Video Article

# Measurement of Cellular Chemotaxis with ECIS/Taxis

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## Abstract

Cellular movement in response to external stimuli is fundamental to many cellular processes including wound healing, inflammation and the response to infection. A common method to measure chemotaxis is the Boyden chamber assay, in which cells and chemoattractant are separated by a porous membrane. As cells migrate through the membrane toward the chemoattractant, they adhere to the underside of the membrane, or fall into the underlying media, and are subsequently stained and visually counted [1]. In this method, cells are exposed to a steep and transient chemoattractant gradient, which is thought to be a poor representation of gradients found in tissues [2].

Another assay system, the under-agarose chemotaxis assay, [3, 4] measures cell movement across a solid substrate in a thin aqueous film that forms under the agarose layer. The gradient that develops in the agarose is shallow and is thought to be an appropriate representation of naturally occurring gradients. Chemotaxis can be evaluated by microscopic imaging of the distance traveled. Both the Boyden chamber assay and the under-agarose assay are usually configured as endpoint assays.

The automated ECIS/Taxis system combines the under-agarose approach with Electric Cell-substrate Impedance Sensing (ECIS) [5, 6]. In this assay, target electrodes are located in each of 8 chambers. A large counter-electrode runs through each of the 8 chambers (**Figure 2**). Each chamber is filled with agarose and two small wells are the cut in the agarose on either side of the target electrode. One well is filled with the test cell population, while the other holds the sources of diffusing chemoattractant (**Figure 3**). Current passed through the system can be used to determine the change in resistance that occurs as cells pass over the target electrode. Cells on the target electrode increase the resistance of the system [6]. In addition, rapid fluctuations in the resistance represent changes in the interactions of cells with the electrode surface and are indicative of ongoing cellular shape changes. The ECIS/Taxis system can measure movement of the cell population in real-time over extended periods of time, but is also sensitive enough to detect the arrival of a single cell at the target electrode.

*Dictyostelium discoidium* is known to migrate in the presence of a folate gradient [7, 8] and its chemotactic response can be accurately measured by ECIS/Taxis [9]. Leukocyte chemotaxis, in response to SDF1α and to chemotaxis antagonists has also been measured with ECIS/Taxis [10, 11]. An example of the leukocyte response to SDF1α is shown in **Figure 1**.

## Video Link

The video component of this article can be found at https://www.jove.com/video/3840/

## Protocol

## 1. ECIS/Taxis Electrode Preparation

1. The gold surface of the ECIS/taxis electrode array (consisting of 8 chambers per slide) is first stabilized by pre-treatment with sterile 10 mM cysteine in deionized water ($dH_2O$) for 15 min at room temperature under sterile conditions.
2. Aspirate the cysteine solution from each electrode chamber, rinse 3 times with sterile $dH_2O$, and replace with 250 µl of complete medium (RPMI 1640, 10%FBS, 25 mM HEPES buffer).
3. Connect the electrode array to contact pins on instrument array holder to perform electrode check.
4. Chambers that are properly connected will be highlighted in green on the computer screen. If the chambers are highlighted in red, they are not connected and the electrode should be re-positioned in the array holder to establish contacts for all chambers.
5. Once all chambers are connected, click on the "check" button in the software controller to determine the initial resistance and capacitance values for each chamber. Resistance values should be between 1600 and 2000 ohms, and capacitance values should be between 5 and 6 nanofarads for an 8 chamber array. If a chamber is not within these acceptable parameters, the individual chamber should not be used to collect data.
6. Disconnect the electrode from array holder to prepare the individual agarose chambers. Aspirate medium from chambers and wash each chamber 3 times with sterile $dH_2O$.

## 2. Preparing Agarose Chambers

1. In a sterile 15 ml polypropylene conical tube, measure 0.03 g of Seakem GTG agarose and add 1 ml sterile PBS. Replace tube cap loosely, and melt the agarose in autoclave for a 2 min liquid cycle (melting in a microwave is not effective for this assay). This short time will not melt the conical tube. For less fastidious cells that do not require serum for culture, (e.g. *Dictyostelium discoideum*) 0.5% agarose can be made in the appropriate medium and melted in a microwave. The autoclave is necessary for cells that require serum, as this will be denatured if melted in microwave.
2. Add 5 ml of complete RPMI medium warmed in a water bath (55 °-60 °C) directly to the hot agarose, to a final concentration of 0.5% agarose and pipet up and down gently to mix.
3. Pipet 300 µl of melted agarose medium into each chamber, replace the ECIS/taxis array cover and allow to gel at room temperature.
4. Pipet remaining agarose into cap of 15 ml tube to assess agarose gelling. This excess agarose will also be used in part 4: Cutting wells into agarose.

