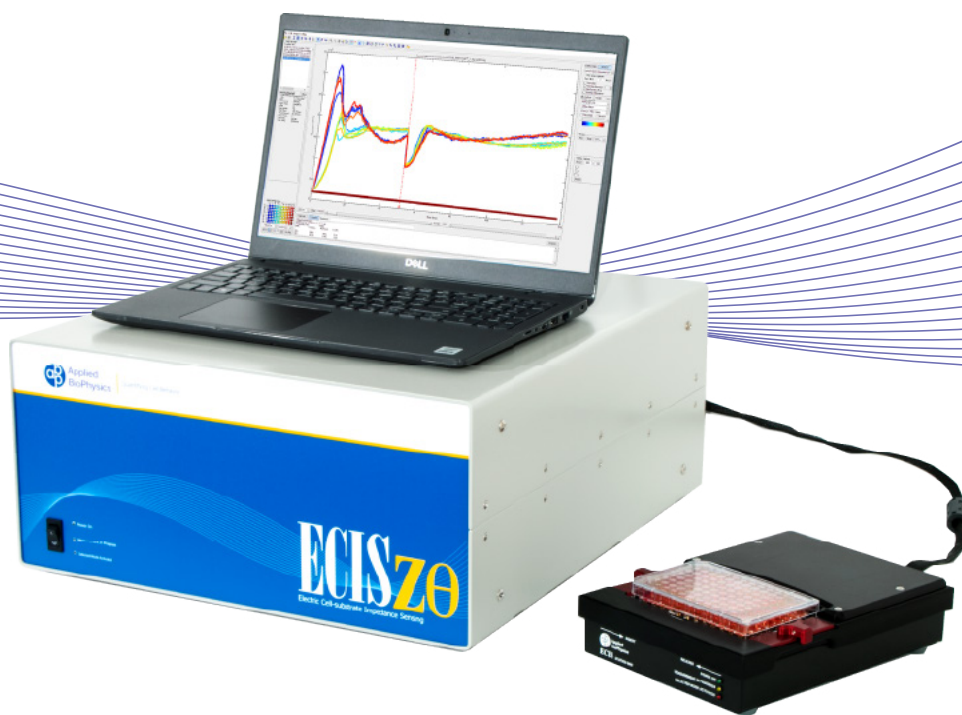


# Installation and Operation Manual

## ECIS<sup>®</sup> Z-Theta



# Contents

<b>ECIS Theory.....</b>	<b>5</b>
How AC Frequencies Reveal Cell Behavior.....	6
<b>System Installation.....</b>	<b>7</b>
Inspection .....	7
Equipment Installation.....	8
Computer & Software Installation.....	9
Calibration with 16-well Test Array.....	11
Calibration with 96-well Test Array.....	11
<b>ECIS Arrays.....</b>	<b>13</b>
ECIS Cultureware.....	13
Standard 8-well Arrays.....	13
Standard 96-well Arrays.....	14
<b>Starting an Experiment.....</b>	<b>15</b>
How to Choose an Array.....	15
Array Specifications.....	15
Conditioning the Arrays.....	16
Precoating with Proteins.....	16
Electrode Stabilization.....	17
Cysteine Treatment.....	18
Software Electrode Stabilization.....	19
Inoculation of Arrays.....	20
Setting Up Time Course Measurements.....	21
Starting an Experiment with a 16-well Station.....	22
Starting an Experiment with a 96-well Station.....	24
Controls During Data Acquisition.....	26
<b>Wounding &amp; Electroportion.....</b>	<b>27</b>
Wound Healing Protocol.....	27
Electric Fence.....	34
Electric Fence Protocol with Expert Features.....	35
<b>8-Well Transfilter Array.....</b>	<b>37</b>
Preparation of the 8-well Transfilter Array.....	37
8-well Transfilter Array Protocol.....	38
<b>Analyzing Data.....</b>	<b>41</b>
Use of the Toolbar Icons.....	41
Time-Series Options.....	43
Averaging Multiple Wells.....	43
Error Bars.....	44
Coloring the Wells and Graphs with 8-well Array.....	45
Coloring the Wells and Graphs with 96-well Array.....	45
<i>Color Map</i> .....	45
<i>Color Based Filtered Data</i> .....	46
<i>Selection and Deselection of Wells</i> .....	46
Different Means to Plot 96-well Data.....	47
<i>3D Map of Wells</i> .....	48

2D map of Columns/rows.....	48
Array Map.....	49
Overlay Map.....	49
Text Matrix.....	50
Returning to the Well Configuration Colormap.....	50
Exporting Data and Graphs.....	50
How to Make Use fo the Multiple Frequency Time Course Data.....	51
Determining the Best AC Frequency by Examination.....	51
Analyzing Frequency Scan Data Using Ratio of Cell to Cell-free Data.....	52
<b>Data Modeling.....</b>	<b>55</b>
General Notes About Understanding ECIS Model.....	55
The ECIS Model.....	55
Modeling Software.....	58
Selecting the Cell-free Data.....	60
Getting Cell-free Data from Another Data Set.....	61
Choosing the Data to be Modeled.....	61
The Validity of the Modeled Data.....	63
<b>Menus.....</b>	<b>67</b>
File Menu.....	67
Open.....	67
Database.....	67
Recent Files.....	68
Import 1600R Data.....	68
Open Demo Data.....	68
Export Graph.....	68
Export Data.....	68
Copy To.....	69
Close.....	69
Exit.....	69
Edit Menu.....	69
Copy Graph.....	69
Export Graph.....	69
Copy Comments.....	69
Full Screen.....	69
Default Well Info.....	69
Copy Well Info.....	69
Paste Well Info.....	69
Append Dataset.....	69
Combine Datasets.....	69
Color Palette.....	70
Error Bars.....	70
Number of Error Bars.....	70
Use Graph Time Format.....	70
Filter Noise Spikes.....	70
Temperature/CO2.....	70
Display.....	70
Auto Z/R/C Frequency Select.....	70

<i>Running Average Size</i> .....	70
<i>Plot Style</i> .....	70
<i>Set Zero Time</i> .....	71
<i>Legend</i> .....	71
Acquire Menu.....	72
<i>Setup New Expt</i> .....	72
<i>Activate All Wells</i> .....	72
<i>Resume Paused Expt</i> .....	72
<i>SetOutputDirectory</i> .....	72
<i>Find Instrument</i> .....	72
<i>ECIS Instrument Options</i> .....	72
<i>Default Expt. Type</i> .....	72
<i>Set ECIS COM Port</i> .....	72
<i>Set Flow COM Port</i> .....	72
<i>Set Scan Frequencies</i> .....	72
<i>Set Water Bath/Incubator COM Port</i> .....	73
<i>Expt. Sounds</i> .....	73
<i>Deep Diagnostics</i> .....	73
<i>Saver</i> .....	73
Help Menu.....	73
<i>Application Help</i> .....	73
<i>Show Expert ToolBar/Menu Items</i> .....	73
<i>Getting Started</i> .....	73
<i>Manual</i> .....	74
<i>Open Log File</i> .....	74
<i>Check for Updates</i> .....	74
<i>www.biophysics.com</i> .....	74
<i>License Agreement</i> .....	74
<i>About</i> .....	74
<b>Additional Information</b> .....	<b>75</b>
How to Order Arrays.....	75
CompanyHistory.....	75

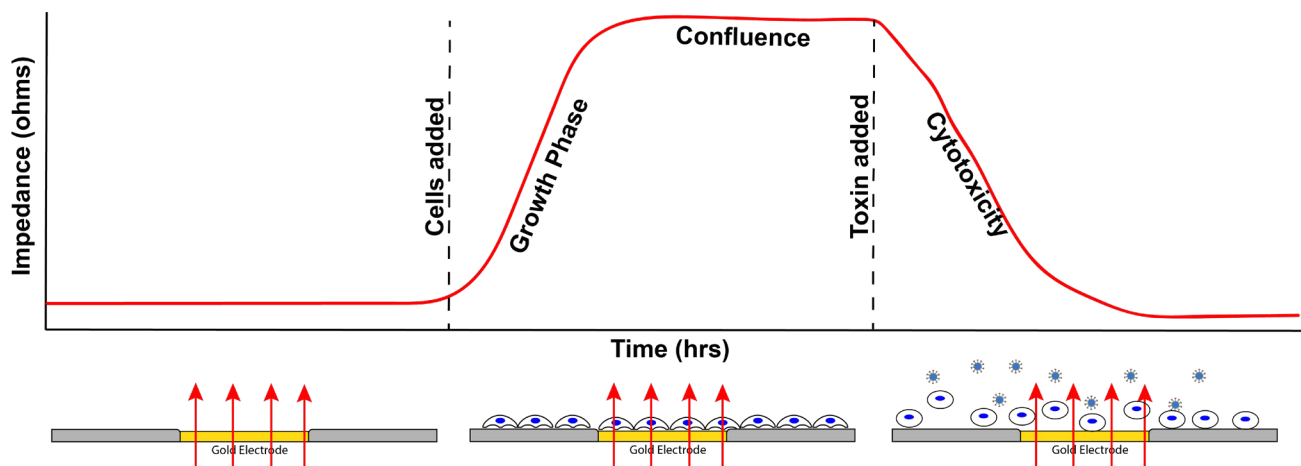
# ECIS Theory

## ECIS Description

The ECIS electronics are contained in two separate units, the Station Controller (Z0) 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).

## ECIS Theory

Electric Cell-Substrate Impedance Sensing (ECIS) is a method to monitor cell behaviors in vitro using a small alternating current (I) that is applied across an electrode pattern at the bottom of ECIS arrays, resulting in a potential (V) across the electrodes which is measured by the ECIS instrument. The impedance (Z) caused by the cell layer covering the electrodes due to the insulating membranes of the cells can be determined by using Ohm's law  $Z = V/I$ . As cells grow over the electrodes, the current is impeded in a manner related to the number of cells covering the electrode and is presented in graphical format of Impedance vs Time (figure 1).



**Figure 1:** Graphical representation of impedance over time caused by cells covering the gold electrodes of an ECIS array.

## How AC Frequencies Reveal Cell Behavior

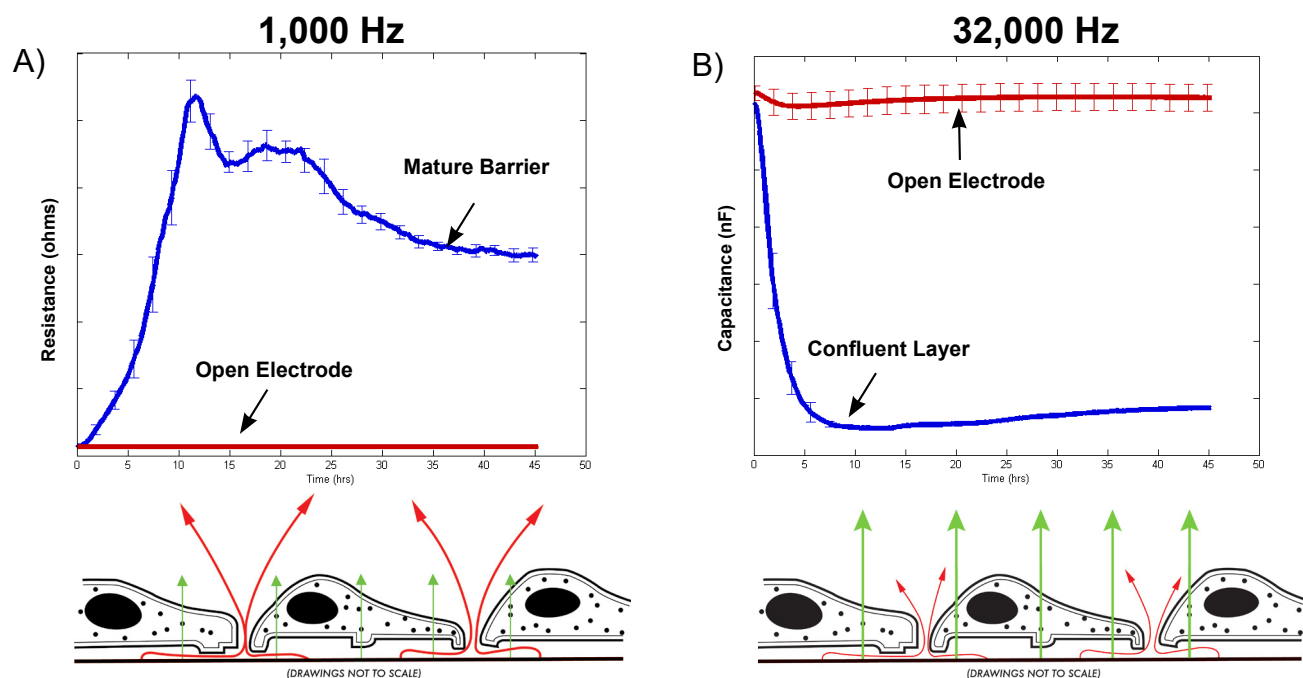
Many cell behaviors can be monitored using the ECIS method including proliferation, cytotoxicity, barrier function/TEER, migration, attachment and more. The ECIS Z-Theta instrument measures “complex impedance” where the impedance is broken down into its components (**resistance** and **capacitance**), and quantitative information through varying AC frequencies about the cell behavior can be obtained (Giaever and Keese PNAS 1991).

To understand why AC frequency is important in complex impedance, we have to consider how frequency affects the current paths of cell-covered electrodes. (Note: the total current is maintained constant and voltage changes are measured.)

At relatively low frequencies ( $< 4,000\text{Hz}$ ) most of the current flows in the solution channels under and between adjacent cells (red lines) and constitutes the *resistive* portion of the total complex impedance (figure 2A).

At higher frequencies ( $> 32,000\text{ Hz}$ ) more current now capacitively couples directly through the insulating cell membranes (green lines) and constitutes the *capacitive* portion of the total complex impedance (figure 2B).

The high frequency impedance is more affected by cell-coverage, whereas the low frequency responds more strongly to changes in the spaces under and between the cells.

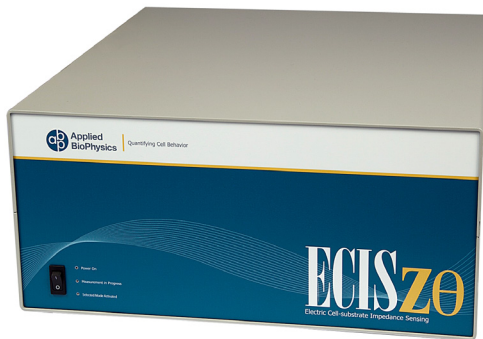


**Figure 2:** Frequencies reveal different cell behaviors in the same sample. At low AC frequencies, the resistive portion of impedance is caused by the current flowing underneath and through cell junctions making it useful for barrier function analysis(A), whereas at high AC frequencies, the capacitive portion of impedance is caused by the current coupling through the cell membranes and is useful for proliferation to confluence and cytotoxicity assays (B).

# System Installation

## Inspection

When receiving the ECIS Z-theta system, inspect the package(s) for damage to the exterior or interior of the carton and components. If damages are found, please contact the carrier for the procedures on claiming damages or missing items or contact Applied BioPhysics. The following items should be included in the shipment unless specified otherwise. If no damages are found and items are complete, remove contents and proceed to setup



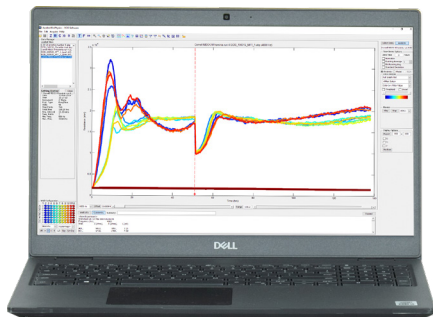
**Z-Theta Station Controller**



**96-well Array Station**



**16-well Array Station**



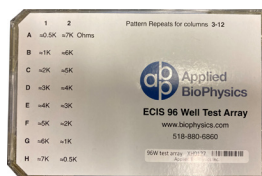
**PC Laptop with ECIS Software**



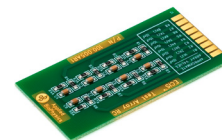
**USB Cable**



**Power Cable**



**96-well Validation Array**



**8-well Validation Array**

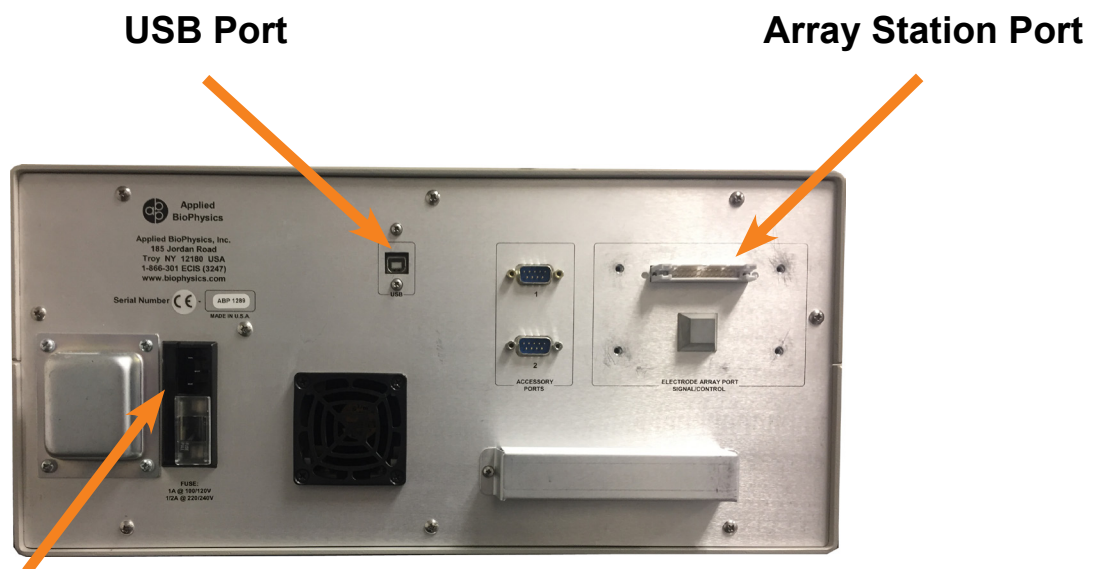


## Equipment Installation

1. Unpack your station and place it in the plastic bag Ziploc bag. Ensure the plastic bag is sealed tightly. Place the array station inside the incubator for at least 2 hours or until the station's temperature has come to equilibrium with the incubator temperature. This will prevent water from condensing inside your station.
2. Unpack the station controller and set it next to the incubator opposite the hinge side or closest to the accessory port. Unpack the laptop and set on top of the station controller. The laptop should be positioned on top of the ECIS Z $\theta$
3. Find the auxiliary port in the incubator and feed the cable from the array station through the port or through the front door and to the back of the Station Controller.

