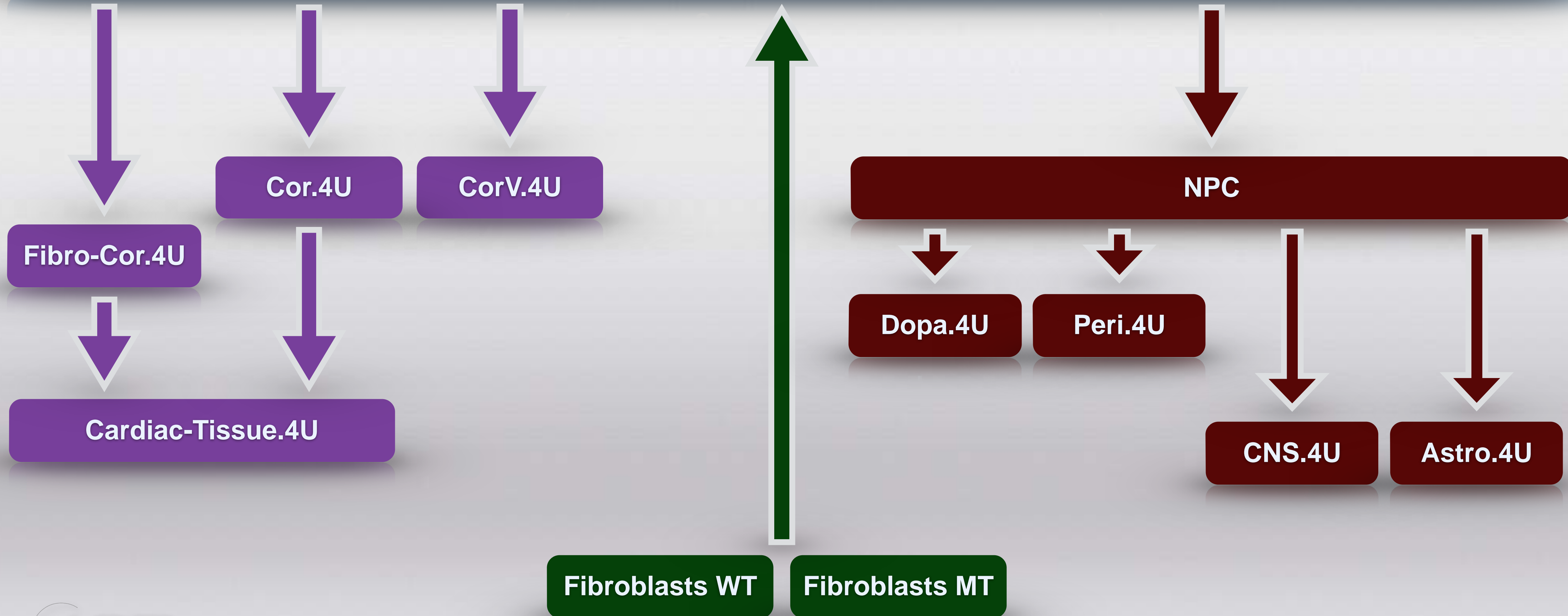




**Axiogenesis growing portfolio on human iPSC-derived cells**

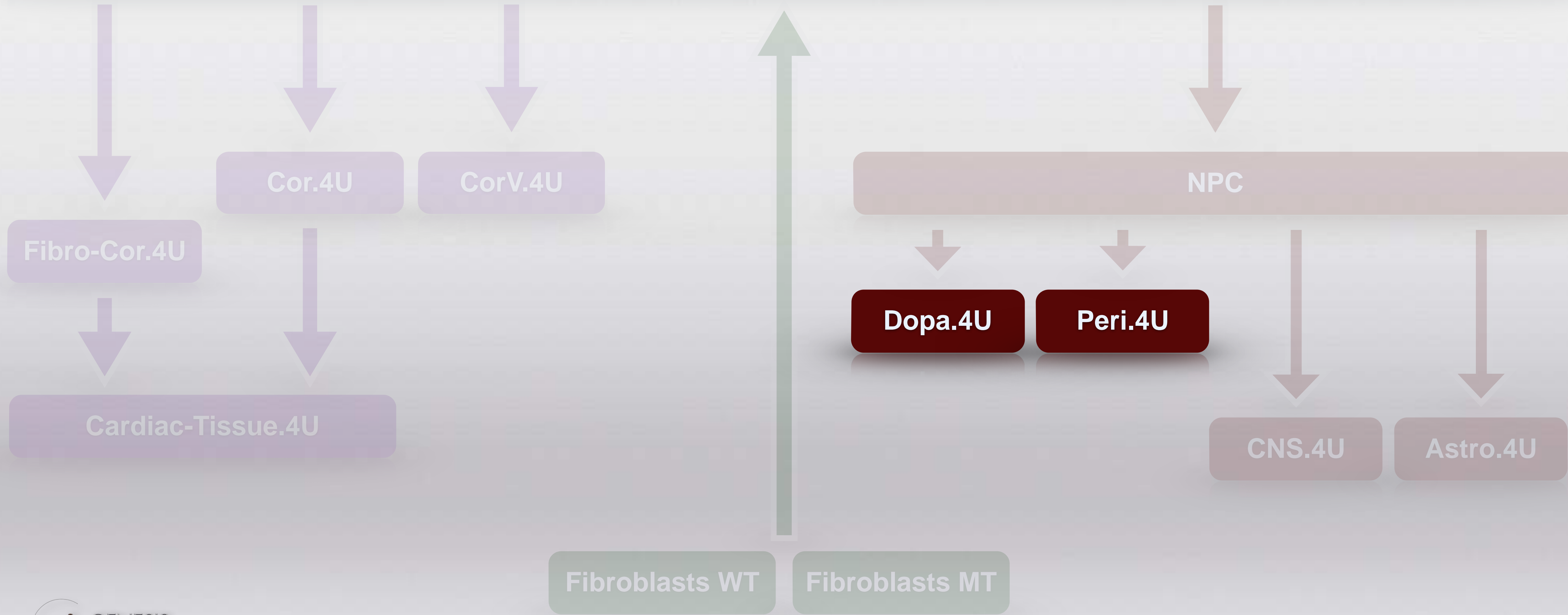
# Overview

hiPSC (from Axiogenesis/from clients/CRISPR/...)



# Overview (Current Products)

hiPSC (from Axiogenesis/from clients/CRISPR/...)



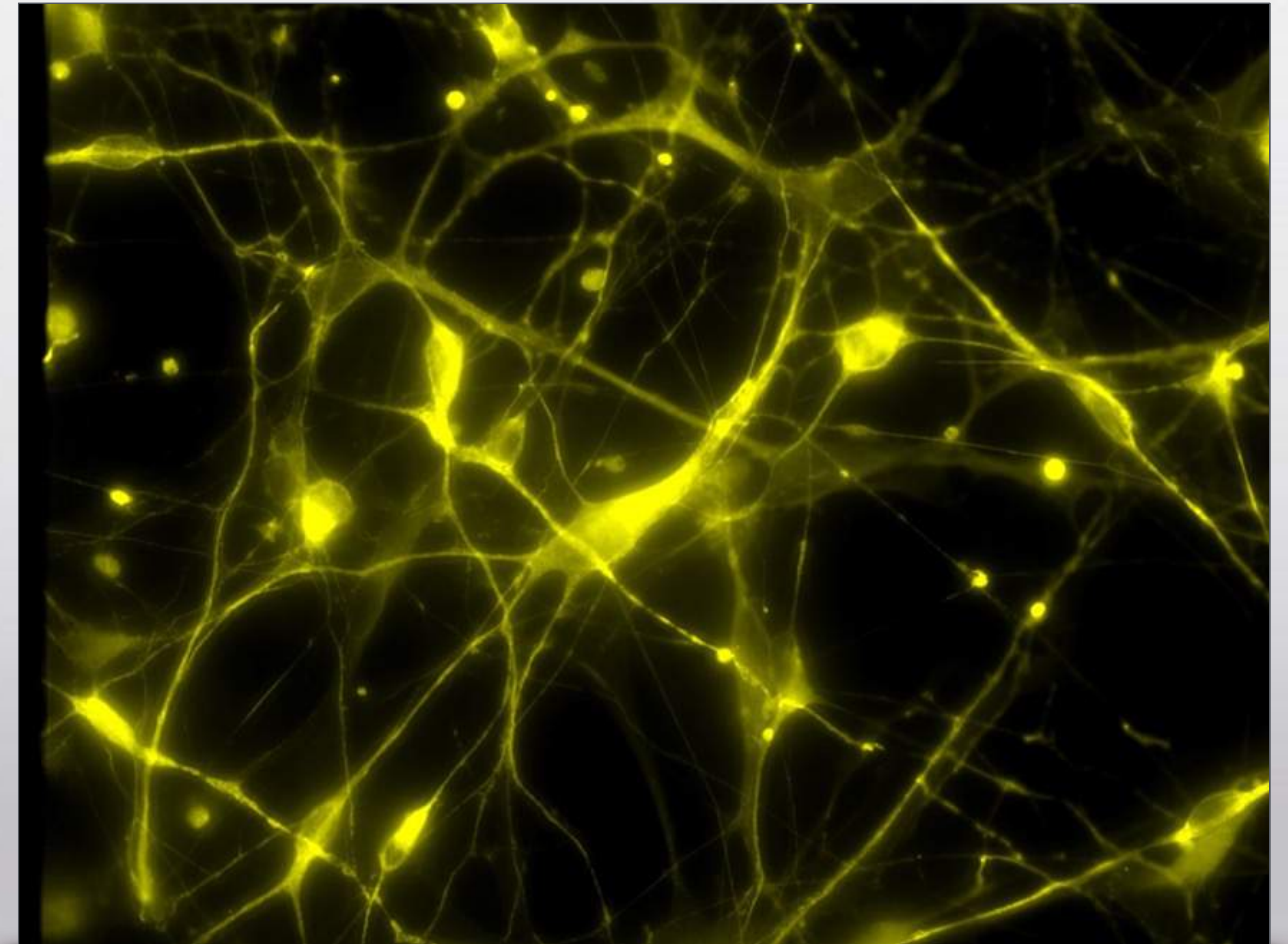
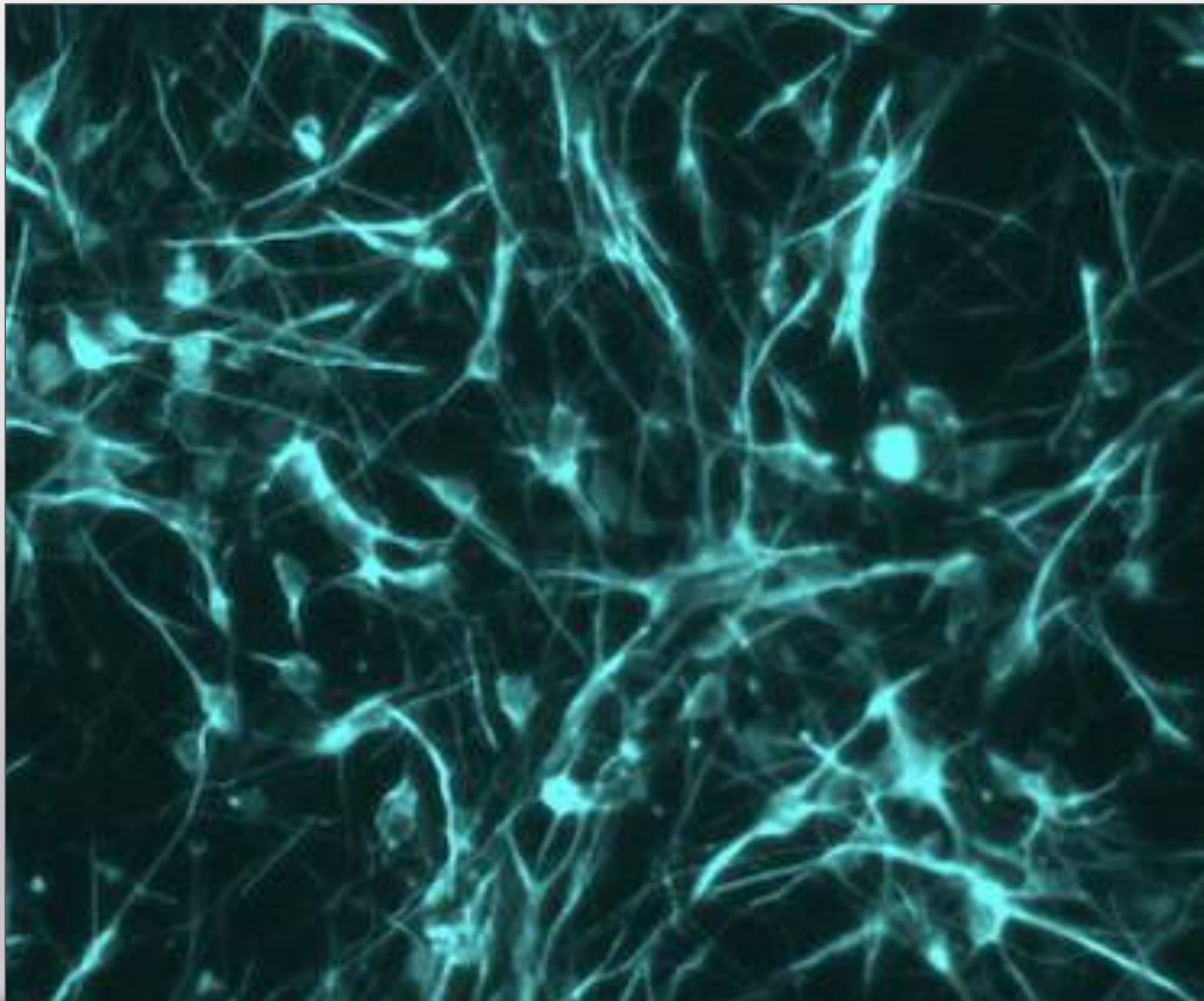
Fibroblasts WT

Fibroblasts MT



# Current „Neural-Products“

## Dopa.4U - Dopaminergic Neurons



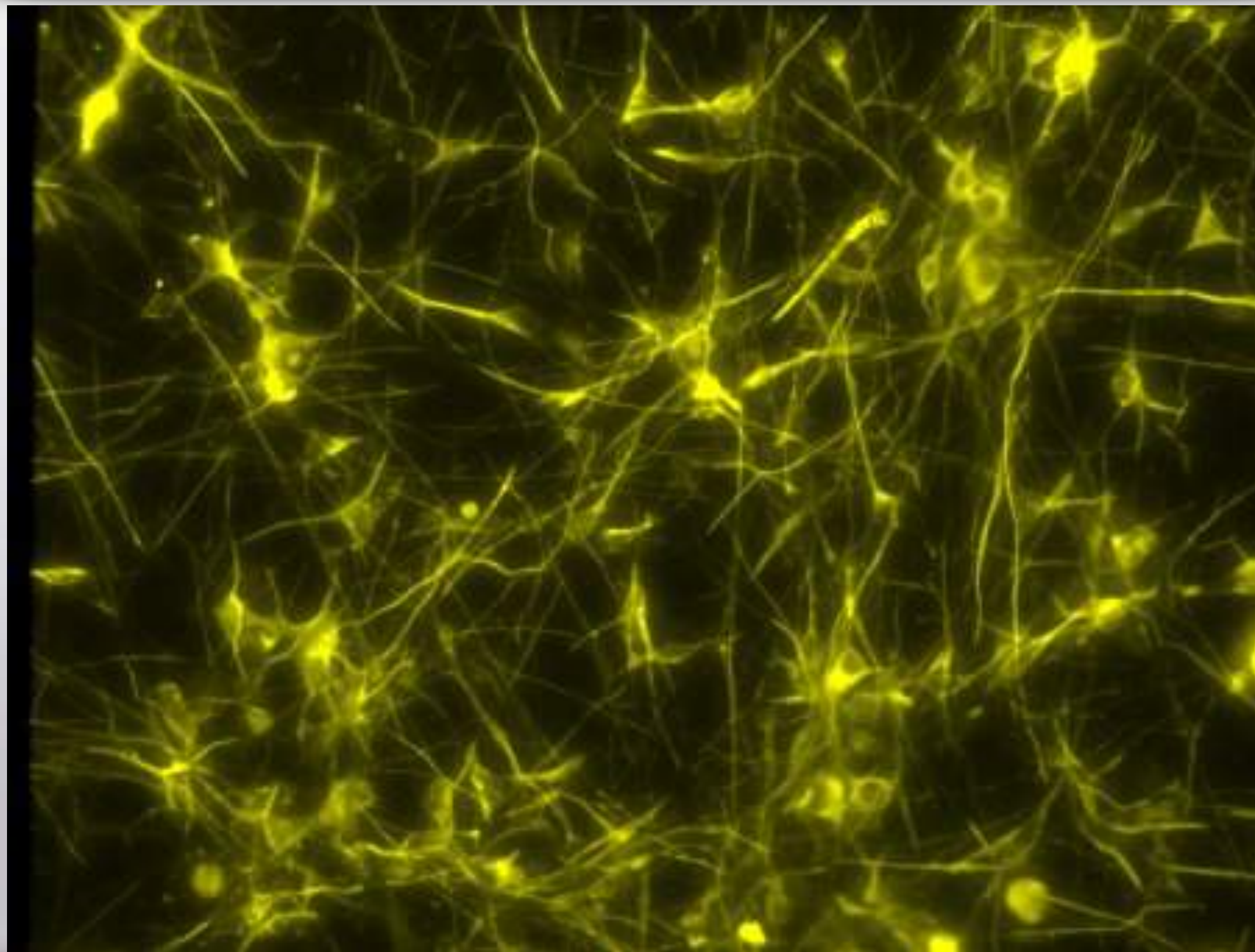
## Peri.4U - Peripheral Neurons



# Dopa.4U - Dopaminergic Neurons

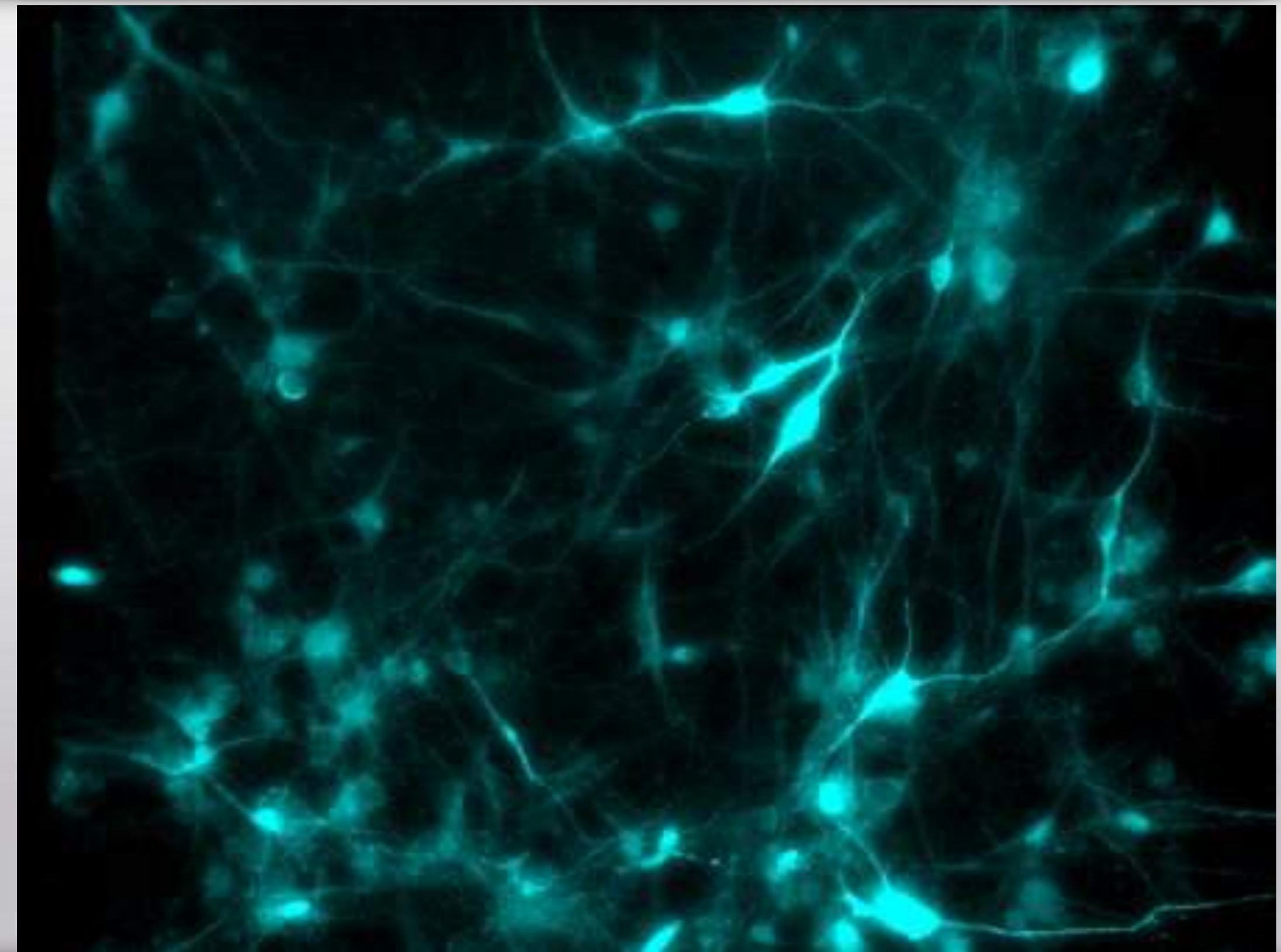
## Beta-3-tubulin

(neuron-specific microtubuli)



## Tyrosine Hydroxylase

(dopaminergic neuron-specific protein)

