Wide range of nanomaterials designed for biomedical applications

Magnetic nanoparticles (MNPs)

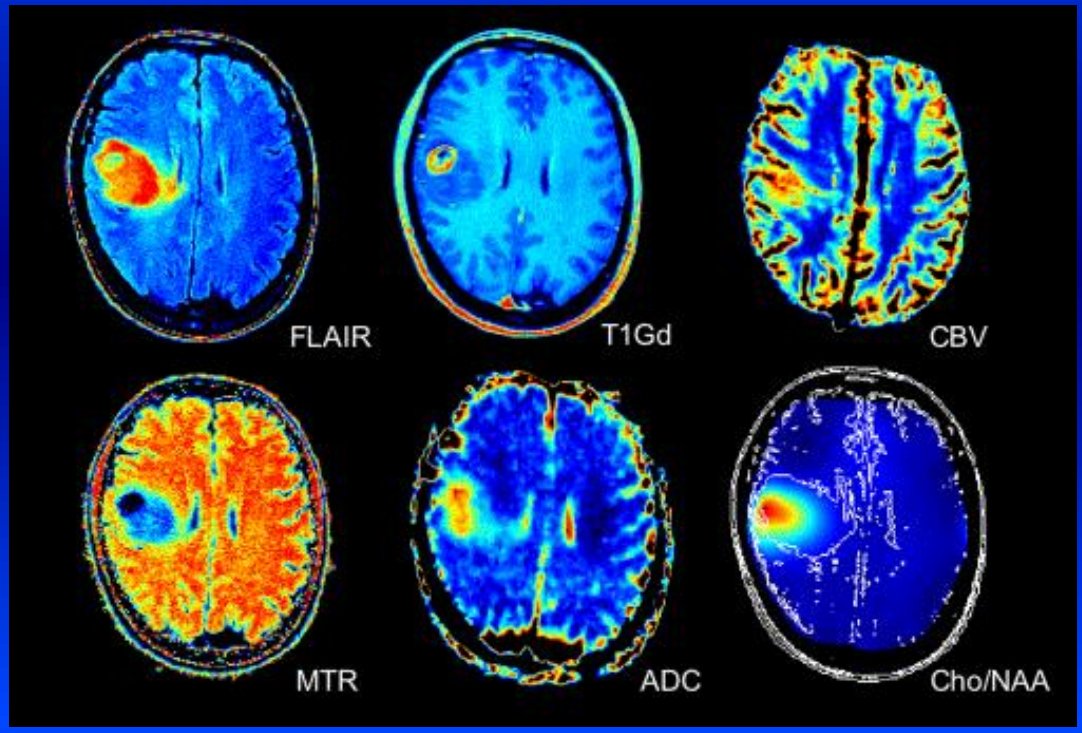
- Can be detected in magnetic resonance imaging
- Can be coated with biocompatible materials due to their reactive surface

Among the various fields of application of MNPs, our experience has focused on:

- Potential use of MNPs in multimodal diagnostic imaging

- Possibilities of confinement of MNPs in predetermined sites by external magnetic fields

Use of MNPs in multimodal diagnostic imaging



MNPs

MRI contrast enhancement agents in T2-weighted magnetic resonance imaging (MRI),

More interestingly

MNPs

Carriers on which to build molecular probes with high specificity

↓

Diagnostic imaging with multi-modal approaches

Each imaging modality has some advantages
(e.g., high resolution for MRI, high detection sensitivity for PET),

To supply multimodal molecular probes with integrated functions

↓

Significant synergy of information.

Indium-111 or Technetium-99m-labelled leukocyte scans

Diagnostic tools widely employed in the study of several inflammatory and infective diseases.

Limitations:

- Low resolution of gamma cameras
- Lack of structural reference points which allow for correct localisation of the lesions.

Technetium-99m-labelled leukocyte scans

Co-acquisition of single-photon emission computed tomography and computed tomography (SPECT-CT)

Improvement localisation of inflammatory/infective processes,

SPECT-TC withTechnetium-99m-labelled leukocyte scans

Limitations:

The 24-h acquisition is necessary in the study of the most frequent of these processes (bone infections)

↓

Short physical half-life of the used radionuclide (6h)

↓

Low count rate at this time with deterioration of images

Initial attempt to develop a positron-emitting specific tracer for inflammatory and/or infective diseases

In vitro labelling of leukocytes with 2-deoxy-2-[18F]fluoro-D-glucose (^{18}F-FDG)

Limitations:

The short half-life of ^{18}F (110 min) does not permit monitoring of the bio-distribution of the labelled cells for the required period of time (up to 18–24 h)

Labelling of granulocytes by engulfment of these cells with chitosan-coated MNPs previously labelled with ^{64}Cu

↓

A probe which emits positrons for a longer period and allows high-resolution images

↓

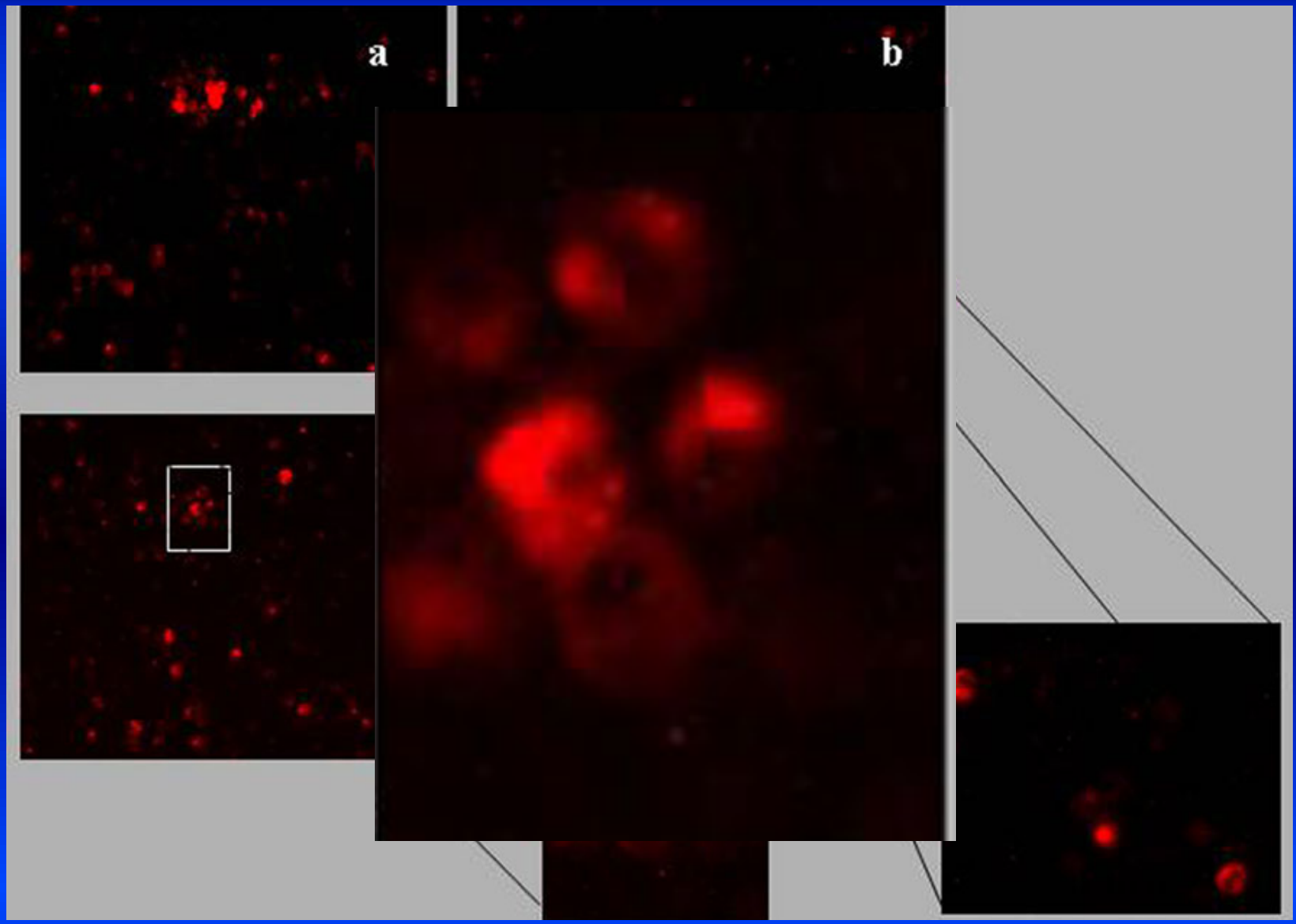
A radiopharmaceutical suitable for dual imaging (PET-MRI) of inflammatory/infective diseases.

