Il laboratorio See-Life - una risorsa per l'imaging preclinico su modelli animali.

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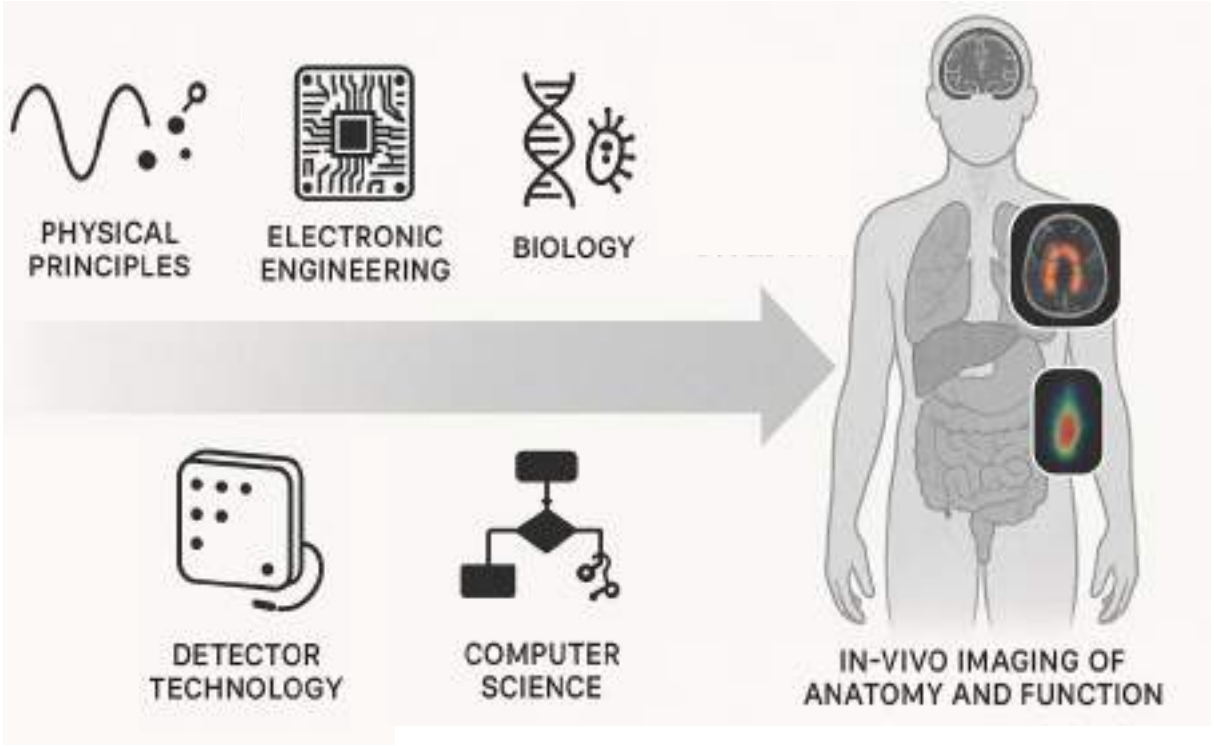
CISUP

Finanziato dall'Unione europea NextGenerationEU

Ministero dell'Università e della Ricerca

What's Biomedical Imaging?

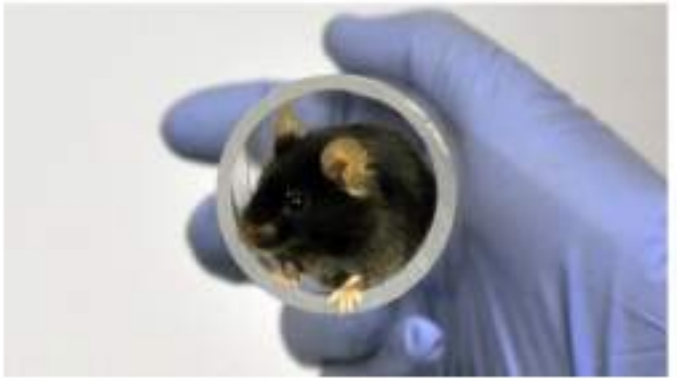


Biomedical imaging *is a key tool for* ***understanding*** *physiological and pathological processes, supporting both preclinical research and personalized medicine.*

Translational Role of Small Animal Imaging

***Animals** are used in scientific research to <u>model</u> what happens in the <u>human body</u>.*





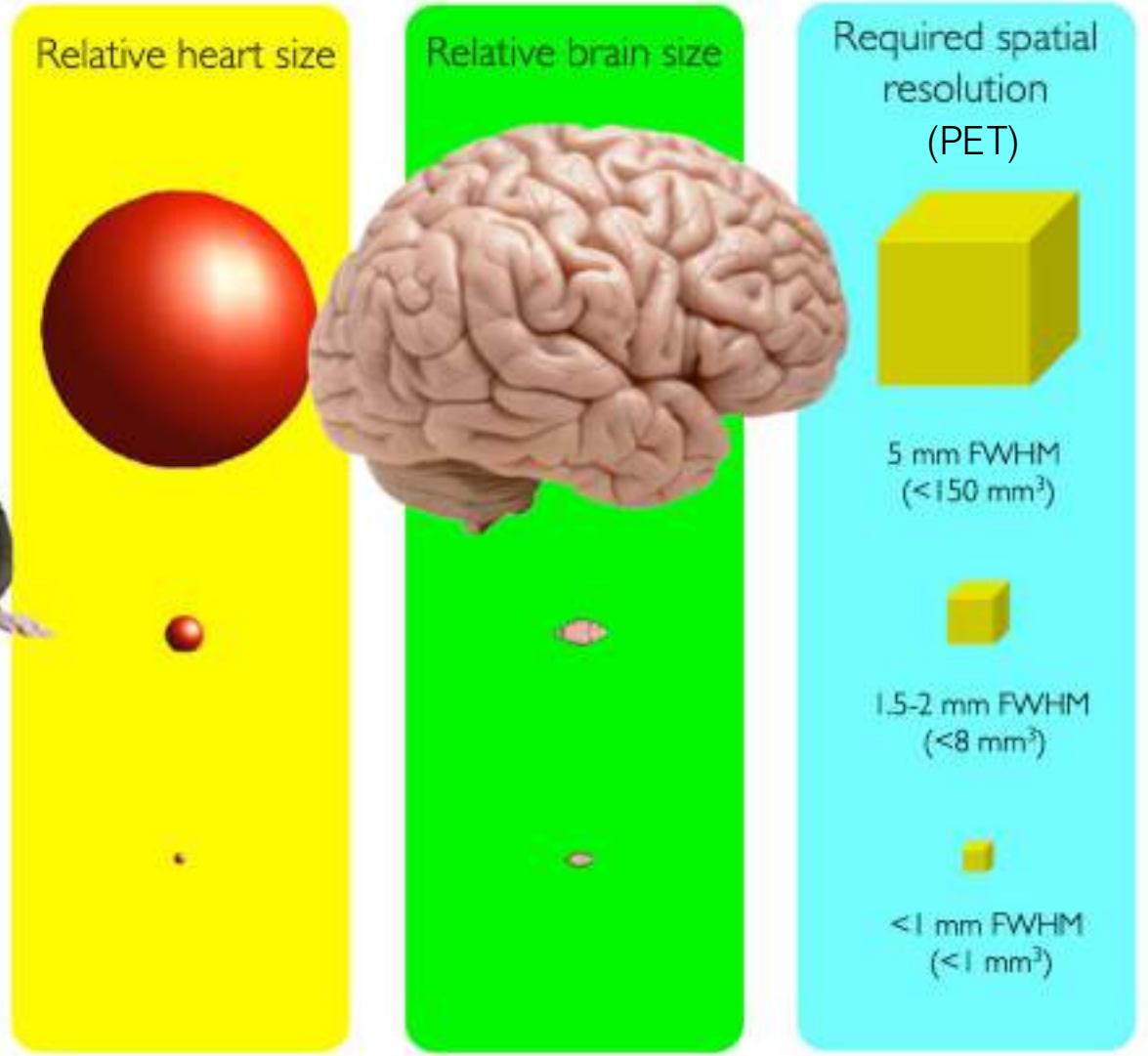
- Animal models are now chosen for their genetic and pathophysiological similarities to human conditions
- Small animal imaging enables real-time, in-vivo investigation of disease mechanisms.
- Supports precision medicine by validating molecular and functional biomarkers that predict human responses.
- Combining omics data, AI-driven image analysis, and computational modeling allows for personalized therapeutic predictions.

Spatial resolution challenges

Human body: ~70 kg
Heart mass: ~300 g
Aorthic cannula Ø: ~30 mm
Brain cortex apex – temporal lobe: ~105 mm

Rat body: ~200 g
Heart mass: ~1 g
Aorthic cannula Ø: 1.5 - 2.2 mm
Brain cortex apex – temporal lobe: ~10 mm

Mouse body: ~20 g
Heart mass: ~0.1 g
Aorthic cannula Ø: 0.9 - 1.3 mm
Brain cortex apex – temporal lobe: ~6 mm



Molecular imaging on small animals

A. PET Imaging on rats using ^{18}F-FDG showing glucose metabolism

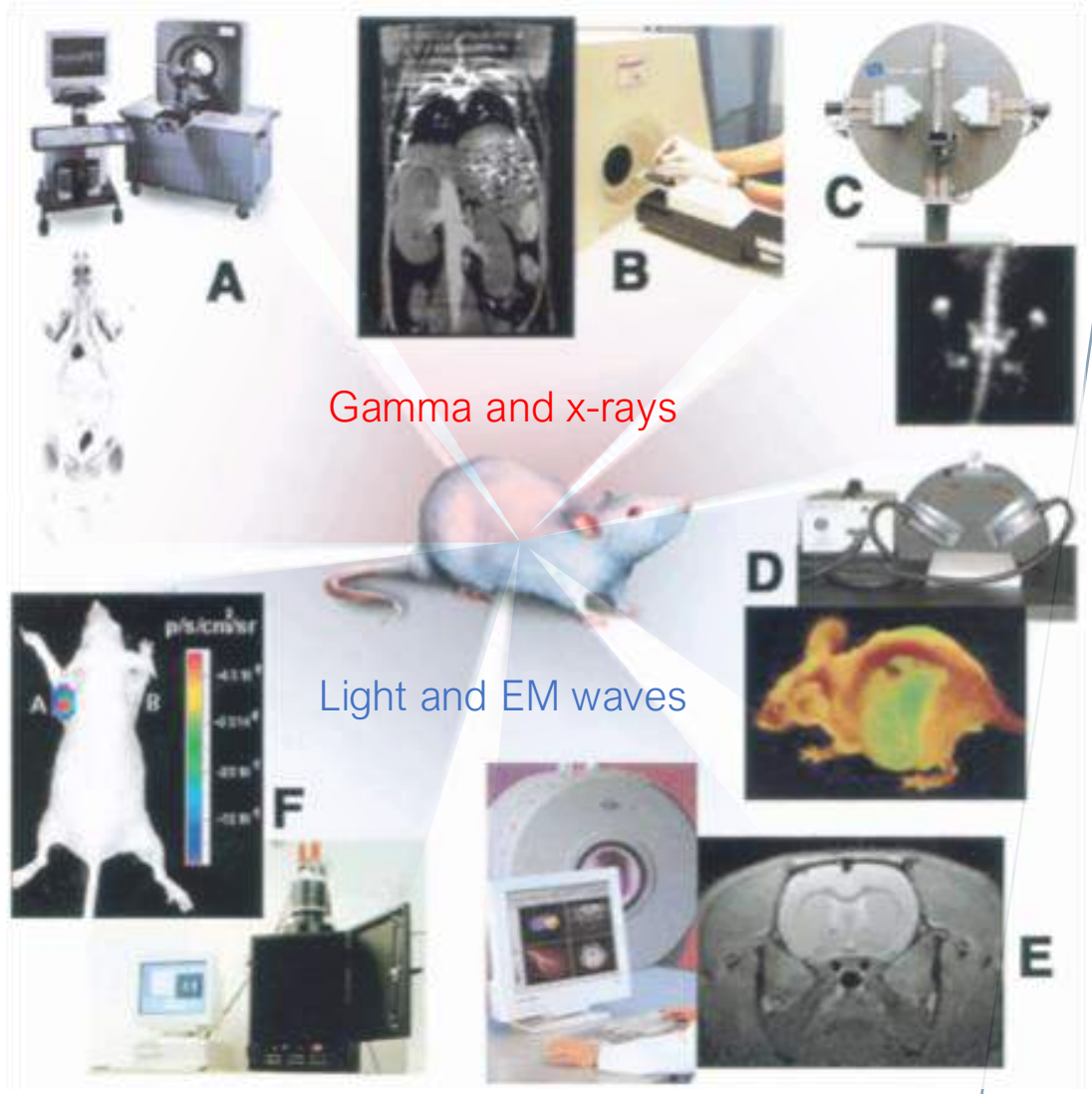
B. CT Imaging of a mouse abdomen after the injection of a contrast agent

C. SPECT Imaging of a mouse abdomen after the injection of ^{99m}Tc "methylene diphosphonate" showing the accumulation of the tracer in bones.

D. Optical Imaging of a mouse showing the fluorescence of GFP from liver, abdomen, spinal cord, and brain due to the presence of cancer cells.

E. MRI image T2-weighted of a mouse brain.

F. Bioluminescence optical imaging of a mouse superimposed to the picture of the animal.



Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Massoud and S.S.Gambhir GENES & DEVELOPMENT 17:545–580, 2003

International context

EURO BIOIMAGING

The European Research Infrastructure for Biological and Biomedical Imaging

We are proud to be part of a Euro-BioImaging Node, providing open access to imaging technologies & expertise

- ✓ *Euro-BioImaging is a distributed research infrastructure with Nodes across Europe*
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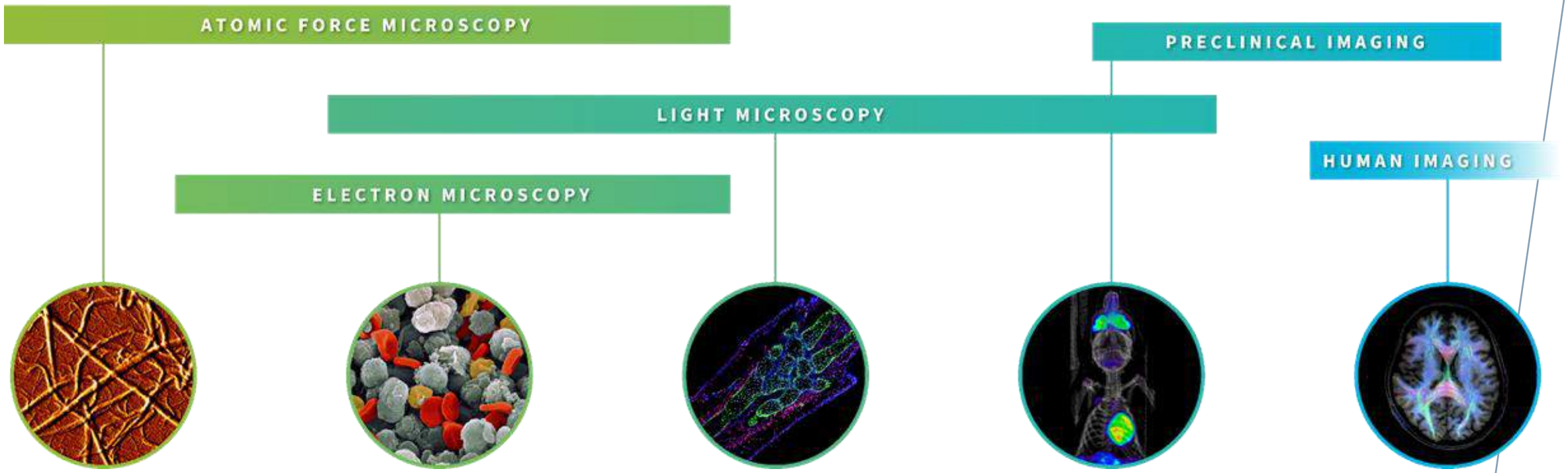


How to get access?

INITIAL CONSULTATION

ACCESS REQUEST

SCIENTIFIC ADVICE

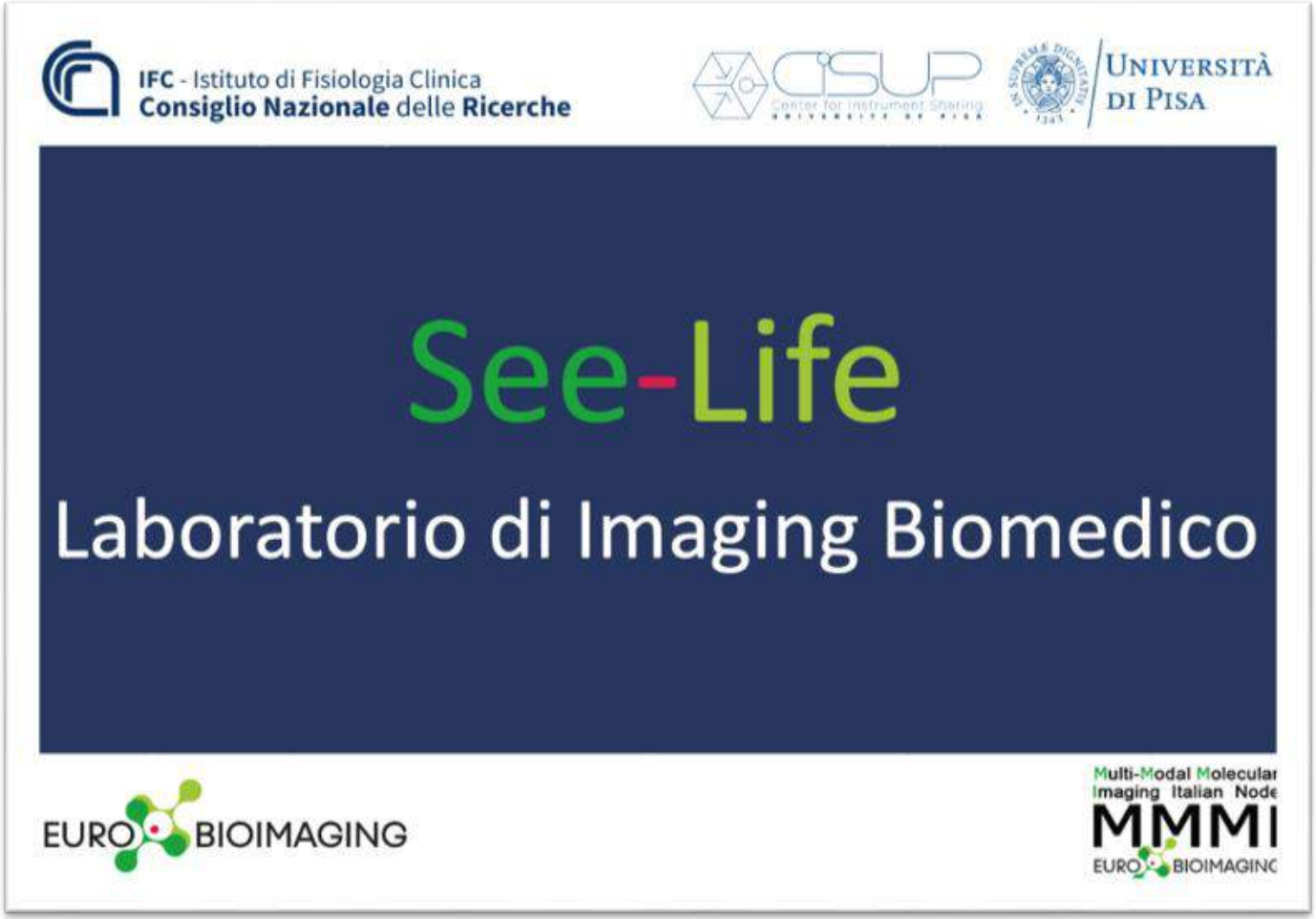
TECHNICAL ADVICE

SERVICE PROVISION

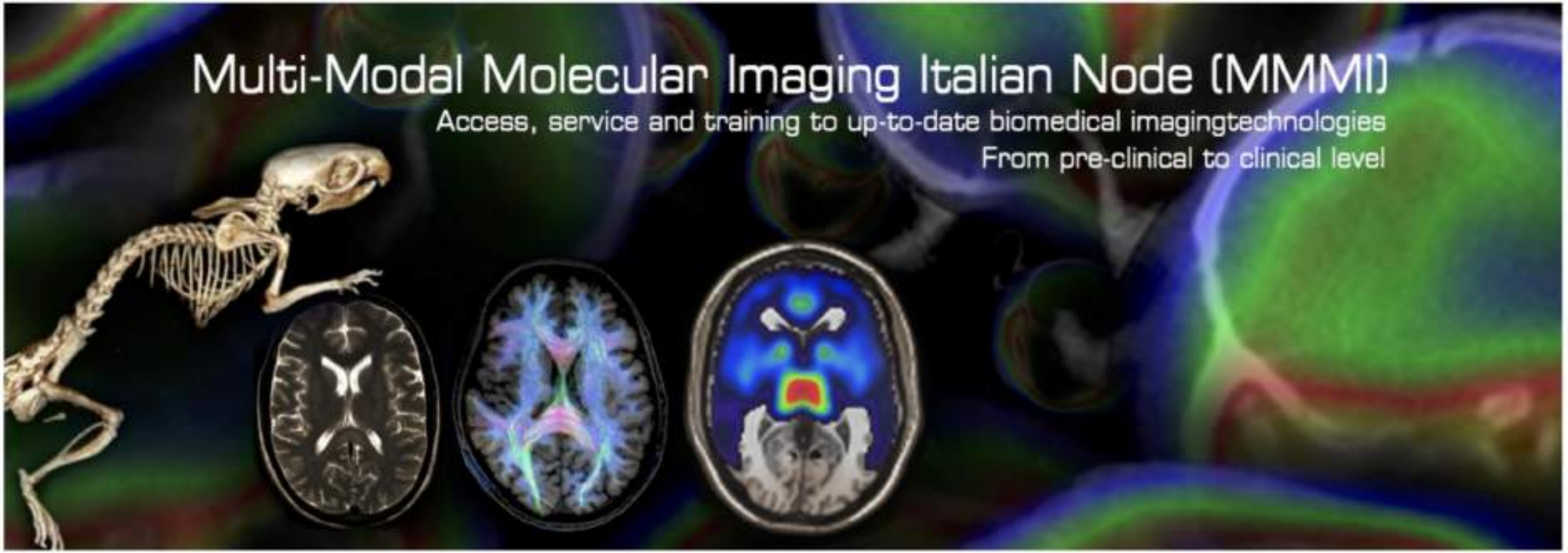
Visit www.eurobioimaging.eu

- ✓ Apply for access to biological and biomedical imaging technologies
- ✓ Apply for access to image data services for analysis, FAIRification and data sharing
- ✓ Find imaging training courses at Euro-BioImaging Nodes
- ✓ Find out about funding opportunities and Euro-BioImaging event
- ✓ Contact us for consultation and advice

