USER'S GUIDE

C-Pace EM 100

Manual Revision 1.6
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ION OPTIX

Disclaimer: This is an electrical device. There is an inherent risk of electrical shock if used improperly. Please take the necessary precautionary measures that are commonplace for any electrical device. It is solely intended for the applications outlined in this document. The manufacturer is not liable for any misuse, or any injury incurred because of misuse.

Always unplug device before checking fuse!

Disclaimer: This product is intended for research purposes only. It is not certified for clinical applications (including diagnostic purposes). Use of this product in uncertified applications is in violation of FDA regulations.

Contents

1 Introduction

The C-Pace EM is the third generation of the IonOptix C-Pace line of multi-channel stimulators, the premier chronic cell culture stimulation device on the market. With each generation, IonOptix has strived to create more innovative and effective devices to prevent the dedifferentiation of fully committed adult cardiomyocytes and other tissues while in culture. Now with the C-Pace EM, we have added features for stem cell biologists who want to incorporate electrical and mechanical stimuli into their stem cell differentiation protocols.

The C-Pace EM provides all the various electrophysiology features such as frequency variation, insertion of periodic off beats, and programmable frequency changes that were available in the earlier C-Pace EP. Likewise, it still accepts TTL triggering and provides TTL outputs for integration with other devices.

However, the C-Pace EM is now equipped with new, enhanced high voltage output banks. These banks continue to allow the stimulation pulse output voltage (+/- 40 V), frequency (0.010 to 99 Hz), and duration (0.4 ms to 10 ms) to be easily adjusted from the front panel and saved in non-volatile memory for quick start-up like the C-Pace EP. But in addition, the banks on the C-Pace EM also allow the instrument to control the new C-Stretch module. With eight independent high voltage banks, the C-Pace EM can be configured with any combination of eight different C-Dish or C-Stretch modules, all of which can be controlled from the front panel and the new C-Pace Navigator software as described below.

1.1 C-Dishes

The C-Pace EM will drive our full family of C-Dish electrode assemblies. The enhanced high voltage banks allow use of the 24 well dishes without modifying the front panel as was required with the C-Pace EP. The C-Dish electrode assemblies are available for most popular tissue culture plate brands in the 4, 6, 8, 12, and 24-well formats. This permits chronic electrical stimulation in a variety of different applications.

1.2 C-Stretch

In addition to our current line of C-Dish products, the C-Pace EM supports a completely new “dish” type, the C-Stretch. The C-Stretch permits exploration of electrical and mechanical stimulation in a single device. It has been designed to house cell cultures within specialized PDMS chambers. These chambers can be mechanically stretched up to 20% while being simultaneously electrically paced. Like the C-Dish, the C-Stretch uses carbon electrodes to minimize electrolysis byproducts and can be paced with all the familiar biphasic waveform parameters.

With the C-Stretch, investigators can add a mechanical component on top of the electrical pacing for customized exercise, arrhythmia, or any other specialized protocol. The timing of the electrical pulse can be offset from the stretch protocol and frequency of the combined electromechanical waveforms can be randomized to model pathophysiologic conditions such as arrhythmia.

1.3 C-Pace Navigator

The complete system includes the C-Pace Navigator software which features a user-friendly GUI designed to manage protocols directly from any Windows computer equipped with a USB port. Any changes made on the software are reflected immediately on the front panel display, while changes to the front panel interface will be updated in the C-Pace Navigator software.

1.4 Features

- The C-Pace EM has been designed to operate the C-Stretch which is capable of combined electromechanical stimulation of up to six individual flexible culture wells.
- The EM version maintains all the functionality of the EP version, including the ability to operate C-Dish electrode assemblies.
- The C-Pace Navigator software provides a user-friendly GUI to easily manage stimulation protocols directly from a Windows PC.
- Any changes made on the software are reflected immediately on the C-Pace EM display, and vice versa.
- Fully programmable multi-step sequence protocols can have up to five different pacing and stretching steps – useful for exercise protocols.
- Fully customizable trapezoidal stretch waveform with adjustable expansion, holding and contraction phases – useful for constraining systolic and diastolic dwell times.
- Program electrical pulse to occur at any time during stretch protocol, including with randomized variability – useful for mimicking arrhythmia.
- Multiple temperatures are reported in real-time to assess device and cell health.
- Carbon electrodes and biphasic pulses minimize electrolysis byproducts in the culture media.
- Thin ribbon cables connecting the C-Pace EM and C-Dish or C-Stretch ensure incubators maintain a solid seal.
- Short circuit and current limit protection of electronics.
- Reed relays prevent leakage current passing into wells.

1.5 Specifications-at-a-Glance

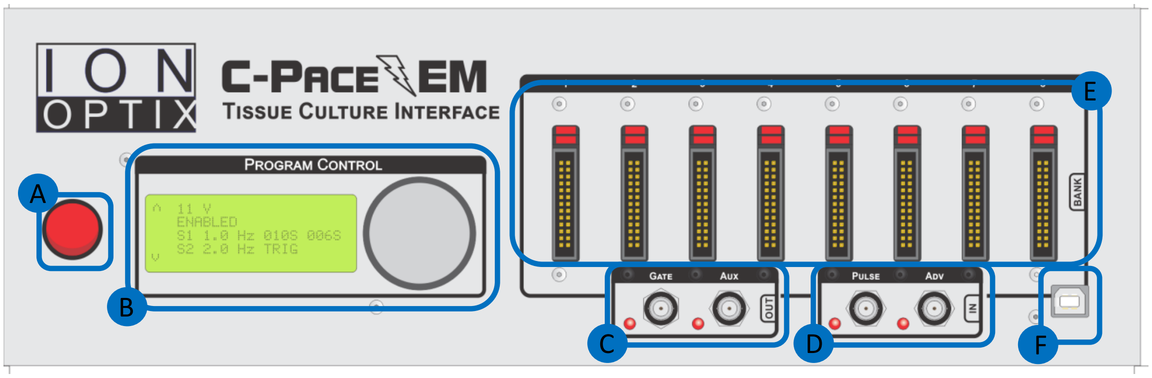
- **Digital adjustment of electrical pacing parameters:**
    - Pacing frequency: 0.01-99Hz
    - Pulse duration: 0.4 - 10 ms
    - Voltage: up to +/- 40 V
    - Current up to 240 mA for C-Dish and 1000 mA for C-Stretch
    - TTL input / output logic for integration with experiments
- **Digital adjustment of mechanical stretching parameters:**

    - Loading frequency: 0.1 to 10 Hz
    - Stretching range: 0 - 20%
    - TTL input / output logic for integration with experiments
- **Management of protocols available in the software:**
    - Pulse offset as a percentage of any mechanical phase
    - Programmable ratio of pulse to stretch
    - Randomized variability of stretch as a percentage of frequency
    - Real-time seven-point reporting of temperature

2 Hardware

The following sections describe the hardware aspects of the C-Pace EM. The menus and programming of the device are discussed in section *3 Software: LCD Menus* below.

2.1 Device Front Panel



Above is a figure showing the front panel of the C-Pace EM. There are a number of individual areas highlighted in the figure:

- **A.** Power Switch
- **B.** LCD Interface & Encoder Knob
- **C.** Digital Outputs
- **D.** Digital Inputs
- **E.** High Voltage Banks
- **F.** USB Port

Each element of the front panel is further discussed below.

2.1.1 Power Switch

Power to the C-Pace EM is turned on and off with the switch (A) 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. If the LCD does not light up, or there appear to be other issues, please refer *Appendix 1: Troubleshooting* below.

