“Techniques in Cellular and Molecular Neurobiology”

International Master’s Degree in Neuroscience

Lesson 2

DIPARTIMENTO DI SCIENZE DELLA VITA

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Technics in Cellular and Molecular Neurobiology

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provisional

TOPICS – in progress

1) Presentation and Scientific Method
2) The biological problem
3) Scientific Model in Neurobiology
    - Descriptive Neurobiology and/or mechanisms research
4) Experimental manipulations
    - Genetic
    - Pharmacological

Biological problems in Neuroscience

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Trends Cogn Sci. 2015 April ; 19(4): 173–175. doi:10.1016/j.tics.2015.01.007.

The unsolved problems of neuroscience

Ralph Adolphs
California Institute of Technology, Pasadena, CA, USA

Note that some future questions build on prior ones: we need to understand psychiatric illnesses before we can cure them, and whole-brain microscopic-resolution imaging of the zebrafish brain (100 000 neurons; done, although temporal resolution will improve [4]) needs to come before we do the same for the mouse brain (70 000 000 neurons), let alone the human brain (80 000 000 000 neurons).

The unresolved problems of neuroscience

The unsolved problems of neuroscience

Problems that are solved, or soon will be:

I How do single neurons compute?

II What is the connectome of a small nervous system, like that of *Caenorhabitis elegans* (300 neurons)?

III How can we image a live brain of 100 000 neurons at cellular and millisecond resolution?

IV How does sensory transduction work?

The unresolved problems of neuroscience examples… personal perspective

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Problems that we should be able to solve in the next 50 years:

V	How do circuits of neurons compute?
VI	What is the complete connectome of the mouse brain (70 000 000 neurons)?
VII	How can we image a live mouse brain at cellular and millisecond resolution?
VIII	What causes psychiatric and neurological illness?
IX	How do learning and memory work?
X	Why do we sleep and dream?
XI	How do we make decisions?
XII	How does the brain represent abstract ideas?

The unresolved problems of neuroscience examples… personal perspective

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Problems that we should be able to solve, but who knows when:

XIII How does the mouse brain compute?

XIV What is the complete connectome of the human brain (80 000 000 000 neurons)?

XV How can we image a live human brain at cellular and millisecond resolution?

XVI How could we cure psychiatric and neurological diseases?

XVII How could we make everybody's brain function best?

The unresolved problems of neuroscience examples… personal perspective

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Problems we may never solve:

XVIII How does the human brain compute?

XIX How can cognition be so flexible and generative?

XX How and why does conscious experience arise?

Meta-questions:

XXI What counts as an explanation of how the brain works? (and which disciplines would be needed to provide it?)

What are Scientific Models?

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- Models are a representation or description designed to make a particular part/feature of the world easier to understand, define, quantify, visualize, or simulate by using multiple forms of data.
  - Models help us understand.
  - Explain or analyze concepts.
  - Represent things too small or too large.
  - Explain past or the present; predict the future.

What are Scientific Models?

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Physical Models

- Models you can see and touch. (3D or 2D)
- Used to represent very large (organism) or small (cell) objects.
- Limitation: Looks similar, but does not function in the same way as the original.

What are Scientific Models?

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Mathematical Models

- Models are made up of mathematical equations and data. They help us process large amounts of data.
  - Simple models – formula for how fast phenome occours
  - Complex models (computer) – population growth
- Limitation: Only as accurate as the data inputted.

What are Scientific Models?

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Conceptual (Diagram) Models

- Used to understand large and complex processes and how they work.
  - Biochemical Pathways
  - Organs – tissue description
  - Nervous system representation
- Limitations: Gives limited information, shown in 2D.

