



CALIFORNIA SCIENCE & ENGINEERING FAIR 2018 PROJECT SUMMARY

Name(s) Kalyan Nath	Project Number 38792
Project Title Phasor Characterization of "Hidden" Huntington Inclusions	
Objectives/Goals Normally, the presence of Huntington's disease (HD) is marked by an aggregation (inclusion) of the diseased huntingtin protein, which is visible via standard electron microscopy. However, research report that these inclusions may not be visible always. Hypothesis: The Fluorescence Lifetime Imaging (FLIM) technique, used in this study, will reveal the "hidden" inclusions in diseased cells, and "g" value analysis will show the difference between healthy and diseased cells. Methods/Materials PC12 cells (rat neuronal cells), Green Fluorescent Protein(GFP), SimFCS (FLIM analysis tool) I transfected 16 cells with the diseased (97q) huntingtin protein. Then, I tagged these diseased as well as 13 healthy (25q) huntingtin protein with GFP. After imaging the cells with the Carl Zeiss microscope, I fed them into the SimFCS program, developed by Dr. Gratton, which created a phasor plot of all the pixels of the image. From this graph, I could pinpoint the location of "hidden" inclusions and determined the difference in "g" values between diseased and healthy huntingtin protein. Results After combing through each data point, I isolated specific points which highlighted the "hidden" inclusions graphically. The x-coordinate of these points represented the "g" values for the particle of interest. The average "g" value of the diseased inclusions was .4646 and the healthy protein was .5839. From the phasor plot, I also determined the range of "g" values: for Healthy inclusion: 388 - .672; for hidden diseased inclusion: .665-.951. Conclusions/Discussion The findings addressed the study's hypothesis. Researchers may now use FLIM to visualize the "hidden" inclusions, and use the g-value ranges that I derived, to determine whether they are looking at a diseased inclusion or a healthy protein. This procedure will allow them to diagnose the disease in patients who would have previously left undiagnosed, hence untreated. In future, researchers may use magnetic resonance imaging to view the inclusions.	
Summary Statement My project aimed to use Fluorescent Lifetime Imaging to reveal the existence of Huntington's Disease in cells that do not visibly display signs of the disease.	
Help Received My mentor, Mrs. Sara Sameni helped prepare the cells on the weekends when I was not available and verify that my data seemed logical.	