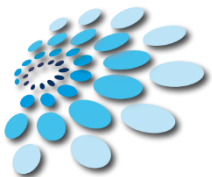


“ Techniques in Cellular
and Molecular
Neurobiology ”

International Master's Degree in Neuroscience

Lesson 8



DIPARTIMENTO DI
SCIENZE DELLA VITA

Gabriele Baj
gbaj@units.it



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Image J



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

or simply type : imagej or



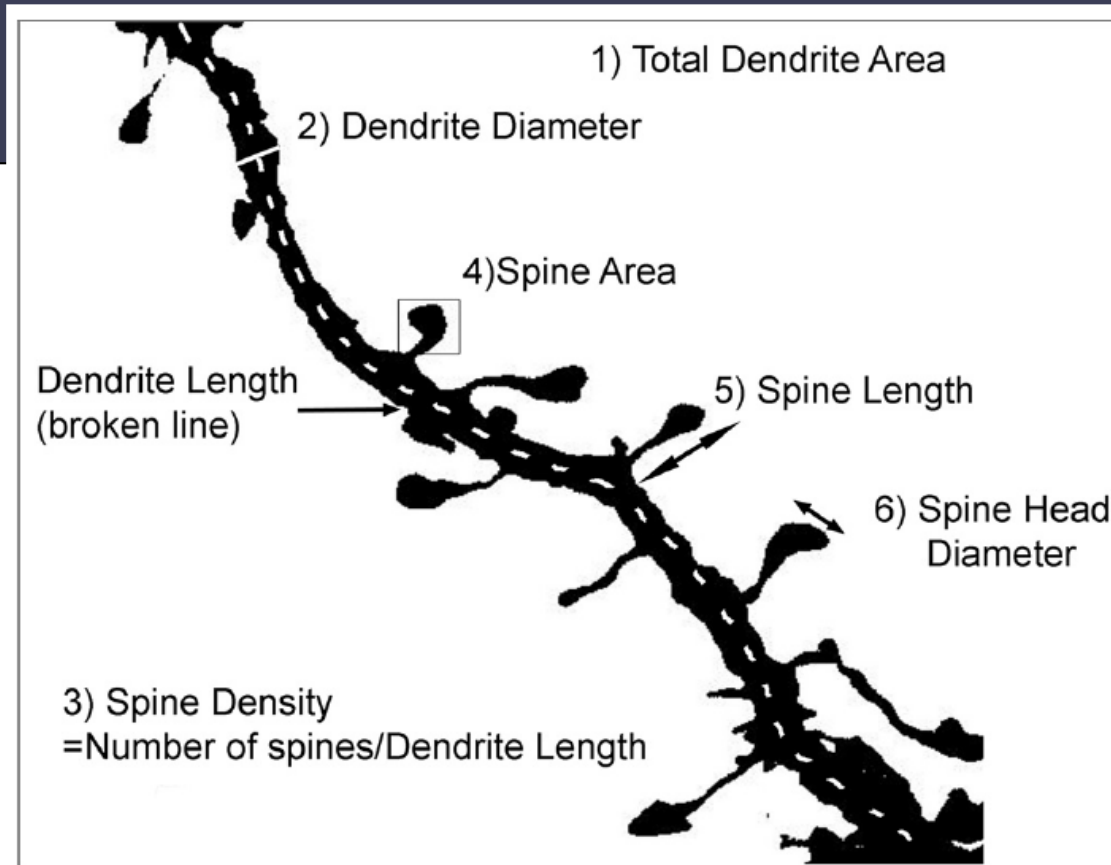


Fig. 1. Neuronal dendrite and spine measurement by Image J analysis. A typical dendrite segment from a pyramidal neuron is shown, and the six quantification parameters labeled as follows. 1) Total dendrite area is measured by drawing a box around the whole image; 2) dendrite diameter is obtained by drawing a line across the dendrite thickness at a place of average width; 3) spine density is the total number of spines divided by the dendrite length; 4) spine area is measured by drawing a box around the whole spine; 5) spine length uses the broken line tool to measure the length; and 6) spine head diameter again uses the broken line tool in Image J to measure the diameter across the head of the spine.

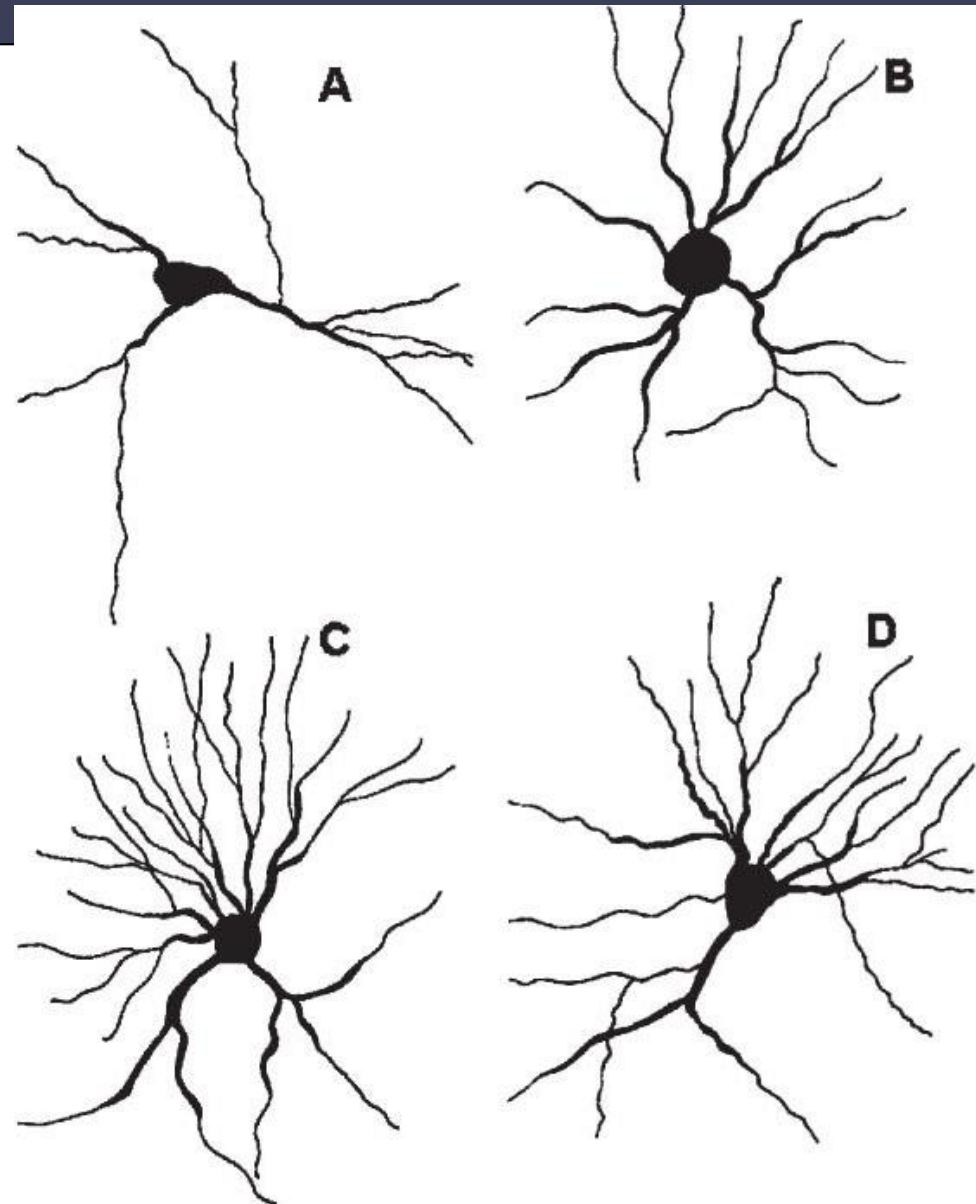
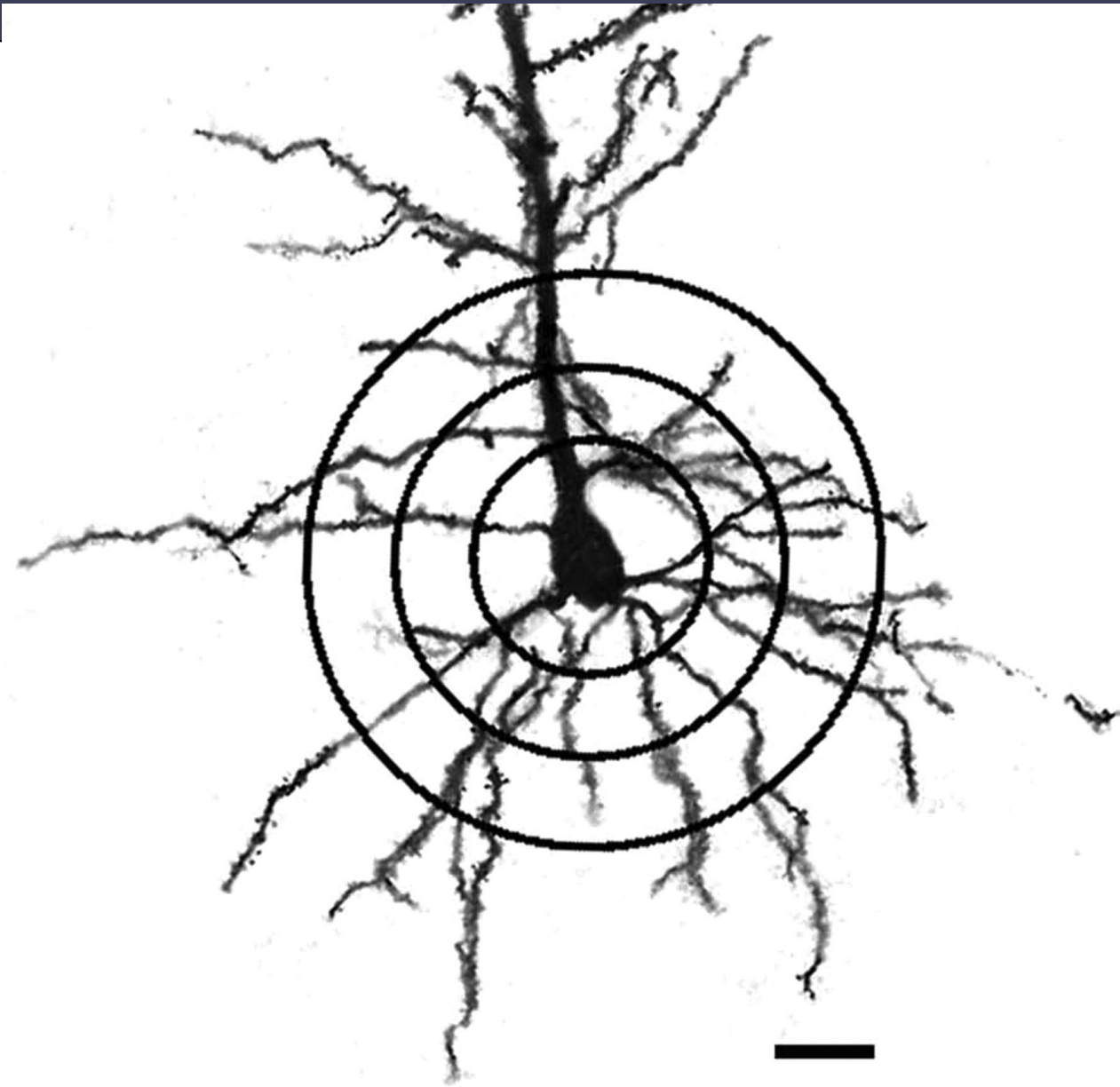


Figure 5. General histological types of neurons and associated glial cells. A) multipolar neuron, B) multipolar neuron, C) multipolar neuron, D) multipolar neuron.



