^{3}H IMAGING

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on behalf of:

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acknowledgments to:

Michael Campbell, Bettina Mikulec & Lukas Tlustos,
representing the MEDIPIX2 collaboration for the fruitful cooperation

Outline

introduction

- Radioactive labeling
- Standard devices for biological applications

two complementary pixel sensors

- MEDIPIX2
- MIMOSA5

playing the game

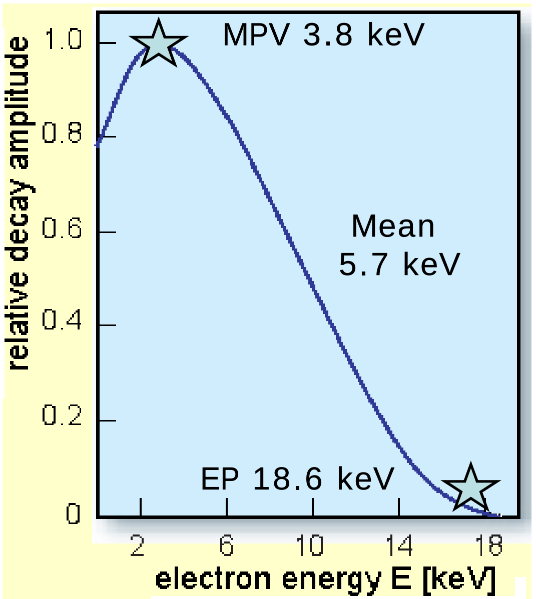
conclusions & outlook

Biological Sample Imaging

Radioactive labeling is a widely used **tracking method** to follow the distribution and uptake of bioactive molecules throughout the body of animal models, allowing the localization of specific cell populations.

Standard Markers: **^{3}H - ^{14}C - ^{32}P - ^{33}P**

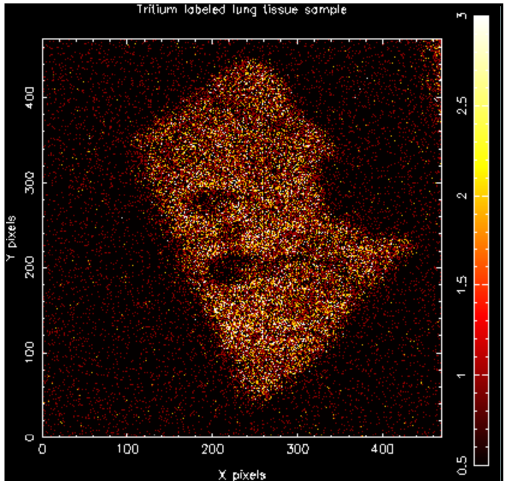
^{3}H is the **favourite** and also the most **challenging**



- **Biological reason:**
  very common in organics molecules

- **Image quality:**
  low energy→NO blurring due to the particle penetration in the substrate

- **Short range** → NO air & NO dead layers
- **Low energy** → LOW noise
- **Very Low activity** → Very Long exp. time



Slice of a (poor) rat lung tissue where amminoacid was labelled with ^{3}H to identify spot of damaged alveolar epitelium

- a stable detector
- a possible enormous quantity of data… taking into account an image can require up to 10^{5}-10^{6} frames to get a proper quality.

Standard Devices

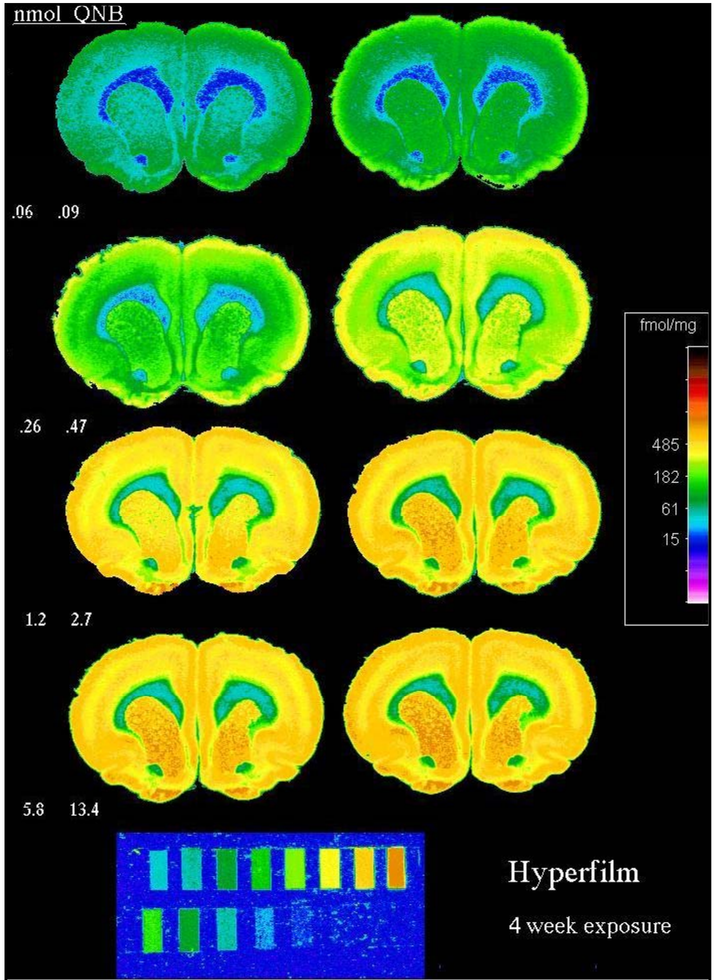
Films

- **Disposable & cost effective**
- **Dynamic range covering a few orders of magnitude (~ 3)**
- **Very high granularity**
- **Poor detection relative efficiency**

Phosphor imaging plates

- **Sensitivity two orders of magnitude higher wrt films (e.g. ~ 30 $^{dpm}/_{mm^2}$ on ^{14}C, for the BAS Fuji system)**
- **Reusable (but the full apparatus is quite expensive)**
- **5 orders of magnitudes dynamic range**
- **Lower image resolution wrt films**

Both of them are *Integration Devices* (i.e. no spectrometric capabilities and different labels cannot be distinguished)



4 weeks exposure

Specs of the ideal sensor

- High sensitivity to low energy electrons (i.e. no entrance window, good Charge Collection Efficiency, low noise, high discrimination against cosmic rays and thermal noise)
- High duty cycle
- Effective on-line sparsification & data reduction
- Good granularity
- **Large** (extremely large!) area (a standard experiment consists in about 60 slices ~130 cm^2)

LOWER the EXPOSURE TIME & Get the image in real time

SELECTION GUIDE BY TECHNICAL OVERVIEW – Microscale Autoradiography Standards

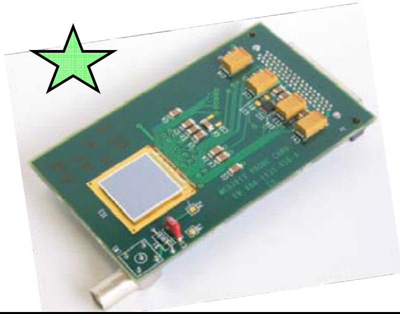
[^{3}H] Microscales (RPA506) Bq/mg	nCi/mg	(RPA507) Bq/mg	nCi/mg	[^{14}C] Microscales (RPA504) kBq/g	nCi/g
4048.0	109.4	588.0	15.90	31.89	862
2468.0	66.7	290.0	7.84	25.90	700
1487.0	40.2	180.0	4.87	19.39	524
872.8	23.6	74.4	2.01	12.91	349
525.4	33.5	37.0	1.00	8.55	231
320.4	8.7	17.8	0.48	4.44	120
175.0	4.7	9.62	0.26	2.18	59
110.6	3.0	3.74	0.10	1.11	30

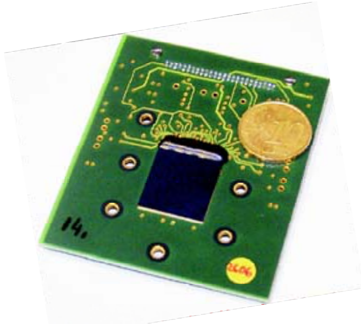
Note: Typi

Amersham ^{3}H calibration standards

1 week exposure (film)

Two complementary pixel sensors





	MEDIPIX2	MIMOSA 5
Technology	Hybrid	Monolithic
Active Volume	0.3 mm thick, fully depleted->drift	0.015 mm thin, not depleted->diffusion
Architecture	Binary (double threshold) + counting (13 bits)	Full analog
	500 transistors/cell	3 transistors/cell
Granularity	Moderate (55 µm)	High (17 µm)
Active area	≈ Cm scale edge	≈ Cm scale edge
Main advantage	• stability (leakage current compensation) • high duty cycle • simple off-line analysis	• granularity • full analog info • detectability@1KeV (3 rms)
Main disadvantage	• granularity • No analog info • noise floor at ≈ 3 keV	• large data volume (sparsification required!) • low duty cycle • Non trivial off-line analysis

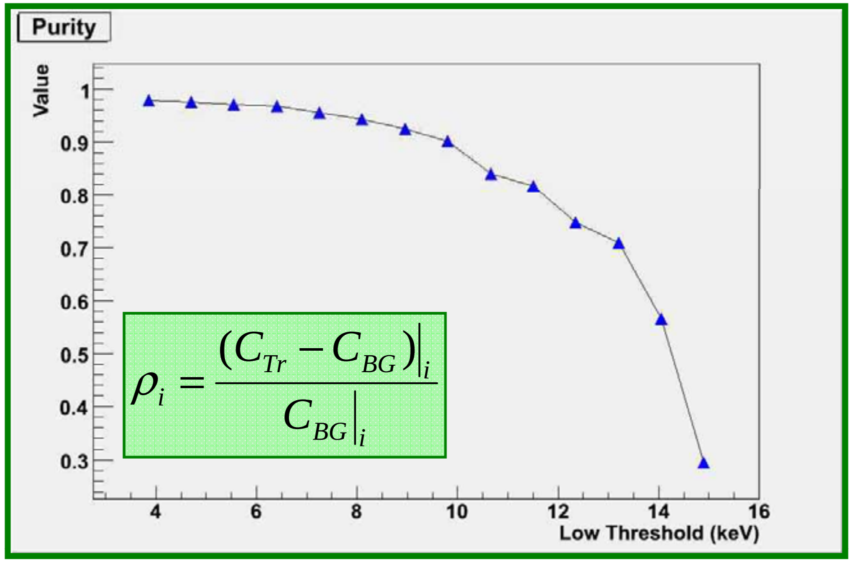
Grateful thanks going to Bettina Mikulec, Lukas Tlustos, Michael Campbell and to all the **Medipix2** collaboration for the fruitful cooperation

