



UNIVERSITÀ
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Department of Physics

Master's Degree in Physical Sciences

**QUAD TimePix3 detector
calibration for ^{10}B concentration
and spatial distribution
measurement in biological samples**

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Academic Year 2025 – 2026

Abstract

Boron Neutron Capture Therapy (BNCT) is a hadron therapy with a selectivity acting at the single cell level with characteristics that are complementary to proton and carbon-ion therapy.

BNCT uses the neutron capture reaction on ^{10}B to produce two energetic ions that release all of their energy approximately within the borders of the single cell where the reaction takes place. To properly exploit the therapeutic selectivity, the ^{10}B enriched capture agent must be loaded preferentially by tumor cells thanks to dedicated and highly engineered vectors. In this scenario, it is important to quantify the selective accumulation of ^{10}B in tissues in order to properly link the delivered dose to the biological effects both on the healthy and carcinogenic tissues. For this purpose, several techniques are currently being used and developed for ^{10}B concentration and spatial distribution measurement both at the clinical and preclinical level.

The TimePix3 is a high spatial resolution hybrid pixelated detector that allows to simultaneously carry out a measurement of Time of Arrival (ToA) and Time over Threshold (ToT) with high spatial resolution, dictated by the pixel pitch of $55\text{ }\mu\text{m}$. This allows to detect and identify the particles produced by the ^{10}B neutron capture reaction using PID techniques. Furthermore, it is possible to juxtapose several TimePix3 chips to allow for a larger sensitive area. Such a configuration has been used in this thesis work in the form of a QUAD TimePix3 detector, a 2×2 array of TimePix3 chips.

At Pavia University Physics Department, in collaboration with the ENEA institute and the Frascati Laboratory of INFN, the exploitation of a QUAD TimePix3 detector has been carried out for several years. Pilot and promising information about TimePix3 capability of working in a neutron field for BNCT as well as imaging capabilities on ^{10}B enriched samples have been collected in previous measuring campaigns, as reported in [31].

In this framework, the purpose of this thesis is to explore the possibility of using a QUAD TimePix3 detector to simultaneously measure concentration and visualize spatial distribution of ^{10}B in organic matrices at low concentrations, compatible with those expected in clinics and basic experiments of BNCT.

The thesis is organized into 4 chapters. The first one is a brief overview of the most diffused techniques adopted worldwide for ^{10}B quantification and imaging. Apart from describing the different methods, comparison of the main characteristics of each technique is reported.

Chapter 2 deals with the description of the instruments used during this thesis. It starts by giving a general introduction on the MediPix family of pixel detectors (of which the TimePix3 is part) and in particular it focuses on the characteristics of the TimePix3. Then, a brief description of the neutron irradiation facilities that were exploited. Finally, a description of the experimental setup and data acquisition tools is given.

Chapter 3 is the core of the thesis. Indeed, it describes the experimental efforts carried out to positively calibrate the QUAD TimePix 3 detector for its routinely use in research and preclinical BNCT studies for ^{10}B quantification and imaging. To do this, two sets of measurements were carried out at the *Laboratorio di Energia Nucleare Applicata* (LENA) of the university of Pavia. The first in the *Prompt Gamma Neutron Activation Analysis* (PGNAA) facility with a highly thermalized and collimated neutron beam. In this facility, it was performed a first attempt at the calibration curve, but due to the relatively low neutron flux and the limits to perform a long irradiation for each of the samples, it was not possible to get a satisfactory calibration curve.

Nonetheless, this set of measurements was useful as a confirmation of the imaging capability of the detector and as a qualitative assessment of the linear response of the ^{10}B concentration range 0-100 ppm by the detection system.

For the second set of measurements, the experiment was transferred in the thermal column of the reactor. In it, the neutron flux can reach 10^3 times more than in the PGNAA facility, but the beam is not collimated.

The measurements taken in the thermal column allowed to get a preliminary, but finally reliable calibration curve for the analysis of thin tissue samples deposited on Mylar supports and expected to contain an unknown amount of ^{10}B .

The raw results of the calibration curve obtained in the LENA thermal column required a careful statistical analysis of the data as well as of the detection system. To support the analysis, Quantitative Neutron Autoradiography was performed on the samples used for the calibration, in particular to confirm their microscopic features as ^{10}B standards.

Finally, this calibration was then tested against biological samples containing unknown concentrations of ^{10}B . The ^{10}B concentrations that the TimePix3 technique yields are compatible with the results obtained on twin samples using another consolidated technique such as ICP-OES. The comparison with twin samples measured with Alpha Spectrometry yielded lower concentration values than expected, but this can be imputed, at least partially, to the state of conservation of the sample. Chapter 4 closes the work reporting the main outcomes as well as the limits iden-

tified during the projects, the latter presently under further investigation to suggest mitigations or solutions.

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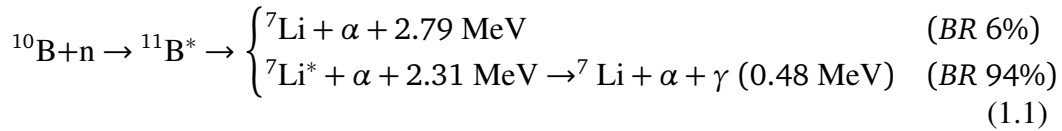
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Chapter 1

Boron Measurement Techniques for BNCT

1.1 Introduction

Boron Neutron Capture Therapy (BNCT) is a hadron therapy that exploits the ability of the non-radioactive isotope of Boron, ^{10}B (19.8% natural abundance), to capture neutrons in the reaction 1.1 to selectively destroy tumor cells.



Indeed, ^{10}B has a particularly high cross-section (see Figure 1.1) for the capture of thermal ($\leq 0.5 \text{ eV}$) and epithermal ($0.5\text{eV} < E \leq 10\text{keV}$) neutrons; therefore, by accumulating a sufficient amount of ^{10}B atoms exclusively within tumor cells (typically of the order of a few $\mu\text{g/g}$), it is possible to selectively and lethally irradiate only the diseased tissue.

This approach spares healthy tissues, which suffer negligible or otherwise manageable effects due to both a lower ^{10}B accumulation (typically 1/3 of the concentration found in tumor cells) and the lower biological effectiveness of the reactions

Particle	E_k (MeV), BR 94%	E_k (MeV), BR 6%
α	1.47	1.78
^7Li	0.84	1.01
γ	0.48	-

Table 1.1: Energies of the reaction products depending on the Branching Ratio.

