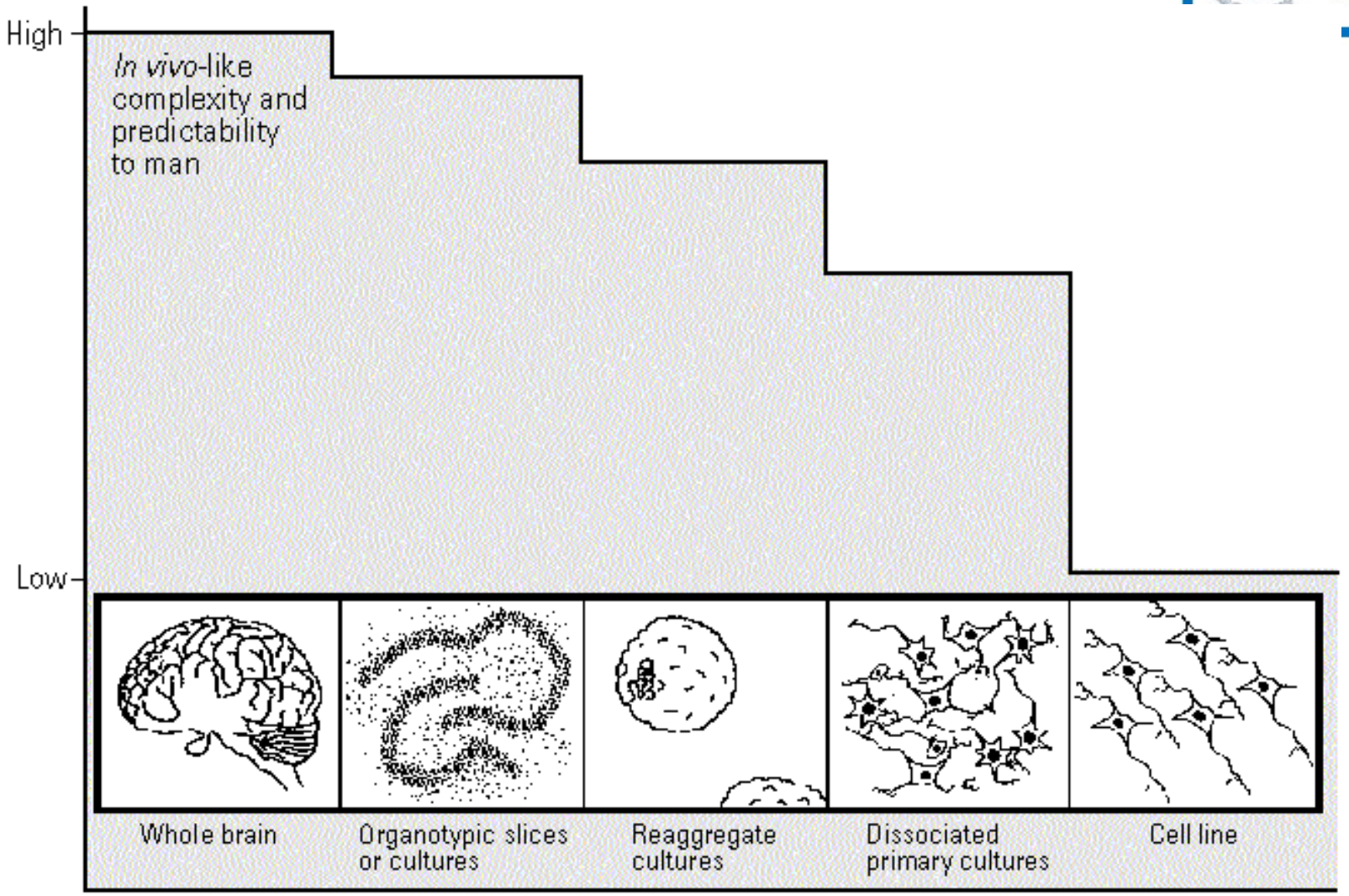
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

Techniques in cellular neurobiology

Lesson 2

Gabriele Baj
gbaj@units.it

MODELs IN NEUROBIOLOGY



Cell Lines

(i) the scientific method,

(ii) signaling processes involved in cellular differentiation, and

(iii) the use of pharmacological agents to manipulate a cell culture system.

Cell Lines

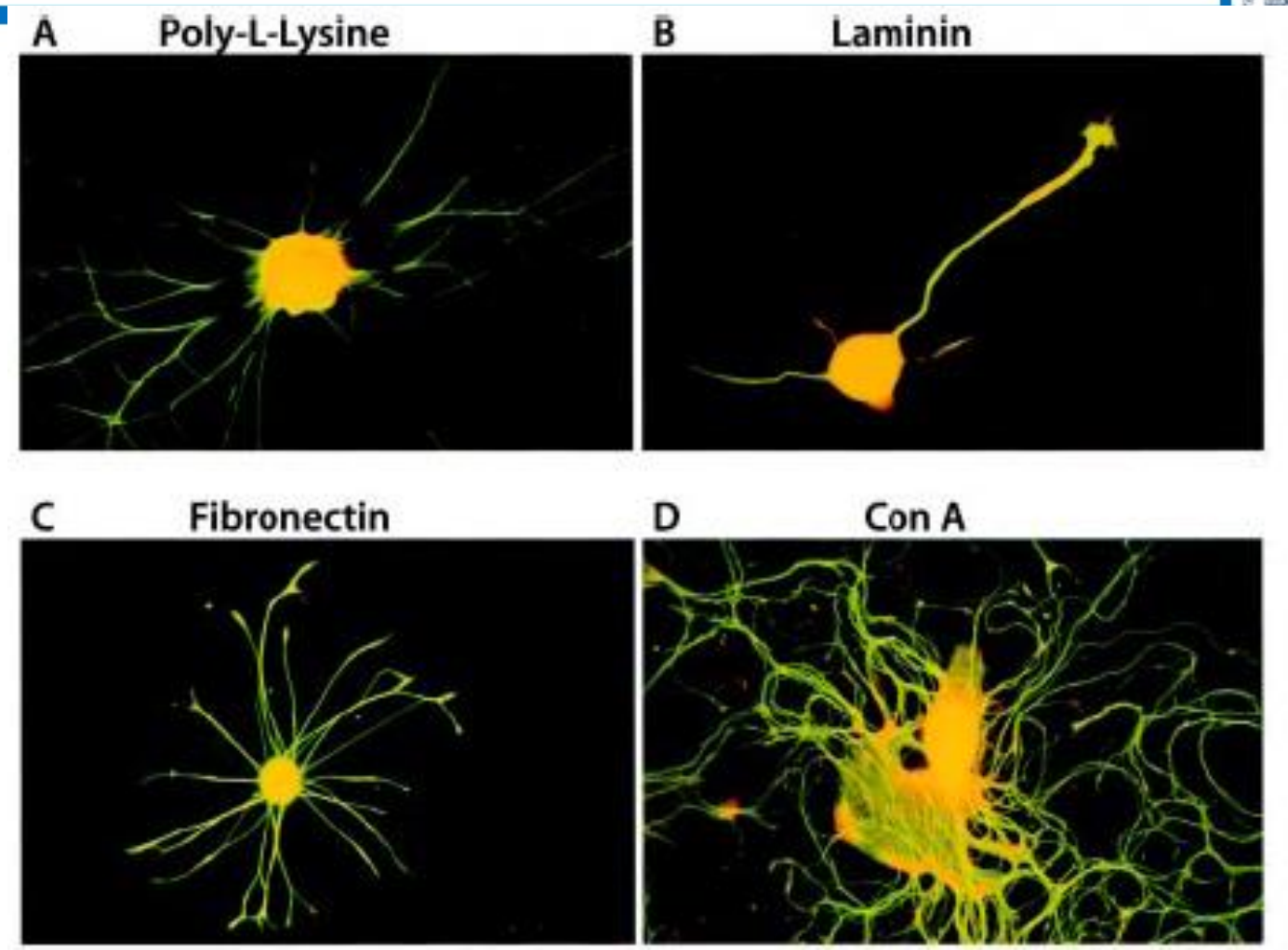
Advantages	Disadvantages
Controlled physiochemical environment (pH, temperature, oxygen, carbon dioxide, osmotic pressure, etc.)	
Controlled and defined physiological conditions (constitution of medium, etc.)	10 times more cheap for the same quantity of animal tissue
Homogeneity of cell types (achieved through serial passages)	Unstable aneuploid chromosome constitution
Economical, since smaller quantities of reagents are needed than in vitro	Requirement of controlled and defined physiological conditions

Advantages and Disadvantages of Primary cells and cell Lines

Features	Primary cells	Cell Lines
1. Ploidy	Diploid Euploid	Heteroploid Aneuploid
2. Transformation	Normal	Transformed
3. Tomourigenicity	Non-tumourigenic	No Tumourigenic
4. Anchorage Dependence	Yes	No
5. Contact Inhibition	Yes	No
6. Density Limitation of Growth	Yes	No

Features	Primary cells	Cell lines
7. Mode of Growth	Monolayer	Monolayer of Suspension.
8. Maintenance	Cyclic	Steady State Possible.
9. Serum Requirement	High	Low
10. Cloning Efficiency	Low	High
11. Markers	May be tissue specific	Chromosomal, enzymic
12.Special Functions	May be retained	Often lost
13. Growth Rate	Slow	Rapid
14. Yield	Low	High
15. Control Features.	Generation Number in vivo markers	Strain Characteristics

Primary cells



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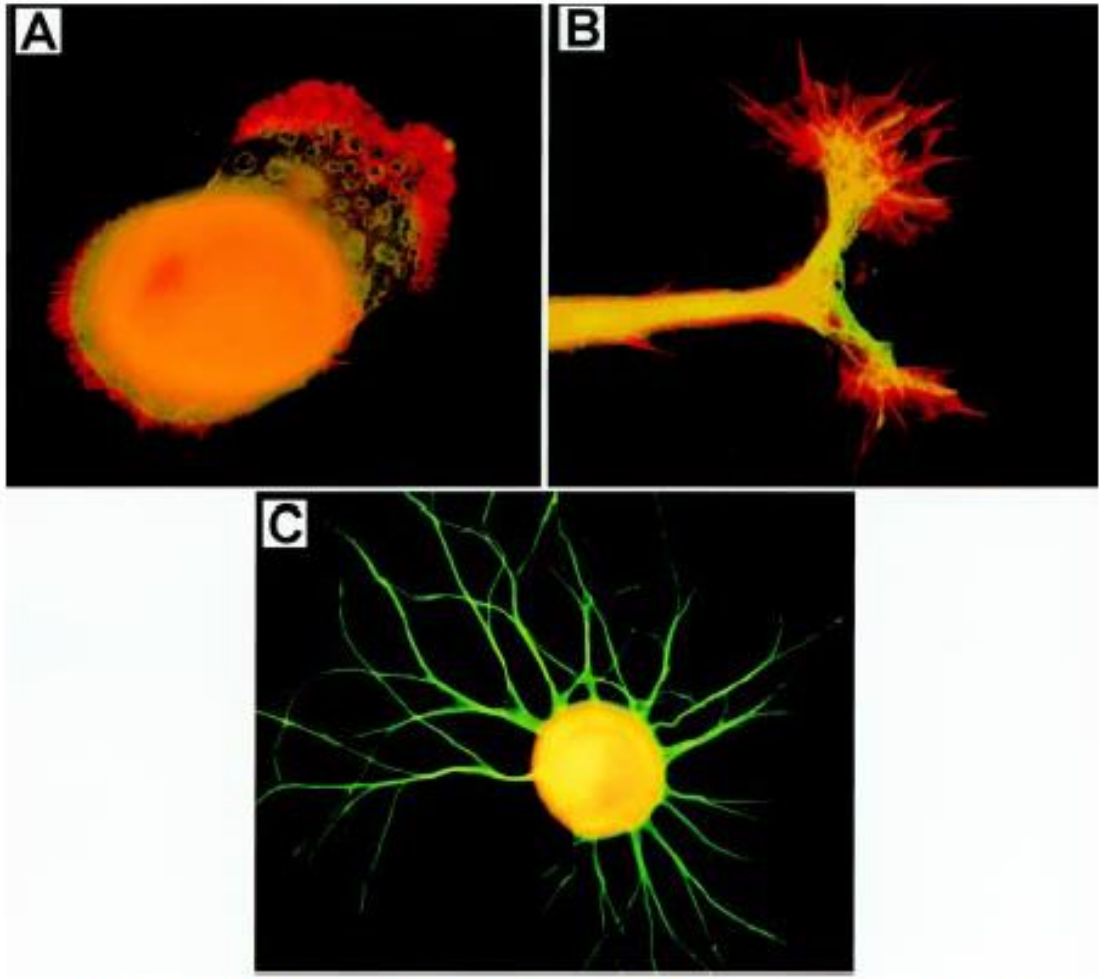


Fig. 4. Actin and microtubular organization at various stages of neurite outgrowth. A) During early sprouting, actin (*red*) is most prominent at the peripheral domain. Note that at this time point (1–2hrs of culture), microtubules (*green*) are not well organized. B) Actin dominates the peripheral domain of the growth cones, whereas microtubules are now well organized within the neurite (*green/yellow* – 12–18hrs *in vitro*). C) After 24hrs in cell culture, microtubules make up the core of cytoskeletal elements.

Primary cells

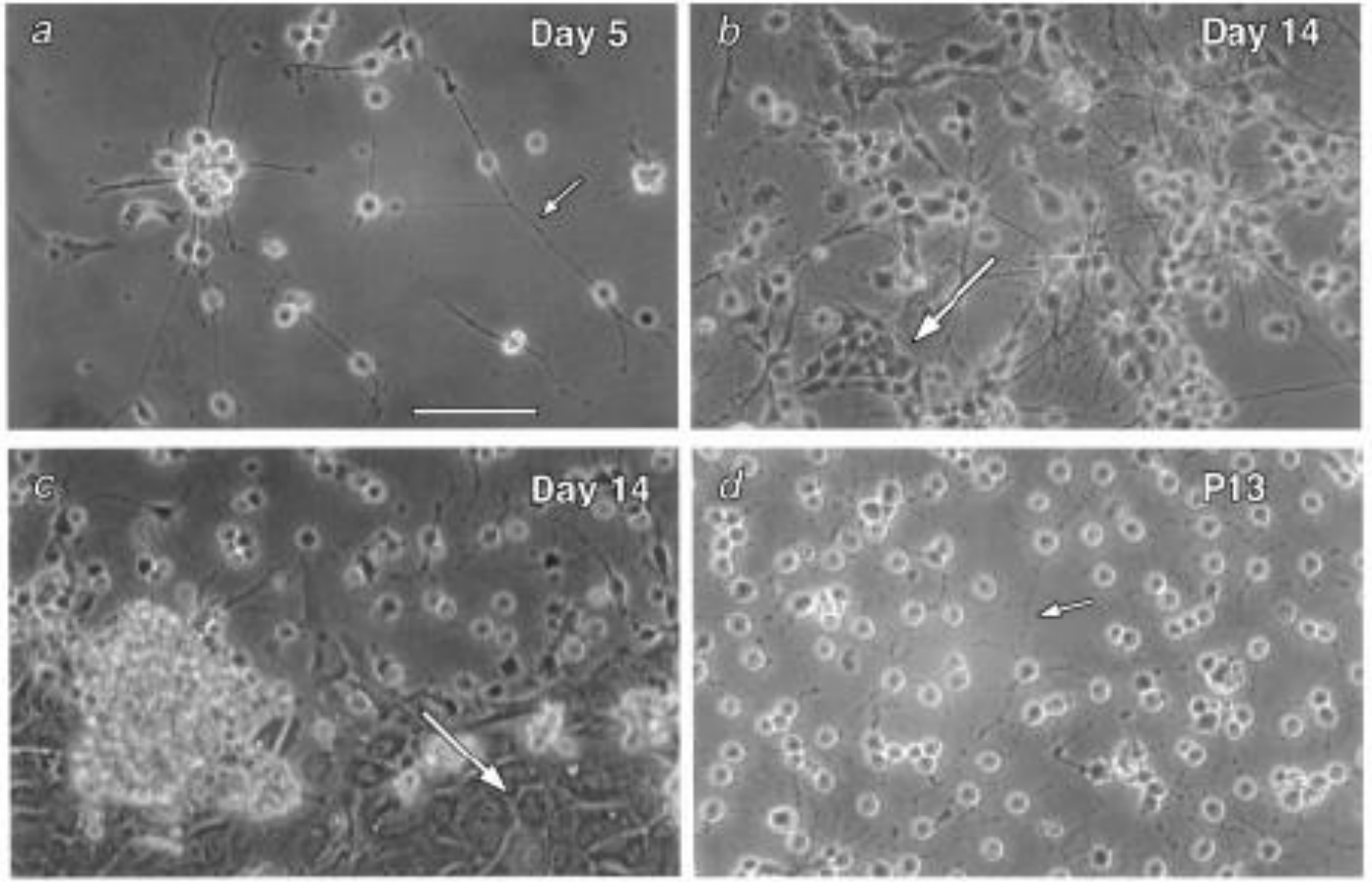


Fig. 2. Morphology of adult rat neural stem cells cultured in serum-free N2 medium containing FGF-2. (*a*) Proliferating cells can be seen by 3–5 days in vitro (DIV). Stem cells have small phase bright cell bodies and two or more long processes (small arrows in *a*, *d*). (*b*) By 14 DIV, a large number of stem cells are present. (*c*) The cultures also contain flat cells (indicated by long arrows) which do not stain for any stem or precursor cell markers. Small phase bright cells seem to generate on top of these flat cells. (*d*) Mostly stem cells are present in the passaged cultures. Scale bar: 100μm

Neural stem cells

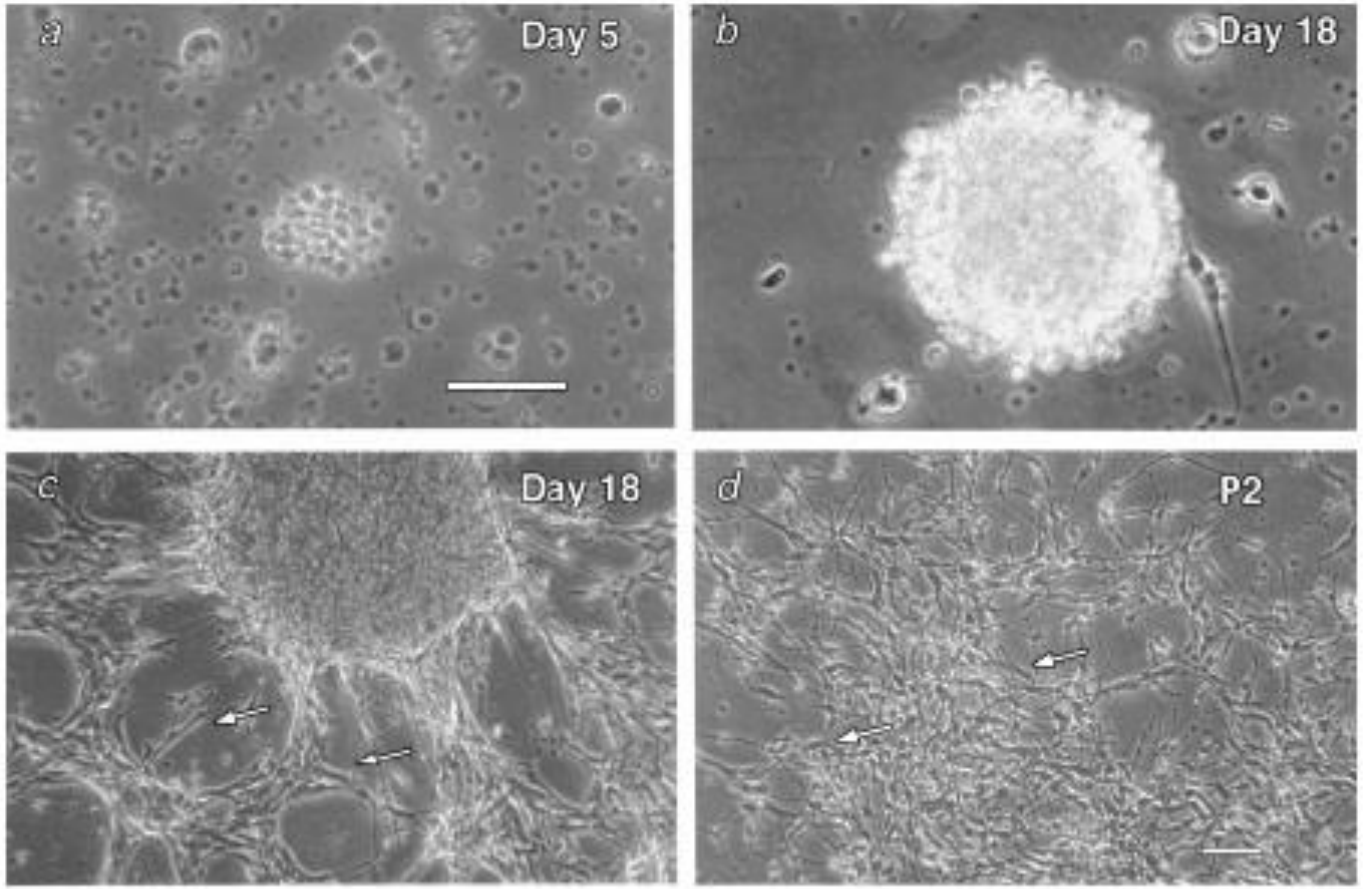


Fig. 3. Morphology of adult mouse neural stem cells cultured in serum-free N2 medium containing EGF, FGF-2 and heparin. (*a*) Neurospheres are visible by 5 DIV. With time in culture neurospheres increase in size (*b*) and some of the spheres attach to the substratum (*c*). Cells stream out of the spheres and grow as monolayer. (*d*) Upon passage attached cells grow as monolayers. Mouse stem cells have more elongated cell bodies (arrows in *c*, *d*) and smaller processes than rat stem cells. Scale bar: 100 µm

Neuroscience

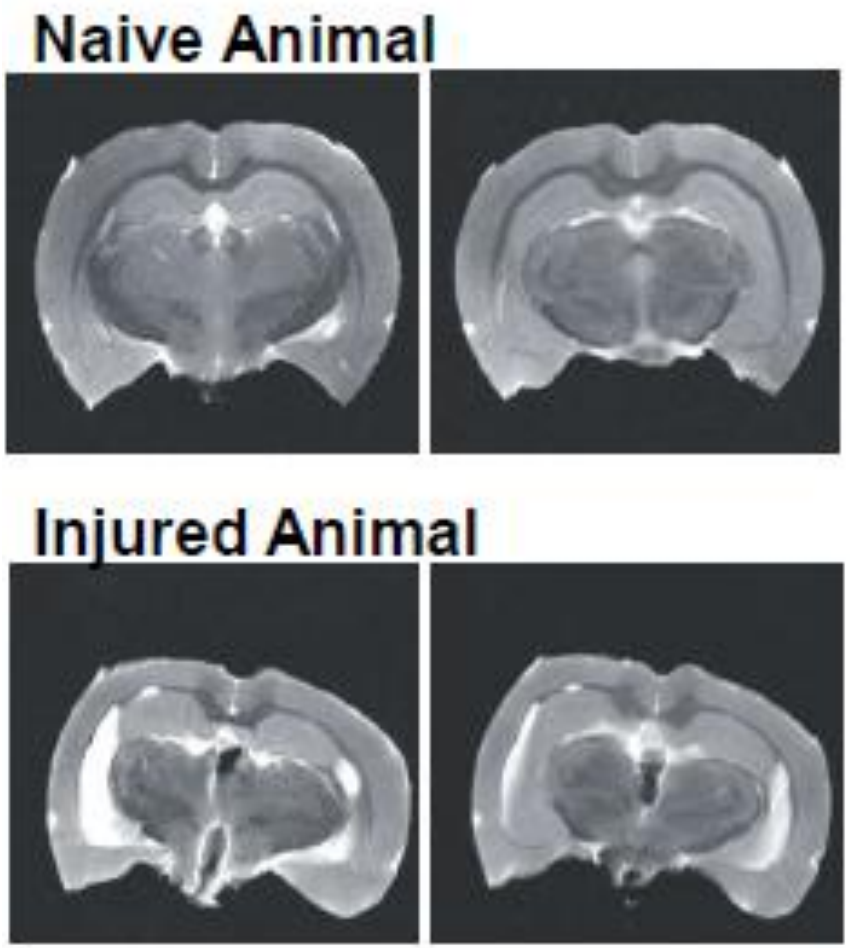
- biological study of the brain
- interdisciplinary field that involves many levels of study from the
  - molecular level
  - cellular level (individual neurons)
- small assemblies of neurons like cortical columns
- larger subsystems : subserves visual perception
- large systems : cerebral cortex or cerebellum
- the highest level the nervous system as a whole

Neuroscience is a field of study that deals with:

- structure, function,
- development, genetics,
- biochemistry, physiology,
- pharmacology,
- pathology of the nervous system,
- study of behavior and learning is also a division of neuroscience.

