

OrganoTEER[®]

User manual

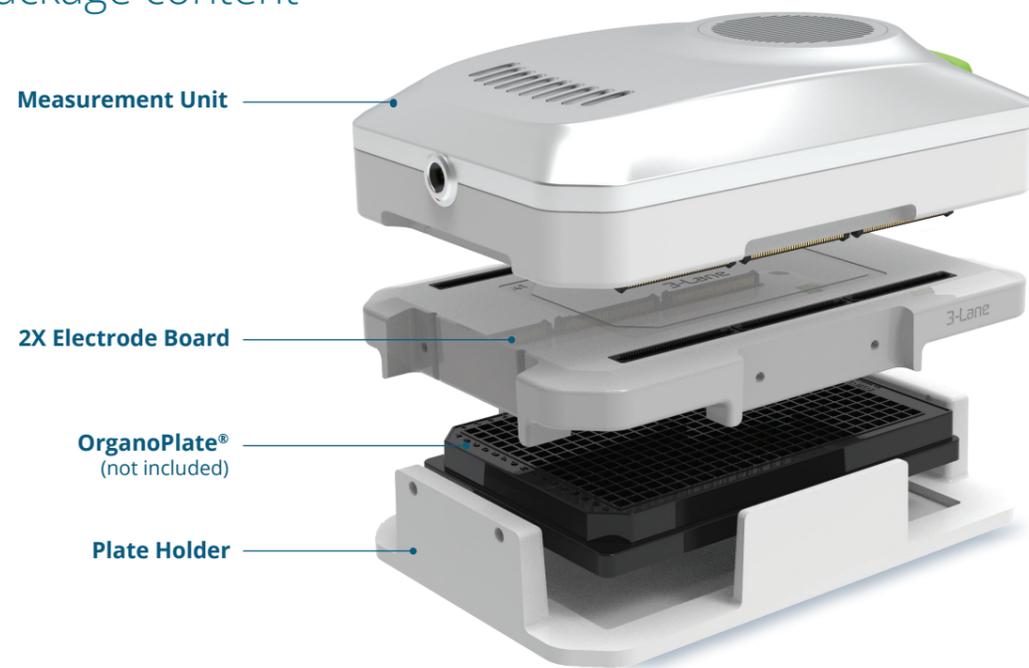
MIMETAS

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1. Package content



Connection Hub with:

1. Data cable
2. Power cable
3. USB cable



Electrode Board Stand



Cleaning Solution



Single-Well Plate



Software



- User manual
- Quick Start Guide
- Terms & Conditions

2. Intended use

This instrument is intended for assessing barrier function of a cell layer by performing transepithelial electrical resistance (TEER) spectroscopy over a selected frequency range. Before setting up the OrganoTEER®, an OrganoPlate® with fully grown tubules (cultured against ECM) is needed. For protocols on culturing tubular tissue models in the OrganoPlate®, please refer to protocols on mimetas.com/support.

At this point, OrganoTEER® is intended only for measuring TEER in the OrganoPlate®. Please consult with Mimetas before using the OrganoTEER® for any other purposes. Using the OrganoTEER® for any other unauthorized applications will result in voiding the warranty. The OrganoTEER® is intended for research applications only and was developed specifically for interrogating cells in the OrganoPlate® platform. The OrganoTEER® was designed to operate at room temperature under general laboratory conditions and in CO₂ incubators at temperatures between 5°C and 40°C under non-condensing conditions.

TEER values obtained by measuring cells with OrganoTEER® in OrganoPlates® and normalized per area (in $\Omega \text{ cm}^2$), can be only very approximately compared with normalized TEER values obtain with other TEER systems and cell culture platforms. As a rule of thumb, comparison of normalized TEER values tends to be more accurate at higher TEER values (e.g. $> 1000 \Omega \text{ cm}^2$). However, due to current density gradient along the cell layer, user normalized TEER values across different platforms can differ very substantially in the low (under several hundred $\Omega \text{ cm}^2$) TEER range.

3. Materials required

- OrganoPlate® with a tubule of live cells against ECM
- Cell culture medium
- Repeating multichannel pipet and tips
- Ethanol (70%)
- Aspirator

4. Setting up the OrganoTEER®

Upon arrival of the OrganoTEER®, please inspect the package for any damage caused by the shipment. If there is no visible damage, unpack the box and check for the presence of all components. In case of any missing or damaged component, please contact Mimetas support directly via mimetas.com/support.

Keep all parts of the OrganoTEER® system on a flat, dry and clean surface. Connect the power cable to the Connection Hub and plug the adaptor into an appropriate electric outlet. Connect the USB cable to the Connection Hub and plug it into one of the laptop's USB ports.



Connect the data cable to the other side of the Connection Hub. Please ensure alignment of the orientation of the male and female plug before gently pushing in the connector. Do not force connection of misaligned connectors.