**\*NOTE: DO NOT PLUG THE ARRAY STATION INTO THE CONTROLLER WITHOUT CHECKING THAT THE POWER IS OFF\*\***


4. Unscrew the cover of the array station port.
5. Plug the array station cable into the back of the station controller.
6. Fasten the cover plate over the array station cable with the screws provided.
7. Plug in the power cable.
8. Plug in the USB cable to the back of the Z-Theta Controller
9. Take the station out of the plastic bag. Make sure each clamp is plugged into the back of each station. The station can now be placed back inside the incubator.
10. Plug in the Main Z-Theta Power Cord to the power outlet and turn on the power switch.
  - a. You will hear 4 Trumpet sounds after the power switch is turned on

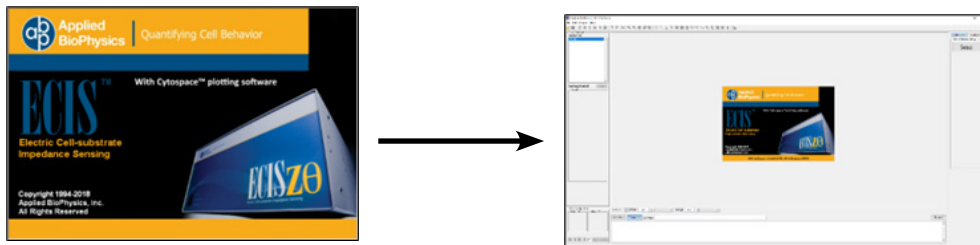


**Power Cable Plug-in**

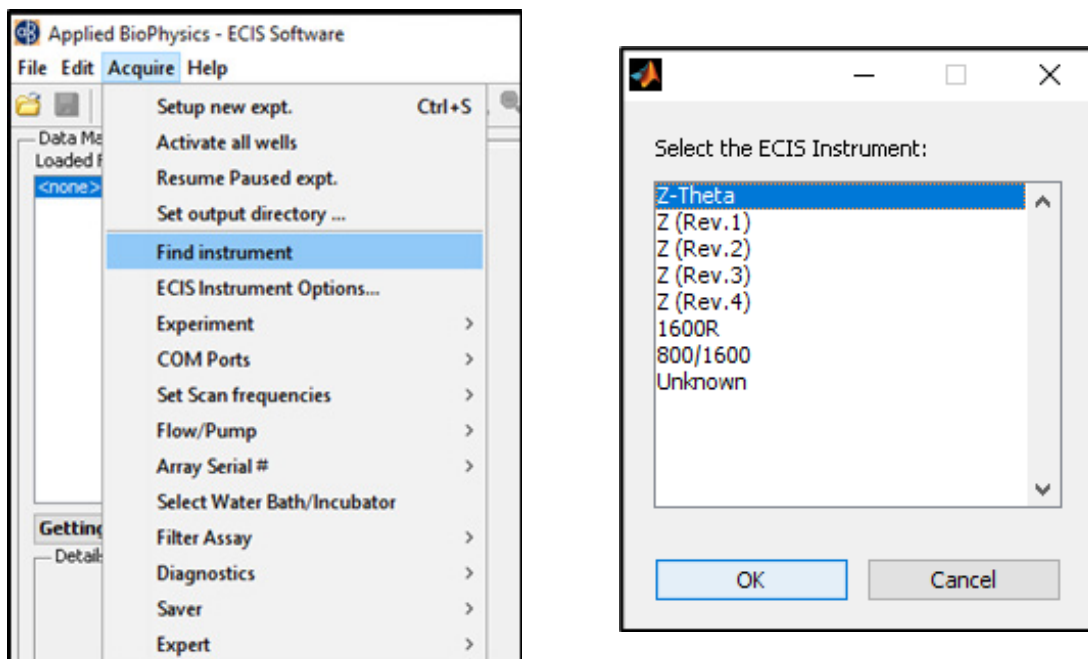


## Computer & Software Installation

1. Start and boot the laptop.
  - a. Plug in the power cable for the laptop to ensure it does not turn off or run out of batteries during an experiment.
2. Once the laptop has booted and the user is logged in, connect the USB cable to the computer via any open USB port. The other end was already plugged into the Z-theta in previous Step 9
3. Find the ECIS software icon and double click 
4. The following loading screen will pop up followed by the program opening screen.

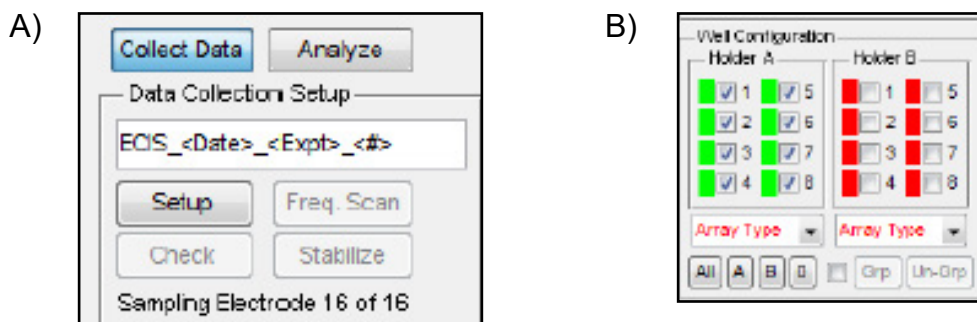


5. Once the software loads, select **Acquire** → **Find Instrument** from the pull-down menu.
  - a. A pop-up window will appear asking you to confirm your system type. Select the correct system and then click OK.



## Calibration with Test Array for 16-well Station

1. 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.
2. 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.
3. Click on the **Setup** button (A). 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 (B).



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

4. With the test array properly in place, click the **Check** button. 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.
5. 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 resistance and capacitance:

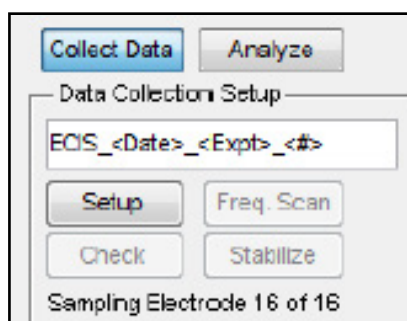
Test Array Well Number	Approximate Resistance (ohms)	Aproximate Capacitance (nanoFarads)
1	1000	10
2	2000	10
3	3000	10
4	4000	10
5	5000	10
6	6000	10
7	7000	10
8	8000	10

## Calibration with Test Array for 96-well Station

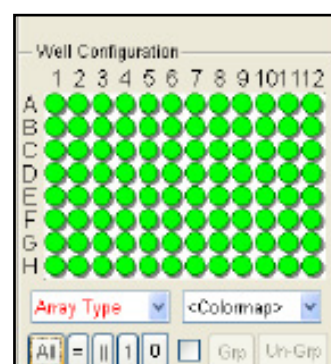
### 96 Well Station

1. Place the ECIS Test Array supplied with the instrument on the bed of electrodes of the 96 Well Station and slide the retaining clamps inward to clamp while pushing down on the array. Double click the ECIS icon to bring up the main menu.
2. Click on the **Setup** button (A). 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 (B).

A)



B)



\*If any wells are red, please remove and reinsert the test array and then click the **Setup** button once again.

3. 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. At the completion of the run, the values for the 96 electrodes will be displayed. These values will show the impedance, resistance and capacitance of each well. *Note: the 96-well test array has no capacitors so values of "C" should read "none."*

Test Array Row	Approximate Resistance (ohms) Column 1	Aproximate Resistance (ohms) Column 2
A	500	7000
B	1000	6000
C	2000	5000
D	3000	4000
E	4000	3000
F	5000	2000
G	6000	1000
H	7000	500

\*Pattern repeats for columns 3-12



# 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  $\sim 0.8 \text{ cm}^2$  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  $\sim 0.3 \text{ cm}^2$  and holds a maximum volume of  $\sim 400$  microliters. An array of electrodes are located on the bottom of 96-well ECIS arrays and will clamp onto the electrode pin bed on the 96-well array stations by pressing down on the array and sliding the two side clamps inward to secure the array.

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

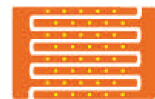
The **8W1E** ECIS array has eight wells with one active electrode per well (250 micrometer diameter) and a large counter electrode.



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



The **8W10E+** ECIS array 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.



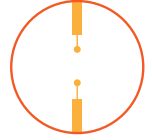
The **8W20idf** ECIS array has a total electrode area of  $3.985 \text{ mm}^2$  located on inter-digitated fingers to provide measurements of cells. Each well has a substrate area of  $0.8 \text{ cm}^2$  and a maximum volume of  $600 \mu\text{L}$ . On average, with a confluent layer, approximately 4000 to 8000 cells will be measured by the electrodes.



## Standard 96 Well Arrays

### Custom Arrays

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



The **96W10idf** ECIS array has 96 wells and an Inter-digitated finger configuration. The total electrode area is 2.09 mm<sup>2</sup> which measures a maximum of 2000-4000 cells.



The **96W20idf** ECIS array has 96 wells and an Inter-digitated finger configuration. The total electrode area is 3.985mm<sup>2</sup> which measures a maximum of 4000-8000 cells.

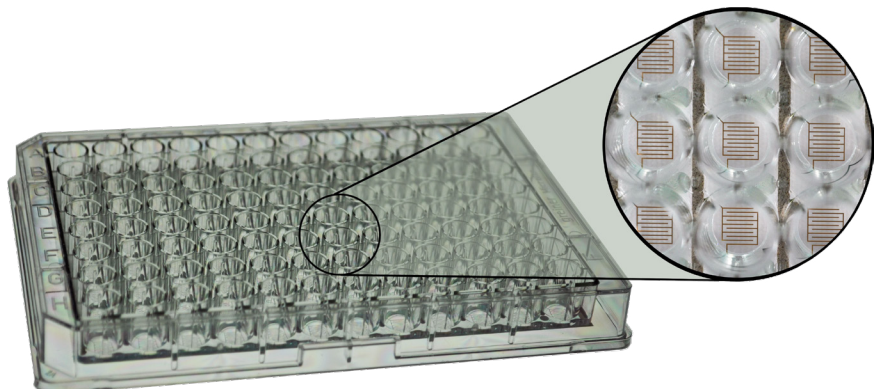


The **8W flow** ECIS arrays have flow channels with independent electrodes. Each electrode is 250 micrometers in diameter and is ideal for shear stress assays when used in conjunction with a peristaltic pump.

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



**8-Well Array**



**96-Well Array**

# Starting an Experiment

## How to Choose an Array

### 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

Array Type	Wells	Electrodes per Well	Electrode Area (mm <sup>2</sup> )	Shape	Transparent
8W1E	8	1	0.049	Circular	Yes
8W10E	8	10	0.49	Circular	Yes
8W10E+	8	40	1.96	Circular	Yes
8W20idf	8	Interdigitated fingers	3.985	IDF	Yes
96W1E+	96	2	0.256	Circular	Yes
96W10idf	96	Interdigitated fingers	2.09	IDF	Yes
96W20idf	96	Interdigitated fingers	3.985	IDF	Yes



## 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.

### **Pre-Coating Electrodes with Defined Protein Coats**

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:

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. This method will essentially clean the gold electrodes of any molecules that might have bound to the gold surface. Attention to this will minimize electrode drift during an experimental run. Electrode stabilization can be conducted with two separate methods:

1. **Cysteine Treatment**
2. **Software Electrode Stabilization**

*\*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. The following table represents expected values of each type of ECIS array that has been fully stabilized. If values aren't reaching these levels, repeat stabilization.*

Cell-Free Wells	Resistance @ 4,000 Hz	Capacitance @ 4,000 Hz
8W1E	1700-1800	5-6
8W10E	350-400	50-60
8W10E+	200-250	50-60
8W20IDF	80	100-120
96W1E+	1700-1800	5-6
96W10IDF	200-250	50-60
96W20IDF	80	100-120

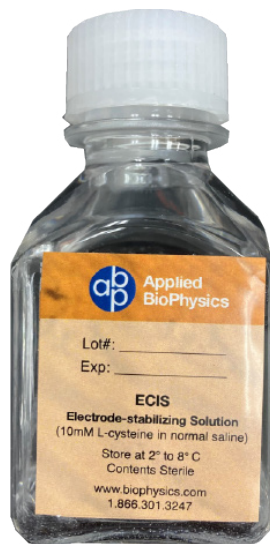
# Starting an Experiment

## 1. Electrode Stabilization: Cysteine Treatment

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 ~15 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.*

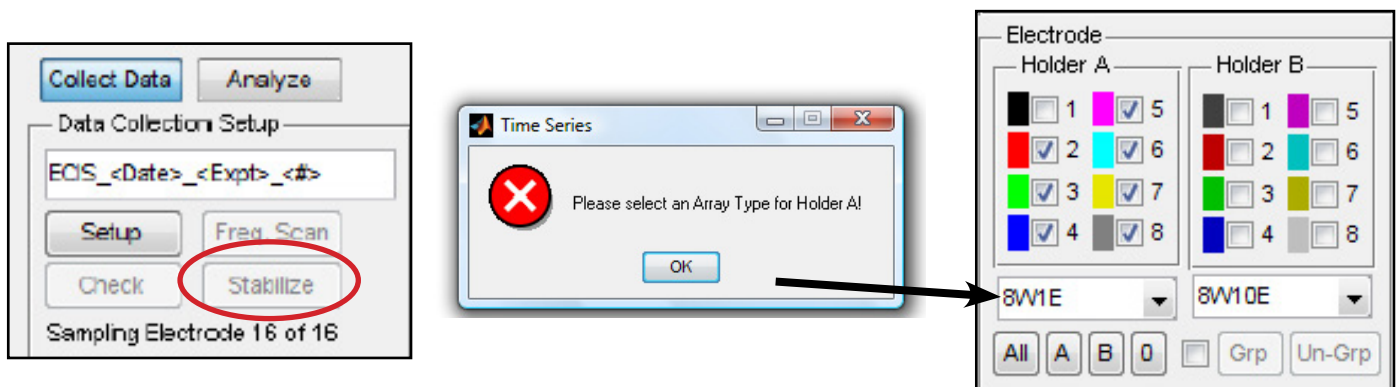


## 2. Software Electrode Stabilization

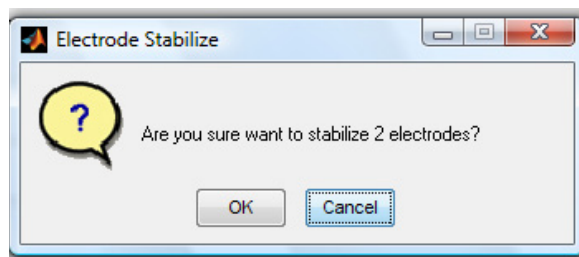
The ECIS software includes a feature that allows one to condition or stabilize electrodes electrically before they are inoculated. It can be used in conjunction with cysteine treatment if the cysteine treatment alone doesn't reach desired values.

**This process should never be activated for wells containing cells as cell damage may result.**

1. Click the **Stabilize** button, the system will first check to see that the type of array has been specified. This must be done before proceeding.



2. 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), The results will appear and then become a permanent part of the comments for the experiment (see page 17 for clean electrode values).

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  $0.8 \text{ cm}^2$  for 8W wells and  $0.3 \text{ cm}^2$  for 96W wells. A typical inoculation to give a confluent cell layer would be approximately  $10^5 \text{ cells/cm}^2$ , 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  $\sim 2.5 \times 10^5 \text{ cells/ml}$
- **96W arrays:** 300 microliters per well of cell suspension at  $\sim 1.2 \times 10^5 \text{ cell/ml}$

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 convection 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.

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.

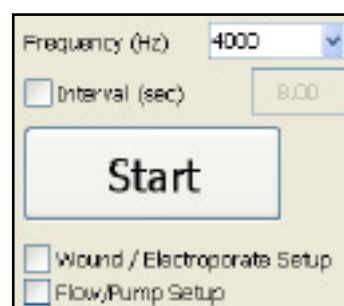
## Setting up Time Course Measurements

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



A screenshot of a software menu for selecting time course measurement programs. It contains three radio button options: 'Multiple Freq. / Time (MFT)', 'Single Freq. / Time (SFT)' (which is selected), and 'Rapid Time Collect (RTC)'.