TOTAL Brain imaging an example: MRI

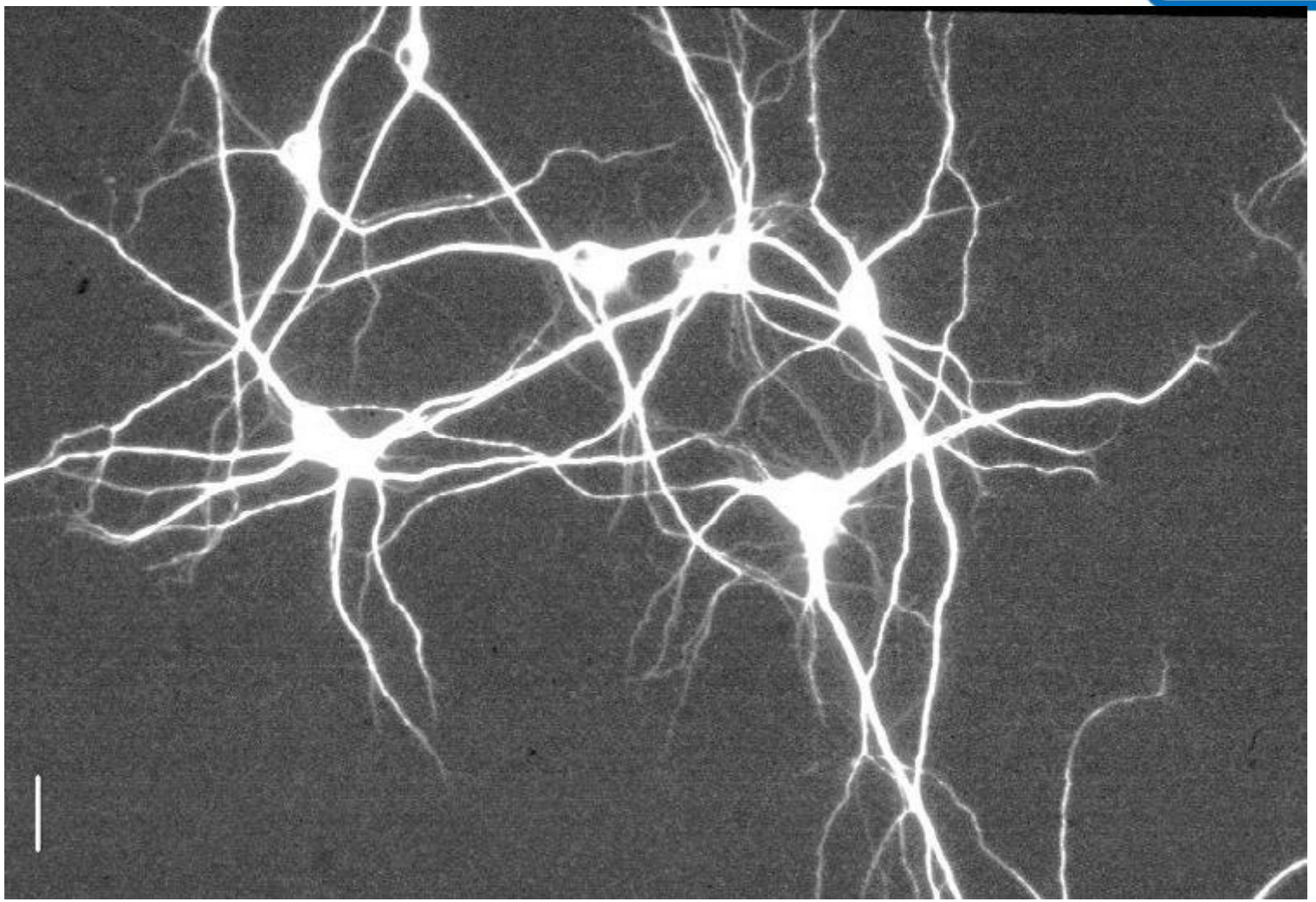
• Want to observe the same animal repeatedly over an extended period of time.
• Advantages:
– Non-Invasive
– Whole brain imagining
– White and Gray Matter
• Disadvantages:
– Slow (hours)
– Spatial Resolution (tops out around 1 x 1 x 1 um)
– Cost
– Space



Experimental Read-OUT in cellular neurobiology

- Primary culture (directly from animal tissue)
- Extended culture (multipassage culture) – cell strain
- Established (transformed) cell lines
- Organotypic cultures

- Primary culture (directly from animal tissue)



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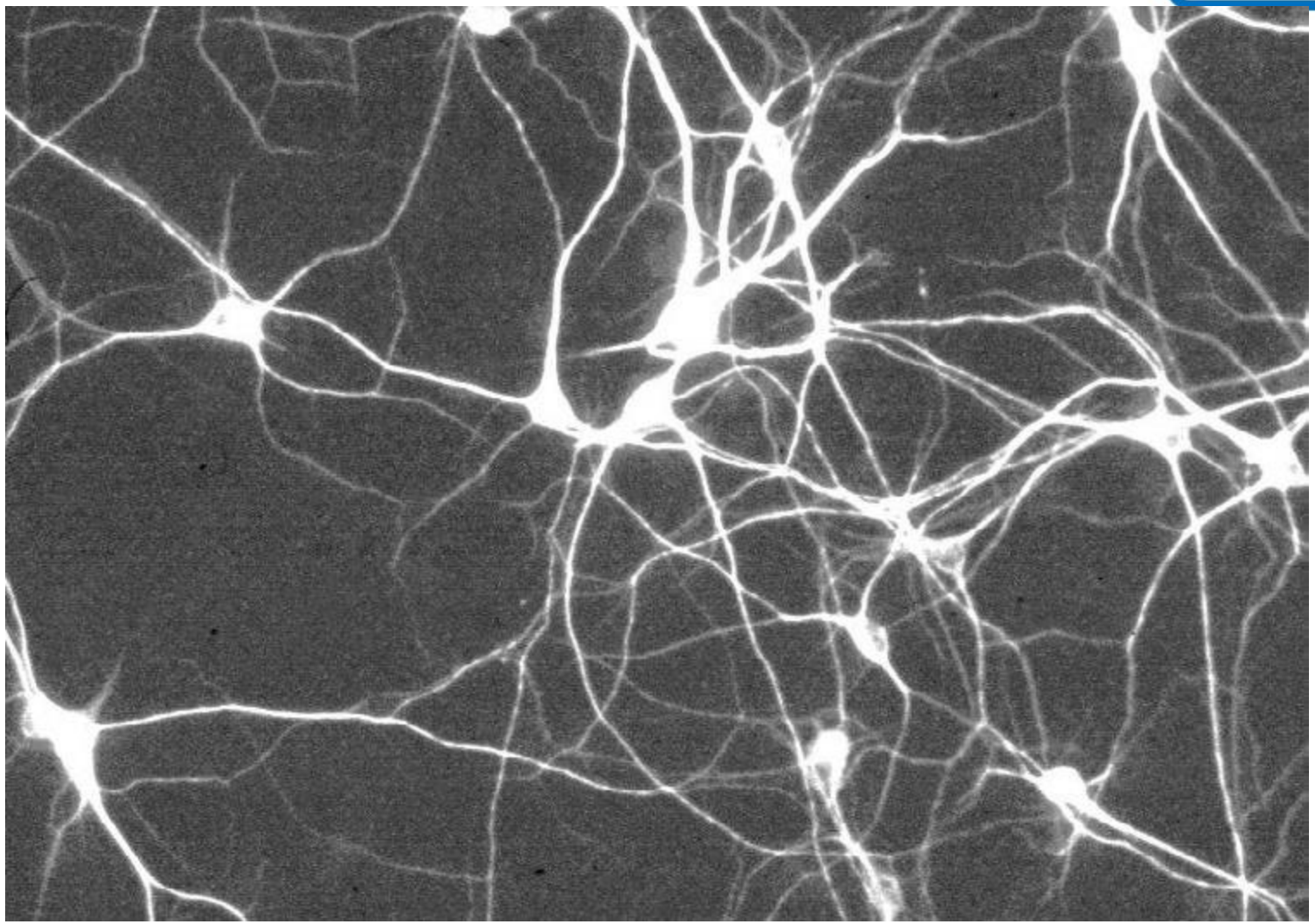

COMMON READ OUT primary cell line

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Protein

1. Western BLOT (limitation due to cell amount)
2. Immunocito
3. ELISA (minor limitations due to cell amount)
4. Overexpression (limitation due to transfectability)

1.Downregulation (limitation due to transfectability)

COMMON READ OUT primary cell line

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RNA

1. PCR
2. Real Time-PCR
3. Northern Blotting
4. InSitu Hyb

COMMON READ OUT primary cell line

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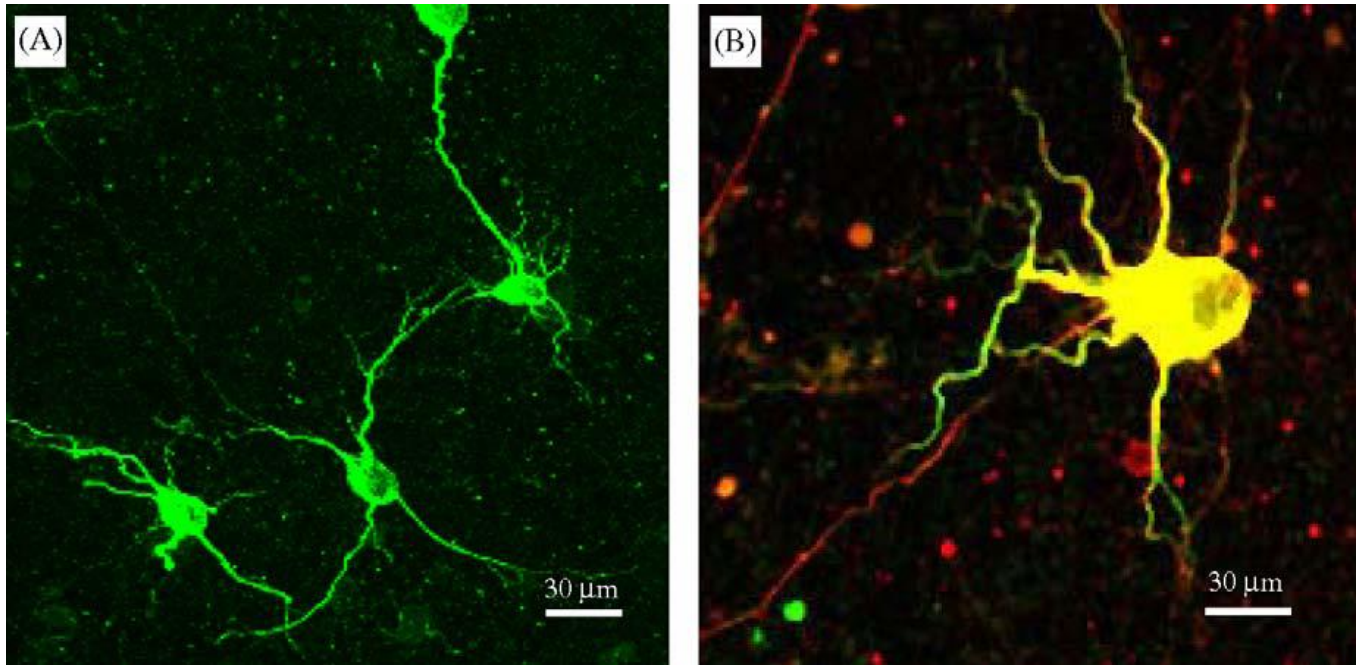
Electrophysiology on “fake” network

- single celll
- field potential



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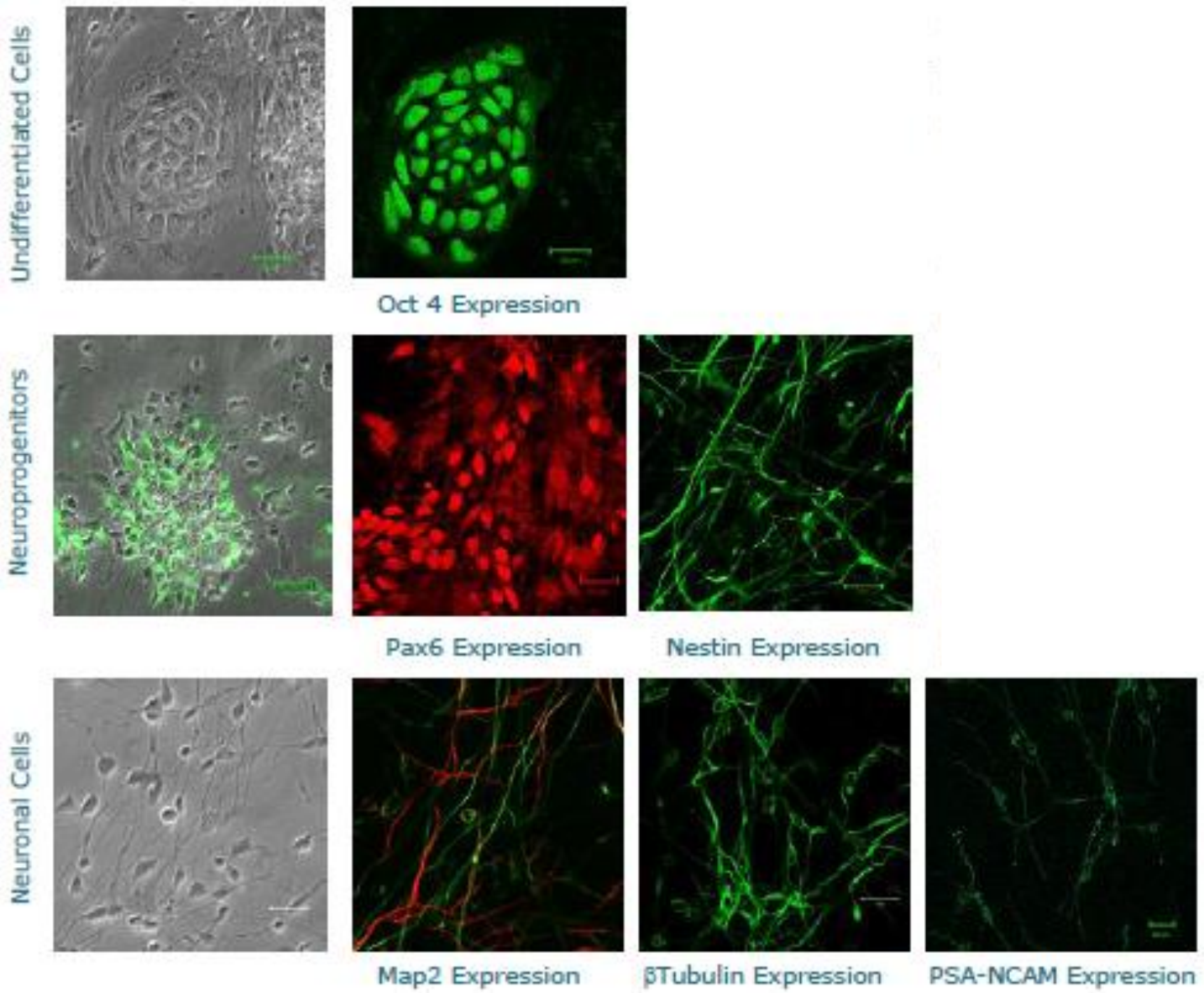
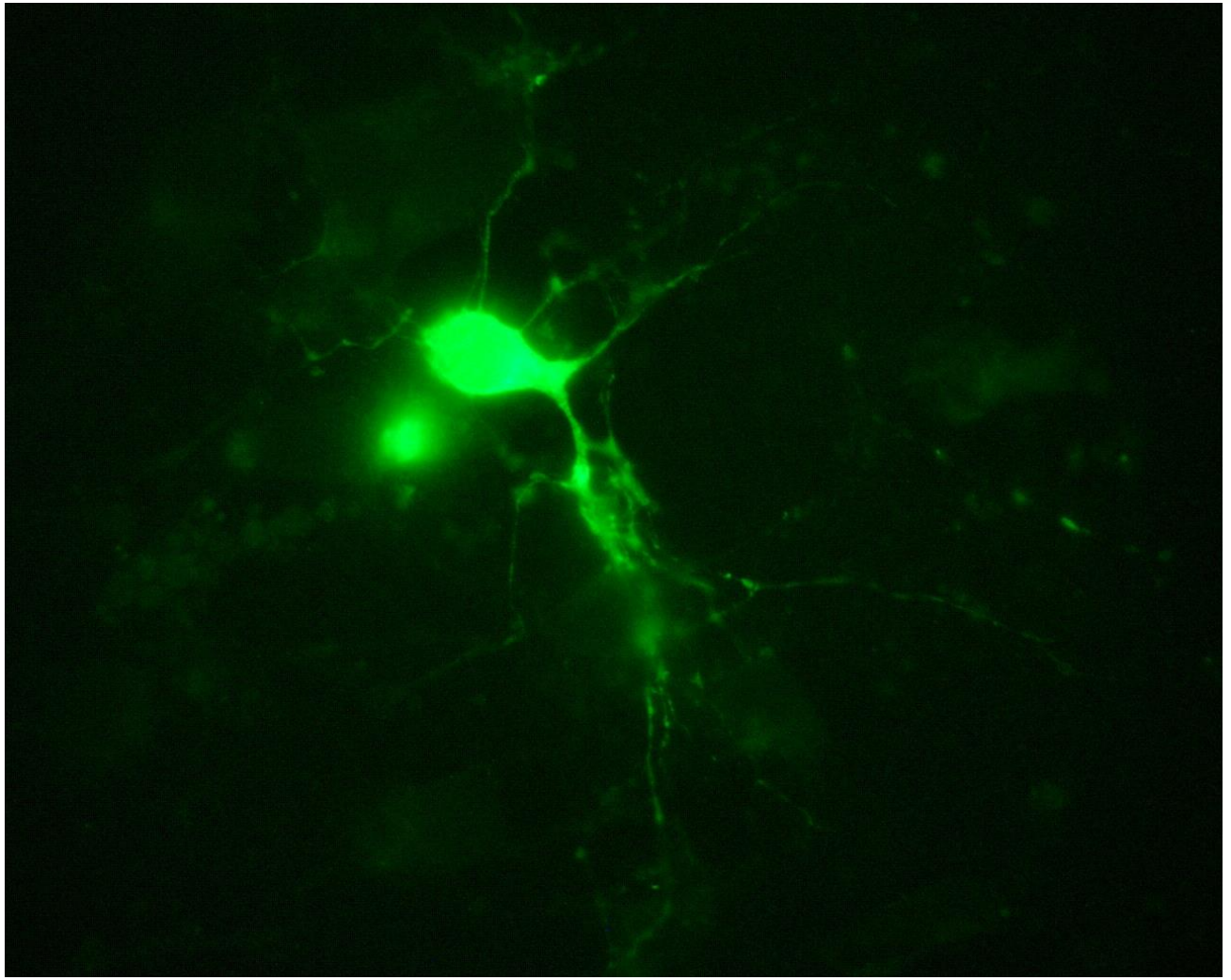
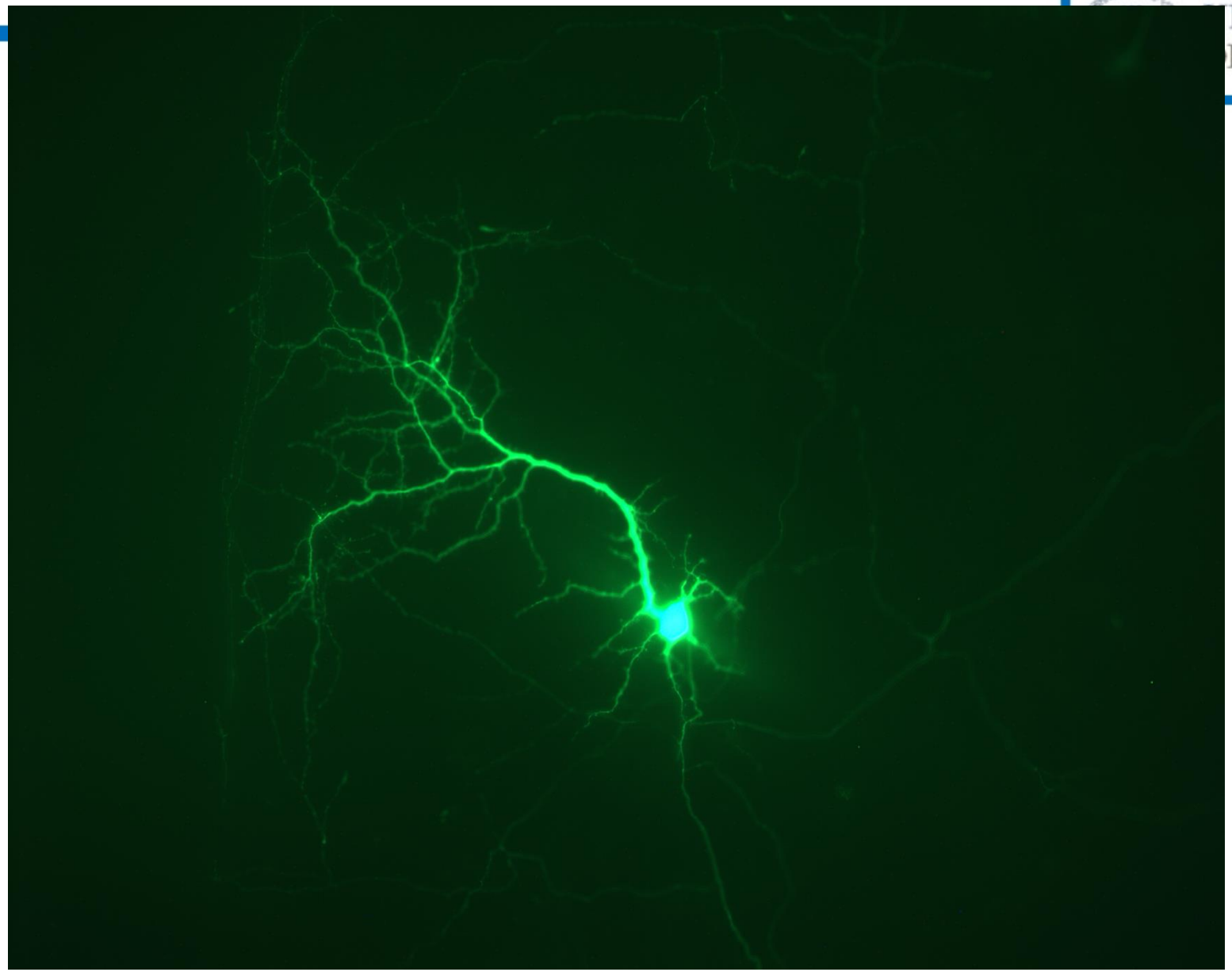


primary cell line





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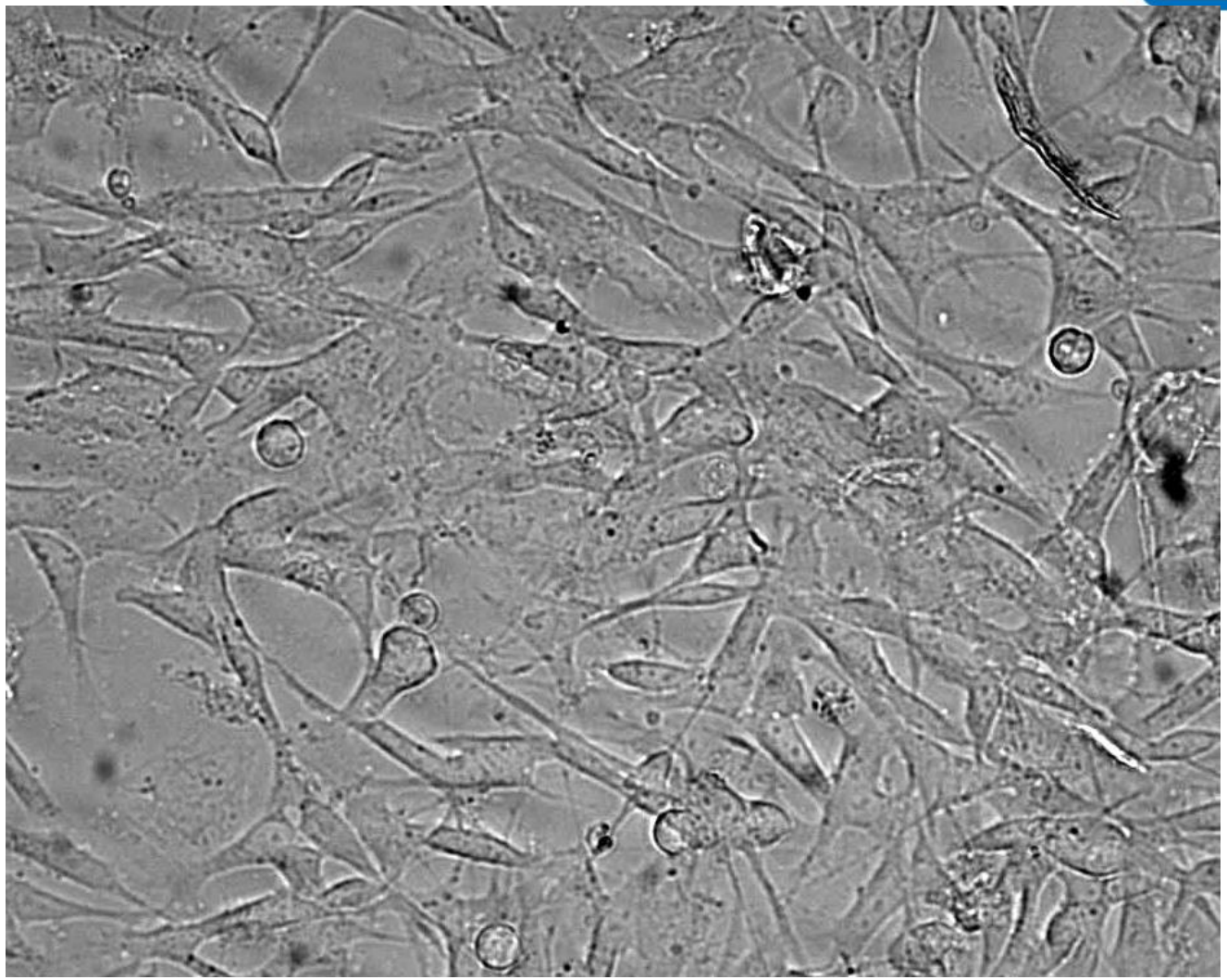