2.1.2 LCD Interface & Dial Encoder

Menu choices and stimuli parameters can be selected via the LCD screen and the **LCD Encoder**, located directly next to the display. To permit one-handed adjustment, the encoder knob can be turned as well as clicked. In general, turning the encoder will perform a scroll function and clicking the dial will perform a select function. Complete documentation on programming the device starts in section *3 Software: LCD Menus* below.

2.1.3 Digital Outputs

There are two digital output BNC connectors available on the C-Pace EM, **Gate Out** and **Aux Out**. These outputs permit synchronizing other devices to the C-Pace EM via TTL signaling that are timed with the electrical pacing. Both the **Gate Out** and **Aux Out** digital outputs are ultimately synchronized to a single well from a single bank. That well is termed the **Sync Channel**. This well is controlled by the **Sync Channel** setting (see section *3.1.7 below*).

2.1.3.1 Gate Out

This TTL output will emit a pulse exactly matching the duration of the *entire* high voltage output pulse seen by the **Sync Channel**. The rising edge of the **Gate Out** 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 **Gate Out** BNC connector will light up for the duration of the **Gate Out** pulse. Thus if the **Sync Channel** is set to Well 1, then the **Gate Out** will emit a pulse every time Well 1 sees a stimulation pulse.

2.1.3.2 Aux Out

This TTL output is only used in **Sequence Mode** (see sections *3.1.2 Operating Mode* and *3.3 Sequence Edit Menu* below) to indicate the beginning of a sequence step. It must be enabled in the

Sequence Edit Menu of the desired step via the **Aux Out Enabled** setting as described in section <u>*3.3.3 Aux Out Enable below*</u>. The exact behavior of the **Aux Out** signal depends on whether the sequence step is a **Pulse Train** or a **Delay**.

If the step is a **Pulse Train**, the **Aux Out** pulse will occur in sync with the high voltage stimulus pulse applied to the **Sync Channel** in that step. It uses the same logic as the **Gate Out** signal so you will see that the **Aux Out** matches **Gate Out**, but only for the first pulse in the step.

In the case of a **Delay** step, the **Aux Out** pulse will go high at the beginning of the delay and remain high until the delay step has ended. Thus, if the delay is for 10 seconds, the **Aux Out** pulse will also be 10 seconds long.

In both cases, the LED next to the **Aux Out** BNC connector will light up for the duration of the **Aux Out** pulse.

Note that because the **Aux Out** output uses the **Sync Channel** setting to determine exactly when to emit its TTL pulse, it is very important that the **Sync Channel** is actually in a bank that is using **Sequence Mode**. Otherwise you will never see pulses. Of course, if there are multiple banks running in **Sequence Mode**, only the one containing the **Sync Channel** will generate **Aux Out Pulses** even if other banks have **Aux Out Enabled** steps.

2.1.4 Digital Inputs

The C-Pace EM has two available digital input BNC connections, **Pulse In** and **Advance ("ADV") In**. These inputs permit other devices to control aspects of the C-Pace EM behavior. These inputs are dedicated to specific purposes as described below.

While these inputs will only have an effect if specifically enabled in the pacing protocol, they can be used simultaneously. For example, one group of banks could be configured to use **Pulse In** and a second group could be configured to use **Advance In**.

2.1.4.1 Pulse In

This TTL input 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 occurs approximately 600 µs after the rising edge of the **Pulse In** input. The LED next to the **Pulse In** BNC connector will light up during TTL high.

2.1.4.2 Advance In

This TTL input is only used in **Sequence** mode. It is used to end a sequence step that is being timed with the **Trigger** option. This works with either a **Pulse Train** or a **Delay** step.

The rising edge of a pulse on this input will end the current step and start the next step in the sequence if the trigger option has been selected in the **Sequence Edit Menu**. If the step is a **Pulse Train**, the high voltage pulse train will finish with the current pulse before switching to the next step. 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 **Advance In** BNC connector will light during TTL high.

2.1.5 High Voltage Banks

The C-Pace EM supports up to eight independent high voltage banks (HVB). The HVBs can drive either the C-Dish electrode assemblies or the C-Stretch module. The device will automatically recognize whether the C-Dish or the C-Stretch is connected upon initialization.

Note: If you are using C-Dish assemblies with the 10 pin connector, you will need to manually set the C-Dish configuration for each bank as described in *Appendix 3: Changing the HVB Channel Count* below.

Each HVB has a voltage range of up to +/- 40 V and high current amplifier that generates the stimulation pulse. The amplifier current limit is programmable and is set to 240 mA for C-Dish banks and 2 A for C-Stretch banks. These output current limits have been shown experimentally to be sufficient for all standard C-Dish and C-Stretch configurations.

Each HVB has a series of reed relays interposed between the amplifier and the attached C-Dish/ C-Stretch. These relays multiplex the stimulation pulse onto different physical stimulation circuits and assure zero leakage current when in the off state.

The exact manner in which the stimulation protocol is delivered to each well in a culture dish depends on the dish itself. In some cases, each well is individually stimulated in sequence (e.g. 4 well C-Dish). In other cases, groups of wells are stimulated in parallel, group by group, until all the wells have been stimulated (e.g. high well count C-Dishes). Lastly, the C-Stretch has all 6 wells stimulated at once in parallel.

This fact is important to consider if you are trying to synchronize well stimulation to other events because the well you are looking at may or may not be stimulated at the same moment as other trigger signals in the system.

2.1.5.1 Connectors

The C-Pace EM HVB uses a 26-pin ribbon cable connector to drive all C-Dish / C-Stretch configurations.

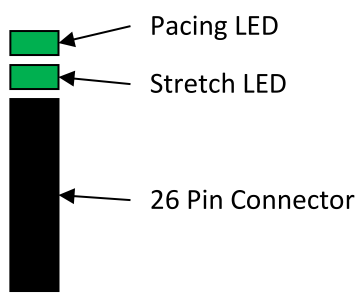
All C-Dishes have a 10-pin connector; thus you will need a 26 to 10 pin ribbon cable when attaching C-Dishes to a C-Pace EM.

For the C-Stretch, there is a small “dongle” box that has a 26-pin connector on one side and a 24-pin connector on the other. You will need two cables in this case, a 26 to 26 pin ribbon cable and a 24 to 24 pin ribbon cable.

In all cases, the cabling is a ribbon cable thin enough to maintain a solid seal when the incubator is closed. If you are unsure whether you are using the correct cable, contact IonOptix for assistance.

2.1.5.2 Indicator LEDs

The block of two LEDs above each connector is the **Indicator LEDs**. The top LED, the **Pacing LED**, is used to indicate the actual electrical pacing. The bottom LED is the **Stretch LED**. It is used to indicate the mechanical stretching. These two LEDs can also blink together to indicate error states.



2.1.5.2.1 Pacing LED

The **Pacing LED** is used to indicate the operating status of the high voltage stimulation output. In all situations, the **Pacing LED** will turn green when a well is being paced. The LEDs are programmed to match the frequency of the electrical pacing. Thus, if you are driving a 4 well dish, you will see 4 rapid blinks on each stimulation cycle. Moreover, the length of the blink is determined by the stimulation pulse width.

The **Pacing LED** is also used to indicate any warnings or errors that the stimulation circuit detects as shown in the following table.

