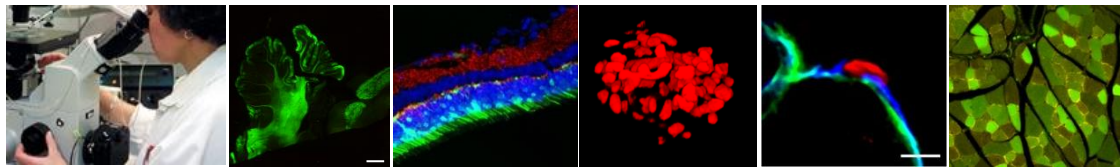


La Bio-imagerie en fluorescence pour l'exploration tissulaire et cellulaire, du nouveau sur la plateforme APEX



APEX

Anatomical Pathology

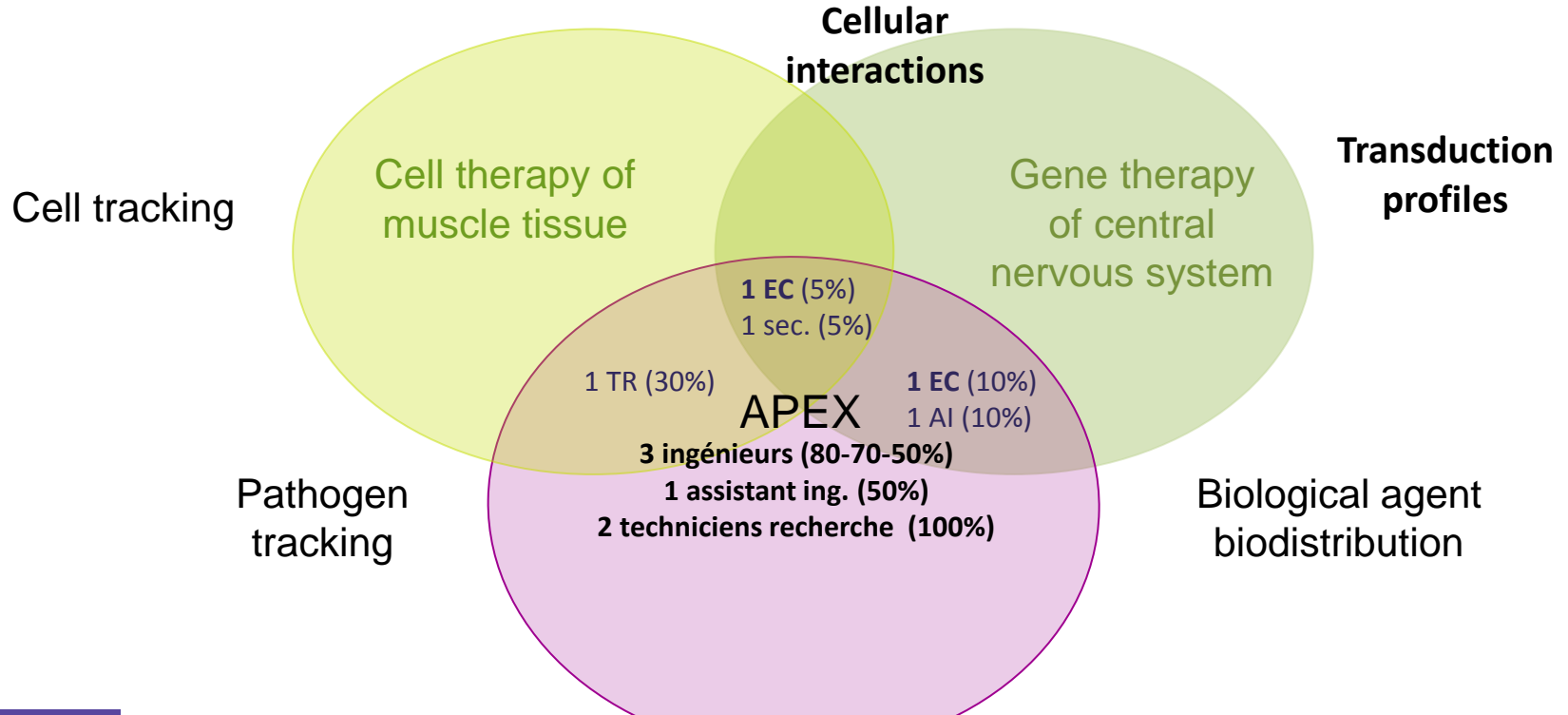
Expertise for Research

Laurence Dubreil / Thibaut Larcher

UMR703 PAnTher

Physiopathologie Animale et bioThérapies du muscle et du système nerveux central

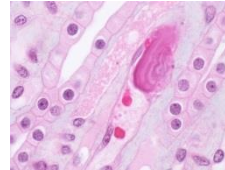
- ❖ #15 permanent staff members
- ❖ # 4 PhD students
- ❖ Headed by M-A. Colle and K. Rouger



APEX

Synergy between two fields of expertise

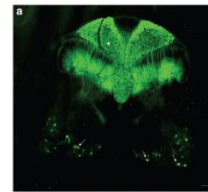
- Veterinary pathology



Autopsy
Lesionnal investigation
Diagnosis
Histomorphometry

Thibaut.larcher@nantes.inra.fr

- Fluorescence bio-imaging



Cellular typing
Co-labeling
3D imaging
Spectral imaging
Image analysis

Laurence.dubreil@oniris-nantes.fr

Scientific fields

One World, One Medicine, One Health

❖ Validating animal models

- Spontaneous
- Induced (chirurgical, targeted mutagenesis)

❖ From tissue and cell tracking to physiopathogeny

- Multilabelling
- Highly sensitive detection

❖ Ecotoxicology

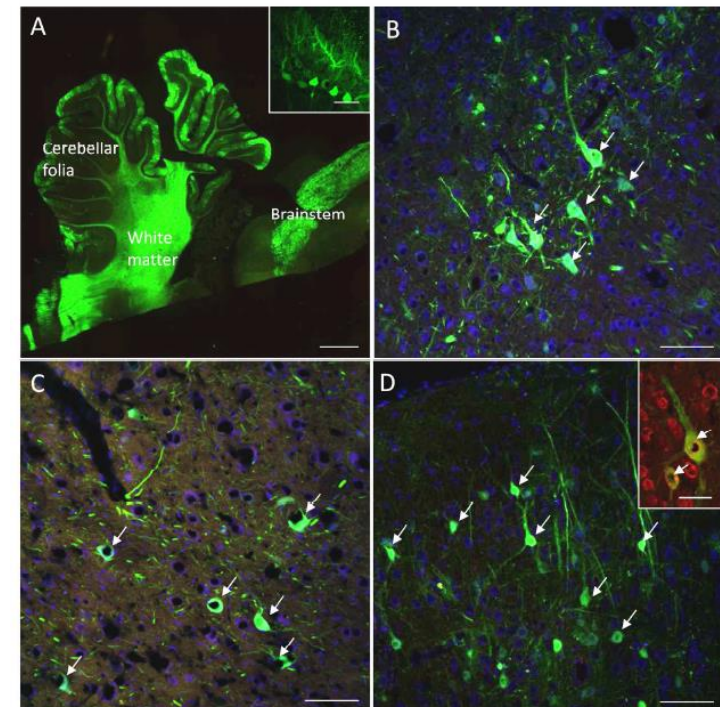
- Whole-organism studies

❖ Preclinical testing

- Efficacy and safety evaluation



Animal models of chikungunya disease for gaining insight into persisting arthralgia physiopathogeny



scAAV9 intracisternal delivery to the central nervous system of a feline model of moto-neuron disease

