



CENTRE D'IMAGERIE BIOMÉDICALE

Techniques of small animal imaging

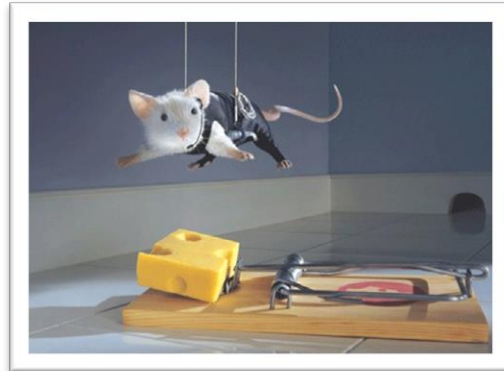


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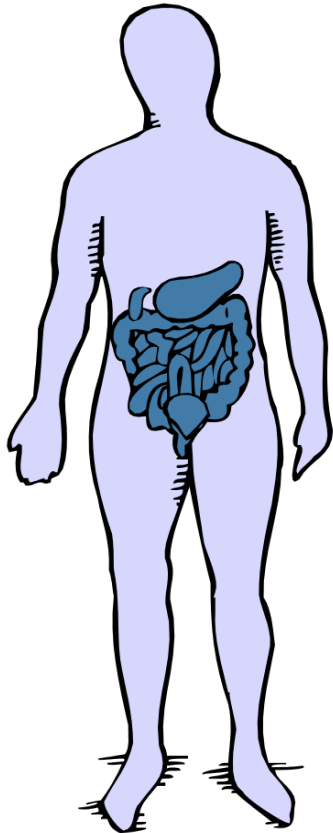
HUG  
Hôpitaux Universitaires de Genève



Agenda

- The Bio-imaging and the 3Rs (Refine, Reduce, Replaces),
- The Micro-PET/SPECT/CT : Generalities,
 - The radio-isotopes,
 - The radio-tracers.
- Progress of an experience: from the protocol to the image !
 - Description of the material used on the platform Micro-Pet,
 - The animals preparation :
 - The anesthesia,
 - The injections and catheterisations.
 - The images analysis.
- Example of Micro-PET/SPECT/CT applications.

From human to animal Translational Imaging !

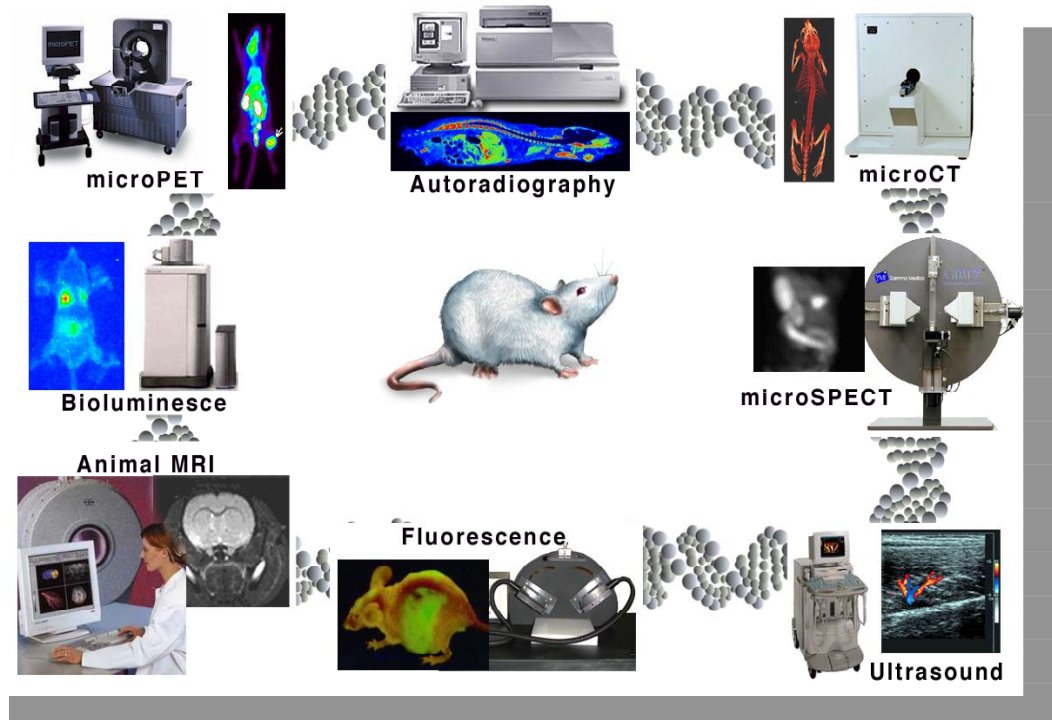


75kg



20g

3750:1



- Resolution : **Our MicroPet approx. 1.2 mm / CT approx. 80 μ m,**
- **Very good Sensitivity.**

The MicroPet or NMRI.....the powerful techniques for the 3Rs !!!



Save number of animals !



And it's not painful !! Not invasive !!



Example in oncology



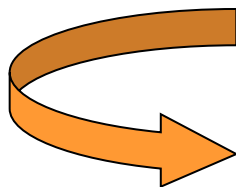
Nod Scid + S.C. Tumor



Swiss Nude + Ascite

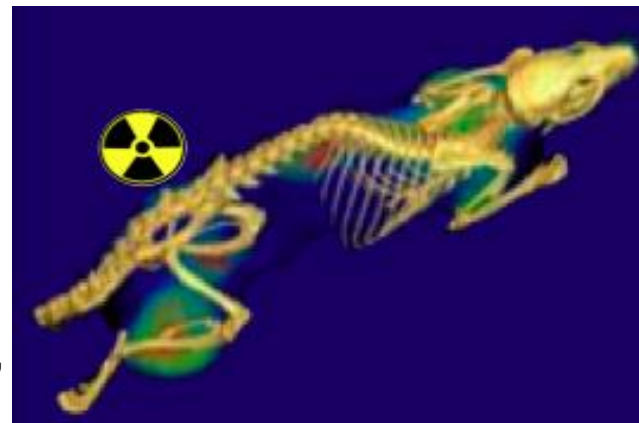
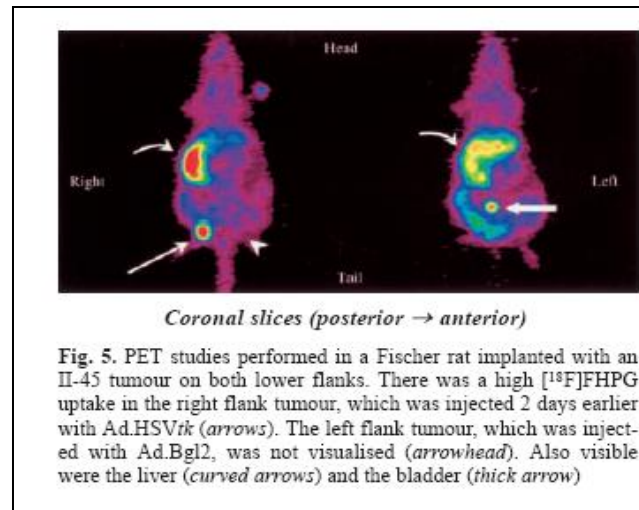
Without Imaging

Models of tumors limited (SC, not-orthotopic model), **obligation to sacrifice animals** to follow the growth of tumors and the efficiency of the product, the significant number of animals.....

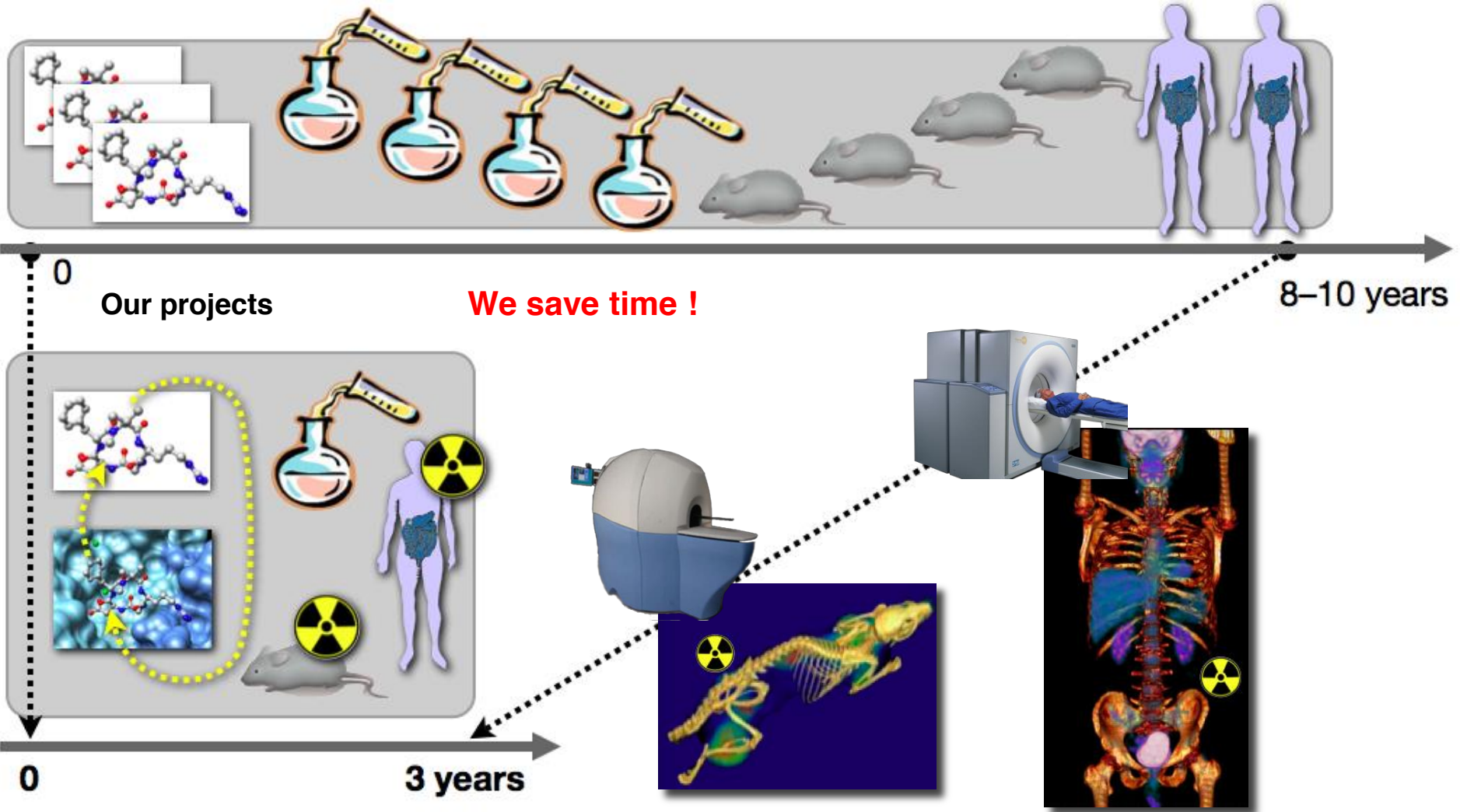


With Imaging

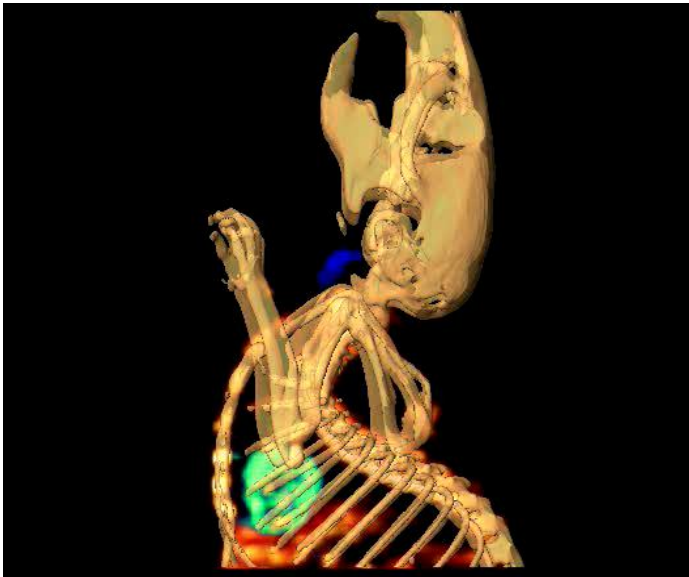
Use orthotopic models, (tumors of prostate, lung, pancreas, etc.) **with not invasive method, quantitative**, reduces the number of animals....



Standard Développement



The PET/SPECT/CT Imaging : Generalities.



PET: Positron Emission Tomography

SPECT: Single Photon Emission
Computed Tomography

CT: Computed Tomography

Micro-PET/SPECT/CT



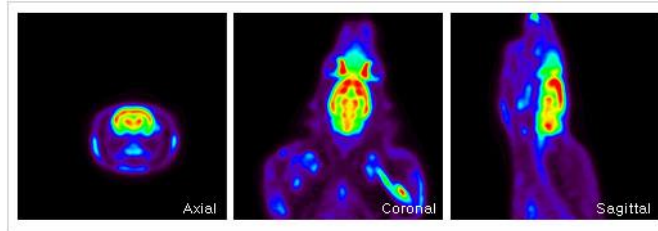
+



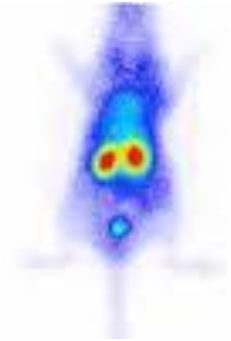
What's my name?
Tiger or rabbit ???



Positron emission tomography (PET) **uses positron emitters (F-18, C-11,...)**, this positron annihilates with an electron in the body of a patient and thereby **creates two gamma-rays** under an **angle of 180°**. The beautiful thing of PET are these two gamma-rays. When two detectors (in the ring) detect a ray at the same line, you can draw a virtual line between them (180°C!!). On the intersection of all the different "lines" lies your source of radiation. This gives PET a **good sensitivity and resolution** and **quantification possibilities**.



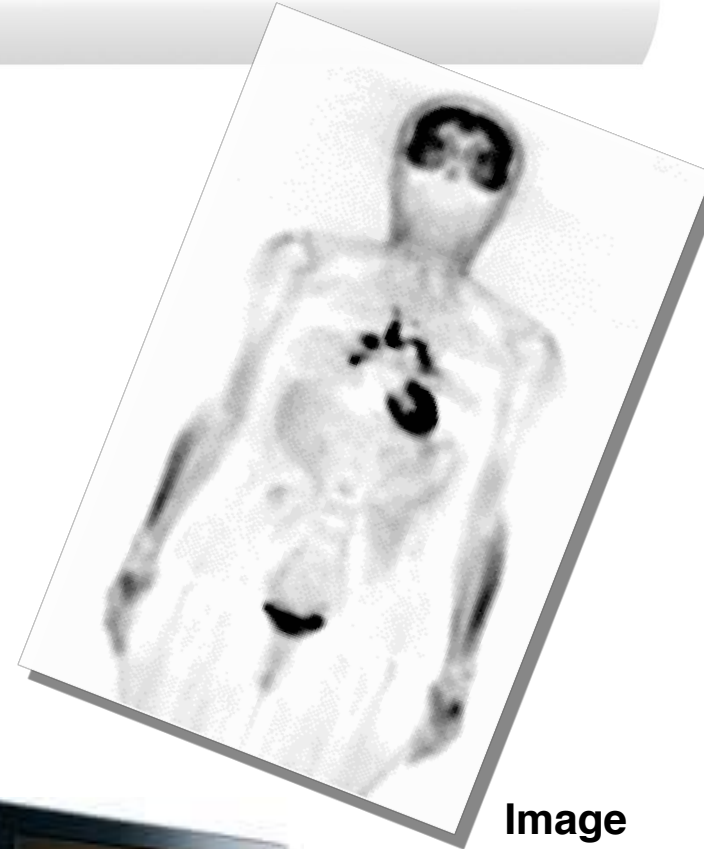
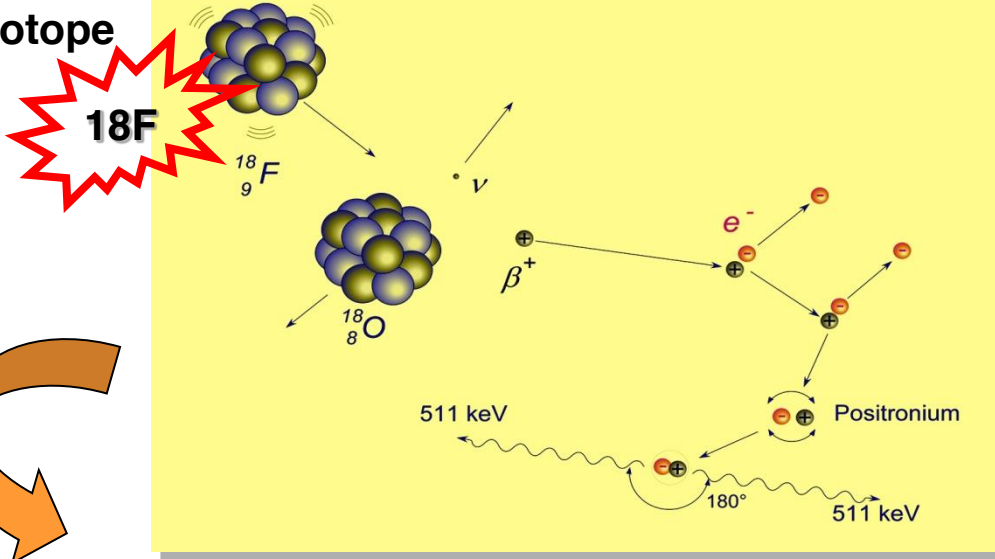
Single photon emission computed tomography (SPECT) **uses also isotopes emitters (99mTc, I123)** but **creates just one gamma ray**, so reconstruction is more difficult and **the image less beautiful**. Only rays that fall straight on the detector are detected (otherwise you won't know from which direction it came), so the camera has to rotate to make a full image.



Computed tomography (CT) is used to generate a **3D images** of the inside of an object from a large series of two-dimensional **X-rays** images taken around a single axis of rotation. You **can't see the soft tissues very well**.



The Isotope



Image

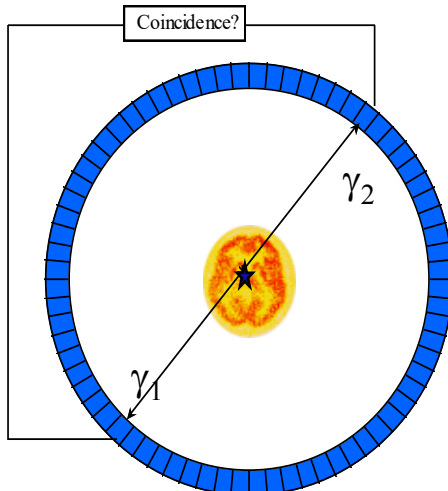
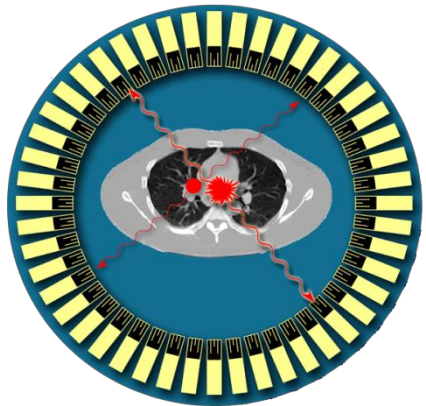
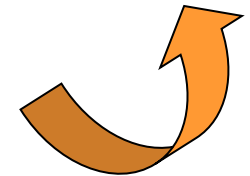


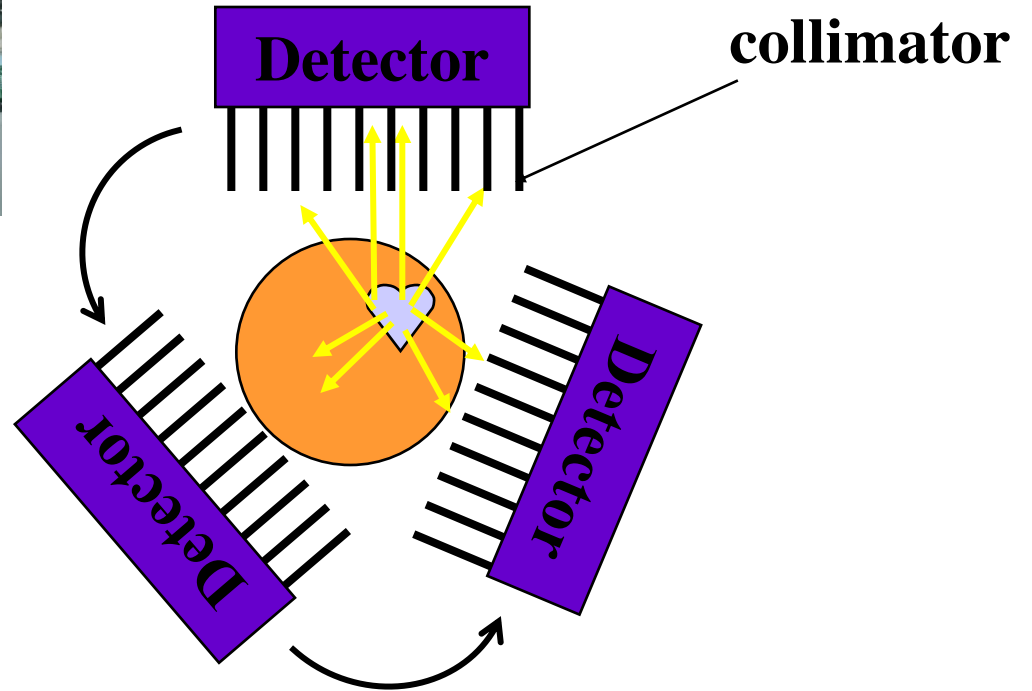
Image reconstruction



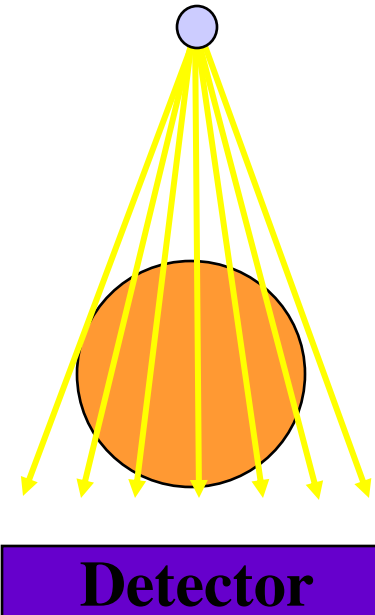
Coincidence measure in PET Scan



Head of the SPECT



- The radiations are transmitted since an external source (x-ray tube) through the animal.
- Do not to use too dense materials (metals, hard plastics etc.) because they will create artefacts on the image.



