



Association Française
des Sociétés de Services et d'Innovation



Centre de recherche
FRANCAIS

afssi.fr

Les membres AFSSI ont la parole ”

WEBINAIRE



Le partenaire incontournable de **vos innovations**

Proche de chez vous



AMYLGEN : Your partner for the discovery of new drug candidates for the treatment of CNS diseases



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Les membres AFSSI
ont la parole ”

WEBINAIRE

Qui suis-je ?
La réponse
en 20 min

AFSSI
Association Française
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Activity

As a preclinical CRO, Amylgen proposes rodent models of neurodegenerative and psychiatric diseases allowing the rapid testing of new drugs with validated predictive value.

Furthermore, Amylgen evaluates nutraceutical products aiming the field of "brain health" or "healthy brain ageing".



Amylgen

- Amylgen was created in 2009 as a spin off of the University of Montpellier
- In 2014, Amylgen moved to its own A1 certified housing animal facility
- Facilities include an extensive platform of behavioral analyses and fully equipped biochemistry and IHC laboratory for *in vitro* and *ex vivo* experiments

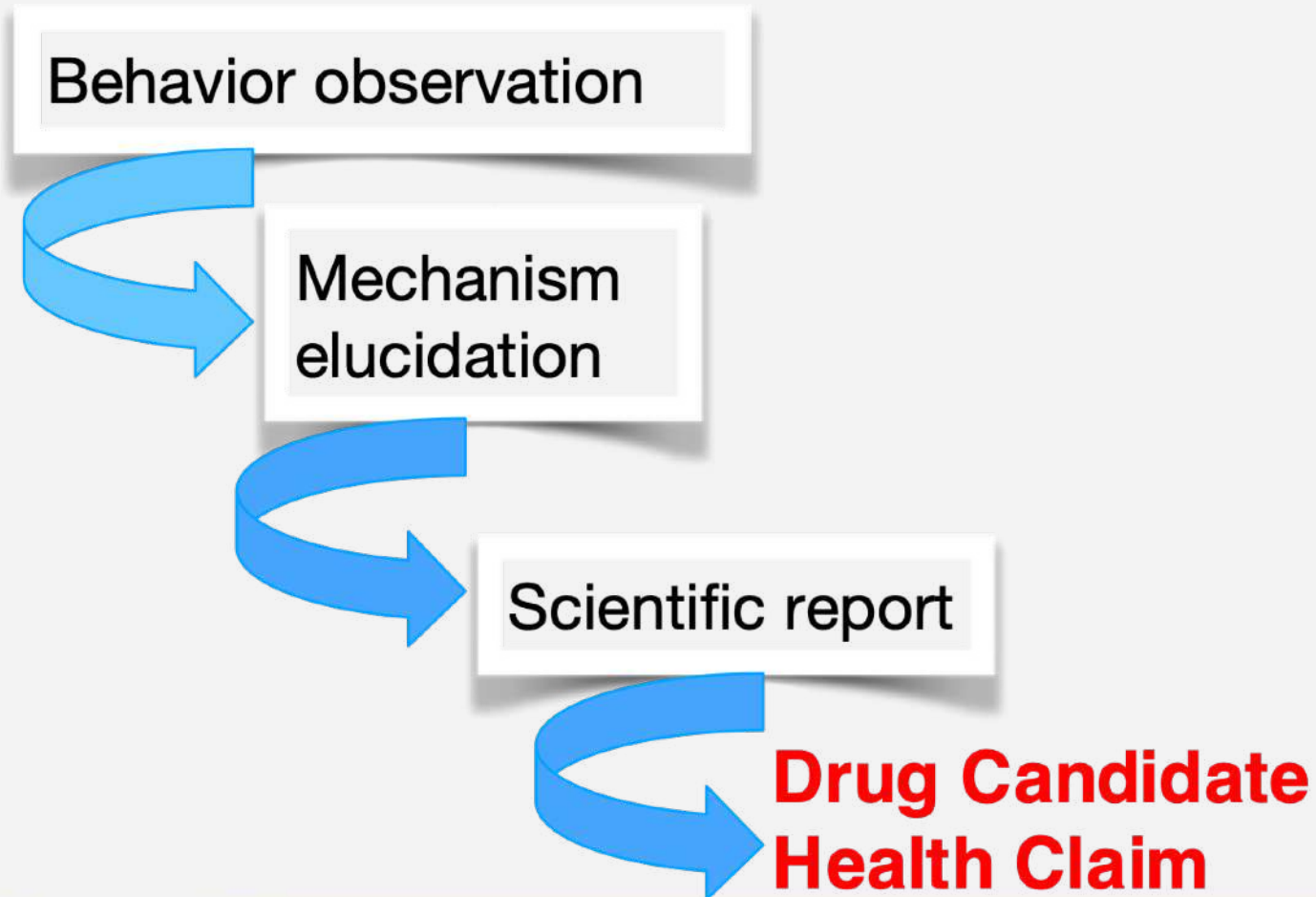
Amylgen' preclinical offer

Amylgen is at your service to provide quick POC of the efficacy of your product in improving **brain health**





Amylgen's preclinical offer





Areas of interest

- Brain ageing preservation
- Memory enhancement
- Brain development
- Antidepressant/antianxiety effect
- Protection against neurodegenerative diseases



Animal models

Neurodegenerative diseases

Alzheimer's disease:

*Sporadic pathology (SAM)
5XFAD Tg mouse
Active tau PFF fibrils (under dev)*

Parkinson's disease:

*6-hydroxydopamine (6-OHDA)
 α -synuclein overexpression*

Amyotrophic Lateral Sclerosis:

*SOD1*G93A mouse model*

Huntington's Disease:

R6/2 mouse model

Mood and psychiatric disorders

Depression, Anxiety

Chronic restraint stress

Schizophrenia:

*MK801-induced hyperactivity
D-amphetamine-induced hyperactivity
Phencyclidine-induced hyperactivity
PPI (under dev)*

Sleep deprivation

Effects on memory



Cognitive & Memory deficit

*MK-801-induced cognitive deficit
Scopolamine-induced amnesia
Phencyclidine-induced amnesia*

Brain development or ageing

*Senescence Accelerated Mice (SAM)
D-galactose (D-Gal) intoxication model
Normal young or Aged animals
Neurogenesis*

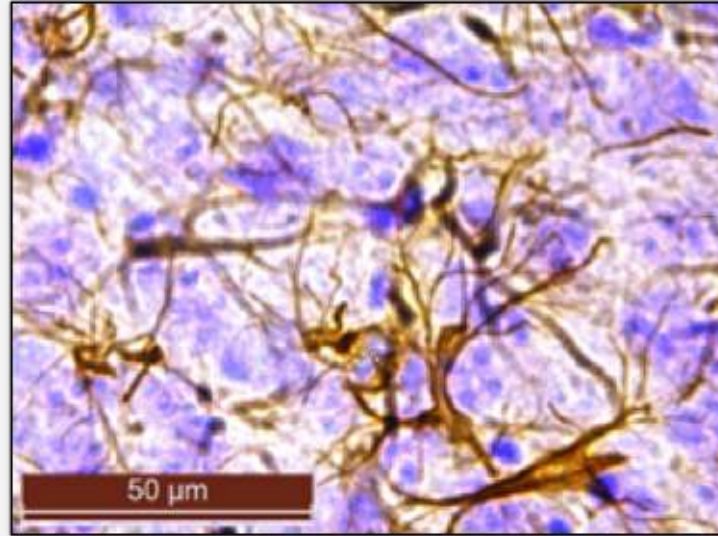
Attention deficit

Prenatal stress in rats

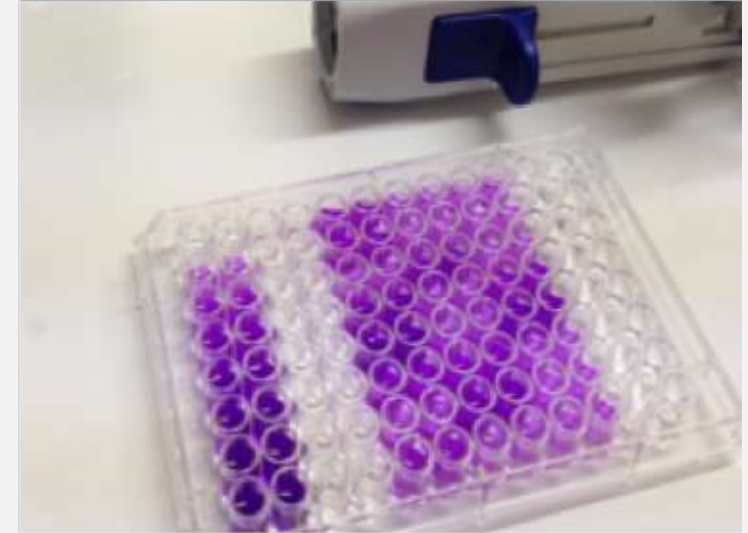
Fully integrated services



Behavioral analyses



Histology & Immunohistochemistry analyses



Molecular & Biochemical analyses



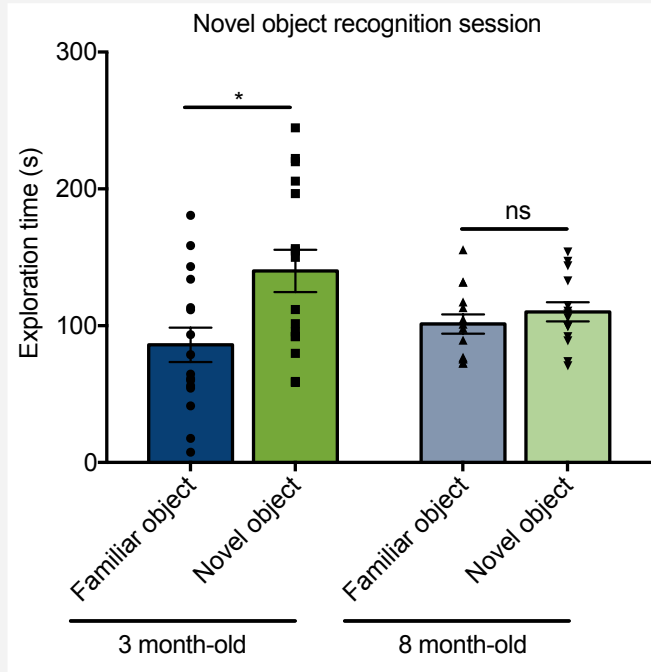
Senescence Accelerated Mice (SAM) model



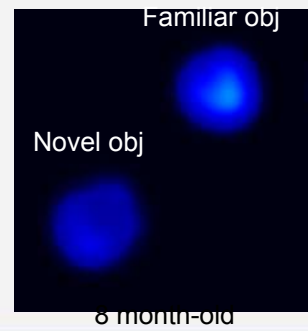
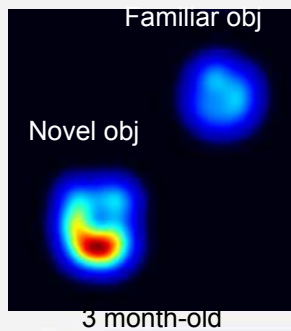
SAMP/8 show cognitive and physiological hallmarks of ageing at 12 months of age whereas SAMR/1 control line age normally (24-30 months).

Maurice, Roman et al., Brain Res., 1996

NOR



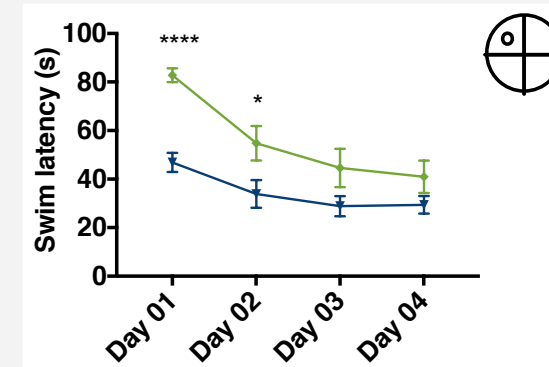
Test session (retention interval :24h)



SAM

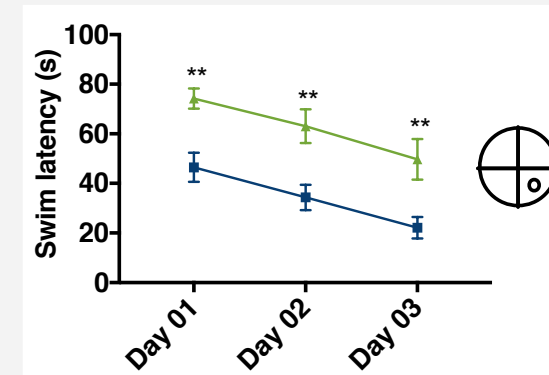
MWM

Learning session



■ SAMP8 (6 month-old)
▲ SAMP8 (10 month-old)

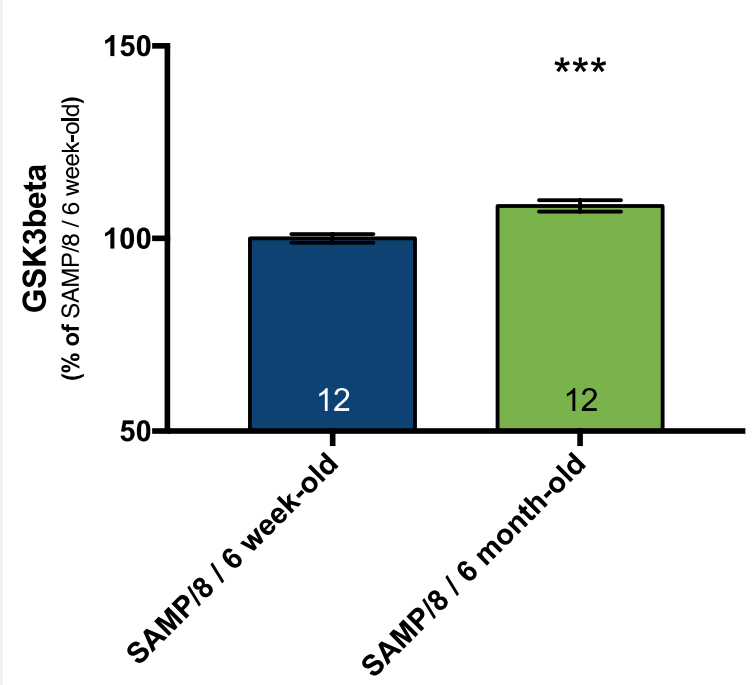
Reversal session





SAM

GSK3 beta (Cortex)



