Fluorescence Bio-imaging practical courses

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

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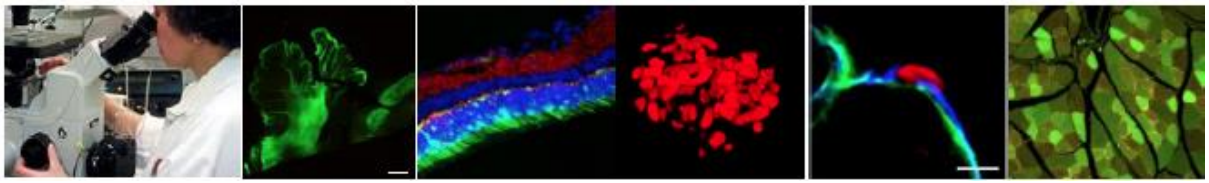


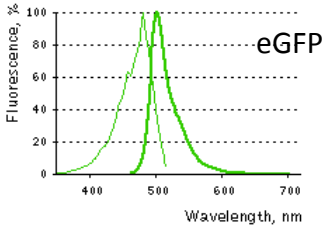
Phenotyping of brain transduced nervous cells

1- Cryosections of brain
2- Observation of GFP expression in brain section
    - Wildefield 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
    - Wildefield 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

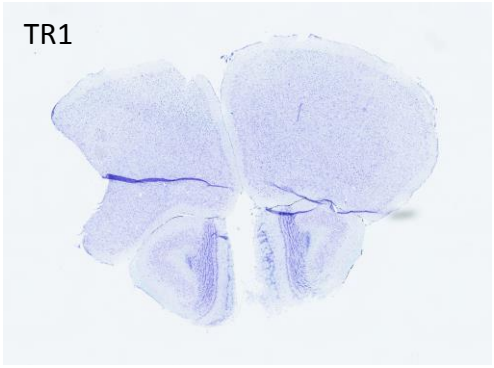
Spectra :

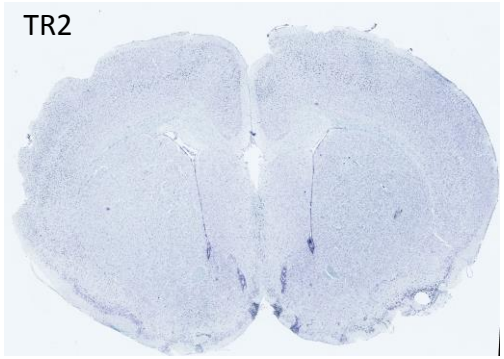


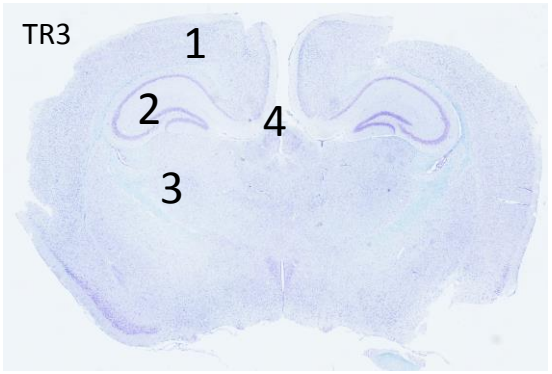
Laser : argon ion laser 488nm
Emission Band pass : 500-530 nm

