



APPLIED BIOPHYSICS, INC.

2018 ECIS USER HANDBOOK

ECIS[®] Handbook

Table of Contents

Choosing the Right Array	1
Small Electrode Arrays.....	1
Large Interdigitated Finger Arrays	2
Array Preparation	3
Cysteine Treatment.....	3
Electrode Stabilization.....	4
Coating the Electrodes	5
Step by Step Array Preparation & Coating Guide.....	6
Inoculating with Cells.....	7
How to Prevent Convection Effects	8
Cell Numbers	9
ECIS Software Options	10
Multiple Frequency Time (MFT)	10
Single Frequency Time (SFT)	10
Rapid Time Collection (RTC).....	11
Adding Compounds.....	12
Starting an Experiment.....	14
Step1: Checking Your Array	14
Step 2: Configure and Run the ECIS Software	15
Step by Step Quick Experiment Guide	16

Choosing the Right Array

Choosing the correct array for your application is an important first step before starting an experiment and can greatly impact the measurements.

There are 2 broad categories of arrays: Small electrode arrays (1E, 10E, 10E+) and large interdigitated finger arrays (10idf, 20idf). Each type of array has an optimal application, but some arrays can be used successfully for multiple applications.

Small Electrode Arrays

8W1E or 96W1E+: Cells are measured on a single 250 micrometer diameter active electrode and two 354 micrometer electrodes respectively. With this small population of cells, one is able to observe the random non-synchronous motions of cells in culture as fluctuations of the impedance signal. This movement has been termed “micromotion” and its measurement is unique to the ECIS technology. Although the 1E or 1E+ array is the best choice to maximize these measurements, micromotion is evident on the 8W10E and, to a lesser extent, on the 8W10E+ arrays.

The **8W1E** and **96W1E+** arrays are optimal for wound healing migration or micromotion assays.

8W10E or 8W10E+: Cells are measured on ten and forty 250 micrometer electrodes respectively. These arrays are an excellent choice when one wants to average over a larger number of cells, but still have high sensitivity to more subtle changes. These arrays are commonly used for barrier function analysis, signal transduction assays and many other assays, where synchronous changes in cell morphology are expected. They also are an excellent choice for the ECIS extravasation assay.

The **8W10E+** is optimal for barrier function/permeability assays.

Large Interdigitated Finger Arrays

96W10idf, 8W20idf, or 96W20idf: Cells are measured on large interdigitated finger electrodes. The **96W10idf** array offers a similar performance to the **8W10E+** because the total area of electrodes is equal. The **8W20idf** and **96W20idf** have electrode areas twice as large as the **8W10E+** and **96W10idf** and so are very good at measuring cell proliferation and attachment and spreading of cells with or without ECM proteins. Measurements with these arrays are less sensitive to cell distribution on the bottom of the well and average over a very large substrate area. These arrays are commonly used for barrier function analysis, signal transduction assays and many other assays, where synchronous changes in cell morphology are expected.

The **8W10idf** is optimal for barrier function/permeability assays.

The **8W20idf** and **96W20idf** are optimal for proliferation or cell attachment and spreading assays.

Array Preparation

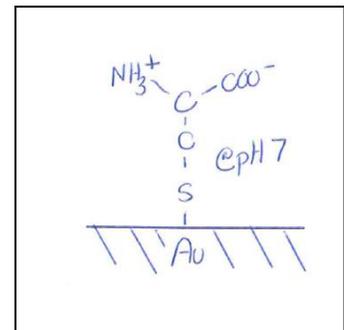
Preparing your array before cell inoculation is an important step to maximize consistent and low-variable results well to well.

Arrays are oxygen plasma etched the day they are shipped from Applied BioPhysics. This step sterilizes the arrays and also cleans the gold resulting in clean, pristine electrode. Over time, small molecules in the atmosphere adsorb to the gold surfaces resulting in increased electrode impedance and resistance – lower capacitance. When exposed to tissue culture medium, these molecules gradually desorb and the impedance values eventually return to those associated with clean electrodes. To assure this takes place in a timely manner, we strongly recommend that researchers take measures to clean the gold before protein-coating and/or cell inoculation. There are two approaches to accomplish this – **cysteine treatment** and **electrical stabilization**.

Cysteine Treatment

Our preferred way to clean the electrodes is with the amino acid **L-cysteine**. This treatment cleans and modifies the electrode surfaces enhancing experimental repeatability with minimum variation between ECIS wells.