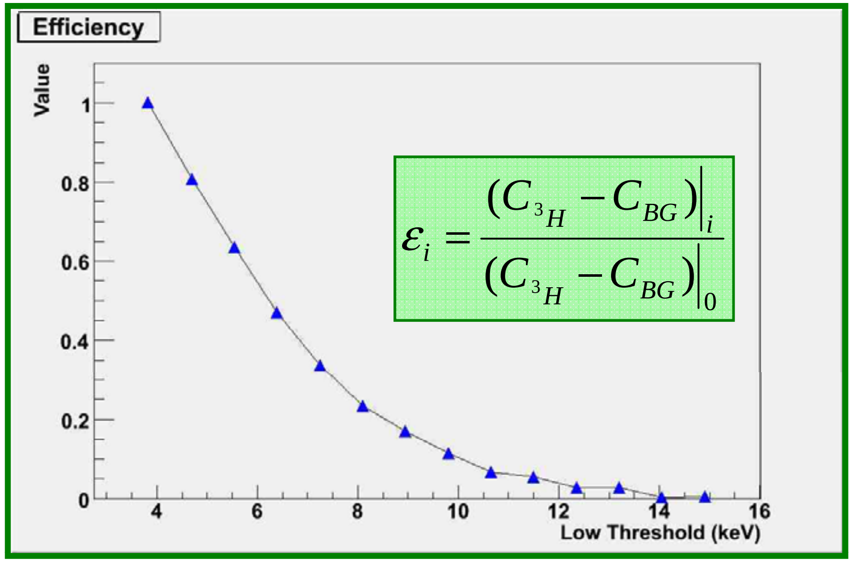
Medipix2 Optimal Working Point

The Medipix2 optimal working point has been defined relying on the measurements of the Efficiency and the Purity recorded for different ***Low Threshold*** values using a "high flux" Tritium source

$$3800\ ^{nCi}/_{mg} \ \text{->}\ 500\ ^{counts}/_{mm^2 \cdot s}$$

The **Purity** and the **Efficiency** are both decreasing due to the reduced fraction of the Tritium spectrum that is detected as the threshold is increased, together with a possibly flat spectrum of the left-over noisy pixels.





Low Threshold = 3 keV

Medipix2 Optimal Working Point

The flat field by the uniform flux of cosmic rays was reduced in a twofold way:

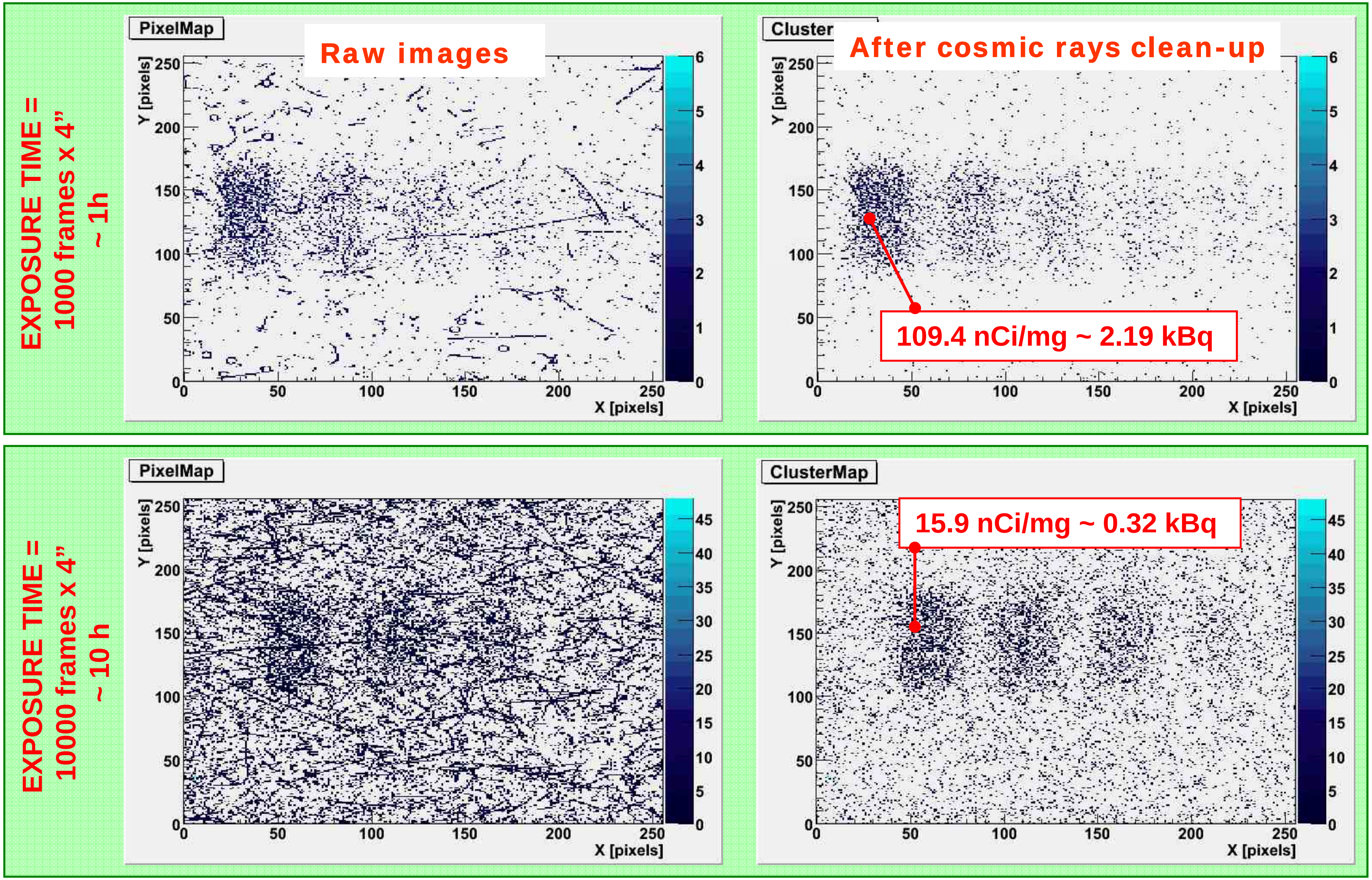
- Setting the ***High Threshold*** of the discrimination window at ~**20keV**, i.e. at the end point of the ^{3}H spectrum. Cosmic rays crossing the detector at a nearly orthogonal direction are expected to deposit ~110keV (most probable value), making them clearly distinguable.

- By a dedicated cluster shape analysis identifying track like clusters by inclined cosmics.

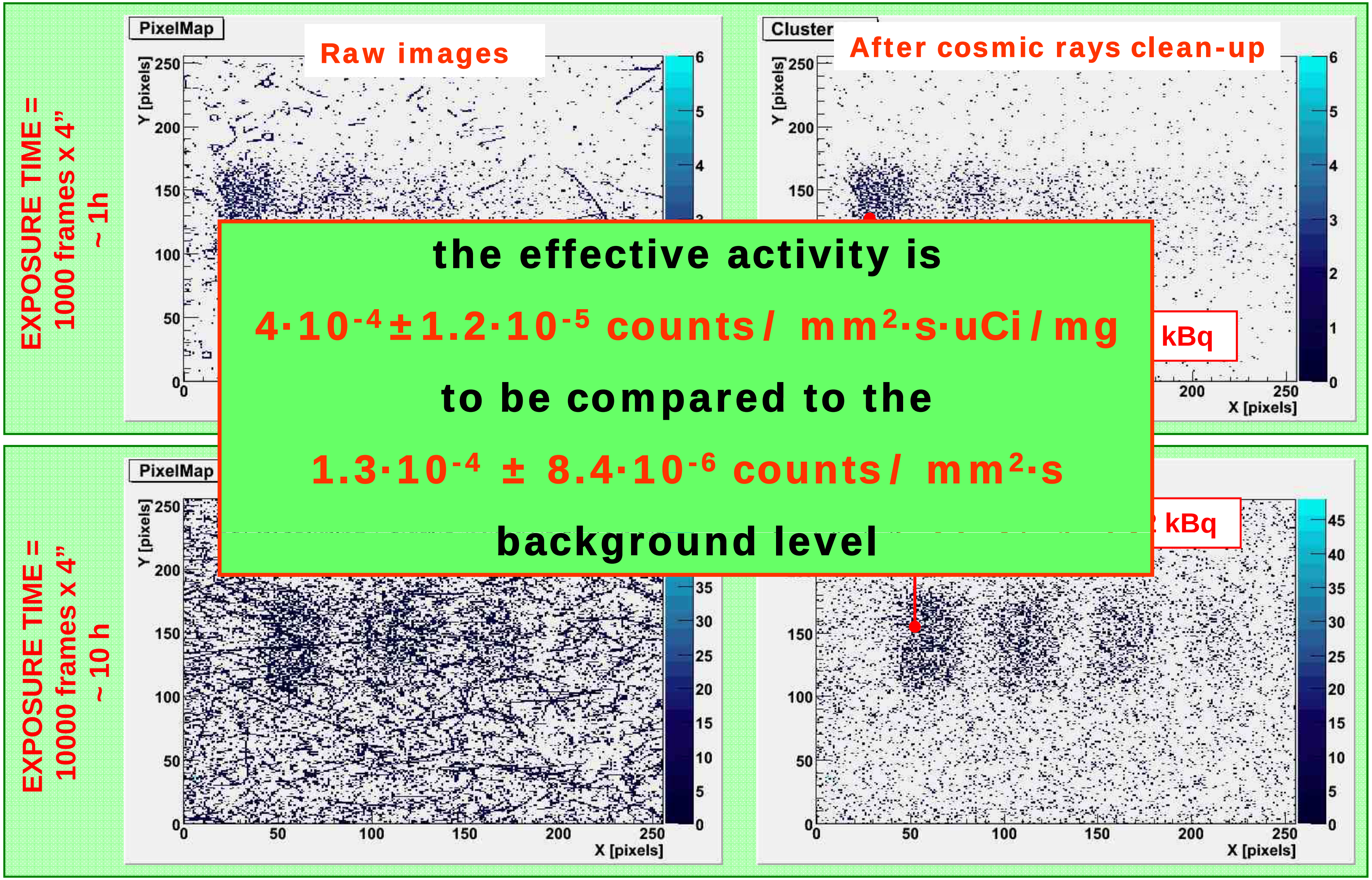
the net result is a reduction in the background contamination

