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## PHARMACY

### Development of an Imaging Analysis Algorithm for Fluorescence-Based High Content Screening in Parkinson's Disease

**Student researcher: Ryan Arlinghaus, Senior**

Parkinson′s disease (PD) is the second most common neurodegenerative disorder. It affects over half a million individuals in the U.S., with another 50,000 diagnosed every year. PD is characterized by motor dysfunction caused by a progressive loss of dopaminergic neurons. Currently, there are no treatments that slow the progression of the disease, which highlights the need for the development of new therapies.

High content screening (HCS) is a drug discovery method that could be leveraged in cellular models of PD to assess the therapeutic viability of thousands of compounds. However, HCS is challenging as it requires rapid analysis of large volumes of data from images of fluorescently labeled cells.

In our study, carried out in the laboratory of Dr. Chris Rochet, we are developing an automated protocol for use in HCS. Our HCS will monitor cellular uptake of fluorescently labeled, misfolded protein aggregates called fibrillar seeds, which promote protein accumulation and clumping. Fibrillar seeds are believed to be the species responsible for the propagation of neuropathology in the brains of PD patients by inducing abnormal protein folding and aggregation in neurons, as is also true for other neurodegenerative diseases. Synthetic preformed fibrils (PFFs) labeled with fluorescent tags are administered to neuron cell cultures, and specific uptake of these fibrils in primary midbrain neurons is monitored (see image). Recent plugins developed within ImageJ, an image analysis program, have been used to automatically recognize regions of co-localized fluorescence and calculate average intensity from these regions. We are designing fluorescence-based methods and image analysis plugins that can be used to visualize and quantify the presence of PFFs in the interior of the cell. Our HCS approach will be used to identify protective compounds with the potential to prevent PD progression.

*Graduate student mentor Sayan Dutta writes: "Ryan's research addresses a key piece of a bigger puzzle of finding effective drug molecules to slow down Parkinson's disease. His current efforts in designing a fluorescent probe and image analysis algorithm are making our high content screening project move forward significantly."*

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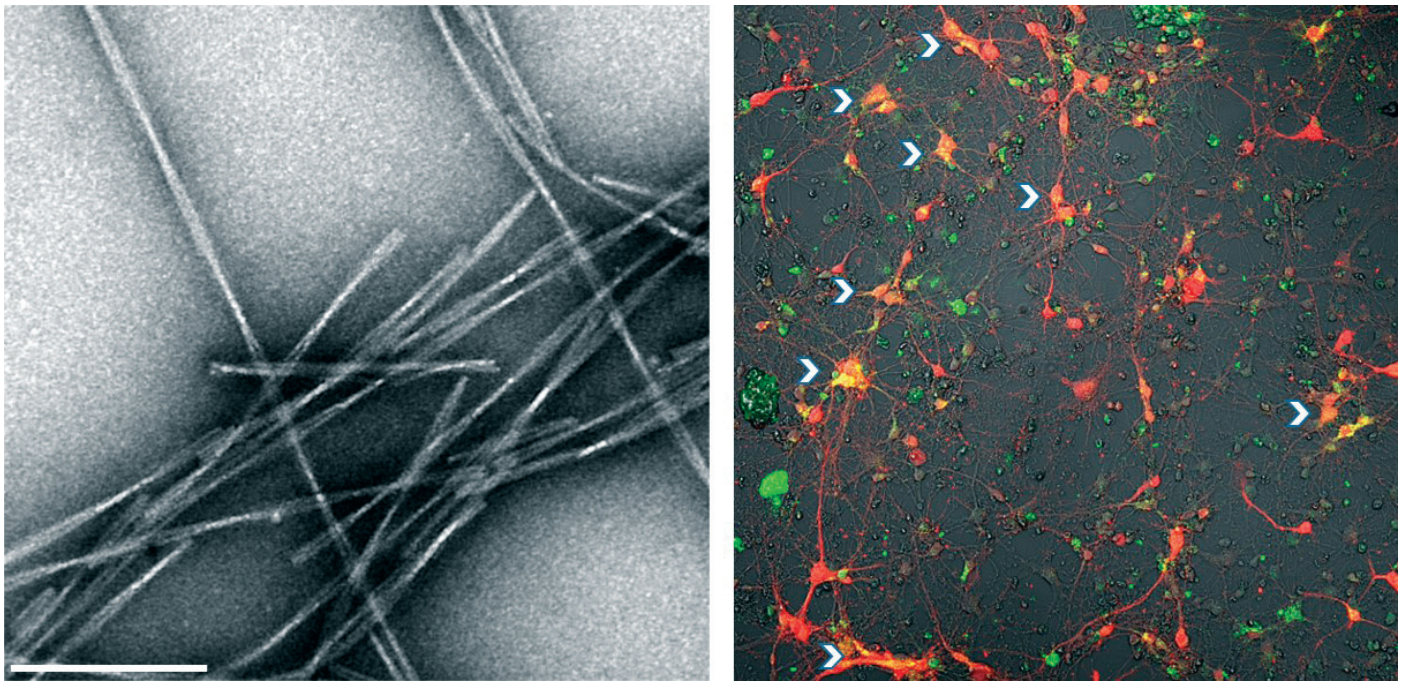

Evidence of aSyn PFF uptake in primary midbrain neurons. (Left) Transmission electron microscopy images of fibrils formed by unmodified human WT aSyn; scale bar = 200 nm. (Right) Primary midbrain cultures transduced with virus-encoding RFP downstream of the neuron-specific synapsin promoter were treated with PFFs derived from recombinant human WT aSyn labeled with Alexa 488. After 24 hrs, the cultures were treated with trypan blue for 15 min to quench extracellular fluorescence and imaged via confocal microscopy. Green fluorescent puncta corresponding to internalized, Alexa 488-labeled aSyn were detected in neurons that appear red because of RFP expression (the yellow color corresponds to colocalized green and red signals). (Image retrieved with help of Dr. Ranjan Sengupta and Sayan Dutta.)

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