New Perspectives in Molecular Imaging of Cardiovascular Diseases

F. Garibaldi - INFN – Roma1

- molecular imaging: the role of radionuclides techniques

- building an open, flexible system

- cardiovascular diseases (diagnosis and therapy)
  - detecting vulnerable atherosclerotic plaque
  - stem cell therapy of heart infarction

- conclusions and outlook

Collaboration

Istituto Superiore di Sanita'

E. Cisbani
S. Colilli
R. Fratoni
F. Garibaldi
TESA M. Gricia
M. Lucentini
F. Santavenere
S. Torrioli

Farmaco G. Marano
M. Musumeci
Farmaco

Ematologia M. Baiocchi
Oncologia L. Vitelli

INFN - Roma1

F. Cusanno
M.L. Magliozzi

Jefferson Lab (DOI)

S. Majewski
D. Weisemberger
B. Kross
J. Proffit

Johns Hopkins University

B.Tui
Y Wang

University of Rome

G. De Vincentis

Molecular Imaging

- "… the *in vivo* characterization and measurement of biologic processes at the cellular and molecular level.

- - It sets forth to probe the molecular abnormalities that are the basis of disease rather than to image the end effects of these molecular alterations.

- Imaging of specific molecular targets enables:

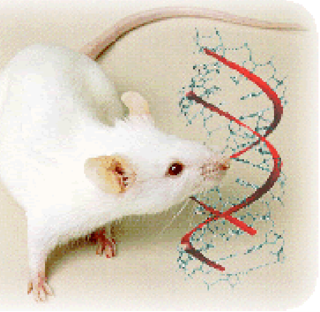
  ✓ earlier detection and characterization of disease;

  ✓ earlier and direct molecular assessment of treatment effects;

  ✓ more fundamental understanding of disease processes.

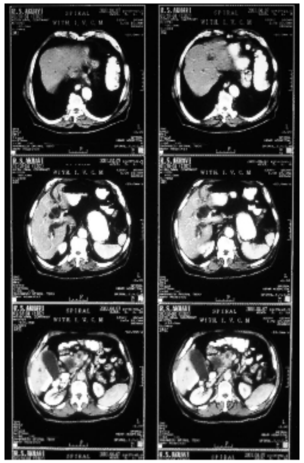
• The rat and <u>mouse</u> host a large number of human diseases
  * Opportunity to study disease progression / therapeutic response
    ✓ under controlled conditions
    ✓ non-invasively
    ✓ in same animal
    ✓ repetitively

Mice advantages: small size, rapid gestation period large litter size, low maintenance costs. Moreover, the mouse genome has been extensively characterized. Gene-targeted "knock-out" and transgenic overexpressionexperiments are performed using mice, rather than rats, but submm spatila resolutin needed!



Molecular Imaging Modalities

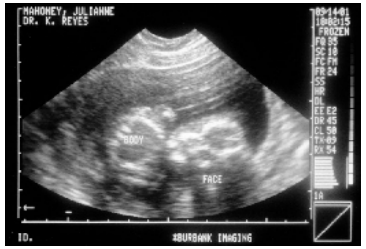
CT



A

Tissue Densit
20-50 µm

Ultrasound



A F

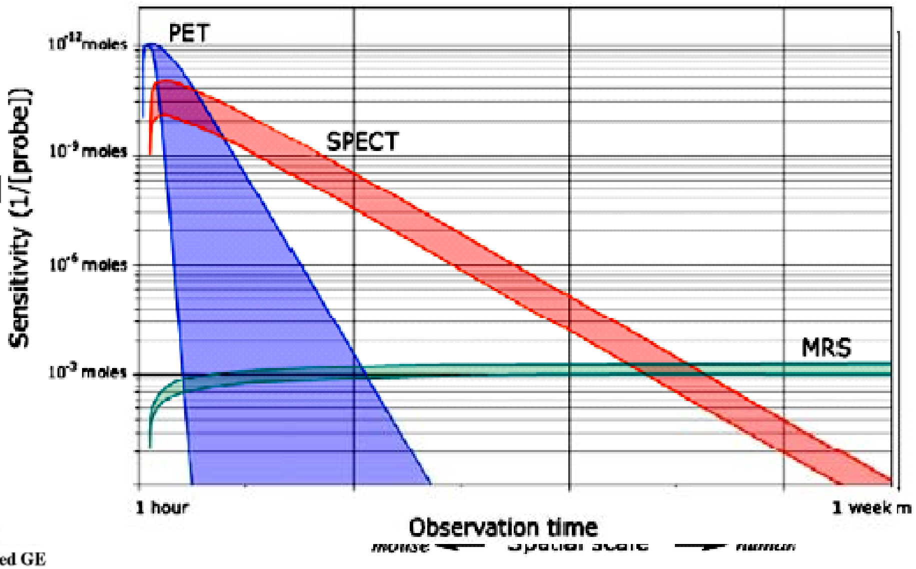
Structure
0.1 mm
Doppler

Optical
(Bioluminescence, fluorescence)

A M

Topography
µm to mm
~10^3 cells
≠ quantitative

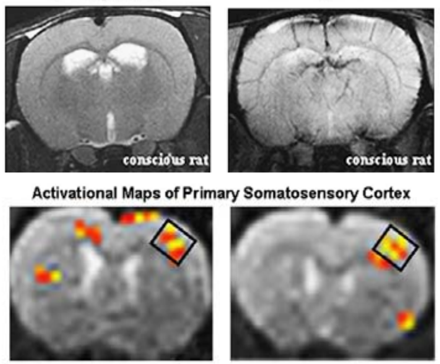
Unique !!



MRI

4.7T, Dual Coil, Coil,
T1 Weighted SE

4.7T, Dual
T2 Weighted GE

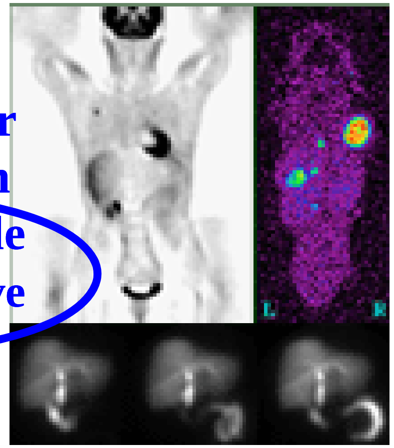


A F M

H Concentration
0.1 mm
BOLD, DCE
β-galactocidase
0.1 µmole H / µmole ^{31}P

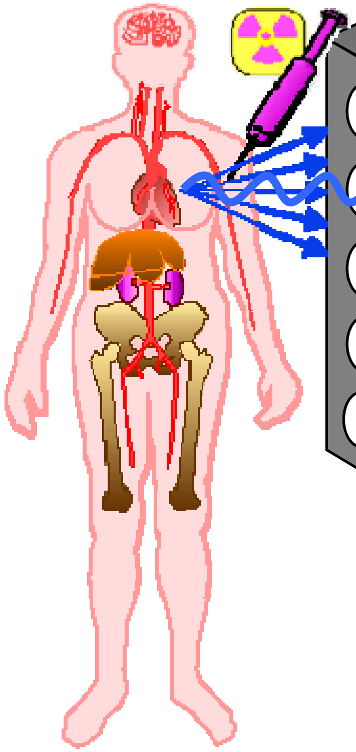
PET/SPECT

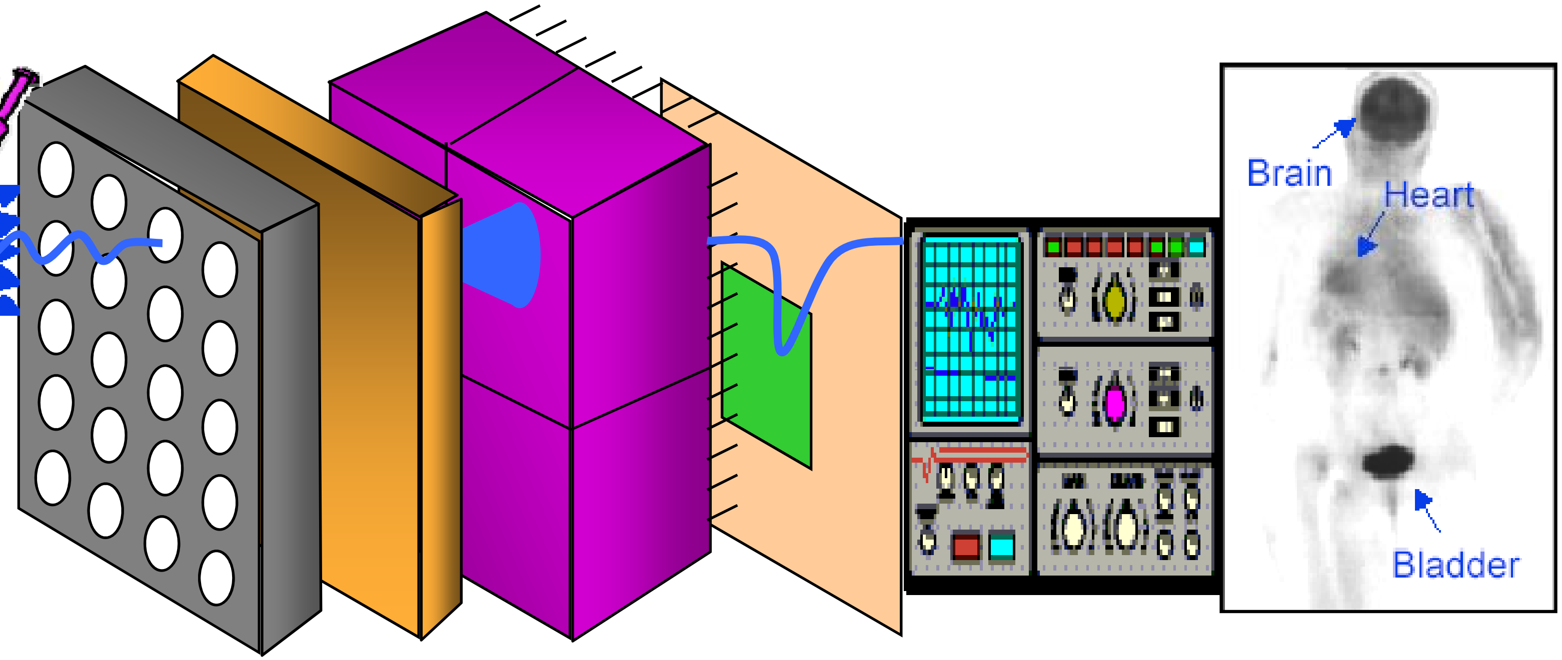
F M



Radiotracer
~1-2 mm
<10^{-12} mole
= quantitative

Single Photon Detector Module





Patient injected with radioactive drug.

Drug localizes according to its metabolic properties.

Gamma rays, emitted by radioactive decay, that exit the patient are imaged.

