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System Installation
**ECIS Description**

The ECIS electronics are contained in two separate units, the **Station Controller (Z or Zθ)** that is located outside of the incubator and the **Station** (either 16W or 96W) that is placed within the incubator space. The Station is connected with a six foot long flat cable that exits the incubator either through an established port or between the rubber seal and glass interior incubator door. This cable is plugged into a socket on the back panel of the Station Controller that is then covered with a shield / strain relief cover. The Station Controller contains most of the electronics including the oscillator supplying the AC signal and sophisticated impedance measuring circuits. The array station provides additional signal processing circuits and switching mechanisms to change the measurement from well to well. The instrument is capable of simultaneously measuring both the resistance and capacitance of the ECIS electrodes over a broad range of AC frequencies. In addition, the electronics only accept signals having the same frequency as that of the source oscillator and is said to “lock” to the signal, eliminating most extraneous electrical noise. The switch on the front panel turns on all ECIS electronics. On the back panel, along with the Station socket, is a USB socket for communication and a receptacle for power (110-240 V, 50-60 Hz).

**Station Controller Installation**

Place the ECIS Station Controller next to the tissue culture incubator. The unit can be placed in a vertical configuration if desired; simply remove the four feet from the base and screw them into the four threaded locations on the right side of the instrument (these openings are filled in the new unit with machine screws that can be removed).

**Array Station Installation**

Place the ECIS array station within the incubator space. To avoid unwanted condensation, keep the array station and clamps in a sealed plastic bag until it has warmed to incubator temperature. Then, remove the array station from the bag and follow the instructions below.

Insert a shelf in the CO₂ incubator cavity near the access port and place the ECIS array station on the shelf. Thread the flat cable carefully through the incubator port* (*handle this plug at the end of the cable with care until it is finally connected to the Station Controller). Bring the flat cable to the back of the Station Controller. Adjust the cable to leave some slack in the incubator cavity. Using a split stopper or other pliable device, provide an airtight seal by the lead into the incubator space. Carefully insert the 26-pin plug into the port on the back panel of the Station Controller observing the key way to assure correct positioning. Once firmly in place, be certain the cable runs vertically down from the socket and attach the cable cover to the back panel. Secure this by tightening the four knurled screws, such that the cable is pinched between the two rubberized pads to provide strain relief. **Note:** if the incubator lacks a port, the leads can be brought out of the incubator door using the glass inner door and gasket to make a seal.
Computer & Software Installation

The laptop PC (or computer and monitor) and printer should be placed a short distance away from the ECIS instrumentation such that all leads will reach their destinations without undue stress.

As required, connect the keyboard, mouse, monitor, and printer to the appropriate locations on the computer. If questions arise, please check the manuals supplied with these devices. ECIS software is already installed on the PC before shipment. If it becomes necessary to reload the software please contact us and we can guide you through the process. To reload the software, it will be necessary to have the ECIS setup disk that was supplied with the instrument. Be certain to store this in a safe place. Finally, connect the USB port on the Station Controller to any USB port on the PC with the cable supplied.

The Incubator Setup (if this option was ordered with your system)

Following the instructions supplied in the manual accompanying the incubator; connect the device to a CO2 source and a standard AC power outlet. If the incubator has a water jacket, follow instruction in the incubator manual to fill the jacket with either distilled or tap water (about 7 gallons with the 2 cubic foot unit). Do not use deionized water. Fill the pan in the bottom of the incubator cavity with distilled water, and following the manual instructions set the temperature and CO2 concentration as desired. Although usually not necessary, it is always a good practice to calibrate both temperature and CO2 levels from time to time. Once the incubator has reached the normal operating temperature, the overtemp control on the back of the instrument can be set per the manual's instructions.

The system is now ready for use.
Getting Started with ECIS Arrays
**ECIS Cultureware**

The standard 8 well ECIS disposable arrays consist of gold film electrodes delineated with an insulating film and mounted on a 20 mil optically clear Lexan® polycarbonate substrate. The eight well top assembly is made of polystyrene. The gold layer is sufficiently thin (approx. 50 nm) to allow microscopic observation of the cells using a standard inverted tissue culture microscope. Each well has a surface area for cell attachment and growth of ~0.8 cm² and holds a maximum volume of about 500 microliters. Gold pads at the edge of the array connect electrodes to the ECIS electronics via contact with spring-loaded pins within the electrode array station.

The standard 96 well ECIS disposable arrays consist of gold electrodes delineated with an insulating film and mounted on opaque fiberglass supports. The 96 well top assembly is made of polystyrene. Each well has a surface area for cell attachment and growth of ~0.3 cm² and holds a maximum volume of ~400 microliters. A PCI connector on the top edge of the array connects electrodes to the ECIS electronics via contact with a special socket on the electrode array station.

We maintain a large supply of arrays for shipment from our facility. When arrays are ordered, a final oxygen plasma etching procedure is used to both clean the gold surfaces and to sterilize the arrays before they are packed and shipped.

**Standard 8 Well Arrays**

The **8W1E** ECIS array has eight wells with one active electrode per well (250 micrometer diameter) and a large counter electrode.

The **8W10E** ECIS array has eight wells with 10 active electrodes connected in parallel and a large counter electrode.

The **8W10E+** ECIS array is the latest addition to our standard array portfolio. This array has two sets of 20 electrodes mounted on inter-digitated fingers to provide measurements of cells upon a total of 40 electrodes. The electrical properties are similar to the 8W10E array, but sample even more regions of the wells and greater numbers of cells substantially quieting the impedance fluctuation due to random micromotions of the cells.

**Standard 96 Well Arrays**

The **96W1E** ECIS array has 96 wells with one active electrode per well. Each electrode is 250 micrometers in diameter.

The **96W10E+** ECIS array has 96 wells with 20 active electrodes per well. Each electrode is 355 micrometers in diameter (twice the area of the 1E electrode). The array has electrical properties similar to those of the 8W10E+. 
Custom Arrays

The **8W1F** ECIS array has one flow channel with 8 independent electrodes. Each electrode is 250 micrometers in diameter.

The **8W1E DD** ECIS array has 8 wells with one active electrode per well. The wells come in pairs with electrodes of 250, 100, 50, & 25 micrometers in diameter.

The **8W2x1E(Medusa)** ECIS array has 8 wells with 2 active electrodes per well. Each electrode is 250 micrometers in diameter. This array is unique because you get 16 separate, independent measurements with one array.

The **Chemotaxis** ECIS array has 8 wells with one active line electrode per well. Each electrode is 200 micrometers in Length. You fill the wells with agarose and then punch two holes on either side of the line electrode (where the two gold dots are), you add your cell suspension on one side of the electrode in the punched hole, and add your chemoattractant to the other hole. A gradient forms and you can monitor your cells as they cross the electrode in the direction of the chemoattractant.*


We will also work with customers to build arrays to their specifications if possible. Please contact us for more information regarding this service.

How to Choose an Array

The **8W10E** and **8W10E+** multiple electrode arrays contain 10 and 40 small electrodes respectively rather than one at the base of each well. The **96W10E+** arrays have 20 electrodes but with twice the area of the electrodes from the 8 well arrays. These can be useful for some ECIS measurements you may be attempting.

**Advantages of the multiple electrode arrays:**

1. The multiple electrode arrays average the ECIS response over 10 to 40 times the number of cells observed using a single electrode, giving a more statistically relevant result.

2. The arrays sample the ECIS response over different spatial regions of the well bottom. This means that differences in experimental values resulting from uneven inoculation density or from convection flow disturbances along some areas the well bottom tend to be averaged out.

3. With these arrays, the micromotion behavior of the cells, although a measurement of importance in many ECIS experiments, is smoothed out. This can be of importance in experiments where
micromotion sometimes obscures the general response of a cell layer to the addition of an active agent.

Advantages of the single active electrode arrays:

(1) Fluctuating impedance signals as a means to detect and study cell micromotion will be enhanced with single electrode measurements.

(2) Study of single cells or populations of very few cells will be facilitated with the single electrodes.

(3) The ECIS programs involving electroporation or cell wounding may require the single electrode arrangement to achieve the higher electric fields depending upon the characteristics of the cells being studied.

(4) Using a single electrode allows one to microscopically observe the very cells that are responsible for the ECIS impedance changes.

---

**Array Specifications**

<table>
<thead>
<tr>
<th>Array Type</th>
<th>Wells</th>
<th>Electrodes per Well</th>
<th>Diameter(µm)</th>
<th>Shape</th>
<th>Transparent</th>
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</thead>
<tbody>
<tr>
<td>8W1E</td>
<td>8</td>
<td>1</td>
<td>250</td>
<td>Circular</td>
<td>Yes</td>
</tr>
<tr>
<td>8W10E</td>
<td>8</td>
<td>10</td>
<td>250</td>
<td>Circular</td>
<td>Yes</td>
</tr>
<tr>
<td>8W10E+</td>
<td>8</td>
<td>40</td>
<td>250</td>
<td>Circular</td>
<td>Yes</td>
</tr>
<tr>
<td>96W1E</td>
<td>96</td>
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<td>250</td>
<td>Circular</td>
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</tr>
<tr>
<td>96W10E+</td>
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<td>20</td>
<td>355</td>
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<tr>
<td>8W1F</td>
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<td>8</td>
<td>250</td>
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<tr>
<td>8W1E DD</td>
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<td>1</td>
<td>250, 100, 50, 25</td>
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<tr>
<td>8W2x1E(Medusa)</td>
<td>8</td>
<td>2</td>
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<tr>
<td>Chemotaxis</td>
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<td>1</td>
<td>2mm x 25µm</td>
<td>Line</td>
<td>Yes</td>
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</table>
Starting an Experiment
Calibration with Test Array

**Eight Well Station**

Place the ECIS Test Array supplied with the instrument into the left-hand side (A side) of the array holder such that the nine contact pads line up with the nine gold contact pins. Turn the knurled stainless steel knob clockwise, lowering the pins to the contact pads, until finger tight. From the Desktop, double click the ECIS program icon to bring up the main menu.

