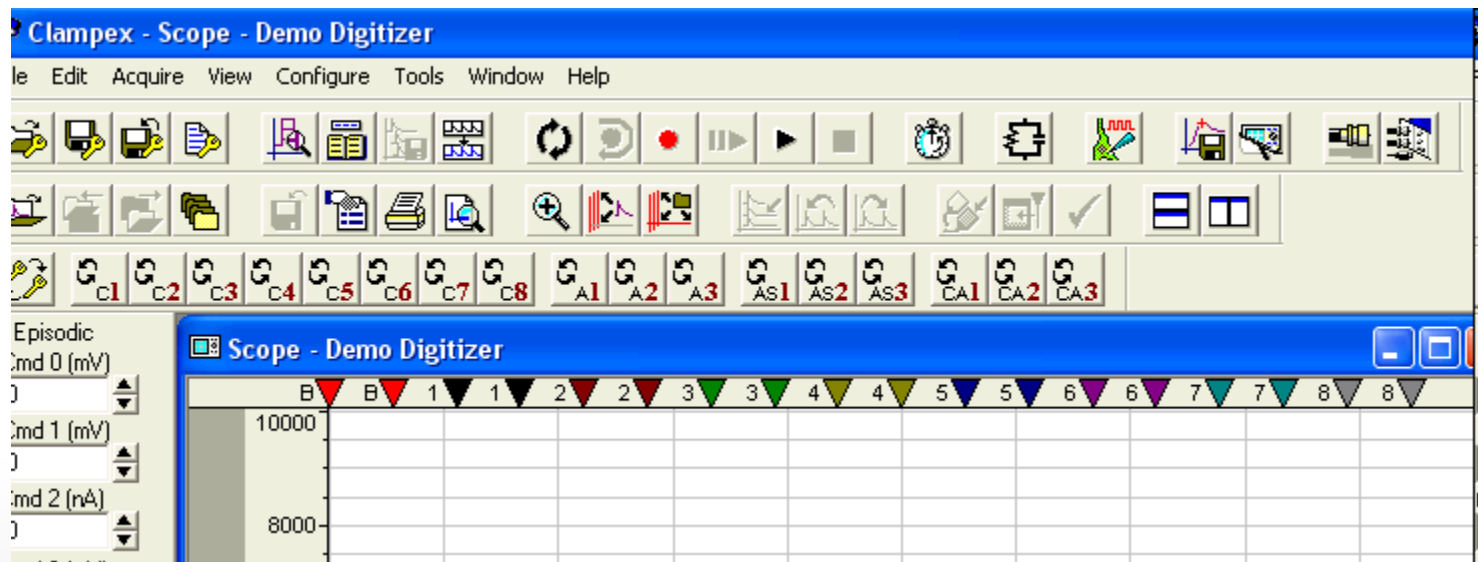
From  % To  %

- ✓ Maximum rise slope (pA/ms)
- ✓ Time of maximum rise slope (ms)
- ✓ Maximum decay slope (pA/ms)
- ✓ Time of maximum decay slope (ms)

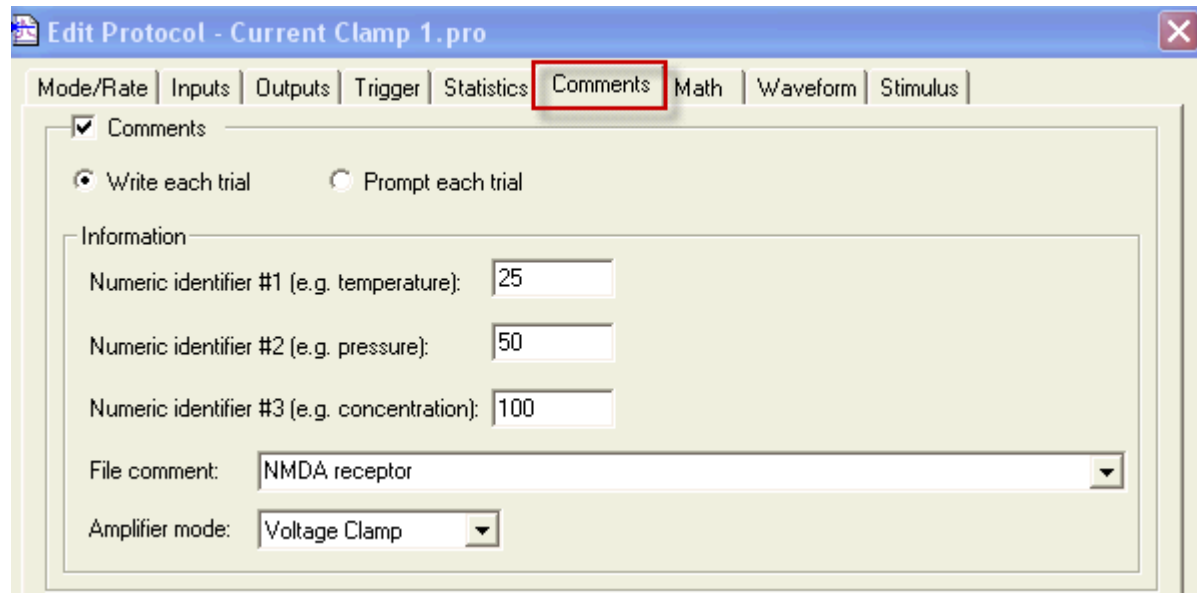


# Q & A

- How many search regions can be applied during data acquisition?
- How?

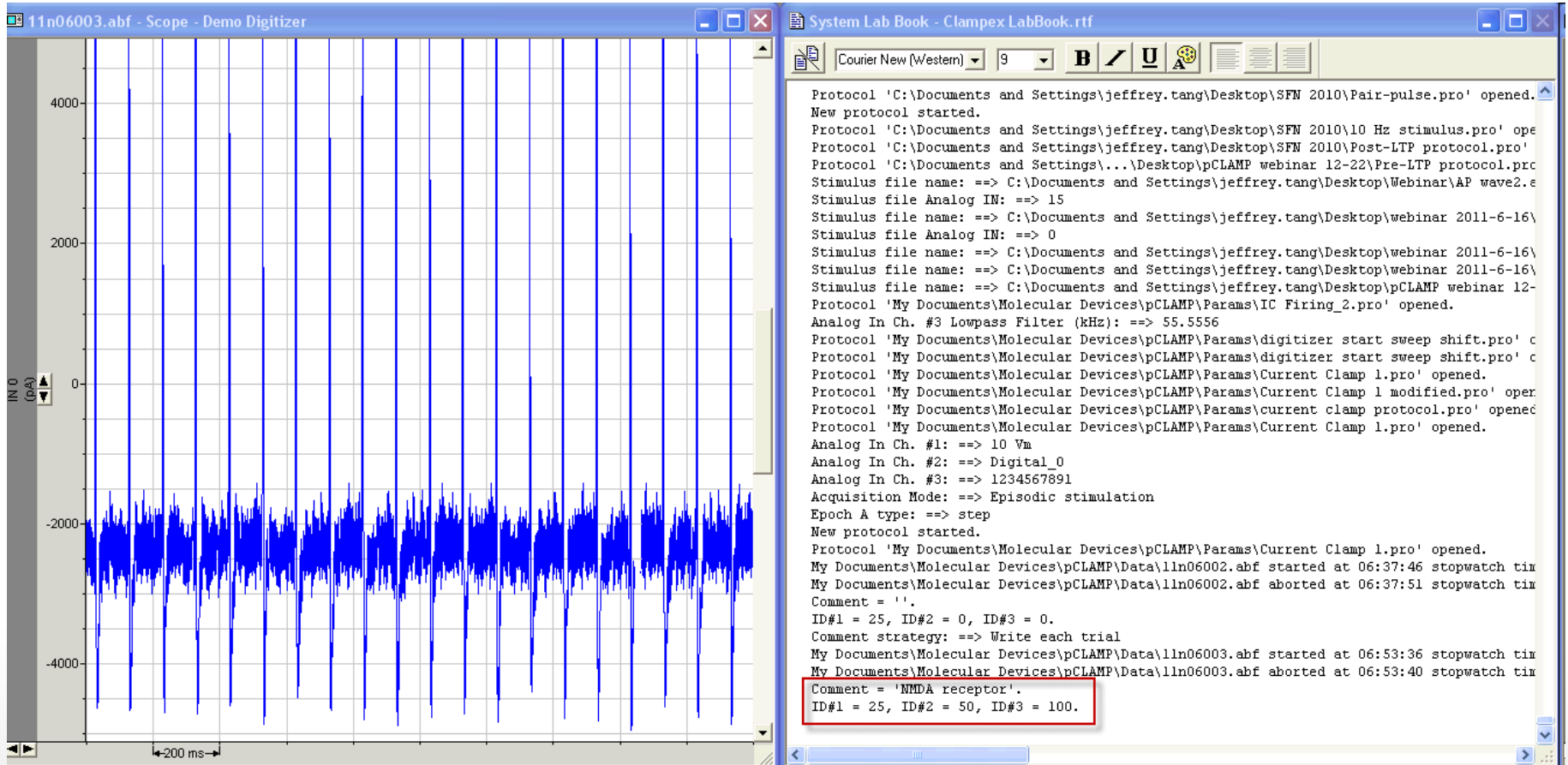


# Comments

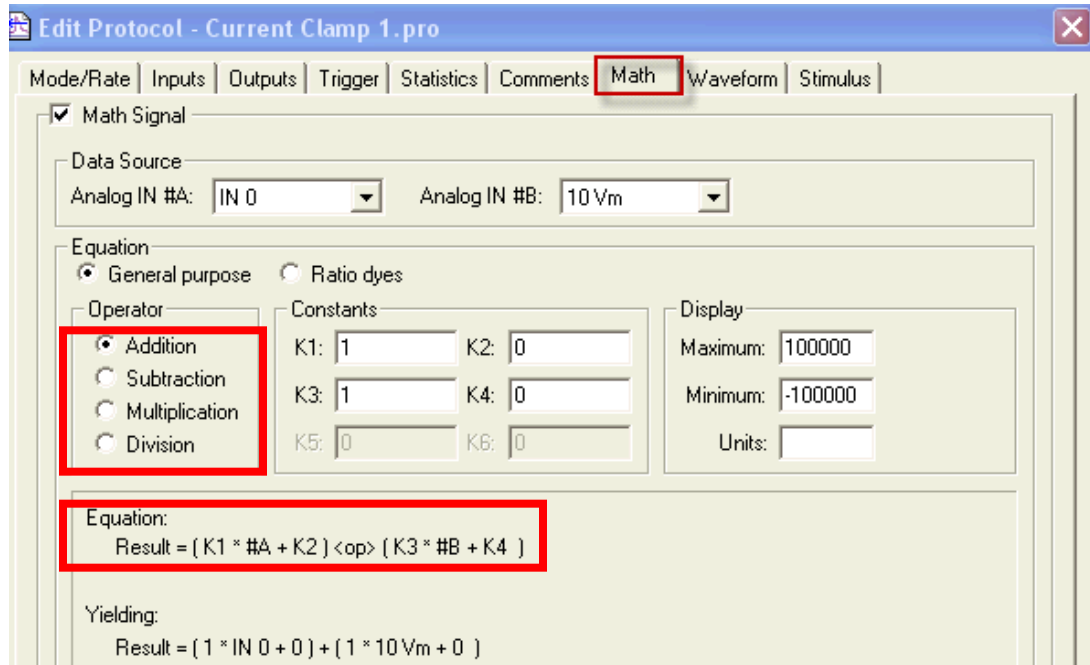


- The comments can be written automatically to every data file
- The comments are stored with data file information in the file header, viewed from [File / Properties](#), and can also be displayed in the [Data File Index](#).

# Comments

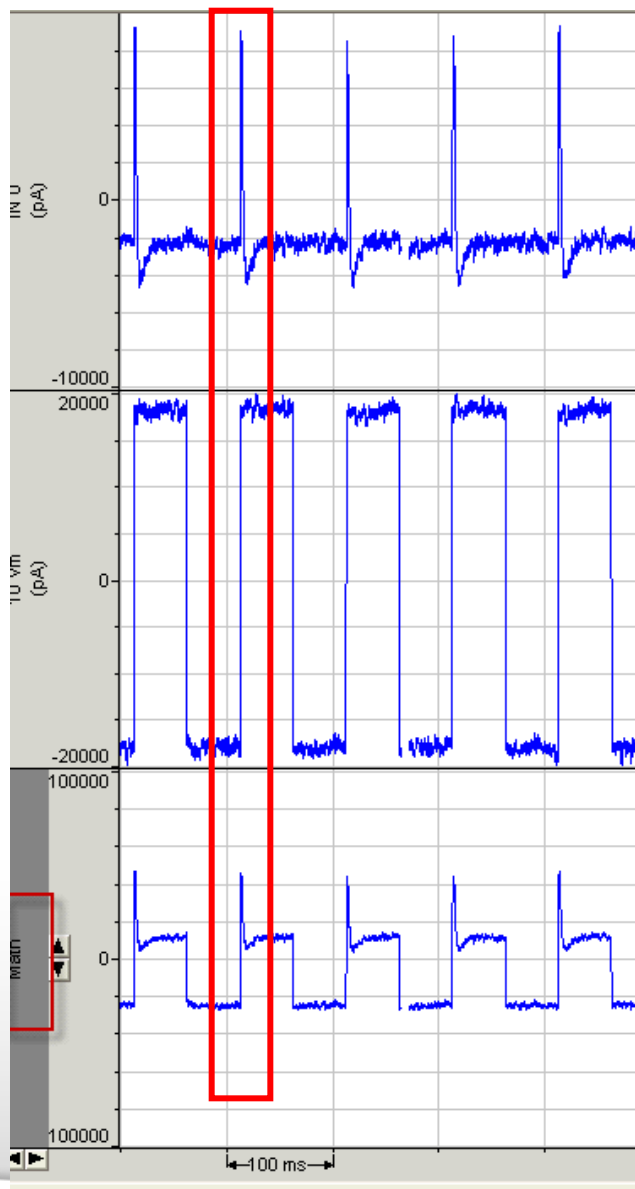


# Math



- Allows arithmetic calculation between two analog input signals
- Calculate on-line ratios, products, sums and differences.

# Math



# Waveform

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | **Waveform** | Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform  Digital Outputs

Epochs  Stimulus file

Intersweep holding level:

Epoch Description	A	B	
Type	Step	Off	0
Sample rate	Fast	Fast	F
First level (mV)	112	0	0
Delta level (mV)	-20	0	0
First duration (ms)	100	0	0
Delta duration (ms)	0	0	0
Digital bit pattern (#3-0)	1111	0000	0
Digital bit pattern (#7-4)	0000	0000	0
Train rate (Hz)	0	0	0
Pulse width (ms)	0	0	0

Number of sweeps = 10

Channel #0 Channel #1 Channel #2 Cha

112 mV  
92 mV  
72 mV  
0 mV

100 ms

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview



# Epochs

**Edit Protocol - (untitled)**

Mode/Rate | Inputs | Outputs | Trigger | Statistics

Waveform Analog OUT: Cmd 0 Info

Analog Waveform

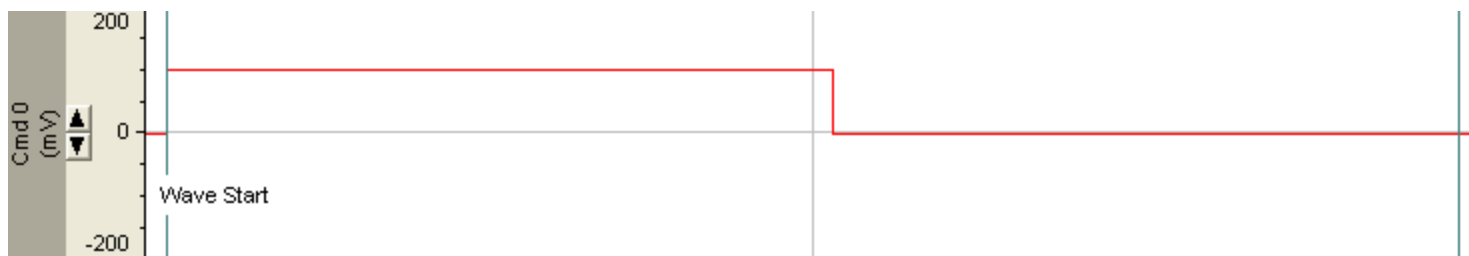
Epochs  Stimulus file

Intersweep holding level: Use holding ▼

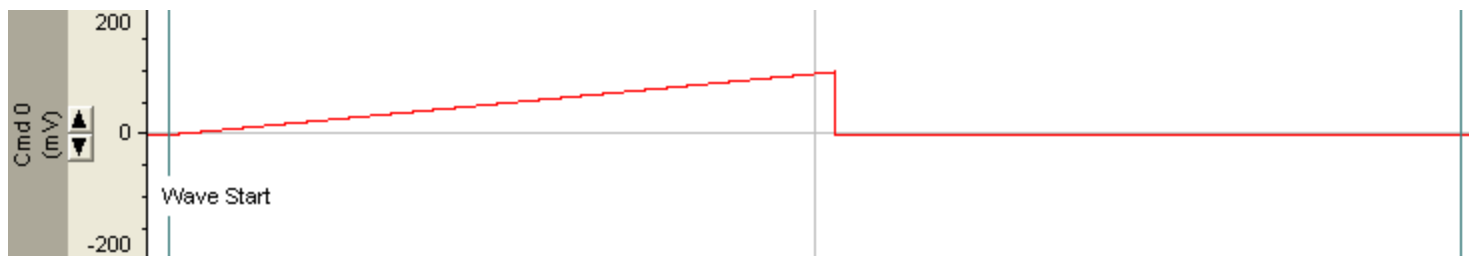
Epoch Description	A	B	C
Type	Step	Off	Off
Sample rate	Off		
First level (mV)	✓ <b>Step</b>		
Delta level (mV)	Ramp		
First duration (ms)	Pulse train		
Delta duration (ms)	Biphasic train		
Digital bit pattern (#3-0)	Triangle train		
Digital bit pattern (#7-4)	Cosine train		
Train rate (Hz)			
Pulse width (ms)	0	0	0

# Default waveforms

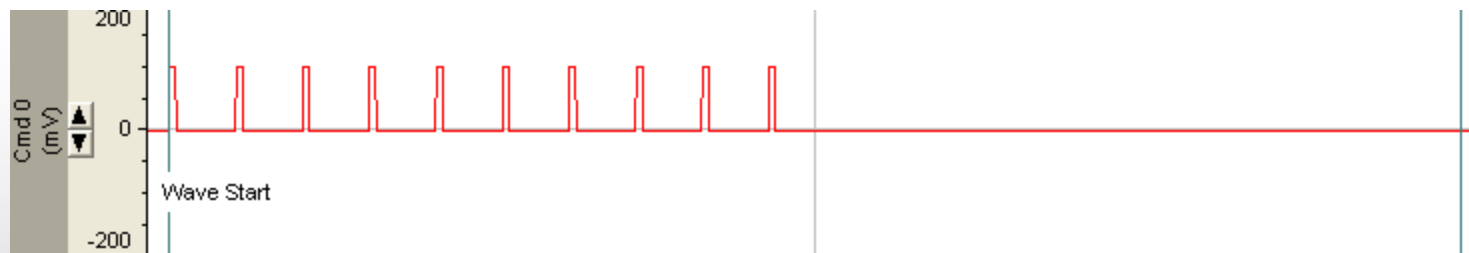
Step:



Ramp:

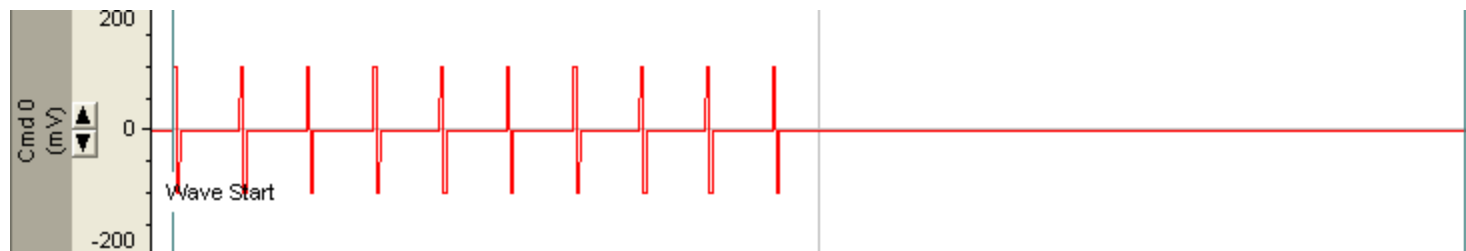


Pulse train:

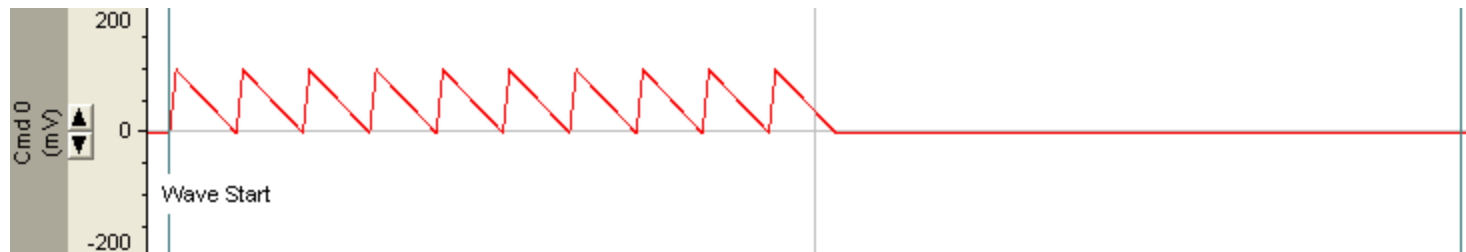


# Default waveforms

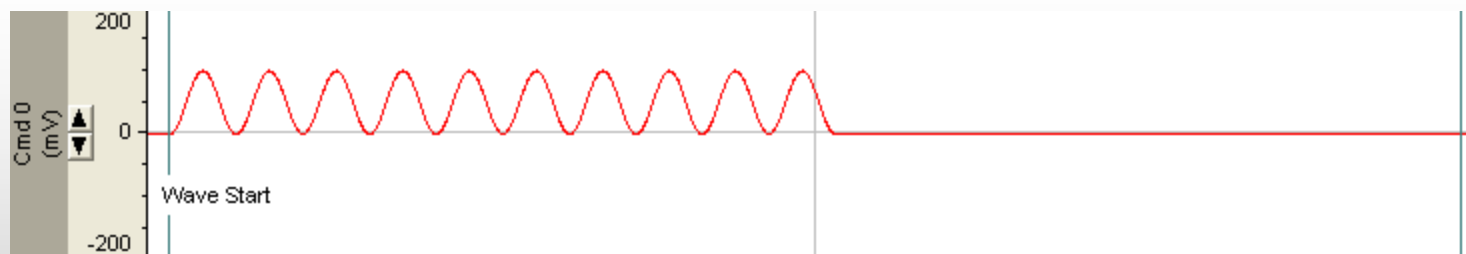
Biphasic train:



Triangle train:



Cosine train:



# Stimulus File

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform Info

Epochs  Stimulus file

Intersweep holding level: Use holding

Digital Outputs Info

Active high logic for digital trains Info

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type										
Sample rate										
First level (mV)										
Delta level (mV)										
First duration (ms)										
Delta duration (ms)										
Digital bit pattern (#3-0)										
Digital bit pattern (#7-4)										
Train rate (Hz)										
Pulse width (ms)										

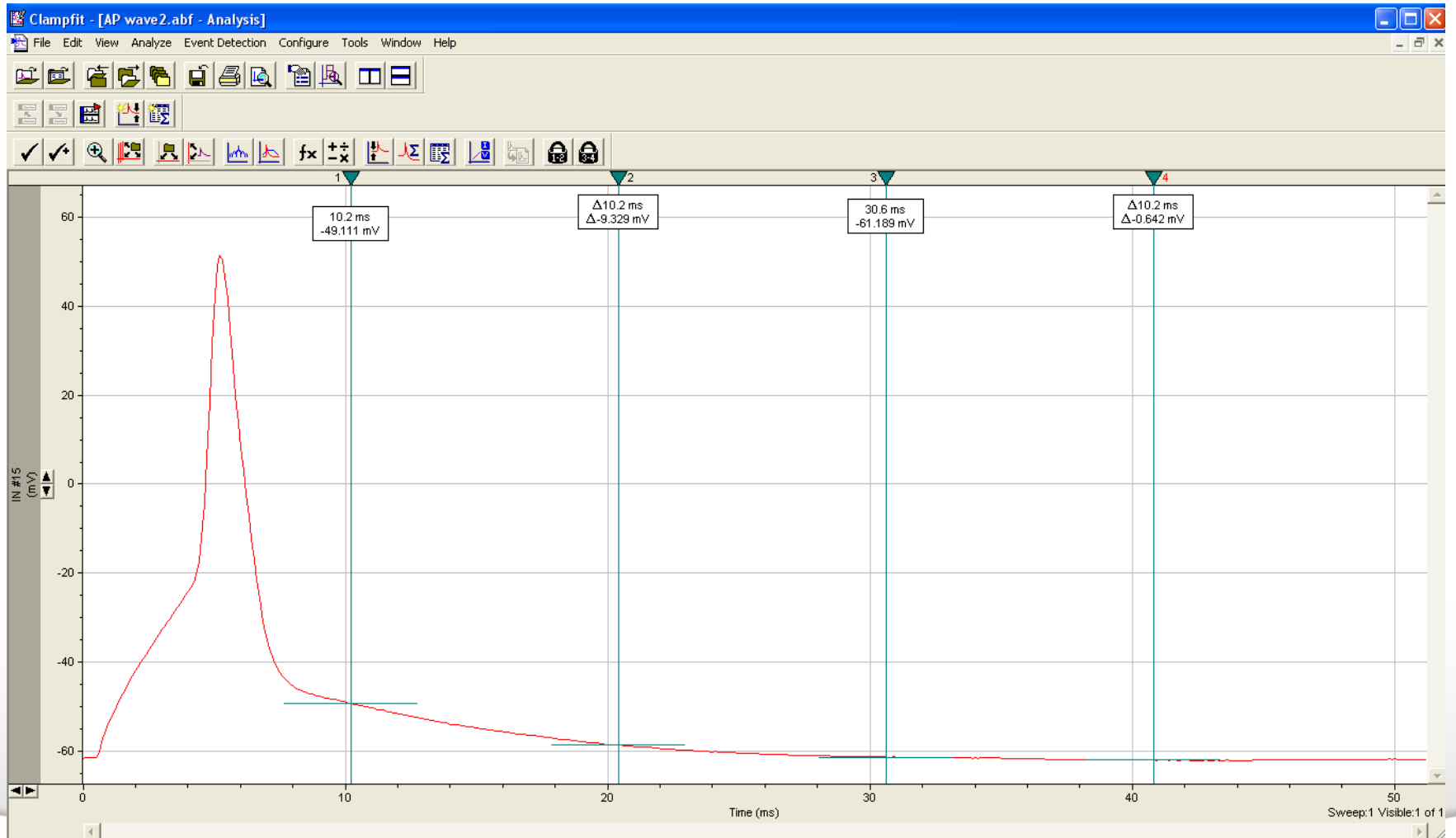
Number of sweeps = 10 Allocated time:

Stimulus File... Stimulus file not selected.

Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

# Stimulus File



# Stimulus File

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform Info

Epochs  Stimulus file

Intersweep holding level: Use holding ▼

Digital Outputs Info

Active high logic for digital trains Info

Intersweep bit pattern: Use holding ▼

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type										
Sample rate										
First level (mV)										
Delta level (mV)										
First duration (ms)										
Delta duration (ms)										
Digital bit pattern (#3-0)										
Digital bit pattern (#7-4)										
Train rate (Hz)										
Pulse width (ms)										

Number of sweeps = 10 Allocated time:

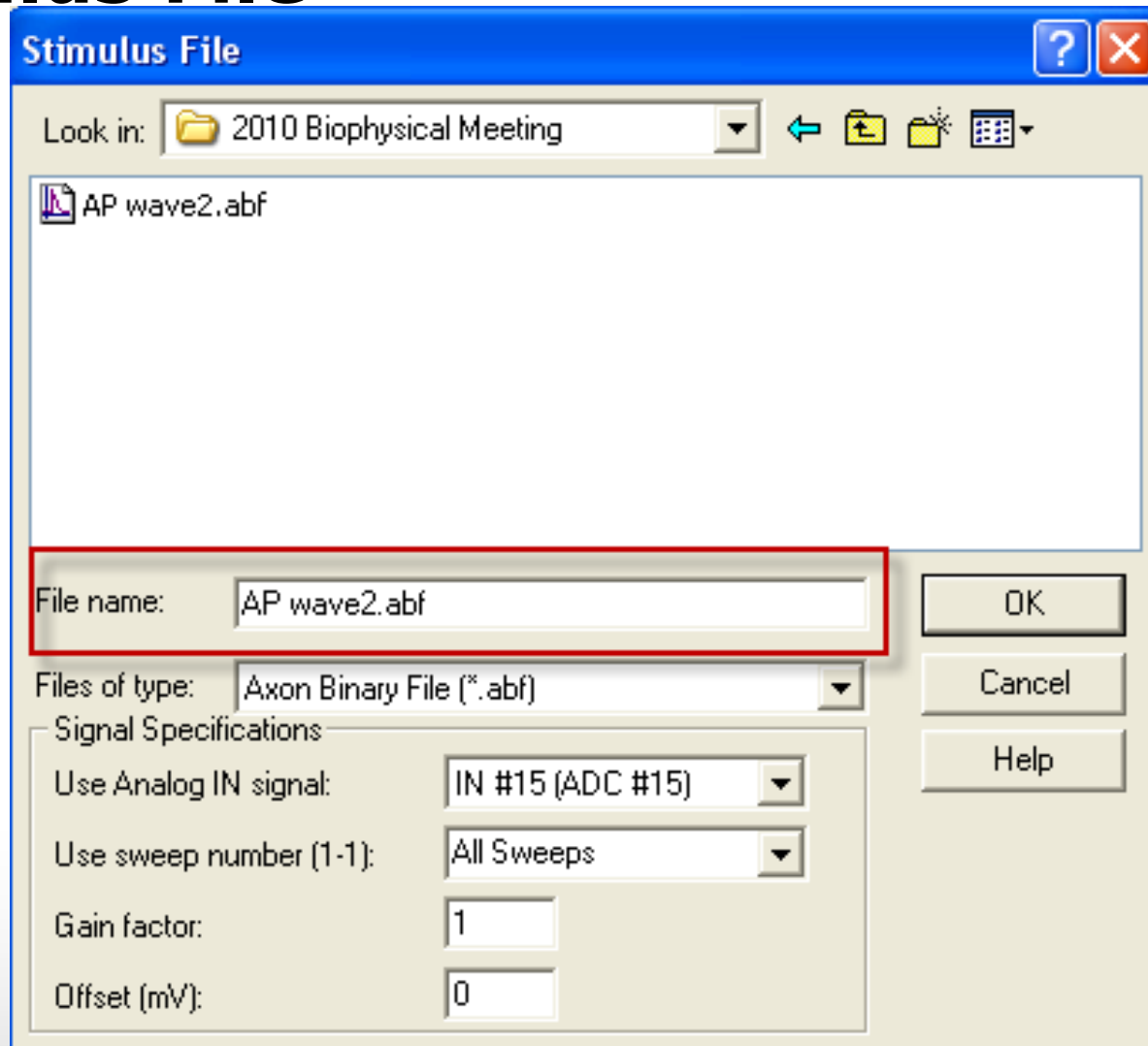
Stimulus File... Stimulus file not selected.

Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview

# Stimulus File



# Stimulus File

Waveform Analog OUT: Cmd 0 Info

Analog Waveform Info

Epochs  Stimulus file

Intersweep holding level: Use holding

Digital Outputs Info

Active high logic for digital trains Info

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type										
Sample rate										
First level (mV)										
Delta level (mV)										
First duration (ms)										
Delta duration (ms)										
Digital bit pattern (#3-0)										
Digital bit pattern (#7-4)										
Train rate (Hz)										
Pulse width (ms)										

Number of sweeps = 10 Allocated time:

**Stimulus File...** Stimulus file 'AP wave2.abf', all sweeps, from Analog IN channel #15.

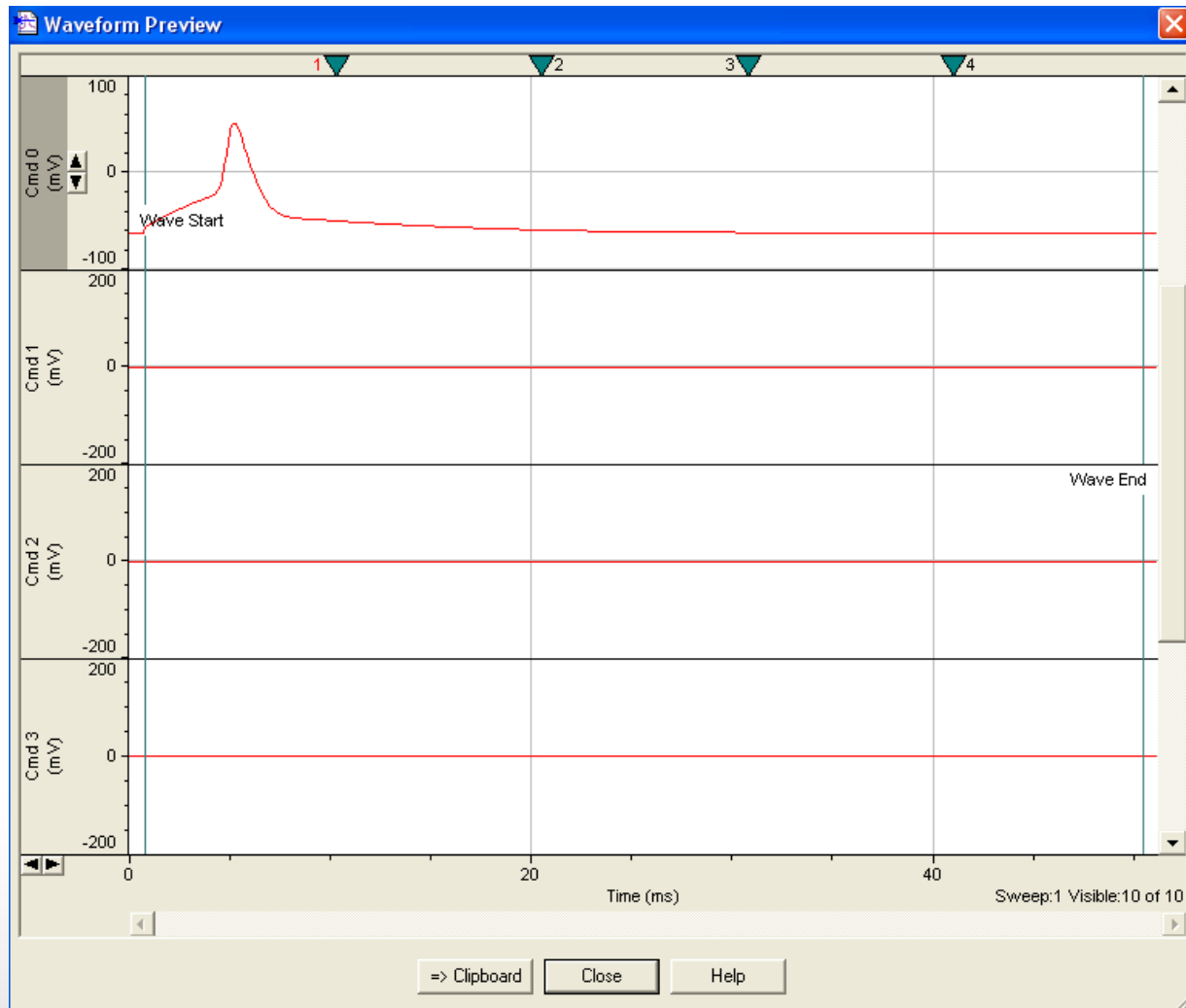
Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview



# Action Potential Waveform



# Digital Outputs

**Edit Protocol - (untitled)**

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform

Digital Outputs

Epochs  Stimulus file

Intersweep holding level: Use holding ▼ Info

Active high logic for digital trains Info

Intersweep bit pattern: Use holding ▼

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Off	Off	Off	Off	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	112	0	0	0	0	0	0	0	0	0
Delta level (mV)	-20	0	0	0	0	0	0	0	0	0
First duration (ms)	100	0	0	0	0	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	1111	0000	0000	0000	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0	0	0	0	0	0	0
Pulse width (ms)	0	0	0	0	0	0	0	0	0	0

Number of sweeps = 10 Allocated time: 106.2 of 200 ms

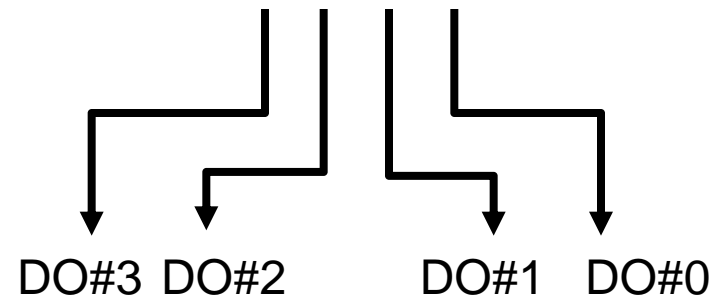
Stimulus File... Final level -68.00 mV  
First duration 100.00 ms (1000 samples)

Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

# Digital Bit Pattern

Digital bit pattern (#3-0): 0101



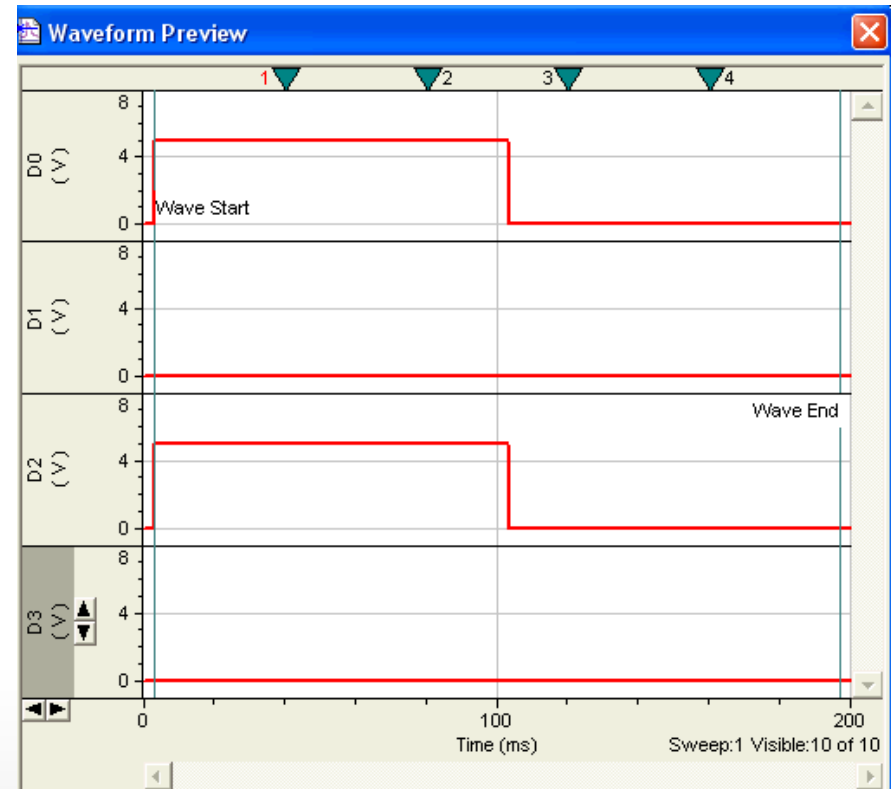
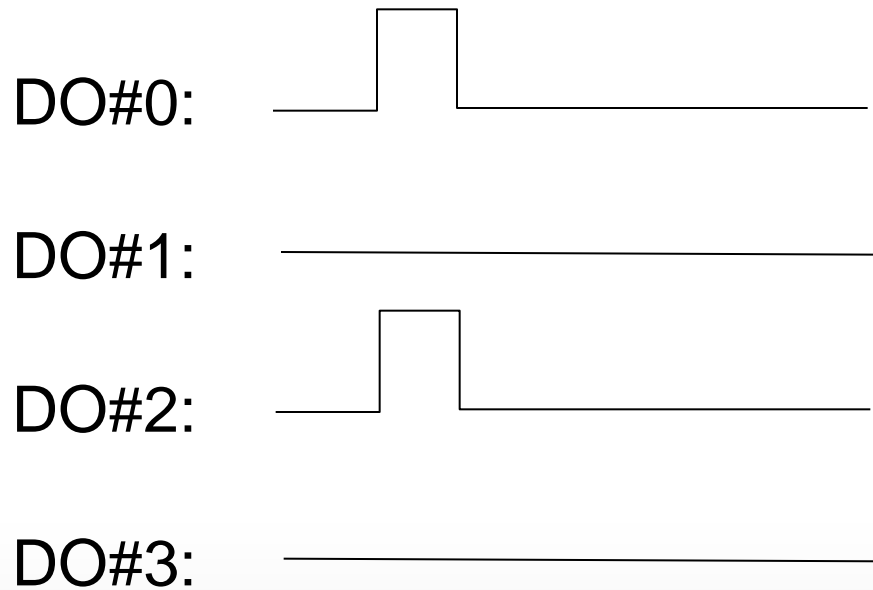
1 → High, single TTL (5V)

0 → Low, No single TTL (5V)

an asterisk, \* → A pulse train of TTL (5V)

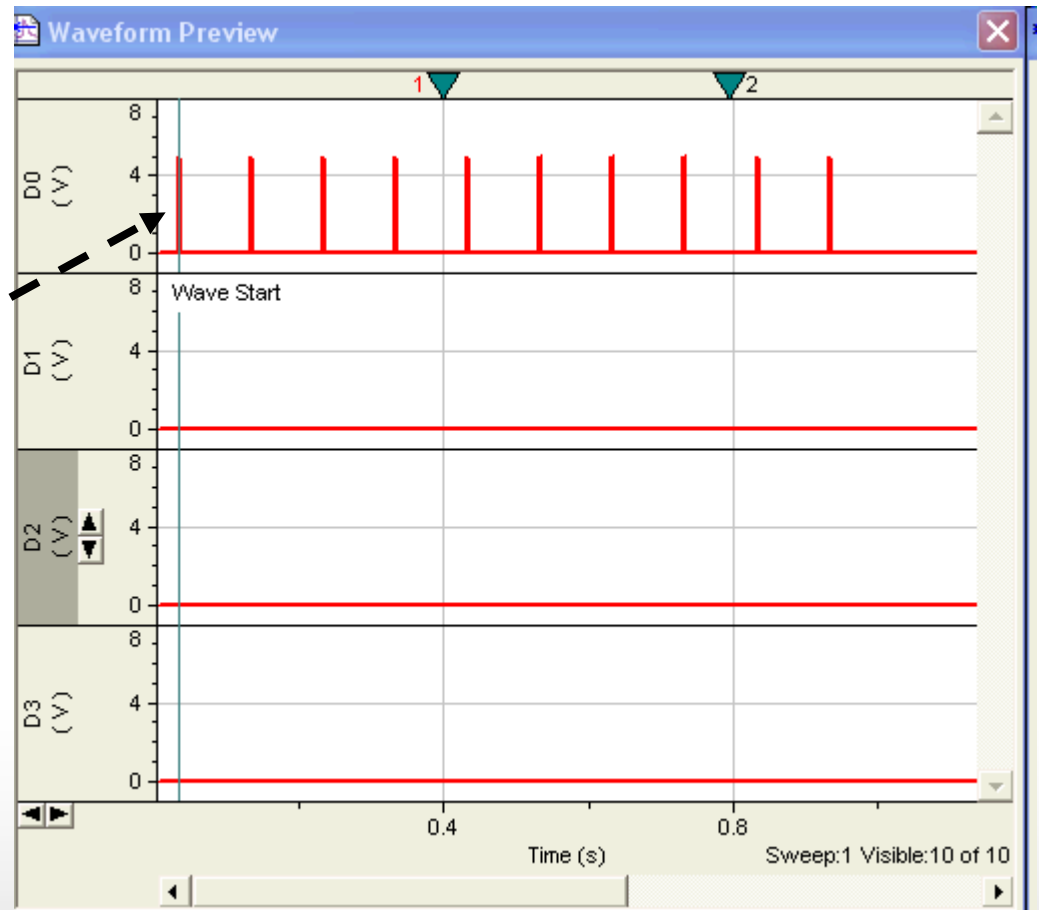
# Digital Bit Pattern---Single Pulse

Digital bit pattern (#3-0): 0101



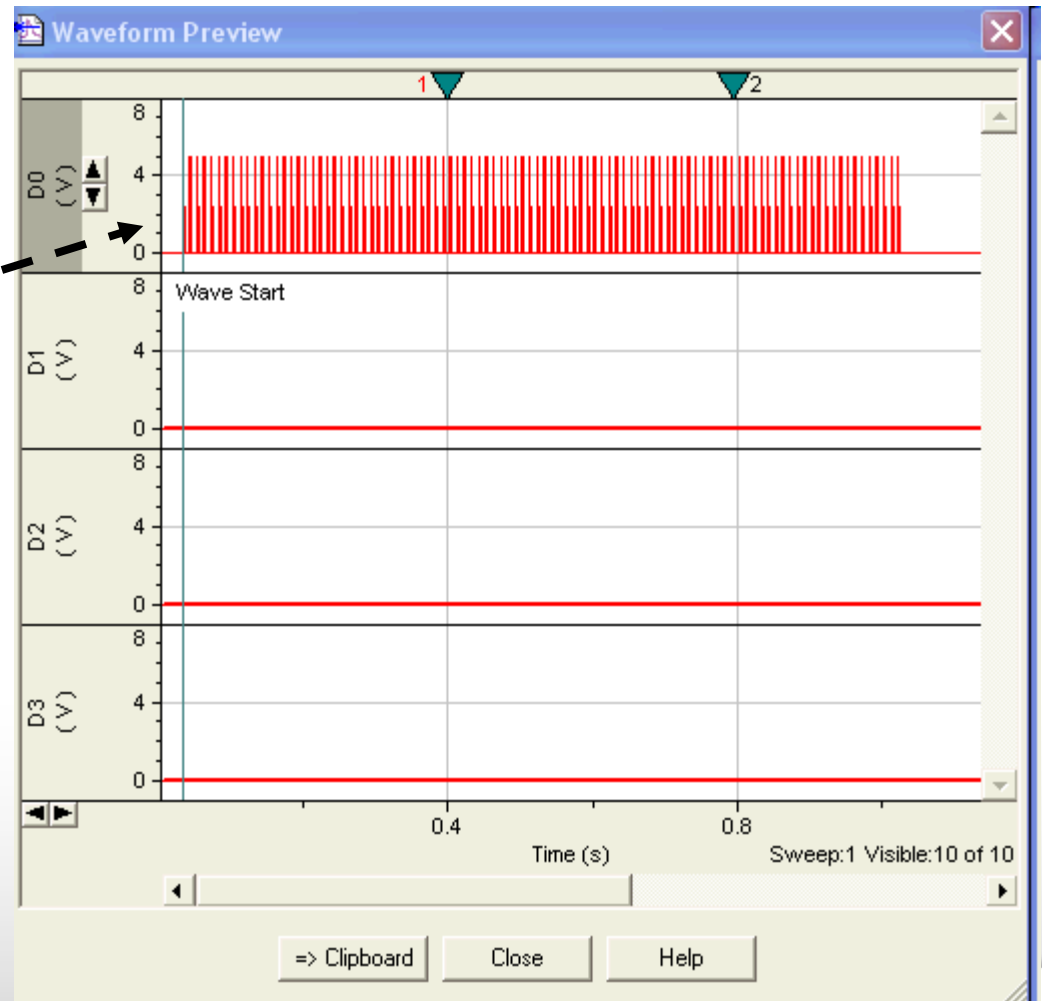
# Digital Bit Pattern---Train Pulse

Digital bit pattern (#3-0)	000*
Digital bit pattern (#7-4)	0000
Train rate (Hz)	10
Pulse width (ms)	1