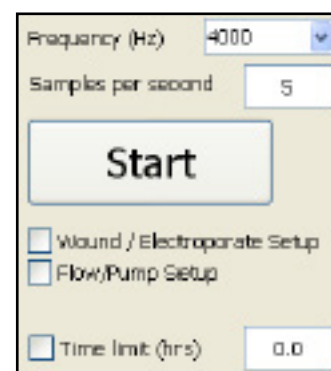
- Multiple frequency (**MFT**) runs. With the Z  $\theta$  instrument, the program will measure each well at 11 different predefined frequencies for 8 well arrays and 7 different frequencies for 96 well arrays. The time between points can be selected, but the minimum time will depend upon the number 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.



A screenshot of the 'Multiple Freq. / Time (MFT)' setup window. It features a 'Frequency (Hz)' dropdown menu set to 4000, an 'Interval (sec)' input field set to 8.00, a large 'Start' button, and two checkboxes for 'Wound / Electroporate Setup' and 'Flow/Pump Setup'.

- 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.



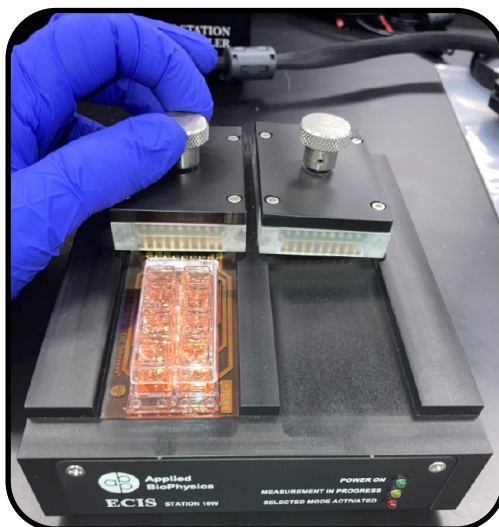
A screenshot of the 'Rapid Time Collect (RTC)' setup window. It features a 'Frequency (Hz)' dropdown menu set to 4000, a 'Samples per second' input field set to 5, a large 'Start' button, checkboxes for 'Wound / Electroporate Setup' and 'Flow/Pump Setup', and a 'Time limit (hrs)' input field set to 0.0.


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.

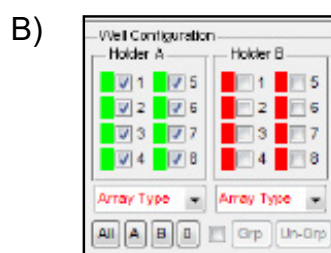
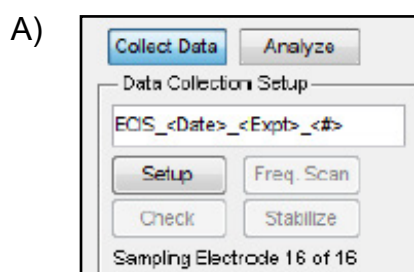
# Starting an Experiment

## Starting an Experiment with a 16-well Array Station

1. Prewarm 16-well station by placing inside Ziploc bag and into incubator for 30 minutes before starting experiment.
2. Stabilize gold electrodes of desired ECIS Array wells with 200  $\mu$ l of 10 mM L-Cysteine for 15-30 minutes and rinse (see page 18).
3. Coat electrodes by adding preferred substrate protein for adsorption to each well following manufacturer's instructions and rinse (optional step).
4. Add 400  $\mu$ l of media to each desired well to be used.
5. Insert ECIS array(s) into A, B, or both sides of 16-well array station ensuring that the gold contact pads at the end of the array align with the contact pins of the 16-well station clamp.
6. Turn the knurled stainless steel knob clockwise, lowering the pins to the contact pads, until finger tight.

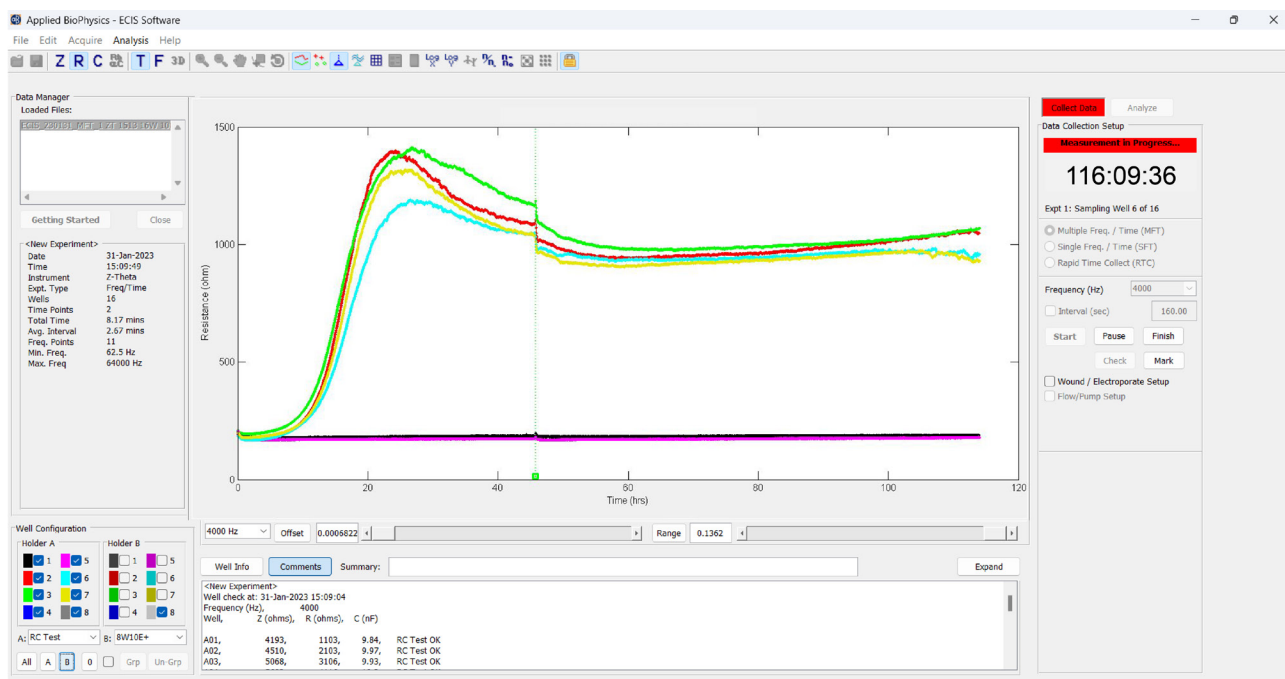


7. From the Desktop, double click the ECIS program icon to bring up the main menu. 
8. Click on the **Setup** button (A). This quickly measures the impedance of the array and then reports whether or not an open circuit has been detected. You should see green for properly connected wells (with media) and red for absence of arrays or media or an incorrectly connected array.





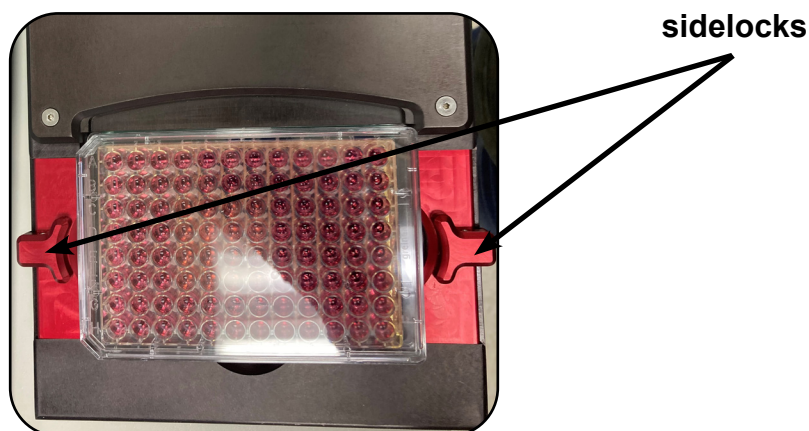
9. Next, click the **Check** button. This measures the impedance of each well at the default 4000 Hz and will display a window with values of impedance (Z), resistance (R), and capacitance (C). This allows the user to ensure that the electrodes are clean. For cell-free electrodes, reference values for each type of array are listed on page 17.  
  
\*If electrode values (mainly capacitance) don't meet expected values, use **Stabilize** button to further clean the electrodes (see page 19)
10. Inoculate desired wells with 400  $\mu$ l of cell suspension using prewarmed media (see page 20) and repeat steps 5-9.
11. Select timecourse measurement : Multifrequency timecourse (**MFT**), single frequency timecourse (**SFT**), or rapid timecourse (**RFT**) (see page 21)
12. Click **Start** to begin the experiment. This option will prompt a save screen to name and save the experimental file to the desired file location.
13. Click **Pause** when needing to remove the array from the array station for addition of compounds, etc. After connecting the array back into the array station, click **Check** to confirm values of properly connected array and then click **Resume** to allow the software to continue recording data.
14. Click **Finish** when experiment is completed.




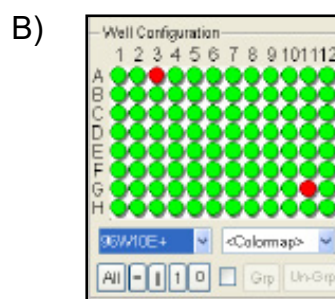
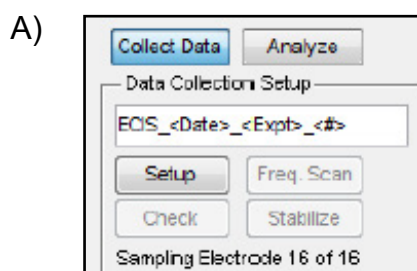
# Starting an Experiment

## Starting an Experiment with a 96-well Array Station

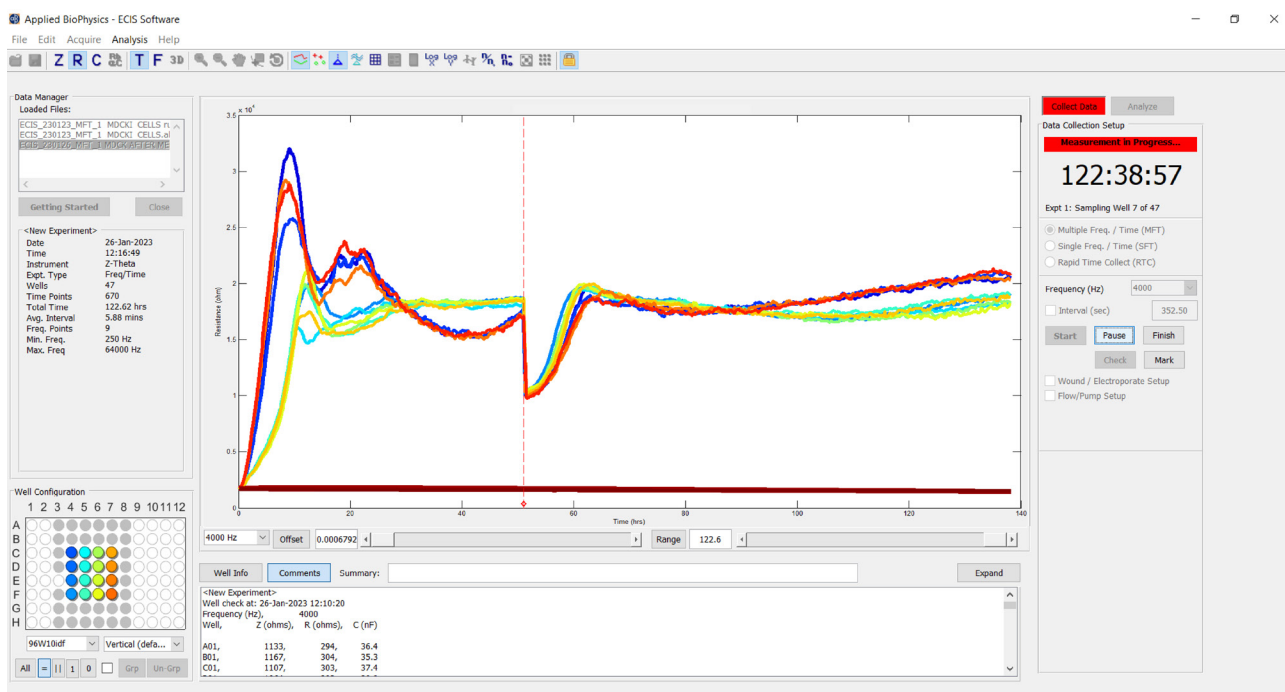
1. Prewarm 96-well station by placing inside Ziploc bag and into incubator for 30 minutes before starting experiment.
2. Stabilize gold electrodes of desired ECIS Array wells with 100-200  $\mu$ l of 10 mM L-Cysteine for 15-30 minutes and rinse (see page 18).
3. Coat electrodes by adding preferred substrate protein for adsorption to each well following manufacturer's instructions and rinse (optional step).
4. Add 250  $\mu$ l of media to each desired well to be used.
5. Insert ECIS 96-well array into the 96-well array station positioning well A1 in the upper left corner.
6. While pressing down on top of array, slide each sidelock inwards over the edge lip of the base of the array.



7. From the Desktop, double click the ECIS program icon to bring up the main menu. 
8. Click on the **Setup** button (A). This quickly measures the impedance of the array and then reports whether or not an open circuit has been detected. You should see green for properly connected wells (with media) and red for absence of arrays or media or an incorrectly connected array.



9. Next, click the **Check** button. This measures the impedance of each well at the default 4000 Hz and will display a window with values of impedance (Z), resistance (R), and capacitance (C). This allows the user to ensure that the electrodes are clean. For cell-free electrodes, reference values for each type of array are listed on page.....  
  
\*If electrode values (mainly capacitance) don't meet expected values, use **Stabilize** button to further clean the electrodes (see page 19)
10. Inoculate desired wells with 250  $\mu$ l of cell suspension using prewarmed media (see page 20) and repeat steps 5-9.
11. Select timecourse measurement : Multifrequency timecourse (**MFT**), single frequency timecourse (**SFT**), or rapid timecourse (**RFT**) (see page 21)
12. Click **Start** to begin the experiment. This option will prompt a save screen to name and save the experimental file to the desired file location.
13. Click **Pause** when needing to remove the array from the array station for addition of compounds, etc. After connecting the array back into the array station, click **Check** to confirm values of properly connected array and then click **Resume** to allow the software to continue recording data.
14. Click **Finish** when experiment is completed.

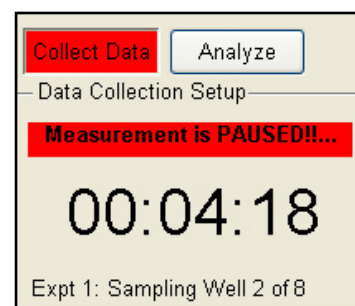
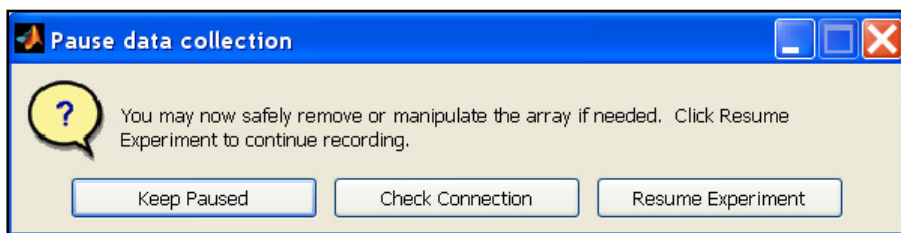
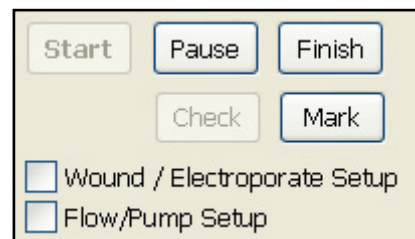


# Starting an Experiment

## Controls During Data Acquisition

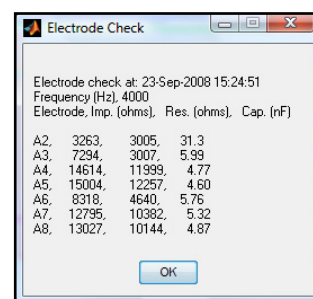
During data acquisition, in addition to using the tool bar at the top of the screen to alter the displayed information (see details below) one can **Pause** data acquisition or **Mark** data files.

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**.

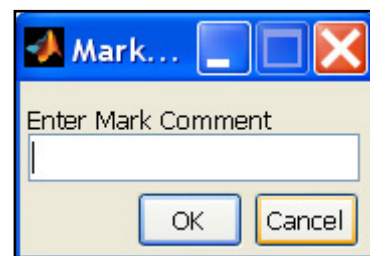
If the array has been removed from the Station during the Pause, it is very important to be certain the array is connected to the Station before resuming data acquisition. If one fails to have the array correctly positioned, a large meaningless impedance spike will be a part of the data set. To avoid 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.



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



# Wounding & Electroporation

## Wounding and Electroporation (Optional)

The system is equipped with the Elevated Field Module (**EFM**) and 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.

## Wound Healing/Electroporation Protocol

The basic ECIS Wound Healing assay inoculating the electrodes with a cell suspension, automatically wounding the cell monolayer with an electrical current, and monitoring the dynamics of cell migration and spreading via impedance measurements.

## Choosing an Array Type

Any ECIS array is capable of conducting the wounding current, but the different electrode geometries will generate different effective potentials across the cells. We recommend the 8W1E or the 8W2LE arrays for ECIS Cell Migration Assays as these array types are capable of generating the highest potentials across the cells which allows for less variable results.

The 8W10E arrays data is the combined response of 10 wounding and migration sites.