Place the other end of the cable, unconnected, near your working area and proceed to 5. Performing a measurement with the OrganoTEER®

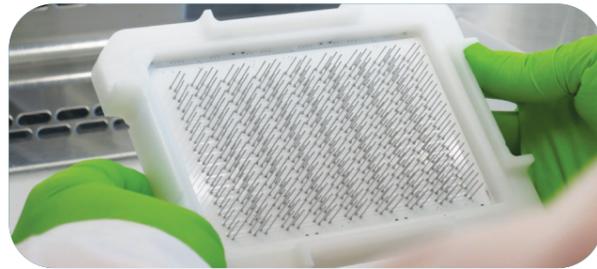
5. Performing a measurement

Cleaning the Electrode Board

Select the Electrode Board you will be using for the experiment.

NOTE: Wear protective gloves and do not touch the electrodes when manipulating the Electrode Board. Handle the board by holding its white body.

In a sterile environment (e.g. in a biosafety cabinet), clean the Electrode Board by spraying the electrodes with 70% ethanol. Hold the board electrodes facing down and ensure all electrodes are wetted. Let the Electrode Board dry in the storage rack for at least 30 minutes (without lid) before using it for measurements.



Preparing the OrganoPlate® cell culture for TEER measurements

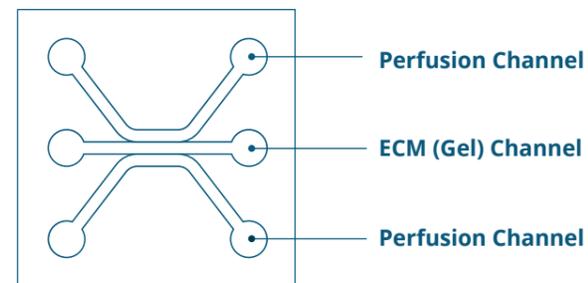
There are two ways to measure an OrganoPlate® using the OrganoTEER®. The first is to perform a TEER measurement in a flow cabinet, the other inside the incubator. The former is used for point measurements at room temperature, while the latter is used for time-lapse measurements at 37°C.

To measure the TEER of tubules in an OrganoPlate® using the OrganoTEER®, the electrodes of the Electrode Board need to be in contact with the liquid on the apical and basal side of the tube. To ensure proper contacting, an appropriate volume of conductive liquid (media/PBS/

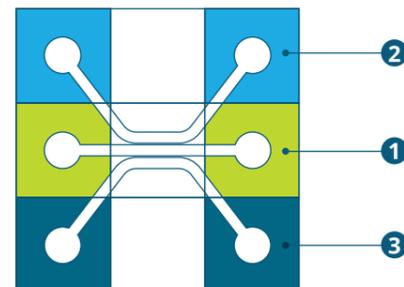
HBSS) needs to be present in:

- the gel inlet and outlet wells
- the inlet and outlet wells of the perfusion channel where the tubule is present.

A volume of 50µl per well is recommended. The minimum volume is 20µL. Ensure no air bubble is blocking the inlet and outlet holes, using a low volume pipet to dislodge trapped bubbles.



OrganoPlate® 3-lane 40



1. Add 50µL medium to the gel inlet and outlet
2. Add 50µL medium to the top channel (tubule in top channel)
3. Add 50µL medium to the bottom channel (tubule in bottom channel)

Installing the OrganoPlate® for TEER measurement

It is important to equilibrate the temperature of the OrganoPlate® prior to measurement. 30 Minutes before measuring, place the OrganoPlate® inside the Plate Holder in static position in a sterile environment. Ensure correct orientation and placement of the OrganoPlate® in the Plate Holder.

Note: The temperature of the barrier tissue during measurements affects the observed TEER. Always perform measurements at the same temperature, and ensure proper equilibration of the plate, medium and instrument before each measurement.

After 30 minutes, remove the lid and directly proceed with next step.



Take a clean, dry, and sterile Electrode Board from the board rack. Ensure correct orientation of the OrganoPlate® in respect to the Electrode Board by referring to the markings onto the Electrode Board. Slide the Electrode Board vertically inside the Plate Holder on top of the OrganoPlate®. The frame should slide in with no resistance. If the board does not slide down, do not force it, but ensure all parts are correctly placed and retry.



Take the Measurement Unit, align the connectors of the Measurement Unit and the Electrode Board, then snap the parts together. Press down firmly to ensure good connection.



Check from the side whether the OrganoTEER® is correctly connected. The OrganoTEER® is correctly installed when no gap is visible between parts.



