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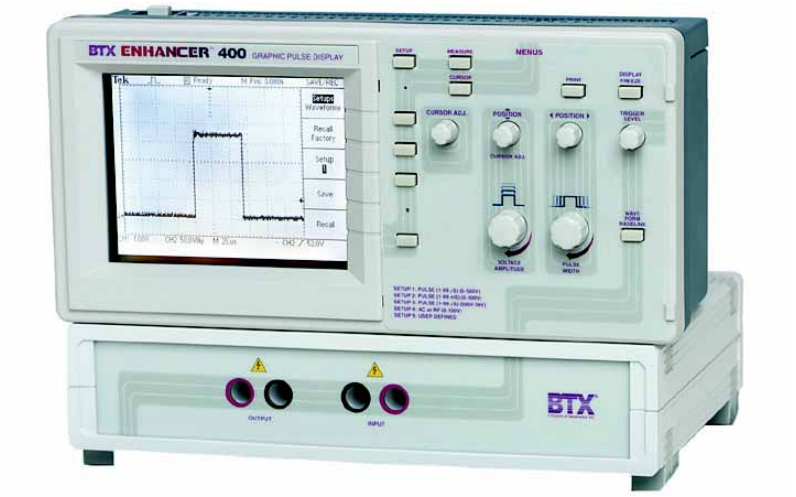
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ENHANCER™ 400

Instruction Manual



Ask for: P/N 00-900045-02 Rev D
when ordering new manuals

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A Division of Genetronics, Inc.

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Warranty

The BTX Instrument Division of Genetronics, Inc. (Genetronics) warrants that the BTX Enhancer™ 400 is free of defects for a period of two (2) years from time of delivery from an authorized Genetronics Distributor. If any defects covered by this warranty appear within the above period, Genetronics shall have the option of repairing or replacing the equipment at its expense. Such repair or replacement shall be the customer's exclusive remedy for breach of warranty or for negligence. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than Genetronics without Genetronics' express and prior approval, (c) used in violation of instructions furnished by the BTX Instrument Division of Genetronics, Inc.

Manufacturer shall not be liable for any special or consequential damages or for loss, damage or expense (whether or not caused by or resulting from Manufacturer's negligence) directly or indirectly arising from use of the instrumentation sold hereunder either separately or in combination with any other equipment or from any other cause.

The above warranty shall be in lieu of and excludes all other expressed or implied warranties of merchantability, or fitness for any purpose, or otherwise. Without limiting the generality of the foregoing, Manufacturer shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence.

Any malfunction not caused by operator abuse will be corrected at no charge for parts and labor by Manufacturer. All service under the warranty will be made at the Genetronics, Inc. San Diego, CA facilities or at another location approved by Genetronics, Inc. Owner will ship instrument prepaid to San Diego, CA. Manufacturer will return the instrument after servicing, freight prepaid to owner's address.

Warranty is VOID if the instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by Manufacturer.

Warranty is VOID if parts or connections not manufactured by Manufacturer are used with a BTX instrument.

NOTE: ***UNDER NO CONDITIONS SHOULD THE INSTRUMENT OR ACCESSORIES BE RETURNED WITHOUT PRIOR APPROVAL AND A RETURNED GOODS AUTHORIZATION (RGA) NUMBER FROM MANUFACTURER***

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Customer Service

For any inquiry or request for repair service, contact the BTX Instrument Division of Genetronics, Inc. Customer Service group in writing or by the following:

Phone: 1-800-289-2465 (858) 597-6006

FAX: (858) 597-9594

E-mail: cust@genetronics.com.

If outside the United States and Canada, call (858) 597-6006 or contact your nearest BTX Distributor.

Technical Service

BTX is the ultimate resource for technical information on the use of high voltage electric fields for performing high efficiency cell fusion, embryo manipulation, gene transfer, bacterial transformation and general electroporation of molecules and drugs into cells. We constantly track and monitor all scientific publications in this area. Our Technical Service group extracts and enters pertinent information, such as results and parameters from these papers into a Database Management System. The resultant Electronic Genetics® Database can be accessed and searched on any combination of the field identifiers.

For technical assistance or information, contact the BTX Instrument Division of Genetronics, Inc. Technical Service group:

Toll free telephone:	(800) 289-2465 (858) 597-6006
Fax:	(858) 597-9594
E-mail:	tech@genetronics.com

If outside the United States and Canada, call (858) 597-6006 or contact your nearest BTX Distributor.

BTX®

A Division of Genetronics, Inc.

11199 Sorrento Valley Road

San Diego, CA 92121-1334 U.S.A.

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General Safety Summary

Review the following safety precautions to avoid injury and prevent damage to this product or any products connected to it. To avoid potential hazards, use this product only as specified.

Only qualified personnel should perform service procedures.

To Avoid Fire or Personal Injury

Use Proper Power Cord. Use only the power cord specified for this product and certified for the country of use.

Connect and Disconnect Properly. Do not connect or disconnect probes or test leads while they are connected to a power source.

Ground the Product. This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making connections to the input or output terminals of the product, ensure that the product is properly grounded.

Observe All Terminal Ratings. To avoid fire or shock hazard, observe all ratings and markings on the product. Consult the product manual for further ratings information before making connections to the product.

Do Not Operate Without Covers. Do not operate this product with covers or panels removed.

Use Proper Fuse. Use only the fuse type and rating specified for this product.

Avoid Exposure To Circuitry. Do not touch exposed connections and components when power is present.

Do Not Operate With Suspected Failures. If you suspect there is damage to this product, have it inspected by qualified service personnel.

Provide Proper Ventilation. Refer to the manual's installation instructions for details on installing the product so it has proper ventilation.

Do Not Operate in Wet/Damp Conditions.

Do Not Operate in an Explosive Atmosphere.

Keep Product Surfaces Clean and Dry.

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Safety Terms and Symbols

These terms may appear in this manual:

⚠	**WARNING.** Warning statements identify conditions or practices that could result in injury or loss of life
⚠	**CAUTION.** Caution statements identify conditions or practices that could result in damage to this product or other property.

Terms on the Product. These terms may appear on the product:

DANGER indicates an injury hazard immediately accessible as you read the marking.

WARNING indicates an injury hazard not immediately accessible as you read the marking.

CAUTION indicates a hazard to property including the product.



Protective (Earth) Terminal



ATTENTION Refer to Manual



WARNING High Voltage

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Enhancer™ 400
Electrical and Technical Specifications

Display

Type: 4.7 in (120mm) diagonal liquid crystal
Resolution: 320 horizontal by 240 vertical

Power Source

Voltage: 100 - 240 Vac, 50 - 60 Hz, CAT II
Power: < 20 W
Fusing: 1 A, T rating 250 V (internal)

Environmental

For Indoor Use Only

Operating Temperature: 0º C to + 50º C
Cooling: Convection through top and bottom vents
Humidity: < 90% relative humidity

Mechanical

Height:	22cm	(8.63”)
Width:	30.5cm	(12.0”)
Depth:	12.1cm	(4.75”)
Weight:	3.2Kg	(7.1 lbs)

High Voltage Module

Maximum Voltage: Input: 3,000 Volts Peak
Maximum Pulse Length: 99µs @ 3,000 Volts Peak
99ms @ 500 Volts Peak
Transient Over voltage: CAT II
Fusing: 0.1A, 250V, FB (internal)

Pollution Degree 2

(Not to be operated in conductive pollutants atmosphere)

High Voltage (HV) Cables

Length: 1 Meter
Voltage Rating: 3,000V pk with retractable sheath
Wire Gauge: 16 AWG

General Specifications

Certifications and Compliance’s

Over voltage Category:	Examples of Products in this Category	
	CAT III	Distribution-level mains, fixed installation
	CAT II	Local-level mains, applications, portable equipment
	CAT I	Signal levels in special equipment or parts of equipment, telecommunications, electronics

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Enhancer™ 400 Operational Troubleshooting

The Enhancer 400 has an intuitive self test that automatically checks electronic functionality upon start up. If this Enhancer 400 displays an error on startup, please contact the BTX Technical Service Group, as outlined on page ii of this Instruction Manual. For other problems see table below.