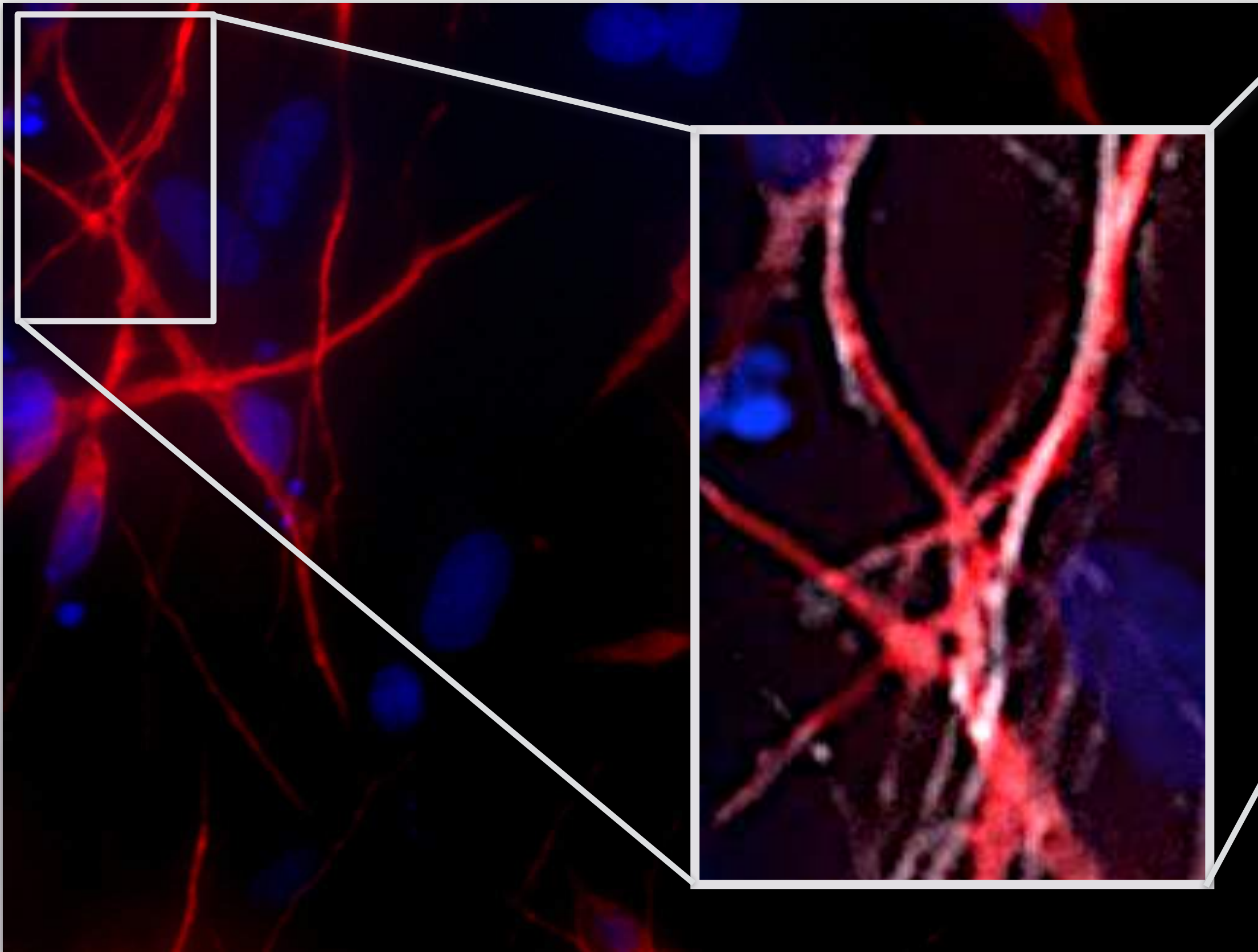




# Dopa.4U - Dopaminergic Neurons

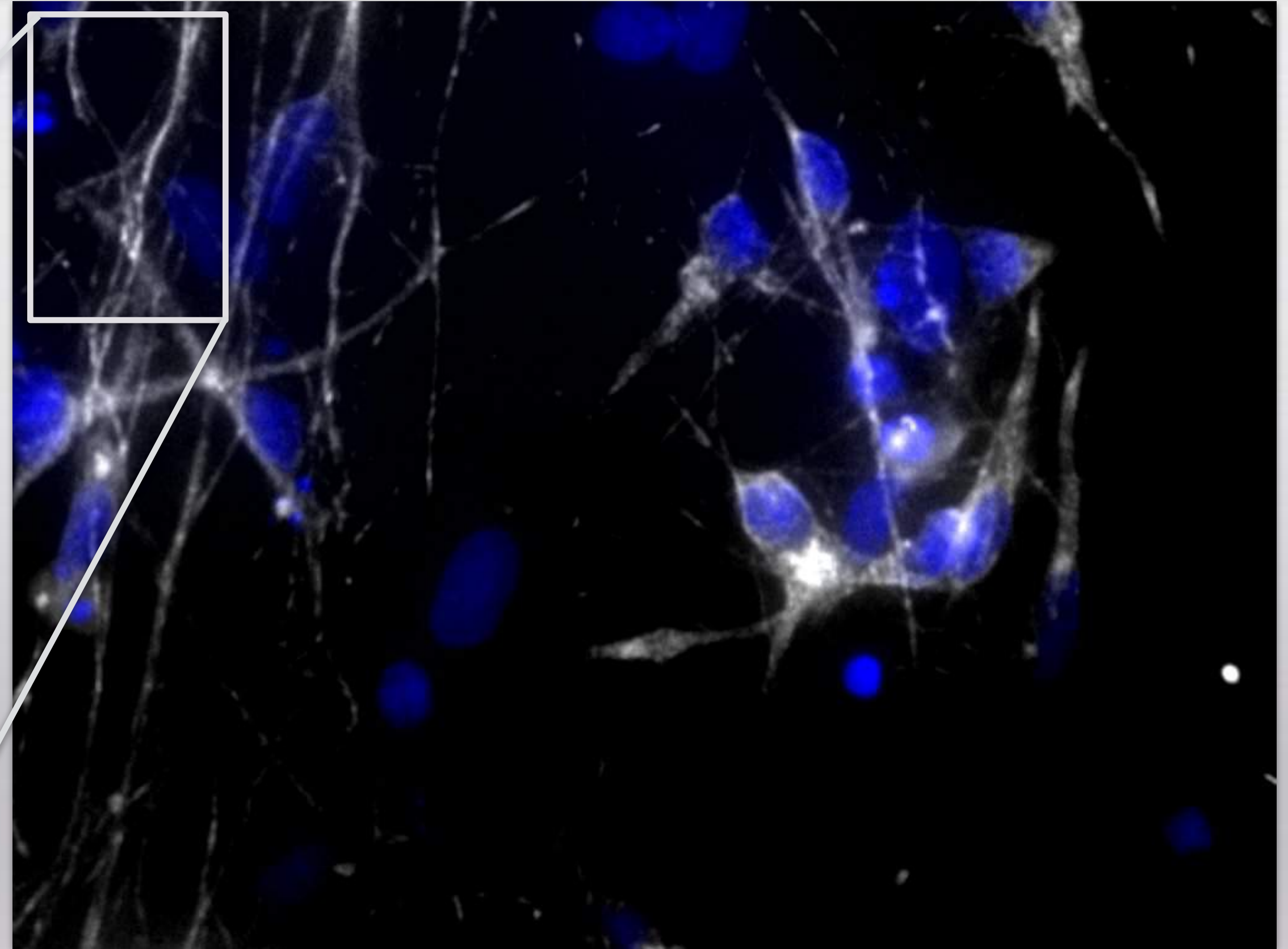
## MAP-2

(neuron-specific microtubule-associated protein 2)



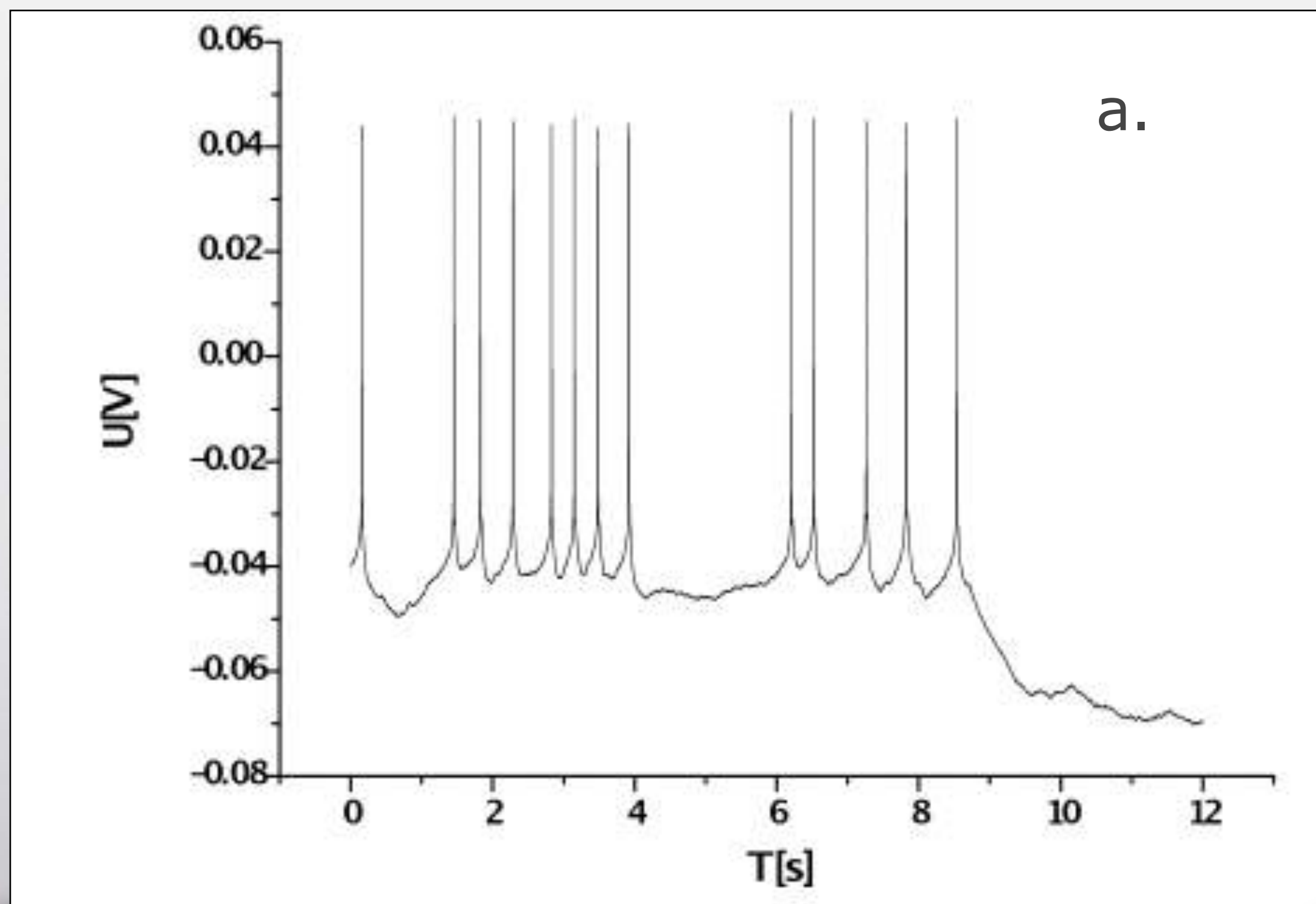
## Synaptophysin

(presynaptic)

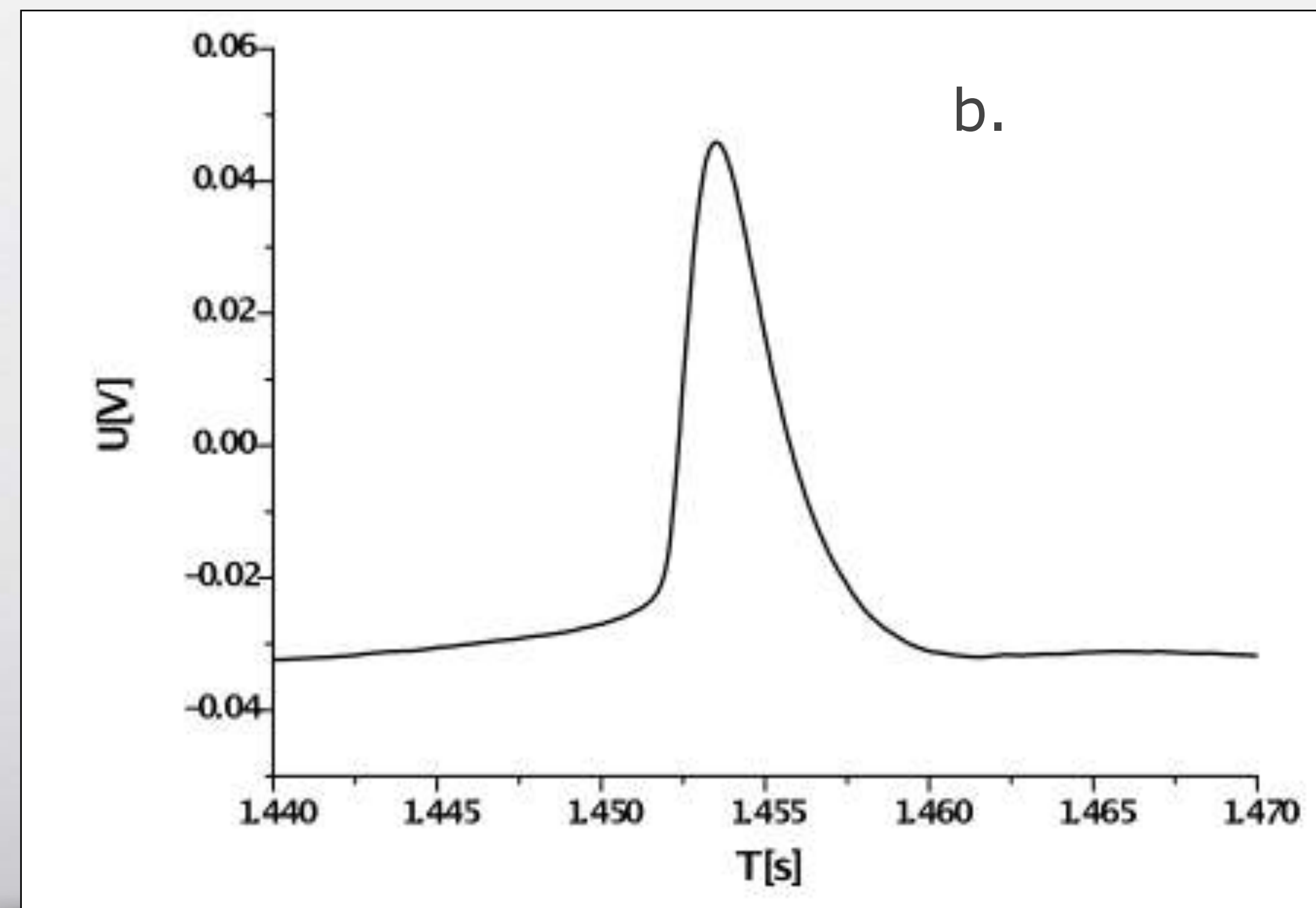




# Dopa.4U - Patch Clamp - Current clamp mode (whole cell configuration)



Spontaneous action potentials in current clamp mode  
(resting potential of an inactive cell is around -80 mV)

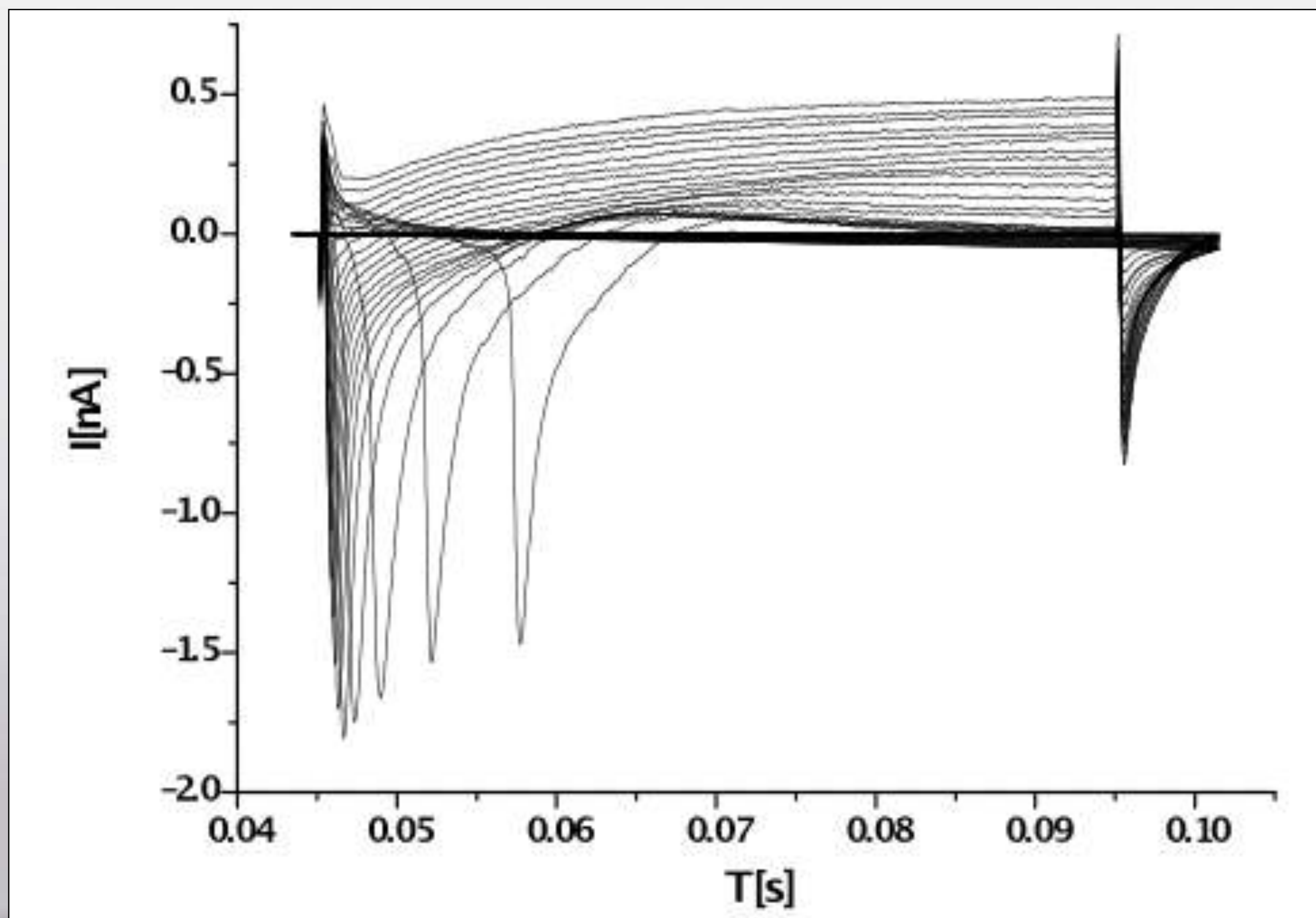


Spontaneous action potential (higher magnification)

Dopa.4U cells exhibit spontaneous action potentials



## Dopa.4U - Patch Clamp - Voltage clamp mode (whole cell configuration)

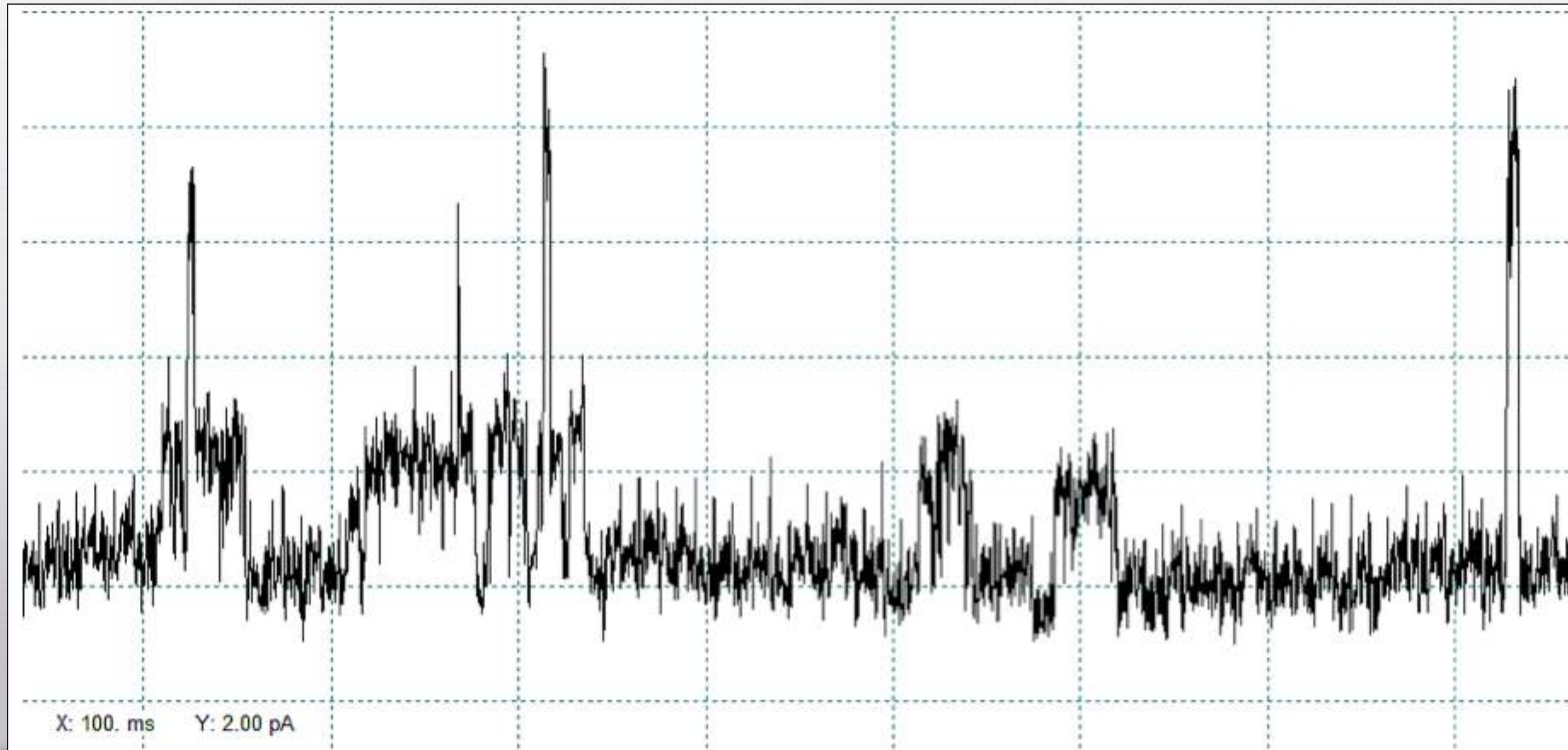


Overlay of current traces showing the responses to voltage pulses from -60 mV to +60 mV in 5 mV increments.

Dopa.4U exhibit Sodium Currents (0,5 nA - 2 nA)



