Molecular Devices

Together through life sciences.

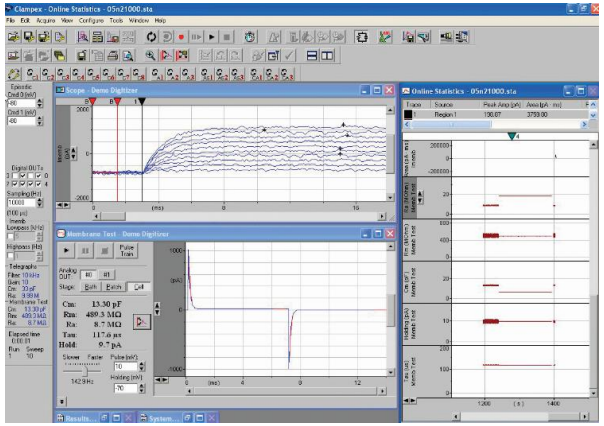
pCLAMP workshop

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



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Flow of recording signal

Axopatch 200B

Amplifiers

Axoclamp 900A

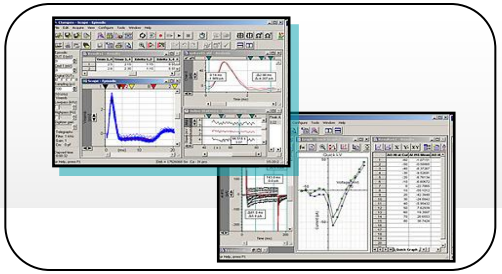
MultiClamp 700B

Biological signals:
current and voltage

Digital
Conversion

Digidata 1440A

Digitizer



pCLAMP 10
Software

Software

Electrophysiology rig

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Measurement Techniques

- Extracellular voltage recording
  - Single unit and field potential recording in brain or brain slices, electrocardiagrams, 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

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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

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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…

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A Walkthrough of Protocol Editor in Clampex

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Golden Tips that help

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

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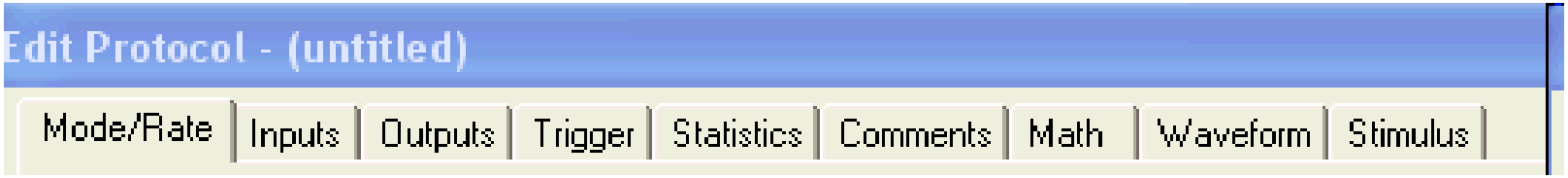
Agenda



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

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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

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Telegraphs

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

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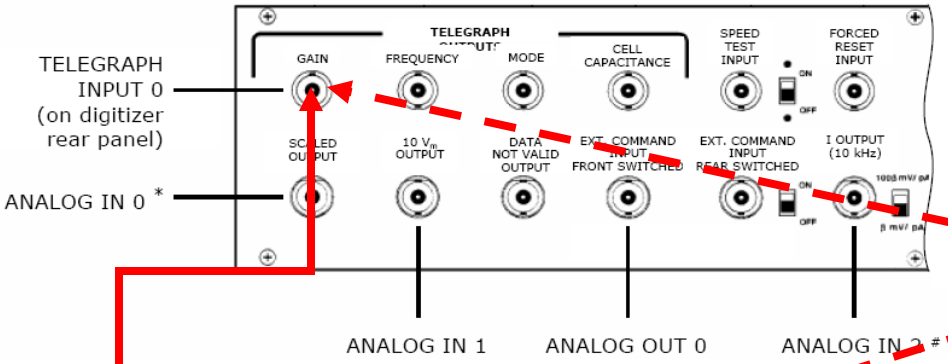
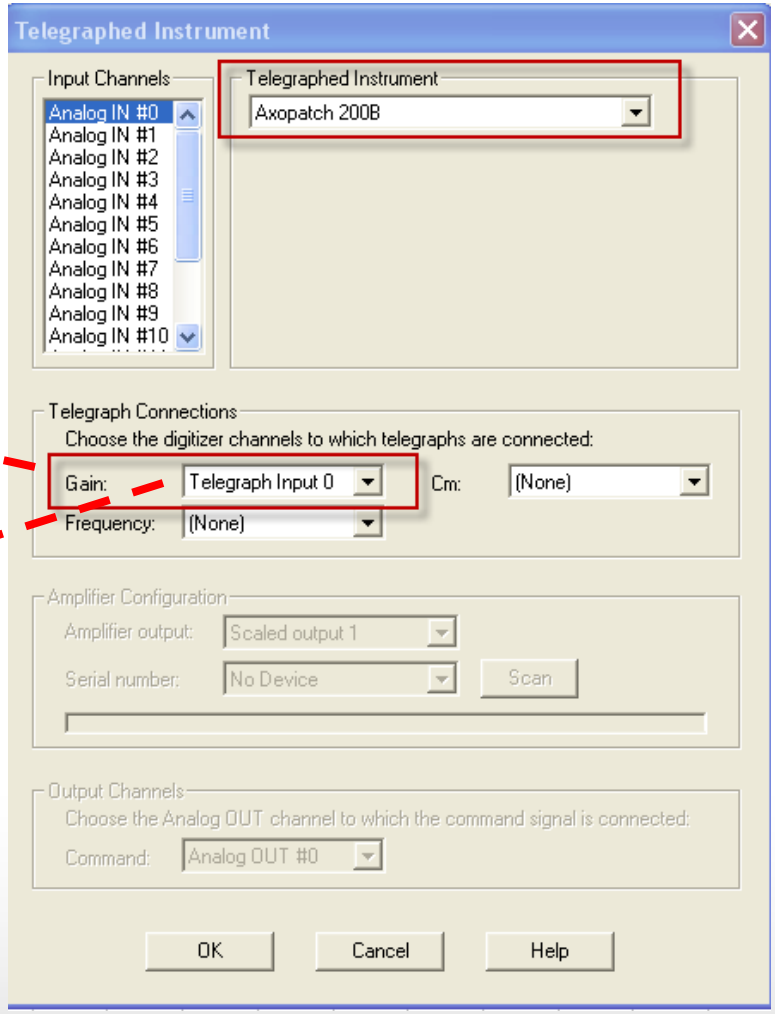
Telegraph setting in AxoPatch™ 200B

Axopatch Connections 3





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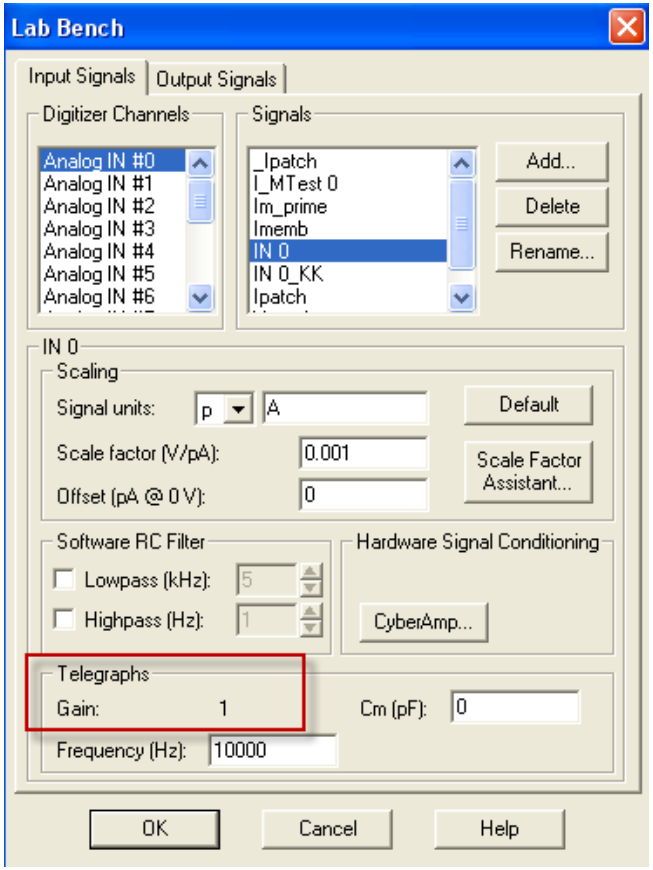
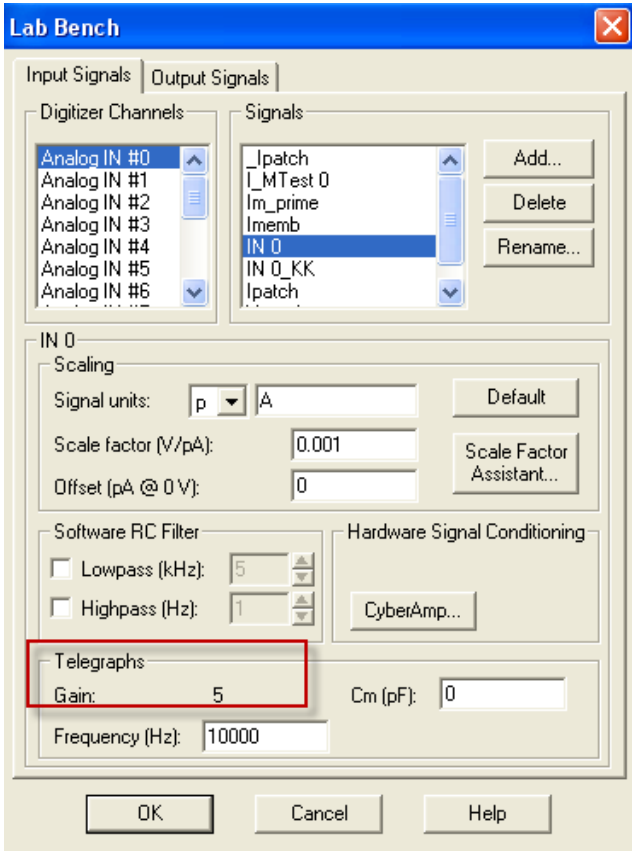
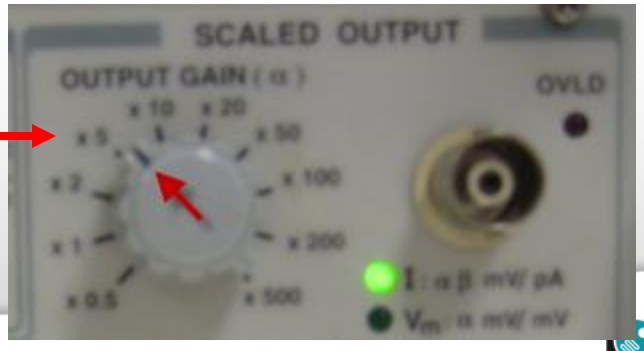
Molecular Devices

Telegraph setting in AxoPatch™ 200B







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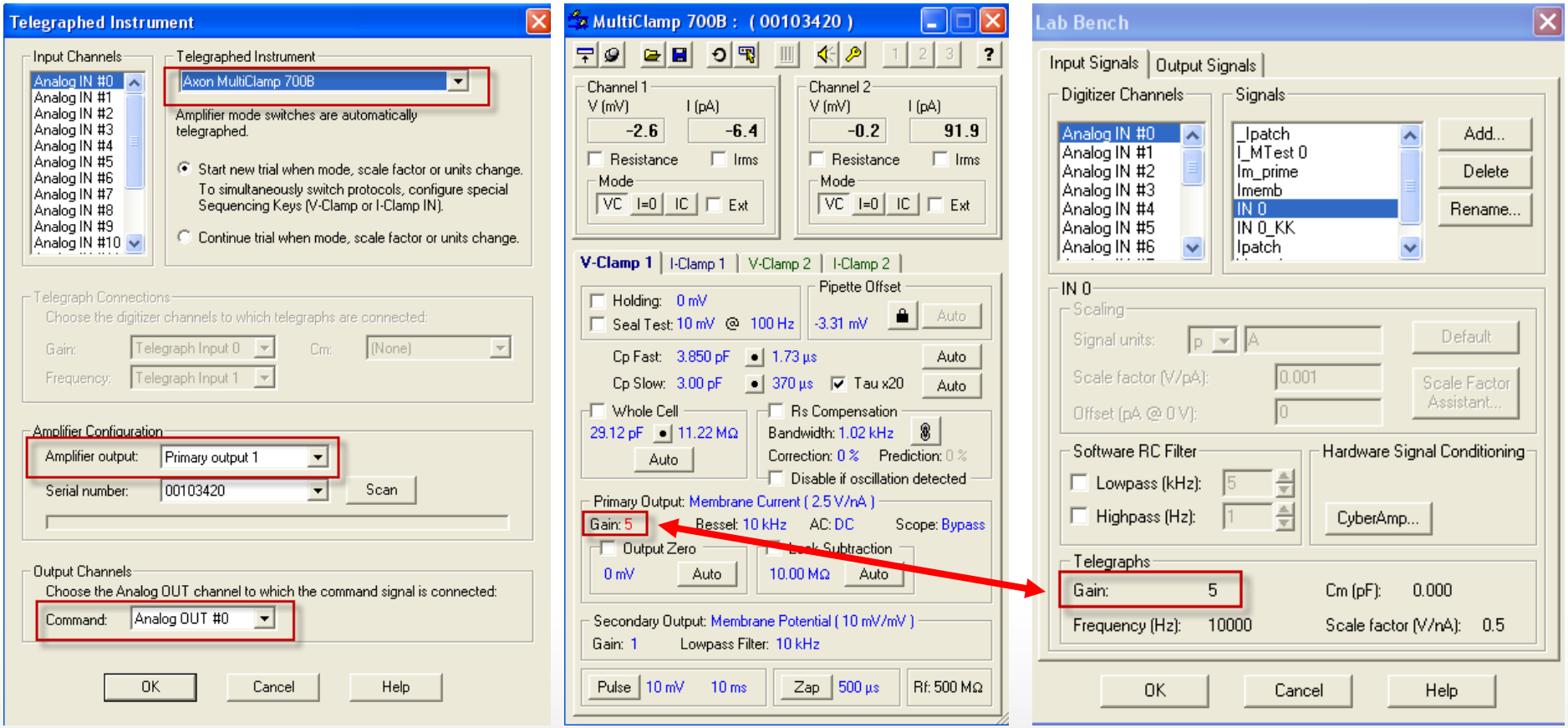
Telegraph setting in MultiClamp™ 700B



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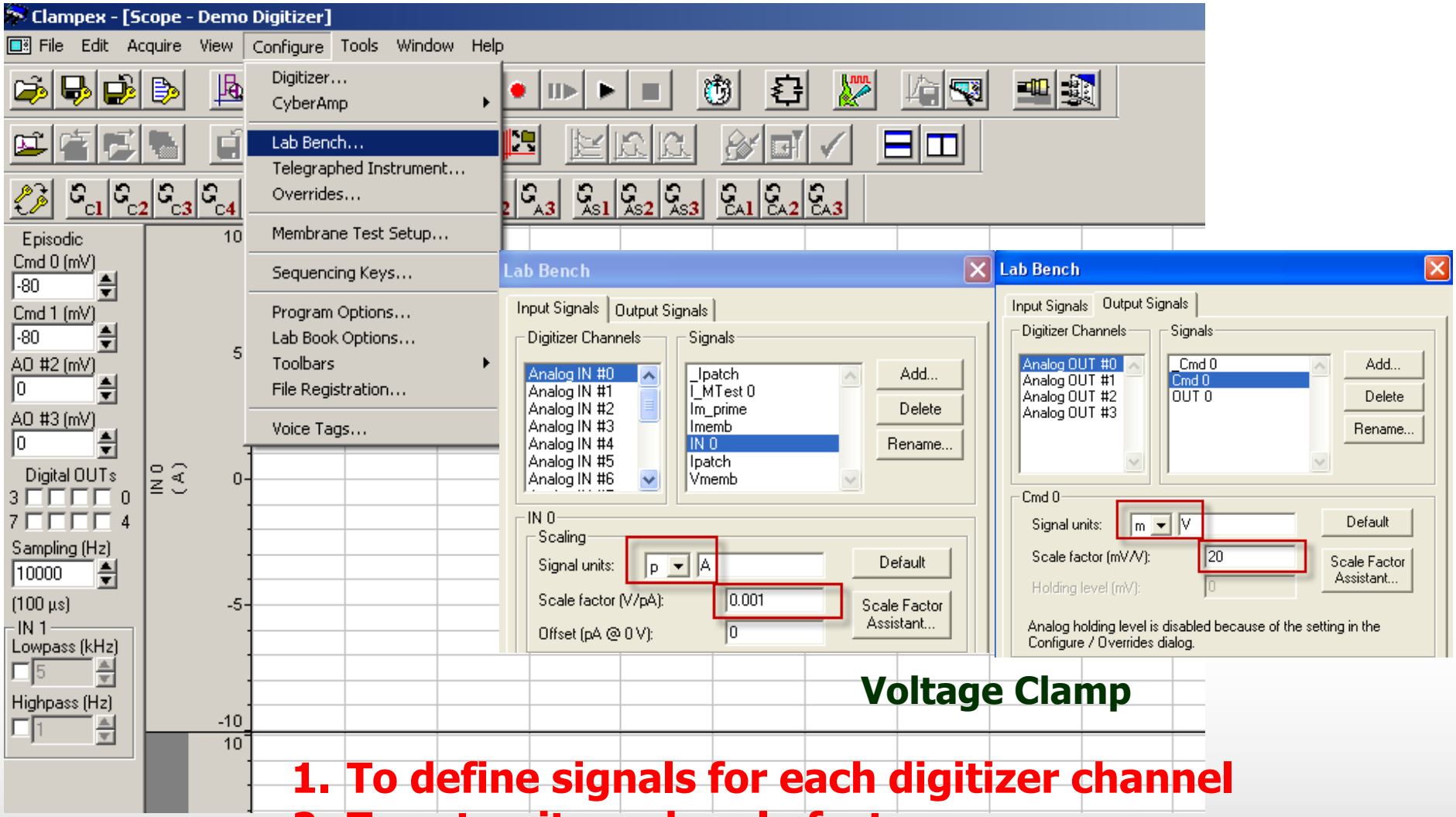
Lab Bench setting in AxoPatch™ 200B



Voltage Clamp

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

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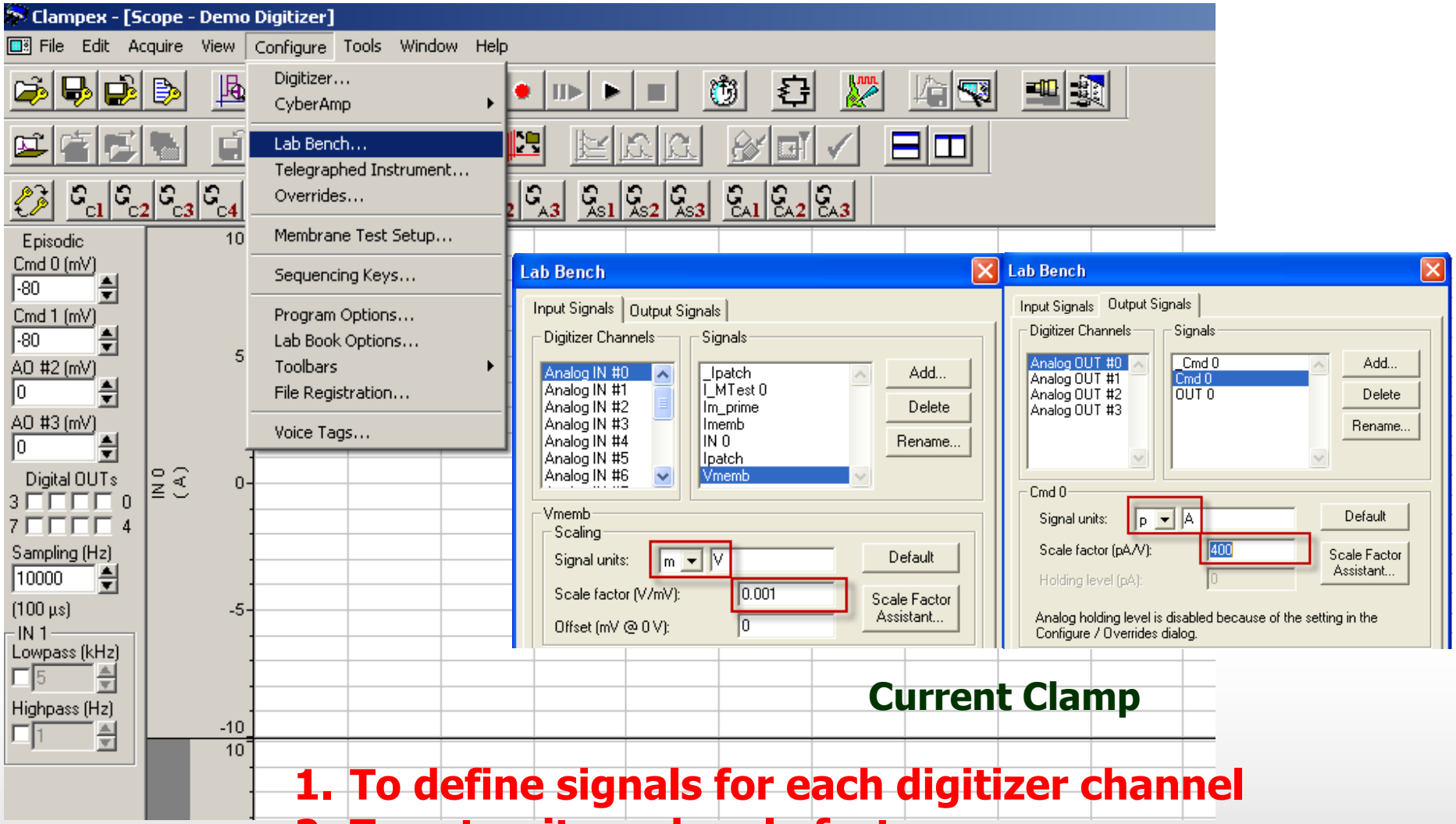
Lab Bench setting in AxoPatch™ 200B



Current Clamp

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

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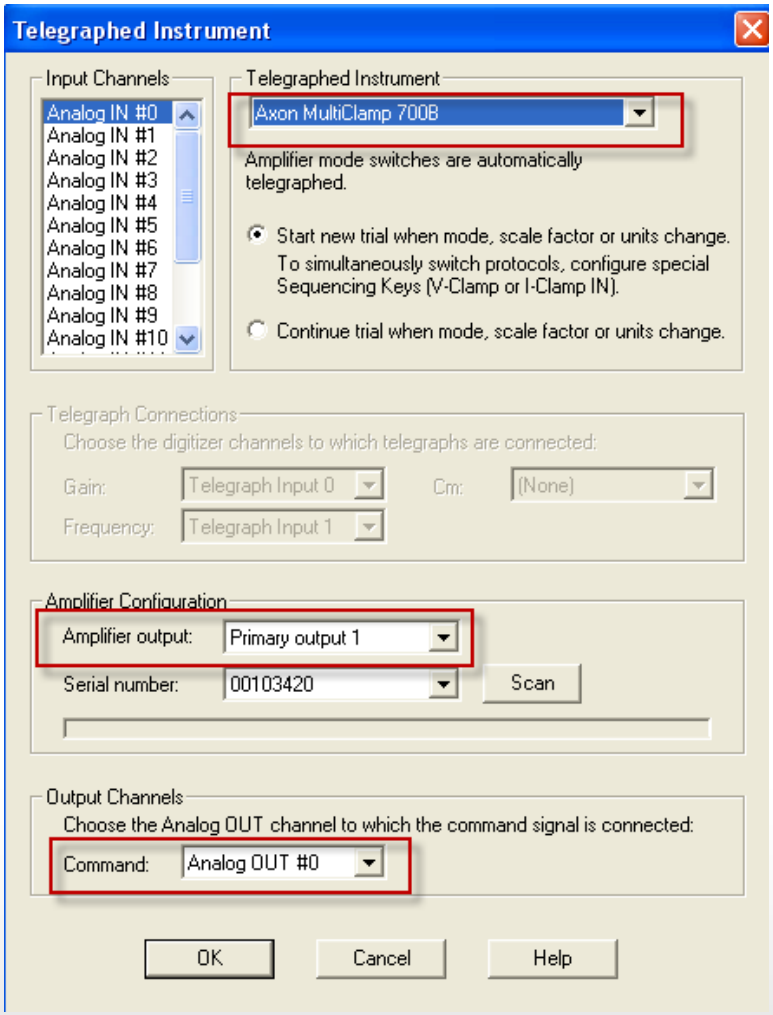
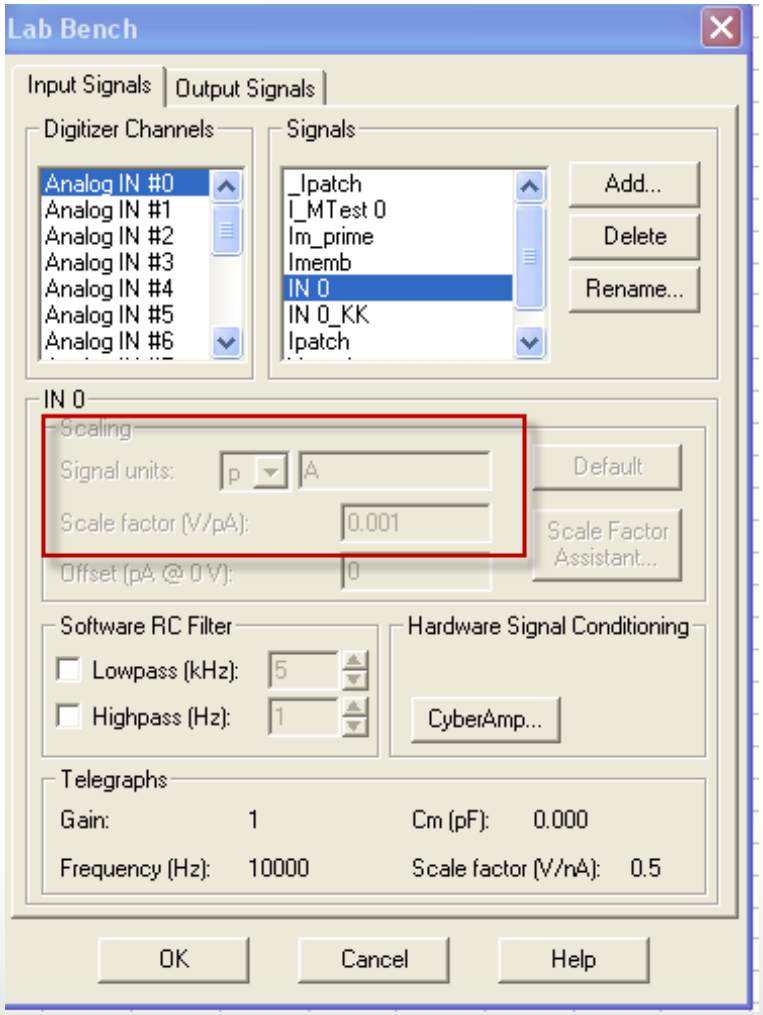
Lab Bench setting is telegraphed in MultiClamp™ 700B





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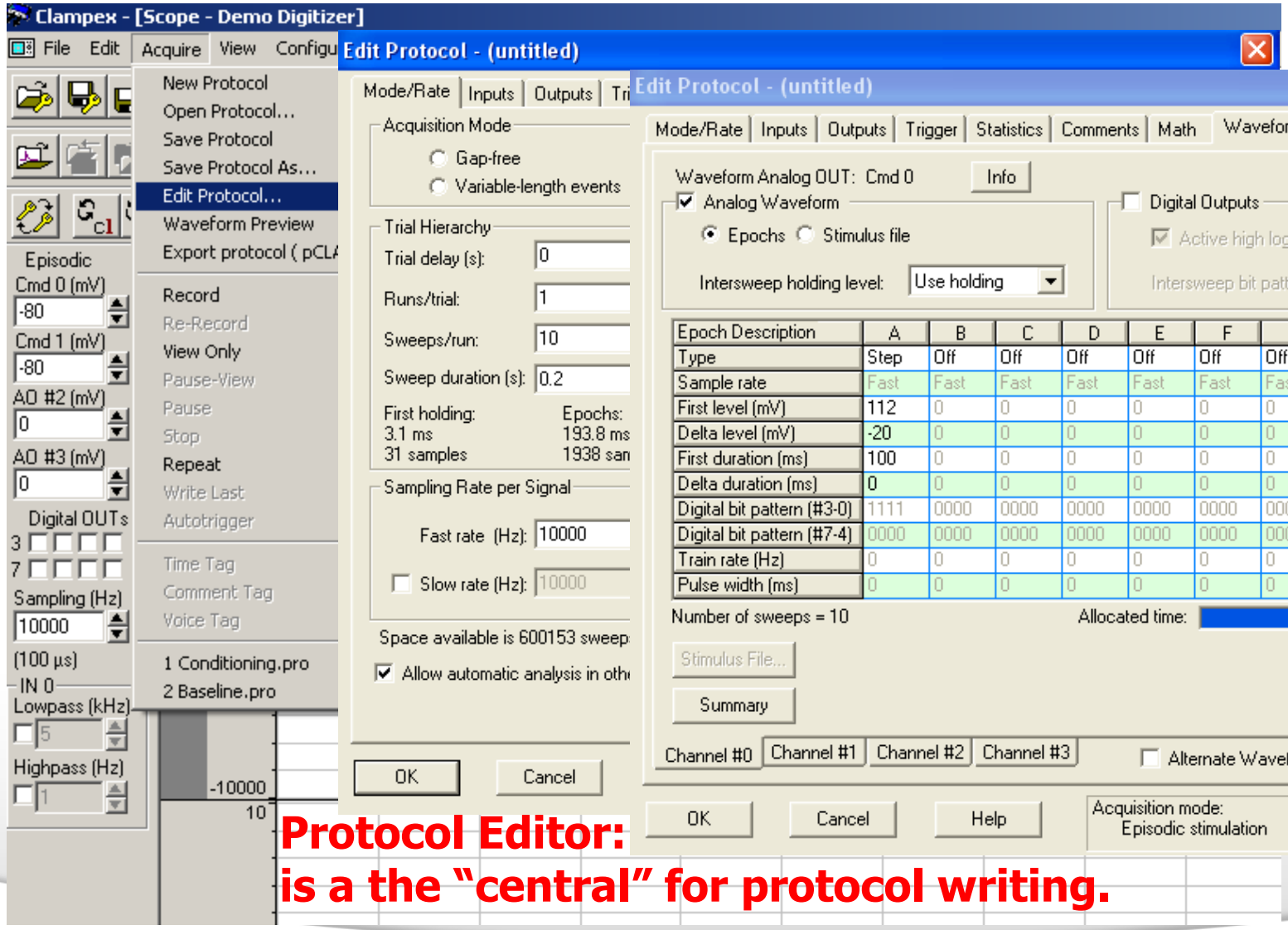
Protocol Editor



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

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Acquisition Mode?

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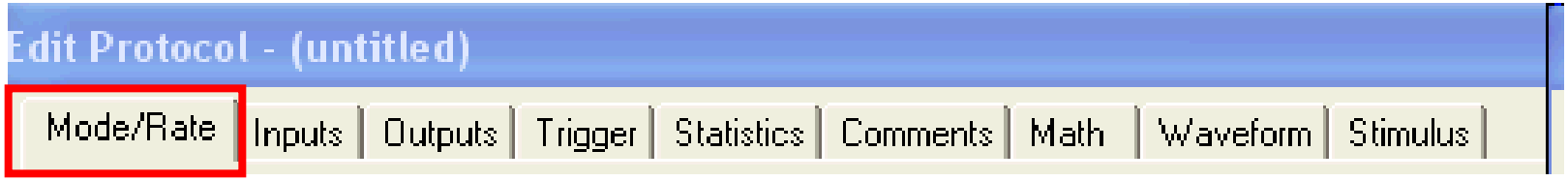
Mode/Rate



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

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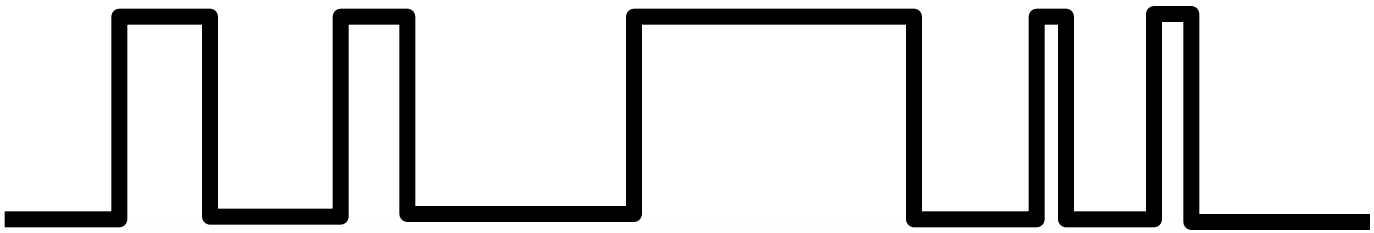
Gap-free Mode

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



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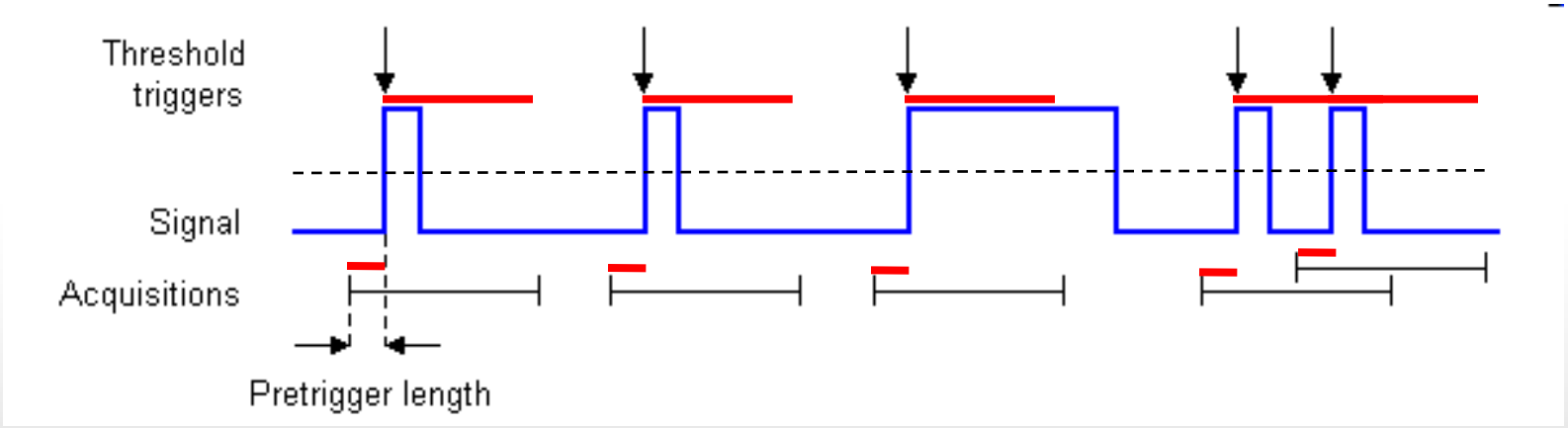
Fixed-length Events Mode

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



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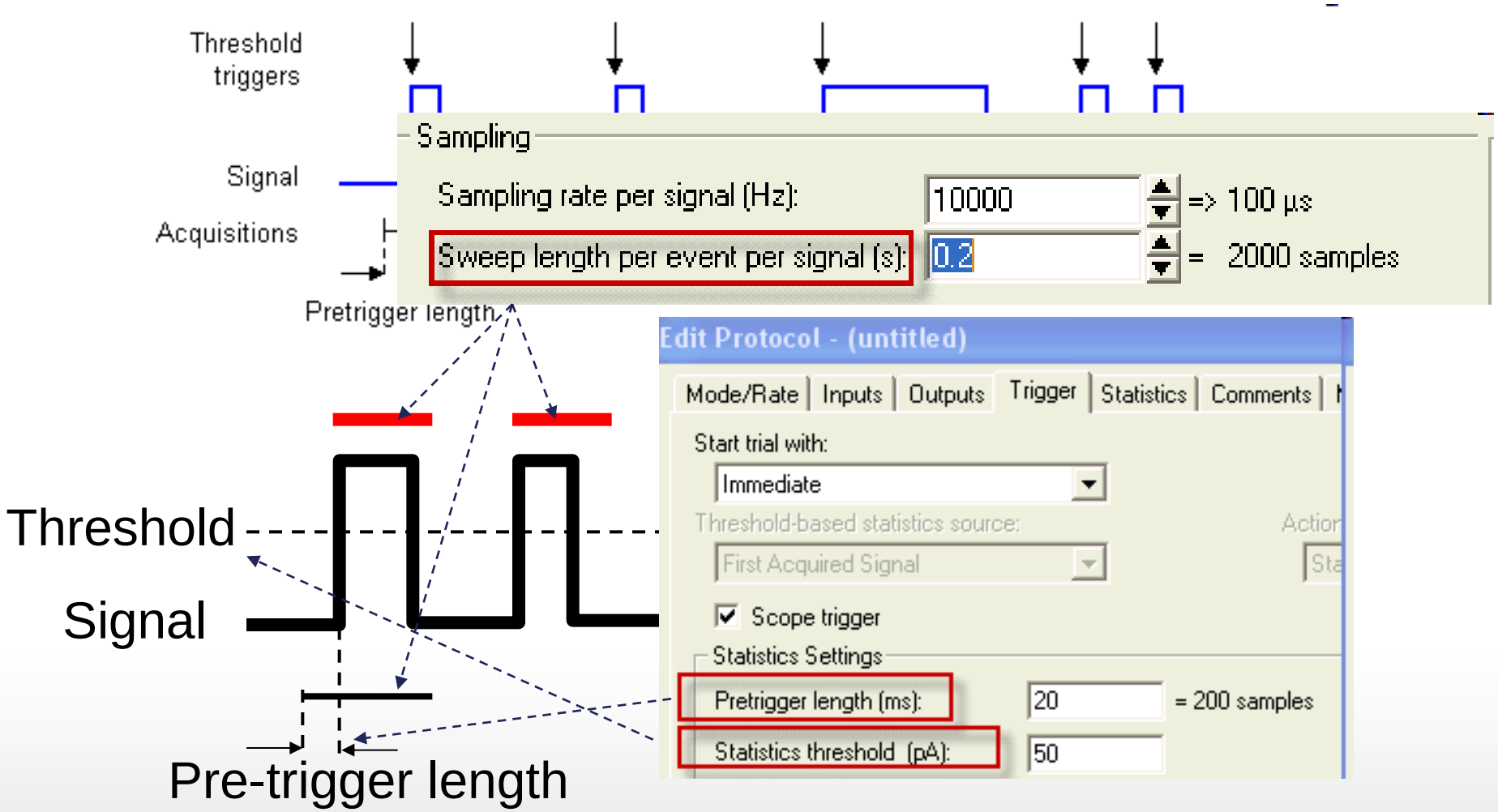
Fixed-length Event Mode



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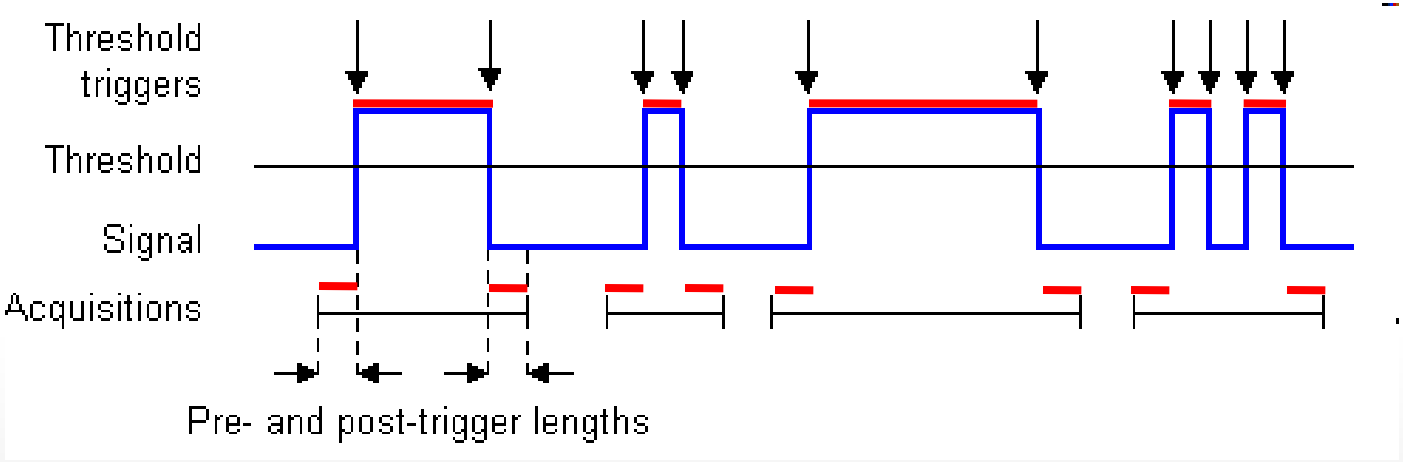
Variable-Length Events Mode

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



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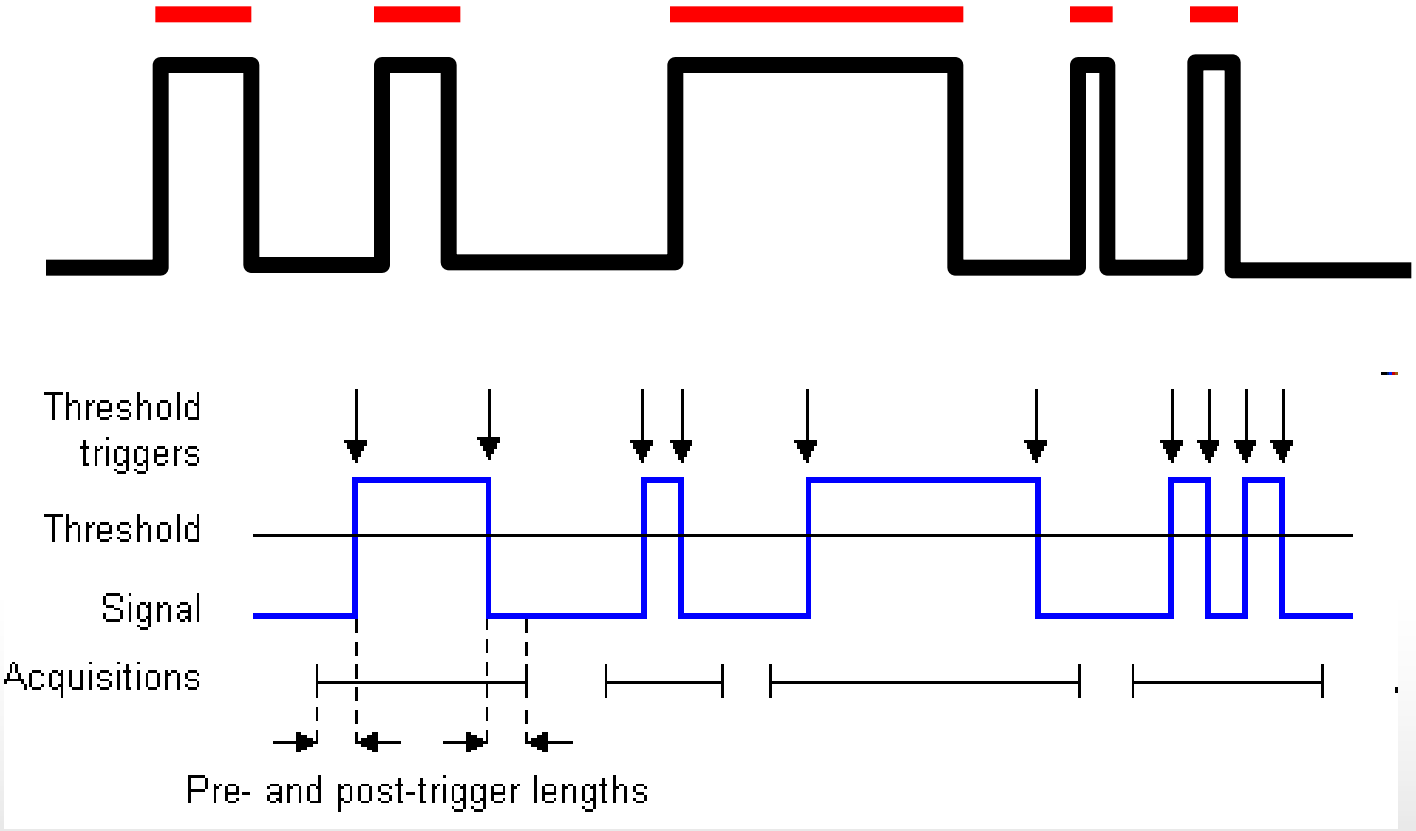
Variable-Length Events Mode



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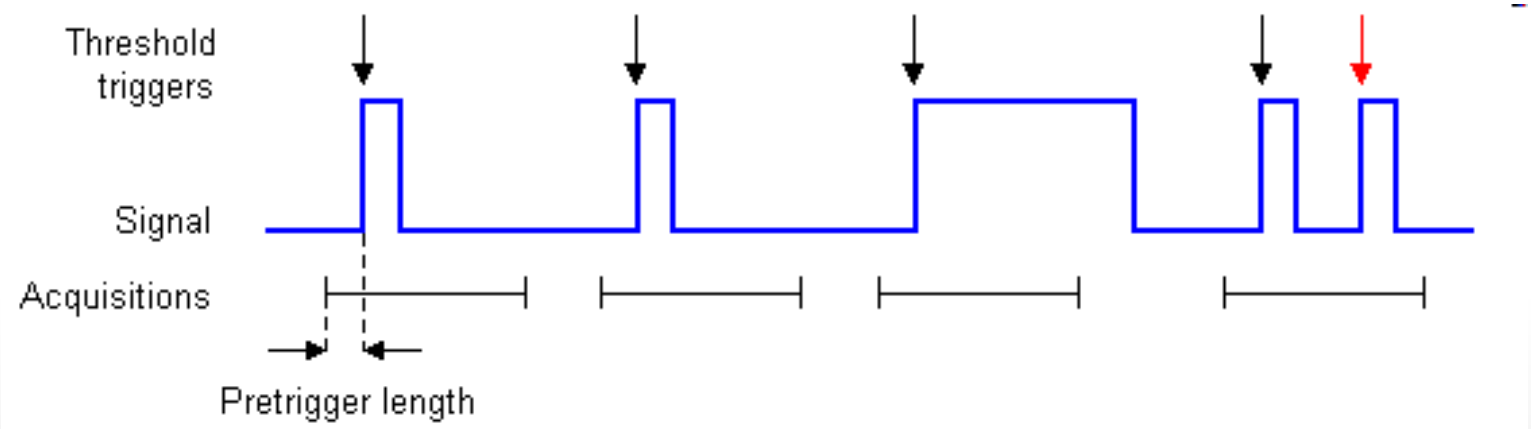
High-Speed Oscilloscope Mode

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



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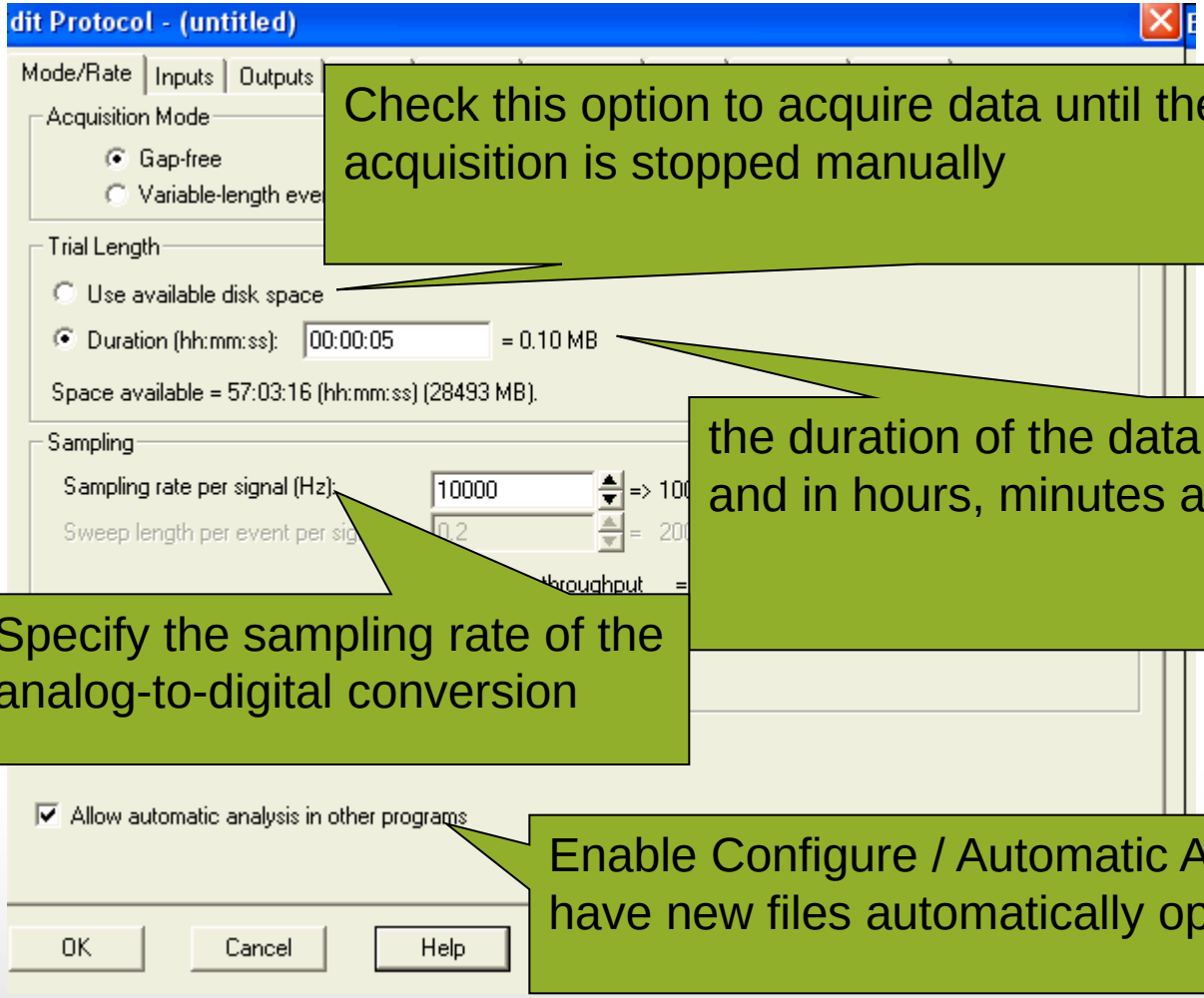
Passive acquisition modes



Check this option to acquire data until the disk is full, or until acquisition is stopped manually

the duration of the data acquisition. in megabytes, and in hours, minutes and seconds

Specify the sampling rate of the analog-to-digital conversion

Enable Configure / Automatic Analysis in Clampfit to have new files automatically open in Clampfit.

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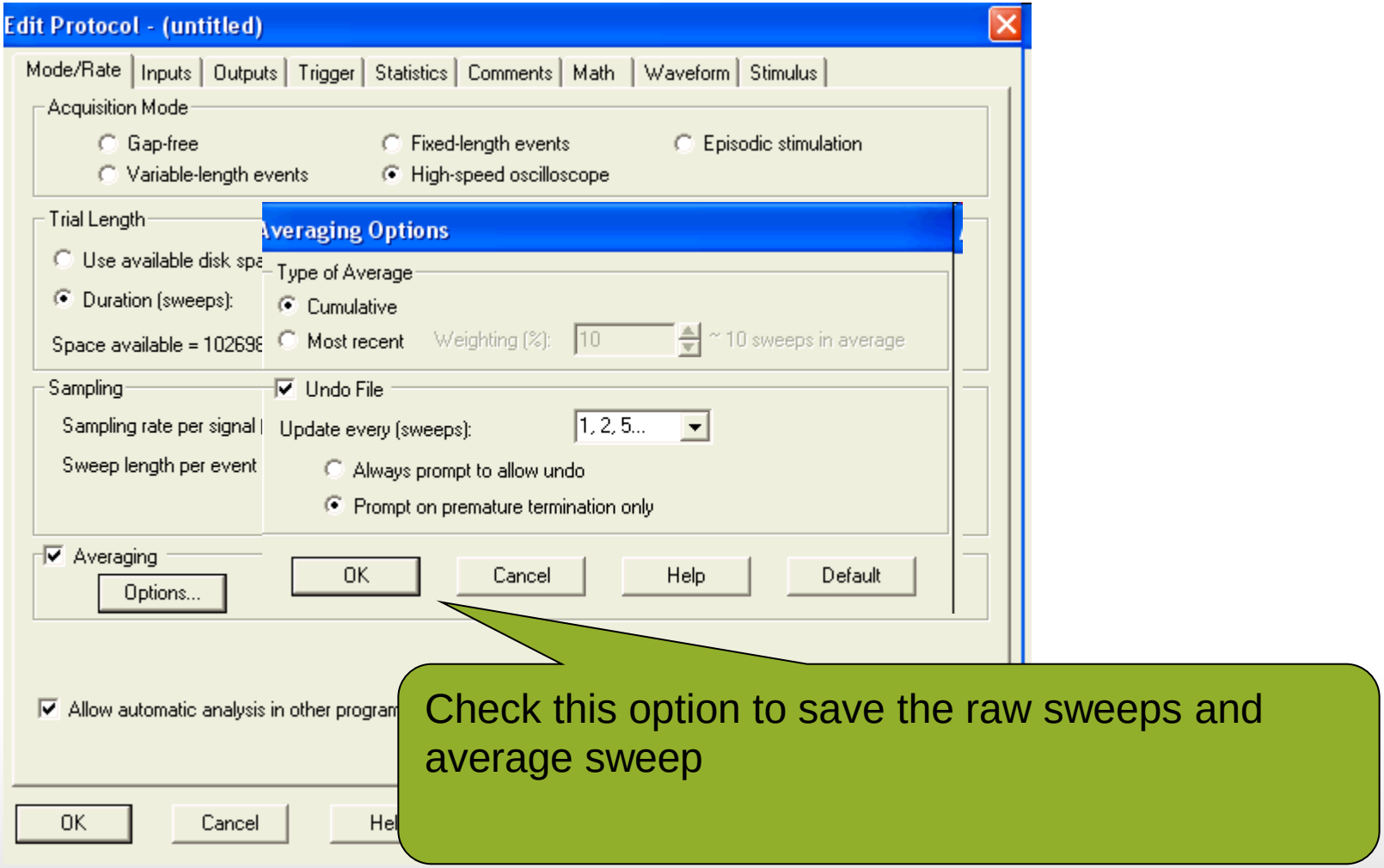
High-speed Oscilloscope Mode



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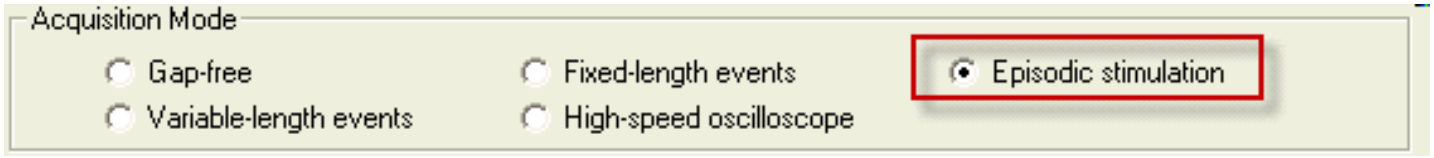
Episodic Stimulation Mode



- 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.

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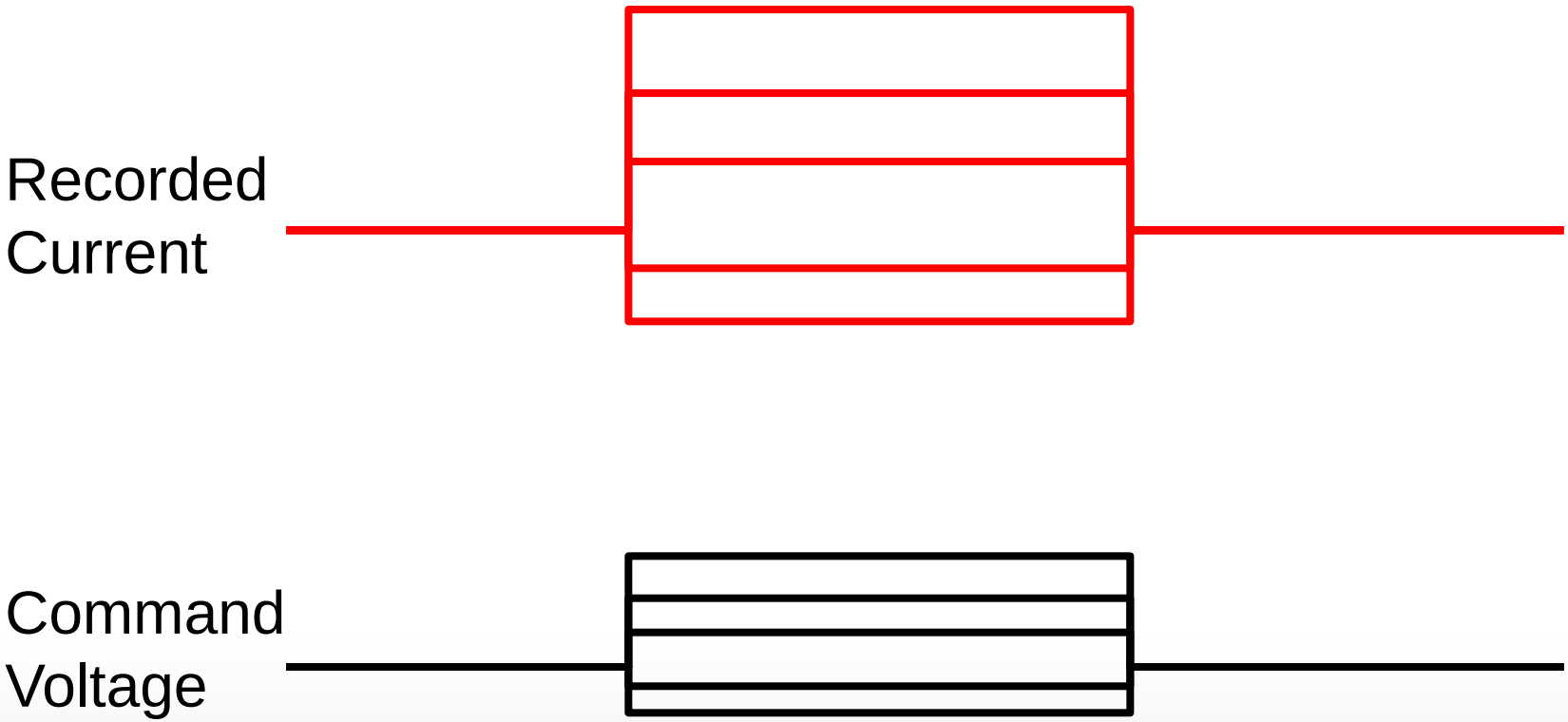
Episodic Stimulation Mode



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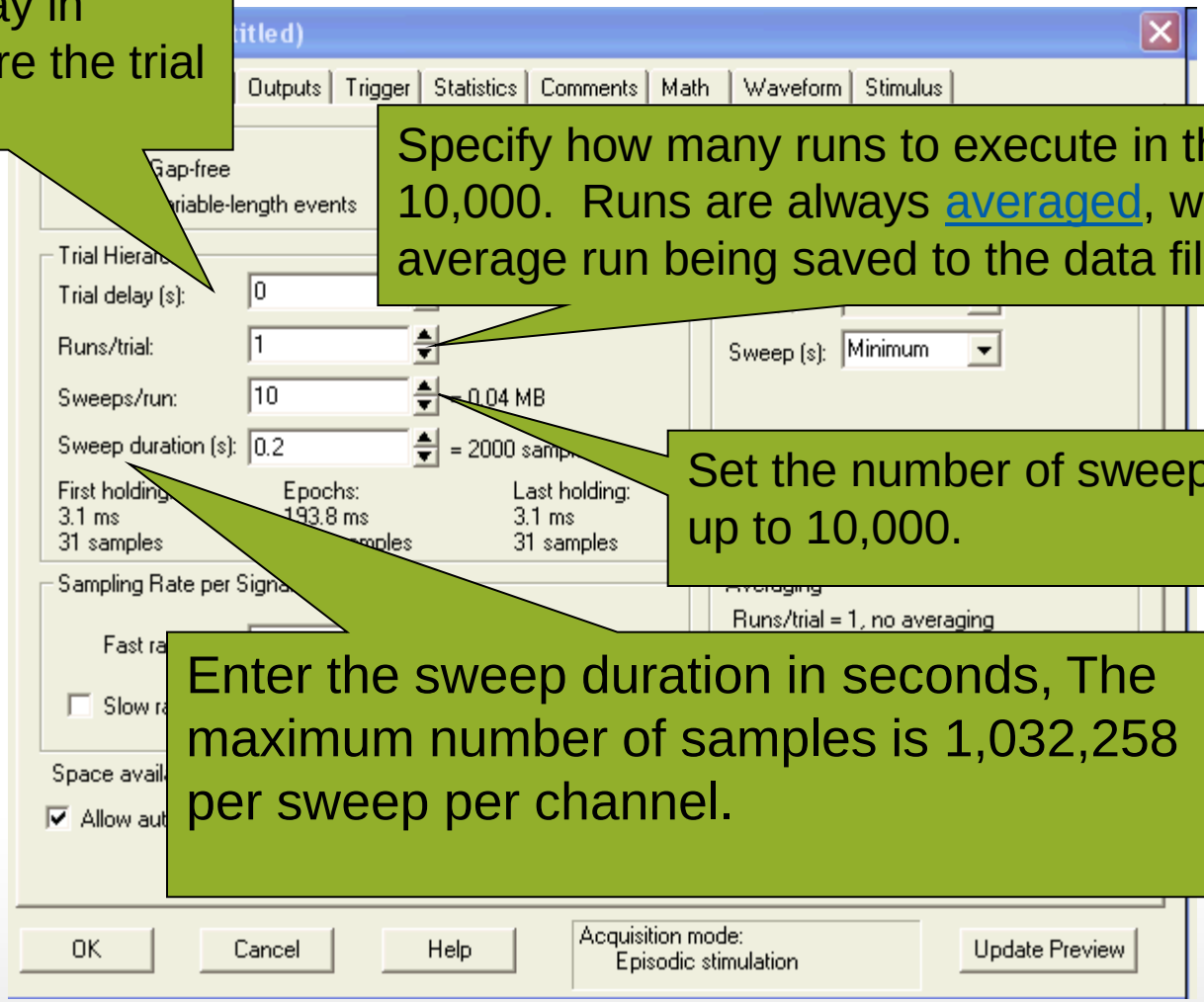
Episodic Stimulation Mode

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.

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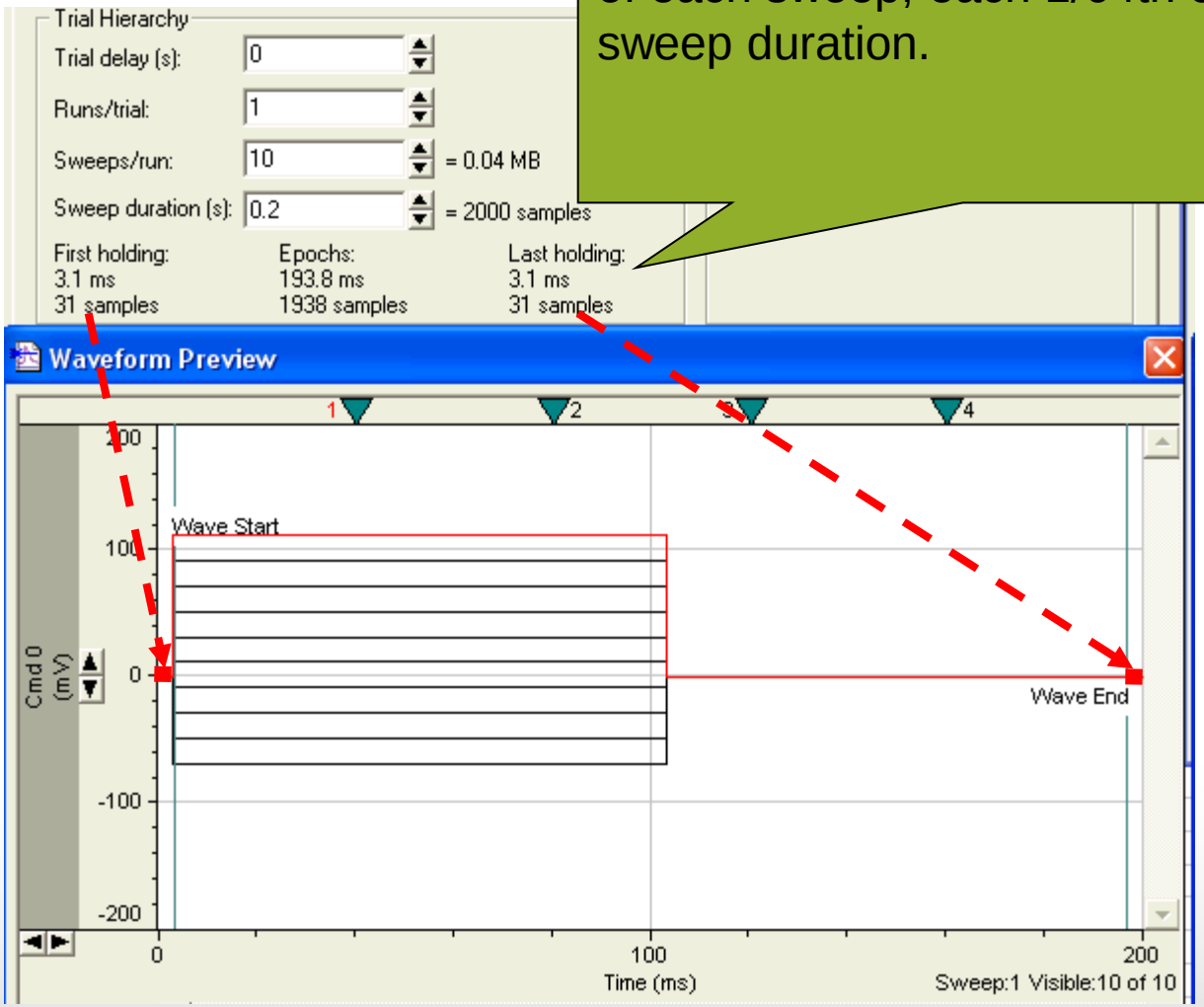
Episodic Stimulation Mode---Holding Periods

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



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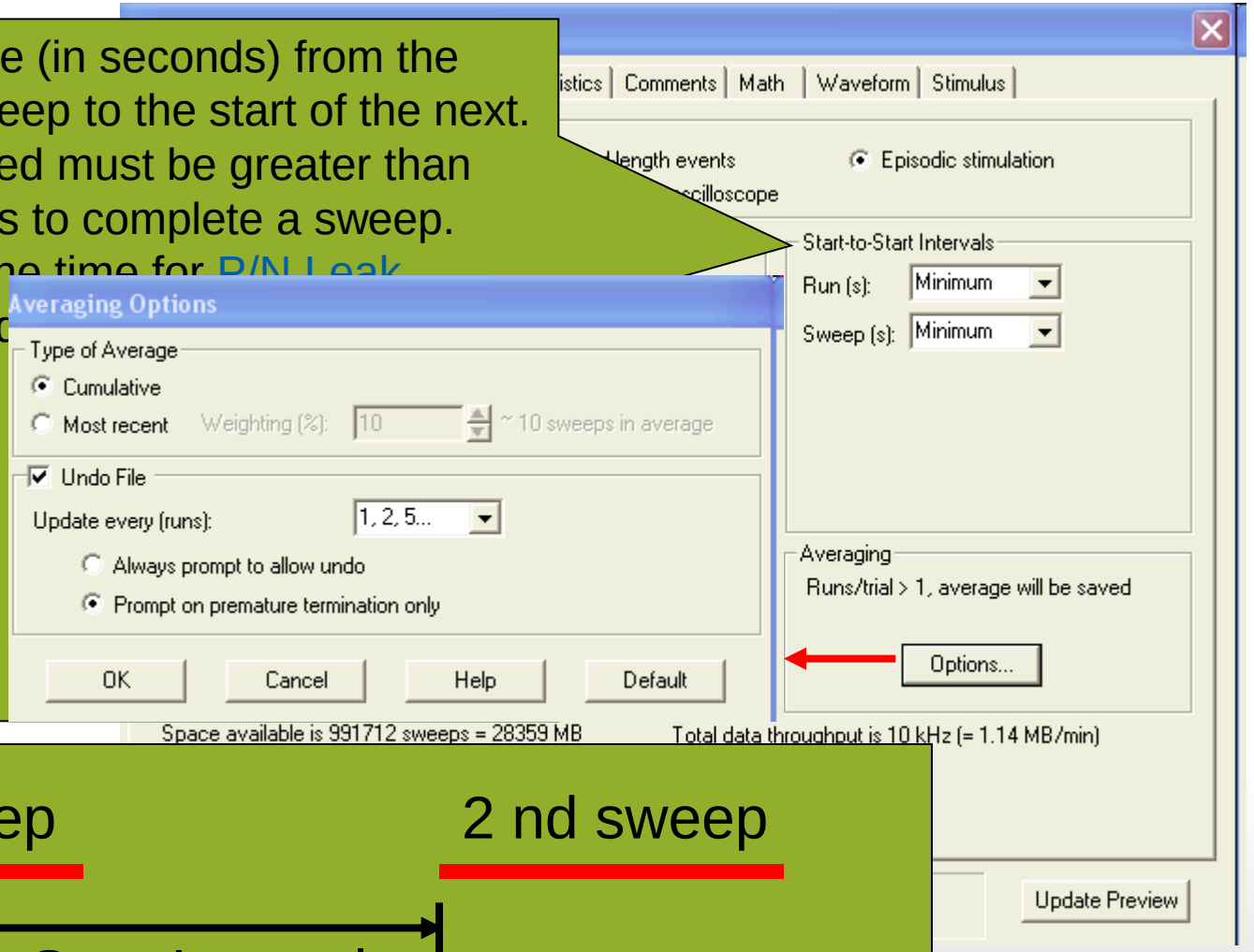
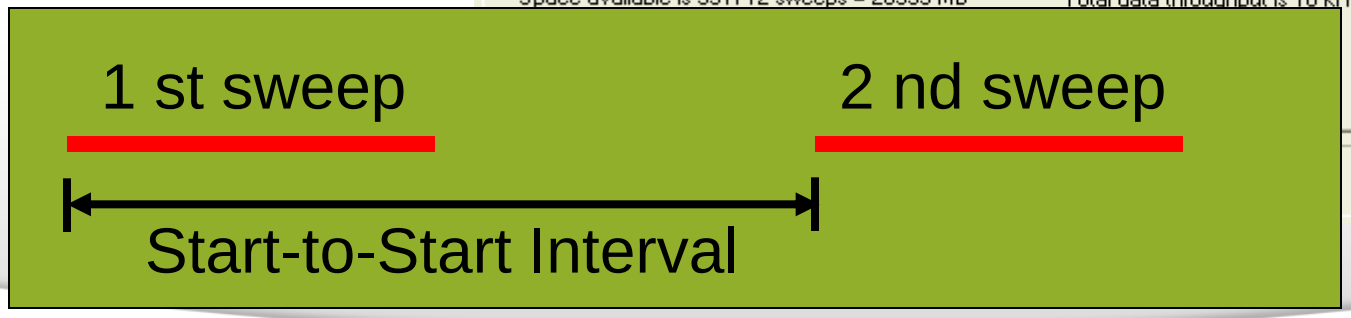
Episodic Stimulation Mode

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





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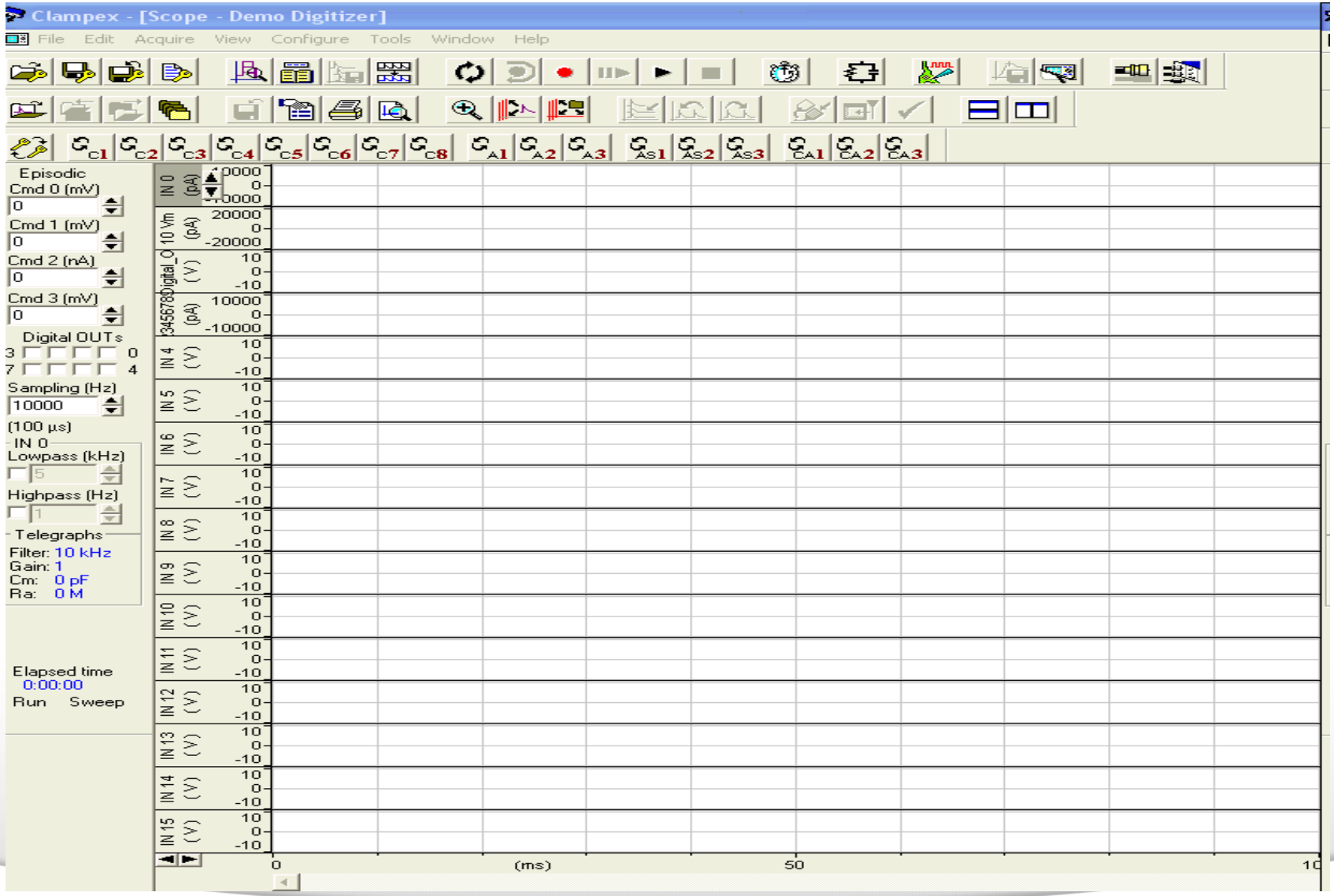
Inputs



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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.