Phagocytic capacity of granulocytes for MNPs

Use of MNPs with a diameter of 150 nm, containing a fluorescent dye covalently bound to chitosan.

Localisation of the MNPs within the cells.

Granulocytes engulfed with chitosan-coated MNPs previously labelled with fluorescent dye.



Computerised expansions of frames b and c show intensively stained cytoplasm. The stain-free profile of the nuclei demonstrates the internalisation of MNPs.

Labelling of MNPs

Chitosan coated MNPs were washed with saline-isotonic solution and recuperated by magnetic decantation;

10 mg MNPs were allowed to react with 16 µg ^{64}Cu [$^{64}Ni(p,n)$ at 12-9 MeV, specific acivity 56 MBq/ µg] in 500 µl saline solution acidified at pH 5.5 for 30 min. at room temperature;

Resulting specific activity of MNPs was 75 MBq/mg

Labelling of Granulocytes

- Pellets of granulocytes were obtained from peripheral blood;
- MNPs engulfment by granulocytes was carried out;
- Assessment of granulocyte-engulfed viability by the trypan blue exclusion (TBE) test was performed at 5 min, 2 h and 4 h;
- Assessment of the release of ^{64}Cu from labelled granulocytes in plasma was achieved by measuring the radioactivity of both cellular pellet and supernatant solution.

Binding capacity of chitosan-coated MNPs for cationic metal.

MNPs (mg)	ml	µg Cu^{++}	µg Cu^{++} bound/mg MNPs
5	0.5	15.5	1.4
5	0.5	58.25	1.6
10	0.5	15.5	1.4
10	0.5	58.25	1.5
20	0.5	15.5	0.8
20	0.5	58.25	1.4
		µg $^{64}Cu^{++}$	
10	0.5	16.5	1.4

The amount of Cu^{++} chelated captured per mg of MNPs is constant and independent of the reagent concentrations.

Specific activity: 75 MBq/mg MNPs

Granulocytes labelling yield = 75%

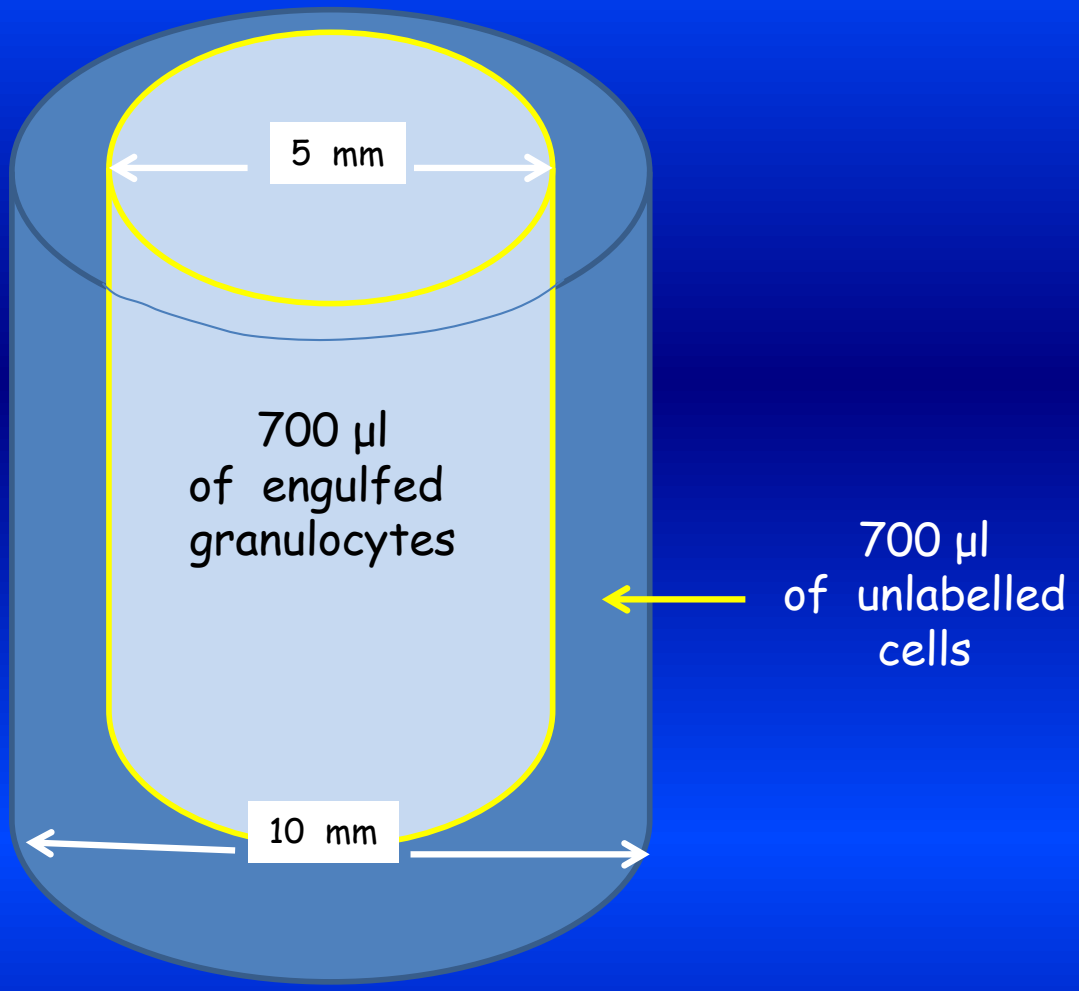
More than 90% of the engulfed cells were shown to be viable to the TBE test

