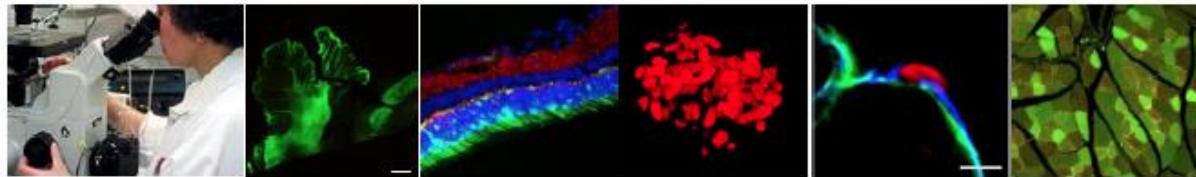




Fluorescence Bio-imaging practical courses

Gene GFP transfert in the brain after intracerebral administration by using AAV-eGFP

Laurence Dubreil/Steven Nedelec/Johan Deniaud/Candice Babarit/Helicia Goubin





Phenotyping of brain transduced nervous cells

- 1- Cryosections of brain
- 2- Observation of GFP expression in brain section
 - Widefield fluorescence microscope
- 3- Observation of KB stain brain section and mapping of brain structure
 - Brightfield microscope
- 4- Immunolabeling of nervous cells
 - Neurons
 - Microglial cells
 - Oligodendrocytes
 - Astrocytes : GFAP
- 5- Observations of immunolabeling and colocalisation with GFP
 - Widefield fluorescence microscope
 - Confocal microscope
- 6- Separation of autofluorescence from specific fluorescence signal by spectral confocal microscopy

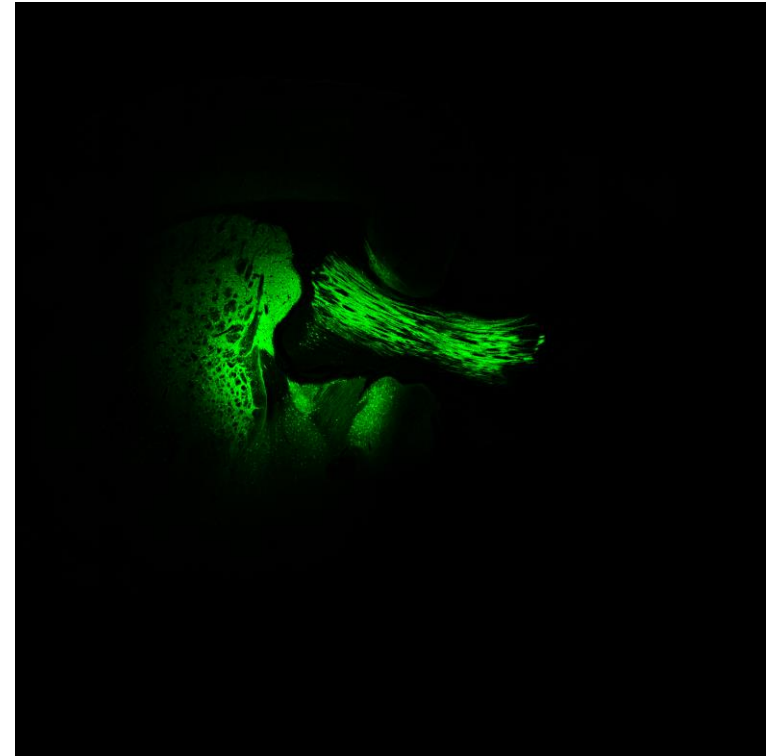
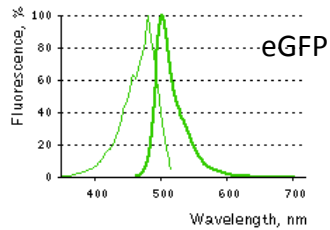
Mapping of GFP expression in the brain section



