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Tracing radioactive molecular  
markers with pixel detectors

presented by Maurizio Conti



# *Outline*

- ♣ Autoradiography techniques
- ♣ Pixel detectors in autoradiography
- ♣ *BETA view* system
- ♣  $\beta$  sources studies: simulation and experimental
- ♣ Specific biomedical applications
- ♣ Future developments



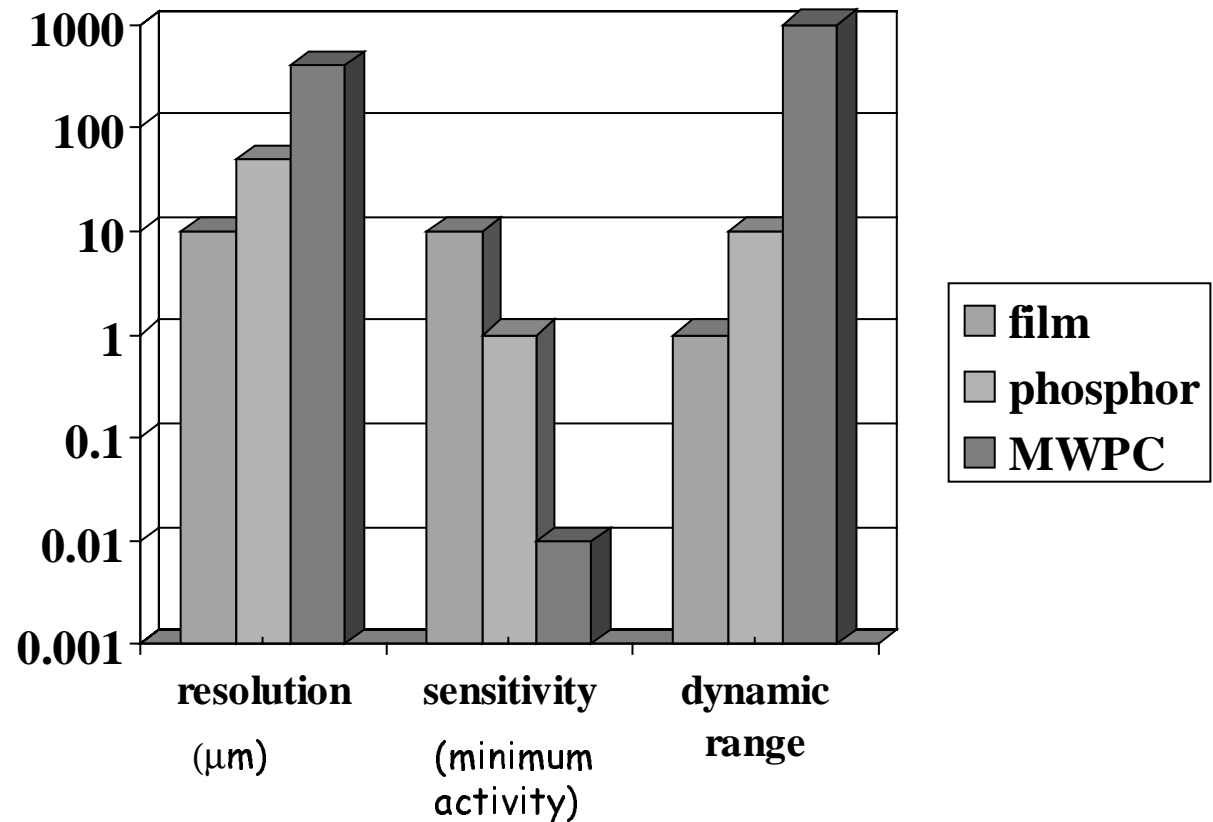
# *Autoradiography*

- Biological sample with a molecular marker labeled with radioactive ( $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ , etc)
- Sample positioned on the detector
- Image is created via counts accumulation on the detector
- Marker is located and if possible quantified

# *Main commercial systems*

Other key parameters:

- portability
- developing time and real time imaging
- dynamic studies capability

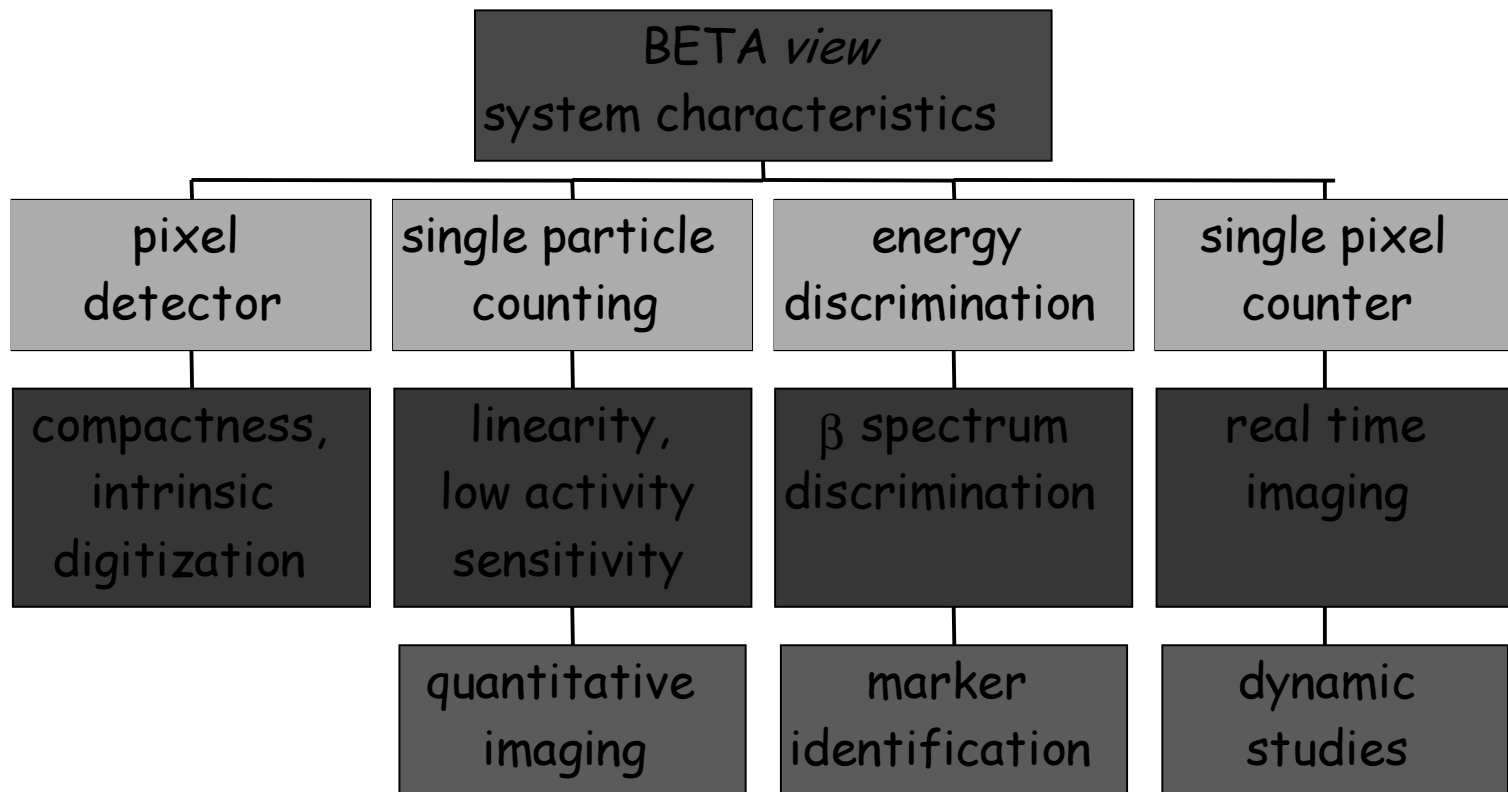




## *Ongoing research*

- MWPC+image intensifier+CCD
- Scintillator+Si pixel detectors
- MicroChannelPlates
- Silicon strips
- .....
- GaAs/Si pixel detectors

# *Solid state pixel detectors*





## *Special applications*

- DNA hybridization probe array
  - identification of genetic mutations
- Viral infection identification
  - in situ hybridization of viral genetic material
- Kinetics of biological mechanisms
  - protein/amino acid uptake and localization