## 3. Constructing and Sharpening the Cannula Well Cutting Tool

1. Sharpen two 14 gauge blunt end cannulae, using a 5/64" bit. Rotate the tip of the drill bit in each cannula opening with a slow speed drill, making certain the drill bit cuts entire interior edge of each cannula opening to form a sharp cutting edge (**Figure 4A**).
2. Drill two holes through 1"x 2"x ¼" plexiglass (**Figure 4B-D**) with a 5/64" bit in a drill press. Insert each sharpened 14 gauge cannula so it protrudes an equal distance through each plexiglass hole.

## 4. Cutting Wells into Agarose

1. Sterilize the 14 gauge cannulae by lightly flaming the tips. Prior to cutting wells in the ECIS chamber, allow the cannulae to cool to room temperature, or cool the cannulae by touching the agarose in the cap of the 15 ml tube. If the temperature of cannula is too high, the agarose will melt as the chamber wells are cut, rendering the wells misshapen and defective.
2. Align the cannula pair above the two gold dots surrounding the target electrode in each chamber (**Figure 2c and 5**). Insert the cannula vertically, without any horizontal movement, stopping upon gentle contact between the cannula and the electrode surface. Carefully remove the cannula, again without any horizontal movement. Placement of additional wells for opposing gradients is also possible with a reconfiguration of the cannula jig.
3. *Gently* aspirate the agarose plug left by the cannula, using a sterile 9" Pasteur pipette, connected to low vacuum pressure, with a vacuum trap catch agarose waste.
4. Add 300 µl of complete RPMI media to the surface of each chamber at 37°C, for 2 min to saturate the agarose, and then gently aspirate the media from the surface and the wells without disturbing the agarose.

## 5. Loading the Wells

1. Each well is capable of holding a volume of 7 µl. To one well in each chamber, add 7 µl of cells suspension. For Jurkat T cells, use 2 x$10^7$ cells/ml. These cells will respond to 7 µl of 200 ng/ml SDF-1α diluted in incomplete RPMI, which lacks serum. Use complete media alone as a negative control.
2. After the wells are loaded, view each chamber of the array with an inverted microscope to ensure that all wells were cut and filled properly. Cells should be in within the confines of the well's boundary. If they are seen in both wells, or are spreading beyond cell well border, then a gap has formed under the agarose. Note the improperly set up wells, as data cannot be reliably interpreted from those chambers.

## 6. Data Collection

1. Place the electrode in the array holder and re-connect the gold pads on the electrode array to the spring-loaded pogo pins of the holder and click "setup". The wells that are properly connected will be highlighted in green. If the wells are red, they are not connected and the electrode should be repositioned in the array holder.
2. Select "8WE1" (8 well 1 electrode) from the drop down menu in the software panel to indicate the array configuration.
3. Select "check" in the software control panel to determine the initial resistance and capacitance values for each well. The resistance values should be between 1600 and 2000 ohms, while the capacitance values should be between 5 and 6 nanofarads.
4. Select multiple frequencies to measure resistance at several frequencies. There is a choice of customizing frequencies, or using a set of recommended frequencies. The standard multiple frequency acquisition settings capture data at 11 pre-set frequencies (52.5, 125, 250, 500, 1,000, 2,000, 4,000, 8,000, 16,000, 32,000 and 64,000 Hz) at a rate of 8 wells/min. The time interval between measurements can also be customized. Collecting multiple frequency data allows for analysis of capacitance, impedance, and resistance. Collecting resistance values at 4,000 Hz is usually sufficient for mammalian cell chemotaxis studies, but the additional frequencies may provide information in other contexts[12,13].
5. Total experiment duration can be set, on the right hand panel, or the experiment can be stopped manually, by clicking "finish" once cells have produced resistance that are indicative of arrival at the electrode. The time it takes for cells to arrive at the electrode depends on the cell type used.
6. Click "Start" to initiate data collection.

## 7. Data Management

1. Although the data can be exported to Excel, the ECIS 1600R software, which accompanies the ECISθ system is the most efficient method to analyze ECIS/Taxis data.