SPECT :

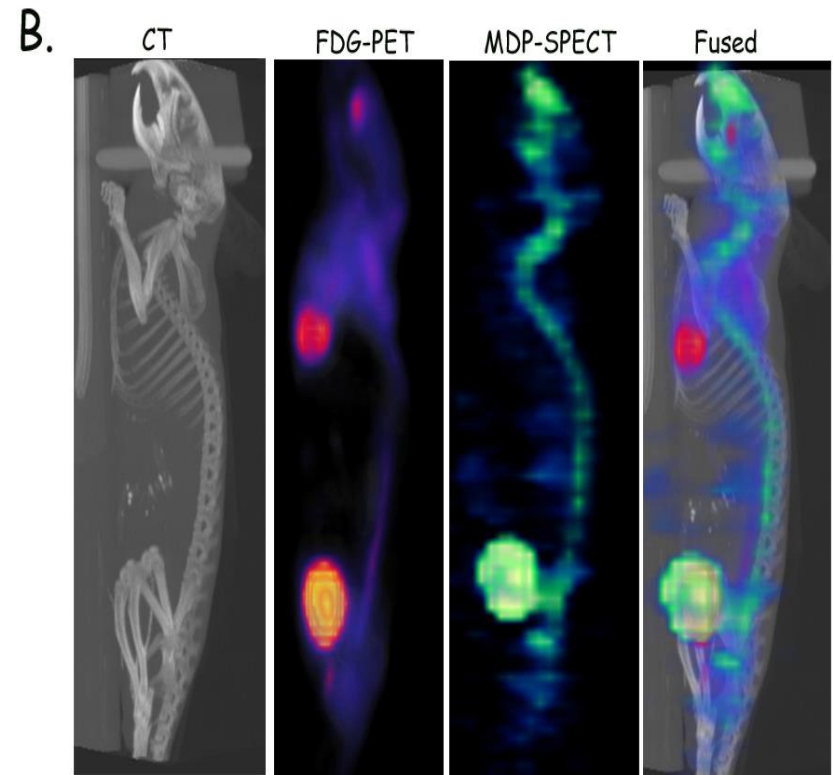
- +** : No need of Cyclotron (Commercials radioactives sources: Iode123, Thallium, Tc- 99m ...), simpler chemistry, **SPECT is less expensive** than PET, for less equipment is involved and fewer staff is required to perform the test.
- : More time for the SPECT analysis, poorer resolution and sensibility (poorer image), molecules **are not "natural"**.

PET :

- +** : Good sensitivity and resolution and quantification possibilities. Molecules used as tracers **are more "Natural"** (In the body Carbon, Oxygen, Nitrogen, Fluorine....)
- : Need of Cyclotron

The +++ ...the mix of
PET/SPECT/CT !!!

Tri-modalities



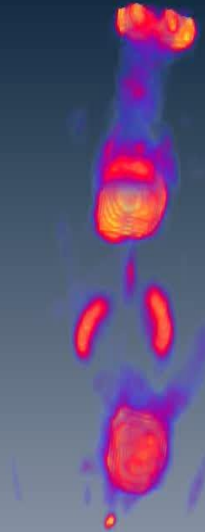
CT

Useful to localize the target organ !

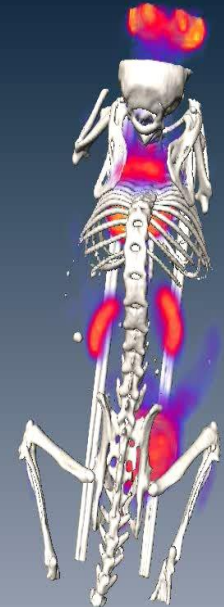


PET

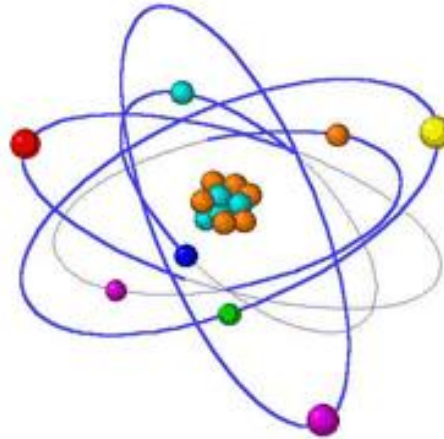
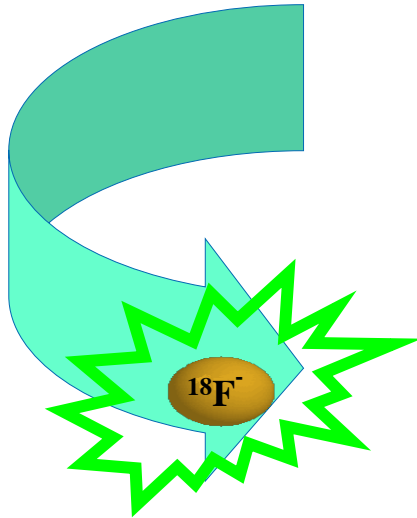
Useful to record the metabolism !



Fusion PET/CT



The radio-isotopes



Isotope	Half-life	Nuclear reaction	Emission
^{18}F	109.8 min	$^{18}\text{O}(p,n)^{18}\text{F}$	97% β^+ ; 3% EC
^{11}C	20.39 min	$^{14}\text{N}(p,\alpha)^{11}\text{C}$	100% β^+
^{13}N	9.965 min	$^{16}\text{O}(p,\alpha)^{13}\text{N}$	100% β^+
^{15}O	122.2 s	$^{14}\text{N}(d,n)^{15}\text{O}$	100% β^+
^{64}Cu	12.70 h	$^{64}\text{Ni}(p,n)^{64}\text{Cu}$	43% EC; 18% β^+ ; 39% β^-
^{68}Ga	67.63 min	$^{68}\text{Ge}/^{68}\text{Ga}$ generator	89% β^+ , 11% EC
^{82}Rb	1.273 min	$^{82}\text{Sr}/^{82}\text{Rb}$ generator	95% β^+ ; 5% EC
^{124}I	4.18 d	$^{124}\text{Te}(p,n)^{124}\text{I}$	77% EC; 23% β^+
^{76}Br	16.2 h	$^{76}\text{Se}(p,n)^{76}\text{Br}$	55% β^+ ; 45% EC
^{86}Y	14.74 h	$^{86}\text{Sr}(p,n)^{86}\text{Y}$	68% EC; 32% β^+
^{45}Ti	3.08 h	$\text{Sc}(p,n)^{45}\text{Ti}$	85% β^+ ; 15% EC
^{55}Co	17.53 h	$^{54}\text{Fe}(d,n)^{55}\text{Co}$	76% β^+ ; 24% EC

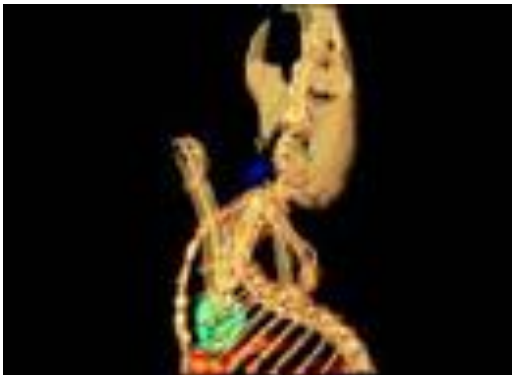
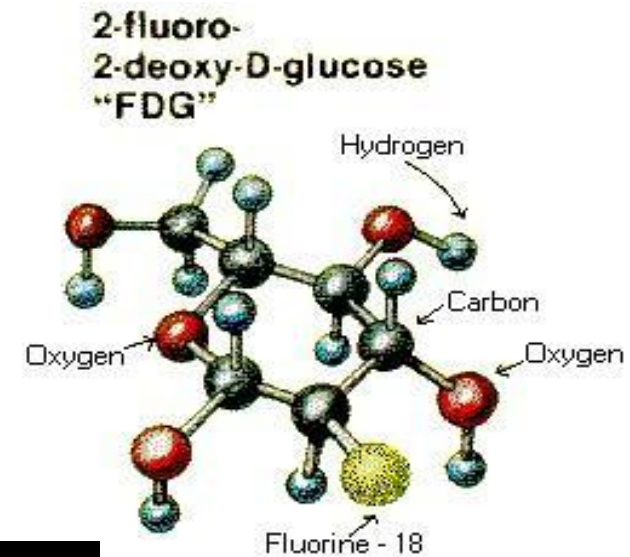


SPECT Radioisotopes

Isotopes	Half Life	Energy	Significance
Tc^{99m}	6hr	140KeV	Mostly commonly used Isotope, optimal energy and $T_{1/2}$, mature labeling techn.
I^{123}	13hr.	159Kev	Same as above
I^{125}	60d	25KeV	Longitudinal studies, Ab tracking;
In^{111}	2.8d	171Kev 245KeV	Cell tracking, RBC labeling;
Tl^{201}	3.04d	166KeV	Blood flow, oncology,

The radio-Tracers

[¹⁸F]FDG





Synthesis of chemistry



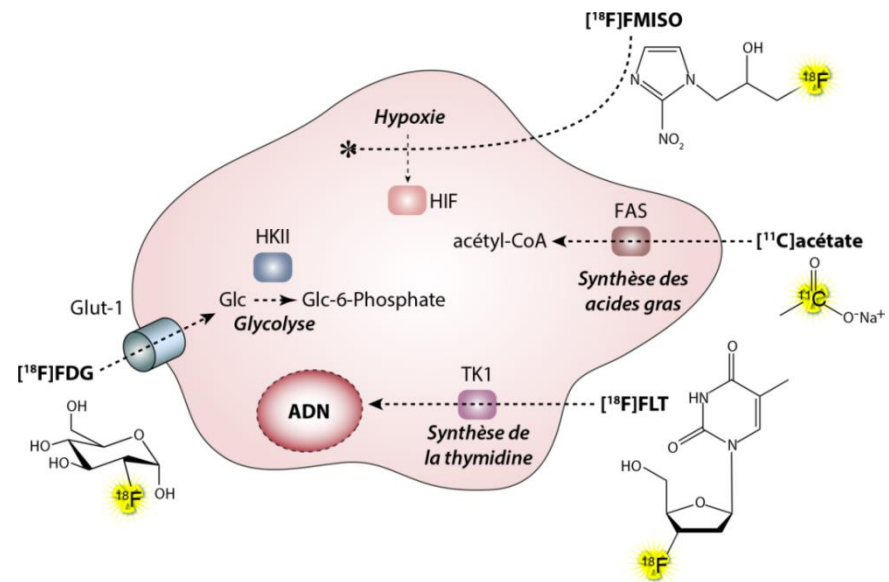
The synthesis is made by modules, completely automated, placed in shielded units.

Examples of PET radio-tracers

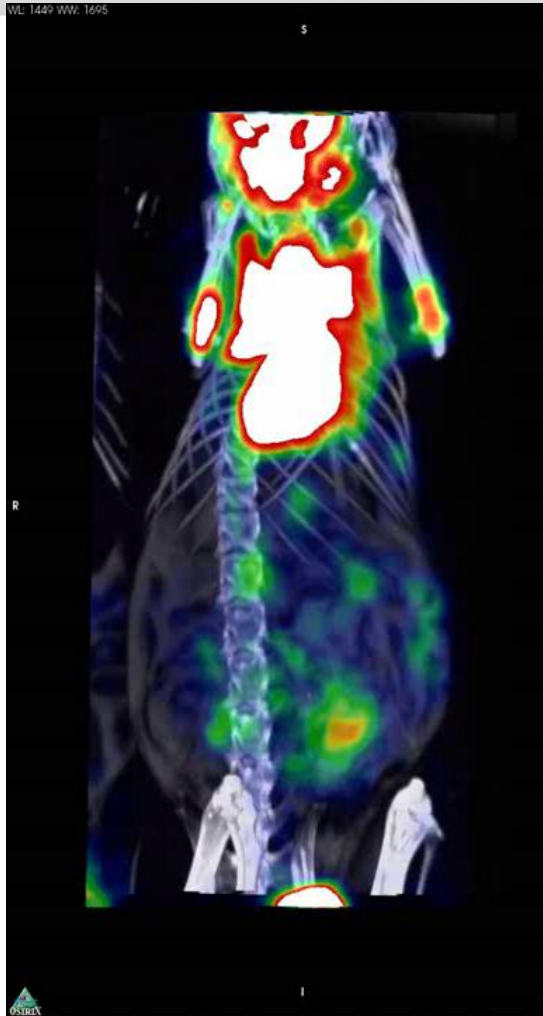
ISOTOPE	T _{1/2} min	Reaction	Target	Tracer	Applications
¹⁸F	110 min	¹⁸ O (p,n) ¹⁸ F	[¹⁸ O]H ₂ O	[¹⁸F]-FDG	Tumors Metabolism
¹⁸F	110 min	¹⁸ O (p,n) ¹⁸ F	[¹⁸ O]H ₂ O	[¹⁸F]-FDopa	Parkinson, Neuro endocrin tumors
¹⁸F	110 min	¹⁸ O (p,n) ¹⁸ F	[¹⁸ O]H ₂ O	[¹⁸F]-Fluoride	Bone Metastasis
¹¹C	20 min	¹⁴ N (p,α) ¹¹ C	N ₂	[¹¹C]-Acetate	Oxydative heart metabolism, prostate cancer
¹¹C	20 min	¹⁴ N (p,α) ¹¹ C	N ₂	[¹¹C]-Choline	prostate cancer
¹³N	10 min	¹⁶ O (p,α) ¹³ N	H ₂ O	[¹³N]-NH₃	Heart perfusion
¹⁵O	2 min	¹⁴ N (d,n) ¹⁵ O	N ₂	[¹⁵O]-H₂O	Brain perfusion

Examples of PET radio-tracers for tumors applications

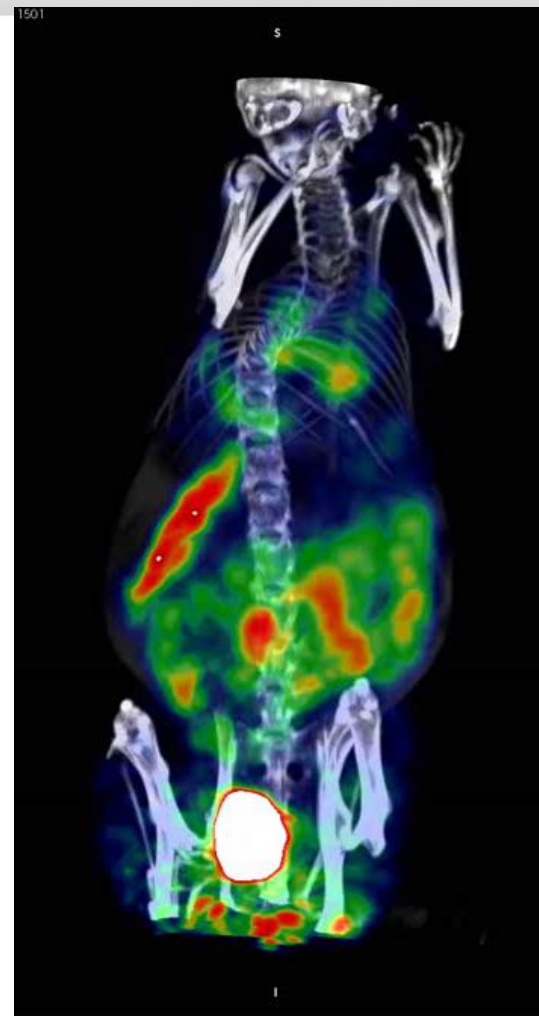
- **(¹⁸F)FDG (Glucose metabolism),**
- **(¹⁸F)FLT (Proliferation),**
- **(¹⁸F)FMISO (Hypoxic status),**
- **(⁶⁸Ga) PSMA (PSMA receptors),**
- **(⁶⁸Ga) DOTATATE (Somatostatin receptors),**
-



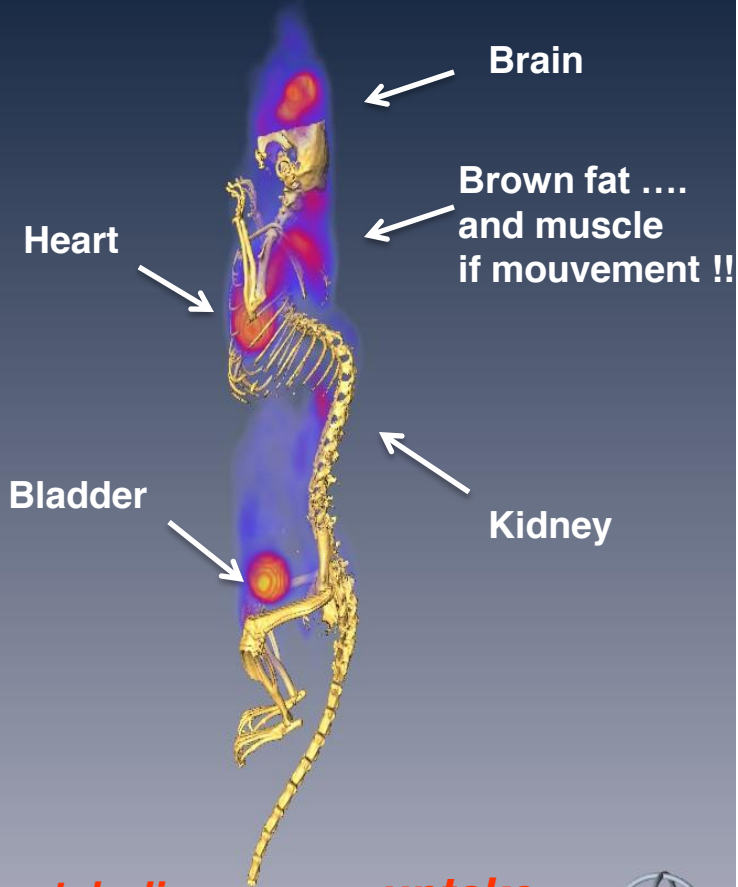
The F18-FDG image compare to F18-FLT image



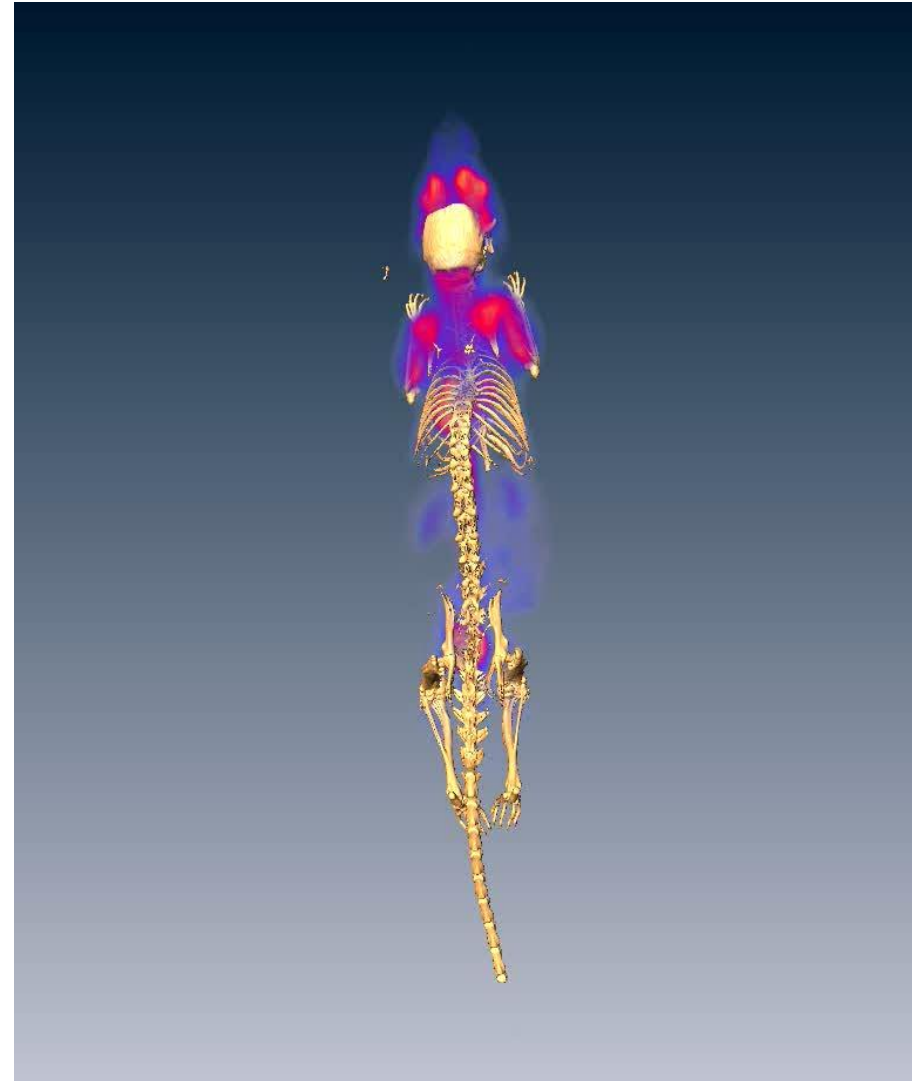
F18FDG



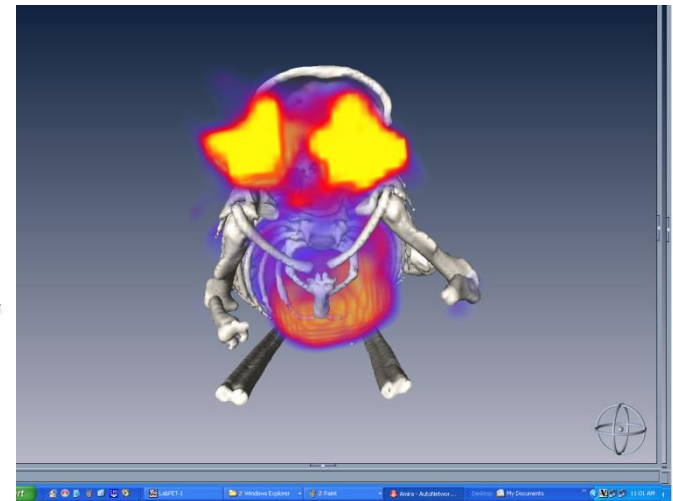
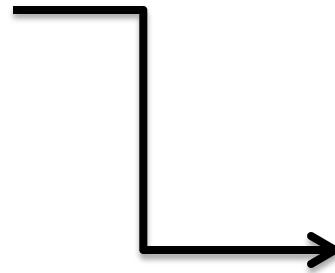
F18FLT



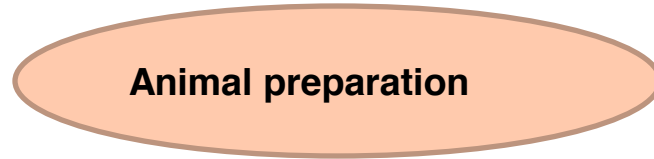
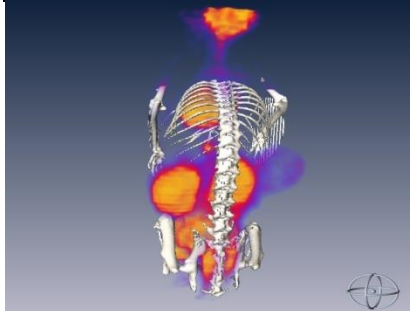
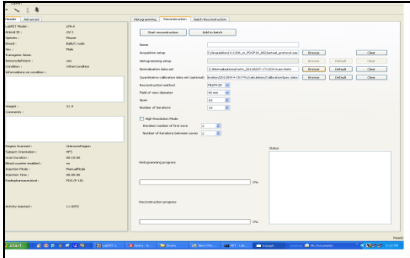
**All metabolics organs uptake
FDG !!**



Progress of an experiment: from the protocol to the image!



Progress of an experiment : The protocol



Approx.30min



5 min to 6h
(moy. 1h)



1 h to 6h
(moy. 2h)



5min to 1h
(moy. 30min)



Total
de 2h à 24h
(moy. 3h)



Important parameters to determine before the experiment

- Ways of injections (IP, IV, SC, Oral ..),
- Dynamic injections = without waiting time (Brain, heart) or waiting time to determine (tumors, inflammation),
- Type of tracer the best adapted to the model,
- Dose to inject (MBq),
- PET / SPECT / CT or simply PET, or simply CT, or simply SPECT.