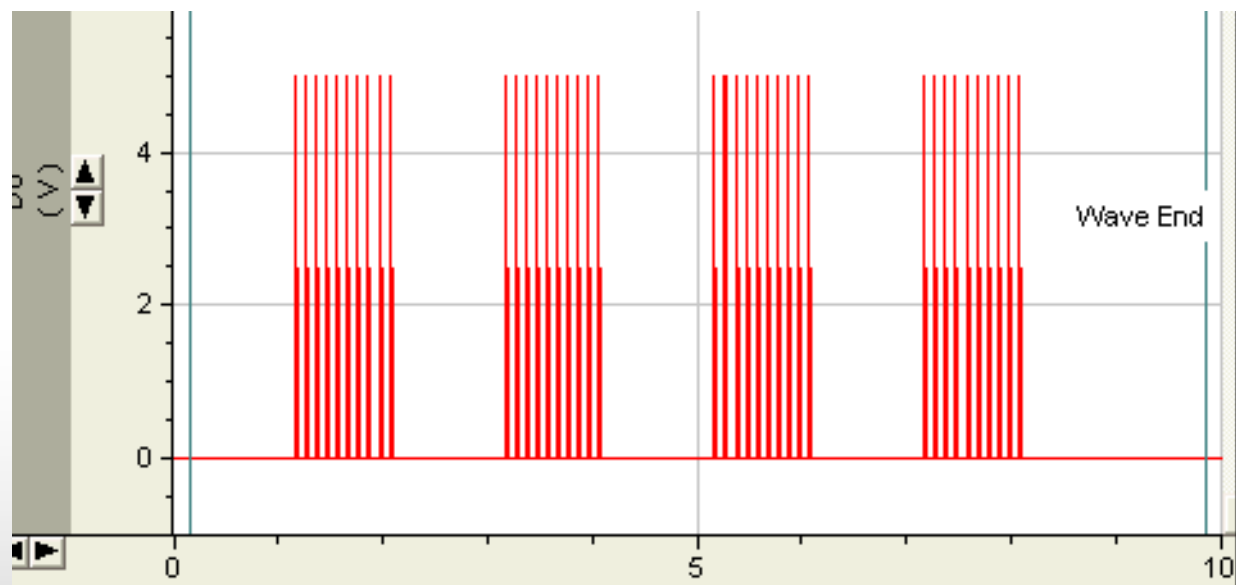
# Digital Bit Pattern---Train Pulse

Digital bit pattern (#3-0)	000*
Digital bit pattern (#7-4)	0000
Train rate (Hz)	100
Pulse width (ms)	1

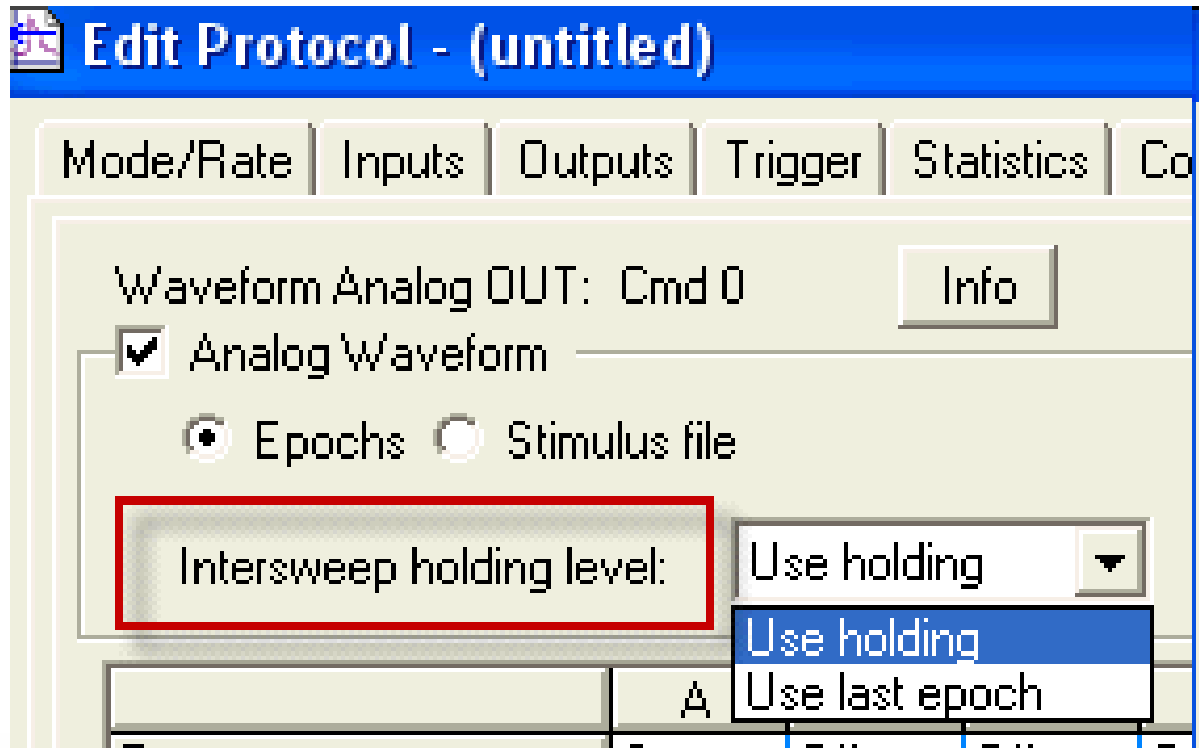


# Digital Bit Pattern

	A	B	C	D	E	F	G	H	I	J
Type	Step	Pulse	Step	Pulse	Step	Pulse	Step	Pulse	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	0	0	0	0	0	0	0	0	0	0
Delta level (mV)	0	0	0	0	0	0	0	0	0	0
First duration (ms)	1000	1000	1000	1000	1000	1000	1000	1000	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	000*	0000	000*	0000	000*	0000	000*	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	10	0	10	0	10	0	10	0	0
Pulse width (ms)	0	1	0	1	0	1	0	1	0	0

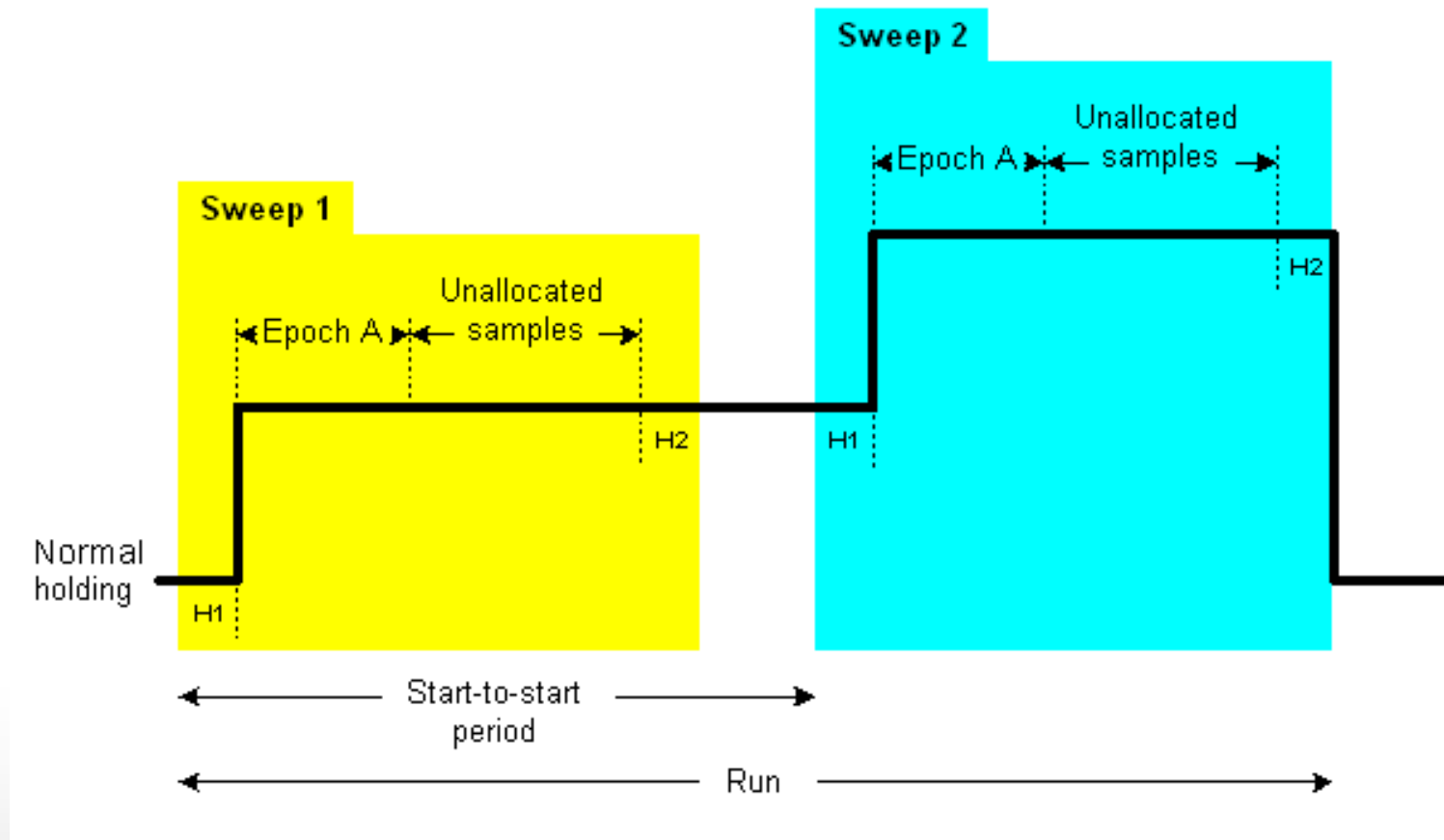


# Intersweep Holding Level

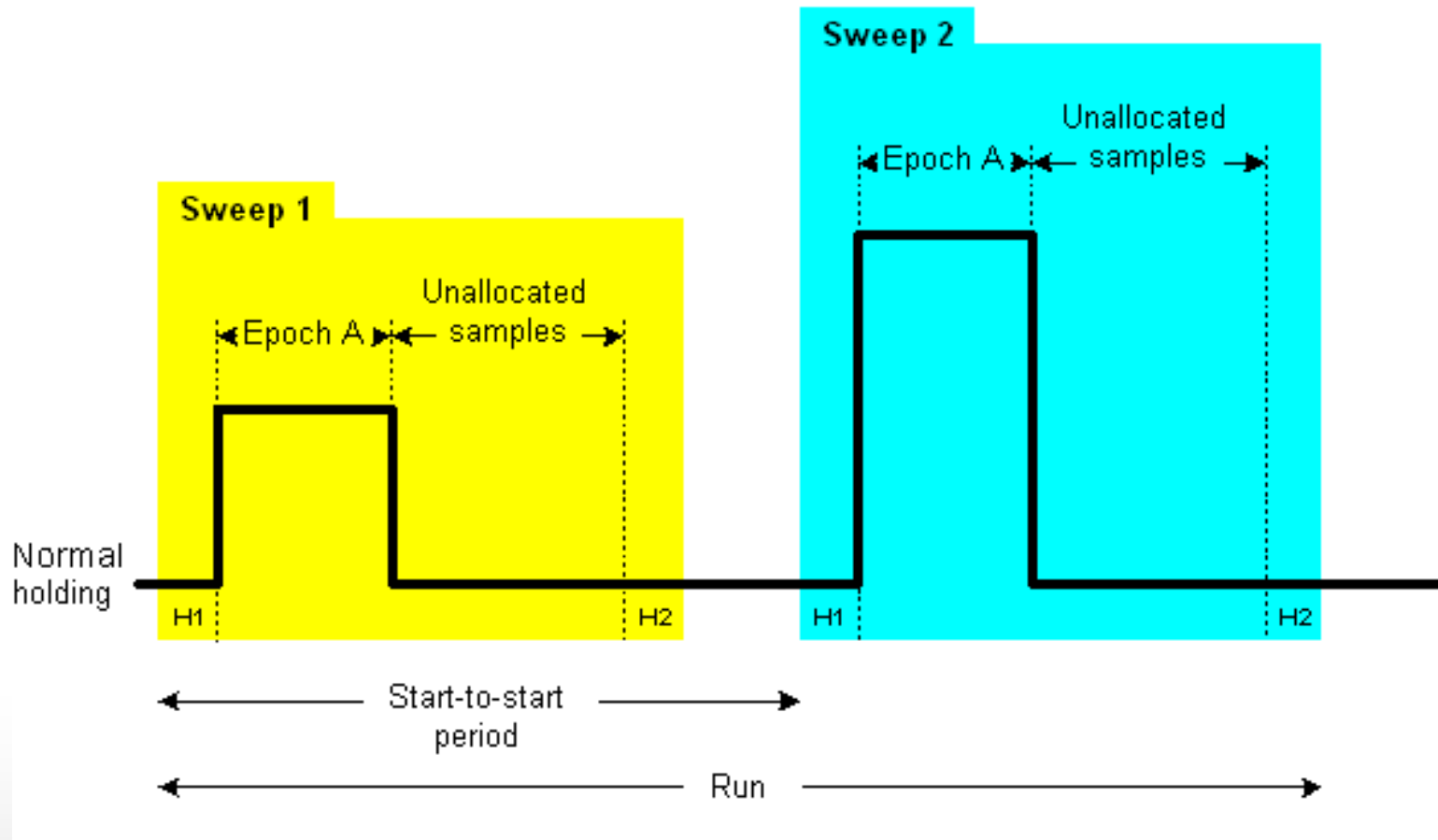




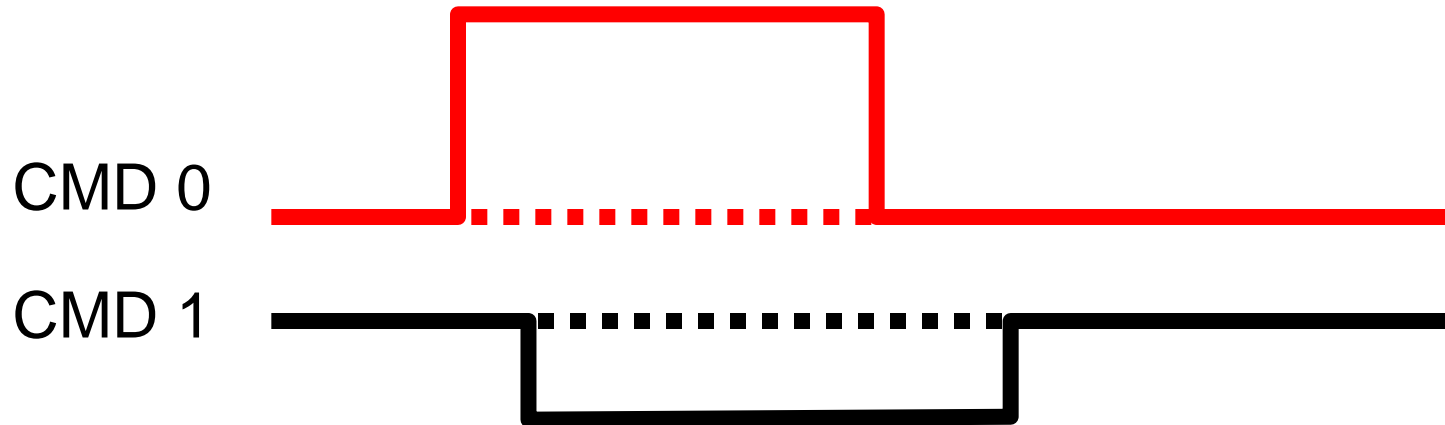
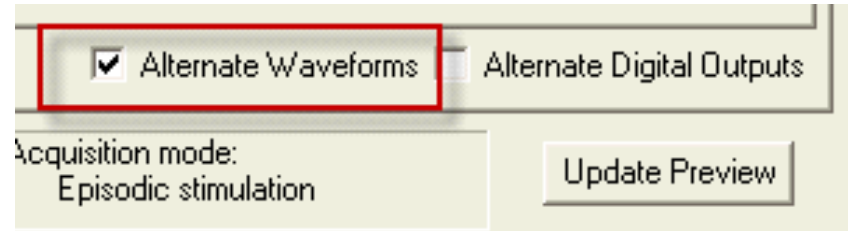
# Intersweep Holding Level---Use last epoch



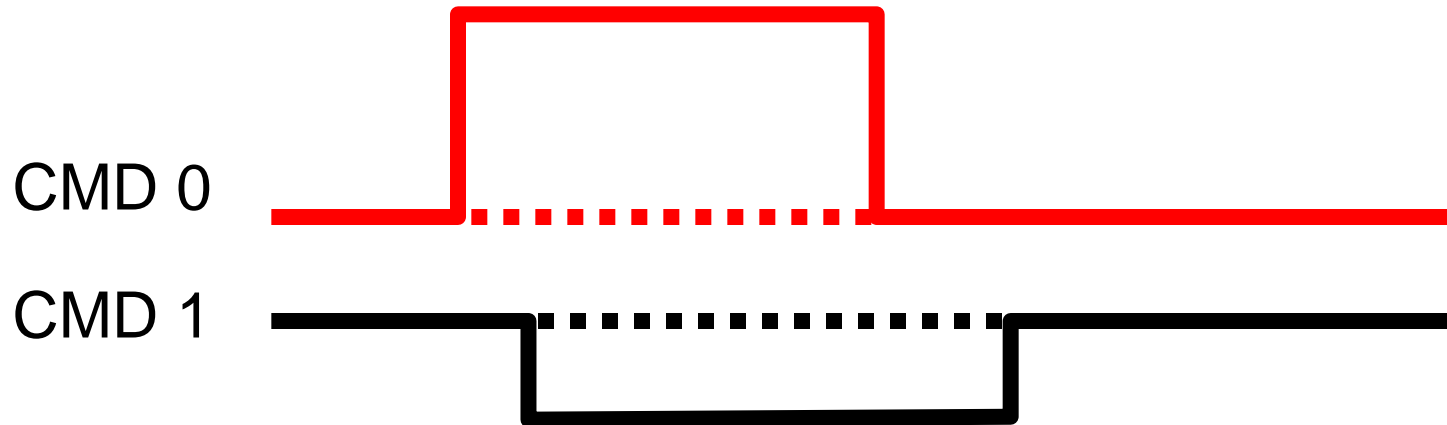
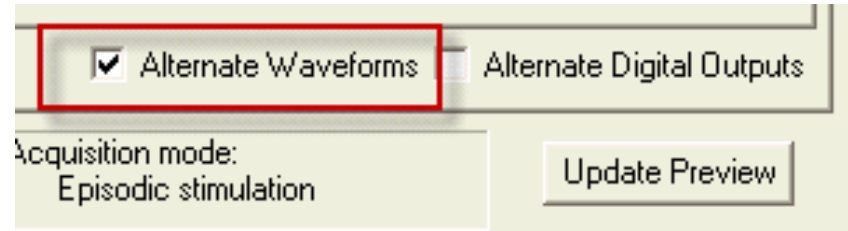
# Intersweep Holding Level---Use Holding



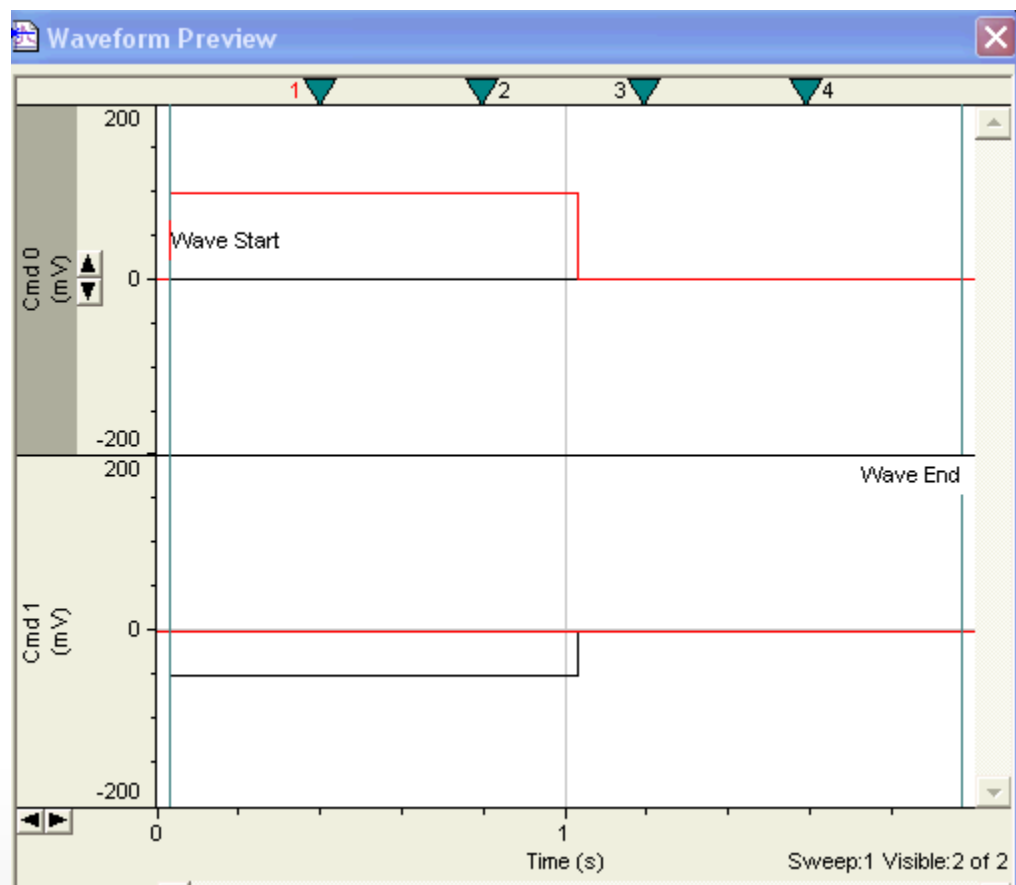
# Alternative Waveform



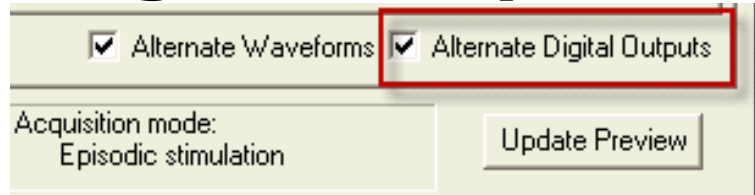
# Alternative Waveform



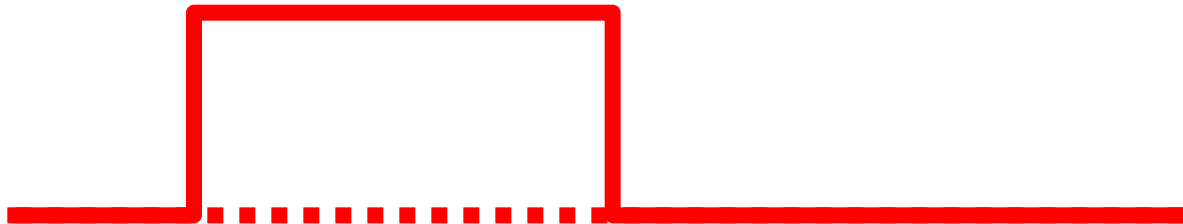
# Alternative Waveform



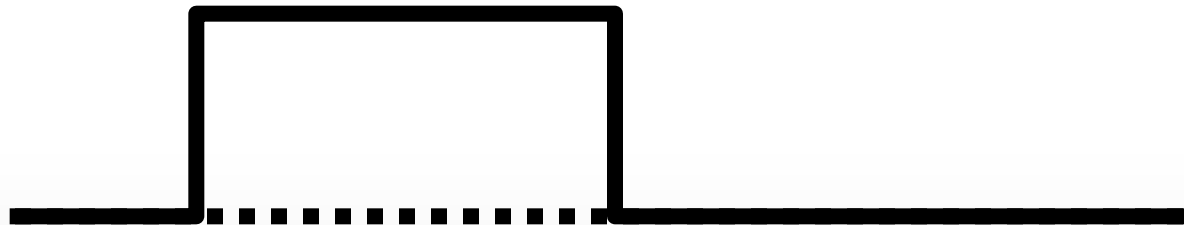
# Alternative Digital Outputs



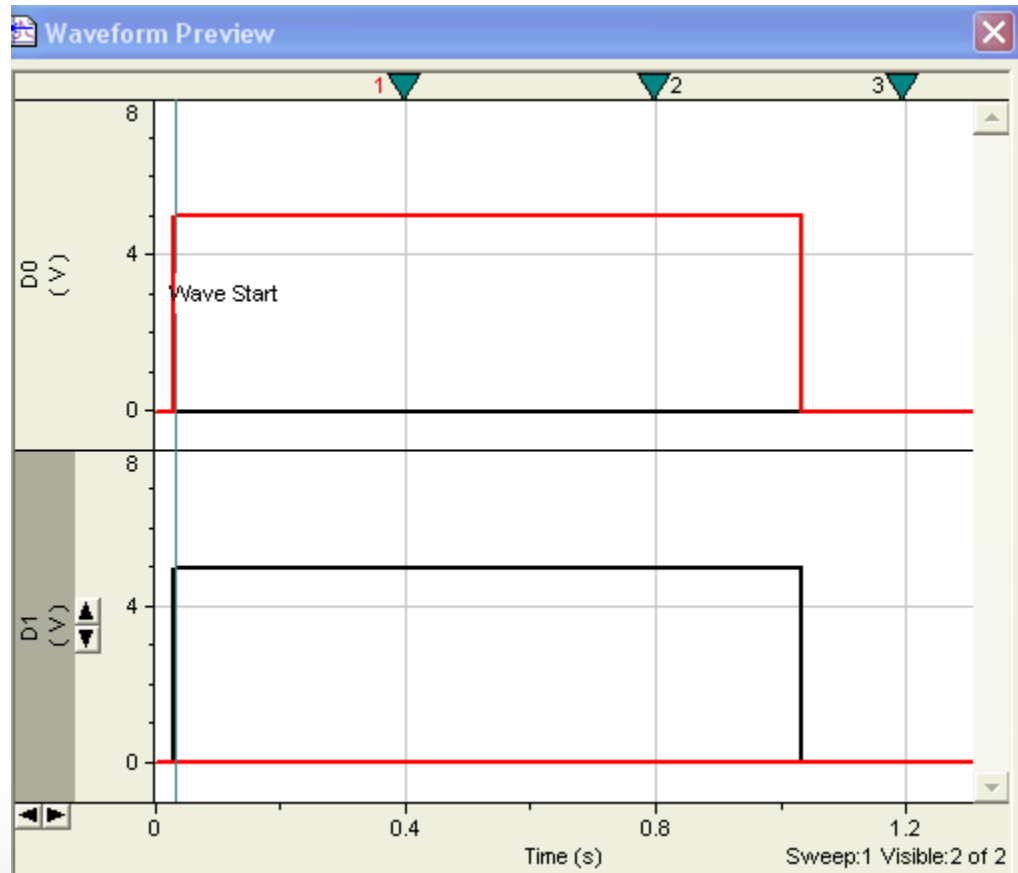
DO# 0



DO# 1



# Alternative Digital Outputs

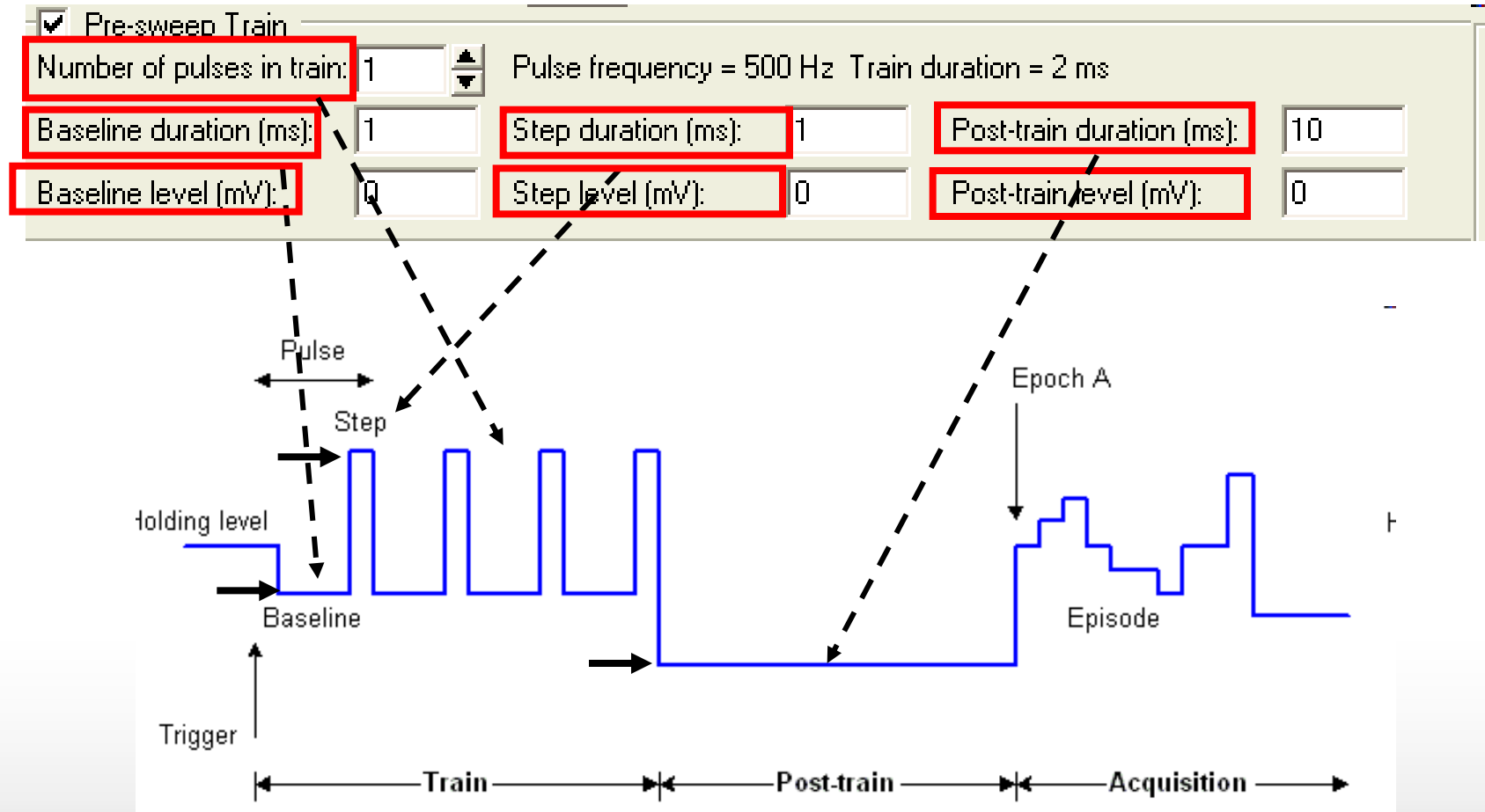


# Pre-sweep Train

- A pre-sweep train consists of repeated square waveform pulses
- Conditioning trains
- No acquisition occurs

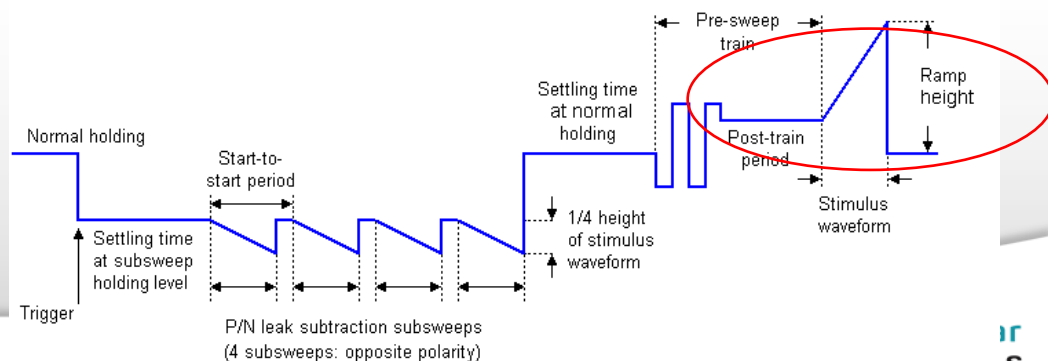


# Pre-sweep Train



# P/N Leak Subtraction

- Leak subtraction corrects for the passive responses by applying test subsweeps before or after the main stimulus waveform
- The technique is called P/N subtraction, in which N subsweeps each 1/Nth of the amplitude of the main stimulus waveform are applied.
- Example: Number of subsweep (N)=4, the stimulus waveform (P)= 80 mV, the pulse of each subsweep=80/4=20 mV



# P/N Leak Subtraction

P/N Leak Subtraction

Apply to Analog IN signal: IN 0

Number of subsweeps: 4

Subsweep start-to-start (ms): Minimum

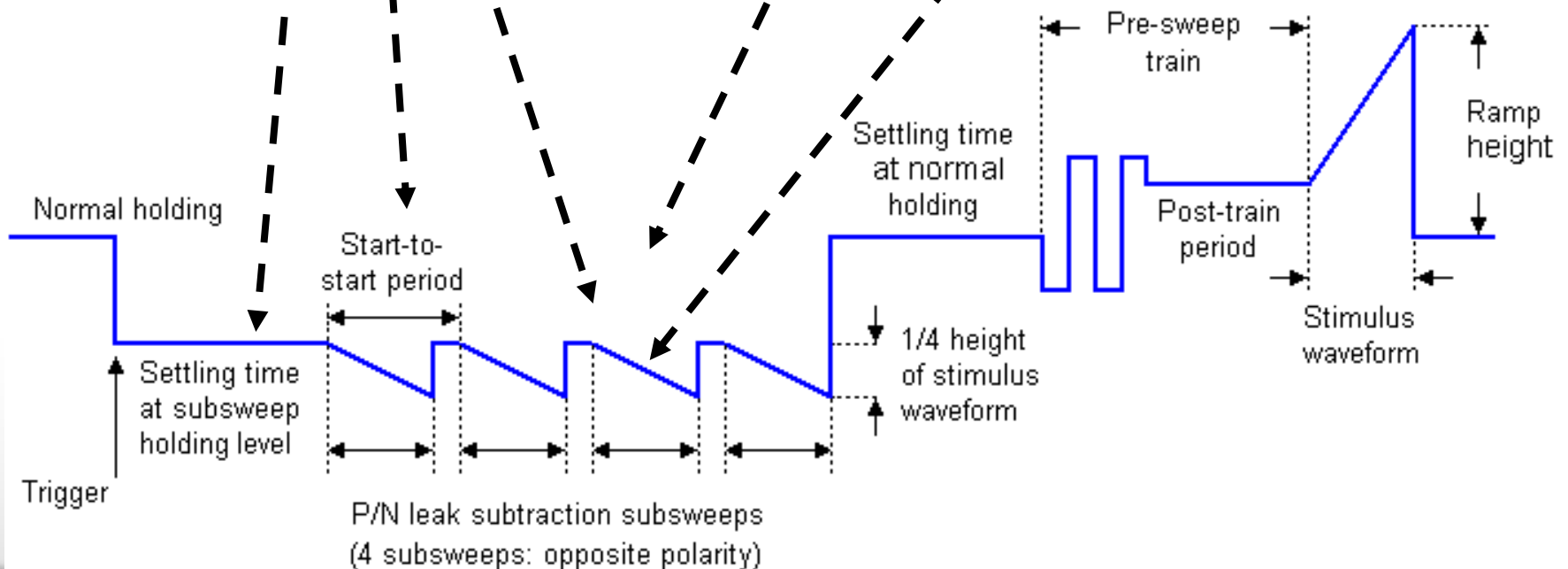
Settling time (ms): 100

Execution:  Before  After

Polarity:  Same as waveform  Opposite to waveform

Subsweep holding level (mV): 0

Show corrected sweep data

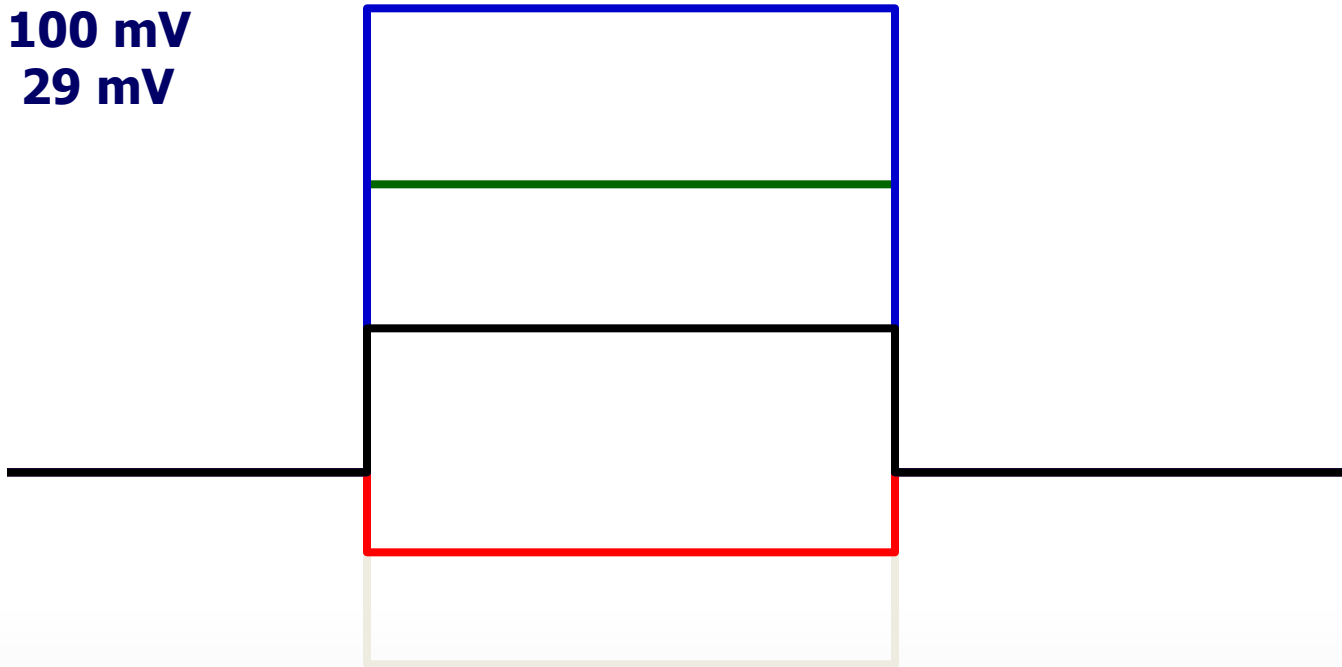


# User List

- Customizing output features
  - Analog
  - digital
- Overrides the generalized settings made elsewhere in the Protocol Editor.

# User List---Holding Level

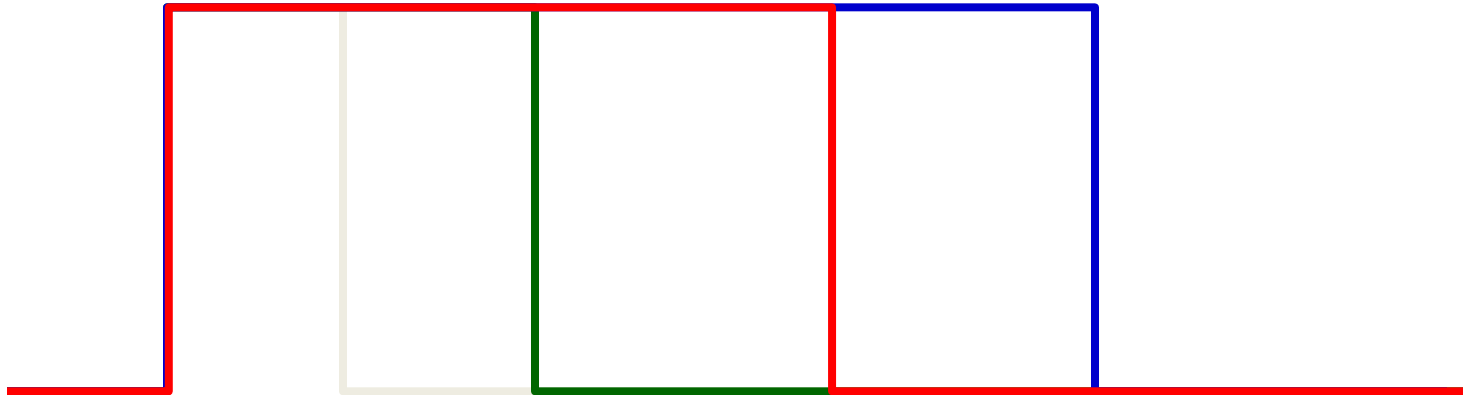
- Sweep 1: -50 mV**
- Sweep 2: 65 mV**
- Sweep 3: -20 mV**
- Sweep 4: 100 mV**
- Sweep 5: 29 mV**



**You can set the arbitrary delta values for the waveform holding levels.**

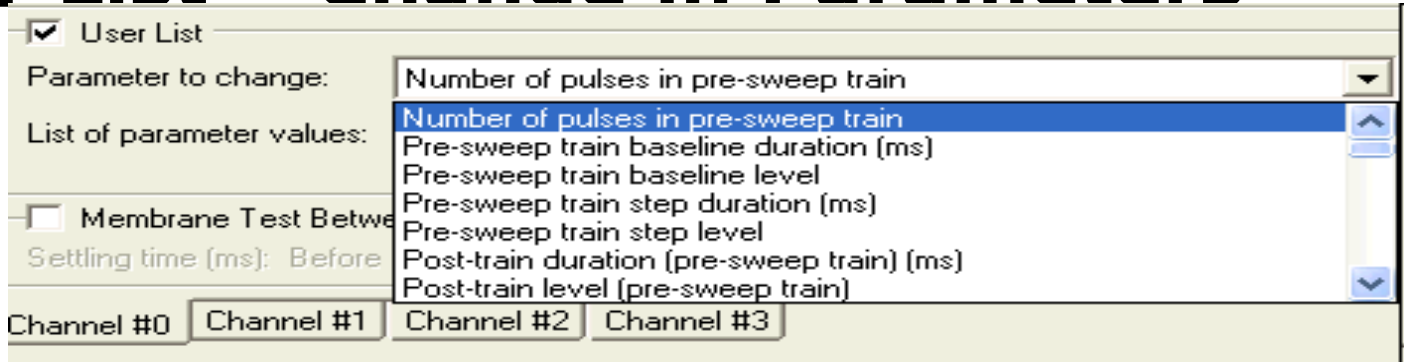
# User List---Epoch Duration

**Sweep 1: 22 ms**  
**Sweep 2: 44 ms**  
**Sweep 3: 100 ms**  
**Sweep 4: 75 ms**



**You can set the arbitrary delta values  
for the waveform sweep durations.**

# User List---Change in Parameters



- Epoch A–J level
- Epoch A–J duration
- Epoch A–J digital pattern
- Epoch A–J train period
- Epoch A–J train pulse width
- Time between sweep starts (s)
- Inactive analog OUT holding level
- Digital intersweep holding level
- Number of P/N subsweeps
- Number of pulses in pre-sweep train
- Pre-sweep train baseline duration (ms)
- Pre-sweep train baseline level
- Pre-sweep train step duration (ms)
- Pre-sweep train step level
- Post-train duration (pre-sweep train) (ms)
- Post-train level (pre-sweep train)

# Lists of parameter values

- The list of values for the A Epoch level might be:
  - -50, 65, -20, 100, 29
- The list of values for the A Epoch duration might be:
  - 22, 44, 100, 75



# Membrane Test Between Sweeps

Trial Hierarchy

Trial delay (s): 0

Runs/trial: 1

Sweeps/run: 100 = 1.91 MB

Sweep duration (s): 1 = 10000 samples

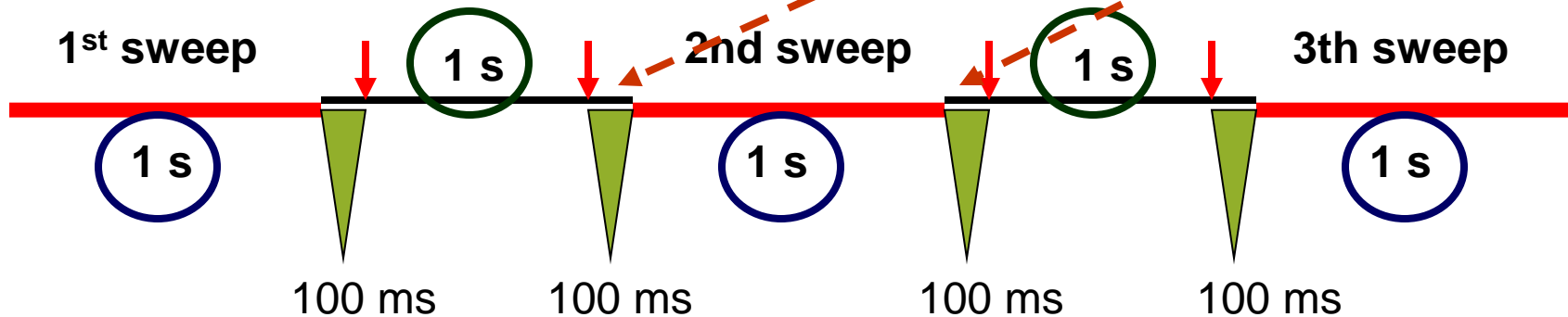
Start-to-Start Intervals

Run (s): Minimum

Sweep (s): 2

Membrane Test Between Sweeps

Settling time (ms): Before 100 After 100



↓ = Membrane test



# pCLAMP™ 10 data acquisition software: Key features review

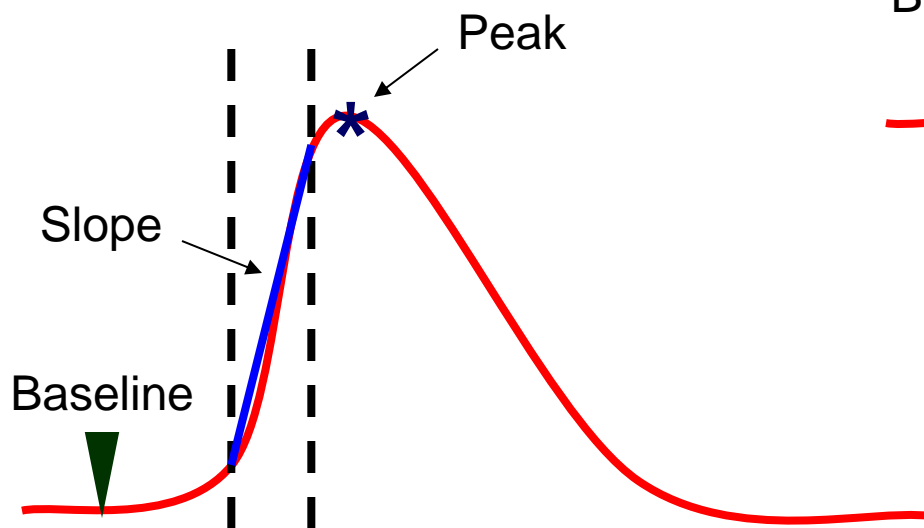
# Key features of Clampex 10

- Online Statistics
- Sequencing keys
- User list
- Membrane test between sweep
- Protocol editor
- LTP assistant
- Stimulus file
- Triggering external devices