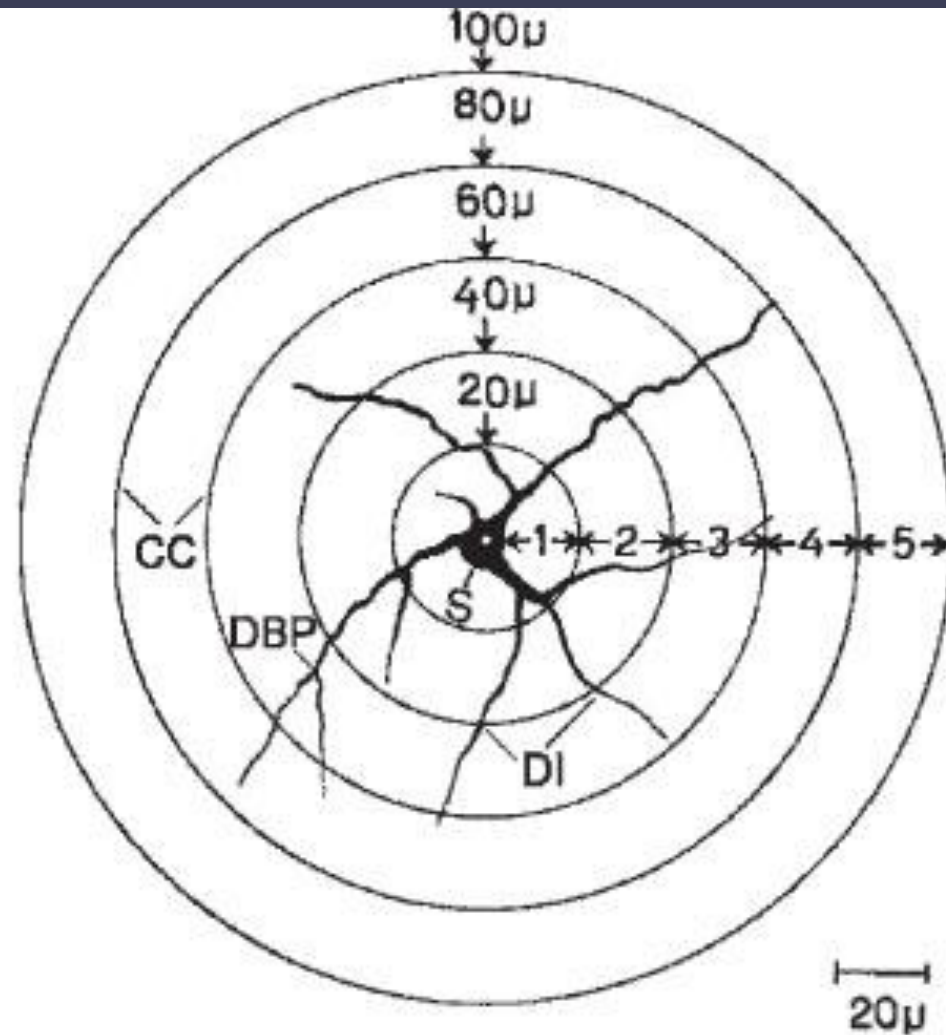
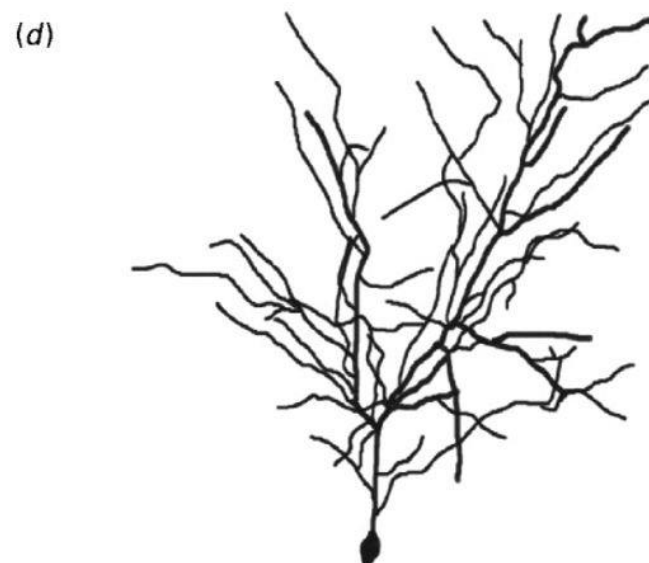
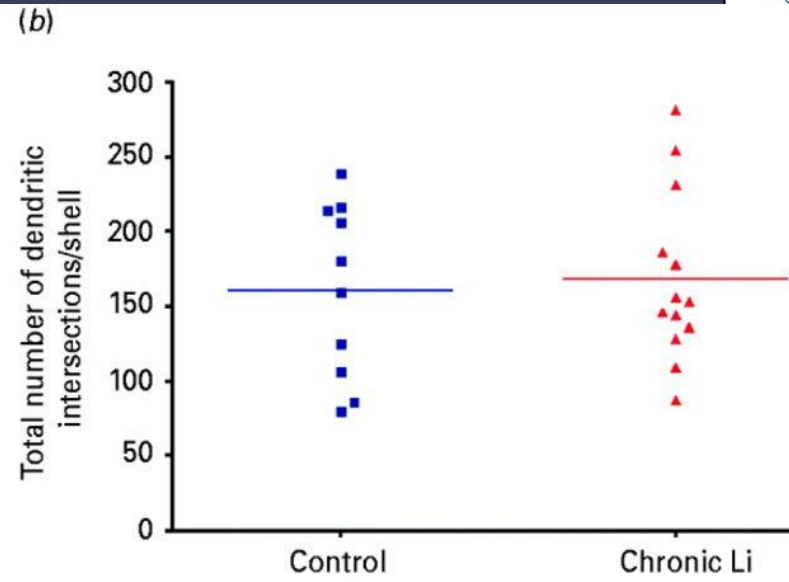
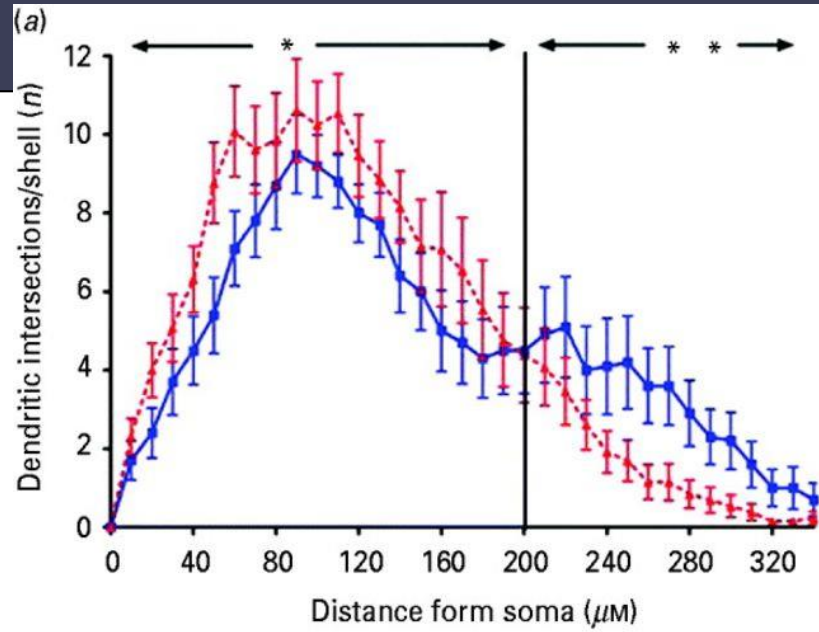
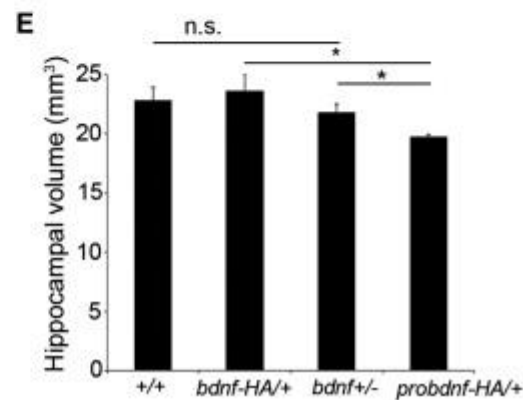
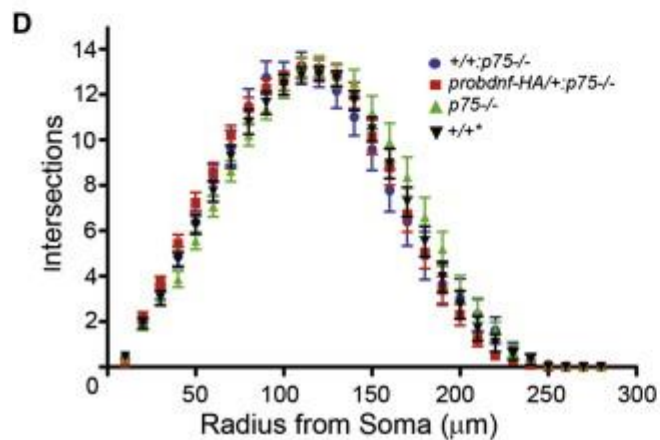
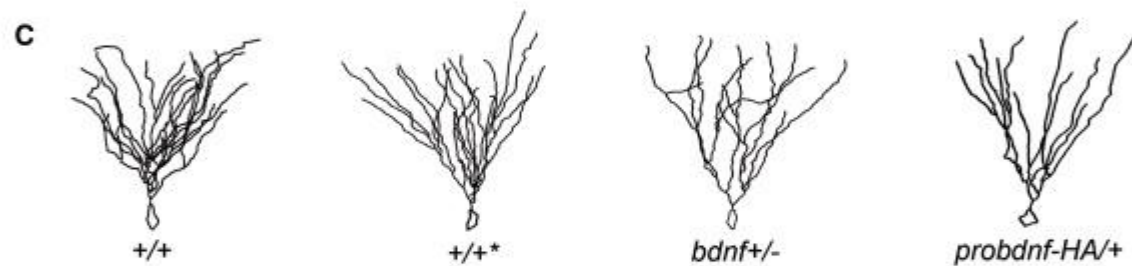
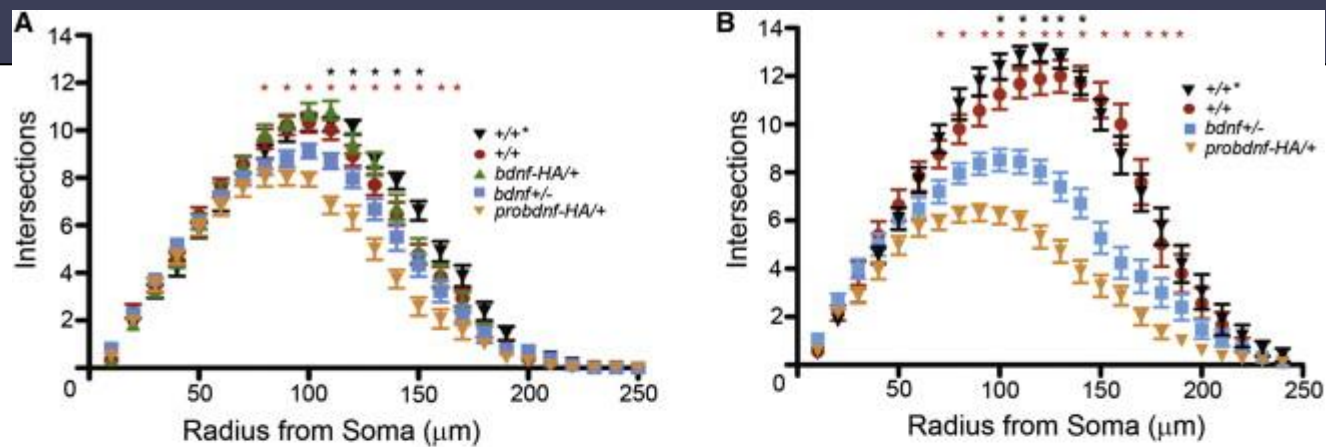
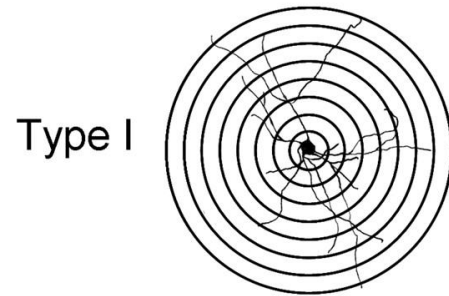


Figure 1 - Diagram of an amygdaloid neuron and the scheme of dendritic quantification. DBP – Dendritic branching points; DI – Dendritic intersections; CC – Concentric circles; S – Soma; and 1 to 5 – Concentric zones.