Scientific MODEL

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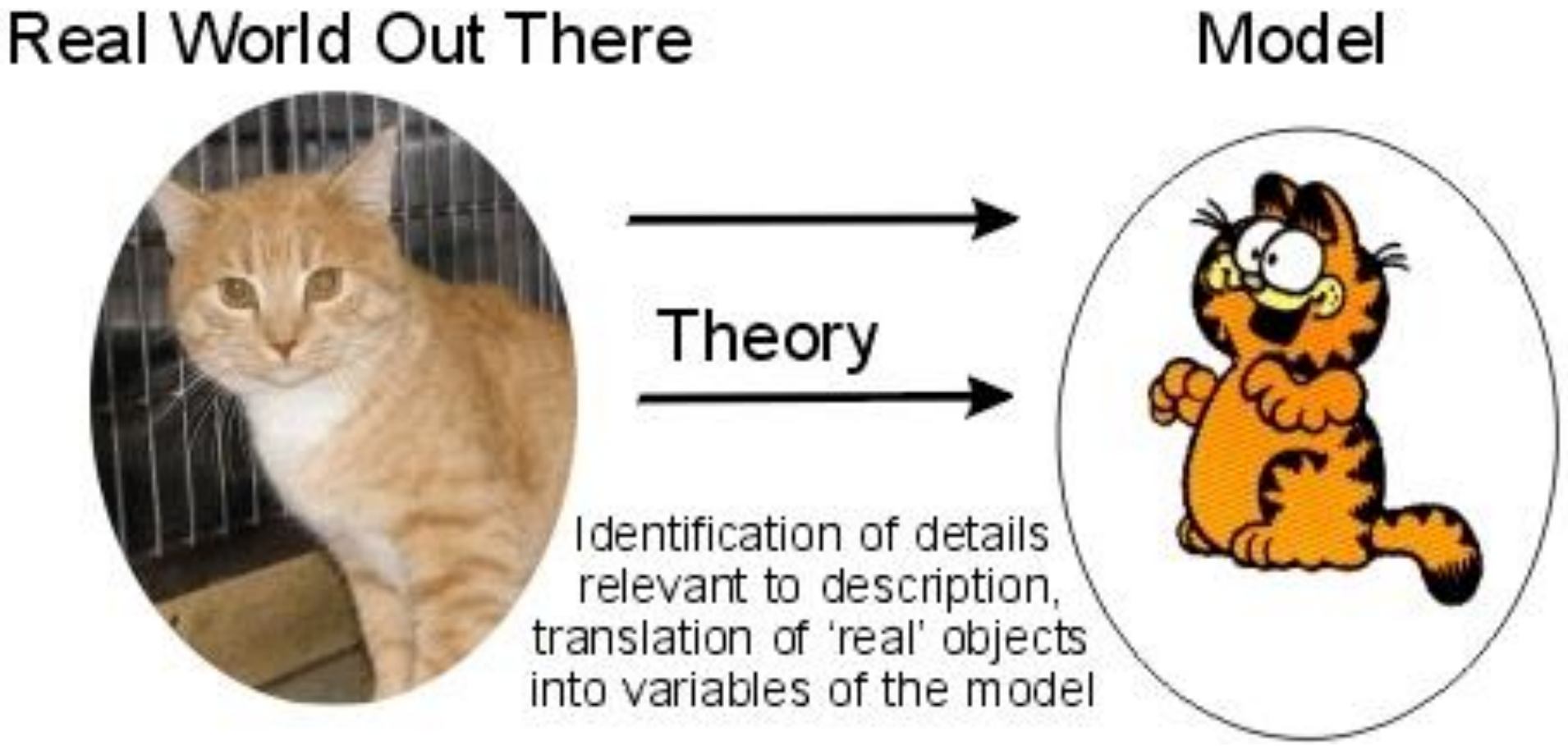
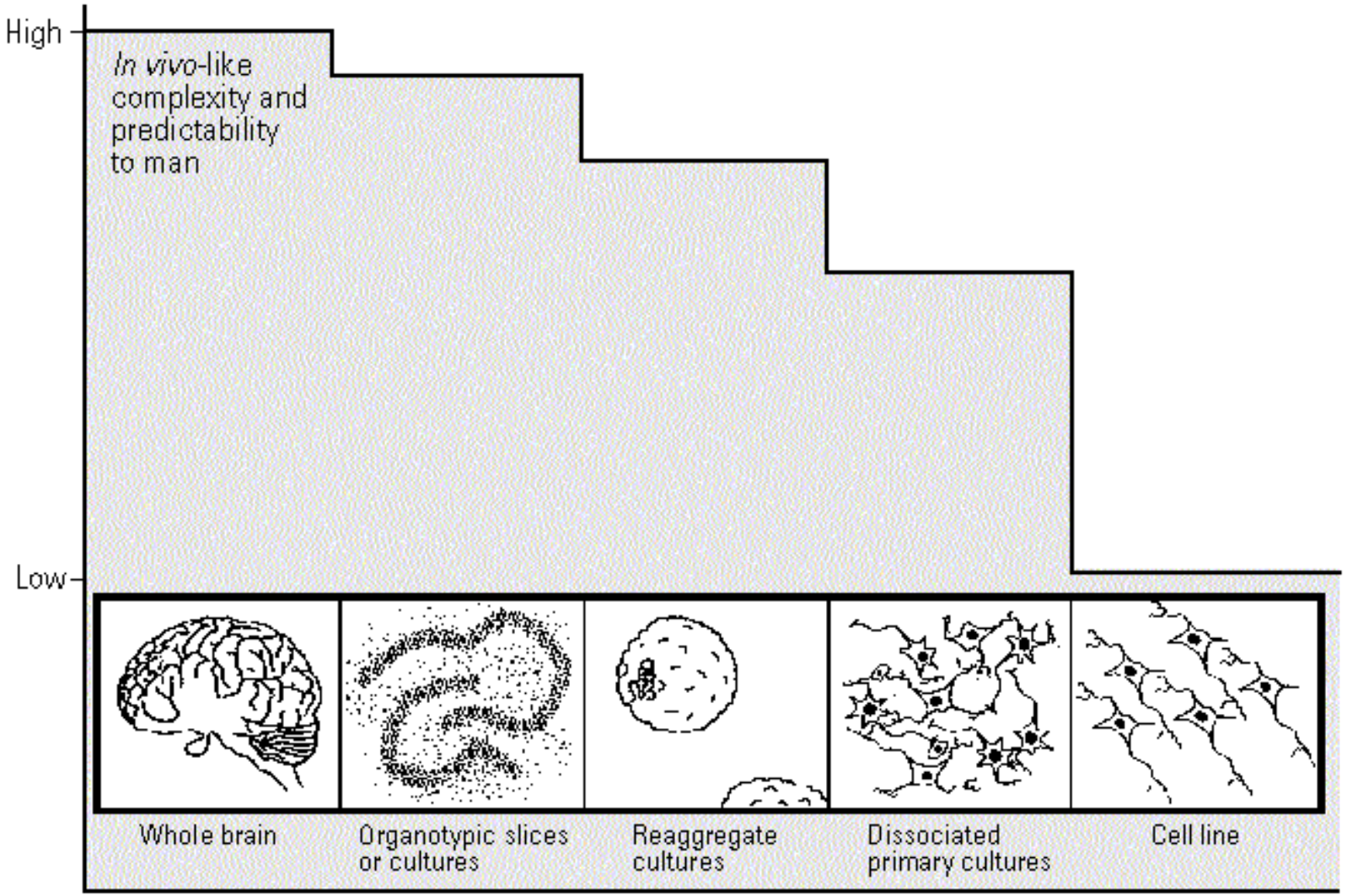