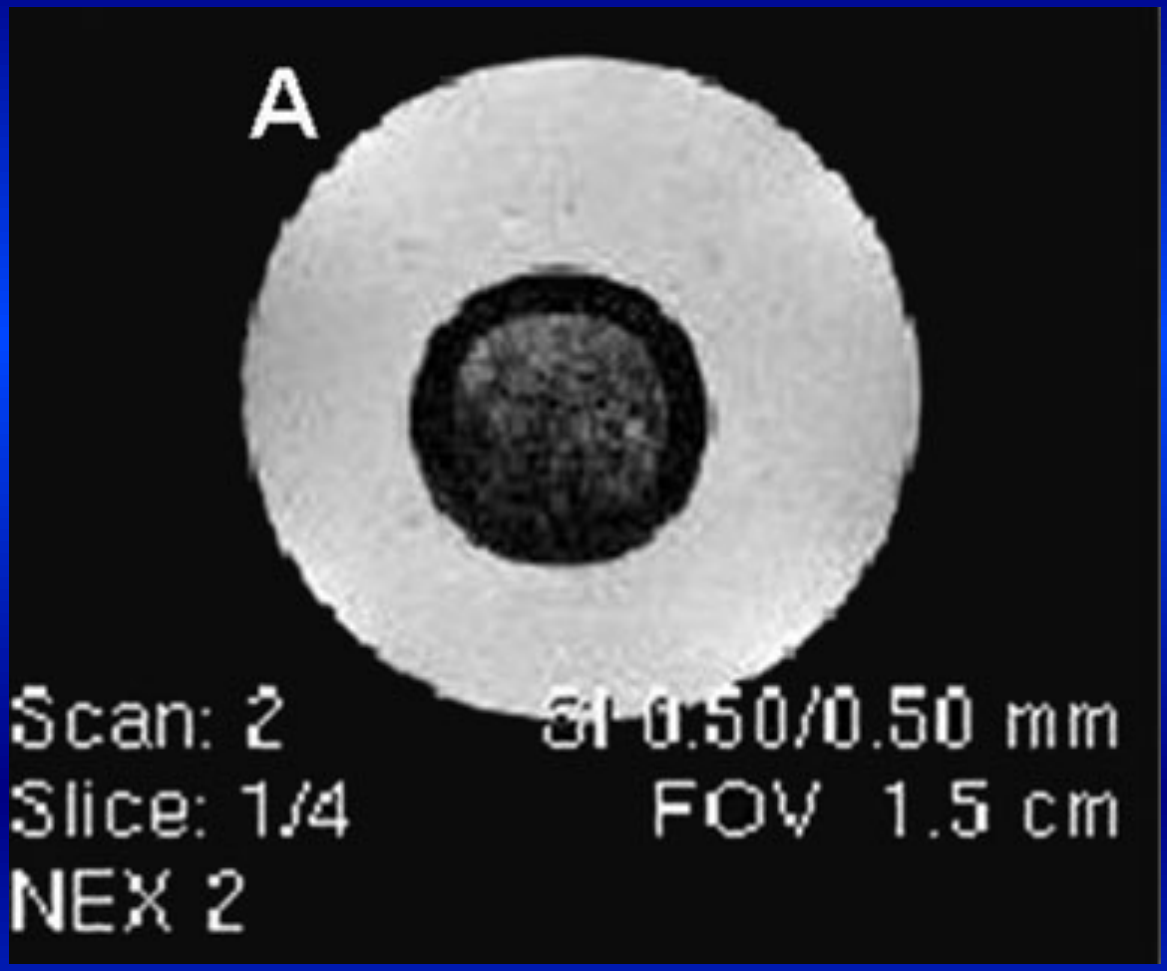
Amounts of ^{64}Cu released from engulfed granulocytes suspended into human plasma

Time (h)	0.5	1	2	4
Release (%)	8	15	18	20

The cells retain the engulfed radioactivity in vitro for a time sufficient for clinical use

Visualisation of engulfed cells by means of MRI instrument operating at 9.4 T equipped with a micro-imaging probe





Magnetic resonance imaging showing the different contrast between engulfed granulocyte suspension (inner circle) and non-engulfed granulocyte suspension (outer circle).

Conclusions

- This study describes for the first time a simple, reliable and fast method for labelling human Granulocytes with ^{64}Cu MNPs;

- If these studies are confirmed by in vivo experiments, this method may be used in bimodal imaging to simultaneously obtain PET and MR imaging of infections and inflammations

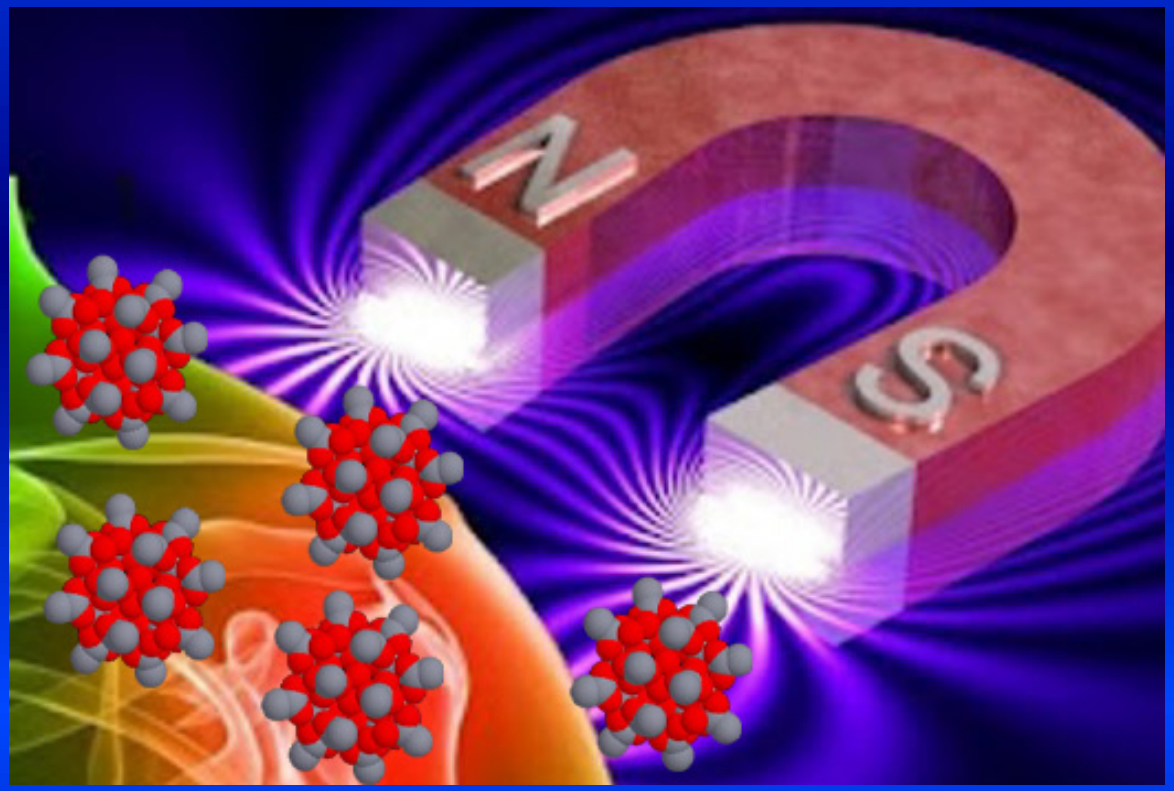
Possibilities of confinement of MNPs in predetermined sites by external magnetic fields

