Applied BioPhysics

CP96 MANUAL

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The ECIS® CP96 uses microampere, high frequency 48KHz alternating current (AC) to follow the capacitance of gold electrodes used as cell substrates. As a growing monolayer of cells forms upon the electrodes, the capacitance decreases, and this information is used to calculate the relative cell coverage (CELLX). CELLX is reported from 0 to 100 units, where 0 represents a cell-free electrode and 100 represents an electrode fully covered with a confluent layer of cells. To make the complete measurement it is necessary to know the capacitance of the cell-free electrodes (zeroing). These data can be collected in advance of the experimental run or later, by maintaining at least one well cell free throughout the experiment. During data collection, the change in capacitance due to the cells is measured and returned as CELLX values.

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Setting up the CP96

System includes:

- CP96 Station
- Station Controller with power supply
- Laptop PC with CP96 software installed
- CP96 Validation Array
- 1 USB cable
- 1 LEMO cable
- 96W20idf arrays

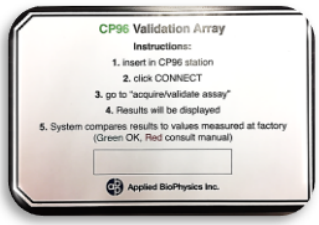
Station Controller

Station

LEMO Cable

USB Cable

Power Cable



Validation Array

1. **System Setup**
   a) Remove components from packaging.
   b) Connect the power cable to the Station Controller, and plug the power supply into wall outlet (If there is concern about quality of power, an Uninterruptible Power Supply (UPS) is recommended).
   c) Connect the laptop to Station Controller with the USB cable.
   d) Connect the Station Controller to the CP96 Station with the Lemo cable using Unit A port on back of Station Controller (the slide switch should be in the L position for the CP96 instrument)
   e) Connect power to laptop and turn laptop on to log in.
   f) Enter the username: ECIS User (password is not required).

2. **Mount the Validation Array in the CP96 Station**
   a) On the CP96 Station, slide the two retaining clamps outward.
   b) Insert the Validation Array using correct orientation.
   c) Push down on one side of Validation Array and slide the retaining clip inward to hold the plate down; repeat on the opposite side.

3. **Start CP96 Software**
   a) Double-click the CP96-A icon and allow loading time.
   b) Press **Connect** and wait for attached wells to be recognized
   c) All 96 wells should appear green in the Well Map. If any wells appear red, reseat Validation Array and re-press **Connect**.
   d) Repeat until all wells appear green in Well Map.

4. **Validate Array**
   a) Select **Acquire > Validate Assay** from the Menu bar.
   b) Enter the serial number found on the Validation Array when prompted.
   c) Values of calculated CellX (degree of cell coverage) from the Validation Array will be shown in the display. These are compared to measured values when the instrument was setup and tested at the factory. Wells will appear green* if values are within 2% of factory values

**If wells appear yellow or red, try remounting and connecting validation array. or contact customer service*

5. **Placing the CP96 Station in a humid incubator**
   a) Seal the CP96 Station in supplied poly bag.
   b) Allow the Station to reach incubator equilibrium (2 hours)
   c) Remove the Station from the poly bag and store it in the incubator

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Running a CP96 Experiment

1. Preparing the 96W20idf Array

a) Recommended Step: Coat well substrate with desired matrix proteins (follow protein manufacturer's recommendations/protocols).

b) Place 0.2 ml of ABP Electrode-stabilizing Solution (10 mM cysteine in sterile water) in each empty well.

c) Incubate array at room temperature for at 30 minutes or more.

d) Rinse wells twice w/sterile water or saline.

e) Set up experimental protocol and fill wells with either cell-free growth medium or prepared cell suspensions (0.3 ml per well recommended). All media should be prewarmed to incubator temperature.

f) Clamp the array into the CP96 Station (Refer to Step 2 "Setting Up CP96"). Note: to facilitate array insertion and removal it may be useful to briefly remove the Station from the incubator space.

