# Systems neuroengineering

*the use of engineering tools and technologies to image, decode, and modulate the brain in order to comprehend its functions and to repair its dysfunction.*


1) Technical systems for researching the brain and behavior

Neuroscience & Cognitive Science

Engineering

**Analysis**
How does the brain work?

2) Analyzing & Modeling Neural Data

3) Neuro-inspired Technical Systems

**Synthesis**
How can we use neuroscientific knowledge to build better technical systems?

4) Brain-Machine Interfaces

*Adapted from: Berberich N., Cheng G. (2020) Kognitive Systeme und Neurorobotik. In: Mainzer K. (eds) Philosophisches Handbuch Künstliche Intelligenz. Springer Reference Ge*

# Systems neuroengineering

*the use of engineering tools and technologies to image, decode, and modulate the brain in order to comprehend its functions and to repair its dysfunction.*


## Engineering Principles:

- ❑Signal processing (analysis, manipulation, filtering, amplification modulation)
- ❑Control systems (design, implementation to stabilize process or output)
- ❑Instrumentation (design & development of devices)

# Systems neuroengineering

*the use of engineering tools and technologies to image, decode, and modulate the brain in order to comprehend its functions and to repair its dysfunction.*


**Neuroimaging**

Measure cerebral activities: ranging from the direct neuronal output of the brain to the metabolic requirements of its function.

Study neurovascular relationships occurring within the brain

**Neural interfacing technologies**

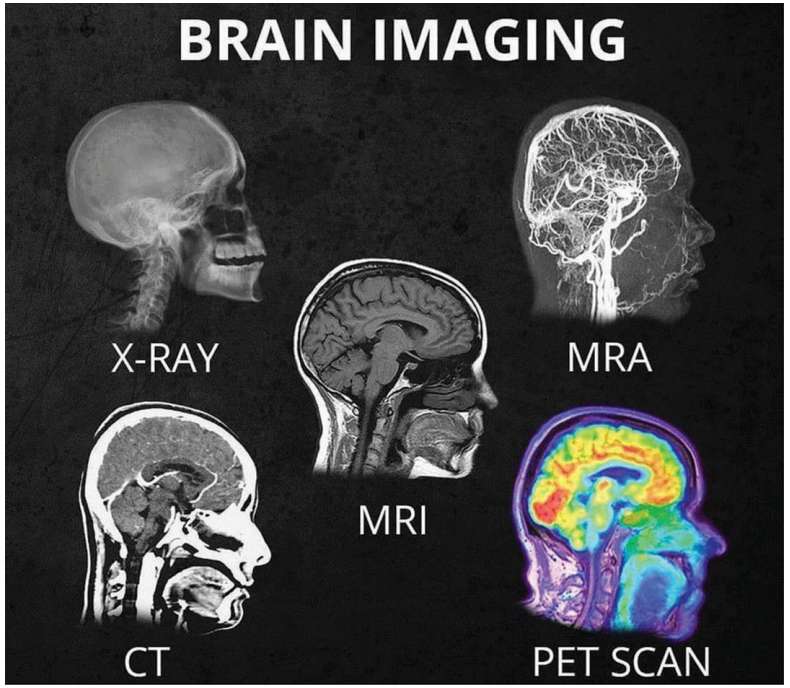
Decoding the neural activity related to daily bodily functions

Neural code: specific functions elicit a stable and repeatable sequence of activity and neural interfacing tech allows to detect these patterns and determine the corresponding behavior or operation

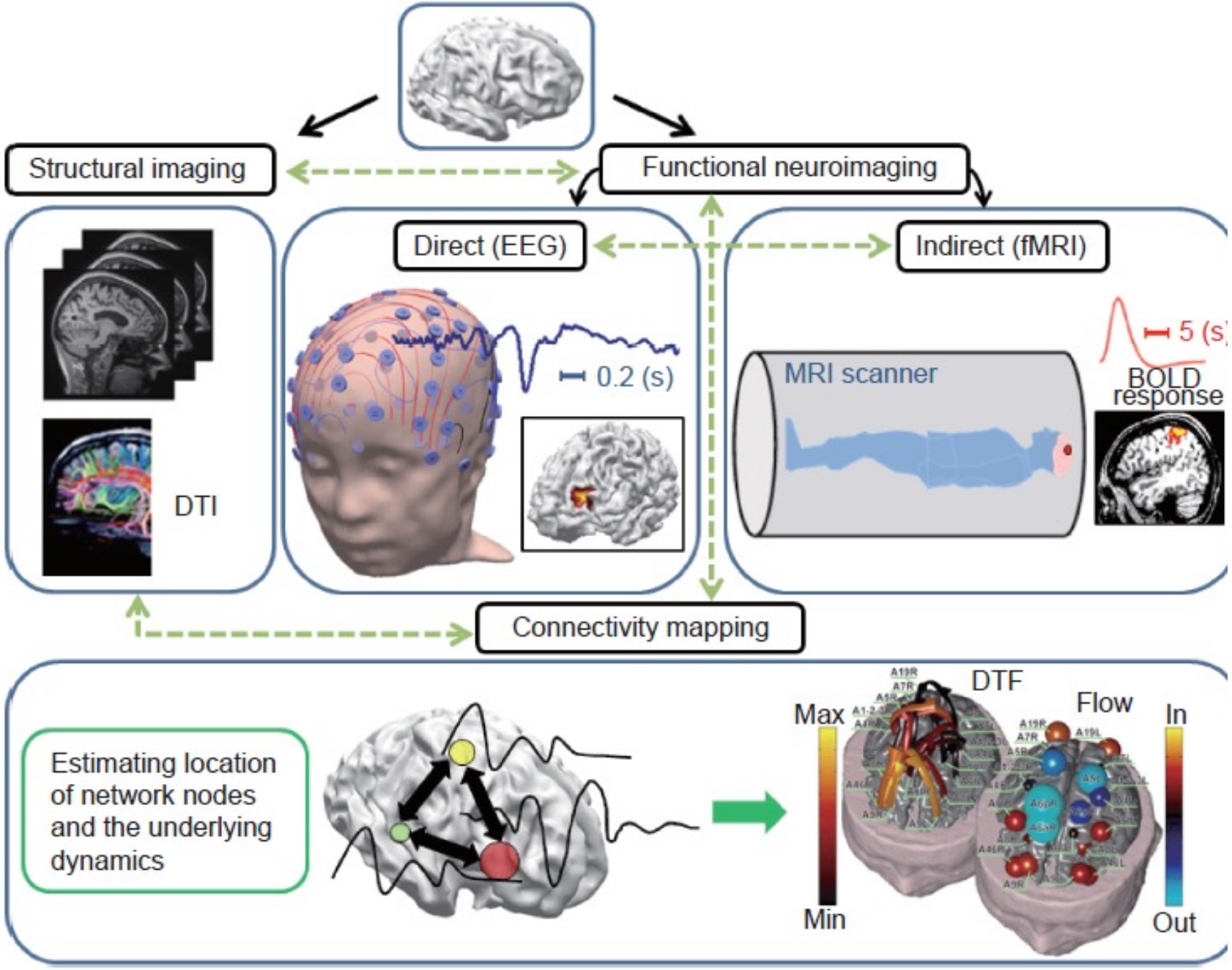
Advantages: can be used to detect electrophysiological responses of a cellular or system activity (single unit vs EEG)

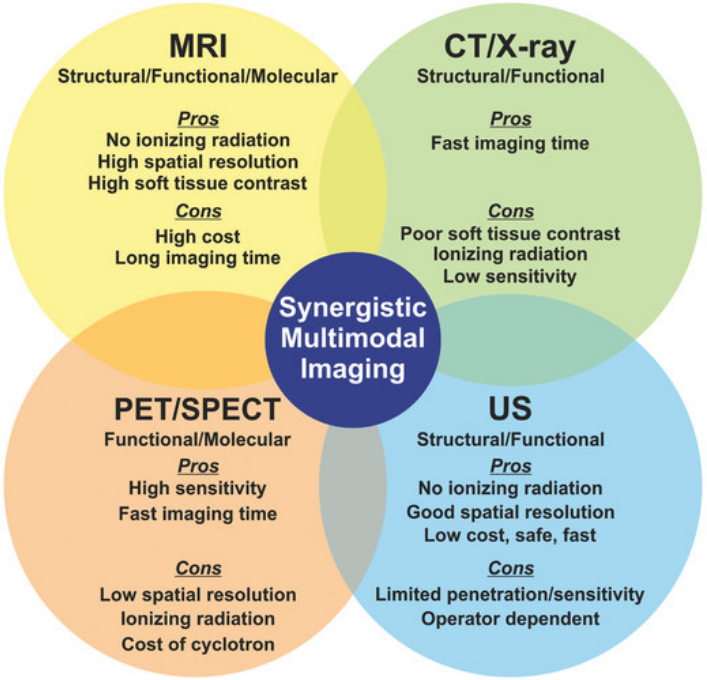
**Neuromodulation**

Alter irregular activity by stimulating the brain using a variety of electrical, optical, and sonic approaches with the goal of stabilizing the system to a healthy state



# Neuroimaging modalities



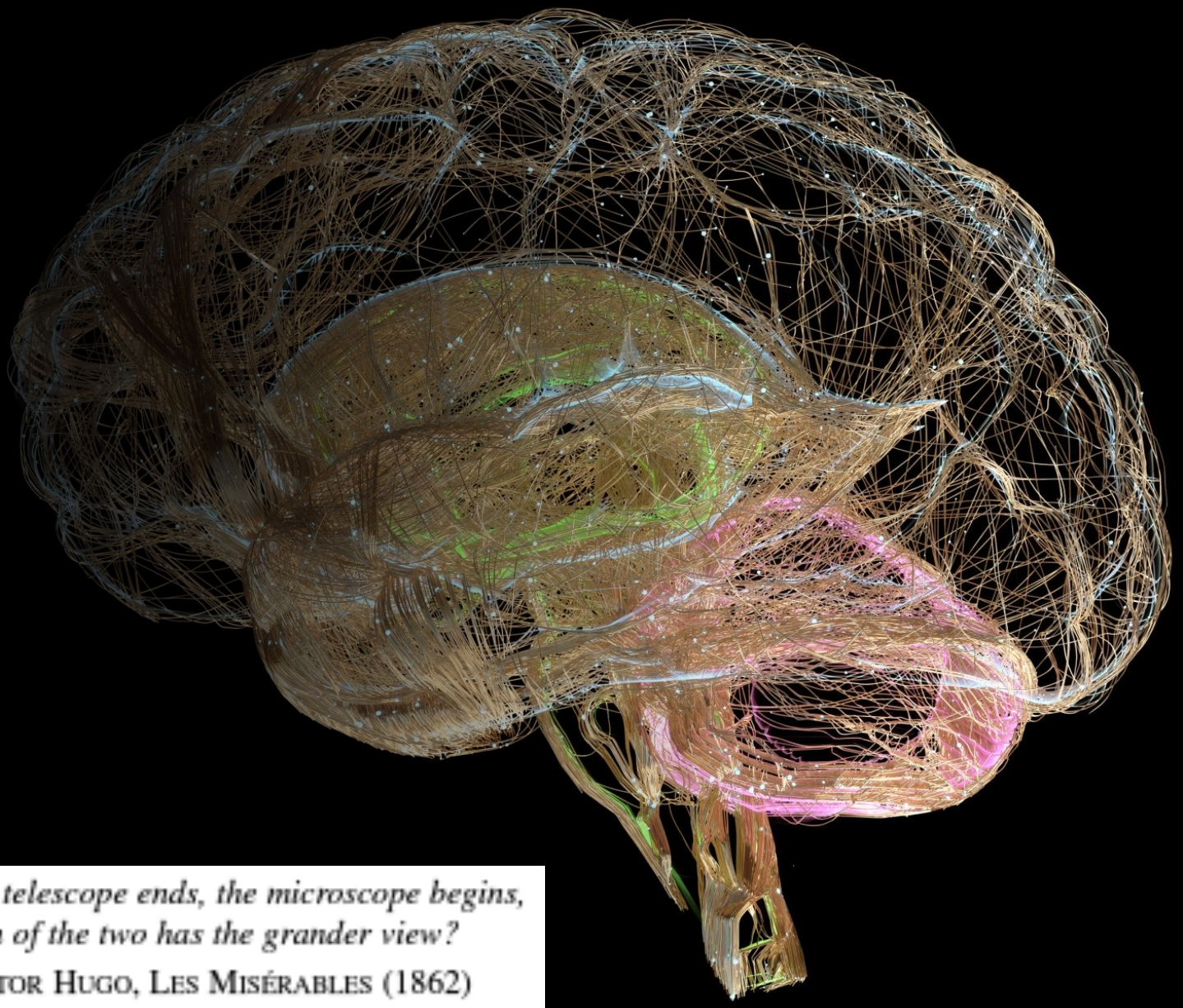




*Edelman et al., 2015 J. Engineering, Review*
*Rieffel et al., 2015, Small, Review*