Integrated offer for tissue phenotyping

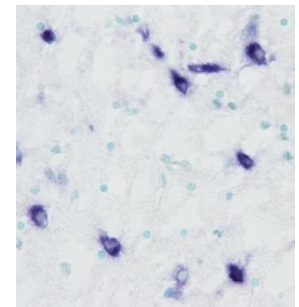
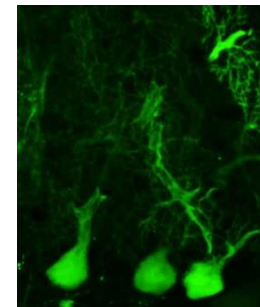


- ❖ Tissue sampling
- ❖ Tissue trimming
 - Vibrating cutting device
 - Cryostat
 - Microtome
 - Ultramicrotome
- ❖ Topographic staining
- ❖ Immunostaining and histoenzymology
- ❖ *In situ* hybridization

>1100 blocks and >2200 slides per year

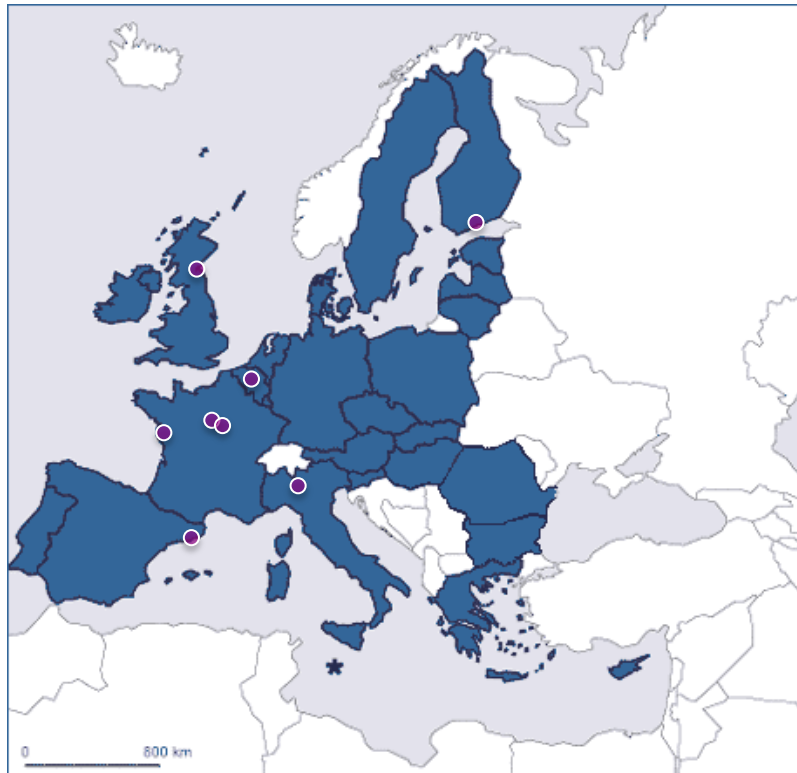


- ❖ Fluorescence bio-imaging
- ❖ Histomorphometry



APEX & other open platforms in Europe

With at least 1 ECVP/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)



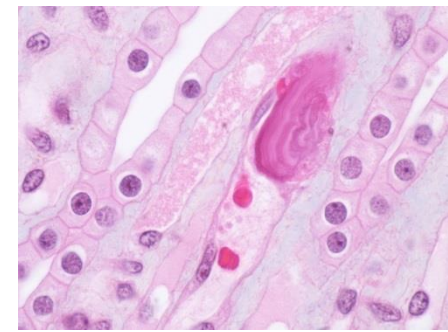
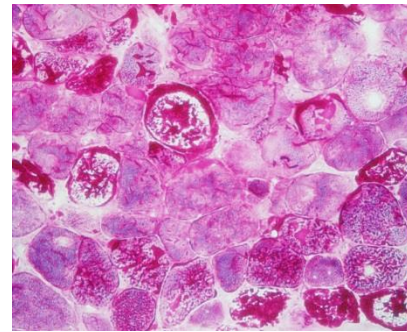
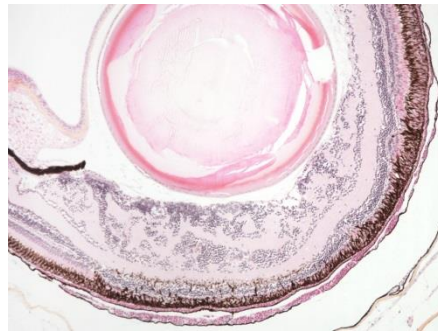
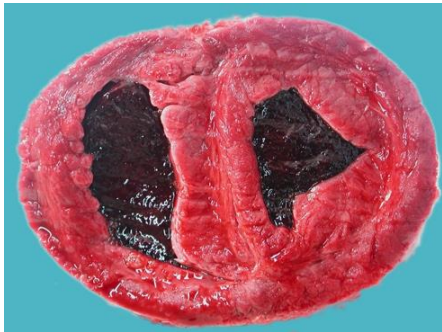
VIB – Center for the Biology of Disease (Louvain)



Field of expertise:
Animal model or **Target species**

Expertise in pathology, what's for ?

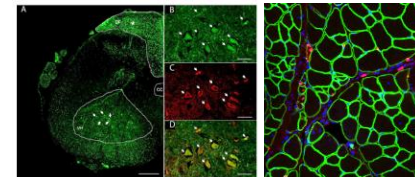
- ❖ (Anatomical) Pathology: study of morphological changes (lesions) of tissues and organs associated to diseases
- ❖ A medical speciality (recognised training program for veterinarians of 3 to 3.5 years in France)
- ❖ A veterinary pathologist should be useful to identify...
 - normal versus abnormal appearance
 - significant versus insignificant lesion
 - interspecies variations
 - uncommon lesions
 - primary lesions versus secondary lesions



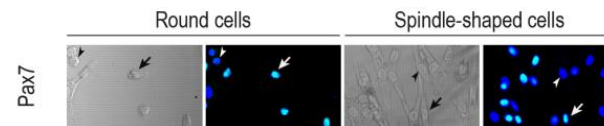
Expertise in tissue exploration by fluorescence bio-imaging, what's for?