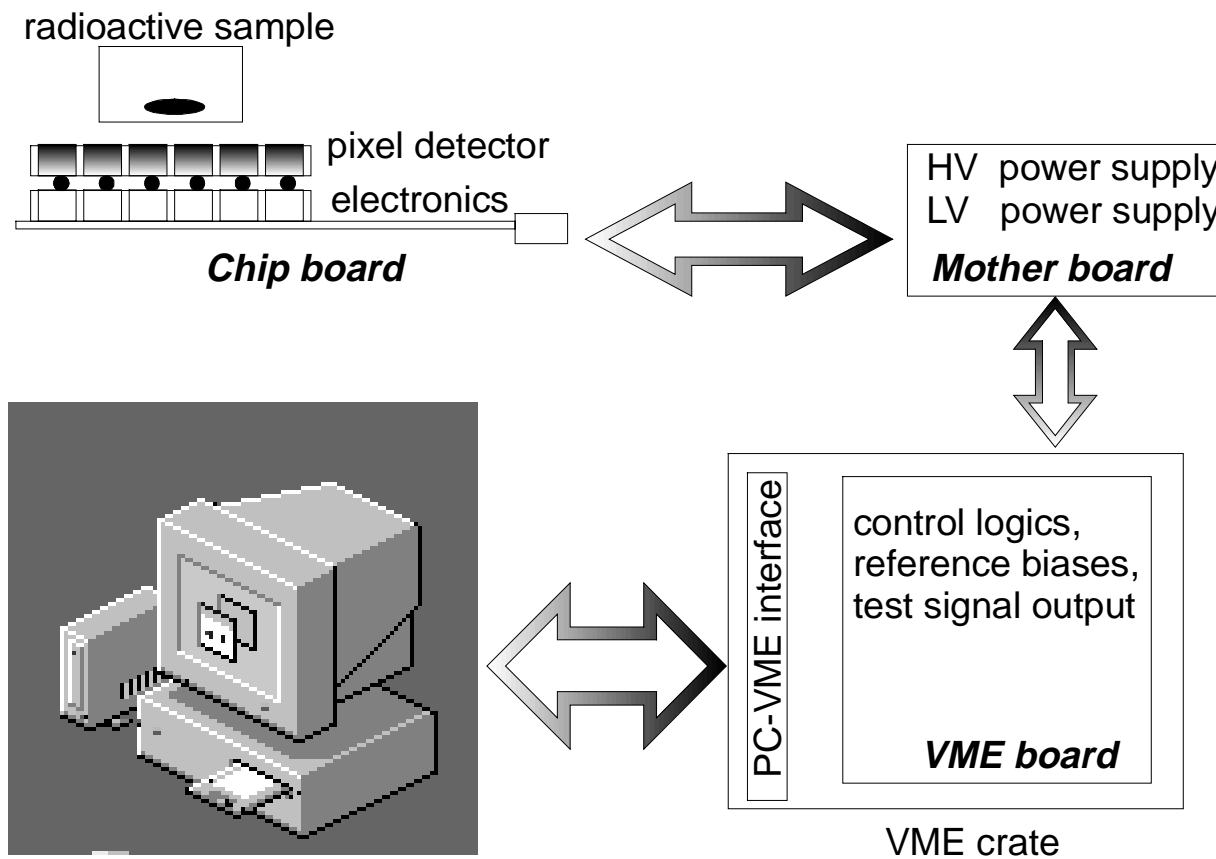


## • *System description*

- GaAs pixel detectors : 64x64, 170  $\mu\text{m}$  size, 200  $\mu\text{m}$  thick
- Bump-bonded to Medipix\* front-end electronics:
  - low noise, low threshold analog electronics
  - 15-bit counter under each pixel
- VME board/crate for control and read-out, bias and reference voltages
- Control and image presentation on PC (NI interface and LabWindows CVI software)
- 3D (1 $\mu\text{m}$ ) translation system to move the sample
- Acquisition time: N frames (from 1s to several h)
- \* Medipix: CERN, INFN, Univ. Freiburg, Univ. Glasgow.



# Data Acquisition System



# *Detector and front-end chip*

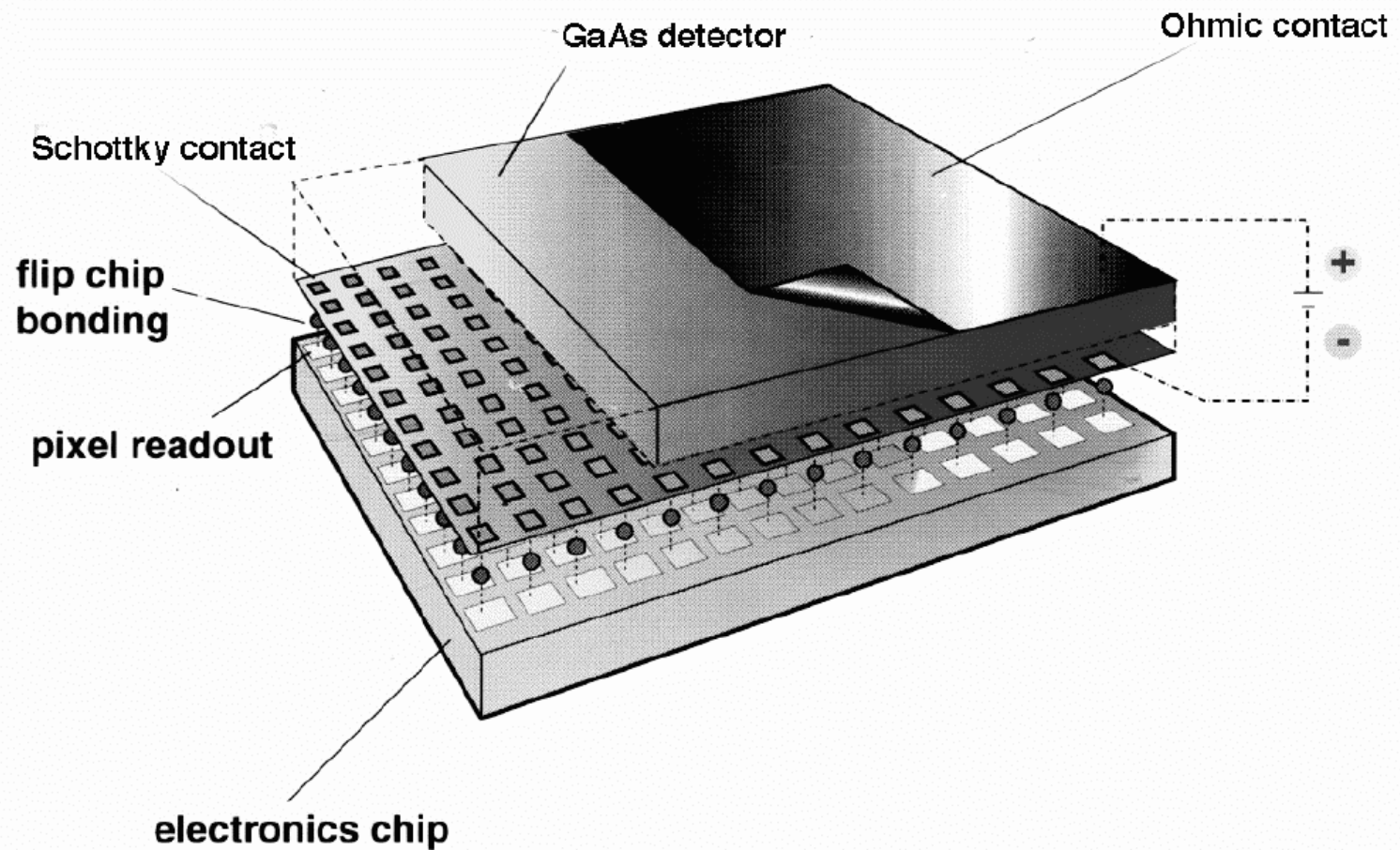
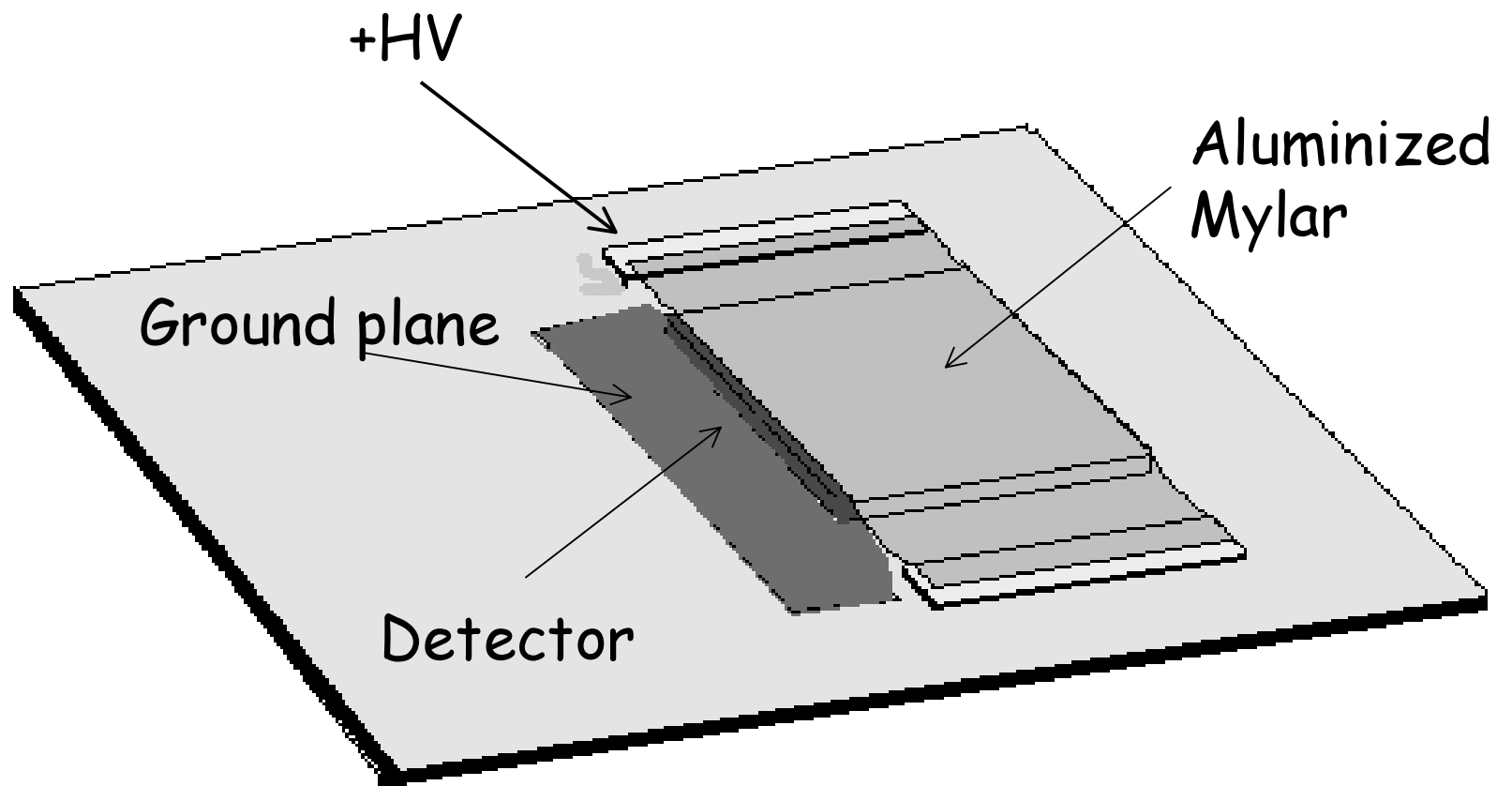