2. Starting ECIS CP96 Software and Setting up a Zero Reference

a) Double-click CP96-A icon to open software.

b) Press **Connect** to test connection of each well (shown on Well Map).

- All wells with medium should appear green; wells without medium or are not connected will appear red.
- If necessary , reseat well plate and retest connection until all filled wells are green.

c) Allow the array to equilibrate with incubator conditions – generally about 15-30 minutes using prewarmed medium

d) Press **Zero** to set the zero reference. A popup window will allow three options (discussed in next section of manual):

- **Create Zero File**: A new zero reference point is recorded and stored for each active well.
- **Load Zero File**: A zero reference point from a previous dataset will be loaded.
- **Cell Free Wells**: Choose cell-free wells to be used as a zero reference.

e) A diagram will be displayed showing estimated values of CellX for each well based upon a typical capacitance for cell-free electrodes. As a reference, green color is used to point out wells showing low CellX values; orange and red colors denote medium and high values for CellX respectively.

f) Select cell-free well(s) on the Well Map; a white center will be shown for each selected well

g) Once zero reference is set, the **Start** button will become active.

3. Running a Time Course Experiment

a) Press **Start** to begin the time course . Results will be presented in a graph where CellX values are plotted against experimental time.

b) During the experiment, **Pause** may be pressed to halt data collection for cell treatment, etc. (the clock remains running) If the array has been removed from the Station during the pause, before resuming data collection, press **Check** to verify that proper contact has been made to all active wells being used. Press **Resume** to continue data collection.

a) To end experiment, press **Finish**.

Setting up a Cell-free Zero Reference

More information on the methods of Zeroing

1. Create Zero File

In most cases this approach is recommended as this zero measurement will provide unique values for each of the wells, helping to eliminate any subtle well-to-well variations in the measurements. In this method, the 96W20idf array is prepared with both cell-free and inoculated wells and is then placed in the Station. Before the start of any significant cell spreading*, all wells are measured to create the zero file.

*Note: If this is not possible due to the nature of the experiment, all zero measurements can first be made using cell-free media in all wells and these data called up after wells have been inoculated using the **Load Zero File** command.

Procedure:

a) Prepare a 96-well array adding 0.3 ml of pre-warmed medium or cell suspension to each well being used. (if doing cell proliferation measurements we suggest using a cell suspension concentration of 25 x10^3 cells/ml or 7,500 cells /well)

a) Clamp the array in the Station in the proper orientation (well A1 in the upper left corner).

a) Select **Connect** to confirm connections are in place. Upon completion of the measurement, all wells being used should be marked green. If for some reason contact is not properly achieved, the wells will be marked red. If this occurs, check wells for media and be certain the plate is properly clamped into place.

b) Allow a short time for the array to equilibrate to incubator conditions (15 to 30 min*), and then click **Zero** and then **Create Zero File**. (*if working with only media in all wells, longer times may be used**)**

c) Accept the name suggested by the software or provide another name for the file, and click **Save** to begin the zero measurements. The newly recorded zero reference file will have the extension PRZ.

d) Upon completion of the zero measurement, an estimated CellX value for each well will be displayed and you will be asked to identify cell-free wells. These cell-free wells will be used to correct for any non-cellular changes during an experiment e.g. incubator temperature changes, evaporation of medium from wells, etc.

e) When **Start** is selected, these zero reference values and the marked cell-free wells will be automatically used in calculating the displayed CellX values.

***Note**: Any PRZ zero file can be called up for later use by clicking **Load Zero File** and selecting the appropriate file. The CellX values of the previously measured zero will be displayed, and these values will then be used when **Start** is selected. Be aware that drifts in cell-free electrode values from the previous zero run and the current time course will be displayed as part of the CellX values.*

Setting up a Cell-free Zero Reference

2. Cell-free Wells

In this simplified zero referencing procedure, there must be at least one well that is cell free (additional wells are recommended). All remaining wells can be inoculated with cells or even have complete cell layers in place. Unlike the "create zero file" method described above, where each well has its own zero reference recorded, this method applies only the selected cell-free wells as a zero reference for all wells. This method does not correct for subtle well-to-well variations but will correct for any drift in the CellX values due to changes taking place in all wells, e.g. incubator temperature changes and evaporation.