❖ Cell and tissue phenotyping

- Multi-labelling
- Colocalization

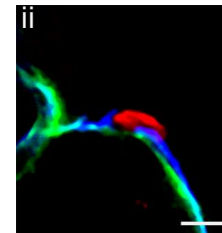


Motorized wide-field fluorescence microscopy, 80i Nikon



❖ Cell tracking

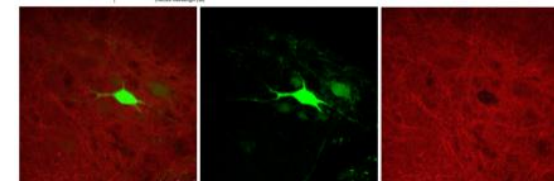
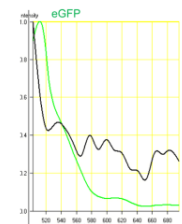
- Multi-labelling
- 3D imaging



Confocal laser scanning microscope
Nikon C1

❖ Tracking of molecules

- Multi-labelling
- Spectral imaging
- Online linear unmixing



GFP + Noise

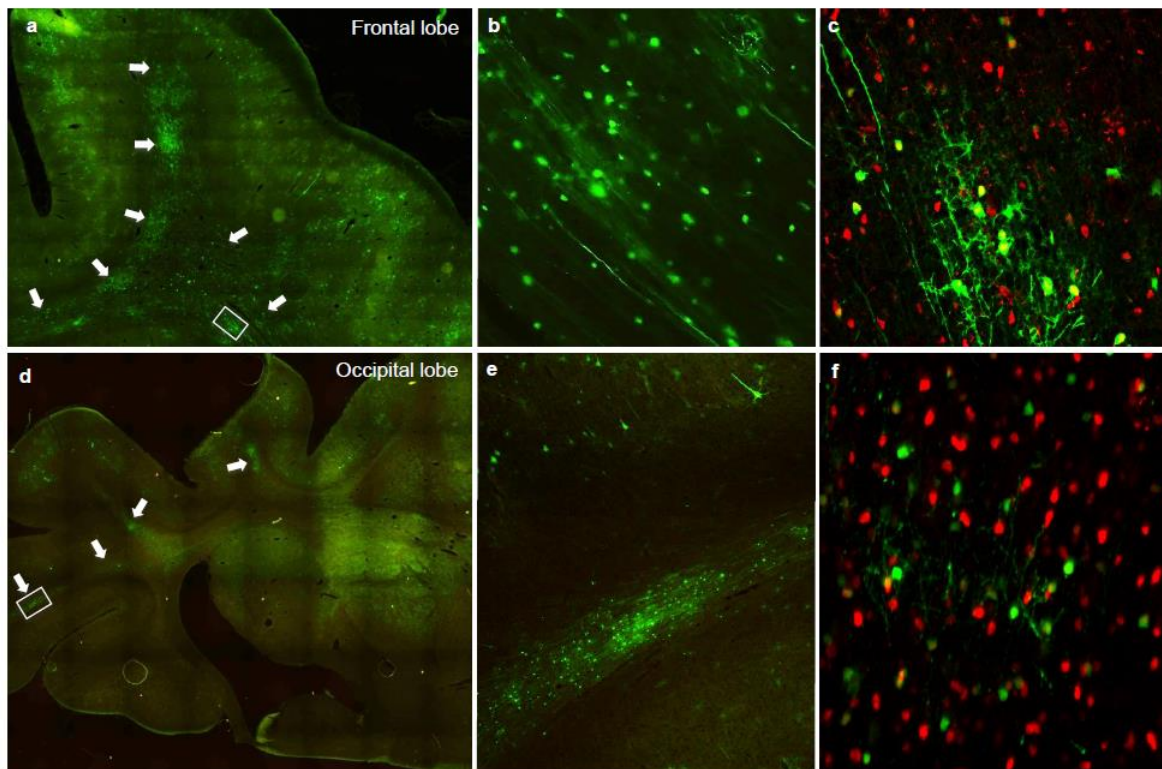
GFP

Noise

Spectral confocal microscope
LSM 780 Zeiss with GaAsps detectors

Cellular and tissular phenotyping of SNC

Origin of demand = U1089 Inserm Nantes, gene therapy of SNC



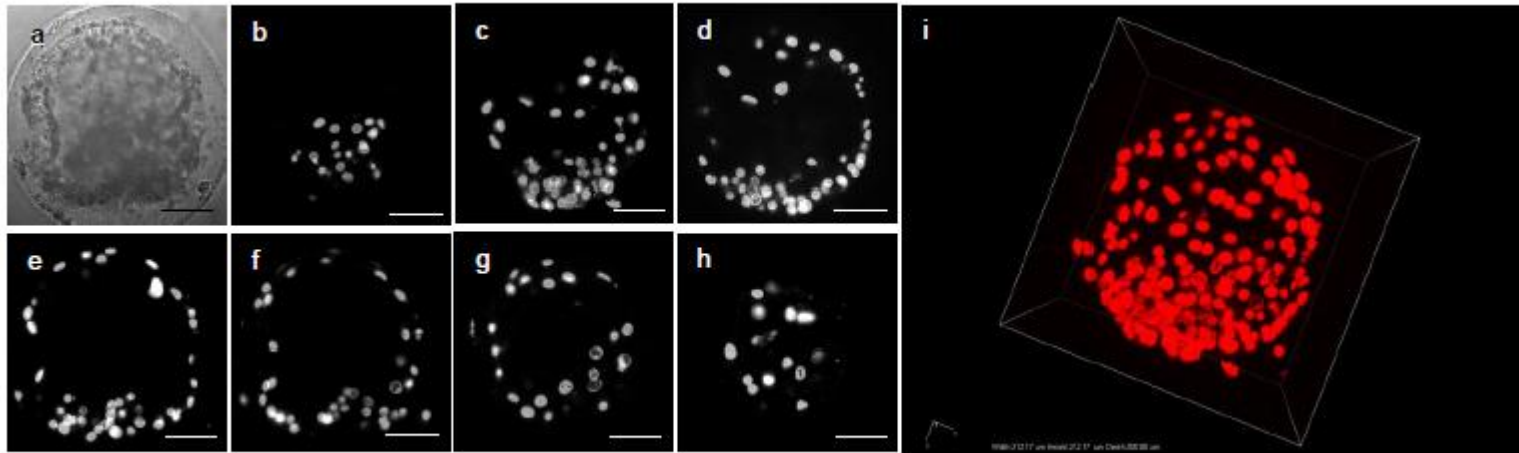
APEX offers :

