

Flocel

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Trans-Endothelial/Epithelial Electrical Resistance (TEER)

A favored approach to analyzing cell culture modules is Trans-Endothelial/Epithelial Electrical Resistance (TEER), which provides a rapid evaluation of the cell layer's integrity by measuring impedance across its boundary. The *DIV-BBB 2.0* comes equipped with a TEER Measurement Unit and four docking platforms for cell culture modules, which can all be tested concurrently. Trials can be performed at sampling frequencies between 0.1 Hz and 1 kHz, with frequency resolution of 0.1 Hz, for any duration of time; this data is automatically logged to an Excel or .csv save file of the user's preference. These systems are controlled via the *DIV-BBB 2.0 User Interface* software package which is distributed from an online source after purchase of the system.

TEER Measurement User Interface:

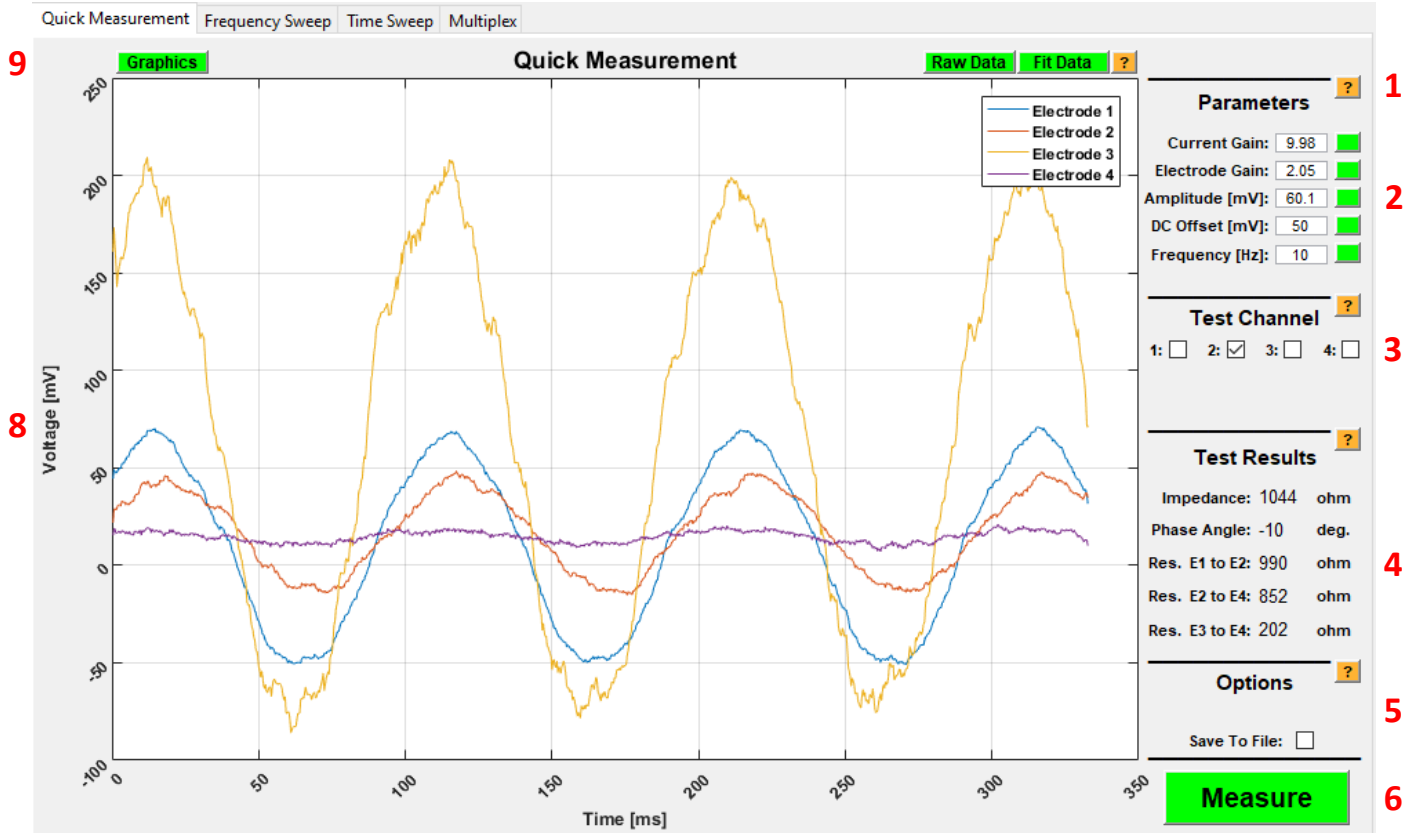
The *TEER Measurement User Interface* is a standalone program created in MATLAB. There are four testing screens the user can navigate between without risk of interrupting active tests, which are as follows:

1. *Quick Measurement:* Adjust testing parameters for the TEER (e.g., current gain, DC offset, etc.), and view plotted electrode data and impedance/phase angle values from one selected cell culture module.
2. *Single-Timepoint Frequency Sweep / Frequency Sweep Over Time:* Perform either a single-timepoint sweep of a user-defined range of frequencies on one cell culture module, or -if the "Time Sweep" checkbox is activated- select to have this frequency sweep repeated at user-defined time intervals.
3. *Single-Frequency Time Sweep:* Perform a single-frequency, single module test for a user-defined span of time, with impedance and phase angle being plotted as a smoothed value over time.
4. *Multiplex (if multiplexed TEER is purchased):* Perform the same functions as described in 2. and 3. with up to four simultaneous and independently-controlled cell culture modules. Results will be plotted and saved strictly based on the active channels that are selected under the **Test Channels** section.

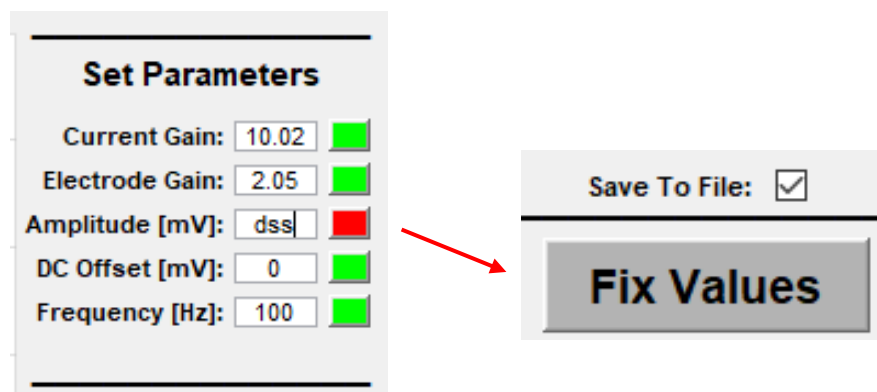
When a test is performed, the *TEER Measurement User Interface* controls the application of a sinusoidal waveform of controlled voltage and frequency from the jumper wire labeled "1" to the cell culture module. This excitation signal travels to the interepithelial resistance barrier, where transmembrane impedance is recorded and measured. This process may occur with up to four active channels containing cell culture modules.

Quick Measurement:

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1. *Help Buttons* – These appear next to every significant section of the program, and outline much of the information discussed in this manual. They are your best guide to conducting a test or interpreting results.
2. *Parameters* – These parameters are applied to all other test types, unless otherwise specified on a different test page. A green button indicates that the currently displayed value has been accepted. A gray button indicates that a new value has been typed in, but the button has not yet been clicked and the new value has not been accepted. A red button indicates that the user input is non-numeric and must be changed before a test can be conducted (as shown below). Hitting the “Enter” key does *not* register a new user value, this is only accomplished by clicking the button next to each individual parameter.