2. The software data analysis tool bar contains the radio buttons Z, R, and C. These determine whether impedance, resistance, or capacitance, respectively, is plotted on the vertical axis against the x-axis (elapsed time).
3. ECIS/Taxis data is most efficiently displayed as resistance over time at 4,000 Hz. Rapid fluctuations in resistance, or microtransients (**Figure 1**), are evidence of cellular movement over the electrode. Compiling, or binning the data points so that not all points are graphed, is not recommended for ECIS/Taxis, since it will average microtransients out of the data.
4. Once the graph has been made with the ECIS1600R software, it can be exported by choosing "export graph" from the file menu, and saved as a .tif or .jpg file.

## 8. Representative Results

Cellular chemotaxis is indicated by an increase in resistance at 4,000 Hz. The arrival of cells on the target electrode is also indicated by the appearance of rapid fluctuations in resistance called microtransients, or micromotion as described in Opp *et. al*., 2009 (**Figure 1**)[14]. A negative result is indicated by consistent, or a slight decrease, in resistance at 4,000 Hz, as seen in red in **Figure 1**. The absence of microtransients is also a sign of an absence of cell movement to the electrode. The increase in resistance is directly proportional to the number cells that cross the electrode[10]. The addition of monoclonal antibody specific for a chemoattractant or toxins known to interfere with G protein coupled receptors can block chemotaxis[11].

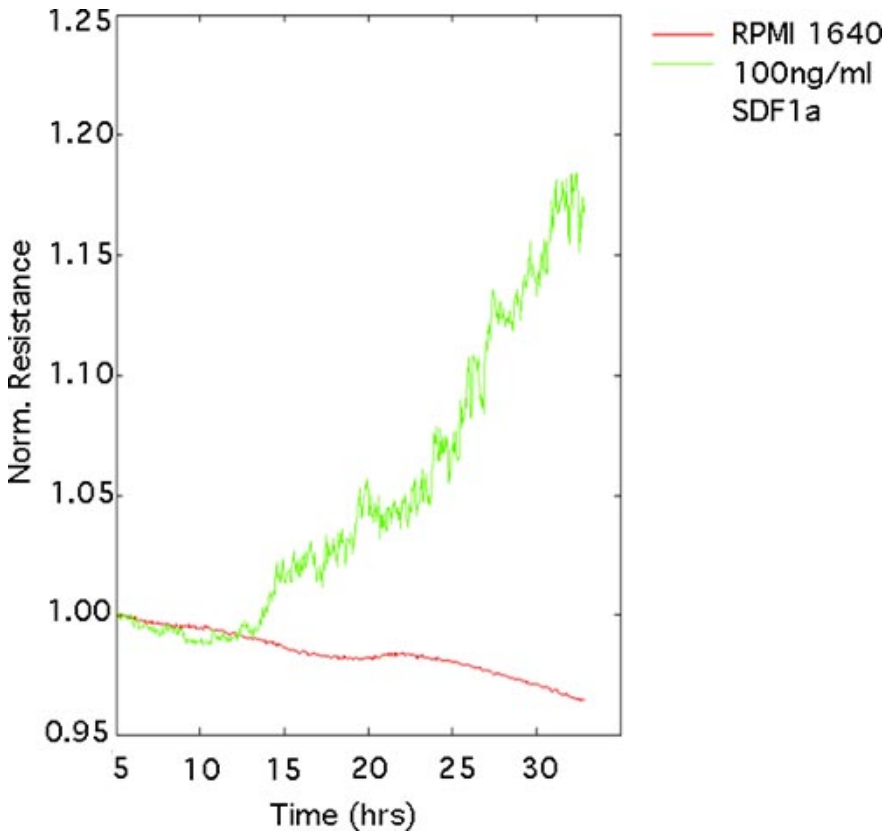


**Figure 1. Chemotaxis of Human Jurkat T cells in response to SDF1α.** Human Jurkat T cells ($2.0x10^6$ cell/ml) were exposed to a gradient of SDF-1α (starting concentration 100 ng/ml) or RPMI 1640 as a negative control. The normalized resistance at 4000 Hz was graphed. Human Jurkat T cells moved in response to SDF-1α, as evidenced by the increase in resistance and microtransients, while no movement was seen with exposure to RPMI 1640.

