



APEX, une plateforme d'anatomie pathologique vétérinaire et de phénotypage tissulaire/ cellulaire au service de la recherche

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HAL Id: hal-01594224

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Submitted on 5 Jun 2020

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Jeudi 3 avril 2014
à Saint-Malo

APEX, une plateforme d'anatomie pathologique
vétérinaire et de phénotypage tissulaire/
cellulaire au service de la recherche

Intervenants : Thibaut Larcher, Laurence Dubreil



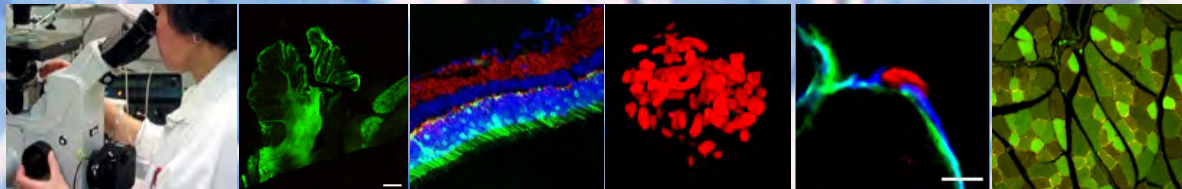
École Nationale
Vétérinaire, Agroalimentaire et de l'Alimentation
Santé et alimentation au cœur de la vie



APEX

Veterinary pathology and fluorescence bio-imaging for animal tissue exploration

Thibaut LARCHER – Laurence DUBREIL



APEX structure

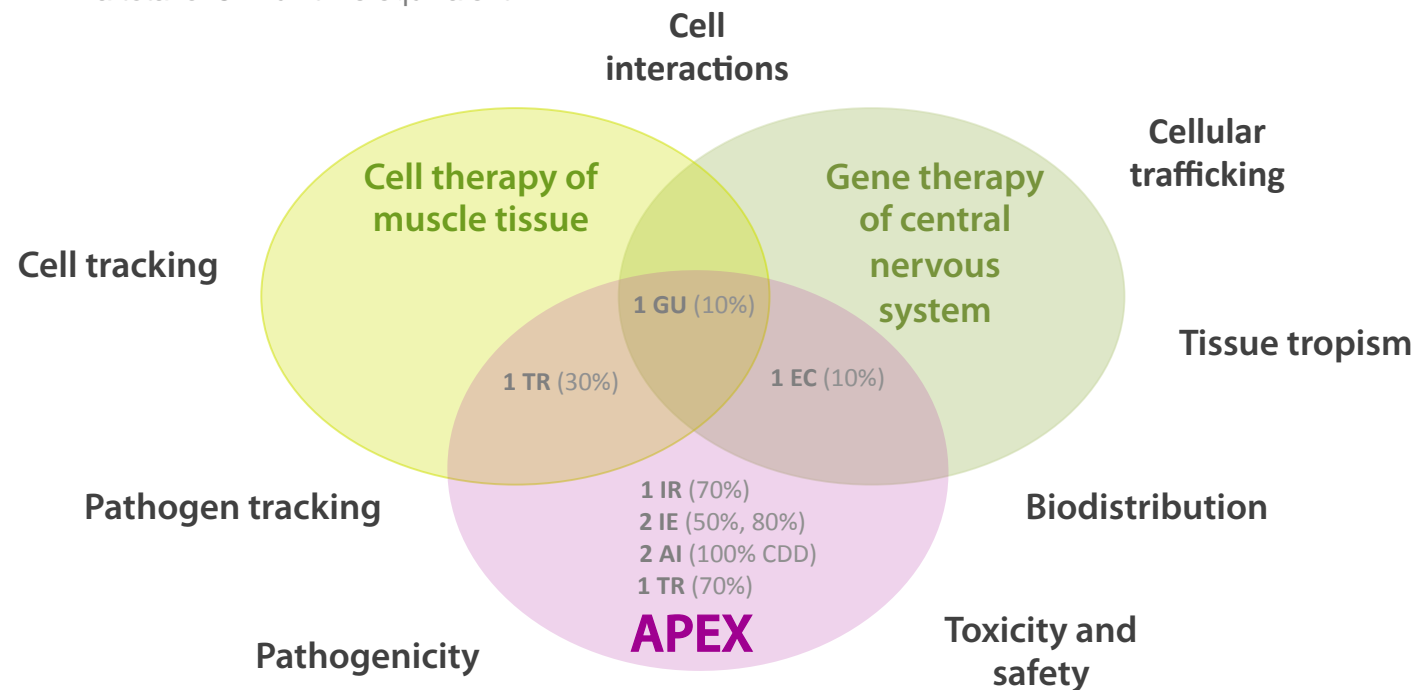
❖ APEX belongs to UMR703 PAnTher INRA-Oniris, Nantes

- #15 permanent staff and 4 PhD students
- headed by Marie-Anne COLLE and Karl ROUGER
- 2 own research axes



❖ APEX, an open platform dedicated to tissue exploration

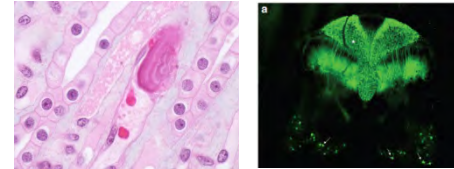
- 2 veterinary pathologists (ECVP diplomates) including a neuropathologist
- 2 engineers (fluorescence bio-imaging and hybridization in situ)
- a total of 5.2 full-time equivalent



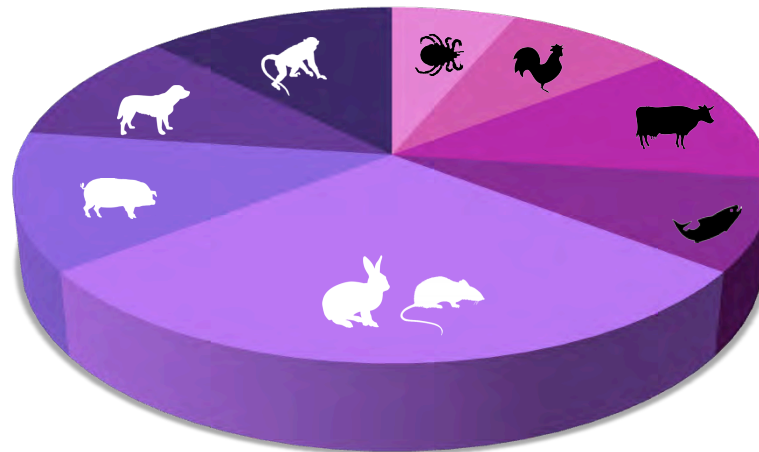
APEX: two main original features

❖ Synergy between two fields of expertise

- Veterinary pathology
- Fluorescence bio-imaging

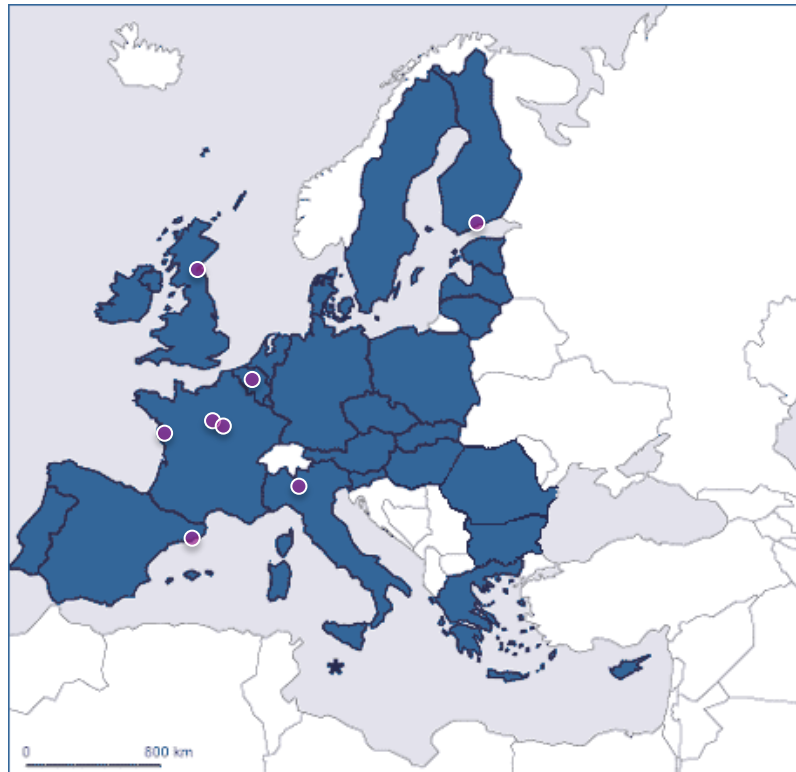


❖ A large spectrum of investigated animal species including **animal models** and **target species**