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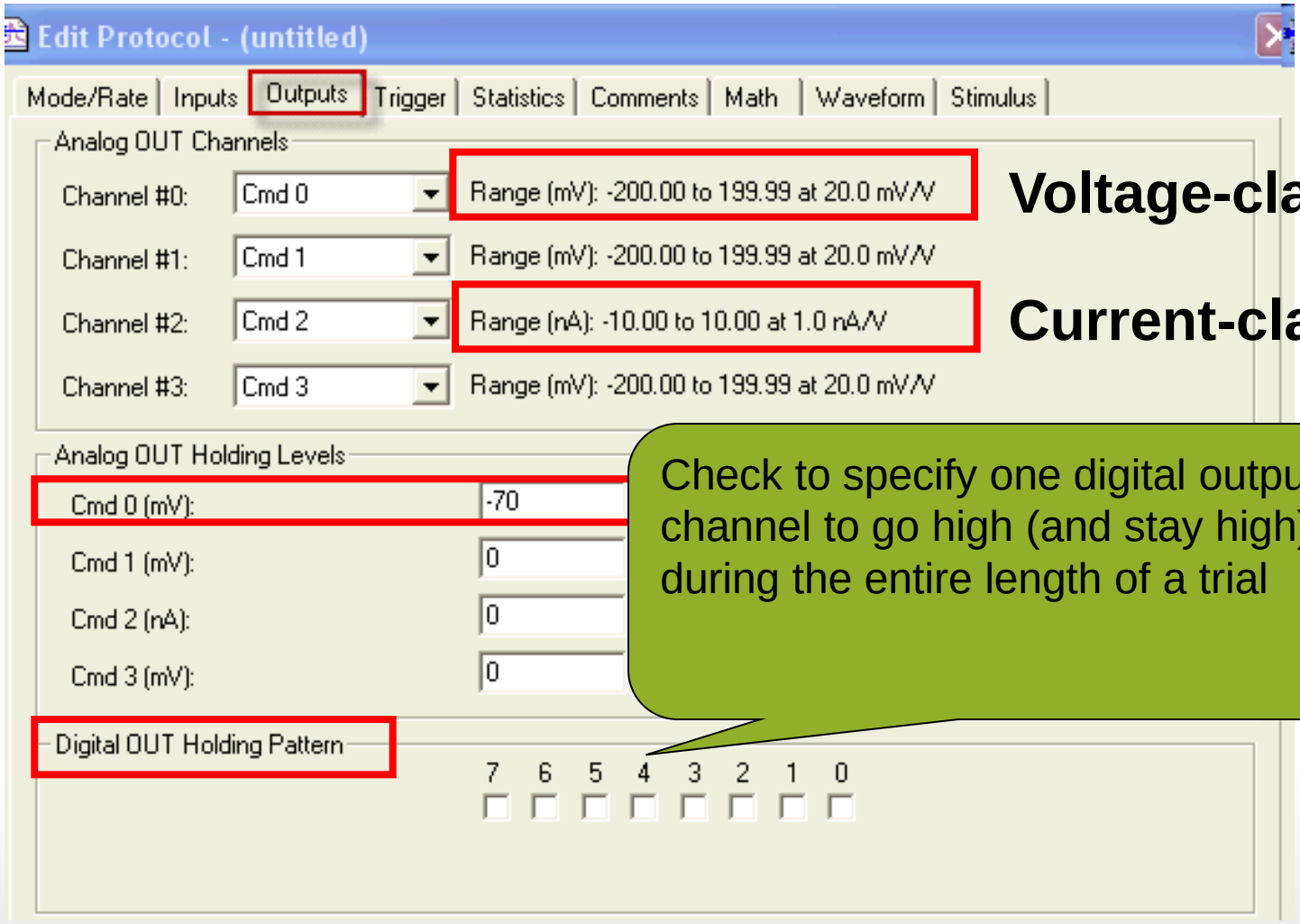
Outputs



Voltage-clamp

Current-clamp

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

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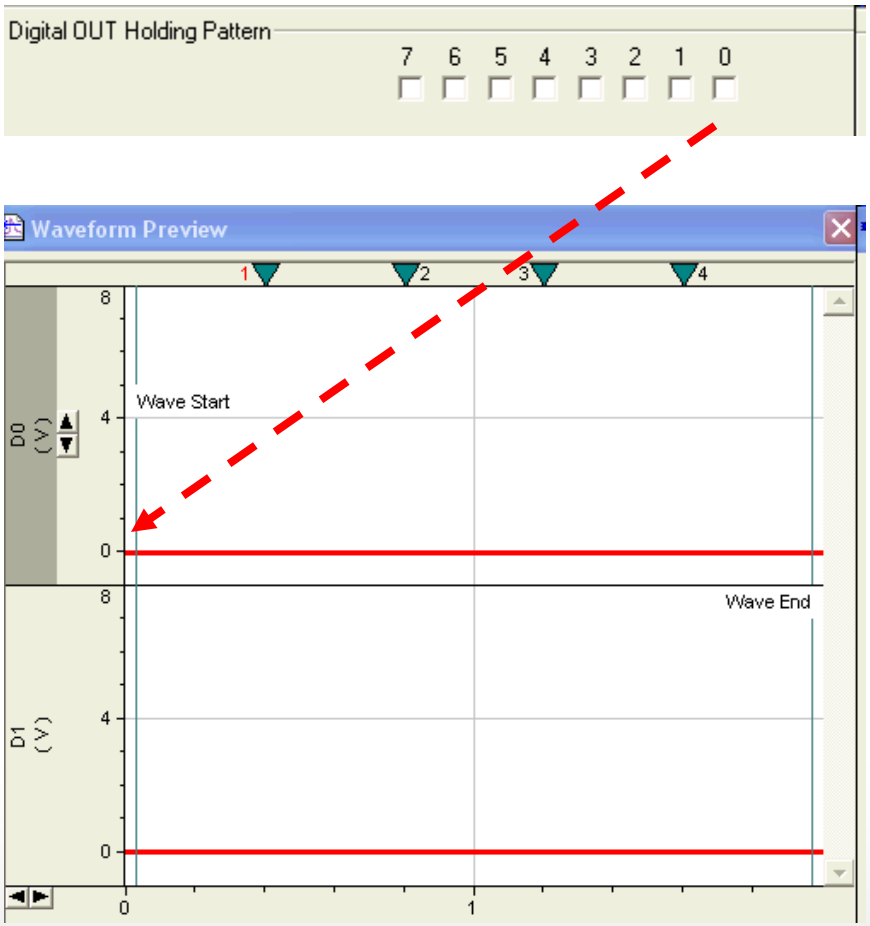
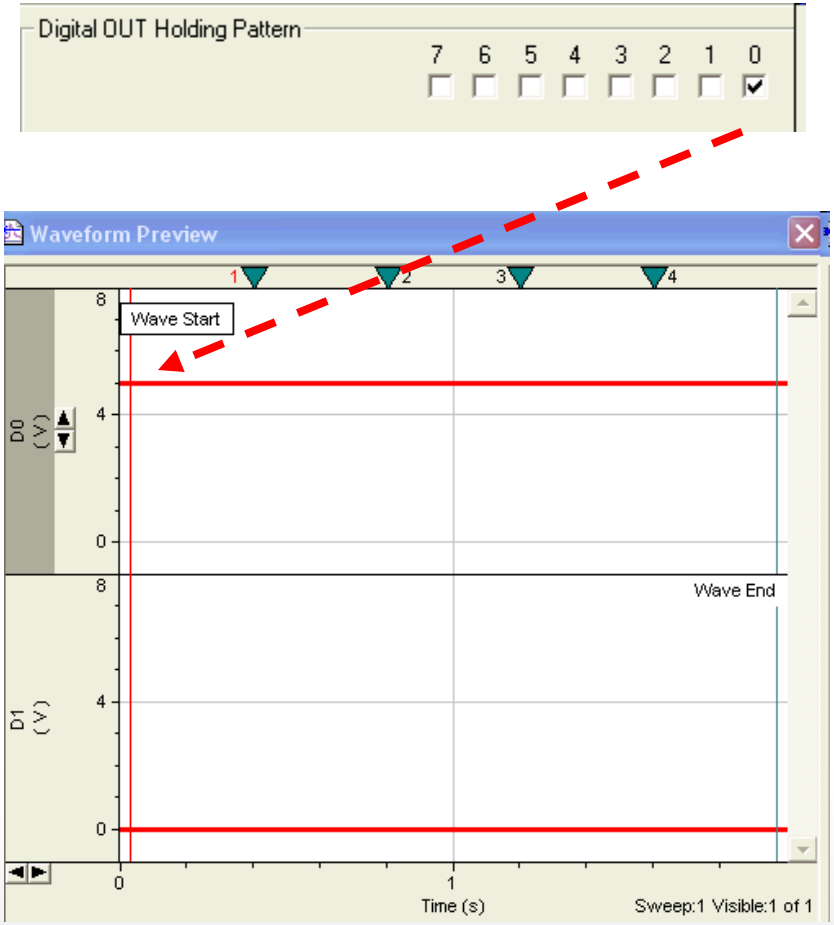
Digital Out Holding Pattern





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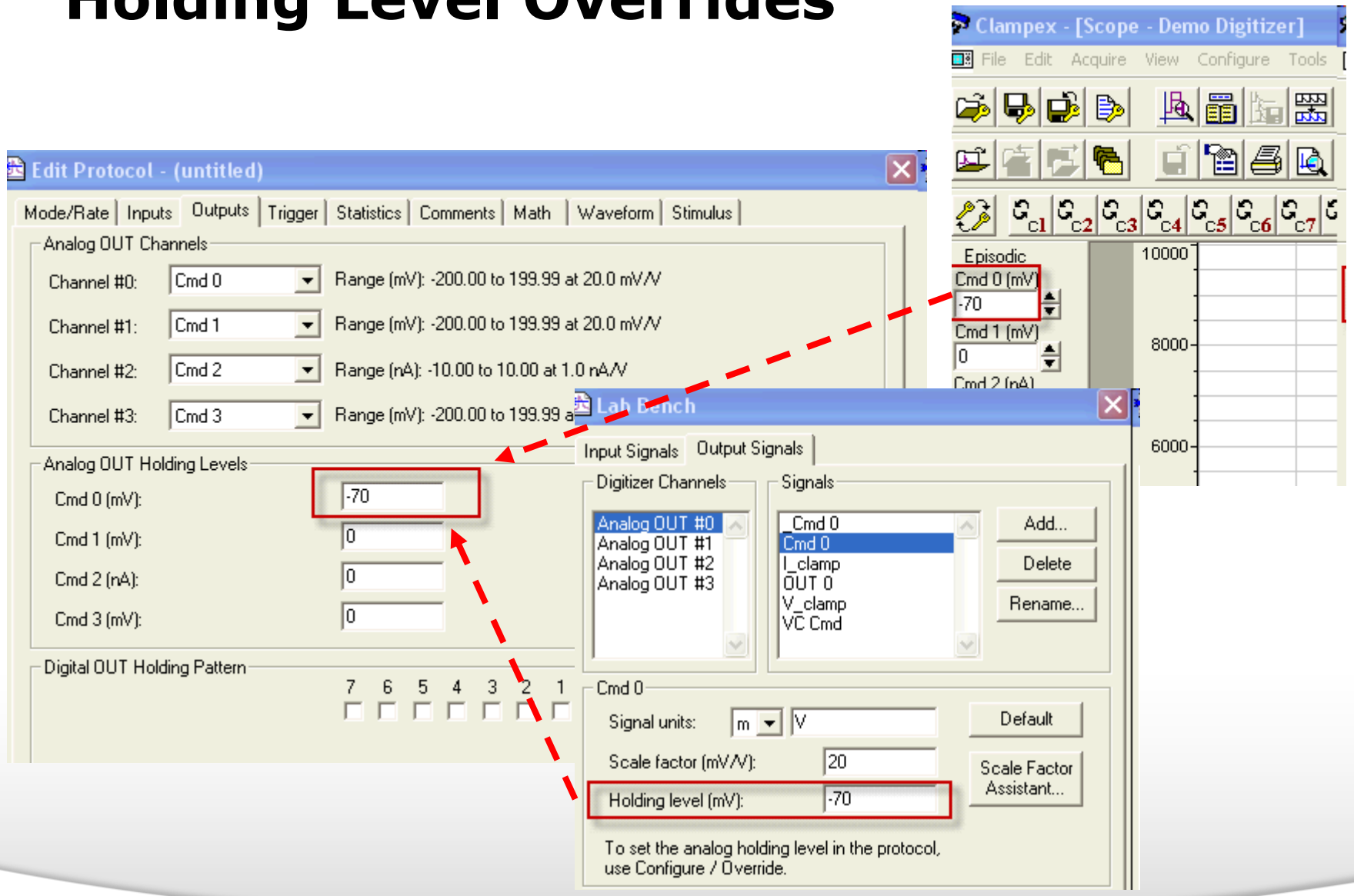
Holding Level Overrides



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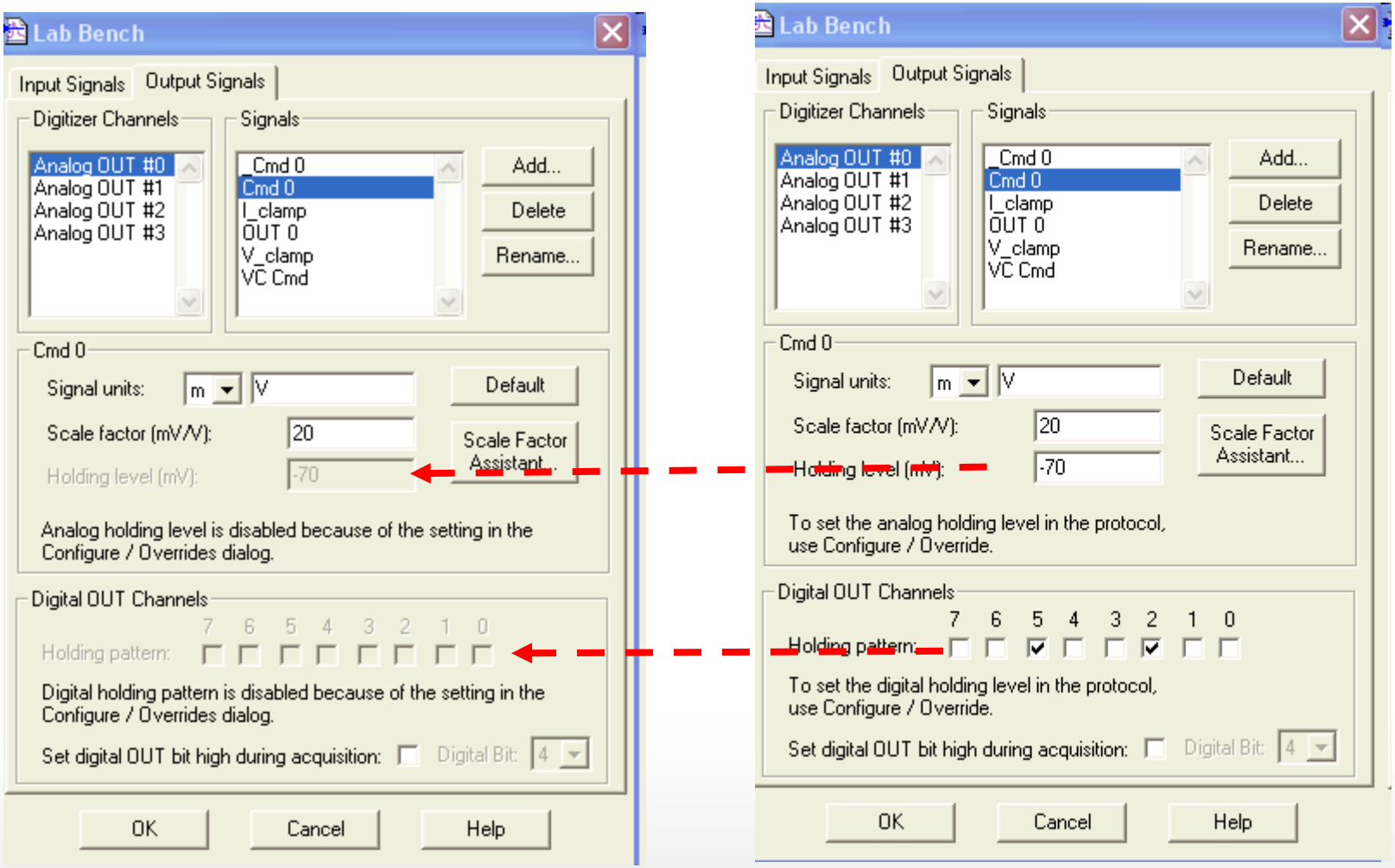
Molecular Devices

Holding Level Overrides



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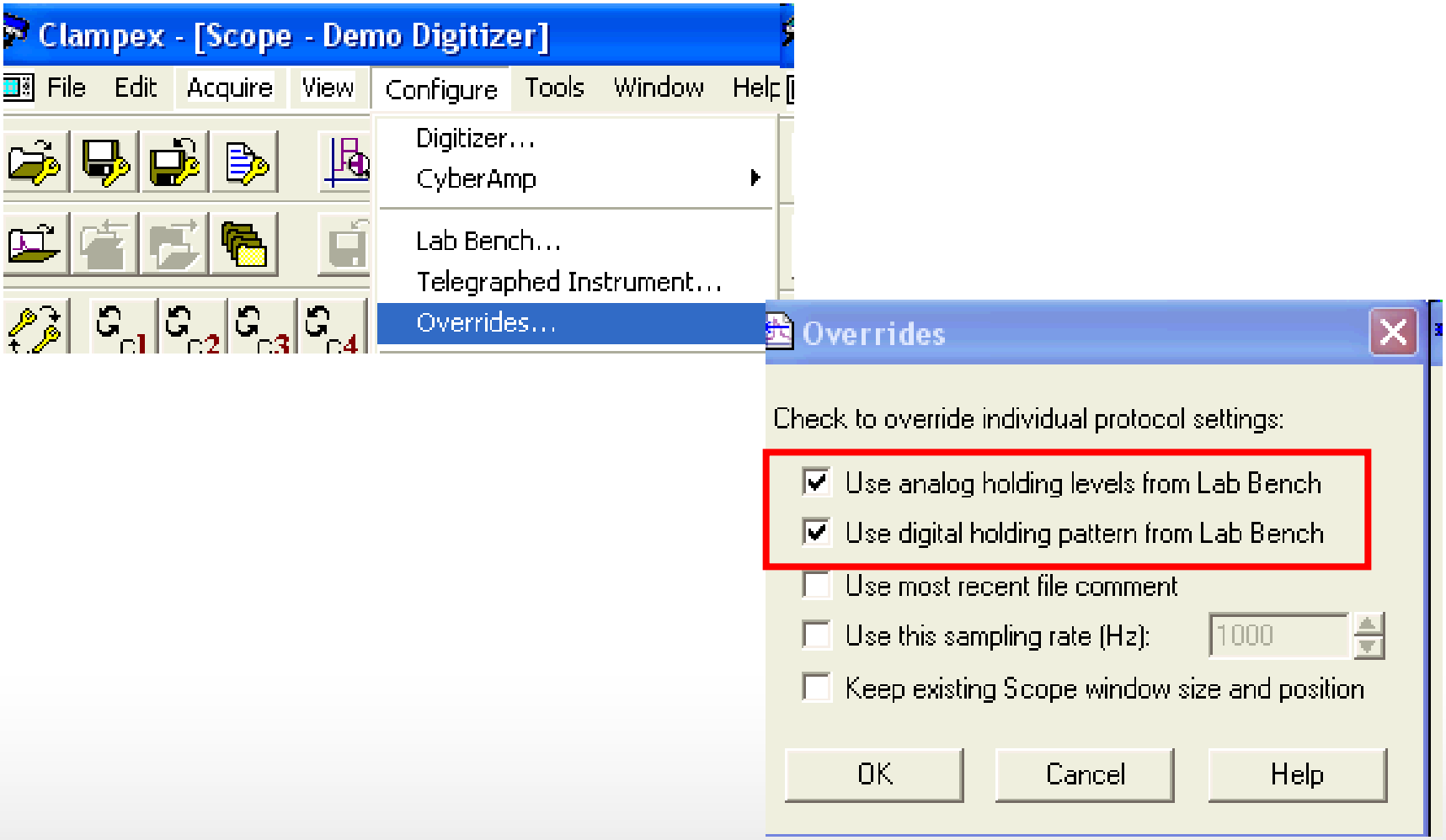
Holding Level Overrides



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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

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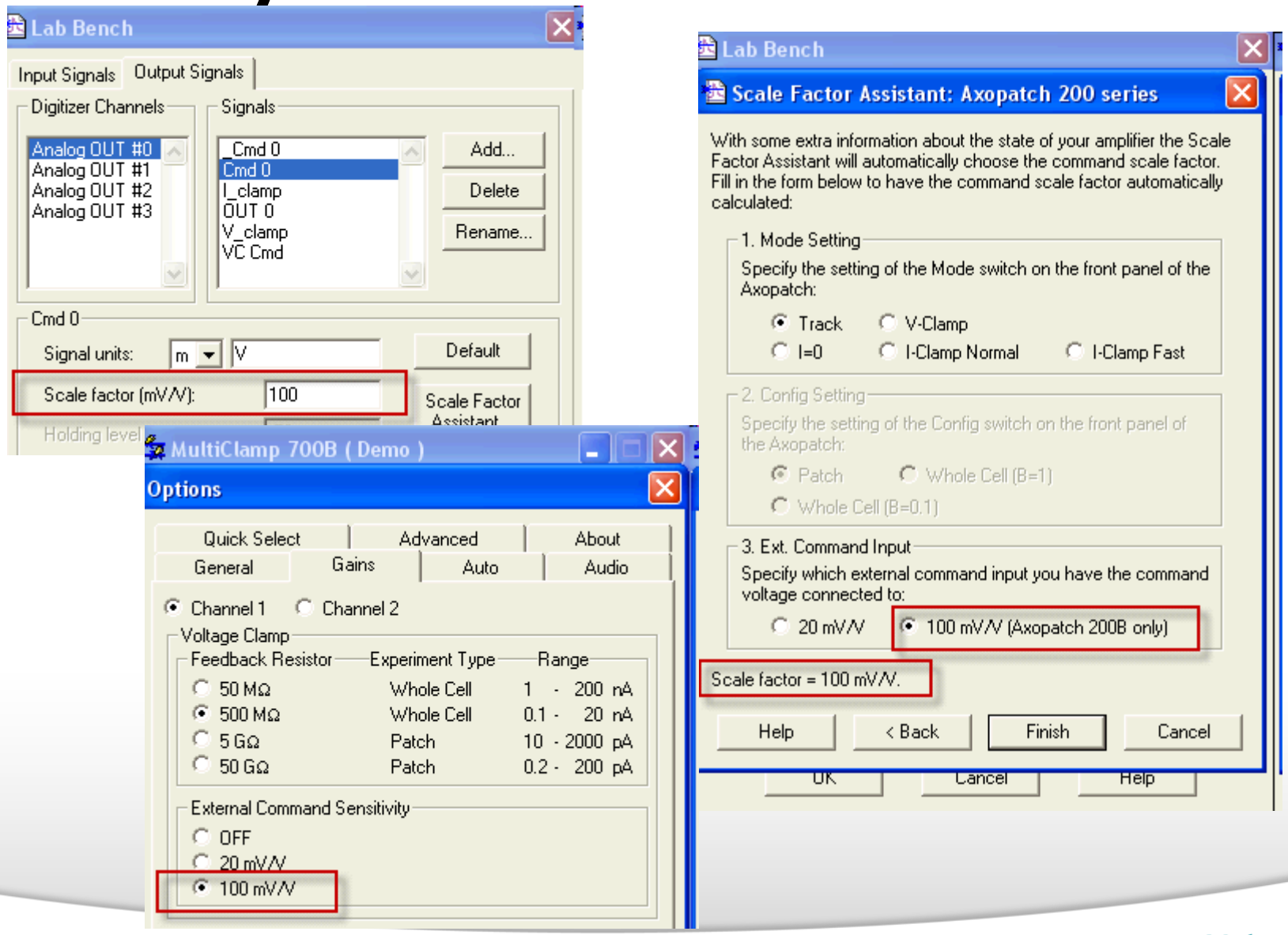
Scale Factor/ External Command Sensitivity



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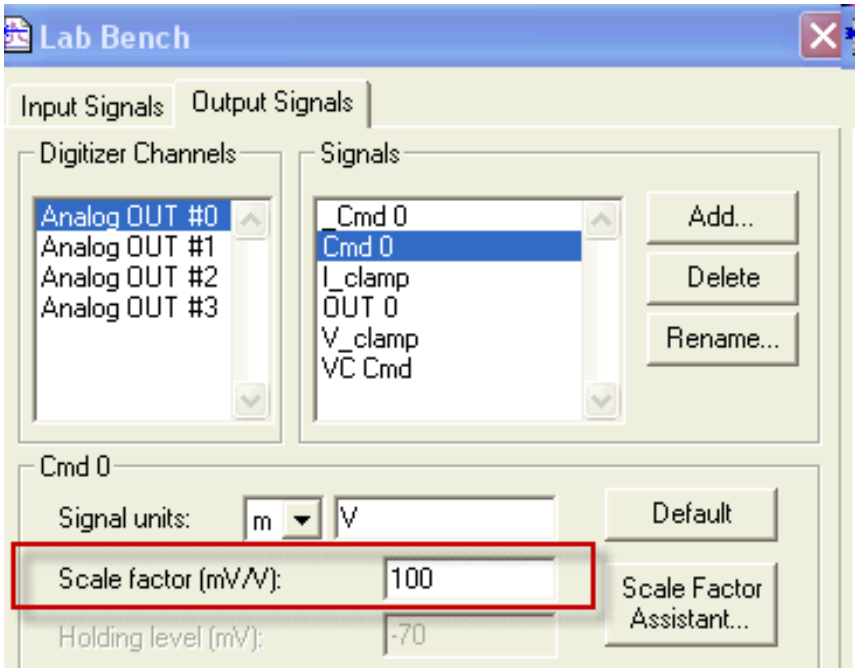
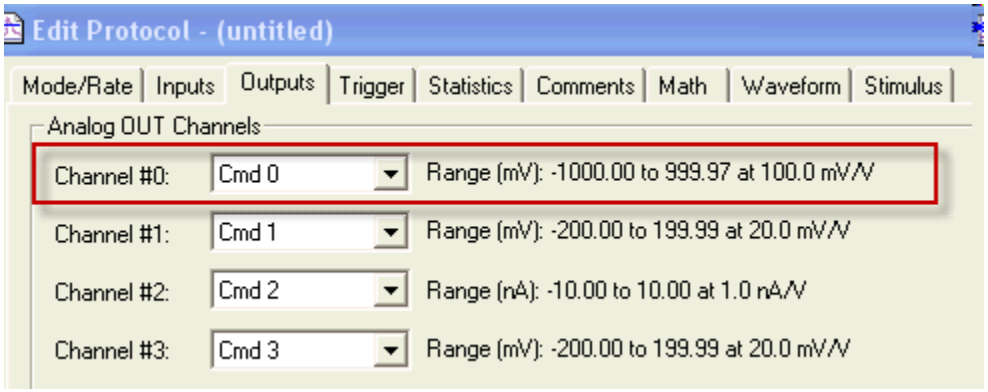
Scale factor/Output





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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.

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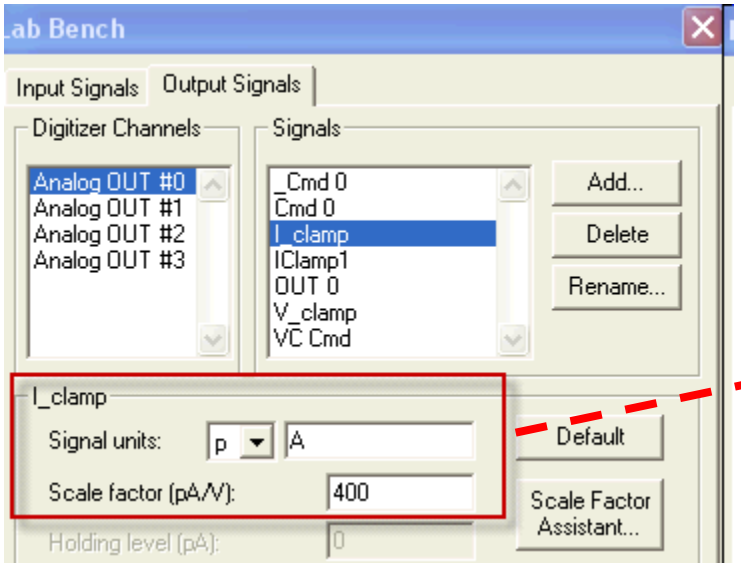

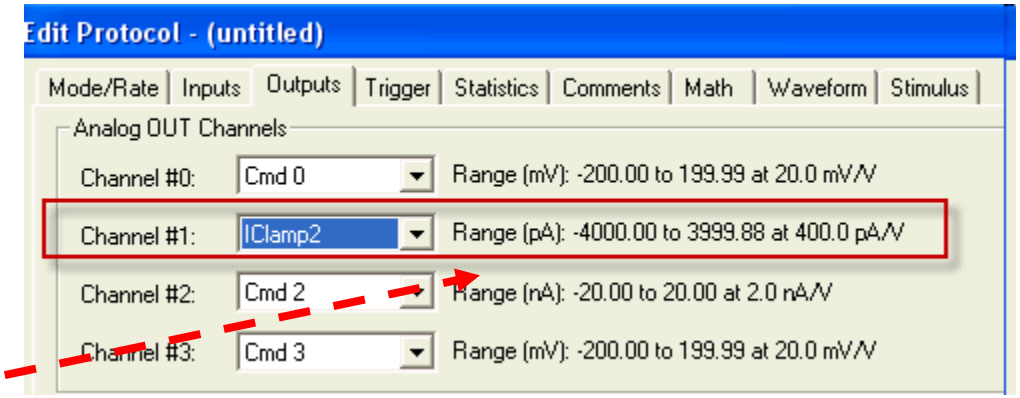

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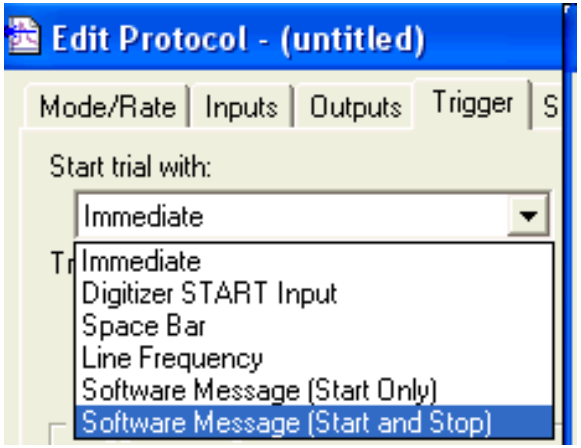
Trigger



- **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**

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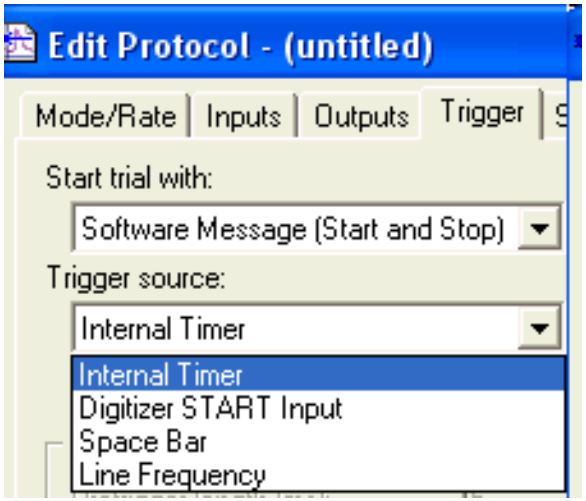
Trigger



- **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).**

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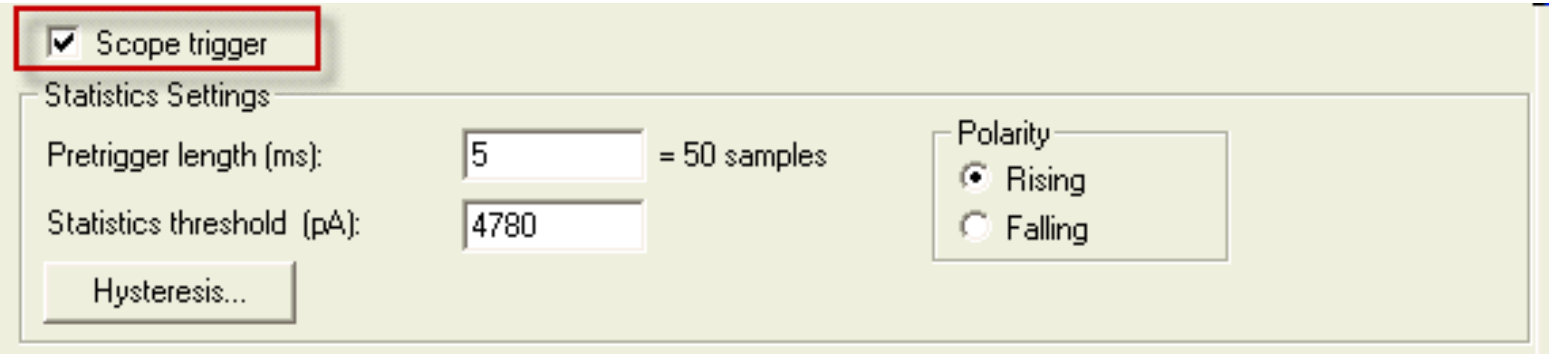
Scope Trigger



- 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)

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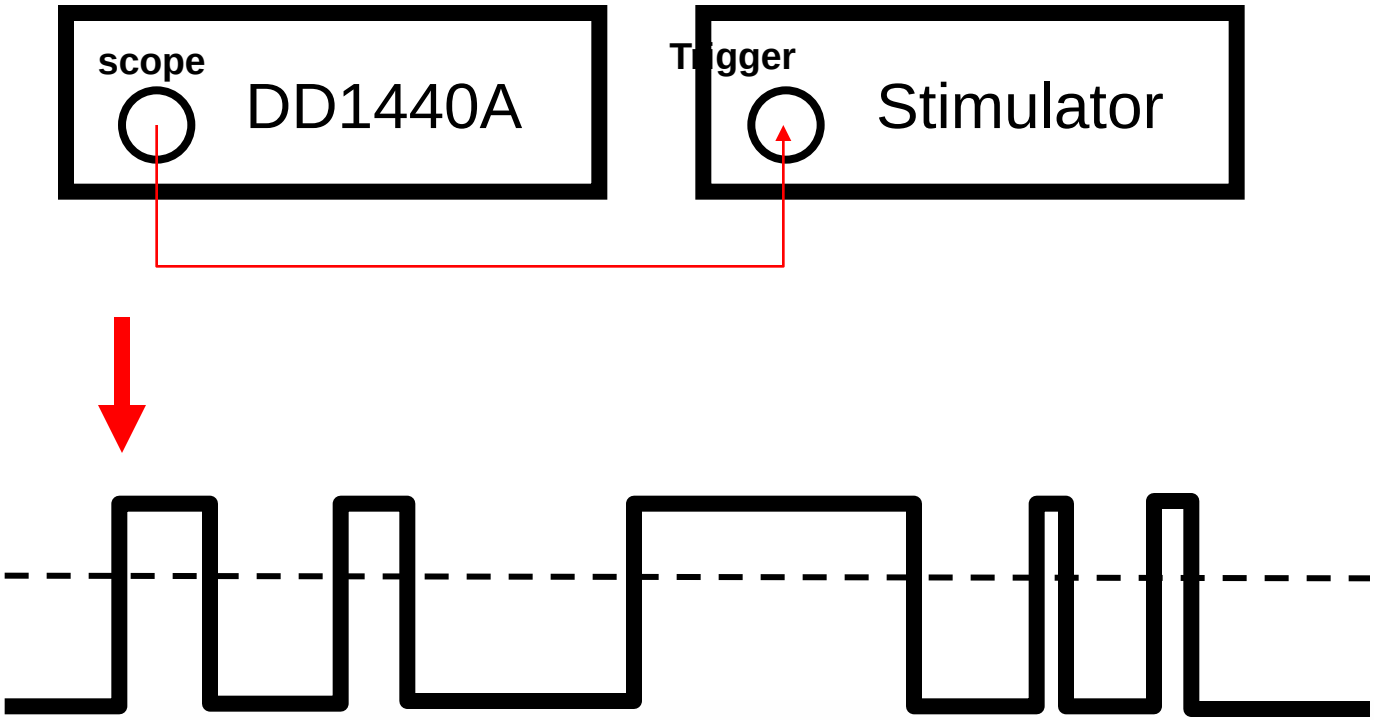
Scope trigger



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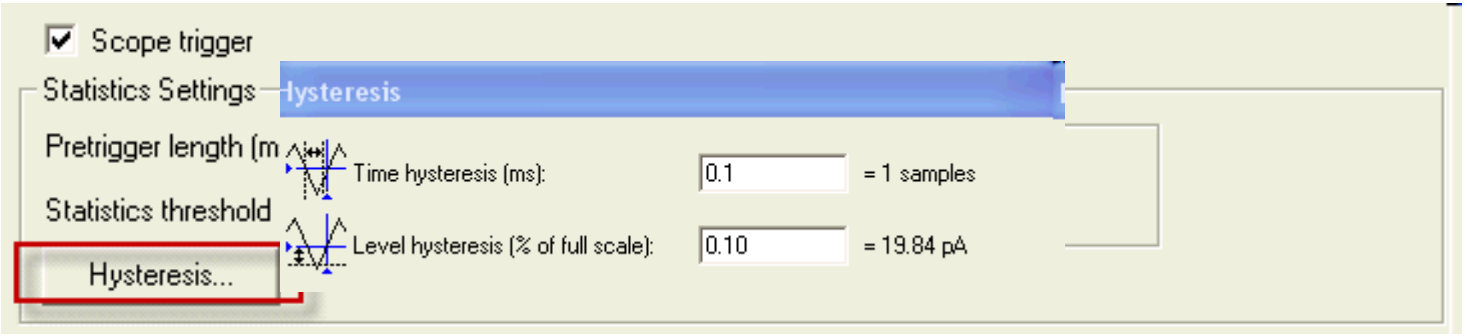
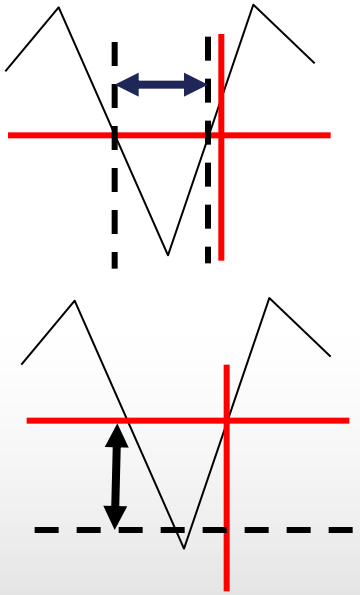
Hysteresis



- 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).



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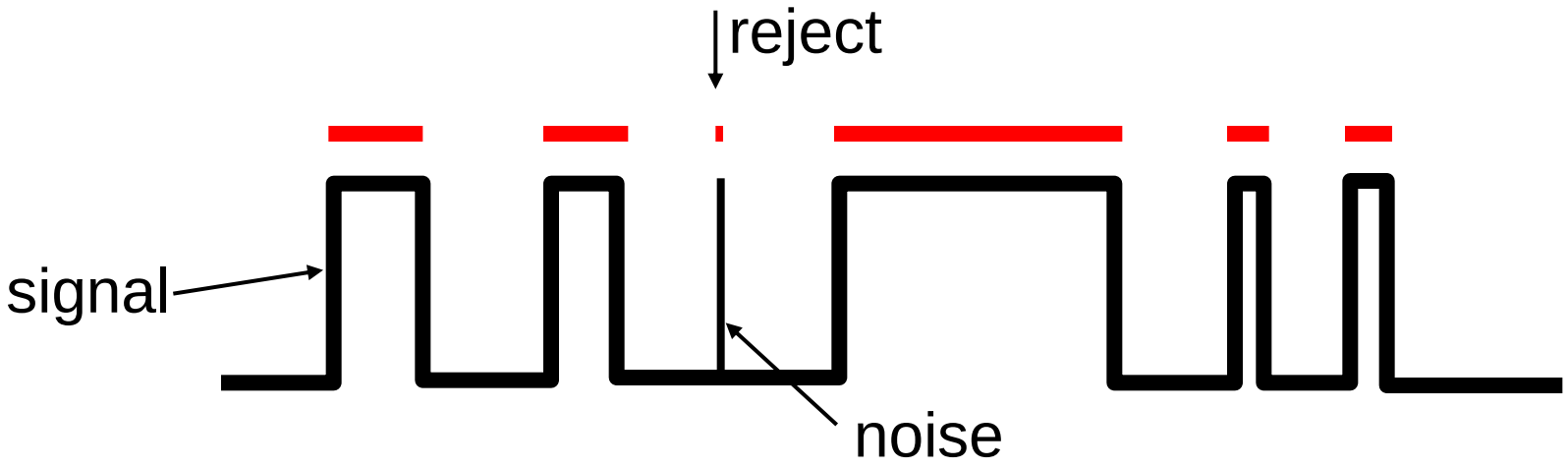
Time hysteresis



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

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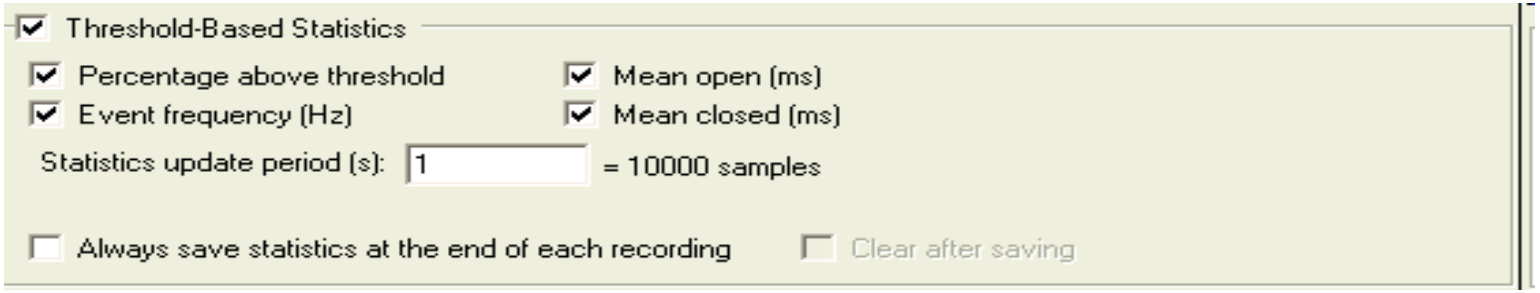
Threshold-Based Statistics



- 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.

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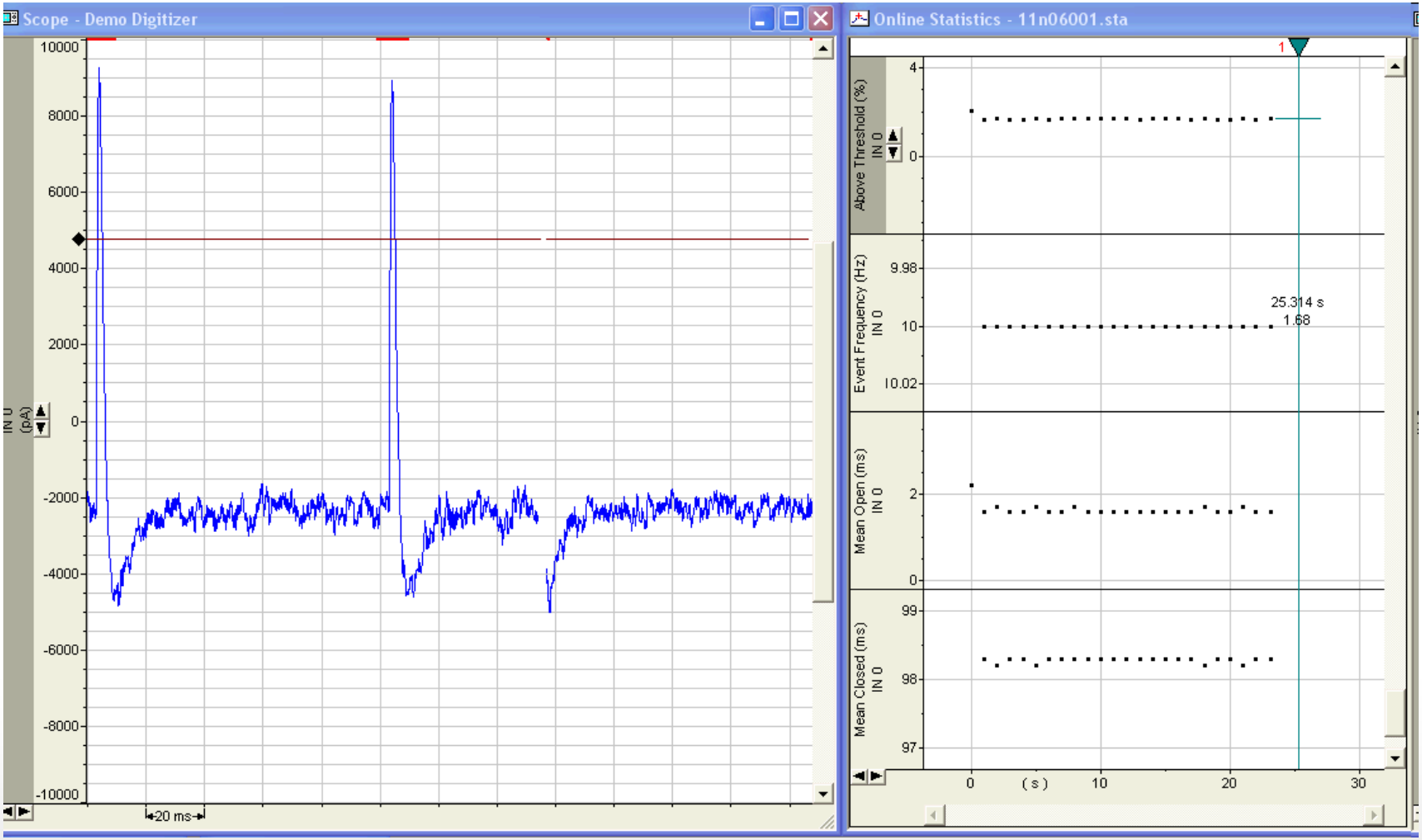
Threshold-Based Statistics



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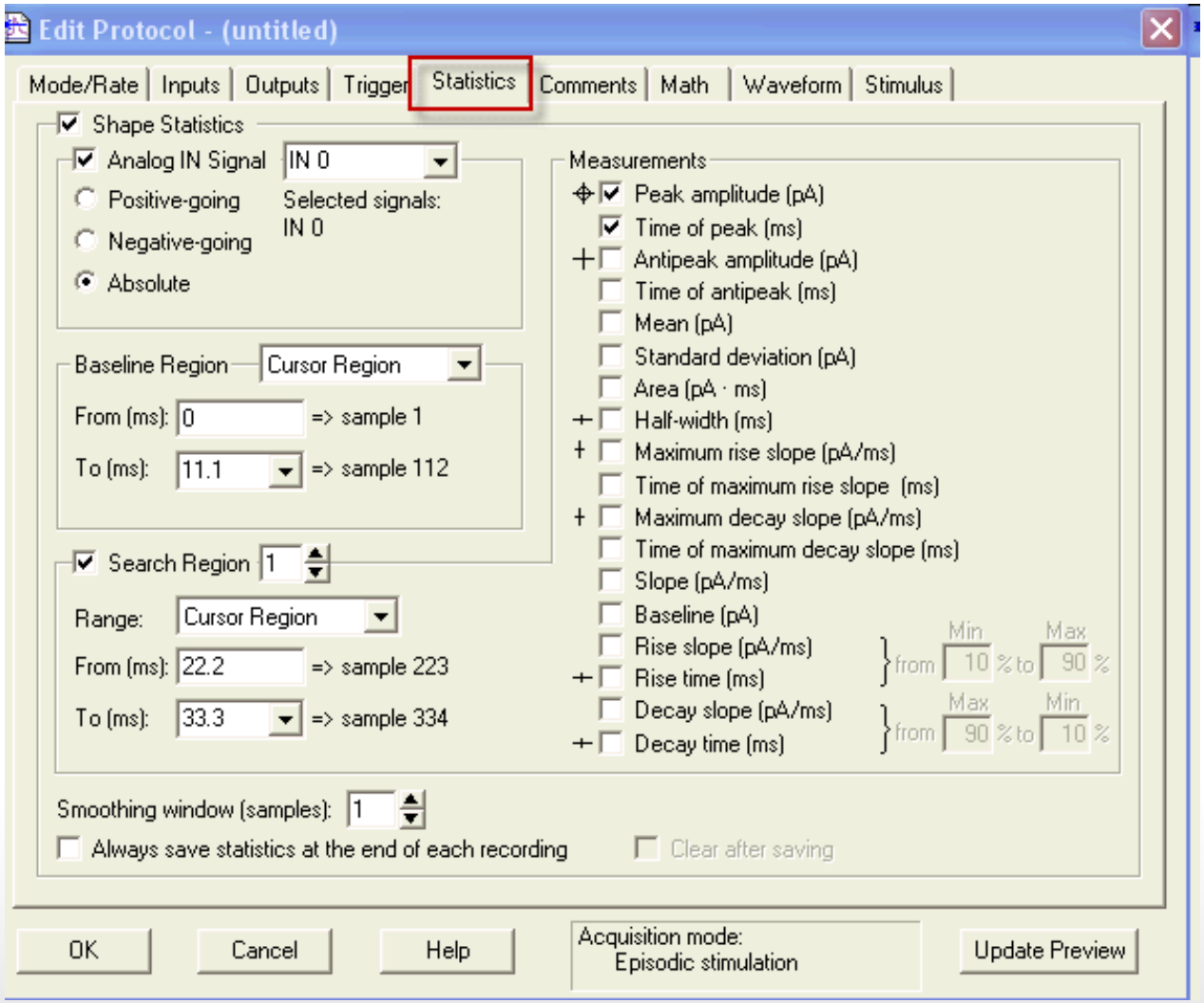
Statistics



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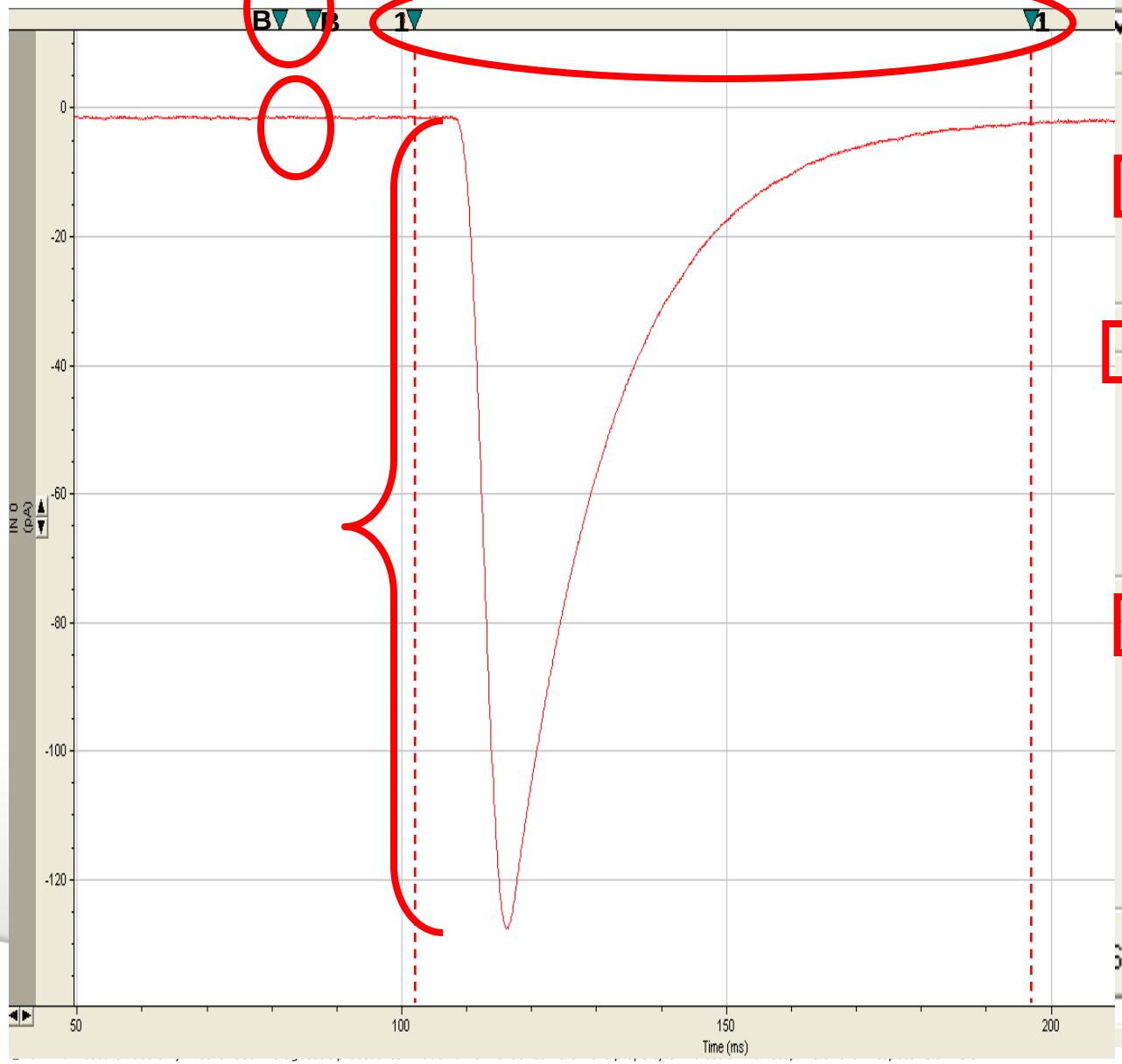
Statistics



Shape Statistics

Analog IN Signal IN 0

Positive-going

Negative-going

Absolute

Selected signals: IN 0

Baseline Region Cursor Region

From (ms): 0 => sample 1

To (ms): 11.1 => sample 112

Search Region 1

Range: Cursor Region

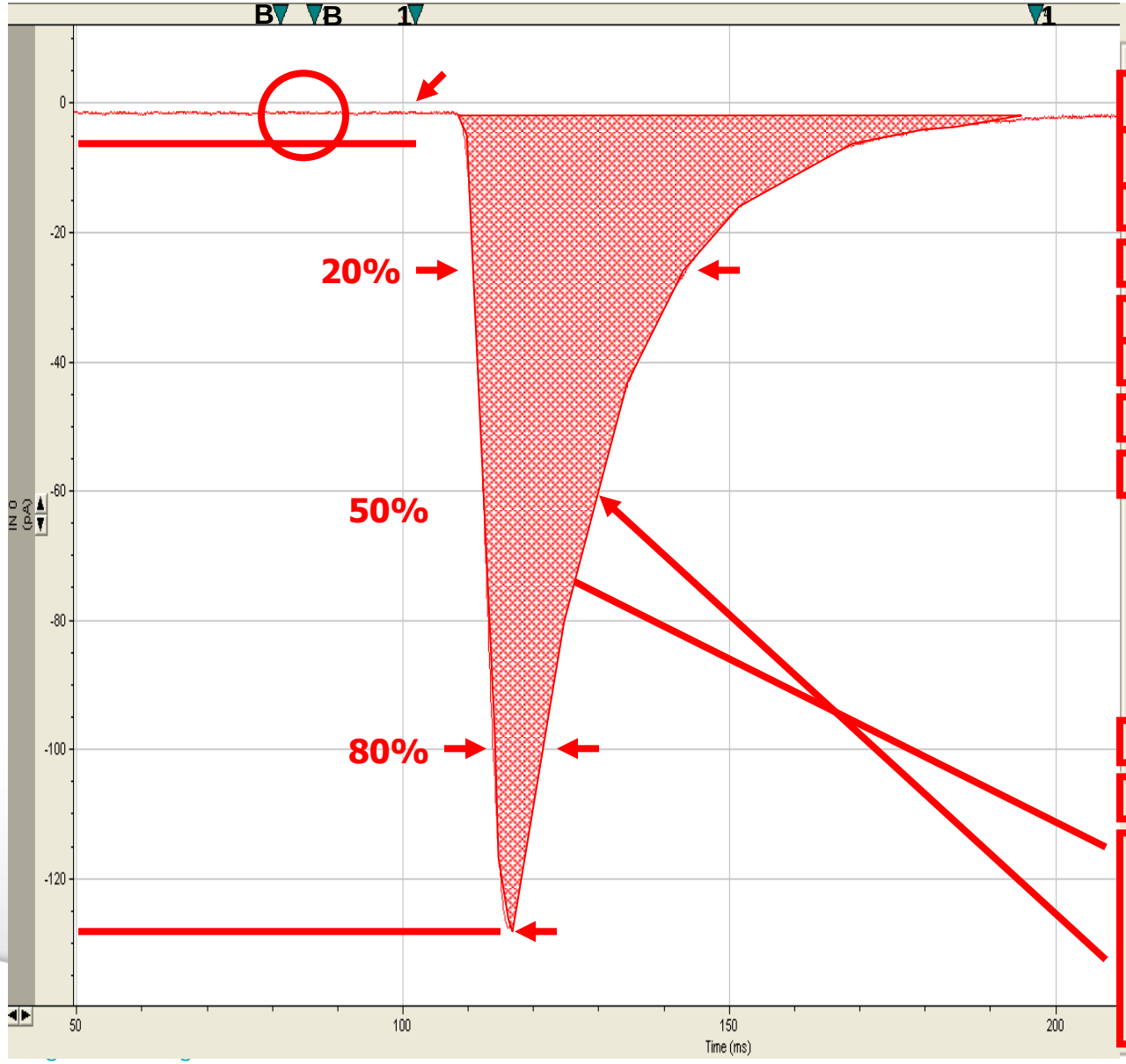
From (ms): 22.2 => sample 223

To (ms): 33.3 => sample 334

Smoothing window (samples): 1

Always save statistics at the end of each recording

Statistics



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)

	Min		Max	
From	20 %	To	80 %	
From	Max 80 %	To	Min 20 %	

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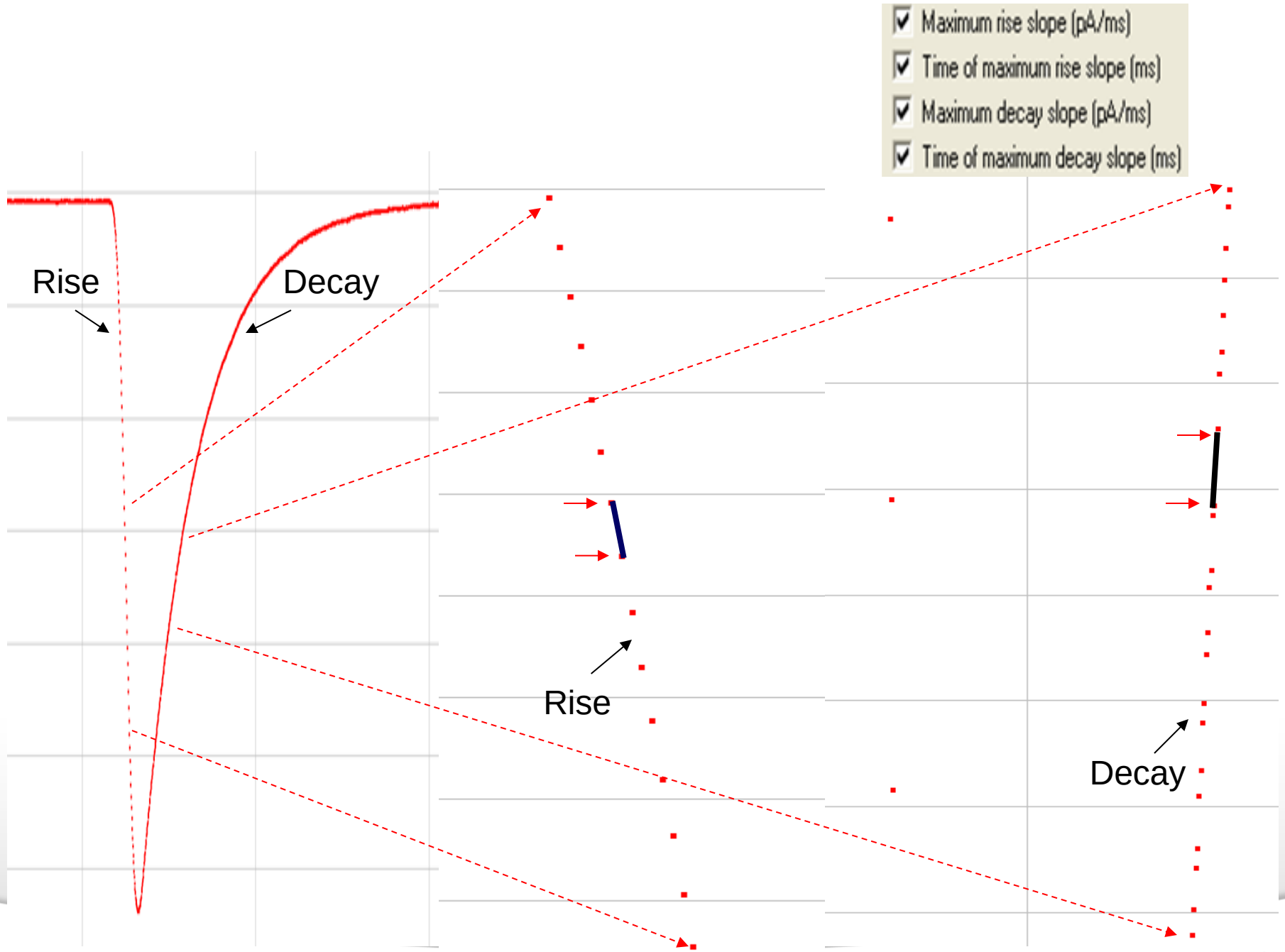
Devices



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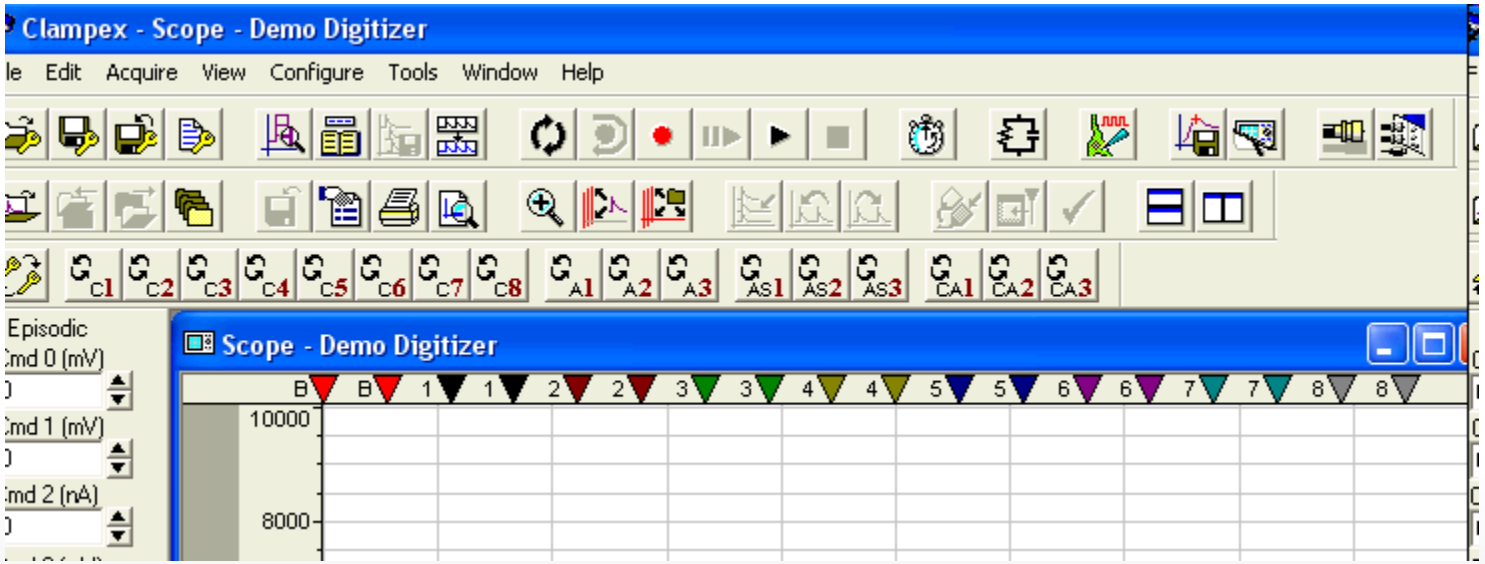
Q & A

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



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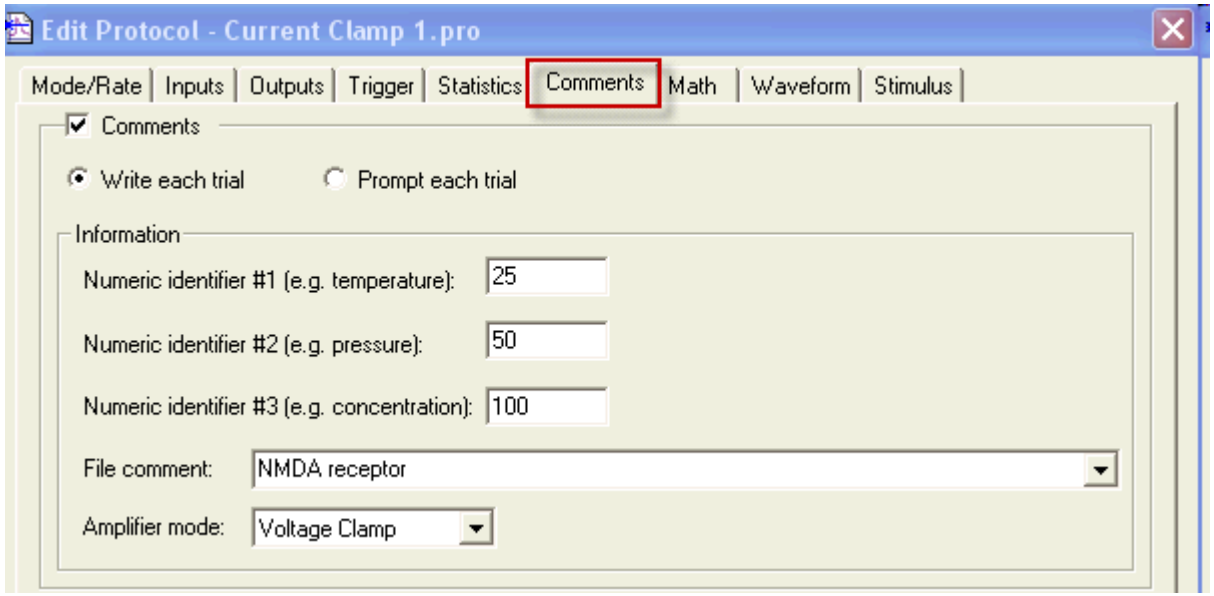
Comments



- 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.