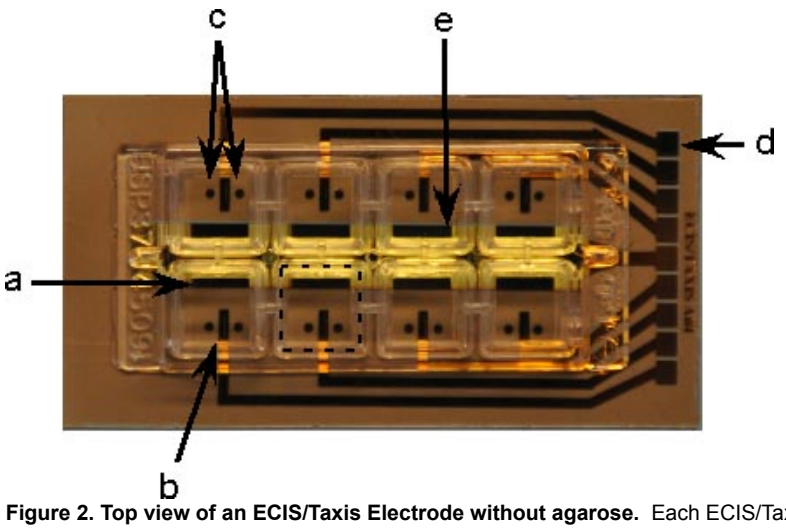


**Figure 2. Top view of an ECIS/Taxis Electrode without agarose.** Each ECIS/Taxis electrode is comprised of 8 individual chambers. Each chamber shares a large electrode (a), and contains an individual target electrode (b). Each chamber also has 2 gold circles (c), which are used as a guide when cutting the wells in the agarose with the cannulae. The gold squares (d) are the contact pads that connect to the pogo pins in order to apply the AC current to the electrode. The individual target electrodes are each in circuit with larger counter electrode (e).

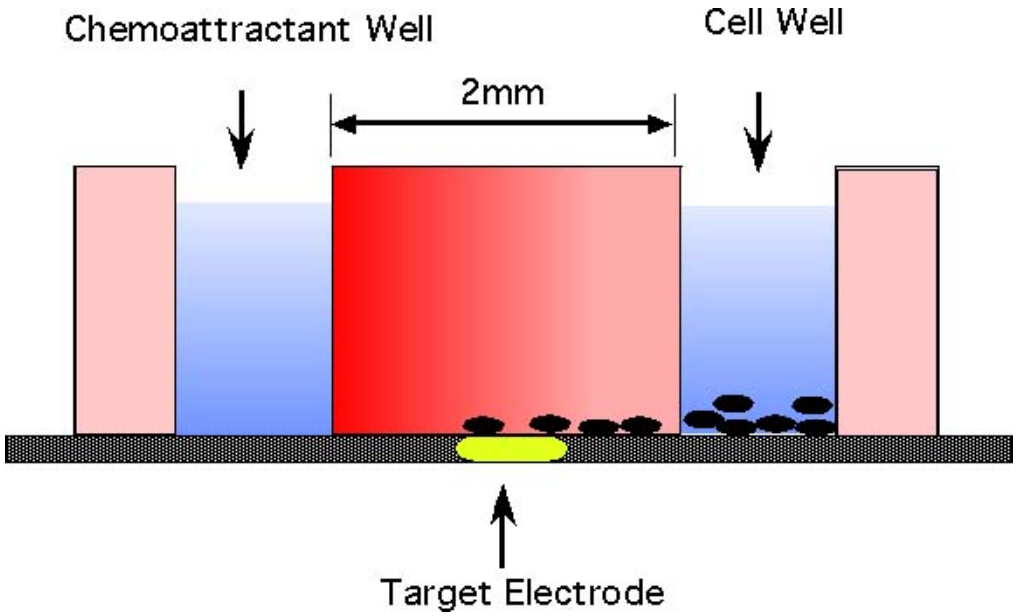


**Figure 3. Two Wells are cut into each agarose-filled chamber.** When the chemoattractant is added to one well it diffuses to create a gradient in the agarose, with the highest concentration of chemoattract closest to the chemoattractant well. The cells travel beneath the agarose toward higher concentrations of chemoattractant and can pass over the target electrode. As the cells cross the target electrode, an increase of resistance is recorded by the ECIS 1600R software.