To accomplish this, the well should be flooded with a 10 mM sterile solution of L-cysteine in distilled H₂O. The solution can easily be prepared in your laboratory and filter sterilized, or the solution can be purchased from Applied BioPhysics*. After exposure to the cysteine, the solution can be rinsed out of the wells, proteins adsorbed if desired, and the wells inoculated with cells. The time for the cysteine exposure depends upon the history of the array, for relatively newly etched arrays often the reaction is completed in only a few minutes, but to be certain the reaction has gone to completion, an hour-long exposure is recommended for all arrays.



The cysteine will form a covalent sulfur-gold linkage with the electrode surface likely displacing any unwanted small molecules that have adsorbed to gold surface over time

and stabilizing the impedance. This cysteine layer provides a hydrophilic substrate that is excellent for protein adsorption and ultimately cell attachment and spreading.

8 well arrays: Add 200ul for 15 minutes

96 well arrays: Add 100ul for 15 minutes

***NOTE:** You can purchase Electrode Stabilizing Solution in 50mL or 250mL bottles.

50mL – Catalog Number: C-001

250mL – Catalog Number: C-002

Electrode Stabilization

An alternative way to clean the electrodes uses the ECIS **electrical stabilization** method. This feature is built into the ECIS software. One simply fills wells with complete medium, clicks on the wells to be stabilized and then starts the process by clicking the stabilization command. This process usually takes several minutes depending upon the number of wells treated and the condition of the electrodes—so please be patient. In addition, following electrical stabilization about 30 minutes equilibration is required for the electrodes to settle into their final impedance values. Waiting this period helps minimize impedance drift during an experimental run and establishes good reproducibility from one experiment to another. This equilibration period is not required with cysteine and so following cysteine treatment one can immediately begin inoculation.

Note: Electrical stabilization should only be used on cell-free electrodes

Coating the Electrodes

Coating the electrodes with an ECM protein can be integral to the success of the cell attachment and your experiment.

ECIS electrodes shipped from Applied BioPhysics have no macromolecular coatings. When culture medium is added to the arrays proteins and other large molecules in the medium immediately adsorb to the very wettable gold surface as they do with any uncoated tissue culture dish. Often one wishes to define the substrate by coating it with proteins – for example collagen, gelatin, fibronectin or other ECM proteins.

We suggest using the following protocol: Prepare a solution of the desired protein at 100 micrograms per ml or more in 0.15M NaCl. We have found that the use of phosphate buffer (PBS) can seriously interfere with the adsorption of some proteins, so if a buffer is required, a mild Tris solution (e.g. 0.01M) is recommended. The electrode arrays are stable under acidic conditions, so when coating with collagen there is no problem using solutions containing acetic acid.

To coat the electrode, place the protein solution in the bottom of the well for 10 minutes or more. If the protein is valuable, lower protein concentrations can be used with increased times for adsorption. In addition, when using a 1E array it is only necessary to coat the small active electrode (250 micrometer diameter). Just a few microliters can be carefully applied to this small spot, as only cells upon the active electrode are observed in the ECIS measurement.

Once adsorption has taken place, an approximate mono-molecular layer of the protein will coat the surface. You can safely rinse the protein solution from the well with sterile medium, saline or water without concern of removing the adsorbed layer. Medium can now be added and inoculation begun.

Remember, the first macromolecules the substrate is exposed to will be the final coating that the cells first encounter. With this in mind, here is the suggested sequence of steps to prepare the array for inoculation with a defined protein coat. If you are not pre-coating the array with protein the *italicized blue lines* can be ignored.

NOTE: We do not recommend drying the protein solutions in place as it can foul or damage the electrodes.

Step by Step Array Preparation & Coating Guide

Using cysteine treatment alone

1. *Add the protein solution to coat the substrate (wait for protein to adsorb).*
2. *Rinse the protein solution from the well*
3. Flood the wells with the cysteine solution
4. Rinse the wells
5. You can now add medium or a cell suspension

Using electrical stabilization alone

1. *Add the protein solution to coat the substrate (wait for protein to adsorb)*
2. *Rinse the protein solution from the wells*
3. Add medium to the wells
4. Run electrical stabilization
5. You can now remove the medium and add the cell suspension

Using both cysteine treatment and electrical stabilization in combination

1. *Add the protein solution to coat the substrate (wait for protein to adsorb).*
2. *Rinse the protein solution from the well*
3. Flood the wells with the cysteine solution
4. Rinse the wells
5. Add medium
6. Run electrical stabilization

Inoculating with Cells

Careful inoculation is the key to good reproducibility from well to well and from experiment to experiment.