The 8W2OE array provides for a longer migration distance, at two oblong wounding/migration sites, and generates potentials across the cells approximately that of the 8W10E arrays.

Table 1 summarizes the types of electrodes that we offer for wounding experiments.

Catalog Number	Description	Migration Distance
8W1E*	8 Wells w/1 circular electrode each	125 $\mu\text{m}$
8W10E	8 Wells w/10 circular electrodes each	125 $\mu\text{m}$
8W2LE	8 wells w/2 linear electrodes each	75 $\mu\text{m}$
8W2OE	8 wells w/2 oblong electrodes each	250 $\mu\text{m}$
96W1E+	96 wells w/2 circular electrodes each	125 $\mu\text{m}$

\*Recommended for pilot experiments

## Wound Healing/Electroporation Protocol (cont'd)

### SETTING UP THE EFM PARAMETERS

1. Open the ECIS Software and click **Set-up**
  - a. Ensure all wells with cells and media light up Green
  - b. Choose your **Array Type** from the dropdown menu  
\*8W1E PET is recommended
2. Check the box next to **Wound/Electroporate Set-up**
  - a. This should enable the wounding feature.
3. Check the radio button next to **Wound or Electroporate**
  - a. This will enable the default recommended settings for your array type.
  - b. We recommend trying the default settings for your 1st experiment and adjusting as needed.
4. Set the **Wound Time**
  - a. Enter the time in seconds you want the high current pulse to last
  - b. Each well will be exposed to the high current for this duration
  - c. For Electroporation, wound for no longer than 0.2 seconds
5. Set the **Wound Current**
  - a. The higher the current the more disruptive for the cell layer.
  - b. Increasing the current may increase the chance of a complete wound on the electrode
6. Set wound **Frequency**
  - a. The lower the frequency the more disruptive for the cell layer.
  - b. Decreasing the frequency may increase the chance of a complete wound on the electrode.
  - c. **NOTE:** Going lower than 20,000 Hz will increase the chance of damaging the electrode along with the cells.

Wound ☒ Electroporate ☐

Wound Time (sec) 20

Wound Current (uA) 5000

Frequency (Hz) 60000

☐ Delay Until Hour

Activate

### INITIATE WOUNDING

1. Choose the wells you want to wound from the well menu on the lower left of the software.

**NOTE:** You can Activate the EFM/wounding immediately – OR delay until a specific time. Skip to the section (A or B) of your choice

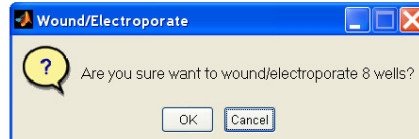


## Wound Healing/Electroporation Protocol (cont'd)

### **Option A: Activate NOW**

#### 2. Click **Activate**

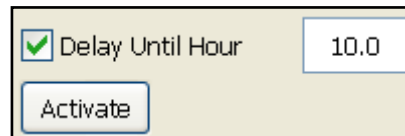
- a. A dialog box will pop up to check that you have the correct number of wells selected



- b. Choose OK if everything is correct or Cancel if you need to make a change
- c. Clicking OK will immediately complete the action and wounding will begin!
- d. Only wells that have a check mark will be wounded/exposed to the high current.

### **Option B: Delay Activation until a specified time**

#### 3. Check Box Next to **Delay Until Hour**

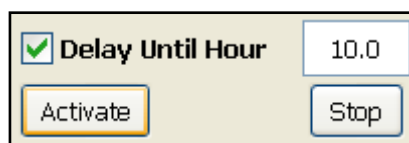


#### 4. Enter the specified time into the time box next to Delay Until Hour.

- a. This number corresponds to the experimental hour on the x axis (time) – not the time until wounding is completed.
- b. For example – if 20 is put into the box – the system will wait until hour 20 of the experiment and activate the wounding. If the experiment is 10 hours in – it will be 10 hours until wounding it is activated. A countdown clock will note the time until wounding.

#### 5. Click **Activate**

- a. A dialog box will pop up to check that you have the correct number of wells selected
- b. Choose OK if everything is correct or Cancel if you need to make a change
- c. Clicking OK will immediately complete the action and a countdown clock to wounding will begin!
- d. Click STOP to cancel wounding at any point before the specified time.

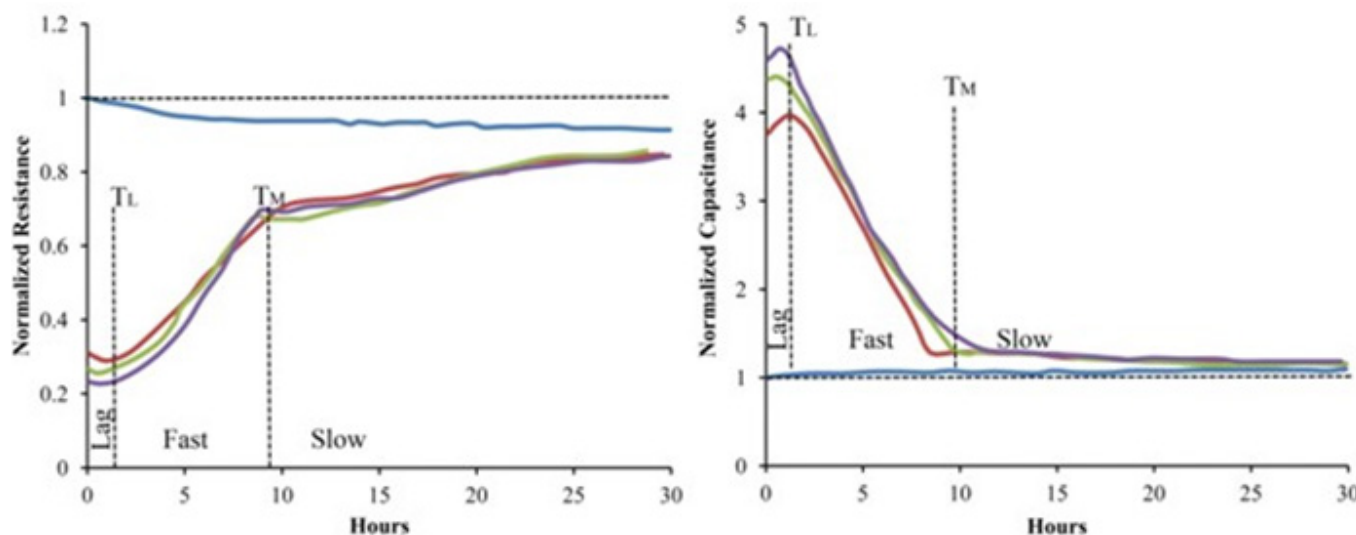




## Wound Healing Protocol (cont'd)

### DATA ANALYSIS

The ECIS Wound Healing Assay is a recovery after irreversible electroporation experiment and has three ECIS phases; lag, fast recovery, and slow recovery. We currently correlate these phases with a cell transition, cell migration, and re-establishment (annealing) of cell-cell interactions respectively. Of the three phases of recovery, only the transition and migration phases would be observable by microscopy-based methods. The annealing phase is only observable with ECIS and best resolved by monitoring resistance at low frequencies (<4000 Hz). The transition and migration phase are best measured by Capacitance or Impedance at high frequency (>40,000 Hz). At high frequencies the measurement is less sensitive to cell-cell interactions resulting in impedance or capacitance curves dominated by cell migration.



*Figure 1- ECIS Cell Migration Assay showing recovery as a function of normalized resistance and normalized capacitance. Data is normalized to the control well starting value. The three phases of recovery after irreversible electroporation are demarcated in both instances. Confluent BSC-1 cells were wounded by 2.5 V for 5 sec at 40,000 Hz just prior to the start of ECIS recordings.*

The proper units for cell migration experiments are distance per unit time. ECIS Wound Healing Assays directly measure the recovery of impedance in a very precise cell free area. Full recovery of the impedance consists of both the migration phase and the annealing phase. To convert to distance per unit time requires that the migration phase be differentiated from the annealing phase. In general, this is accomplished by analyzing the recovery by Capacitance or Impedance at high frequencies where the annealing phase is a minor contributor in the recovery.

## Wound Healing Protocol (cont'd)

Once the migration phase has been identified the conversion to distance per time is made by dividing the migration distance of the array type (Table 1) by the time between the wound and end of the migration phase (TM). This number will most closely compare with optical measurements in typical scratch assays. However, as this number incorporates the transition phase (TL), it underestimates the actual migration rate of the cells. A truer estimate of a cells migration rate would use the time interval from the beginning of the migration phase to the end (TM – TL).

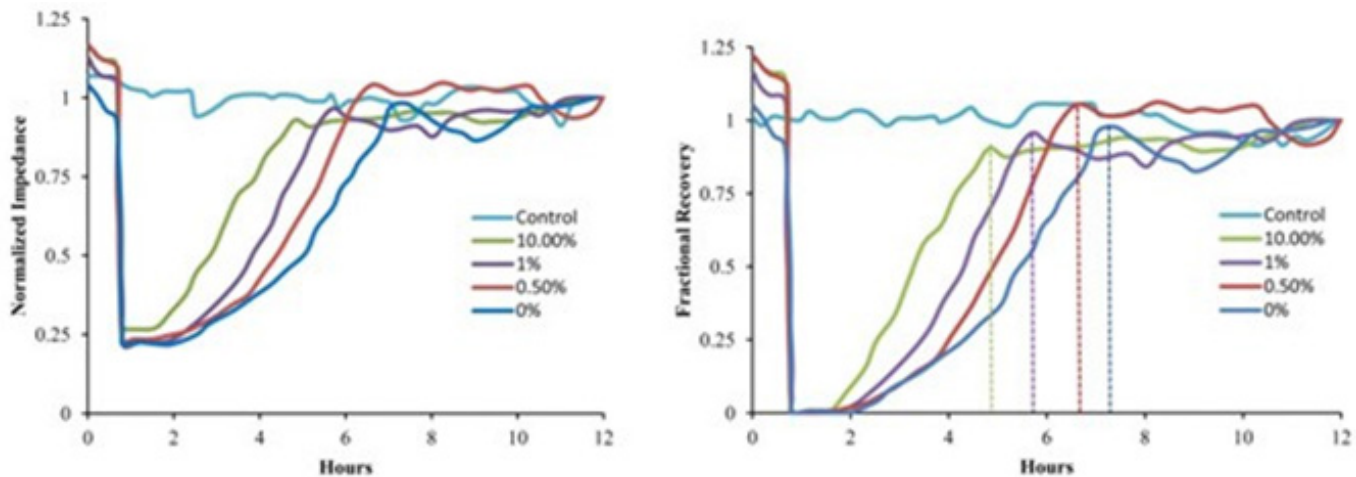


Figure 2- ECIS Cell Migration curves shown as change in normalized impedance as a function of time (A) or fractional recovery as a function of time (B). Data is derived from (10) of BSC-1 cells recovering after a 2.5 V 15 second pulse at 40,000 Hz. Cells were cultured in normal media supplemented by the indicated amount of serum.

If only ECIS based data will be compared, then just the time interval between wounding and a common end point is sufficient for comparing data sets across experiments. As long as the electrode size is kept constant the migration distance is always the same. Thus, TM becomes a sufficient metric by which to characterize the cell migration.

For displaying the results of migration data, it is generally preferable to show the richness of the time course data.

Typically, people report their cell migration experiments in graphical format like that of figure 2. Here wells recover from the wounding pulse in a dose dependent manner on the percentage of serum in the media. While showing the results as normalized impedance, resistance, or capacitance is acceptable, it is generally more informative to express results as a fractional recovery. The advantage of this transformation is that recovery measured by capacitance (figure 2b), which occurs by going from large values to low, can be inverted and then has a display similar to impedance or resistance which go from low values to high. The following table shows the conversion formulae for converting impedance, resistance, and capacitance to fractional recovery units.

### Wound Healing Protocol (cont'd)

Impedance Value	Formula to Convert to Fractional Recovery
Impedance	$Z(t)-Z(\text{min})/[Z(0)-Z(\text{min})]$
Resistance	$R(t)-R(\text{min})/[R(0)-R(\text{min})]$
Reactance	$C(\text{max})-C(t)/[C(\text{max})-C(0)]$

$Z(t)$ ,  $R(t)$ , and  $C(t)$  are the impedance, resistance or capacitance value at time =  $t$ .  $Z(\text{min})$  and  $R(\text{min})$  are the minimal impedance and resistance values immediately after wounding.  $C(\text{max})$  is the maximal capacitance value directly after wounding.  $Z(0)$ ,  $R(0)$ , and  $C(0)$  are the initial impedance, resistance, or capacitance values immediately prior to wounding.

### OPTIMIZING WOUNDING PARAMETERS

The ECIS Wound Healing Assay is accomplished by creating a cell free area on the electrode by electrically killing the cells on the electrode. This is accomplished by generating a large potential across the cells, generating pores in the cell membrane. The theory explaining this phenomenon is electroporation and for the wounding application we are generating irreversible electroporation. An introduction to this technology is given in a review by Boris Rubinsky 19.

Briefly, the cells are killed by pores forming in the cell membrane that exist long enough to induce lysis of the cell. To induce these pores a sufficient potential for a critical time must be sustained across the cells. However, the exact potentials or time durations vary with cell types, so optimal conditions need to be found for each cell type. In addition, in some instances over electroporation can cause fusion of the cells or damage the electrode. Thus, we recommend that the minimum conditions to ensure complete wounding be used for ECIS Cell Migration Assays.

## Wound Healing Protocol (cont'd)

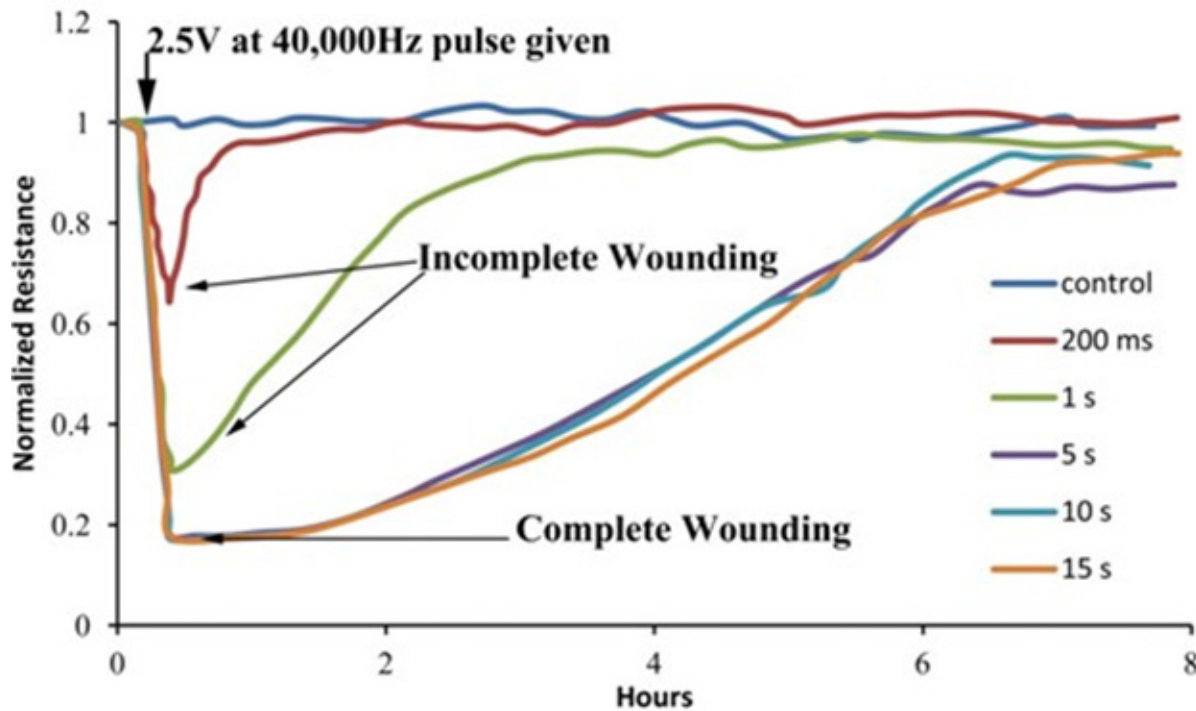


Figure 3- 2.5 V pulse was given to BSC-1 cells growing on 8W1E electrodes for the indicated time. Curves are normalized to the Resistance just prior to wounding.

To establish the minimum conditions for complete cell wounding by irreversible electroporation, an initial experiment like that of figure 1 should be carried out. In this experiment a current is selected to deliver 2.5 V at 40,000 Hz to an 8W1E array for 0.2, 1, 5, 10, and 15 seconds in different wells. The trace corresponding to 200ms and 1s illustrates incomplete wounding. Complete wounding occurs when the 2.5 V pulse is applied for 5 seconds or longer. Subsequent ECIS Cell Migration Assays for these cells should therefore have 2.5 V at 40,000 Hz for 5 Seconds as their wounding parameters.

**Note:** For some cell types that are resistant to electrical wounding, repeated 10 second pulses have proven to be more effective than a single 30 second or longer pulse. If you are finding that you need longer high voltage pulses in order to wound your cells, we recommend trying repeated pulses. In some instances, it may be necessary to apply the high voltage pulse in a low osmolarity lysis medium.

## 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 (A). 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 (B). To turn off the EF, click the *Electric Fence* button again.