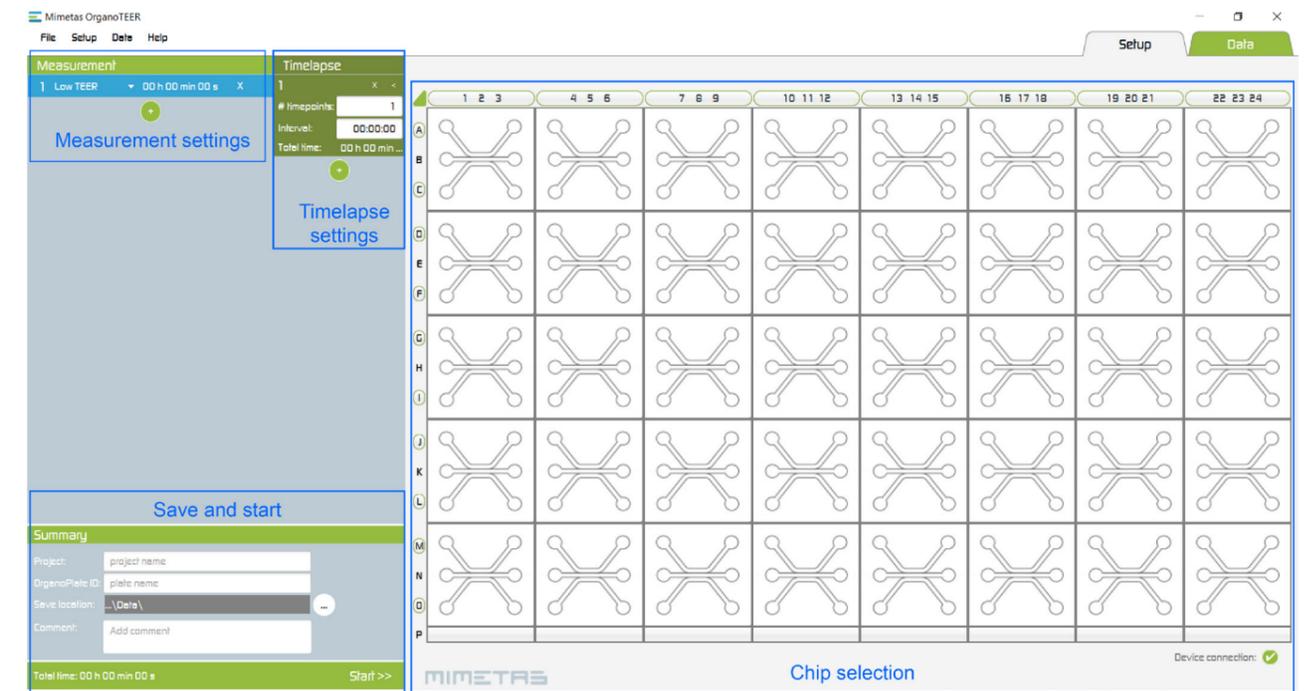
You can now connect the open-ended data cable to the Measurement Unit. Make sure the connector has the red dot aligned with the red dot of the plug. For proper recognition of the device by the software, first fully connect your device before switching the power button on the Connection Hub. A small LED should light up at the front of the Connection Hub.



6. Setting up a measurement

After connecting the device with the OrganoPlate® inside, start the OrganoTEER® software by clicking on the shortcut on the desktop. The setup window will appear. A small icon at the bottom right will show your connection with the device.

To set up your measurement protocol, you'll need to enter the measurement settings, select the measurement settings and corresponding chips and press start. Please refer to the following sections to properly configure those panels.



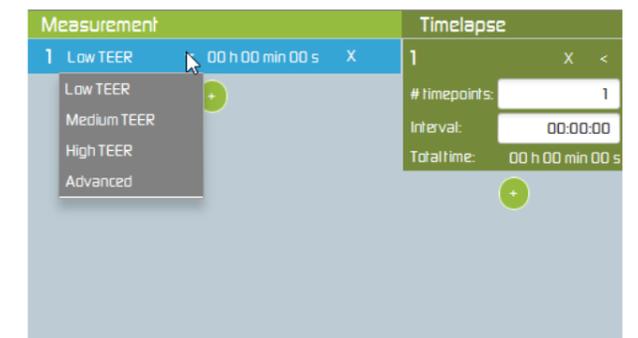
Measurement settings

At the top left you'll find the Measurement settings panel. To run a standardized setup configuration, select the Low, Medium or High TEER from the drop-down menu. The setups are designed to fit the following TEER ranges:

- Low 2-50 Ohm.cm²
- Medium 25-500 Ohm.cm²
- High 500-5000 Ohm.cm²

Each Measurement option will take approximately one minute to measure all top and bottom perfusion channels, or 80 tubules, and less than half a minute if only top or bottom tubules are selected.

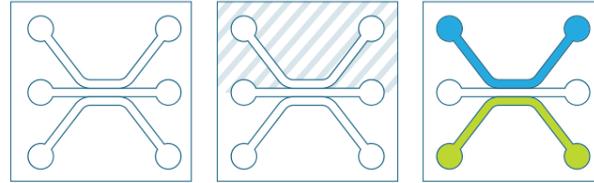
In the case where different tubules have different expected TEER - e.g. in a coculture with two different cell types in the top and bottom perfusion channels of a chip - you can create additional measurement configurations by clicking on the + button. Select the corresponding chips you'd like to measure with the selected measurement setup in the chip selection panel. The chips will change to the colour of the measurement setting.



For designing custom measurement parameters, select Advanced from the drop-down menu. Please look at "An in-depth look into frequency settings" (Annex 2) to design your own parameters.