Click on the **Setup** button. This quickly measures the impedance of the device and then reports whether or not an open circuit has been detected. With the test array in the A slot and nothing on the B side you should see the Well Configuration area light up green on the A side indicating connections and red on the B side indicating open circuits.

If the A side wells are not all green, adjust the placement of the test array and click Setup again.

With the test array properly in place, click the Check button. If the instrument is a Z Theta, measurements of the test array will be made using the default frequency of 4000 Hz. At the completion of the run, the values for the eight electrodes will be displayed showing impedance as well as the series resistance and capacitance. Next, remove the test array from side A and insert in to side B and repeat this procedure for the B side. Each test array will be slightly different due to the tolerance of the RC components, but wells should have the following approximate values for impedance, resistance and capacitance:

<table>
<thead>
<tr>
<th>Test Array well number</th>
<th>Approx. Resistance (ohms)</th>
<th>Approx. Capacitance (nanoFarads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>3000</td>
<td>10</td>
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<tr>
<td>4</td>
<td>4000</td>
<td>10</td>
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<tr>
<td>5</td>
<td>5000</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>6000</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>7000</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>8000</td>
<td>10</td>
</tr>
</tbody>
</table>
If the instrument is a Z, measurements of the test array will be made using the default frequency of 16,000Hz. The following approximate values should be obtained for the impedance:

1410, 2240, 3160, 4120, 5100, 6080, 7070, 8060 ohms

Be sure to check out both the A and B sides.

**96 Well Station**

Place the ECIS Test Array supplied with the instrument on the sled of the 96 Well Station and move the stainless steel lever to the right to insert the edge of the card into the connecting socket. Double click the ECIS icon to bring up the main menu.

Click on the **Setup** button. This quickly measures the impedance of the device and then reports whether or not an open circuit has been detected. If wells are properly connected the Well Configuration area will show those connected wells green and any unconnected wells as red. If any wells are red please removed and reinsert the test array using the stainless steel lever and then click the Setup button once again.

With the test array properly in place, click the **Check** button. This will make measurements of the test array using the default frequency of 4000 Hz for a Z Theta instrument and 16,000 Hz for the Z instrument. At the completion of the run, the values for the 96 electrodes will be displayed. These values will show the impedance of each well and, if measurements are being made using a Z Theta instrument, will also break the impedance down into that resulting from a series circuit of a resistor and capacitor.
You can also check out the instrumentation using the ECIS arrays. As in any ECIS experiment with the consumable arrays, we recommend electrodes first be treated with the electrical stabilization method or treated with cysteine (see below in this manual). Next, aseptically place 200 microliters of medium in each well of an array. Place the array in the array station. Double click the ECIS icon to bring up the main menu and run a **Setup/Check** of the electrode values and let the instrument gather impedance data. *Should contact not be made* you will see the electrode display light up red for the disconnected wells.. Usually this problem can be solved by simply releasing and reinserting the array.

A 96W1E+: note wells A3 and G11 are without medium or are not making proper contact with the array holder and show up red in the figure.
**Conditioning the Arrays**

The final step in the manufacturing process of the arrays involves exposure of the slides to oxygen plasma that both cleans the electrodes and sterilizes the chambers and lids. The oxygen plasma effectively burns away any residues of the insulating film and other organic compounds that have bound to the surface of the gold and leave the electrodes clean with a high capacitance. Over time, small molecules in the atmosphere, e.g. plasticizers, bind to the gold surface resulting in a decrease in capacitance. When the arrays are filled with culture medium, these molecules desorb and the impedance over time returns to acceptable values for protein-coated gold.

**Precoating with Proteins**

<table>
<thead>
<tr>
<th>Pre-Coating Electrodes with Defined Protein Coats</th>
</tr>
</thead>
<tbody>
<tr>
<td>The ECIS gold electrodes are pristine and have no coating when the arrays are shipped from Applied BioPhysics. When culture medium is added, the protein and other large molecules in the medium will immediately adsorb to the very wettable gold surface as they do to any standard tissue culture dish (note: this will take place even after reacting the gold with cysteine). If you wish to precoat the electrodes with fibronectin or other extracellular matrix proteins to enhance cell attachment and spreading, we suggest the following protocol:</td>
</tr>
</tbody>
</table>

Make up a solution of the protein at 200 micrograms per ml* in 0.15M NaCl. The use of phosphate buffer can seriously interfere with the adsorption of some proteins and should be avoided. If a buffer is essential, a mild Tris solution (e.g. 0.01M) is recommended.

To coat the electrode, simply place a small amount of the protein solution in the bottom of the well and allow it to remain in place for 10 minutes or more. If the protein is valuable, with the 8W1E arrays, it is only necessary to coat the small active electrode (250 micrometer diameter), and as little as 10 microliters or less can be carefully applied to this very small spot at the bottom of each well. Once the adsorption has taken place, an approximate monomolecular layer of the protein will be coating the surface and will not be removed by rinsing. If desired you can rinse the protein solution from the well with sterile medium, saline or water. Medium can now be added and inoculation or electrode stabilization begun. The effect of adsorbed proteins sometimes produces dramatic changes in the dynamics of cell attachment and spreading.

*If the protein is in plentiful supply, e.g. gelatin, solutions of 1 mg/ml can of course be used, but again avoid phosphate buffers and rinse the wells before inoculation.

- **We do not recommend drying the protein solutions in place** as it can foul or damage the electrodes.
Electrode Stabilization

Before running ECIS experiments stabilizing the electrode impedance values is recommended. Attention to this will minimize electrode drift during an experimental run.

The ECIS software includes a feature that allows one to condition or stabilize electrodes electrically before they are inoculated. This new proprietary method essentially replaces the need to use freshly etched electrodes. It can be used in conjunction with cysteine treatment (see below), but we have found that cysteine treatment is generally not needed with the instrument-stabilized electrodes. To use this feature we suggest the following order be used – note many of these steps are optional and are labeled as such. Please also note that if specific protein coats are to be applied they must be adsorbed before the electrode is exposed to the culture medium.

1. Cysteine treat the wells (optional)

2. Pre-coat electrodes with protein to be used (optional). As in all operations, if one wishes to adsorb a specific protein this must be done before the addition of serum-containing medium, as the gold and surrounding substrate will adsorb the first macromolecules they encounter.

3. Add 200 microliters of complete medium to each well and place the array in the array holder. Use Setup/Check to ascertain the array is properly connected.

4. Stabilize the electrodes.

5. A Check* (optional) can now be run to check the value of the cell-free wells.

*Upon checking the impedance values of the empty electrode you will notice the electrode stabilization procedure has elevated the capacitance and reduced the resistance of the electrodes to that associated with freshly etched or cysteine-treated arrays. Following electrode stabilization, the 1E well capacitance will normally be over 5 nF and over 50 nF for the 10E and 10E+ arrays. If you are planning to study the attachment and spreading of cells and these values have not been achieved, you may elect to rerun the Stabilize electrode feature before inoculation.
Cysteine Treatment (optional)

This involves treating each well with ~200 microliters of a 10 mM solution of cysteine in water (commercially available as ECIS electrode-stabilizing solution). After exposing the wells to the solution for ~10 minutes at room temperature, this solution can be aspirated from the well followed by at least two rinses of sterile distilled water. The wells can then be coated with proteins or have medium added for immediate use; alternatively, the rinsed wells can be allowed to dry and stored for future use.

Note – the cysteine treatment should be done before the application of any protein layers to the electrode.

The -SH residue of the cysteine will form covalent linkages with the gold, and this organic layer coating the gold will remain in place and maintain the electrode capacitance to that of new arrays.

As described above, it is recommended to use either electrical stabilization of electrodes or cysteine treatment before beginning ECIS experiments. If you are using cysteine alone, first treat the wells with cysteine solution, then, if desired, carry out the protein-coating procedure. If you plan to use the electrical stabilization feature, protein coat the wells, and then fill with medium before actuating the electrode stabilization feature.

Inoculation of Arrays

The base of the ECIS well has a surface area of about 0.8 cm² for 8W wells and 0.3 cm² for 96W wells. A typical inoculation to give a confluent cell layer would be approximately $10^5$ cells/cm²; however, this can vary considerably depending on the actual cell type being used.

To obtain an even coating of cells that will achieve confluence following attachment and spreading, we suggest preparing a monodisperse cell suspensions and using the following cell concentrations and volumes:

8W arrays: 400 microliters per well of cell suspension at ~$2.5 \times 10^5$ cells/ml,

96W arrays: 300 microliters per well of cell suspension at ~$1.2 \times 10^5$ cell/ml,
These volumes and the inoculation densities are only guidelines and can be changed without affecting the experiment. It is, however, generally suggested during measurements to have a well volume of at least 150 to 200 microliters for all array types – volumes below this could interfere with current flow and add small amounts of impedance unrelated to the cell measurement.

To obtain good reproducibility, especially using the 1E arrays, it is most important to obtain a uniform inoculation of the well bottom. What you want to establish is an even "snowfall" of cells upon the well bottom assuring that every small 250 micrometer diameter electrode in each well sees approximately the same number of cells falling upon its surface. This can be facilitated by following two important guidelines:

1. First, upon preparing the cell suspension, try to avoid clumping of cells. For some cell lines this is not difficult but for others, particularly if they have been attached and spread for long periods of time, clumping is not so easily avoided. What you want to achieve is a clearly monodisperse cell suspension.

2. Temperature can also contribute to an uneven cell distribution upon inoculation comes about due temperature considerations. If the cell suspension is colder than incubator conditions, the small ECIS wells will be heated from the bottom forming a convention cell, where medium rises in the center and falls back down the walls of the wells. Because of this flow, as cells attempt to fall down upon the central region, they are swept upward. The overall effect is that the cell density becomes reduced in the central regions of the well and a confluent layer does not form over the central electrodes upon inoculation. This problem can be largely avoided by making sure the ECIS array and cell suspension added to the wells are at incubator temperatures. We have even tried using cell suspensions warmed a degree or two higher than incubator temperatures with success.