Problem	Solution
No waveform displayed	1. Verify that trigger status reads "Ready". If the status reads "Auto", press the soft switch for "Recall". If the status reads "Stop", press the "DISPLAY FREEZE" key 2. Verify that your trigger level does not exceed the set voltage on your pulse generator 3. Verify that the setup program in use fits your expected waveform 4. Verify that horizontal trigger is on screen
Pulse profile does not have the appropriate resolution	1. Adjust voltage amplitude and pulse width knobs as needed 2. Adjust the horizontal and vertical position as needed
Waveform is off screen	1. Adjust voltage amplitude and pulse width knobs as needed 2. Adjust the horizontal and vertical position as needed
Waveform is characterized by a spike or series of spikes on the rising edge of the pulse	Normal display for some instrumentation and pulse delivery into certain loads. Check with BTX Technical Service for more information
Voltage displayed following automatic voltage measurement is greater than the set voltage	One or more spikes may characterize rising edge of pulse. Use manual voltage measurement
Display windows are not displaying the appropriate functions	1. Verify you are in the appropriate menu by checking the menu status 2. Toggle the soft switch corresponding to the window in question
Printer not printing	1. Verify appropriate printer cable connections 2. Refer to Printer Setup
Waveform not uploading into WaveStar	1. Verify appropriate computer cable connections 2. Refer to Optional Computer Interface Setup
All Other Problems	Contact BTX Technical Service Group

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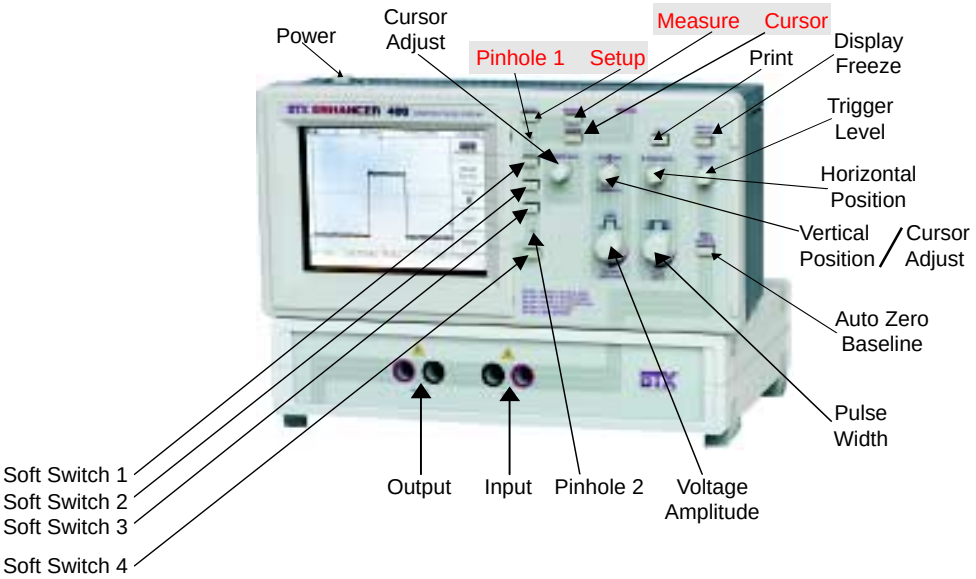
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Quick Start

Enhancer™ 400 Controls



Power Cord

Use only harmonized cords furnished with your Enhancer™ 400. Plug into an easily accessible power receptacle. The power cord can then be disconnected in an emergency shutdown. Ensure that the receptacle used is properly grounded to provide safe operation. Use a power source that delivers 100 to 240 Vac at 50 or 60 Hz. Variations of the power source by +/- 10% are inconsequential.

High Voltage (HV) Cables

Use only the set of patch cords furnished with your Enhancer™ 400. Replace any frayed or cut cords to prevent arcing to personnel or equipment. Turn off generators before unplugging patch cords from the Enhancer 400.

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Installing The Enhancer™ 400

- Set the Enhancer 400 on a stable surface. Allow a clearance of one inch from the top surface of the case to allow proper cooling.
- Plug in power cord.
- Connect output of your pulse generator to the input terminals of the Enhancer 400 using the HV input cables provided. (Refer to diagram 2)
- Turn on the instrument. The switch is located on the top left panel.
- Wait for the confirmation that all self tests of installed modules have passed.

  (approximately 8 seconds)
- Press the SETUP menu key (front panel top center). The screen displayed in Diagram 1 will appear.
- Press the Soft Switch for “SETUP” until the “2” appears in the window. Lock in the setup by pressing the soft switch for “Recall”.
- Proceed with functional check.

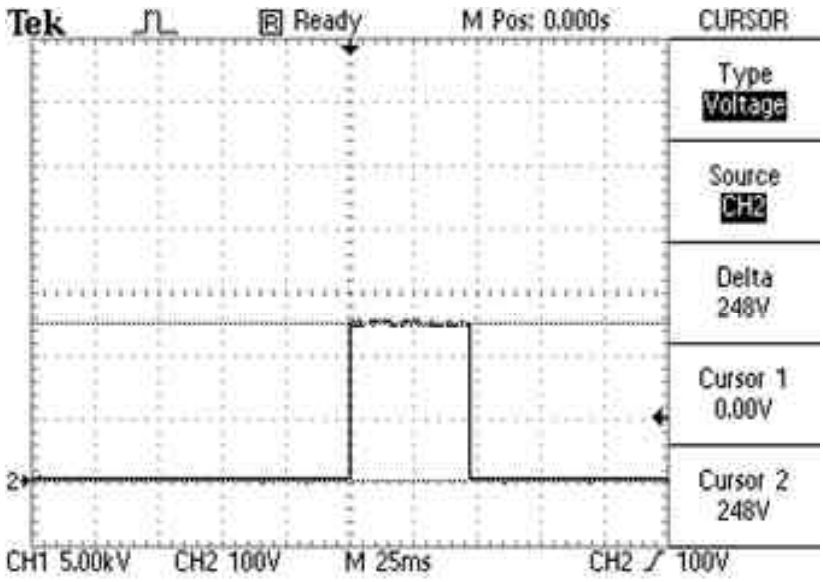
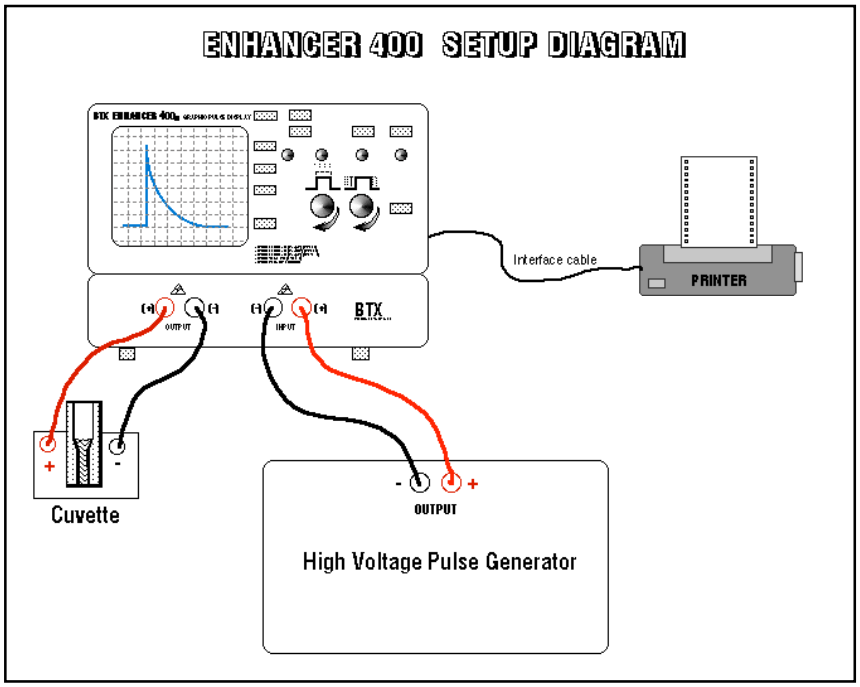


Diagram 1

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Functional Checks

Functional Check using Exponential Decay Wave Generators
Set DC voltage at about 200V and capacitance/resistance to values that will lead to a pulse with a time constant of approximately 50 msec. Follow the generator instructions for the initiation of the pulse. Verify that the Enhancer™ 400 has recorded the pulse and is displaying it on the screen.

Functional Check using Square Wave Generators
Set DC voltage at about 200V and a pulse length of 50 msec. Follow the generator instructions for the initiation of the pulse. Verify that the Enhancer 400 has recorded the pulse and is displaying it on the screen.

You are now ready to proceed with your experimentation.

