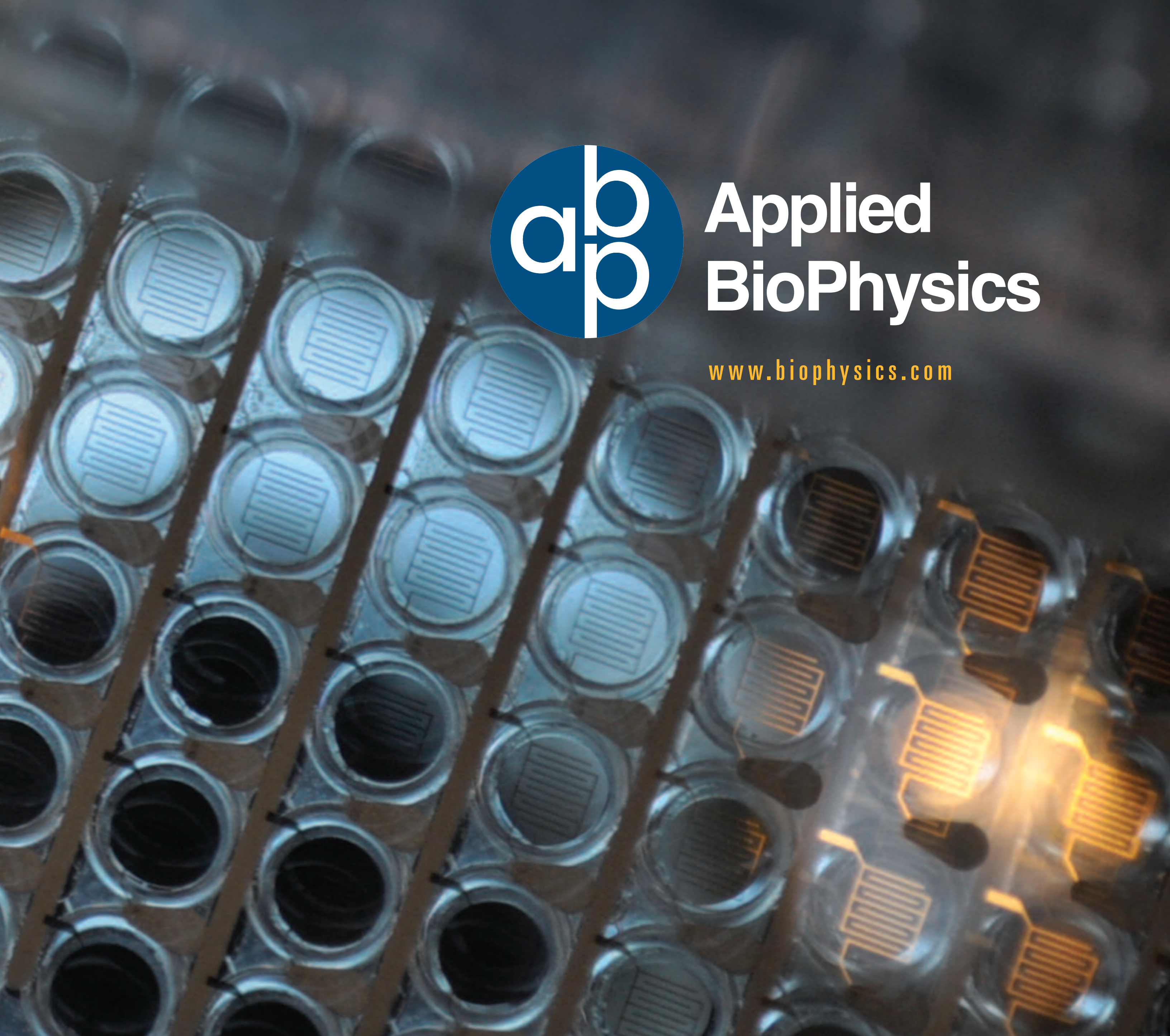
ECIS

ELECTRIC CELL-SUBSTRATE IMPEDANCE SENSING

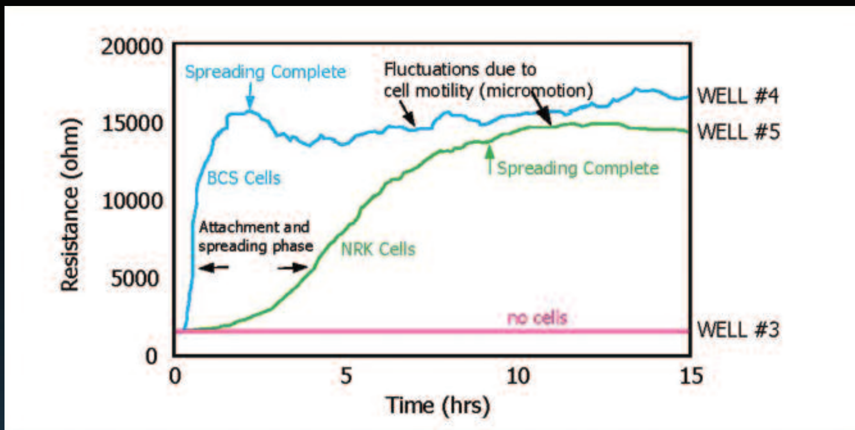
PRODUCT GUIDE

Applied BioPhysics

www.biophysics.com

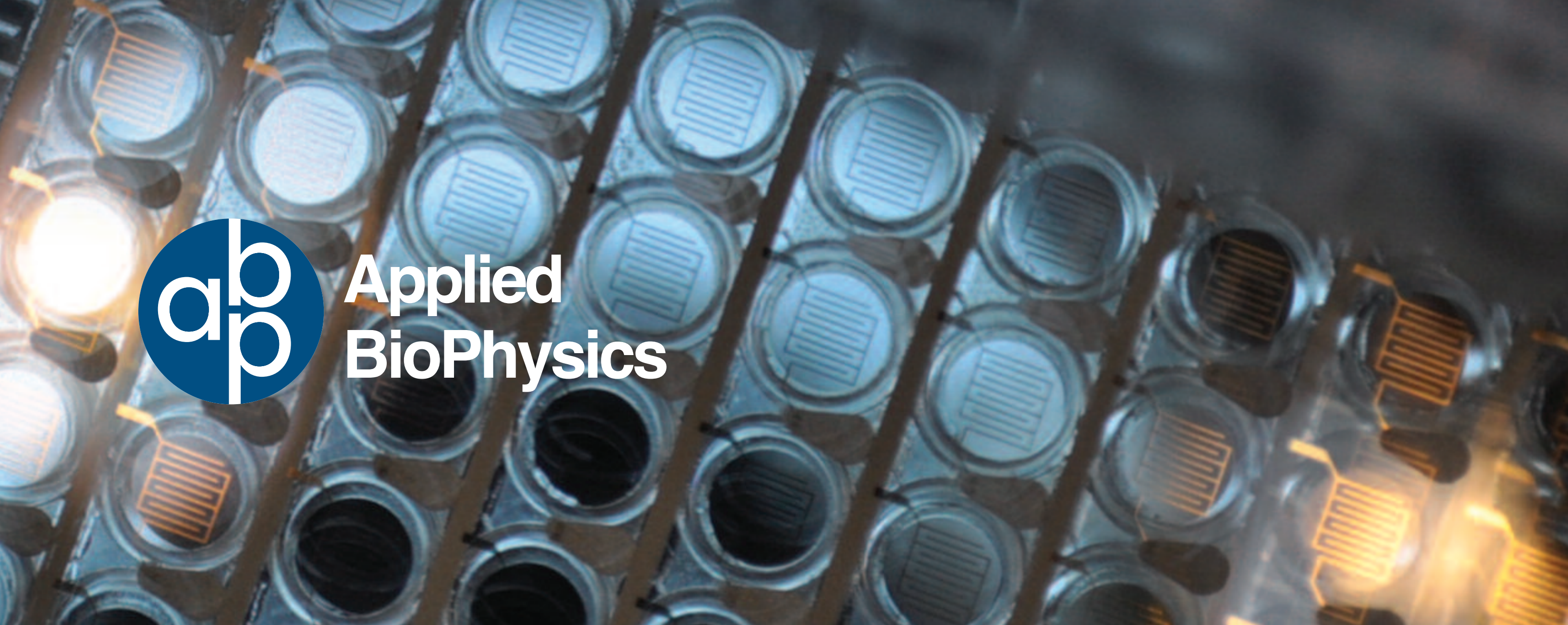


ECIS Time Course Measurements



Cells can be sampled as often as several times per sec to as slowly as desired. Each impedance reading is plotted as a point, in Ohms or nanofarads (C), verses time. The total acquisition time is user controlled and can range from a fraction of an hour to several days. The above plot is a measurement of cells attaching and spreading in two different wells of an array. Well #4 are BCS cells and Well #5 are NRK cells. The electrode in each well was sampled at a few second intervals for 15 hours.

Following inoculation at time zero, impedance increases as the cells attach to the electrode and begin spreading. The impedance continues to increase until the cells reach confluence at 2 hours for the BCS cells and 10 hours for the NRK cells. The small fluctuations in the curves are due to micromotion from the constant movement of the monolayer of cells on the electrode.



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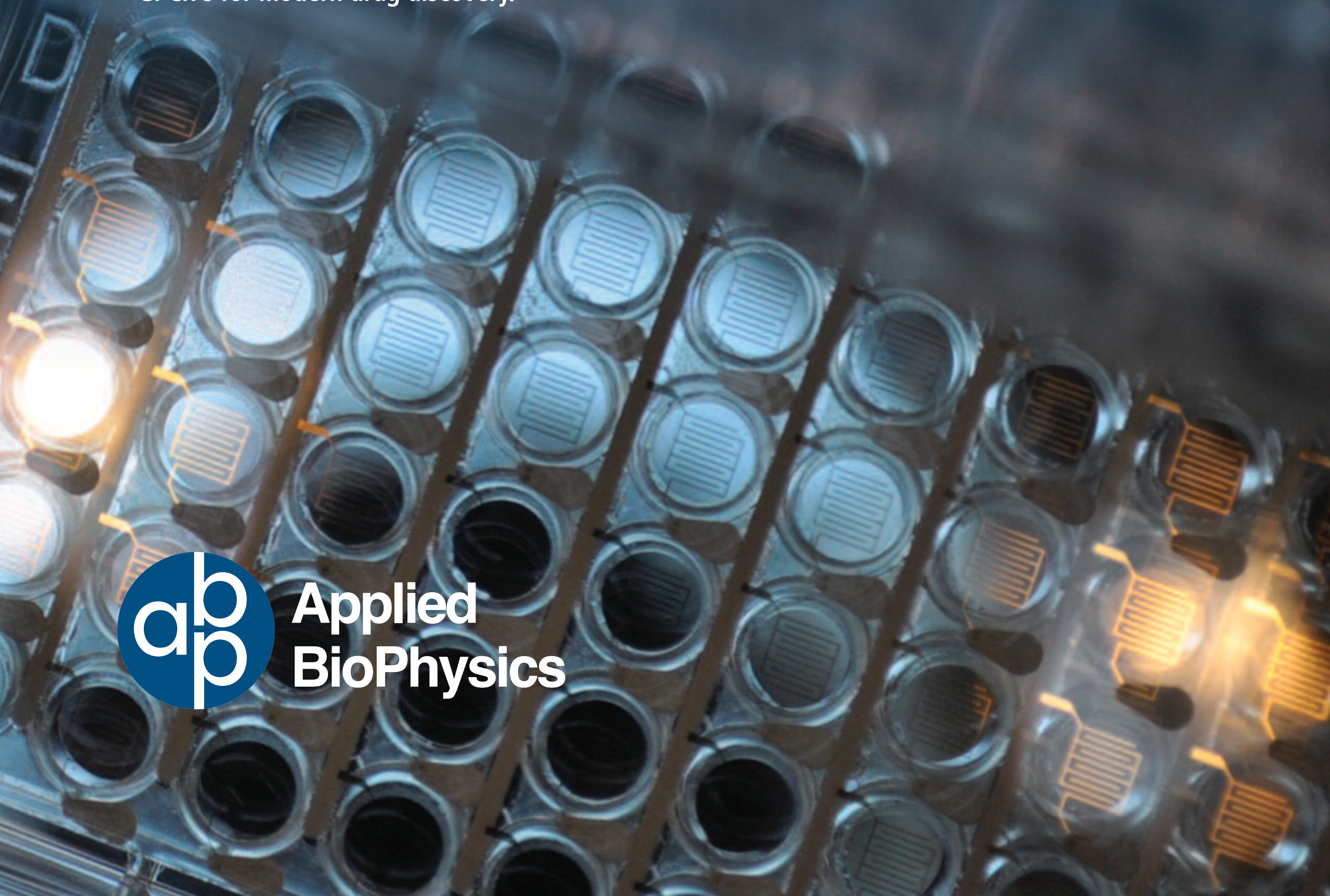
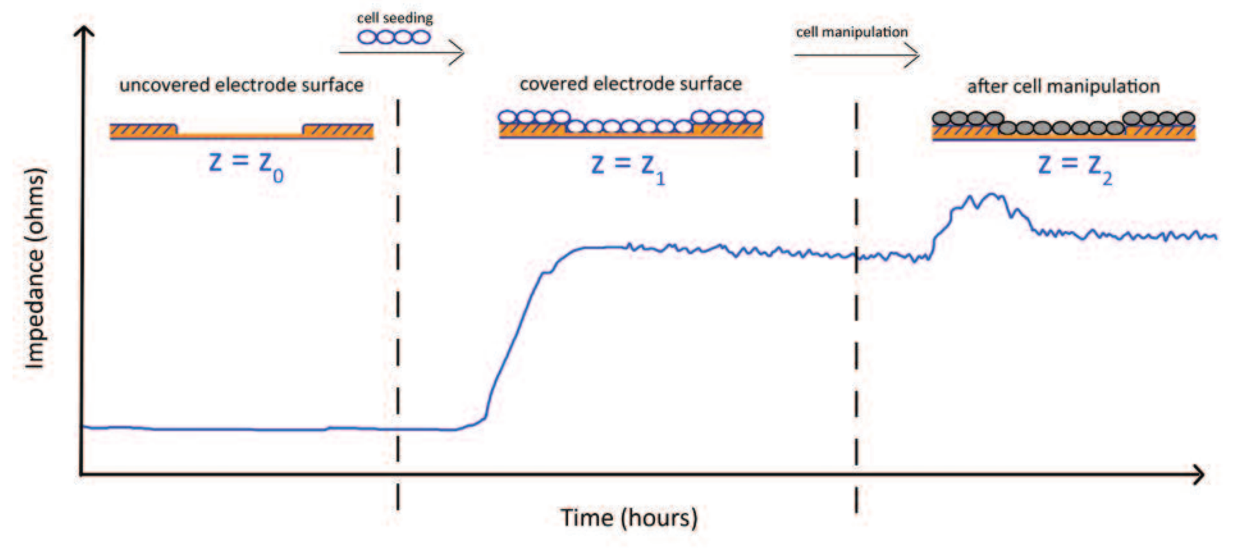
1 what is ECIS

ECIS® (Electric Cell-substrate Impedance Sensing) is a real-time, impedance-based method to study many of the activities of cells when grown in tissue culture. These include morphological changes, cell locomotion, and other behaviors directed by the cell's cytoskeleton. Impedance-based cell monitoring technology was invented by Drs. Ivar Giaever and Charles R. Keese who formed Applied BioPhysics, Inc. to commercialize ECIS® and other biophysical technologies.