If at any point in the inoculation process, the array is removed from the instrument, it is always necessary to do a Setup or Check upon reinsertion of the array in the holder to be certain contact with the slide has been properly reestablished.

Note: Although many cell culture studies are done using confluent layers, the Zθ system is able to measure the activities of sparse cultures and even detects the presence of single cells on the active electrode.
Acquiring Data
Using the ECIS Software

Clicking the ECIS screen icon will bring up the graphical interface shown above:

With the “Collect Data” button highlighted, click the **Setup** button to connect to the ECIS instrument. Once a successful connection to the instrument is made, the system is ready to begin impedance measurements.
Electrode Preparation

Before the arrays to be used for an experiment are loaded into the Station 16W, they may be first coated with proteins (for example gelatin, fibronectin, etc.) as described on page 16 and then filled with medium; alternatively, they may be simply filled with medium without applying a specific protein coat.

Initial Setup with 8W arrays

Slide the array(s) into one of the slots of the Station 16W and click the Setup button.

This will cause the instrument to make a series of rapid impedance measurements to determine if arrays are properly connected to the system. The results of these measurements cause the array diagram in the lower left hand position of the screen to change – lighting up green for properly connected arrays and red for the absence of an array or an incorrectly connected array.
If wells of an array light up red but are intended to be connected, try repositioning the array such that the nine gold square pads are properly contacted by the spring-loaded pogo pins. Repeat the Setup command. Remember that wells without medium will be measured as not connected.

**Initial Setup with 96 Well arrays**

Place the array on the sled of the 96W Station and slide the insertion lever all the way to the right moving the array into the device and making contact with the connector at the top of the plate. Click the Setup button; this will cause the instrument to make a series of rapid impedance measurements to determine if arrays are properly connected to the system. The results of these measurements cause the array diagram in the lower left hand position of the screen to change – lighting up green for properly connected arrays and red for the absence of an array or an incorrectly connected array.

If wells of an array light up red but are intended to be connected, try removing and reinserting the array by moving the lever to the left and then right. Repeat the Setup command. Remember that wells without medium will be measured as not connected.
Stabilization (optional, but recommended procedure)

We recommend stabilizing the wells, as this tends to clean the gold surfaces reducing unwanted drift in impedance during an experimental run. This process should never be activated for wells containing cells as cell damage may result. Upon clicking the Stabilize button, the system will first check to see that the type of array has been specified. This must be done before proceeding.

Once the type of array has been selected and the wells marked, stabilization will proceed after verifying the number of wells to be treated. Impedance values can be verified at any time by clicking the Check button (the default frequency is 4000 Hz for the Z-Theta and 16000 Hz for the Z, but any other frequency can be selected for this measurement). The results will appear and then become a permanent part of the comments for the experiment.
**Cell Inoculation**

Following stabilization, the array can be removed from the array station and inoculated with cells. When using arrays with only one active electrode per well (1E arrays) it is very important that the suspension be monodisperse, without clumps of cells, and that the temperature of the suspension be pre-warmed to incubator temperatures. This will help assure a uniform distribution on the substrate and the electrodes.

Once the array is replaced in the Station, another **Setup** should be run to check the connection to the array. This can be followed by an electrode **Check** to confirm the initial impedance values of the wells. The system is now ready to begin a time course measurement.

_____________________________________________________________________

**Note:** more details regarding electrode stabilization and cell inoculation can be found on pages 18 and 19 respectively.
Setting up Time Course Measurements

There are three different time course programs that can be selected.

- Multiple frequency (MFT) runs. With the Z θ instrument, the program will measure each at well at 11 different predefined frequencies for 8 well arrays and 7 different frequencies for 96 well arrays. The Z instruments use 8 frequencies for both types of arrays. The time between points can be selected, but the minimum time will depend upon the number of wells being measured, as the measurement requires a few seconds to complete each selected well. The minimum interval between points will be listed, but a longer time can be specified.

- Single frequency (SFT) runs follow selected wells and measure each at the frequency specified. As with MFT, the interval between points and the total length of the run can be specified in the menu if desired.

- Rapid time collect (RTC) can be run when rapid data collection is necessary. This measurement is useful to track very rapid changes in impedance (e.g. during signal transduction). Only a single well is followed with points taken rapidly – the default time between points is 0.2 seconds (5 points per second). The maximum data rate for the Z-Theta is approximately 25 samples per second. The total length of the run can be specified if desired, otherwise the instrument will continue to take and record data until the Finish button is clicked.

Once the Start button is selected, you will be given the opportunity to name the file where data will be stored, or you can use the name selected by the system which includes the date, the type of data and the number of the run for that date and type.
## Controls During Data Acquisition

During data acquisition, in addition to using the tool bar at the top of the screen to alter the displayed information (see details below) one can **Pause** data acquisition or **Mark** data files.

Clicking the **Pause** button will cause the instrument to break from data acquisition but continue to run the experimental clock (the time display will blink on and off when data is not being acquired). During this period one can manipulate the array or remove it from the Station to carry out drug addition, feeding, etc.

To resume data collection immediately, click **Resume Experiment**.

If the array has been removed from the Station during the Pause, it is very important to be certain the array is connected to the Station before resuming data acquisition. If one fails to have the array correctly positioned, a large meaningless impedance spike will be a part of the data set. To avoid this, before giving the final restart command, click **Check Connection** and look at the data to make sure it is similar to that the system has been collecting. The dialog shown to the right is for the Zθ, if a Z instrument is used, only the impedance will be reported.

If necessary, one can reposition the array in the Station until the data looks OK. Now being certain that connection has been properly made one can safely click the **Resume** button that will resume data acquisition.

Clicking **Mark** will place a mark on the graph and allow an associated comment to be entered.

Most of the menu items and toolbar controls will be locked during data collection to ensure stability of the system.
**Wounding and Electroporation (Optional)**

If your system is equipped with the Elevated Field Module (EFM) it is capable of electroporating or wounding the cells on the small active ECIS electrodes. In this mode, the current applied to the ECIS arrays results in a low voltage drop (a few volts), so there is no danger of electrical shock as in most electroporation equipment. In spite of the low voltage, the electric fields (V/cm) are high. This is due to the small geometry of the active electrode that results in most of the field being applied directly across the cell layer. These currents and the resulting electric fields are sufficiently high to form pores in the cell membranes and, when applied for longer periods of time, can wound and kill the cells situated on the small electrode.

To call up this function click the **Wound / Electroporate Setup** box and the following controls appear.

The boxes contain values for time, wound current (voltage for 1600R instruments) and frequency. The default values that initially appear have been selected based on the type of array being used. For electrical current, the default conditions are 1400 and 5000 microamperes for 1E and 10E/10E+ arrays respectively. Default values for time depend upon whether one wishes to wound (20 sec) or electroporate (0.1 sec), and finally for all operations, the AC frequency initially defaults to 60 kHz. These may not be the optimum values for the cell type being used, and it may prove advantageous to experiment with different values to refine conditions for different cell lines, medium, etc. For this purpose one can specify different times, and there are drop down menus to adjust the current and frequency. In general it is best to use high frequencies (>20,000 Hz) to maintain uniform high fields across the cells and to avoid any damage to the gold film electrodes of the array. If the type of array is changed during the process of wounding or electroporating, it is recommended to click again in the **Wound / Electroporate Setup** box to lock in the proper default values for that type of array.

Once these conditions are set, go to the Well Configuration panel and have check marks only in those wells to receive the high fields. When this is completed click in the **Activate** button. Upon doing this you will be reminded of the number of wells to be affected (just a check).

Clicking OK will commence the high field application to each well.
The option of delaying the application of the wounding (or electroporation) current is also available. This is useful if the cell layer is not ready for wounding but one cannot be present to run the wounding commands. In the example below we have chosen to have the wounding current applied at hour 10 of the experimental run.

Again, after choosing the time, current and frequency of wounding, indicate the wells to be altered and click **Activate**. After confirming that the number of wells is correct, the system is set to perform the delayed wound. The following dialog will appear to indicate how to cancel the delayed wound, or to perform a separate wound operation.

At any time before the experimental hour is reached (10 hours in this case) it is possible to abort the wounding action by clicking **Stop**.

To see the current settings for the delayed wound, just place the pointer over the **Stop** button.
Electric Fence (Optional)

The EFM module also includes the "Electric Fence" functionality. The Electric Fence (EF) applies a short pulse to the selected electrodes on a regular basis during data collection (e.g. every 5 minutes). This prevents the cells from growing onto the electrodes. When the layer is confluent the electric fence can be turned off and the rate of cell migration onto the empty electrodes can be monitored. This is particularly useful with cell types that may resist the normal wounding mechanism as the cells never have a chance to attach to the electrode. This also means that the data collection should begin and the electric fence applied as soon as the cells are added to the wells.

The Electric Fence setup is available from the Wound/Electroporate options. The desired wells should first be selected, then click Electric Fence. The default time interval is 5 minutes, and the EF will be applied after a full time point is collected. The frequency and current levels have been preset for the selected well type. Note that normal Wounding/Electroporate functionality and Pausing are still available while the EF is on, but these will temporarily interrupt the EF application.

It is recommended to select the wells and setup the Electric Fence prior to starting the experiment. Enter the interval and then click Electric Fence. The EF will not be applied until the experiment is started. When the EF is setup the button and selected wells will turn orange as shown below. To turn off the EF, click the Electric Fence button again.
Analyzing Data
Analysis of Data Files

Upon exiting data acquisition or opening previously acquired files, data can be graphed in a variety of ways. The key to many of these manipulations is the tool bar at the top of the screen.

Use of the tool bar icons

The use of these various icons will be described in groups.

The first four symbols determine whether impedance (Z), resistance (R), capacitance (C) or modeling parameters (RbaC) are plotted on the vertical axis. For the Z instruments only Z is available.

