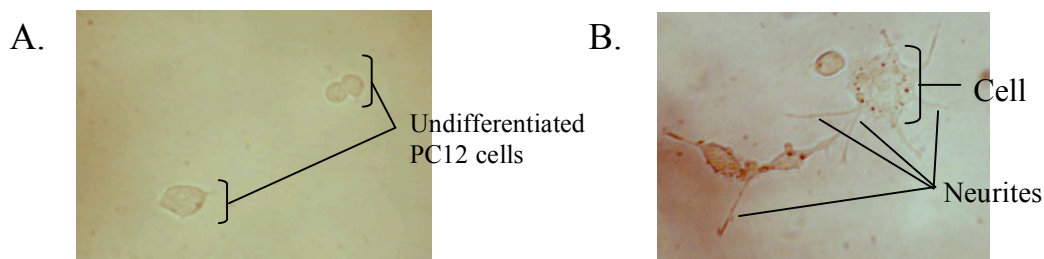


## Scanning Electrochemical Microscopy Imaging of Model Neurons

Cellular damage due to oxidation has been observed in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. Scanning Electrochemical Microscopy (SECM) is a technique well suited to study the effects of oxidative damage on cells such as neurons. The electrochemical analysis of model neurons using SECM can provide a unique view of cellular activity and can be used to observe effects on neuronal signaling. It is possible to detect the release of neurotransmitters such as dopamine, norepinephrine, and serotonin from individual cells using these electrochemical techniques (Cannon *et al.*, Borland *et al.*, Travis *et al.*, Adams *et al.*). The ultramicroelectrode of the SECM can also focally generate the molecules that cause cellular oxidative damage and then the electrode can be used to measure neurotransmitter release from those neurons. We are using SECM in collaboration with the J.E.Baur lab at Illinois State University as a method to investigate the chemical signaling processes that occur between model nerve cells, looking particularly at the effects of oxidative stress and its relationship to neurodegenerative diseases.

### Cell Culturing Model Neurons

Rat Pheochromocytoma cells (PC12 cells) have been used as model neurons for years and the growth patterns of these cells are well documented (Greene *et al.*). PC12 cells have served as model neurons because of some of the unique properties that they exhibit. First, they synthesize, store, and release two common neurotransmitters: dopamine and norepinephrine. PC12 cells release these neurotransmitters in response to potassium ions, acetylcholine, or nicotine, just as a neuron would. Furthermore, PC12 cells exhibit two growth patterns. When they are grown as undifferentiated cells, they divide approximately every 24-48 hours and they have a roughly spherical cell shape (Figure 1A). Second, when PC12 cells are exposed to nerve growth factor, they exhibit a different growth pattern. Initially, they cease cellular division. Afterwards, they flatten out and develop long projections known as neurites (Figure 1B). The neurites often grow to be in close contact with a neighboring cell, resembling a neuronal synapse. If the nerve growth factor is removed, the PC12 cells will eventually return to their roughly spherical shape and resume cell division (Greene *et al.*).



**Figure 1A.** The photograph of undifferentiated PC12 cells shows the roughly spherical shape of the dividing cells.

**B.** After treatment with nerve growth factor, PC12 cells show characteristics of differentiated nerve cells: they flatten out and develop neurites towards neighboring cells.

