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on behalf of BNCT group, Pavia University & INFN-Pavia

Misura del ^{10}B tramite autoradiografia neutronica:imaging della distribuzione e quantificazione in campioni cellulari e tessuti

NEPTUNE kick-off meeting, 14/12/2018, LNS-INFN, Catania

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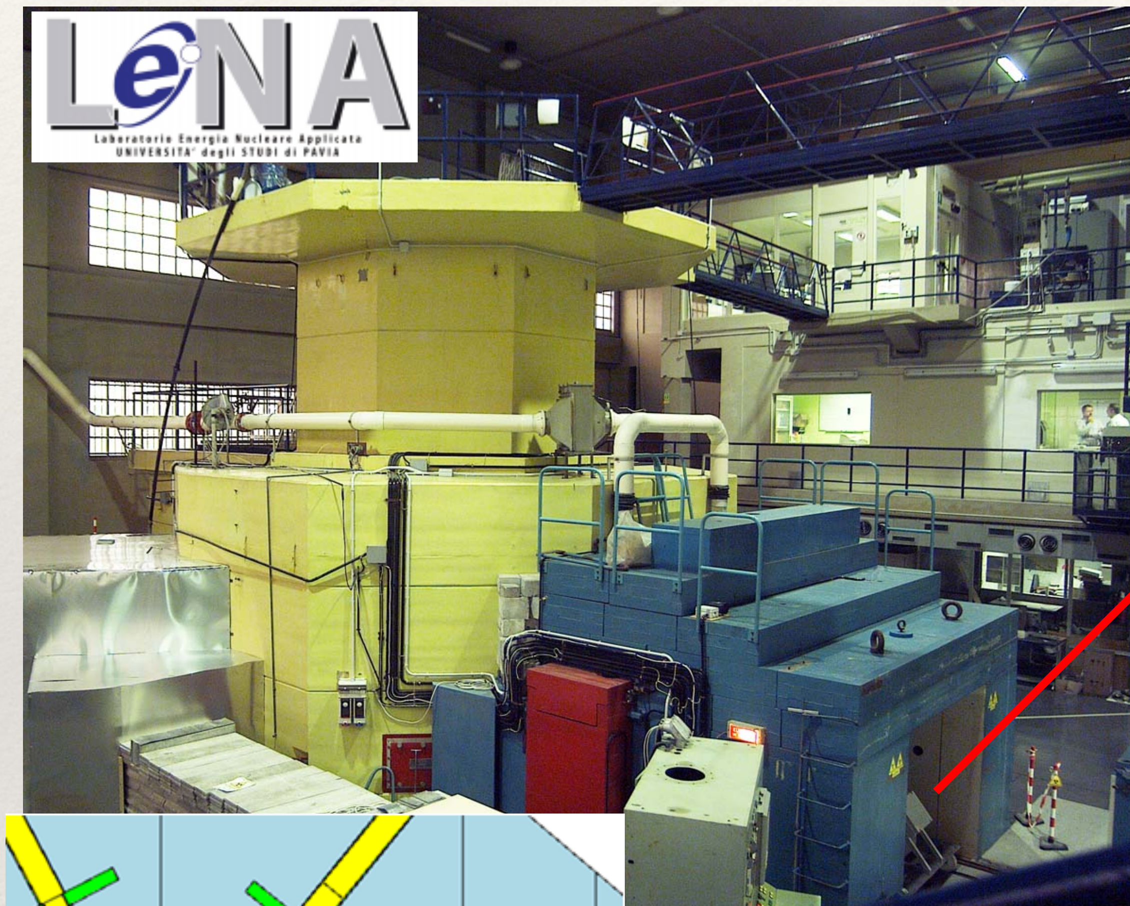
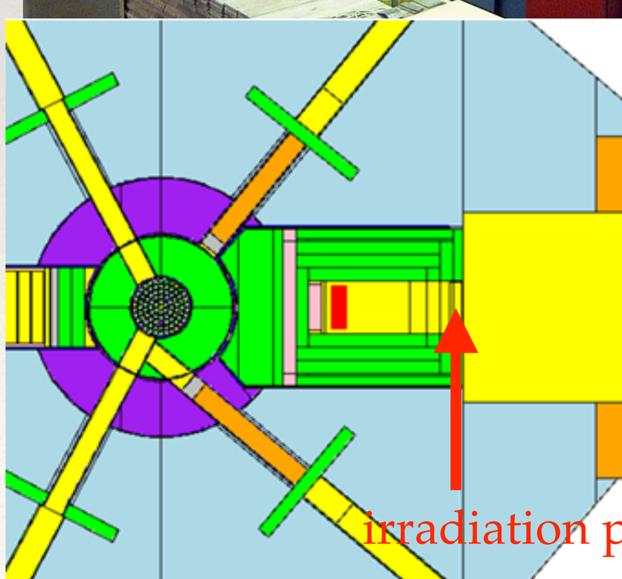
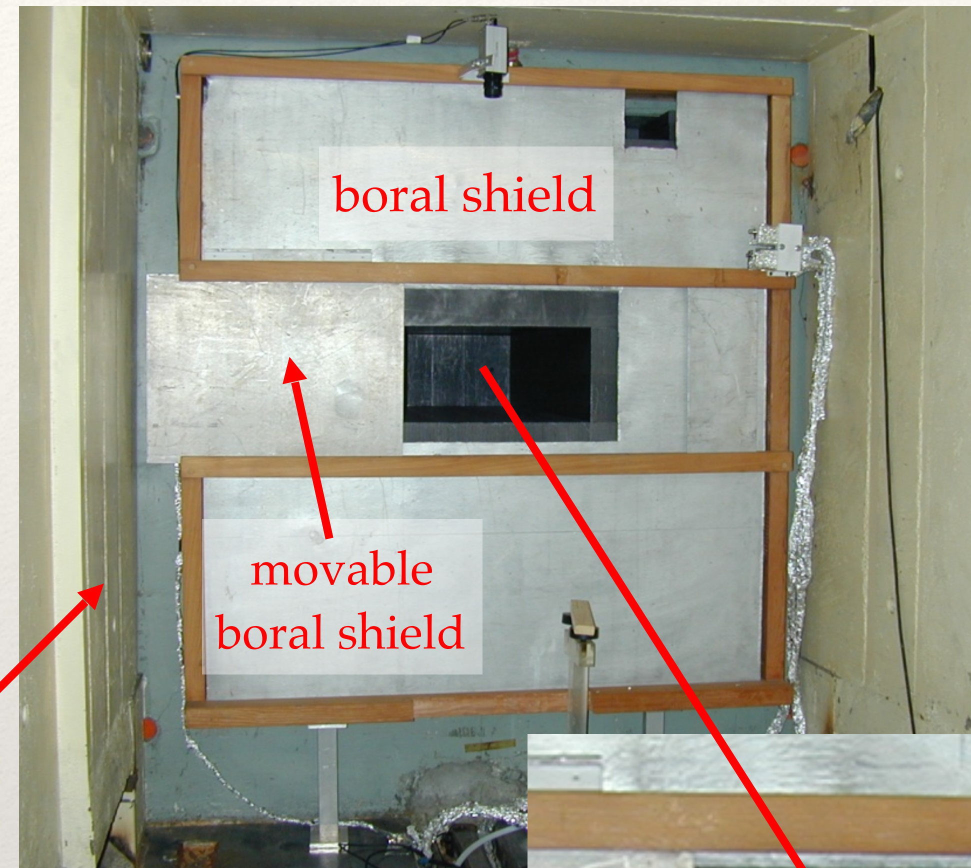
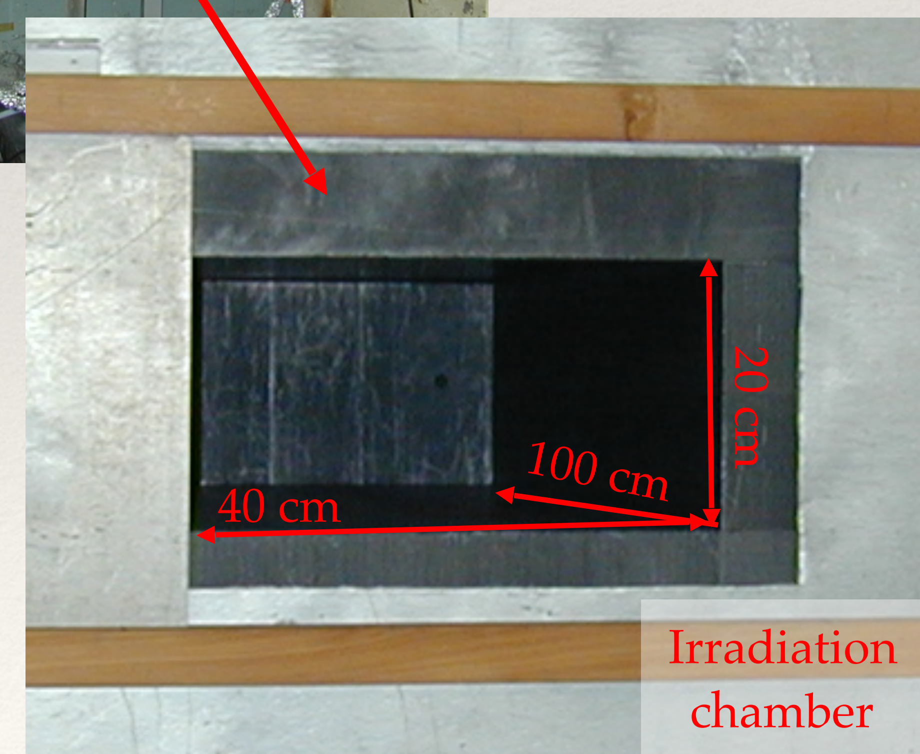
The thermal neutron facility at Pavia University

TRIGA Mark II research nuclear reactor, open pool type; steady state operation up to 250 kW









In air thermal neutron flux, at sample irradiation position: about $2 \cdot 10^{9}$ $cm^{-2}s^{-1}$

^{10}B measurement in biological samples

MACROSCOPIC TECHNIQUES

AES

Atomic Emission Spectroscopy

(0.1 μg B / ml; at least 50 mg sample, dispersed in a noble gas (Ar) plasma; destructive technique)

PGNAA

Prompt Gamma Neutron Activation Analysis

(down to 1 μg ^{10}B)

Mean concentration of ^{10}B inside solid tissues and blood?