The See-Life laboratory



Multi-Modal Molecular Imaging Italian Node



UNIVERSITÀ DI TORINO

IFC - Istituto di Fisiologia Clinica
Consiglio Nazionale delle Ricerche

Fondazione Toscana Gabriele Monasterio

UNIVERSITÀ DI PISA

IBB

OSPEDALE SAN RAFFAELE

IBSBC

SYNLAB IRCCS SDN

BCU BIO CHECKUP
Innovative Diagnostic Imaging

UNIVERSITÀ DI PISA

The early days of μ(S)PET imaging - the YAP-(S)PET

The YAP-(S)PET with 4 rotating detectors

First imaging procedure at IFC-CNR

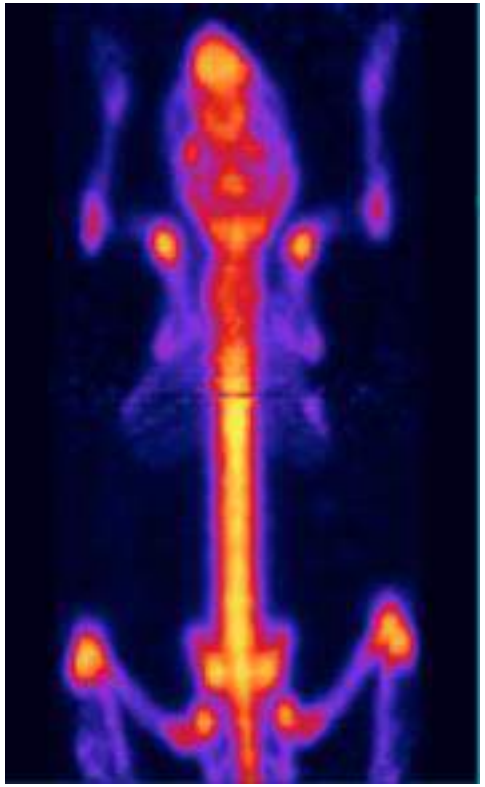
The early days of μ(S)PET imaging - the YAP-(S)PET

Commercial version by I.S.E. s.r.l. installed at the HSR Milan

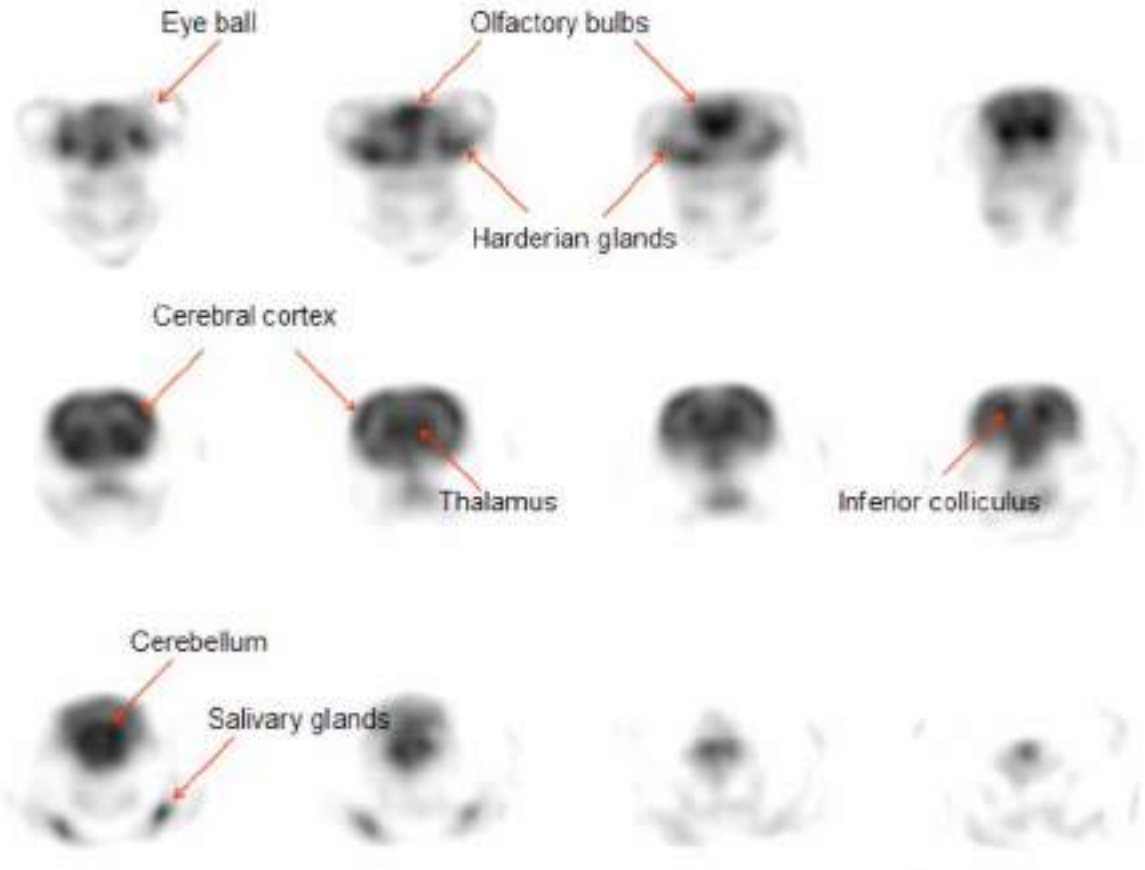
First imaging procedure at IFC-CNR

The early days of μ(S)PET imaging - the YAP-(S)PET

The YAP-(S)PET with 4 rotating detectors



$^{18}F^-$ imaging in mouse

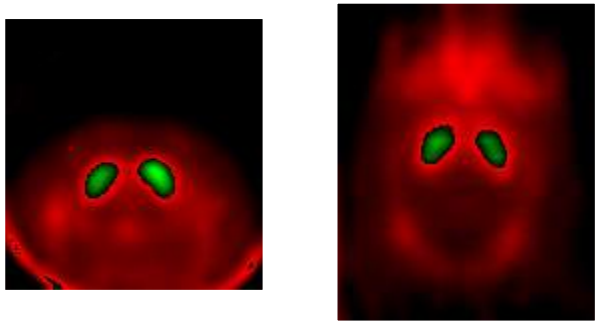


^{18}F-FDG imaging in rat brain

Case study – drug development

- Normal rats were compared with rats with receptor blocking by monitoring the activity in the striatum, using a high-affinity dopamine D2 receptor ligand ^{18}F-Fallypride

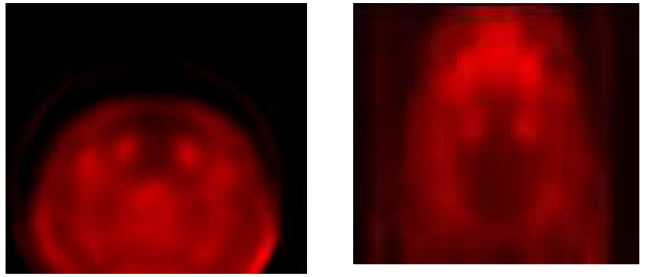
Normal Rat



Transaxial section

Horizontal section

Rat treated with receptor blocking (amisulpride)



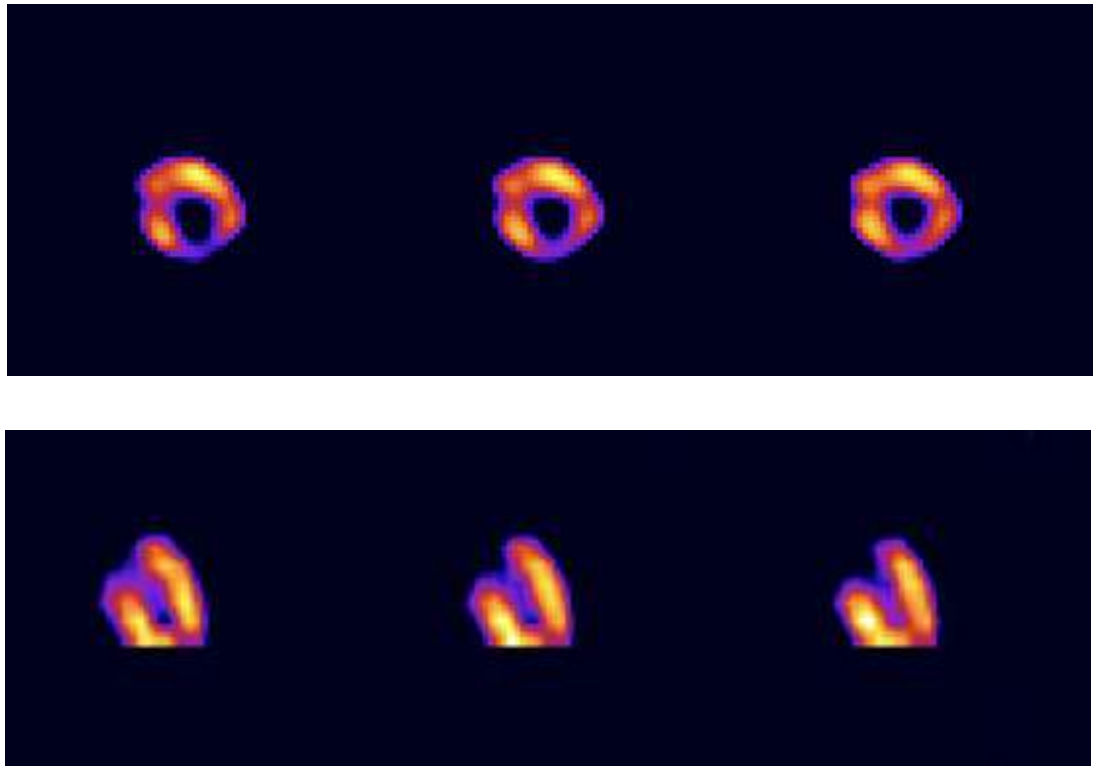
Transaxial section

Horizontal section

- Blocking dopamine receptors can help reduce overactive dopamine signaling, which is thought to contribute to psychotic symptoms.

The early days of μ(S)PET imaging - the YAP-(S)PET

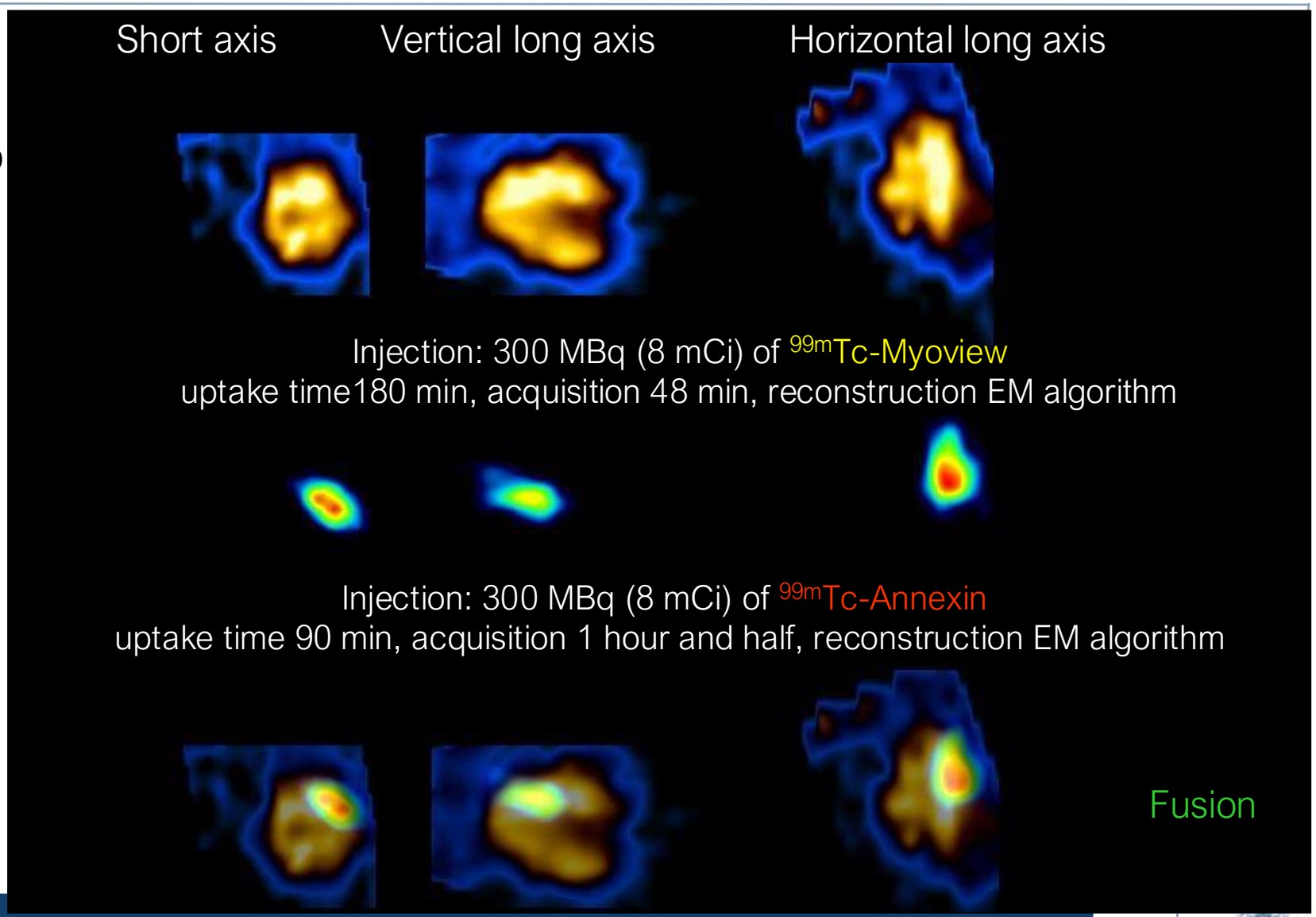
The YAP-(S)PET with 4 rotating detectors



^{99m}Tc-Myoview in rat heart

Case study – Ischemia and reperfusion

- SPECT image of a rat model of heart ischemia and following reperfusion to evaluate the damage.
- Myoview trace the heart perfusion
- Annexin trace the cellular apoptosis

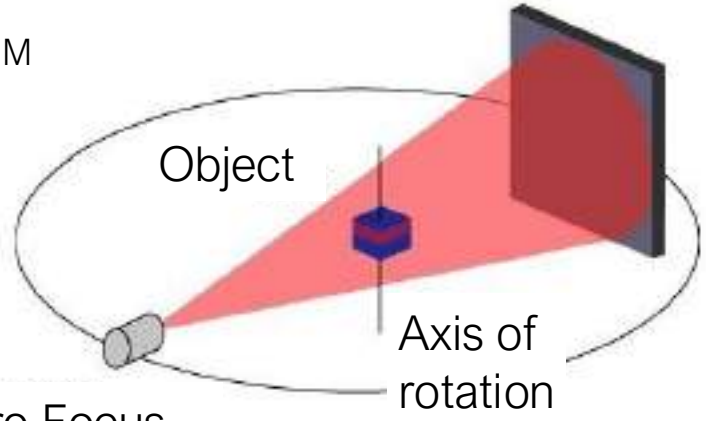


CT imaging to add morphology to function

- PET and SPECT imaging lack morphological information
- CT imaging provides direct 3D estimation of attenuation coefficients
- The typical micro-CT geometry is the “cone-beam”

X-ray source
- Fixed tungsten anode
- Maximum voltage: 60 kV
- Maximum power: 10 W
- Measured focus size: 7 μm FWHM
- Beam aperture: 32°

Micro Focus x-ray source

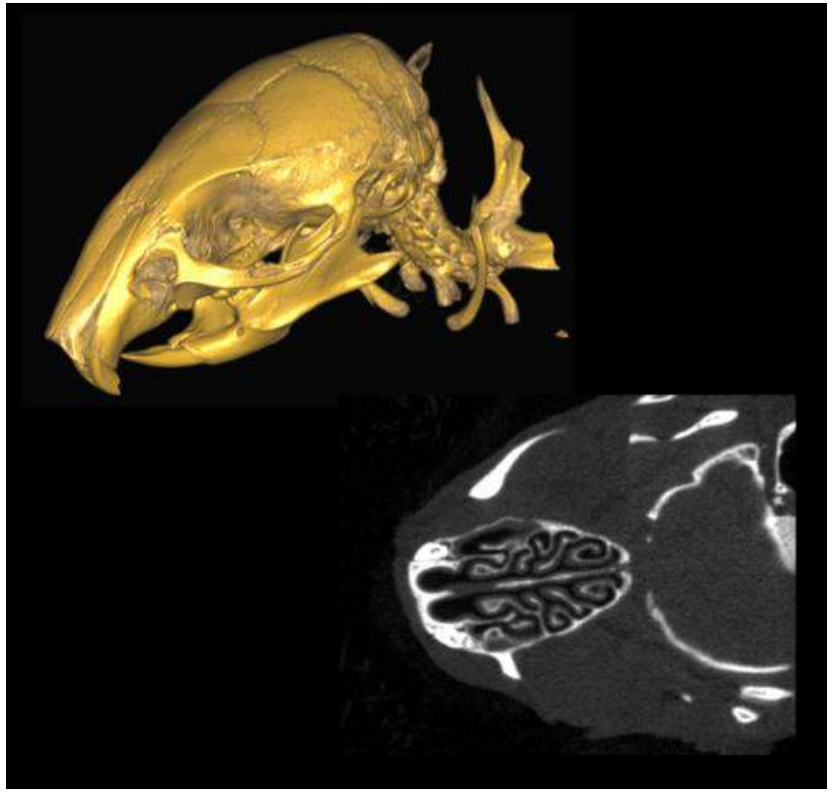


Flat panel detector

X-ray detector
- 1024 x 2048 pixels (48 μm each)
- 5 cm x 10 cm active area
- GADOX scintillator
- Maximum frame rate 2.7 fps