$4.4 \cdot 10^{-4} \pm 1.5 \cdot 10^{-5}$ counts / $mm^2 \cdot s$ → $1.3 \cdot 10^{-4} \pm 8.4 \cdot 10^{-6}$ counts / $mm^2 \cdot s$

MEDIPIX2: imaging the ^{3}H standards



MEDIPIX2: imaging the ^{3}H standards



the effective activity is

$4\cdot10^{-4}\pm1.2\cdot10^{-5}$ counts/ mm$^{2}\cdot$s$\cdot$uCi/mg

to be compared to the

$1.3\cdot10^{-4}\pm8.4\cdot10^{-6}$ counts/ mm$^{2}\cdot$s

background level

Mimosa V: the analog information

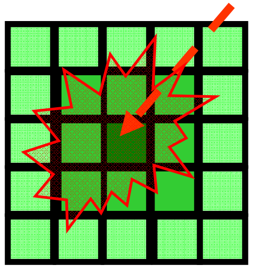
The major benefit the Mimosa V is offering is the **Analog Information.**

Because of the extremely low activities of the ^{3}H biological samples, images results from the integration of the information contained in a number of **1Mpixel** frames typically between **10^5-10^6**.

SPARSIFICATION ALGORITHMS: As of today sparsification algorithms are applied frame by frame during the data processing after the full matrix is transferred to the equipment computer.

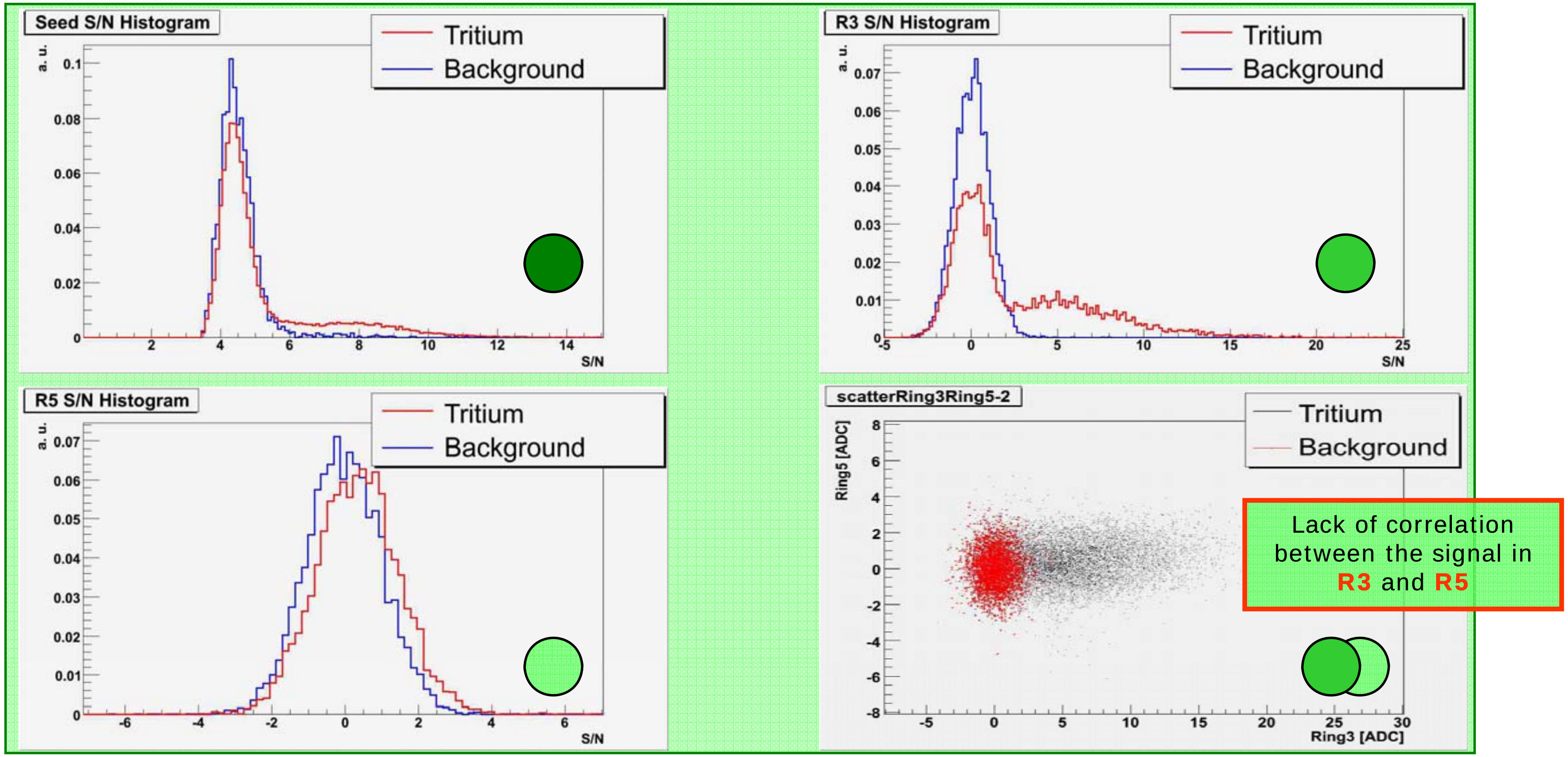
DUTY CYCLE ~2.5%

Mimosa V: where's the ^{3}H info?



The off-line data analysis is essentially based on three quantities: the signal in the **Seed** pixel, in the ring made by the 8 neighboring cells (**R3**) and in the outer most 16 pixels ring (**R5**).

^{3}H "info" is carried mainly in **Seed** and **R3**



Mimosa V: the noise

Standard **noise** values at room temperature for the MIMOSA family corresponds to an ENC **~30 e-** r.m.s., to be compared to a most probable value of **1000 e-** signal from ^{3}H decays.

High detection efficiency may be expected, irrespective of the diffusion over a 5x5 pixel cluster of the charge carriers generated by the ionizing particle. As a matter of fact, the large cluster size may be used; both directly and indirectly to reject the noise generated hits.

But… on the other hand the discrimination against cosmics rays is rather poor, since the signal in 15 um thick sensitive volume is de facto equivalent to ^{3}H.

off-line **Bayesian analysis** on analog signal of any triggered event

Mimosa V: the bayesian analysis

Bayesian analysis: signal enhancement against the hypotesis of beiing the hit generated by the noise (the method is descrived in G. Borisov and C. Mariotti, Nucl. Instr. and Meth. A **372** (1996) 181-187).

• the signal over noise spectra (**Seed**, **R3**, **R5**) for a blank run are considered has reference distributions.
• the corresponding cumulative distributions I_S, I_{R3}, I_{R5} are constructed:

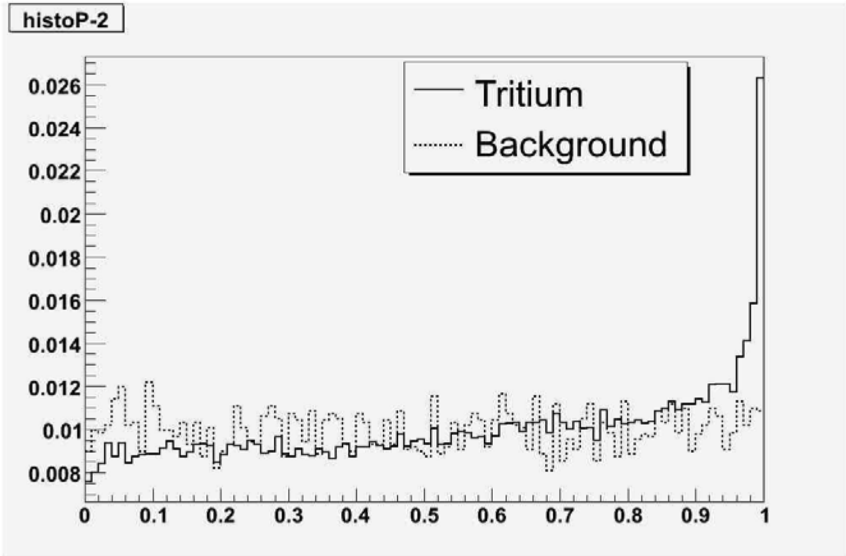
$$I_i(\tilde{x}_i) = \int_{-\infty}^{\tilde{x}_i} h_i(x_i)\,dx_i \qquad h_i(x_i)$$: signal over noise distributions

• the quantity (P) combining the three I_i information's is constructed as follows:

$$P = \Pi \cdot \sum_{i=0}^{2} \frac{(-\log \Pi)^i}{i!}, \Pi = I_S \cdot I_{R3} \cdot I_{R5}$$

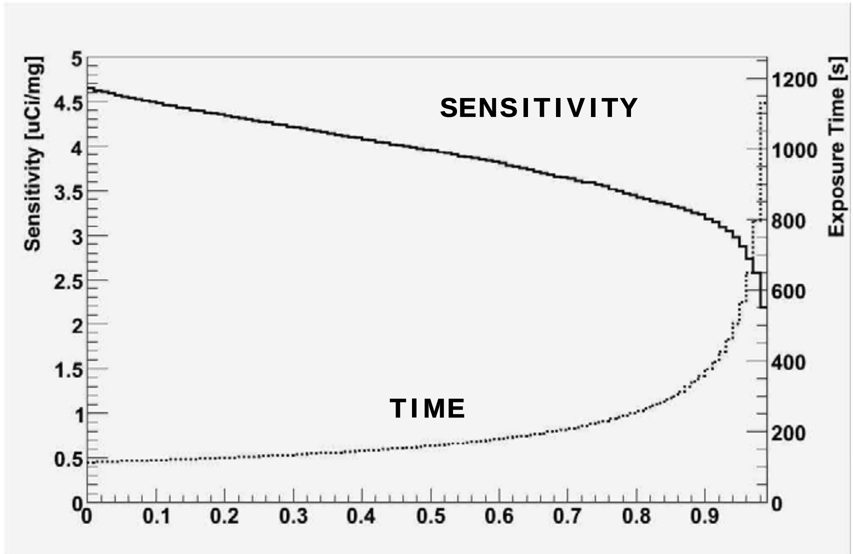
in order to have flat spectrum for blank runs.

