Flocel Inc.

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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 TEER and Phase Angle across its boundary. The *DIV-BBB 2.0* comes equipped with a TEER Measurement Unit and four 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, amplitude, *etc.*), and view plotted TEER / Phase Angle calculations from one selected cell culture module at a time.
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 TEER and Phase Angle being plotted as an averaged 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 blue/black jumper wire to the cell culture module. This excitation signal travels to the interepithelial resistance barrier, where transmembrane resistance and phase angle are measured. This process may occur with up to four active channels containing cell culture modules.

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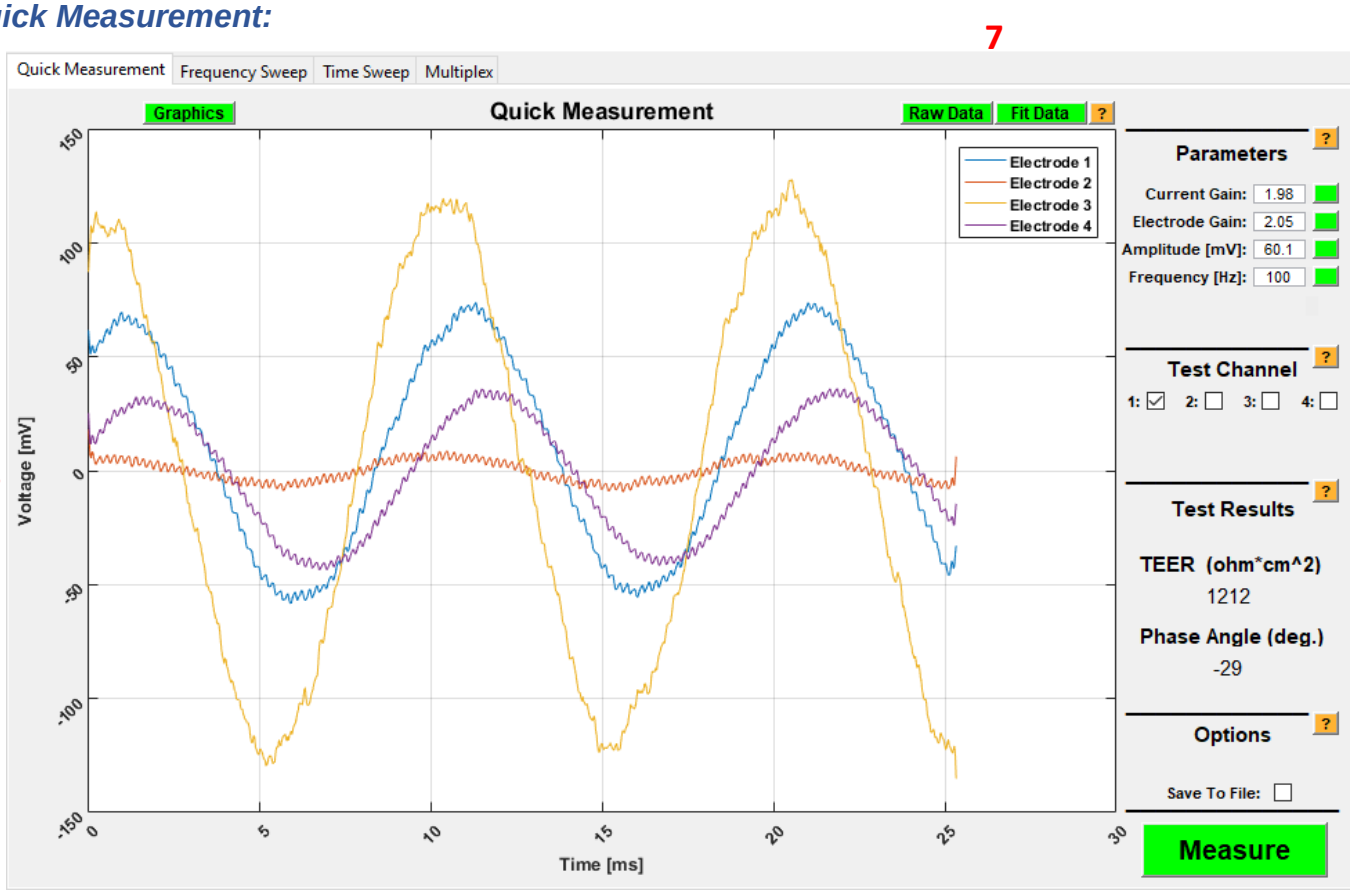
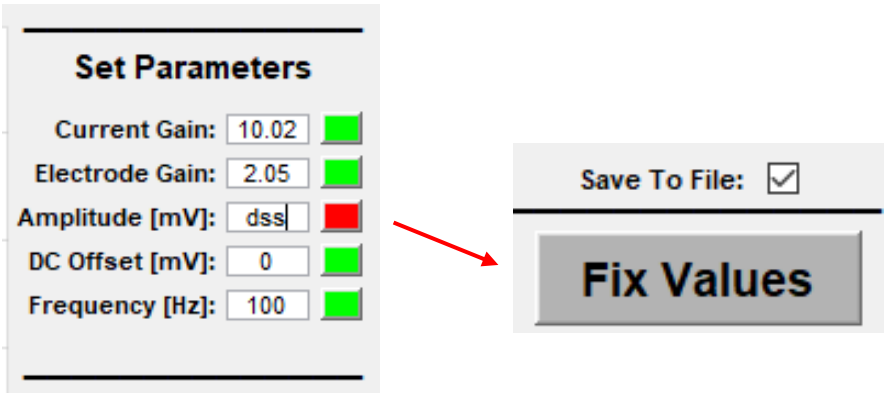
Quick Measurement:



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.



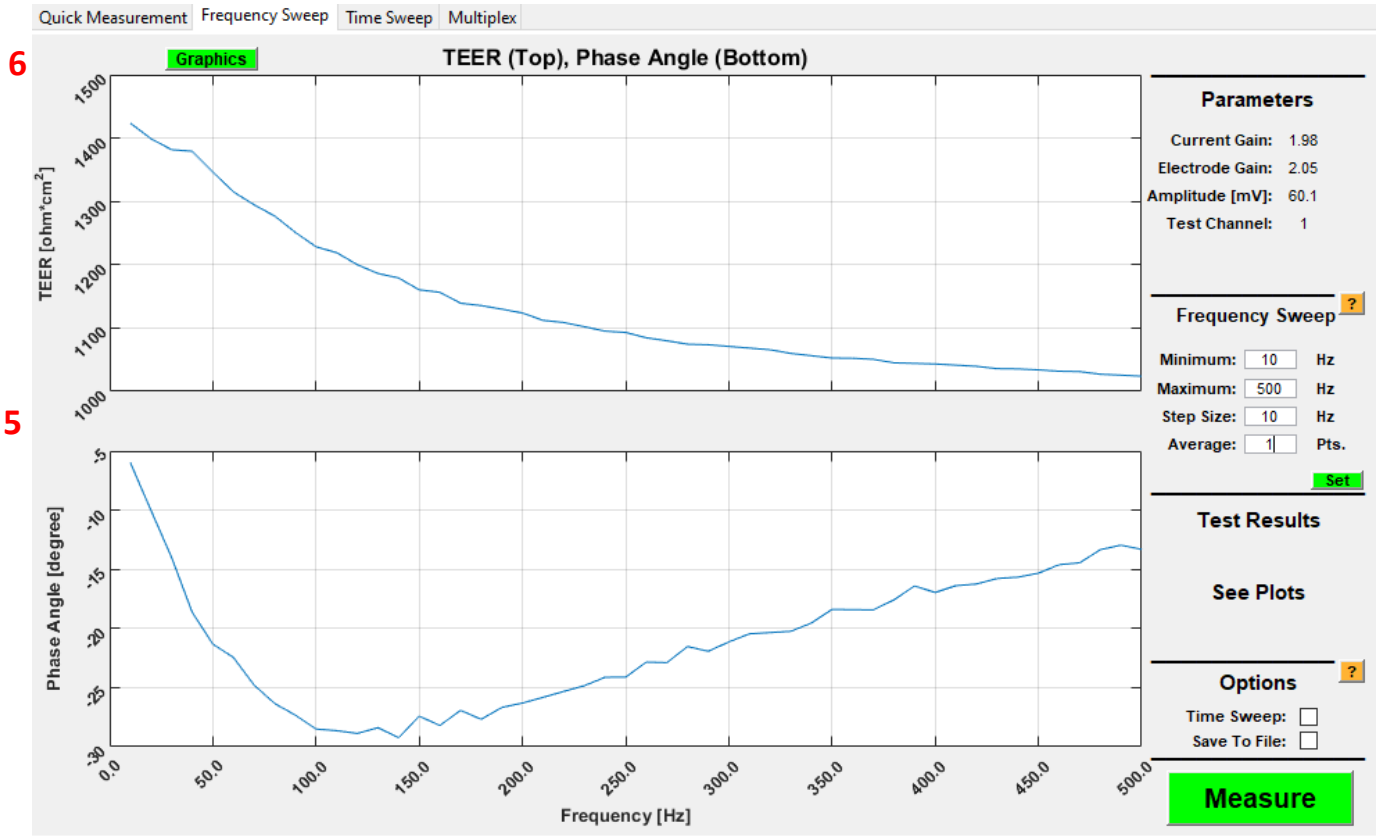
3. *Test Channel* – Select a cell culture module to test. This feature does *not* throw a warning if you attempt to test the wrong module; the user must ensure they have properly numbered each module before testing.
4. *Test Results* – Presents the TEER and Phase Angle calculations of each measurement for this test page. This field is refreshed with each test as long as the Graphics button is active. If the Save To File box has not been selected and the user wishes to record measurement data, it must be manually recorded.
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 a 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 without electrode gain, and calculates amplitude and phase angle.
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.