# Online Statistics

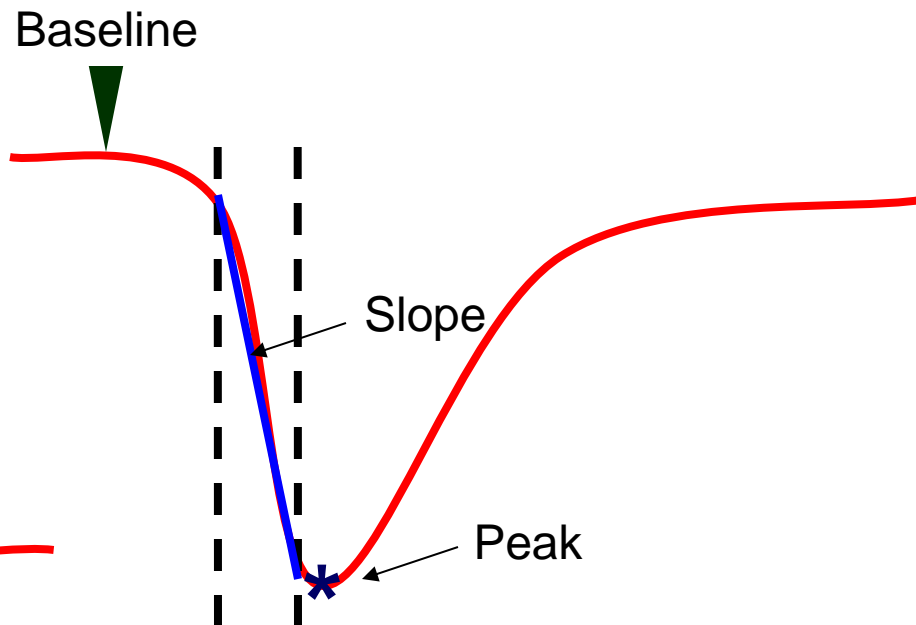
- Measure various parameters of evoked events such as peaks, slopes, areas, and rise time
- Available in episodic and oscilloscope modes
- Up to 8 search regions

## Outward signal

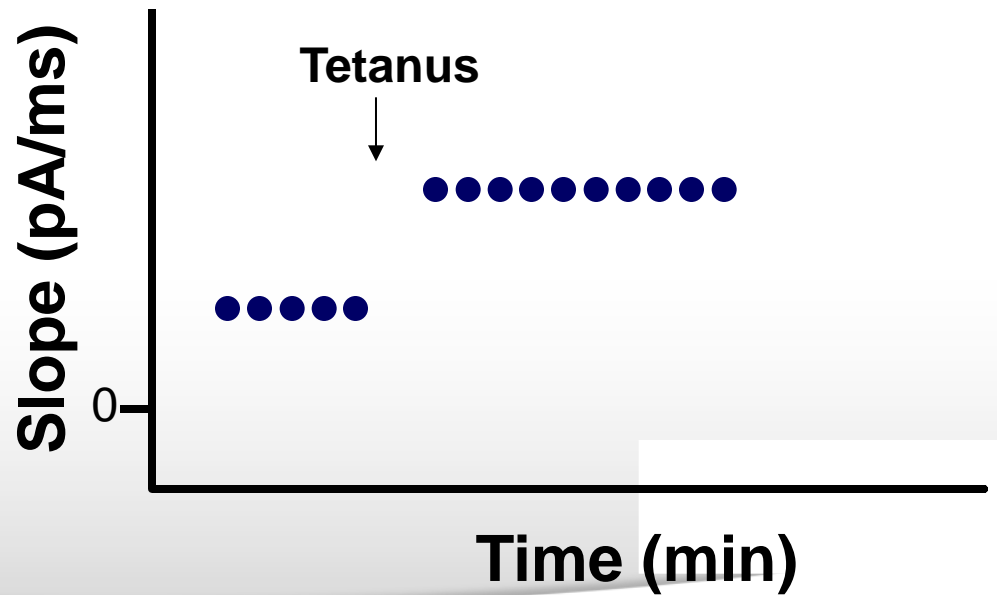
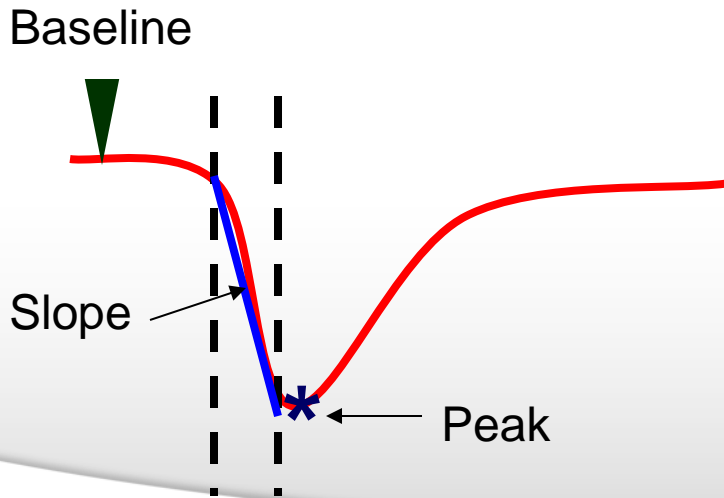
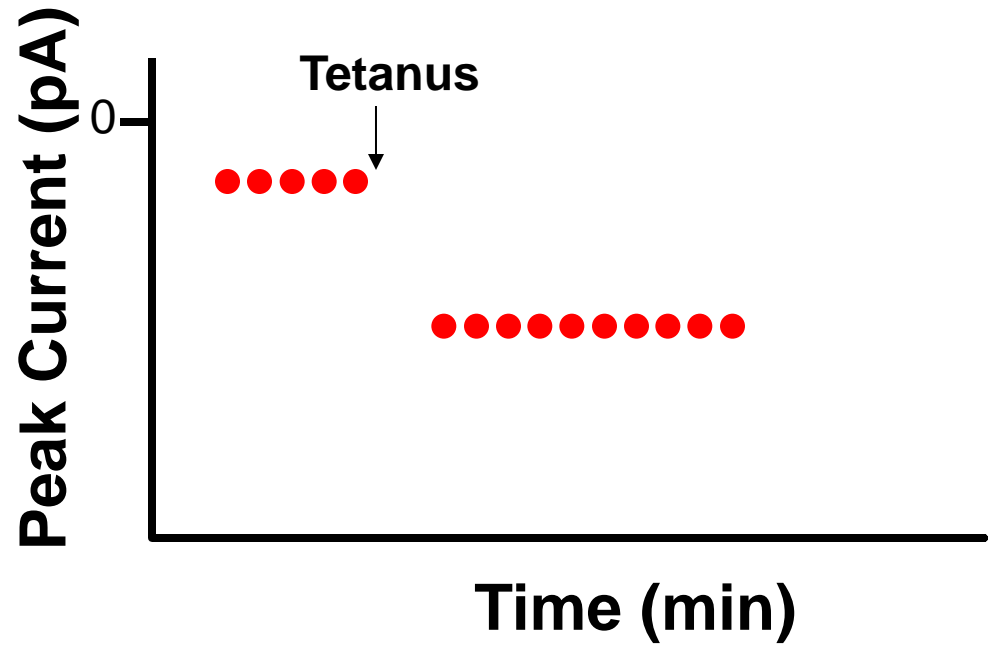
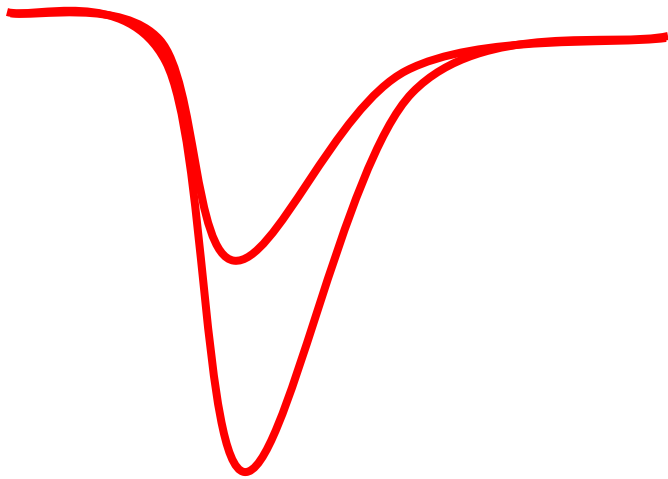


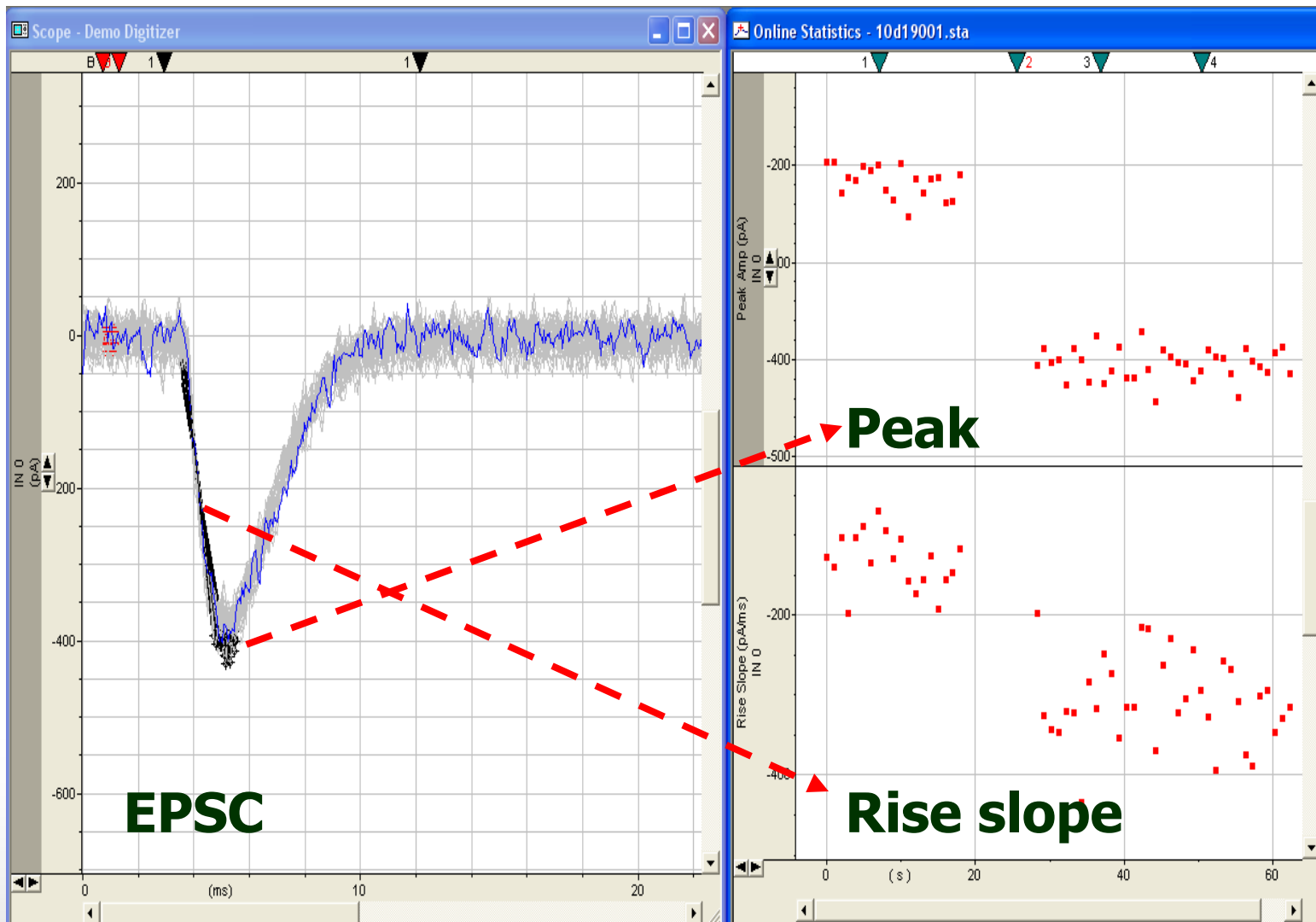
Example:  
Excitatory postsynaptic potential (EPSP)

## Inward signal



Example:  
Excitatory postsynaptic current (EPSC)  
Population spikes  
Field EPSP





Clampex - 10d18005.abf - Scope - Demo Digitizer

File Edit Acquire View Configure Tools Window Help

- New Protocol
- Open Protocol...
- Save Protocol
- Save Protocol As...
- Edit Protocol...**
- Waveform Preview
- Export protocol ( pCLAMP 9 compa
- Record
- Re-Record
- View Only
- Pause-View

**Edit Protocol - Post-LTP and Membrane**

Mode/Rate Inputs Outputs Trigger **Statistics** Comments Math Waveform Stimulus

Shape Statistics

Analog IN Signal IN 0

Positive-going Selected signals: IN 0

Negative-going

Absolute

Baseline Region Cursor Region

From (ms): 0.75 => sample 16

To (ms): 1.6 => sample 33

Search Region 1

Range: Cursor Region

From (ms): 3.15 => sample 64

To (ms): 9.55 => sample 192

Smoothing window (samples): 1

Always save statistics at the end of each recording  Clear after saving

Measurements

- Peak amplitude (pA)
- Time of peak (ms)
- Antipeak amplitude (pA)
- Time of antipeak (ms)
- Mean (pA)
- Standard deviation (pA)
- Area (pA · ms)
- Half-width (ms)
- Maximum rise slope (pA/ms)
- Time of maximum rise slope (ms)
- Maximum decay slope (pA/ms)
- Time of maximum decay slope (ms)
- Slope (pA/ms)
- Baseline (pA)
- Rise slope (pA/ms) } from 10 % to 90 %
- Rise time (ms)
- Decay slope (pA/ms) } from 90 % to 10 %
- Decay time (ms)

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview



# Clampex - 10d18005.abf - Scope - Demo Digitizer

File Edit Acquire View Configure Tools Window Help

- New Protocol
- Open Protocol...
- Save Protocol
- Save Protocol As...
- Edit Protocol...**
- Waveform Preview
- Export protocol ( pCLAMP 9 compa

Record  
Re-Record  
View Only  
Pause-View

Episodic  
Cmd 0 (mV)  
0  
Cmd 1 (nA)  
0

## Edit Protocol - Post-LTP and Memb.pro

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Shape Statistics

Analog IN Signal IN 0  
Selected signals: IN 0

Positive-going  
 Negative-going  
 Absolute

Baseline Region: Cursor Region

From (ms): 0.75 => sample 16  
To (ms): 1.6 => sample 33

Search Region 1

Range: Cursor Region

From (ms): 3.15 => sample 64  
To (ms): 9.55 => sample 192

Smoothing window (samples): 1

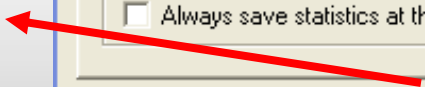
Always save statistics at the end of each recording  Clear after saving

Measurements

- Peak amplitude (pA)
- Time of peak (ms)
- Antipeak amplitude (pA)
- Time of antipeak (ms)
- Mean (pA)
- Standard deviation (pA)
- Area (pA · ms)
- Half-width (ms)
- Maximum rise slope (pA/ms)
- Time of maximum rise slope (ms)
- Maximum decay slope (pA/ms)
- Time of maximum decay slope (ms)
- Slope (pA/ms)
- Baseline (pA)
- Rise slope (pA/ms) } from Min 10 % to Max 90 %
- Rise time (ms)
- Decay slope (pA/ms) } from Max 90 % to Min 10 %
- Decay time (ms)

OK Cancel **Help** Acquisition mode: Episodic stimulation Update Preview

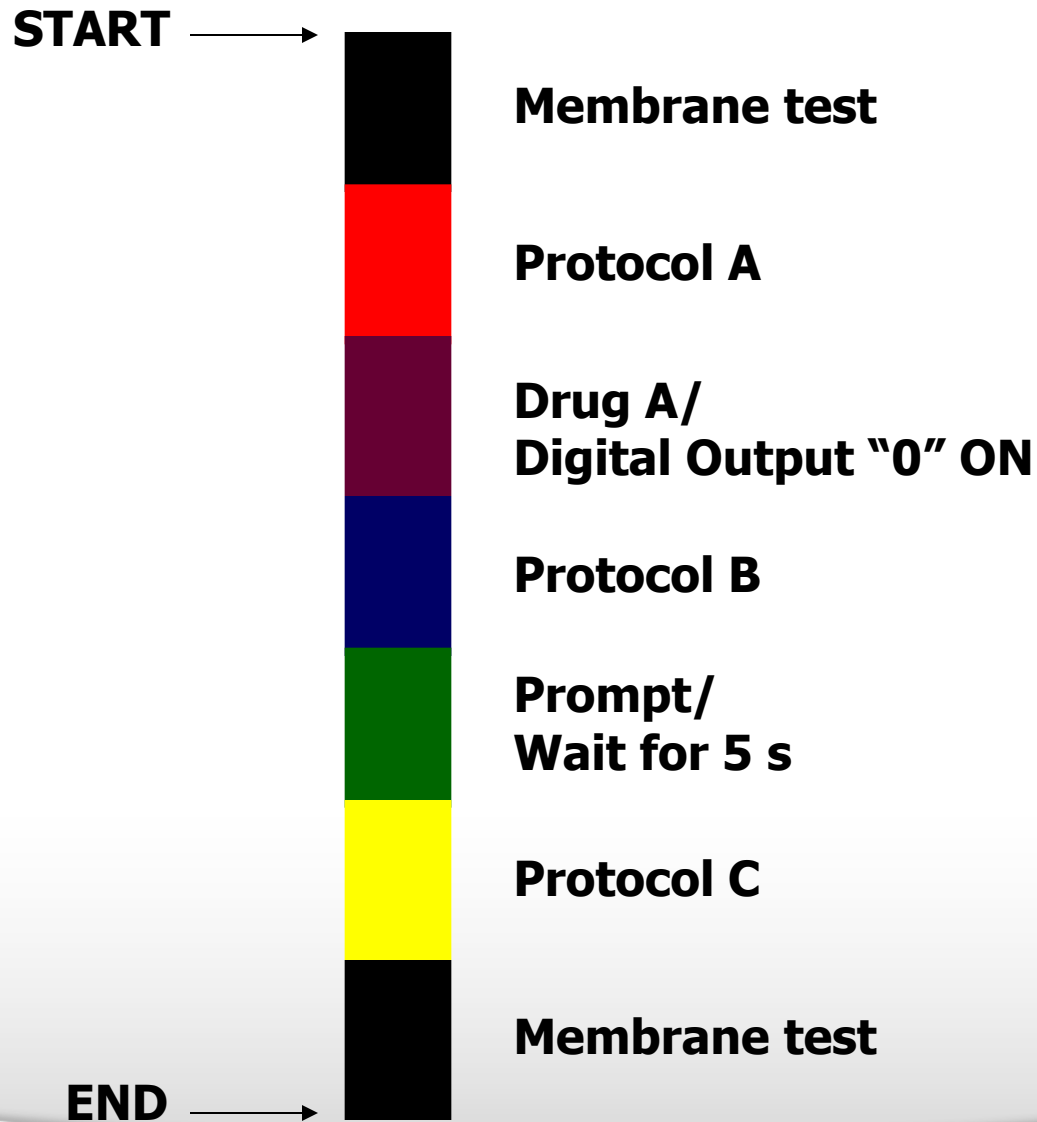
Click "Help" for feature description



# Sequencing Keys

- **Sequencing keys command allows you associate events, or a sequence of event, with a keystroke.**
  - **Set various digital outputs**
  - **Change the holding levels**
  - **Insert a comment tag**
  - **Start a Membrane Test**
  - **Load or run a protocol**
  - **Display a prompt**
- **Use sequencing keys to link one event to another, and run an experiment in an “**automated**” fashion.**

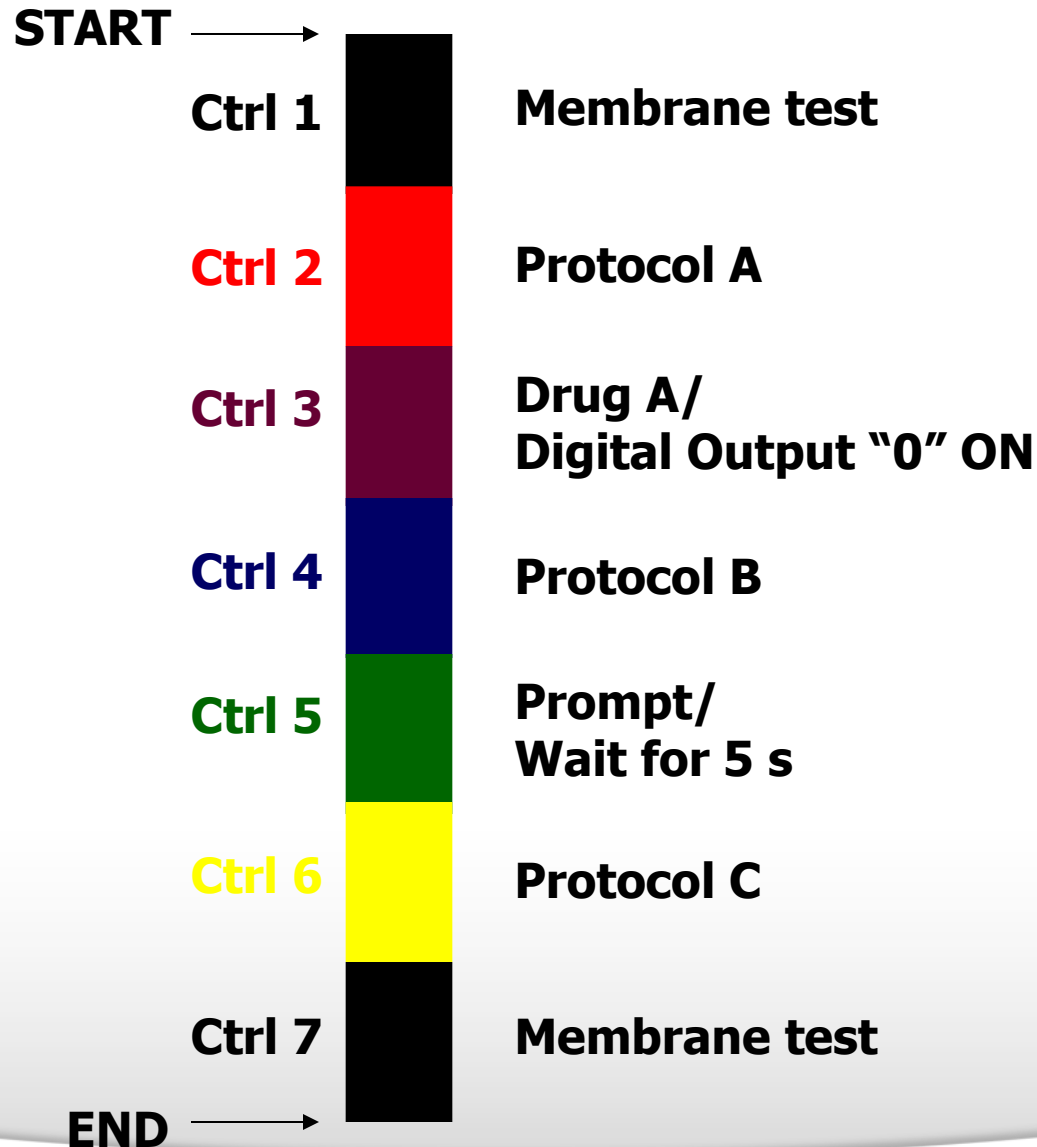
# An experimental protocol



# Sequencing Keys

- The key strokes and tool button identifiers used for sequencing keys are:
  - <Alt + 0> through <Alt + 9> (i.e. hold down the Alt key and press the relevant numeral)
  - <Alt + Shift + 0> through <Alt + Shift + 9>
  - <Ctrl + 0> through <Ctrl + 9>
  - <Ctrl + Alt + 0> through <Ctrl + Alt + 9>
  - <Ctrl + Shift + 0> through <Ctrl + Shift + 9>
- 50 sequencing keys can be triggered in one time.

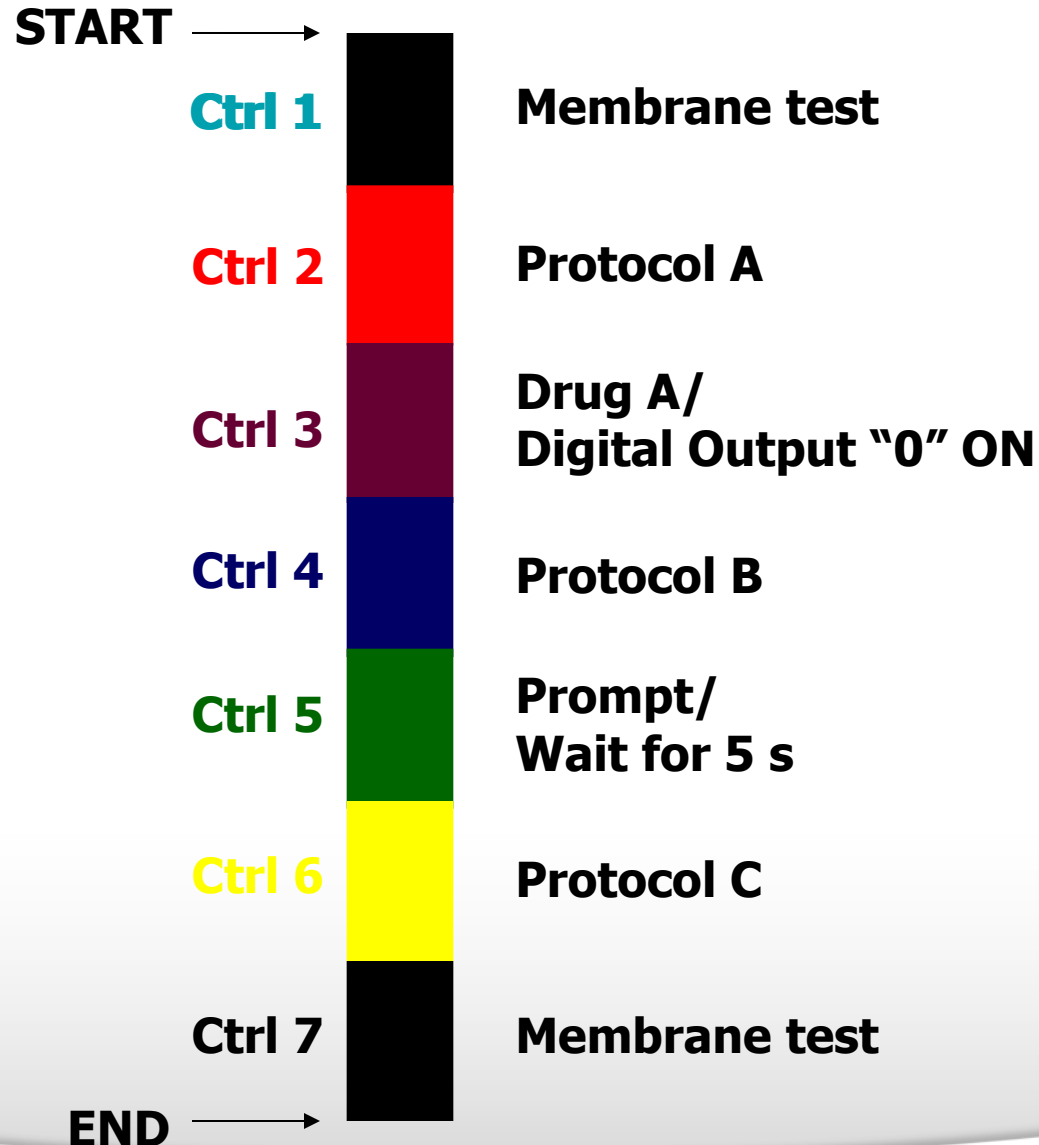
# Set sequencing keys

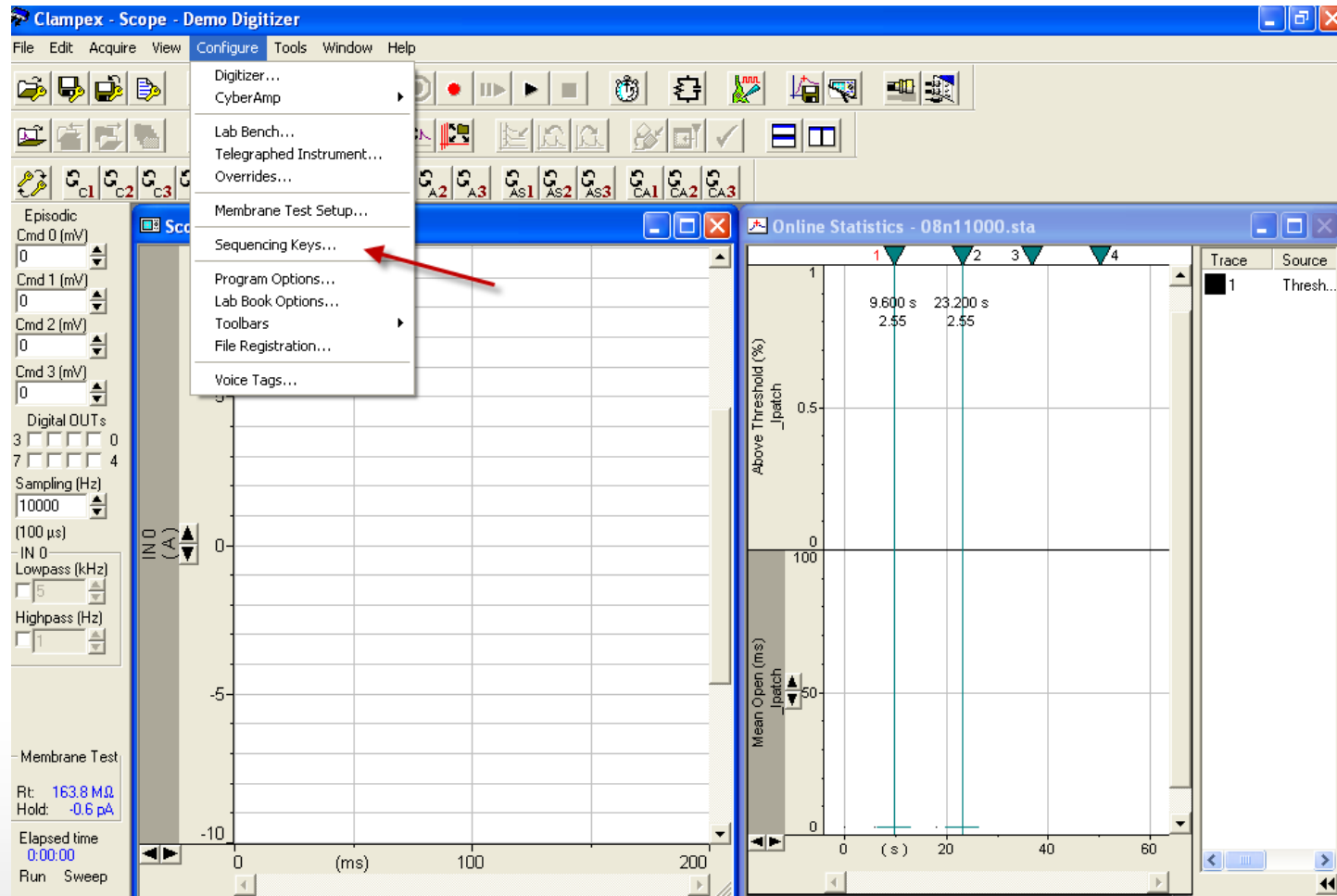


# Sequencing Keys

- Only the key for the first element needs to be triggered to start off the entire sequence.

# Run a protocol in an automated fashion







## Sequencing Keys - LTP22

Key	Next Key	Type	Description
Ctrl+1	Ctrl+2	Protocol	Record using 'ES 16 sweeps.pro'. 1 reps. Pr
Ctrl+2	Ctrl+3	Protocol	Record using 'ES Ramps.pro'. 1 reps. Protoc
Ctrl+3	Ctrl+4	Protocol	Record using 'ES Sine.pro'. 1 reps. Protocol
Ctrl+4	None	Protocol	Record using 'ES Fancy Waveform 01.pro'.

Close  
Help  
Add...  
Remove  
Copy...  
Properties...  
Verify  
=> Clipboard  
New Set...  
Save Set...  
Open Set...  
Options...

# User List

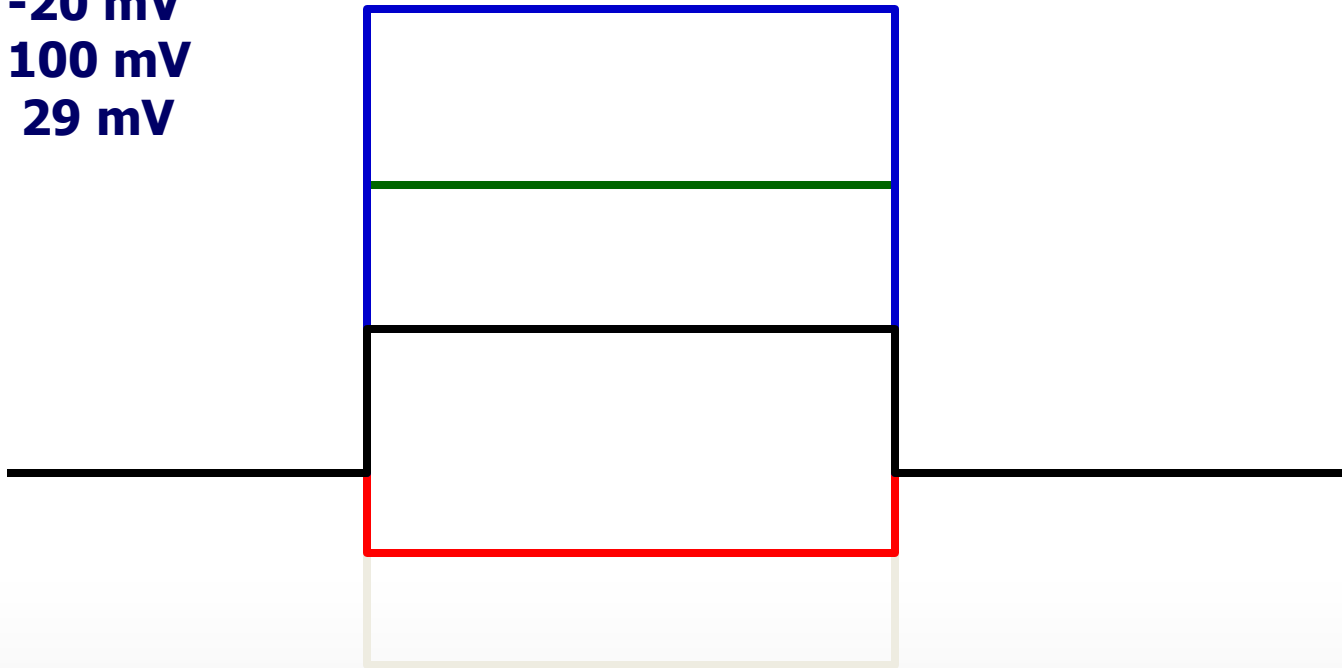
- Provide a way of customizing one of a range of analog and digital output features.
- It overrides the generalized settings made elsewhere in the Protocol Editor.

# User List

- With User List you can select one of a range of parameters offered, to configure on a sweep-by-sweep basis.
- This function allows you to set specific values for each sweep in a run. You can also set a sequence of values and have this repeat.

# Change in Epoch holding levels

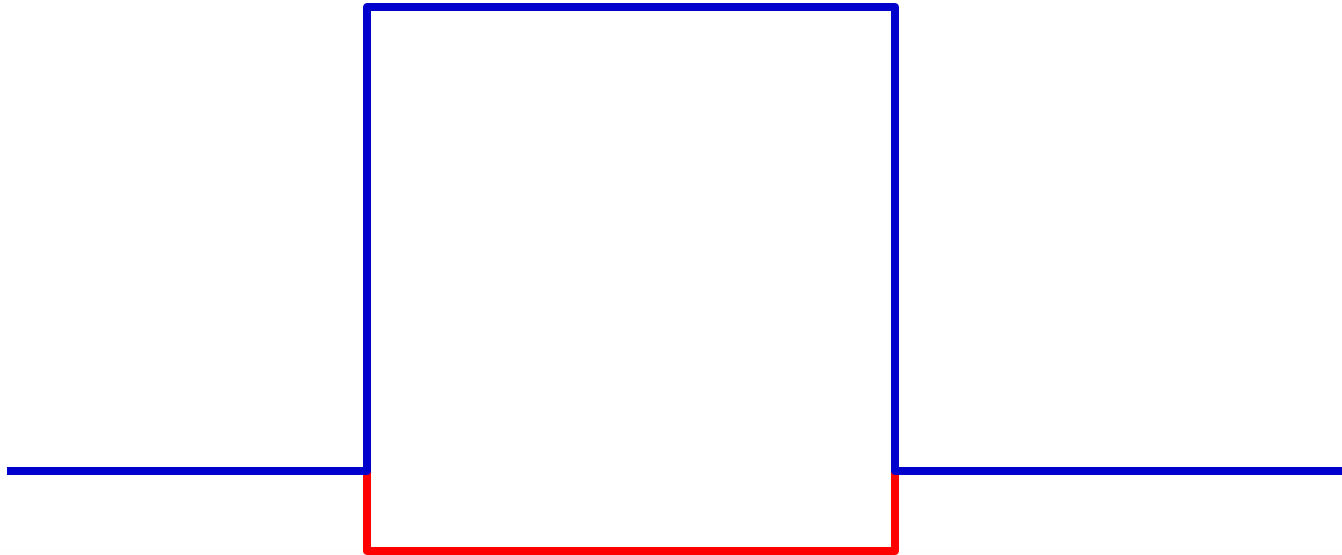
- Sweep 1: -50 mV**
- Sweep 2: 65 mV**
- Sweep 3: -20 mV**
- Sweep 4: 100 mV**
- Sweep 5: 29 mV**



**You can set the arbitrary delta values for the waveform holding levels.**

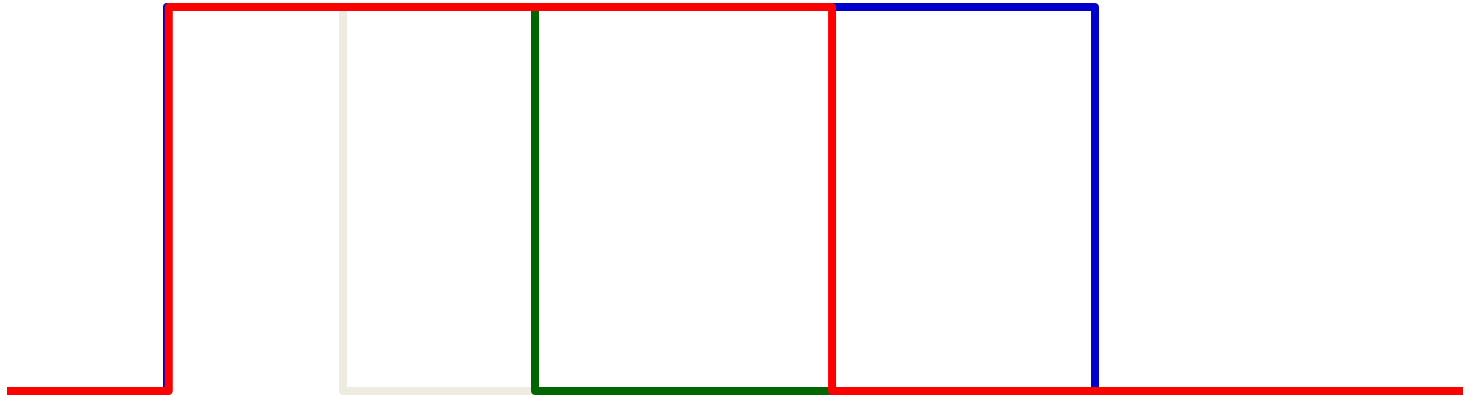
# Change in Epoch holding levels

**Sweep 1: -20 mV**  
**Sweep 2: 100 mV**  
**Repeat...**



# Change in Epoch durations

**Sweep 1: 22 ms**  
**Sweep 2: 44 ms**  
**Sweep 3: 100 ms**  
**Sweep 4: 75 ms**



**You can set the arbitrary delta values  
for the waveform sweep durations.**

# Change in Epoch durations

**Sweep 1: 22 ms**

**Sweep 2: 75 ms**

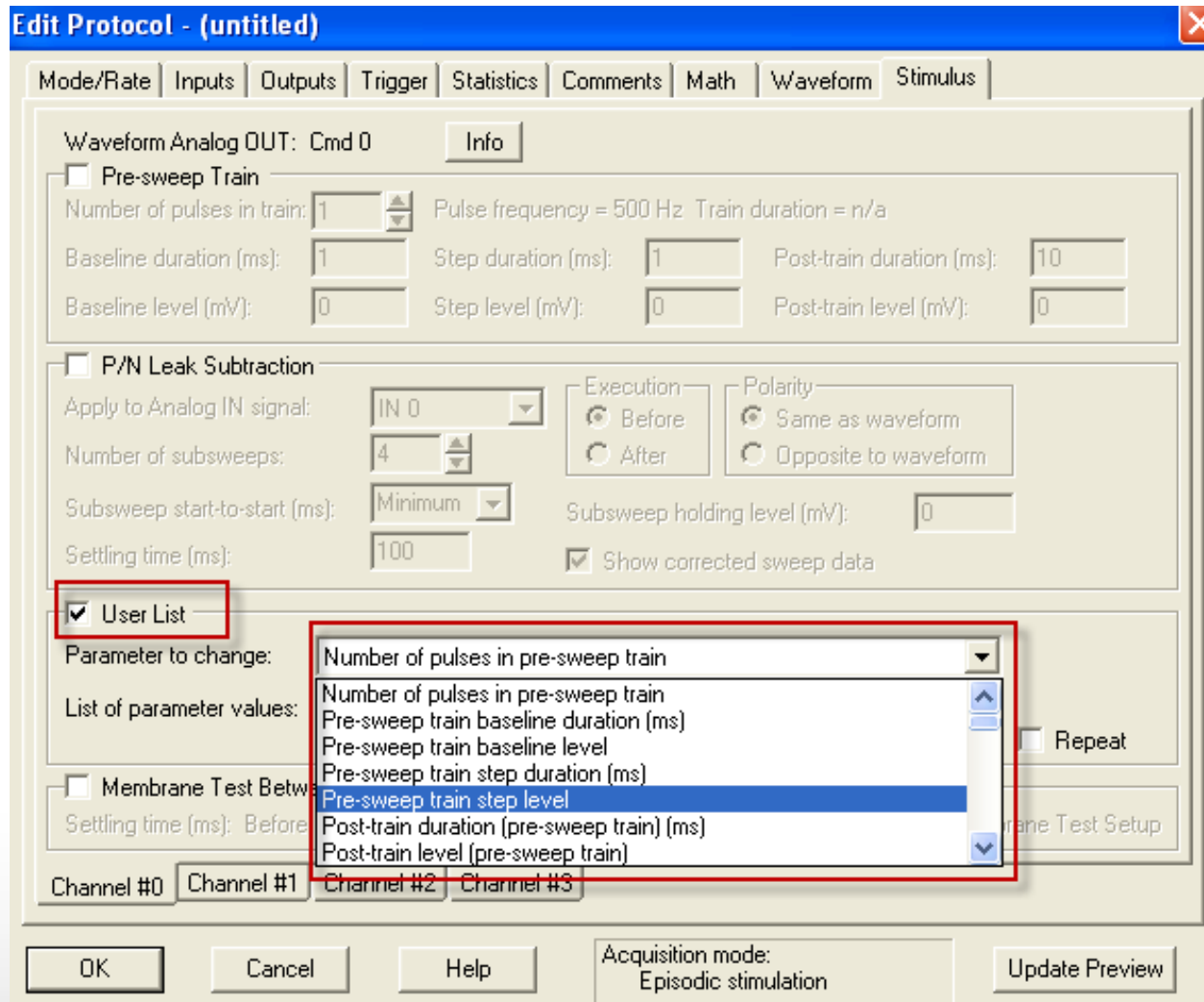
**Repeat...**



# Parameter to Change

- Epoch A–J level
- Epoch A–J duration
- Epoch A–J digital pattern
- Epoch A–J train period
- Epoch A–J train pulse width
- Time between sweep starts (s)
- Inactive analog OUT holding level
- Digital intersweep holding level
- Number of P/N subsweeps
- Number of pulses in pre-sweep train
- Pre-sweep train baseline duration (ms)
- Pre-sweep train baseline level
- Pre-sweep train step duration (ms)
- Pre-sweep train step level
- Post-train duration (pre-sweep train) (ms)
- Post-train level (pre-sweep train)

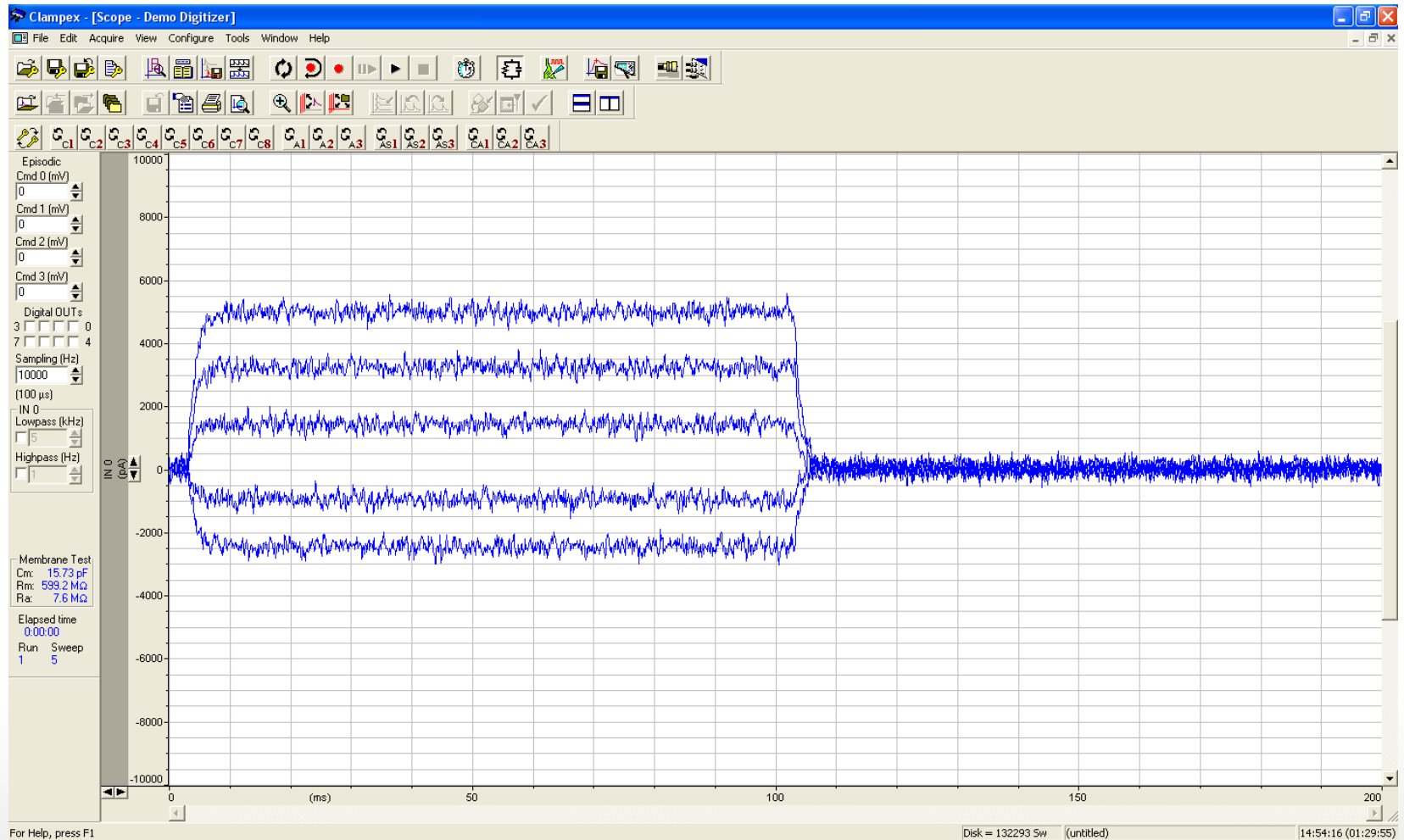




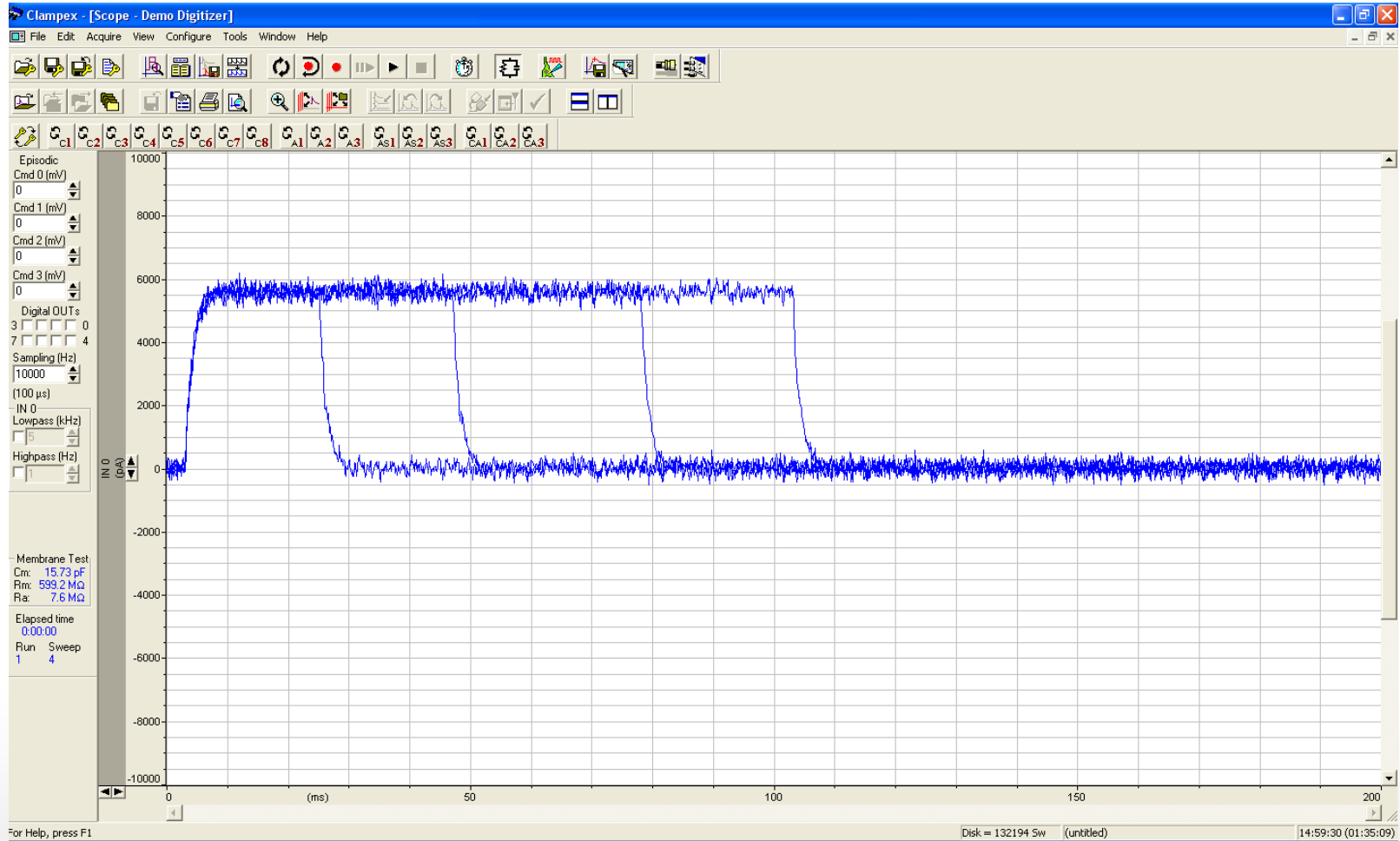
# Examples: List of Parameter values

- The list of values for the A Epoch level might be:
  - -50, 65, -20, 100, 29
- The list of values for the A Epoch duration might be:
  - 22, 44, 100, 75

# Change in Epoch holding levels



# Change in Epoch durations



# Membrane Test Between Sweeps

# Membrane Test

- **Access resistance,  $R_a$**
- **Membrane resistance,  $R_m$**
- **Membrane capacitance,  $C_m$**
- **Time constant,  $\tau$**
- **Holding current,  $I_{hold}$**

Edit Protocol - Post-LTP protocol.pro

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Acquisition Mode

Gap-free       Fixed-length events       Episodic stimulation

Variable-length events       High-speed oscilloscope

Trial Hierarchy

Trial delay (s): 0

Runs/trial: 1

Sweeps/run: 100 = 3.81 MB

Sweep duration (s): 1 = 20000 samples

First holding:	Epochs:	Last holding:
15.6 ms	968.8 ms	15.6 ms
312 samples	19376 samples	312 samples

Start-to-Start Intervals

Run (s): Minimum

Sweep (s): 1

Sampling Rate per Signal

Fast rate (Hz): 20000 => 50  $\mu$ s

Slow rate (Hz): 20000 => 50  $\mu$ s

Averaging

Runs/trial = 1, no averaging

Options...