A)

B)

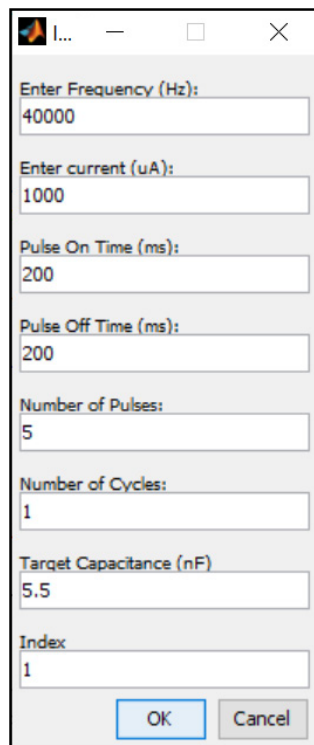
## Electric Fence Protocol with Expert Features

The electric fence switches on and off pulsing a high current at a defined interval until you turn the feature off. You can alter the electric fence parameters by turning on the expert features.

To Activate the Electric Fence parameters:

1. Go to **Acquire --> Show Expert Toolbar**
2. Check the box next to **Wound/Electroporate Option**
  - a. This should enable the wounding feature. It's possible you can skip this step if you have already activated this feature.
3. Set the **Pulse Frequency**: Add the desired time in minutes in the box next to **Electric Fence**.
  - a. Recommended: Put 2 in the box. This will pulse the wells every ~2 minutes.
4. Choose the wells you want to fence and click **OK**

The default parameter window will pop up letting you choose the frequency, current, and number of cycles.



A screenshot of a software window titled 'I...' with standard Windows window controls (minimize, maximize, close). The window contains several input fields for configuring electric fence parameters. The values entered in the fields are: 40000 for Frequency (Hz), 1000 for current (uA), 200 for Pulse On Time (ms), 200 for Pulse Off Time (ms), 5 for Number of Pulses, 1 for Number of Cycles, 5.5 for Target Capacitance (nF), and 1 for Index. At the bottom of the window are 'OK' and 'Cancel' buttons.

Parameter	Value
Enter Frequency (Hz):	40000
Enter current (uA):	1000
Pulse On Time (ms):	200
Pulse Off Time (ms):	200
Number of Pulses:	5
Number of Cycles:	1
Target Capacitance (nF)	5.5
Index	1

### Electric Fence Protocol with Expert Features (cont'd)

5. Choose the desired current parameters (1000uA is Default)
  - a. **Recommended: Increase the current from 1000uA to 1500uA (for a 1E)**
  - b. You can increase the current up to a maximum of 3000uA (1E), 6000uA (10E)
  - c. Be aware High Current and Low Frequency can destroy the electrodes.
6. Choose the desired frequency parameters (default 64,000Hz)
  - a. **Recommended: Decrease the Frequency to 32,000Hz**
  - b. You can decrease the frequency to a minimum of 20,000Hz
  - c. Be aware high current and low frequency can destroy the electrodes.
7. Choose the number and duration of the pulses
  - a. This will determine how many pulses will occur each cycle (ie: 5 or 10 or 15 etc.)
  - b. You can choose how long each pulse lasts (ie: 200 msec on, 200 msec off)
8. Choose the number of cycles
  - a. This will determine how many times the system cycles through the number of pulses chosen in Step 7
  - b. If you choose 10 pulses in Step 7 and Choose 3 cycles in step 8 – the system will pulse on and off 10 times and then repeat that for a total of 3 times/cycles.

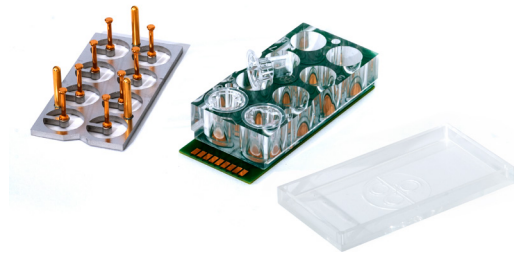
**Electric Fence Tip: Seed your cells at 50% of usual seeding density to prevent a large snowfall of cells making initial attachments early. We recommend seeding < 40,000 per well when doing an electric fence protocol (Standard seeding recommendation is 80,000/well). We find this works better than doing a quick wounding at the beginning, although that is another option you can try to ensure starting values are at normal open well values.**



# 8-well Transfilter Array

## **The ECIS® 8-well Transfilter Array**

The ECIS® 8-well Transfilter Array (**8wTFA**) is designed to measure the trans epithelial electrical resistance (TEER) of cell layers grown on filter inserts. The 8wTFA accepts up to eight standard 24-well cell culture inserts (6.5 mm) and is comprised of three parts: (1) the baseplate with addressable gold electrodes, (2) the gold-plated stainless steel common dipping pin adapter (DPA) and (3) a clear lid.



Cell culture inserts drop into the wells of the baseplate, and the DPA is placed such that each pin is inserted into the inner well of the cell culture insert. Electrical contact to the DPA is accomplished with three gold pins that drop from the DPA to the base of the baseplate. The lid then covers the wells including the DPA to keep the contents sterile.

The 8wTFA fits into either side of the standard ECIS® 16 Well Array Station and two 8wTFAs may be run simultaneously. Alternatively, an 8wTFA and a normal ECIS® 8-well array may also be run together. When asked to identify the array type, choose 8W Filter for the 8wTFA.

### **Preparation of the 8wTFA Adapter**

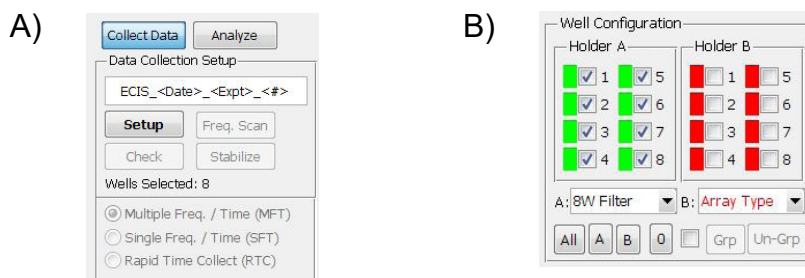
The 8wTFA baseplate can be cleaned with any tissue culture compatible detergent and rinsed with distilled water. We recommend soaking the DPA in 1% tissue culture compatible detergent at 50-70° C for at least one hour and then sterilized by autoclaving. Alternatively, one can flood the wells and expose the DPA to a 70% isopropyl alcohol solution. After this treatment the wells and DPA should be thoroughly rinsed with sterile water and allowed to dry.

*Note: do not sterilize using bleach (sodium hypochlorite) solutions.*

Cell culture inserts can remain untreated or be treated prior to measurement with matrix proteins; please follow the filter manufacturer's guidelines for these treatments. For TEER measurements we generally use filters having a pore size of 0.4 micrometers diameter. For this example we are using the Corning Transwell® Permeable Supports (#3413).

### 8-Well Transfilter Experiment Protocol

1. Pipet 1,000 microliters of medium in the large outer wells that will be used for the experiment and, using sterile forceps, place the membrane inserts into the wells.
2. Pipet 200 microliters of medium into the inner wells being careful not to damage the filter with the pipet tip.  
\*The media volumes suggested above can be varied somewhat and may be different when using other commercial brands of membrane inserts. The important considerations when filling the wells are that:
  - The dipping electrode's hemisphere tip is submerged
  - The two compartments are only connected through the filter and never by an overflow between the inner and outer chambers
3. With the filters in place and medium in both inner and outer wells, place the sterile dipping electrodes into the culture inserts with the three DPA guide pins matching the associating holes on the baseplate and place the lid on the device.
4. Then insert the complete 8wTFA into one side of the ECIS® 16-Well Array Station.
5. Open the ECIS software and click **Setup** (A).
  - All wells of the 8wTFA in which you have placed medium should appear green in the lower left hand Well Configuration display. If any wells appear red, check that the 8wTFA is inserted correctly into the array station and that medium levels in the inner wells are sufficiently high to submerge the dipping electrode tip. If necessary, add medium to the inner well.
6. Select the array type for sides A and B.; for TEER measurements with the 8wTFA select 8W Filter (B).



7. Click **Check** using **125 Hz** frequency
    - a. Manually change the check frequency below **Time Course Options**
    - b. For clean dipping pins, a cell-free value should have a resistance of ~150 +/- 50 ohms
- \*We recommend equilibrating the electrodes and filter membranes for 30 minutes or longer in the tissue culture incubator before making ECIS measurements.

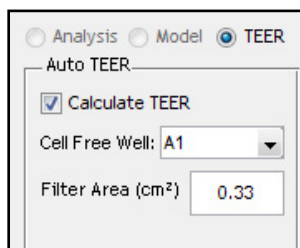
### 8-Well Transfilter Experiment Protocol

8. Remove the 200 microliters of medium from the inner well and replace this with 200 microliters of warm cell suspension to provide the desired cells/cm<sup>2</sup> density.
  - In the experimental setup be certain to include a cell-free membrane insert that receives medium without cells as this is required for TEER analysis.
9. Select Multifrequency timecourse (**MFT**)
  - TEER should be analyzed at 125 Hz for Resistance
  - Cell proliferation to confluency should be analyzed at 64,000 Hz Capacitance
10. Click **Start** to begin the experiment. This option will prompt a save screen to name and save the experimental file to the desired file location.
11. Click **Pause** when needing to remove the array from the array station for addition of compounds, etc. After connecting the array back into the array station, click **Check** to confirm values of properly connected array and then click **Resume** to allow the software to continue recording data.
12. Click **Finish** when experiment is completed.

### Analysis of Completed TEER Data

Upon completion of the experiment, open the data file for analysis. If you did not set the array type to 8W Filter you can do that at this point.

With this array selection, the Auto TEER feature shown below becomes available.



Select the filter without cells (A1 in this case), input the filter area in cm<sup>2</sup> (0.33 cm<sup>2</sup> for the Corning Transwell®) and click on the **Calculate TEER** box. The system will automatically carry out the calculations and present the TEER values in ohm-cm<sup>2</sup> as a function of time using the 125 Hz frequency of the data set. For more precise TEER resistance readings, we recommend viewing the resistance (R) frequency at 125 Hz. If one wishes to view cell growth to confluency, view the data under capacitance (C) with a frequency at 64,000 Hz.

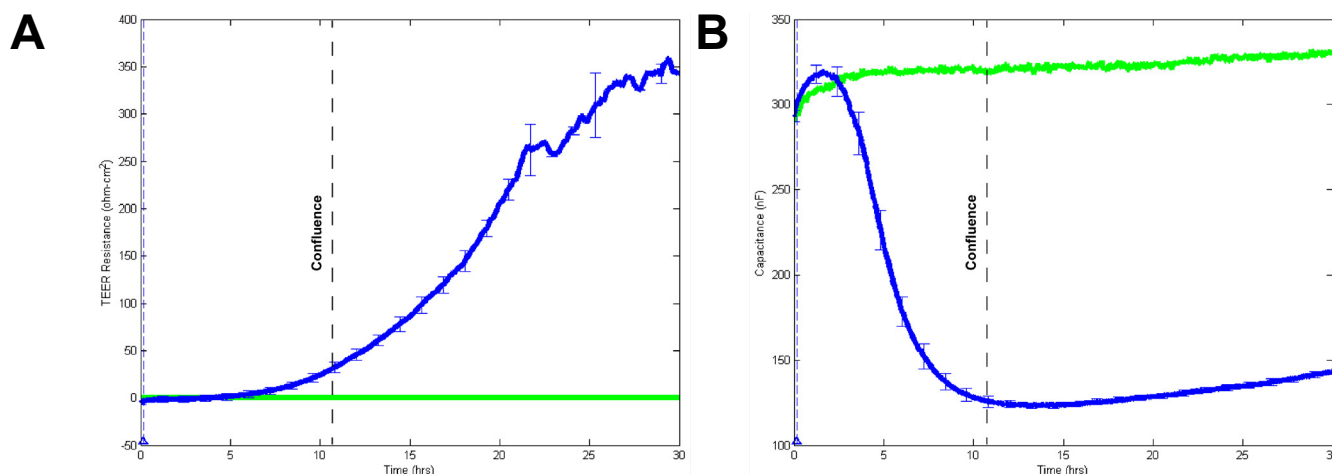
If desired, other frequencies can be examined using the frequency selection feature beneath the left side of the graph.

## 8-well Transfilter Array

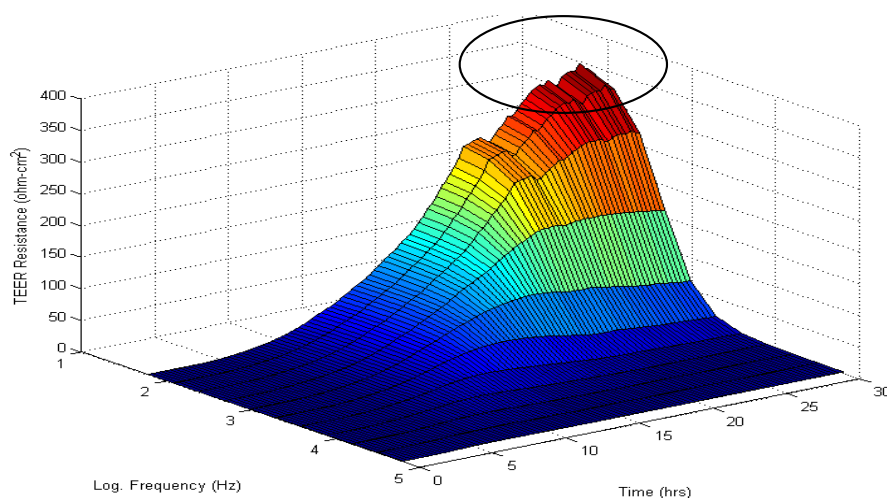
*Note: the reported TEER values will fall off at high frequencies as more current will flow capacitively through the cell membranes rather than through the paracellular space.*

Graphs can be exported (under Display Options) or the graphed TEER data can be stored as a comma separated variable (.csv) file by clicking on the File tab, then Export data and Graph Data.

### Sample Data



**Figure 1:** A heavy inoculation of MDCK II cells in complete DMEM with 10% FBS using Corning® Transwells (0.4 micrometer pores, 108 pores/cm<sup>2</sup>). A) The plot shows changes in TEER as a function of time for MDCK II cells (Blue) vs cell-free filters (Green) analyzed using the ECIS Auto TEER software. B) The plot of the same run showing changes in capacitance (nF) until confluence at ~11 hours.



**Figure 2:** A 3D plot of the previous run is shown looking at the data from the blue trace (1.0X inoculation). Notice how the lowest frequencies (circled) are in good agreement with curves peaking around 350 ohm-cm<sup>2</sup>. As frequencies get higher, the TEER that is reported appears lower, as more current now capacitively couples through the cell membranes rather than flowing through the paracellular path.

# 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





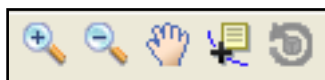
The use of these various icons will be described in groups






The first four symbols determine whether impedance (Z), resistance (R), capacitance (C) or modeling parameters (Rb, Alpha, Cm) are plotted on the vertical axis.

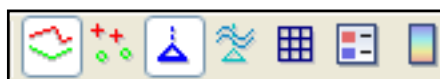
The next three symbols determine whether the horizontal axis is time (T) or, providing the data has been collected in the MFT mode, frequency (F), or a three dimensional plot (3D) where frequency and time form two of the axes.






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 .




## Analyzing Data

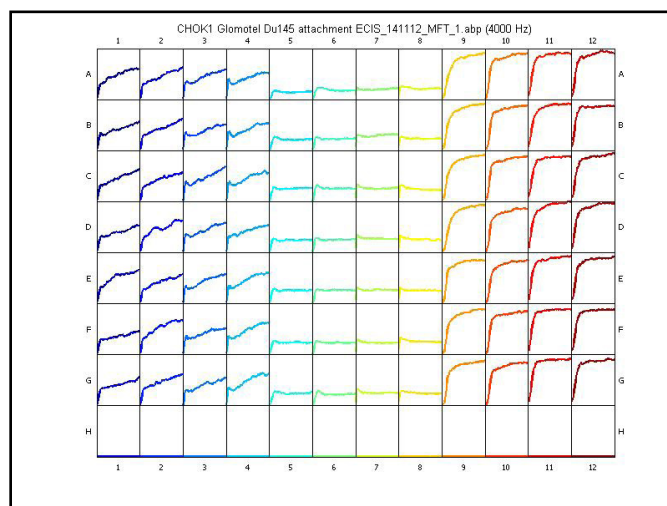
The next two symbols allow one to change to log scales for the x and y axes and back to normal linear scales. The error bar symbol  plots or removes error bars on data that has been grouped and averaged (described below). These error bars are plus and minus one standard deviation and are plotted at regular intervals along the graph. The  $n/n_0$  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  $n-n_0$  icon  plots the difference between the data and the time zero reference. If both icons are selected, the calculation used is  $n/n_0 - 1$ , commonly referred to as Cell Index.




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 last icon is the lock icon  which restricts the menu and toolbar items available when performing data collection.



## Time-Series Options

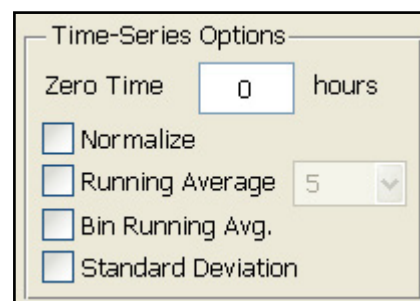
**Zero Time** enables the origin of the x-axis to be moved to the specified time.