The ECIS® approach has been applied to numerous investigations including measurements of the invasive nature of cancer cells, the barrier function of endothelial cells, in vitro toxicity testing as an alternative to animal testing, and signal transduction involving GPCR's for modern drug discovery.



HOW TO QUANTIFY CELL BIOLOGY

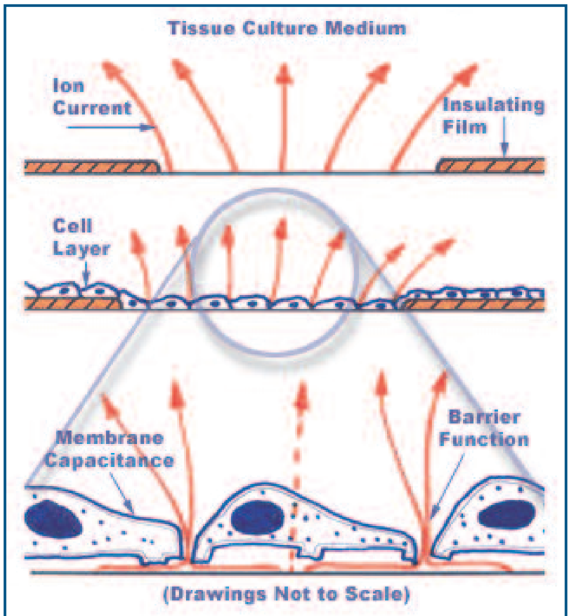


Schematic of an ECIS® experiment starting with a cell free electrode. Once cells are added their presence causes a rise in impedance which plateaus once the cells have reached confluency. At this point the cells can be perturbed and the resulting changes in impedance due to changes in cell behavior are monitored.

Cell function modulates cell morphology. ECIS® is capable of detecting and quantifying morphology changes in the sub-nanometer to micrometer range. In ECIS® a small alternating current (I) is applied across the electrode pattern at the bottom of the ECIS® arrays (direct current cannot be used). This results in a potential (V) across the electrodes which is measured by the ECIS® instrument.

The impedance (Z) is determined by Ohm's law $Z = V/I$. When cells are added to the ECIS® Arrays and attach to the electrodes, they act as insulators increasing the impedance. As cells grow and cover the electrodes, the current is impeded in a manner related to the number of cells covering the electrode, the morphology of the cells and the nature of the cell attachment.

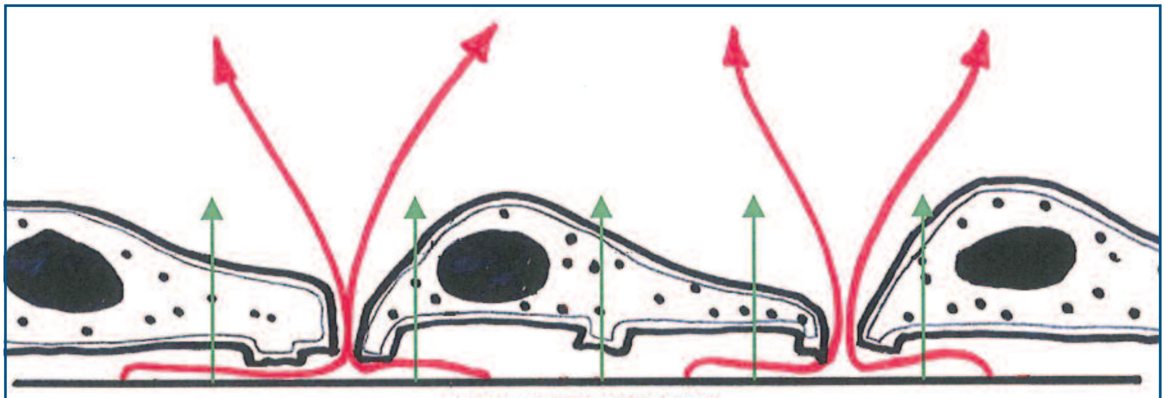
When cells are stimulated to change their function, the accompanying changes in cell morphology alter the impedance. The data generated is impedance versus time.



ECIS® electrodes are defined by small openings in an insulating film covering the gold surface. Once cells fill these openings the current flow from the gold surface is restricted.

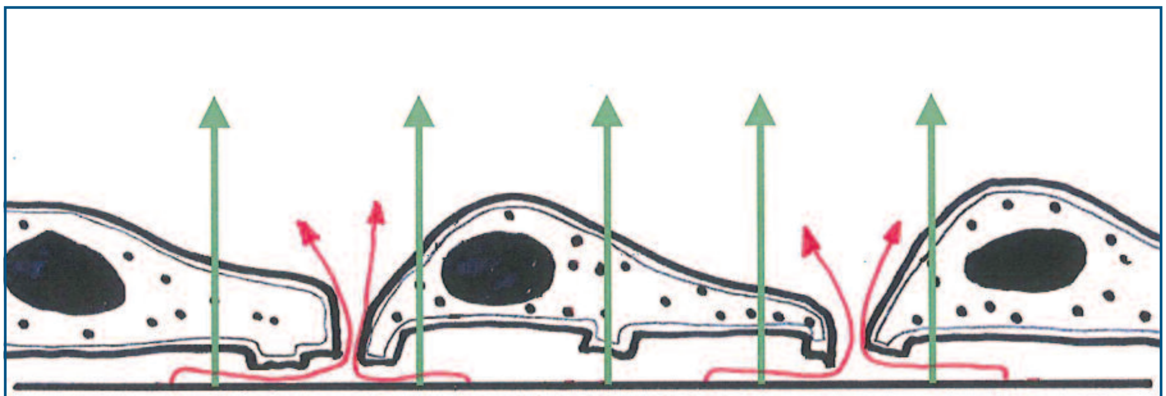
HOW FREQUENCIES REVEAL CELL BEHAVIOR

To understand why AC frequency is important in ECIS® we have to consider how frequency affects the current paths of cell-covered electrodes. (Note: the total current is maintained constant and voltage changes are measured.) At relatively low frequencies (< 2,000Hz) most of the current flows in the solution channels under and between adjacent cells (red lines).



Current flow at low AC frequencies is via paracellular pathways.

At higher frequencies (> 40,000 Hz) more current now capacitively couples directly through the insulating cell membranes (green lines).



Current flow at high AC frequencies is via transcellular pathways.

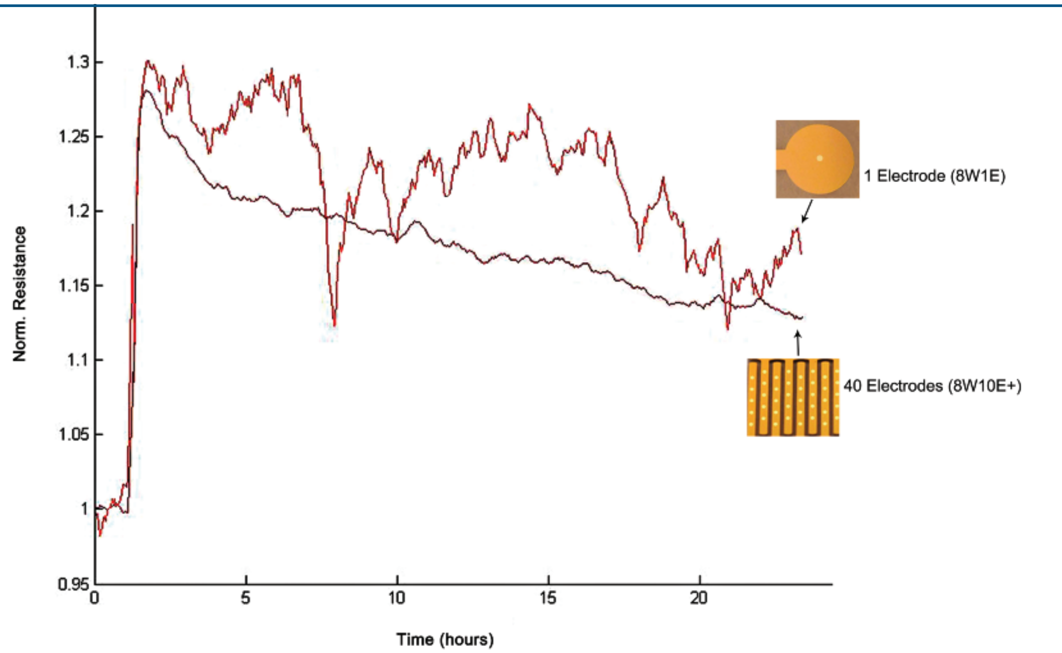
The high frequency impedance is more affected by cell-coverage, whereas the low frequency responds more strongly to changes in the spaces under and between the cells.

With the more advanced ZΘ instrument, where the impedance is broken down into its components (resistance and capacitance), quantitative information about the cells can be obtained by modeling (Giaever and Keese PNAS 1991).

Using impedance data at multiple AC frequencies the ECIS® model calculates time course changes in:

- The barrier function (permeability) of the cell layer
- The degree of constricted flow of current under the cells
- The cell membrane capacitance

HOW ELECTRODE DESIGNS REVEAL ASPECTS OF CELL BEHAVIOR



Response of confluent cell layers to the addition of fresh medium. The plot shows the normalized resistance change as a function of time; the starting values for the 1E plot is 11,500 ohms and for the 10E+ is 1,300 ohms.

Small Electrodes

Small electrodes (1E, 10E, 10E+ type arrays) and their layout within the wells ensure that all current passes through the cell monolayer. This allows the ability to analyze data with the ECIS® modeling software to determine barrier function, cell membrane capacitance as well as the spacing between the cell basal membrane and electrode.

Keeping the total surface area of the electrodes small also allows for a relatively low AC current to generate the large electric field necessary to either electroporate or kill the cells in migration experiments.

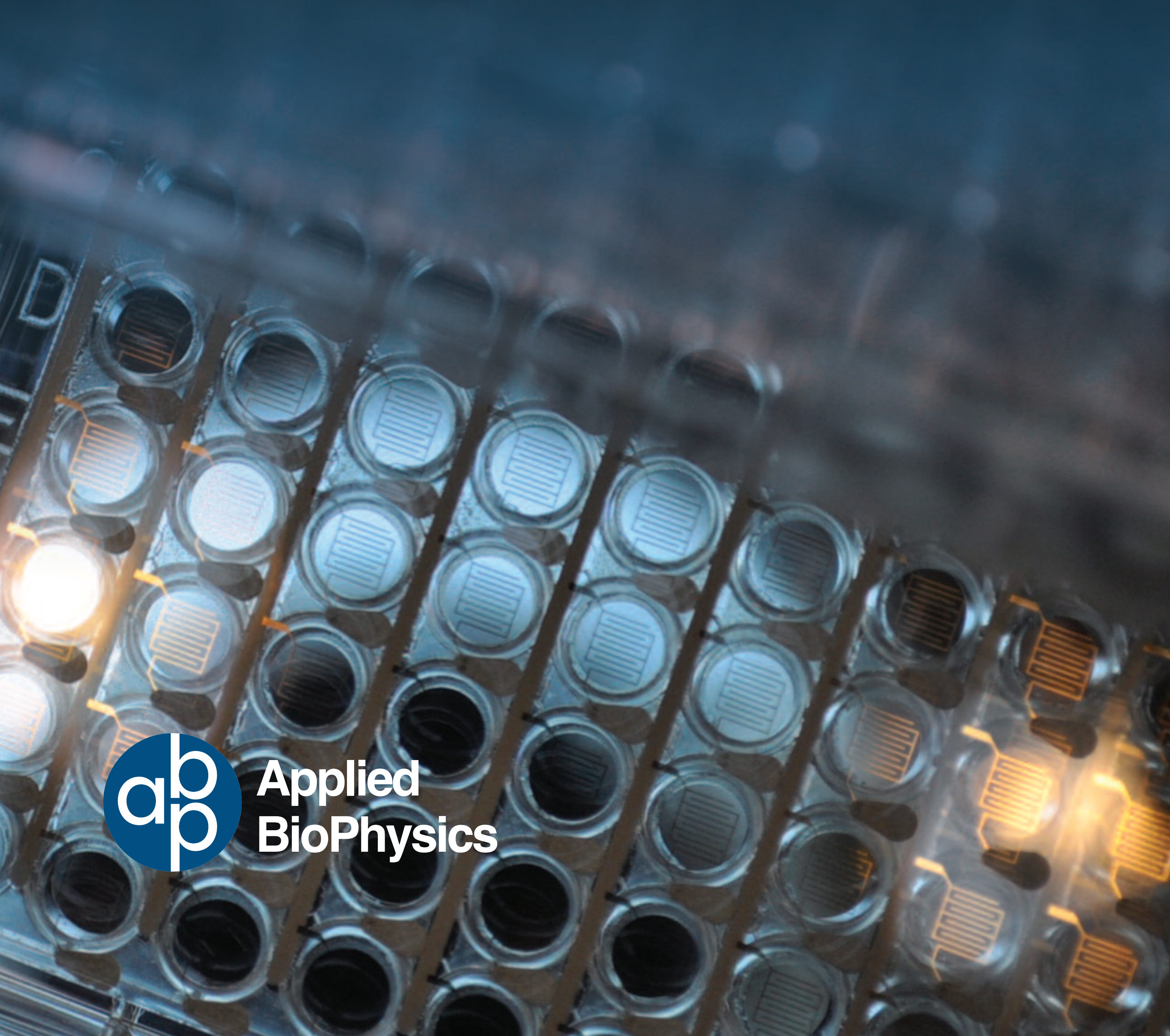
Small electrodes also provide the ability to monitor the uncorrelated nano-scale morphological changes of individual or small populations of cells (<100), while larger or multiple electrodes provide the averaged morphological response of many cells (1000+).

Large Electrodes

Some experimental protocols, such as cell proliferation, require sparse inoculations leading to a variance of cell density at the bottom of the well. Large electrodes (CP Array) or a large collection of small electrodes (10E+ Array) increases the sampling size resulting in less variability.

2 applications

By quantifying cell behavior ECIS® characterizes the life cycle of the cell: attachment and spreading of cells, growth into a confluent monolayer, the dynamics of the monolayer, reactions to stimuli, and finally cell death.