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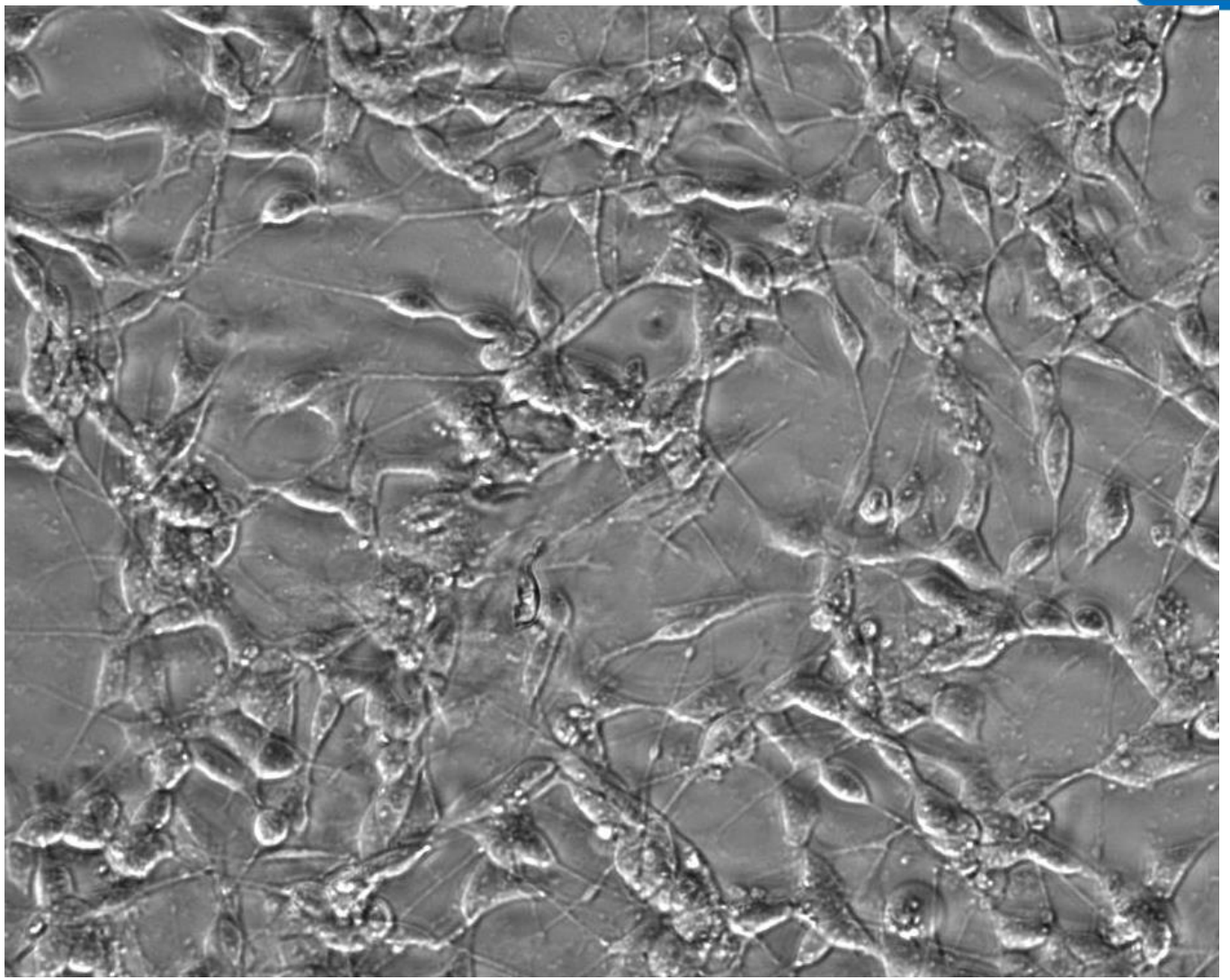

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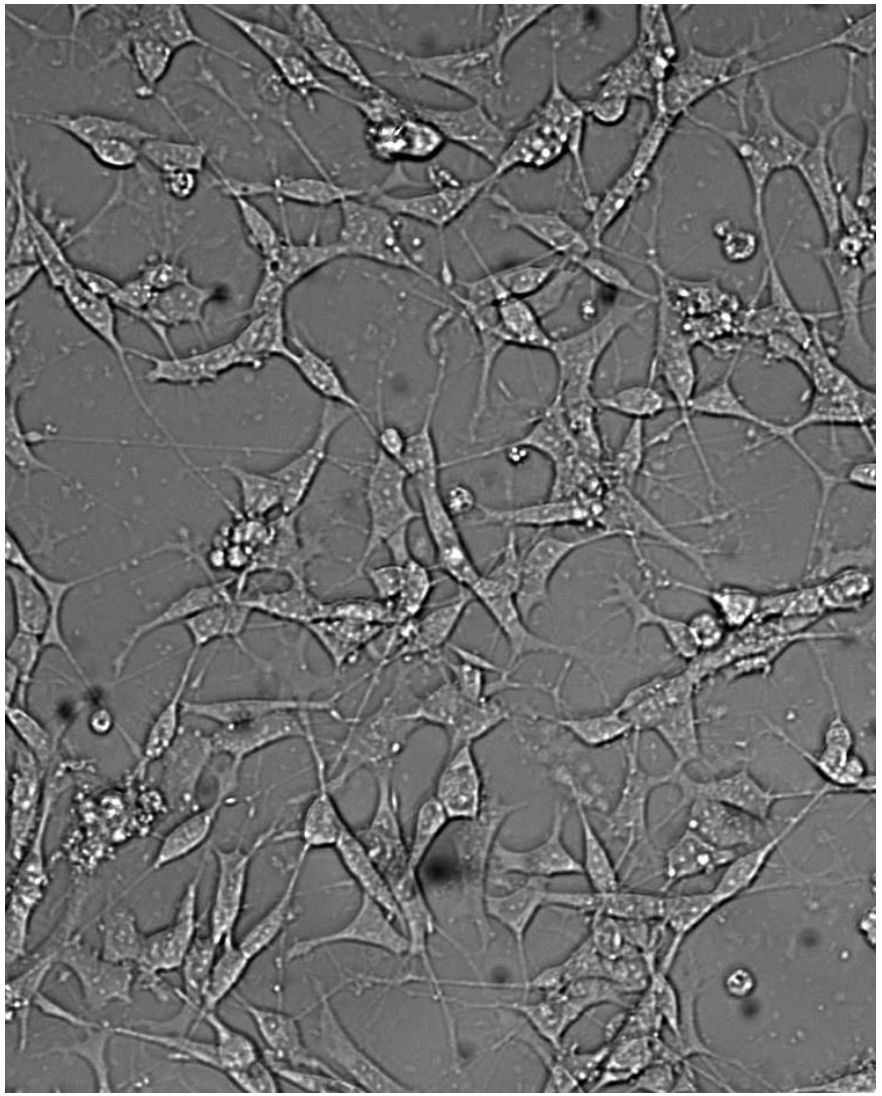

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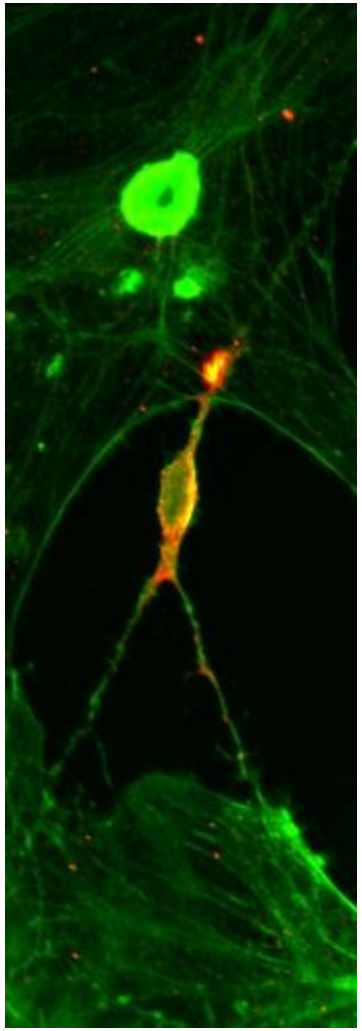

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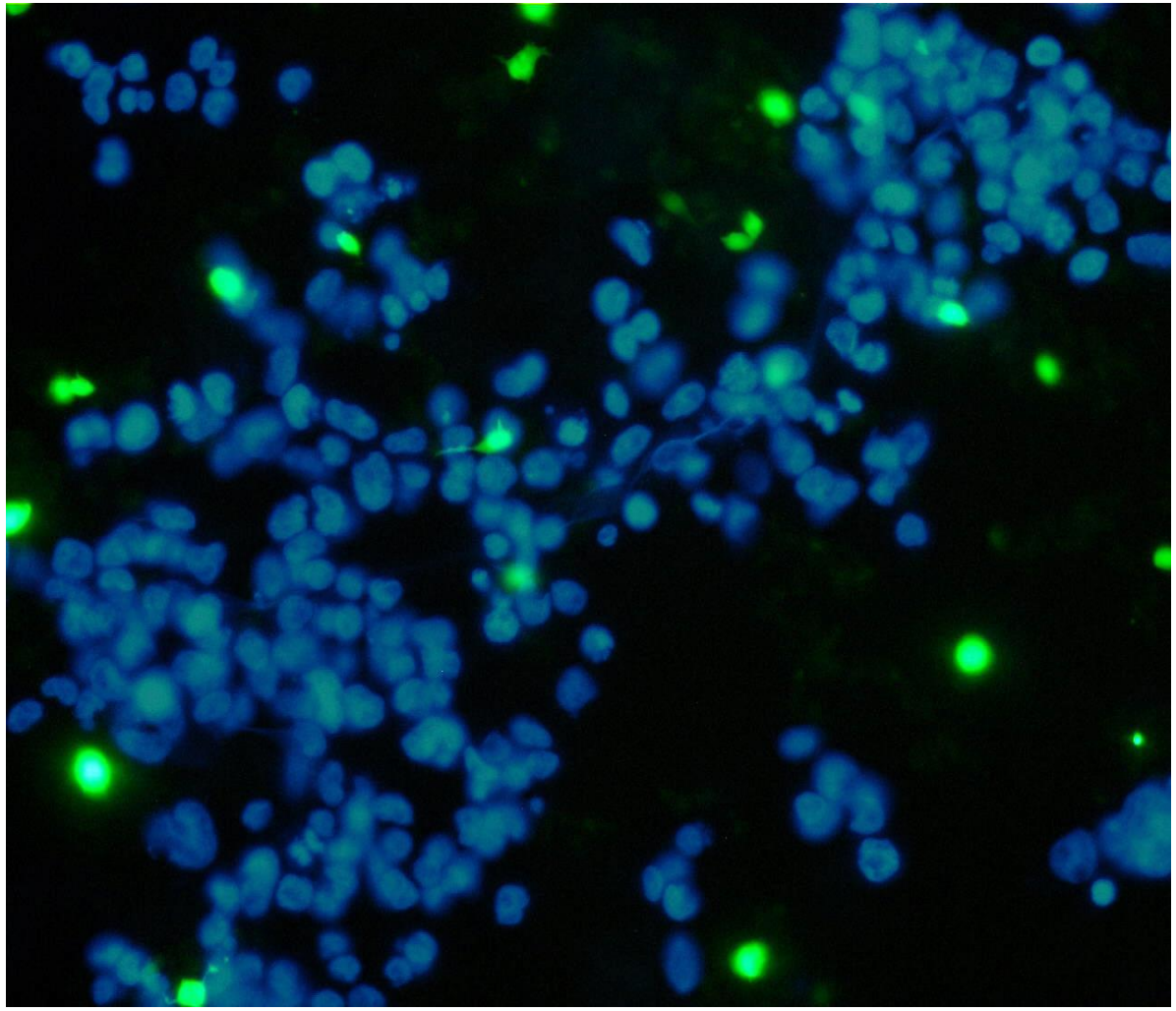

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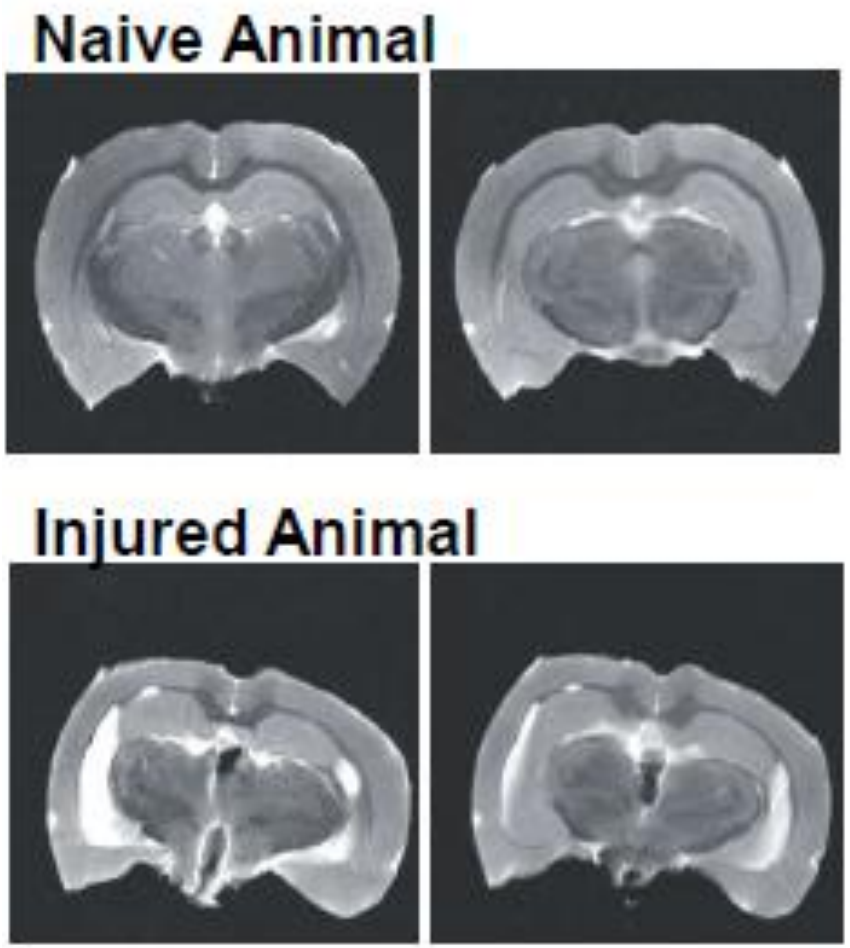

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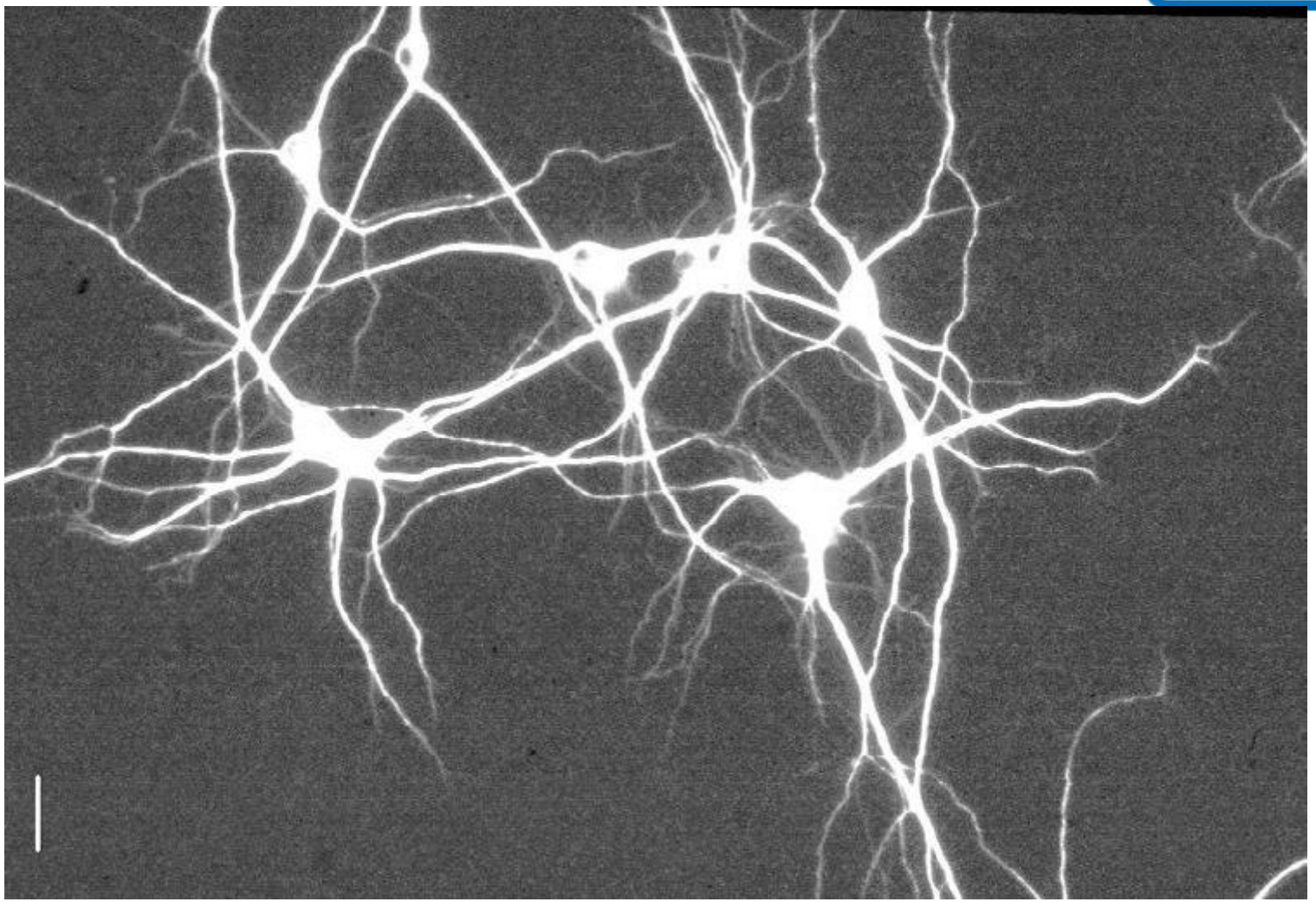

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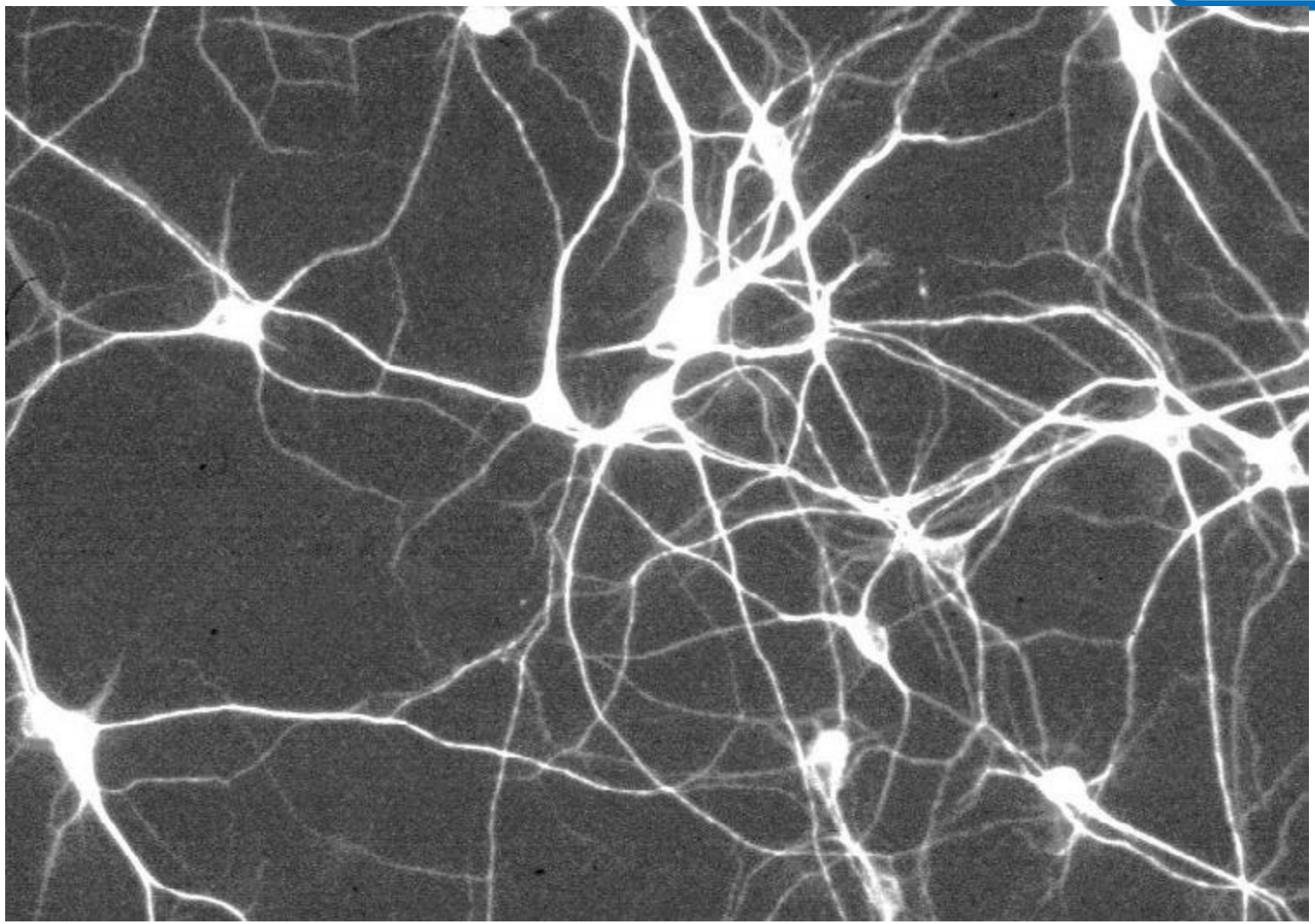