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

Bucher and al, Gene Therapy, January 2014 accepted

Embryon 3D imaging and image analysis

Origin of demand : *sécurité sanitaire des biotechnologies de la reproduction, Oniris*

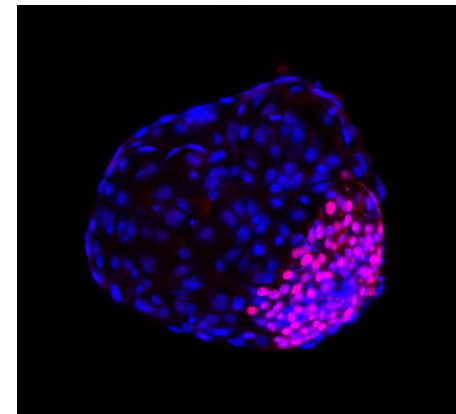


APEX offers :

Development for embryo imaging

Training : multilabeling, image analysis

Confocal microscopy, 3D imaging

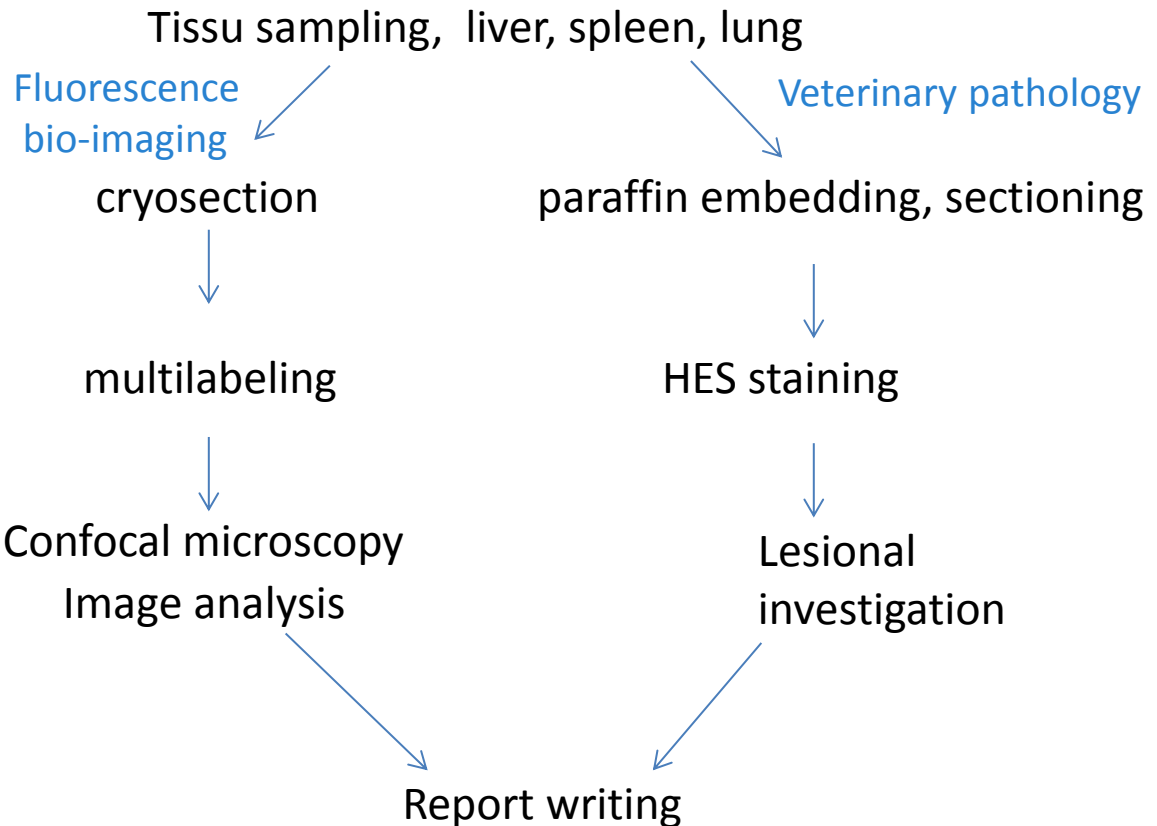
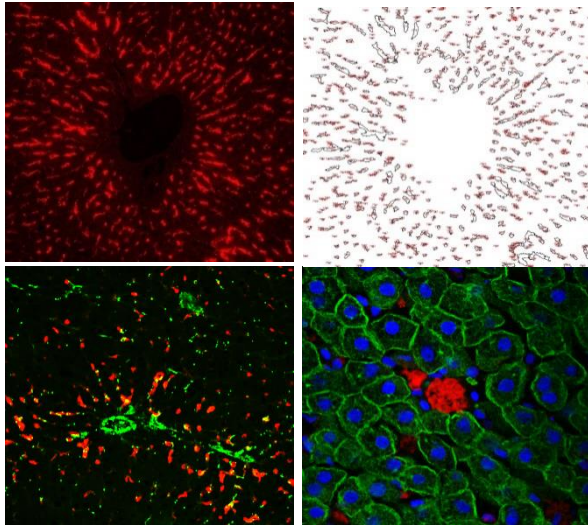


Moreno D and al., Reproduction, Fertility and Development 26(1): 154. January 2014.

Molecular tracking in rat liver

Origin of demand : *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 since november 2013 at APEX

- 6 raies laser : 405, 458, 488, 514, 561, 633 nm
- 32 détecteurs GaAsps spectraux et 2 PMT
- 1 TPMT/DIC
- Acquisition confocale FRAP

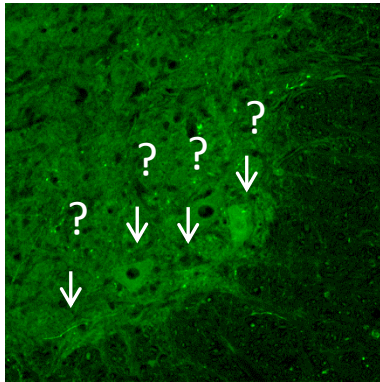
Multilabeling, spectral imaging, spectral unmixing



A spectral confocal microscope, a powerful tool to separate specific signal from autofluorescence