Figure courtesy of M. Snoeys

# *Detector biasing technique*



# VLSI front-end cell (Medipix)

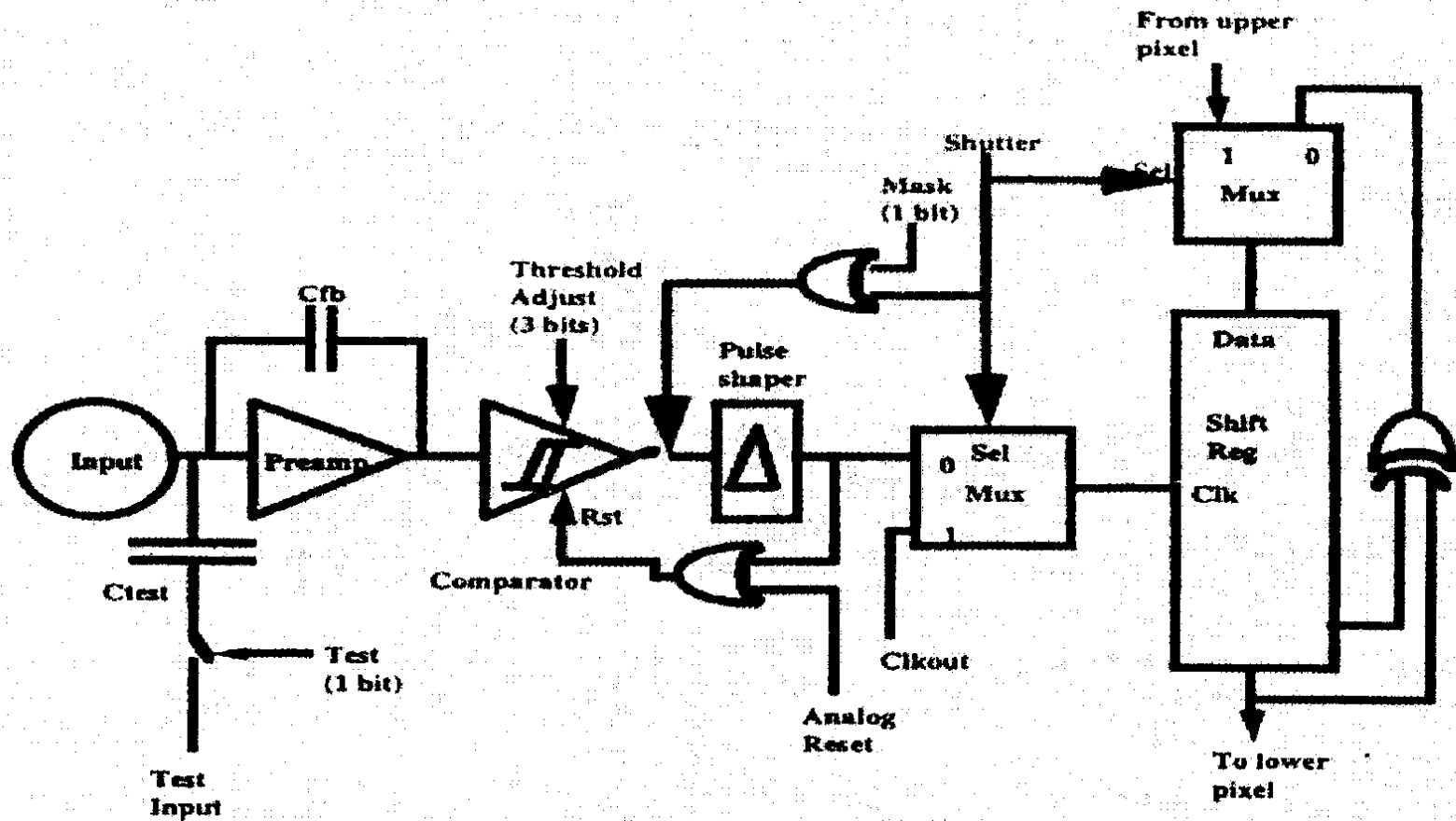
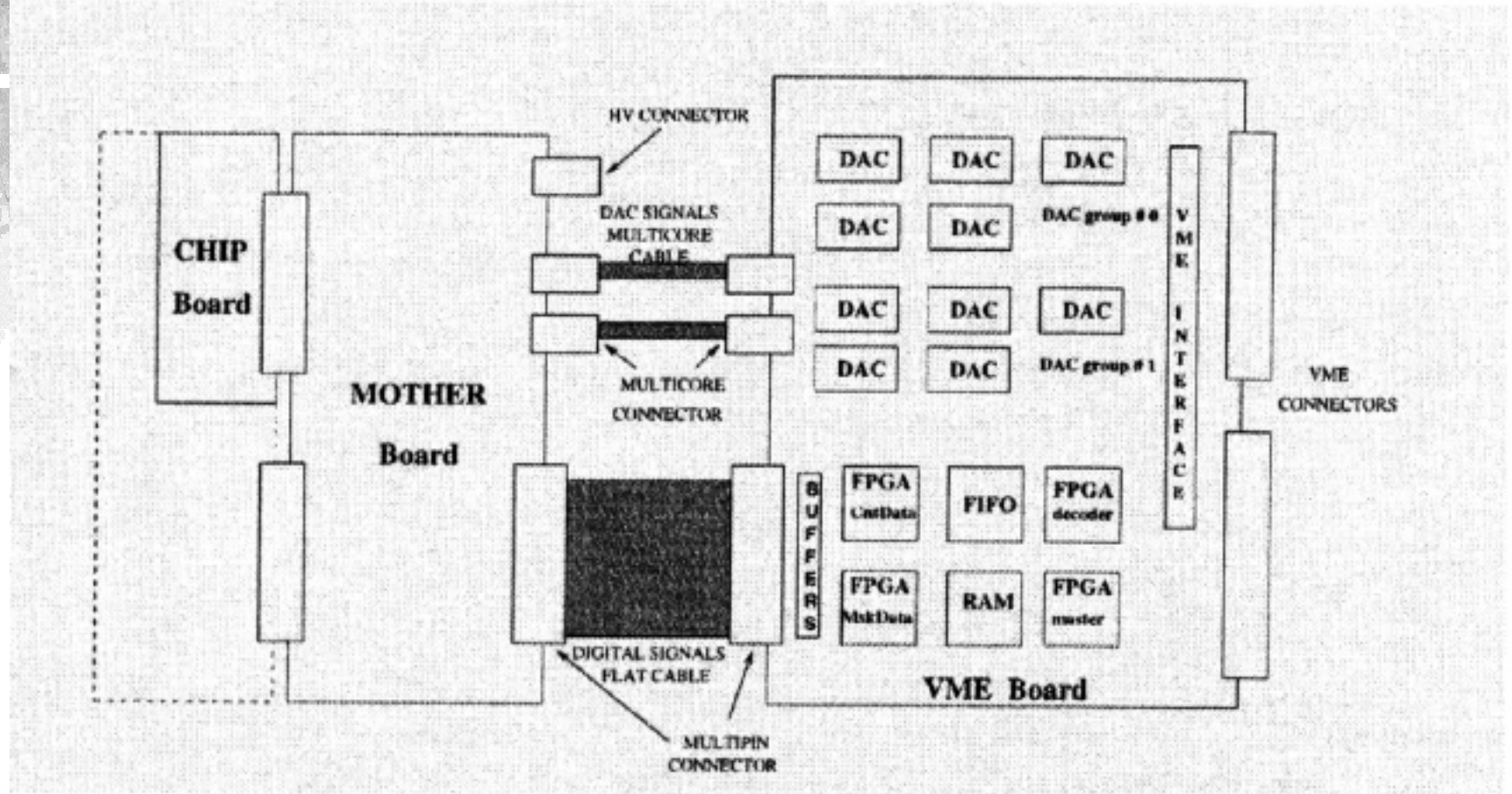


Figure courtesy of M. Campbell

# Read-out schematics



# *Experimental set-up*





## *Experimental set-up info:*

- Detector (GaAs) HV bias 300-400 V
- Assemblies were slightly cooled (16-19 °C)
- Threshold set from 25 keV (6000 electrons) to 35 keV (8400 electrons)
- Threshold adjusted pixel by pixel to get better uniformity
- Background noise counts of about 0.25 cps/cm<sup>2</sup>
- Acquisition time: multiple frames of 10 minutes

# Real-time image build-up

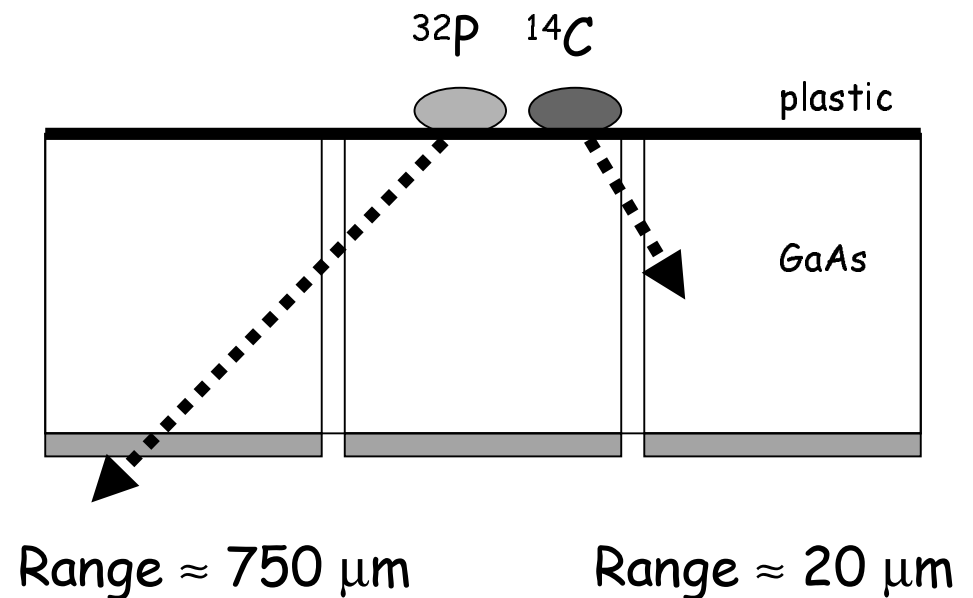
The screenshot displays the Medipix software interface. The main window shows a real-time image build-up on a grid. The grid has a central dark spot. The axes are labeled from 0 to 63. A green cursor is positioned at Row 56, Column 3, with Counts 0. The interface includes several control panels: Acquisition (tau0: 1, Acquisition Time, Trigger), ANIN (ANIN Cycles, ANIN Delay, ANIN Period), Zoom (Zoom, Restore), Row & Column profile (Profile), Image Settings (Settings), and Load & Save (Load..., Save..., C14\_30~1.dat). A 'Turn On Medipix' button is circled. The bottom right shows a 'gol' button. The status bar at the bottom indicates 'LabWindows/CVI' and the time '7:20 PM'.