- GFP

Laser : argon ion laser 488nm
Emission Band pass : 500-530 nm

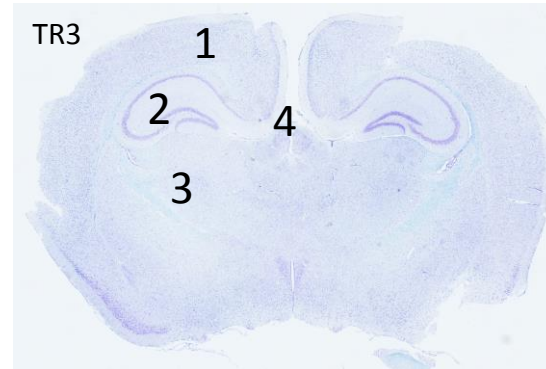
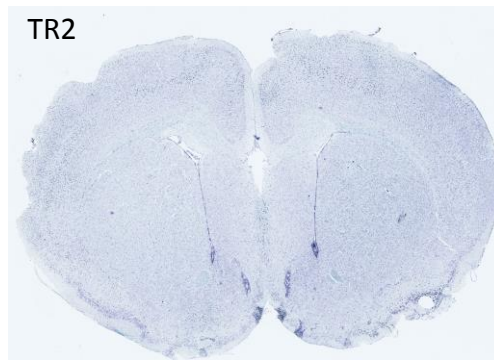
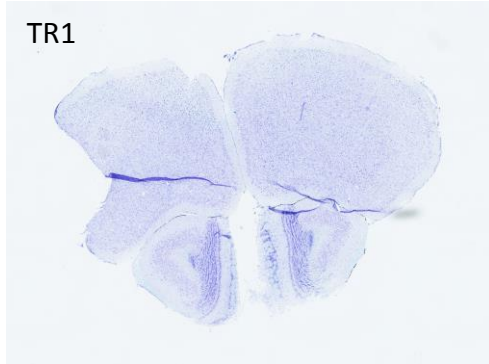
Spectra :



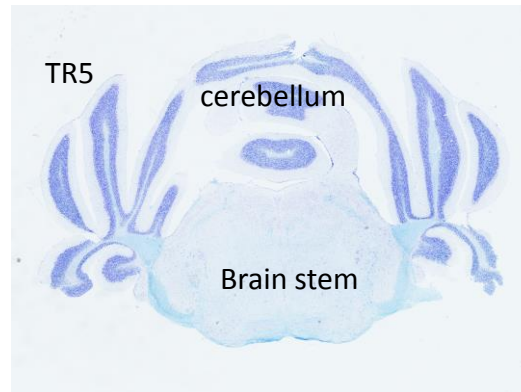
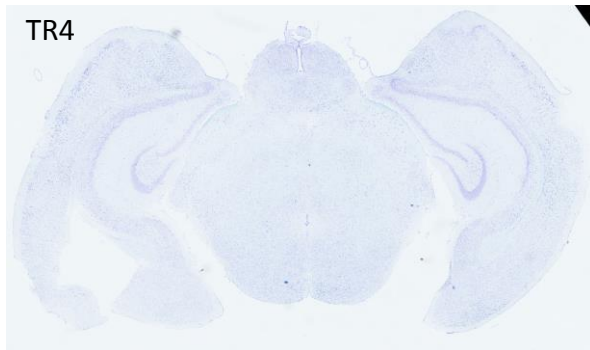