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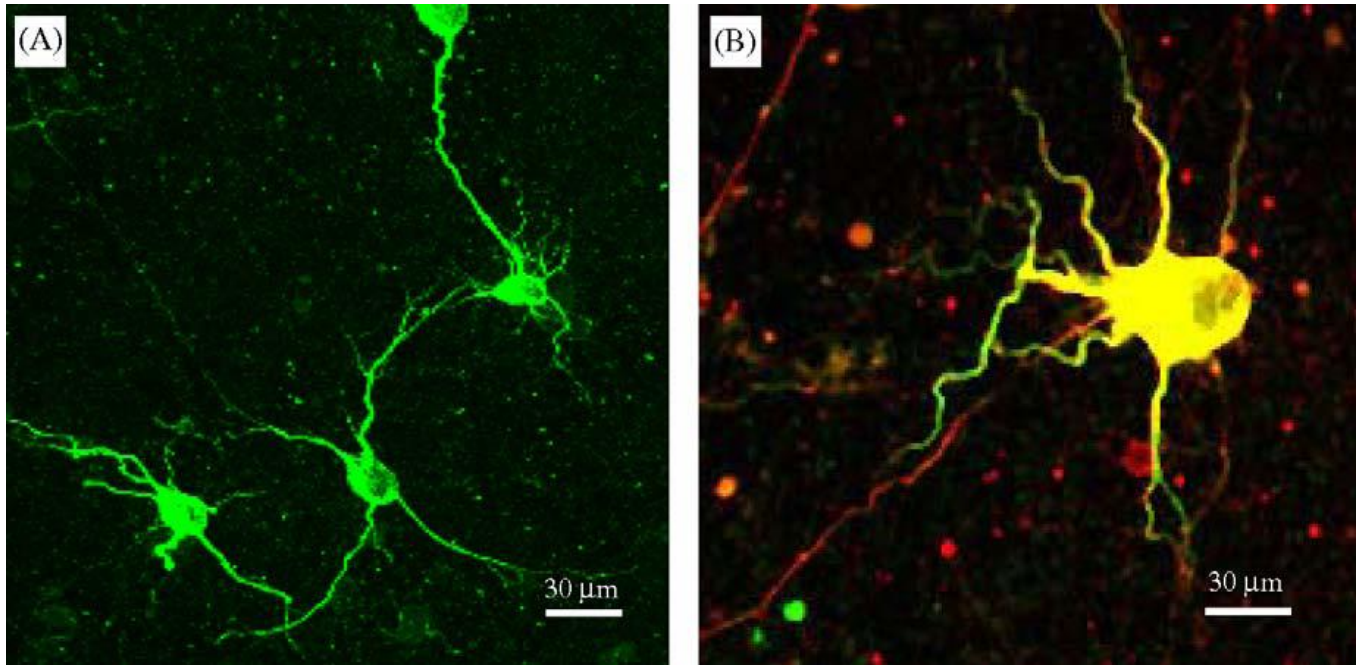

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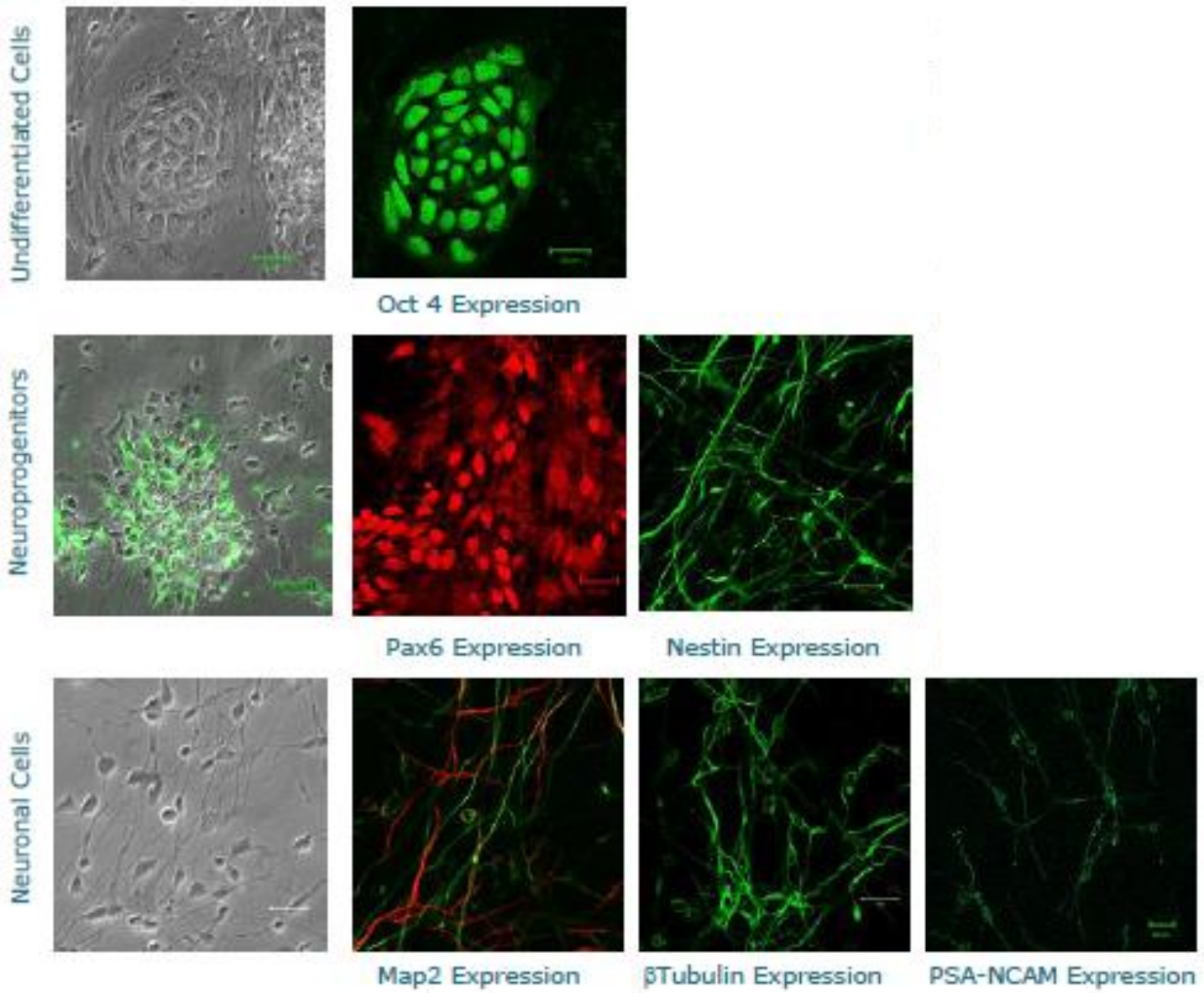

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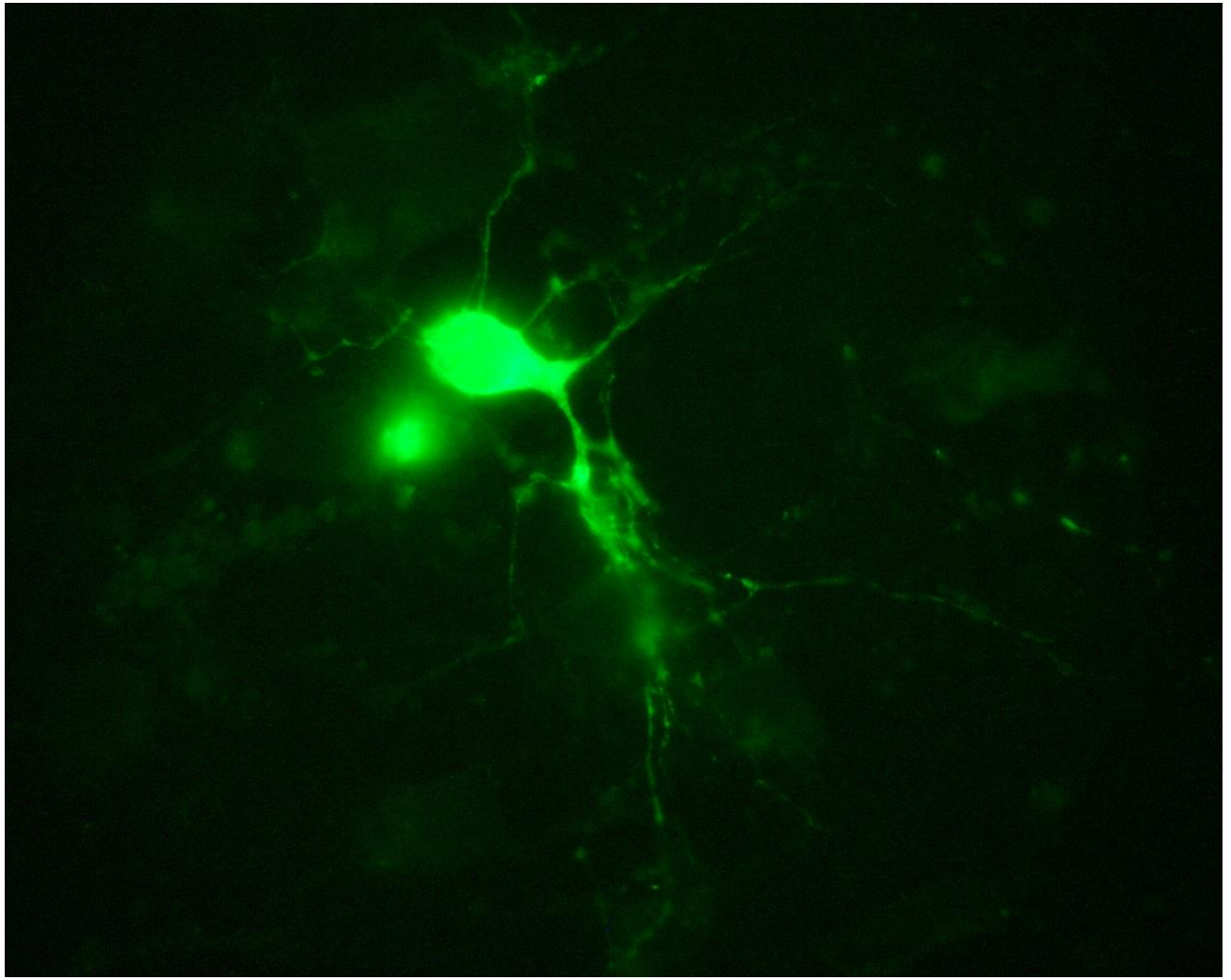

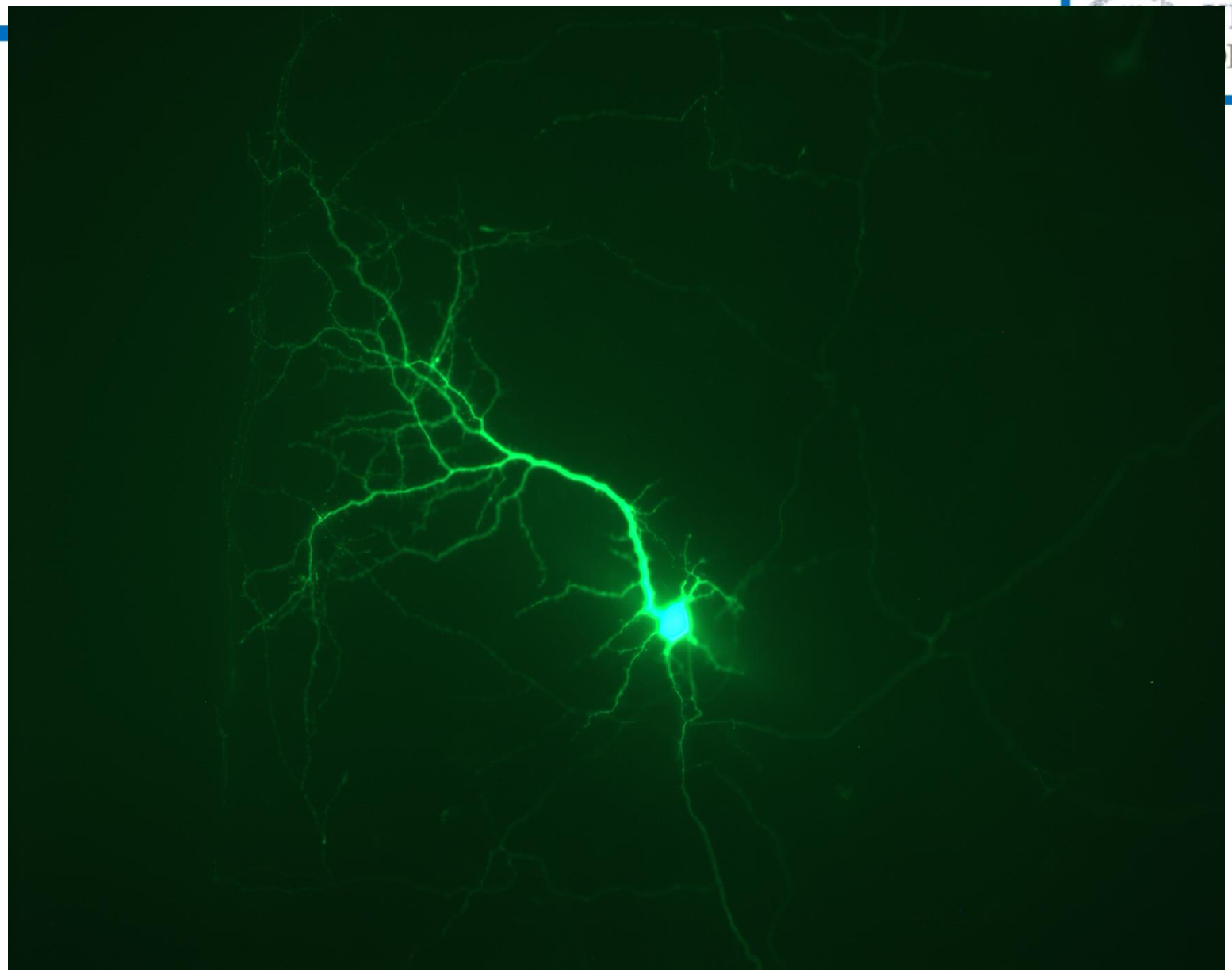

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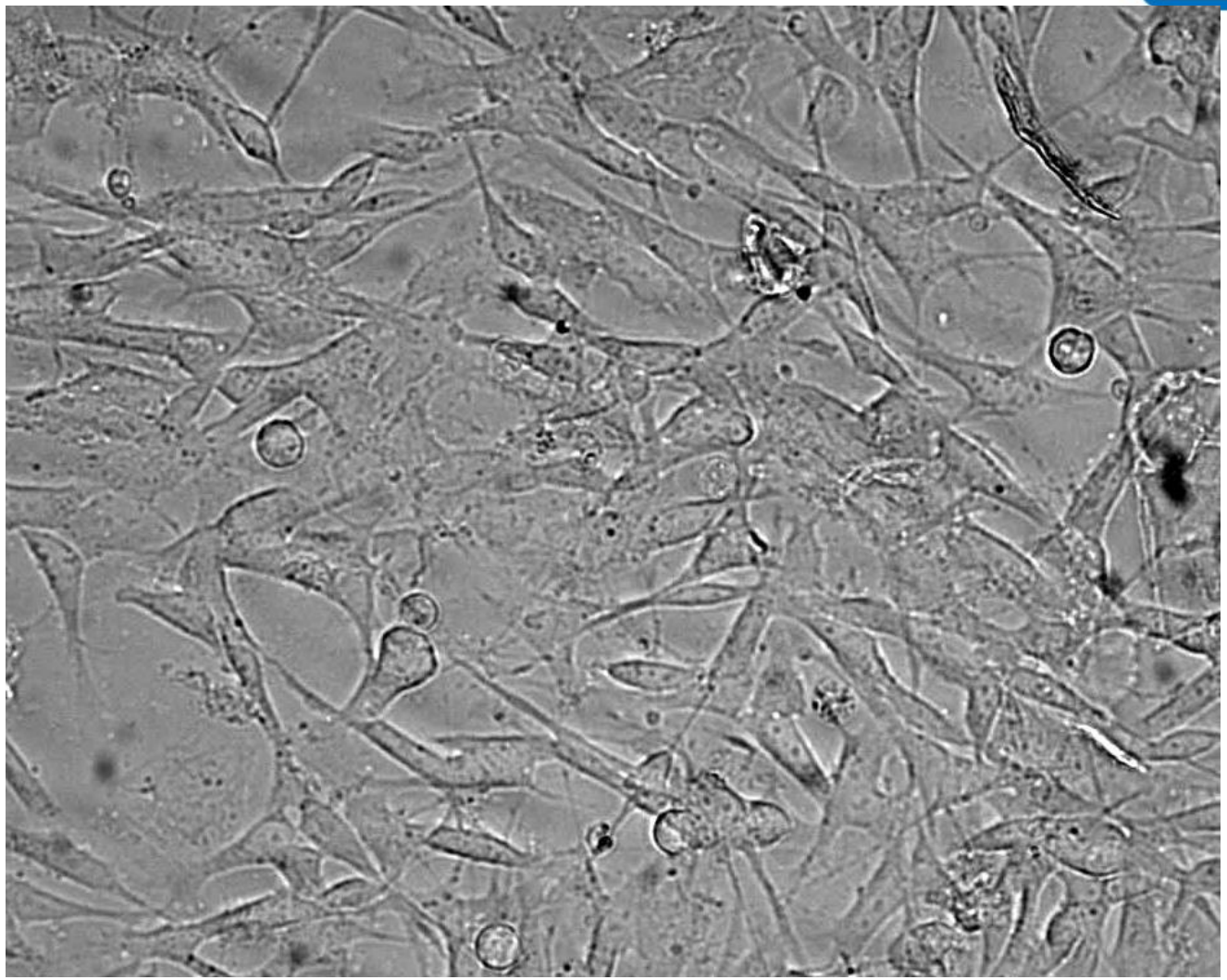

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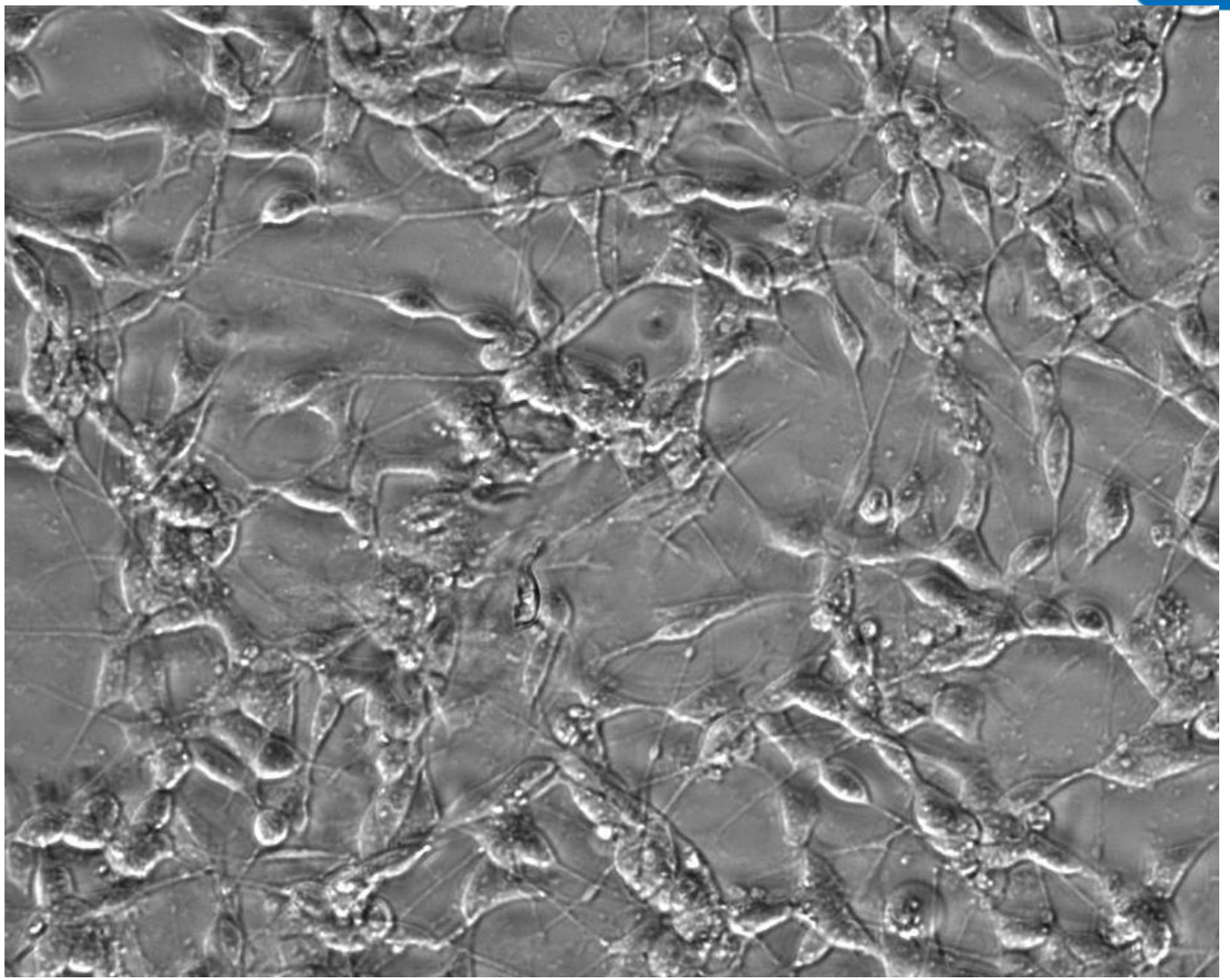

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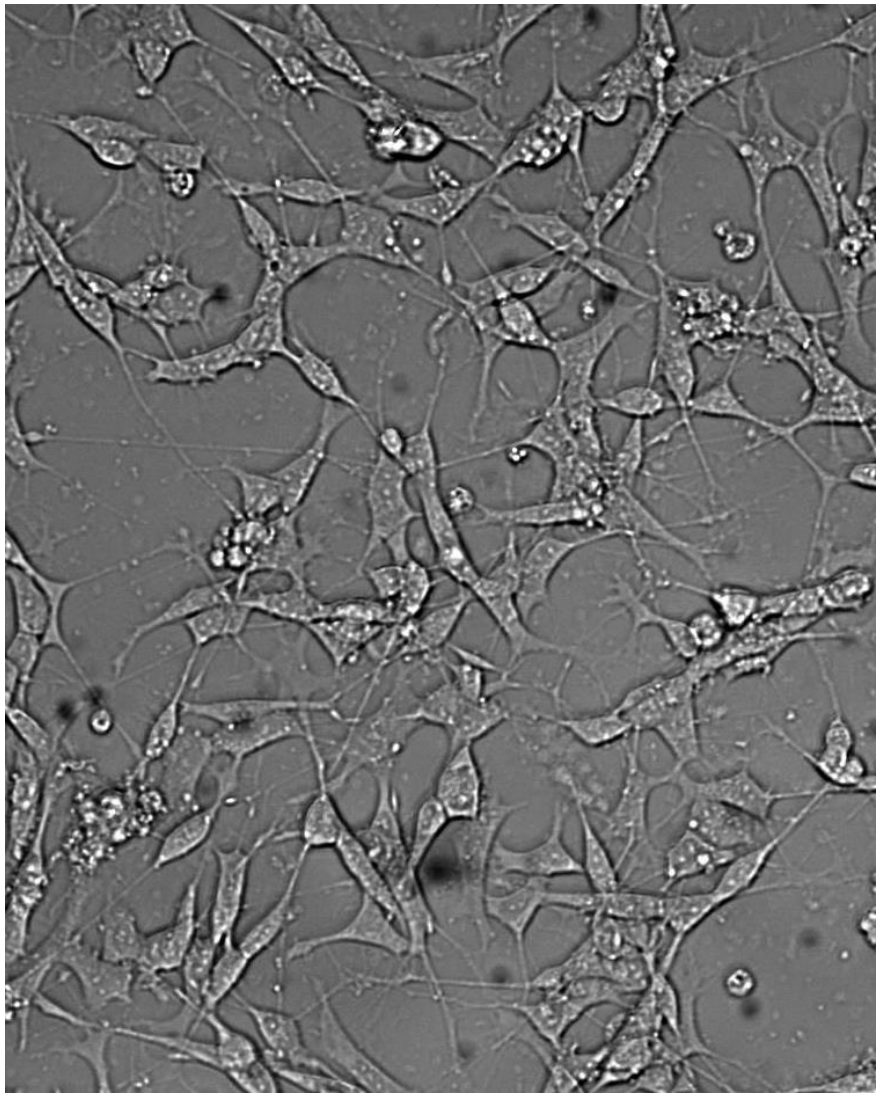
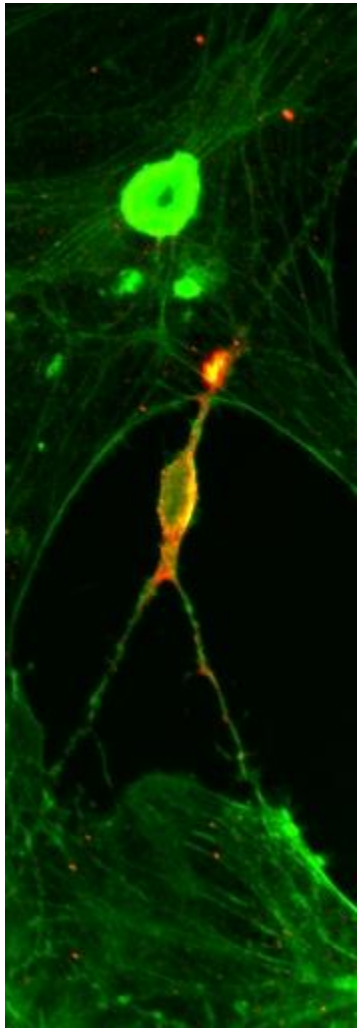