Condition	Pacing Indicator Blinking	Background
Normal Operations		
Current Limiting		
Short Circuit Condition		blinking

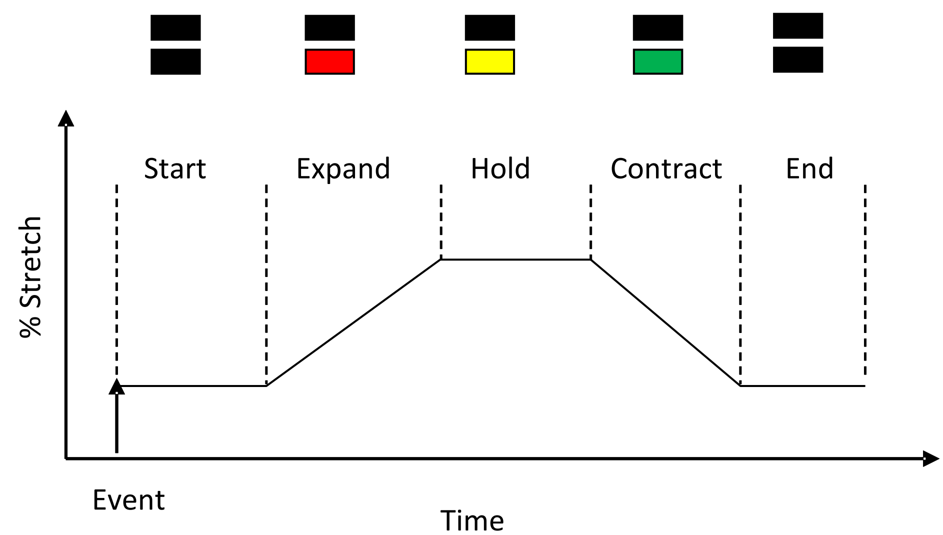
In the normal operating condition, the green blinking that indicates an active high voltage output happens against a black (i.e. unlit) background.

However, if the circuit is *current limiting*, the background will become yellow. That is, the normal state of the LED will be yellow with it still blinking green when the high voltage stimulus pulse occurs. In this state, the system will be delivering a lower voltage than the user has programmed. The system will remain in this state until the resistance of the bath is increased (usually by reducing the solution level) or the voltage is lowered such that the current requirement is below the limit.

If the system detects a *short circuit* the green blinking will be shown on top of a blinking red background. The background will be blinking once per second regardless of the pacing frequency. This will continue until the short circuit condition is removed.

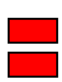
2.1.5.2.2 Stretch LED

The **Stretch LED** is used to indicate the mechanical stretching. The LED will turn red to indicate the chambers are being expanded, yellow to indicate holding the stretch, and green to indicate contraction, and will turn off in the start and end phases.



2.1.5.2.3 Fatal Error

If both the **Pacing LED** and the **Stretch LED** turn solid red, the bank has encountered a fatal error. This is usually caused by a component on the board going bad, or by the board overheating. Please power cycle the device; if the red LED is still present, please contact IonOptix at **info@ionoptix.com**.



Fatal Error Indication

2.1.6 USB Port

Any Windows-based laptop equipped with a USB connector may connect to the C-Pace EM via the USB port. The C-Pace Navigator software allows the user to manage the stimulation protocols via a user-friendly graphic user interface (GUI). The software can control any aspect of the device that can be controlled via the front panel in addition to being able to save entire device protocols to file and load them back into the device. See the C-Pace Navigator manual for more information.

2.2 Device Back Panel

2.2.1 Power inlet

The use of an IEC 60320 C14 inlet which permits the C-Pace EM to be used globally with the appropriate local power cable. The power supplies are all wide input and can accept voltages from 85 to 265 V AC.

2.2.2 Fuse

A compartment in the power entry module holds two active 3 Amp/250V, 5x20mm slow blow fuses. You must unplug the power cord in order to access the compartment.

Always unplug the device before checking the fuse!

2.3 Device Expansion (adding HVBs)

The C-Pace EM can be configured with up to eight HVB driver boards. Each driver board plugs into a slot on the main board. There is no configuration needed to add an HVB to a C-Pace EM chassis.

If you are adding an HVB to an old C-Pace EP chassis, you will need to program the desired slot ID into the HVB before plugging it in to the main board. Programming the ID is explained in *Appendix 3: Changing the HVB Channel Count* below.

In either case, plugging the driver board into the main board is a straightforward process but requires a partial disassembly of the device.

2.4 Timing Details

To be able to use the C-Pace EM effectively it is important to understand how the waveforms are generated with respect to timing. This is particularly important when using the C-Stretch module or attempting to synchronize multiple banks to each other or to other devices.

2.4.1 Pacer Events

The pacer **Event** is the fundament timing signal used to trigger all voltage and stretch waveforms. This signal can be generated either internally, or externally via the TTL Lock operating mode. In either case, this signal is the “time zero” for both voltage and stretch waveforms. Thus, when you program a frequency via one of the LCD menus, you are programming the **Event** frequency.

The term **Event Window** describes the span of time between two **Events**. It defines the amount of time to execute a waveform and is used to determine the exact timings of a stretch waveform.

2.4.2 Synchronization

Each HVB has its own dedicated microcontroller. This processor controls both the voltage waveform and the stretch protocol. The HVB microcontroller has a special architecture that permits extremely accurate timings to be achieved on each individual bank. Because each bank is individually programmable and controlled by its own microcontroller it is, in general, not possible to have multiple banks run in complete synchronicity even when running the same protocol.

The main C-Pace EM chassis also has its own microcontroller. This processor responds to user input and issues commands to the high voltage banks. In particular, it issues the commands to switch between sequence steps. This process is less accurate than the individual waveforms generated by each bank. As a result, you may notice some very small jitter (microseconds) if you were to look on an oscilloscope.

If you wish to have reasonably precise synchronization between banks in terms of **Event** frequency, you should use TTL Lock mode. This will let you control the **Event** frequency externally and all banks will be triggered at the same time.

If you wish to synchronize sequences, you should use the ADV input.

2.5 Voltage Control

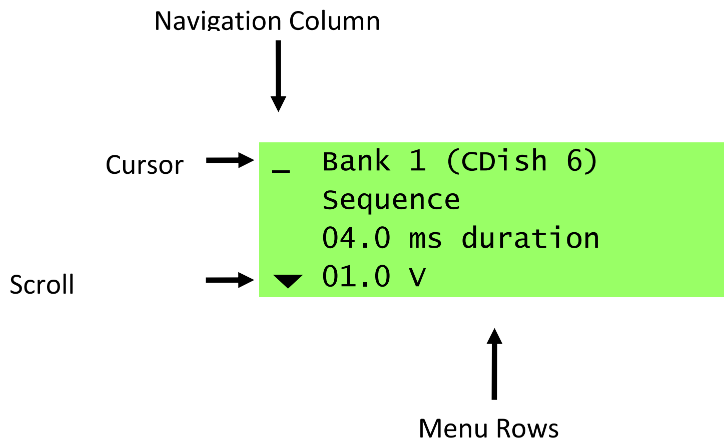
This device does produce high voltages. We take measures to assure that high voltage is not present on any output at unexpected times. All high voltage lines are run through normally-open reed relays. This assure that the banks are disabled on power off to eliminate the chance of undesired high voltage pulses on power on. These relays also ensure a true open circuit between pulses to eliminate leakage currents.

Furthermore, the new HVB design now includes real-time programmable current limiting. Each HVB constantly monitors the output current and clips the voltage when it detects that the current has exceeded the limit. It also detects short circuit conditions and can withstand this condition indefinitely. The front panel LEDs for each HVB are used to indicate the current limit and short circuit conditions so that the user can take appropriate action. This is a major improvement over the C-Pace EP which did not have any indication of entering the current limiting state.

3 Software: LCD Menus

This section describes the front panel menus. As mentioned above, all programming functions outlined below can be controlled via the C-Pace Navigator software as well.

There are several different menu screens in the C-Pace EM and all operate on a similar principal. Below is a sample menu.



To edit an element on a given row, first turn the **Menu Encoder** knob to move the cursor down the **Navigation Column** to the row in question. Push the knob to select and move the cursor into the row.