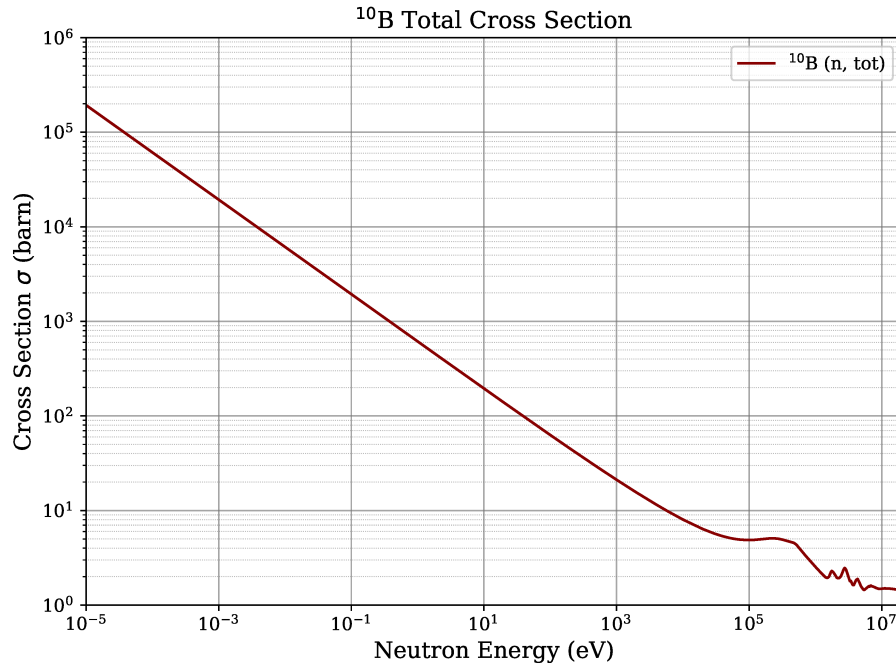


Figure 1.1: Neutron capture cross-section of ^{10}B . Data taken from [11].

induced by thermal and epithermal neutrons on tissue constituents (specifically nitrogen and hydrogen).

Returning to the ^{10}B capture reaction, the biological damage is primarily caused by the two charged products, the α particle and the recoiling lithium nucleus ^7Li . The energy is distributed between the two reaction products as reported in Table 1.1 .

These are high-LET (Linear Energy Transfer) particles capable of depositing all their energy within micrometric regions centered around the neutron capture point. In fact, the sum of the tissue ranges of the alpha particle and the lithium ion is approximately $10\ \mu\text{m}$, an order of magnitude that closely corresponds to the average size of individual cells.

It is therefore possible to define BNCT as a **binary therapy** that requires two distinct components:

- A thermal or epithermal **neutron source** (depending on the depth of the tumor).
- A **pharmacological vector** capable of delivering boron selectively to cancer cells.

1.1.1 Neutron Source

Regarding the neutron source, it must comply with specific standards (see Table 1.2) established by the International Atomic Energy Agency (IAEA) in 2001 [17] and updated in 2023 [18], which address four main aspects:

- **Spectrum:** To optimize the capture reaction, the neutrons must be thermal ($E \leq 0.5$ eV) or epithermal ($0.5 \text{ eV} < E \leq 10$ keV). Their ratio can vary depending on the depth of the tumor to treat, since thermal neutrons are stopped (and thus give more dose) in more superficial tissues.
- **Flux:** This relates to the necessity of irradiating patients within a clinically reasonable timeframe (≤ 1 h per irradiation session). For typical boron concentrations in cancer tissues (25-35 ppm), an epithermal flux of $\phi_{epi} \geq 5 \cdot 10^8 \text{ n}/(\text{cm}^2 \text{ s})$ is recommended.
- **Contamination:** This includes contamination from both fast neutrons and gamma rays. Both contribute to the non-therapeutic dose, and it is thus strictly recommended to keep them as low as possible.
- **Directionality:** This parameter quantifies the degree of beam collimation. High directionality is crucial for confining the irradiation field strictly to the target volume and affords greater flexibility in patient positioning, allowing an increased distance from the beam exit port. In addition, a highly collimated beam is necessary for the treatment of deep-seated tumors. As epithermal neutrons penetrate the tissue, elastic scattering with hydrogen nuclei causes the beam to thermalize and lose its initial spatial collimation. From a design perspective, directionality is one of the most challenging parameters to optimize due to an inherent trade-off: enhancing beam collimation requires restrictive moderating assemblies, which inevitably attenuate the overall available neutron flux.

Beam quality component	Symbol or definition	Reference value
Therapeutic epithermal flux	ϕ_{epi}	$\geq 5 \cdot 10^8 \text{ cm}^{-2} \cdot \text{s}^{-1}$
Thermal to epithermal flux ratio	ϕ_{th}/ϕ_{epi}	≤ 0.05
Beam Directionality	J/ϕ_{epi}	≥ 0.7
Fast neutron dose per unit epithermal fluence	$D_H / \int \phi_{epi}(t) \cdot dt$	$\leq 7 \cdot 10^{-13} \text{ Gy} \cdot \text{cm}^{-2}$
Gamma dose per unit epithermal fluence	$D_\gamma / \int \phi_{epi}(t) \cdot dt$	$\leq 2 \cdot 10^{-13} \text{ Gy} \cdot \text{cm}^{-2}$

Table 1.2: Reference neutron beam quality factors. Data taken from [18].

Until a few years ago, the only facilities capable of producing a beam meeting these standards (especially the flux) were nuclear reactors. In the last decade, how-

ever, significant efforts have been directed toward the development of accelerator-based neutron sources.

These facilities offer several advantages over nuclear reactors.

First, accelerator-based facilities produce a naturally softer neutron spectrum, peaked around a specific energy of the order of 1-2 MeV, depending on the converter and the accelerator energy, for the most commonly used endothermic reaction (see Table 1.3). This makes the beam easier to moderate compared to the harder nuclear reactor spectrum, which can reach energies up to 10 MeV (Figure 1.2).

