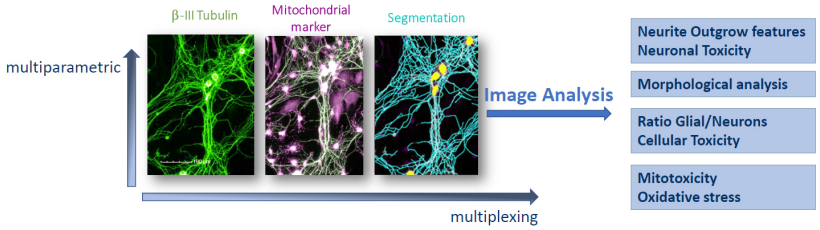
Applications of High Content Screening in Early Drug Discovery

High content screening (HCS) is a powerful approach that combines cell based assays and image analysis to study and characterize the effect of bioactive compounds on biological pathways within the complex cellular environment. Axxam's high-content platform can track multiple phenotypes at the cellular and subcellular levels to get insights into pathways and mechanisms of action involved in several human neurodegenerative diseases, such as Alzheimer's, Parkinson's and neuronal inflammation. Multiple assays can be customized for many disease models, and new assays can also be developed upon request.

Neuropathic Pain Platform

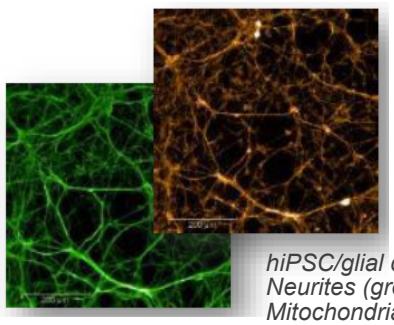
Neuropathic pain is often associated with cancer chemotherapy as side effect, limiting the dose that can be administered to patients, thus reducing the efficacy of the chemotherapy treatment. It is important to identify neuroprotective agents able to mitigate neuropathic pain to increase the efficacy of drug treatments.



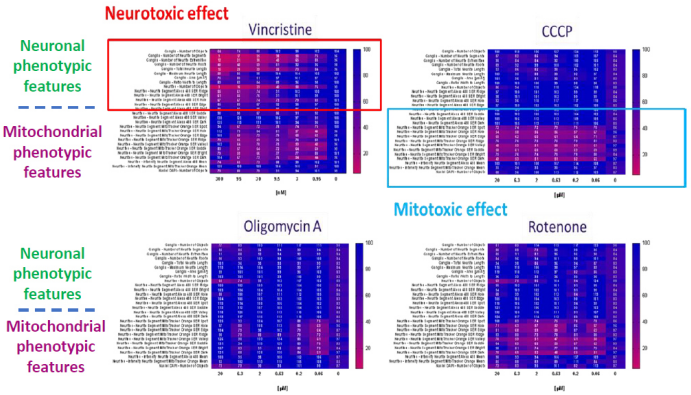
Key information from phenotypic analysis (inc. cell painting)

Relevant cell models for neuropathic pain: primary and iPS-derived sensory neurons

A miniaturized in vitro assay for neuropathic pain has been established with both rat primary sensory neurons and human iPSC derived sensory neurons in co-culture with glial cells. This assay can be used to assess the neuroprotective effect of drugs on neurotoxicity and mitochondrial toxicity.



hiPSC/glial co-cultures: Neurites (green), Mitochondria (red)



Assay on Dorsal Root Ganglion

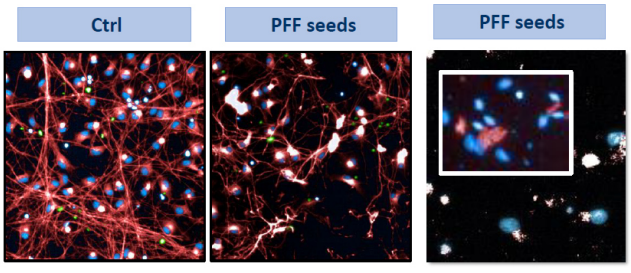
Project under NGN-PET - IMI2-2015-07-03
Grant agreement no. 116072

Neurodegeneration / Inflammation Platform

Our platform can be adapted and used for several different neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. One common hallmark of neurodegeneration processes is the formation of pathological aggregates.