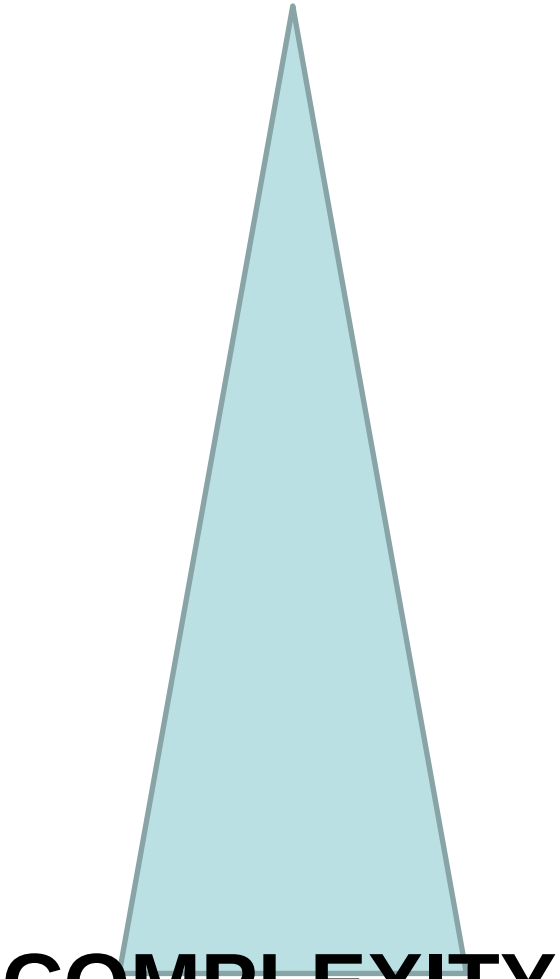
The model is a simplified version of the real world out there, simplified in the sense that it deals only with a limited amount of details.