The next three symbols determine whether the horizontal axis is time (T) or, providing the data has been collected in the MFT mode, frequency (F), or a three dimensional plot (3D) where frequency and time form two of the axes.

These symbols allow one to zoom in on the data (+) or return to full scale (-). The small hand is for panning to drag the graph to regions of interest (note: clicking the Offset button below the graph will return the figure to its initial appearance). The next symbol is the data cursor – this allows one to click on and specify a particular point in the graph as shown below. Note that the x and y value of the point will be listed. This tool becomes particularly important in the modeling section of this manual.

The rotate icon is to aid in looking at 3D plots.

The next six icons modify the graph display. The first two allow you to choose between lines and/or points in graphing. The symbol with the triangular base calls up or removes marks (blue) that have been placed in the data set or have been automatically added for elevated field operations (red) or pauses in the data acquisition (green). The forth icon with the cyan triangle and wavy lines shows flow marks when using a pump system. Grid lines can be added or removed from the graph
with the grid symbol 📋. The sixth icon displays a legend that aids in identifying curves when several wells are being plotted 🌈. The last icon is a color bar for use with 3D plots 🌈.

The next two symbols allow one to change to log scales for the x and y axes and back to normal linear scales. The error bar symbol 🌈 plots or removes error bars on data that has been grouped and averaged (described below). These error bars are plus and minus one standard deviation and are plotted at regular intervals along the graph. The n/n₀ icon 🌐 normalizes the curve (divides all values by that obtained at the time listed in the zero time box). Note this defaults to time zero where all curves would be 1.00 at time zero. The n-n₀ icon 🌐 plots the difference between the data and the time zero reference. If both icons are selected, the calculation used is n/n₀ – 1.

The full screen symbol 🌐 takes whatever is shown in the graphic field and presents it filling the full screen; clicking this again returns to the standard graphing field.

The thumbnail view symbol 🌐 presents each well as a separate graph. From both this view, as well as from the well configuration in the lower left hand portion of the screen, wells can be selected or deselected. When the thumbnail icon is clicked again, the screen returns to the standard single graph just showing those wells selected. Wells not selected are shown with a gray background in the thumbnail view. The thumbnail view is most useful when using a 96W Station; typical thumbnail views are shown below for a 96 well plate.

The thermometer icon is for displaying recorded temperature and/or CO₂ data from supported water baths or incubators. This icon is only displayed when a dataset contains supported data.

The last icon is the lock icon 🗝️ which restricts the menu and toolbar items available when performing data collection.
**Time-Series Options**

**Zero Time** enables the origin of the x-axis to be moved to the specified time.  

**Normalize** duplicates the tool bar $n/n_0$ command and uses the Zero Time as the reference time.  

**Running Average** will smooth the data using the specified number of data points in the drop-down menu.  

**Bin Running Avg.** will reduce the number of data points by displaying every $n$-th point from the running average with length $n$.  

**Standard Deviation** will plot a bar graph showing the standard deviation of each selected well for the time interval specified on the graph.

**Averaging Multiple Wells**

The values plotted for any number of wells can be averaged. This is accomplished in the Well Configuration menu at the lower left side of the screen. Here we see eight wells have been measured (A). Below is an example of how to average wells 5, 6, 7 and 8 (B). After clicking on these wells, click the small box at the base of the menu. This activates the grouping controls. Now the group button (Grp) can be clicked, and the wells are averaged and represented on the plot as a single color (C).

This process can be continued to group wells 1 and 2 and wells 3 and 4 as shown.
Clicking the small center box mark will toggle between individual wells plots and grouped data.

To group wells differently, first click **Un-Grp** to ungroup the selected wells.

If **Grp** is clicked when all selected wells are already grouped, then a single super group of all selected wells will be formed.

The grouping feature is also used in an identical manner with 96 well plates.

If the original data is plotted with normalization activated prior to clicking the grouping check box, then the normalized data will be grouped and averaged. The normalization controls will then be inactive when viewing the grouped data. If the data is plotted without normalization prior to grouping, then the grouped data can be normalized.

**Error Bars**

Error bars representing plus and minus one *Standard Deviation* can be added to the averaged curves by clicking the $\pm$ icon. The bars are spaced at regular intervals with the default number of bars being 25. This can be changed from the menu **Edit | Number of Error Bars**.

Alternatively the *Standard Error of the Mean* (SEM) can also be plotted by selecting the option from the **Edit | Error Bars** menu. The SEM is the standard deviation divided by the square root of the number of data points used in the calculation.

Here we see a section of averaged data that has had the error bars inserted.
Grid lines can be added and legends to this figure with the appropriate icons from the tool bar. In the case we see wells A1, A2 and A3 have been averaged as indicated in the legend on the right.

Coloring the Wells and Graphs with 8 Well Array

The colors of the 8 well arrays can be customized either by selecting a different colormap from the Edit | Colormap menu. The current options are Standard, Alternate, Jet, HSV, Rows, Columns, Pairs, Eights and Manual. Select the one that best matches the array setup and graph display. To manually specify the color of each well, right click on the color swatch next to the well, select Change Color, and select a color from the color palette. The user specified colors can be recalled by selecting the Manual colormap option.

Coloring the Wells and Graphs with 96 Well Plates

Colormap

Because of the large number of wells, there are a variety of ways to color the wells (and their data plots) to end up with a color scheme such that wells can be easily distinguished and to aid in filtering and selecting relevant data. The system initially defaults to a vertical system where each column is a given color but the degree of saturation of the color changes for each well of the column resulting in 96 different shades. This scheme can be changed to horizontal, again with 96 shades. It is also possible to have the 96 shades presented across the two different diagonals of the pattern. Finally for experiments where all of the wells in a row or column have
identical experimental conditions, all wells of a row or column can be colored with the same color.

**Color based filtered data**

It is also possible to color wells and their graphs by filtering the data. The various filters are listed in a drop down menu. Wells can be colored based on the time course data of Z, R or C at the frequency selected and for the time interval selected. These filters include:

- size of start or end value
- maximum or minimum value over the time period selected
- maximum minus the minimum value (largest overall change)
- size of the rise or fall over a 1 hour period
- size of the integrated value over the time course selected
- size of the standard deviation of the parameter for the selected time

The filtered data can be colored in two different manners:

**Filter Value**: in this case, the smallest and largest value of all wells for which data was taken are used to define the end points of the color spectrum shown. Intermediate values are then chosen based on a linear scale between these two end points. With this system of coloring, it is possible that many wells (having values in a narrow range of the spectrum) will have similar colors.

**Order Index**: in this case, the filtered parameter for all wells used in the experiment are put in rank order from lowest to highest. These are then spread evenly over the spectrum giving each well a unique color.
**Selection and Deselection of wells**

Once a filter has been implemented, various wells can be added (selected) or deselected and the colors will adjust automatically to the new collection of wells. In addition, filtered values can be used to select or deselect wells for plotting. This is accomplished using the threshold control.

Click the **Threshold** box and move the slider across the spectrum from blue to red. As this is done, at first all of the wells will be selected, but then the lowest value wells will begin to be deselected and the colors will change accordingly. As the slider is moved further to the right, additional wells will be deselected. In this manner one can narrow in on the desired wells. Clicking **Invert** will reverse the pattern and the previously deselected wells will be graphed.

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**Different Means to Plot 96 Well Data**

With both the 16 well and 96 well stations, data can be plotted as a full graph, as well as separate graphs (thumbnail plots). In both of these methods, data (e.g. Z, R and C) are plotted as a function of time.

With 96 wells it is useful to also plot the value of filtered data as a function of well position. To this end there are several additional plotting formats that are listed below the full graph and thumbnail graph plot choices.

These various methods are shown below where location of the wells and the value from the filter are displayed in different manners.
3D Map of wells

2D Map of Columns (or rows)
**Array Map**

**Overlay Map**
Returning to the well configuration colormap

Once wells have been selected using the filters described above, if desired, the filter can be turned off and the well colors set by the colormap in the well configuration field at the lower left hand side of the display.

Exporting Data and Graphs

Data can be exported in a variety of formats and imported into programs such as Microsoft, Excel or PowerPoint. From the File menu select Export Data and then one of the following options:

All Data – exports the entire dataset as a CSV, ABP or MAT file.
Selected Wells/Time – exports only the selected wells and time range as a CSV, ABP or MAT file.
To Excel (Selected) - exports only the selected wells and time range to an Excel XLS file.
Graph Data – exports the data that is currently displayed on the graph as a CSV file.
Compress/Zip – will zip the file and add the .zip extension to make the file smaller for transferring.

The graph itself can be exported to the clipboard as a bit map by clicking File | Export Graph. The Display Options at the lower right can be used to set the graph dimension and the aspect ratio. The X and Y axis limits can also be set.
Clicking Export will copy the graph to a new window where the graph can be saved, or arranged with other exported graphs.
Clicking Redraw will force the current graph to update.
How to Make Use of the Multiple Frequency Time Course Data

Both the Z and the Zθ instruments can take time course data at multiple AC frequencies. In the case of the Z instrumentation there are 8 preset frequencies (1k, 2k, 4k, 8k, 12k, 24k, 32k, 64k Hz). For the Zθ system there are 11 frequencies for 8 well arrays (see figure below for listing) and 7 frequencies for 96 well arrays (the four lower frequencies are not used). In general, unless very rapid collection of data is required, we recommend all experiments be done using these multiple-frequency options. In this manner data is likely to be taken at or near the optimum frequency for an experiment. In addition, one is also able to perform an analysis of frequency scans and, with the Zθ instruments, modeling to see time course changes in barrier function (see below) can be conducted.

Determining the best AC frequency by examination

If one is gathering data using the multiple frequency time (MFT) course settings, it is easy to determine the frequency where cells show the maximum response to a treatment or other condition. Ideally, compare the ratio of the cell to cell-free measurement. The maximum response for Z, R and C will likely occur at different frequencies.