Mapping of brain structure on KB brain section

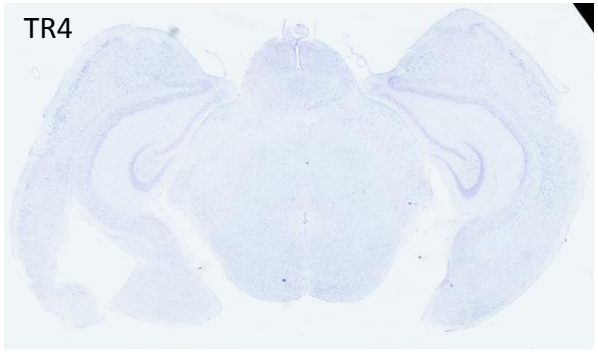
Klüver and Barrera staining

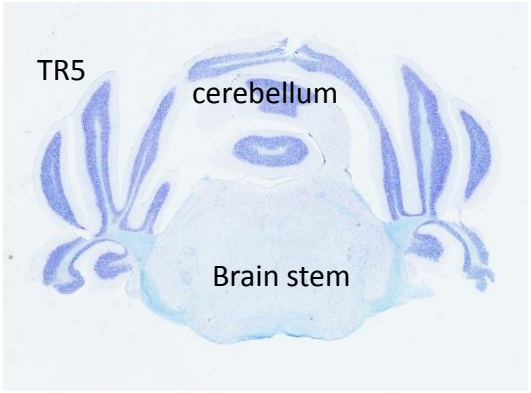




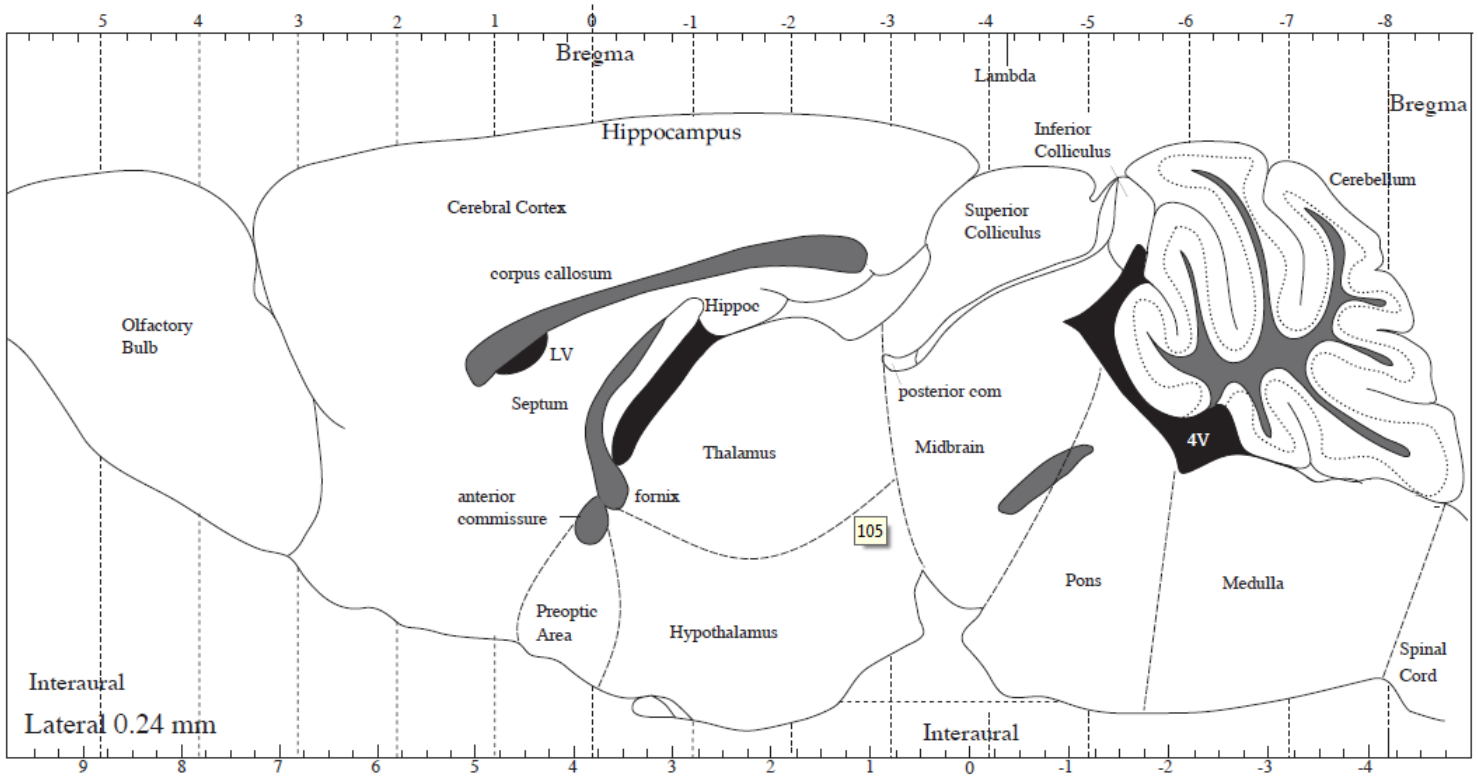


1- cerebral cortex
2- hippocampus
3- thalamus
4- corpus callosum





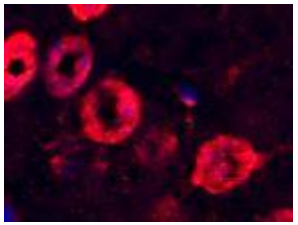
Sagital brain section



Immunolabeling of brain nervous cells

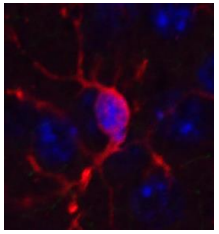
- Neun : neuronal marker

  - electrically excitable cell



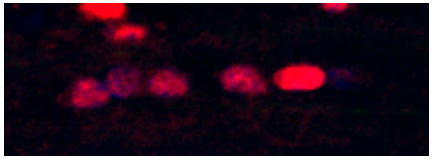
- Iba1: microglial cells marker

  - resident macrophages of the brain