Space available is 24114 sweeps = 50071 MB      Total data throughput is 20 kHz (= 2.29 MB/min)

Allow automatic analysis in other programs

OK    Cancel    Help    Acquisition mode: Episodic stimulation    Update Preview

Stimulus

sin duration = 2 ms

Post-train duration (ms): 10

Post-train level (mV): 0

Polarity

Same as waveform

Opposite to waveform

ing level (mV): 0

ted sweep data

Repeat

Membrane Test Between Sweeps

Settling time (ms): Before 100    After 100    For other settings, use Configure / Membrane Test Setup

Channel #0    Channel #1    Channel #2    Channel #3

OK    Cancel    Help    Acquisition mode: Episodic stimulation    Update Preview

Trial Hierarchy

Trial delay (s): 0

Runs/trial: 1

Sweeps/run: 100 = 3.81 MB

Sweep duration (s): 1 = 20000 samples

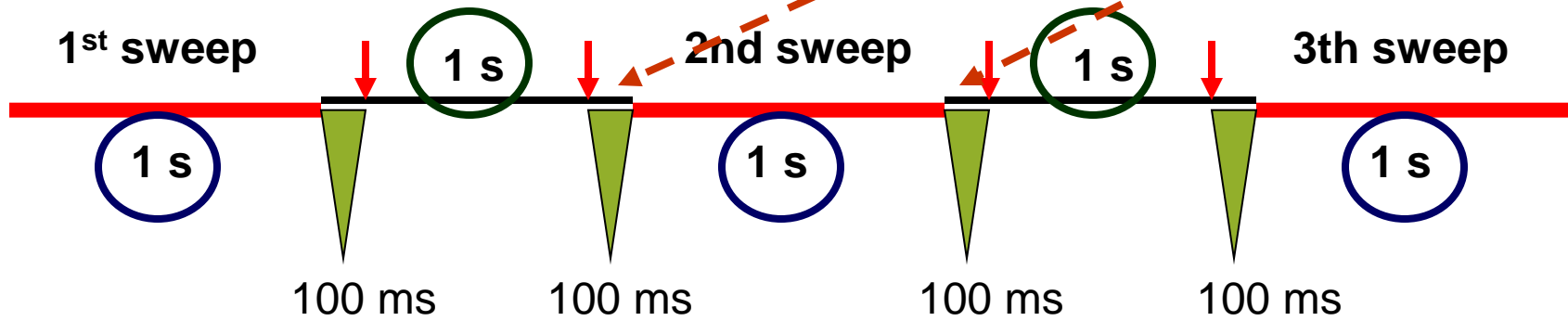
Start-to-Start Intervals

Run (s): Minimum

Sweep (s): 1

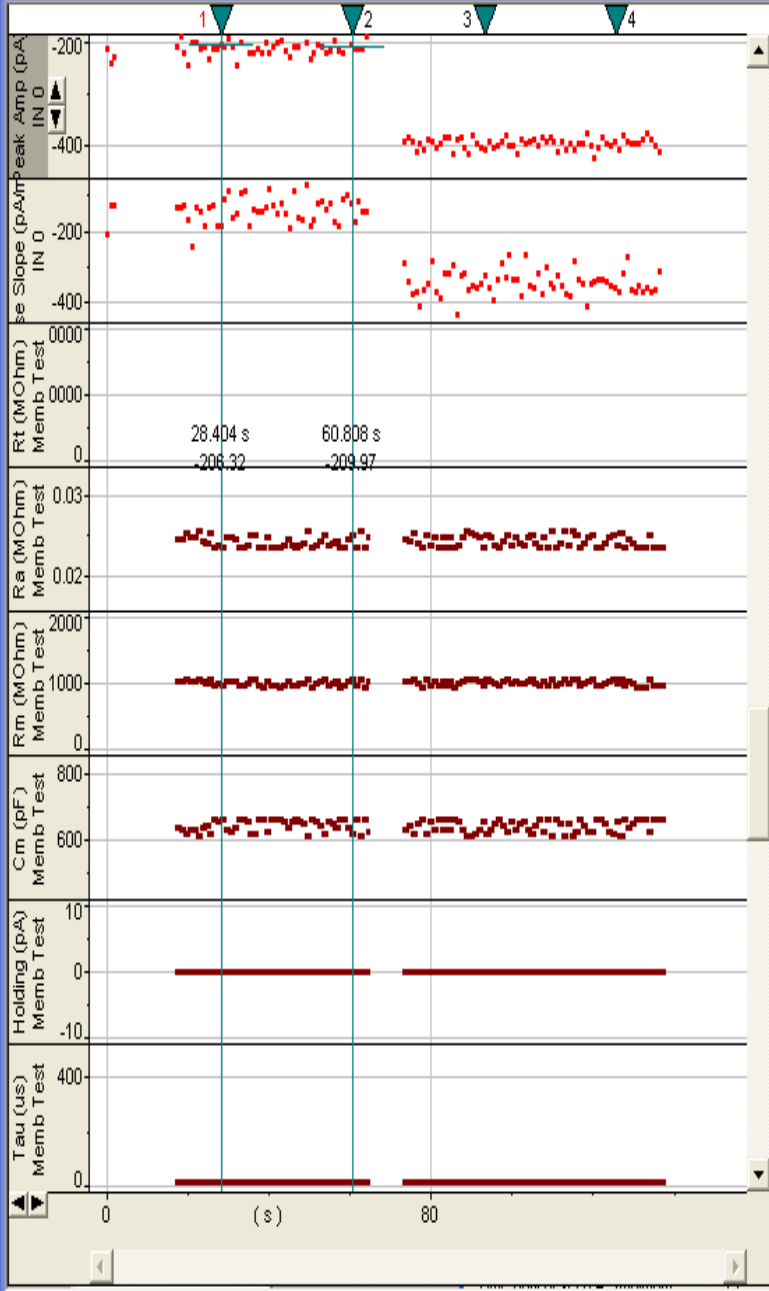
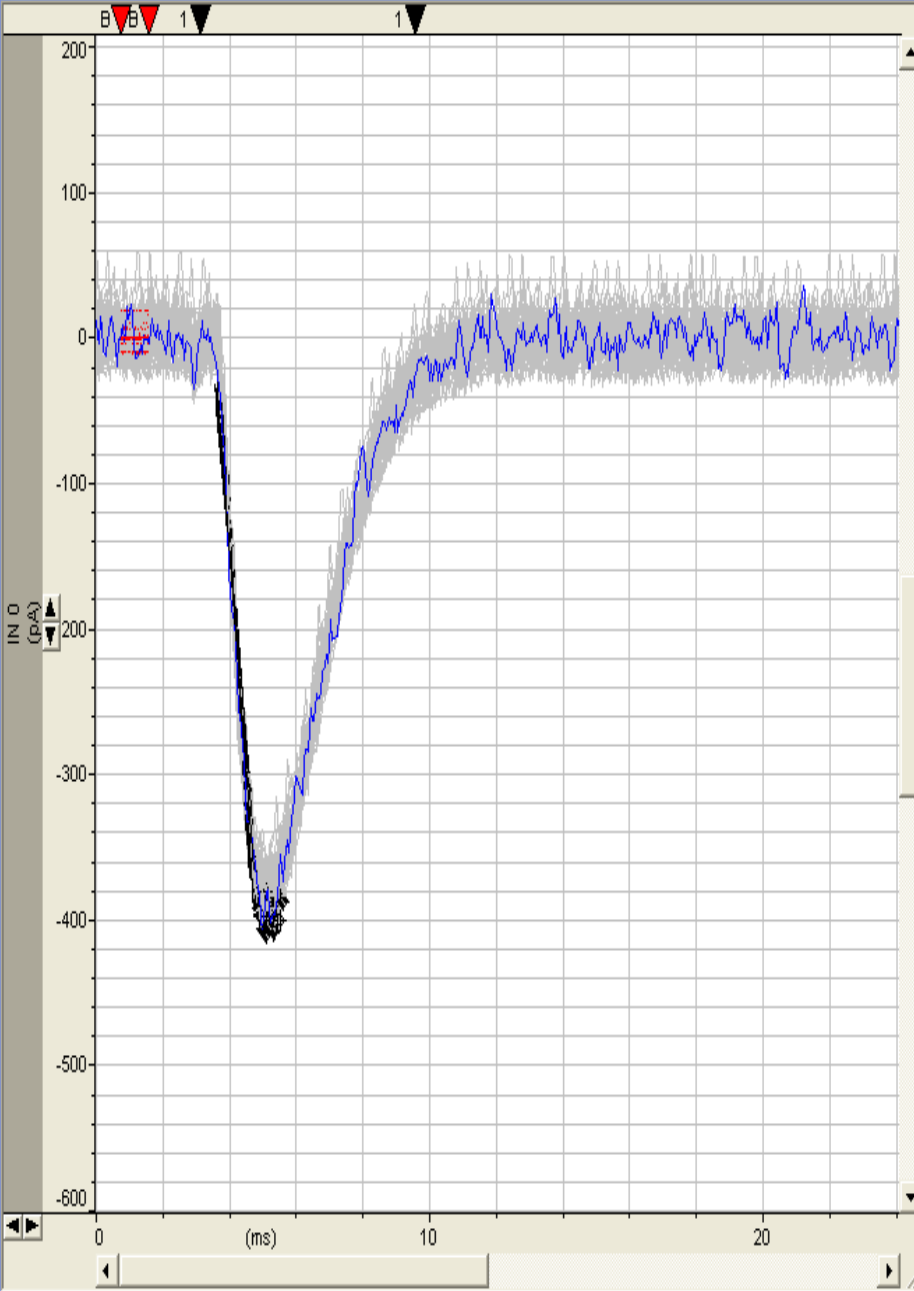
Membrane Test Between Sweeps

Settling time (ms): Before 100 After 100



↓ = Membrane test





Trace	Source
1	Region 1
2	Memb...

**Clampfit - [Results1 - Results]**

File Edit View Analyze Format Event Detection Configure Tools Window Help

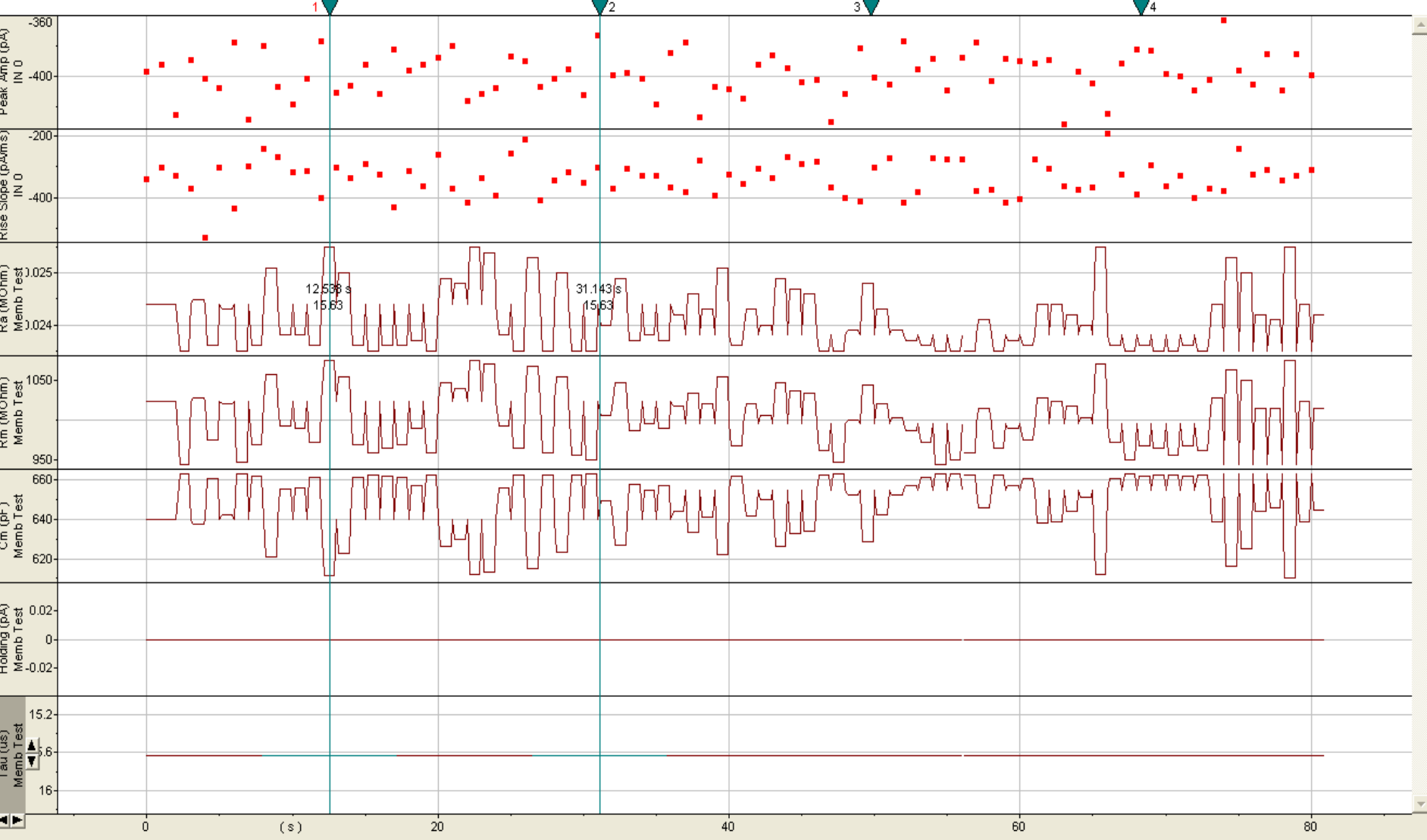
- New
  - New Data File Index
- Open Data... Ctrl+O
  - Open Data Options...
  - Open Data File Index...
  - Open Other
    - Lab Book...
    - Results & Statistics...
- Close Ctrl+W
  - Close All

**Clampfit - [Results1 - Results]**

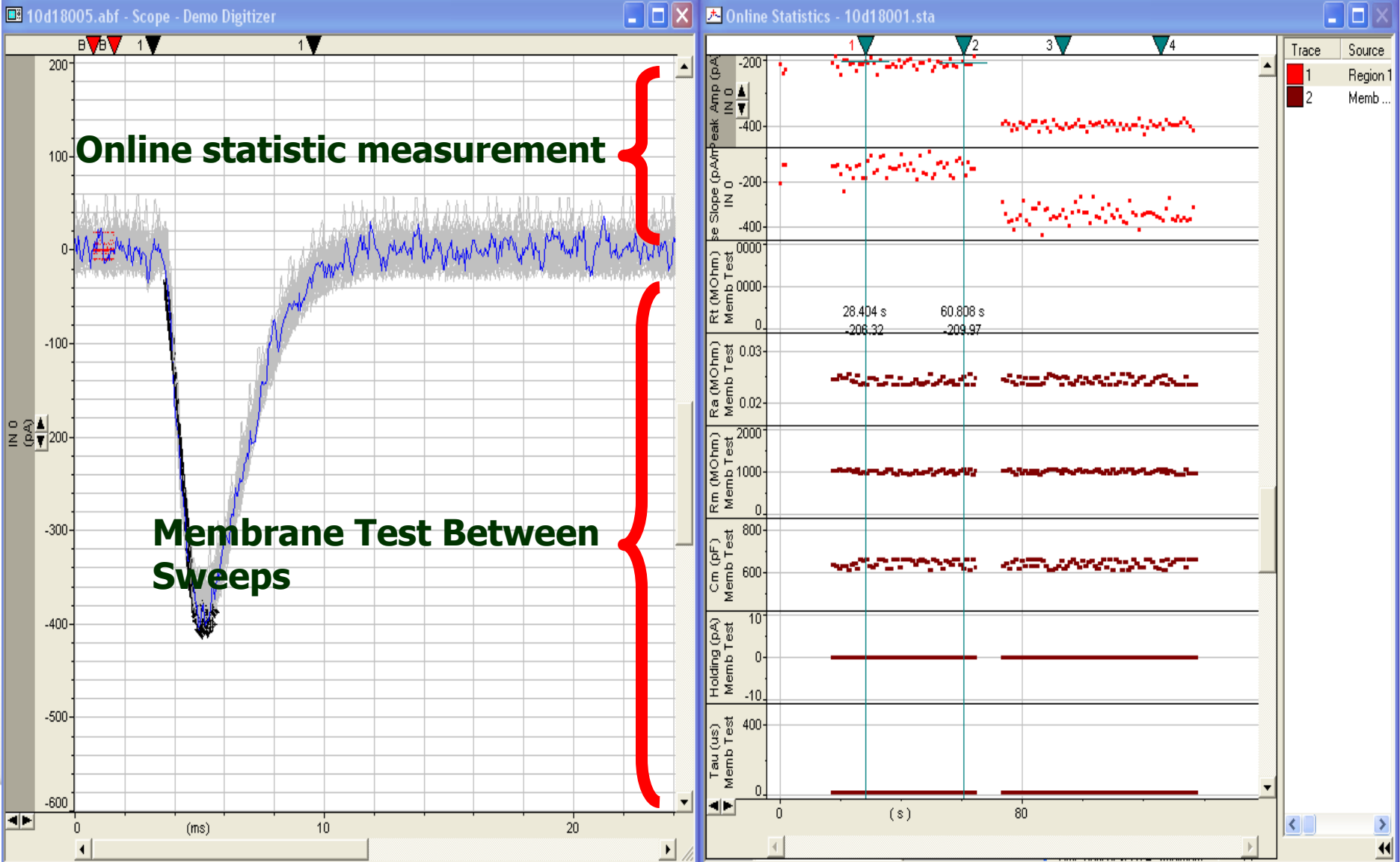
File Edit View Analyze Format Event Detection Configure Tools Window Help

	Time (s)	Region 1-IN	Region 1-OUT	Memb Test 0	Memb Test 0-Mem	Memb Test 0	Memb Test	Memb Test
1								
2								
3	6.70476e-00	-396.618	-341.907	0.0244	1023.98	640.185	0	15.625
4	1.00001	-392.27	-304.071	0.0244	1023.98	640.185	0	15.625
5	2.00001	-425.617	-327.669	0.0244	1023.98	640.185	0	15.625
6	3.00001	-389.135	-369.928	0.0244	1023.98	640.185	0	15.625
7	4.00001	-401.418	-527.35	0.0244	1023.98	640.185	0	15.625
8	5.00001	-407.63	-301.137	0.0244	1023.98	640.185	0	15.625
9	6.00001	-377.328	-433.741	0.0244	1023.98	640.185	0	15.625
10	7.00001	-429.17	-300.389	0.0244	1023.98	640.185	0	15.625
11	8.00001	-380.015	-241.361	0.0244	1023.98	640.185	0	15.625
12	9.00001	-407.305	-269.556	0.0244	1023.98	640.185	0	15.625
13	10	-418.625	-317.582	0.0244	1023.98	640.185	0	15.625
14	11	-402.032	-312.375	0.0244	1023.98	640.185	0	15.625
15	12	-377.067	-402.452	0.0244	1023.98	640.185	0	15.625
16	13	-411.306	-303.043	0.0244	1023.98	640.185	0	15.625
17	14	-406.689	-337.691	0.0244	1023.98	640.185	0	15.625
18	15	-392.543	-291.797	0.0244	1023.98	640.185	0	15.625
19	16	-411.435	-325.913	0.0244	1023.98	640.185	0	15.625
20	17	-382.614	-429.453	0.0244	1023.98	640.185	0	15.625
21	18	-396.152	-312.506	0.0244	1023.98	640.185	0	15.625
22	19	-392.563	-363.627	0.0244	1023.98	640.185	0	15.625
23	20	-387.604	-260.169	0.0244	1023.98	640.185	0	15.625
24	21	-379.512	-369.29	0.0244	1023.98	640.185	0	15.625
25	22	-416.414	-416.725	0.0244	1023.98	640.185	0	15.625
26	23	-412.133	-336.846	0.0244	1023.98	640.185	0	15.625
27	24	-407.789	-392.96	0.0244	1023.98	640.185	0	15.625
28	25	-387.002	-255.915	0.0244	1023.98	640.185	0	15.625
29	26	-390.243	-214.245	0.0244	1023.98	640.185	0	15.625
30	27	-406.754	-407.541	0.0244	1023.98	640.185	0	15.625
31	28	-401.839	-342.663	0.0244	1023.98	640.185	0	15.625
32	29	-395.766	-317.118	0.0244	1023.98	640.185	0	15.625
33	30	-412.66	-351.936	0.0244	1023.98	640.185	0	15.625
34	31	-372.564	-304.056	0.0244	1023.98	640.185	0	15.625
35	32	-399.646	-371.95	0.0244	1023.98	640.185	0	15.625
36	33	-397.646	-307.246	0.0244	1023.98	640.185	0	15.625
37	34	-401.371	-330.406	0.0244	1023.98	640.185	0	15.625
38	35	-418.472	-330.846	0.0244	1023.98	640.185	0	15.625
39	36	-384.828	-367.603	0.0244	1023.98	640.185	0	15.625
40	37	-377.506	-380.116	0.0238	995.964	654.885	0	15.625
41	38	-427.499	-278.578	0.0238	995.964	654.885	0	15.625
42	39	-406.899	-393.753	0.0238	995.964	654.885	0	15.625

.sta



# Summary



# Stimulus File

- Stimulus Files are used when the Waveform Editor cannot create the desired waveform.

# Edit Protocol - (untitled)



Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0

Info

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holding

Digital Outputs

Active high logic for digital trains

Info

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Off	Off	Off	Off	Off	Off	Off	Off	Off
Sample rate	Off			Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	0			0	0	0	0	0	0	0
Delta level (mV)	0			0	0	0	0	0	0	0
First duration (ms)	0			0	0	0	0	0	0	0
Delta duration (ms)	0			0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000			0000	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000			0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0			0	0	0	0	0	0	0
Pulse width (ms)	0	0	0	0	0	0	0	0	0	0

Number of sweeps = 10

Allocated time: 106.2 of 200 ms

Stimulus File...

Final level -68.00 mV  
First duration 100.00 ms (1000 samples)

Summary

Channel #0 | Channel #1 | Channel #2 | Channel #3

Alternate Waveforms  Alternate Digital Outputs

OK

Cancel

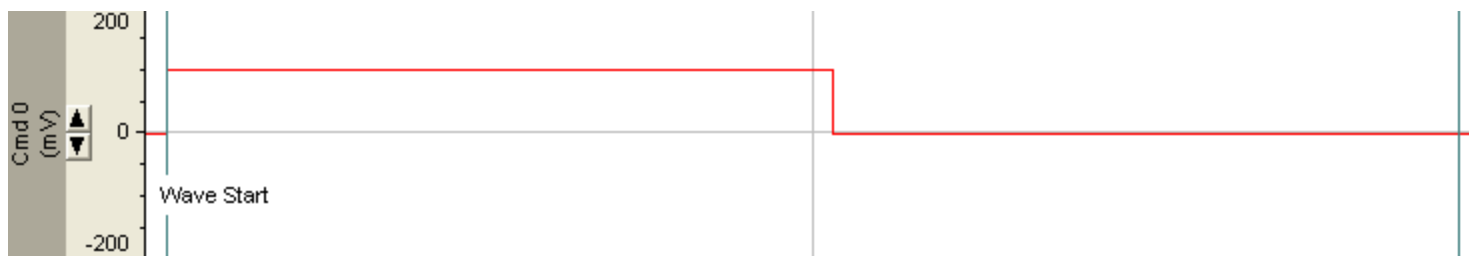
Help

Acquisition mode:  
Episodic stimulation

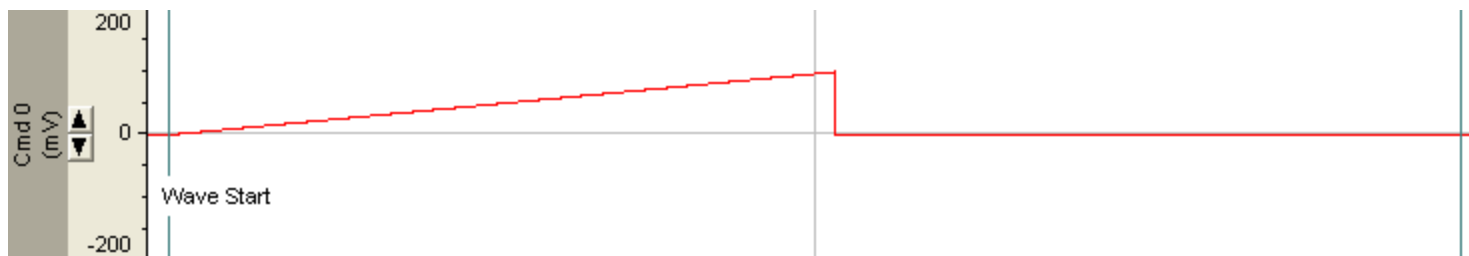
Update Preview

# Default waveforms

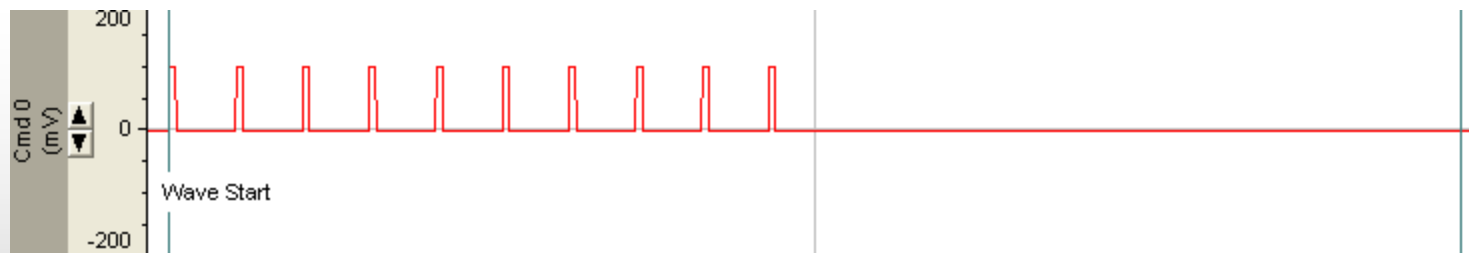
Step:



Ramp:



Pulse train:

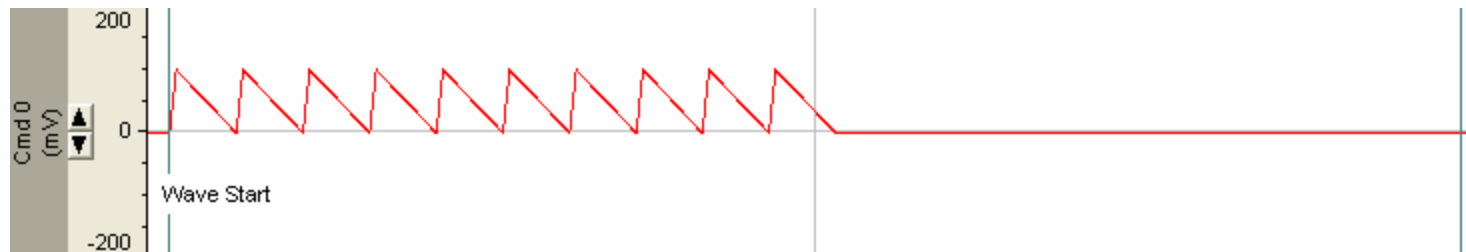


# Default waveforms

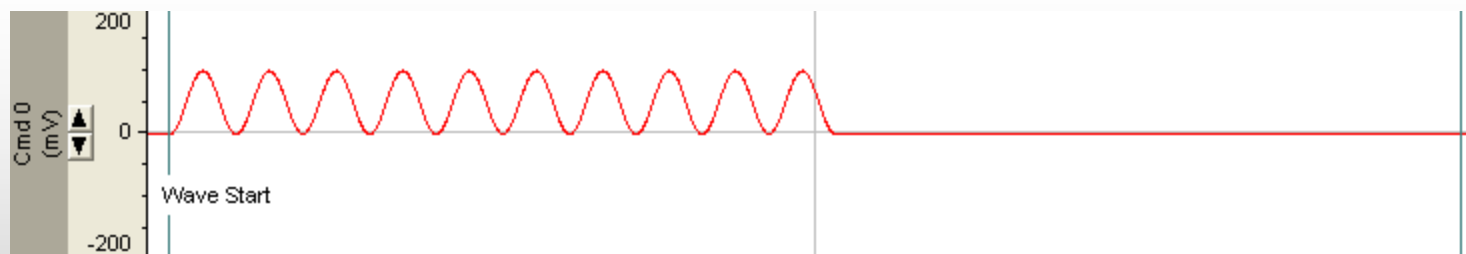
Biphasic train:



Triangle train:

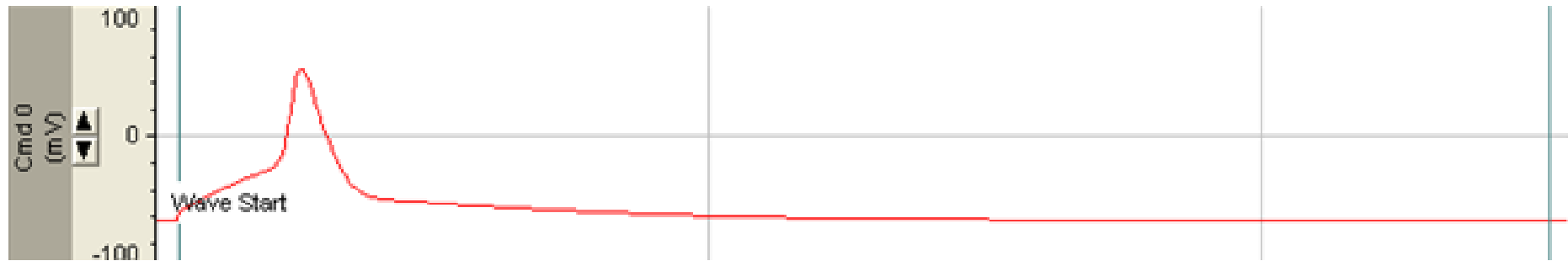


Cosine train:





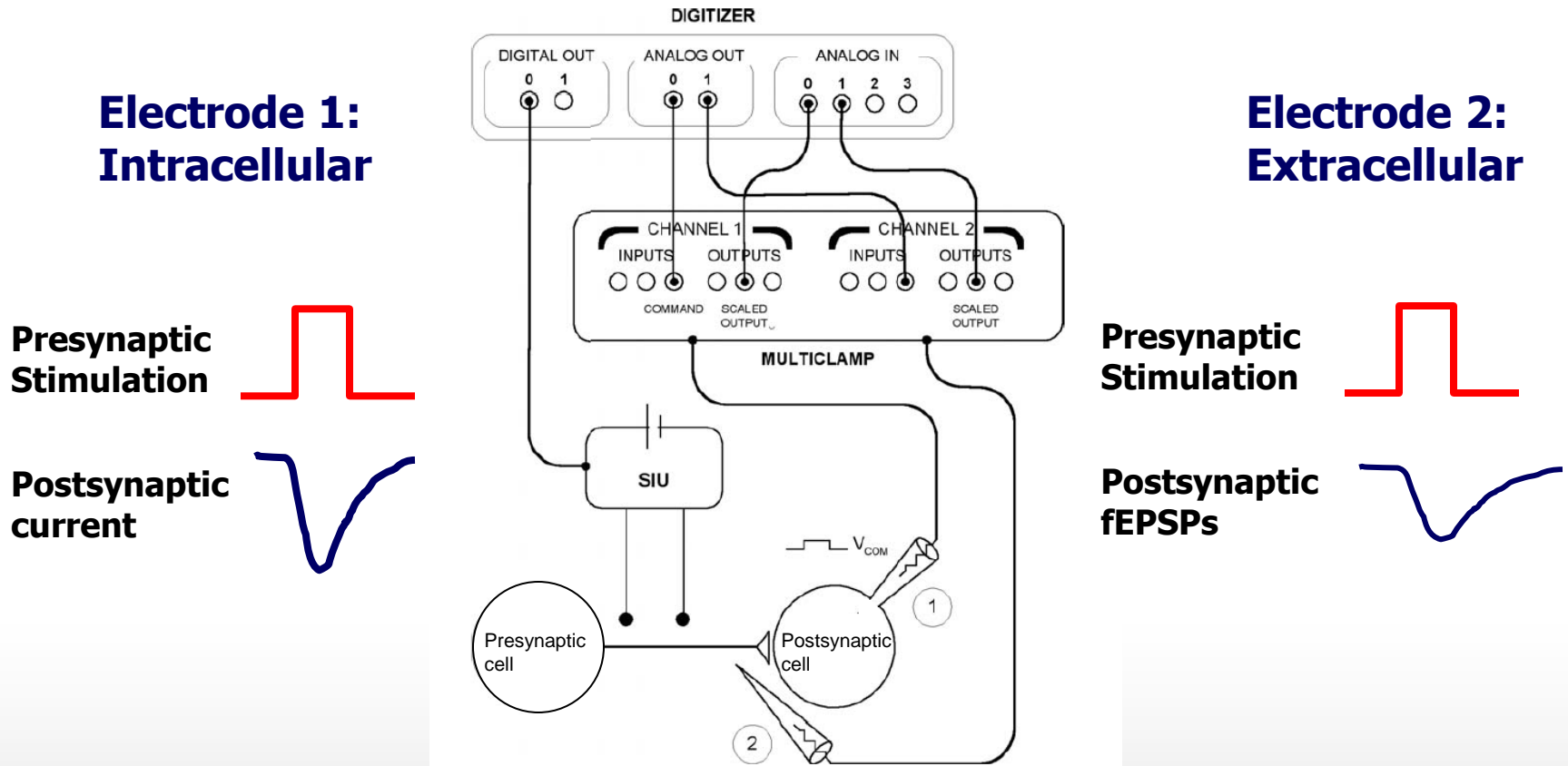
# How to create an action potential waveform?



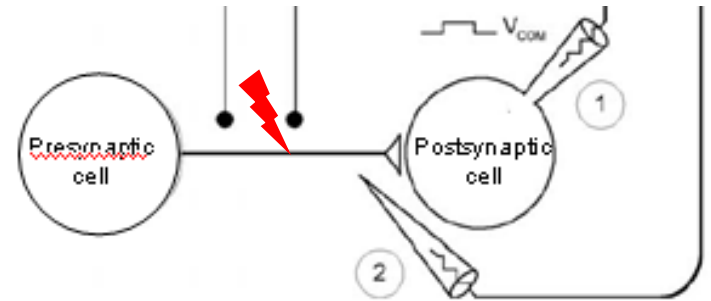
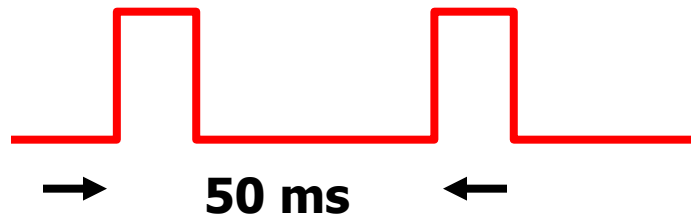


# Advanced Tips for Protocol Writing in the Clampex Data Acquisition Module

# Whole cell and Extracellular Recordings

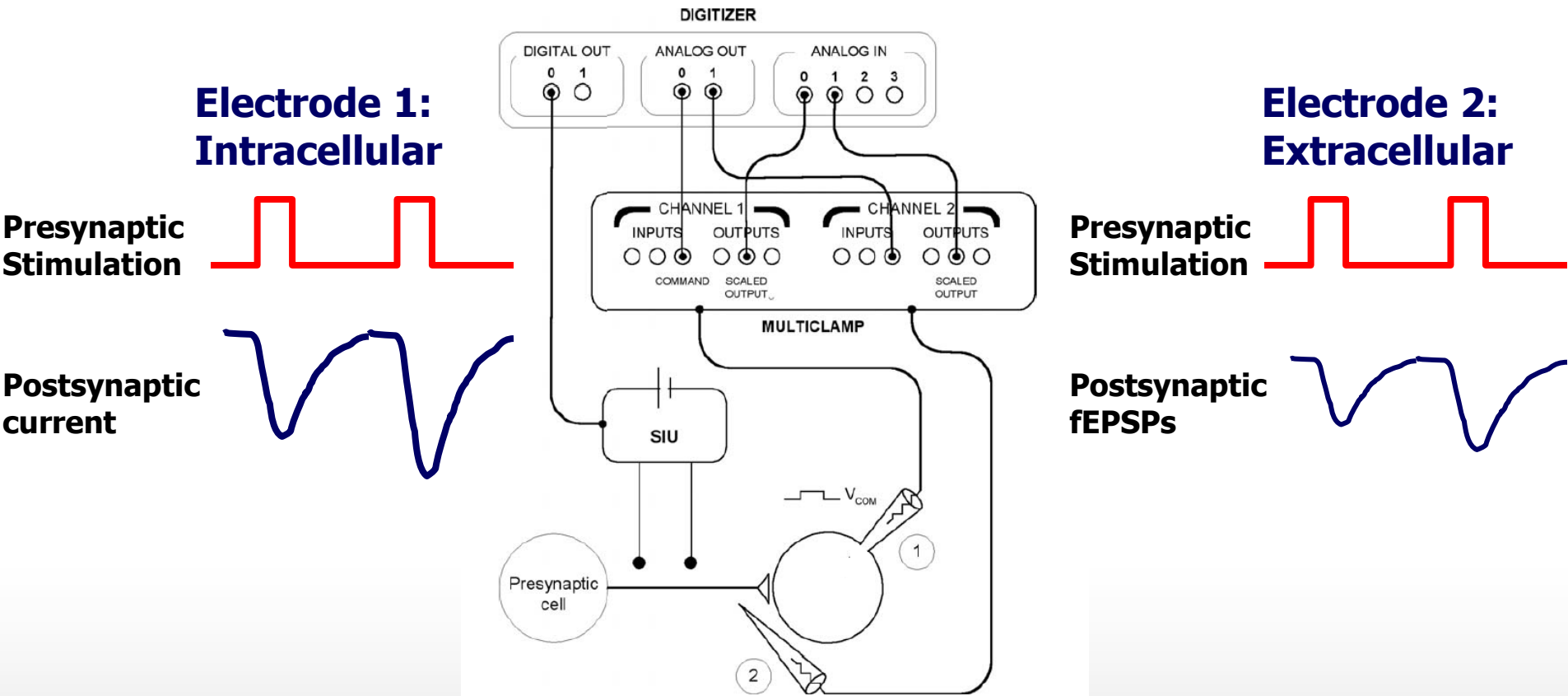


# Pair-pulse stimulation



**Application: Synaptic plasticity, release probability in brain slice preparation**

# Pair-pulse Stimulation: Presynaptic afferents



# Pair-pulse stimulation

Edit Protocol - (untitled)

Mode/Rate Inputs Outputs Trigger Statistics Comments Math Waveform Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform  Digital Outputs

Epochs  Stimulus file

Intersweep holding level: Use holding

Active high logic for digital trains Info

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Step	Step	Step	Step	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	-80	-80	-80	-80	-80	0	0	0	0	0
Delta level (mV)	0	0	0	0	0	0	0	0	0	0
First duration (ms)	100	1	49	1	50	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	0001	0000	0001	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	0	0	0	0	0	0	0	0	0
Pulse width (ms)	0	0	0	0	0	0	0	0	0	0

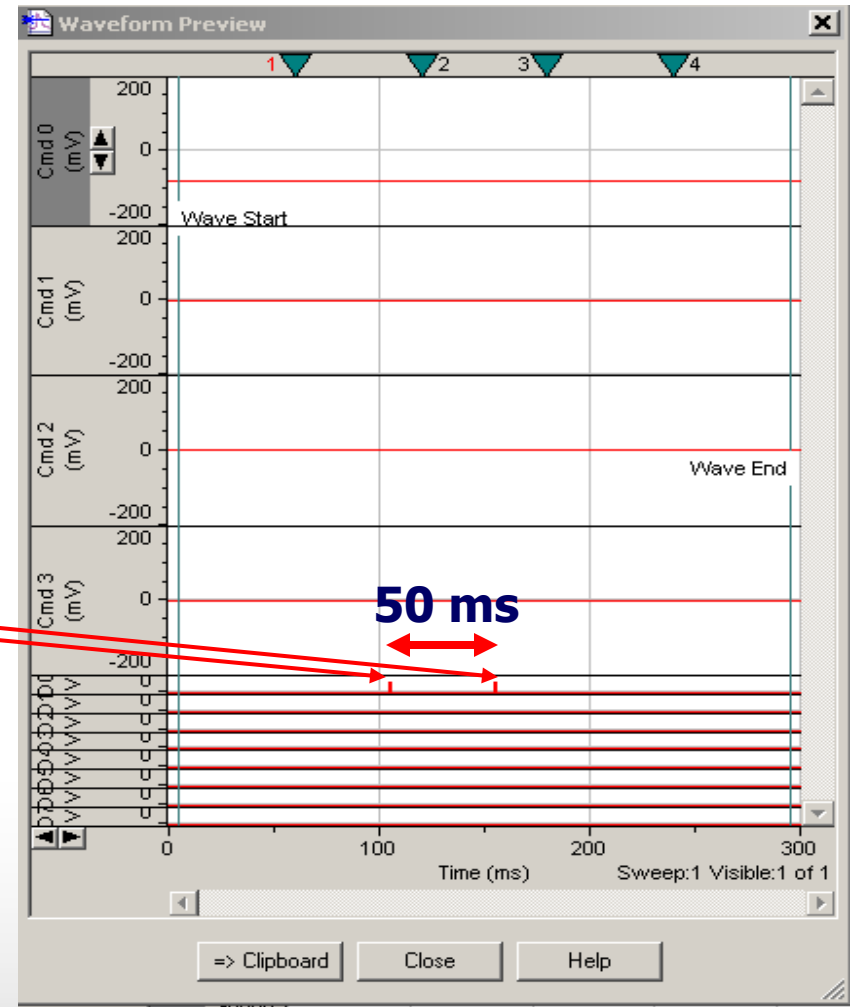
Number of sweeps = 10 Allocated time: 210.2 of 300 ms

Stimulus File... First duration 49.00 ms (490 samples)

