User's Guide

C-Pace EP

C-Pace EP with optional 6-well C-Dish electrode assembly

Manual Revision 7.2
August 2011

ION OPTIX

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Introduction

Overview

The C-Pace EP is a multi-channel stimulator designed for chronic stimulation of bulk quantities of cultured cells in incubators. On top of the basic features offered in the original C-Pace, the EP version introduces the abilities to provide constant variation in frequency, to insert periodic off beats, and to program frequency changes. It can also accept TTL triggering and provide a TTL output for use in collected experimental data.

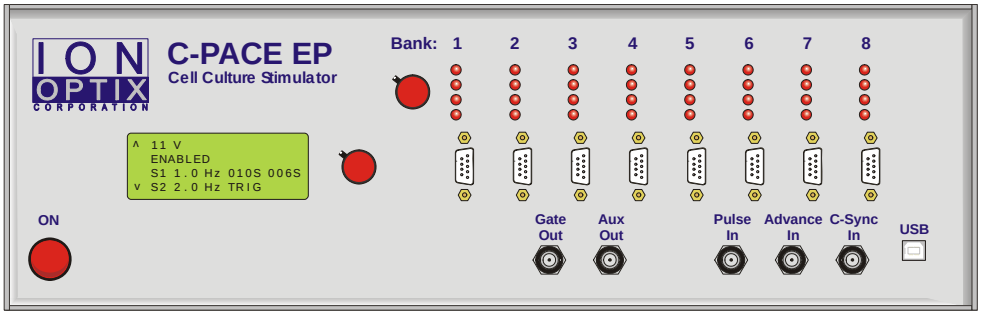
It has been shown that chronic stimulation of cultured cardiac myocytes prevents de-differentiation, maintaining the contractile properties of the cell for much longer than unstimulated cells. The C-Pace EP features up to eight independent banks with four, six or eight channels each, allowing for the pacing of cells in a large number of wells (each channel paces one well). The ability to emit bipolar stimuli greatly reduces problems with electrolysis at the electrodes and facilitates cell stimulation. Output voltage (+/- 40 V), frequency (.010 to 99 Hz), and duration (.4 msec to 24 msec) for each bank can all be easily adjusted from the front panel and are saved in non-volatile memory for quick start-up.

Features

- Digital adjustment of:
  - Frequency (0.010-99Hz)
  - Pulse duration (0.4-24 msec)
  - Voltage (up to +/- 40 V and 240 mA)
- Bi-polar pulses
- Current up to 240 mA
- Short circuit and open circuit protection of electronics
- Works in conjunction with IonOptix C-Dish™ Culture Dish Electrode Assemblies that fit standard Nunclon rectangular 4-well and 8-well dishes, a variety of 6 well Culture Plates, and discrete 35mm 6-well dishes
- C-Pace EP connects to C-Dishes™ through a thin ribbon cable to allow an incubator door to close around it and maintain a good seal.
- Digital inputs and outputs
- Programmable multi-step protocols

Hardware

Front Panel



User Interface

Menu choices and current parameters are displayed on a 20x4 character LCD screen. To permit one-handed adjustment, we used **Encoders** (or dials) that can be turned as well as clicked. Encoder 1 is directly next to the display. In general, turning Encoder 1 will perform a scroll function and clicking the dial will perform a select function. On the LCD display, the cursor indicates current position, and carets indicate that the menu continues beyond the display and continuing to turn the encoder will perform a scrolling function. Encoder 2 is next to the pacing indicator LEDs. Turning this encoder will cause a green light to scroll through the pacing LEDs, indicating the well that the Gate Out and Aux Out will lock to. Clicking this encoder performs no function.

High Voltage Section / Well Number Adjustment

The high voltage section of the C-Pace EP consists of up to 8 independent banks with four, six, or eight channels each. (It is possible to change the number of channels the bank is set to by changing the setting of the dipswitch in the far corner of the High Voltage Board. Begin with all switches in the "ON" position. The numbers 4, 6, and 8 are written on the board next to switches 1, 2, and 3 respectively. Turn off the switch that corresponds to the desired number of wells. See Tech Note at end of manual for details.) Identical pulses are sent sequentially through the channels of a bank. The high voltage amp is connected to each channel through its own reed relay, to ensure complete open circuit between pulses. The amplifier

provides an output of up to +/-40 V and up to 240 mA. This output has been shown experimentally to be sufficient for the standard rectangular and 35mm wells we support.

