CALIFORNIA STATE UNIVERSITY SAN MARCOS

PROJECT SIGNATURE PAGE

PROJECT SUBMITTED IN PARTIAL FULLFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

PROJECT TITLE: The Investigation of Specific Genetic Alleles and External Factors as a Determinant of Increased Neural Cell Cytotoxicity in Alzheimer 's Disease

AUTHOR: Cherie Handley

DATE OF SUCCESSFUL DEFENSE: August 2, 2019

THE PROJECT HAS BEEN ACCEPTED BY THE PROJECT COMMITTEE IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOTECHNOLOGY.

Patrick McDonough, PhD	Patrick McDonough	7/31/2019
PROJECT COMMITTEE CHAIR	SIGNATURE	DATE
Betsy Read, PhD	Betsy Read	8/7/2019
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Tetsuya Kawamura, PhD		8/2/19
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ABSTRACT

The Investigation of Specific Genetic Alleles and External Factors as a Determinant of Increased Neural Cell Cytotoxicity in Alzheimer 's Disease

VALA Sciences

Cherie Handley

Professional Master's Degree Program

Cal State University San Marcos

Objective Alzheimer's disease is a neurodegenerative disorder in the aged population in which memory is lost. It is a leading cause of dementia. Pathologically, the ailment is characterized by the aggregation of beta-amyloid plaques and tau neurofibrillary in the brain. There is no cure however researchers are investigating the cause of the disease, be it genetic or environmental, as a way to lead to a cure. Live cell imaging provides scientists with the ability to observe live cells via time-lapse photography. The objective of this project is to investigate the role of environmental compounds or genetics in the pathogenesis of the disease. The lab accomplishes this by developing a cell-based cytotoxicity assay using Kinetic Imaging Cytometers (KIC). These instruments are capable of imaging fluorescently labeled proteins and cell structures. Here we analyze cellular action potentials as well as calcium ion transients. Additionally, post-KIC we stain the cells using Immunocytochemistry (ICC) to observe cellular components of interest. The key to healthy neuronal cell function is to maintain cellular activities at metabolically non-disruptive levels. Our instrument provides a resource in studying the molecular and biochemical pathways for potential therapies.

Methods We investigate the feasibility of developing our assay for use on various cell types as well as to observe the effects of potentially therapeutic or cytotoxic compounds. Upon completion of the feasibility studies our lab will optimize assay parameters, including controls. The lab sourced human induced pluripotent stem cells (hi-PSC) derived iGlutaneurons, excitatory neuronal cell lines. Additionally, we derived other cell types as needed. The cells are exposed to selected compounds at varying doses. We plan to assay isogenic cell lines to study differences among ApoE isotypes and their roles in neuropathology. VALA researchers measure cellular responses post-treatment via quantification of Calcium and Voltage fluorescent indicator signals followed by ICC.

Results The mean peak area for Particulate Matter smaller than 2.5 micrometers, $PM_{2.5,}$ when exposed to ANOVA results, a p-value of 1.68E-03. Dunnet method indicates a statistically significant response at 100 ug/mL dose.

Conclusions $PM_{2.5}$ is significantly different among doses when neurons were exposed for 24 hours. The 24-hour exposure to $PM_{2.5}$ results in increased Calcium signals in our neuronal cell line but eventually at the highest dose was below 0-dose control. There was no significant difference in action potentials. Treatments with ApoE3 and ApoE4 demonstrated a cytotoxic effect with increasing dose. Intracellular calcium eventually waned to below 0 treatment control at the highest dose. Analysis of the raw data is a work in progress. The threshold can vary between active and dead cells. We continue our on-going assay development.

The Investigation of Specific Genetic Alleles and External Factors as a Determinant of Increased Neural Cell Cytotoxicity in Alzheimer ’s Disease

Cherie Handley| Teacher’s name | CSU San Marcos

Are genetic or external factors attributable to Alzheimer's Disease?

Is it feasible to develop a biological assay to differentiate contributing causes of the disease?

Alzheimer’s Disease is a form of dementia marked by a chronic decline in mental processes and can manifest as mild, moderate, or severe.

National Institutes of Health

- Memory
- Daily Living

Physical and mental symptoms of impaired cognitive functions manifest as a person ages.

- Finding the Correct Words to Communicate
- Impaired Decision Making
- Impaired Reasoning
- Loss of Memory
- Inability to Learn New Concepts
- Physical Sensory Decline
- Fitful Behavior

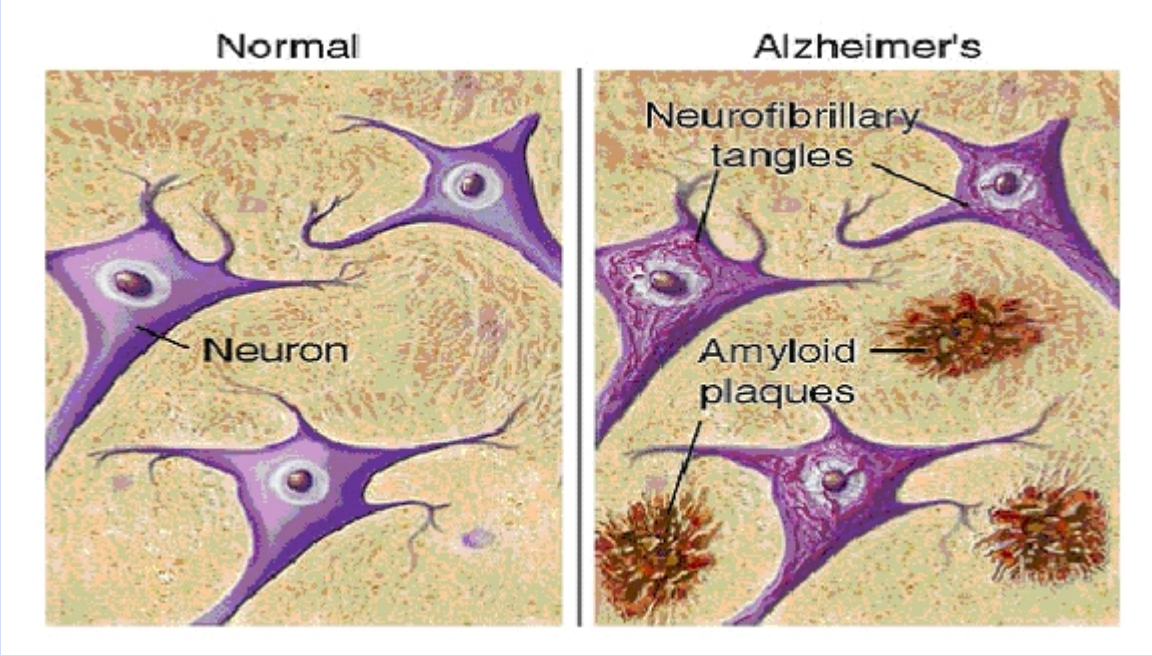
Clinical pathology characterization has revealed traits upon examination of AD brains.

Plaques

- Beta-amyloid

Tangled Fibers

- Tau NFT



Causes stem from genetic or environmental factors.

Genetic

- Mutation

Environmental

- Compounds

Options for Therapy are used in an attempt to treat the symptoms.

- Treatments for Sleep Changes:

- Treatments for Memory

    Cholinesterase Inhibitors
    Memantine

- Treatments for Behavior

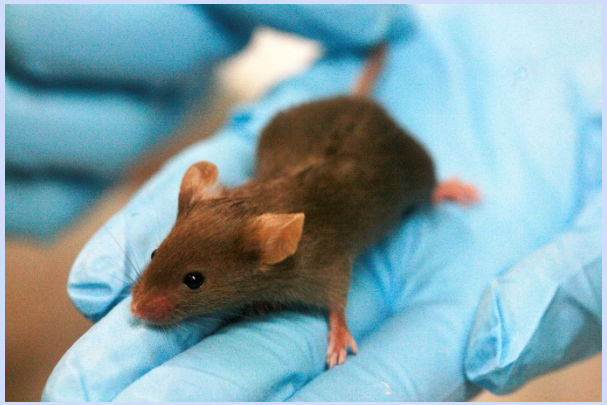
Currently there is no cure for the disease.

Our focus is on the development of an assay to aid researcher's in the study of Alzheimer's Disease.

The assay will help determine if the ApoE status is a determinant in developing AD

Gene Variant	Nucleotide Sequence	Amino Acid	Risk
APOE3	tgc	Cysteine	Normal
APOE4	cgc	Arginine	Increased

Historically, biomedical research is performed on non-human species such as monkeys, mice, insects, viruses, or tumor cell lines to name a few.

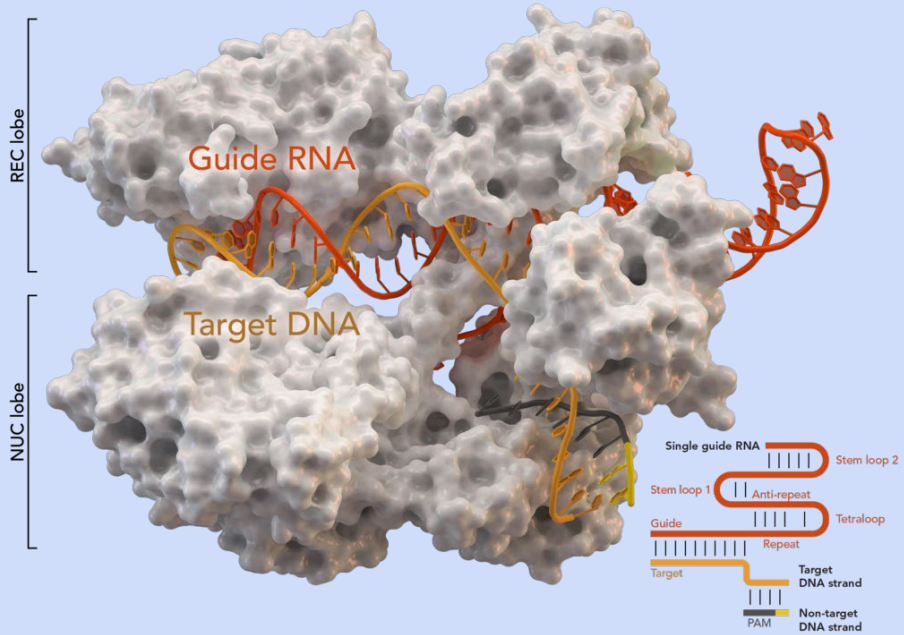


Drawbacks:

- Physiological Variation among species
- Chromosomal abnormalities in tumor cell lines can cause complications

Stem cells provide science the opportunity to study a healthier cell model when compared with tumor derived cell lines.

- Create Specific Cell Models:



- Reduce exposure to toxins during drug screening

Specific Aim #1 is to source our Neuronal Cell Lines.