Mimosa V: the bayesian analysis



the spectra of P is **flat** for **noise clusters**, while it is strongly **asymmetric** for **^{3}H hits**.

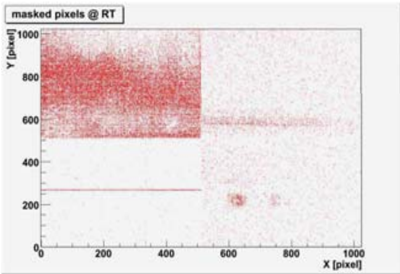
The optimal discrimination value for P has been defined trading off the effective sensitivity, defined as the specific activity resulting in a counting rate in excess of 3 noise counting rate fluctuations, and required exposure time.

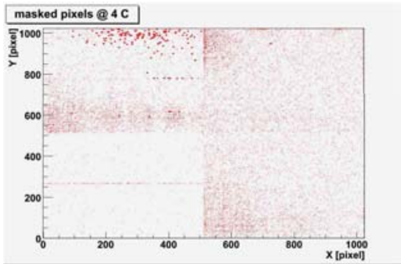


MIMOSA 5: analysis @ different T

Background analysis @ different Temperatures

SYSTEMATIC noise





masked Bad Pixels @ RT

8 %	1.1 %
0.3 %	0.7 %

masked Bad Pixels @ 4°C

2.2 %	1.2 %
0.1 %	1.1 %

STOCASTIC noise

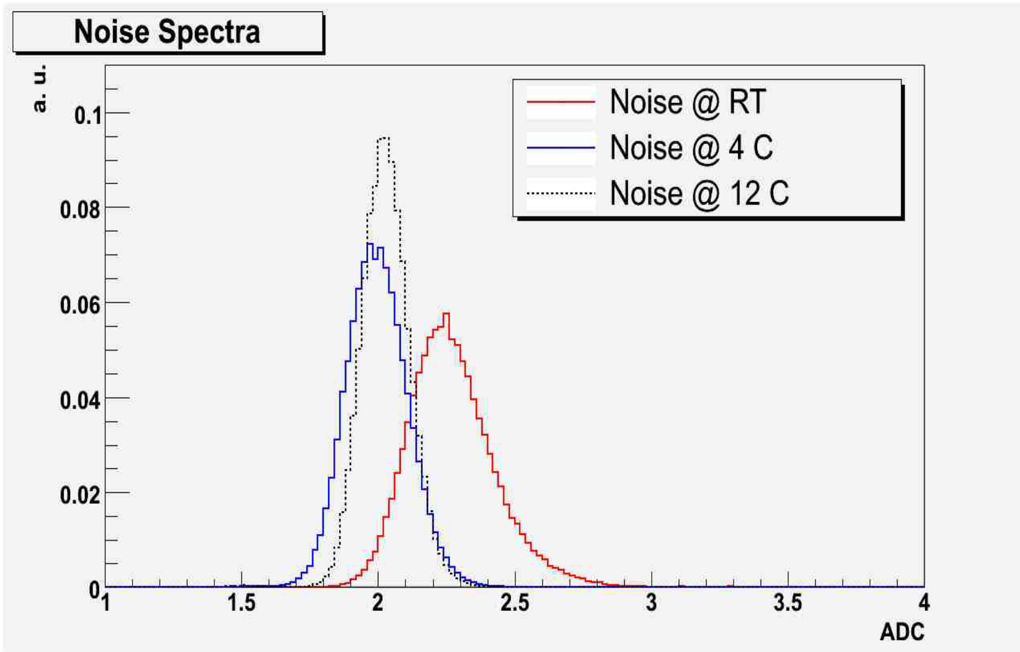
Analyzing an empty run with same selection rules as in a Tritium run…

Temperature	Hits x mm-^{2}sec^{-1}
@ RT	4.7
@ 12° C	1.9
@ 4° C	0.9

you may expect that a selection in sigma units will result in a more efficient ^{3}H selection (for instance, a 3 sigma cut leads to a different fraction of retained ^{3}H if the sigma is 1 or 3 keV)

Mimosa V: background analysis

STOCASTIC noise

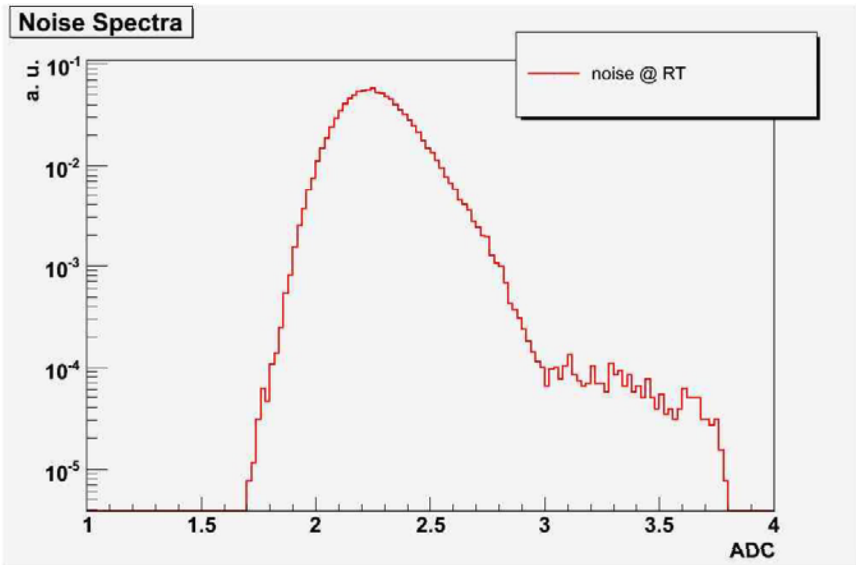


as expected the mean noise value is going down with the temperature

but… a better look can reveal some surprises…

Mimosa V: background analysis

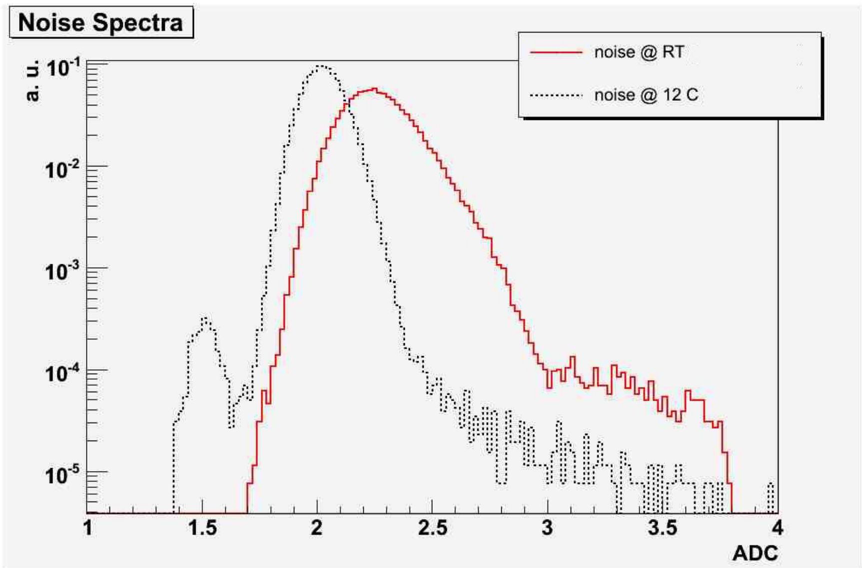
STOCASTIC noise



if you look at the noise distribution at room temperature (in log scale), a non gaussian shoulder is clearly visible. This is the contribution of the "bad" pixels singing lound too often and introducing a systematic effect.

Mimosa V: background analysis

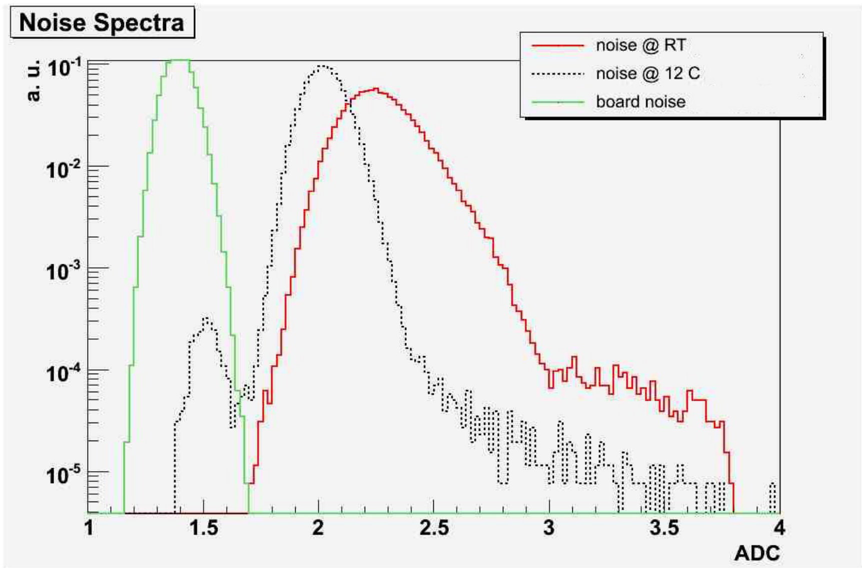
STOCASTIC noise



going down in T is actually removing a great part of these bad pixels, but another contribution pops up on the left hand side tail. This seems to be the minimum noise value achievable by the system.