Progress of an experiment : The protocol

Important: sheet for recording datas

Date: 28/09/17 Project: Arthrite (Guillaume) CT parameters: x: 174 y: 274 z: 286
 Operator: Radiotracer: F18 FDG Magnification / Frames: 1,3/1024 Voxel size (µm):

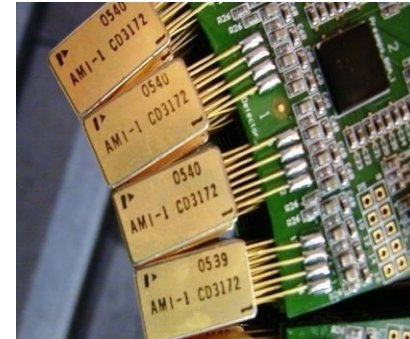
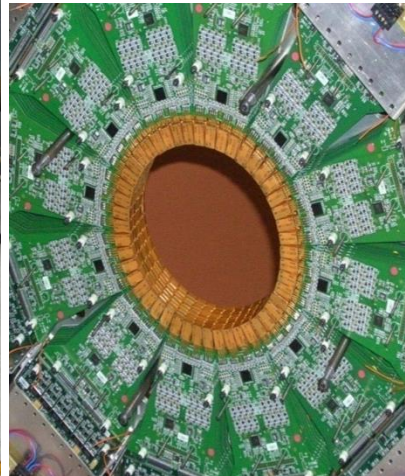
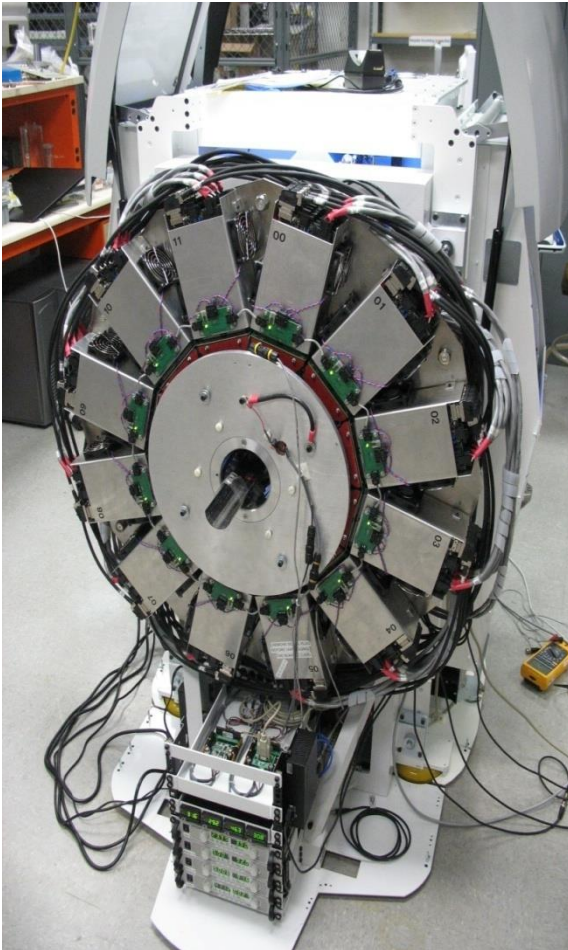
Souris à femur 1
 chauffage IFR + lit
 lit 3 souris sur dos

Cage	N° Animal / Identification	Weight (g)	Inj. Route / Success	Activity in syringe before inj. (MBq) / Time	Activity left in syringe (MBq) / Time	Tracer injection time	Time PET Start	Uptake Duration (min)	Acquisition Duration (min)	Name of PET File (Suffix-x.00x)	Name of CT File	Remarks	Position of animals
High Score cage 1	W01R Rouge	26,2	IR RO OK	8,67 10H43	1,16 11H32	11H30	12H32 12H42	1H02 1H12	2x10 min	001 002	CT1	Ces 4 pattes dans FOV 5 pattes arrières pattes Avants (62,62)	2 3R 1R
"	W02R	26,3	"	8,87	1,13	"	"	"	4	"	"	"	"
"	W03R	29,8	"	8,83	1,02	"	"	"	4	"	"	"	"
Cage 2 Int. Sco	1V	28,2	"	8,71 11H34	1,11 12H11	12H06	13H08 13H19	1H02 1H13	"	003 004	CT2	" (2 ?) " (2 62,98)	3B 1V 2V
Cage 1 Int. Sco	2V	26,2	"	8,20	1,08	"	"	"	"	"	"	"	"
Cage 1 Low Sco	3B	25,6	"	8,76	1,34	"	"	"	"	"	"	"	"
Cage 2 Int.	3V	28,2	"	8,59 12H42	1,25 12H48	12H38	13H42 13H55	1H04 1H17	"	005 006	CT3	" 2(56,36) " 2(62,62)	2B 3V 1B
" Low	1B	26,3	"	8,40	0,85	"	"	"	"	"	"	"	"
" Low	2B	26,8	perdu 1 goutte	7,91	1,11	"	"	"	"	"	"	"	"
Cage 3 Int.	1W	31,5	OK	8,84 12H51	1,22 13H16	13H12	14H13 14H31	1H02 1H14	"	007 008	CT4	" 2(55,54) " 2(63,17)	3W 1W 2W
"	2W	30	"	9,19	1,25	"	"	"	"	"	"	"	"
"	3W	29,2	"	9,10	1,42	"	"	"	"	"	"	"	"

Description of the material used on the platform Micro-PET /SPECT/ CT



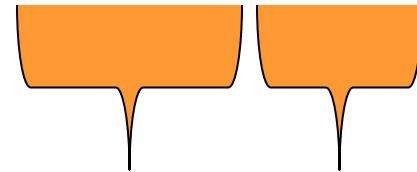
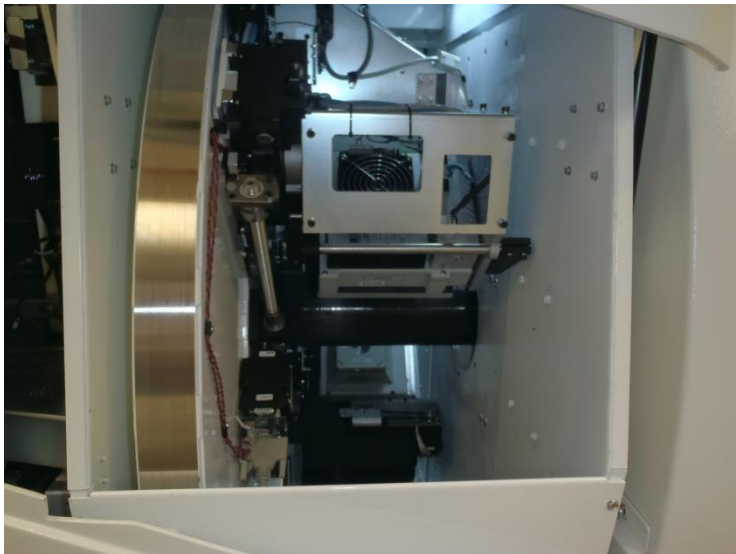
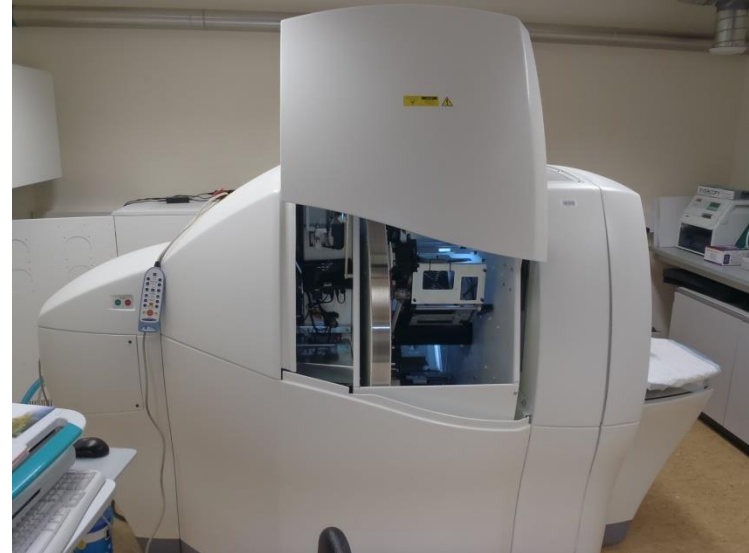




Avalanche

Photo-

Diode

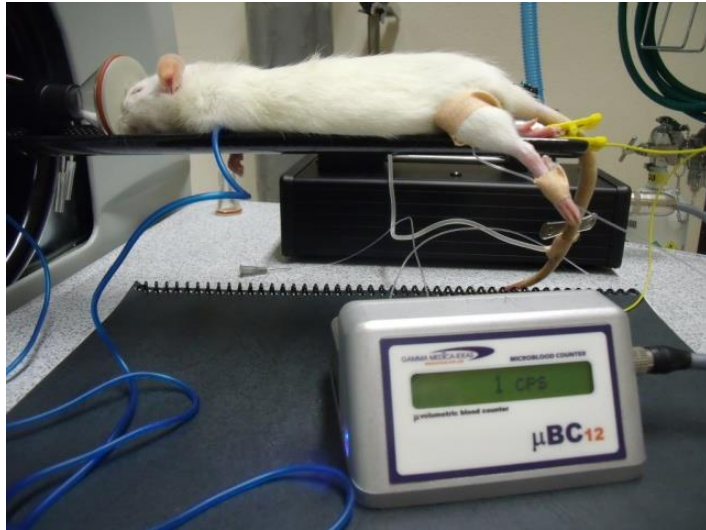


**SPECT
et
CT**

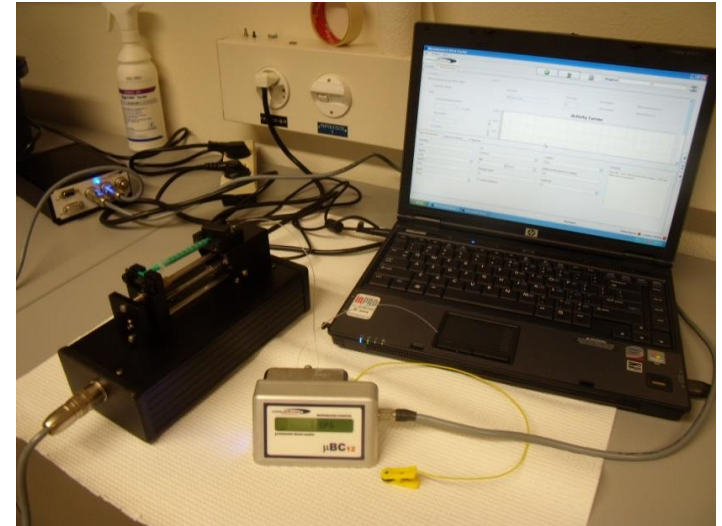
PET





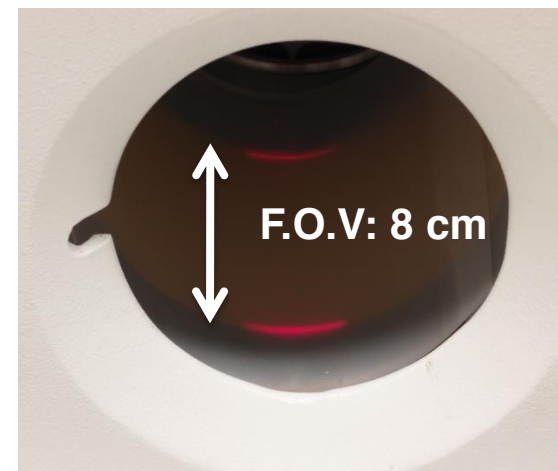
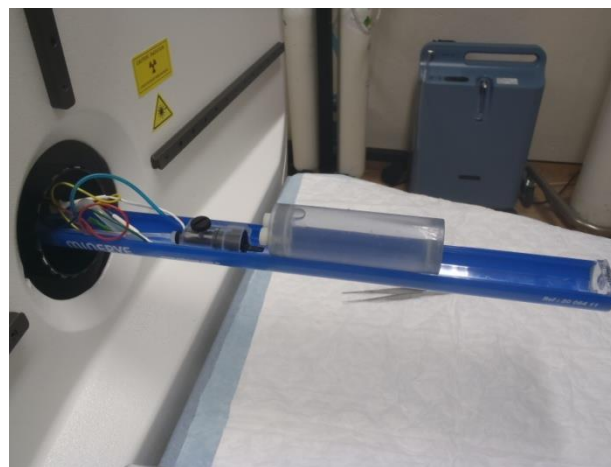
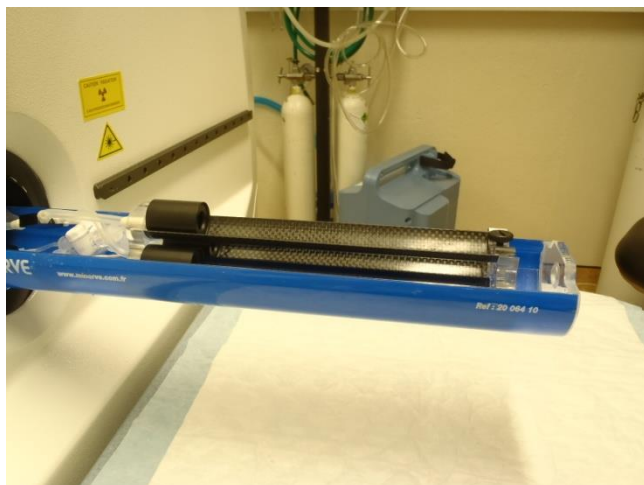
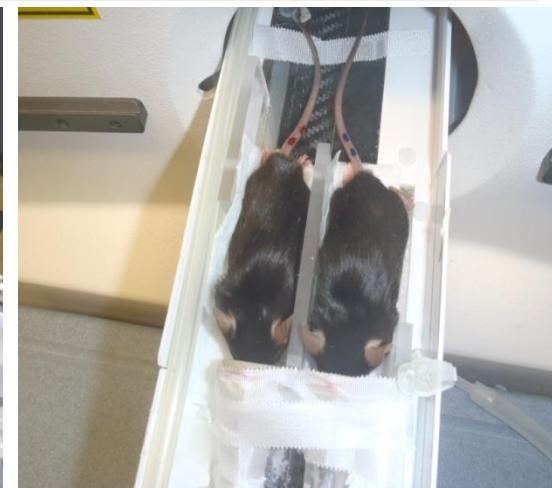
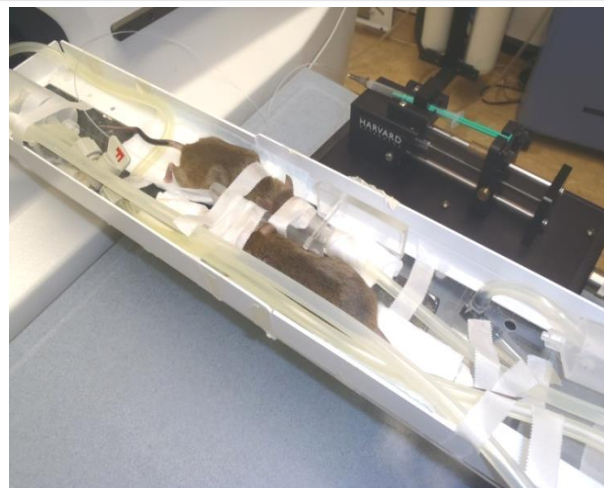


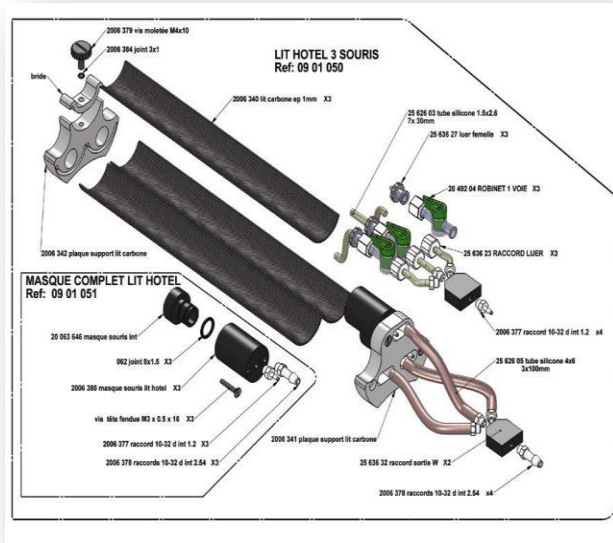
The Gamma Micro-blood counter:
Measure the rate of radioactivity in the blood in time.



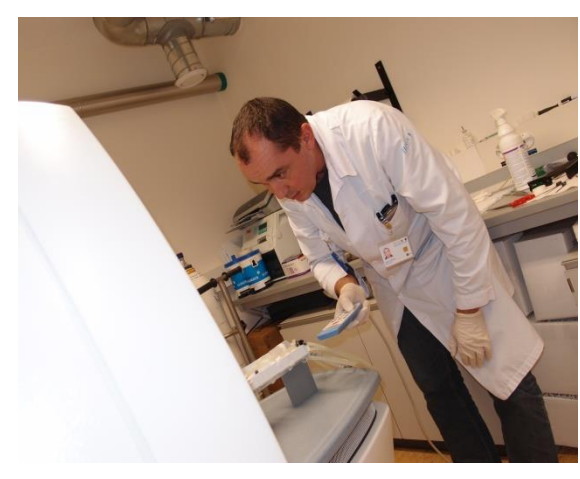
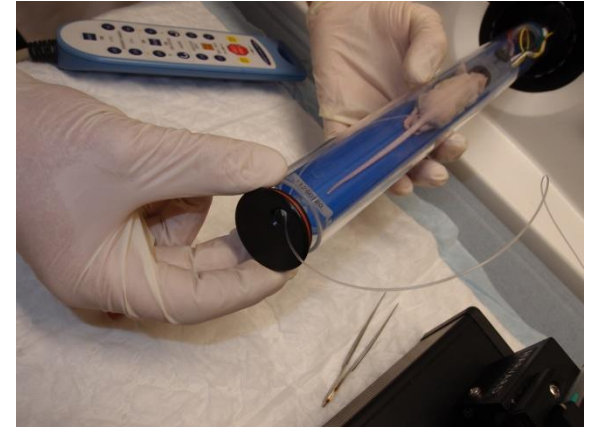
Perfusion Pump:
Remote injection to avoid the radiation.

The various beds and the field of view (F.O.V.)

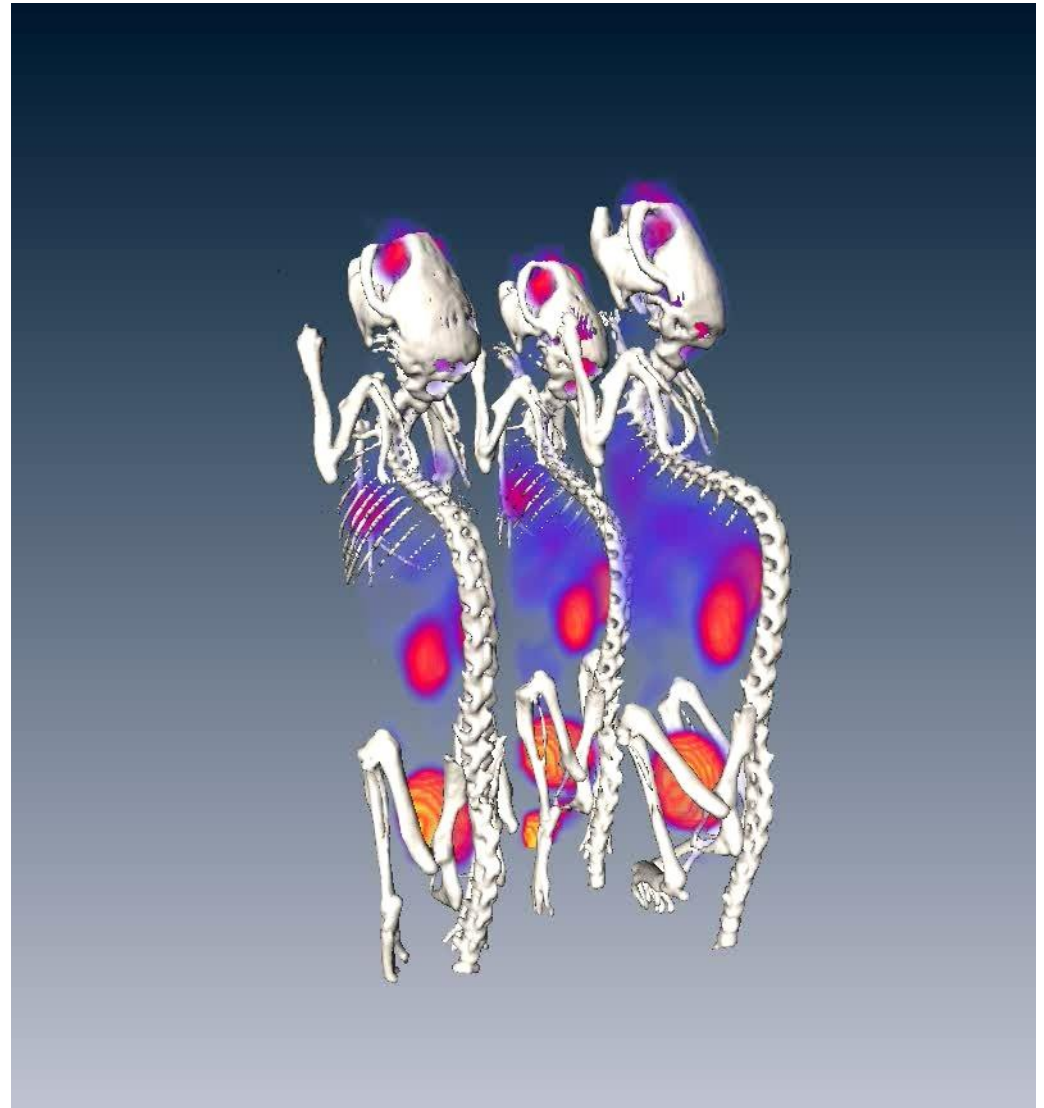


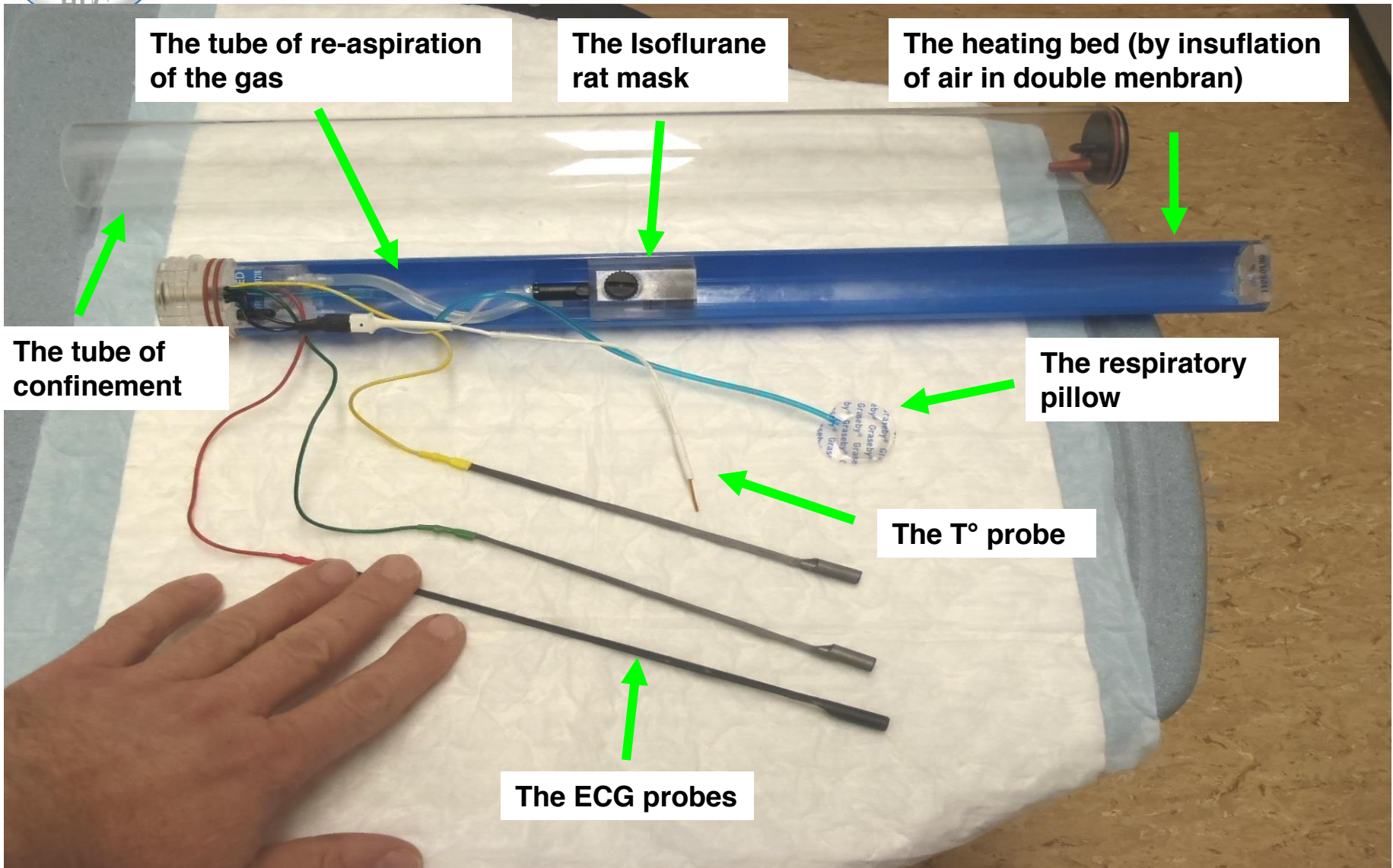


The implementation of animals



.....results x 3 !!