Once a row is selected, the first element is typically automatically selected for editing. Turn the **Menu Encoder** knob to change the value of the element. If there is more than one editable element on a row, click the knob again to move to the next element. Clicking the knob when the cursor is on the last element will move it back out to the **Navigation Column**.

While the LCD has only 4 lines of text, the menus can be of arbitrary length. When the menu is longer than 4 rows, black triangles will show the directions the cursor can be moved to scroll up or down. Below are three figures illustrating a six-row menu and how the scrolling works. The green rows represent the rows of the menu currently displayed on the LCD, while the gray lines represent the “hidden” lines. Arrows at either the top or bottom of the **Navigation Column** indicate that more menu is available.

Top of the Menu	Middle of the Menu	Bottom of the Menu
Line 1 Line 2 _ Line 3 ▼ Line 4 Line 5 Exit	Line 1 ▲ Line 2 Line 3 _ Line 4 ▼ Line 5 Exit	Line 1 Line 2 ▲ Line 3 Line 4 _ Line 5 Exit

When a triangle disappears, there are no more rows of the menu available in that direction.

As a small aside, notice that the figures above show **Exit** as the last line in the menu. This will be the case for all menus except the Main Menu. Selecting the **Exit** row will always return to the previous menu.

3.1 Main Menu

_ Bank 1 (CDish 6)	Line 1: Bank Selection
Sequence	Line 2: Operating Mode
04.0 ms duration	Line 3: Stimulus Pulse Duration
▼ 01.0 V	Line 4: Stimulus Voltage
Temperature	Line 5: Temperature Menu
Disable	Line 6: Bank Enable/Disable
Sync Chan: None	Line 7: Sync Channel
Mode/Stretch Option(s)	Line 8(+): Mode or Stretch Specific Options
Bank Options	Last Line: Bank Options Menu

When the device starts you will see a menu like the above example. This is the **Main Menu** for a bank, in this case **Bank 1**. Each bank has its own menu to set the stimulation parameters for that bank. The majority of the options are set via this **Main Menu**. The length of the main menu will change depending on the bank configuration (C-Dish or C-Stretch) and the mode settings for the bank. However, the first six lines are always the same.

The stretch specific options that are available for C-Stretch configurations are tightly integrated into the mode options. The stretch options are by-and-large identical regardless of the mode and, therefore, are treated as a group later in section *3.4 Stretch Menu Options* below.

3.1.1 Bank Selection

```
_  Bank 1 (CDish 6)
   Sequence
   04.0 ms duration
▼  01.0 V
   Temperature
```

The first line in the Main Menu indicates the current bank, that is, the bank being edited. To select the current bank, move the cursor to the first line and click. This will move the cursor under the '**B**' in **Bank**. Turn the knob to scroll through available banks. Click again to select a bank. The cursor will move back to the first column. The type of attached dish, either C-Dish or C-Stretch, will appear in parentheses next to the bank number. If it is a C-Dish, it will further indicate the well count. This helps to differentiate one bank from the next and is also useful for troubleshooting purposes.

Note: If a bank is not showing up, it means the main board hasn't been able to communicate with it. See *Appendix 1: Troubleshooting* for more information.

3.1.2 Operating Mode

```
   Bank 1 (CDish 6)
_  Sequence
   04.0 ms duration
▼  01.0 V
   Temperature
```

The second line of the Main Menu indicates the Operating Mode of the current bank. There are 3 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 Event Pacing** (see *4.1 Irregular Event Pacing*) is available in this mode.

Sequence — The **Sequence Mode** should be selected if the user wishes to make programmable frequency changes, including single off beats, 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 Event Pacing** is also available in this mode (see *4.1 Irregular Event Pacing*).

TTL Lock — In this mode, every TTL pulse on the **Pulse In** input will initiate a pacer **Event**. This makes the bank a complete slave to the device that is driving the **Pulse In** input.

Note: This mode is not available for C-Stretch Banks.

There are two other options in the **Operating Mode** setting that are not really modes but rather are commands that will set the mode.

Re-Initialize This option will reload factory defaults of all bank values. The factory default is Basic Mode.

Copy Previous Bank This option saves the user from having to re-enter 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. As described in section *2.4.2 Synchronization* above, each HVB runs from its own clock. Thus bank to bank synchronization is not possible without using TTL Lock.

Note: Stretch parameters will not be copied to a bank with a C-Dish. However, pacing and mode parameters will be copied.

To lock two or more banks together:

- Set the **Sync Channel** to the first channel in the master bank (see *2.1.3 Digital Outputs* above),
- Set the slave bank to **TTL Lock**
- Connect a BNC cable between **Gate Out** and **Pulse In.**

There will still be a small delay (450 (+/-10) µsec) between master and slave.

Note: If you only have one bank, this command will not be present.

3.1.3 Stimulus Pulse Duration

```
  Bank 1 (CDish 6)
  Sequence
  04.0 ms duration
▼ 01.0 v
  Temperature
```

The third line in the Main Menu determines the duration of the biphasic stimulation pulse. The duration of the pulse and can be adjusted between 0.4 and 10 ms for all bank configurations (C-Dish or C-Stretch). The upper range of the duration is dependent on the current pacing frequency, variability settings, and well count and is recomputed when any of those settings change. The bank will reflect the changes as the duration is tuned.

Note: To reduce electrolysis byproducts, the duration should be kept as short as possible. Standard values are in the 2 - 10 ms range.

3.1.4 Stimulus Voltage

```
▲ Sequence
  04.0 ms duration
  01.0 V
▼ Temperature
  Disabled
```

The fourth line in the Main Menu determines the electrical stimulation pulse voltage. The voltage can be adjusted from 0 – 40 V. To reduce electrolysis byproducts, the pulses that are emitted are all square biphasic pulses, so half of the chosen duration will be positive, half negative. There is a coarse adjustment of 1 V and a fine adjustment of 0.1 V that can be selected by clicking the encoder to move to each decimal place. If the pacing is enabled while tuning the voltage, the banks will immediately reflect the changes.

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 require different voltages. For adult cardiomyocytes, the four well dishes will need voltages in the 32 - 40 V range, the 35 mm discrete dishes about 8 – 10 V, the 6-well multi-dishes about 10 – 14 V, and the 8-well dishes about 16 – 20 V. Observing the cells with a microscope is the best way to select the appropriate voltage.

3.1.5 Temperature

```
▲ 04.0 ms duration
  01.0 V
  Temperature
▼ Disabled
  Sync Chan: None
```

The fifth line in the **Main Menu** takes you to the **Temperature Menu**, illustrated below.

```
  HVDrv-P : 35.6
  HVDrv-N : 37.3
  HVxB   : 30.8
  Exit
```

There are several different temperatures shown and an exit option to get you out of this screen. The temperature values, in units of degrees Celsius, are for informational purposes only and cannot be edited. In general, if a particular temperature crosses a threshold that indicates a problem, the system will shut down and indicate an error state with both LEDs showing solid red.

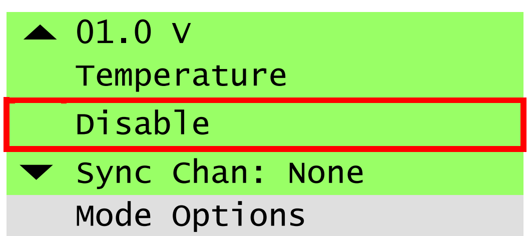
If the device attached to the bank is a CDish, this screen will display the below three values.

Parameter	Description	Typical Value	Error Threshold
HVDrv-P	The temperature of the positive high-voltage, high-current drive transistor.	35	80
HVDrv-N	The temperature of the negative high-voltage, high-current drive transistor.	35	80
HVxB	The ambient temperature in the vicinity of the bank inside the C-Pace main chassis.	30	60

If the bank is attached to a C-Stretch, there will be the following additional values.