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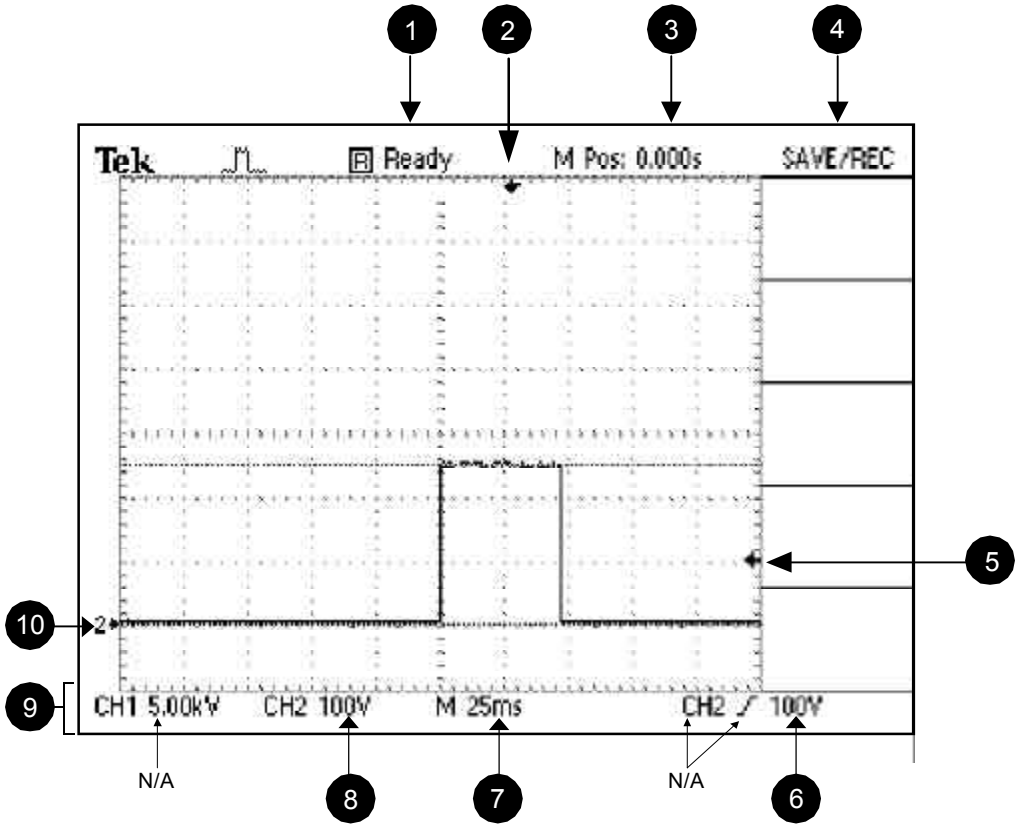


Figure 3

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Instrument Controls

The front panel is divided into three areas: Display, Soft Switches, and Advanced Controls (refer to Fig. 3).

Display

The display will show the pulse and the Enhancer™ 400 settings.

Become familiar with the location and significance of the following instrument settings:

1. **Trigger status:** Will show one of four messages:

   Ready: The Enhancer 400 is waiting for a pulse or signal.

   Trig'd: The Enhancer 400 is processing a pulse or signal.

   Stop: The Enhancer 400 has "frozen" a pulse on the screen.

   Auto: This setup is not used for electroporation purposes.

2. **Horizontal trigger position:** Beginning of the pulse display. The default position should be in the horizontal center of the screen.
3. **Horizontal trigger position (Readout):** "Time delay between the horizontal center of the screen and the beginning of the pulse".
4. **Menu Mode**
5. **Trigger level:** Visual indicator showing the voltage level at which the Enhancer will start recording a pulse. If the voltage is set below this value, a pulse will not be recorded. The trigger level may be adjusted.
6. **Trigger level (Readout):** "Voltage value of the trigger".
7. **Main Time Scale:** Read in µs or ms. Each grid corresponds to this many units.
8. **Voltage Scale:** Read in volts. Each vertical grid corresponds to this many volts.
9. **Instrument Settings Window**
10. **"2":** indicates where the baseline point lies. (0 volts).

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Soft Switches

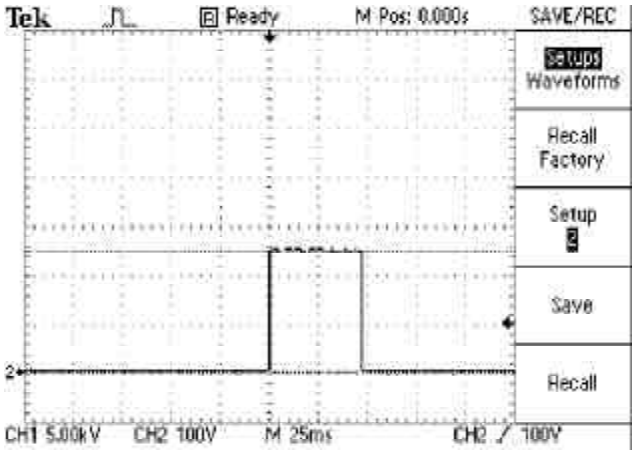
There are four apparent and one “hidden” soft switches (Pinhole) located on the right edge of the display. The lack of markings indicates that the function of soft switch changes with the menu selected. The function of a soft switch is read on the display itself.

Each pressing of a red labeled menu key triggers the display of a specific set of soft switches. The highlighted portion of a soft switch indicates which function is active. Subsequent pressing of a soft switch will usually toggle the function selected.

Setup Menu

Press the Setup key to display the Setup menu

The **Setup** menu enables the following display:
(Refer to Fig. 4)



The function of the soft switches under the setup are:

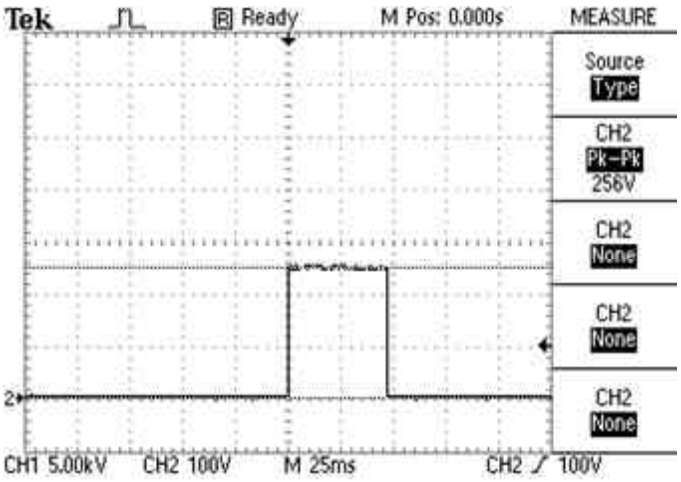
Setups or Waveforms:	This toggle action selects between the instrument setup routine or an actual Wave form to be saved or recalled.
Recall Factory:	Programs the instrument to a factory setting. This setup is not used for electroporation purposes.
Setup:	Instrument configurations stored in 5 memories. The active setup is indicated by the highlighted number. Activation is triggered after the pressing of the “Recall” soft switch. Suggested setup application are printed on the overlay.
Save:	Saves a displayed waveform or current instrument configuration to a memory location. This soft switch is purposely hidden To prevent the accidental erasure of a preprogrammed setup.
Recall:	Locks in the configuration stored in the setup program.

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Measure Menu
Press the Measure key to display the Measure menu

The **Measure** menu enables the following display:

(refer to Fig 5)



The functions of the Soft Switches under the Measurement menu are:

Source: Selects a channel for measurement. The highlighted portion should always show CH2.

Type: Selects the type of measurement from a single waveform. The user can select up to five measurements prior to acquiring a waveform. Measurements available are:

Cyc RMS: Provides a true RMS measurement of one complete cycle of the waveform.

Mean: Provides the arithmetic mean voltage over the entire waveform record.

Period: Provides the time elapsed during one cycle.

Pk-Pk: Provides the absolute difference between the maximum and minimum peaks.

Freq: Provides the frequency of the waveform. It is the mathematical inverse of the period.

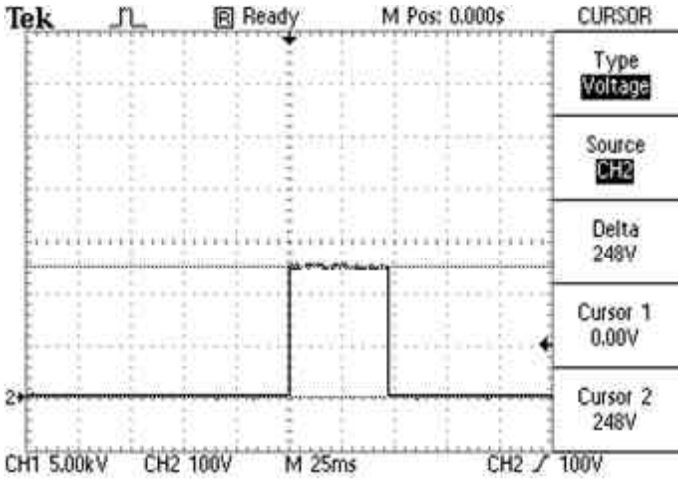
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Cursor Menu

Press the Cursor key to display the Cursor menu

The **Cursor** menu is an electronic ruler that facilitates the precise measurement of voltage and time. The menu enables the following display.

(refer to Fig. 6):



The functions of the soft switches are:

Type: Toggles between Voltage, Time and Off positions.

Source: Selects the channel where the cursor measurements are being made. This should always be CH2.

Delta: Displays the difference between the two cursor positions.

Cursor 1: Displays the difference between ground and the cursor 1 or between the trigger point and the cursor 1 Position is controlled by the leftmost Cursor Adj. knob.