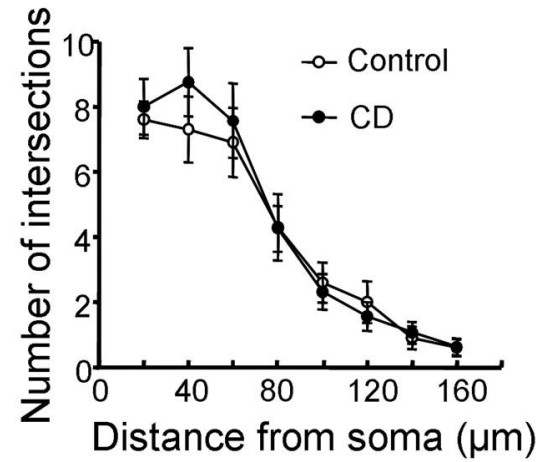




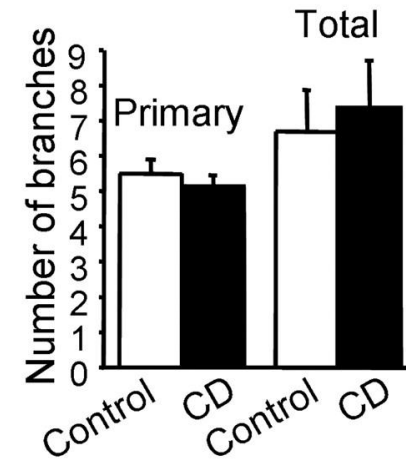
A



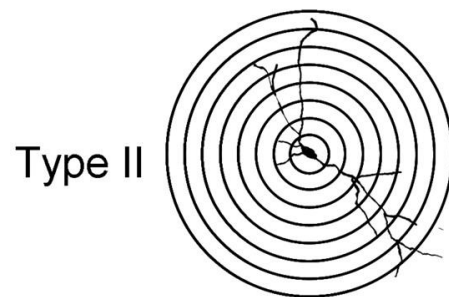
B



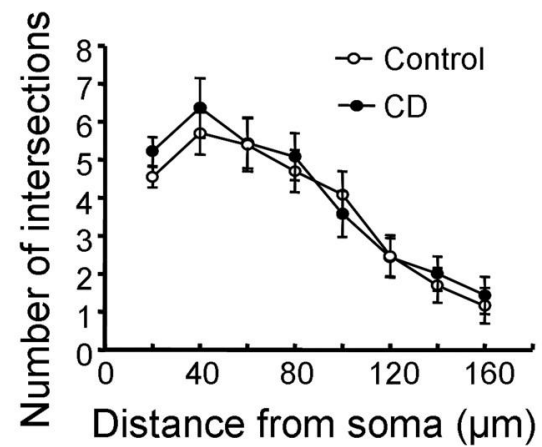
C



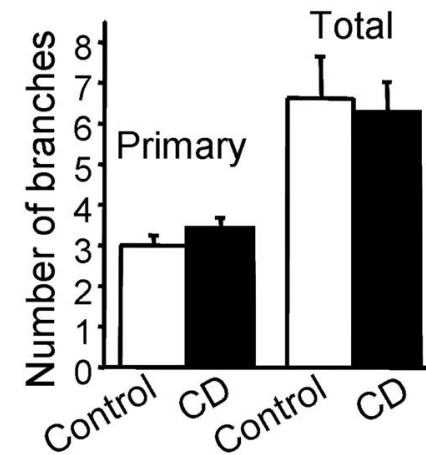
D

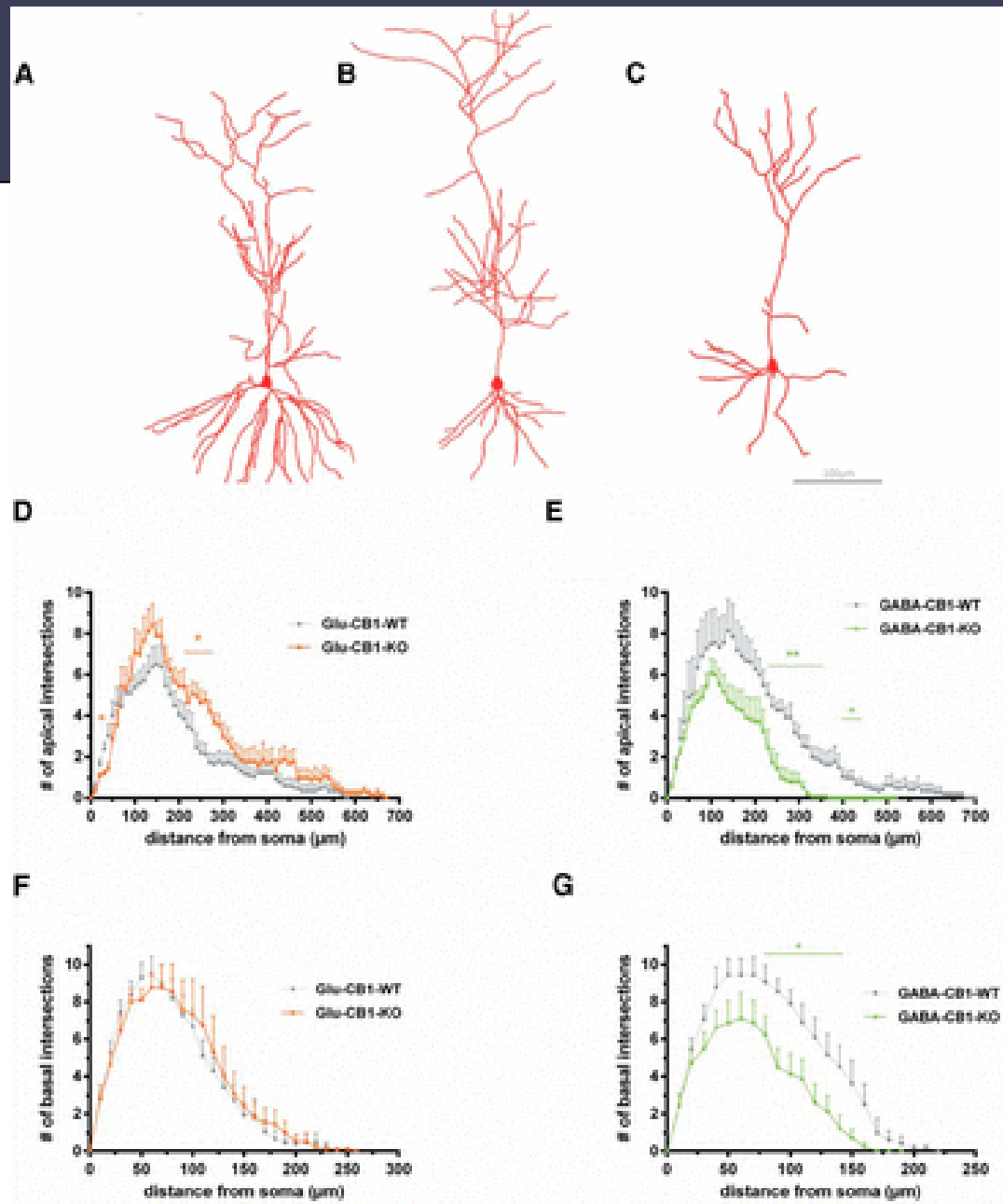


E

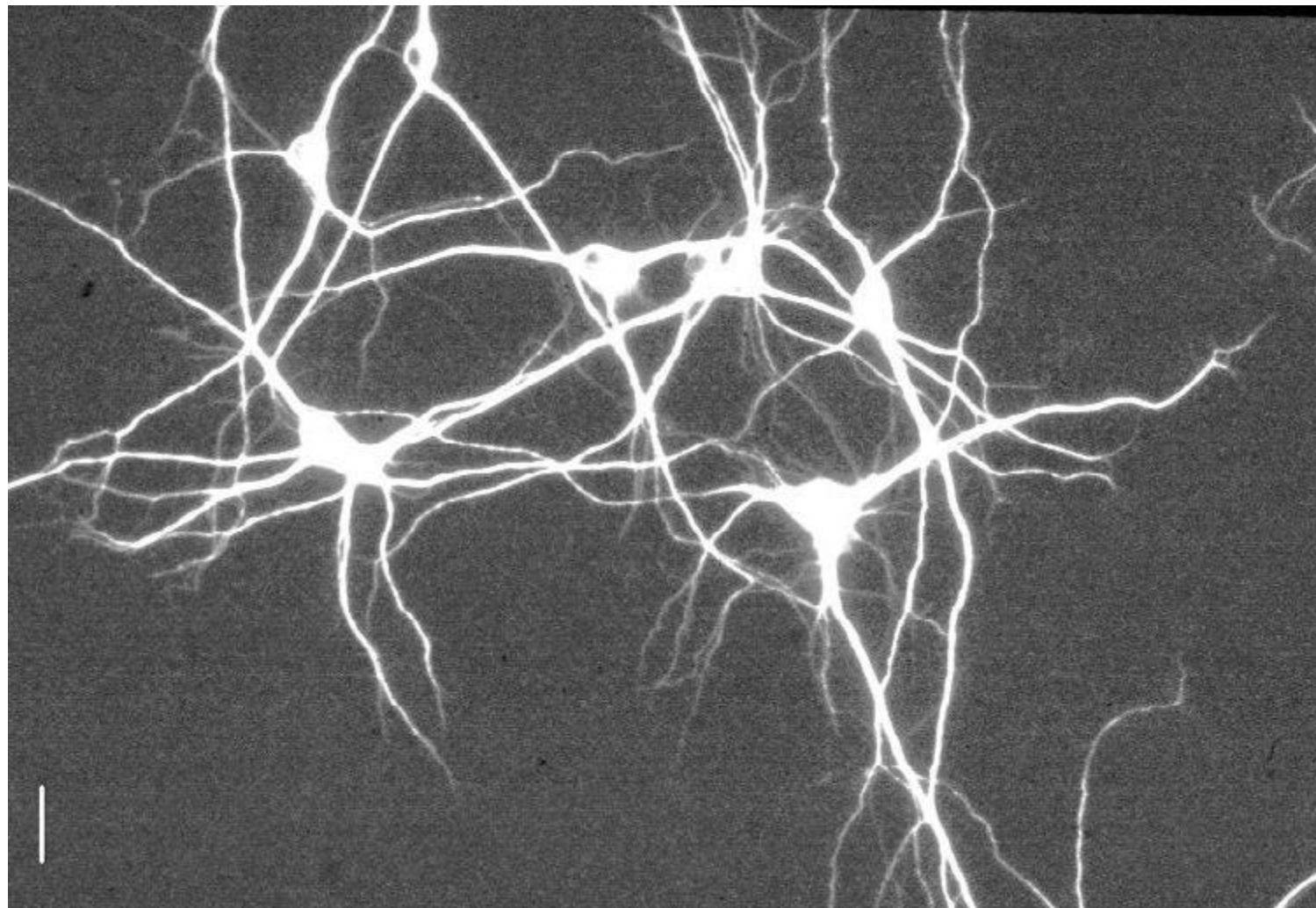


F

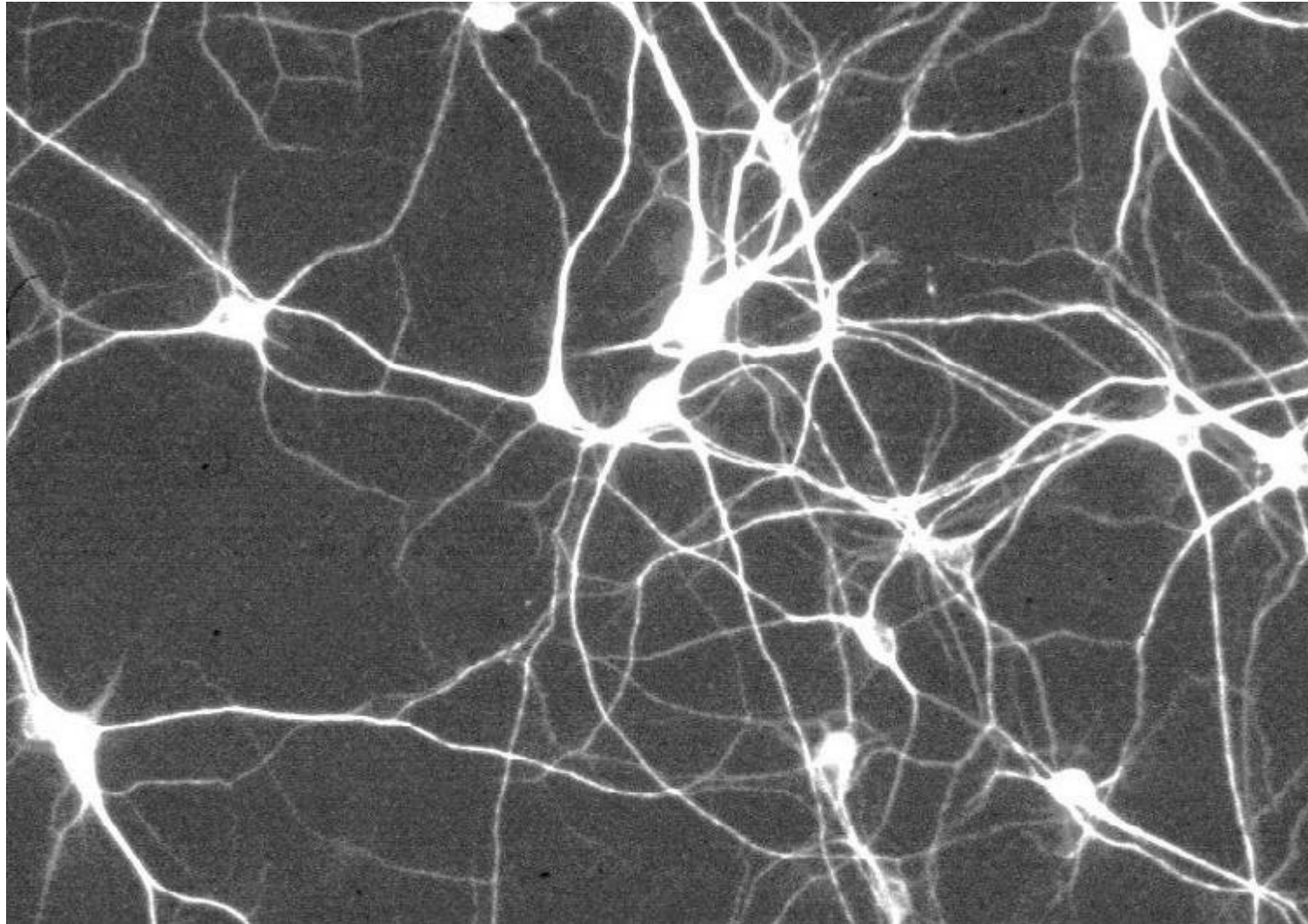


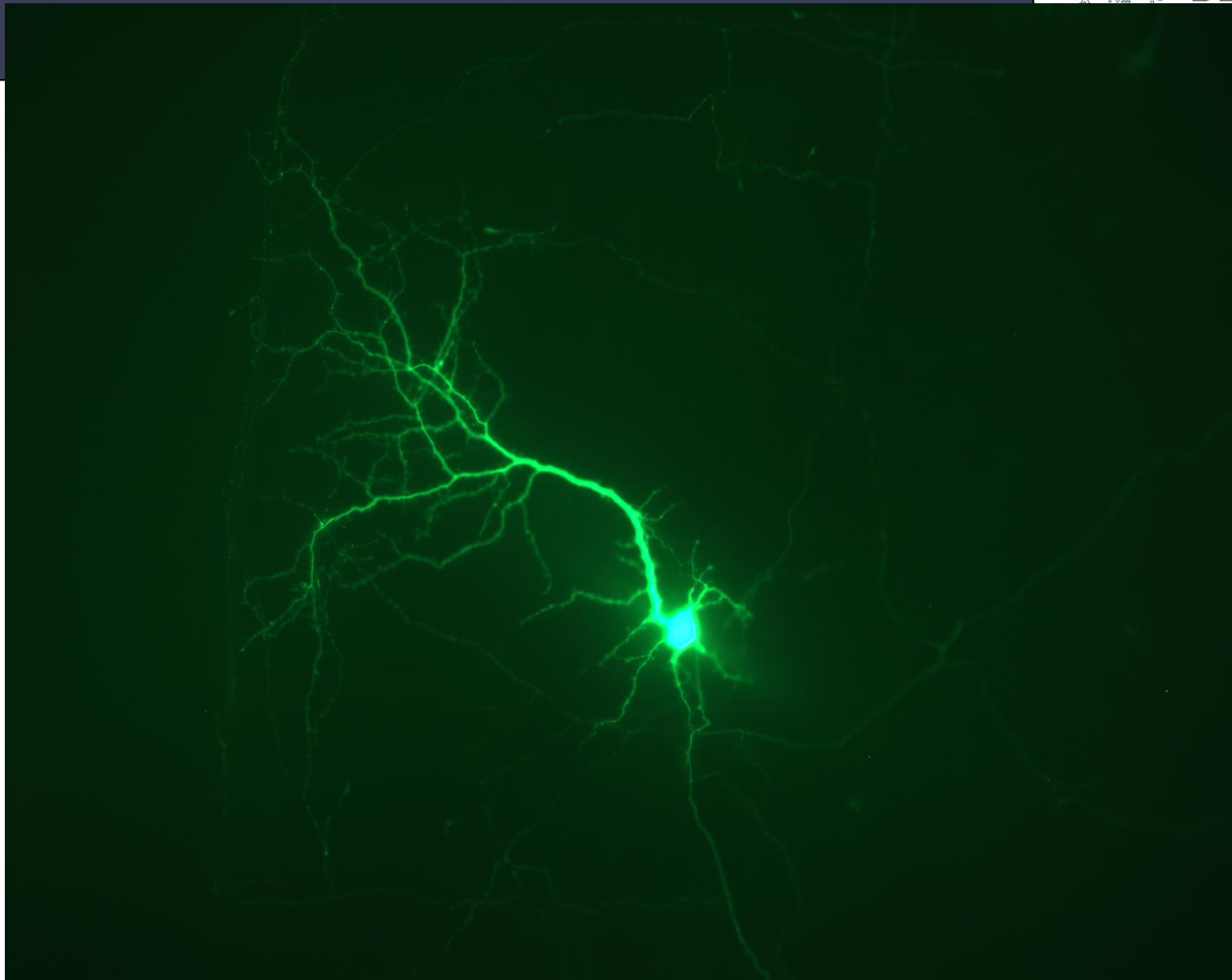


- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)

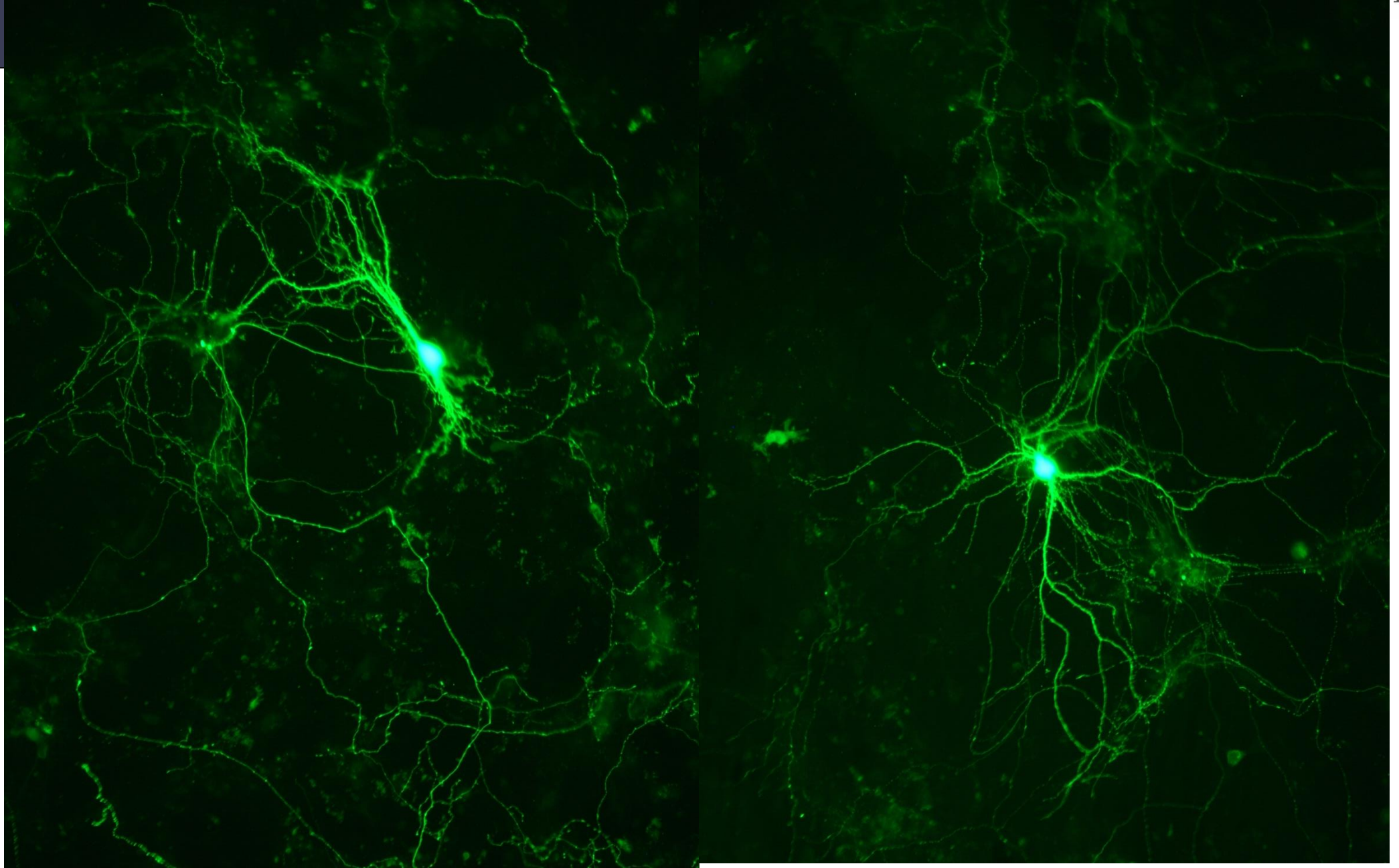




Neurons



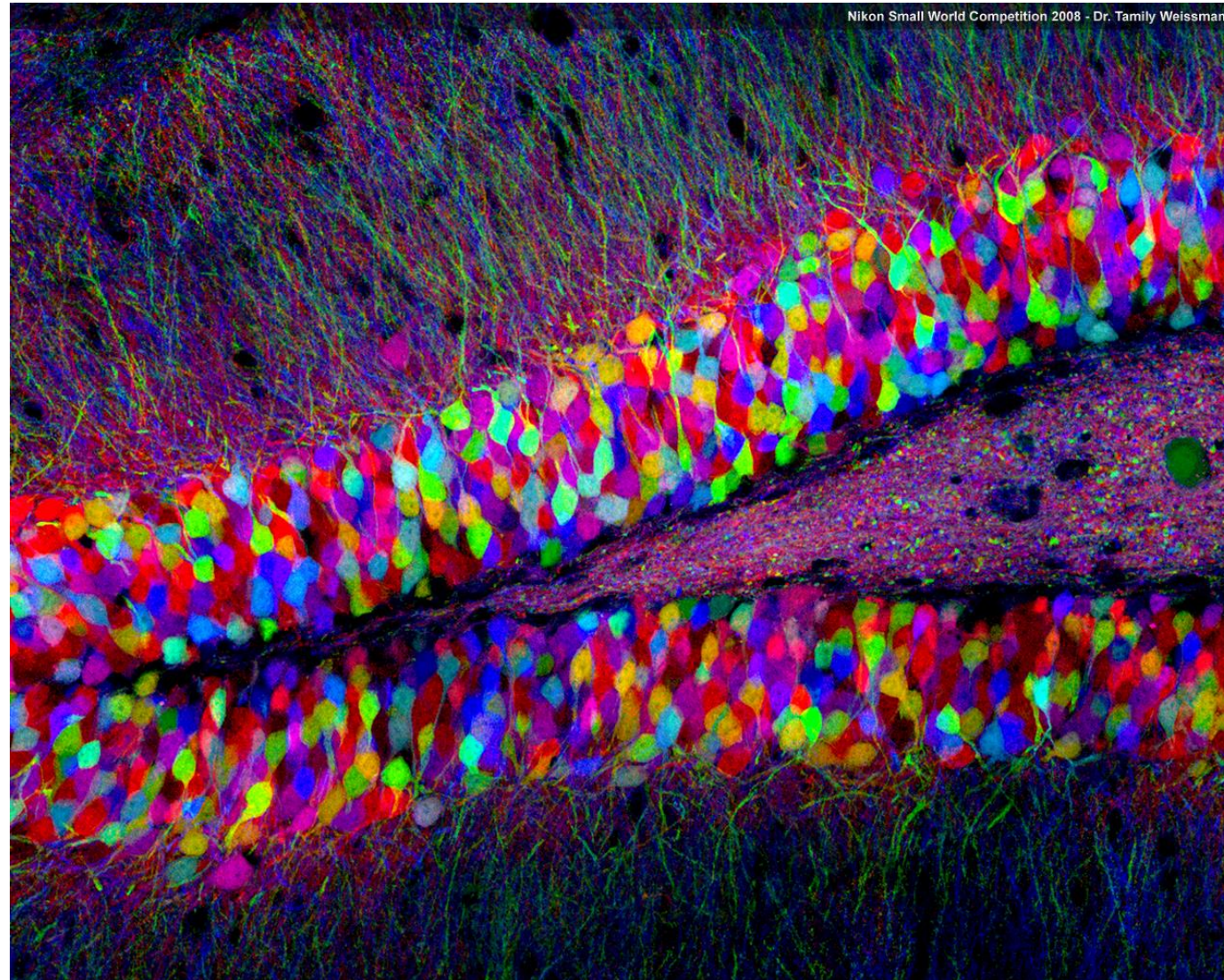
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Brainbow MODEL