Primary cells



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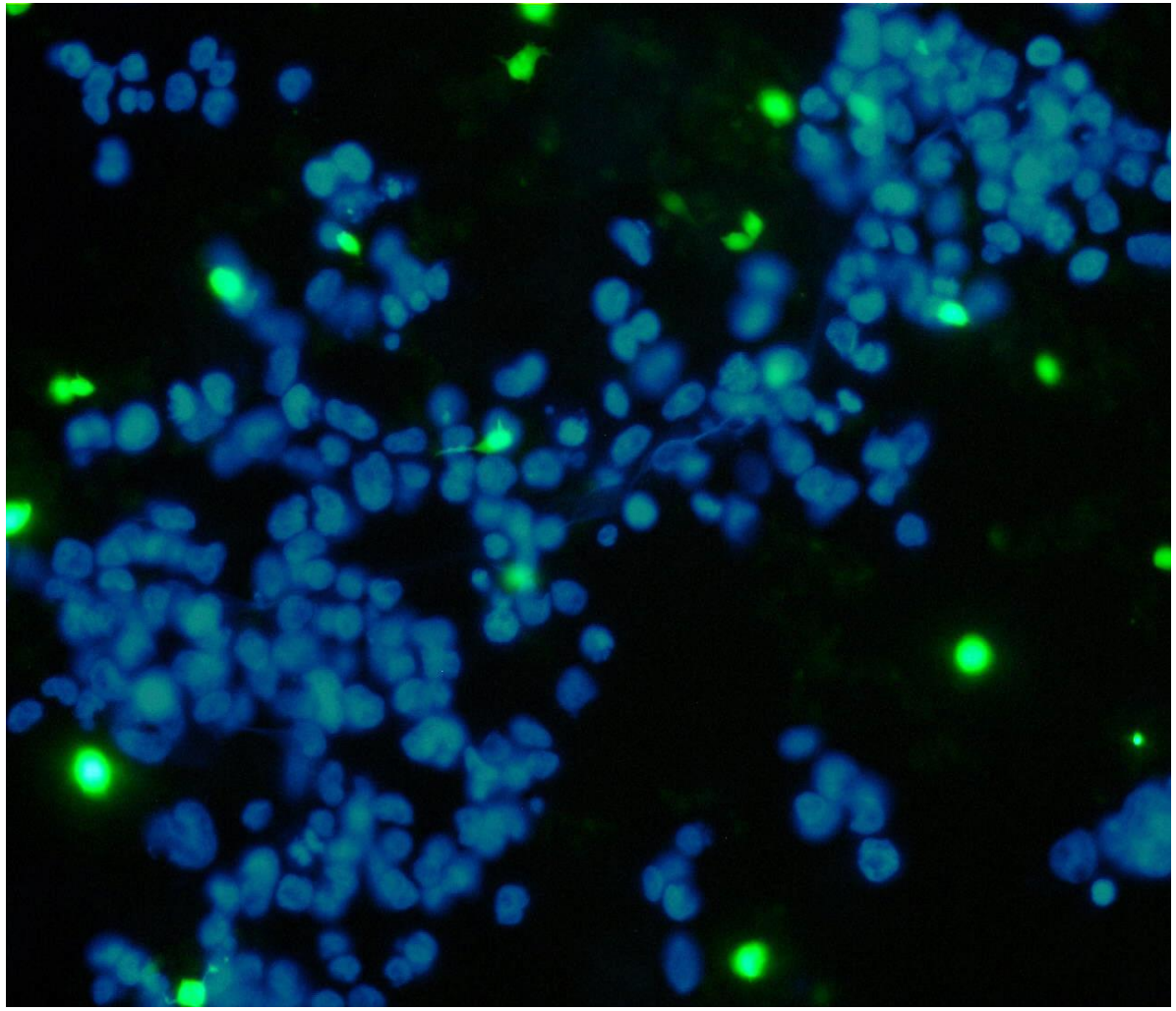

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Neuroscience read out

Neuroscience *in vivo* :

1) behavioral
2) metabolism
3) toxicology
4) electrophysiology

etc

NB (In vivo only on KNOWN TARGET)

Neuroscience *in vitro* :

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TARGETS

DNA

RNA

PROTEIN

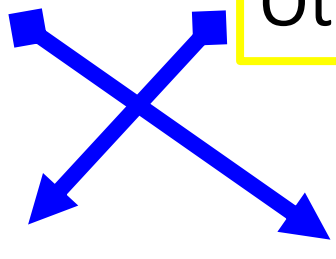
Other elements

DNA – Protein

RNA-Protein

PROTEIN-Protein

Other elements



UNKNOWN TARGET

KNOWN TARGET

Neuroscience *in vitro* : a NOT complete list

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DNA Microarray Methods

- DNA Microarray Maker
- cDNA production
- Random Priming
- *in situ* Hybridization
- Genome-wide response to Glucose Consumption
- Chuck Close and DNA Micorarrays

Genomic Circuits Methods

- Plasmids with inducible promoters
- CAT Assays
- GFP and reporter proteins/genes
- Growth Curves
- Homologous Recombination
- Brain Anatomy

Neuroscience *in vitro* : a NOT complete list

Proteomics Methods

- Domain Functions
- Yeast Two Hybrid
- *Arabidopsis*
- ICAT (silent version)
- Transposons
- Cre / *lox* P recombination
- Epitope Tags
- Barcode knockout yeast
- Biotin and Avidin binding
- Affinity Chromotography
- Kinase and enzyme assays
- Relative Sizes
- RNAi (RNA interference)
- Mass Spectroscopy
- Visualization of Data

Neuroscience *in vitro* : a NOT complete list

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Genomic Medicine Methods

- Pathology/Histology Slides
- Karyotypes
- Immunoprecipitation (silent version)
- PCR

SDS-PAGE and Coomassie Staining

- Western Blot
- Southern Blot
- Northern blot
- Immunofluorescence
- Chromosomal Walking
- RFLP
- Knockout Mouse and Homologous Recombination
- Liposomes

Neuroscience *in vitro* : a NOT complete list

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Sequence Methods, Acquisition, and Analysis

- Capillary Electrophoresis
- Chromatogram
- Cycle sequencing
- Dendrogram
- Elephant Skin Lesions
- ELISA (see animated version)
- FACS (Fluorescence Activated Cell Sorting)
- Knockout Mouse and Homologous Recombination
- Liposomes
- Nested PCR
- Northern Blot
- PCR
- Pulse-field Gel Electrophoresis
- Real-time PCR
- RT-PCR (reverse transcriptase-PCR)
- SDS-PAGE and Coomassie Stain of Protein Gel
- Southern Blot
- Western Blot
- Whole-Genome Sequencing
- X-Ray film

Neuroscience *in vitro* : a NOT complete list

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UNKNOWN TARGET

BIOINFORMATICS

http://www.ebi.ac.uk/

MicroARRAYS

- mRNA
- miRNA
- ncRNA
- SNP
- Protein

Biochemical read out

ALL TARGETS

Biochemical read out

2 Gel-Electrophoresys

2 Hybrid Screen

Biochemical read out

High-throughput screening

screening

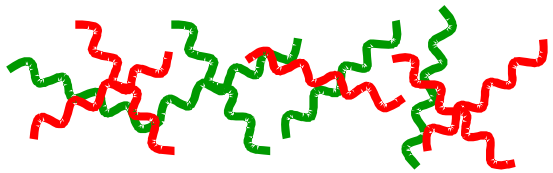
Biochemical read out

etc

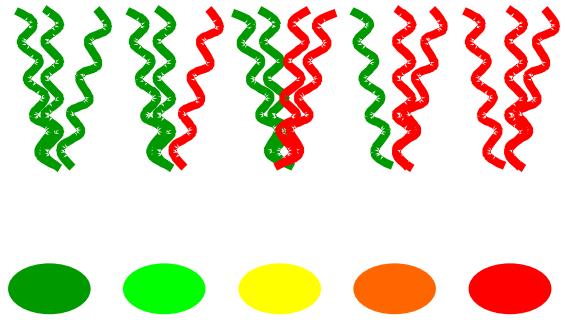
mRNA

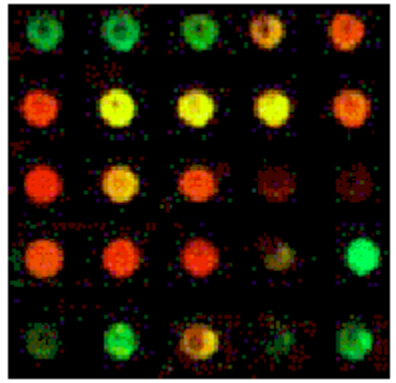
Reverse transcription

Labeled 1st strand cDNA

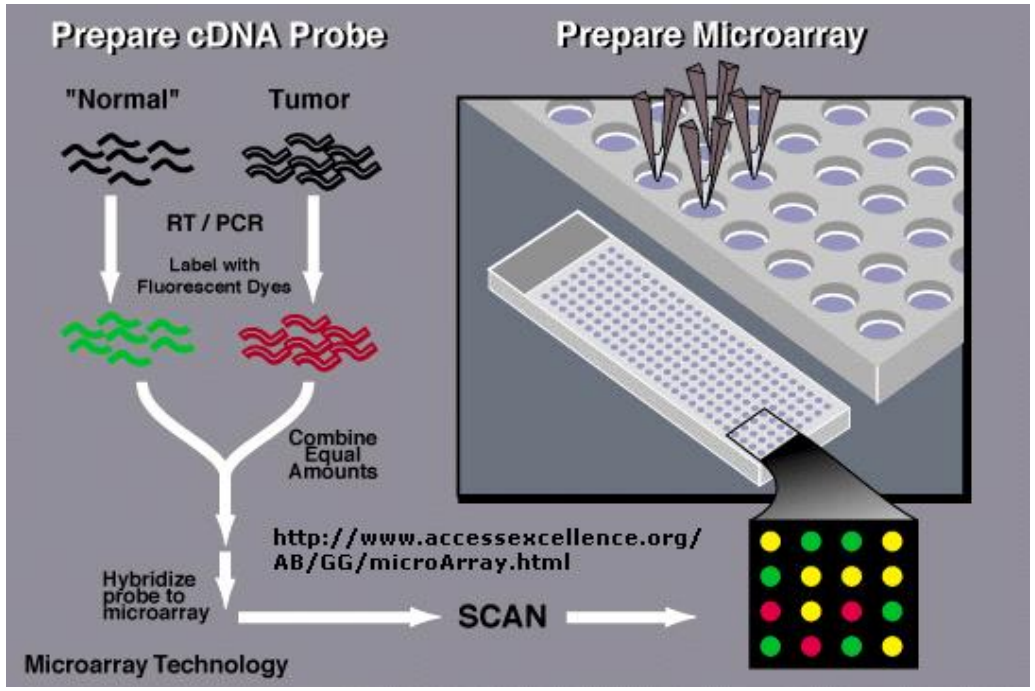


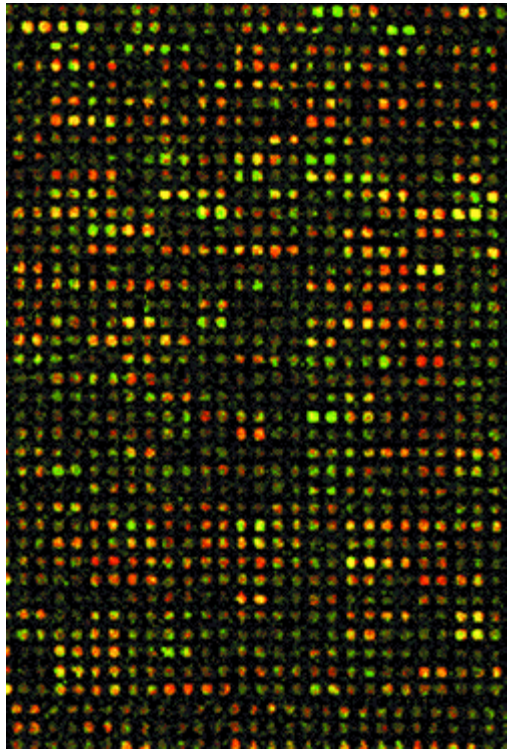
Hybridization on the cDNA microarray



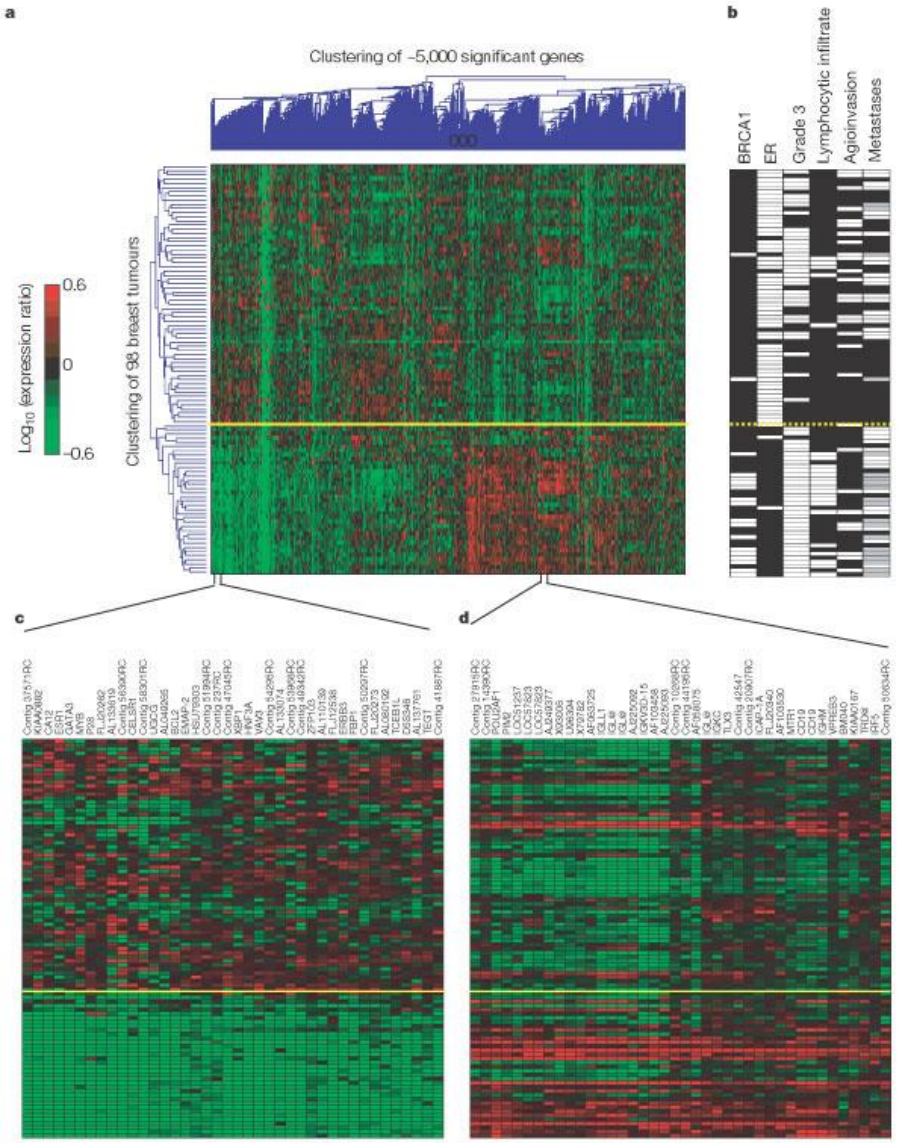


cDNA Microarray





cDNA Microarray read out



Available microarrays
also
for RNAs and Proteins

Protein

2D-Gel electrophoresis

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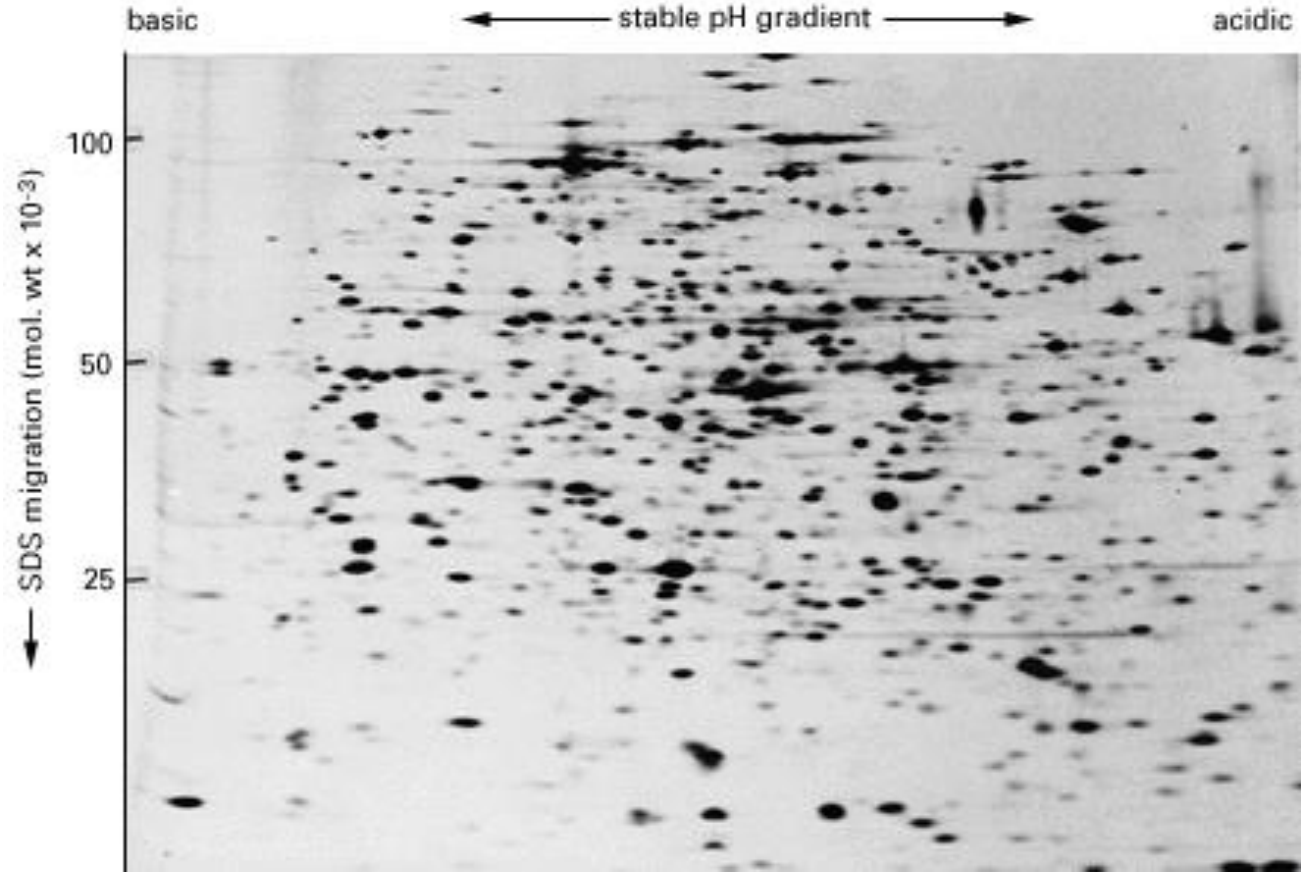


Neuroscience *in vitro* : a NOT complete list

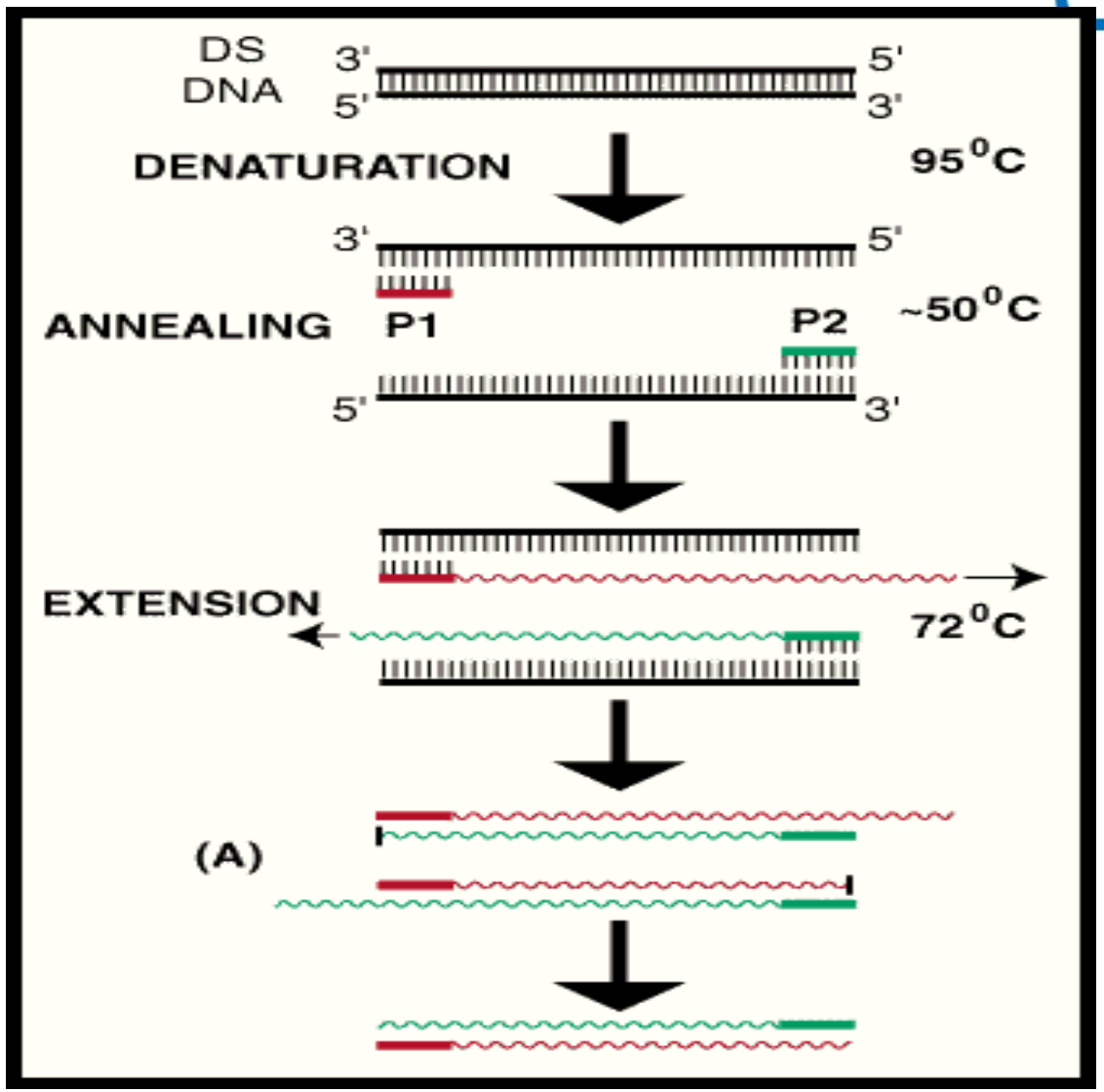
KNOWN TARGET

BIOINFORMATICS

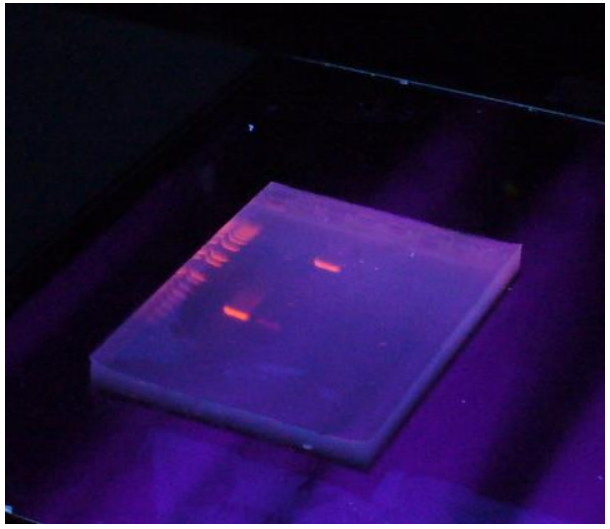
ALL TARGETS

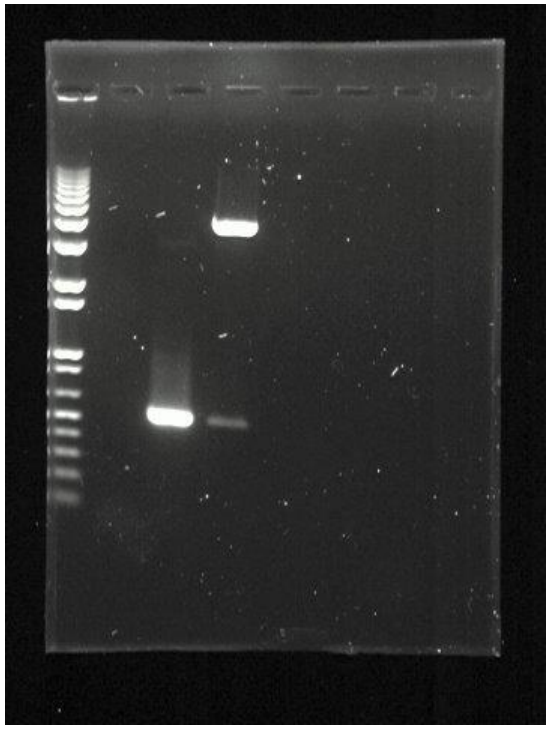
DNA	PCR	Biochemical read out
	Southern Blot	Biochemical read out
	Sequencing	Biochemical read out