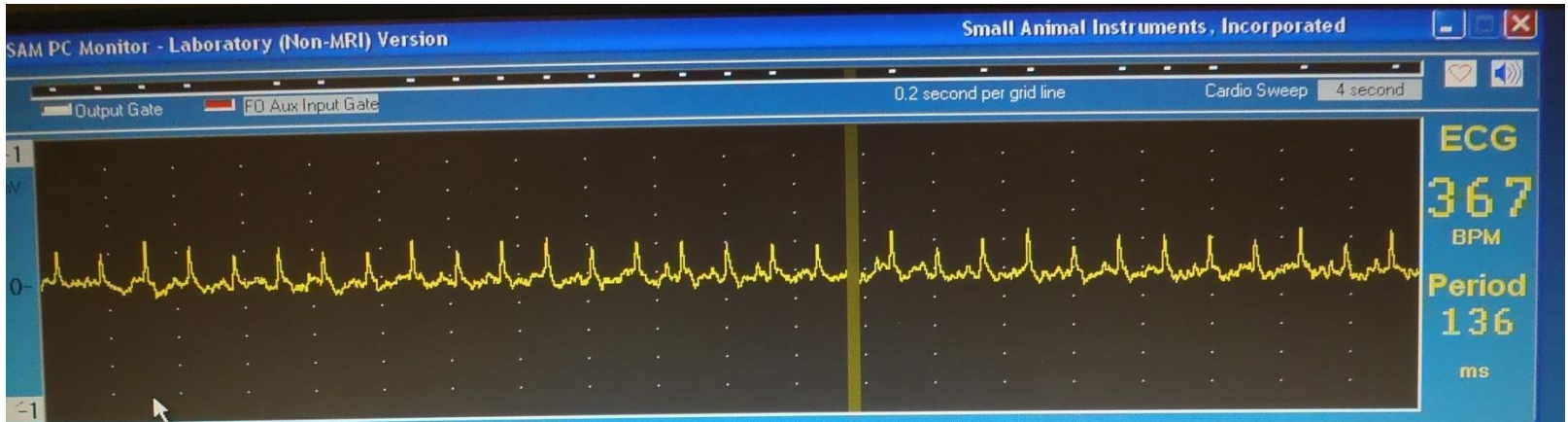




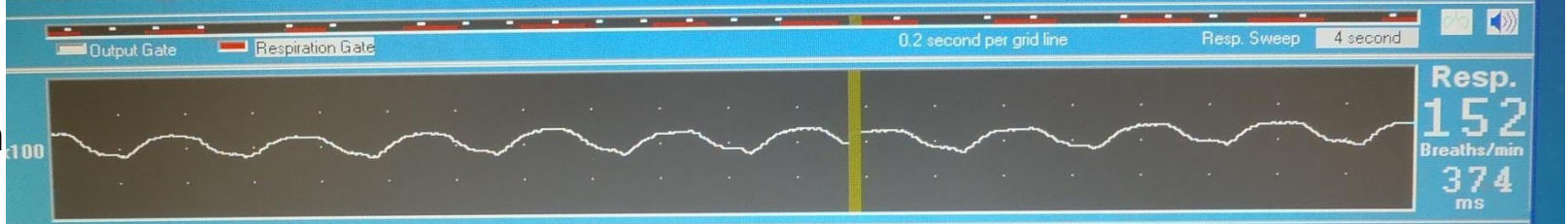


The monitoring console

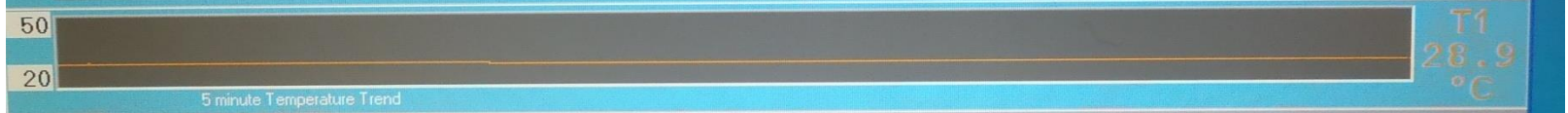
ECG



Respiration

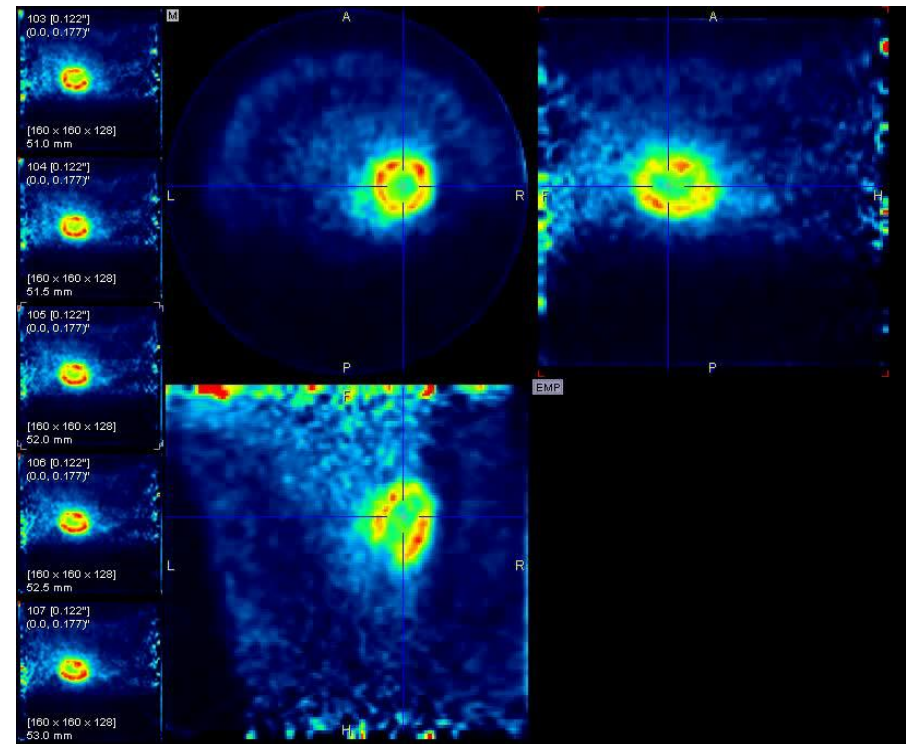
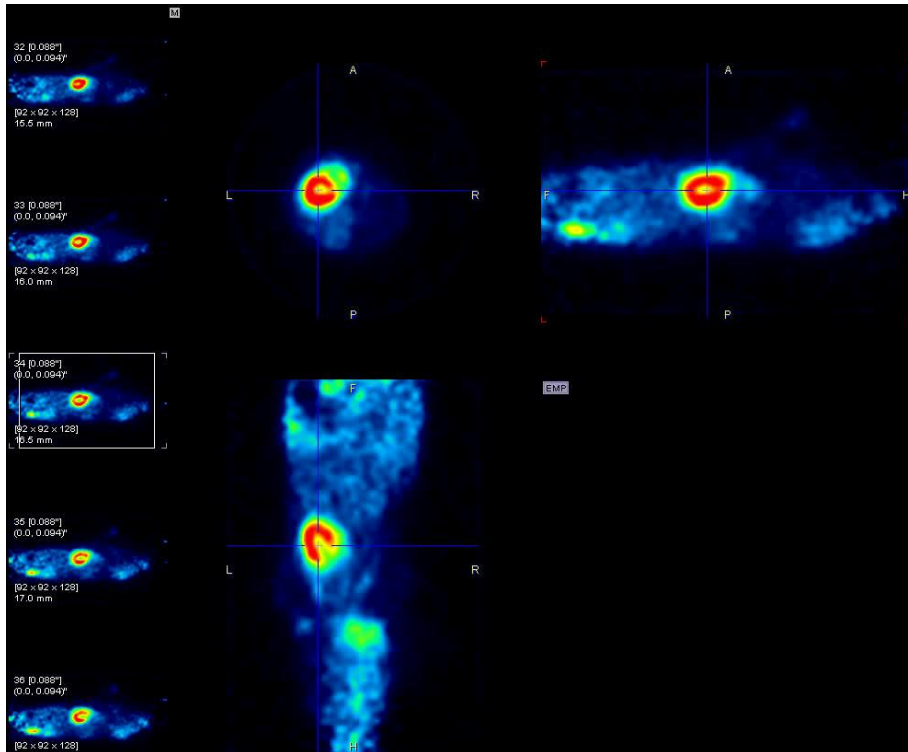


T°



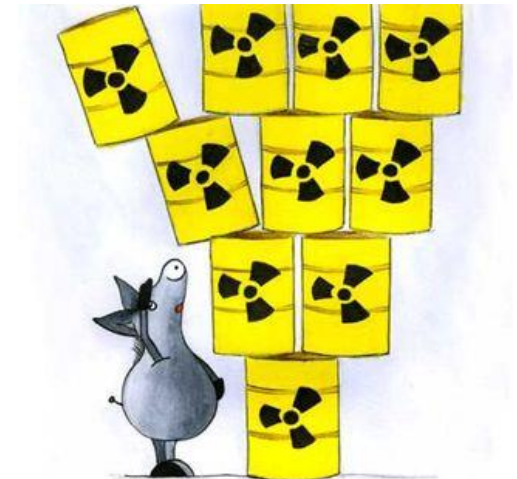
The Gating !

Heart perfusion and cardiac muscle contractility studies





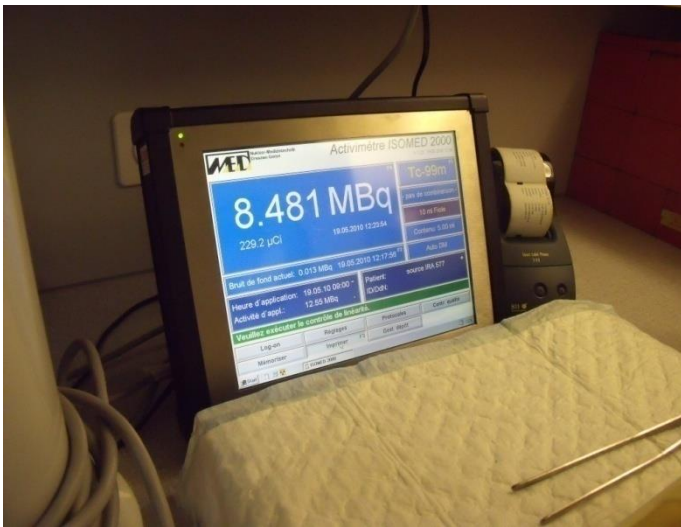
Lead boxes



F.BRUNET WWW.NUCLEAIRE-NONMERCI.NET



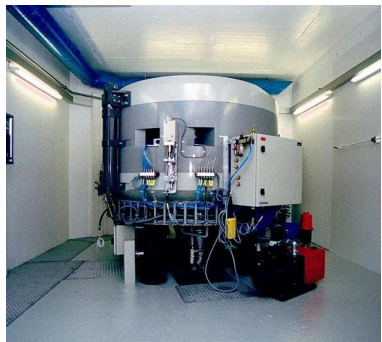
Doses preparation



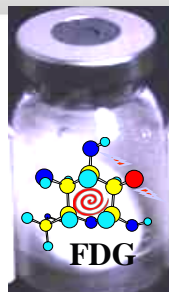
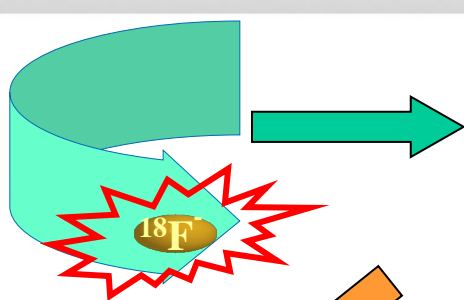
Activimeters



At the end we verify the contamination of surface with an activimeter



Cyclotron production:
about **250 GBq of ^{18}F**



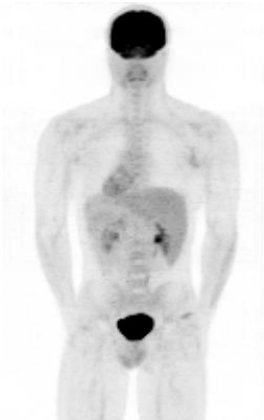
Synthesis of
about **120 GBq (^{18}F)FDG**

Human injection
~ **400 MBq**

Mouse injection
~ **3.7 MBq**

Rat injection
~ **9.25 MBq**

Other species ??
to adapt





Ventilated cages to keep the sterility.





Radioactive Diminution (~24h)

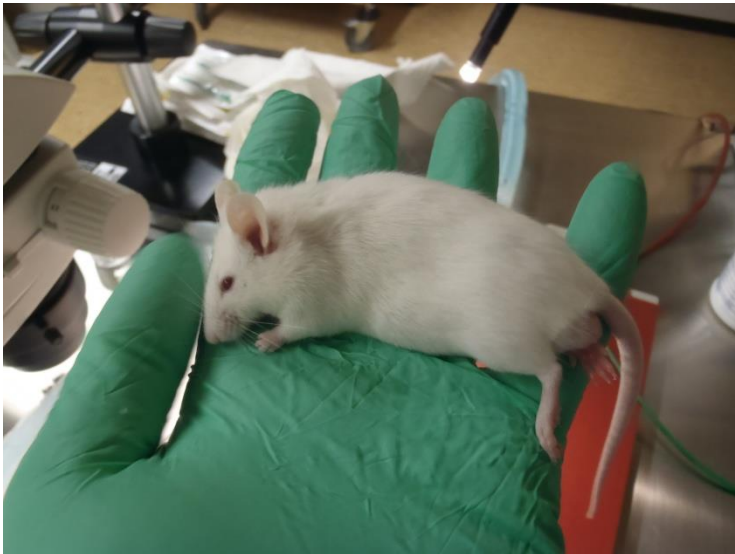
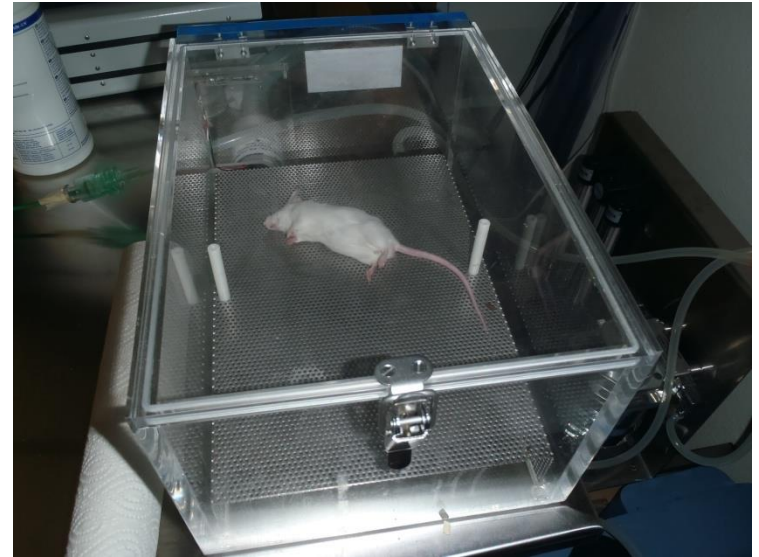


Ready for the
second
injection !!!!

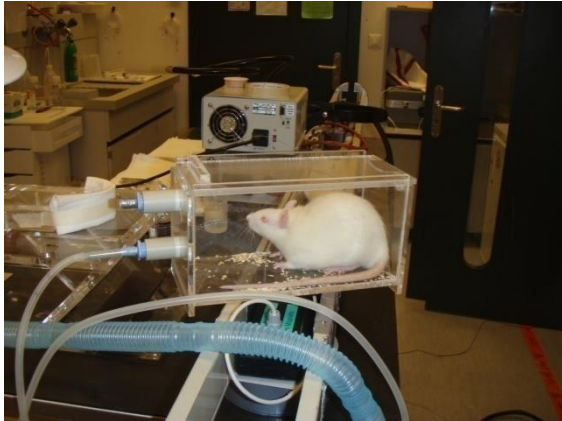
The animal facility : it is just 3 racks of ventilated cages (1 rat and 2 mice) for the radioactive diminution. Then animals are transferred in the big animal facility of CMU.

Animals preparation: anesthesia, injections, catheterisations...

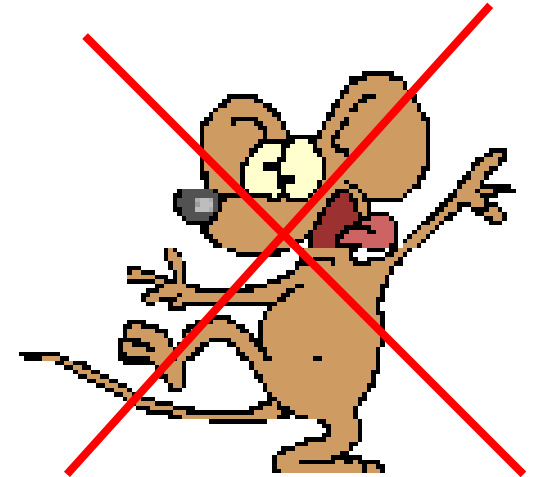








It's important to have a good anesthesia to **avoid movement** during the Scan !!!! (if movements artefacts in the images)

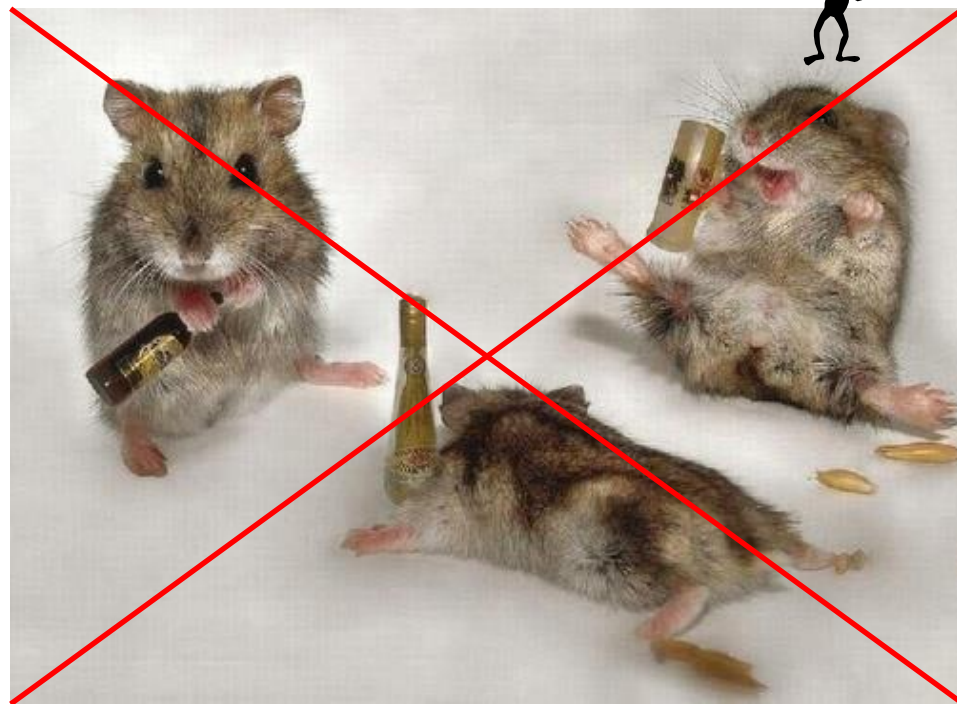




It is important to have a good anesthesia and to have **quiet animals** before the anesthesia to avoid the movements and not disturb the imaging !!!

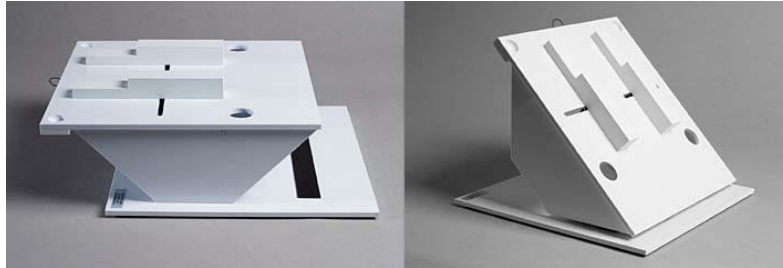
(Consumption of glucose if muscles are in movement)

So no festivities before the analysis!!!!