APEX & other open platforms in Europe

With at least 1 ECVP or ACVP diplomate



CReSA (Barcelona)



Finnish Centre for Laboratory Animal Pathology (Helsinki)



Human histopathology and animal models (Pasteur Institute, Paris)



MIRCen (INSERM-CEA, Fontenay-aux-Roses)



Moredun (Penicuik)



Mouse & Animal Pathology Lab (Milan)



VIB – Center for the Biology of Disease (Louvain)



An integrated technical offer for tissue phenotyping

❖ Tissue sampling

❖ Tissue trimming

- Vibrating cutting device
- Cryostat
- Microtome
- Ultramicrotome

>1100 blocks and >2200 slides per year

❖ Topographic staining

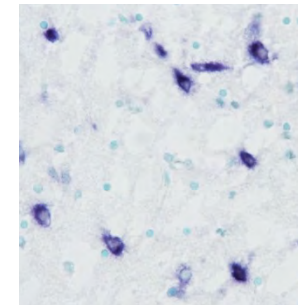
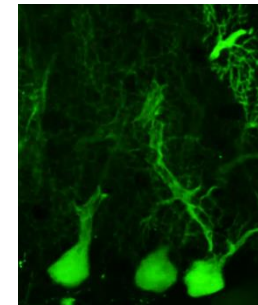
❖ Immunostaining and histoenzymology

❖ *In situ* hybridization



❖ Fluorescence bio-imaging

❖ Histomorphometry



Training and support

❖ Development

- Multi-labelling (IHC/ISH)
- Tracking
- 3D imaging

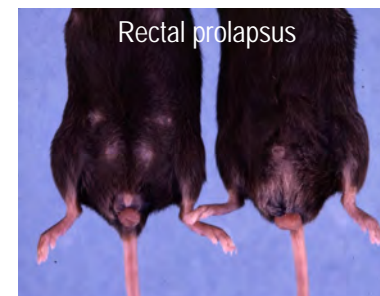
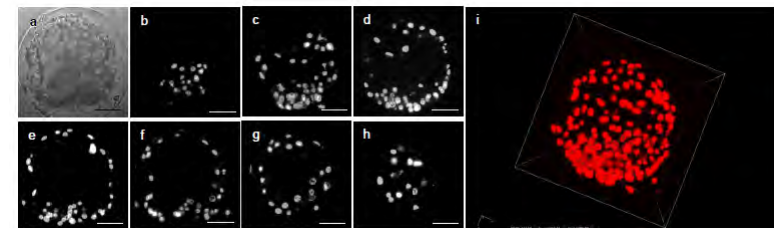
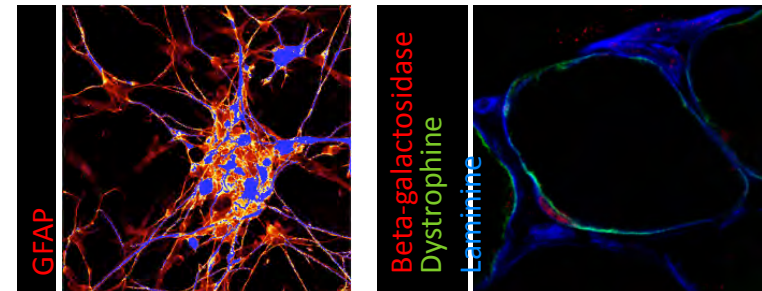
❖ Training

- Tissue sampling on lab animals
- Lesion recognition and description
- Fluorescence bio-imaging
- Histomorphometry
- Workshops (Imaris, Perkin Elmer)
- Tracking
- 3D imaging

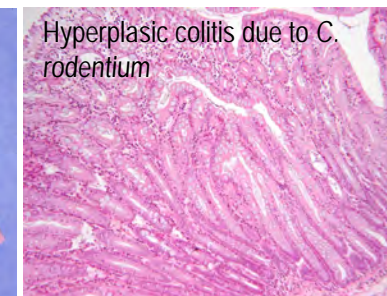
10 individual training sessions since 2010 and 6 seminars for research actors
A mean of 25 days per year dedicated to training

❖ Necropsy and histological diagnosis

About 15 diagnosis each year regarding morbidity or mortality in lab animal housing facilities



Rectal prolapsus



Hyperplasic colitis due to *C. rodentium*

Access

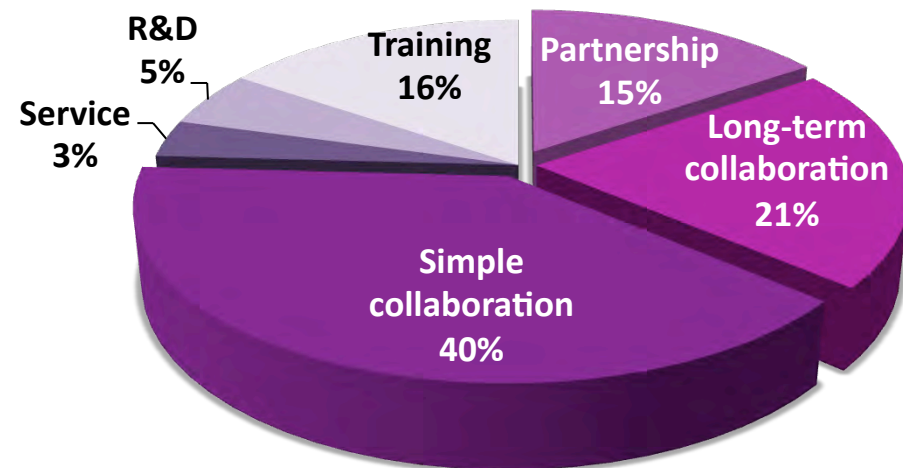
❖ About 20 studies per year

❖ Sources of requests

- INRA-Oniris (other than UMR703) = 52 %
- Other academics = 37 %
- Privates = 11 %

User guideline and costs available online:
www6.inra.fr/anatomie_pathologique_sante_animale

- ❖ Partnership (3 on-going ANR, 1 ICSA, 1 European program)
- ❖ Scientific collaborations (about 10 co-authorships per year in scientific journals)
- ❖ Services
- ❖ Development
- ❖ Training and support



Scientific fields

One World, One Medicine, One Health

❖ Validating animal models

- Spontaneous
- Induced (surgical, targeted mutagenesis)

❖ From tissue and cell tracking to physiopathology

- Multilabelling
- Highly sensitive detection

❖ Ecotoxicology

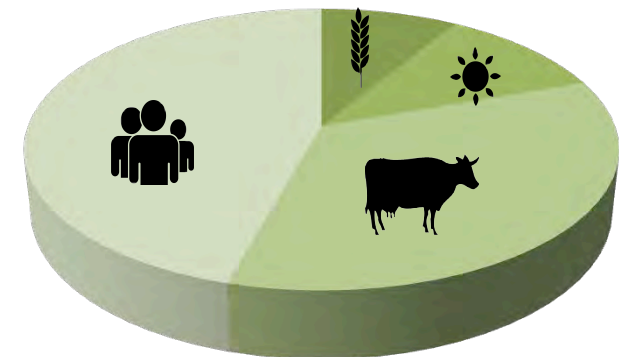
- Whole-organism studies

❖ Preclinical testing

- Efficacy and safety evaluation



BIOTHÉRAPIES ET
PHYSIOPATHOLOGIE ANIMALE



- Alimentation
- Environnement
- Santé animale
- Santé humaine



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Expertise in veterinary pathology



Expertise in pathology, what's for ?