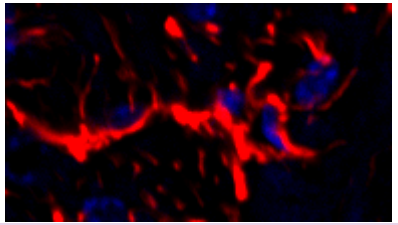
- Olig2 : Oligodendrocytes marker

  - 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

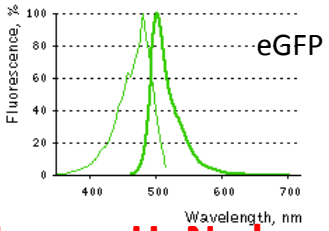


Colocalisation of GFP and cells immunolabeled

- GFP

Spectra :

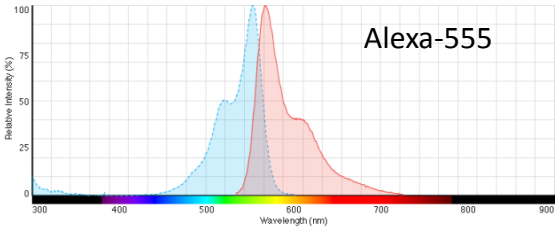
Laser : Argon ion laser 488nm
Emission Band pass : 500-530 nm



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

Spectra :

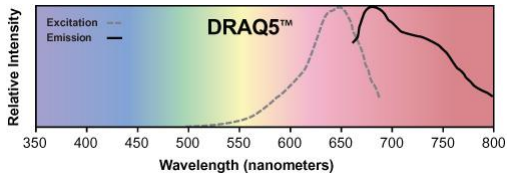
Laser : HeNe laser 543nm
Emission Band pass: 575-615 nm



- DRAQ5 (nuclei)

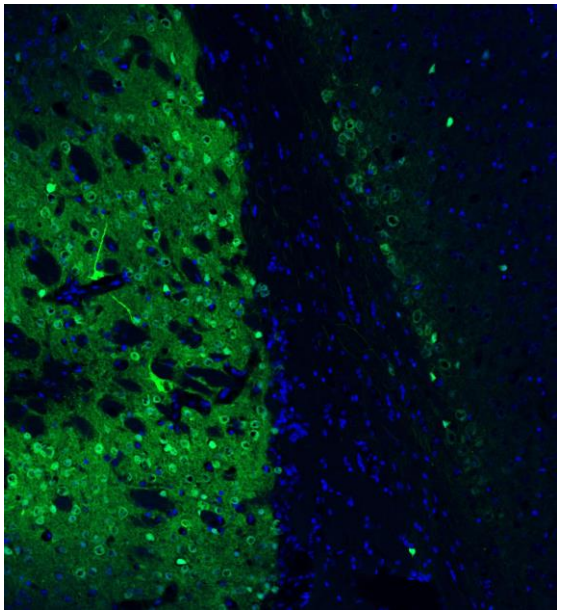
Spectra :