1) Example : spinal cord

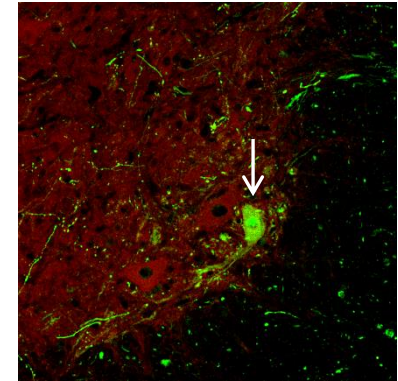
Confocal channel mode



Lambda acquisition
from tissue control

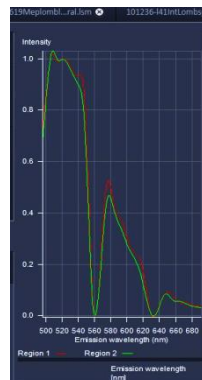
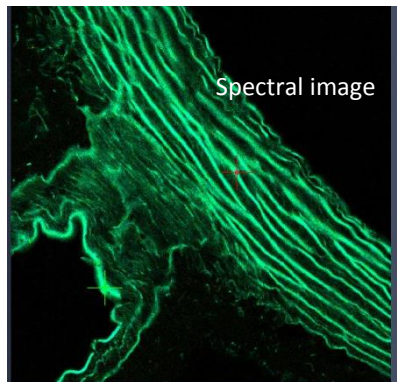


Online fingerprinting



Neurone GFP+
Autofluorescence

2) Example : aorta



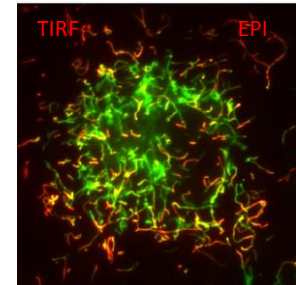
— GFP spectra
— Autofluorescence

autofluorescence spectra
of elastic fibers

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

❖ Membrane trafficking, TIRF imaging

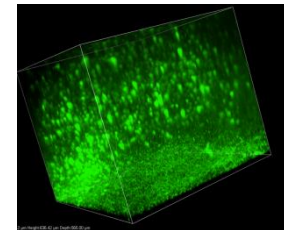
- High resolution at the membrane level (80 nm)
- Trafficking of molecules and organelles to and from the cellular membrane
- Binding of ligands to their membrane-bound receptors



Kolikova *et al.* (2006) BCB

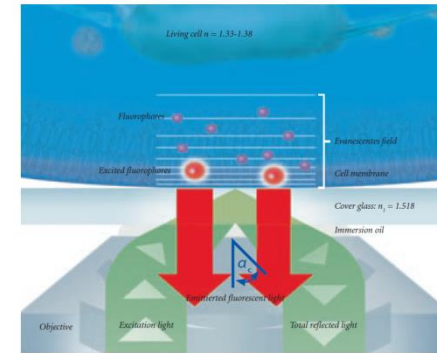
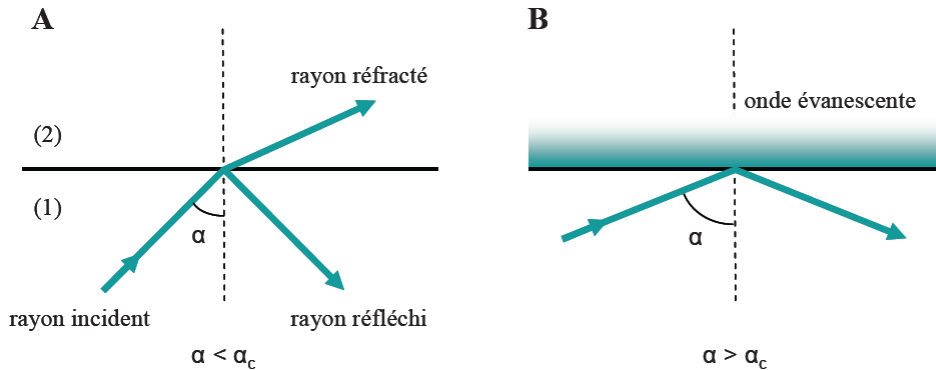
❖ Phenotyping of living tissues, Biphotonic imaging

- 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



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

objX63, NA 1,46

Digital EMCCD Camera

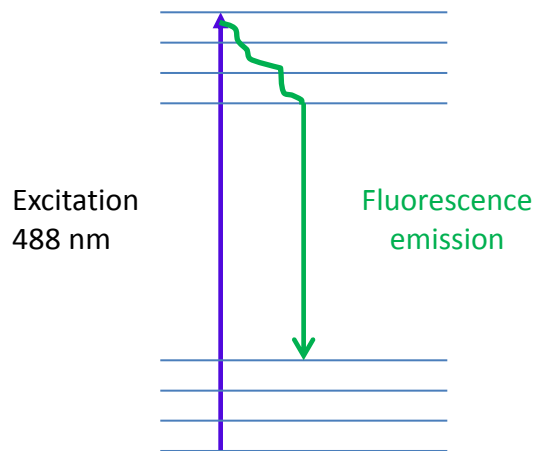


ROLERA[™]
em-c²

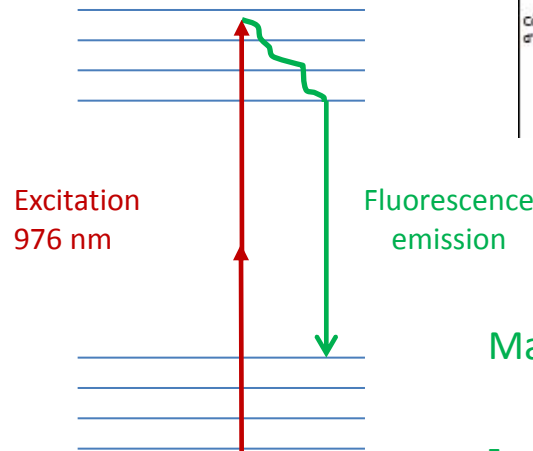
Biphotonic imaging, what's for in tissular and cellular exploration in vivo?