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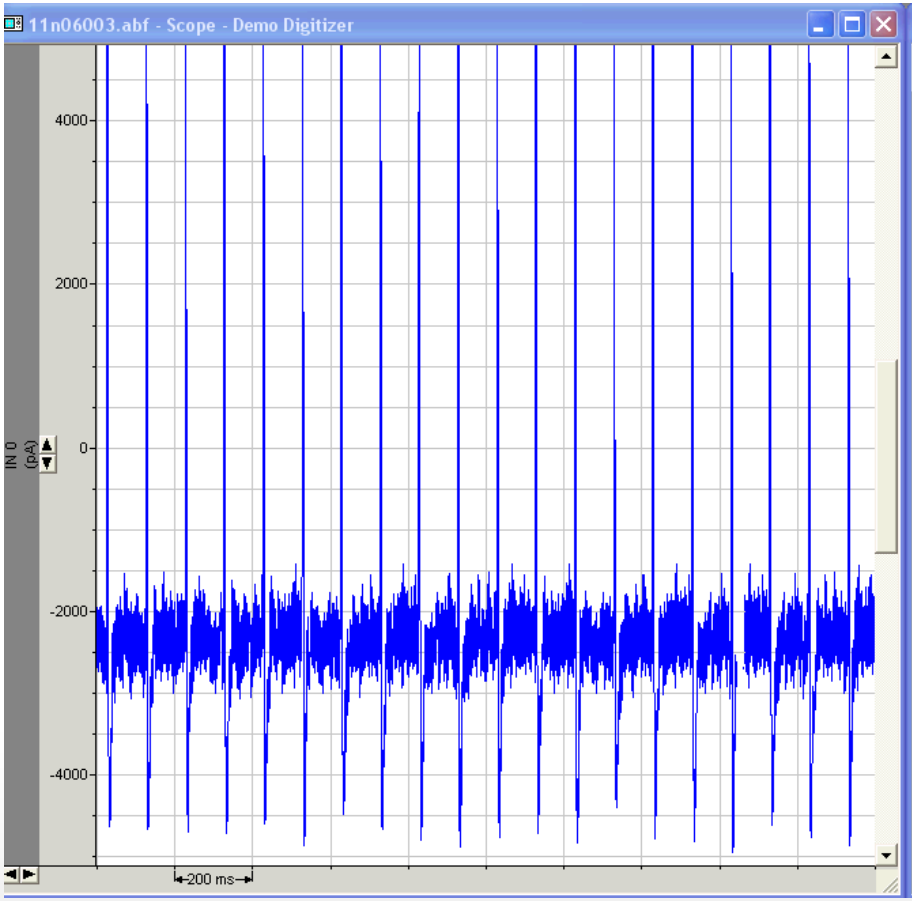
Comments