By default the optimal frequencies are:
- Resistance (R) @ 4000Hz
- Impedance (Z) @ 16000Hz
- Capacitance (C) @ 64000Hz

This can be examined by simply changing the frequency and examining the data. The frequency can be changed by clicking on the check box next to the listed frequency at the lower left of the graphing area. From this listing simply choose the AC frequency at which you wish to display the data and the graph will automatically update. The frequency list shown below is for the Zθ instruments.
Analyzing frequency scan data using the ratio of cell to cell-free data

The frequency where cells have the largest effect upon the impedance parameters can be determined by directly comparing cell data with that obtained from cell-free electrodes. Again, it is first necessary to have gathered MFT data (the recommended protocol for most experiments).

First you must choose the cell-free data. There are several ways in which this can be done. If cell-free data is in the data file you are analyzing (e.g. a cell free well or a cell-free point at the start of an inoculation) you can simply click on the Find button in the Modeling menu and the software will choose the well and time point it finds most representative of cell-free data.

You can also choose a particular point by moving the data cursor to that point on the graph and clicking the Set button. If you have no cell-free data in the file being analyzed, open a file with data from the same type of array and medium where cell-free points are available. Now, using Find or the data cursor, obtain the cell-free data as described above. When you return to the original data set of interest, transfer this cell-free information by clicking the Get button.

With the cell-free data selected, next select a point in the data set where the cells are thought to be confluent or nearly confluent. Again with the data cursor, choose a point. Now click on the F (frequency) button in the tool bar and the display will change to display both the cell free data and the cell data for the point your have chosen. If both are not displayed chose Both in the Freq. Scan Modeling controls.

Note that the Cell-Free curves are drift corrected such that the capacitance at the lowest frequency matches that of the Cell measurement. Therefore multiple Cell-Free curves may be displayed.
Illustrated above is a plot of frequency vs. resistance presented as a log-log plot. The upper trace is the data for the cells – the lower the cell free. Examining the curves, it is apparent there is a larger separation between $10^3$ and $10^4$ Hz. To better determine the maximum difference, click on the Ratio (C/F) selection. This will now show the ratio of the cell to cell-free data versus the log of the AC frequency. Normally both the R and Z plots of this sort will show a peak. Moving the data cursor to this region of the graph will list the AC frequency. The C ratio plot (Zθ systems only) will always show the maximum effect of the cells at the highest frequency measured, where the cells very effectively block free flow of current to the gold electrode. Notice this ratio is less than 1.0 as C is decreased by the presence of the cells. Below are the three ratio plots (Z, R and C) obtained for two wells that were selected.
With these calculations done, click 3D to see how these ratios change during the time course of the experiment. The data shown above is an experiment where cells were inoculated at time zero. Here we see the change in the cell to cell-free resistance ratio as a function of both time and the log of the AC frequency. (Note: the color and texture of the 3D graphs can be changed by clicking on the Plot Lines and Plot Points tool bar functions)
Modeling Data
(1600R and Zθ Instruments Only)
General notes about understanding the ECIS model

To completely understand the ECIS model, a mathematical background that is often not part of the training of cell biologists is necessary. That being said, this does not mean that a reasonable understanding and appreciation of the model parameters that are detected with an AC impedance measurement cannot be gained. Just as a full understanding of optics is not necessary to properly use a microscope, a background in electrical engineering is not needed to properly use ECIS and its modeling capabilities. In using the ECIS model, as in most of science, the work that underpins its derivation, has been peer reviewed and tested by many scientists since its introduction in 1991. It is an accepted means to explore cell morphology and allows one to use the impedance data to calculate parameters that can be directly related to cell morphology. The measurement of complex impedance and modeling is unique to ECIS and is not a part of normal TEER measurements or simple impedance measurements.

If you wish to study this modeling approach in detail, please consult the PNAS paper [Giaever and Keese, PNAS 88, 7896 (1991)], where the model is developed. In the paper, after developing the model, its validity is confirmed by comparing calculated values of resistance and capacitance of cell-covered electrodes with those measured using ECIS over three magnitudes of AC frequency (100 to 100,000 Hz).

A simplified description of the model and its use is described below.

The ECIS model

There are some restrictions that apply in using the ECIS model. First, the model is only valid for confluent cell layers (no open regions in the gold); in addition, to employ the model it is also necessary to measure the resistance and capacitance of cell-free electrodes and cell-covered electrodes at multiple AC frequencies. In the model cells are represented as disk shaped objects having insulating membrane surfaces and filled with conducting electrolyte as diagrammed in the figure.
Since the focal adhesion plaques of cells represent a very small fraction of the total ventral surface of the spread cell, cells are treated as hovering a small distance (h) above the electrode which serves as their substratum. In calculating how the impedance changes due to this cell coverage, we assume that the resistance and capacitance of the gold surface itself does not change but rather that the measured capacitance and resistance change, because the cells alter the path of the current flow as shown by the arrows in the figure. The main source of the impedance change can be attributed to the fact that some current must flow through the narrow spaces between the cells and the electrode, and that current then flows out through the narrow spaces between the cells (barrier resistance). Some AC current can pass directly through the cells since their insulating membranes serve as capacitors themselves (suggested with the dotted arrow in the figure). The model determines three parameters by fitting the theory to the experimental curves. These parameters are:

1. Barrier function, \( R_b \), which can range from 0 to approximately 100 ohm-cm\(^2\) or more. This is an important parameter for studies of endothelial and epithelial cells permeability.

2. Another parameter is called alpha (\( \alpha \)). Alpha is associated with the impedance due to the constricted current flow beneath the cells. It is related to the radius of the cell and its average height above the substratum (h). ECIS provides perhaps the most sensitive means available to study changes in this region beneath the cells. If one estimates the radius of the cells from microscopic images, it is possible to use alpha to evaluate the average distance between the electrode (the substrate) and the basal membrane of the cell. The units of alpha are (ohm-cm\(^2\))\(^{0.5}\).

3. Finally, it is possible to determine the average capacitance of the cell plasma membranes, \( C_m \). With the current model, it is not possible to distinguish between the apical and basal membrane and so this number, in microfarads/cm\(^2\), treats the two membranes as having identical values.

These three parameters are automatically calculated from data with the ECIS software as described below.
Note: For cell spreading studies we recommend trying capacitance measured at >40,000 Hz [Wegener, et. al., Exp. Cell Res., 2000]. At this high frequency for many cell types, the capacitance will change in a nearly linearly manner with the amount of open area of the electrode, and ECIS basically gives back coverage information similar to that observed in a microscopic view of the cells. A single open electrode will have a capacitance at this frequency of about 6.0 nF. With a complete confluent layer of cells in place, this will be reduced to maybe 0.8 nF. (The capacitance does not go to zero in this situation, as some current capacitively couples through the cell’s plasma membranes which act as capacitors in series with the electrode.)

If this linear dependence upon cell capacitance and open electrode area is established, then one can determine the fractional area covered by the cell population. If one has a cell layer giving a capacitance of 2.5 nF, the fraction of the electrode covered with cells is given approximately by:

\[
\text{Fractional area} = \frac{C_{\text{open}} - C_{\text{cells}}}{C_{\text{open}} - C_{\text{confluent}}}
\]

Or in this case,

\[
\text{Fractional area covered by cells} = \frac{6.0 - 2.5}{6.0 - 0.8} = 0.67 \ (\text{or 67%})
\]

Please note that this means to determine the fractional coverage is true for most cell lines. With any untested cell line, ECIS coverage measurements should always be initially verified with microscopic observations.
**Modeling Software**

The ECIS Zθ software can calculate time course changes in the three model parameters from time course data taken using multiple frequencies (MFT files). To accomplish this it is first necessary to specify the impedance of a cell-free electrode to be used in modeling the time course data. The source of this data can be from the file that is to be modeled or from any other file providing it is using the same type of array (1E, 10E or 10E+).

The modeling options can be found by selecting the ‘Analyze’ button. If using 96-wells, also select the ‘Modeling’ radio button.

Pressing the ‘Model’ button, will bring up a dialog to simplify the process of specifying the cell-free reference. Alternatively, the ‘Find’, ‘Set’, and ‘Get’ buttons can be used to manually define the cell-free data.

Before beginning modeling:
- Select only the wells to be modeled
- Also select any cell-free wells
- Select only the desired time-range to be modeled
- If required, use the data cursor to select the cell-free reference well and time-point.

Note: The impedance of the cell free reference should be less than the data to be modeled (at all frequencies). The software will automatically correct for any capacitance drift.

![Modeling dialog](image)

*Opens the Modeling dialog that simplifies modeling setup and processing.*
Default display of modeling dialog:

In specifying a new **Cell-Free Reference** there are 3 options to choose from:

- **CFR1: Media-only well**: Use when at least one well is left cell-free during the experiment (i.e. media only). This is recommended to get an accurate reference and to be able to track any changes in the reference over time.
- **CFR2: Well is cell-free at specified time**: Use if part of the experiment run contains cell-free conditions (e.g. at the start of experiment just after inoculation, or immediately after wounding)
- **CFR3: All wells are cell free at specified time**: Use specified time point but use cell-free conditions in each well. Each well will have a different reference.

With each option use the **Find** button to automatically find the best cell-free well and/or time-point from the selected wells and update **Current Selection**

Alternatively if the Cell-Free Reference has been previously set use **Previously Set Reference**:

- **PSR1: Use currently set reference**: Use cell-free reference already set from previous modeling
- **PSR2: Get reference Set in another dataset**: Use cell-free reference that was just set using **Set Ref** button from another dataset
- **PSR3: Use factory default**: Use factory reference for the particular array type (use only if no other cell-free reference is available)
Freq. Plot  
Displays the current cell-free reference frequency scan. The scan should be monotonically decreasing and level off at the highest frequencies.

Set Ref.  
Sets the cell-free reference and closes the dialog, allowing the reference to be transferred to another dataset.

Model  
 Begins the modeling processing for the selected wells and time range, using the specified cell-free reference. This can take some time depending upon the amount of data to process, and the display will update periodically.

