



 biospace lab

μ imager

Application Notes

## Comprehensive functional imaging of metabolism, perfusion and interstitial fluid on histopathologic slices of infarcted myocardium

*S. Poussier<sup>1</sup>, F. Maskali<sup>1</sup>, P. Y. Marie<sup>1</sup>, F. Carbillet<sup>1</sup>, A. Bertrand<sup>1</sup>, P. Olivier<sup>1</sup>, F. Plenat<sup>2</sup>, L. Antunes<sup>2</sup>, D. Meng<sup>2</sup>, H. Boutley<sup>1</sup>, G. Karcher<sup>1</sup>, S. Maitrejean<sup>3</sup>.*

*1.Laboratoire de Biophysique et Médecine Nucléaire, 2.Laboratoire d'Histopathologie Expérimentale et Moléculaire. Facultés de médecine de Nancy - BP184 – 54505 Vandoeuvre les Nancy. 3.Biospace Instruments, Paris.*

*Contact : sylvain.poussier@medecine.uhp-nancy.fr*

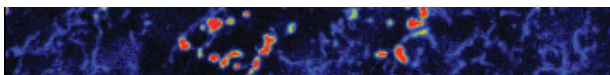
### Goal :

Histopathologic slices from infarcted hearts allow the assessment of the repartition of:

- (i) structural abnormalities (fibrosis development, cellular loss, oedema...) and
- (ii) proteins, hormones and receptors involved in the subsequent repairing process (immuno-histology, in situ hybridization, ...). However, it is likely that these results depend on local variations in the functional status of myocardium and especially on the levels of tissue perfusion and cell viability. If available, such functional information might markedly enhance the understanding of histopathologic analyses.

In vivo, three main techniques may be used for assessing metabolism and perfusion of myocardium:

- (i) glucose uptake analyzed with PET-<sup>18</sup>FDG,
- (ii) tissue perfusion analyzed using SPECT tracers such as <sup>99m</sup>Tc-Sestamibi or <sup>201</sup>Tl and
- (iii) extracellular fluid volume analyzed with MRI tracers such as DTPA evidencing the reduction in the volume of cell compartment and/or the fibrosis development.



All these tracers may be labelled with  $\beta$  emitter tracers. Therefore, using a high resolution imager for  $\beta$  emitters, the  $\mu$  IMAGER<sup>TM</sup>, it was postulated that the distribution of such tracers could be, simultaneously, precisely imaged and quantified on histopathologic slices with a high spatial resolution ( $\sim 20\mu\text{m}$ ). The  $\mu$  IMAGER<sup>TM</sup> is a high resolution radioactive disintegration imaging/counting system that allows the recording of data in the list mode. This records includes spatial coordinates as well as the time at which each disintegration occurs.

The aim of this study was to determine whether a global and comprehensive functional imaging of metabolism, perfusion and interstitial fluid could be provided by the  $\mu$  IMAGER<sup>TM</sup> on histopathologic slices of infarcted myocardium from rats. In a first step, the ability of the  $\mu$  IMAGER<sup>TM</sup> to separate activities provided by 3 different tracers ( $^{18}\text{F}$ ,  $^{99\text{m}}\text{Tc}$  and  $^{111}\text{In}$ ), using the time information recorded by the instrument, was tested on phantoms.

## Material and methods :

### *Phantom study*

Four cylindrical solid phantoms, containing homogeneous concentrations of isotopes, were made. Three were filled with only one isotope ( $^{18}\text{F}$ ,  $^{99\text{m}}\text{Tc}$  or  $^{111}\text{In}$ ) and the fourth was filled with a mixture of the three isotopes. Isotopes were initially added to a solution containing: 2ml Acrylamide-Bis-Acrylamide at 40%, 14 $\mu\text{l}$  Ammonium Persulfate liquid and 1,2 $\mu\text{l}$  N,N,N',N'-Tetramethylethylenediamine(Temed), with the following activities:

- + 16.4 MBq of  $^{18}\text{F}$  in a 0.4 mL solution
- + 29.6 MBq of  $^{99\text{m}}\text{Tc}$  in a 0.3 mL solution
- + 0.5 MBq of  $^{111}\text{In}$  in a 0.4 mL solution
- + a mixture of 4.1 Bq of  $^{18}\text{F}$ FDG, 9.9 MBq of  $^{99\text{m}}\text{Tc}$  and 0.2 MBq of  $^{111}\text{In}$  in a 0.3 ml solution

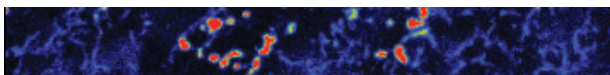
Each of the 4 solutions were placed into a cylindric mold 7 mm in diameter and frozen at -80°C. Solid slices of 14  $\mu\text{m}$  thick were obtained using a cryostat (LEICA CM 3050S). The image acquisition was started 2 hours later.

### *Animal study*

Myocardial infarct injury was induced by coronary ligation: after left lateral thoracotomy in animals under general anaesthesia and tracheal intubation, the left anterior descending artery was tied by means of a 7-0 prolene suture.

One to 3 months later, radiotracers were injected intravenously (penienne vein) and under a slight general anesthesia (intra-peritoneal injection of 0.4 mL of pentobarbital):

- + a 0.2 mL solution containing 55.2 MBq of  $^{18}\text{F}$ FDG was injected 1 hour after 1,2mL of glucose feeding and 1 hour prior to animal sacrifice,
- + a 0.3 mL solution containing 23.4 MBq of  $^{99\text{m}}\text{Tc}$ -Sestamibi was injected immediately after  $^{18}\text{F}$ FDG injection,
- + a 0.6 mL solution containing 15.6 MBq of  $^{111}\text{In}$ -DTPA was injected 10 min before animal sacrifice.



The animal was killed by injection of 4 mL of pentobarbital and a thoracotomy was immediately performed for excising and freezing the heart.

Histopathologic slices of 8  $\mu\text{m}$  thick, oriented along the horizontal long-axis of the heart, were obtained using a cryostat LEICA CM 3050S. The image acquisition was started 2 hours later.

### *Imaging protocol*

Phantom slides were exposed during 20 hours, whereas histopathologic slices were exposed 48 hours. Although long exposure gives better results, it has been shown that isotope separation can be accurately performed with much shorter exposure (six hours). The three  $\beta$  emitter distributions were separated according to the following technique : first, activity versus time curves was computed at each pixel using time information recorded in the list file. Then, these curves were compared to a weighted sum of three decay curves which half periods are respectively, 108 mn ( $^{18}\text{F}$ ), 6.02 hours ( $^{99\text{m}}\text{Tc}$ ) and 2.8 days ( $^{111}\text{In}$ ). The three weights obtained for each pixel correspond to the number of disintegrations of each isotope. Once this calculation had been made, three new list files were generated by adding to the already existing information (coordinates, time), the probability for each information to be issued from Fluor (first file), Technetium (second file), or Indium (third file). The time decay of the three new files follow exactly the natural decay curves of the corresponding isotopes.

### *Phantom study results*

In the phantom study, the separation of the 3 isotopes could be obtained with a precision on quantitation better than 5%.

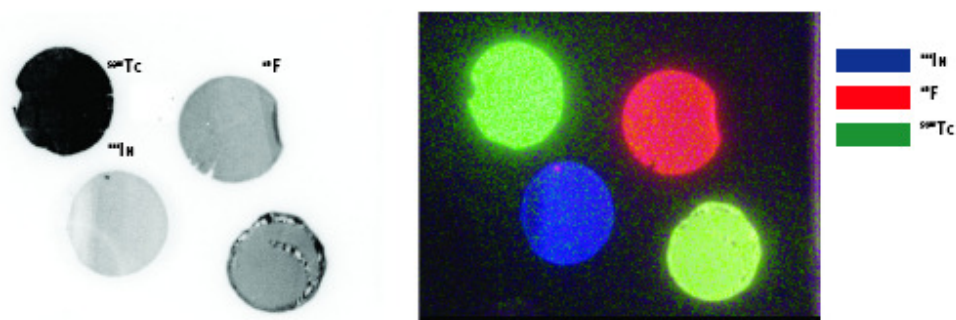
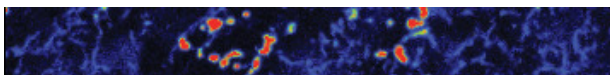