Laser : HeNe laser 633nm Long Pass : 650 nm

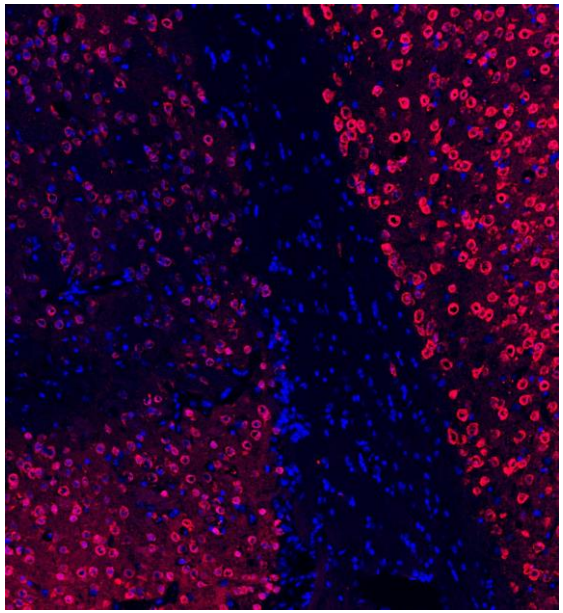


GFP expression in brain neurons, channel acquisition

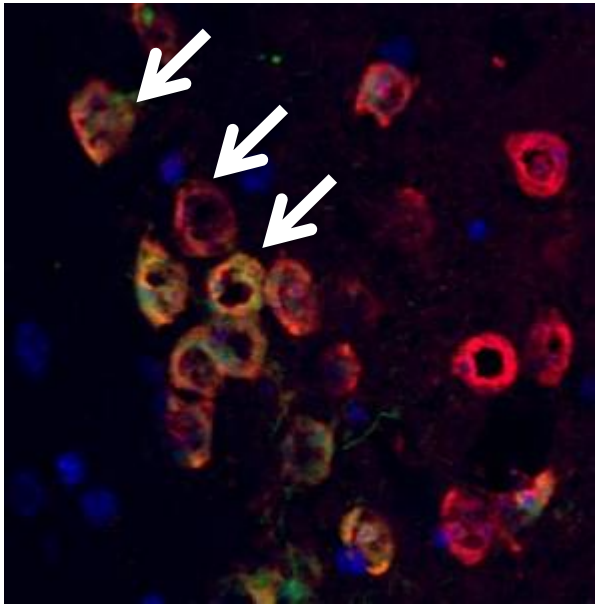
GFP



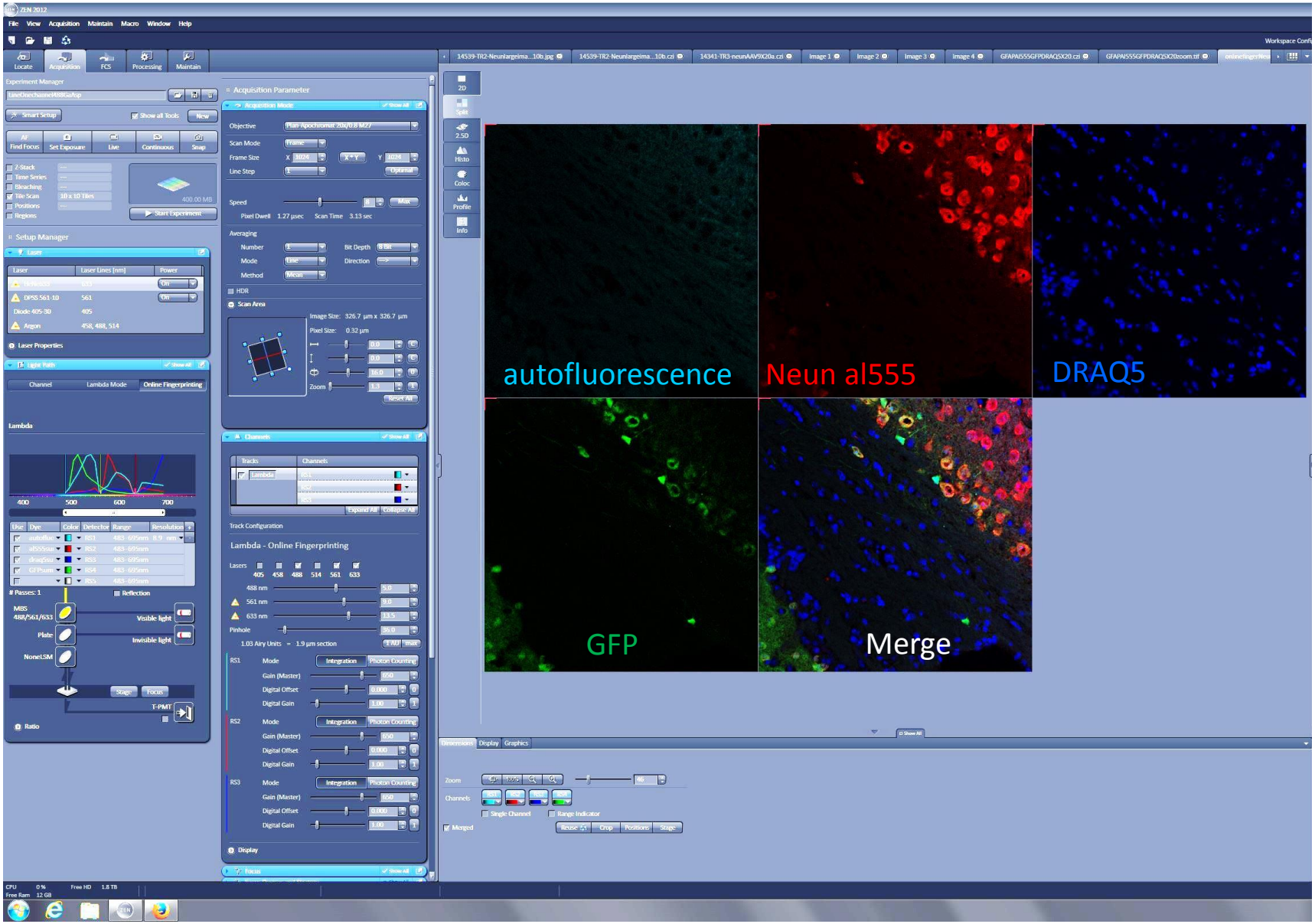
Neun



Merge



GFP expression in brain neurons, spectral imaging



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