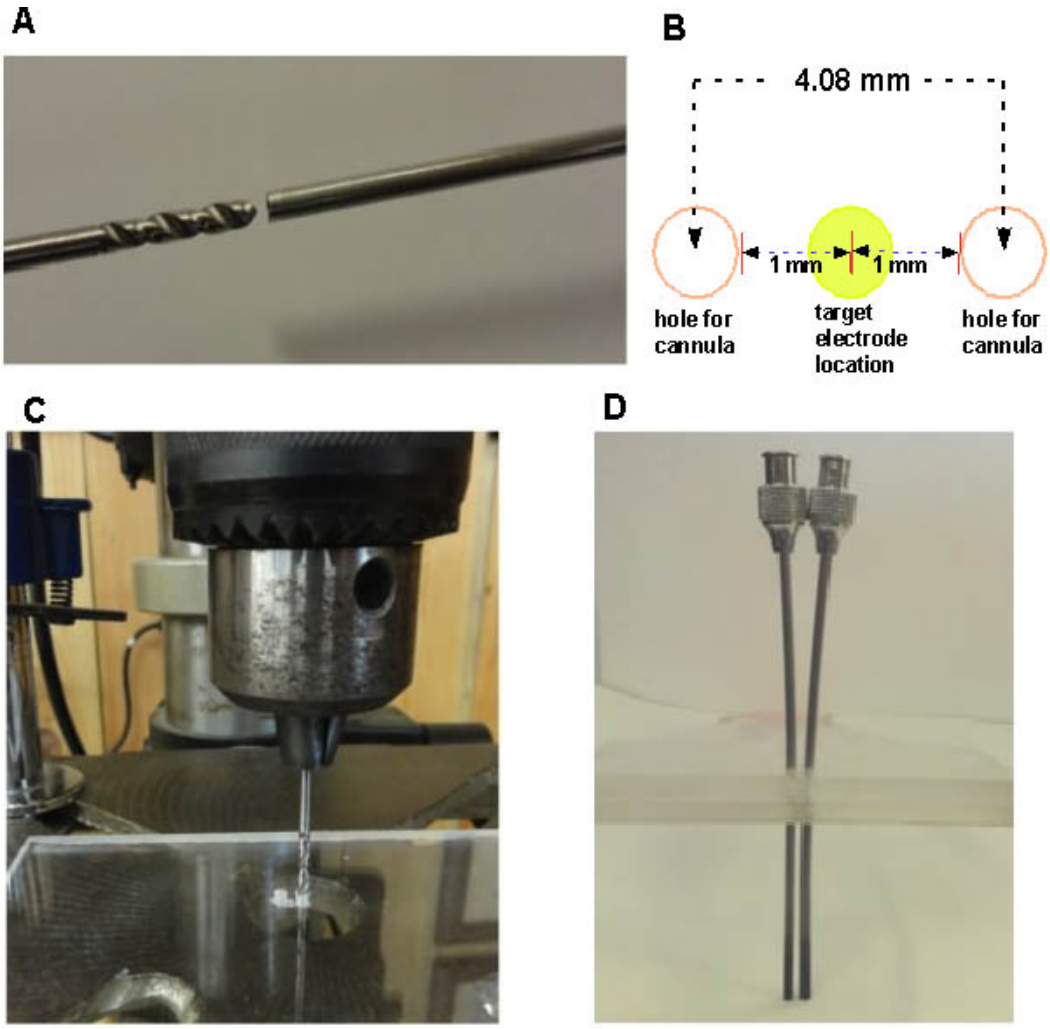


**Figure 4. Insertion of two 14 gauge cannulae into plexiglass. A)** A 5/64" drill bit is used to sharpen the cannula tip. **B)** Two holes must be drilled into plexiglass to accommodate the 14 gauge cannulae according to the layout shot. **C)** Use a drill press to ensure the holes are perpendicular to the pleixglass surface. **D)** Two sharpened Cannulae are inserted through ¼" plexiglass, 2 mm apart (measured from inner edges). These sharpened cannulae are carefully inserted into agarose to cut the wells.