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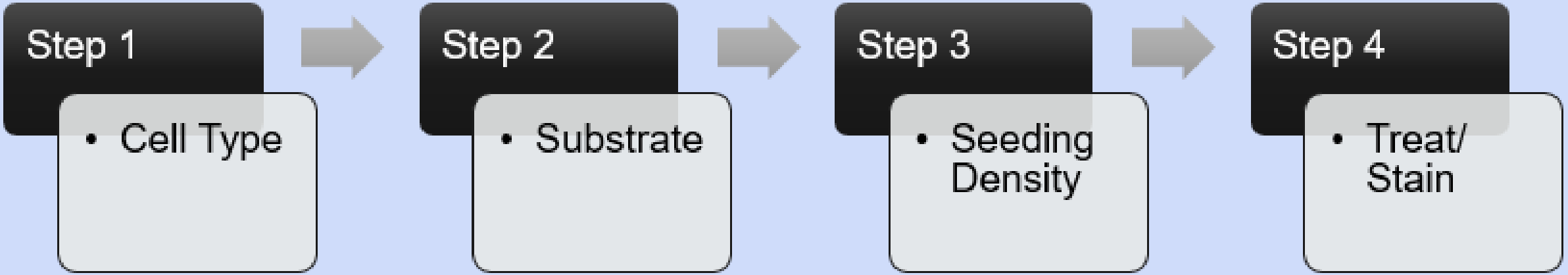
Controlled variables	**Independent variable**	**Dependent variable**
• Isogenic Cell Line	• Engineered Isogenic Cell Line	• Treatment

The most important materials we used are listed below.

Reagents	Equipment	Consumables
Cell Lines	Water Baths	Pipets, Tips
Growth Media	37C Incubator	Culture Vessels
Supplements	Biological Safety Cabinet	0.22 micron filters
Primary Antibodies	Microscope	Centrifuge Tubes
Secondary Antibodies	Centrifuge	
Loading Dyes	Cell Imager	
PBS	Refrigerator	
70% Ethanol	Freezers	

The procedure entails 8 steps to form a conclusion on the feasibility of developing the assay.



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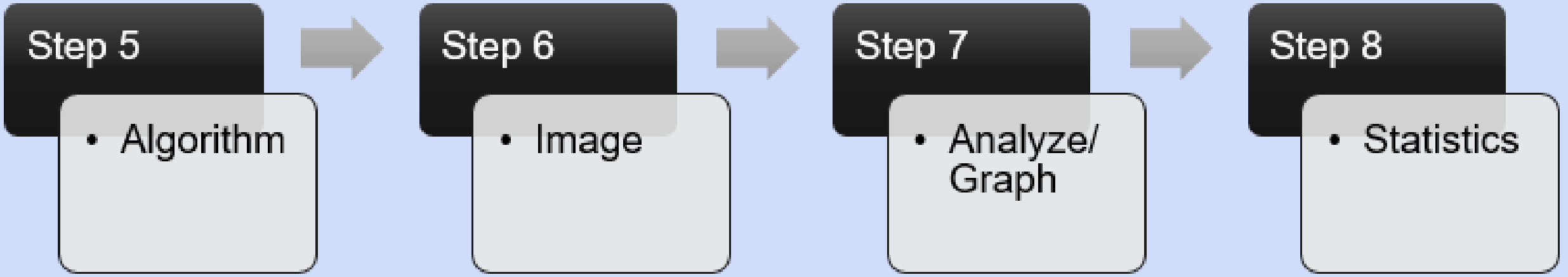
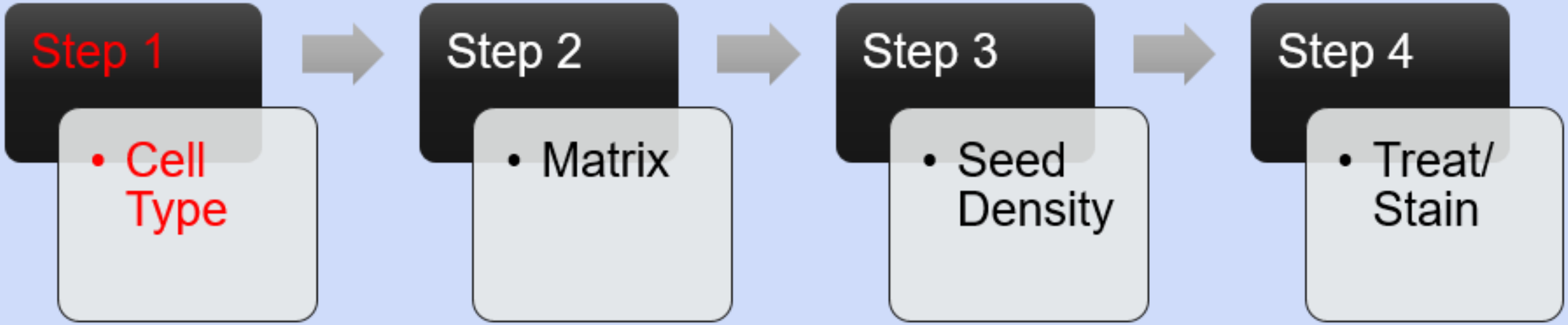
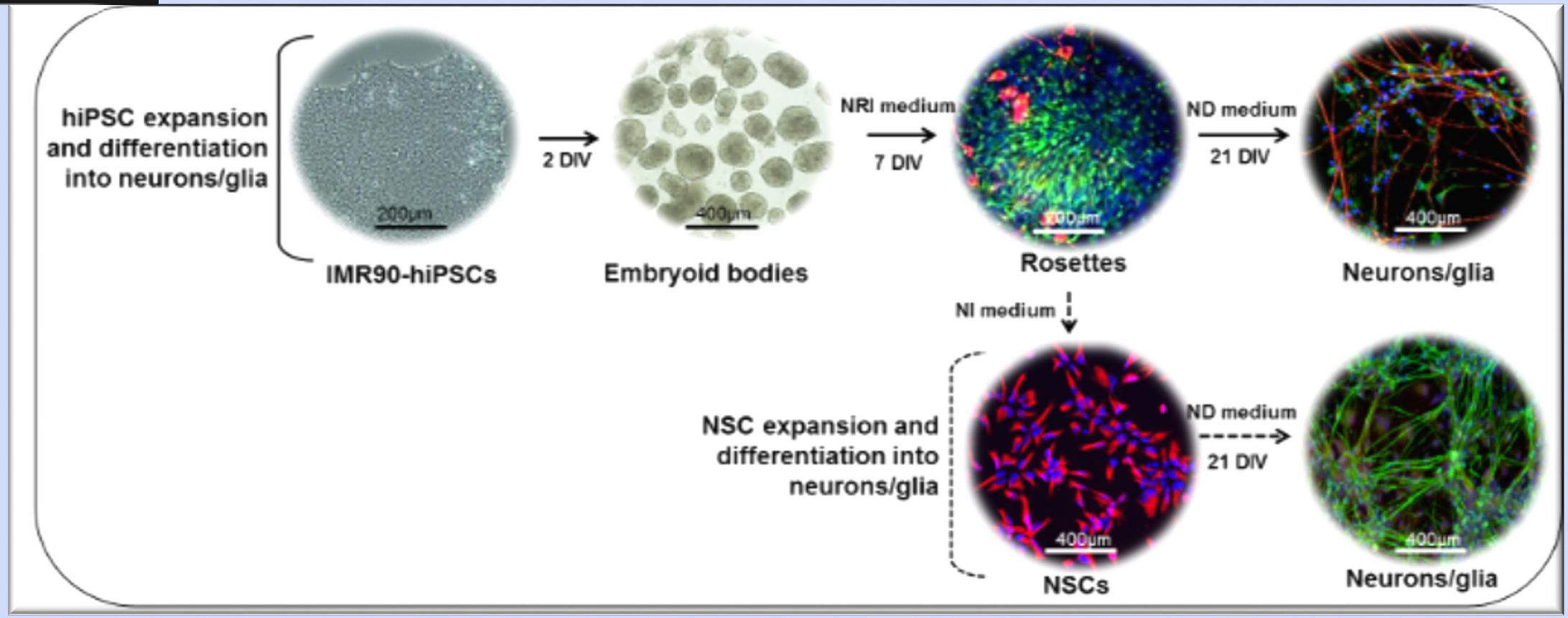


We passage our Induced pluripotent stem cells and differentiate to form specific cell types and we purchase Neurons.



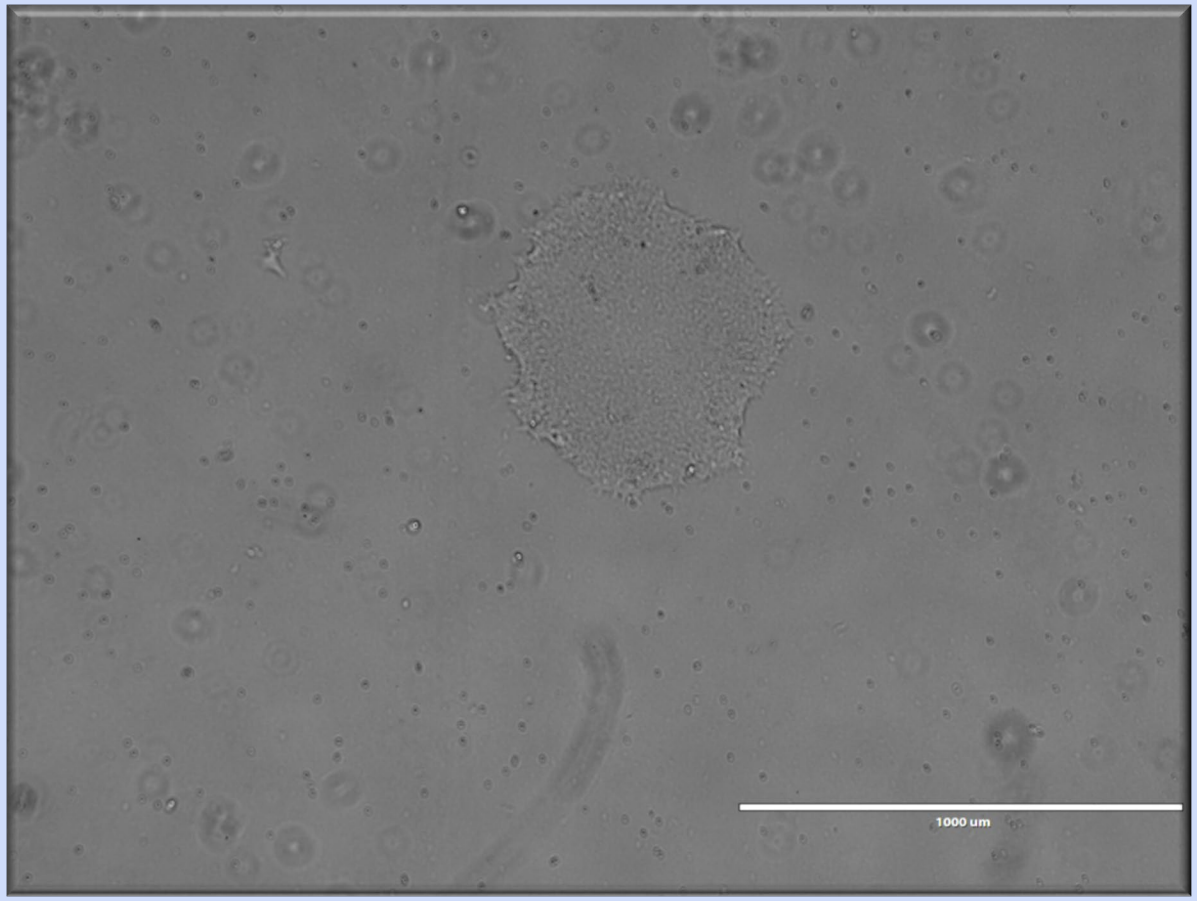
We select cell types based on the portion of the protocol we are currently focusing our efforts: Neurons.