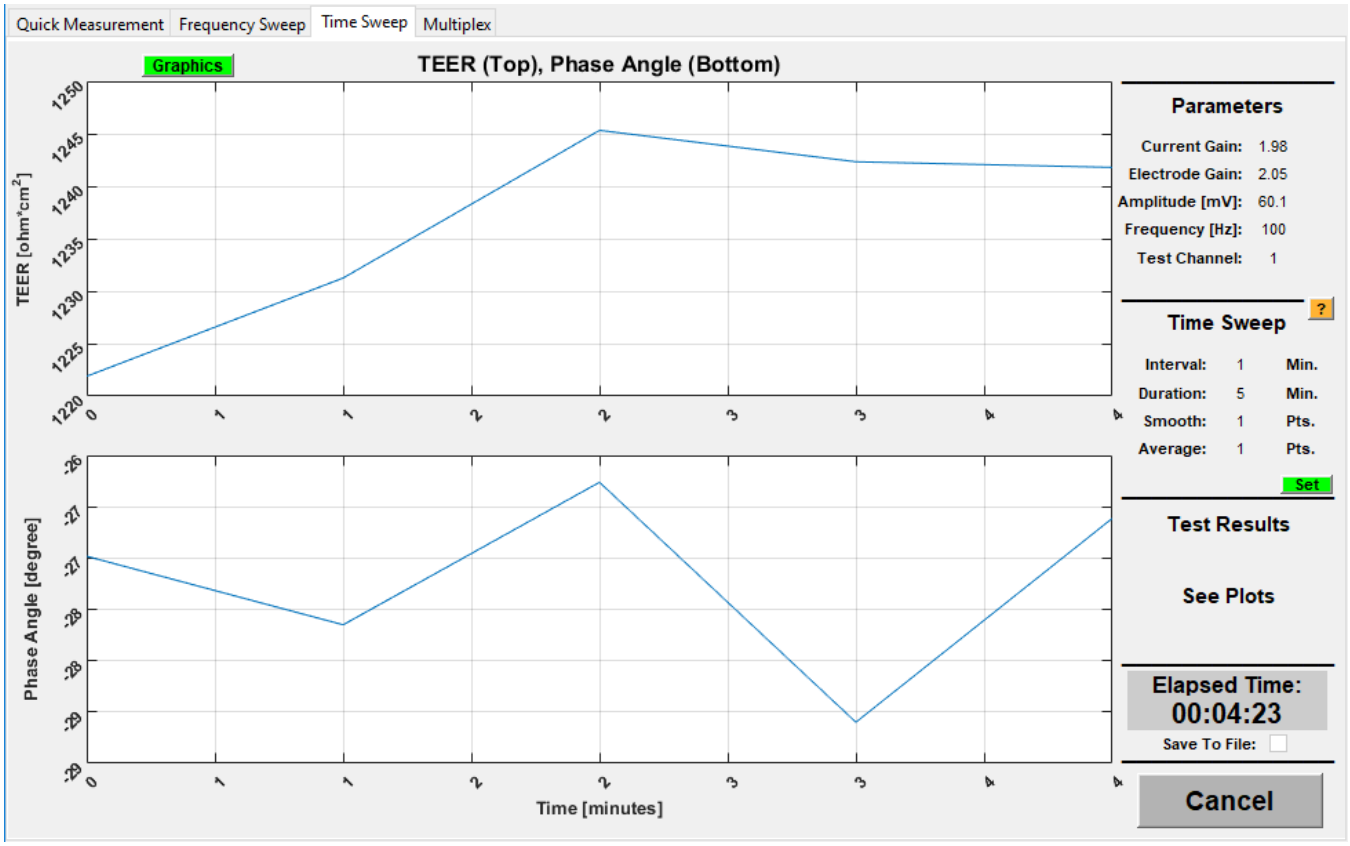
Note: It is important -particularly during tests with numerous data refreshes (i.e., a multiplex time sweep lasting longer than 12 hours) to turn off the Graphics buttons when they are not needed! The Graphics buttons should be turned off on all test pages, and only flipped on when the user is checking 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:



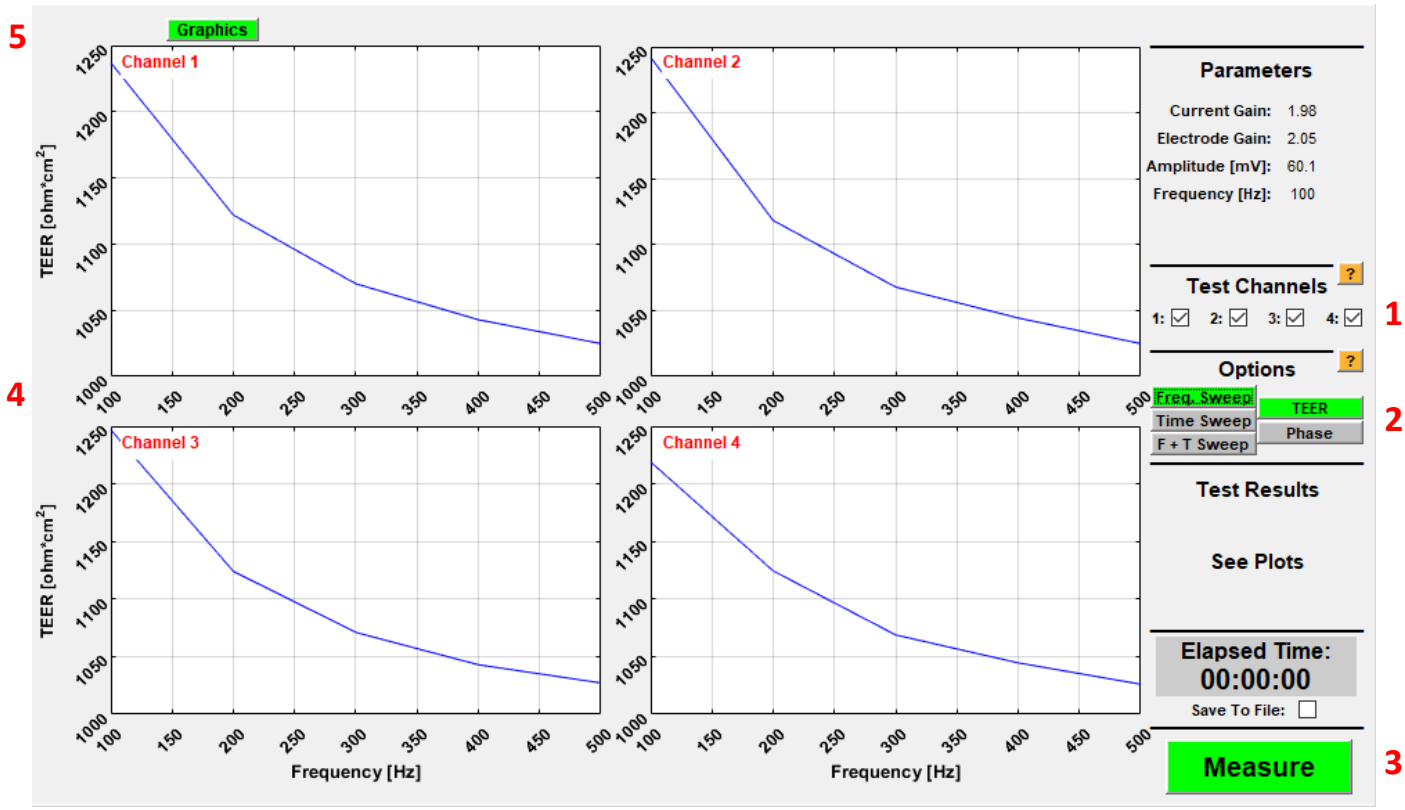
1. *Parameters* – Displays the parameter values that were set on the Quick Measurement test page. This includes the active test channel, but not frequency (this is determined as a frequency sweep range in 2).
2. *Frequency Sweep* – Select values for the minimum frequency, maximum frequency, step size between each test, and the number of tests that are averaged together for each individual frequency.
3. *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.**
4. *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.
5. *TEER and Phase Angle Plots* – Each data point represents the average value of TEER (top plot, in units of ohm*cm^2) and Phase Angle (bottom plot, in units of degrees) for that respective frequency.
6. *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. *TEER and Phase Angle Plots* – Each data point represents the average value of TEER (top plot, in units of ohm*cm^2) and Phase Angle (bottom plot, 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):