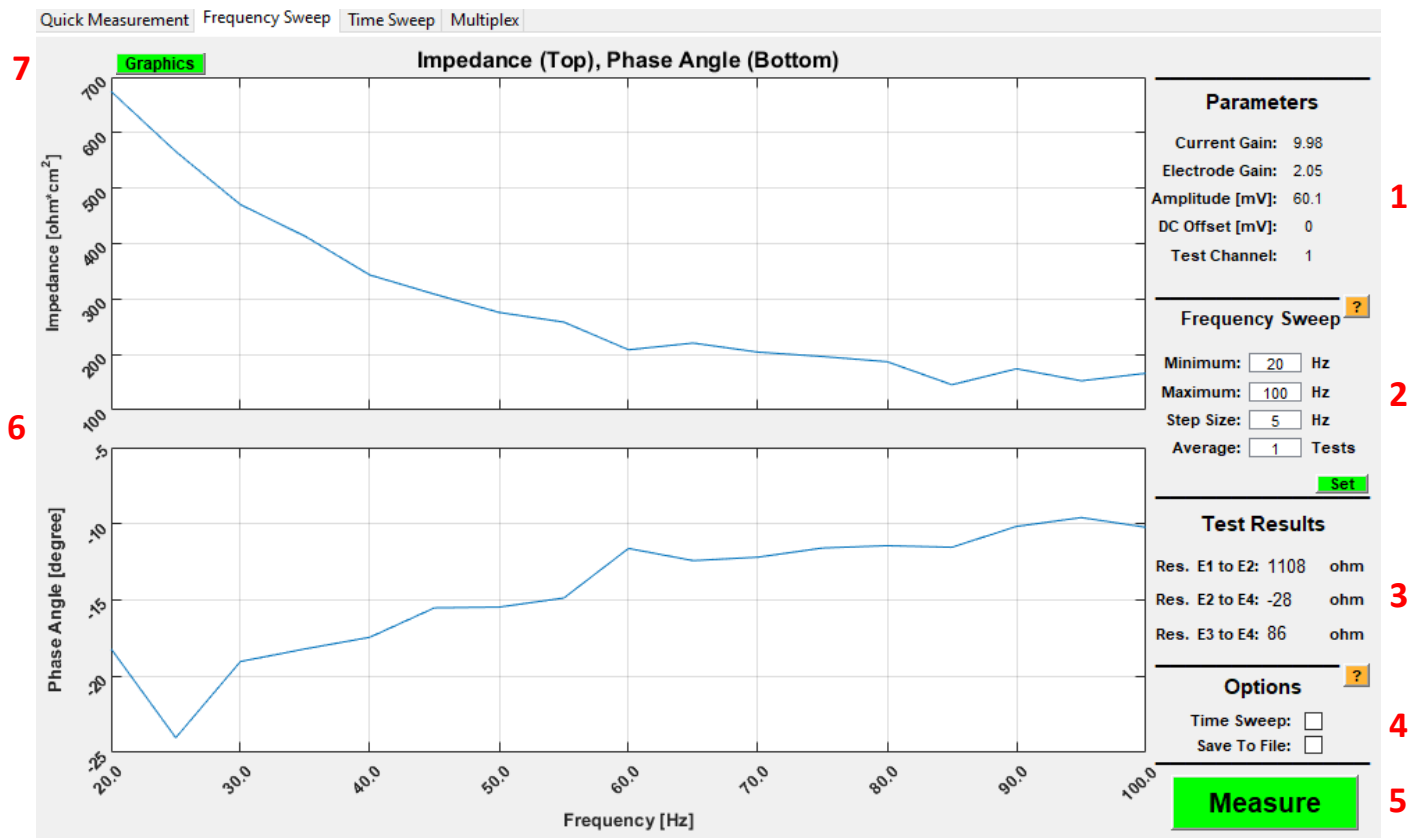
3. *Test Channel* – Select a cell culture module to test. This feature does *not* throw a warning if you attempt to test the wrong module; the user must ensure they have properly numbered each module before testing.
4. *Test Results* – Presents the results of each measurement for this test page. This field is refreshed with each test if the Graphics button is active. If the Save To File box was not checked and the user wishes to record this data, it must be manually recorded. The values presented in this section are as follows:
 - *Impedance*: Total resistance across the epithelial cell barrier, measured in $\text{ohm}\cdot\text{cm}^2$.
 - *Phase Angle*: Shift in phase between excitation signal and response signal, measured in degrees.
 - *Res. E1 to E2*: This resistance indicates whether there is leakage in the gasket securing the fibers.
 - *Res. E2 to E4*: “Cross voltage” value of the cell culture module.
5. *Save To File* – This box must be checked in order to log data from a given test. If this box is checked and the Measure button is clicked, the user will be prompted to select a file save location before a test is run. The two file options for data logging are comma-separated values (.csv) and Excel spreadsheet (.xlsx). If a test is canceled before it has run to completion and the Save To File box is checked, the program will save all data that had been recorded up to that point; any unused rows of the save file will be zeros.

Note: An error will occur if you attempt to save to a file name that is already created and open on your desktop at the time of testing. Although the software is designed to catch this error without losing the test data, it is best to close out any previous files before saving a file of the same name.