PCR


DS
DNA
3'
5'
5'
3'
DENATURATION
95°C
3'
5'
ANNEALING
P1
P2
~50°C
5'
3'
EXTENSION
72°C
(A)

PCR

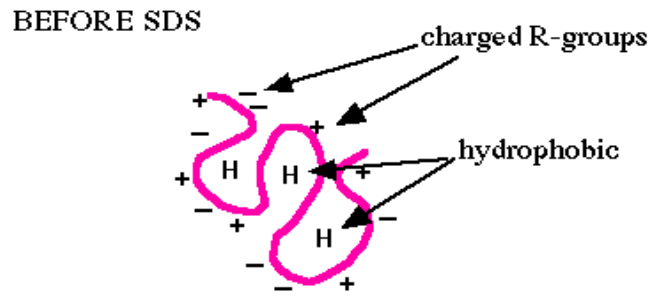


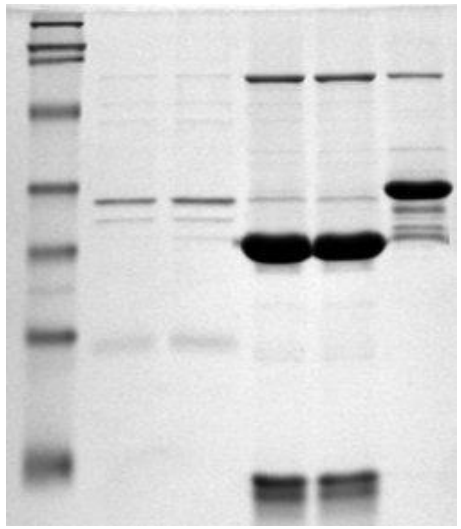


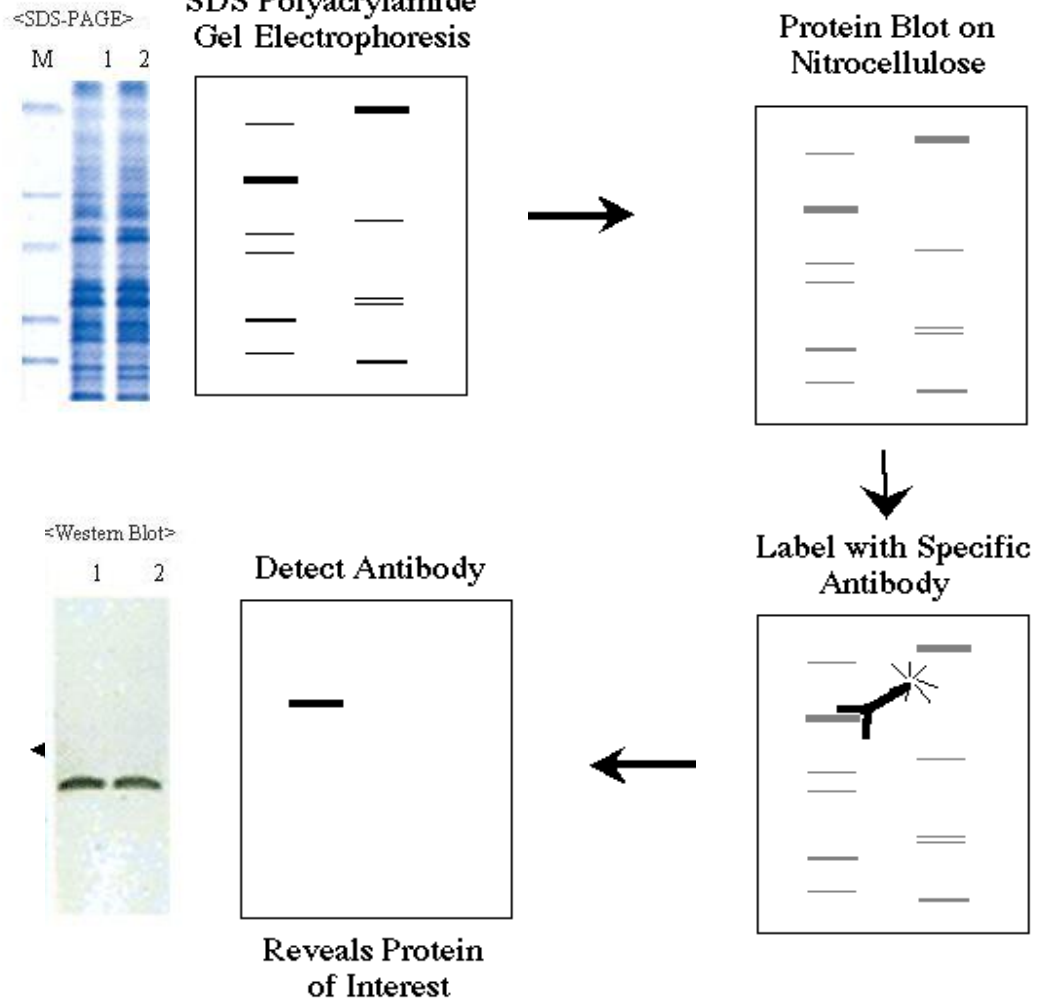
Western Blot

- Western blots allow investigators to determine the molecular weight of a protein and to measure relative amounts of the protein present in different samples.
- Proteins are separated by gel electrophoresis, usually SDS-PAGE.
- The proteins are transferred to a sheet of special blotting paper called nitrocellulose or PVDF.
- The proteins retain the same pattern of separation they had on the gel.

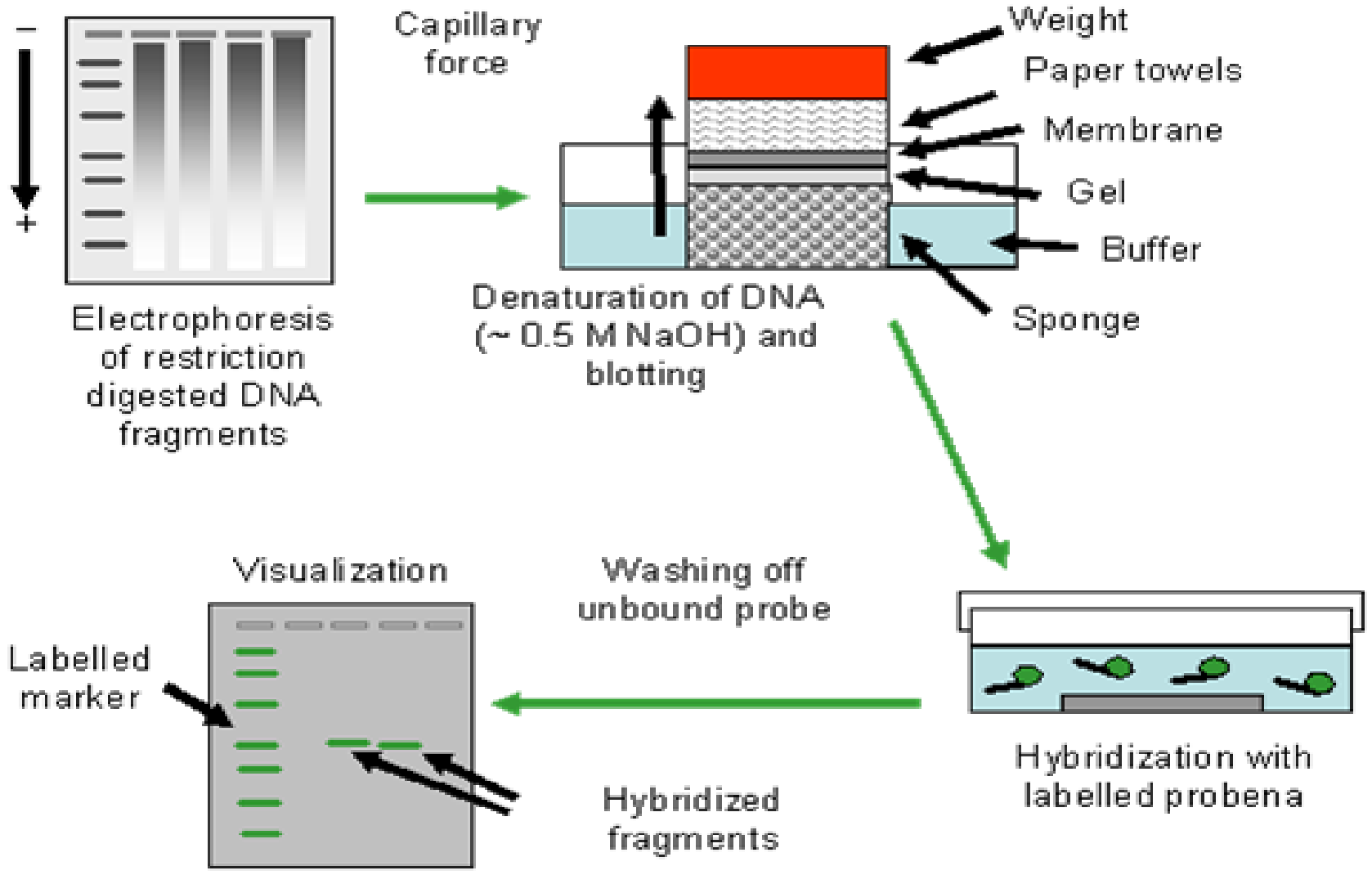
Western Blot







Southern blot



Southern blot

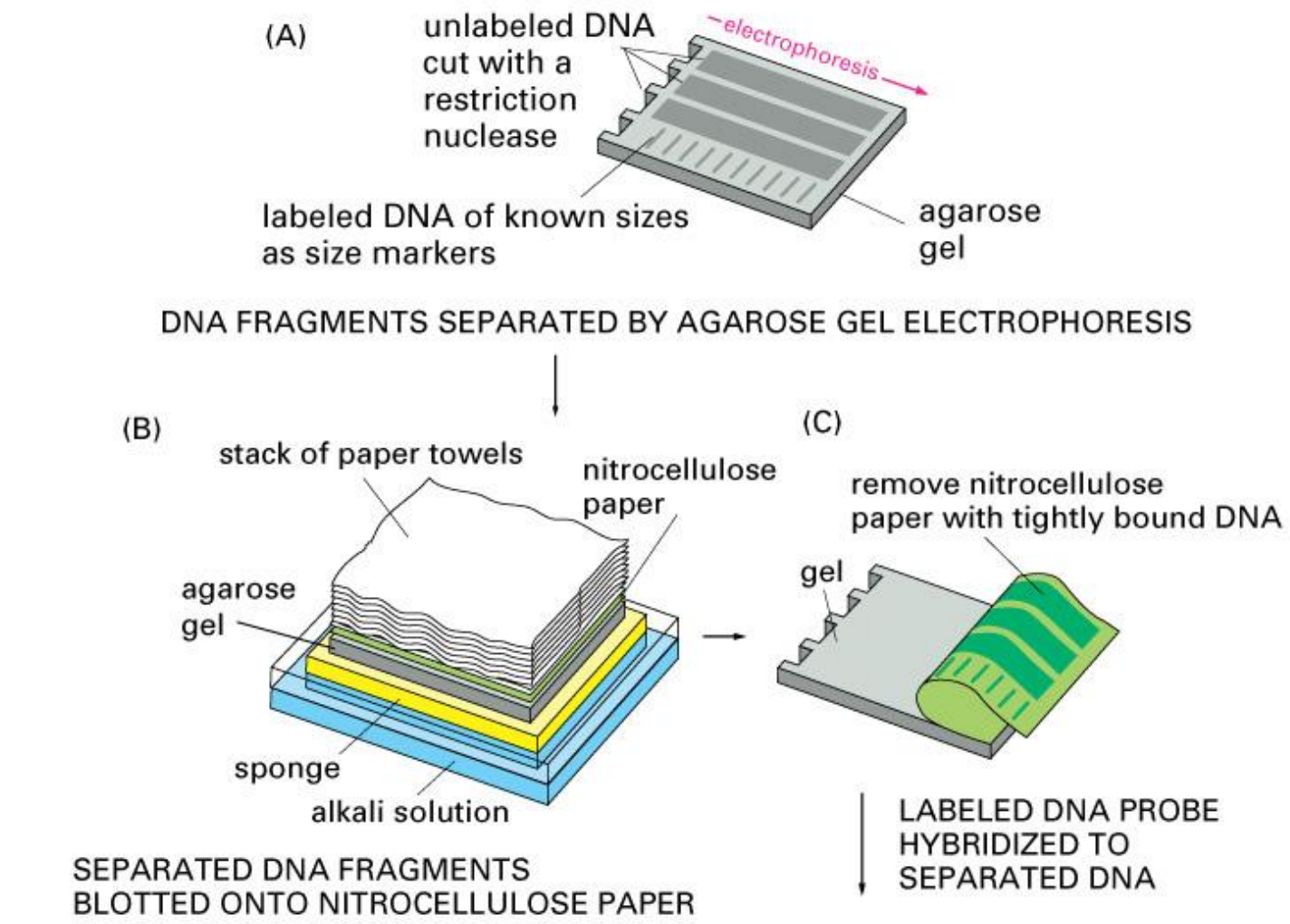


Figure 10-14 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Neuroscience *in vitro* : a NOT complete list

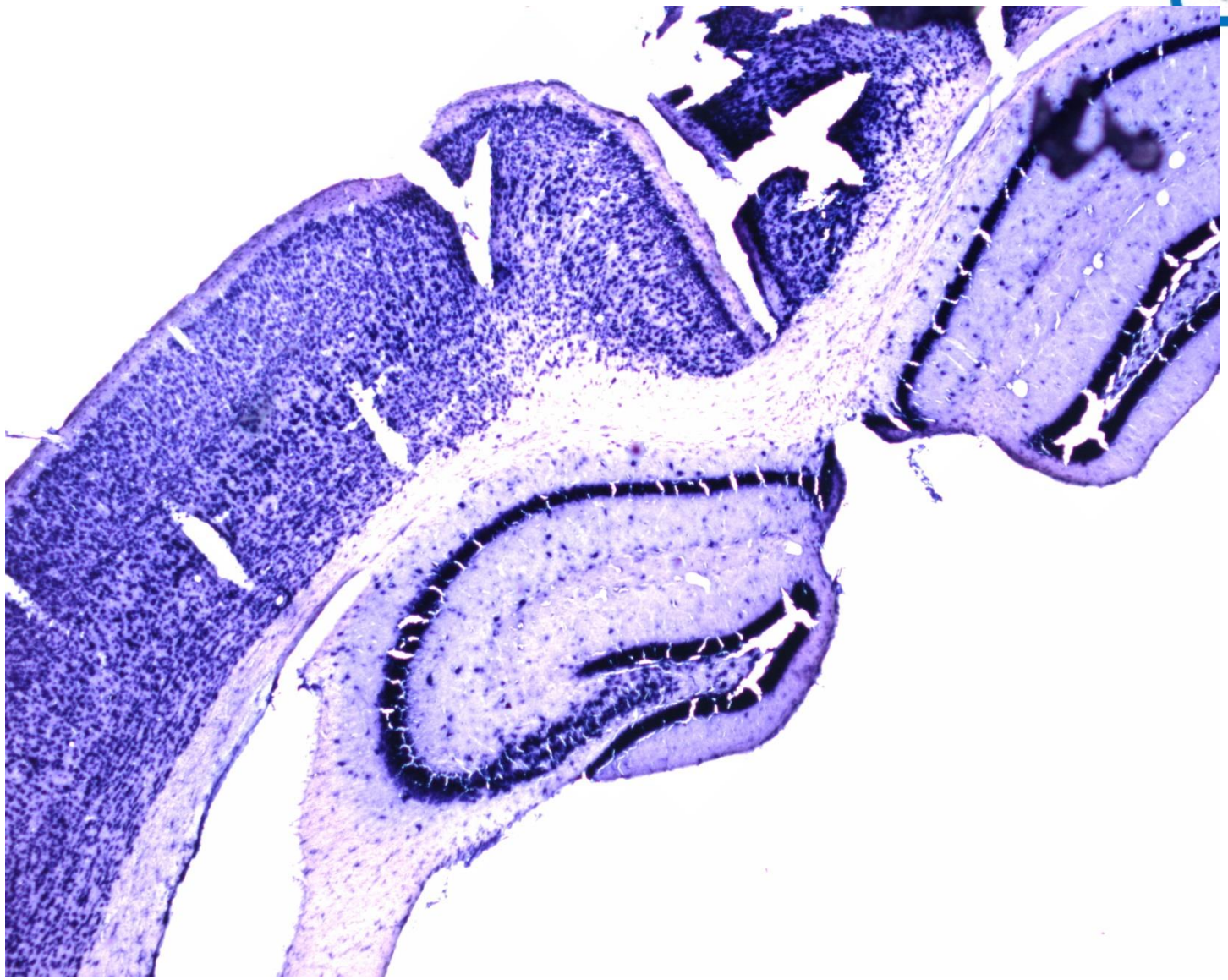
KNOWN TARGET

BIOINFORMATICS

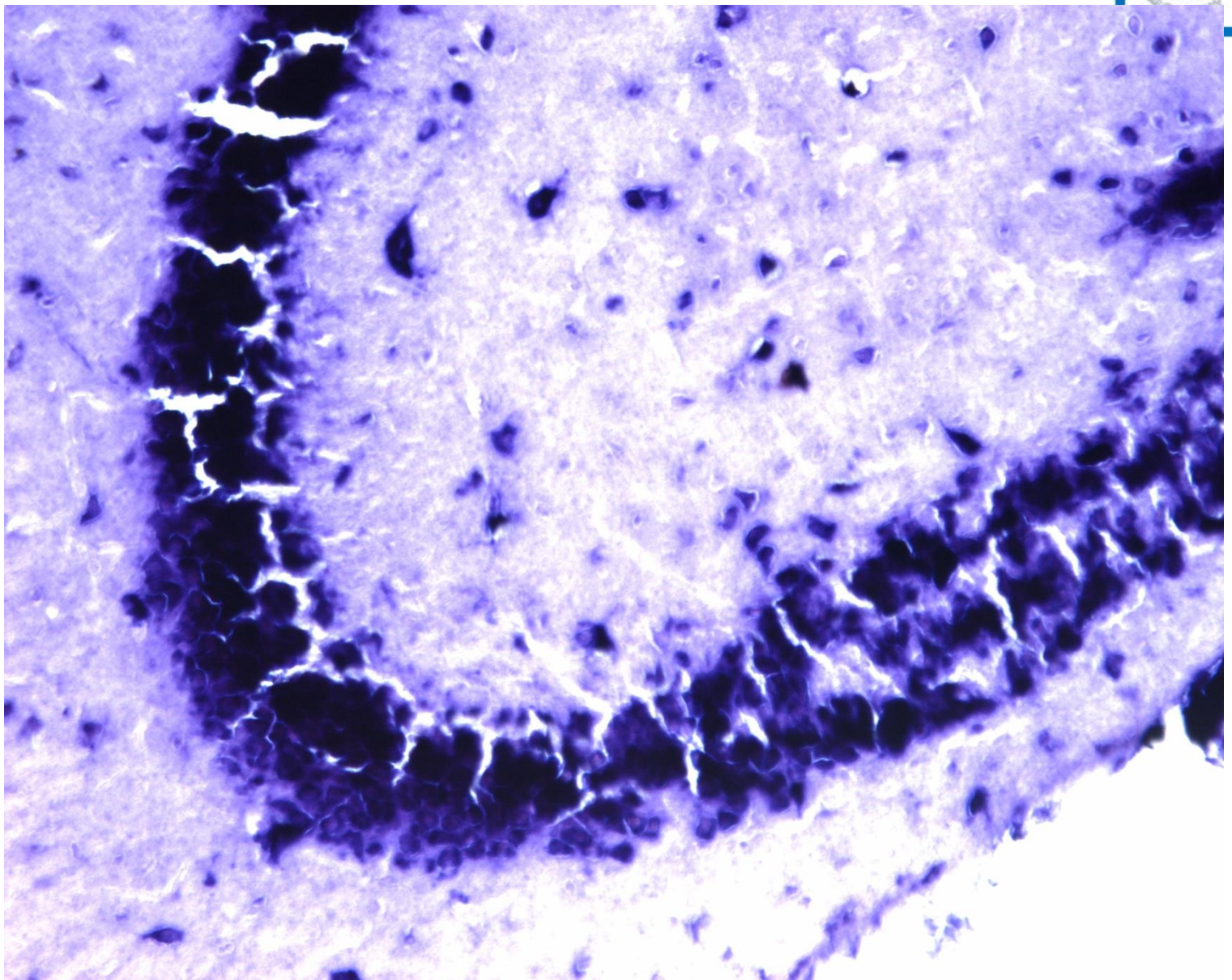
ALL TARGETS

RNA	PCR	Biochemical read out
	Northern Blot	Biochemical read out
	Sequencing	Biochemical read out
	In situ	subcellular read out