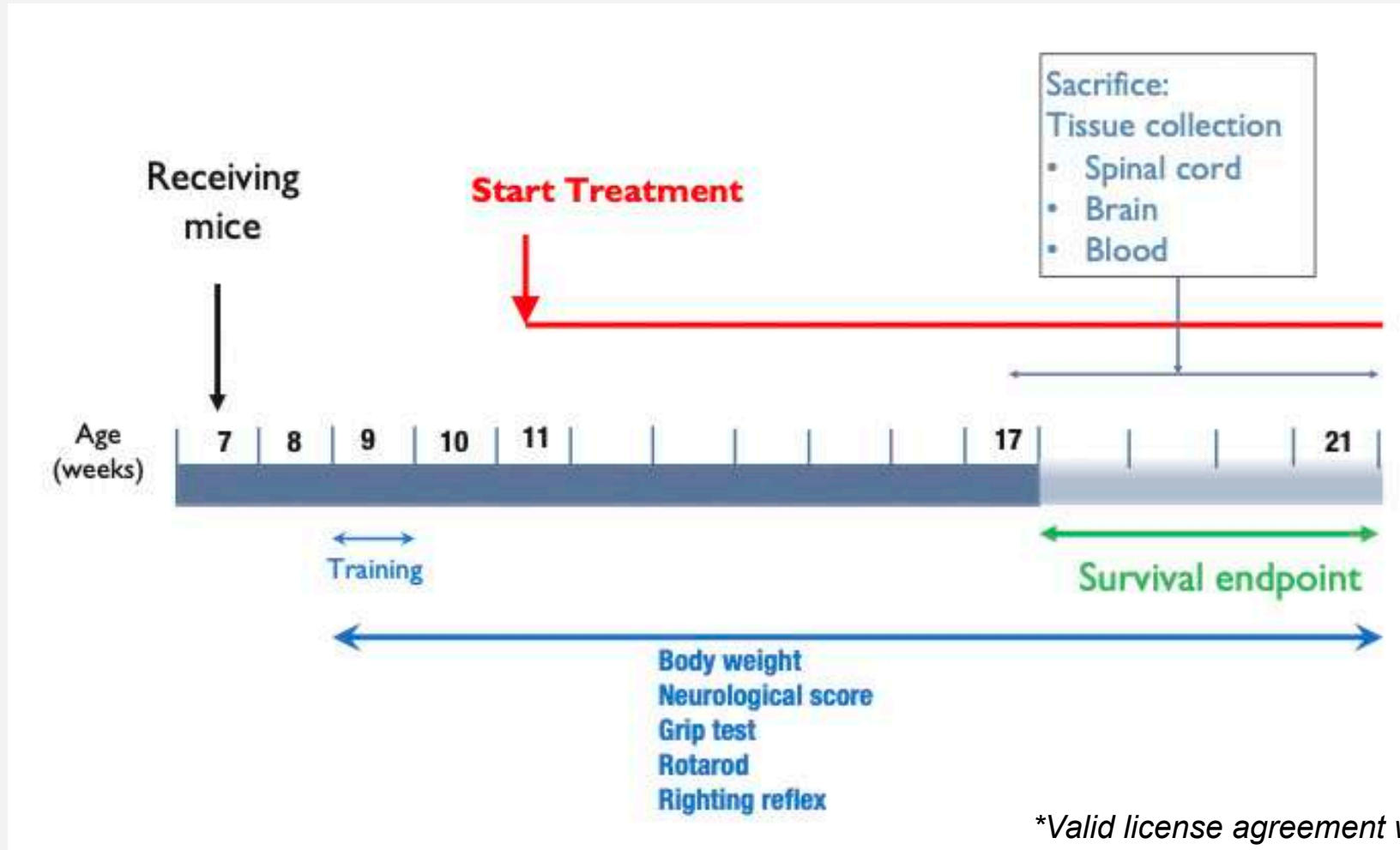


SAM develop a sporadic form of Alzheimer's disease

Besides important deficits in memory, at 8 months of age, SAM display:

- Anxiety
- Increase of various markers of
 - Oxidative stress (LPO)
 - Vascular inflammation (VCAM1)
 - Apoptosis (caspase 3, caspase 12)
 - AD markers: $A\beta_{1-42}$ and hyperphosphorylated Tau protein

Amyotrophic Lateral Sclerosis (ALS): SOD1*G93A mice



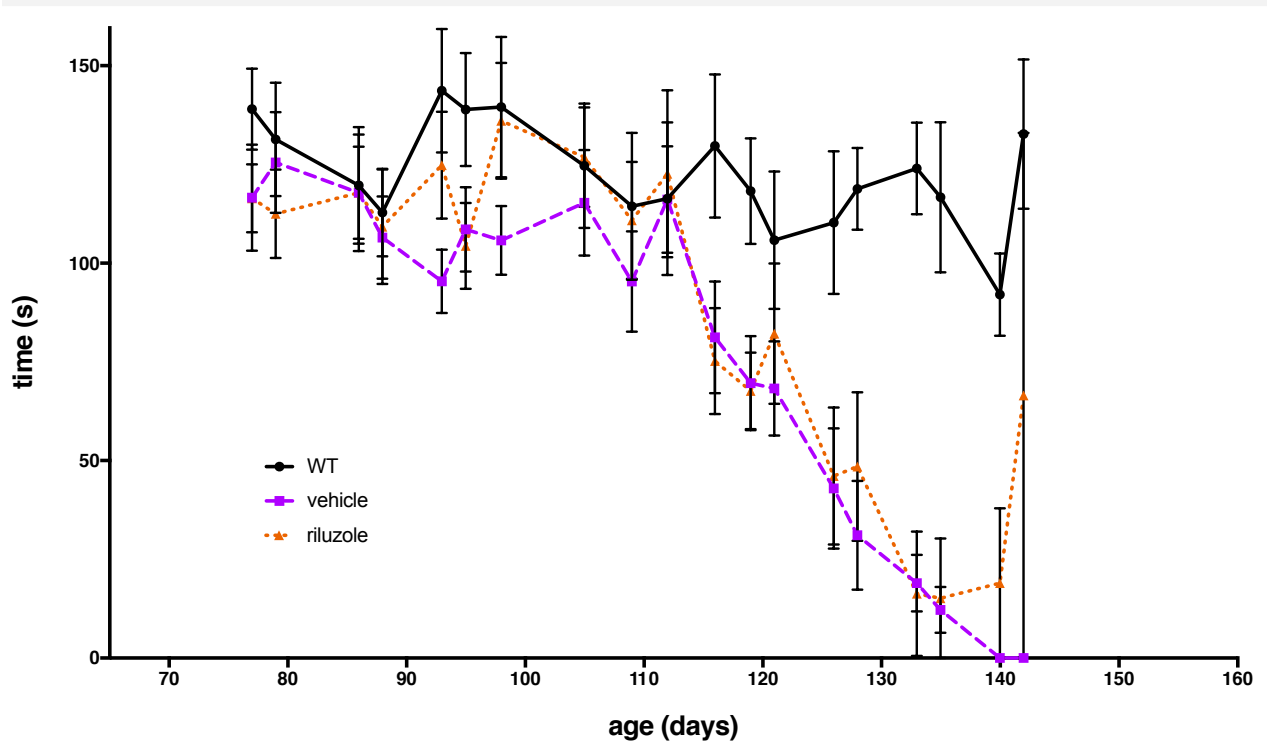
**Valid license agreement with Northwestern University*

ALS

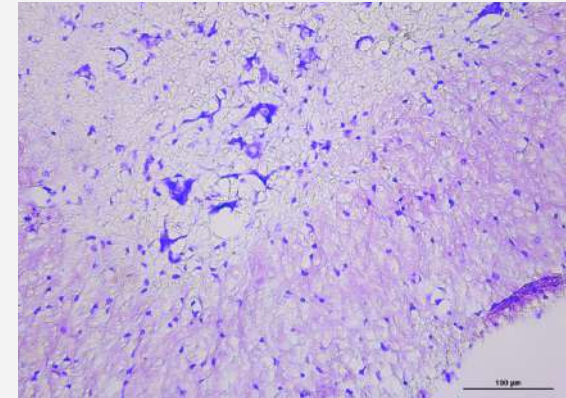
Rotarod

Spinal cord (ventral horn)

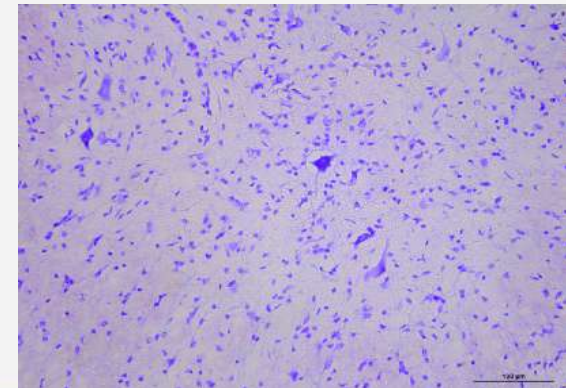
@ 21 weeks of age



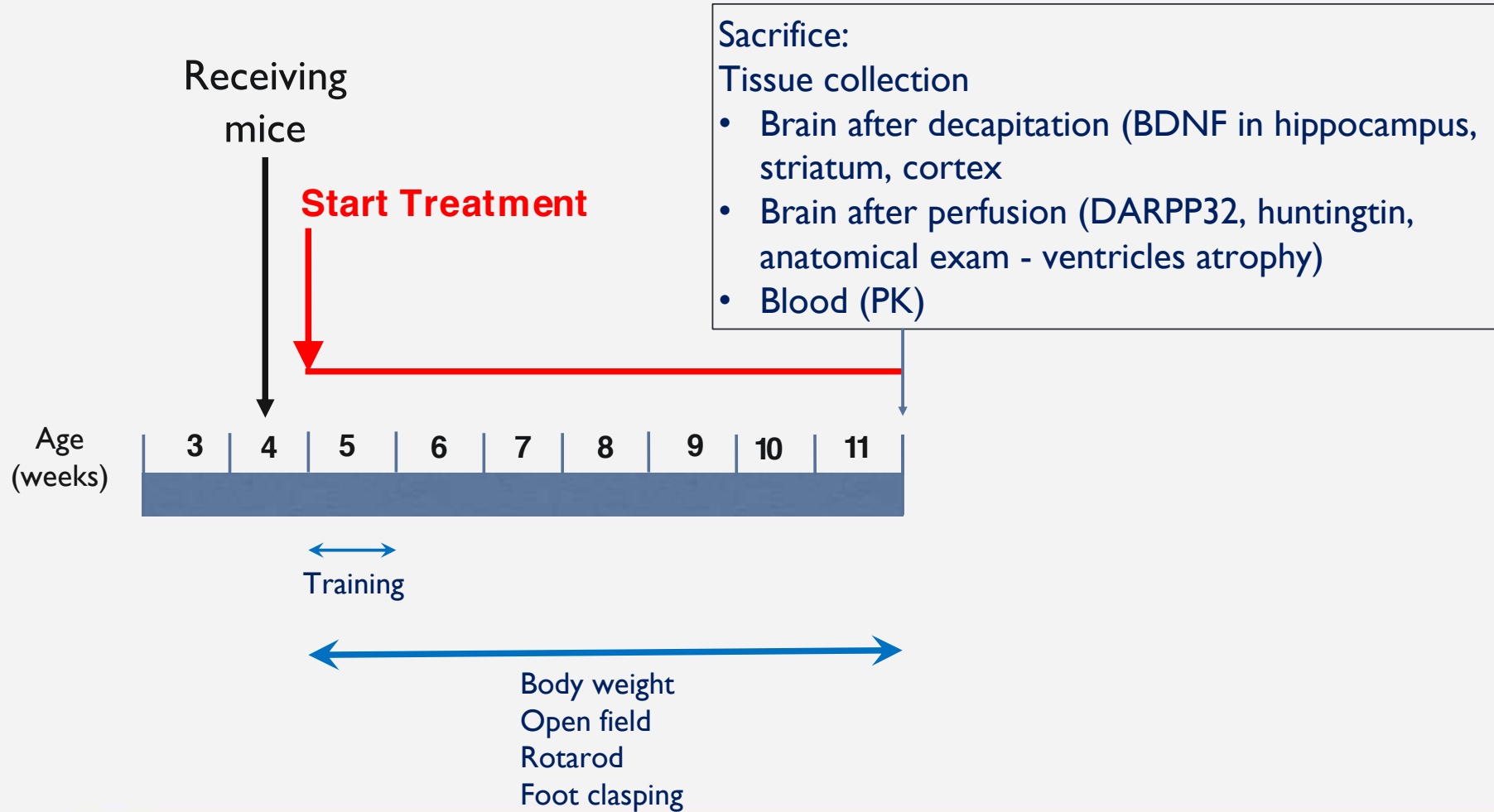
WT- veh



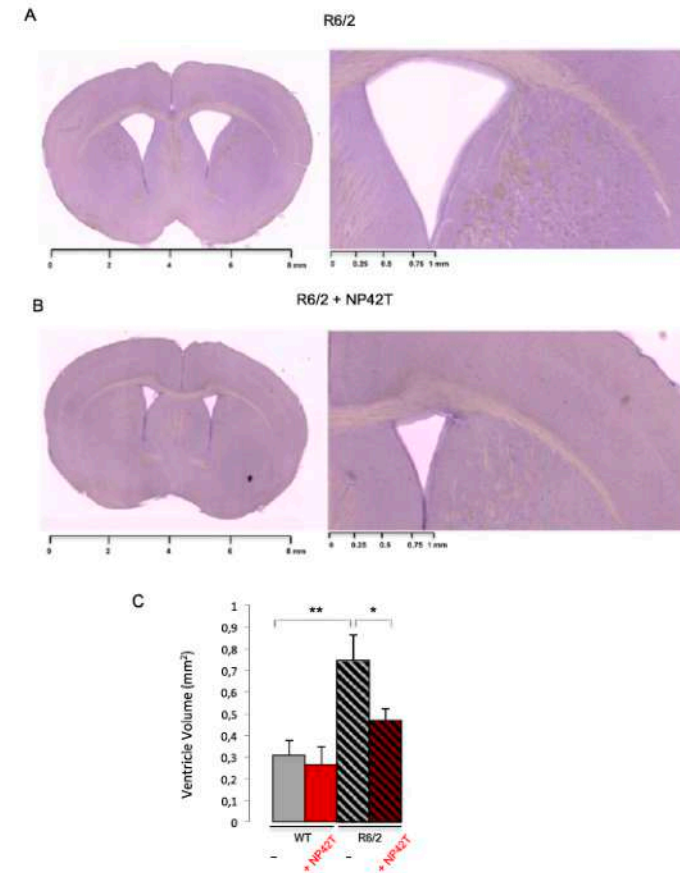
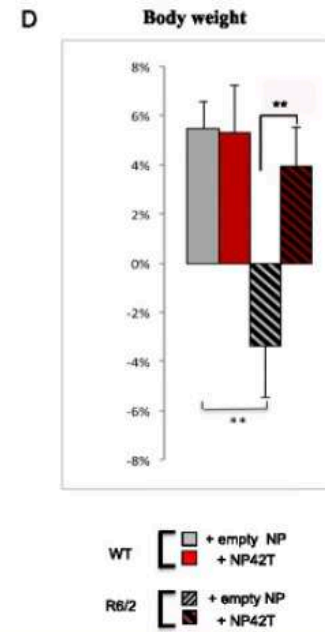
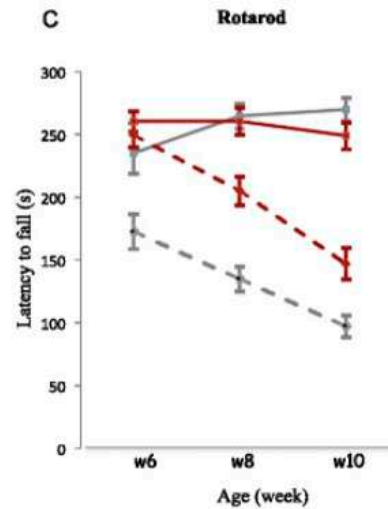
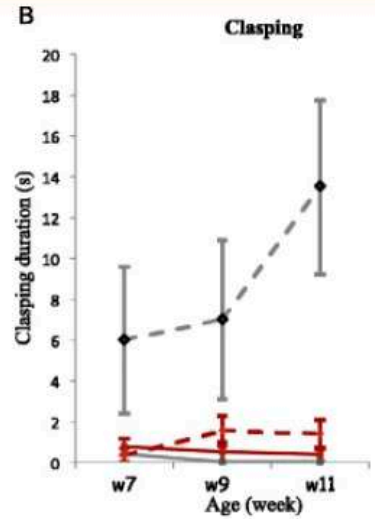
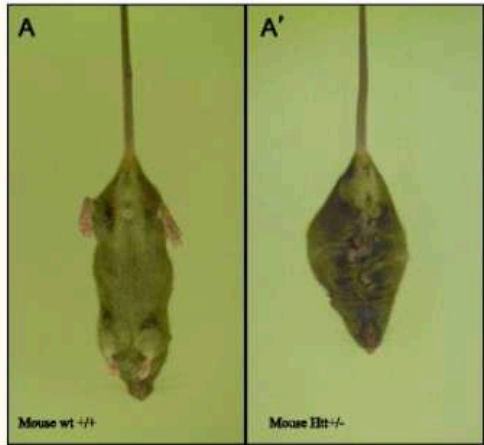
SOD- veh