Parameter	Description	Typical Value	Error Threshold
Cells	The temperature inside the cell chamber. This should be very near to the ambient temperature of the room or incubator containing the C-Stretch device.	37	-
Motor	The temperature of motor that is driving the stretch protocols.	49	80
Motor-Drv	The temperature of motor driver that is controlling the motor.	49	60
ECM-Brd	The temperature of the main electronics board inside the C-Stretch.	42	60

3.1.6 Enable/Disable Status



The sixth line in the **Main Menu** enables and disables the electrical pulse trains and (if applicable) the stretch for a bank. When a bank is **Disabled**, reed relays in the high voltage path are opened, ensuring a completely open circuit. These relays are also opened between pulses to prevent leakage of voltage.

With a C-Stretch, the motor will return the dishes to a state of zero percent stretch when **Disable** is selected.

3.1.7 Sync Channel

```
▲ Temperature
  Disabled
  Sync Chan: None
▼ Mode Options
  Bank Options
```

The seventh line in the **Main Menu** is used to set the **Sync Channel** of the entire C-Pace EM device. Both the **Gate Out** and **Aux Out** digital outputs are ultimately synchronized to **Sync Channel** as documented in *2.1.3 Digital Outputs* above.

The **Sync Channel** is indicated by the number given in this setting. This value can be **None**, or a number between **1** and the number of wells in the attached C-Dish. If a C-Stretch is attached to the bank, the options will be **None** or **1**.

None indicates that this bank is not used to determine the **Sync Channel**. If you switch a **Sync Channel** setting away from **None**, you will remove the setting from which ever bank had it previously since there is only one **Sync Channel** for the entire device.

3.1.8 Mode Specific Menu Lines

Starting on line 8, the Main Menu structure depends on the selected **Operating Mode** setting. These mode specific options are described below.

3.1.8.1 Basic Mode Preset Lines

```
▲ 01.0 V
  Enabled
  Sync Channel: None
_ 1.0*2.0 3.0 4.0 Hz
  Bank Options
```

The **Basic Mode Presets** are shown on the eighth line of the menu. **Basic Mode** is designed for general stimulation purposes under manual control of the stimulator properties. If you want more complex stimulation protocols, you should use **Sequence Mode**.

When the C-Pace EM is operating in **Basic Mode**, you will have access to 4 different predefined sets of stimulation parameters. Each **Preset** is identified by its **Event Frequency** in the menu line.

There is always an active **Preset** that determines the current operating parameters of the stimulator. The active **Preset** is identified with a ***** in the menu line following the selected frequency.

You can switch between presets at any moment, and you can edit the parameters of any preset, either active or non-active. To modify or select a **Preset**, click the encoder button when on the **Preset** line. Then turn the knob to move the cursor horizontally over the present you want to work with. Double-click on a preset to activate it or single-click to enter the **Basic Edit Menu**. The settings of the **Basic Edit Menu** are described in section *3.2 Basic Edit Menu* below.

3.1.8.2 Sequence Step Menu Lines

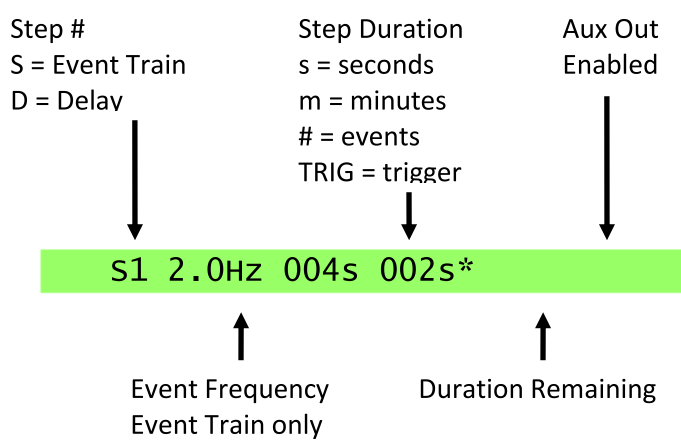
```
▲ S1 2.0Hz 004s 002s*
_ S2 1.0Hz 001#
  …
  End
  Bank Options
```

Line 8(+): Sequence Steps. **End** is a sequence step that ends the sequence (unless you have 5 steps in which case **S5/D5** is the last step).

The **Sequence Mode Steps** start on Line 8 of the main menu and can extend for up to 5 lines. **Sequence Mode** is designed for complex stimulation protocols. If you want general stimulation functionality, you should use **Basic Mode**.

When the C-Pace EM is operating in **Sequence Mode**, the stimulator will automatically cycle through a series of sequence steps. Each step is a different set of stimulation parameters. You can have a protocol with up to 5 different steps. This feature lets you generate complex stimulation waveforms such as arrythmias and offbeats.

When in **Sequence Mode**, each step will appear on its own line of the **Main Menu**. Each line offers a summary of the protocol and also displays the amount of time left in the step. The figure below explains the elements of this summary.



To edit a particular step, scroll the cursor to the line to be edited and click to go to the **Sequence Edit Menu**. The settings of the Sequence Edit Menu are described in section *3.3 Sequence Edit Menu* below.

Note that **End** is a step. Editing that step will let you convert it from an **End** step into a real step thus adding a step to the sequence.

An Example

```
▲ S1 2.0Hz 004s 002s*
_ S2 1.0Hz 001#
  End
```

The example above shows a two-step protocol. In the first step (labeled **S1**) pulses are output for 4 seconds at a rate of 2.0 Hz. The asterisk at the end of **S1** indicates that a TTL pulse will be output on the **Aux Out** BNC connector on the first pulse of the step if the **Sync Channel** is a member of the bank. (See *2.1.3 Digital Outputs* above).

In the second step (**S2)**, a single 1.0 Hz **Event** is generated, and then the sequence ends, at which point the sequence will loop back to the beginning.

The result of this sequence is a continuous **Event** train with an offbeat every 5 seconds.

The **002s** marker in the row for **S1** indicates it is the currently executing step **S1** and that there are 2 seconds remaining.

Please note that if the bank is **Enabled**, the sequence will continue to loop over the steps forever until **Disabled**. There is no way to program a “stop step” except via an external trigger. See section *3.3.4 Duration* below for more information.

3.1.8.3 TTL Lock Lines

```
▲ Temperature
  Enabled
  Sync Channel: None
  Bank Options
```

In the **TTL Lock Mode**, there are no Main Menu options. The bank is a slave to the external device connected to the **Pulse In** input. A digital pulse seen on the **Pulse In** input will immediately generate a pacer **Event**. This will in turn trigger an electrical stimulus pulse. The **Pulse In** input will be “blind” for the duration of the electrical pulse or stretch cycle. Any digital signal that appears during the blind period will be ignored.

Note: TTL Lock is not available for C-Stretch Banks.

3.1.9 Bank Options

```
▲ Disable
  Sync Chan: None
  Mode Options
  Bank Options
```

Regardless of the operating mode, the very last option on the **Main Menu** is the **Bank Options**. Selecting this will take you to the **Bank Options Menu**, explained in section *3.5 Bank Options Menu* below.

3.2 Basic Edit Menu

_ Toggle to Select	Line 1: Select
1.0 Hz	Line 2: Event Frequency
00 %	Line 3: Variability
Stretch Options	Lines 4-13: Stretch Options (if C-Stretch is connected)
Exit	Last Line: Exit

The **Basic Edit Menu** is very simple and has the options explained below.

3.2.1 Select

Select **Toggle to Select** to activate this group and return to the **Main Menu**.