### Scanning Electrochemical Microscopy (SECM)

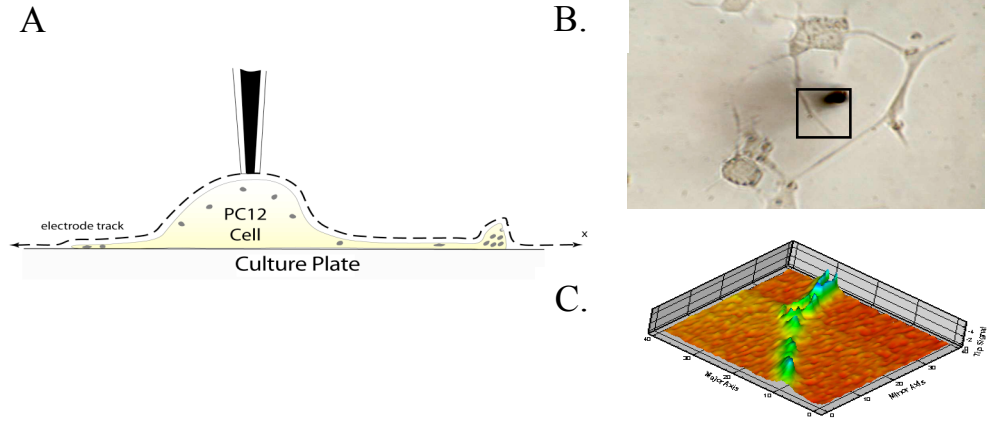
The SECM is a powerful tool that can be used to study the relationship between oxidative stress and neurotransmitter release. The SECM can be used in two distinct modes: 1) constant distance, and 2) generator/collector. In the constant distance mode, the tip of the ultramicroelectrode is used to obtain information on the topography of the cell. In the generator/collector mode, the electrode can be used to generate the reactive oxygen species (ROS) as well as to detect the presence of an electroactive agent, such as a neurotransmitter. (Amemiya *et al.*, Sun *et al.*). Because the ultramicroelectrode probe can be used both to generate the reactive oxygen species and to measure to release of neurotransmitter, it should be possible to detect early changes in neurotransmitter release as well as cellular morphological changes in response to oxidative stress.

In the constant distance mode, the SECM uses negative feedback to maintain the electrode at a constant distance from the substrate surface (Figure 2). All movement of the electrode tip is controlled by an extremely sensitive positioner that is interfaced to the instrument. The feedback loop is used to maintain the distance from the surface, and as the electrode tip moves across the surface of the cell, the data of the position of the tip is recorded. As the instrument collects data on the movement of the electrode tip, it generates a topographical map of the surface (Kurulugama *et al.*, Liebetrau *et al.*).

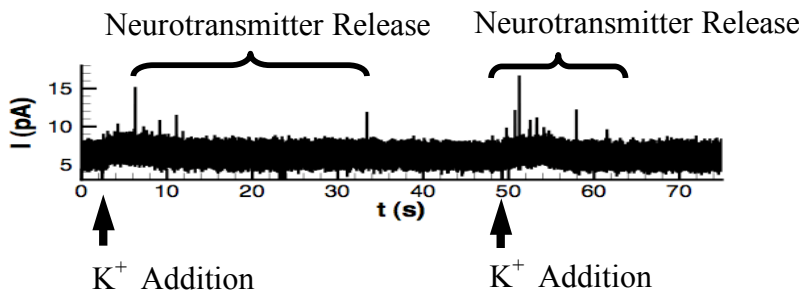
**Figure 2. A.** Cartoon diagram of constant distance imaging showing how the ultramicroelectrode tracks across the surface of a differentiated PC12 cell.

**B.** Photograph of differentiated PC12 cells. The highlighted square shows the area of the scanned image. The dark spot in the top right corner is the ultramicroelectrode (5  $\mu\text{m}$  carbon fiber).