Connectors

The C-Pace EP is connected to the stimulation electrodes via 9-connector ribbon cables. The cable is thin enough to close the incubator door on and maintain a good seal.

Pacing Indicators

There is one LED for each channel that will turn red to indicate a high voltage pulse. It will remain on for the duration of the pulse.

Digital Inputs

Advance In

This is only used in Sequence mode. The rising edge of a TTL pulse at the 'Advance In' input will initiate the next step if the trigger option has been selected in the Sequence Edit menu. The output pulse train will finish with the current pulse before switching steps. This means that the first pulse of the next step will not actually be synchronized with the Advance In input, but will occur at the end of the period of the previous pulse. Multiple banks can use the same trigger. The LED next to the BNC will light during TTL high.

Pulse In

This is only used in TTL Lock mode. The rising edge of a TTL pulse at the "Pulse In" input will trigger High Voltage output pulses on all banks set to TTL Lock. The duration of the pulse remains programmable and is independent of the duration of the TTL input. Due to the settling time required by the closing of the relays, the pulse on the first well will occur 450 (+/-10) us after the rising edge of the TTL input. The LED next to the BNC will light during TTL high.

TTL Outputs / Gate Selection

Gate Selection

There are two TTL outputs that can be locked to any well by adjusting the encoder next to the Pacing indicator LEDs. As the knob is turned, the green light will move from well to well, indicating the bank and well the output pulse will be locked to.

Gate Out

This output will emit a pulse train of TTL pulses exactly reflecting the high voltage output pulse train. The rising edge of the TTL pulse corresponds with the beginning of the high voltage pulse and the falling edge corresponds to the end of the high voltage pulse. The LED next to the BNCs will light during TTL high.

Aux Out

This is only used in Sequence mode to indicate the beginning of a step and must be enabled in the sequence edit menu of the desired step as shown below. In the case of a pulse train, the TTL pulse will occur in sync with the first pulse of the well the gate is locked to. In the case of a delay, the pulse will go high at the beginning of the delay, and remain high until the delay step has ended. The LED next to the BNC will light during TTL high.

USB

NOT CURRENTLY FUNCTIONAL: Will allow the user to program and save protocols from a computer interface.

Power on/off switch

Power to the C-Pace EP is turned on and off with the switch located on the lower left half of the front panel. Flipping the switch to the 'on' position will cause the switch to light, and, after about 1 second, the LCD display will light up.

Back Panel

Power inlet

The C-Pace EP has a Universal Wide Range Input power supply, with UL, CSA, TUV, and CE mark approval. This means it can be used with power grid voltages ranging from 85 to 265 V AC.

Fuse

Always unplug device before checking fuse! In devices with a separate fuse holder, the C-Pace EP uses a 1 ¼ inch 3Amp fuse. Push and turn the fuse holder cover to open. In devices with combined power entry and fuse modules, a compartment in the power entry module holds two active 3 Amp/250V, 5x20mm slow blow fuses.

Internal Hardware

Pulse Train Control

The C-Pace EP uses micro-controllers to process the interface and control the pulse train. Variables that are programmed are saved in non-volatile memory for quick and easy start up. Banks are disabled on power off to eliminate the chance of undesired high voltage pulses on power on. Reed relays ensure an open circuit between pulses.

High Voltage Banks

The C-Pace EP can be configured with up to eight High Voltage Banks (HVB), which function as daughter boards to the main board. An additional bank may be added by matching the ID on the HVB with the proper slot and plugging it in to the main board. It can be done in lab, but does require a fairly complete disassembly and reassembly of the device.

Trouble-Shooting

Please let me know if you have had problems so I can continue to improve the device. Also feel free to contact me if the following suggestions don't fix the problem.

Kate Barber

kate@ionoptix.com

617-696-7335

Always unplug device before opening box!

No Power

Make sure the power cord is plugged in firmly and connected to an active outlet. Unplug and check fuse. If necessary, replace with 3 Amp 5x20mm slow blow fuses (older devices with detached fuse holders use 1 ¼ inch fuses).

Always unplug device before opening box!

Blank Display

The main board's micro-controller chip may not be in its socket firmly. Press down on the chip with the handwritten label to make sure it is making good connection. Also check the cable to the display.