Mimosa V: background analysis

STOCASTIC noise

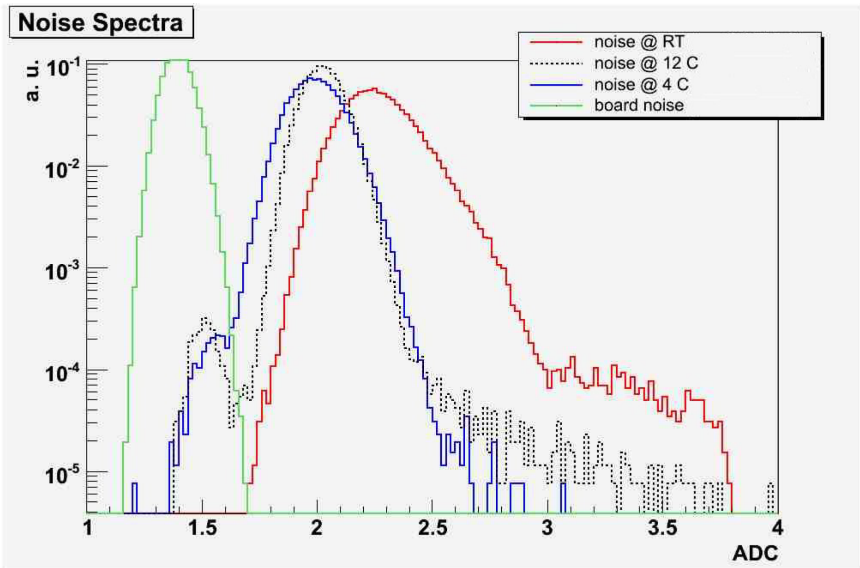


if you study the noise of the full system without the sensor, you get noise distribution that is of course temperature independent being the DAQ system located outside the cooled box peaked at 1.4 ADC.

So the second peak popping out at 12° C is probably the sum of the DAQ system noise and the temperature independent contribution of the sensor noise.

Mimosa V: background analysis

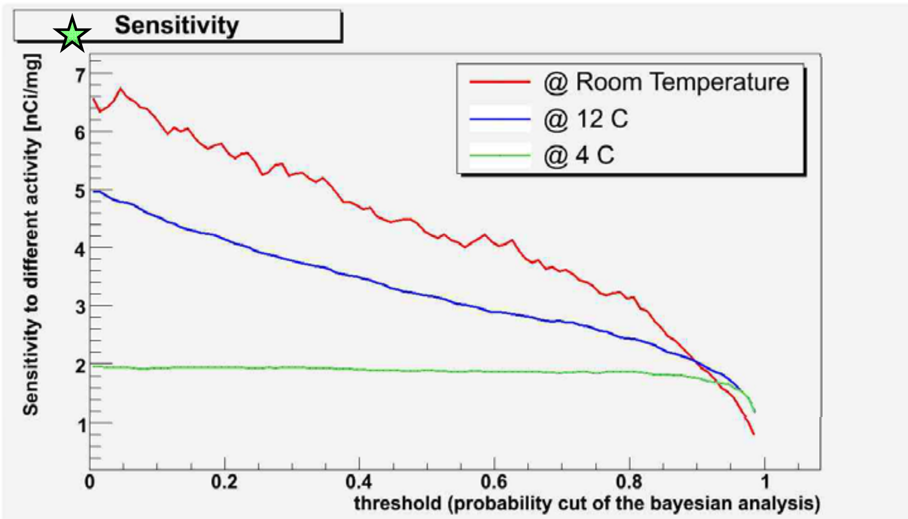
STOCASTIC noise



going down further in temperature is completely removing the systematic contribution and moving the gaussian core towards the "minimum" peak.

Mimosa V: background analysis

STOCASTIC noise



☆ defined as the specific activity resulting in a counting rate in excess of 3 noise counting rate fluctuations

increasing the cut, the sensitivity is going down (true at RT and at 12° C, but not at 4° C); at 4° C the sensitivity is flat as the bayesian algorithm is no more discriminating between noise and signal. It seems that there some signal in this noise!

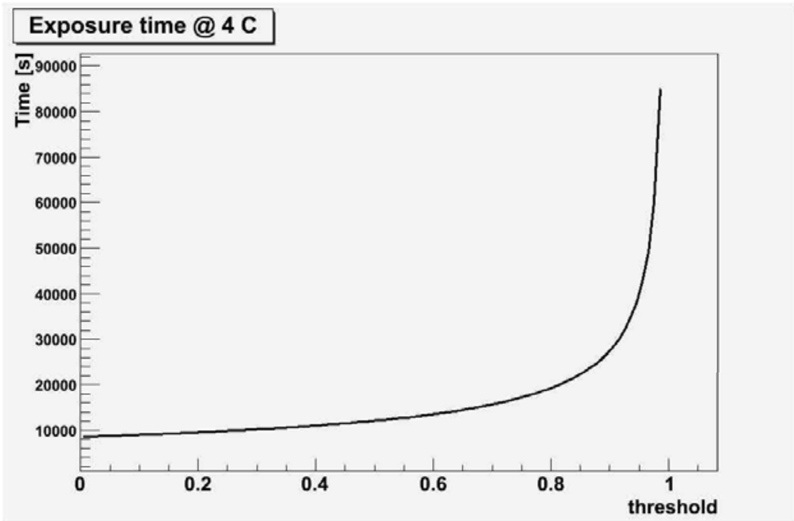
~ 10^{-4} hits/mm^2s

In fact, the cosmic ray flux, usually negligible compared with source rate and standard exposure time, it becomes important when you have very low rate and extremely long exposure time.

Mimosa V: background analysis

STOCASTIC noise

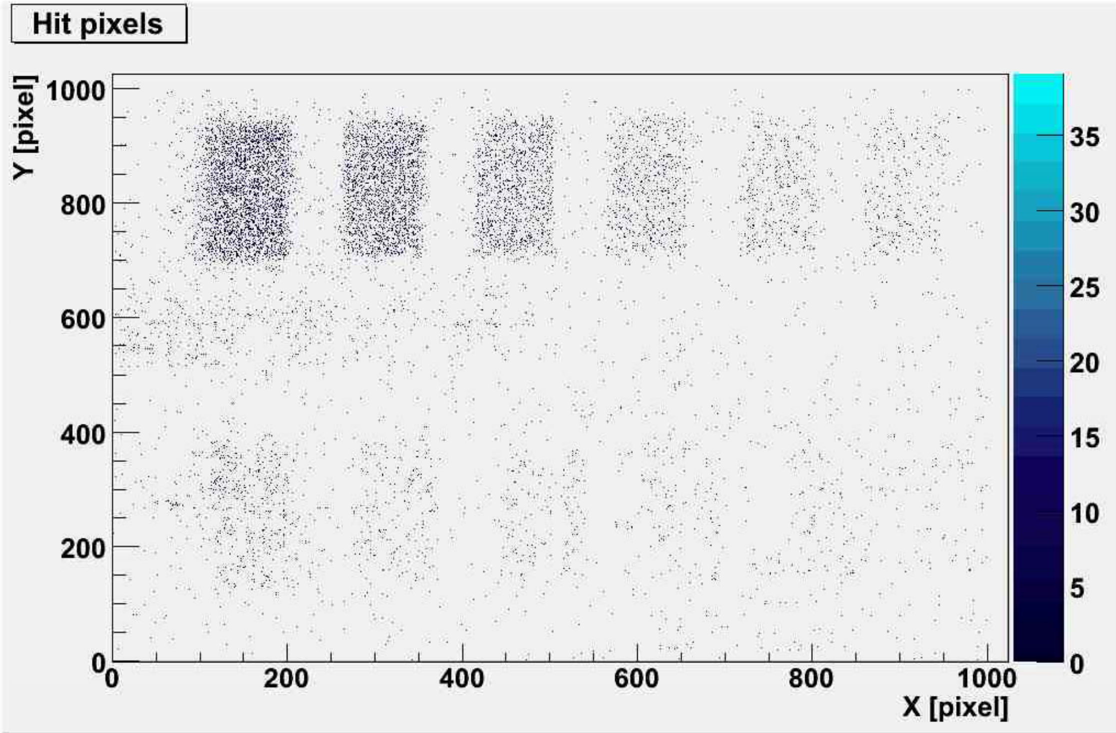
...but there is also the back side of the medal. If size matters (and we all know it matters), time matters as well.



Increasing the probability cut is indeed increasing the sensitivity but this doesn't come for free, you have to pay to in exposure time!

Mimosa V: signal analysis

Hitmap @ 4° C of Amersham ^{3}H calibration standards



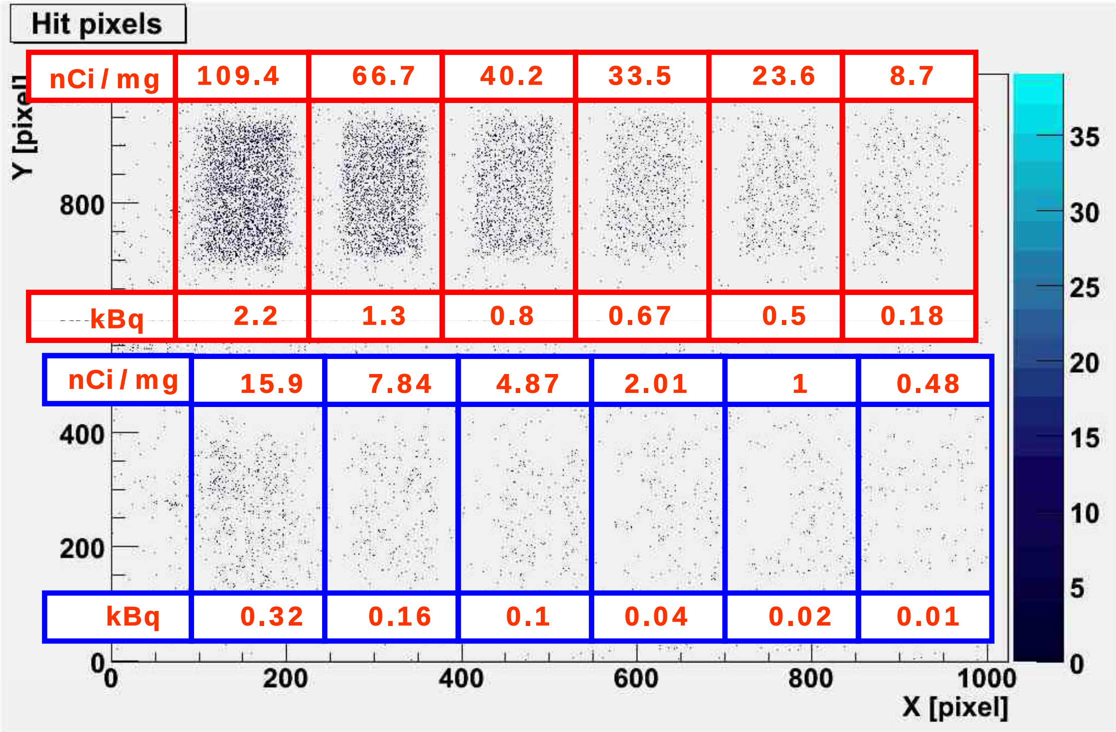
Exposure time: ~3700 s corresponding to ~41 hours wall clock time!