**Table 1 Brain Mapping Methods Used in the Study of Human Health and Disease, along with the Types of Measurements They Provide and Some of the Clinical Situations in Which They May Be of Use**

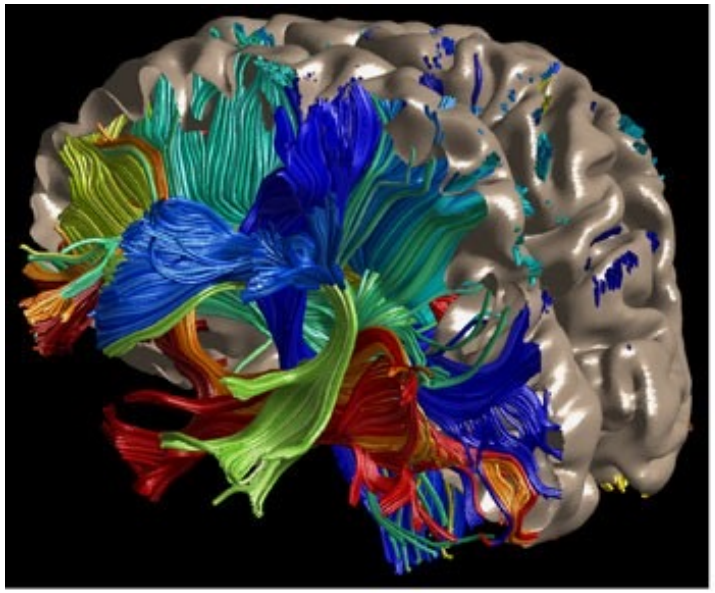
| Method | Measurements provided | Disorders | Advantages | Limitations |
|---|---|---|---|---|
| X-ray computed tomograhy (CT) | 1. Brain structure<br>2. Blood–brain barrier integrity | 1. Acute/chronic hemorrhages<br>2. Acute trauma<br>3. General screening of anatomy<br>4. Focal or generalized atrophy<br>5. Hydrocephalus | 1. Excellent bone imaging<br>2. 100% detection of hemorrhages<br>3. Short study time<br>4. Can scan patients with ancillary equipment<br>5. Can scan patients with metal/electronic devices | 1. Ionizing radiation<br>2. Poor contrast resolution |
| Magnetic resonance imaging (MR) | 1. Brain structure<br>2. Brain and cervical vasculature<br>3. Relative cerebral perfusion<br>4. Chemical concentrations<br>5. Fiber tracts<br>6. Blood–brain barrier integrity | 1. Acute ischemia<br>2. Neoplasms<br>3. Demyelinating disease<br>4. Epileptic foci<br>5. Degenerative disorders<br>6. Infections<br>7. Preoperative mapping | 1. High spatial resolution<br>2. No ionizing radiation<br>3. High resolution<br>4. High gray–white contrast<br>5. No bone–generated artifact in posterior fossa<br>6. Can also perform chemical, functional, and angiographic imaging | 1. Long study duration<br>2. Patients may be claustrophobic<br>3. Electronic devices contraindicated<br>4. Acute hemorrhages problematic<br>5. Relative measurements only |
| Positron emission tomography (PET) | 1. Perfusion<br>2. Metabolism<br>3. Substrate extraction<br>4. Protein synthesis<br>5. Neurotransmitter integrity<br>6. Receptor binding<br>7. Blood–brain barrier integrity | 1. Ischemic states<br>2. Degenerative disorders<br>3. Epilepsy<br>4. Movement disorders<br>5. Affective disorders<br>6. Neoplasms<br>7. Addictive states<br>8. Preoperative mapping | 1. Can perform hemodynamic chemical, and functional imaging<br>2. Quantifiable results<br>3. Absolute physiologic variables can be determined<br>4. Uniform spatial resolution | 1. Ionizing radiation<br>2. High initial costs<br>3. Long development time for new tracers<br>4. Limited access<br>5. Low temporal resolution |
| Single-photon-emission computed tomography (SPECT) | 1. Perfusion<br>2. Neurotransmitter integrity<br>3. Receptor binding<br>4. Blood–brain barrier integrity | 1. Ischemic states<br>2. Degenerative disorders<br>3. Epilepsy<br>4. Movement disorders | 1. Can perform hemodynamic, chemical, and functional imaging<br>2. Widely available | 1. Ionizing radiation<br>2. Relative measurements only<br>3. Nonuniform spatial resolution<br>4. Low temporal resolution |
| Xenon-enhanced computed tomography (XECT) | 1. Perfusion | 1. Ischemic states | 1. Uses existing equipment | 1. Ionizing radiation<br>2. High xenon concentrations have pharmacalogic effects |
| Spiral computed tomography (CT angiography, CTA) | 1. Vascular anatomy<br>2. Boney anatomy | 1. Vascular occlusive disease<br>2. Vascular and boney anatomy only | 1. Provides high-resolution vascular images | 1. Ionizing radiation<br>2. Vascular and boney anatomy only |
| Electroencephalography surface (EEG) | 1. Electrophysiology | 1. Epilepsy<br>2. Encephalopathies<br>3. Degenerative disorders<br>4. Preoperative mapping | 1. No ionizing radiation<br>2. High temporal resolution<br>3. Widely available<br>4. Can identify epileptic foci | 1. Low spatial resolution<br>2. Weighted toward measurements |
| Magnetoencephalography (MEG) | 1. Electrophysiology | 1. Epilepsy | 1. No ionizing radiation<br>2. High temporal resolution<br>3. Can identify epileptic foci | 1. Low spatial resolution |
| Transcranial, magnetic stimulation (TMS) | 1. Focal brain activation | 1. Preoperative mapping | 1. No ionizing radiation<br>2. Potential for therapy<br>3. Can be linked to other imaging methods (PET fMRI) | 1. Low spatial resolution<br>2. Has produced seizures in certain patient groups |
| Optical intrinsic signal imaging (OIS) | 1. Integrated measure of blood volume, metabolism, and cell swelling | 1. Intraoperative mapping | 1. No ionizing radiation<br>2. High temporal resolution<br>3. High spatial resolution | 1. Complex signal source<br>2. Invasive only (intraoperative) |

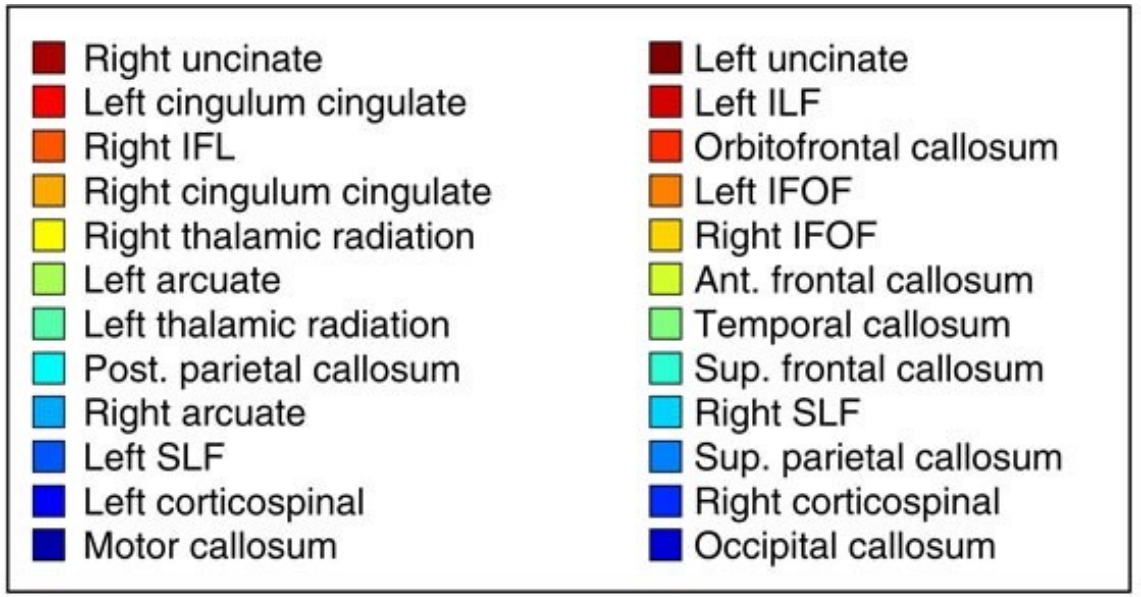
# Visualizing neural structure and function

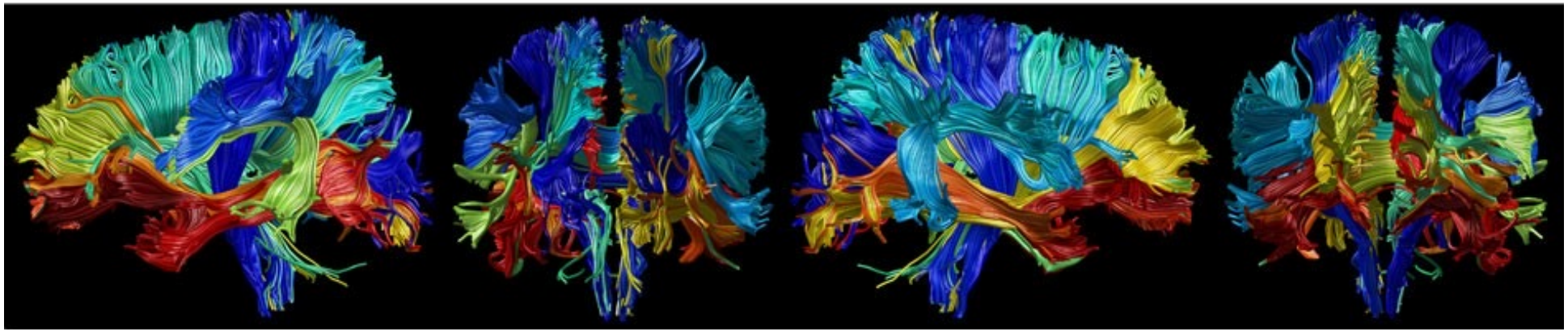


*Where the telescope ends, the microscope begins, which of the two has the grander view?*