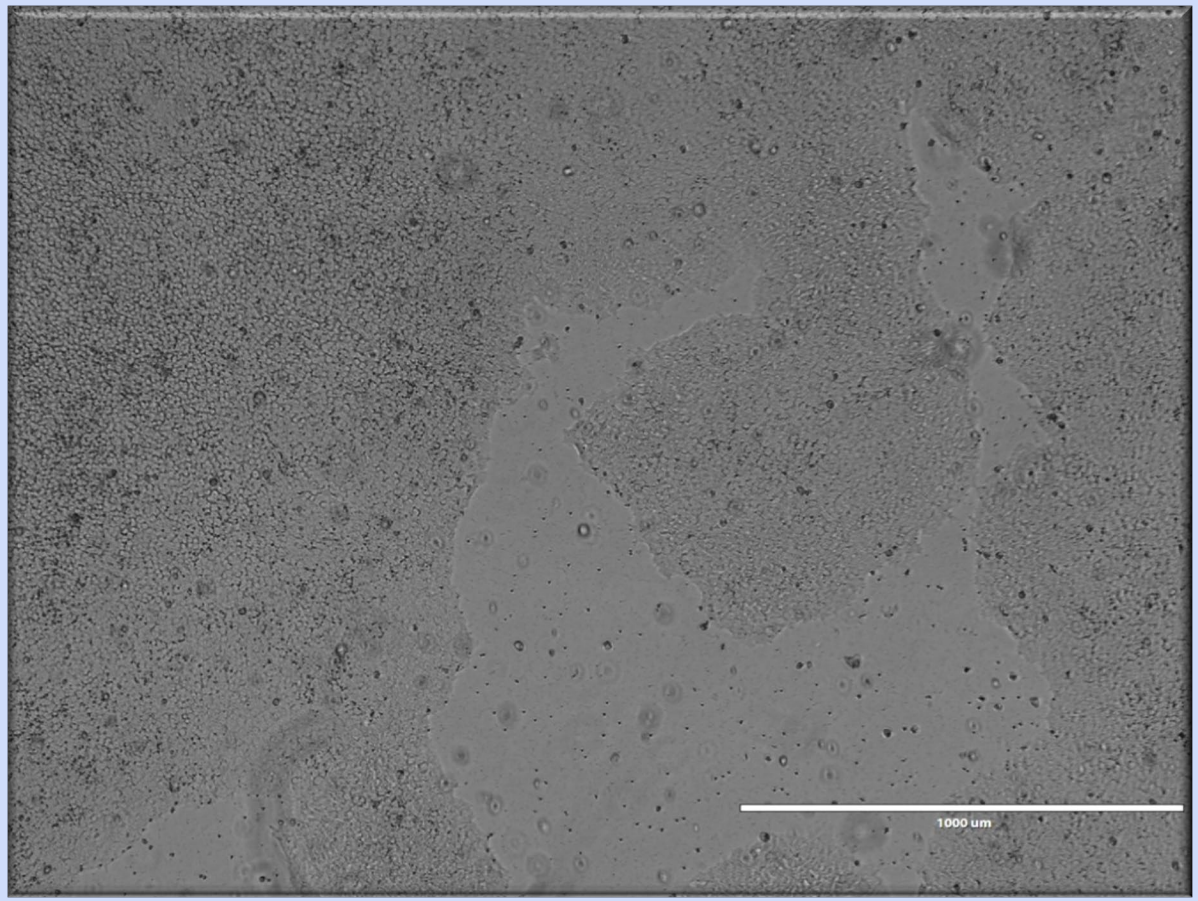
Step 1



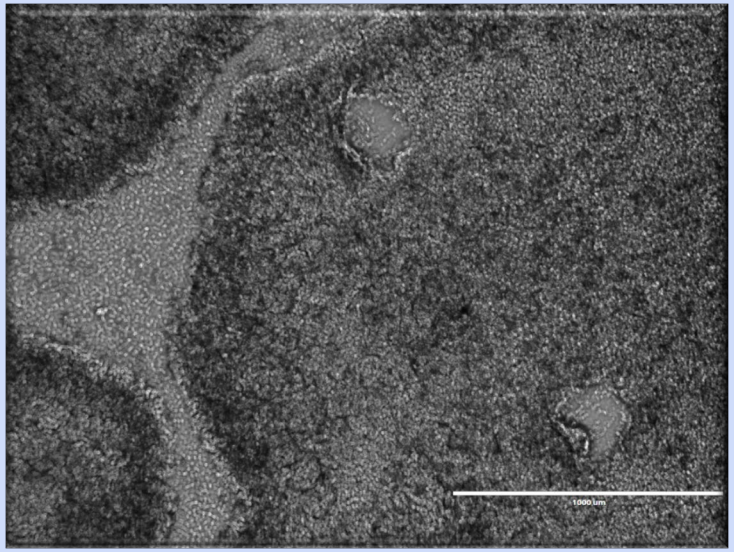
JOVE Developmental Biology Image Link

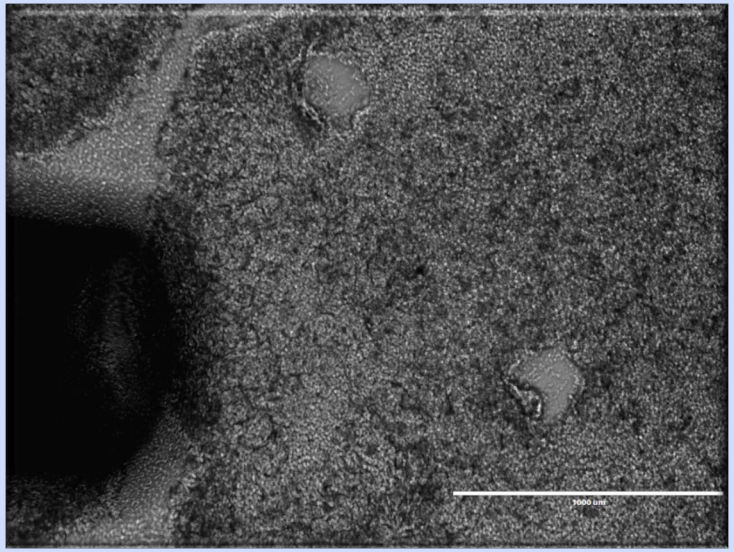
Observations below reveal expansion of iPS colonies and partial differentiation into specific cell types.

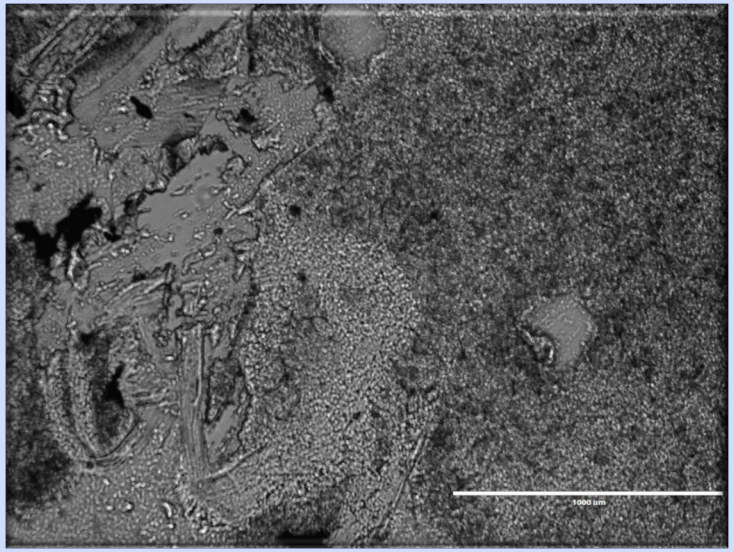




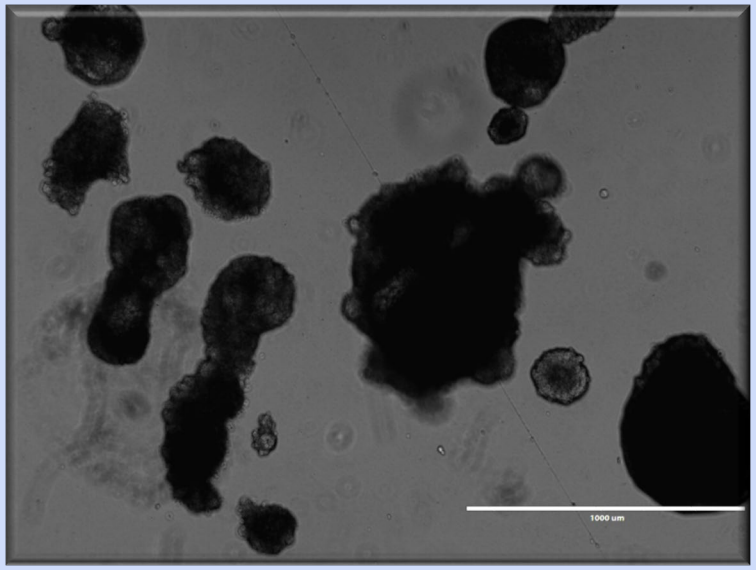
Stem cell colonies displaying loss of pluripotency via differentiation were selectively removed from culture.