Intubation Cannula



Intubation table



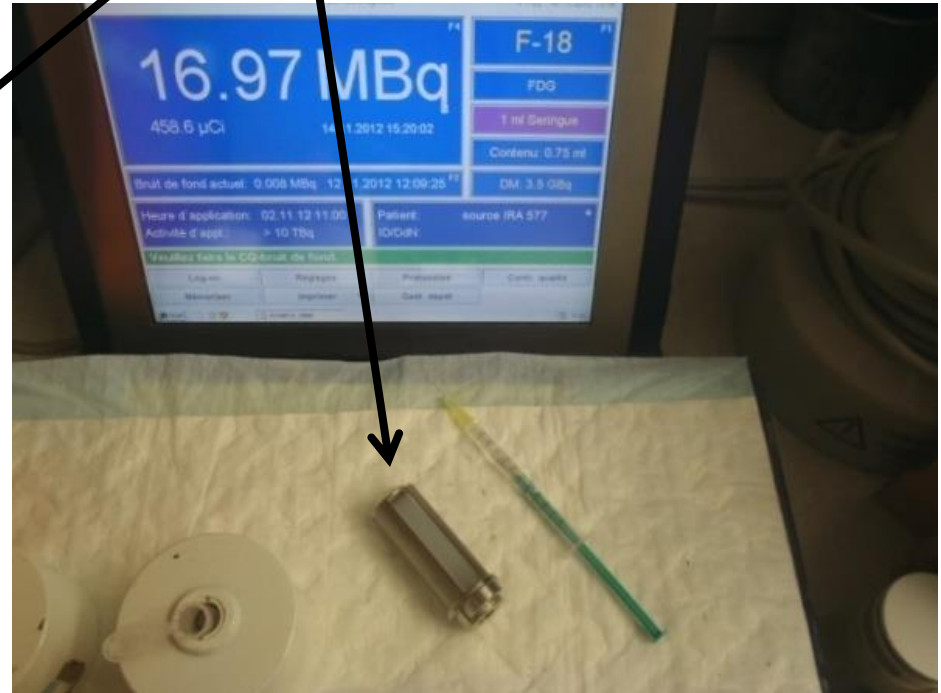
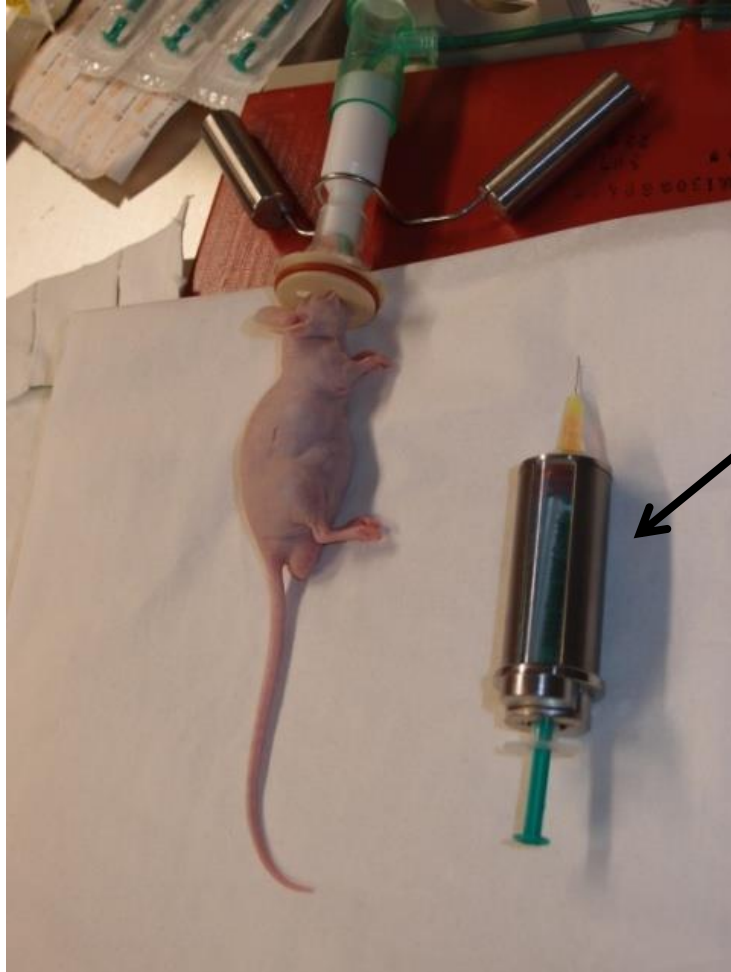
Intubation via tracheal way



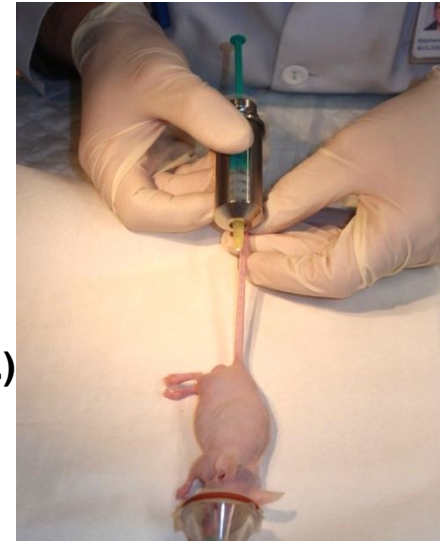
Ventilator:
Depending of the animal weight you have to regulate the BPM and the Flow.

The tracers injections

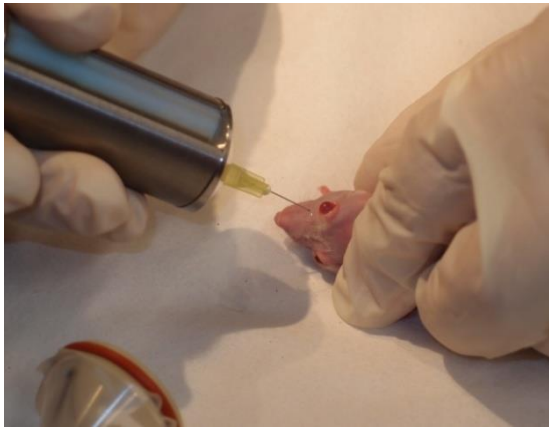
Difficulty of the injection: Lead syringe (heavy and size +++) and speed to protect the experimentator against radiations.



The different sites of tracers injections



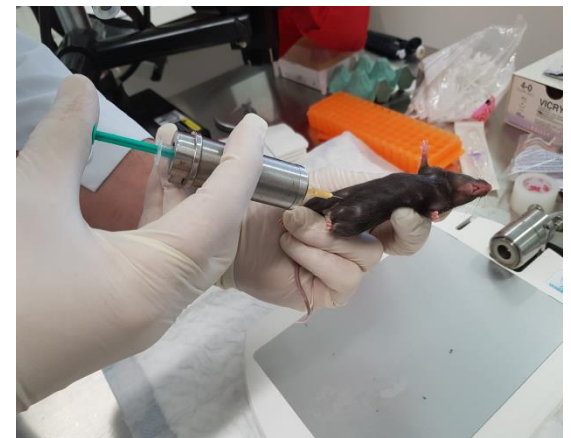
←
Caudal Vein (I.V.)
→



Rétro-orbital Sinus (I.V.)

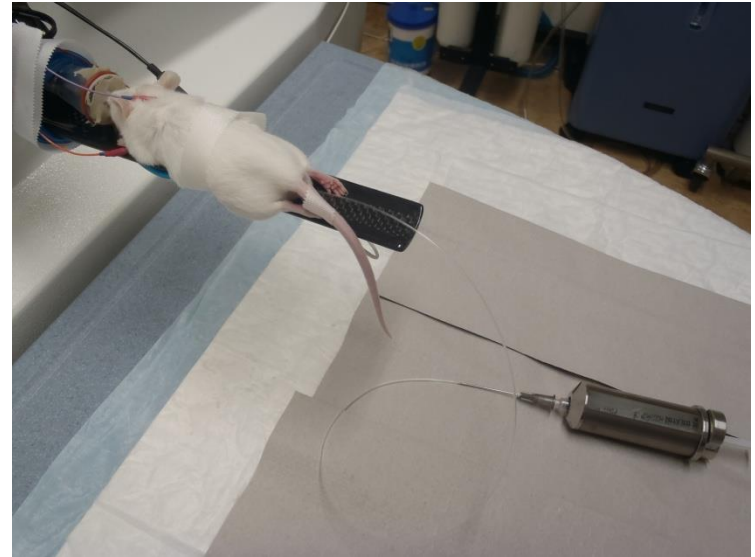
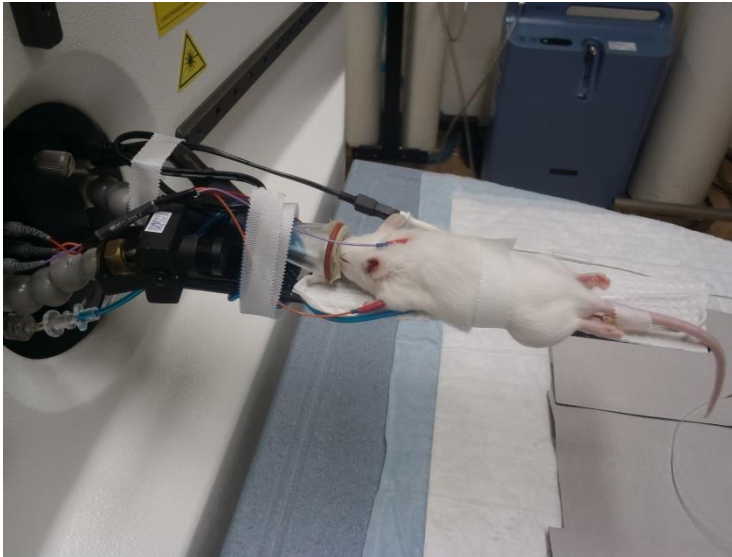


Femoral Vein (I.V.)

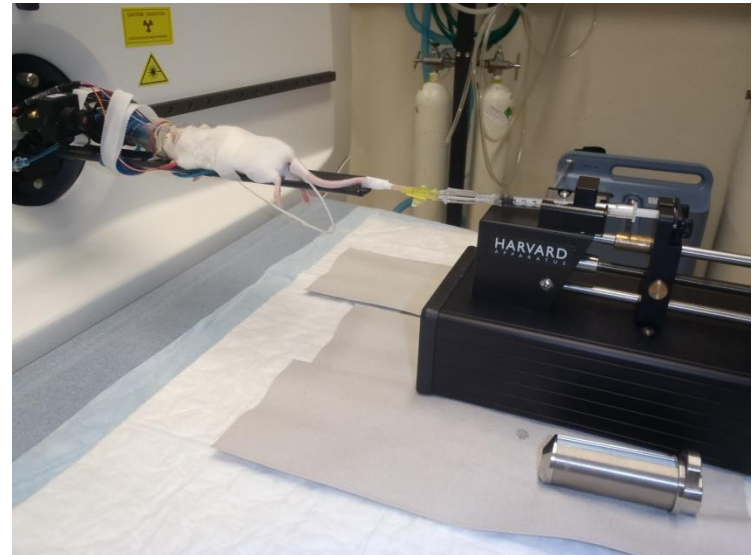
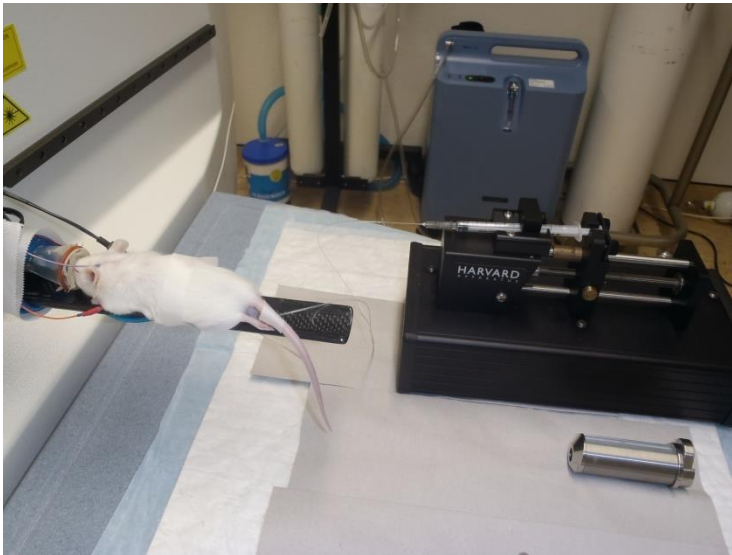


Intra-Peritoneal (I.P.)

Manuals injections



**Automatics injections
(For dynamics injections)**



The catheterisations : Tail vein or Femoral vein



Surgery table



Induction box



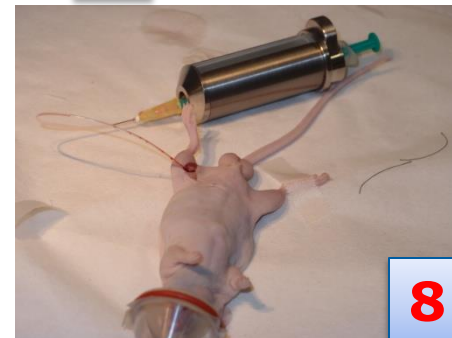
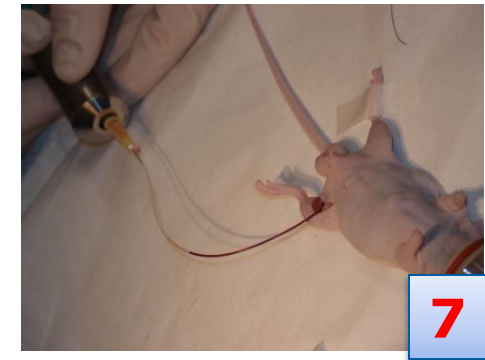
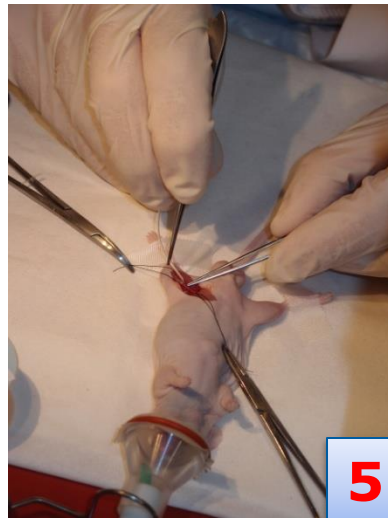
Heating pad



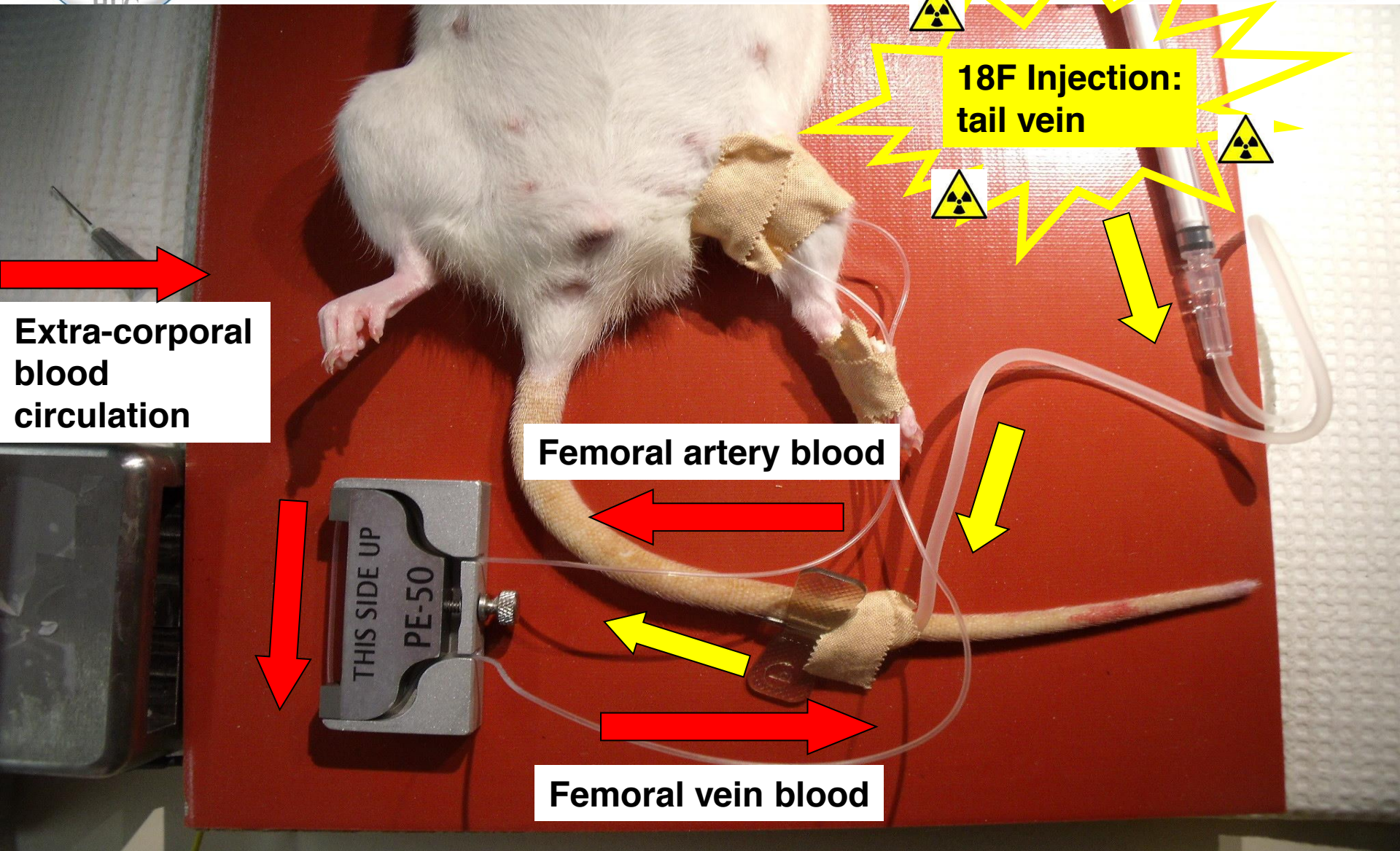
**Tail vein and Femoral vein for tracer injection.
Femoral artery for blood sampling and to follow the blood pressure.**

**Tail vein for non- terminal experiment
Femoral artery for terminal experiment**

Femoral catheterisation in mice



The Loop surgery (Femoral Artery / Vein)

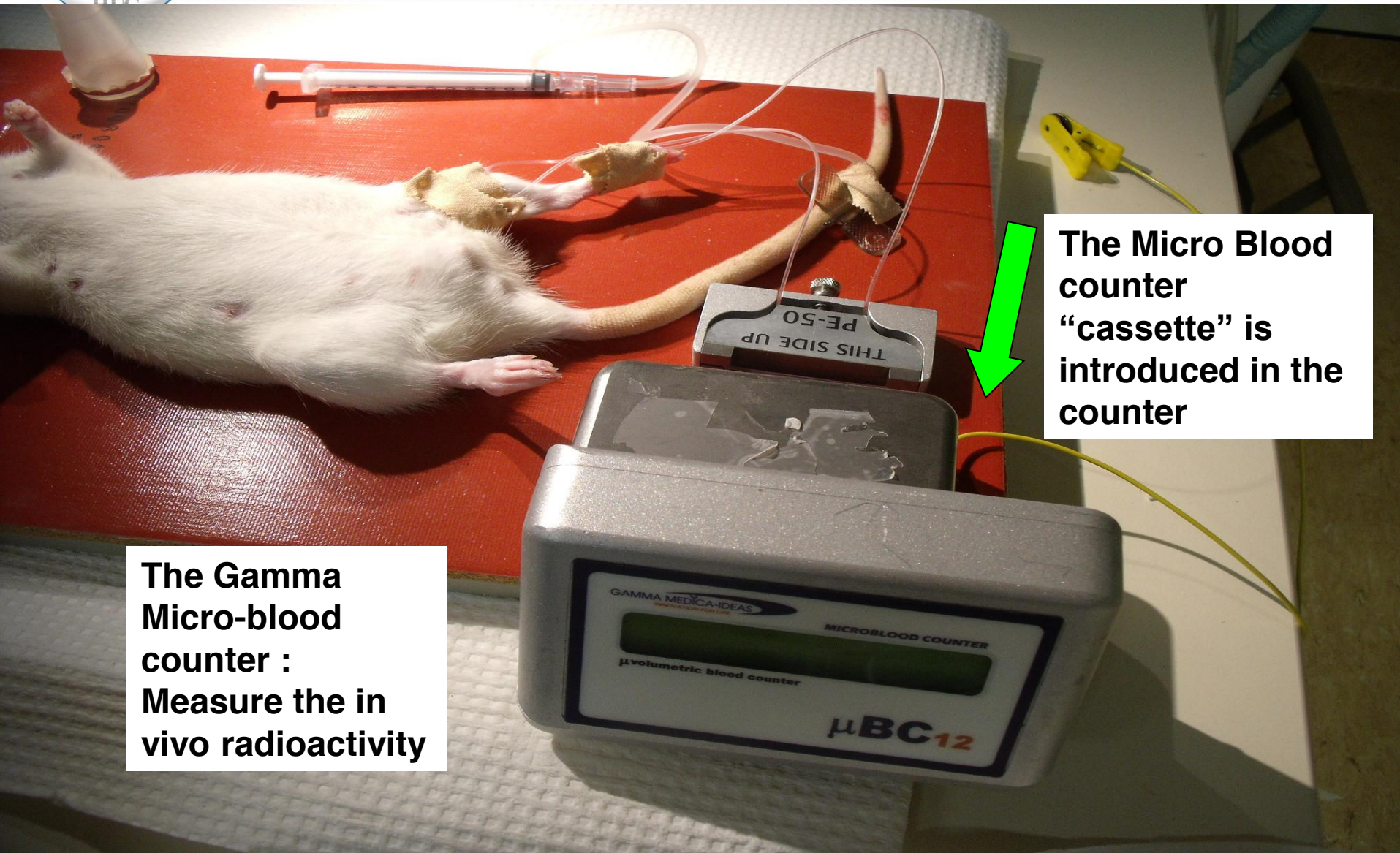


**18F Injection:
tail vein**

**Extra-corporal
blood
circulation**

Femoral artery blood

Femoral vein blood



**The Gamma
Micro-blood
counter :
Measure the in
vivo radioactivity**

**The Micro Blood
counter
“cassette” is
introduced in the
counter**

The images analysis

The Standard Uptake Value (S.U.V.)

S.U.V. ??

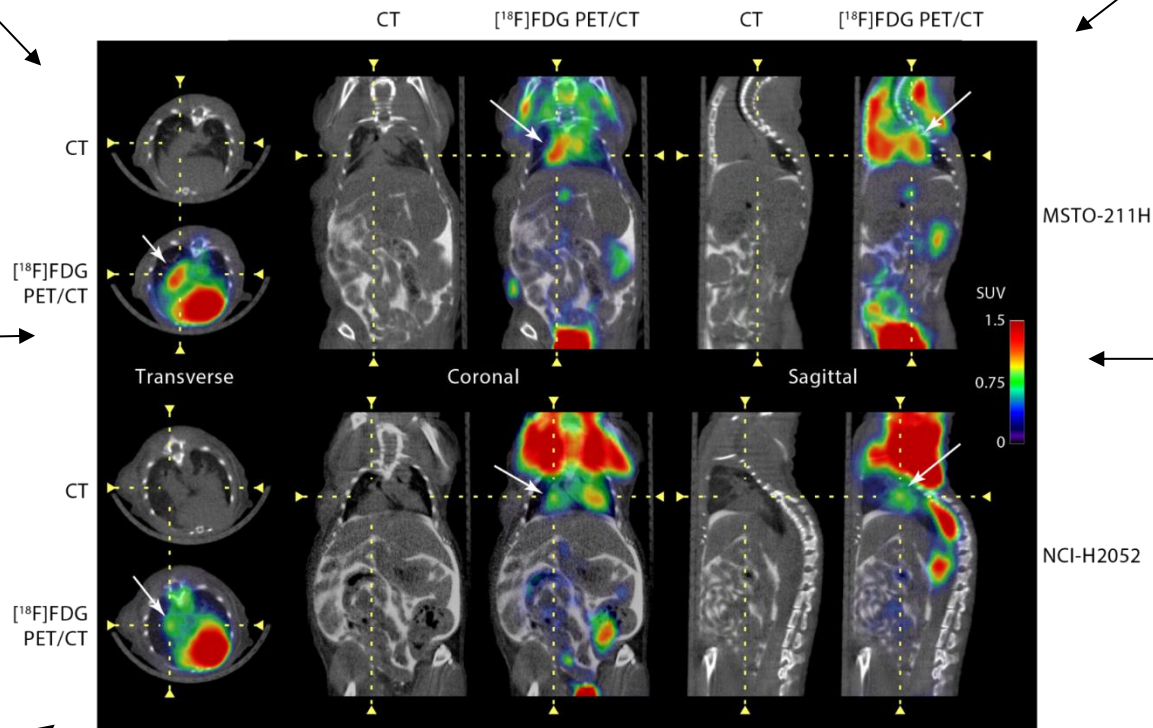
S.U.V. ??