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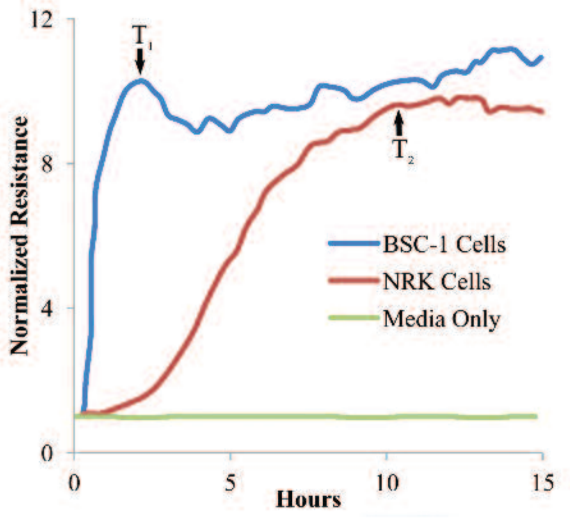
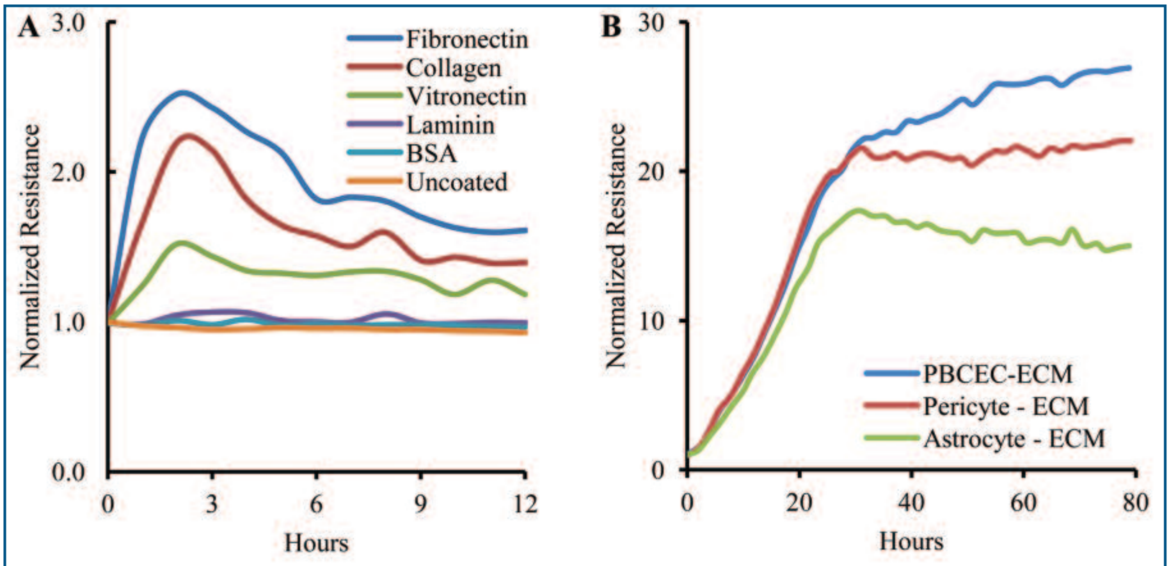
ATTACHMENT AND SPREADING



ECIS® graph of the attachment and spreading of BSC-1 and NRK cells onto ECIS 1E type electrodes. Cells were seeded at density of 105 cells/cm2, and a confluent monolayer was achieved at T1 and T2 for BSC-1 and NRK cells respectively. The fluctuations that exist after these time points are due to nano-scale cellular activity.

ECIS® experiments start with freshly seeded cells attaching to the ECIS® electrodes at the bottom of the wells. As cells attach to the surface they begin to spread, increasing the amount of cell area in contact with the ECIS® electrode and the measured impedance. ECIS® provides a continuous real-time measurement of this process, quantifying both the rate of change and the final value of the impedance. Published examples of attachment assays include dependencies upon ECM proteins, genetic manipulation of junction and signal transduction proteins, and binding competition with anti-integrin antibodies or the tetrapeptide RGDS.

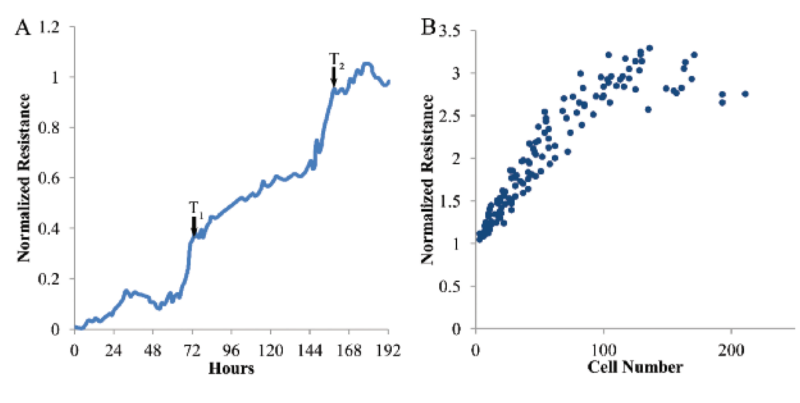
Traditional "counting attached cells assays" can only quantify the number of cells attached to any ECM coating. ECIS® assays give feedback on the strength of the attachment of the cells to the ECM. The transparent nature of the electrode mean results can be normalized to resistance per cell or per cell area by means of optical inspection. Longer term assays can probe not just the effect of ECM proteins on attachment, but functional consequences of ECM derived from different sources (see B below).



Graphs depicting the attachment and spreading dynamics of (A) renal vascular smooth muscle cells and (B) porcine brain capillary endothelial cells. In (A) ECIS® arrays were pretreated with the indicated matrix proteins prior to cell seeding. In (B) prior to recording, pericytes, astrocytes, or cerebral endothelial cells were allowed to grow to confluency in ECIS® arrays. The cells were then removed by trypsinization and porcine brain endothelial cells introduced to the wells and their attachment and spreading dynamics were measured. Data of (A) derived from Balasubramanian, L. et al., 2008 Am. J. Physiol. Cell Ph. 295:C954 and (B) from Hartmann, C. et al., 2007 Exp. Cell Res. 313:1318.

CELL PROLIFERATION

As cells proliferate two factors act to change the impedance: cell number and cell morphology. In most instances the cells grow asynchronously and the impedance gradually increases until a maximum when cells become confluent. The impedance change is approximately linear with cell number while the cells are sub-confluent. If the cells are synchronized, then the progression of cell morphological changes associated with the cell cycle can be visualized as a series of peaks and valleys in the impedance plot. Compounds affecting cell growth can be introduced before or after cells have attached to distinguish changes in growth from the ability of cells to attach to the substrate.

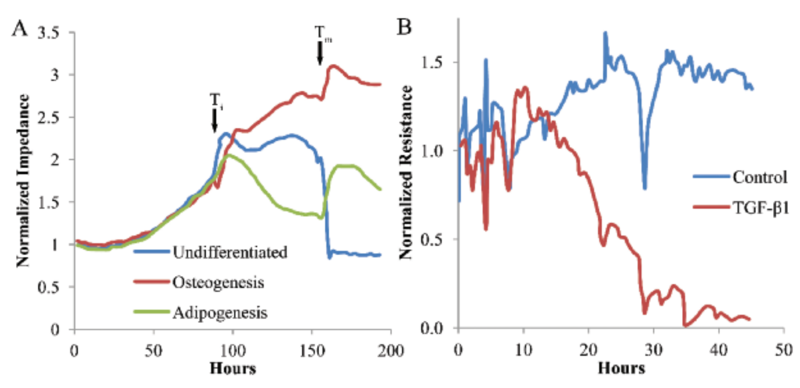


ECIS® graphs of cell growth and the relationship between measured resistance and cell numbers. In (A) primary brain micro capillary endothelal cells are seeded at time zero and continously measured over the ensuing week. Plataus indicated at T1 and T2 represent time points at which the cell population has doubled. (B) A linear correlation of resistance with the number of cells on the electrode exists below a saturation density of approximately 100 cells per electrode. Data derived Bernas, M.J. et al., 2010 Nat. Protocols 5:1265 (A) and from Xiao, C. & Luong, J.H.T., 2003 Biotechnol. Progr. 19:1000

DIFFERENTIATION AND STEM CELL BIOLOGY

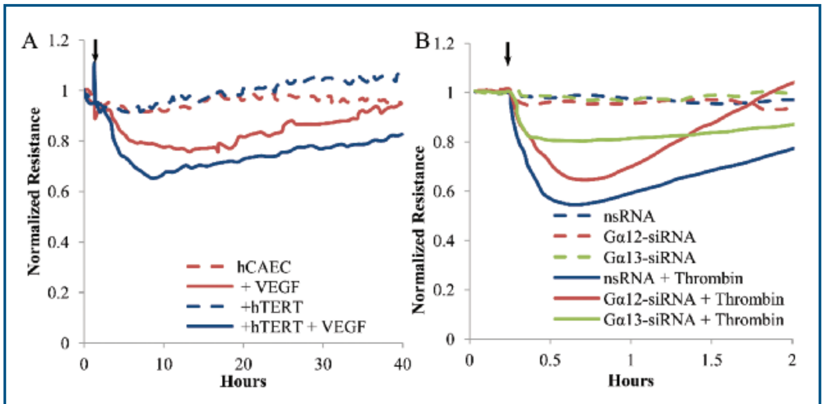
When cells differentiate they change their behavior allowing ECIS® to follow the events of cell differentiation. While most tools available to characterize stem cells preclude their further use, the label-free non-invasive nature of ECIS® allows for subsequent use of characterized stem cells. Thus stem cells can be selected for functional abilities before their use in research or therapy.

This ability to characterize cells by their function allows ECIS® to be a powerful tool in phenotypic drug discovery. It use is not limited to changes in phenotypes but in also assuring the quality of cell stocks or assurance that differentiated cells have not reverted



ECIS® graph of differentiating cells. In (A) adipose-derived stem cells are monitored before and after differentiation is induced at T1. The distinct differences between the differentiated cell types persists beyond the first media change at Tm. In (B) NMuMG (murine mammary gland) cells are monitored after the addition of 5ng/ml TGF-β1 (red line) at 0 hours. The decline in normalized resistance between 10 and 30 hours correlates with the transition of the NMuMG cells from having endothelial characteristics to mesenchymal. Data derived from Bagnaninchi, P.O. & Drummond, N., 2011 PNAS 108:6462 (A) and Schneider, D., et al., 2011 BBA 1813:2099 (B).

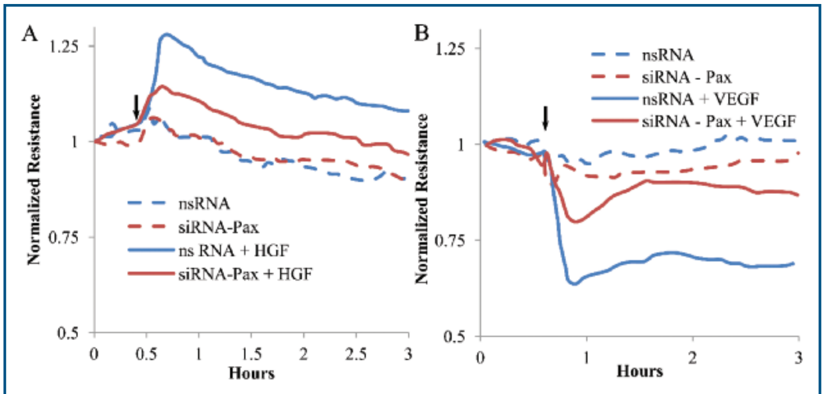
BARRIER FUNCTION



ECIS® graphs of barrier function in HCAEC (A) and human pulmonary aortic endothelial cells (B). In (A) over-expression of human telomerase reverse transcriptase enhances the ability of VEGF to reduce barrier function in human coronary artery endothelial cells. In (B) HPAEC were incubated with siRNA to Gα12 and Gα13 or treated with nonspecific RNA and were stimulated with thrombin (50 nM) at the time marked by the arrow. Data derived from Baumer, Y. et al., 2011 Exp. Bio. Med. 236:692 (A) and (B) Birukova, A. et al., 2004 FASEB J. 18:1879

Epithelial cells and endothelial cells regulate the passage of molecules across cell layers. Diseases, especially vascular disease, occur when this function is impaired. Passage of molecules across an endothelial or epithelial cell layer occurs in two ways; actively by transport through the cell or passively by diffusion in the para-cellular space. ECIS® measurements of the resistive portion of impedance at frequencies below 5kHz are very sensitive to changes in the barrier function of these cell types. ECIS® has been used to demonstrate the effects of many regulating molecules including VEGF, thrombin, TNFalpha, and sphingosine-1-phosphate.

SIGNAL TRANSDUCTION

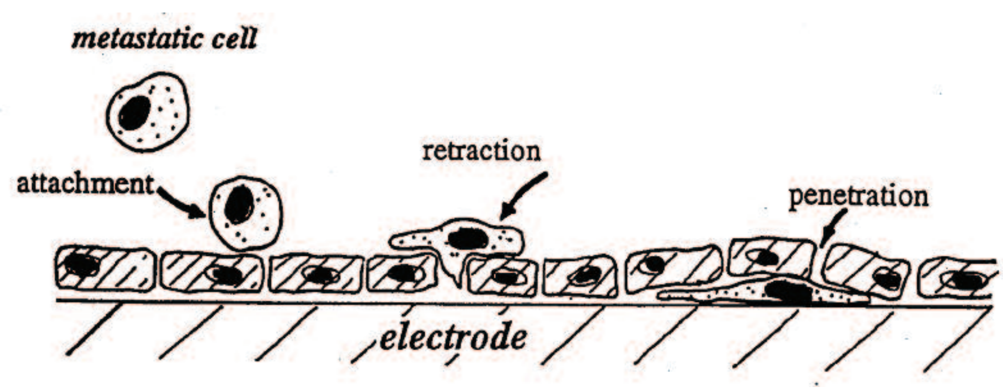


ECIS® graphs for studying the intersecting signal transduction pathways of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in pulmonary endothelial cells. (A) The barrier enhancing effect of HGF (added at arrow) is attenuated by the loss of paxcillin due to siRNA knockdown. (B) Barrier loss due to VEGF addition (arrow) is also attenuated by the loss of paxcillin due to siRNA. Data derived from Birukova, A. et al., 2009 Am. J. Resp. Cell Mol.40:99

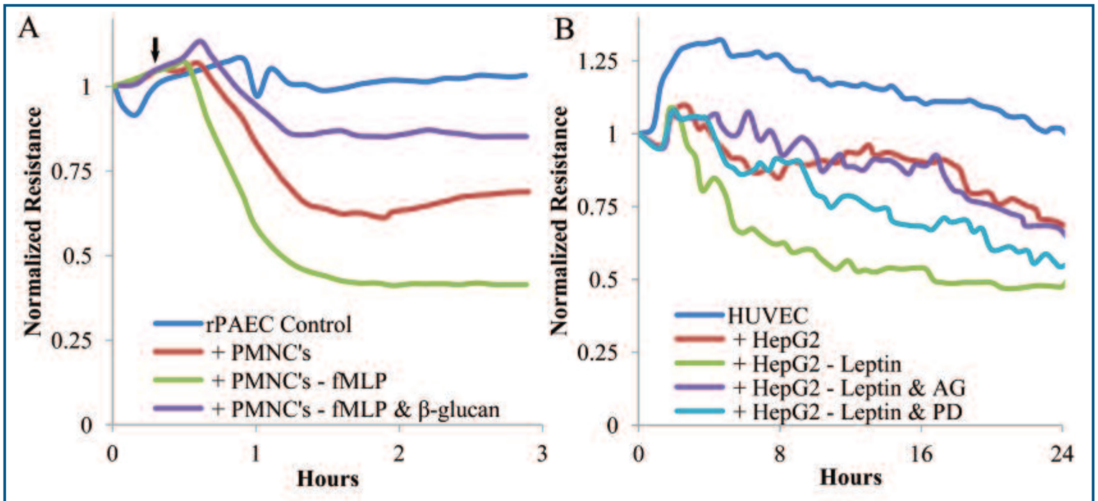
Many compounds of interest are those molecules that specifically bind to cell-surface receptors. When ligands bind to their receptors they initiate signal transduction pathways resulting in a cellular response. ECIS® monitors cellular responses to ligands directly, avoiding false positives, providing reliable information regarding the efficacy of compounds. ECIS® is especially useful to monitor the signal transduction pathways activated by G protein coupled receptors (GPCR). GPCR activation, regardless of the second messenger, results in alterations of the cell's cytoskeletal elements, causing morphological changes. This is precisely the type of cell behavior detected in real time and with great sensitivity by ECIS®.