Reaction	E_{th} (MeV)	E_{in} (MeV)	Total Production (n/(mA s))	Fraction (%) $E_n \leq 1$ MeV	E_{max} (keV)	E_{min} (keV)
${}^7\text{Li}(p, n){}^7\text{Be}$	1.880	1.880	0	100	30	30
		1.890	6.3×10^9	100	67	0.2
		2.500	9.3×10^{11}	100	787	60
		2.800	1.4×10^{12}	92	1100	395
${}^9\text{Be}(p, n){}^9\text{B}$	2.057	2.057	0	100	20	20
		2.500	3.9×10^{10}	100	574	193
${}^9\text{Be}(d, n){}^{10}\text{B}$	0	0	0	50	3962	3962
		1.500	3.3×10^{11}	50	4279	3874
${}^{13}\text{C}(d, n){}^{14}\text{N}$	0	0	0	75	4974	4964
		1.500	1.9×10^{11}	70	6772	5616
${}^{12}\text{C}(d, n){}^{13}\text{N}$	0.327	0.327	0	100	4	3
		1.500	6.0×10^{10}	80	1188	707
$d(d, n){}^3\text{He}$	0	0	0	0	2451	2451
		0.120	3.3×10^8	0	2898	2123
		0.200	1.1×10^9	0	3054	2047
$t(d, n){}^4\text{He}$	0	0	0	0	14050	14050
		0.150	4.5×10^{10}	0	14961	13305

Table 1.3: Reactions used in accelerator-based BNCT facilities. E_{th} is the threshold energy of the reaction, E_{in} is the beam energy, E_{max} is the maximum energy of produced neutrons, and E_{min} is the minimum. The reactions currently used in clinical facilities are the endothermic ones on Lithium and Beryllium. Data taken from [28].

Another critical aspect is that accelerator-based BNCT (AB-BNCT) is more suitable for clinical environments compared to reactor-based BNCT (RB-BNCT): accelerators are already widely employed in clinical frameworks (e.g., conventional hadron therapy) and therefore benefit from established legal and regulatory frameworks allowing their installation in or near hospitals.

In addition, nuclear reactors are subject to extremely strict regulations, making their installation, operation, and decommissioning much more complex and costly

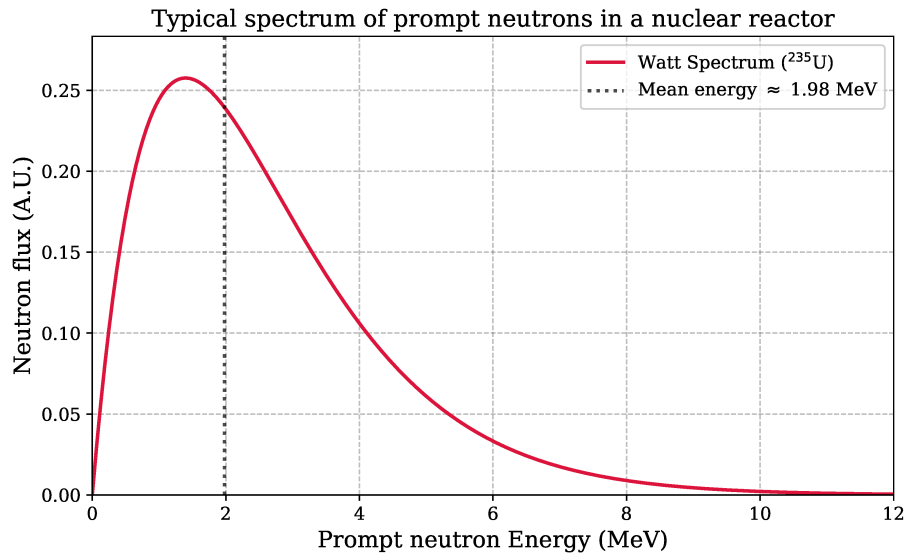


Figure 1.2: Typical neutron spectrum in a nuclear reactor core, generated using the Watt distribution.

than those of an accelerator.

Lastly, public acceptance must be taken into account: a nuclear reactor is generally not perceived as a clinical facility, and its installation could face significant opposition from both the public administration and the general public.

1.1.2 Boron Carriers

Boron carriers must meet specific technical requirements [18, 4]:

- **Concentration and selectivity:** it must have a high tumor uptake (25-35 ppm of ^{10}B) so that the neutron irradiation can be expected to produce the antitumor effect. Moreover, this accumulation must be selective to the tumor, thus, a tumor-to-normal tissue ratio (T/N) and a tumor-to-blood ratio (T/B) ≥ 3 are required.
- **Rapid clearance:** it needs to persist in tumor for at least several hours during neutron irradiation and be rapidly cleared from the system (normal tissues and blood) after the irradiation.
- **Low toxicity:** it must be non-toxic and it has to comply with the international guidelines for neoplastic agents set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [15].

Currently, there are only two compounds that meet these requirements and are approved for clinical use: Boronophenylalanine (BPA) and Sodium Borocaptate (BSH). These compounds have been clinically employed for decades (since 1987 and 1967, respectively [19]). However, both of these drugs exhibit limitations regarding biodistribution and selectivity. Specifically, BPA accumulates well in tumor cells but has a low T/N; BSH, on the other hand, has a high T/N ratio, but it predominantly accumulates in the extracellular matrix rather than being internalized by the tumor cells [10].

For this reason, extensive research is underway to develop what are known as third-generation boron delivery agents. To effectively develop these new molecules and further study the ones already at our disposal, reliable techniques capable of measuring the concentration and spatial distribution of boron within tissues are required. Such techniques can then be applied in clinical dosimetry to aid the development of integrated radiobiological models to be used in treatment planning systems (TPS).

The next section will describe the most widespread and commonly used of these techniques. The core focus of this thesis will then be the development of a novel measurement technique based on the TimePix3 detector, which is uniquely capable of simultaneously providing information on both the concentration and the spatial distribution of boron within a given sample.

1.2 Boron-10 Measurement Techniques

This section provides an overview of the main techniques used to measure the concentration and spatial distribution of ^{10}B in both clinical and preclinical applications (see Table 1.4).

To fully exploit the selectivity of BNCT, it is fundamental to employ techniques that allow the characterization of boron at different scales, ranging from its macroscopic distribution in tissues down to the cellular and subcellular levels. Furthermore, precise quantification of boron concentration within these tissues is essential.

These measurement techniques can be grouped into three main categories [7]:

- **Concentration measurement techniques:** These are used both in preclinical settings to assess boron uptake in tissues, and in clinical settings to verify the patient's suitability for treatment prior to irradiation.
- **Micro-imaging techniques:** These are necessary to study the selective accumulation and distribution of boron at the cellular and subcellular levels.