Cursor 2: Same function as cursor 1. Position is controlled by the right most Cursor Adj. knob, labeled Position/Cursor Adj.

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Advanced Controls

The advanced controls are used to shape and control the waveform displayed on the screen. The current setup of the Enhancer™ is hence modified by the action of advanced controls. The current setup is saved when the Enhancer is turned off. However, the current setup will be overwritten if a numbered setup is recalled.

Voltage Amplitude: Controls the voltage sensitivity of the Enhancer thus altering the height of the waveform. The voltage sensitivity is indicated on the bottom line of the display as CH2 Vrms where V is the value in volts/division.

Pulse Width: Controls the time base (x-axis) of the Enhancer. This control will modify the horizontal size of the display.*

Position: Controls the position (X and Y-axis) of the whole waveform. Center the horizontal trigger prior to changing the “Pulse Width” to ensure proper waveform display.

Trigger Level: Modifies the voltage level at which the Enhancer starts the recording of a waveform. The setpoint is graphically indicated on the screen by an arrow on the right margin. A numerical value is also displayed on the right hand side of the bottom line.

Autozero Baseline: Erases the displayed waveform and leaves behind a straight line.

Display Freeze: Locks a waveform on the screen preventing the accidental erasure of an experimental pulse. The freeze is active when the word STOP has replaced READY in the top middle screen.

Note: *Several functions become inactive in the Frozen mode.*

* Adjust the horizontal trigger to the center screen position prior to changing the pulse width to ensure proper waveform display.

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Printer Setup

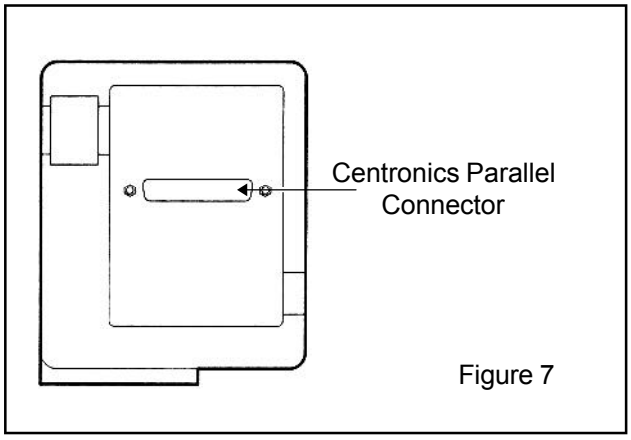


Figure 7

1. Connect a parallel port printer (Centronics) to the Enhancer printer port located on the back of the instrument. The cable should be included in the system package. Turn on the Enhancer and the printer.
2. Push the Utility Menu switch located behind the pinhole #1 below the save/recall menu switch, using a blunt object such as a paper clip.
3. Press the Hard Copy Setup soft switch. Select the appropriate printer by pressing the Format soft switch until the desired printer format is highlighted. The printer selection is automatically saved. A list of printers compatible with the Enhancer 400 is: Epson, ThinkJet, DeskJet, LaserJet, BMP, PCX, EPSIMAGE, INTERLEAF, DPU411 (v.1.04 or later), DPU412 (v. 1.00 and later).

   These printers must <u>NOT be</u> Windows only.
4. Test the setup by pressing the PRINT key. The Enhancer will be printing an exact duplicate of the screen.

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Computer Interface Setup

The back of the Enhancer™ 400 will contain 3 connectors. The top one is a 9 pin RS-232 serial connector. The center connector is a parallel printer port (Centronics). The operation of the printer port is described in the Printer Setup section. The lower connector is for GPIB (General Purpose Instrument Bus) and is not used for electroporation purposes.

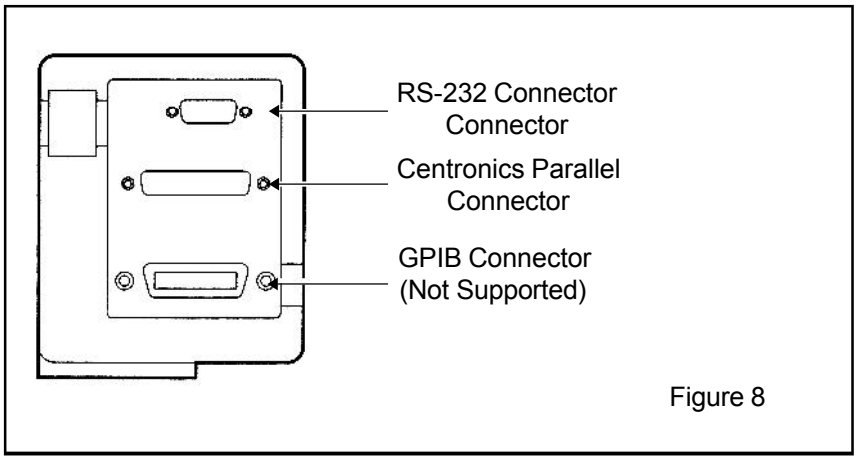


Figure 8

Enhancer™ 400 Computer Interface

WaveStar™ Installation and Use

Setup

1. Install the WaveStar software using the installation instructions provided in the software envelope.
2. Connect the 9-pin female end of the provided cable to the rear of the Enhancer 400 in the port-labeled “RS-232 port”.
3. Connect the other female end of the cable to the available 9-pin serial port on the rear of your PC. Your PC may have a 25-pin serial port in the rear. If this is the case, the furnished adapter can be used for connection to your 25-pin port.
4. The available serial port is one of the following: COM1, COM2, COM3, or COM4. Verify the available serial port on your PC.

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5. Once you have verified your available serial port, you must make sure it has the proper settings.
6. The serial port settings can be found under the port properties in the Control Panel menu for your PC.
7. The settings must be as follows:

Baud rate:	9600 bits per second
Data flow control:	Hardflag
Parity:	None
EOL string:	CR

8. Turn on the Enhancer™ 400.
9. Run the WaveStar™ program.
10. Select the Instrument pull-down menu and choose Select. This should activate the Instrument Select dialog box.
11. From the instrument list, select Tek TDS 210 and click OK
12. From this same dialog box, click on the RS-232 settings tab, and choose either COM1, COM2, COM3, or COM4 for your port. Remember, this must match your available serial port on the rear of your PC. This port will be the connection to the Enhancer™ 400. Now press the TEST button with your mouse.
13. If everything is connected properly, you will receive a dialog screen that states, “RS-232 connection OK”. Press OK.
14. If you receive a timeout error, then double check your connections, and look at the PC settings for its port. It must match with the software’s settings. Also check to see if you selected the right oscilloscope. The default scope for this software is the TDS 220. You must select TDS 210.

Acquiring the Waveform onto the PC Monitor

1. Apply a pulse to the ENHANCER™ 400. Press the Display Freeze key on your Enhance 400.
2. Go to the monitor on your PC and click on the Instrument pull-down menu and select Acquire. The waveform will be displayed on your PC.

Note: ***It may take up to 10 seconds to upload a waveform.***

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Exporting the Waveform to your PC

1. Click on the format pull-down menu and select graticule.
2. Click on the color tab.
3. From this tab, change copy from color to black lines on white.
4. From this same tab, change Print from Color to Black Lines on White. Press OK. These changes will allow you to print the waveform with a white background and a black waveform.
5. Click on the Edit pull-down menu.
6. Select Copy from the Edit menu. The waveform is now on your clipboard.
7. Run your post-processing program.
8. Click on the Edit pull-down menu of your post-processor, and select Paste. This will paste your waveform onto an empty space or page of your program.
9. Save the document in your post-processor immediately. You can save the wave form in WaveStar™, but you cannot retrieve it at a later time within WaveStar. A good suggestion is to save the document with the waveform immediately after you paste.

Added Features

Refer to section under measurement of key parameters for additional computer interface instructions. There are many added features to the WaveStar™ package. These features are shown but disabled on your version of WaveStar. If you are interested in finding out more about this software package and taking full advantage of its potential, contact Genetronics Customer Service (800) 289-2465.

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Monitoring

The following Setup Programs are available for use:

Setup 1: PULSE (1-99 µS) (0-500V)
Setup 2: PULSE (1-99 ms) (0-500V)
Setup 3: PULSE (1-99 µs) (500V-3kV)
Setup 4: AC/RF (0-100V)
Setup 5: USER DEFINED

Monitoring an Exponential Decay Wave Pulse

Recommended Setup Programs: Setup 2 for LV applications, Setup 3 for HV applications

1. Refer to Enhancer™ 400 Setup Diagram, interface and initialize all instruments. Proceed with Functional Check.
2. Choose an appropriate setup program that closely matches the expected experimental voltage and time constant by pressing the Setup Menu key, then pressing the Setup soft switch until the setup number appears in the window.
3. Lock-in setup program by pressing the Recall soft switch.
4. For the pulse to be monitored, the voltage must be greater than the trigger level position indicated on the screen. To increase or decrease the trigger level, adjust the trigger position with the Trigger Level Knob. Change the voltage resolution by adjusting the Voltage Amplitude Knob as needed. Change the pulse length resolution by adjusting the Pulse Width Knob as needed. Change the horizontal baseline position by adjusting the Cursor Adjustment Knob as needed.
5. Deliver a pulse and visualize the pulse profile. Make adjustments as desired. (These adjustments will be saved for the next experiment unless a setup change is initiated with the setup and recall soft switches).
6. To print following the display of a pulse, press the Print key after form feeding paper into the printer.
7. To upload data to your computer using the printer interface, refer to use of the Enhancer™ 400 computer interface, pg. 11.