Micro X-ray imaging - The XaltHR

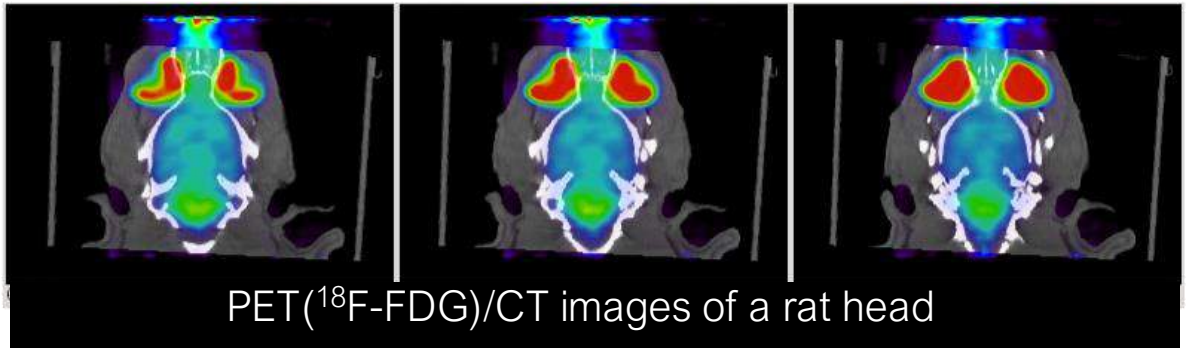
Micro X-ray imaging - The XaltHR



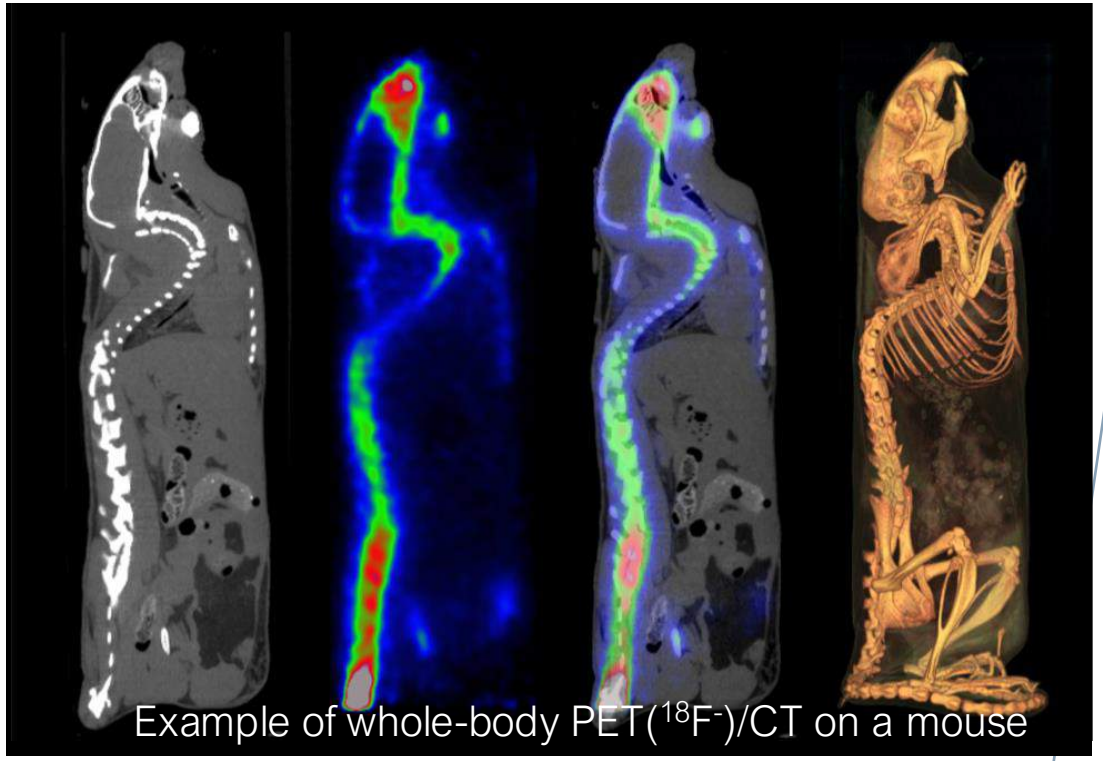
In-vivo mouse head

Image courtesy:
Daniele Panetta
IFC-CNR, Pisa, Italy

First PET/CT images

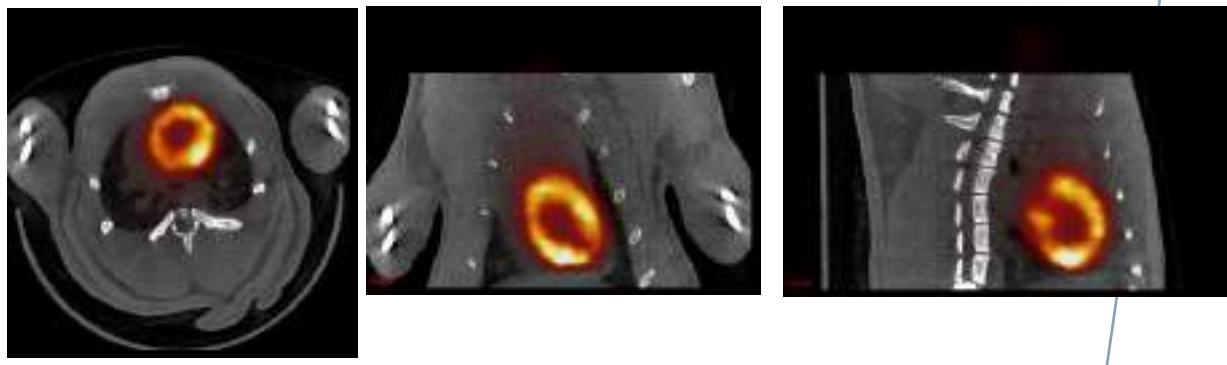


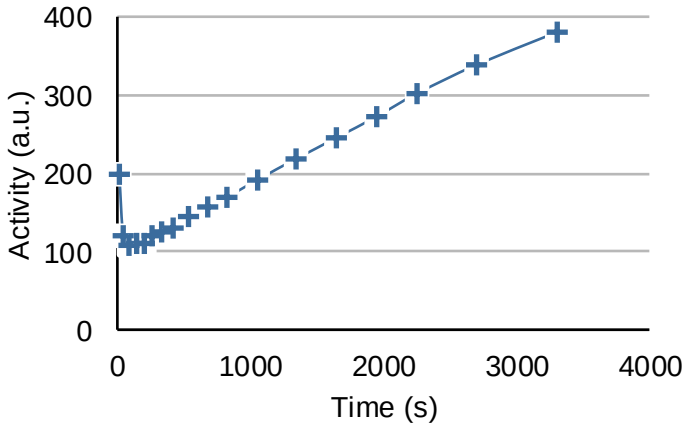
PET(^{18}F-FDG)/CT images of a rat head



Example of whole-body PET(^{18}F^{-})/CT on a mouse

First PET/CT images

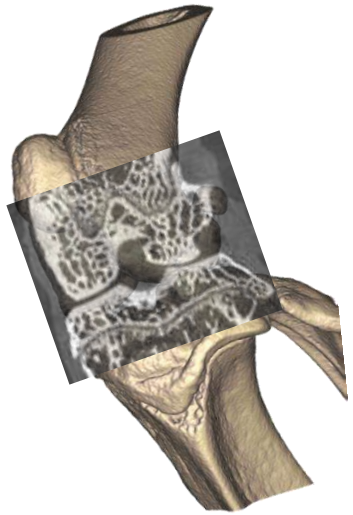




Dynamic study of FDG uptake in the rat heart

Micro X-ray imaging - The XaltHR

Atherosclerosis/
Vascular imaging



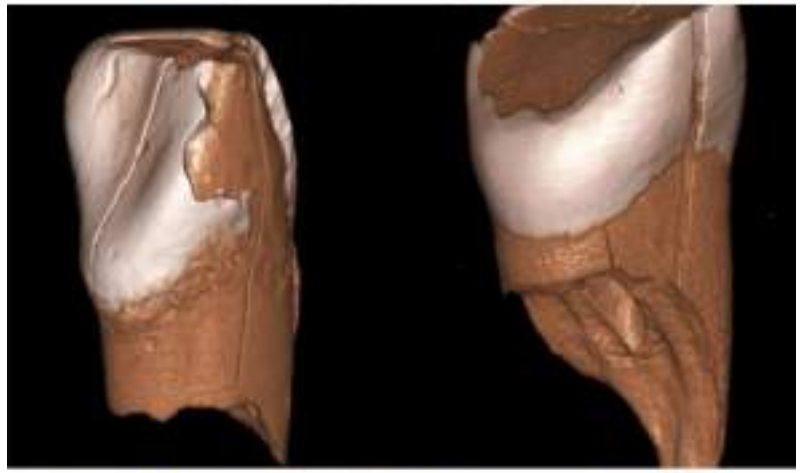
Regenerative
medicine/Osteoporosis

Small fossils
Non-destructive testing
(NDT)

Focus

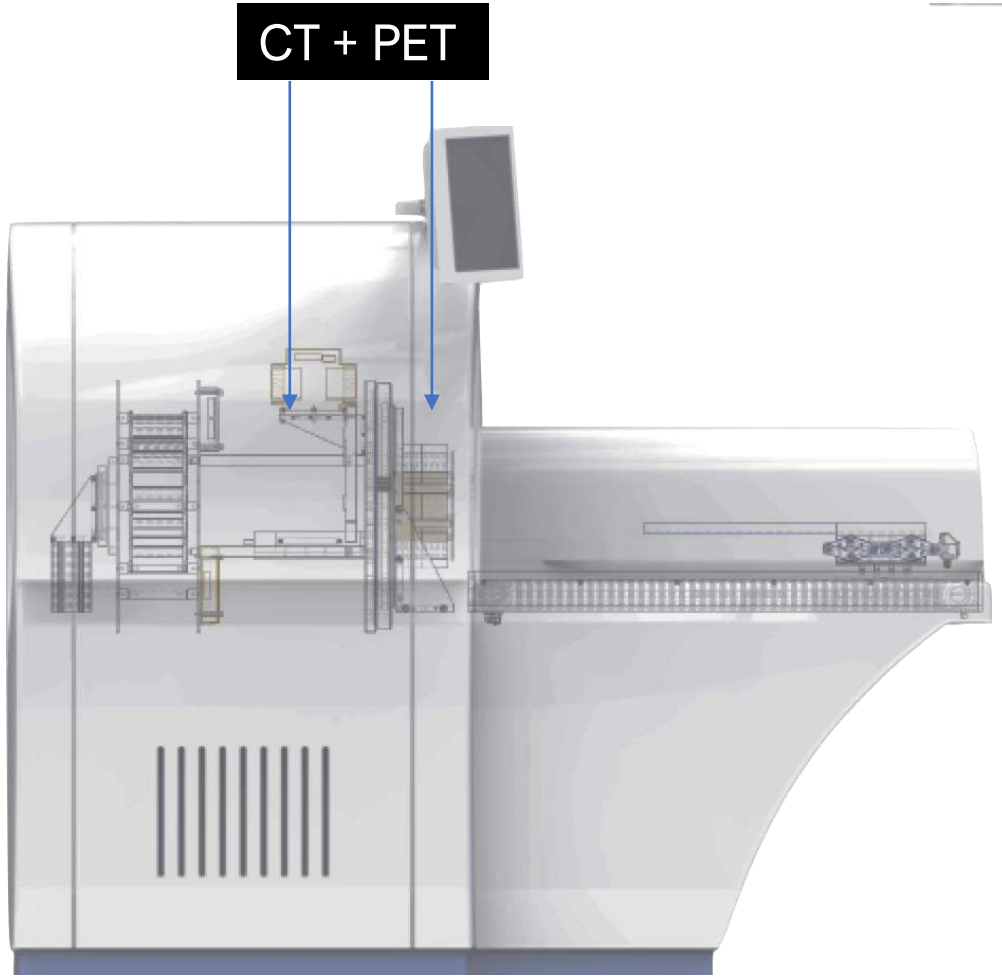
Storia Nuovi indizi sulla fine dei Neanderthal

Due denti da latte rinvenuti in Liguria e in Veneto apparterrebbero ai primi Sapiens giunti in Europa 42 mila anni fa. Una civiltà che, con le sue innovazioni tecnologiche, avrebbe contribuito alla scomparsa dei Neanderthal.



Le copie digitali tridimensionali degli incisivi studiati. A sinistra, quello di Riparo Bombrini; a destra, quello di Grotta di Fumane. Daniele Ronetta, Istituto di fisiologia clinica del CNR di Pisa

A fully integrated PET/CT - The Inviscan IRIS



A fully integrated PET/CT - The Inviscan IRIS

inviscan
Imaging systems

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ULTIMATE PRECLINICAL PET/CT IMAGING

PET/CT for mouse and rat

PET/CT with large bore size

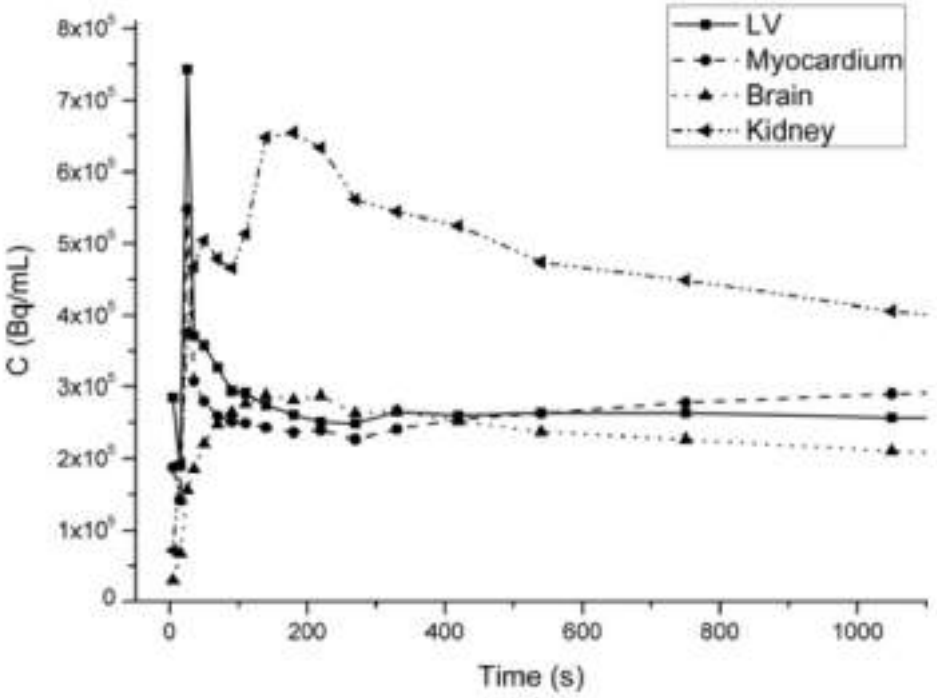
https://inviscan.fr/index.html

A fully integrated PET/CT - The Inviscan IRIS

3.7 MBq of ^{18}F-FDG Dynamic scan

Image taken with the Inviscan IRIS PET/CT

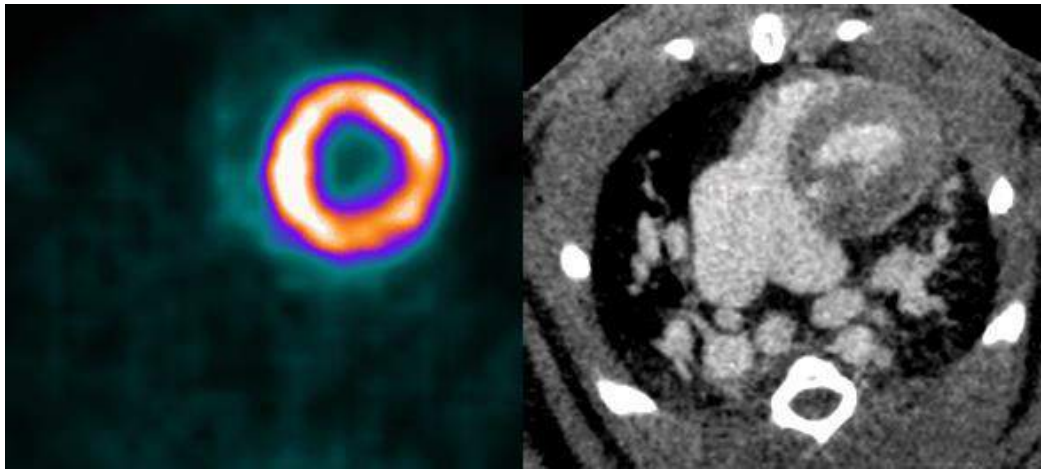
A fully integrated PET/CT - The Inviscan IRIS

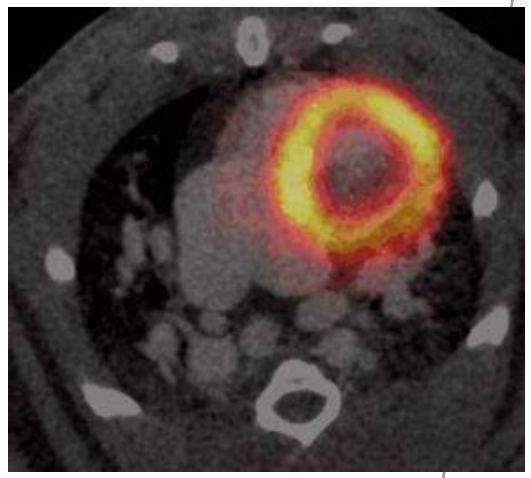


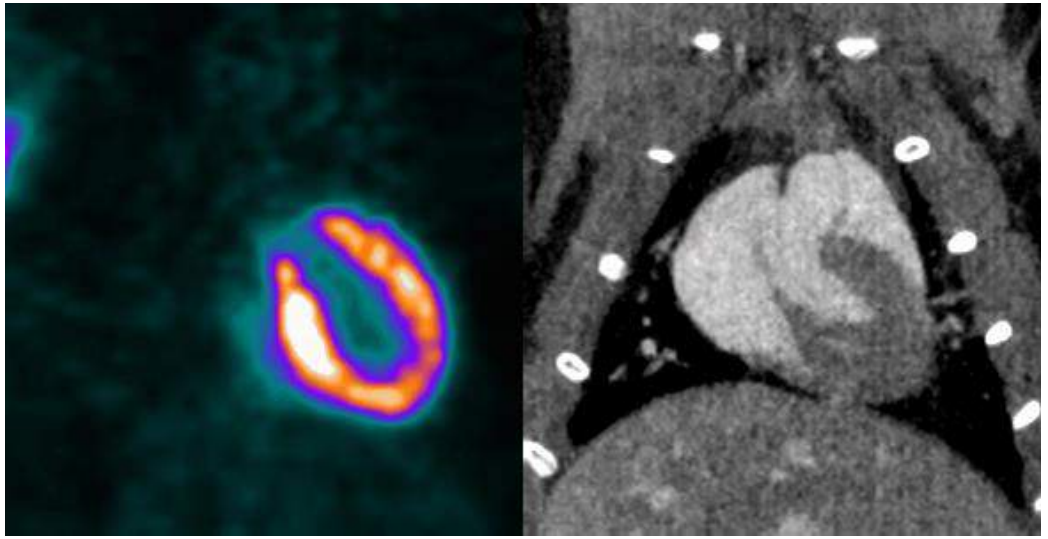
3.7 MBq of ^{18}F-FDG Dynamic scan
(15 s minimum reconstructed time frame)
Image taken with the Inviscan IRIS PET/CT

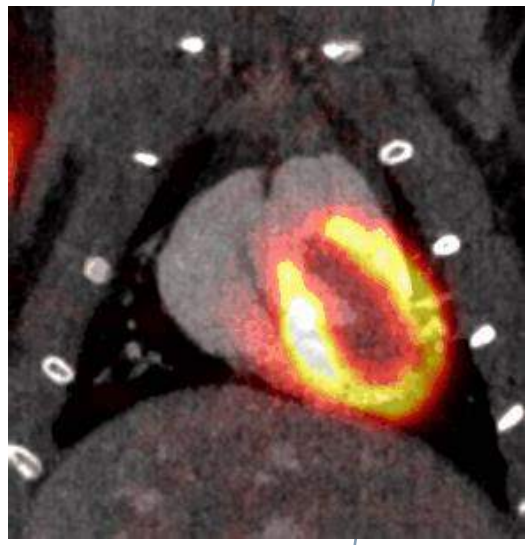
A fully integrated PET/CT - The Inviscan IRIS