Always unplug device before opening box!

Stuck on "IonOptix"

If the display show's IonOptix for more than a few seconds, it means the main board is unable to communicate with the HVBs.

1) It is possible that one or more High Voltage Boards have come loose in their sockets. Take the bottom of the C-Pace off and push up on the main board to push the High Voltage Boards more firmly into their sockets.

2) A slave board's micro-controller chip may not be in its socket firmly. Press down on the chips with the handwritten labels to make sure they are making good connection.

3) The ID is set with the dipswitch labeled "BANK" on the top of the High Voltage Board. It's possible that the setting has gotten changed by mistake. The setting should match the table below with "O" meaning the switch has been pressed down on the "Open" side or is pulled to the "Off" position (depending on the dipswitch). 'C' is "Closed" or "On".

Number of the Slot on the Front Panel	Number on Dip Switch			
	1	**2**	**3**	**4**
1	O	O	O	O
2	C	O	O	O
3	O	C	O	O
4	C	C	O	O
5	O	O	C	O
6	C	O	C	O
7	O	C	C	O
8	C	C	C	O

Always unplug device before opening box!

Not Displaying a Bank

If line 1 has been selected, and scrolling fails to show a bank, it means the main board has not been able to communicate with that bank. See “Stuck on IonOptix” above.

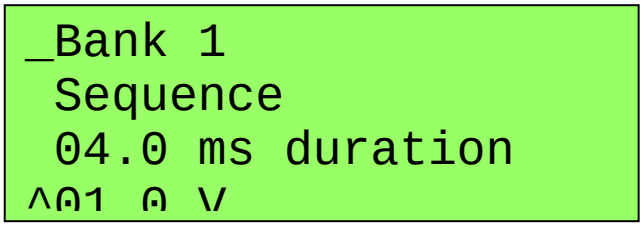
Other odd behavior

Probably reflects a software bug. Powering off and on will likely help. If not, try selecting Re-Initialize on the second line of the main menu. This will restore all values to factory defaults. They can then be reset. Please let me know if you see any odd behavior (kate@ionoptix.com).

Software

Controlling the Pulse Train

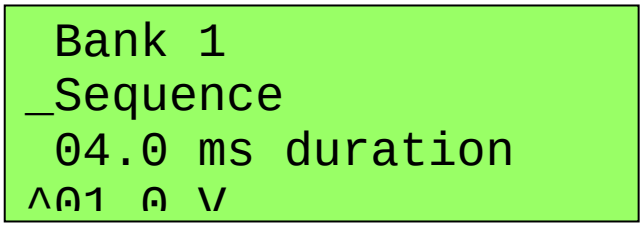
Line 1: Bank Selection



To select the bank to be edited, click on the first line, and turn the knob to scroll through available banks. Click to select.

Trouble Shooting: If a bank isn't showing up, it means the main board hasn't been able to communicate with it. See trouble shooting in the hardware section.

Line 2: Mode of Operation



Each bank has 4 different modes of operation.

Basic This is the easiest mode in which to start a bank pacing at a single frequency and make changes manually. Irregular pulses (see Irregular Pulses) are available in this mode.

Sequence The Sequence Mode should be selected if the user wishes to make programmable frequency changes, including single offbeats, delays, and rate changes. The advancement to the next step in the protocol can be triggered manually, from an external TTL signal, or from an internal timer. Irregular pulses (see Irregular Pulses) are also available in this mode.

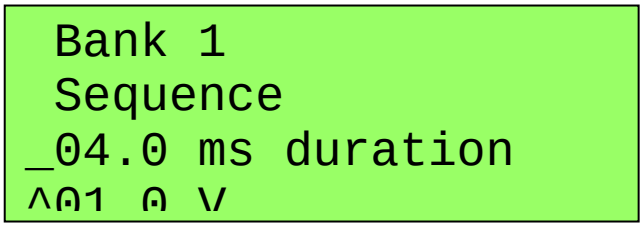
TTL Lock In this mode, a TTL pulse on the "Pulse In" input will initiate a single high voltage pulse.

Re-Initialize This option will reload factory defaults of all bank values.

Copy Previous Bank This option saves the user from having to reenter frequency information. It is a one-time copy of information, and does not provide a permanent link. Once the information is copied, the mode and frequency information will be displayed and can be adjusted further. The ENABLE/DISABLE status, voltage, and pulse duration are not copied and must be set for each bank.