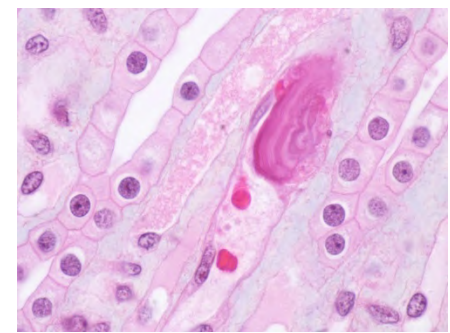
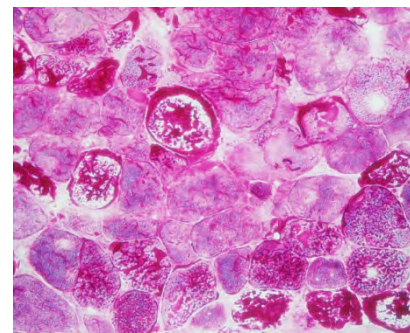
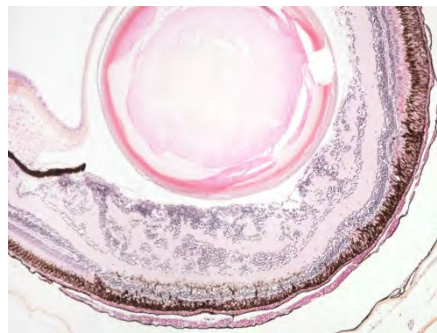
❖ (Anatomical) Pathology: study of morphological changes (lesions) of tissues and organs associated to diseases

❖ A medical specialty

- with a recognized training program for veterinarians of 3 to 3.5 years in Europe (ECVP)

❖ A veterinary pathologist should be useful to identify...

- normal versus abnormal appearance
- significant versus insignificant lesion
- primary lesions versus secondary lesions
- uncommon lesions
- interspecies variations





Animal model validation

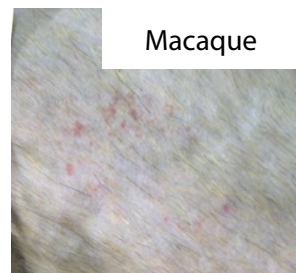
Example of chikungunya virus infection



- ❖ Facing emerging or re-emerging diseases: chikungunya outbreak in 2005-2007
- ❖ Need of animal models for physiopathogeny and for preclinical testing
- ❖ Validation of 2 animal models: macaque (in collaboration with SIV, CEA, Fontenay aux Roses) and adult mouse (in collaboration with QIMR, Brisbane, Australia)



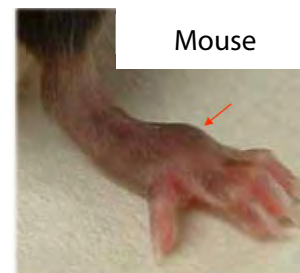
Human



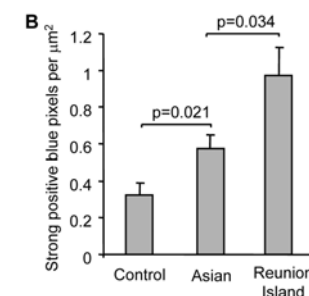
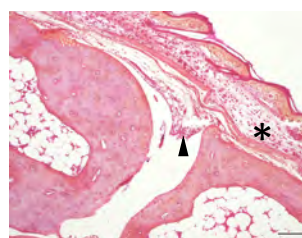
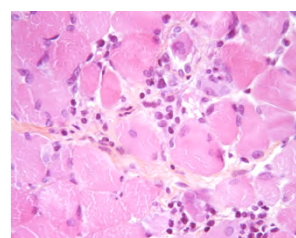
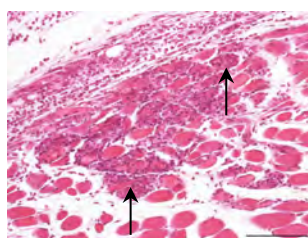
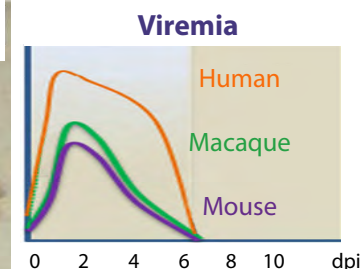
Macaque



Human



Mouse



Labadie K, Larcher T et al. *J Clin Invest*, 2010, **120** : 894-906

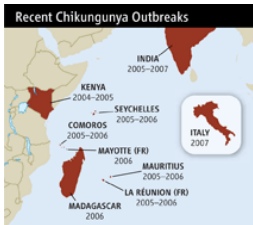
Gardner J et al. *J Virol*, 2010, **84** : 8021-32

Rudd PA et al. *J Virol*, 2012, **86** : 9888-98



Animal model evaluation

Example of chikungunya virus infection

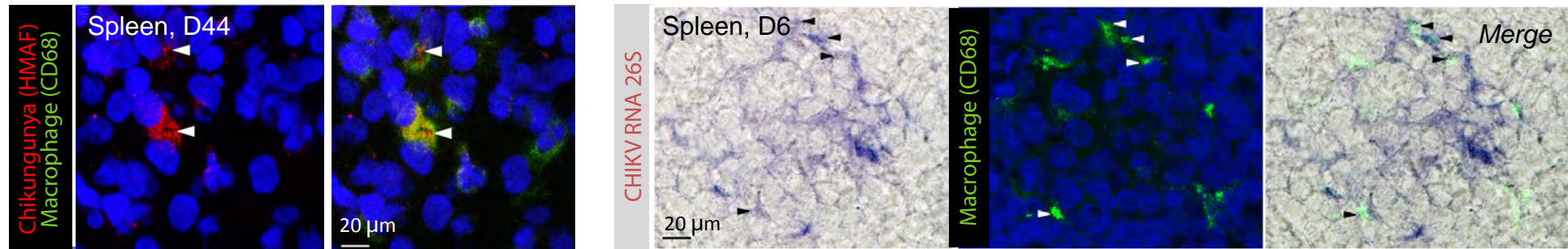


❖ Physiopathogeny of persisting arthralgia in chikungunya virus infection

- Multilabelling for infected cell and tissue identification (in situ hybridization and immunohistochemistry)
- Highly sensitive detection for reservoir identification

❖ Preclinical testing

- Efficacy and safety evaluation of a vaccine strain



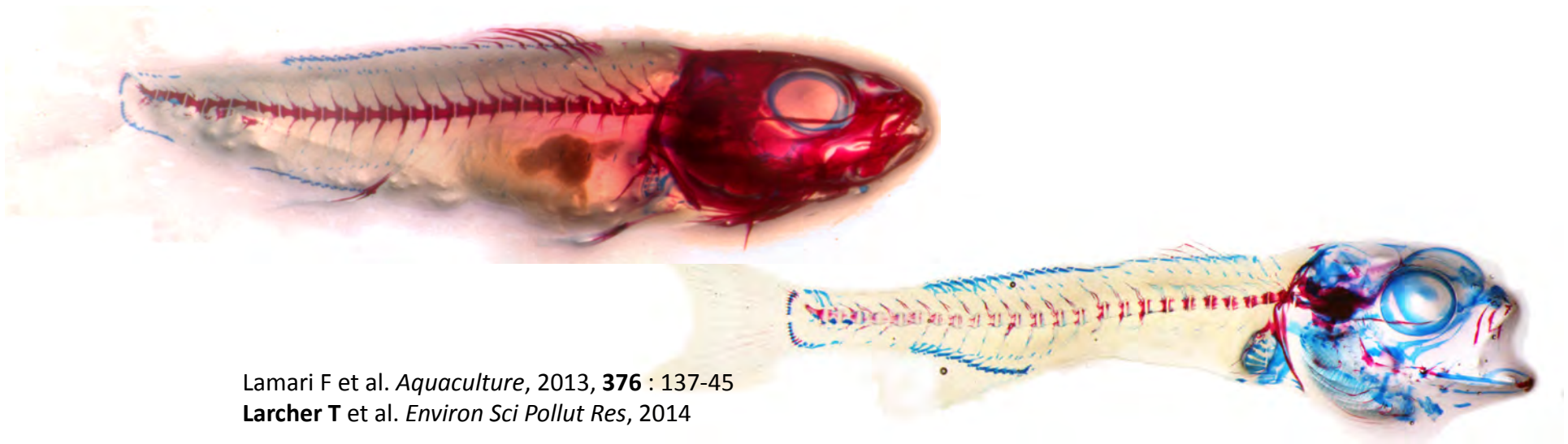
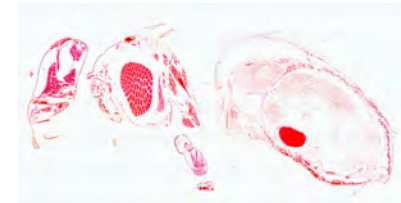
Labadie K, Larcher T et al. *J Clin Invest*, 2010, **120** : 894-906