MODELs IN NEUROBIOLOGY



Classification of tissue cultures based on the origin of the cells

- Extended culture (multipassage culture) – cell strain and or (transformed) cell lines
- STEM CELLS and iPSCs
- Primary culture (directly from animal tissue)
- STEM CELLS and iPSCs
- Organotypic cultures
- Animal Model



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:

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

of neuroscience.

Neuronal cell culture in Neurobiology

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Methods Mol Biol. 2013 ; 1078: 1–8. doi:10.1007/978-1-62703-640-5_1.

General overview of neuronal cell culture

Jennifer Gordon, **Shohreh Amini**, and **Martyn K. White**
Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA 19140

Experimental Read-OUT in cellular neurobiology

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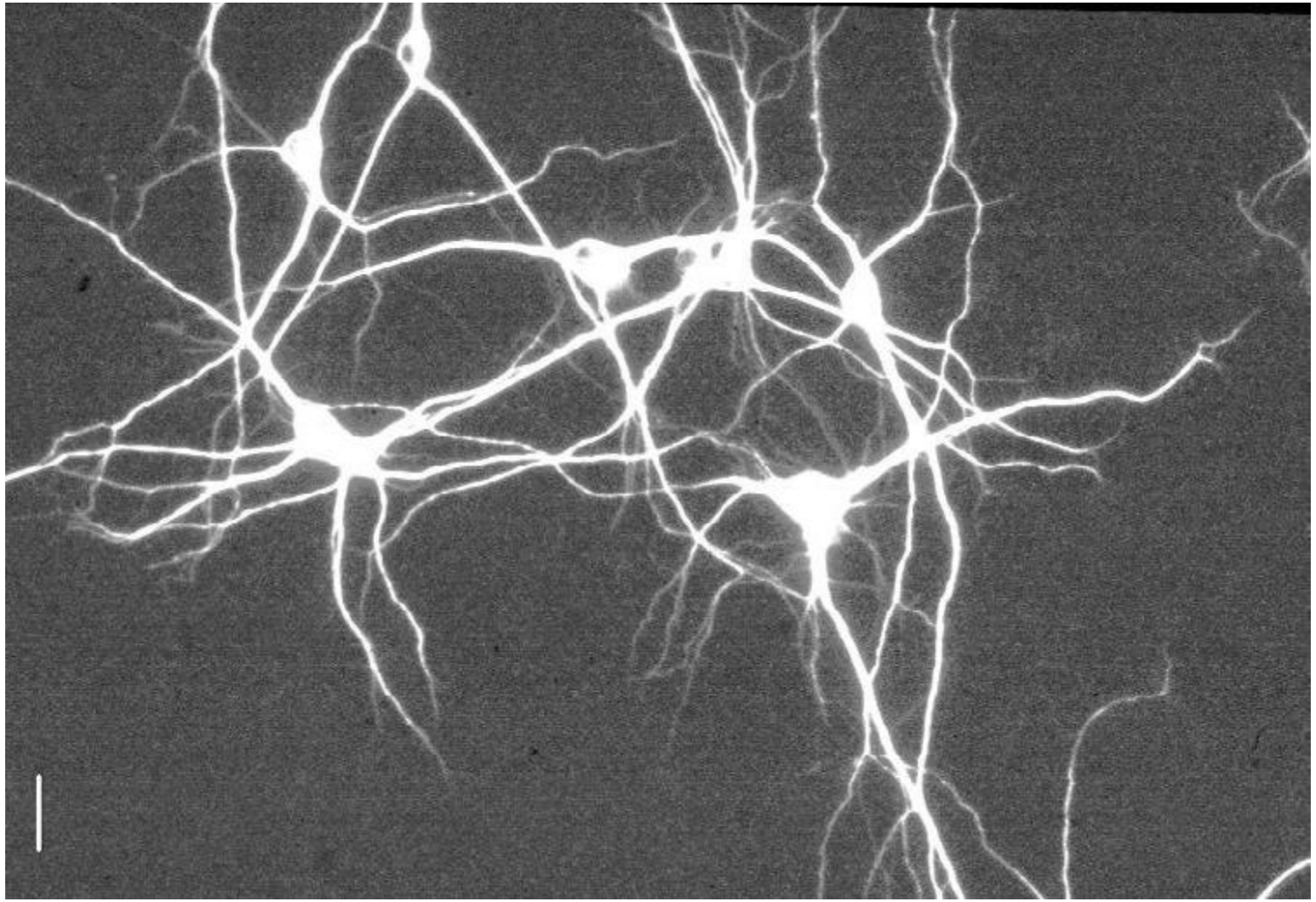
- Primary culture (directly from animal tissue)