Principe

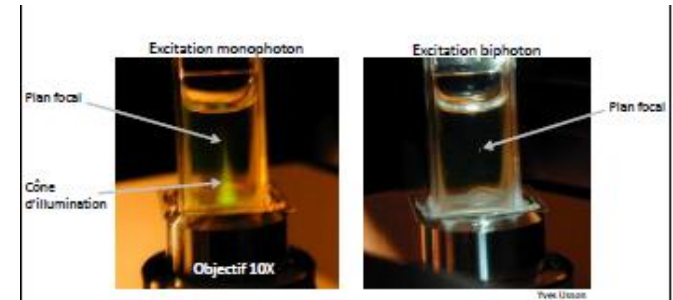
1Photon excitation



2Photon excitation



Femtosecond laser (infrared laser beam 680-1080 nm)



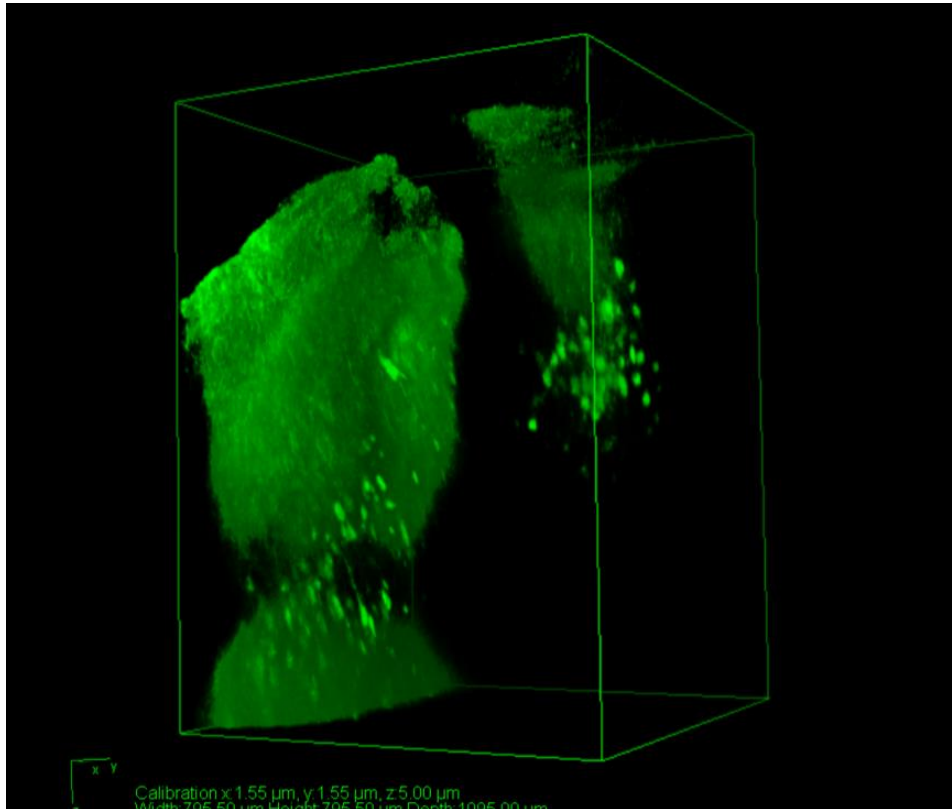
Major Inputs of 2Photon excitation:

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

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

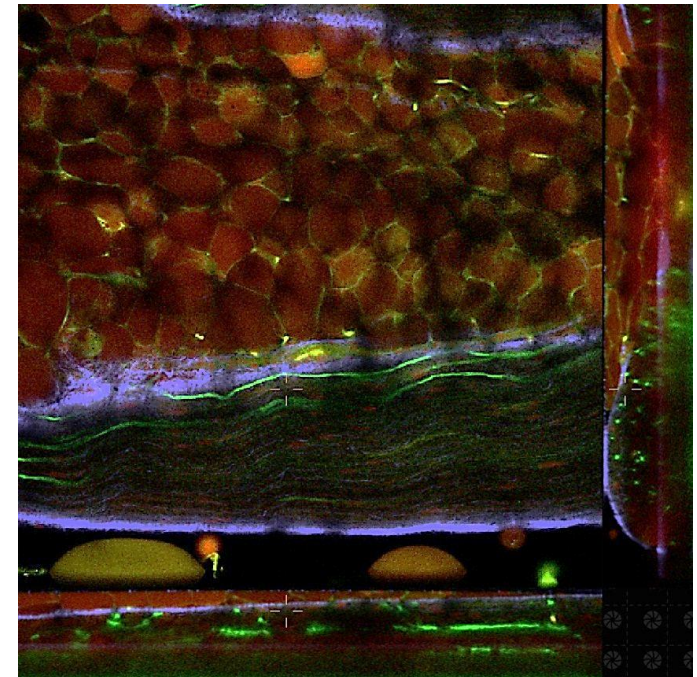
Major Input : deeper tissu penetration (2X with biphotonic microscopy)

Scaled brain GFP expression A1RPM, Nikon, MiFoBio 2012



Second harmonic generation signal ($\lambda/2$), molecular conformation and orientation (fibrillar collagen).

sciatic nerve from a mice



Collagen matrix : second harmonic generation

L. Dubreil, APEX UMR 703

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

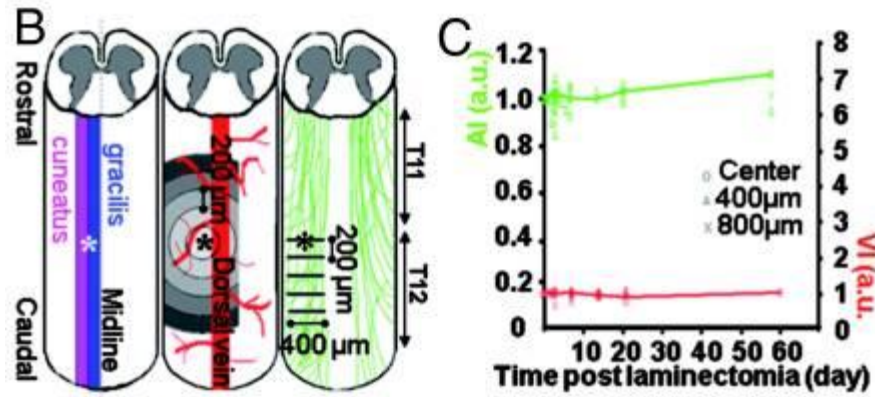
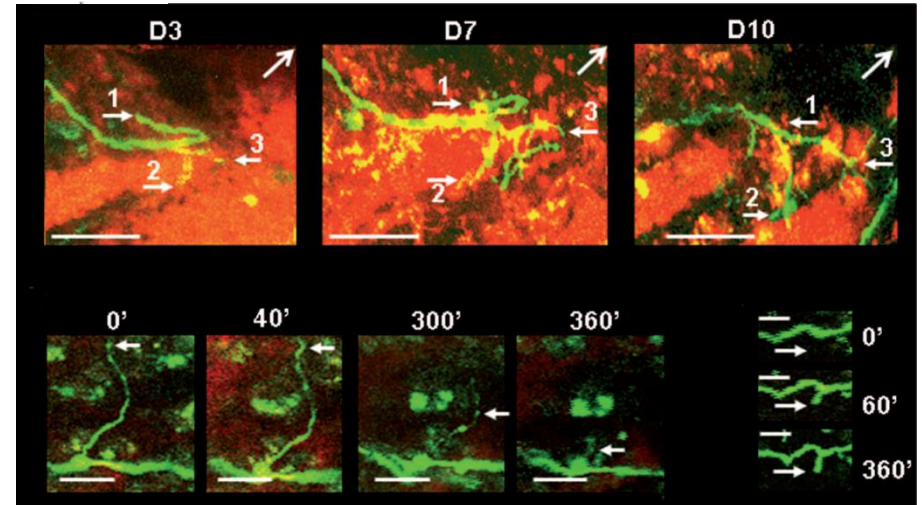
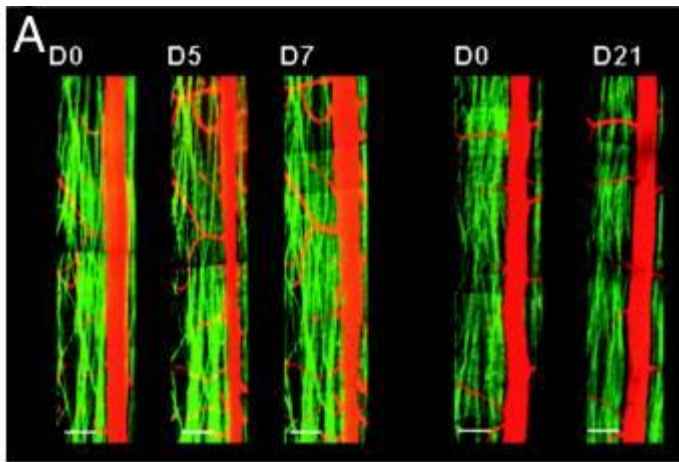
[Proc Natl Acad Sci U S A.](#) 2009 Jun 9;106(23):9459-64