Toxicopathology and ecotoxicology

LALLEMAND

- ❖ Physiopathological consequences of an exposure to environmental pollutants
 - Animal models (zebrafish) and sentinel species (bee, European flounder)
- ❖ Multi-disciplinary investigations
- ❖ Whole-body pathological examination
 - Ontogenesis and developmental abnormalities



Lamari F et al. *Aquaculture*, 2013, **376** : 137-45
Larcher T et al. *Environ Sci Pollut Res*, 2014



Toxicopathology and ecotoxicology

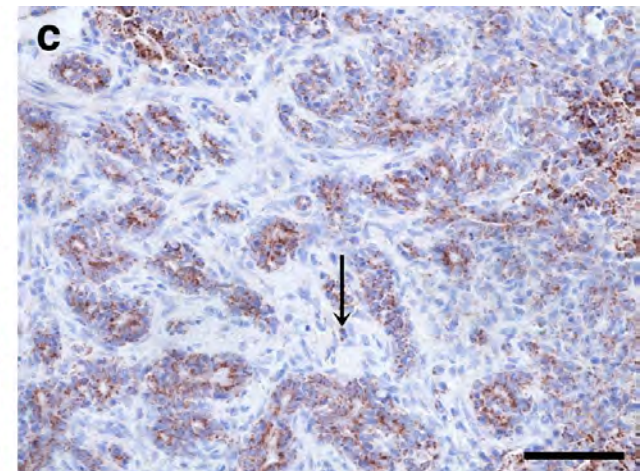
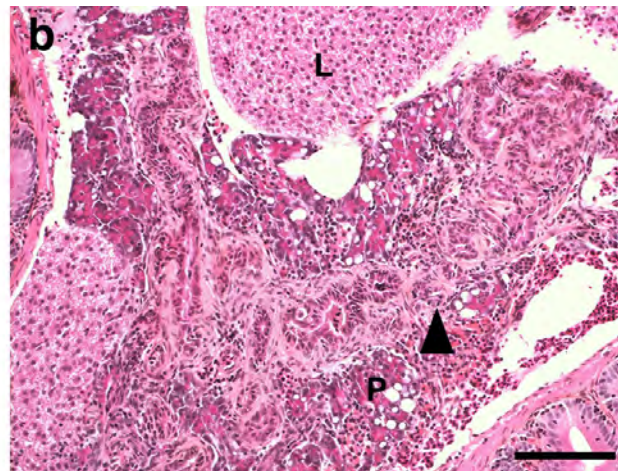
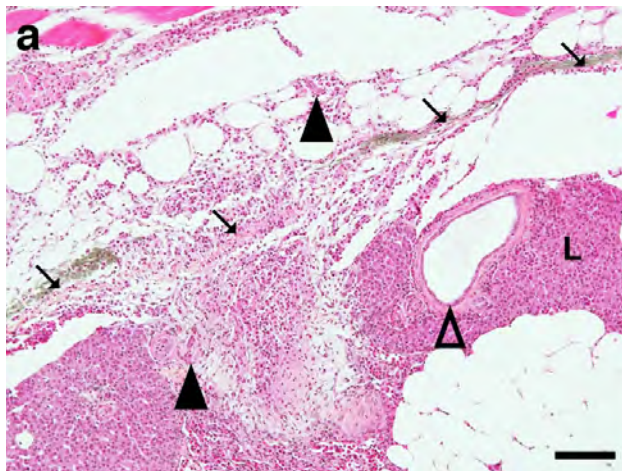
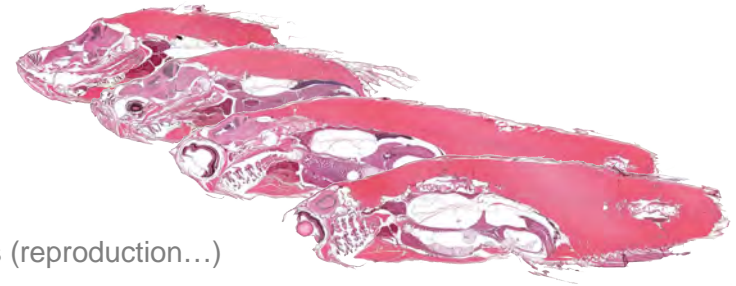
❖ Physiopathological consequences of an exposure to environmental pollutants

- Animal models (zebrafish) and sentinel species (bee, European flounder)

❖ Multi-disciplinary investigations

❖ Whole-body pathological examination

- Ontogenesis and developmental abnormalities
- Carcinogenesis
- Tissular evidence of physiopathological processes (reproduction...)



Larcher T et al. *Environ Sci Pollut Res*, 2014



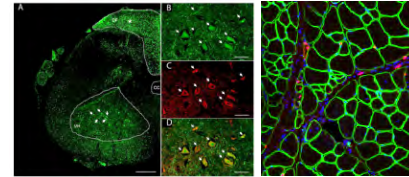
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Expertise in fluorescence bio-imaging

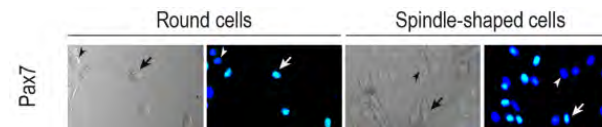
Expertise in tissue exploration by fluorescence bio-imaging, what's for?

❖ Cell and tissue phenotyping

- Multi-labelling
- Colocalization

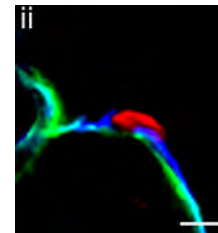


Motorized wide-field
fluorescence microscopy,
80i Nikon



❖ Cell tracking

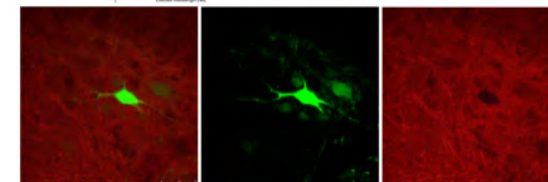
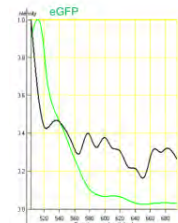
- Multi-labelling
- 3D imaging