Boron spatial distribution at cellular and sub cellular level?

MICROSCOPIC TECHNIQUES

Alpha spectroscopy

(0.5 μg - 1 mg ^{10}B)

Neutron autoradiography

1. low resolution (down to 100 μm).
2. high resolution (1-2 μm, 0.1 μg ^{10}B)

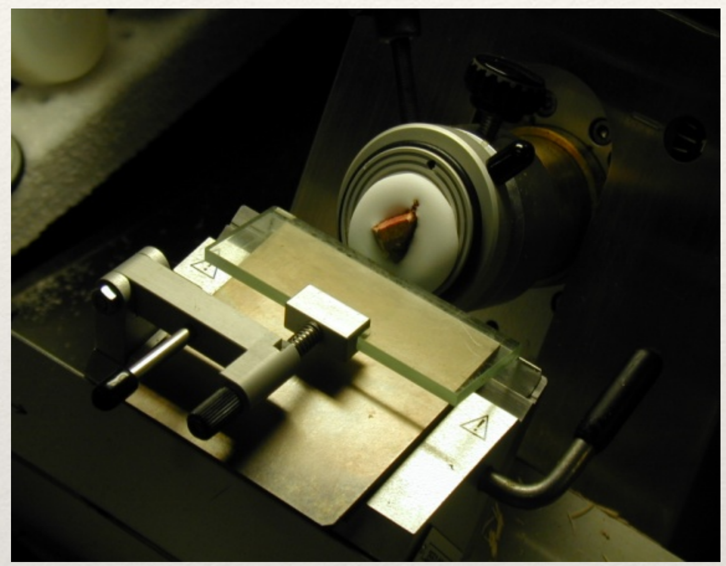
SIMS

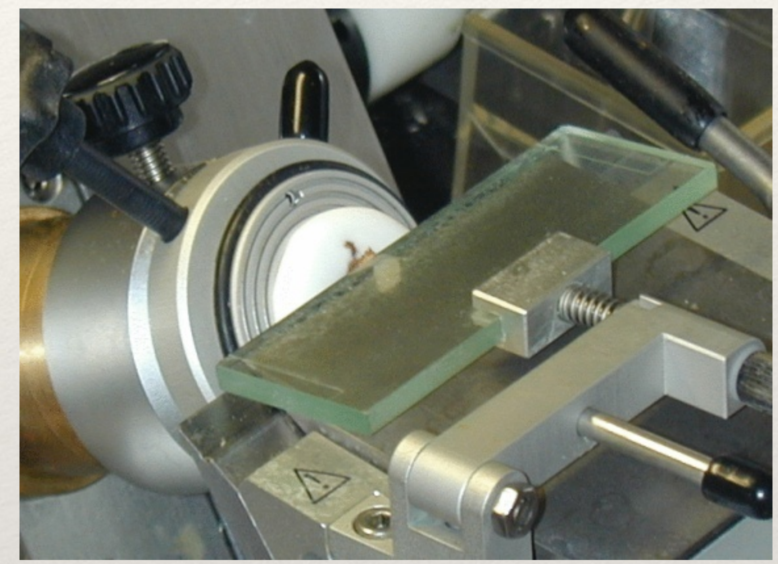
Secondary Ion Mass Spectroscopy

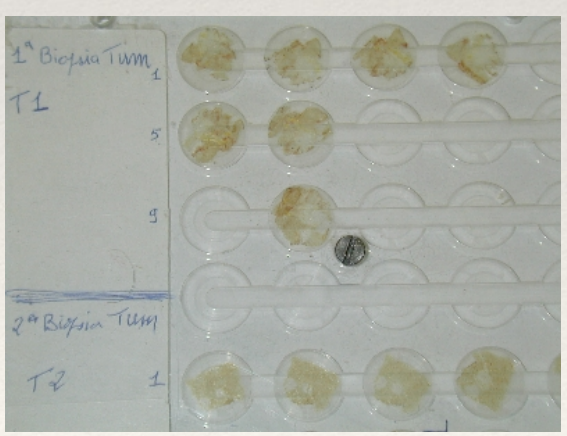
sub-μm resolution

Sample preparation

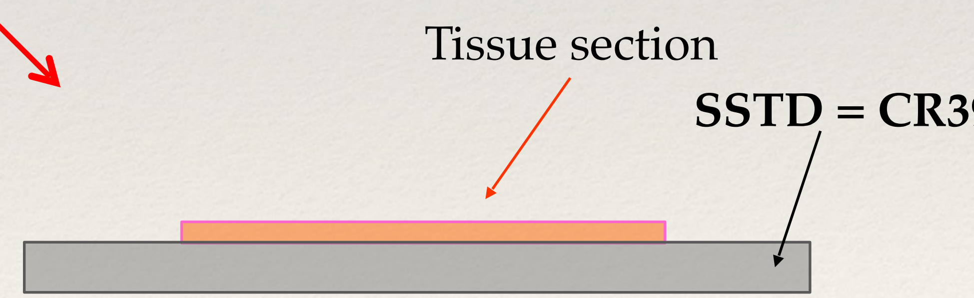
- Solid tissue biopsy (taken from patient, animal model, etc…)
- Criostatic cut of the thin sample (10-60 μm slide)





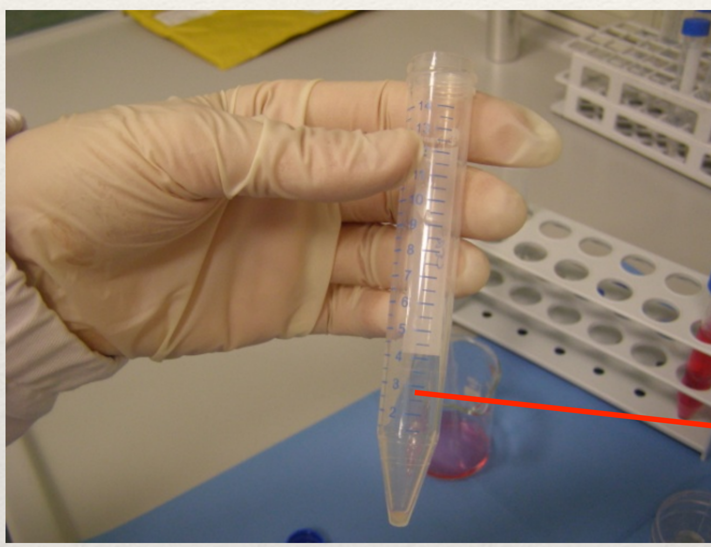


Tissue slices deposited over mylar disks
(α-spectrometry)

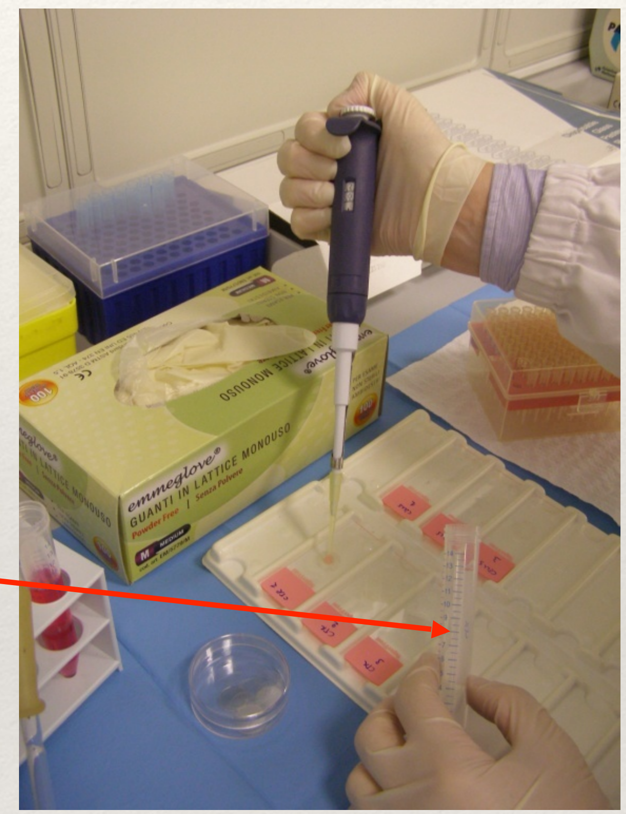


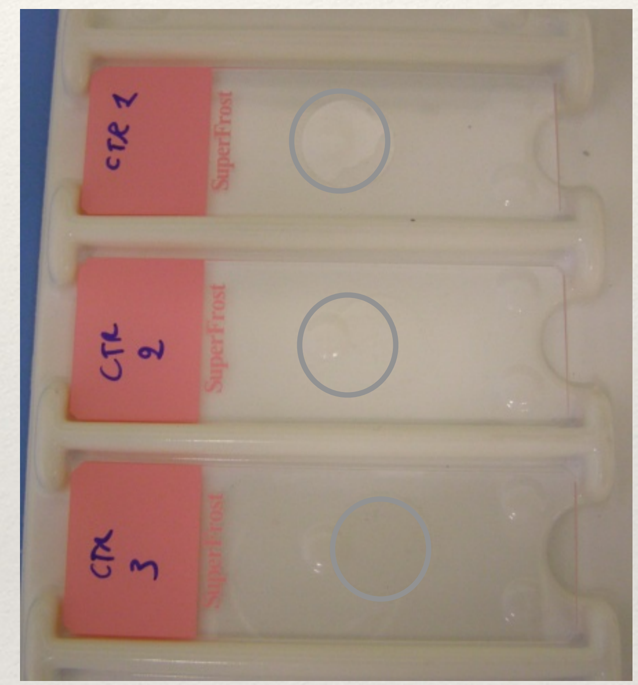
Simplified sketch of sample prepared for
neutron autoradiography