Monitoring a Square Wave Pulse

Recommended Setup Programs: Setup 1 or 3 for µs pulse ranges, Setup 2 for msec pulse ranges.

1. Refer to Enhancer™ 400 Setup Diagram, interface and initialize all instruments. Proceed with Functional Check.

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2. Choose an appropriate setup program that closely matches the experimental voltage and pulse length by pressing the Setup Menu key, then pressing the Setup soft switch until the setup number appears in the window.
3. Lock setup program by pressing the Recall soft switch.
4. For the pulse to be monitored, the voltage must be greater than the trigger level position indicated on the screen. To increase or decrease the trigger level, adjust the trigger position with the Trigger Level Knob. Change the voltage resolution by adjusting the Voltage Amplitude Knob as needed. Change the pulse length resolution by adjusting the Pulse Width Knob as needed. Change the horizontal baseline position by adjusting the Cursor Adjustment Knob as needed.
5. Deliver a pulse and visualize the pulse profile. Make adjustments as desired. These adjustments will be saved for the next experiment unless a setup change is initiated with the setup and recall soft switches.
6. To print following the display of a pulse, press the Print key after form feeding paper into the printer.
7. To upload data to your computer using the printer interface, refer to use of the Enhancer™ 400 computer interface, pg. 11

Monitoring Alternating Current and Radio Frequency

Recommended Setup Programs: Setup 4

1. Refer to Enhancer™ 400 Setup Diagram, interface and initialize all instruments. Proceed with Functional Check.
2. Choose Setup 4.
3. Lock that setup program by pressing the Recall soft key.
4. For the pulse to be monitored, the voltage must be greater than the trigger level position indicated on the screen. To increase or decrease the trigger level, adjust the trigger position with the Trigger Level Knob. Change the voltage resolution by adjusting the Voltage Amplitude Knob as needed. Change the pulse length resolution by adjusting the Pulse Width Knob as needed. Change the horizontal baseline position by adjusting the Cursor Adjustment Knob as needed.
5. Deliver a pulse and visualize the pulse profile. Make adjustments as desired. These adjustments will be saved for the next experiment unless a setup change is initiated with the setup and recall soft keys.
6. To print following the display of a pulse, press the Print key after form feeding paper into the printer.
7. To upload data to your computer using the printer interface, refer to use of the Enhancer 400 computer interface, pg. 11.

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Note: *For fusion experiments utilizing both AC alignment and DC pulsing, use the above instructions to monitor the AC alignment and calculate the appropriate AC parameters. Follow the Monitoring a Square Wave Pulse instructions to monitor the DC pulse and calculate the appropriate DC parameters. The Enhancer™ 400 will not display multiple DC pulses. In order to visualize the AC alignment and DC pulse, set the trigger level above AC alignment amplitude.*

Monitoring Guidelines for BTX Generators

ECM®2001

Follow instructions in the previous section for AC alignment and DC square wave pulse monitoring. If you have a printer that has been configured to work with the ECM 2001, it cannot be interfaced with the Enhancer™ 400, and will only print from the ECM 2001. Alternatively, a printer configured for the Enhancer 400 will not interface with nor print from the ECM 2001.

ECM®200

Use Setup 5

Follow instructions in the previous section for AC alignment and DC square wave pulse monitoring. Use Model 461, or 1296 and the standard interface cables to interface the ECM®200 with the Enhancer 400.

Important Note: *If a setup program other than Setup 5 is used in conjunction with the ECM200, pulse profiles will be inverted on the monitor.*

ECM®830 ElectroSquarePorator

Follow the instructions in the previous section for square wave pulse monitoring.

T820 ElectroSquarePorator™

Follow the instructions in the previous section for square wave pulse monitoring.

T800 ElectroSquarePorator™

Use Model 461, or 1296 and the standard interface cables to interface the T800 with the Enhancer 400. Follow the instructions in the previous section for square wave pulse monitoring.

ECM®630, ECM®600, ECM®399, ECM®395, T720, T700

Follow the instructions in the previous section for exponential decay wave pulse monitoring.

T300, T100

Use Model 461, or 1296 and the standard interface cables to interface the pulse generator with the Enhancer™400. Follow the instructions in the previous section for exponential decay wave pulse monitoring.

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Saving a Waveform

1. Enter the SETUP menu by pressing the key SETUP.
2. Press soft switch 1 to highlight the word “Waveform”. This puts the Enhancer™ in the waveform saving mode.
3. Select the source of the waveform to be saved by pressing soft switch 2. CH 2 must be selected. CH 1 and MATH are invalid choices.
4. Waveforms can be saved to Ref A or to Ref B. The location is selected by pressing soft switch 3. The waveform will be saved by pressing the soft switch located behind pinhole 2.
5. The saved waveform is displayed only if Ref A or Ref B has been selected using soft switch 4. The displayed waveform will appear on the screen in low contrast gray. The baseline of the saved waveform is indicated by an A or a B on the left margin of the screen.

Measurement of Key Parameters

Electroporation-DC Parameters

Automatic Peak Output Voltage Measurement

1. After monitoring a pulse, enter the measurement menu by pressing the Measure key.
2. Verify that the top right window has the word “Type” highlighted. If the word “source” is highlighted, press the top soft switch once.
3. Press the second soft switch until the word “Pk-Pk” appears in the second window of the screen. The peak voltage measurement will then appear in the same window.

Note: Some combinations of electrode and buffers will allow some overshooting on the rising edge of a pulse. The Enhancer 400 will then measure the maximum voltage achieved during the overshoot. A manual measurement of the voltage using the cursor menu would then be indicated.

Manual Peak Voltage Measurement

1. Stabilize the display by pressing the Display Freeze key.
2. Press the Cursor Menu key.
3. Press the soft switch for “Type” until “Voltage” is indicated.
4. Verify that the second window is indicating “Source CH2”.

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5. Move Cursor 1 to the 0-volt baseline by turning the Cursor Adjust knob.
6. Move Cursor 2 to the top of the waveform by turning the vertical Position/ Cursor Adj. knob located above the Voltage Amplitude knob. The voltage measurement is indicated in the middle window labeled “Delta”.
7. The display can be re-enabled by pressing the Display Freeze key.

Field Strength Calculation

1. Obtain the value of the peak output voltage applied.
2. The field strength is obtained using the following equation:

$$E = Vp/d$$

Where E is the Field Strength in kV/cm or V/cm, Vp is the voltage in kV or V, and d is the chamber gap in cm.

Pulse Length Measurement

1. Stabilize the display by pressing the Display Freeze key.
2. Press the cursor menu key
3. Press the soft switch for “Type” twice. The top window will have changed from “Off” to “Voltage” to “Time”.
4. Verify that the second window indicates “Source CH2”.
5. Move the left most vertical bar (Cursor 1) to the beginning of the pulse by turning the Cursor Adjust knob.
6. Move the right most vertical bar (Cursor 2) to the end of a square pulse or to the point where an exponential decay curve reaches 1/e (0.37) of the peak voltage. The right cursor is moved by rotating the Position / Cursor Adj. knob located above the voltage amplitude knob.
7. The time measurement is indicated in the window labeled “Delta”.
8. The display may be re-enabled by pressing the Display Freeze Key.

Electrofusion-AC/RF Parameters

Automatic AC/RF Amplitude Measurement

1. Enter the measurement menu by pressing the Measure key.
2. Verify that the top right window has the word “Type” highlighted. If the word “source” is highlighted, press the top soft switch once.
3. Press the second soft switch until the word “Pk-Pk” appears in the second window of the screen. The peak voltage measurement will then appear in the same window.

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Manual AC/RF Amplitude Measurement

1. Stabilize the display by pressing the Display Freeze key.
2. Press the Cursor key.
3. Press the soft switch for “Type” until “Voltage” is indicated on the screen.
4. Verify that the second window is indicating “Source CH2”.
5. Move Cursor 1 to the bottom peaks of the AC waveform by turning the Cursor Adj. knob.
6. Move Cursor 2 to the top peaks of the AC waveform by turning the Position / Cursor Adj. knob located above the Voltage Amplitude knob. The Peak-to-Peak voltage measurement is indicated in the middle window labeled “Delta”.