Image courtesy:
Daniele Panetta
IFC-CNR, Pisa, Italy

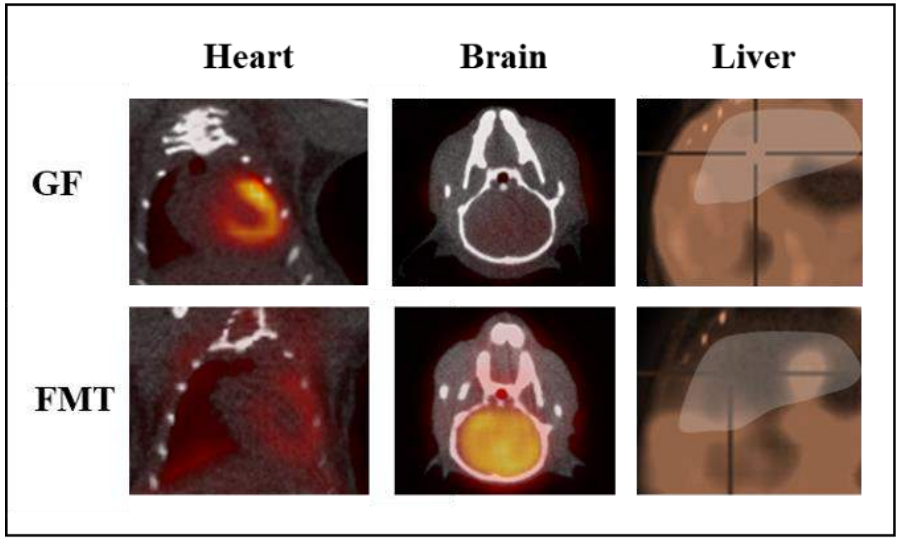


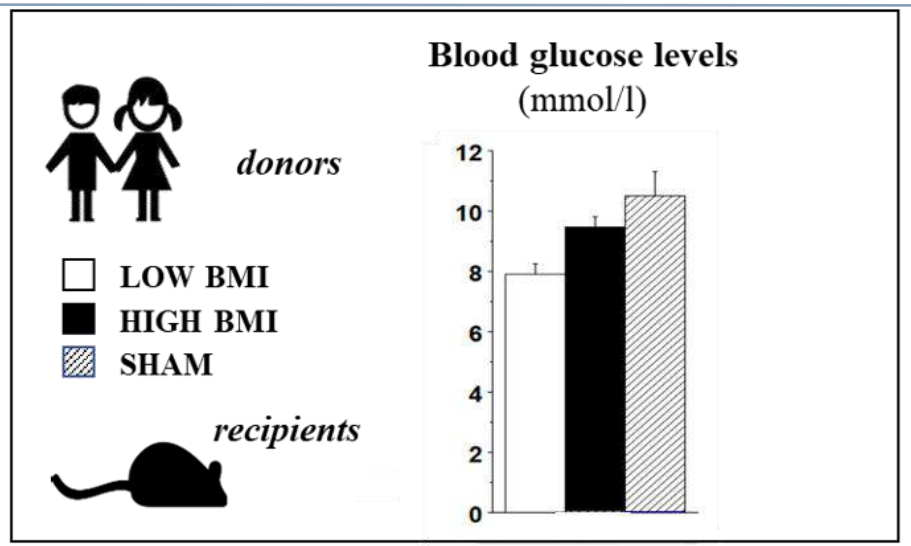


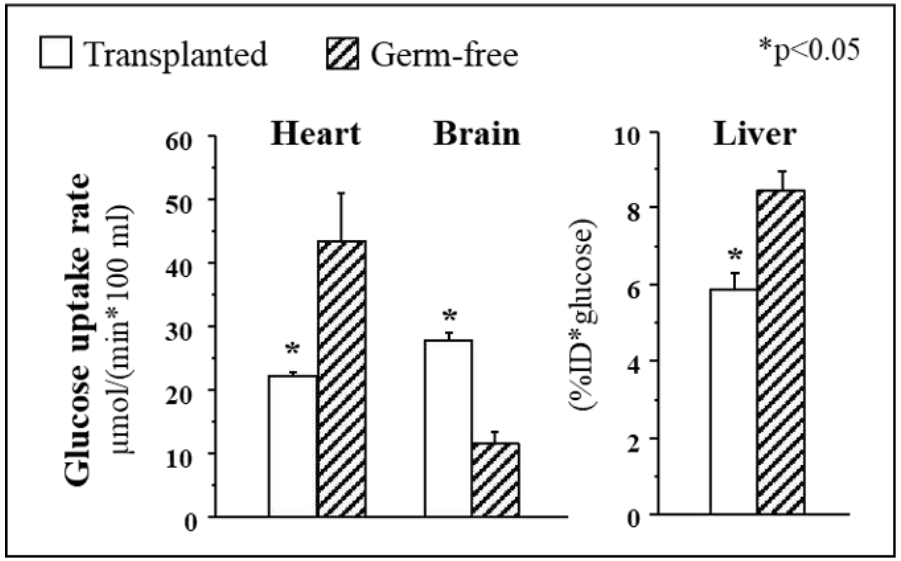


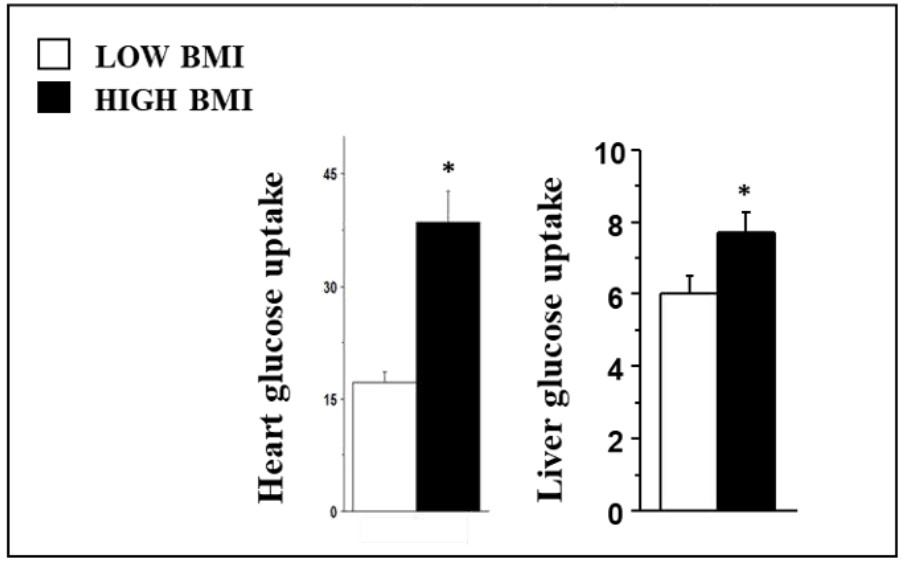


Long-lasting effects of human microbiota in vivo in the surrogate host









Data courtesy:
Patricia Iozzo
IFC-CNR, Pisa, Italy

CNR IFC RADIO

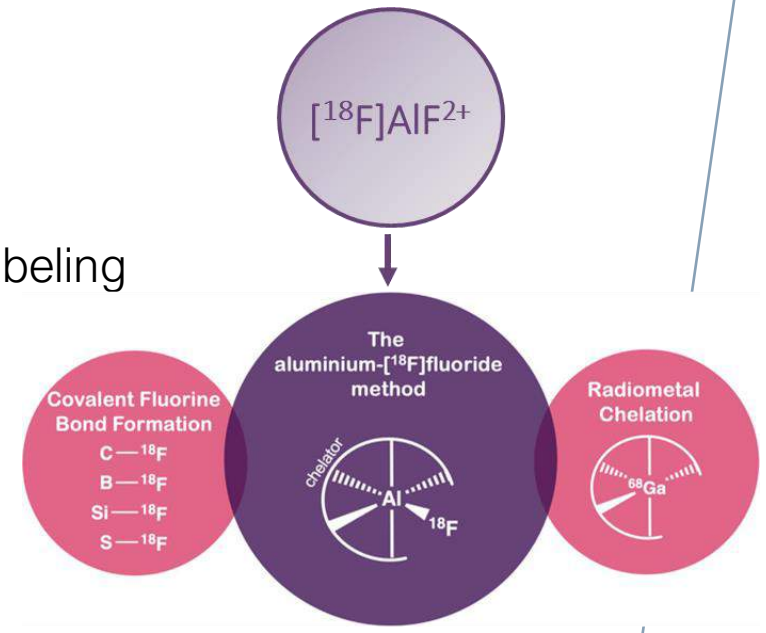
IFC - Istituto di Fisiologia Clinica
Consiglio Nazionale delle Ricerche

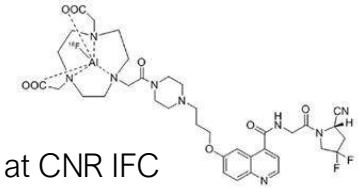
- Key Expertise for Radiochemical Development for PET and Theranostic Radiopharmaceuticals in Metabolic and Oncological Applications

16 MeV biomedical cyclotron at CNR IFC

Synthesis and characterisation of new Radiotracers

- Radionuclide Production with Biomedical Cyclotron
- Labeling Chemistry
- Selection of chelators or prosthetic groups for molecular labeling
- Development of radiopharmaceuticals targeting metabolic pathways or tumor-specific biomarkers





Example of labelling of radiopharmaceuticals at CNR IFC

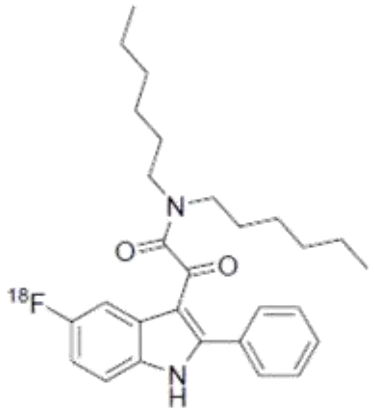
Università di Pisa

Development of non-standard PET tracers

- Development of non-standard PET tracers targeting emerging biological pathways—such as:
  - TSPO
  - TAAR1
  - GLP-1R
  - SGLT transporters
  - PD-1/PD-L1
  - selective kinase mutations

Development of non-standard PET tracers

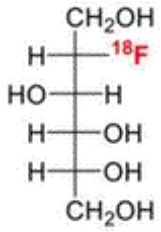
- Development of non-standard PET tracers targeting emerging biological pathways—such as:
  - TSPO (Neuroinflammation)
  - TAAR1 (Neurotrasmission)
  - GLP-1R (Neuroprotection)
  - SGLT transporters
  - PD-1/PD-L1
  - selective kinase mutations



[^{18}F]2-(5-fluoro-2-fenil-1H-indol-3-il)-2-osso-N,N-dietilacetamide

- Ligands for such novel targets facilitate real-time, non-invasive assessments of pharmacodynamics and target engagement, which are critical for both drug development and clinical decision-making.

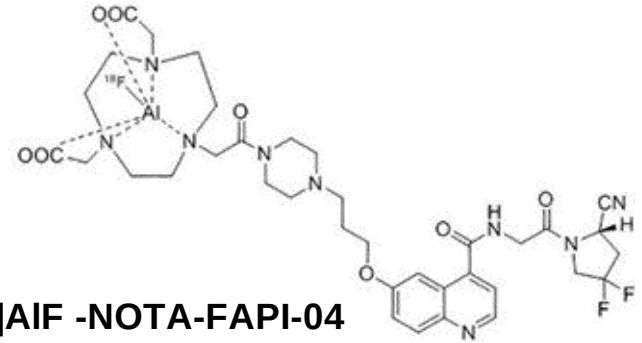
Development of non-standard PET tracers



[^{18}F]2-fluoro-2-deoxy-sorbitol ([^{18}F]FDS)

Mapping the permeability of the BBB

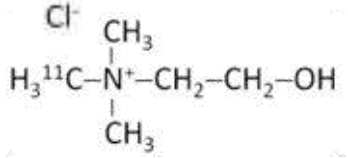
^{18}F



[^{18}F]AlF -NOTA-FAPI-04

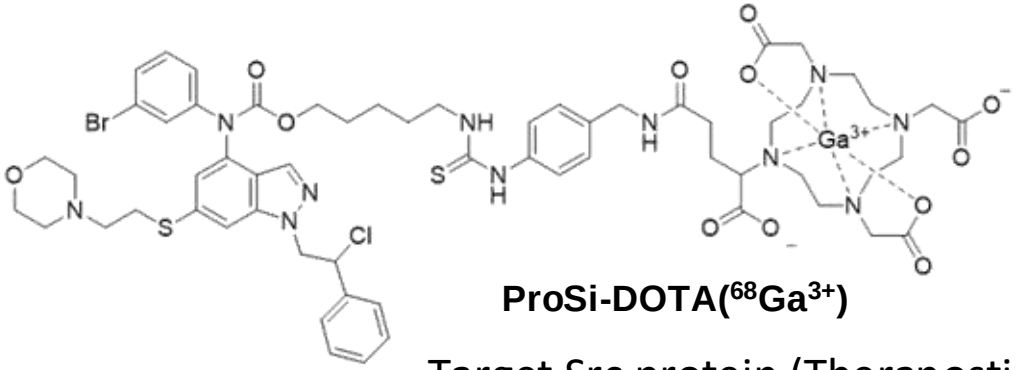
Expressed in the tumour microenvironment (Glioblastoma)

^{11}C



[^{11}C]choline chloride

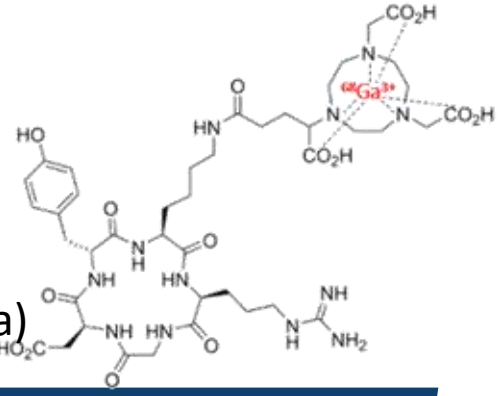
Metabolism of Phospholipids



ProSi-DOTA(^{68}Ga^{3+})

Target Src protein (Theranostic)

^{68}Ga

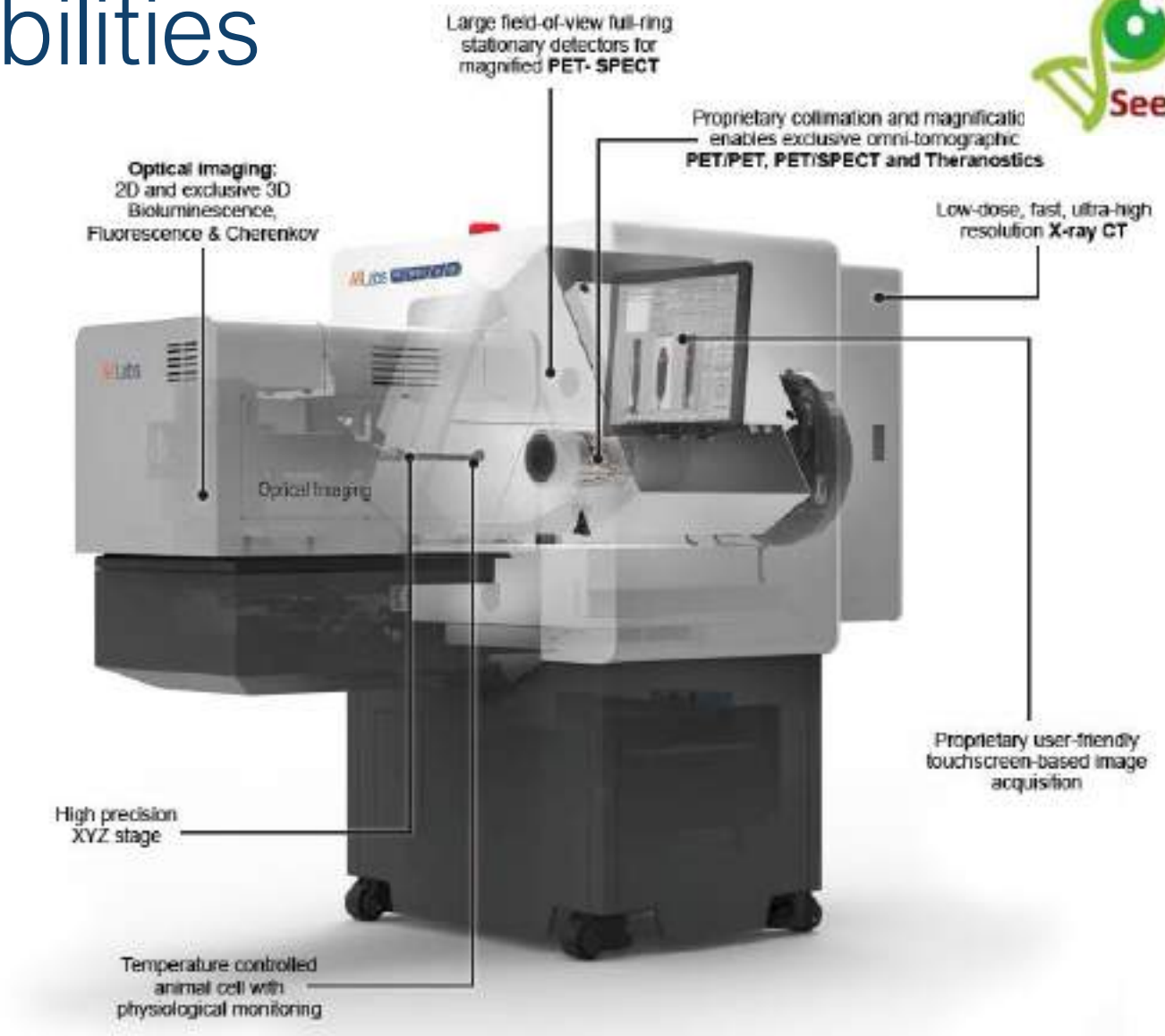


^{68}Ga-NODAGA-RGD

Angiogenesis (Stroke and glioblastoma)

Expanding the possibilities

- MiLabs SPECT/CT/OI
  - Stationary multi-pinhole SPECT
  - Variable magnification microCT
  - Optical imaging
    - 2D fluorescence/bioluminescence/ cherenkov
    - 3D FLT/BLT



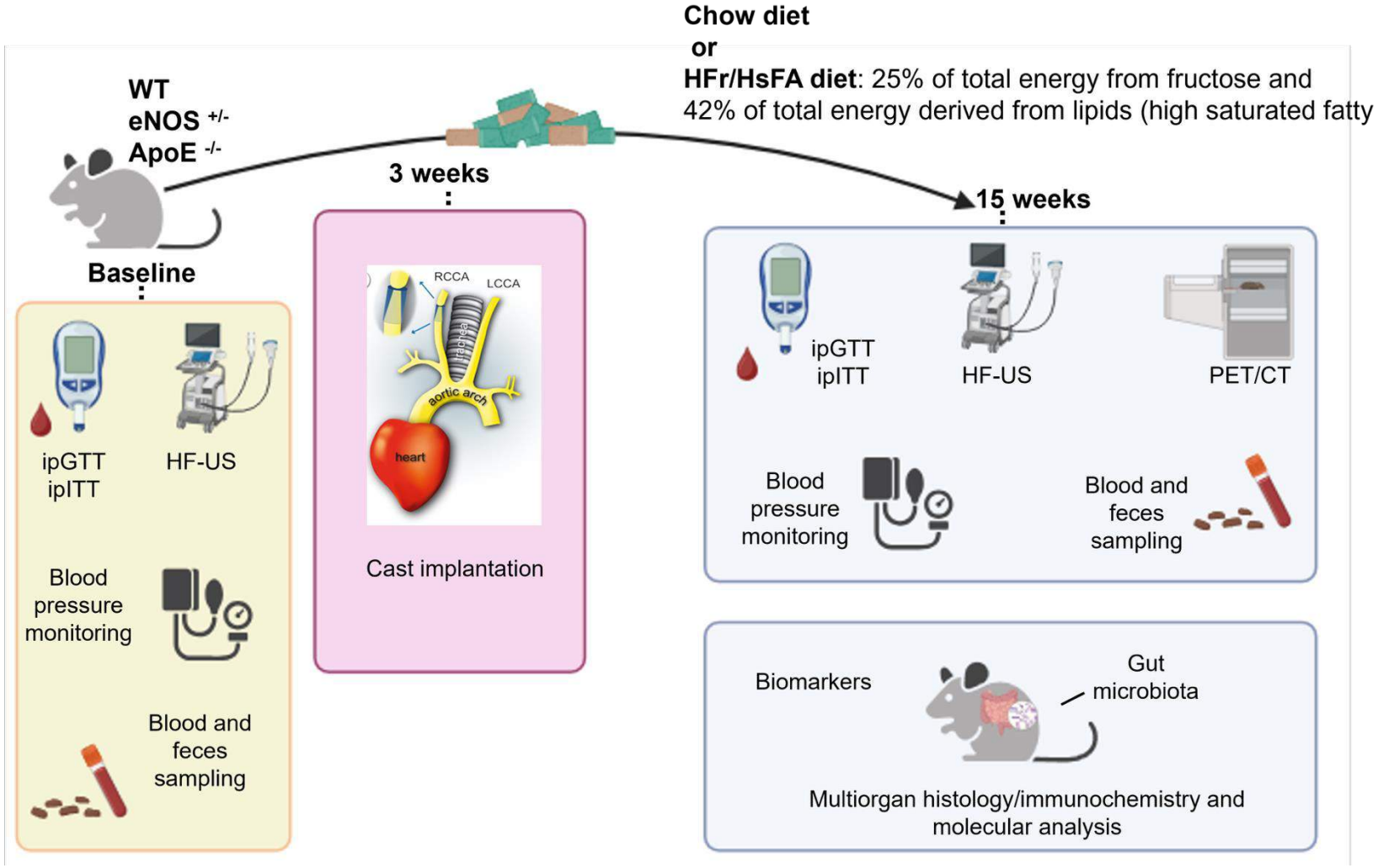
Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani
Università di Pisa

Installation at Laboratorio di Imaging Biomedico

2D optical imaging system on the docking station

SPECT/CT and 3D optical imaging system

High resolution carotid plaque imaging in murine models of atherosclerosis

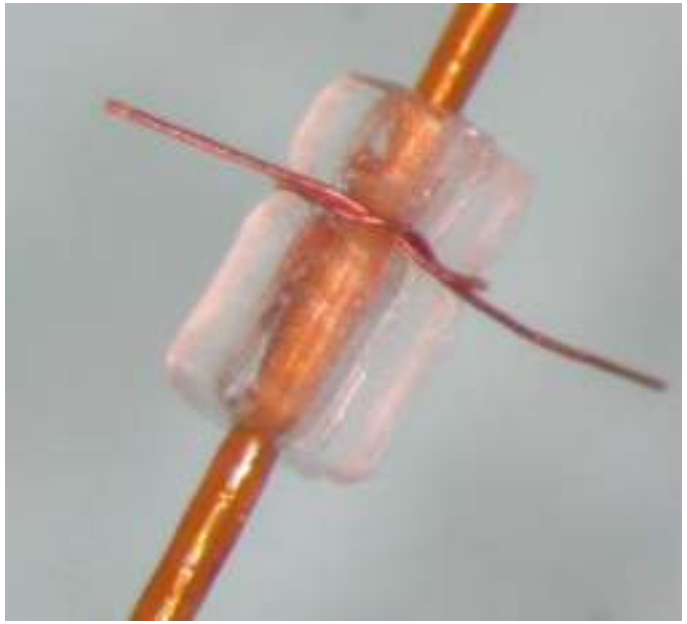


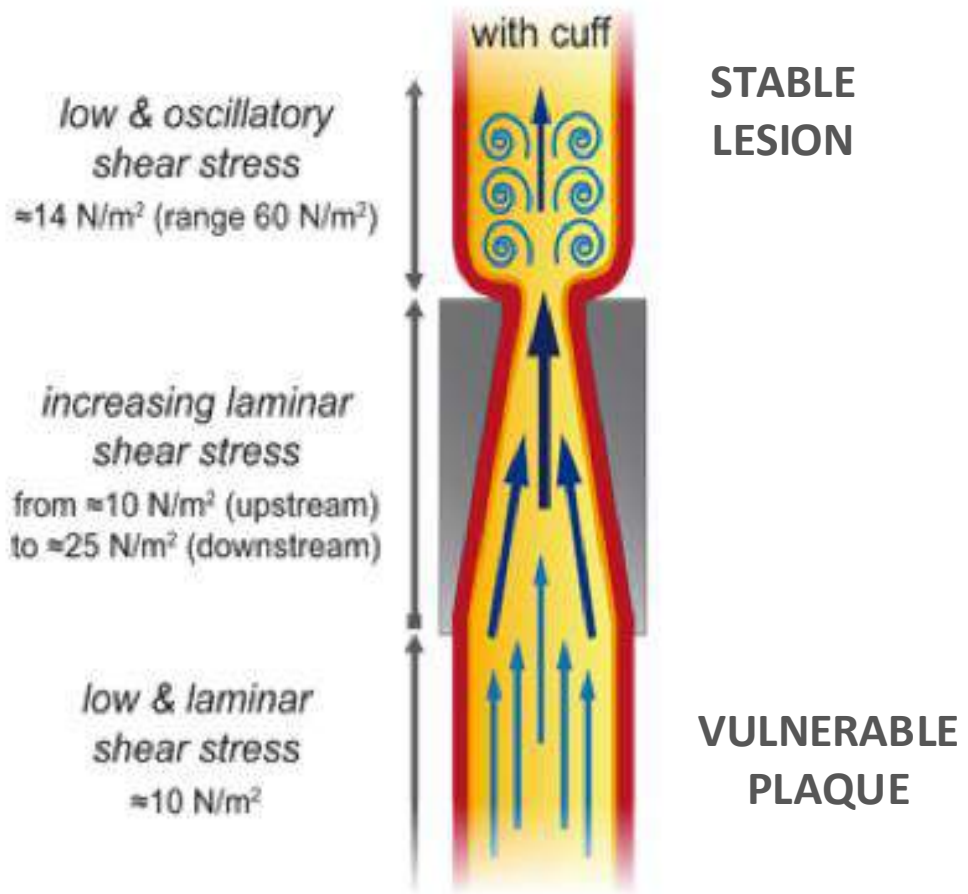
TNA Approved Application

Courtesy, Dott.ssa Claudia Kusmic CNR-IFC. PRIN 2022WBSR95 -Authorization number 527/2024-PR