Mimosa V: signal analysis

Hitmap @ 4° C of Amersham ^{3}H calibration standards

0.5 mg/dot

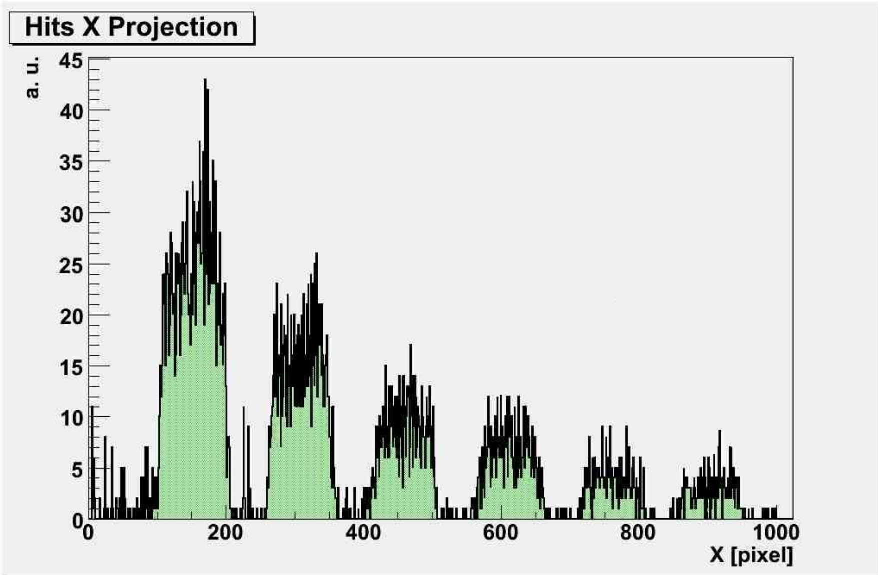
1 nCi = 37 Bq

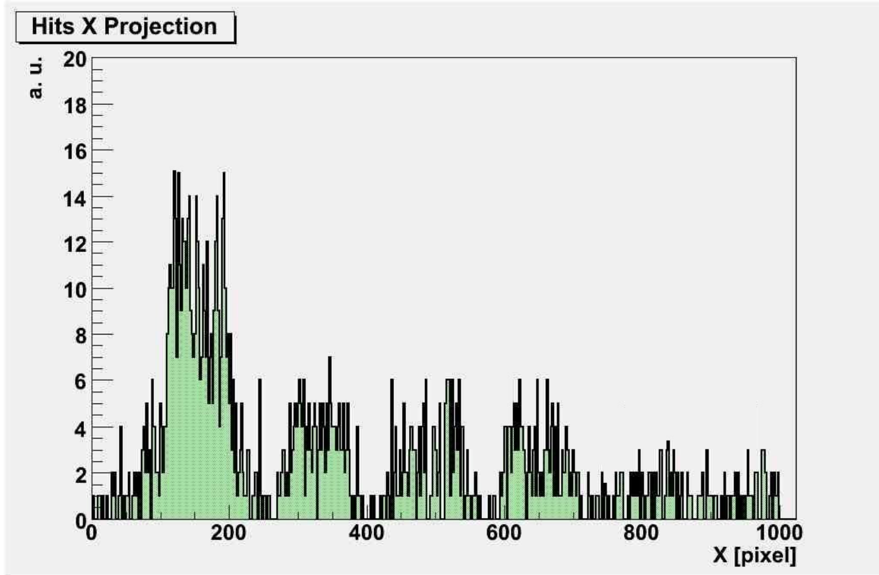


Exposure time: ~3700 s corresponding to ~41 hours clock wall time!

Mimosa V: signal analysis

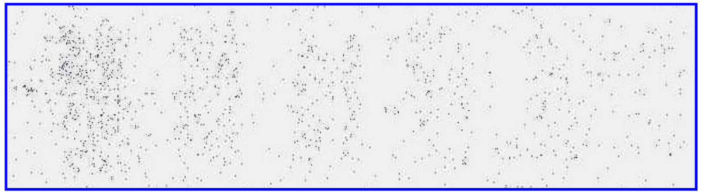
Hitmap @ 4° C of Amersham ^{3}H calibration standards





kBq	2.2	1.3	0.8	0.67	0.5	0.18

kBq	0.32	0.16	0.1	0.04	0.02	0.01



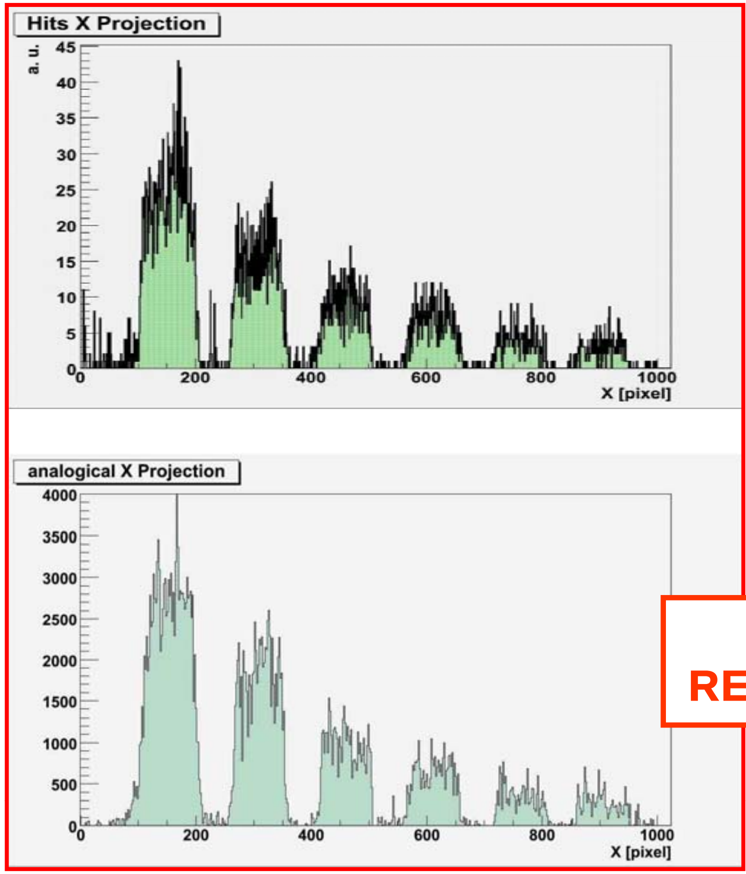
Exposure time: ~3700 s corresponding to ~41 hours clock wall time!

Mimosa V: image optimisation

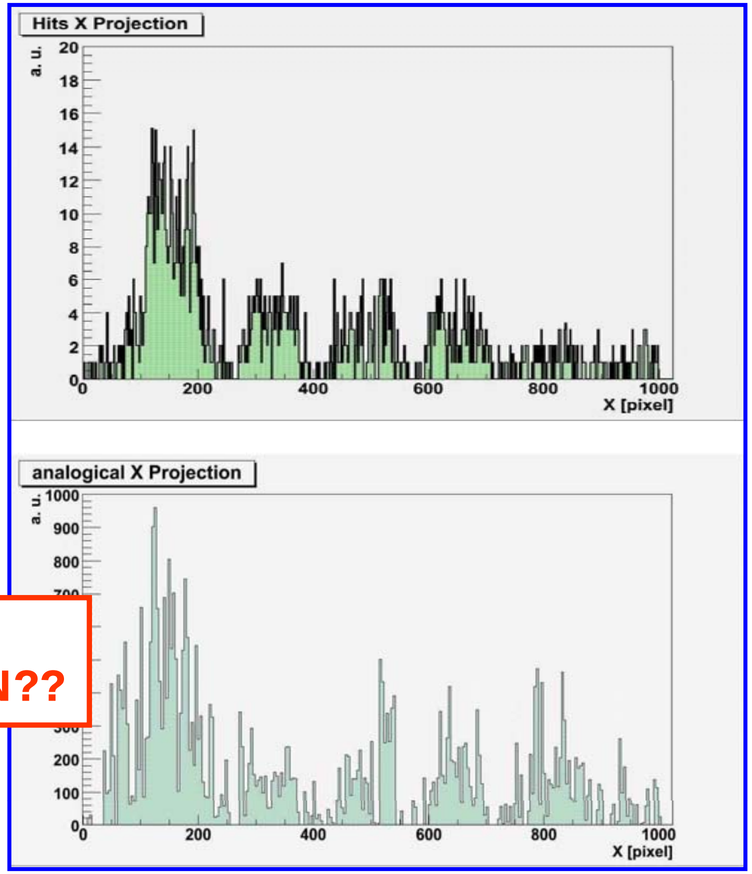
Different ways to build up the image:

- Binary info of any cluster;
- Analog signal for each pixel in the cluster;

MOST INTENSE ^3H SOURCE

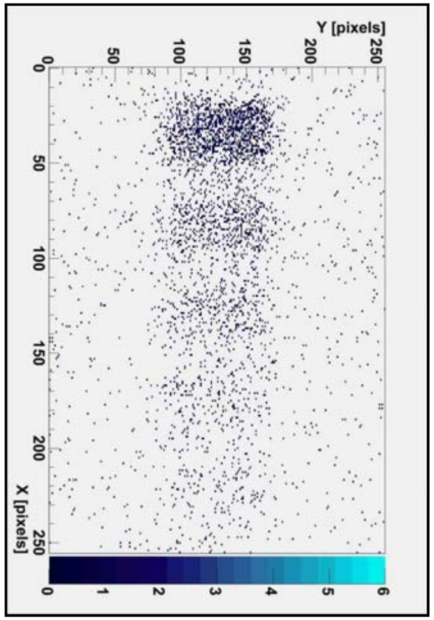


LESS INTENSE ^3H SOURCE



BETTER RESOLUTION??