# Dopa.4U - Patch Clamp - Measurement of single channels



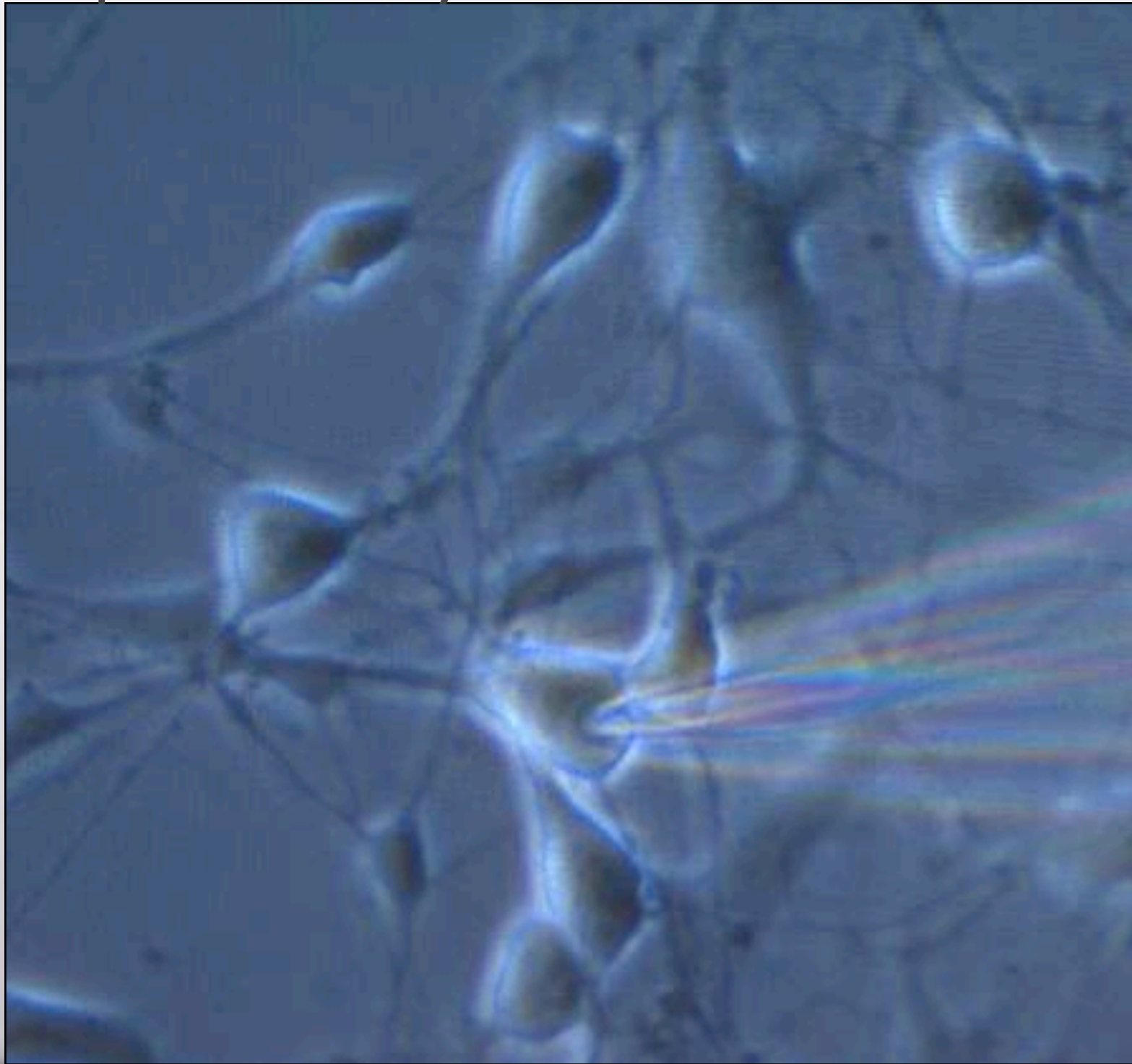
X: 100. ms Y: 2.00 pA

X: 100. ms Y: 2.00 pA

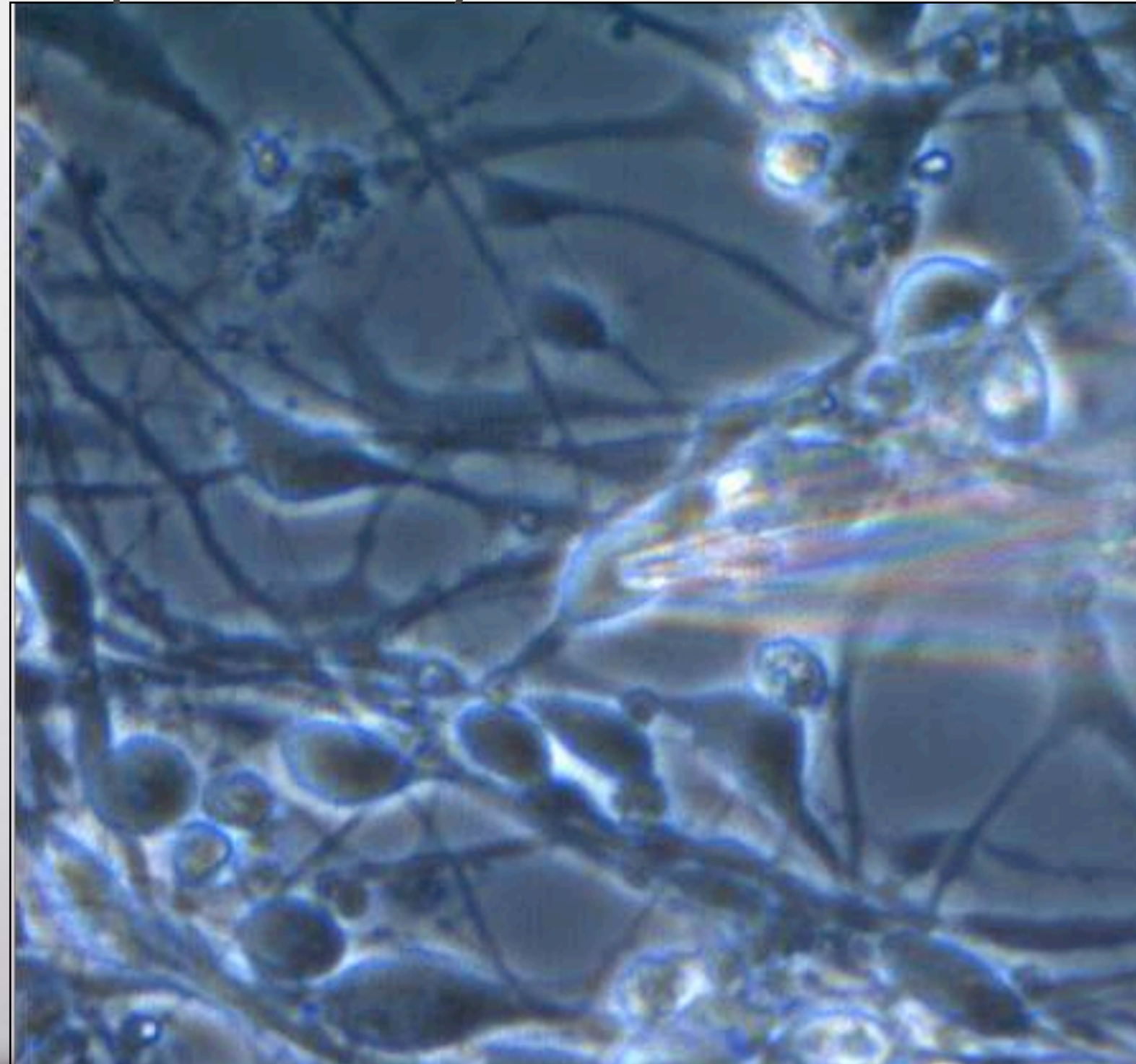


# Dopa.4U - Patch Clamp - Measurement of Long-term Cultures

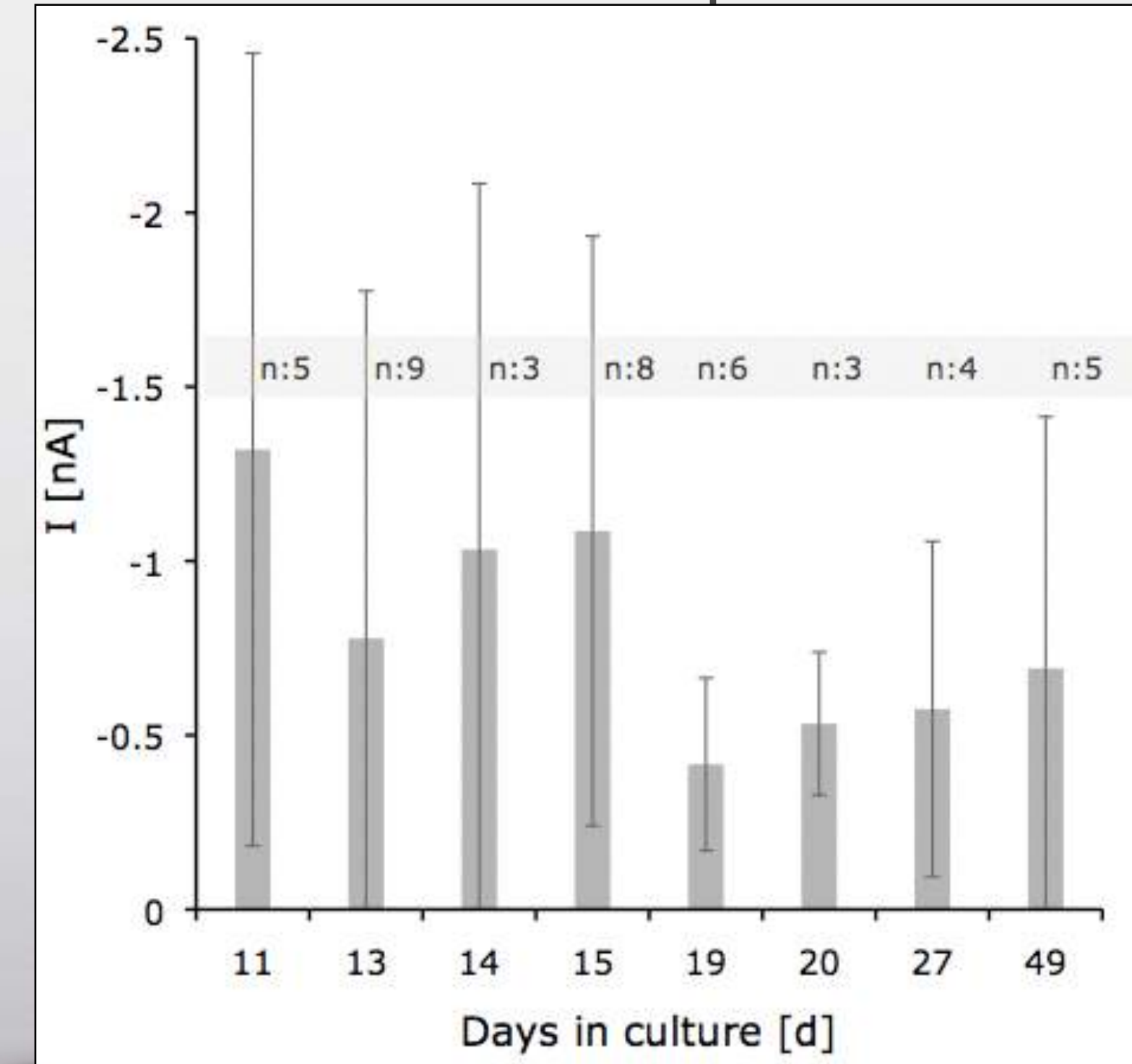
Dopa.4U: 15 days in culture



Dopa.4U: 49 days in culture



Sodium Currents of Dopa.4U



Dopa.4U were seeded (30.000 cells/ cm<sup>2</sup>) onto cover slips and measured after 11 to 49 days in culture.

Dopa.4U cells maintain sodium currents during long-term culture



## Dopa.4U - MEA Recordings

MEA recording station Axion Biosystems



Maestro System, 12 well MEA

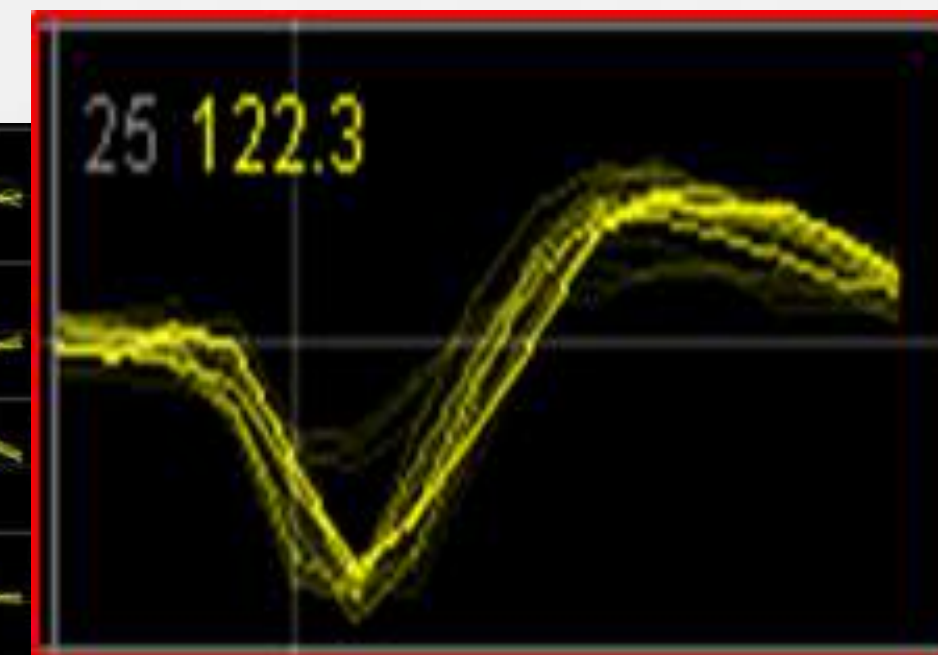
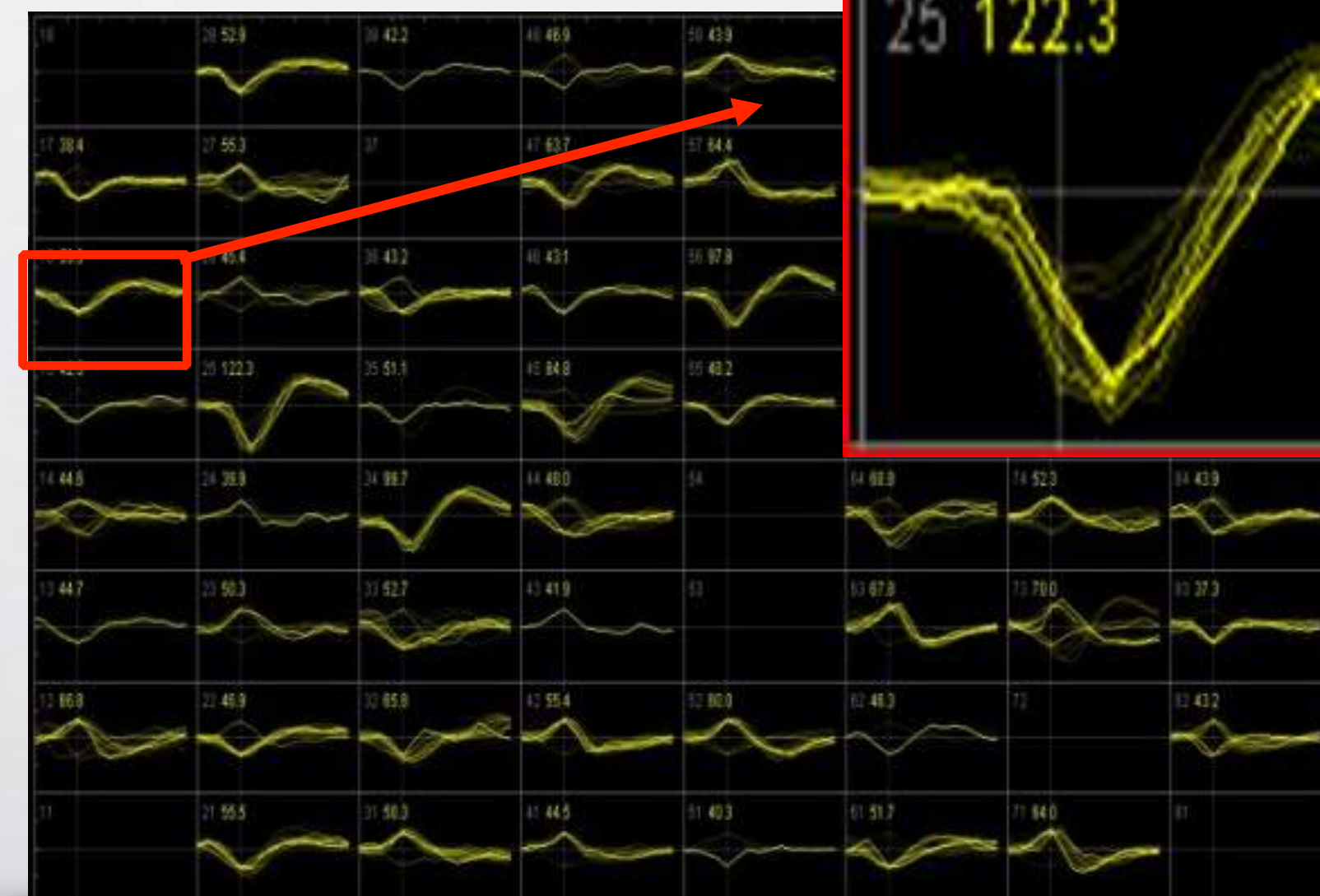
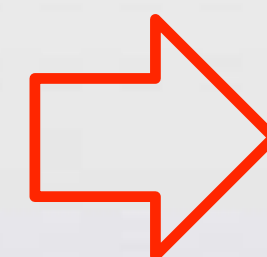
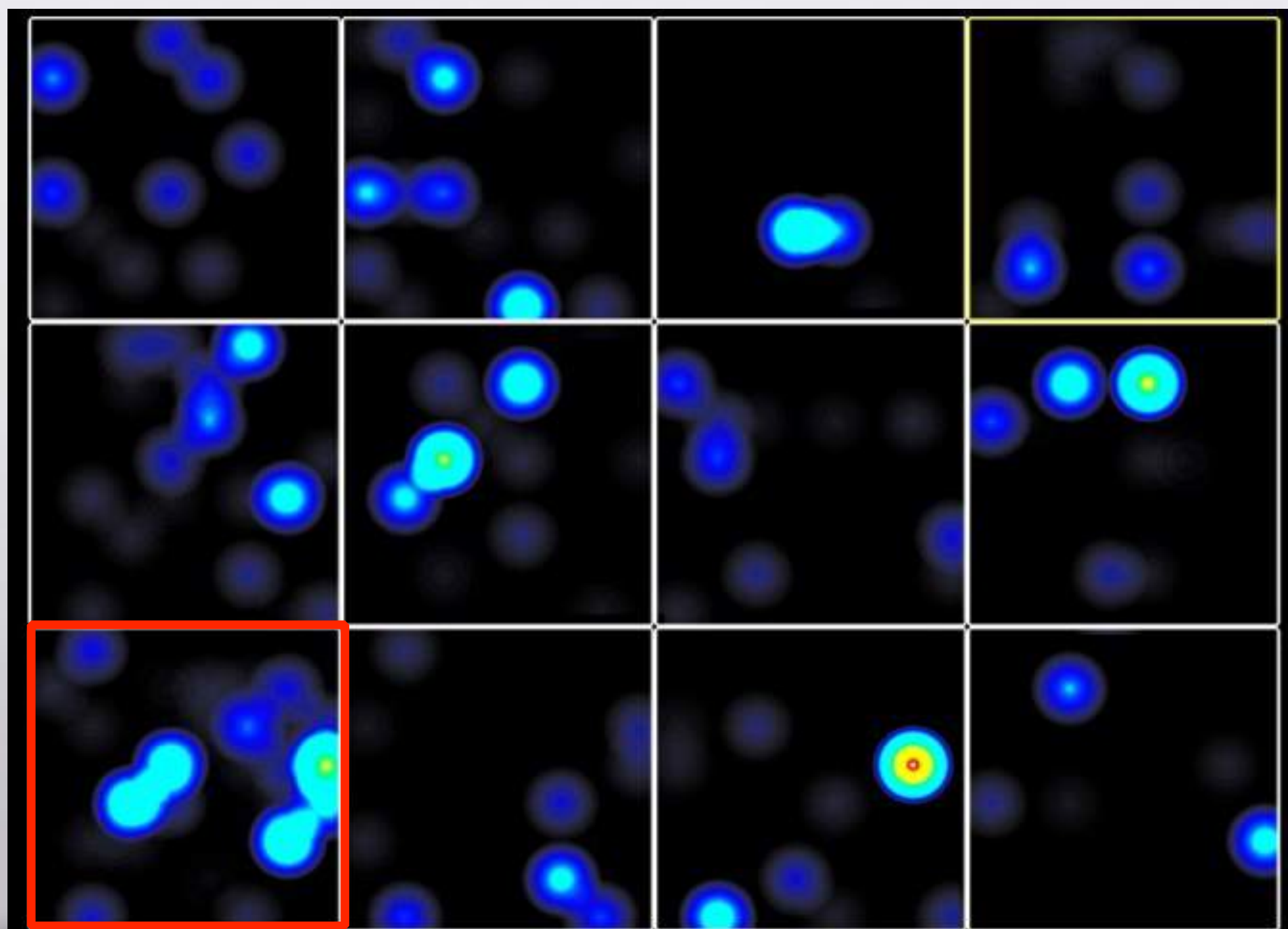
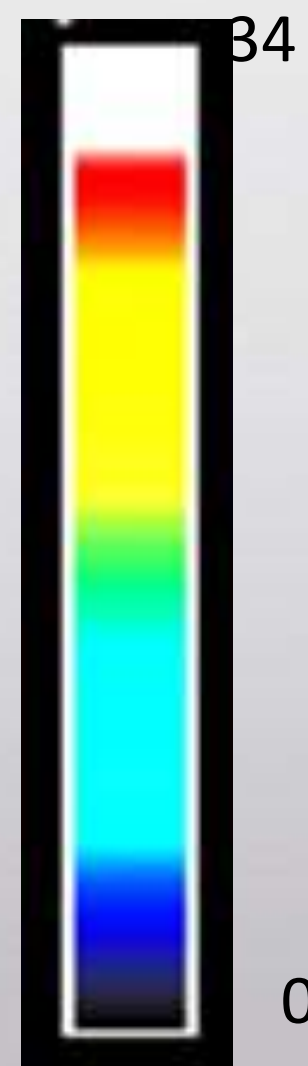




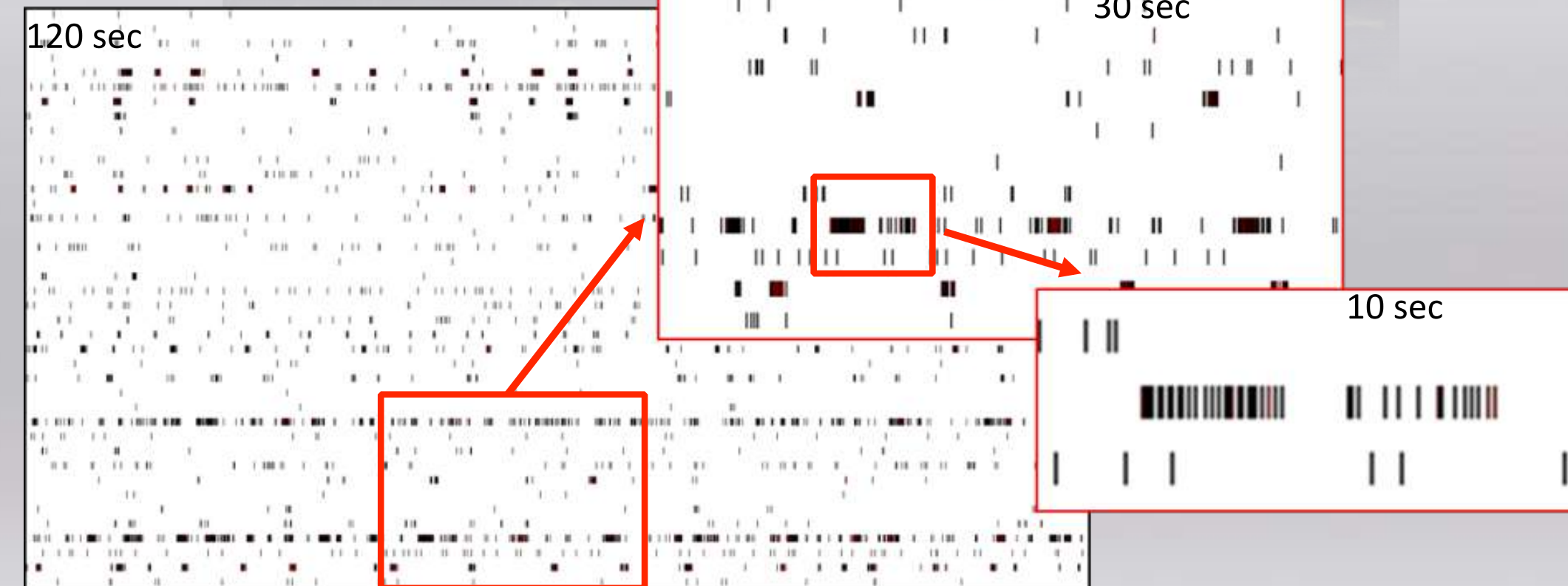
# Dopa.4U - MEA Recordings

## 12 Wells – Heatmap (spontaneous activity)

spikes/sec



### Bursting neurons



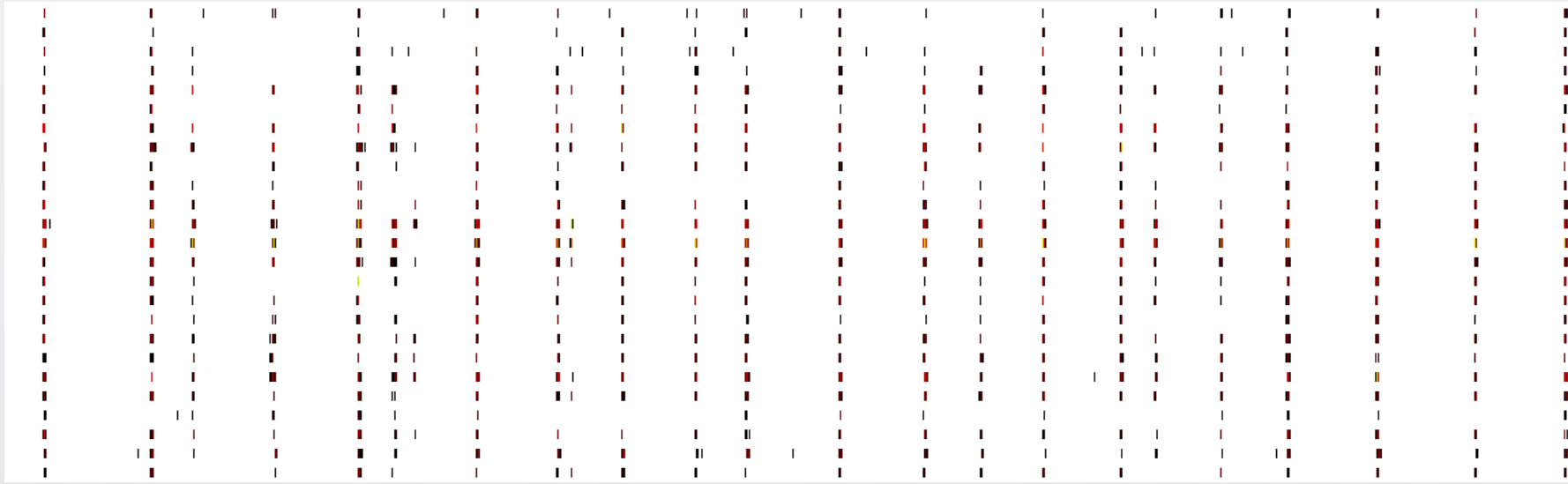
activity of one well (7div)

activity of one well (7div)



# Dopa.4U - Comparison of the Spontaneous Activity Patterns:

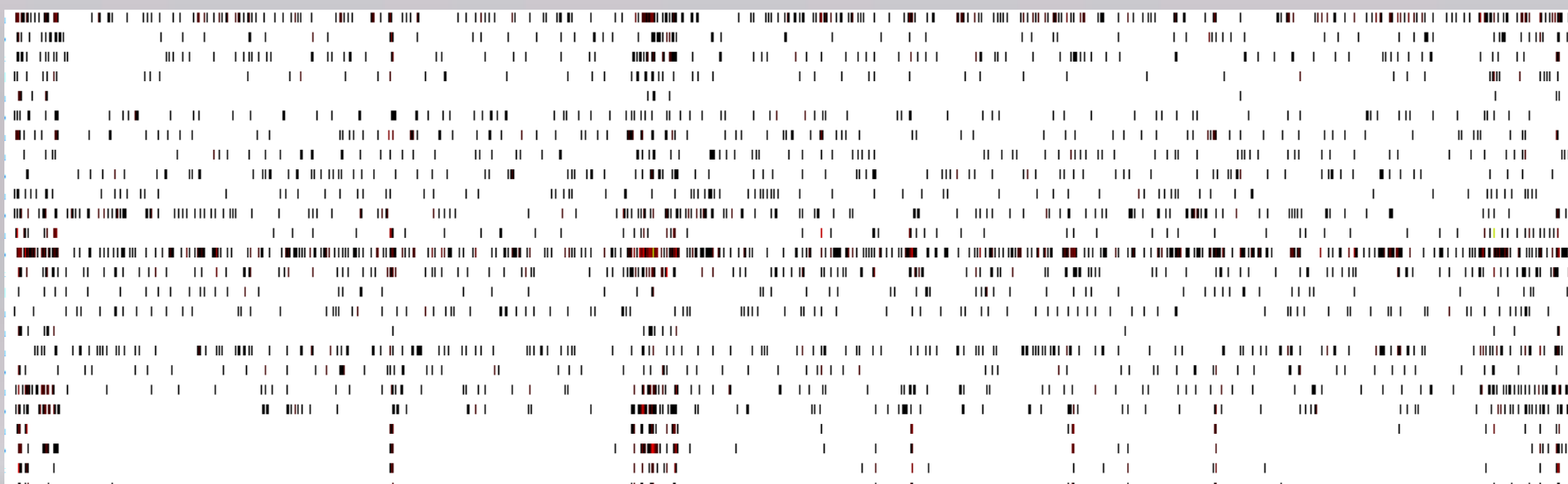
## Primary Mouse Frontal Cortex – 28 days in vitro