AC/RF Duration Measurement

1. Stabilize the display by pressing the Display Freeze key.
2. Adjust pulse width knob so that entire AC wave is displayed.
3. Press the Cursor key.
4. Press the soft switch for “Type” until “Time” is indicated on the screen.
5. Verify that the second window is indicating “Source CH2”.
6. Move Cursor 1 to the beginning of the AC pulse by turning the Cursor Adj. knob.
7. Move Cursor 2 to the end of the AC pulse by turning the Position / Cursor Adj. knob located above the Voltage Amplitude knob. The AC/RF duration measurement is indicated in the middle window labeled “Delta.” The unit for pulse duration is in seconds.

Frequency Measurement

1. Stabilize the display by pressing the Display Freeze key.
2. Press the Cursor key.
3. Press the soft switch for “Type” until “Time” is indicated on the screen.
4. Verify that the second window is indicating “Source CH2”.
5. Move Cursor 1 to the middle of one positive peak by turning the Cursor Adj. knob.
6. Move Cursor 2 to the middle of the next positive peak by turning the Position / Cursor Adj. knob located above the Voltage Amplitude knob. The frequency of the AC waveform is indicated in the middle window labeled “Delta”. The unit for frequency is Hertz (Hz).

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AC to DC Ramp Time Measurement

1. If used with ECM® 2001, the Time Scale, which is at the bottom center of the screen, should be adjusted to as low as 250µs.
2. Stabilize the display by pressing the Display Freeze key.
3. Press the Cursor key.
4. Press the soft switch for “Type” until “Time” is indicated on the screen.
5. Verify that the second window is indicating “Source CH2.”
6. Readjust the Time Scale to 25µs.
7. Move Cursor 1 to the end of the AC pulse by turning the Cursor Adj. knob.
8. Move Cursor 2 to the beginning of the square pulse by turning the CURSOR ADJ knob located above the Voltage Amplitude knob. The Ramp Time measurement is indicated in the middle window labeled Delta

WaveStar™ Measurements

1. Go to the Cursor section of the WaveStar screen, which is located on the upper right hand side.
2. Select Type and four different options will appear.

a). **Vertical** is used to measure a length of time. This can be useful for measuring pulse length.

Exponential Pulse: Pulse length may be measured by moving one cursor to the beginning of the pulse, and by moving the other cursor to the point where the pulse has decayed to 1/e of its original peak value. Click and drag to move the cursor. Pulse Length is displayed next to the heading “dX”.

Square Pulse: Pulse length may be measured by moving one cursor to the beginning of the pulse, and by moving the other cursor to the end of the square pulse. Click and drag to move the cursor. Pulse length is displayed next to the heading “dX”.

b). **Horizontal** is used to measure the voltage.
Exponential and Square Pulse: Voltage delivered may be measured by placing one cursor at the bottom of the pulse, and placing the other cursor at the top of the pulse. Click and drag to move the cursor. The voltage is displayed next to the heading “dY”.

c). **Paired** may be used to measure either Voltage and/or Pulse length. Paired contains two vertical lines, and each vertical line has a small horizontal bar which automatically traces the curve.

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Exponential Pulse: Voltage may be measured by clicking and dragging one cursor to the beginning of the pulse. The peak voltage is displayed next to the heading “Y”. Pulse length may be measured by leaving this first cursor at the beginning of the pulse, and then dragging and clicking the other cursor to the point where the pulse has decayed to 1/e of its original peak value. This 1/e value needs to be calculated by the user. Move the cursor until the value next to the heading “Y” displays this 1/e value. Once this is done, the pulse length is found next to the heading “dX”.

Square Pulse: Voltage may be measured by clicking and dragging one cursor to the beginning of the pulse. The peak voltage is displayed next to the heading “Y”. Pulse length may be measured by leaving this first cursor at the beginning of the pulse, and then dragging and clicking the other cursor to the end of the square pulse. Pulse length is found next to the heading “dX”.

Experiment with this cursor. It can be quite useful.

d). **Crosshair** may be used, however it is not as simple or as accurate to use as the above mentioned.

3. The data achieved by using the cursors appear on the PC screen and automatically appear when the waveform is copied to another program and/or printed.

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Electroporation / Electrofusion Theory

Electroporation is the application of controlled, pulsed electric fields to biological systems. If the biological system contains a lipid bilayer, which is the case if the system is a suspension of cells or liposomes, the pulsed electric field may overcome the field potential of the lipid bilayer, resulting in a reversible breakdown of the bilayer and a resulting formation of temporal pores in the membrane. The pores formed are of the order of 40-120 nm. Most pores reseal within a few seconds, after allowing the transfer of materials into and out of the cells.

During a typical electroporation process, target cells and molecules are mixed together. An electroporation pulse is delivered, resulting in the formation of temporal pores. Before the pores reseal, the target molecules are observed to enter the cells. Upon re-sealing of the pores, the molecules become incorporated within the cell. The eventual target site depends on the application; molecules can remain in the cytoplasm, interact with the membrane, and move into the nucleus, for example.

Applications for electroporation include permeabilization of virtually all cells to a wide variety of molecules and ions. The most common applications for electroporation are the transformation or transfection of cells with DNA or RNA. Other applications for electroporation include electroactivation, electroinsertion of proteins into cell membranes and electroextraction of molecules from cells. Although electroporation has mainly been used as a research tool, recent work has demonstrated its potential for clinical applications. Some areas being explored include electrochemotherapy which involves electroporation for delivering chemotherapeutic agents directly to tumor cells, encapsulation of drugs/genes into cells for their use as carrier systems, transdermal delivery of drugs/genes, gene therapy and delivery of drugs/genes with an electroporation catheter.

Electrofusion. Cell or protoplast fusion may occur during the electroporation process, if the cells/protoplasts are brought into physical contact prior to the delivery of the pulsed electric field. In the electrofusion process, cells/protoplasts may be brought into contact through an alternating current (AC) alignment. The AC current causes a dielectrophoresis, which may result in the formation of pearl chains of cells/protoplasts. After delivery of the direct current pulse, pores that have been formed in close juxtaposition may re-seal upon one another. If the process results in an intact hybrid, electrofusion has occurred. Commonly a second round of AC alignment is employed following resealing, in an attempt to “compress” or stabilize the hybrid, and increasing the efficiency of the process.

Applications for electrofusion include embryo manipulation, hybridoma formation, and transgenic plant production.

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Applications of the Enhancer™ 400

The Enhancer 400 is designed as a universal graphic display and monitor and can be used with all standard electroporation and electrofusion instruments, to monitor and display pulse profiles, and to calculate delivered voltage, field strength, and pulse length. The Enhancer 400 may be used for all the following applications.

Electroporation and electrofusion generators can be characterized by waveform.

Exponential waveform generators, such as the BTX ECM® 630, ECM® 600 and the BTX ECM® 399, ECM® 395, and BTX™ T 720 deliver an exponentially decaying pulse. The length of such a discharge waveform is commonly characterized by the time required for the initial voltage to decay to 1/e of the initial value.

To achieve a desired pulse length, appropriate resistance and capacitance must be selected on the instrument. Voltage may be directly set on the instrument.

Square wave generators, such as the BTX ECM 830, T 820 and ECM® 2001 deliver square wave pulses of fixed pulse length, which may be directly adjusted on the instrument. The square wave pulse is produced by a partial discharge of a large capacitor, which requires the interruption of high currents against high voltages. Typically, voltage, pulse length, and number of pulses are all directly set on the instrument. Square wave pulses have well defined electric field amplitude, are both effective and also relatively mild to cells.

AC and radio frequency (RF) generators, such as the BTX ECM 2001, deliver a sinusoidal wave, and are used to cause dielectrophoresis, in which cells may be brought together for fusion. Parameters which typically may be set include amplitude, duration, and frequency.

Electroporation (EP)

Bacteria and Yeast Electroporation

The most common application is transformation. Field strength and pulse length are critical parameters for reporting, optimization, and troubleshooting bacterial and yeast applications. (Use the Enhancer 400 for field strength and pulse length calculation)

Mammalian Cell Electroporation

Electroporation has been used successfully to introduce many different molecule types into cells. Most commonly, electroporation is used for the processes of transfection, in which nucleic acid (DNA and RNA), is introduced into cells.

Electroporation can be used to deliver oligonucleotides into cells for anti-sense applications. It can be used to deliver proteins into cells, even large enzymes such as restriction enzymes, and antibodies, for various purposes.

Peptides have also been electroincorporated. Smaller molecules, such as dyes, sugars, and dNTPS have been incorporated into cells and liposomes.

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Electroporation has been used to study cellular activation processes, by electropermeabilizing cells to Ca^{2+}, Mg^{2+}, and Na^{+}. Electroporation is also used to electroinsert proteins into the cell membrane. Finally, electroporation has been used to introduce drugs, such as the chemotherapeutic agent bleomycin, into cancer cells, in vitro and in vivo.