Sample preparation



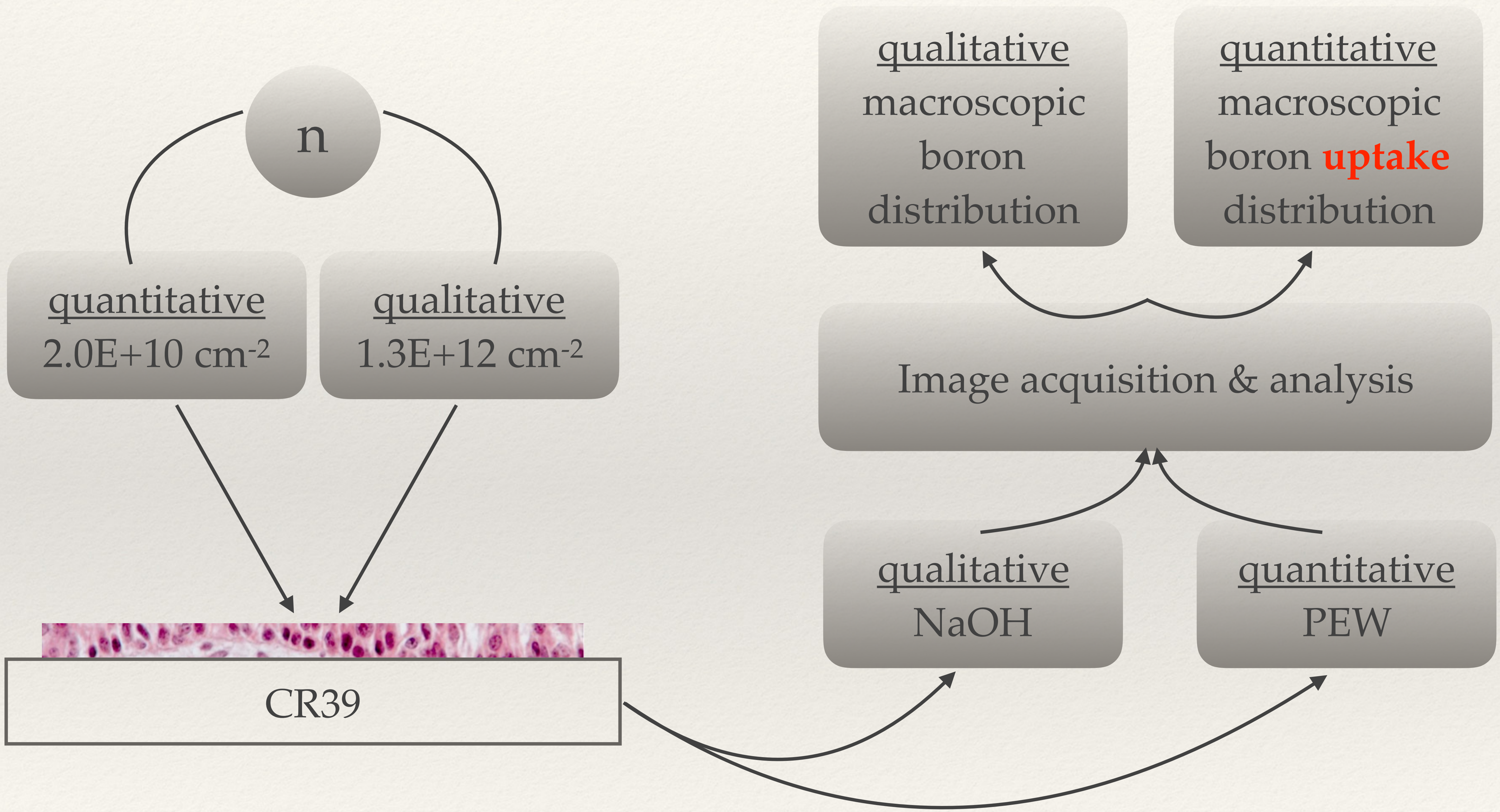
1) Cell suspension (from adherent as well as suspended cell cultures)





2) Deposition of a fixed aliquot on mylar disks

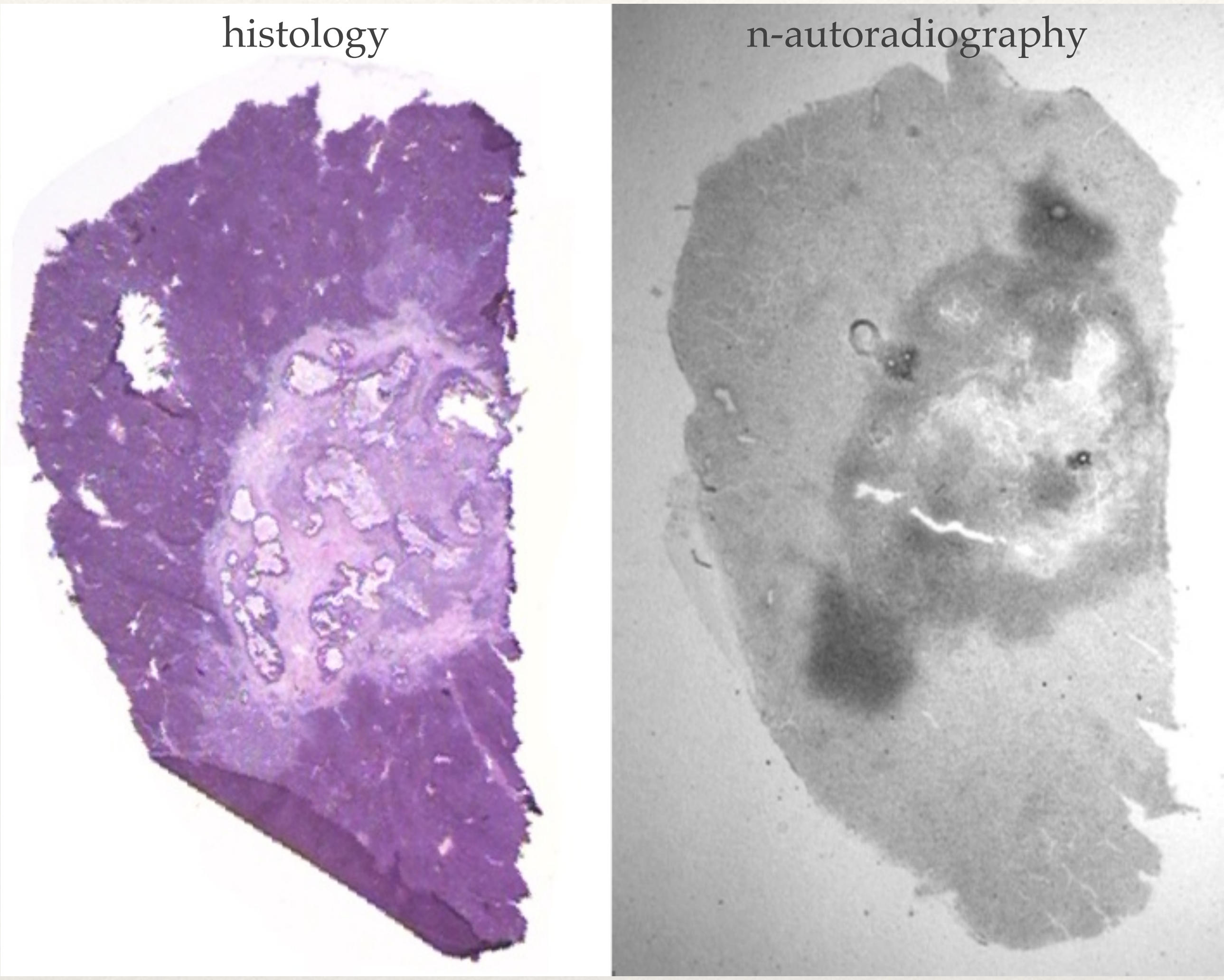
Analysis workflow of n-autoradiography



n-autoradiography

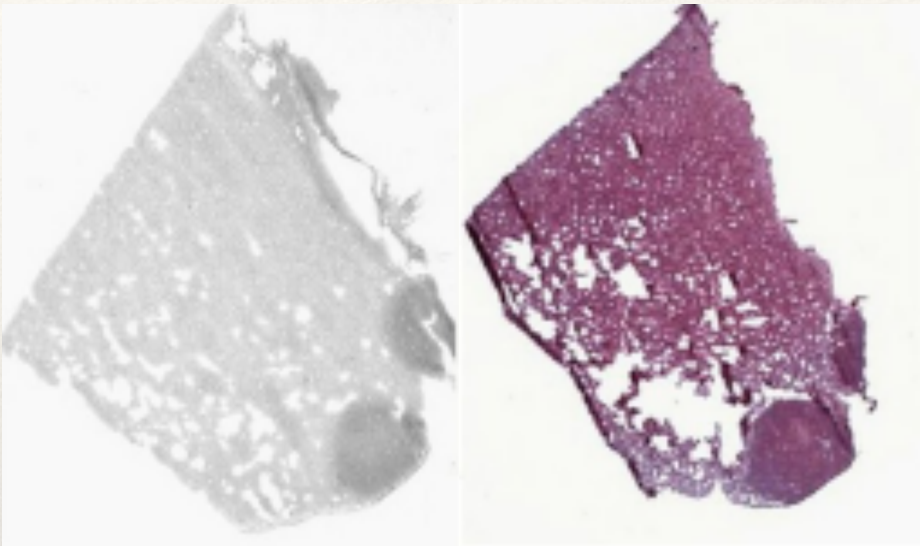
- The thermal neutron flux at the sample irradiation position equals to $2 \cdot 10^9$ $cm^{-2}s^{-1}$; ^{10}B concentrations between 1 and 100 ppm can be pointed out with irradiation times ranging from 10 up to 100 minutes
- The α particles coming from the $^{10}B(n,\alpha)^7Li$ reaction leave latent tracks on the SSTD, which can be visualized by an appropriate etching procedure.
- The etching agent (NaOH, PEW) concentration, the solution temperature during etching and its duration in time are the parameters to be varied in order to optimise the boron imaging (quantitative vs qualitative)
- The image of the etched SSTDs are then acquired by a Leica stereomicroscope and analysed