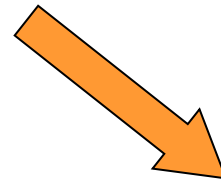
S.U.V. ??

S.U.V. ??

S.U.V. ??

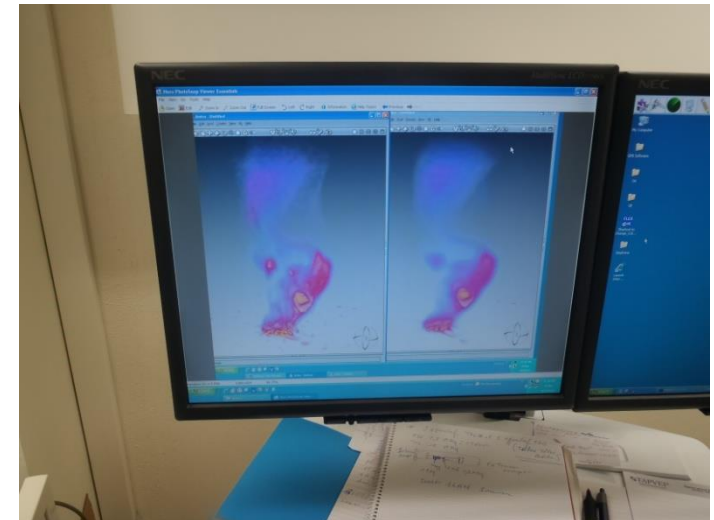
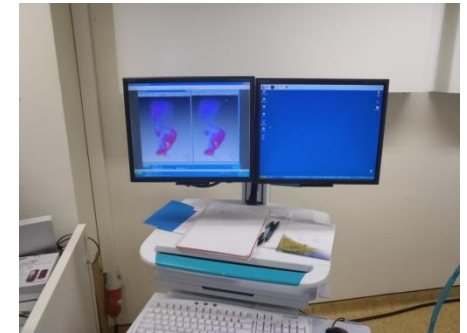
S.U.V. ??





Softwares for imaging :

- Vivid
- Pmod
- Osirix



Computers:

- 1 Mac for the PET
- 1 PC for the SPECT
- 1 PC for the CT

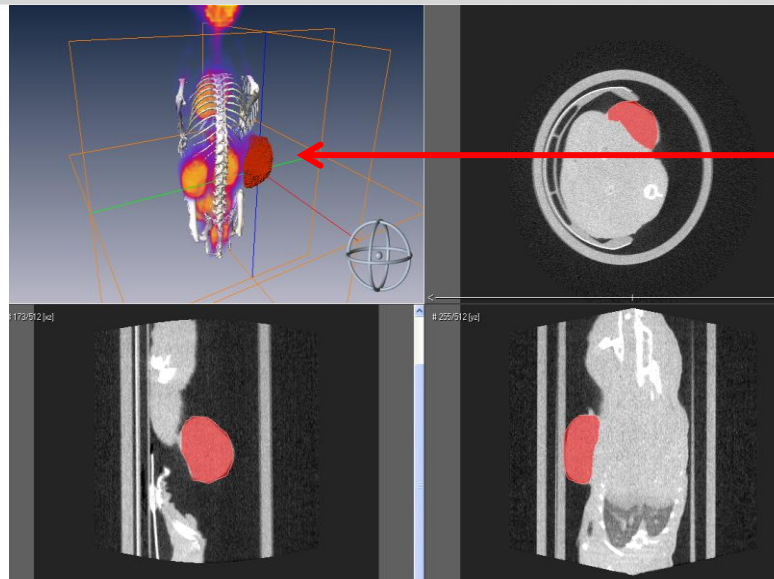
Backups:

- 1 hard disc for the PET
- 1 hard disc for the SPECT the CT

The Standard Utake Value (S.U.V.)

Body slices before quantification and Uptake calculation S.U.V. (Standard Uptake Value)

Tumor volume, 3D's calculation (ml)

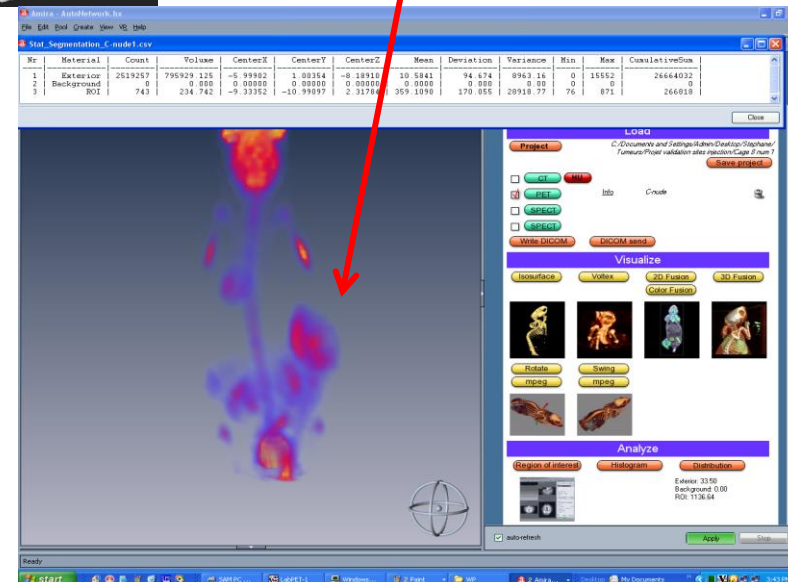


Tumor

S.U.V. (Standard Uptake Value)

$S.U.V. = \text{Activity concentration (KBq/ml)} / (\text{injected dose (KBq)} / \text{animal weight (g)})$.

The conversion of the images in SUV allows to normalize the images and to make them comparable from a subject to an other one, and from an examination to an other one.

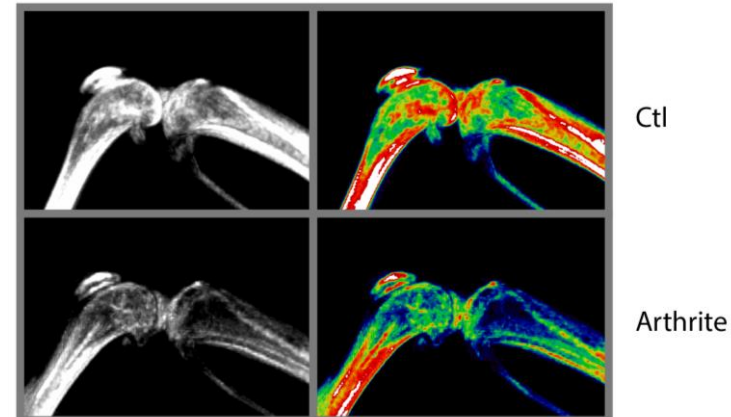
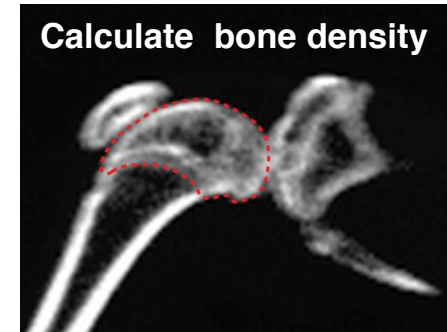
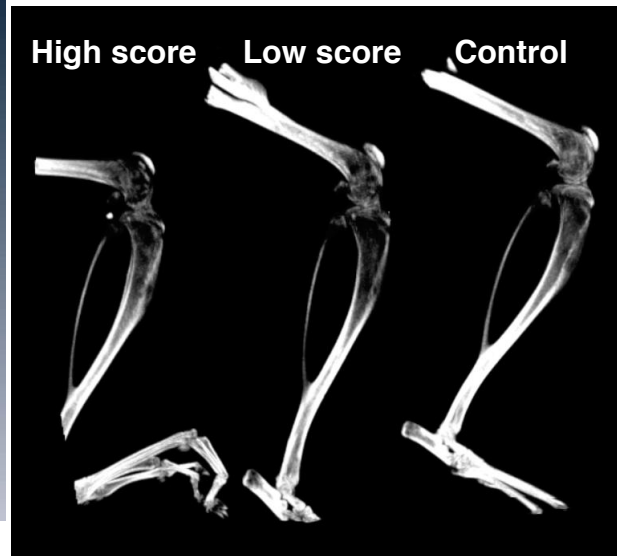


Micro-PET/SPECT/CT applications



Example of protocol : Variation of the bone density in a **mice model of arthritis** (Collaboration Marine Lacroix / Novimmune SA, Geneva) :

- D0 establishment of the arthritis model,
- D0 à D? injection of anti-arthritis products and follow the evolution of the arthritis by scoring,
- D30 **CT's scans and follow the bone density on excised organs (visualisation and quantification of the arthritis in the knees of animals),**



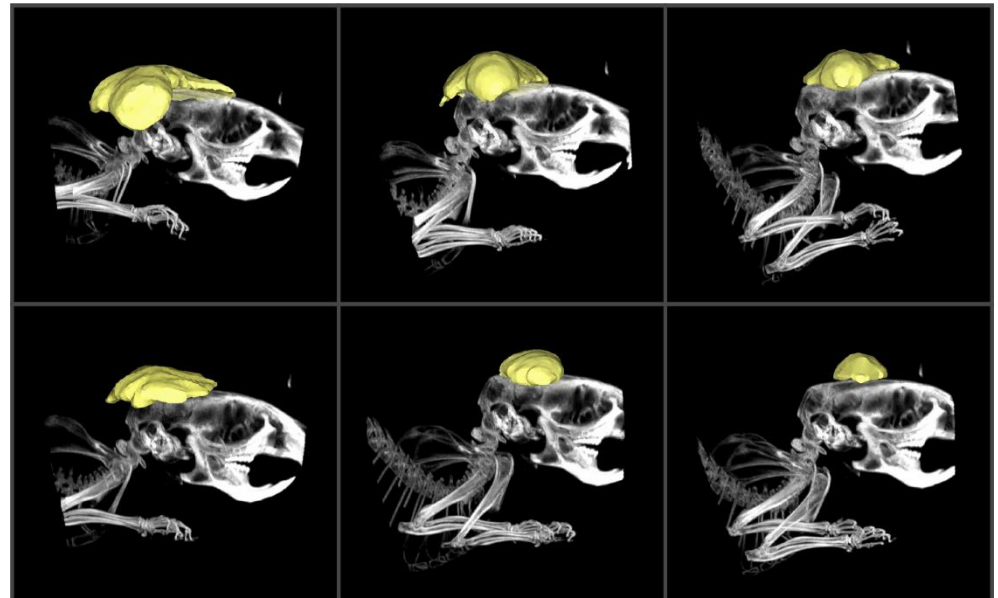
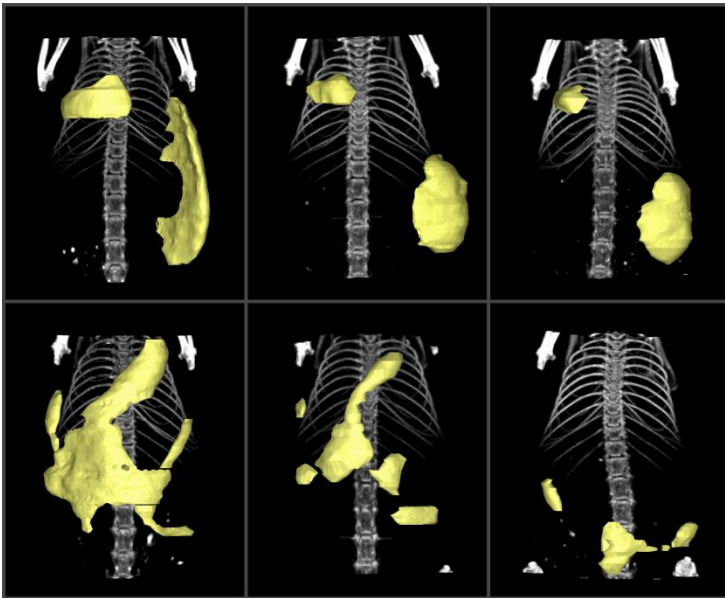
Example : Observation of the variability of the fat density (human fat) in **mouse model** (Collaboration Dr Dominik Lévine / H.U.G, Geneva) :

- D0 Human fat implantation in Nude mice treated or not with a drug (TTT 3),
- D1, D30 et D60 **CT scans and follow the fat density (visualisation and quantification of the fat implants),**

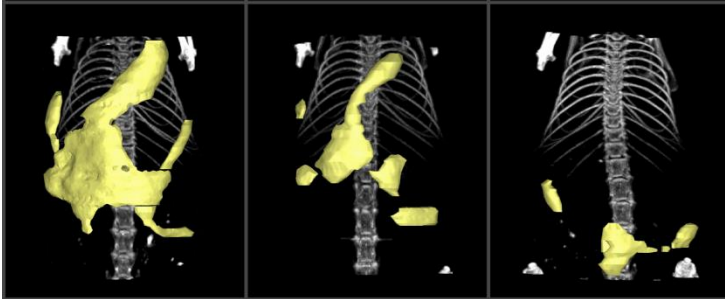
Back implant

Head implant

Ctl 1



TTT 3



Day 1

Day 30

Day 60

Day 1

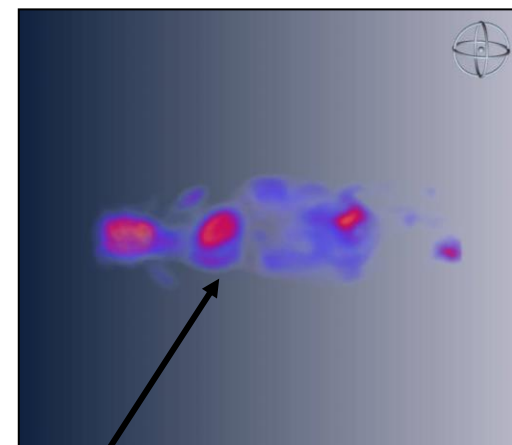
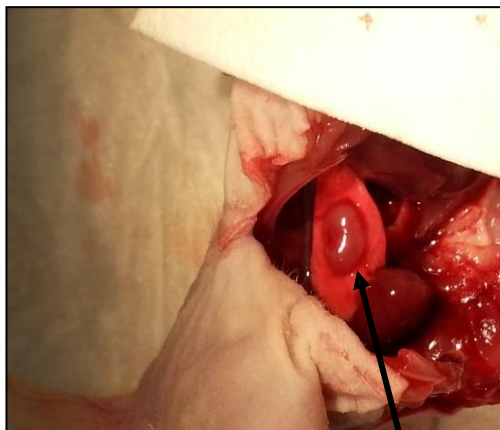
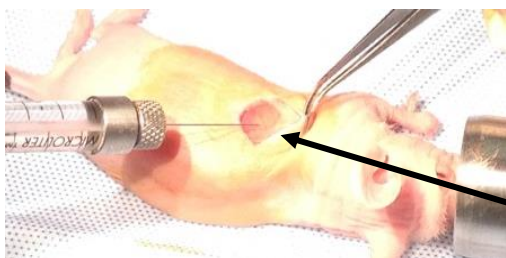
Day 30

Day 60

Example of protocol: Monitoring of orthotopic pleural mesothelioma models (Collaboration Dr Véronique Serre-Beinier, H.U.G and Dr Didier Colin, H.U.G.)

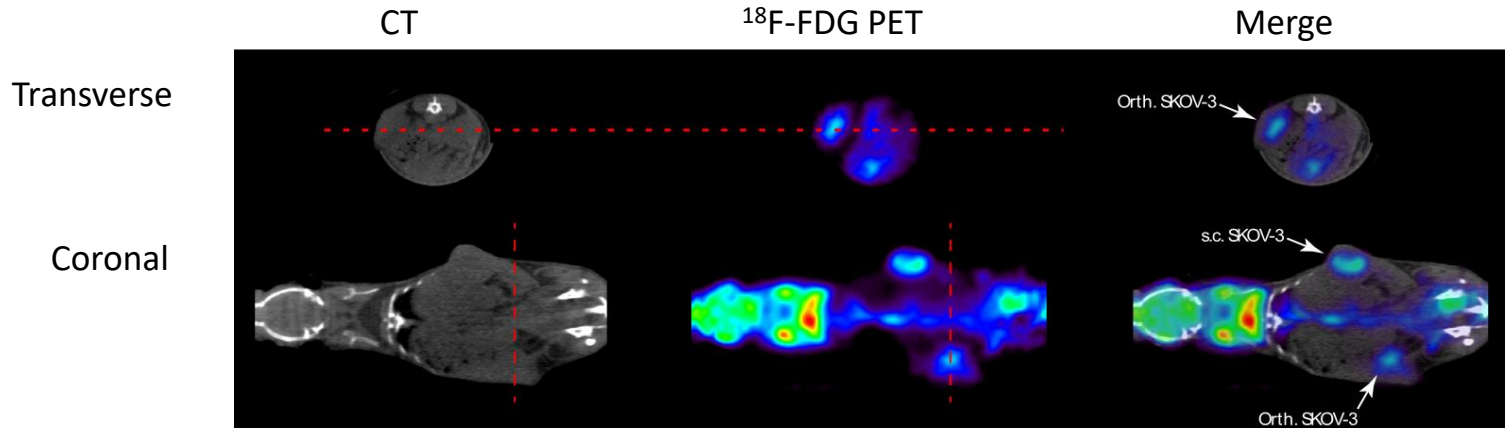
- J-15 Human cancer cell cultures,
- J0 injection orthotopic of tumors in the lungs,
- J2 in J? Scan **F18FDG or/and F18FLT** for tumors growth and quantification,
- J+2 Efficiency of treatments (**visualization and quantification**),

Immunodeficient mice Nude (accept transplant human cancer cells)

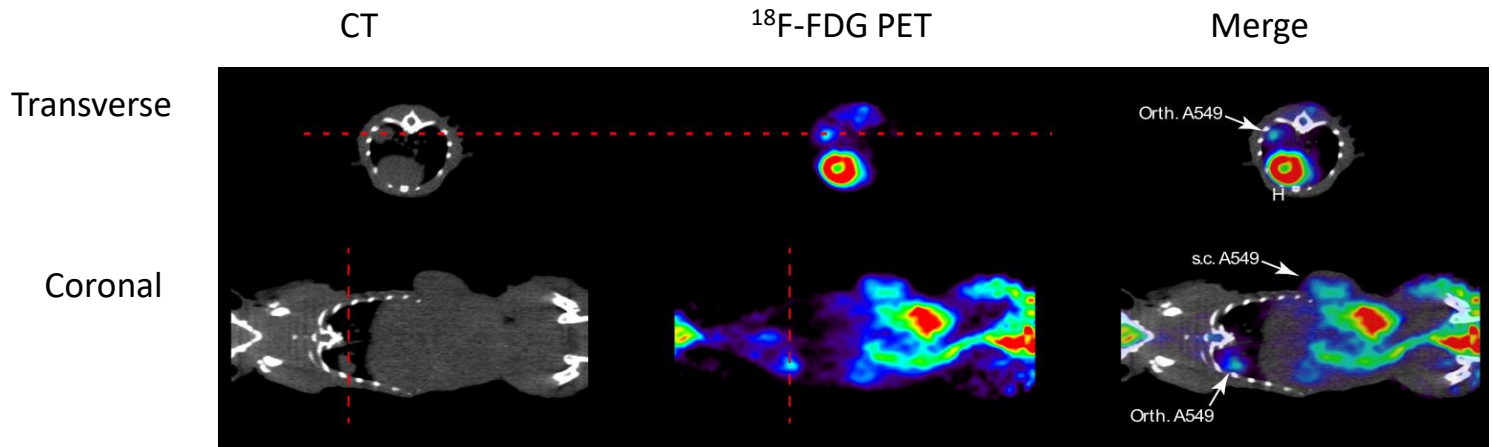


Example:
Orthotopic mesothelioma tumor
(intra-pleural injection)

Ovary

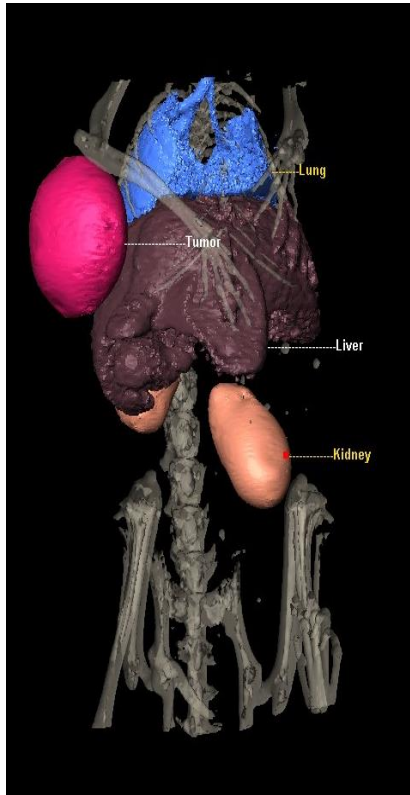
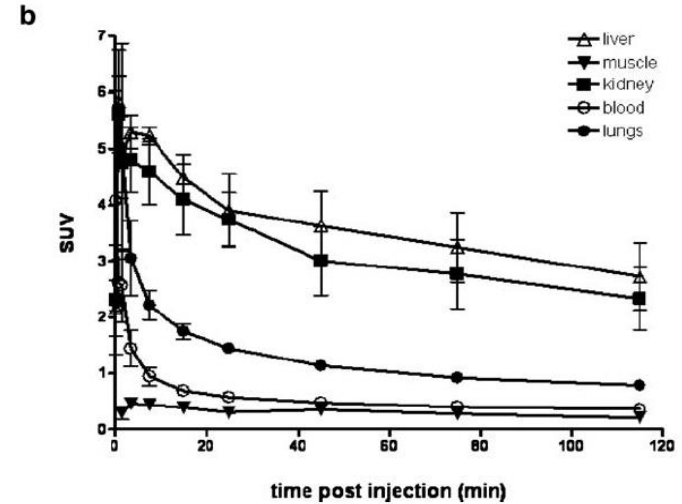
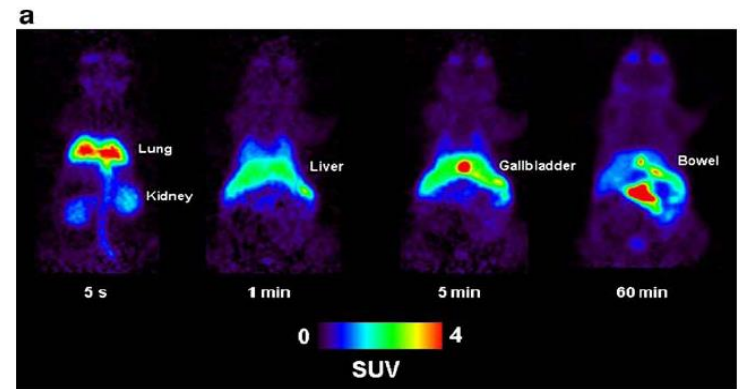


Lung



Bio-distribution and drugs tracability

Fig. 2 Biodistribution of [^{18}F] gefitinib in mice. **a** Normal (nontumor-bearing) mice were subjected to a microPET/CT scan after intravenous injection of 7.4 MBq (200 μCi) of [^{18}F] gefitinib. **b** Quantitative analysis of [^{18}F] gefitinib uptake in various tissues over time. Error bars indicate 1 SD