—Victor Hugo, Les Misérables (1862)





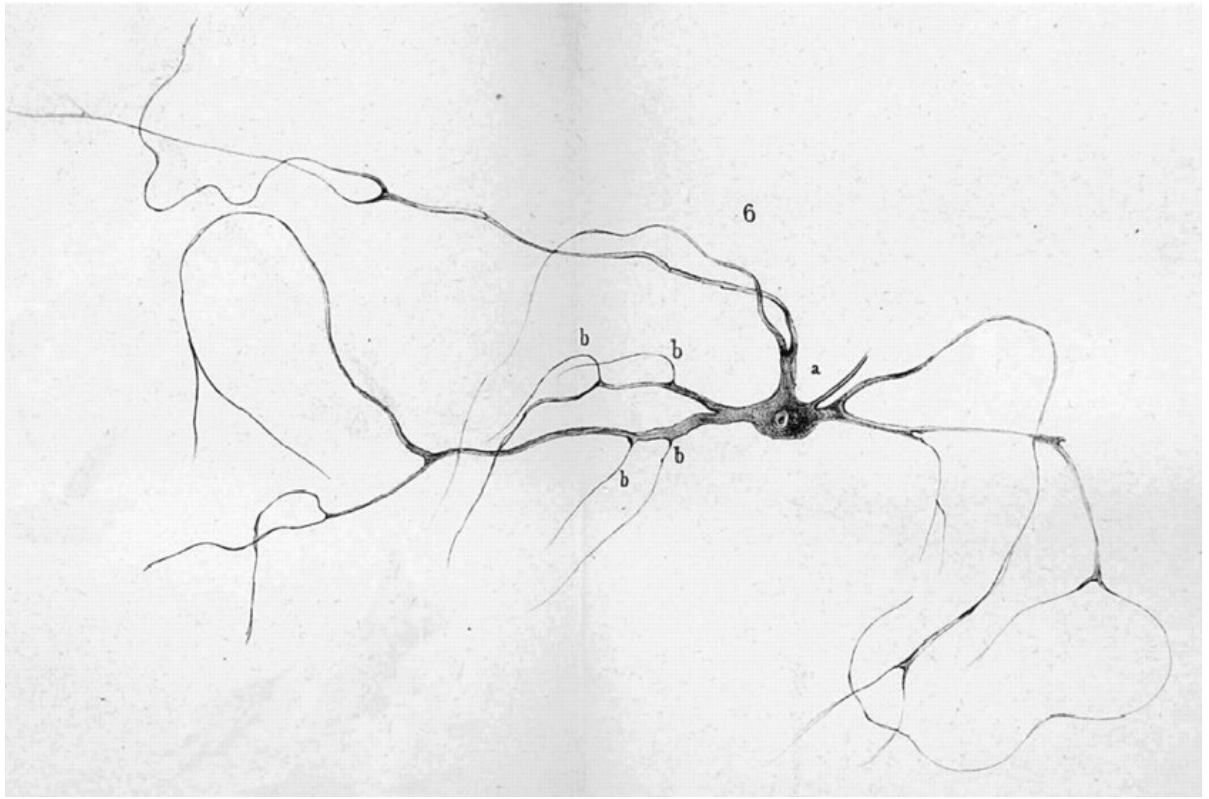



*Yeatman et al., Nat. Com., 2014*

# Otto Friedrich Karl Dieters

1834-1863

**Introduced potassium dichromate to harden the neural tissue**



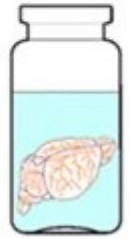

https://jralonso.es/2015/11/08/la-celula-ramificada/

# Tissue preparation

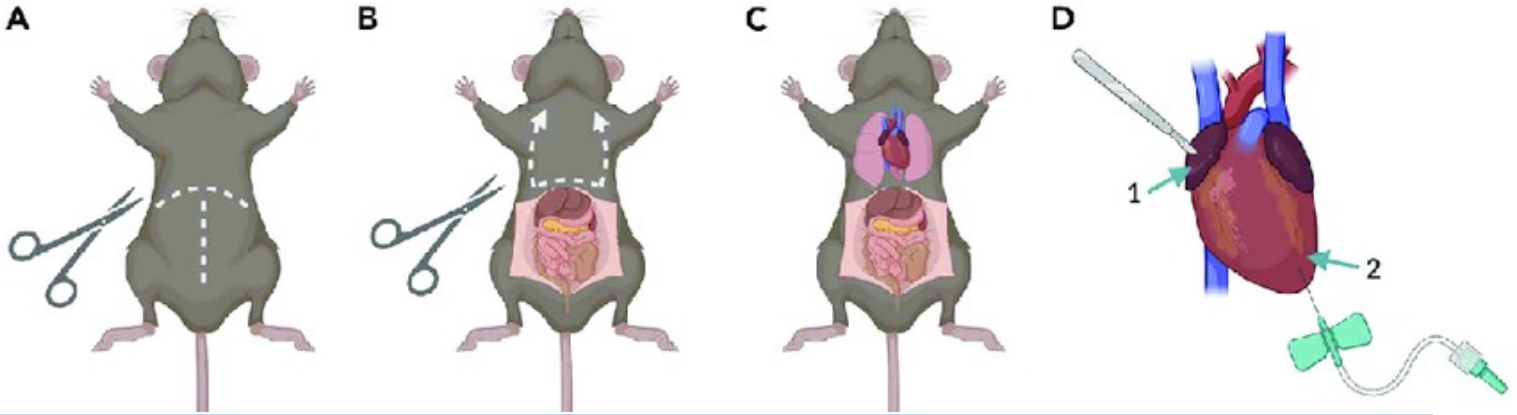
Fixation | Embedding | Sectioning

Fixation is a chemical process used to preserve, stabilize, and strengthen biological specimens for histological procedures and microscopic analysis.

Immersion



Perfusion



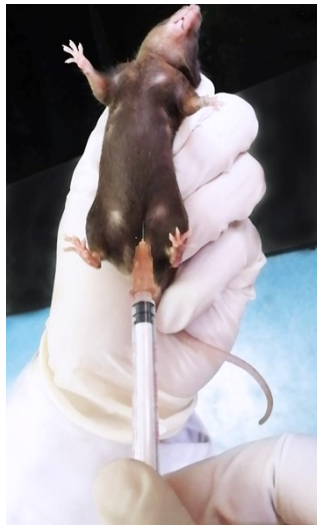

| Fixative Type | Name | Key Features |
|---|---|---|
| Cross-linking | Formaldehyde | Preserves morphology, widely used, may cause DNA-protein crosslinks |
| | Paraformaldehyde | Similar to formaldehyde, but purer and more potent |
| | Glutaraldehyde | Excellent for preserving ultrastructure, but can distort morphology |
| | Osmium Tetroxide | Used in electron microscopy, good for preserving lipids and cell membranes |
| Dehydrating | Methanol | Causes protein denaturation and lipid dissolution, good for preserving RNA |
| | Acetone | Rapidly penetrates tissues, good for preserving lipids, can cause shrinkage |