**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.

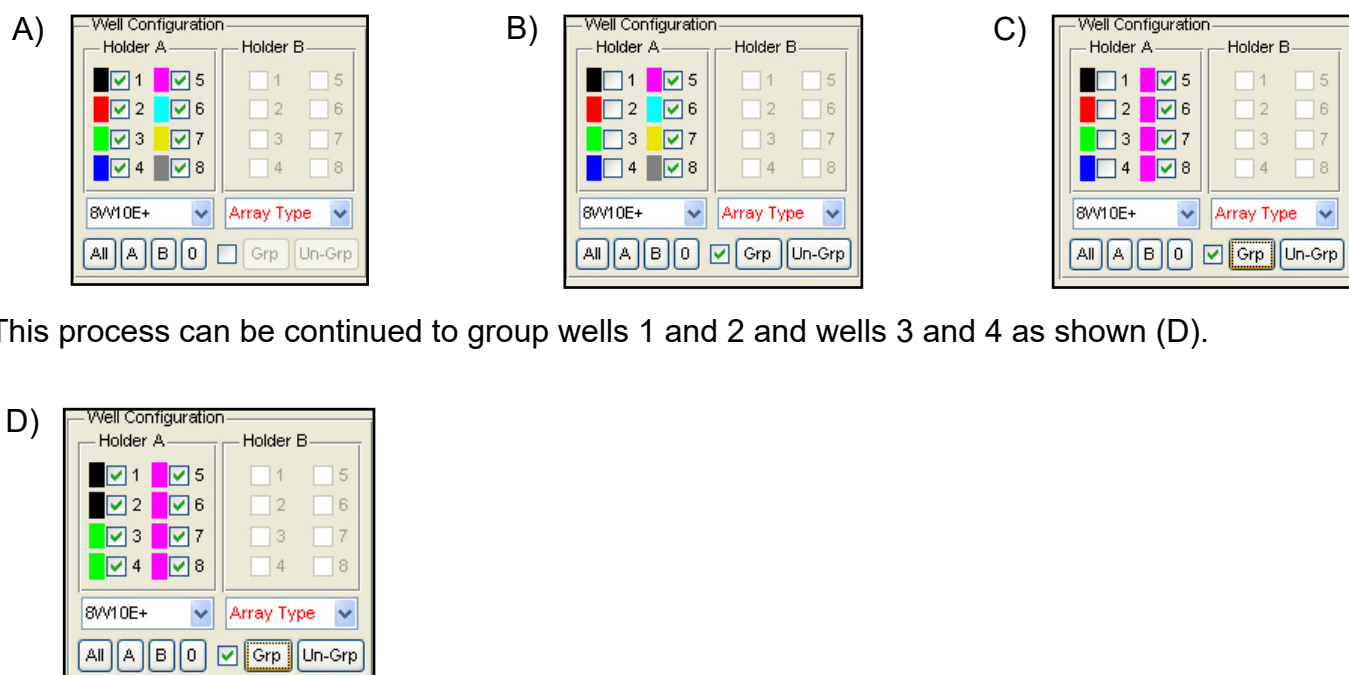
**Bin Running Avg.** will reduce the number of data points by displaying every n-th point from the running average with length n.

**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 (D).

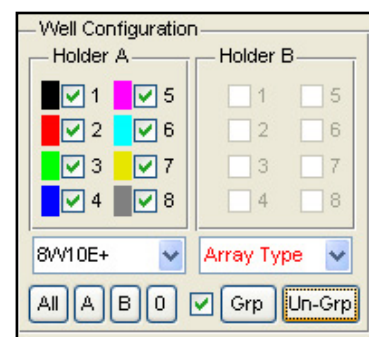


## Analyzing Data

Clicking the small center box mark will toggle between individual wells plots and grouped data.

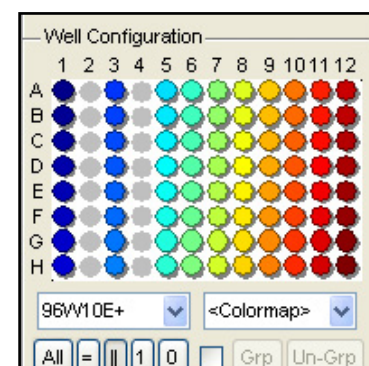
To group wells differently, first click **Un-Grp** to ungroup the selected wells.

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.

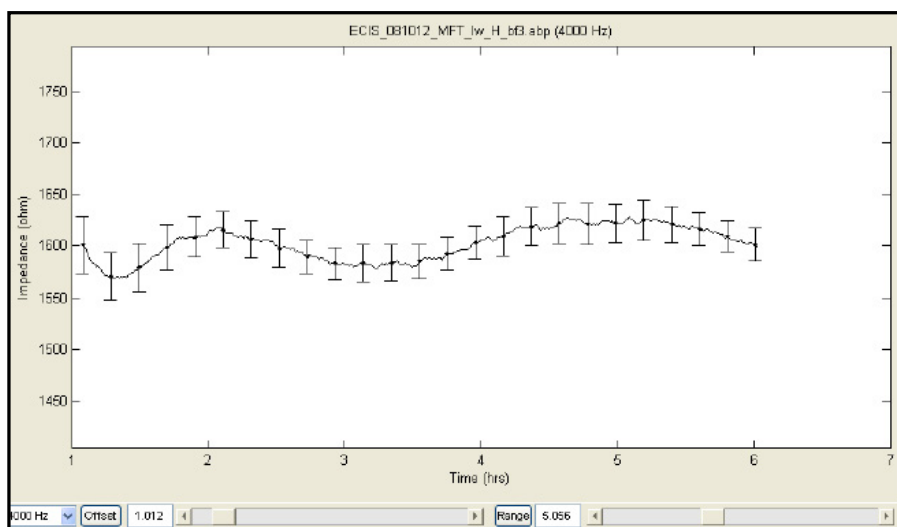


### Error Bars

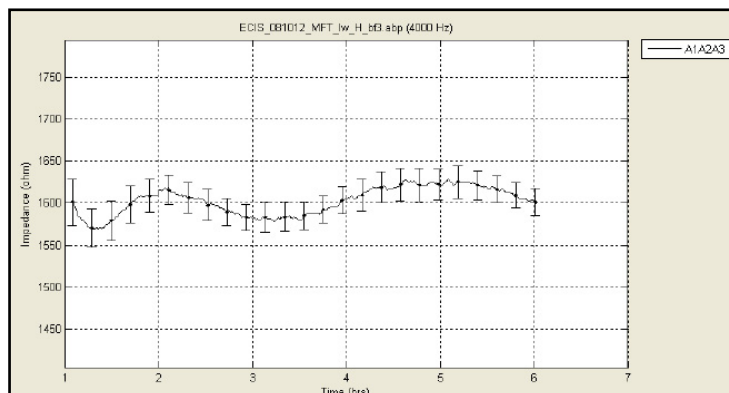
Error bars representing plus and minus one Standard Deviation can be added to the averaged curves by clicking the icon. The bars are spaced at regular intervals with the default number of bars being 25. This can be changed from the menu **Edit | Number of Error Bars**.

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.

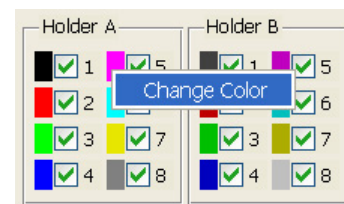


Grid lines can be added and legends to this figure with the appropriate icons from the tool bar. In the case we see wells A1, A2 and A3 have been averaged as indicated in the legend on the right.



## Coloring the Wells and Graphs with 8 Well Array

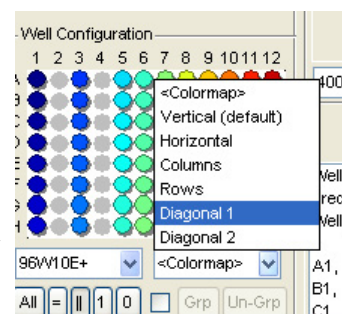
The colors of the 8 well arrays can be customized either by selecting a different colormap from the Edit | Colormap menu. The current options are Standard, Alternate, Jet, HSV, Rows, Columns, Pairs, Eights and Manual. Select the one that best matches the array setup and graph display. To manually specify 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

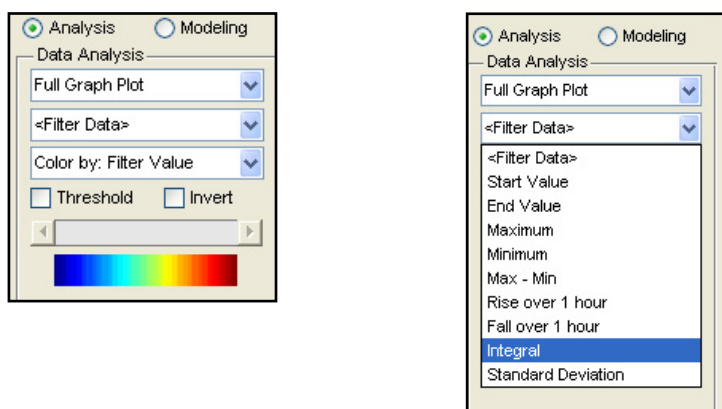
Because of the large number of wells, there are a variety of ways to color the wells (and their data plots) to end up with a color scheme such that wells can be easily distinguished and to aid in filtering and selecting relevant data. The system initially defaults to a vertical system where each column 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:

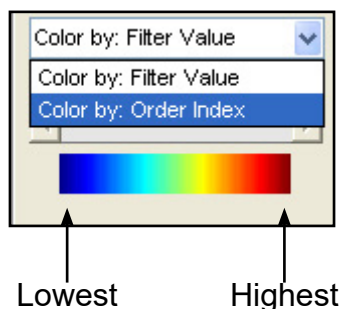
- size of start or end value
- maximum or minimum value over the time period selected
- maximum minus the minimum value (largest overall change)
- size of the rise or fall over a 1 hour period
- size of the integrated value over the time course selected
- size of the standard deviation of the parameter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

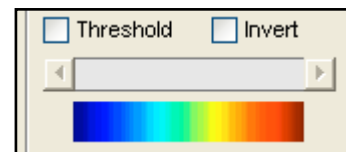
**Order Index:** in this case, the filtered parameter for all wells used in the experiment are put in rank order from lowest to highest. These are then spread evenly over the spectrum giving each well a unique color.



## 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.

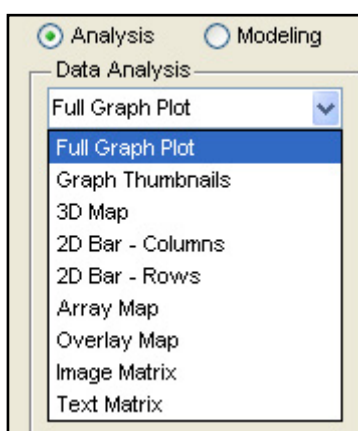


## Different Means to Plot 96 Well Data

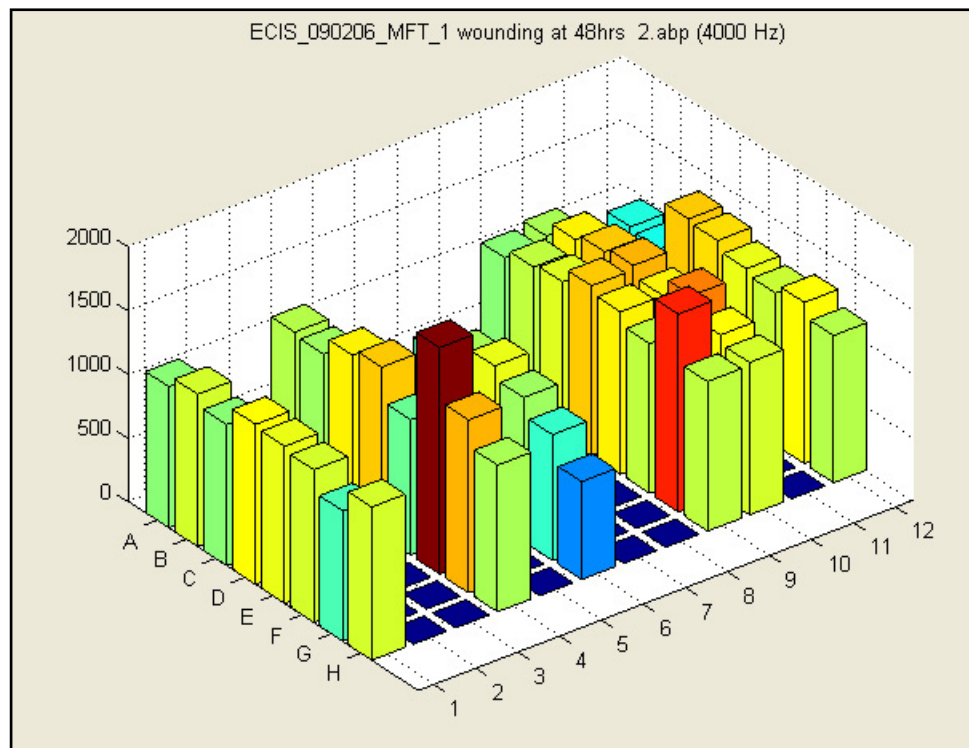
With both the 16 well and 96 well stations, data can be plotted as a full graph, as well as separate graphs (thumbnail plots). In both of these methods, data (e.g. Z, R and C) are plotted as a function of time.

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.

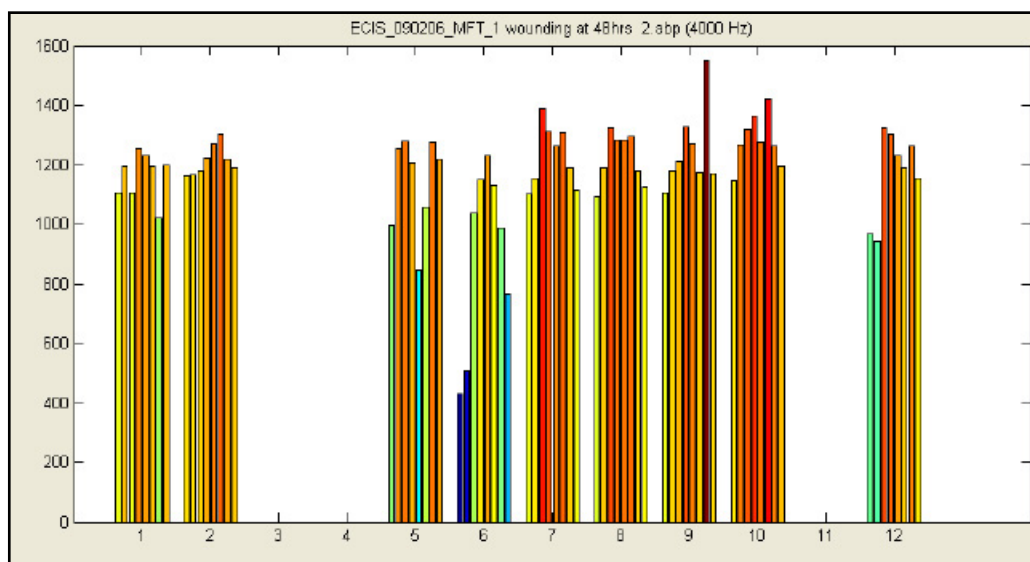
These various methods are shown below where location of the wells and the value from the filter are displayed in different manners.