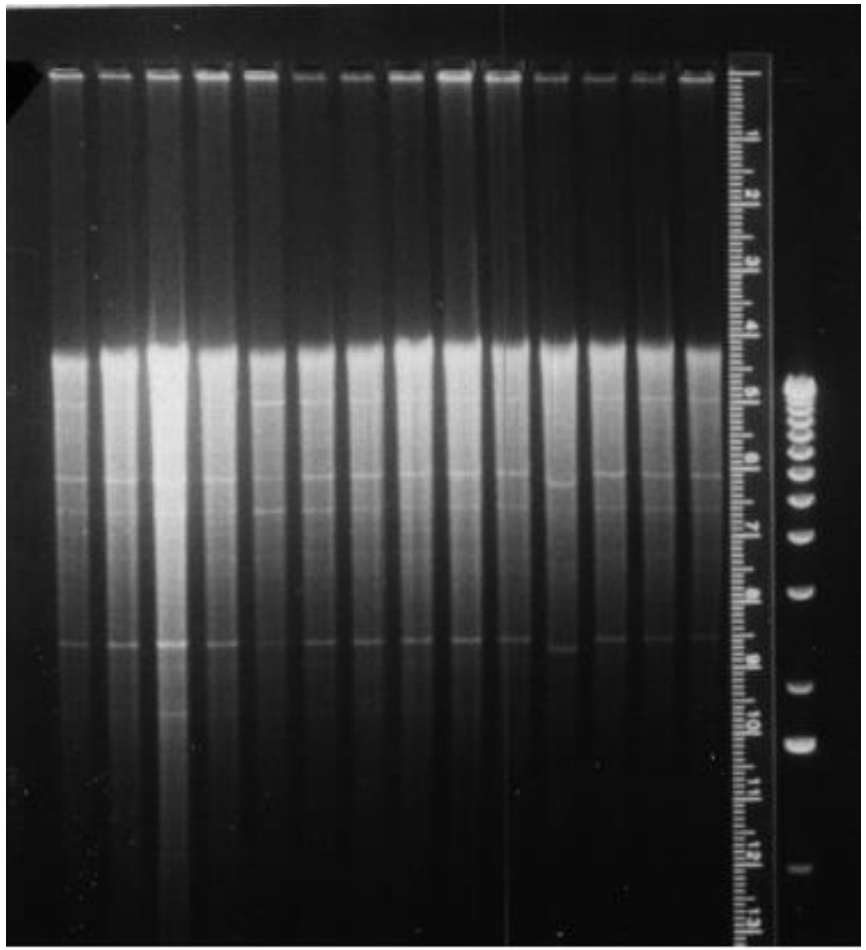
In situ

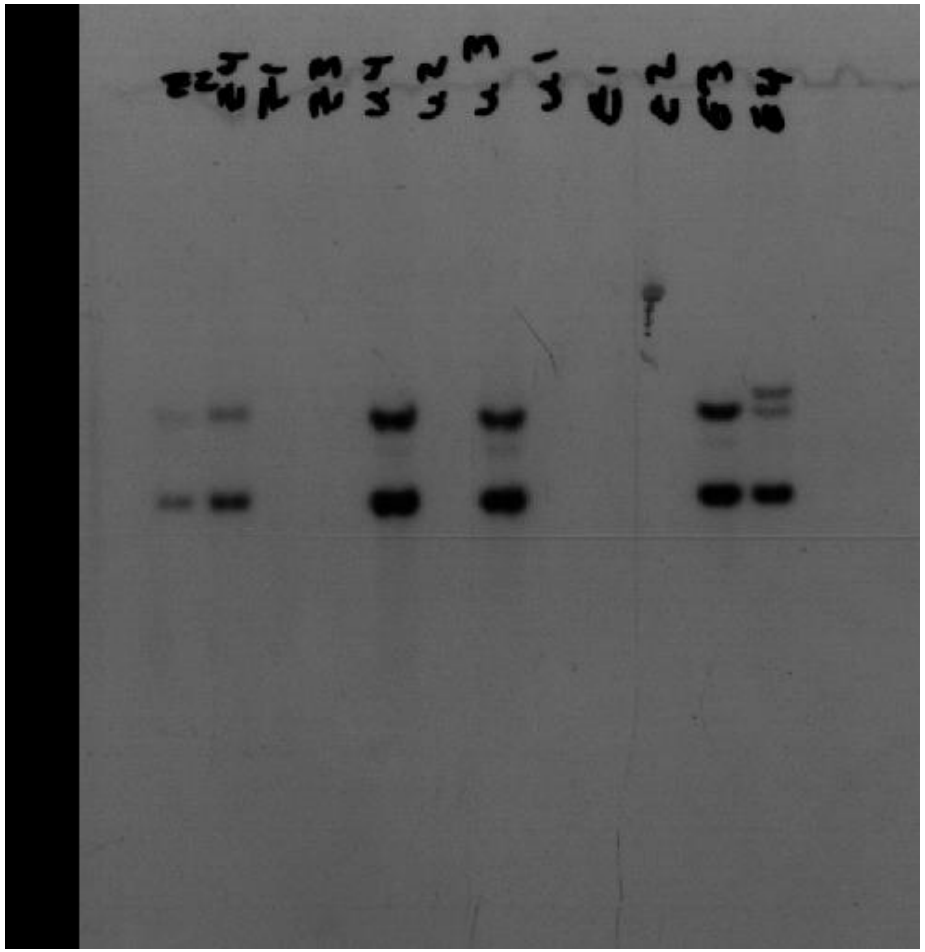


In situ



Southern/Northern blot





Comparison of Southern, Northern and Western blot hybridization

Blot type	Target	Probe	Applications
Southern	DNA	DNA or RNA (Agarose Gel)	mapping genomic clones estimating gene numbers, etc
Northern	RNA	DNA or RNA (Formaldehyde agarose gel)	RNA sizes and abundance (gene expression level)
Western	Protein	Antibodies (Polyacrylamide gel)	protein size and abundance (gene expression level)

Neuroscience *in vitro* : a NOT complete list

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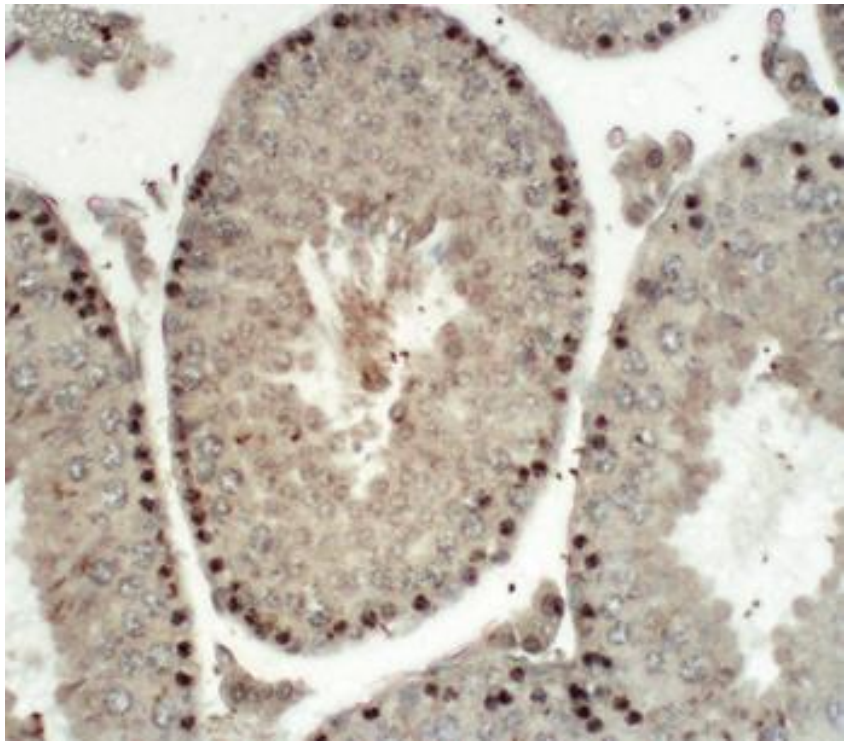
KNOWN TARGET

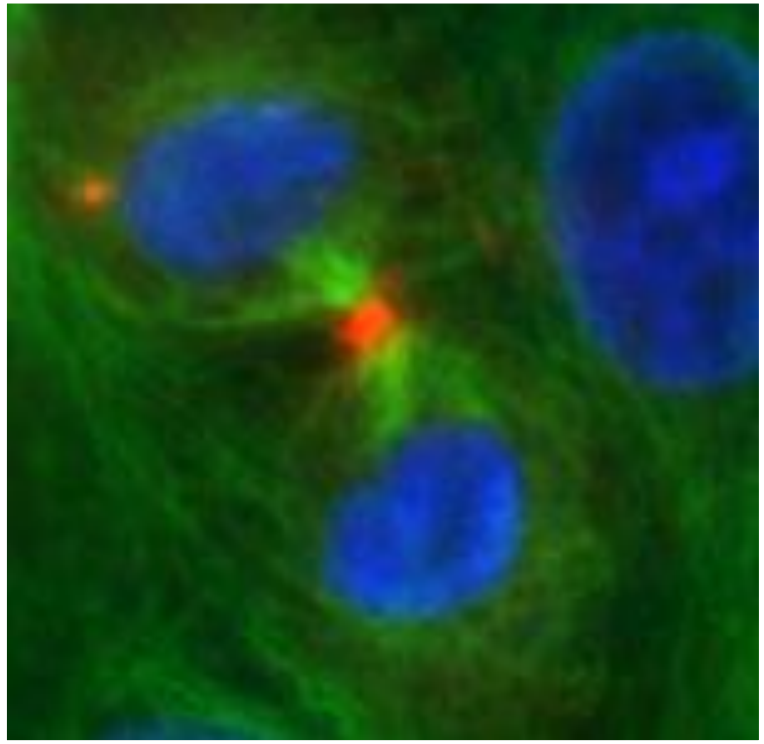
BIOINFORMATICS

ALL TARGETS

Protein	Western Blot	Biochemical read out
	Elisa	Biochemical read out
	Sequencing	Biochemical read out
	Immuno Istochemistry	subcellular read out
	Immuno Citochemistry	subcellular read out

Immunohistochestry
Immunocytochemistry





Neuroscience *in vitro* : a NOT complete list

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KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

BASAL CONDITION "MODIFIED CONDITIONS"

DNA-Protein

Chromatin immunoP

Biochemical read out

RNA-Protein

Co immunoP + RT PCR

EMSA

SuperShift

Gel Shift

Biochemical read out

Protein-Protein

FRET

subcellular read out

Neuroscience *in vitro* :The choice of a Model

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BASAL CONDITION **"MODIFIED CONDITIONS"**

- Cell Lines

Biochemical read out

subcellular read out

Biochemical read out

subcellular read out

- Primary culture

Biochemical read out

limited by the *availability*

MORFOLOGICAL READ OUT

subcellular read out

MORFOLOGICAL READ OUT

subcellular read out

- Primary culture

from Transgenic/

KO/KIN Animal

Biochemical read out

MORFOLOGICAL READ OUT

subcellular read out

Biochemical read out

MORFOLOGICAL READ OUT

subcellular read out

Neuroscience *in vitro* :The choice of a Model

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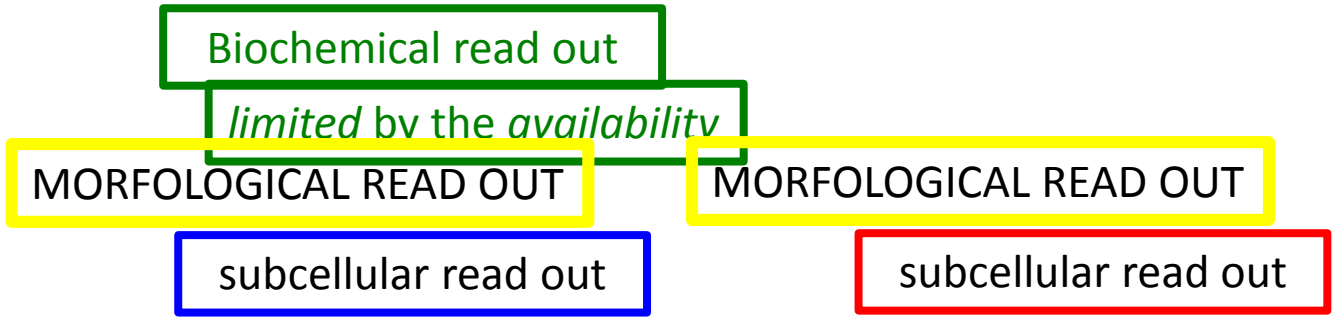
BASAL CONDITION **"MODIFIED CONDITIONS"**

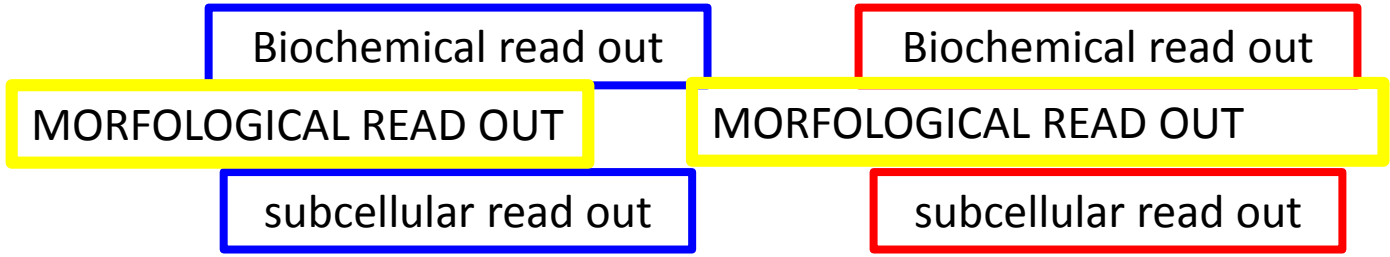
- Organotypic cultures



- Organotypic cultures

from Transgenic

KO/KIN Animal



Neuroscience *in vitro* :The choice of a Model

- Cell lines = NO REAL NEURONS

NO REAL MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE → FUNCTION

Neuroscience *in vitro* :The choice of a Model

- Primary culture = NO REAL TISSUE

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE FUNCTION

LIMITED TO SINGLE FAMILY OF CELLS

Neuroscience *in vitro* :The choice of a Model

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- Organotypic slice= NO REAL BRAIN

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE FUNCTION

LIMITED TO SINGLE TISSUE

Consider Technical LIMITATIONS

Neuroscience *in vitro* :The choice of a Model

- Animal = NO HUMAN BRAIN

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE → FUNCTION

LIMITED TO SINGLE ANIMAL SPECIEs

Consider many Technical LIMITATIONS

Neurons

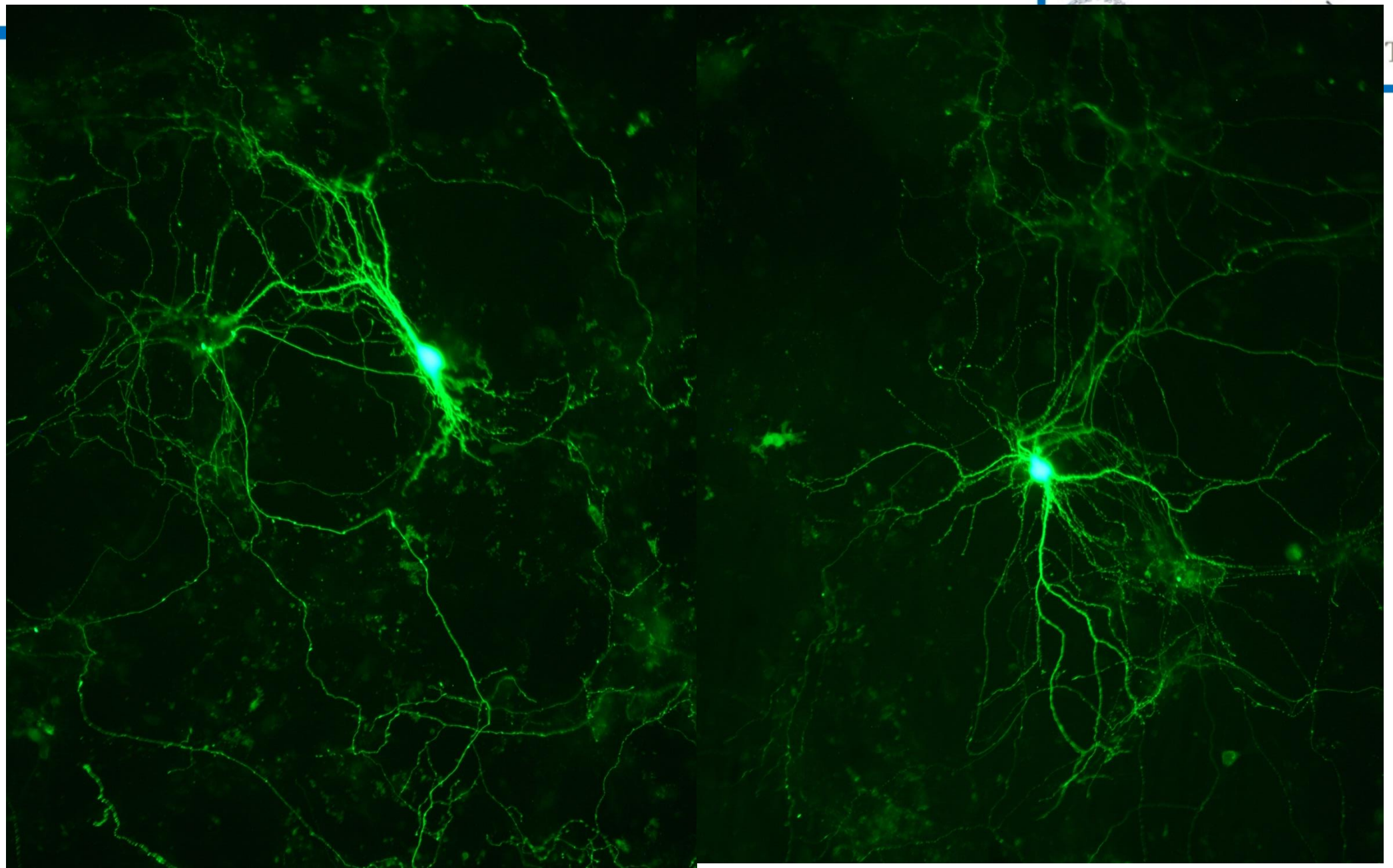

Image J

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http://rsbweb.nih.gov/ij/

or simply type : imagej on g

Standard Microscopy

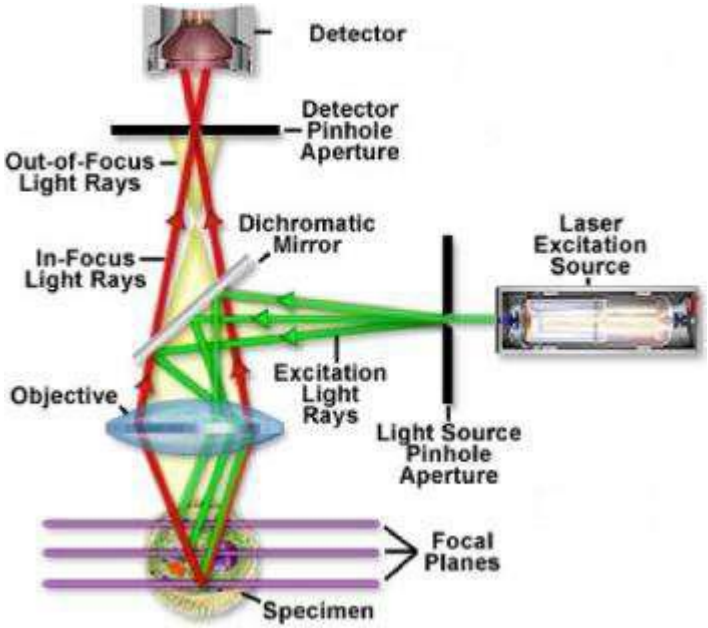
Epi-fluoresence

– Advantages:

- Fast (biological reactions)
- Full Field
- Affordable

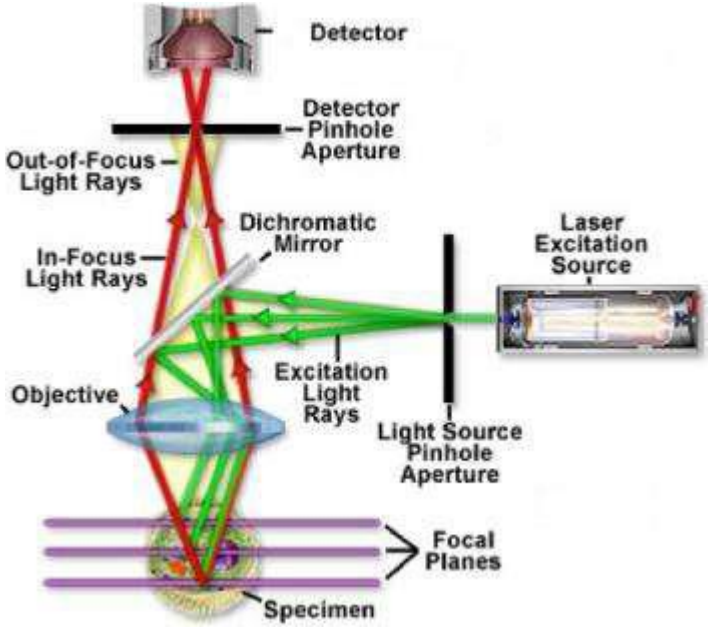
– Disadvantages:

- Lower resolution
- High background
- Photobleaching and photoxic



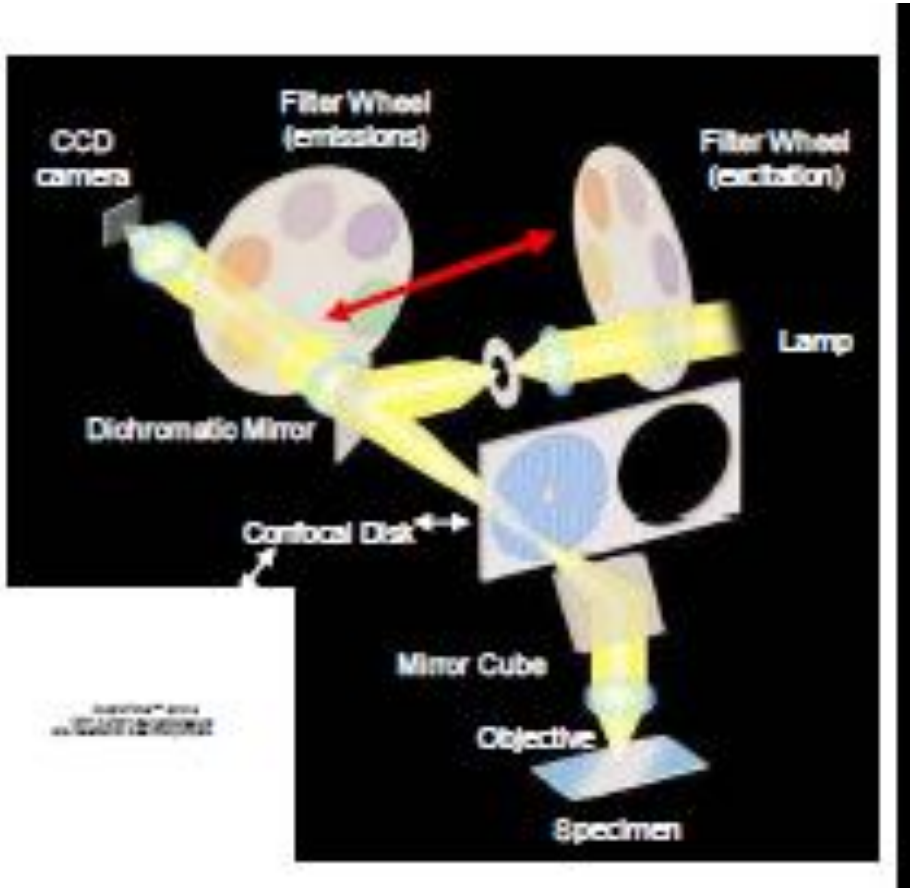
Scanning Confocal Microscopy

– Advantages:
• High resolution
• 3D overlay
– Disadvantages:
• Photobleaching and phototoxic
• Slow