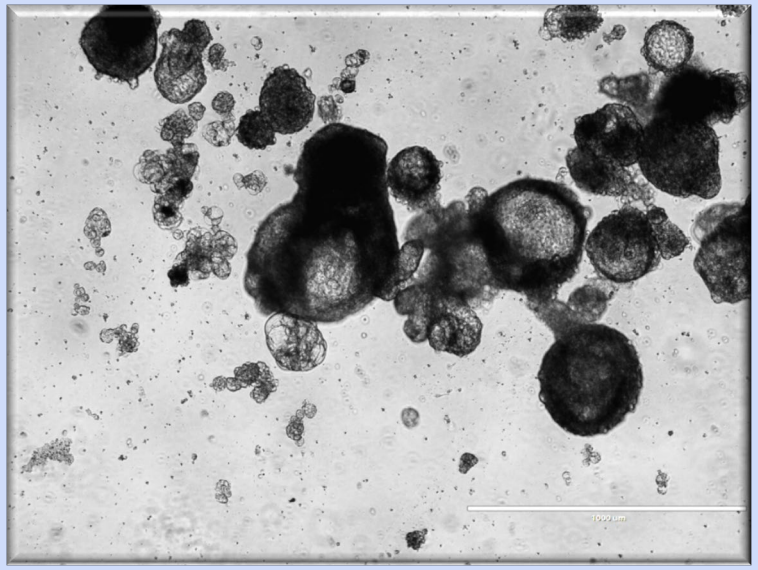


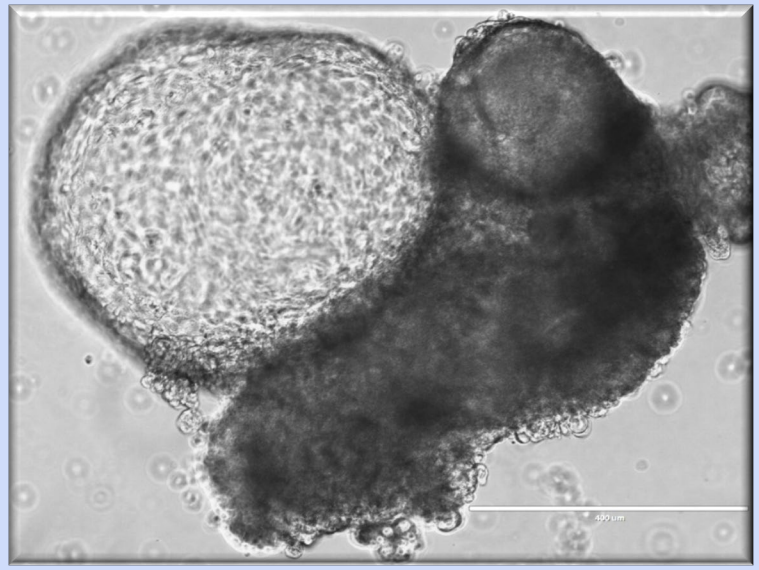




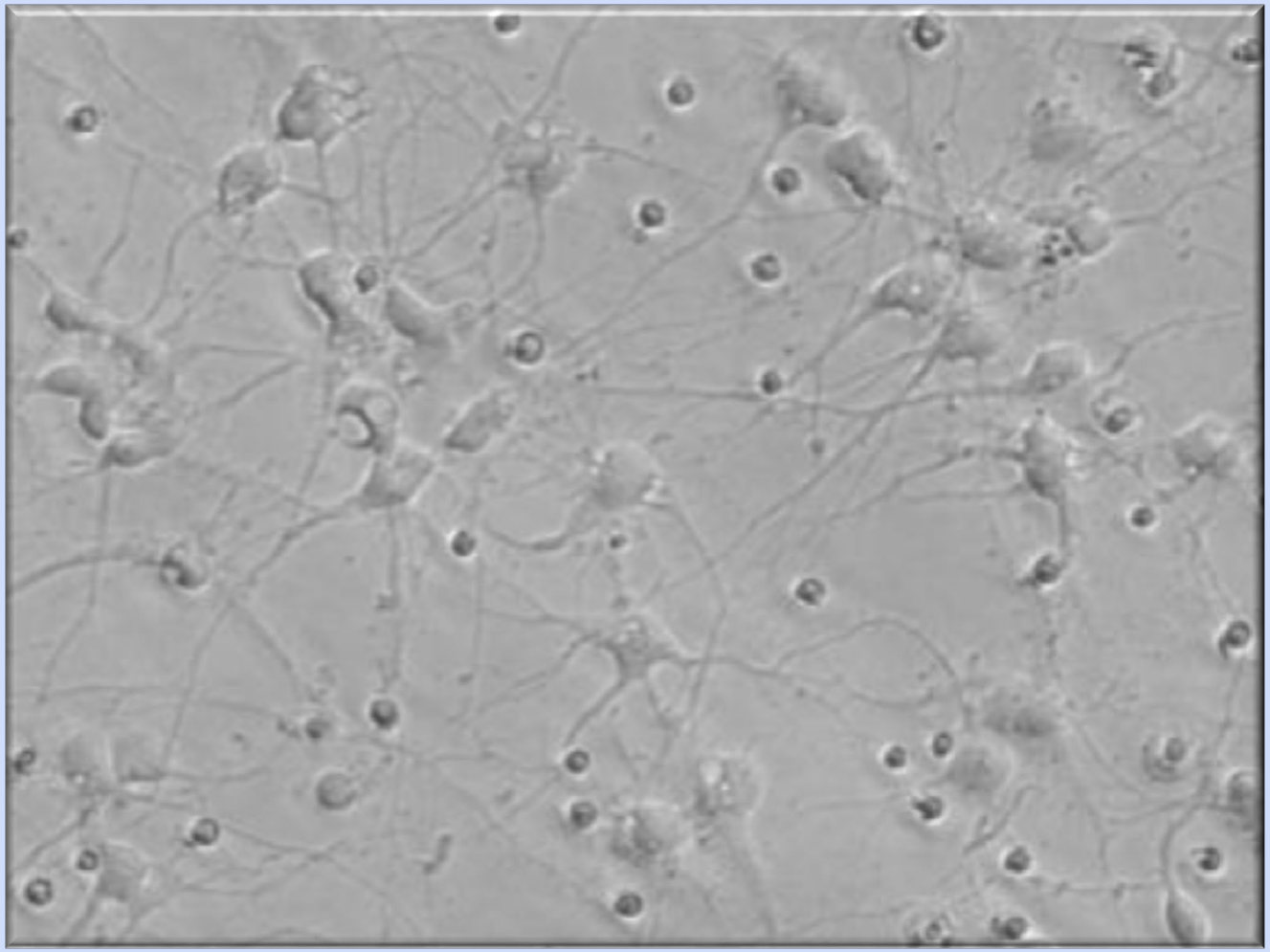
Observations below reveal expansion of iPS colonies and partial differentiation into specific cell types.



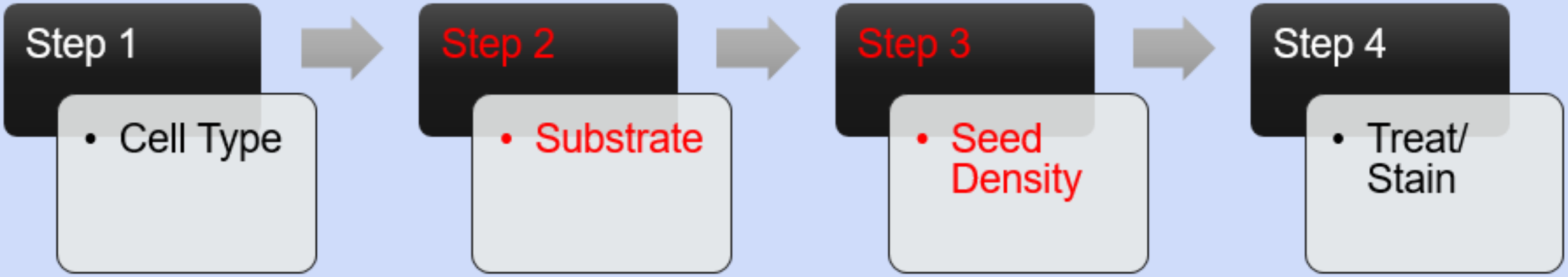




A graphical presentation of certain iPS derived neurons displaying typical cell morphology used during this aspect in the development of our assay.



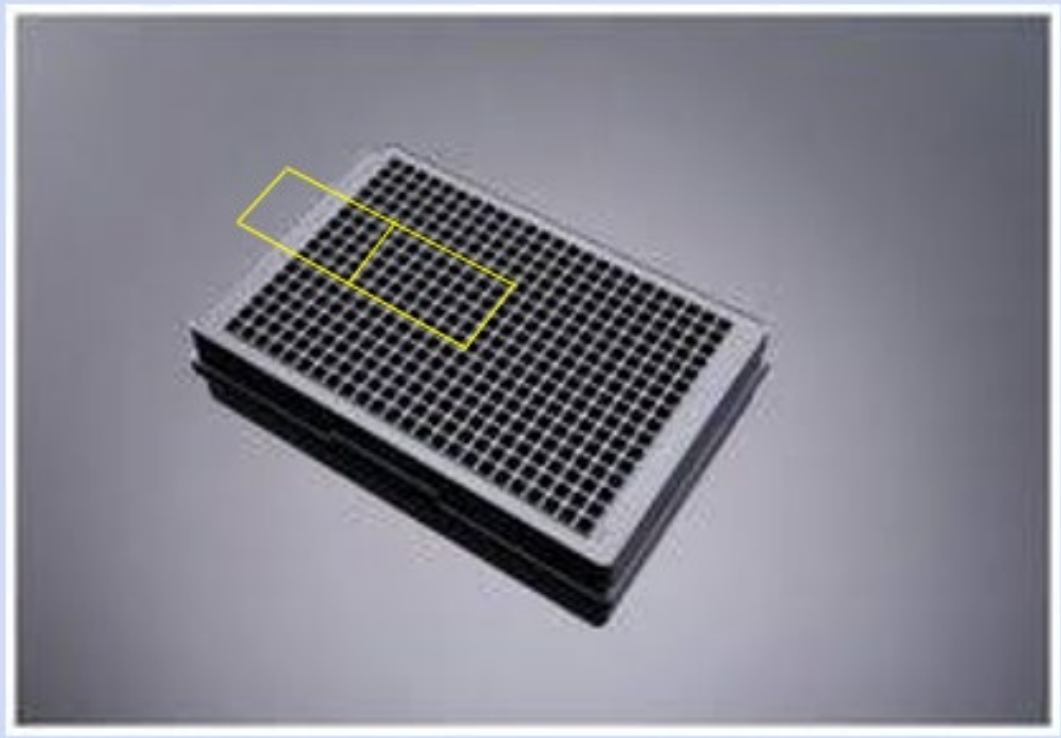
Following stem cell expansion and differentiation or thaw and maintenance we optimize our substrate.