Whole area		Selected area	
Total counts	Average counts	Total counts	Average counts
1207	0.29	986	1.52



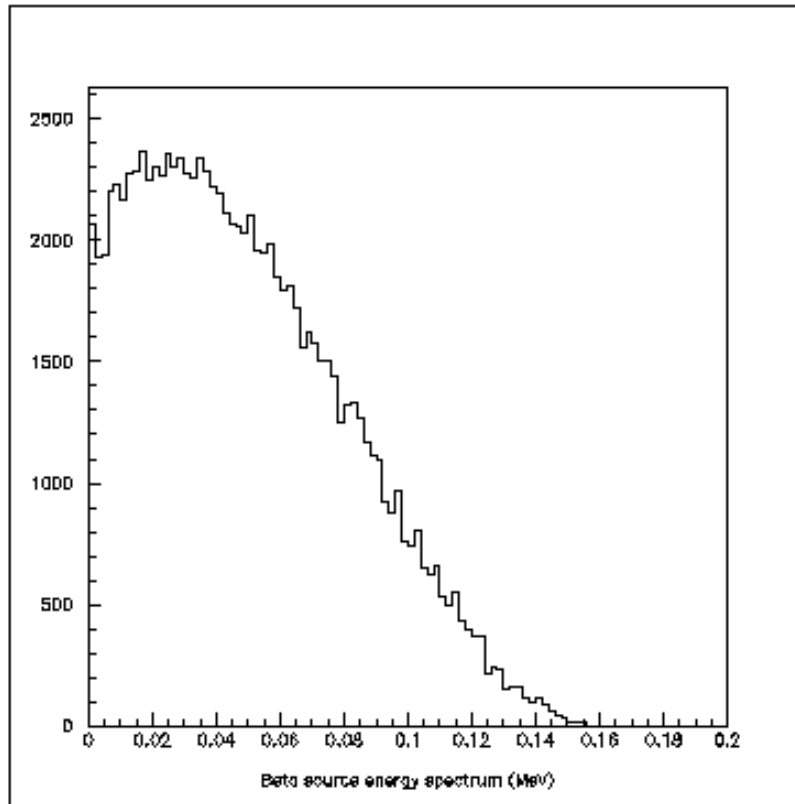
# Simulation studies

- $\beta$  markers:  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$
- effect of pixellated structure:
  - efficiency
  - pixel counts (multiplicity)
  - spatial resolution
- geometry:
  - 10  $\mu\text{m}$  plastic
  - 200  $\mu\text{m}$  GaAs
  - 170  $\mu\text{m}$  pixel size
- pixel threshold :
  - 25 and 35 keV

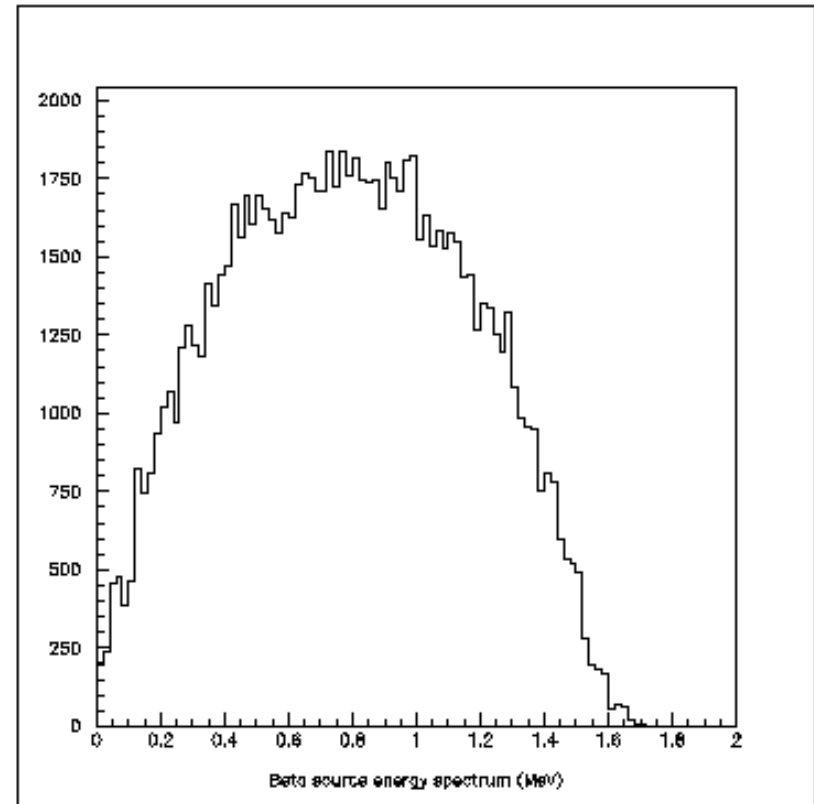


# $\beta$ Spectra

$\beta$  markers:  $^{35}\text{S}$  and  $^{14}\text{C}$   
end point about 150 keV

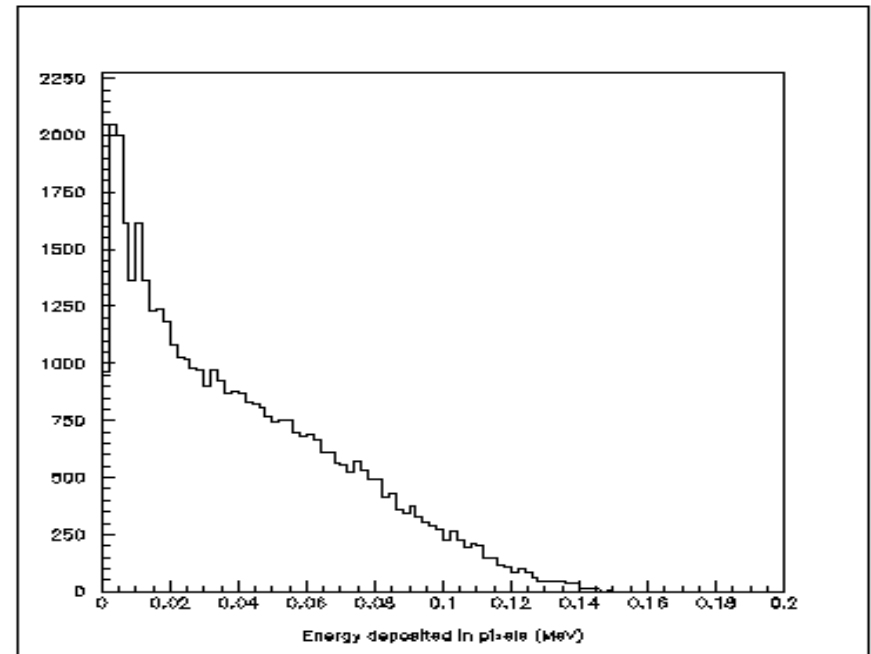


$\beta$  marker:  $^{32}\text{P}$   
end point about 1.7 MeV



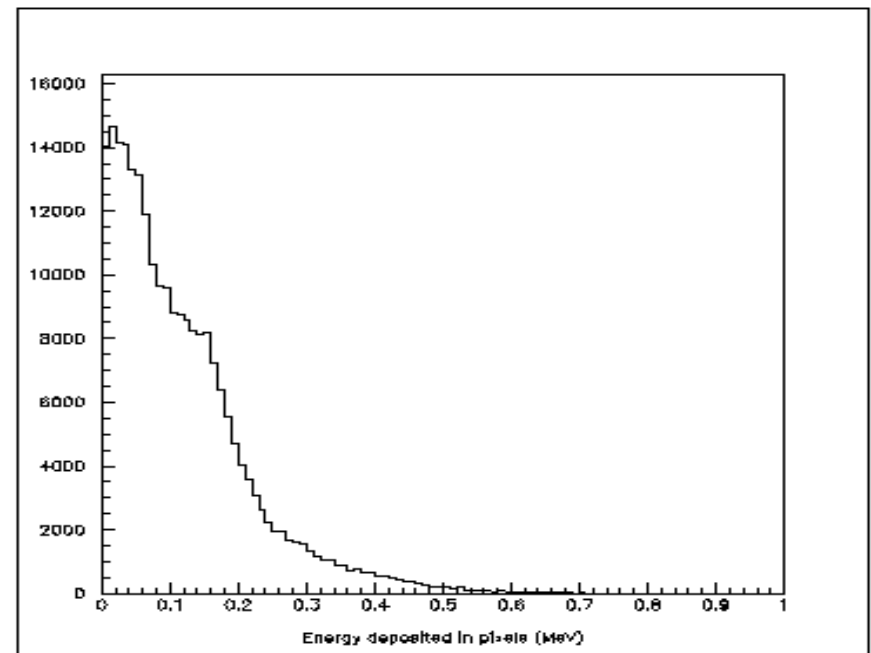
# *Simulation: $^{14}\text{C}$ and $^{35}\text{S}$*

- detection efficiency (threshold 25 keV):  
 $26.6 \pm 0.2 \%$
- pixels hit by one particle:  $1.005 \pm 0.001$
- detection efficiency (threshold 35 keV):  
 $21.6 \pm 0.1\%$
- pixels hit by one particle:  $1.002 \pm 0.001$