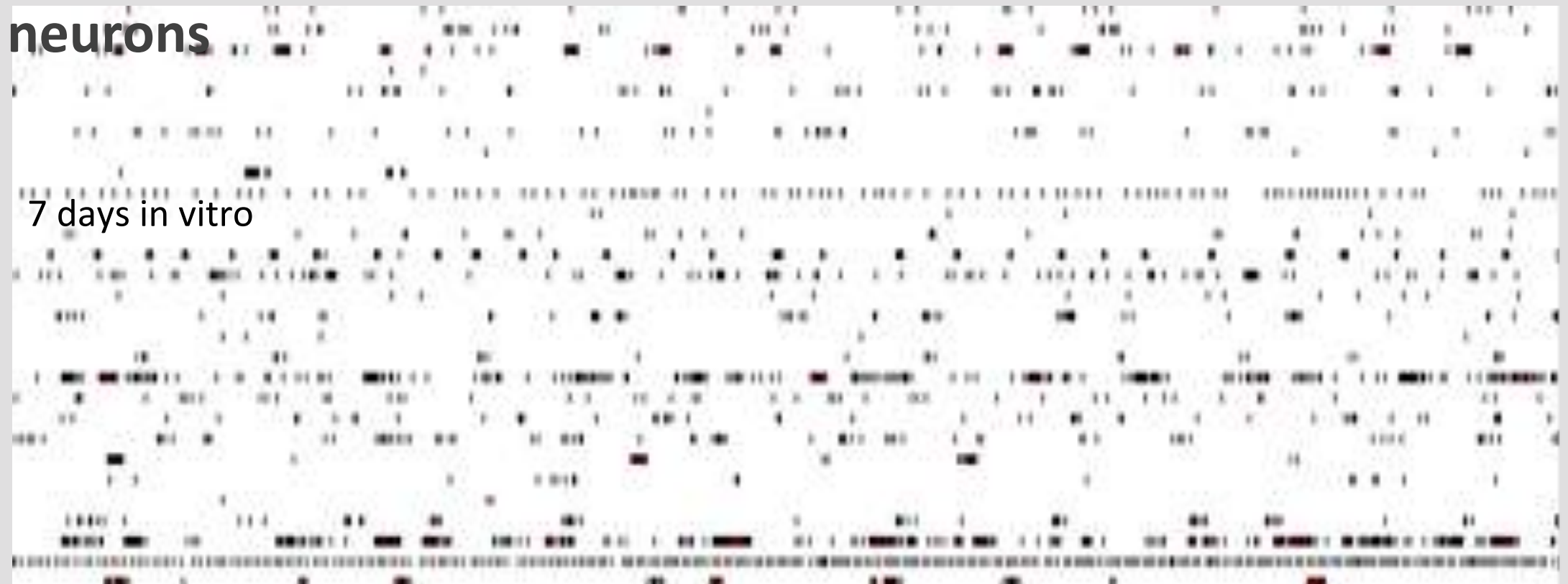
## Primary Mouse Midbrain – 28 days in vitro



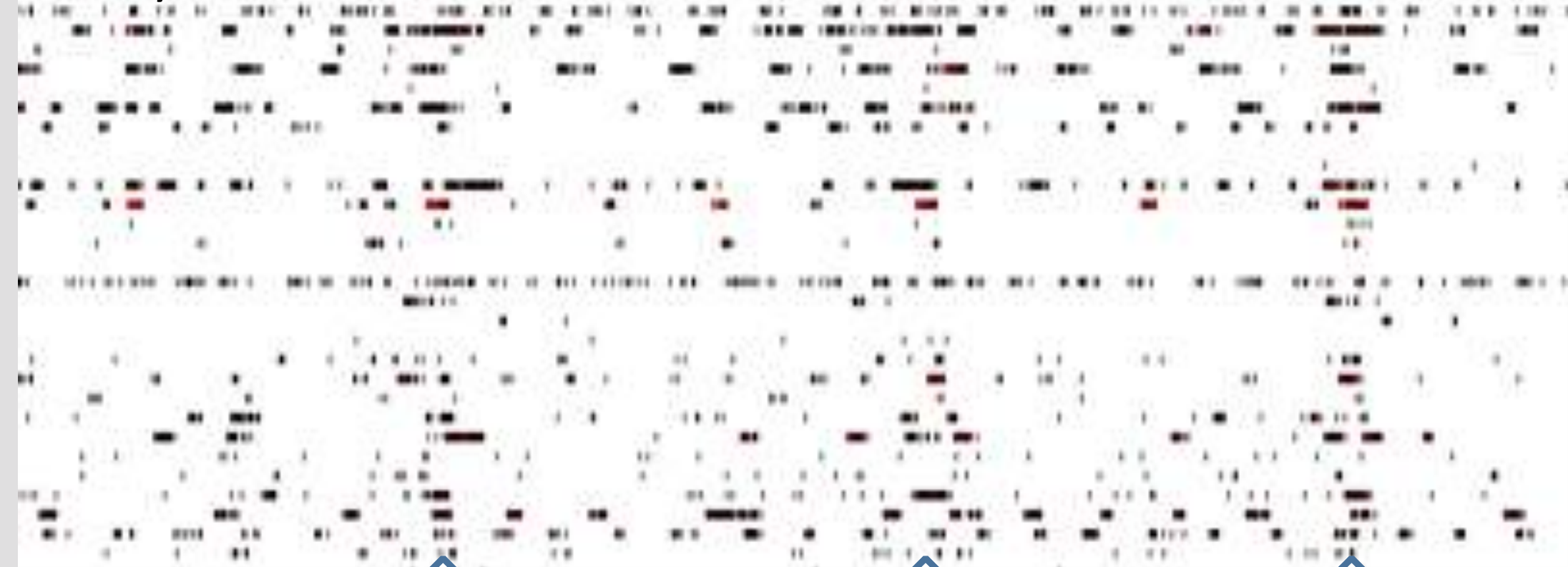
## Primary Mouse Hypothalamus – 28 days in vitro



## Dopa.4U human iPSC-derived dopaminergic neurons



## 14 days in vitro



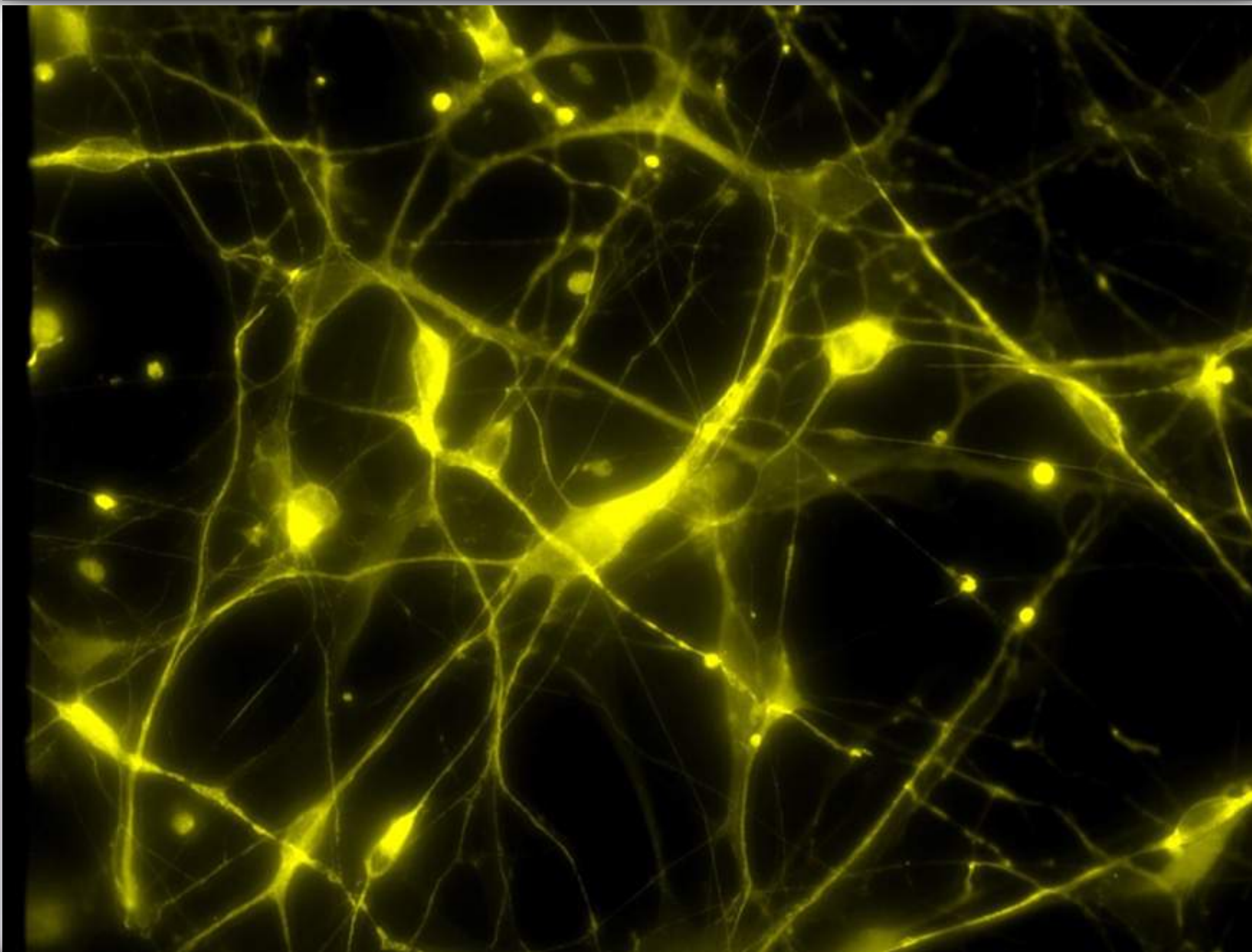
60 sec



# Peri.4U - Peripheral Neurons

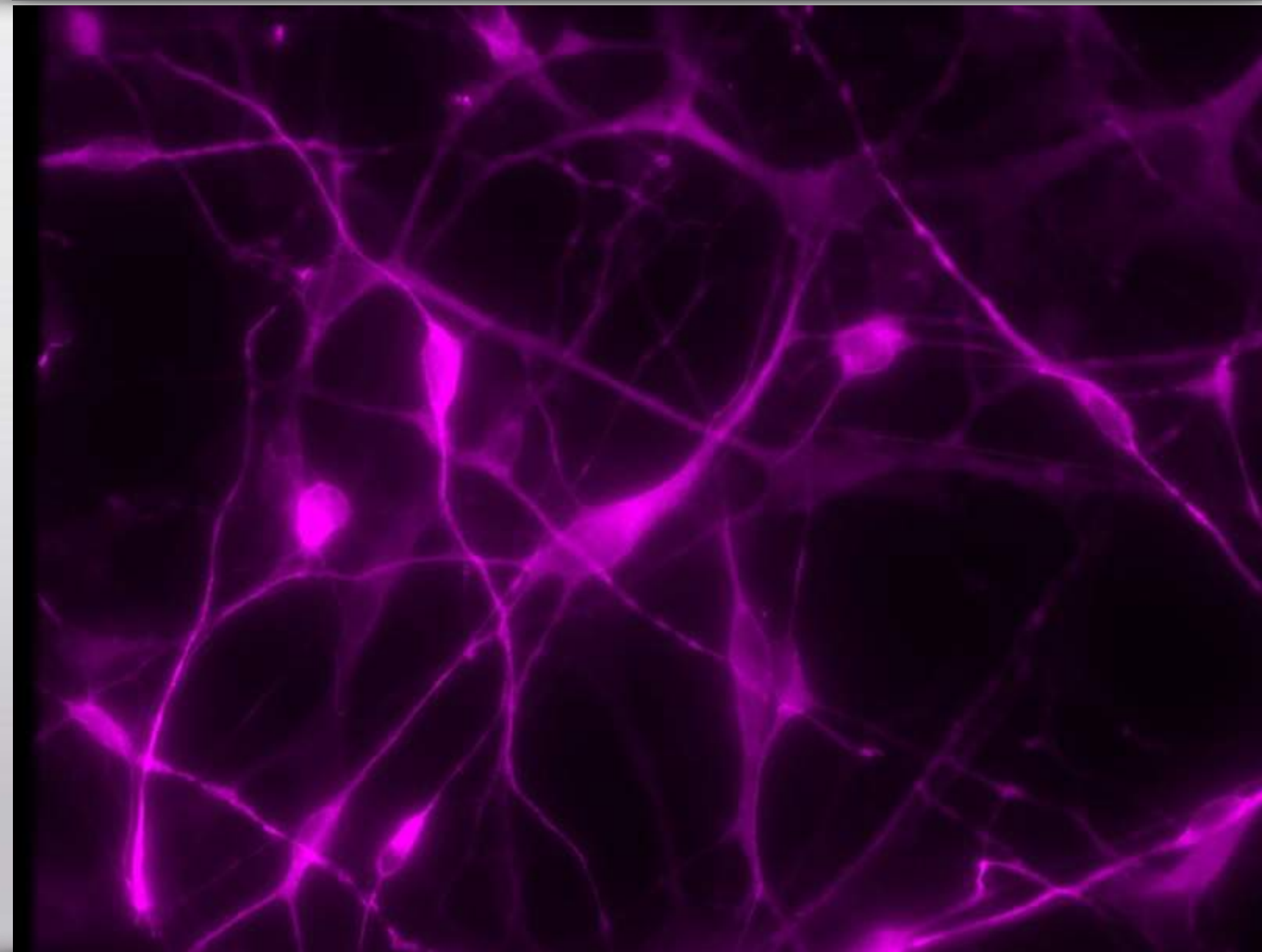
## Beta-3-tubulin

(neuron-specific microtubuli)



## MAP-2

(neuron-specific microtubule-associated protein 2)

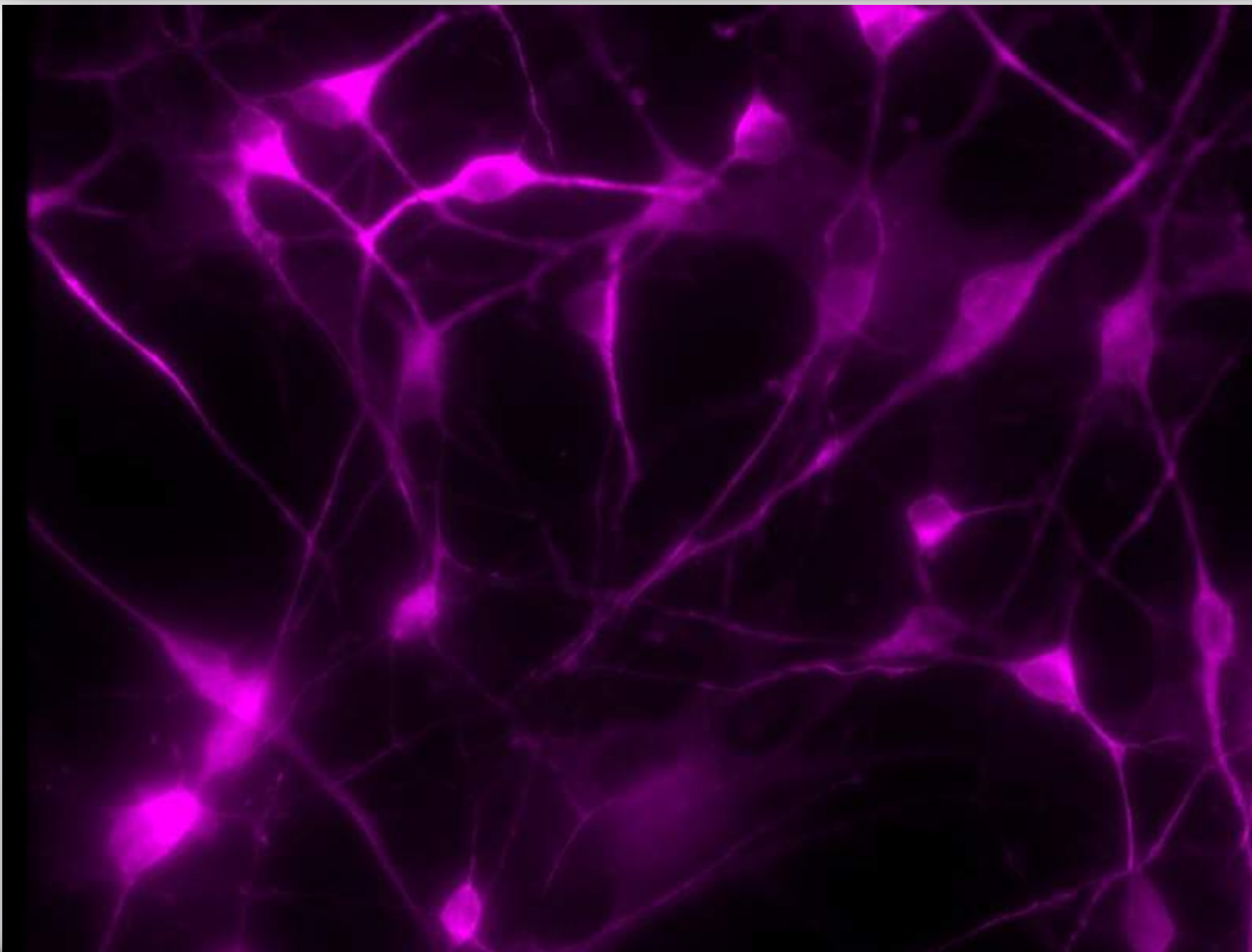




## Peri.4U - Peripheral Neurons

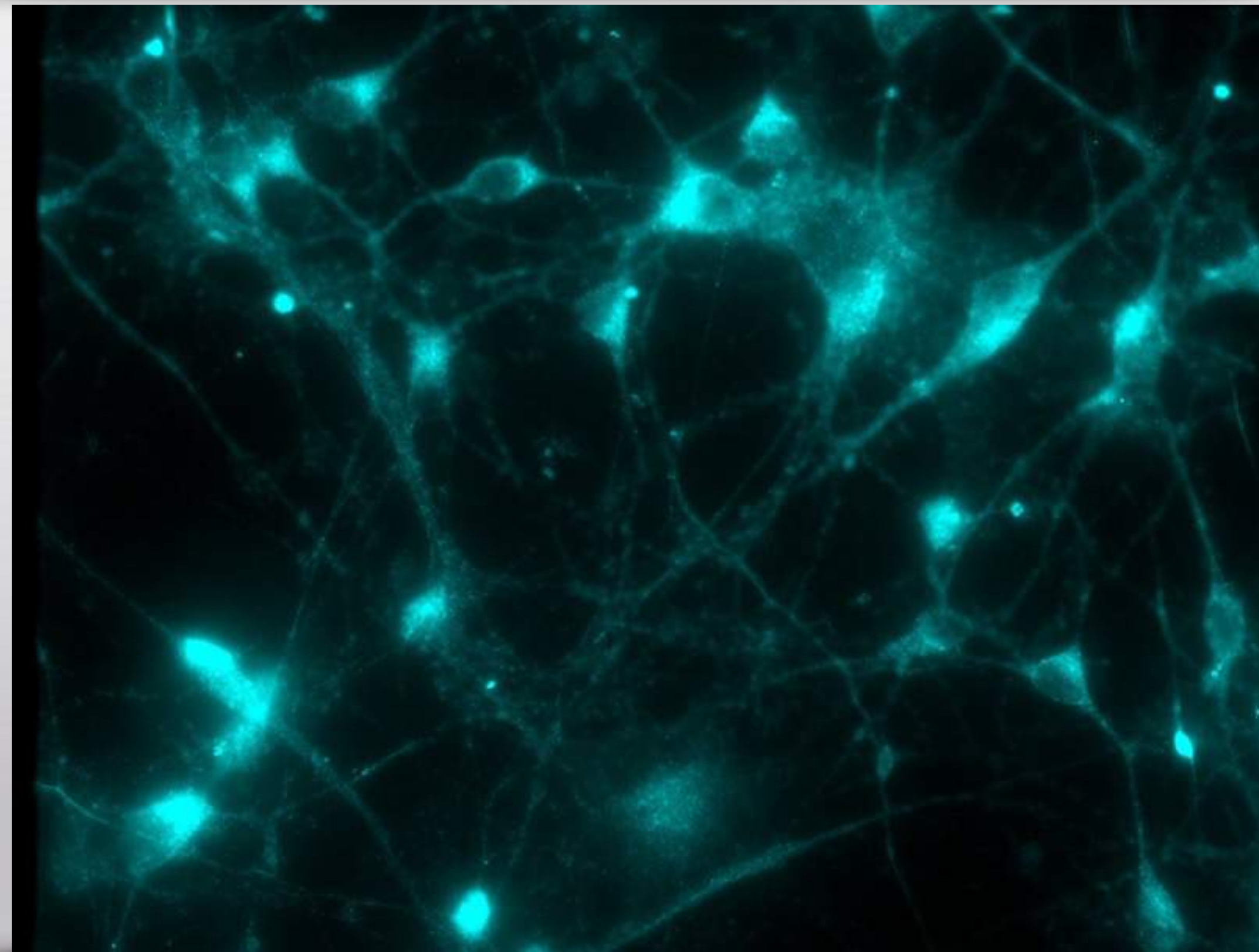
### MAP-2

(neuron-specific microtubule-associated protein 2)



### Peripherin

(peripheral neuron-specific intermediate filament)