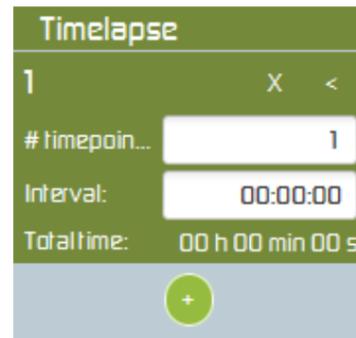
Selecting chips

Chip selection can be done one by one, per row and per column, or by clicking the small triangle at the top left of the chip selection window. By clicking it multiple times it will select respectively: all tubes, all top tubes, all bottom tubes and deselect all.



Timelapse settings

To perform multiple measurements over time, enter the required number of measurements and interval in the Timelapse Settings panel. By default, this is set to single measurement. For advanced measurement procedures, multiple timelapses can be queued by clicking “+” button below the timelapse panel. For an interval below 10 minutes, the number of timepoints is limited to 20, to ensure constant conditions for your OrganoPlate®.



Finally, at the summary window of the screen enter the measurement details. Entering a Project name and Plate name will generate a default save location. If desired, add a comment to your measurement. The total time your measurement takes is showed next to the start button.

Starting a measurement

Click on the “Start” button at the bottom of the summary window to run the measurement protocol. The window will switch to the “Data” tab, starting the measurement sequence as defined in the setup window. A progress bar and percentage indicator show the progress of the measurement. Once the measurement is completed, the TEER values will be shown in the table.



7. Analysing & exporting data

Basic analysis can be performed and exported within the Mimetas OrganoTEER® software environment. Once the measurement is finished, you’ll see the TEER values for the measured tubes in Ohm.cm² in a heatmap. All data can be found in the destination folder selected along with an .xls file containing TEER, cell capacitance as well as channel resistance for further processing. The measurement settings and device information are also included. Additionally, you can export a copy of this result sheet to a folder of your choice by clicking on export. A separate file with temperature information is also exported to the destination folder.



Upon selecting one or multiple TEER values, a bar graph of the values will be plotted in the Preview window. Deselection can be done by clicking “Deselect Chips” or by pressing “Esc”.



After clicking “Average”, the average of the selected values will be plotted as a single bar with standard deviation. Clicking inside the bar allows plotting data from the calculated chips. To go back to the averaged bar graph, press Esc. A TEER value can only be attributed to one average group.



Averaging can be done multiple times to create an overview of the different groups of TEER values.



When your project contains multiple time points, you can scroll through the timepoints using the horizontal bar at the bottom.

The switch button at the top of the Preview window will allow you to switch between a bar graph and a time curve, in which you can plot single values or group averages as well.

Finally, use the export button to save a .xls sheet containing the initial result data as well as the averaged group data for further processing in your data processing software of choice.

Multiple parameters are extracted from the impedance spectra measured on each selected chip. Using the Data menu, you can change the displayed parameter.

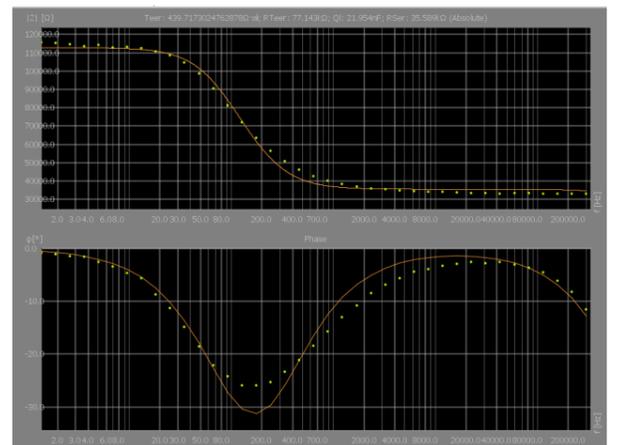
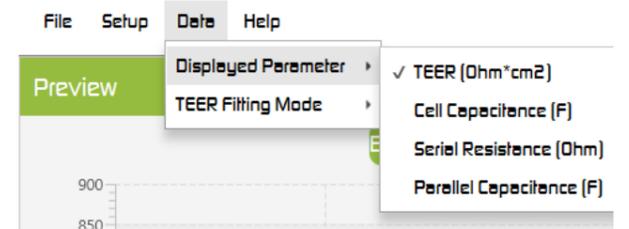
TEER: the tubule electrical resistance in Ohm.cm².

Cell Capacitance: This parameter is also a property of the tubule (see Annex 1) and is displayed in Farad. It can be corrected to an area independent value in Ohm/cm² by dividing it by the area of the ECM supported part of the membrane (approximated 0.0057cm²)

Serial Resistance: This is the electrical resistance of the chip itself. It is dependent on the geometry of the microfluidic channel as well as the conductivity of the media

Parallel Capacitance: This value is associated with the parasitic component of the system, and can be useful in determining the limit of detection for each chip (see Annex 1)

By double clicking on an acquired value in the data tab, you can visualize the impedance spectra associated with the tubule. The green dot represents the measured absolute impedance (top graph) and phase (bottom graph) measured at various frequency points. The Orange line represents the estimated spectra based on an equivalent electrical model composed of the TEER, cell capacitance, series resistance and parallel capacitance.