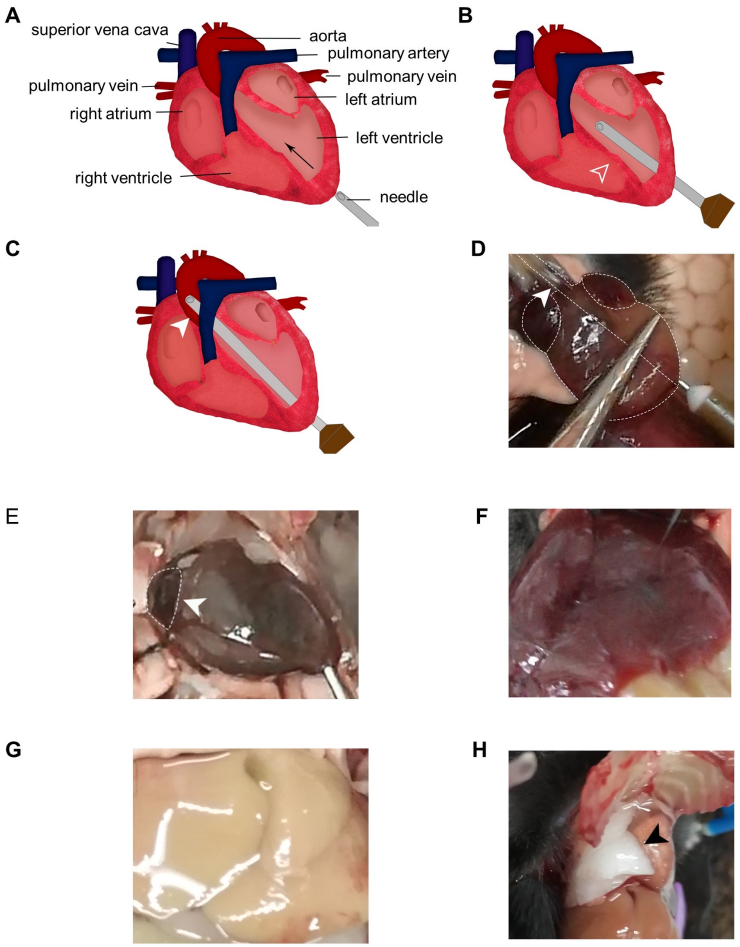
# Tissue preparation

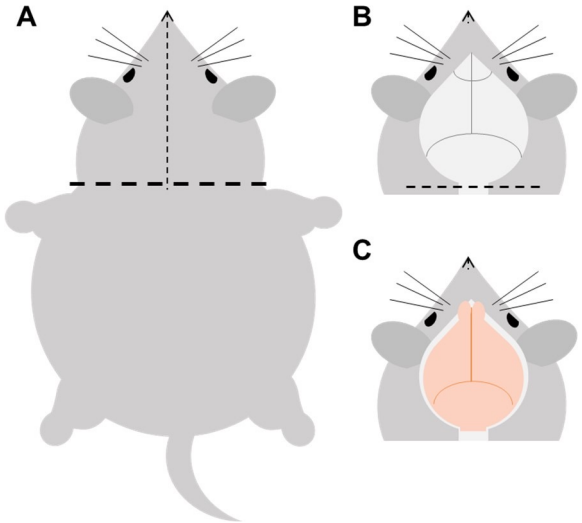
Fixation

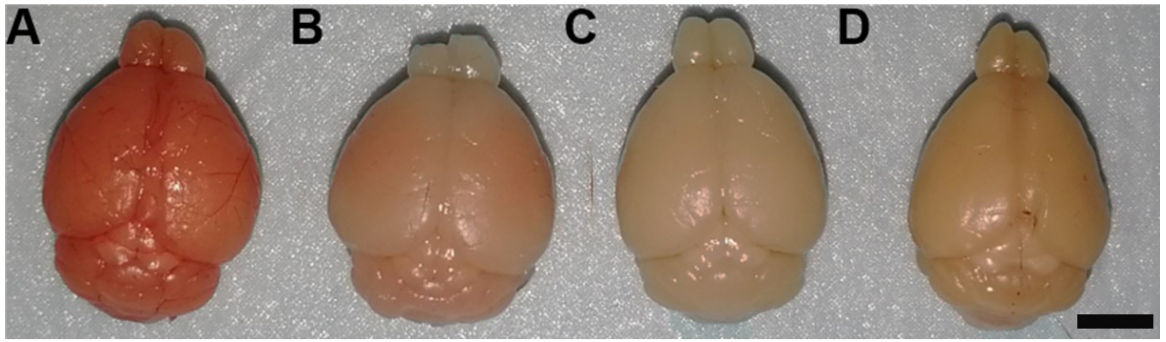
Embedding

Sectioning





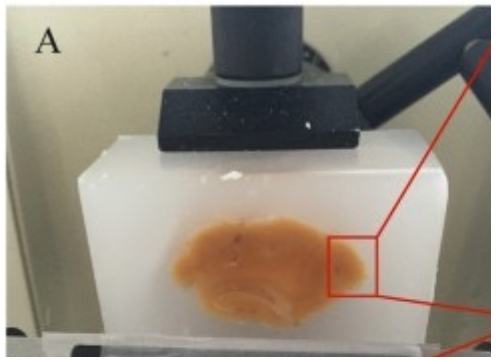

*Wu et al., Bio Protocol, 2021*

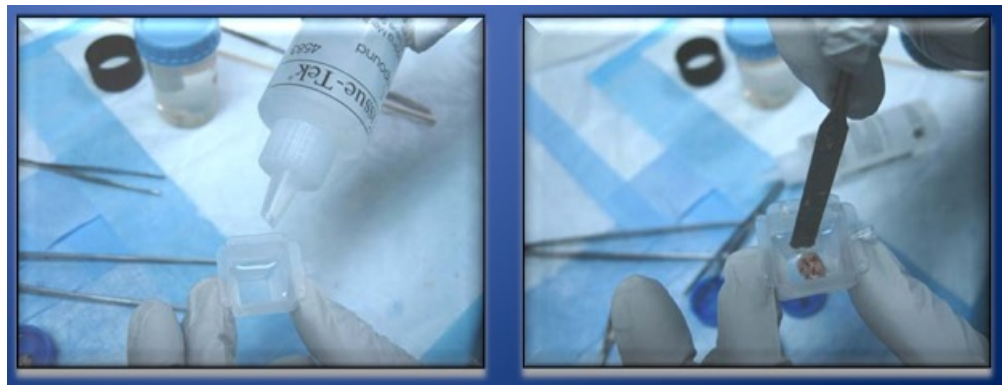
# Tissue preparation

Fixation | Embedding | Sectioning

This process stabilizes the tissue's structure and makes it easier to cut

| Embedding Medium | Key Features |
|---|---|
| Paraffin Wax | Most commonly used, good for routine histology and immunohistochemistry, requires deparaffinization before staining. |
| OCT (Optimal Cutting Temperature) Compound | Used for frozen sections, good for preserving enzymes and antigens, does not require deparaffinization. |
| Resin (e.g., Epoxy Resin) | Used for electron microscopy, provides excellent resolution, but can be difficult to cut. |
| Gelatin | Good for light microscopy, particularly for large specimens or whole organs. |
| Agar | Useful for small, delicate specimens that may be distorted by other embedding media. |