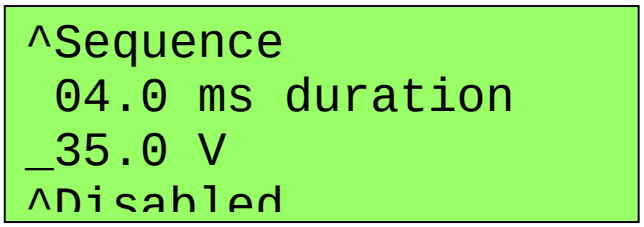
Note: This option does not lock the pulse trains of multiple banks together. To lock banks together, set the gate to the master bank (see Gate Selection), set the slave bank to TTL Lock, and connect a BNC cable between "Gate Out" and "Pulse In". There will still be a small delay (450 (+/-10) usec) between master and slave.

Line 3: Duration



This adjusts the duration of each pulse between .4 and 24 msec. The banks will reflect the changes as the duration is tuned. To reduce electrolysis byproducts, the pulses that are emitted are all square bipolar pulses, so half of the chosen duration will be positive, half negative. To further reduce byproducts, the duration should be kept as short as possible. Standard values are in the 4-10 msec range. The upper range of the duration is recomputed each time to take current frequency and variability settings and number of wells into account.

Line 4: Voltage

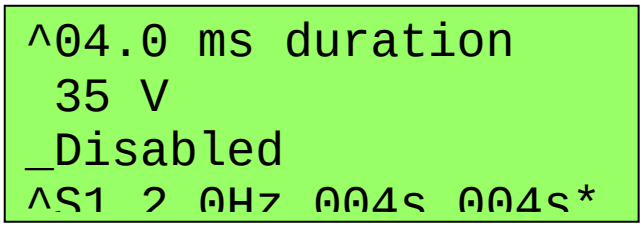


This adjusts the voltage of each pulse from 0-40V. To reduce electrolysis byproducts, the pulses that are emitted are all square bipolar pulses, so half of the

chosen duration will be positive, half negative. There is a coarse adjustment of 1V and a fine adjustment of .1 V. The banks will reflect the changes as the voltage is tuned.

Note: It is worth experimenting with the voltage setting. Many labs find they get best end results by selecting a voltage which initially has a relatively low percent capture (50% or so) following the theory that this method pre-selects the healthiest cells and avoids the damage to them caused by excessive voltage. The different configurations of the C-Dishes will need different voltages. For MyoCytes, the four well dishes will need voltages in the 32-40 range, the 35 mm discrete dishes about 8-10V, the 6 well multidishes about 10-14V and the 8 well dishes about 16-20V. Watching the cells with a microscope is the best way to select the appropriate voltage for your cells.

Line 5: Enable/Disable Status



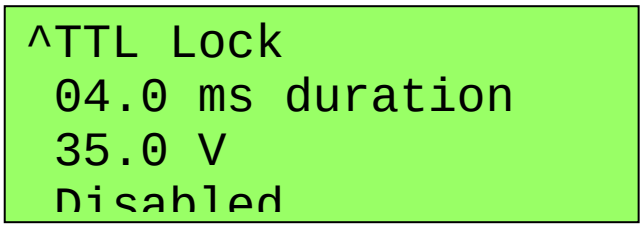
This setting initiates and ends the pulse trains. When a bank is Disabled, reed-relays in the high voltage path are opened, ensuring complete open circuit. These relays are also opened between pulses.

Frequency Control

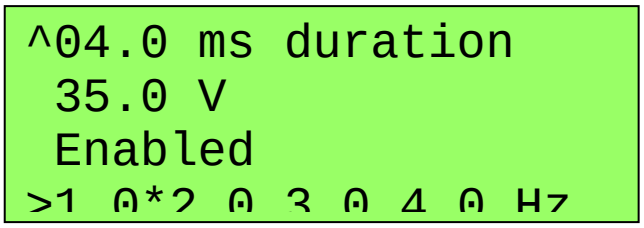
Lines 6-10 vary, depending on the mode of operation selected in line2.

TTL Lock

In this mode, all frequency control is external, so these lines are not present.