Disconnecting the Measurement Unit from the Electrode Board

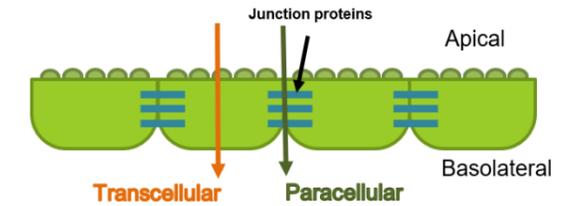
When your measurement has finished, disconnect your Measurement Unit from the Electrode Board. This can be done by pulling the green handle on the side of the Measurement Unit to the right. Clean the Electrode board as described earlier and use Electrode Board Stand for safe storage. Use the 1 well plate to keep it sterile outside of the flow hood.

Take your OrganoPlate® out of the Plate Holder, replace your sterile lid on the OrganoPlate® and continue your experiment as desired.



8. Annex 1: Understanding impedance data

An epithelial or endothelial layer is composed of cells linked by junction proteins. These junction proteins have an affinity for small hydrophilic molecules and form the **paracellular pathway**. Hydrophobic molecules will tend to cross through the cell itself and form the **transcellular pathway**.



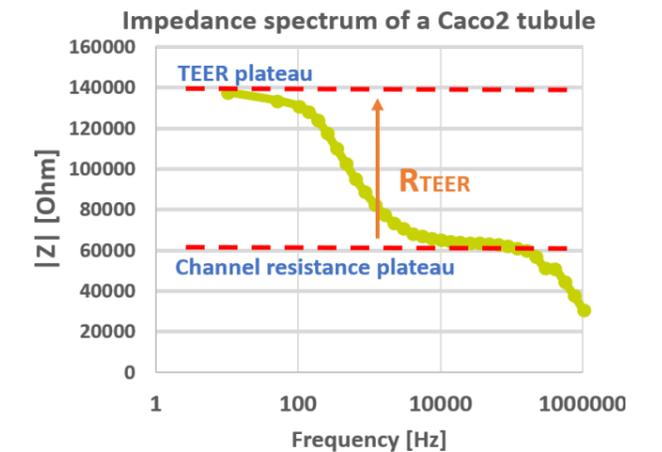
An **impedance** is the measure of **electrical conductivity** of a tissue to an alternative current (AC) for a **given frequency**. An impedance spectrum consists of multiple impedance points measured in a certain frequency range.

When measuring the impedance spectrum of a tubule in the OrganoPlate®, both the paracellular and transcellular pathway, as well as the chip channel length and width will influence the output.

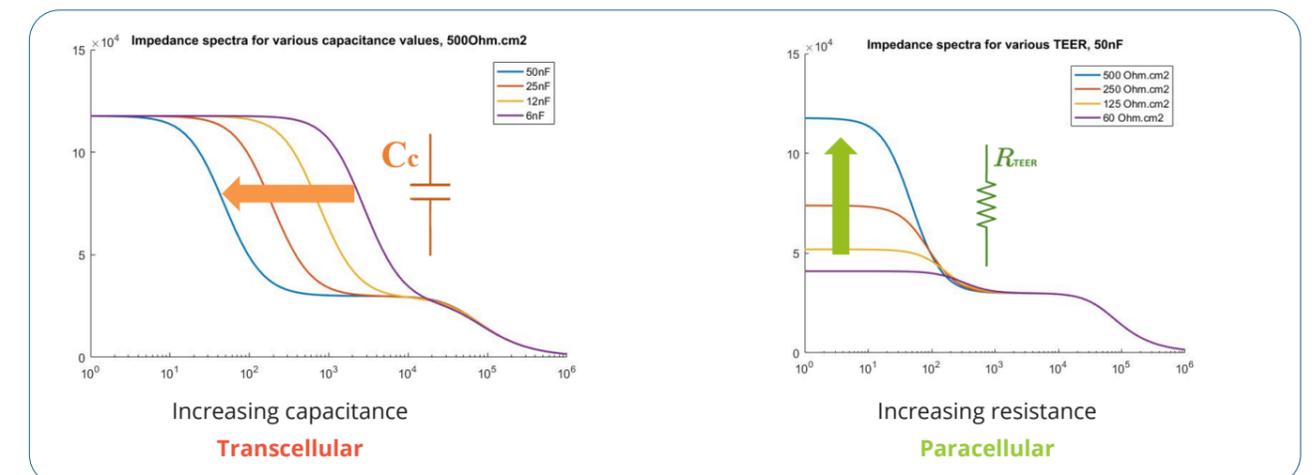
The paracellular pathway contributes to the impedance as an electrical resistance, commonly called **Trans Epithelial/endothelial Electrical Resistance**, or **TEER**. On the other hand, the transcellular pathway is associated to an electrical capacitance, called **cell Capacitance**, or **C_c**. The TEER influence can be recognized as an **upper plateau in the low frequency** part of the spectrum. Past the TEER plateau, a second plateau can be found, which correspond to the OrganoPlate® **channel electrical resistance**.

The frequency at which the plateau change happen will vary depending on both the **TEER** and the **C_c**. Additionally, the frequency at which plateau shift, called cut off frequency, can be calculated described in the formula on the right.