Technique	Acronym	Detection limit	Imaging capability
Inductively Coupled Plasma Spectrometry	ICP-OES	15-30 ppb (parts per billion) [18]	No
	ICP-MS	1-3 ppb in biological matter, 0.5 ppb in human serum [18]	No
Prompt Gamma Neutron Activation Analysis	PGNAA	0.04-10 ppm [18]	No
Prompt Alpha Neutron Spectrometry	PANS	0.5 ppm [18]	No
Neutron Capture Radiography	NCR	0.01-0.1 ppm [18]	5-10 μm [7]
Secondary Ion (Neutral) Mass Spectrometry	SIMS	0.1-10 ppb [18]	$\leq 10 \mu\text{m}$ [7]
	laser-SNMS		
Electron Energy Loss Spectrometry	EELS	$\gtrsim 10$ ppm [35]	0.5 μm [7]
Prompt Gamma Single Photon Emission Computed Tomography	PG-SPECT	10 ppm [18]	4-6 mm [22]
Positron Emission Tomography	PET	$\gtrsim 1$ ppm [18]	2-4 mm [22]
Nuclear Magnetic Resonance	MRI	20 ppm [18]	Yes

Table 1.4: Summary of techniques used for ^{10}B concentration measurements and their detection limits and imaging capability. Data taken from [7, 18, 22, 35].

- ***In vivo* macro-imaging techniques:** These are currently under development to measure boron uptake in individual patients, thereby enabling personalized treatment planning, and to monitor boron concentration *online* during treatment.

In a clinical setting, the primary goal of measuring boron is to verify that a patient who has been administered a boron delivery agent is eligible for irradiation. Therefore, such techniques must meet three main clinical requirements:

- **High sensitivity:** Given the typical boron concentrations required for treatment, these techniques must be sensitive down to the parts per million (ppm) level.
- **Rapid execution:** The measurements must be performed in the time window between the drug infusion and the actual treatment to confirm feasibility. Consequently, they must provide results within a short timeframe, typically on the order of a few minutes.

- **Non-invasiveness:** In most cases, obtaining a direct histological sample (biopsy) from the tumor just before treatment is not feasible. Thus, techniques must either utilize minimally invasive samples (such as blood or urine) or operate entirely non-invasively without requiring physical samples from the patient.

Additionally, there are several advanced capabilities that are highly desirable for future clinical routine:

- ***In vivo* imaging:** The ability to map the spatial distribution of boron directly within the patient, allowing for more personalized treatment planning.
- **Real-time (*online*) monitoring:** The ability to dynamically track boron concentration and spatial distribution during the irradiation process. A notable example of this approach is Prompt Gamma Single Photon Emission Computed Tomography (PG-SPECT, see subsubsection 1.2.3.1), which is currently still in the experimental phase and has not yet been integrated into clinical practice.

1.2.1 Concentration Measurement Techniques

The therapeutic dose in BNCT is primarily delivered by the boron neutron capture reaction within tissues. Accurate knowledge of the boron concentration is therefore fundamental for calculating the dose delivered to both tumor and healthy tissues.

Due to the difficulty of obtaining a tumor biopsy and the unavailability of an *in vivo* imaging technique, it is not possible to directly measure the boron concentration in tissues. Consequently, monitoring relies on measuring the boron concentration in the blood before, during, and after irradiation, and subsequently applying specific conversion factors based on the administered drug and its expected pharmacokinetics.

The two primary techniques currently used to measure macroscopic boron concentration are Inductively Coupled Plasma Spectrometry (ICPS) and Prompt Gamma Neutron Activation Analysis (PGNAA).

1.2.1.1 Inductively Coupled Plasma Spectrometry

Inductively Coupled Plasma Spectrometry (ICPS) techniques rely on the conversion of a sample into a plasma state through the application of a radiofrequency (RF) field. Two main variants of this technique are commonly used in BNCT:

- ICP-OES (Optical Emission Spectrometry, also known as Atomic Emission Spectrometry, AES).

- ICP-MS (Mass Spectrometry).

Both techniques share the same sample preparation and ionization process; they differ only in the detection method employed.

Samples are collected from the patient in either solid (histological tissue) or fluid (blood or urine) form. The former is typically used in preclinical pharmacokinetic studies, whereas the latter is the standard routine in clinical facilities to verify that the therapeutic boron concentration has been reached prior to irradiation.

The sample is appropriately prepared and dissolved into a solution. Upon injection into the ICPS apparatus, it is nebulized into an aerosol (usually with Argon). Once initially ionized by an electrical discharge, this aerosol is introduced into a radiofrequency field, where it is completely converted into a plasma state.

At this stage, the measurement is carried out. In the case of ICP-OES, the spectrometer measures the specific wavelengths of light emitted by the continuous excitation and relaxation of atoms and ions within the plasma. Conversely, ICP-MS couples the plasma generator to a mass spectrometer, separating the ionized atoms based on their mass-to-charge ratio (m/z) to determine the elemental and isotopic composition.

Both techniques require very small sample volumes (~ 1 ml) and offer an extremely low detection limit, reaching down to the parts per billion (ppb) scale. The primary drawback is that these techniques are destructive, meaning the sample is entirely consumed during the measurement. In addition to that, the only information that they give is the average over the sample, which is sufficient for liquid samples (i.e. blood) taken before irradiation, but it is a disadvantage when studying the pharmacokinetics using samples from biopsies.

Comparing the two, ICP-OES is more cost-effective and generally requires less stringent sample preparation. However, ICP-MS is significantly more sensitive and offers the crucial advantage of discriminating the specific isotopic composition (i.e., distinguishing the therapeutic ^{10}B isotope from ^{11}B).

This is one of the most widely used techniques in clinical settings, primarily because it does not require neutron irradiation of the sample.

1.2.1.2 Prompt Gamma Neutron Activation Analysis

Prompt Gamma Neutron Activation Analysis (PGNAA) is a technique that enables the analysis of the elemental composition of a sample by studying the characteristic de-excitation gamma rays emitted by nuclei immediately following neutron capture (prompt gammas). Indeed, a nucleus that absorbs a neutron is often left in an excited state and promptly decays to a lower energy level. The target element can be identified by the characteristic energy of the emitted γ -ray.

The concentration of a given element can also be determined by looking at the intensity of the peak, which is then compared against a standard sample of known concentration.

In the context of BNCT, the specific signal used for PGNAA is the 478 keV γ -ray emitted during the de-excitation of the ^7Li nucleus, which is produced in an excited state in about 94% of the neutron capture events. Because this is a recoiling nucleus, the resulting γ -ray peak experiences Doppler broadening.

PGNAA offers a relatively fast measurement (~ 5 min per sample) with an acceptable detection limit on the order of parts per million (ppm). The primary drawbacks are that it requires relatively large sample volumes (≥ 1 ml) and, most importantly, necessitates a dedicated neutron beam for the activation process.

An advanced, spatially resolved extension of this technique is Prompt Gamma Single Photon Emission Computed Tomography (PG-SPECT), which will be discussed in detail in subsection 1.2.3.1.

1.2.1.3 Prompt Alpha Neutron Spectrometry

Prompt Alpha Neutron Spectrometry (PANS) is an analytical technique based on the direct spectroscopy of the heavy, charged particles emitted during the thermal neutron capture reaction, namely the α particles and the recoiling ^7Li ions.