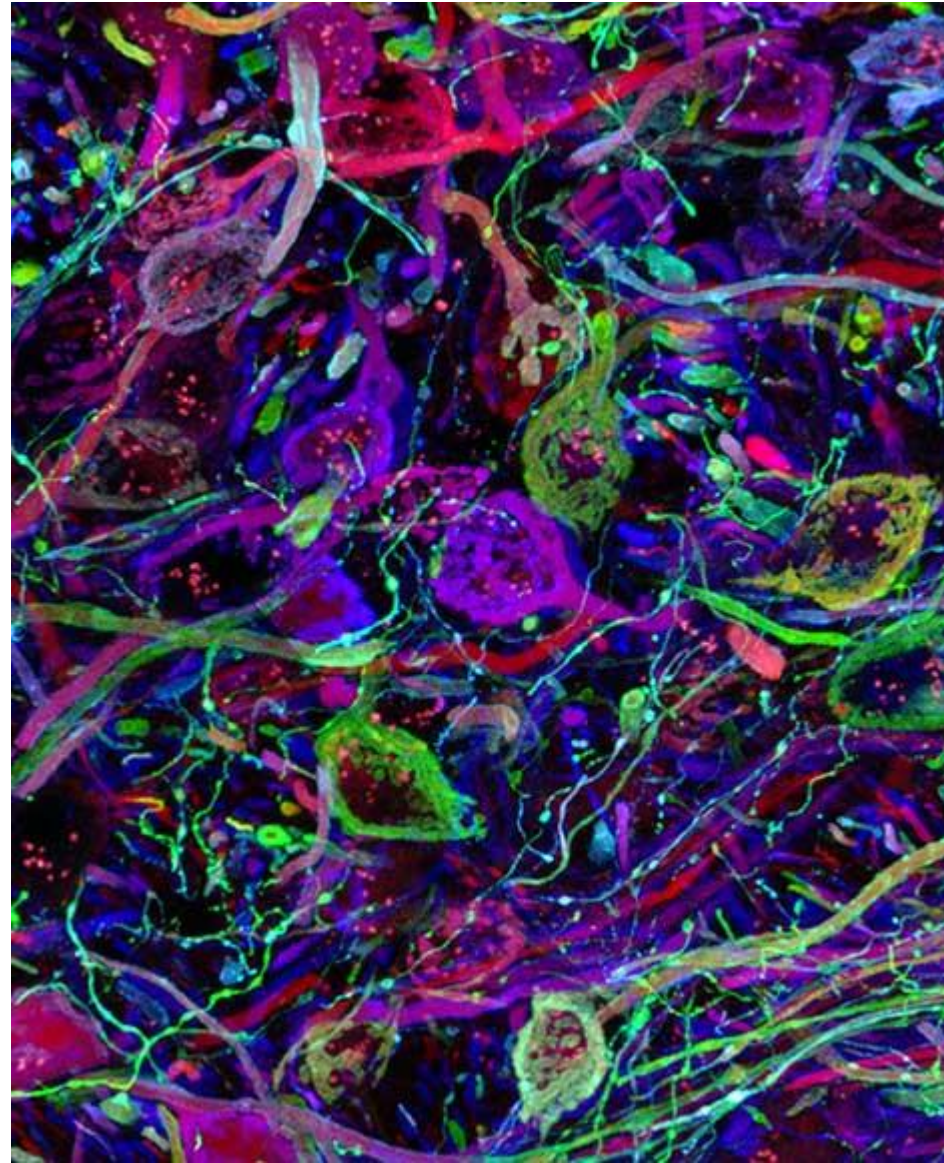
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Brainbow MODEL



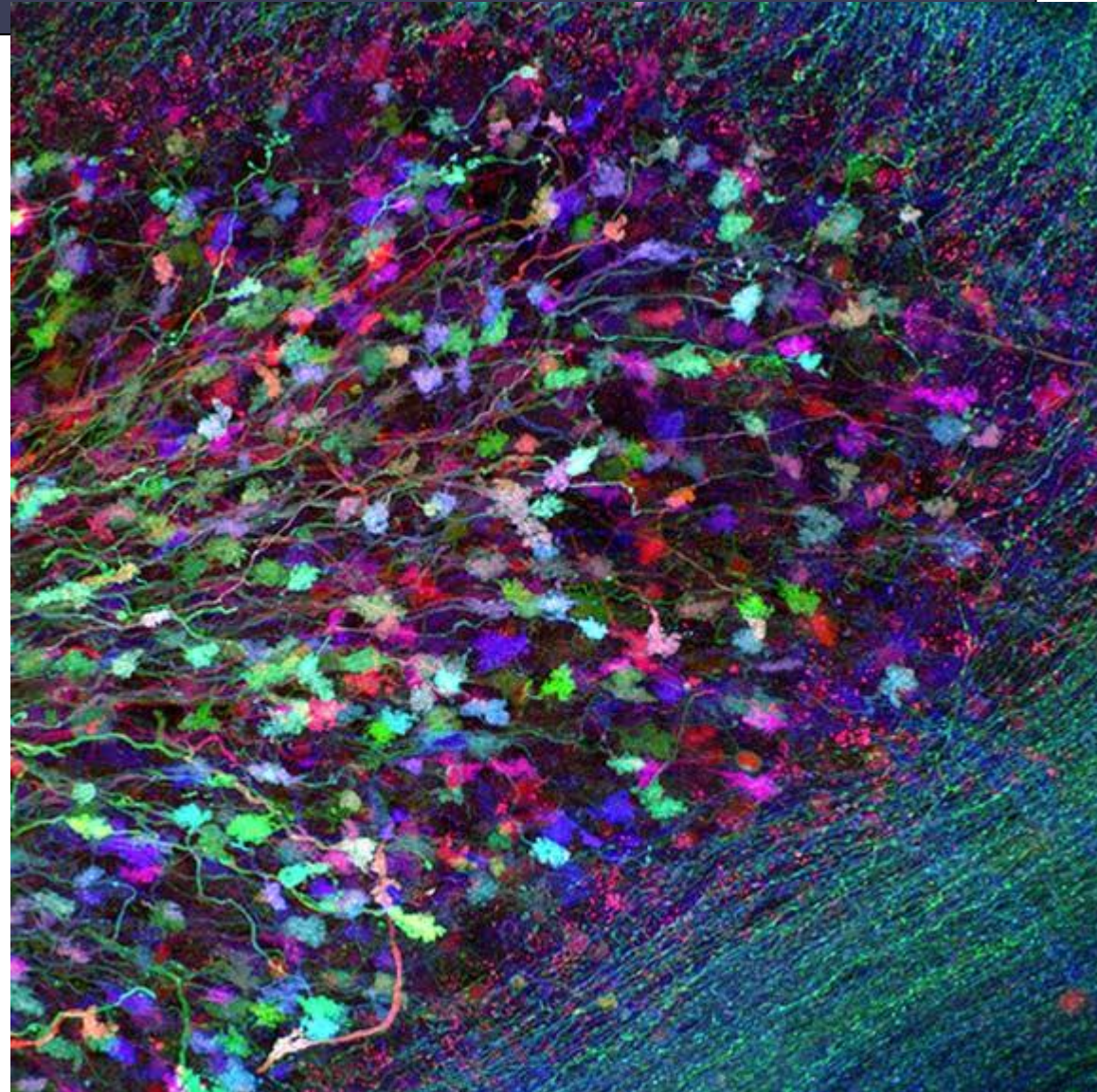
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Brainbow MODEL



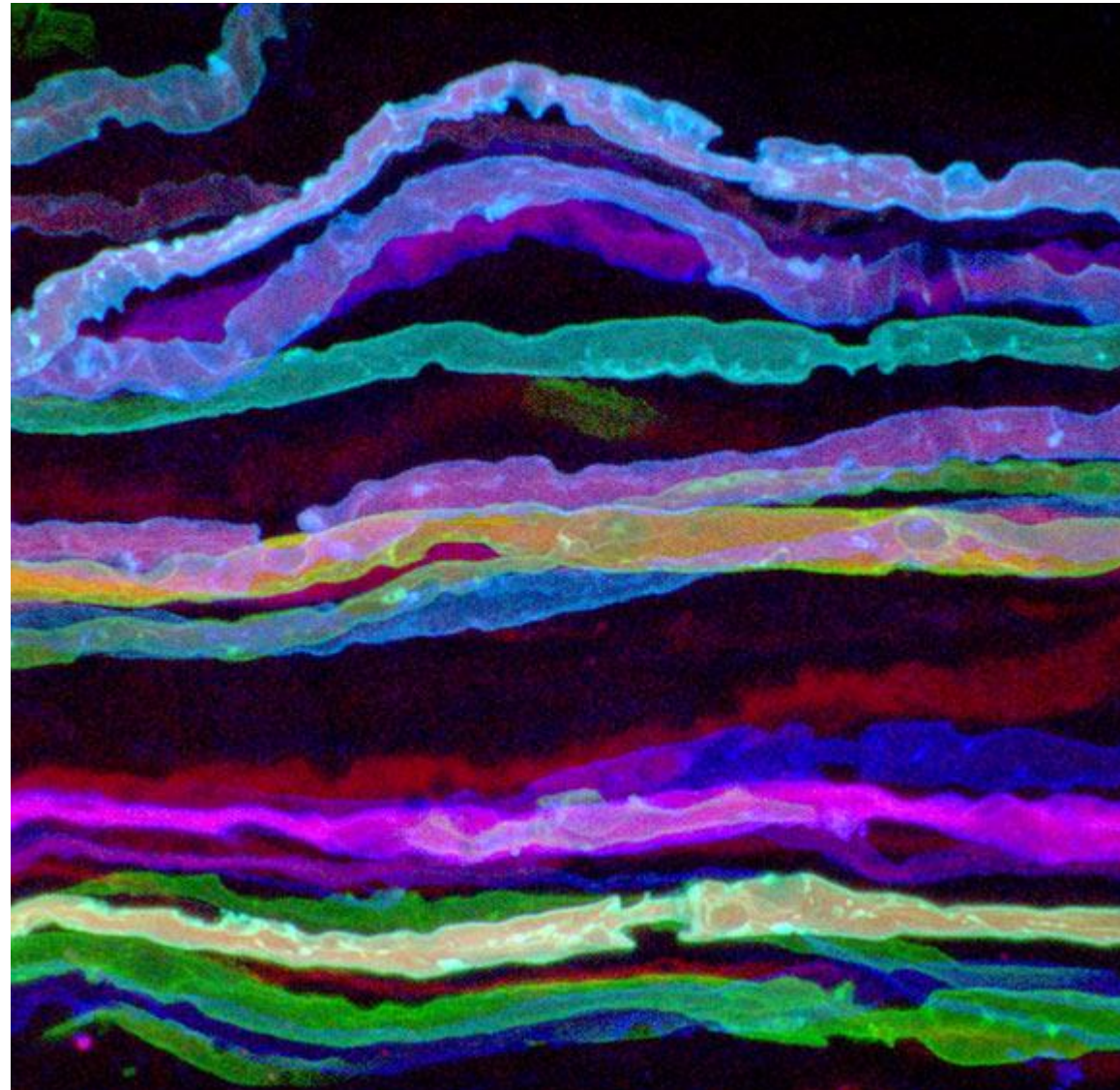
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Brainbow MODEL



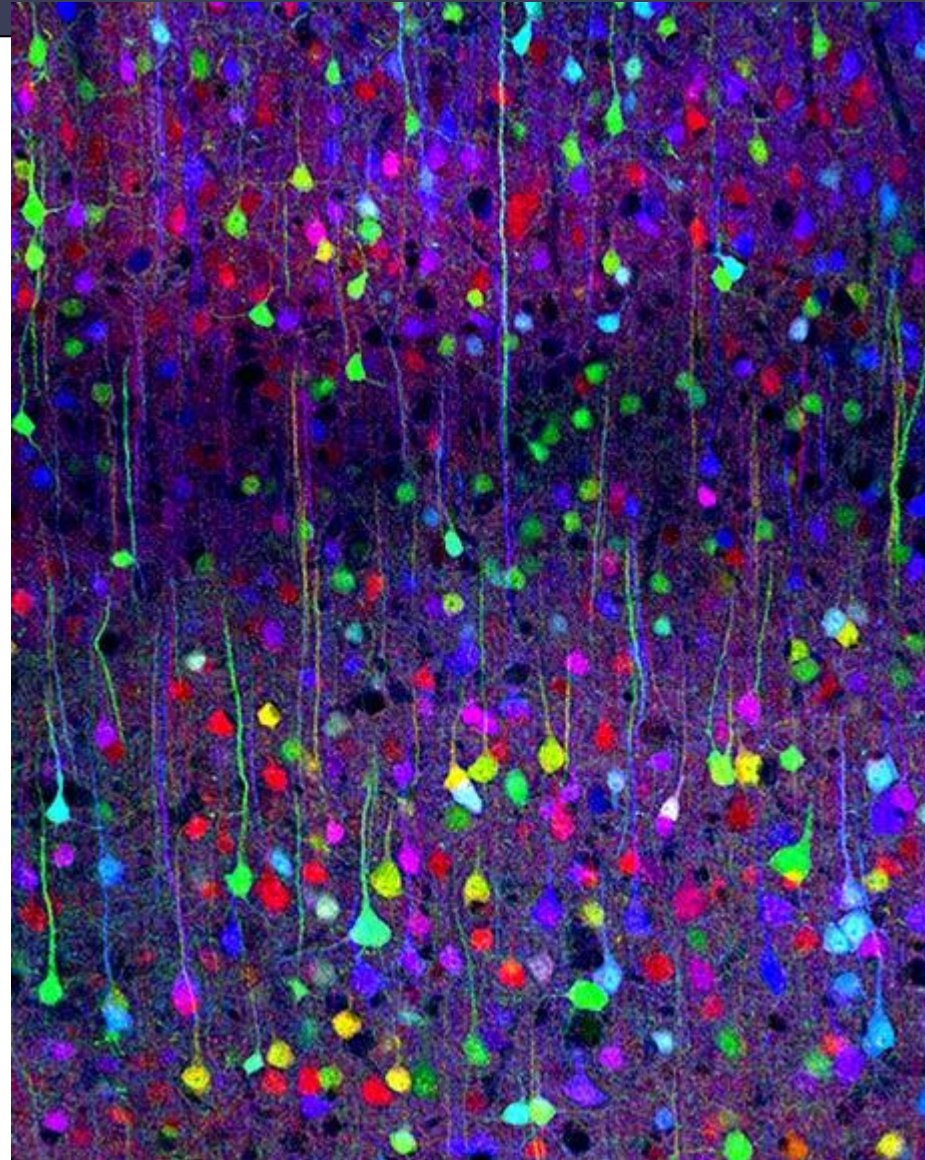
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Brainbow MODEL