- Extended culture cell strain and/or established (transformed) cell lines

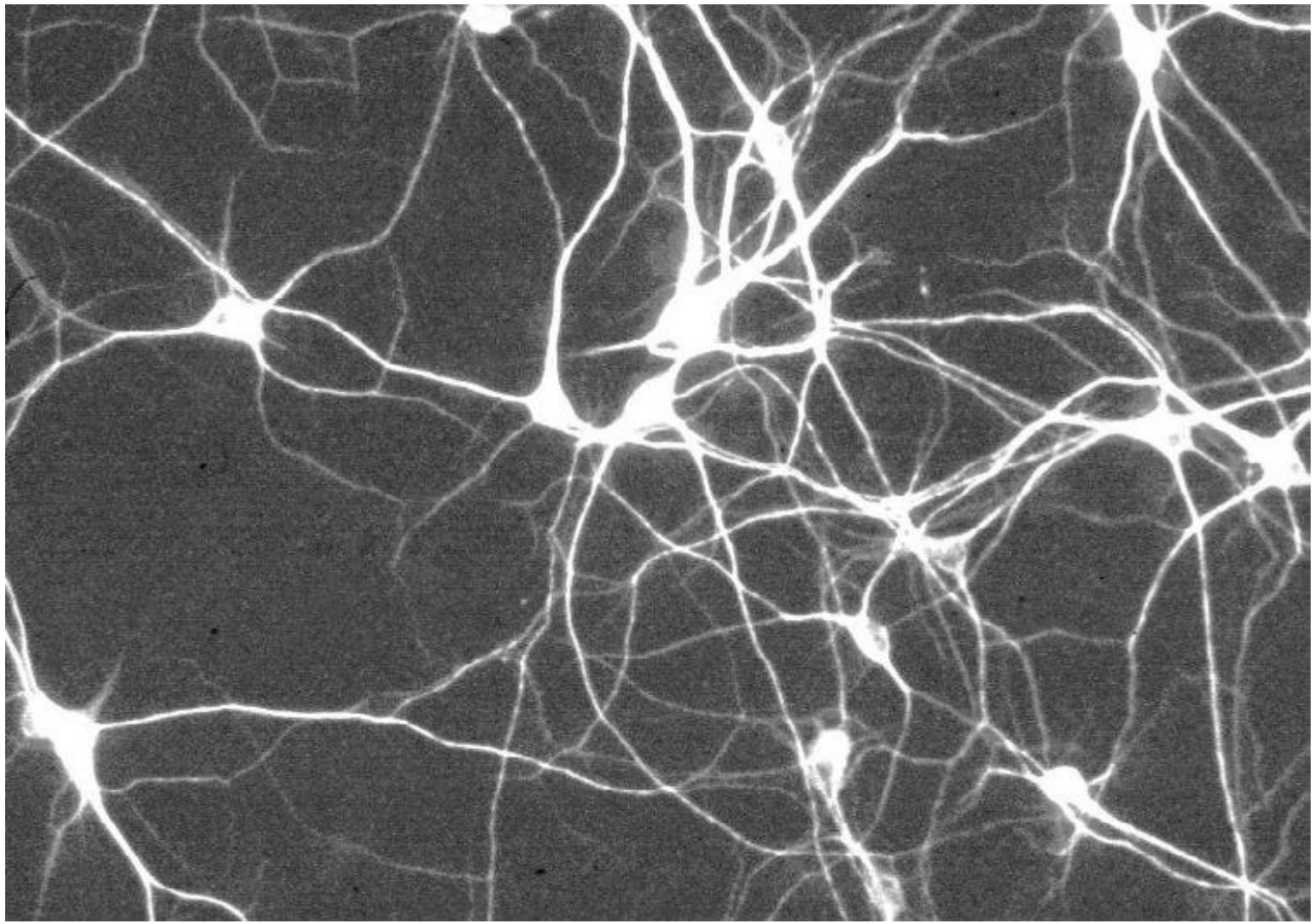
- Stem cells / IPSCs

- Organotypic cultures

- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)



• Primary culture (directly from animal tissue)

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COMMON READ OUT primary cell line

Protein

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

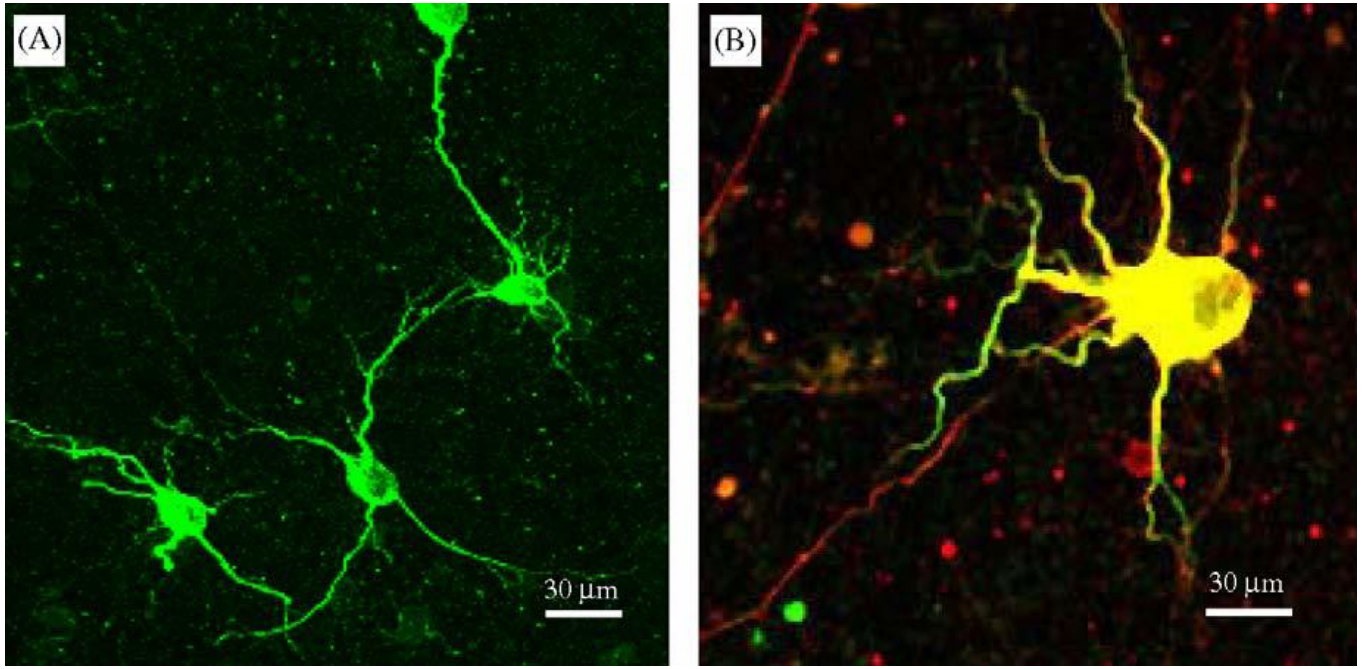
- Primary culture (directly from animal tissue)