CELL INVASION

By quantifying cell behavior, ECIS® can give new insight into how invasive cells and pathogens cross endothelial and epithelial monolayers. By simultaneously monitoring both barrier function and cell viability, ECIS® can distinguish between transmigration mechanisms that leave the monolayer intact from those that disrupt the cell layer. Published examples include metastatic cell and leukocyte trans-endothelial migration, as well as the migration of pathogens such as yeast, anthrax, streptococcus, plasmodium, trypanosomes, and spirochetes.



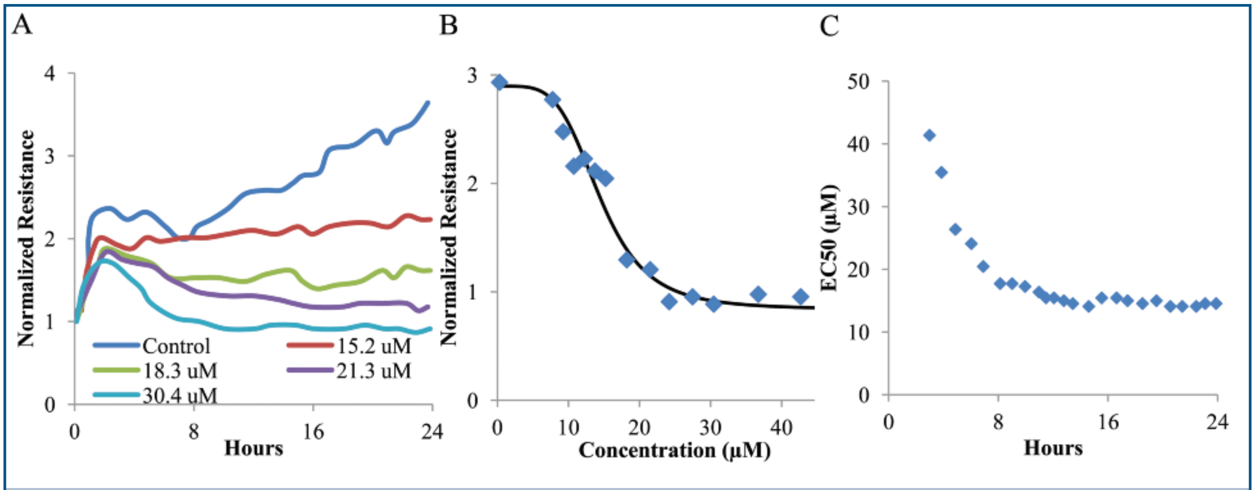
A drawing depicting the extravasation process as observed in tissue culture. A layer of normal endothelial cells is disturbed invading metastatic cells.



ECIS® graphs monitoring the response of confluent rat pulmonary aortic endothelial cells (A) or human umbilical vein endothelial cells (B) after being challenged by non-adherent cells. In (A) peripheral mononuclear cells are untreated, treated with the chemoattractant fMLP, or treated with fMLP and β-glucan and then added to the confluent rPAEC layer (arrow). In (B) HepG2 hepato-carcinoma cells are untreated, treated with leptin or cotreated with leptin and the JAK/STAT inhibitor AG490 (Calbiochem) or the MAPK inhibitor PD098059 (Sigma). After treatment, the carcinoma cells are added onto the confluent HUVEC layer just prior to recording. Data from (A) Tsikitis, V.L., et al., 2004 J. Immun. 173:1284 and (B) Saxena, N.K. et al., 2007, Cancer Res. 67:2497.

CELL TOXICITY

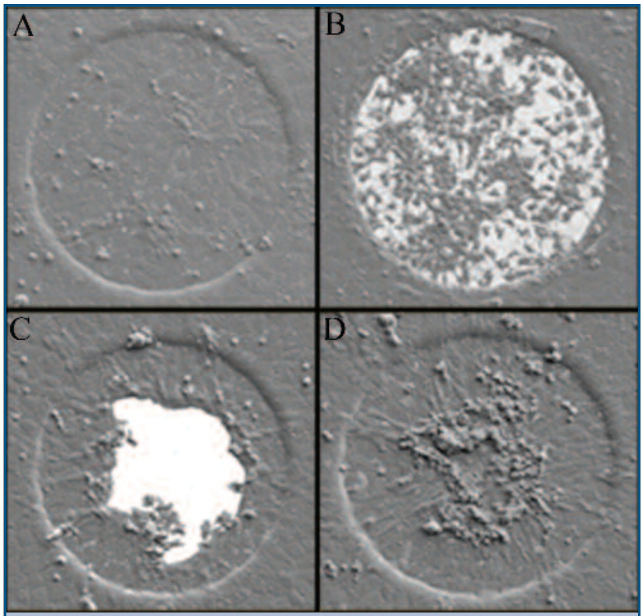
Traditionally, toxicity assessments involve animal studies that are both time-intensive and costly. The ECIS® system has been used specifically to assess the cytotoxicity of a variety of toxicants. ECIS-based toxicity tests are far superior to simple cell death assays, because cell function is also monitored. This is important as a change in cell function is not necessarily a cytotoxic event but could be toxic to the individual organism. Cells can be treated in suspension with a toxicant, and then their ability to adhere and grow on the ECIS® electrodes can be monitored. Alternatively, the impedance of established cell monolayers can be monitored after toxicant addition. Analysis of cell micromotion has been shown to enhance the detection of some toxicants by a factor of 10.



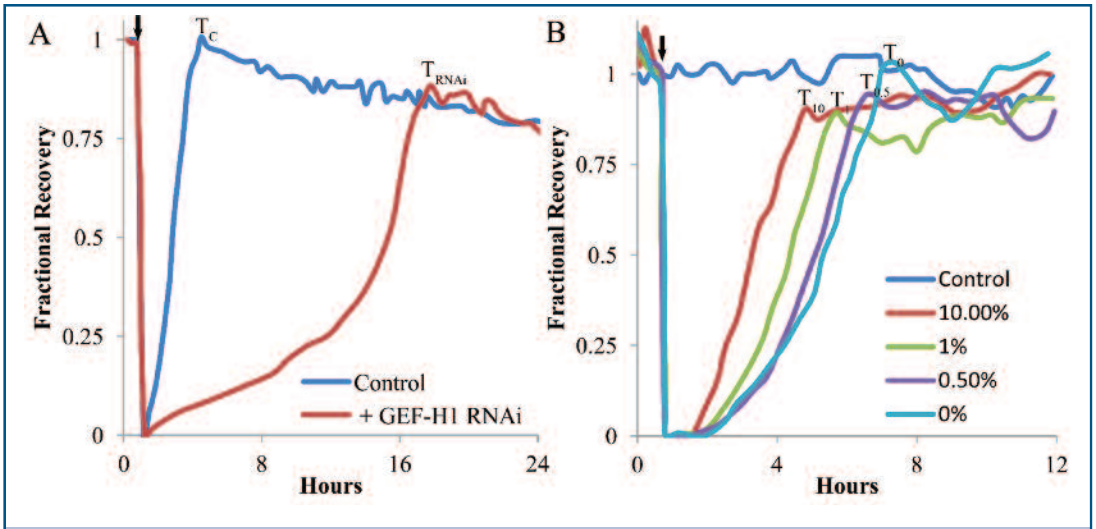
Generation of time dependent EC50 curve of benzalkonium chloride toxicity in V79 cells. In (A) ECIS® curves of fibroblastic V79 cells treated with increasing concentrations benzalkonium chloride. (B) Hill plot of ECIS® response vs concentration evaluated at 16 hours after the start of the experiment. (C) Plot of EC50 concentrations as a function of time after the calculation of EC50 at a series of timepoints. Data derived from Xiao, C. et al., 2002 Anal. Chem. 74:5748

AUTOMATED CELL MIGRATION

ECIS instruments include an elevated field mode allowing for electroporation and wounding. The ECIS® wound is precisely defined, as it includes only those cells on the electrode. Additionally, with ECIS® the ECM protein coating is not scraped off and is unaffected by the current.

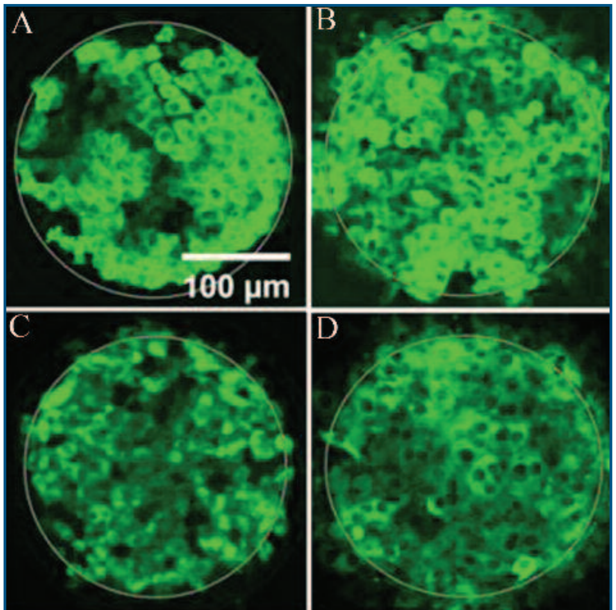


Scanning electron micrographs of the ECIS electrode at time points just prior to (A), just after (B), 4 hours after (C), and 8 hours after (D) the application of a high field pulse across the ECIS electrodes.



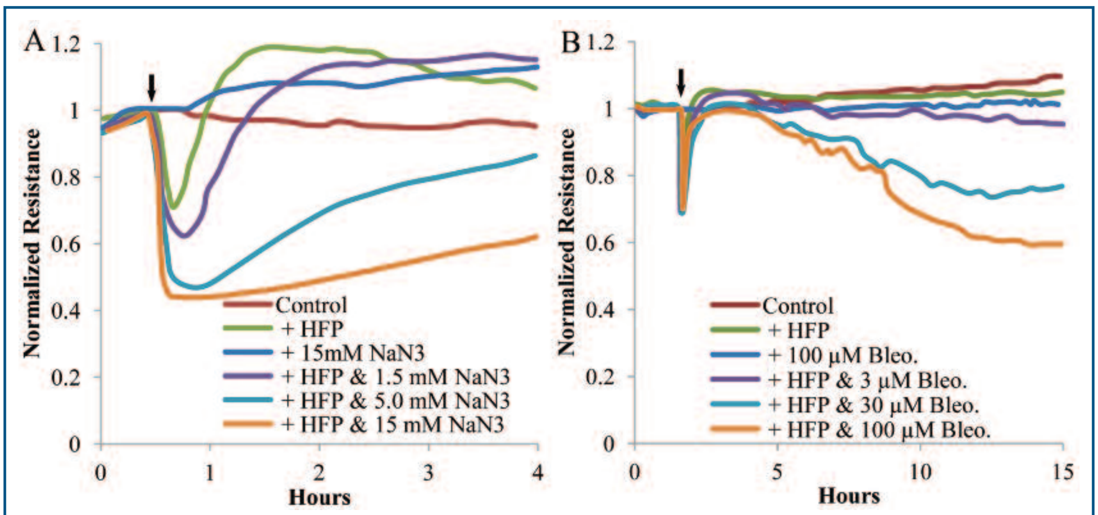
ECIS® graphs of recovery after wounding by applying a high field pulse across the ECIS® electrodes (arrows). In (A) retinal pigment epithelial cells lacking the guanine nucleotide exchange factor GEF-H1 show a prolonged recovery time (TRNAi) as compared to their non-treated controls (TC). In (B) BSC-1 cells were grown to confluency, and 20 hours prior to the experiment the complete media was replaced with media containing the indicated amount of serum. Recovery times (T10,T1,T0.5,T0) were inversely correlated with amount of serum. Control cells were maintained in 10% serum and did not receive a high field pulse. Data derived from (A) Tsapara, A. et al., 2010 Mol. Bio. Cell 21:860 and (B) Keese, C.R., et al., 2004 PNAS 101:1554.

IN SITU ELECTROPORATION & MONITORING



Direct flourescent imaging of the ECIS® electrode after 250kD FITC-labeled Dextrans were introduced into (A) NRK, (B) HEK-293, (C) CHO, and (D) NIH-3T3 cells by ECIS® in situ electroporation.

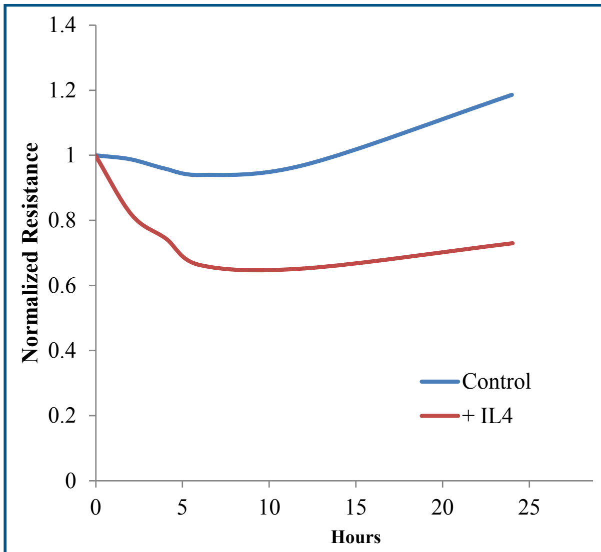
ECIS® instruments include an elevated field mode allowing for electroporation and wounding. ECIS® in situ electroporation of adherent cells is more convenient and less disturbing for anchorage-dependent cells than standard electroporation techniques. In addition, it offers the added benefit that the cellular response can be studied immediately after pulsing. Membrane-impermeable toxins, inhibitors or other bioactive compounds can be introduced into the cytoplasm of adherent cells. Monitoring the cell response in real-time, from minutes to weeks, opens up new strategies to study such complex processes as signal transduction, cell differentiation, cell proliferation and apoptosis.