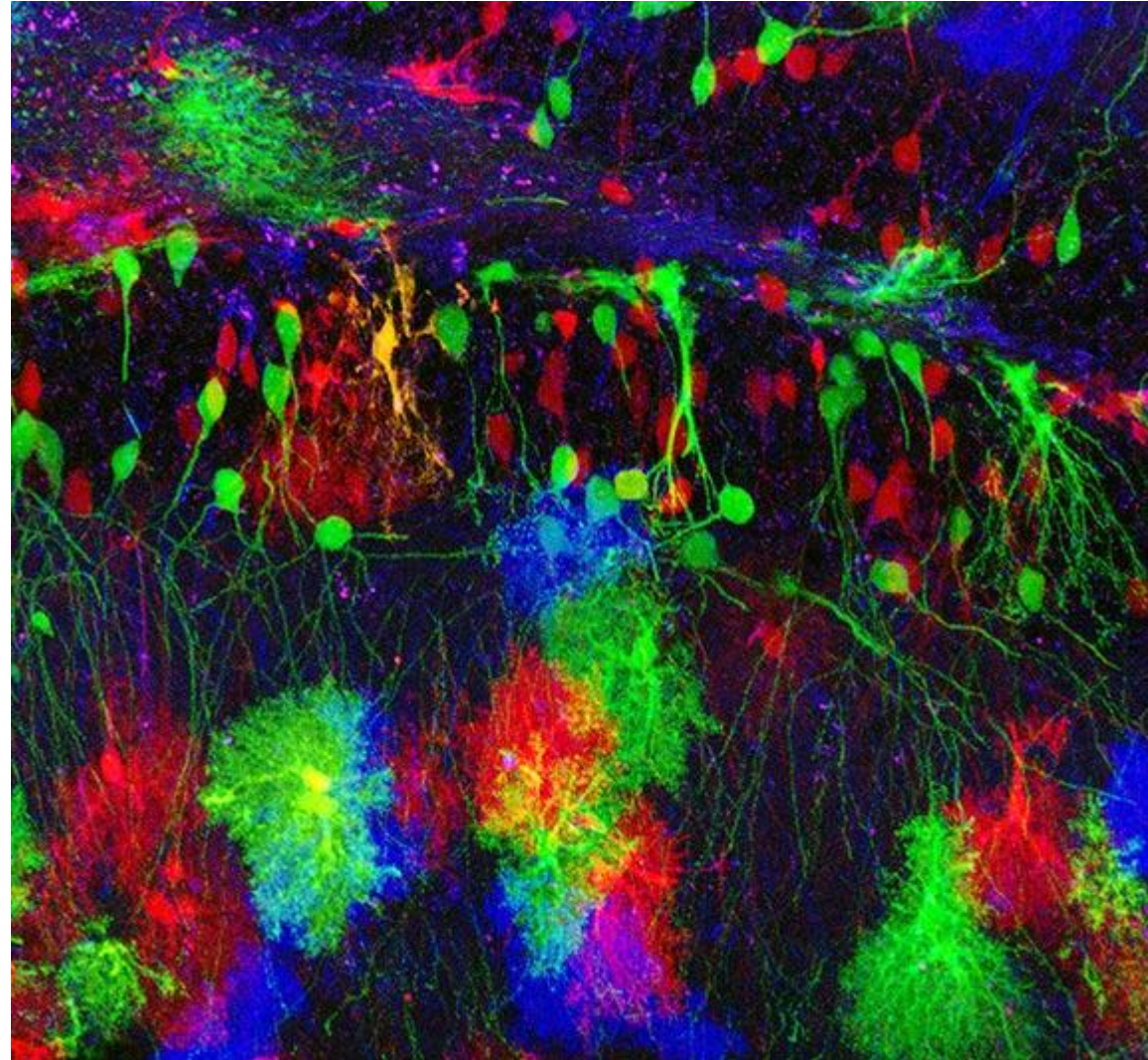
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Brainbow MODEL



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Neuroscience read out



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Neuroscience *in vivo* :

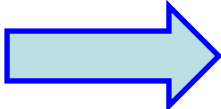
- 1) behavioral
- 2) metabolism
- 3) toxicology
- 4) electrophysiology

etc

NB (In vivo only on KNOWN TARGET)

- Cell lines = NO REAL NEURONS

NO REAL MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION

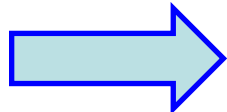
- Primary culture = NO REAL TISSUE

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION
LIMITED TO SINGLE FAMILY OF CELLS

- Organotypic slice= NO REAL BRAIN

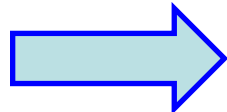
MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION
LIMITED TO SINGLE TISSUE

Consider Technical LIMITATIONS

- Animal = NO HUMAN BRAIN

MORPHOLOGICAL READ OUT
IN NEUROSCIENCE

STRUCTURE  FUNCTION
LIMITED TO SINGLE ANIMAL SPECIES

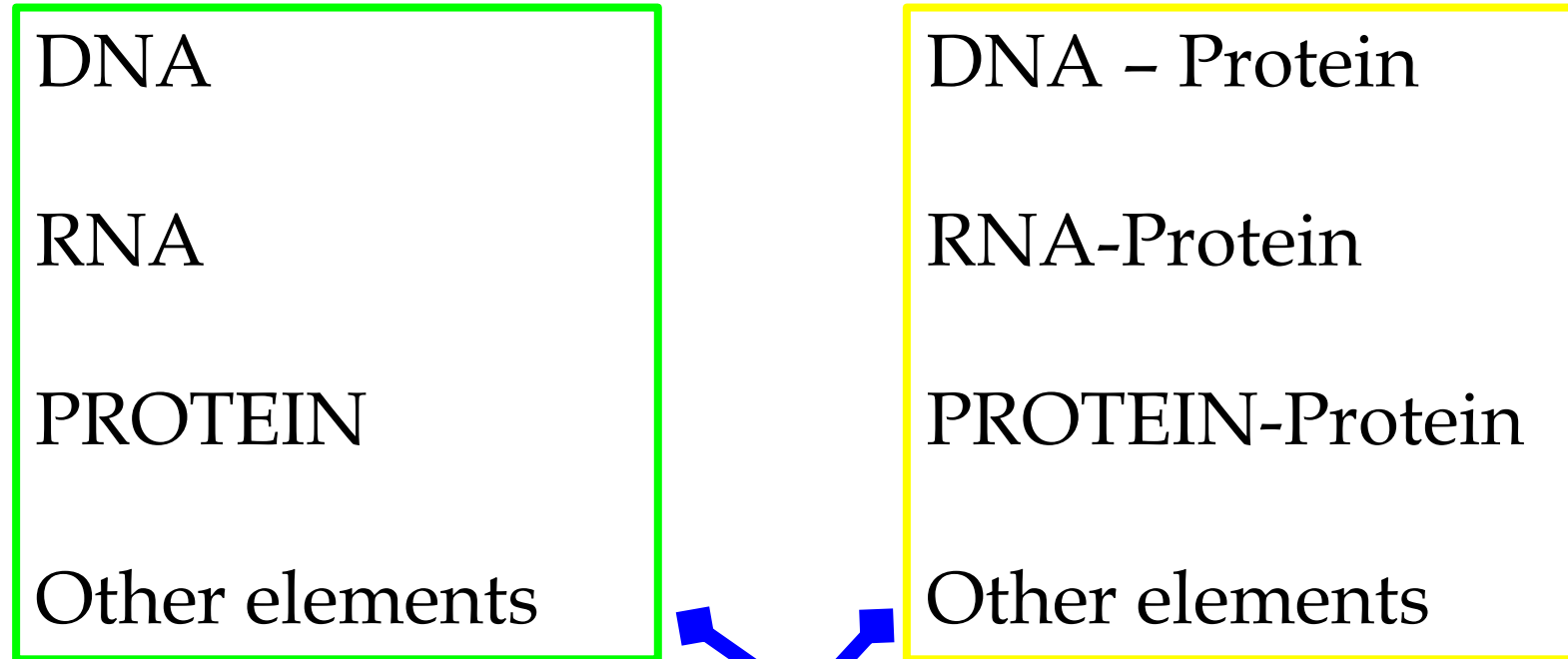
Consider many Technical LIMITATIONS

Neuroscience *in vitro* :



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TARGETS



UNKNOWN TARGET

KNOWN TARGET

Neuroscience *in vitro* : a NOT complete list



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DNA Microarray Methods

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

Genomic Circuits Methods

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

Neuroscience *in vitro* : a NOT complete list



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Proteomics Methods

- Domain Functions Known target
- Yeast Two Hybrid Unknown target
- Cre / lox P recombination Known target
- Biotin and Avidin binding Known target
- Affinity Chromotography Known target Unknown target
- Kinase and enzyme assays Known target
- RNAi (RNA interference) Known target
- Mass Spectroscopy Unknown target

Genomic Methods

- Pathology/Histology Slides Unknown target
- Karyotypes Known target Unknown target
- Immunoprecipitation
- PCR Unknown target
- SDS-PAGE Known target Unknown target
- Coomassie Staining Unknown target
- Western Blot Known target
- Southern Blot Known target
- Northern blot Known target
- Immunofluorescence Known target
- Chromosomal Walking Unknown target
- RFLP
- Knockout Mouse and Homologous Recombination Known target
- Liposomes

Neuroscience *in vitro* : a NOT complete list



- Capillary Electrophoresis Known target Unknown target
- ELISA (see animated version) Known target
- FACS (Fluorescence Activated Cell Sorting) Known target
- Knockout Mouse and Homologous Recombination Known target
- PCR Known target
- Real-time PCR Known target
- RT-PCR (reverse transcriptase-PCR) Known target
- Whole-Genome Sequencing Unknown target

UNKNOWN TARGET

BIOINFORMATICS

<http://www.ebi.ac.uk/> Biochemical read out

MicroARRAYS

mRNA

miRNA

ncRNA

SNP

Protein

Biochemical read out

ALL TARGETS

2 Gel-Electrophoresys

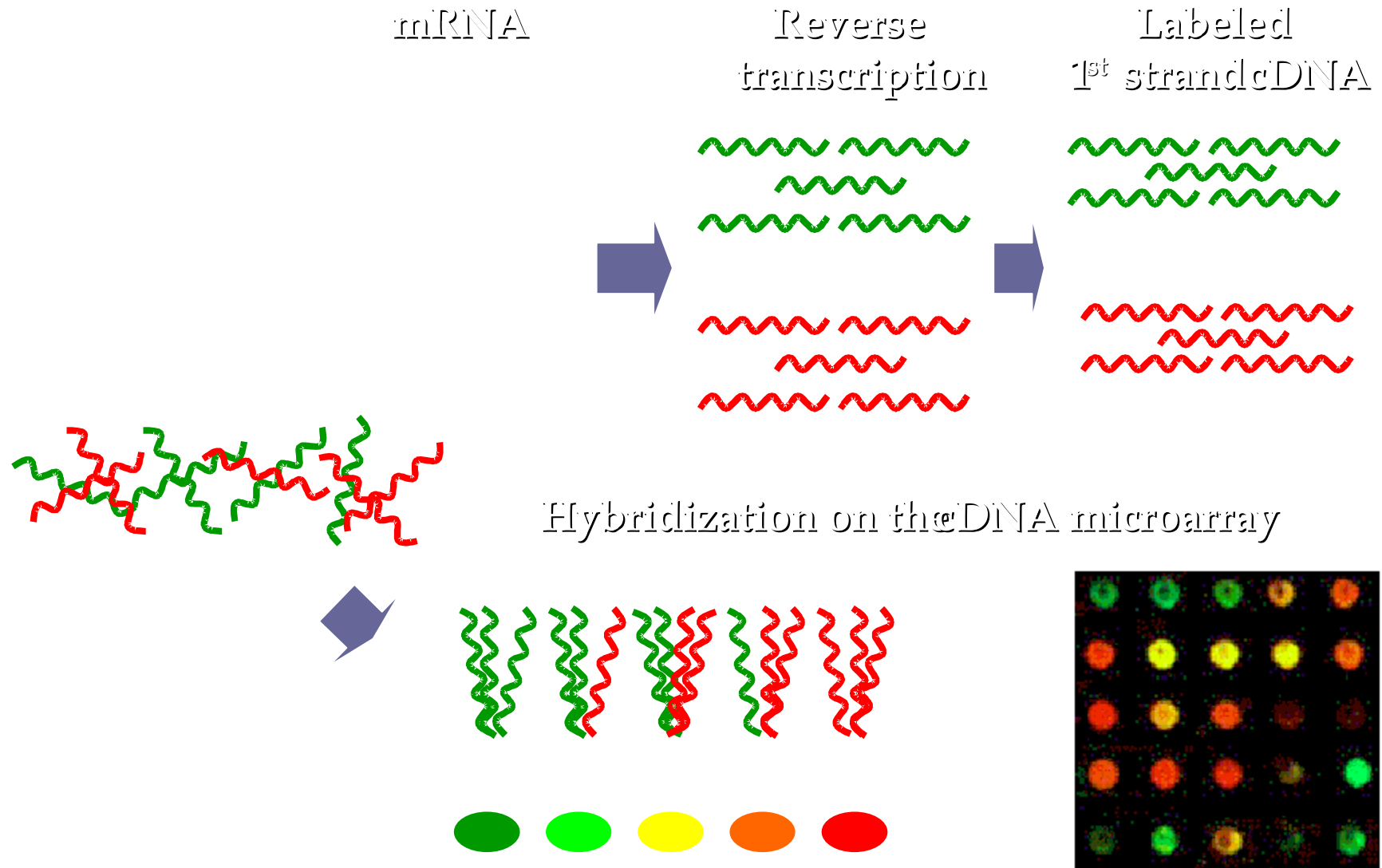
2 Hybrid Screen

Biochemical read out

High-throughput screening
screening

Biochemical read out

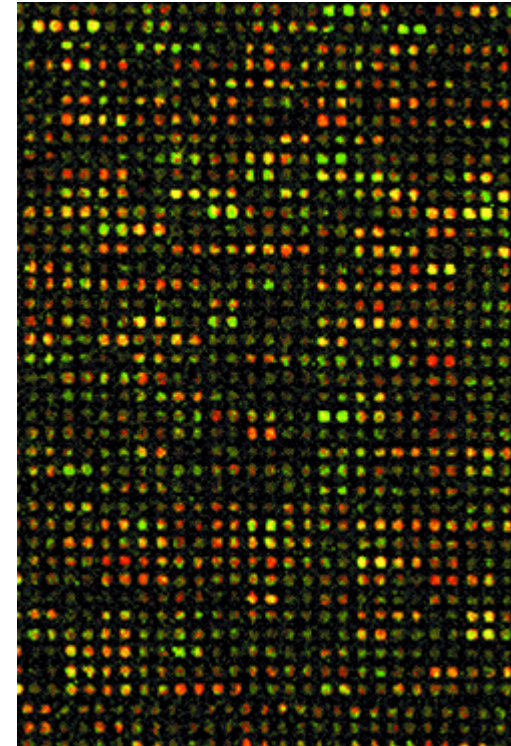
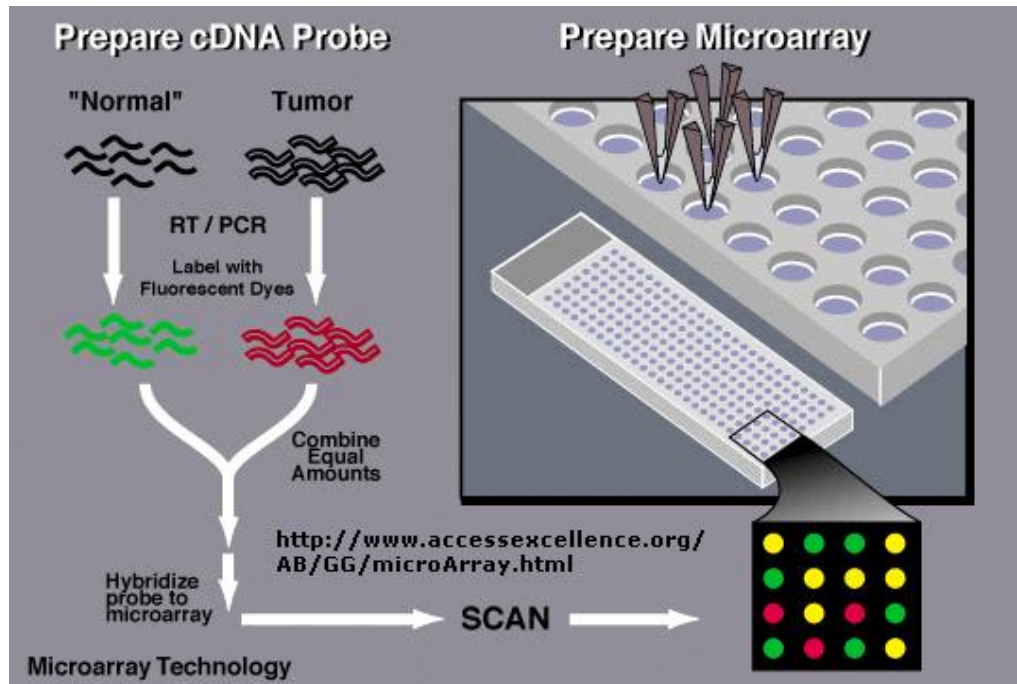
etc