Confocal laser
scanning
microscope
Nikon C1

❖ Tracking molecules

- Multi-labelling
- Spectral imaging
- Online linear unmixing



GFP + Noise

GFP

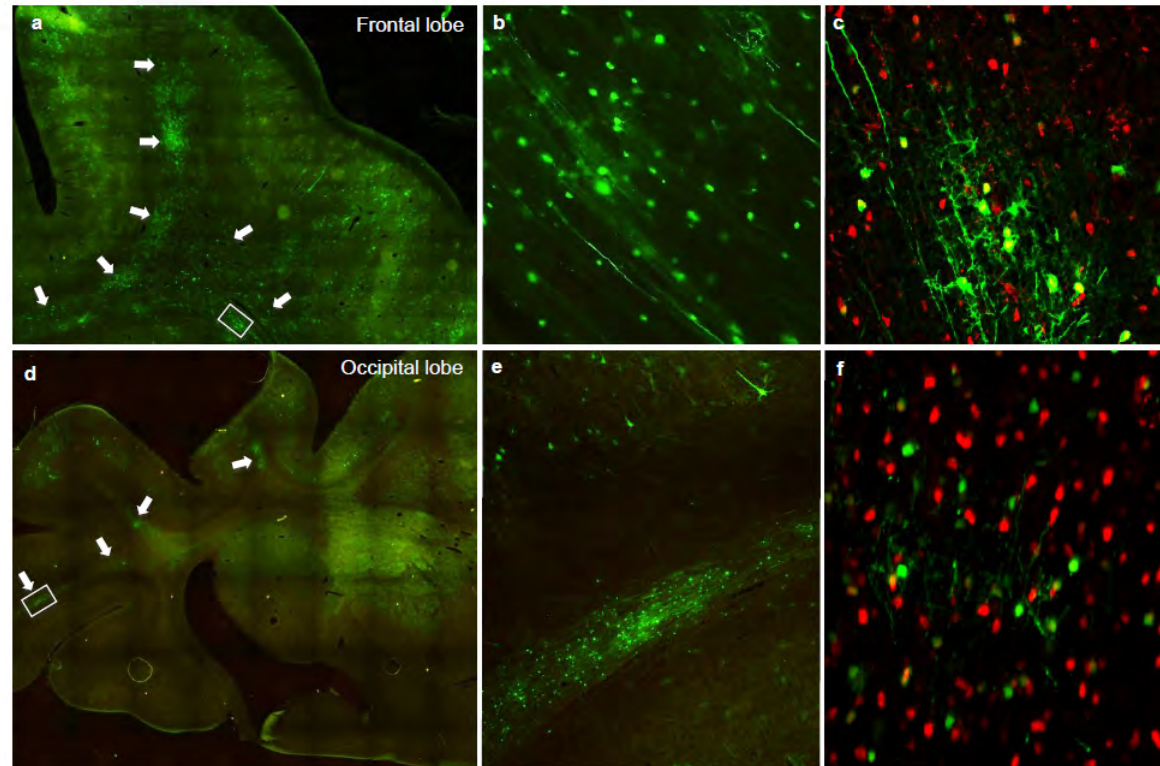
Noise

Spectral confocal microscope
LSM 780 Zeiss with GaAsps detectors

Cellular and tissular phenotyping of SNC

Origin of request: U1089 Inserm Nantes, gene therapy of SNC

Inserm
Institut national
de la santé et de la recherche médicale



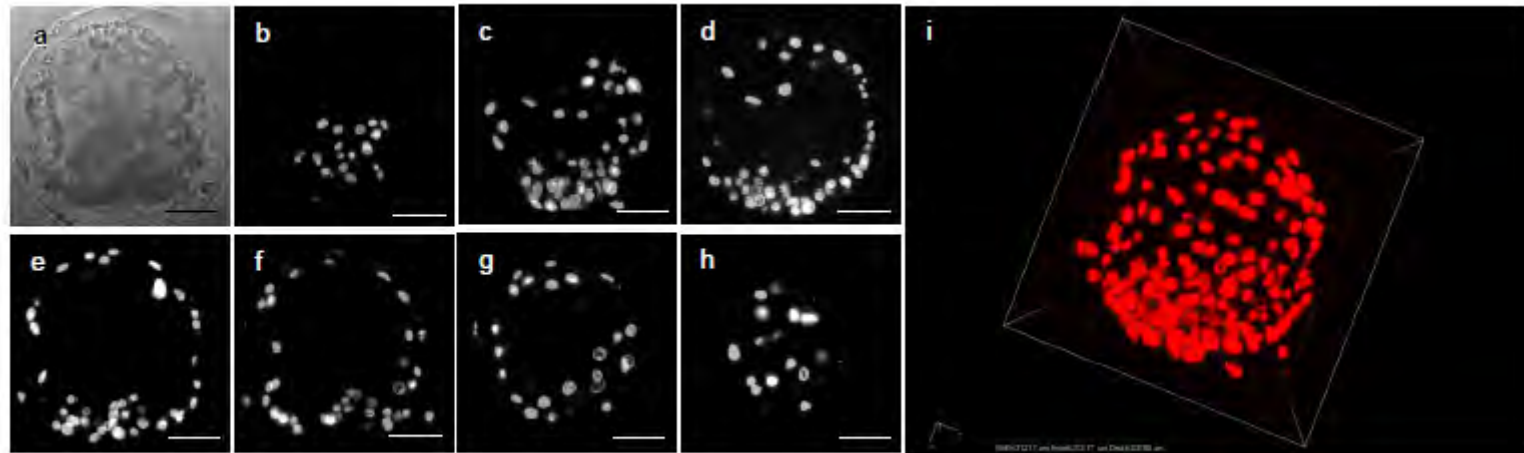
APEX offers:

- Training : tissue sampling, tissue trimming, multilabeling, fluorescence bio-imaging
- Confocal microscopy, Morphometry, Neuropathology.

Bucher and al, Gene Therapy, 2014,1-7.

Embryon 3D imaging and image analysis

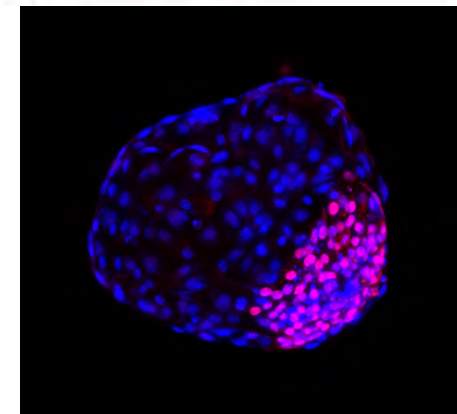
Origin of request : Unit « *sécurité sanitaire des biotechnologies de la reproduction* », Oniris



APEX offers :

- Development for embryo imaging
- Training : multilabeling, image analysis
- Confocal microscopy, 3D imaging