System Lab Book - Clampex LabBook.rtf

Courier New (Western) 9

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New protocol started.
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Protocol 'C:\Documents and Settings\jeffrey.tang\Desktop\SFN 2010\Post-LTP protocol.pro'
Protocol 'C:\Documents and Settings\...\Desktop\pCLAMP webinar 12-22\Pre-LTP protocol.prc
Stimulus file name: ==> C:\Documents and Settings\jeffrey.tang\Desktop\Webinar\AP wave2.e
Stimulus file Analog IN: ==> 15
Stimulus file name: ==> C:\Documents and Settings\jeffrey.tang\Desktop\webinar 2011-6-16\
Stimulus file Analog IN: ==> 0
Stimulus file name: ==> C:\Documents and Settings\jeffrey.tang\Desktop\webinar 2011-6-16\
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Stimulus file name: ==> C:\Documents and Settings\jeffrey.tang\Desktop\pCLAMP webinar 12-
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Analog In Ch. #3 Lowpass Filter (kHz): ==> 55.5556
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\digitizer start sweep shift.pro' c
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\digitizer start sweep shift.pro' c
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\Current Clamp 1.pro' opened.
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\Current Clamp 1 modified.pro' oper
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\current clamp protocol.pro' opened
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\Current Clamp 1.pro' opened.
Analog In Ch. #1: ==> 10 Vm
Analog In Ch. #2: ==> Digital_0
Analog In Ch. #3: ==> 1234567891
Acquisition Mode: ==> Episodic stimulation
Epoch A type: ==> step
New protocol started.
Protocol 'My Documents\Molecular Devices\pCLAMP\Params\Current Clamp 1.pro' opened.
My Documents\Molecular Devices\pCLAMP\Data\11n06002.abf started at 06:37:46 stopwatch tim
My Documents\Molecular Devices\pCLAMP\Data\11n06002.abf aborted at 06:37:51 stopwatch tim
Comment = ''.
ID#1 = 25, ID#2 = 0, ID#3 = 0.
Comment strategy: ==> Write each trial
My Documents\Molecular Devices\pCLAMP\Data\11n06003.abf started at 06:53:36 stopwatch tim
My Documents\Molecular Devices\pCLAMP\Data\11n06003.abf aborted at 06:53:40 stopwatch tim
Comment = 'NMDA receptor'.
ID#1 = 25, ID#2 = 50, ID#3 = 100.
```


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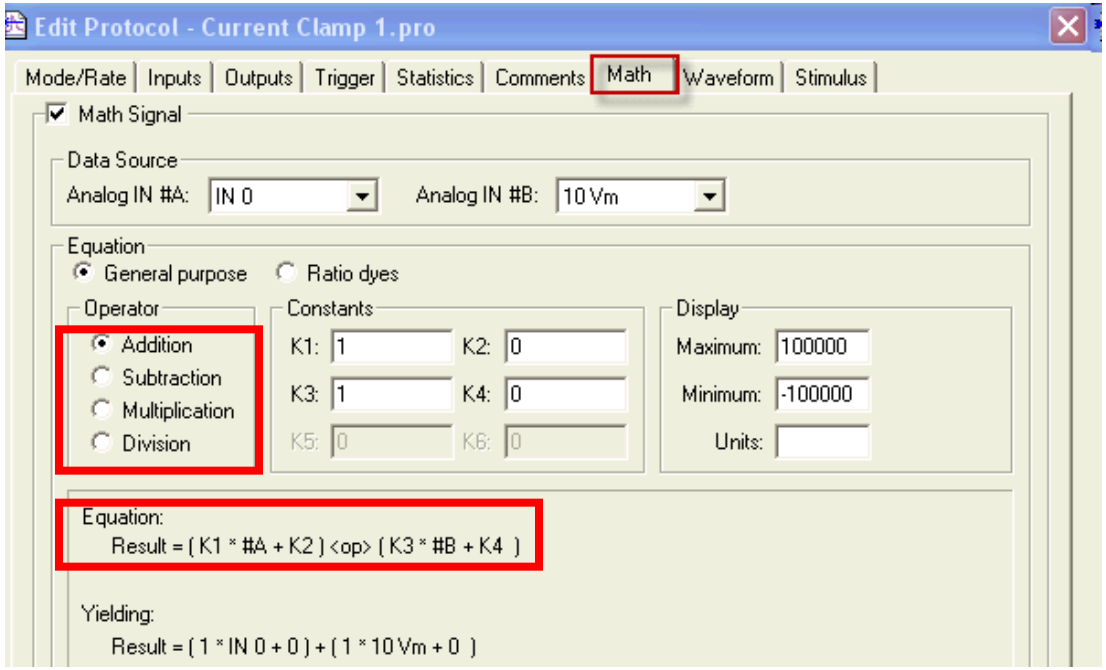
Math



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

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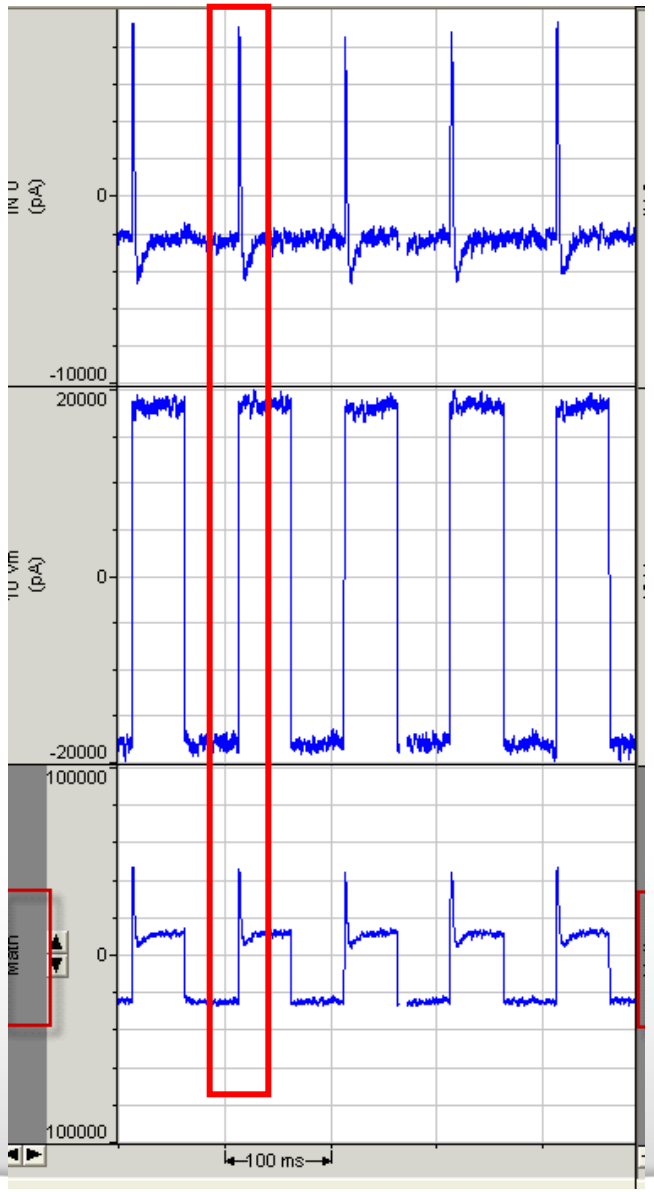
Math



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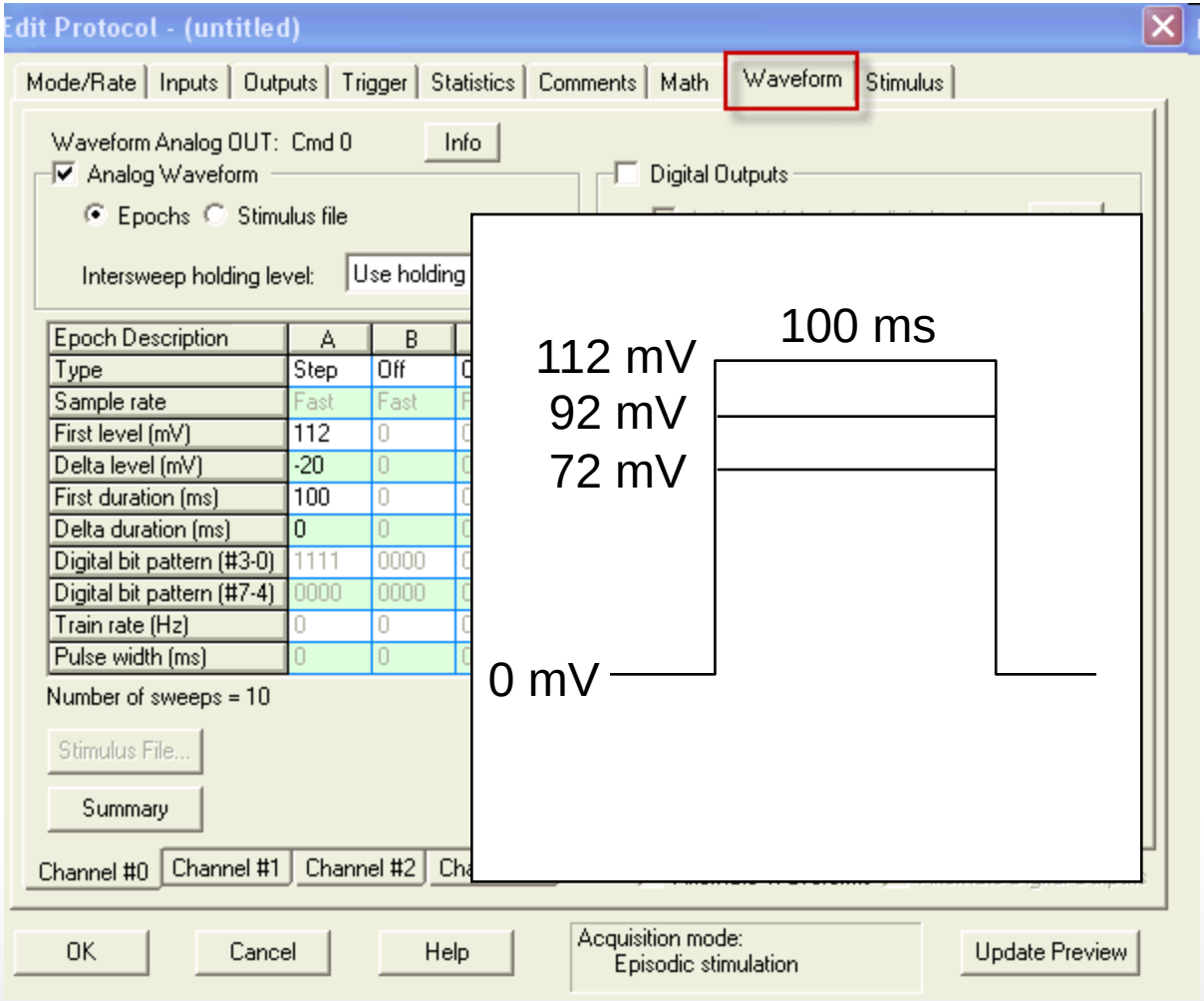
Waveform



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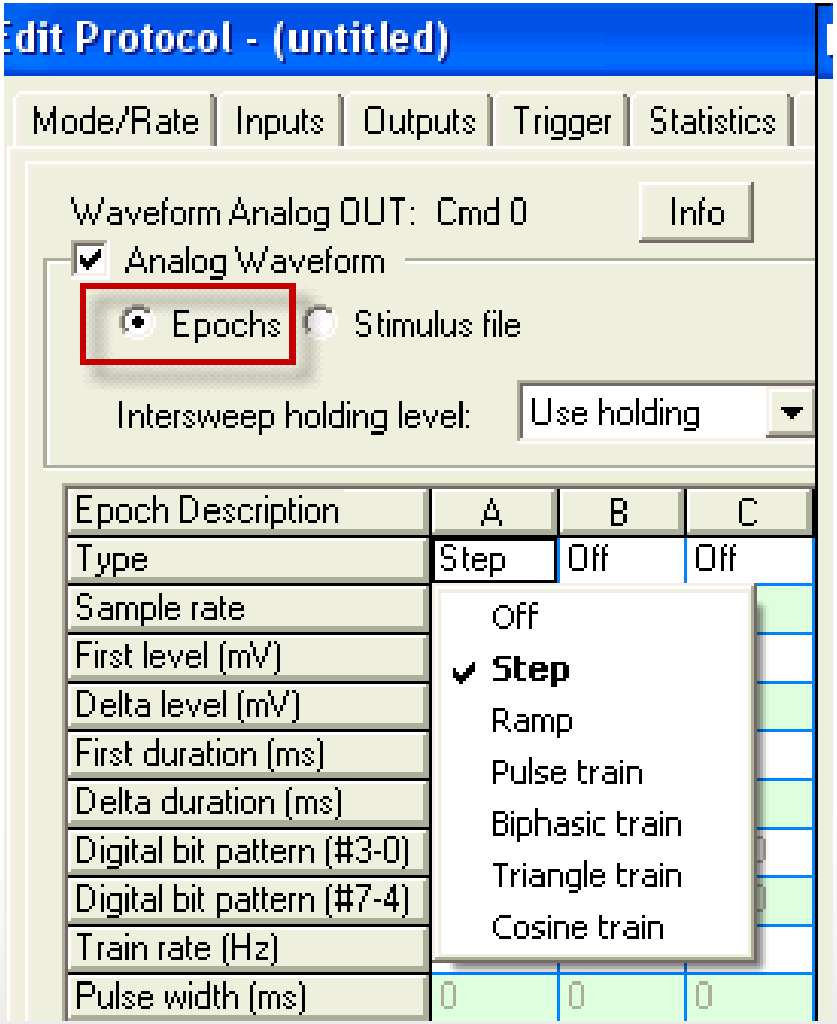
Epochs



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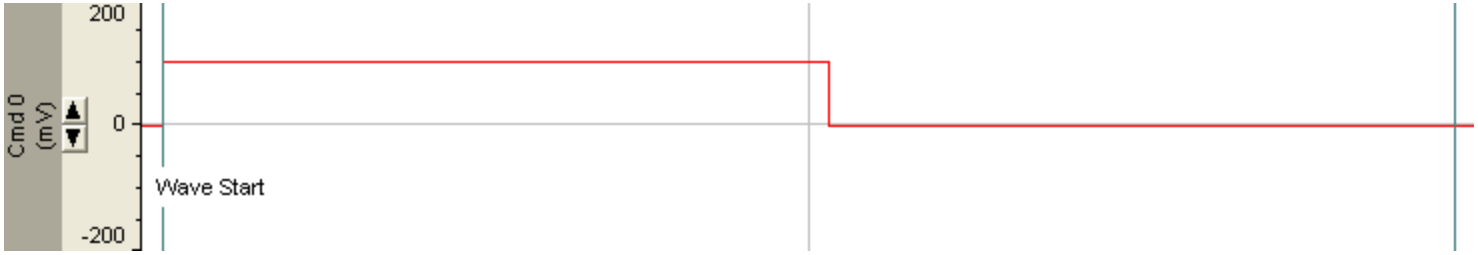
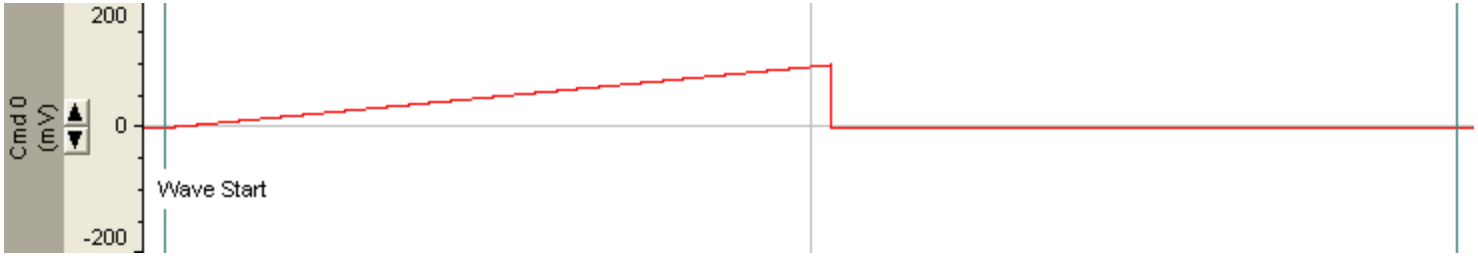
Default waveforms

Step:



Ramp:



Pulse train:

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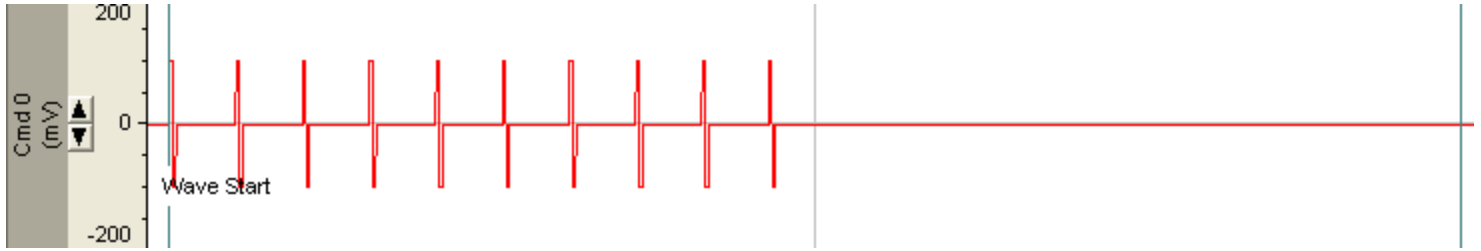
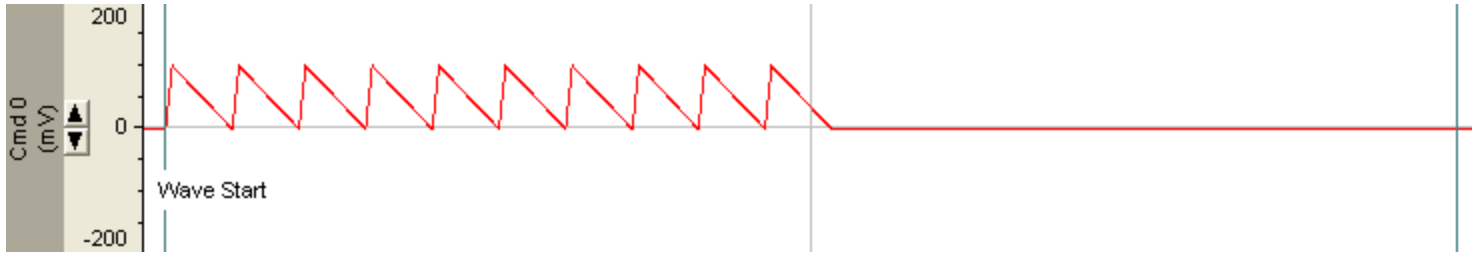
Default waveforms

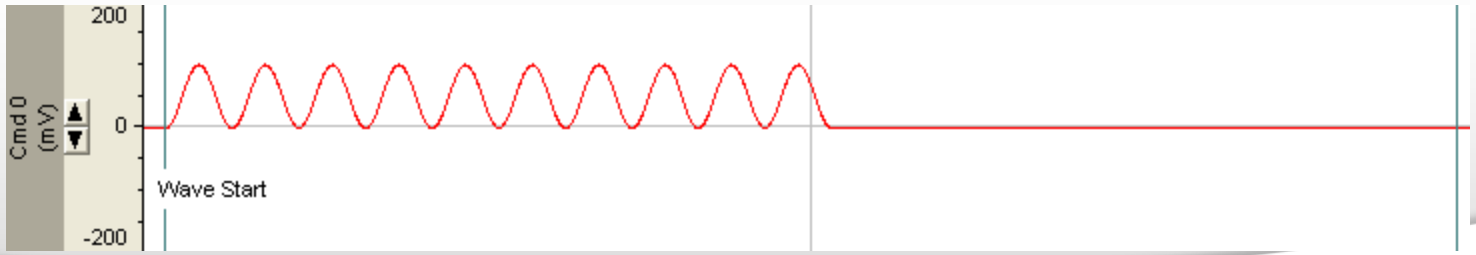
Biphasic train:



Triangle train:



Cosine train:



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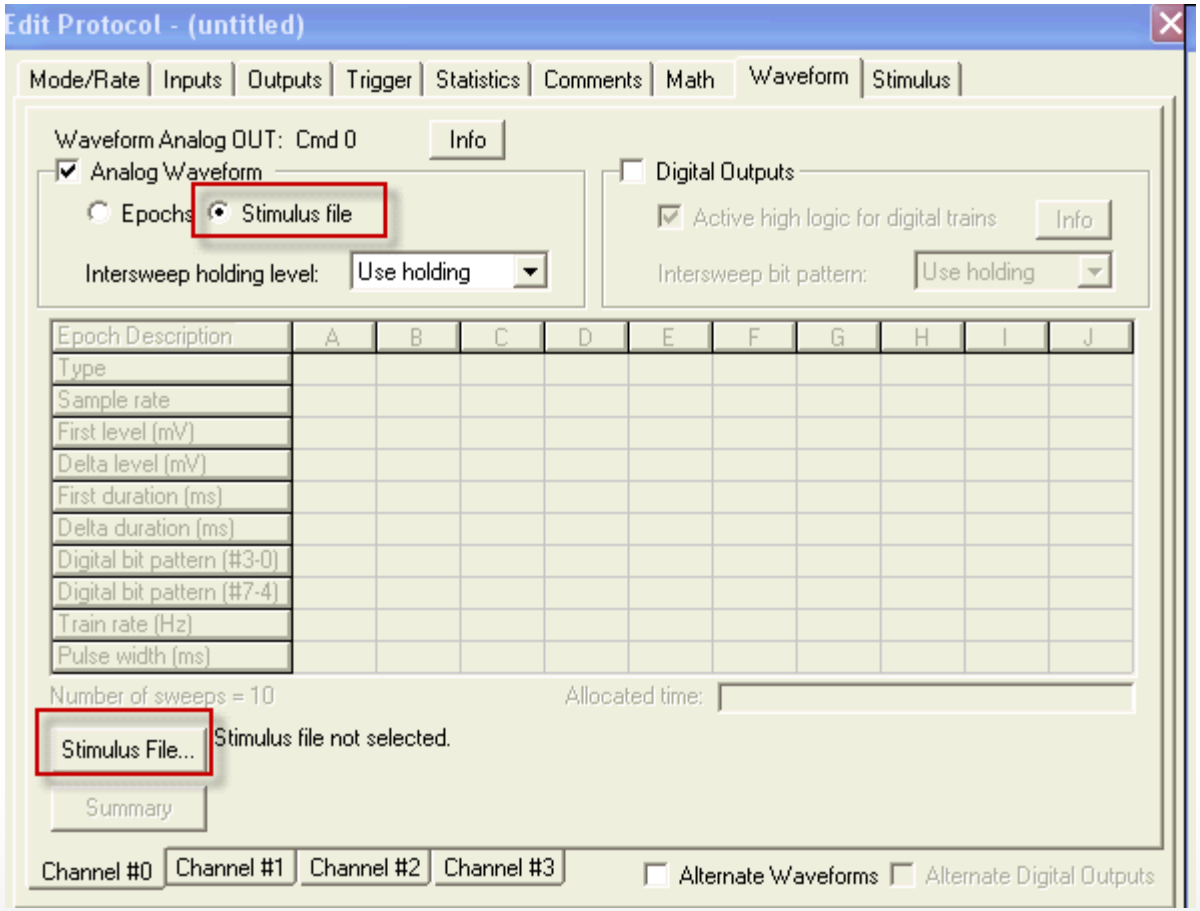
Stimulus File



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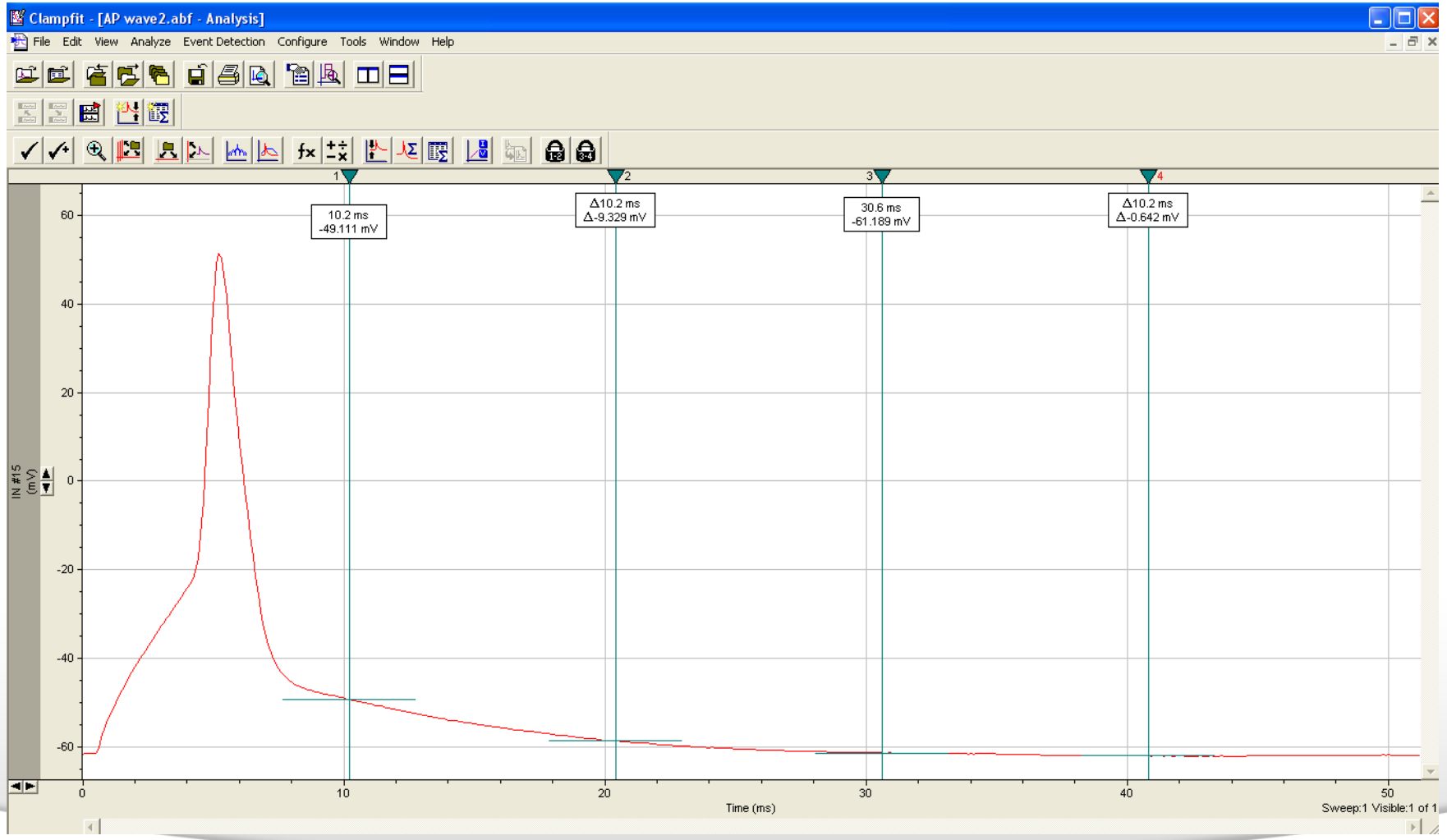
Stimulus File



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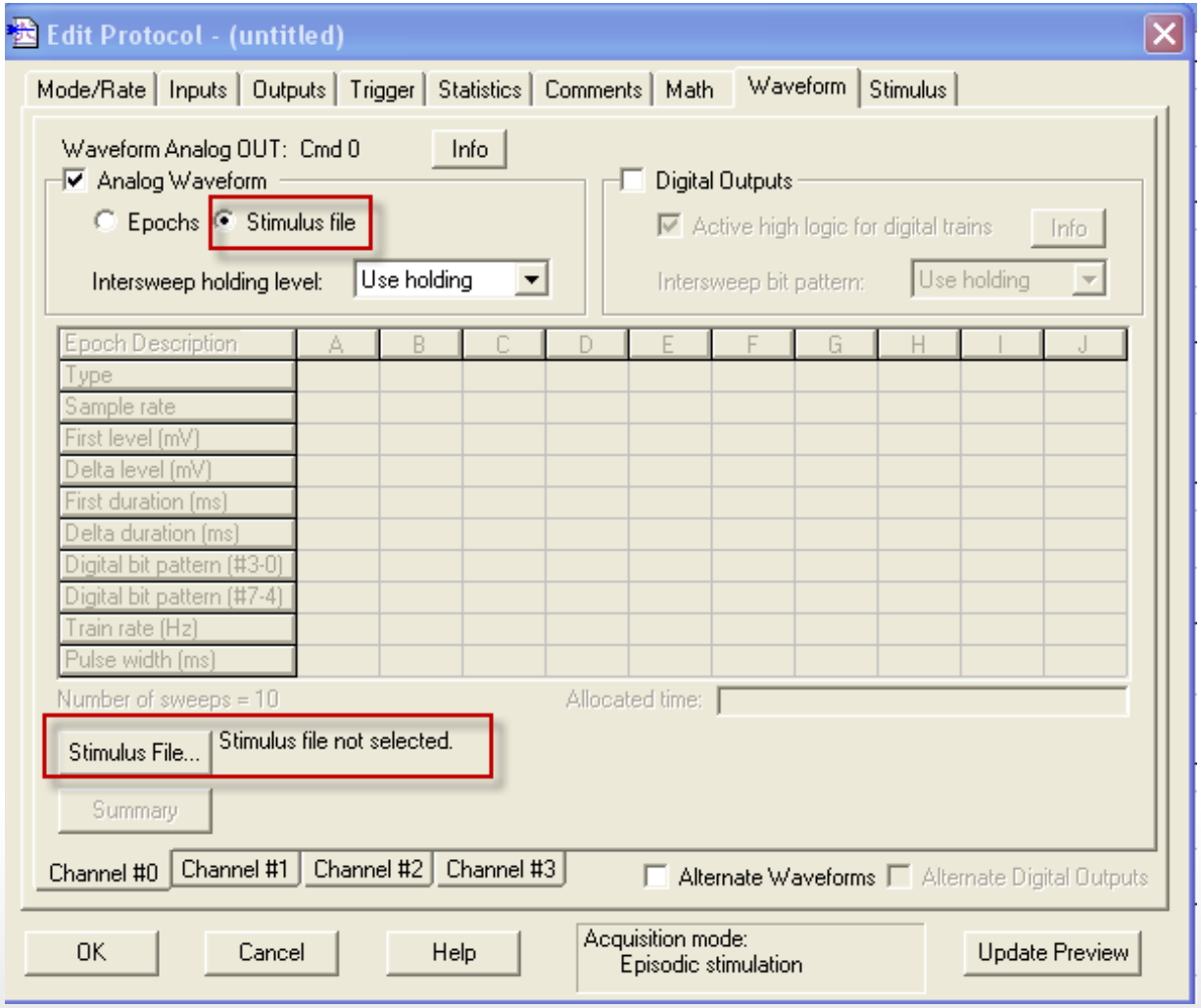

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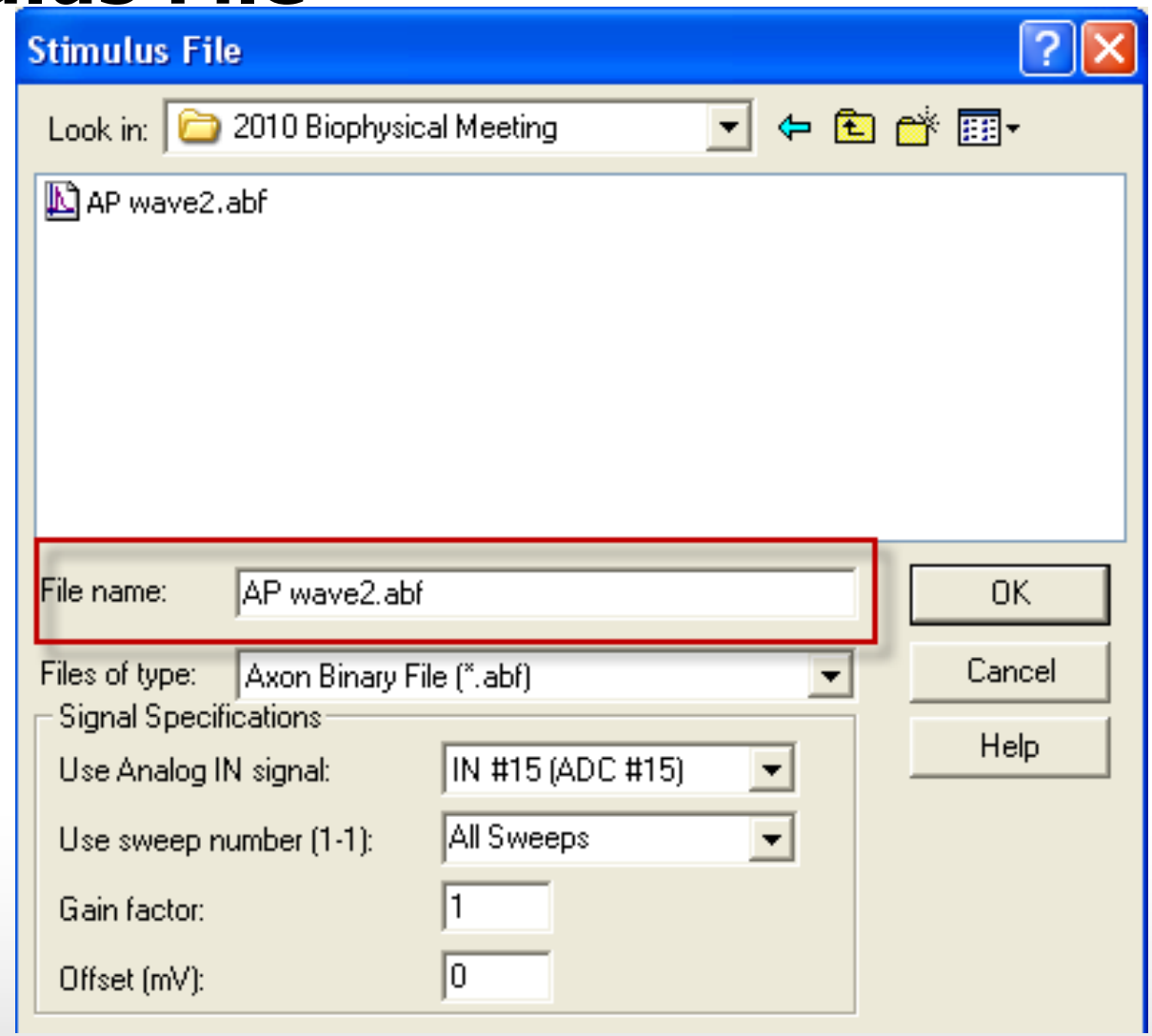
Stimulus File



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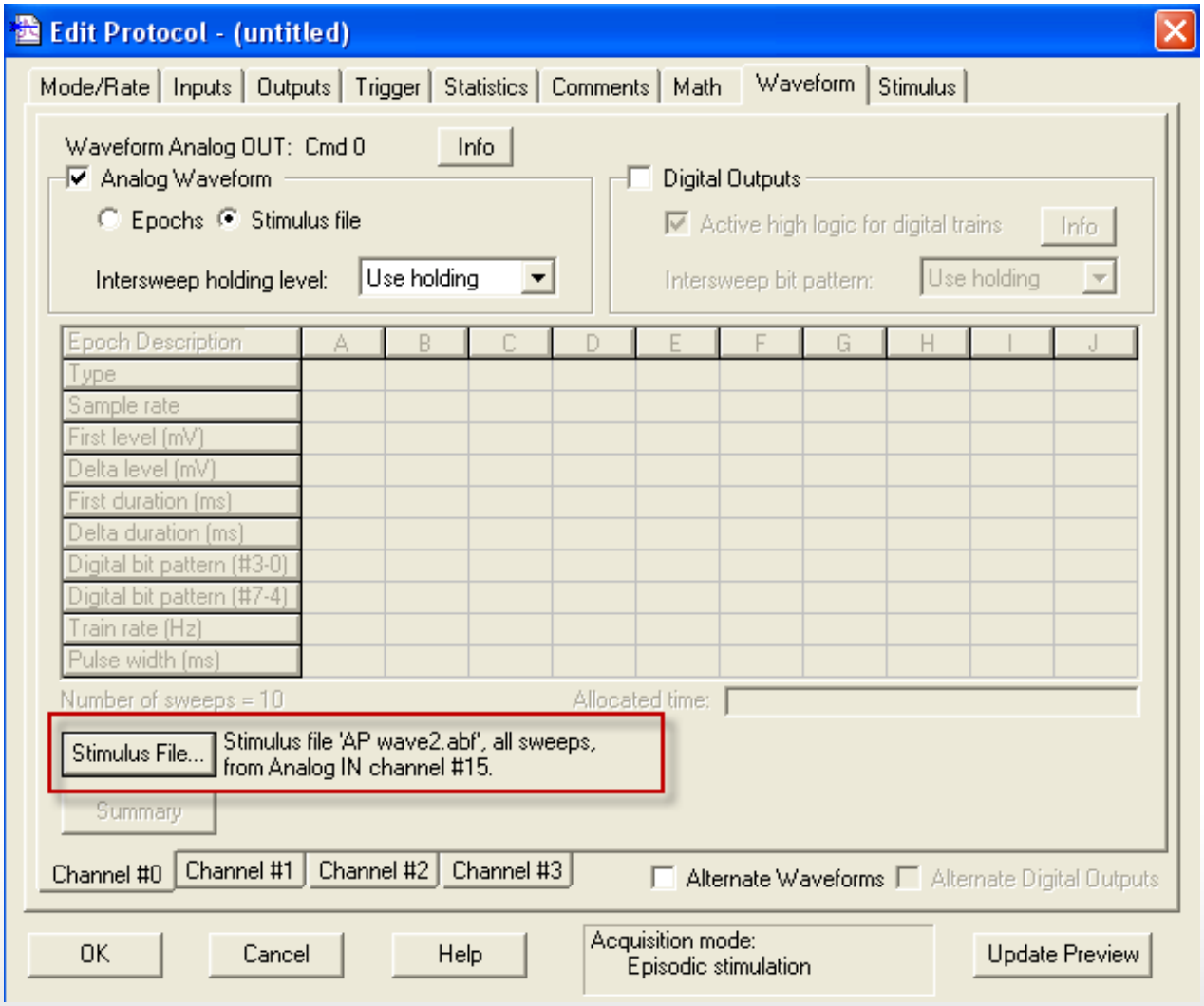
Stimulus File



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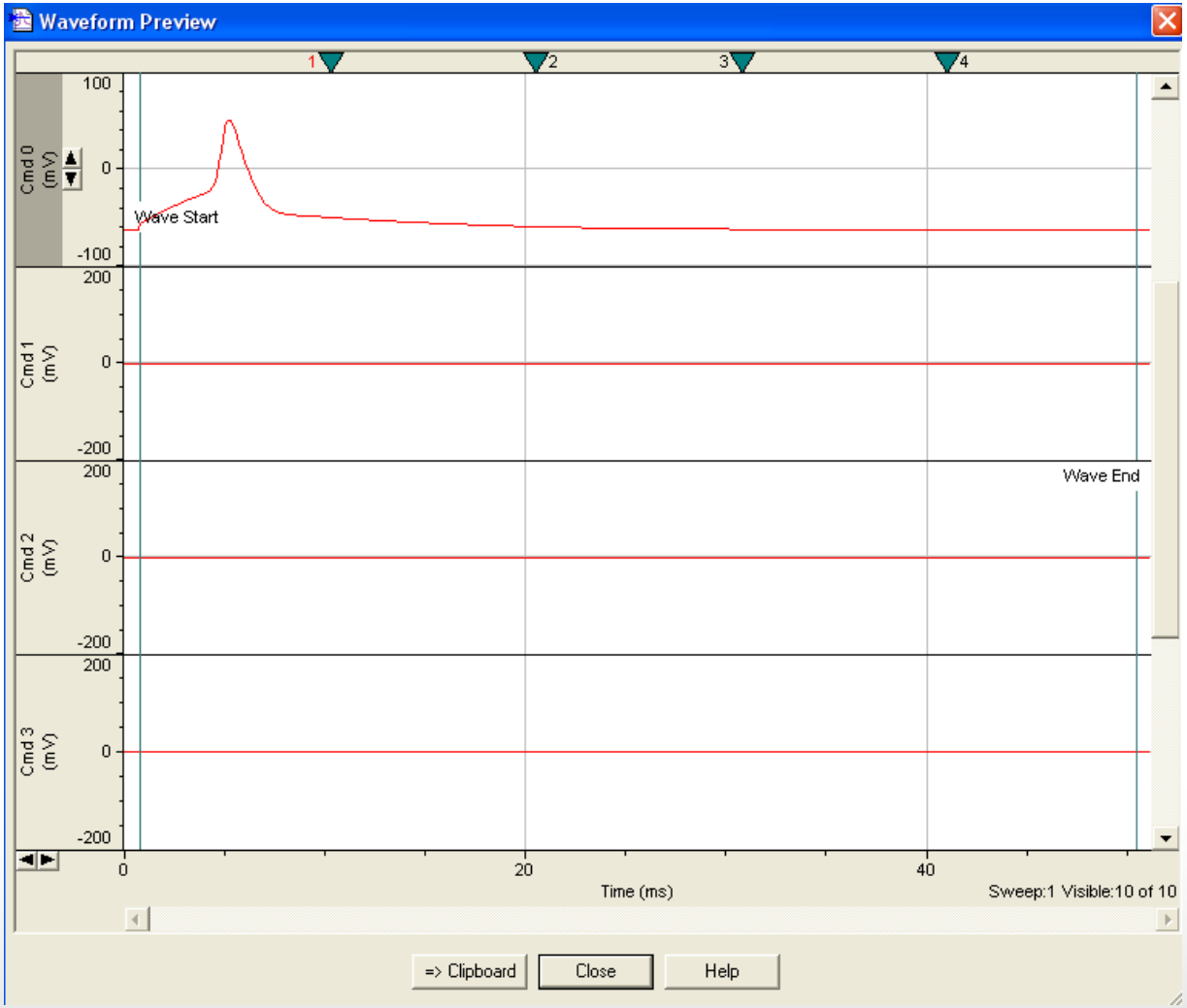
Action Potential Waveform



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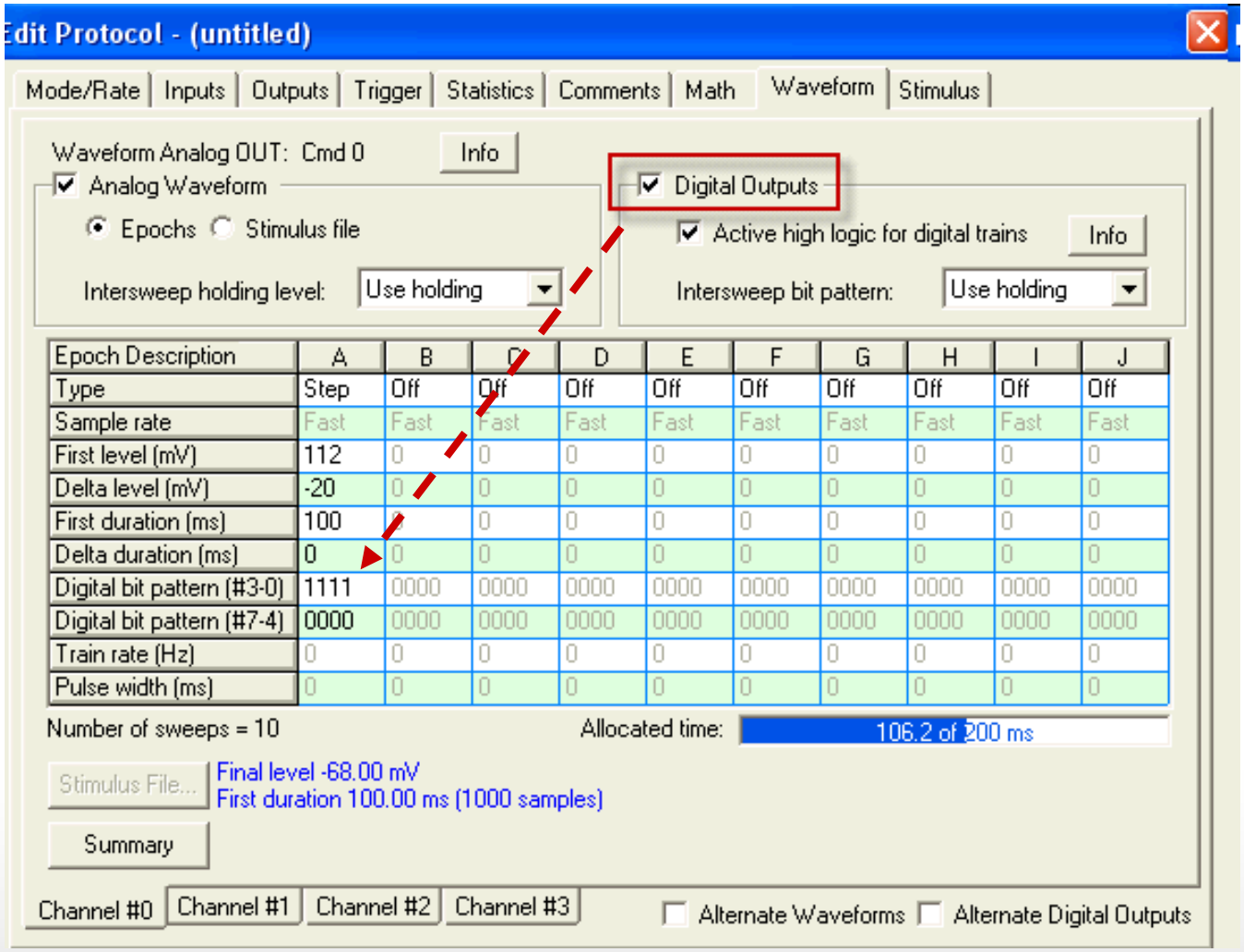
Digital Outputs



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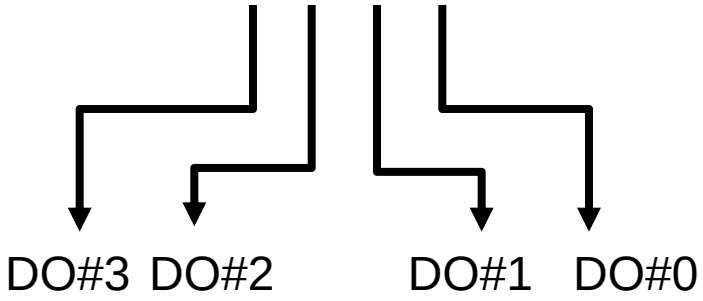
Digital Bit Pattern

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)

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Digital Bit Pattern---Single Pulse

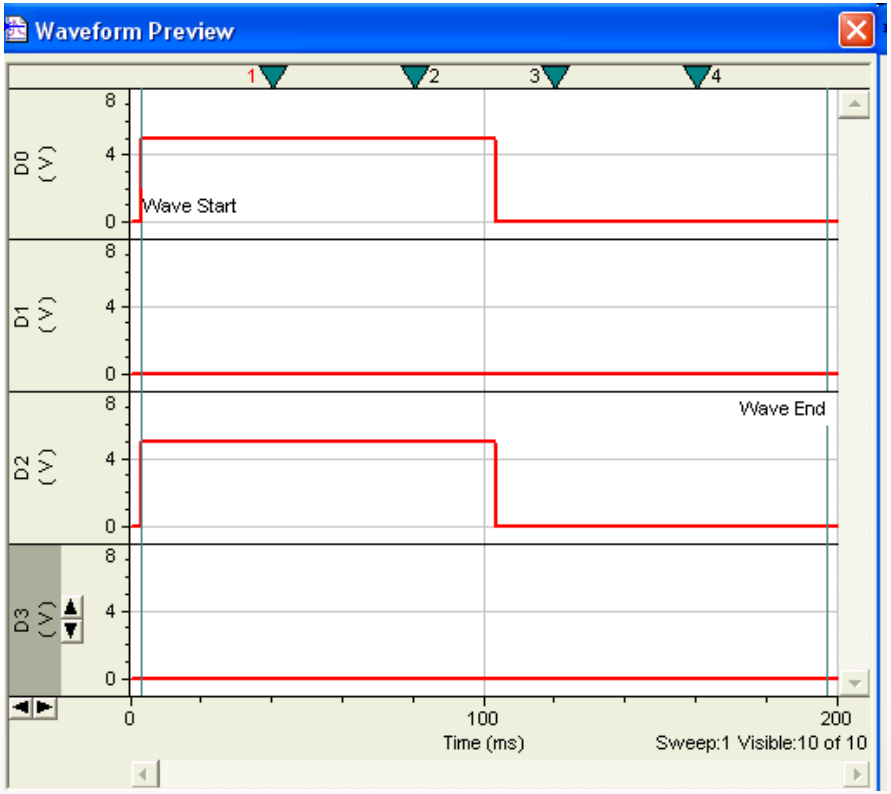
Digital bit pattern (#3-0): 0101

DO#0:

DO#1:

DO#2:

DO#3:



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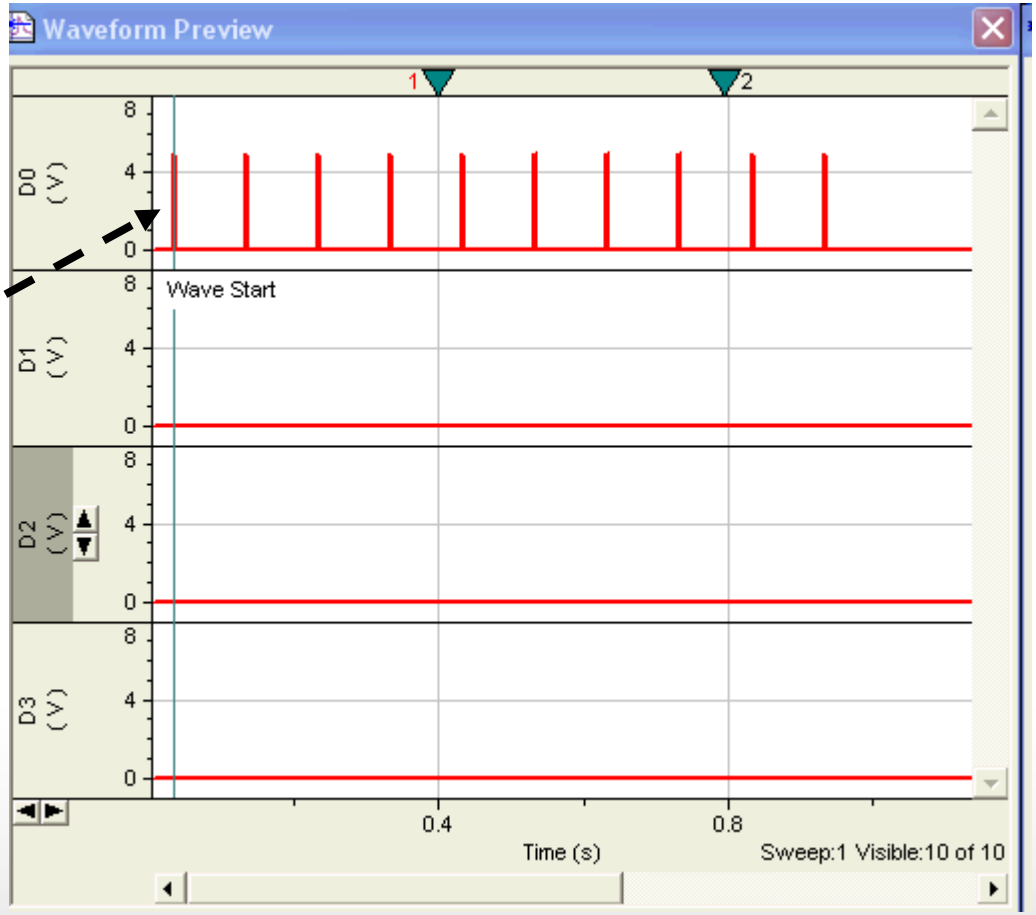
Digital Bit Pattern---Train Pulse

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



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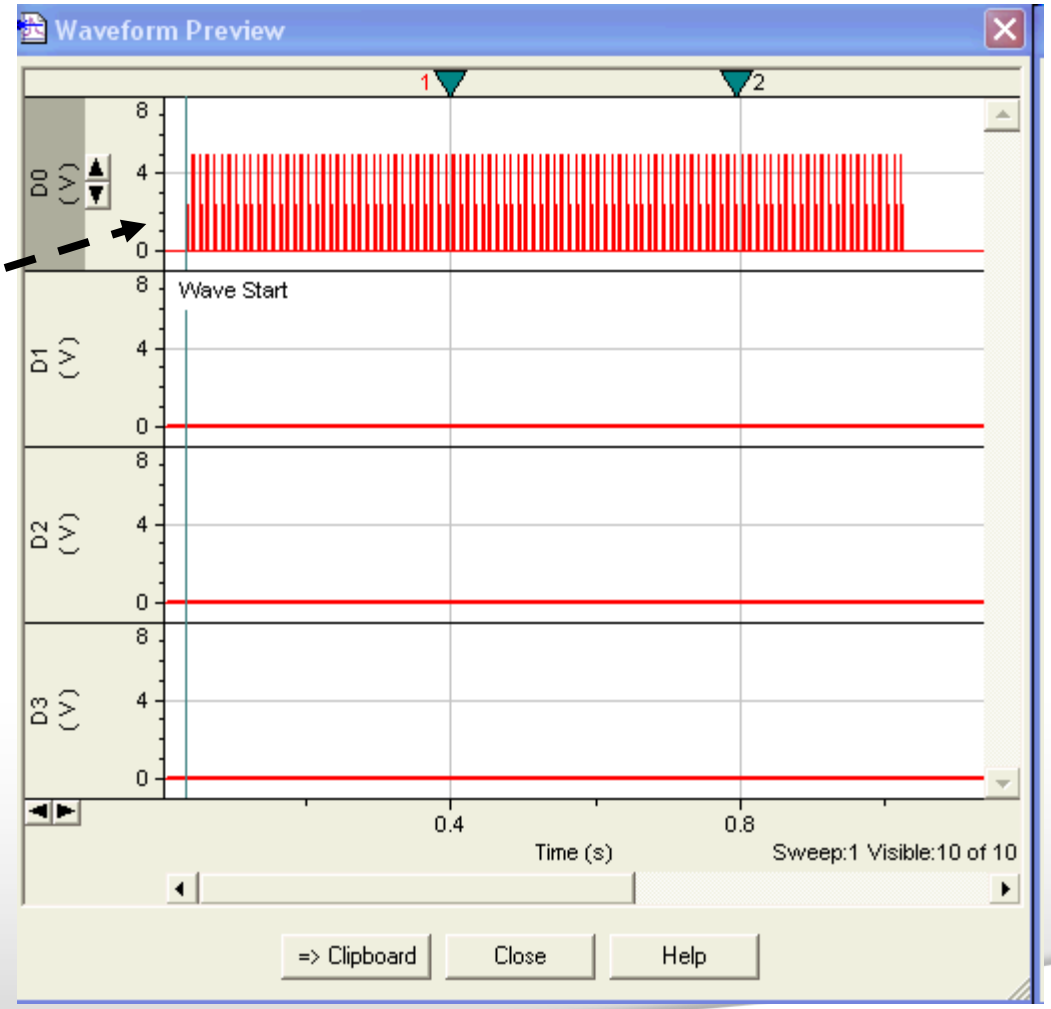
Digital Bit Pattern---Train Pulse

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



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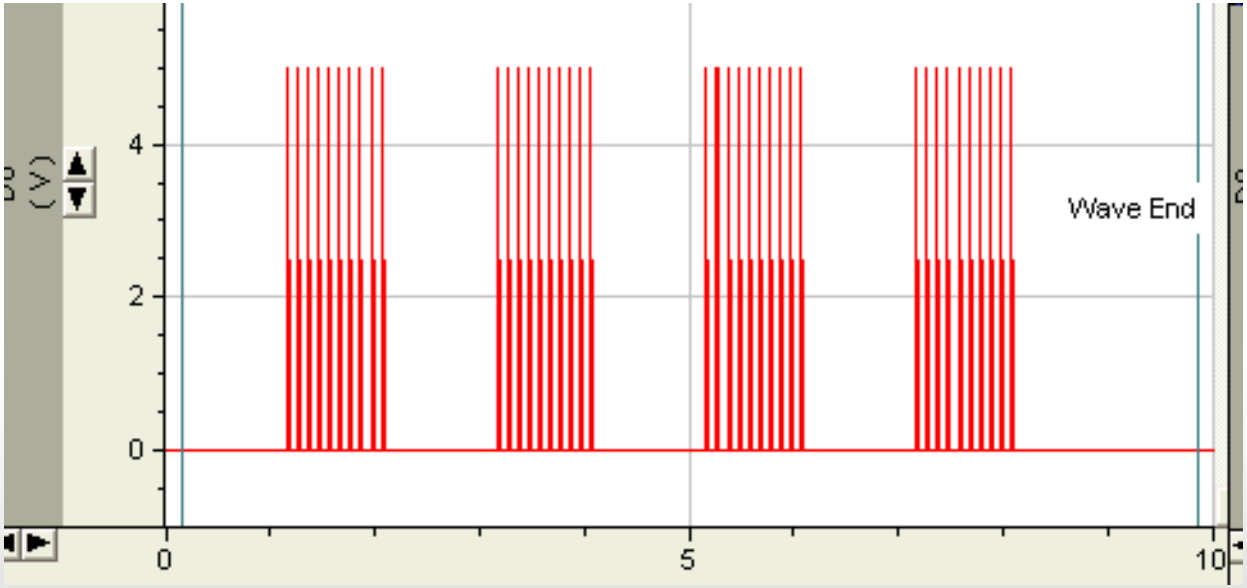
Digital Bit Pattern

	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



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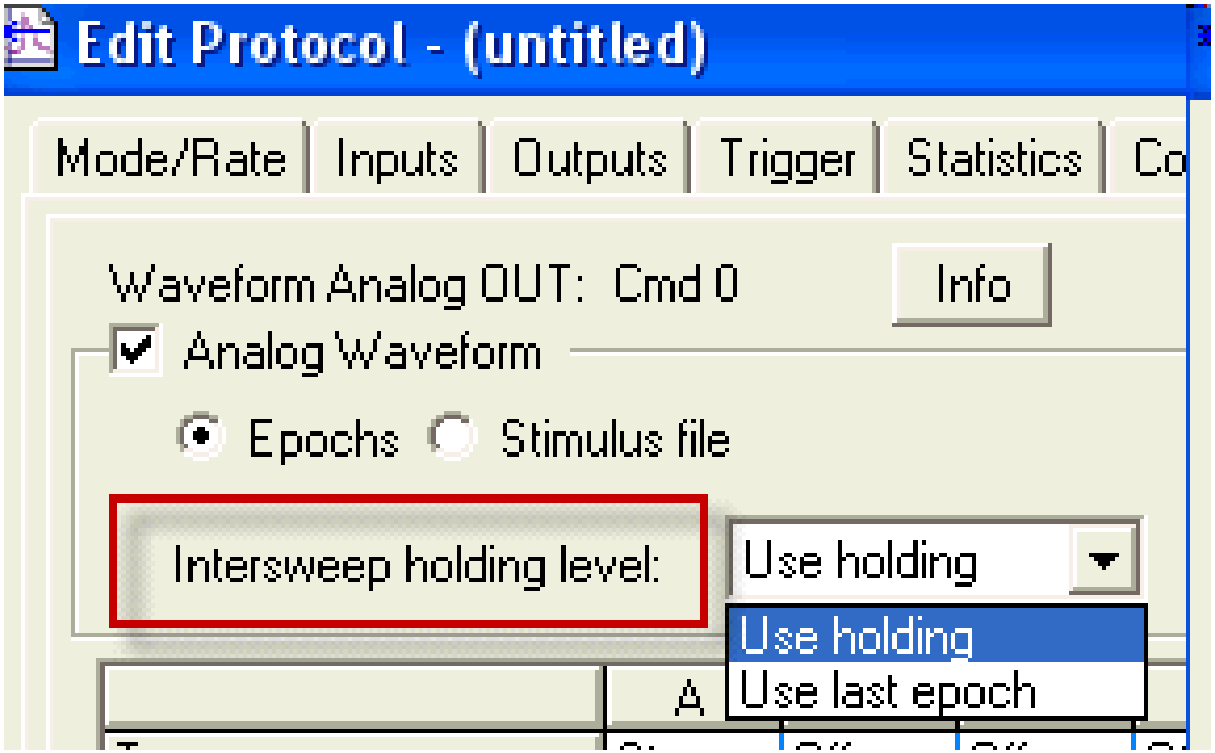
Intersweep Holding Level



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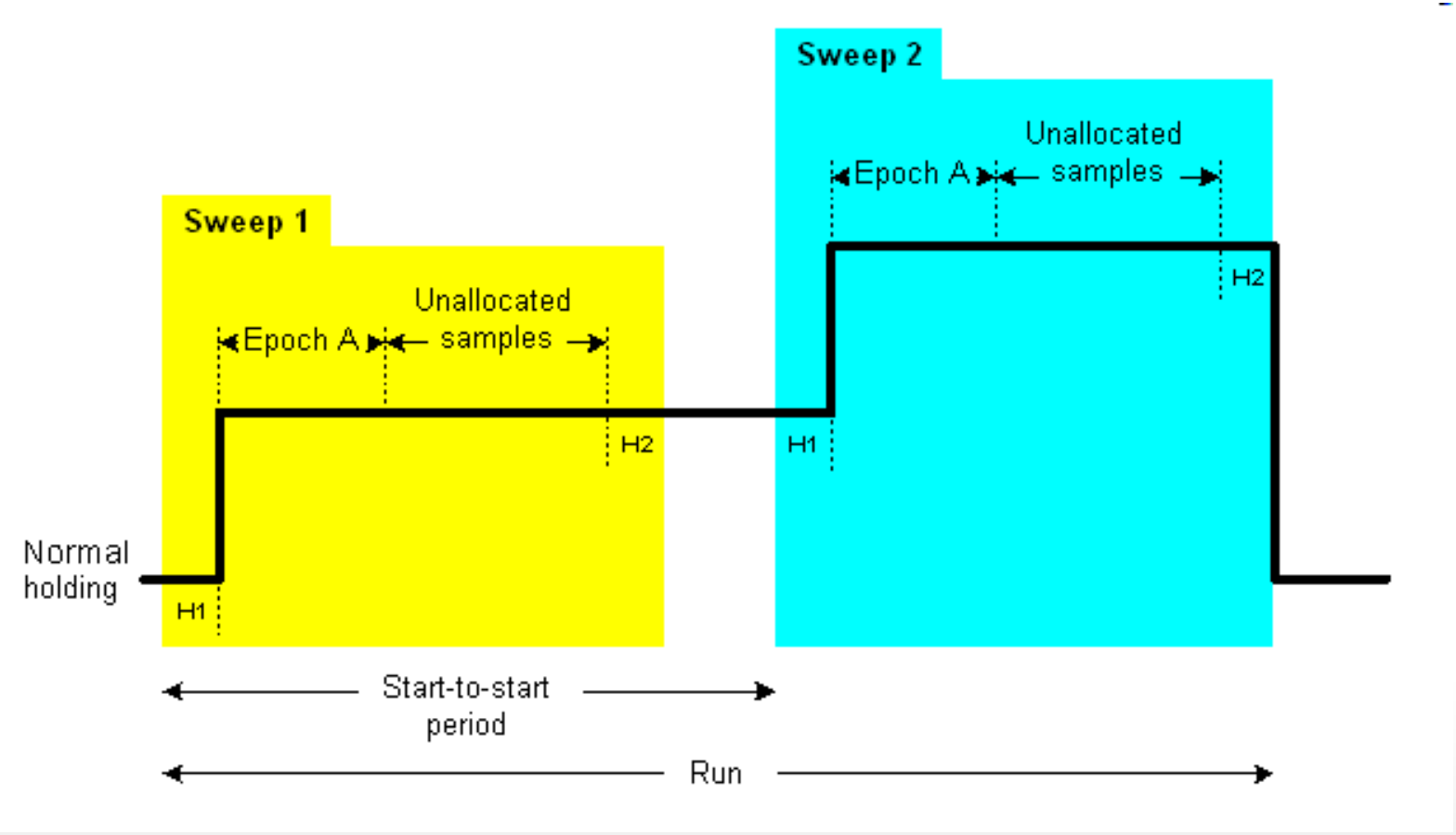
Intersweep Holding Level---Use last epoch



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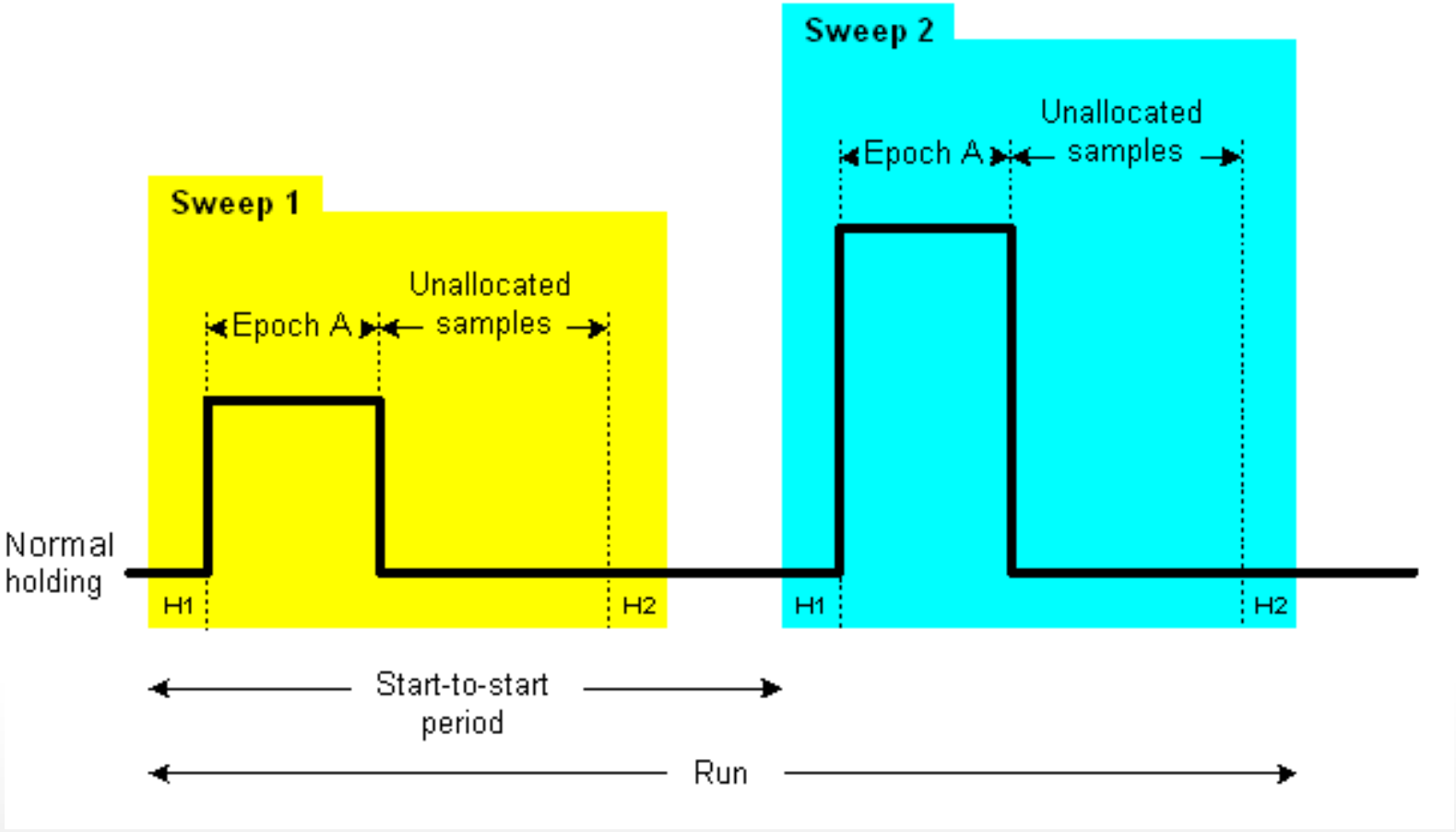
Intersweep Holding Level---Use Holding



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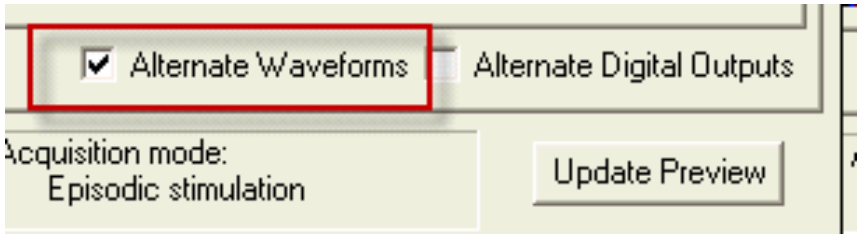
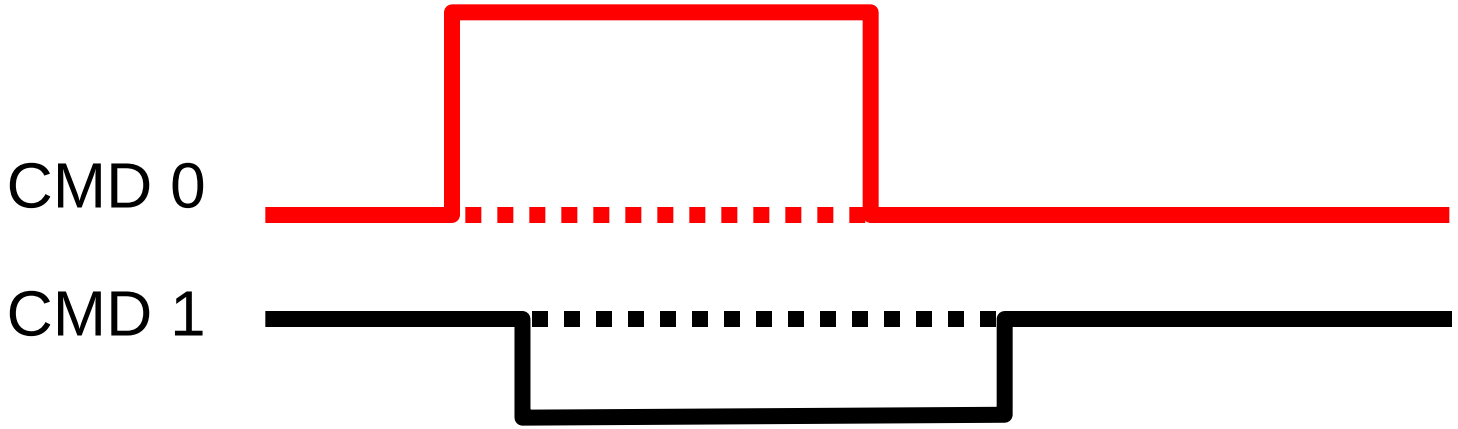
Alternative Waveform





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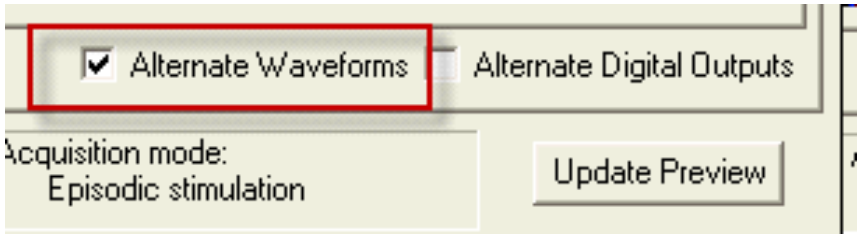
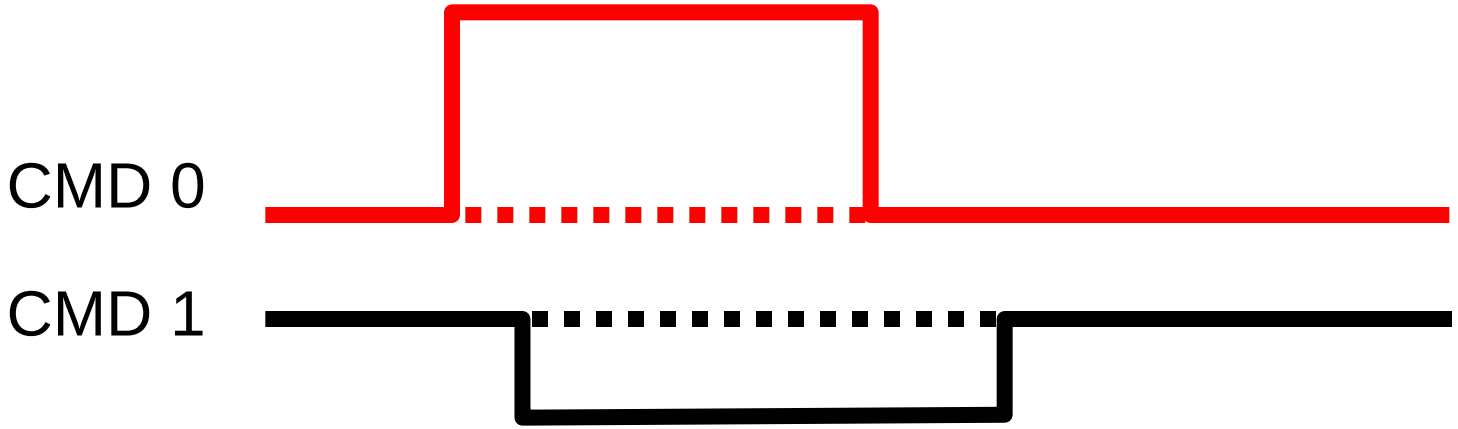
Alternative Waveform





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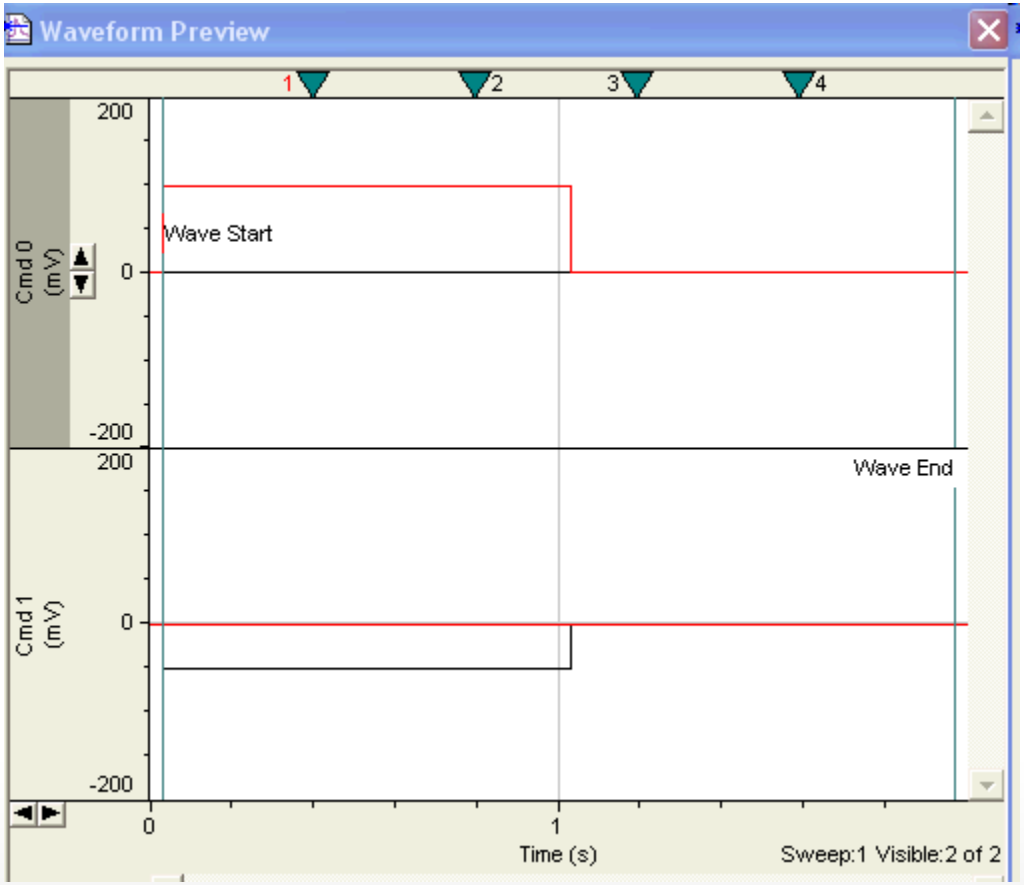
Alternative Waveform



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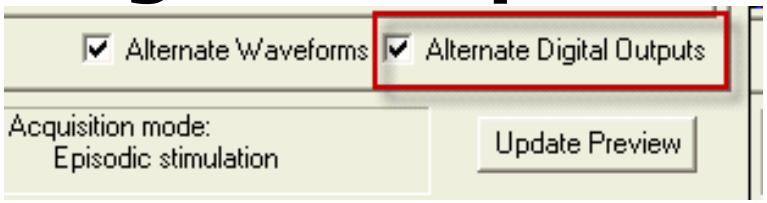
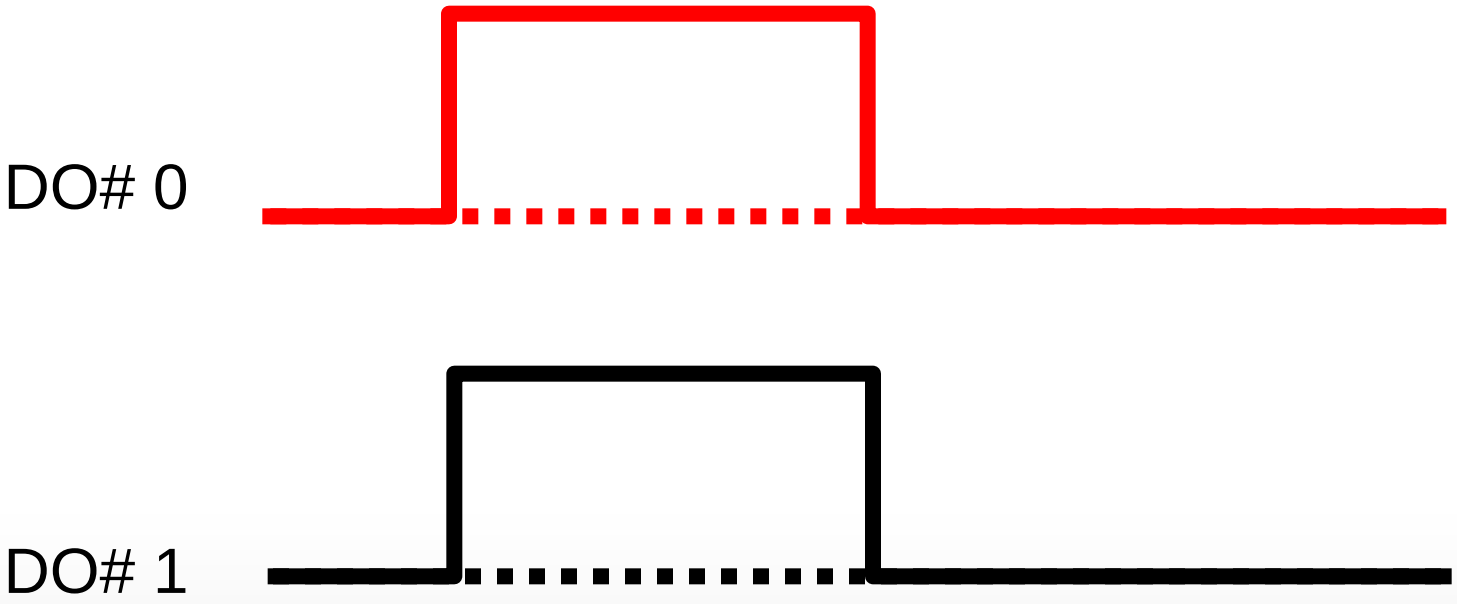
Alternative Digital Outputs