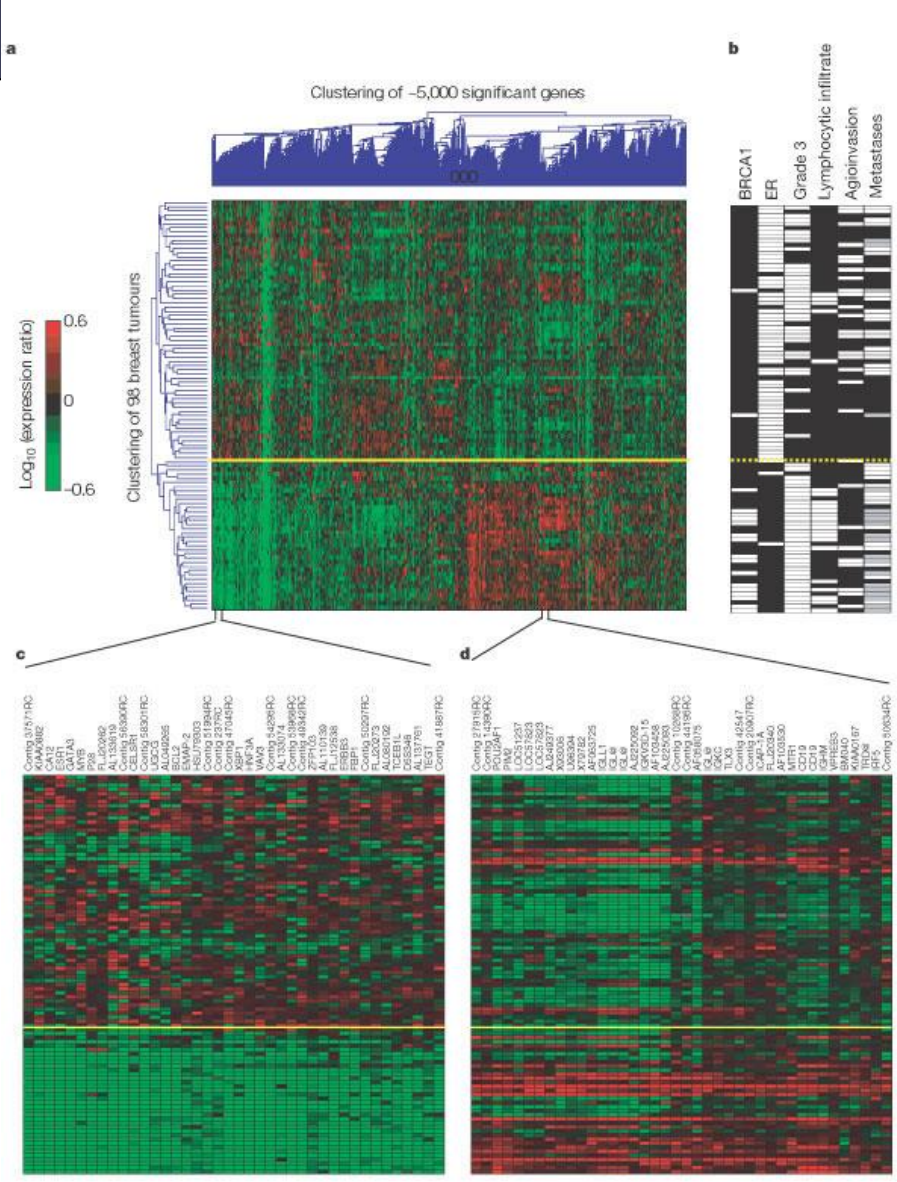
cDNA Microarray



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cDNA Microarray read out



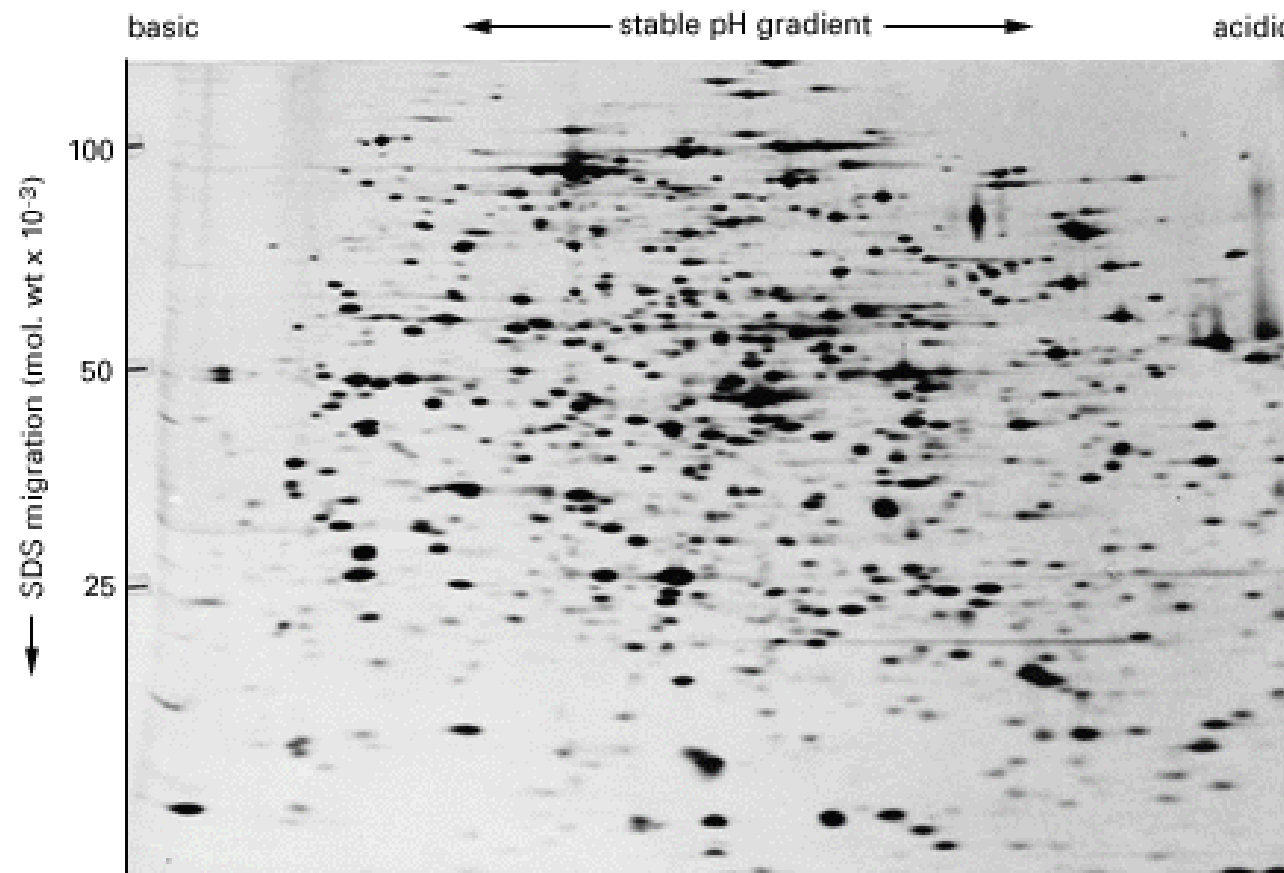
Available microarrays
also
for RNAs and Proteins

2D-Gel electrophoresis



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Protein



KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

DNA

PCR

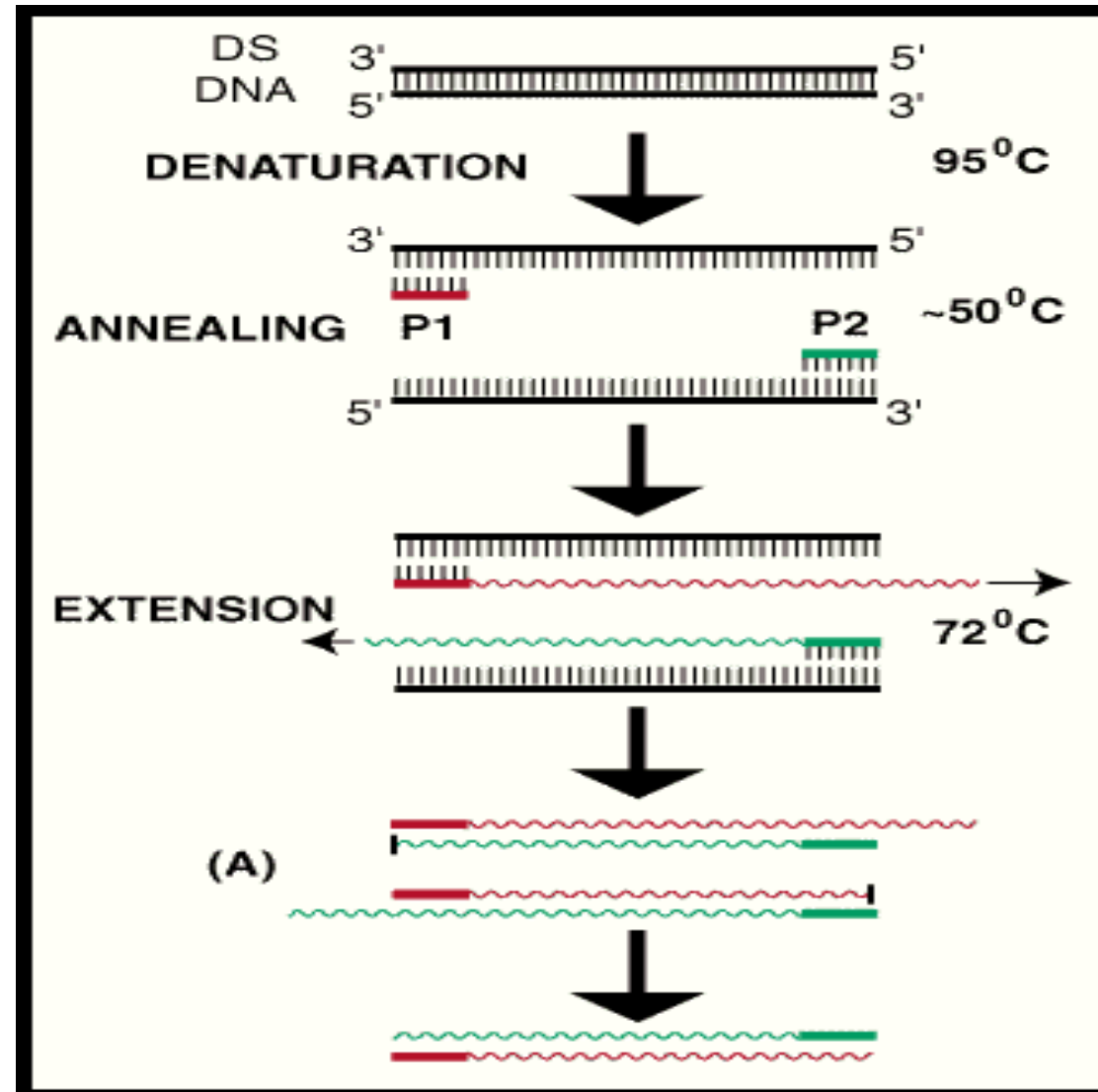
Southern Blot
Sequencing

Biochemical read out

Biochemical read out

Biochemical read out

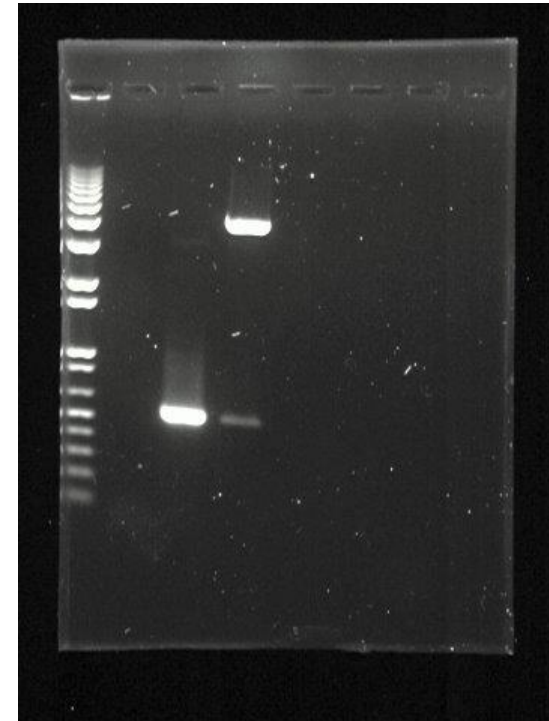
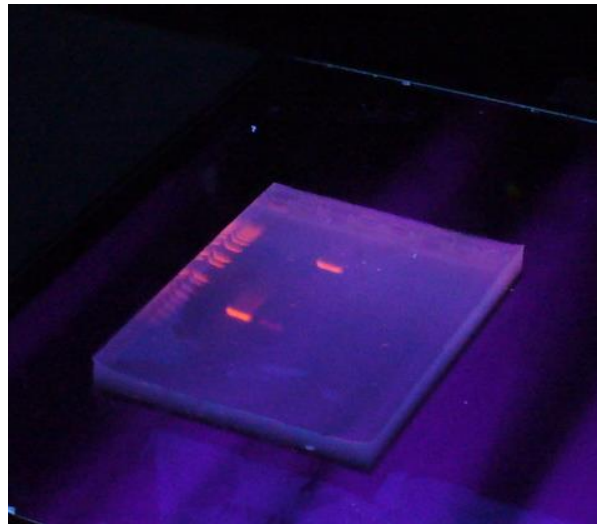
PCR



PCR



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Western Blot

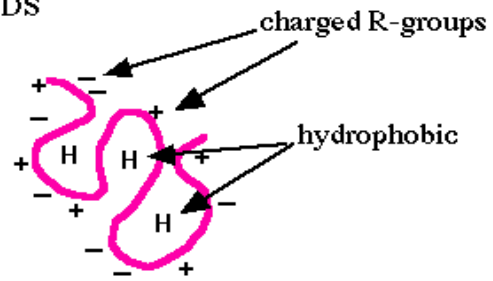


- Western blots allow investigators to determine the molecular weight of a protein and to measure relative amounts of the protein present in different samples.
- Proteins are separated by gel electrophoresis, usually **SDS-PAGE**.
- The proteins are transferred to a sheet of special blotting paper called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