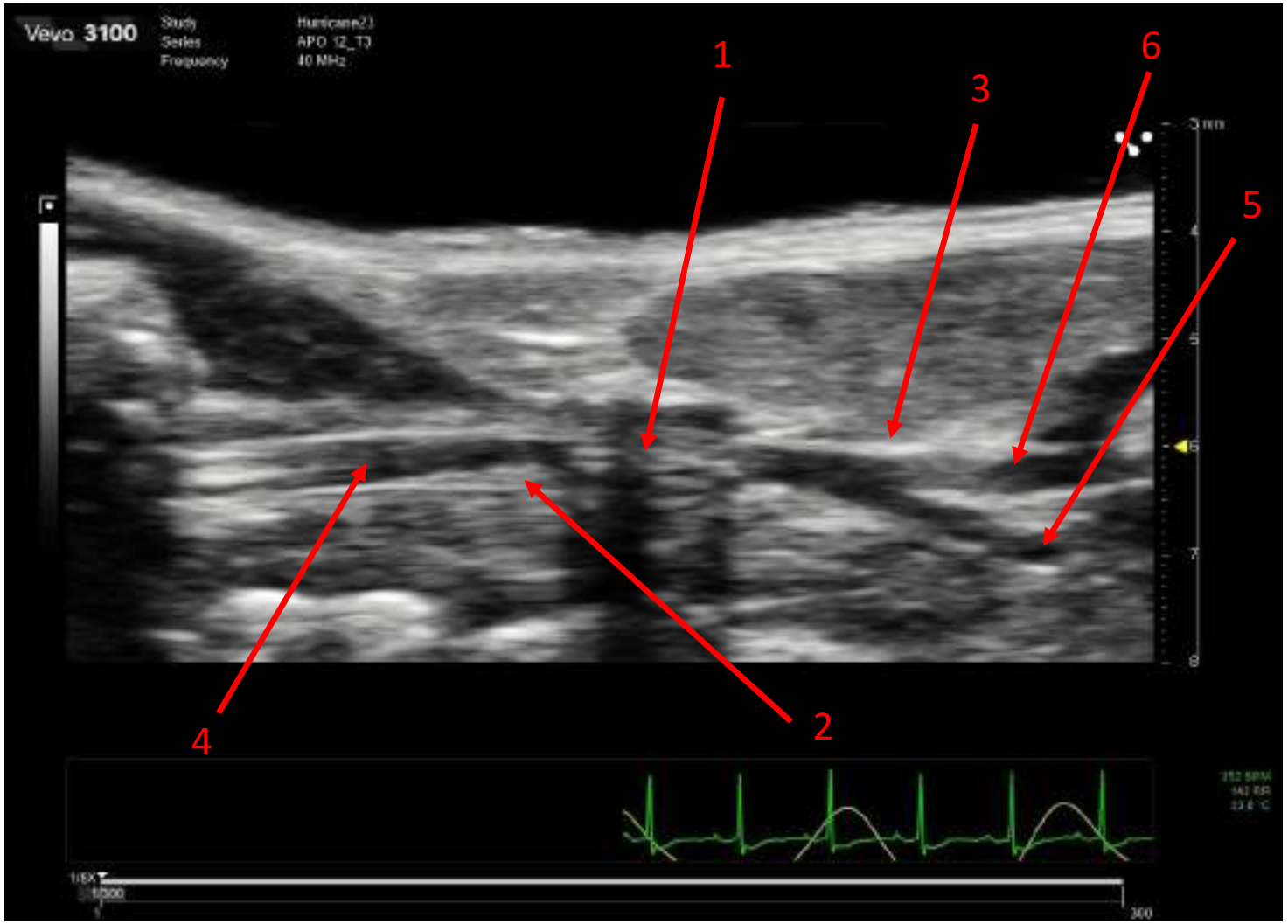
Clinical protocol

- Specific murine model characterized by a controlled development of carotid atherosclerotic plaques (genotype + diet + flow modifier)





Ecography images

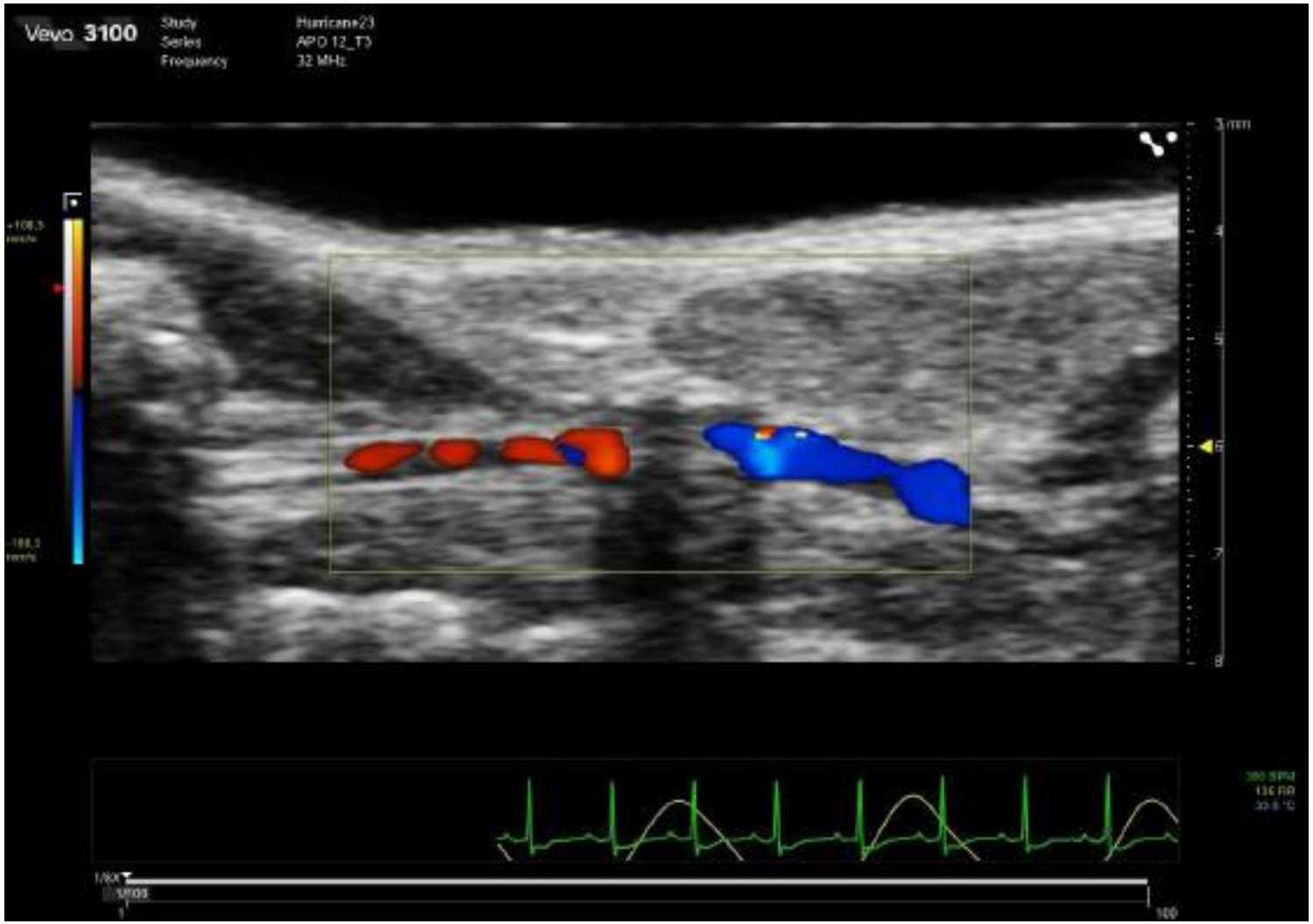


Longitudinal section of right common carotid artery B-mode ultrasound mode (morphological)

1) Constrictor
2) Plaque
3) Bulb
4) Common carotid artery
5) Internal carotid artery
6) External carotid artery

The presence of plaque on the far wall in the area proximal (and adjacent) to the hemodynamic constrictor can be appreciated. According to the theory underlying the hemodynamic model, plaque should be vulnerable in nature.

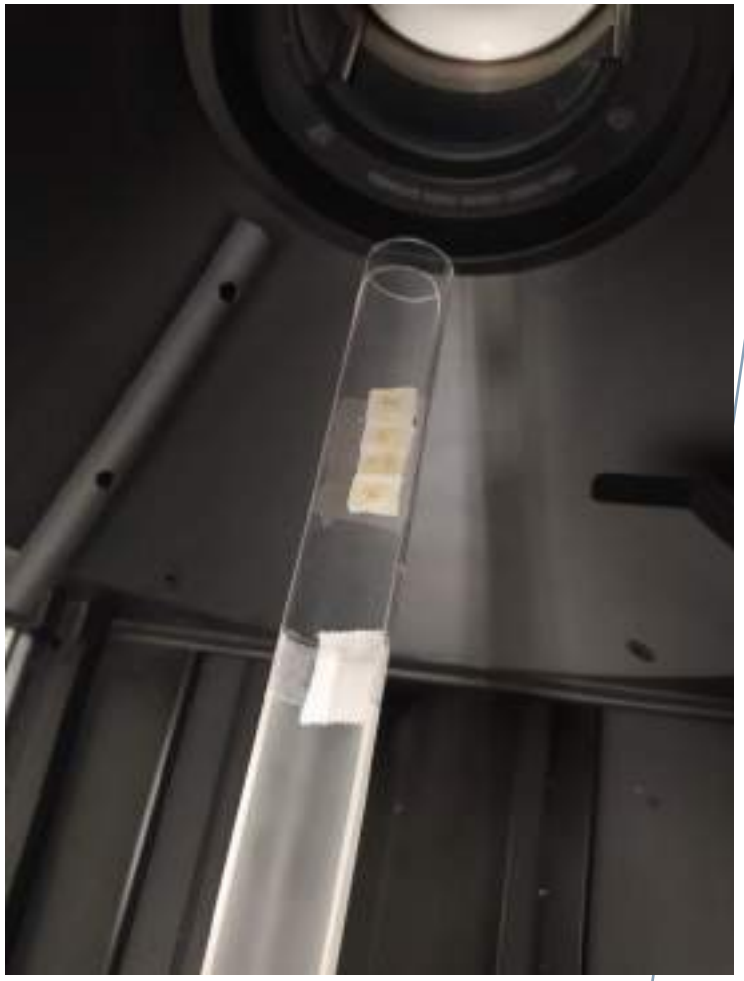
Ecography images



- Longitudinal section of right common carotid artery
- Color-mode ultrasound mode (flows)
- It can be appreciated how the presence of plaque on the far wall in the area proximal (and adjacent) to the hemodynamic constrictor induces alterations in laminar blood flow, with a deviation of direction with respect to the axis of the vessel and partial inversion, as documented by the presence of a blue signal superimposed on the red one at the plaque (particularly in the systolic phase).

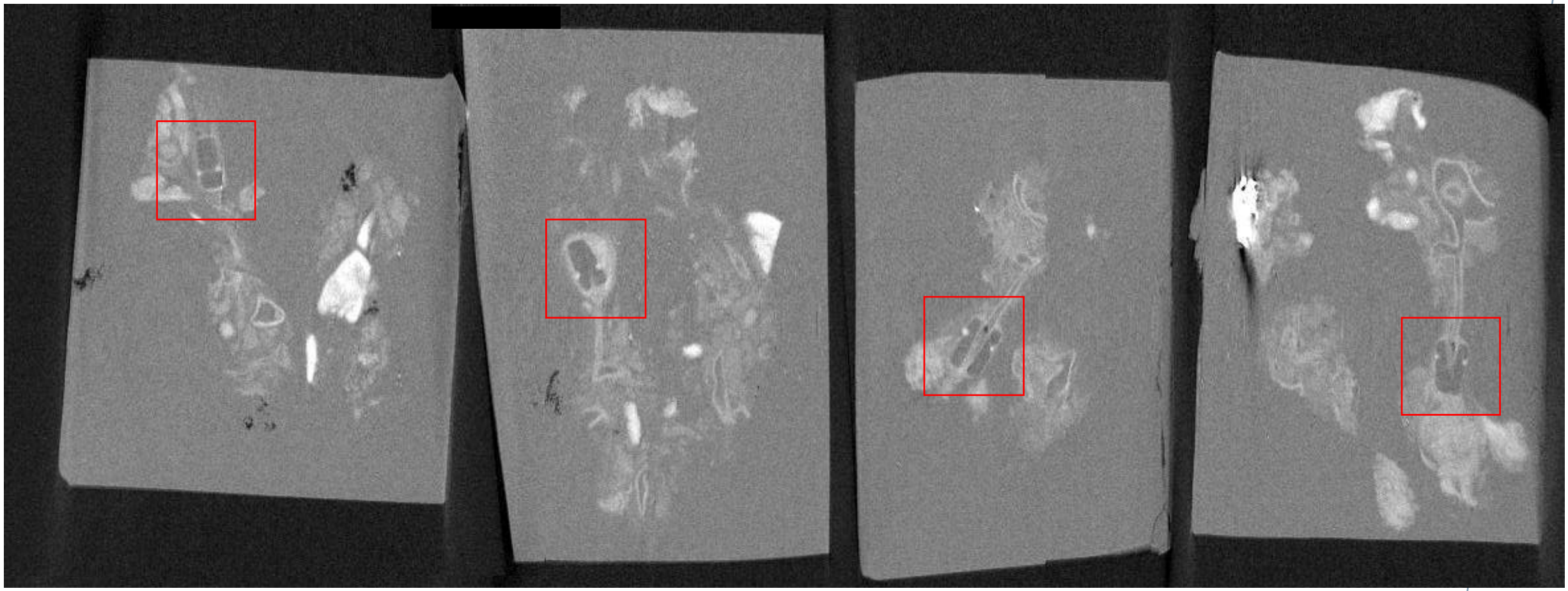
CT images

- Carotids are extracted and embedded in paraffin
- Samples are scanned with the maximum resolution and minimum kVp for approx. 2 h per sample
- Is calcium present in the plaque?



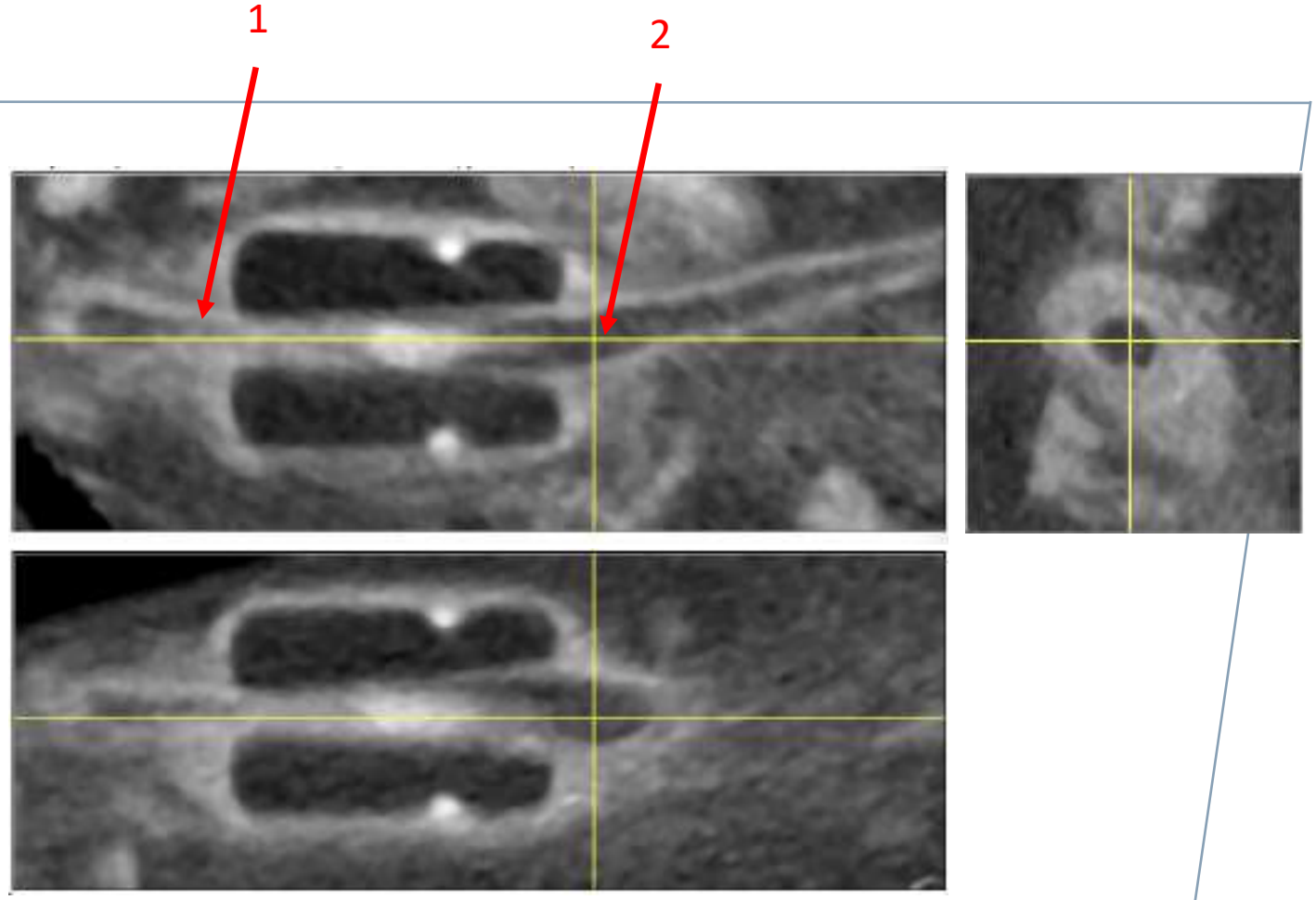
CT images

- Constrictors are identified in the CT images re-aligned

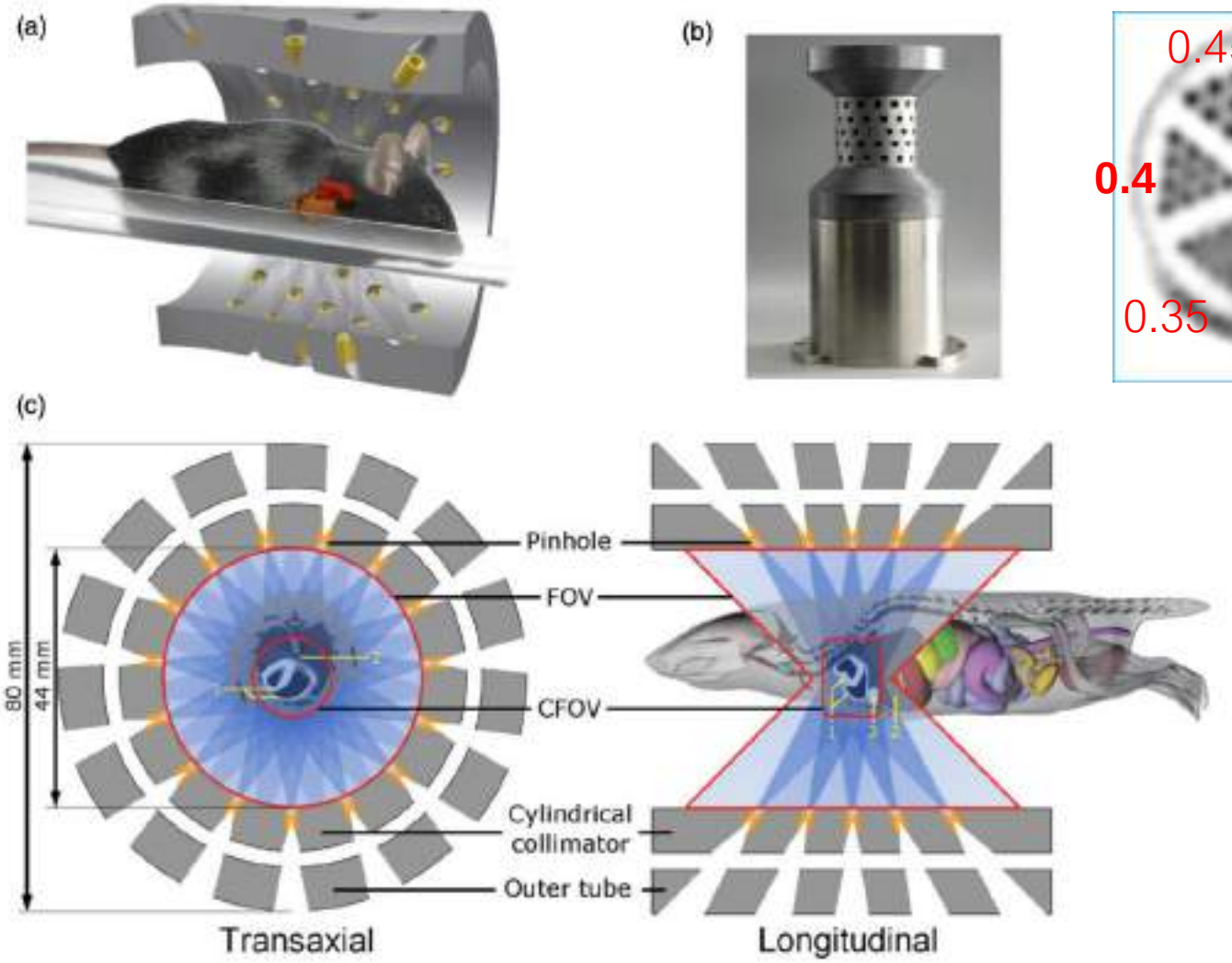


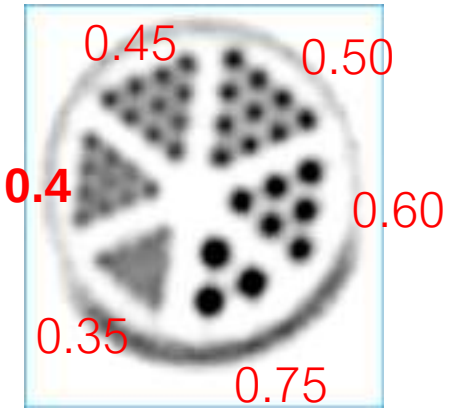
CT images

- The presence of calcium plaques and wall and lumen size are studied in the proximal (1) and distal (2) regions.
- No calcium is identified at this stage of the disease progression