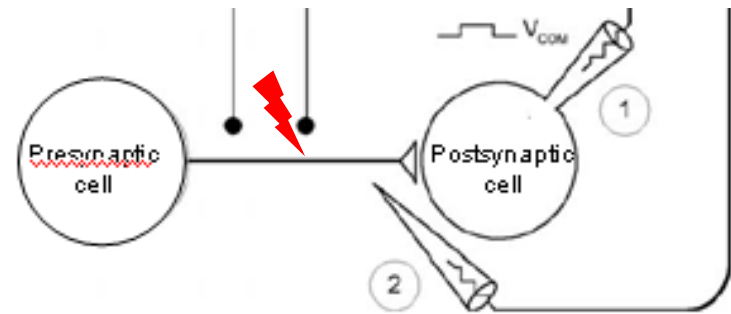
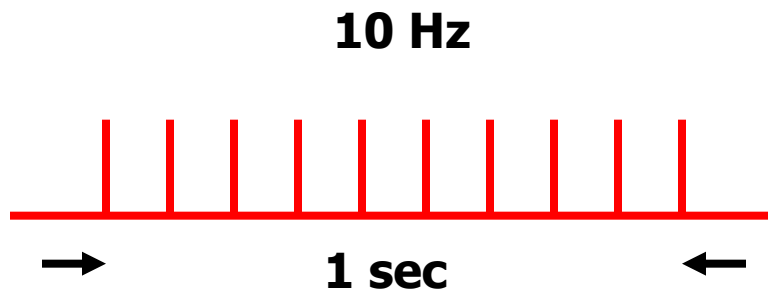
Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview



# Train stimuli



**Application: Synaptic plasticity, LTD  
in brain slice preparation**

# Train stimuli-10 pulses

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holding

Digital Outputs

Active high logic for digital trains

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Pulse	Step	Off	Off	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	-80	-80	-80	0	0	0	0	0	0	0
Delta level (mV)	0	0	0	0	0	0	0	0	0	0
First duration (ms)	100	1000	100	0	0	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	0000*	0000	0000	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	10	0	0	0	0	0	0	0	0
Pulse width (ms)	0	1	0	0	0	0	0	0	0	0

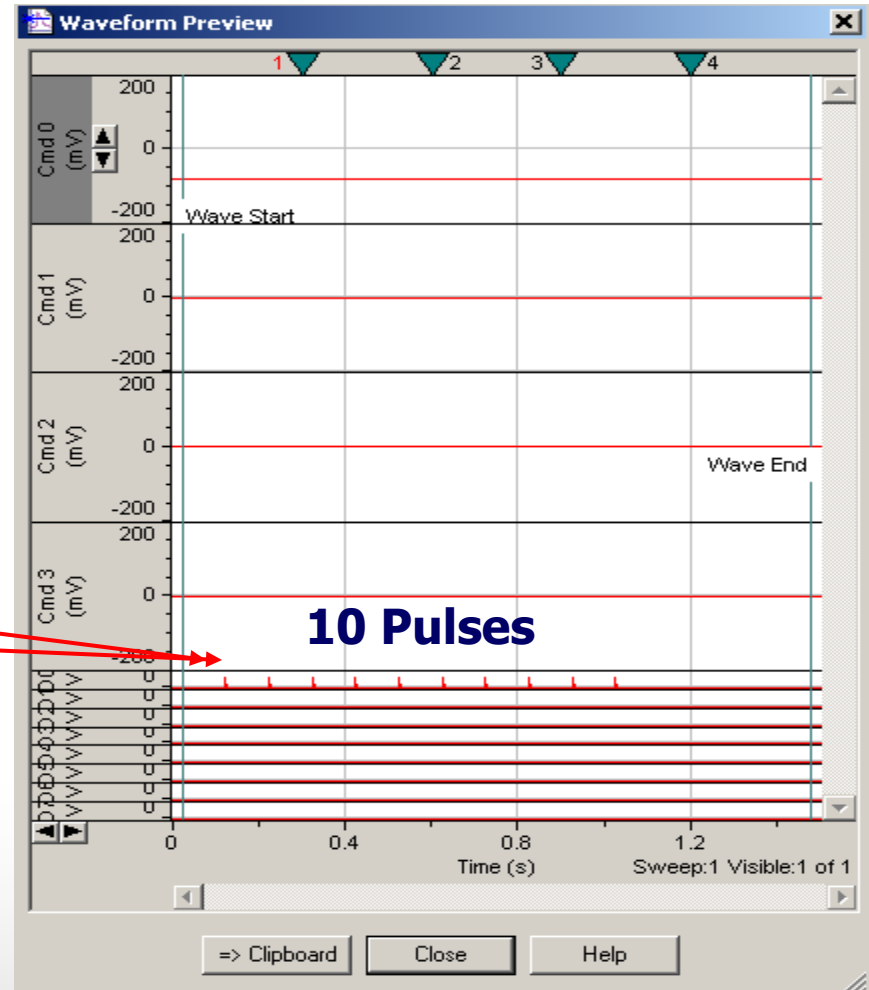
Number of sweeps = 10      Allocated time: 1246.8 of 1500 ms

Stimulus File... First duration 1000.00 ms (10000 samples)      Pulse count 10  
Train rate 10.00 Hz (1000 samples)  
Pulse width 1.00 ms => 1 % (10 samples)

Summary

Channel #0 Channel #1 Channel #2 Channel #3       Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help      Acquisition mode: Episodic stimulation      Update Preview





# Train stimuli-50 pulses

**Edit Protocol - (untitled)**

Mode/Rate | Inputs | Outputs | Trigger | Statistics | Comments | Math | Waveform | Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform Info  Digital Outputs

Epochs  Stimulus file

Intersweep holding level: Use holding ▼ Intersweep bit pattern: Use holding ▼

	A	B	C	D	E	F	G	H	I	J
Type	Step	Pulse	Step	Off	Off	Off	Off	Off	Off	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	-80	-80	-80	0	0	0	0	0	0	0
Delta level (mV)	0	0	0	0	0	0	0	0	0	0
First duration (ms)	100	1000	100	0	0	0	0	0	0	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	1000*	0000	0000	0000	0000	0000	0000	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	50	0	0	0	0	0	0	0	0
Pulse width (ms)	0	1	0	0	0	0	0	0	0	0

Number of sweeps = 10 Allocated time: 1246.8 of 1500 ms

Stimulus File... First duration 1000.00 ms (10000 samples) Pulse count 50  
Train rate 50.00 Hz (200 samples)  
Pulse width 1.00 ms => 5% (10 samples)

Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview

**Waveform Preview**

Cmd 0 (mV) Cmd 1 (mV) Cmd 2 (mV) Cmd 3 (mV)

Wave Start Wave End

**50 Pulses**

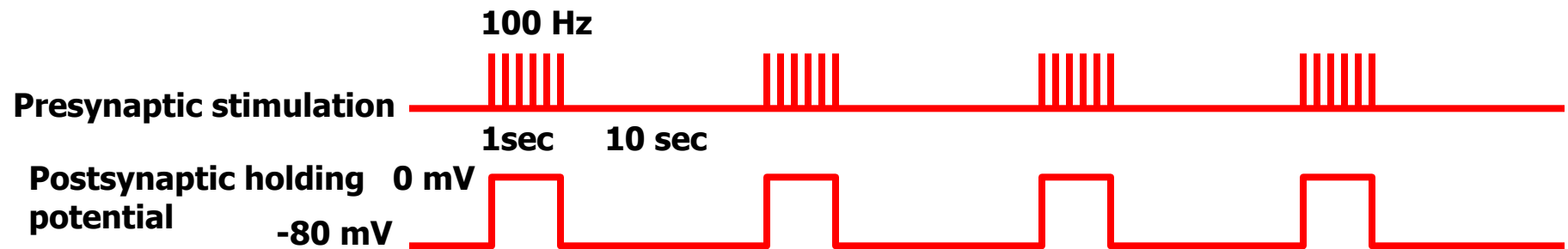
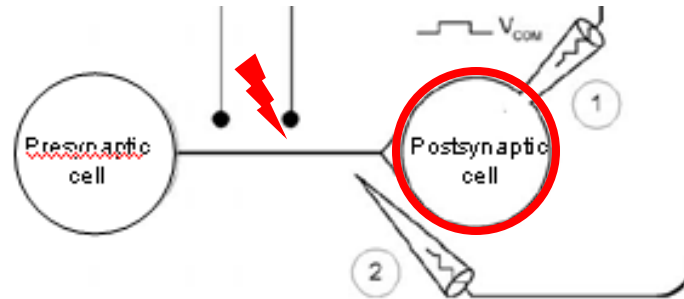
Time (s) Sweep: 1 of 1 Visible: 1 of 1

=> Clipboard Close Help

# Summary

- **Telegraphing/Lab Bench settings**
- **Writing protocols for**
  - **Pair-pulse stimulation/Digital Output**
  - **Train stimulation**

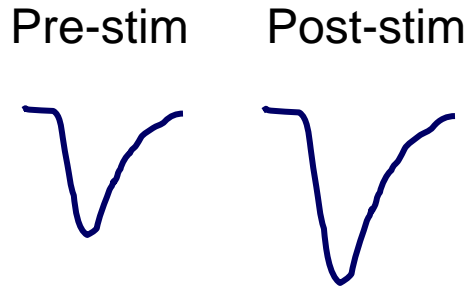
# Tetanus Stimulation protocol



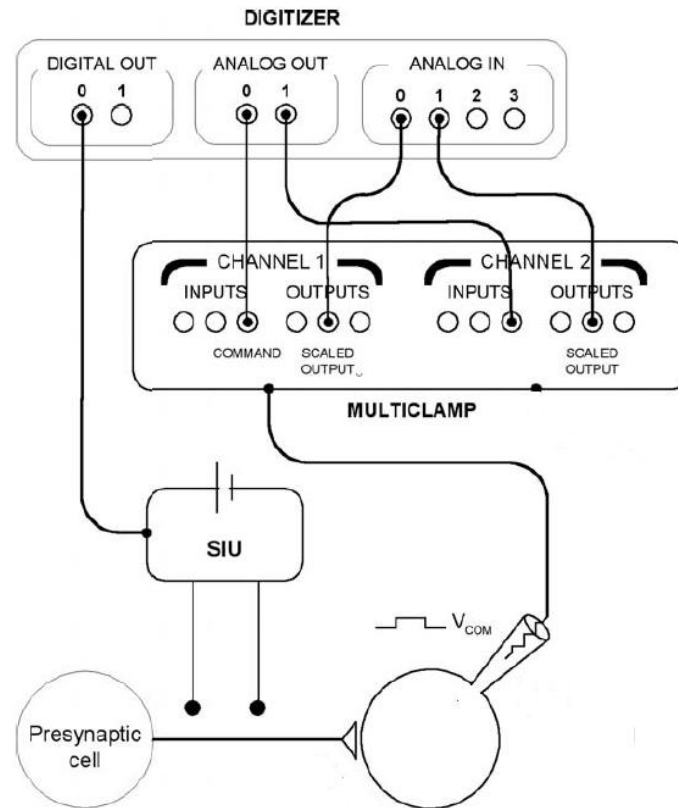
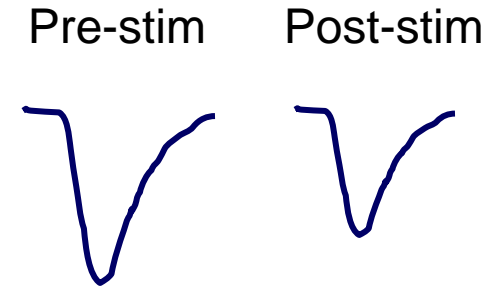
**Application: Synaptic plasticity, LTP/LTD in brain slice preparation**

# Tetanus Stimulation and Postsynaptic Depolarization

**LTP:**



**LTD:**



100 Hz

1sec      10 sec

Presynaptic stimulation

Postsynaptic holding potential      0 mV  
-80 mV

# Tetanus Stimulation and Postsynaptic Depolarization

Edit Protocol - (untitled)

Mode/Rate Inputs Outputs Trigger Statistics Comments Math Waveform Stimulus

Waveform Analog OUT: Cmd 0 Info

Analog Waveform  Digital Outputs

Epochs  Stimulus file

Intersweep holding level: Use holding

Active high logic for digital trains Info

Intersweep bit pattern: Use holding

Epoch Description	A	B	C	D	E	F	G	H	I	J
Type	Step	Step	Step	Step	Step	Step	Step	Step	Step	Off
Sample rate	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast
First level (mV)	-80	0	-80	0	-80	0	-80	0	-80	0
Delta level (mV)	0	0	0	0	0	0	0	0	0	0
First duration (ms)	100	1000	10000	000	10000	1000	10000	1000	100	0
Delta duration (ms)	0	0	0	0	0	0	0	0	0	0
Digital bit pattern (#3-0)	0000	000*	0000	000*	0000	000*	0000	000*	0000	0000
Digital bit pattern (#7-4)	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000
Train rate (Hz)	0	100	0	00	0	100	0	100	0	0
Pulse width (ms)	0	1	0	0	1	0	1	0	0	0

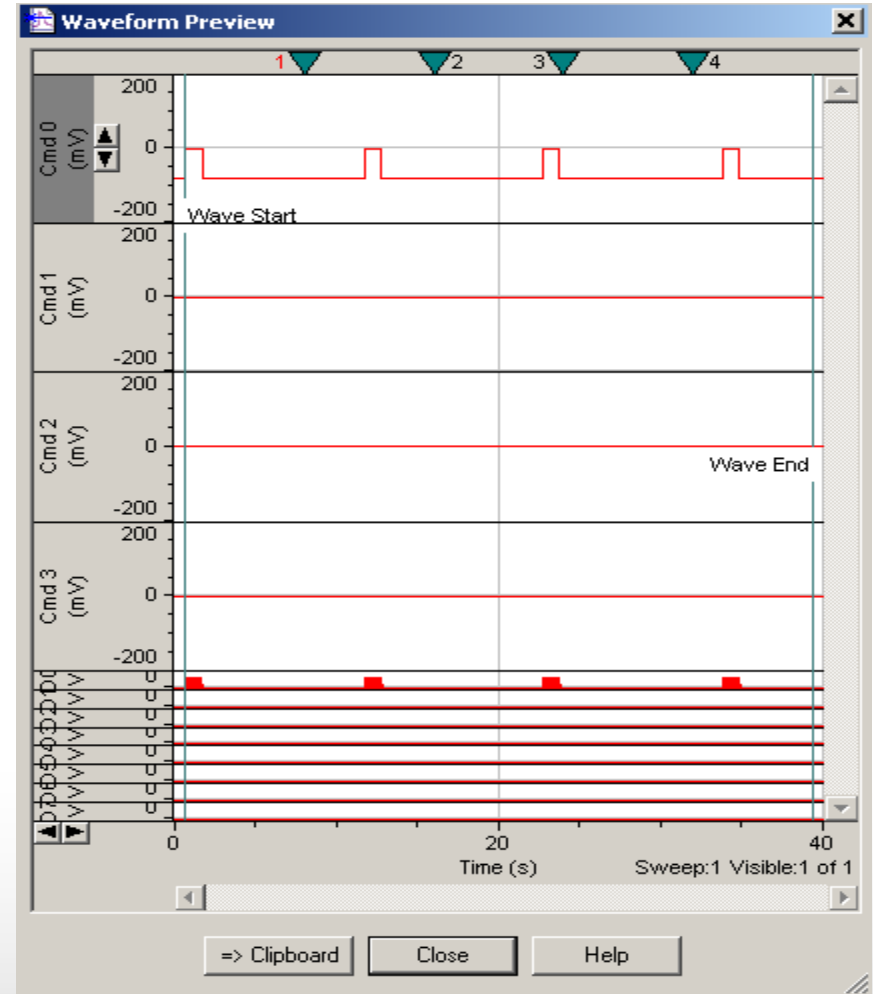
Number of sweeps = 10 Allocated time: 35450 of 40000 ms

Stimulus File... First duration 100.00 ms (1000 samples)

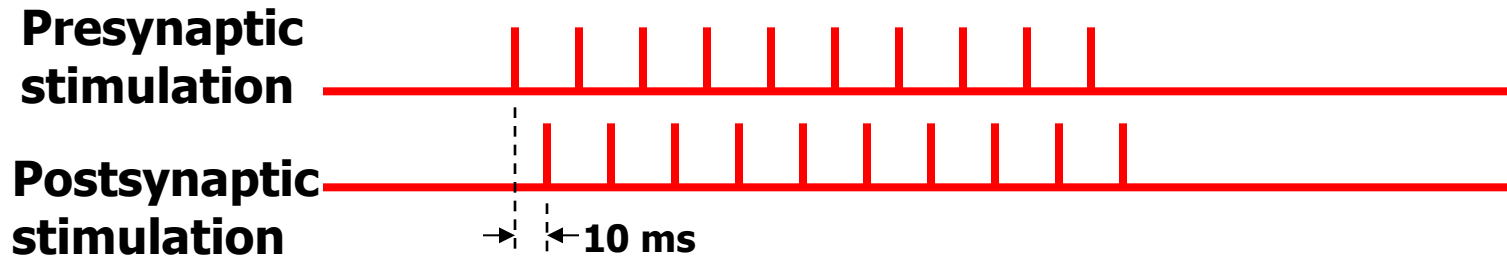
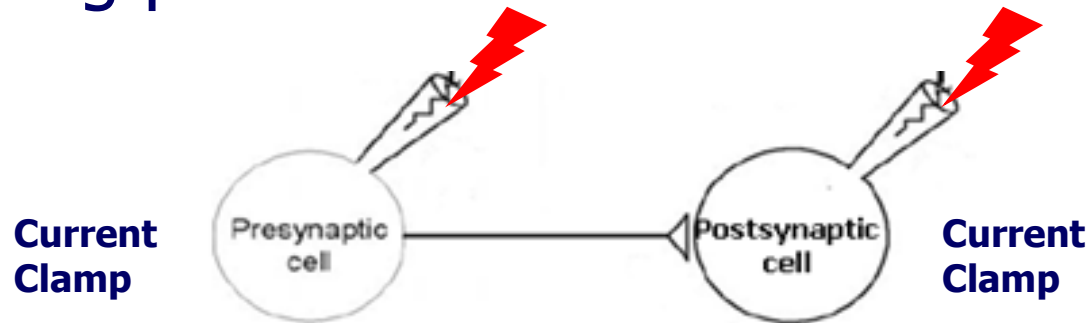
Summary

Channel #0 Channel #1 Channel #2 Channel #3  Alternate Waveforms  Alternate Digital Outputs

OK Cancel Help Acquisition mode: Episodic stimulation Update Preview

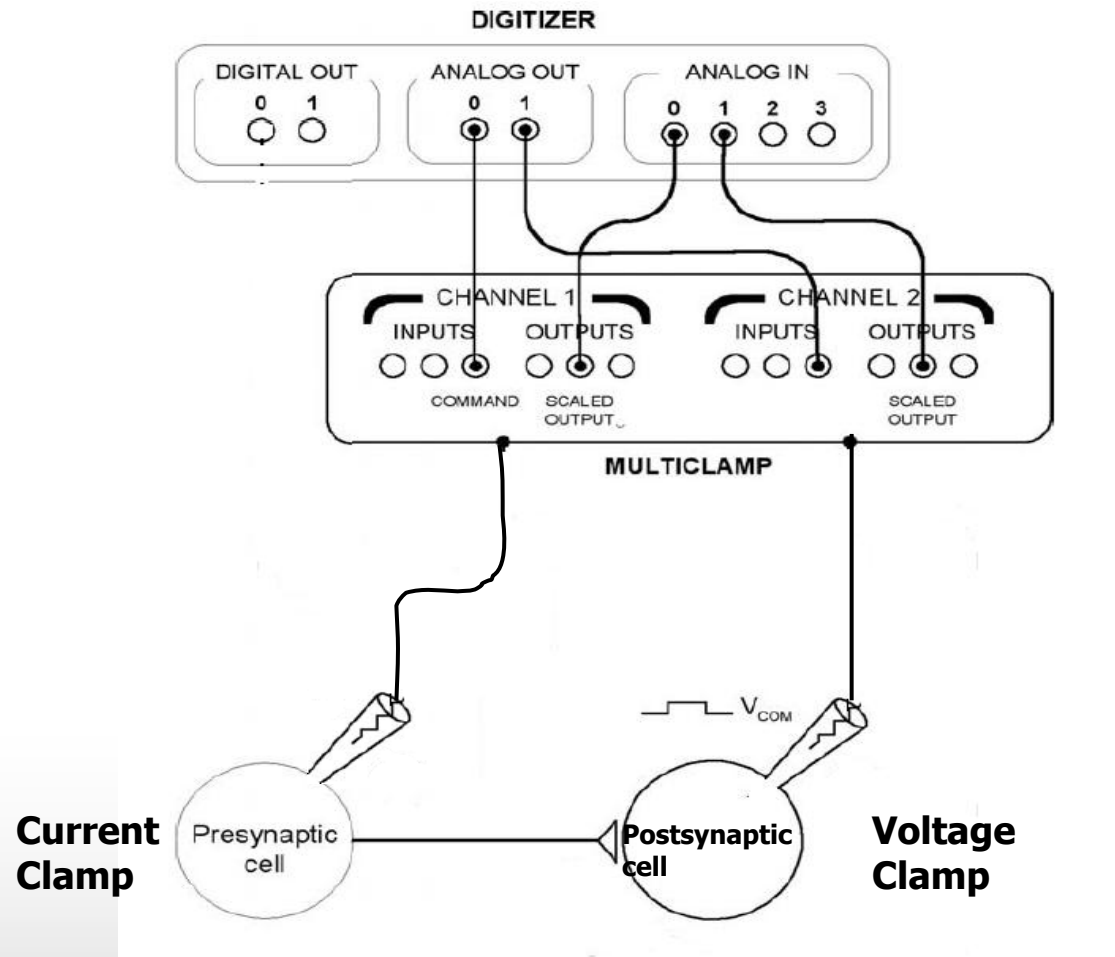


# Spike timing protocol

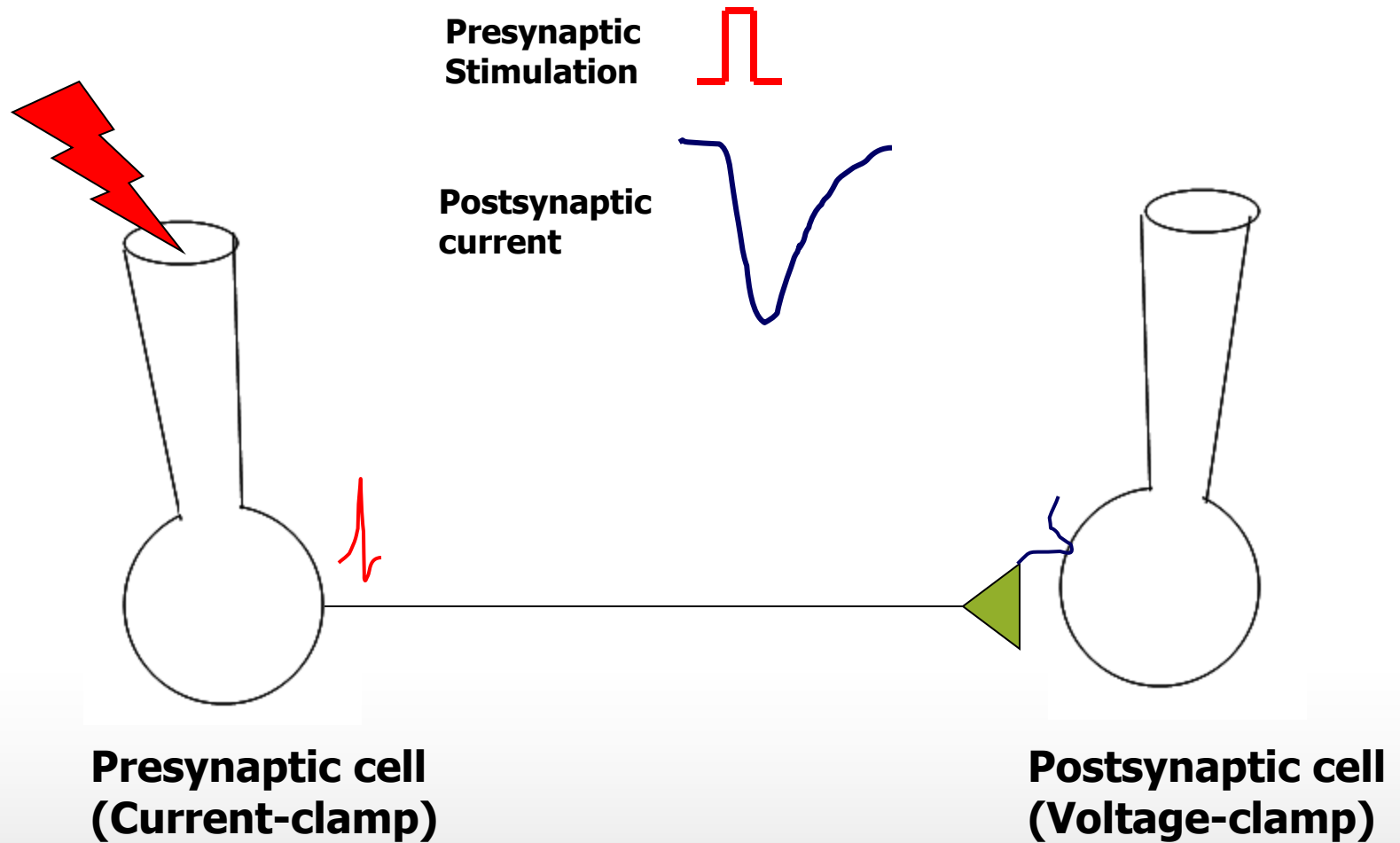


**Application: Synaptic plasticity, LTP/LTD in brain slice preparation and culture cell**

# Dual Whole-cell Patch-clamp Recordings

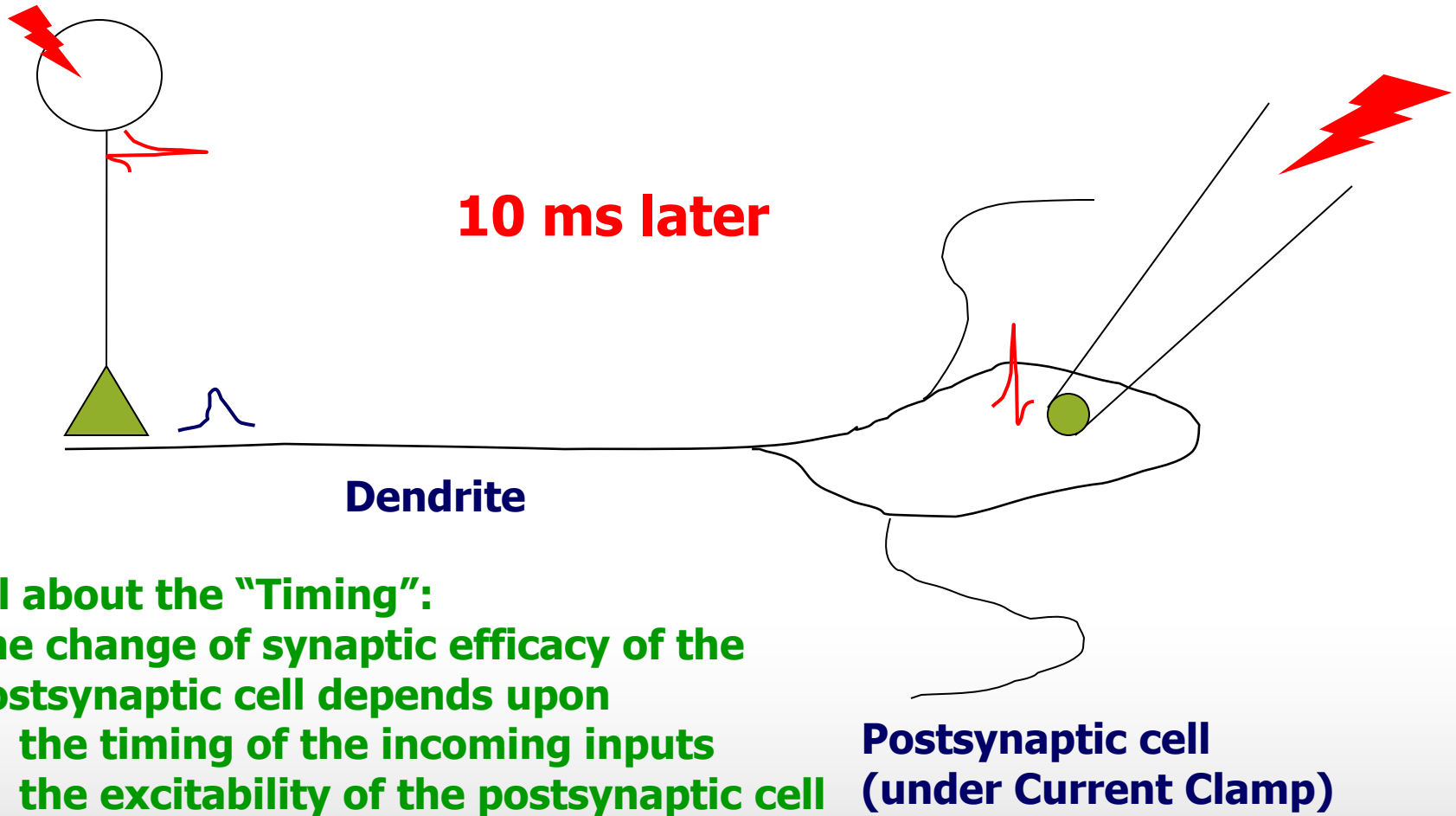


# Dual Whole-cell Patch-clamp Recordings

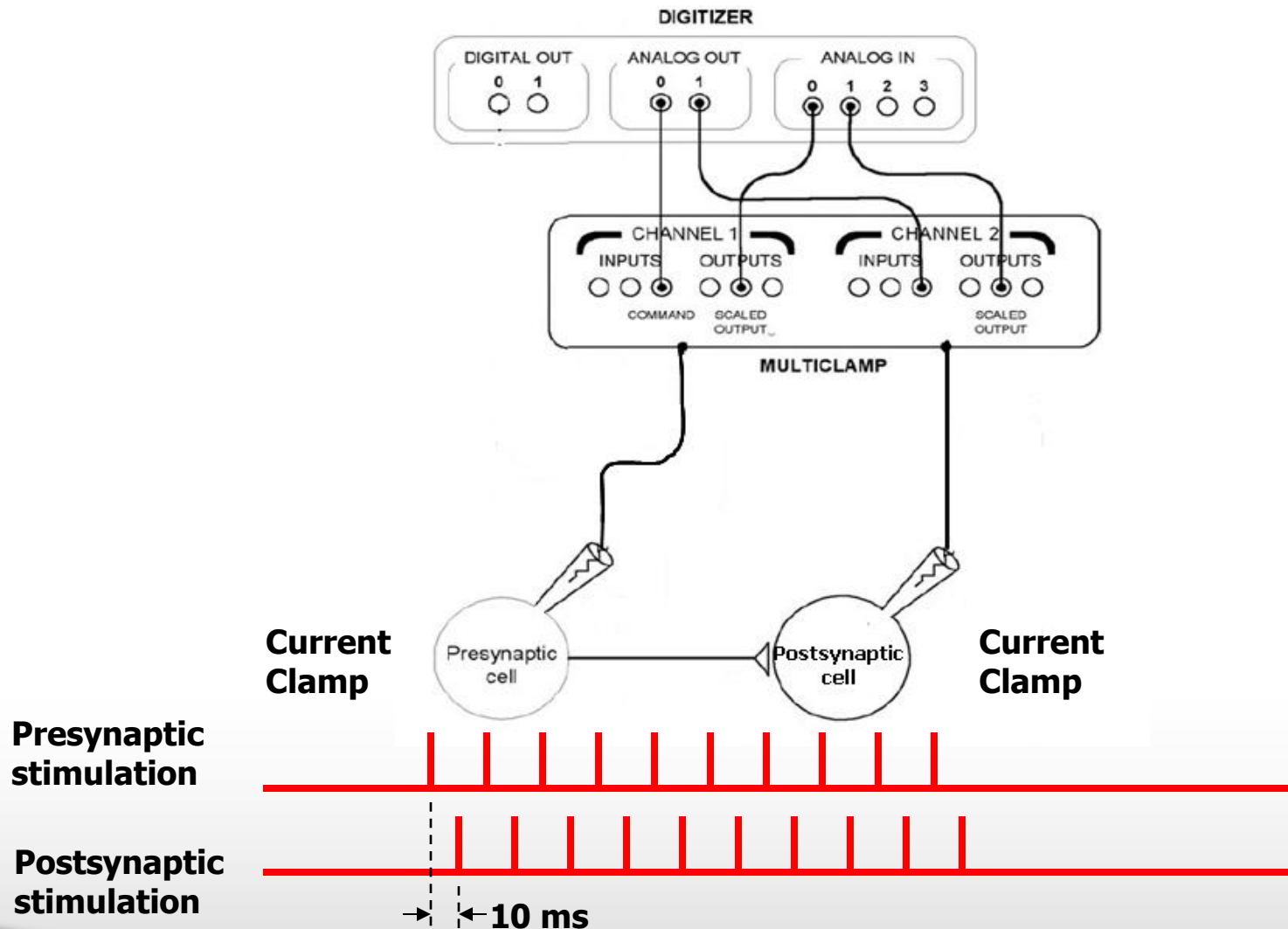




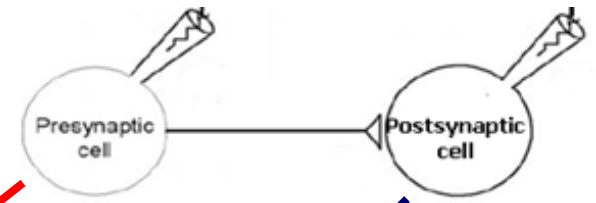
# Spike timing protocol: EPSP precedes Action Potential



# Spike timing protocol: EPSP precedes Action Potential



# Spike timing protocol: EPSP precedes Action Potential



**Edit Protocol - (untitled)**

Mode/Rate | Inputs | Outputs | Trigger | Sta

Waveform Analog OUT: I\_clamp

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holdin

Type	A	B
Sample rate	Fast	Fast
First level (pA)	0	1000
Delta level (pA)	0	0
First duration (ms)	50	1000
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	1111	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	10
Pulse width (ms)	0	1

Number of sweeps = 1

Stimulus File... First duration 1000.00 ms

Summary

Channel #0 Channel #1 Channel #2 C

OK Cancel He

**Edit Protocol - (untitled)**

Mode/Rate | Inputs | Outputs | Trigger | Sta

Waveform Analog OUT: I\_clamp2

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holding

Type	A	B
Sample rate	Fast	Fast
First level (pA)	0	1000
Delta level (pA)	0	0
First duration (ms)	60	1000
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	0000	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	10
Pulse width (ms)	0	1

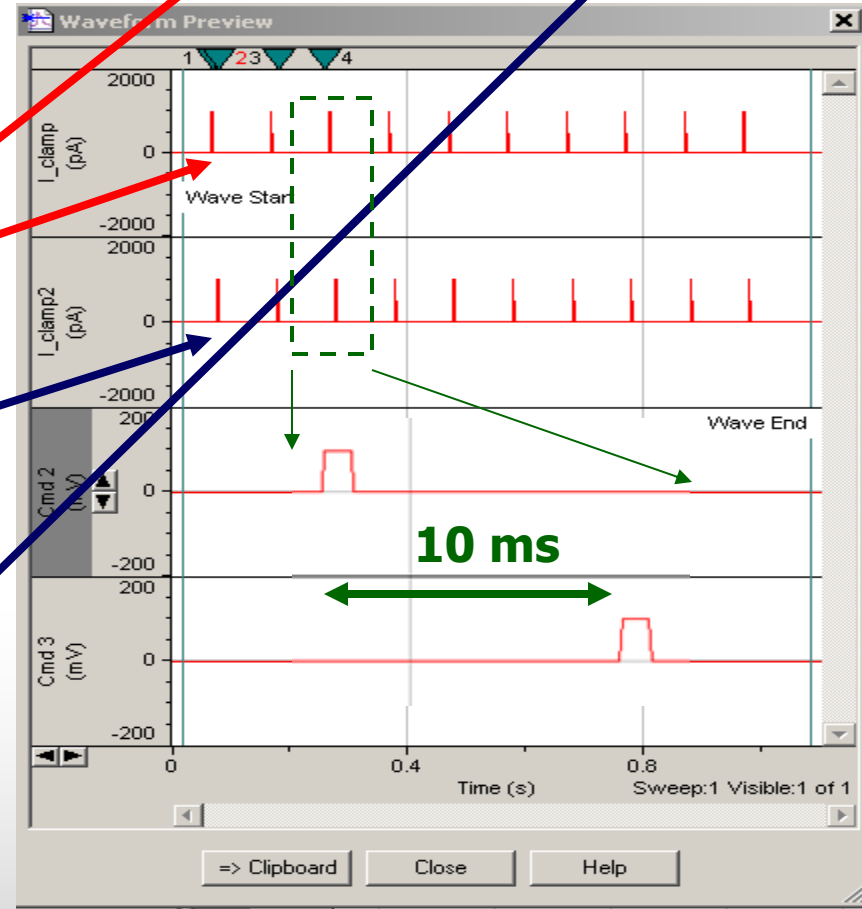
Number of sweeps = 1

Stimulus File... First duration 1000.00 ms

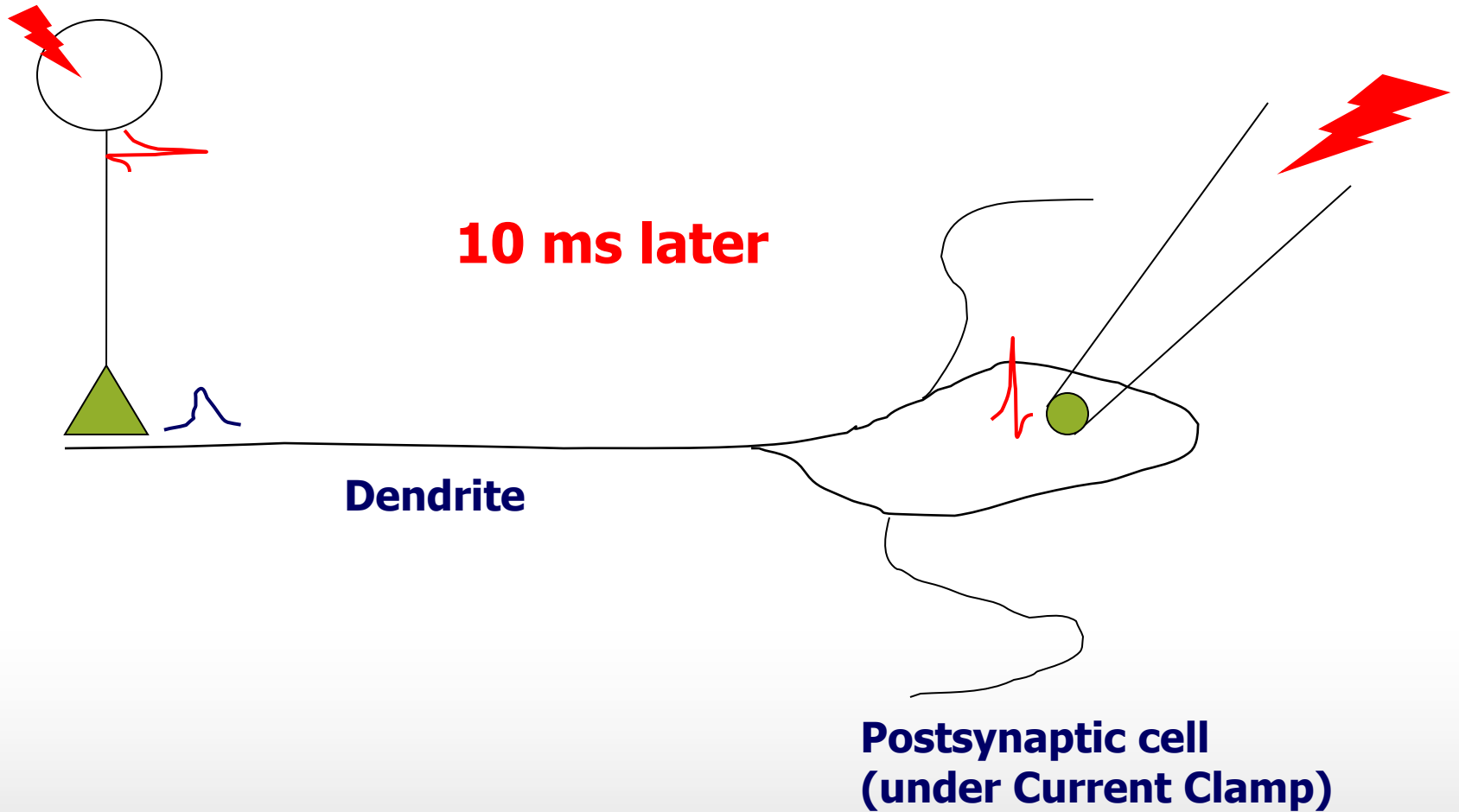
Summary

Channel #0 Channel #1 Channel #2 Ch

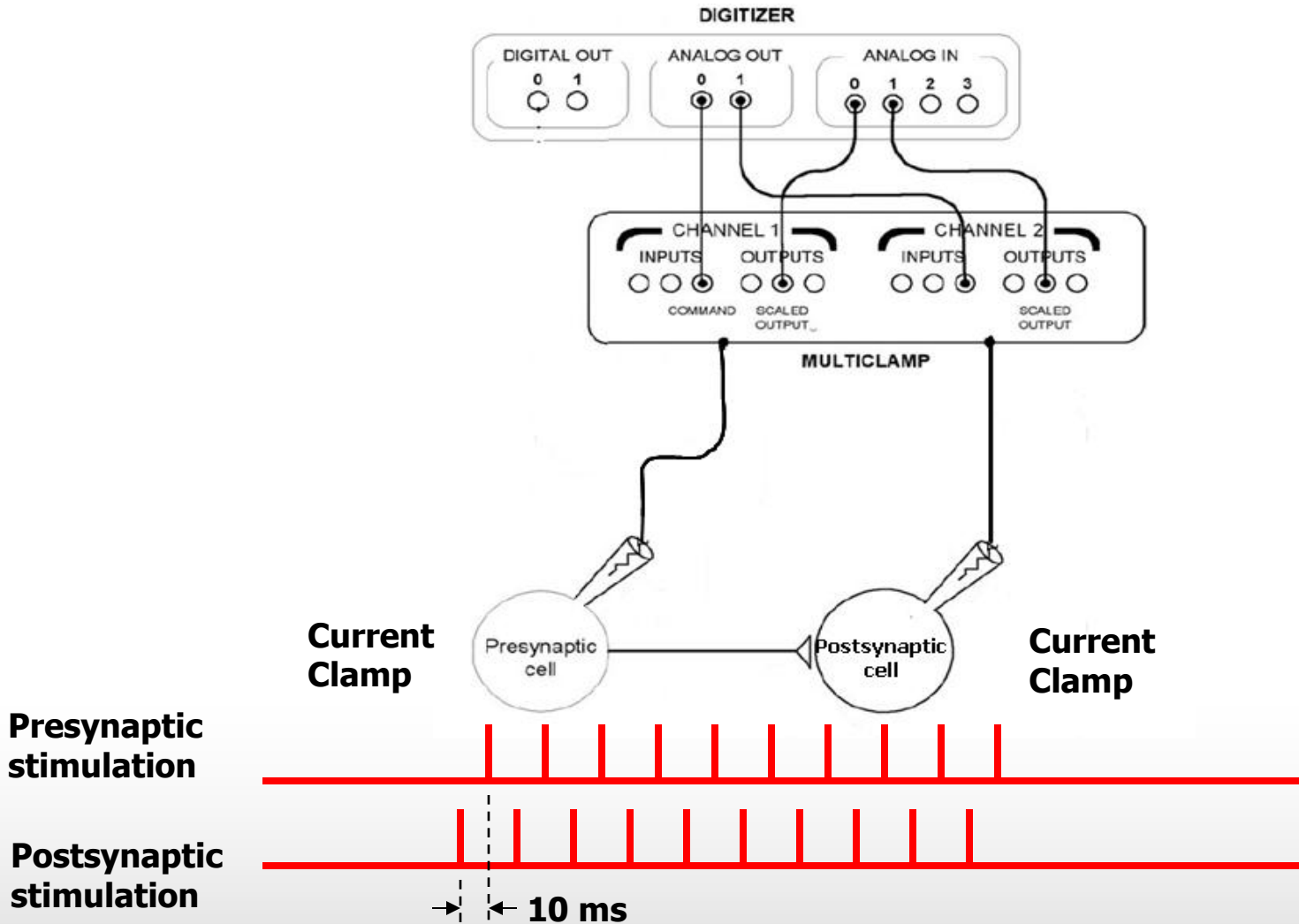
OK Cancel Help



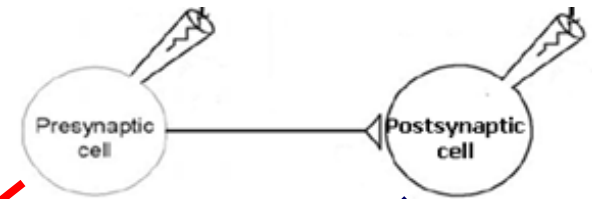
# Spike timing protocol: Action Potential precedes EPSP



# Spike timing protocol: Action Potential precedes EPSP



# Spike timing protocol: Action Potential precedes EPSP



Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Sta

Waveform Analog OUT: I\_clamp

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holding

Type	A	B
Sample rate	Fast	Fast
First level (pA)	0	1000
Delta level (pA)	0	0
First duration (ms)	60	1000
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	1111	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	10
Pulse width (ms)	0	1

Number of sweeps = 1

Stimulus File... First duration: 60.00 ms (60)

Summary

Channel #0 Channel #1 Channel #2 Cl

OK Cancel Hel

Edit Protocol - (untitled)

Mode/Rate | Inputs | Outputs | Trigger | Sta

Waveform Analog OUT: I\_clamp2

Analog Waveform

Epochs  Stimulus file

Intersweep holding level: Use holding

Type	A	B
Sample rate	Fast	Fast
First level (pA)	0	1000
Delta level (pA)	0	0
First duration (ms)	50	1000
Delta duration (ms)	0	0
Digital bit pattern (#3-0)	0000	0000
Digital bit pattern (#7-4)	0000	0000
Train rate (Hz)	0	10
Pulse width (ms)	0	1

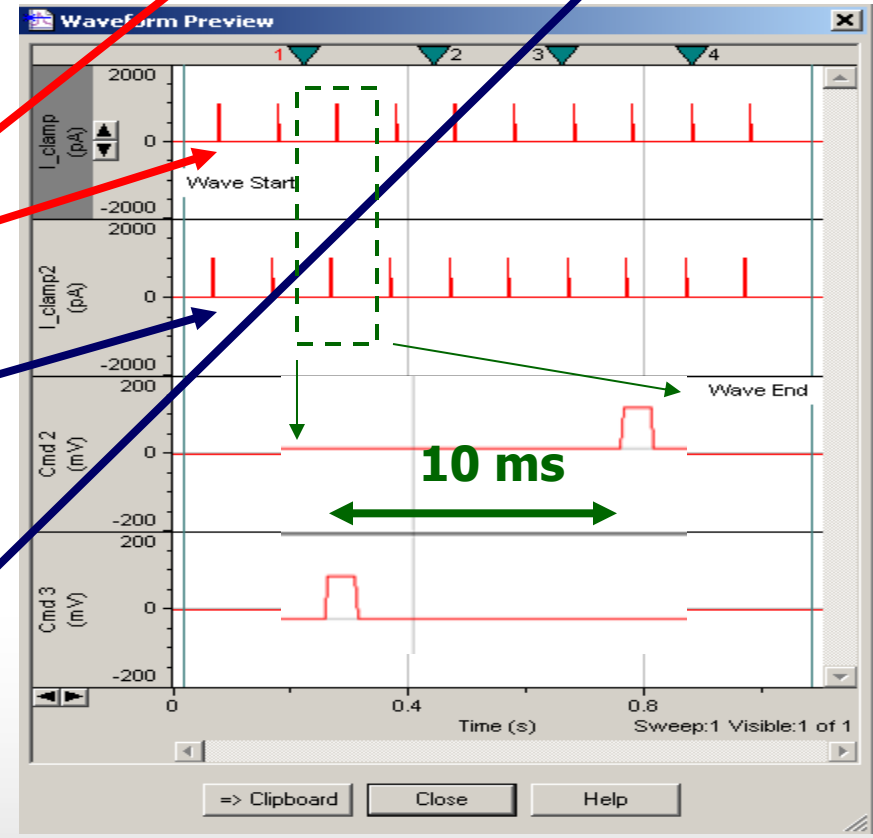
Number of sweeps = 1

Stimulus File... First duration: 50.00 ms (50)

Summary

Channel #0 Channel #1 Channel #2 Cl

OK Cancel Hel





# Action Potential Analysis in Clampfit

# Frequent Ask Questions in Action Potential Analysis

- How can I measure the frequency of action potentials?
- The baseline of my recording is drifting. It is hard to analyze the spikes.
- How do I sort the spikes?
- My recording is too noisy. It is hard to retrieve the spikes from the noises?
- When I do the event search, how can I avoid the large spike of stimulation artifacts?
- How do I perform a phase plot of  $dV/dt$  vs  $V$  in Clampfit?
- How do I calculate the cardiac action potential duration at 90% repolarization ( $APD_{90\%}$ )?



