New microscopy method opens window on previously unseen cell features

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Despite the sophistication and range of contemporary microscopy techniques, many important biological phenomena still elude the precision of even the most sensitive tools. The need for refined imaging methods for fundamental research and biomedical applications related to the study of disease remains acute.

Nongjian (N.J.) Tao and his colleagues at the Biodesign Institute at Arizona State University have pioneered a new technique capable of peering into single cells and even intracellular processes with unprecedented clarity. The method, known as electrochemical impedance microscopy (EIM) may be used to explore subtle features of profound importance for basic and applied research, including cell adhesion, cell death (or apoptosis) and electroporation—a process that can be used to introduce DNA or drugs into cells.

This new investigative tool is expected to make significant research inroads, improving drug discovery for diseases like cancer, furthering the study of host cell-pathogen interactions, and refining the analysis of stem cell differentiation.

The group's research appears in today's issue of the journal *Nature Chemistry*.

As Tao explains, the method builds on the advantages of a powerful existing technology known as electrochemical impedance spectroscopy (EIS). Here, an AC voltage is applied to an electrode and the current

response is measured as a change in impedance. (Impedance is defined as opposition to alternating current and extends the idea of electrical resistance to AC circuits.)

In addition to permitting observation of DNA, proteins, viruses and bacteria, EIS allows other subtle phenomena occurring at the electrode's surface to be imaged, including molecular binding events. Modifications of the EIS method have been applied to the study of other cellular processes including cell spreading, adhesion, invasion, toxicology and mobility.

A further attraction of the technique is that unlike fluorescence imaging, EIS is a so-called label-free technology, making it non-invasive to the sample under study. No fluorescent labeling particles or dyes—which can often interfere with normal cellular function—are required.

EIS however has one Achilles heel—it can't provide good spatial resolution. As Tao explains "Our technology provides high spatial resolution, making it possible to image and study single cells and subcellular processes, and detect and anayze biomolecules in a high density microarray format."

Obtaining good spatial resolution through conventional EIS would either require the use of multiple electrodes monitoring the surface to be studied, or a single electrode that mechanically scans across the surface. Both of these strategies have serious limitations that make them impractical. Tao and his colleagues have taken a different approach, combining EIS with another robust imaging technology based on surface plasmon resonance.

Surface plasmon resonance or SPR imaging is an optical detection process. Under proper conditions, polarized light striking a thin layer of gold, will cause free electrons to absorb the incident light particles,

converting them into a surface plasmon wave, which propagates across the gold layer's surface, much like a wave on water. Perturbations of this delicate wave by target molecules cause alterations in the reflective properties of the incident light. These changes can be recorded and translated into an image.

Using SPR, simultaneous events over the entire surface of a biochip can be studied in real time, without the need for multiple electrodes. The method developed by Tao—known as electrochemical impedance microscopy (EIM)— differs from conventional EIS in that it does not measure current, but rather, uses plasmon resonance to detect impedance changes optically, dramatically enhancing spatial resolution of observed features. In addition to the EIM image, the new technique produces simultaneous optical and SPR imagery, which provide useful complementary information.

EIM allows for sub-micron spatial resolution of biological phenomena. Two cell processes in particular were observed in the current study: apoptosis and electroporation. Both of these phenomena require not only good spatial resolution but the ability to monitor fast-changing events in real time—something EIM excels at, using a specialized video camera to record rapid cellular events.

Apoptosis or cell death is of critical research significance. It is a central element in homeostasis and tissue/organ development. A better understanding of the cellular mechanisms of apoptosis is also critical for cancer research, and for the design of cancer therapies, which often attempt to induce apoptosis in malignant cells.

Tao and his group induced cell death in cervical cancer cells through the application of two molecules: MG132 and TRAIL—an apoptosis-inducing ligand. EIM imaging yielded detailed information of the successive stages of apoptosis, which include cellular shrinking and

condensation followed by the fragmentation of nuclear material and eventual disintegration of the cells, with SPR and EIM imagery providing a complementary record of events. As Tao notes, before this study, such detailed information was only obtainable through fluorescent staining or electron microscopy.

Electroporation was also observed through EIM. Here, a voltage pulse is applied to a cell, causing a sudden increase in the conductivity and permeability the cell's plasma membrane. This valuable technique can be used to insert a molecular probe to monitor a cell's interior, or to introduce a cell-altering drug or segment of coding DNA. Once again, complementary information provided by optical, SPR and EIM combined to give a much more complete picture of this process, with the EIM images revealing the most dramatic changes over time. "We are excited by its potential for mapping out local activities of many celluar processes, such as ion channel activities and drug-cell interactions. "

Continued work will further refine this label-free, non-invasive microscopy technique, offering fresh insights into previously elusive cellular events.

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