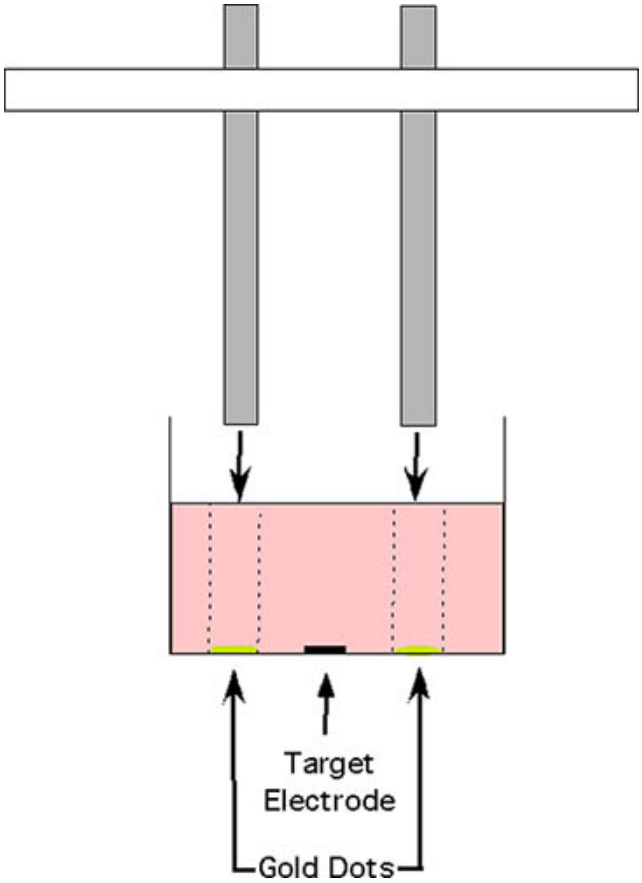


**Figure 5. Aligning cannulae with gold dots in ECIS/Taxis chamber.** To cut wells, the sharpened cannulae must be aligned with the gold dots flanking the target electrode on the bottom of the ECIS/Taxis chamber. The cannulae should be inserted vertically, without any horizontal movement, and removed in the same manner.

## Discussion

Novel characteristics of the ECIS/Taxis assay include its ability to automate the collection of real-time data as cells respond to chemoattractant. While the most commonplace application of this technology is to measure cellular responses to individual chemotactic gradients, or to gradients comprised of mixtures of chemotaxis agonists and antagonists, the ECIS/Taxis approach is also amenable to variations to these configurations that could be quite helpful in the assessment of cellular responsiveness. There is good evidence that overlapping or sequential gradients can influence cellular behaviors in novel ways. Moreover, it is likely that these more complex gradients are the norm *in situ* [12, 13].

The ECIS/Taxis assay can enable modeling of these different gradient configurations in ways that are not possible with other technologies. For example, by adding additional wells, one can distribute the orientation of the gradient(s) in relation to the cell well and target electrode. It is also possible to configure the assay to place cells in one of the wells as the source of chemotactic factors.

When setting up the assay, it is important to maintain complete hydration of the gel that overlays the target and counter electrodes in each chamber, and to cut wells that have the correct spatial relationship to the target electrode. Moreover, the bottom of the cell well needs to be contiguous with the thin film of liquid that forms under the agarose layer; it is in this film that the cells will move in response to the overlying gradient. To do this, one must aspirate all of the agarose plug without leaving gel fragments behind. The removal of the agarose plug cut by the cannula can create a tunnel between the two wells, which has the effect of deforming the gradient and allowing the cells to move freely across the target electrode. It is crucial that the vacuum pressure used to aspirate the gel plug is low to avoid compromising the agarose well integrity.

We have found that variations in the percentage of agarose used in the gel can differentiate cells that express cytoskeletal defects from wild-type cells, suggesting that this manipulation can be used to interrogate the forces that cells can exert on their environment. If the gel dehydrates during culture, changes will occur that influence the experimental results, including a relative increase in gel rigidity that would slow cell movement and an increase in solute concentration that would decrease total system impedance.

The assay is compatible with the measurement of movement by leukocytes and other cell types that do not allow current flow through the cell body. This is not a universal characteristic of cells: nerve cells and some epithelial cells (for example) could transmit current through the cell body and would thus produce far lower resistance values per individual cell.

While these approaches are specifically defined to detect cellular responses to chemotactic gradients, it is easy to imagine the assay as similarly applicable to studies involving metastasis and the extracellular signals that enhance metastatic cell movement. Moreover, there are additional array configurations that can be used in other cell-substrate interaction studies.

## Disclosures

David A Knecht and Michael A Lynes have an issued patent for the ECIS/Taxis technology, which as been licensed by the University of Connecticut to Applied Biophysics, Inc.

## Acknowledgements

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