Examples of qualitative n-autoradiography

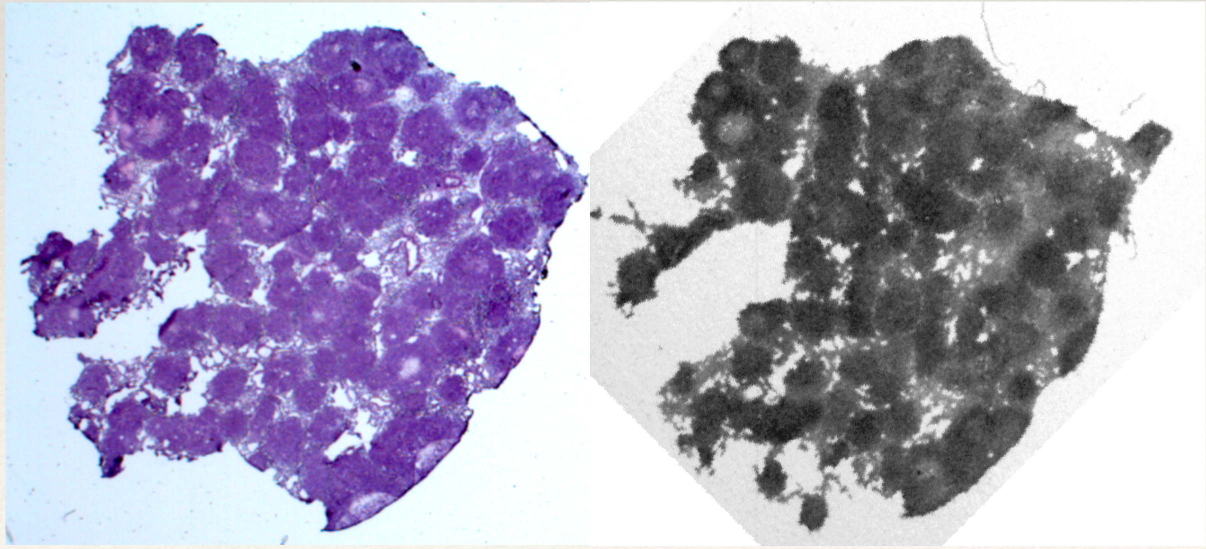


Human liver tissue affected by multiple metastases (treated with f-^{10}BPA)

n-autoradiography histology



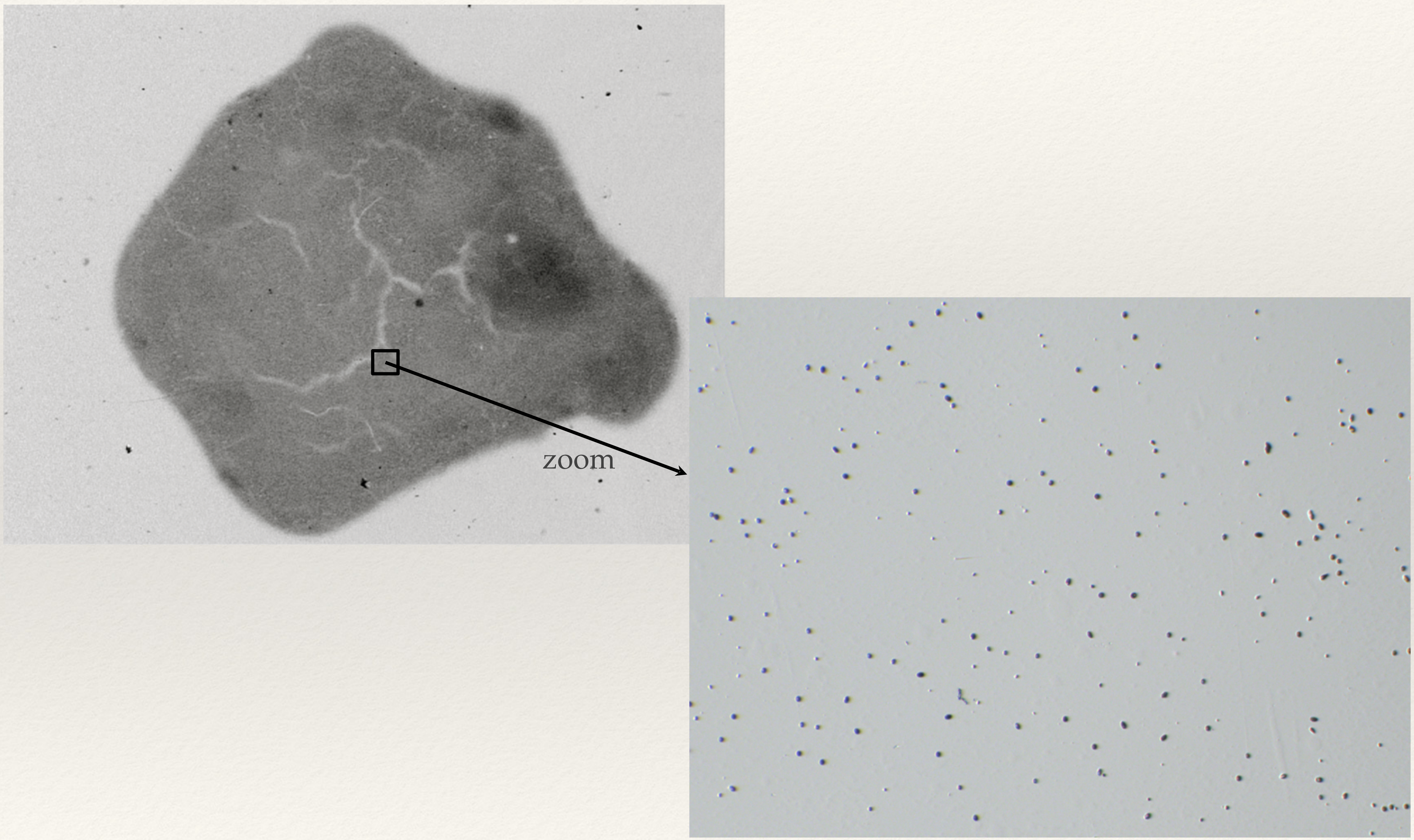
Rat lung tissue affected by multiple metastases (treated with f-^{10}BPA)



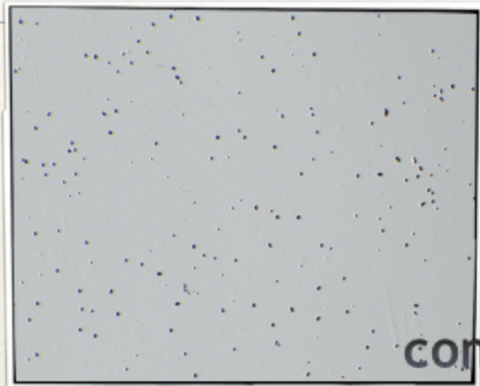
histology n-autoradiography

REFs:
S.Altieri et al., ARI 2008, 66:1850-1855
S.Bortolussi et al., ARI 2011, 69(2):394-398

Quantitative n-autoradiography



Quantitative n-autoradiography



CR39

6.5 N NaOH @ 70°C

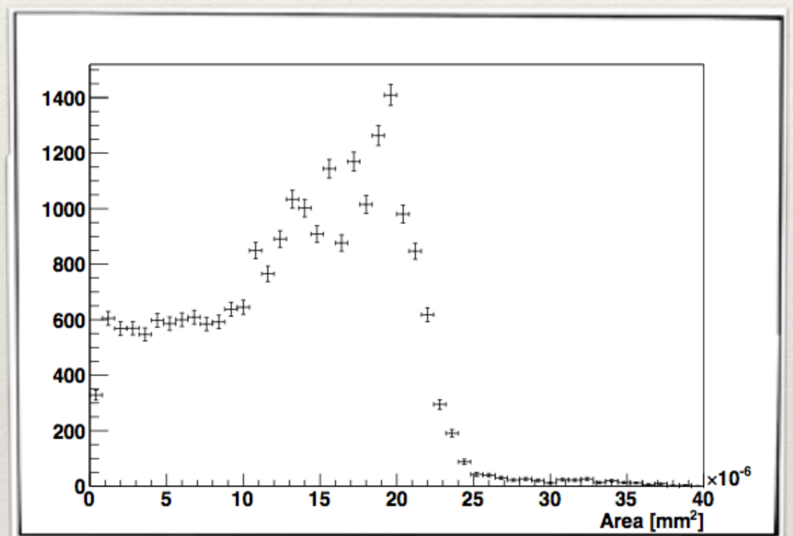
125’

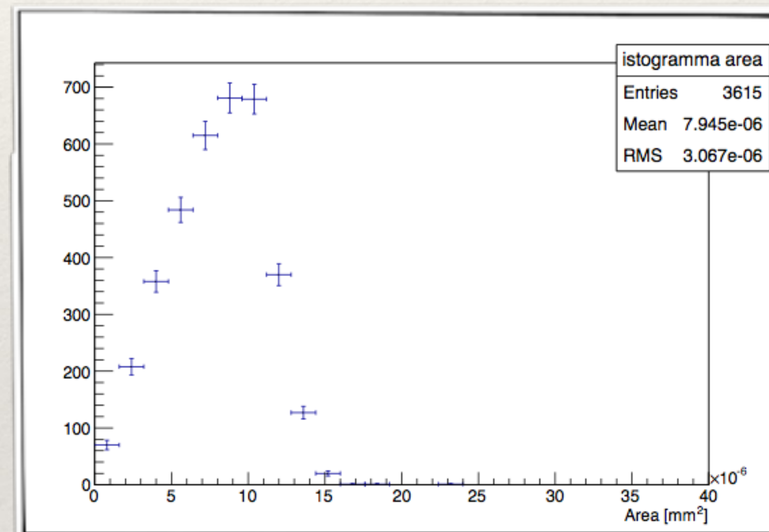
contaminazione p^+ da N+n

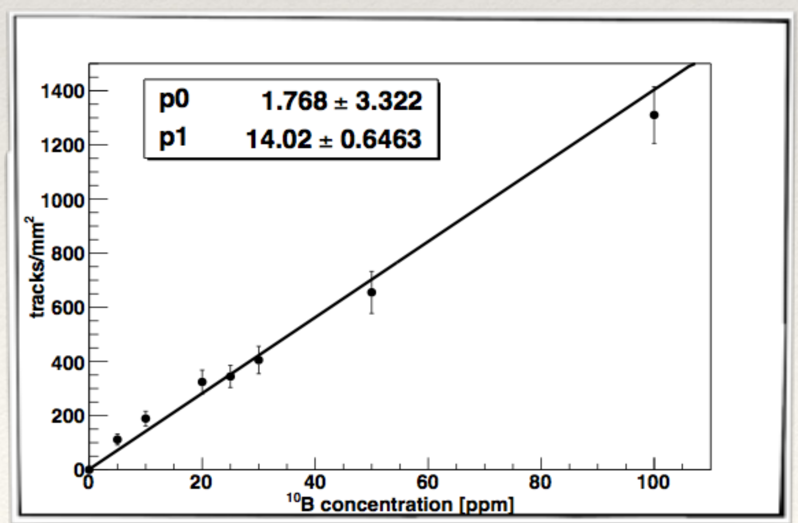
PEW (15% KOH + 40% C2H5OH)