Magnetic properties of NPs make possible to hypothesise a confinement of MNPs by external magnetic fields,

Delivery of radionuclides and/or chemical substances near to biological target

Possible monitoring of the delivery by PET, MRI SPECT or planar scintigraphic imaging.

Our study was undertaken to explore this possibility of confinement using ^{99m}Tc-labelled MNPs.



Preparation o f MNPs

- A mixture of ($Fe_2(SO_4)_3$) and ($Fe(NH_4SO_4)_2$) in distilled water was dripped in a solution of ammonium hydroxide.
- Reaction was allowed to proceed for 15' under stirring at 0 °C.
- MNPs were then washed with water and saturated sodium phosphate solution and submitted to dialysis against distilled water.
- MNPs were functionalised with 3-aminopropyl-trietoxysilane (APES) in 01. M phosphate buffer 5mM EDTA, pH 8.0,
- A solution of 3-iminothiolane was added under stirring for 1 hr at room temperature.
- MNPs were washed by magnetic decantation with the same buffer solution.

Labelling o f MNPs

2.5 mg thiolated MNPs in 1 ml of 0.1 M phosphate buffer, pH 8, were allowed to react for 1 hr with 444 MBq of ^{99m}Tc previously submitted to reduction with 10 mg of dithionite.

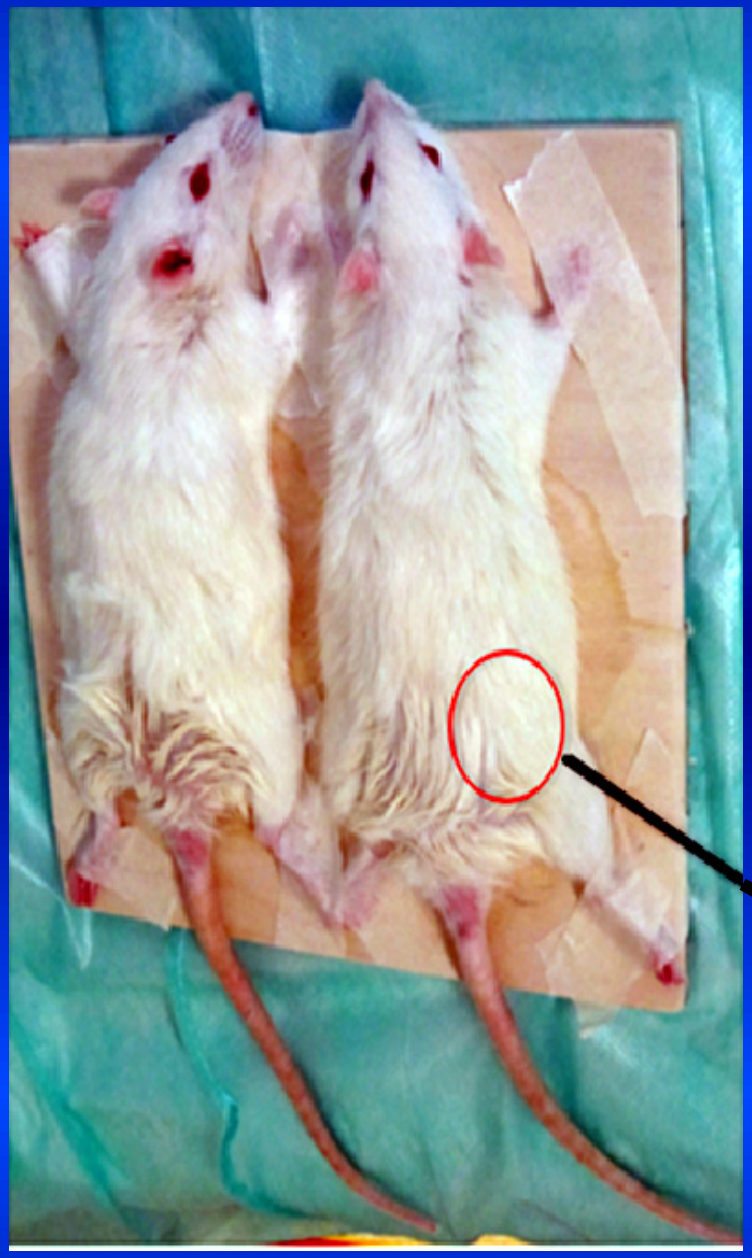
Labelling yield = 70%

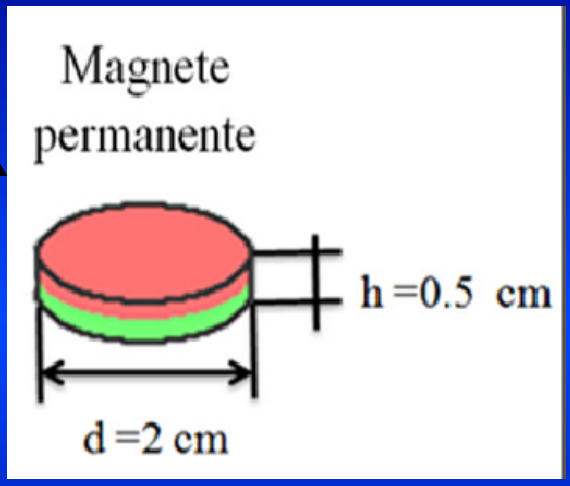
Specific activity = 124 MBq/mg

Animal studies

- Adult Wistar rats were anesthetized by intraperitoneal injection;
- Two rats were fixed with a tape on a wooden board;
- A Neodimio magnet was firmely fixed under the upper part of the right posterior leg of one of the two;
- Two hundred fifty microliters of a suspension of MNPs containing 74 MBq ^{99m}Tc, were slowly injected into the caudal vein of the animals.