Mapping of brain structure on KB brain section

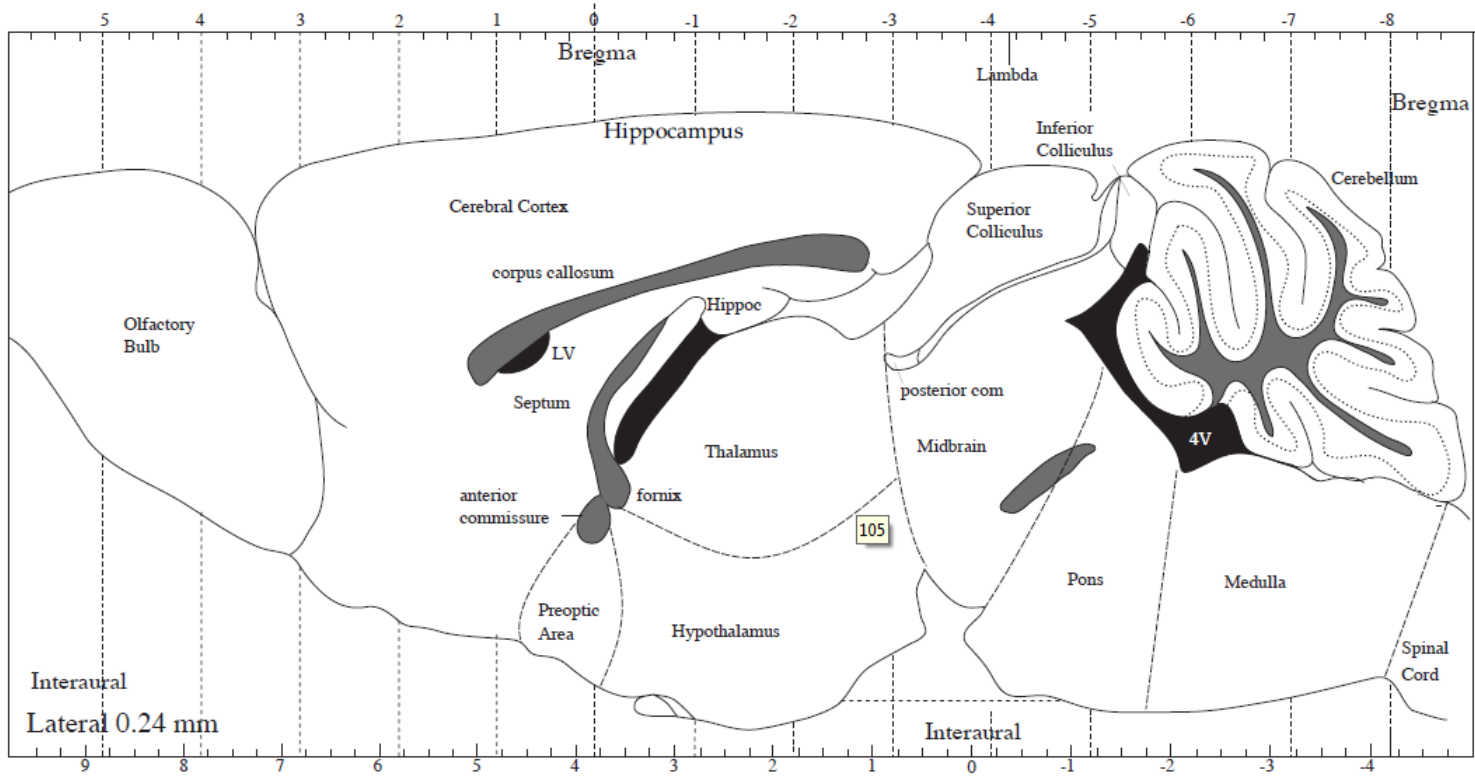
Klüver and Barrera staining



- 1- cerebral cortex
- 2- hippocampus
- 3- thalamus
- 4- corpus callosum



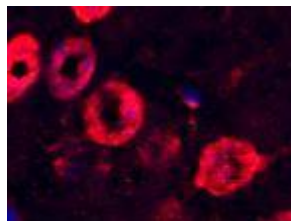
Sagittal brain section



Immunolabeling of brain nervous cells

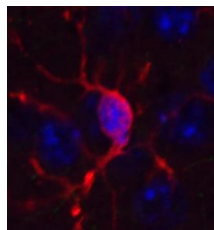
- Neun : neuronal marker

- electrically excitable cell



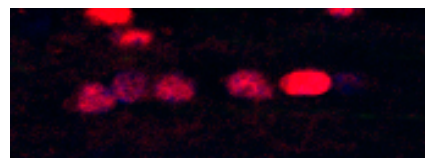
- Iba1: microglial cells marker

- resident macrophages of the brain



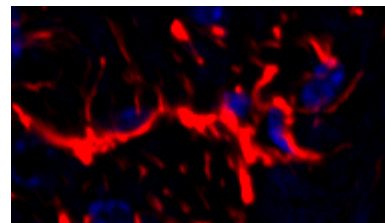
- Olig2 : Oligodendrocytes marker

- support and insulation to axons in the central nervous system



- Glial fibrillary acidic protein (GFAP) : Astrocytes Marker

- many functions : form the blood-brain barrier,
- provision of nutrients, role in the repair



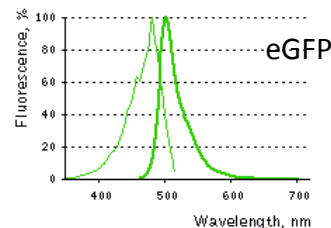
Colocalisation of GFP and cells immunolabeled



- GFP

Spectra :

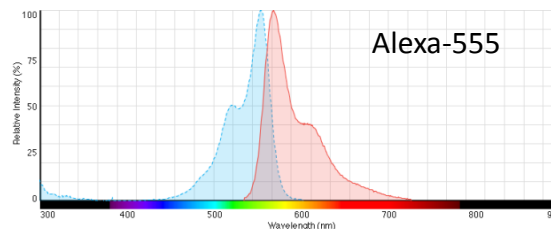
Laser : Argon ion laser 488nm
Emission Band pass : 500-530 nm



- Alexa-555 (neun, Iba1, olig2, GFAP)

Spectra :

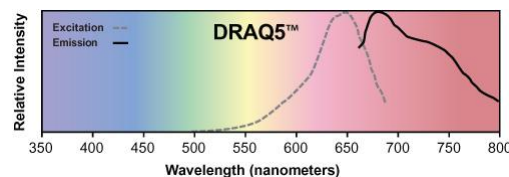
Laser : HeNe laser 543nm
Emission Band pass: 575-615 nm



- DRAQ5 (nuclei)

Spectra :