For all applications, use the Enhancer™ 400 to display critical electroporation pulse profiles and calculate actual peak voltage, field strength, and pulse lengths. Optimize and troubleshoot experiments with the Enhancer™ 400.

The use of low impedance buffers such as PBS may result in a voltage drop so that the actual peak voltage delivered to samples may be less than the set voltage. Use the Enhancer 400 to calculate the actual field strength experienced by the sample.

With exponential decay generators, monitoring is necessary to identify the pulse length, or time constant, since this parameter may be very much dependent on the impedance of the sample (sample load). Use the Enhancer 400 to calculate the pulse length.

When using complex and custom electroporation applicators and chambers, which includes BTX models PP35-2, 366, 372, 747, 840, etc., the electroporation waveform may be altered. Use the Enhancer 400 to verify the integrity of the pulse profile.

Plant Protoplast Electroporation
Electroporation has been used to introduce molecules into plant protoplasts, pollen, and most recently, direct transfer into plant tissue has been carried out, in essence, in vivo. Use the Enhancer 400 to display key electroporation pulse profiles and calculate actual peak voltage, field strength, and pulse lengths, enabling optimization and troubleshooting.

Other Electroporation Applications
Use the Enhancer 400 to assist in optimization and troubleshooting (voltage, field strength, and pulse length) for other cell types and applications, including the following Embryo Manipulation and In Vitro Fertilization techniques:

1. Transgene incorporation, in which simple transfection of fish embryos has resulted in transgenic zebrafish.
2. Utilization of sperm as biological DNA carriers, in which pulsed fields cause the complexing of DNA to sperm, which then act as carriers upon fertilization.
3. Acrosome enhancement in which an exponential decay pulse enhances the acrosome reaction and facilitates fertilization.
4. Embryonic Stem Cell Chimeras, in which embryonic stem cell transfection, followed by micromanipulation into host blastomere, has resulted in chimeric mice.

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5. Parthogenesis, in which a repetitive DC pulse stimulates an unfertilized egg to activate and divide as if fertilized, resulting in haploid and diploid embryos.

6. Tetraploid embryos may be obtained if parthenogenesis is carried out at the 2 cell stage.

Electrofusion (EF)

Embryo Manipulation

Embryo Manipulation includes nuclear transplantation experiments, in which blastomeres are inserted by micromanipulation through the zona pellucida into the perivitelline space of a surrogate enucleated egg. After application of an AC wave to facilitate compression and alignment, a single DC square wave fuses the pair, and subsequent pulses activate the resulting nuclear transplant embryo. Use the Enhancer™ 400 to monitor and optimize both AC alignment (AC amplitude, duration, and frequency) and the DC fusion (DC voltage, field strength, and pulse length).

Hybridoma Formation

Electrofusion has been used by researchers as a replacement for polyethylene glycol (PEG), in the production of monoclonal antibodies by hybridomas. Microelectrofusion of hybridomas is used to generate hybrid hybridomas, or quadromas, secreting bifunctional antibodies, without the use of drug selection markers, resulting in higher yields than PEG without the delays of drug selection. Use the Enhancer 400 to monitor and optimize both AC alignment (AC amplitude, duration, and frequency) and the DC fusion (DC voltage, field strength, and pulse length).

Plant Protoplast Fusion

The process of electrofusion has been used extensively to fuse plant protoplasts, resulting in viable hybrid offspring. Use the Enhancer 400 to monitor and optimize both AC alignment (AC amplitude, duration, and frequency) and the DC fusion (DC voltage, field strength, and pulse length).

Other Electrofusion Applications

Electrofusion has been used in nuclear transfer, cloning, adoptive immunotherpay, etc. Use the Enhancer™ 400 to monitor and optimize both AC alignment (AC amplitude, duration, and frequency) and the DC fusion (DC voltage, field strength, and pulse length).

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Appendix A: Optimization Strategies

General

The success of electro cell manipulation (ECM) lies in selecting appropriate ECM systems capable of delivering the pulses suitable for the cell being electro-manipulated. One or several pulses of the appropriate field strength, pulse length and wave shape may be required for this purpose.

The key to success with electroporation-based technologies involves a proper combination of biological, physical, chemical, and pulse parameters. In general, cells must be in mid-logarithmic growth for optimal electroporation. Various temperature regimens have been described. It has been shown that a variety of chemical techniques may increase electroporation and electrofusion efficiencies, including addition of EDTA, “intracellular salts”, and serum prior to or after the pulse. Optimizing protocols abound. Analysis of these optimization regimens has lead to proposals of “universal” protocols, involving very limited optimization over a narrow range.

Optimizing with the Enhancer™ 400

Use the Enhancer 400 to assist in protocol optimization through its monitoring, display, and calculation capabilities.

Electroporation

1. Vary the voltage in order to vary the field strength, keeping other parameters constant. Assay sample for both viability and endpoint. Plot the field strength versus both viability and endpoint and extrapolate the optimal field strength and voltage. Use the Enhancer 400 to calculate peak voltage and field strength.

2. Vary the capacitance/resistance/sample volume at the optimal voltage setting in order to vary the pulse length for exponential decay instruments. Directly vary square wave instrument pulse length. Assay sample for both viability and endpoint. Plot the pulse length versus both viability and endpoint and extrapolate the optimal pulse length/parameters. Use the Enhancer 400 to calculate pulse length.

3. For multiple pulsing systems/protocols, vary the number of pulses at the optimal field strength and pulse length. Assay sample for both viability and endpoint. Plot the number of pulses versus both viability and endpoint, and extrapolate the optimal number of pulses. Use the Enhancer 400 to verify field strength, pulse length, and number of pulses.

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Electrofusion

AC Alignment

1. Set AC amplitude and frequency. Initiate alignment manually, monitoring the process visually on an inverted microscope. When alignment is optimal, discontinue alignment and initiate DC fusion. Use the Enhancer™ 400 to visually verify the AC, AC amplitude, and AC frequency.
2. If AC amplitude is unknown, initiate alignment manually, and begin increasing the amplitude from 0 V, until optimal cell motion is observed. When alignment is optimal, discontinue alignment and initiate DC fusion. Use the Enhancer 400 to visually verify the AC, calculate amplitude, and verify frequency.
3. Record duration of AC alignment for further experiments in an automatic mode. Use the Enhancer 400 to verify the AC duration.

DC Fusion

1. Vary the voltage in order to vary the field strength, keeping other parameters constant. Assay sample for both viability and fusion yields. Plot the field strength versus both viability and fusion yields and extrapolate the optimal field strength and voltage. Use the Enhancer 400 to verify voltage and field strength.
2. Vary the pulse length at the optimal voltage. Assay sample for both viability and fusion yield. Plot the pulse length versus both viability and fusion yields and extrapolate the optimal pulse length. Use the Enhancer 400 to verify pulse length.
3. For multiple pulsing systems/protocols, vary the number of pulses at the optimal field strength and pulse length. Assay sample for both viability and fusion yields. Plot the number of pulses versus both viability and fusion yields, and extrapolate the optimal number of pulses. Use the Enhancer 400 to verify field strength, pulse length, and number of pulses.

Appendix B: Experimental Troubleshooting

1. **Arcing:** Verify electrical component functionality. Verify properties of cell sample (do cells need to be washed? Is the buffer appropriate for application?). Verify properties of transfectant/molecule (Is the buffer appropriate for application?). Try reducing the voltage until arcing is no longer a problem. Use the Enhancer 400 to verify pulse profile, actual peak voltage, and pulse length during an arc.
2. **Low or no transfection efficiency, or incorporation:** Verify physical, biological, and chemical parameters. Verify delivery of the pulse and pulse parameters. Is the voltage correct? Chamber gap? Pulse length or appropriate instrument settings? Number of pulses? If so, follow Optimization Guidelines outlined in Appendix A. Use the Enhancer™ 400 to verify pulse delivery, peak voltage, field strength and pulse length.

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3. **Low viability:** Verify physical, biological, and chemical parameters. Verify delivery of the pulse and pulse parameters. Is the voltage correct? Chamber gap? Pulse length or appropriate instrument settings? Number of pulses? If so, reduce voltage, pulse length, or number of pulses and re-optimize protocol to improve viability as outlined in Appendix A. Use the Enhancer 400 to verify pulse delivery, peak voltage, field strength, pulse length, and number of pulses.
4. **No/negligible alignment:** Verify physical, biological, and chemical parameters. Verify frequency and amplitude. Re-optimize amplitude and duration as outlined in Appendix A. Use the Enhancer 400 to verify AC, frequency, and amplitude.
5. **Low fusion yield:** Verify physical, biological, and chemical parameters. Troubleshoot alignment as outlined in 4. Verify delivery of DC fusion pulse, voltage, field strength, and pulse length. Re-optimize the DC fusion parameters as outlined in Appendix A. Use the Enhancer 400 to verify DC pulse, voltage, field strength, pulse length, and number of pulses.