The impact of varying TEER and C_c can be seen in the graph below. An **increase in TEER** correlates with a **higher TEER plateau**, while an **increase in C_c** correlates with a **lower cut-off frequency**.



$$f_c = \frac{1}{2\pi \cdot R_{TEER} \cdot C_c}$$



9. Annex 2: An in-depth look into frequency settings

The software supports custom settings for a frequency sweep adapted to your cell type. Please read the Annex “Understanding impedance data” to understand the weight of each parameter.

The parameters with the most influence on the results will be the start and end frequency (Minf and Maxf) of the sweep, the number of steps and the precision/averaging settings.

The starting frequency (Minf) should be chosen so as to include the TEER plateau in the impedance spectrum. If Minf is too high, the TEER plateau will be outside of the measured range, resulting in a poorly fitted values. The default setting of the end frequency (Maxf=300kHz) is appropriate for most measurements. While it can be changed, no significant improvement in measurement quality or speed can be achieved.

The lower the starting frequency, the more time will be spent measuring. The estimated time per measurement is shown above the setting area.

The “Steps” setting affects at how many points across the frequency spectrum the conductivity is measured, more points typically improving the fitting. The “Precision” setting affects how precise each individual point is measured (higher is more precise) and “Averaging” affect how often each individual point is measured. Higher settings in these parameters will improve dataquality, but will also increase the measurement time.

The precision should be set between 0.0 and 2.0. The averaging number corresponds to the number of measurements to be done per chip and averaged. We recommend increasing the precision and leave the averaging at 1.



Measurement		
1	Advanced	00 h 00 min 00 s
Minf [Hz]	Maxf [Hz]	Steps
1000.0	1000000.0	51
Amplitude [mV]	Precision	Averaging
100.0	0.0	1

A table of recommended settings for different TEER ranges is depicted below:

TEER ranges	Minf	Maxf	Precision	Steps	Amplitude	Averaging
<20 Ohm.cm ²	1000Hz	150kHz	1.0	100	100	1
20-100 Ohm.cm ²	100Hz	150kHz	0.5	50	100	1
100-500 Ohm.cm ²	10Hz	150kHz	0.0	50	100	1
>500 Ohm.cm ²	1Hz	150kHz	0.0	30	100	1

10. Annex 3: Cleaning an Electrode Board

The Electrode Board is composed of 480 stainless steel electrodes, which needs to be handled and cleaned with care.

NOTE: Avoid touching the electrodes and always handle the boards by holding the white casing. Store the boards in the appropriate rack or in a base with a one well plate in place to avoid damage.

There are three aspects of maintaining Electrode Board cleanliness and sterility:

- Soaking in MilliQ water/PBS/ HBSS to remove residue from the electrodes.
- Ethanol rinse before and after performing a TEER measurement
- Electrode fouling removal by dipping in the Mimetas Electrode Board cleaning solution

Ethanol rinse before and after performing a TEER measurement

Before measuring:

Spray the board with a generous amount of 70% ethanol in a sterile working environment and let the Electrode Board dry in the storage rack for a minimum of 30min before using it for measurements.

After measuring:

It is recommended to soak the Electrode Board in MilliQ water/PBS/HBBS using the 1 well plate provided with your board, to avoid carry-over or contamination, especially when using toxic compounds in your experiment.

Place the 1 well plate in the Plate Holder, fill it up to the rim with your washing solution (MilliQ water/PBS/HBBS) and insert your Electrode Board onto the frame. Leave the electrodes submerged for 5 minutes, remove the board, discard the washing solution and repeat. After the repetition, spray your Electrode Board with 70% ethanol as in the *Before measurement* step.

Electrode fouling removal by dipping in Mimetas Electrode Board cleaning solution

Following proper instruction will ensure optimal lifetime for your Electrode Board. However, when using the board frequently and/or in an incubator environment for extended amount of time, calcium deposit can appear on the electrode. When you notice such fouling, you can clean the electrodes in an anti-fouling agent.

To do so, place your one well plate into the frame, and fill it with a 20:1 solution of MilliQ water and Mimetas Electrode Board cleaning solution. Immerse the electrode in the solution and leave it in there for 15 minutes. Do not leave it for longer than 20 minutes.

Immerse the electrodes in the water for two times 10 minutes, include refreshing of the water inbetween.

Finally, remove the electrode, discard the MilliQ and perform an ethanol rinse as describe in this annex.

11. FAQ about OrganoTEER®

Before measurement

How much influence have different medium compositions on TEER measurements?

Medium composition will influence the channel resistance of your chip (see Annex 1 for detailed explanation about the mechanism behind TEER measurements in the OrganoPlate). This should not affect your TEER measurements. Difference in pH and temperature however can have an influence on the TEER values.

What is the minimum volume needed for TEER measurements in the OrganoPlate®?

The recommended volume is 50µL and the minimum volume is 20µL. With volumes lower than 50µL, special attention should be given to avoiding bubbles at the microfluidic inlets.

Do different volumes affect the TEER values?

If the minimum required medium volume of 20µL is maintained in all inlets and outlets of the measured tubules and the gel channel, variations of medium volume do not have a significant effect of the measured TEER values.