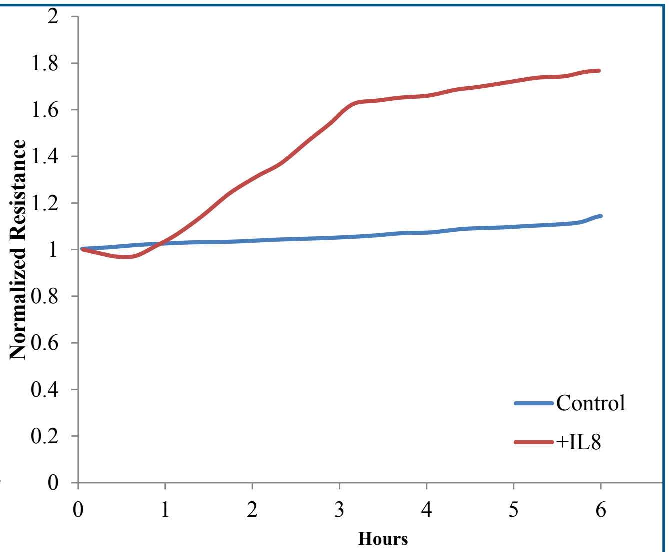


NRK cells incubated with different concentrations of sodium azide (A) or bleomycin (B) at the concentrations indicated and subject to in situ electroporation (Arrowhead) by a high field pulse (HFP). Control cells remained entirely untreated, whereas +HFP cells were electroporated only. A third control set was incubated with the highest concentration of toxin but never electroporated. Derived from Stolwijk, J.A. et al., 2011 Biosens. Bioelectron. 26:4720

INFLAMMATION

Inflammation is the body's response to pathogens, adverse stimuli such as toxins or ischemia, and physical injury. During an inflammatory response cytokines and interleukins are secreted to guide immunological cells to the site of infection or wounding. Key targets of these molecules are epithelial and endothelial cells which are often the site of injury or need to allow the passage of immune cells to the site of injury. Acute inflammation is generally healing in nature and generally understood. Chronic inflammation is the lack of proper immune regulation and is the core dysfunction for many diseases, including asthma, arthritis, inflammatory bowel disease, cancer, and allergies. Chronic inflammation and its causes are less well understood. ECIS offers a number of cell based assays used to study the inflammatory process. ECIS recovery-after-wounding assays allow for the discovery of molecules which aid in the process of tissue repair. ECIS barrier function assays specifically measure the response of epithelial and endothelial cells to secreted cytokines and can give indirect information about the binding of immune cells to the epithelium or endothelium. Also our newly introduced trans-Filter adapters can be used to study the 3D migration of cells across a Matrigel® layer.



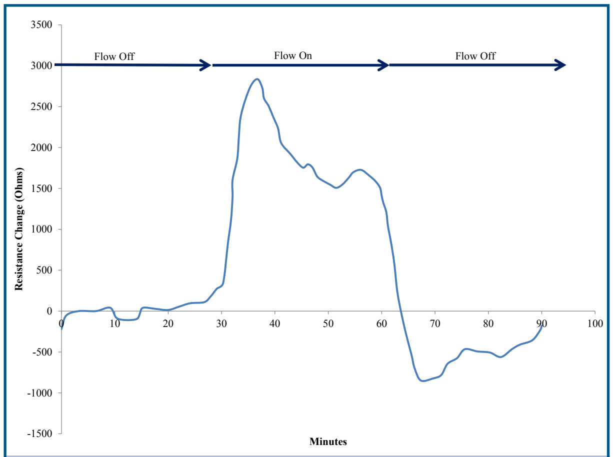


ECIS assays for immunology. In an ECIS barrier function assay (A) bronchial airway epithelial cells respond to the Th2 cytokine il-4 by a drop in resistance. In an ECIS recovery-after-wounding assay(B) keratinocytes demonstrate a faster rate of recovery when treated with the cytokine il-8. Data from (A) derived from Ramirez-Icaza, G. et al. 2004 J. Clin. Immunol. 24:426 and (B) from Jiang, W. G., et al. 2012 Exp. Ther. Med. 3:231.

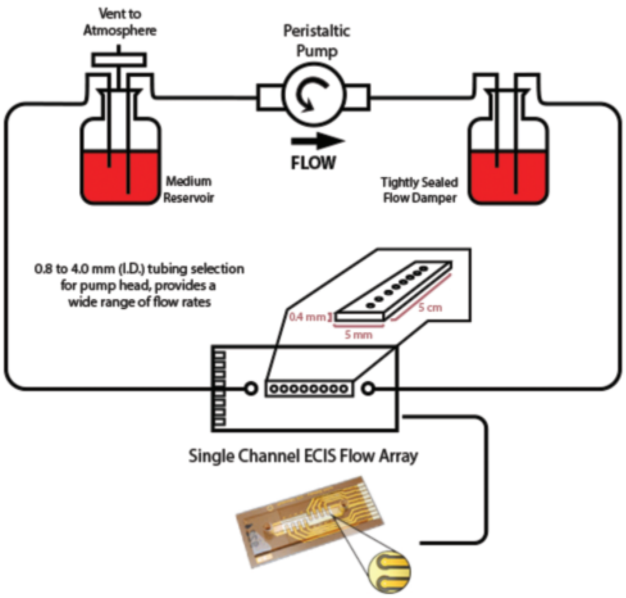
FLOW BASED ASSAYS

In their natural environment, endothelial cells are constantly exposed to physical and biochemical stimuli that can alter cell permeability. Laminar shear stress due to blood flow is a principal regulator of systemic endothelial cell gene expression, morphology, and the production of soluble mediators. Its importance is highlighted by pathological processes associated with reduced or absent laminar shear stress, including atherosclerosis. Endothelial transport of macromolecules has been shown to be responsive to flow shear stress, hydrostatic pressure, thermal shock, and agonists such as histamine and thrombin. The ECIS pFlow solutions combines different pump technologies with ECIS flow arrays to allow researchers to study endothelial permeability in vitro under complex shear flow conditions.

The pFlow systems use peristaltic pumps to create shear stress conditions within ECIS flow arrays. The ECIS system then monitors continuously the TEER of cell monolayers exposed the shear stress conditions. This allows for the dynamic changes in TEER to be recorded due to changes in flow rates, addition of vasoactive compounds under flow conditions, or the introduction of secondary cells. Stock ECIS flow arrays create laminar shear stress across the cells. Turbulent flow can be created by customizing the ECIS flow arrays at reasonable costs.



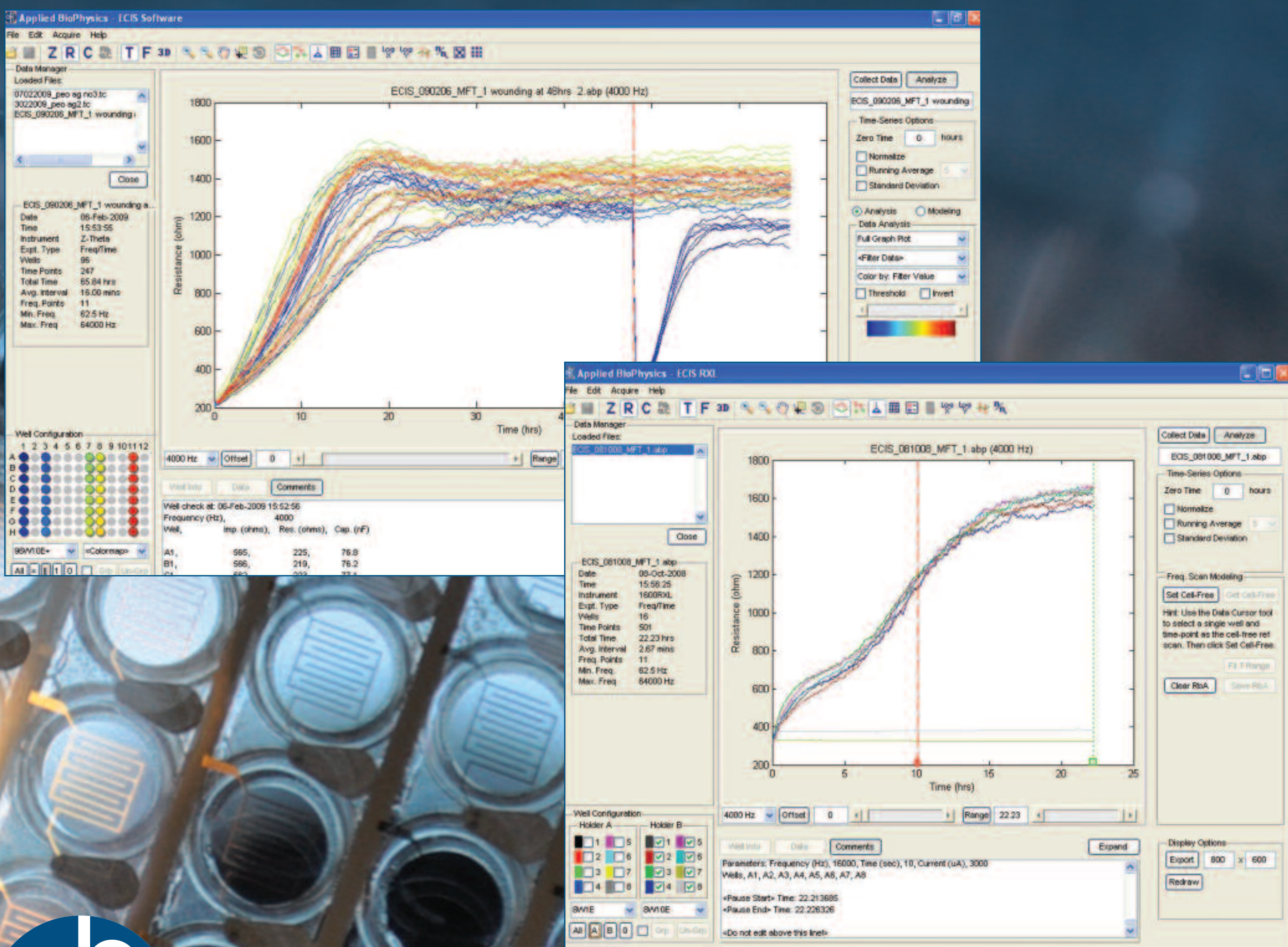
Step increases in fluid shear stress cause rapid enhancement of barrier function in lymphatic endothelial cell monolayers. Changes in TER (y-axis) of HMLEC-d monolayers were recorded under no-flow conditions for 30 minutes, after which a step increase in laminar flow was applied, generating a shear stress of 10 dynes=cm2. The flow was then turned off after 30 min and changes in TER recorded for another 30 minutes under no-flow conditions. A rapid increase in TER occurred after the step increase was applied. TER then gradually decreased but remained elevated until the flow was turned off, which caused a rapid drop. Data taken from Breslin, J.



3 instruments and accessories

From our all-in-one system to our more function-specific systems, the ECIS line of instruments include different models that range in performance and capability.

Applied BioPhysics has developed a number of accessories that integrate with the ECIS® line of instruments. We offer the following items either directly from Applied BioPhysics or in conjunction with third party vendors.



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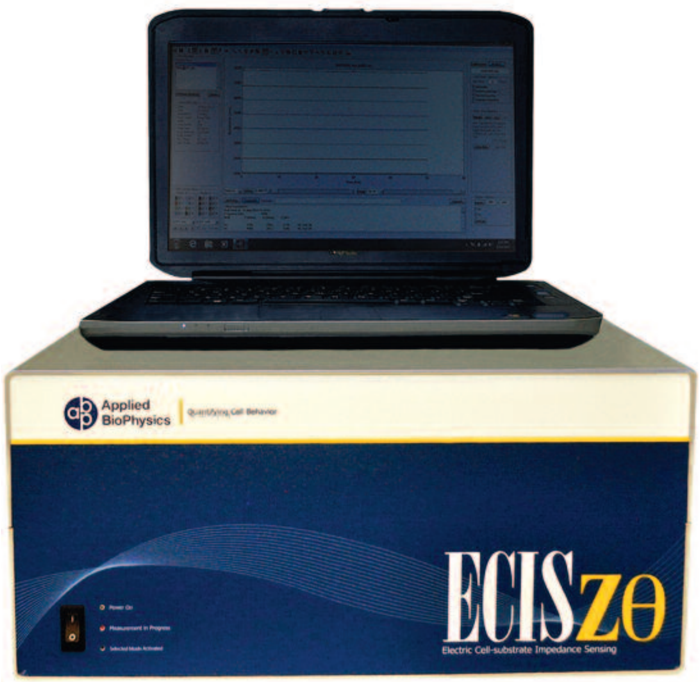


ECIS® ZØ

The ECIS® ZΘ (Z Theta) is our most advanced instrument capable of noninvasively monitoring cell behavior in real-time. The turn-key package consists of a ZΘ System Controller, 16 and/or 96 well Array Station, and computer with integrated software running on Windows. The system also includes our exclusive elevated field mode (EFM) to carry out automated cell migration and electroporation studies.

The ECIS® ZΘ interprets complex impedance as series resistance and capacitance and reports these values at any AC frequency. Data gathered from confluent cell monolayers at multiple AC frequencies can also be mathematically modeled to present time course changes in three parameters related to actual cell morphology. These parameters include changes in the barrier function (permeability) of the confluent layer, averaged apical and basal membrane capacitance and the close contacts between the basal cell membrane and the cell substrate.

System Includes:

- 16 and/or 96 well station located inside CO2 incubator
- External control module
- Laptop PC
- ECIS control, acquisition, and display software
- Elevated field module (EFM) for automated cell migration and electroporation
- Twelve 8-well and/or six 96-well consumable electrode arrays

16 AND 96 WELL ARRAY STATIONS

ECIS® Array Stations connect to the back of the ECIS® Z and ZΘ Station Controllers. They are usually placed inside a tissue culture incubator and have been designed to operate at high humidity conditions.

A flat shielded cable from the Array Station exits the incubator by the rubber seal of the inner glass door or through an incubator port, if available. LED's show the state of the device while looking through the incubator window.