1. Collimator

Only gammas that are perpendicular to imaging plane reach the detector

2. Scintillator

Converts gammas to visible light

3. Photodetector

Convert light to electrical signal

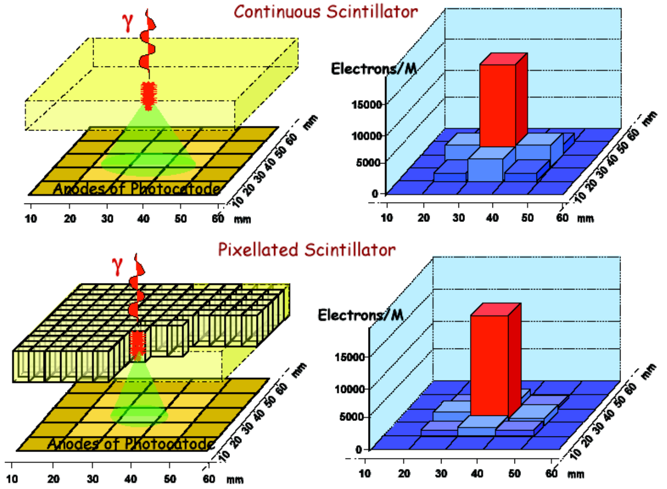
4. Readout Electronics

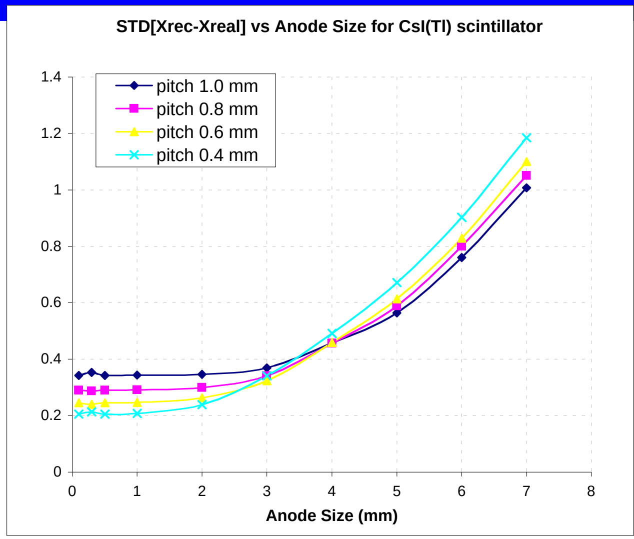
Amplify electrical signal and interface to computer

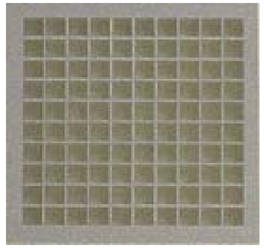
5. Computer decoding procedure

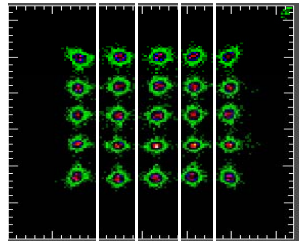
Elaborate signal and gives image output

Importance of pixel identification



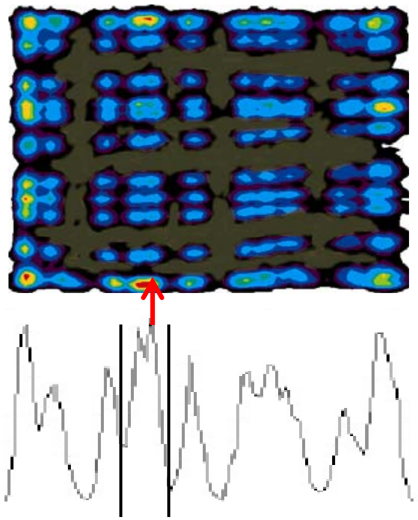




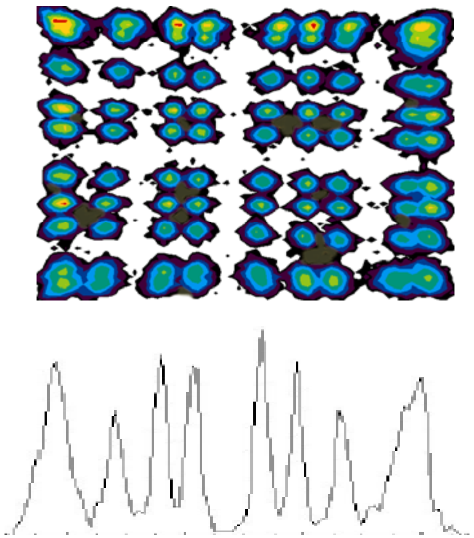


good pixel identification is fundamental for correct digitization affecting spatial resolution and contrast

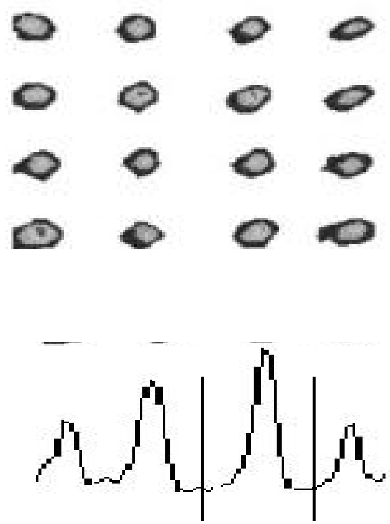
C8 strips



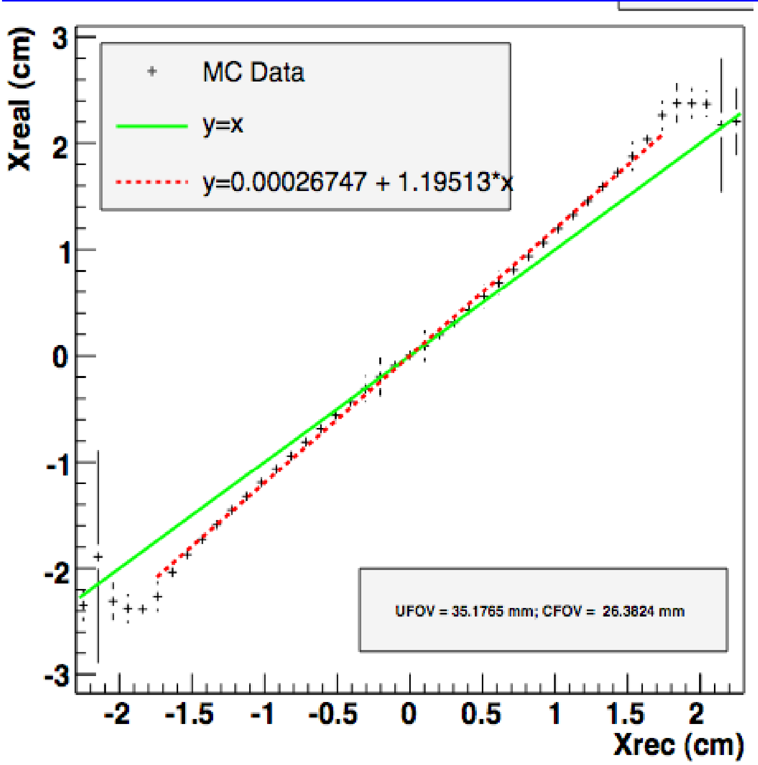
M16 (4 x 4) mm²



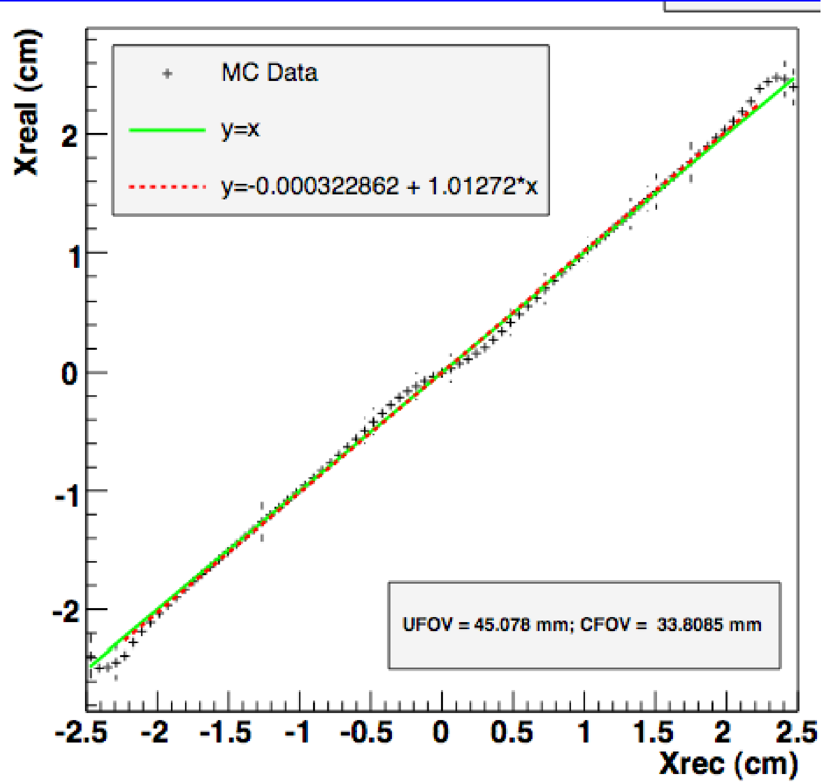
M64 (2 x 2) mm²

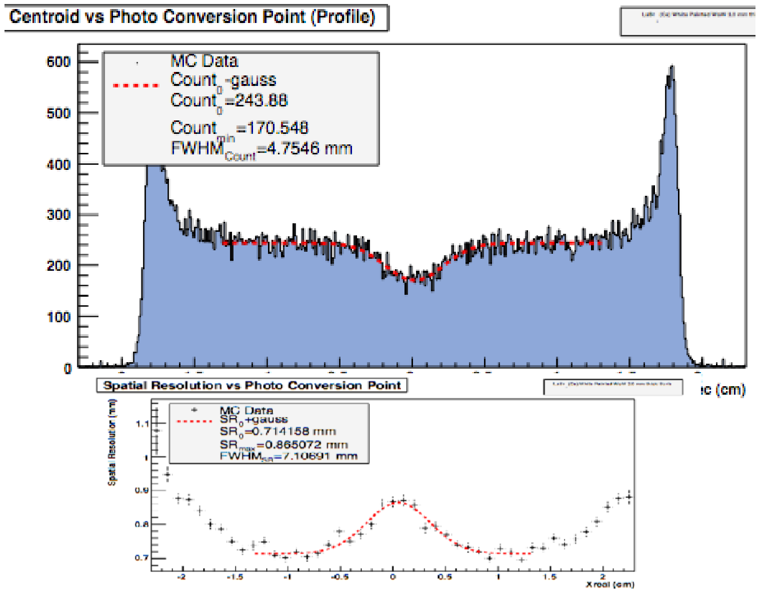


Labr3 Continuoum different performances for different window treatment, diffusing (a), absorbing (b)

