Confocal microscopy, a major tool for the cell and gene therapy of the muscle and nervous central system.

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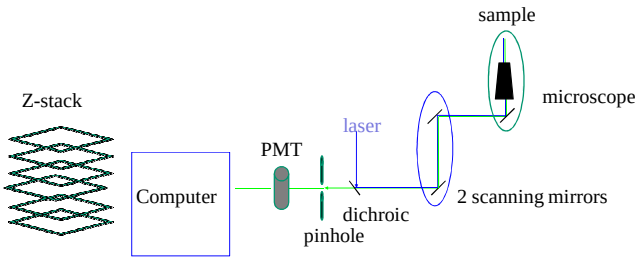
Confocal Microscopy a Major Tool for the Cell and Gene Therapy of Muscle and Central Nervous System

UMR 703 PAnTher INRA/Oniris
Animal pathophysiology and biotherapy for muscle and nervous system diseases
Oniris, Nantes-Atlantic College of Veterinary Medicine and Food Sciences

The specific research areas of the UMR 703 are focused on cell and gene therapies of Duchenne Muscular Dystrophy (DMD) and Pompe disease (glycogenosis type II), a lysosomal storage disease with both CNS and muscle involvement. The unit has also developed a pathology core APEX. Histopathological expertise is the main mission of APEX by providing pathology and phenotypic analysis to support both academic and private research teams. Confocal microscopy is a major tool to explore cells and tissues in biotherapy development and pathophysiology characterization.
In confocal microscopy, the presence of a pinhole allows the elimination of the background information away from the focal plane and the acquisition of serial optical sections from thick specimens to build .

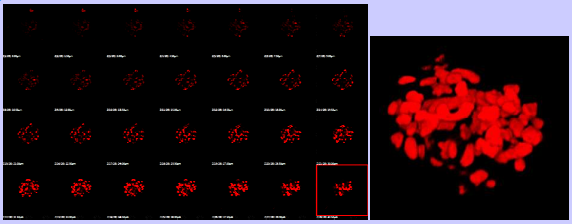
Simplified schematic representation of confocal microscope



Examples of confocal microscopy applications in muscle and nervous central system biotherapies:

1. Confocal 3D imaging of cultured cells, brain, muscle and retina;
2. Cartography of gene expression in brain, after injection of AAV-egfp;
3. Quantification of colocalization in cells;
4. Spectral separation of GFP specific signal from autofluorescence by linear unmixing.

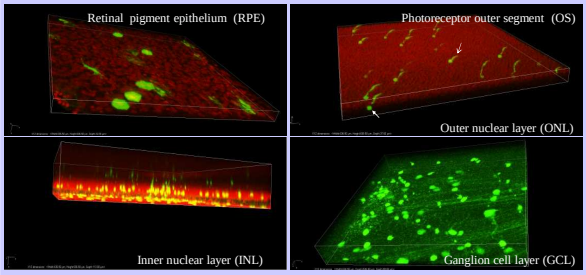
1 Confocal 3D imaging of cultured cells



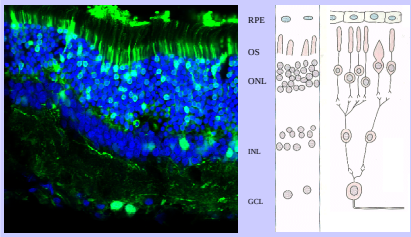
Z stack from cultured Mustem cells. Topro3 DNA staining.
MuStem cells proliferate in suspension as myospheres.

Am J Pathol 179(5): 2501-18, 2011

Confocal 3D imaging of a rat retinal wholemount



Profil GFP expression in the retina after IV injection of AAV10-egfp



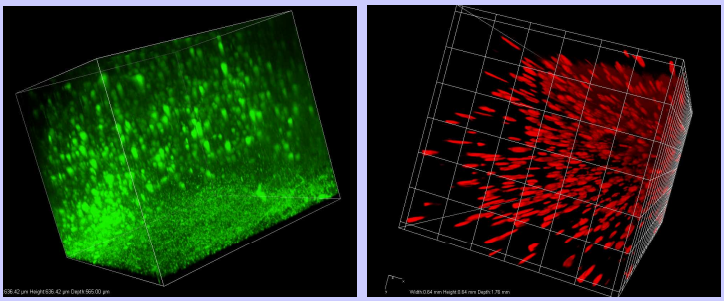
Cryosections of retina transduced ganglion cells are underestimated compared to the numerous transduced ganglion cells observed on retinal wholemount (non invasive method).

1 Confocal 3D imaging of brain and muscle specimens treated by Scale medium*

The tissue transparency was increased by using Scale*, a chemical reagent, allowing greater depth of imaging to explore transgene expression in brain or nuclear distribution in muscle after gene or cell therapy.

Scaled brain
GFP expression

Scaled muscle
Topro3 DNA staining

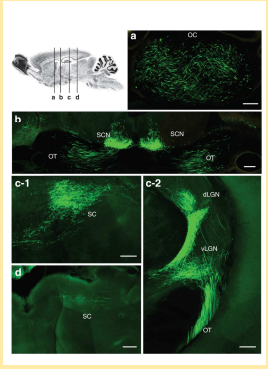


Depth 565 µm

Depth 1760 µm

Description of Scale medium in Nat Neurosci 14:1461-1468, 2011

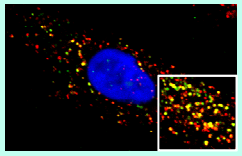
2 Profil of AAV8-egfp expression in brain after subretinal injection



Cartography of GFP expression in brain was possible by using confocal microscope to analyse thick coronal slices of 100µm.

Mol Ther 6(5): 916-2, 2008

3 Colocalization of α-glucosidase (green) and lysosomes (lamp2, red) in fibroblaste from patient affected with type II glycogenolyse

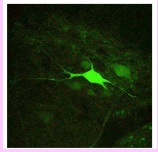


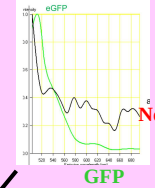
Colocalized pixels

Quantification of colocalization
Pearson's correlation (Rr) = 0.683
Overlap coeff Manders (R) = 0.744
Colocalization coeff. M1 = 0.800
Colocalization coeff M2 = 0.999
(Plugin colocalization image j)

4 Spectral imaging and the linear unmixing, a power tool to separate the signal of fluorophores having close spectra

Image without unmixing





Spectral imaging and linear unmixing separate specific eGFP signal from noise (autofluorescence) generated by chemical fixation of the tissues