3.2.2 Event Frequency

As discussed earlier, there is an internal signal in the C-Pace EM called the pacer **Event** that triggers the start of both the high voltage stimulus pulse and, for a C-Stretch, the mechanical stretch cycle. The base frequency of the **Event** is dictated by this setting.

If the edited group is activated, the banks will reflect the changes as the frequency is tuned. The frequency range is dependent on the bank type as shown in the table below.

Bank Configuration	Frequency Range
C-Dish	0.01 Hz to 99 Hz
C-Stretch	0.1 Hz to 10 Hz

Note: The pace frequency range may be additionally truncated as a result of the current pulse duration, number of channels, and the **Irregular Event Pacing Variability** setting (max. frequency including variability = channels * (duration + 0.5 ms)). Because of motor velocity limits, the stretch frequency range may also be truncated based upon the contract, hold, and release phases as well as other parameters.

3.2.3 Variability

This line sets the maximum **Irregular Event Pacing Variability** of the pulse train. Setting this element to a non-zero value will enable **Irregular Event Pacing** for this step. For most purposes, this should be set to 00%. For a detailed explanation of **Irregular Event Pacing**, please see the section *4.1 Irregular Event Pacing* below.

Stretch Only

As with **Sequence Mode**, the **Event Window** defined by the **Frequency** and **Variability** can be limited based on the stretch parameters, in particular the amount of stretch requested. There is a maximum velocity that the stretch can achieve which limits the minimum period of the **Event Window** (and thus the maximum **Event** frequency). If you are bumping against a maximum frequency that is less than what is in the table, it is likely because of the amount of stretch.

3.2.4 Stretch Options

When a C-Stretch is connected, the stretch options for the group will appear after the variability. These options are explained in section *3.4 Stretch Menu Options* below.

3.2.5 Exit

Selecting **Exit** returns you to the **Main Menu** without activating the frequency.

3.3 Sequence Edit Menu

_ Toggle to Select	Line 1: Manual Activation	All
Pulse Train	Line 2: Step Type	All
Aux Out Enabled	Line 3: Aux Out Enable	Event Train, Delay
▼ seconds 004s	Line 4: Duration	Event Train, Delay
Hz 1.0 00%	Line 5: Frequency/Variability	Event Train
Stretch Parameters	Lines 6-15: Stretch Param-eters	Event Train (C-Stretch only)
Exit	Last Line: Exit	All

The **Sequence Edit Menu** permits setting the parameters for a single step in a sequence. This menu, like the **Main Menu**, is of variable length depending on the **Step Type** selection.

The figure above shows the full set of options, which are only available when the **Step Type** is **Event Train**. For other **Step Types**, some number of options will not be available and the list will be collapsed. These restrictions are noted above and in the documentation for each option below.

3.3.1 Manual Activation

```
_  Toggle to Select
   Pulse Train
   Aux Out Enabled
▼  seconds 004s
```

Click to **Toggle to Select** to activate this step and return to main menu. This permits you to manually change the normal execution of the step list.

3.3.2 Step Type

```
   Toggle to Select
_  Pulse Train
   Aux Out Enabled
▼  seconds 004s
```

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

Pulse Train Indicates a series of pacer **Events** of a specific frequency will be generated for the duration of this step. These **Events** will trigger electrical stimulation pulses and/or stretch cycles as appropriate for the bank.

Delay Indicates that no **Events** will be generated 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. You can change any step in a sequence to **End** at any time. For example, if you have a sequence with 5 steps and you change the third step to **End**, you will now only have 2 steps.

3.3.3 Aux Out Enable

```
   Toggle to Select
   Pulse Train
_  Aux Out Enabled
▼  seconds 004s
```

Note: This option is only available for Pulse Train or Delay steps.

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 **Sync Channel** has been adjusted to select the bank (see *3.1.7 Sync Channel* above). In the case of a pulse train, the TTL pulse will occur in sync with the first **Event** for 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.

3.3.4 Duration

```
▲  Pulse Train
   Aux Out Enabled
_  seconds 004s
▼  Hz 1.0 00%
```

Note: This option is only available for Pulse Train or Delay steps.

This option sets the duration of the step. There are four options: number of minutes, number of seconds, number of **Events**, and trigger (for TTL).

minutes and seconds Set period of time on this line. An internal timer will count down the designated period of time. The output pulse 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 occurred.)

pulses **Pulses** is only an option if the step type is a **Pulse Train**. In this case, the number of **Events** generated by the bank is counted down and the step ends when the last **Event Window** expires. As noted before, the **Event**

Window is the time between **Events** and is determined by the **Event** frequency. For example, the **Event Window** for 1 Hz **Events** is 1 second whereas the **Event Window** for 2 Hz **Events** is 0.5 seconds.

Note: The **Event** 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 offbeat followed by a delay, two pulses 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** connection or until the user manually double clicks on a step line to use the **Activate Step** function. The output **Event** train will finish the current **Event Window** before switching steps. This means that the first **Event** of the next step will not actually be synchronized with the **Advance In** input but will occur after the current **Event Window** expires.

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.

3.3.5 Frequency

```
▲ Aux Out Enabled
  seconds 004s
_ Hz 1.0 00%
  Exit
```

Note: This option is only available for **Pulse Train** steps.

In a sequence the **Frequency** for a step determines the rate at which pacer **Events** are generated for the step. It may be set either in terms of frequency or in terms of period by selecting either **Hz** or **s**. This option is the first element of the line and will be selected for editing when you enter the line.

The next two elements are the coarse and fine adjust for the value. If the step you are editing is also currently the active step, the bank will reflect the changes as the frequency is tuned.

The fourth and final element in the **Frequency** setting is the **Irregular Event Pacing Variability** of the **Event** train. Setting this element to a non-zero value will enable **Irregular Event Pacing** for this step. For most purposes, this should be set to 00%, which will make the **Event** train perfectly periodic. For a detailed explanation of **Irregular Event Pacing**, please see section *4.1 Irregular Event Pacing* below.

The frequency range is dependent on the bank type as shown in the table below.

Bank Configuration	Frequency Range
C-Dish	0.01 Hz to 99 Hz
C-Stretch	0.1 Hz to 10 Hz

Furthermore, the range may be additionally truncated as a result of the current pulse duration, number of channels, and the **Irregular Event Pacing Variability** setting.

Stretch Only

As with **Basic Mode**, the **Event Window** defined by the **Frequency** and **Variability** can be limited based on the stretch parameters, in particular the amount of stretch requested. There is a maximum velocity that the stretch can achieve which limits the minimum period of the **Event** Window (and thus the maximum **Event** frequency). If you are bumping against a maximum frequency that is less than what is in the table, it is likely because of the amount of stretch.

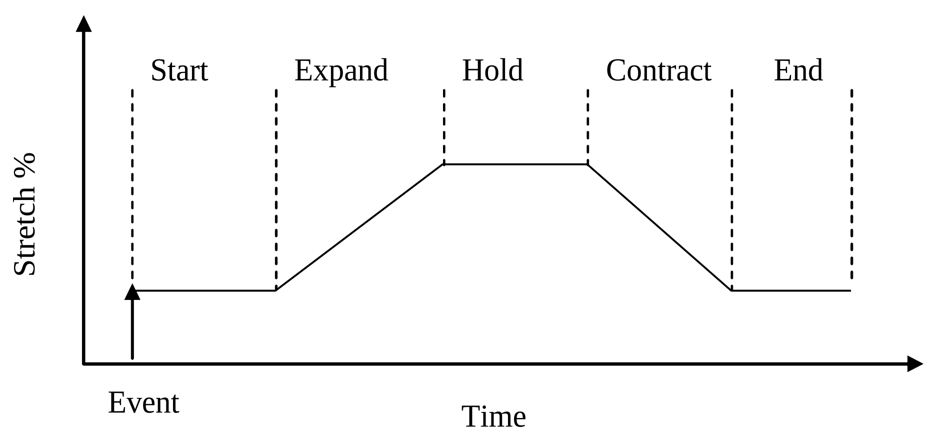
3.3.6 Exit

```
▲ Aux Out Enabled
  seconds 004s
  Hz 1.0 00%
_ Exit
```

Exit returns you to the **Main Menu** without activating the frequency

3.4 Stretch Menu Options

The C-Pace EM stretch system follows a simple cycle of stretching and relaxing the cell chambers between two user programmable amounts of stretch. This cycle is initiated by the pacer **Event** and continues as long as the bank is enabled. The initiation of the stretch cycle can be delayed from the pacer **Event** which gives the waveform 5 different phases as illustrated in the graph below.