Autopsies / protected dissections

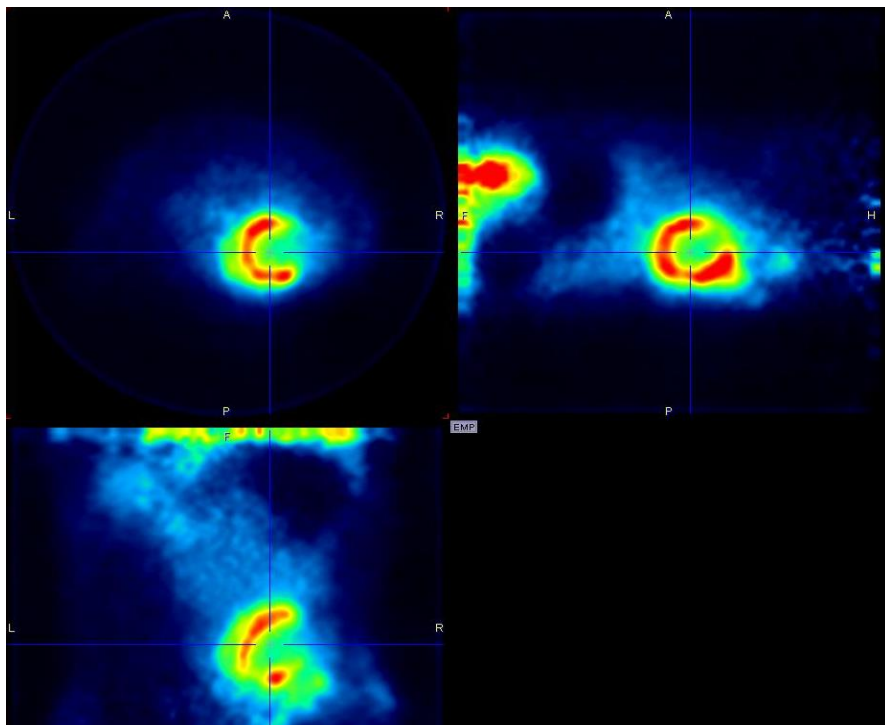


Gamma counter

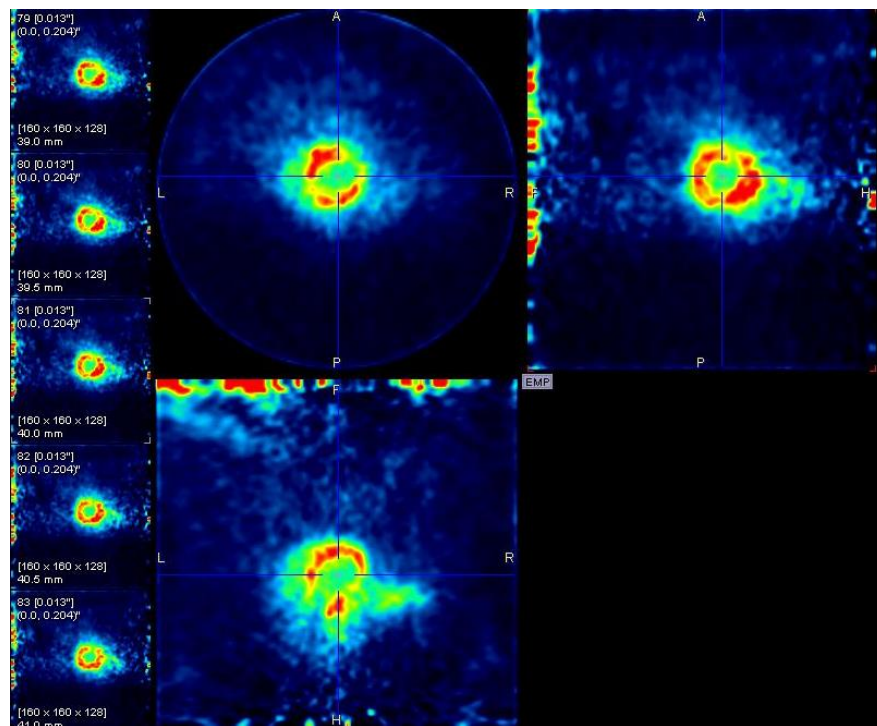
Example: protocol with rat Fisher (Collaboration Prf. Walpoth H.U.G):

- J0 partial ligation of the coronary (create an infarct),
- J2 first scan **F18FDG** and **NH3** (**visualization and quantification infarct**),
- J3 patch surgery implantation (for re-vascularization of the infarcted zone).
- J15 the second scan **F18FDG** and **NH3** (**visualization and quantification of the infarcted zone**).

J2 Heart + infarct



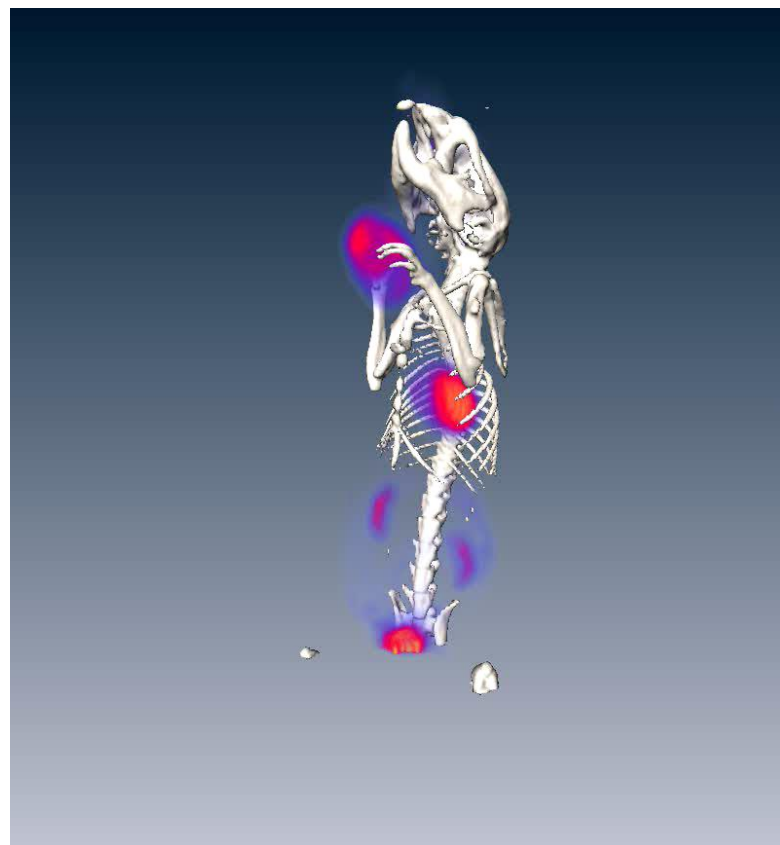
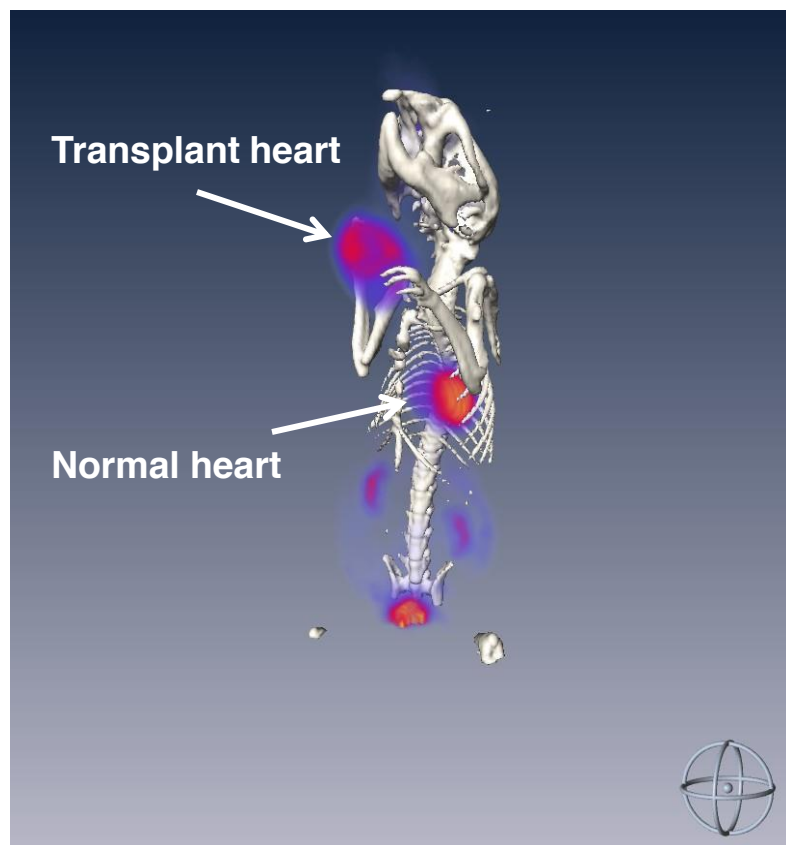
J15 Heart + patch



Example : Heart transplant in mice (Collaboration Dr Bedat Benoit H.U.G).

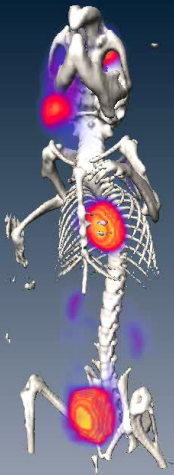
Effect of the TLR4 monoclonal antibody on the heart transplant viability.

- D0 Transplantation of a « second heart » on the neck of the animal (allogenic or isogenic grafts),
- D5, D7 and D13 scans with **F18FDG (visualisation et quantification of the heart transplant viability)**,
- D0 à D15 test by injection of graft rejection drugs.



The same thing but we follow the same mouse during the time !
(Isogenic graft without treatment)

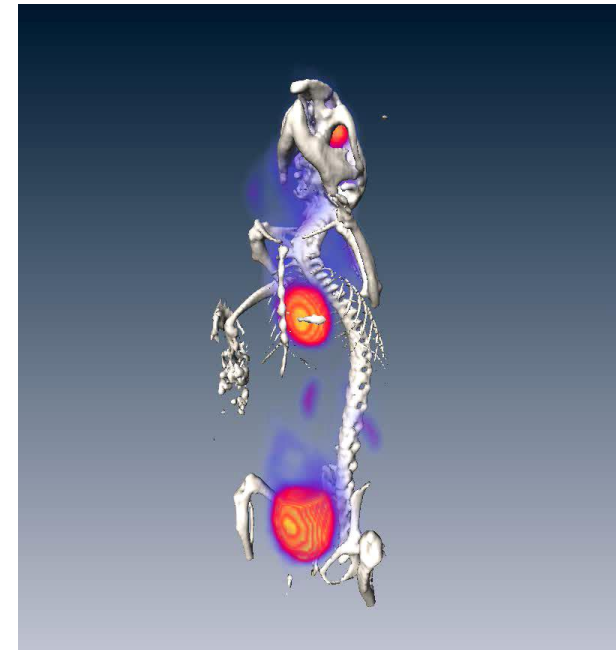
D5



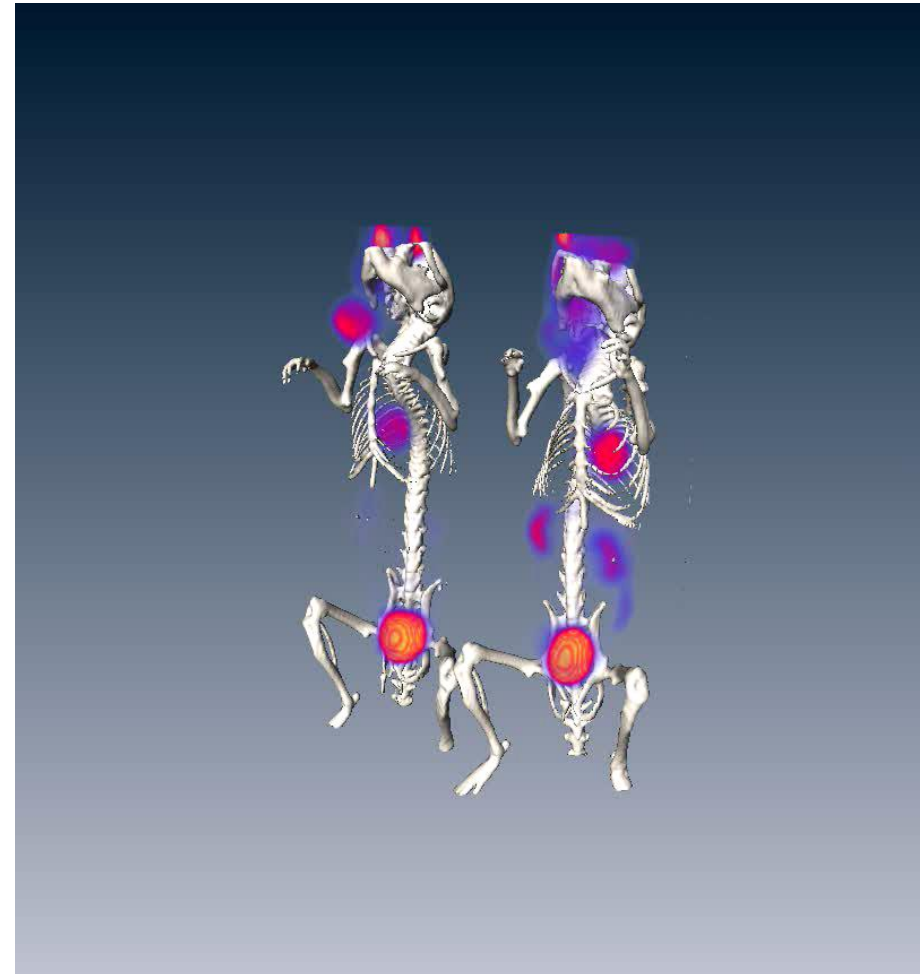
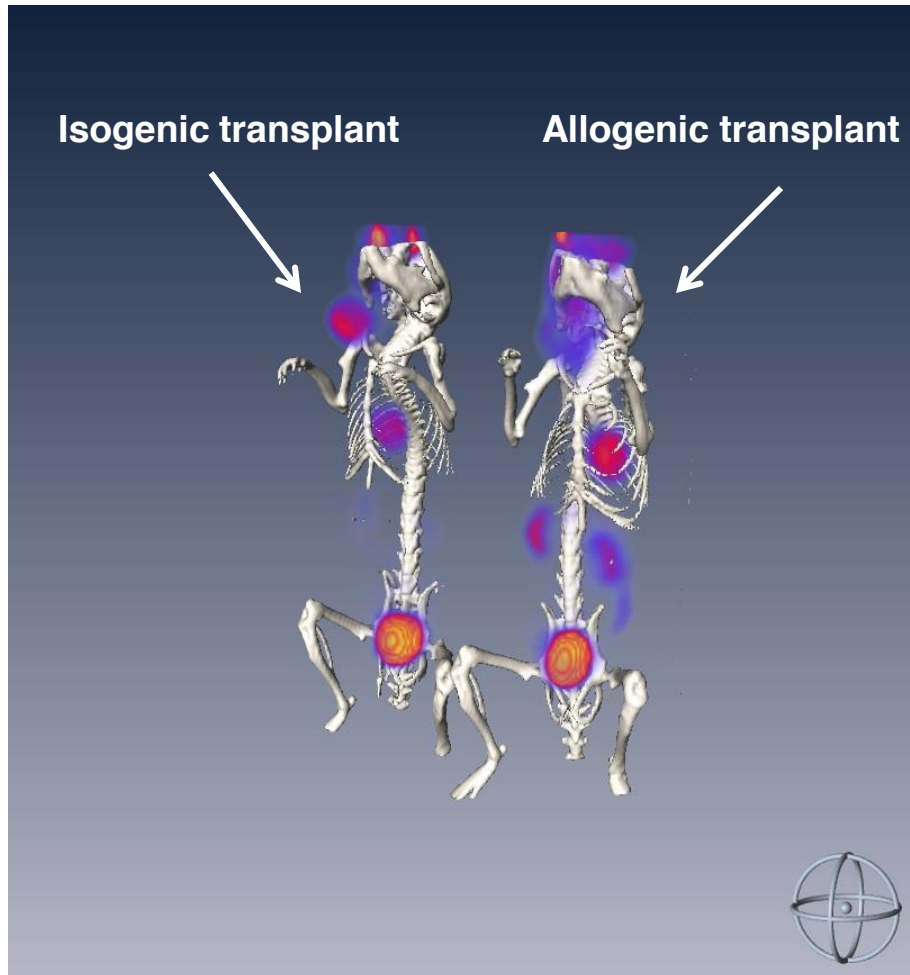
D7



D13



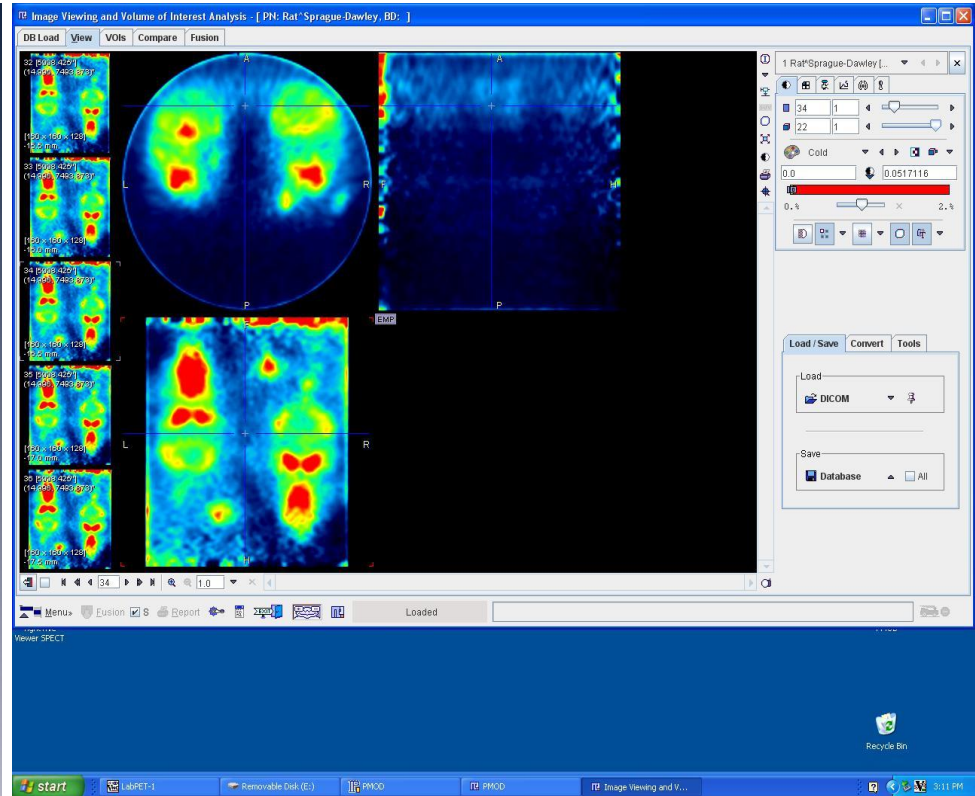
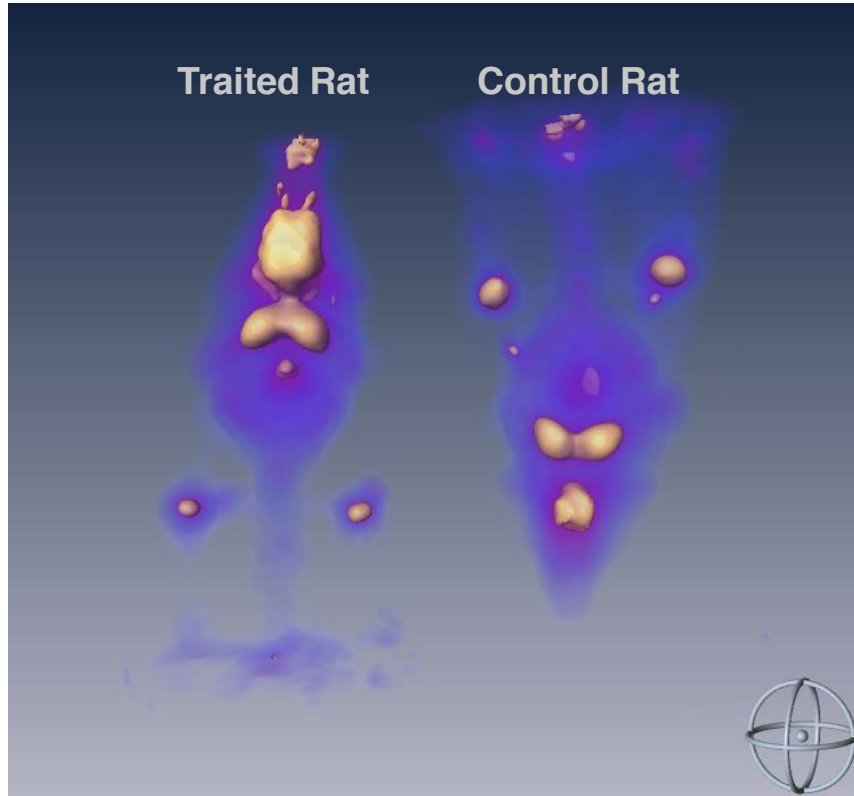
Here is the comparison (with the double bed) between an isogenic mouse and an allogenic mouse (without treatment).



Study on rat (Collaboration Dr Nathalie Ginovart, H.U.G. Belle-idée)

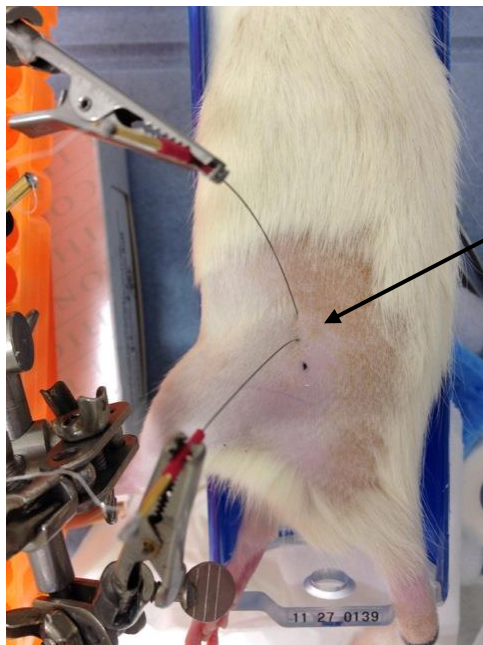
Model of Schizophrenia:

- J0 to J14 T.H.C. (Cannabis) treatments by injections I.P.,
- Scan of 2 rats by bed with specific tracer **F18 Fallypride** (**Visualization and quantification of the Dopamine receptors in Striatum**),
- At the same time as the MicroPet, we make behavioral tests revealing the state of Schizophrenia.