Procedure:

a) Prepare a CP96-well plate having at least one well without cells. Use the same medium and volume in the cell-free wells as in the cell-containing wells (generally 0.3 ml). Allow the cells to growth to a desired coverage. In this procedure, the single well or group of wells will be identified by the researcher as cell-free. The average values of these wells will be used in calculating the CellX values for the cell-containing wells.

b) Once one wants to begin measurements, clamp the plate into the Station as described above, and select **Connect** to confirm connections are in place. Use the toolbar selection tools if using a partial group of wells. All wells with media should be marked green.

c) Click **Zero** and then select **Cell-Free Wells**. The instrument will then read all wells and present the estimated CellX value for each well (the cell-free wells should show low CellX values). Next, select the cell-free wells by clicking on those wells in the lower-left panel of the display – their centers will be marked white. (Clicking a second time will deselect the well.)

d) Click **Start** to begin time-course measurements.

3. Zeroing for Quick Read

The Quick Read option is used to provide a rapid estimate of the CellX values of each selected well using a fixed cell-free value and a reasonable value for the confluent cell readings. This approach will not provide a time course, but rather a single estimated measure of the current CellX value of each well. (note: the reactance values used for cell-free and confluent electrodes in making this estimate can be viewed and changed under the **Acquire** dropdown menu and choosing **CellX Parameters** – values are in ohms).

Procedure:

a) Clamp the array in place as described above and select **Connect** to confirm connections. Use the toolbar selection tools if using a partial group of wells. All wells with media should be marked green.

b) Click the **Quick Read** button. The system will read all wells and display the estimated CellX values in a well map format.