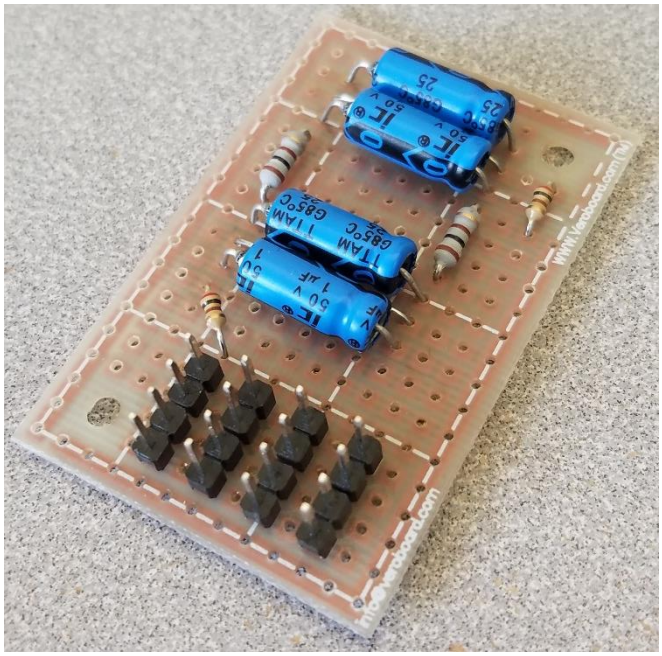


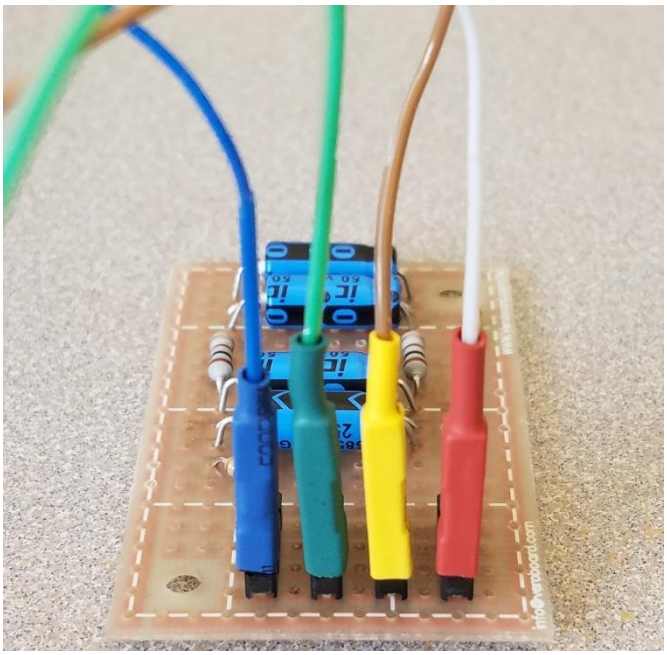
1. *Test Channels* – Select which cell culture module channels are active. Any unselected channels will have their respective plots "grayed out" to indicate that the plots will not be refreshed with test data (see above).
2. *Options* – Select whether the tests being conducted are a single-timepoint frequency sweep, single-frequency time sweep, or a frequency sweep over a duration of time, as well as whether the plots will display TEER 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 TEER and Phase Angle plot types being displayed 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. *TEER and Phase Angle Plots* – Each data point represents the averaged value of TEER (in units of ohm*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 data being displayed on the plots can be toggled between TEER and Phase Angle before, during or after testing.
5. *Graphics* – Toggle on or off the automatic refresh of visual data for the Multiplex test page.

Example Protocol with the TEER Measurement Unit

Running a Frequency Sweep with the TEER Starter Kit (TSK):

For those who wish to familiarize themselves with operating the TEER Measurement Unit before setting up their cell culture modules, we have provided a simple test circuit board to interact with: the *TEER Starter Kit (TSK).* This circuit mimics the frequency-dependent behavior of the cell barrier and allows the end user to demonstrate or practice with the *TEER Measurement User Interface* virtually anywhere, with little setup required.





Each horizontal row of electrodes (as viewed from left to right in the images above) can be used to service one electrode connection cable; thus, one *TSK* board can be used to connect and test all four TEER Measurement Unit test channels simultaneously. It is important to connect these electrode cables as shown above (from left to right: blue/black, green, yellow, red) or else the *TSK* will not provide the expected signal. When operating the *TEER Measurement User Interface* with this circuit board connected, running a frequency sweep between 10 Hz – 500 Hz should result in a plot closely resembling that shown in the Figure on page 4.

***NOTE*: Please make sure to connect the electrode cables to the *TEER Starter Kit* before plugging in the TEER Measurement Unit, and avoid touching the *TSK* board when powered on, to minimize risk of damage from static discharge! Additionally, please keep the *TEER Starter Kit* away from wet surfaces.**

Examples of Save File Structure

Excel Spreadsheet (.xls) File Structure:

	A	B	C	D	E	F	G	H	I
1	**Time of Test:**	1/17/2018 17:21		**Resistances [ohm]:**					
2	**Test Channel:**	2		**Electrode 1 to 2:**	1014.504067				
3	**Current Gain:**	9.984375		**Electrode 2 to 4:**	218.5654928				
4	**Electrode Gain:**	2.046316964		**Electrode 3 to 4:**	78.66551452				
5	**Amplitude [mV]:**	29.8828125		**Impedance [ohm*cm^2]:**	311.7350745				
6	**Frequency [Hz]:**	100		**Phase Angle [degree]:**	-8.277490989				
7	**DC Offset [mV]:**	0							
8									
9	**Time [ms]:**	**Electrode 1, Raw [mV]:**	**Electrode 1, Fit [mV]:**	**Electrode 2, Raw [mV]:**	**Electrode 2, Fit [mV]:**	**Electrode 3, Raw [mV]:**	**Electrode 3, Fit [mV]:**	**Electrode 4, Raw [mV]:**	**Electrode 4, Fit [mV]:**
10	0	20.80078125	0	20.80078125	1.449577733	14.6484375	-1.243399287	11.1328125	-0.082673391
11	0.057	16.50390625	1.270414645	15.4296875	1.731798246	44.7265625	-0.275126409	5.76171875	-0.006490403
12	0.082	11.1328125	1.827196167	8.984375	1.85489974	59.765625	0.149739346	1.46484375	0.026931585
13	0.107	7.91015625	2.383526856	4.6875	1.977543565	50.09765625	0.574568154	-3.90625	0.060346927
14	0.133	8.984375	2.961484977	3.61328125	2.104575122	42.578125	1.016238248	-1.7578125	0.095082972
15	0.158	18.65234375	3.516470487	10.05859375	2.226191601	18.9453125	1.440666698	2.5390625	0.128459197
16	0.183	30.46875	4.07058836	19.7265625	2.3472588	25.390625	1.864739686	13.28125	0.161803727
17	0.209	34.765625	4.645803596	25.09765625	2.472553903	17.87109375	2.305286044	16.50390625	0.196439572
18	0.234	29.39453125	5.197727816	24.0234375	2.5924081	24.31640625	2.728309561	14.35546875	0.229693943
19	0.259	19.7265625	5.748369574	18.65234375	2.71162266	37.20703125	3.150659909	6.8359375	0.26289164
20	0.284	13.28125	6.297593008	12.20703125	2.830168167	49.0234375	3.572232879	0.390625	0.296024473
21	0.309	13.28125	6.845262605	7.91015625	2.948015373	47.94921875	3.992924453	-1.7578125	0.329084266
22	0.335	17.578125	7.413045423	5.76171875	3.069804454	47.94921875	4.429397131	0.390625	0.363380203
23	0.36	27.24609375	7.957126633	11.1328125	3.186137155	42.578125	4.84796906	7.91015625	0.396265723
24	0.385	25.09765625	8.49924454	18.65234375	3.301683724	61.9140625	5.265344826	15.4296875	0.429053471
25	0.411	27.24609375	9.060820715	21.875	3.420987584	43.65234375	5.698036036	19.7265625	0.463040316
26	0.436	21.875	9.598519281	21.875	3.534842259	23.2421875	6.112652648	16.50390625	0.495603551
27	0.461	24.0234375	10.13384956	15.4296875	3.647824765	14.6484375	6.525761054	12.20703125	0.528044503