6. *Measure* – For the Quick Measurement test page, this button runs a single-frequency scan on the selected channel. Raw (unfitted) plot data and Test Results will be displayed at the end of each test.
7. *Plot Types* – These buttons appear only after the first test has been conducted and data has been collected. “Raw Data” displays the unfiltered waveforms with their respective gains applied. “Fit Data” fits each data trace to an ideal sinusoidal waveform. This function removes the electrode gains, calculates amplitude and phase angle, and replots these waveforms with the user-defined DC voltage offset.
8. *Plot Data* – A single plot displays the fitted and unfitted data as voltage (mV) over time (ms).
9. *Graphics* – Toggle on or off the automatic refresh of visual data for the Quick Measurement test page.

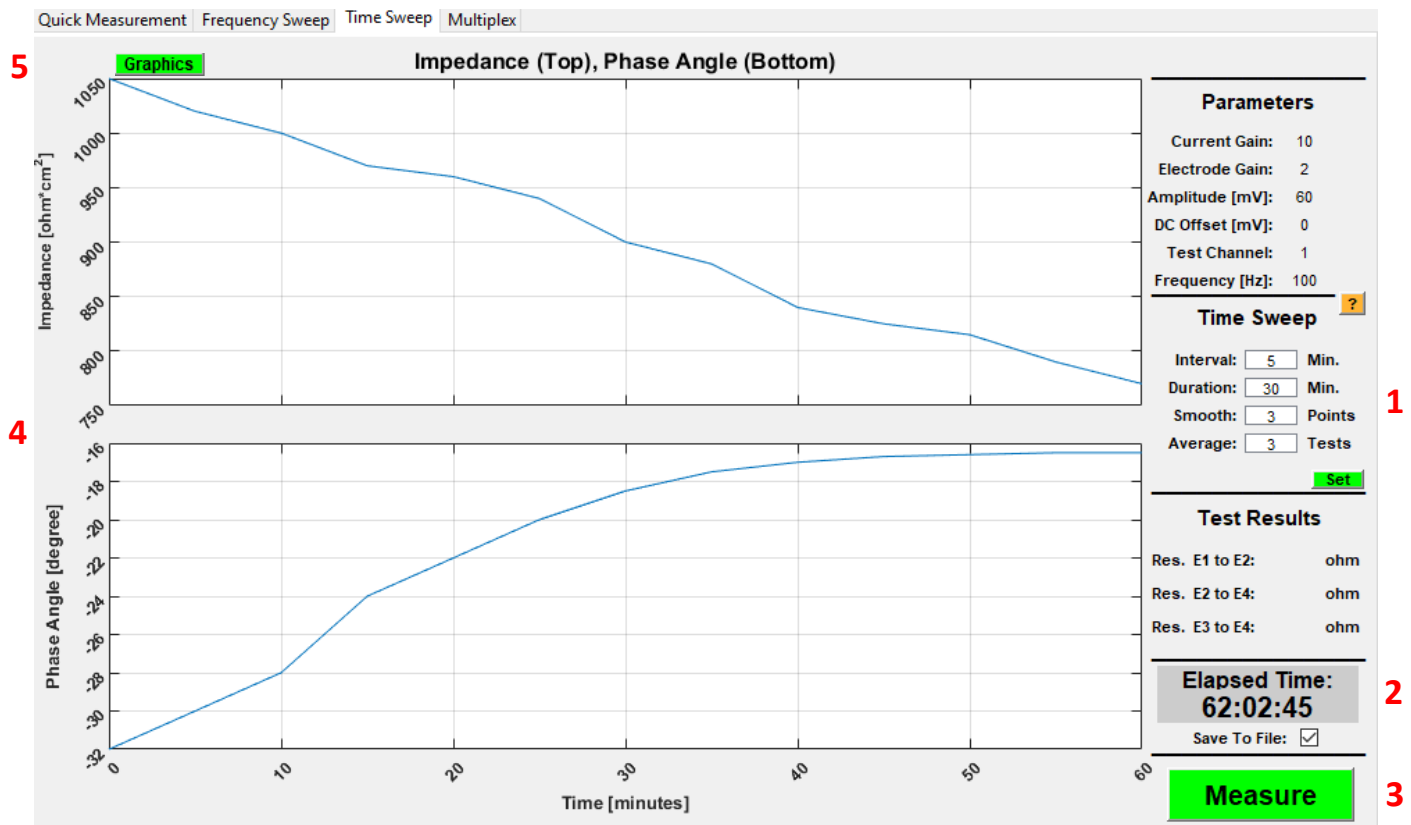
Note: It is important -particularly during tests with numerous data refreshes (i.e., a multiplex time sweep lasting longer than 12 hours) to turn off the Graphics buttons when they are not needed! The Graphics buttons should be turned off on all test pages, and only flipped on when the user is checking a test in progress or calibrating parameters on the Quick Measurement test page. Leaving the Graphics on for an extended time will eventually begin to lag the program. If a test is being conducted over multiple days, this lag may accumulate enough to affect the timing properties of the program and put the experiment at risk. Every test in the TEER Measurement User Interface has been ensured to operate smoothly when the Graphics are used in moderation throughout a test; thus, Flocel, Inc. is not liable for any errors, crashes or losses of data that occur due to the visual refresh feature being left active for an extended amount of time.