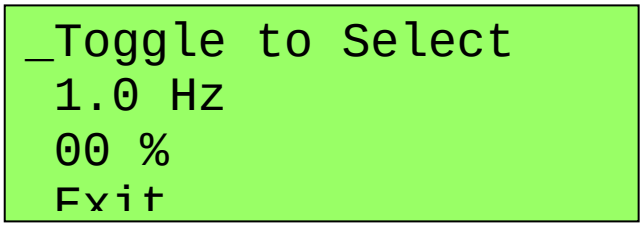


Basic



In Basic Mode, line 6 is the last line and consists of 4 frequencies in Hz written side by side. An asterisk is written after the frequency that is currently activated. When the knob is turned, the cursor will scroll horizontally through this line. Double click to activate the frequency, or single click to enter the Basic Edit Menu.

Basic Edit Menu



First Line Click to Activate this frequency and return to main menu.

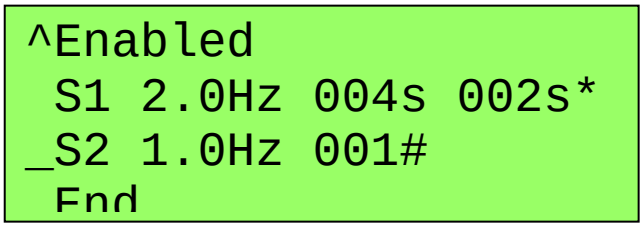
Second Line Turn the knob to edit the frequency. There is a coarse and fine adjust. If the edited frequency is the one activated, the banks will reflect the changes as the frequency is tuned. The frequency has a range of .01 Hz to either 99 Hz, or the maximum possible given pulse duration, number of channels, and the variability setting (max frequency including variability = channels * (duration +.5 ms)).

Third Line This line sets the maximum variability of the pulse train. For most purposes, this should be set to 00%. The maximum is determined by pulse duration, number of channels, and the frequency setting. For irregular pacing, please see the Irregular Pacing section.

Forth Line Exit returns you to the main menu without activating the frequency

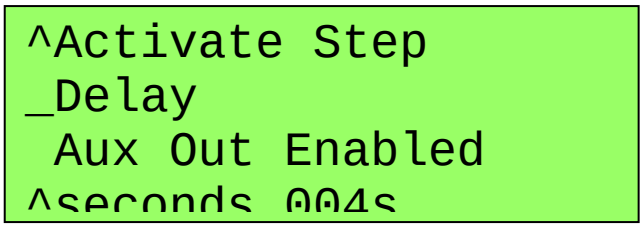
Sequence Edit Menu

Sequence



Sequence mode offers the user the ability to program multistep (up

to 5 steps) protocols. The main menu presents a summary of the protocol and displays the current timer. Scroll the cursor to the line to be edited and click to enter the Sequence Edit Menu. The example above shows a two-step protocol. Pulses are output for 4 seconds at a rate of 2.0 Hz, a single 1.0 Hz pulse is output, and then the sequence ends, at which point the pulse train will loop back to the beginning. A continuous pulse train with an offbeat every 5 seconds is the result. The "002s" marker in the upper right reflects that there are 2 seconds still remaining in step S1. The asterisk at the end of S1 indicates that a TTL pulse will be output on the "Aux Out" BNC on the first pulse of the step if the gates are locked to that bank. (See Gate Selection)



First Line Click to Activate this Step and return to main menu.

Second Line There are three step types that can be selected on this line.

Pulse Train Indicates a series of High Voltage pulses of a specified frequency will be output for the duration of this step.

Delay Indicates the High Voltage outputs will remain off for the duration of this step.

End Indicates that the protocol has ended and a return to the beginning of step 1 will immediately follow.

Third Line

End In the case that "End" was selected on the second line, the third line is the last line of the edit menu and simply Exits back to the main menu.

In all other cases

The "Aux Out" output is enabled or disabled on this line. If enabled, a TTL pulse will be output at the beginning of this step IF the gate selection knob has been adjusted to select the bank (See Gate Selection). In the case of a pulse train, the TTL pulse will occur in sync with the first pulse of the well the gate is locked to. In the case of a delay, the pulse will go high at the beginning of the delay, and remain high until the delay step has ended.