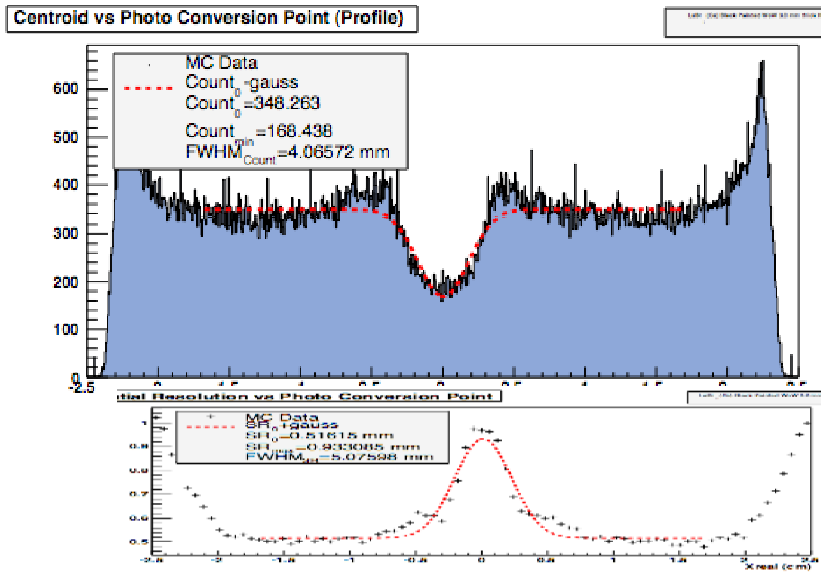


a





b



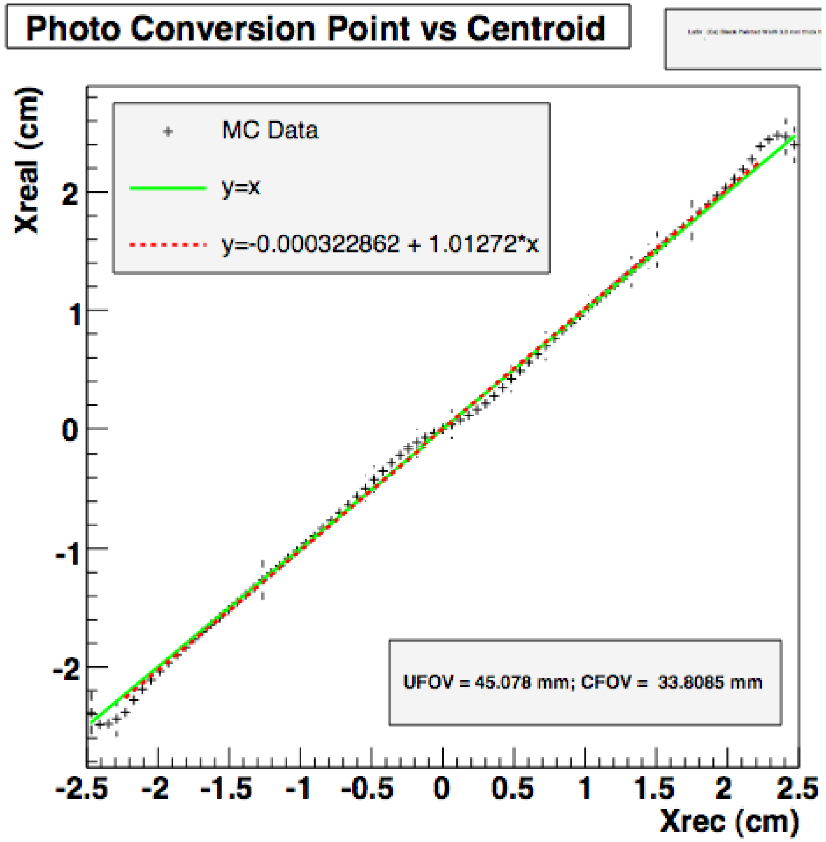
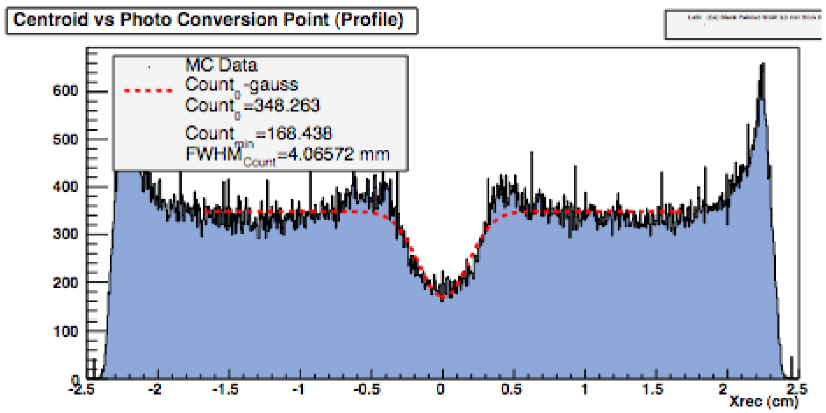
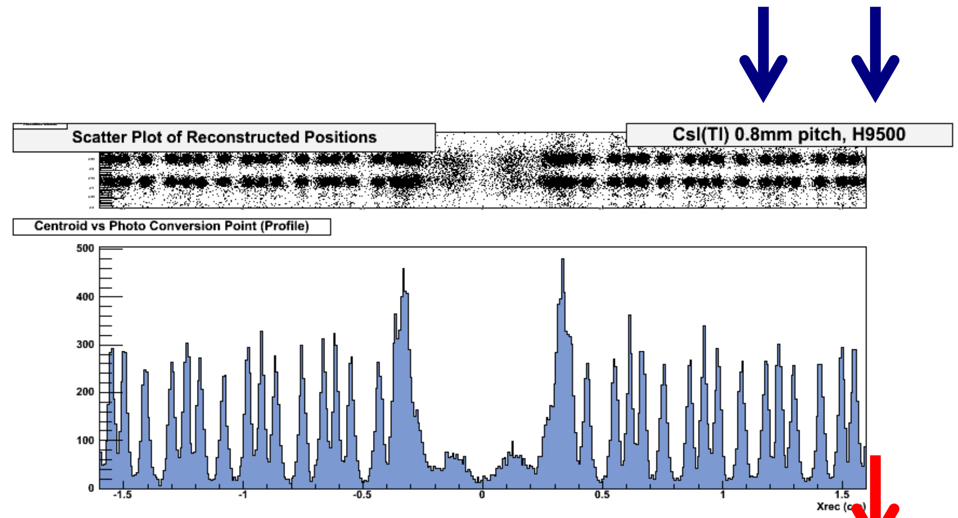
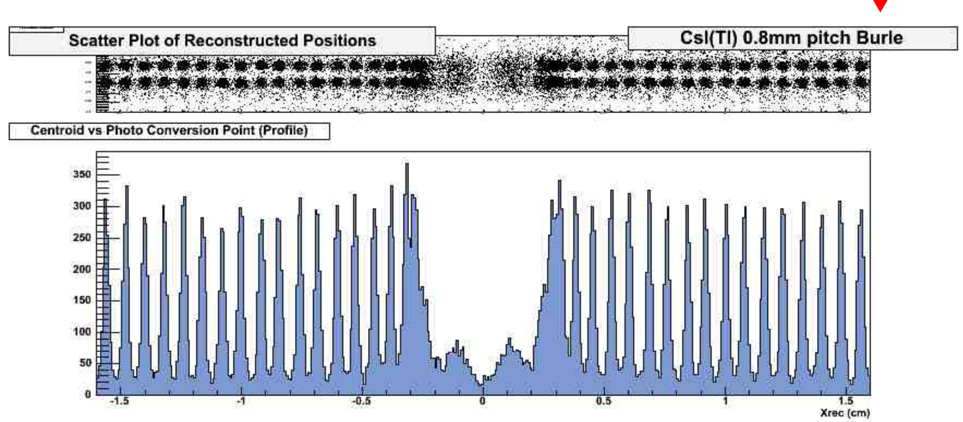
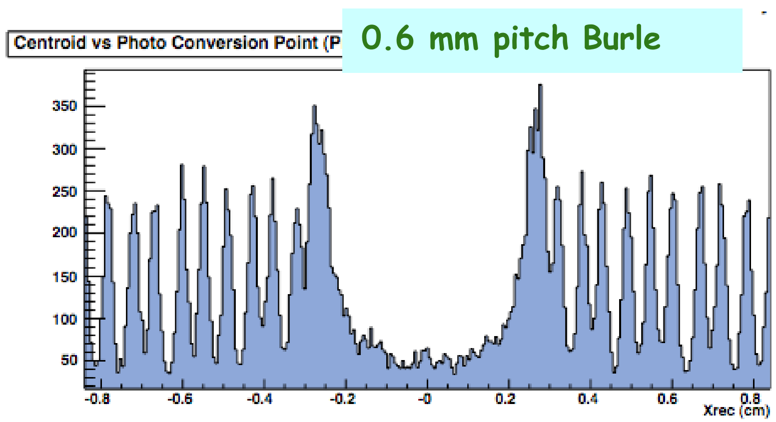


Photo Conversion Point vs Centroid
MC Data
y=x
y=-0.000322862 + 1.01272*x
UFOV = 45.078 mm; CFOV = 33.8085 mm
Xreal (cm)
Xrec (cm)


Centroid vs Photo Conversion Point (Profile)
MC Data
Count_0-gauss
Count_0=348.263
Count_min=168.438
FWHM_Count=4.06572 mm
Xrec (cm)


Scatter Plot of Reconstructed Positions
CsI(Tl) 0.8mm pitch, H9500
Centroid vs Photo Conversion Point (Profile)
Xrec (cm)


Scatter Plot of Reconstructed Positions
CsI(Tl) 0.8mm pitch Burle
Centroid vs Photo Conversion Point (Profile)
Xrec (cm)

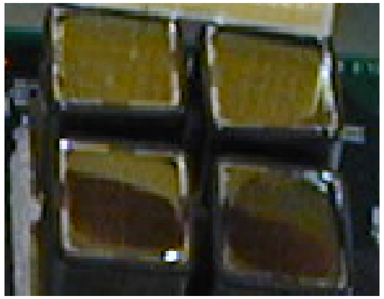

Centroid vs Photo Conversion Point (P
0.6 mm pitch Burle
Xrec (cm)

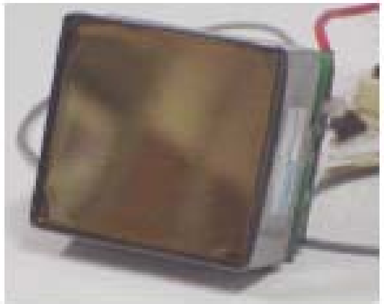
spatial resolution and linearity

- number of p.e.
- light spread
- light sampling

- scintillator thickness
- granularity

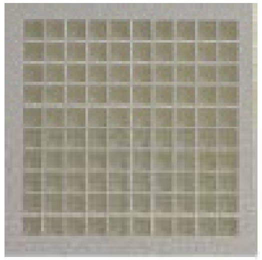
- R5900 C8 strips, 4 mm
- R5900 M16 4x4 mm2
- R5900 M64 2x2 mm2
- H8500 6x6 mm2
- H9500 3x3 mm2





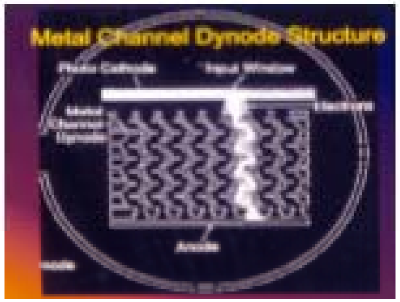
C8,
M16,
M64

H8500
H9500



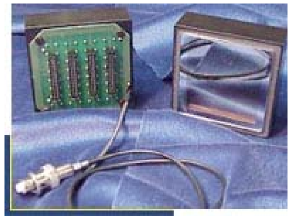
NaI(Tl): 1.2 pitch, 1.5 pitch
6 mm thick

CsI(Tl) 0.4 -1.0 mm pitch



Hamamatsu PSPMT's

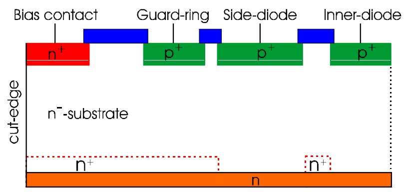
MCP (Burle)
1.5x1.5 mm2

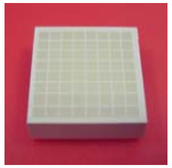


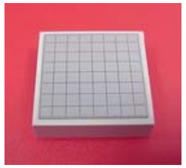
$LaBr_3$

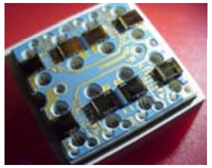
readout of channels individually is important
but
number of channels high

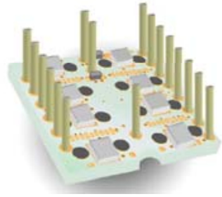
Silicon Drift Detectors

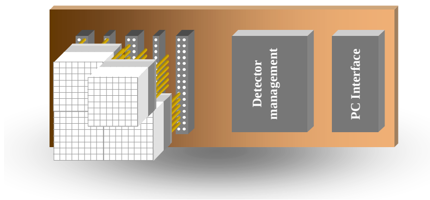




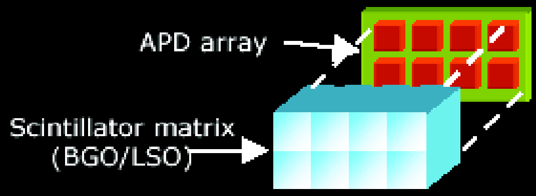


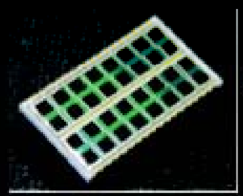






APD





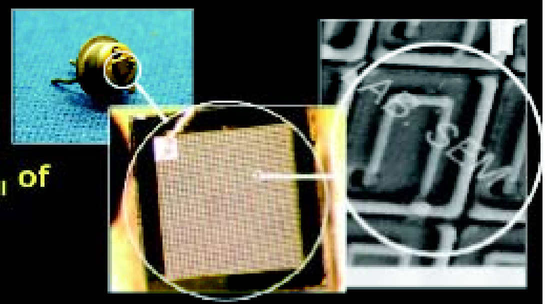
Implemented on MADPET II

+ High spatial resolution
+ No Pile-up
+ No scattering in the crystals
- Expensive
- Many channels
- Difficult tuning

SiPM

SiPM are p-n diodes operating in **Geiger mode**, which means that the bias voltage is above the diode breakdown voltage.