Animal studies





Scintigraphic methods

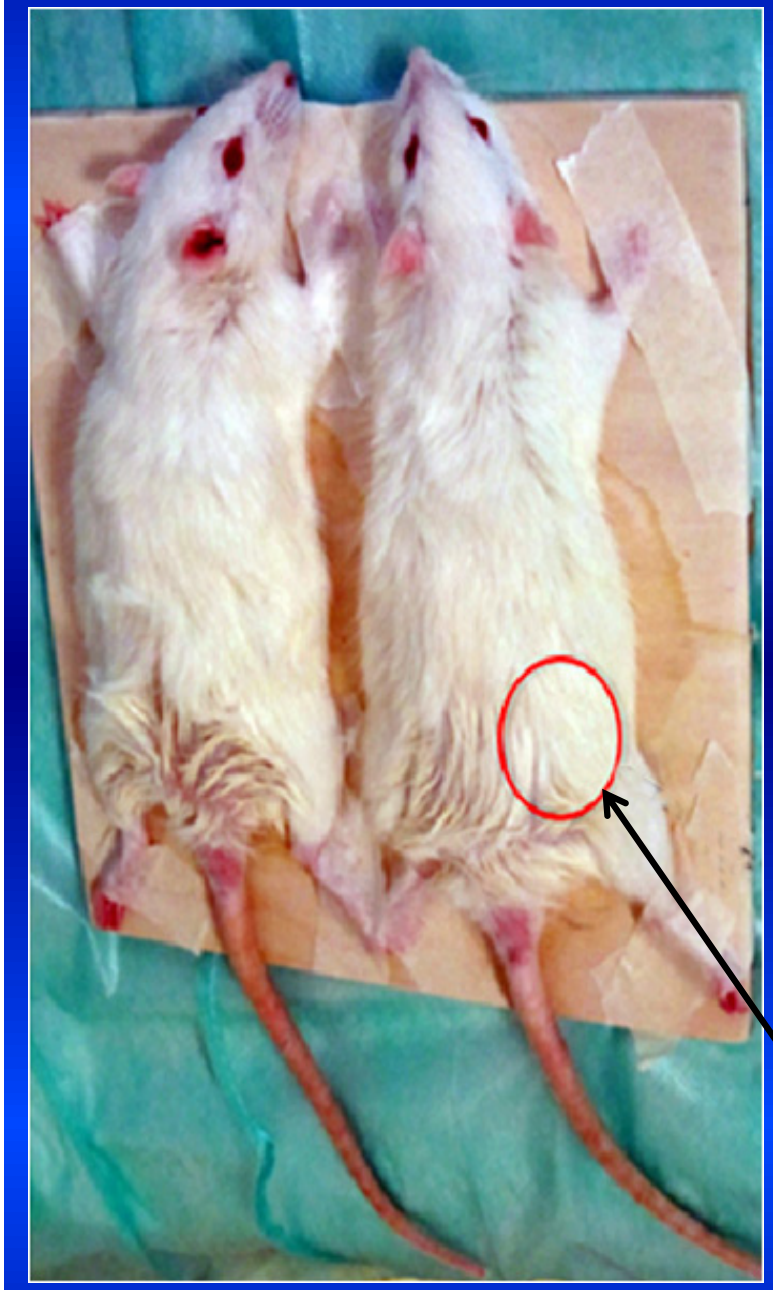
- A dynamic acquisition (1 image every 30 seconds for 30 min.) was performed by a gamma-camera equipped with a high resolution collimator;

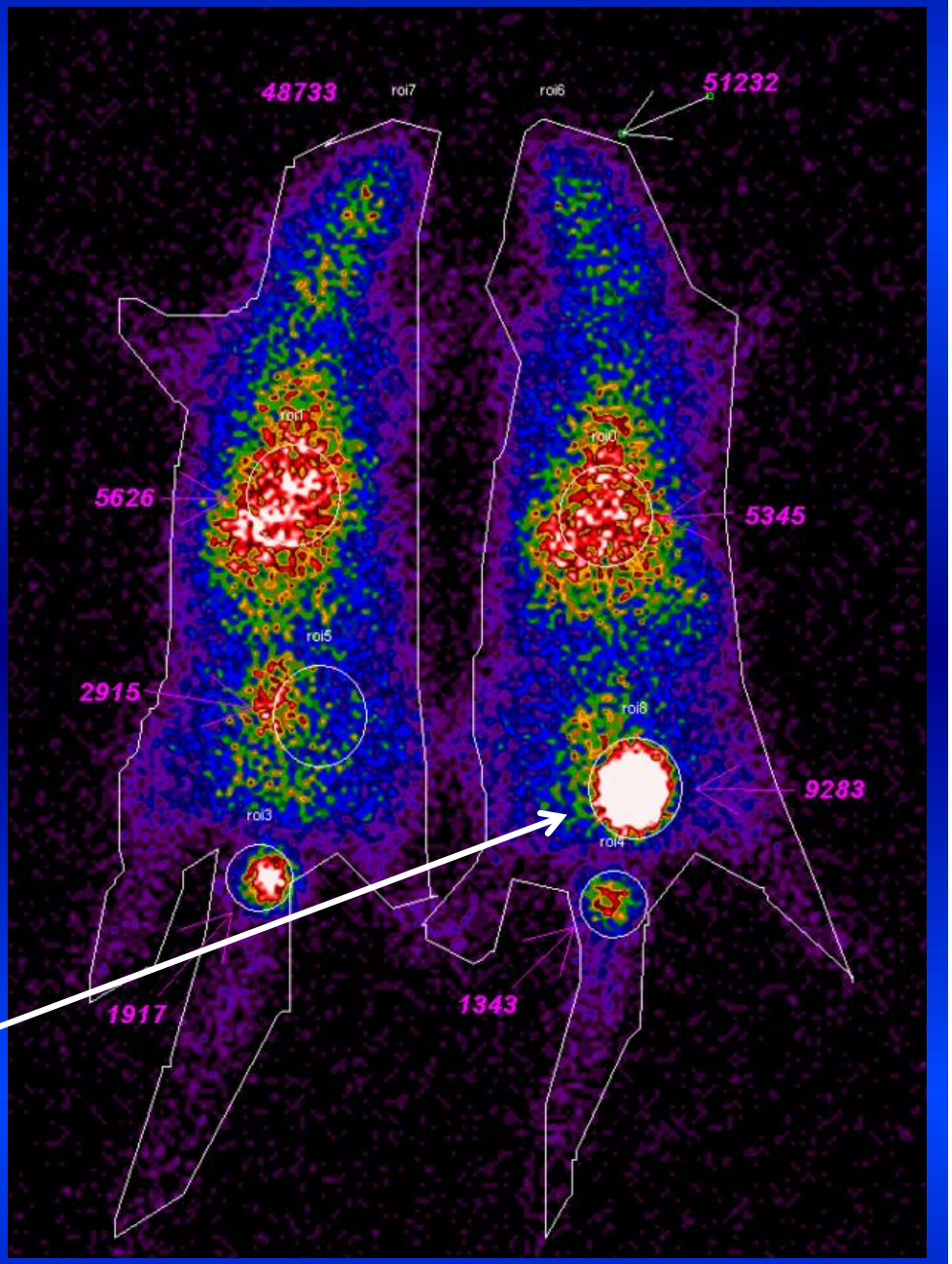
- A static acquisition was carried-out at 45';