10’

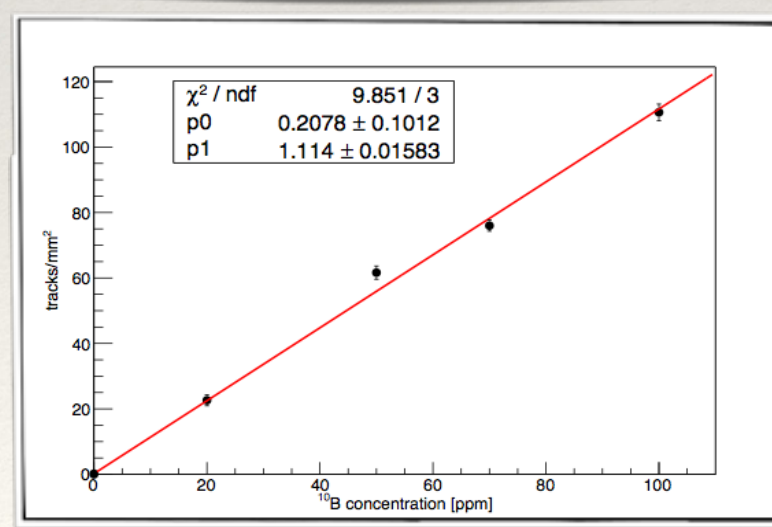
fluttuazioni statistiche ridotte

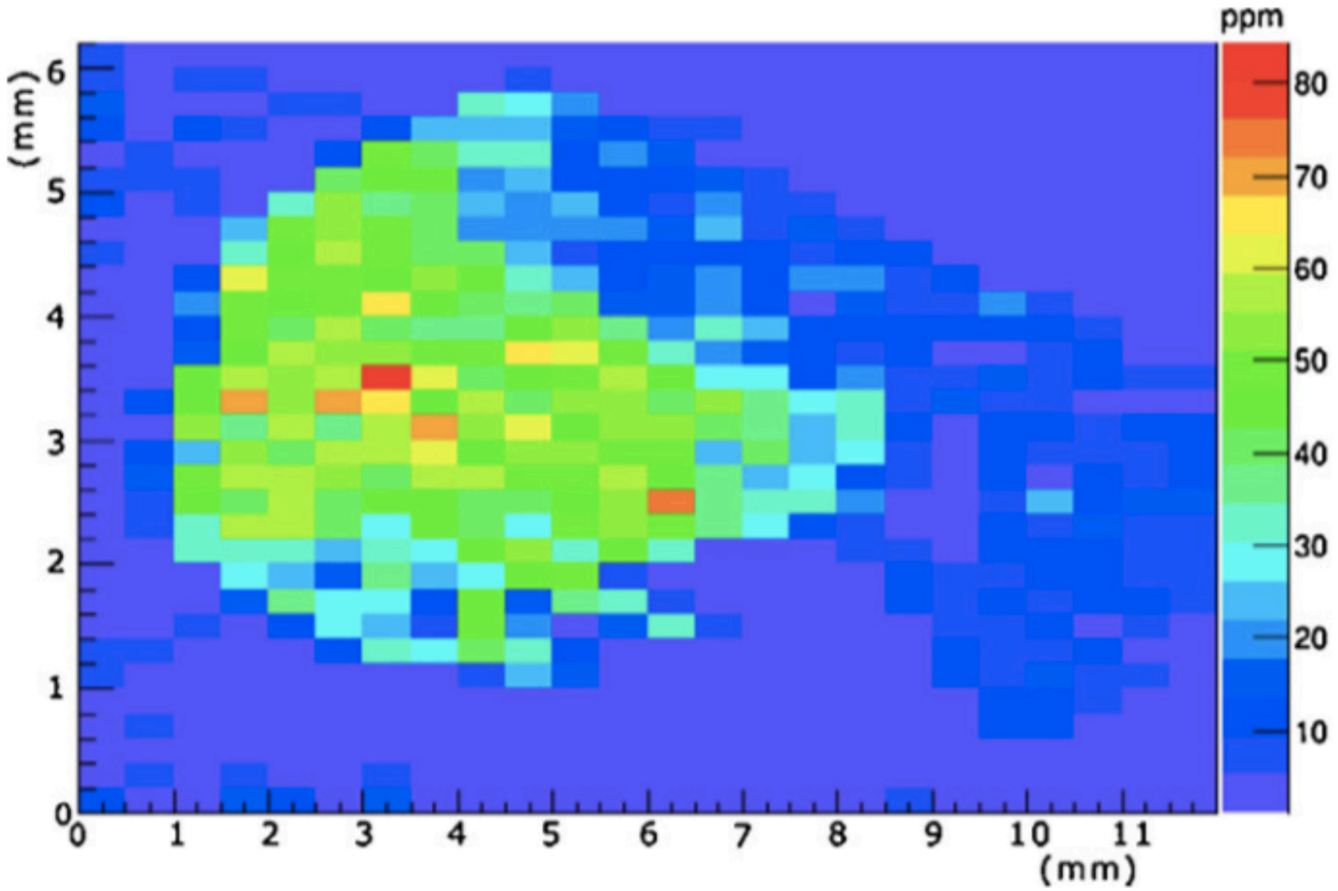


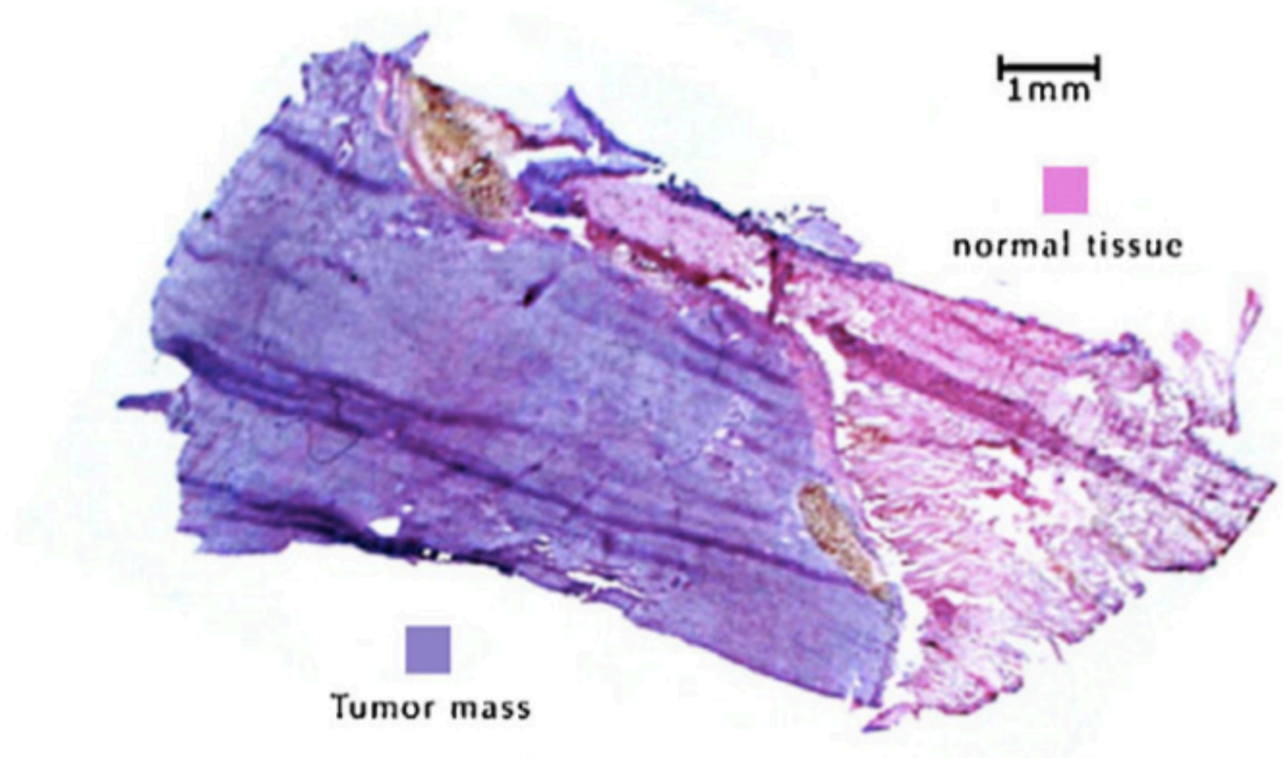




REFs:
M.Gadan et al., NIM-B 2012, 274:51-56
I.Postuma et al., Rep Pract Oncol Radiother 2016, 21(2):123-128