In this measurement setup, thin sections of biological tissue (typically frozen and cut to a thickness of $\sim 70\ \mu\text{m}$) are irradiated with a thermal neutron beam under vacuum. As the capture reactions occur, the emitted charged particles are detected and their energy is measured.

Since the emitted particles lose a portion of their kinetic energy traveling through the tissue itself, the resulting energy spectrum recorded by the detector is not composed of sharp Gaussian peaks, but rather presents a continuous profile. To determine the boron concentration, a specific residual energy interval is selected (e.g., between 1100 and 1350 keV [5]) where the collected events are expected to be exclusively α particles.

By calibrating the counts within this energy window against samples of known concentration, it is possible to accurately calculate the absolute ^{10}B concentration. A significant advantage of this technique is its ability to measure boron concentration in heterogeneous samples. Although the spectrometry itself provides an average concentration, the measurement can be coupled with neutron autoradiography (see subsection 1.2.2.1) to obtain microdistribution information. This combined approach enables researchers to precisely separate the boron concentration values of viable tumor cells from those of healthy or necrotic tissues.

The primary disadvantages of this method are the requirement for a dedicated thermal neutron beam during the irradiation phase, and the necessity to carry out the measurements under vacuum conditions.

1.2.2 Micro-Imaging Techniques

The curative efficacy of BNCT depends heavily on the macroscopic boron concentration within both tumor and normal tissues. However, this is not the only determining factor; a deeper understanding of how boron is distributed at the single-cell level is essential to identify exactly where the capture reactions take place and which intracellular structures are most severely damaged.

Therefore, investigating the microdistribution of ^{10}B within tumor and healthy cells at a subcellular level is crucial for evaluating the true biological effectiveness of any BNCT boron carrier. The primary techniques employed to measure boron at this micrometric scale include Neutron Capture Radiography (NCR), Secondary Ion Mass Spectrometry (SIMS), and Electron Energy Loss Spectroscopy (EELS). These techniques offer a spatial resolution in the μm range, making them highly capable of localizing boron at both the cellular and subcellular levels.

1.2.2.1 Neutron Capture Radiography

Neutron Capture Radiography (NCR), also known as Neutron Autoradiography, is a technique that enables the simultaneous measurement of both the spatial distribution and the absolute concentration of ^{10}B .

In this method, a thin slice of the sample (typically 60-70 μm thick) is deposited onto a Solid State Nuclear Track Detector (SSNTD). The assembly is then irradiated with thermal neutrons, after which the SSNTD undergoes a chemical etching process. This procedure enlarges and highlights the microscopic tracks left by the heavy reaction products (the α particle and the recoiling ^7Li ion) that can then be analyzed with a microscope. If the tissue section is also histologically stained, it becomes possible to directly correlate the position of the nuclear tracks with their origin at the cellular and subcellular levels.

Furthermore, by evaluating the track density and applying an appropriate system calibration, the macroscopic boron concentration within the sample can be accurately quantified.

This technique can be performed in two main variants:

- **Qualitative Neutron Autoradiography:** this technique consists in irradiating the sample with a high thermal neutron flux for a long period of time. This aids in visualizing the spatial distribution of boron, in particular spotting areas of anomalous concentration, but cannot be calibrated to measure the concentration of boron due to the concentration of etch pits being too high, thus leading to track overlapping.
- **Quantitative Neutron Autoradiography:** this technique consists in irradiating the sample with a lower neutron flux for a short period of time. This

approach partially sacrifices visualization details, but the lower concentration of tracks allow to linearly correlate it to the concentration of boron.

Both techniques provide high-resolution imaging of boron microdistribution in relatively small biological samples, with a concentration detection limit of 0.1-10 ppm.

The primary drawback of NCR is the lengthy processing time required to obtain the results, largely dictated by the chemical etching phase of the SSNTD and the analysis with the microscope, which typically is of the order of a few hours.

1.2.2.2 Secondary Ion Mass Spectrometry

Secondary Ion Mass Spectrometry (SIMS) is another analytical technique based on mass spectrometry. Unlike ICP-MS, the ions in SIMS are produced by bombarding the sample with a focused primary ion beam under high vacuum. This bombardment causes atoms and molecules to be ejected from the surface, a phenomenon known as *sputtering*. A fraction of these ejected particles are ionized (secondary ions) and are subsequently analyzed by a mass spectrometer.

This technique enables the measurement of the spatial distribution of boron by raster-scanning the sample with the primary ion beam. Consequently, the spatial resolution is primarily limited by the focal spot size of the beam, allowing modern instruments to achieve sub-micrometric resolution.

This level of precision makes it possible to study boron distribution directly at the subcellular level. To accomplish this, specific biological markers are utilized to differentiate distinct cellular compartments, such as the nucleus, the cytoplasm, and the cell membrane, allowing the boron signal to be superimposed on the cellular architecture.

A notable limitation of traditional SIMS is its relatively low sensitivity, as only a minuscule fraction of the sputtered particles are naturally ionized and thus detectable. To overcome this, laser Secondary Neutral Mass Spectrometry (laser-SNMS) can be employed. This variant uses a high-intensity laser to post-ionize the neutral sputtered particles, drastically increasing the overall sensitivity of the technique.

The main disadvantage of SIMS and its variants is the strict requirement to operate under high-vacuum conditions, which significantly complicates sample preparation, especially for biological and histological tissues. Moreover, this technique requires specific and expensive equipment and the expertise to use it, which are not widespread.

1.2.2.3 Electron Energy Loss Spectroscopy

Electron Energy Loss Spectroscopy (EELS) is an analytical technique based on the inelastic scattering interactions between a primary electron beam and the electron clouds of the sample's constituent atoms. The incident electrons lose a specific amount of energy that is strictly characteristic of the atomic or molecular energy levels they interact with.

By raster-scanning the sample with the electron beam, similar to the process described for SIMS, it is possible to accurately map the spatial distribution of boron. This approach achieves a remarkably high, sub-micrometric spatial resolution.

The primary drawbacks of EELS are its relatively low sensitivity (~ 10 ppm) and the strict requirement for ultra-thin sample preparation (typically ~ 100 nm thick). Furthermore, unlike mass spectrometry techniques, EELS lacks the capability to discriminate between different boron isotopes.

1.2.3 In Vivo Macro-Imaging Techniques

In order to have a better understanding of the dose delivered to the patient, it is necessary to employ techniques that allow for the personalization of the treatment plan beforehand and the monitoring of the dose during irradiation.