- The analysis of dynamic acquisition was performed by region of interest and relative time-activity curves over heart, liver, spleen, kidneys and area subtended by the magnet;

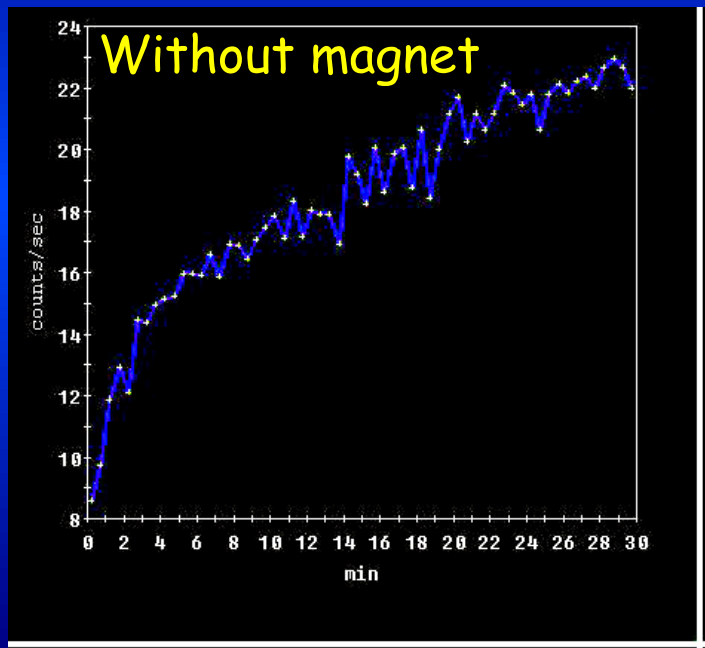
- The percentages of total activity detected in each organ were also calculated from 45 min. static acquisition.