Display of Cell-Free Reference

To manually specify the cell-free reference, the ‘Find’, ‘Set’, and ‘Get’ buttons are available.

Selecting the cell-free data

If the data set being analyzed has cell free data you wish to use, simply click Find, and a point will be automatically selected. If the file containing the data to be modeled has cell-free information at time zero (just prior to cell-inoculation), simply clicking the Set button will choose this time (time 0) and use the values obtained for each well when that specific well is being modeled. If cell-free data is found
later in the same file – for example by trypsinization followed by data collection - one can specify this point with the data cursor.

The cell-free data will now be used when doing the time course calculations for all of the wells when modeling is called up.

**Getting cell-free data from another data set**

It is also possible to take the cell-free data from another experimental run. Simply call up a file with cell-free data using the same type of array as the data to be modeled (1E, 10E, 10E+…). Now, as described above using the **Find** feature or choosing the point with the data cursor, select the cell-free data and click **Set**. To continue with modeling it is necessary to now return to the data set of interest and click **Get**.

**Choosing the data to be modeled**

Next, with the data set of interest open, choose the well(s) to be modeled, and using the Offset and Range sliders beneath the graph, limit the starting time and the length of time to be modeled respectively. The modeling calculations assume that there are no areas of the gold electrode freely open to solution, hence, only data where the cell layer is confluent can be correctly modeled.
Clicking the Fit T Range button, will begin the calculations.

As the calculation proceeds you will see the various values of the model parameters displayed both in graphical form and in the numerical display boxes.
The Validity of the Modeled Data

The lower the values for Drift Correction and Model Fit RMSE (root mean square error), the better the model has been able to fit your data.

Remember, the model is only valid for confluent layers of cells – should openings be present in the cell monolayer that can be easily discerned in a microscopic view, Rb may be correctly listed as zero, however, the membrane capacitance will be artificially high* and alpha values may not be correct. Model parameters falling in this category should be disregarded.

*Membrane capacitance calculated for confluent cell layers is rarely greater than 4 microfarads/cm².

To view the model fit over time with respect to Z, R or C, select the appropriate time view and check ‘Plot Model Fit’. The model fit will be plotted as a dashed line.

In addition to looking at changes in the model parameters versus time, one can plot impedance, R or C versus the log of the frequency at a given time point and see how well the calculated values fit these curves. Change to frequency plots using the tool bar at the top of the screen.

One can now look at the experimental data for one time point and show both this and the cell-free data.
You can now “slide” along the data set using the offset button to find an area of interest, or select the data point to be modeling from the time course data using the data cursor function. Upon clicking **Fit Current** and checking the **Plot RbA fit** box, the values for the parameters will be listed in the Modeling Results, and circles will be added to the graph showing the calculated values for the frequency scan data.
By clicking **Auto fit**, data will be automatically fitted as the offset value is changed with the slider beneath the graph.

Clicking the **Ratio (C/F)** will show the ratio of the cell data divided by the cell-free data for the impedance, R or C. These data do not require modeling and are valid for any point in the data set. The ratio data can also be presented in 3D, where the ratio is plotted as a function of time and the log of the AC frequency.
The ratio data can also be modeled where circles show the calculated ratio values, - but at the risk of overstating this - please keep in mind that these modeled points are only relevant for confluent cell layers.
Menus
**File Menu**

**Open...**
Launches the standard ‘File Open’ dialog to load data files. Multiple files can be selected and loaded at once.

The following file formats are supported:
- ABP: Standard file from latest ECIS software
- ABP.ZIP: A zipped ABP file
- CSV: Comma separated value file exported by ECIS
- TC: Old ECIS 1600 file
- AB: Old ECIS 9600 file
- MAT: ECIS Matlab file
- FIG: Matlab Figure file

Please contact ECIS support if you cannot open a valid ECIS file.

If you wish to import an ECIS 1600R file you must first convert it to CSV using the ‘Import 1600R data...’ function (see below).

**Database...**
Opens the file database which stores information about recently used datasets. The ‘open folder’ icon on the toolbar also opens the database.

To load a dataset, click the checkboxes under ‘Select’, and click ‘Load’. For files not in the database, click ‘Browse...’, to launch the File Open dialog. Once the files are selected, click ‘Load’.

---

**ECIS File Database**

<table>
<thead>
<tr>
<th>Select</th>
<th>Directory</th>
<th>File</th>
<th>Type</th>
<th>Hours</th>
<th>Wells</th>
<th>Date</th>
<th>Summary</th>
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<td></td>
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</table>
The entries in the database can be sorted using the drop-down menu at the bottom left. The sort order can be reversed by checking the ‘Reverse’ option.

Selected entries can be cleared from the database using the ‘Clear’ button.

**Recent files**
Allows one of the 10 most recent files to be quickly loaded. Selecting ‘<more…>’ opens the file database.

**Import 1600R data…**
Uses the previous 1600R software to convert files to CSV prior to loading. This process is only required once for each file, as File | Open can then be used to load the CSV. The previous 1600R software must be installed in the default location (C:\ECIS1600R) (PC Only).

Supported files are:
- AD: Additive
- AT: Attachment
- FR: Frequency Scan
- MI: Micro-Motion

Please contact ECIS support if you cannot open a valid ECIS file.

**Open demo data…**
Launches the file open dialog in the demo data directory (if demo data is available).

**Export Graph…**
Copies the current graph display to a new window, which can then be resized and exported in a variety of formats. The graph can be reloaded (but not the data), if exported as a FIG.

**Export Data…**
Enables the current dataset to be exported in a different format such as CSV, or Matlab (MAT). A modified dataset can also be resaved as ABP to retain maximum data precision.

*All Data…*
Exports entire dataset.

*Selected Wells/Time…*
Exports only selected wells and current time range.

*To Excel (Selected)...*
Exports to Excel XLS format the selected wells and time-range. Requires Excel to be installed with valid license (PC Only).

*Graph Data…*
Exports actual data values on current graph as CSV.
Compress/ZIP...
Compresses current dataset into a zip file with extension .zip.

Copy To...
Copies the original data file to a new location (e.g. external drive). Faster than re-exporting.

Close
Close current dataset.

Exit
Close all datasets and exit application.

Edit Menu

Copy graph
Copies current graph to clipboard.

Export Graph...
Copies the current graph display to a new window, which can then be resized and exported in a variety of formats. The graph can be reloaded (but not the data), if exported as a FIG.

Copy comments
Copy dataset comments to clipboard.

Clear comments
Clear dataset comments

Full screen
Hide GUI components and have graph take entire window.

Default Well Info
Set default column headings for well info

Copy Well Info
Copy well info so it can be pasted into a different dataset

Paste Well Info
Paste well info into current dataset (dataset must be in data collection mode)

Append Dataset
Append a second dataset to the end of the current dataset. Both datasets must have the same number of wells and frequencies. This is useful if an experiment was interrupted, and was restarted creating a second dataset. The time gap between datasets will be included for accurate display.
Combine Datasets
Enables multiple 16 well datasets to be combined into a single dataset using the 96 well format (up to 6 datasets). The datasets must have the same number of frequencies and should be of the same array type. The data is displayed relative to the experiment start time.

Color Palette
Allows the colors associated with each well to be changed. Current options are Standard, Alternate, Jet, HSV, Rows, Columns, Pairs, Eights and Manual. With 8 well arrays, right-click on the color swatch in Well Configuration and select **Change Color** to manually select the desired color.

Error Bars
Specify what the error bars signify. Either 1 Standard Deviation or 1 Standard Error of the Mean (Standard Deviation divided by the square root of the number of wells used in the calculation).

Number of Error Bars
Set the number of error bars when displaying grouped data (default is 25).

Use Graph Time Format
Display the Time axis of the graph in Days, Hours, Minutes and Seconds.

Filter noise spikes
Filters out individual noise spikes that exceed 1% of the surrounding values and replaces them with the median of the neighborhood. This is only typically required with old 1600 instrument data.

Temperature/CO2
Displays saved temperature and/or CO2 when collected from supported water bath or incubator.

Display
Display the temperature/CO2 data. The following display options can then be toggled.
- All Temp. – display all temperature data
- All CO2 – display all CO2 data
- Set Temp
- Water Bath Temp.
- Probe Temp
- Set C02
- Actual CO2
- Toggle oC/oF – toggle between degrees Celsius and Fahrenheit

Auto Z/R/C Freq. Select
Automatically change the frequency when selecting Z, R and C.
- Resistance (R) @ 4000Hz
- Impedance (Z) @ 16000Hz
- Capacitance (C) @ 64000Hz
This is where the measurement gives the greatest response.
**Running Avg. Size**
Manually specify the number of data points to be used when calculating the running average.

**Plot Style**
Manually specify line style, line width and marker style for each well plotted (8 and 16 wells only).

Line Styles:
- solid
- dotted
- dash dot
- dashed

Line Width: In pixels

Marker Styles:
. point
○ circle
x x-mark
+ plus
* star
s square
d diamond
v triangle (down)
^ triangle (up)
< triangle (left)
> triangle (right)
p pentagram
h hexagram

Once the plot styles have been manually specified, they can be all applied (Use All Styles) or individually (Use Line Style, Use Line Width, Use Marker Style).

**Set Zero Time**
Manually specify the zero time of the graph from the dataset.

**Legend**
Set the legend entries for each well. Use the Legend toolbar button to toggle the Legend display.
Acquire Menu

Setup new expt.
Setup a new experiment. Same as the Setup button or pressing ‘Ctrl-S’. Can be used if the Setup button becomes disabled for some reason.

Activate all wells
Enables all the wells to be selected by overriding the automatic selection performed during Setup. The wells will turn from red to green, but must be manually selected to be included. It is recommended that a Check be performed to confirm that the wells have valid impedances.

Resume Paused expt.
Resume a previously paused experiment. Same as the Resume button and can be used if the Resume button becomes disabled for some reason.

Set output directory...
Set the default output directory for saved data files. A suggestion is to create and use ‘C:\ECISData’.

