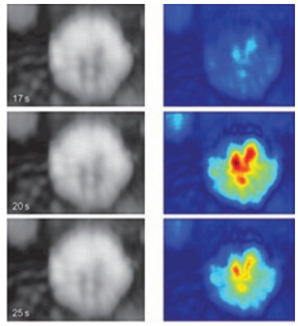
MICROSCOPY

Imaging impedance

A label-free microscopy technique based on electrochemical impedance offers a new way of studying electrochemical processes in single cells.

Many types of microscopic electrochemical reactions take place in cells, from electron transfer in respiration and photosynthesis to neurotransmitter release. Whereas several powerful electrochemical techniques have been devised to detect these events, these methods have not had the capability of spatial resolution. "This is very important if you want to study [single] cells, which are heterogeneous," notes Nongjian Tao of Arizona State University.



Electroporation of a single cell. The process was monitored by SPR imaging (left) and EIM (right) at indicated times. Reprinted from *Nature Chemistry*.

There are two solutions to achieving spatial resolution, but both face challenges in practice. In one, the electrode could be scanned across the surface, but this is a relatively slow process and is limited by a tradeoff in resolution and sensitivity. Another is to use a very-high-density array of electrodes, but this is subject to interconnection problems and cross-talk between electrodes.

Tao and his colleagues now report a new label-free electrochemical imaging method, which they call electrochemical impedance microscopy (EIM). Impedance, which is related to resistance, is defined as voltage over current response; it is typically measured by applying a voltage to an electrode and monitoring the current. But EIM does not use an electrode to measure current. Instead, it measures an optical signal. "The trick here is how we make a connection between optical signal and current," says Tao. Tao and his colleagues exploited the technology known as surface plasmon resonance (SPR), "which has an interesting property that people have not explored." Though SPR is well known as a technology to monitor binding interactions, it is also sensitive to surface charge density changes. Tao's insight was that "if you can measure charge density without measuring current directly, then you can obtain the localized impedance using an optical approach."

In SPR imaging, the sample is placed on a thin metal film; when the metal layer is hit with a laser beam, surface plasmon waves are generated and can be detected as an image with a camera. The resonant angle of the reflected beam of light is sensitive to changes in the refractive index near the metal surface, which is why SPR is particularly useful for detecting binding interactions. But the metal film can also serve as a working electrode for electrochemical detection. In EIM, a voltage is applied to the metal film; this results in surface charge density modulation, which can be optically recorded in the SPR signal. The SPR images can then be converted into electrochemical images that map the local conductivity and electrical polarizability, reflecting changes in the ionic distribution and cell structure, respectively.

In their first report describing the EIM technology, Tao and his colleagues demonstrated imaging of single cells undergoing apoptosis and electroporation; both processes result in cell-polarization changes. "When cellular processes cause a change in the distribution of ions, you can measure a change in impedance," explains Tao. As cell polarizability and conductivity cannot be easily imaged using other microscopy techniques, EIM should have unique applications. Tao envisions using EIM to study ion channels, image pathogen-host cell interactions and stem cell differentiation, and also for label-free detection of the readout of DNA and protein microarrays.

Another unique property of EIM is that it allows an electrochemical map as well as a SPR imaging map to be recorded simultaneously, which can provide complementary information. The researchers' current setup has sub-micrometer spatial resolution and millisecond temporal resolution. With faster cameras, they hope to image extremely fast electrochemical events in single cells.

Allison Doerr

RESEARCH PAPERS
Wang, W. *et al.* Single cells and intracellular processes studied by a plasmonic-based electrochemical impedance microscopy. *Nat. Chem.* advance online publication (23 January 2011).