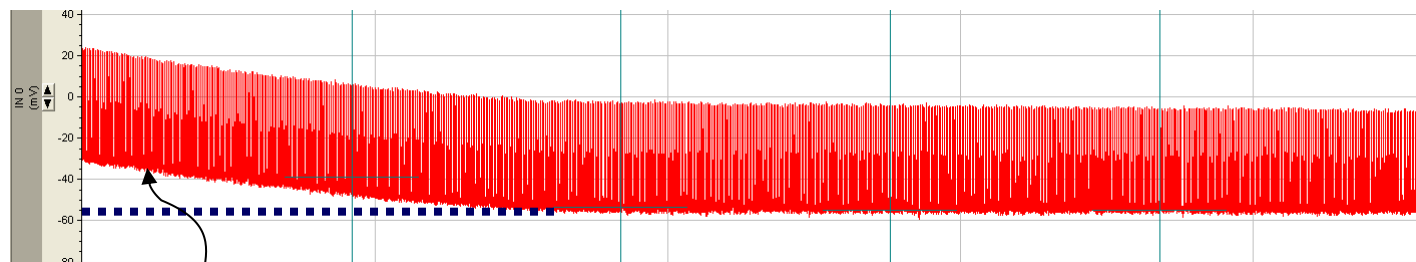
# What we learn today

- Baseline adjustment
- Event Search
- Event sorting
- Noise/Event rejection
- Spike alignment
- Combine trace
- Phase plot
- Action potential analysis

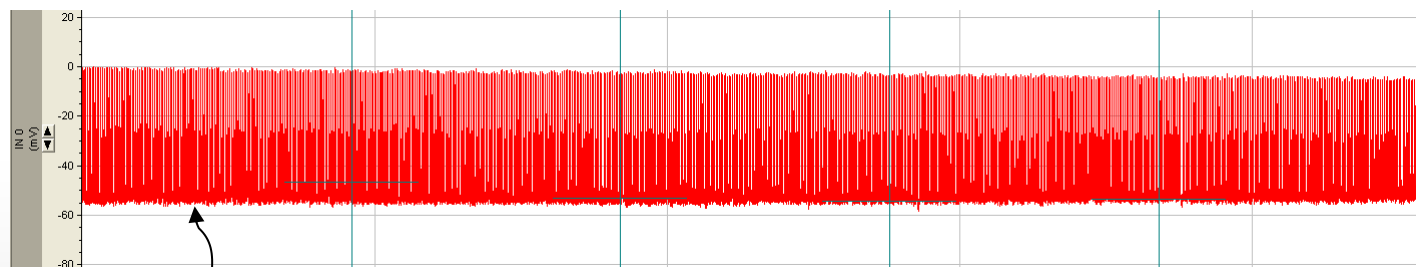
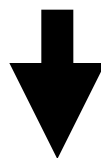
# Features in Clampfit

- Baseline adjustment
  - Manual baseline adjustment
- Search event
  - Event Detection/Threshold Search
- Event sorting
  - Event Detection/Threshold Search
- Spike alignment
  - Time shift
- Phase plot/Combine trace
  - Arithmetic
- Action potential analysis
  - Statistics

# Baseline adjustment

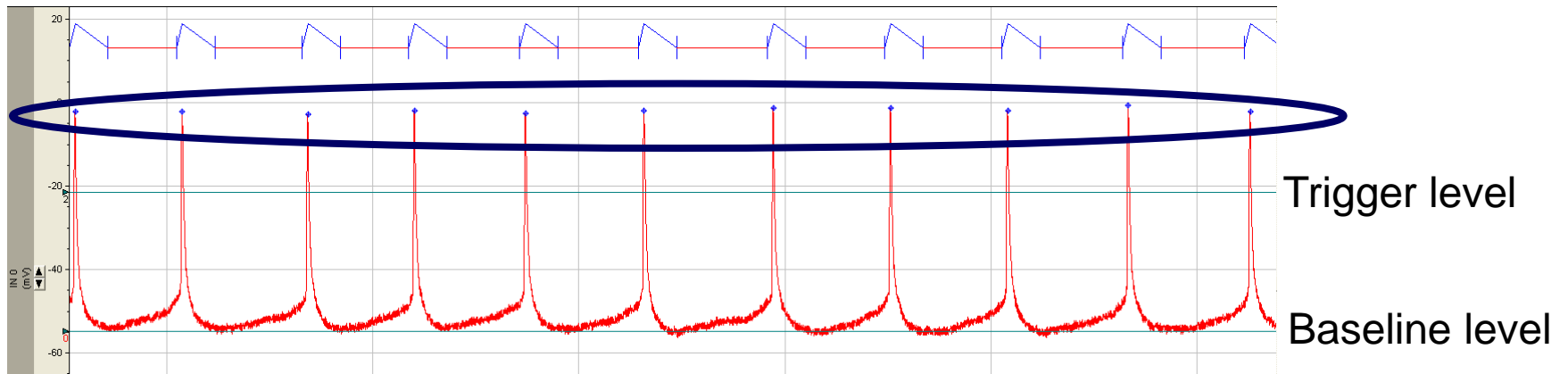


Drifting baseline



Stable baseline

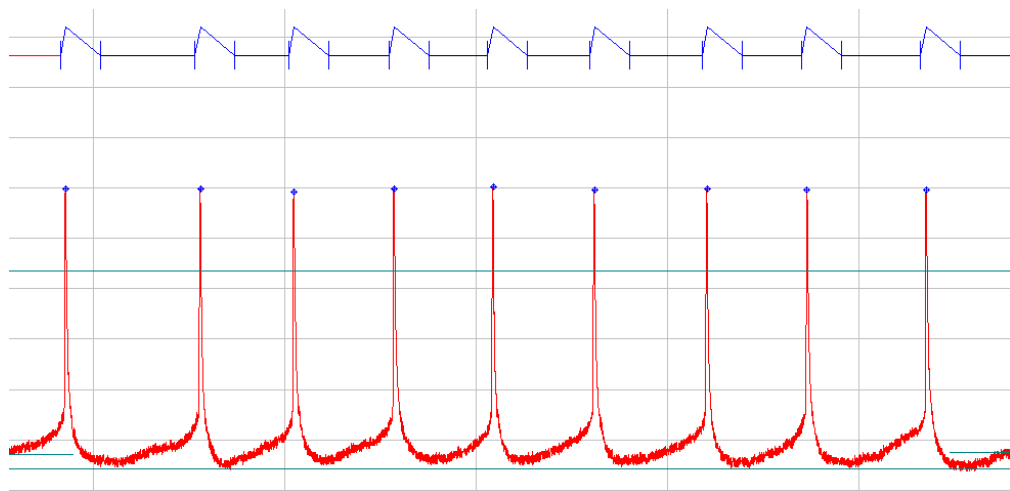
# Event Search



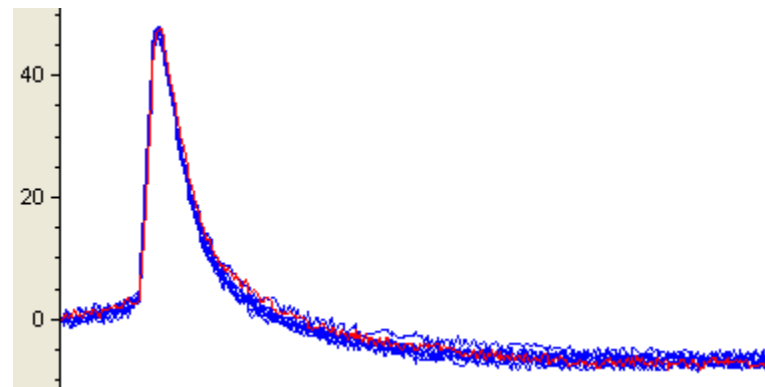
If an event crosses the trigger level, the event will be accepted.

# Event Sorting

- Extract events from a gap-free file and transform them into sweeps.



Gap-free files

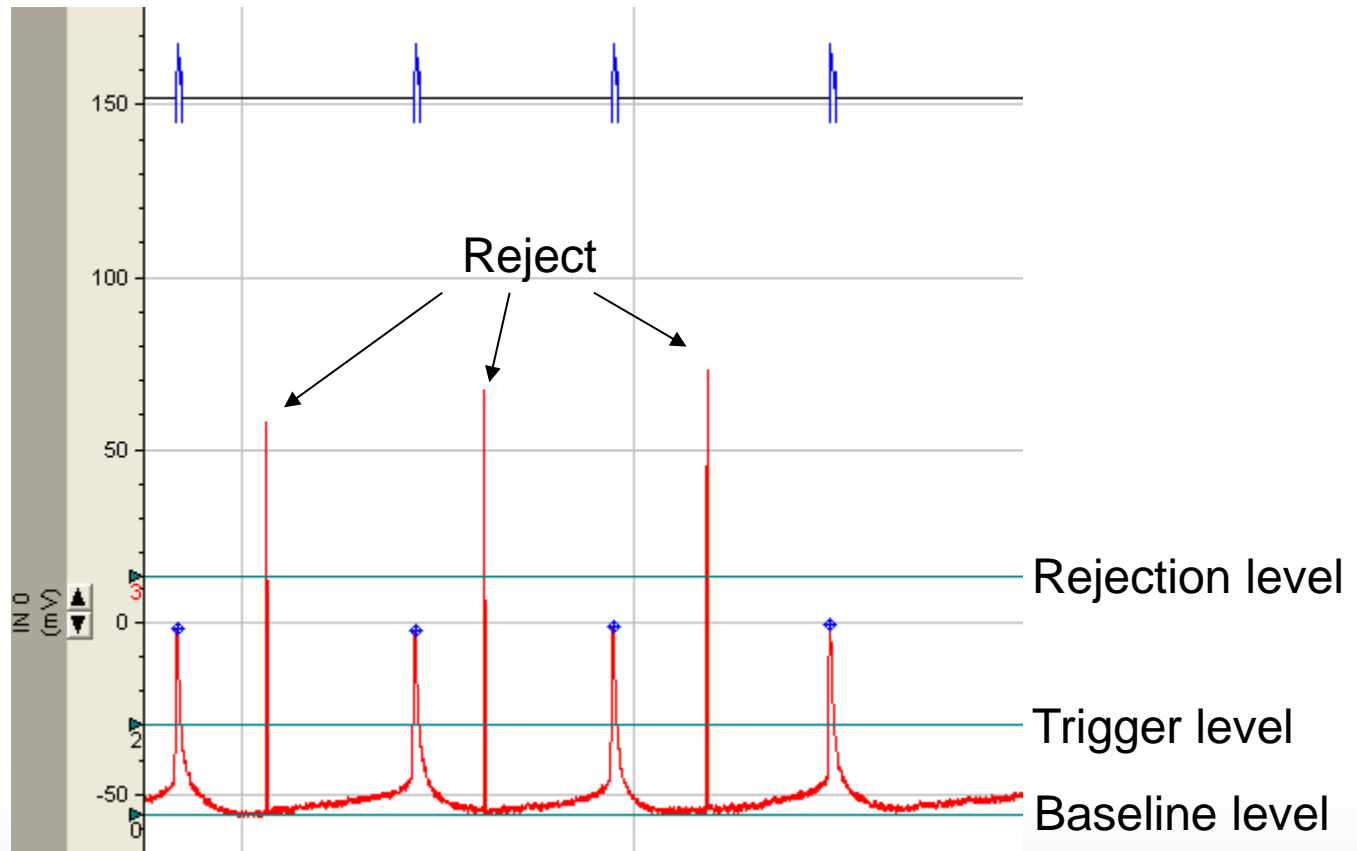


Sweep files

# Noise/Event Rejection

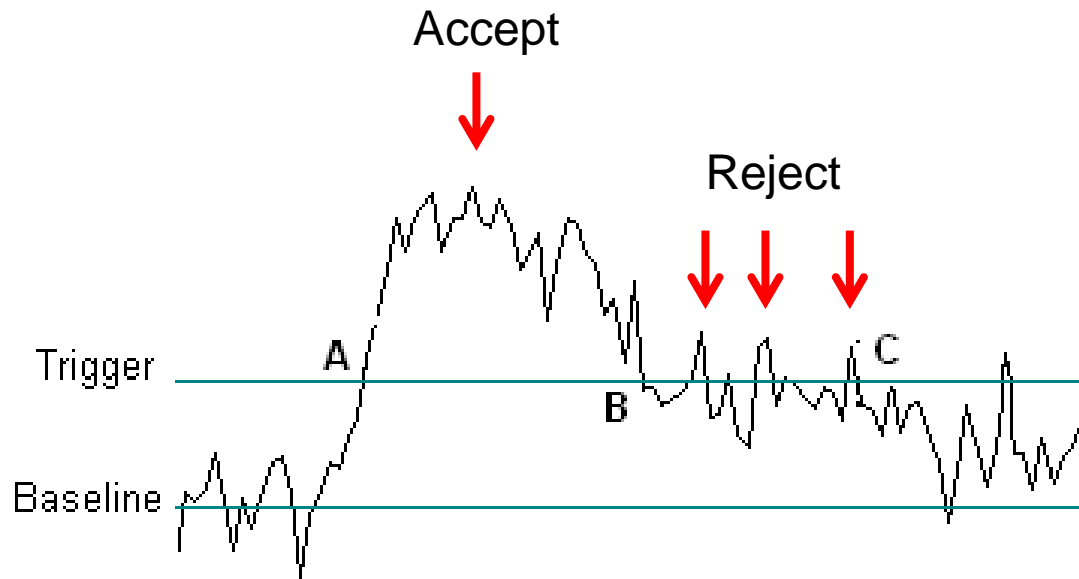
- Event rejection based on amplitude
- Noise rejection based on short duration
- Event rejection based on event length

# Noise/Event Rejection based on amplitude



If an event crosses the rejection level, the event will be rejected.

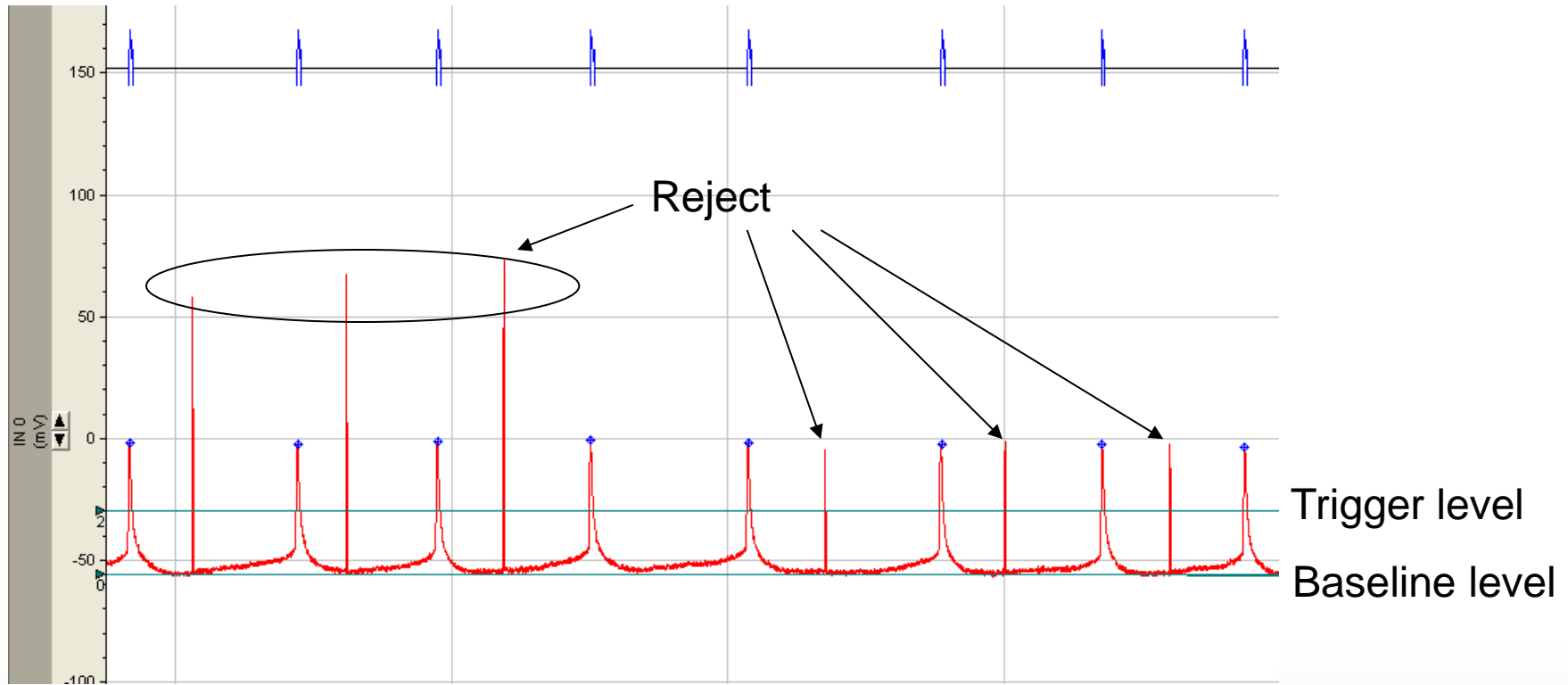
# Noise/Event Rejection based on too short duration



Noise duration in ms can be set to reject noise spikes.

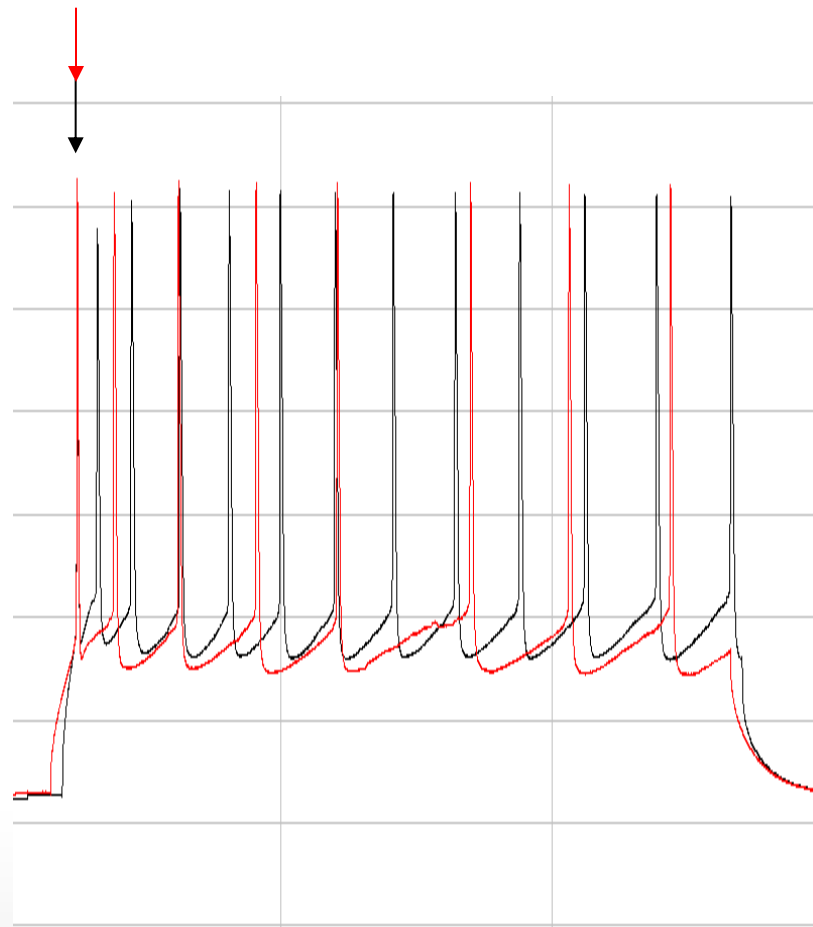
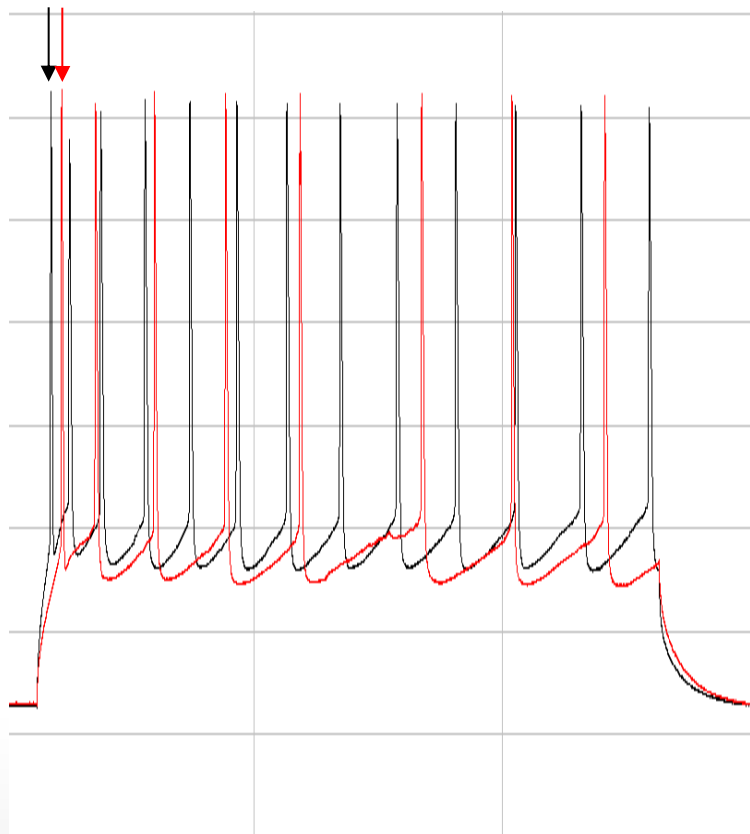


# Noise/Event Rejection based on event duration

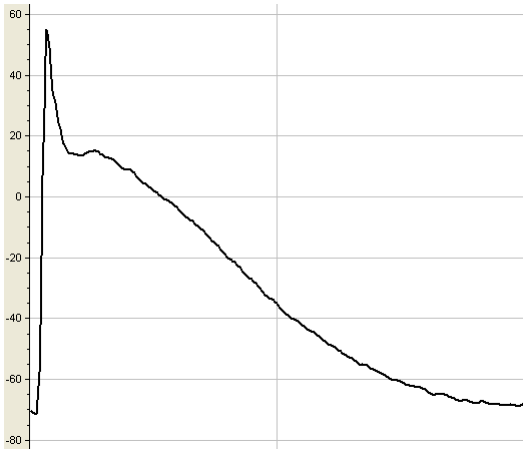


You can select Min allowed duration to eliminate short events.  
You can select Max allowed duration to discard events that are too long.

# Peak Alignment

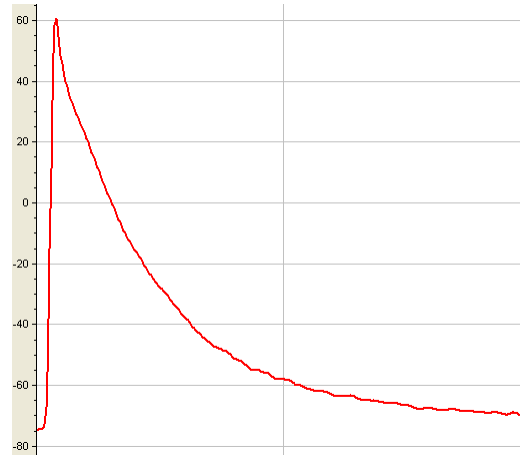


# Combine traces



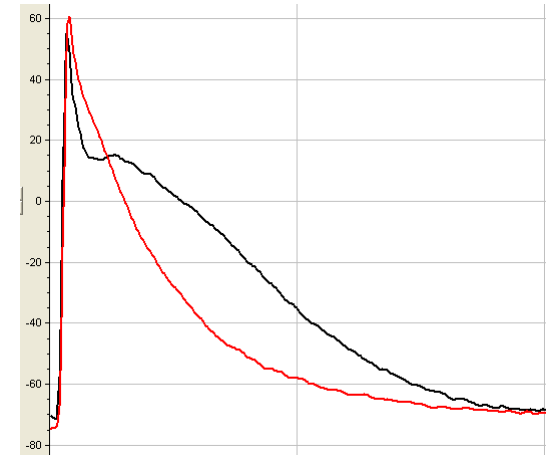
Trace in File A

+



Trace in File B

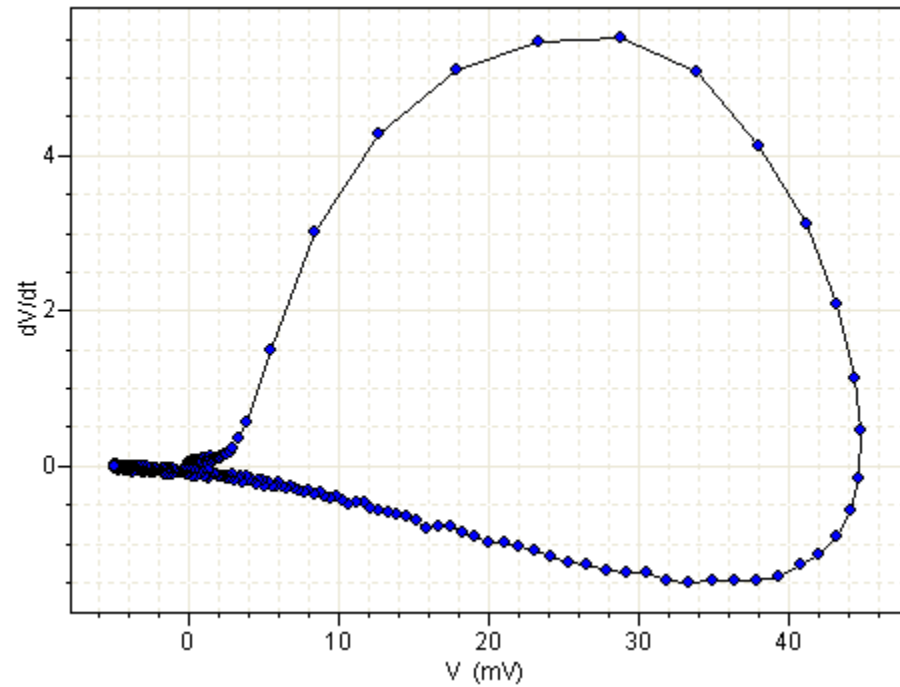
=



Trace combined

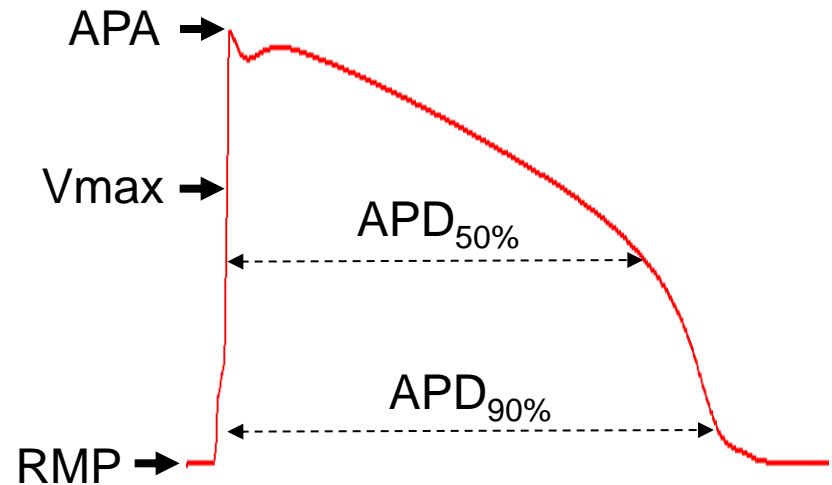
# Phase Plot

- Plot  $dV/dt$  versus  $V$

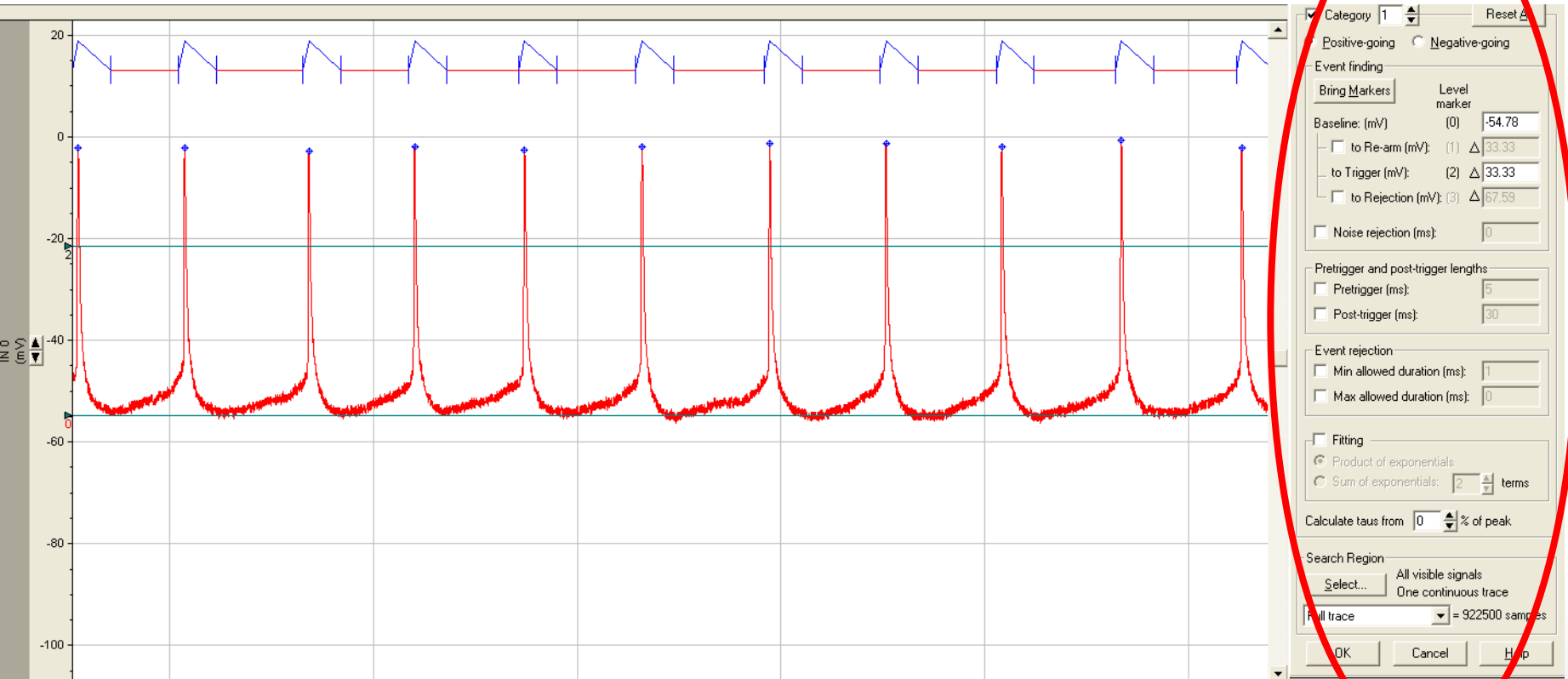


# Cardiac Action Potential Analysis

- Resting membrane potential (RMP)
- Action potential Amplitude (APA)
- Action Potential Duration (APD)
- Max Slope of depolarization (Vmax)



# Event Detection/Threshold Search



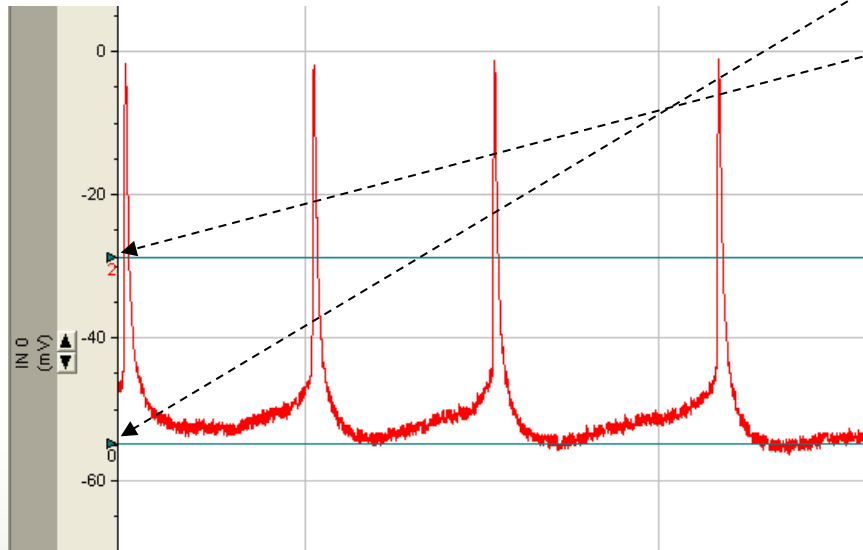
## Set category

- Each category represents a unique search criterion. There are up to 8 different categories
- The positive- and negative-going describe the location of peak relative to baseline.
  - Action potential is a positive-going event



# Bring markers

- Bring markers
  - Baseline marker
  - Trigger marker



Event finding

Bring Markers

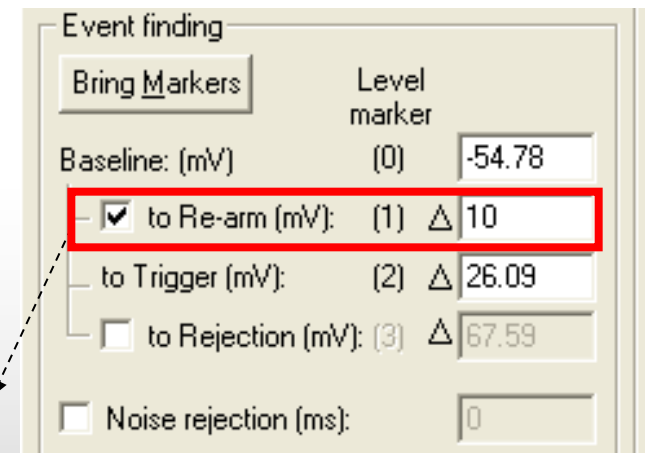
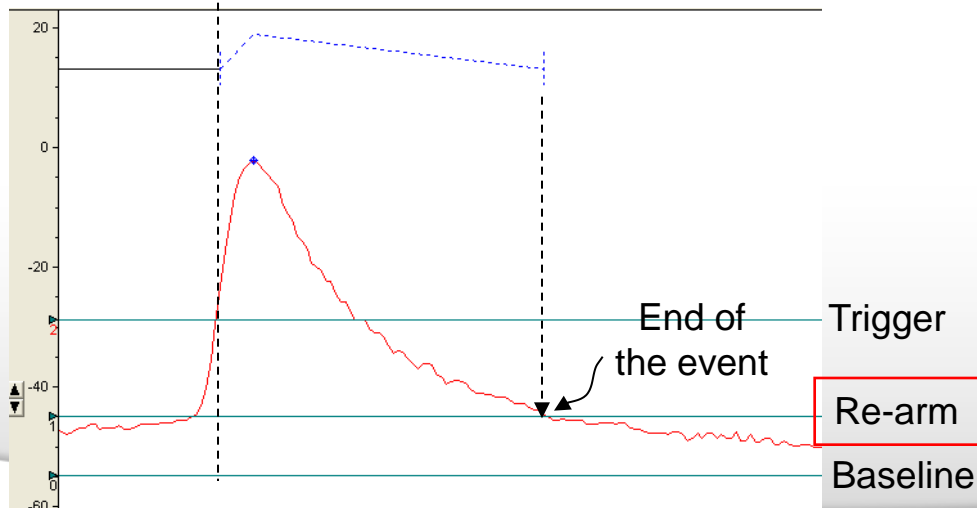
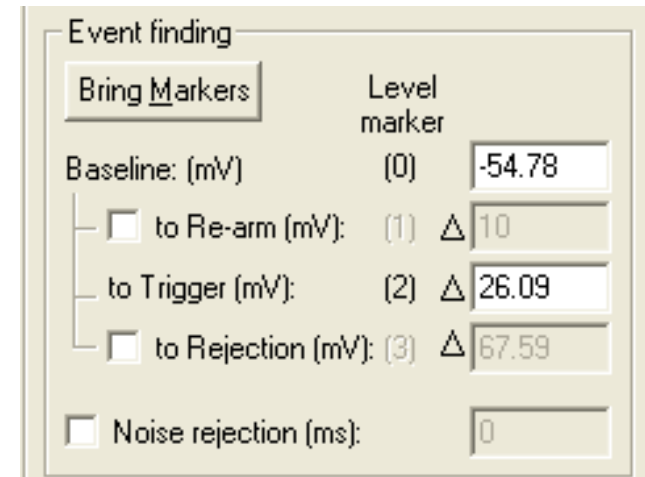
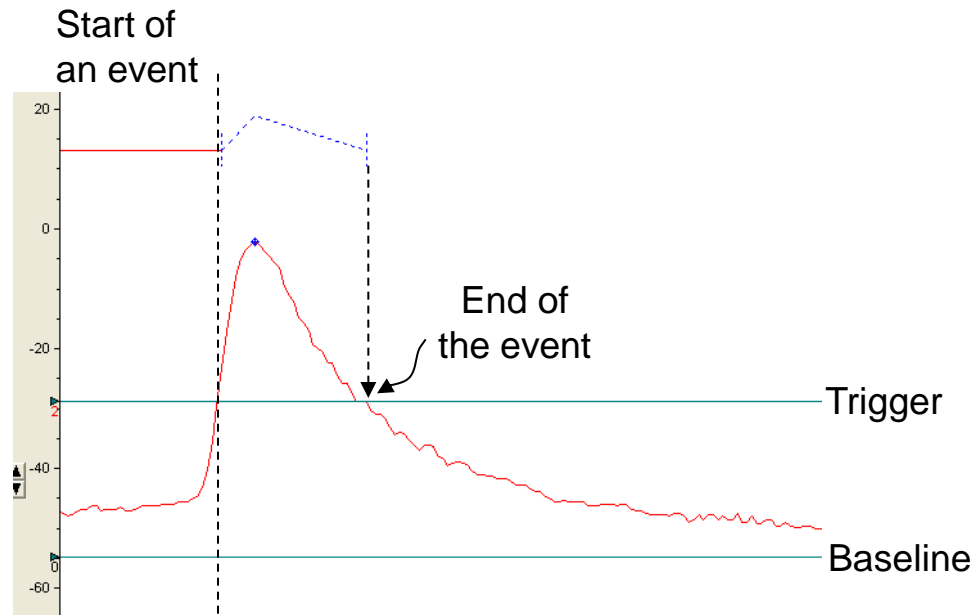
	Level marker	
Baseline: (mV)	(0)	-54.78
<input type="checkbox"/> to Re-arm (mV):	(1) $\Delta$	33.33
<input type="checkbox"/> to Trigger (mV):	(2) $\Delta$	26.09
<input type="checkbox"/> to Rejection (mV):	(3) $\Delta$	67.59
<input type="checkbox"/> Noise rejection (ms):		0

Trigger marker

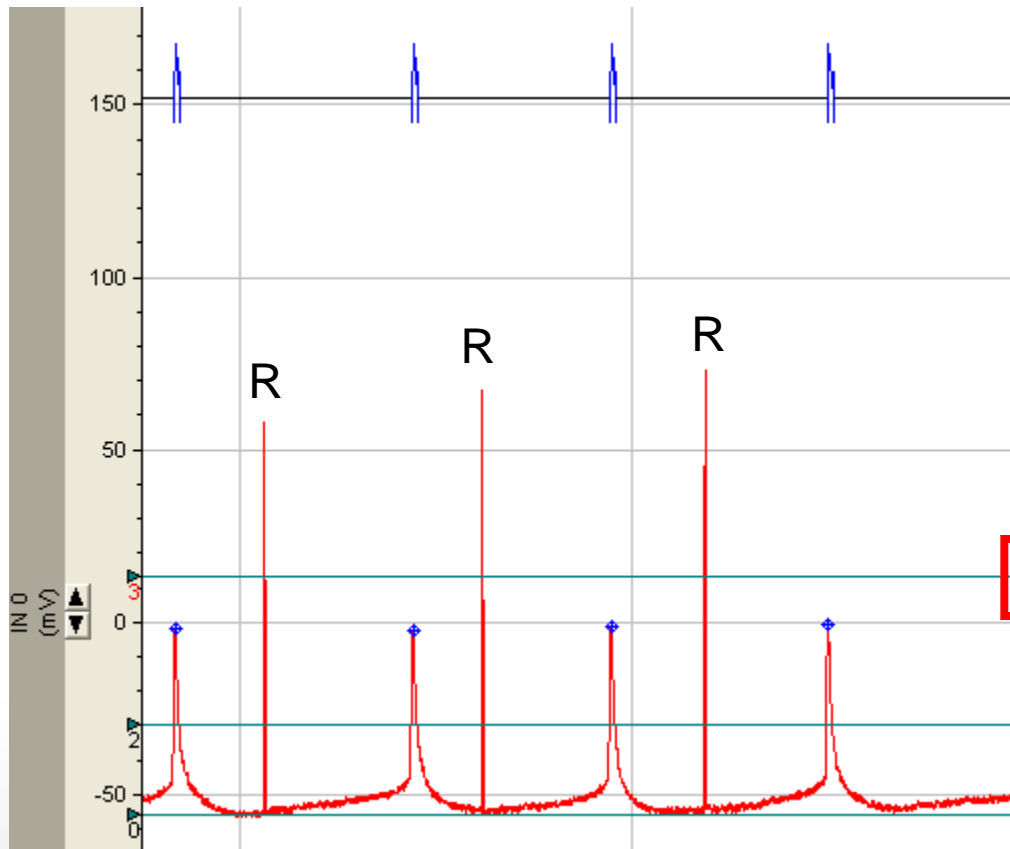
Baseline marker



# Re-arm



# Event Rejection



Event finding

Bring Markers

	Level marker	
Baseline: (mV)	(0)	-55.57
<input type="checkbox"/> to Re-arm (mV):	(1) $\Delta$	10
<input type="checkbox"/> to Trigger (mV):	(2) $\Delta$	26.09
<input checked="" type="checkbox"/> to Rejection (mV):	(3) $\Delta$	69.09
<input type="checkbox"/> Noise rejection (ms):		0

Rejection level

Trigger level

Baseline level

# Noise Rejection

Event finding

Bring Markers

Level marker

Baseline: (mV) (0) -55.46

to Re-arm (mV): (1)  $\Delta$  28.36

to Trigger (mV): (2)  $\Delta$  13.63

to Rejection (mV): (3)  $\Delta$  75.8

Noise rejection (ms): 5

Event finding

Bring Markers

Level marker

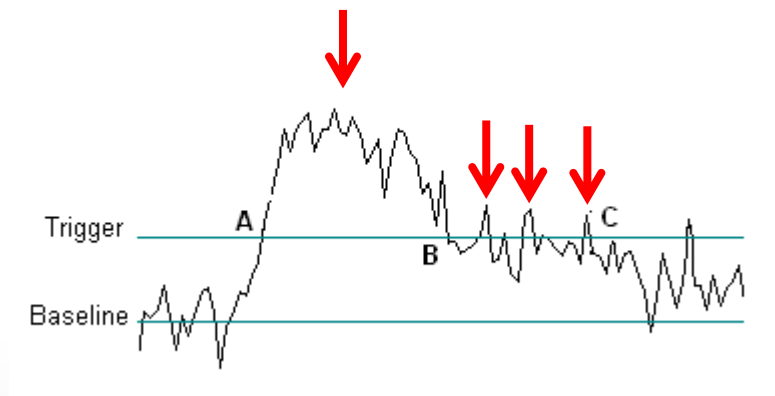
Baseline: (mV) (0) -55.46

to Re-arm (mV): (1)  $\Delta$  28.36

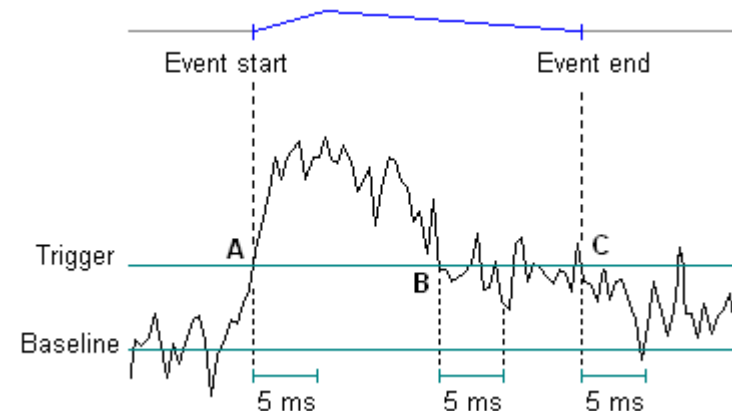
to Trigger (mV): (2)  $\Delta$  13.63

to Rejection (mV): (3)  $\Delta$  75.8

Noise rejection (ms): 5



Event Start and End  
without Noise rejection



Event Start and End  
with 5 ms Noise rejection

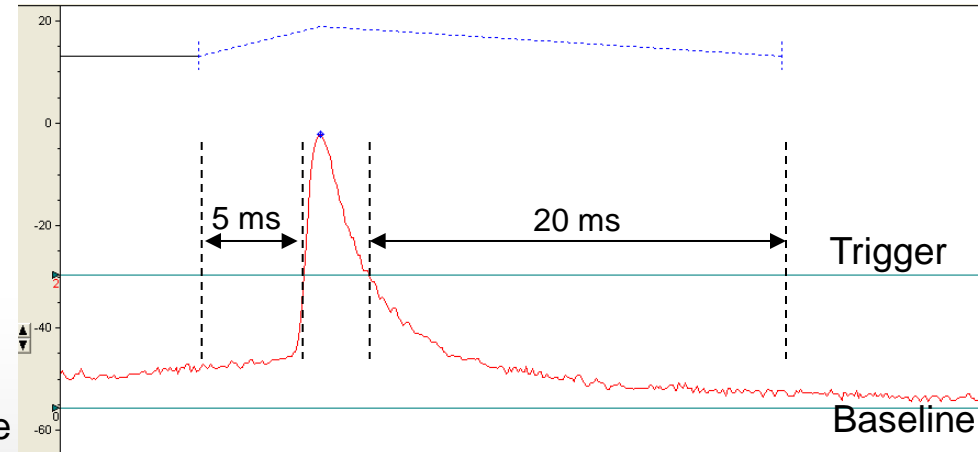
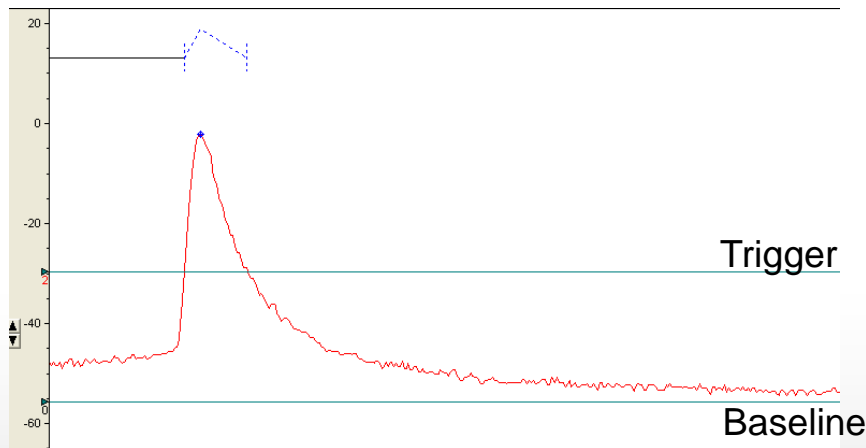
# Pre-trigger and Post-trigger