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

Unité Mixte de Recherche 6216, Centre National de la Recherche Scientifique, Université de la Méditerranée, Institut de Biologie du Développement de Marseille-Luminy, Case 907, Parc Scientifique de Luminy, 13288 Marseille Cedex 9, France

Major input : Imaging of living tissu



Adult transgenic Thy1GFP-M mice
Rhodamin B isothiocyanate-Dextran

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

Imaging of second harmonic generation signals : A tool to explore fibrosis (collagene type I)

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}

⁽¹⁾ EA 4427 SeRAIC - INSERM U522, IFR 140, Université de Rennes 1, Hôpital Pontchaillou, 35033 Rennes, FRANCE

⁽²⁾ Institut de Physique de Rennes, UMR UR1-CNRS 6251, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes, FRANCE

⁽³⁾ Liver Department Hôpital Pontchaillou, 35033 Rennes, FRANCE

⁽⁴⁾ Department of Pathology, Hôpital Pontchaillou, 35033 Rennes, FRANCE

⁽⁵⁾ EA SeRAIC - INSERM U620, IFR 140, Université de Rennes 1, 35043 Rennes, FRANCE

⁽⁶⁾ Laboratoire de Spectrométrie et Optique Laser, EA 938, Université de Bretagne Occidentale, 29238 Brest, FRANCE

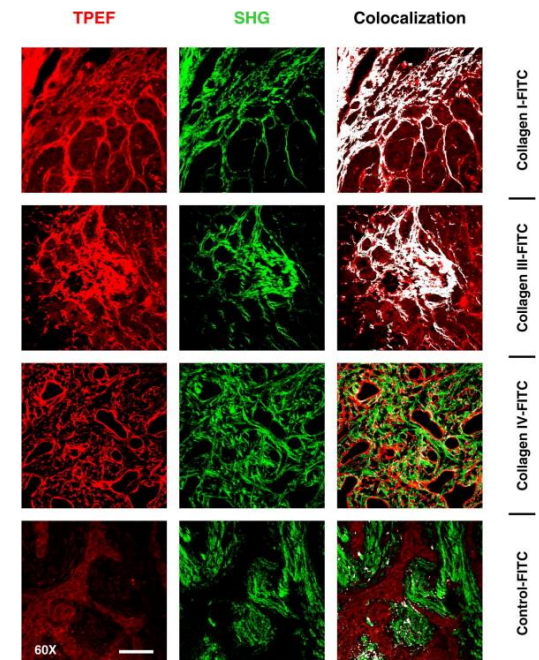
⁽⁷⁾ Université Européenne de Bretagne, 5 Boulevard Laënnec, 35000 Rennes, FRANCE

We first validated the selectivity of SHG signals for fibrotic deposits (fibrillar collagen type I and III) in human liver by establishing a strong correlation between SHG and immunochemistry assays.

Both frozen fixed or paraffin embedded tissues can be used

SHG scoring gives the opportunity to quantify fibrosis regression in response to an antifibrotic treatment for validating experimental protocols especially for cirrhotic patients

SHG imaging and fibrillar collagen scoring will be applicable to endoscopic systems by using optic fibers coupled with multiphoton microscopy, thus leading to a less invasive procedure for fibrosis evaluation