28	0.487	19.7265625	10.68793878	10.05859375	3.76437141	39.35546875	6.953684329	4.6875	0.561644772
29	0.512	16.50390625	11.21802884	3.61328125	3.875488488	68.359375	7.363400365	1.46484375	0.593811482
30	0.537	18.65234375	11.74535102	1.46484375	3.985649348	76.953125	7.771299591	0.390625	0.625831678
31	0.563	25.09765625	12.29069006	5.76171875	4.09917307	62.98828125	8.193479335	7.91015625	0.658968767
32	0.588	32.6171875	12.81196309	12.20703125	4.207299223	59.765625	8.597360706	13.28125	0.690665617
33	0.613	35.83984375	13.33007495	19.7265625	4.314387288	59.765625	8.999120806	19.7265625	0.722192056
34	0.638	33.69140625	13.84489782	20.80078125	4.420410842	72.65625	9.398660509	16.50390625	0.753540305
35	0.663	26.171875	14.35630467	17.578125	4.525343726	62.98828125	9.795881233	14.35546875	0.784702629
36	0.689	20.80078125	14.88440845	12.20703125	4.633289114	89.84375	10.20642547	7.91015625	0.816905952
37	0.714	13.28125	15.38845629	5.76171875	4.735917046	83.3984375	10.59861221	1.46484375	0.8476652
38	0.739	15.4296875	15.88870726	2.5390625	4.83737646	95.21484375	10.9881839	-1.7578125	0.878215298
39	0.765	25.09765625	16.40480788	5.76171875	4.941627783	87.6953125	11.39046111	1.46484375	0.909757434
40	0.79	37.98828125	16.89693154	15.4296875	5.040626116	73.73046875	11.77440077	11.1328125	0.939857553
41	0.815	45.5078125	17.38488614	24.0234375	5.13838075	80.17578125	12.15543527	19.7265625	0.969725777
42	0.841	42.28515625	17.88780696	26.171875	5.238700329	72.65625	12.54852832	19.7265625	1.000534813
43	0.866	35.83984375	18.36688448	22.94921875	5.333843364	105.9570313	12.92334558	14.35546875	1.029907182
44	0.891	30.46875	18.84143025	16.50390625	5.427670352	108.1054688	13.29497419	4.6875	1.059025436
45	0.916	25.09765625	19.31132717	8.984375	5.520158145	101.6601563	13.66332248	-1.7578125	1.087882391
46	0.941	25.09765625	19.77645932	3.61328125	5.61128392	95.21484375	14.02829954	-6.0546875	1.116470928
47	0.967	28.3203125	20.25501891	5.76171875	5.704585735	107.03125	14.4042027	-2.83203125	1.145910686
48	0.992	36.9140625	20.71007634	12.20703125	5.792863646	113.4765625	14.76202415	4.6875	1.173929849
49	1.018	42.28515625	21.1779139	21.875	5.883156165	109.1796875	15.13029347	13.28125	1.202762422
50	1.044	42.28515625	21.64009975	22.94921875	5.971878657	85.546875	15.49452499	17.578125	1.231274015
51	1.07	35.83984375	22.09651055	19.7265625	6.059007445	81.25	15.85462151	15.4296875	1.259457021
52	1.095	30.46875	22.52980753	12.20703125	6.141260537	86.62109375	16.1968792	10.05859375	1.286239188