The latter requires a system capable of monitoring the boron concentration and position in the body in real time. This can be achieved by a technique such as Prompt Gamma Single Photon Emission Computed Tomography (PG-SPECT). The former requires a non-invasive technique that allows imaging of the pharmacokinetics. Currently, the only technique available at the clinical level to study pharmacokinetics is Positron Emission Tomography (PET), but Magnetic Resonance Imaging (MRI) is under investigation as a technique that does not involve ionizing radiations.

1.2.3.1 Prompt Gamma Single Photon Emission Computed Tomography

As anticipated, Prompt Gamma Single Photon Emission Computed Tomography (PG-SPECT) is a technique that uses the 478 keV γ emitted by the recoil ^7Li . The aim is to use this to perform a SPECT imaging during irradiation.

By surrounding the patient with a detection system optimized to detect the 478 keV γ it is possible to reconstruct a real time tomographic image of the boron distribution.

This technique shows technical limitations, mainly due to the higher energy of the photon involved with respect to conventional SPECT (which requires different materials in order to optimize detection) and the intense gamma background, always present whenever a neutron field is turned on, which significantly decreases

the signal to noise ratio.

Despite having been under investigation for many years by several BNCT groups, a fully equipped PG-SPECT detector for clinical practice has not yet been developed.

1.2.3.2 Positron Emission Tomography

Positron Emission Tomography (PET) is an imaging technique that uses a β^+ radionuclide (often referred to as a *radiotracer*) to track the uptake of a specific substance in the body.

This is achieved by detecting the two back-to-back 511 keV photons emitted during positron annihilation as coincidence events.

This technique enables the study of the pharmacokinetics of a boron carrier. However, a key prerequisite is that the molecule must allow for the attachment of a radiotracer without significantly altering its chemical properties, ensuring that its natural uptake remains unaffected.

Among the boron carriers currently available, BPA and BSH, only the former can be tracked using PET via its ^{18}F -BPA derivative. Conversely, it has not yet been possible to synthesize a viable BSH variant incorporating a PET tracer.

One of the primary requirements for the new generation of boron carriers is to possess *theranostic* properties (the ability to be used for both therapy and diagnostics), meaning they must be trackable *in vivo* using a radiotracer or other imaging modalities.

Since this technique involves the use of a radiopharmaceutical (^{18}F -BPA) in a non-therapeutic framework, the actual amount of molecule injected in the patient is several orders of magnitude lower than the one actually used for therapy. This could lead to results that do not take into account possible effects of saturation that could happen during the treatment.

1.2.3.3 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive *in vivo* imaging technique that offers the notable advantage of not relying on ionizing radiation.

Both stable boron isotopes possess a non-zero nuclear spin ($I = 3$ for ^{10}B and $I = 3/2$ for ^{11}B), meaning they are intrinsically NMR-active and can theoretically be detected using MRI.

While direct measurement is possible, these isotopes have a relatively low gyromagnetic ratio, resulting in inherently weak signals. Furthermore, their transverse relaxation time (T_2) is extremely short, which means the signal decays very rapidly and must be acquired in very short times before it is lost.

Alternatively, boron can be tracked utilizing *chemical shift* imaging (CSI). This technique enables the identification of specific molecules by detecting variations in the local magnetic field induced by the electron clouds surrounding the nuclei within the compound.

A major area of current investigation for MRI in BNCT is the development of theranostic molecules. Specifically, researchers are exploring the possibility of conjugating the boron delivery agent with an MRI contrast agent (such as gadolinium) or a NMR-active element (like fluorine) to boost the signal intensity.

As discussed in the context of PET imaging, the functionalization strategy using fluorine can be successfully applied to BPA. New ^{10}B carriers have been developed taking into consideration the possibility to link them with commercial Gd-based contrast agents [8].

1.2.4 Final Remarks

In conclusion, a wide array of measurement techniques is currently available to evaluate boron concentration and distribution, providing crucial data for both pre-clinical research and clinical BNCT practice.

While these established methodologies have formed the backbone of BNCT research for decades [35], and despite undergoing significant technological improvements over the years, their fundamental detection paradigms have remained largely unchanged. Most traditional methods force a compromise, excelling either in macroscopic quantification or in high-resolution spatial imaging, but rarely offering both simultaneously in a streamlined process.

To bridge this gap, the aim of this thesis is to investigate and develop a novel measurement technique based on the TimePix family of pixel detectors. By exploiting the advanced sensing capabilities of these pixelated detectors, this innovative approach enables the combination of precise spectrometric analysis (similar to PANS) with the ability to directly and accurately map the spatial microdistribution of boron within a sample (similar to NCR).

Chapter 2

Materials and Methods

2.1 MediPix Family of Pixel Detectors

Pixelated detectors have been under development at CERN since the 1980s.

In particular, this section examines the MediPix family of pixel detectors, of which the TimePix is part, that emerged from several CERN collaborations that began in the 1990s and continue to this day.

2.1.1 MediPix

The MediPix1 collaboration was started in order to transfer the knowledge derived from the years of R&D on detectors for the Large Hadron Collider (LHC). This led in 1997 to the development of the MediPix chip.

The MediPix chip is a *hybrid pixel detector*, which means that the sensitive detector and the readout electronics are separate entities and then put together via the *bump-bonding* technique (Figure 2.1).

It consists in using solder micro-bumps (typically gold, tin-lead alloy or indium) of diameter $\sim 20\ \mu\text{m}$ to physically and mechanically weld the two components.

The advantage of hybrid detectors is the possibility to use the same electronics with different kinds of sensitive detectors, allowing the use of the same ASIC (Application-Specific Integrated Circuit) in different applications.

This detector was made up of a 64×64 array of pixels, with a pitch of $170\ \mu\text{m}$, while the ASIC is based on CMOS technology.

The sensitive detector can thus be chosen depending on the specific application of the detector.

This detector could only operate in counting mode, and its main use was conceived for biomedical applications, in particular for X-ray imaging.