Western Blot



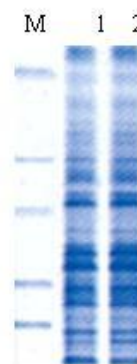
BEFORE SDS



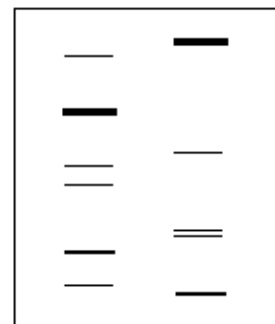
AFTER SDS



<SDS-PAGE>



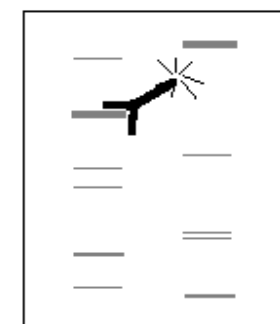
SDS Polyacrylamide
Gel Electrophoresis



Protein Blot on
Nitrocellulose



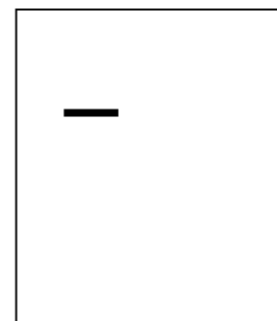
Label with Specific
Antibody



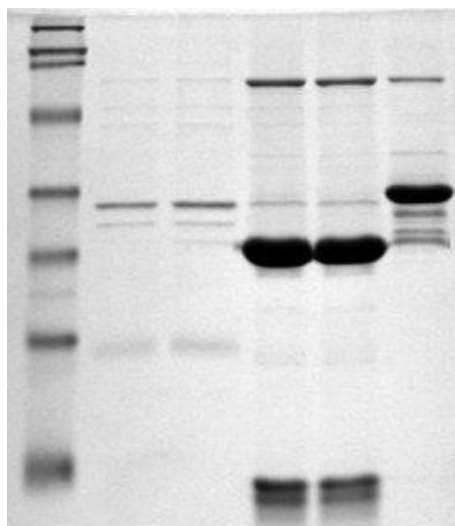
<Western Blot>



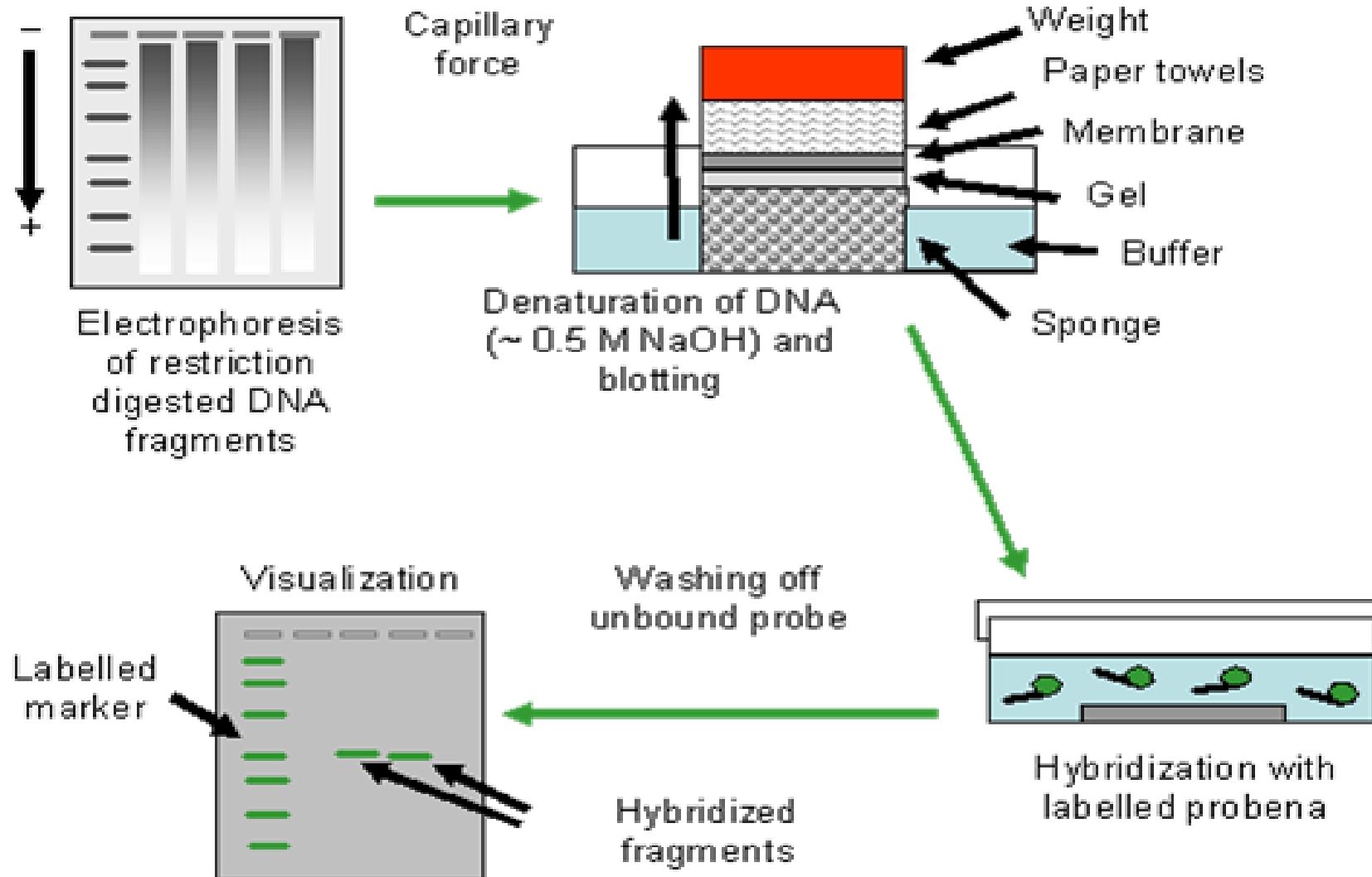
Detect Antibody



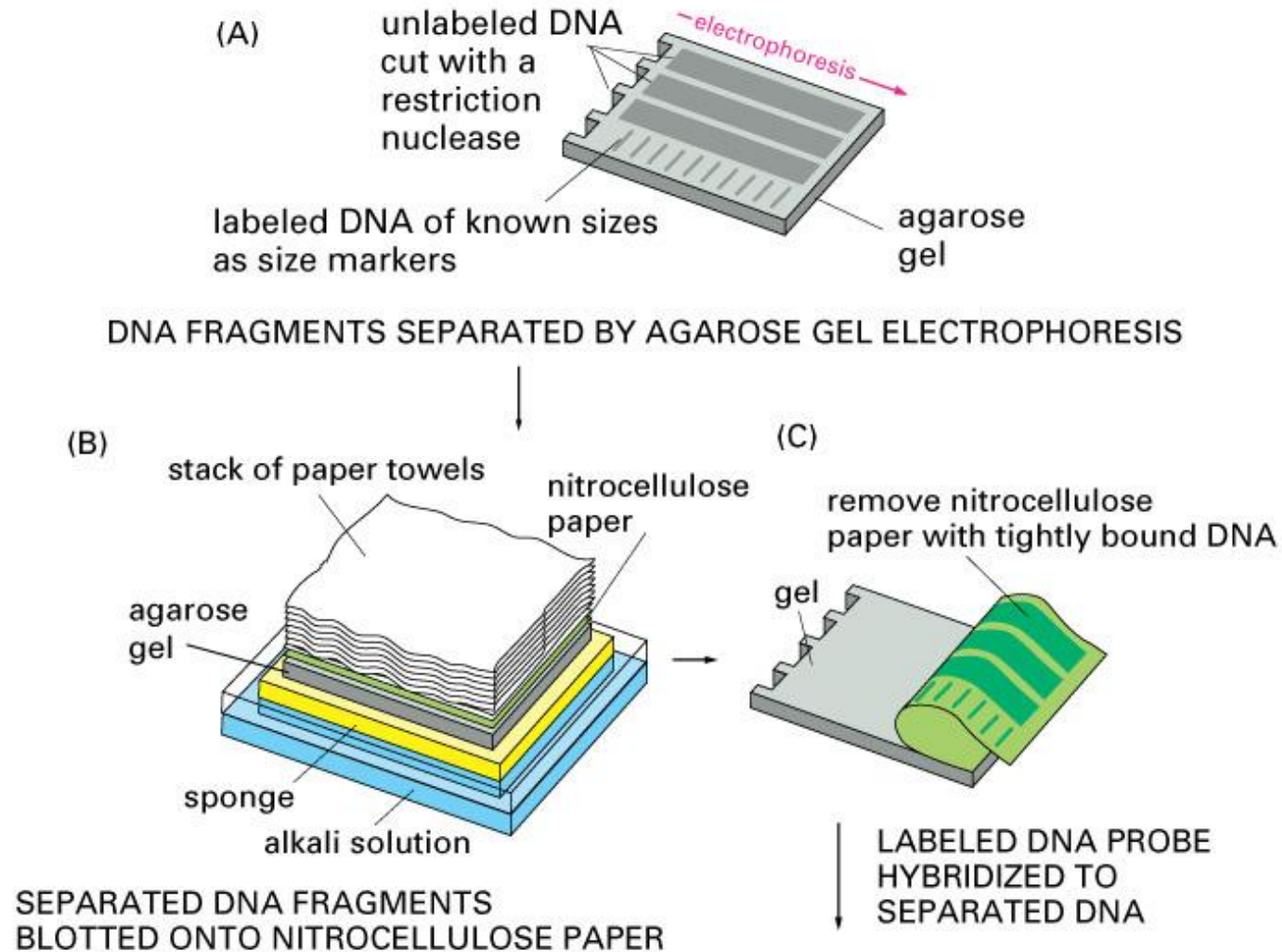
Reveals Protein
of Interest



Southern blot



Southern blot



KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

RNA

PCR

Northern Blot
Sequencing

In situ

Biochemical read out

Biochemical read out

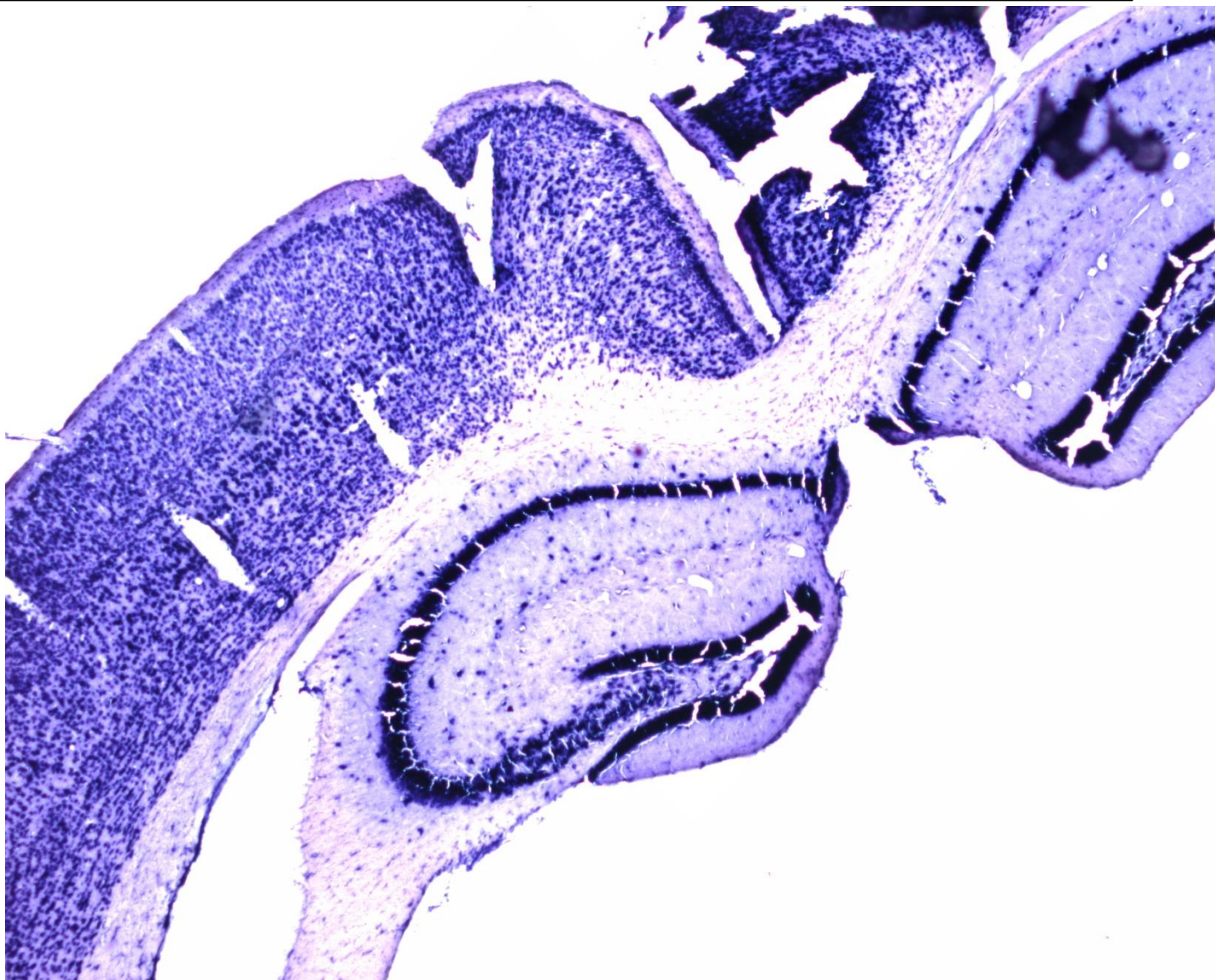
Biochemical read out

subcellular read out

In situ



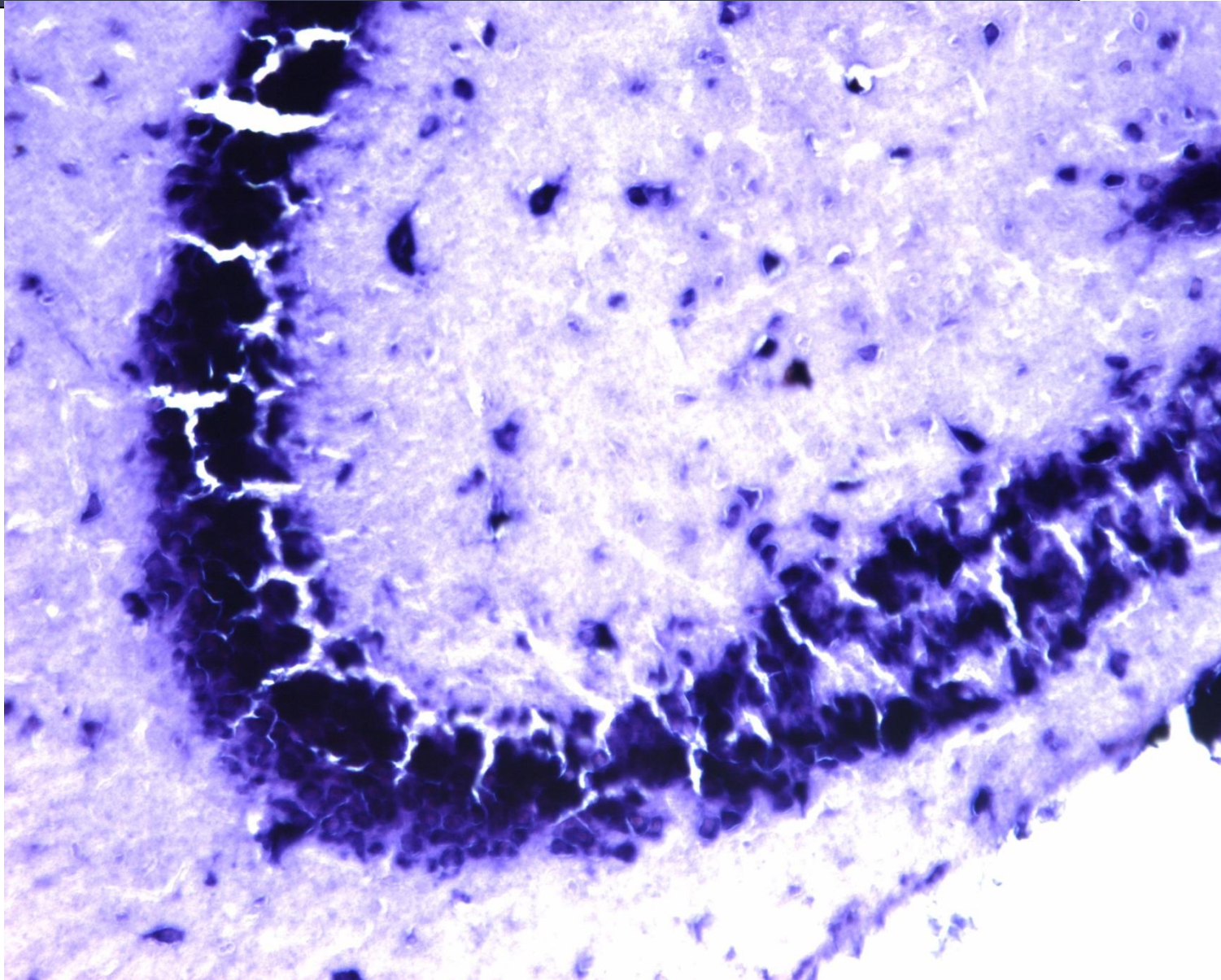
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In situ