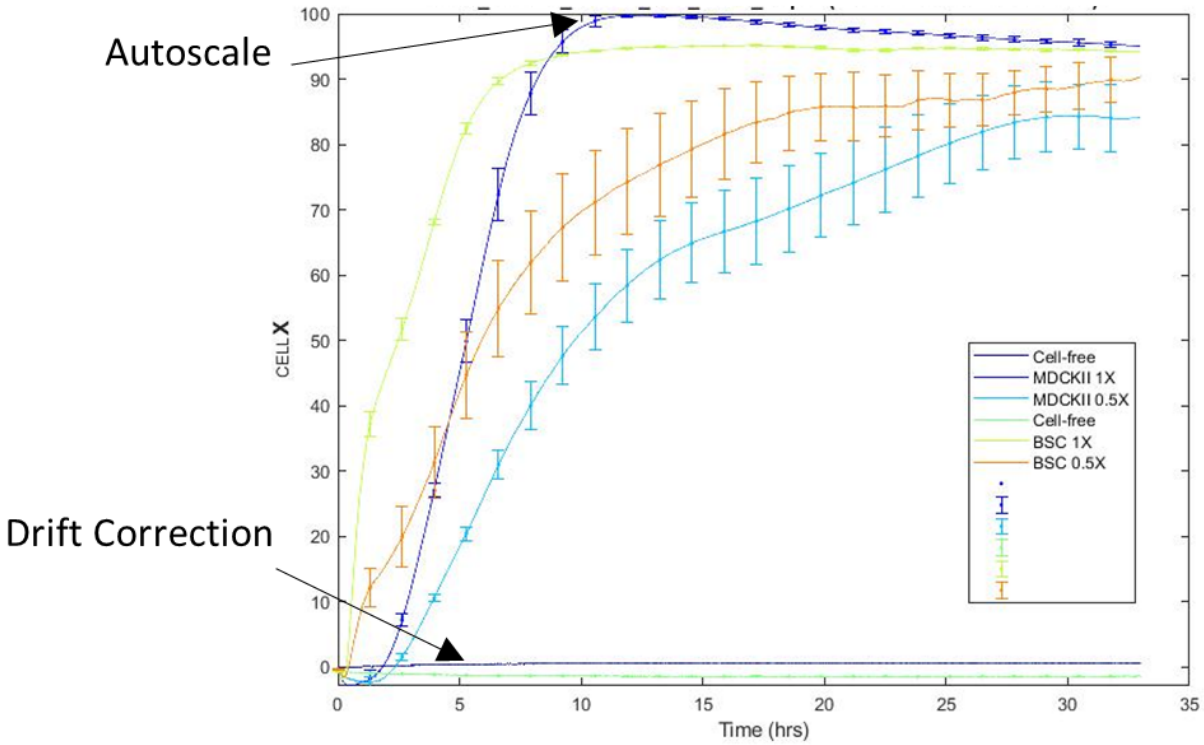
CP96 Autoscale and Drift Correction

The CP96 electronics measure capacitance at 48,000 Hz to ultimately calculate the CellX values (see ***Technical Notes*** section).

There are two adjustments to set the value for confluent cell-covered electrodes and cell-free electrodes to 100 and 0 respectively - namely **Autoscale** and **Drift Correction**.

1) **Autoscale**

When the inoculated electrodes display the confluence plateau in CellX (figure) or are verified microscopically to be confluent, the user can adjust the CellX value to 100 (complete coverage) by clicking the **Auto Scale** icon in the tool bar menu. This will rescale the Y-axis and set the highest CellX value to 100.



Comparison of cell growth rates of MDCKII and BSC-1 cells seeded at 1X and 0.5X relative concentrations. CellX values were obtained using the ECIS CP96 Cell Population Monitor.

2) Drift Correction:

After the user completes the initial zeroing (i.e. "Create Zero File"), the software will prompt the user to select cell-free wells. Since a variety of factors can cause slight drifts in the CellX measurements, the "drift correction" will keep the selected cell-free wells averaged at a CellX value of 0, and will subtract the correction from all the wells being measured. This can be accomplished by clicking the **Drift Correction** icon in the tool bar or can be selected under Acquire → Zero/Cell Free Ref. → Drift Correct.

CellX Values (Technical Notes)

How CellX values are determined:

The CP96 monitors cell population changes by following the reactance of gold electrodes using a weak 48,000 Hz AC signal.

This reactance, measured in ohms, is first converted to capacitance:

$$C=1/(2\pi Xf) \quad (1)$$

X is the reactance and f the AC frequency.

The intrinsic capacitance of the gold electrode without cells in tissue culture medium is approximately 10 mF/cm^2. For the electrodes of the 96W20idf, the active electrode area is approximately 0.01 cm^2, so the capacitance of the cell-free array electrode ($C_{electrode}$) is about 100 nF.

Cell layers by themselves have a combined intrinsic capacitance across the apical and basal plasma membranes of approximately 1 mF/cm^2. The capacitance of a confluent cell layer over the electrode (C_{memb}) is about 10 nF.

When cells spread upon the gold substrate, the membrane capacitance is effectively in series with the capacitance of the gold. For a complete confluent layer, this combined series capacitance is given by the equation below.

$$C_{total} = (C_{electrode} \times C_{memb})/(C_{electrode} + C_{memb}) \quad (2)$$

For capacitance values, considered above, of 100 nF and 10nF for the electrode and cell layer respectively, the combined series capacitance becomes 9.1 nF.