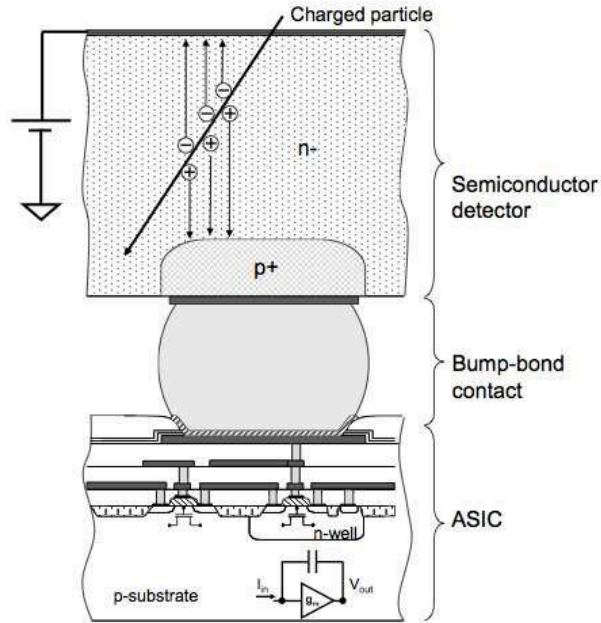


Figure 2.1: Section of a MediPix single pixel. It is clear to be seen how the electronics and sensitive area are separate and connected by the bump bond. From [34]

2.1.2 TimePix1

Given the success of the MediPix1 collaboration, the MediPix2 collaboration was formed in 1999, leading to the development of the MediPix2 chip.

The main improvement with respect to the MediPix1 is concerning the pixels, which were upgraded to an array of 256×256 square pixels with a pitch of only $55 \mu\text{m}$.

In this collaboration, another ASIC was also designed: the TimePix1. Basically, the signals were digitized using the Time over Threshold (ToT) technique, based on an internal clock oscillating at a frequency of tens of MHz. This new architecture allows to operate in three modes:

- **Counting** (or *MediPix*): when the signal crosses the threshold (set by the user) an internal register is increased by one. This mode can be used for X-ray imaging.
- **Time of Arrival (ToA)**: whenever the signal crosses the threshold, a clock starts incrementing an internal register until the end of that acquisition frame. By counting the number of clocks registered and knowing the clock's frequency it is possible to reconstruct when the signal arrived.

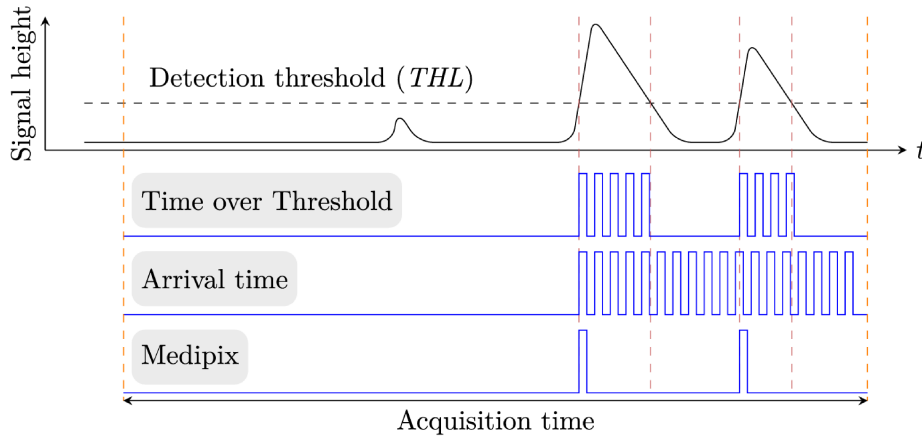


Figure 2.2: TimePix1 operation modes. From [14].

- **Time over Threshold (ToT):** the clock is registered only during the time the signal stays over the threshold. Since the signal is amplified and shaped (all signals are electronically processed in order to have the same shape), this measure is correlated to the energy deposited in that pixel.

The schematic of the TimePix operation modes is given in Figure 2.2.

The TimePix data collection is *frame-based*. This means that each time an acquisition window ends, the signal is collected sequentially from all the matrix, independently from the fact that a pixel recorded the signal or not. Furthermore, a pixel fired by an event remains unavailable to detect additional hits for the entire duration of the frame acquisition and the time required for data transfer. The latter constitutes a dead time that can vary from a minimum of $300 \mu\text{s}$ to over 10 ms, depending on the matrix occupancy level and the number of registered bits.

2.1.3 TimePix3

The TimePix1 chip showed to be extremely versatile in numerous field: from biomedical [9], to ambient monitoring [30] and even aerospace [2].

However, it still had two main limitations:

- It can only be set to perform an operation mode at a time (thus limiting the amount of information that is possible to get from each event).
- The long dead time due to the frame-based architecture that causes a significant loss of events during the readout.

These limitations were addressed during the MediPix3 collaboration (2005), which gave us the MediPix3 and TimePix3 chips.

In particular, the TimePix3 chip has the same pixel structure of the TimePix1 (array 256×256 , pitch $55 \mu\text{m}$), but allows to record simultaneously the information of ToA and ToT for each pixel. Moreover, a 640 MHz clock has been added to a coarse 40 MHz clock in order to increase the timing resolution to 1.56 ns. These and other characteristics of this chip are reported in Table 2.1

CMOS technology	130 nm, 8-metal stack
Pixels	256×256
Pixel size	$55 \times 55 \mu\text{m}$
Acquisition modes	Charge and time Time only Event counting and integral charge
Zero suppressed readout	Yes
Dead time per pixel	ToT Pulse time + 475 ns
Timing resolution	1.5625 ns (640 MHz)
On-chip power pulsing	Yes
Output bandwidth	Up to 5.12 Gbps ($8 \times 640 \text{ Mbps}$)
I/O	SLVS, 8b/10b, 8 output links for data

Table 2.1: Timepix3 features. Adapted from [25].

Another important improvement that has been made is the data-driven readout architecture. This means that the transfer of information of a hit is started as soon as the hit happens, instead of waiting for the whole matrix to be read out like in the frame-based architecture of the TimePix1. This allows for faster readouts with respect to the frame-based architecture that was characteristic of the TimePix1 chip and a lower dead time. In particular, this is true up to a pixel occupancy (the number of pixels that are hit during an acquisition window) of 50% (as can be seen in Figure 2.3).

The readout of the chip is carried out through micron-scale electrical interconnects referred to as *wire bonds*.

Readout time can be calculated using the following equation:

$$T_{\text{readout}} = \frac{N_{\text{pixels}} \cdot \text{bits}_{\text{pixel}}}{BW} \quad (2.1)$$

where N_{pixels} is the number of pixels being readout, $\text{bits}_{\text{pixel}}$ the number of bits per pixel and BW the total output bandwidth of the chip. For frame-based architecture N_{pixels} is the total number of pixels (for a single chip 65536) and $\text{bits}_{\text{pixel}}$ is 28, while in data-driven mode N_{pixels} is the actual number of pixels that were hit, while $\text{bits}_{\text{pixel}}$ is 28+16 (due to address overhead for each packet).