Each cycle begins with the **Start** phase. The length of the chamber at this phase is determined by the **Preload** setting as described below. The cycle then proceeds to the **Expand** phase where the tissue is stretched to a length determined by the **Load** setting. The tissue then rests at the **Load** length during the **Hold** phase. The **Contract** phase is next and returns the tissue to the **Preload** length where it rests for the **End** phase until the next pacer **Event** fires.

Notice that the **Preload** condition corresponds to the shortest length of the tissue while the **Load** condition corresponds to the longest length of the tissue.

	Preload:	05%	Line 1: Start and End Phase Stretch Magnitude
_	Load:	15%	Line 2: Hold Phase Stretch Magnitude
	Start:	00%	Line 3: Start phase duration, absolute/relative selection
▼	Expand:	40%	Line 4: Expand phase duration
	Hold:	10%	Line 5: Hold phase duration
	Contract:	40%	Line 6: Contract phase duration
	End:	10%	Line 7: End phase duration (read-only)
	P Trig:	Contract	Line 8: Stim Pulse Trigger Option
	P Delay:	-10%	Line 9: Stim Pulse Delay
	P Ratio:	01:01	Line 10: Stim:Stretch Ratio

Above is the stretch menu. There are 10 options which fall into 3 broad groups: the Stretch Amounts, the Stretch Phase Durations, and the Pacing Options.

3.4.1 Stretch Amounts

	Preload:	05%
_	Load:	15%
	Start:	00%
▼	Expand:	40%

The shortened and stretched lengths of the tissue are set via the first two menu lines. The shortened length is set by the **Preload** parameter and corresponds to the amount of stretch in the **Start** and **End** phases of a stretch cycle. The stretched length is set by the **Load** parameter and corresponds to the amount of stretch in the **Hold** phase.

These values are both specified as a percent stretch where 0% indicates no stretch. You can calculate the percent stretch via the following equation:

*percent contraction=(long-resting)/(resting)*100*

Note: the stretch amounts are based on the movement of the posts that hold the PDMS wells. Compliance in the PDMS wells themselves causes the actual stretch of the well to be somewhat lower than the programmed stretch amount. This deviation from the ideal varies based on the well itself.

3.4.2 Stretch Phase Durations

```
▲ Start:     00%
_ Expand:    40%
  Hold:      10%
▼ Contract:  40%
  End:       10%
```

There are 5 phases to the stretch waveform, as shown earlier. You can individually program the width of each phase. The widths are specified as a percentage of the **Event Window** period. Thus, if the **Event** frequency were 1 Hz, 50% would represent 500ms.

It is unnecessary to specify the duration for the **End** phase as its duration is simply whatever amount of time is left over after the other phases have been set.

The duration of any static phase (**Start**, **Hold**, or **End**) can be set to zero. The dynamic phases will have minimum durations that are determined by the waveform shape and the stretch amounts.

3.4.3 Pacing Options

```
▲ End:       10%
_ P Trig:    Contract
  P Delay:   -10%
  P Ratio:   01:01
```

The **Pulse Trigger** (**P Trig**) and **Pulse Delay** (**P Delay**) settings together determine when the electrical stimulus pulse will be presented relative to the stretch waveform. The **Pulse Trigger** is always the beginning of a phase. Thus, the selections for the trigger are simply the five phases as illustrated in the table above.

Once the trigger is selected the **Pulse Delay** is applied to arrive at the exact moment the stimulus pulse will start. The delay can be positive or negative. A positive value puts the pulse after the trigger while a negative value will put it before the trigger. The values are bounded such that the pulse will never precede the start phase or follow the end phase. The units for the delay are the same as those selected for the **Stretch Phase Durations**, namely a percentage of the **Event Window**.

The **Pulse Ratio** setting determines how often an electrical stimulus pulse is generated relative to the stretch protocols. The ratio is of the form:

pulse:stretch

A ratio of 1:1 indicates that every pacer **Event** will generate a stretch and a pulse. A value of 1:2 indicates that while stretches will occur on every **Event**, pulses will only be generated on every other **Event**. A ratio of 2:1 indicates the reverse: a pulse on every **Event** but a stretch on every other **Event**.

A ratio of 0:x results in stretches only while a ratio of x:0 results in pulses only.

3.5 Bank Options Menu

```
Bank Type : CDish 6
Stim Mode : Classic
FW 163: Prop v.3
Exit
```

Here you can view information about the bank, and depending on the firmware in the bank, set parameters that control the bank functionality. As always, selecting Exit will take you back to the main menu.

3.5.1 Bank Type

```
Bank Type : CDish 6
Stim Mode : Classic
FW 163: Prop v.3
Exit
```

The **Bank Type** menu line simply tells you the type of dish attached to the bank.

3.5.2 Stim Mode

```
Bank Type : CDish 6
Stim Mode : No Leak
FW 163: Prop v.3
Exit
```

The Stim Mode menu line tells you the **Stimulation Mode** the bank is using. There are two options: **Classic** and **No Leak**. Depending on the firmware version of the bank, this parameter may be editable. Otherwise, it tells you the hard-coded mode which depends on the firmware version and the **Bank Type**.

3.5.2.1 Classic Stim Mode

It is not recommended to use **Classic** mode for new experiments. It is only included to permit reproducing results from old experiments.

The **Classic** stimulation mode is the "one well at a time" stimulation pattern used by all C-Pace devices manufactured before approximately 2017. In this mode the relays on the high voltage board are closed in pairs designed to target each well in a culture dish independently. Unfortunately, a design flaw created a situation where other wells in the dish were also receiving a portion of the stimulation voltage. This flaw lead to the creation of the **No Leak** stimulation mode. The **No Leak** mode is the default for all current devices and the **Classic** mode exists only for comparative tests and experimental compatibility with old devices.

3.5.2.2 No Leak Stim Mode

The **No Leak** stimulation mode corrects the flaw in the **Classic** mode and assures that each well in a culture dish receives exactly one stimulation pulse of the correct voltage. It does this by stimulating multiple wells in parallel to eliminate the secondary current paths that compromised the **Classic** mode approach. This does require more current from the stimulation amplifier and therefore the current limit is increased appropriately.

This is now the default stimulation mode for all C-Pace devices.

3.5.3 FW

```
Bank Type : CDish 6
Stim Mode : No Leak
FW 163: Prop v.3
Exit
```

The **FW** line reports the raw firmware version number reported by the high voltage bank (in the example above, 163) before the colon. After the colon it decodes that raw number to indicate the type of processor and the firmware revision it is running.

3.5.4 Exit

```
Bank Type : CDish 6
Stim Mode : No Leak
FW 163: Prop v.3
Exit
```

Exit returns you to the **Main Menu**.