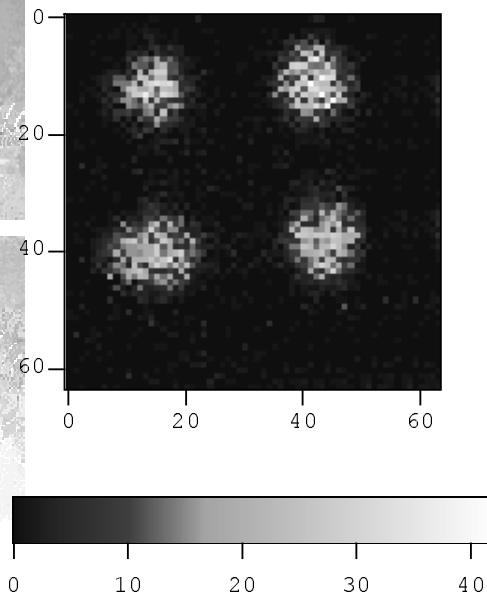


# *Simulation: $^{32}\text{P}$*

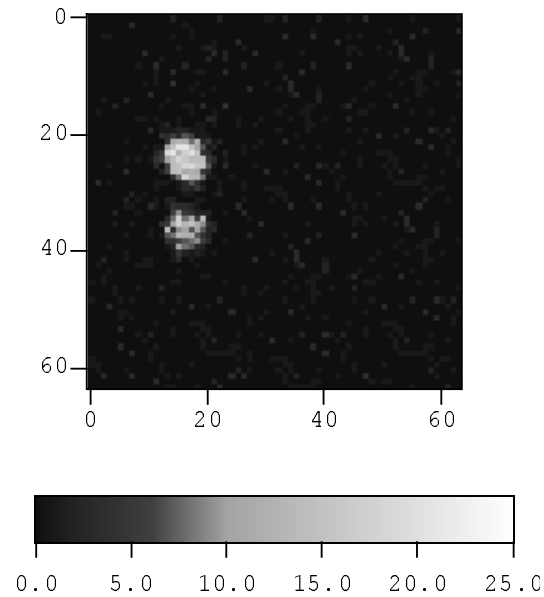
- detection efficiency (threshold 25 keV):  
 $85.6 \pm 0.1 \%$
- pixels hit by one particle:  $2.367 \pm 0.003$
- detection efficiency (threshold 35 keV):  
 $82.8 \pm 0.1\%$
- pixels hit by one particle:  $2.27 \pm 0.07$



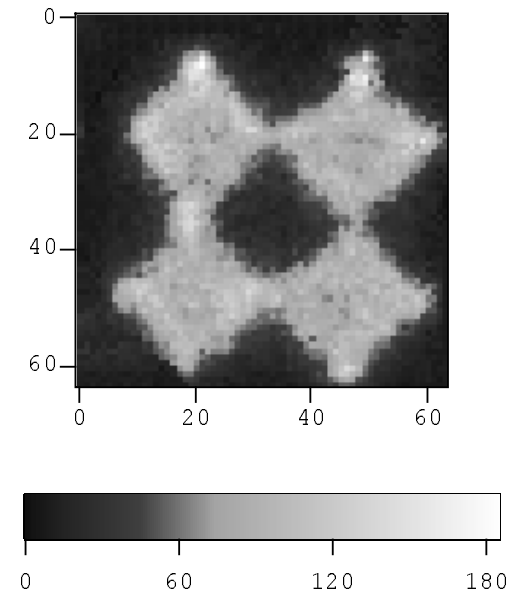
# *$\beta$ sources tests*



- $^{14}\text{C}$  (50 Bq)
- 5 mm grid
- 30 min

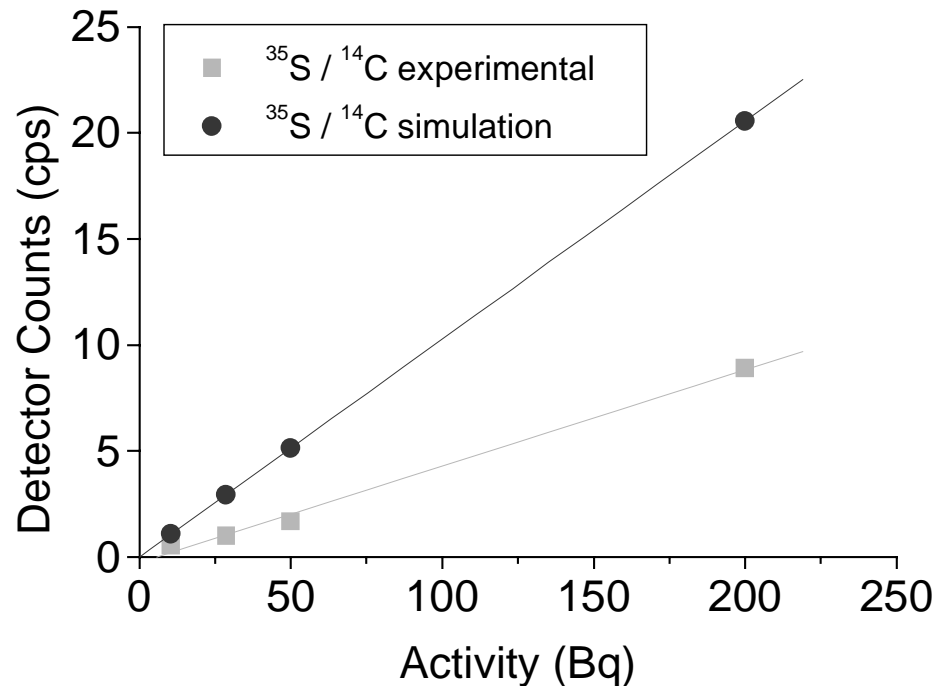


- $^{35}\text{S}$  (10.44 Bq)
- 2 mm grid
- 30 min



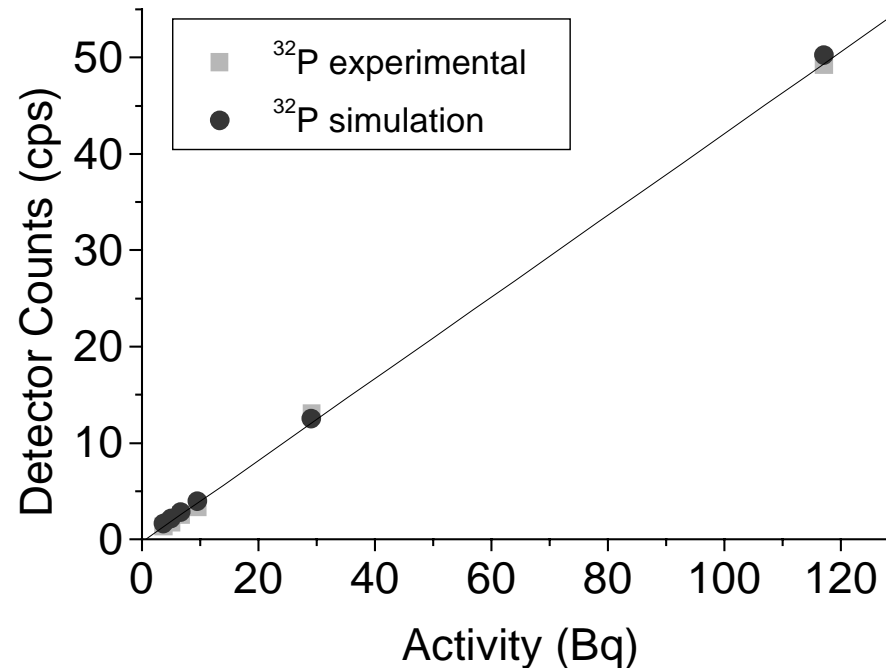
- $^{32}\text{P}$  (29.2 Bq)
- 5 mm grid
- 30 min

# Linearity ( $^{14}\text{C}$ and $^{35}\text{S}$ )



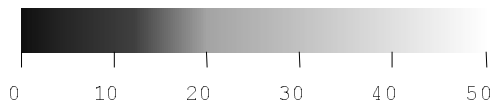
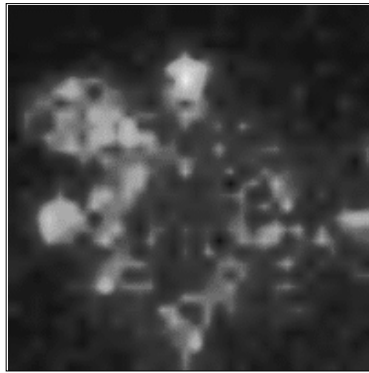
- Measured detection efficiency  $9.1 \pm 0.4 \%$
- Calculated detection efficiency  $20.56 \pm 0.01\%$

# Linearity ( $^{32}\text{P}$ )

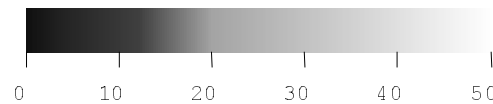
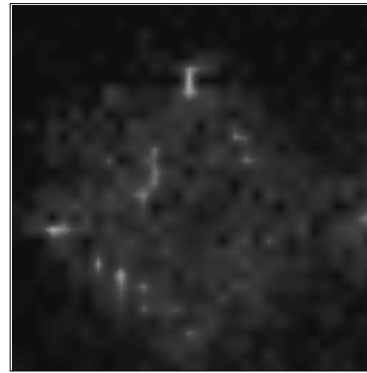


- Measured detection efficiency  $84.8 \pm 1.2 \%$
- Calculated detection efficiency  $84.8 \pm 0.1 \%$

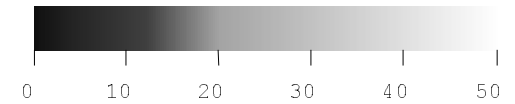
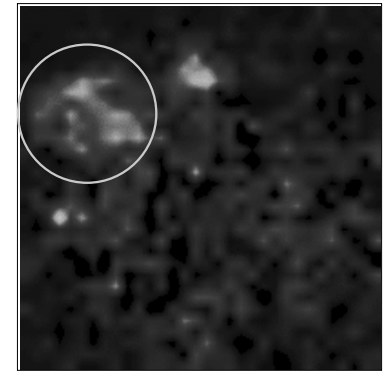
*Experiments with 2 markers:  
 $^{35}\text{S}$  and  $^{32}\text{P}$*



35 keV threshold



70 keV threshold



Subtraction

$^{32}\text{P} \approx 1.65 \text{ cps}$

$^{35}\text{S} \approx 0.43 \text{ cps}$



# *DNA probe array*

- ① Probe-array of known gene sequences
- ② Unknown target sample is radio-labeled
- ③ Hybridization probe-array / target
- ④ Detector locates the marker

→ Laboratory tool to develop clinical tests for :

- multi-gene analysis (interaction between genes in disease development)
- research of human gene mutations in patients
- research of viral gene mutations

