Improving the "Brainbow" System for Multi-color Cellular Labeling using Epitope-Tags and Antibodies

Abstract

Researchers have recently created a line of genetically modified mice whose neurons express differential levels of three spectrally distinct fluorescent proteins: red, green, and blue. The use of colors helps to distinguish processes and increases resolution, thereby allowing for a broader range of studies which involve neuronal connectivity and its development. However, this method is limited by the fact that fluorescent protein expression is weak during the first ten postnatal days, when circuits are remodeling. Furthermore, even in adult mice, the intensity of the fluorescent signal may not be strong enough to discern certain thin processes.

In order to address these issues, I developed a new method using modified versions of three spectrally distinct GFP variants that can be simultaneously labeled with antibodies. I have shown that the constructs including the antibody tags reliably maintain the endogenous Brainbow stochastic color distribution in vitro. We have successfully electroporated and labeled mouse retinal ganglion cells with each of the modified proteins, thereby demonstrating that the constructs are functional in vivo. We show that antibody staining maintains strong fluorescence intensity throughout the labeled cell, whereas the endogenous fluorescence is very bright around the nucleus but dim in the distal regions of the axonal arbors, where the concentration of the proteins are lowest. However, the preliminary data show that the antibodies did not fully recreate the Brainbow stochastic color distribution.

These results are promising and provide the motivation to continue and create a new mouse line which expresses these modified fluorescent proteins, which contain antibody attachment sites, or epitope tags, in the Brainbow construct. In order to acquire any type of connectivity information from the confocal data, researchers also need software capable of correctly segmenting and identifying connections in neuronal networks. We have developed algorithms designed to extract such information from the microscopy data in a more time efficient manner than previous computation methods.