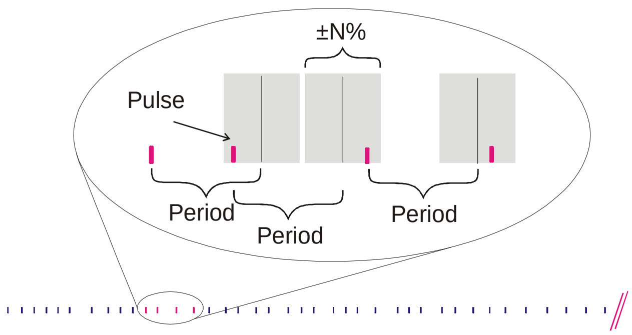
4 Examples

This section contains several examples to assist in setting up common EM protocols.

4.1 Irregular Event Pacing

```
_  Toggle to Select
   1.0 Hz
   50% Variability
   Exit
```

The **Irregular Event 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.



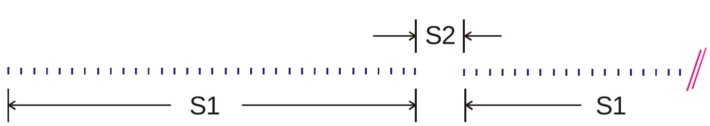
4.2 Arrhythmia - Insert an Offbeat Pulse at a Fixed Interval

```
▲  Sync Chan: None
   S1 2.0Hz 004s 002s
_  S2 1.0Hz 001#
   End
```

Define **S1** (step 1) of the **Sequence** to be a pulse train with desired frequency and length.

Define **S2** (step 2) of the **Sequence** to be a pulse train with desired frequency and duration of one pulse.

Define **S3** (step 3) of the **Sequence** to be the **End** of the **Sequence**.



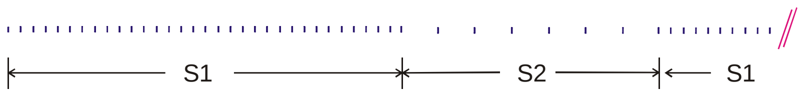
4.3 Exercise Protocol - Multiple Pulse Trains with Individual Frequency and Duration

```
▲ Sync Chan: None
  S1 2.0Hz 060m
_ S2 1.0Hz 660m 212m
  End
```

Define **S1** (step 1) of the **Sequence** to be a pulse train with desired frequency and length.

Define **S2** (step 2) of the **Sequence** to be a pulse train with desired frequency and length.

Define **S3** (step 3) of the **Sequence** to be the **End** of the **Sequence**.



4.4 Examples Using the Digital Inputs and Outputs

The examples in this section illustrate uses of the C-Pace EM's digital outputs and inputs. These are described in detail in *2.1.3 Digital Outputs* and *2.1.4 Digital Inputs* above.

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

```
▲ TTL Lock
  04.0 ms duration
  35.0 V
_ Temperature
```

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 connector. The rising edge of a pulse will initiate the high voltage pulses. There is a 450 (+/-10) µs 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.

4.4.2 Advance In: Initiate an Event with an External TTL

```
▲ Sync Chan: None
_ 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 at 450 (+/-10) µs 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.

4.4.3 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.

4.4.4 Aux Out: Control an External Piece of Hardware

```
▲ Toggle To Select
_ Delay
  Aux Out Enabled
▼ seconds 004s
```

Select **Aux Out Enabled** on the desired step.

```
▲ Sync Chan: 1
  D1 004s 002s*
_ S2 1.0Hz 060m
  End
```

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

Make sure the **Sync Chan** is set to the well you want to trigger the external device. Typically, this would be well 1 unless there is a reason to do otherwise.

A TTL signal will now be output on the **Aux Out** BNC connector 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.

5 Appendices

5.1 Appendix 1: Troubleshooting

Below are possible problems with their solutions.

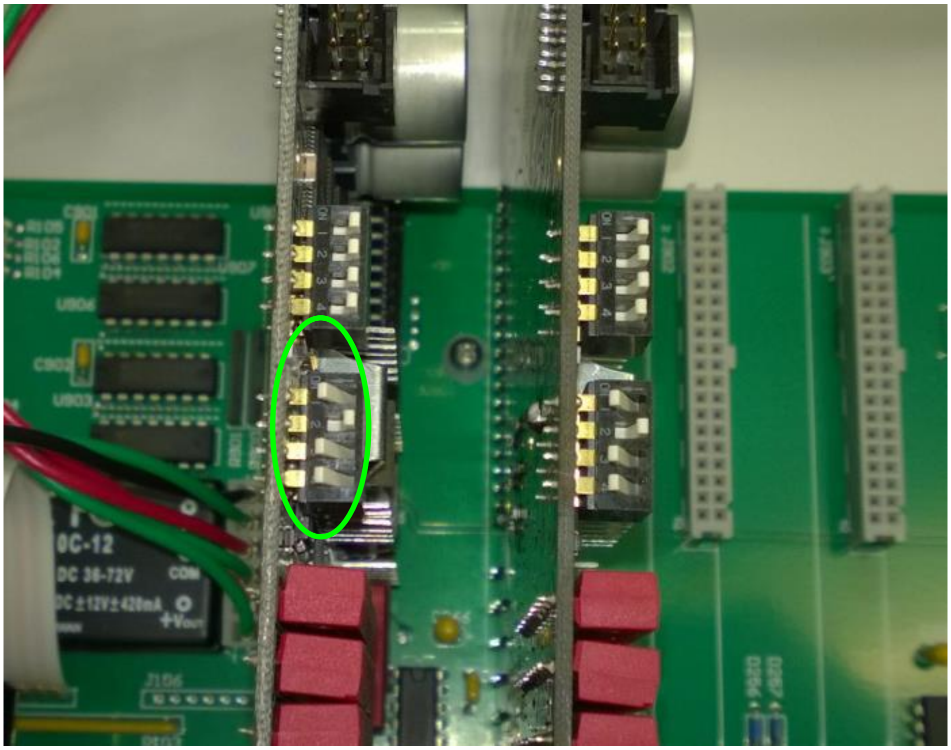
Problem	Solution
No Power	Make sure the power cord is plugged in firmly and connected to an active outlet. Unplug and check fuse. If necessary, replace with 3 Amp 5x20mm slow blow fuses (older devices with detached fuse holders use 1 ¼ inch fuses).
Blank Display	**Always unplug device before opening box!** 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.
Stuck On "IonOptix"	**Always unplug device before opening box!** 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 a good connection.
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	Possibly 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 us know if you see any odd behavior via the contact information below.

Please contact us if you experience any problems or require any clarifications on the device functionality at info@ionoptix.com or 617-696-7335.

5.2 Appendix 2: Media/Voltage Recommendations

Plate Type	Recommended Media Volume	Typical Voltage Range
4 well multiwell plate	5-7 mL	30-40 V
6 well multiwell plate	2-3 mL	9-13 V
6 individual 35mm dishes	2-3 mL	7-10 V
8 well multiwell plate	2.5 – 4 mL	12-17 V
12 well multiwell plate	1-1.25 mL	4-6 V
24 well multiwell plate	.5-.75 mL	3-4 V

5.3 Appendix 3: Changing the HVB Channel Count



Always unplug device before opening box!

Use a Phillips screwdriver to take the top off the C-Pace EM. You will see the High Voltage Banks mounted vertically. The dip switch closest to the red relays (circled above in yellow) sets the C-Dish type the bank is configured to drive. Set the dip switch to reflect the settings below for desired number of wells. The banks in the above picture are set for a 6-well dish. (Note: Plugging in a C-Stretch will automatically override this setting.)

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

Please note that while the terms wells and channels are used a bit interchangeably in this section and on the circuit boards, strictly speaking the channels are the electrical signals coming from the HVB while the wells are the actual chambers in the culture plate. While it is generally the case that channel 1 corresponds to well 1, it is not always the case. Please check your C-Dish documentation for more information.

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