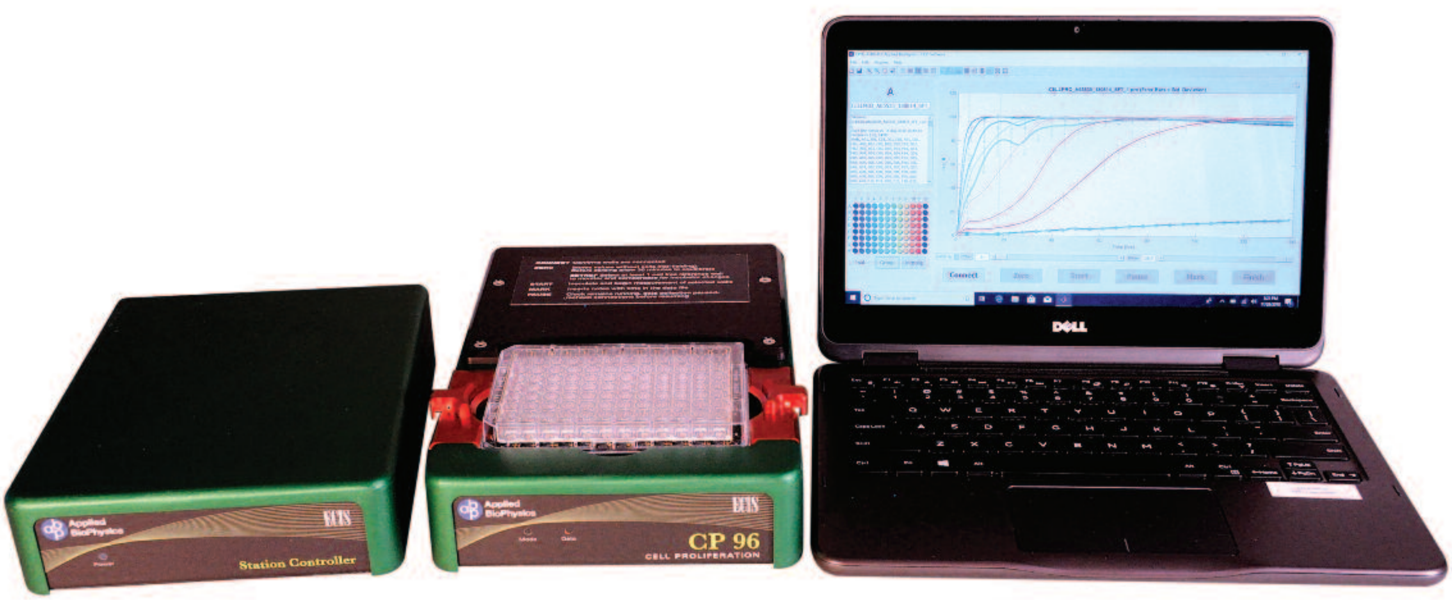
The 16 Well Array Station provides electrical contact for two 8 well ECIS® arrays. The 96 Well Array Station provides electrical contact for a single 96 well ECIS® Array.

TEER24

This system provides repeatable, label free automated TEER measurements to electrically monitor the barrier function of epithelial and endothelial cells as they are grown in normal CO2 tissue culture incubators. Data is collected continuously and reported as real-time changes in barrier function of cell layers in 10-1000 ohm-cm^2.

System Includes:

- TEER 24 station located in CO2 incubator
- Stainless steel plate assembly with 24 gold plated dipping pins
- External control module
- Laptop PC
- ECIS acquisition, control and display software
- Validation test array
- Four TEER24 consumable electrode arrays

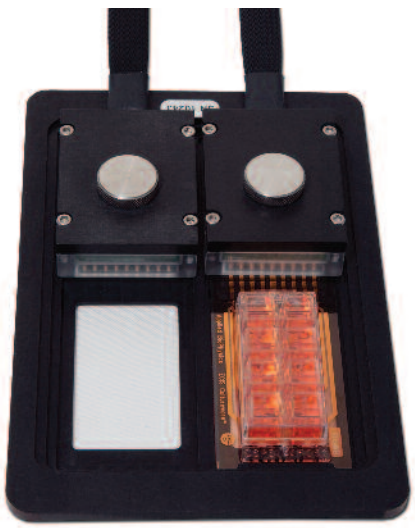


CP96 Cell Proliferation

The complete turn-key CP 96 system provides a means to carry out reproducible, label-free, automated cell proliferation measurements. Cell-proliferation is continuously monitored as cells grow in a normal CO2 tissue culture incubator, and data are reported as real-time changes in percent cell coverage.

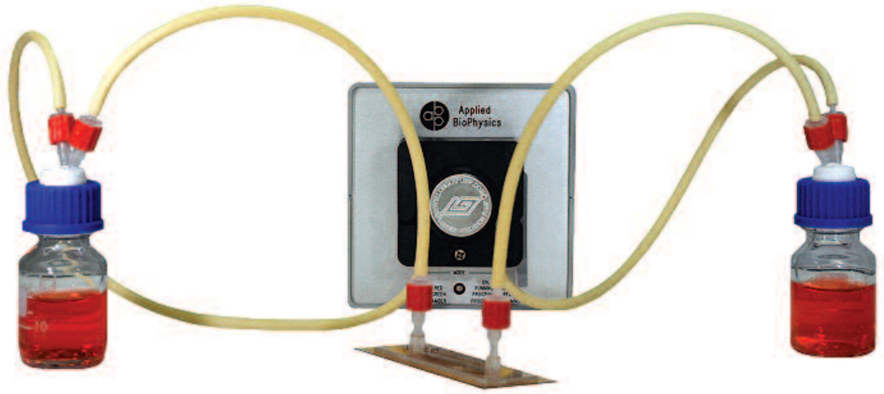
System Includes:

- CP 96 station located in CO2 incubator
- External control module
- Laptop PC
- ECIS acquisition, control and display software
- Validation test array
- Six 96-well consumable electrode arrays



ARRAY STAGE HOLDER

This device allows simultaneous ECIS® and optical measurements but is simply a platform and does not provide a chamber for heating and atmospheric control as with our Stage Incubator. The Array Stage Holder accommodates two 8 well ECIS® arrays (not compatible with ECIS® Flow Arrays) and fits on the stage of an inverted tissue culture microscope.



P-FLOW PERISTALTIC PUMP

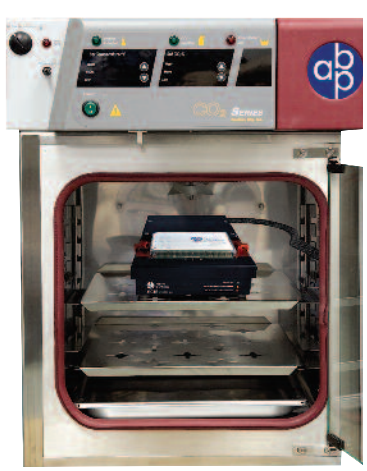
The Model ECIS p-Flow can be controlled manually to adjust flow rate from 0 20ml/min with a minimum flow rate of 70ul/min. Direction of the pump can be adjusted via a toggle switch or via the ECIS software (V 1.2.151 or higher).

The pump can also be controlled automatically from within the ECIS software (V 1.2.150 or higher) using USB or RS232. The pump can be programmed to run continuous or ramp up and down. It can store complex programmed flow profiles and run them disconnected from the PC. Each pump has a unique serial number so multiple pumps can be run from one PC.

For additional application information see Flow Based Assays, page 16.

Performance specifications:

- Maximum Flow Rate with 3mm ID tubing: 20ml/min
- Minimum Flow Rate with 3mm ID tubing: 70ul/min (1.2uL/sec)
- Flow Rate resolution with 3mm ID tubing: 70ul/min from -20ml/min to +20ml/min
- Max ramp rate 0-20ml/min : 150msec (forward or reverse)
- Full forward to full reverse, -20ml/min to +20ml/min : 400msec
- Pump Speed: -60 to +60 RPM stepper driven motor
- Pump Resolution: 1024 increments/rev
- Control Input: USB or RS232



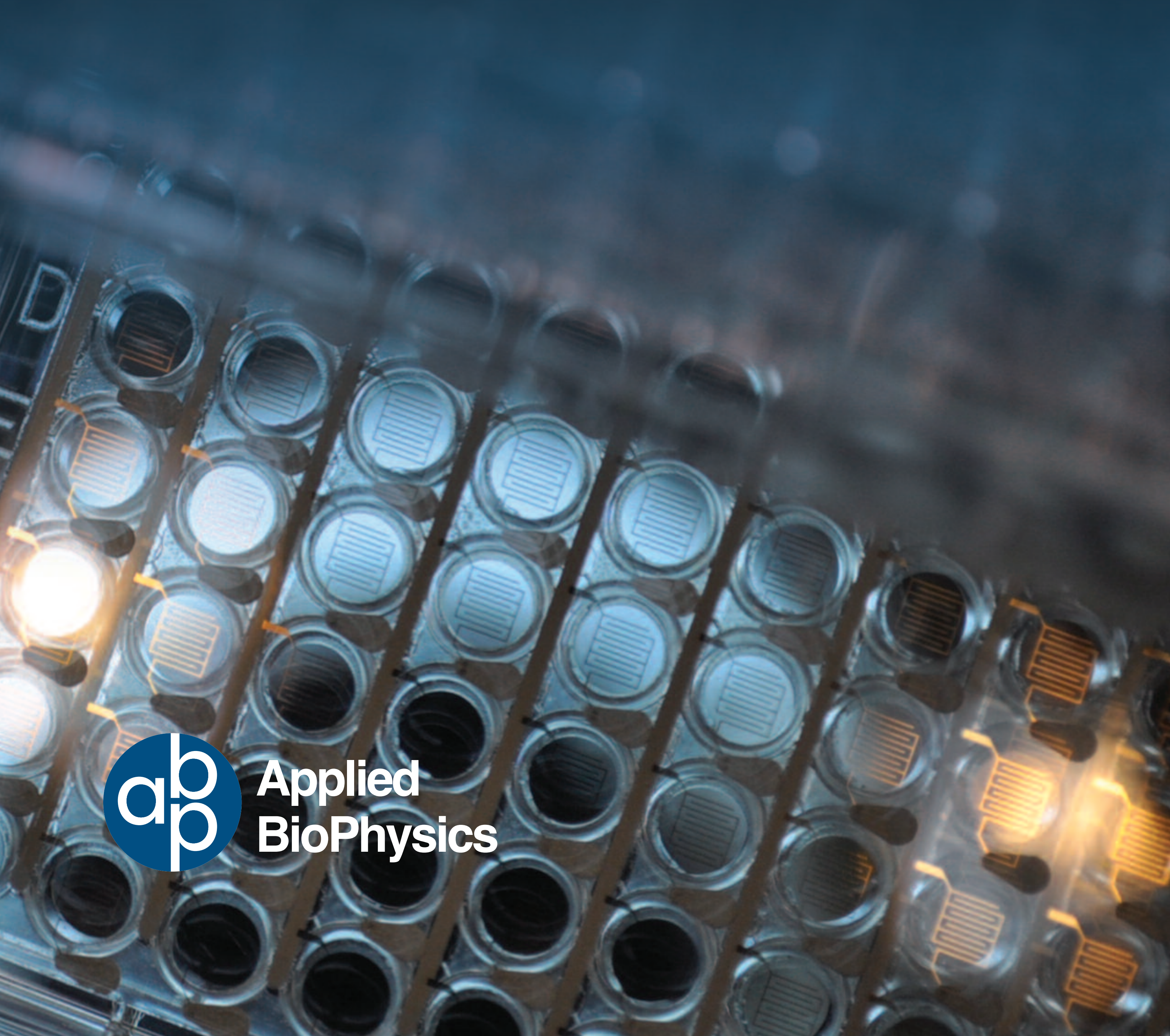
CO_2 TISSUE CULTURE INCUBATOR

The ECIS® array holder is normally located in a tissue culture incubator to provide the normal growth requirements for cells. Since thermal and CO_2 fluctuation affect the behavior of the cells and can show up in ECIS® measurements, we recommend the use of a dedicated incubator that is only opened and closed occasionally during data acquisition.

The incubator has an exterior footprint of approximately 24 × 24 inches (28 inches in height) and a 1.8 cubic foot interior chamber. A port, with a special seal into the chamber, is provided for the ECIS® leads as well as for tubing if one is using the ECIS® flow module.

4 arrays

ECIS® Cultureware consists of patterned gold electrodes on thin plastic films bonded to different well configurations including 8 well arrays, 96 well microtiter plates, or specialized flow arrays from ibidi GmbH. The gold layer is thin enough to allow microscopic observation of the cells using a standard inverted tissue culture microscope



Applied BioPhysics

STANDARD 96 WELL ARRAYS

ARRAY	ELECTRODES PER WELL	ELECTRODE AREA (mm^2)	NUMBER OF CELLS MEASURED WHEN CONFLUENT	WELL VOLUME (µL)
96W1E+	2	0.256	100-200	300
96W10idf	idf	2.09	2000-40000	300
96W20idf	idf	3.985	4000-8000	300

STANDARD 8 WELL ARRAYS

ARRAY	ELECTRODES PER WELL	ELECTRODE AREA (mm^2)	NUMBER OF CELLS MEASURED WHEN CONFLUENT	WELL VOLUME (µL)
8W1E*	1	0.049	50-100	600
8W10E*	10	0.49	500-1000	600
8W10E+*	40	1.96	2000-4000	600
8WCP20idf	idf	3.985	4000-8000	600

SPECIALTY ARRAYS

ARRAY	ELECTRODES PER WELL	ELECTRODE AREA (mm^2)	NUMBER OF CELLS MEASURED WHEN CONFLUENT	WELL VOLUME (µL)
8W2x1E	2x1	2x0.049	50-100	600
8W1CXE	1	0.049	50-100	600
8W2LE	2	0.20	200-400	600
8Wµ1E+	4	0.196	200-400	600
2W4x10E	4x10	4x0.49	2000-4000	600

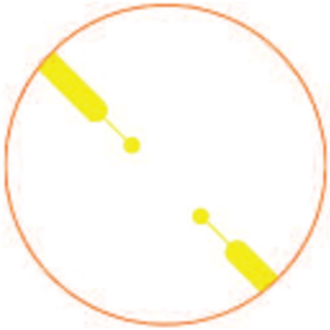
FLOW ARRAYS

ARRAY	ELECTRODES PER WELL	ELECTRODE AREA (mm^2)	NUMBER OF CELLS MEASURED WHEN CONFLUENT	CHANNEL/RESERVOIR VOLUME (µL)	CHANNEL HEIGHT X WIDTH (mm)
1F8x1E	8x1 (1 channel)	0.049	50-100	90/60	0.36 x 5
1F8x10E	8x10 (1 channel)	0.49	500-1000	90/60	0.36 x 5
6F1E	1 (6 channels)	0.049	50-100	45/60	0.66 x 5
6F10E	10 (6 channels)	0.49	500-1000	45/60	0.66 x 5
1F2Yx10E	8x4x2 (30&45 degree sides**)	0.49	500-1000	165/60	0.66 x 5

**four electrodes are equal in size to 8W10E

*available in PET and PC
idf: interdigitated fingers
PET: Polyethylene terephthalate, standard thickness 0.25mm
PC: Clear ploycarbonate substrate, standard thickness 0.51mm or high numerical aperture (HNA) series of 0.13mm
PCB: Printed circuit board, standard thickness 1.55mm

96W STANDARD ARRAYS



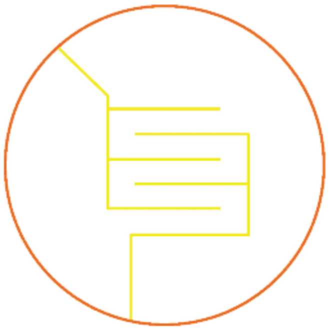
96W1E+

Each of the 96 wells in a standard plate configuration contains two circular 350µm diameter active electrodes on a transparent PET substrate. As with other 1E arrays, a major use of this array is for the ECIS wound-healing assays where the small electrodes assures the high current pulse will result in complete cell killing.