Assessing Pathological Aggregates

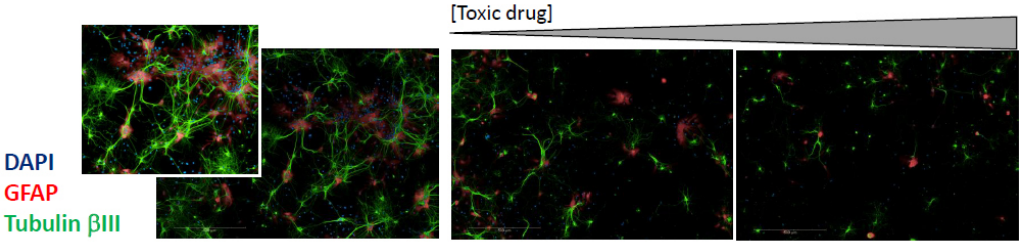
Pathological aggregates can be assessed by immunostaining with a specific antibody and/or marker and the cells analyzed for the number of aggregates present in the cell population upon treatments.



Images of hiPSC derived neurons analyzed for the onset of pathological aggregates (white spots, middle panel; red spots right panel)

Neurite Outgrowth Analysis to Reveal Neurotoxicity

A co-culture of hippocampal primary cells and primary glial cells treated with different neurotoxic and mitotoxic drugs, shows the reduction of neurites, indicating the onset of neurological disorders.



Primary hippocampal cells in co-culture with primary glial cells. Glial cells identified by red and neurons by green staining, respectively.

Effect of increasing toxic drug concentration leads to neurite reduction

Cells provided by Neuro-Zone Srl (https://www.neuro-zone.com)

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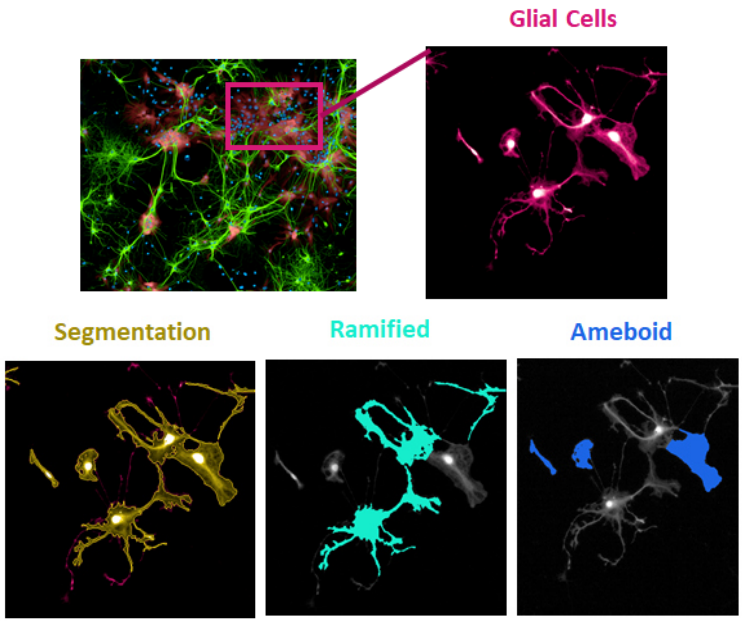


Images showing glial cells with different shapes

Cells provided by Neuro-Zone Srl (https://www.neuro-zone.com)