Huntington's Disease: R6/2 mice



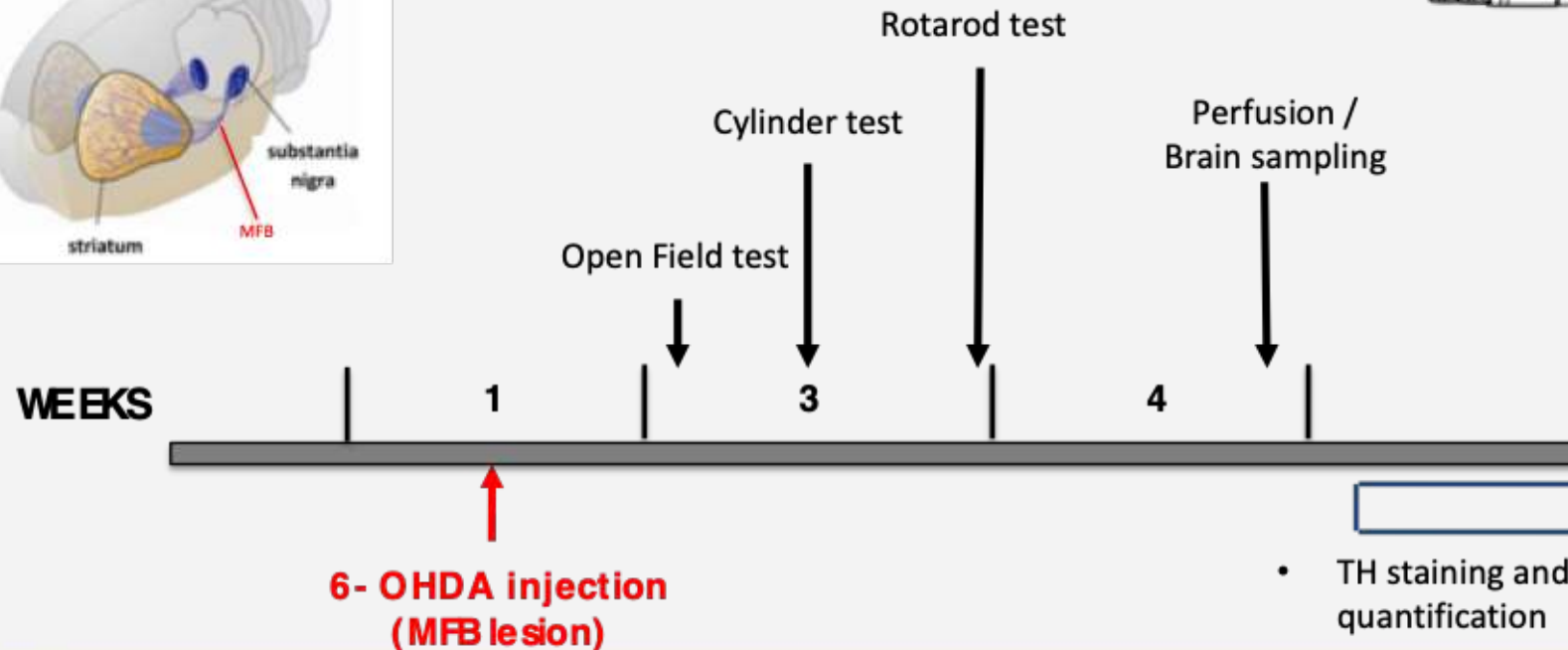
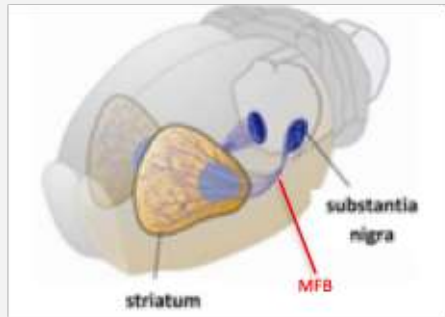
Huntington's Disease: R6/2 mice



Arribat et al., 2014

Parkinson's Disease: 6-hydroxydopamine (6-OHDA)

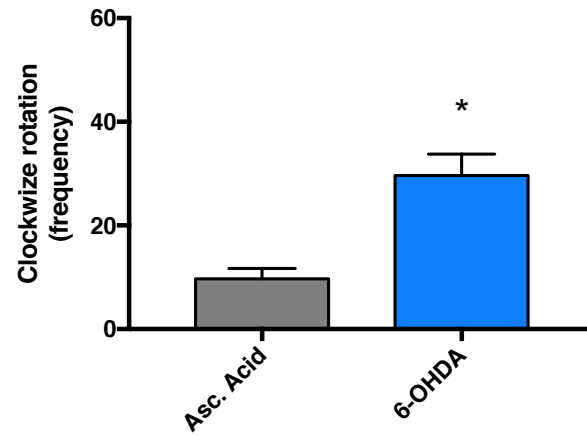
✓ Unilateral 6-hydroxydopamine (6-OHDA) injections into the medial forebrain bundle (MFB) containing the ascending nigrostriatal fibers



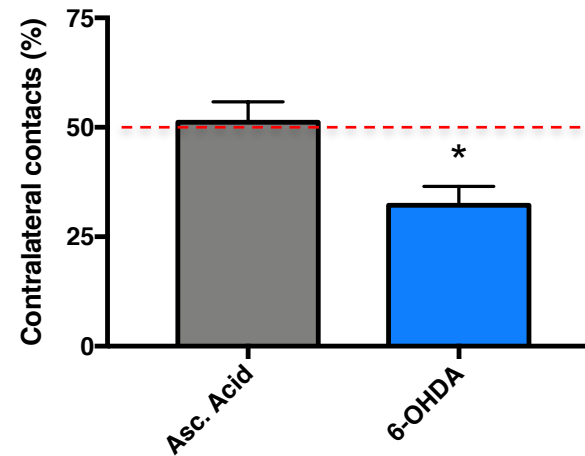


Parkinson's Disease: 6-hydroxydopamine (6-OHDA)

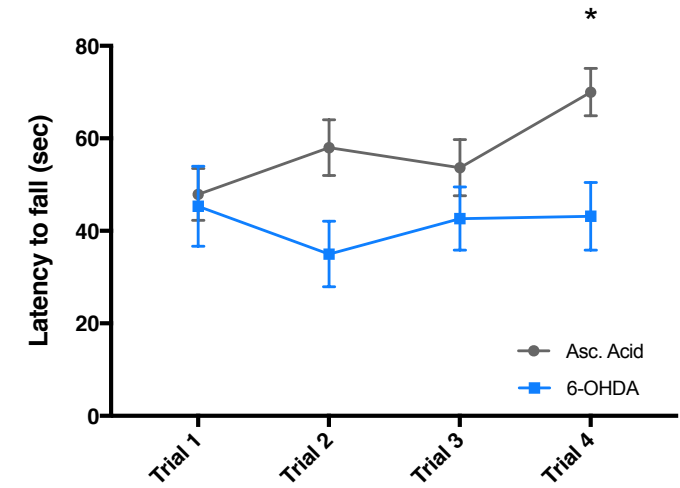
Ipsilateral rotation



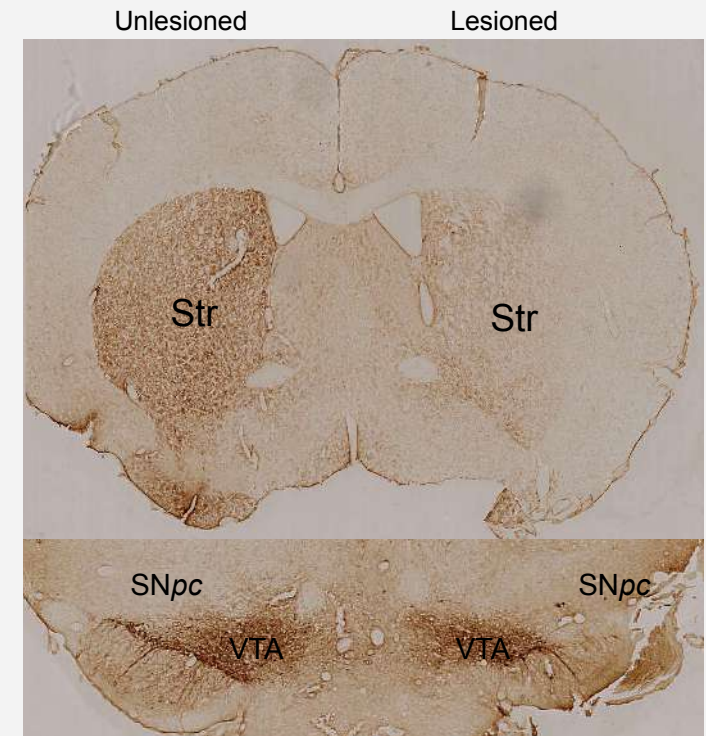
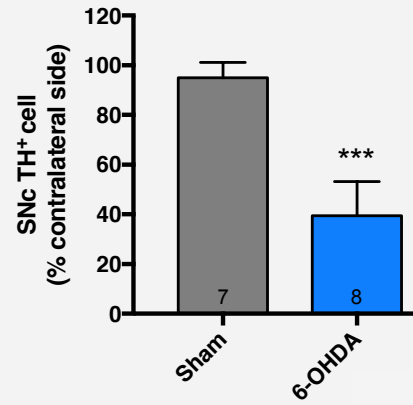
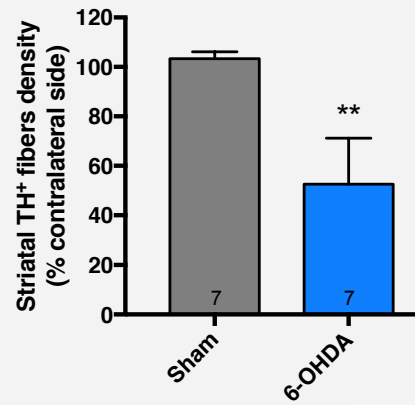
Forelimb asymmetry



Motor coordination

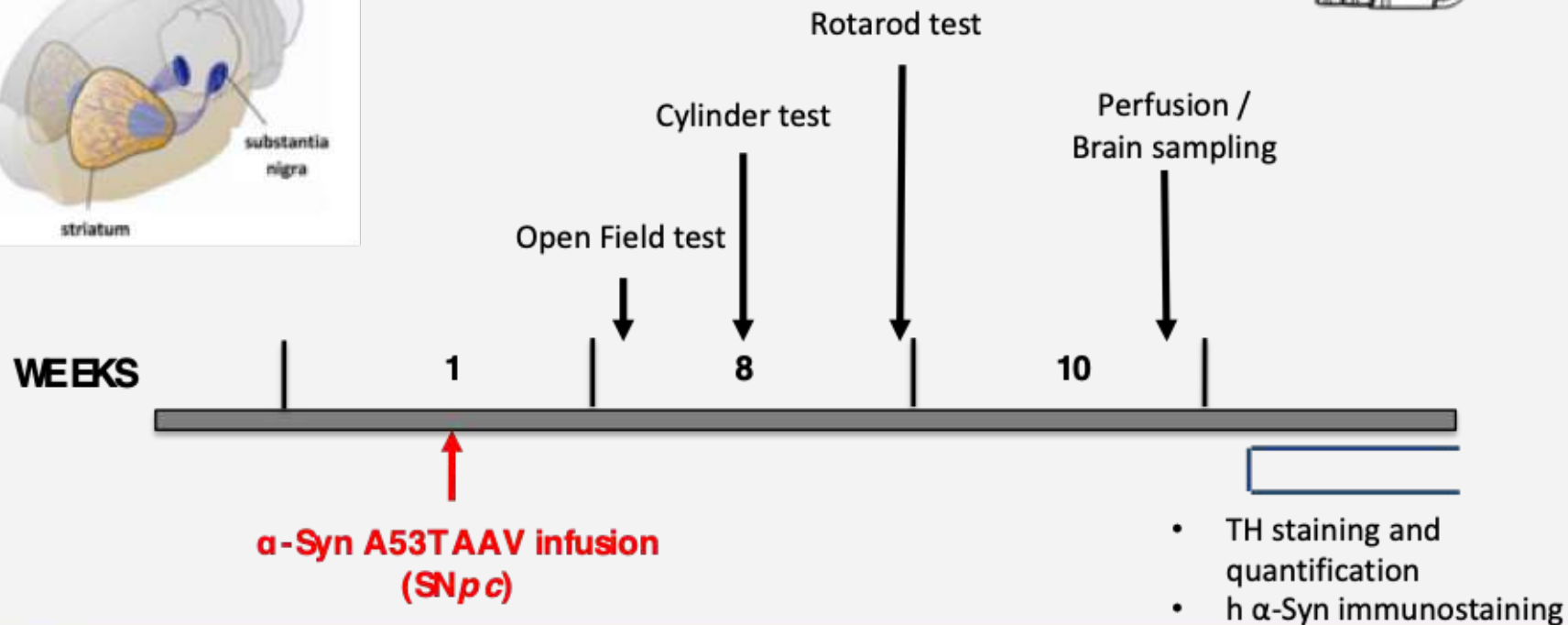
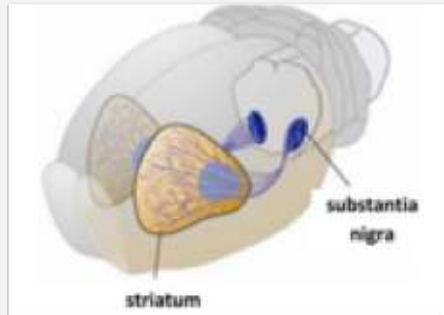