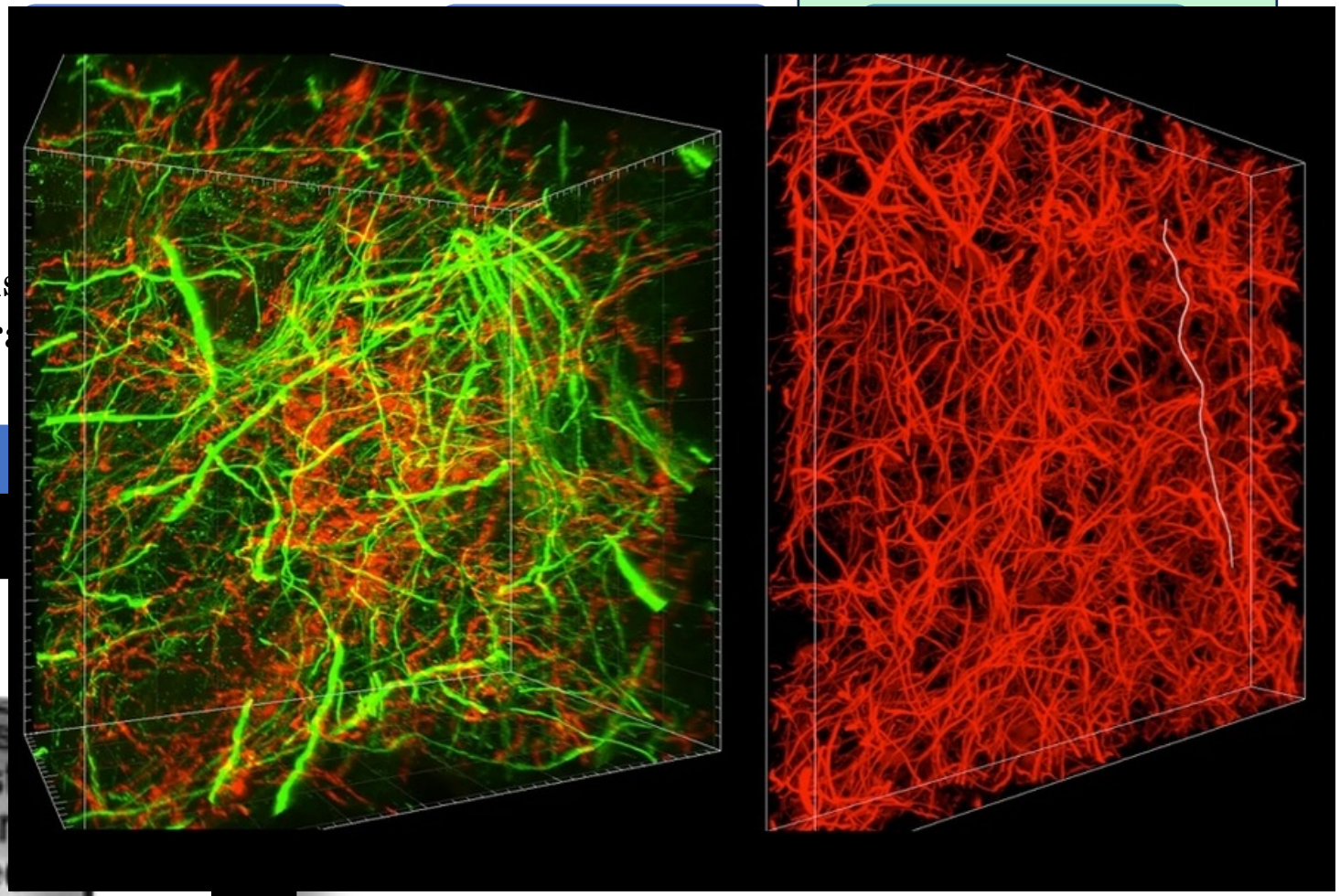


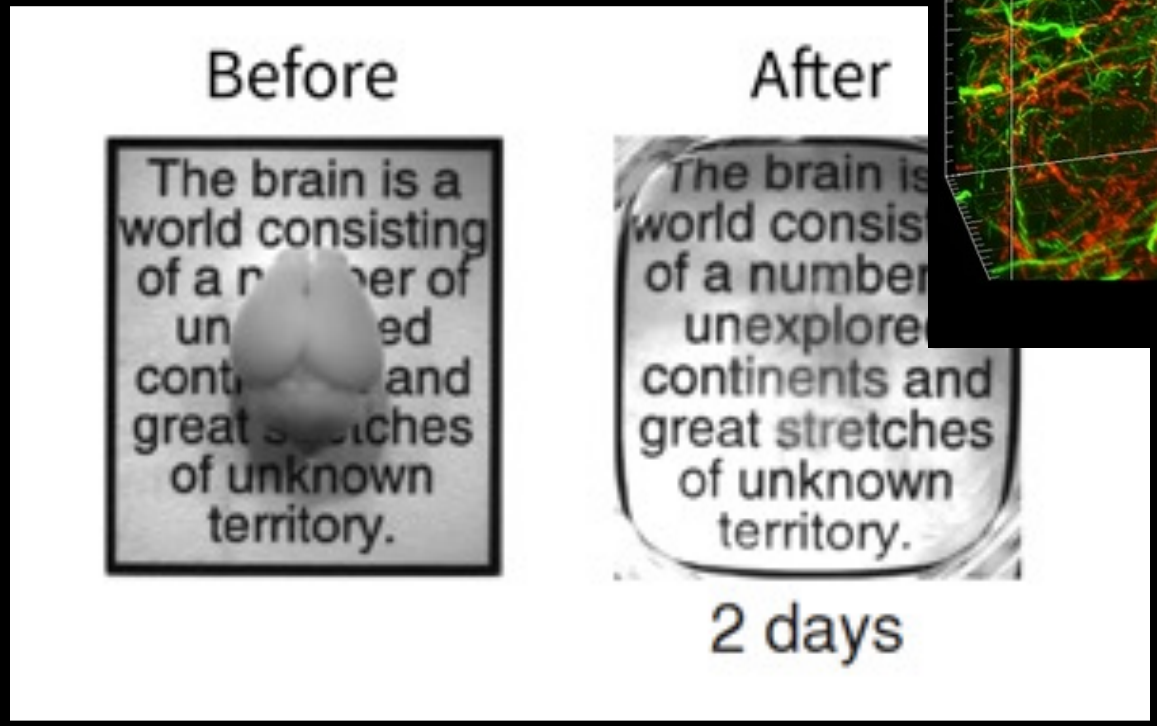



# Tissue preparation

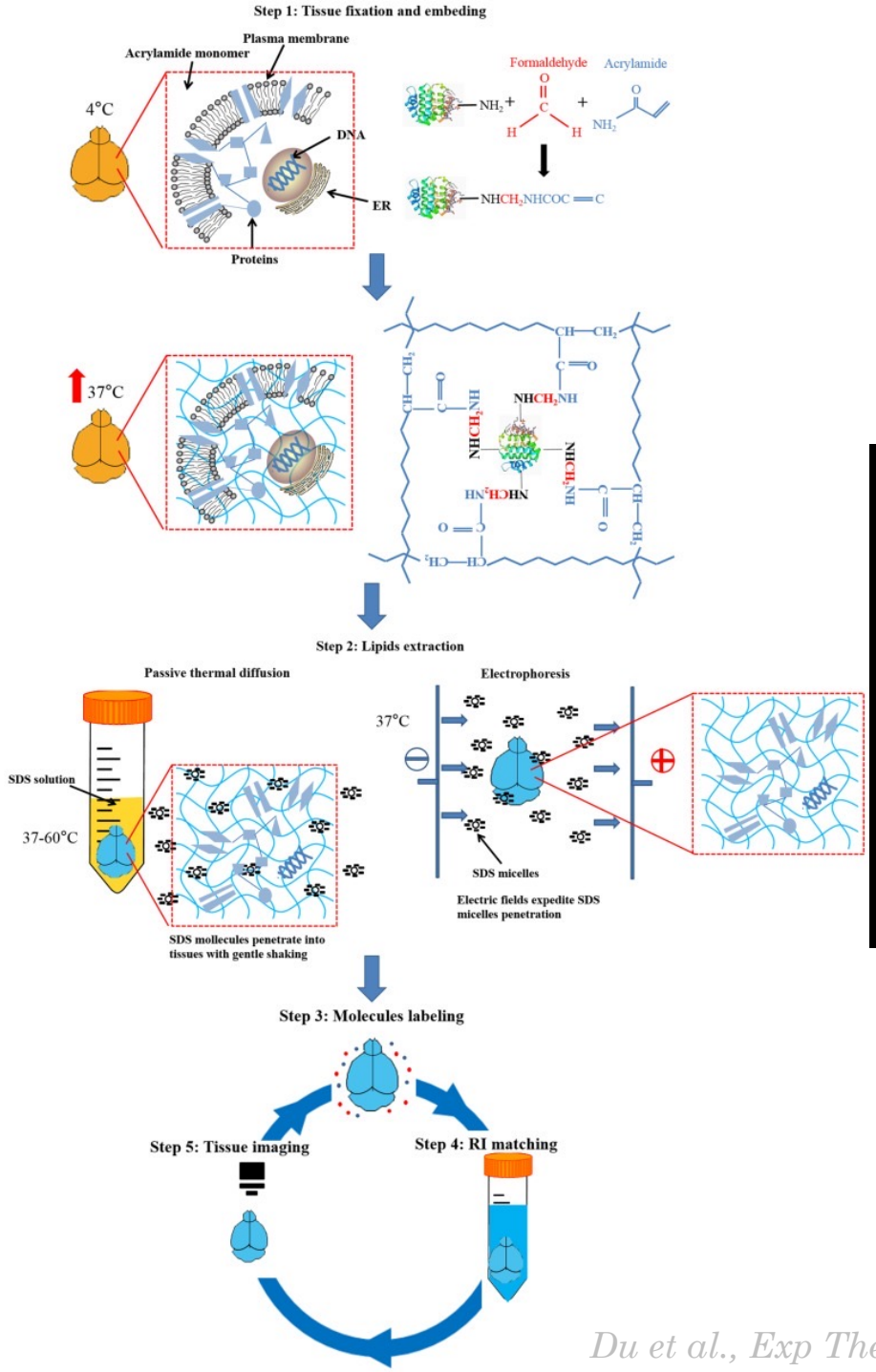
grants access to cells within the slice for his
reagents but also facilitates the study of br
structures under a microscope.

| Sectioning Method | Essential Features |
| --- | --- |





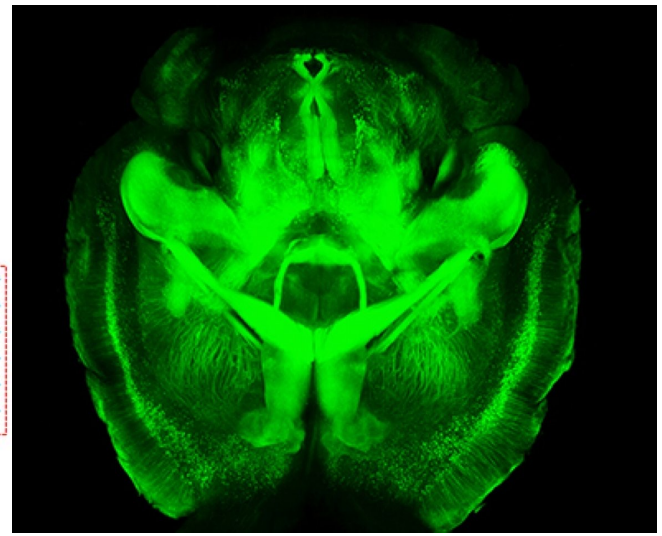

**CLARITY** – **C**lear **L**ipid-exchanged **A**crylamide-hybridized **R**igid **I**maging/immunostaining/*in situ* hybridization-compatible **T**issue h**Y**drogel

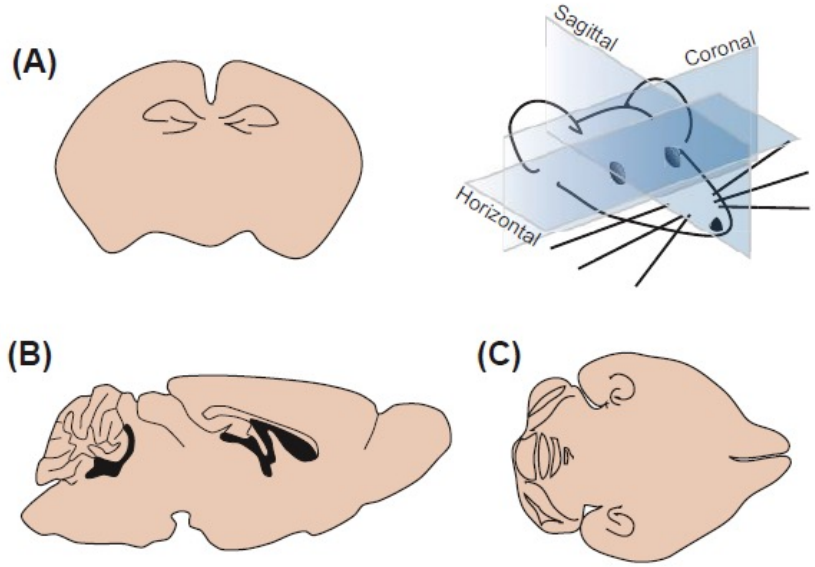



Fixation

Embedding

Sectioning






**CLARITY** – **C**lear **L**ipid-exchanged **A**crylamide-hybridized **R**igid **I**maging/immunostaining/*in situ* hybridization-compatible **T**issue h**Y**drogel

*Du et al., Exp Ther Med, 2018*

Engineering principles in tissue processing

- Mechanical Principles
- Material Properties
- Resonance and Vibrations
- Thermal Considerations
- Optics and Imaging
- Bio-compatibility