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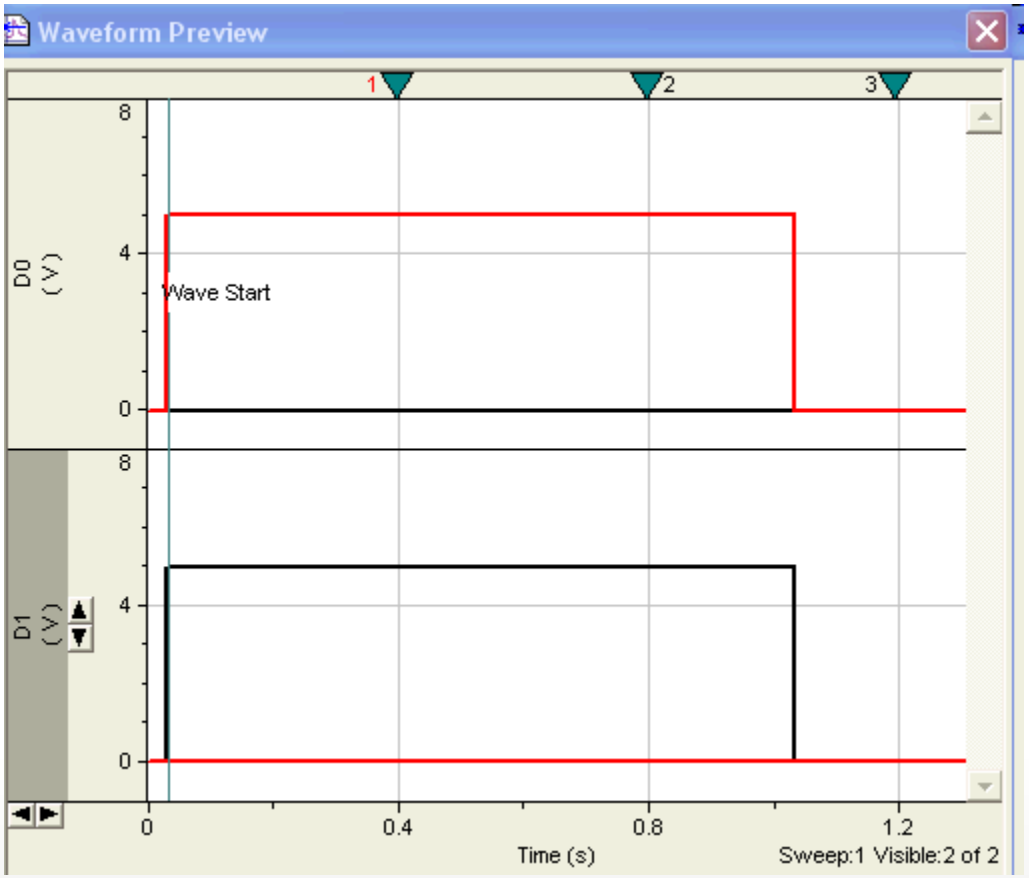
Alternative Digital Outputs



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Pre-sweep Train

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

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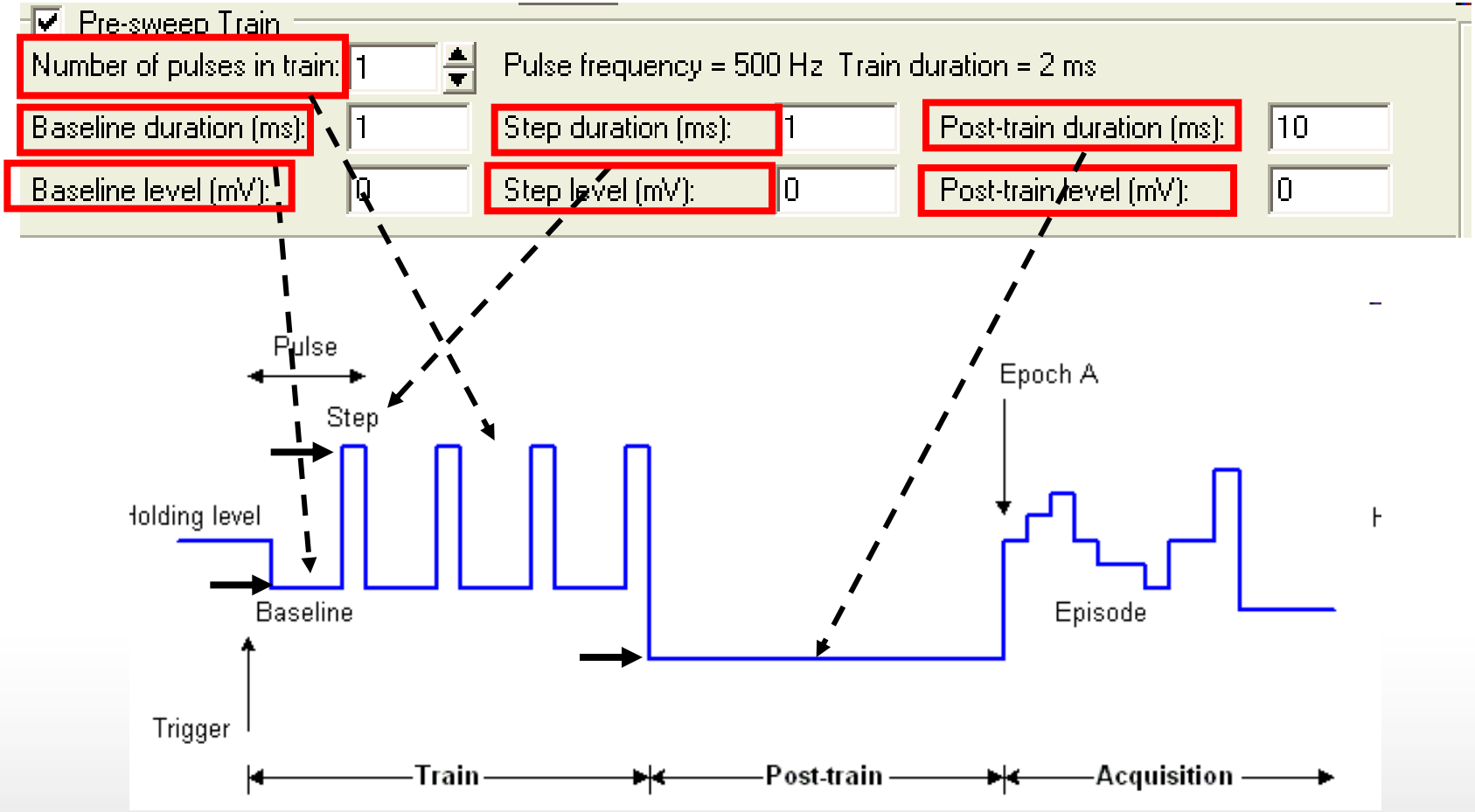
Pre-sweep Train



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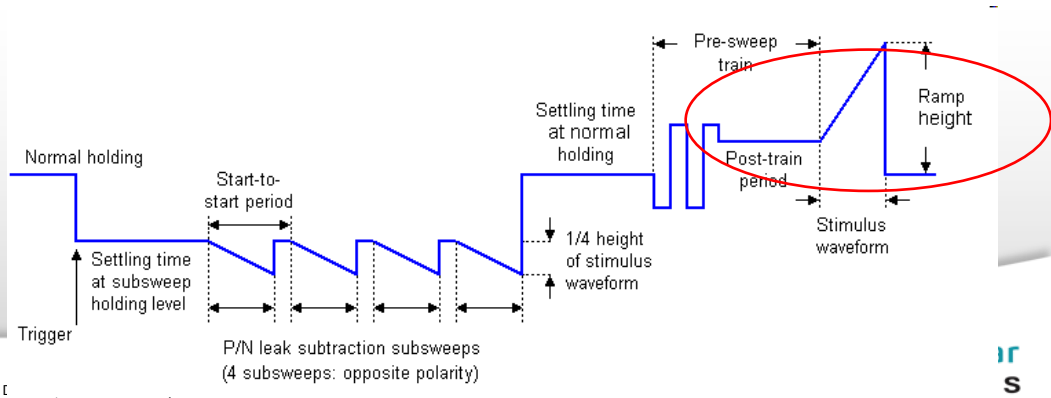
P/N Leak Subtraction

- 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



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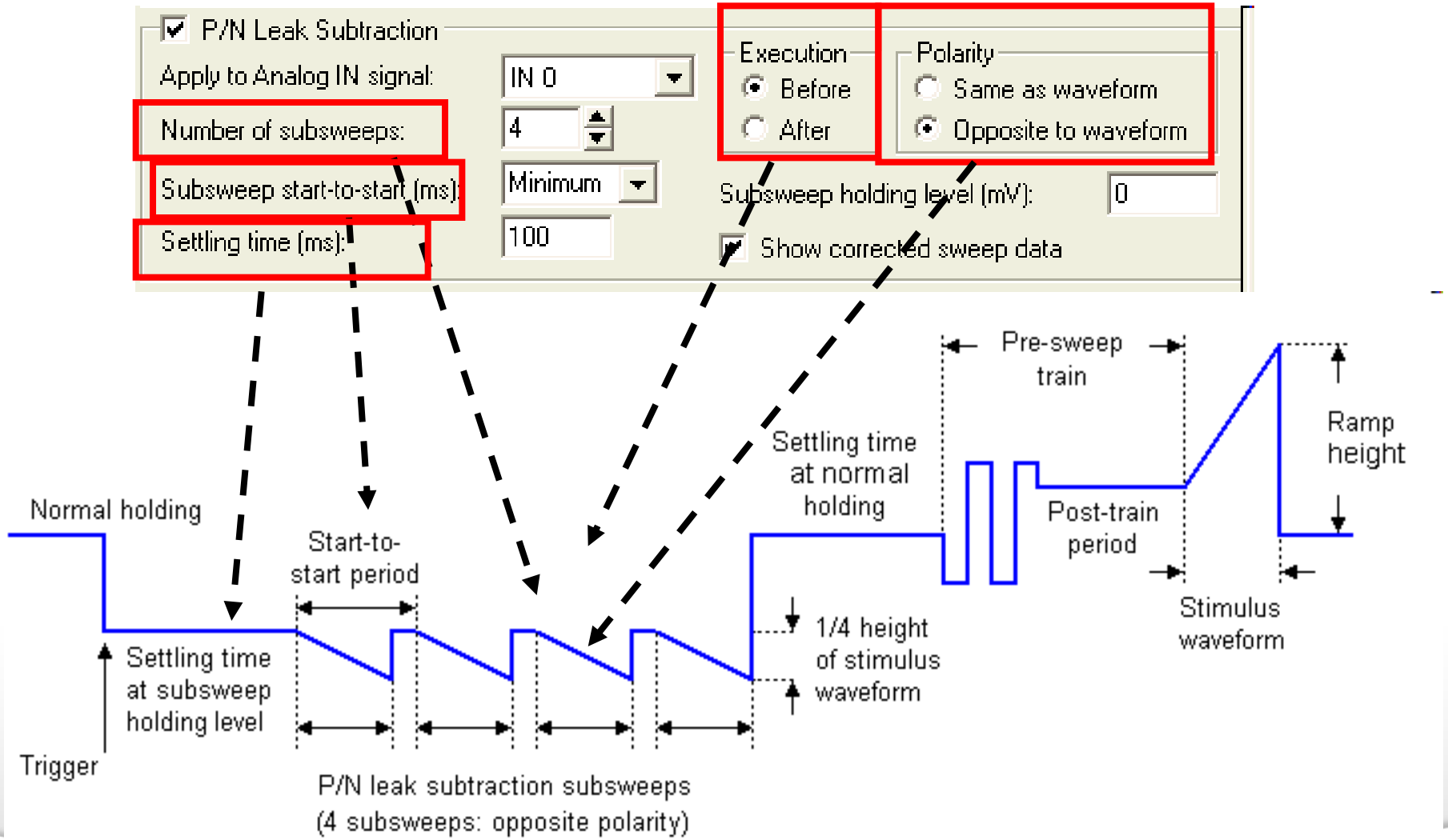
P/N Leak Subtraction



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User List

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

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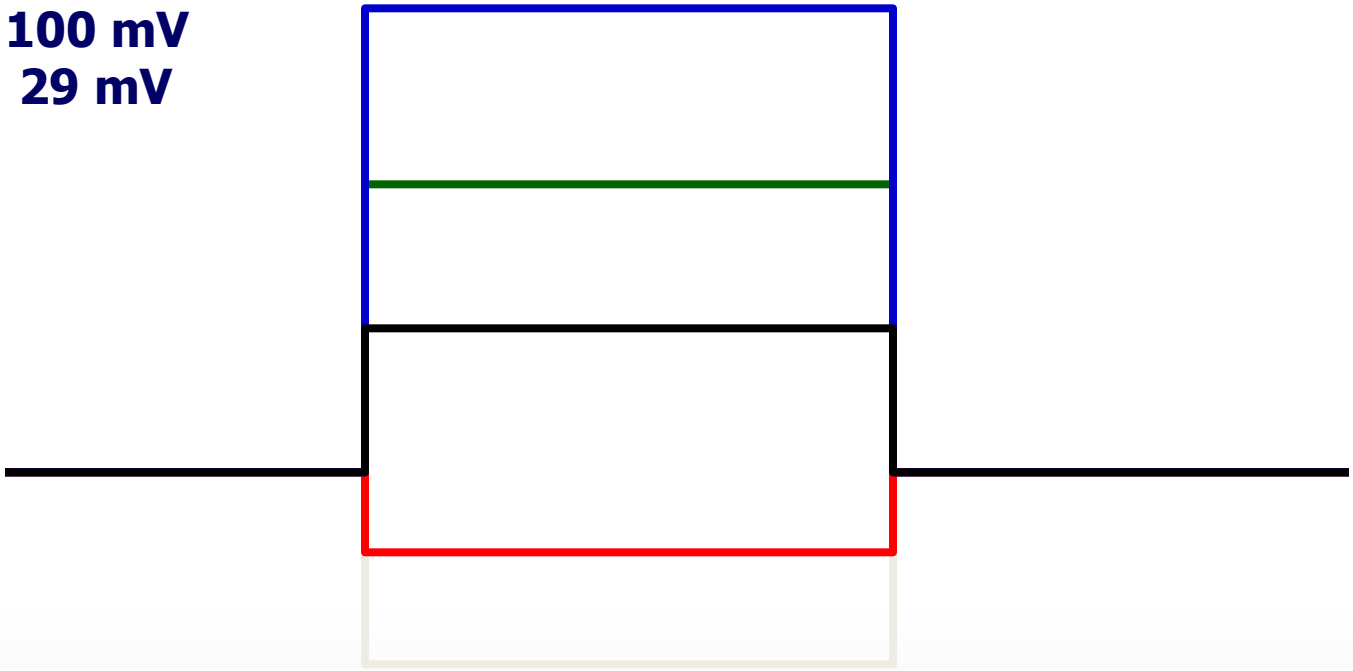
User List---Holding Level

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.

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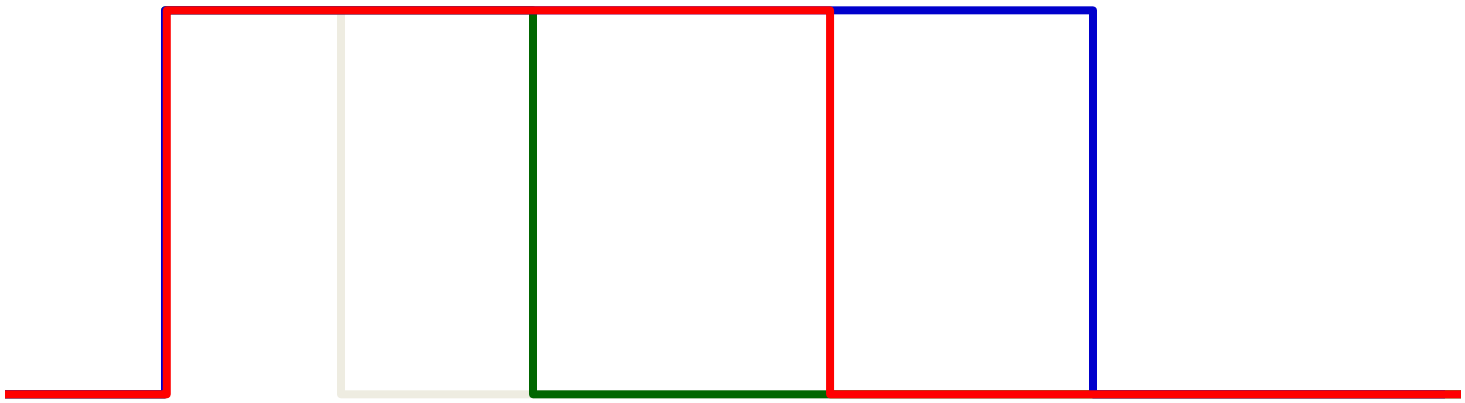
User List---Epoch Duration

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.

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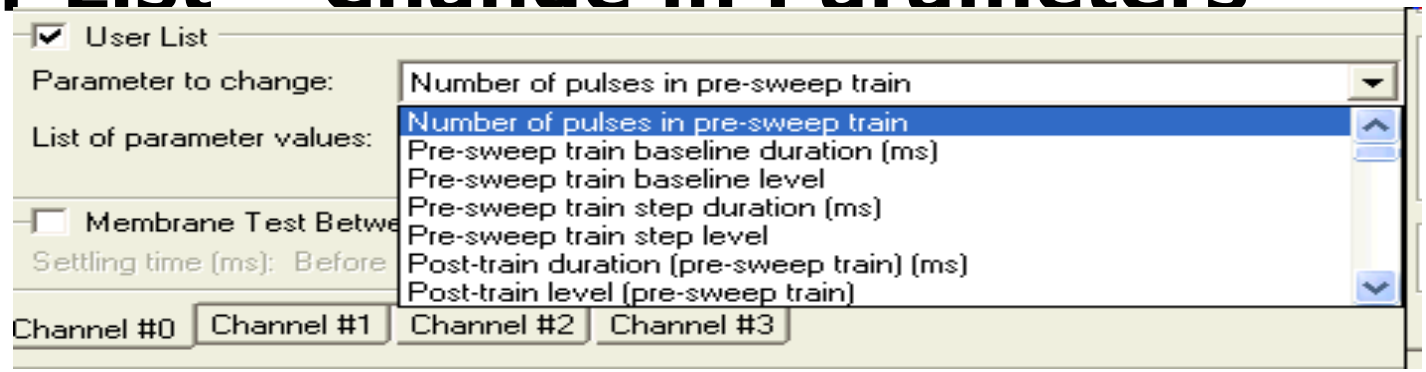
User List---Change in Parameters



- 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)

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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

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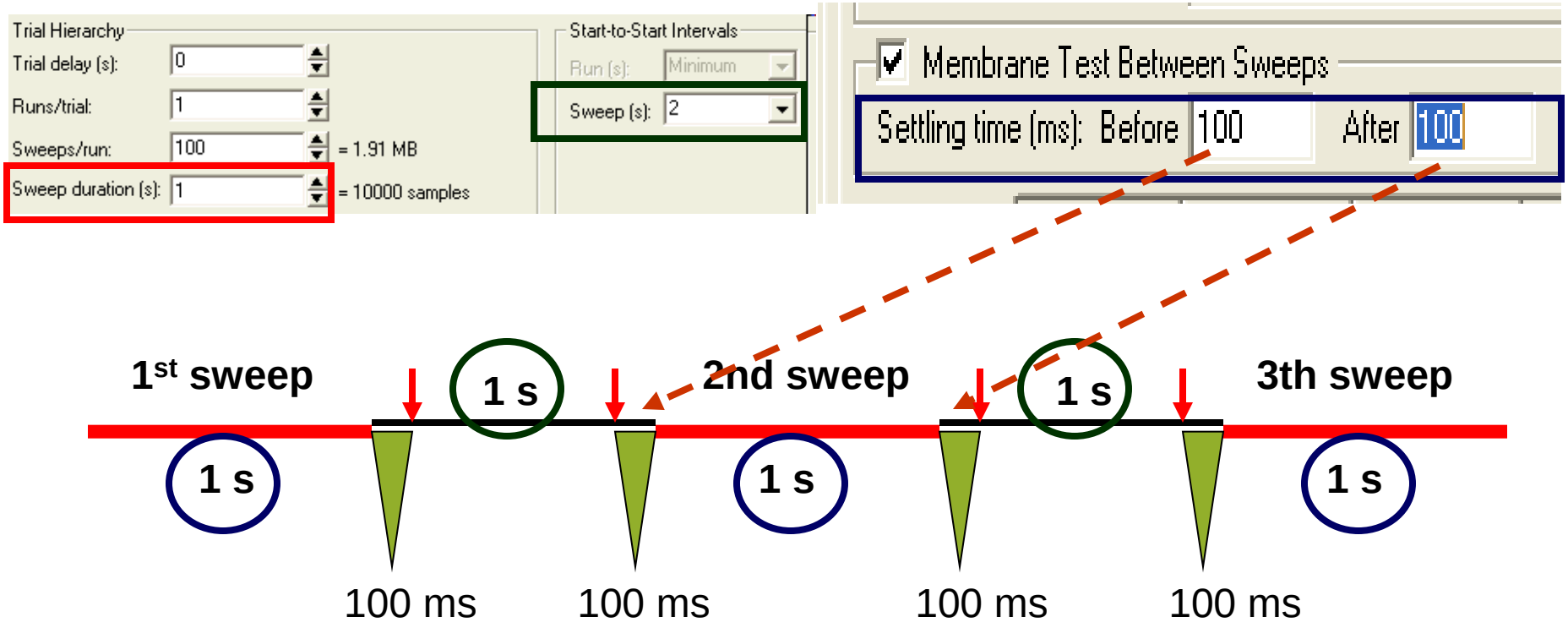
Membrane Test Between Sweeps



= Membrane test

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pCLAMP™ 10 data acquisition software: Key features review

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Key features of Clampex 10

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

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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

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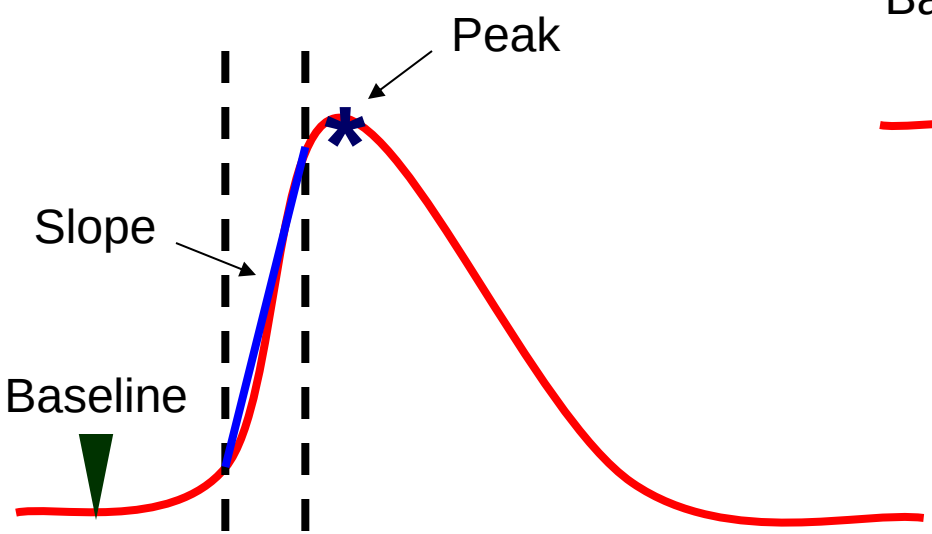
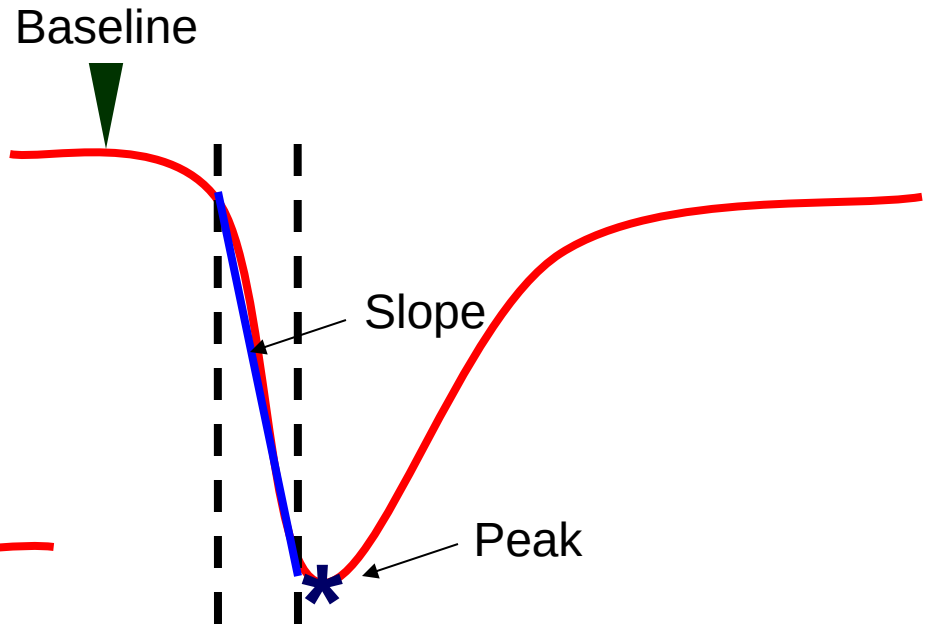
Outward signal



Example:
Excitatory postsynaptic potential (EPSP)

Inward signal



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

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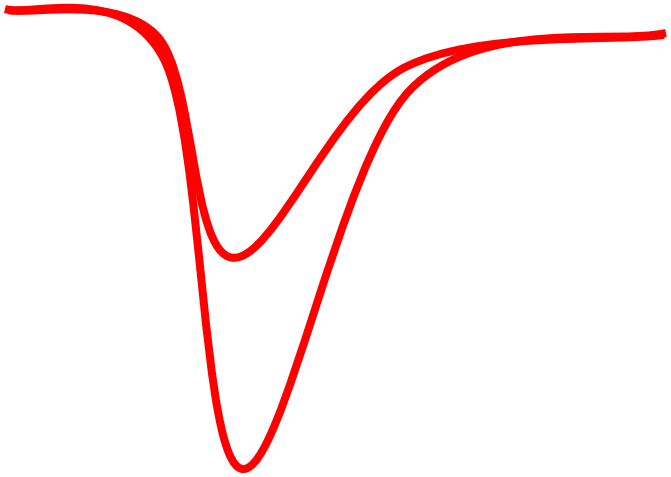

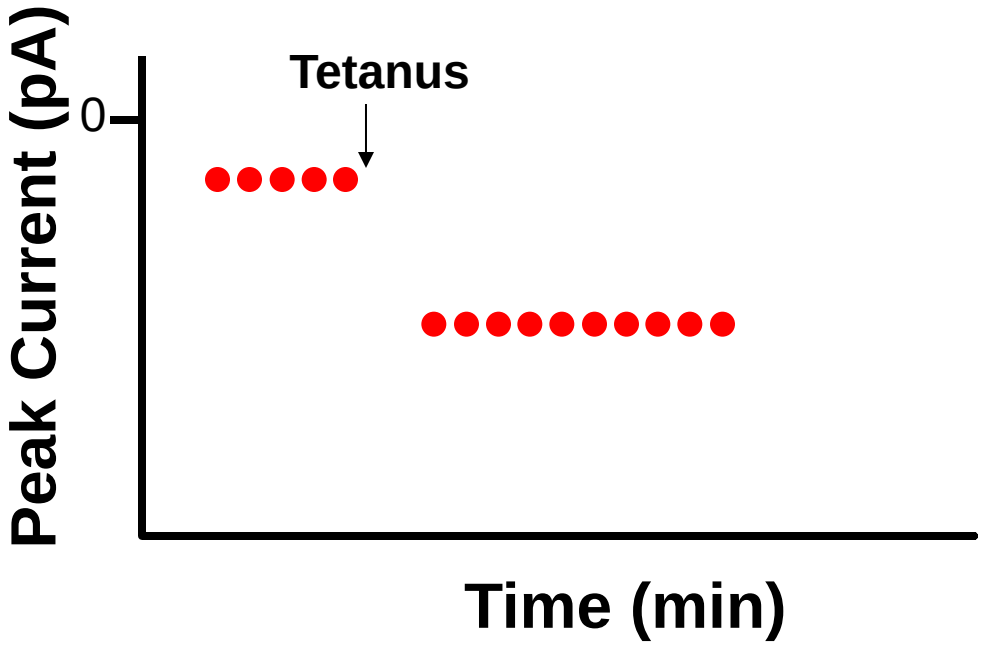

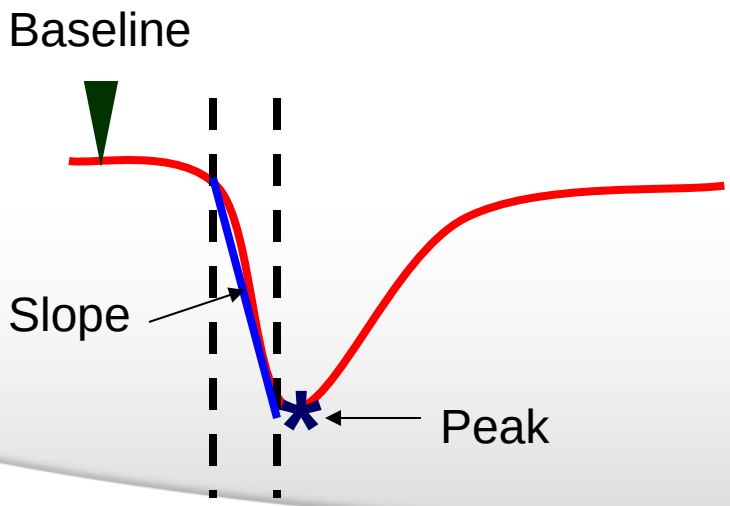

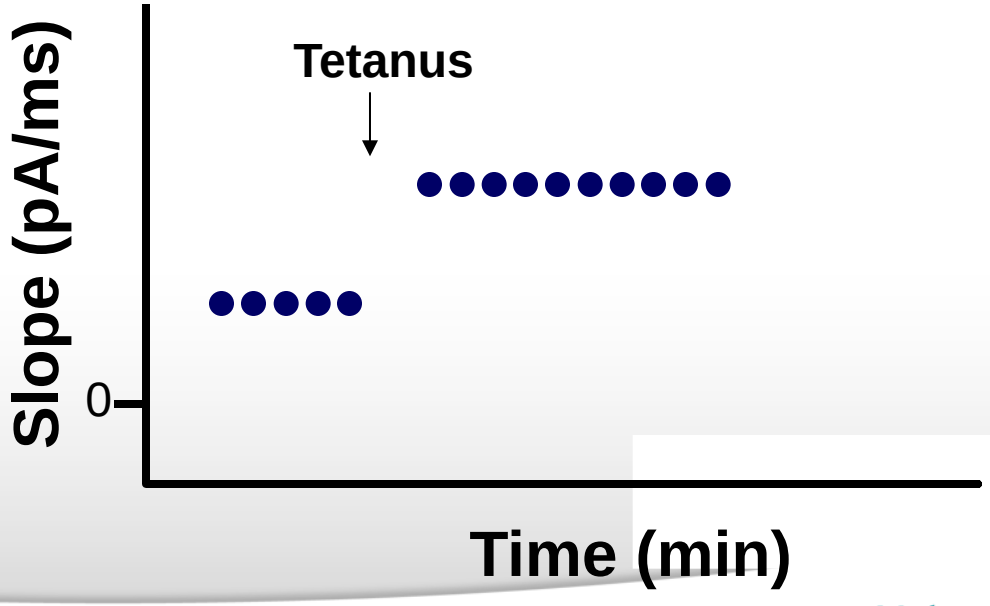

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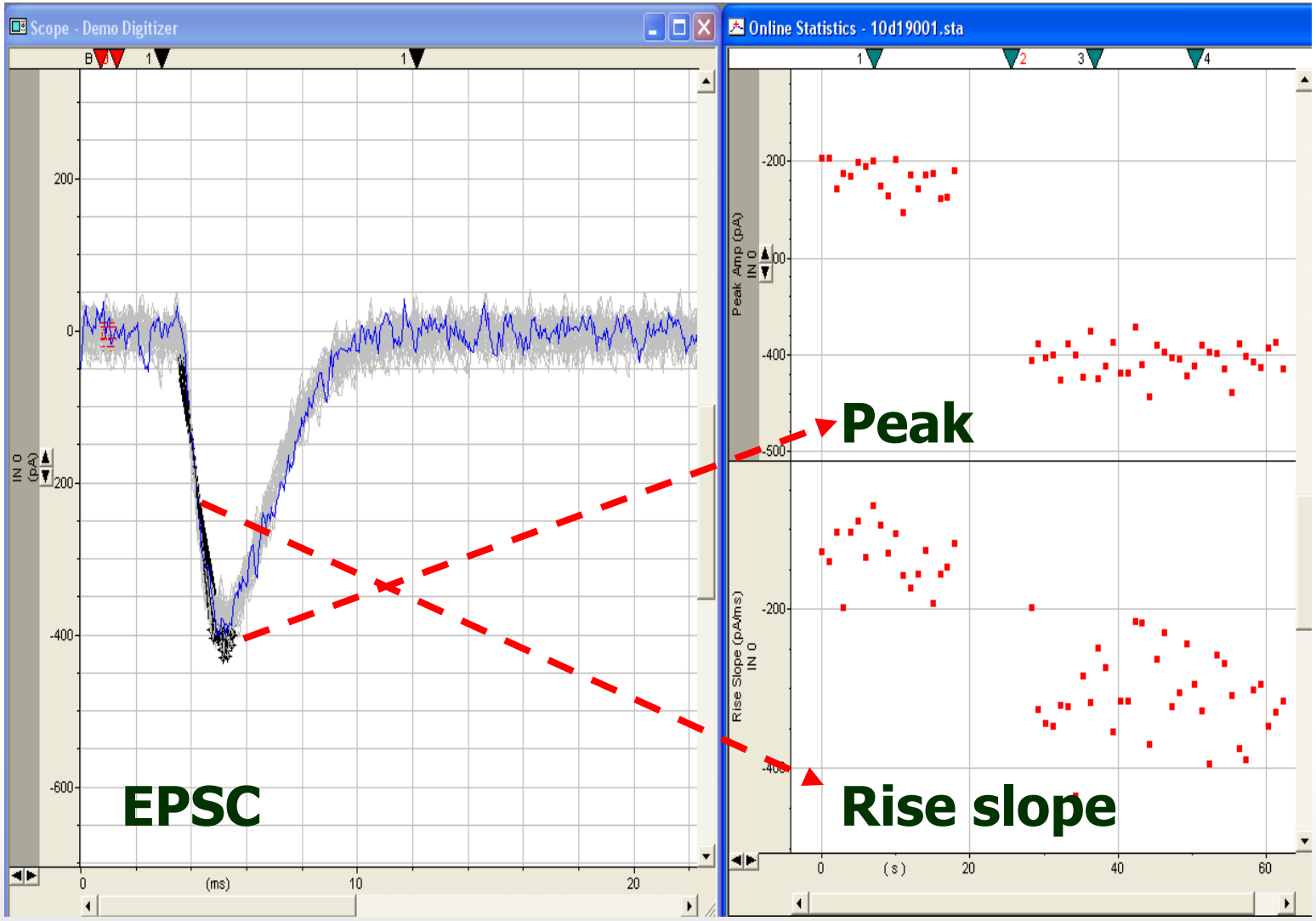

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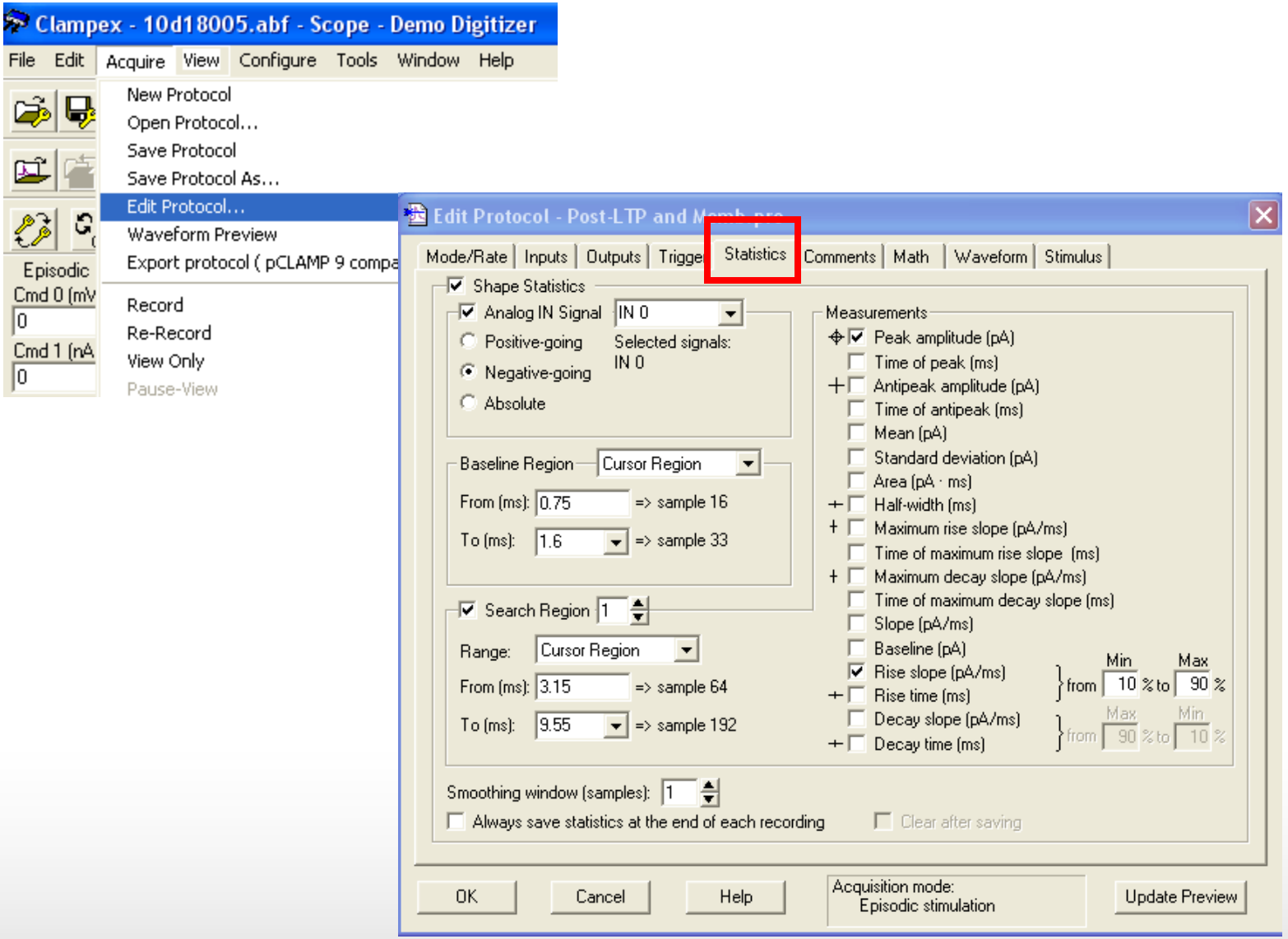

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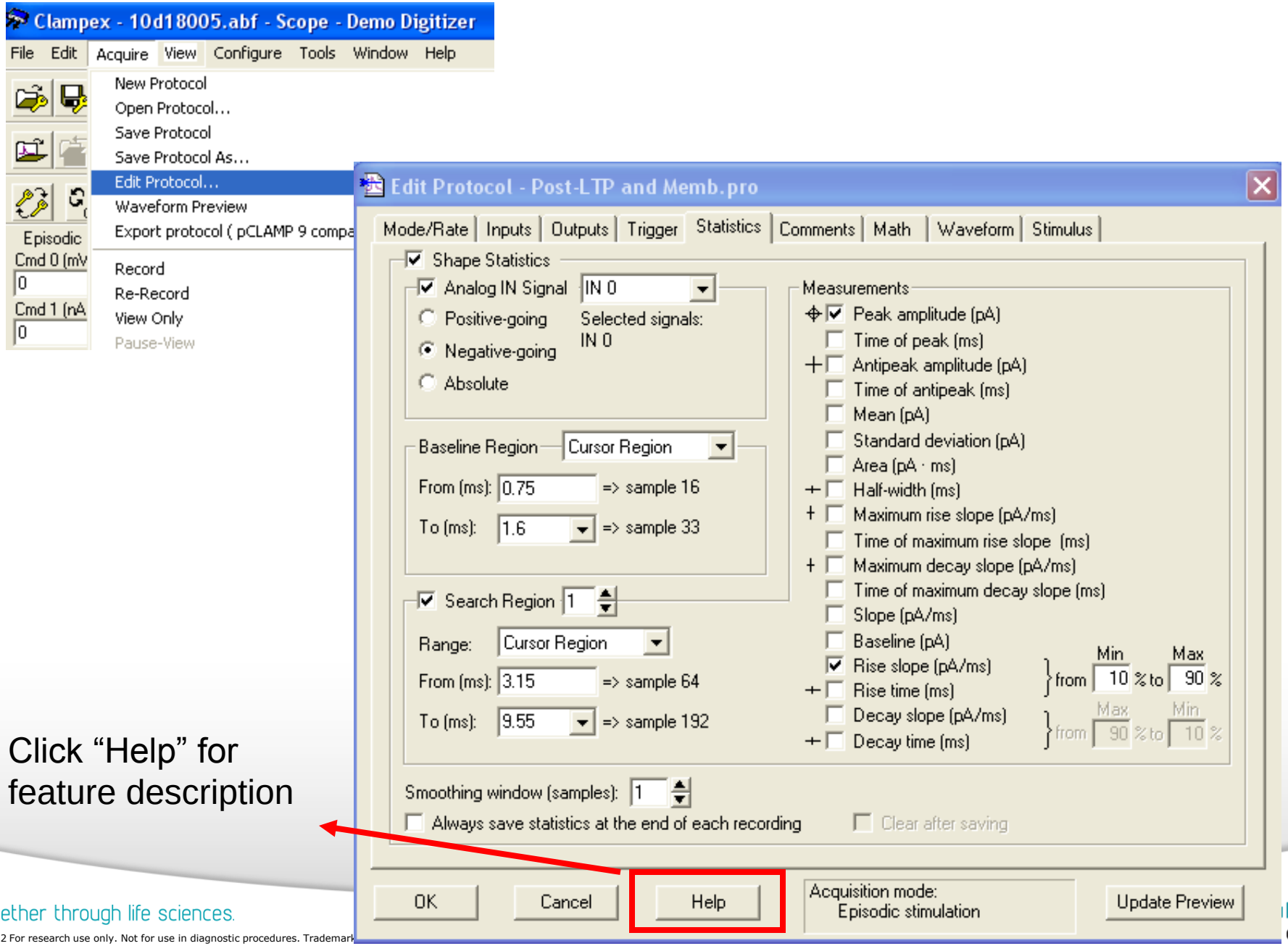

Click “Help” for feature description

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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.**

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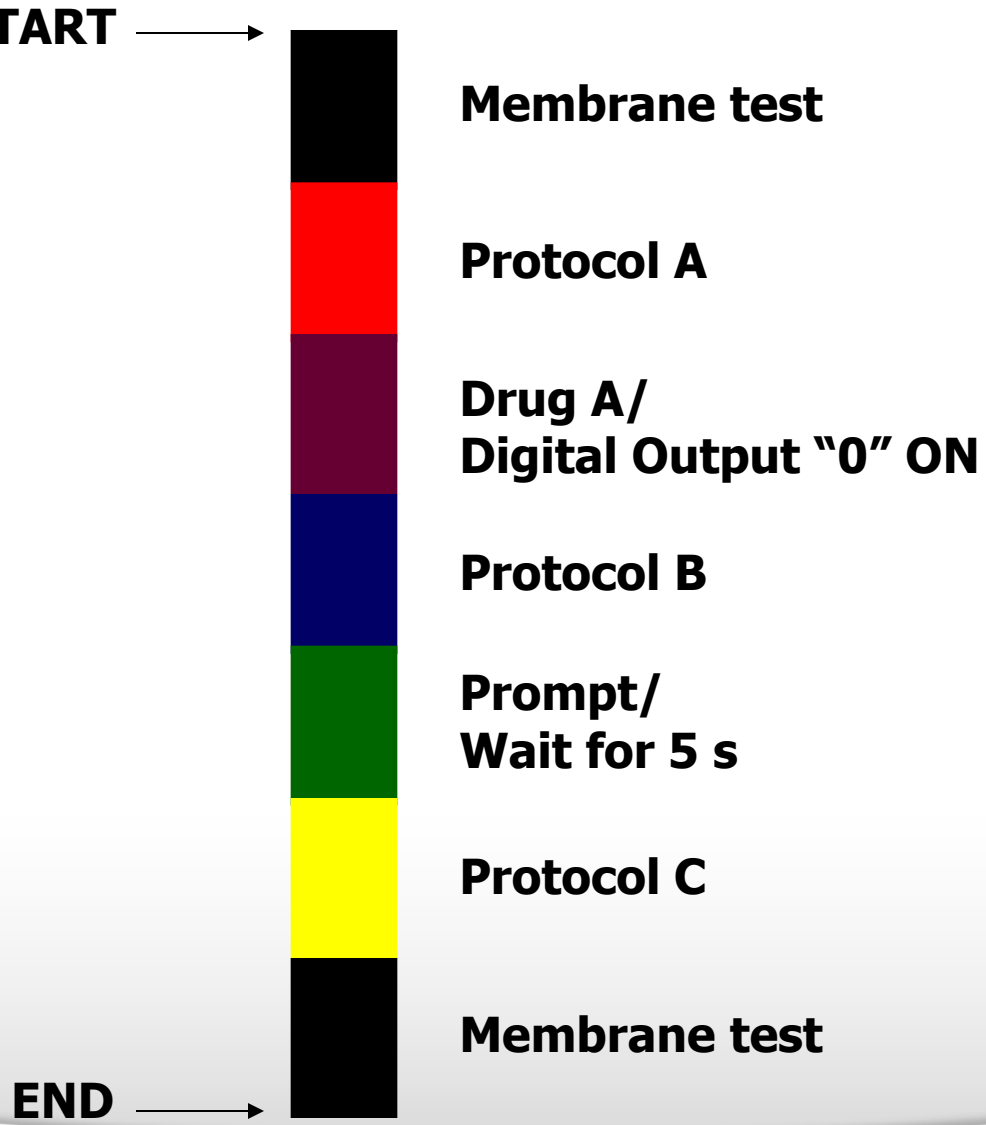
An experimental protocol



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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.

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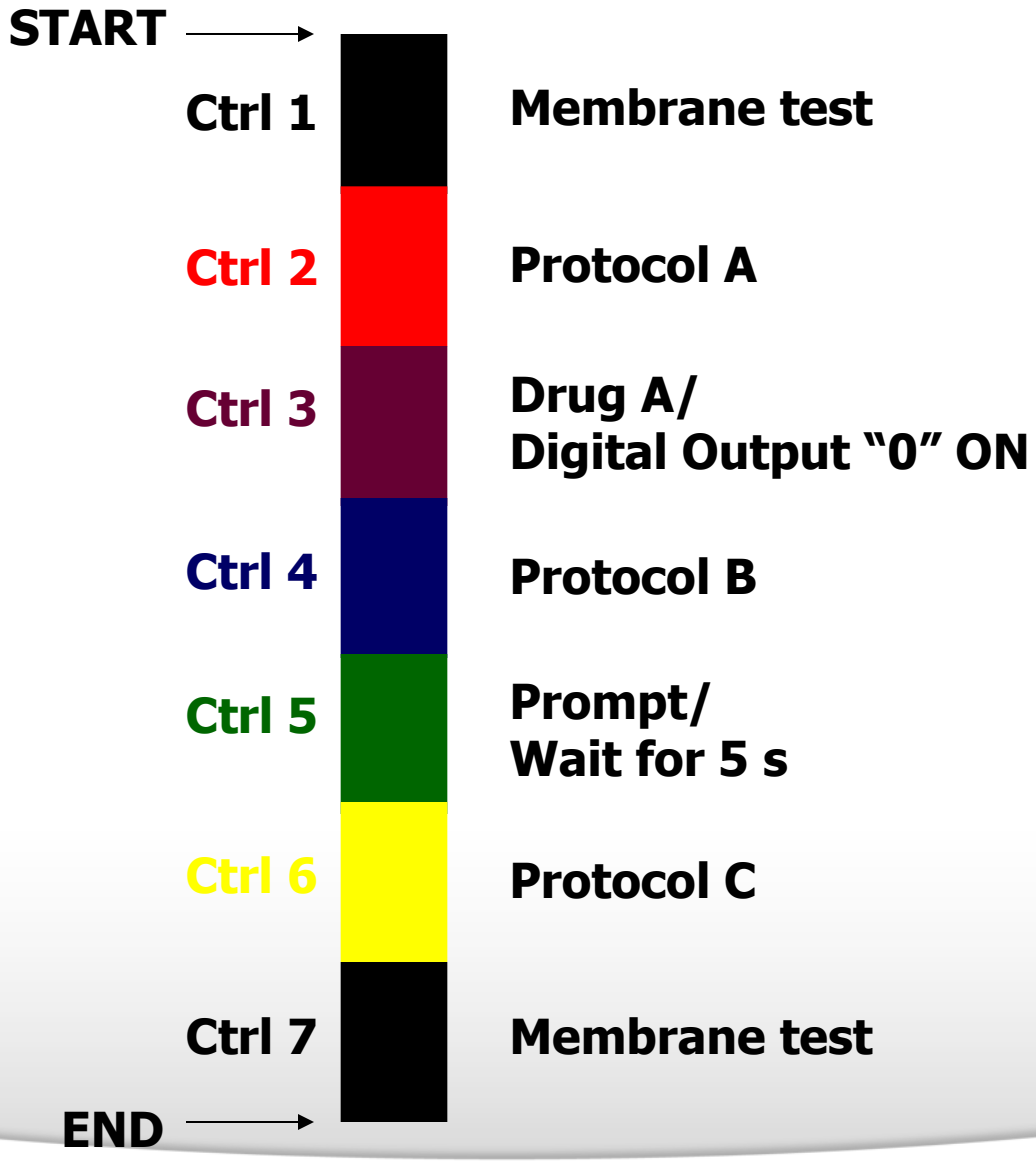
Set sequencing keys



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Sequencing Keys

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

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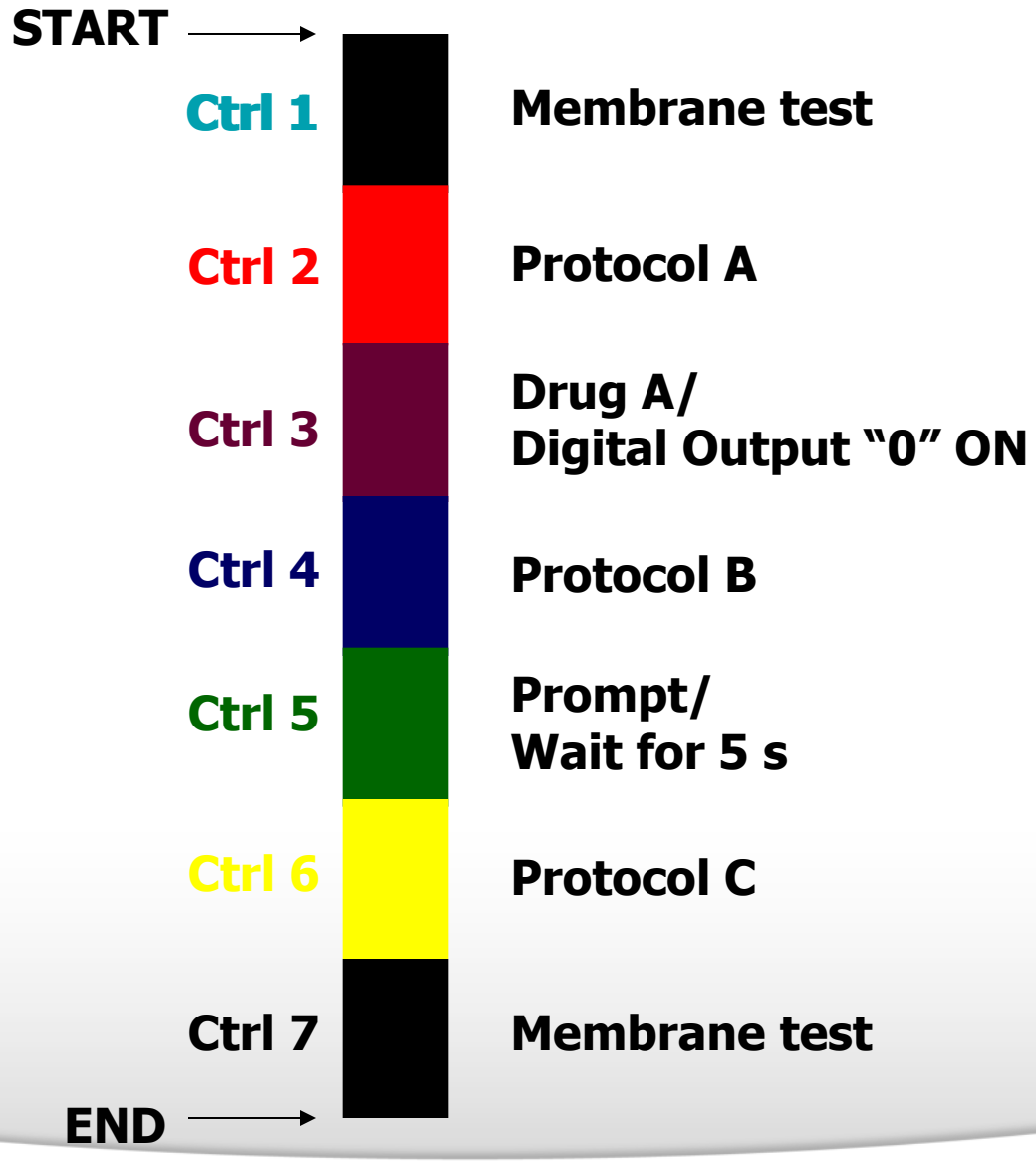
Run a protocol in an automated fashion



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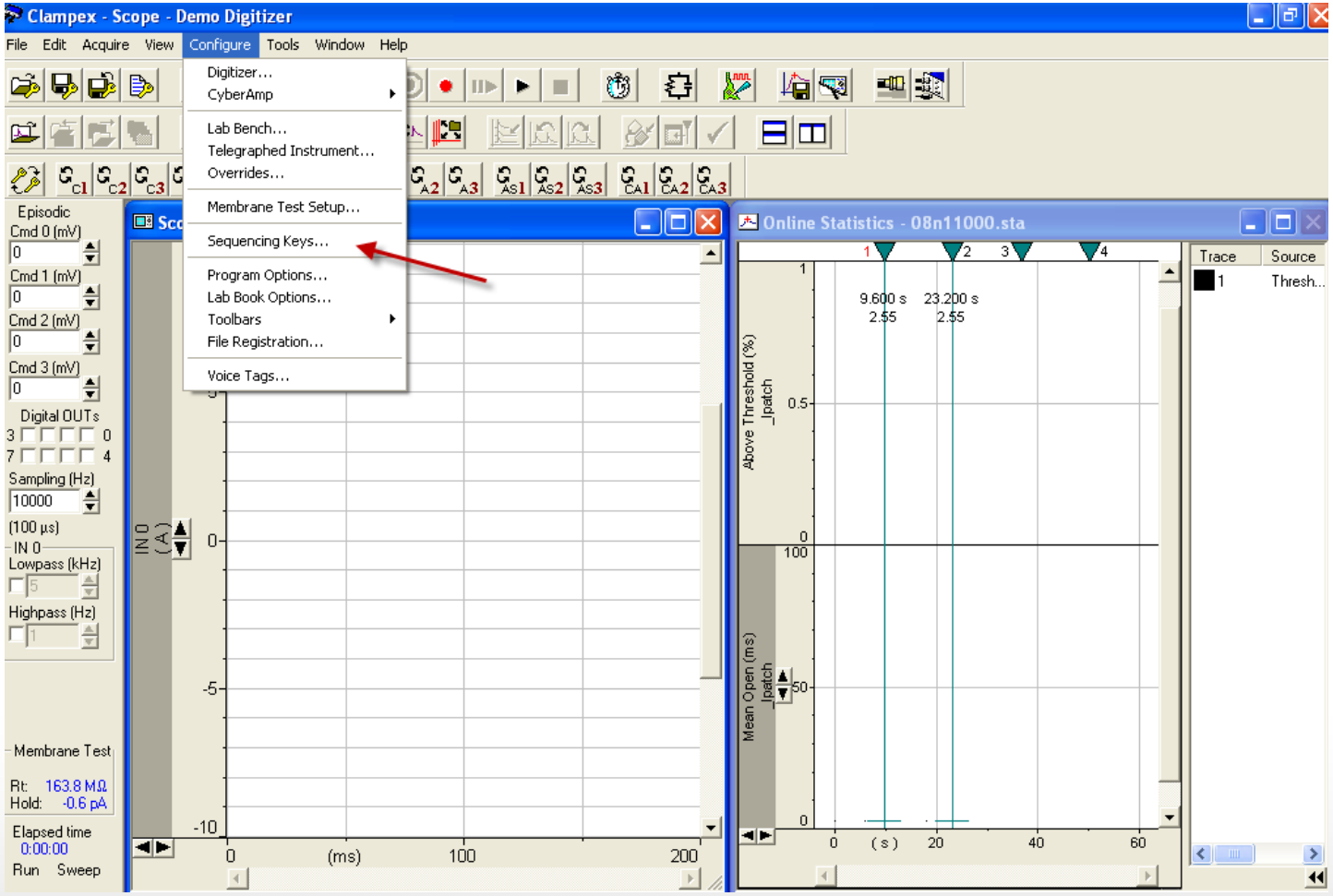

Together through life sciences.

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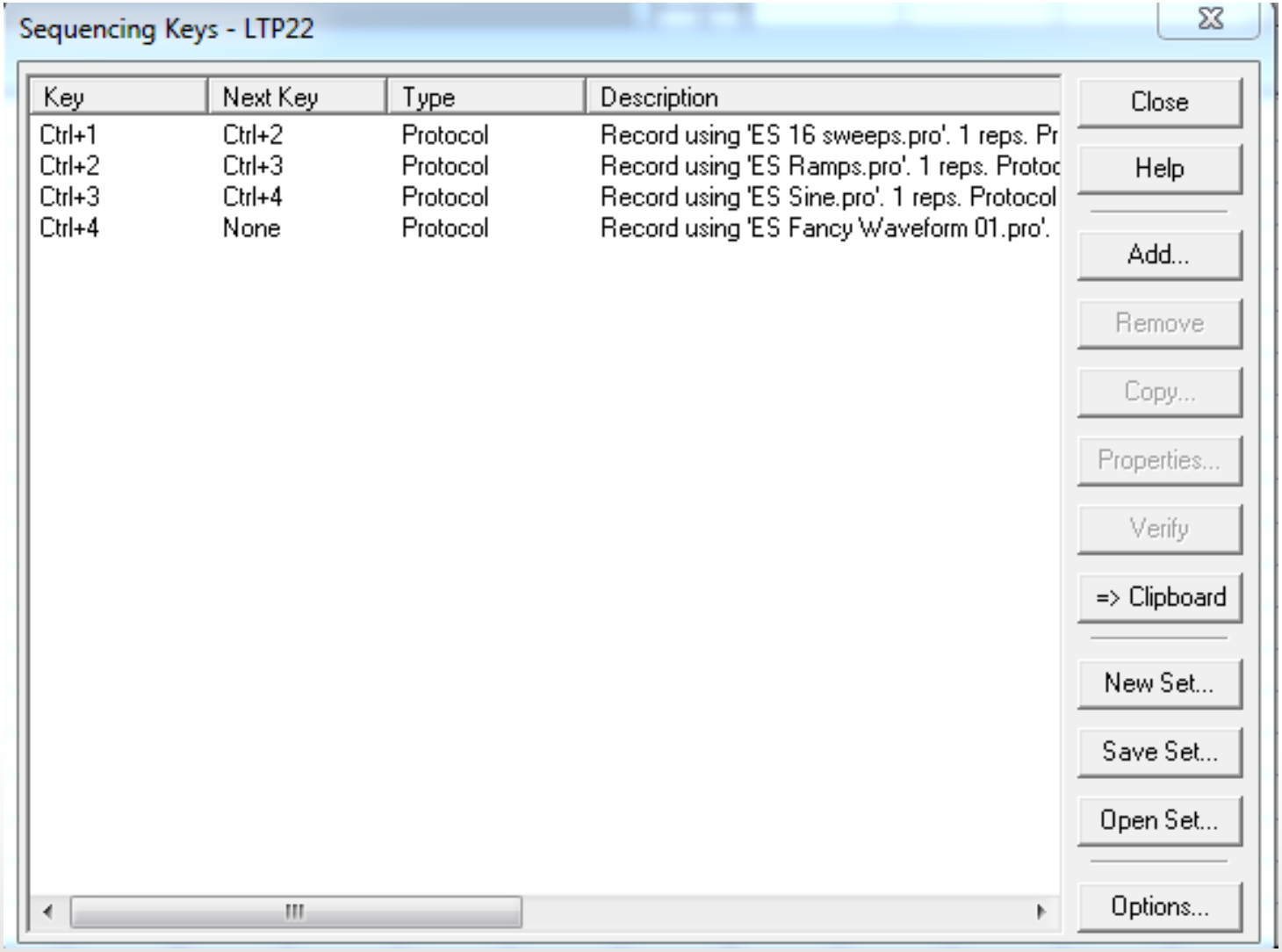
Molecular Devices



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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.

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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.

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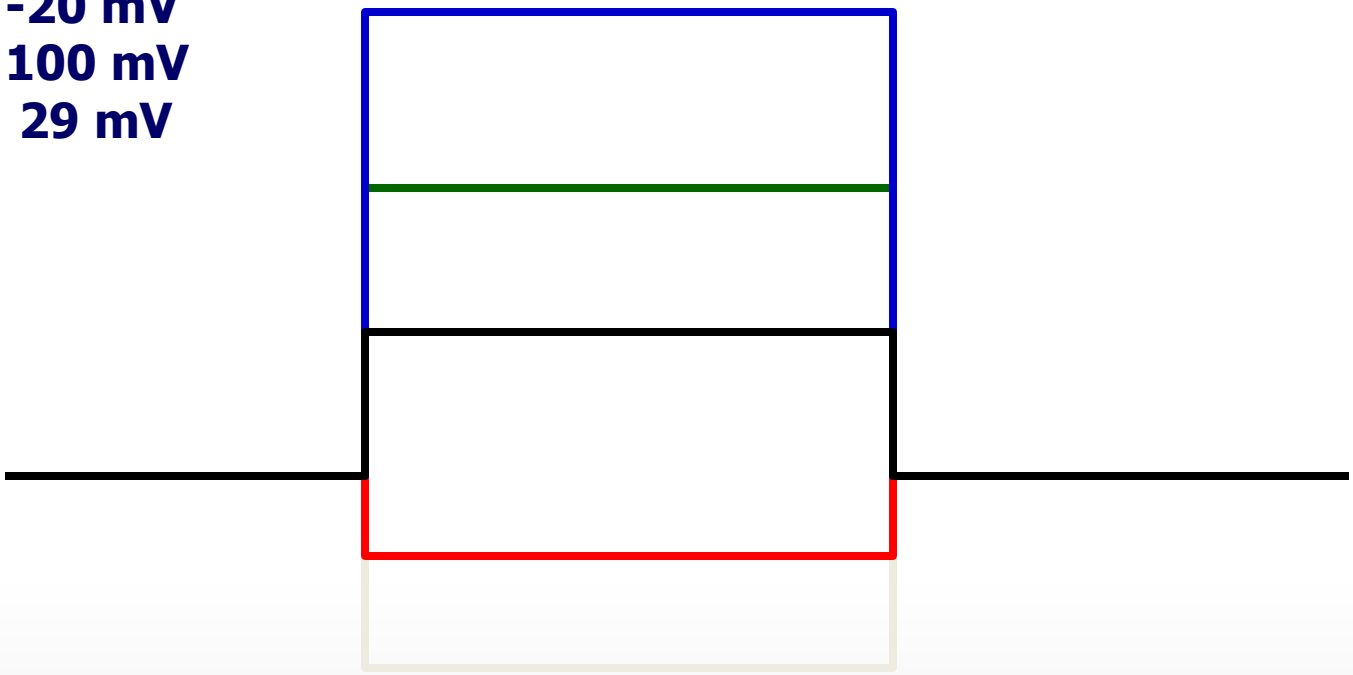
Change in Epoch holding levels

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.

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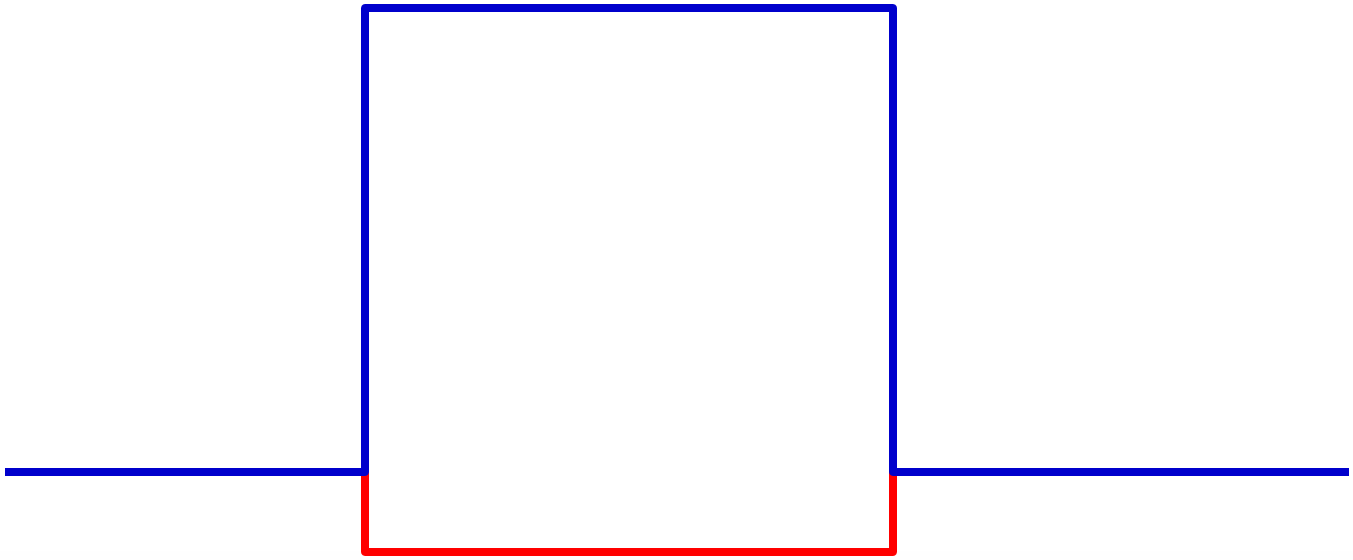
Change in Epoch holding levels

Sweep 1: -20 mV
Sweep 2: 100 mV
Repeat…



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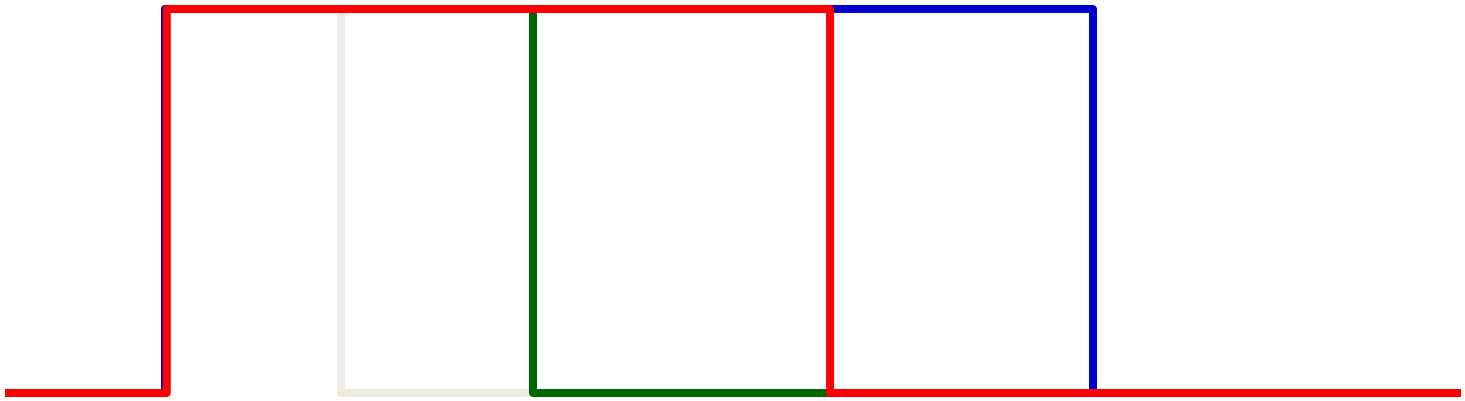
Change in Epoch durations

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.

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Change in Epoch durations

Sweep 1: 22 ms
Sweep 2: 75 ms
Repeat…

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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)

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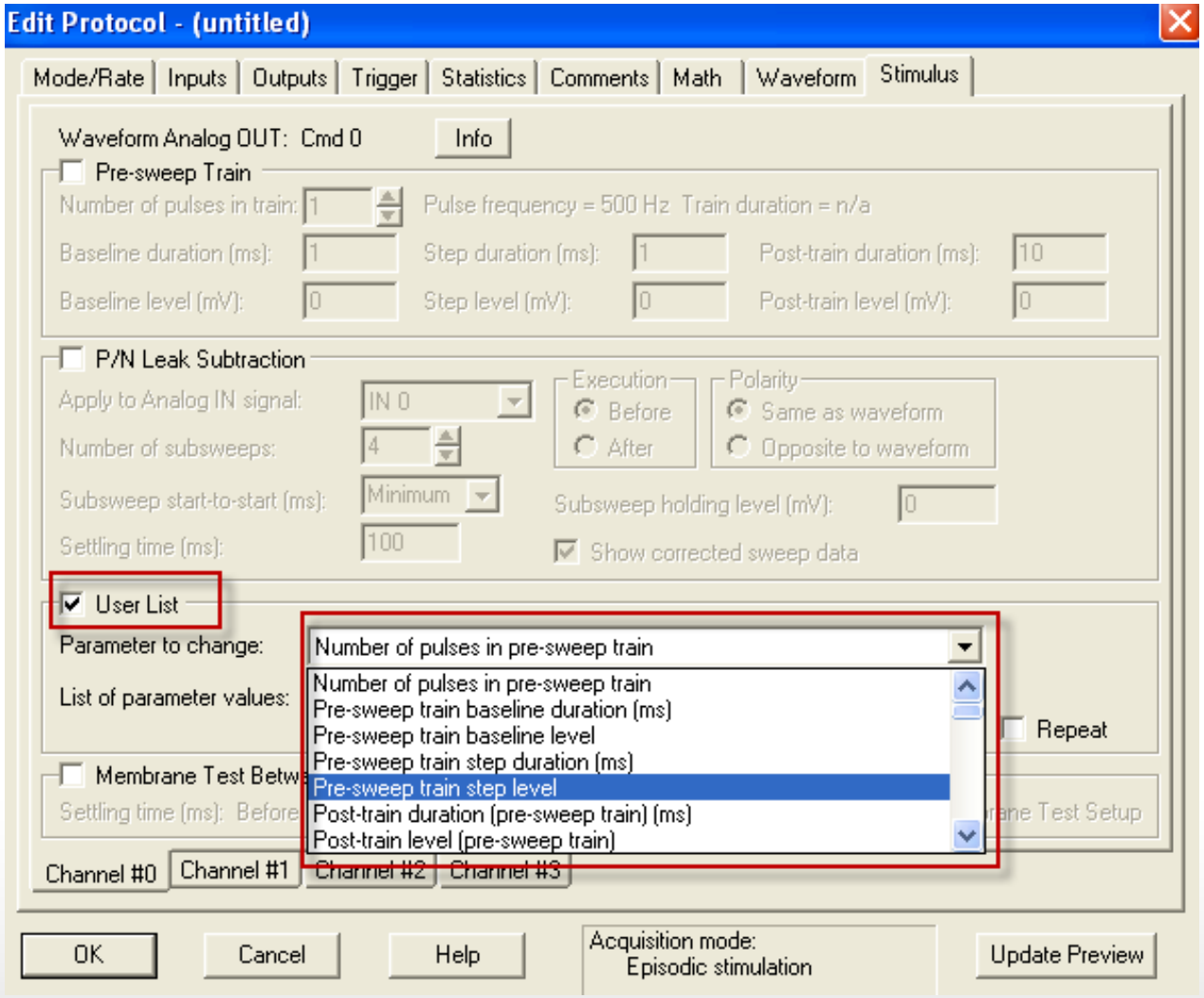

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Examples: List of Parameter values

- 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

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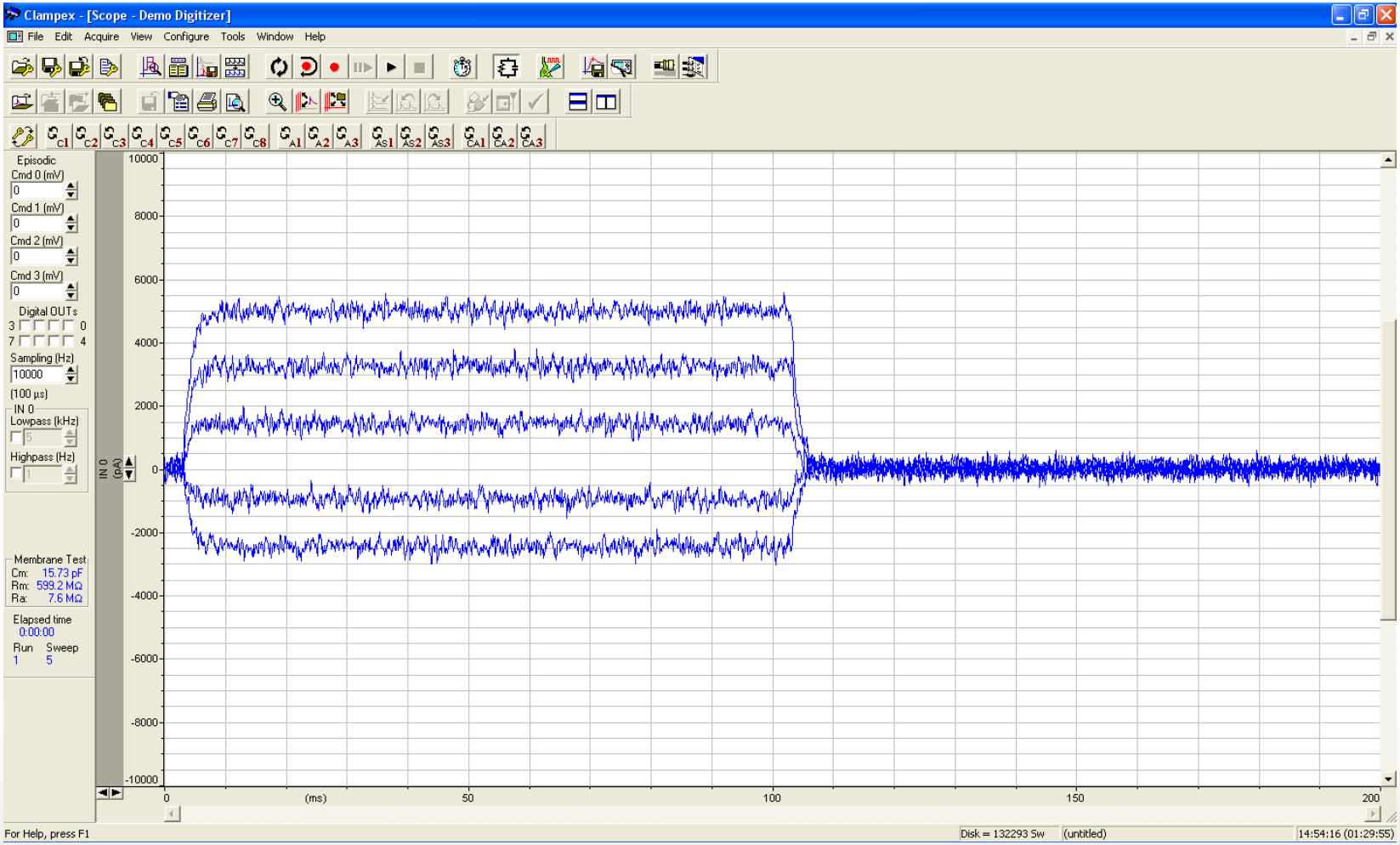
Change in Epoch holding levels



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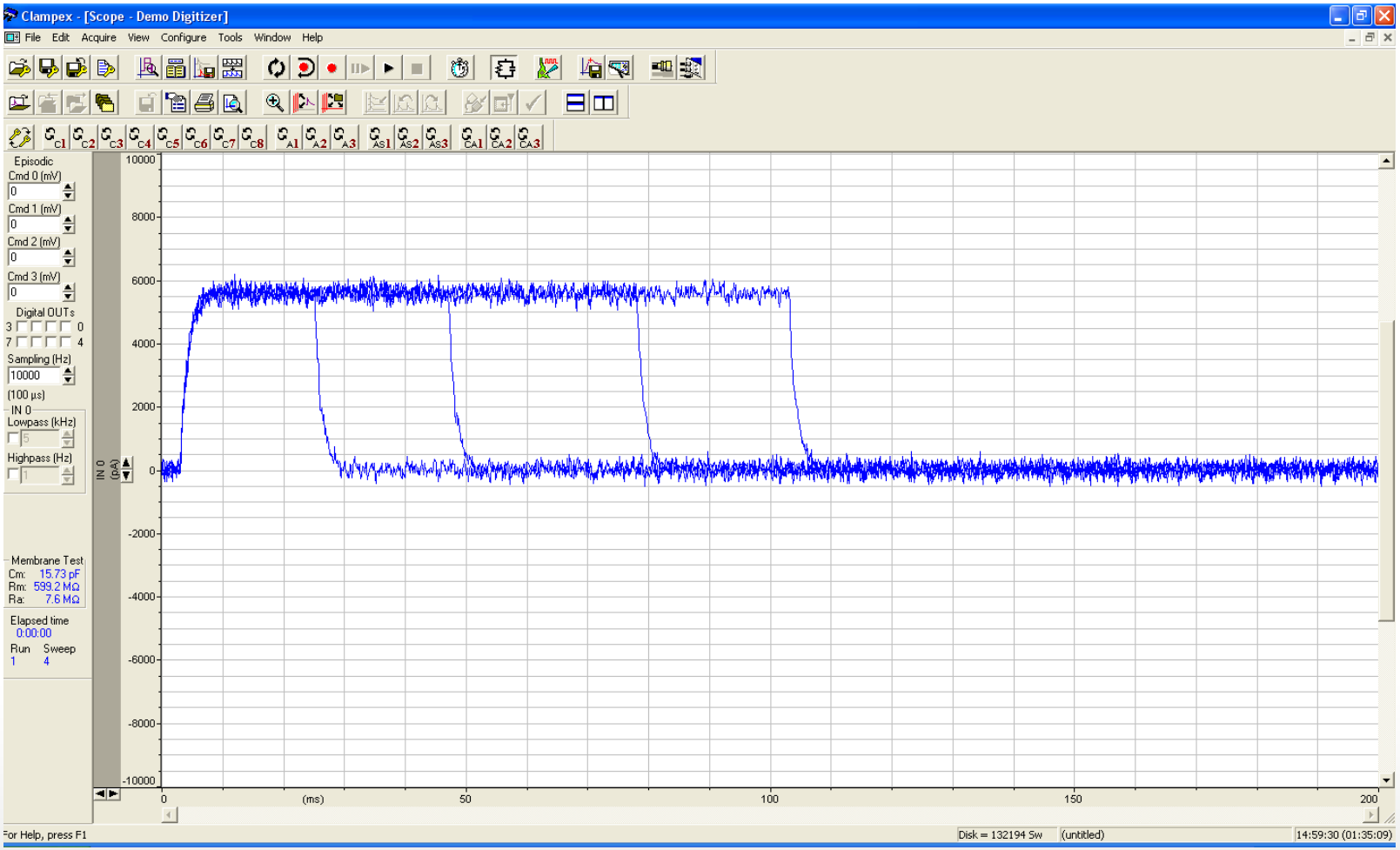
Change in Epoch durations



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Membrane Test Between Sweeps

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Membrane Test

- **Access resistance, Ra**
- **Membrane resistance, Rm**
- **Membrane capacitance, Cm**
- **Time constant, Tau**
- **Holding current, Hold**

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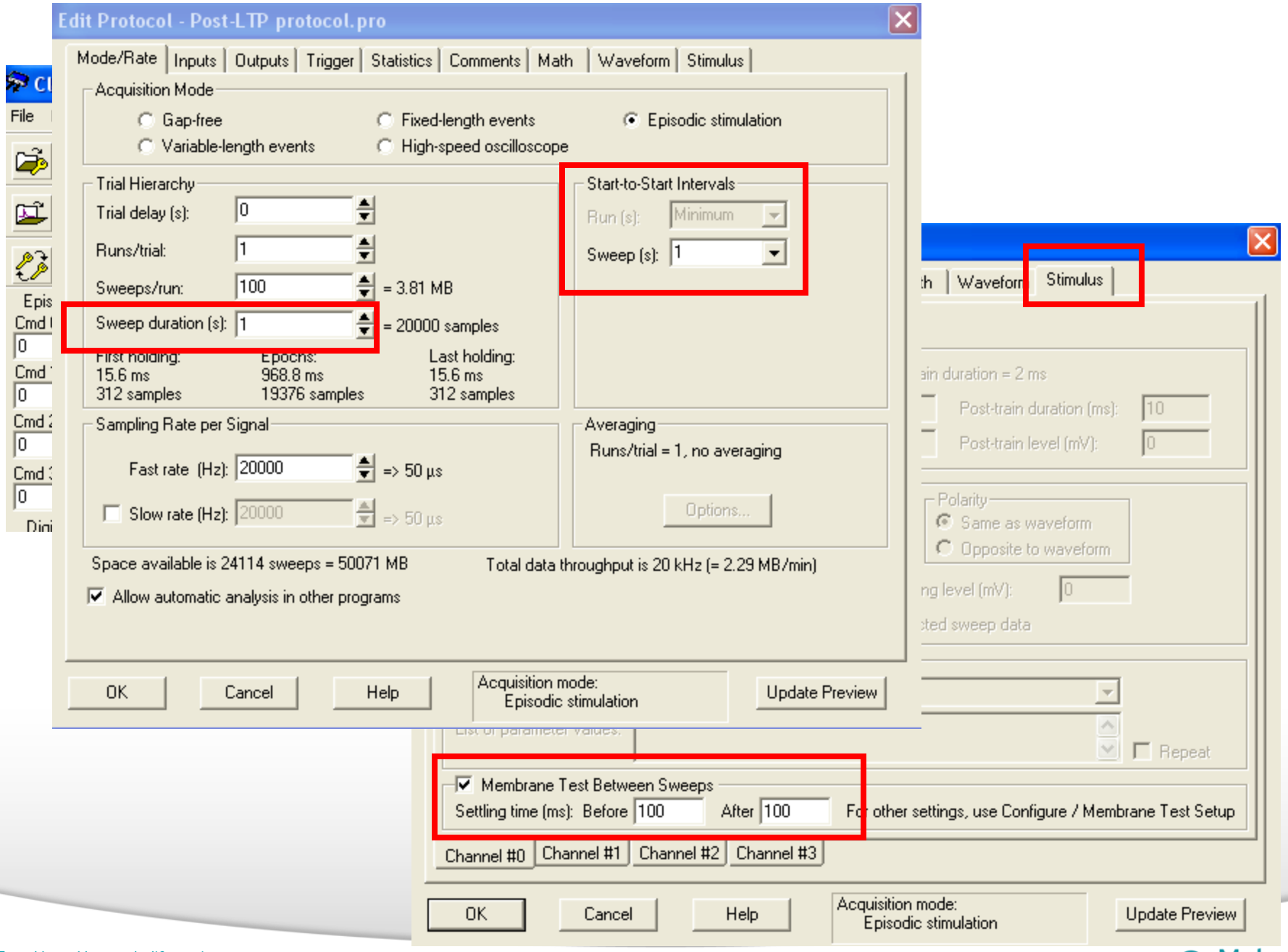

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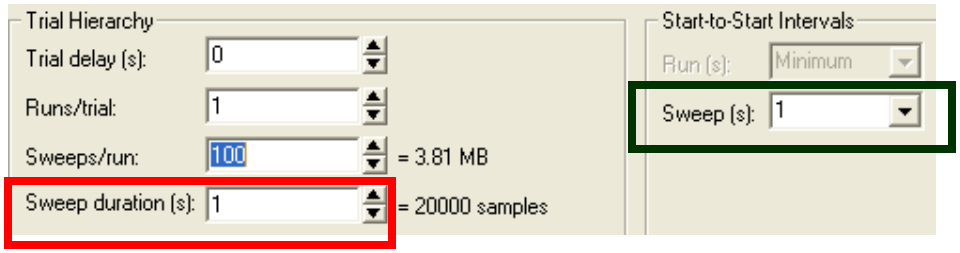

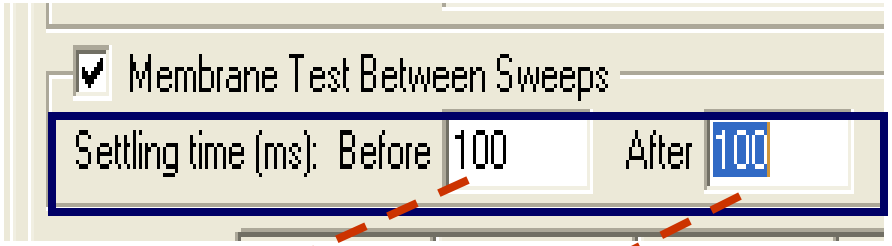

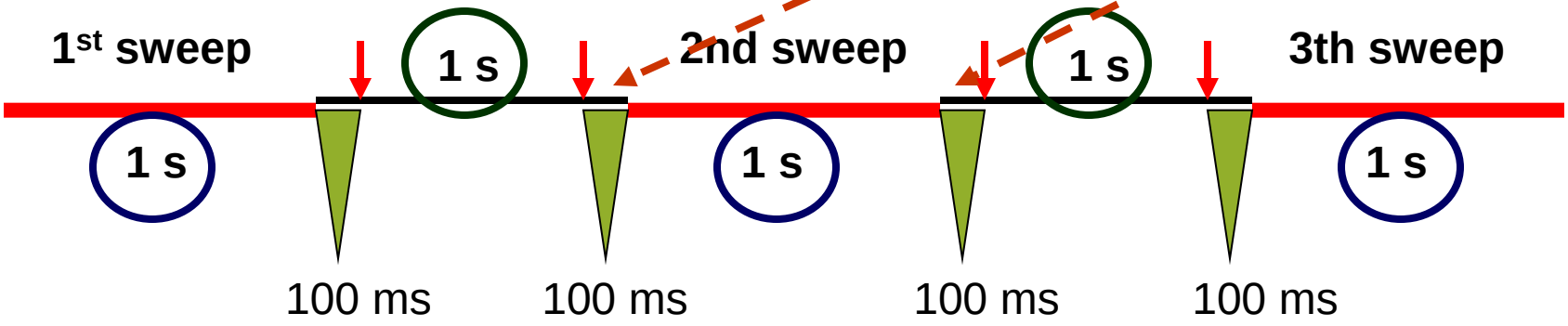

= Membrane test

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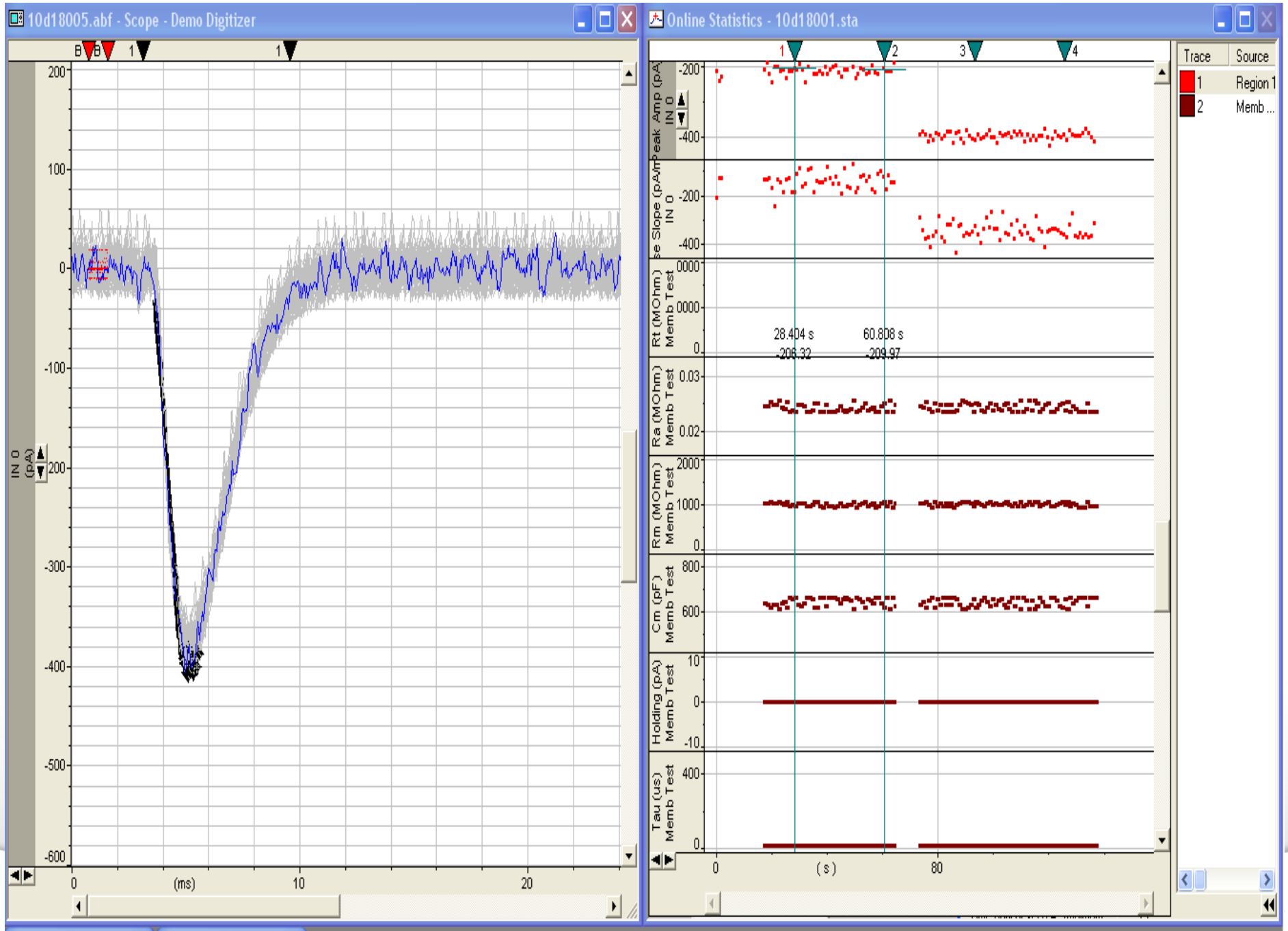