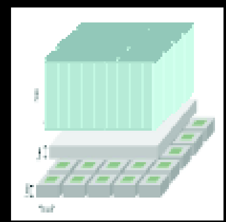
In this way output is independent from input:
⇨ the surface is divided into **m cells** (~1000/m²)



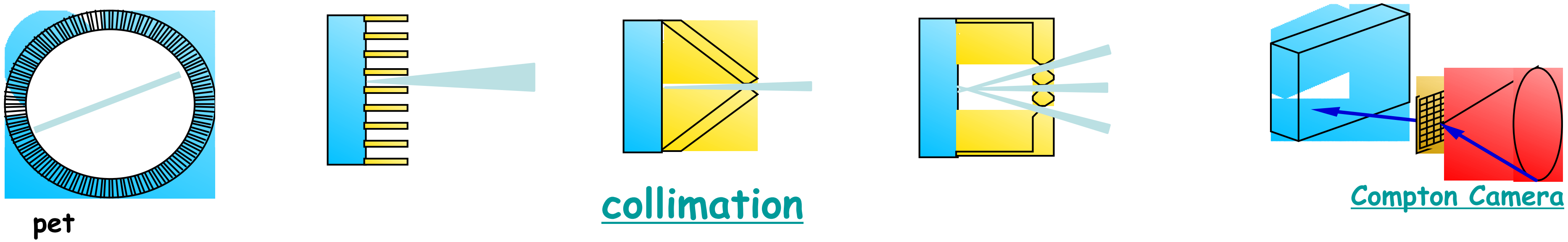
Signal ∝ N_{cell} of hit cells

+ High gain
+ Low noise
+ Good proportionality if $N_{photon} < N_{cell}$

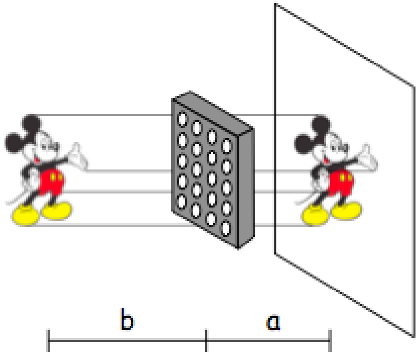
An array of SiPMs can be used for "individual" readout, instead of PSMPT

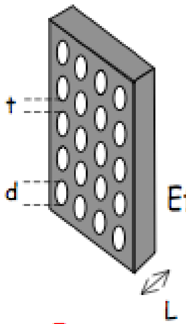


Si Pin diode: high QE, simple, economics, but no gain ! noise etc



Parallel Holes



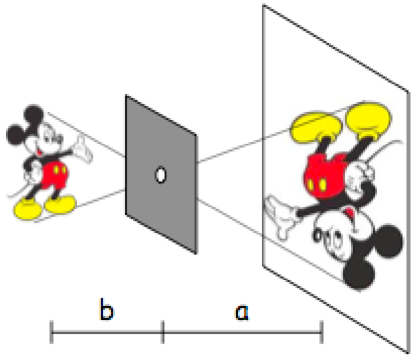


$$\text{Resolution} = \frac{d}{L}(L+b)$$

$$\text{Efficiency} \propto \left(\frac{d}{l+b}\right)^2 \left(\frac{d}{d+t}\right)^2$$

- Resolution & Efficiency inversely related

Pinhole

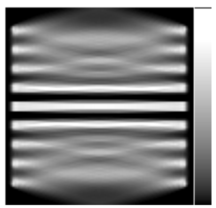


$$\text{Resolution} = d\left(1+\frac{1}{M}\right)$$

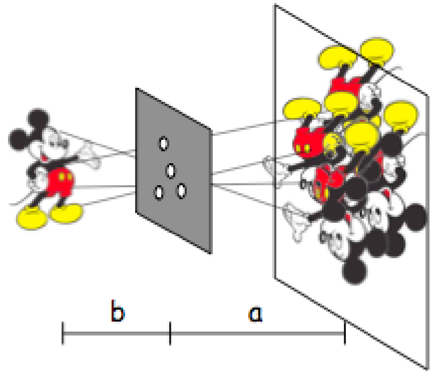
$$M = \frac{b}{a}$$

$$\text{Efficienza} \propto \left(\frac{d}{4b}\right)^2$$

- Better Resolution
- Little Field of View
- Low Efficiency



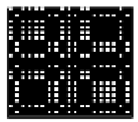
Coded Aperture



$$\text{Resolution} = d\left(1+\frac{1}{M}\right)$$

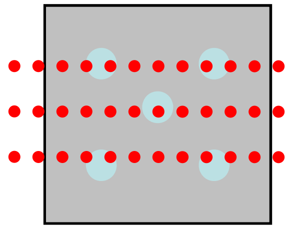
$$M = \frac{b}{a}$$

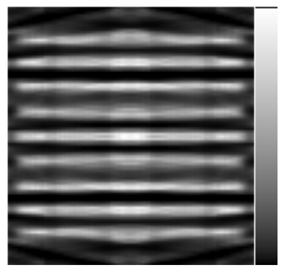
$$\text{Efficienza} \propto N\left(\frac{d}{4b}\right)^2$$



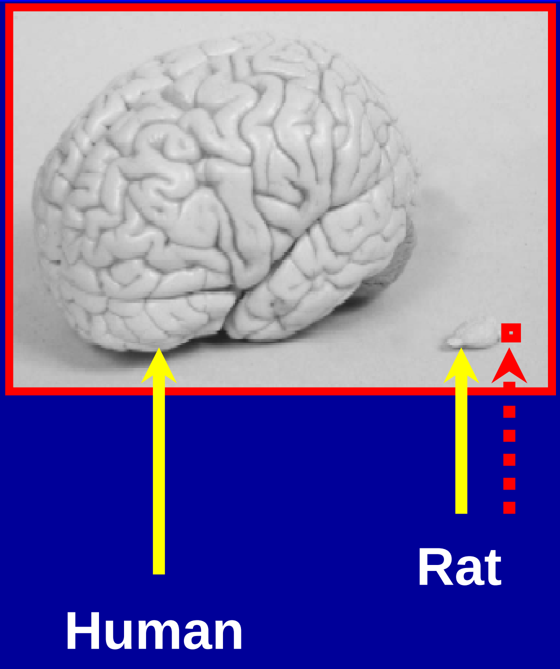
- Better Resolution
- High Efficiency
- Complicated Reconstruction

Multipinhole

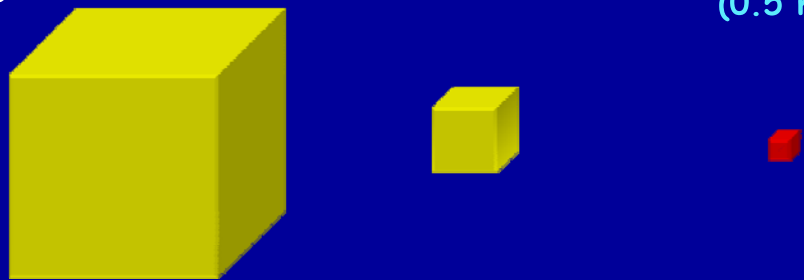




Performances not good enough for imaging biological process in vivo in small animals (mice)



	Man	Rat	Mouse
Body weight	~70 kg	~200 g	~20 g
Brain (cortex apex-temporal lobe)	~105 mm	~10 mm	~6 mm
Heart	~300 g	~1 g	~0.1 g
Aorthic cannula (□)	~ 30 mm	1.5 - 2.2 mm	0.9-1.3 mm (0.5 mm)



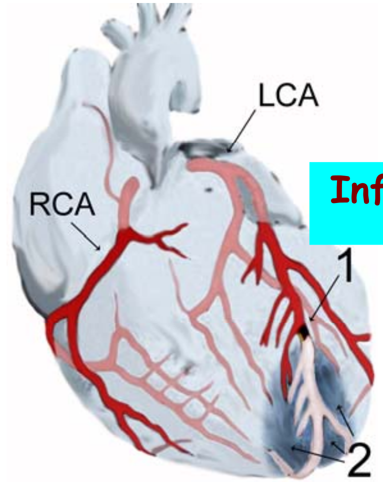
6 mm FWHM ($200\ mm^3$)	2 mm FWHM ($8\ mm^3$)	1 mm FWHM (1 mm3))

Required spatial resolution:

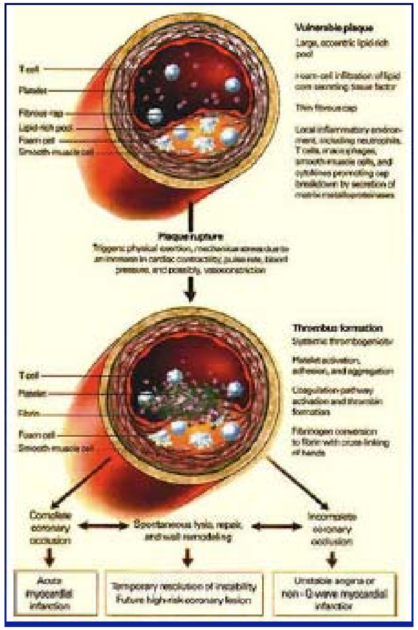
- Small size detectors (high pixellization)
- Individual detectors or "perfect" coding

→ **Submillimiter spatial resolution, high sensitivity needed**

Cardiovascular diseases



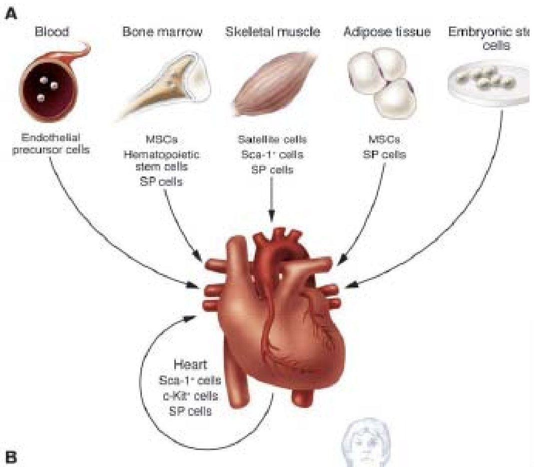
Infarction

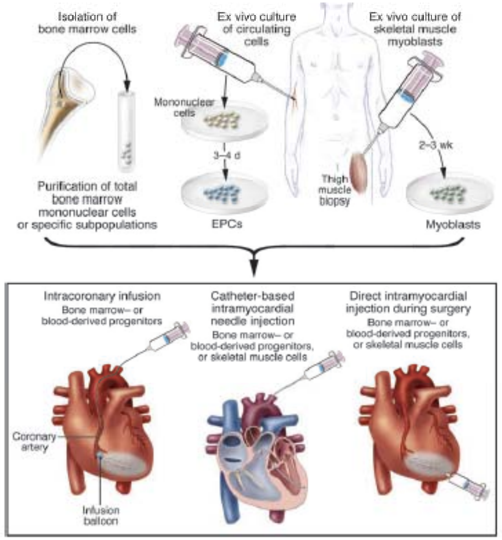


Diagnosys

Detection of cause of occlusiom

➲ ***Imaging* in vivo: detection of vulnerable plaques**





Theraphy

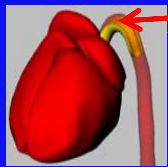
Stem cell theraphy for cardiac repair

➲ ***Imaging* in vivo: monitoring stem cells diffusion, differentiation, grafting, looking at the effects**

APPROACH

- Transgenic mouse model
  - *APOE-/- mice*
  - Spontaneous growth of atherosclerotic plaques accelerated by fatty diet
- Imaging agent
  - ^{99m}Tc-HYNIC-*Annexin-V* (Binds to apoptotic cells)