It has been shown that this combined capacitance measured at high AC frequency decreases in a linear manner with the fractional area covered by the cells [Wegener et.al. *Exp Cell Res* **259**, 158 (2000)]. This allows CellX values to be calculated from the measured capacitance (Cm) as:

$$CellX = [(C_{electrode} - Cm)/(C_{electrode} - C_{memb})] \times 100 \quad (3)$$

CellX Values

Calibrating the CellX parameters to give 0 and 100 for open and cell-covered electrodes respectively

Setting open electrodes to give a CellX of 0

The calculation of CellX as described above requires a value for the capacitance of the cell-free electrodes in culture medium ($C_{electrode}$). This is generally about 100nF for a newly fabricated array, but overtime, molecules in the atmosphere may adsorb to the gold surfaces resulting in somewhat lower capacitance. This can be corrected by exposing the gold electrode to a 10 mM sterile solution of L-cysteine in distilled water. The solution can be prepared and filter sterilized in the laboratory or purchased from Applied BioPhysics (Electrode-Stabilizing Solution). This cysteine treatment is highly recommended to enhance experimental repeatability with minimum variation in cell-free capacitance between ECIS wells.

Once CellX data is being collected the Drift Correction tool can be used to automatically set the highest measured capacitance to that for $C_{electrode}$ in the formula calculating CellX (equation 3). This well will then display a CellX value of zero.

Setting confluent cell layer to give a CellX of 100

The calculation of CellX also requires a value for the capacitance of a confluent cell layer (C_{memb}). This quantity depends upon the actual capacitance of the cell membranes and how closely the combined electrode and membrane capacitance mimic a simple parallel circuit. In general there is little variation in this quantity from one cell type to another, but it is possible to make a correction using the Autoscale tool.

Once a confluent layer is achieved for at least one of the wells, the Autoscale tool can be selected. This will result in using lowest capacitance measured for C_{memb} in the formula to calculate CellX (equation 3) and the confluent wells will now have a maximum CellX value of 100.