Frequency Sweep:



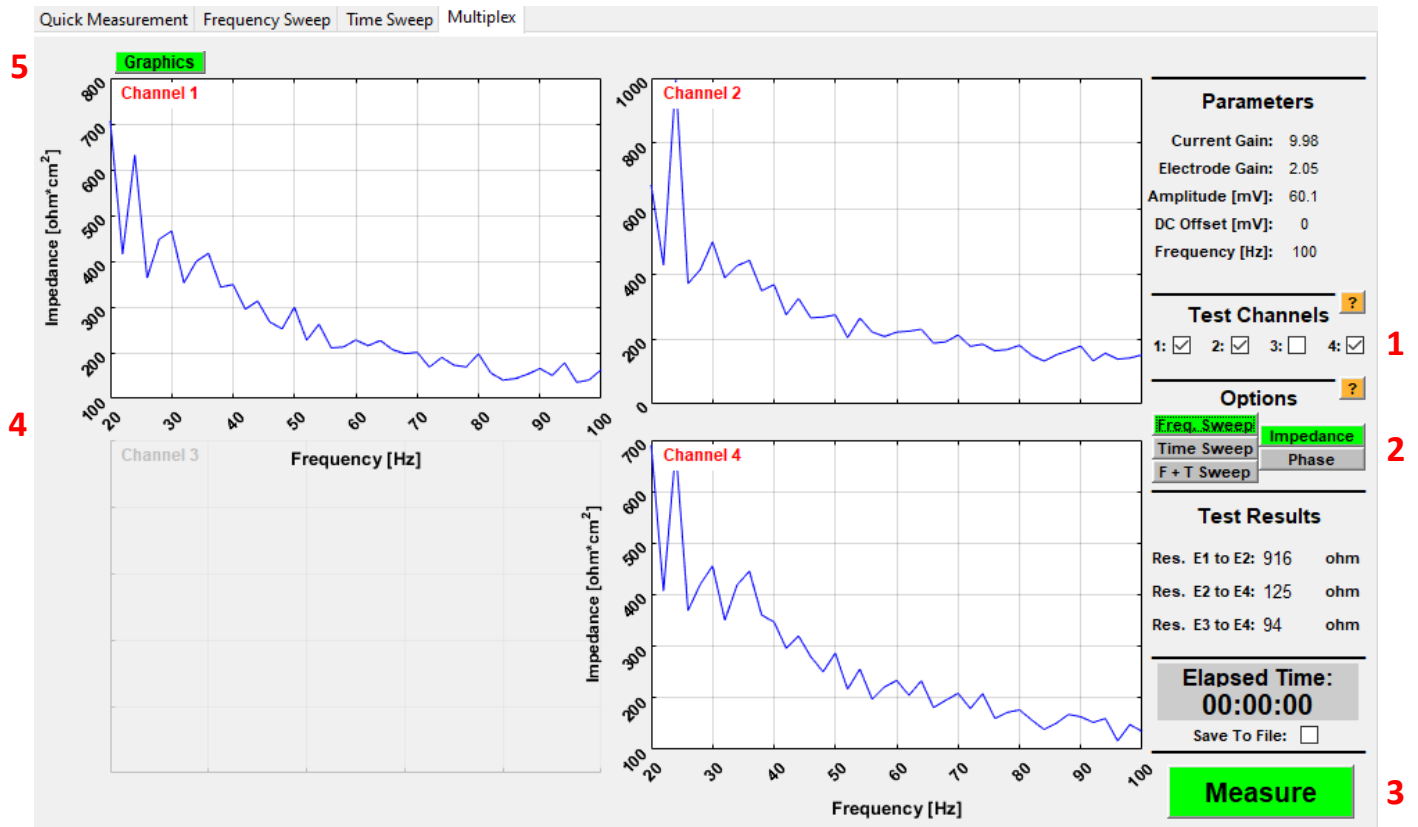
1. *Parameters* – Displays the parameter values that were set on the Quick Measurement test page. This includes the active test channel, but not frequency (this is determined as a frequency sweep range in 2).
2. *Frequency Sweep* – Select values for the minimum frequency, maximum frequency, step size between each test, and the number of tests that are averaged together for each individual frequency.
3. *Test Results* – Resistance values for each individual test; these values are *not* averaged.
4. *Time Sweep* – The user-defined frequency sweep is conducted over a span of time. These sweeps occur in line with the **Frequency Sweep** and **Time Sweep** parameters, each of which need to be re-selected once the *Time Sweep* box has been checked. Once the frequency sweep range has been input, the program will calculate the minimum time interval necessary to complete each sweep (you can't run 1,000 frequency scans every minute!). **Although the test itself must be started by clicking the Measure button on the Time Sweep test page, all data will be plotted on the Frequency Sweep test page.**
5. *Measure* – For the Frequency Sweep test page, this button runs a sweep through each frequency selected in the range from 2, and averages the number of tests selected by the Average value.
6. *Impedance and Phase Angle Plots* – Each data point represents the average value of impedance (top, in units of ohm*cm²) and phase angle (bottom, in units of degrees) for that respective frequency.
7. *Graphics* – Toggle on or off the automatic refresh of visual data for the Frequency Sweep test page.