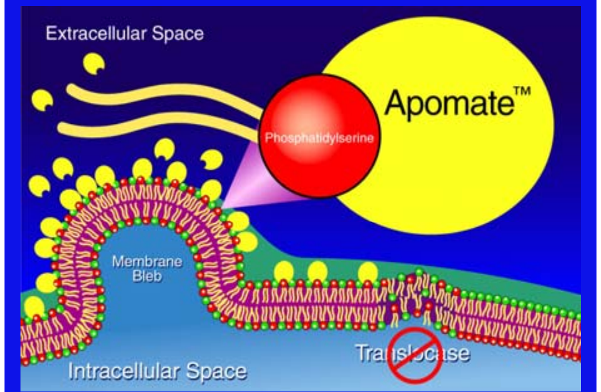
plaque



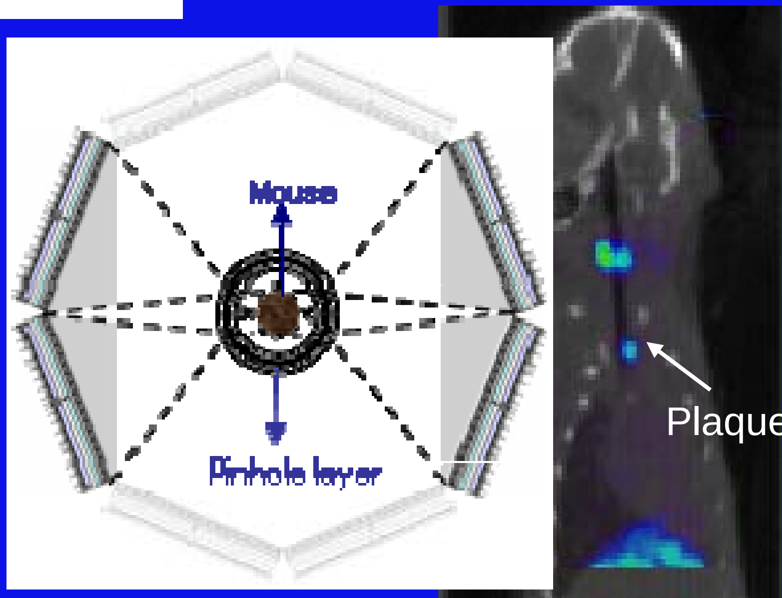
- ~ 2 mm diameter aorta
- ~ 0.5 X 1 X 4 mm3
- Total activity: ~ 1 microCi, (0.05% of average injected dose of ~2 mCi)

^{99m}Tc-Annexin-V SPECT images

Excised aorta from 37 weeks mouse

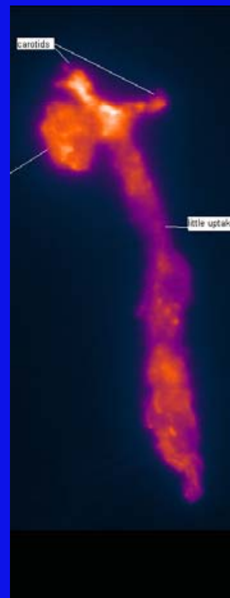


Apomate™: Trade name for Hynic Annexin V, North American Scientific, Inc.

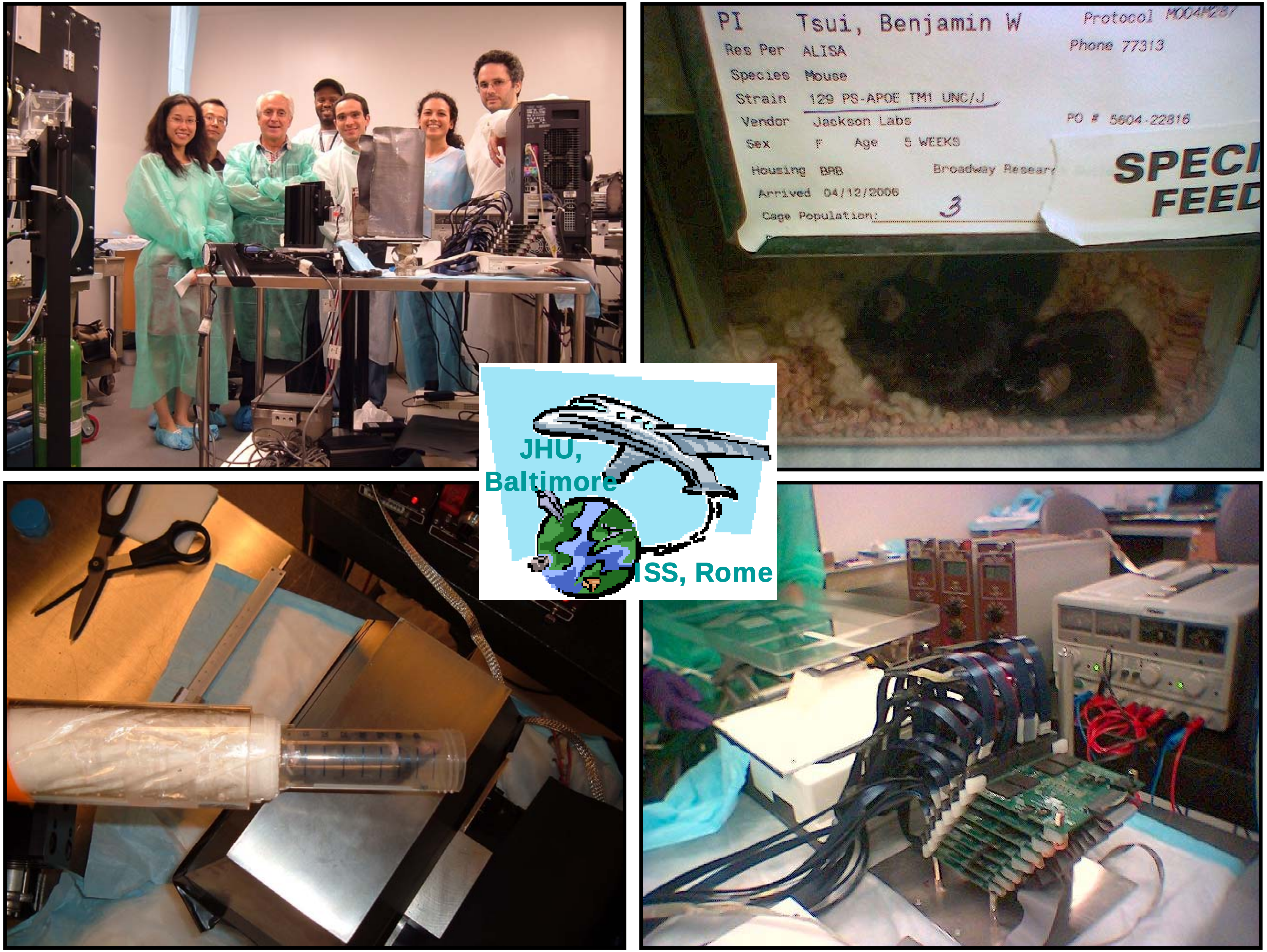


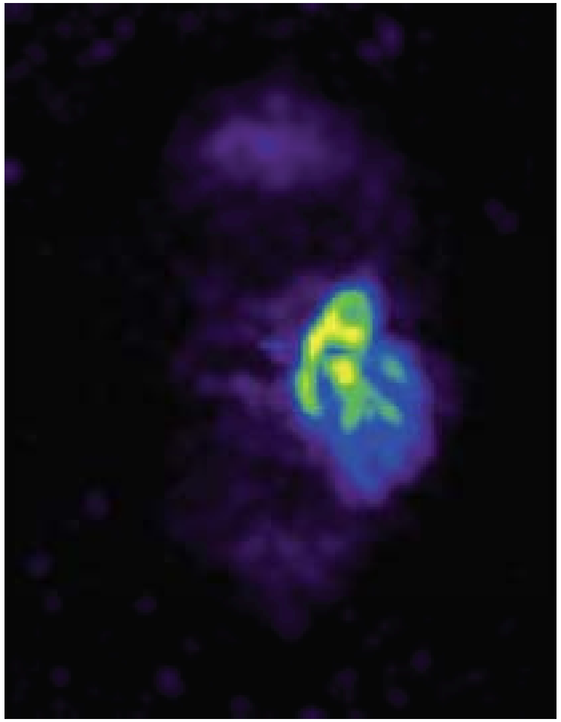
20 weeks

Photo

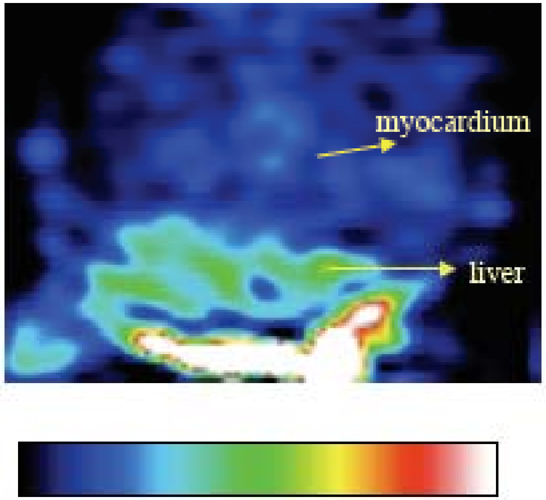


autoradiography


PI Tsui, Benjamin W
Phone 77313
Res Per ALISA
Species Mouse
Strain 129 PS-APOE TM1 UNC/J
Vendor Jackson Labs
PO # 5604-22816
Sex F Age 5 WEEKS
Housing BRB
Arrived 04/12/2006
Cage Population: 3
JHU, Baltimore
ISS, Rome

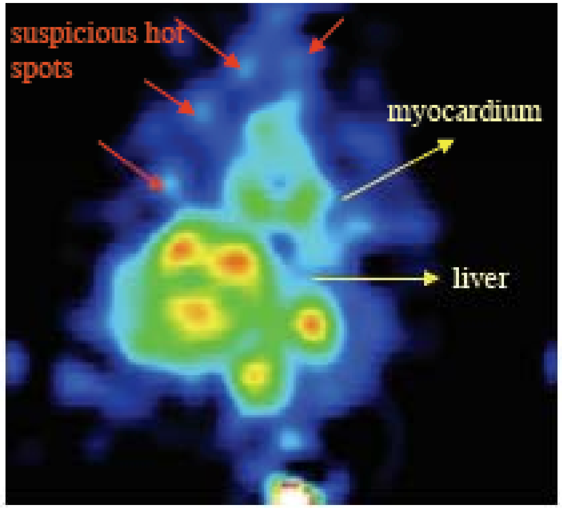


a. Topo di 6 settimane



b. Topo di 25 settimane

control



c. Topo di 25 settimane

Fig. 25. Immagini di topi geneticamente modificati (APOE -/-) cui e' stata iniettata Annexin V marcata con Tc-99. a. Uptake solo dal rene; b. non c'e' uptake da placche, si vede chiaramente il miocardio c. Si vede chiaramente l'uptake da placche aterosclerotiche

nature

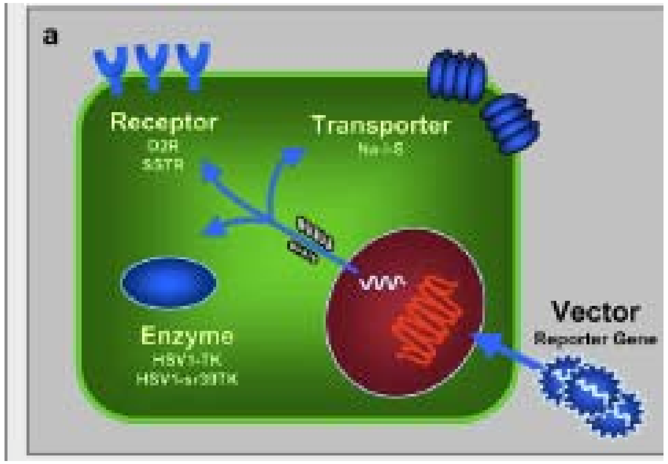
Develop and validate imaging tools for novel cell therapies (e.g. immune cells or stem cels) allowing tracking of cells and assessment of cell fate (e.g. viability, differentiation, migration and therapeutic effects, that could be tested in animal models with a view towards translational medicine. The development tools should provide specific and quantifiable information with respect, for example, to cell homing, functional read-outs, tracking of differentiation or immune system response .FP7

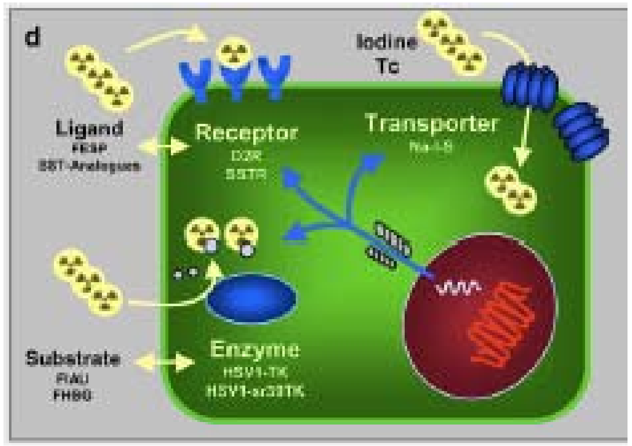
These reports underscore the need for a greater understanding of the mechanisms underlying stem cell biology and cellular reparative therapy, and their potential uses in the post-infarction state.