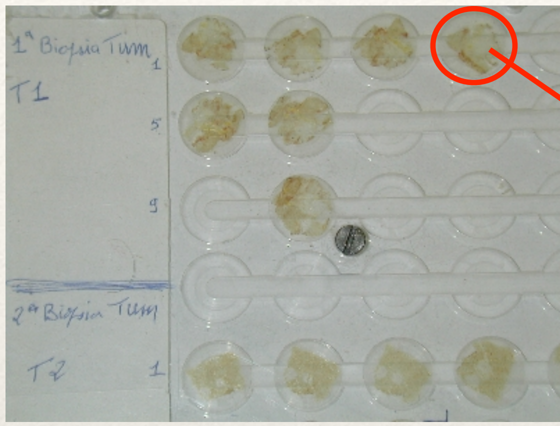




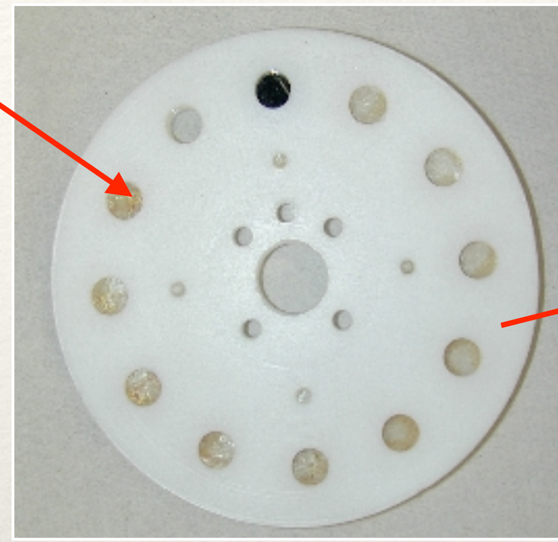


I.Postuma et al., Rep Pract Oncol Radiother 2016, 21(2):123-128

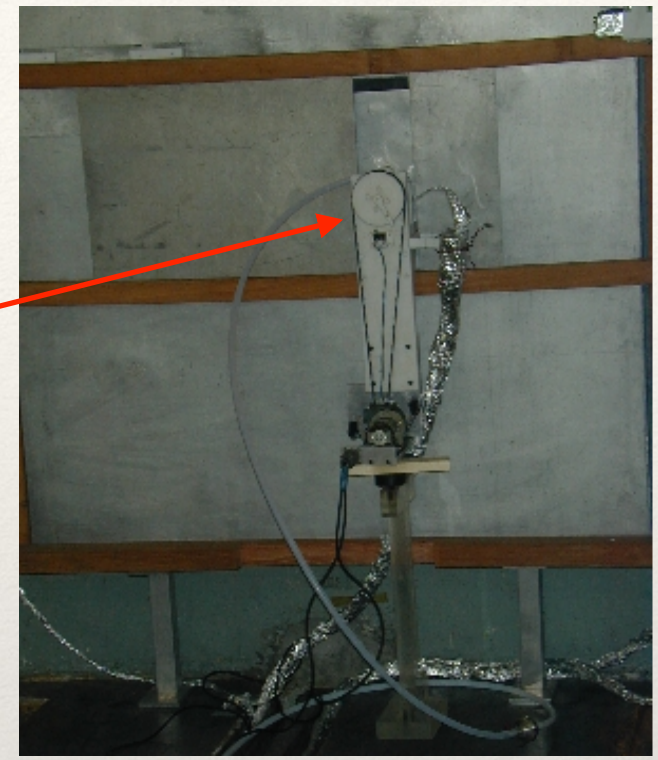
Alpha-spectrometry



solid tissue samples on mylar



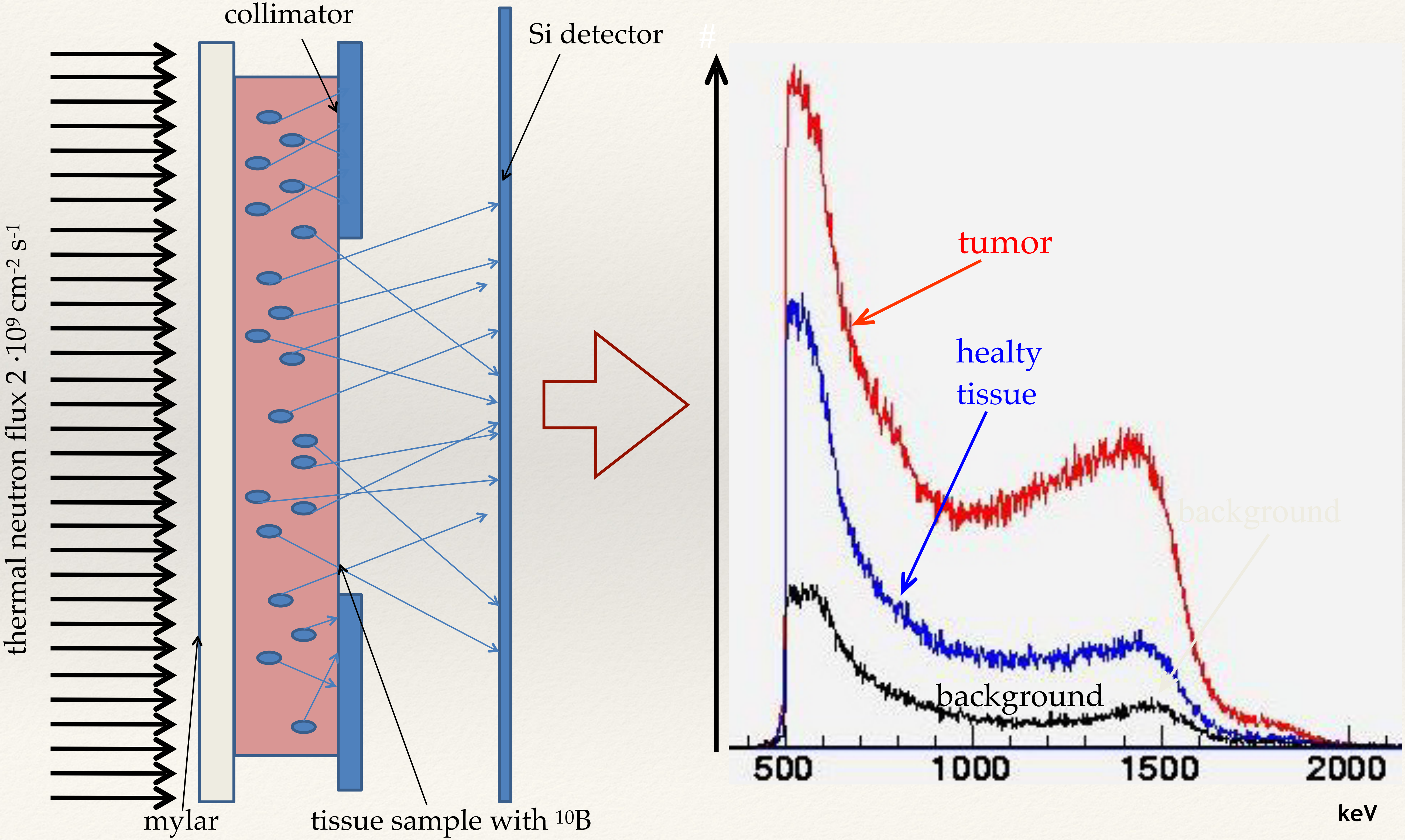
remotely rotating samples holder



samples holder ready for data acquisition inside the Pavia TRIGA thermal column

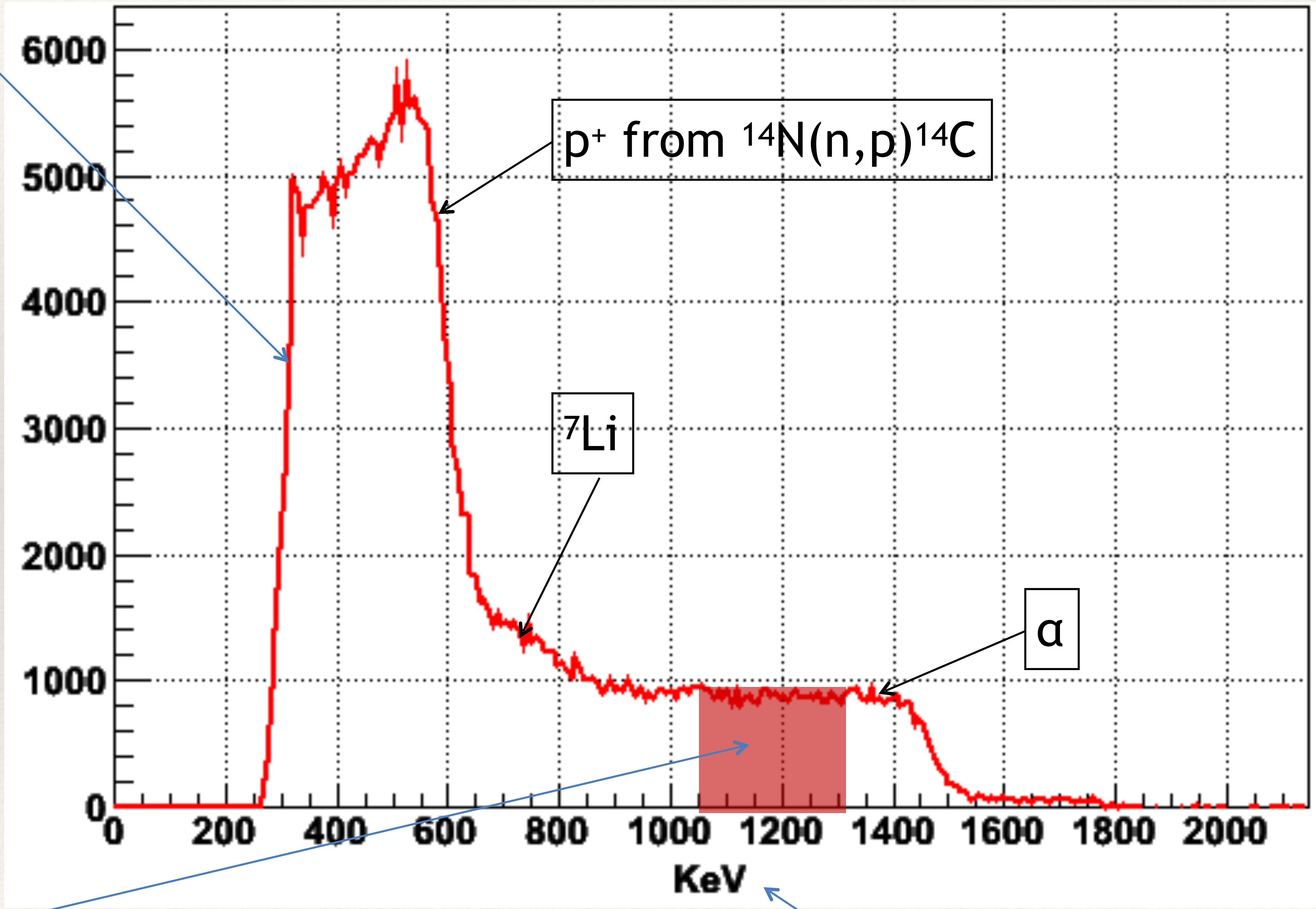
- 10 minutes irradiation at the maximum reactor power (250 kW) for each sample to collect a statistical significant charged particle spectrum
- certified ^{10}B superficial implant (NIST) for energy calibration and to know the thermal neutron flux at the sample irradiation position