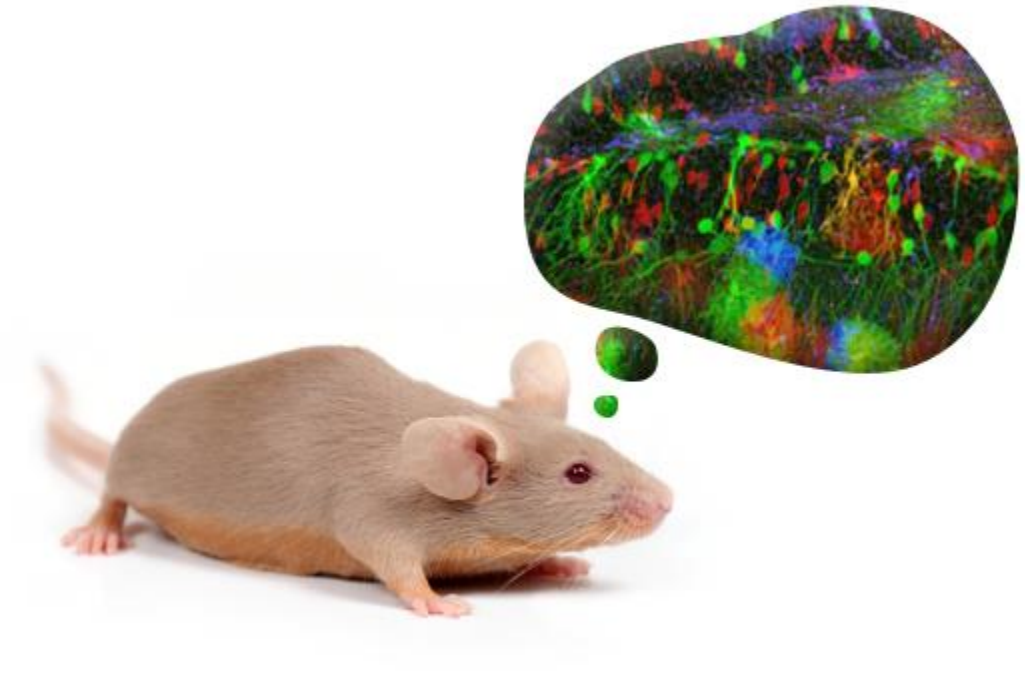


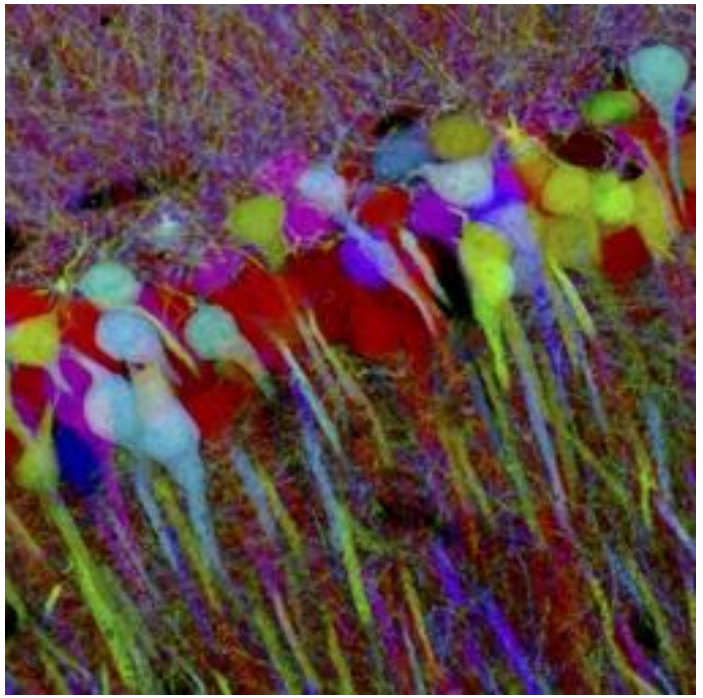
Spinning Disk Confocal Microscopy

– Advantages:
• High resolution
• Full field
• Fast (better than video rate)
• Great for biological reactions
– Disadvantages:
• Photobleaching and phototoxic
• More expensive, more technical



Brainbow MODEL





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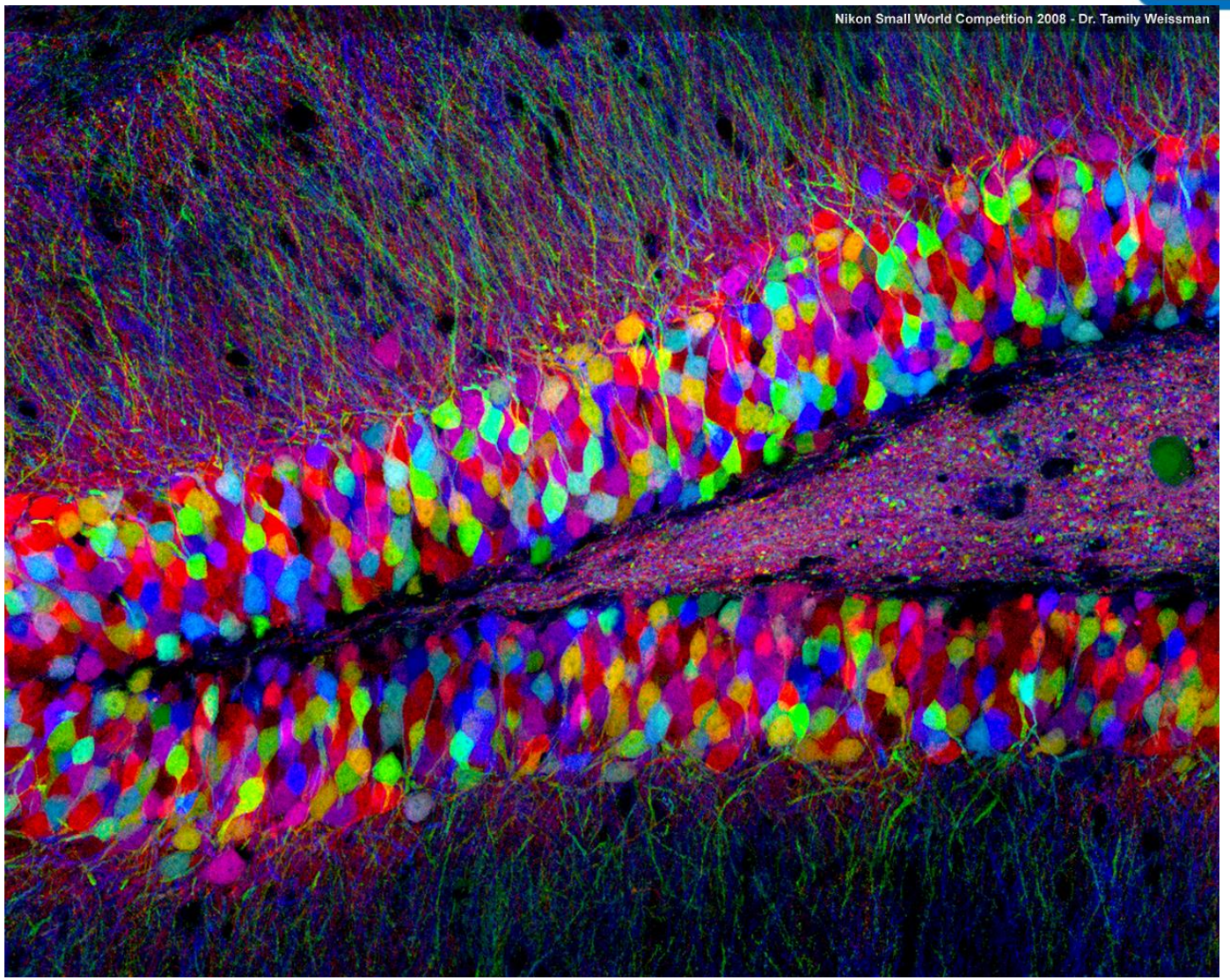

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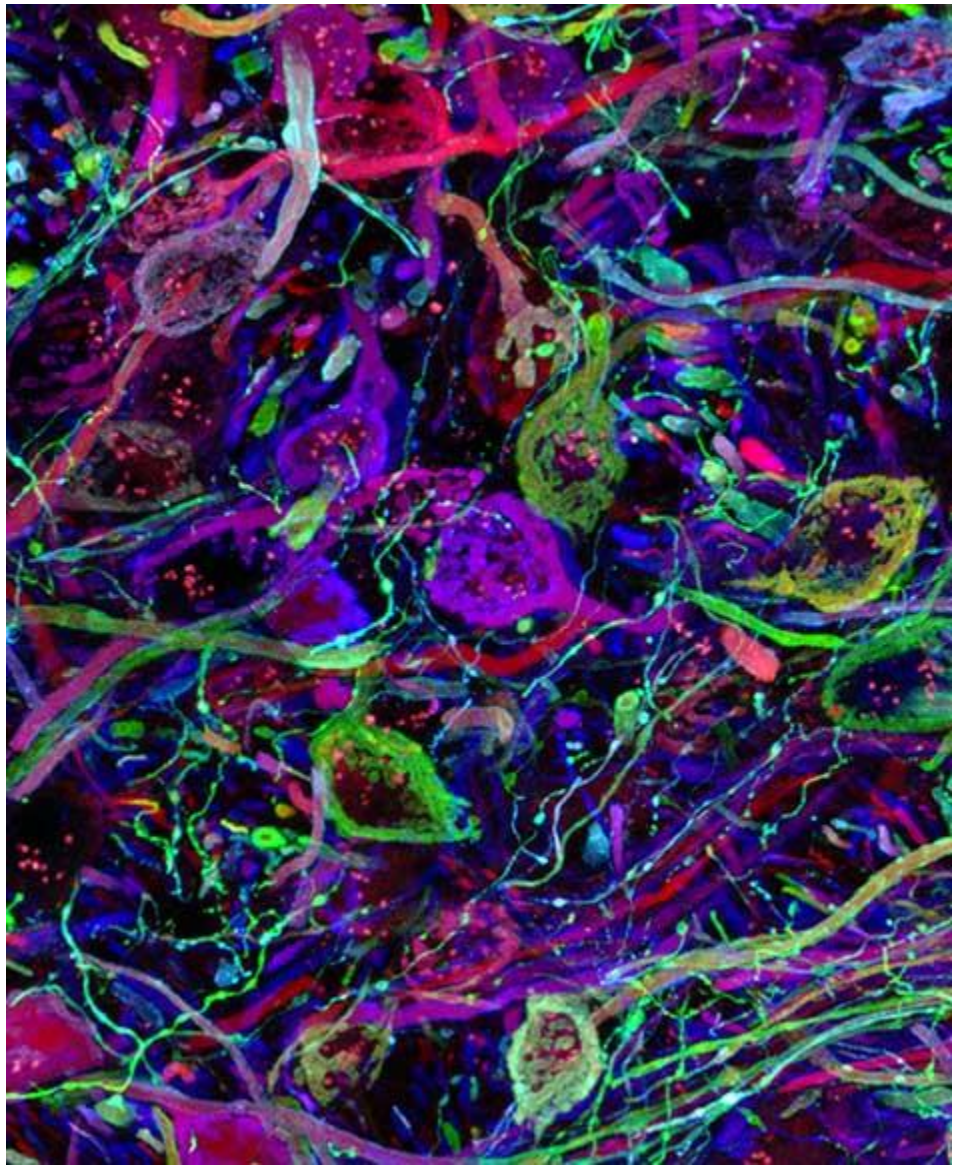

Brainbow MODEL

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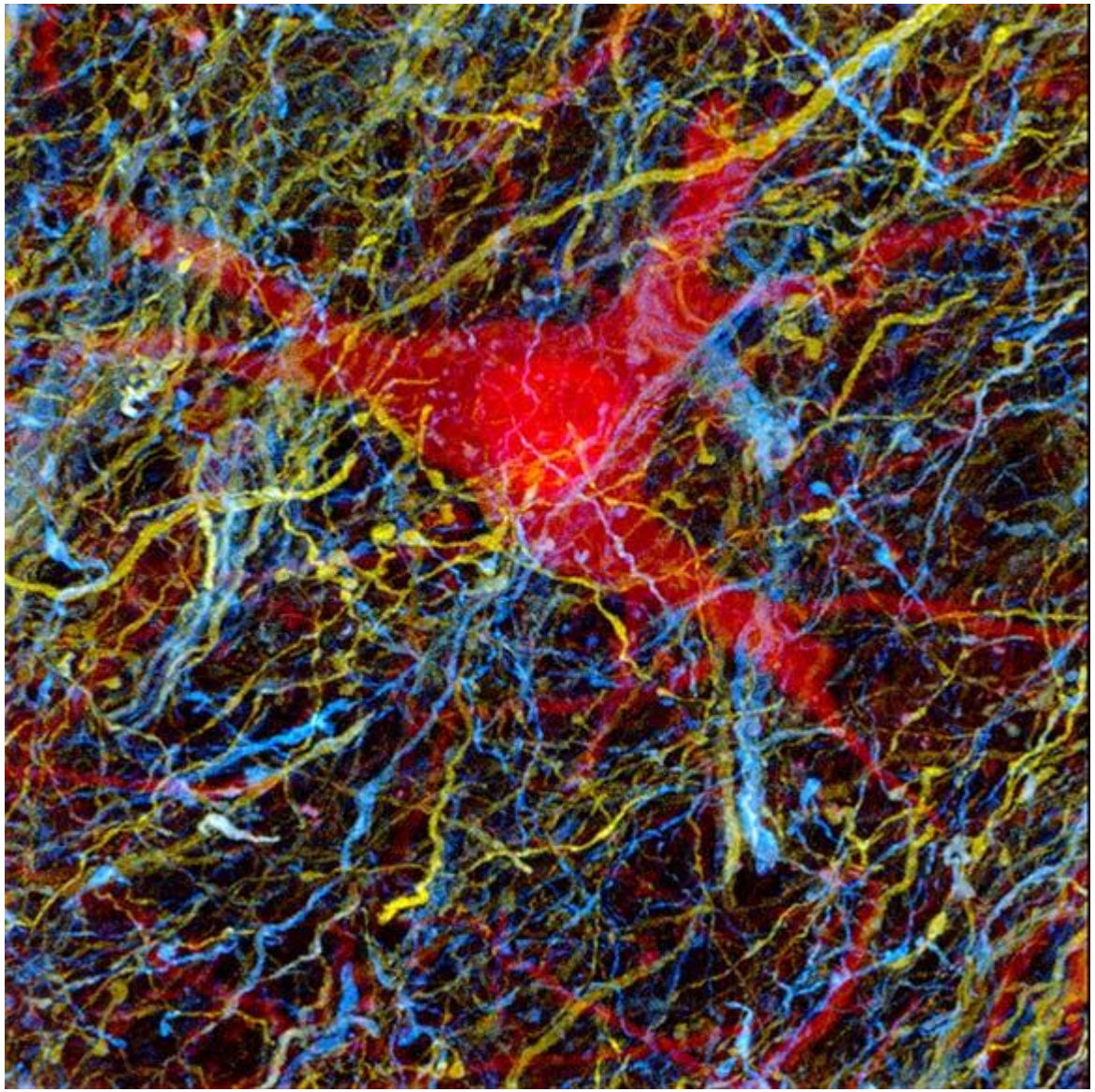

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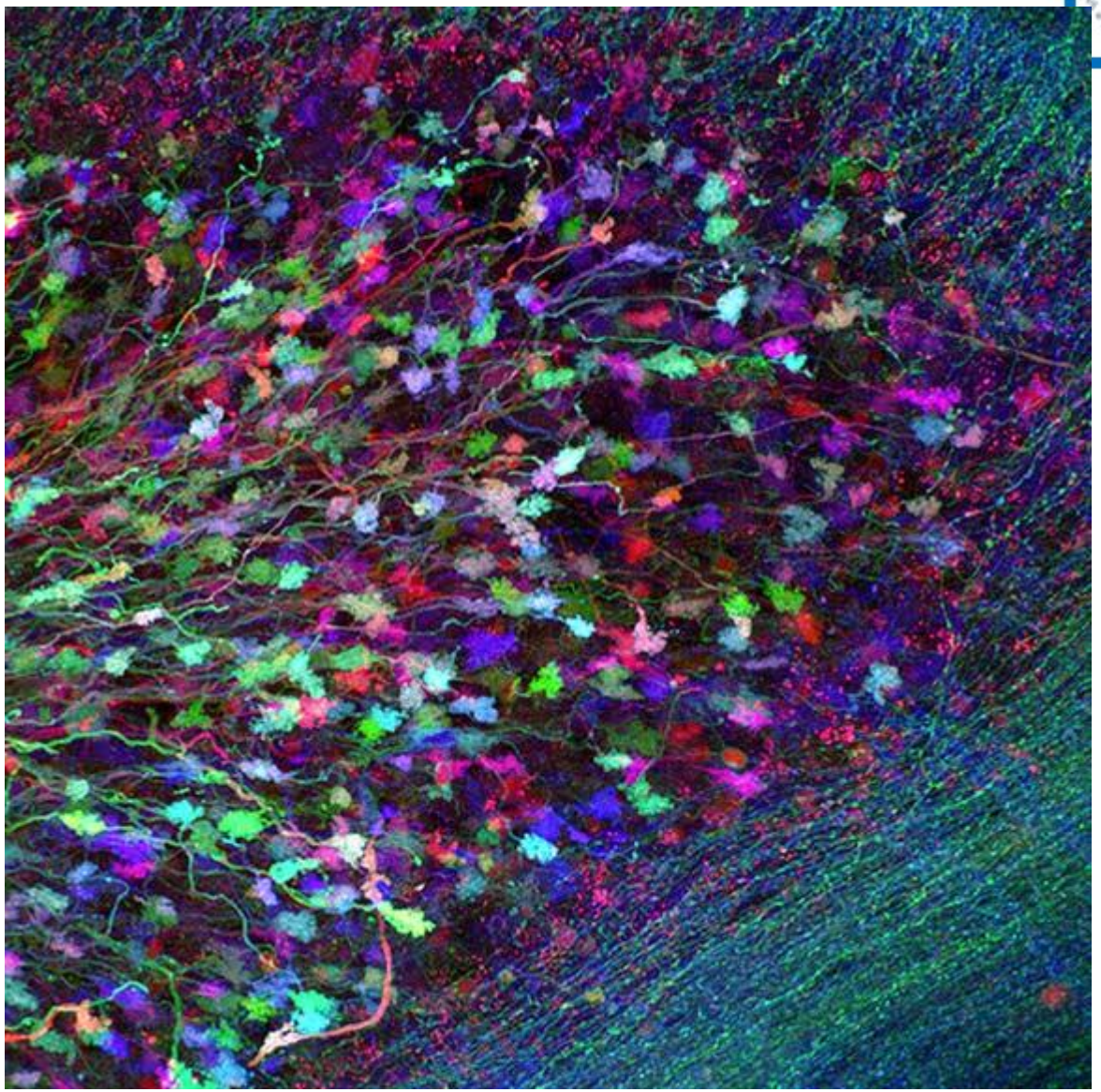

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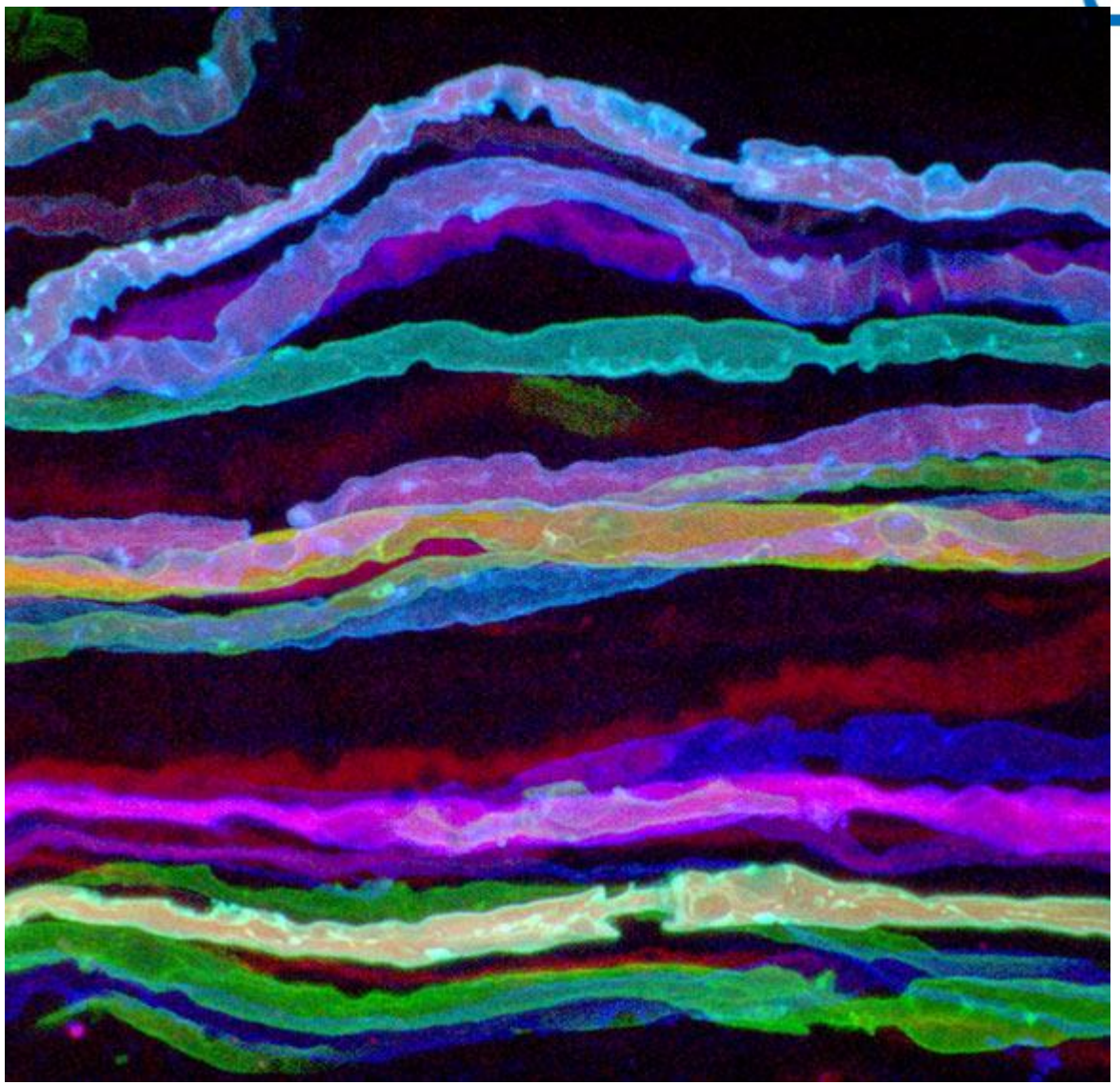

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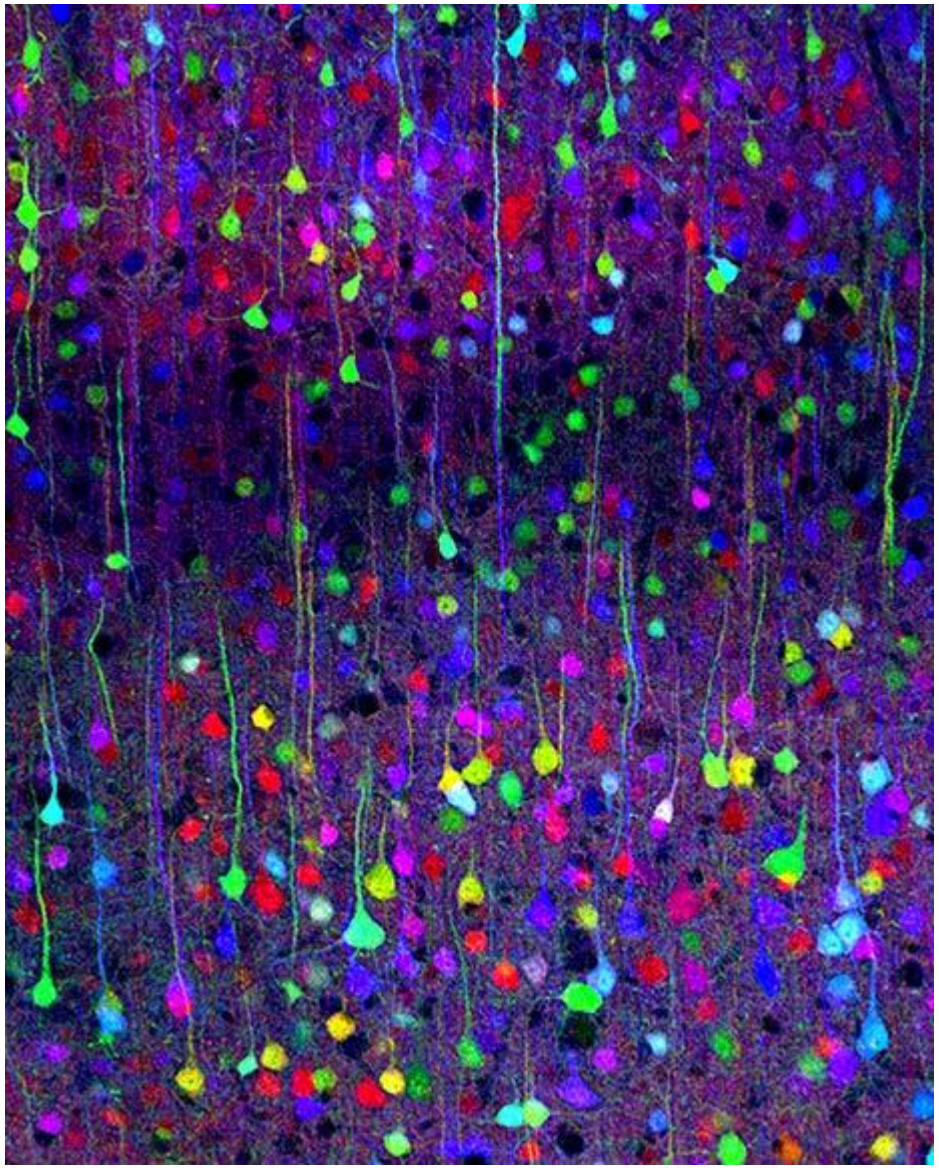

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