It is most important to obtain a uniform inoculation of the array well bottom. To accomplish this, wells should receive an even "snowfall" of cells so that each electrode has approximately the same number of cells falling on its surface - this can be facilitated by attentively **following some important guidelines:**

1. Make up a monodisperse cell suspension.

For some cell lines this is easily achieved, but for others, particularly if cells have been attached and spread for long periods of time, clumping takes place and longer trypsinization may be needed. The goal is to achieve a clearly monodisperse suspension of single cells.

2. Keep the suspension agitated

It is also important to keep the cell suspension uniform so each well receives approximately the same number of cells; agitate the cell suspension frequently to prevent settling of the cells.

3. Add the suspension to an empty well

If possible, avoid mixing the cell suspension with liquid already in the well. It is best to remove all media from wells before adding the cell suspension such that no mixing within the wells is required. If a cell suspension must be added to liquid already in the well, thorough mixing of the two solutions is essential.

4. Allow the cells to settle and attach with the array outside of the incubator

Temperature considerations are often overlooked and can be extremely important when dealing with cell distribution in small wells.

If the temperature of the cell suspension is lower than the temperature of the incubator, when placed in the incubator, the wells will be heated from the bottom. This will cause a convection cell to form, where medium rises in the center and falls back down the walls of wells. Due to this flow, as cells attempt to fall to the central region of the well, they are swept upward. The overall effect is that the cell density becomes reduced in the central regions of the well. This is very undesirable and critical with the 1E arrays and 10E arrays with their centrally located measuring electrodes.

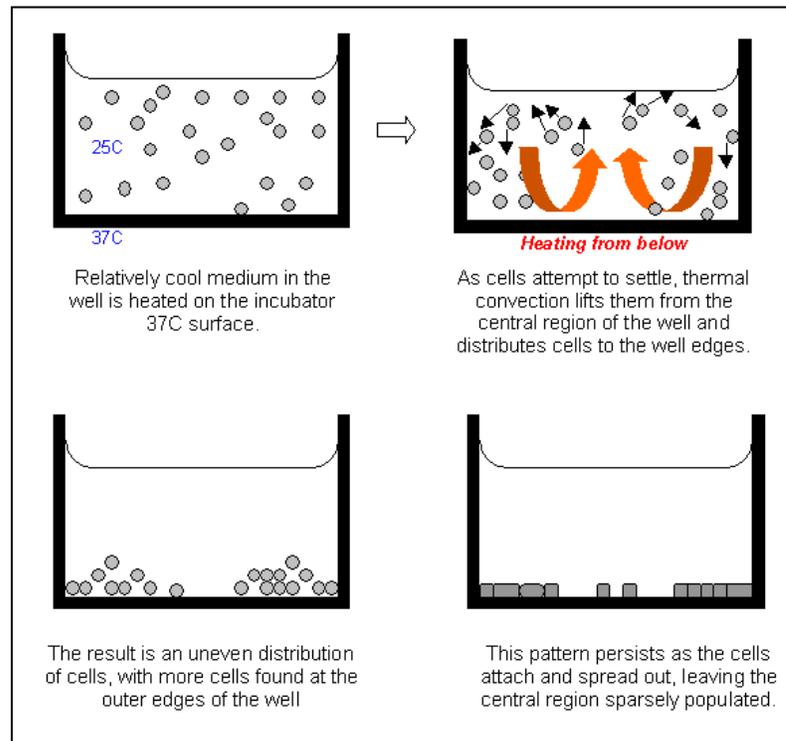


FIGURE 1 An example of the convection effect on a cell suspension with a lower temperature than the temperature of the incubator.

How to Prevent Convection Effects

1. Simply inoculate the ECIS arrays outside of the incubator using room temperature medium, and then wait 20 to 30 minutes before placing the array in the incubator space. Since there is no heating from below, there is no thermal convection, as the cells settle uniformly over the entire substrate and begin to attach to the surface. We recently started using this approach, and the results have been most satisfactory. CO₂ dependent medium would experience some pH increase out of the incubator in this protocol, and one could consider ways to avoid this. In our hands, however, we have not found

a pH drift to be a problem, as once in the incubator space, we observe normal cell attachment and spreading impedance data.

