Volume 2, Issue 2

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MetaMatters

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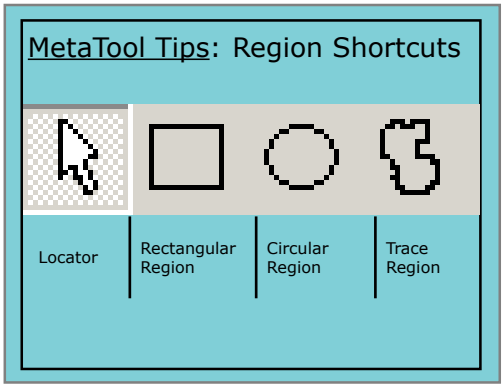
Inside this issue:



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FOCUS: Graphing Astrocytic Calcium Intensity with MetaMorph® Software

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Changes in intracellular calcium are one of the most widely used mechanisms of signaling in cells and are involved in processes such as cell movement, neurotransmitter release, cell division, and more. Many efforts have been made to monitor these calcium fluctuations both *in vivo* and *in vitro* with fluorescent calcium indicators. We have used a non-ratiometric indicator, X-Rhod-1, and 2-photon microscopy to study intrinsic *in vivo* astrocytic calcium signaling in a mouse model of Alzheimer's disease. Dysfunctional astrocytric Ca^{2+} signaling has been found in several diseases or disorders associated with the brain, such as epilepsy, traumatic brain injury, and Alzheimer's disease. Due to the significance of Ca^{2+} in astrocytic signaling, the observed dysfunctions are believed to play an important role in the pathology of such disorders.

We have used the "graph intensities" MetaMorph application to analyze the astrocytic calcium signaling observed in a mouse model of Alzheimer's disease. An advantage to using MetaMorph software is the ability to import T-Series sets as their native 12-bit format, thus maintaining all information collected. Our data are collected as .tiff files, which are easily handled by MetaMorph software, and opened as a tiff image sequence. Once opened in the software, we applied pseudocoloring to the series. (***con't pg.3***)

FOCUS: Graphing Astrocytic Calcium Intensity with MetaMorph® Software

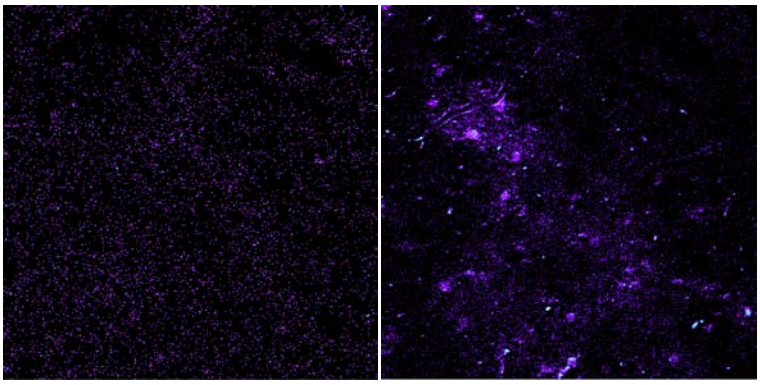


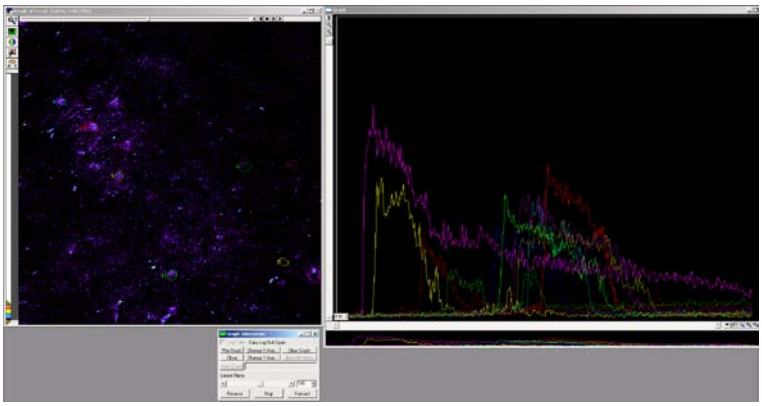
Figure 1- The ΔF/F0 for a non-signaling frame (A), and a frame containing multiple astrocytic signals (B).