This proposal aims to elucidate the mechanism of action behind stem cell activity in cardiac regeneration, an area of tremendous contemporary pre-clinical and clinical activity. This ambitious proposal targets one of the most important questions arising in this field using a multiple way approach. The complex and innovative strategy to elucidate the mechanisms of stem cell mediated cardiac repair is highly valuable and could be beneficial for further development of pragmatic therapeutic strategies in this clinically relevant area. Such approach is complemented by the intention to develop multimodal molecular imaging technologies, primarily integrating SPECT with MRI. This technically difficult and challenging task would, if successfully accomplished, open new possibilities for the whole area of interest. Some aspects of this and other working tasks would,

- Optical 10e15 to 10e17 mol/l
- PET, SPECT 10e11 to 10e12 mol/l
- MRI 10e5 mol/l

direct labeling: labels may be diluted upon cell division, making these cells invisible; and labels may efflux from cells or may be degraded over time.





alternative approach: stable transfection of cells with a reporter gene, such as herpes simplex virus type-1thymidine kinase (HSV1-tk), whose expression can be visualized using a radioactive PET or SPECT reporter probe

(phosfphorilates --> TK --> triphosphate --> cells)

PET and SPECT imaging can be used to assess cell trafficking, function, and efficacy, using methods which are easily translatable to humans. Reporter gene approaches are particularly valuable, as they provide information not only on cell trafficking, but also on cellular function and survival

Dual labeling

- Optical imaging techniques provide high spatial resolution and permit tracking of stem cells but are limited to preclinical use
- Magnetic resonance imaging methods permit good spatial resolution but limited detectability
- Nuclear techniques, including reporter genes and direct cellular radiolabeling, afford very good detectability but more limited spatial resolution

A multimodality approach using combined PET or SPECT and MRI agents may ultimately prove most useful in clinical settings.

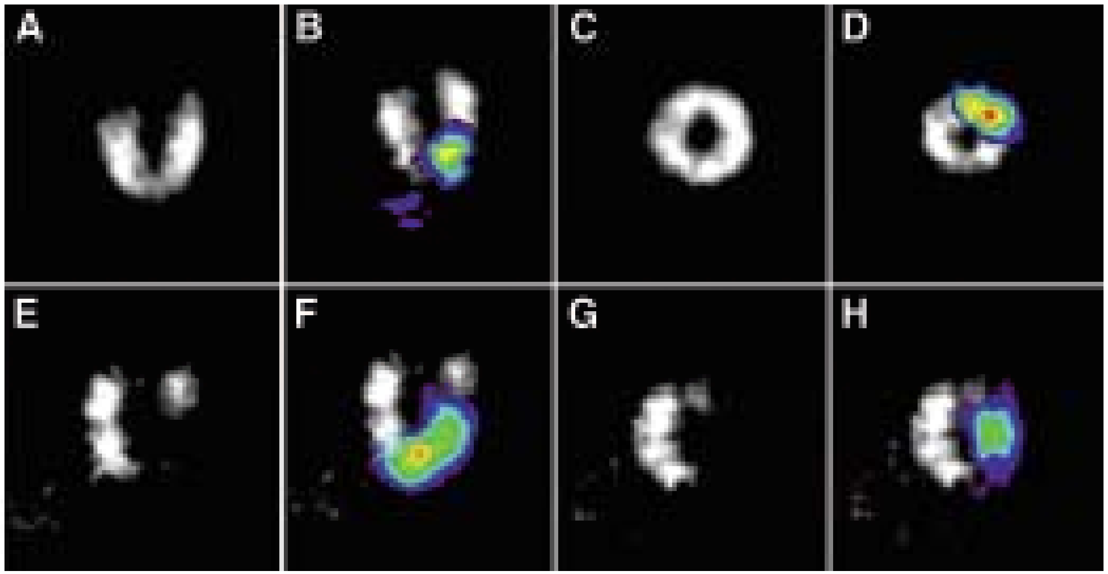


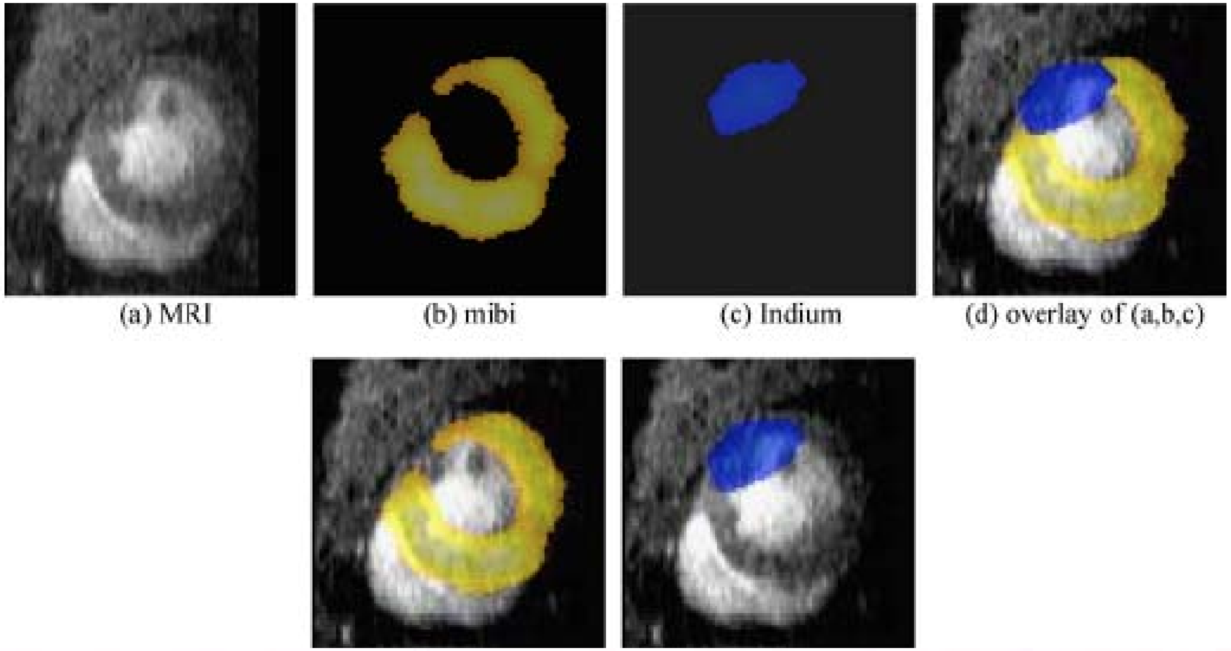
FIGURE 1. Cardiac long- and short-axis SPECT images of normal (A and C) and infarcted (E and G) heart using perfusion tracer ^{99m}Tc-sestamibi. ^{111}In signal (color) was overlaid on gray-scale ^{99m}Tc-sestamibi images for normal (B and D) and infarcted (F and H) heart.

P. Acton and al.

and Multimodality

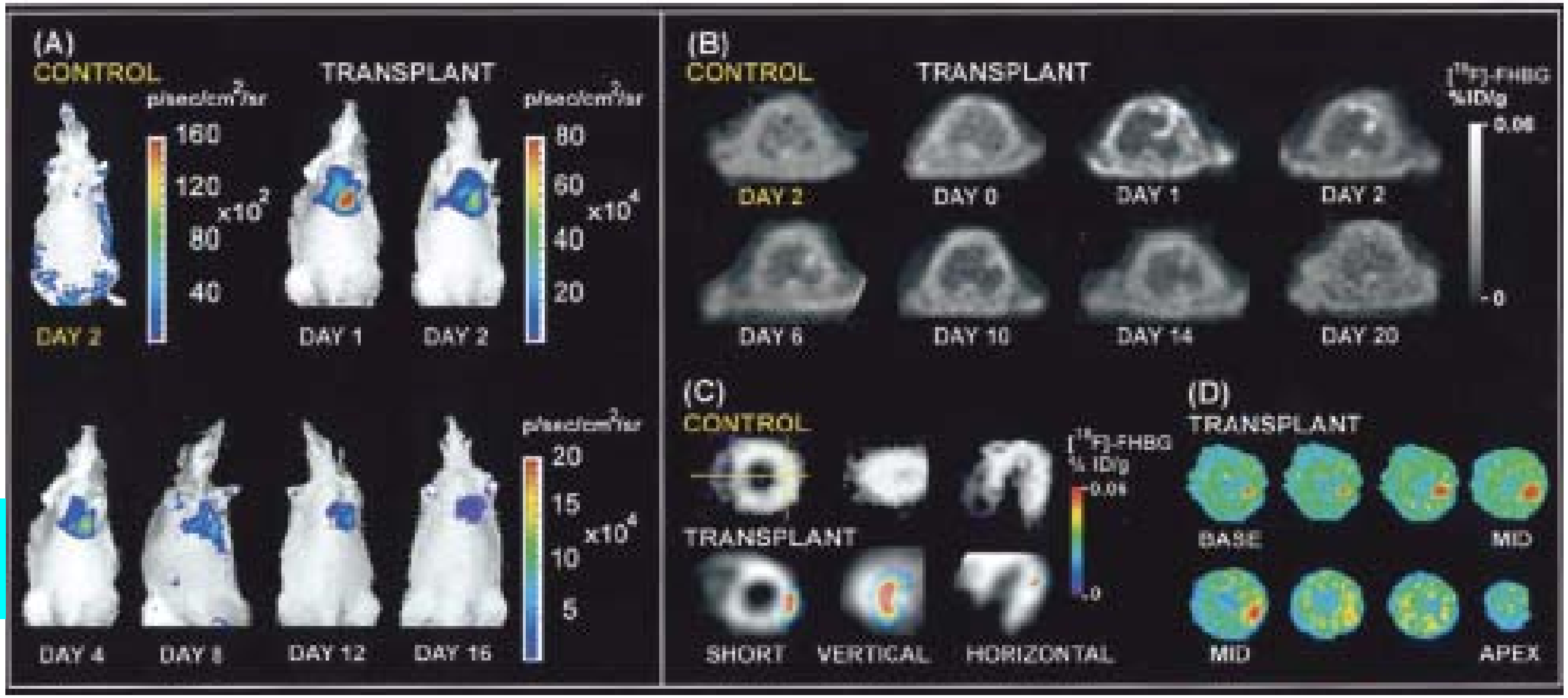
(Zhou, Acton)

SPECT/MRI



OPTICAL/PET

(Gambir)