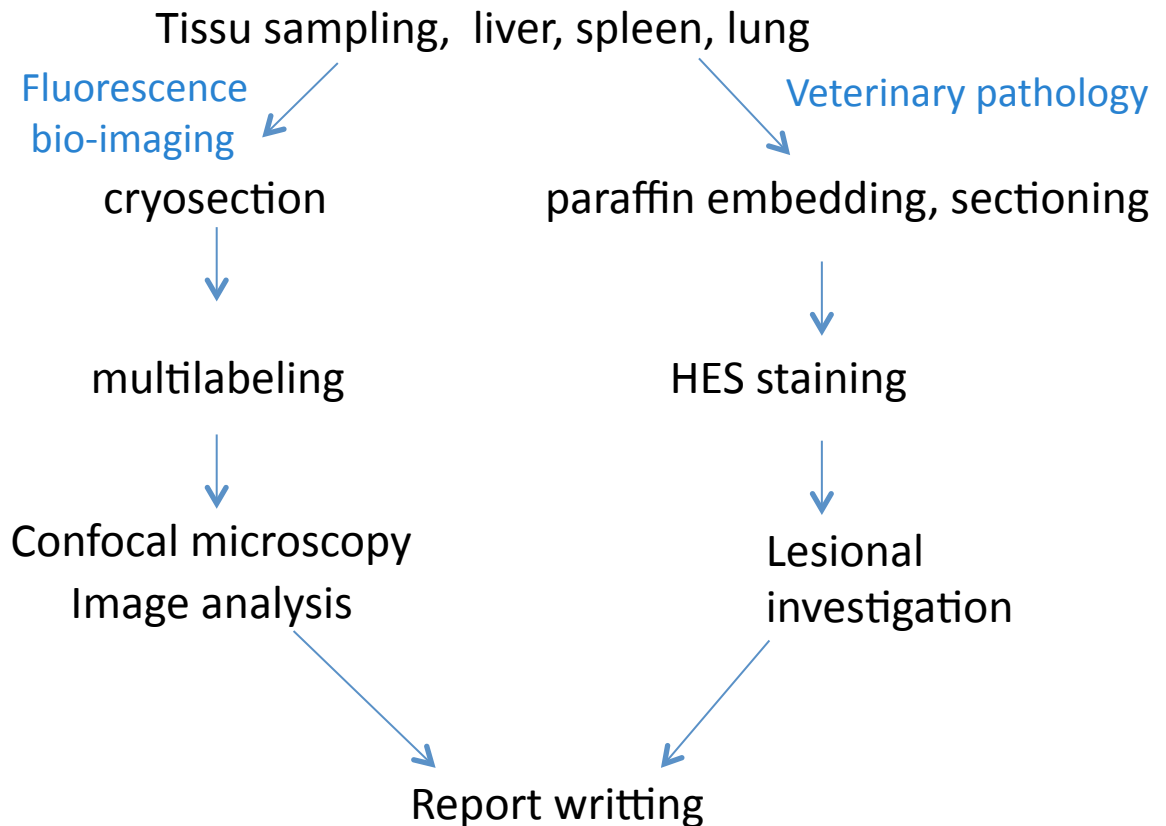
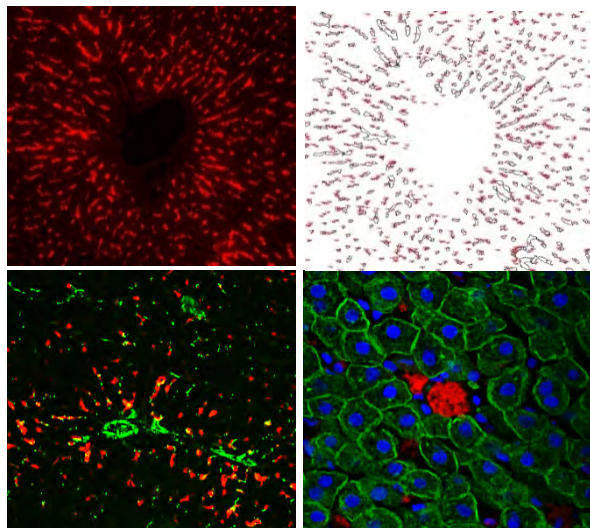
Moreno D and al., *Reproduction, Fertility and Development* 26(1): 154. January 2014.



Molecular tracking in rat liver

Origin of request : *private company*

Injection substance
X-rhodamine
t15min, t2h, t4h...



APEX offers: Veterinary pathology expertise and tissular exploration by fluorescence bio-imaging

A spectral confocal microscope LSM 780 ZEISS since 11-2013 at APEX

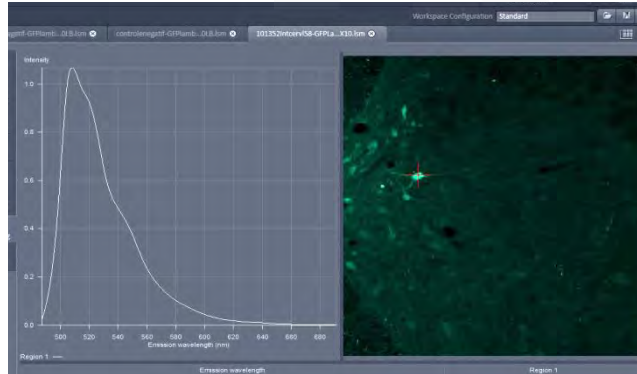
- 6 lasers beam : 405, 458, 488, 514, 561, 633 nm
- 32 spectral detectors GaAsps and 2 PMT
- 1 TPMT/Differential Interference Contrast
- Confocal acquisition FRAP