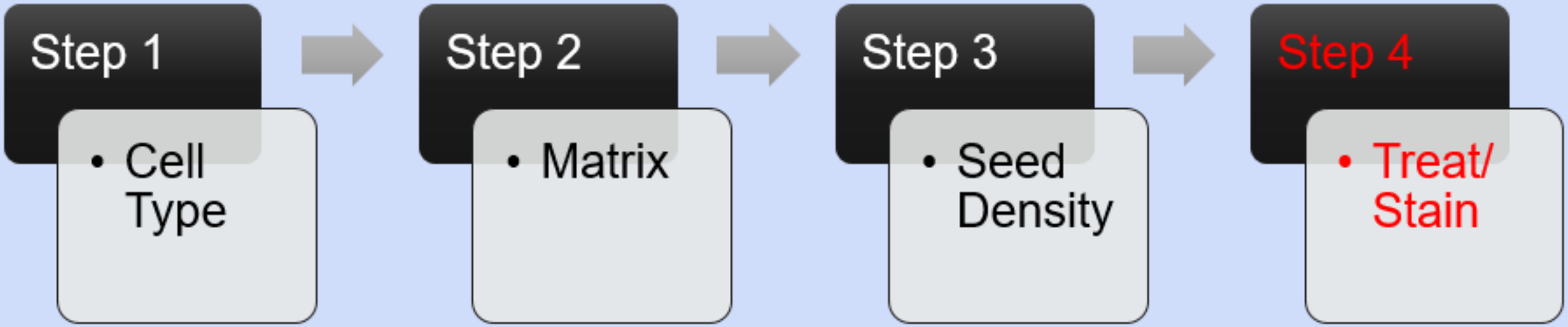
Following selection of target cell type, we plated derived cells on 2 matrix types in 384-well plates to determine the optimum substrate after which we optimized cell density per well.

Step 2

- Tissue Culture Treated
- PEI/Matrigel
- Other Substrates



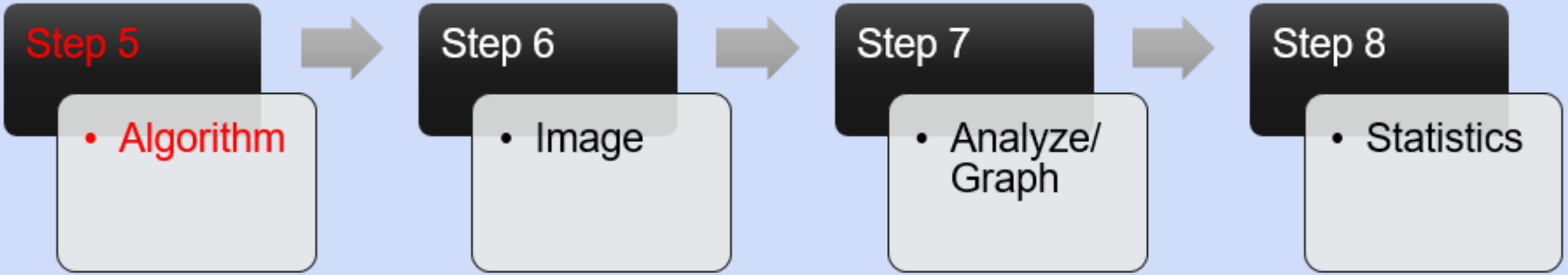
The next step is to treat and stain our cells to observe for Calcium and Voltage.



Using selected compounds we treated the cells temporally and observed for differential effects.

Treatment	Time	Dose
1	4 Hours	0, 6.25,12.5, 25, 50, 100 ug/mL
	24 Hours	
2	4 Hours	
	24 Hours	
3	24 Hours	0, 12.5, 25, 50 ug/mL

Immuno-fluorescent probes are used to visualize specific markers.



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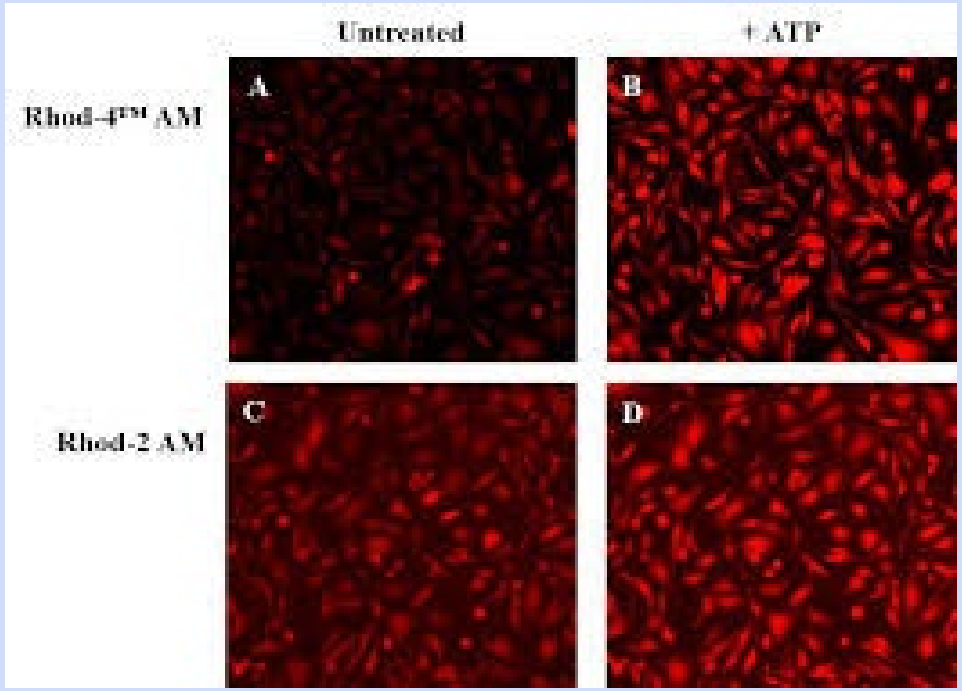
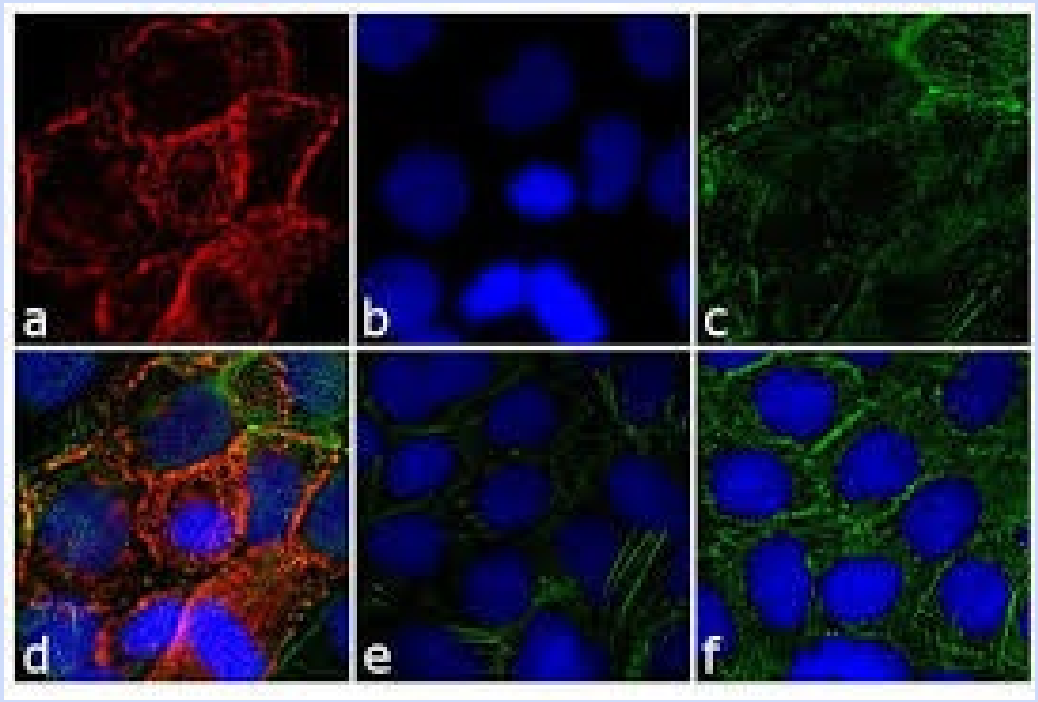
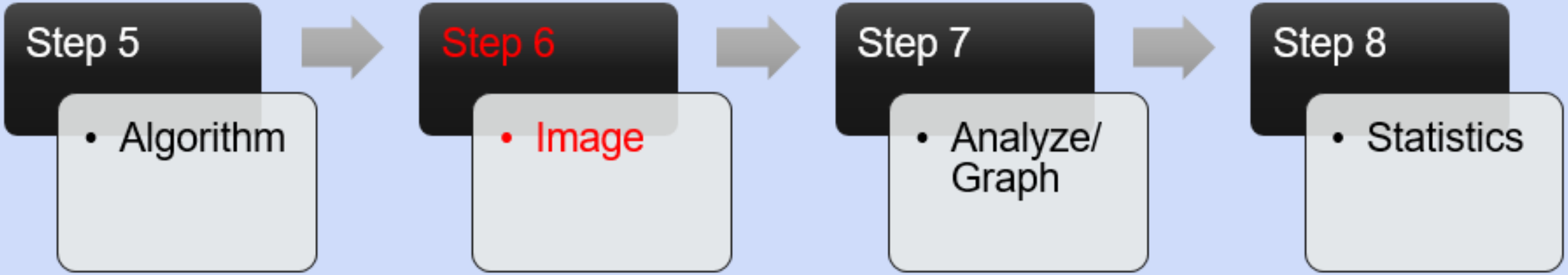
Kinetic Imaging