What influence does temperature have on the TEER measurements?

Changes in temperature has been observed to affect the TEER of epithelial and endothelial cell layers. This is a real biological effect and not a measurement artefact. This effect poses a requirement for stable measurement temperature. Whether the measurement is performed at room temperature or inside an incubator, maintaining a constant temperature is critical for generating reproducible datasets.

Do I really need to equilibrate the temperature in the plate before the TEER measurement?

Yes, whether it is at room temperature or in an incubator, it is highly recommended to stabilize the temperature of your plate prior to performing a measurement to achieve reproducible results.

Are probes used in a barrier integrity assay interfering with the TEER measurement?

Fluorescent non-ionic compounds should not have any direct effect on the TEER measurements itself. Cytotoxic compounds will affect barrier integrity and result in a decrease of the TEER value.

What are the proper controls for a TEER measurement in the OrganoPlate®?

Absolute TEER is extracted from the raw impedance spectra without the need for a baseline correction as in the case in single frequency point methods. You should nevertheless use negative and positive controls as an essential part of any experiments used to validate your results.

Why does the OrganoTEER® software display Low TEER instead of zero values?

The OrganoTEER® lower limit of detection is not zero, but the lowest value that can be given with confidence by the fitting algorithm. The device displays Low TEER in the software interface but will write 0 in the data sheet for easier processing. The lower limit of detection depends on the measurement setting as well as the TEER and capacitance values. For better results, select the measurement setting that is suited to your expected measurement range.

Setting up the OrganoTEER®

How can I see that the OrganoTEER® is connected before placing my plate in the device?

The OrganoTEER® software beta will automatically detect a TEER Measurement Unit upon starting, without the need of an OrganoPlate® being present. Make sure to connect the Electrode Board to the Measurement Unit prior to powering up the device. The software then automatically detects the connection. A connectivity marker on the lower right side of the Setup panel will inform you whether the OrganoTEER® is connected.

Do bent electrodes have an influence on TEER measurements?

Bent electrodes are usually a result of improper handling. Do not let any solid object or fingers come in contact with the OrganoTEER® electrodes. If you observe a bent electrode in your device, use a plastic tweezers to straighten it. Excessively damaged or missing electrodes will require replacing the Electrode Board.

Measuring with the OrganoTEER®

How do I know if the OrganoTEER® settings are correct for my cells?

The Quick start setups have a recommended range. The "Low TEER" setup works well below 50 Ohm.cm², the "Medium TEER" setup is adapted to TEER from 20 to

500 Ohm.cm² while the "High TEER" setup is best used for TEER consistently above 400-500 up to 5000 Ohm.cm². Please refer to the Annex 2 "An in depth look into frequency sweep" for further information.

Are TEER measurements invasive for my cells?

A TEER measurement by itself will not harm the cells. If you observe loss of barrier integrity, review the protocol to ensure you followed all steps correctly. Make sure ethanol is completely dried out from the electrode board prior to assembly with the OrganoPlate®. Verify proper experimental conditions and settings for your model (e.g., rocker settings, ambient temperature, relative humidity if incubator is used, etc.)

Data analysis

How can I check if my TEER values are correctly calculated?

Refer to Annex 1 "Understanding impedance data" to visually verify your data.

Can I spot holes in my cell barrier with a TEER measurement?

Holes in your tubule will have an influence on the TEER, but it will be indistinguishable from a general loss of tubule leak tightness. Please refer to fluorescence-based Barrier Integrity assay (mimetas.com/page/assays#barrier) for visually identifying discontinuity in cell layer confluency.

Can I distinguish from the TEER data a tight tubule with an isolated hole from an overall leaky tubule?

TEER is a measure of permeability of an entire tubule and does not provides an insight into the distribution of permeability along the tubule.

Is the OrganoTEER® measuring the resistance of the entire 3D tubule formed in the OrganoPlate® or the cells layer formed against the ECM only?

TEER is measured across the cells against the ECM. The remainder of the tubule has little effect on the measured TEER values.

What will be the influence of different ECM gels on TEER measurements?

The type of ECM used has effect on the gel stretching, affecting the surface area of the meniscus against which the cell layer grows. This can induce variation in TEER values between different ECMs.

Does using different ECMs with different meniscus surfaces require adjustments in surface normalization of the TEER values expressed in Ohms.cm²?

The estimated meniscus area for collagen type I in concentration of 4 mg/mL is ~0.0057 cm². Variations in the meniscus "stretching" and subsequent differences in the surface area of the tubule in contact with ECM can influence the absolute TEER value. If needed, correcting for different ECM meniscus length can be done based on microscopic imaging the meniscus extension and geometric shape.

Should I normalize the data if I want to compare the effect of treatment on different cell lines?

Absolute TEER values can greatly depend on the cell type and culture model used. Using percentage changes in TEER due to a treatment is therefore recommended.

12. Safety instructions

WARNING! Switch off the power and disconnect the power supply before lifting or moving the OrganoTEER® for service or maintenance to avoid electrical shock.

WARNING! DO NOT use the OrganoTEER® in hazardous conditions or expose it to hazardous materials/chemicals for which the device was not intended.