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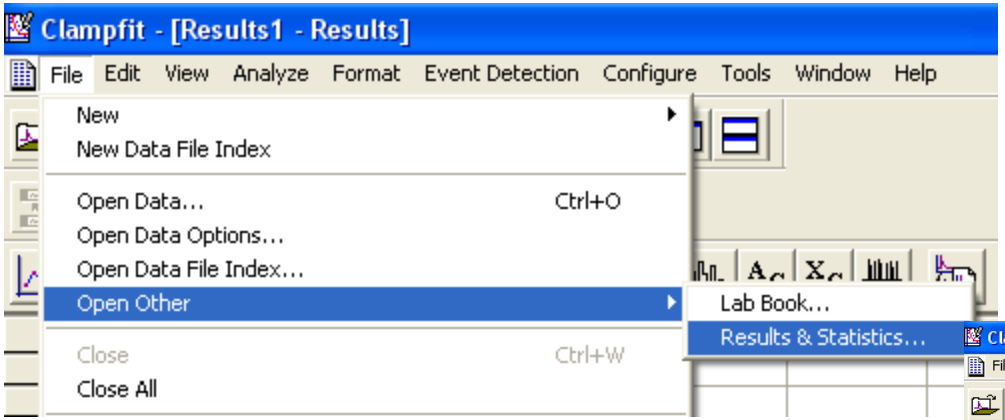

Clampfit - [Results1 - Results]

File Edit View Analyze Format Event Detection Configure Tools Window Help

	Time (s)	Region 1-IN	Region 1-IN 0	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

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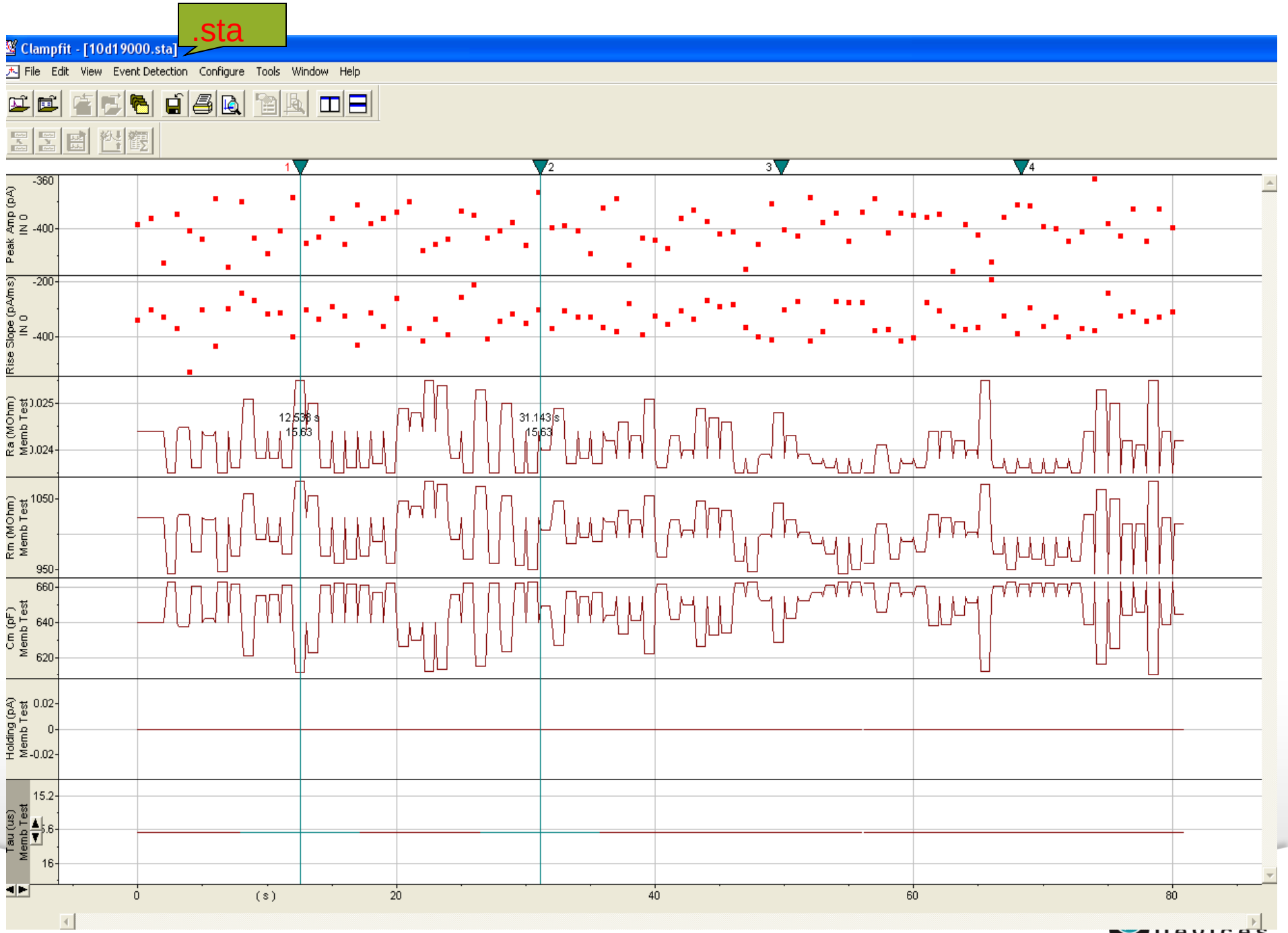

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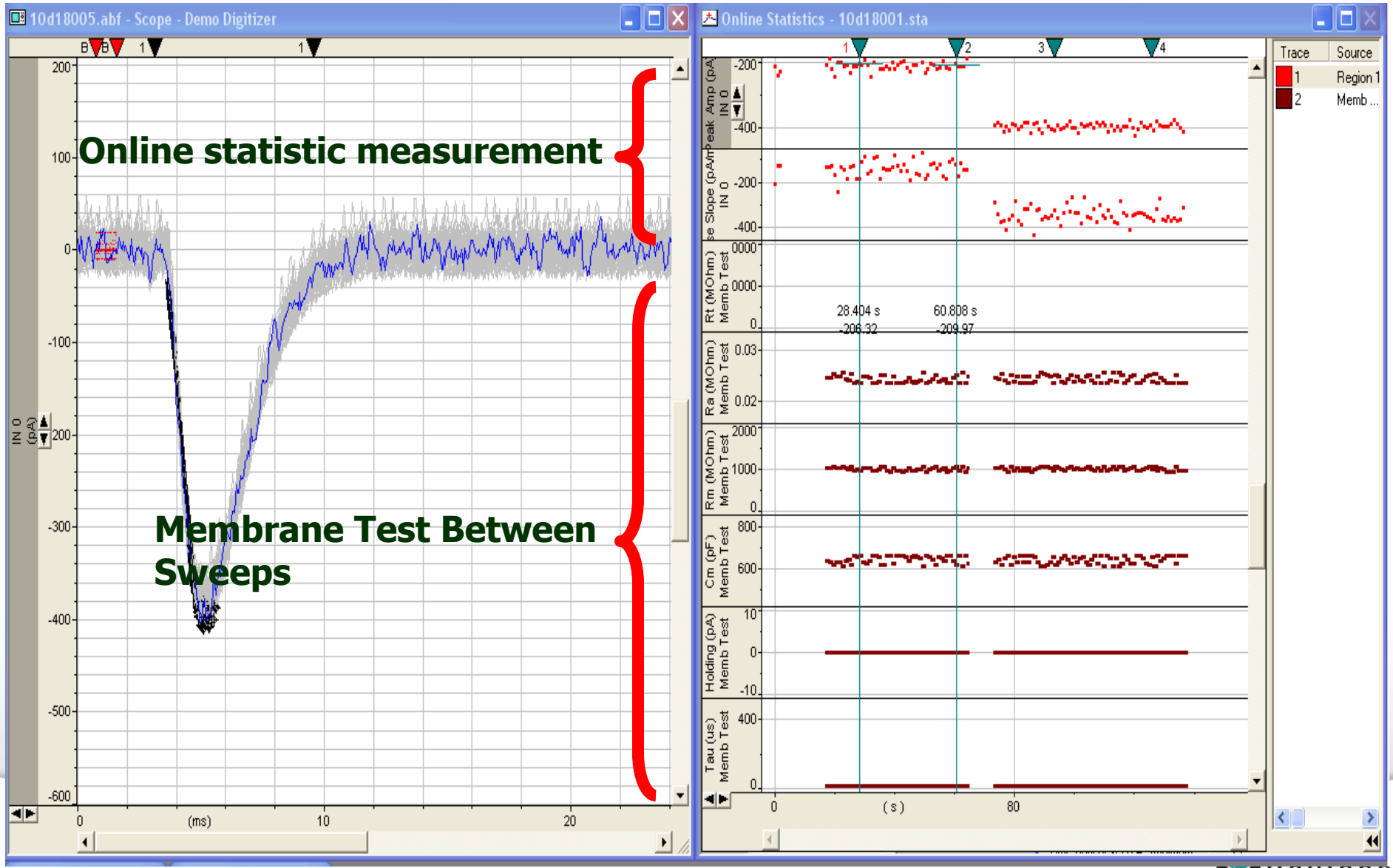
Summary



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Stimulus File

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

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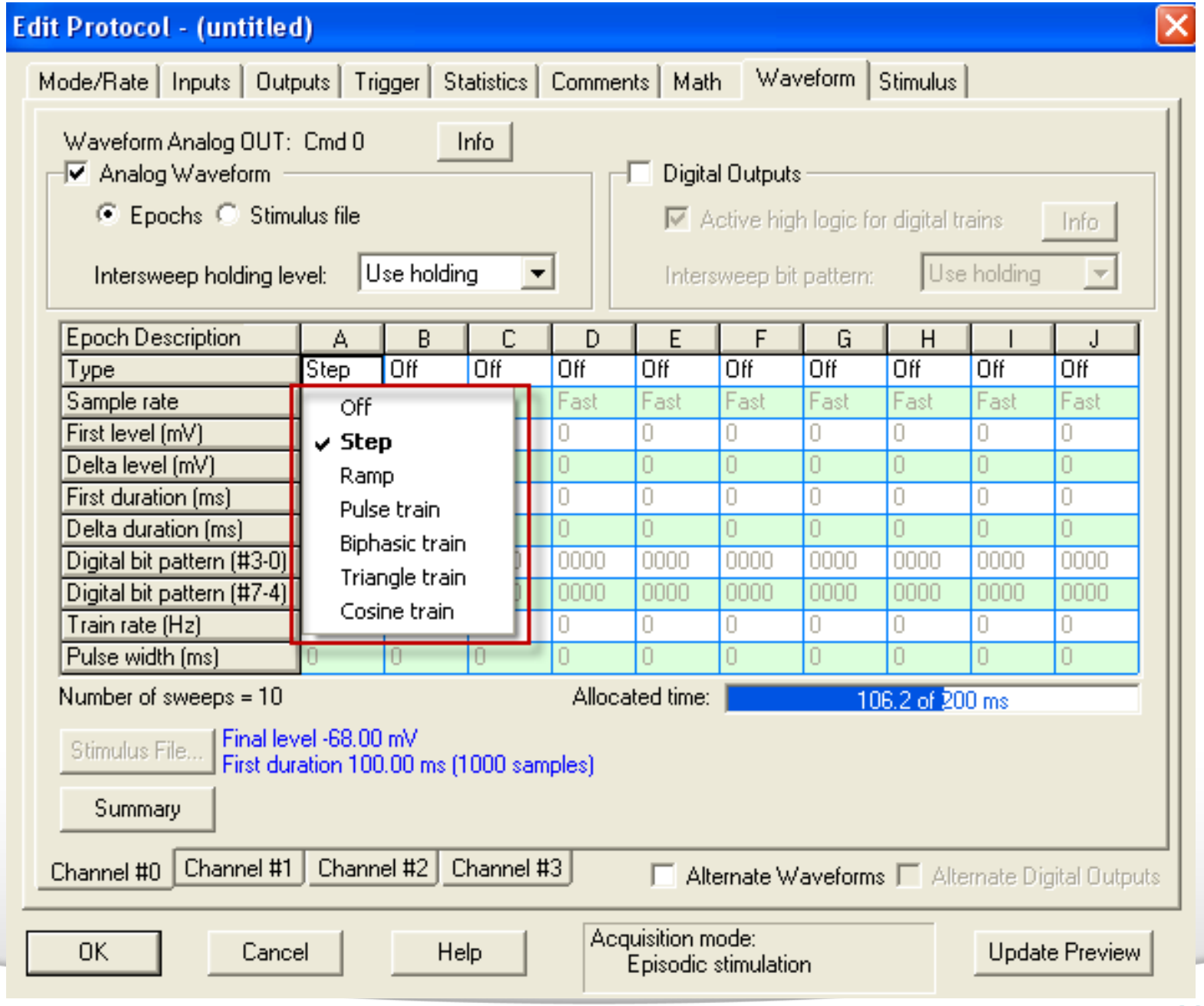

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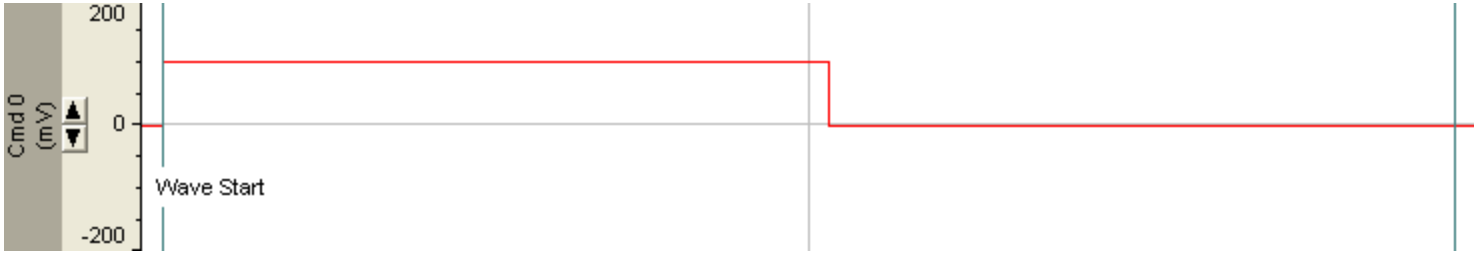
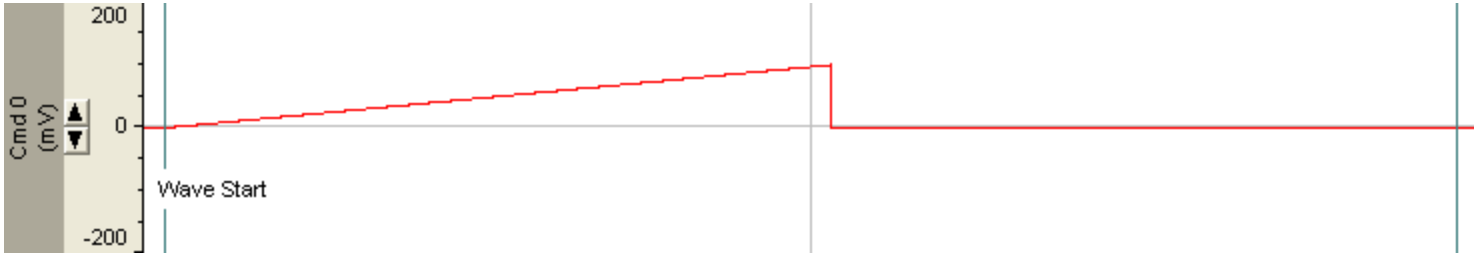
Default waveforms

Step:



Ramp:



Pulse train:

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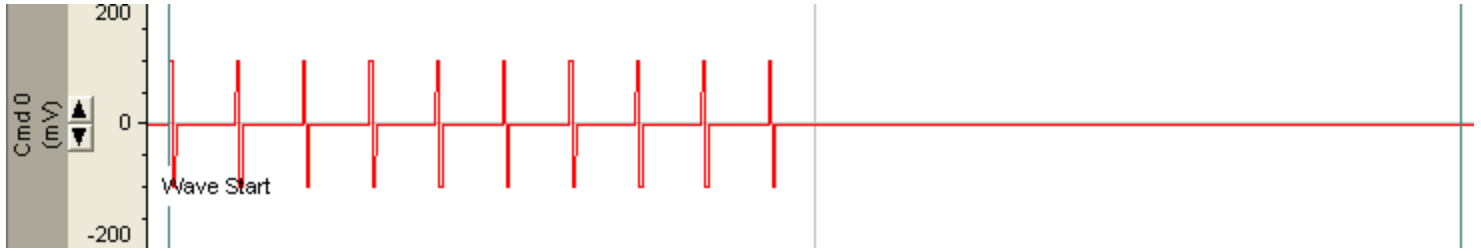
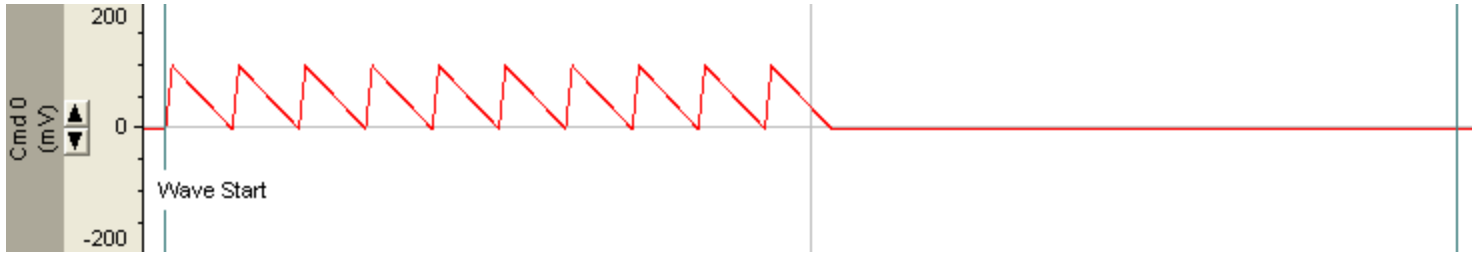
Default waveforms

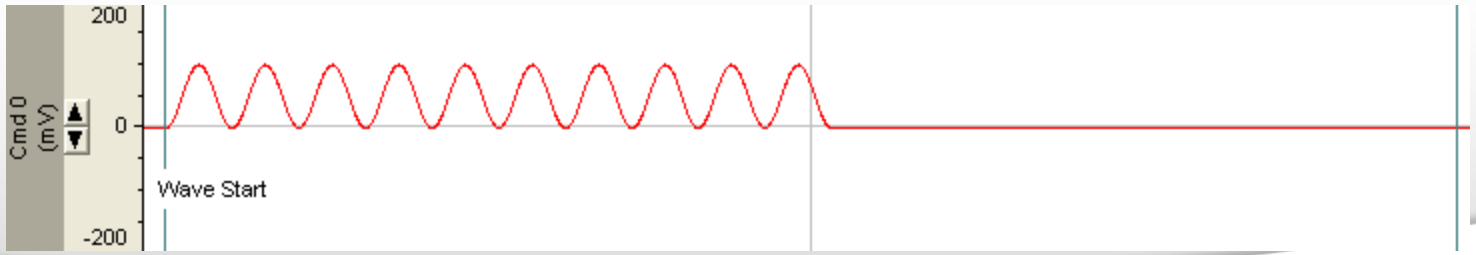
Biphasic train:



Triangle train:



Cosine train:



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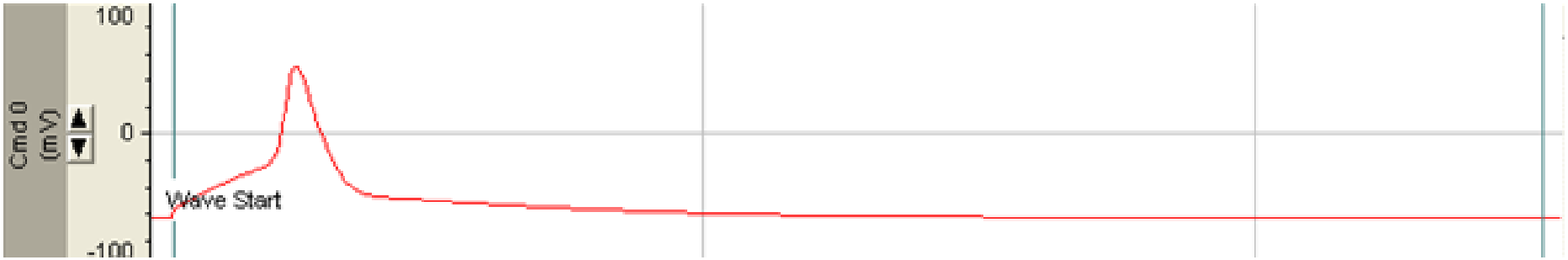
How to create an action potential waveform?



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Advanced Tips for Protocol Writing in the Clampex Data Acquisition Module

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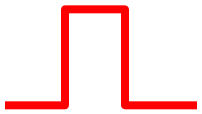
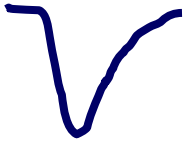
Whole cell and Extracellular Recordings

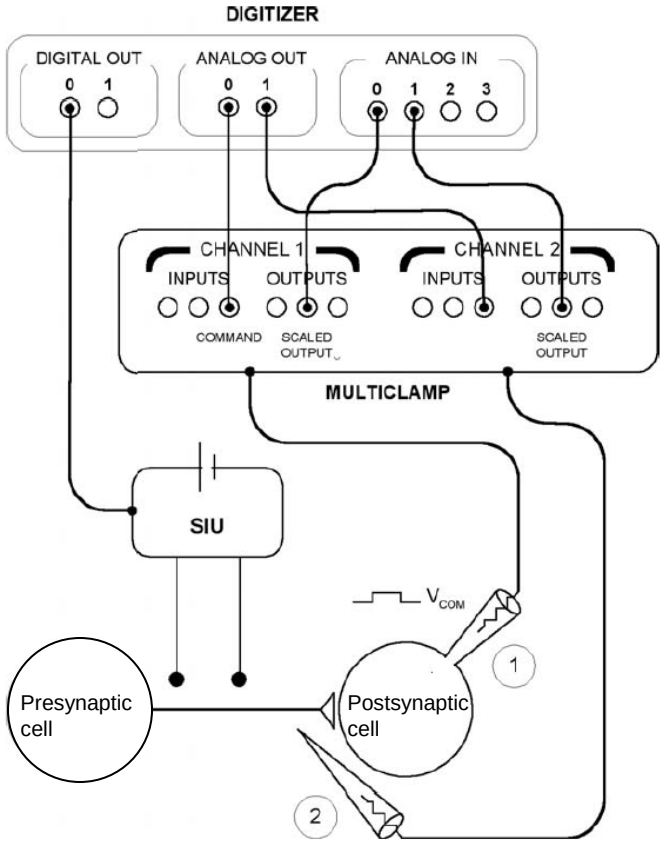
Electrode 1: Intracellular

Presynaptic Stimulation



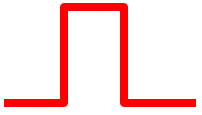
Postsynaptic current





Electrode 2: Extracellular

Presynaptic Stimulation



Postsynaptic fEPSPs

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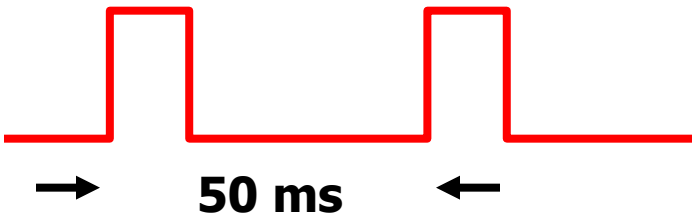
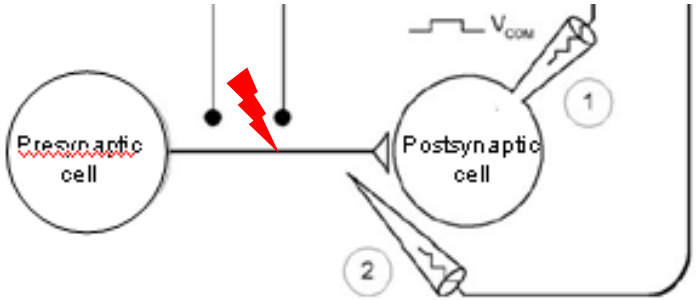
Pair-pulse stimulation





Application: Synaptic plasticity, release probability in brain slice preparation

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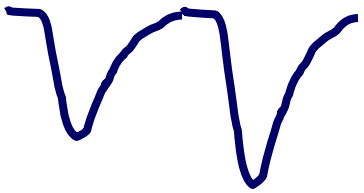
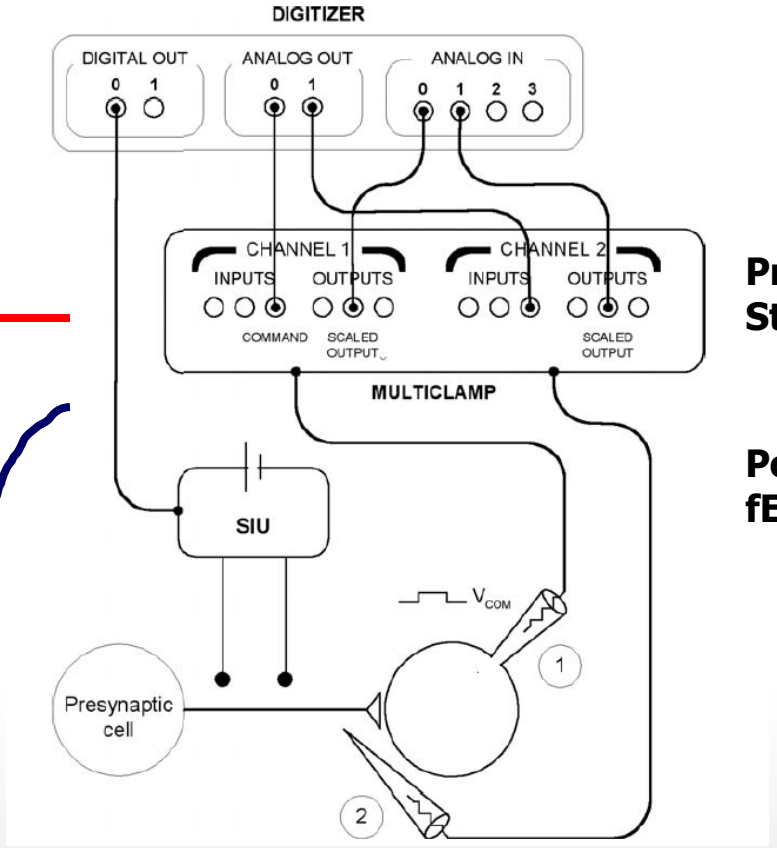
Pair-pulse Stimulation: Presynaptic afferents

Electrode 1: Intracellular

Presynaptic Stimulation

Postsynaptic current





Electrode 2: Extracellular

Presynaptic Stimulation

Postsynaptic fEPSPs

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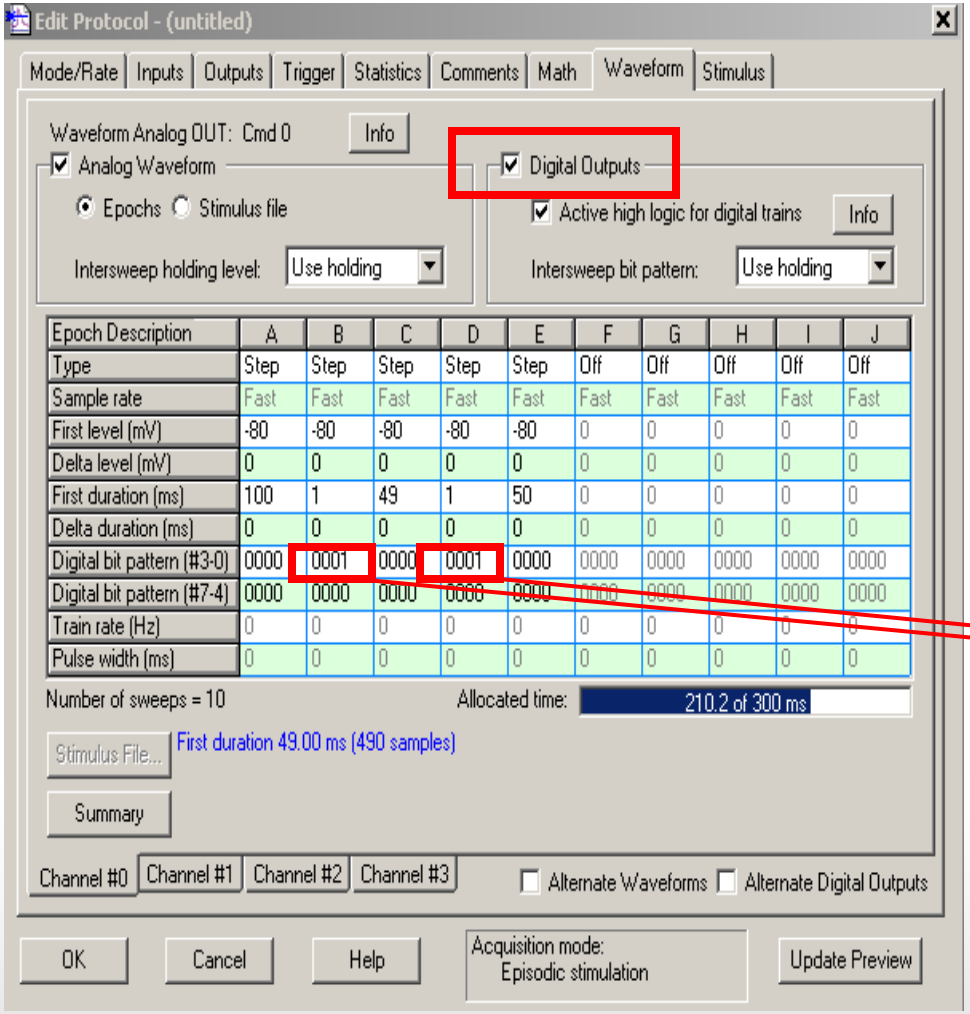
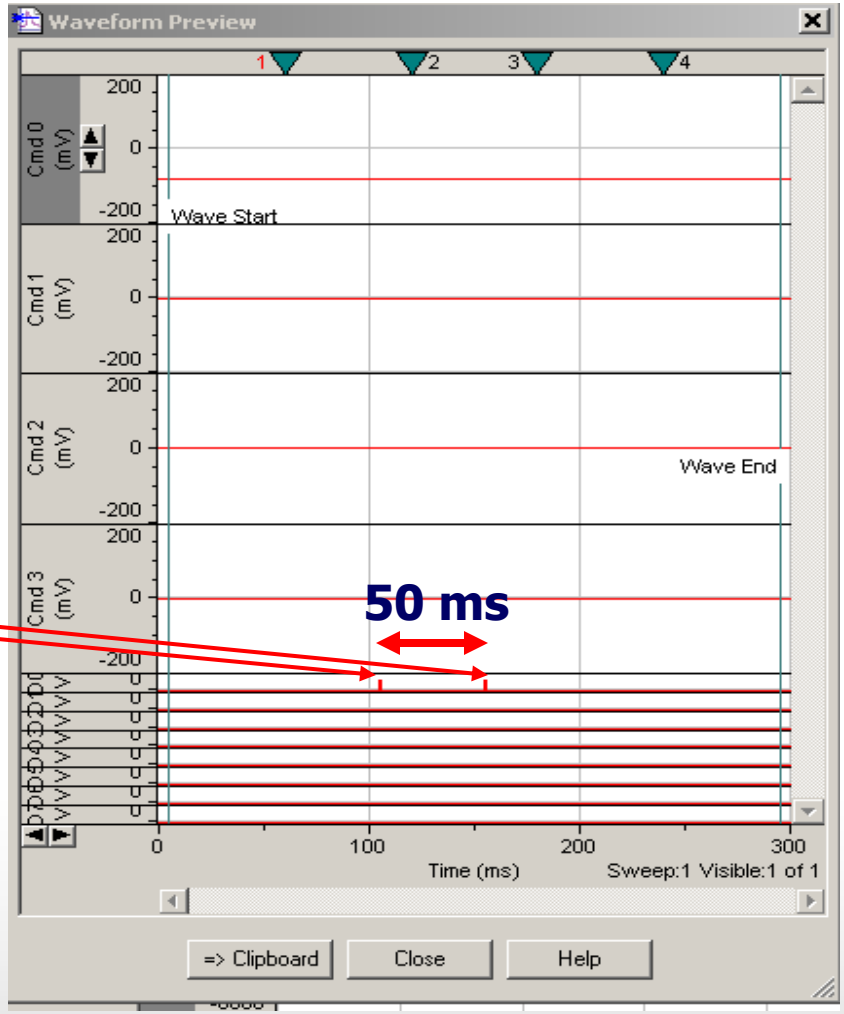
Pair-pulse stimulation





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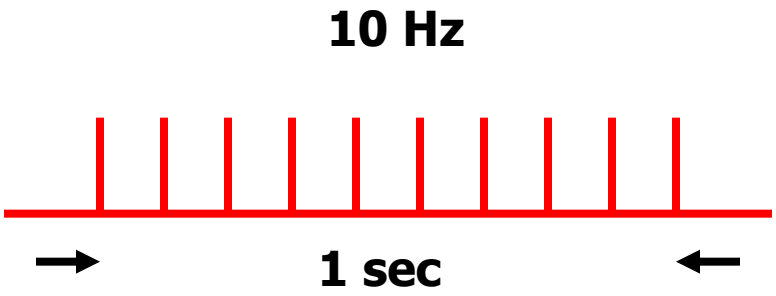
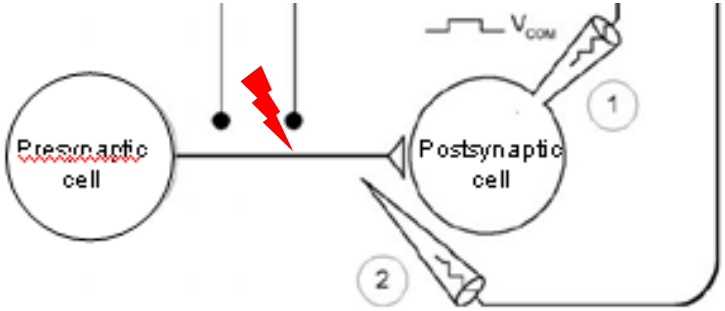
Molecular Devices

Train stimuli





Application: Synaptic plasticity, LTD in brain slice preparation

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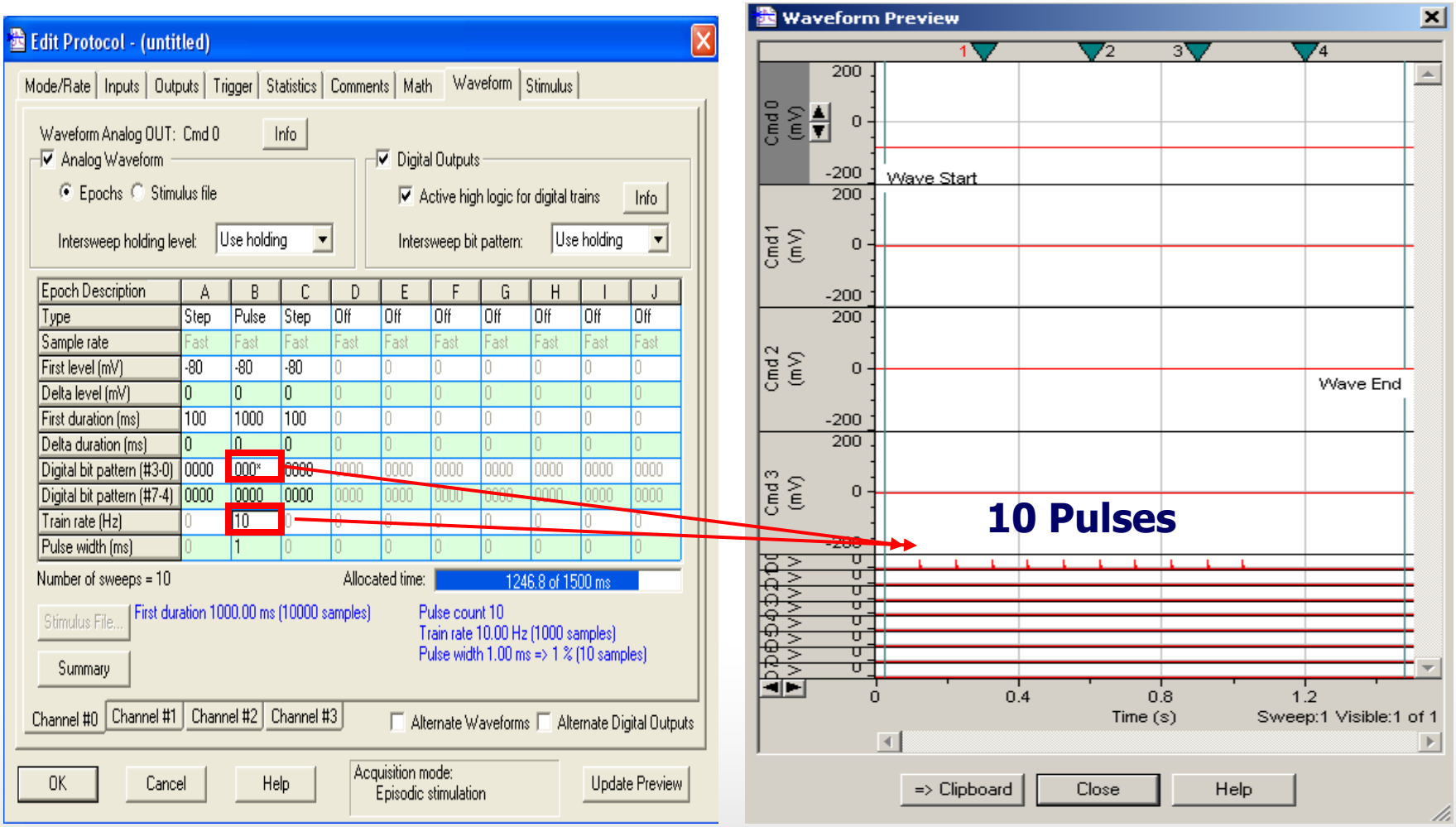
Train stimuli-10 pulses



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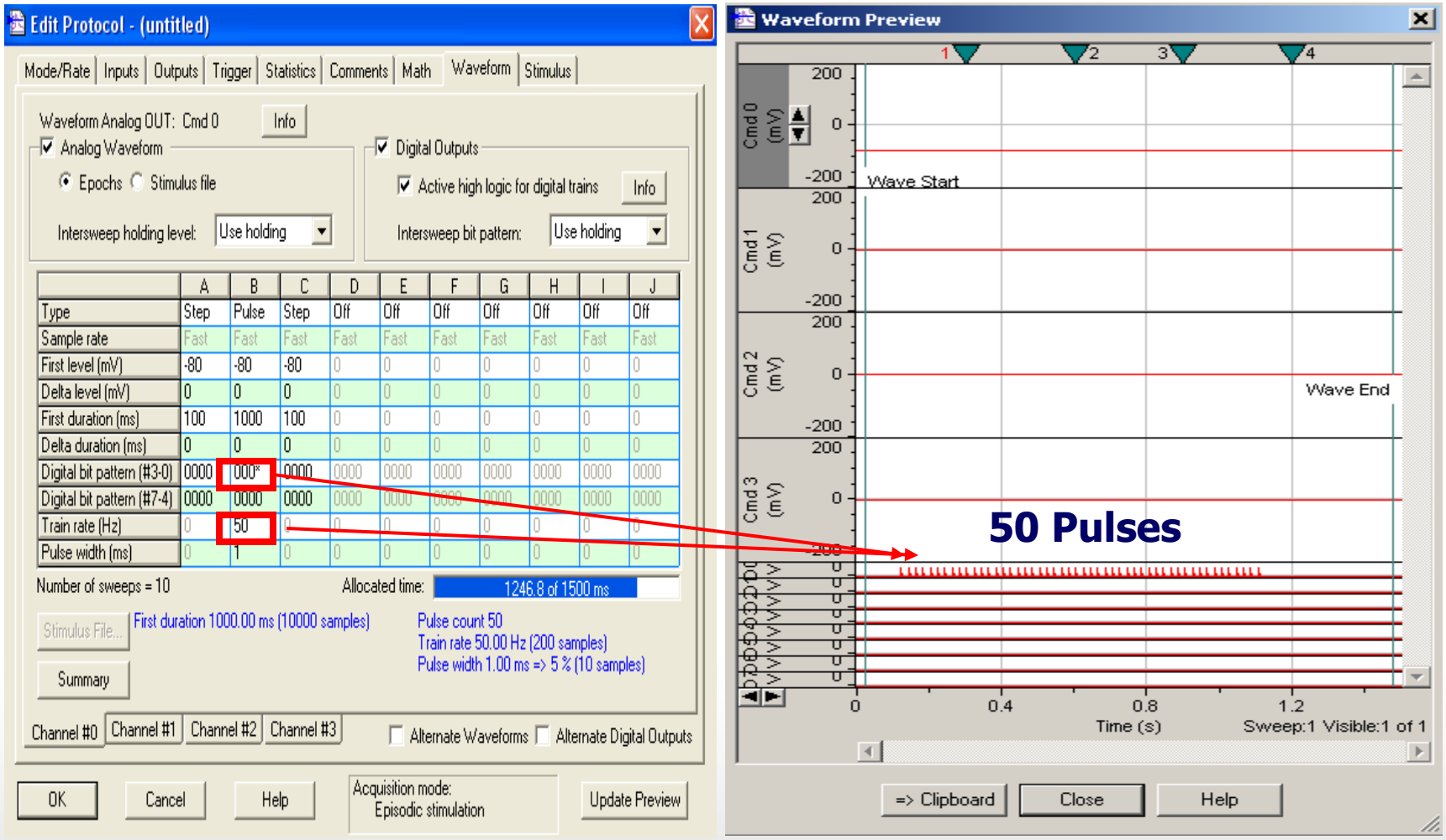
Molecular Devices

Train stimuli-50 pulses



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Molecular Devices

Summary

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

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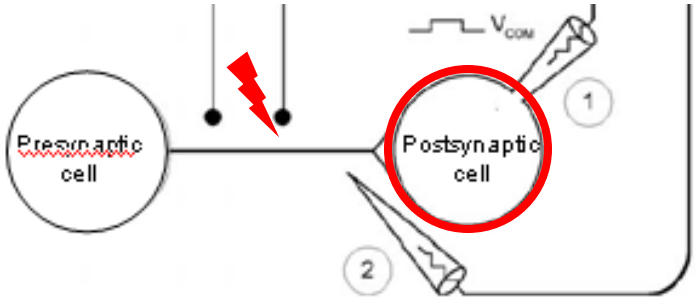
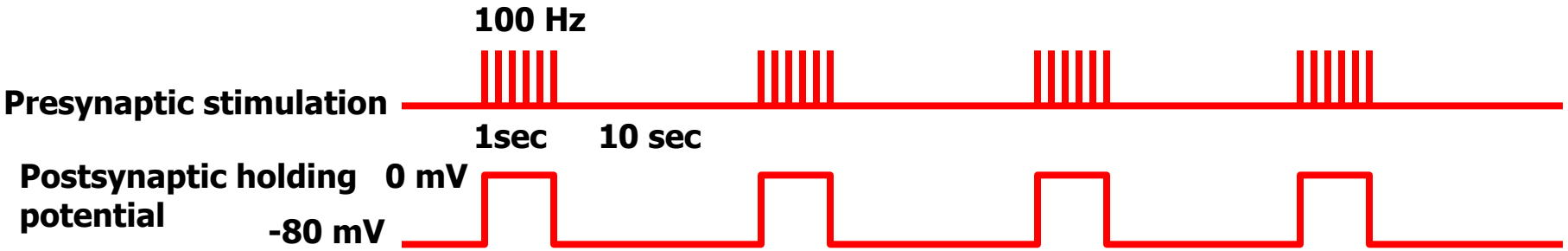
Tetanus Stimulation protocol





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

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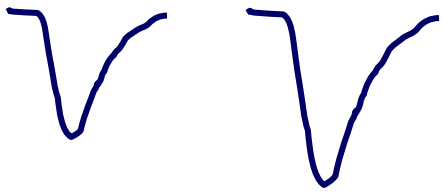
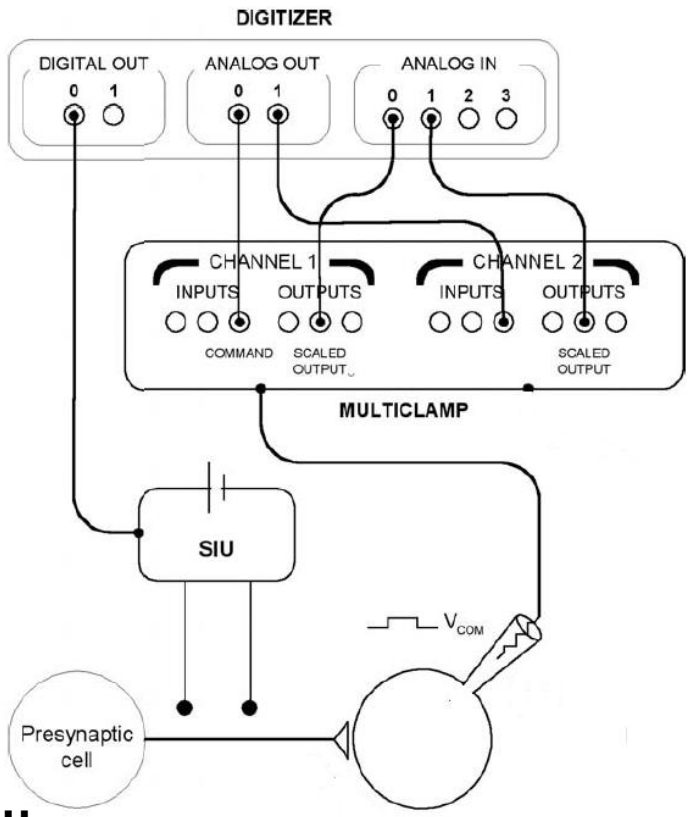
Tetanus Stimulation and Postsynaptic Depolarization

LTP:

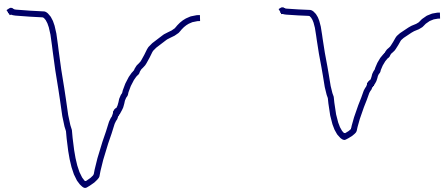
Pre-stim Post-stim

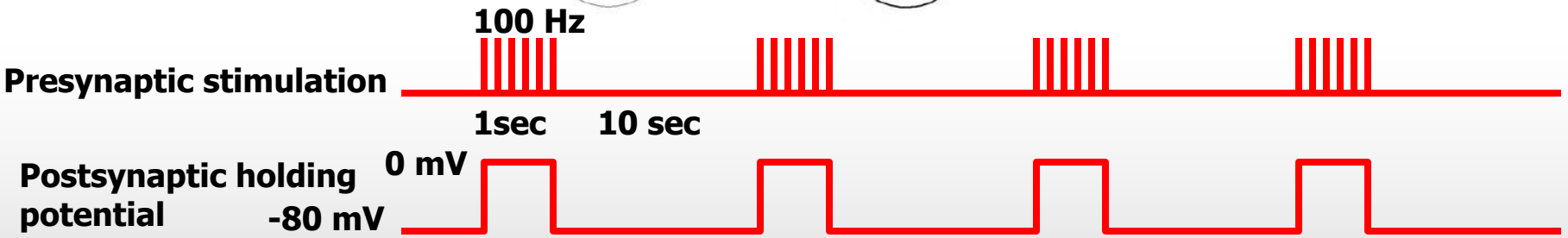




LTD:

Pre-stim Post-stim





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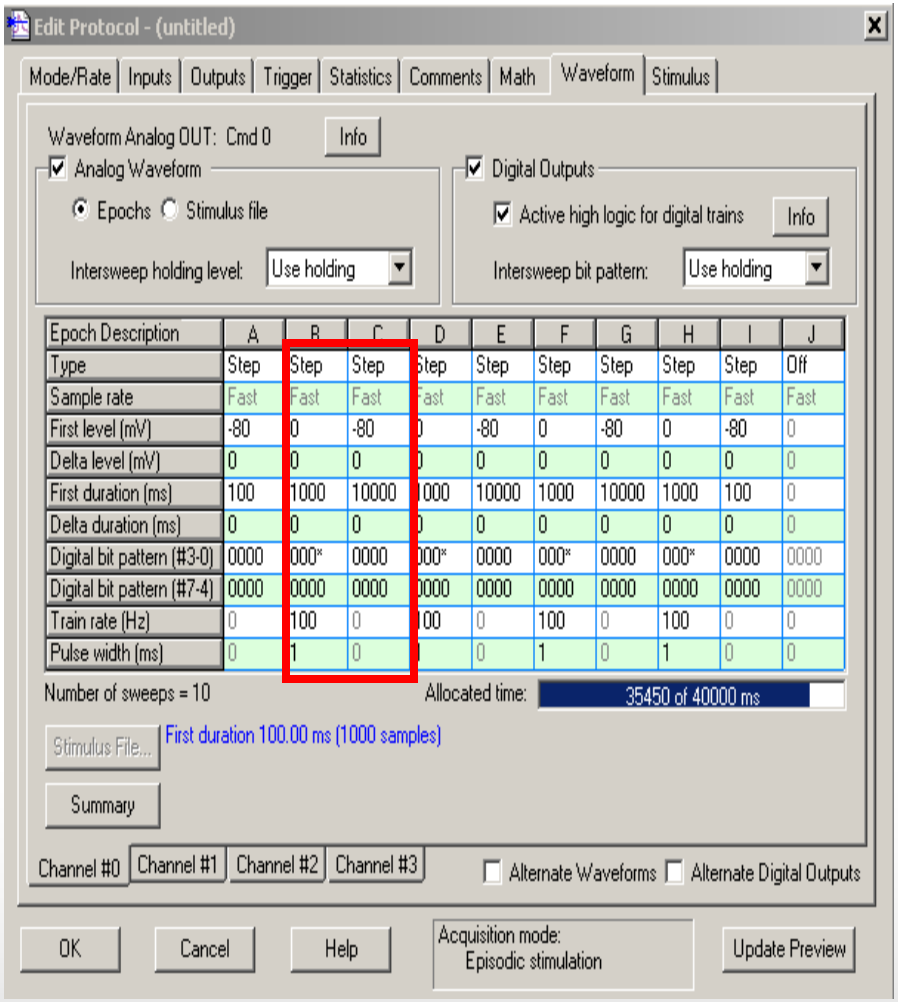
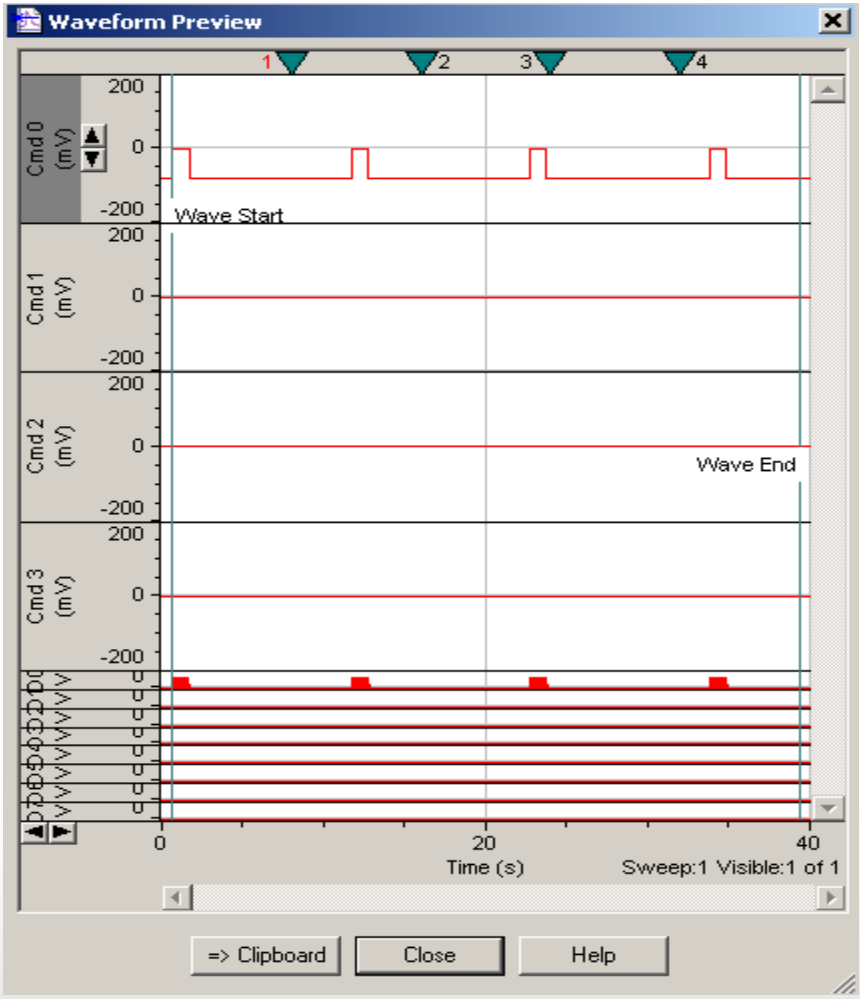
Tetanus Stimulation and Postsynaptic Depolarization





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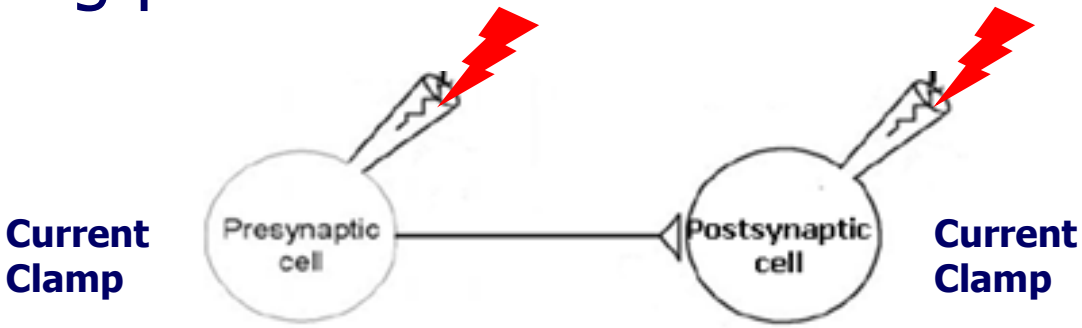
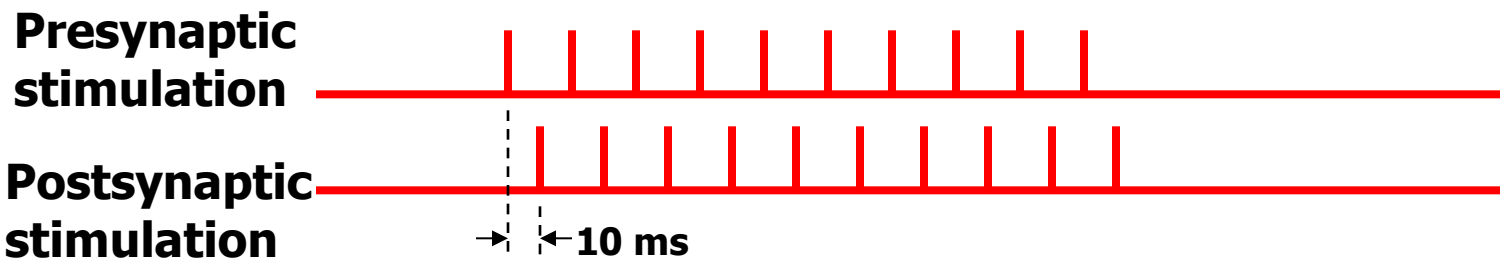
Molecular Devices

Spike timing protocol





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

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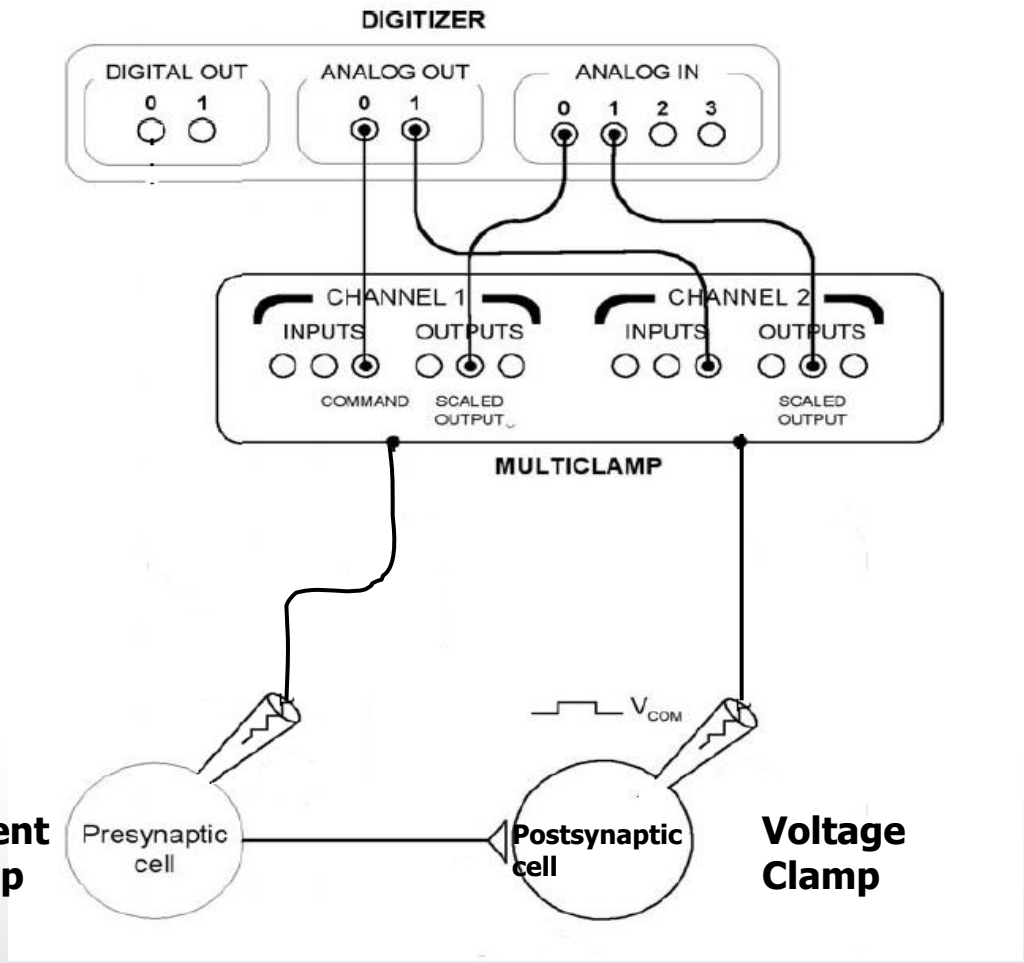
Dual Whole-cell Patch-clamp Recordings



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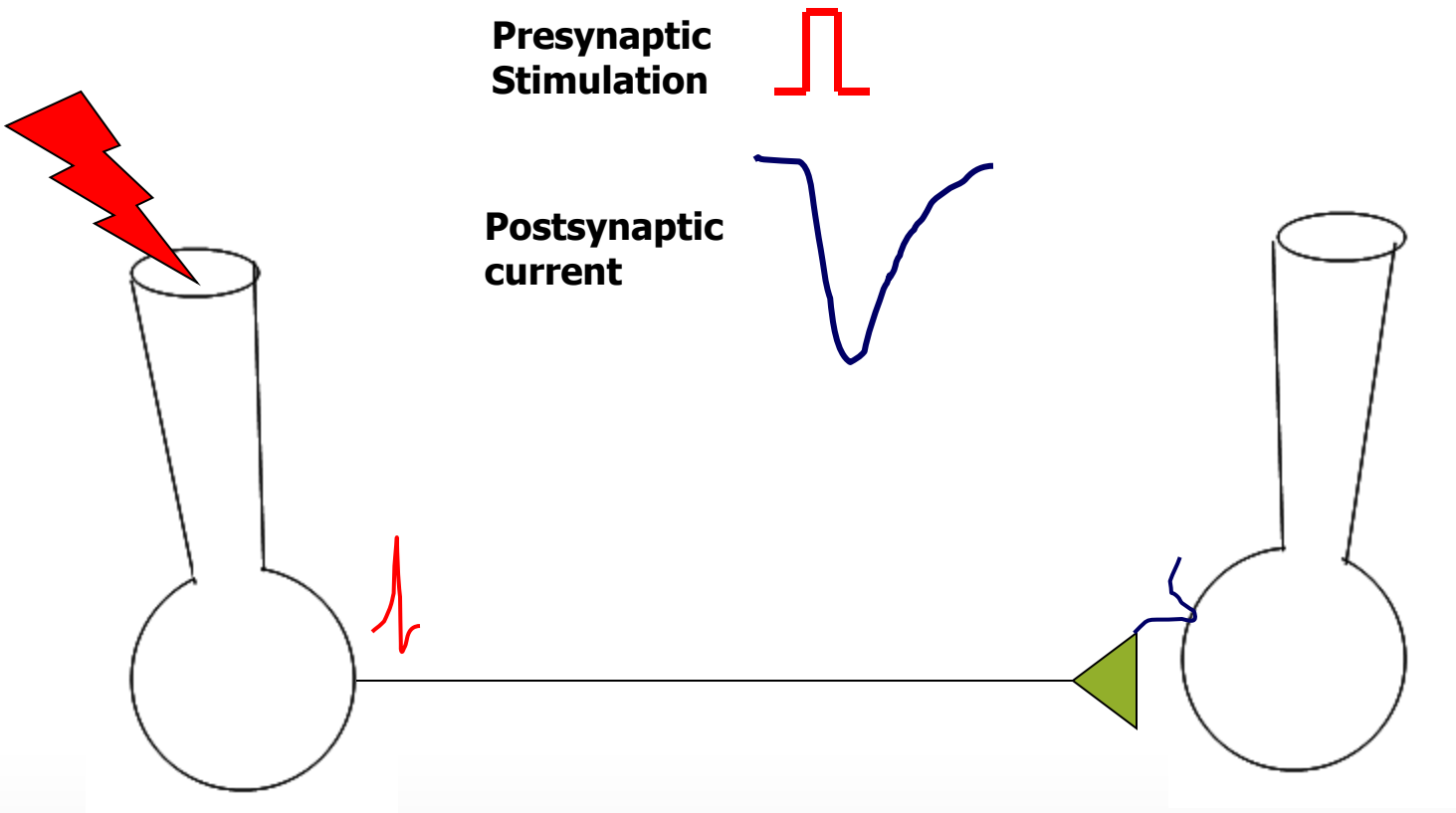
Dual Whole-cell Patch-clamp Recordings



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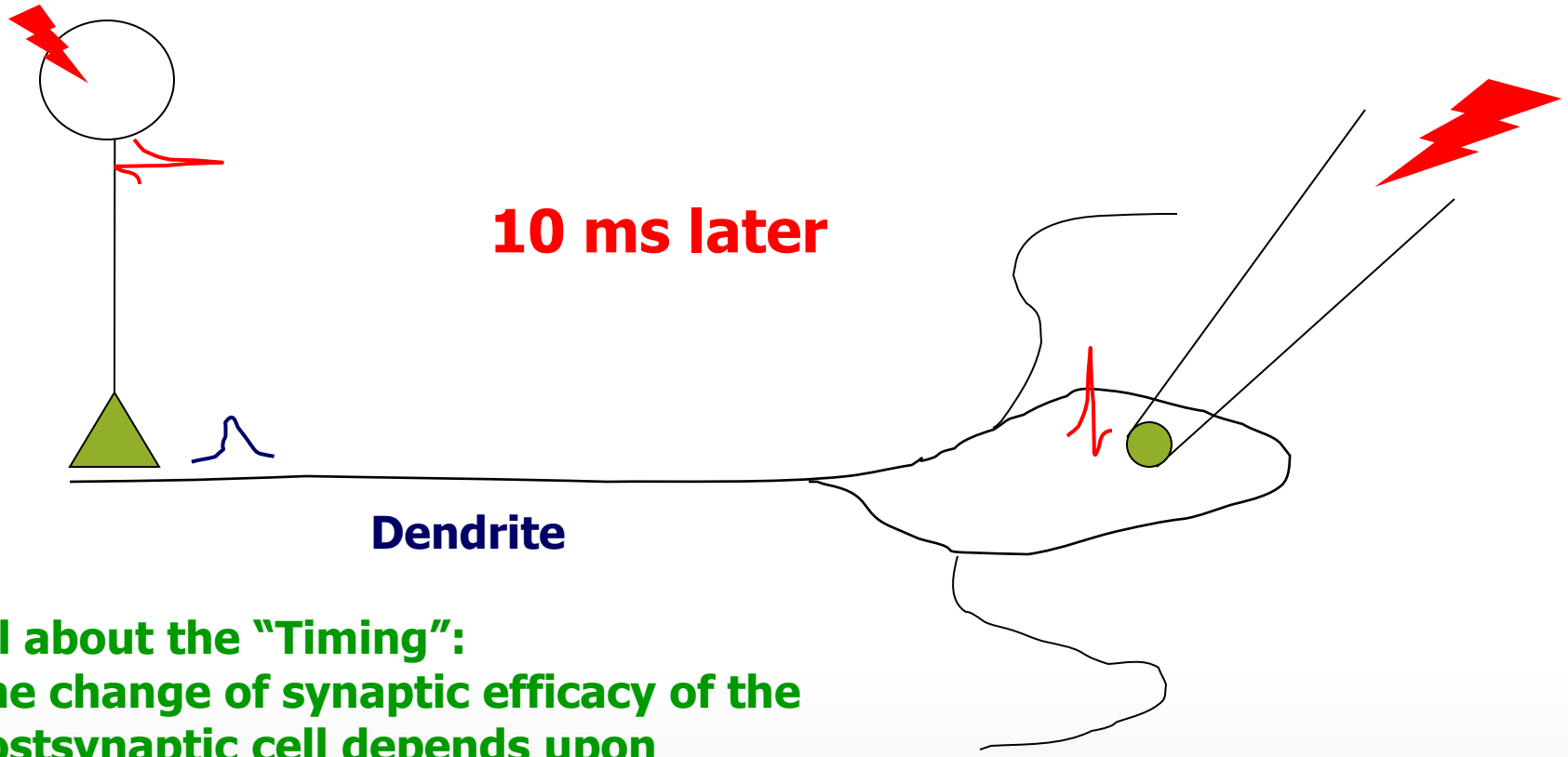
Spike timing protocol: EPSP precedes Action Potential



All about the "Timing":
The change of synaptic efficacy of the postsynaptic cell depends upon

1. **the timing of the incoming inputs**
2. **the excitability of the postsynaptic cell**

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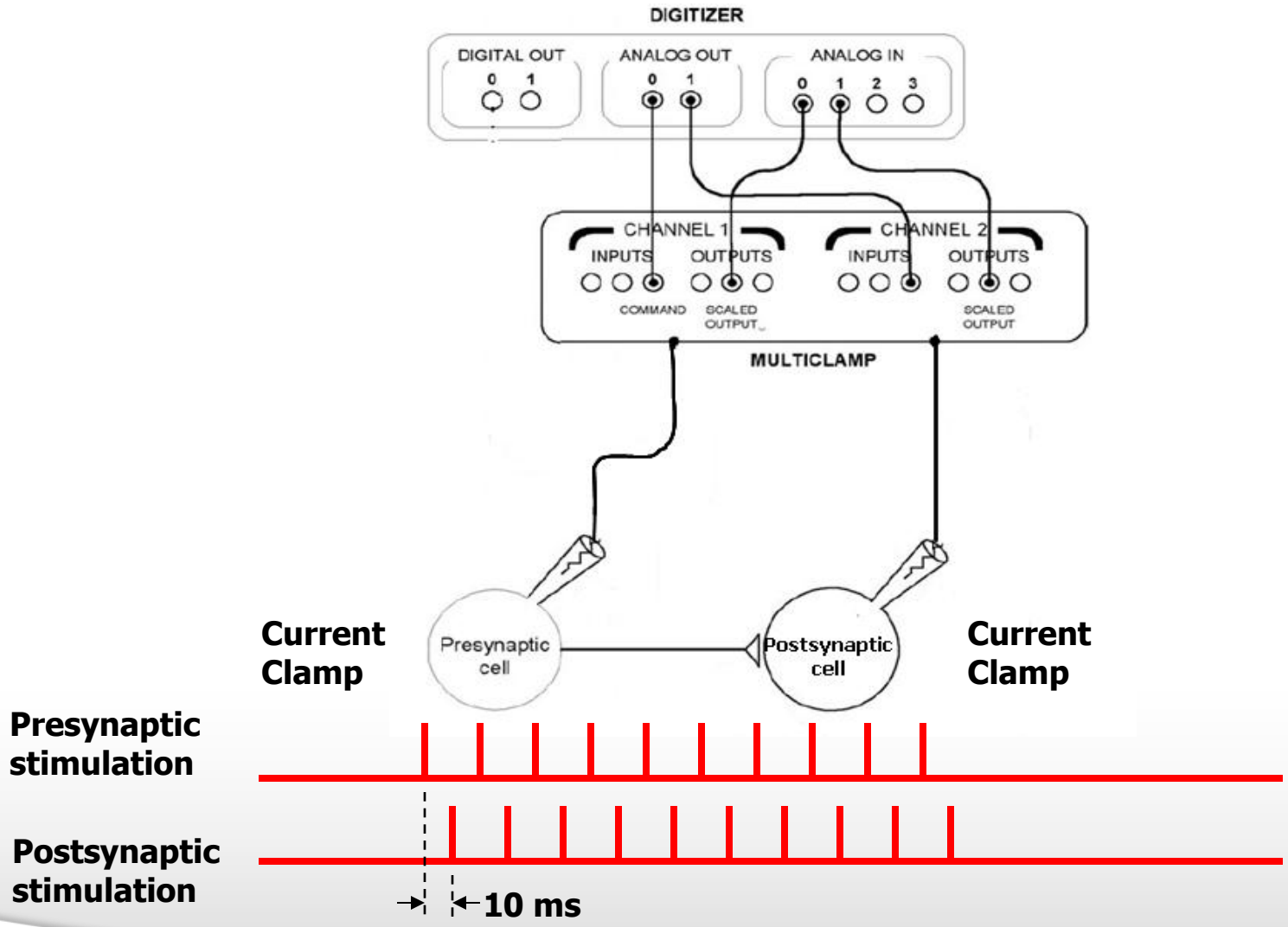
Spike timing protocol: EPSP precedes Action Potential



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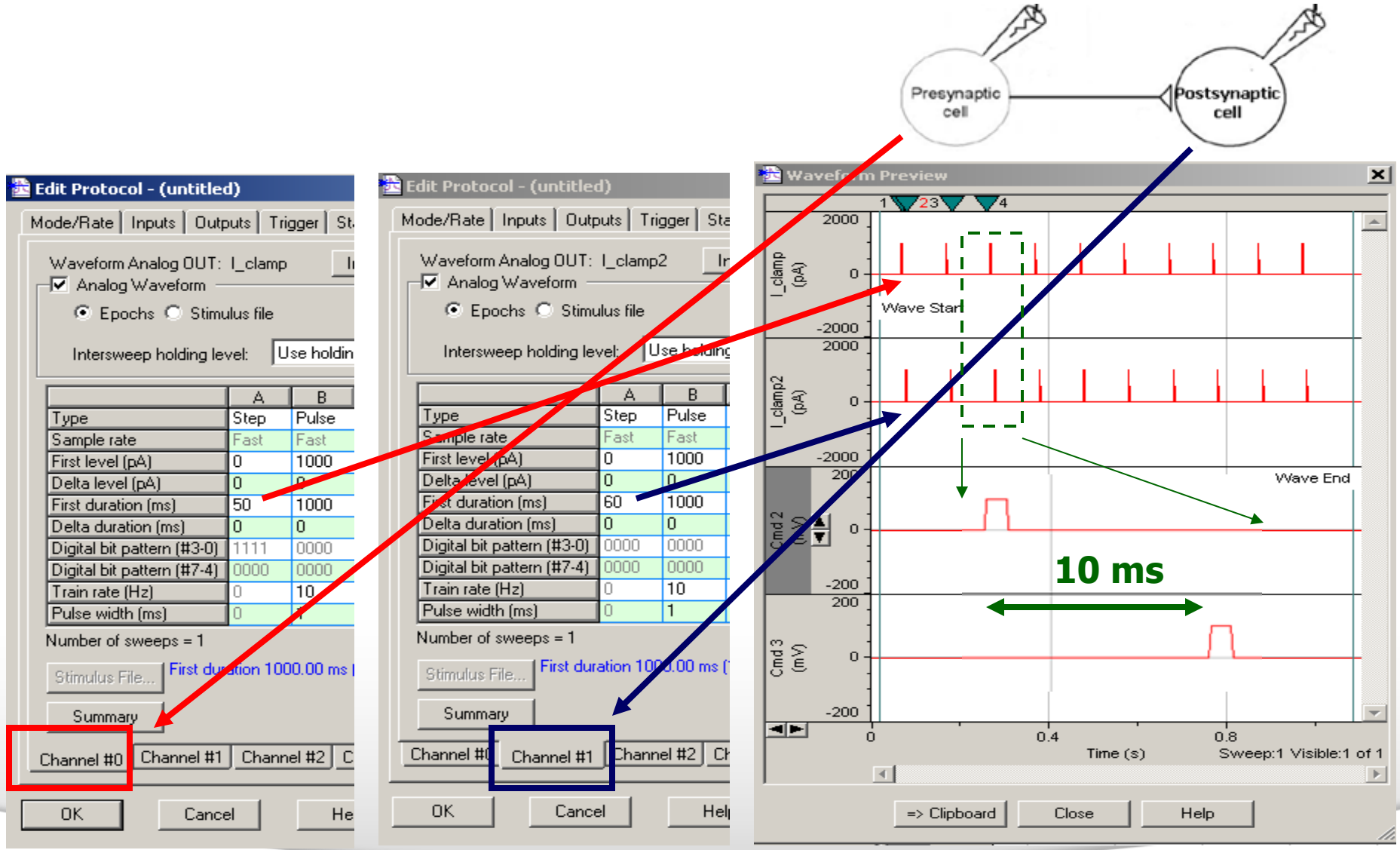
Spike timing protocol: EPSP precedes Action Potential



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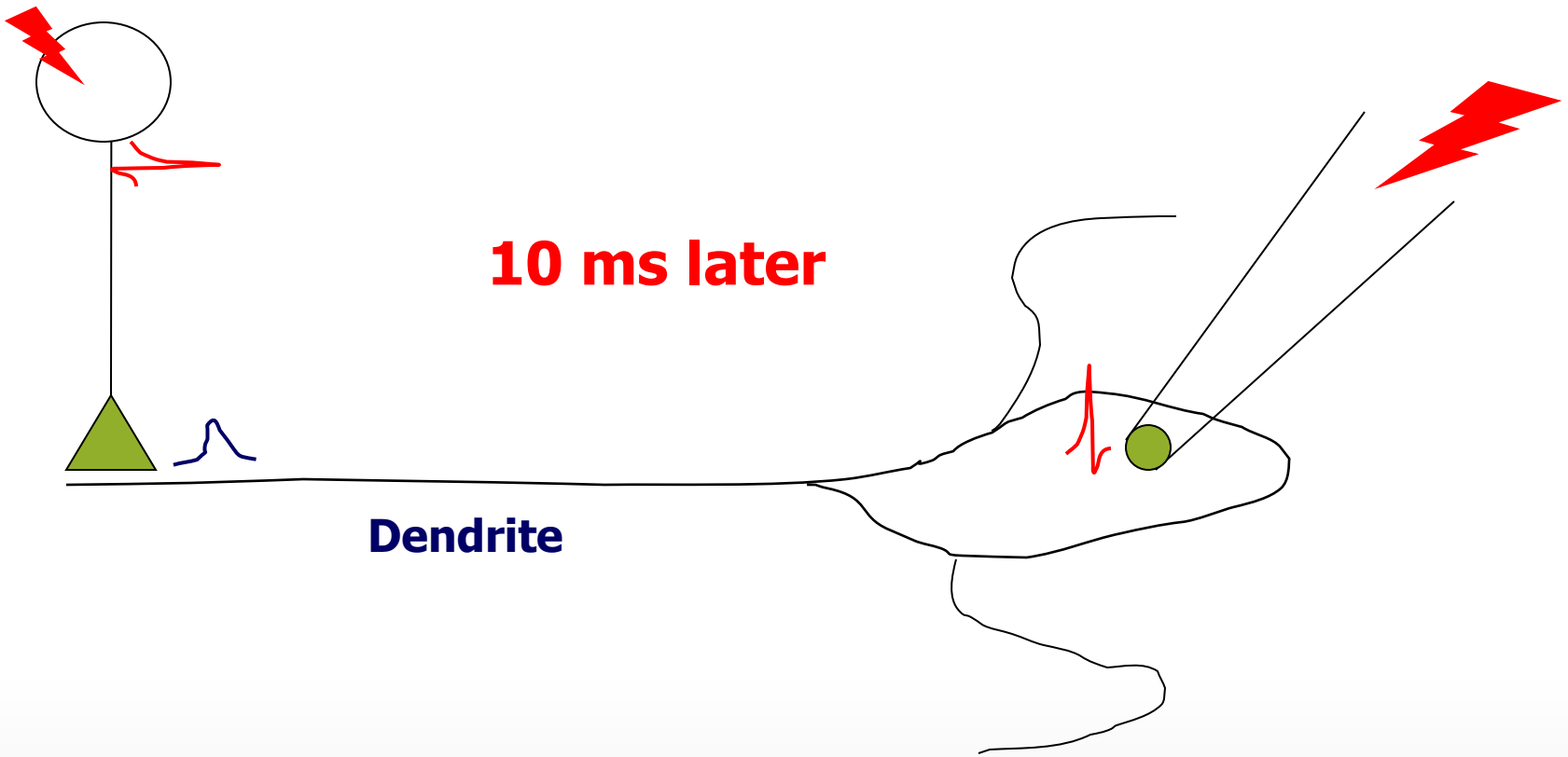
Spike timing protocol: Action Potential precedes EPSP



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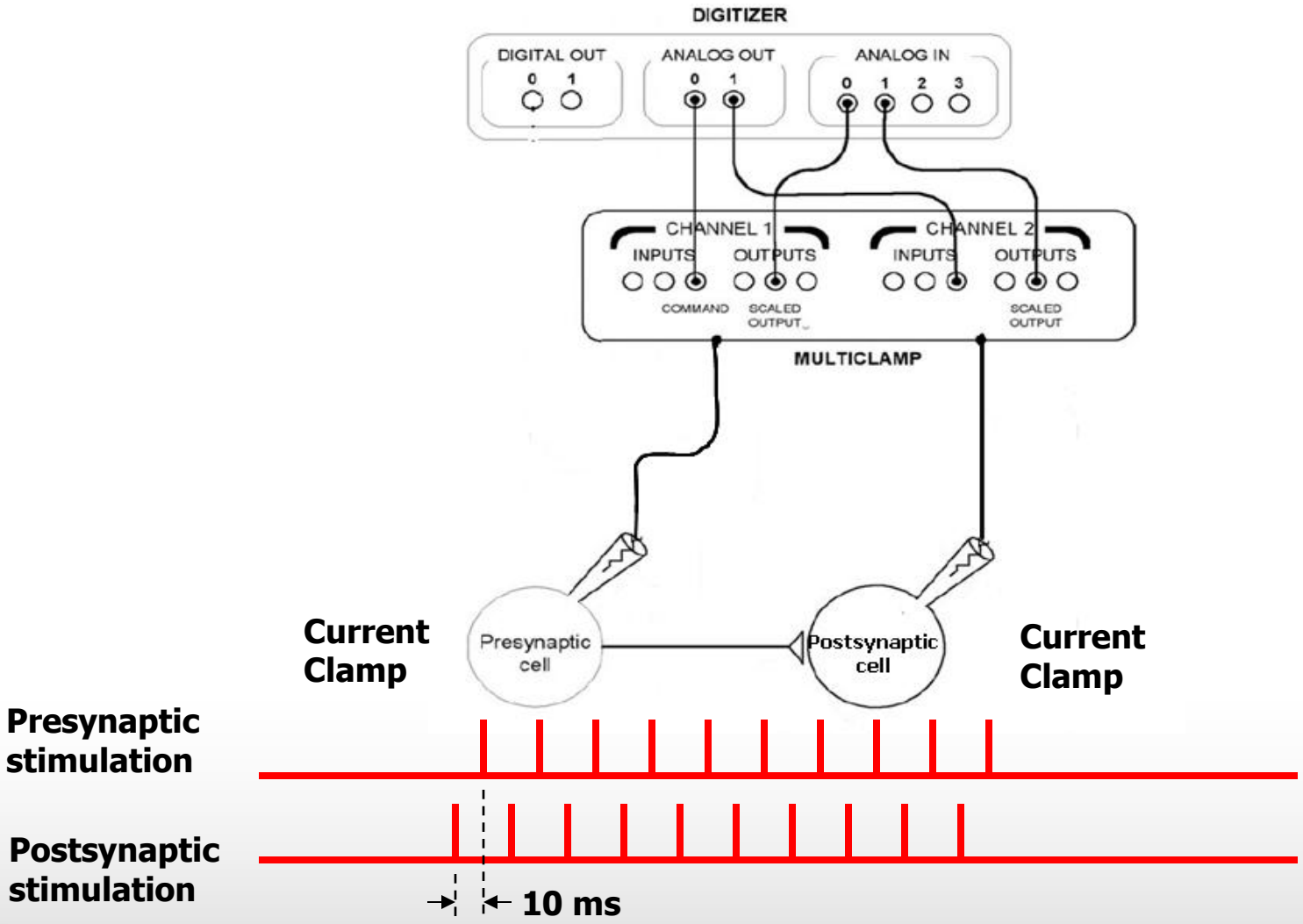
Spike timing protocol: Action Potential precedes EPSP



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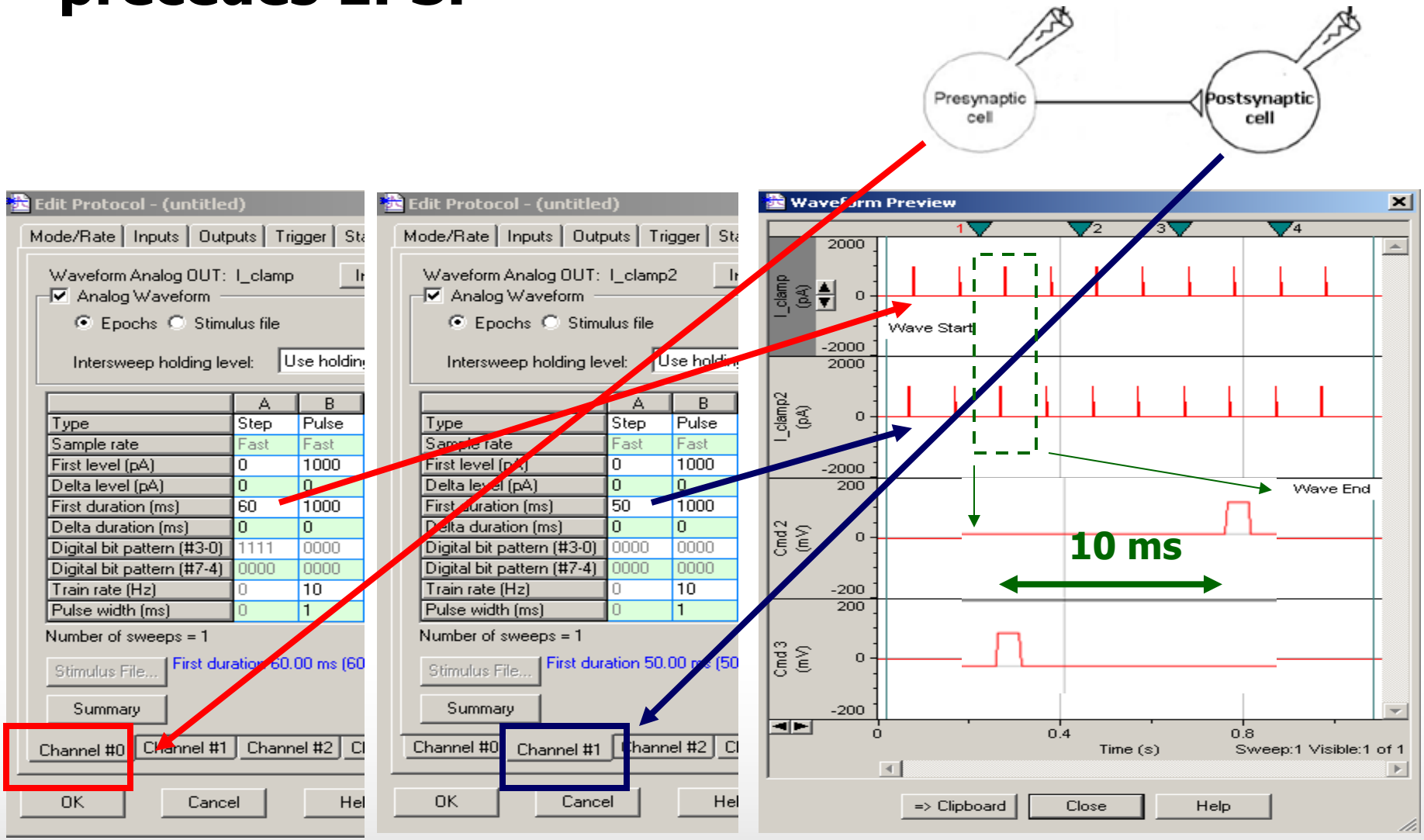
Spike timing protocol: Action Potential precedes EPSP



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Action Potential Analysis in Clampfit

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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 ($APD_{90\%}$)?

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What we learn today