RNA

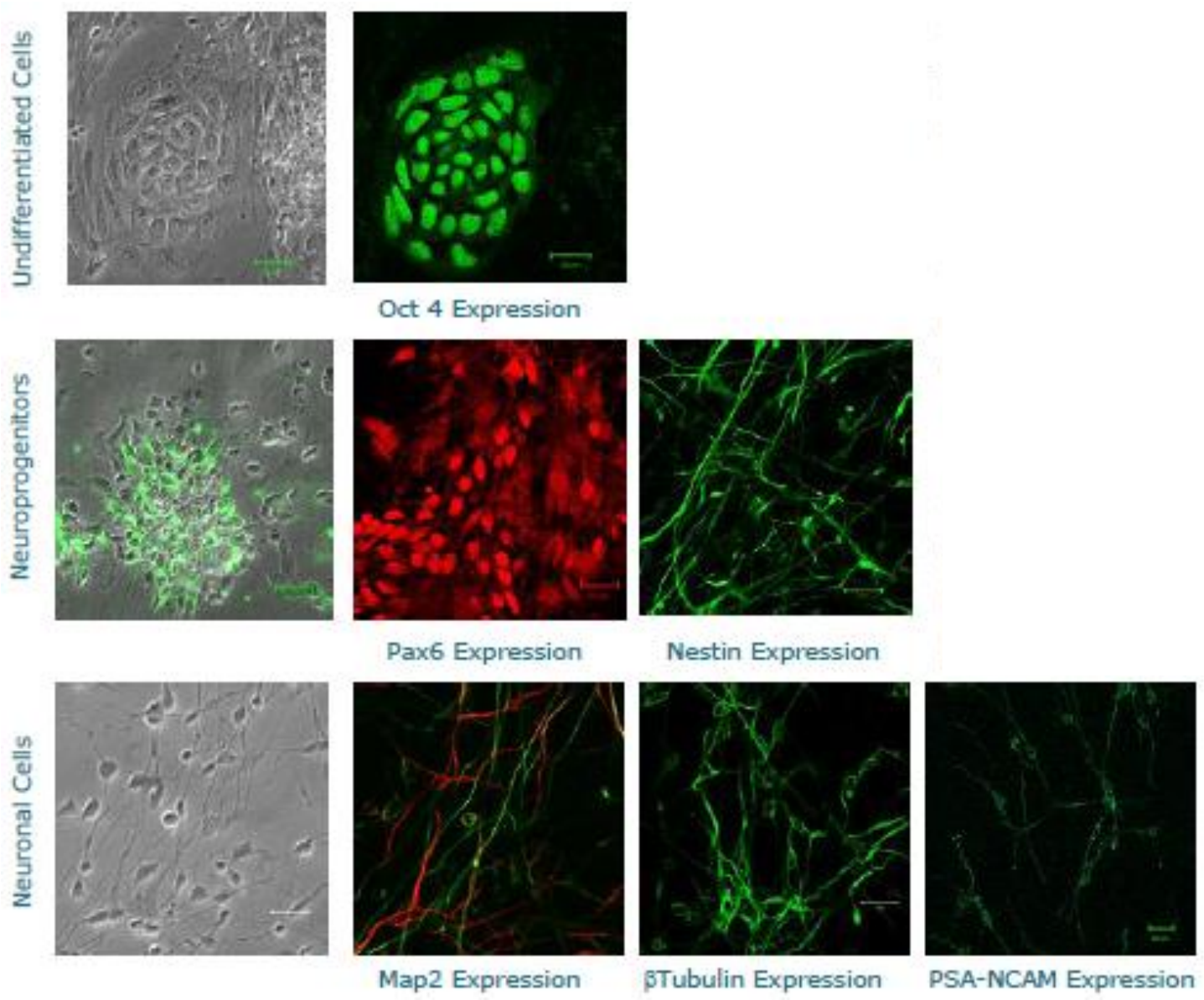
1. PCR
2. Real Time-PCR
3. Northern Blotting
4. InSitu Hyb