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CP96 Software Toolbar icons

Zoom In/Out: Zoom in/out on areas of graph

Pan: Grab and drag on graph

Data Cursor: Display values of data points

Select All Wells: Select all wells in well map

Select Well Rows: Select rows in well map

Select Well Columns: Select columns in well map

Select Individual Well: Select wells individually in well map

Clear All Wells: Clear all selected wells in well map

Plot Lines: Display lines across data points in graph

Plot Points: Display individual data points in graph

Display Marks/Pause: Display marks and pause points in graph

Grid Lines: Display gridlines in graph

Insert Legend: Insert data legend in graph

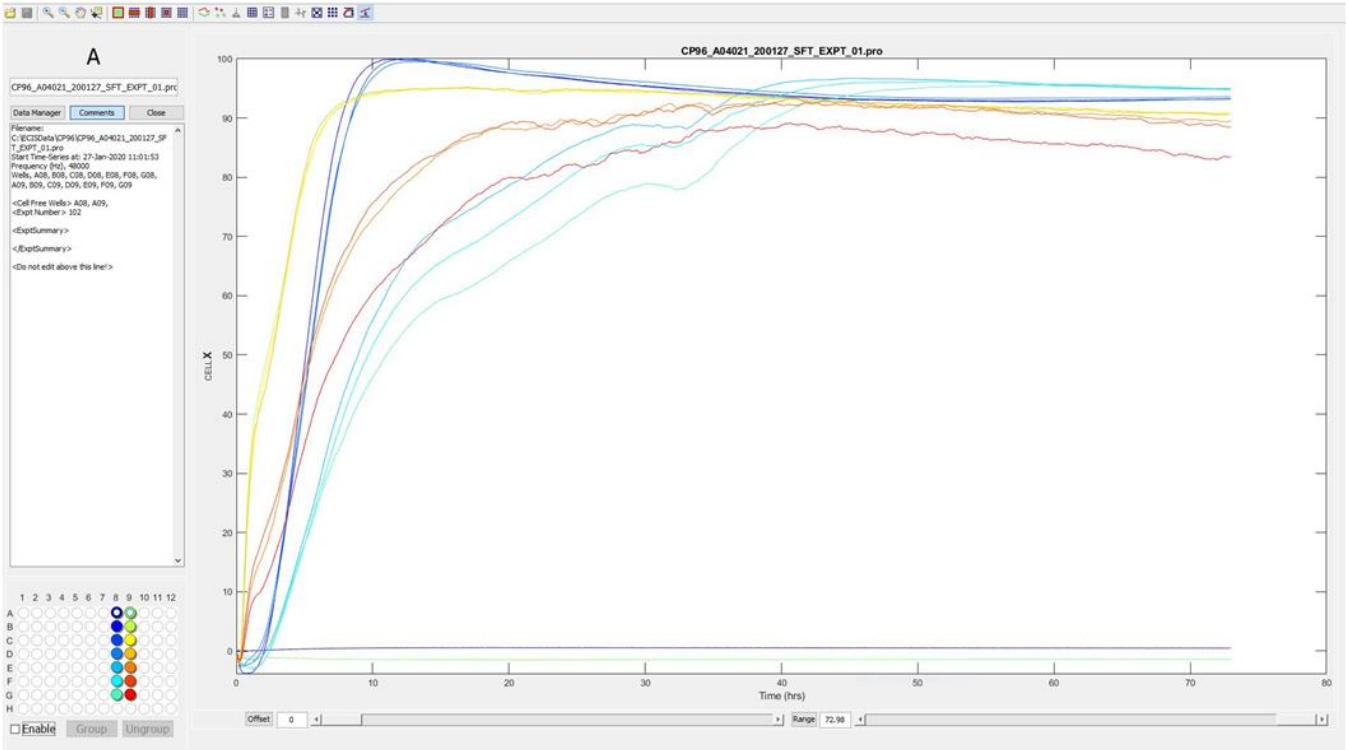
Display Error Bars: Display error bars on grouped data

Full Screen: Make graph full screen

Thumbnail View: Display each well data in separate thumbnail plot

CellX Auto-Scale: Corrects CellX scale of 100 to highest data point

CellX Drift Correct: Corrects CellX 0 value to cell-free wells for drift correction



Menu bar commands

FILE

Open	Loads a previous experiment.
Recent Files	Loads a recent experiment.
Export CELLX	Exports the current experiment to a .csv file.
Close	Close the current experiment.
Exit	Ends the program.

EDIT

Copy Graph	Copy image of the current grap
Export Graph	Exports the current graph in a figure format (jpg, tif, etc)
Color	Palette Select the well color scheme.
Error Bars	Adjust the error bar to standard deviation or standard error.

ACQUIRE

CellX Parameter	Adjust Reactance max/min values corresponding to CellX
Setup New Expt.	Resets software to run a new experiment.
Activate All Wells	Overrides well check to activate all wells.
Find Instrument	Set ECIS COM port and look for instrument.
Use Barcode	Scan barcode on plate with barcode reader to identify plate
Validate Assay	Selects the Validation mode of the instrument.
Zero Cell/free Ref.	
• Drift Correct	Sets cell-free wells to 0 value of CellX and adjusts measured data accordingly
• Set Cell-free wells	After clearing all selected cells in well map, select only cell-free wells and resets them
• Display zero ref.	Displays values of the zero reference used
• Plot Raw Data	Plots Reactance values that are used to calculate CellX values
Growth Rate	Calculate growth rates.

HELP

Manual	Open the HTML manual.
Open Log File	Opens the serial log file for inspection.
About	Gives software version and author.

Helpful Tips:

1. **It is very important to minimize temperature changes during the initial setup and medium exchanges. Insure all solutions are pre-warmed to 37^{0}C.**
2. **Before setting the zero-point reference make sure the medium in the CP96 well assembly is at incubator temperatures. Cold medium may cause the zero-point reference to be set too high leading to negative CP96 values.**
3. **When changing medium in a tissue culture hood use a warming plate to keep the CP96 well assembly at 37^{0}C.**