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

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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

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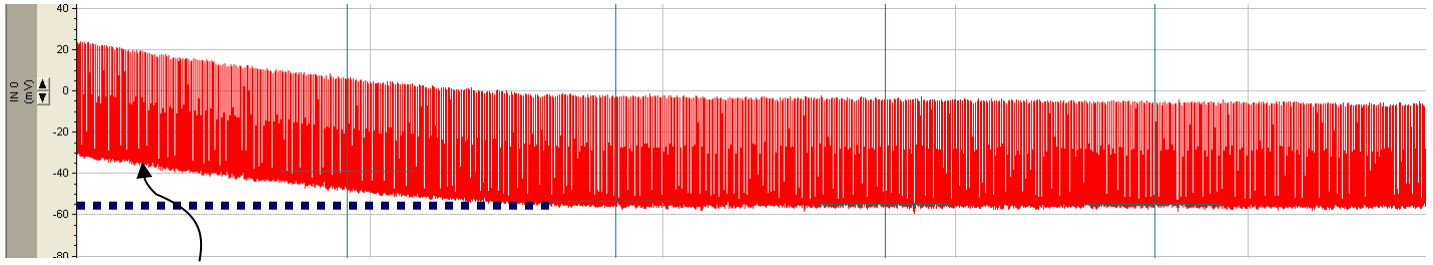
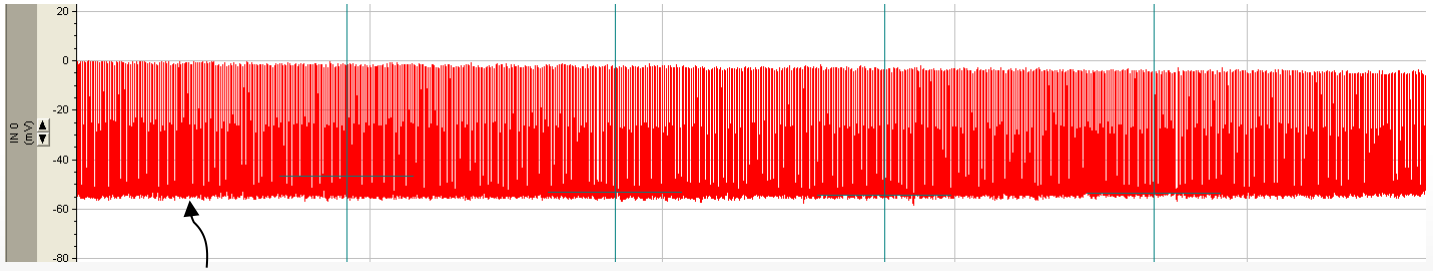
Baseline adjustment



Drifting baseline



Stable baseline

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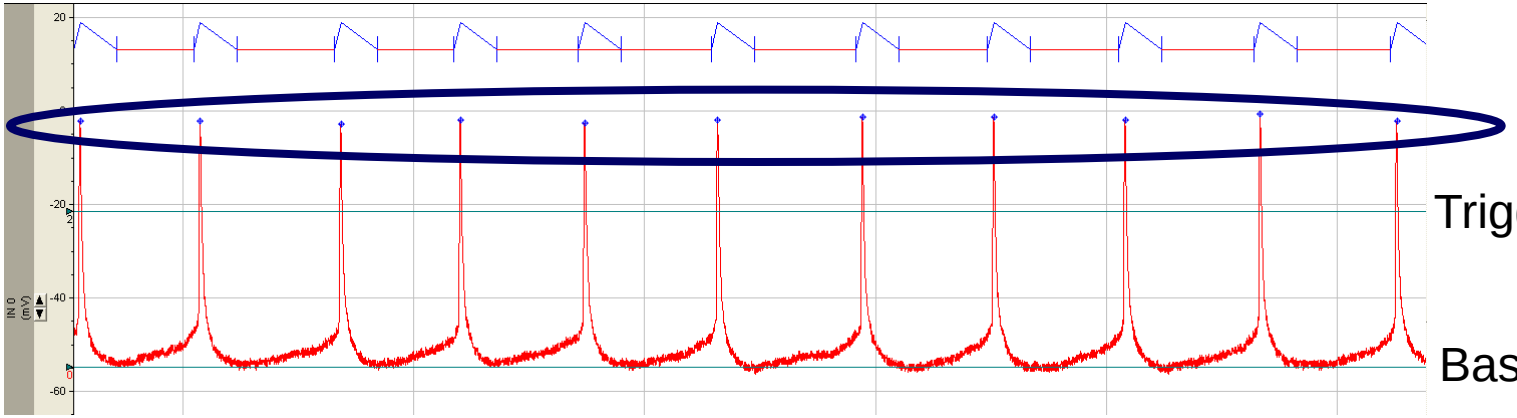
Event Search



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

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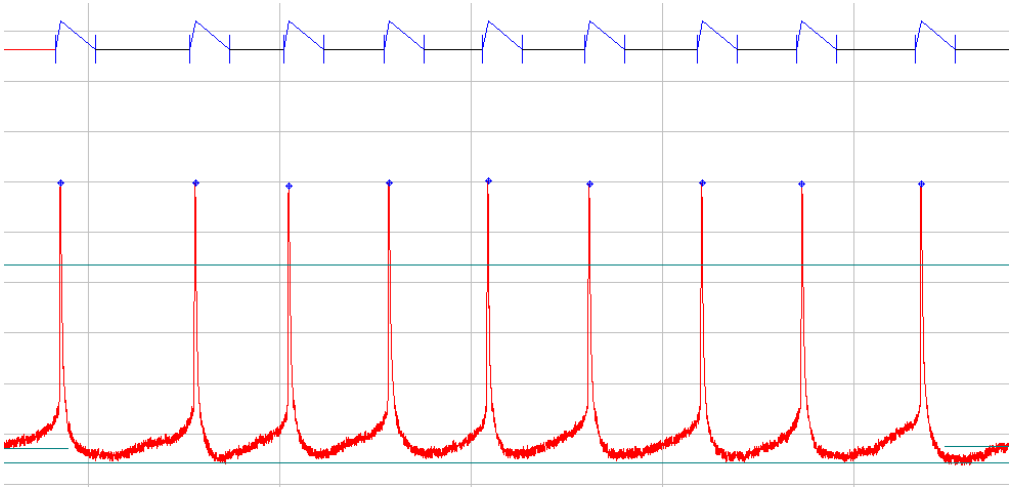
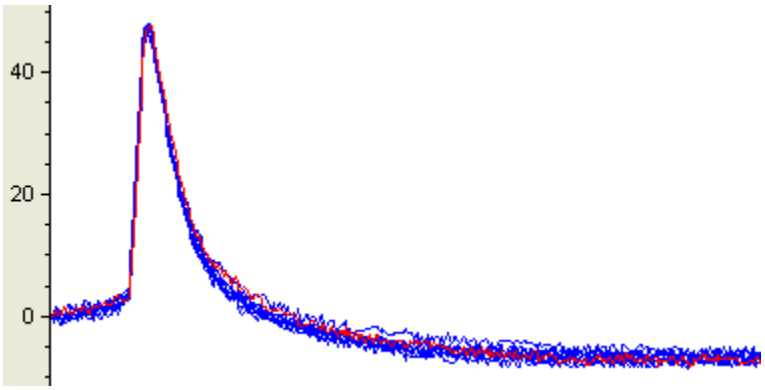
Event Sorting

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



Gap-free files



Sweep files

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Noise/Event Rejection

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

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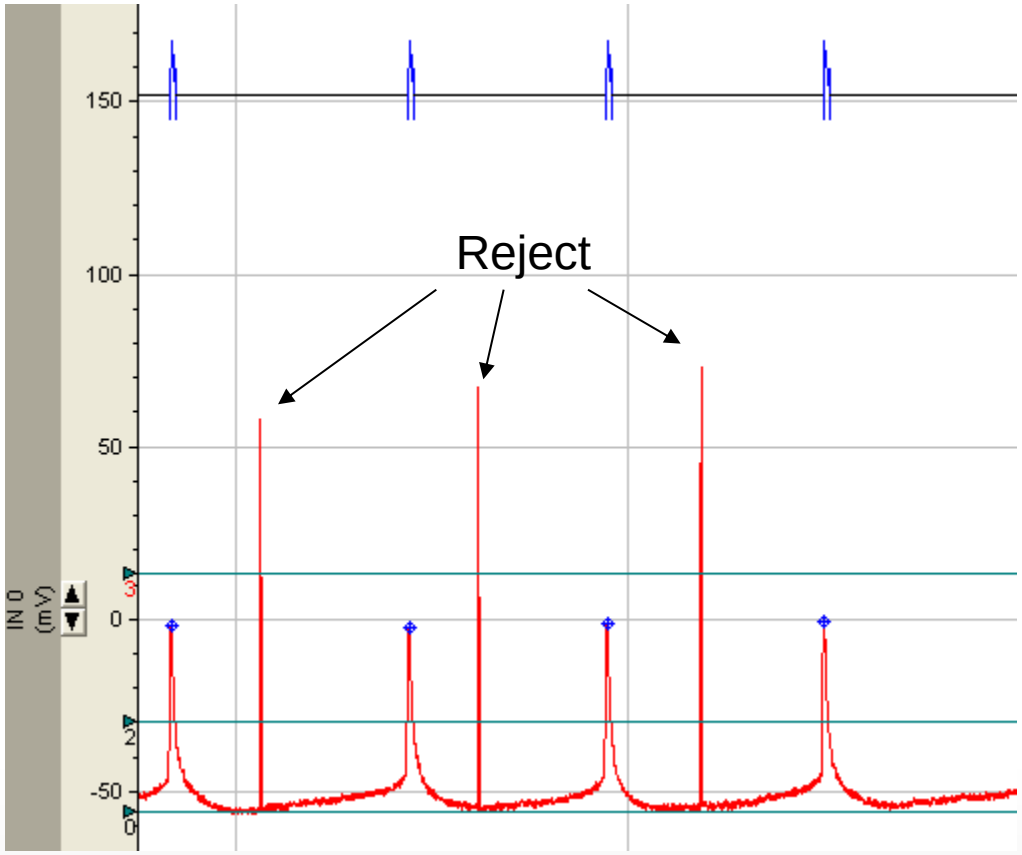
Noise/Event Rejection based on amplitude



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

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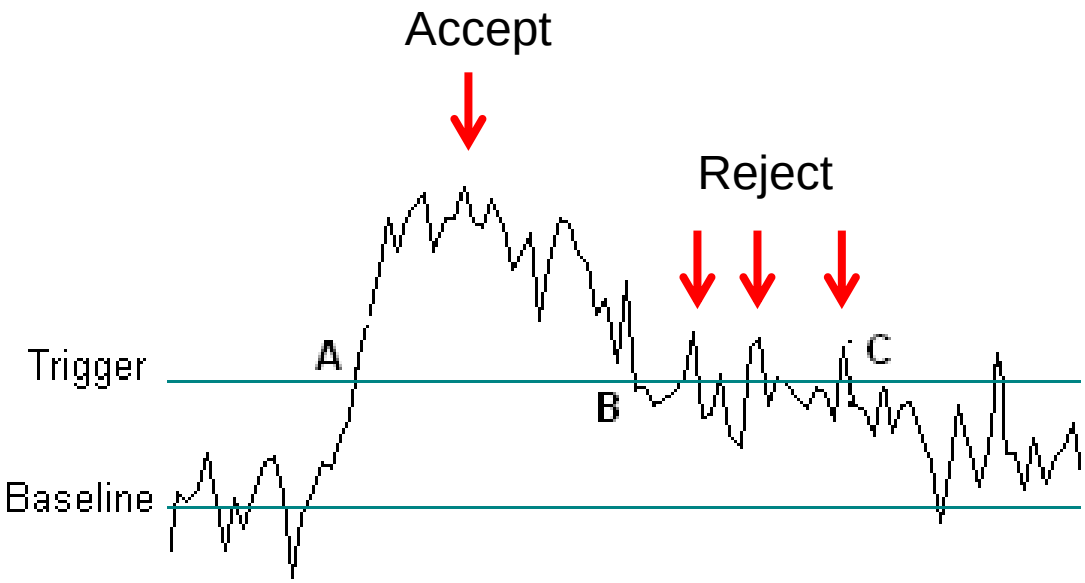
Noise/Event Rejection based on too short duration



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

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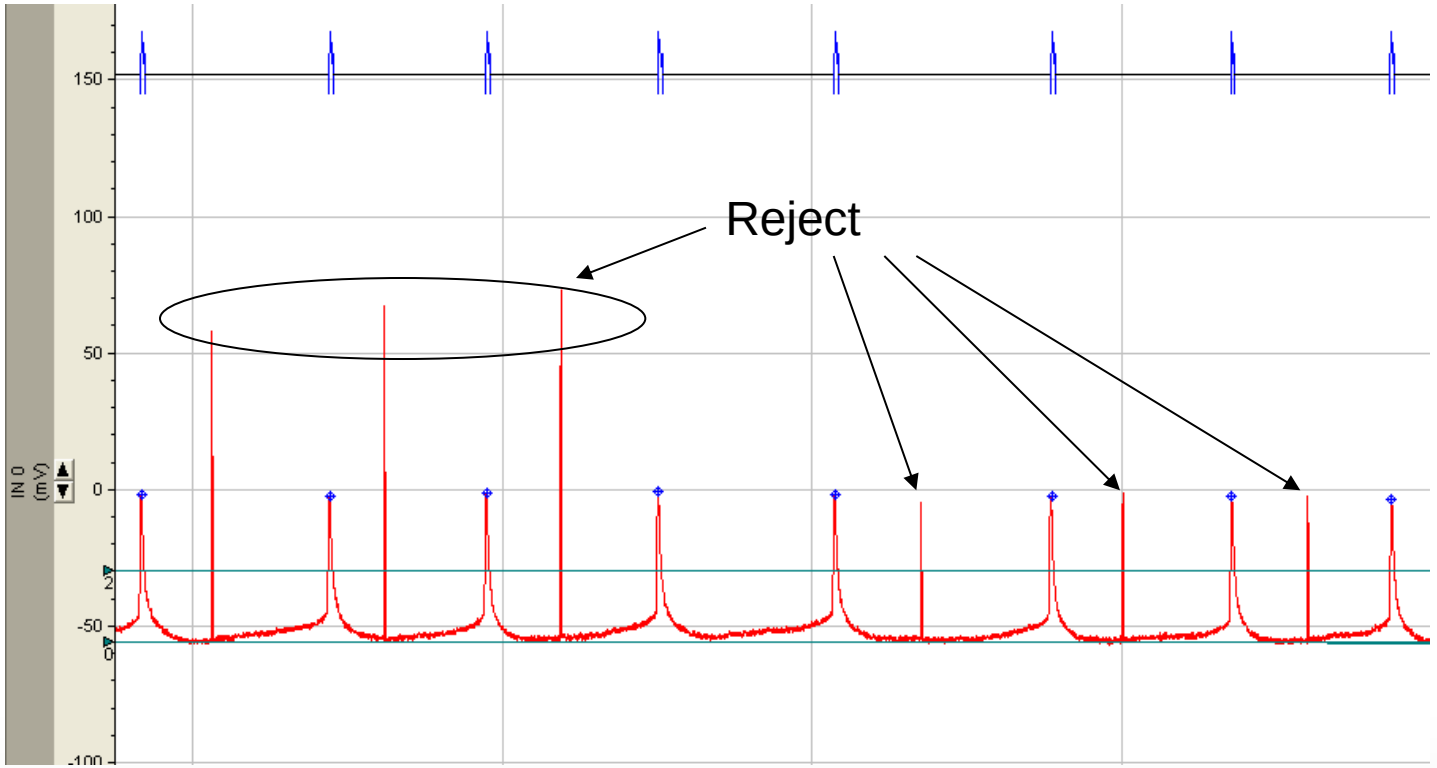
Noise/Event Rejection based on event duration



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

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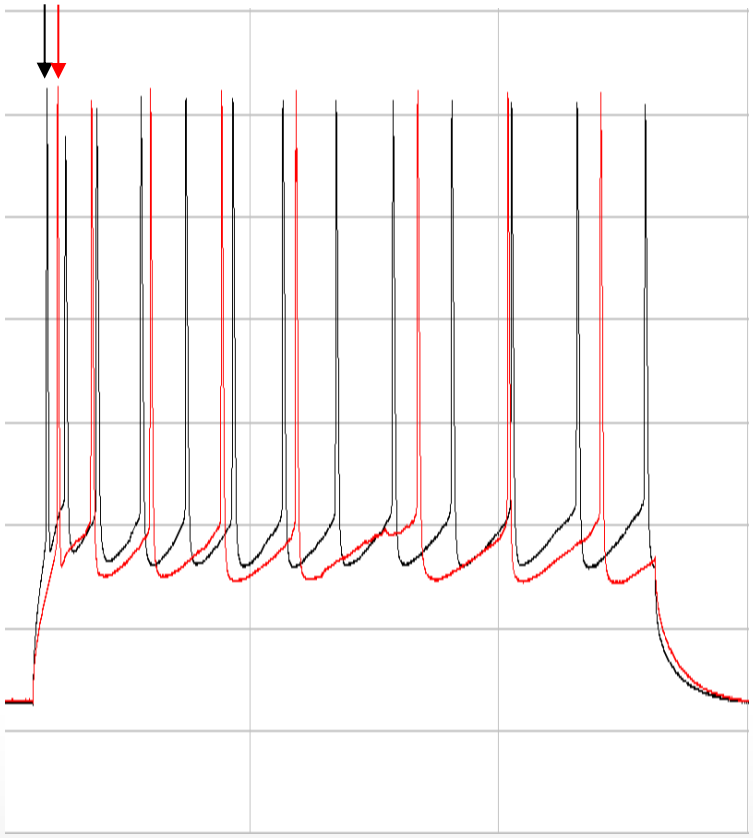
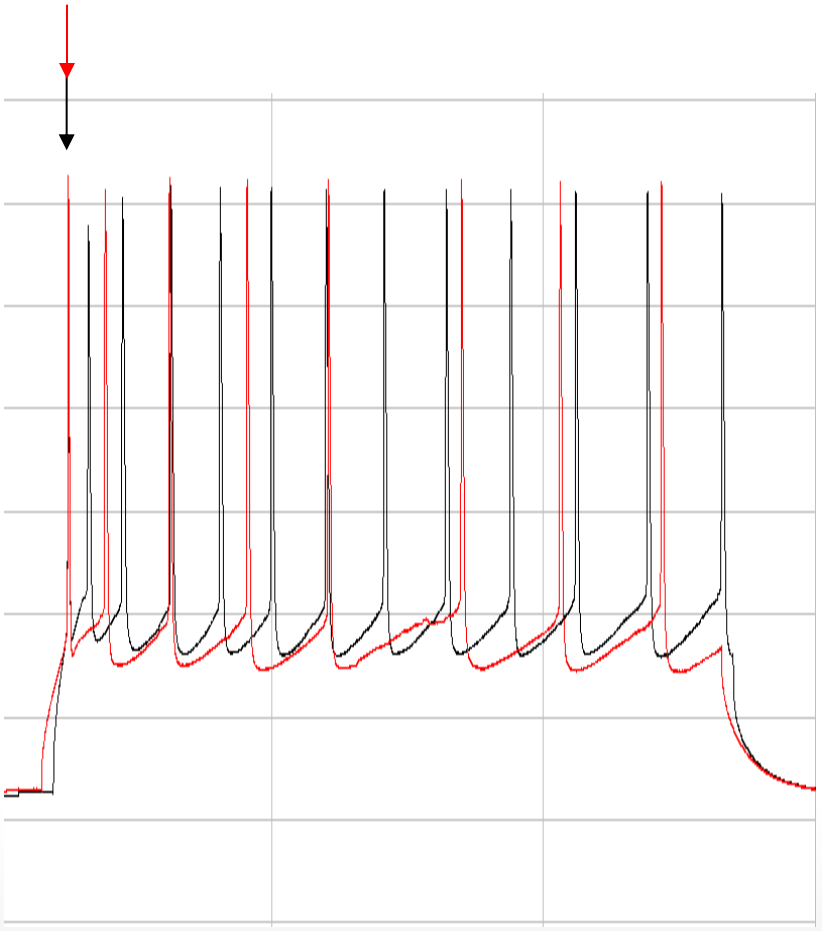
Peak Alignment





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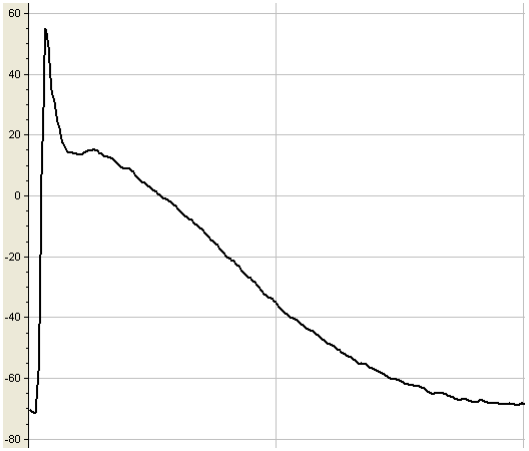
Combine traces



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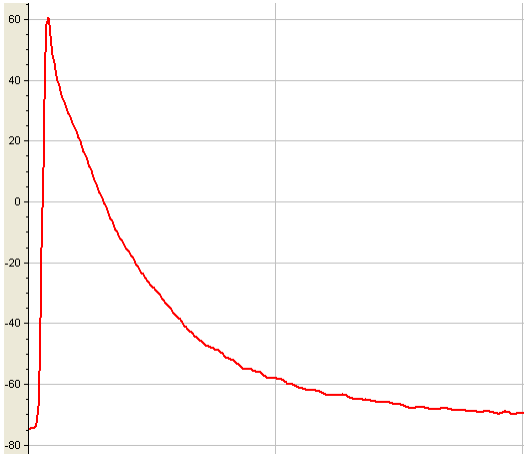

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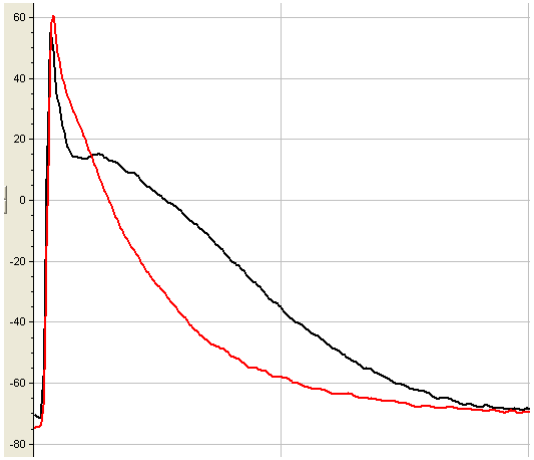

Trace in File A

Trace in File B

Trace combined

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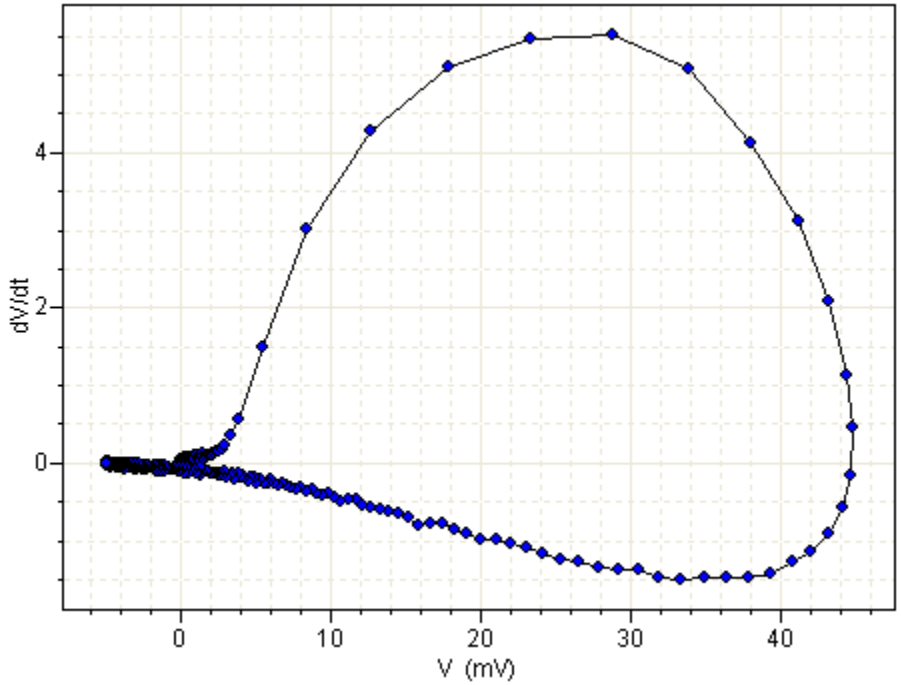
Phase Plot

- Plot dV/dt versus V



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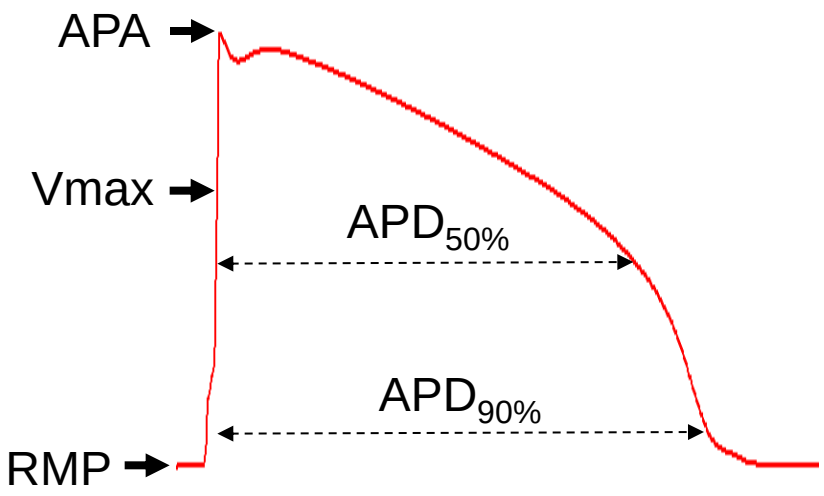
Cardiac Action Potential Analysis

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



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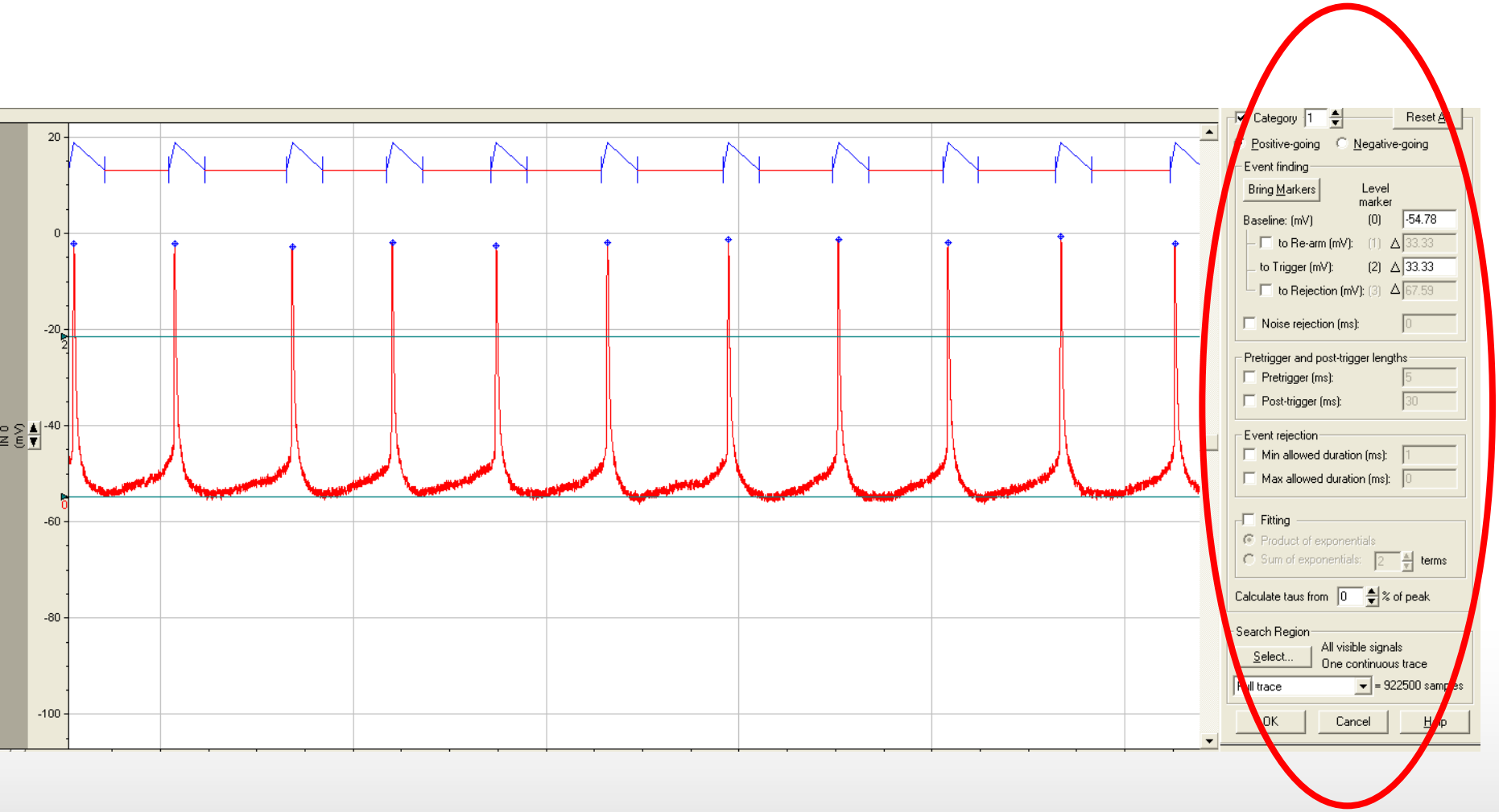
Event Detection/Threshold Search



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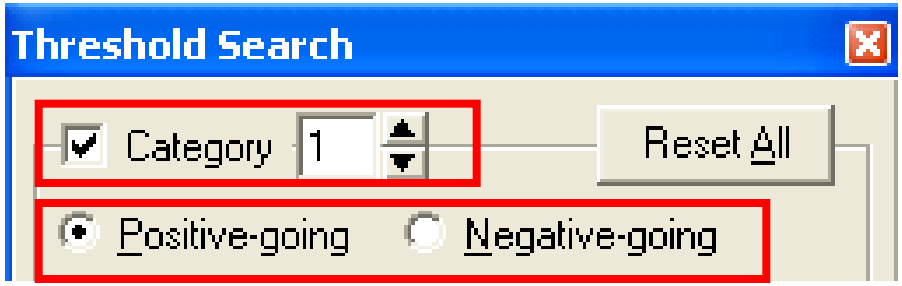
Set category

- 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



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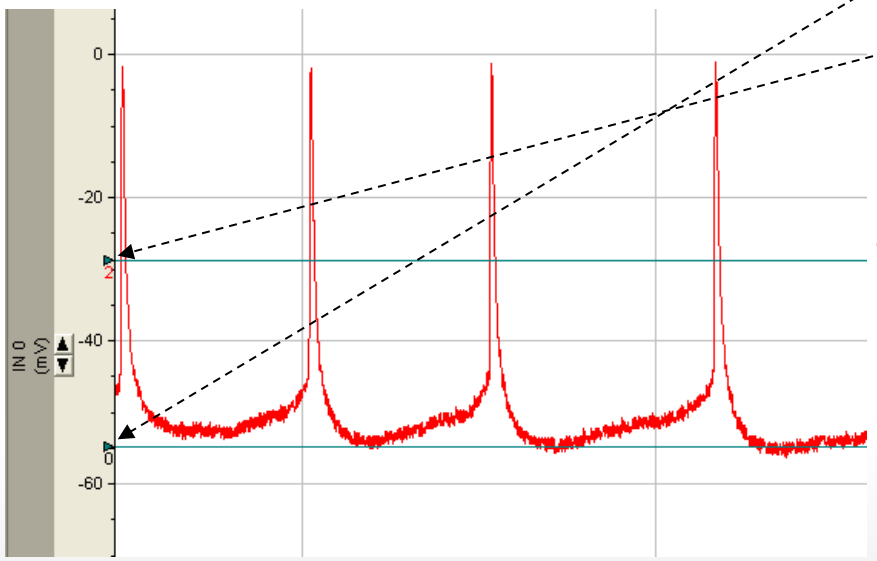
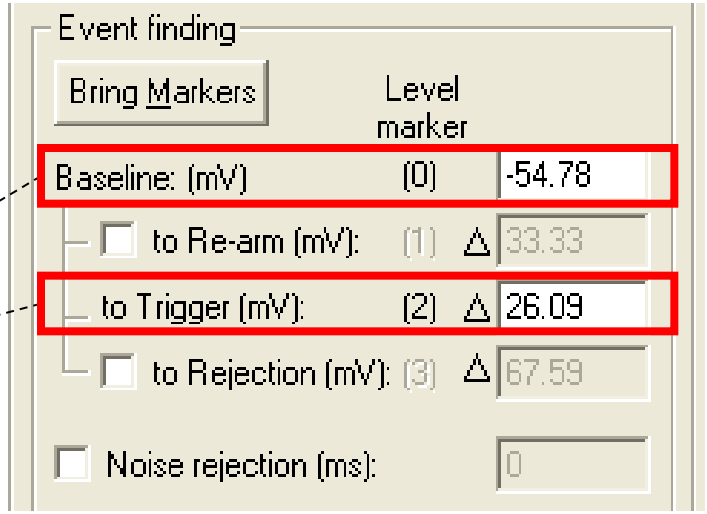
Bring markers

- Bring markers
  - Baseline marker
  - Trigger marker





Trigger marker

Baseline marker

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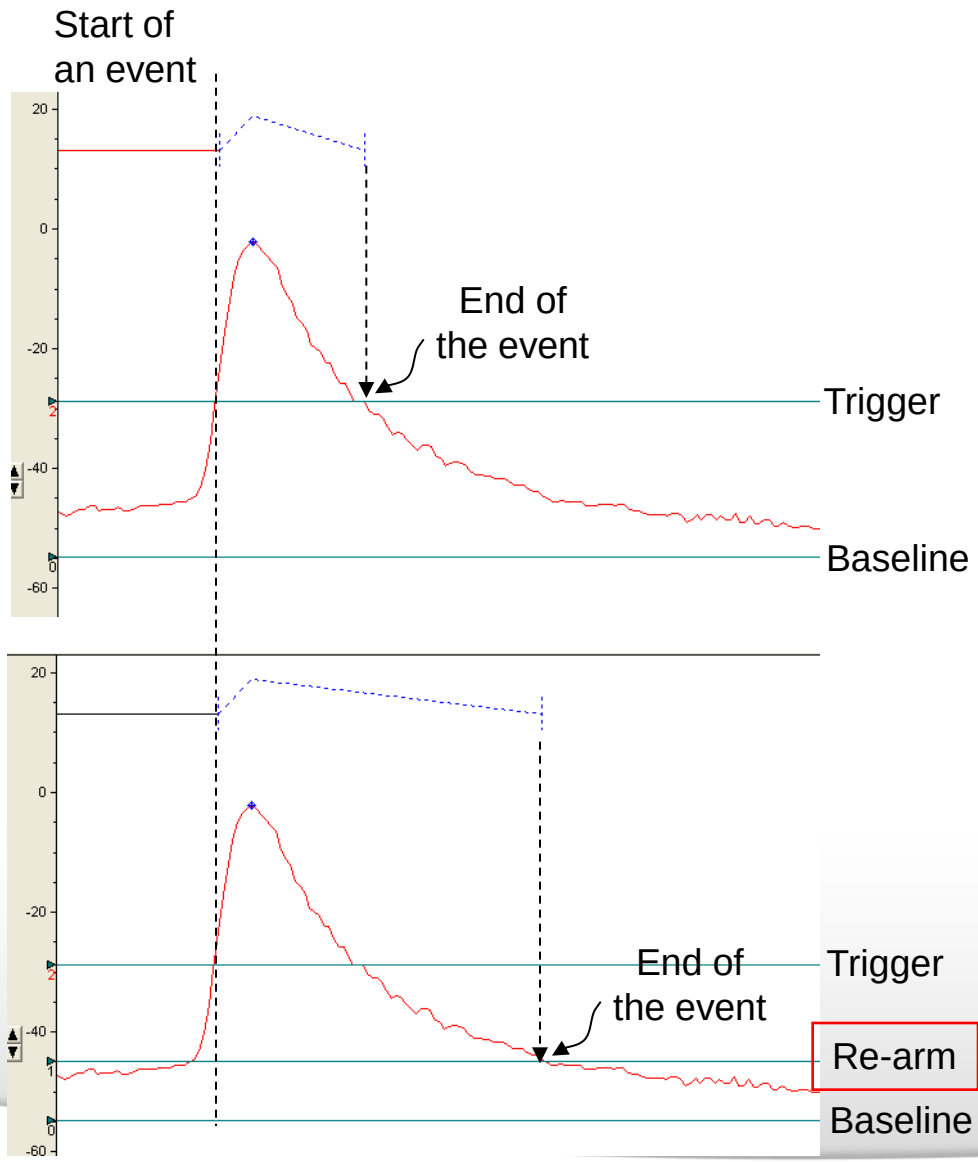
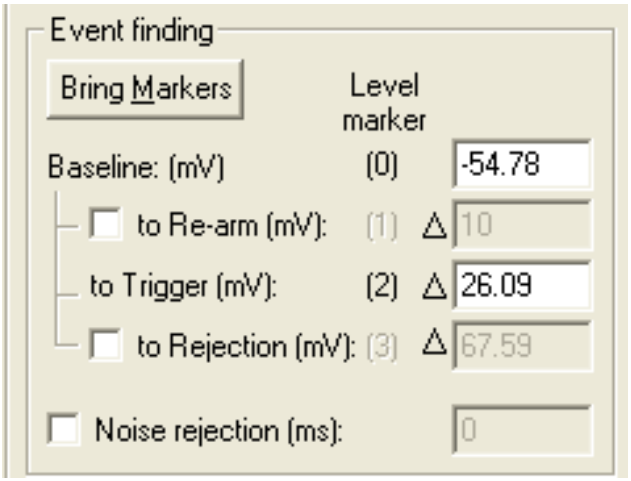
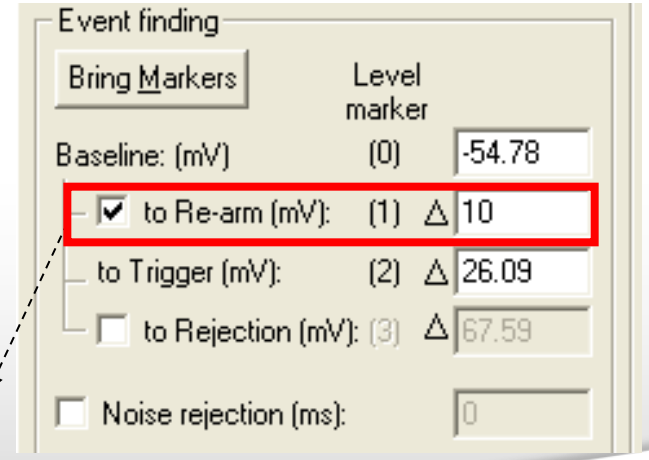
Re-arm







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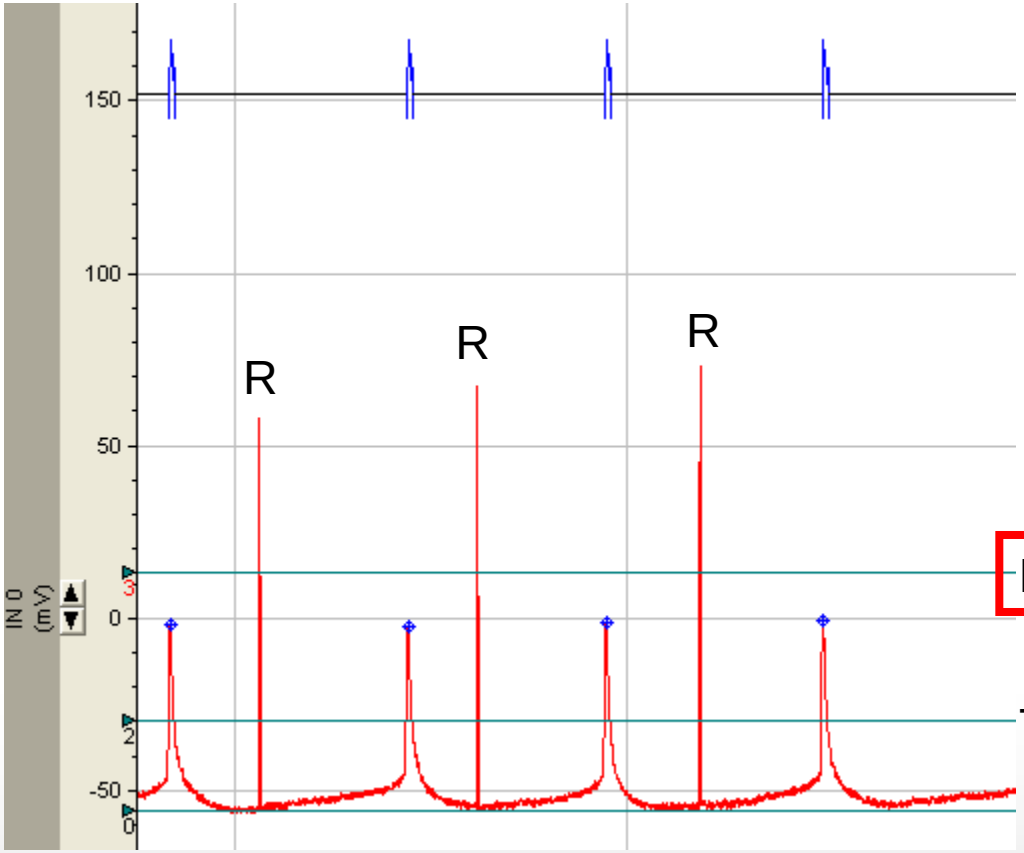
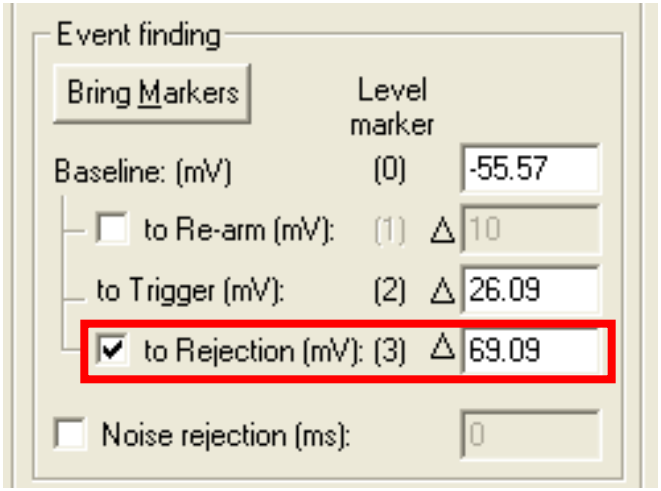
Event Rejection





Rejection level

Trigger level

Baseline level

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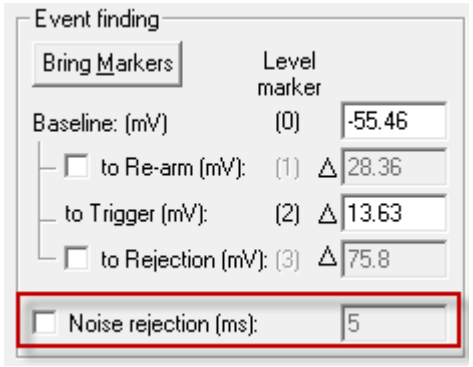
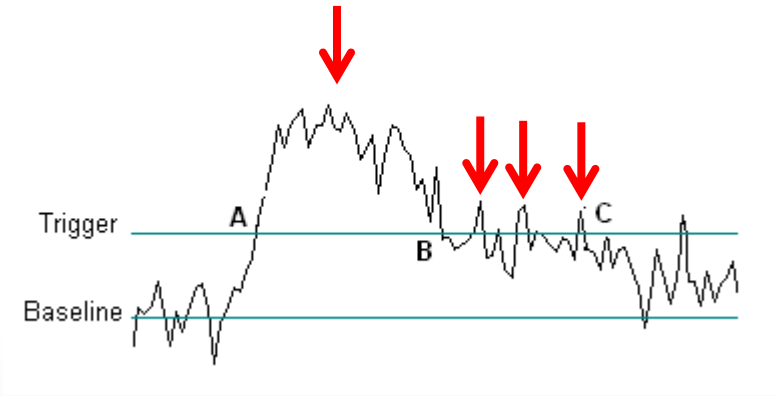
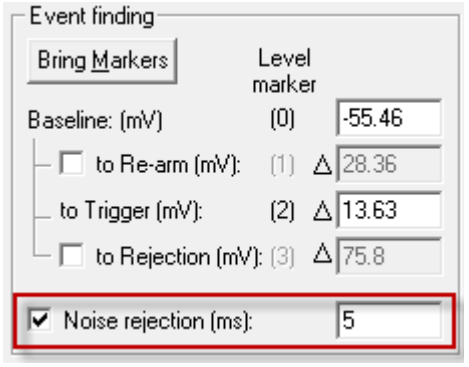
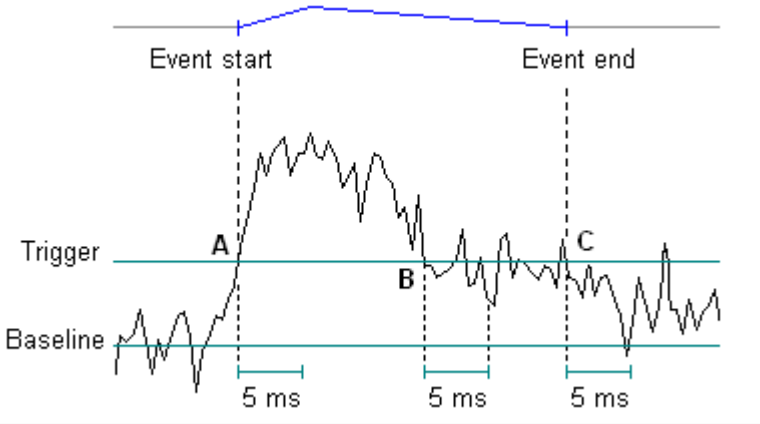
Noise Rejection





Event Start and End
without Noise rejection





Event Start and End
with 5 ms Noise rejection

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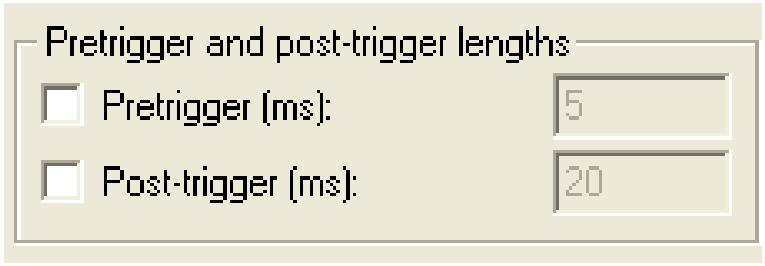
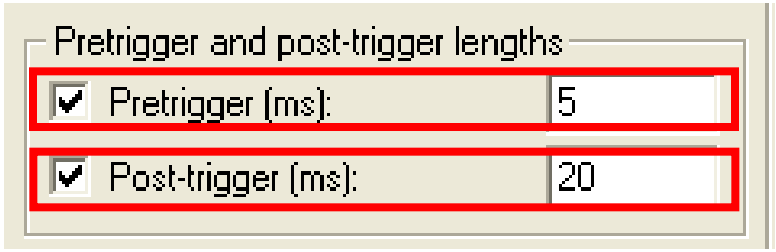
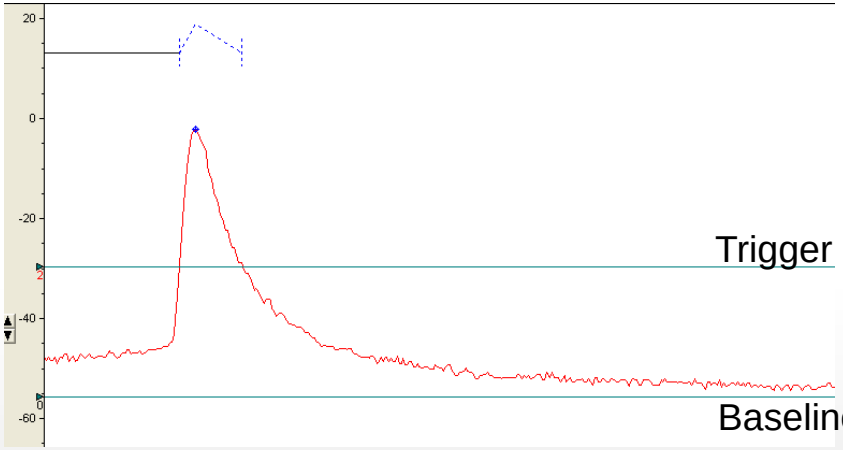
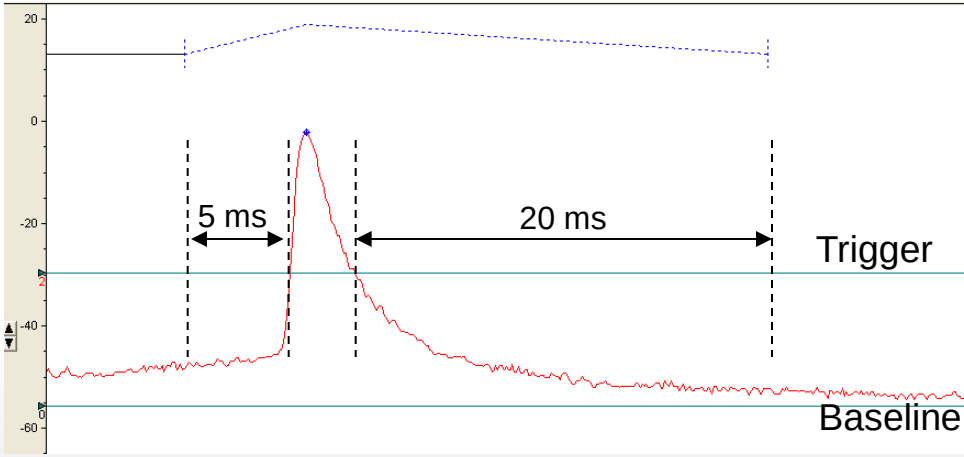
Pre-trigger and Post-trigger









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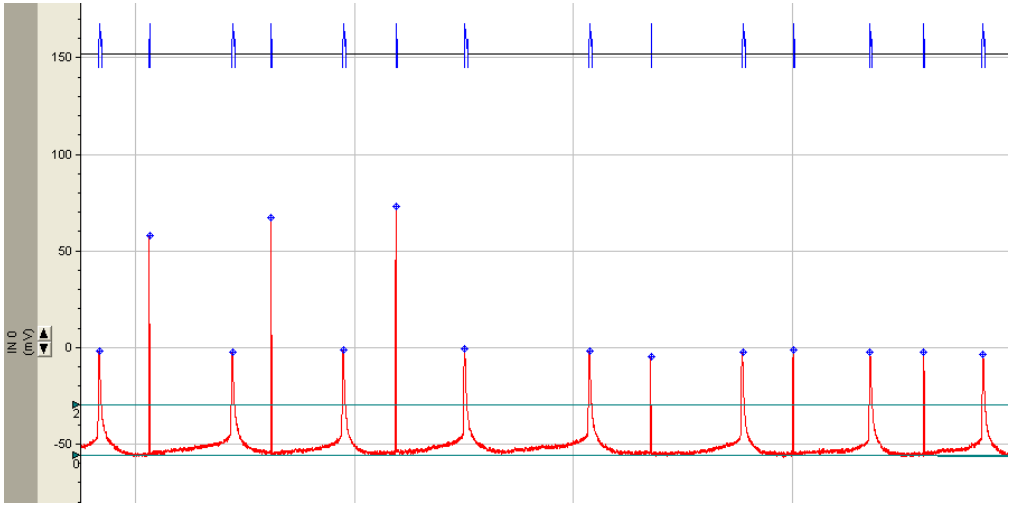
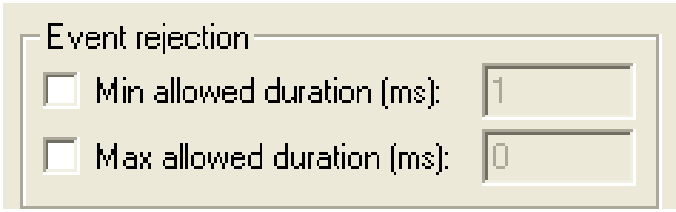
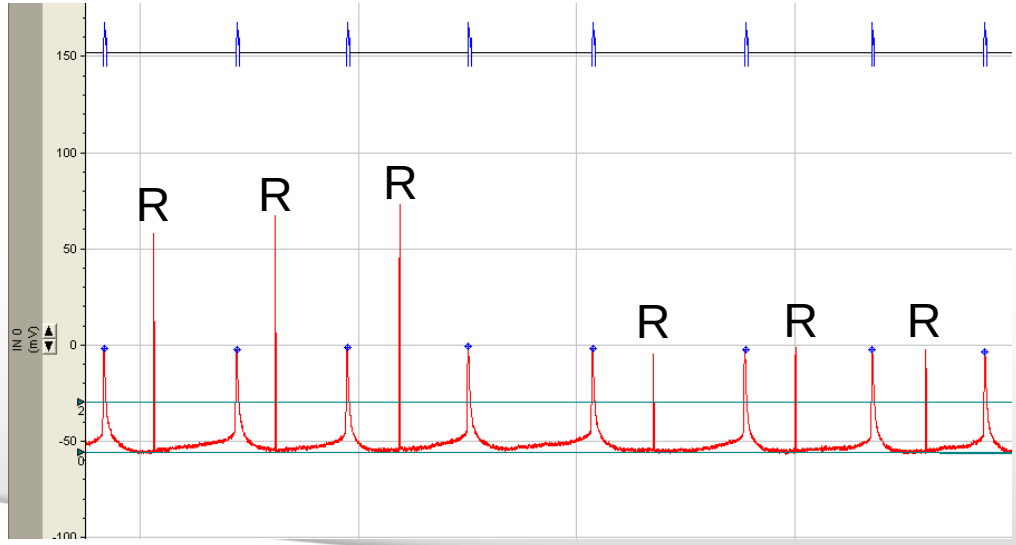
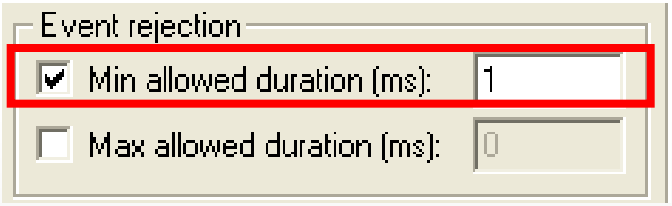
Event Rejection









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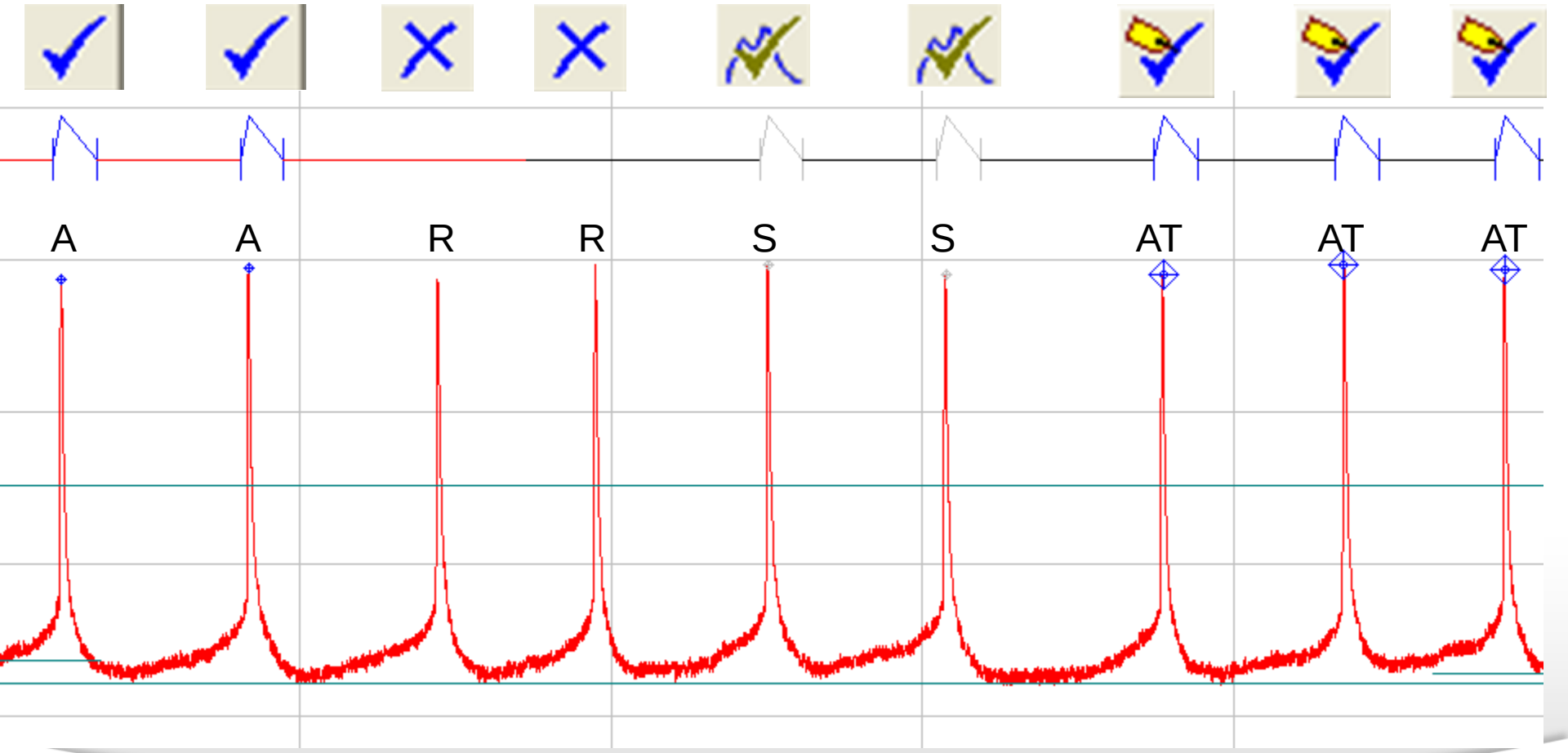
Accept, Reject, Suppress, Accept Tag



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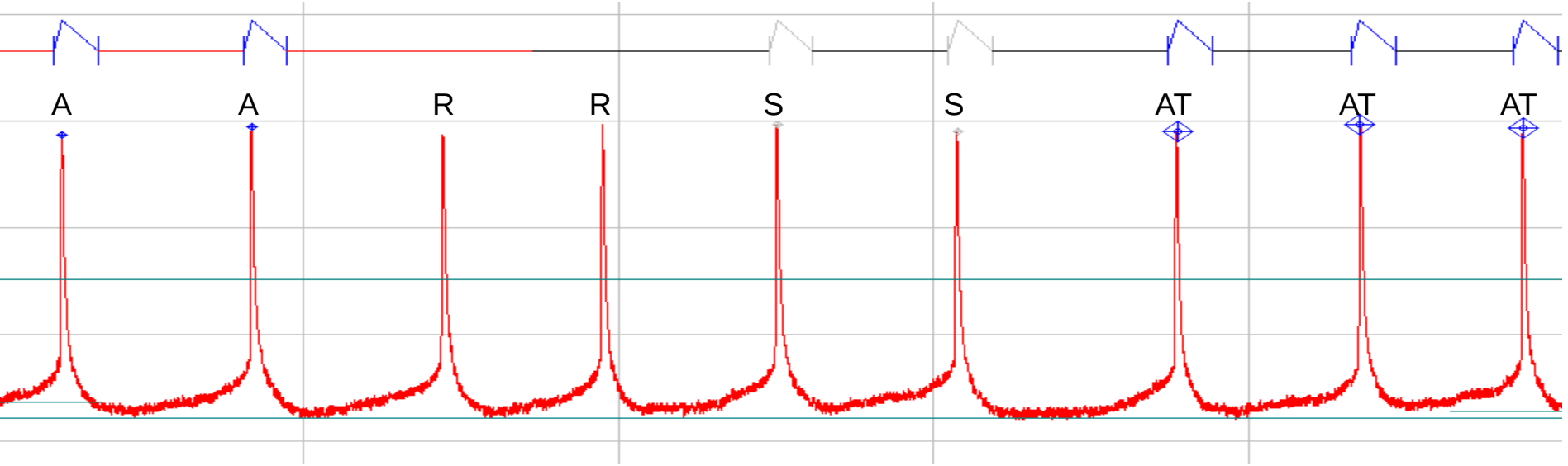
Event States



	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

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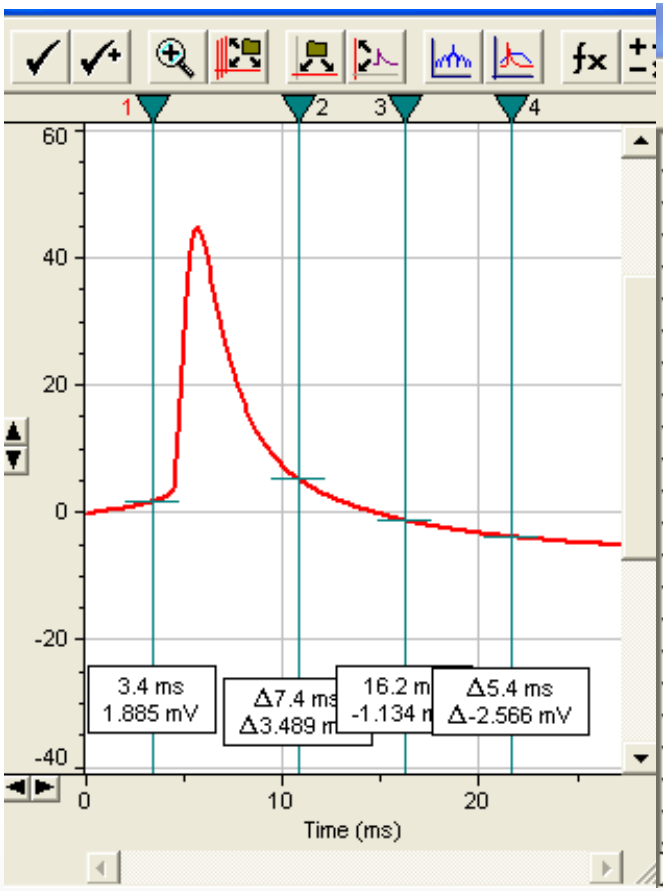
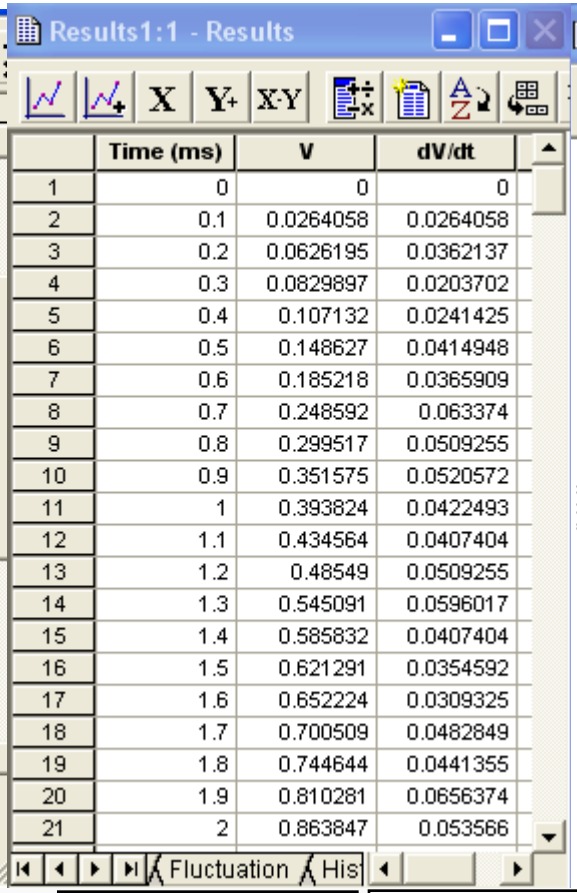
Phase Plot

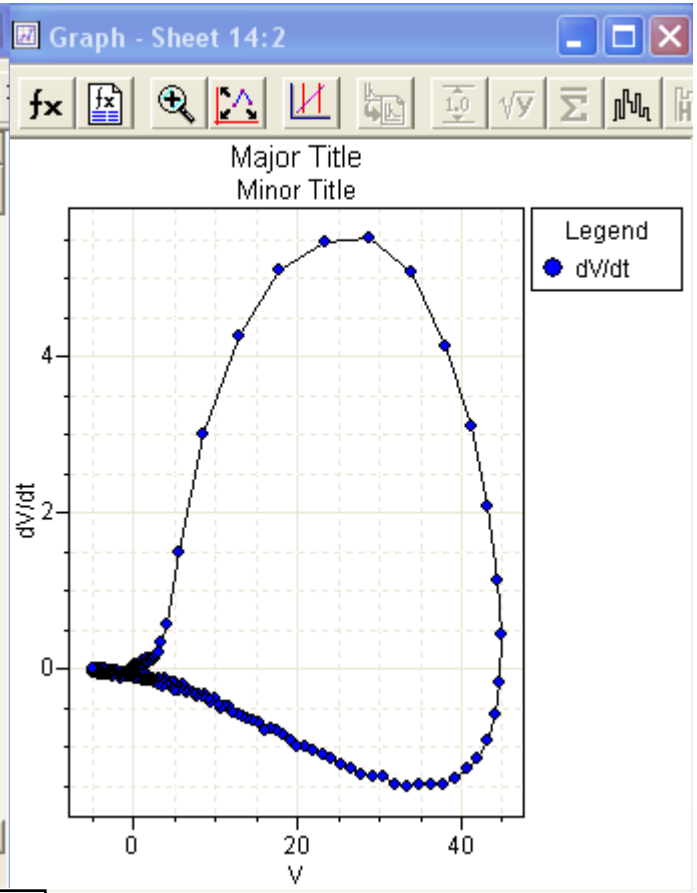


Select the trace



Transfer trace

Column Arithmetic cC=diff(cB)



Plot dV/dt vs V

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Summary

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

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Analysis of Synaptic Events with the Clampfit Data Analysis Module

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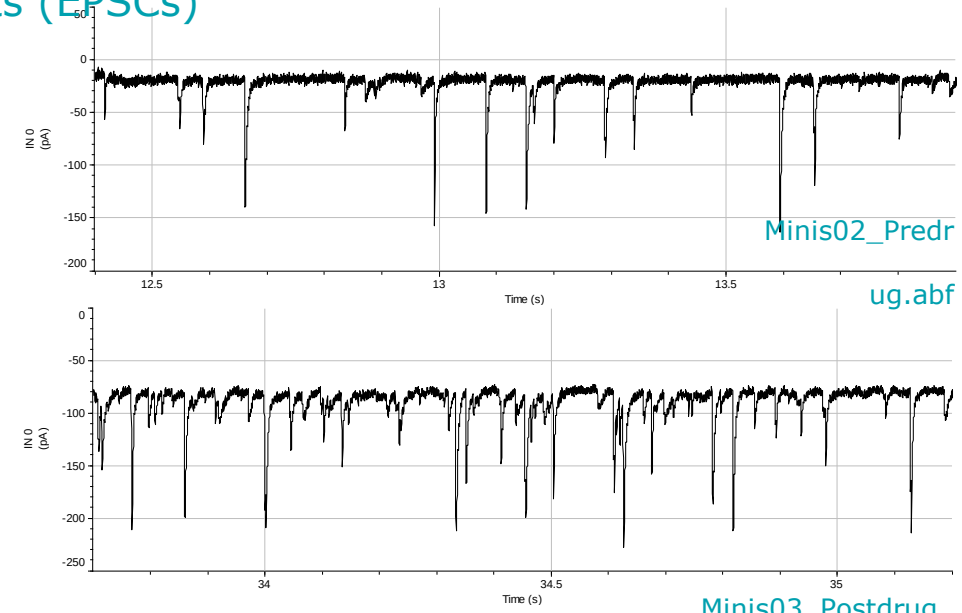
Agenda

- 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

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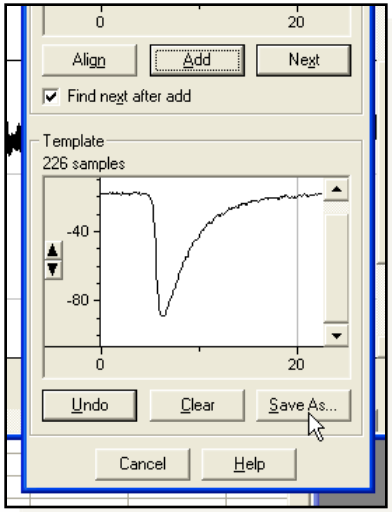
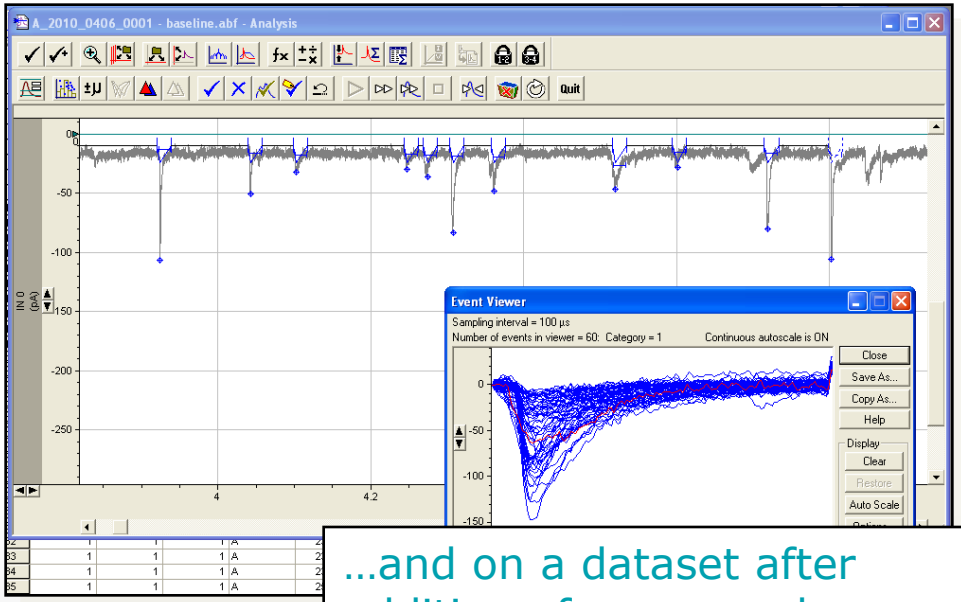
Strategy

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

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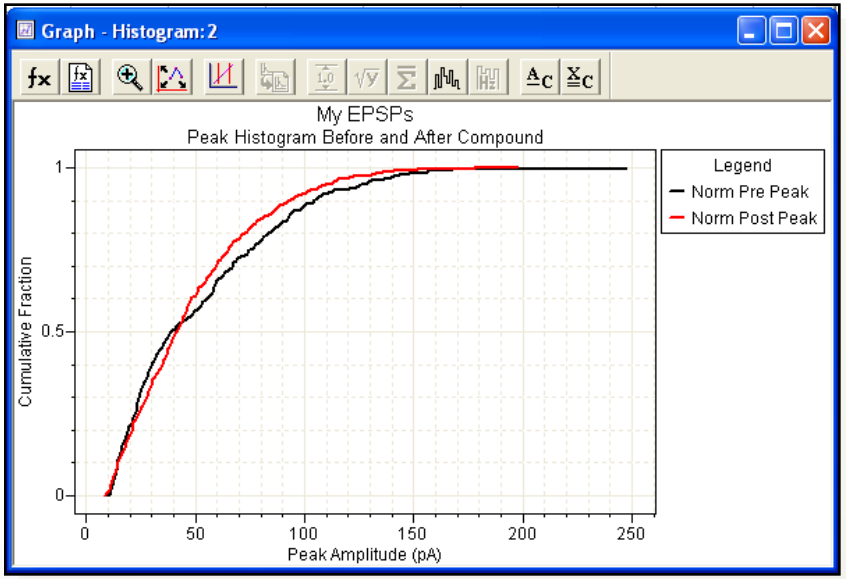
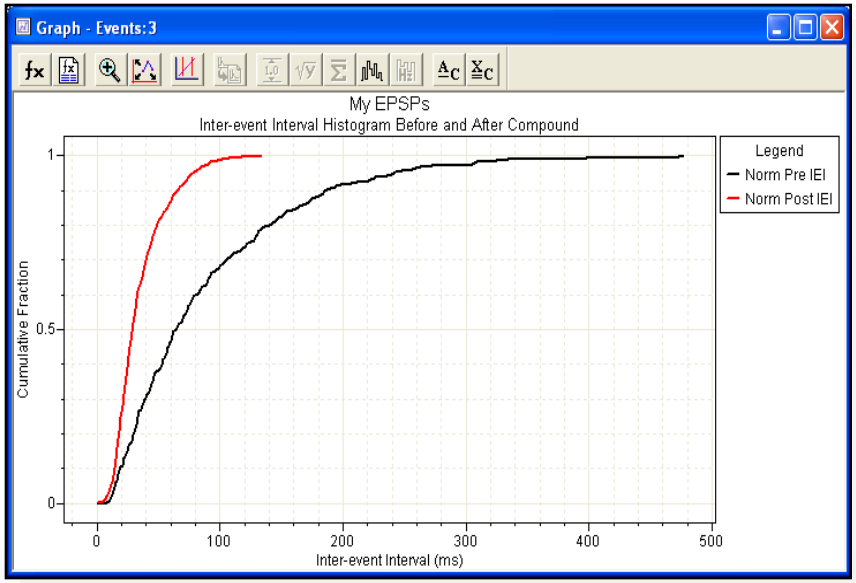
Strategy

Create cumulative histograms of the peak amplitudes…



…and the inter-event intervals.



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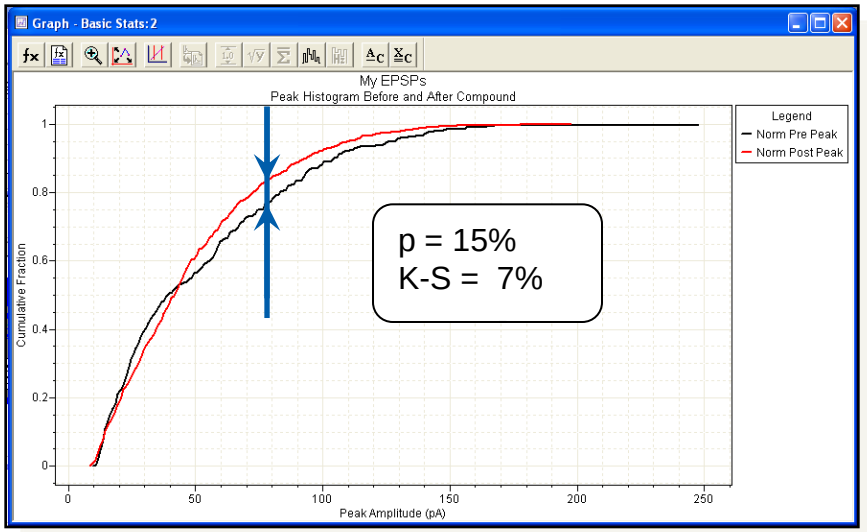
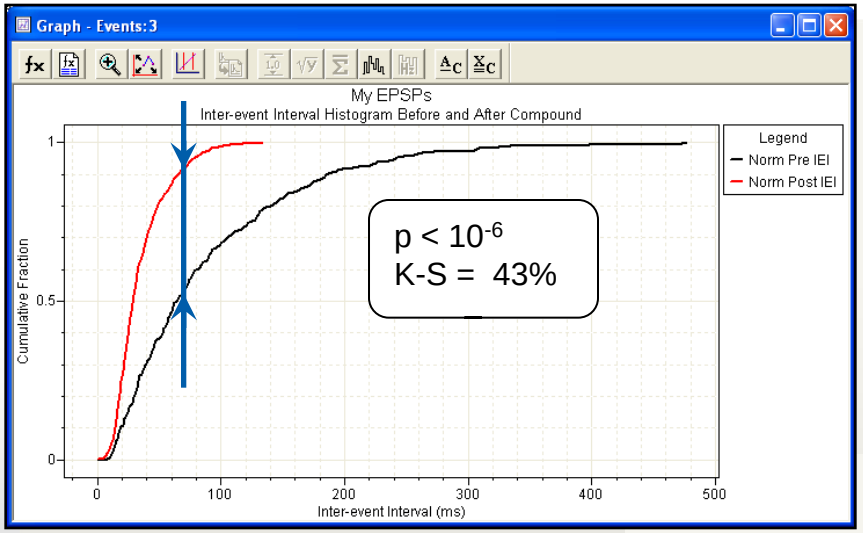
Strategy

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





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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