# Engineering principles in tissue processing

- Conductivity
- Dielectric properties

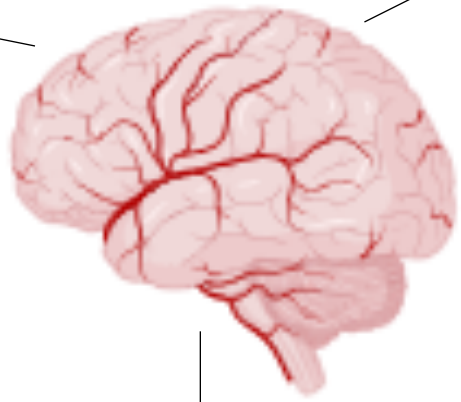
**Electrical properties**

**Mechanical properties**

- Elasticity
- Viscosity
- Anisotropy

**Thermal properties**

- Specific heat capacity
- Thermal conductivity



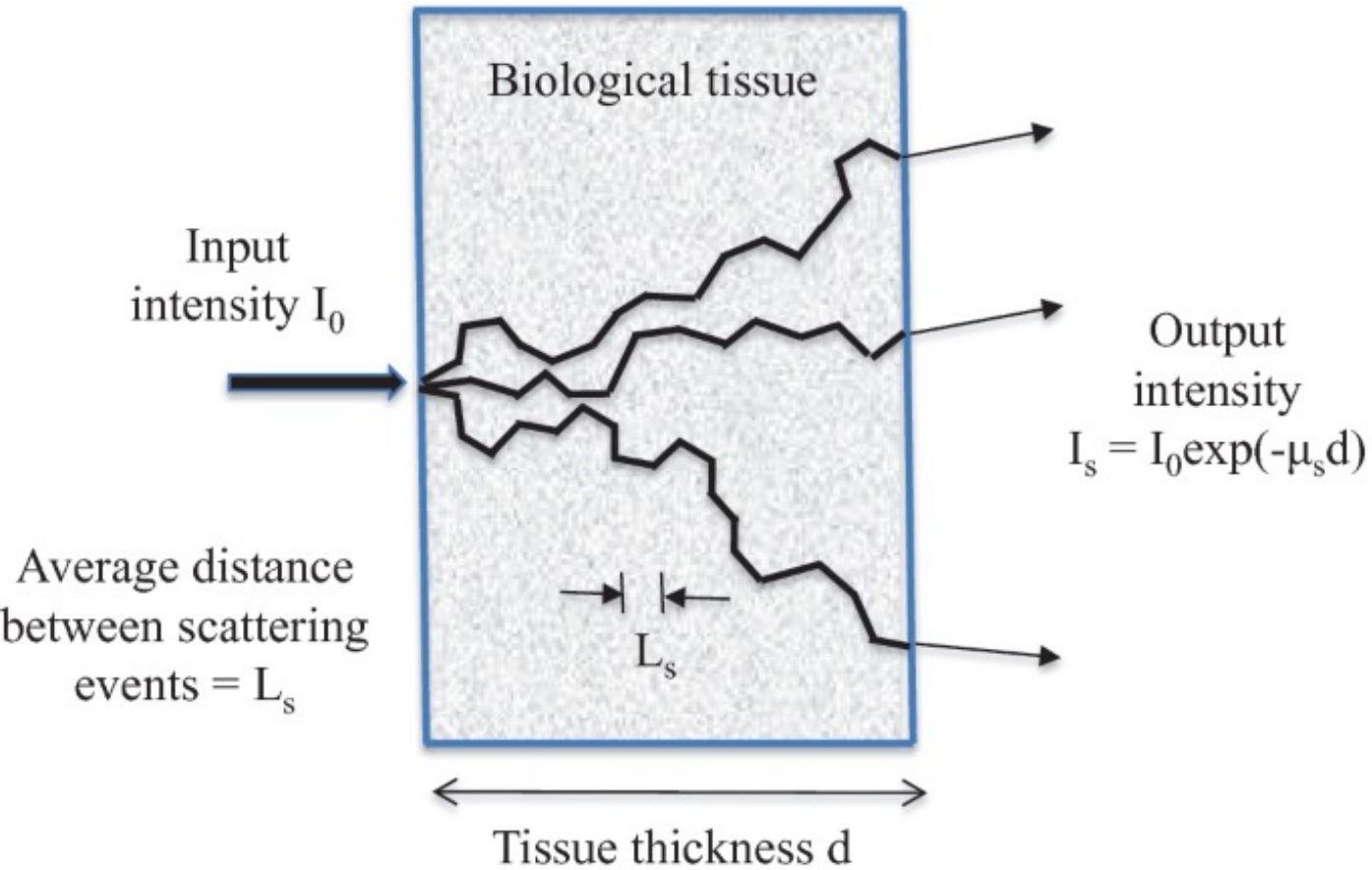
**Hydraulic properties**

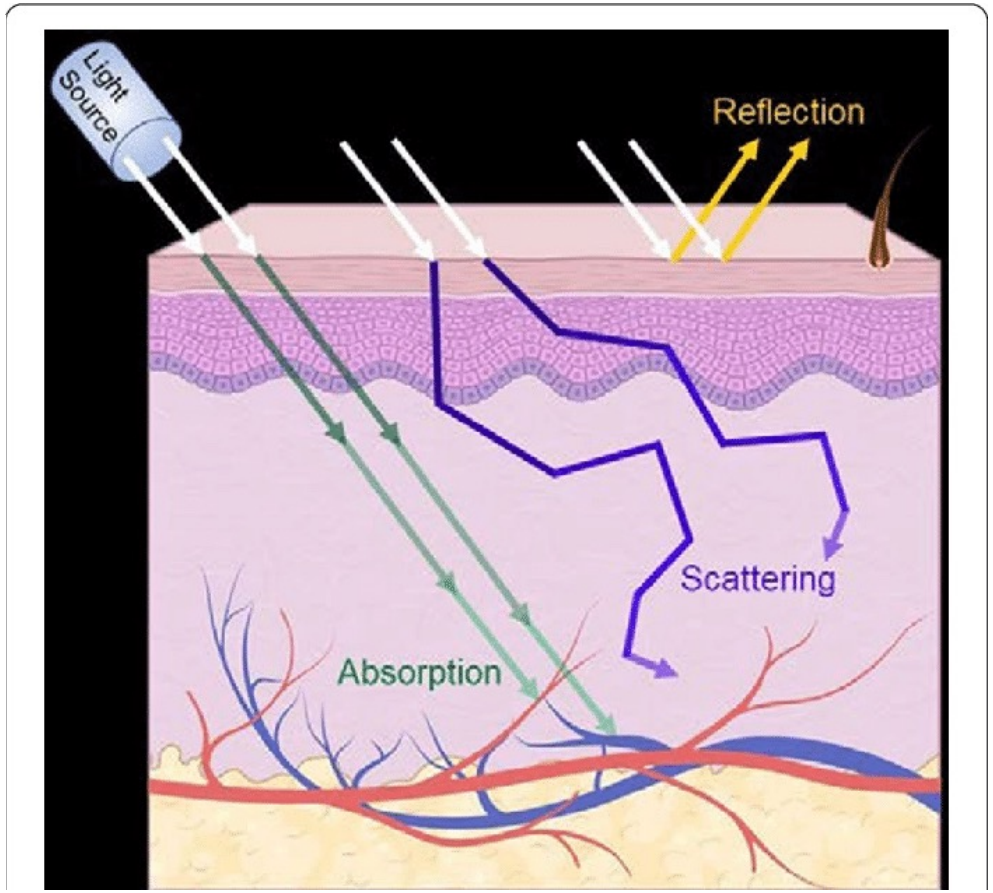
- Permeability

**Biochemical properties**

- Composition
- Degradation

# Light-tissue interraction



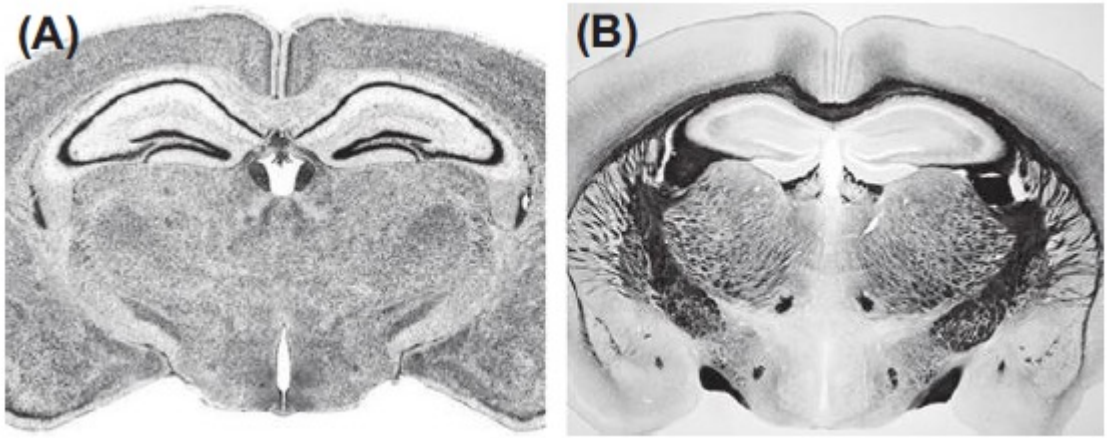




*Keiser, 2022, Biophotonics*

# Visualizing morphology

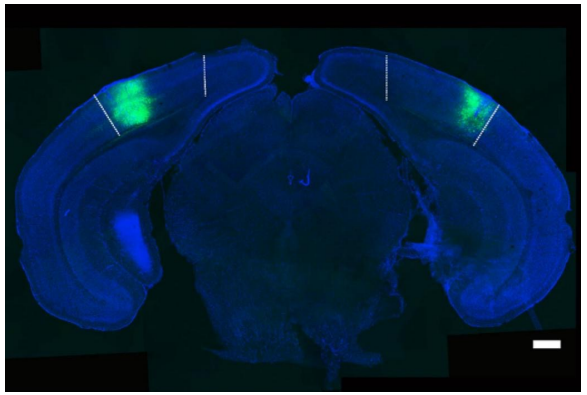
**Key points:**

- Brain sections are transparent due to high water content.
- Various dyes are used to visualize brain structures.
- Dyes increase contrast, enabling visualization of neural systems' features.
- Basophilic stains like hematoxylin and thionine highlight cell bodies by labeling nucleic acids.
- Nissl stains, a type of basophilic stain, label RNA within cells.
- Fluorescent markers like DAPI, Hoechst, and PI are used for cell nuclei in fluorescent microscopy.
- Fiber stains label myelin, aiding in visualizing white-matter tracts.
- Protocols like Weigert's or Weil's methods help in myelin labeling.
- The Golgi stain technique labels individual neurons and their processes.
- Intracellular and juxtacellular labeling methods mark cell bodies/axons post brain fixation and sectioning.

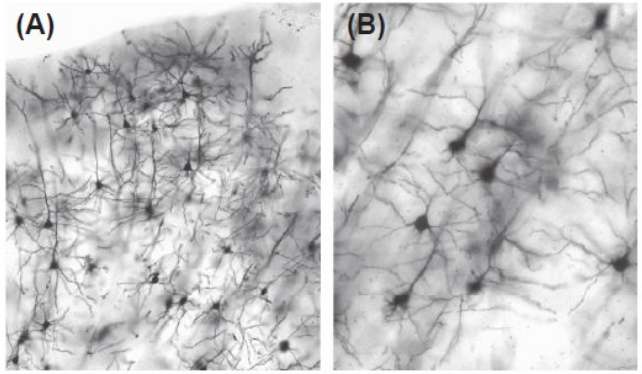



*Rosen, G.D., et al. 2000*

DAPI stain

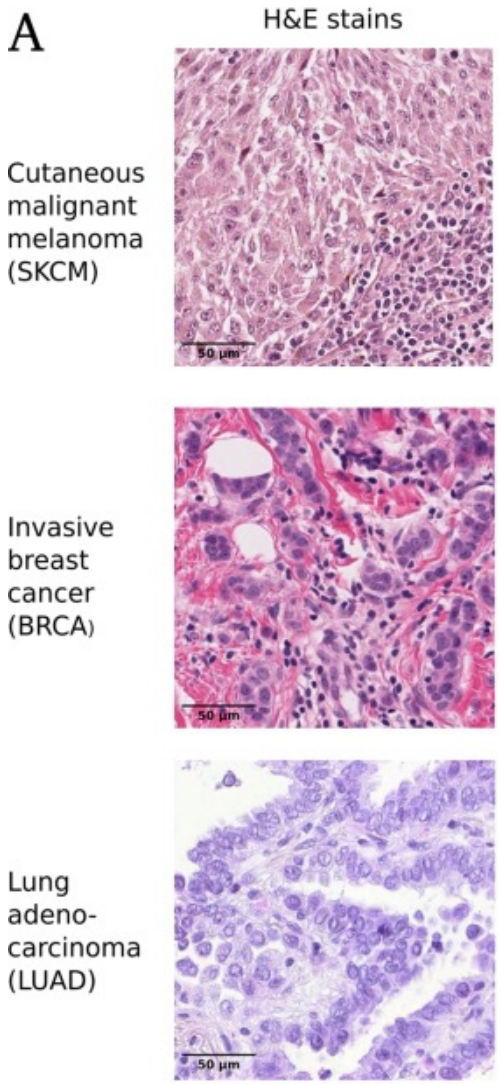


Golgi stain



# Stains used for studying neuroanatomy

| Stain | Use | Appearance | Comments |
|---|---|---|---|
| Cresyl violet (Nissl stain) | Cell nuclei | Blue to purple | Useful for examining cytoarchitecture; stains each type of neuron slightly differently |
| Hematoxylin | Cell nuclei | Blue to blue-black | Often used in combination with eosin; known as H&E |
| Eosin Y | Cytoplasm | Pink to red | Counterstain with hematoxylin; acidophilic stain |
| Thionine (Nissl stain) | Cell nuclei | Blue to purple | |
| Methylene blue | Cell nuclei | Blue | Can be perfused through the brain before fixation |
| Toluidine blue | Cell nuclei | Nucleus is stained blue; cytoplasm light blue | Often used to stain frozen sections |
| DAPI | Cell nuclei | Fluorescent blue | Fluorescent DNA intercalating agent; excited by UV illumination |
| Hoechst (bis-benzamide) | Cell nuclei | Fluorescent blue | Fluorescent DNA intercalating agent; excited by UV illumination |
| Propidium iodide (PI) | Cell nuclei | Fluorescent red | Fluorescent DNA intercalating agent; excited by green light illumination |
| Weigert | Myelin | Normal myelin is deep blue; degenerated myelin is light yellow | Combines hematoxylin with other chemicals to selectively stain myelin |
| Weil | Myelin | Black | Combines hematoxylin with other chemicals to selectively stain myelin |
| Luxol fast blue (LFB) | Myelin | Blue | |
| Golgi stain | Fills neuron cell bodies and processes | Black | Stains individual neurons at random |




*Hagele et al., Nat Sci Rep, 2020*

- Better understand how the brain processes information
- Examine tissue samples for disease
- Study influence of physical impacts on brain tissue

# Visualizing neural circuitry

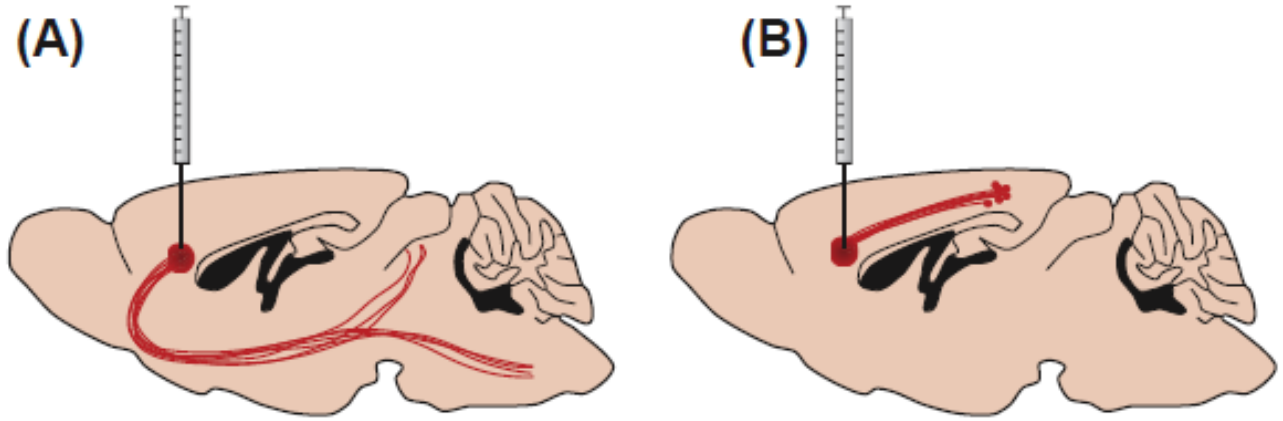



**FIGURE 6.9 Anterograde and retrograde tracers.** (A) Anterograde tracers show efferent projections, revealing regions that *receive* projections from cells in the labeled area. (B) Retrograde tracers show afferent projections, indicating regions that *project to* the labeled area.