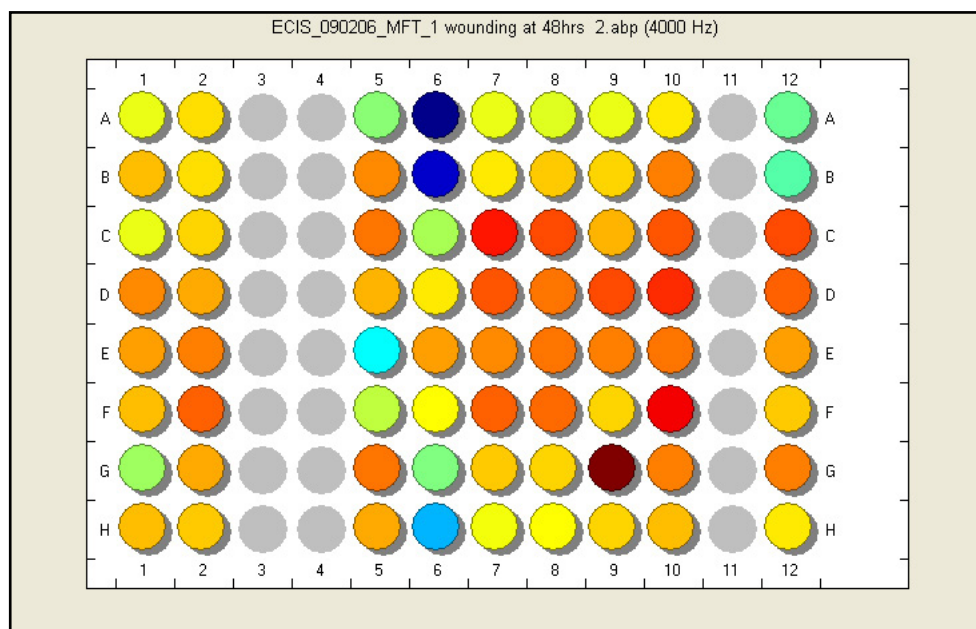
## 3D Map of Wells



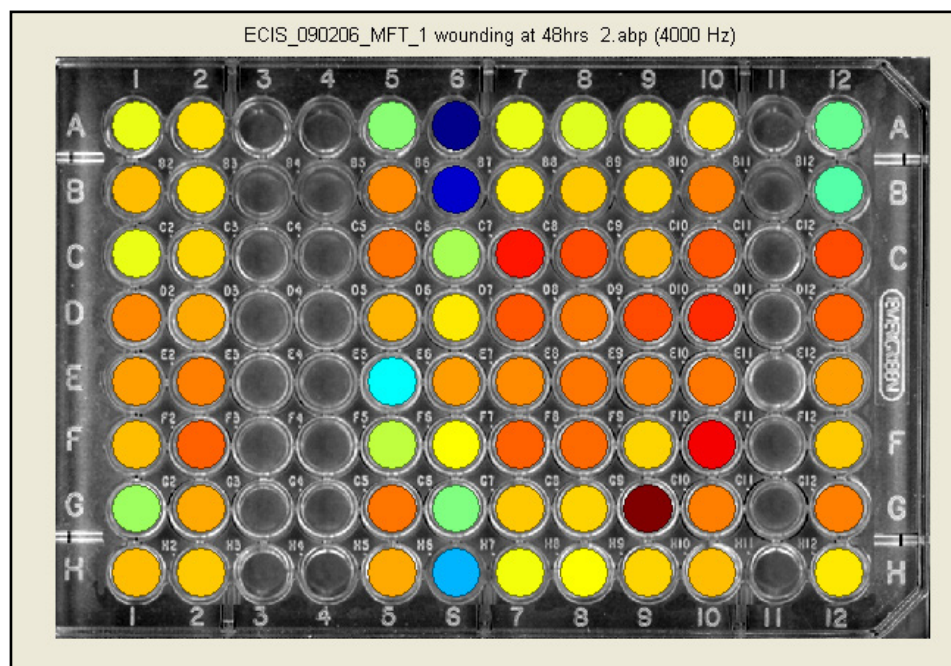
## 2D Map of Columns (or Rows)



## Array Map

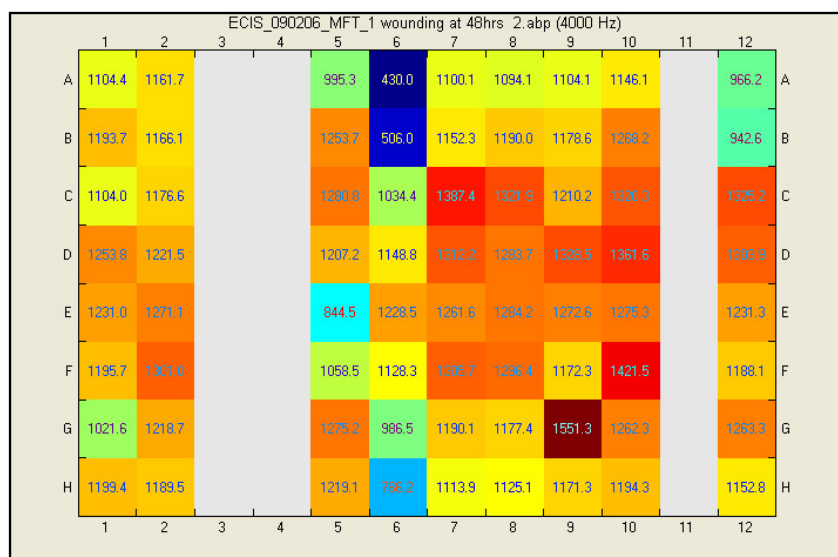


## Overlay Map





## Text Matrix (with both color and value of filter parameter for each well)



## 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.

Display Options

Export
800
x
600

☒ X:
0
to
70

☒ Y:
200
to
2200

Redraw



## How to Make Use of the Multiple Frequency Time Course Data

The Z $\theta$  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 Z $\theta$  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 Z $\theta$  instruments, modeling to see time course changes in barrier function (see below) can be conducted.

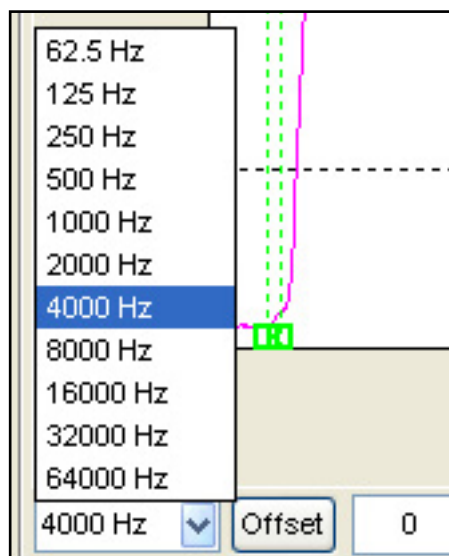
### Determining the best AC frequency by examination

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

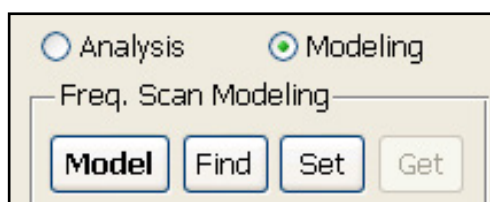
This can be examined by simply changing the frequency and examining the data. The frequency can be changed by clicking on the check box next to the listed frequency at the lower left of the graphing area. From this listing simply choose the AC frequency at which you wish to display the data and the graph will automatically update. The frequency list shown below is for the Z $\theta$  instruments.



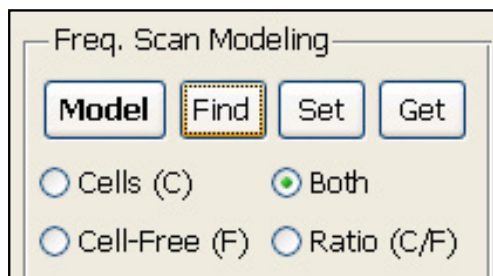
### Analyzing frequency scan data using the ratio of cell to cell-free data

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 cellfree 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.

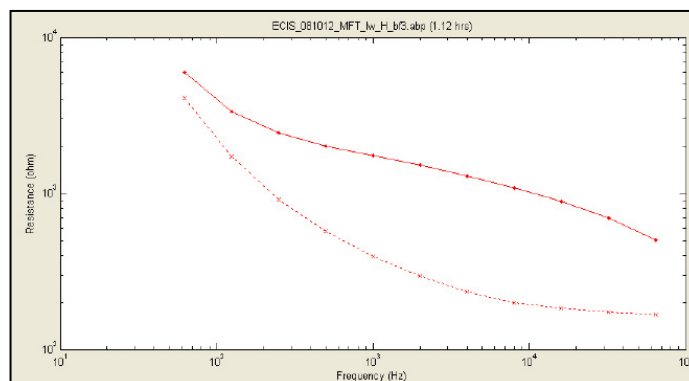


You can also choose a particular point by moving the data cursor to that point on the graph and clicking the Set button. If you have no cell-free data in the file being analyzed, open a file with data from the same type of array and medium where cell-free points are available. Now, using Find or the 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.

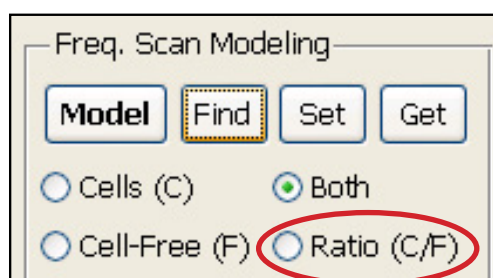


With the cell-free data selected, next select a point in the data set where the cells are thought to be confluent or nearly confluent. Again with the data cursor, choose a point. Now click on the F (frequency) button in the tool bar and the display will change to display both the cell free data and the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

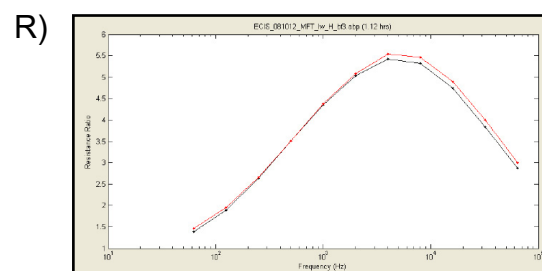
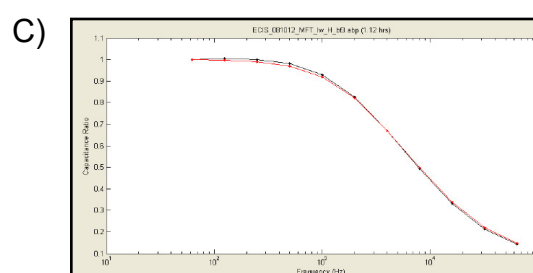
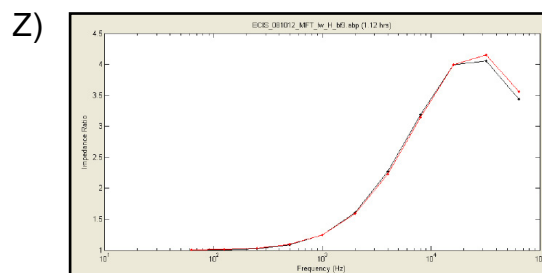
Note that the Cell-Free curves are drift corrected such that the capacitance at the lowest frequency matches that of the Cell measurement. Therefore multiple Cell-Free curves may be displayed.



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.

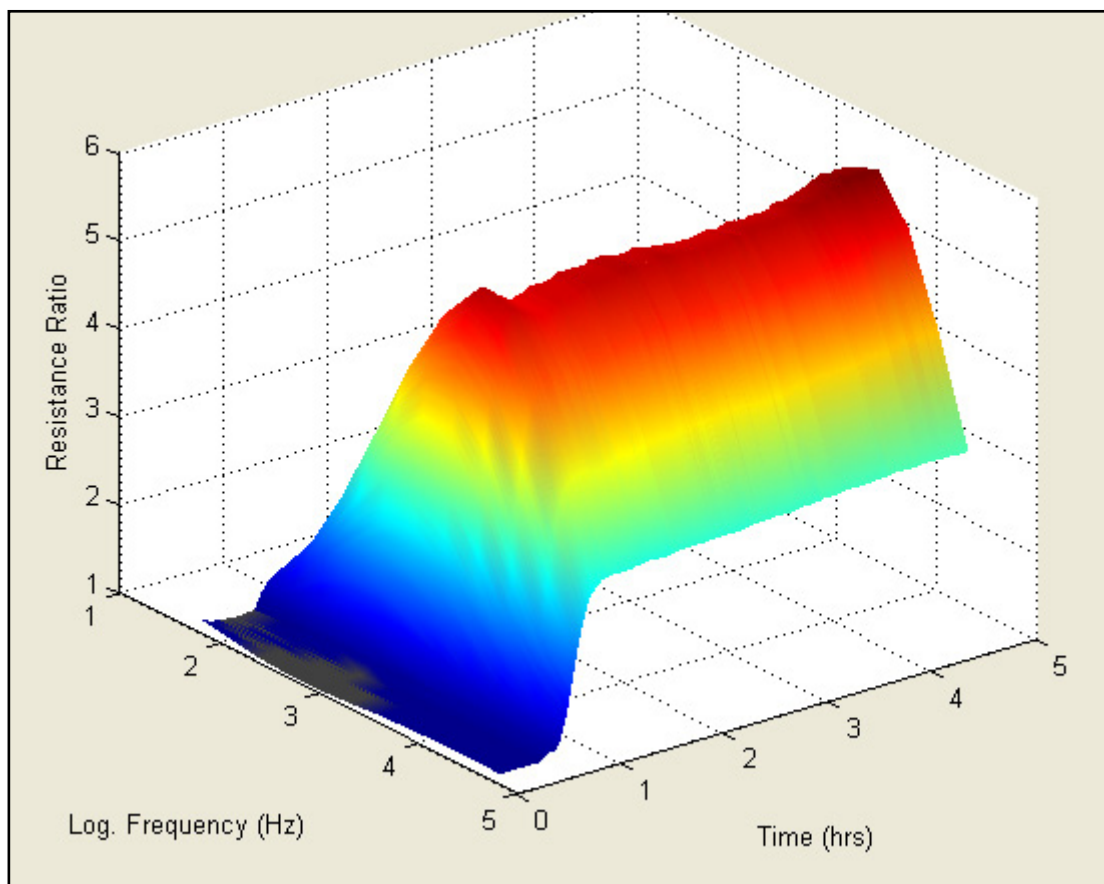


This will now show the ratio of the cell to cell-free data versus the log of the AC frequency. Normally both the R and Z plots of this sort will show a peak. Moving the data cursor to this region of the graph will list the AC frequency. The C ratio plot (Z $\theta$  systems only) will always show the 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.



## Analyzing Data

With these calculations done, click 3D to see how these ratios change during the time course of the experiment. The data shown below 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)



# Data Modeling

## General notes about understanding the ECIS model

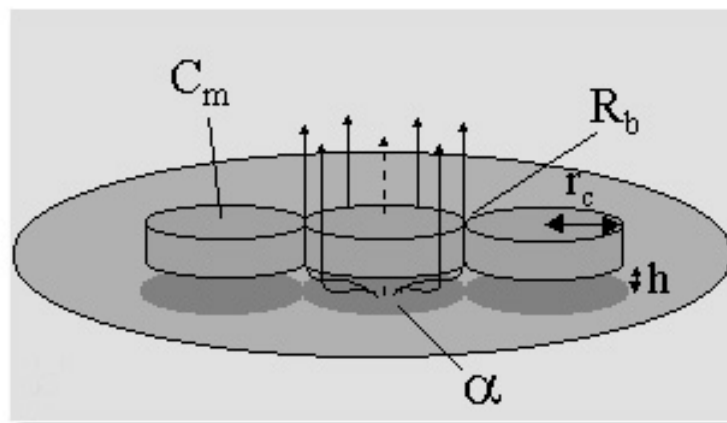
To completely understand the ECIS model, a mathematical background that is often not part of the training of cell biologists is necessary. That being said, this does not mean that a reasonable understanding and appreciation of the model parameters that are detected with an AC impedance measurement cannot 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.



Cells modeled as disks

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, **Rb**, which can range from 0 to approximately 100 ohm-cm<sup>2</sup> 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<sup>2</sup>)<sup>0.5</sup>.
3. Finally, it is possible to determine the average capacitance of the cell plasma membranes, **Cm**. With the current model, it is not possible to distinguish between the apical and basal membrane and so this number, in microfarads/cm<sup>2</sup>, 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:

$$\text{Fractional area} = (\text{Copen} - \text{C cells}) / (\text{Copen} - \text{C confluent})$$

Or in this case,

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

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.

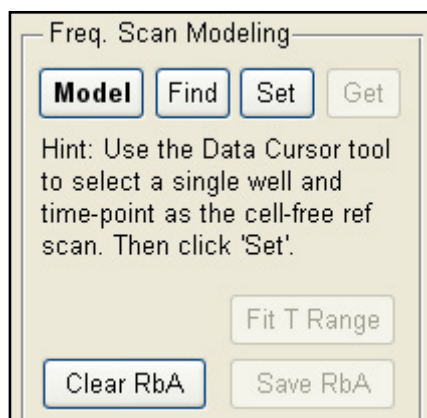


## Modeling Software

The ECIS Z0 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.



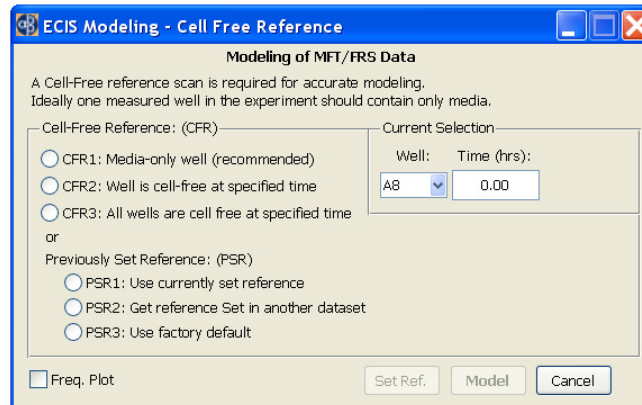
Before beginning modeling:

- 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.

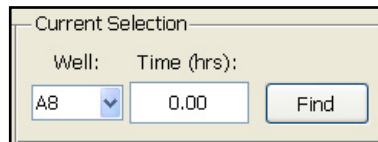
**Model** Opens the Modeling dialog that simplifies modeling setup and processing.

Default display of modeling dialog:



## Current Selection

Displays and allows the user to specify the current cell-free reference well and time-point. These values will be automatically set on startup if the user has selected a well and time-point using the Data Cursor prior to starting Modeling (or if the 'Find' button was used to find a cell-free reference).



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.

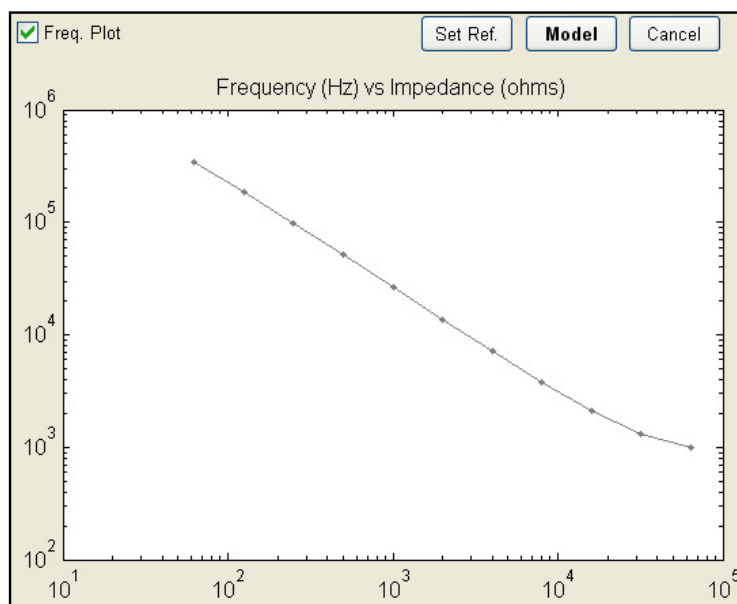
## Set Ref.