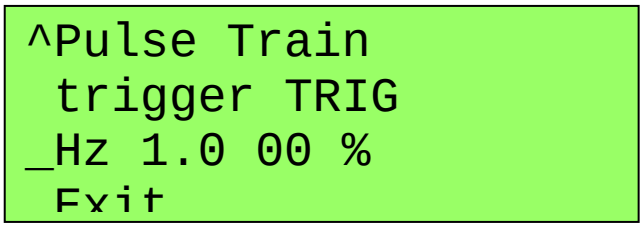
Forth Line The method of determining the duration of the Step is set on this line. There are four options: number of minutes, number of seconds, number of pulses, and trigger.

Minutes and Seconds

Set period of time on this line. An internal timer will count down the designated period of time. The output pulses train will finish with the current pulse before switching steps. (i.e. If the frequency is 0.5 Hz and the timer is set for 3 seconds, the second pulse will take an additional second before the next step is initiated to make a total of 4 seconds. The timer does move to the next step and will begin counting down independently of when the frequency change has actually occurred.)

Pulses Pulses is only an option if the step type is a pulse train. Set number of pulses on this line. In this case, the number of pulses output by the bank is counted down. Note: The pulse occurs at the beginning of the step, followed by the rest of the period. This exact definition is important to the number of pulses that needs to be set and to the interaction with TTL IO. (Example 1: In the case of a protocol ending in a single off beat followed by a delay, two pulses actually have to be specified because the change of period occurs after the first pulse. In the case of the pulse followed by a delay, the period in effect just adds to the delay. Example 2: If the "Aux Out" is enabled in a single offbeat situation, it will fire on the pulse before the offbeat, not following.)

Trigger This setting means that the step will continue until a TTL pulse is input to the "Advance In" BNC or until the user manually double clicks on a step line to use the "Activate Step" function. The output pulse train will finish with the current pulse before switching steps. This means that the first pulse of the next step will not actually be synchronized with the "Advance In" input, but will occur at the end of the period of the previous pulse. There are a few reasons to use this option. It allows an external source to trigger a step change. It also allows an easy way to manually control the switching of steps. By making the last step of a protocol a Delay with a triggered duration, it presents a method of going through the protocol only once, instead of looping back to the beginning.

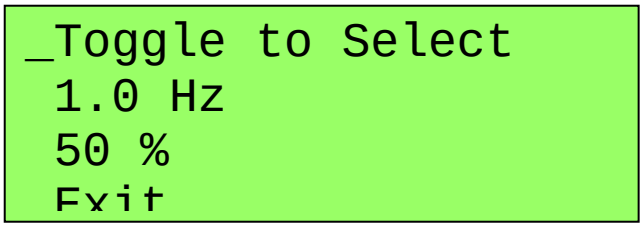


Fifth Line In the case of a Delay, the fifth line simply Exits back to the Main Menu. In the case of a Pulse Train, the fifth line is where the frequency is set. In a sequence the frequency may be set either by frequency or period by selecting either Hz or s. Then, turn the knob to edit the frequency/period. There is a coarse and fine adjust. If the edited frequency is the one activated, the bank will reflect the changes as the frequency is tuned. This line also sets the maximum variability of the pulse train. For most purposes, this should be set to 00%. For irregular pacing, please see the Irregular Pacing section.

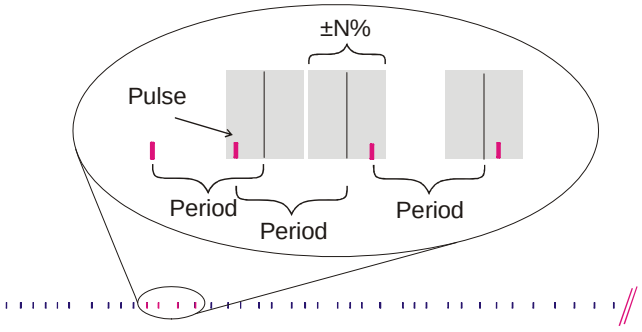
Sixth Line Exit returns you to the main menu without activating the frequency

Examples of Extra Features in the EP version

Irregular Pacing



The irregular pacing feature produces pseudo-random variation of a specified frequency within a definable percentage window and guarantees average effective rate over 100 pulses. For example, if a frequency of 1 Hz with 50% variability is selected, 100 pulses will have occurred after 100 seconds, but the period between any two pulses can be anything from 500 ms to 1500 ms. In actuality, the basis for the variability is a list of numbers from 1 to 100 that were randomly shuffled and saved. Therefore, the pulse train will repeat every 100 pulses, and the same settings will always produce the same pulse train.