Complementary probe

Not-complementary probe

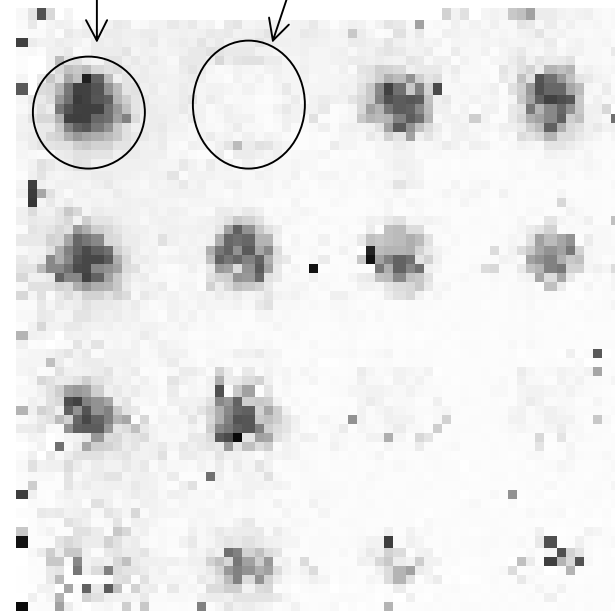


Figure is not an experimental result

# *Diagnosis of viral diseases*

- Quantitative analysis of *in situ* hybridization in degenerative and viral diseases

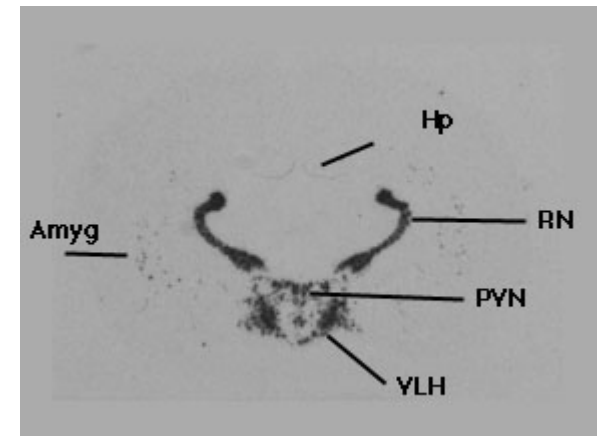
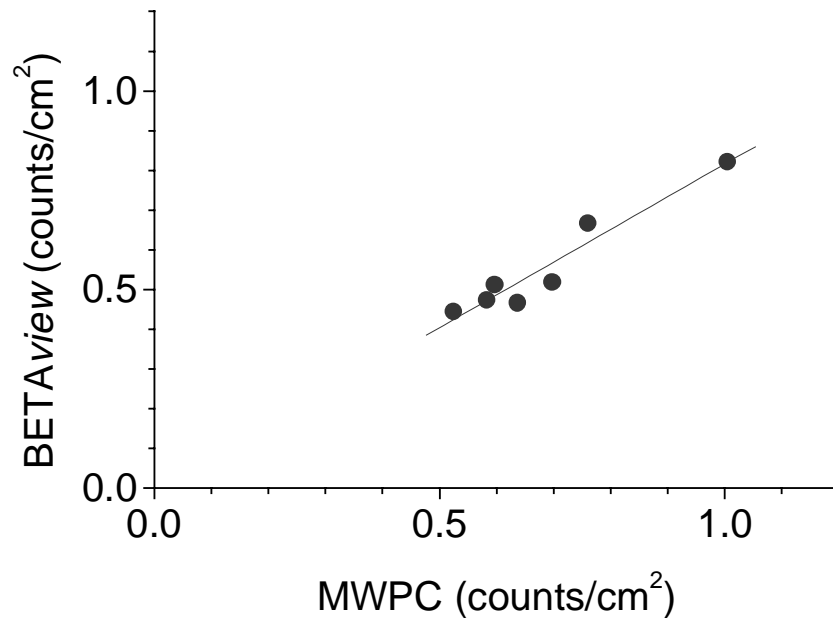


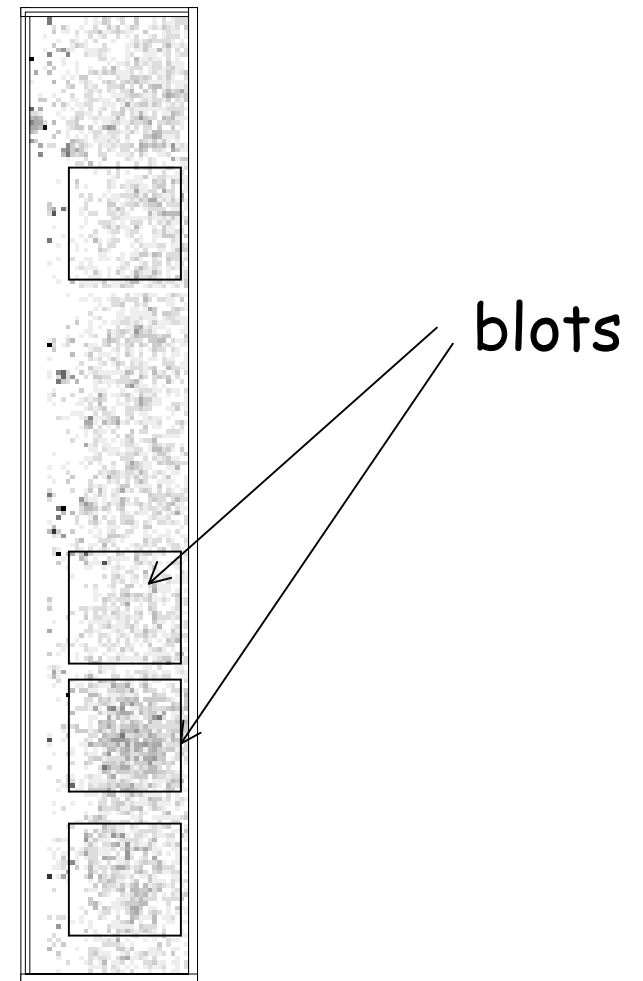
Figure is not an experimental result

- Myocardial diagnostics:
  - ① biopsy
  - ② *in situ* hybridization with viral probe
  - ③ identification of virus in tissue

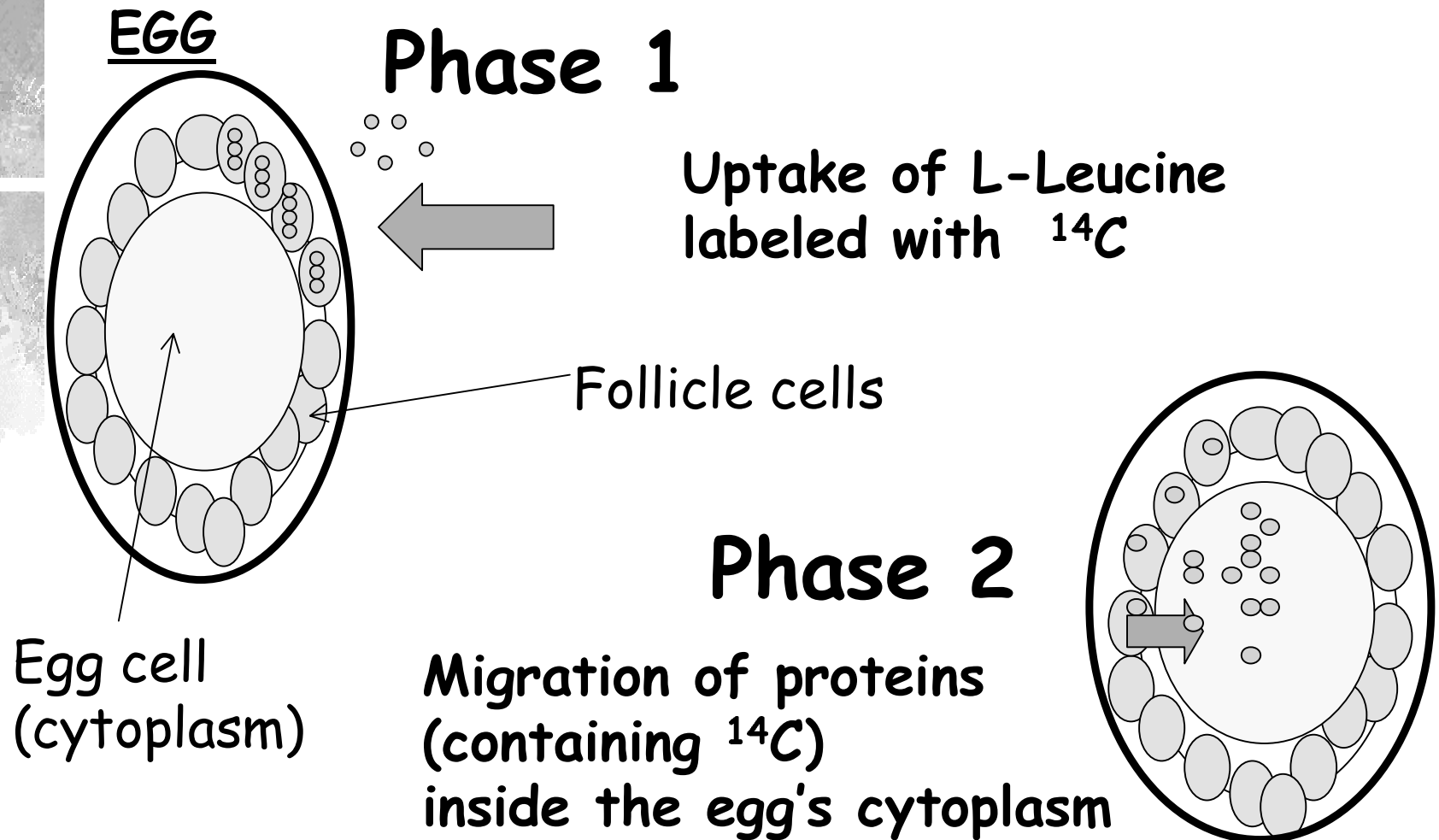
# *Northern blotting: comparison with MWPC (Packard Instant Imager)*