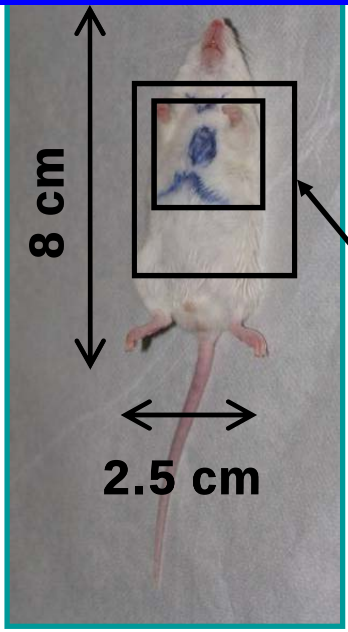


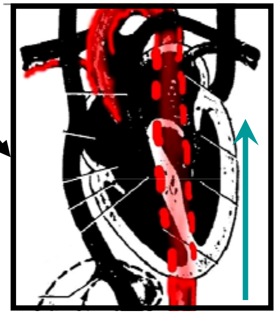
- Detector ~ 100x100 mm^2
  - Intrinsic reaolution: 1.2 - 1.5 mm
  - pinhole (0.5 mm)

rhigh resolution : ~ 0.8 mm

- M= 3 ==> FoV ~ 33 x 33 mm^2

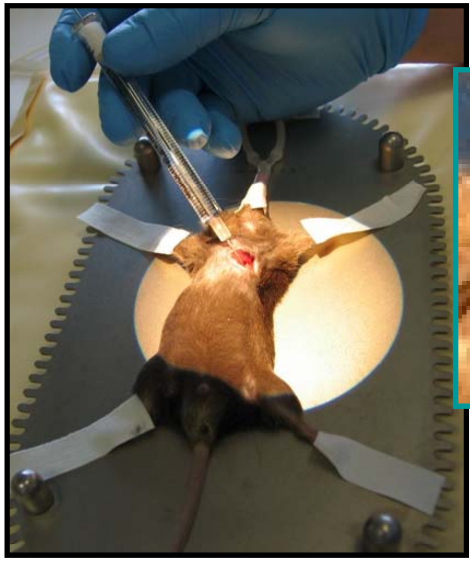
- *Perfusion Imaging*





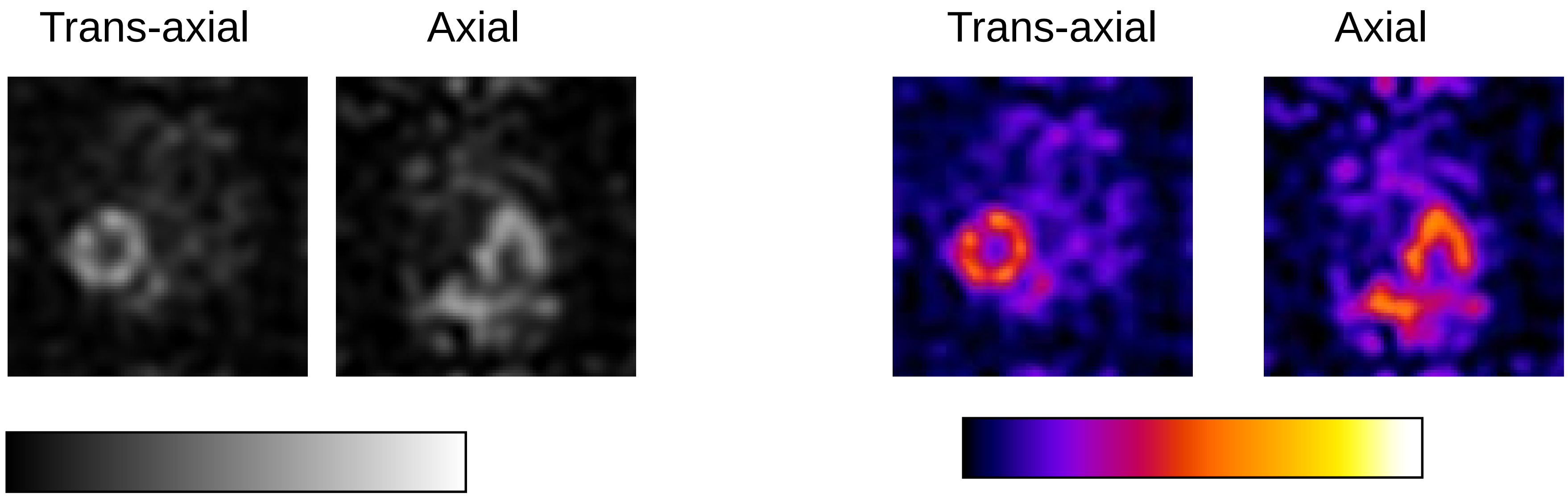
wall thickness
~ 0.8 mm !!!!

- Tracking *and homing* of stem



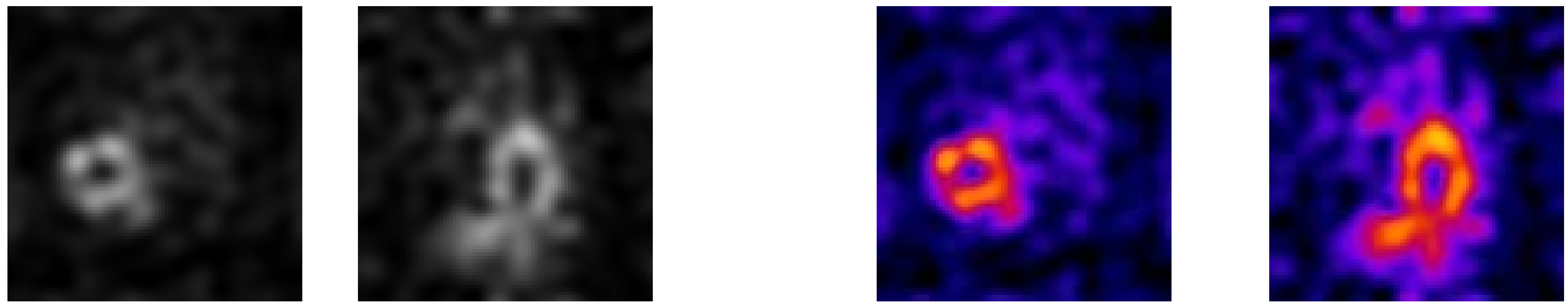
mouse: C57 BL/6, male
age: ~ 12 weeks
weight: ~ 31.5 g
stem cells:~ 6*10^4 murine (SCA+/KIT+ Tc^{99m}-HMPAO (28 microCi)

Reconstruction Images of Mouse Perfusion Scan (I)



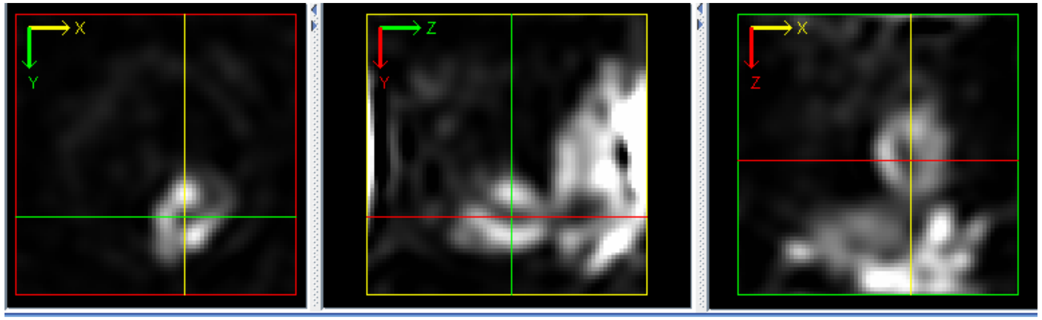
OS-EM, 6 subsets, 2 iterations, post-smoothed by Butterworth filter (cutoff=0.12, order=8), voxel size = 0.25 mm, image dimension 90x90.

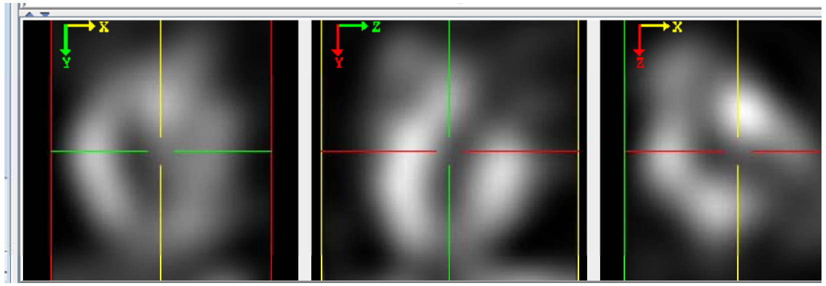
Reconstruction Images of Mouse Perfusion Scan (II)



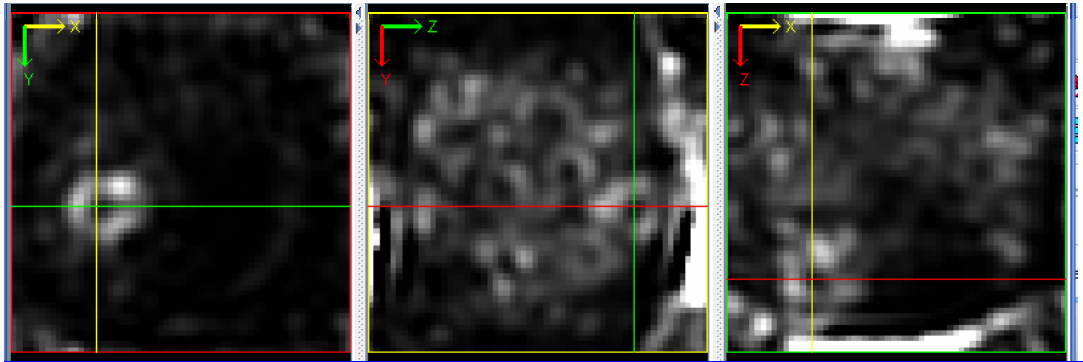
Transverse (X-Y)	Sagittal (Y-Z)	Coronal (X-Z)

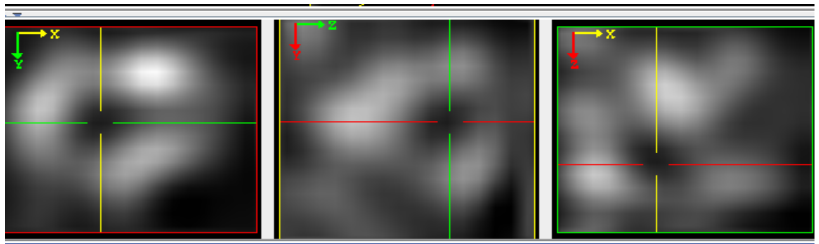
Tail vein injection





Peritoneum injection





It is extremely difficult if not impossible to use the tail vein for radiotracer many times. An alternative route of delivery is needed, but, how much of will arrive to heart? Let's look at the peritoneum.

It works, but there is a price to pay, the uptake is decreased (a factor of ~ 2).

We have to maximize the efficiency

→ More detectors

→ Multipinhole

Conclusions

Importance of molecular imaging in the biomedical research panorama: crucial role of radionuclides techniques

Multidisciplinary approach mandatory

Multimodality ("new" photosensors (SiPm?))