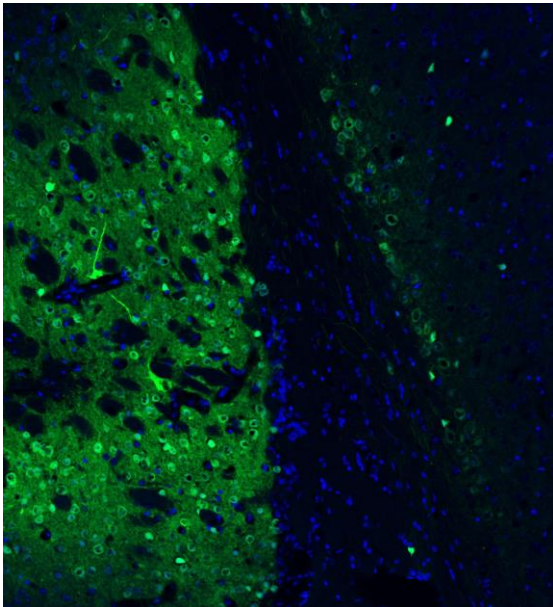
Laser : HeNe laser 633nm Long Pass : 650 nm



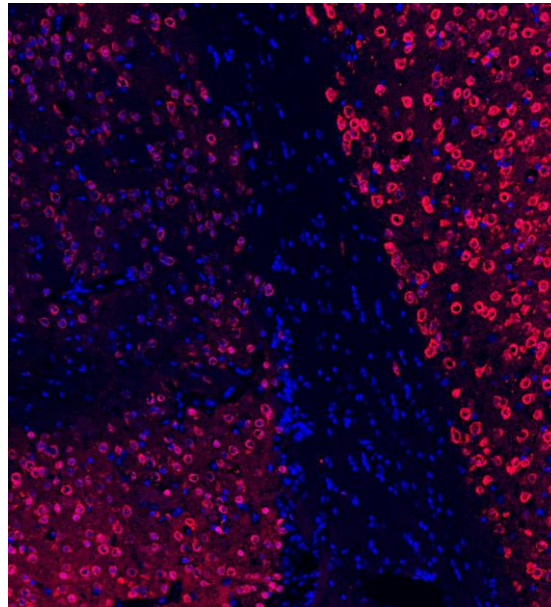


GFP expression in brain neurons, channel acquisition

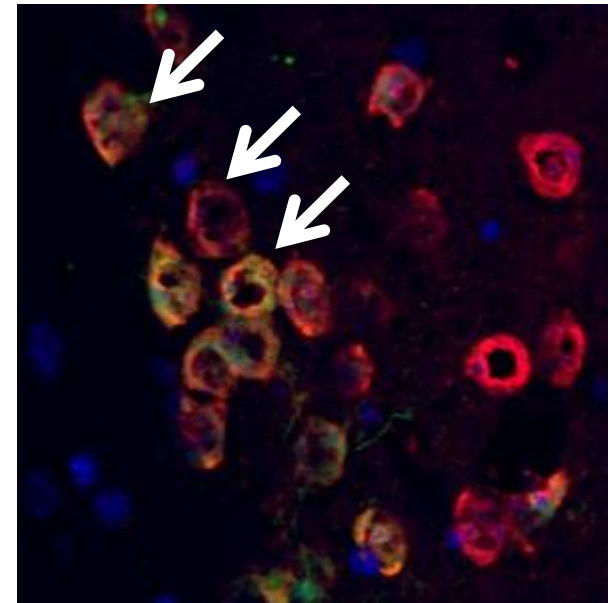
GFP



Neun



Merge



GFP expression in brain neurons, spectral imaging

The screenshot displays the Zen software interface for microscope acquisition and imaging. The main window is divided into several panels:

- Acquisition Parameter:** Shows settings for Objective (Plan ApoChromat 20x/0.75 M27), Scan Mode (Frame), Frame Size (X 1024, Y 1024), Line Step, Speed (Pixel Dwell: 1.27 µsec, Scan Time: 3.13 sec), Averaging (Number, Mode, Method), and Scan Area (Image Size: 326.7 µm x 326.7 µm, Pixel Size: 0.32 µm).
- Channels:** Lists tracks and channels for acquisition.
- Track Configuration:** Details the Lambda - Online Fingerprinting setup, including laser lines (488 nm, 561 nm, 633 nm), pinhole size (1.9 µm section), and detector settings for RS1, RS2, and RS3.
- Experiment Manager:** Includes Setup Manager (Lasers, Laser Properties, Laser Lines) and a Lambda plot showing emission spectra.

The central display area shows a 2x3 grid of fluorescence images:

- Top-left: **autofluorescence** (dark image)
- Top-middle: **Neun al555** (red fluorescence)
- Top-right: **DRAQ5** (blue fluorescence)
- Bottom-left: **GFP** (green fluorescence)
- Bottom-middle: **Merge** (combined image of all channels)
- Bottom-right: A large black area, likely a placeholder for another channel.

The bottom of the interface shows system status (CPU 0%, Free HD 1.8 TB, Free Ram 12 GB) and a Windows taskbar.



Thanks to UMR 703 INRA/Oniris PAnTher

- Marie-Anne Colle
- Karim Bey
- Mathilde Mullard
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