Multilabeling,
Spectral imaging,
Spectral unmixing

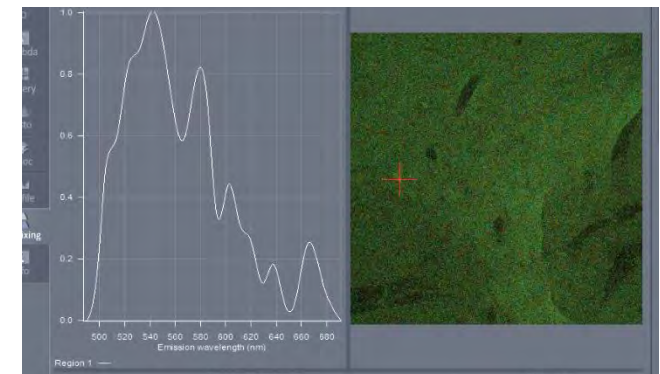


Separation of GFP signal from autofluorescence in spinal cord

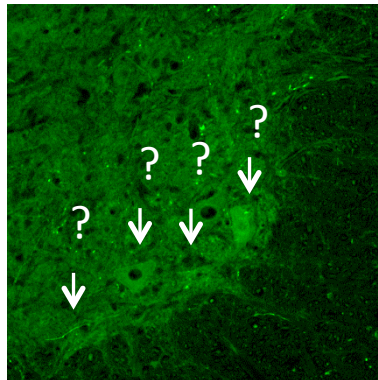
Positive control



Negative control



Confocal channel mode

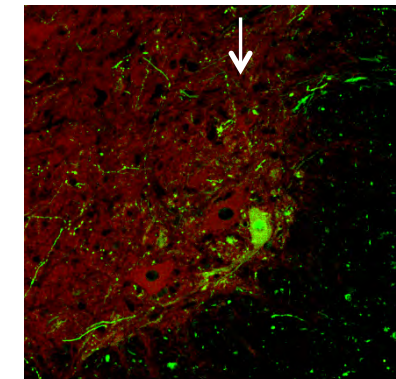


Lambda acquisition from tissue control



— GFP spectra
— Autofluorescence

Online fingerprinting

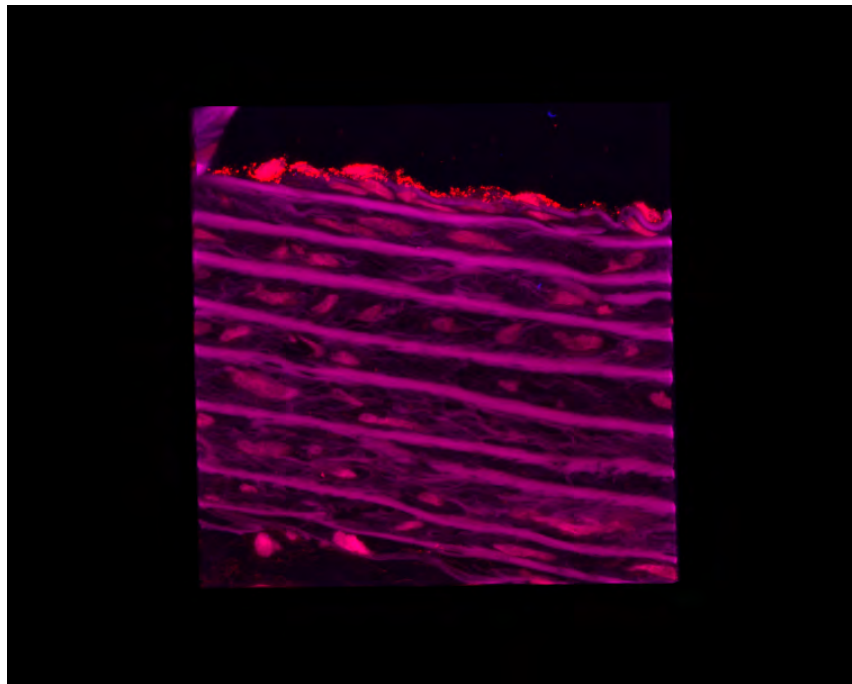


Neurone GFP+
Autofluorescence

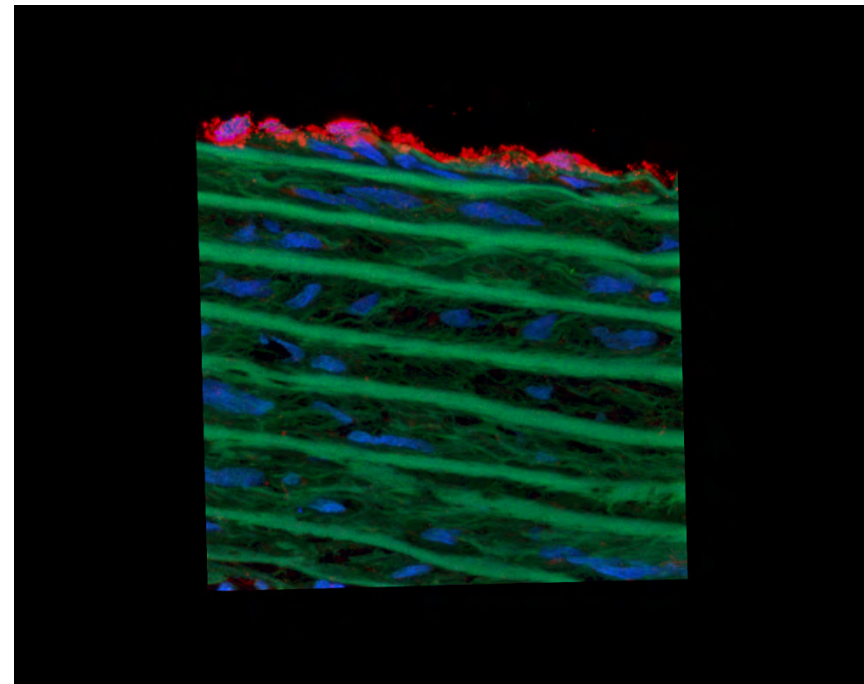
Separation of elastic fibers autofluorescence from multilabeling in aorta

Origin of request : *Unit physiopathologie animale et pharmacologie fonctionnelle, Oniris*

Confocal channel mode



Confocal spectral mode, spectral unmixing



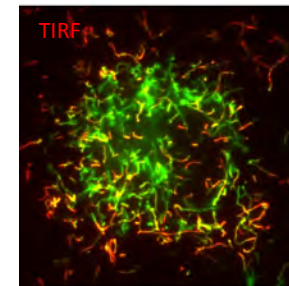
FVIII DAPI Autofluorescence

E. Montaudon, Y. Mallem

New offers in tissue and cell exploration by fluorescence bio-imaging in 2014

❖ Membrane trafficking, TIRF imaging

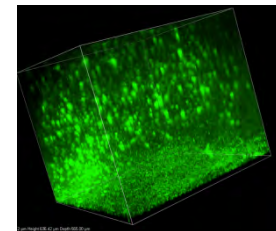
- High resolution at the membrane level (80 nm)
- Trafficking of molecules and organelles to and from the cellular membrane
- Exocytosis, endocytosis
- Binding of ligands to their membrane-bound receptors



Kolikova *et al.* (2006) BCB

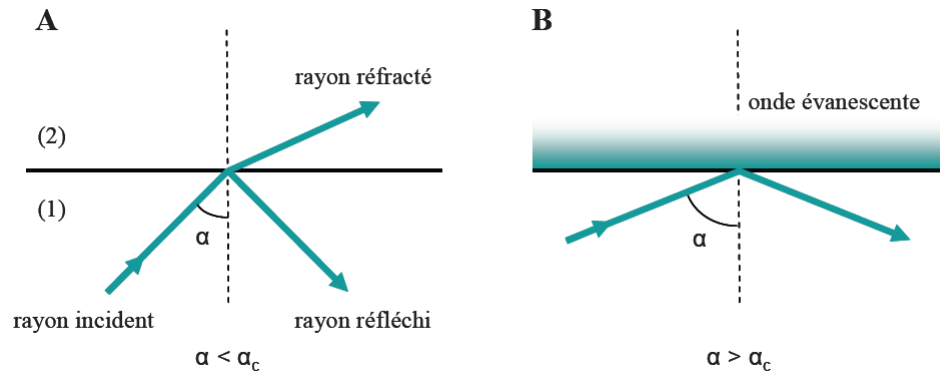
❖ Phenotyping of living tissues, Biphotonic imaging

- Deeper tissue penetration and low phototoxicity by using infrared laser beam
- Intravital microscopy
- Imaging of second harmonic generation signals

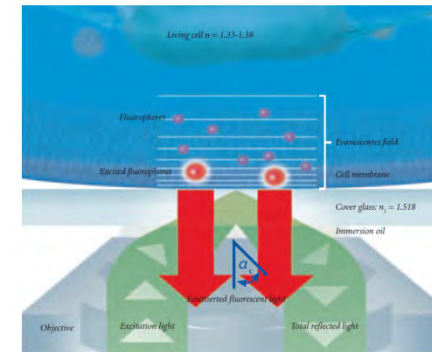


Total Internal reflection fluorescence microscope

TIRF : an evanescent wave to selectively illuminate and excite fluorophores in the region immediately adjacent to the glass-water interface. Resolution 80-100 nm



TIRF at APEX IN APRIL 2014

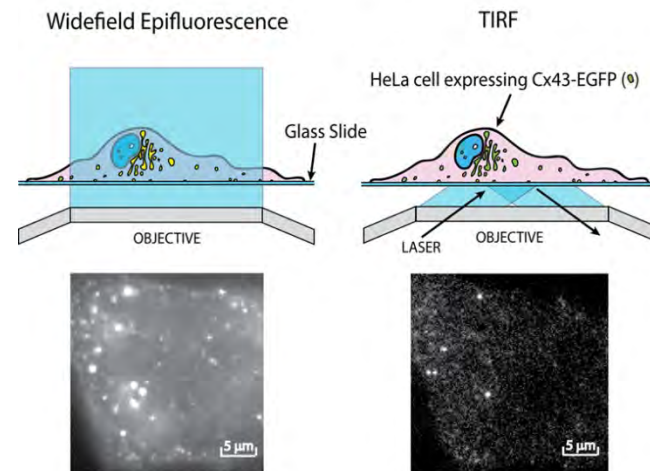


PRINCIPLE OF TIRF MICROSCOPY

- the module can simultaneously be combined with conventional HBO or other white light.



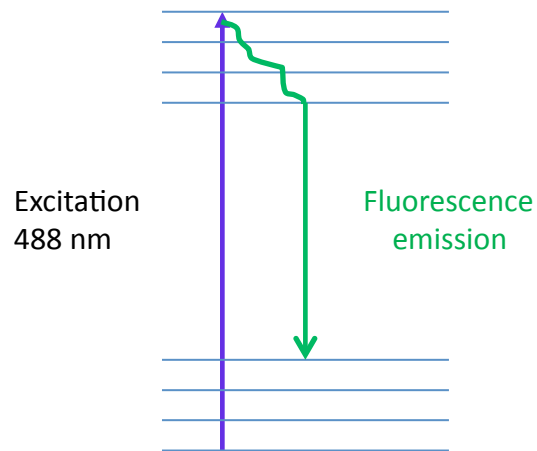
objX63, NA 1,46



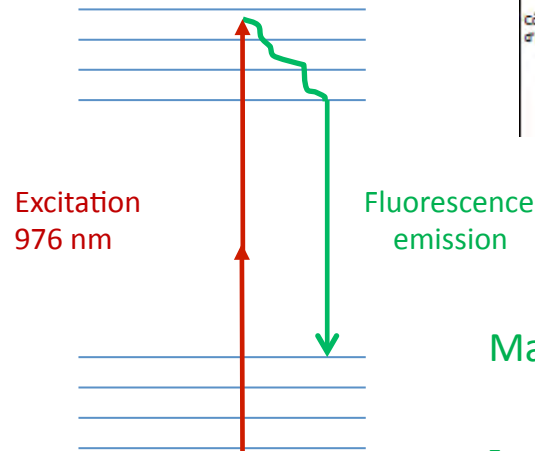
Biphotonic imaging, what's for in tissular and cellular exploration in vivo?