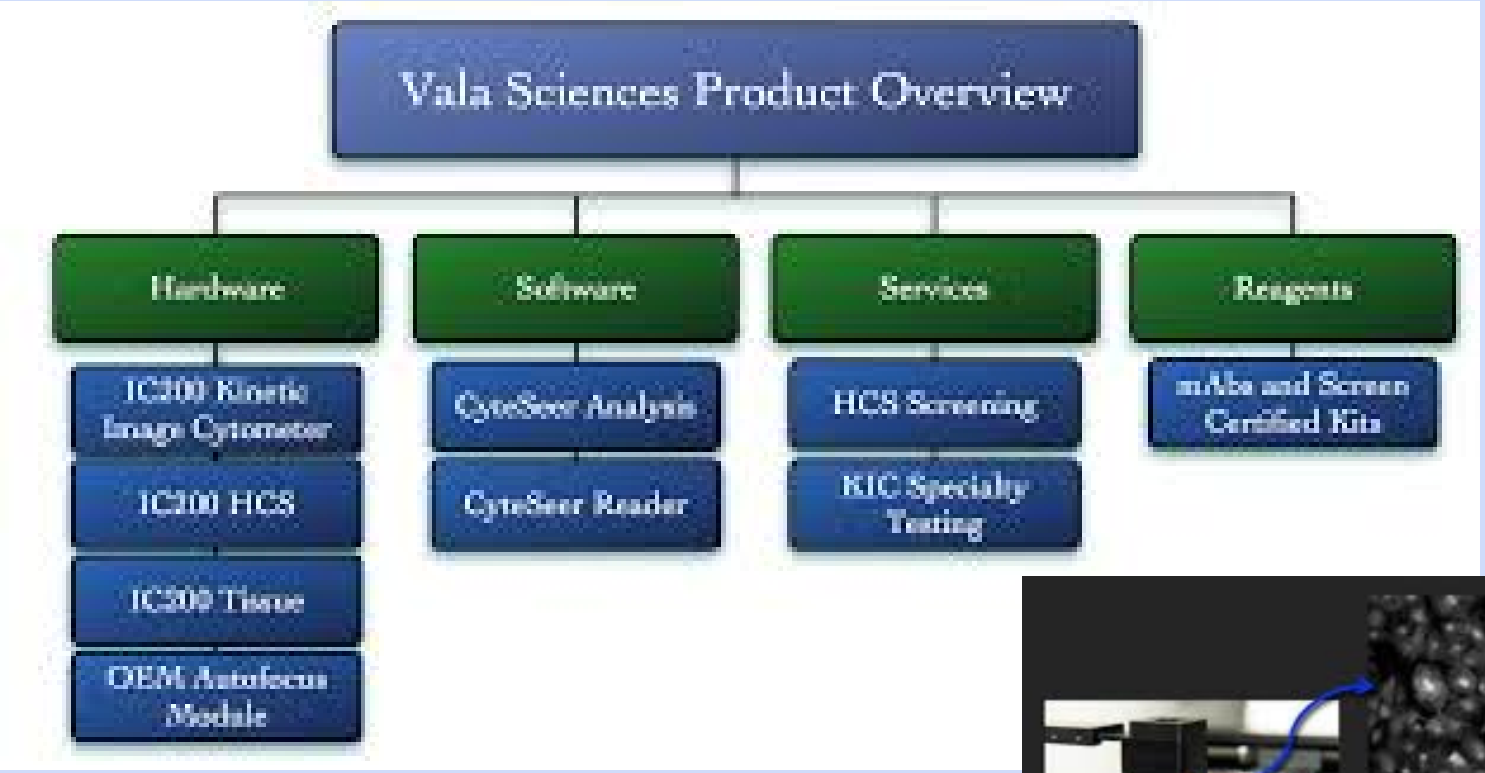


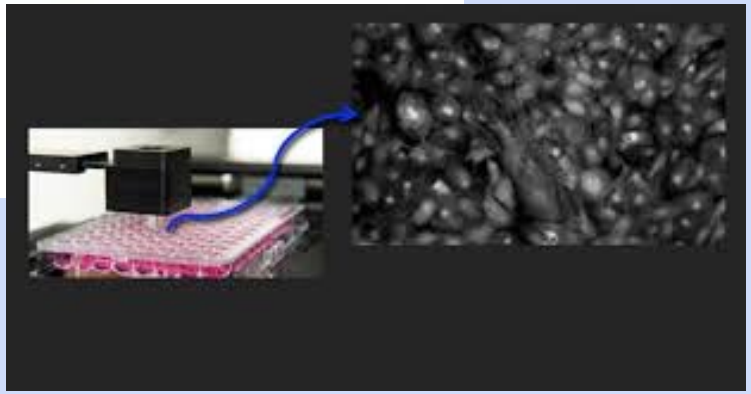
Immunocytochemistry (ICC)



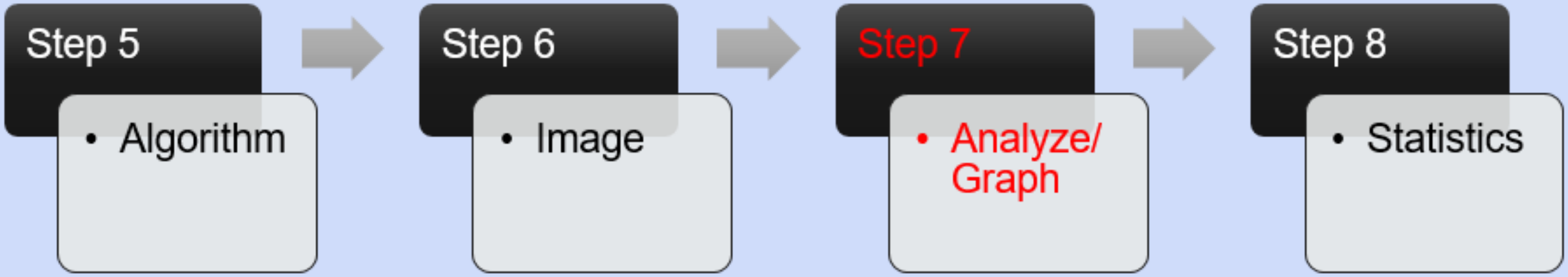

Step 5
• Algorithm
Step 6
• Image
Step 7
• Analyze/ Graph
Step 8
• Statistics

Following labeling of the cells we image the plates using IC200-KIC



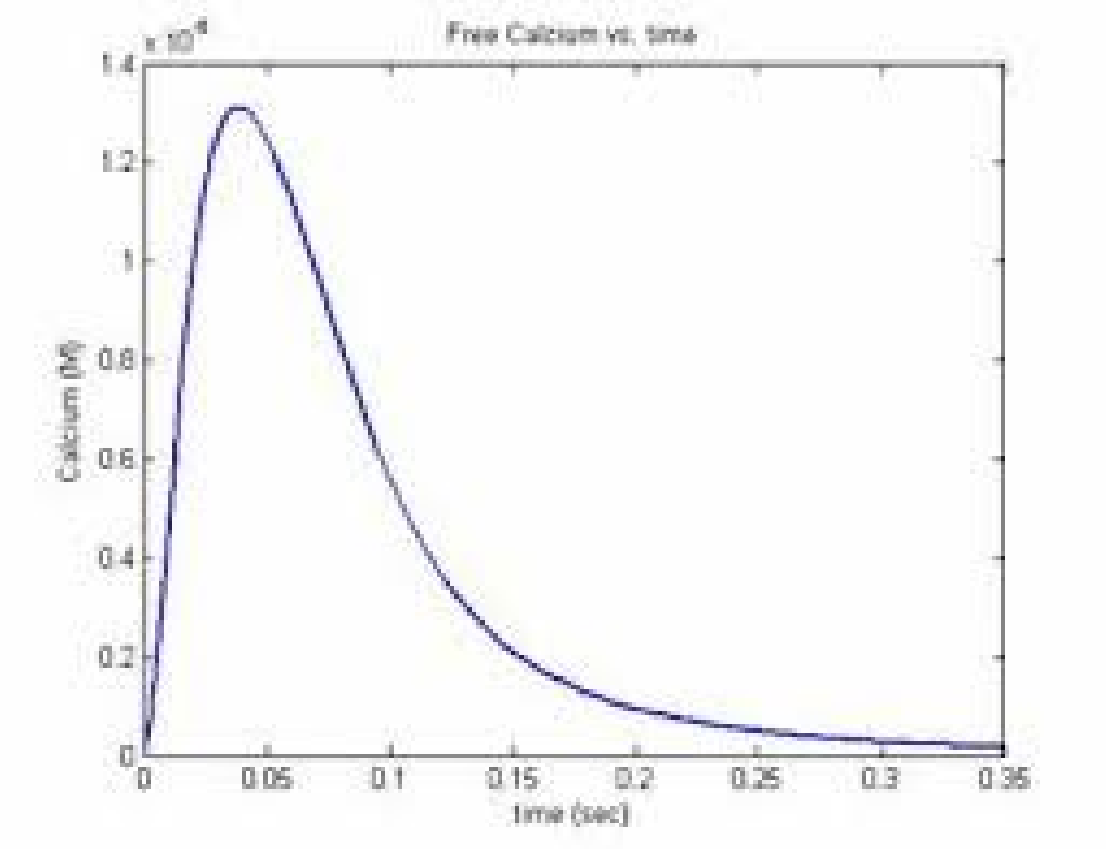


Immuno-fluorescent probes are used to visualize specific markers.



Calcium transients as well as Voltage are quantified per well, graphed and analyzed for each condition.

Calcium



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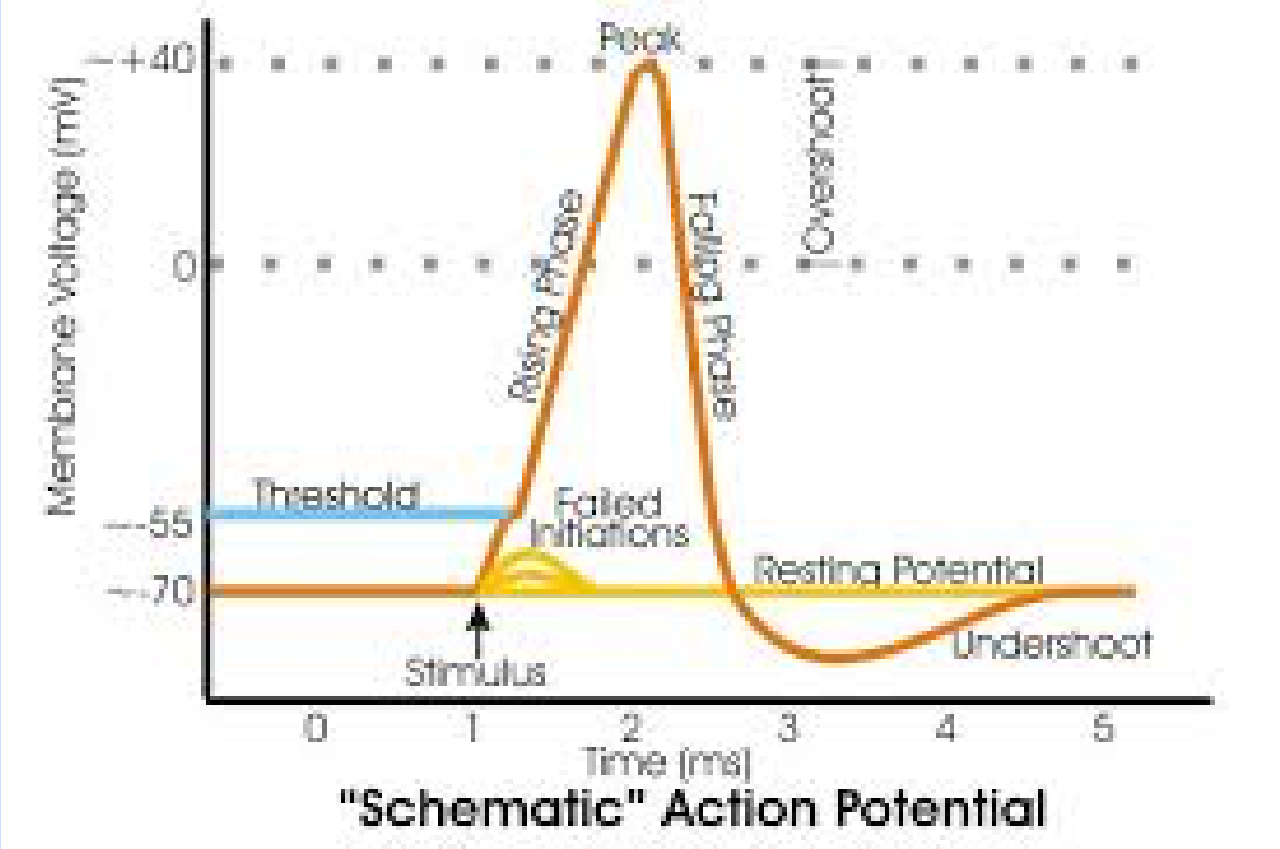
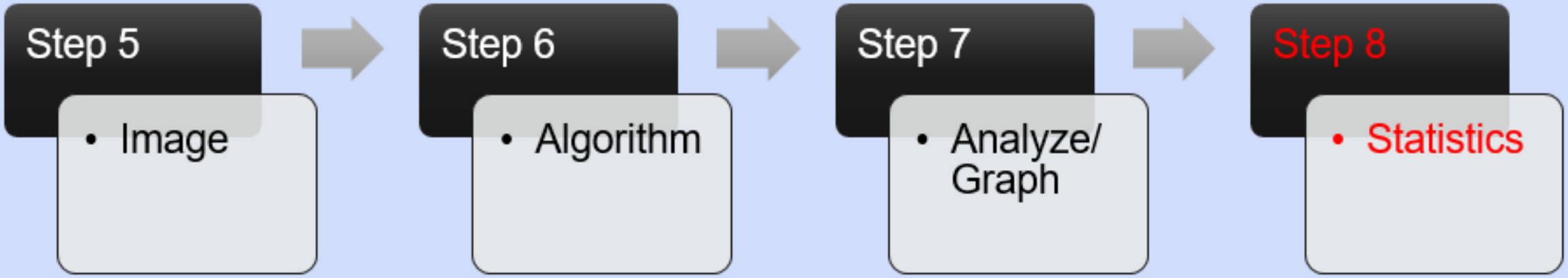
Voltage