Pretrigger and post-trigger lengths

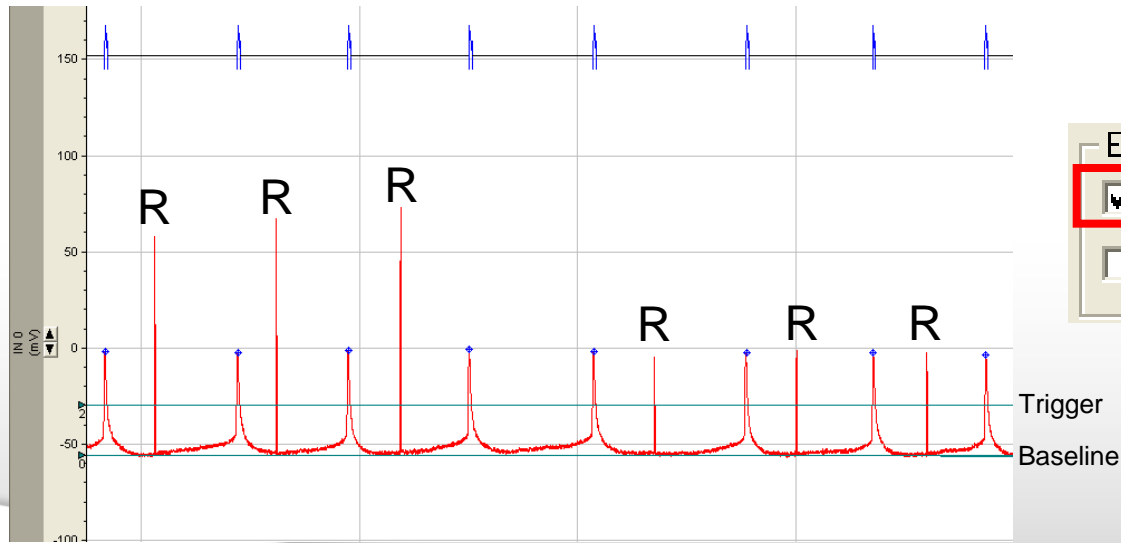
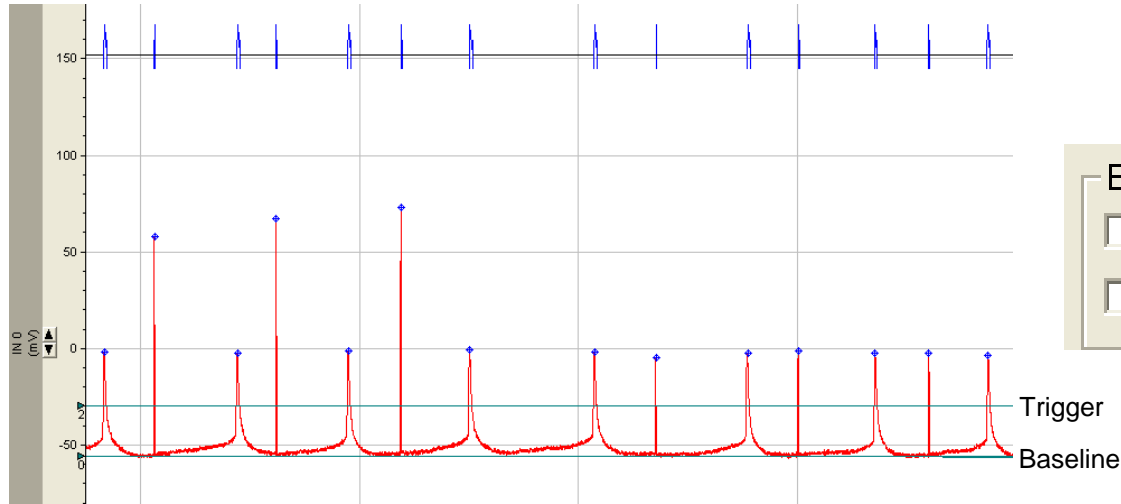
<input type="checkbox"/> Pretrigger (ms):	5
<input type="checkbox"/> Post-trigger (ms):	20

Pretrigger and post-trigger lengths

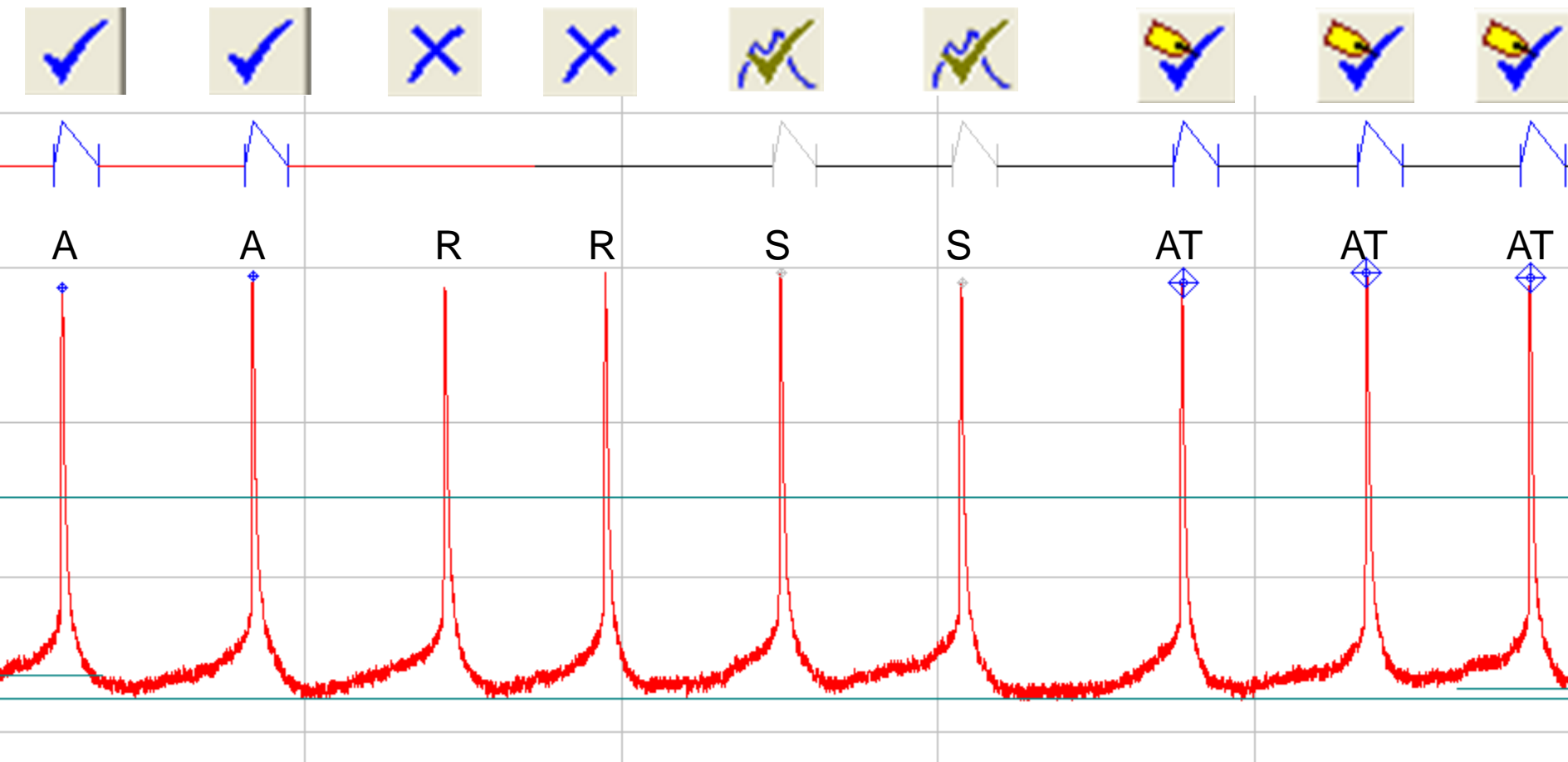
<input checked="" type="checkbox"/> Pretrigger (ms):	5
<input checked="" type="checkbox"/> Post-trigger (ms):	20



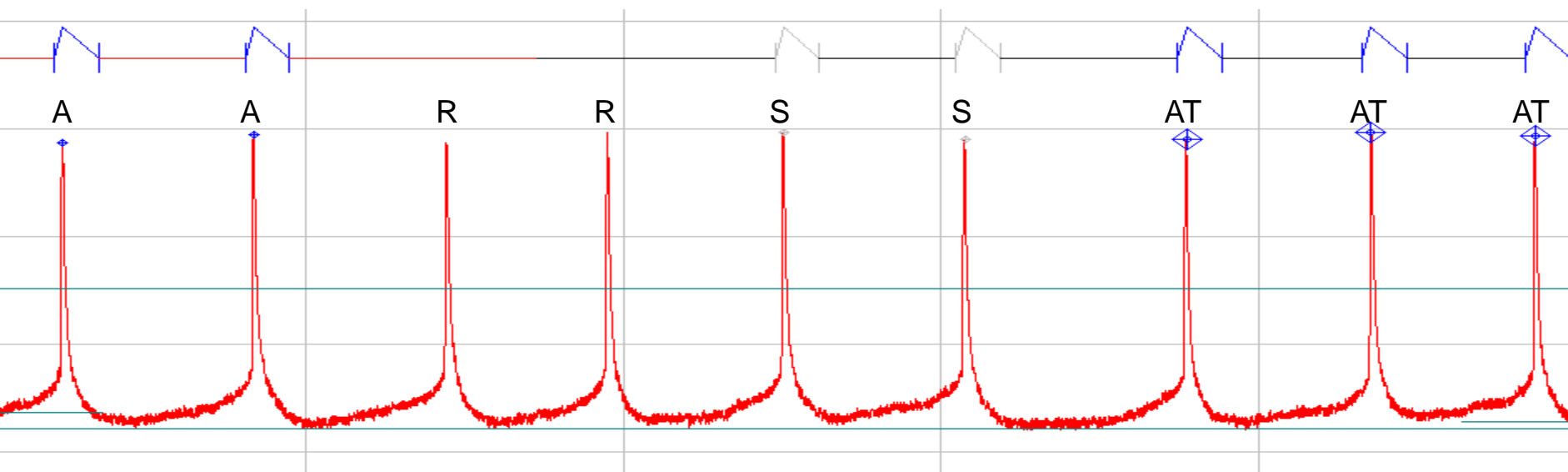
# Event Rejection



# Accept, Reject, Suppress, Accept Tag



# Event States

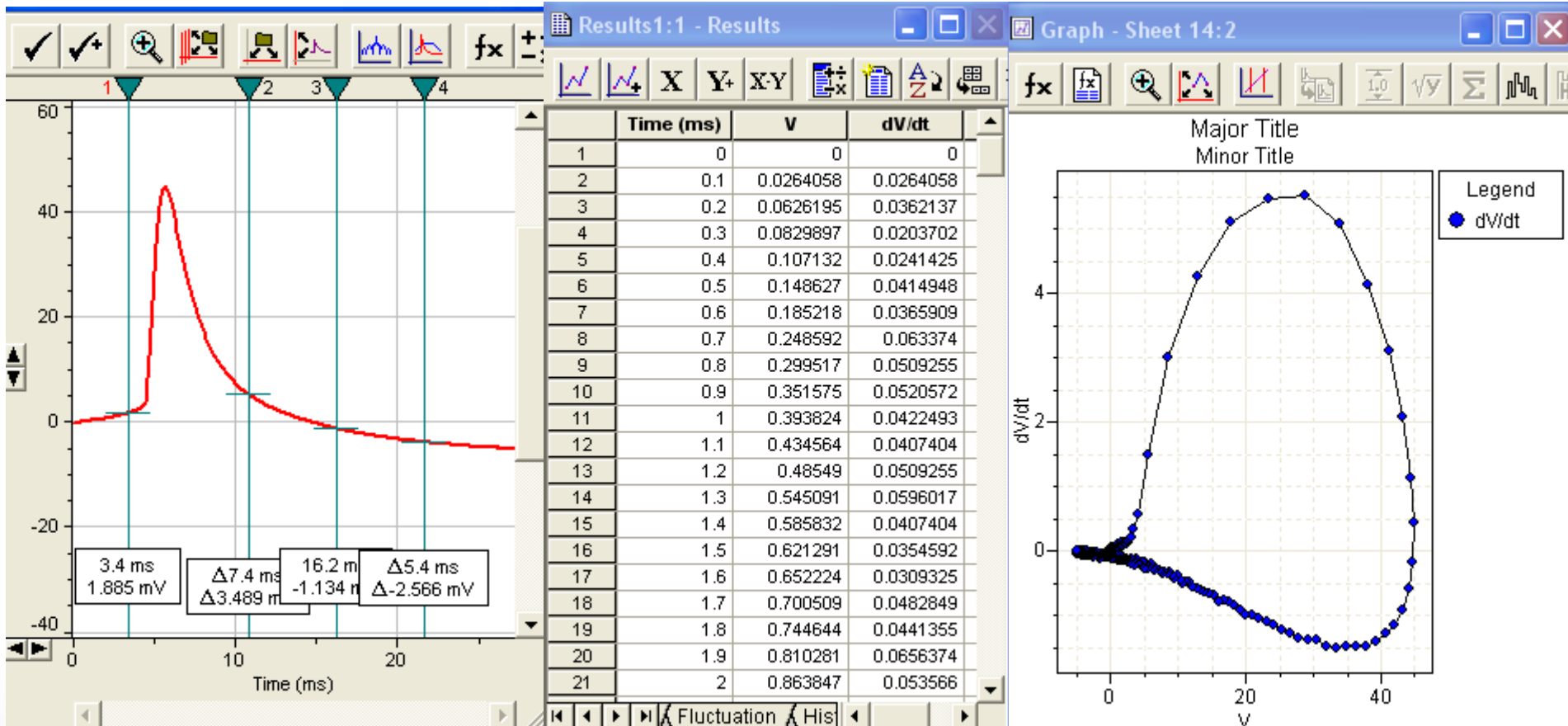


	Trace	Search	Category	State
1	1	1	1	A
2	1	1	1	A
3	1	1	1	AT
4	1	1	1	AT
5	1	1	1	AT

	Trace	Search	Category	State
1	1	1	1	A
2	1	1	1	A
3	1	1	1	S
4	1	1	1	S
5	1	1	1	AT
6	1	1	1	AT
7	1	1	1	AT

Event Detection/Show Suppressed Events

# Phase Plot



Select the trace

Transfer trace

Column  
Arithmetic  
 $cC = \text{diff}(cB)$

Plot dV/dt vs V



# Summary

- Baseline adjustment
- Event Search
- Event sorting
- Noise/Event rejection
- Spike alignment
- Combine trace
- Phase plot
- Action potential analysis

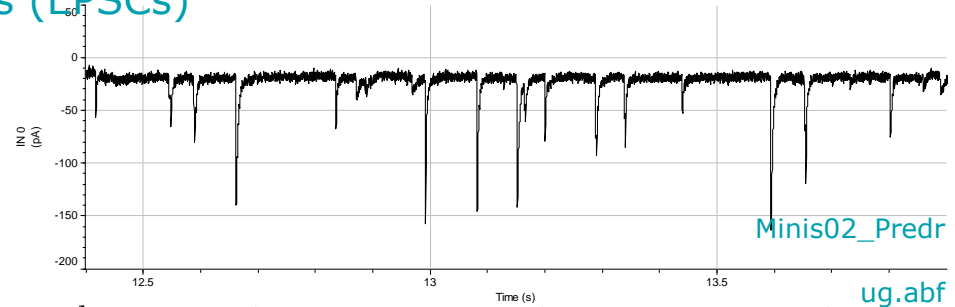


# Analysis of Synaptic Events with the Clampfit Data Analysis Module

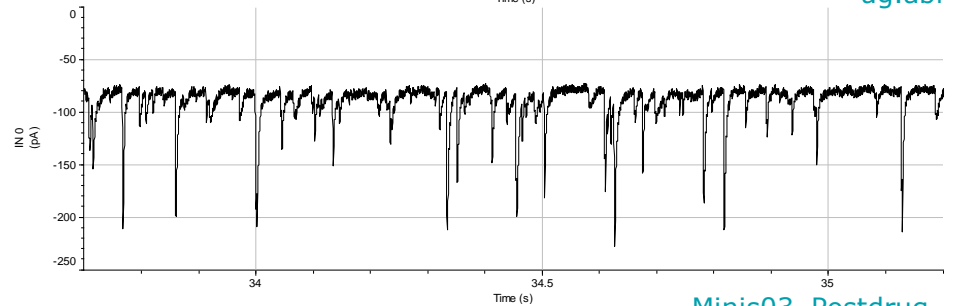
# Agenda

- Postsynaptic miniature currents (EPSCs)

- Pre-drug



- Post-drug



- Are there differences in the amplitude distribution?

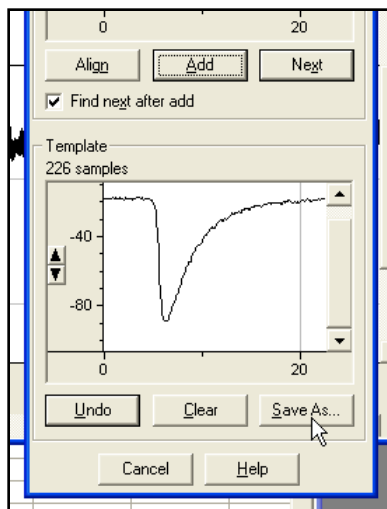
- Post-synaptic mechanism

- Are there differences in the frequency?

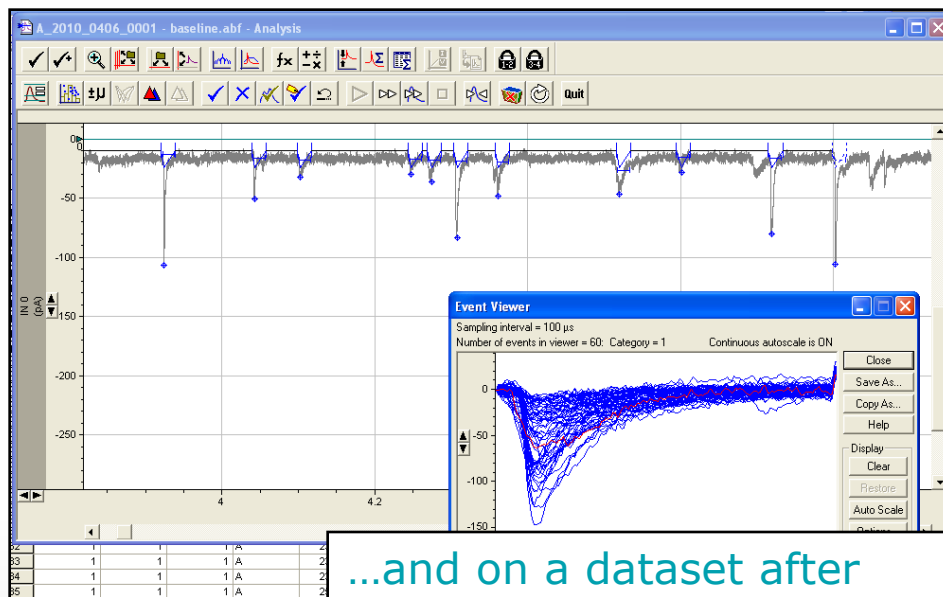
- Pre-synaptic mechanism

# Strategy

Create a template from one of the data files



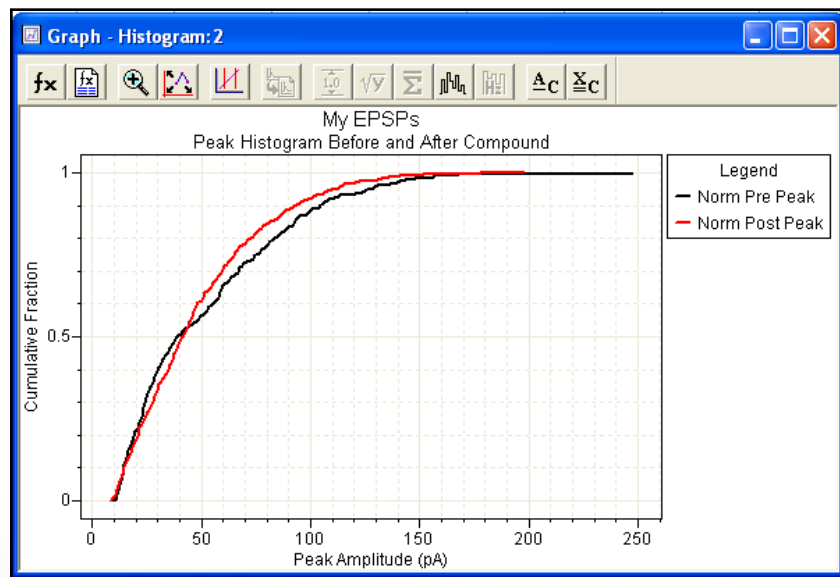
Perform a template search on a data segment before addition of compound...



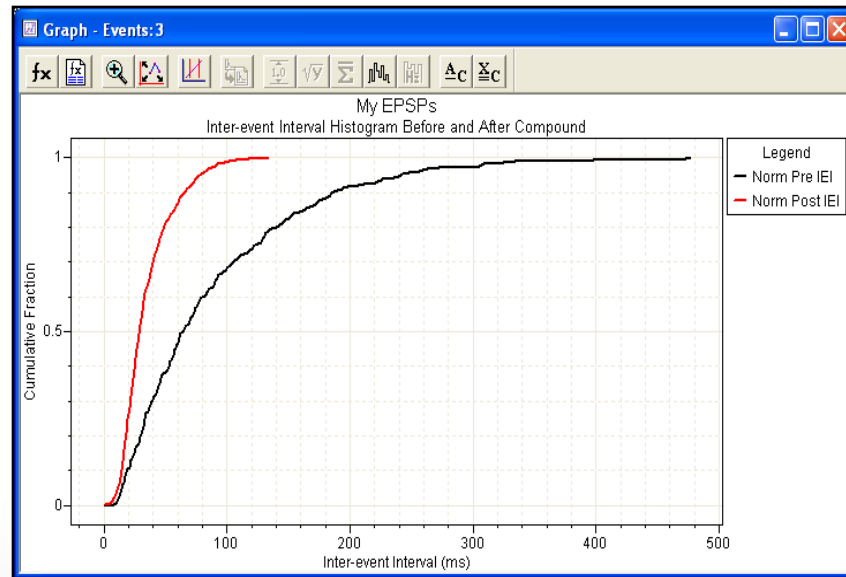
...and on a dataset after addition of compound

# Strategy

Create cumulative histograms of the peak amplitudes...

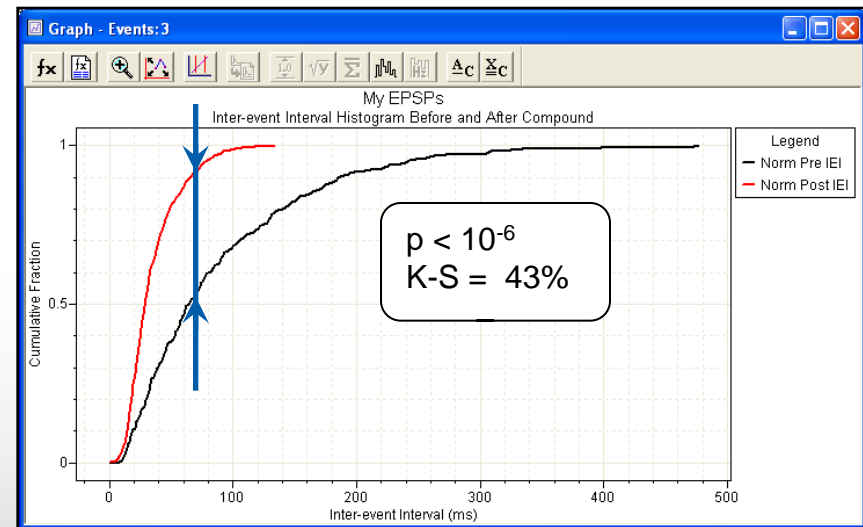
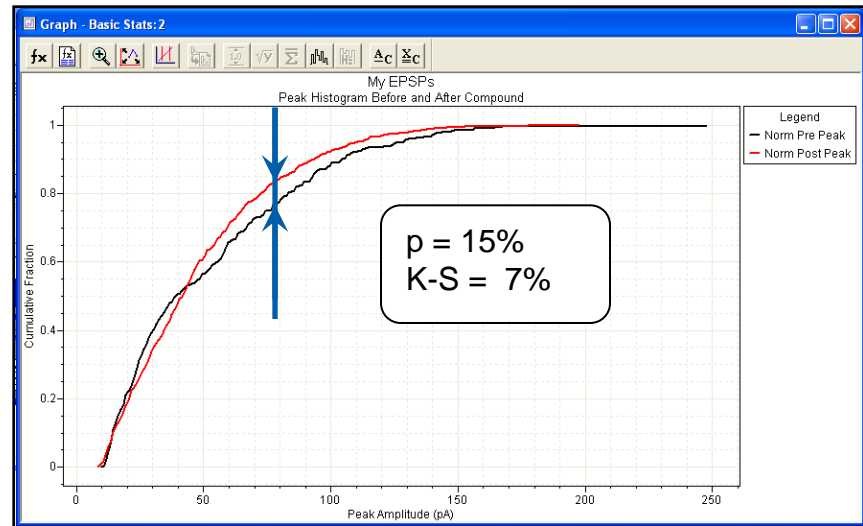


...and the inter-event intervals.



# Strategy

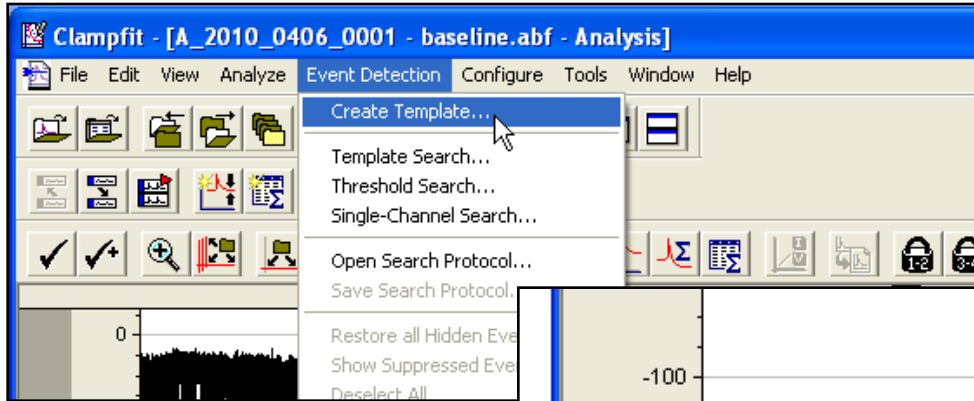
Perform a statistical analysis to determine whether there are differences between the two datasets.



# Clampfit Features Discussed

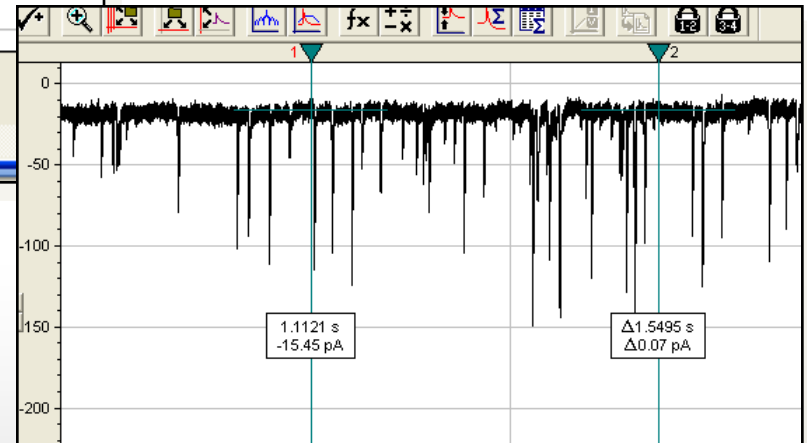
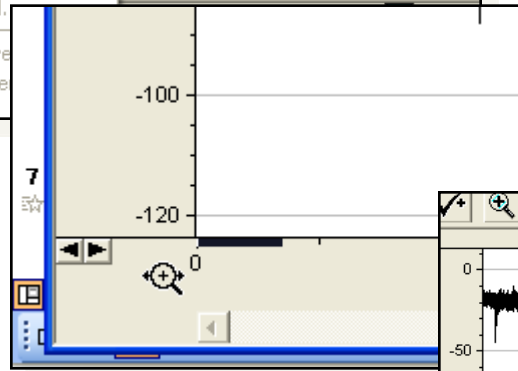
- Event Detection > Create Template
- Event Detection > Template Search
- Move Cursors efficiently
- View > Zoom > Between Cursors
- Analyze > Arithmetic
- Format > Column > Rename
- Format > Rename Sheet
- Analyze > Histogram
- Efficiently creating a graph using X-Y pairs
- Editing a graph
- Analyze > Kolmogorov-Smirnov Test
- Analyze > Basic Statistics

# Creating a Template



Zoom in to  
the first  
few  
seconds

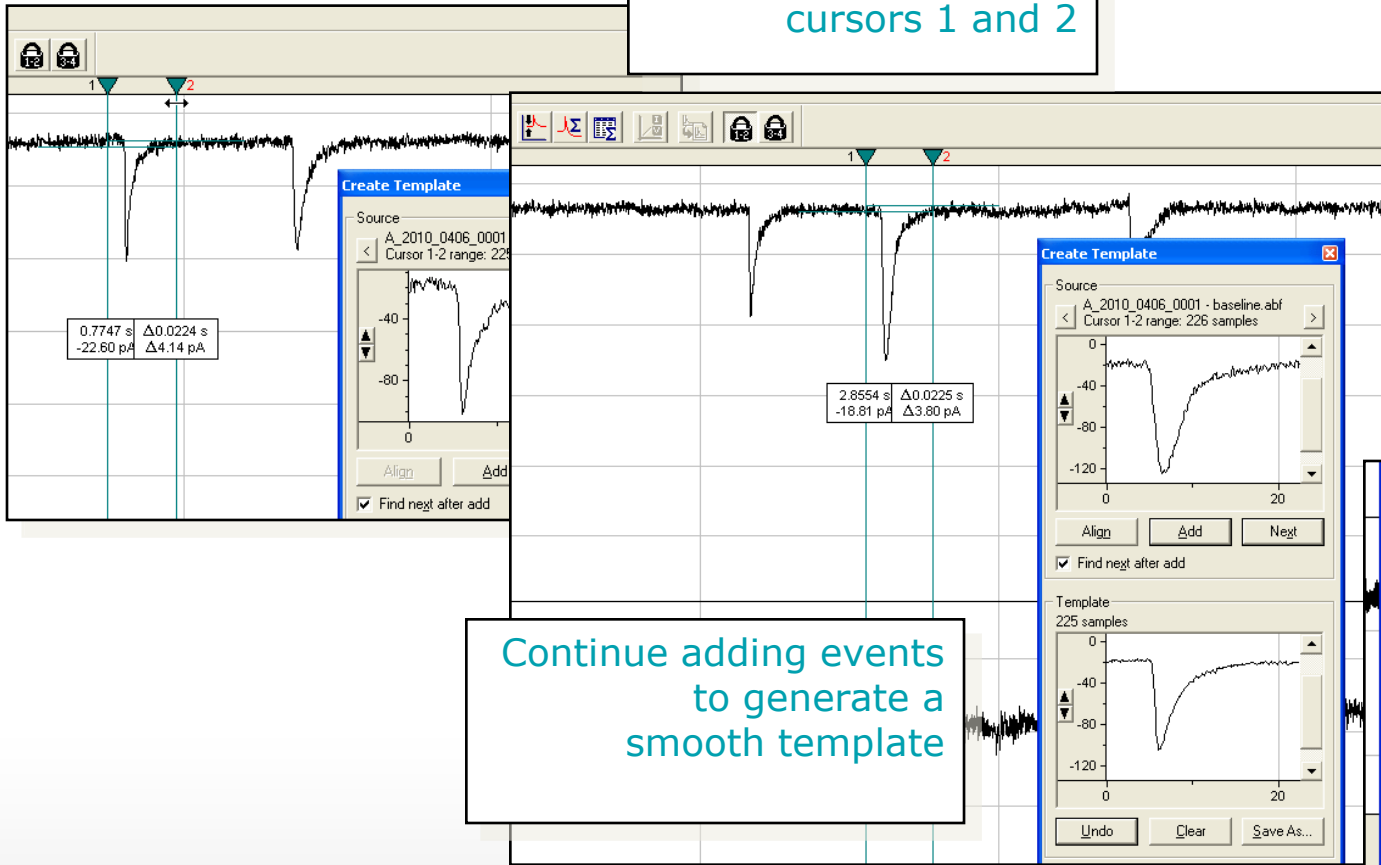
[Shift] + [Click]  
brings the cursors





# Creating a Template

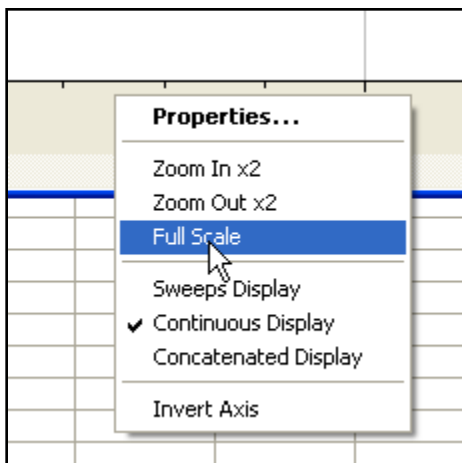
Enclose an event with cursors 1 and 2



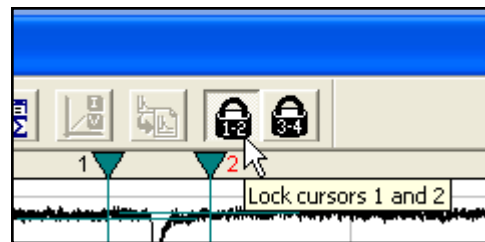
Continue adding events to generate a smooth template

When done, save the template

# Delimit the Analysis Region



Full-scale the time axis,



unlock cursors 1 and 2,

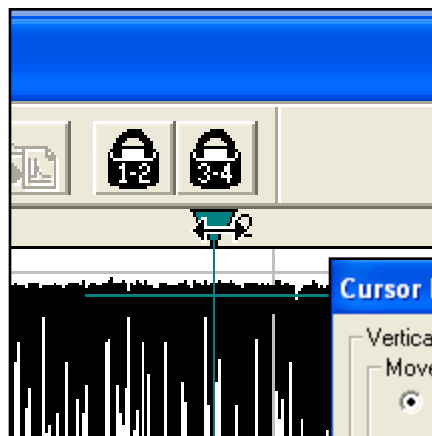


and send cursor **1**...



..."**Home**" to the start of the trace

# Delimit the Analysis Region



Double-click on  
Cursor 2,

**Cursor Properties for Cursor 2**

Vertical Cursor Time Value

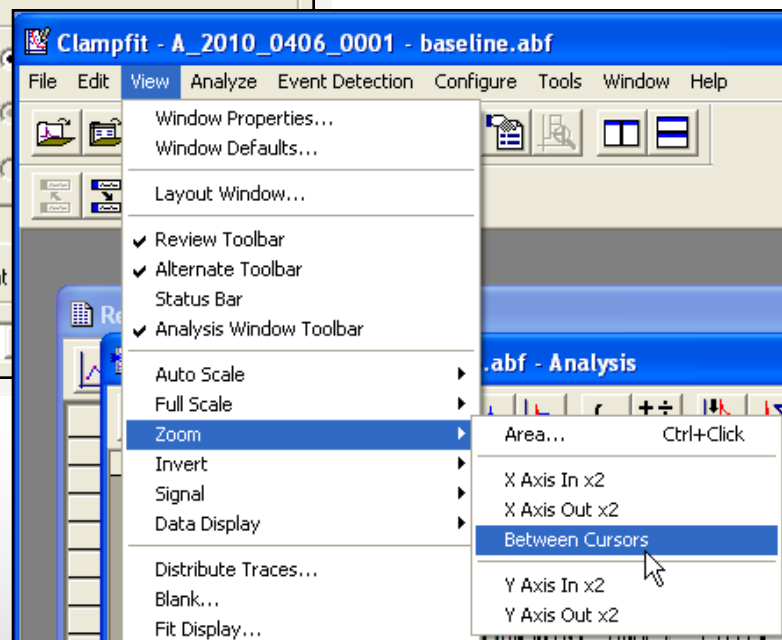
Move To

- Time (s): 30
- Sample number: 960404
- Tag number: 1
- Epoch:
- Keep cursor in view F4 and Shift+F4 repeat

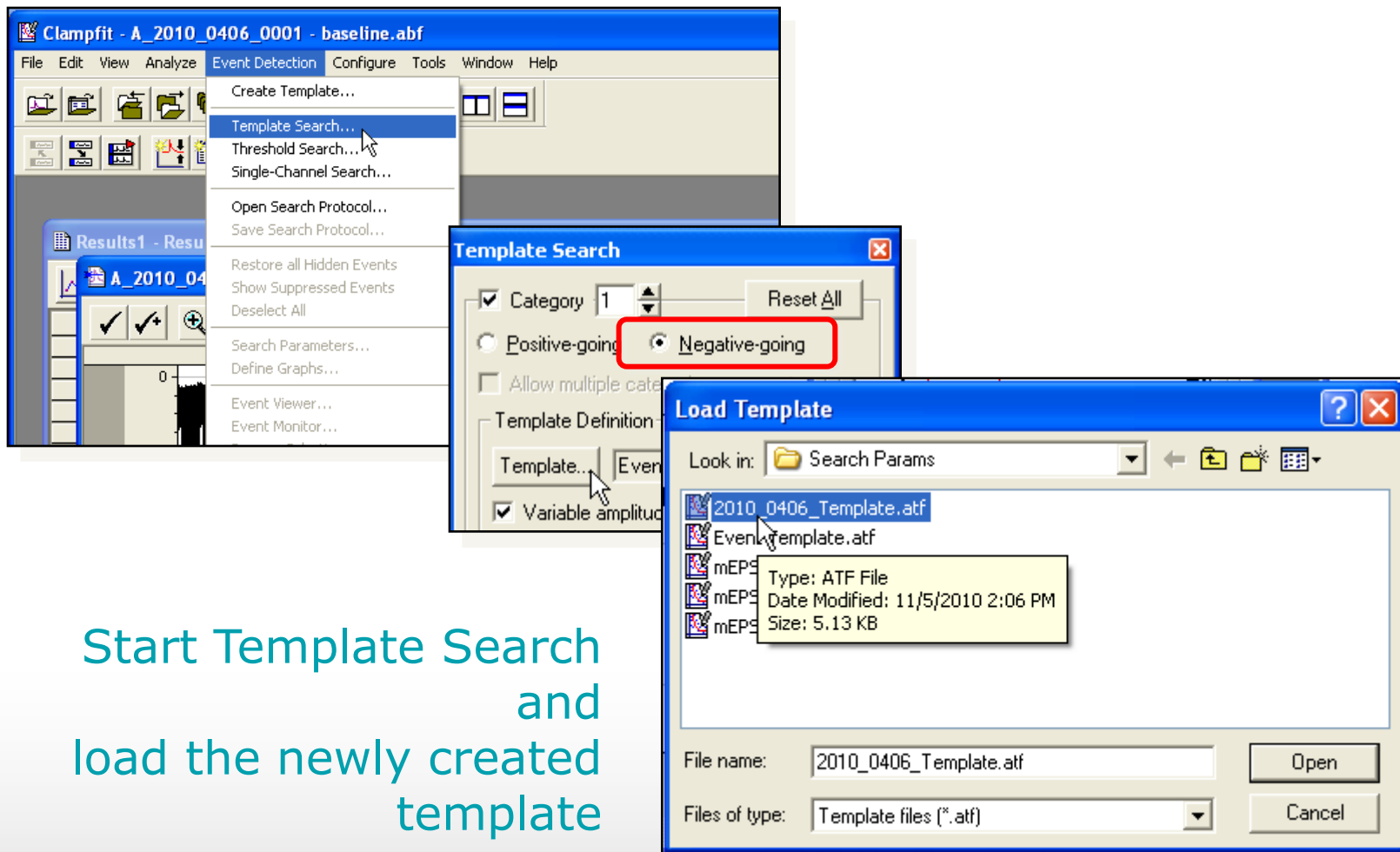
Number of decimal places to show: Default (4)

send it to  
30 s,

...and zoom the  
region we are  
going to analyze

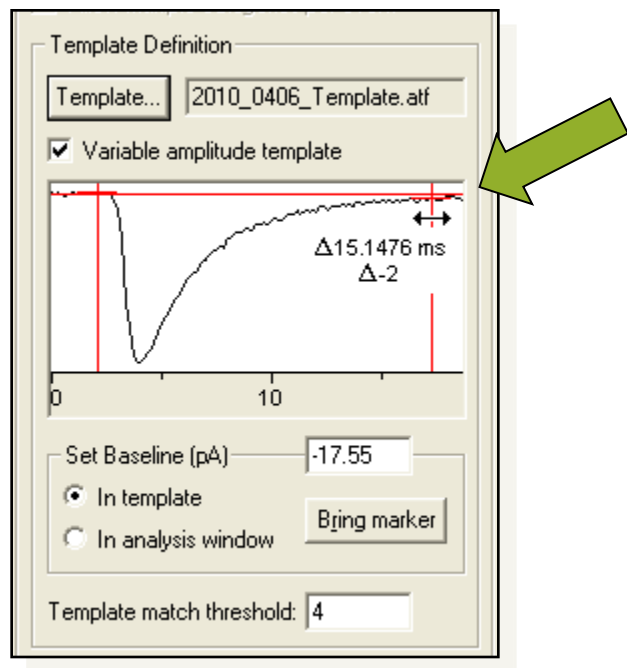


# Template Search

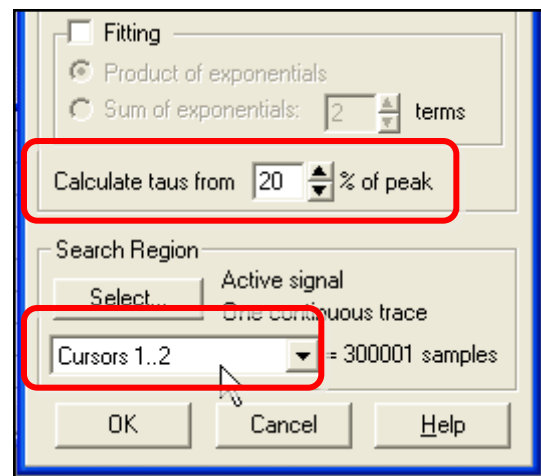


Start Template Search  
and  
load the newly created  
template

# Template Search

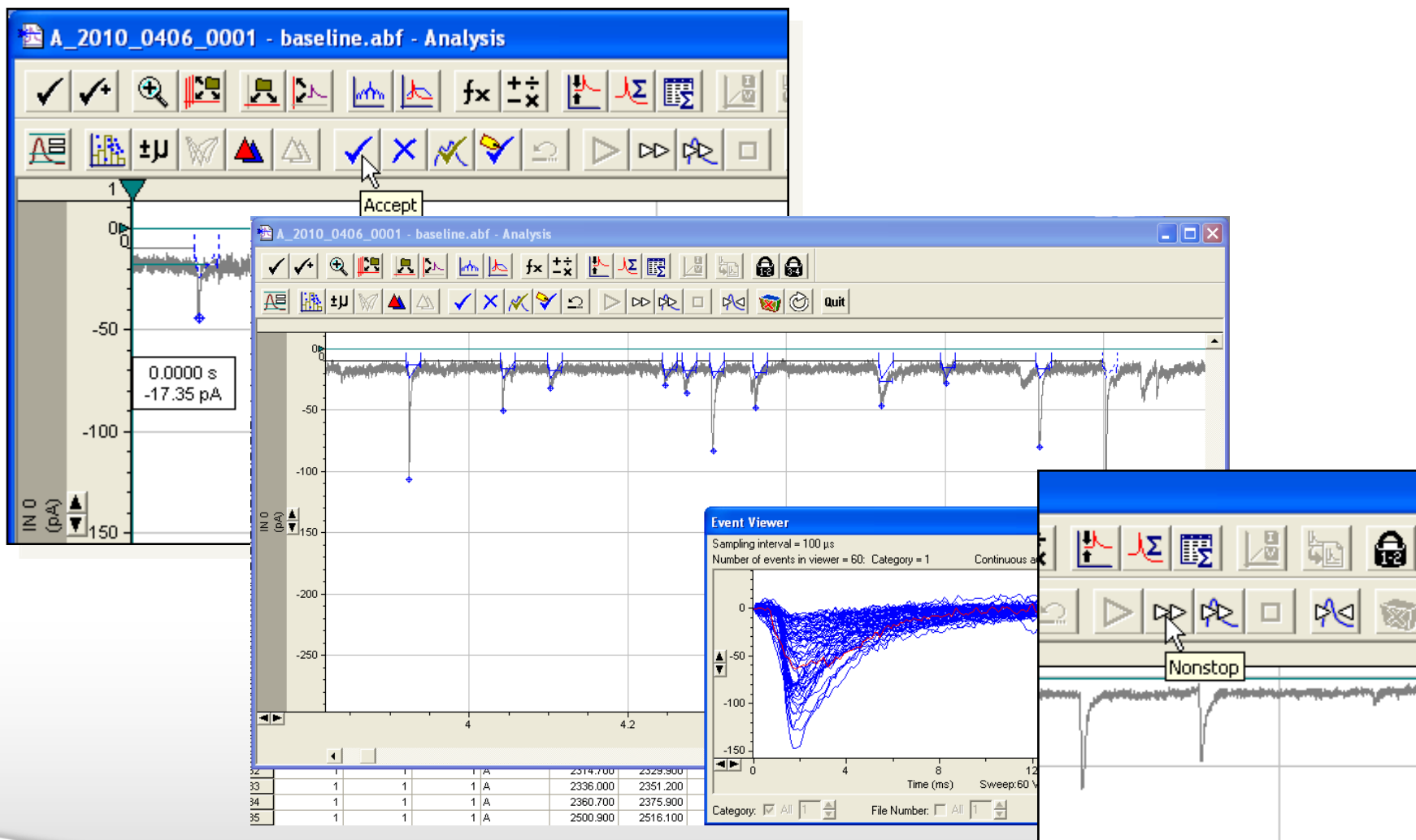


Define the measurement region.



Set taus and analysis region.

# Template Search



# Defining "Sanity-check" Graphs

**Define Graphs**

Graph 1

- Conventional histogram Bin width (ms): 0.1  Auto
- Logarithmic histogram Bins/decade: 10  SqRt N
- Scatter plot  Convert bin counts to frequency

Category:  All 0

Time of peak

Peak amplitude

Graph 2

- Conventional histogram Bin width (pA): 0.1  Auto
- Logarithmic histogram Bins/decade: 10  SqRt N
- Scatter plot  Convert bin counts to frequency

Category:  All 0

Peak amplitude

Decay time

Graph 3

- Conventional histogram
- Logarithmic histogram
- Scatter plot

Graph 4

- Conventional histogram
- Logarithmic histogram Bins/decade: 10
- Scatter plot  Convert bin counts to frequency

OK Cancel

**Clampfit - Results1**

File Edit View Analyze Event Detection Configure Tools Window Help

**Graph - Events:2**

1: Peak Amplitude vs Time Of Peak (Category 1)

2: Peak Amplitude Histogram (Category 1)

Count (N)

Peak Amplitude (pA)

Peak Amplitude (pA)

Time Of Peak (ms)

This graph indicates the absence of an overall trend (run-up or run-down of amplitude or frequency).