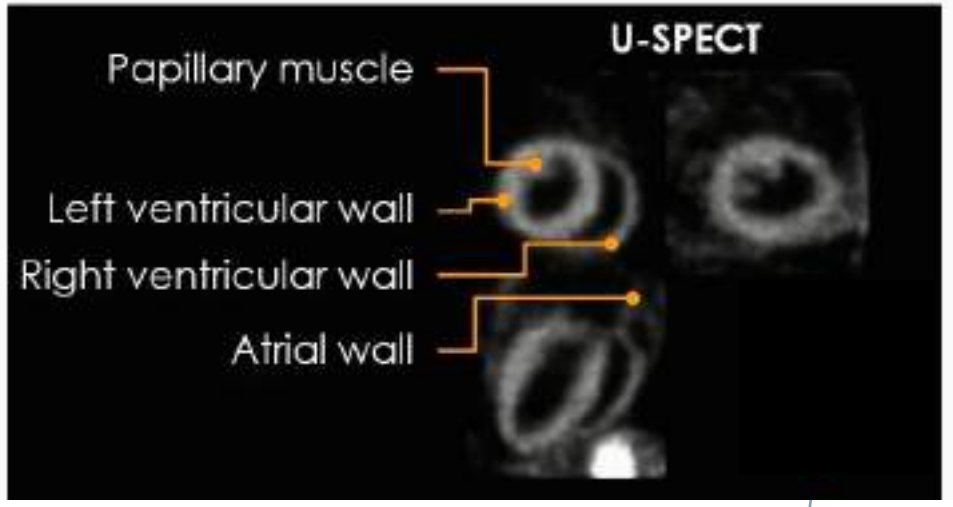


Multi-pinhole collimator approach





Example of **in-vivo** multi-pinhole SPECT imaging in mouse



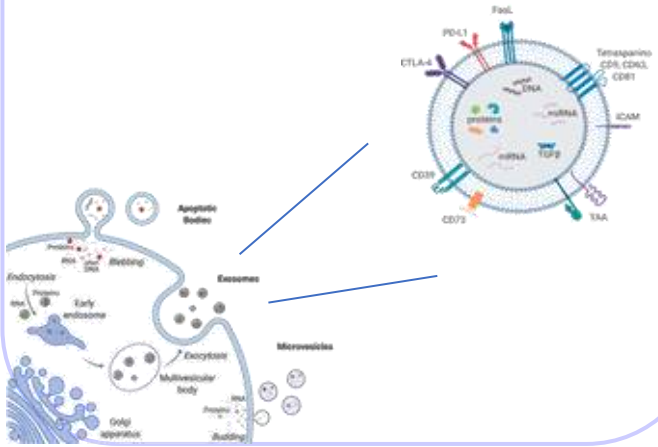
N. Befera, et al. Mol Imaging Biol, 2013

Biodistribution and Trafficking of Exosomes explored in vivo with SPECT

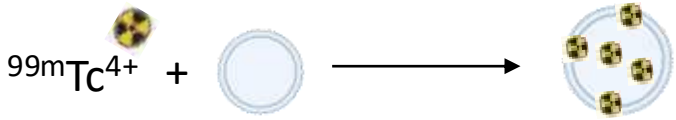
How do exosomes mediate interactions among cells, tissues, and organs?

Exosomes

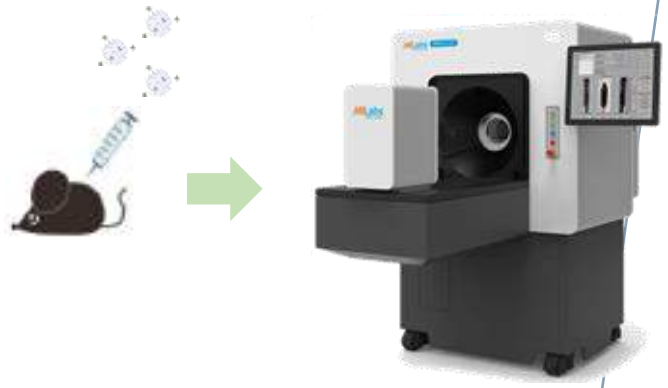
human body "letter carriers"



Exosomes are labelled with $^{99m}Tc^{4+}$



***In vivo* biodistributon Study by SPECT**



Courtesy – Fabio Recchia, Luca Menichetti CNR IFC

Biodistribution and Trafficking of Exosomes explored in vivo with SPECT

How do exosomes mediate interactions among cells, tissues, and organs?

Exosomes

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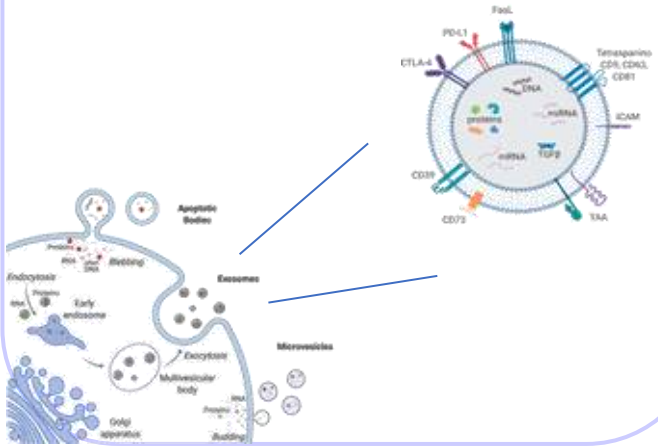
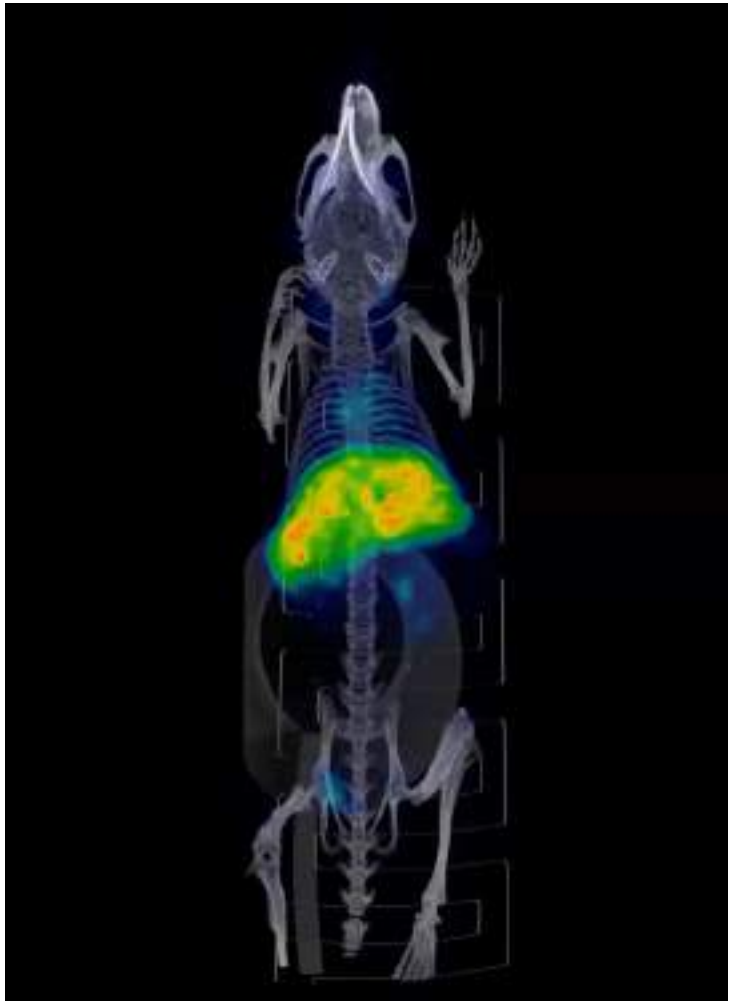
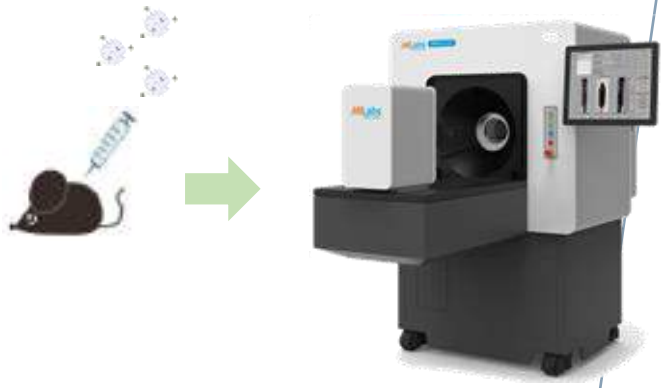




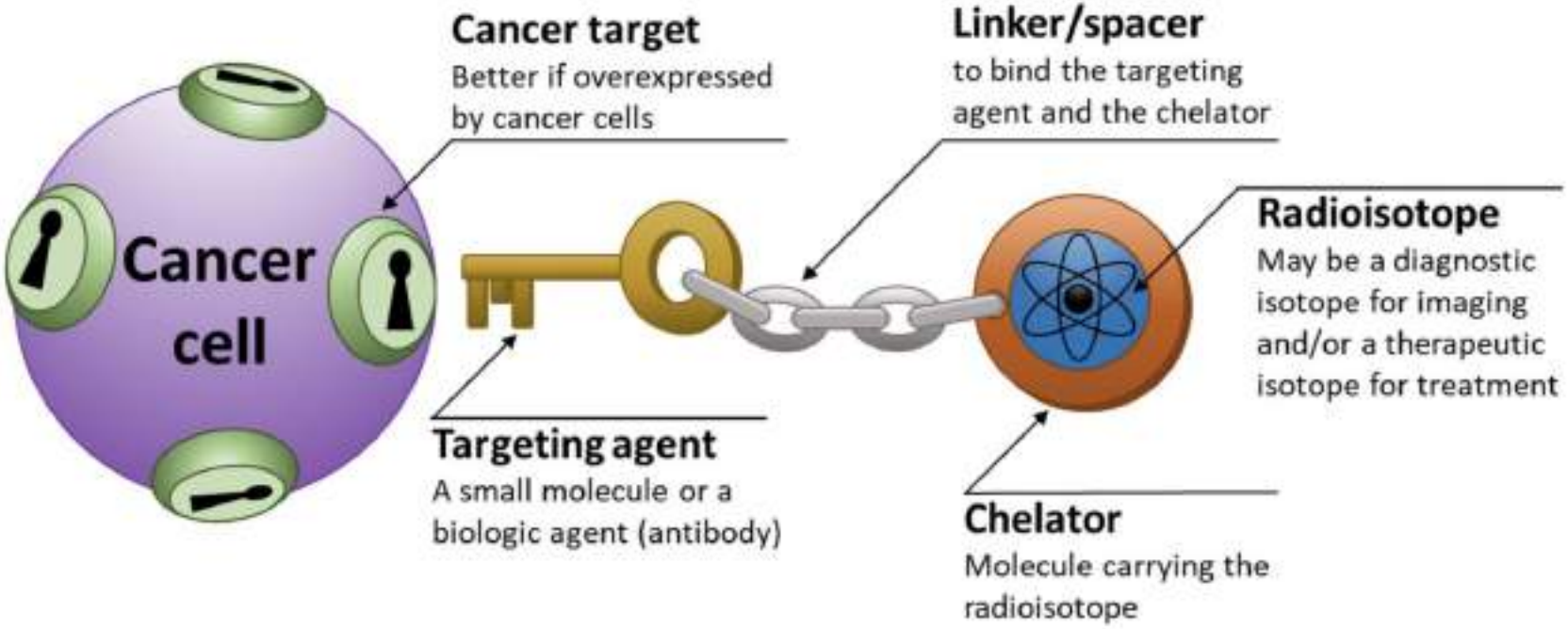
***In vivo* biodistributon Study by SPECT**



Theranostic applications

- beta- or alpha emitters (for therapy) + gamma emission (for imaging)

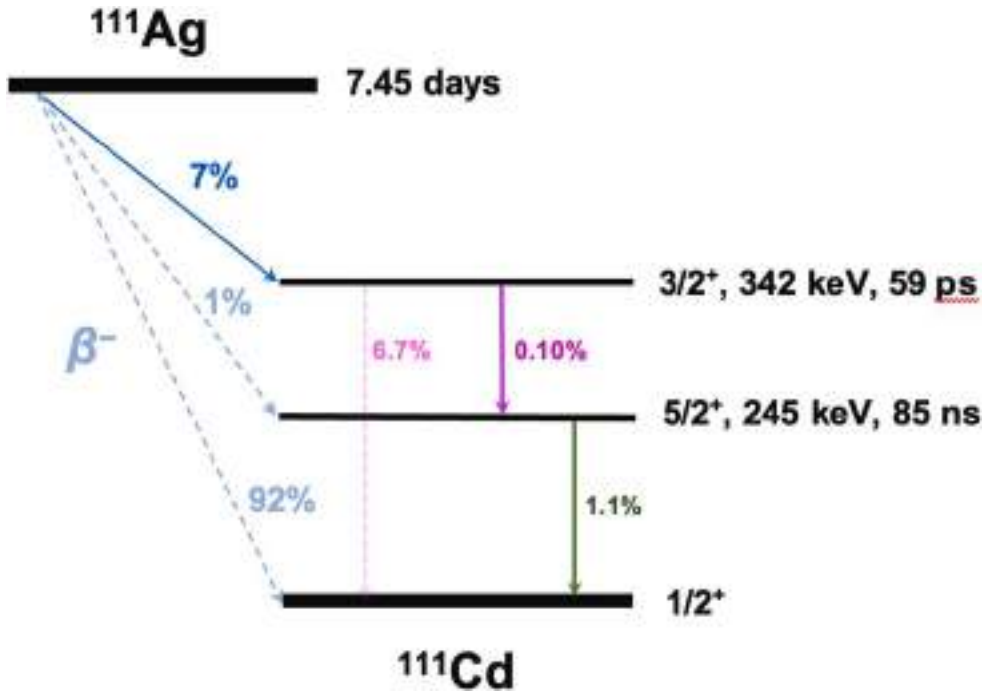
e.g.: Lutetium-177: β-emitter (E_{max} = 498.3 keV) and gamma emitter ($E\gamma_{max}$ = 208 keV), half-life 6.6 days

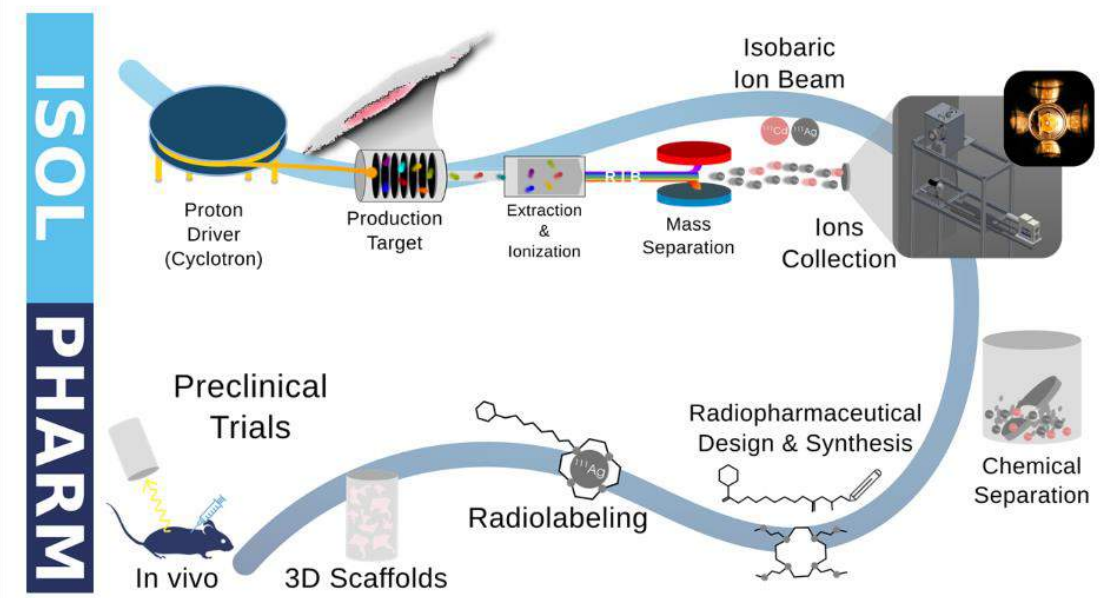


Theranostic applications

- beta- or alpha emitters (for therapy) + gamma emission (for imaging)

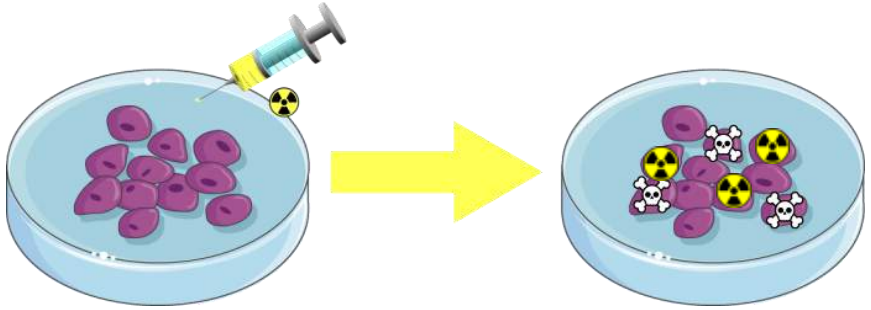
Ag-111: β- (360 (92%) / 223 (7%) / 180 (1%) keV) and gamma (342 (6.7%) / 245 (1.1%), $T_{1/2}$=7.45 d

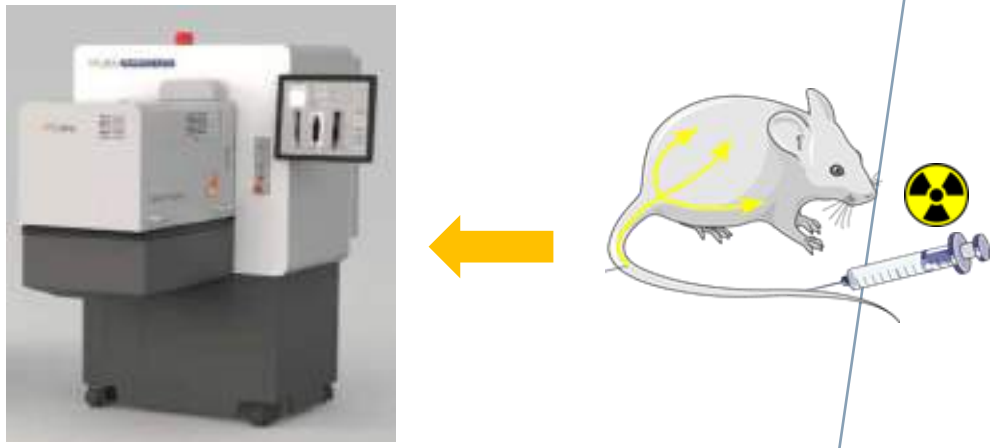




Imaging in theranostic

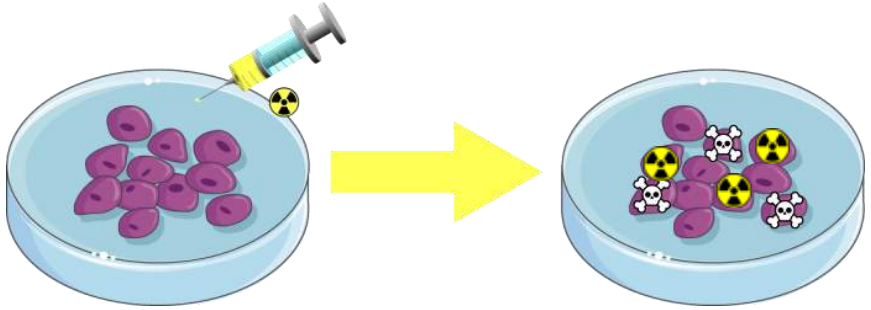
- Cellular imaging
  - Verification of the cellular uptake of the radioisotope
  - Demonstration of the tumour cells damage
- In-vivo imaging
  - Verification of the tissue uptake of the radioisotope in-vivo
  - Demonstration of the effectiveness of the theranostic drug