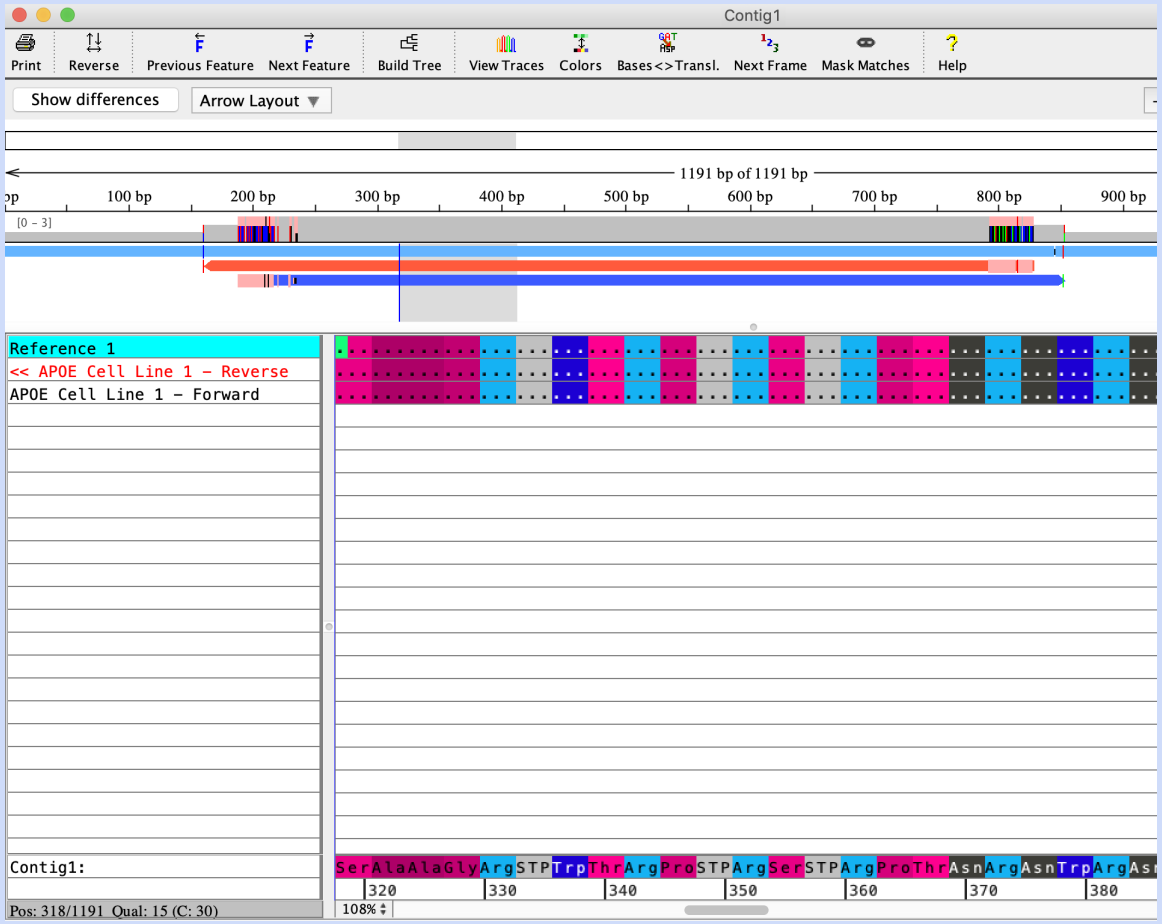


Medical Science Navigator

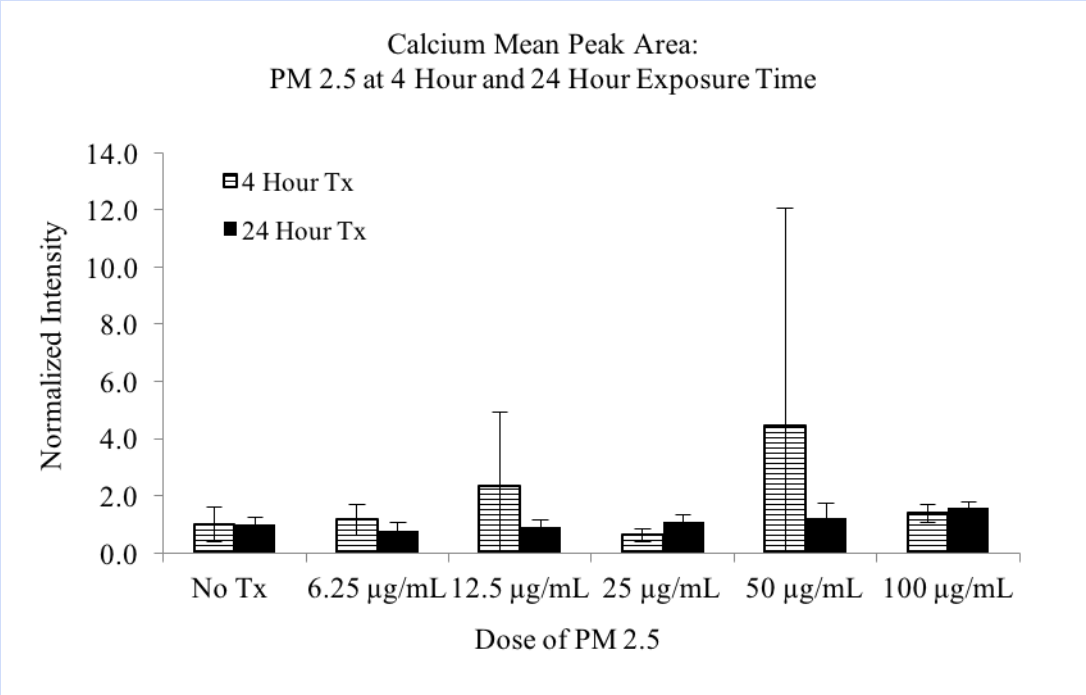
Immuno-fluorescent probes are used to visualize specific markers.

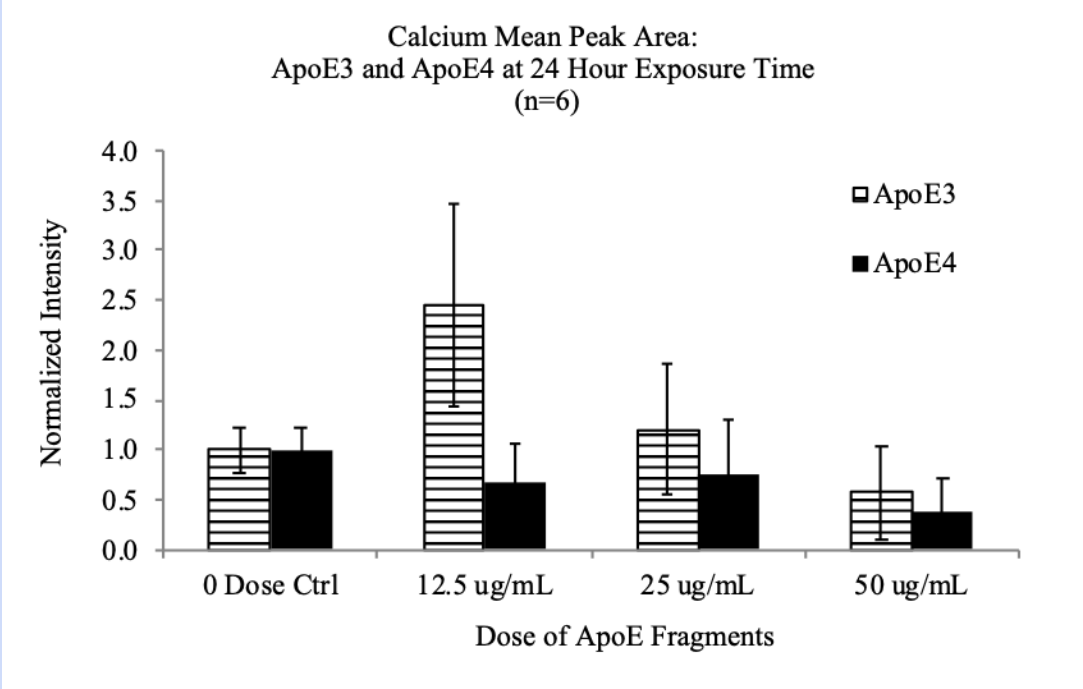


We verified our cell line by sequencing it against a reference gene.