Arrhythmia Protocols

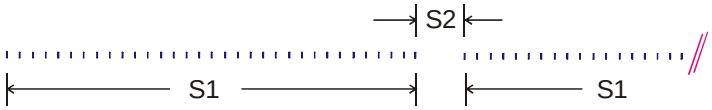
Insert an offbeat pulse at a fixed interval

```
^Enabled
 S1 2.0Hz 004s 002s
_S2 1.0Hz 001#
 End
```

Define step one of the sequence to be a pulse train with desired frequency and length.

Define step two of the sequence to be a pulse train with desired frequency and duration of one pulse.

Define step three of the sequence to be the End of Sequence.



Exercise Protocols

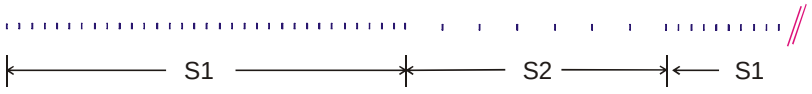
Run multiple pulse trains with individually programmable frequency and duration.

```
^Enabled
 S1 2.0Hz 060m
_S2 1.0Hz 660m 212m
 End of Sequence
```

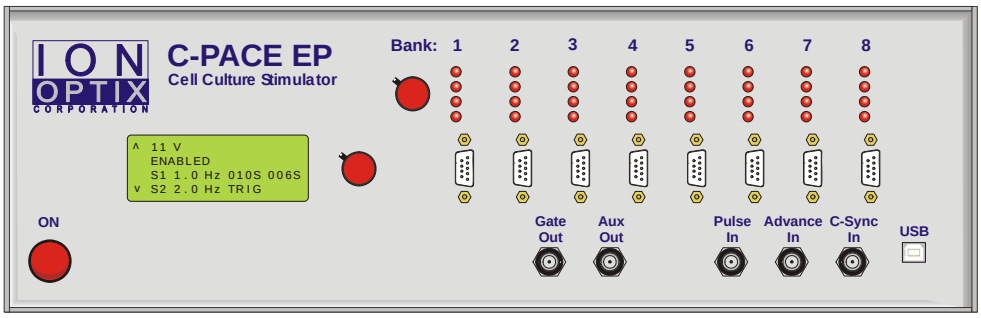
Define step one of the sequence to be a pulse train with desired frequency and length.

Define step two of the sequence to be a pulse train with desired frequency and length.

Define step three of the sequence to be the End of Sequence.



Examples Using the Digital Inputs and Outputs



Pulse In: Synchronize all Banks to an External TTL pulse train

```
^TTL Lock
 04.0 ms duration
 35 V
 ENABLED
```

Set all banks to TTL Lock and make sure the voltage is set and the banks enabled.

Connect a TTL signal into the “Pulse In” BNC. The rising edge of a pulse will initiate the high voltage pulses. There is a 450 (+/-10) usec delay before the first well outputs its pulse and between the well’s pulses due to the closing and opening of the relays. All banks will begin firing at the same time.

Advance In: Initiate an Event with an External TTL

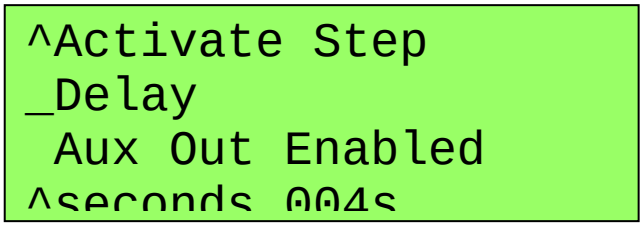
```
^Enabled
_D1        TRIG TRIG
 S2 1.0s   002#
 End
```

The settings shown above create a situation such that no high voltage output pulses will fire until the rising edge of a TTL signal has been sensed at the “Advance In” input. One pulse will be fired beginning 450 (+/-10) usec after this rising edge, and a second pulse will fire one second later. The pulse train will then remain disabled until another TTL signal is sensed.