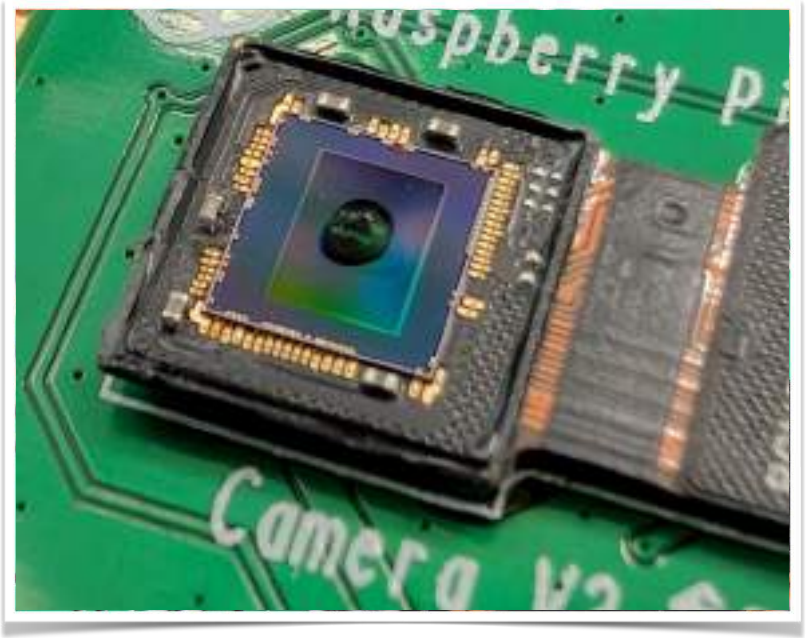
Imaging in theranostic

- Cellular imaging
  - Verification of the cellular uptake of the radioisotope
  - Demonstration of the tumour cells damage

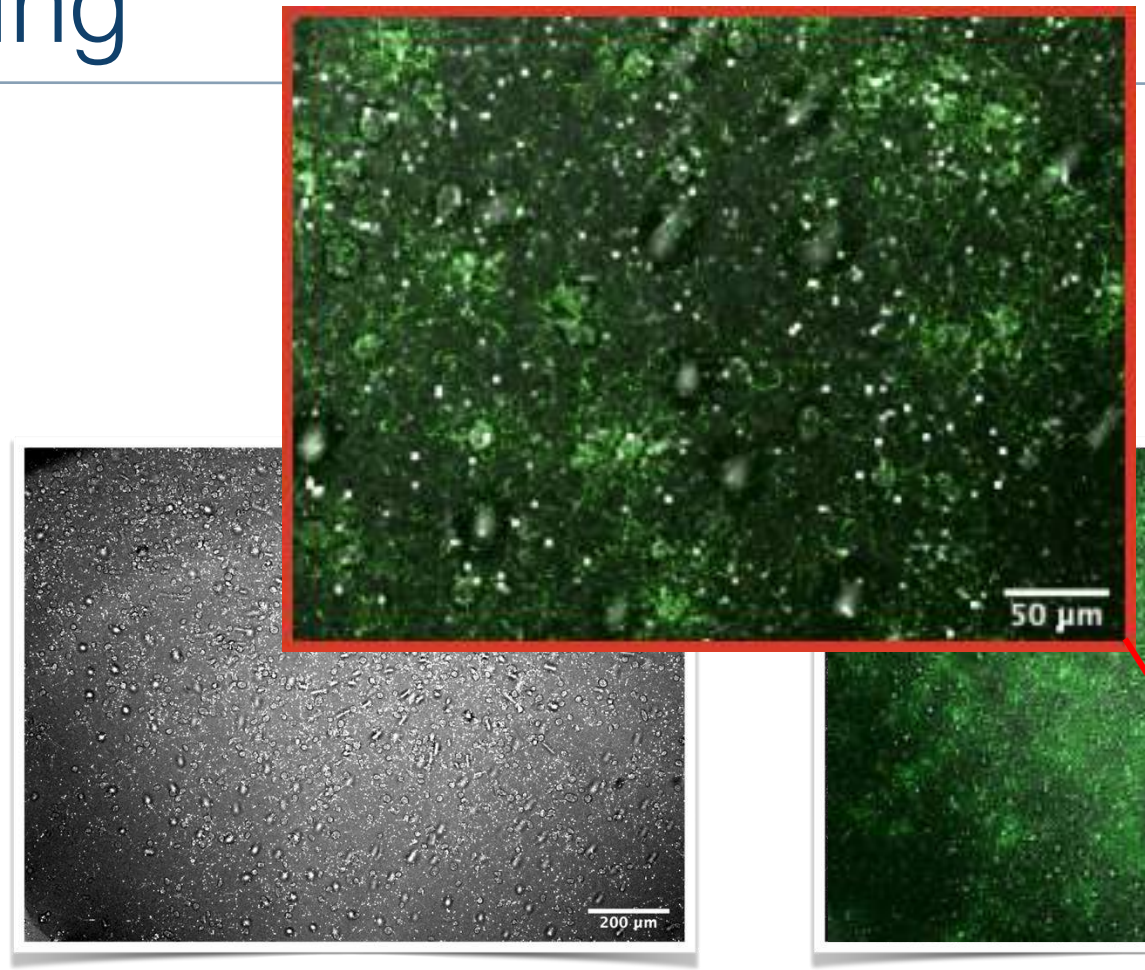


Sensor	IMX219
N. of pixels	3280 × 2464
Sensor size	4.6 mm diagonal
Pixel size	1.12 µm × 1.12 µm
Featured in	PI Camera v2

Cell uptake imaging



The CMOS camera

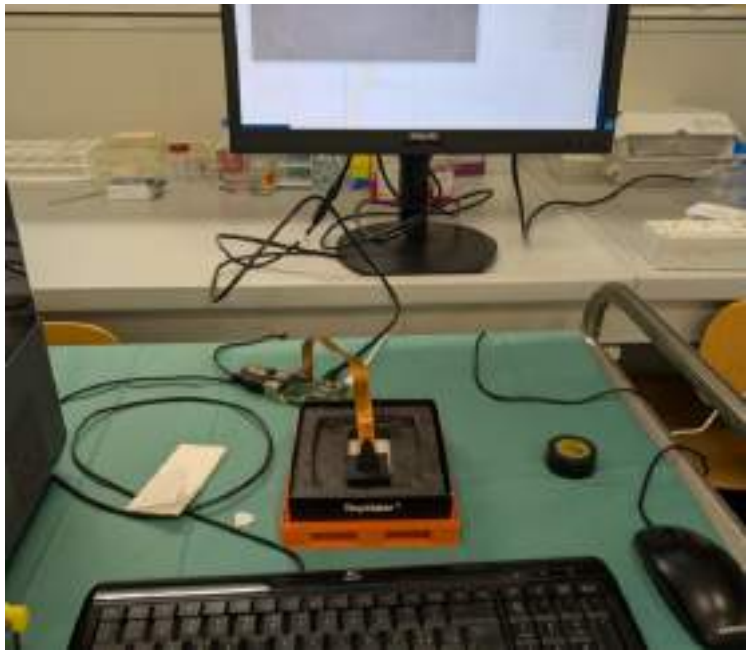


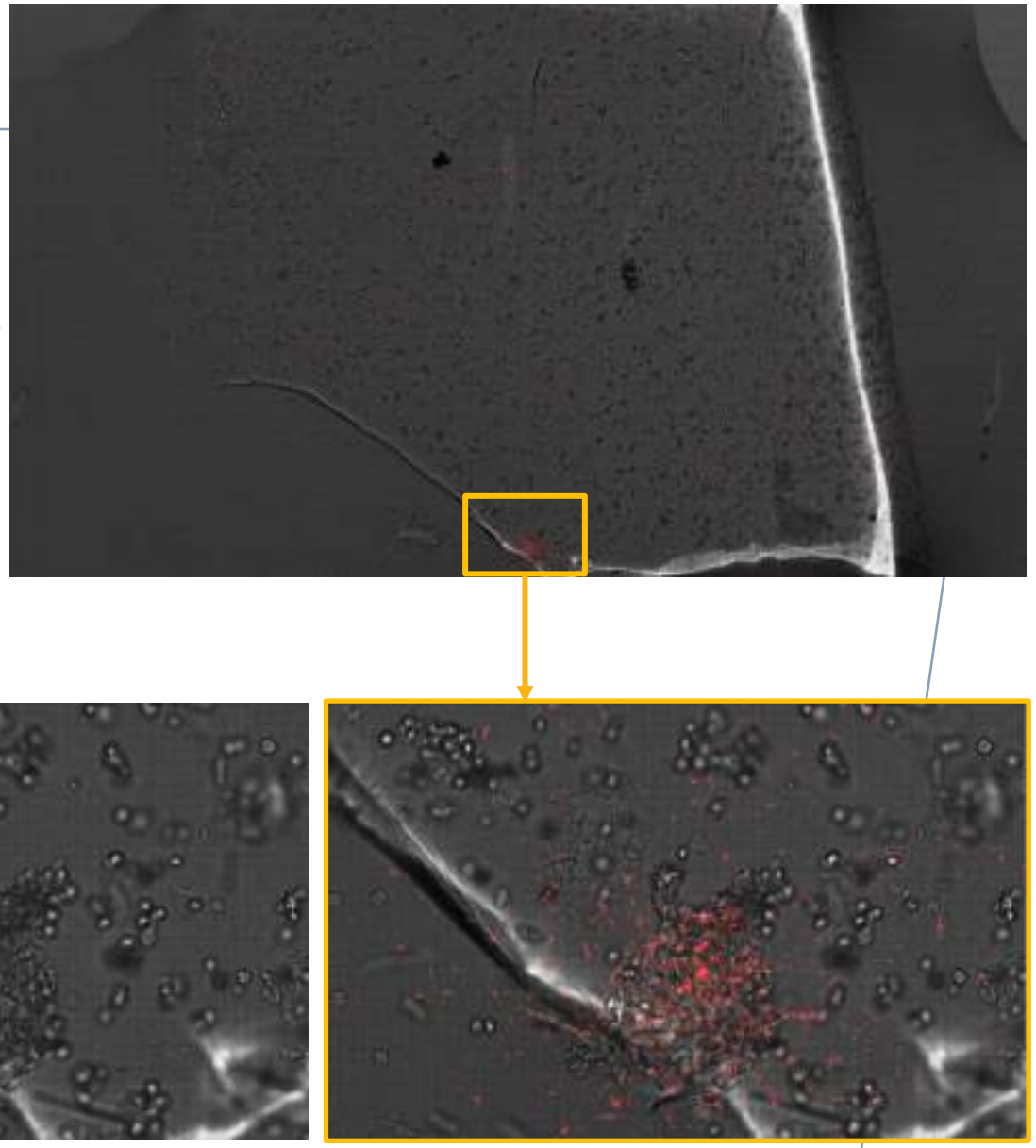
Brightfield image

Positron image

Imaging in theranostic

- Cellular imaging
  - Verification of the cellular uptake of the radioisotope
  - Demonstration of the tumour cells damage

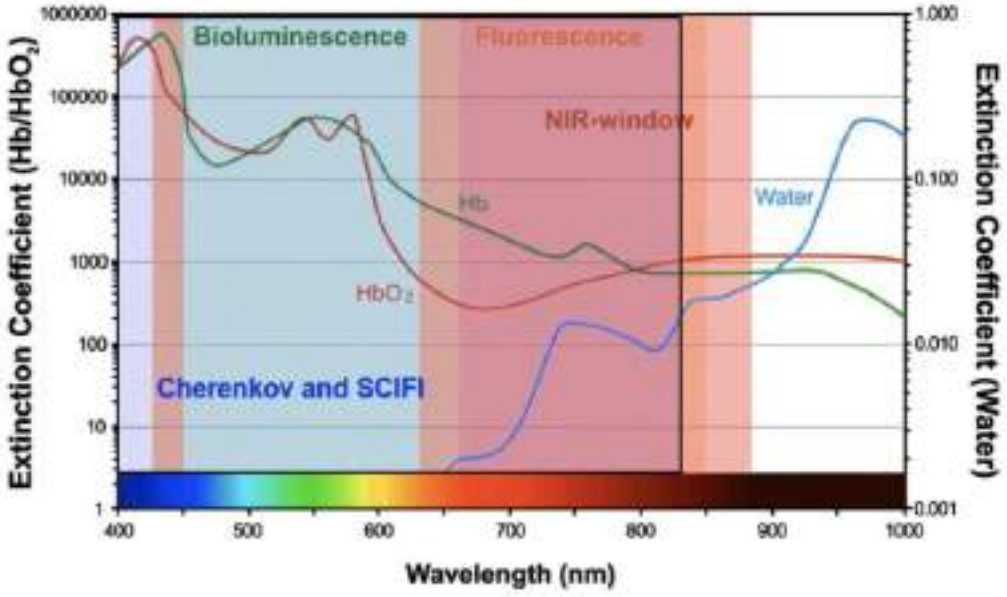




Project funded by INFN – Isolpharm collaboration

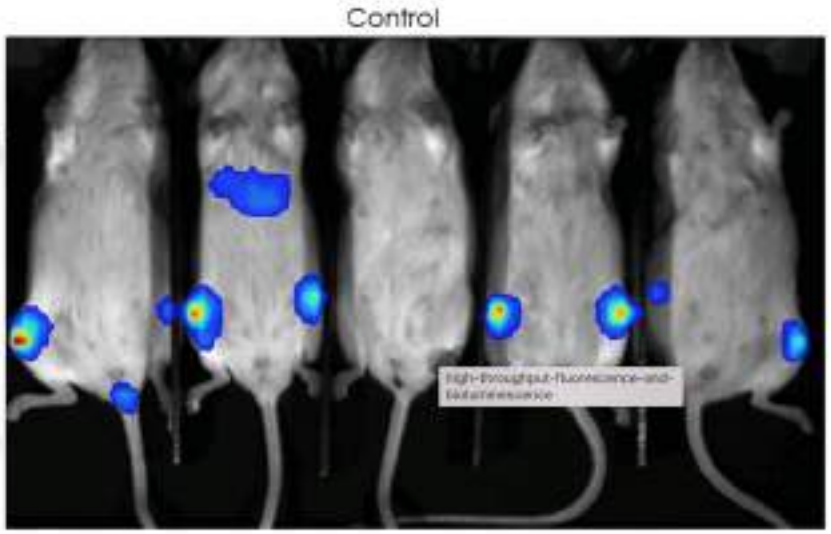
Optical imaging setup

Excellent performance for all *in vivo* Optical Imaging applications including NIR Tomography

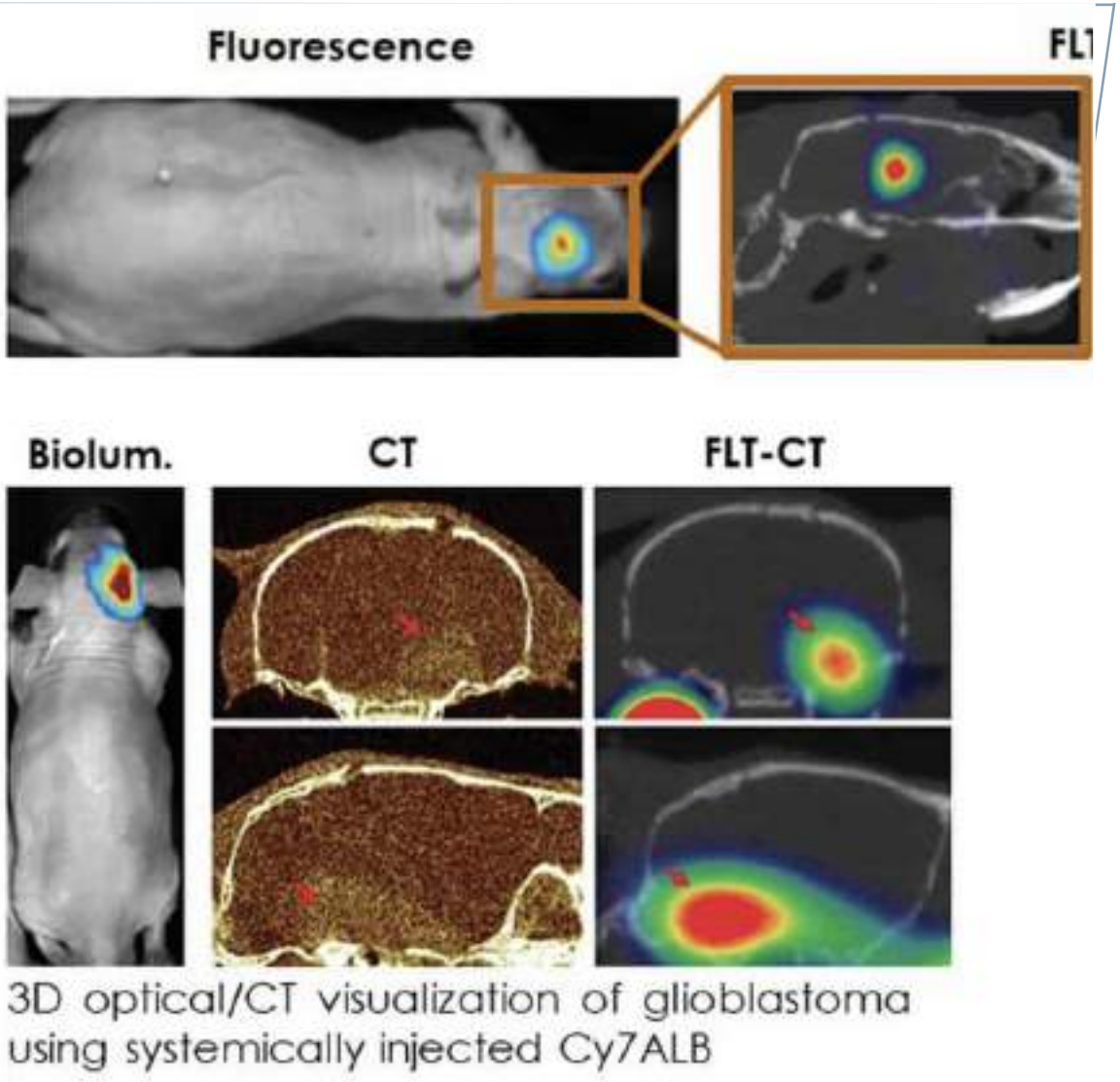


- Bioluminescence and Fluorescence
- NIR-fluorescence
- Cherenkov and Secondary Cherenkov-Induced Fluorescence Imaging (SCIFI)
- **Hybrid FLT-CT uses NIR-fluorescence**

2D Fluorescence / Bioluminescence



Example of 3D optical imaging (FLT)



3D optical/CT visualization of glioblastoma using systemically injected Cy7ALB

Acknowledgements

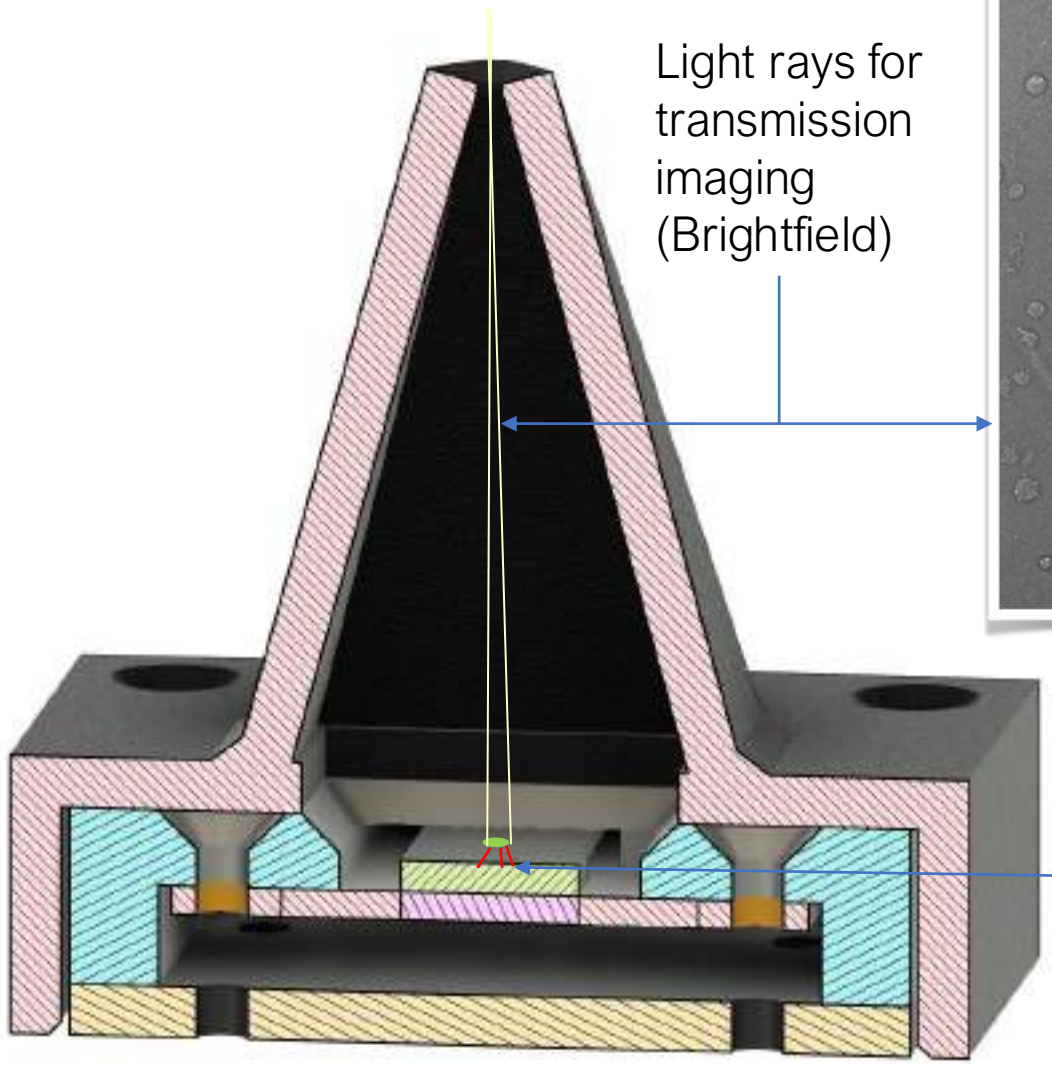
- Alberto Del Guerra
- Giancarlo Sportelli
- Niccolò Camarlinghi
- Simone Capaccioli
- Piero Salvadori
- Patricia Iozzo
- Daniele Panetta
- Luca Menichetti
- Silvia Burchielli

"Io spero che in un giorno non lontano, strettamente integrato nel tessuto universitario ed ospedaliero di questa città, noi vedremo sorgere un grande centro interdisciplinare di ricerca fondamentale e applicata, interamente orientato verso la problematica dell'uomo malato."