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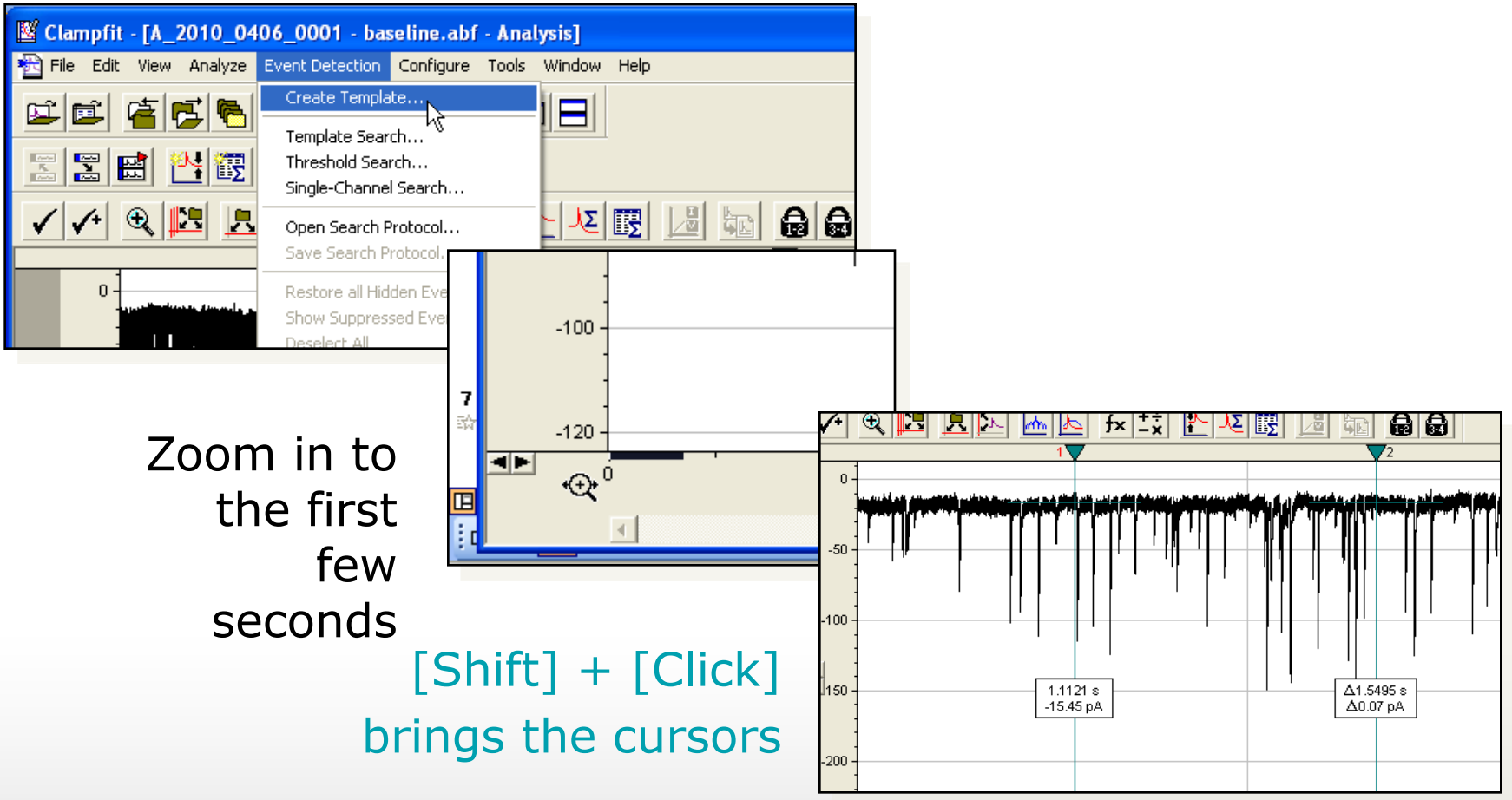
Creating a Template



Zoom in to the first few seconds

[Shift] + [Click] brings the cursors

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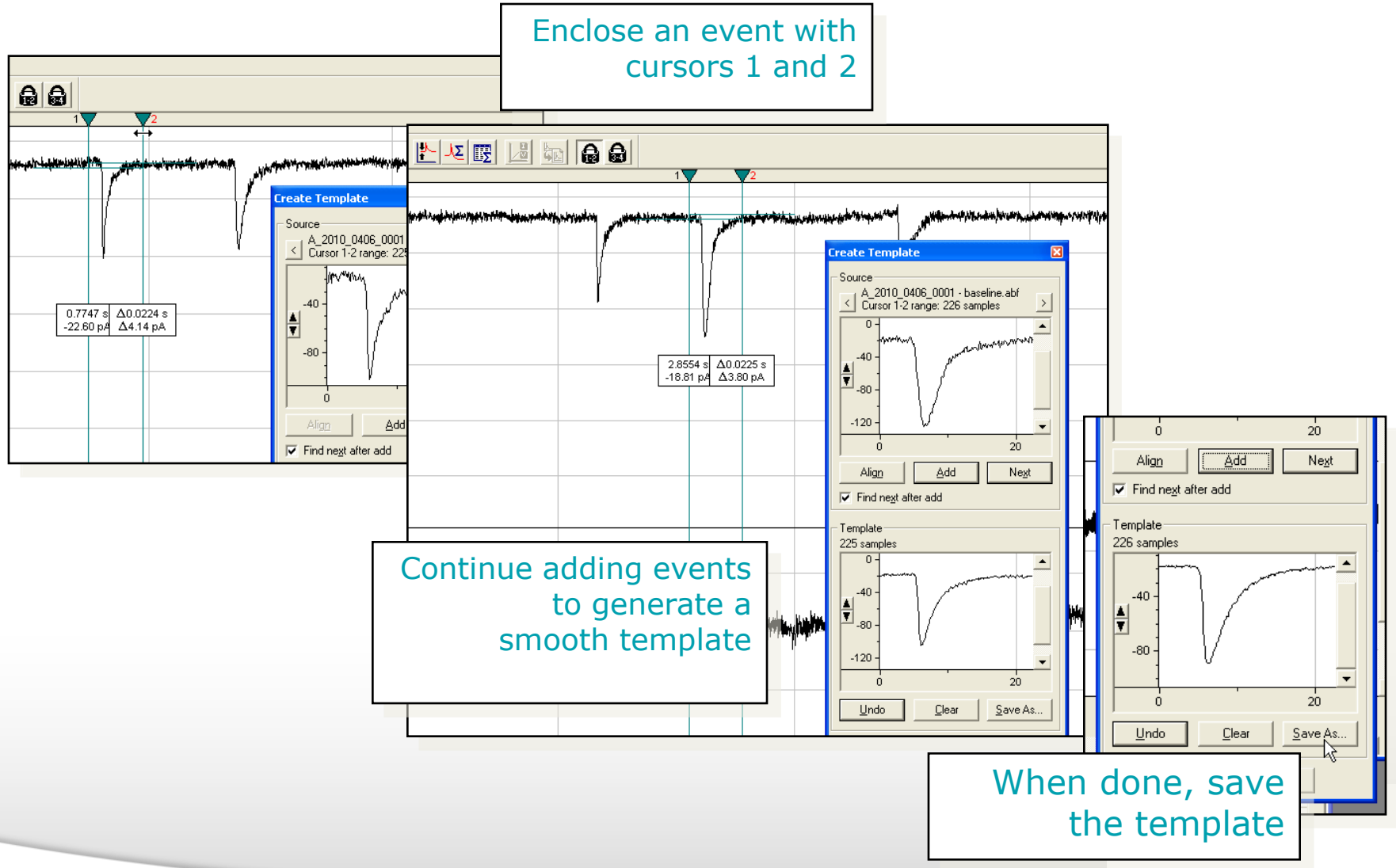
Creating a Template



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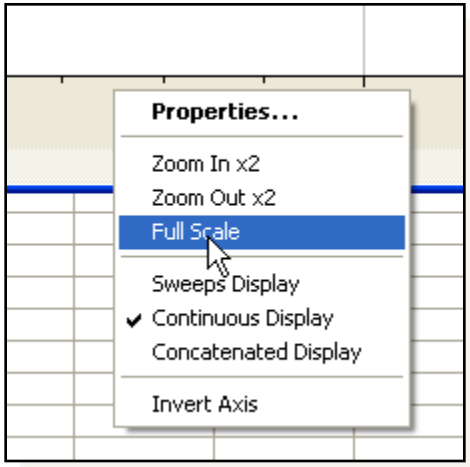
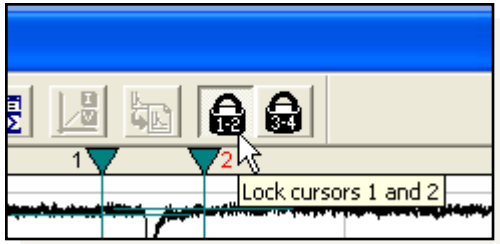
Delimit the Analysis Region



Full-scale the time axis,



unlock cursors 1 and 2,

and send cursor **1**…

…"**Home**" to the start of the trace

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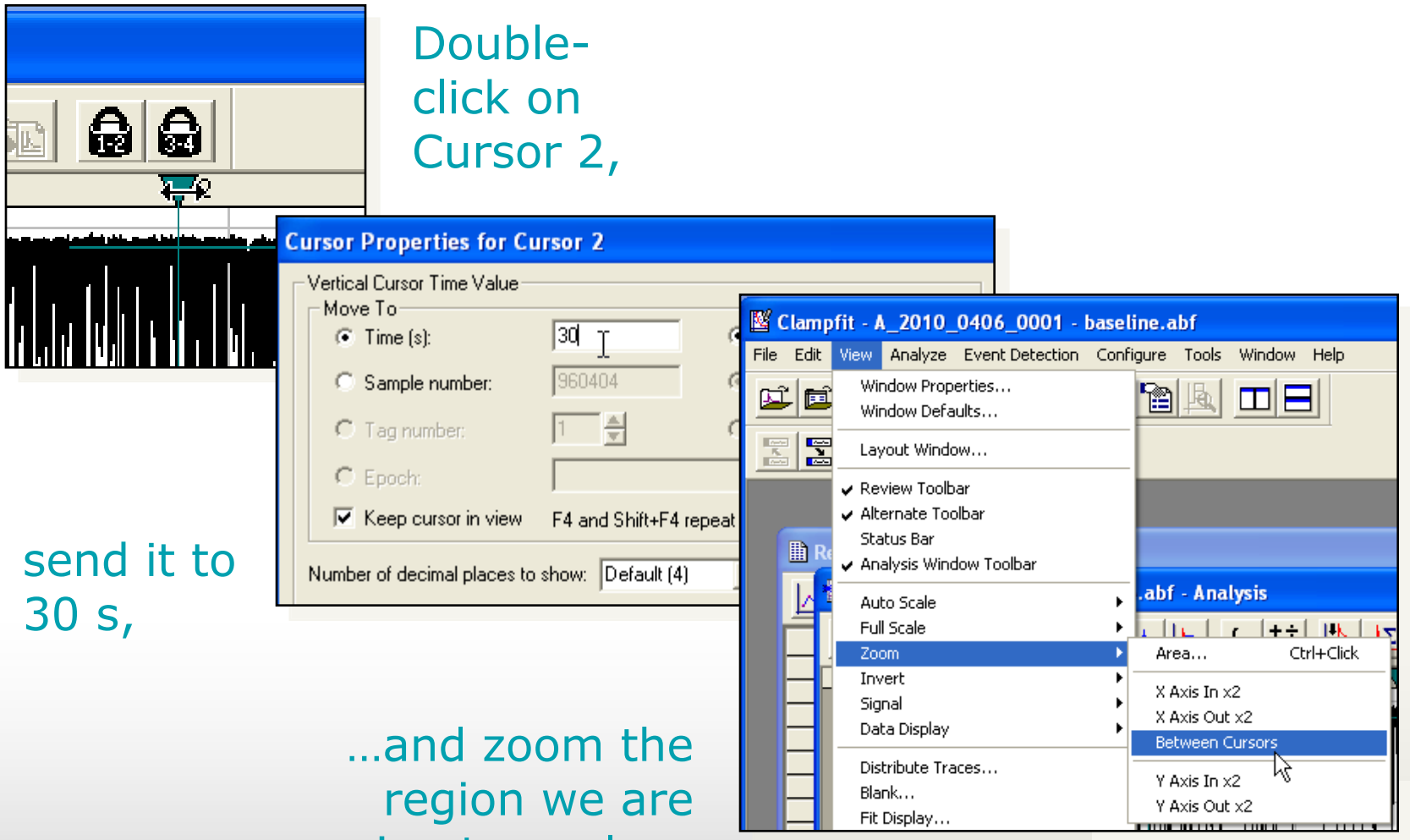
Molecular Devices

Delimit the Analysis Region



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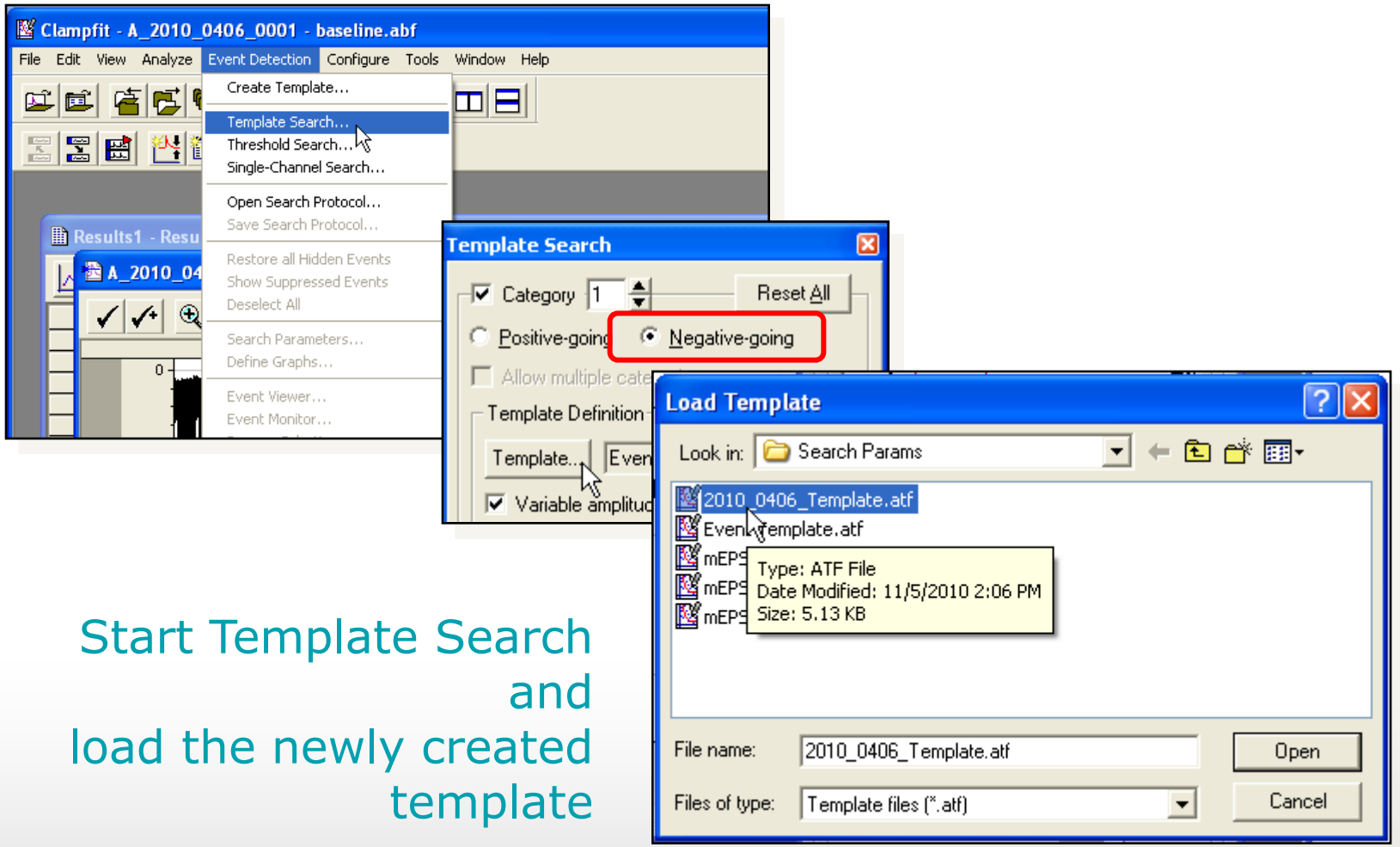
Molecular Devices

Template Search



Start Template Search
and
load the newly created
template

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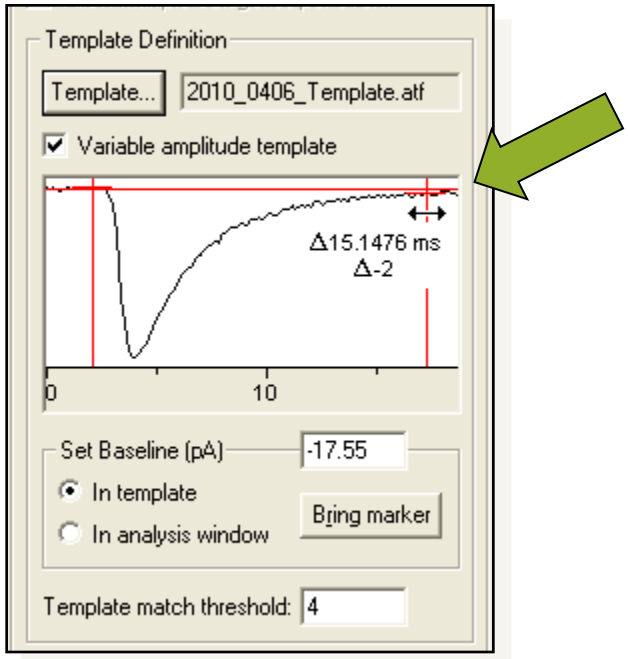
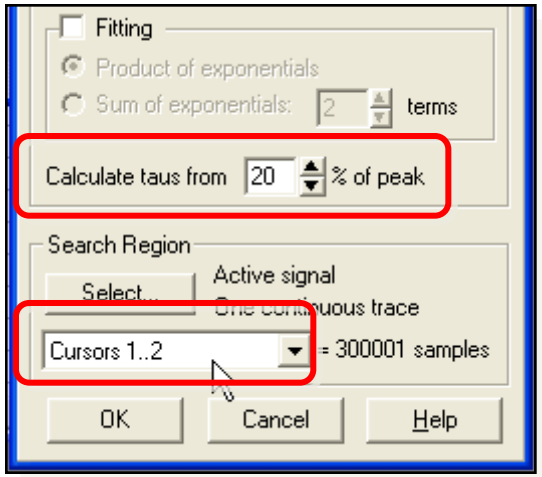
Template Search



Define the measurement region.



Set taus and analysis region.

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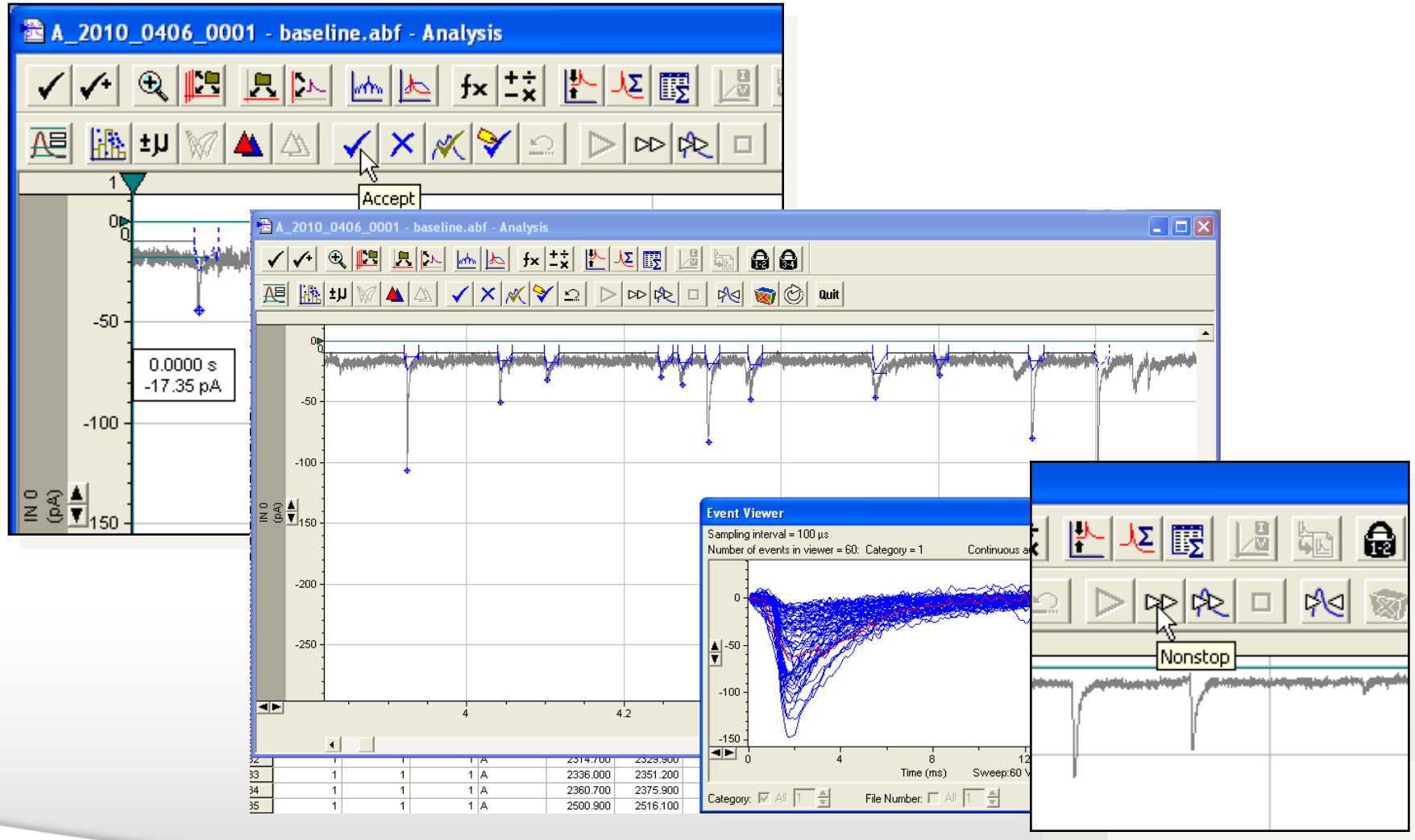
Template Search



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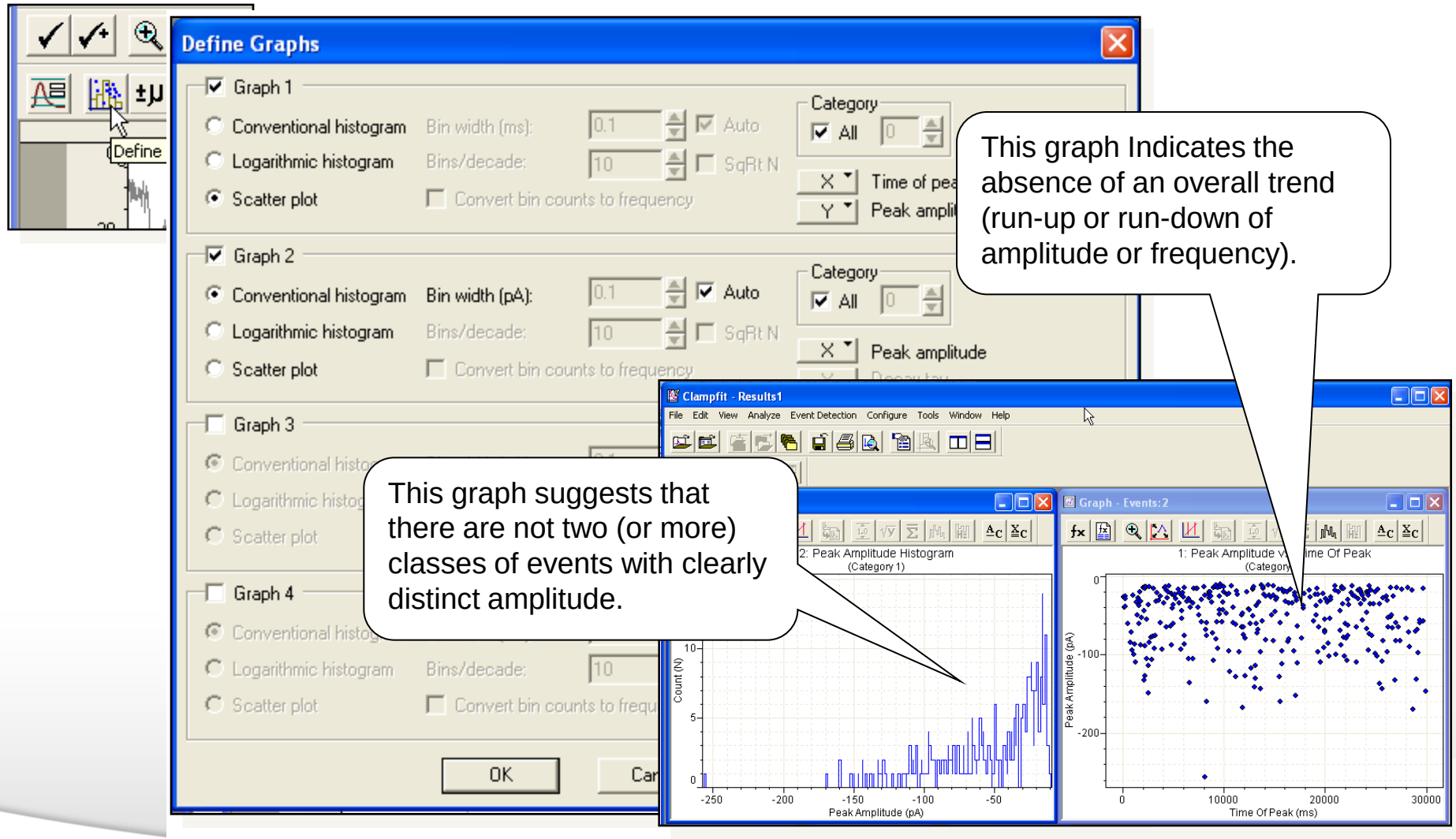
Defining “Sanity-check” Graphs



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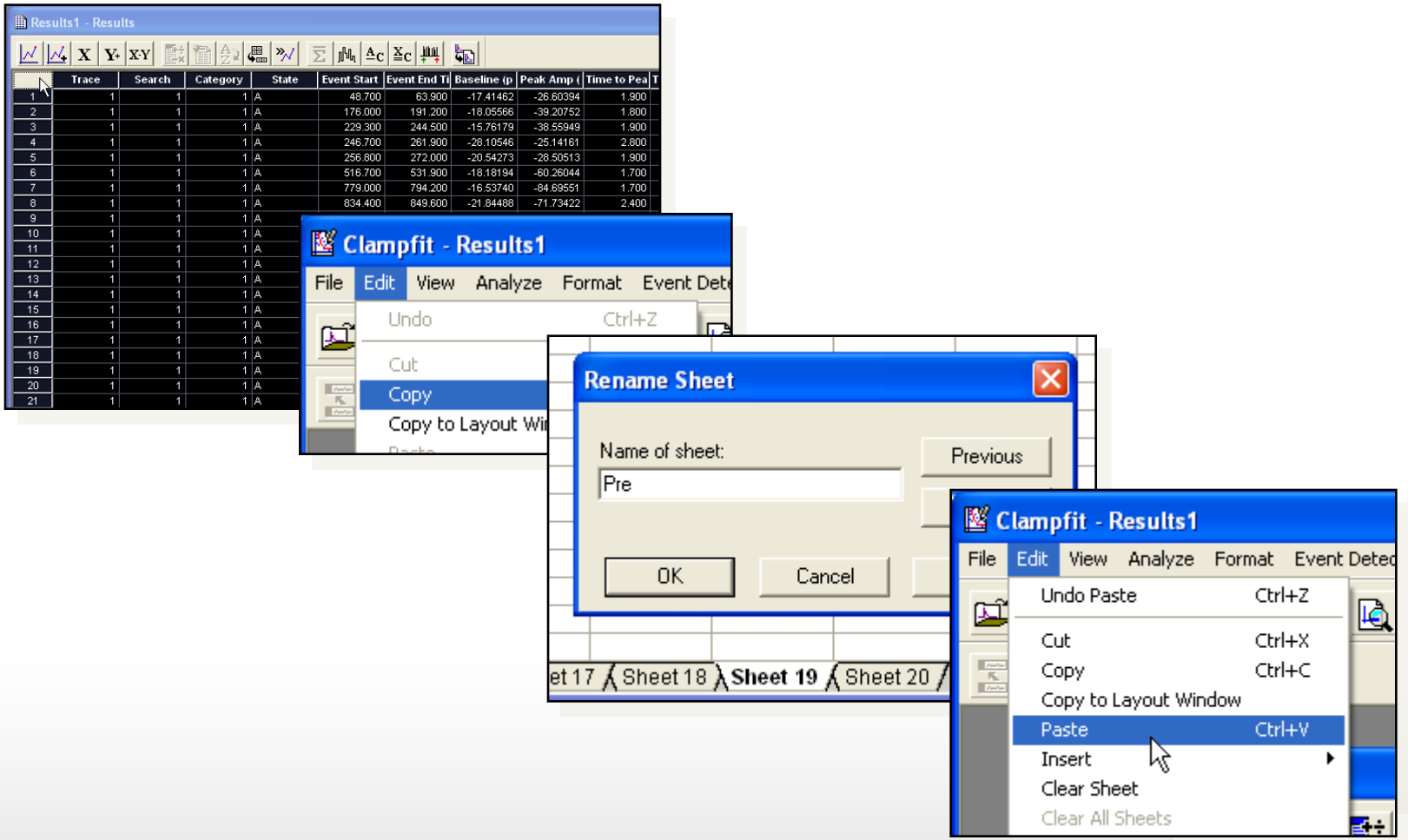
Copying the Pre-compound Results to a New Sheet



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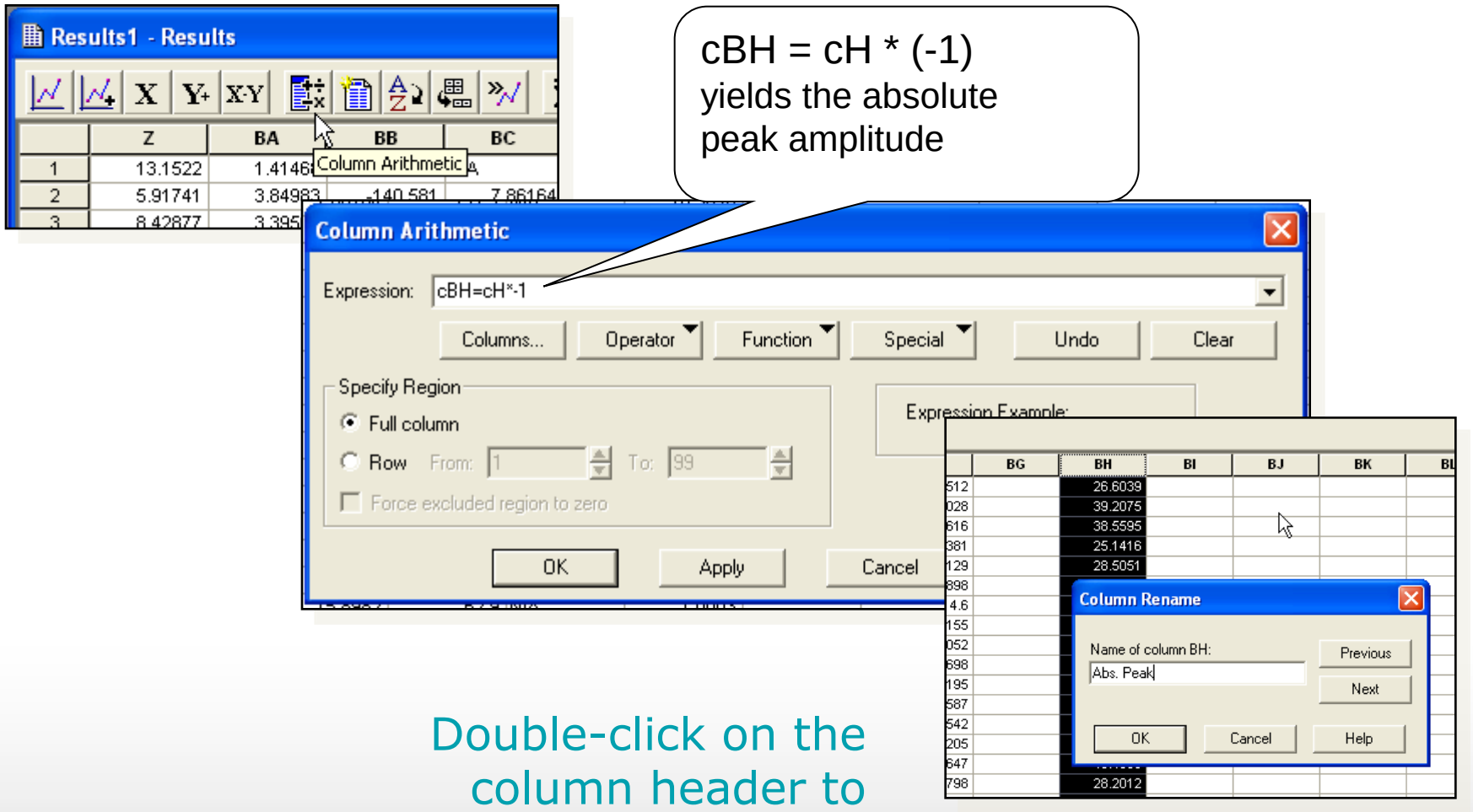
Computing the Absolute Peak Amplitude



Double-click on the column header to rename

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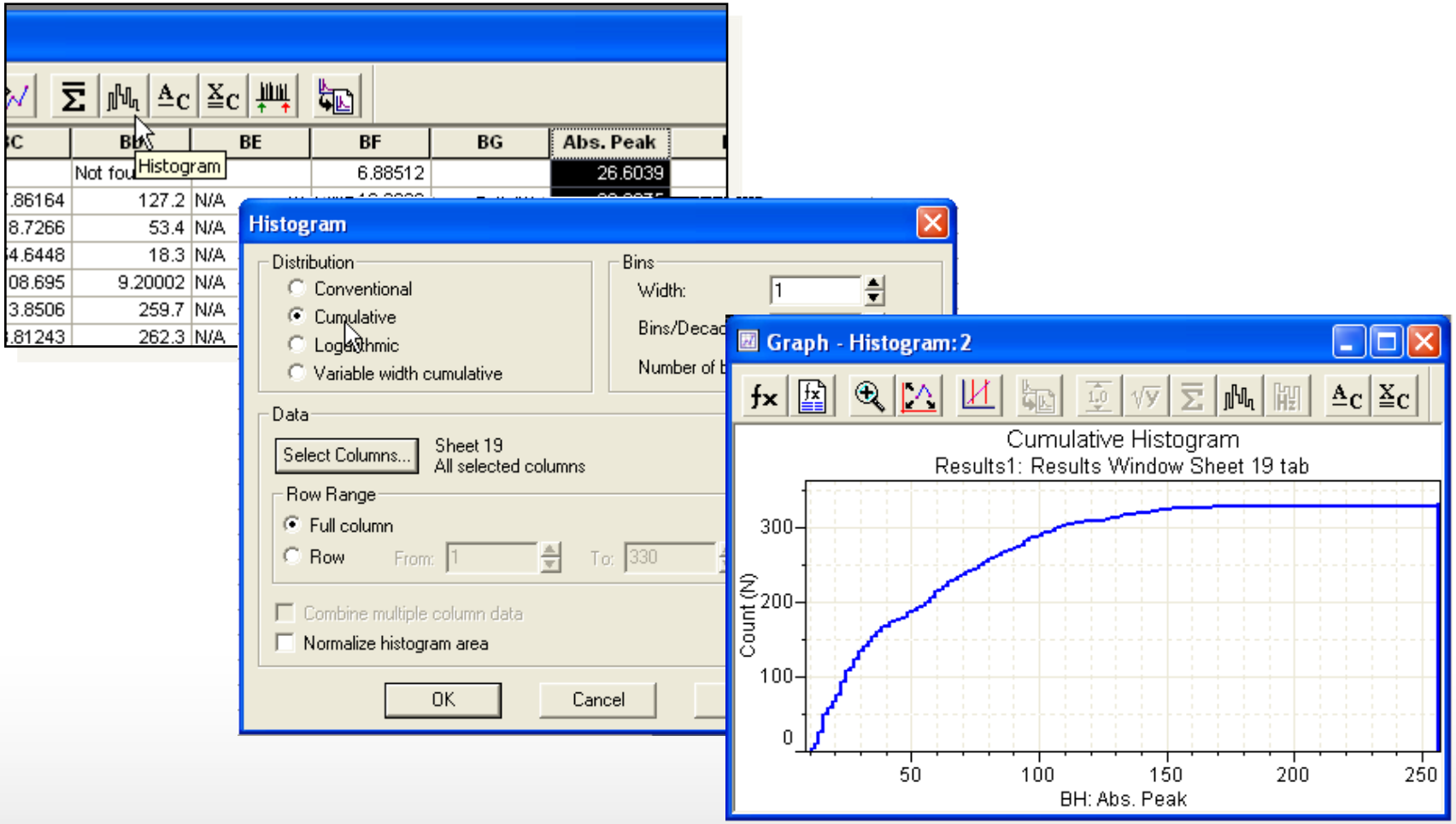
Creating a Cumulative Peak Histogram



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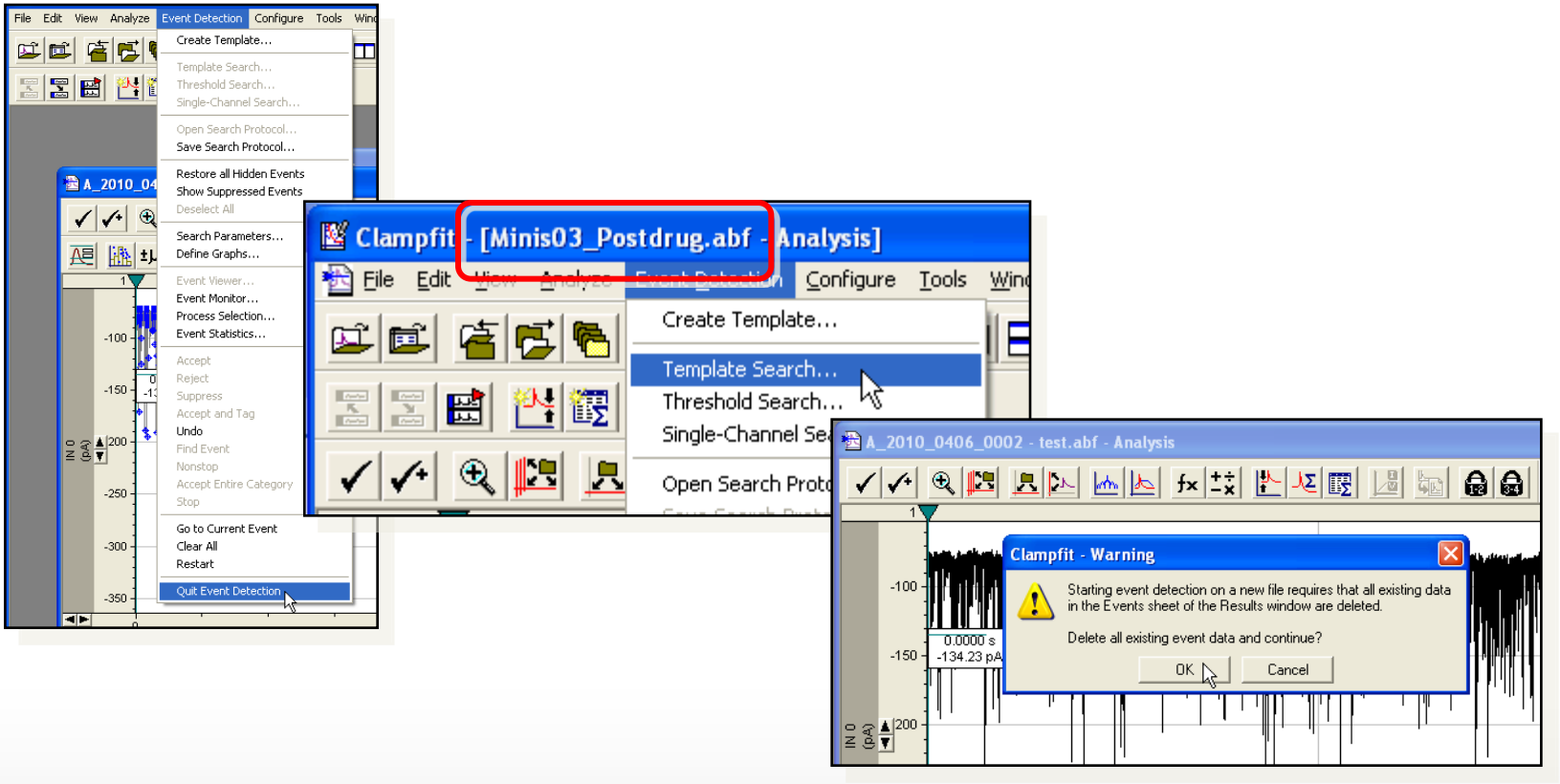
Repeat the Template Search After Compound Addition



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

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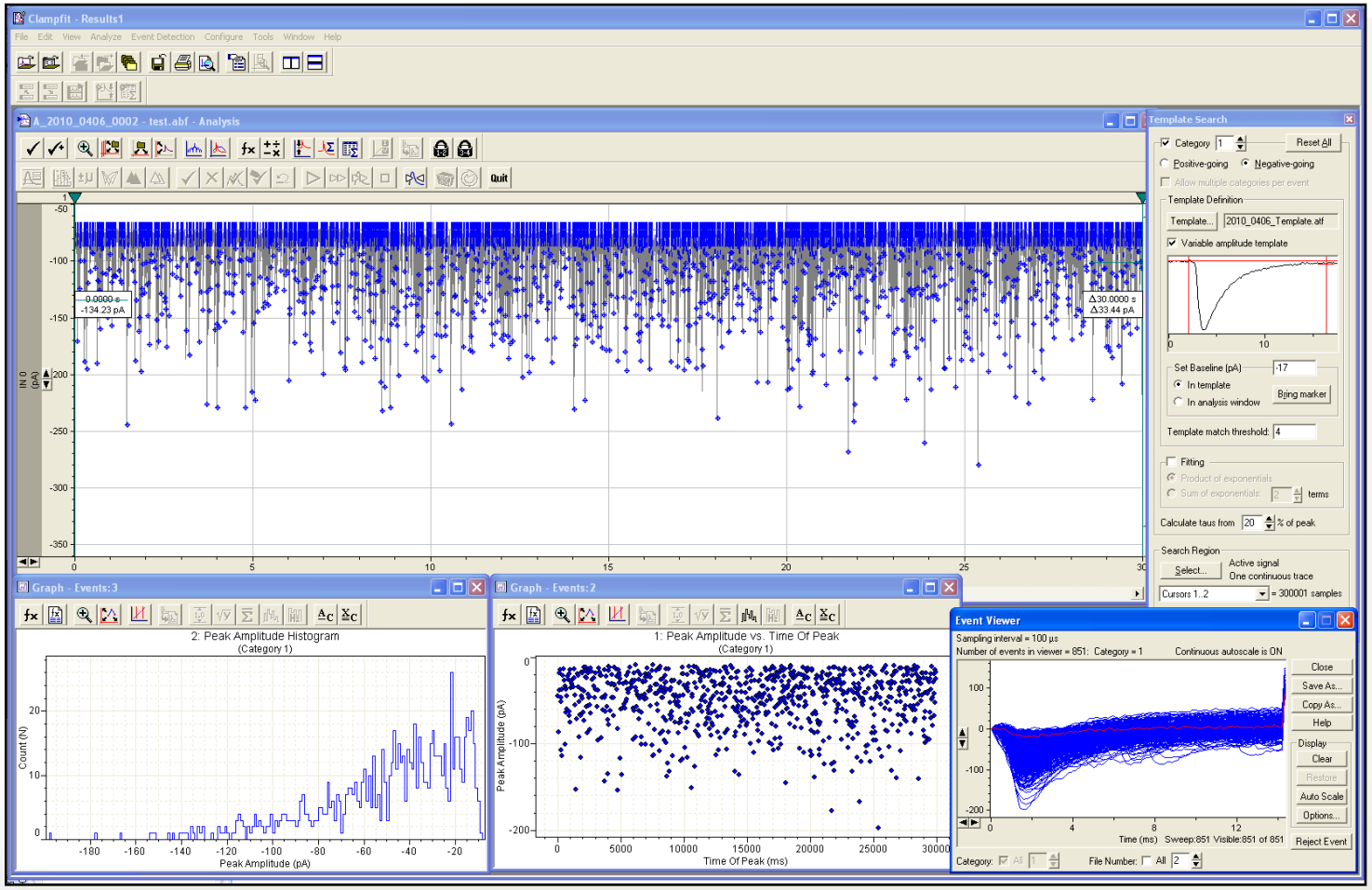
Post-compound Results



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

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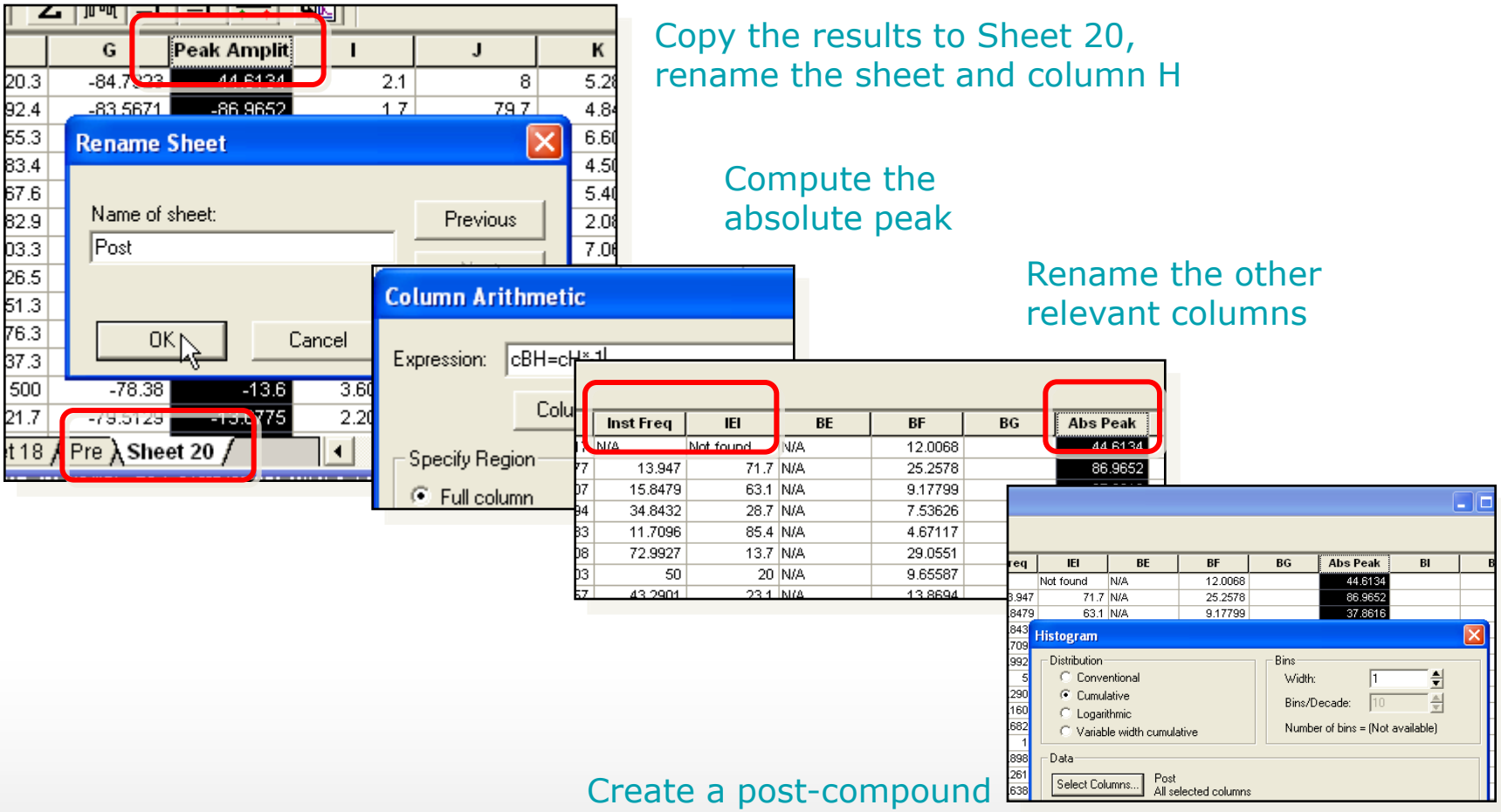
Repeat Post-processing Steps



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

Compute the absolute peak

Rename the other relevant columns

Create a post-compound cumulative histogram

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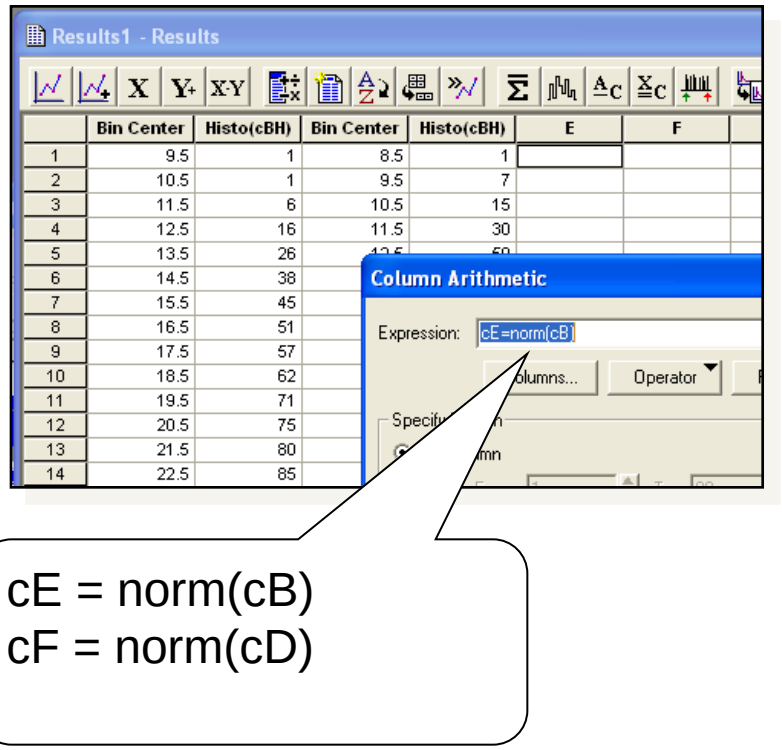
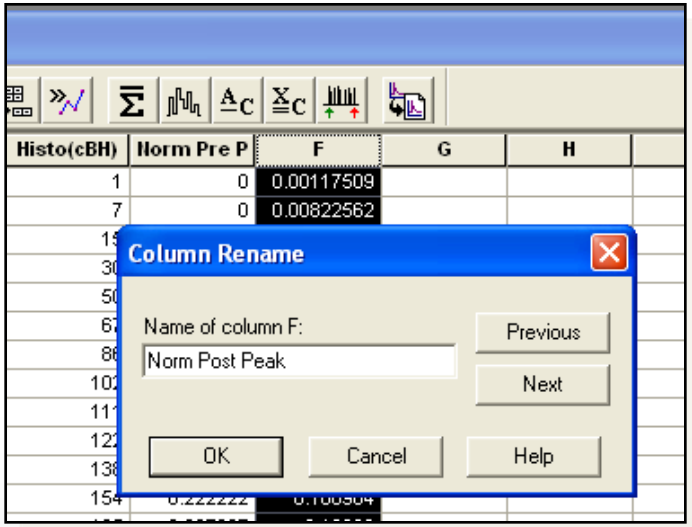
Normalizing the Histogram Values



Normalize the Bin Count columns on the Histogram sheet

cE = norm(cB)
cF = norm(cD)

Rename the two new columns



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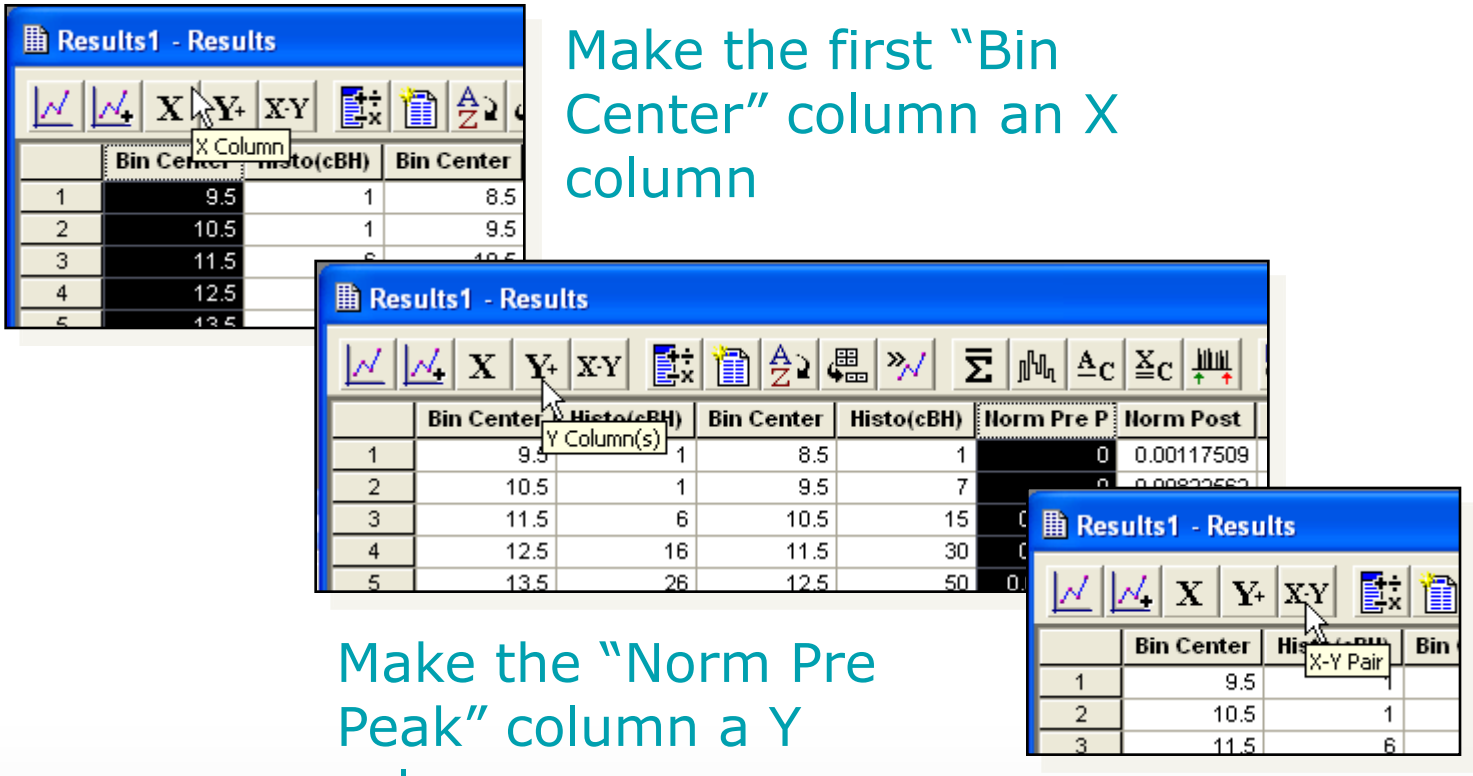
Creating a Combined, Normalized Cumulative Histogram



Make the first "Bin Center" column an X column

Make the "Norm Pre Peak" column a Y column

Define the two columns as an X-Y pair

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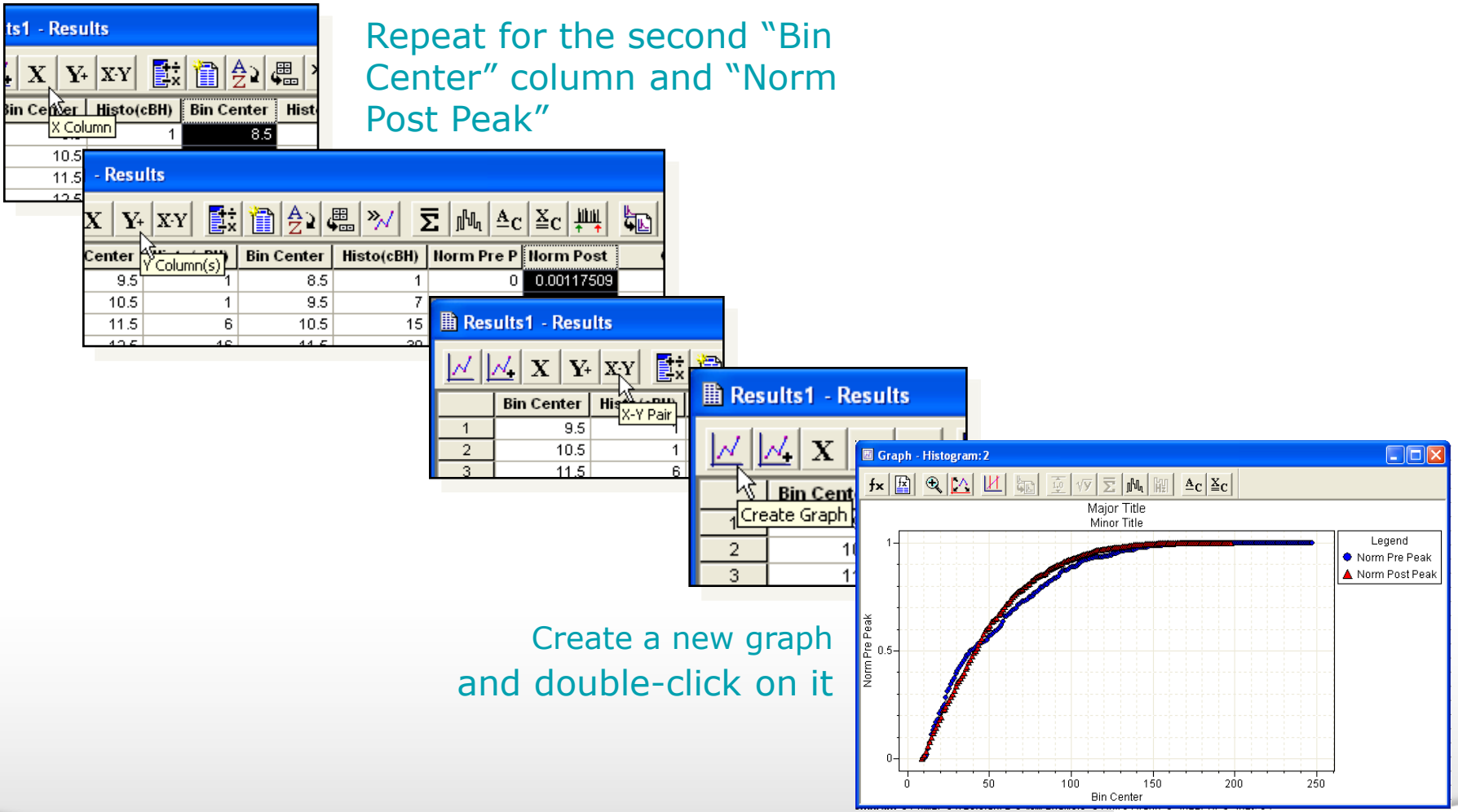
Creating a Combined, Normalized Cumulative Histogram



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

Create a new graph and double-click on it

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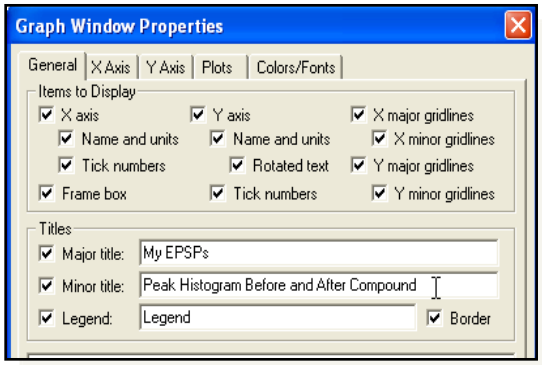
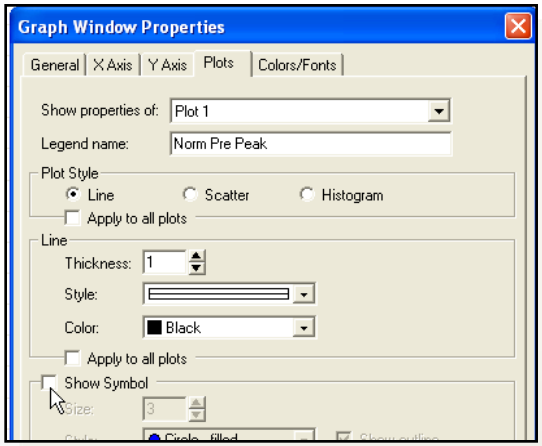
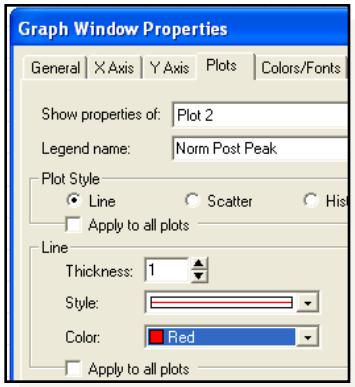
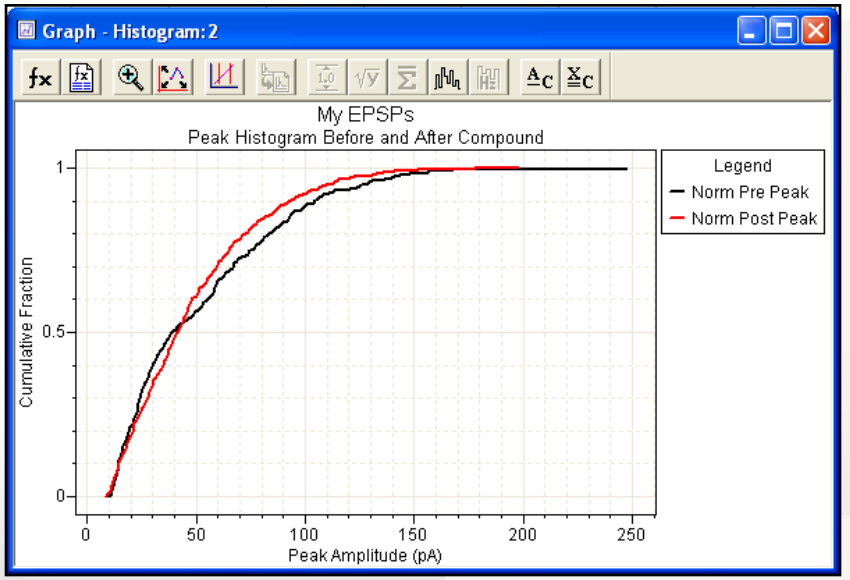
Creating a Combined, Normalized Cumulative Histogram



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







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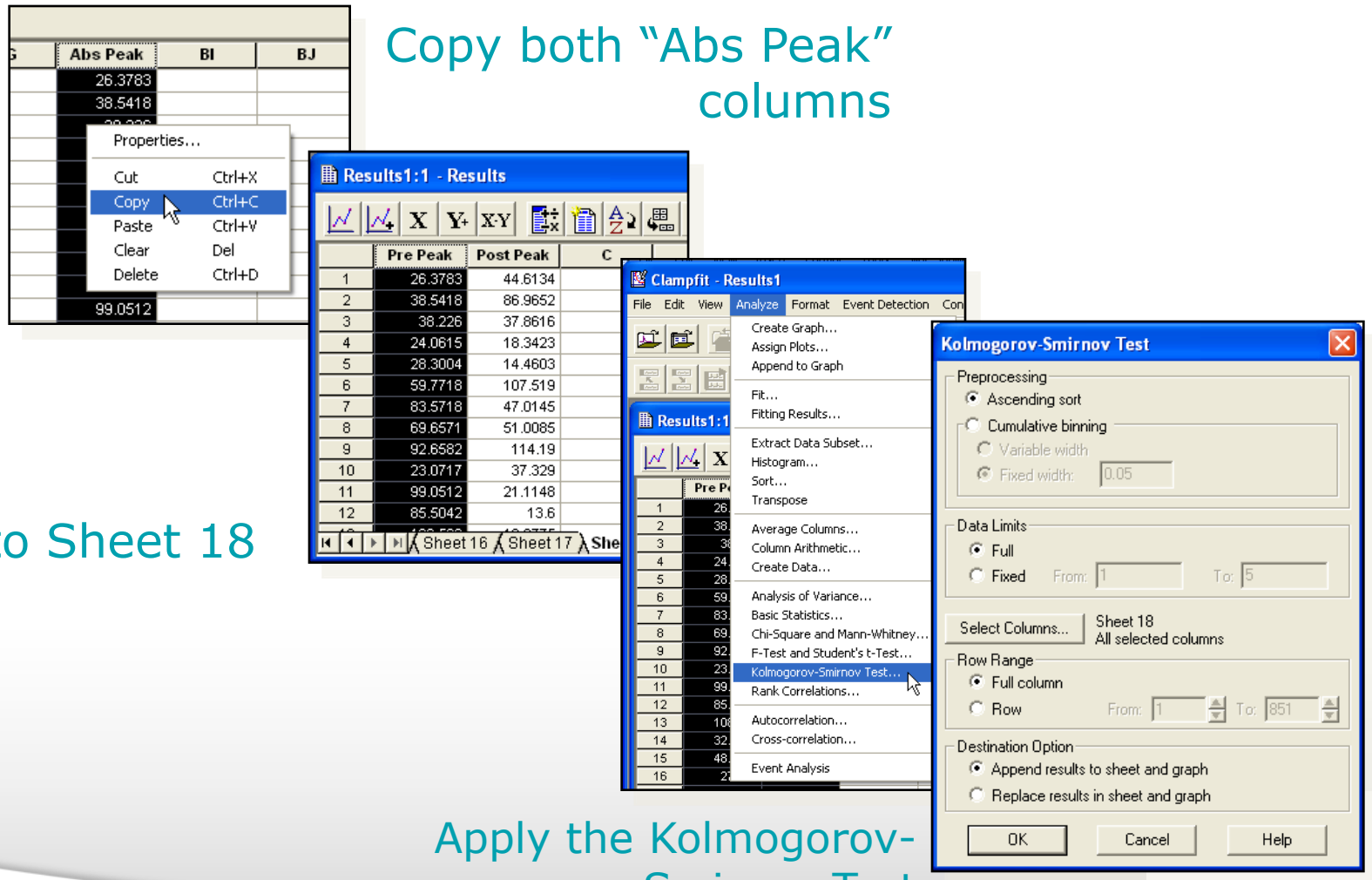
Kolmogorov-Smirnov Test

Copy both “Abs Peak” columns



to Sheet 18

Apply the Kolmogorov-Smirnov Test

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Kolmogorov-Smirnov Test Results

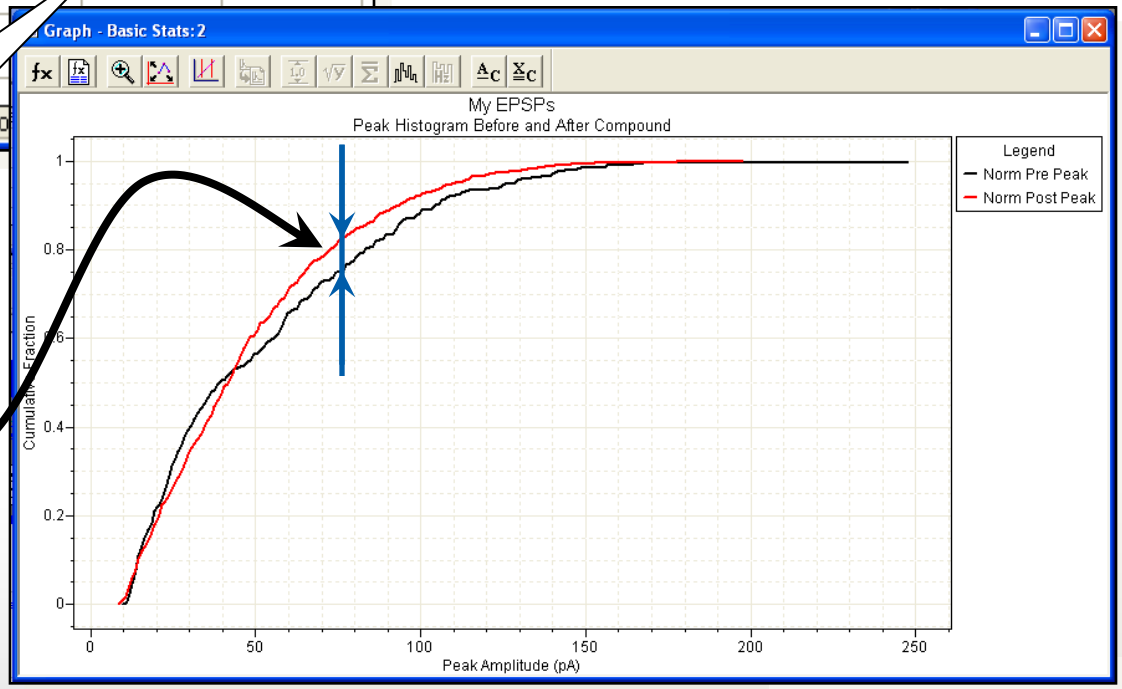
15% probability that the tested samples are from the same universe => no statistically significant difference

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						

Basic Stats | Fit Params | Relatio

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



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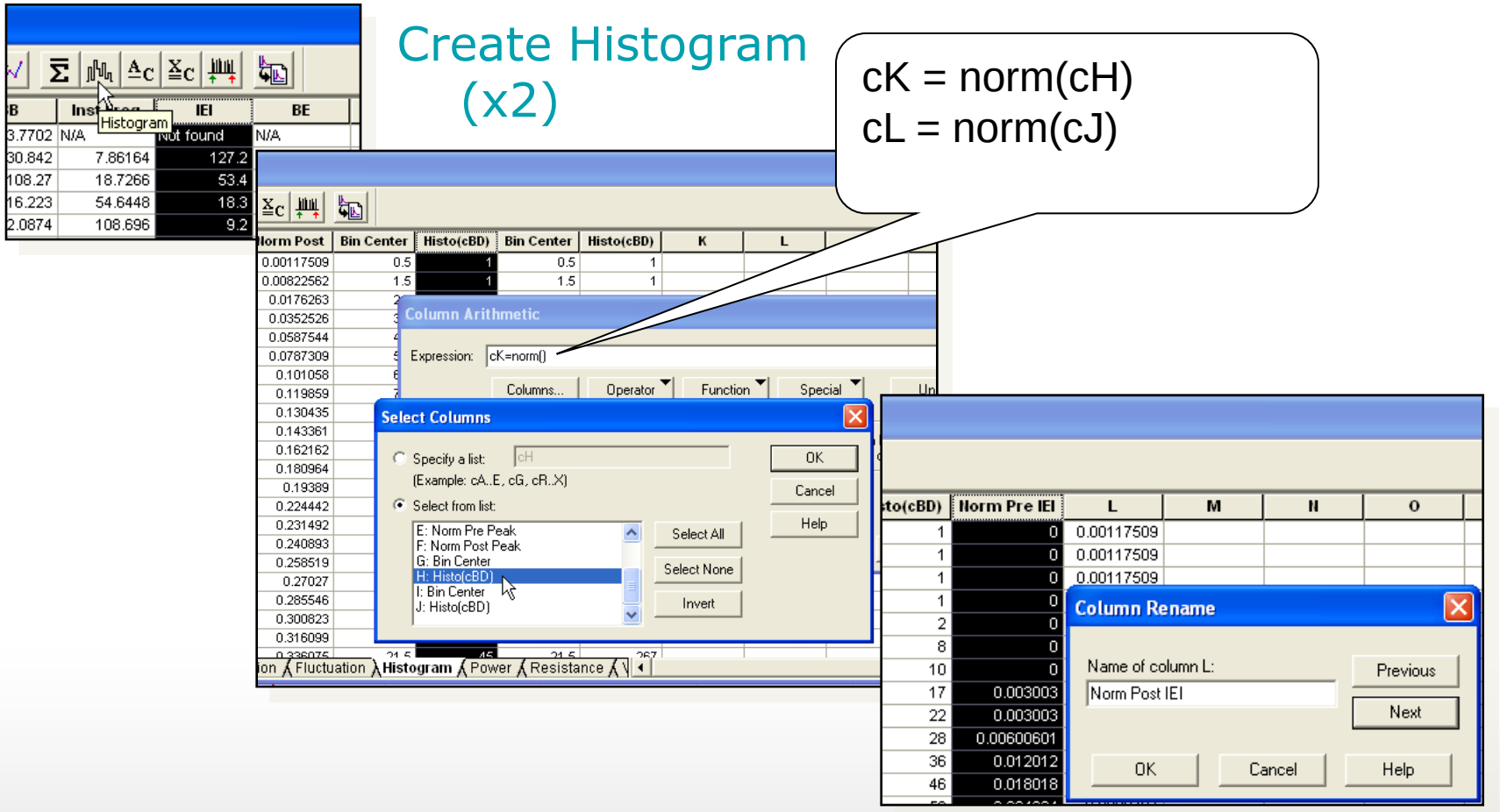
Molecular Devices

Creating Histograms for Inter-event Intervals



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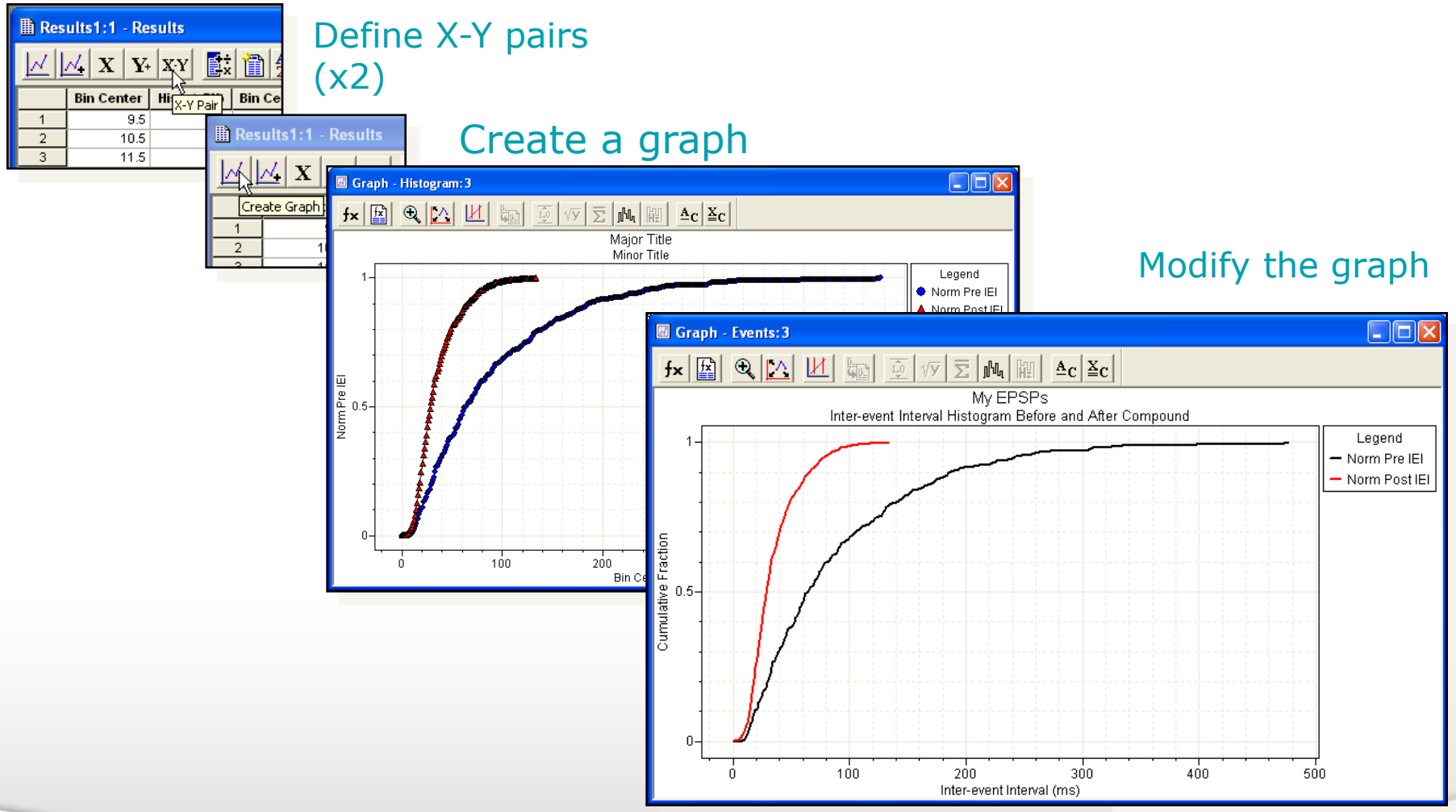
Creating Histograms for Inter-event Intervals



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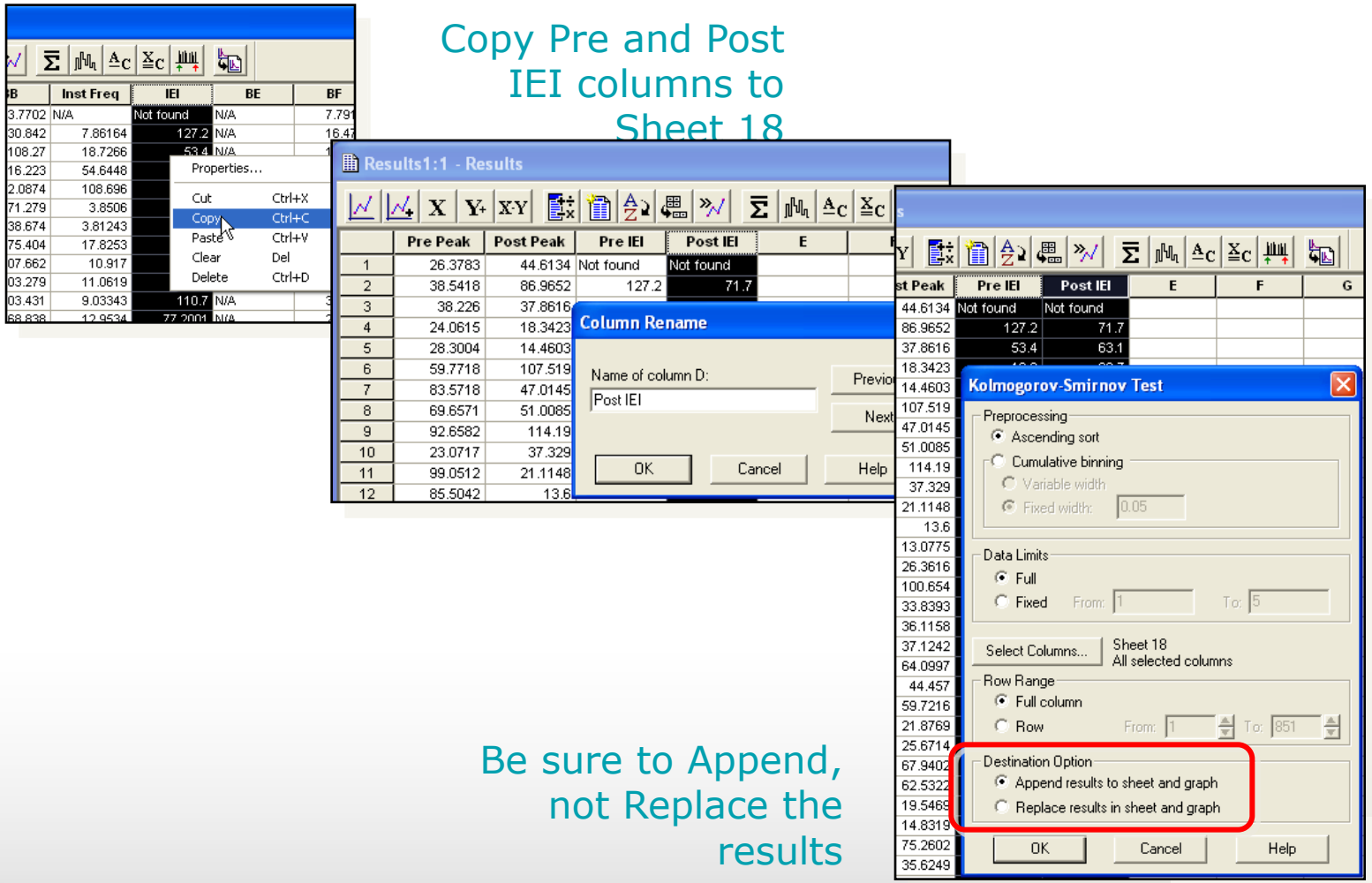
Kolmogorov-Smirnov Test for Inter-event Intervals



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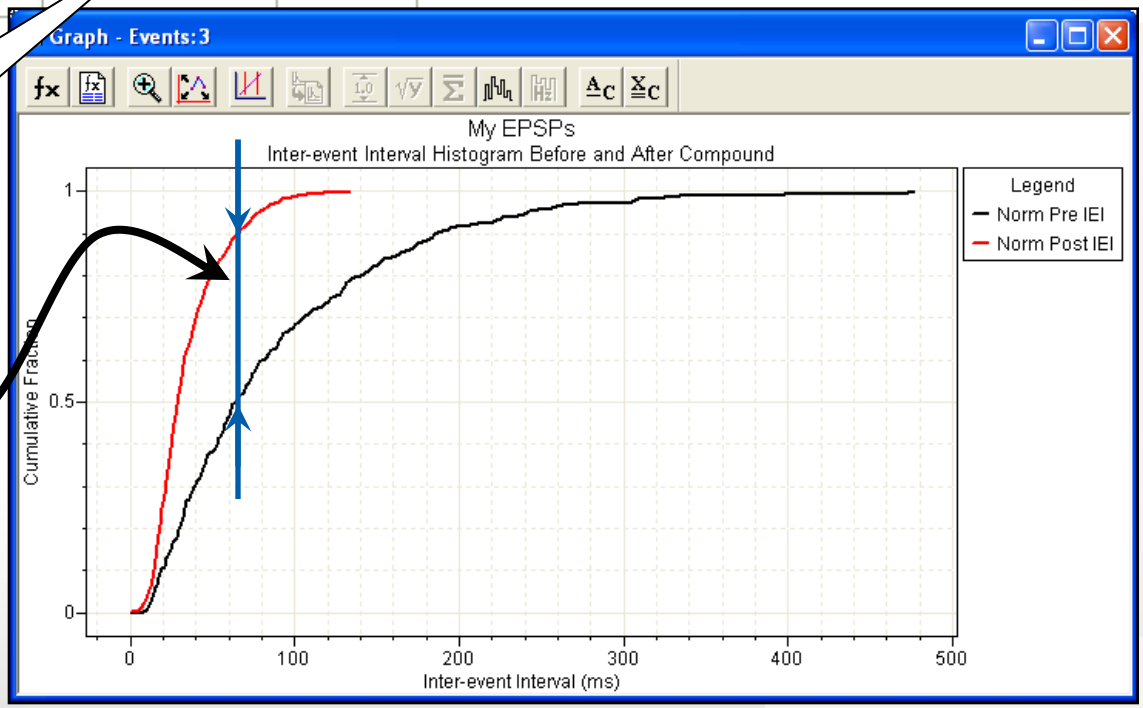
Kolmogorov-Smirnov Test Results

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						

$p<10^{-6}$ => 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:



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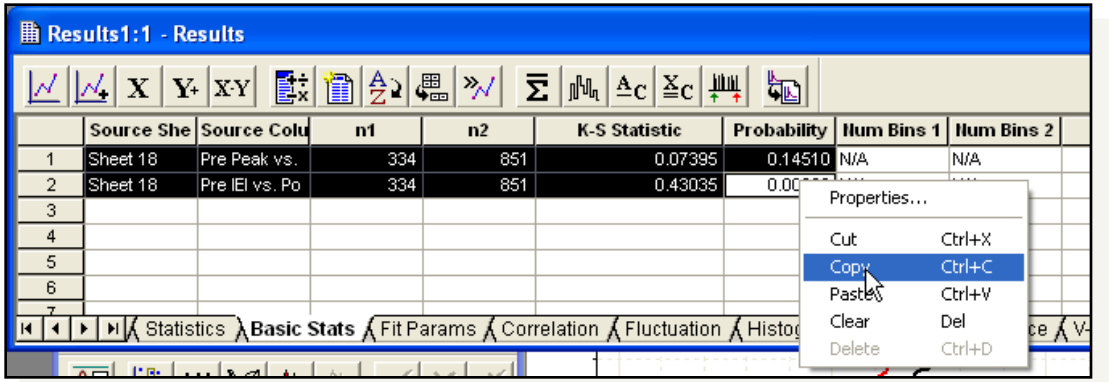
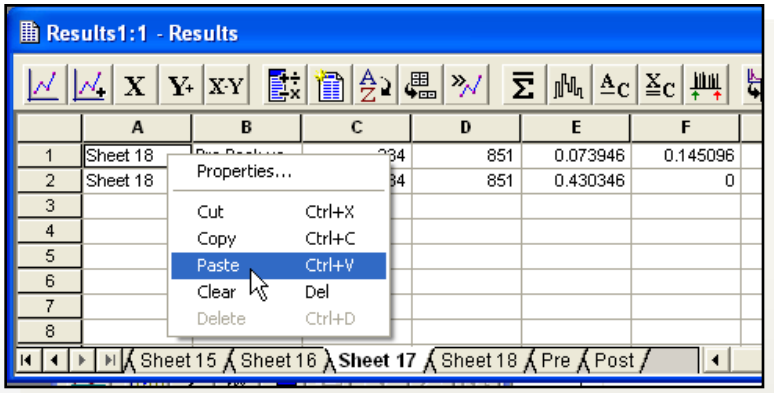
Retain the Kolmogorov-Smirnov Results



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



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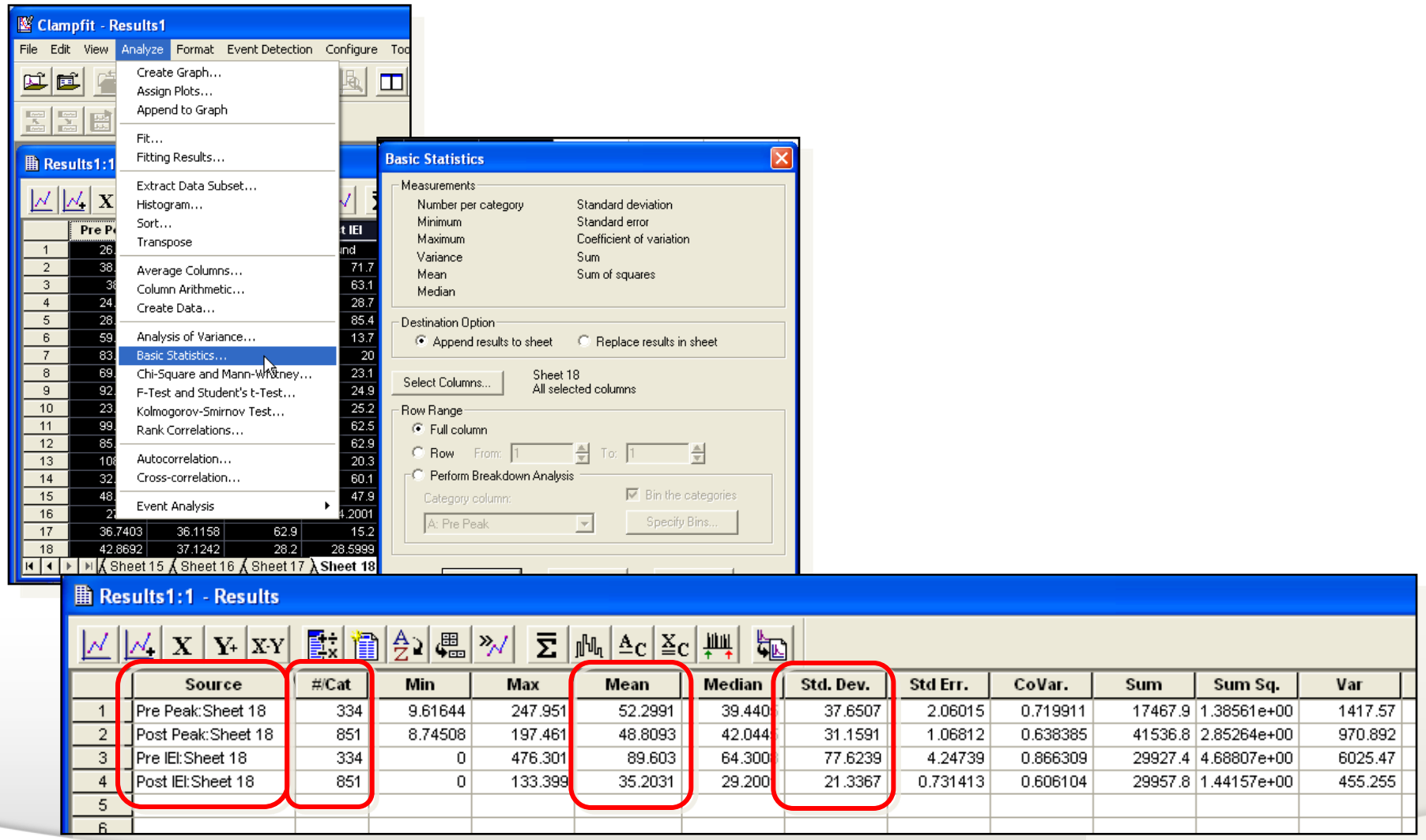
Basic Statistics for Peaks and Inter-event Intervals



	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												

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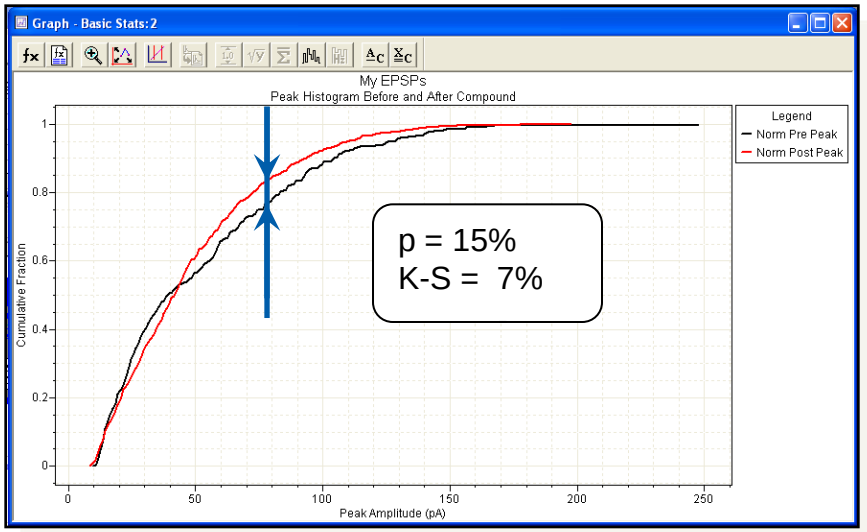
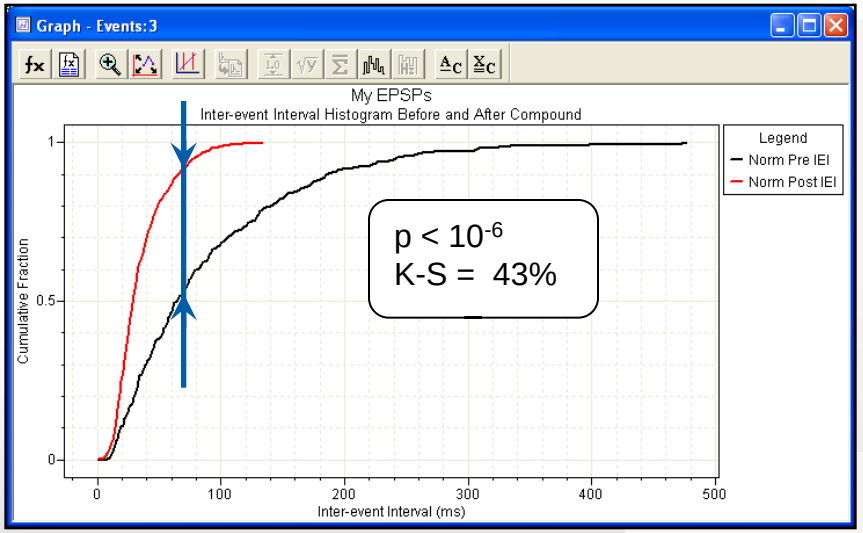
Summary

- 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.



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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

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End

Thank You

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