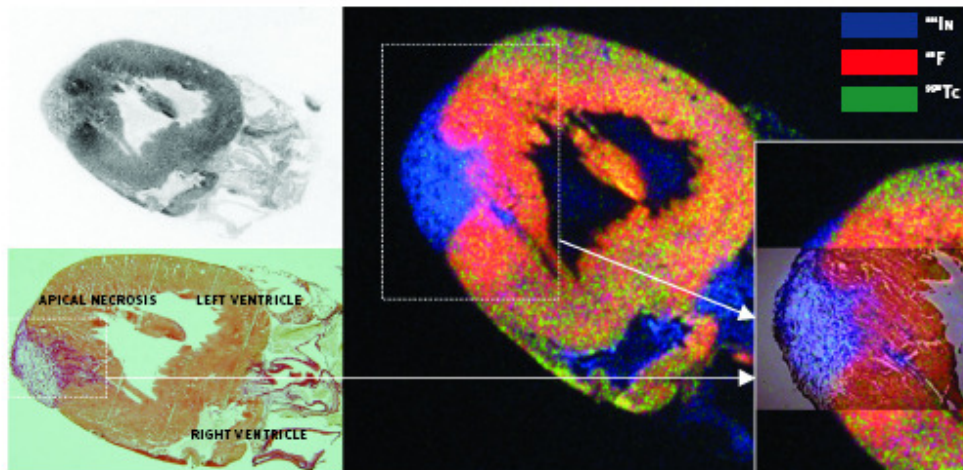
Figure 1 :  $\mu$  IMAGER™ images performed on slices from the 4 solid gels. The total activity is represented in a grey scale on the left image. Activities from each of the 3 isotopes were computed as described, and represented on the right panel by attributing a primary color ( Red, Blue, Green) to each isotope.



### Animal study results

Two experiments, performed in old myocardial infarctions from rats following the above protocol, have given evidence that the  $\mu$  IMAGER™ was able:

(i) to correctly detect the enhanced extra-cellular fluid within fibrotic areas (enhanced activity from  $^{111}\text{In}$ -DTPA within the network of collagen fibres that is represented in blue in Figure 2) and



**Figure 2 :** Images from a histopathological slice of infarcted myocardium from a rat, which was injected by  $^{18}\text{F}$ FDG,  $^{99\text{m}}\text{Tc}$ -Sestamibi and  $^{111}\text{In}$ -DTPA prior to sacrifice. Image acquisition was performed with the  $\mu$ IMAGER™; the slide was then colored with a red sirius solution (lower left panel) revealing in red the fibrosis within the infarcted region. The total  $\mu$  IMAGER™ image is represented in a grey scale in the upper left panel. Distributions from each of the 3 isotopes were computed as described, and represented in the right panel by attributing a primary color ( Red, Blue, Green) to each isotope.

(ii) to image the ischemic and viable area, located around necrosis and characterized by a decrease in perfusion (decreased activity from  $^{99\text{m}}\text{Tc}$ -Sestamibi represented in green in Figure 2) and an enhanced glycolytic metabolism (enhanced activity from  $^{18}\text{F}$ FDG represented in red in Figure 2).

### Conclusion :

This study shows that a global and comprehensive functional imaging of metabolism, perfusion and interstitial fluid of infarcted myocardium from rats can be provided by using multiple markers in vivo and analyzing post mortem the corresponding distribution of each marker on histopathologic slices. Such global functional information is available using the  $\mu$  IMAGER™ and is likely to markedly enhance the understanding of conventional histopathologic analyses.



10, rue Mercœur, 75011, Paris, France  
 t+33 (0)1 55 25 60 60/f+33 (0)1 55 25 60 61  
 185 Alewife Parkway #410, Cambridge, MA02138, USA  
 t+1 330 998 1099/f+(0)1 888 467 3196  
[info@biospace.eu](mailto:info@biospace.eu)/[www.biospace.eu](http://www.biospace.eu)