Alpha-spectrometry



Alpha-spectrometry

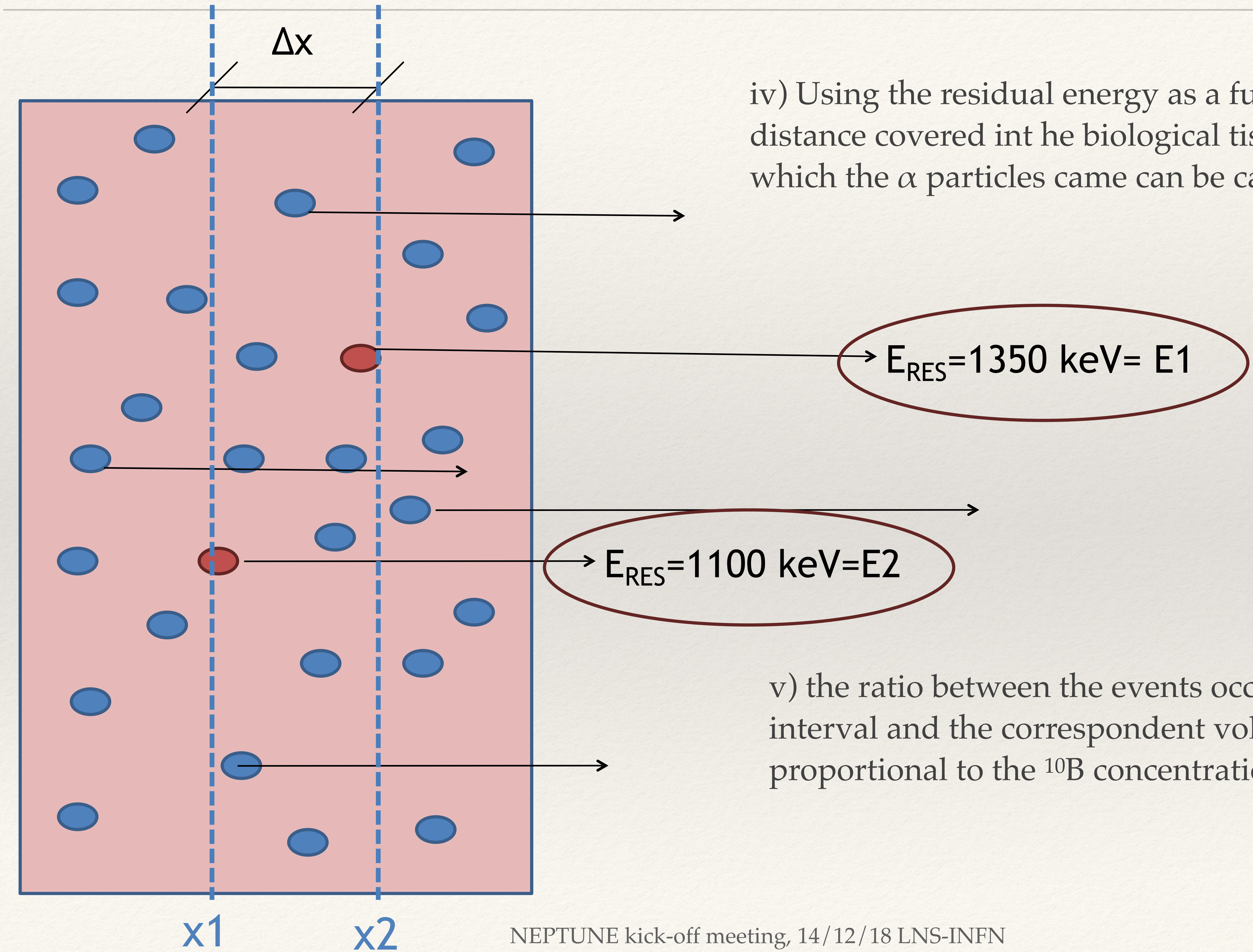
i) The experimental spectra are absorbed spectra because the samples are thick compared to the range of the charged particles in biological material.



iii) α contribution to the spectrum = the highlighted zone represents the α particles that arrive at the detector with RESIDUAL ENERGY BETWEEN 1100 and 1350 keV.

ii) ^{10}B superficial implant (NIST) energy calibration

Alpha-spectrometry



iv) Using the residual energy as a function of the distance covered int he biological tissue, the Δx from which the α particles came can be calculated.

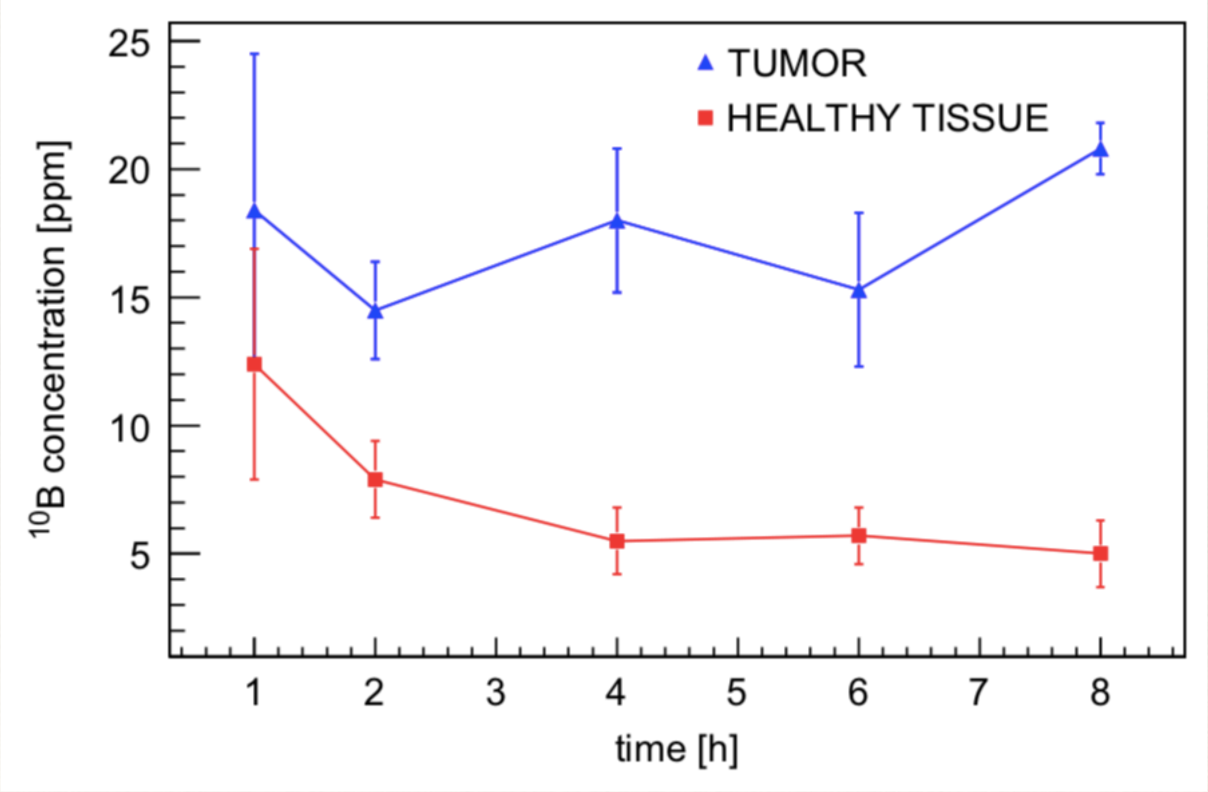
v) the ratio between the events occurring in this interval and the correspondent volume is proportional to the ^{10}B concentration

Alpha-spectrometry

$$(ppm)_F = \frac{K}{\eta\sigma\Phi S}\frac{\Delta E}{\Delta(\rho x)}\frac{A_W}{N_A}\frac{m_{dry}}{m_{fresh}}$$

measured value (for each type of studied tissue)

Where:
K is the number of events in the interval ΔE;
$\Delta E/\Delta(\rho x)$ is the α stopping power in dry tissue;
η is the efficiency of the detection system;
σ is the cross section of the thermal n reaction on ^{10}B;
ϕ is the thermal neutron flux;
S is the surface of the sample seen by the detector;
A_w is the atomic weight of ^{10}B;
N_A is the Avogadro number.