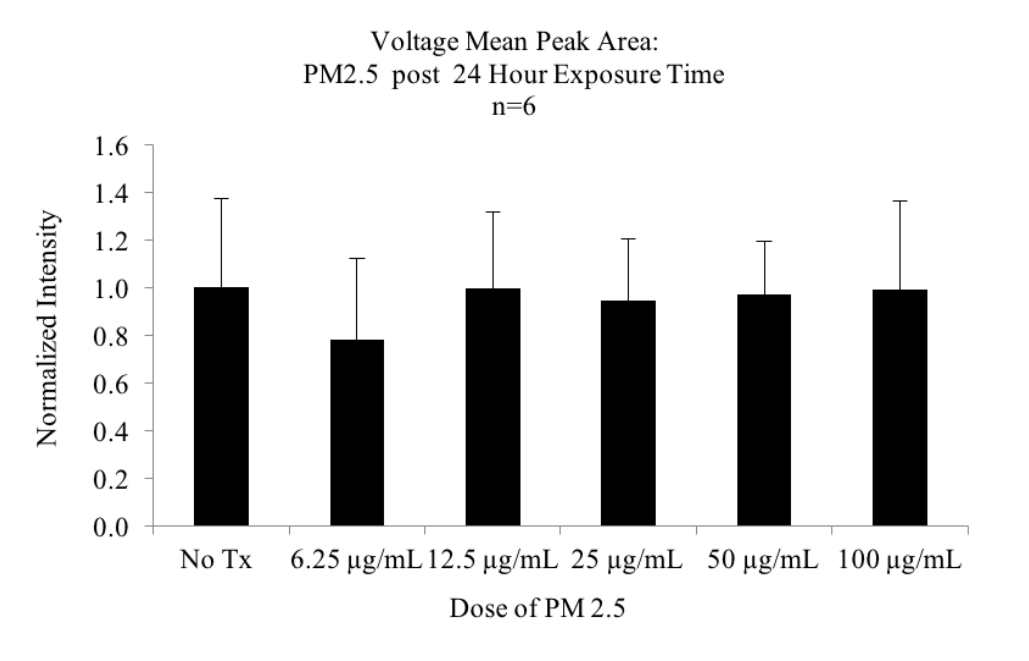


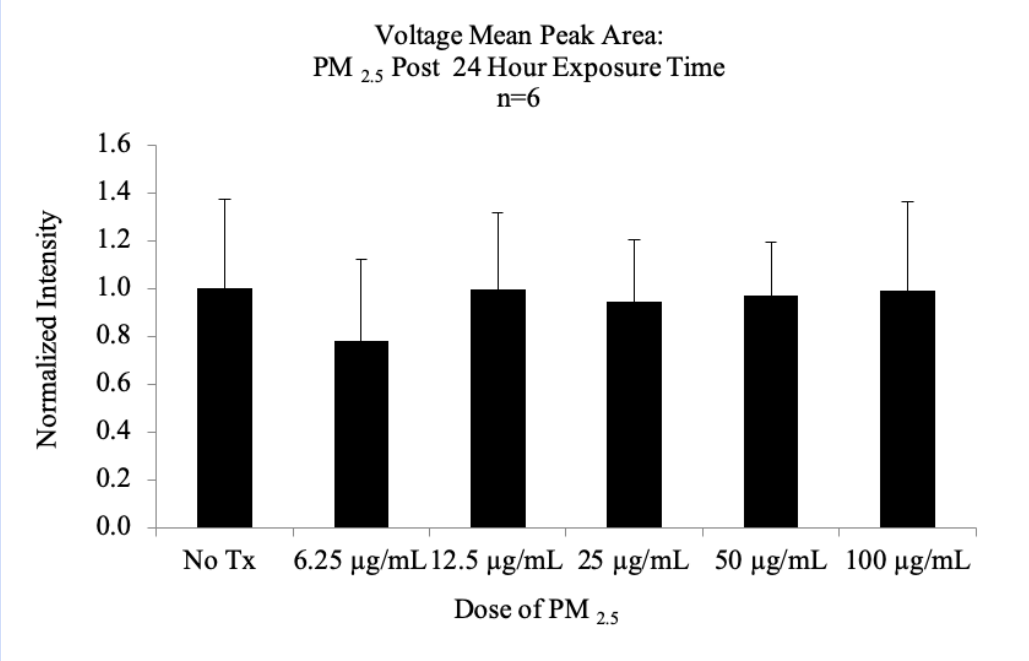
Data/Observations



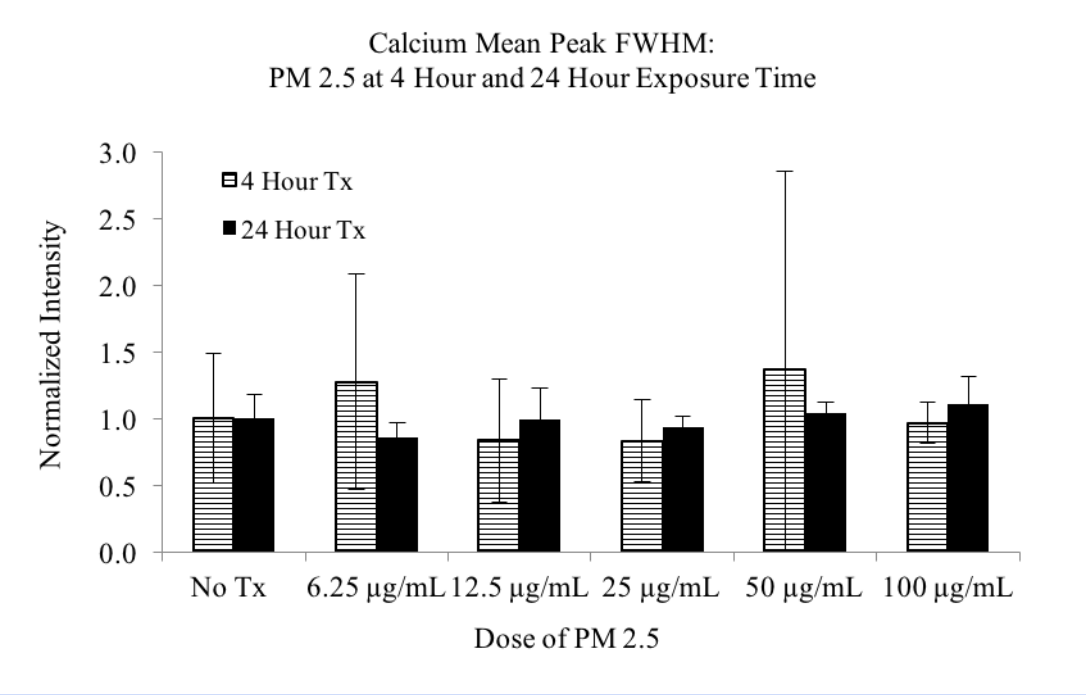


Data/Observations



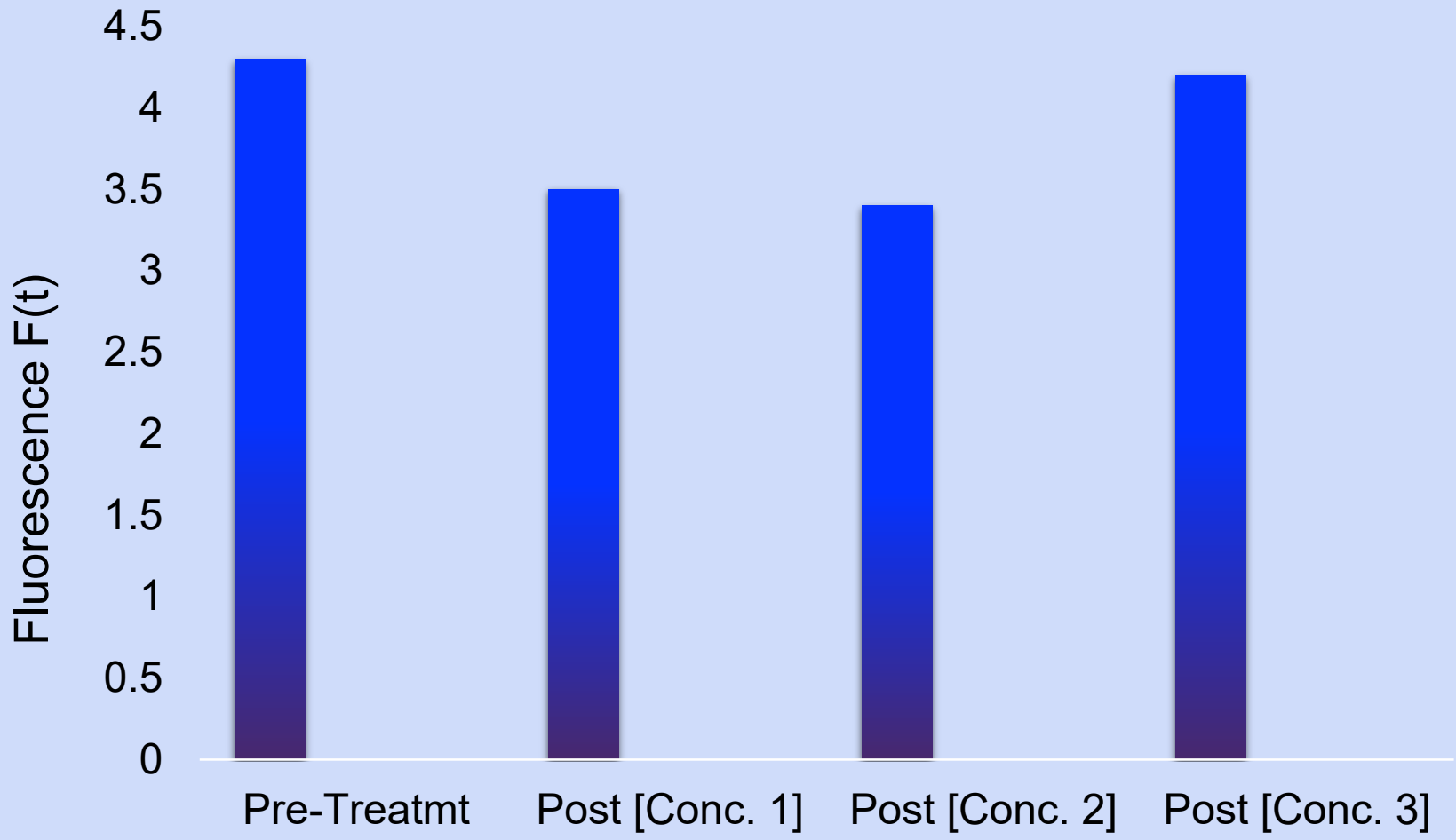


Data/Observations



Statistical analysis of the cells post-treatment with compounds at gradient concentrations is performed.

- One-way ANOVA
- Tukey's Post hoc



Conclusion

- Treatment 1 is significantly different among doses when neurons were exposed for 24 hours. The 24-hour exposure to compound 1 results in increased Calcium signals in our neuronal cell line. Analysis of the raw data is a work in progress. The threshold can vary between active and dead cells. We continue our on-going assay development.

Works Cited

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