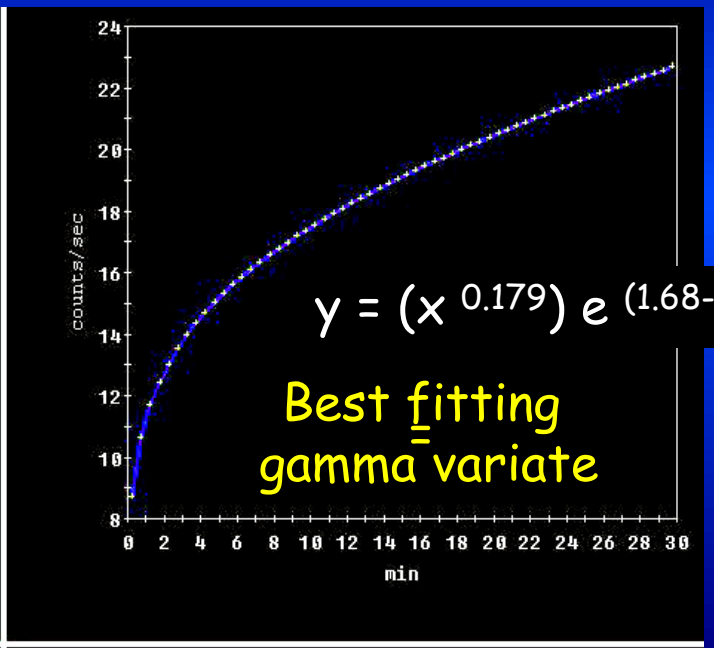
Scintigraphic results





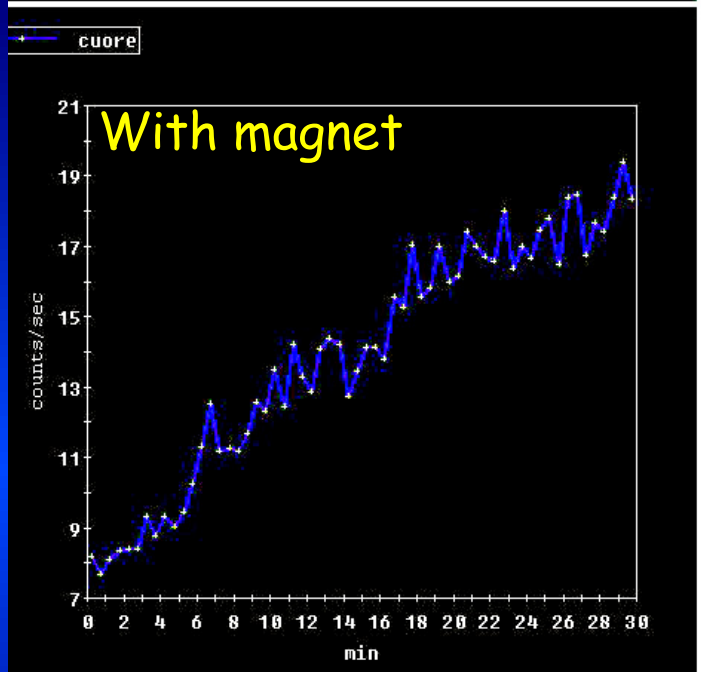
Heart time-activity curves

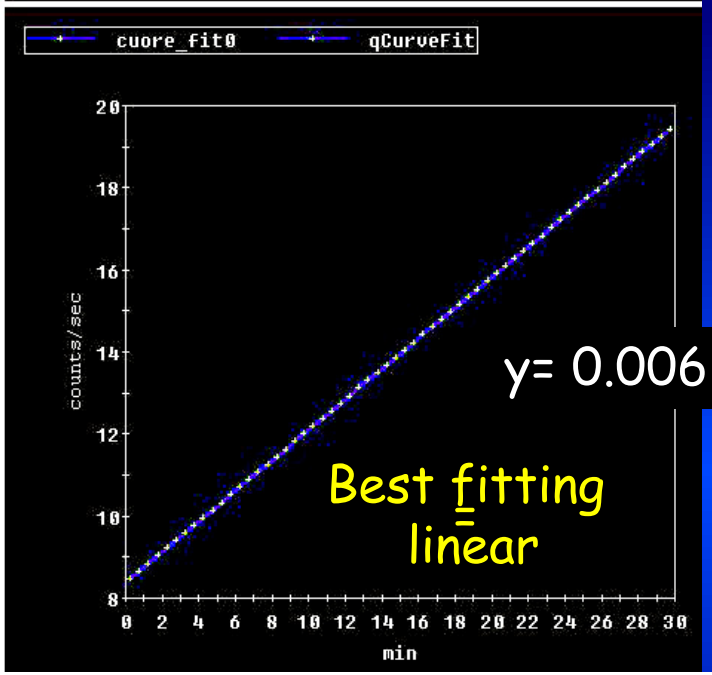




$y = (x^{0.179})\, e^{(1.68-2x/-35882.5)}$

Best fitting gamma variate

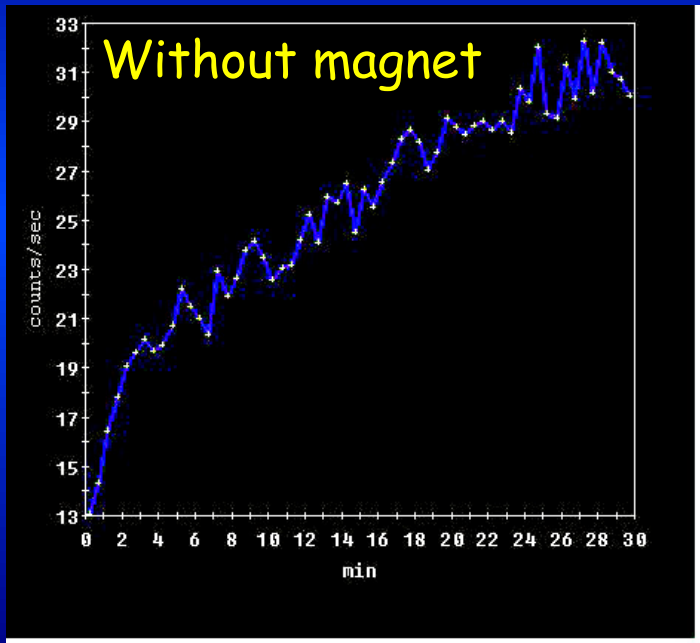


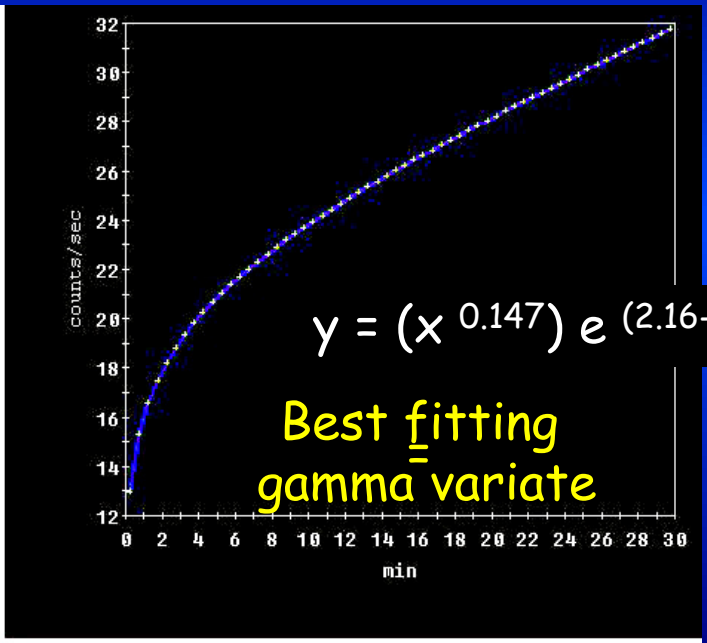


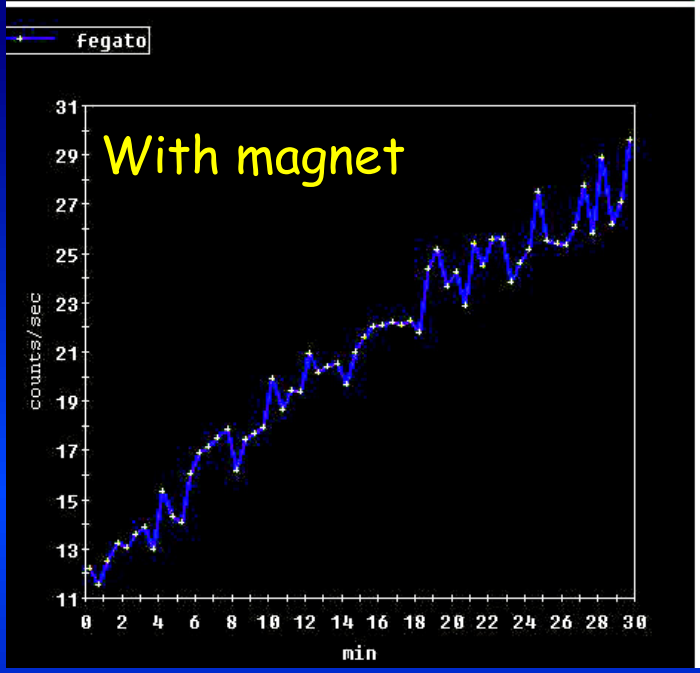
$y = 0.00619x + 8.38$

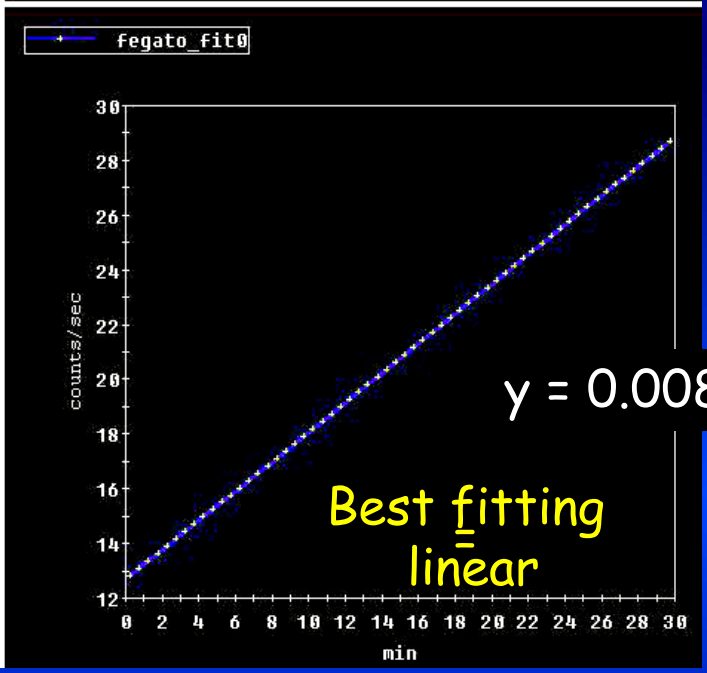
Best fitting linear

Liver time-activity curves

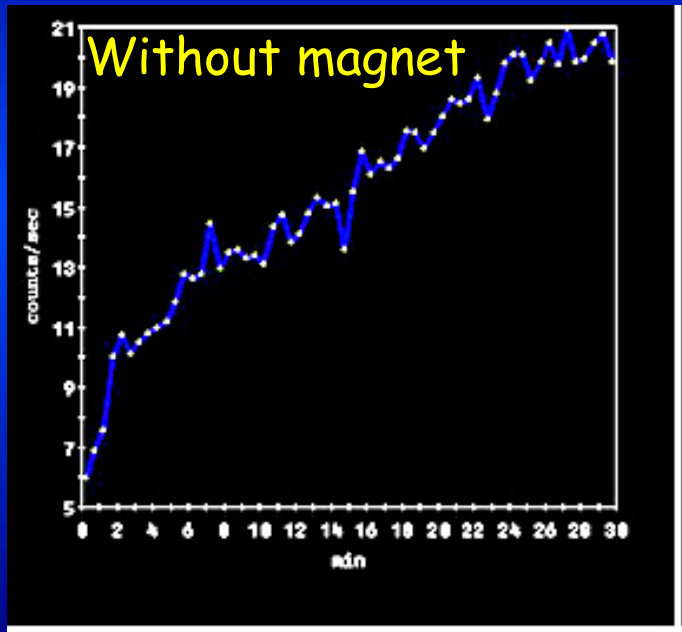


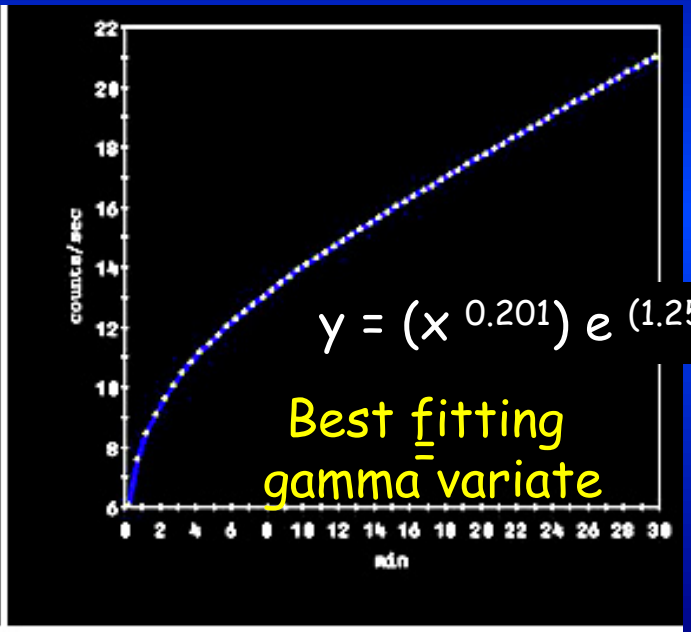






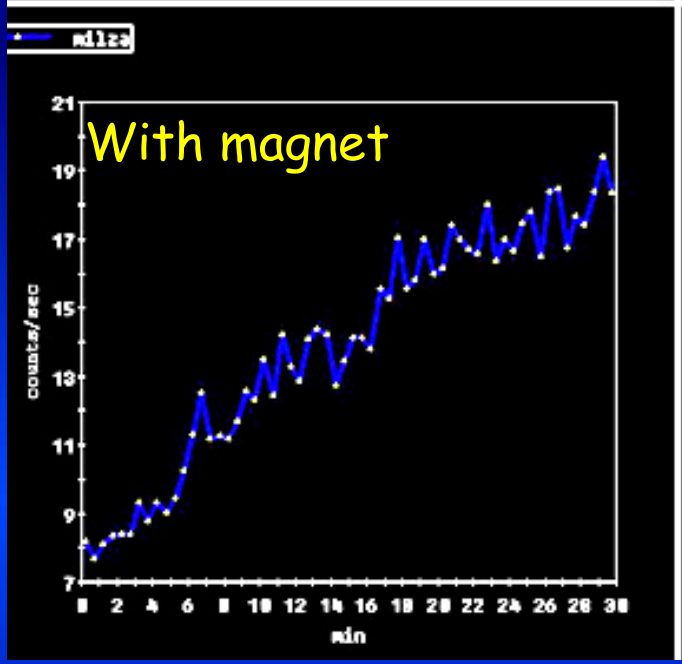
Spleen time-activity curves