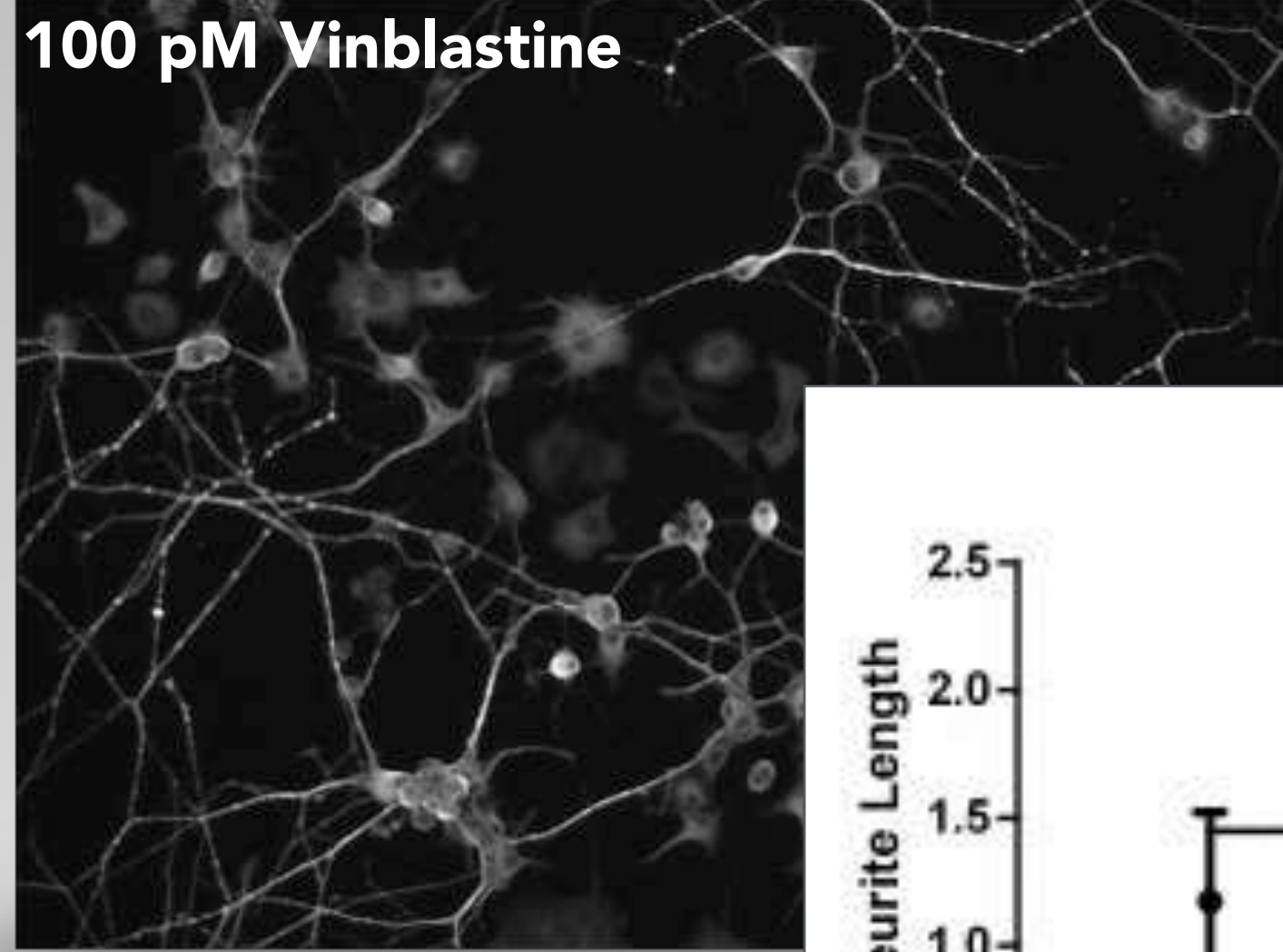




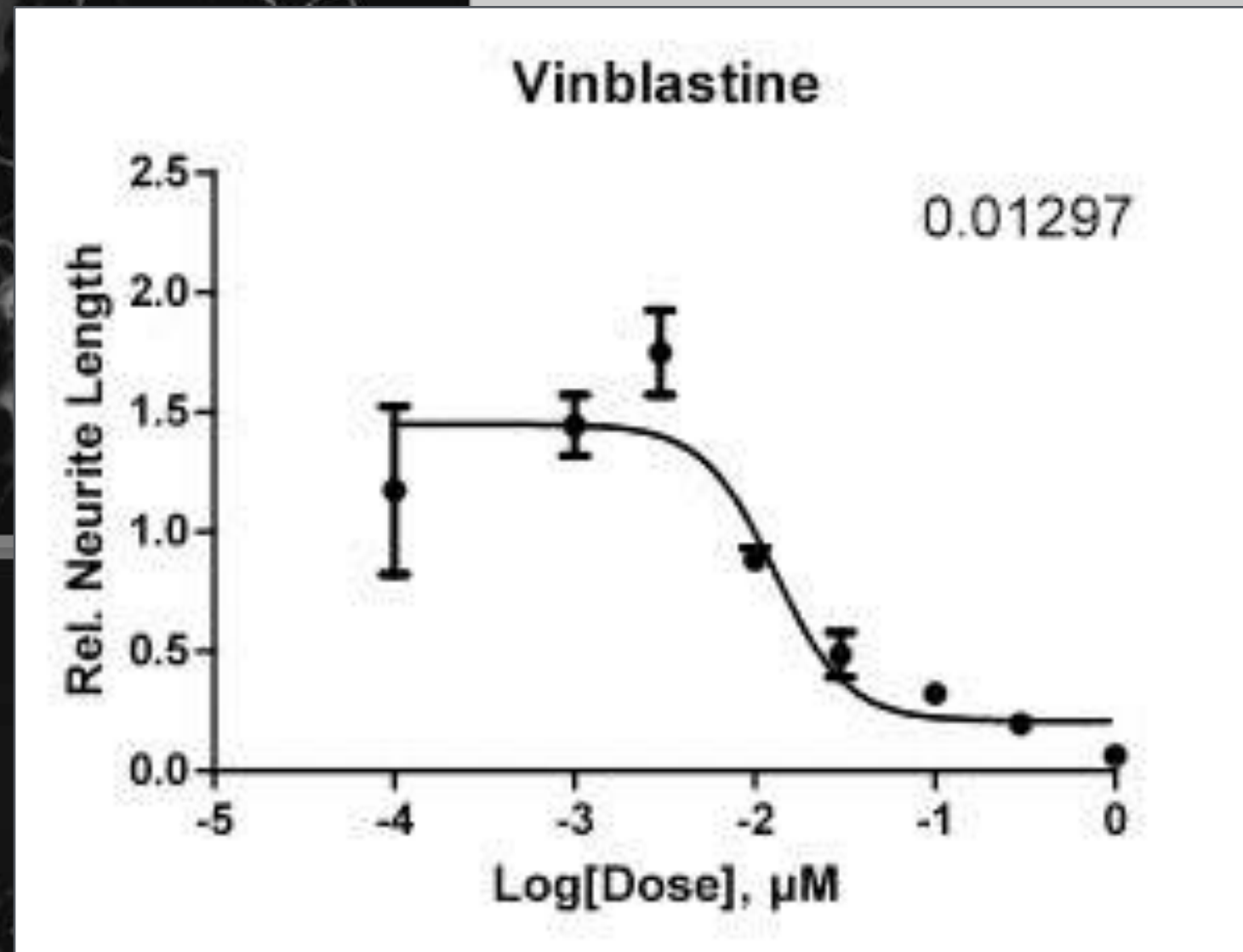
# Peri.4U - Neurite Outgrowth

## PCI2

100 pM Vinblastine

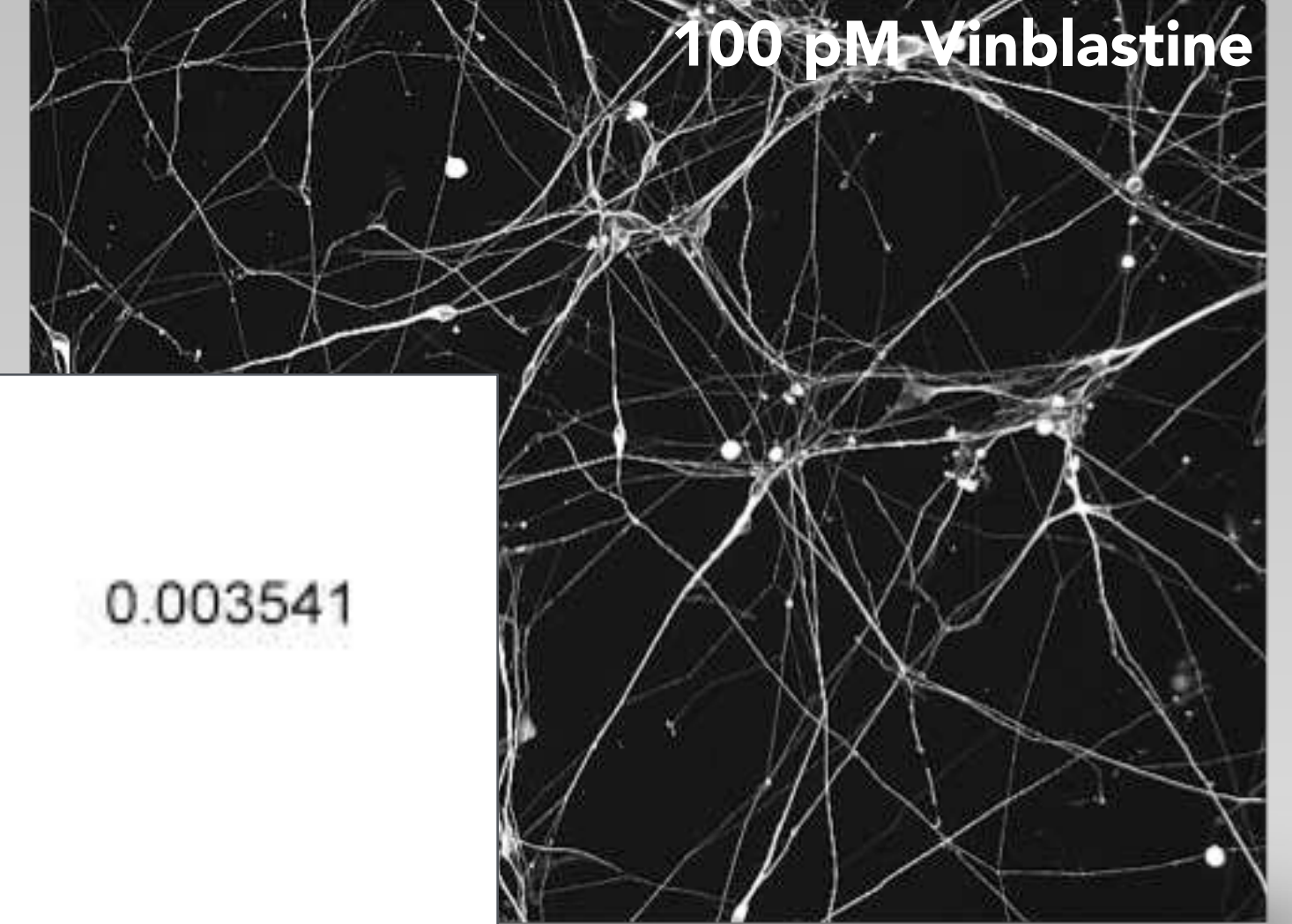


1 μM Vinblastine

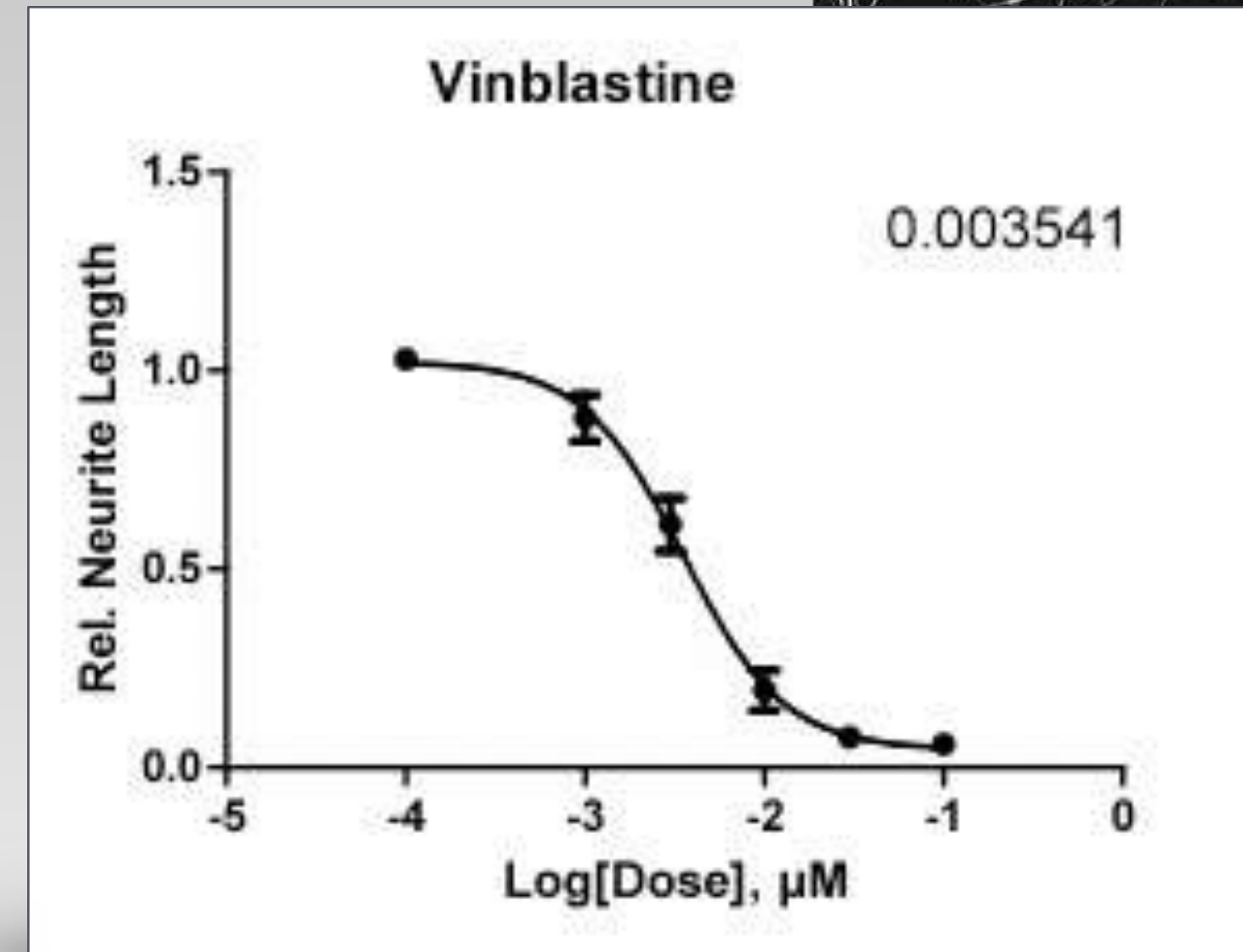
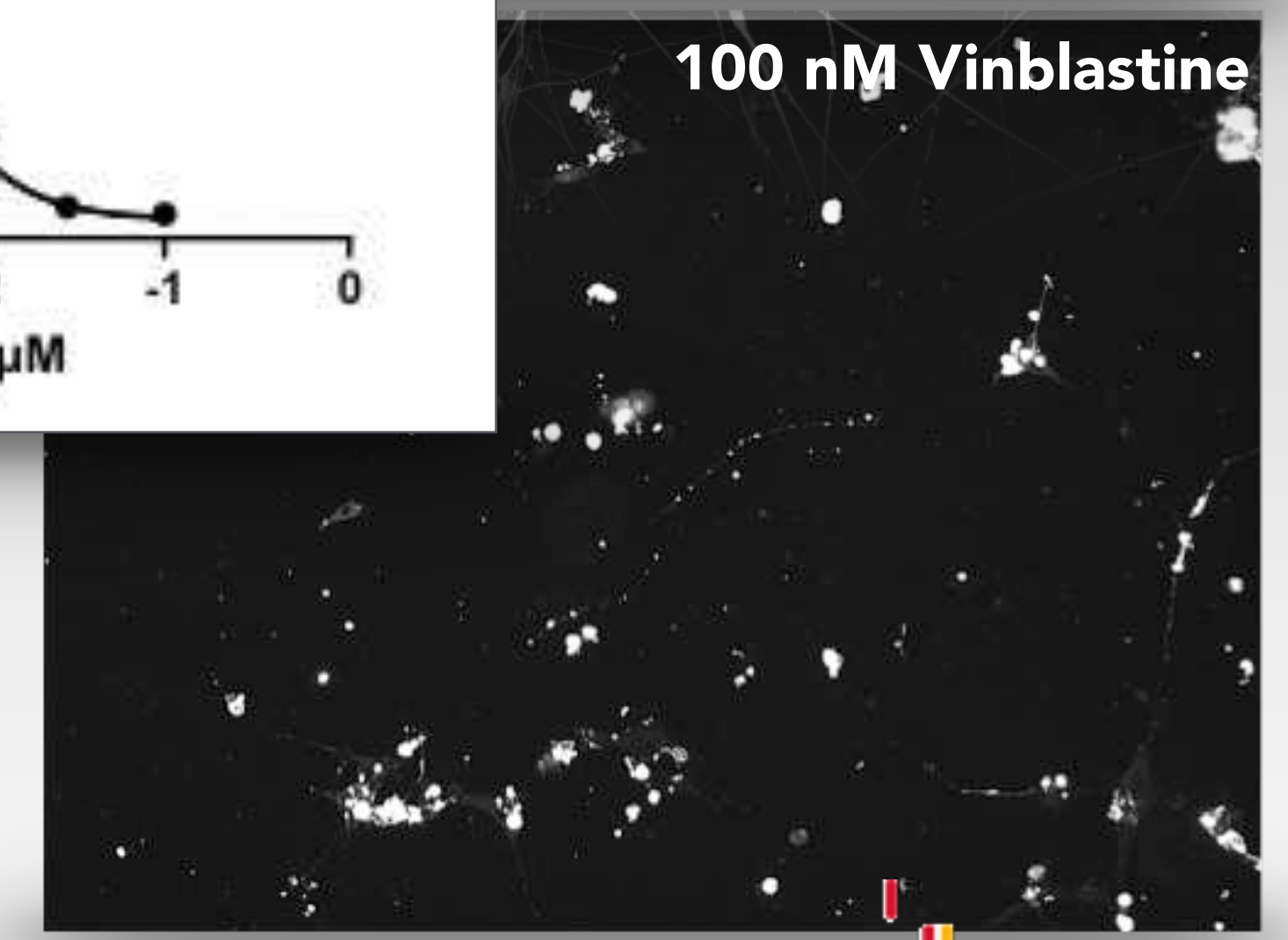


## Peri.4U

100 pM Vinblastine



100 nM Vinblastine

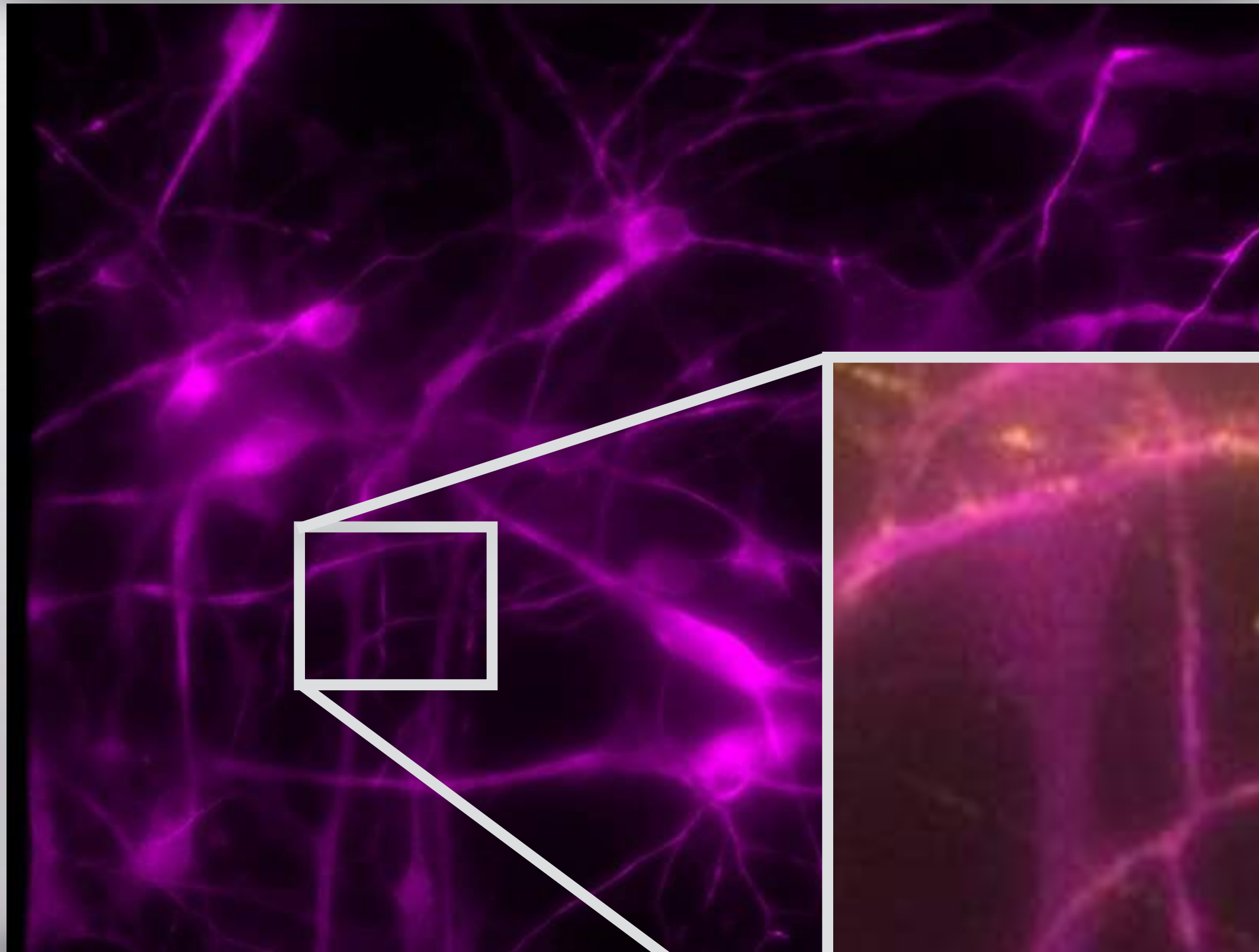




# Peri.4U - Peripheral Neurons

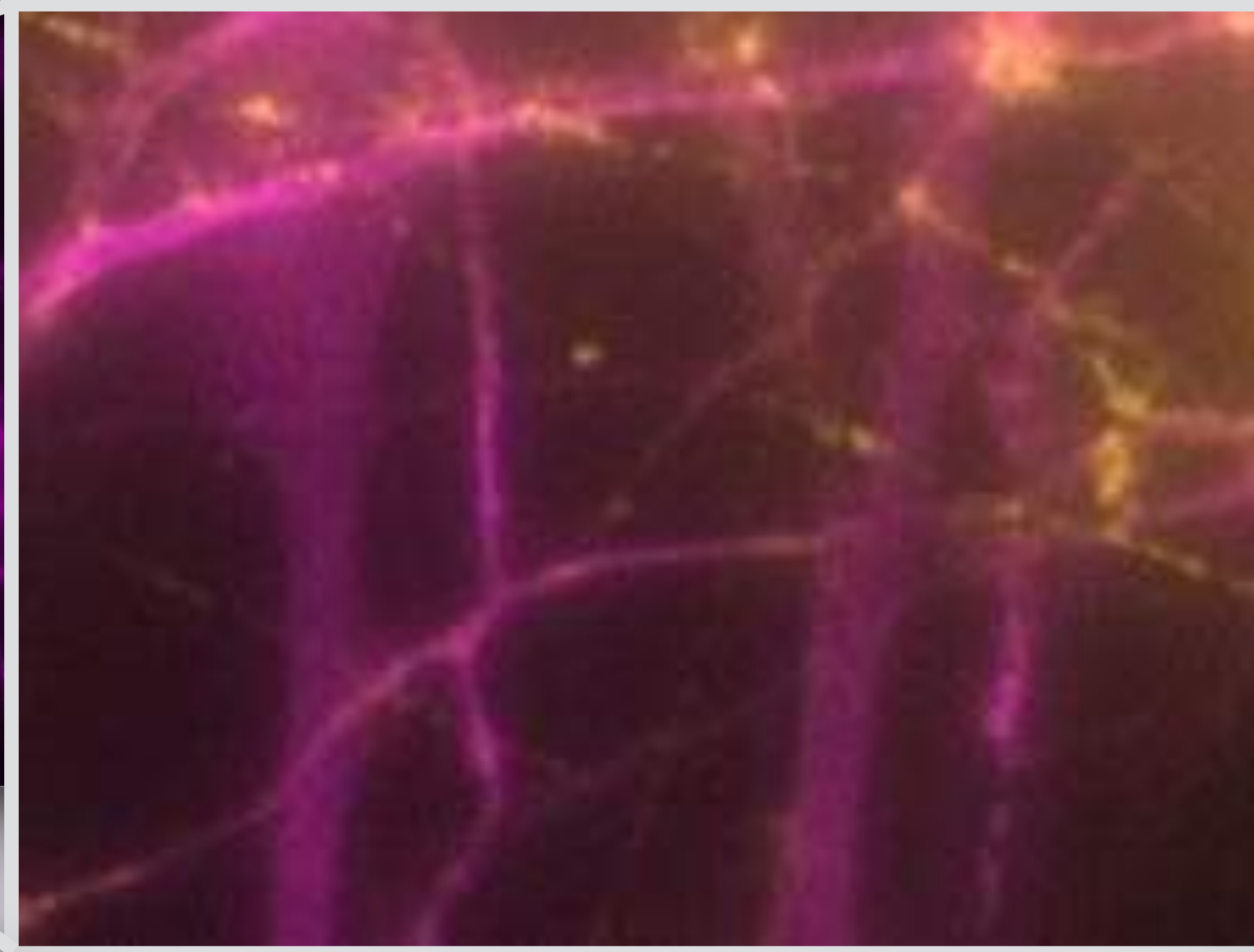
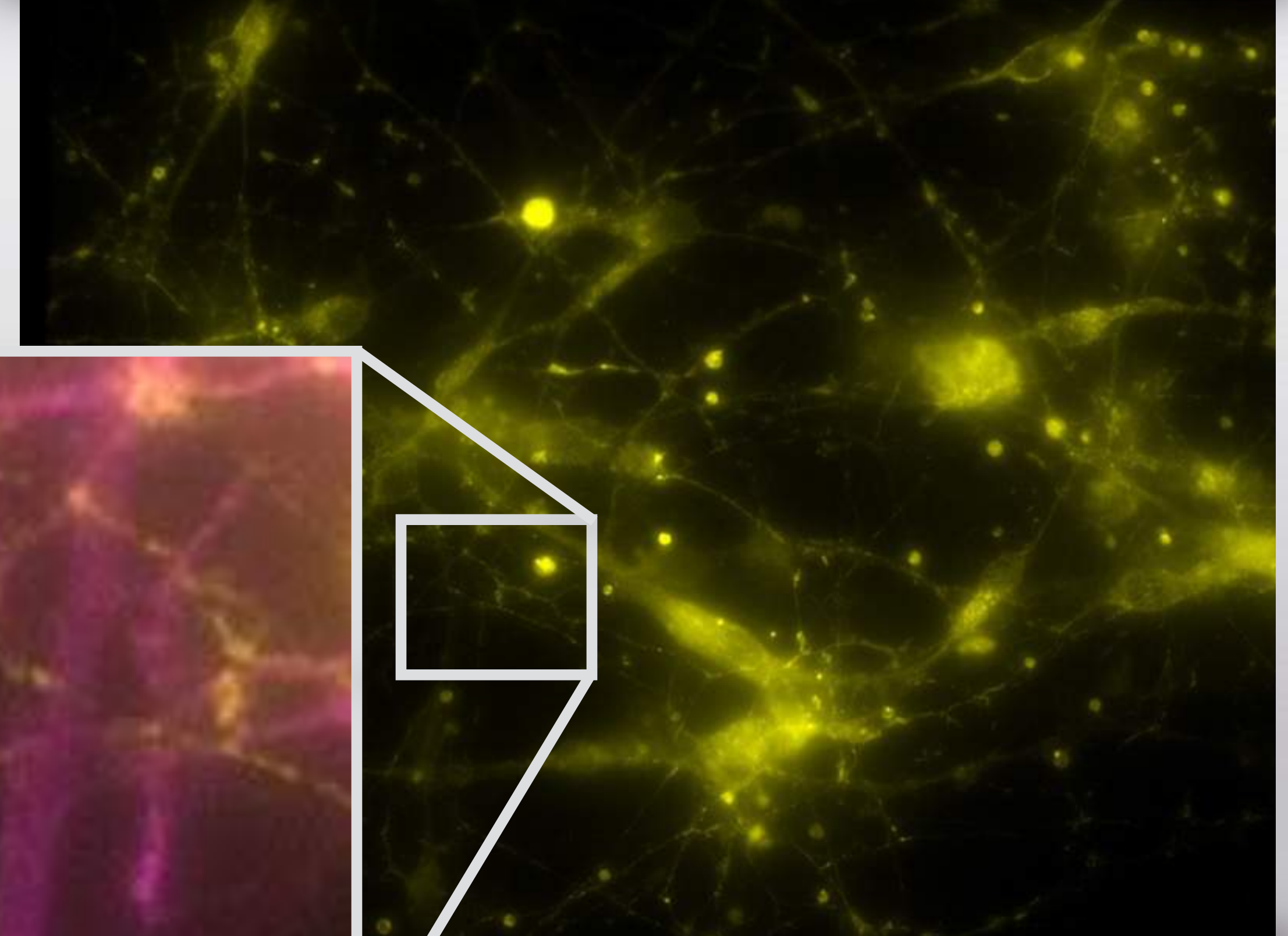
## MAP-2