This graph suggests that there are not two (or more) classes of events with clearly distinct amplitude.

# Copying the Pre-compound Results to a New Sheet

Results1 - Results

	Trace	Search	Category	State	Event Start	Event End T	Baseline (p)	Peak Amp (	Time to Pea	T
1	1	1	1	A	48.700	63.900	-17.41462	-26.60394	1.900	
2	1	1	1	A	176.000	191.200	-18.05566	-39.20752	1.800	
3	1	1	1	A	229.300	244.500	-15.76179	-38.55949	1.900	
4	1	1	1	A	246.700	261.900	-28.10546	-25.14161	2.800	
5	1	1	1	A	256.800	272.000	-20.54273	-26.50513	1.900	
6	1	1	1	A	516.700	531.900	-18.18194	-60.26044	1.700	
7	1	1	1	A	779.000	794.200	-16.53740	-84.69551	1.700	
8	1	1	1	A	834.400	849.600	-21.84468	-71.73422	2.400	
9	1	1	1	A						
10	1	1	1	A						
11	1	1	1	A						
12	1	1	1	A						
13	1	1	1	A						
14	1	1	1	A						
15	1	1	1	A						
16	1	1	1	A						
17	1	1	1	A						
18	1	1	1	A						
19	1	1	1	A						
20	1	1	1	A						
21	1	1	1	A						

Clampfit - Results1

File Edit View Analyze Format Event Det

- Undo Ctrl+Z
- Cut
- Copy
- Copy to Layout Win

Rename Sheet

Name of sheet:

Pre

Previous

OK Cancel

et 17 Sheet 18 Sheet 19 Sheet 20

Clampfit - Results1

File Edit View Analyze Format Event Det

- Undo Paste Ctrl+Z
- Cut Ctrl+X
- Copy Ctrl+C
- Copy to Layout Window
- Paste Ctrl+V
- Insert
- Clear Sheet
- Clear All Sheets



# Computing the Absolute Peak Amplitude

Results1 - Results

	Z	BA	BB	BC
1	13.1522	1.4146	Column Arithmetic	
2	5.91741	3.84983	-140.581	7.86164
3	8.42877	3.395		

$cBH = cH * (-1)$   
yields the absolute  
peak amplitude

Column Arithmetic

Expression:

Columns... Operator Function Special Undo Clear

Specify Region

Full column

Row From:  To:

Force excluded region to zero

OK Apply Cancel

Expression Example:

Column Rename

Name of column BH:

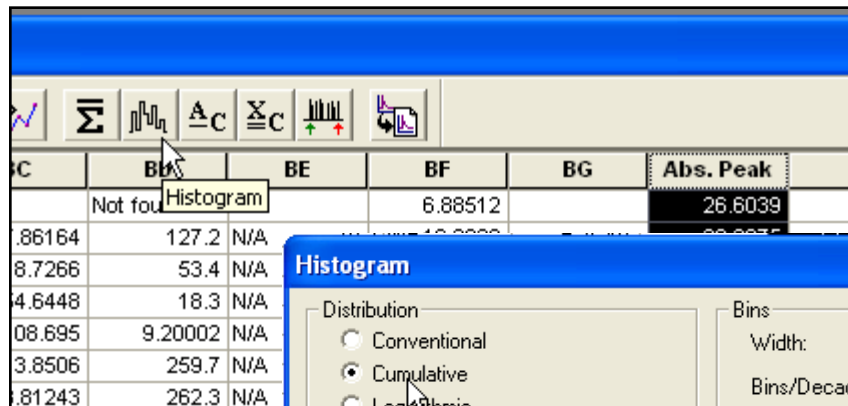
Previous Next

OK Cancel Help

	BG	BH	BI	BJ	BK	BL
512		26.6039				
028		39.2075				
616		38.5595				
381		25.1416				
129		28.5051				
898						
4.6						
155						
052						
698						
195						
587						
542						
205						
647						
798		28.2012				

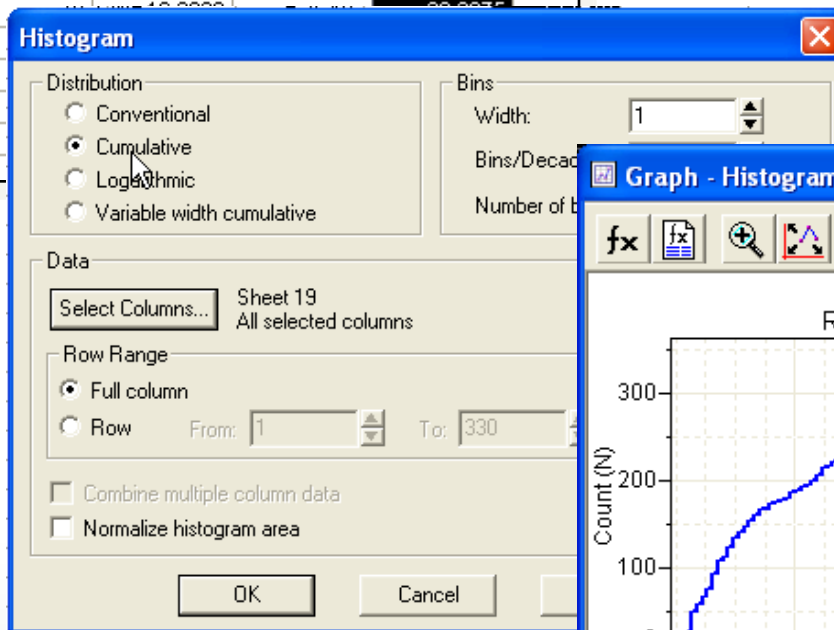
Double-click on the  
column header to  
rename

# Creating a Cumulative Peak Histogram



The screenshot shows a software interface with a toolbar at the top containing various icons, including a histogram icon. Below the toolbar is a data table with columns labeled 'BH', 'BE', 'BF', 'BG', and 'Abs. Peak'. The 'Abs. Peak' column contains numerical values, with the value 26.6039 highlighted. A mouse cursor is hovering over the histogram icon in the toolbar.

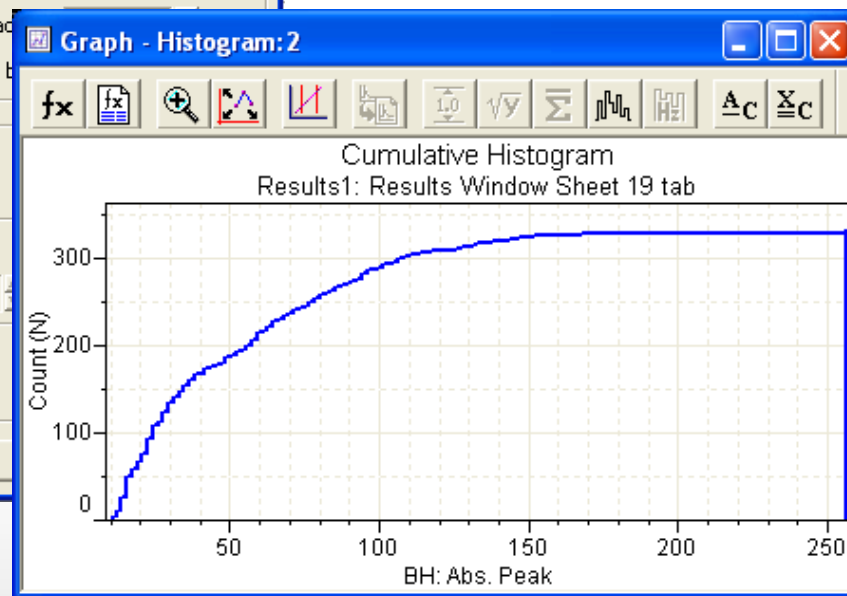
	BH	BE	BF	BG	Abs. Peak
	Not found		6.88512		26.6039
	0.86164	127.2	N/A		
	8.7266	53.4	N/A		
	4.6448	18.3	N/A		
	08.695	9.20002	N/A		
	3.8506	259.7	N/A		
	0.81243	262.3	N/A		



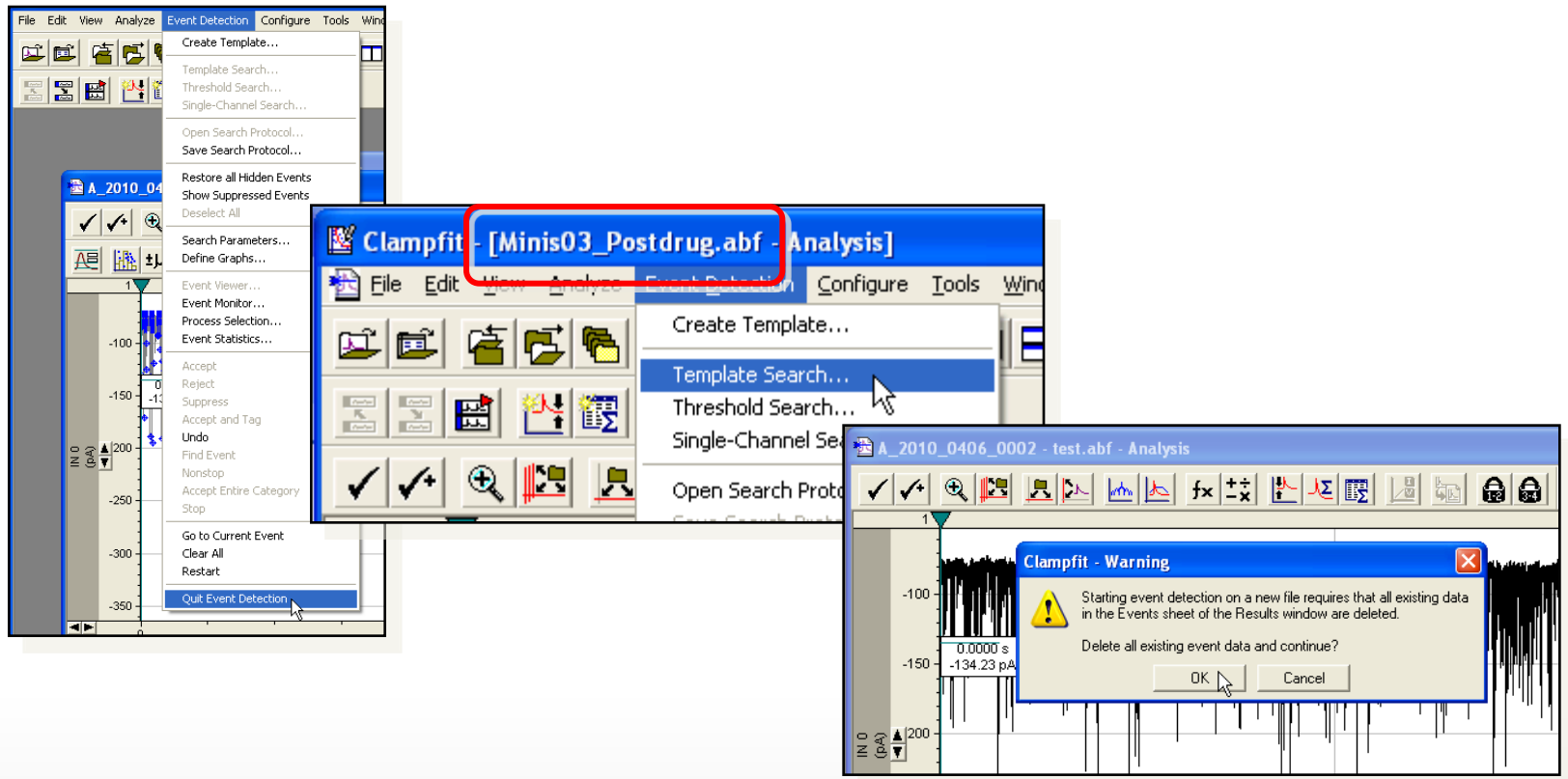
The Histogram dialog box is open, showing the following settings:

- Distribution:**  Cumulative
- Bins:** Width: 1
- Data:** Select Columns... Sheet 19 All selected columns
- Row Range:**  Full column
- Other options:**  Combine multiple column data,  Normalize histogram area

Buttons: OK, Cancel

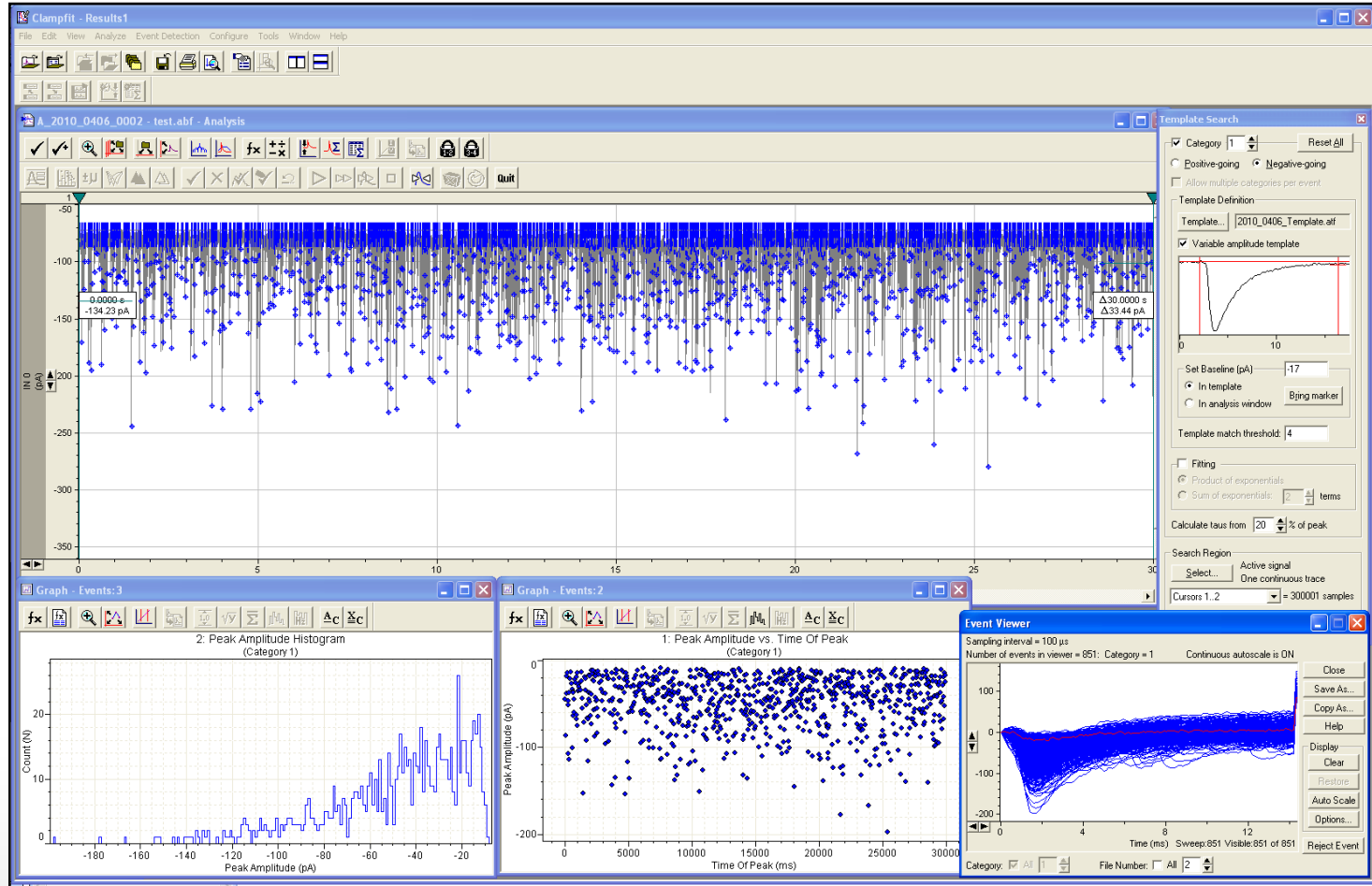


# Repeat the Template Search After Compound Addition



Starting Template Search with a new file erases all results on the Events sheet (but not Sheet 19 )

# Post-compound Results



The post-compound data file  
after completed analysis with  
"sanity check graphs".

# Repeat Post-processing Steps

Peak Amplit

	G	I	J	K
20.3	-84.7923	44.6134	2.1	8
92.4	-83.5671	-86.9652	1.7	79.7
55.3				6.60
83.4				4.50
67.6				5.40
82.9				2.08
03.3				7.08
26.5				
51.3				
76.3				
97.3				
500	-78.38	-13.6	3.60	
21.7	-79.5129	-13.775	2.20	

Rename Sheet dialog: Name of sheet: Post

Column Arithmetic dialog: Expression: cBH=cH\*1

Sheet tab: Pre Sheet 20

Copy the results to Sheet 20,  
rename the sheet and column H

Compute the  
absolute peak

Rename the other  
relevant columns

Inst Freq	IEI	BE	BF	BG	Abs Peak
N/A	Not found	N/A	12.0068		44.6134
13.947	71.7	N/A	25.2578		86.9652
15.8479	63.1	N/A	9.17799		
34.8432	28.7	N/A	7.53626		
11.7096	85.4	N/A	4.67117		
72.9927	13.7	N/A	29.0551		
50	20	N/A	9.65587		
43.2904	23.1	N/A	13.8694		

Histogram dialog: Distribution: Cumulative (selected), Bins: Width: 1, Bins/Decade: 10, Number of bins = (Not available)

Data: Select Columns... Post All selected columns

Create a post-compound  
cumulative histogram

# Normalizing the Histogram Values

The screenshot shows a software interface with a table of histogram data. The table has columns for Bin Center, Histogram values, and calculated columns E and F. A 'Column Arithmetic' dialog box is open, showing the expression 'cE=norm(cB)'.

	Bin Center	Histo(cBH)	Bin Center	Histo(cBH)	E	F
1	9.5	1	8.5	1		
2	10.5	1	9.5	7		
3	11.5	6	10.5	15		
4	12.5	16	11.5	30		
5	13.5	26	12.5	50		
6	14.5	38	13.5	65		
7	15.5	45	14.5	75		
8	16.5	51	15.5	80		
9	17.5	57	16.5	85		
10	18.5	62	17.5	88		
11	19.5	71	18.5	90		
12	20.5	75	19.5	92		
13	21.5	80	20.5	93		
14	22.5	85	21.5	94		

Normalize the Bin Count columns on the Histogram sheet

Rename the two new columns

The screenshot shows the same software interface as the previous one, but with a 'Column Rename' dialog box open. The dialog box is prompting the user to rename column F to 'Norm Post Peak'.

Histo(cBH)	Norm Pre P	F	G	H
1	0	0.00117509		
7	0	0.00822562		

$$cE = \text{norm}(cB)$$
$$cF = \text{norm}(cD)$$

# Creating a Combined, Normalized Cumulative Histogram

Results1 - Results

	Bin Center	Histo(cBH)	Bin Center
1	9.5	1	8.5
2	10.5	1	9.5
3	11.5	6	10.5
4	12.5	16	11.5
5	13.5	26	12.5

Make the first "Bin Center" column an X column

Results1 - Results

	Bin Center	Histo(cBH)	Bin Center	Histo(cBH)	Norm Pre P	Norm Post
1	9.5	1	8.5	1	0	0.00117509
2	10.5	1	9.5	7	0	0.00822662
3	11.5	6	10.5	15	0	0.03283815
4	12.5	16	11.5	30	0	0.12714158
5	13.5	26	12.5	50	0	0.47285842

Make the "Norm Pre Peak" column a Y column

Results1 - Results

	Bin Center	Histo(cBH)	Bin
1	9.5	1	
2	10.5	1	1
3	11.5	6	6

Define the two columns as an X-Y pair

# Creating a Combined, Normalized Cumulative Histogram

ts1 - Results

Bin Center	Histo(cBH)	Bin Center	Hist
X Column	1	8.5	
10.5			
11.5			
12.5			

Repeat for the second "Bin Center" column and "Norm Post Peak"

- Results

Center	Y Column(s)	Bin Center	Histo(cBH)	Norm Pre P	Norm Post
9.5	1	8.5	1	0	0.00117509
10.5	1	9.5	7		
11.5	6	10.5	15		
12.5	46	11.5	20		

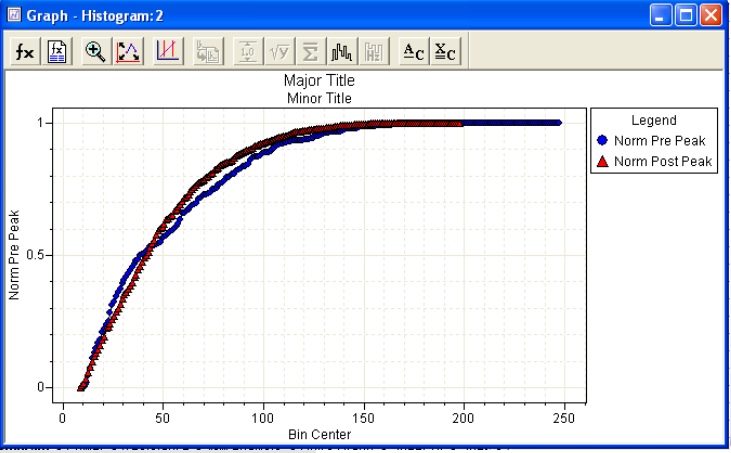
Results1 - Results

Bin Center	Hist
1	9.5
2	10.5
3	11.5

Results1 - Results

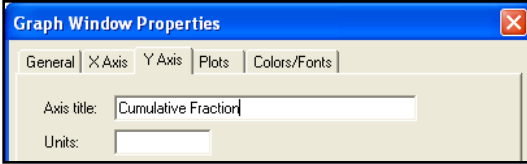
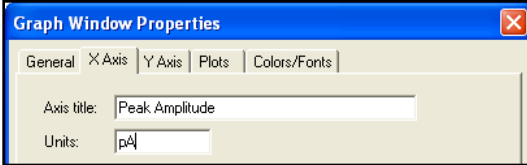
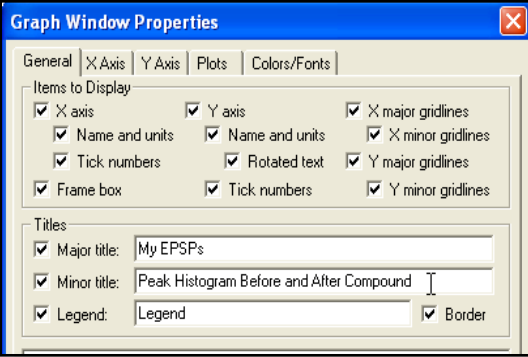
Bin Cent	Hist
1	11
2	11
3	11

Create a new graph and double-click on it

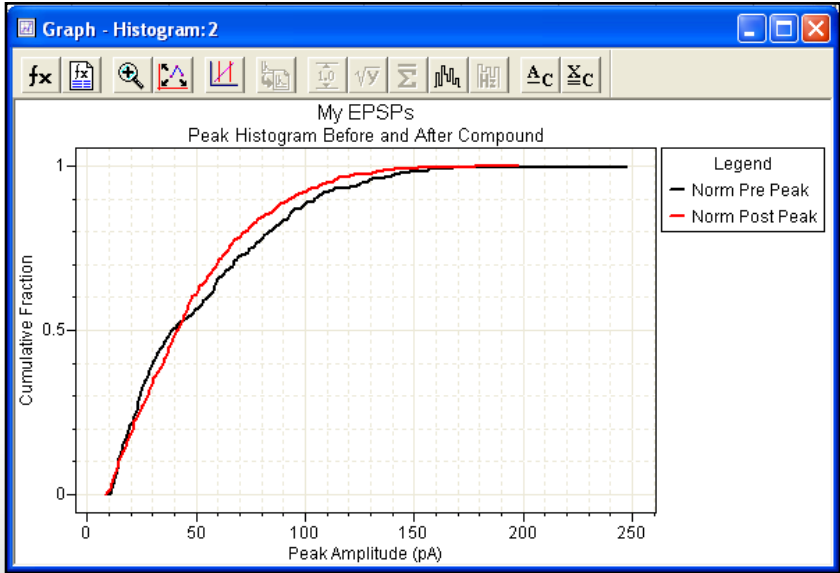
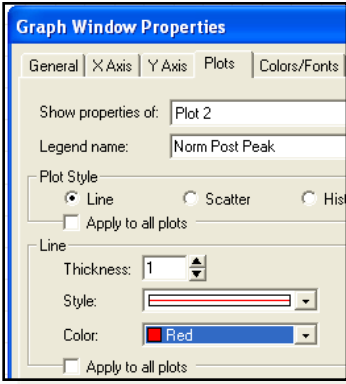
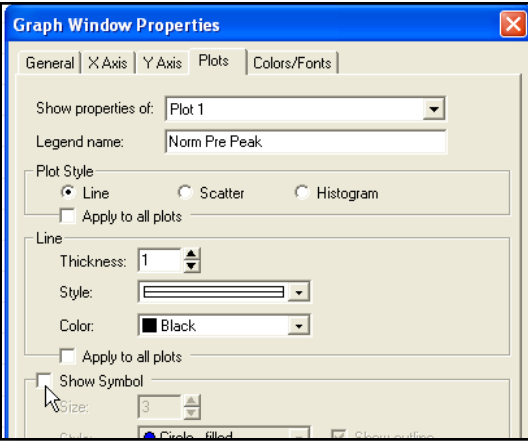




# Creating a Combined, Normalized Cumulative Histogram



Modify Graph titles, axis titles and plots for an appearance as shown



# Kolmogorov-Smirnov Test

Abs Peak	BI	BJ
26.3783		
38.5418		
38.226		
24.0615		
28.3004		
59.7718		
83.5718		
69.6571		
92.6582		
23.0717		
99.0512		
85.5042		

Copy both "Abs Peak"  
columns

to Sheet 18

	Pre Peak	Post Peak	C
1	26.3783	44.6134	
2	38.5418	86.9652	
3	38.226	37.8616	
4	24.0615	18.3423	
5	28.3004	14.4603	
6	59.7718	107.519	
7	83.5718	47.0145	
8	69.6571	51.0085	
9	92.6582	114.19	
10	23.0717	37.329	
11	99.0512	21.1148	
12	85.5042	13.6	

- File
- Edit
- View
- Analyze
- Format
- Event Detection
- Con...

- Create Graph...
- Assign Plots...
- Append to Graph
- Fit...
- Fitting Results...
- Extract Data Subset...
- Histogram...
- Sort...
- Transpose
- Average Columns...
- Column Arithmetic...
- Create Data...
- Analysis of Variance...
- Basic Statistics...
- Chi-Square and Mann-Whitney...
- F-Test and Student's t-Test...
- Kolmogorov-Smirnov Test...**
- Rank Correlations...
- Autocorrelation...
- Cross-correlation...
- Event Analysis

**Kolmogorov-Smirnov Test**

Preprocessing

- Ascending sort
- Cumulative binning
  - Variable width
  - Fixed width: 0.05

Data Limits

- Full
- Fixed From: 1 To: 5

Select Columns... Sheet 18  
All selected columns

Row Range

- Full column
- Row From: 1 To: 851

Destination Option

- Append results to sheet and graph
- Replace results in sheet and graph

OK Cancel Help

Apply the Kolmogorov-Smirnov Test

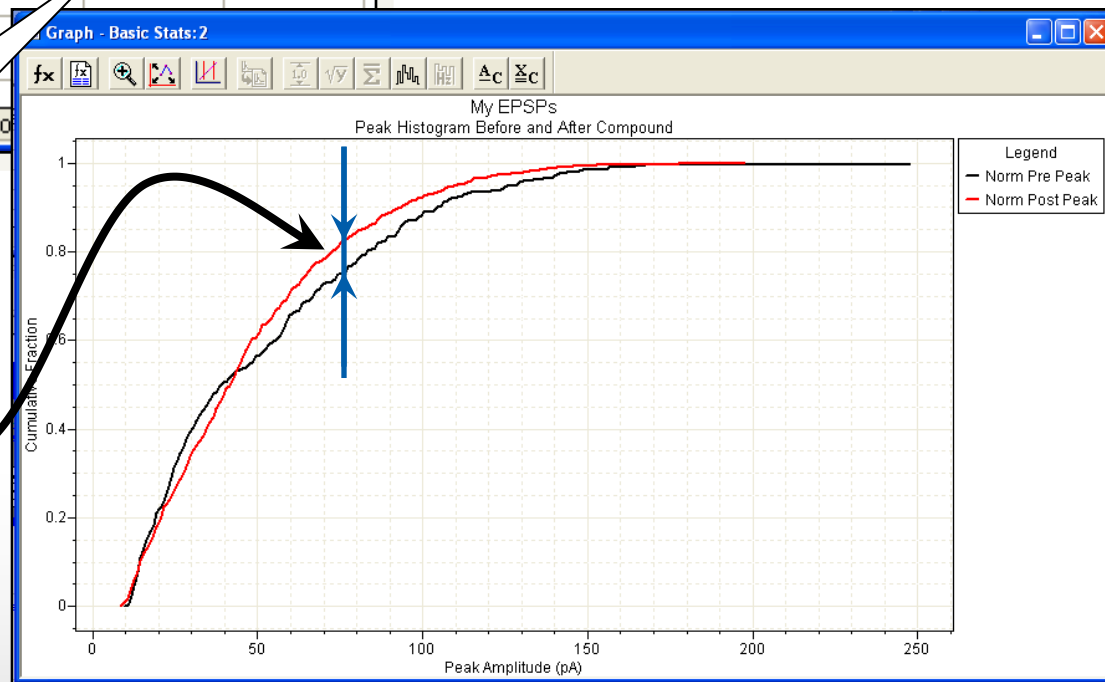
# Kolmogorov-Smirnov Test Results

Results1:1 - Results

	Source She	Source Colu	n1	n2	K-S Statistic	Probability
1	Sheet 18	Pre Peak vs.	334	851	0.07395	0.14510
2						
3						
4						
5						
6						

15% probability that the tested samples are from the same universe => no statistically significant difference

The Kolmogorov-Smirnov Statistic value corresponds to the greatest Y difference between the histograms:



# Creating Histograms for Inter-event Intervals

B	Inst	IEI	BE
3.7702	N/A	not found	N/A
30.842	7.86164	127.2	
108.27	18.7266	53.4	
16.223	54.6448	18.3	
2.0874	108.696	9.2	

Create Histogram  
(x2)

$cK = \text{norm}(cH)$   
 $cL = \text{norm}(cJ)$

Norm Post	Bin Center	Histo(cBD)	Bin Center	Histo(cBD)	K	L
0.00117509	0.5	1	0.5	1		
0.00822562	1.5	1	1.5	1		
0.0176263						
0.0352526						
0.0587544						
0.0787309						
0.101058						
0.119859						
0.130435						
0.143361						
0.162162						
0.180964						
0.19389						
0.224442						
0.231492						
0.240893						
0.258519						
0.27027						
0.285546						
0.300823						
0.316099						
0.336075						

Column Arithmetic

Expression:  $cK = \text{norm}()$

Select Columns

Specify a list:

Select from list:

- E: Norm Pre Peak
- F: Norm Post Peak
- G: Bin Center
- H: Histo(cBD)
- I: Bin Center
- J: Histo(cBD)

Histo(cBD)	Norm Pre IEI	L	M	N	O
1	0	0.00117509			
1	0	0.00117509			
1	0	0.00117509			
1	0				
2	0				
8	0				
10	0				
17	0.003003				
22	0.003003				
28	0.00600601				
36	0.012012				
46	0.018018				

Column Rename

Name of column L:

# Creating Histograms for Inter-event Intervals

Results1:1 - Results

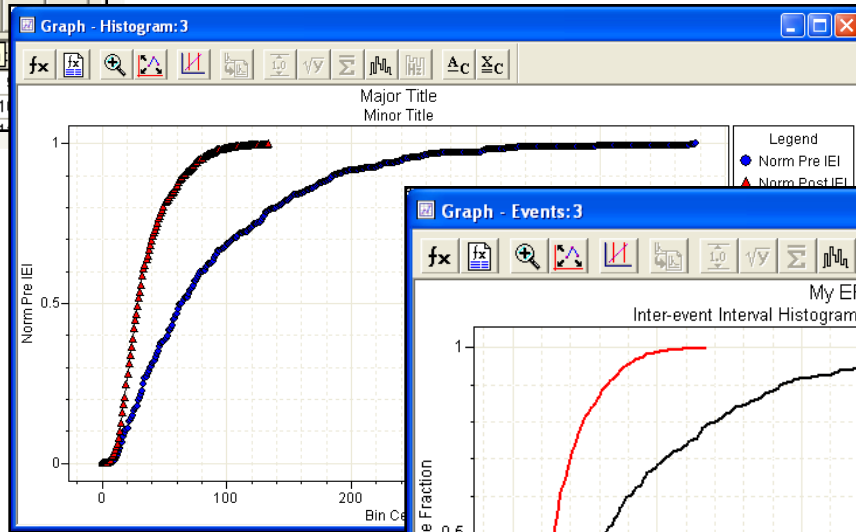
	Bin Center	Hi	X-Y Pair	Bin Ce
1	9.5			
2	10.5			
3	11.5			

Define X-Y pairs  
(x2)

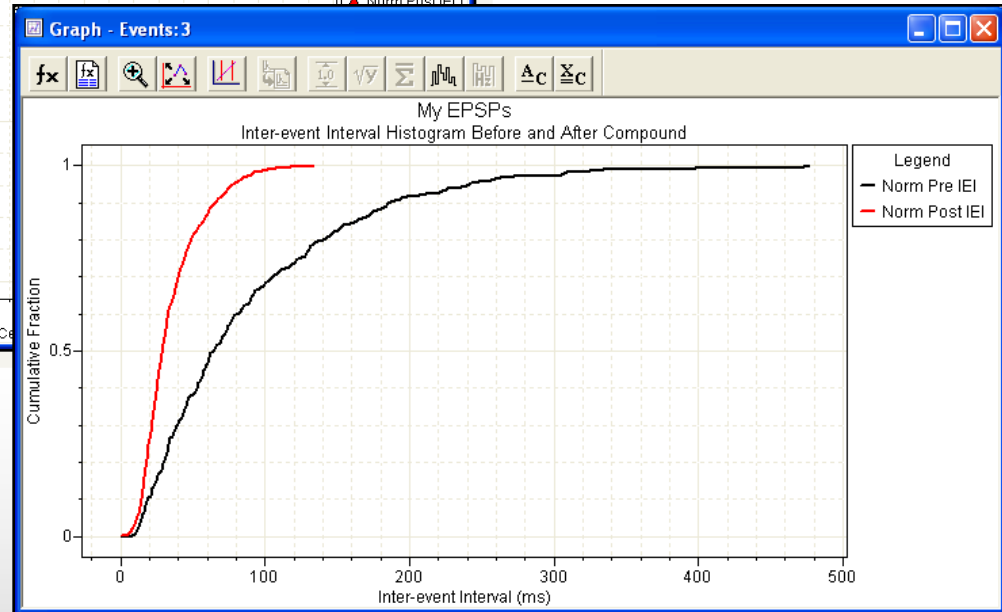
Results1:1 - Results

	Bin Center	Hi	X-Y Pair	Bin Ce
1	9.5			
2	10.5			
3	11.5			

Create a graph



Modify the graph



# Kolmogorov-Smirnov Test for Inter-event Intervals

#B	Inst Freq	IEI	BE	BF
3.7702	N/A	Not found	N/A	7.791
30.842	7.86164	127.2	N/A	16.47
108.27	18.7266	53.4	N/A	
16.223	54.6448			
2.0874	108.696			
71.279	3.8506			
38.674	3.81243			
75.404	17.8253			
07.662	10.917			
03.279	11.0619			
03.431	9.03343	110.7	N/A	
58.838	12.9534	77.2001	N/A	

Copy Pre and Post  
IEI columns to  
Sheet 18

Results1:1 - Results

	Pre Peak	Post Peak	Pre IEI	Post IEI	E
1	26.3783	44.6134	Not found	Not found	
2	38.5418	86.9652	127.2	71.7	
3	38.226	37.8616			
4	24.0615	18.3423			
5	28.3004	14.4603			
6	59.7718	107.519			
7	83.5718	47.0145			
8	69.6571	51.0085			
9	92.6582	114.19			
10	23.0717	37.329			
11	99.0512	21.1148			
12	85.5042	13.6			

Column Rename

Name of column D:  
Post IEI

OK Cancel Help

Kolmogorov-Smirnov Test

Preprocessing

- Ascending sort
- Cumulative binning
  - Variable width
  - Fixed width: 0.05

Data Limits

- Full
- Fixed From: 1 To: 5

Select Columns... Sheet 18  
All selected columns

Row Range

- Full column
- Row From: 1 To: 851

Destination Option

- Append results to sheet and graph
- Replace results in sheet and graph

OK Cancel Help

Be sure to Append,  
not Replace the  
results

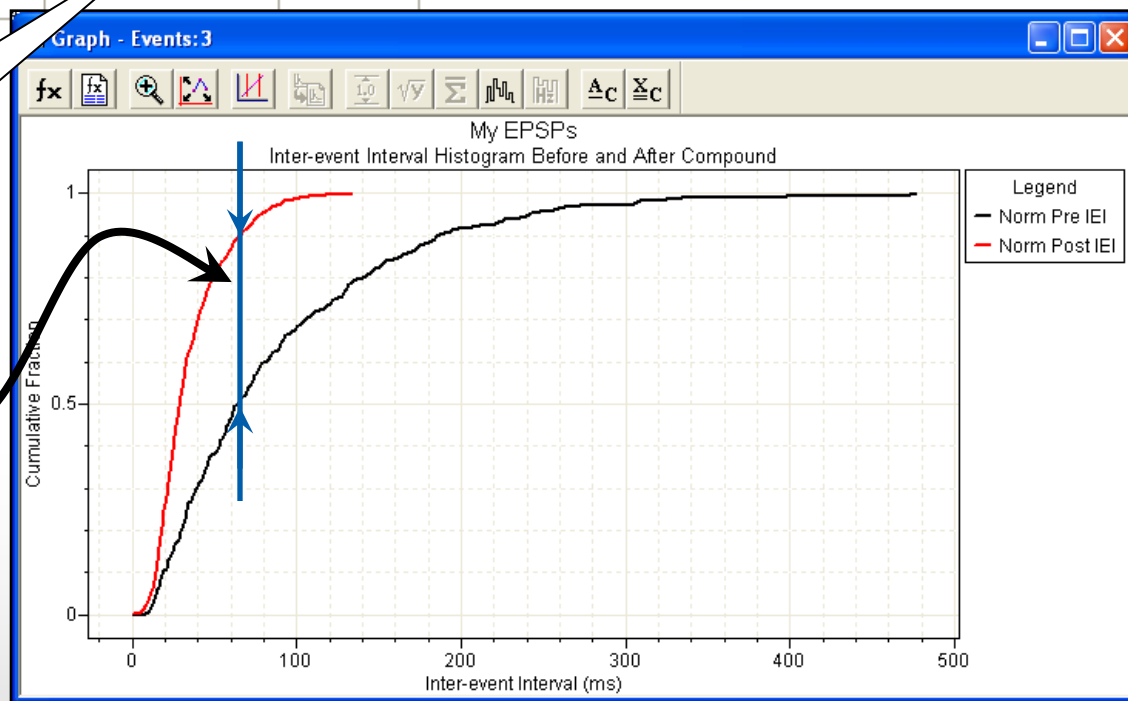
# Kolmogorov-Smirnov Test Results

Results1:1 - Results

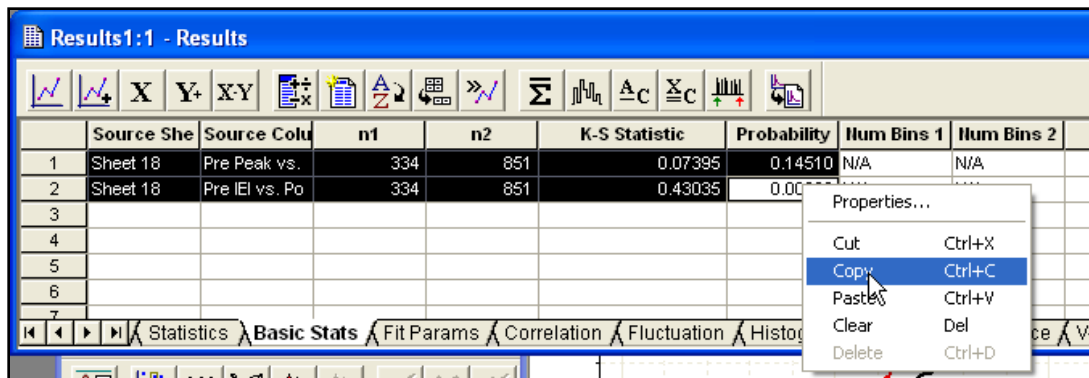
	Source She	Source Colu	n1	n2	K-S Statistic	Probability
1	Sheet 18	Pre Peak vs.	334	851	0.07395	0.14510
2	Sheet 18	Pre IEI vs. Po	334	851	0.43035	0.00000
3						
4						
5						

$p < 10^{-6} \Rightarrow$  highly statistically significant difference between the IEIs recorded in the two conditions

The Kolmogorov-Smirnov Statistic value again corresponds to the greatest Y difference between the histograms:

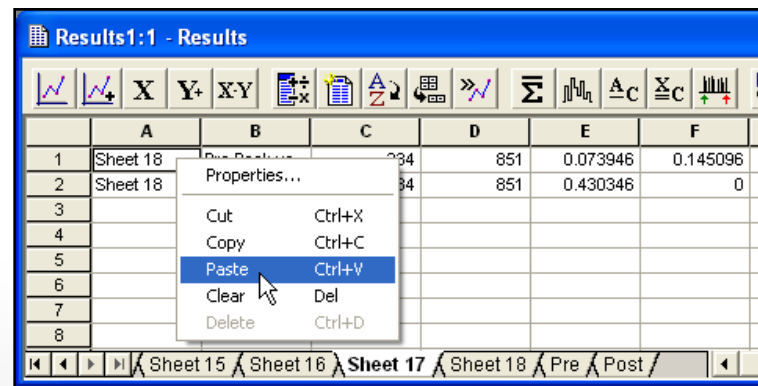


# Retain the Kolmogorov-Smirnov Results



	Source She	Source Colu	n1	n2	K-S Statistic	Probability	Num Bins 1	Num Bins 2
1	Sheet 18	Pre Peak vs.	334	851	0.07395	0.14510	N/A	N/A
2	Sheet 18	Pre IEI vs. Po	334	851	0.43035	0.00		
3								
4								
5								
6								
7								

Copy the results of  
the two K-S tests  
to Sheet 17



	A	B	C	D	E	F
1	Sheet 18	Pre Peak vs.	334	851	0.073946	0.145096
2	Sheet 18	Pre IEI vs. Po	334	851	0.430346	0
3						
4						
5						
6						
7						
8						



# Basic Statistics for Peaks and Inter-event Intervals

The screenshot shows the 'Clampfit - Results1' window. The 'Analyze' menu is open, with 'Basic Statistics...' selected. The 'Basic Statistics' dialog box is also open, showing the following options:

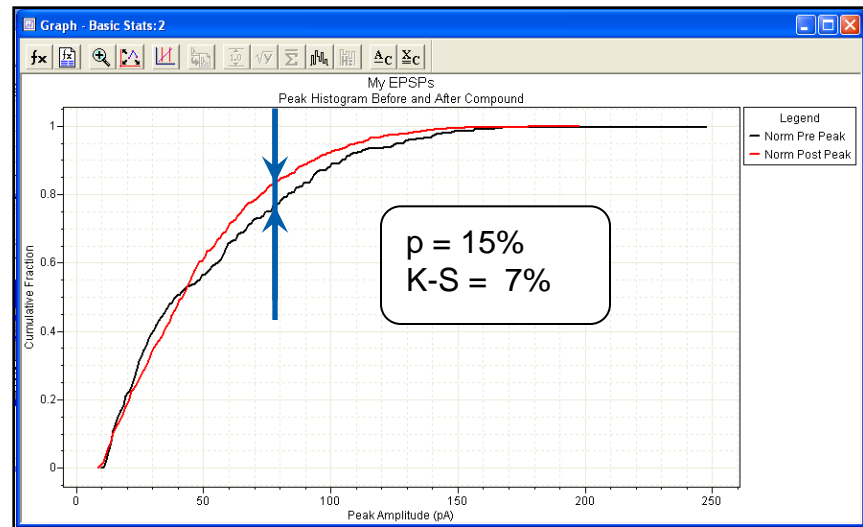
- Measurements:** Number per category, Minimum, Maximum, Variance, Mean, Median, Standard deviation, Standard error, Coefficient of variation, Sum, Sum of squares.
- Destination Option:**  Append results to sheet,  Replace results in sheet.
- Select Columns:** Sheet 18, All selected columns.
- Row Range:**  Full column,  Row (From: 1, To: 1),  Perform Breakdown Analysis.
- Category column:** A: Pre Peak,  Bin the categories, Specify Bins...

Results1:1 - Results

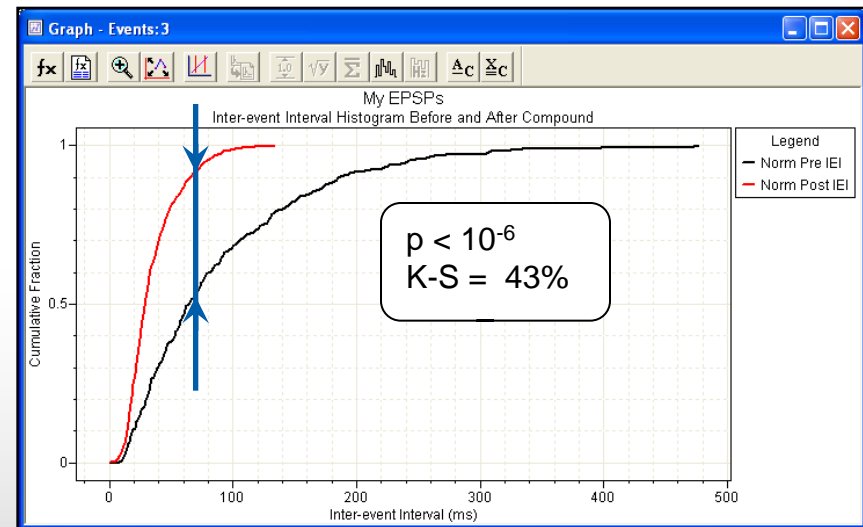
	Source	#/Cat	Min	Max	Mean	Median	Std. Dev.	Std Err.	CoVar.	Sum	Sum Sq.	Var
1	Pre Peak:Sheet 18	334	9.61644	247.951	52.2991	39.440	37.6507	2.06015	0.719911	17467.9	1.38561e+00	1417.57
2	Post Peak:Sheet 18	851	8.74508	197.461	48.8093	42.044	31.1591	1.06812	0.638385	41536.8	2.85264e+00	970.892
3	Pre IEI:Sheet 18	334	0	476.301	89.603	64.300	77.6239	4.24739	0.866309	29927.4	4.68807e+00	6025.47
4	Post IEI:Sheet 18	851	0	133.399	35.2031	29.200	21.3367	0.731413	0.606104	29957.8	1.44157e+00	455.255
5												
6												

# Summary

- Are there differences in the amplitude distribution?
- No.
  - Post-synaptic mechanisms are unaffected.



- Are there differences in the frequency?
- Yes.
  - Pre-synaptic mechanisms are upregulated.



# Clampfit Features Discussed

- Event Detection > Create Template
- Event Detection > Template Search
- Move Cursors efficiently
- View > Zoom > Between Cursors
- Analyze > Arithmetic
- Format > Column > Rename
- Format > Rename Sheet
- Analyze > Histogram
- Efficiently creating a graph using X-Y pairs
- Editing a graph
- Analyze > Kolmogorov-Smirnov Test
- Analyze > Basic Statistics

**End**  
Thank You



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