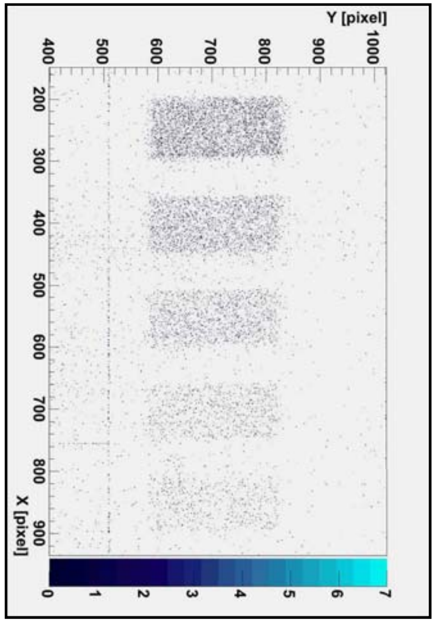
comparison



MEDIPIX2

- 10 h exposure
- background: $\approx 10^{-3}$ events/mm^{2}s
- effective activity:

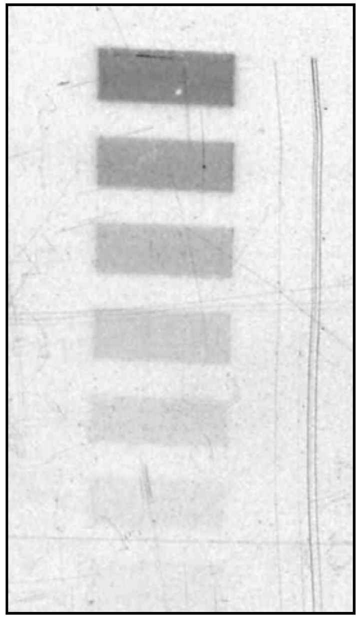
4 x 10^{-4} events/mm^2 s nCi/mg



MIMOSA V

- 14 h exposure (< 1 h effective time)
- background: $\approx 10^{-3}$ events/mm^{2}s
- effective activity:

1,7 x 10^{-3} events/mm^2 s nCi/mg



FILM

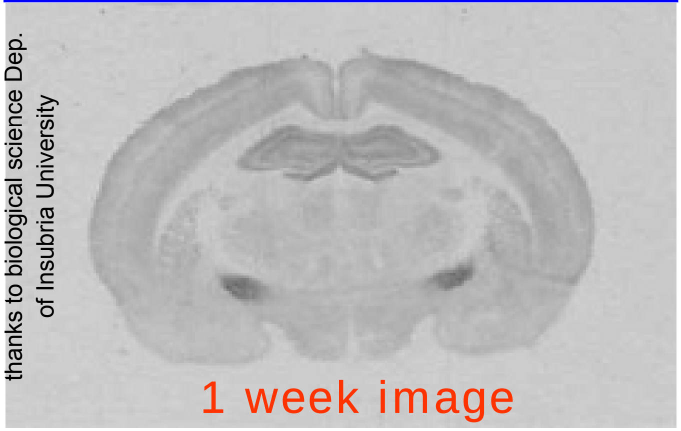
- 170 h exposure

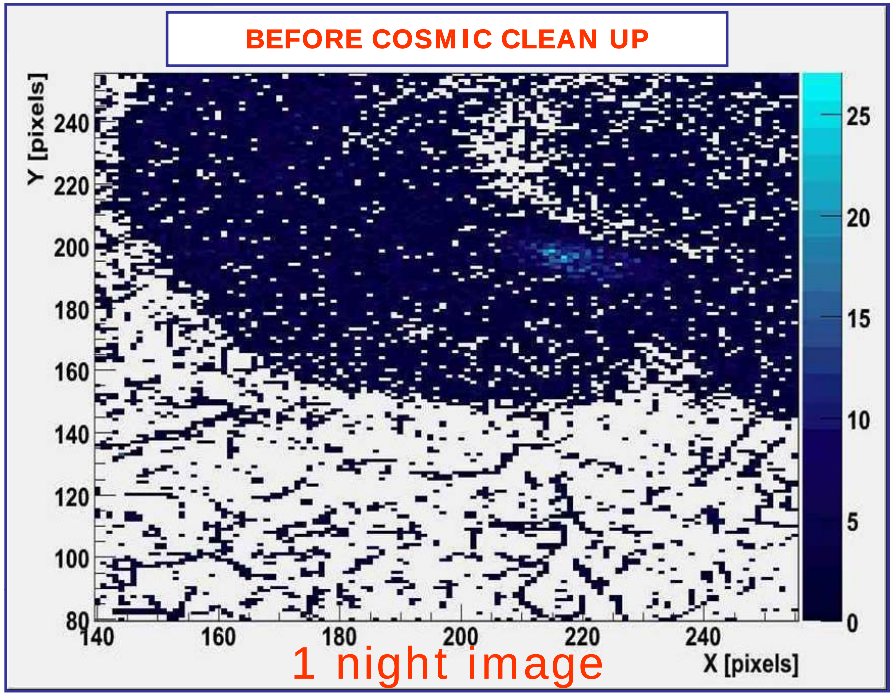
Sensitivity of Phosphor Imaging Screen ≈ 30 dpm/mm^2 = **5 x 10^{-1} events/mm^2 s**

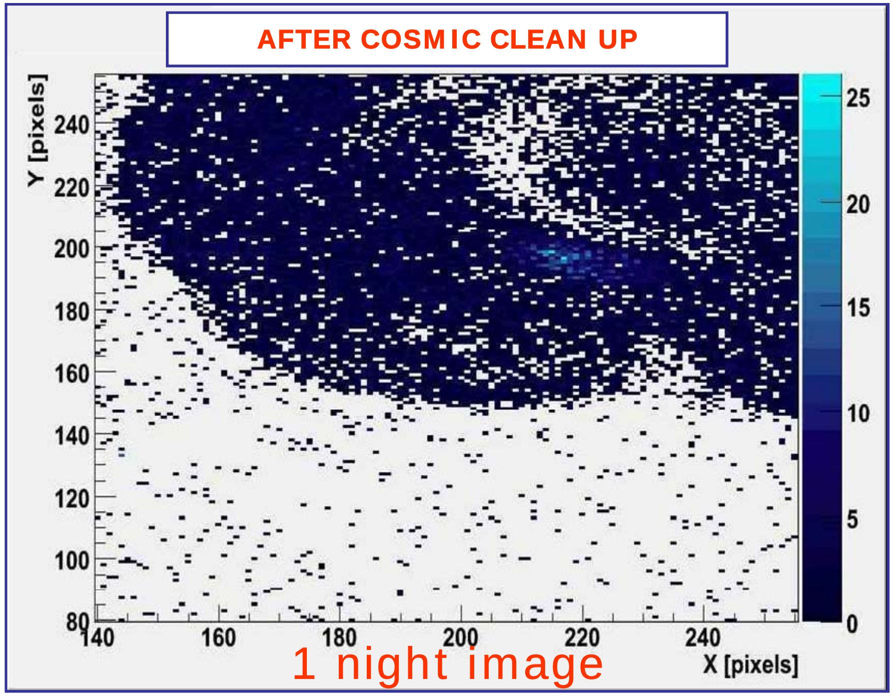
The biologist real life with Medipix2

The biologist real life is different. The goal is not only to recognise a particle, but to take an image!

Tritium labelled slice of mouse brain







The biologist real life with Mimosa V

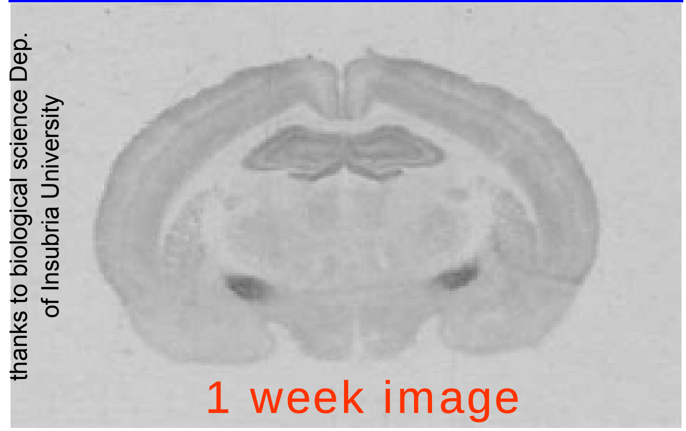
by changing the 2D histo binning it is possible to change its S/N ratio, increasing the contrast of the image.

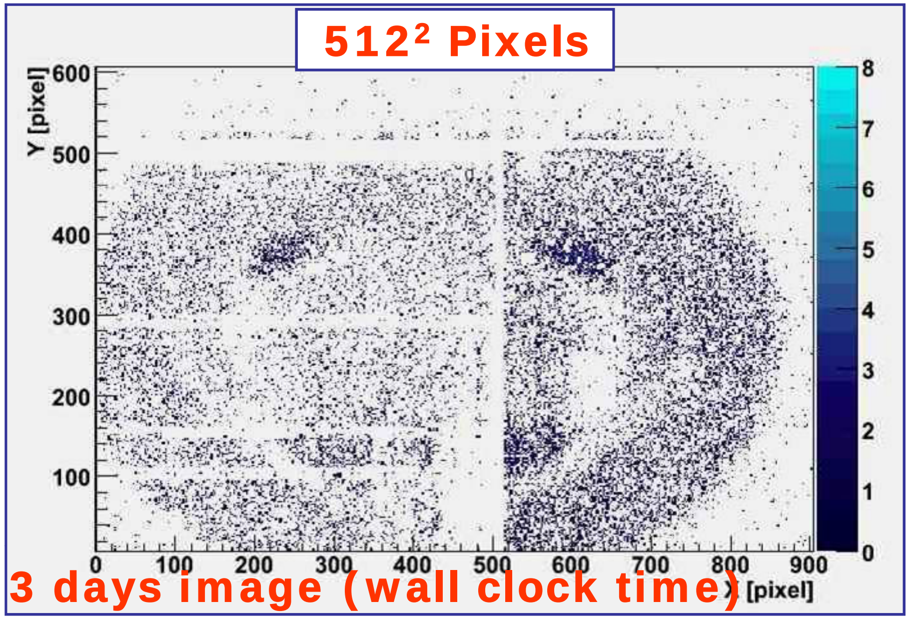
i. e. if there are 16 hits of signal and 4 hits of noise in each pixel you group, you have:

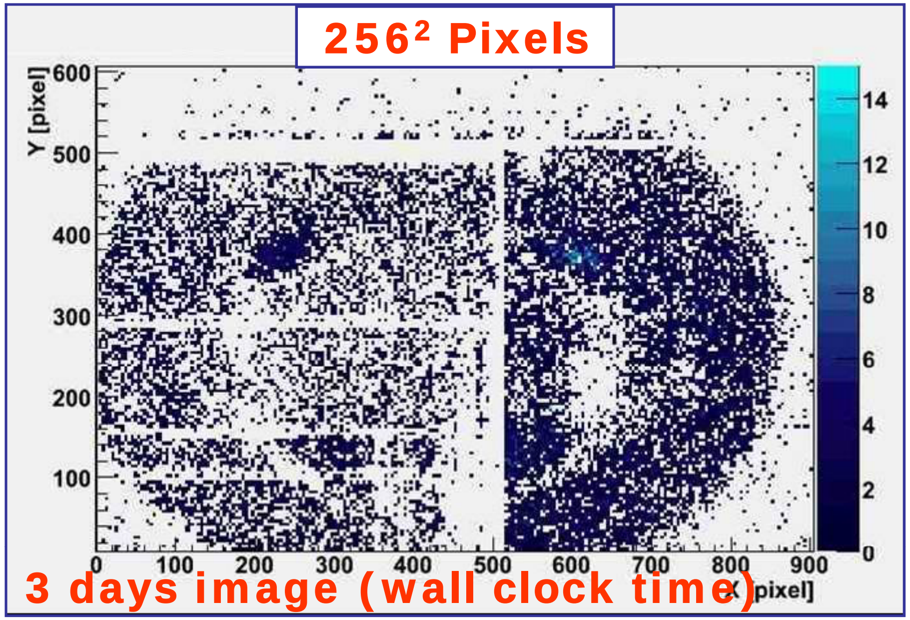
S/N = 16/sqrt(4) = 8 in the original image

S/N = 16x4/sqrt(4x4) = 16 in the rebin image

Tritium labelled slice of mouse brain

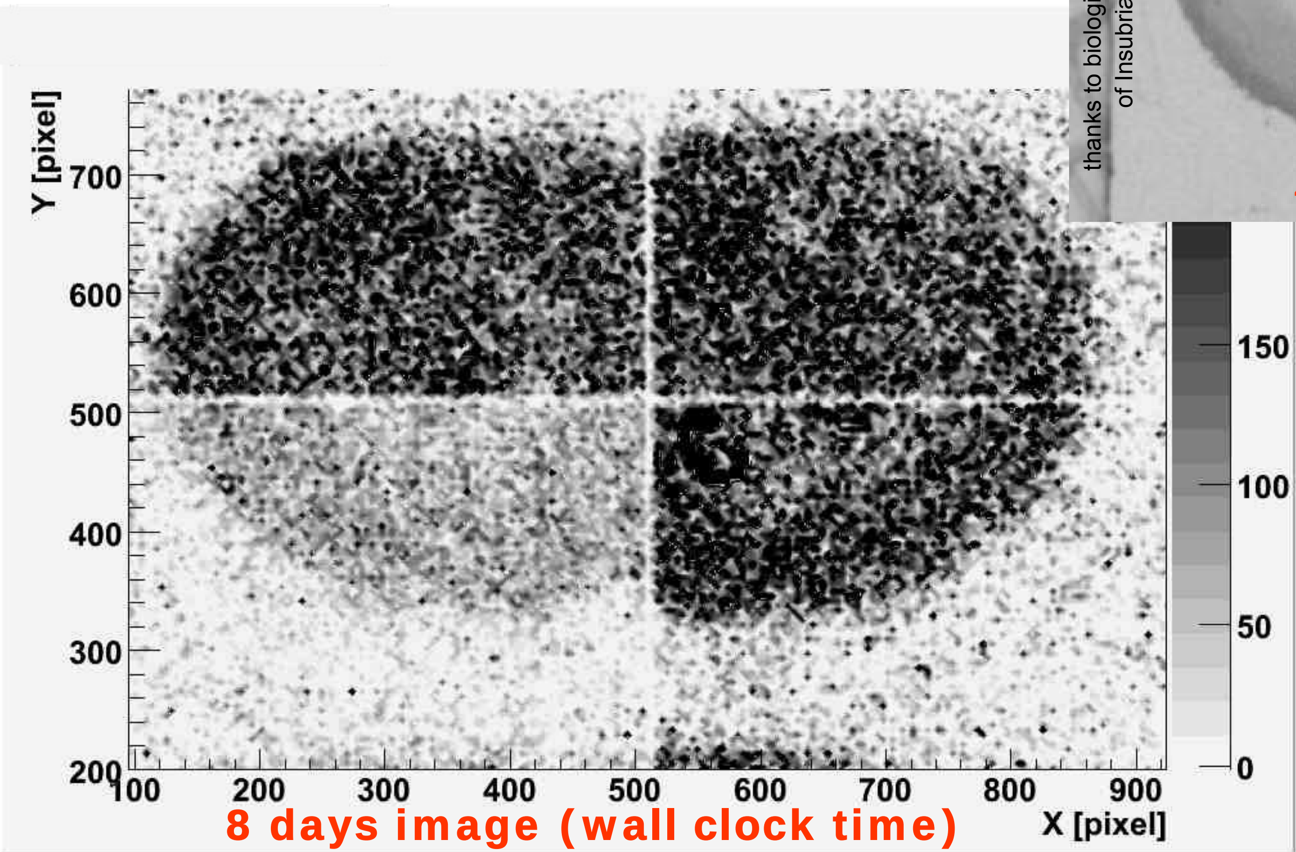




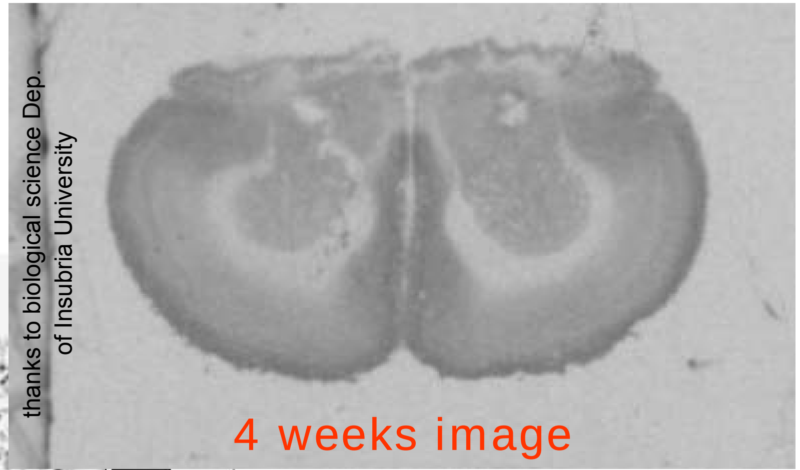


The biologist real life with Mimosa V

distance from the detector (~ 0.2 mm) comparable with the width of 'white ring'



Tritium labelled slice of mouse brain



Conclusions & Outlook

- Proof of concept given!
- Improvement with respect to existing devices:
  - Sesitivity levels -> 10^{-1} counts/$_{mm^2 s}$ to 10^{-3} counts/$_{mm^2 s}$
  - Exposure time -> weeks to hours

- Image quality improvement procedures in progress

for the future…

- The development of a dedicated device is being addressed, with a focus on the sensitivity
- Engineering and real-time signal processing are far from being trivial, in view of the required large area

THANKS FOR YOUR ATTENTION

...

Backup slides

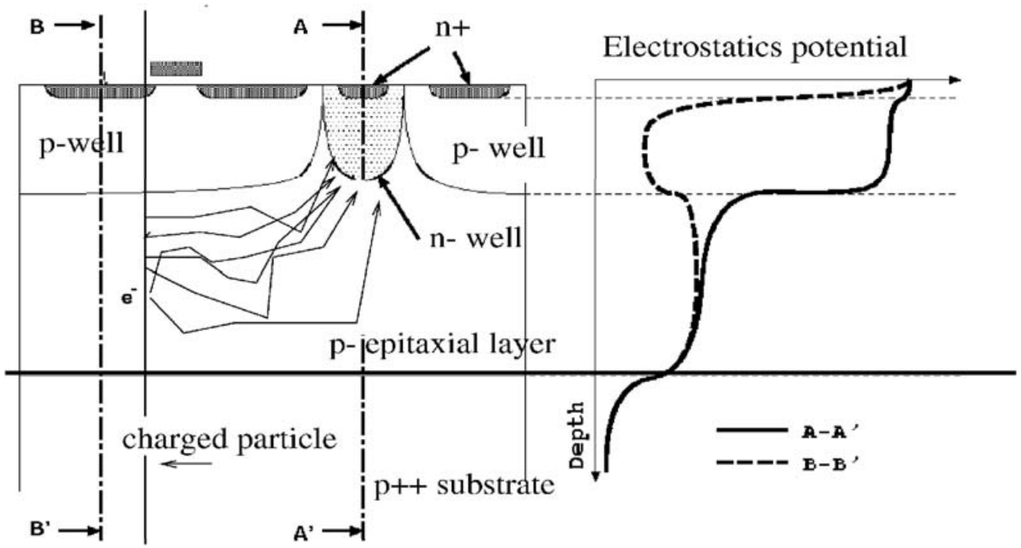
Monolithic Active Pixels Sensor

when a photon or charged particle interacts with silicon in the epitaxial layer, it generates some e^{-}- h^{+} pairs tha t can almost freely diffuse within the crystal lattice since there is no externally applied electric field. The buil-in electrostatic potential due to the doping profile is such that the carge carriers generated inside the epitaxial layer are reflected by the p-well and p^{++}-substrate; by diffusion they get to the collecting diode.

10^{19} at/cm³

10^{14} at/cm³

10^{19} at/cm³



Low resistivity material; not depleted active volume (diffusion instead of drift); active volume = epitaxial layer; extremely high spatial resolution and low noise

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i. e. if there are 16 hits of signal and 4 hits of noise in each pixel you group, you have:

S/N = 16/sqrt(4) = 8 in the original image

S/N = 16x4/sqrt(4x4) = 16 in the rebin image