Neuro-Inflammation Related to Activation of Glial Cells

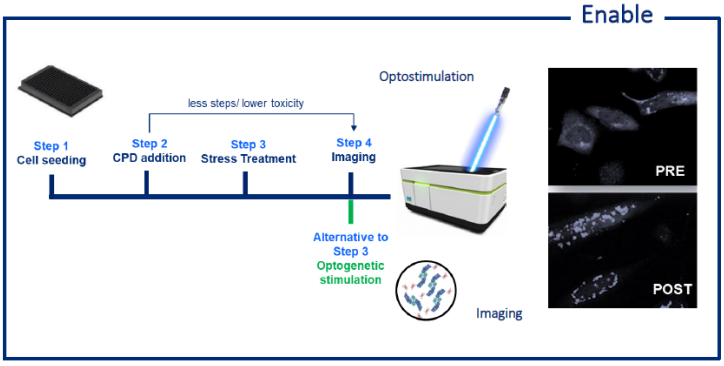
Activation of glial cells also involves a transition of their morphologies. Under physiological conditions they appear starred and ramified, while under stress they start to retreat assuming an ameboid phenotype characterized by an increased motility and phagocytotic activity. Thus, glial cell morphological analysis can be used to evaluate the contribution of glial cells to the progression of neuro-inflammation.

Pro-Inflammatory Markers

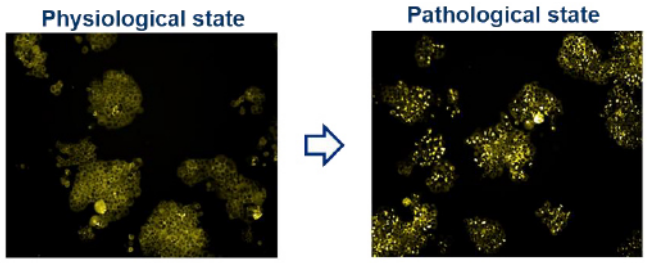
In addition, pro-inflammatory markers, known to increase the expression of inflammation, can be used to follow inflammation, also in relation to different diseases. Compounds that abolish the inflammatory response in glial cells can have a significant therapeutic relevance.

Biomolecular Condensates Platform

Biomolecular condensates are known to be involved in several diseases such as neurodegeneration and cancer. Condensates are organelles without a membrane that regulate many important and complex metabolic pathways, such as protein and RNA interaction, RNA metabolism, DNA replication and also the DNA repair mechanism. Under certain conditions these membrane-less organelles can become insoluble, leading to the formation of pathological aggregates. Axxam has combined its expertise in assay miniaturization with optogenetics and imaging to develop an innovative platform to assess the involvement of these relevant organelles on different pathways and in different diseases.



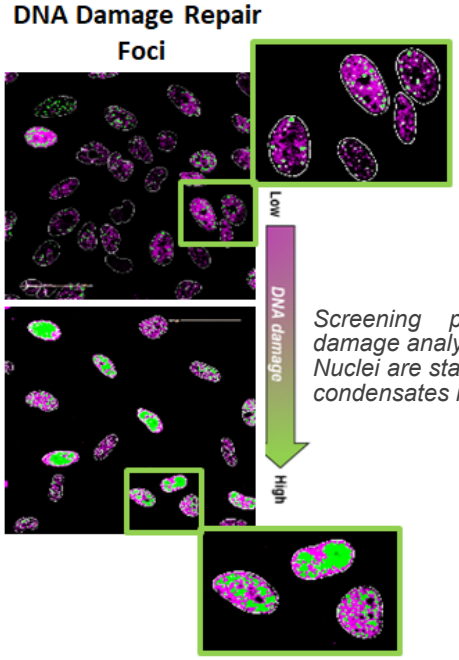
Optogenetic stimulation can be used to induce pathological aggregates



Images showing the physiological and pathological aggregates states of a specific target

Assessment for Compounds able to Modulate the Quantity of Condensates and Aggregates in Cells

Reporter cell lines for a target involved in the onset of pathological aggregates are treated with compounds, followed by stress stimuli (chemical or optogenetic) known to increase the aggregates. The cells are then imaged, and the images analyzed to obtain insight into the mechanism of action of the specific compound.



Screening platform for DNA damage analysis;
Nuclei are stained in pink and the condensates in green

DNA Damage Repair Assay

This assay follows the generation of condensates known to be involved in the mechanism of damaged DNA repair. Cells, upon a genotoxic insult, are stained for the phosphorylated histone H2AX, as a hallmark of DNA damage. A significant increase in the number of DNA-damaged foci is shown as a green signal in the nucleus. These types of analyses can be used to filter out toxic compounds or find new chemotherapy agents.

All data and images generated at Axxam

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