(neuron-specific microtubule-associated protein 2)



## Synaptophysin

(presynaptic)

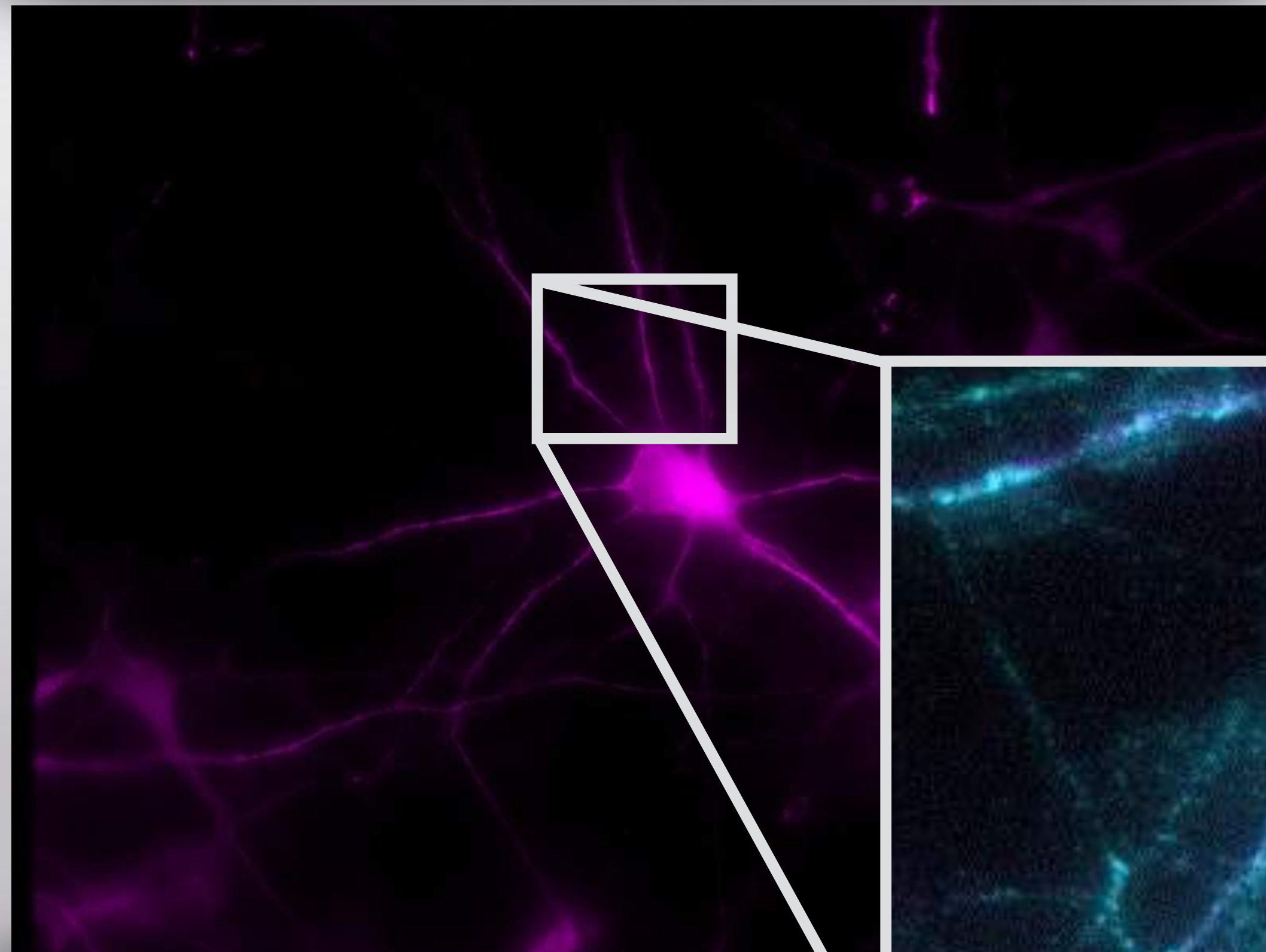




# Peri.4U - Peripheral Neurons

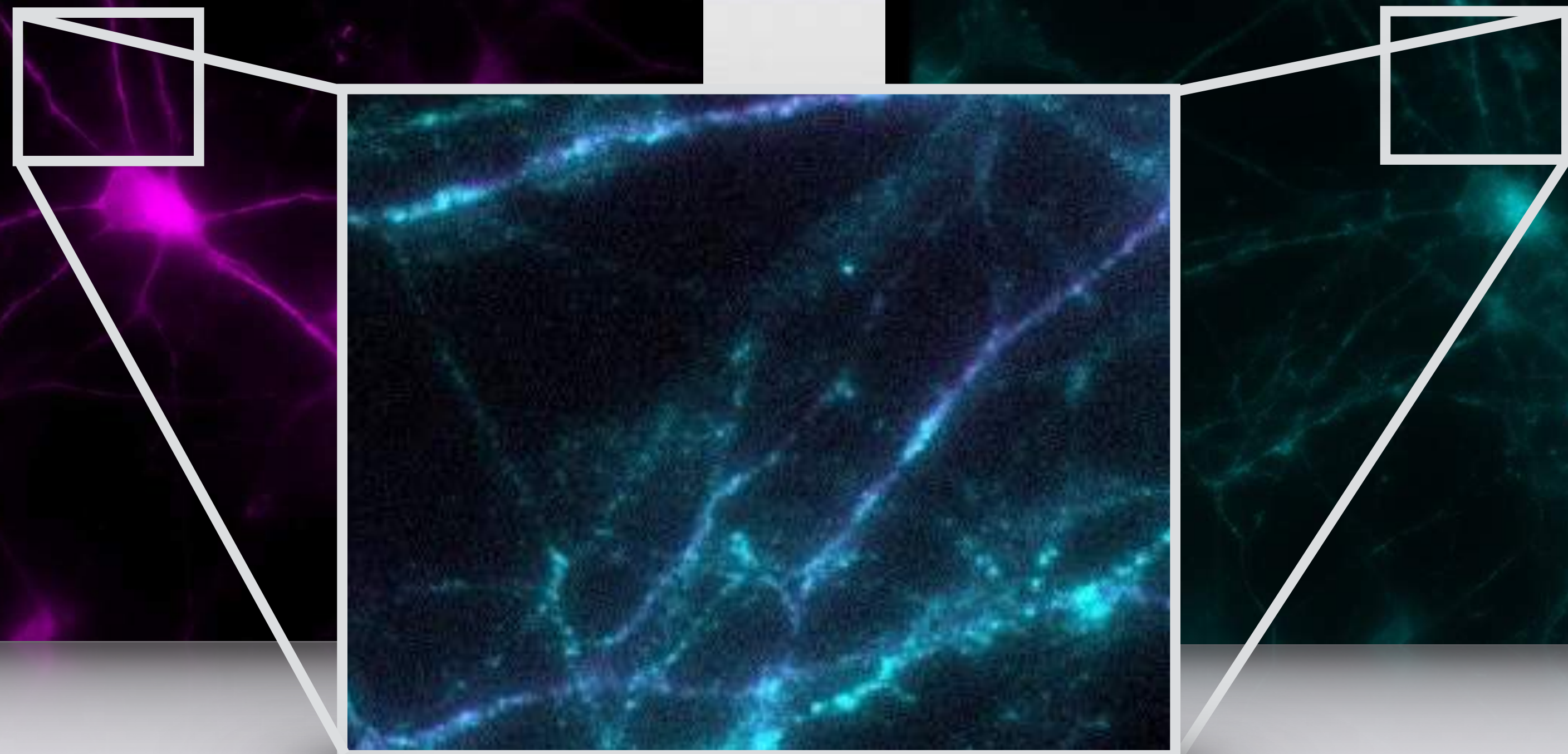
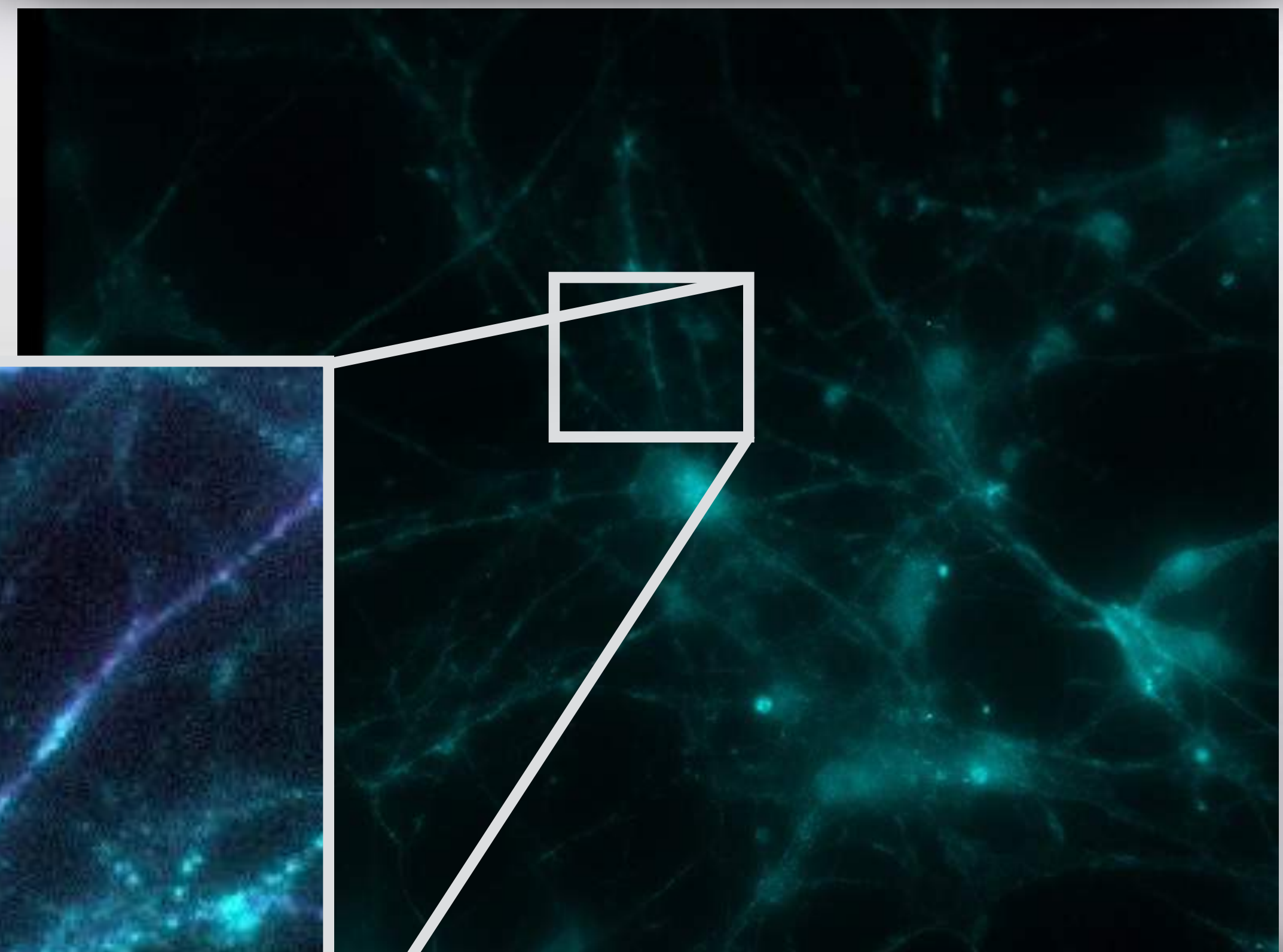
**MAP-2**

(neuron-specific microtubule-associated protein 2)



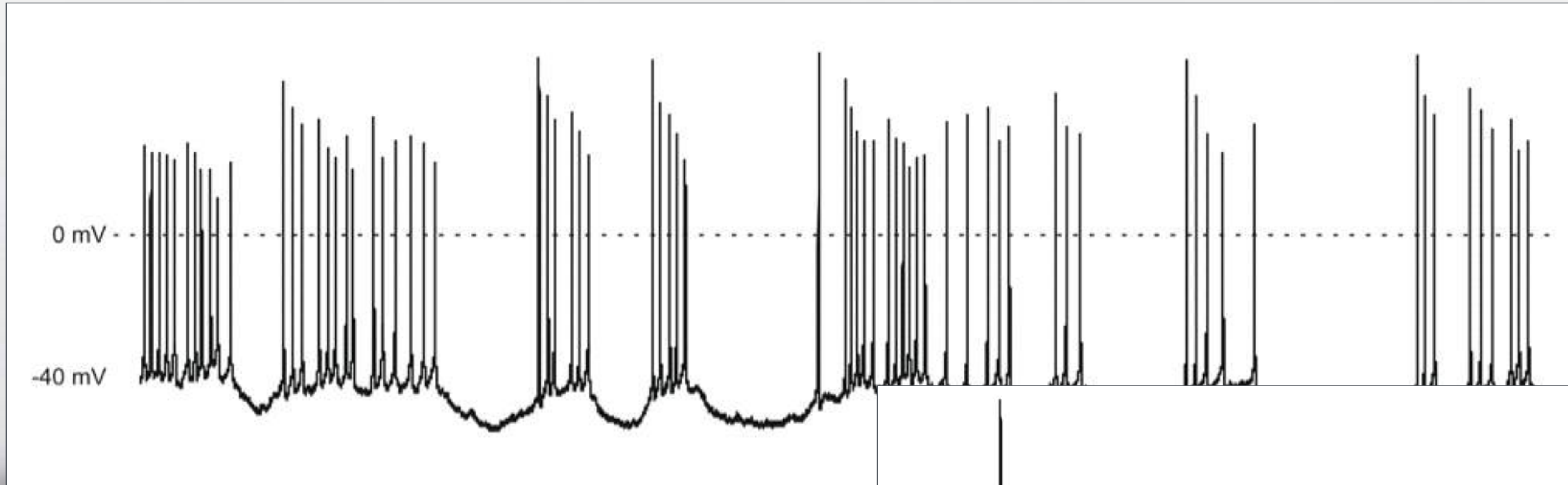
**PSD95**

(postsynaptic)

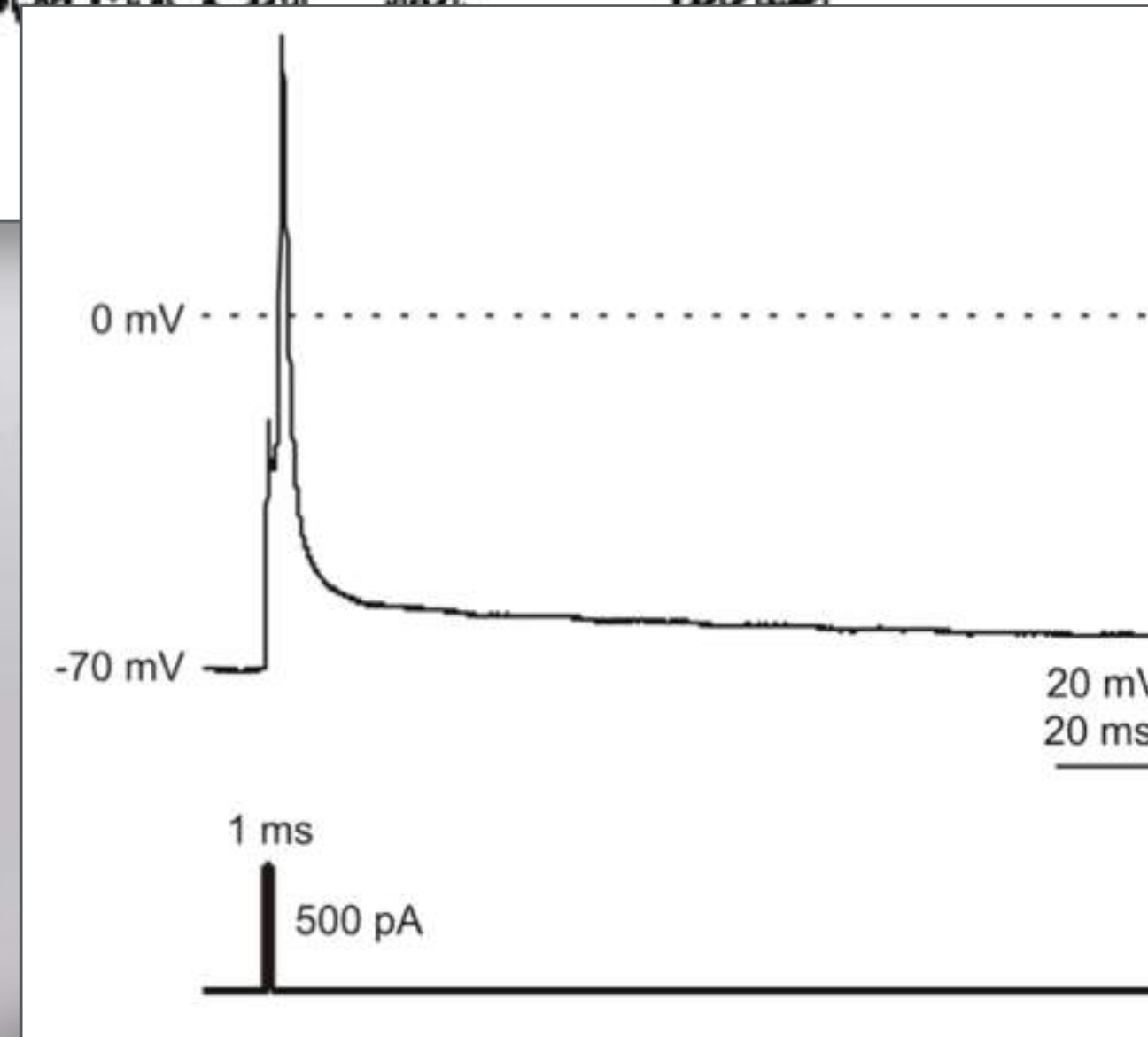




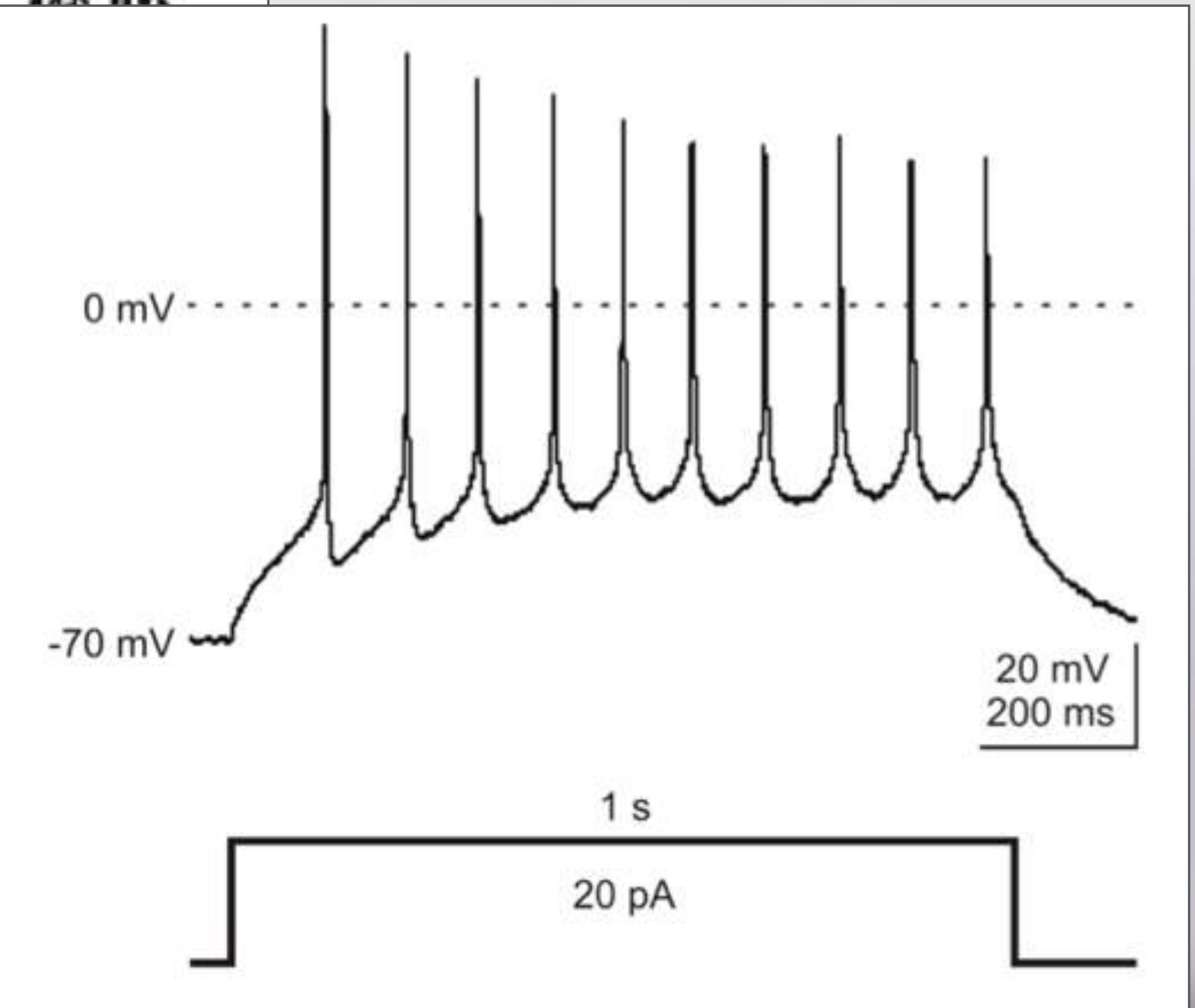
# Peri.4U - Patch Clamp (whole cell configuration)