Only a small population of cells is monitored on the small electrodes resulting in a fluctuating impedance signal due to the random like movement of the cells (micromotion).

Recommended Applications:

- Cell Migration
- In situ Cell Electroporation and Monitoring
- Measurement of micromotion
- Signal transduction assays



96W10idf

Each of the 96 wells has an Inter-digitated finger configuration. The total electrode area is 1.96mm^2 which measures 2000-4000 cells.

Recommended Applications:

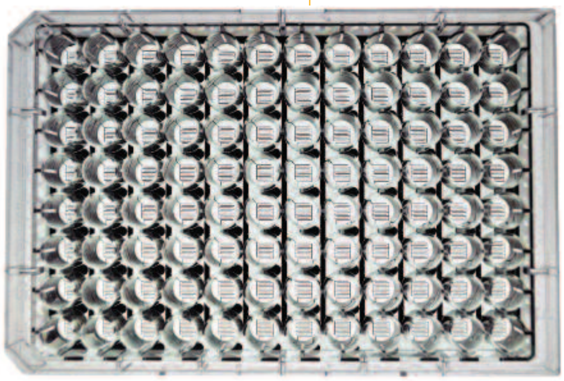
- Signal transduction assays
- Detection of invasion of endothelial cell layers by metastatic cells
- Barrier function
- Cytotoxicity
- Cell differention

96W20idf

Each of the 96 wells has an Inter-digitated finger configuration. The total electrode area is 3.92mm^2 which measures a 4000-8000 cells.

Recommended Applications:

- Cell-ECM protein interactions
- Signal transduction assays
- Detection of invasion of endothelial cell layers by metastatic cells
- Barrier function
- Cell proliferation
- Cytotoxicity
- Cell differention



8W STANDARD ARRAYS

8W1E

Each well contains a single circular 250 µm diameter active electrode. On average, with a confluent cell layer, approximately 50 to 100 cells will be measured by the electrode, but even a single cell can be observed.

Recommended Applications:

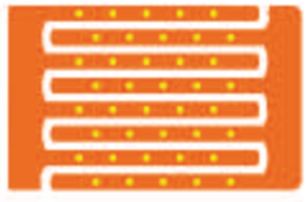
- Signal Transduction
- In situ Cell Electroporation and Monitoring
- Cell Migration / Wound Healing
- Correlated microscopy and ECIS® experiments.

8W10E

Each well contains ten circular 250 µm diameter active electrodes connected in parallel on a common gold pad. On average, with a confluent cell layer, approximately 500 to 1000 cells will be measured by the electrodes.

Recommended Applications:

- Cell Differentiation
- Barrier Function
- Signal Transduction
- Cell Invasion
- Cytotoxicity



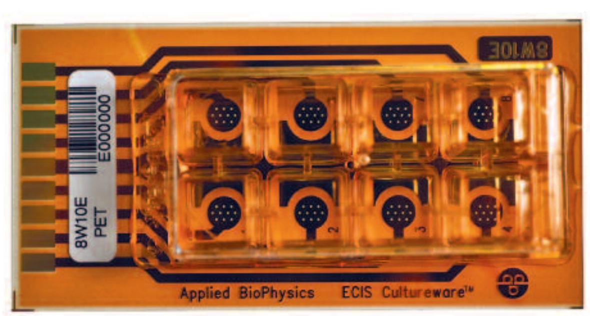
8W10E+

10E+: Each well has two sets of 20 circular 250 µm diameter active electrodes located on inter-digitated fingers to provide measurements of cells upon a total of 40 electrodes. On average, with a confluent layer, approximately 2000 to 4000 cells will be measured by the electrodes.

The 10E+ arrays are designed to monitor larger numbers of cells, sampling over the entire bottom of the well. Because of the relatively high number of cells, impedance fluctuations due to micromotion are largely smoothed out and do not obscure subtle changes in impedance due to the experimental conditions.

Recommended Applications:

- Cell Attachment and Spreading
- Cell Proliferation
- Cell Differentiation
- Cell-ECM Protein Interactions
- Barrier Function
- Signal Transduction
- Cell Invasion
- Cytotoxicity



SPECIALTY ARRAYS

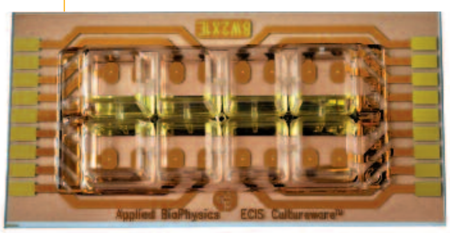
8W2x1E

This array is also called the Medusa array. Each well in this array has two independent single 250 µm diameter active electrodes. The Medusa array is useful for duplicating readings in the same well or to wound/electroporate one electrode while leaving the other as a control within the same well.

When connected to the array holder only the upper four wells are measured. To use the other four wells, the array is turned around and the contact pads at the other end are connected.

Recommended Applications:

- Signal Transduction
- In situ Cell electroporation and Monitoring
- Cell Migration / Wound Healing
- Correlated microscopy and ECIS® experiments.



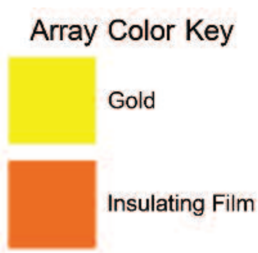
8W20idf

Our special purpose cell proliferation array samples the bottom of the 8 well chamber with our pattered electrodes. As few as 5000 cells per well can be seeded and detected by this array allowing for cell proliferation to be monitored over the course of approximately 5 cell doubling times.

The 8WCP arrays are designed to monitor larger numbers of cells, sampling over the entire bottom of the well. Because of the relatively high number of cells, impedance fluctuations due to micromotion are almost completely smoothed out and do not obscure subtle changes in impedance due to the experimental conditions.

Recommended Applications:

- Cell Attachment and Spreading
- Cell Proliferation
- Cytotoxicity



8W1CXE

Also known as the Chemotaxis array, its use was first described in 2001*. The measuring electrode in this array is a thin gold line 75 µm x 667 µm between two registry marks. The gold line has the same total area as a 250 µm single circular electrode.

In the ECIStaxis assay described by Hadjout et al. the well is flooded with agarose and allowed to polymerize. Once the gel has hardened, wells in the agarose are introduced above the circular registry marks. Chemoattractant is then added in one well, while cells are added to the other. The single electrode is sensitive enough to detect the migration of single cells.

*by Hadjout, N. et al. (2001) **Biotechniques** 31 (5) 1130.

Recommended Applications:

- Cell Chemotaxis

SPECIALTY ARRAYS

8W2LE

Each of the 8 wells contains two linear electrodes with dimensions of 667µm x 150µm in series. Each well thus has an electrode area four times that of our standard 250µm circular electrodes but retains the same impedance values. These arrays were designed for cell migration measurements in which rectangular cell-free areas are generated for direct comparisons with traditional scratch assays.

Recommended Applications:

- Cell Migration / Wound Healing
- Correlated microscopy and ECIS® experiments

8Wµ1E+

Each of the 8 wells contains four 250µm circular electrodes. The placement of the electrodes at the center of the well allows for the use of cloning cylinders to be placed around the electrodes creating micro-wells. The area outside of the cloning cylinder can then be flooded to reduce evaporation from within the micro-wells.

Recommended Applications:

- Barrier
- Signal Transduction
- Cell Invasion
- In situ Cell electroporation and Monitoring
- Cell Migration / Wound Healing
- Correlated microscopy and ECIS® experiments

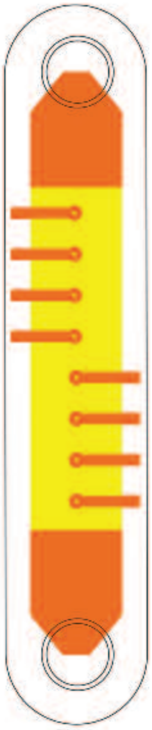
2W4x10E

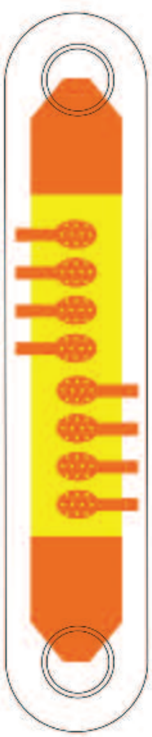
Each of the 2 circular 25 mm diameter wells contains four independent sets of ten 250 µm diameter active electrodes. This array was specifically designed for hypoxia studies to create a large liquid – air interafce for rapid gas exchange. It's design also incoporates a small central area devoid of gold or photoresist allowing for live cell flouresence microscopy.

Recommended Applications:

- Cell Attachment and Spreading
- Cell Proliferation
- Cell Differentiation
- Barrier Function
- Signal Transduction
- Cell Invasion
- Cytotoxicity
- Correlated microscopy and ECIS® experiments

FLOW ARRAYS





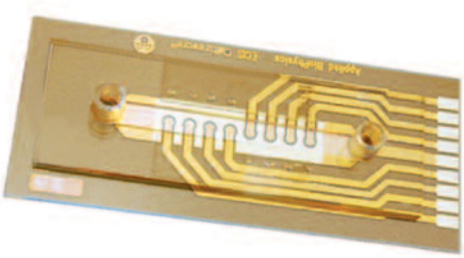
1F8x1E and 1F8x10E

These are specialized Flow arrays with the 1F8x1E having 8 active 250 µm diameter electrodes, and the 1F8x10E having 8 sets of 10 active 250 µm diameter electrodes located in the central region at the base of a flow channel measuring 50 mm in length 5 mm in width and available in 0.36 mm in height with a total channel volume of 90 µL.

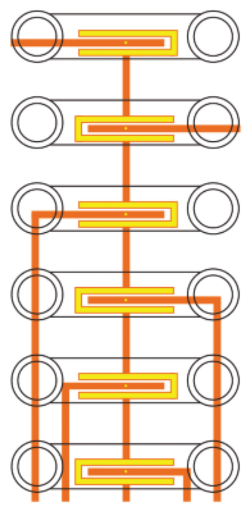
Our flow arrays are designed for ECIS® measurements of cells under perfused conditions or under flow mimicking the shear stress endothelial cells experience in vivo.

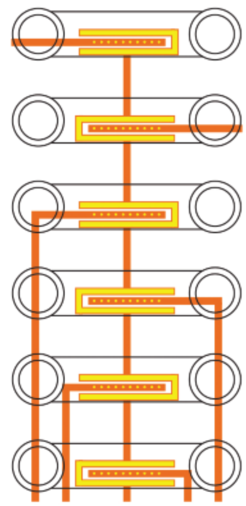
Recommended for the following applications under shear stress conditions:

- Barrier Function
- Signal Transduction
- Cell Invasion
- In situ Cell electroporation and Monitoring
- Cell Migration / Wound Healing
- Cell Proliferation
- Cell Differentiation
- Barrier Function
- Cytotoxicity



Luer lock connection



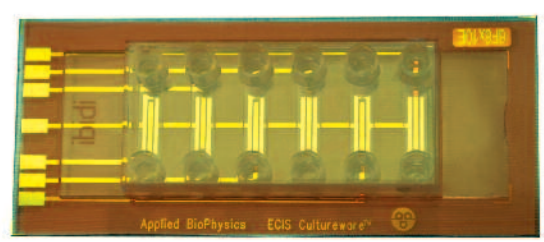


6F1E and 6F10E PC

This flow array allows 6 independent flow assays to be run simultaneously. The channels are 0.66mm in height and 5mm wide with either 1 or 10 active 250um diameter electrodes per channel. Each channel has a 45µL volume with 60µL reservoirs.

Recommended for the following applications under shear stress conditions:

- Barrier Function
- Signal Transduction
- Cell Invasion
- In situ Cell electroporation and Monitoring
- Cell Migration / Wound Healing
- Cell Proliferation
- Cell Differentiation
- Barrier Function
- Cytotoxicity



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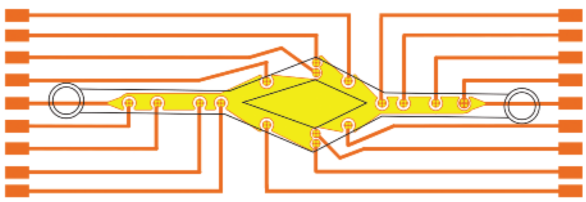
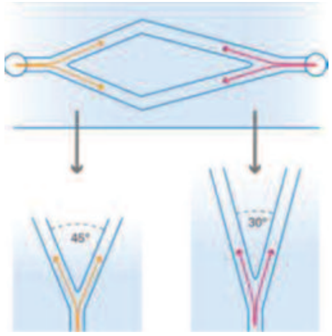


1F2Yx10E PC

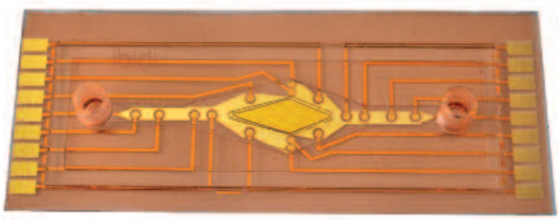


This flow array is intended for bifurcation studies and blood vessel simulation. It splits into 30 degree Y channels in one direction and 45 degree Y channel in the other direction.

This array is double ended with 8 measurement channels available at each end . Eight measurement points, each with 4 circular active electrodes (which are equal in area to an 8W10E), are located along the channel and through the Y portion of the channel. One end of the array is used to monitor the 30 degree Y channel and the other end is used to monitor the 45 degree Y channel. The electrodes are located close in the corners of the flow direction transition points. Each channel has a 165µL volume with 60µL reservoirs. The flow is always laminar, i.e., turbulent flows are not possible. For simulation of turbulence flow we recommend oscillating the flow. Defined shear stress and shear rate levels.

Recommended for the following applications under shear stress conditions:

- Simulation of the bifurcation of blood vessels for arteriosclerosis research
- Rolling and adhesion of leukocytes on endothelial cells cultured under flow
- Cell-cell interaction studies and cell-drug interaction screenings under flow conditions



Luer lock connection

CUSTOM ARRAYS

If you are interested in other electrode sizes and configurations please contact us. We will be happy to work with you on your specific needs.

HOW TO ORDER ARRAYS

We maintain a supply of arrays for shipment from our facility. The arrays are shipped sterile.