- atherosclerosys:
  - looking for smaller plaques "earlier" detection (other mechnisms, other radiotracers)
- stem cells:
  - selecting "right" cells
  - monitoring diffusion, differentiation, grafting etc
  - looking at the effects

===> multimodality (optical, SPECT, MRI,)

Outlook

-the challenge:120 pixel/100 mm, 8 modules 150 X 100 mm^2

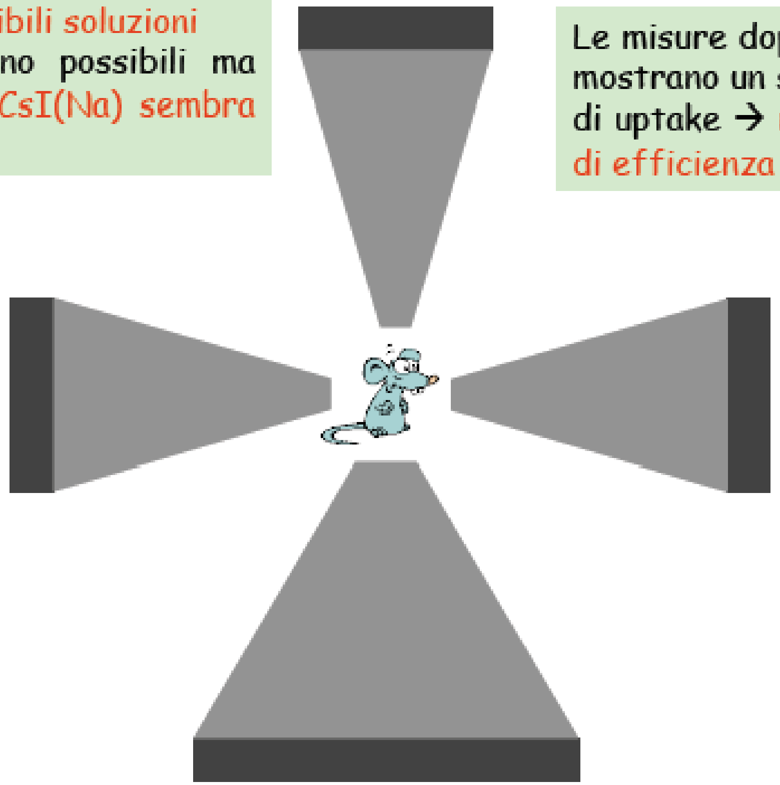
---> FOV 50 x 33 (M=3))

Richiesta per il 2009 →

50×50 mm^2 CsI(Na)
0.8 mm pitch
M = 2, FoV = 25×25 mm^2

Confronto tra 3 possibili soluzioni CsI(Tl) e Labr3 sono possibili ma dievrsi problemi. Il CsI(Na) sembra la soluzione migliore

Le misure dopo iniezione in peritoneo mostrano un significativa diminuzione di uptake → necessita' di valutazione di efficienza con misure su topi

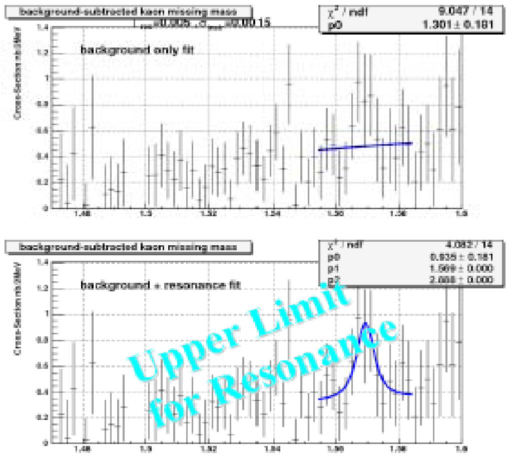


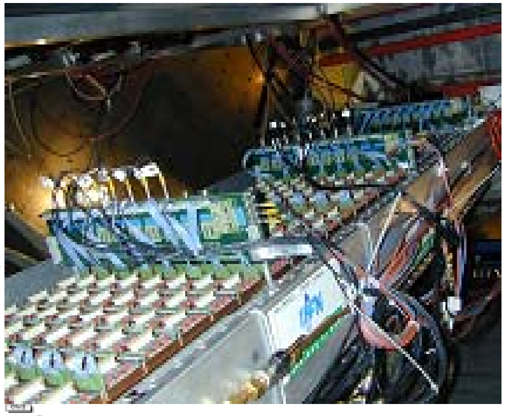
50×50 mm^2 CsI(Tl)
0.8 mm pitch
M = 2,
FoV = 25×25 mm^2

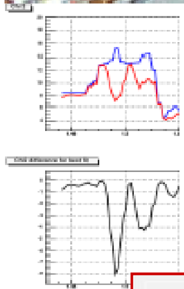
50×50 mm^2 $LaBr_3(Ce)$
4 mm thick,
3 mm thick window
M = 2,
FoV = 25×25 mm^2

100×100 mm^2 NaI(Tl)
1.2 mm pitch
M = 3, FoV = 33×33 mm^2

Θ^{++} Search

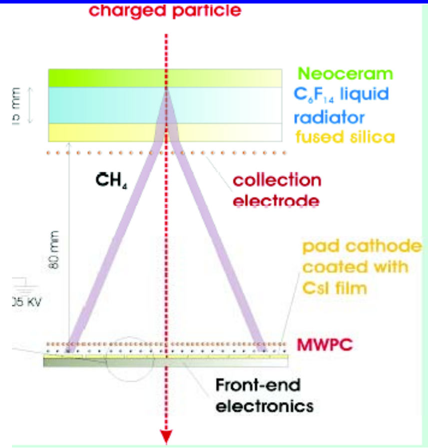


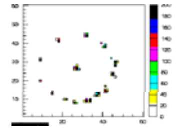




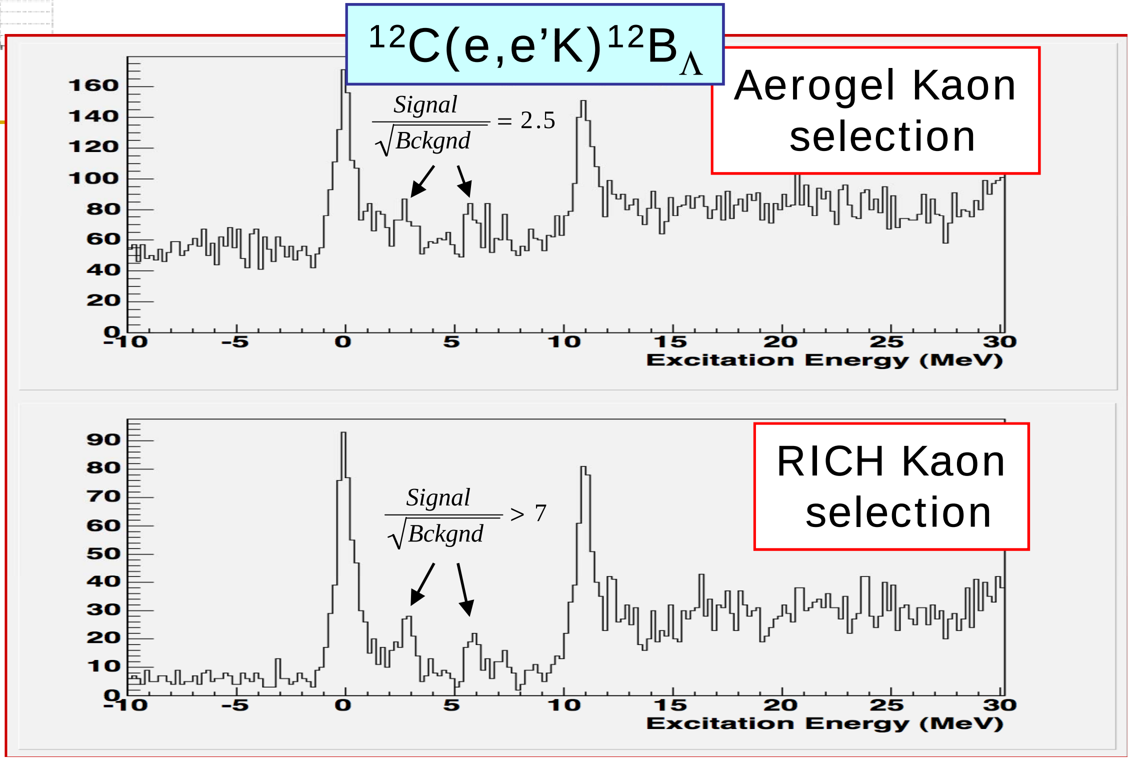
$$\frac{d\sigma}{d\Omega}(\gamma^* p \to \Theta^{++}K^-)|_{\theta=2^\circ} < 3nb$$

Freon/CsI RICH detector (like ALICE)

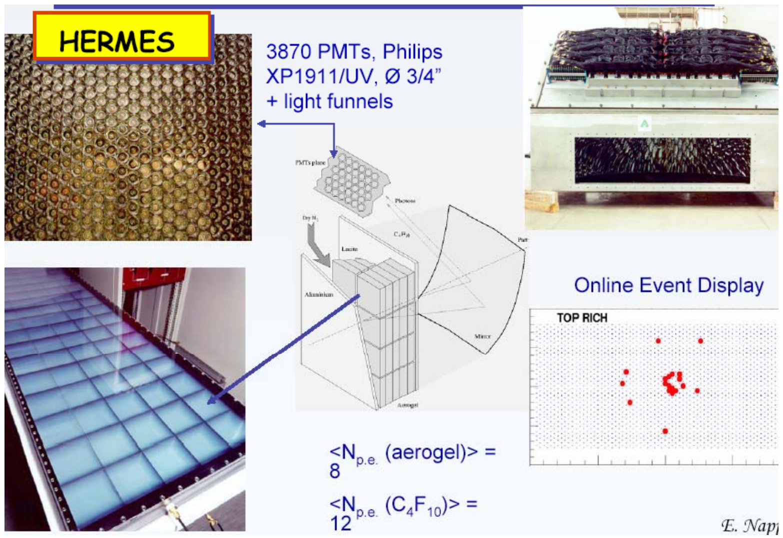




Spectroscopy analysis of $^{12}B_\Lambda$: Aerogel vs. RICH K-selection



Hermes areogel RICH



Important parameters for detectability/visibility

$$SNR = \frac{S - BKG}{\sqrt{S}}$$

efficiency collimation

time (and modality)

uptake (radiopharmacy)

$$X = \frac{\sum_{i=1}^{N_{channel}} c_i X_i}{\sum_{i=1}^{N_{channel}} c_i}$$

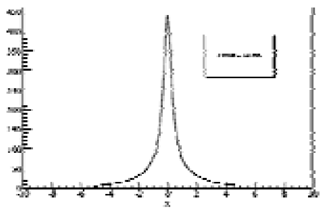
$$IC = \frac{Max - BKG}{Max}$$

detector intrinsic properties

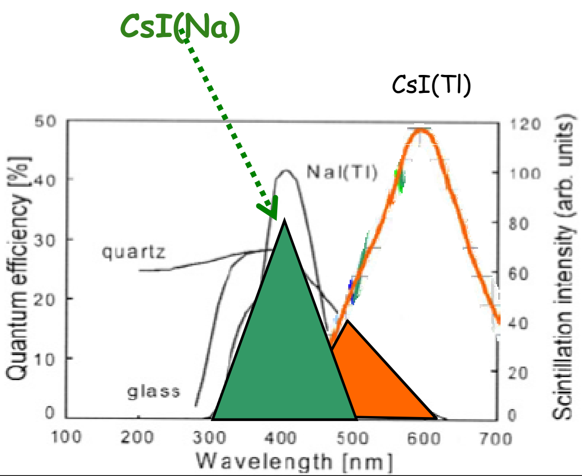
modality (compression)

- scintillator
- pixel dim/n.of pixels
- electronics, DAQ

spatial resolution



. Uniformity of p.h.response (affecs the overall en res. and the energy window seection)



Scintillators

		NaI(Tl)	CsI(Tl)	YAP	LaBr3(Ce)
Density	g/cm^3	3.67	4.51	5.55	5.29
Effective Z		51	54	32	47
Relative Yield	%	100	45	35-40	130
Peak Wavelength	nm	410	550	360	360
$\Delta E/E$ @ 140 keV	%	9	15	20	6
μ @ 140 keV	cm^{-1}	2.7	3.9	1.7	3.0
τ @ 140 keV	cm^{-1}	2.1	3.2	0.9	2.2
Thickness (90% eff)	mm	8.7	6.0	13	

Bialkali PMT

τ/μ fotofraction

$$\sigma_X \propto \frac{\sigma_{X_i}}{\sqrt{N_{p.e}}} \Rightarrow R \equiv FWHM_X \propto \frac{FWHM_{X_i}}{\sqrt{N_{p.e}}}$$

CsI and $LaBr_3$ selected as possible optimal candidates:

- Submillimeter CsI Pixels available
- $LaBr_3$ is very promising (available in tiles of $\sim 5 \times 5$ cm^2)