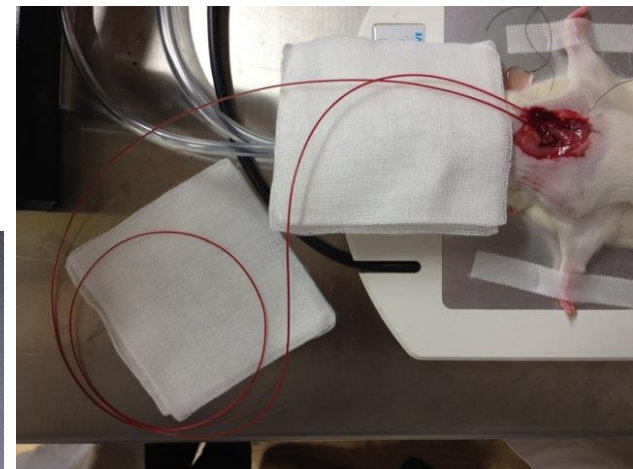
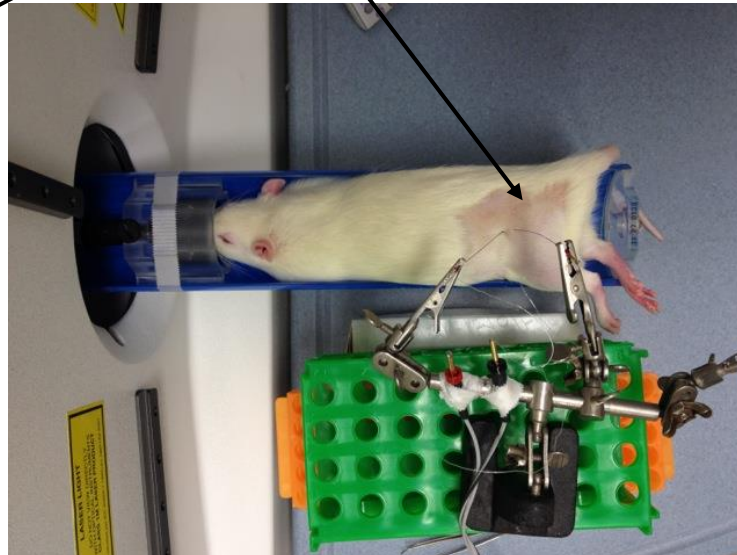


Example : Visualisation of the muscular activity with Acétate C11 on rats (Collaboration Dr Sara Trombella and Prf Osman Ratib H.U.G).

- Loop surgery and back leg stimulation with two electrodes,
- Scans with **Acetate C11 (visualisation et quantification of the muscular activity)**,
- In parallel we record the **level of radioactivity in the blood** with the Microblood counter.



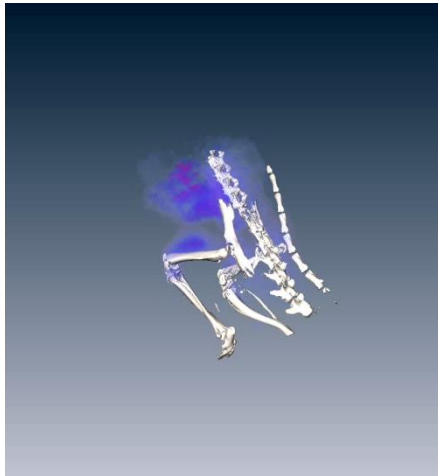
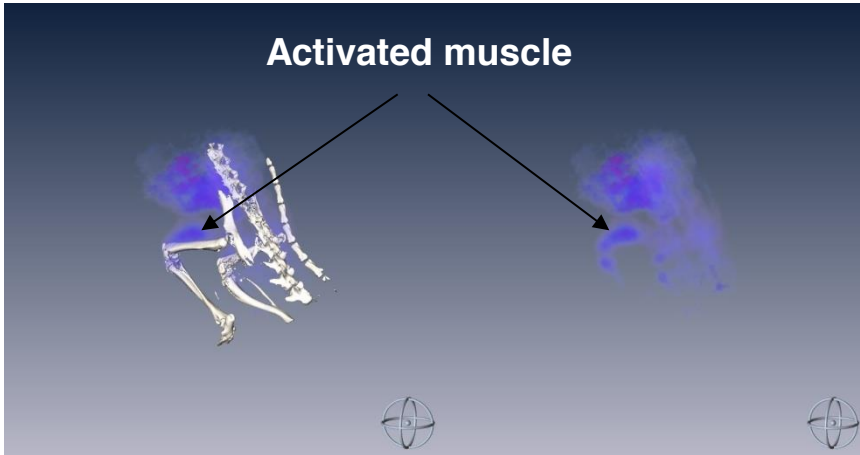
Electrodes
(Physiological stimulation)



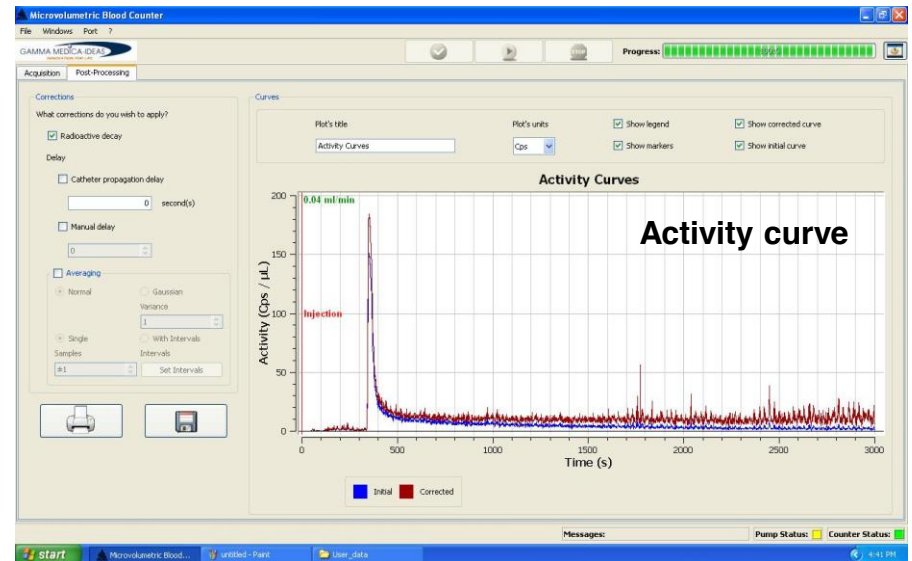
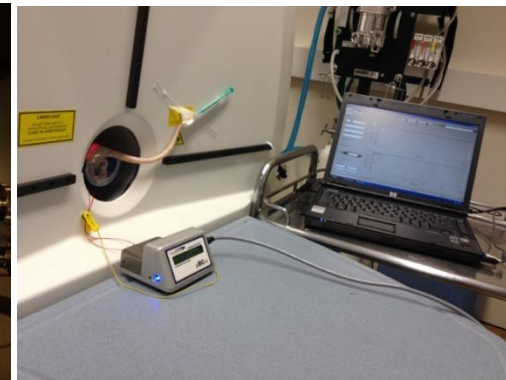
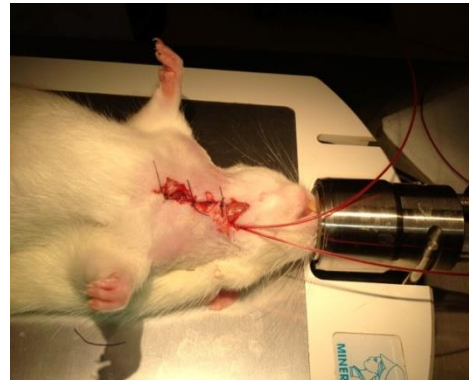
Results:

Pet scan's results

Activated muscle



In parallel Microblood's results (Activity curve in the blood)



Study of the obesity to the rat Lou, Origin of rat staying thin (Collaboration Dr Christelle Veyrat, C.M.U.)

Role of the brown fat: the aim of the brown fat is to regulate the body temperature and to burn the energy brought in excess in the body. Until now, only the rodents and the human newborn children were known to have significant deposits of brown fat. The purpose would be to control it to use its mysterious powers and know if it can provoke a loss of weight.

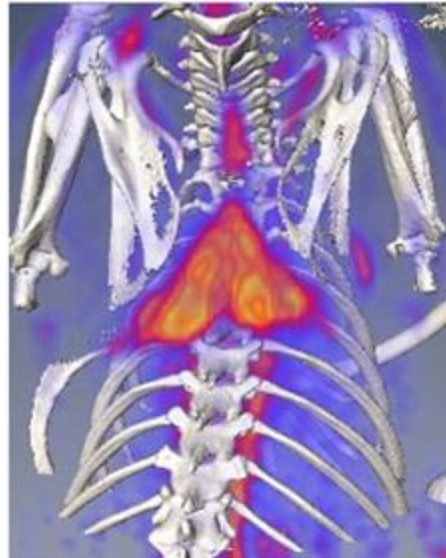
- Scan with **F18FDG** possible because the brown fat consumes some glucose (**visualization and quantification of the brown fat**),
- Possibility of stimulation of the brown fat by the cold.



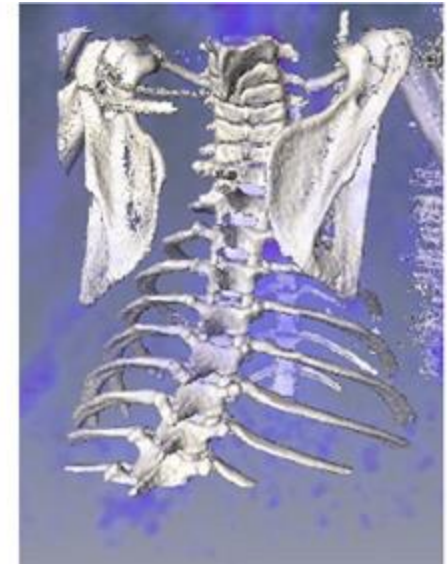
**Lou Rat:
Thin**

**Wistar Rat:
Obese**

At the same age!!



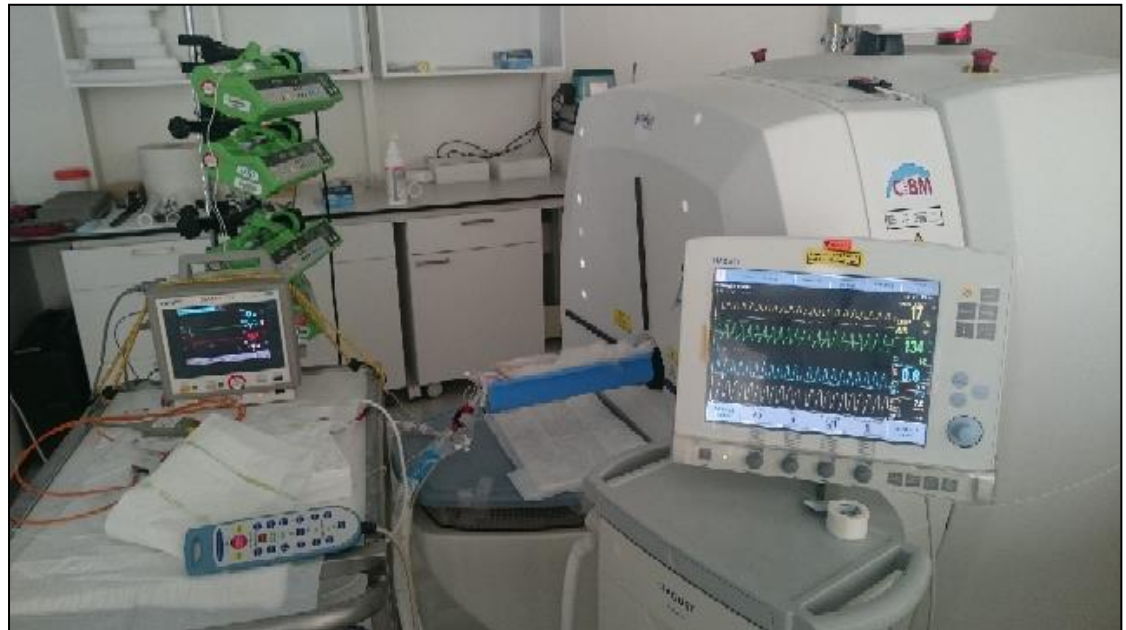
Lou Rat



Obese Wistar Rat

Example: Protocol with young Rabbits (Collaboration Prf. Habre H.U.G):

- Lung hyperventilation during 7h (create lungs inflammation),
- CT scan (Reference site and visualization lungs inflammation),
- SPECT scan Tec99m+Albumine (lungs perfusion),
- PET scan F18FDG (visualization and quantification lungs inflammation),



Results:

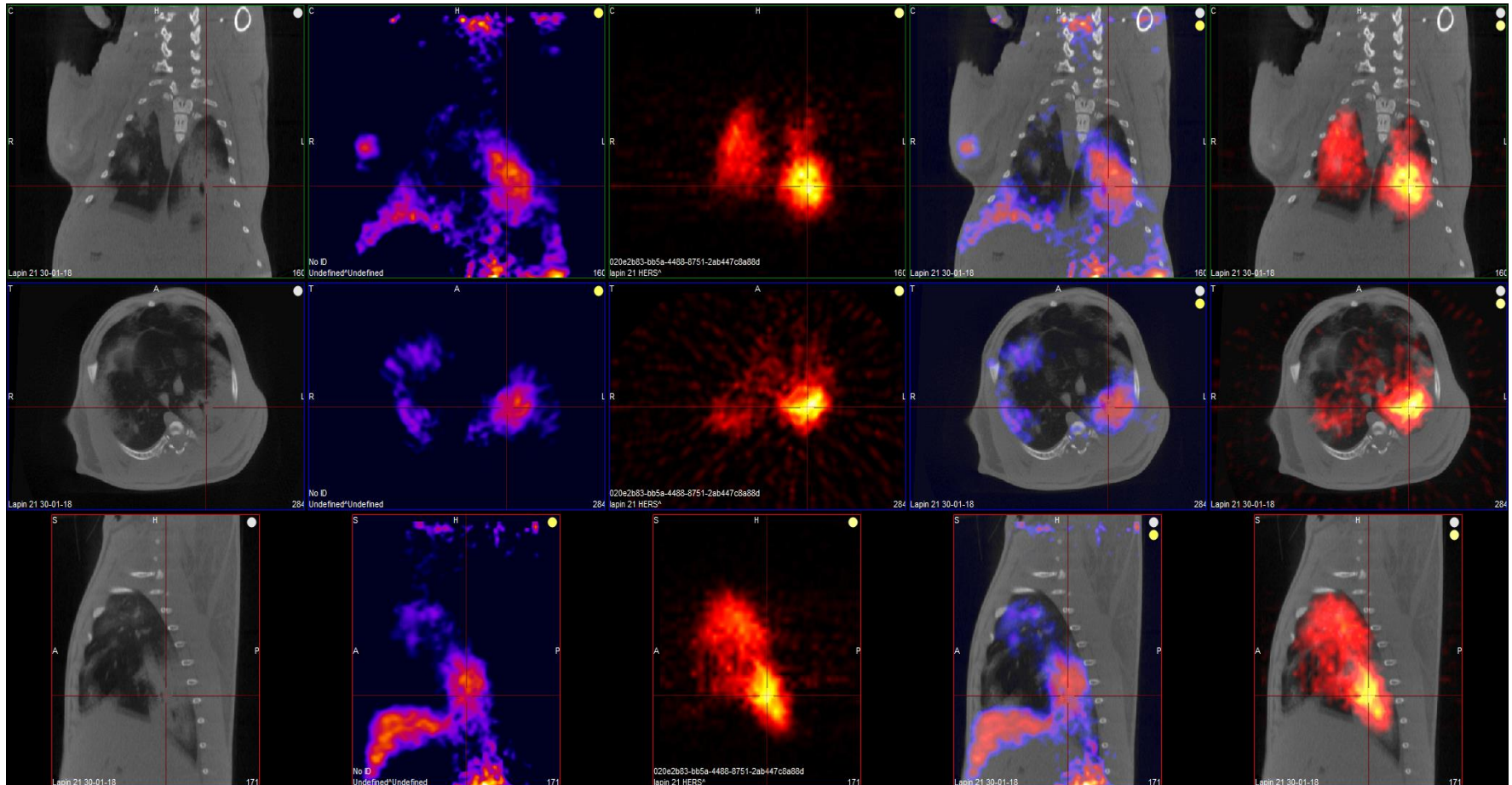
PET (18F FDG) for lungs inflammation metabolism.

SPECT (Tec99m Albumine) for lungs perfusion.

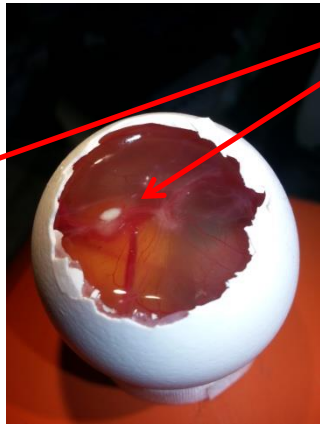
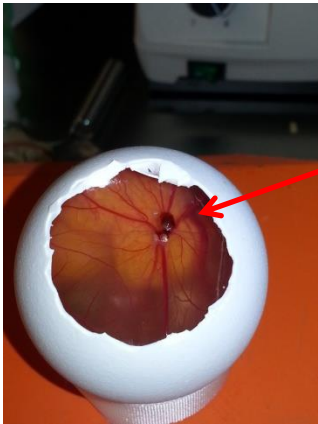
CT

PET/CT

SPECT/CT

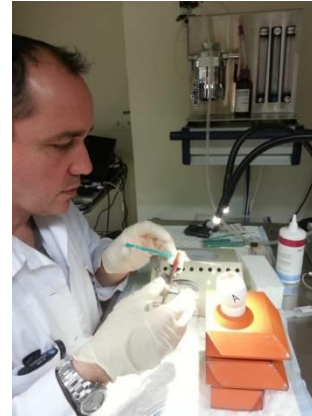


Study of tumor's viability in eggs (Dr. Didier Colin (CIBM))

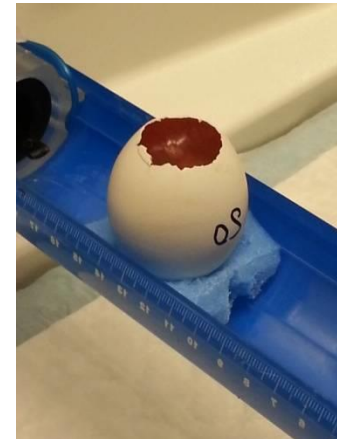
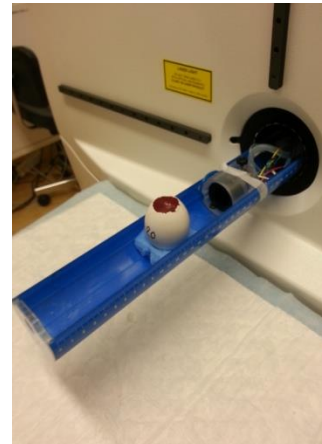


**Tumors
with
angiogenesis**

Eggs at Day 11 incubation + tumors

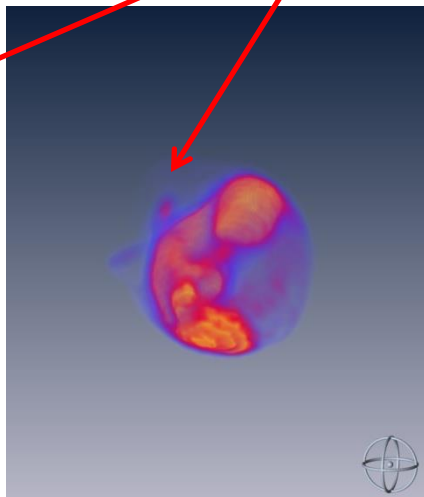
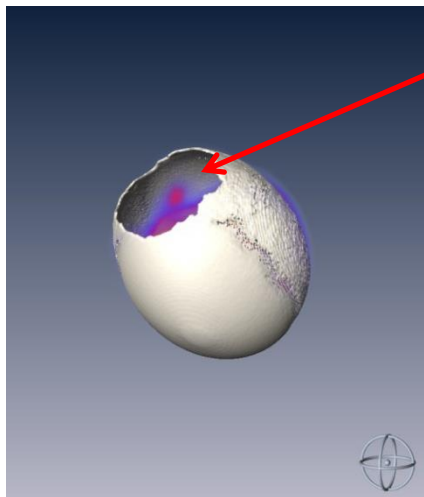
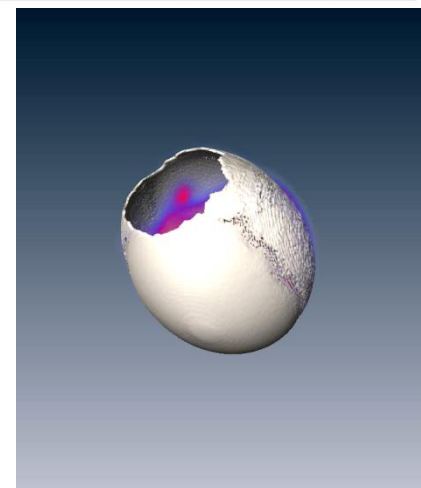
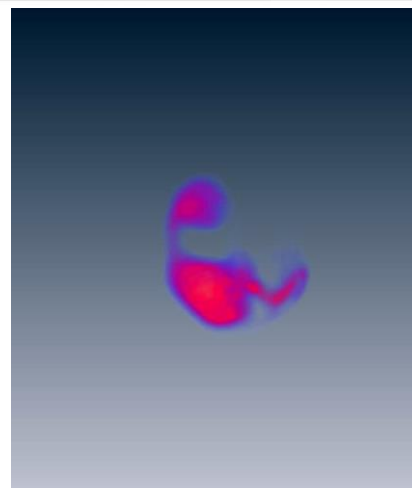
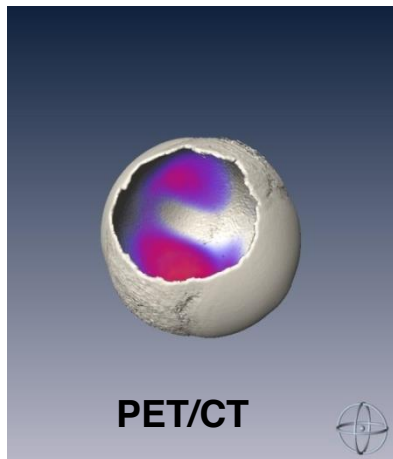


**Injection mix FDG F18 + anti-tumoral drugs in
principal vein**

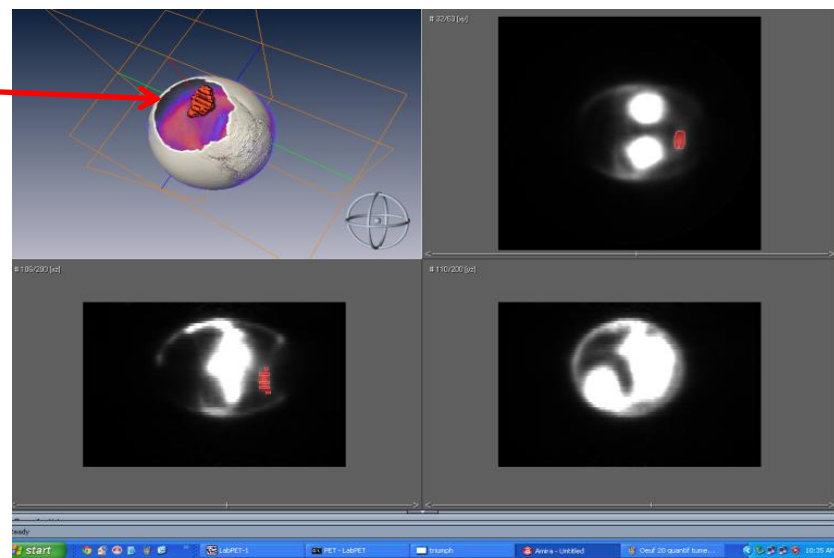


Scan of the egg in PET /CT

Results :



Tumor



Quantification and treatment's effect

.....and the animals !!!

- Pr. Martin Walter, Pr. Osman Ratib, Dr. Didier Colin, (C.I.B.M. Geneva),
- Pr. Rolf Gruetter, (C.I.B.M. Lausanne),
- Cyclotron unit, (H.U.G.),
- All CIBM's collaborators.



Thanks you for your attention !!!

.....And questions ?



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