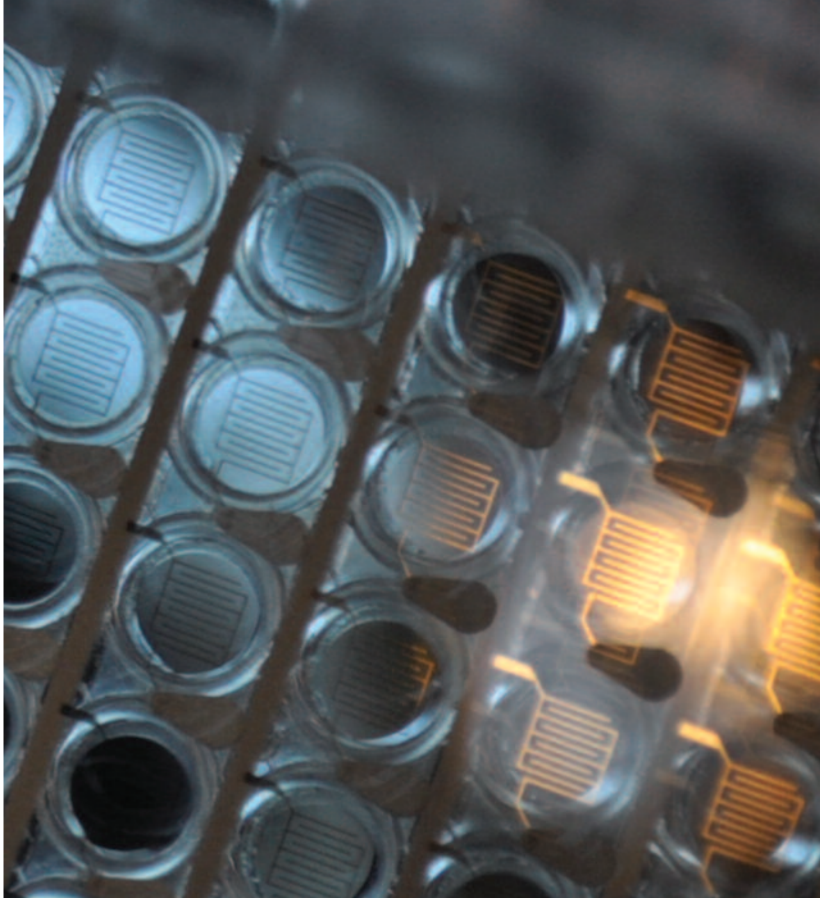
All arrays can be ordered without upper chambers (NC). Gold films are transparent and the upper chambers can be removed following ECIS® measurements to facilitate microscopy including fluorescence measurements

We recommend arrays not to be stored for more than two months.

For ordering, please contact 518-880-6860 or send email to info@biophysics.com.

Please include shipping and billing address.

Credit cards are accepted.



institutions that use ECIS

Australia
University of Sydney
University of South Australia

Austria
Biotec Area Krems
Medical University Graz
Tissue Med Biosciences GmbH & Co
University of Applied Sciences Krems

Belgium
Ghent University
Katholieke Universiteit Leuven

Brazil
Natura Invacao e Technological

Canada
BioPhage Inc
Conseil National de Recherches
Fisheries and Oceans Canada
Millenium Biologix
Ottawa Hospital
St. Michael's Hospital
Université Laval
University of Alberta
University of Montreal
Vertex Pharmaceuticals Incorporated

China
Capital University of Medical Sciences
Central Southern University
Fu Jen Catholic University
Fudan University Medical School
Guangdong Entry-Exit Inspection and Quarantine Bureau
Guangzhou Medical University (GMU)
Inner Mongolia Medical College
Institute of Materia Medica
Jinan University
Shandong Provincial Qianfoshan Hospital
Shanghai Jiao Tong University
Sun Yat-sen University
The Third Xiangya Hospital of Central South University
ZhenJiang First People's Hospital

Denmark
Novo Nordisk

Estonia
Tallinn University of Technology

Finland
Åbo Akademi University
University of Oulu

France
Centre Hospitalier Universitaire de Nice, Inserm, U576
Centre de recherche Cardiovasculaire à l'HEGP, Inserm, U970

Germany
Albert-Ludwigs Universitat
Bayer Schering Pharma AG
Beiersdorf AG
Charite Universitatsmedizin Berlin
CLR Chemisches Lab.
Fraunhofer Institute for Interfacial Engineering & Biotechnology IGB
Friedrich-Alexander-Universität
Friedrich-Schiller-University Jena
Georg-August-Universität Göttingen
Johannes Gutenberg University Mainz
Johann Wolfgang Goethe-University
Ludwig-Maximilians-Universität München
Max-Delbrück-Centrum für Molekulare Medizin (MDC)
MetaGen Pharmaceuticals GmbH
Philipps-Universitat Marburg
Saarland University
Technical University Munchen
Universitat Regensburg
Universitat Wurzburg
University Medical Centre Mannheim
University of Tuebingen
University of Witten/Herdecke
WWU Munster

Hungary
Debreceni Egyetem
Semmelweis University
University of Debrecen Medical School

India
Bengal Engineering and Science University, Shibpur
Central Leather Research Institute
Indian Institute of Science Bangalore
Indian Institute of Technology Madras
Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR)
National Centre for Cell Science
National Institute of Immunology
Osmania University

Ireland
Trinity College Dublin
University College Cork

Israel
Ben Gurion University of the Negev
Haifa University
Rambam Hospital
Teva Pharmaceutical Industries Ltd.

Italy
Analitica DeMori Srl.
European Commission Joint Research Centre
IFOM-IEO Campus

Japan
Astellas Pharma Inc.
Fukui Medical School of Japan
Kawasaki Medical School
National Cancer Center
National Center for Child Health and Development
National Center for Global Health and Medicine Research Institute
National Cerebral and Cardiovascular Center
Okayama University
Osaka City University Medical School
Otsuka Pharmaceutical Co.
University of Tokyo
University of Tsukuba

Korea
DaeJeon Science High School
Chungbuk National University
Gachon University
Jeju National University
Kyungwon University
Yonsei University College of Medicine

Malawi
Queen Elizabeth Central Hospital

Malaysia
University of Malaya

Netherlands
AMC University of Amsterdam
Leiden University Medical Center
Sanquin Research CLB
UMC Utrecht
University Medical Center Groningen
VU University Medical Center

Norway
University of Bergen
University of Oslo

Qatar
Qatar University

Russia
Russian Cardiology Research Center

Spain
Universitat Autonoma de Madrid

Sweden
AstraZeneca
Karolinska University Hospital, Solna
Umeå University

Switzerland
Merck Serono
Novartis Pharma AG
University Hospital Zurich

Taiwan
Academia Sinica Institution
Chang Gung University
Chi Mei Hospital
Food Industry R and D Institute
Fu Jen Catholic University
National Cheng Kung University
National Defense Medical Center
National Chiao Tung University
National Taiwan University
National Taiwan University Hospital
National Yang-Ming University
Tzu Chi College of Technology

United Kingdom
Cardiff University
Novartis Institute for BioMedical Research
Queen's University
University College London
University of Bristol
University of Edinburgh
University of Manchester
University of Portsmouth

United States

Abbott Laboratories
Al Dupont Hospital for Children
Albany Medical College
Albert Einstein College of Med.
Alcon Laboratories
Allergan
Amgen Inc.
Armed Forces Radiobio. Res. Inst.
Bausch & Lomb
Baylor College of Medicine
Beth Israel Deaconess Medical Center
Blood Center of Wisconsin
Blood Systems Research Ins.
Boston Children's Hospital
Brigham and Women's Hospital
Bristol-Myers Squibb
Buffalo State
Capstone Therapeutics Corp.
Case Western Reserve University
Celgene Corporation
Cellular Dynamics International Inc.
Centers for Disease Control and Prevention
Charlie Norwood VA Medical Center
City College of New York
Cleveland Clinic Foundation
Columbia University
Department of Veterans Affairs Medical Center
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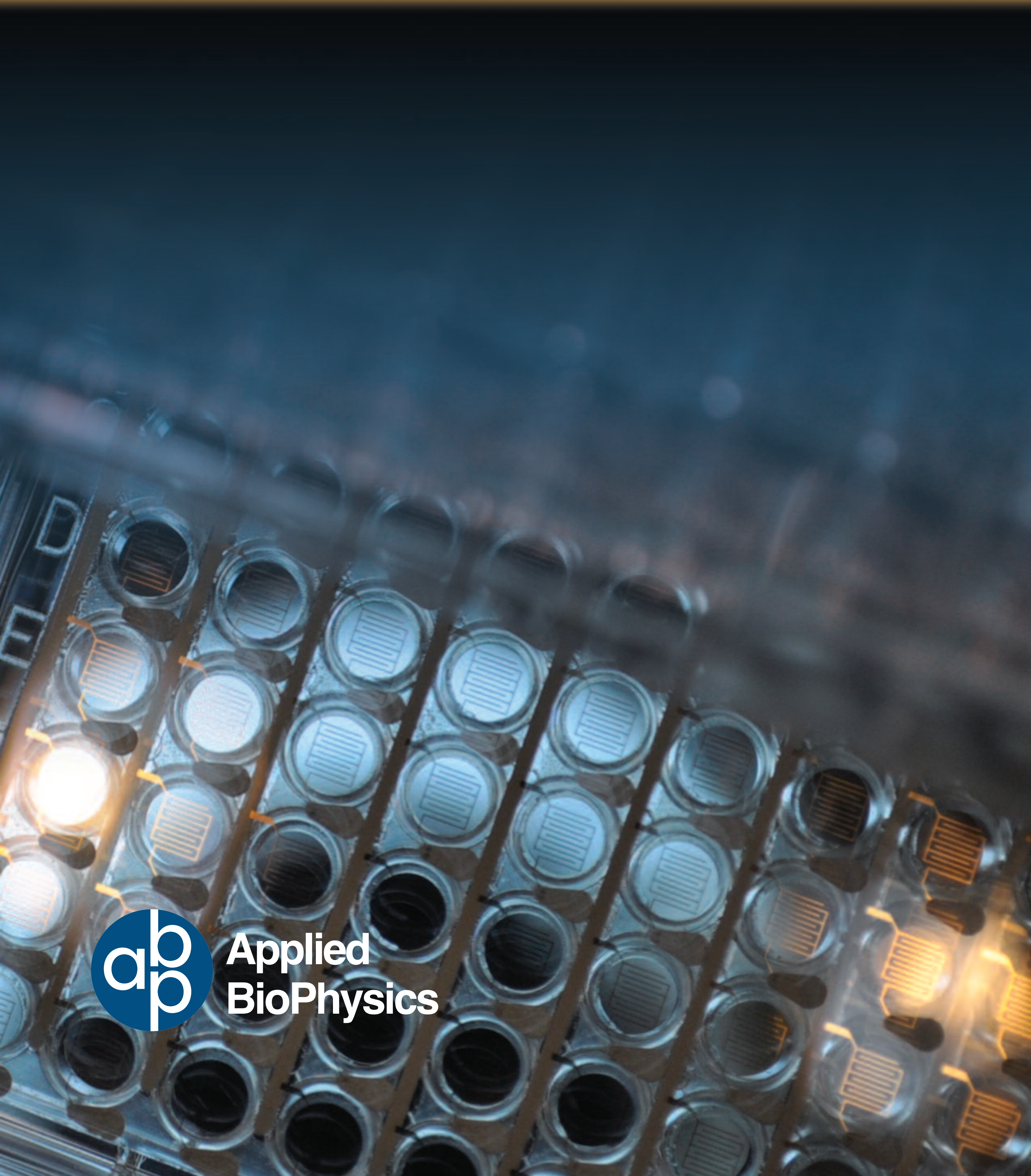
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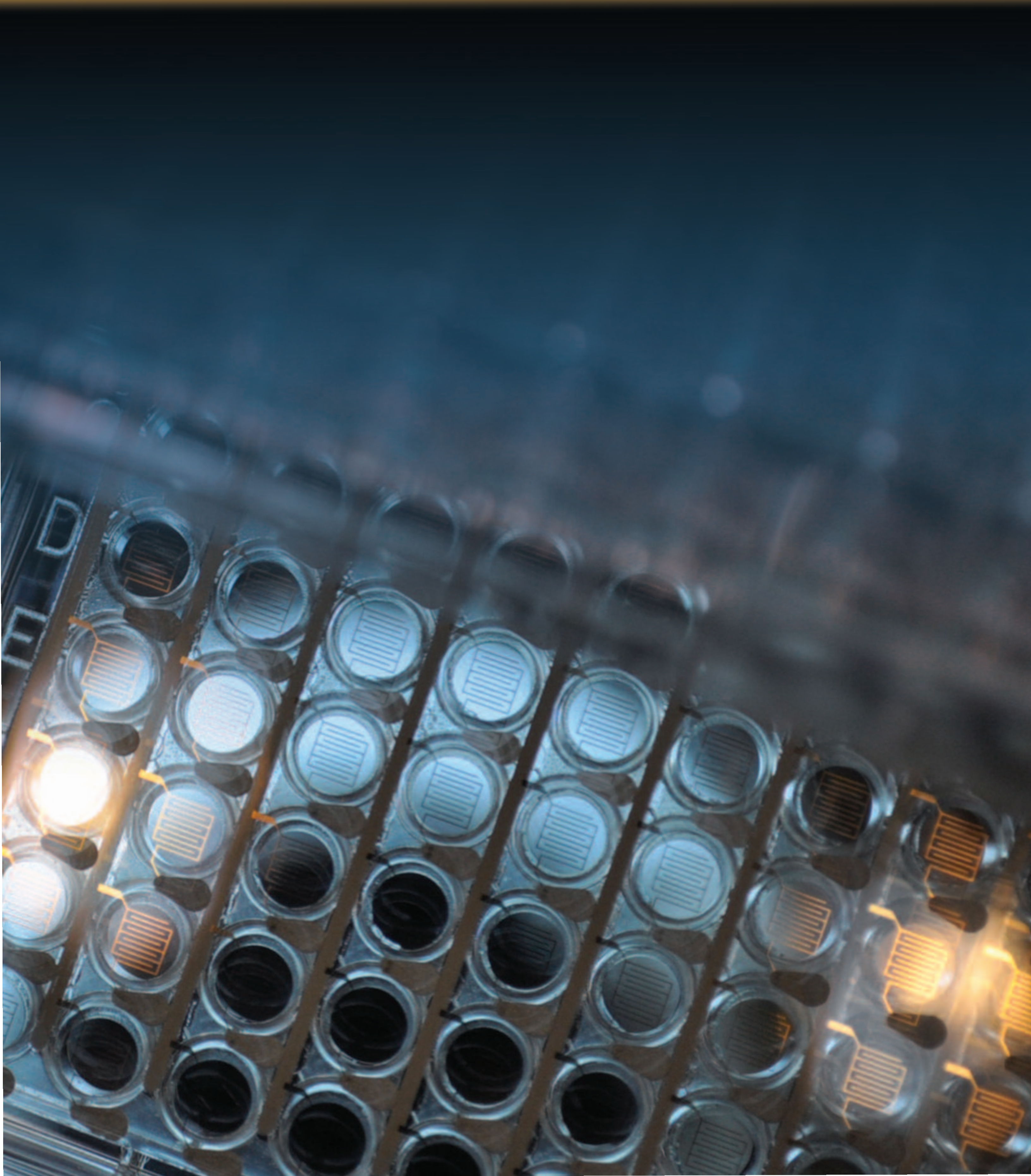

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