Parkinson's Disease: 6-hydroxydopamine (6-OHDA)



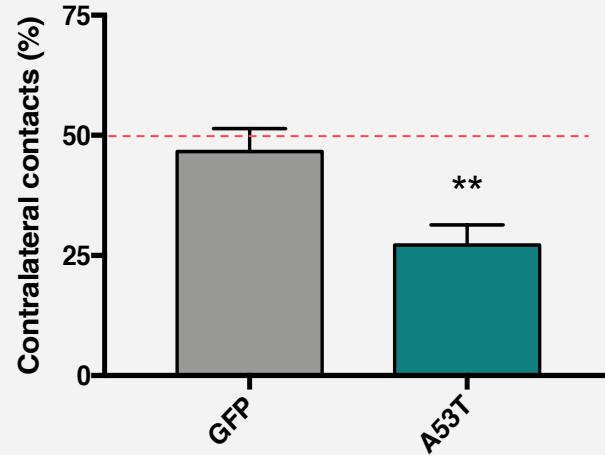
Parkinson's Disease: α -synuclein overexpression

- ✓ Unilateral AAV infusion into the substantia nigra *pars compacta* (SNpc) containing the dopaminergic cells

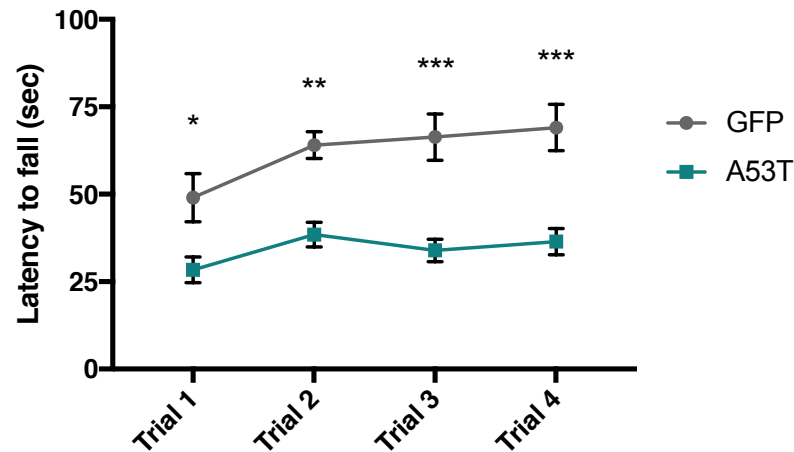


Parkinson's Disease: α -synuclein overexpression

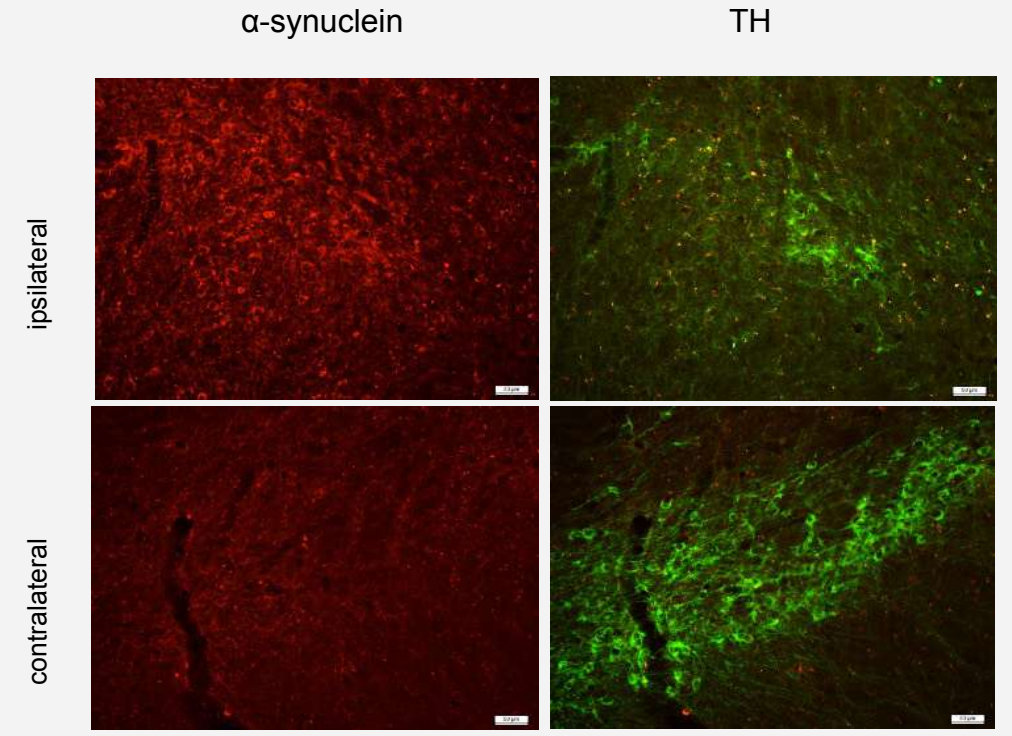
Forelimb asymmetry



Motor coordination



AAV-A53T-a-syn (SNpc - 9 weeks)

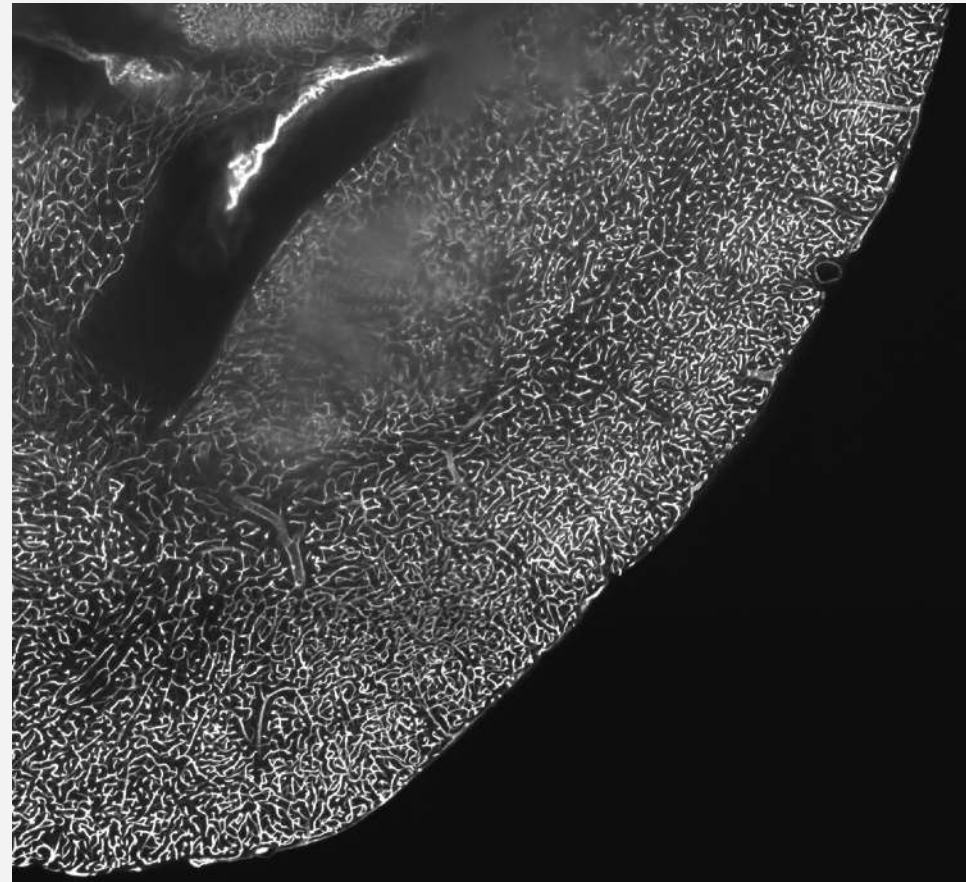




Novel services

Microvascularization analysis in relation with aging or NDDs

- Brain
- Heart
- Eye

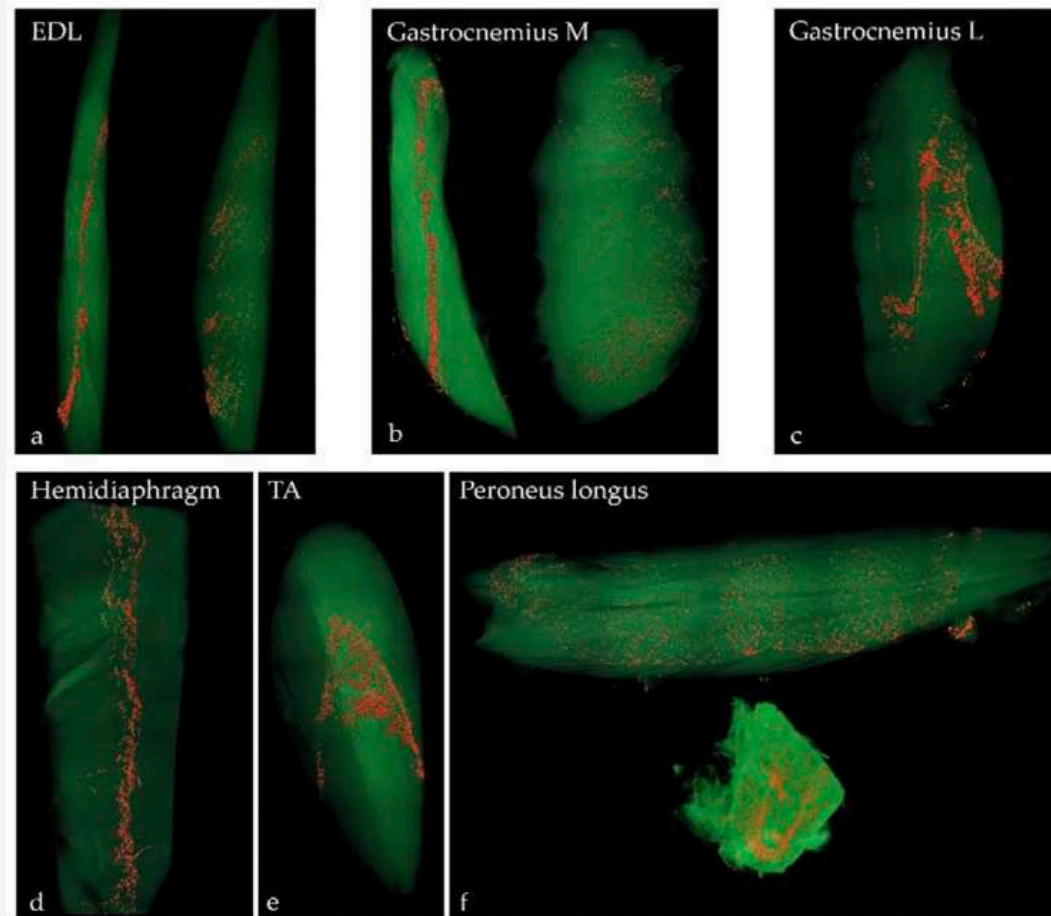


Novel services

Neuro muscular junction quantification

in relation with aging or ALS

- Tibialis
- Gastrocnemius
- Diaphragm



amylgen 



Amylgen's strengths

Competence: *our strong knowledge in CNS diseases and Drug Discovery comes from University and Industrial background*

Flexibility: *we better adapt the experimental designs for your specific needs*

Rapidity: *our challenge is to enable you to move on quickly to the next stage*

Reliability: *our models have enabled both Anavex Life Science and Pharnext to reach quickly the First-in-Man clinical stage*

Network: *we know how to find the experts for each step of your product development*



WEBINAIRE

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Spécial SYNERGIES

6 JUILLET 2021 15H

IMACTIV-3D

en collaboration avec **amylgen** ✓

Nouveau service innovant
pour des tests précliniques en 3 dimensions



- Spin-off du CNRS
- Créée en 2015
- Située à Toulouse
à l'Oncopôle - Campus Santé du Futur

Biologie

Tests biologiques,
préparation des échantillons
(marquage et
transparisation)...

Imagerie

Microscopie à feuille de
lumière, confocale, biphoton,
champ large, lumière
structurée...

Traitement d'image

Restauration, caractérisation,
quantification, visualisation,
realization de médias...

Offre de service modulable



Vos besoins

Nos services



**Modèles *in vitro*
3D**

Modèles 3D commercialisés ou sur-mesure



Imagerie 3D

Plateforme d'imagerie 3D



**Traitement d'image
3D**

Visualisation et reconstruction 3D



**Caractérisation et
Quantification 3D**

Caractérisation et quantification de critères morphologiques, fluorescence, régionalisation...

Offre de service modulable : Notre expertise

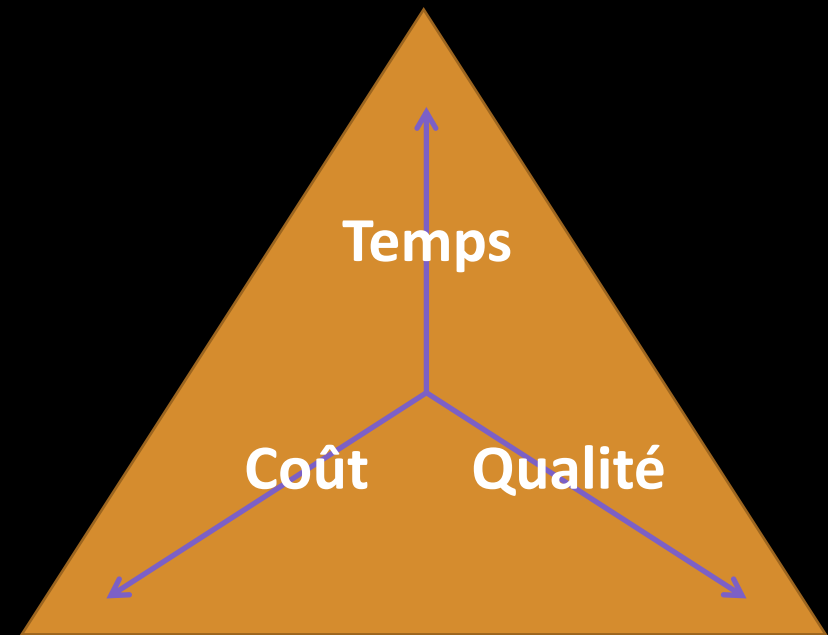


- Préparation des échantillons biologiques
 - Marquage en immunofluorescence
 - Transparisation si nécessaire
 - Préparation pour l'imagerie
- Imagerie en microscopie 2D/3D
 - Conseils pour améliorer vos acquisitions pour la quantification par traitement d'image
 - Prise en charge des phases d'imagerie
- Traitement d'image
 - Développement à façon
 - Transformer une modalité d'imagerie en un instrument de mesure
 - Par méthode mathématique
 - Par Deep learning
 - Transformer des données complexes en informations exploitables
 - Compromis entre la résolution et la quantité de données
 - Compatible avec les coûts des grandes études précliniques ou cliniques