S.Bortolussi et al., ARI 2011, 69(2):394-398

Backup slides

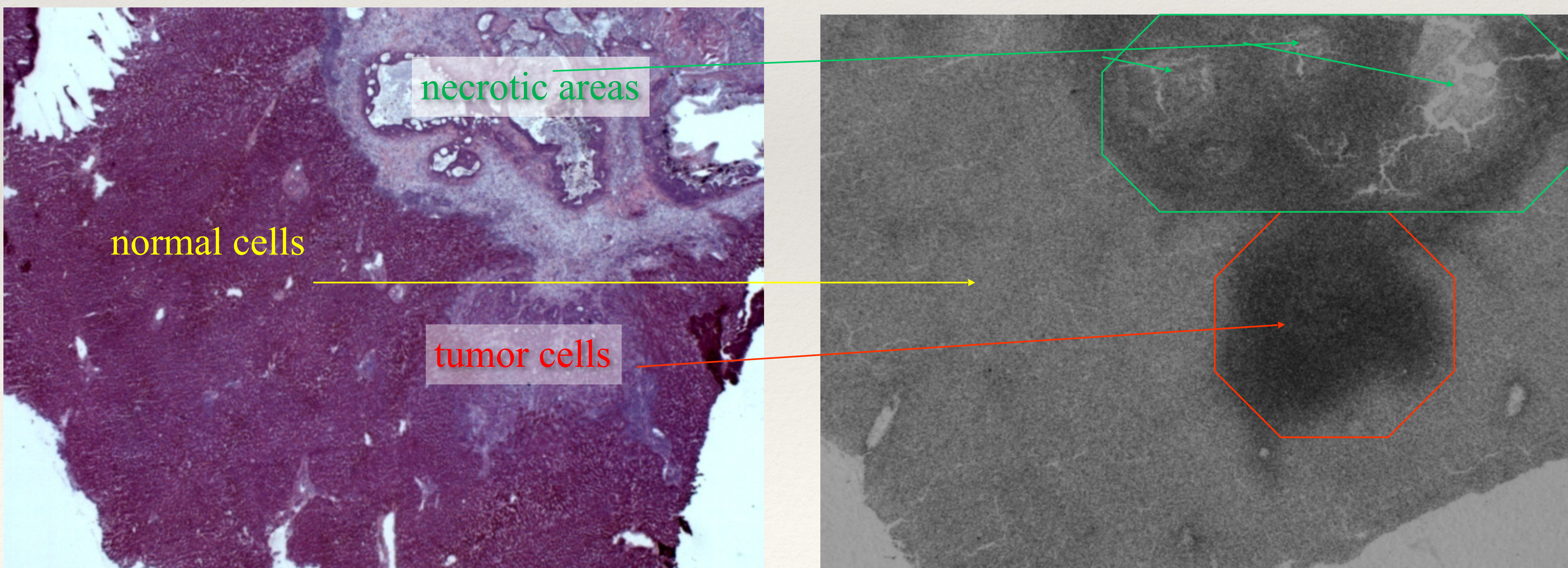
The mixed sample analysis

Inside this sample of a human metastatic nodule we see that within an area of a few squared millimeters we can find: tumour cells, normal cells, necrotic material, …

and neutron radiography shows us that in this sample the boron concentration is very different depending on the tissue type:

histological image

neutron radiography image

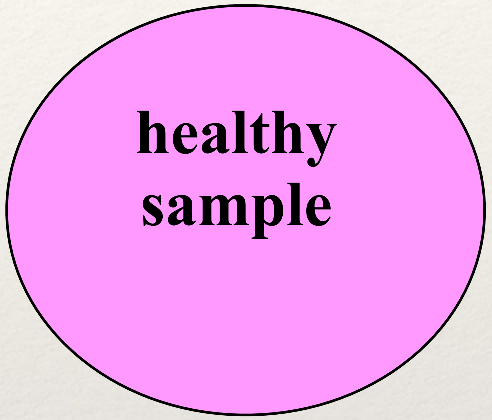


The mixed sample analysis

In order to correctly evaluate the ratio $T = C_T/C_H$ beetwen the concentrations in tumor and in normal cells, it is mandatory to know which kind of tissue we are analyzing; in particular

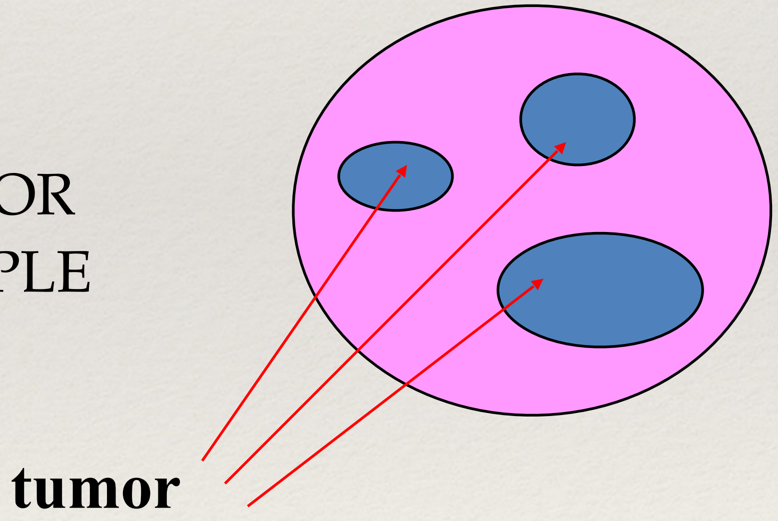
we have to ceck that what we call "the healthy sample" is really healthy

HEALTHY SAMPLE



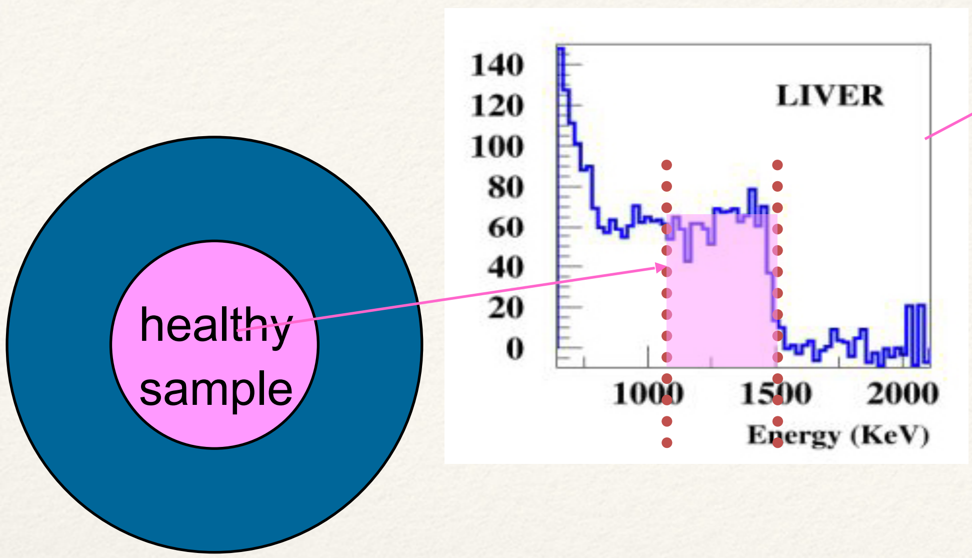
And we have to measure how much tumor is inside the sample containing the tumor

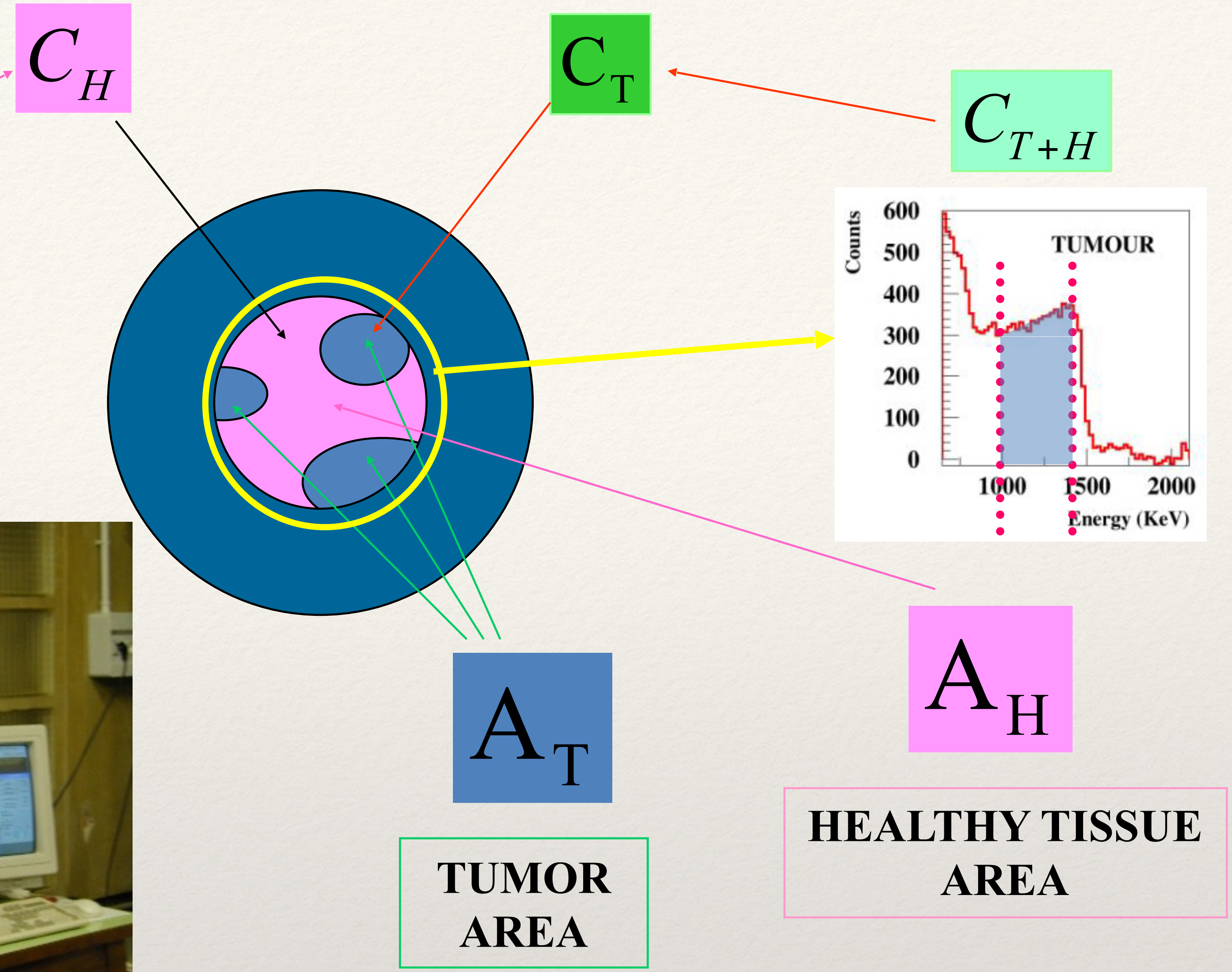
TUMOR SAMPLE



To do this we cut 3 thin slices from both healthy and tumor samples. We use

- the first one to measure boron concentration
- the second one for histopathological analisys
- the last one for boron imaging by neutron autoradiography





Using neutron radiography and biological analisys we measure (by the imaging system) the tumor area and the healthy tissuearea in the mixed sample

Finally by these measured quantity we can evaluate the concentration in the tumor

$$C_T = C_H \frac{A_H}{A_T} \left[\frac{C_{T+H}}{C_H} + \frac{A_T}{A_H} - 1 \right] \Rightarrow T = \frac{C_T}{C_H}$$