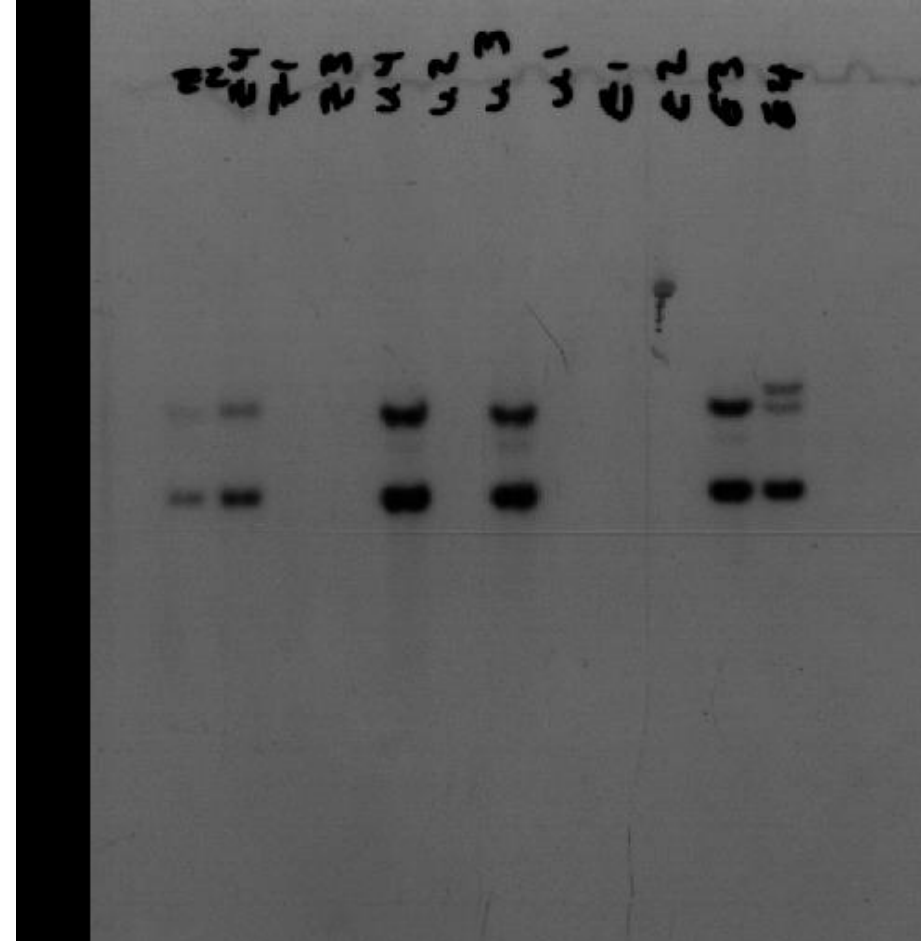
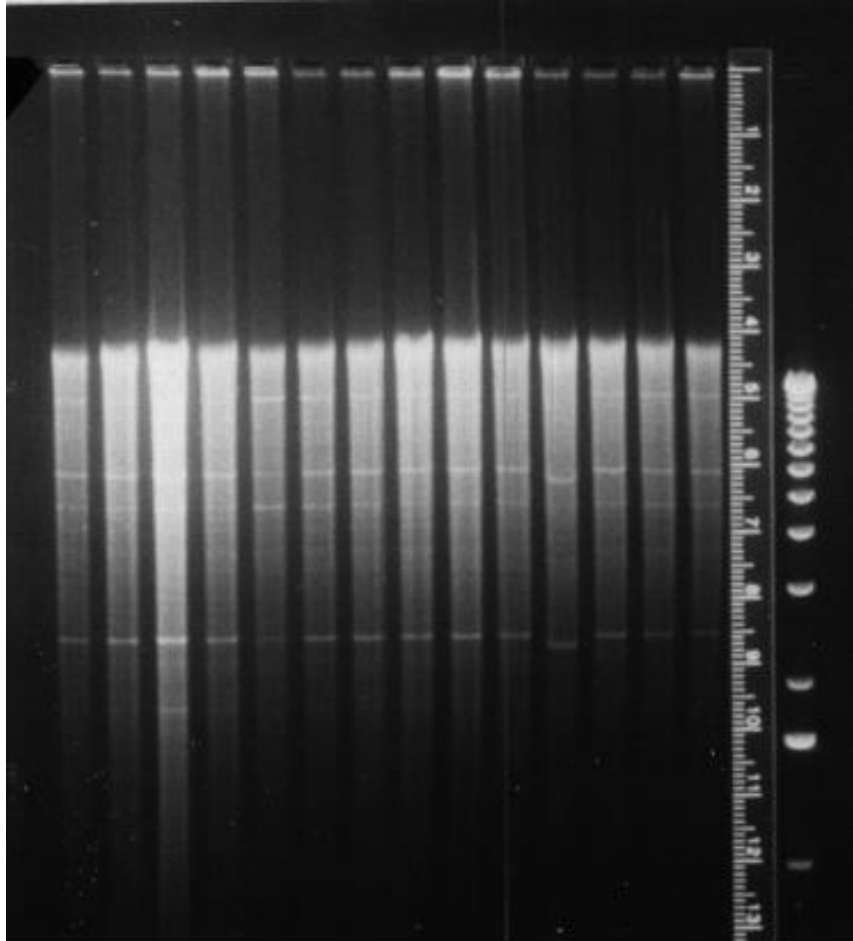
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Southern/Northern blot



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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)

KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

Protein

Western Blot

Biochemical read out

Elisa

Biochemical read out

Sequencing

Biochemical read out

Immuno Istochemistry

subcellular read out

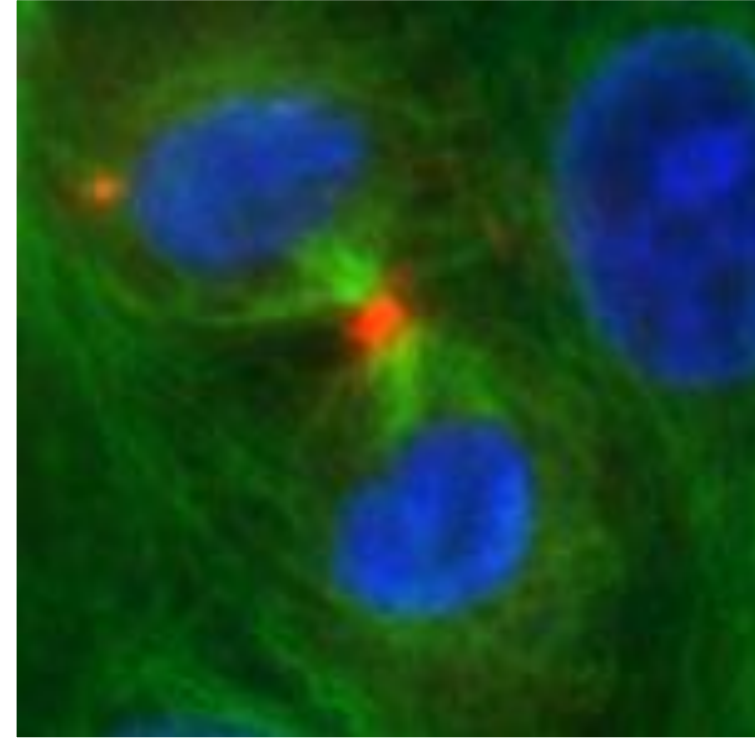
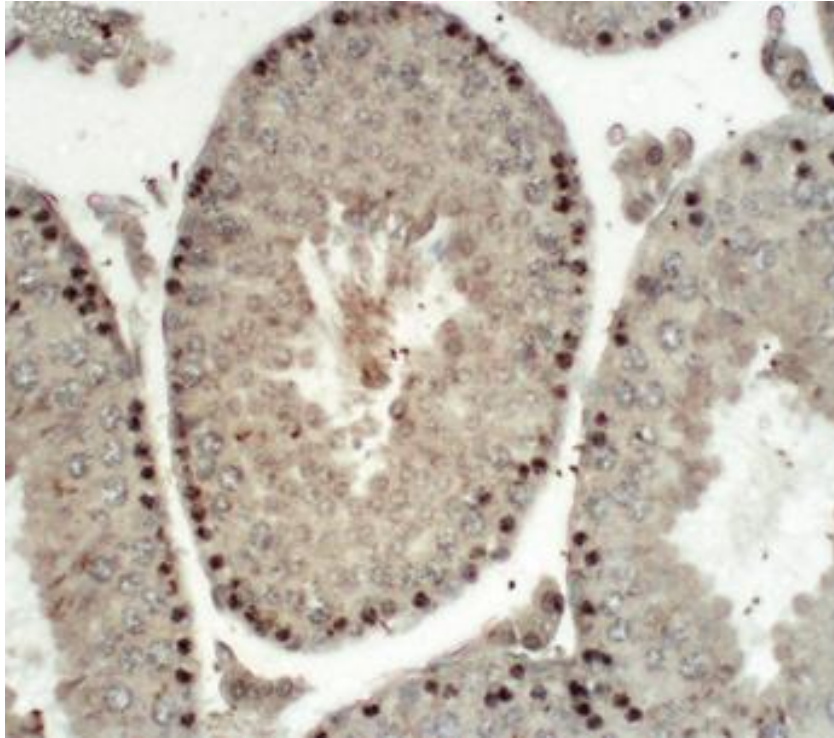
Immuno Citochemistry

subcellular read out

Immunohistochemistry Immunocytochemistry



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KNOWN TARGET

BIOINFORMATICS

ALL TARGETS

BASAL CONDITION
CONDITIONS"

"MODIFIED

DNA-Protein

Chromatin immunoP

Biochemical read out

RNA-Protein

Co immunoP + RT PCR

EMSA

SuperShift

Biochemical read out

Gel Shift

Protein-Protein

FRET

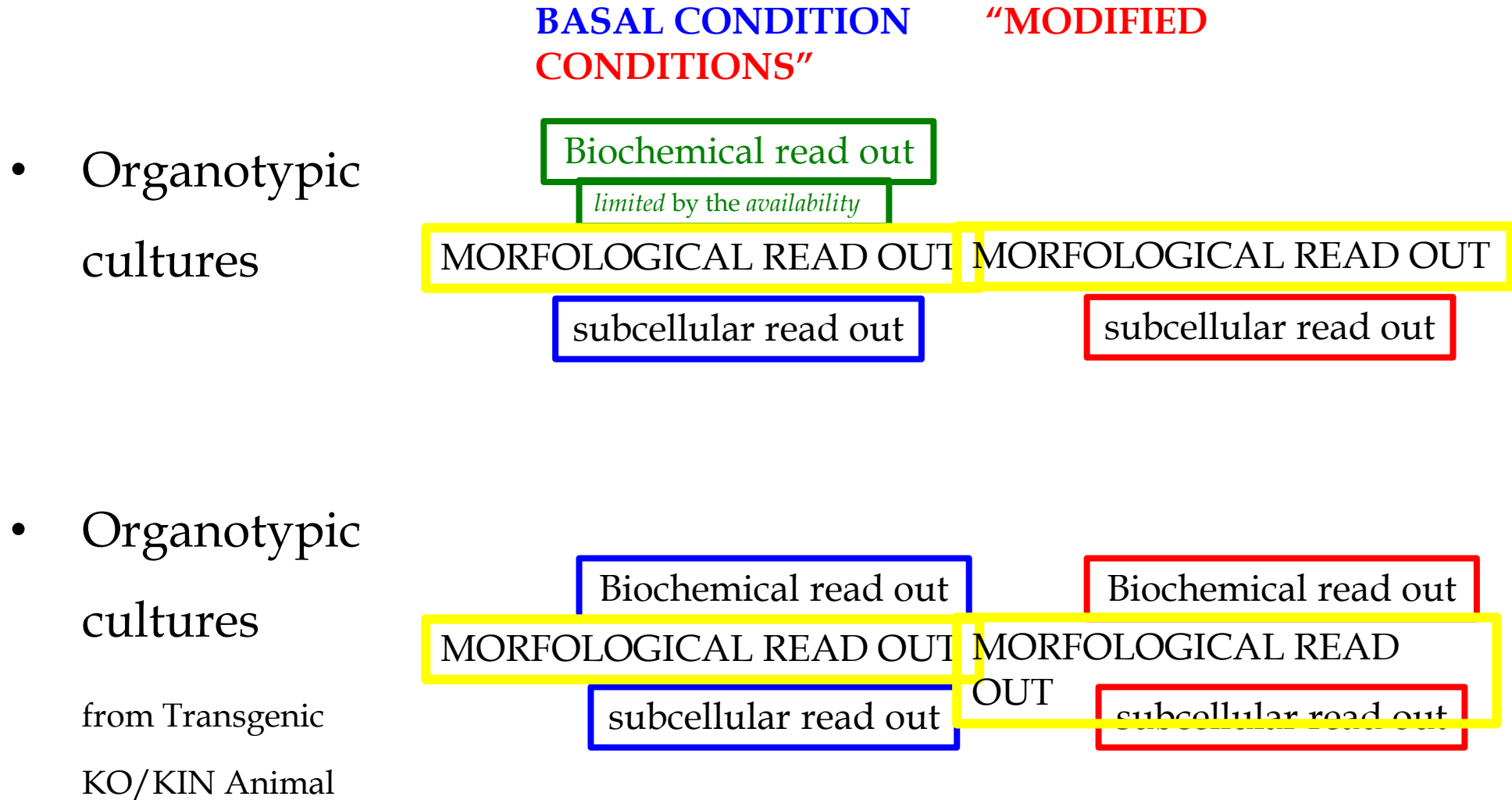
subcellular read out

Neuroscience *in vitro* : a NOT complete list



	BASAL CONDITION CONDITIONS"	"MODIFIED
• Cell Lines	Biochemical read out subcellular read out	Biochemical read out subcellular read out
• Primary culture	Biochemical read out <i>limited by the availability</i> MORPHOLOGICAL READ OUT subcellular read out	MORPHOLOGICAL READ OUT subcellular read out
• Primary culture from Transgenic/ KO/KIN Animal	Biochemical read out MORPHOLOGICAL READ OUT subcellular read out	Biochemical read out MORPHOLOGICAL READ OUT subcellular read out

Neuroscience *in vitro* : The choice of a Model



A fluorescence micrograph showing a dense network of neurons. The neurons are stained with a green fluorescent dye, highlighting their cell bodies and intricate branching processes. Two neurons are particularly prominent, with bright green spots at their cell bodies. The background is dark, making the green structures stand out.

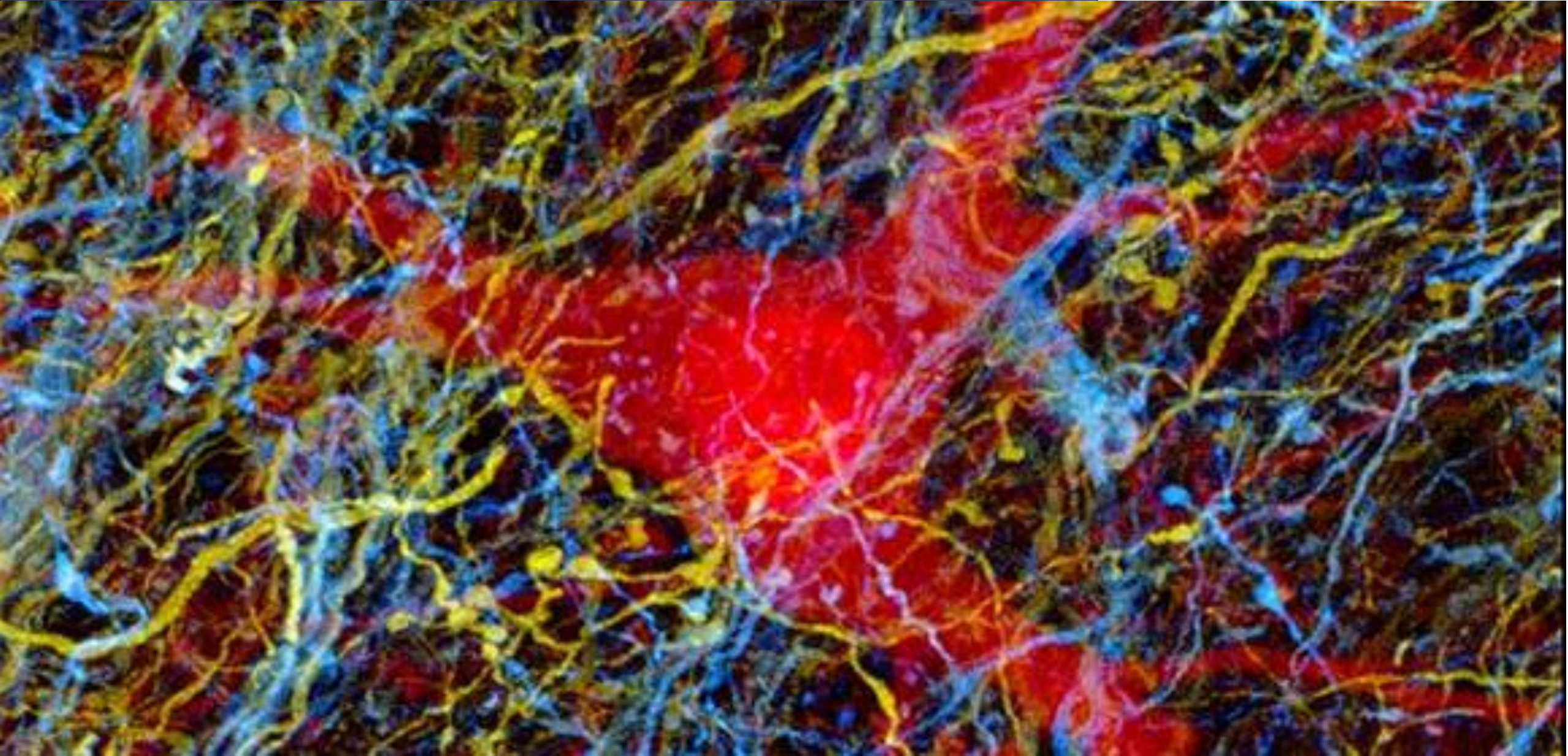
was like study NEURONS

...complicated but
sometimes also funny

Thank you again



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