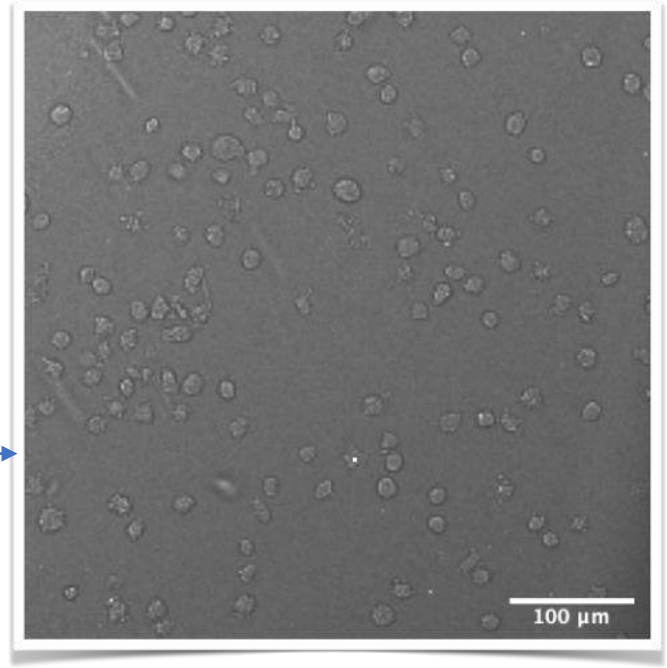
Luigi Donato, 1969

Backup slides

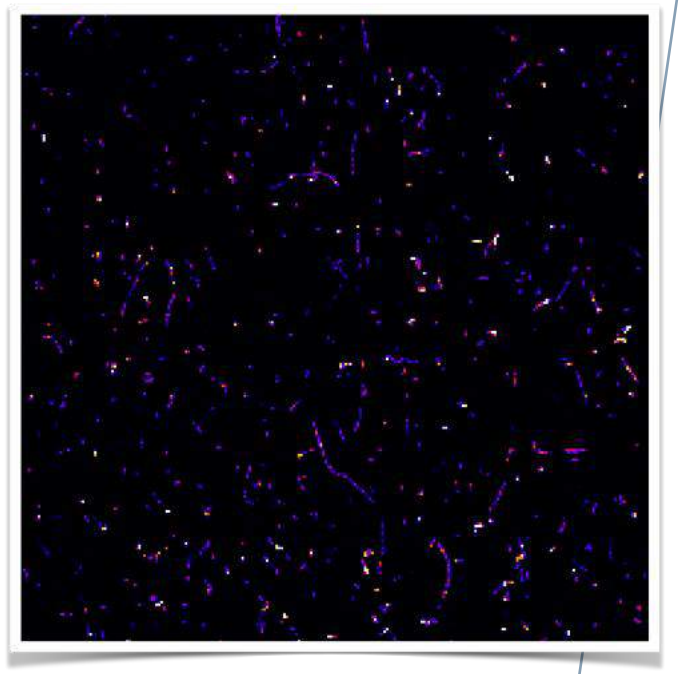
Cell uptake imaging



Light rays for transmission imaging (Brightfield)



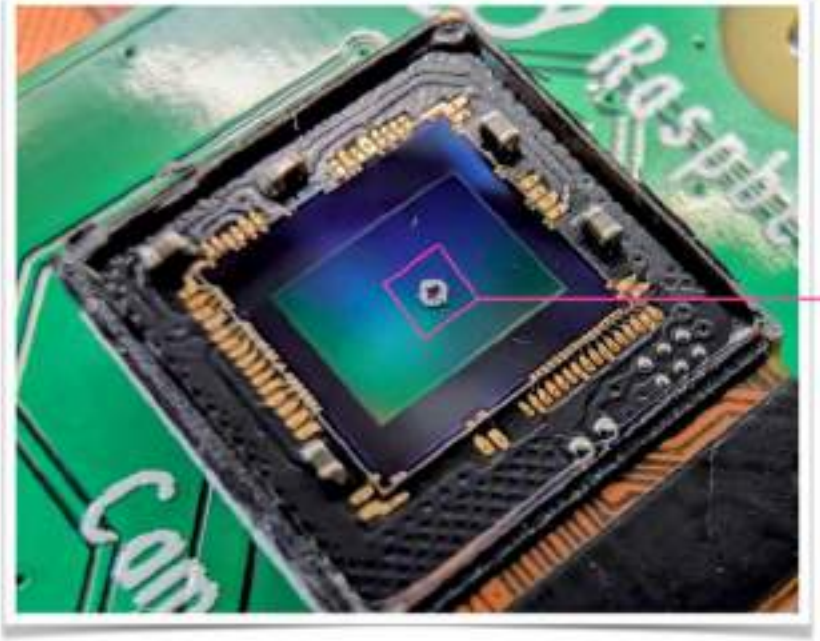
Brightfield image (morphology)



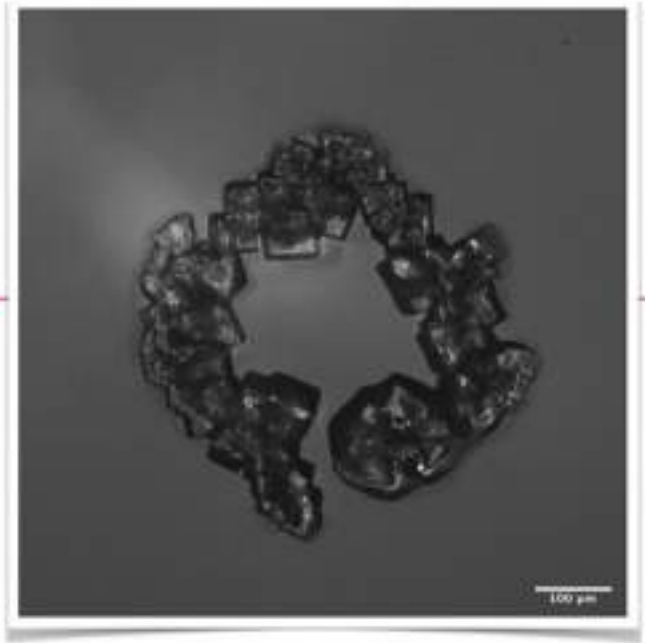
Positron image (tracer uptake)

Positron tracks detected by the sensor

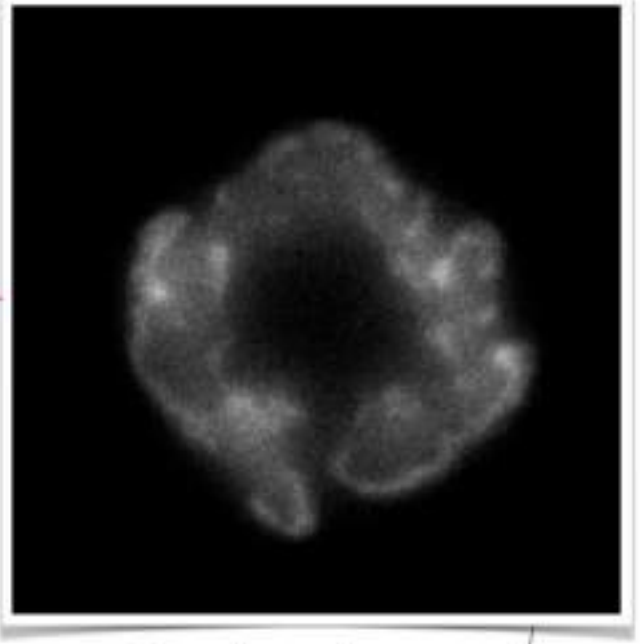
Nuclear imaging at the cellular scale



The CMOS camera

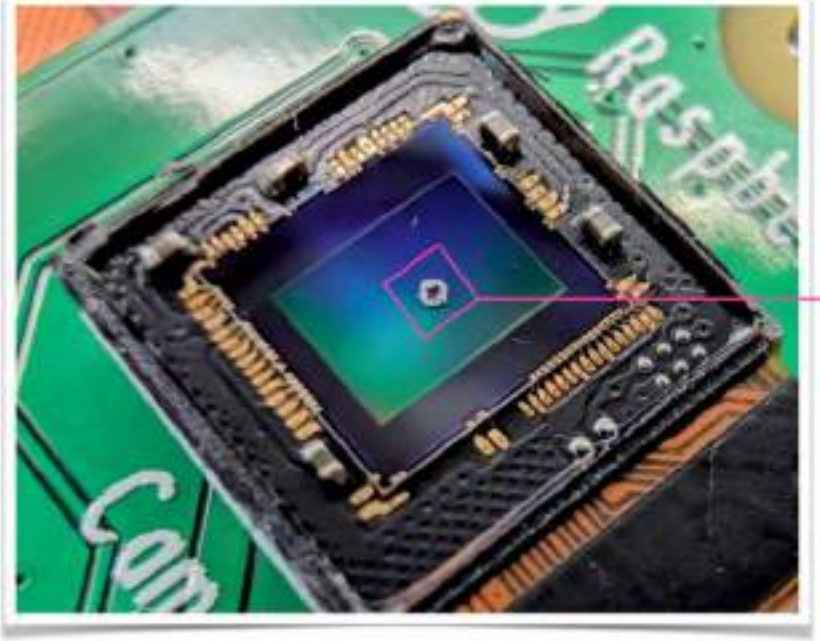


Brightfield image

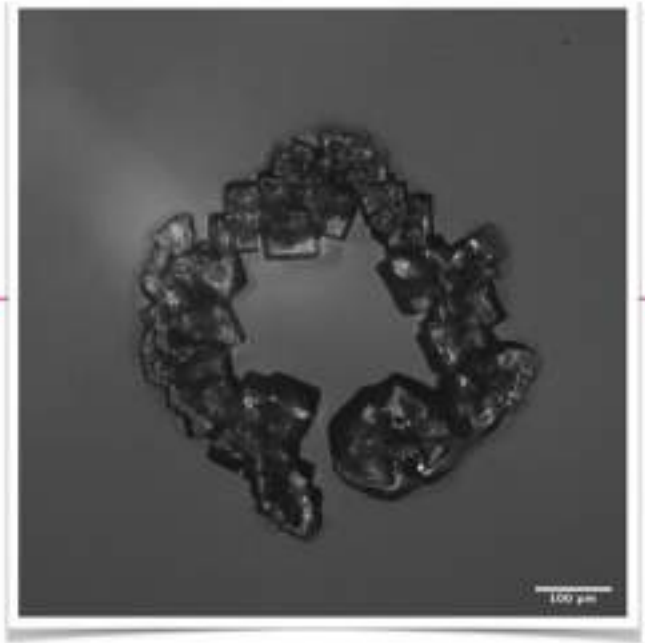


Positron image

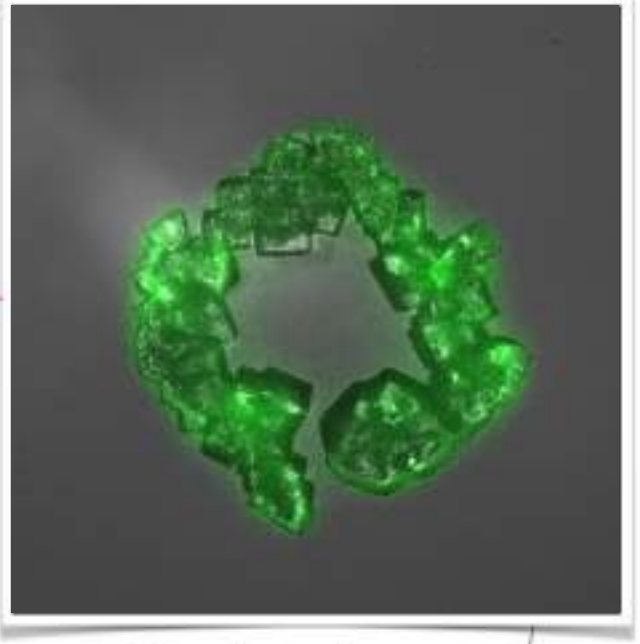
Nuclear imaging at the cellular scale



The CMOS camera

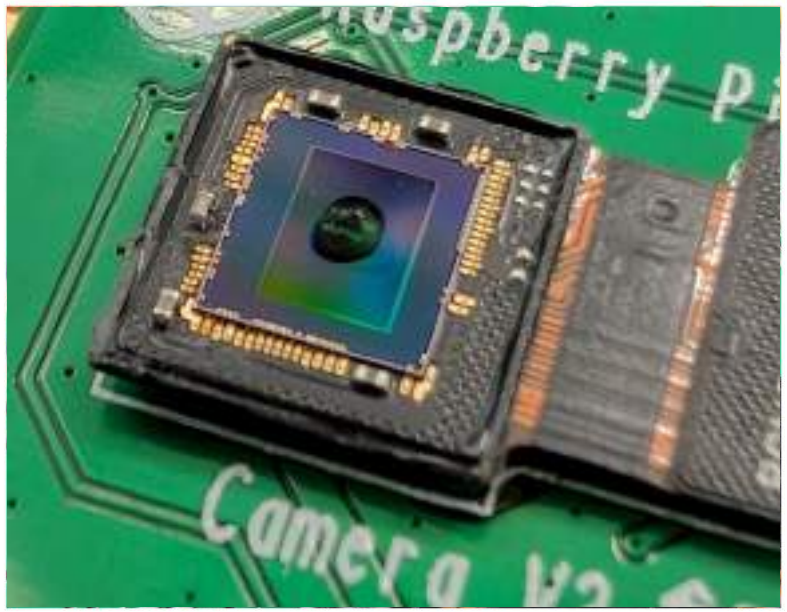


Brightfield image



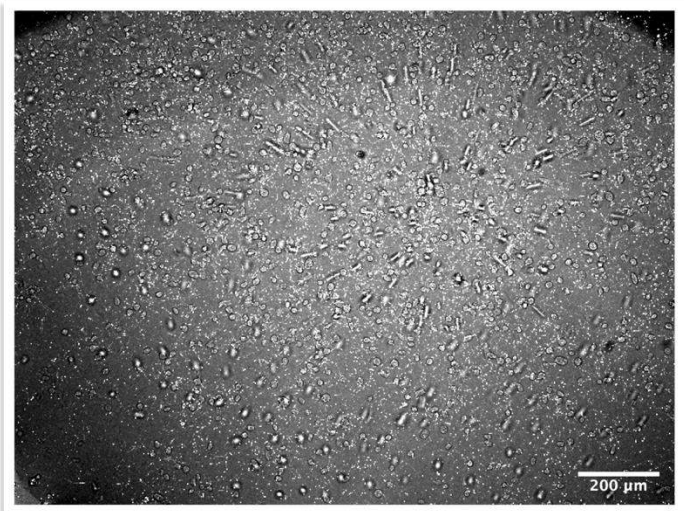
Positron image

Cell uptake imaging



The CMOS camera

Glioblastoma cells imaging



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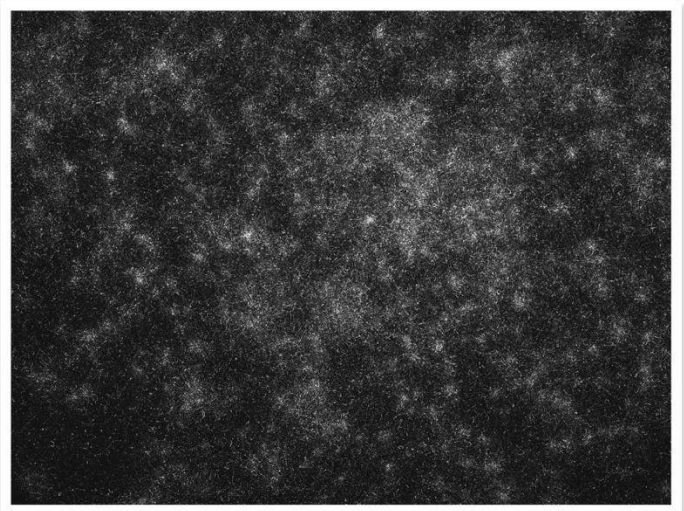
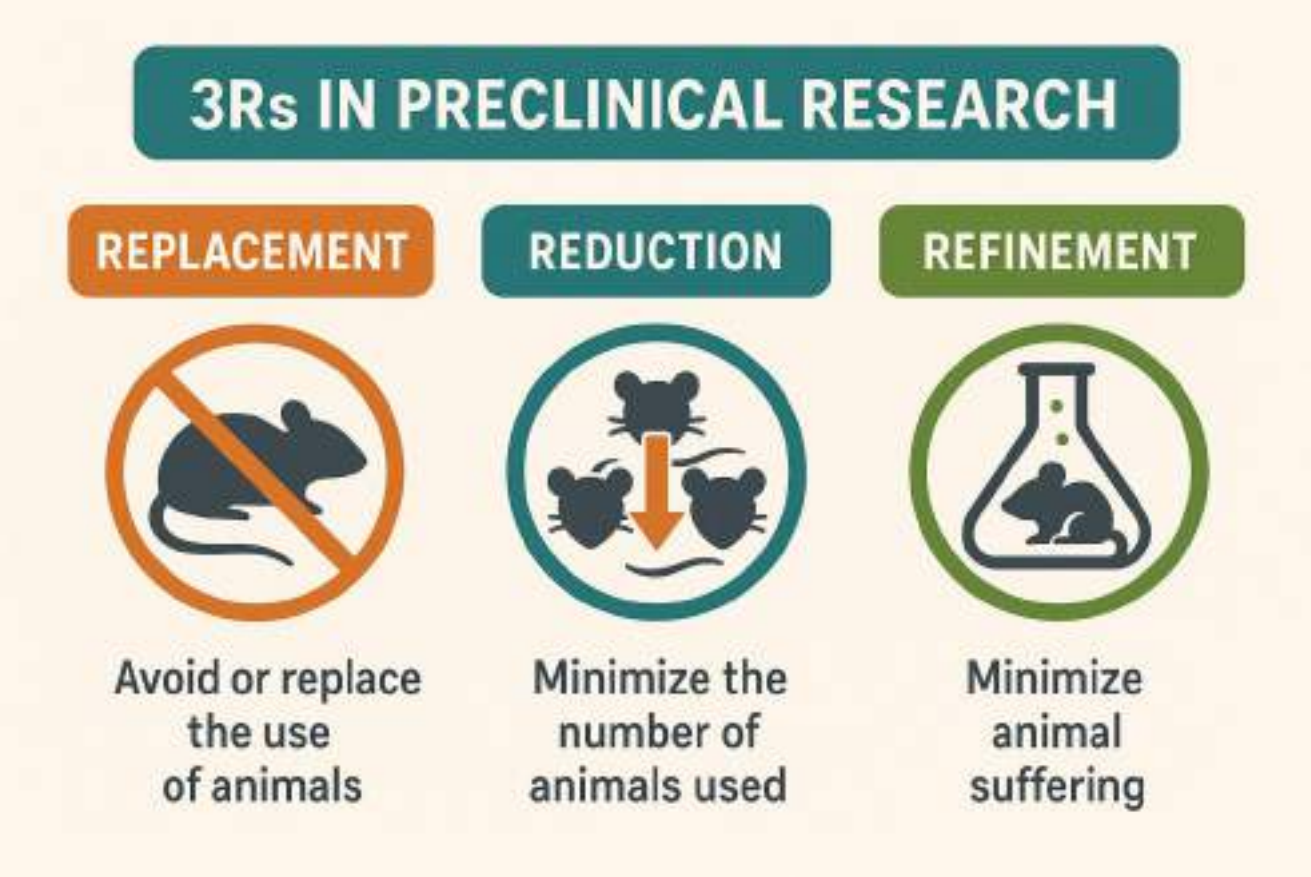


Positron image

The principles of the 3Rs



Replacement

Accelerating the development and use of predictive and robust models and tools, based on the latest science and technologies, to replace the use of animals in addressing important research questions where they would have otherwise been used.

Reduction

Appropriately designed and analysed animal experiments that are robust and reproducible, and add to the knowledge base.

Refinement

Advancing laboratory animal welfare by exploiting the latest in vivo technologies to minimise pain, suffering and distress and improve understanding of the impact of welfare on scientific outcomes.

https://nc3rs.org.uk/who-we-are/3rs