Principle

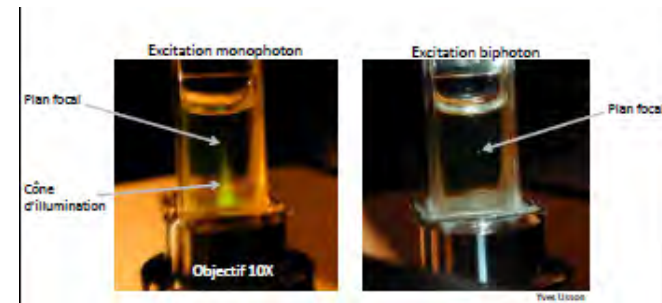
1Photon excitation



2Photon excitation



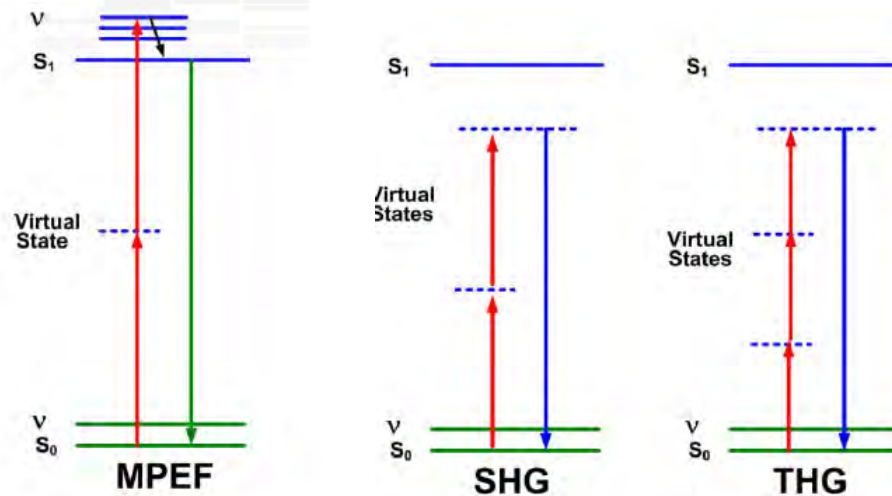
Femtosecond laser (infrared laser beam 680-1300 nm)



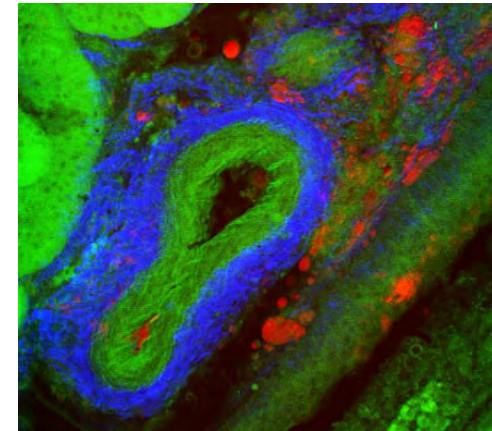
Major Inputs of 2Photon excitation:

- Deeper tissue penetration
- Reduction of the phototoxicity
- Imaging of living tissu
- Imaging of second harmonic signals

Biphotonic imaging, what's for in tissular and cellular exploration in vivo?



- Multiphotonic Emission Fluorescence (MPEF)
- Second Harmonic Generation (SHG)
- Third Harmonic Generation (THG)



Intravital exploration with biphotonic microscopy

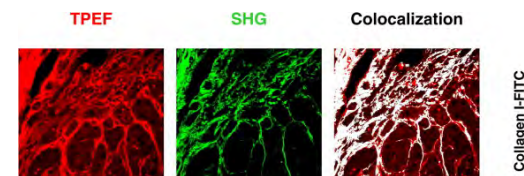
Tissular exploration without labeling :

- Tissue structural changing (SHG, THG): Collagen, microtubules, and muscle myosin; myelin, membranar lipids, markers of pathology and efficacy of treatment for research and diagnostic purpose

Journal of Hepatology 52, 3 (2010) 398-406

Fibrillar collagen scoring by second harmonic microscopy: a new tool in the assessment of liver fibrosis.

Luc Gailhouste ^{1, 2}, Yann Le Grand ^{3, 4}, Christophe Odin ⁴, Dominique Guyader ^{5, 6}, Bruno Turlin ⁷, Frédéric Ezan ¹, Yoann Désille ⁶, Thomas Guilbert ⁴, Anne Bessard ^{1, 8}, Christophe Frémin ¹, Nathalie Theret ⁹, Georges Baffet ^{1, 9}



Intravital exploration with biphotonic microscopy

Tissular exploration with labeling :

- Vasculature imaging (fluorescent probe systemic injection)
- Use of transgenic models expressing fluorescent reporters (GFP, YFP, mCherry ...)

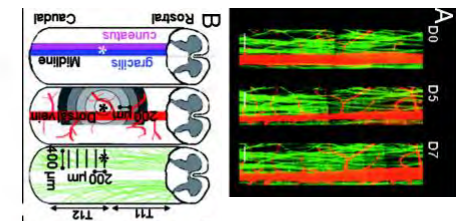
Imaging Single cell in vivo :

- Track the motility of cells within organs in vivo, long terme imaging in the same animal
- Imaging the cells of the immune system in a live animal by transduce fluorescently tagged genes in specific cell populations
- Progression of pathogen in live tissue by GFP-expressing bacteria or virus
- Endocytosis and trafficking through endosomal and lysosomal system

Quantitative analysis by in vivo imaging of the dynamics of vascular and axonal networks in injured mouse spinal cord

Cyril Dray, Geneviève Rougon¹, and Franck Debarbieux

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Interactions with other regional plate-forms



Biotherapy and animal physiopathology

LAV



Viral vector production
for gene therapy

P. Moullier

Inserm

Institut national
de la santé et de la recherche médicale

APEX



Fluorescence bioimaging,
Veterinary pathology

M.A. Colle



Boisbonne center

Animal model housing

J.Y. Deschamp



Training and seminars, exchanges of knowledges

BIBS



Microscopy, MCBL, MET,
AFM

B. Bouchet, C. Alvarado, C. Gaillard



MicroPiCell



Microscopy, MCBL, HCS,
Video microscopy

P. Hulin, S. Nedellec, M. Robard



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CEISAM

Development of fluorescent
probes

E. Ishow

A. Faucon



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Pour en savoir plus...

http://www6.inra.fr/anatomie_pathologique_sante_animale

The screenshot shows a web browser window with the address bar displaying www6.inra.fr/anatomie_pathologique_sante_animale. The page title is "Anatomie pathologique Sante Animale - Accueil". The main banner features the APEX logo, the INRA logo (SCIENCE & IMPACT), and the Oniris logo. The text on the banner reads "APEX cellule d'expertise en anatomie pathologique pour la recherche". Below the banner, the page is divided into several sections:

- Accueil**: A section with the title "APEX : Expertise en anatomie pathologique pour la recherche". It contains a paragraph about the platform's mission and the IBISA logo.
- Actualités**: A section with the title "Actualités" containing three news items:
 - Comparaison de voies d'administration d'un vecteur de thérapie génique**: A paragraph about a collaboration with INSERM U1089, with a link to "Lire la suite".
 - Phénotypage des cellules dendritiques**: A paragraph about a collaboration with UMR707 IECM, with a link to "Lire la suite".
 - Effet de la leptine chez un modèle animal de retard de croissance**: A paragraph about results in PlosOne, with a link to "Lire la suite".
- Nous contacter**: A section with the title "Nous contacter" listing three names: Marie-Anne Collé, Thibaut Larcher, and Laurence Dubreil.
- Le réseau Biogenouest s'agrandit**: A paragraph about APEX joining the Biogenouest network, with a link to "Lire la suite".