Offre de service modulable: Notre apport



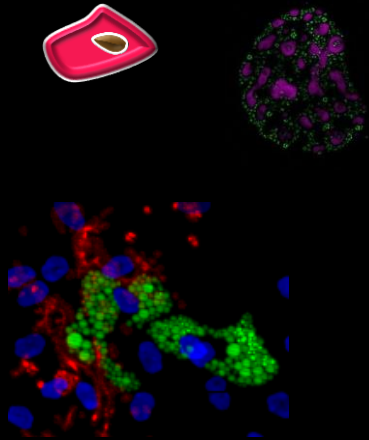
- Pour qui ?
 - Groupes pharmaceutiques
 - En partenariat avec des biotechs
 - Développement de services communs
- Pour quoi faire ?
 - Études *in vitro* ou *ex vivo* en 2D/3D
 - Compréhension des phénomènes biologiques
 - Test d'efficacité des molécules
 - Développement d'une pipeline complete
 - Tout type de modèle biologique
- Nos forces ?
 - Optimisation de vos taches de traitement d'image
 - Automatisation de vos process
 - Amélioration de la robustesse de vos données
 - Diminution du coût de traitement d'image
 - Diminution du temps d'analyse



Gamme d'échantillons pris en charge

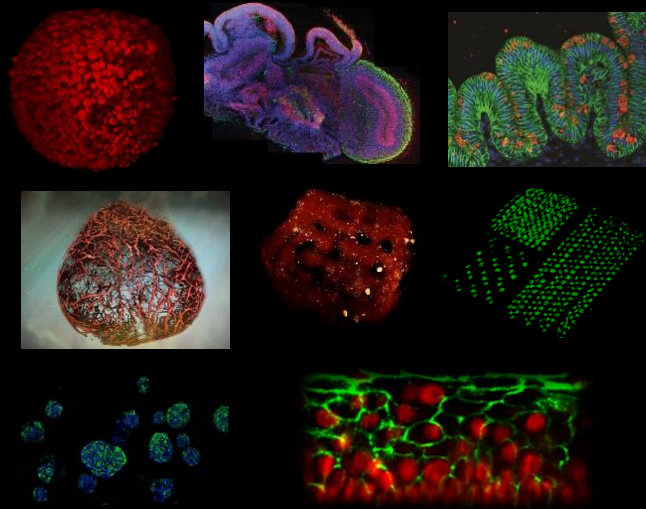


Modèles cellulaires et sub-cellulaires



2D

Modèles cellulaires 3D



In vitro

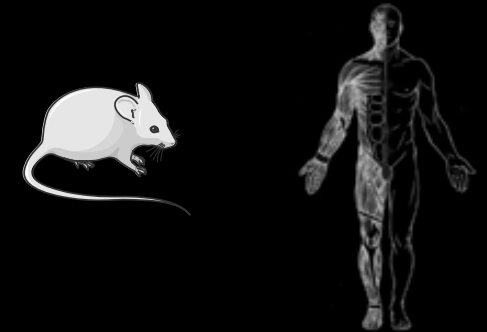
3D

Explants, biopsies



Ex vivo

Animaux, humains



In vivo

Modalités d'imagerie

Microscopie de fluorescence :

- Confocal
- Bi-Photon
- Lumière structurée
- Champ large

Microscopie de fluorescence à feuille de lumière (3D)

Histologie

Imagerie médicale

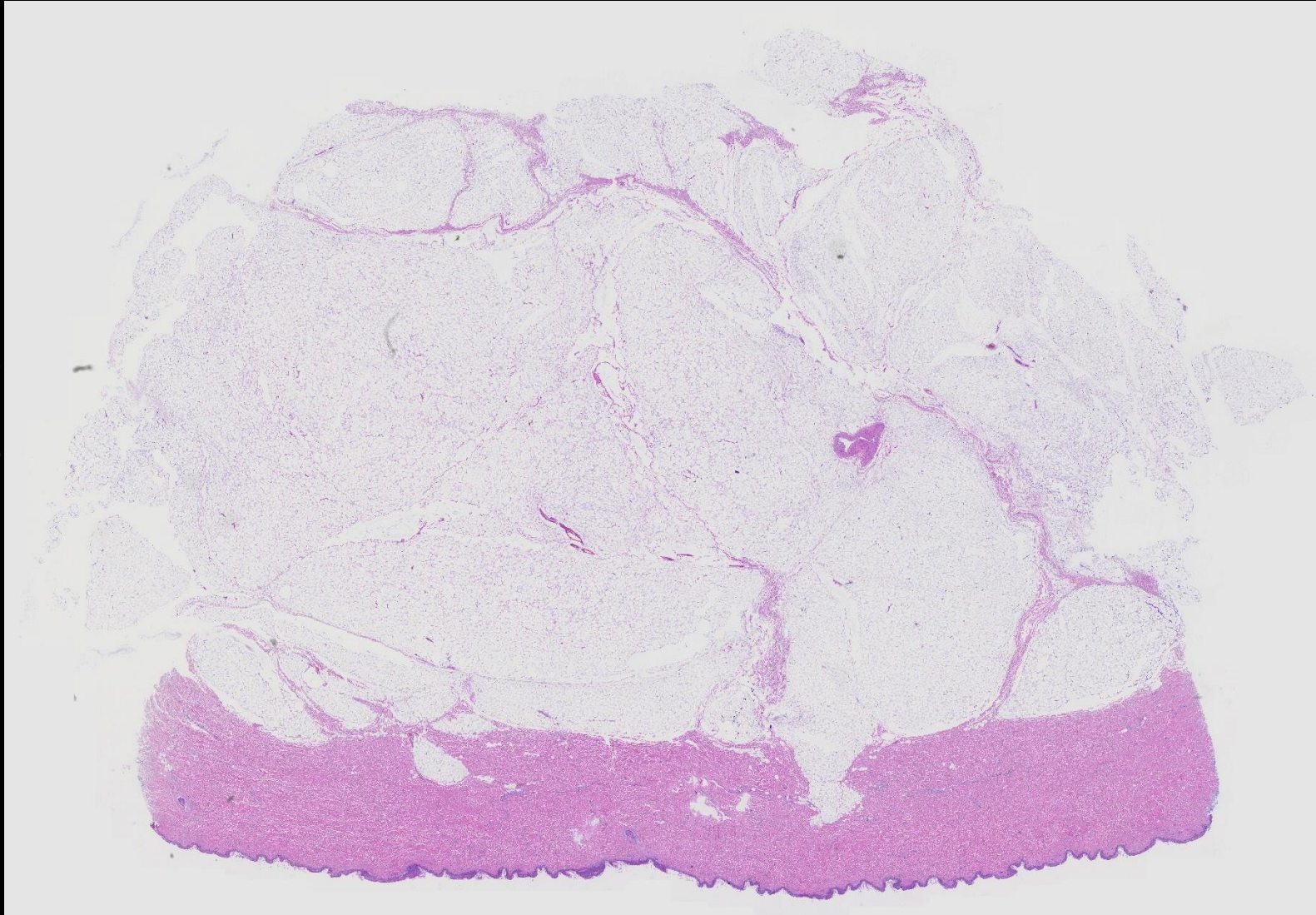
- IRM
- Scanner
- Radiologie
- Echographie
- Tomographie
- ...



IMACTIV-3D

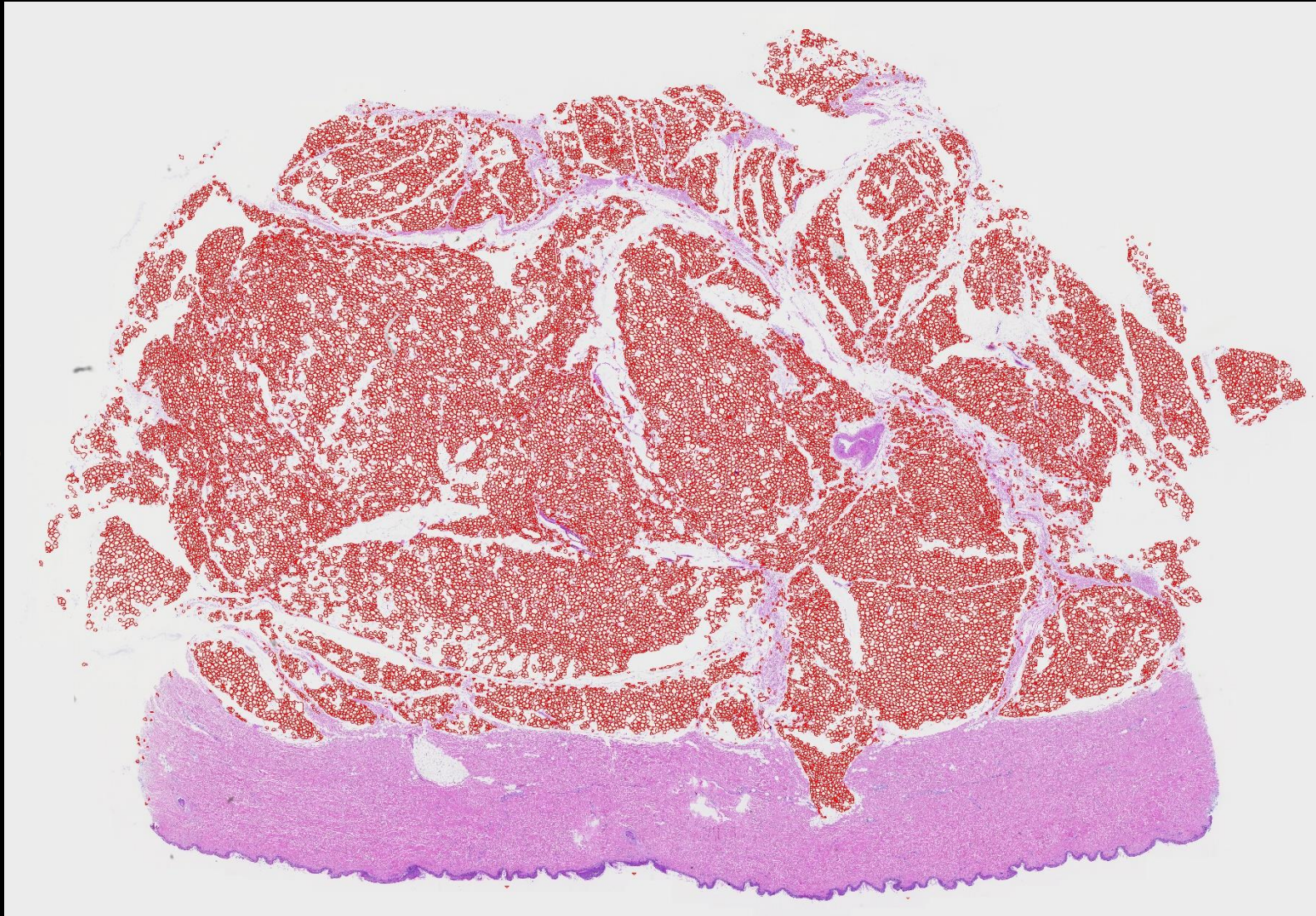
Pourquoi l'imagerie 3D ?

Exemple du tissu adipeux



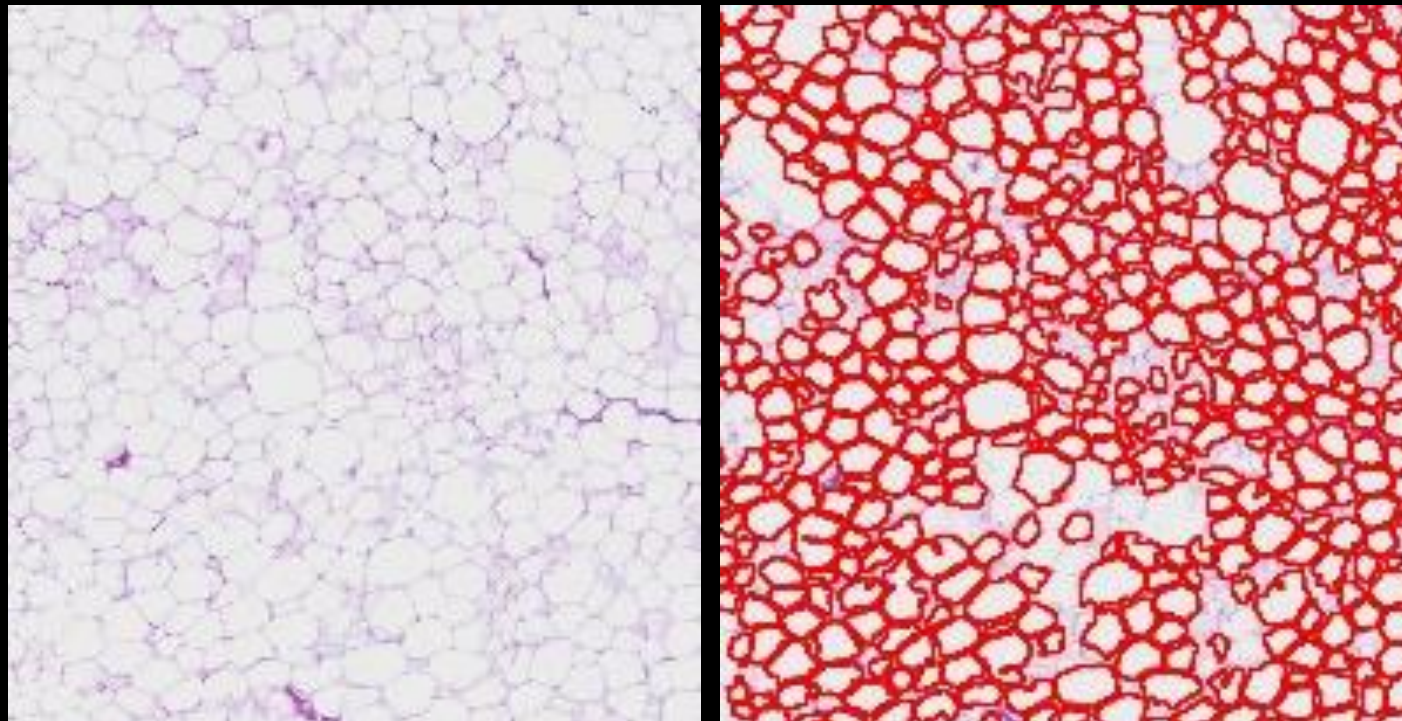
- Comment mesurer la taille des adipocytes ?
- Imagerie par histologie