Appendix C: Glossary of Biological Technical Terms

Alignment
See also Dielectrophoresis and Pearl Chain. A consequence of cells being exposed to an inhomogeneous or divergent electric field, resulting in their movement toward the electrodes, and subsequent alignment or pearl chain formation.

Chambers
Electroporation and Electrofusion Chambers are the devices used to contain the cells/molecules to be fused/transfected. The chambers include the electrodes through which pulse generators deliver the electric field to the cells.

Cloning
In terms of applications for electro cell fusion, cloning refers to the ability to generate identical, viable animals, through processes such as nuclear transplantation.

Compression
See Alignment, Dielectrophoresis, and Pearl Chains. The result of an AC alignment.

Dielectrophoresis
See Alignment

Dimer Formation
The bringing together of 2 cells, through the process of dielectrophoresis, so that they may be fused, resulting in a hybrid. Refer also to Pearl Chain.

Electrofusion (EF) or Electro Cell Fusion (ECF)
Electric field induced cell fusion. A novel physical means to bond two cells together by the application of an electric field pulse of high intensity.

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Electroincorporation
The use of electroporation for the incorporation of molecules or ions into cells, protoplasts, or liposomes.

Electroinsertion
The use of electroporation for the insertion of molecules into lipid bilayer membranes.

Electrolytic
A fluid containing charged molecules is called an electrolyte. Electrolytic properties are associated with such a fluid, such as the ability to conduct current

Electropermeabilization
The use of electroporation to make cells, protoplasts, or liposomes permeable to ions and small molecules in their extracellular environment.

Electroporation
The application of high electric field pulses of short duration to create temporary pores (holes) in the membranes of cells.

Embryo Manipulation
The cloning of animals can be accomplished through embryo manipulation techniques, such as nuclear transfer and electrofusion.

Hybrid
A viable daughter cell resulting from the fusion of two parent cells.

Hybridoma
The fusion of an antibody-producing cell with an immortalized cell, resulting in an immortalized hybrid cell capable of generating antibodies.

Hydrostatic Pressure
The pressure in liquids at rest.

Lipid Bilayer
An assembly of lipid and protein molecules held together by non-covalent interactions. All biological membranes share this common structure.

Microslide
An electrofusion chamber allowing for the observation of the alignment and fusion processes under a microscope.

Osmotic Pressure
The applied pressure required to prevent the flow of solvents of different concentration across a semipermeable membrane.

Pearl Chains
See Alignment and Dielectrophoresis. Chains of cells or vesicles brought into alignment during electro cell fusion, prior to electroporation.

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Pore
A small, mostly transient opening in a cell wall caused by the application of a brief high electric field pulse.

Pressure Gradient
The difference in pressure between two points in a medium.

Protoplasts
The plant cell proper, with the cellulose cell wall removed.

Relaxation Time
The time for a system to reach equilibrium.

Rounding Off
The phenomena of cells of forming a sphere after being fused together.

Transfection
The introduction of nucleic acids into animal cells.

Transformation
The introduction of nucleic acids into microorganisms and plant cells.

Turgor Pressure
The pressure in capillaries.

Appendix D: Glossary of Electrical Technical Terms

Alternating Current (AC)
Current whose amplitude varies with time. AC is used to induce an heterogeneous or divergent electric field during electro cell fusion. The AC current leads to the pearl chain formation which facilitates the fusion of cells.

Amplitude
The instantaneous value of current or voltage in amperes or volts.

Capacitor
A device that stores electric energy in the form of an internal electric field. Energy is delivered when a current flows out of a capacitor. The current normally follows an exponential curve.

Dielectric
A material that has a high resistivity and that can store energy in the form of an electric field.

Dielectric Breakdown
The abrupt change of resistivity in a membrane or a dielectric film. The sudden drop of resistivity allows a much greater current to flow through the structure.

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Dielectric Constant
The ratio of the dielectric value of a material compared to the dielectric value of vacuum.

Direct Current (DC)
Current whose amplitude is constant with time. Direct currents are used to form temporary pores in bi-lipid membranes. Cells may fuse when pores in the membranes of two or more juxtaposed cells reseal after a DC application.

Divergence
The deviation of electric field lines from a parallel homogeneous condition. A highly divergent field has field lines that rapidly change value and direction in the area of interest.

Electric Field
The electric potential difference between two points divided by the distance separating those points. Expressed in volt/cm.

Electric Field Force
The mechanical force acting on any electric charge when placed in an electric field.

Exponential Decay
Non linear waveform typical of capacitor charge and discharge currents and voltage.

Frequency
The number of times that an oscillation goes through a complete cycle in one second. The unit is either cycle/sec or Hertz (Hz).

Homogeneous Electric Field
An electric field where the direction and strength of the field lines are constant. The difference in volts between two points in space.

Heterogeneous Electric Field
An electric field where the direction and strength of the field lines are varying. Potential. The difference in volts between two points in space.

Pulse Length
The length of time that an electric signal is present. In an exponential pulse, the end of the pulse is considered to happen when the waveform has decayed to 1/e of the peak value.

Radio Frequency (RF)
High frequency sinusoidal signal usually used to align cells in pearl chains prior to fusing.

Root Mean Square (RMS)
A mathematical average.

Voltage
The difference of electric potential between two points in space. Expressed in volts.

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Appendix E: Electroporation and Electrofusion Pulse Generator Compatibility

The Enhancer™ 400 is compatible with all common electroporation and electrofusion pulse generators if supplied with the appropriate interface cables. If you have a pulse generator other than those listed, please contact Genetronics BTX Division Technical Service for recommendations. The following is a list of current North America and International Instrument manufacturers with pulse generator models compatible with the Enhancer 400:

- BTX™
- BioRad Laboratories®
- Bridge Laboratories (Kodak-IBI pulse generators)
- eppendorf
- CytoPulse®
- EquiBio (EasyjecT)
- Life Technologies$_{TM}$, Inc.(BRL pulse generators)
- Jouan

Appendix F: Recommended Reading

Eberhard Neumann, Editor, Electroporation and Electrofusion in Cell Biology, Plenum Publishing Corporation, 1989

Michael Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, 1990

Donald Chang, Editor-in-Chief, Guide to Electroporation and Electrofusion, Academic Press, 1992

Jac A. Nickoloff, Editor, Electroporation Protocols for Microorganisms, in Methods in Molecular Biology, Vol 47, Humana Press, 1995

Jac A. Nickoloff, Editor, Animal Cell Electroporation and Electrofusion Protocols, in Methods in Molecular Biology, Vol 48, Humana Press, 1995

Jac A. Nickoloff, Editor, Plant Cell Electroporation and Electrofusion Protocols, in Methods in Molecular Biology, Vol 55, Humana Press, 1995

For further references on specific applications and optimization, please contact Genetronics BTX Division Technical Services.

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Appendix G: Specifications

General Specifications

Certifications and Compliance's	
EMC	Meets requirements of Directive 89/336/EEC for Electromagnetic Compatibility and Low-Voltage Directive 73/23/EEC for Product Safety. Compliance was demonstrated to the following specifications as listed in the Official Journal of the European Communities: **EN 50081-1 Emissions:** EN 55011 Class B Radiated and Conducted Emissions **EN 55082-1 Immunity:** IEC 10004-2 Electrostatic Discharge Immunity IEC 10004-3 RF Electromagnetic Field Immunity IEC 10004-4 Electrical Fast Transient/Burst Immunity
Safety	**Low Voltage Directive 73/23/EEC:** EN 61010-1 Safety requirements for electrical equipment for measurement, control and laboratory use

Appendix H: Product Information & Ordering for Enhancer Model 4001

Description	Model /Part No.
Enhancer 400	4001
High Voltage Cables	06-800073-01, 06-800074-01
Manual (Hardcopy, Electronic)	00-900045-01, 00-900045-02
Wavestar Software	06-800103-01

This product meets the requirements of the European Communities (EC), and complies with EMC Directive 89/336/EEC and 73/23/EEC for product safety.

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Appendix I: General Care and Cleaning

General Care

Do not store or leave the instrument where the LCD display will be exposed to direct sunlight for long periods of time.

⚠ ***CAUTION***. *To avoid damage to the instrument, do not expose it to sprays, liquids or solvents.*

Cleaning

Inspect the instrument as often as operating conditions require.

To clean the instrument exterior, perform the following steps:

1. Remove loose dust on the outside of the instrument with a lint-free cloth. Use care to avoid scratching the clear plastic display filter.
2. Use a soft cloth dampened with water to clean the instrument. Use an aqueous solution of 75% isopropyl alcohol for more efficient cleaning.

⚠ ***CAUTION***. *To avoid damage to the surface of the instrument, do not use any abrasive or chemical cleaning agents.*

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