2. If solution 1 cannot be used, we then recommend using a cell suspension and array that are pre-warmed to incubator temperature and immediately place the array into the incubator upon inoculation. The goal again is to prevent thermal convection by having no heating of the wells taking place during cell settling.

Cell Numbers

If one wishes to achieve confluence after attachment and spreading we **recommend the following inoculation guidelines***.

8 Well Arrays : the substrate area is $\sim 0.81 \text{ cm}^2$. When completely confluent with cells, this surface will have $\sim 80,000$ cells. By adding **400 microliters of a cell suspension at 2.0×10^5 cells/ml** to each well, one should achieve confluence upon attachment and spreading of the cells.

96 Well arrays: the substrate area is 0.3 cm^2 . When completely confluent with cells, this surface will have $\sim 30,000$ cells. By adding **250 microliters of a cell suspension at 1.2×10^5 cells/ml** to each well, one should achieve confluence upon attachment and spreading of the cells.

For cell proliferation measurements or if one wishes cells achieve a natural confluence upon contact inhibition, lower concentrations of cells can, of course, be used for the cell suspension. For example, using 4 fold less cells and assuming a 24 hours generation time, cell confluence should be achieved in ~ 2 days post inoculation. For 10 fold less cells, confluence would be achieved in ~ 3 to 4 days post inoculation

***NOTE:** The numbers above are approximate and dependent on the actual cell line or primary cells used in the array and the viability of cells in the suspensions used for inoculation.

ECIS Software Options

Choosing the correct program in the ECIS software is an important step before starting an experiment and can greatly impact your ability to analyze the data.

Most cell culture experiments involve changes that take place over minutes to hours; however, sometimes more rapid events are followed, e.g. signal transduction. The ECIS software offers three different modes for time course data collection.

Multiple Frequency Time (MFT)

For most experiments, the MFT mode is the best choice. This program will automatically measure each well selected over a range of different AC frequencies. As you become more experienced you will find data taken at different frequencies can reveal different aspects of cell behavior (morphology).

This mode is required for barrier function and permeability assays and is recommended for cell migration, attachment and spreading, cell proliferation, cell extravasion, and any assay where the effects of compounds will be observed at times longer than 15 minutes.

Single Frequency Time (SFT)

The SFT mode enables multiple wells to be monitored at a single frequency at a rate of 0.5 seconds per well.

This mode is recommended to signal transduction and any assay where the effects of compounds will be observed at times shorter than 15 minutes. In addition, if you want to take data very rapidly and only analyze 1 frequency this would be an optimal choice.

Rapid Time Collection (RTC)

For the most rapid data acquisition, the RTC mode collects 10 or more samples per second but is restricted to monitoring a single well at a time and at a single frequency.

This mode is required for monitoring the beating of cardiomyocytes or for any assay where the effects of compounds will be observed at times shorter than 5 minutes.

Adding Compounds

Adding compounds is an important part of almost all experiments. Completing this process with minimal disturbance to your cells can create more consistent and reproducible results.

The ECIS measurement is extremely sensitive, and so, even small responses of the cells to temperature, shear stress, etc. are usually detectable. When adding different compounds care should be taken to minimize these unrelated responses such that the biological effects of the compounds being added are clearly seen. **The following is a suggested protocol:**

1. An hour before the addition of the compound of interest, change the medium over the cells using the medium (or balanced salt solution) that will be present when the compound is added. This will allow the cells time to adjust and equilibrate to the experimental conditions. Of course, this equilibration process can be followed using ECIS to be certain enough time has elapsed to give a steady impedance signal.
2. The compound to be tested is prepared at **2X the final desired concentration** in the same solution as is in the wells. This is equilibrated to incubator temperature. In the doses to be tested, be certain to include a control without the compound.
3. Withdraw 1/2 the medium in the well and add an equal amount of the 2X solution to achieve the final 1X concentration. The addition should be done slowly being certain not to have the cells on the active electrodes exposed to shear stress from the fluid flow.
 - A. **8 Well Arrays:** With 400 microliters in the well, withdraw 200 microliters and then slowly add 200 microliters of the 2X solutions to achieve the final 1X concentration.

- B. 96 Well Arrays:** With 250 microliters in the well, withdraw 125 microliters and then slowly add 125 microliters of the 2X solutions to achieve the final 1X concentration.