DO NOT immerse the OrganoTEER® in liquid for cleaning.

DO NOT place the Connection Hub inside a CO2 incubator.

DO NOT replace the supplied adapter by any other adapter. If necessary, please contact Mimetas for support.

DO NOT place the OrganoTEER® in front of the temperature sensor of the incubator.

DO NOT spray ethanol directly into the cooling fan at the top while cleaning.

DO NOT use any other means for sterilization than 70% ethanol.

DO NOT open the OrganoTEER®. When your device shows signs of mechanical or electrical modifications or damage not related to normal laboratory use, warranty is void.

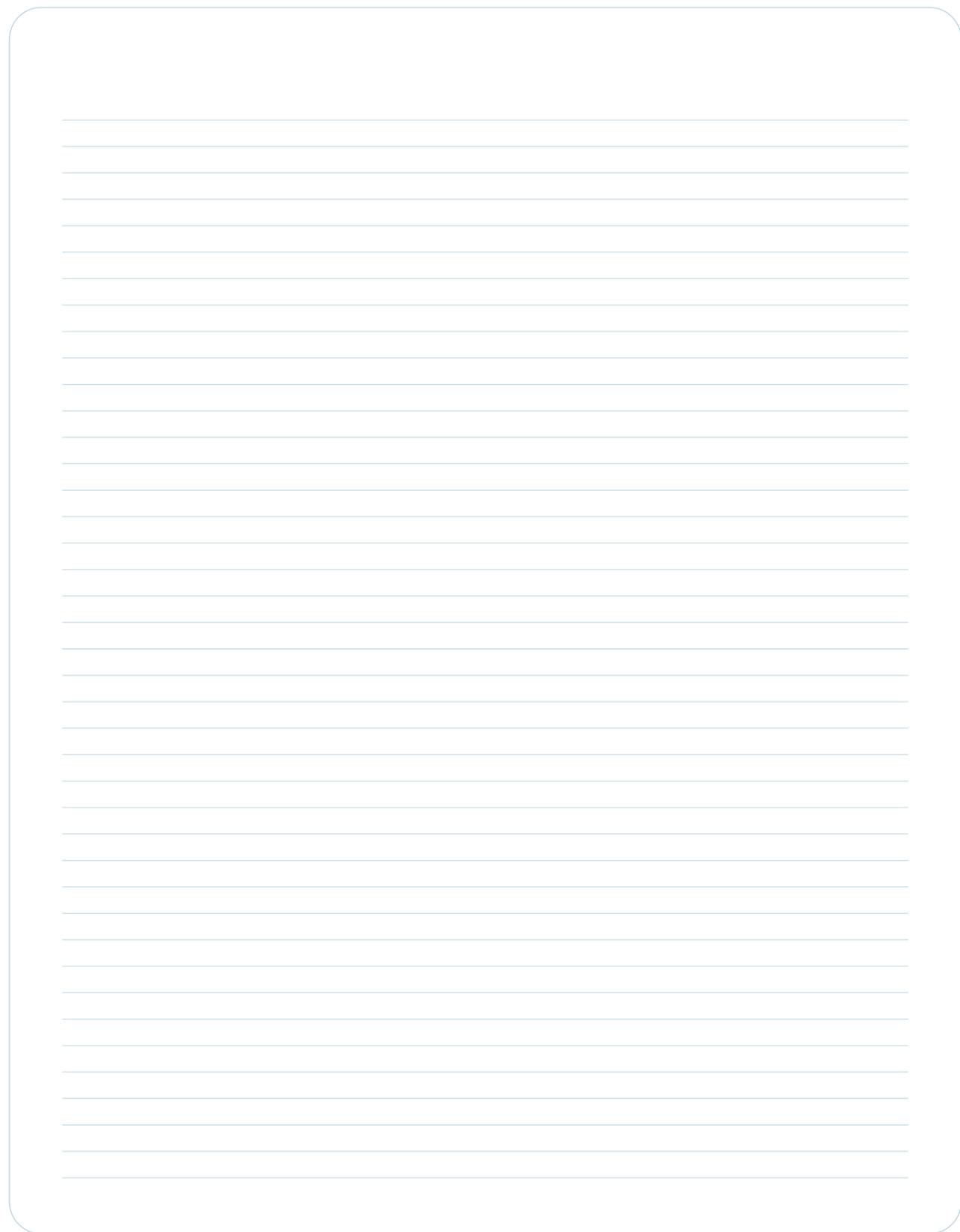
DO NOT place tissue culture plates in close proximity of the OrganoTEER®. The air flow created by the inbuilt fan could negatively affect your culture.

DO NOT place the OrganoTEER® in narrow places to make sure the airflow of the inbuilt fans is not impeded. Leave at least 3 cm (>1 inch) of space available.

DO NOT touch the electrodes of the Electrode Board.

DO NOT autoclave any part of the OrganoTEER®.

17. Notes



A large rectangular area with rounded corners, containing horizontal blue lines for writing notes. The lines are evenly spaced and extend across the width of the box. The box is outlined in a light blue color.