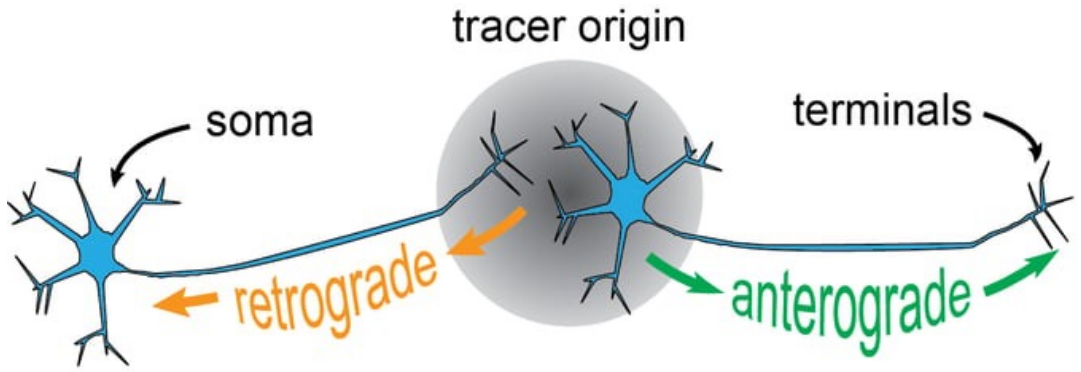
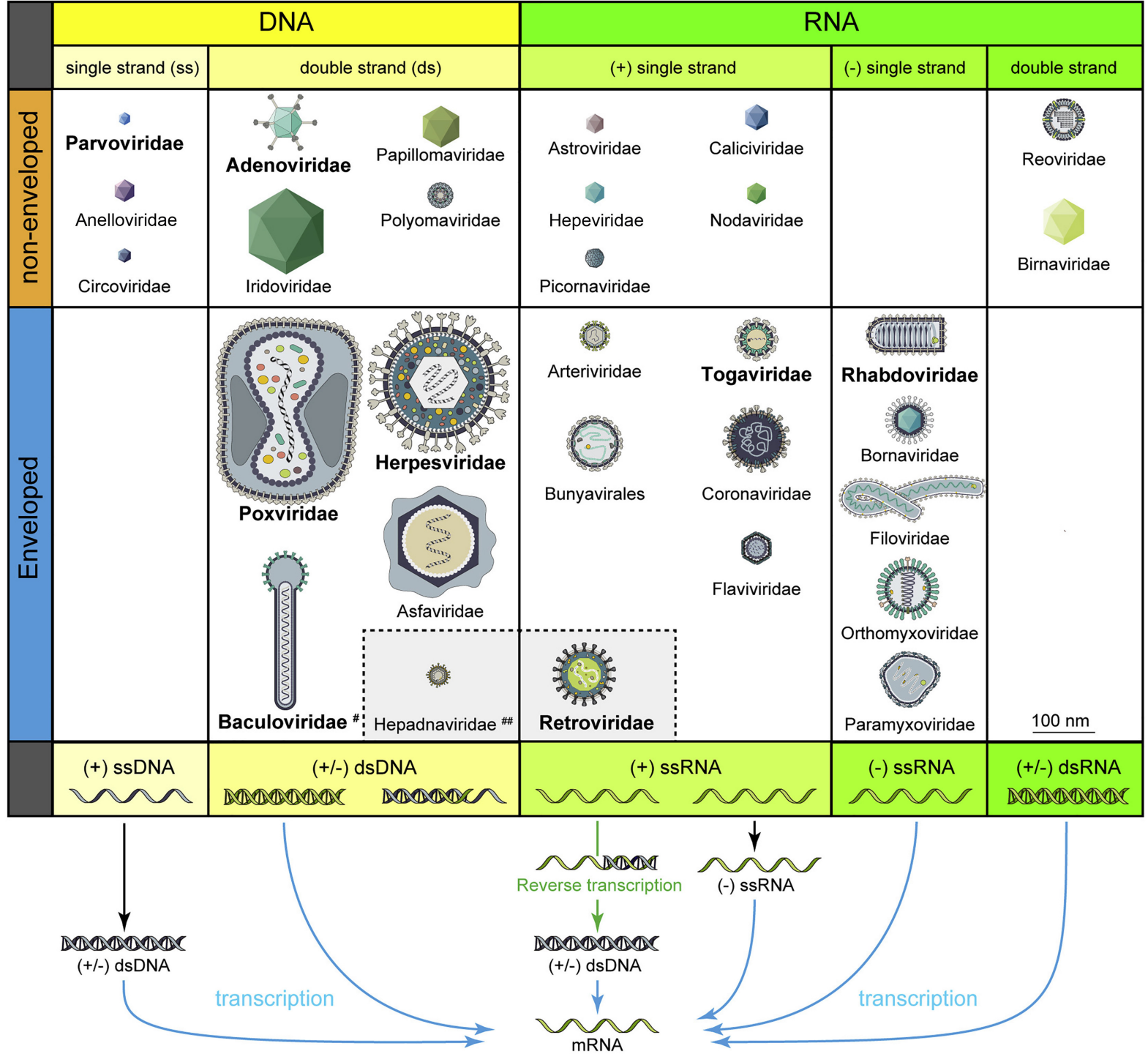


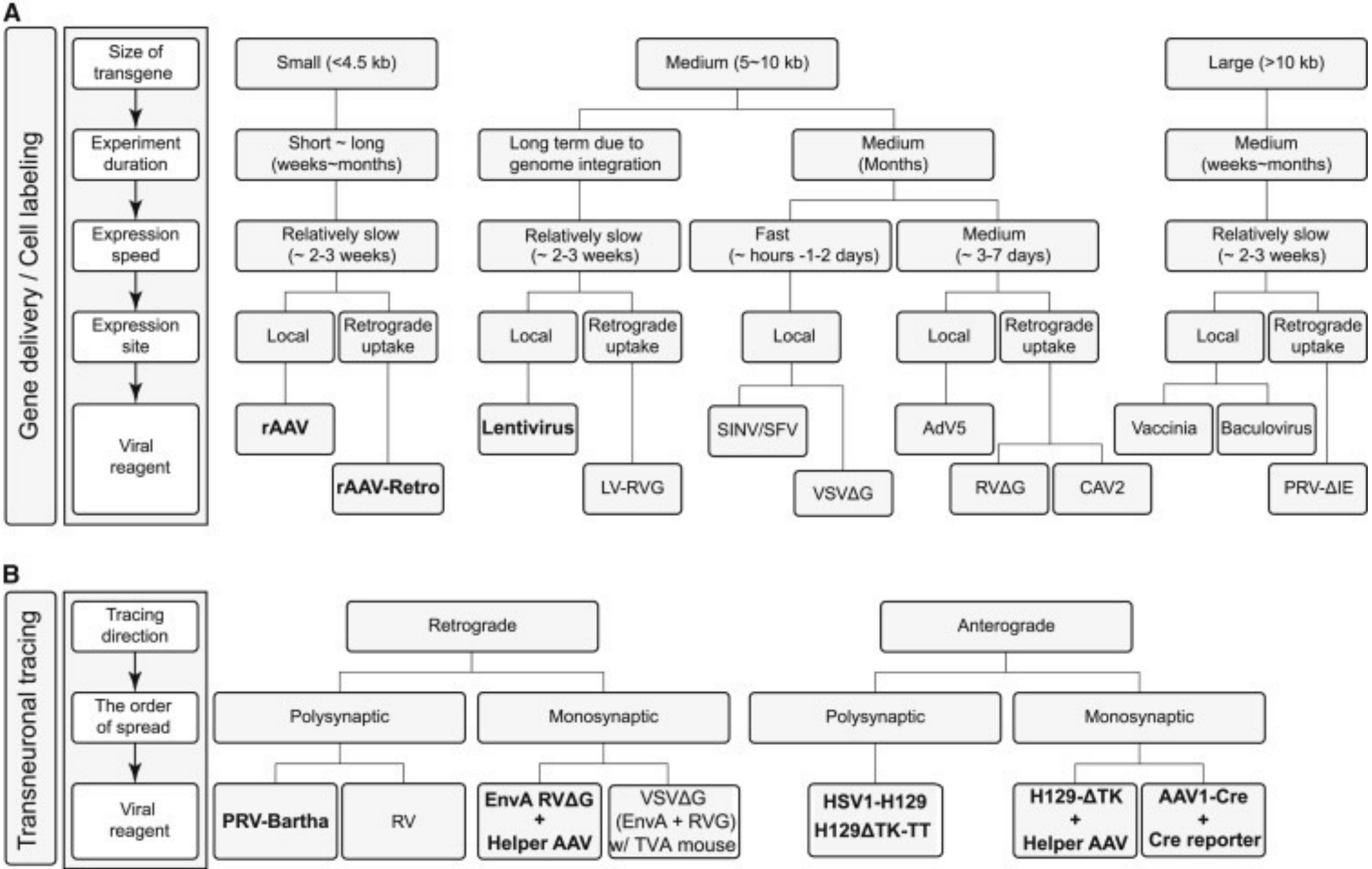

***Figure 2. Depiction of neurons and two types of neuronal tracing.*** *Retrograde tracers (left side, orange) travel within a neuron from the neuron terminals to the soma. Anterograde tracers (right side, green) travel within a neuron from the soma to the terminals. The direction of travel is relative to the site of tracer origin/delivery, which is depicted by a gray circle.*