Spontaneous

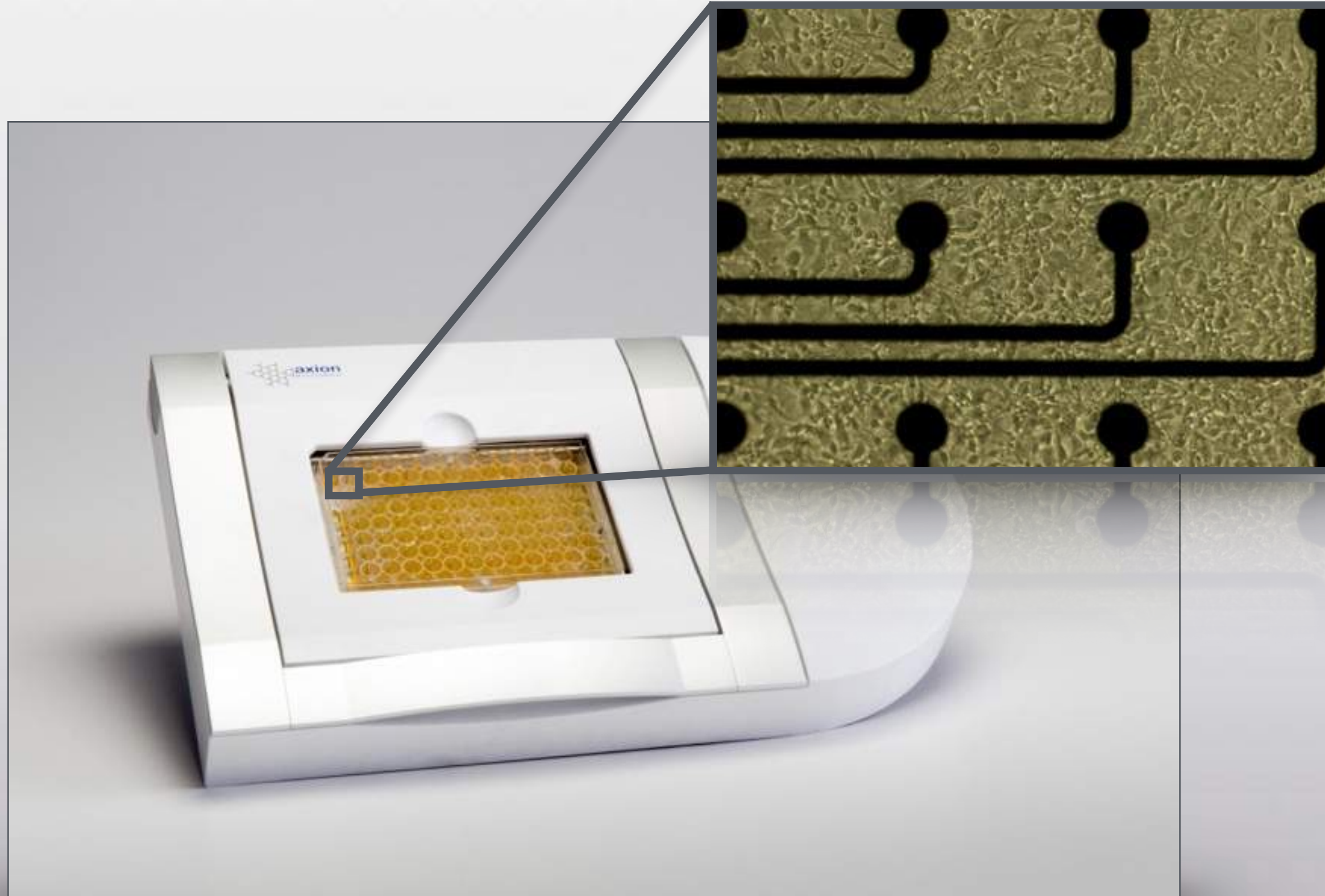


Elicited

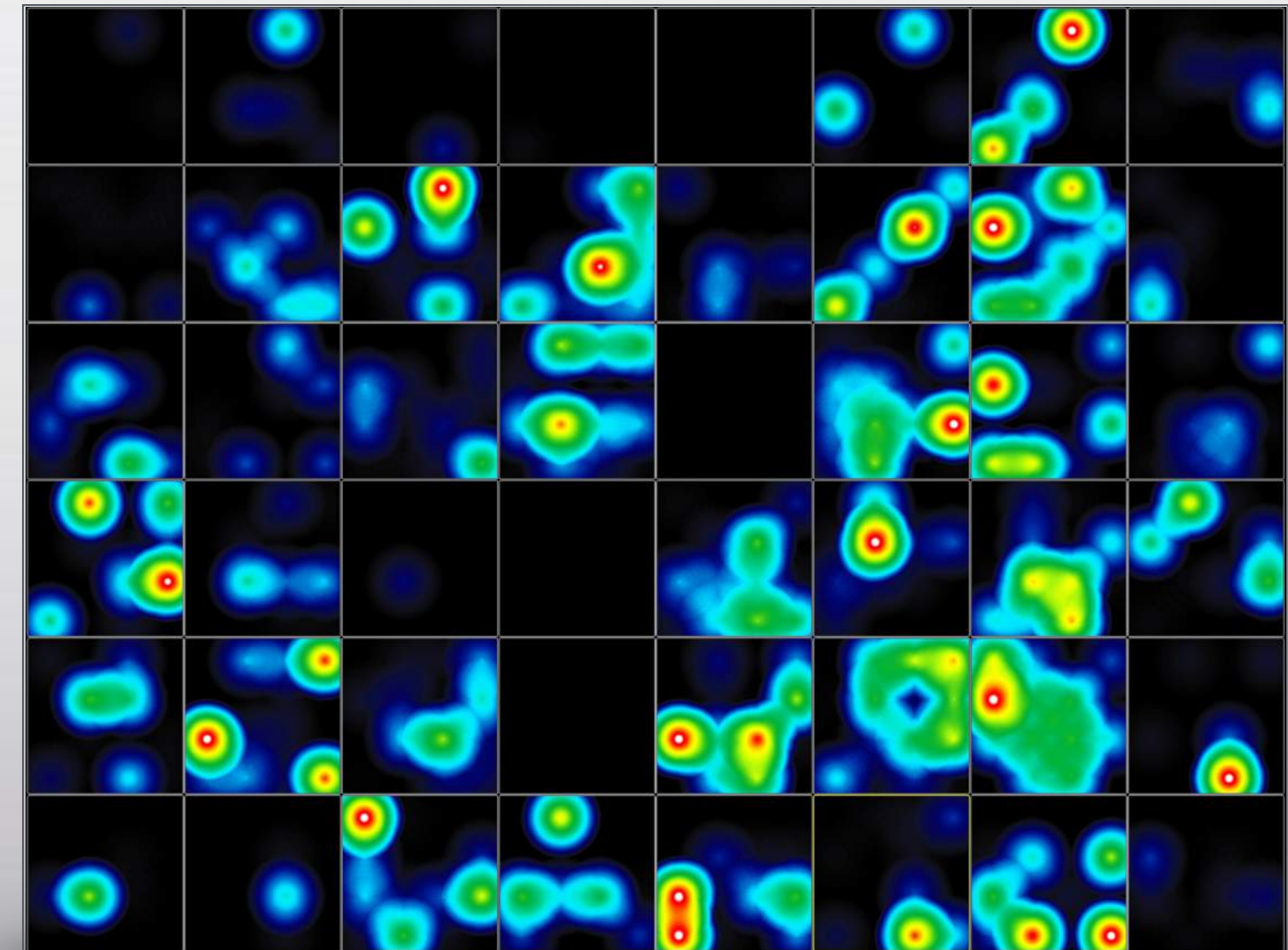




# Peri.4U - MicroElectrode Array (MEA)



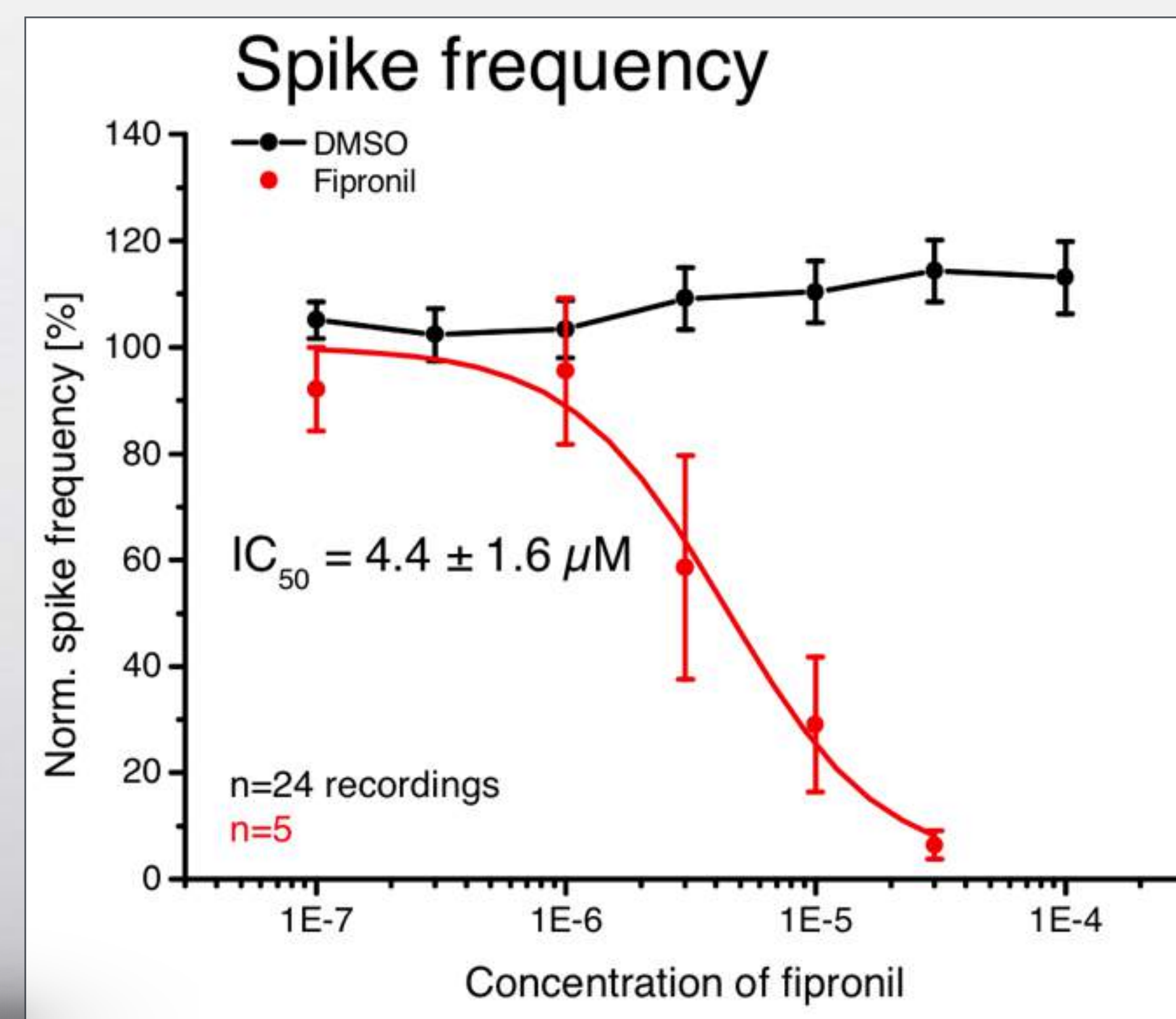
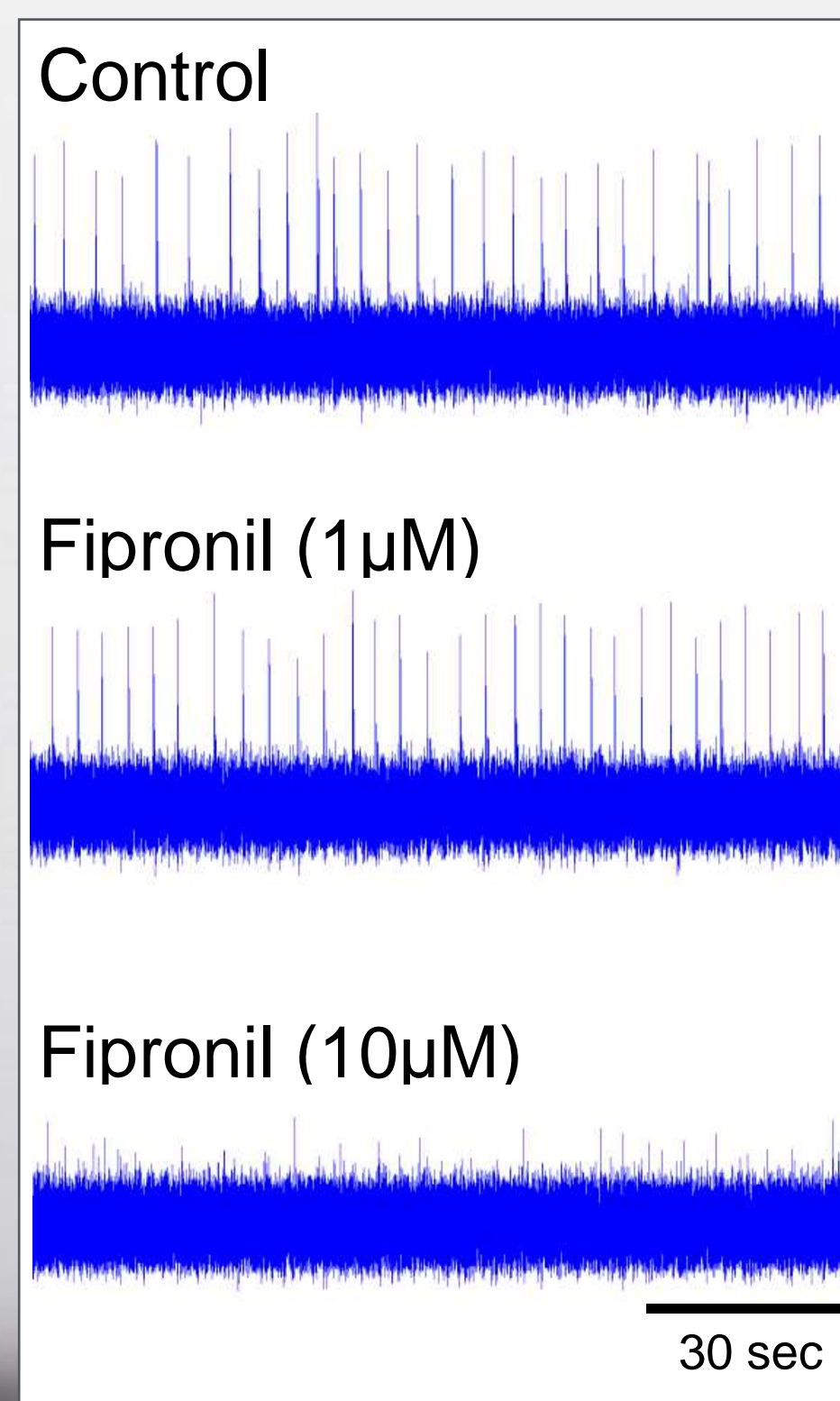
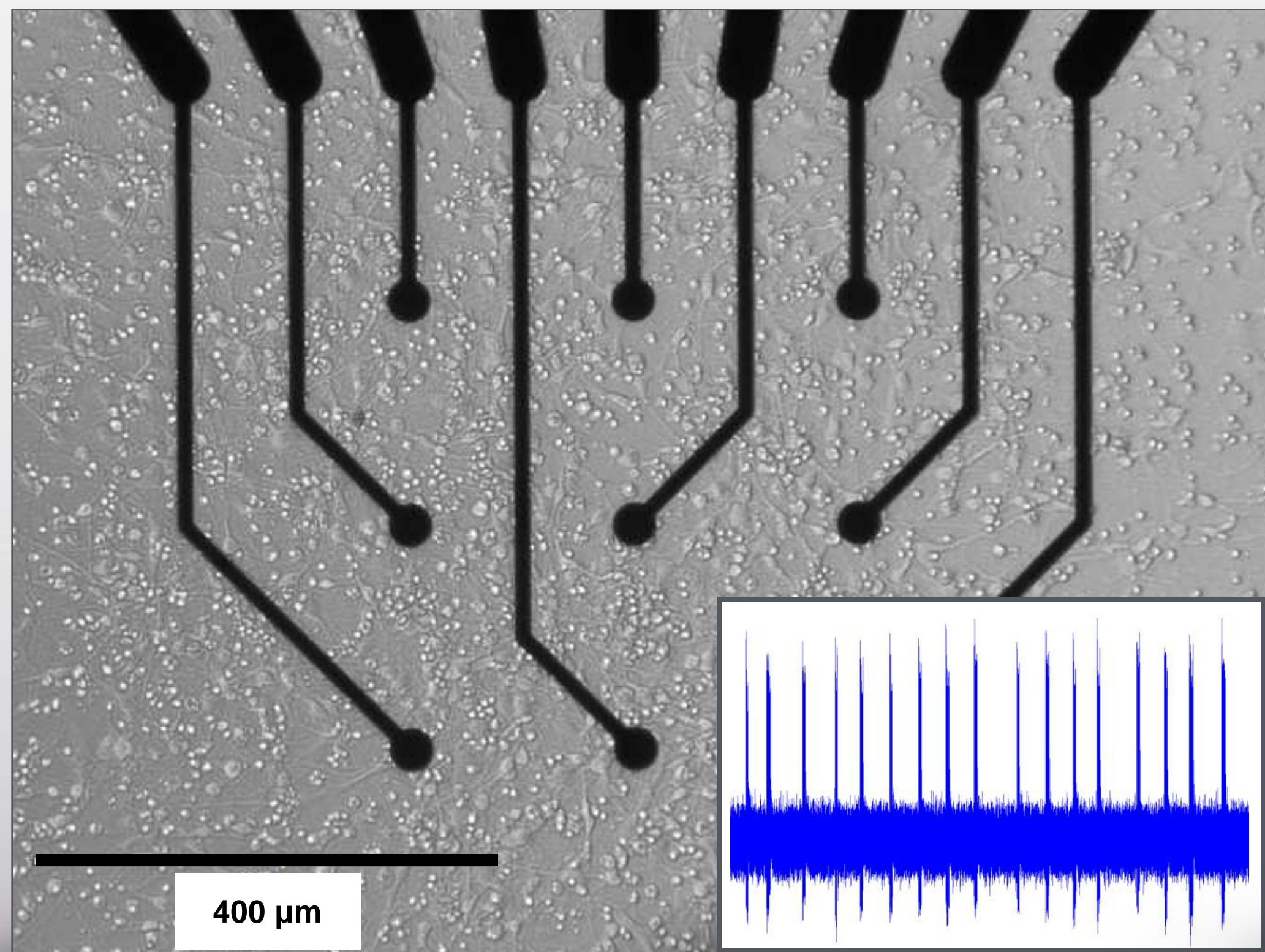
Peri.4U - 3 days *in vitro*



Anthony Nicolini, Axion Biosystems



# Peri.4U - MEA Tox

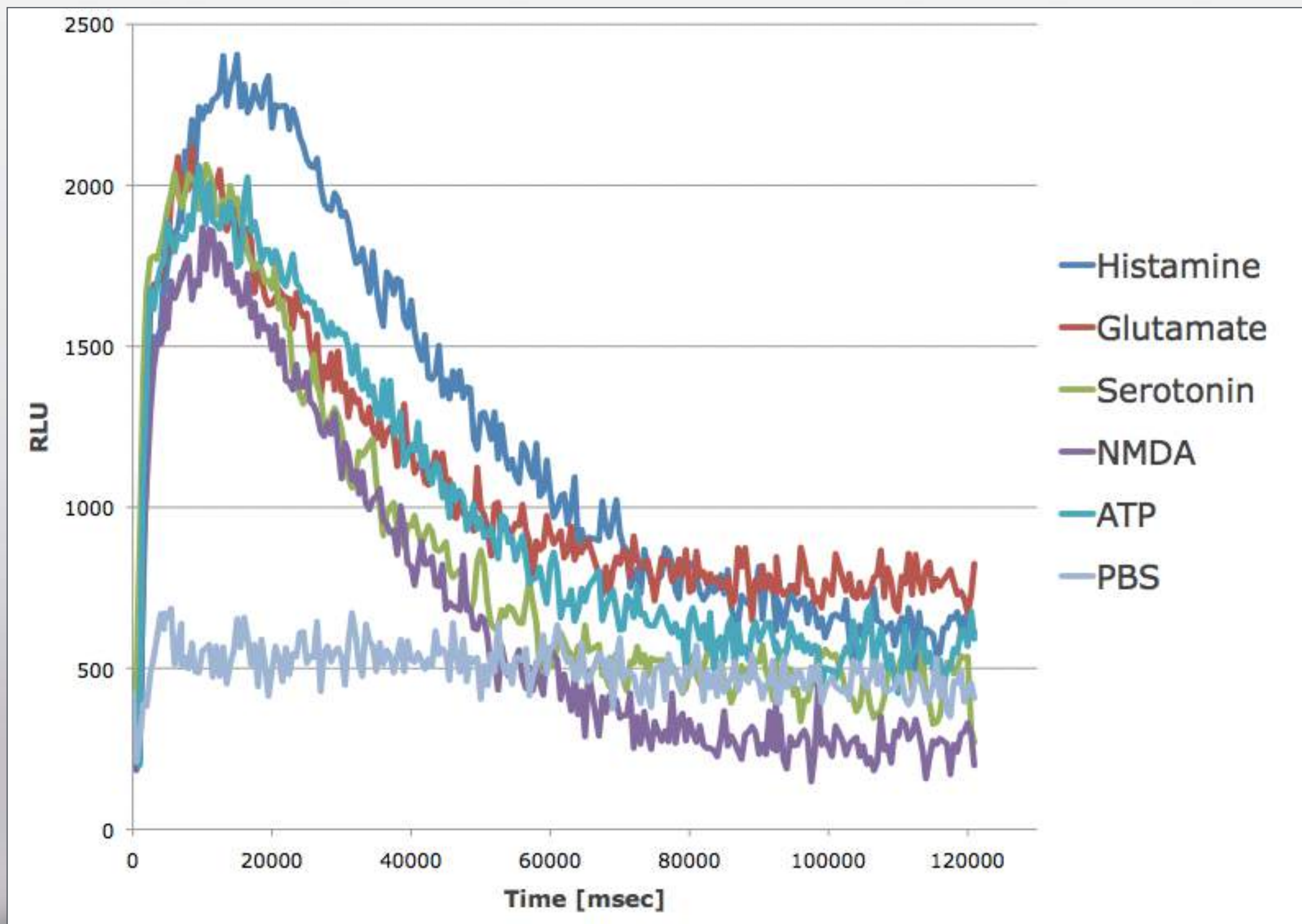


## Decreased Neuronal Activity with Fipronil:

- Rat cortical neurons  $\geq 10\mu\text{M}$
- Other Neuron Supplier  $\geq 50\mu\text{M}$
- Peri.4U  $\text{IC}_{50}=4.4 \pm 1.6\mu\text{M}$



# Peri.4U - Functional receptor activity (Hamamatsu FDSS/ $\mu$ Cell - $\text{Ca}^{2+}$ Transient)

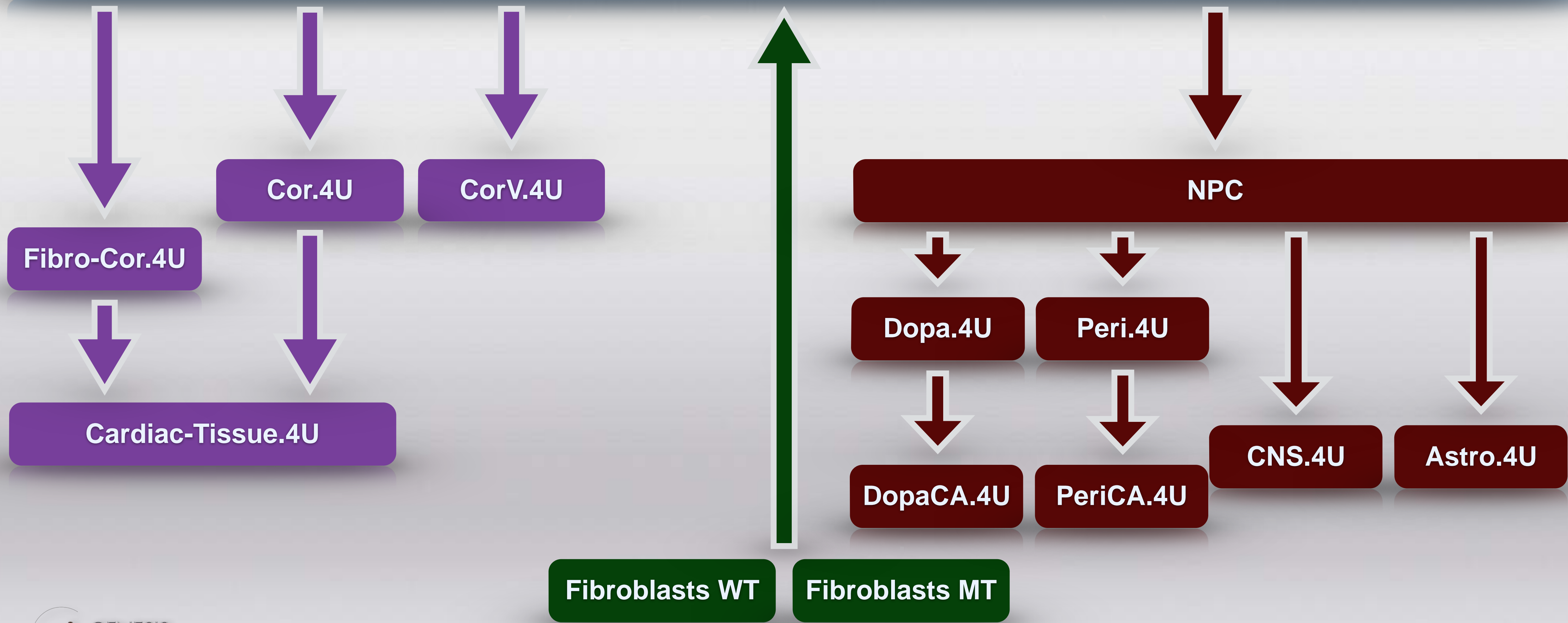


- Histamine (ligand of H3 Receptor expressed in peripheral neurons)
- Glutamate (ligand for metabotropic and ionotropic glutamate receptors as the NMDA receptor in peripheral neurons)
- Serotonin (ligand for 5-HT3 receptor, specific for peripheral neurons)
- NMDA (ligand for NMDA receptor expressed in peripheral neurons)
- ATP (Ligand for purinergic receptors P2RX4 and P2RX7 expressed in peripheral neurons)
- PBS (control)



# Overview ( $\beta$ -Product)

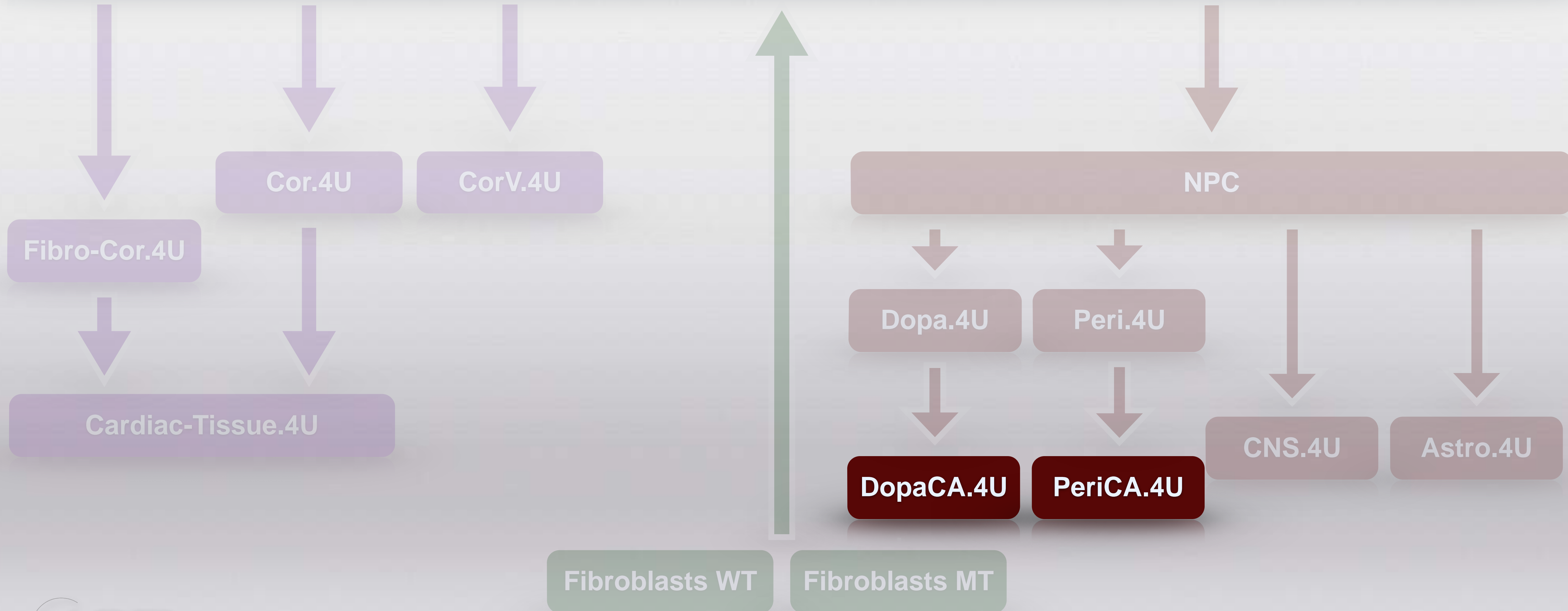
hiPSC (from Axiogenesis/from clients/CRISPR/...)