Types of offers

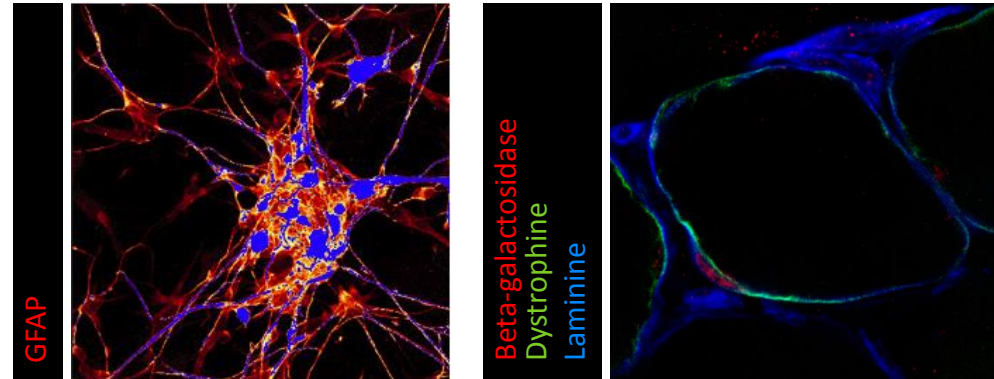
Training and support

❖ Development

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

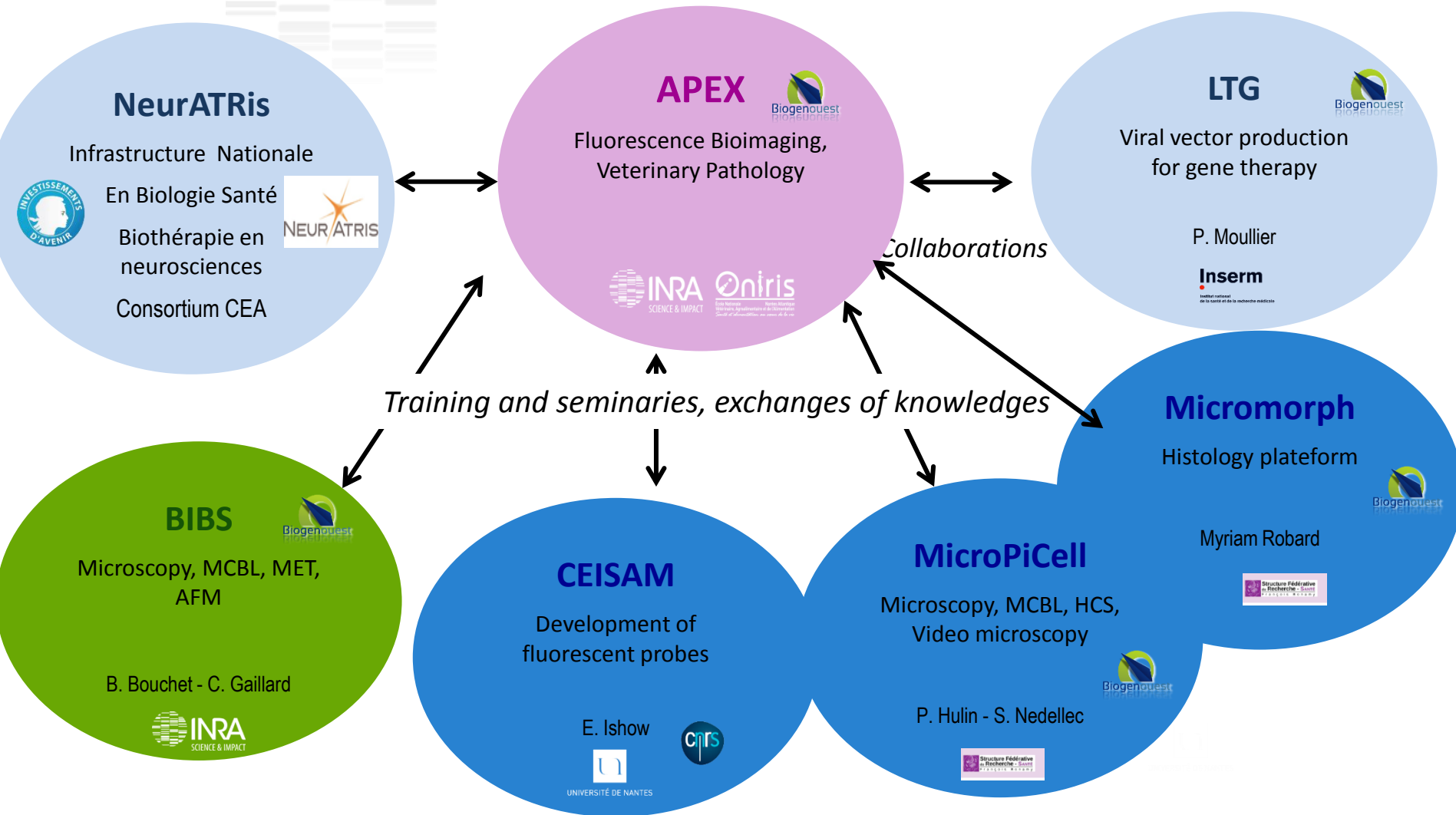
❖ Training

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



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

Interactions with other plate-forms



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 INRA logo (Science & Impact), the text "APEX cellule d'expertise en anatomie pathologique pour la recherche", and the Oniris logo (École Nationale Vétérinaire, Agrobiomérique et de l'Alimentation). Below the banner, the page is divided into sections: "Accueil", "APEX : Expertise en anatomie pathologique pour la recherche", "Actualités", and "Nous contacter". The "APEX" section describes the unit's mission and its association with INRA and Oniris. The "Actualités" section lists three recent publications: "Comparaison de voies d'administration d'un vecteur de thérapie génique", "Phénotypage des cellules dendritiques", and "Effet de la leptine chez un modèle animal de retard de croissance". Each article includes a brief summary, a link to "Lire la suite", and a small image. The "Nous contacter" section lists three contact persons: Marie-Anne Collie, Thibaut Larcher, and Laurence Dubreil. The footer of the page displays the INRA and Oniris logos, along with the text "APEX / Expertise in Veterinary Pathology and fluorescence bioimaging".

Anatomie pathologique Sante Animale - Accueil

www6.inra.fr/anatomie_pathologique_sante_animale

INRA SCIENCE & IMPACT

APEX cellule d'expertise en anatomie pathologique pour la recherche

Oniris École Nationale Vétérinaire, Agrobiomérique et de l'Alimentation

Accueil

APEX : Expertise en anatomie pathologique pour la recherche

Crée en 2006 au sein de l'UMR703 INRA – Oniris, la plate-forme APEX a pour mission principale d'apporter aux équipes de recherche, une expertise en histopathologie animale et en phénotypage tissulaire et cellulaire. APEX associe l'expertise de trois pathologistes vétérinaires, tous diplômés du collège européen de la spécialité, à celle d'ingénieurs spécialisées dans les domaines de bio-imagerie en fluorescence et de biologie moléculaire sur lames.

APEX est une composante de la plate-forme intégrée de «**biothérapie et physiopathologie animale**», labellisée par le GIS IBISA depuis 2008, qui associe le laboratoire de production de vecteurs viraux recombinants (INSERM U1089, IRT, Nantes) et le centre d'expérimentation animale de Boisbonne (Oniris, Nantes). Elle a pour principale mission de répondre aux besoins de la recherche préclinique pour l'évaluation de l'efficacité et de l'innocuité des biothérapies sur des modèles animaux de grande taille.

IBISA

Actualités

Comparaison de voies d'administration d'un vecteur de thérapie génique

Les résultats d'un travail réalisé en collaboration avec l'INSERM U1089 de Nantes, font la couverture de la revue Human Gene Therapy du mois de juillet.



[Lire la suite](#)

Phénotypage des cellules dendritiques

Les conclusions d'un travail réalisé en collaboration avec l'UMR707 IECM d'Oniris sont publiées dans la revue Journal of Immunology.



[Lire la suite](#)

Effet de la leptine chez un modèle animal de retard de croissance

Dans la revue PlosOne viennent de paraître les résultats démontrant le rôle de la leptine dans les processus du développement en général ouvrant de nouvelles perspectives concernant la compréhension...



[Lire la suite](#)

Le réseau Biogenouest s'agrandit

APEX vient de rejoindre le réseau des plates-formes du Grand Ouest en sciences du vivant et de l'environnement.



[Lire la suite](#)

Nous contacter

- Marie-Anne Collie
- Thibaut Larcher
- Laurence Dubreil