Time Sweep:



1. *Time Sweep* – Select values for time interval between tests, duration of testing, number of data points that are smoothed over time, and number of tests that are averaged together at each time point. When the *Time Sweep* box on the Frequency Sweep test page is selected, the Data Smoothing and Averaging parameters will be fixed to 1 in favor of the similar Frequency Sweep parameters. If the user sets the Interval parameter higher than Duration, one initial timepoint will be taken and then the test will cease.
2. *Elapsed Time* – Displays elapsed time in a format of hours, minutes and seconds, as well as a “testing” indicator when the TEER measurement is being recorded and the plots are refreshing with new data. When the *Time Sweep* box on the Frequency Sweep test page is selected, the timer will also show here.
3. *Measure* – For the Time Sweep test page, this button runs a sweep through a user-defined duration of time, averages the appropriate number of tests per time point, and smooths the plot data over time. **This Measure button is also used to begin a *Time Sweep* test on the Frequency Sweep test page.**
4. *Impedance and Phase Angle Plots* – Each data point represents the average value of impedance (top, in units of $\text{ohm}\cdot\text{cm}^2$) and phase angle (bottom, in units of degrees) for that respective point in time.
5. *Graphics* – Toggle on or off the automatic refresh of visual data for the Time Sweep test page.

Multiplex (if multiplexed TEER is purchased):



1. **Test Channels** – Select which cell culture module channels are active. Any unselected channels will have their respective plots “grayed out” to indicate that the plots will not be refreshed with test data (see above).
2. **Options** – Select whether the tests being conducted are a single-timepoint frequency sweep, single-frequency time sweep, or a frequency sweep over a duration of time, as well as whether the plots will display impedance data or phase angle data. The test parameters for frequency sweep and time sweep must be defined in their respective test page; if the values input to either of these test pages is non-numeric, the Set Plot Type buttons will indicate this with a red color until the values are fixed. The impedance and phase angle plot types may be changed before, during or after testing (minding a potential delay if the plot data is partway through a refresh). The *Freq. Sweep*, *Time Sweep* and *F + T Sweep* buttons, however, are locked during a test and can only be changed when the test has concluded.
3. **Measure** – For the Multiplex test page, this button is used to begin all tests outlined in 2., using values defined in that test’s respective parameter page, for any cell culture module channels set as active.
4. **Impedance and Phase Angle Plots** – Each data point represents the averaged value of impedance (in units of $\text{ohm}\cdot\text{cm}^2$) and phase angle (in units of degrees) for that respective point in along the x-axis (frequency in Hz, or time in minutes, depending on the test that has been selected). The plot display can be toggled between impedance data and phase angle data before, during or after testing.
5. **Graphics** – Toggle on or off the automatic refresh of visual data for the Multiplex test page.