Notepad Text Editor (.csv) File Structure:

```
Time of test:   18-Jan-2018 22:10:22
Test Channel:   -
Current Gain:   10.0
Electrode Gain: 2.0
Amplitude [mV]: 60.1
Frequency [Hz]: See Range
DC Offset [mV]: 0


Channel 1:

Elapsed Time [sec]  Frequency [Hz]:   Impedance [ohm*cm^2]:   Phase Angle [degree]:
 5                   10                 988.8813               -13.4495
 11                  10                 890.7551               -12.9078
 16                  20                 645.7655               -18.1578
 22                  20                 619.4148               -19.7518
 246                 10                 978.0574               -14.6376


Channel 2:

Elapsed Time [sec]  Frequency [Hz]:   Impedance [ohm*cm^2]:   Phase Angle [degree]:
 27                  10                 985.8459               -13.8266
 33                  10                 859.4257               -13.173
 38                  20                 632.2547               -17.6808
 43                  20                 578.4083               -19.1346
 268                 10                 975.0731               -13.896


Channel 3:

Elapsed Time [sec]  Frequency [Hz]:   Impedance [ohm*cm^2]:   Phase Angle [degree]:
 49                  10                 991.5811               -14.3165
 54                  10                 875.5241               -15.504
 60                  20                 652.9566               -18.5794
 65                  20                 636.1905               -16.7709
 290                 10                 996.3832               -13.4082


Channel 4:

Elapsed Time [sec]  Frequency [Hz]:   Impedance [ohm*cm^2]:   Phase Angle [degree]:
 71                  10                 970.2044               -14.5509
 76                  10                 869.388                -13.2785
 82                  20                 660.2276               -18.1682
 87                  20                 588.4931               -18.3492
 312                 10                 1000.3273              -13.903
 317                 10                 860.5586               -14.3198
```