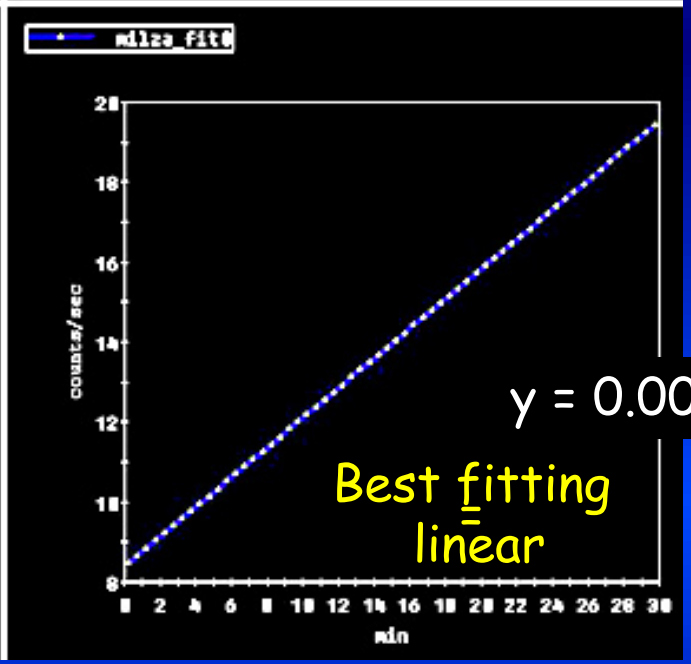




$y = (x^{0.201})\, e^{(1.25-2x/-12691.4)}$

Best fitting gamma variate





$y = 0.00619x + 8.38$

Best fitting linear

Biodistribution of MNPs in the rats at 45 min. in relation to the presence of a magnetic field (% of total radioactivity)

	Area subtended by the Magnet	Liver	Spleen	Hearth
With magnet	26.9	13.76	7.75	6.83
Without magnet		15.98	9.43	10.96

Conclusions

- These results are **absolutely preliminary** and need to be confirmed by other studies

- Within these limits, the application of an external magnetic field seems to be promising for confining in predetermined sites MNPs labelled with radioactive isotopes or other substances