Exemple du tissu adipeux

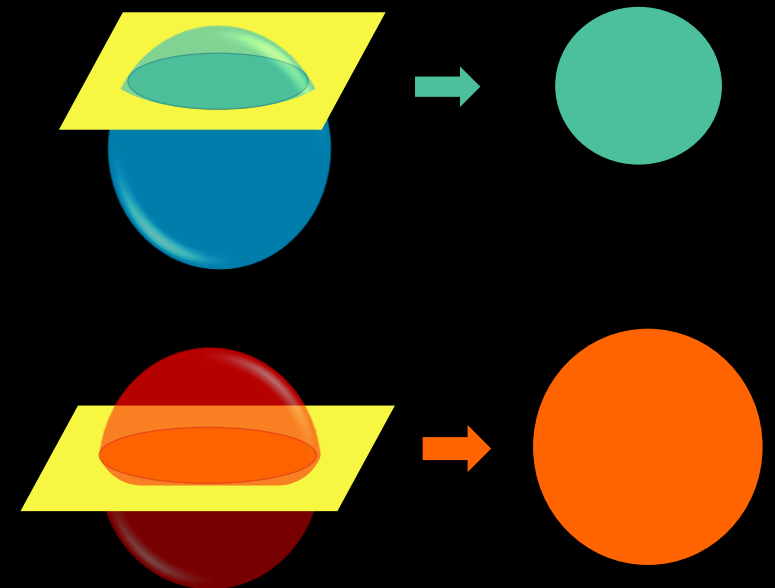


- Reconnaissance automatique des adipocytes
- Calculs de leur taille

Exemple du tissu adipeux

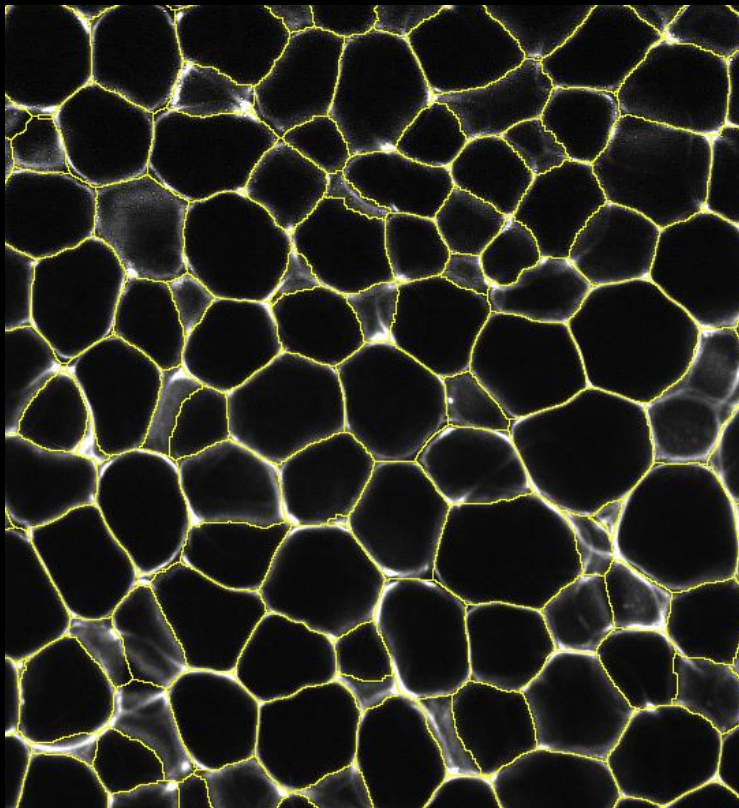


- Adipocyte = Objet sphérique
- Avec l'histologie, les adipocytes ne sont quasi jamais coupés à leur diamètre maximum
- La mesure du diamètre est donc faussée en valeurs absolues
- Mais la grande quantité d'information récoltée rend le test robuste et juste

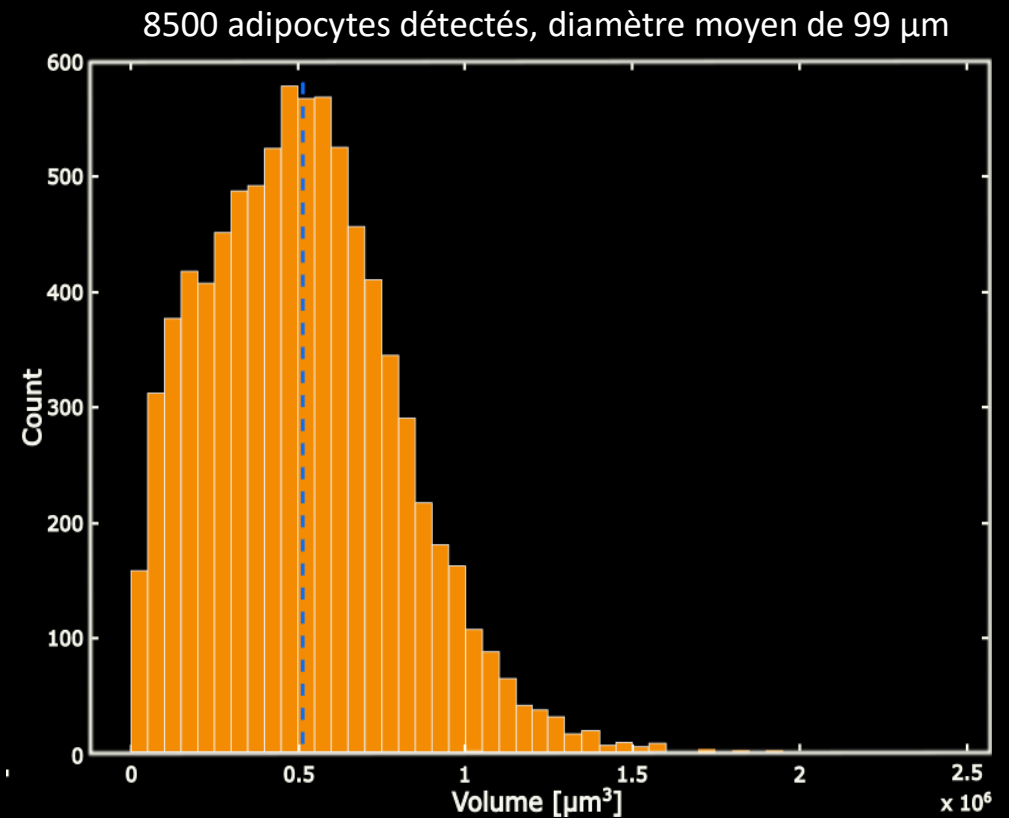


Exemple du tissu adipeux

- Scan 3D scan de l'explant entier
- Équivalent à 1000 coupes en histologie

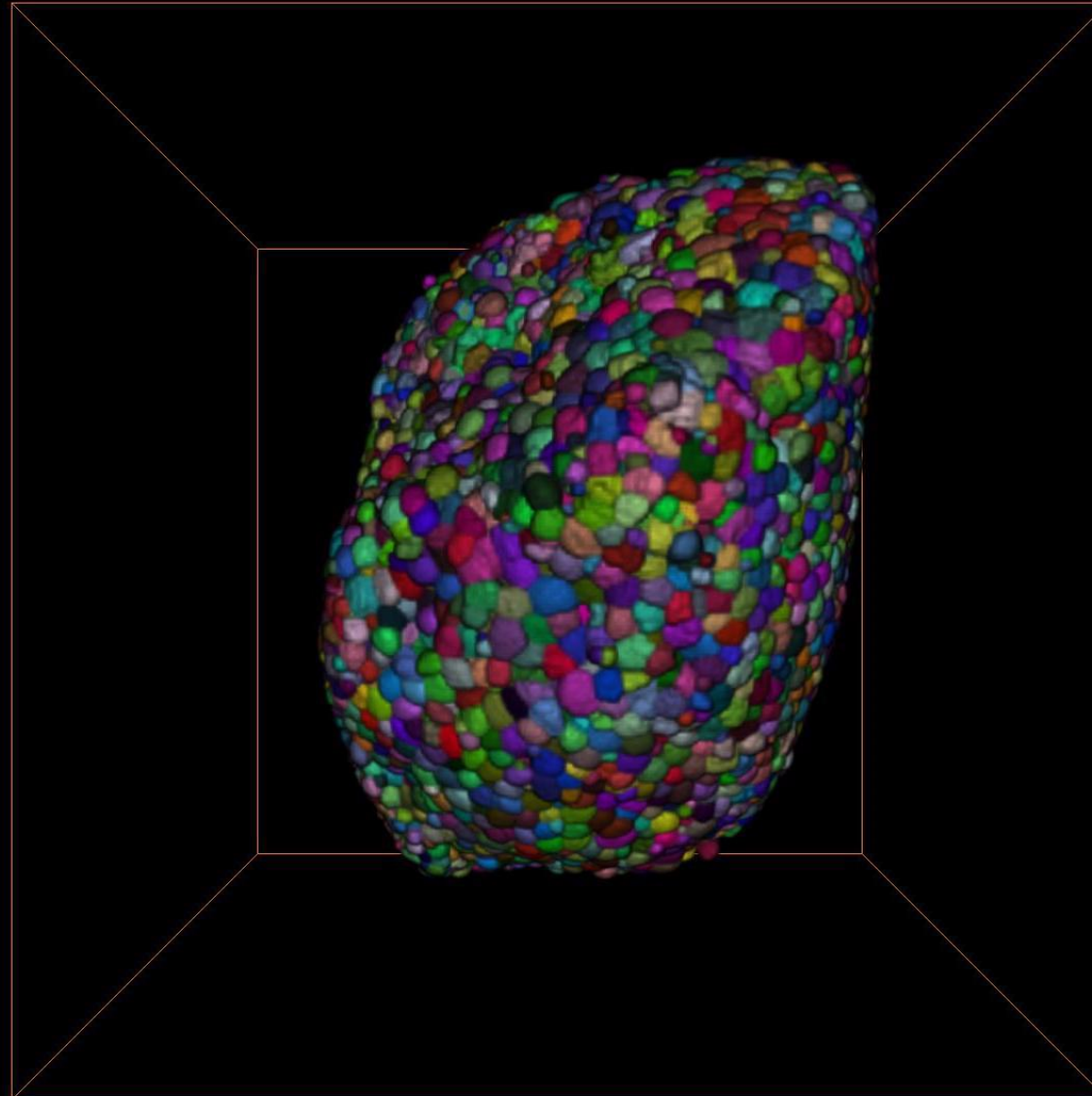


Superposition des données brutes et segmentées



Distribution du volume des adipocytes dans l'explant

Exemple du tissu adipeux



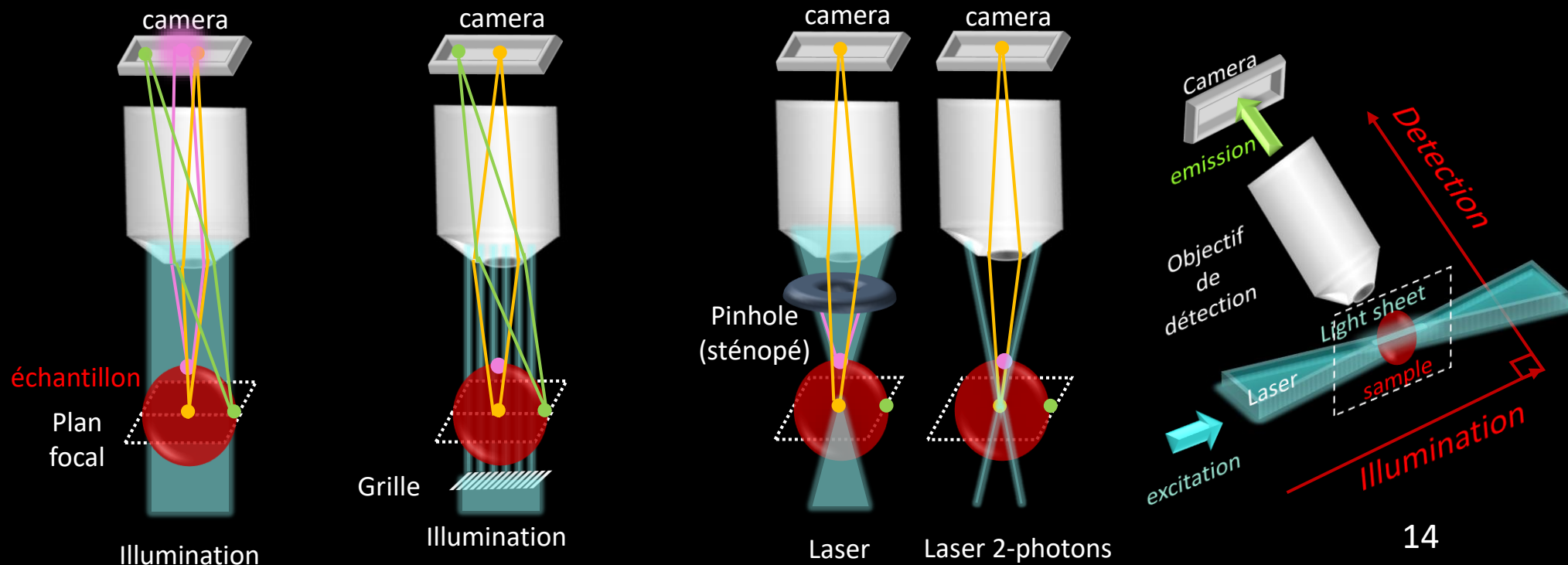


IMACTIV-3D

Quelle technique d'imagerie 3D ?

Comment imager ces objets 3D ?

Microscopie	Champ large	Structured light	Confocal / Bi-photons	Light Sheet
Vitesse	Rapide	Rapide	Lent	Rapide
Pénétration	Faible	Faible	100 à 200 μm	μm au cm
Sectionnement	Non	Calculé	Optique ou focalisé	Épaisseur de la feuille
Photoblanchiment	Non	Non	Oui	Non



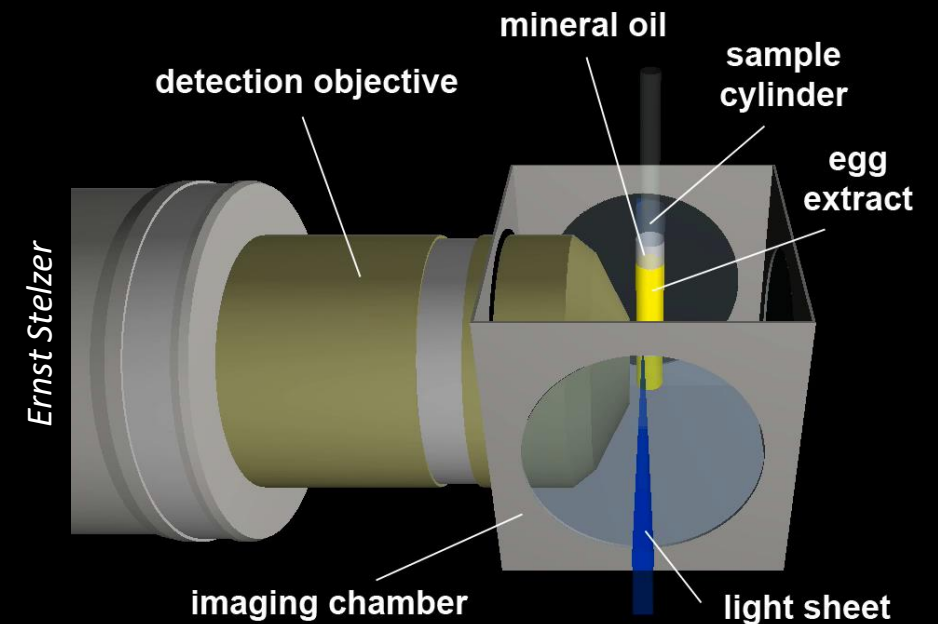
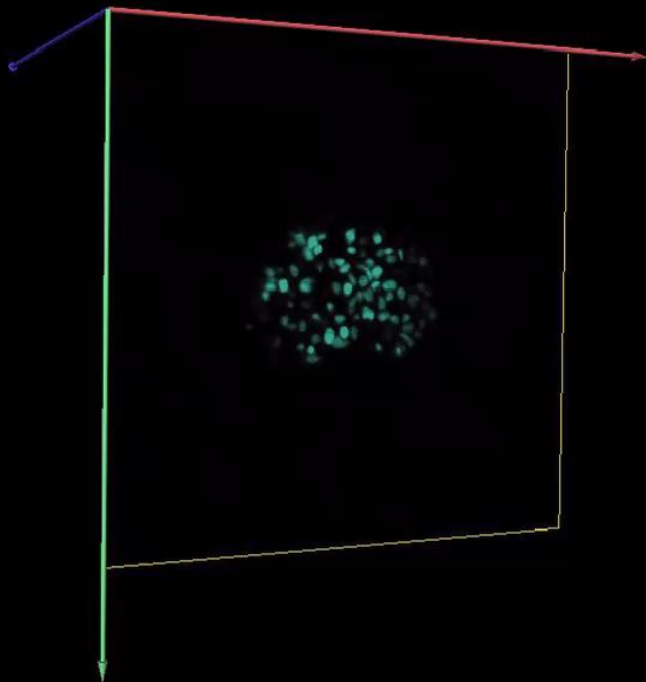