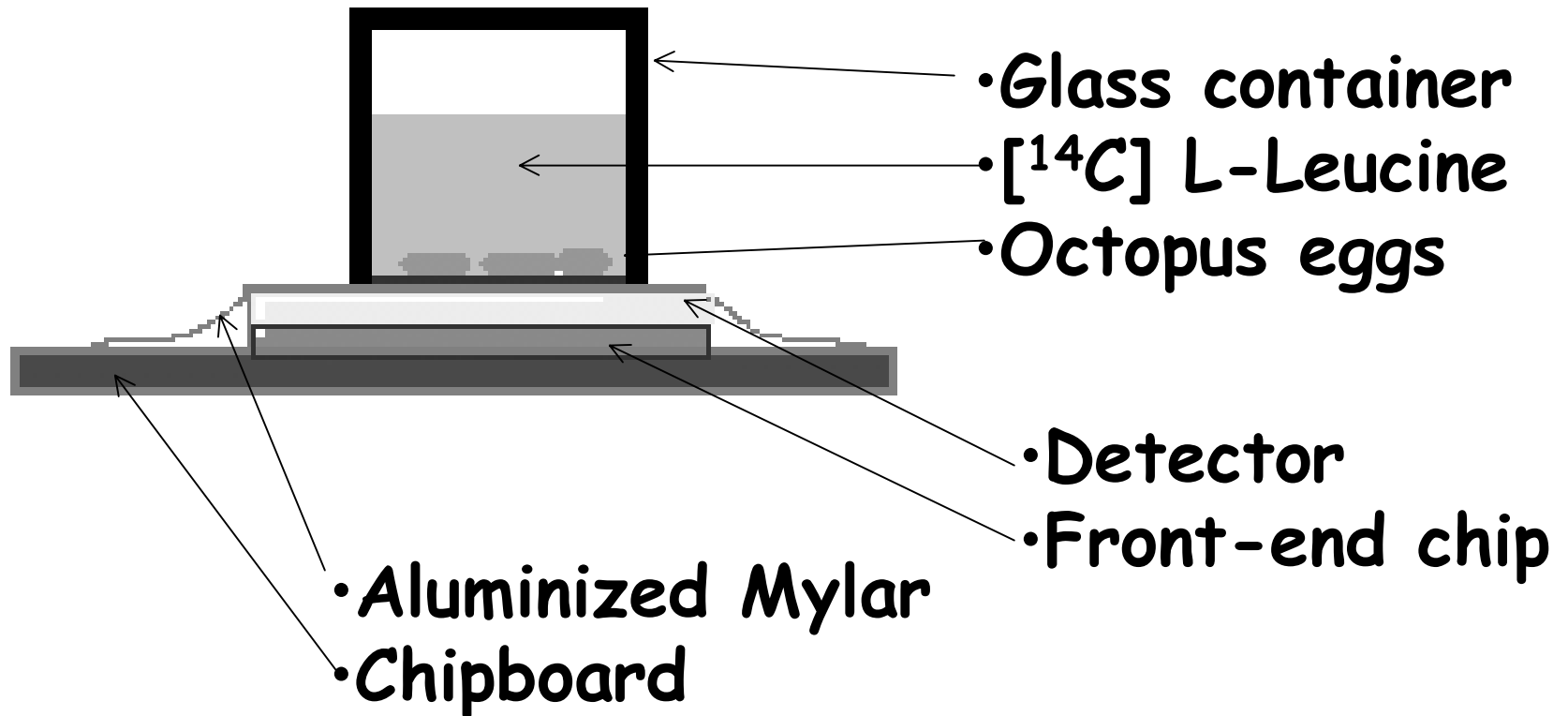
Source : <sup>32</sup>P, 30 minutes  
Relative efficiency = (82 ± 10) %



# Experiment: uptake & internal migration of $^{14}\text{C}$ -labeled aminoacid in Octopus eggs

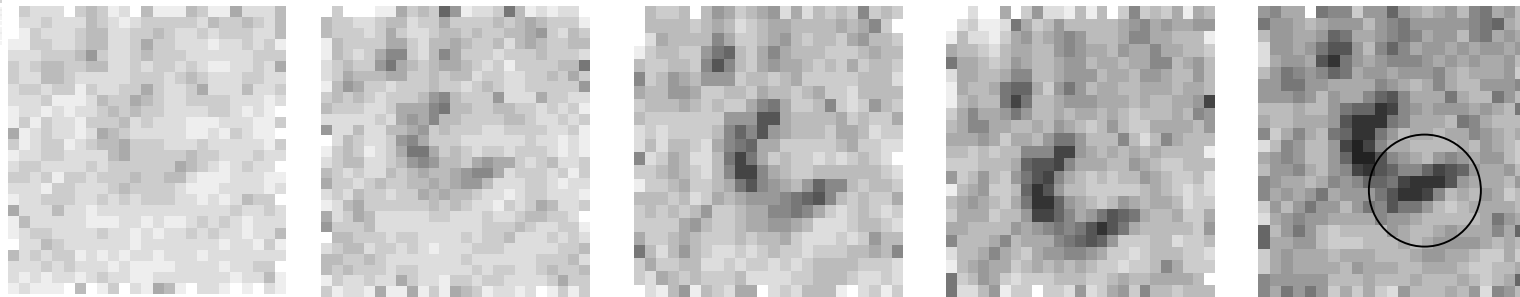


# *Experimental set-up*



*Dynamic studies:  
[<sup>14</sup>C] L-Leucine uptake  
by Octopus eggs*

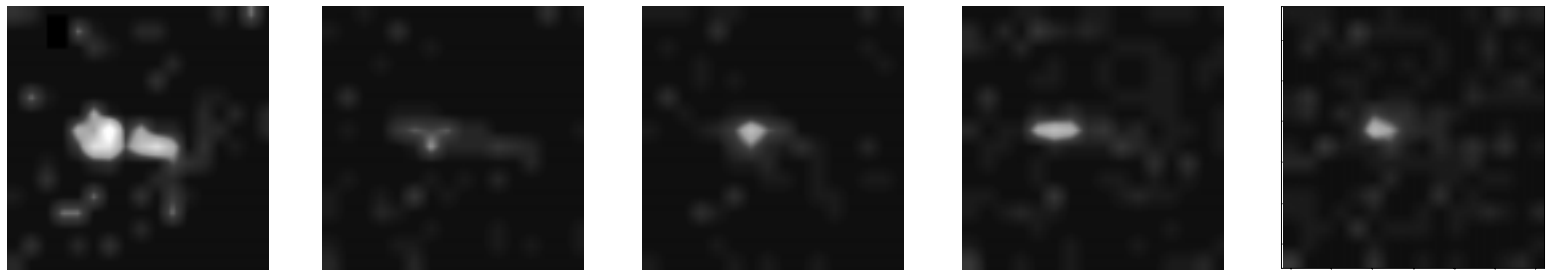
- Marker: [<sup>14</sup>C] L-Leucine
- 30 minute each frame



←→  
2.5 mm

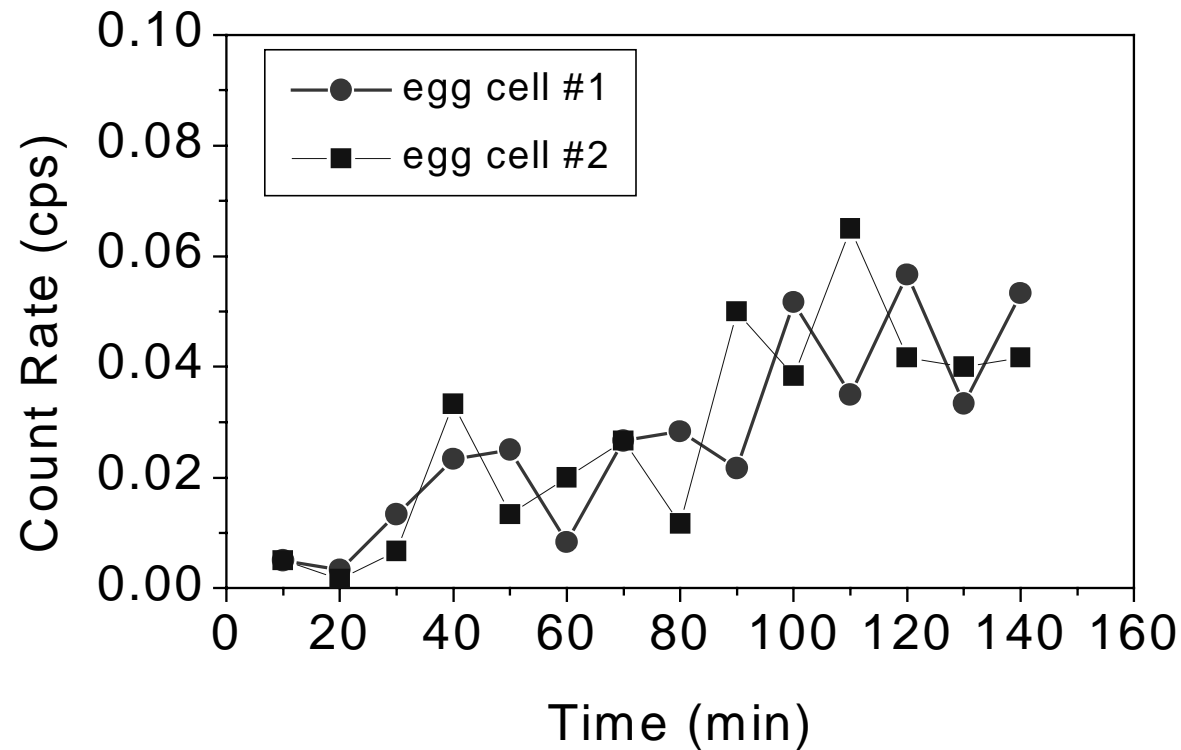
*Dynamic studies: [ $^{14}\text{C}$ ] L-Leucine  
proteins migration  
inside Octopus eggs*