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

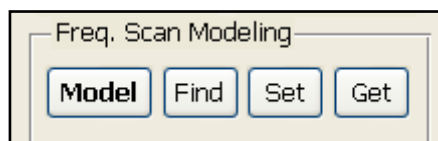
## Model

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

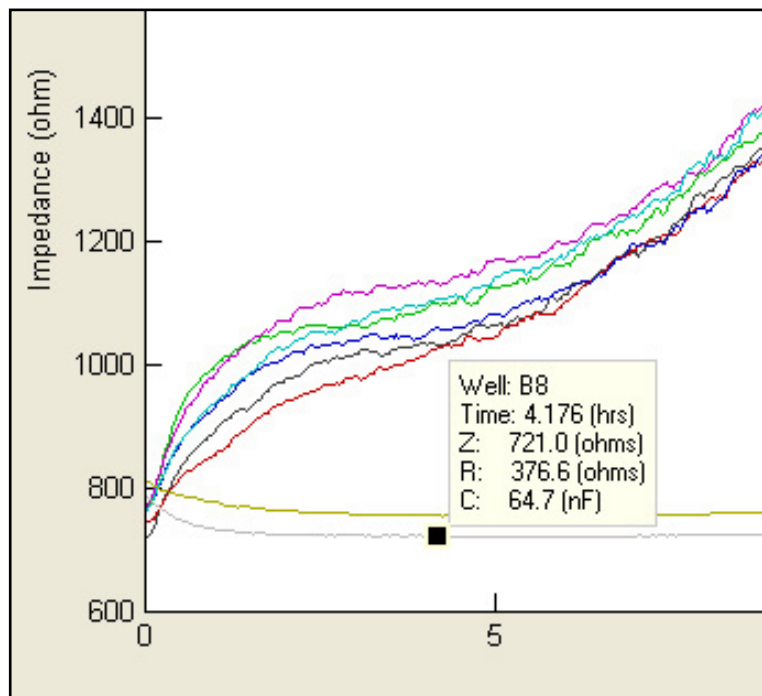


To manually specify the cell-free reference, the **'Find'**, **'Set'**, and **'Get'** buttons are available.



## 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.

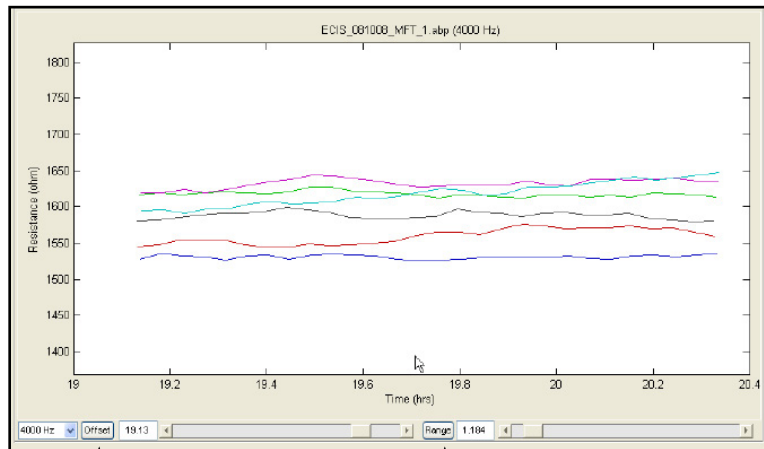
## **Getting cell-free data from another data set**

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.

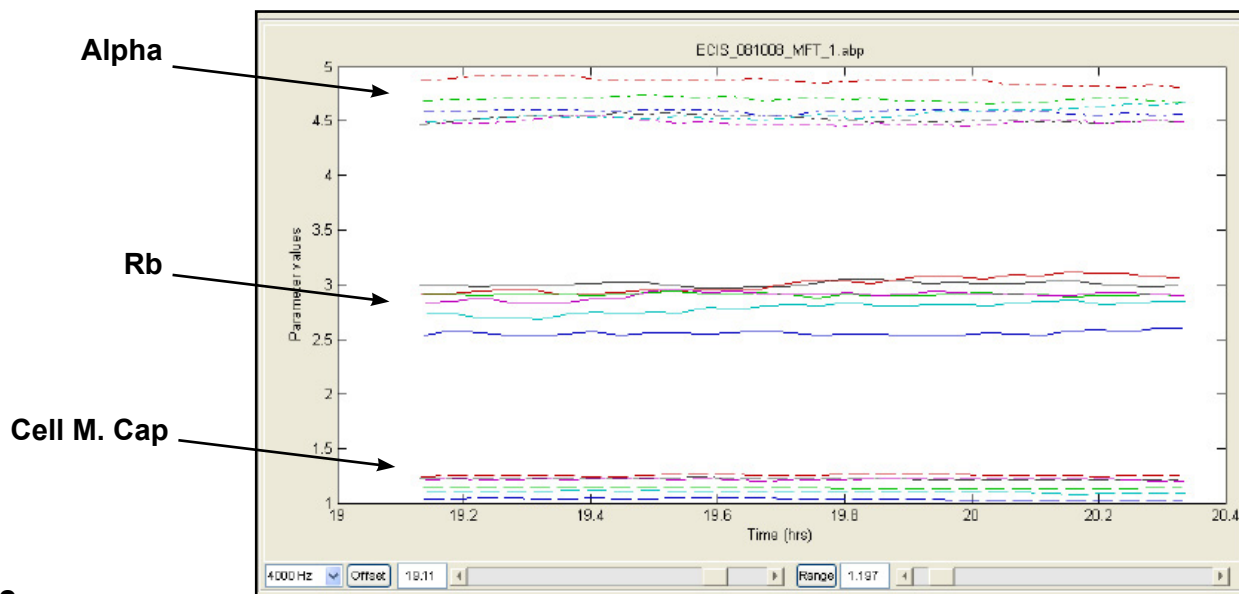
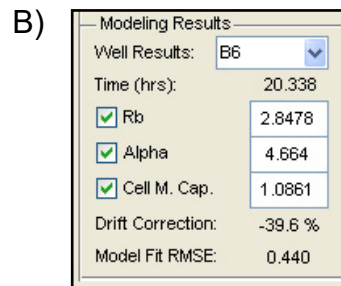
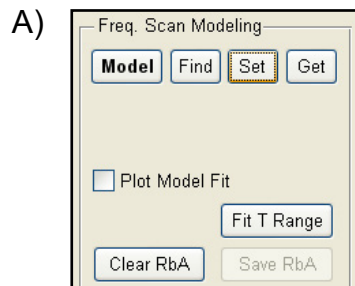
# Data Modeling



Sets Starting Point

Sets Length of Run

Clicking the Fit T Range button (A) 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 (B).

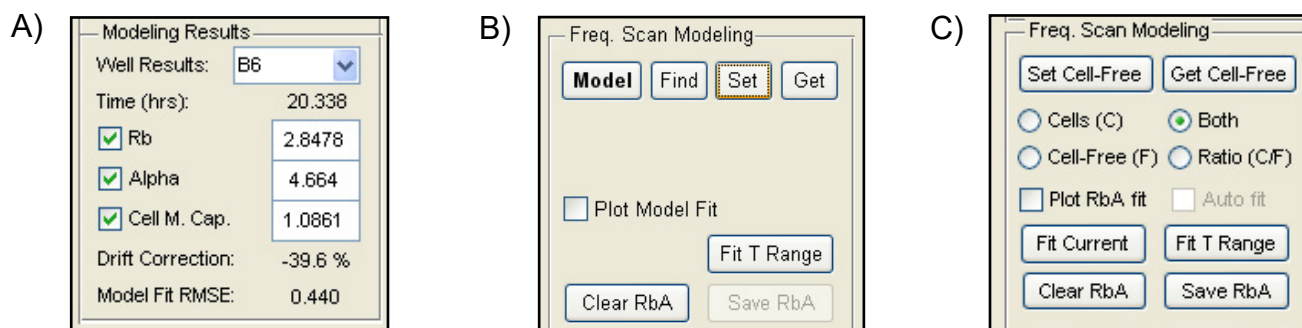


## The Validity of the Modeled Data

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 (A). 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/cm<sup>2</sup>.

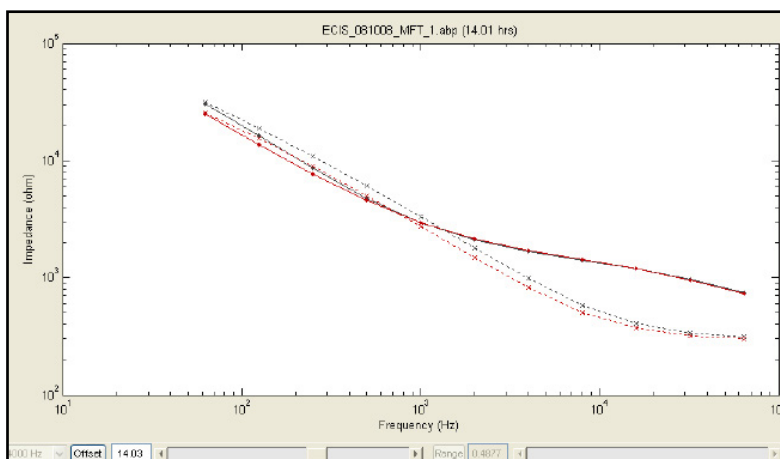
To view the model fit over time with respect to Z, R or C, select the appropriate time view and check 'Plot Model Fit' (B). The model fit will be plotted as a dashed line.



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.

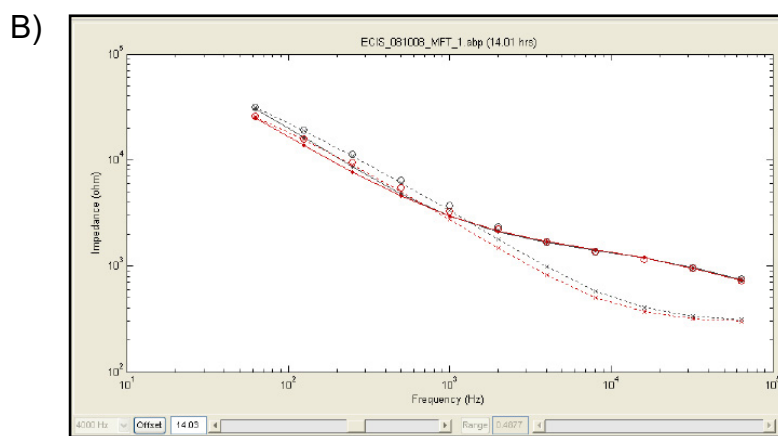
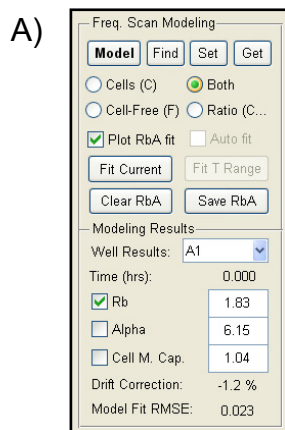


One can now look at the experimental data for one time point and show both this and the cell-free data (C).

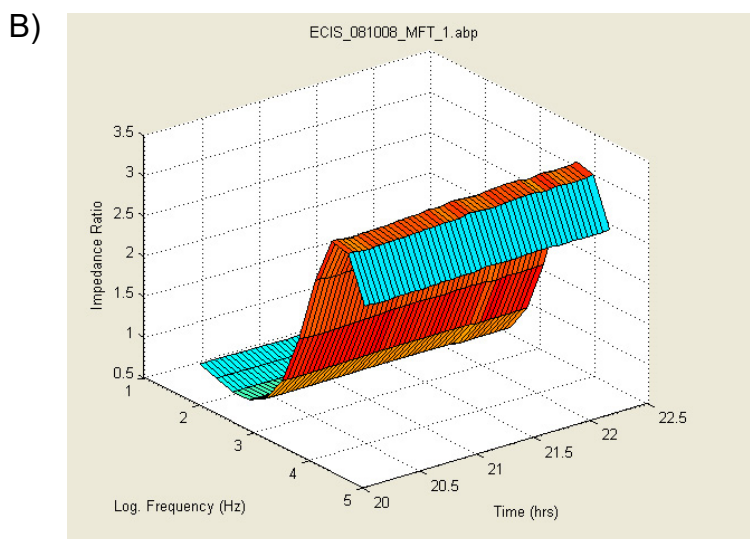
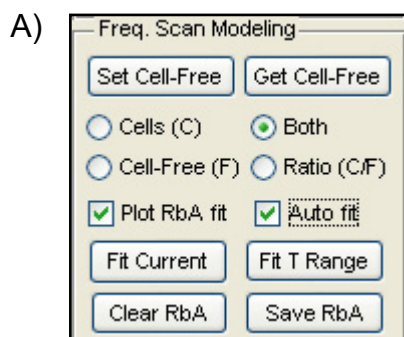


# Data Modeling

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 (A), and circles will be added to the graph showing the calculated values for the frequency scan data (B).

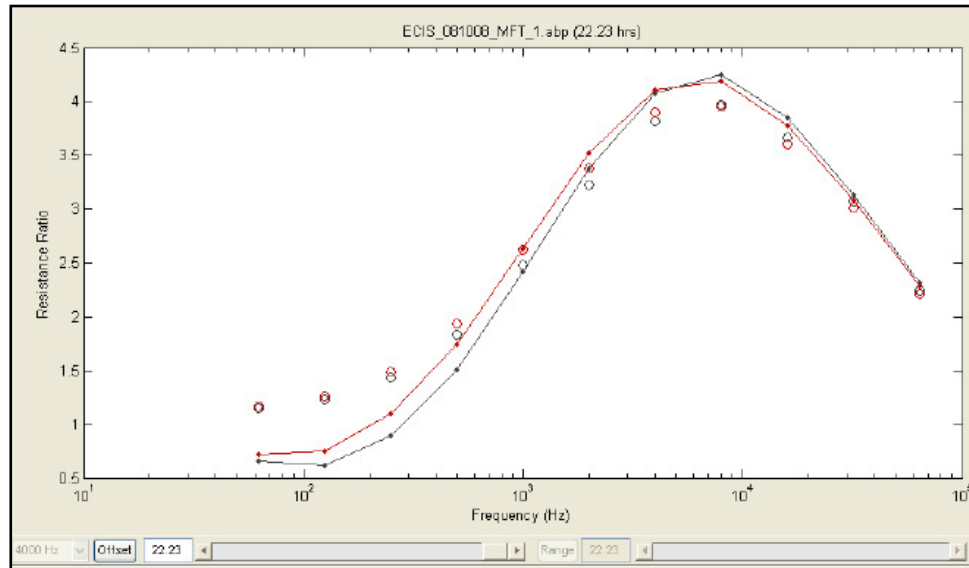


By clicking **Auto fit**, data will be automatically fitted as the offset value is changed with the slider beneath the graph. Clicking the **Ratio (C/F)** will show the ratio of the cell data divided by the cell-free data for the impedance, R or C. These data do not require modeling and are valid for 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 (B).





The ratio data can also be modeled where circles show the calculated ratio values, 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

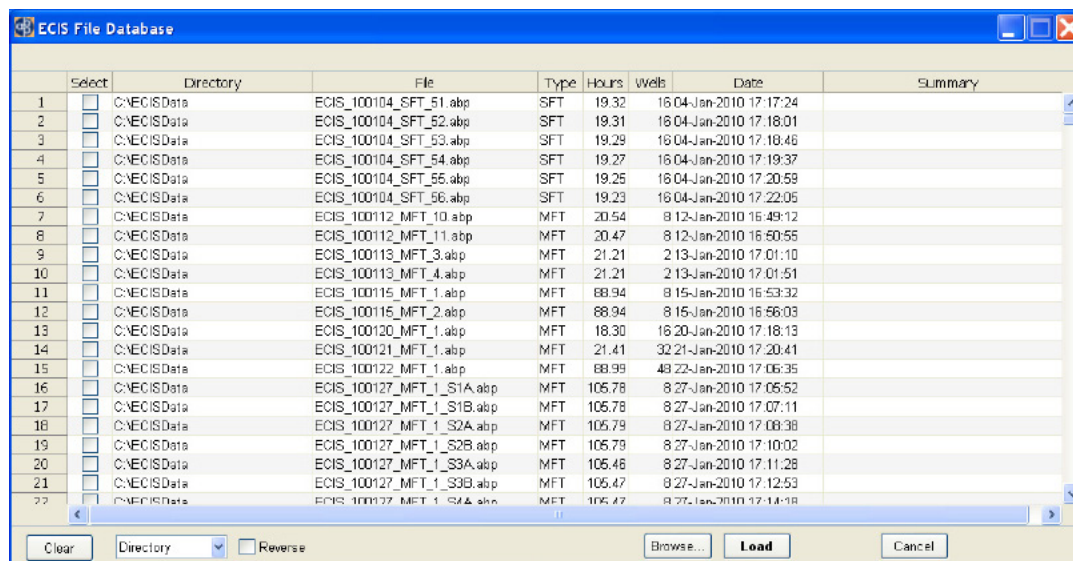
Please contact ECIS support if you cannot open a valid ECIS file.

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'.



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 CO2
- 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
- o 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 be 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 ( $\theta$ ) 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.

## **How to order Arrays**

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

ECIS Arrays can also be ordered through our website at [www.biophysics.com](http://www.biophysics.com)

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](http://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 in the Rensselaer Technology Park.

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. 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.



185 Jordan Road  
Troy NY 12180  
Ph: 518-880-6860  
Fax: 518-880-6865  
1-866-301-ECIS (3247)  
[www.biophysics.com](http://www.biophysics.com)