# Overview ( $\beta$ -Product)

hiPSC (from Axiogenesis/from clients/CRISPR/...)





# PeriCA.4U and DopaCA.4U - Integrated Ca<sup>2+</sup> Sensor

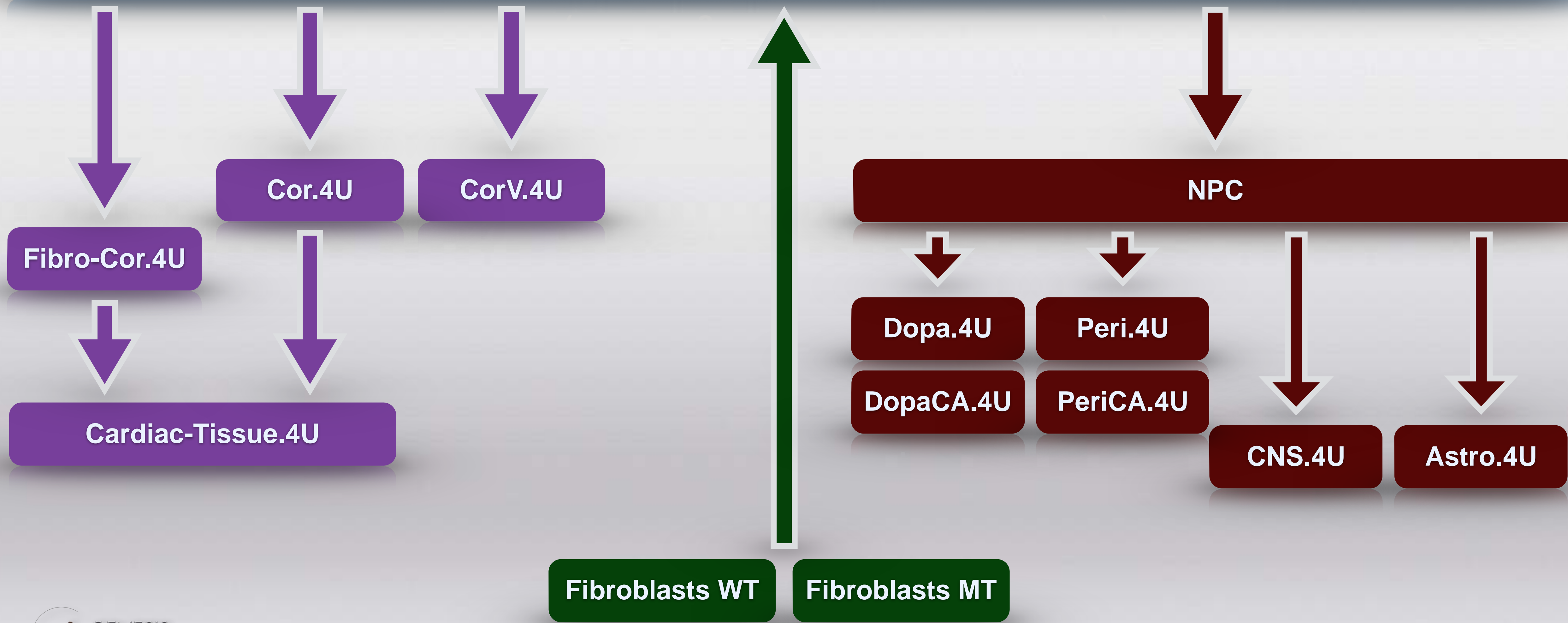


PeriCA.4U: 10  $\mu$ M Ionomycin



# Overview ( $\beta$ -Product)

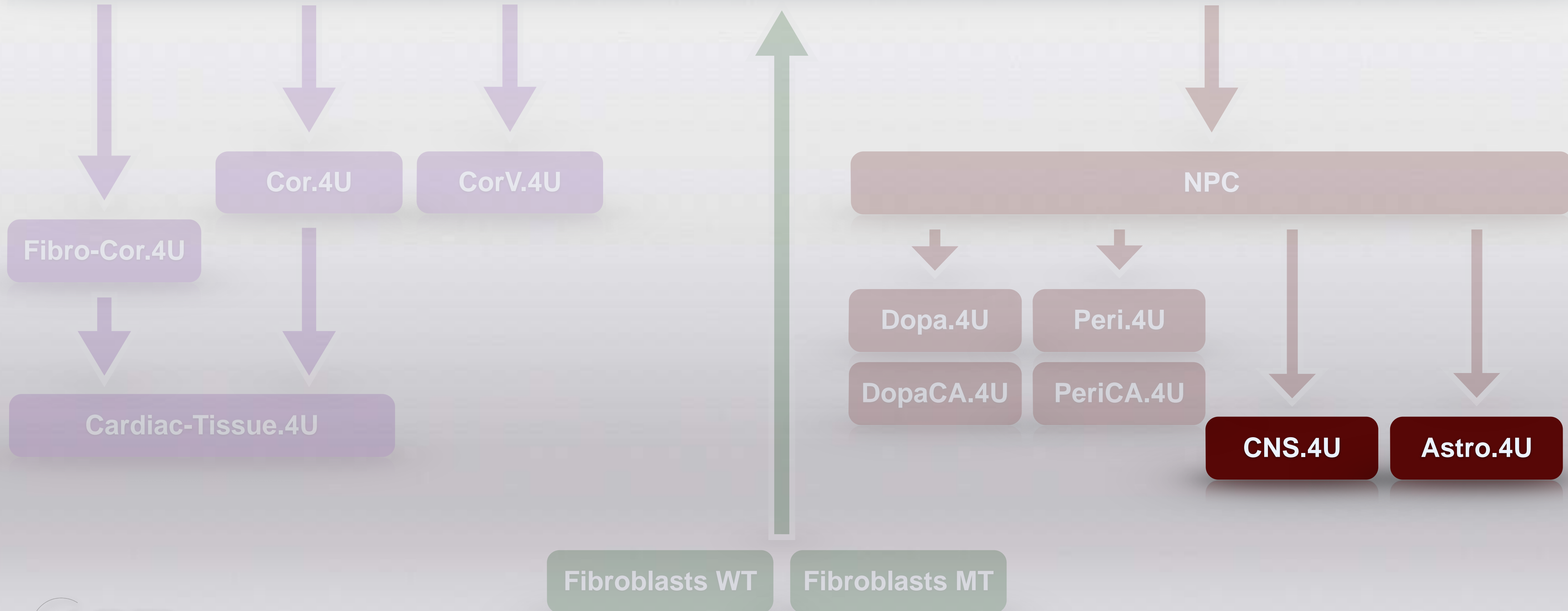
hiPSC (from Axiogenesis/from clients/CRISPR/...)





# Overview ( $\beta$ -Product)

hiPSC (from Axiogenesis/from clients/CRISPR/...)



Fibroblasts WT

Fibroblasts MT

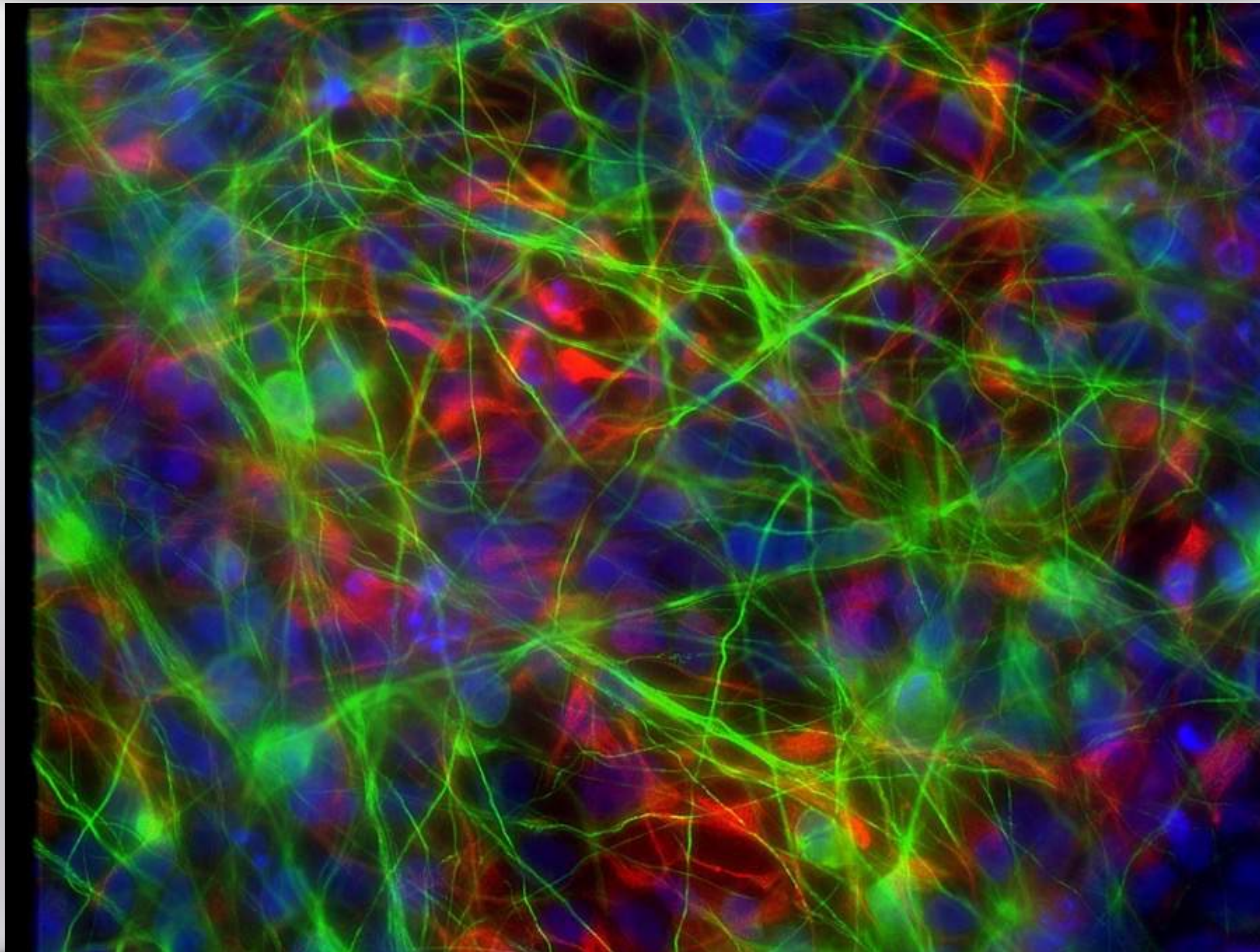


# CNS.4U - human iPSC derived Central Nervous System Cells

Tuji

S100 $\beta$ 

Hoechst



**Neurons / Astrocytes (50% / 50%)**

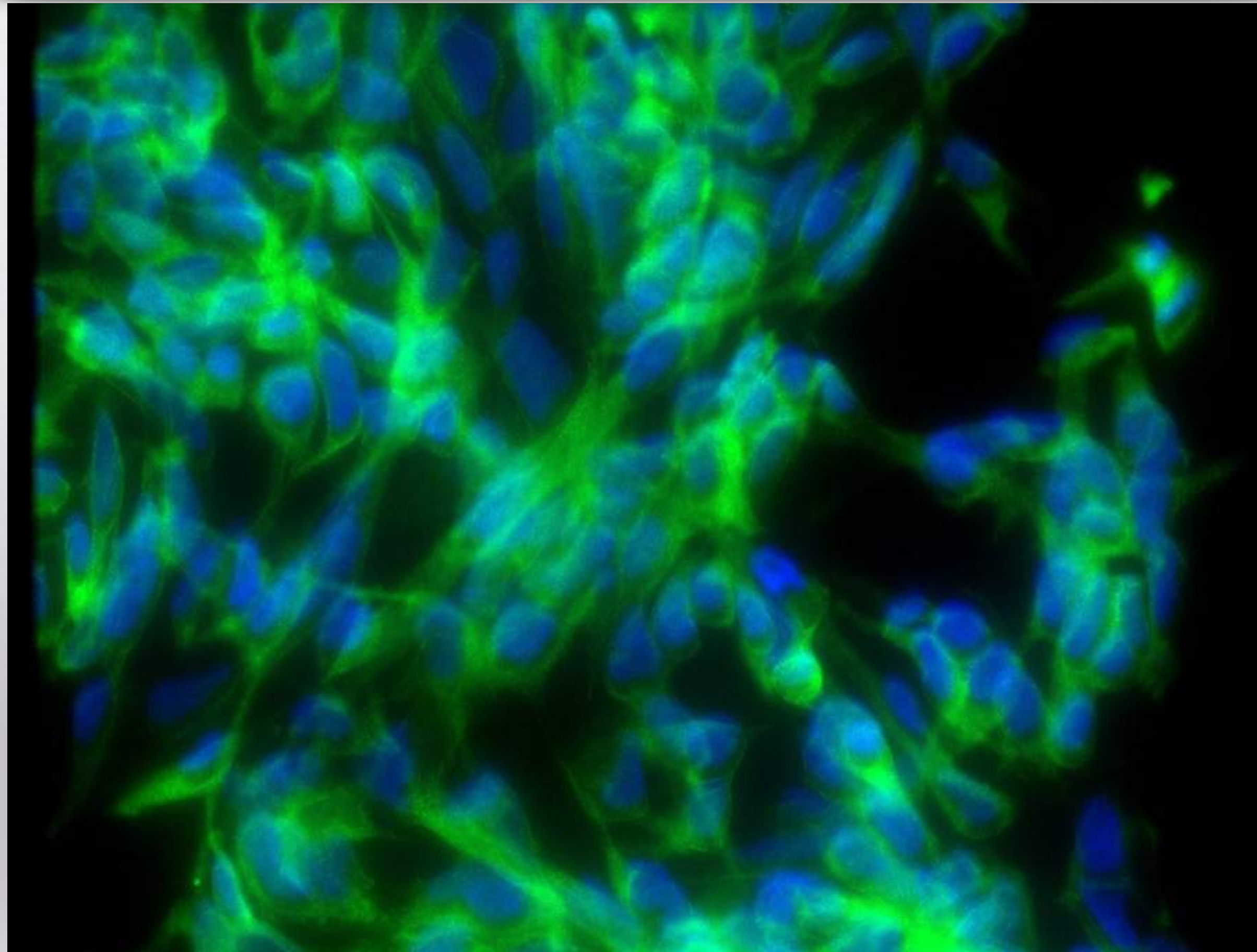
- Glutamatergic Neurons (40-45%)
- GABAergic Neurons (35-40%)
- Dopaminergic Neurons (10-15%)



# Astro.4U - human iPSC derived Astrocytes

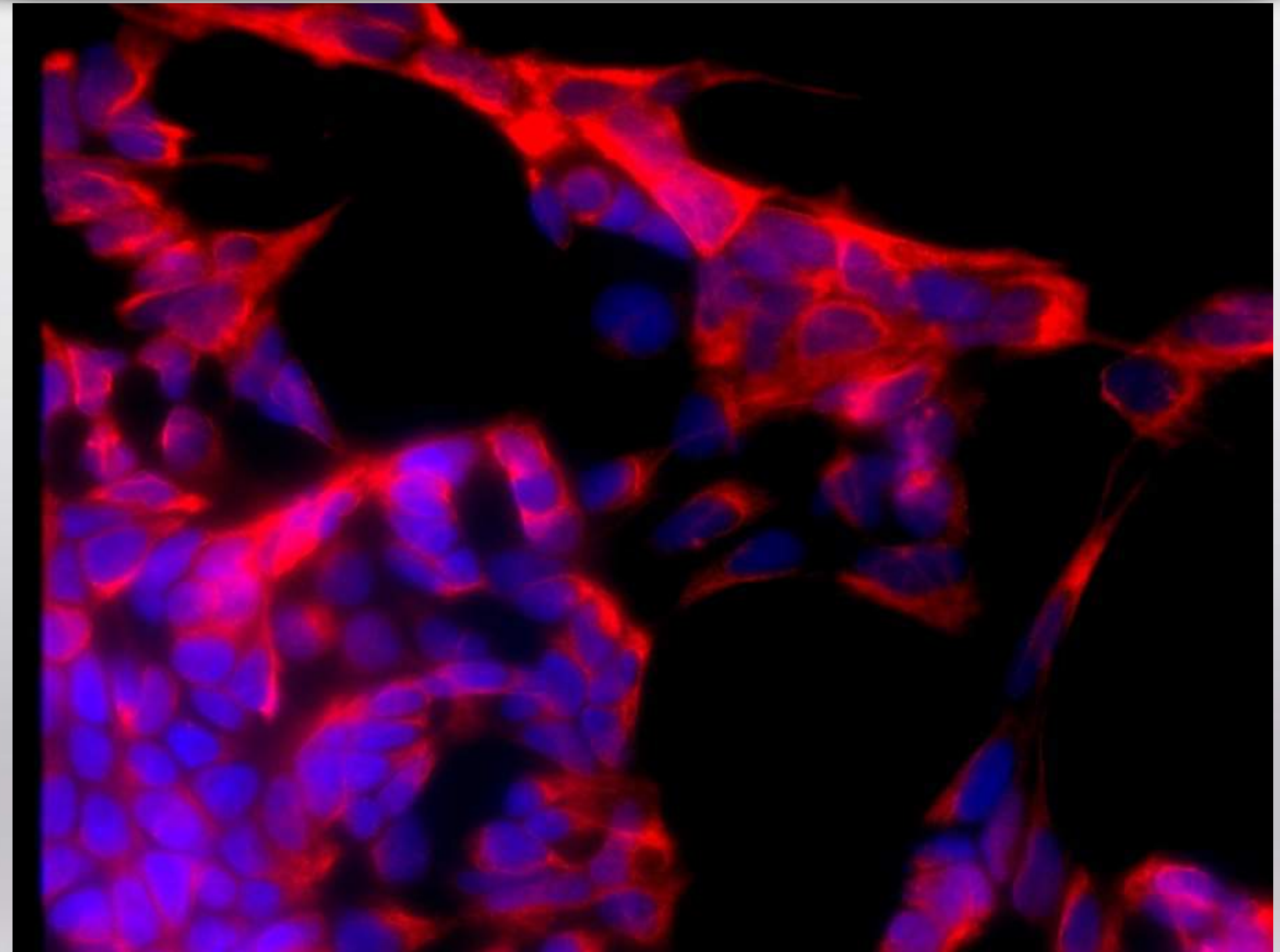
**GFAP**

(Glial fibrillary acidic protein)



**S100B**

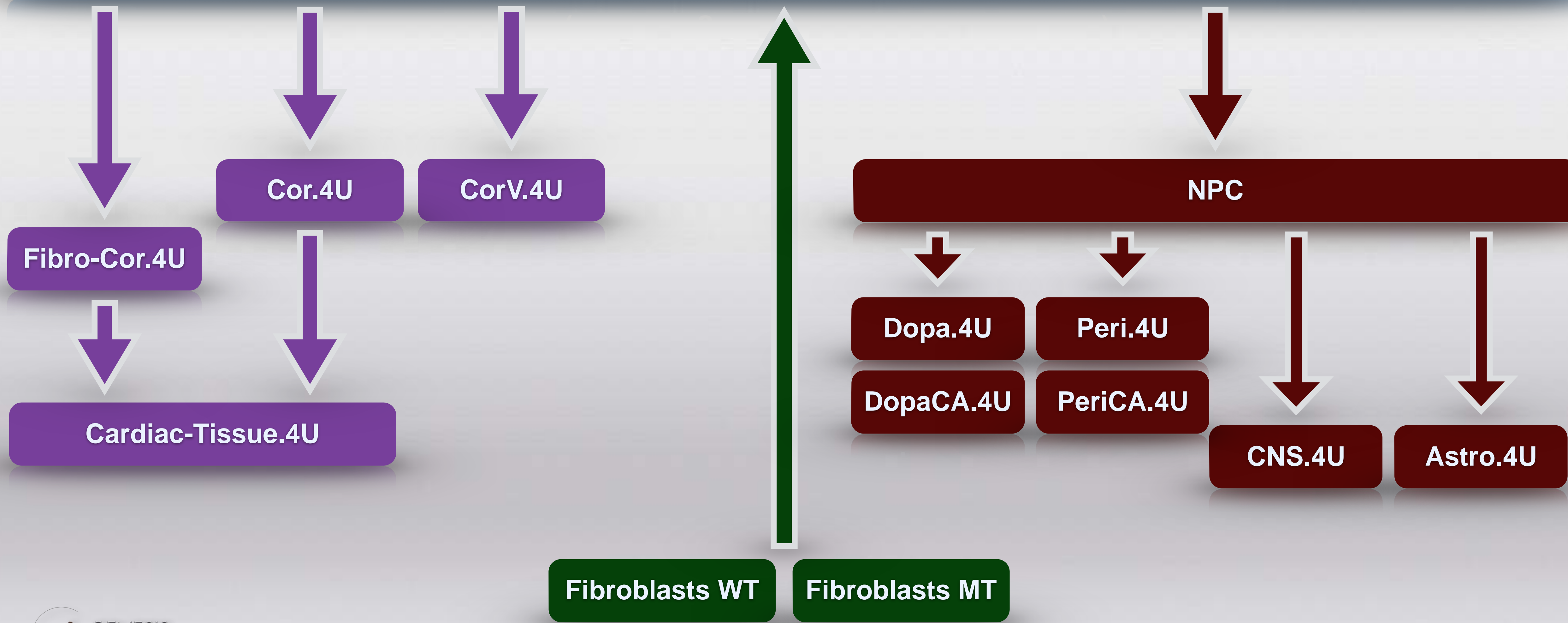
(S100 calcium-binding protein B)





# Overview (Services)

hiPSC (from Axiogenesis/from clients/CRISPR/...)





# Overview (Services)

hiPSC (from Axiogenesis/from clients/CRISPR/...)

## „On Demand“ Cell-Service

Banking & Scale Up

Gene Editing (CRISPR/CAS9)

Patient-derived

Co-development

NPC

Dopa.4U

Peri.4U

DopaCA.4U

PeriCA.4U

CNS.4U

Astro.4U

Fibroblasts WT

Fibroblasts MT



Company

Characterization

Product/Format

Service

**axio** GENESIS

0049-221-998818-0

info@axiogenesis.com

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50829 Cologne, Germany