- Marker:  $^{14}\text{C}$
- 30 minute each frame
- $\approx 0.01$  cps per cell



←→  
2 mm

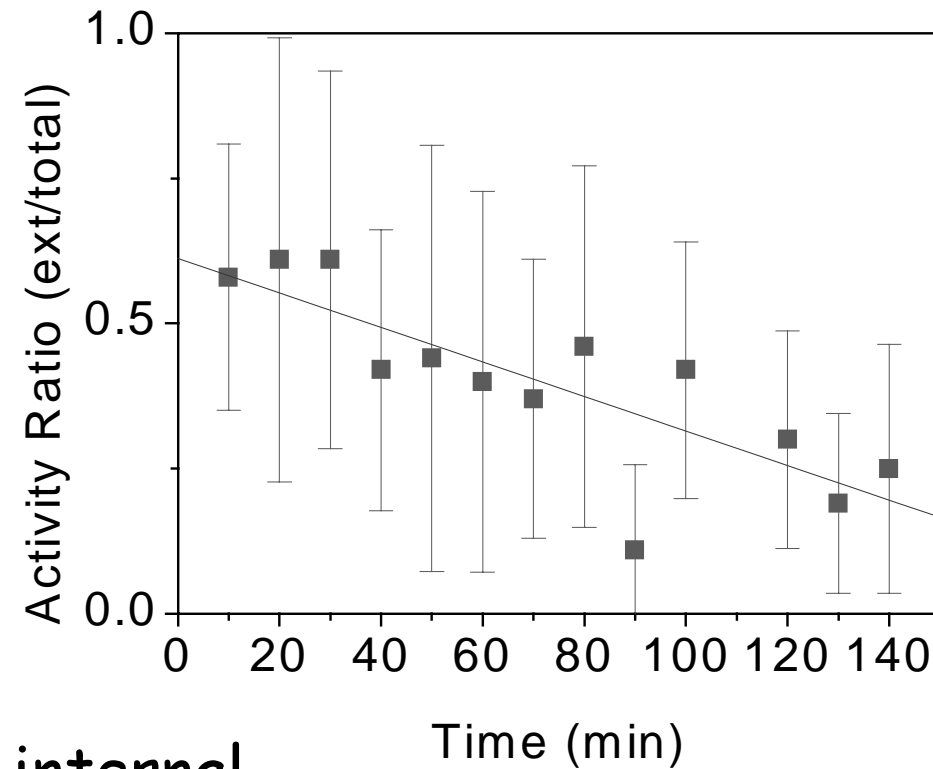
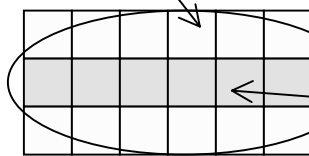
# *Dynamic studies: [ $^{14}\text{C}$ ] L-Leucine uptake in Octopus eggs*





*Dynamic studies: [<sup>14</sup>C]L-Leucine proteins migration inside Octopus eggs*

external



internal



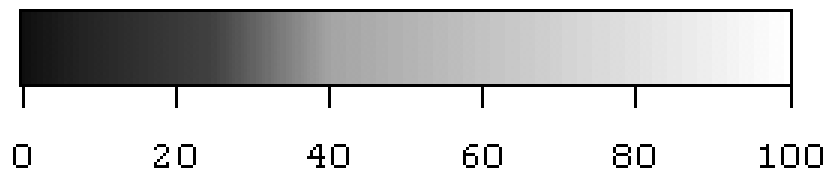
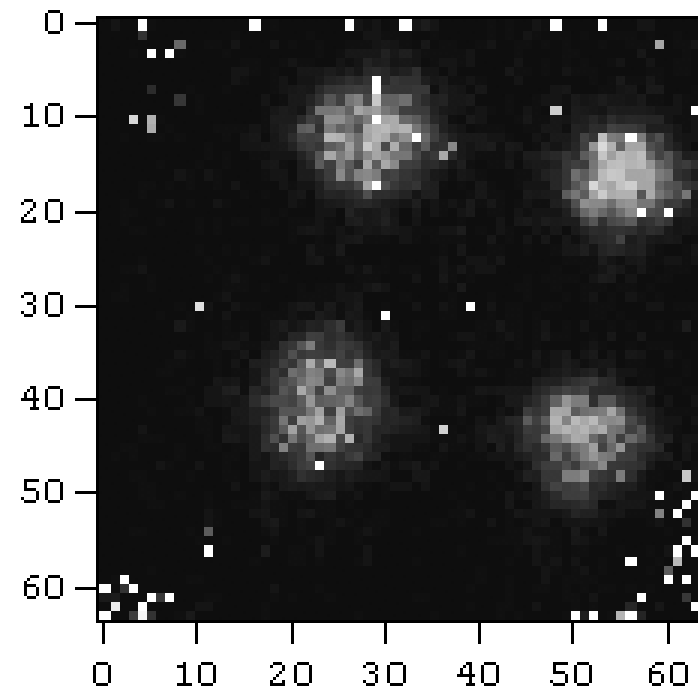
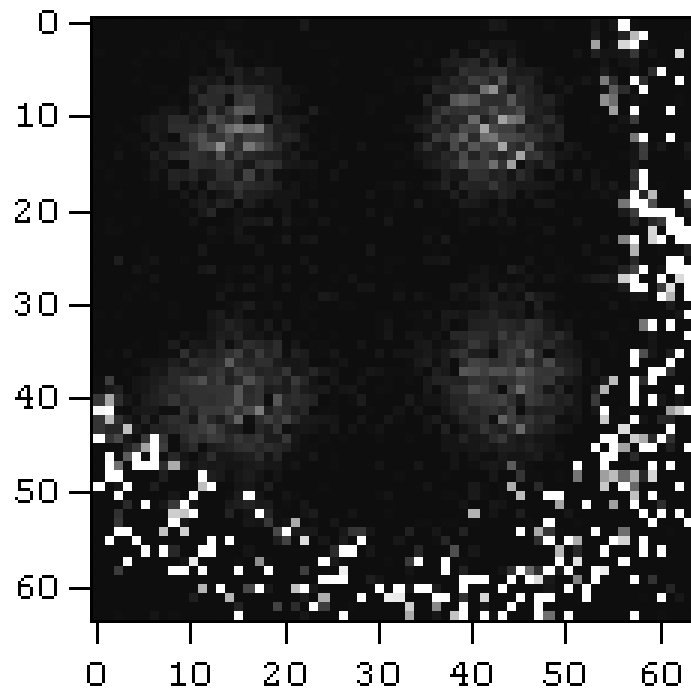
## *Future development:*

- medical and biological tests
- portability (laptop)
- enlarge the area (2D arrays)
- lower noise and better detector uniformity (Silicon detector)
- smaller pixel size (50  $\mu\text{m}$  - Medipix2)

# *Silicon detectors:*

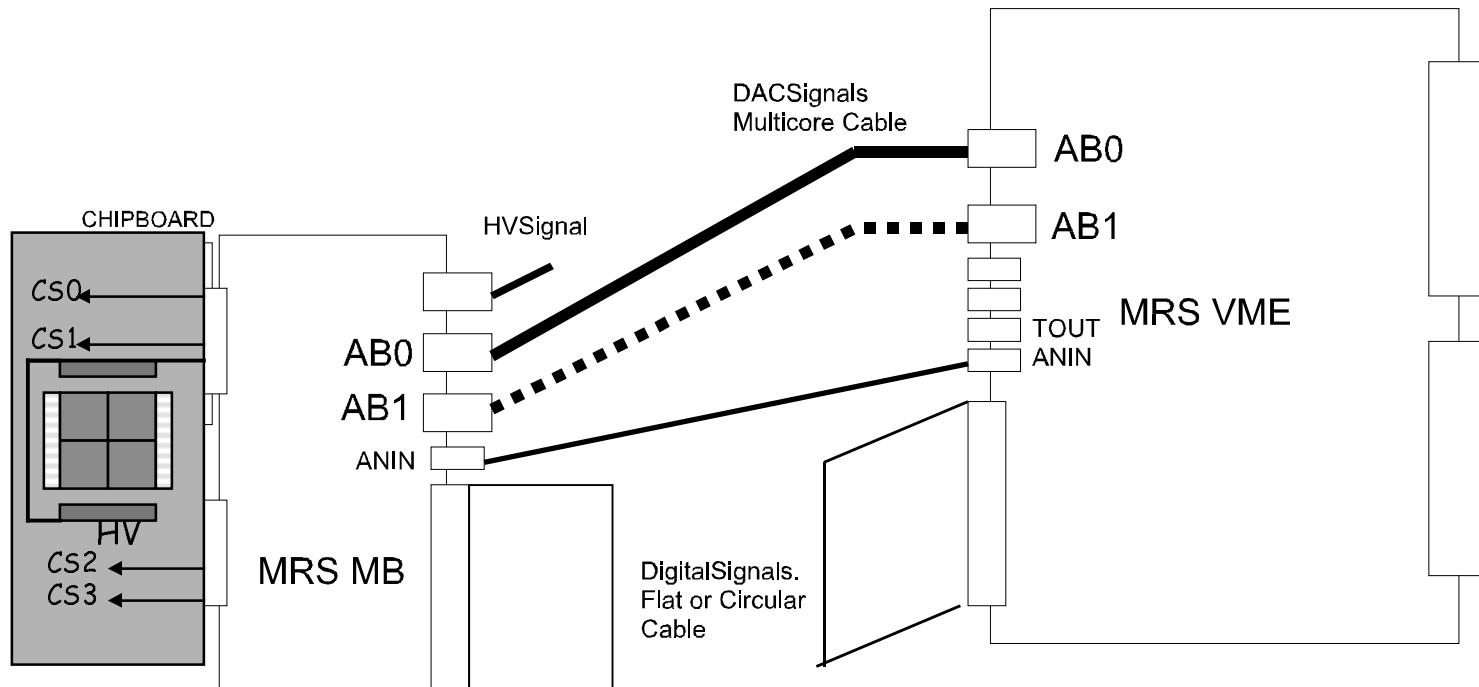
GaAs detector, 4x50 Bq C-14, 30 min.

Si detector, 4x50 Bq C-14, 10 min.



GaAs:  $\sim 2.5$  cps,  $\epsilon \sim 10\%$   
Si:  $\sim 12.5$  cps,  $\epsilon \sim 50\%$

# 4-chip Chipboard





## *Collaborations:*

- *Medipix1-2, CERN + INFN + European Univ.*  
VLSI electronics development
- *Genetic Engineering Center, University of Napoli*      innovative diagnostic genetic tests
- *Molecular Cardiology Lab, University of Roma*      cardiological viral tests
- *Zoology Department, University of Napoli*  
dynamic imaging, basic biological research