Note: If the array is removed from the incubator for this addition, be certain to pause the measurement and work quickly so temperature drops are minimized. Once the array is back in the incubator, do an electrode check to be certain contact has been reestablished and resume data collection

If the compound addition is carried out without removing the array from its holder, it is generally a good idea to pause the measurement during this addition to avoid any unwanted impedance spikes, and once the compound is added to the wells, resume data collection.

Starting an Experiment

Starting an experiment is easy once you have an array prepared and ready!

The ECIS software is easy to use, but there are a few things you need to do before starting to take measurements. If you follow the recommended steps data collection should be easy, fast, and reproducible well to well and experiment to experiment.

Step1: Checking Your Array

Checking your array for correct starting values is an important part of experimental set-up. If all wells begin and correct, and consistent values then consistency and reproducibility is increased well to well.

1. Insert your array with media only in the well in you station. Slide the array(s) into one of the slots of the 16 well station and gently tighten the clamps or secure your 96 well plate in the 96 well station with the red tabs.
2. Click the **Setup** button.

Setup directs the instrument to make a series of rapid impedance measurements to determine if arrays are properly connected to the system. The results of these measurements cause the array diagram in the lower left hand position of the screen to change – **lighting up green for properly connected arrays and red for the absence of an array or an incorrectly connected array.**

3. If wells of an array light up red but are intended to be connected, try repositioning the array.
For an 8 well array – reposition the array such that the nine gold rectangular pads are properly contacted by the spring-loaded pogo pins.

For a 96 well array – make sure the edges of the plate are secured under the red tabs and the plate cannot be moved.

Repeat the **Setup** command.

Remember that wells without medium will be measured as not connected.

4. Click the **Check** button

Check directs the instrument measure the impedance, resistance, and capacitance of each well at the default frequency of 4000Hz. Correct values depend on the type of array you are using.

5. If the wells have the correct starting values, you are now free to rinse the media out of the wells and **inoculate with your cell suspension**.

If the wells do not have the correct values you can move to step 6 and stabilize the electrodes.

6. Click the **Stabilize** button

Stabilize directs the instrument to apply an elevated current to each well to clean the gold surface.

NOTE: The first stabilization will be the most effective. Each additional stabilization will have less effect on the value of the electrodes.

Step 2: Configure and Run the ECIS Software

Choosing your experimental settings is the next step for a successful experiment.

1. **Choose your Program** - MFT, SFT, of RTC

If you choose SFT or RTC you will need to choose a frequency from the drop down menu. Otherwise, MFT will choose a selection of frequencies automatically.

2. Set Interval – **We recommend to use the default interval**

Interval directs the instrument to slow data collection and only begin a new set of data points after the interval has expired. The system will

collect data points in all wells at all selected frequencies – then wait until the interval has passed before beginning another data set collection.

3. Click **Start**
4. Name and Save your data file

NOTE: When you save your data file into a directory this will automatically save every data point taken as long as the system is running. Data is not saved during the finish of the program – but is saved as it is taken to prevent data loss in case of an unintended break in the program.

5. Click **Pause** – if you need to add a compound or change the media

Pause directs the instrument to stop taking data. The clock will still run – keeping track of the duration of the pause – but it will blink to indicate measurements have been paused and no data is being taken.

It is now safe to remove the array from the station.

6. Before resuming the experiment, click **Check** to make sure the array is properly connected before clicking **Resume**.

If the array is not properly connected **Repeat Step 3**

Resume directs the instrument to begin taking data. The clock will stop blinking and data will start to appear in the screen. A Green Bar will appear after a few minutes to indicate when the pause occurred

7. Click **Finish** when you are ready to end the experiment.

Step by Step Quick Experiment Guide

Using cysteine treatment alone:

- 1) Add a protein solution to coat the substrate (wait for protein to adsorb).
- 2) Rinse the protein solution from the well
- 3) Flood the wells with the cysteine solution
- 4) Rinse the wells
- 5) Add Medium to the Wells
- 6) Insert the array into station
- 7) Click “Set-up” and do and electrode “check”
- 8) Make sure starting value are correct

- 9) Rinse Media from Wells
- 10) Inoculate the array with cell suspension (then wait 15 minutes)
- 11) Insert the array into station
- 12) Choose a Program (MFT, SFT, or RTC)
- 13) Click “Start”, Name your file, and begin the experiment!
- 14) Click Pause when you need to add a compound or change the media.
- 15) Remove and then return array to the station
- 16) Click Check before Resuming
- 17) Make sure the array is connected properly
- 18) Resume experiment
- 19) Click Finish when you are ready to stop taking data.