Find instrument
Search all serial ports and look for any ECIS instrument. A dialog box will appear to select the model of ECIS instrument for faster searching. Select ‘Unknown’ if unsure.

ECIS Instrument Options...
Enter temporary and/or permanent codes for ECIS options such as EFM and Flow modules.

Default Expt. Type
Select MFT, SFT or RTC as the default experiment type when the software starts.

Set ECIS COM Port
Manually set the COM port number that the ECIS instrument is connected to.

Set Flow COM Port
Manually set the COM port number that the ECIS Flow unit is connected to. Assumes Flow/Pump unit is connected to Accessory Port 1 on the ECIS instrument.

Set Scan Frequencies
Set the frequencies to use for the MFT experiment type:
- Manual – Enter the frequencies in Hz from low to high (maximum 16 entries)
- 4/16/64 kHz – Quick 3 frequency MFT experiment
- Defaults – Default frequencies for instrument and array type
**Set Water Bath/Incubator COM Port**
Manually set the COM port number that the Water Bath or Incubator unit is connected to. Assumes unit is connected to Accessory Port 2 on the ECIS instrument.

**Select Water Bath/Incubator**
Specify the supported model of Water Bath or Incubator.

**Expt. Time Limit**...
Specify the total time the instrument should collect data during an experiment. By default the value is 0 which indicates the experiment will run until stopped by the user.

**Use mini-array format**
Specifies that the array being used is in the mini-array format, which is rotated compared to a normal 8 well array. The Well Configuration panel will show the wells arranged in the new format. Both A and B holders must use the same array format.

**Update Instrument Code**...
Enables the internal instrument code to be updated using a key provided by Applied BioPhysics technical support.

**Expt. Sounds**
Enable experiment sounds through internal computer speaker.

**Deep Diagnostics**
Perform deep diagnostics on internal instrument hardware. Not available with all instruments.

**Saver**
Available with ECIS Z (version MH4) with the experiment saver installer:
- Directory: Get current directory of saved experiments
- Download Expt: Download specified experiment

---

**Help Menu**

**Application Help**...
Open the help file for the application (PC only).

**Show expert toolbar/menu items**
Activate a variety of toolbar and menu items that should only used by experienced users. These are often undocumented or for specialized purposes. For example, two additional toolbar buttons are displayed that enable the Reactance (X) and Phase (θ) of complex data to be displayed.

**Getting started**...
Open the ‘Getting Started’ PDF.
**Manual**
Open the PDF manual.

**Open Log File**
Open the Log File that contains any error messages generated by the software. Customer Support may request this file for debugging purposes.

**EdgePort USB**
- **Configuration Utility:** Open the EdgePort Configuration Utility.
- **Install Driver v5.30:** Install the latest driver for the EdgePort USB to Serial Device. The default location is C:\Edgeport Drivers. It is recommended to:
  - First unplug the USB cable.
  - Uninstall the previous driver if necessary and reboot.
  - Install the driver.
  - Plug in the USB cable
  - When prompted to search for a driver, specify manual search and select the directory (e.g. C:\Edgeport Drivers). Repeat if prompted multiple times.

**Check for updates**
Check for any update files available via the internet. If any update is to be installed, the current running application must be closed first.

**www.biophysics.com**
Open the Applied BioPhysics website in a browser window.

**License Agreement**
View and accept the software license agreement.

**About...**
View software and hardware version information, and support contact details.
Flow Module (Optional)
General Information

The optional flow module allows one to study cells under flow condition while simultaneously making ECIS measurements. The heart of this system is the ECIS Flow array that has eight ECIS electrodes on the floor of a flow channel that is 5 cm long and 0.5 cm wide with a height of 400 micrometer.

Flow array inoculation
The suggested protocol listed below is that suggested by ibidi Integrated BioDiagnostics, the manufacturer of the channel used with the ECIS flow array.

- Prepare your cell suspension at the desired concentration. Depending upon your cell type, application of a $2-7 \times 10^5$ cells/ml suspension should result in a confluent layer within the channel in 2-3 days.

- If desired, the floor of the channel can be pre-coated with protein following the information provided earlier in this manual for coating standard arrays.

- Method 1: Apply 100 microliters of cell suspension into the end of the channel allowing capillary action to fill the narrow space. Quick dispensing helps to avoid trapped air bubbles. Inject 600 microliters of cell-free medium simultaneously into each reservoir at the ends of the channel to avoid flushing out the cells; avoid tilting the cell to prevent unwanted removal of cells from the channel.

- Method 2: Alternatively, after adding the 100 microliters of cell suspension, one can place the slide in the incubator for the time required for cells to attach to the surface. Once the cells are in place, 1 ml of medium can be placed in one reservoir that will then flow through the channel to replace the channel volume and effectively feed the cells.

- Cover reservoirs loosely with the supplied caps. Do not close them completely until connecting to the pump, as this will prevent gas exchange. Incubate as usual.

Pump and Reservoir setup for flow experiments
The Flow module supports either the Applied BioPhysics pump controller, or the Heidolph Pump Drive (model 5201). The serial cable should be connected to Accessory Port 1 on the ECIS instrument. The Flow module also requires the feature be activated in the software either by a code or by factory programming the instrument. Please contact Applied BioPhysics if you cannot access the flow/pump device or feature.

The location of the pump can either be outside or inside the cell incubator, as the unit is designed for operation in the high humidity of the incubator. If planning to use the incubator space, one should investigate whether the modest heat output of the pump will prevent accurate control of incubator
temperature. We suggest running the pump at its maximum RPM within the incubator for a 24-hour period and observing that the specified incubator temperature is maintained.

All tubing, fittings and valves supplied with your ECIS flow system can be sterilized by standard autoclaving (but not the flow array which is supplied sterile with sterile caps). We suggest assembling as much of the system as possible before autoclaving to minimize any contamination upon final setup. The photo below shows a suggested setup using a Heidolph pump.

The Applied BioPhysics pump controller unit with two pump motors are shown below.
There are two reservoir bottles supplied with the system. Referring to the Heidolph image above:

- The first (left above), referred to as the medium receiver, has three connections - two carrying the flow of medium and another that vents to the air space in the bottle (with an in-line filter if desired).

- The other, referred to as the flow damper, has two connections. This bottle and connectors must be tightly sealed during a run and serves to smooth minor pulsation in the flow resulting from the peristaltic pump action before entry of the medium into the ECIS flow array.

The suggested tubing for the pump head is 1/16” ID (0.8mm) and is supplied with the system. Using a reducing barbed connector, this connects to 1/8” ID tubing (used throughout the outer loop), and this tubing connects to the inlet of the flow damper through an integral ferrule fitting assembly (IFFA). The outlet of the flow damper is connected through another IFFA tubing that connects to one side of the ECIS flow array. The other side of the flow array connects to the inlet of the medium receiver, and the outlet of the medium receiver goes through another reducing barbed connector to the inlet of the pump tubing to complete the loop. The ECIS flow array is held in the ECIS array holder where electrical contact is established.

When using the ECIS program to control the pump, the flow, when facing the pump head, is always from left to right (clockwise rotation of the pump head).

Note that because the flow damper bottle experiences some mild back pressure, it is essential that all connections to this bottle be air tight. To facilitate these connections, the IFFA devices are used to both enter and leave the flow damper bottle. In addition, the flow damper bottle cap should be firmly tightened and all tubing fully over the barbed connectors. We suggest that flow enter the array holder as shown – on the end opposite the pogo pin connections in case a leak should develop at the flow array entrance.

To connect to the ECIS flow array, a barbed fitting is pushed through the thin plastic in the center of the caps supplied with each flow array. The cap in turn is pushed firmly down to make the final seal to the flow array.

*Caution: If it is necessary to remove cap on the flow array after it has been firmly set in place, do not lift on the cap when holding down the thin lower ECIS slide –the upper and lower slide may separate. Instead, remove the cap by lifting it off while pressing firmly on the upper plastic surface that provides the flow channel.*

If using the Heidolph pump, it can both be operated manually using the buttons on the front panel or from within the ECIS software that is described below. When using programmed flow, the pump must be in stopped mode (power should be on but the light by the Start/Stop button off). For any additional information regarding the Heidolph pump, please consult the manual provided by the manufacturer.
**Pump Speeds (flow rate and shear stress)**

The Applied BioPhysics pump can run between 0 and 40 RPM.

The Heidolph peristaltic pump can be run at any rate between 5 and 120 RPM and, of course, can be stopped (0 RPM). If one attempts to input a value between 0 and 5, a **RPM Correction** message will remind the user that these values are not permitted. Similarly if one calls for a pump rotation greater than 120 RPM a menu will appear stating the value is too high.

The maximum flow rate depends upon the tubing size, while the shear stress also depends upon the height of the flow chamber. For a 400um well height, the follow tables apply:

<table>
<thead>
<tr>
<th>Applied BioPhysics Pump Controller</th>
<th>Heidolph Pump Drive 5201</th>
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</thead>
<tbody>
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<td><strong>Tubing Size (mm)</strong></td>
<td><strong>Max Flow Rate (ml/min)</strong></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>0.4</td>
</tr>
<tr>
<td>0.8 mm</td>
<td>1.2</td>
</tr>
<tr>
<td>1.6 mm</td>
<td>4.4</td>
</tr>
<tr>
<td>2.4 mm</td>
<td>9.6</td>
</tr>
<tr>
<td>3.2 mm</td>
<td>16.4</td>
</tr>
<tr>
<td>4.0 mm</td>
<td>23.6</td>
</tr>
</tbody>
</table>

**Calculation of shear stress**

Please note that although the rate of flow is determined solely from the pump’s tubing size and the head rotation rate, the **shear stress** is calculated assuming one is using the standard ECIS flow array. In this array, the flow is directed through a channel 0.5 cm wide and 0.04 cm in height; the length of the channel is 5 cm. Flow throughout all but the very ends of the channel is laminar in nature. The approximate shear stress is calculated using 0.009 P for the viscosity of the medium and employing the following equation:

\[ \tau = \frac{6u\mu}{H} \]

where \(\tau\) is the shear stress in dynes/cm², \(u\) is the velocity of flow through the channel in cm/sec (the flow in ml/min divided by 60 and divided again by the cross sectional area of the channel in cm²), \(\mu\) is the viscosity of the culture medium, and \(H\) is the height of the channel (~0.04 cm).