(***continued from page 2***)

This displays an increase in fluorescence, which indicates an increase in intracellular calcium, as warmer colors. An important step in analyzing any type of intensity over time data is the removal of background noise and the generation of the ΔF/F0 signal. For our data background, F0, we selected the first five frames of the T-Series and compiled an averaged stack image. Using the MetaMorph arithmetic tools, we then subtracted the averaged image from all frames of the T-Series (F-F0), producing ΔF for each frame. Next, we divided all ΔF frames by the averaged image to generate the ΔF/F0 for each image frame (Fig 1). It is from the ΔF/F0 T-Series that we analyze all calcium signal intensities.

The fluorescence intensity over time for each astrocyte must be individually examined, rather than the general change in intensity for the entire image frame. MetaMorph software allows for the selection of multiple astrocytes, or simply regions, within the same frame that can be analyzed simultaneously, which is significantly more efficient than single cell analysis. Individual astrocytes were manually selected with the "trace regions" tool. The region of the astrocyte chosen was the soma, with the trace following the edge of the soma as closely as possible (Fig 2A).

Figure 2, A & B



After the astrocytes of interest were selected, we opened the "graph intensities" module from the "apps" menu. This opened a dialogue box of options entitled "Configure Graph Intensities". For a T-Series such as ours, the options "stack", "plane number", and "average intensity" were selected. After clicking the "ok" button, a new box of parameters, named "Graph Intensities" appeared (Fig 2B). To start graphing the intensities of the selected astrocytes, we hit the "begin" button and the application simultaneously graphed each selected astrocyte into one window (Fig 2C). A right click of the mouse within the graph widow will bring up a menu of options, including graph settings. It is in here that the graph can be customized. Along with graph settings, the right click options menu also includes "show graph data", which will list the intensity values for each astrocyte for each image frame. These data can be logged into Excel for any further analysis.

The "graph intensities" application in MetaMorph software allows us to analyze calcium signals from multiple astrocytes with ease and efficiency. Simultaneous graphing of many cells provides information on dynamics of calcium in the astrocytic network. From this information, it is possible to examine propagation speed of intercellular signaling, distance of signal propagation, and basic kinetics of the intracellular signal. The importance of astrocytic calcium signaling is quickly emerging in a variety of different states of pathology and analysis of such may provide great insights into the mechanisms of diseases and disorders.

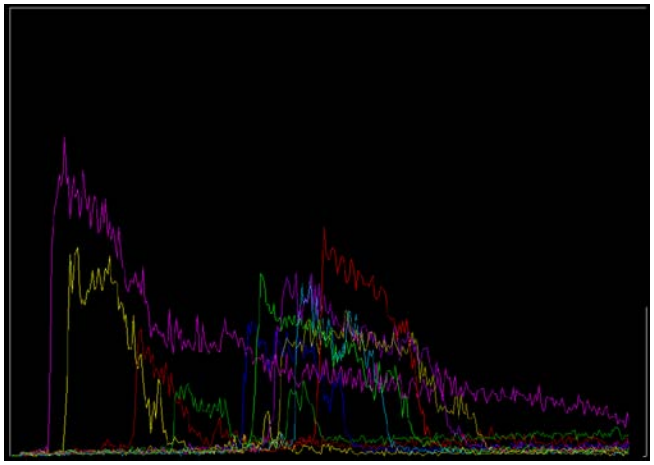


Figure 2C

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→ Note on supported hardware:
Latest SDK from vendor must be downloaded and installed.

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