**C.** SECM image of the neurite in the photograph above.

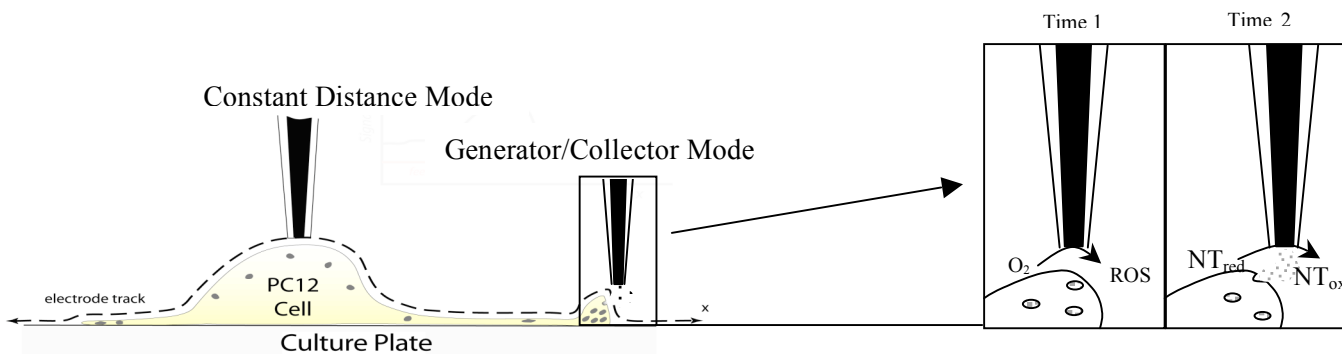


In the generator/collector mode of operation, the electrode is either generating an electroactive agent, such as a reactive oxygen species, or measuring the amount of a particular electrochemically active reagent released from a surface, such as release of neurotransmitter. Neurotransmitters such as dopamine, norepinephrine, and serotonin can be detected electrochemically. When one of these neurotransmitters reacts and loses electrons (known as an oxidation), the process can be detected by a change in current of the nearby electrode (Figure 3). The electrode is set to a potential that provides just enough voltage to oxidize a specific neurotransmitter. When the neurotransmitter is oxidized, it will release electrons and the current of the electrode will momentarily change, generating a spike of current (Cannon *et al.*, Borland *et al.*, Travis *et al.*, Adams *et al.*).



**Figure 3.** Electrochemical detection of neurotransmitter release in model neurons. After treatment with  $\text{K}^+$ , cells release the neurotransmitters dopamine and norepinephrine. The ultramicroelectrode is positioned next to an undifferentiated PC12 cell and detects the release of the neurotransmitters. Each vesicular release is observed as a spike in current.

The two modes of operation of the SECM allows for focal generation of reactive oxygen species and detection of neurotransmitter release with near simultaneous topographical mapping of the cell. (Figure 4).



**Figure 4.** The ultramicroelectrode can operate in two distinct modes. In the constant distance mode, the topography of the cell is determined. In the generator/collector mode, reactive oxygen species (ROS) are generated or electroactive agents, such as neurotransmitters (NT) are detected. Near simultaneous detection of neurotransmitter release and cellular topography can be obtained since the same electrode is used for both processes.

- Adams, K.L., M. Puchades, and A.G.Ewing (2008) In Vitro Electrochemistry of Biological Systems *Annu. Rev. Anal. Chem.* **1**:329-355.
- Amemiya, S., A.J. Bard, F.F.Fan, M. Mirken, and P.Unwin (2008) Scanning Electrochemical Microscopy *Annu. Rev. Anal. Chem.* **1**:95-131.
- Borland, L.M., S. Kottegoda, K.S. Phillips, and N.L. Allbritton (2008) Chemical Analysis of Single Cells *Annu. Rev. Anal. Chem.* **1**:191-227.
- Cannon, D.M. Jr., N. Winograd, and A.G.Ewing (2000) Quantitative Chemical Analysis of Single Cells. *Annu. Rev. of Biophys. Biomolec. Structure* **29**:239-263
- Cohen, G., D. Lewis, and P.M. Sinet (1981) Oxygen Consumption During the Fenton-Type Reaction Between Hydrogen Peroxide and a Ferrous-Chelate (Fe<sup>+2</sup>-DTPA) *J. Inorg. Biochem.* **15**:143-151.
- Greene, L.A., S.E. Farinelli, M.E. Cunningham, and D.S. Park (1999) Methodologies for the Culture and Experimental Use of the PC12 Rat Pheochromocytoma Cell Line. In: *Culturing Nerve Cells* G. Banker, K.Goslin eds., MIT Press, Cambridge, MA pp161-187.
- Kurulugama, R.T., D.O.Wipf, S.A. Takacs, S. Pongmayteegul, P.A.Garris, and J.E.Baur (2005) Scanning Electrochemical Microscopy of Model Neurons: Constant Distance Imaging *Anal. Chem.* **77**:1111-1117.
- Liebetau, J.M., H.M.Miller, J.E.Baur, S.A. Takacs, V. Anupunpisit, P.A.Garris, and D.O.Wipf (2003) Scanning Electrochemical Microscopy of Model Neurons: Imaging and Real-Time Detection of Morphological Changes. *Anal. Chem.* **75**:563-571.
- Slivka, A. and G. Cohen (1985) Hydroxyl Radical Attack on Dopamine *J. Biol. Chem.*
- Sun, P., F.O. Laforge, and M.V. Mirkin (2007) Scanning Electrochemical Microscopy in the 21<sup>st</sup> Century *Phys. Chem. Chem. Phys.* **9**:802-823.
- Travis, E.R. and R.M. Wightman (1998) Spatio-Temporal Resolution of Exocytosis from Individual Cells. *Annu. Rev of Biophys. Biomolec. Struct.* **27**:77-103.