• Primary culture (directly from animal tissue)

Electrophysiology on “fake” network
- single celll
- field potential



- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)

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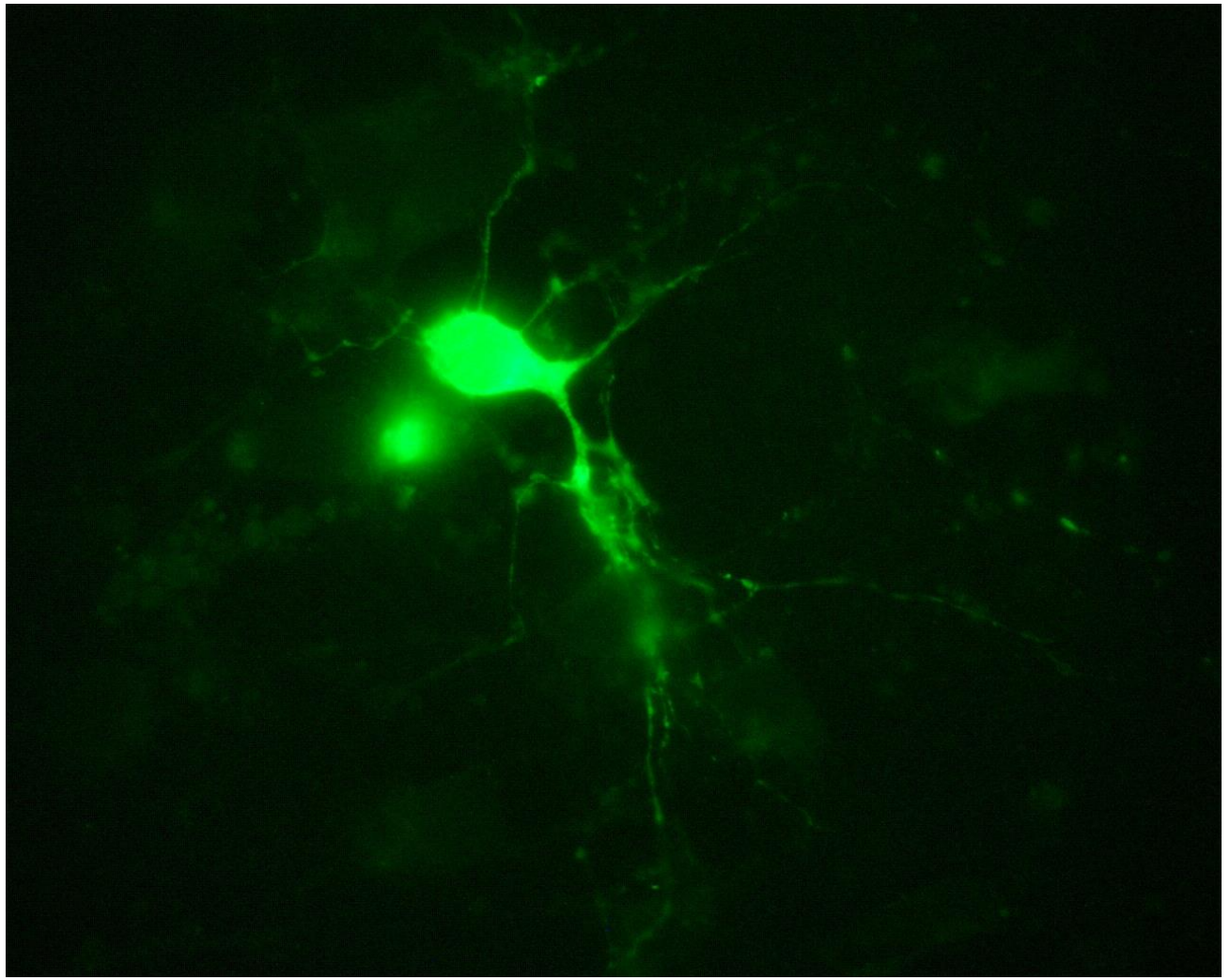

- Primary culture (directly from animal tissue)