IMACTIV-3D

Microscope à feuille de lumière

Microscope à feuille de lumière

Échantillon : Sphéroïde



Partenariat avec ZEISS



- Microscope Z7
- Double illumination
- Double acquisition
- Fluorescence multi-canaux
- Rotation
- 1X à 40X

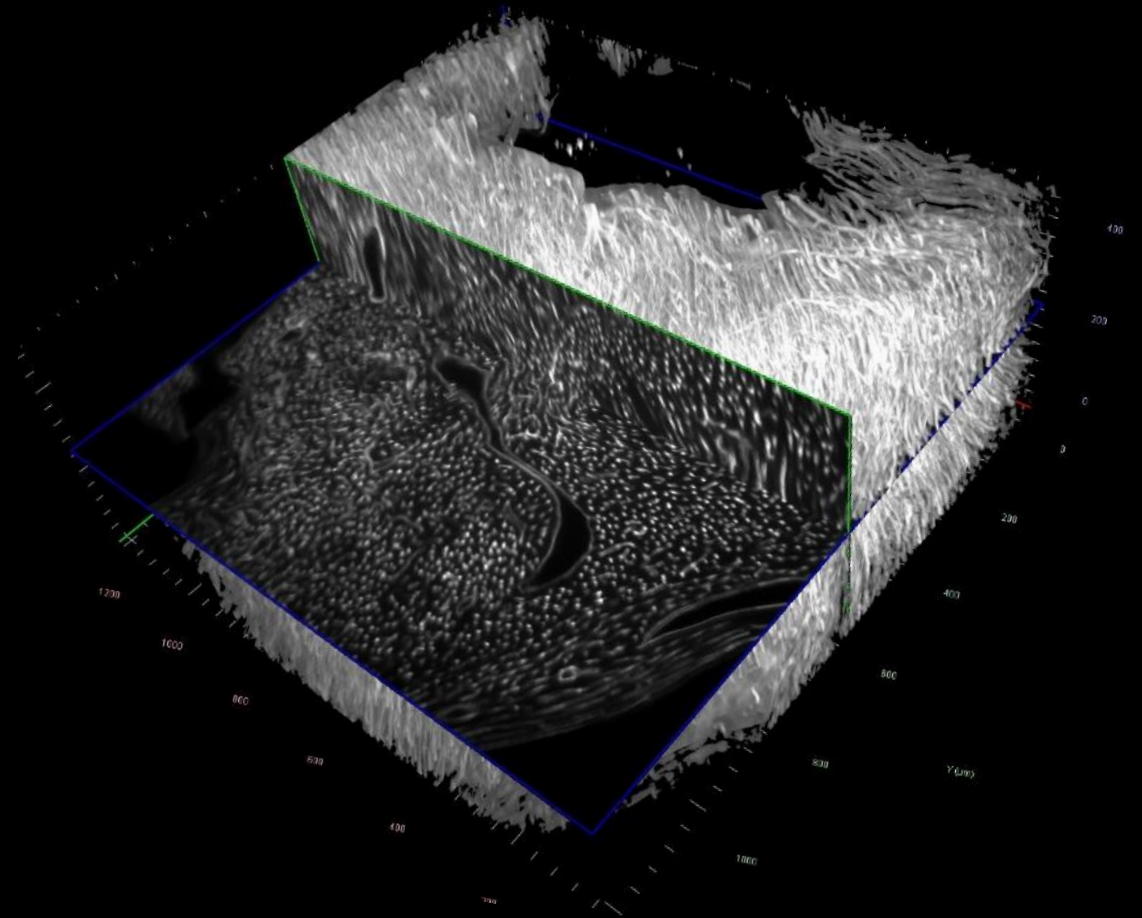
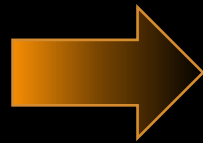
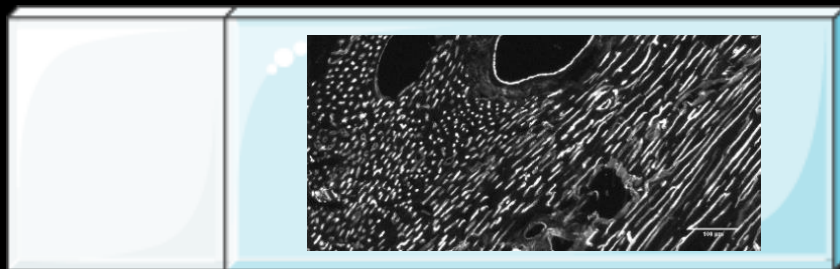




IMACTIV-3D

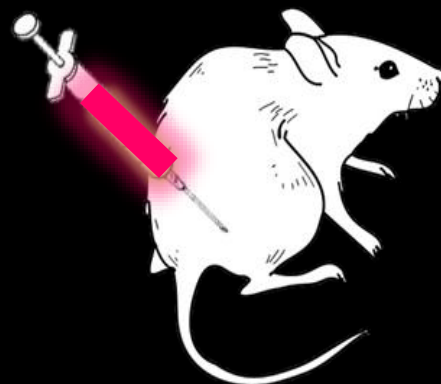
Vascularisation

Vascularisation



Stratégie de marquage

- Marquage avec une lectine fluorescente (lycopersicon couplée avec Dylight-649)
- Injectée *in vivo* par perfusion avant euthanasie



- Fixation de la lectine sur les glycoprotéines des cellules endothéliales
- Marquage des vaisseaux sanguins

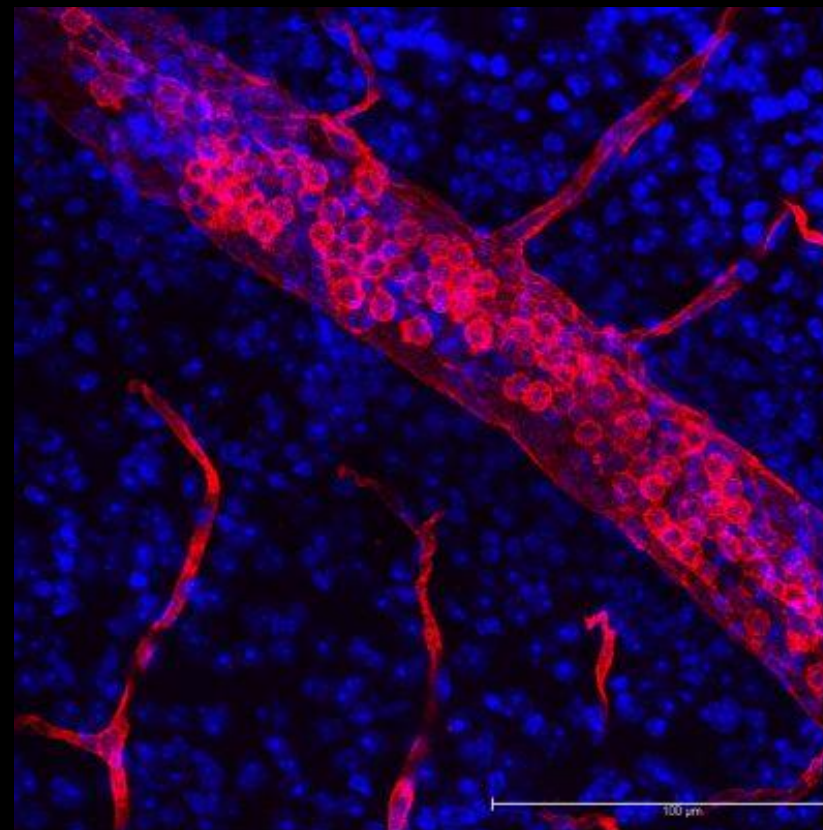


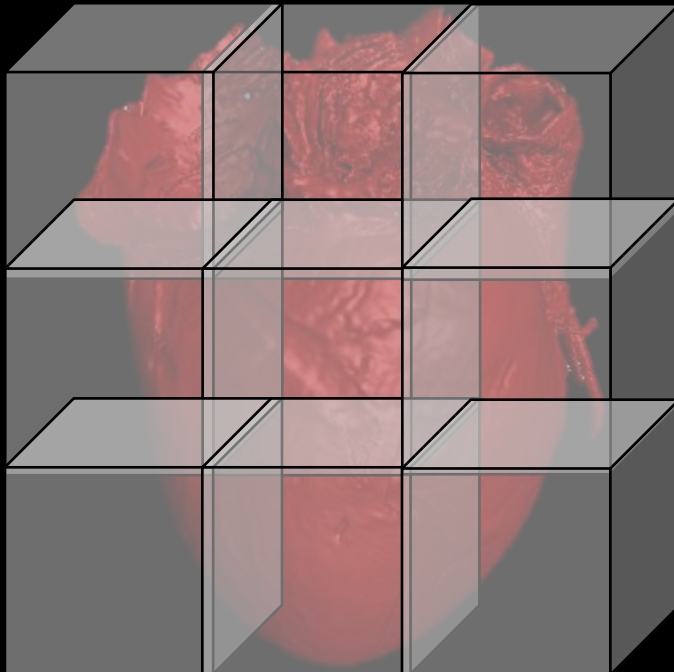
Image d'une coupe disponible sur le site du fournisseur Vectorlabs



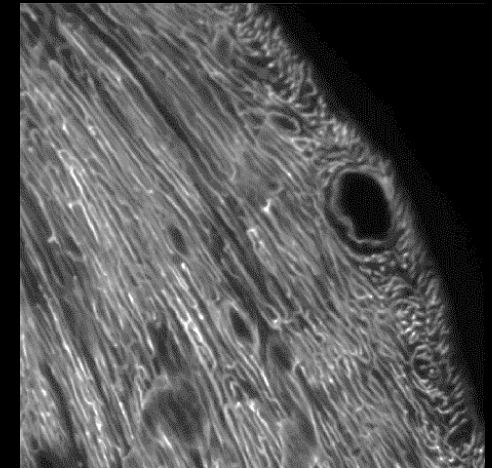
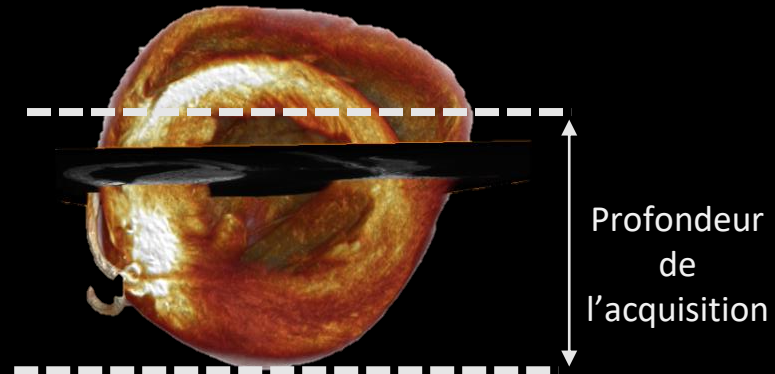
IMACTIV-3D

Vascularisation cardiaque

Stratégie d'imagerie



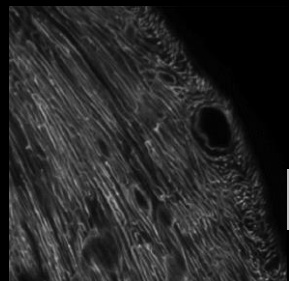
Mosaïque des acquisitions



- Acquisitions à 2X de grossissement
- Acquisition du ventricule gauche entier : $3X \times 3Y = 9$ stacks (en moyenne)
- Mosaïque de champs de vue adjacents
- Chevauchement de 5% entre les champs
- Acquisition jusqu'à la cloison interventriculaire (environ 8mm)