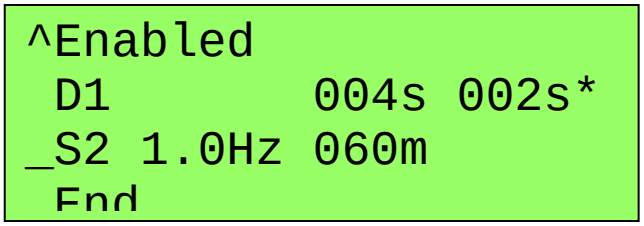
Gate Out: Place Marks in Data Acquisition

The Gate Out will output a TTL concurrent with each high voltage pulse (the rising edge is at the beginning of the pulse, and the falling edge is at the end of the pulse) that is output to the selected well. Select a well by turning the encoder next to the pacing LEDs until the green light is on the desired well.

Aux Out: Control an External Piece of Hardware



Select "Aux Out Enabled" on the desired step.



The Aux Out feature will be marked with an asterisk on the main page.

Turn the Encoder next to the Pacing Indicator LEDs until the green light is on the desired well.

A TTL signal will now be output on the "Aux Out" BNC once an hour. The rising edge will correspond to the beginning of the delay, and the falling edge to the end of the delay, in this case creating a 4 second pulse. If the Aux Out had been enabled on a Pulse Train step, the output pulse would be concurrent both in initiation and duration with the first pulse of the step.

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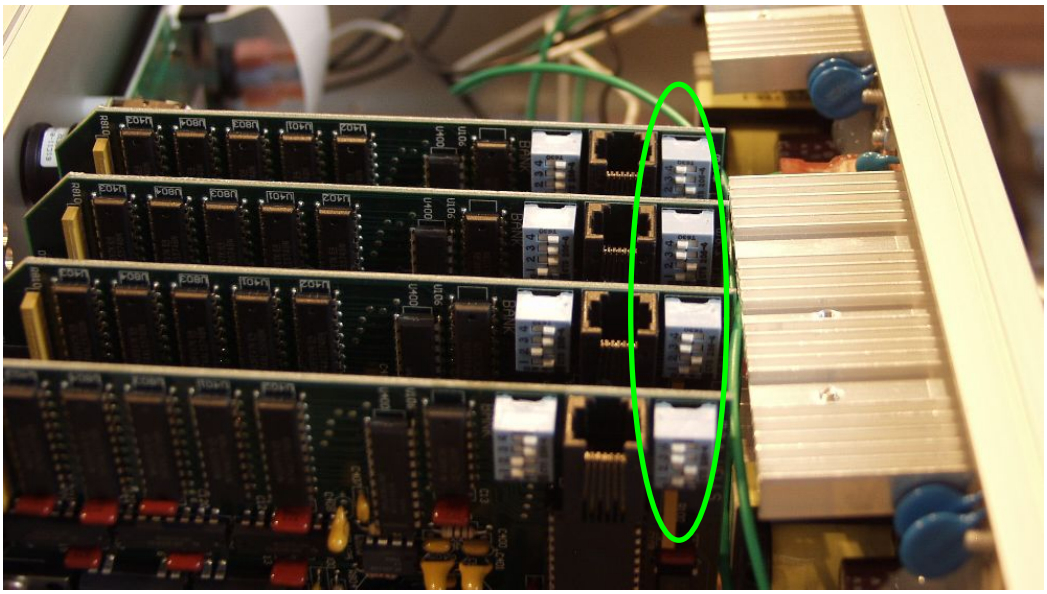
Tech Note: Changing number of wells on a C-Pace or C-Pace EP



UNPLUG DEVICE! Use a Phillips screwdriver to take the top off the C-Pace or C-Pace EP. You will see the High Voltage Banks mounted vertically. The dip switch closest to the edge of the boards and labeled “WELLS” (circled above in green) sets the dish type the bank is configured to drive. You will see that the settings on the switch correlate to the number labeled on the circuit board.

	Number on Dip Switch			
Number of Wells	**1**	**2**	**3**	**4**
4	OFF	ON	ON	ON
6	ON	OFF	ON	ON
8	ON	ON	OFF	ON

Upon power up, the LEDs will cycle through. If each LED lights just one time, that bank is configured for a four well. If they each light once, and then two more patterns occur, it is configured for a six well. If they each light once, and then four more patterns occur, it is configured for an eight well. If you have a C-Pace EP, you can use the second encoder (changes the IO locking) to scroll through more slowly.