Decreasing the height of the flow chamber by a factor of two, will increase the shear stress by a factor of four.
Programming the Pump for Use with ECIS

After the cells have been inoculated and the sterile flow system aseptically connected to the ECIS flow array, you can both control the pump and collect ECIS data from the eight active electrodes within the flow channel. Before beginning programming, be certain the pump is on, but that the Start/Stop light is off (Stop). It is also important that the medium be thoroughly warmed to minimize the formation of air bubbles within the flow channel.

Connecting to the Flow/Pump device

The Flow/Pump feature is activated in the software if the ‘Flow/Pump Setup’ checkbox is available in the Data Collection Setup panel. Ensure the flow/pump device is turned on and connected to the ECIS instrument, then click the checkbox. This will cause the software to connect to the device. If this is the first connection, it may be necessary to specify the model of flow/pump device. If a successful connection is made, then the quick flow configuration panel will appear.

From this panel the pump can be started and stopped by entering the ‘RPM’ and clicking ‘Start’ or ‘Stop’. By entering the appropriate tubing size, the flow rate in milliliters per minute (ml/min) can be specified. If more than one pump is available, then each pump can be selected from the drop down menu. To configure the flow conditions during an experiment, click the ‘Configure’ button to open the full ECIS Flow Configuration dialog (as shown on the next page).

ECIS Flow Configuration

The ECIS Flow Configuration dialog allows the user to setup constant or pulsing flow and to program a flow schedule to run during an experiment data collection. Some functionality and GUI elements may differ between the Applied BioPhysics and Heidolph pump configurations. All items are described for the Applied BioPhysics device.

For each pump check the ‘Pump Attached’ checkbox to activate the features and options. Then enter the well height and the tubing size to enable the flow rate and shear stress to be calculated.

In the Flow Setup panel, enter the desired RPM, Flow rate or Shear Stress and the other values will be determined. Clicking the ‘Max’ button will show the maximum values for the current tubing size and well height. Click ‘Start/Stop’ to start the pump at the desired speed, and click again to stop the pump. Click ‘Set’ to change the pump speed while it is running. Click ‘Stop All’ to stop all running pumps. To change between pumps, use the ‘Pump #’ dropdown.
Pulsating Flow

In addition to continuous flow, the Applied BioPhysics pump may be programmed to provide a limited range of pulsating flow conditions that one might call up to partially simulate the pulsed flow that endothelial cells experience in vivo. In this mode, one first specifies the ramp up time and peak flow desired, then the ramp down time, and minimum flow. A short ramp up time and longer ramp down time may be required to get the desired pulsing effect.

Enter the ramp up time, maximum RPM, ramp down time and minimum RPM in the ‘Pulsing Flow Setup’ panel. Use the buttons marked ‘>’ to transfer the current RPM settings. Click the ‘Pulsing Flow’ check box to start the pulsing flow mode.
**Programmed Pump Flow**

The pump can be run at a constant speed during an experiment simply by specifying a speed and starting the pump as described above. The flow rate can also be changed at any time by manually entering a new pump speed.

Programmed changes in pump speed during an experiment can be achieved using the ‘Pump/Flow Schedule for Experiment’ panel. Simply specify the desired speed/flow rate/shear stress in the ‘Flow Setup’ panel, then enter how long the pump should run for at that speed, and click the ‘Add’ button. The details will be entered in the table for review, and the start time for the next pump change will be updated. The checkbox ‘Use pump/flow schedule during experiment’ will be automatically checked. Uncheck this if the program schedule should not be applied. Click the ‘Clear’ button to clear the table.

Changes to the pump speed will occur if the desired start time has passed at the end of an entire time-point (all wells have been collected). Therefore, the start times may not be exact.

When the experiment ends, the pump will continue at the last programmed speed. To stop the pump enter a speed of 0 at the desired time.

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Some additional information regarding the pulsed flow

The pump does not instantaneously achieve the RPM requested, hence the flow versus time is not a square wave in form. Rather the pump will ramp up to the desired speed. If the length of the pulse is small, the maximum speed may not be achieved before the pump goes to zero flow during the pause. Since the change to zero flow is abrupt, these flow dynamics resemble a saw tooth wave. If the length of the run is longer, such that the desired rate is achieved and maintained, the dynamics will resemble a saw tooth with a plateau phase. One can vary the parameters and see the behavior of the pulsating flow by observing the rotating pump head.

Note: It is possible to specify too small a flow rate or too little a pulse time for the pump to respond – if these conditions are inputted, the user will be notified to change the parameters to fit within the capabilities of the pump.
Water Bath/Incubator (Optional)
The software can collect temperature (and CO₂) data from supported incubators and water bath units. The currently supported models are:

- PolyScience Water Bath/Circulator Model 8012
- New Brunswick Galaxy 14S Incubator

To activate this feature in the software, make sure the unit is turned on and connected to Accessory Port 2 on the ECIS Instrument. Then select from the Acquire menu, ‘Select Water Bath/Incubator’ and specify the model to begin communication.

The ‘Water Bath/Incubator Setup’ check box should now be available. When checked the control panel should appear, and will be different for each device depending upon the available capabilities.

**PolyScience Water Bath**
This device can set the desired water bath temperature and read the internal temperature, and external probe temperature. All three values are recorded with the experiment if the ‘Log Data’ box is checked. Click ‘Update’ to read the current values. The values will be automatically updated during an experiment when read at the end of each time point.

**New Brunswick Galaxy 14S Incubator**
This device can set and read the desired incubator temperature and CO₂ level. All four values are recorded with the experiment if the ‘Log Data’ box is checked. Click ‘Update’ to read the current values. The values will be automatically updated during an experiment when read at the end of each time point.

When an experiment is complete the recorded values can be plotted as a separate graph by selecting the temperature toolbar button 📊. The actual values to plot can be selected from the Edit | Temperature/CO₂ menu. To compare the graphed data with the experimental data, use ‘Export’ to copy the graph into a separate window and then turn off the temperature plot from the toolbar to bring up the experimental data.
Additional Information
How to order Arrays

You can order Arrays via Fax (518) 880-6865 or Phone (518) 880-6860 or (866) 301-ECIS (3247).

Upon ordering Electrode Arrays, please state your P.O. number, along with the quantity and type of Electrode Array (note: there is discount pricing for higher volume orders, please inquire for details). We will also need the shipping and billing addresses; Visa and MasterCard are also accepted.

All shipments are FOB, Troy, NY

Applied BioPhysics, Inc
185 Jordan Road
Troy NY 12180

Phone: (518) 880-6860
Fax: (518) 880-6865
www.biophysics.com
Company History

ECIS or Electric Cell-substrate Impedance Sensing is a novel electrical method to study, in real time, many of the activities of animal cells when grown in tissue culture. These include morphological changes, cell locomotion, and other behaviors directed by the cell’s cytoskeleton.

This technology was invented in 1984, by Drs. Ivar Giaever and Charles R. Keese while working at General Electric Corporate Research and Development, but its commercial potential was not explored as it fell outside of GE’s core interests. In 1991, as the potential applications of the ECIS technology became more apparent, Giaever and Keese formed Applied BioPhysics, Inc. as a private company to develop, commercialize and market the technology. Applied BioPhysics is located next to the Rensselaer Polytechnic Institute campus, where several collaborative research projects are ongoing in the academic laboratories.

Since the formation of the company, the ECIS approach has been applied to numerous investigations including measurements of the invasive nature of cancer cells, the barrier function of endothelial cells, in vitro toxicity testing as an alternative to animal testing, and signal transduction involving GPCR’s for modern drug discovery.
# Instrument Specifications

<table>
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<tr>
<th></th>
<th>ECIS Zθ</th>
<th>1600R Software &amp; Hardware Upgrade</th>
<th>1600R Software Upgrade Only</th>
<th>Current 1600R</th>
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<tbody>
<tr>
<td><strong>Number of wells</strong></td>
<td>16 &amp; 96 Well Stations, interchangeable</td>
<td>16 &amp; 96 Well Stations, interchangeable</td>
<td>16 well array holder</td>
<td>16 well array holder</td>
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<tr>
<td><strong>Single frequency acquisition rate</strong></td>
<td>0.25 seconds per well (16 wells in 4 sec)</td>
<td>0.25 seconds per well (16 wells in 4 sec)</td>
<td>0.50 seconds per well (16 wells in 8 sec)</td>
<td>8 seconds per well (16 wells in 130 sec)</td>
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<tr>
<td><strong>Multi frequency acquisition rate (11 frequencies)</strong></td>
<td>7.5 seconds per well (16 well in 2 min)</td>
<td>7.5 seconds per well (16 well in 2 min)</td>
<td>7.5 seconds per well (16 well in 2 min)</td>
<td>30 sec per well (3 freq only)</td>
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<td><strong>Ability to plot multi-frequency data in 3D (time vs impedance vs frequency)</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td><strong>Rapid acquisition rate (Single well, single frequency)</strong></td>
<td>25 points per second</td>
<td>25 points per second</td>
<td>15 points per second</td>
<td>4 points per second</td>
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<tr>
<td><strong>Electroporation &amp; Wound-Healing Availability</strong></td>
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<td>Yes</td>
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<td><strong>Electrode Stabilization</strong></td>
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<td><strong>Wounding arrays</strong></td>
<td>1E, 10E, 10E+, 96 well</td>
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<tr>
<td><strong>Measurement accuracy</strong></td>
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<td><strong>Frequency range</strong></td>
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<td>25 Hz - 60 kHz</td>
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<td>XP, Vista</td>
<td>XP</td>
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Publications
Selected ECIS Publications