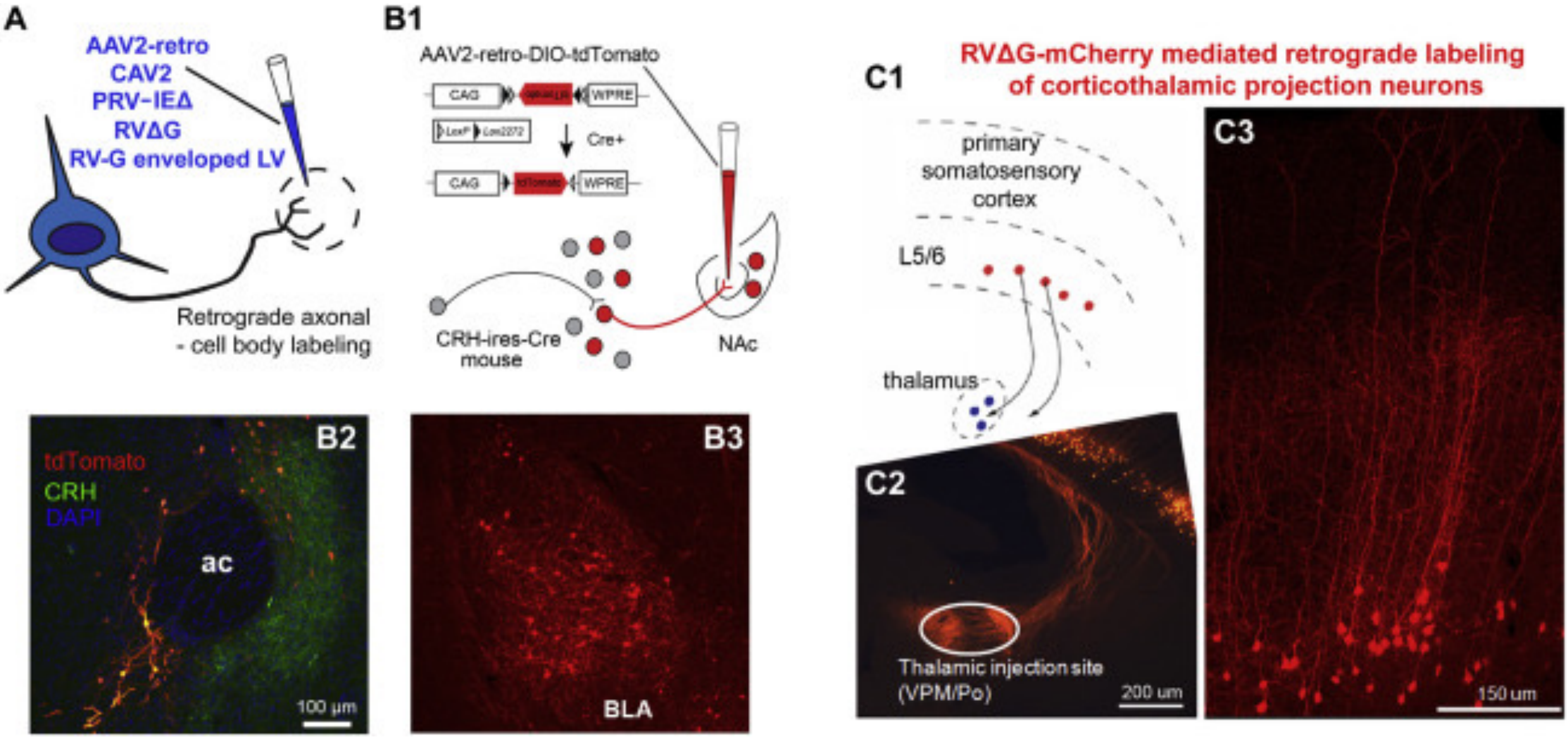
Carter, Matt, and Jennifer C. Shieh. Guide to Research Techniques in Neuroscience, Elsevier Science & Technology, 2015.

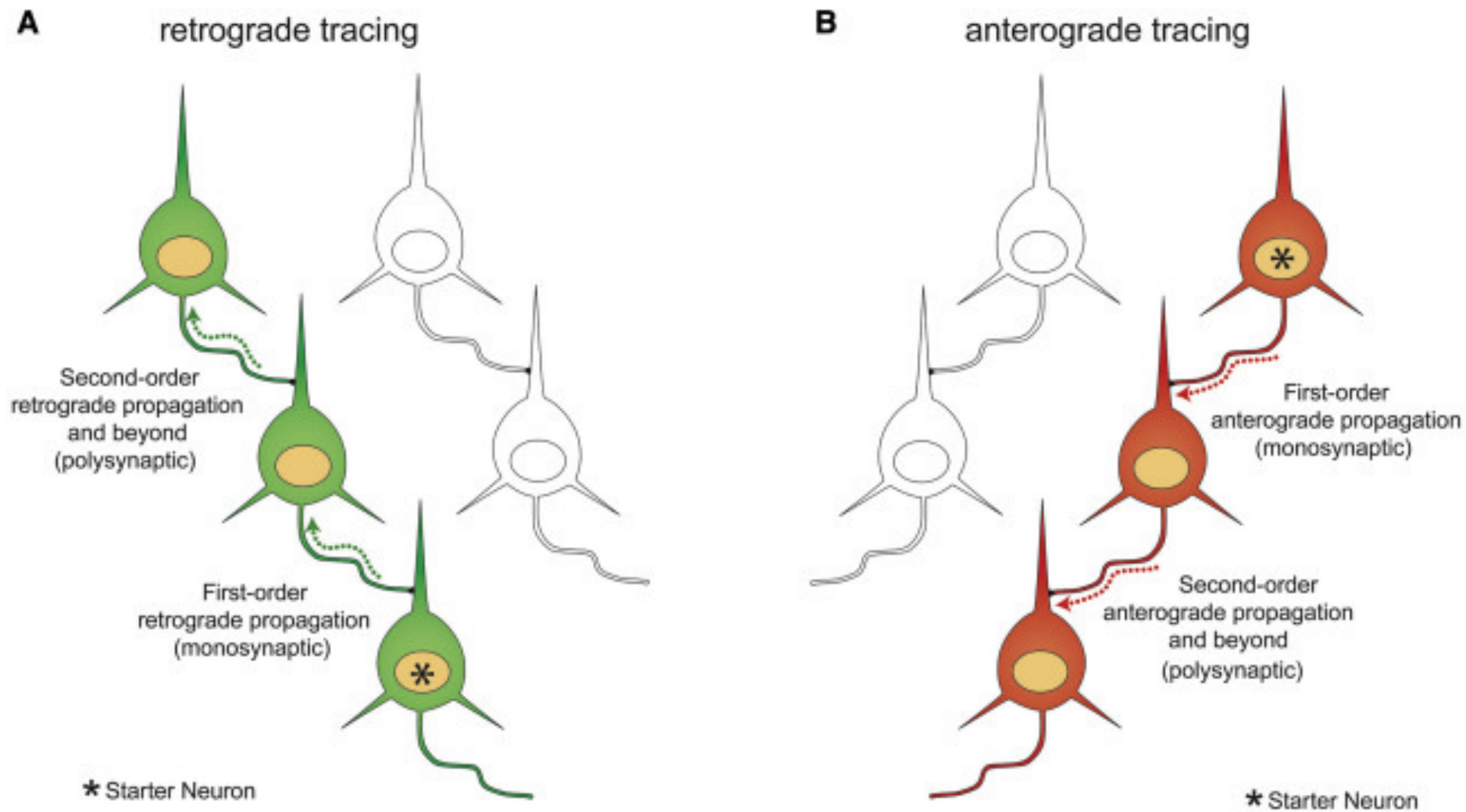
| Tracer | Direction | Comments |
|---|---|---|
| Horseradish peroxidase (HRP) | Retrograde | Produces brown precipitate after reaction with hydrogen peroxide and DAB (diaminobenzidine) |
| Fluorescent microspheres | Retrograde | Available in many different colors; nontoxic |
| Fluoro-gold | Retrograde | Widely used, rapid labeling |
| Diamidino yellow | Retrograde | Produces yellow fluorescence |
| Fast blue | Retrograde | Stable, rapid labeling; produces blue fluorescence |
| Cholera toxin, subunit B (CTB) | Retrograde (may also be anterograde) | May also be anterograde |
| DiI, DiO | Anterograde and retrograde | Lipophilic dye crystals |
| Biotinylated dextran amine (BDA) | Anterograde and retrograde | Widely used; direction of transport depends on molecular weight and pH; can be visualized by EM |
| Phaseolus vulgaris leucoagglutinin (PHA-L) | Anterograde | Plant lectin; can be visualized by EM |

| Tracer | Direction | Comments |
| --- | --- | --- |
| Tritiated amino acids (3H-proline, 3H-leucine) | Transsynaptic (anterograde) | Detected using autoradiography |
| Wheat germ agglutinin (WGA) | Transsynaptic | Plant lectin; anterograde and retrograde transport possible; often conjugated to HRP for detection; transgene encoding WGA can be used to label genetically defined neural circuits |
| Tetanus toxin, fragment C (TTC) | Transsynaptic (retrograde) | Transgene encoding TTC can be used to label genetically defined neural circuits; nontoxic fragment |
| Pseudorabies virus (PRV) | Transsynaptic | Does not infect primates, including humans; bartha strain most commonly used for tracing studies; less virulent, only retrograde transport |
| Herpes simplex virus (HSV) | Transsynaptic | Broad host range, including humans |


DNA
RNA
single strand (ss)
double strand (ds)
(+) single strand
(-) single strand
double strand
non-enveloped
Enveloped
Parvoviridae
Anelloviridae
Circoviridae
Adenoviridae
Papillomaviridae
Polyomaviridae
Iridoviridae
Astroviridae
Caliciviridae
Hepeviridae
Nodaviridae
Picornaviridae
Reoviridae
Birnaviridae
Poxviridae
Herpesviridae
Asfaviridae
Baculoviridae #
Hepadnaviridae ##
Arteriviridae
Togaviridae
Bunyavirales
Coronaviridae
Flaviviridae
Retroviridae
Rhabdoviridae
Bornaviridae
Filoviridae
Orthomyxoviridae
Paramyxoviridae
100 nm
(+) ssDNA
(+/-) dsDNA
(+) ssRNA
(-) ssRNA
(+/-) dsRNA
Reverse transcription
(-) ssRNA
(+/-) dsDNA
transcription
(+/-) dsDNA
transcription
mRNA


A
Gene delivery / Cell labeling
Size of transgene
Experiment duration
Expression speed
Expression site
Viral reagent
Small (<4.5 kb)
Short ~ long (weeks~months)
Relatively slow (~ 2-3 weeks)
Local
Retrograde uptake
rAAV
rAAV-Retro
Medium (5~10 kb)
Long term due to genome integration
Relatively slow (~ 2-3 weeks)
Local
Retrograde uptake
Lentivirus
LV-RVG
Medium (Months)
Fast (~ hours -1-2 days)
Local
SINV/SFV
VSVΔG
Medium (~ 3-7 days)
Local
Retrograde uptake
AdV5
RVΔG
CAV2
Large (>10 kb)
Medium (weeks~months)
Relatively slow (~ 2-3 weeks)
Local
Retrograde uptake
Vaccinia
Baculovirus
PRV-ΔIE
B
Transneuronal tracing
Tracing direction
The order of spread
Viral reagent
Retrograde
Polysynaptic
PRV-Bartha
RV
Monosynaptic
EnvA RVΔG + Helper AAV
VSVΔG (EnvA + RVG) w/ TVA mouse
Anterograde
Polysynaptic
HSV1-H129 H129ΔTK-TT
Monosynaptic
H129-ΔTK + Helper AAV
AAV1-Cre + Cre reporter


A
AAV2-retro
CAV2
PRV-IEΔ
RVΔG
RV-G enveloped LV
Retrograde axonal
- cell body labeling
B1
AAV2-retro-DIO-tdTomato
CAG
WPRE
Cre+
CAG
WPRE
CRH-ires-Cre
mouse
NAc
B2
tdTomato
CRH
DAPI
ac
100 μm
B3
BLA
C1
RVΔG-mCherry mediated retrograde labeling
of corticothalamic projection neurons
primary
somatosensory
cortex
L5/6
thalamus
C2
Thalamic injection site
(VPM/Po)
200 um
C3
150 um


A
retrograde tracing
Second-order
retrograde propagation
and beyond
(polysynaptic)
First-order
retrograde propagation
(monosynaptic)
* Starter Neuron
B
anterograde tracing
First-order
anterograde propagation
(monosynaptic)
Second-order
anterograde propagation
and beyond
(polysynaptic)
* Starter Neuron