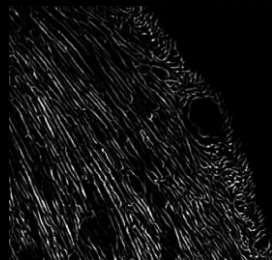
Traitement d'image - processus

Objectif : Quantifier la densité des capillaires dans le ventricule gauche

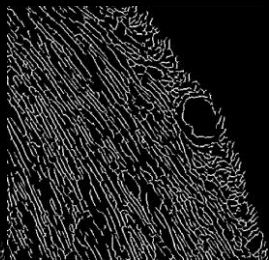


Données brutes

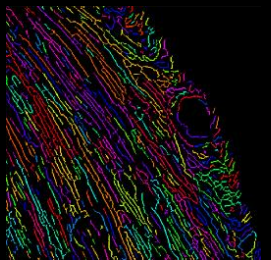
Processus de traitement d'image



Pré-processing



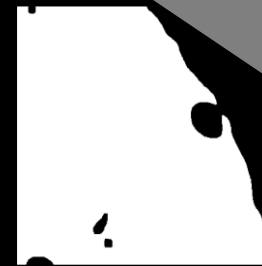
Segmentation



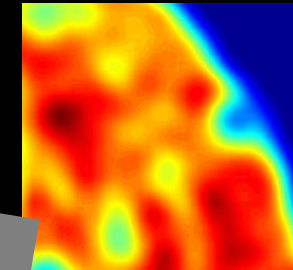
Labellisation



Squelettisation



Estimation du volume utile



Caractérisation qualitative



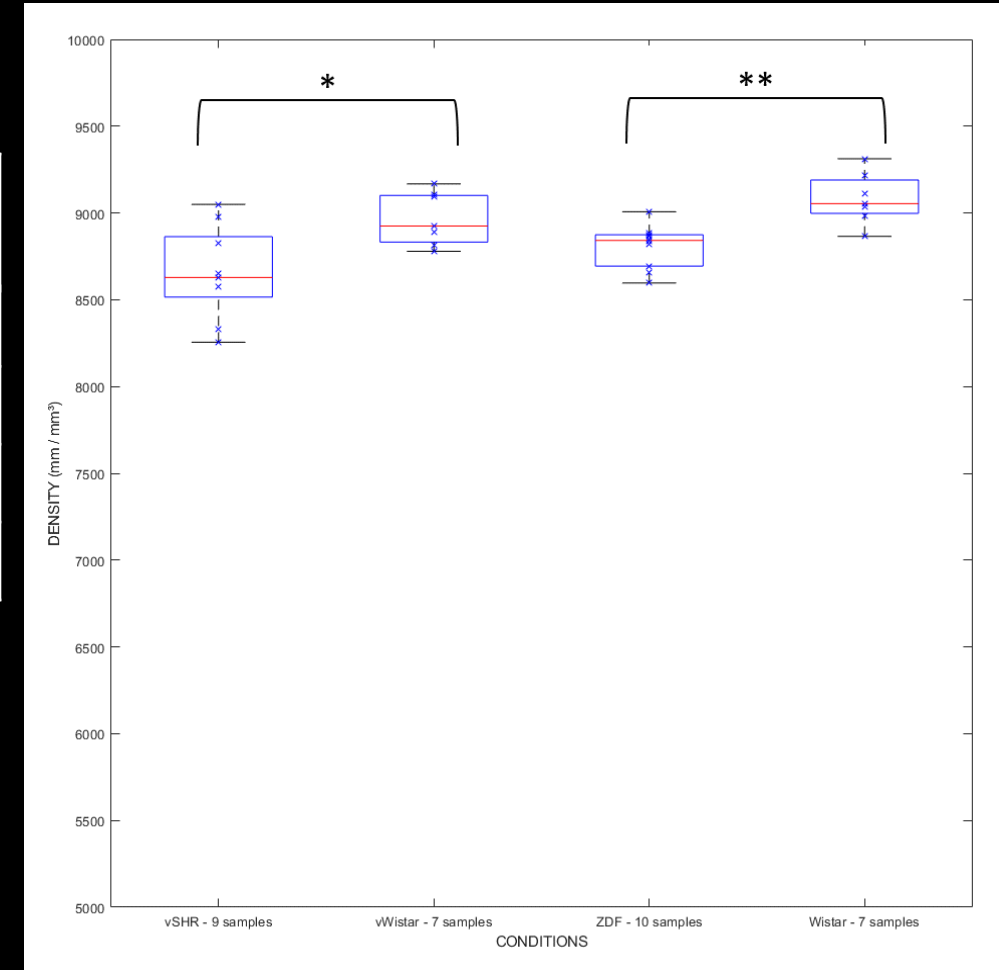
Caractérisation quantitative

Traitement d'image - Résultats



	Nb de cœurs	Densité moyenne	σ
ZDF	10	8809	123
Wistar	7	9083	148
old SHR	7	8658	265
old Wistar	9	8967	154
TOTAL	33		

- Différence entre moyenne Wistar et ZDF: **3,1 %**
- Différence entre moyenne old Wistar et old SHR: **3,5%**
- **σ très faible**





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Autres exemples

Xenogrefe



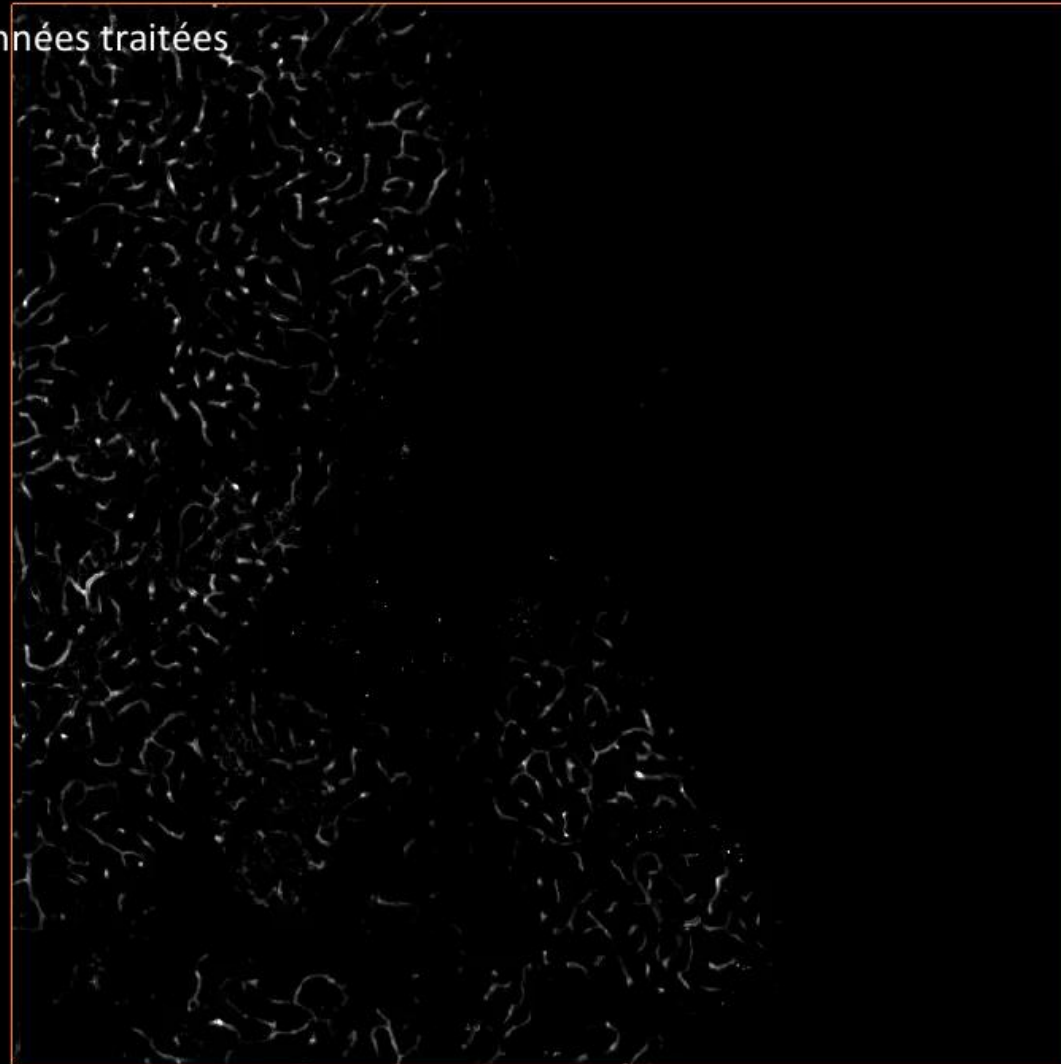
PDAC Δ3-lectin 1X



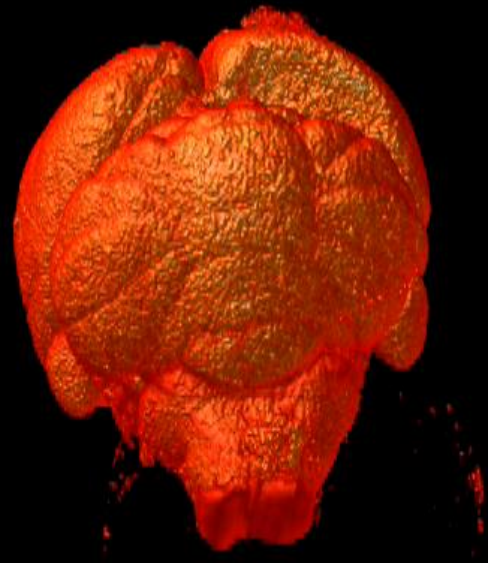
Xenogreffe



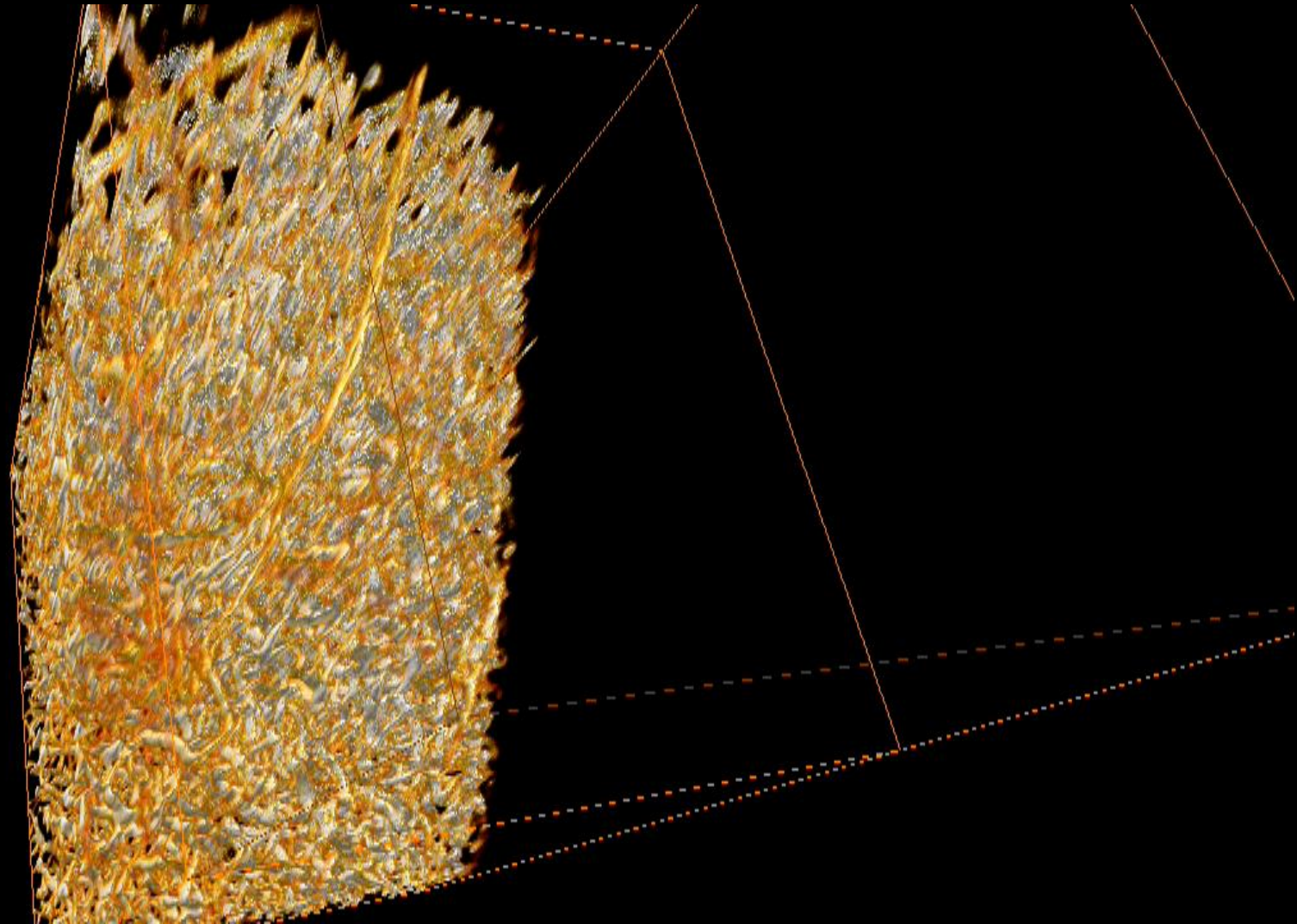
10X - zone "saine" - données traitées



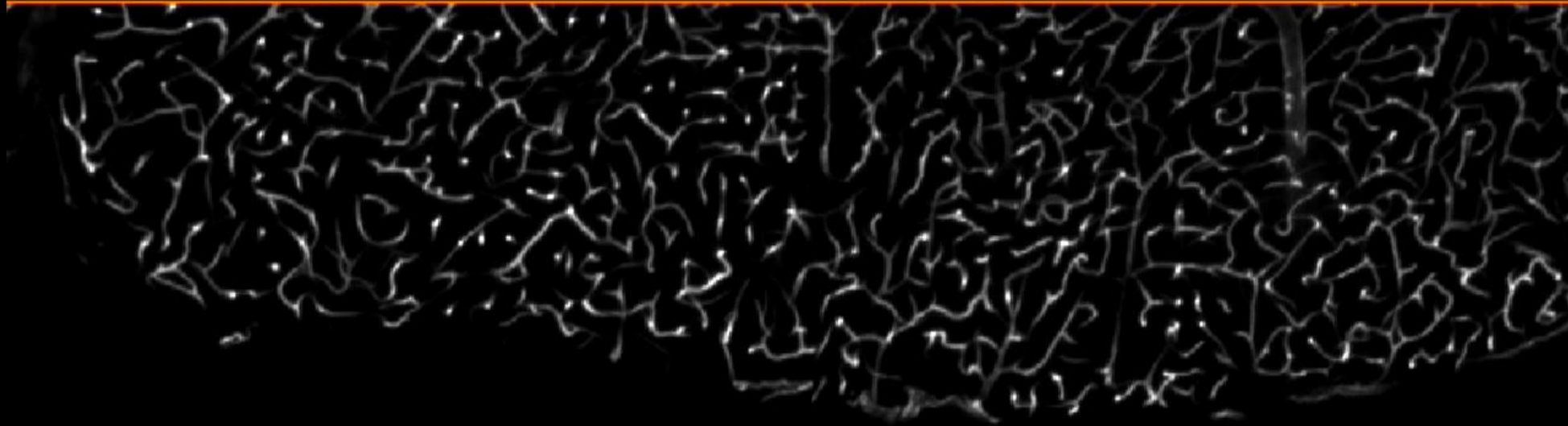
Cerveau



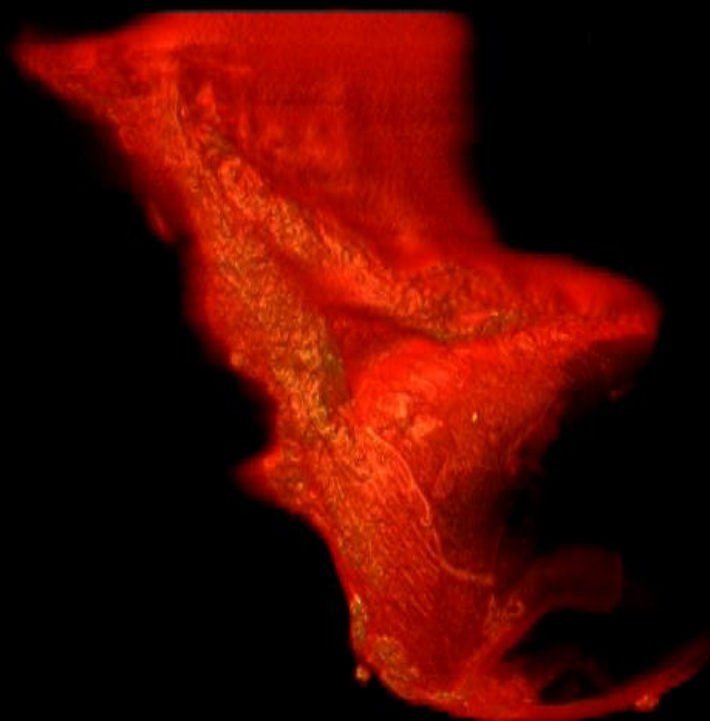
Cerveau



Cerveau



Oeil



Oeil





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Conclusion

Étude de la vascularisation



- Expertises
 - Marquage du réseau vasculaire
 - Méthode de transparence
 - Méthode d'imagerie 3D
 - Méthode de quantification
- Études
 - Vieillesse
 - Maladies vasculaires
 - Maladies neurodégénératives
 - Oncologie

Bureaux et contact



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