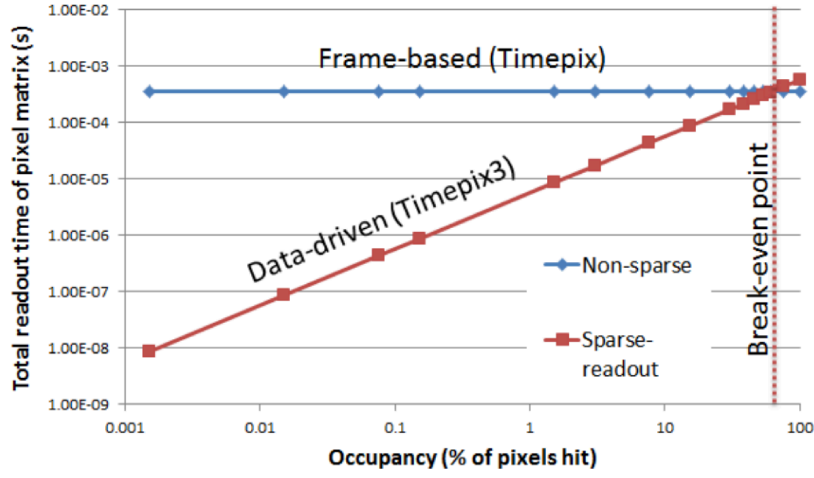


Figure 2.3: Comparison in readout times between the data-driven (packet based architecture of TimePix3) and the frame-based architecture of TimePix. Image from [25].

In order to understand how data transfer occurs, it is necessary to look at the schematics of a single pixel (Figure 2.4). The main component of the analog part consists of a Charge Sensitive Preamplifier (CSP) with a tunable feedback capacitor. The role of the CSP is to amplify and shape the signal. Its output can be described as a triangular wave with a falling edge, having a slope that can be tuned through the feedback capacitor via the I_{krum} parameter on the control panel. After the CSP, the preamplified signal passes through a discriminator, which is based on the global chip threshold and has a 4-bit threshold adjustment. This is used to equalize the response of each pixel in order to compensate for construction inhomogeneities. After this, it is also possible to mask a pixel, which means

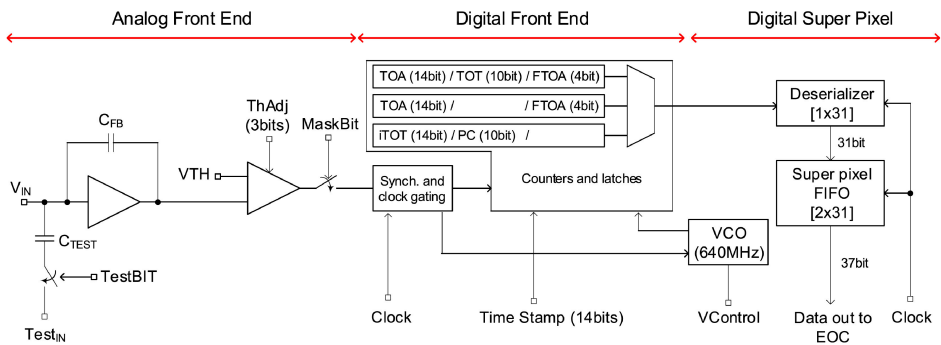


Figure 2.4: Architecture of a single pixel of the TimePix3 detector. The Super-Pixel part is actually shared between 8 pixels. From [3].

excluding it from the readout. This is done when it is not possible to equalize it to the rest of the matrix or it is broken, leading to a continuous and wrong readout. The output signal from the discriminator is synced with the global clock (40 MHz) and the fine clock (640 MHz Voltage Controlled Oscillator) measures the time interval until the next rising edge of the global clock. These give the ToA information in a 14+4-bit counter. Moreover, it is possible to simultaneously record the ToT information in an additional 10-bit counter.

All of this happens in the front-end electronics of each pixel. In the context of sparse readout, in order to minimize the dead time of each pixel, the data is transferred as soon as possible to a back-end digital processing unit called "Super-Pixel". This structure is common to 2×4 pixel groups and has a deserializer that reorders the data and a local memory that allows the storage of events by two pixels at a time in order to be finally taken off-chip. This is what allows to reduce the dead time to a minimum of 475 ns for the TimePix3, a significant improvement with respect to TimePix1.

Since the readout time increases with the pixel occupancy, it is possible to switch between data-driven and frame based via a readout enable signal at the level of the SuperPixel.

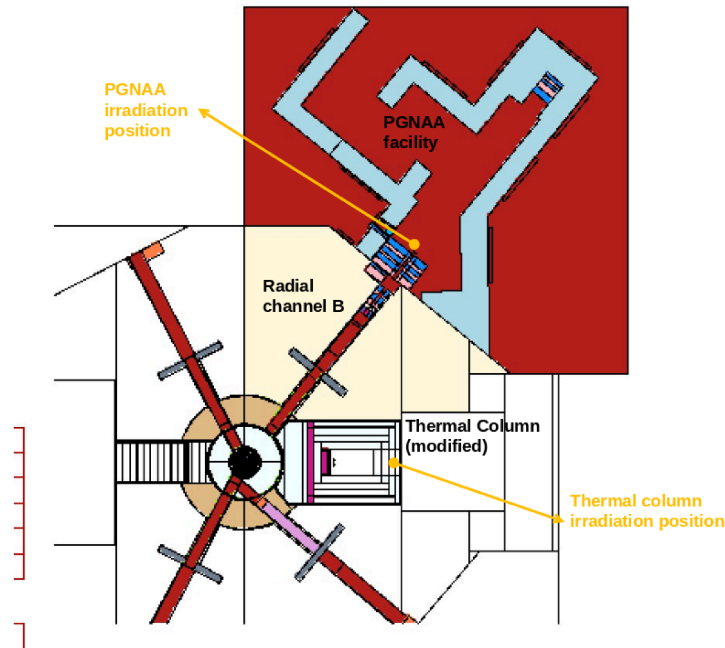


Figure 2.5: Cross-section view of the TRIGA Mark II reactor, with particular focus on the facilities used for this thesis.

2.2 Laboratorio di Energia Nucleare Applicata (LENA)

The measurements for this thesis were carried out at the *Laboratorio di Energia Nucleare Applicata* (LENA) of the University of Pavia. The facility houses a 250 kW TRIGA Mark II nuclear reactor that is used for research purposes to produce neutron spectra.

In particular, two irradiation facilities were used for this work: the Prompt Gamma Neutron Activation Analysis (PGNAA) facility and the Thermal Column, since both provide a highly thermalized beam.

2.2.1 PGNAA Facility

This facility is installed on the radial channel B of the LENA reactor to provide a highly thermalized (Figure 2.6) and collimated neutron beam for PGNAA [27]. The thermalized beam can also be used for BNCT applications

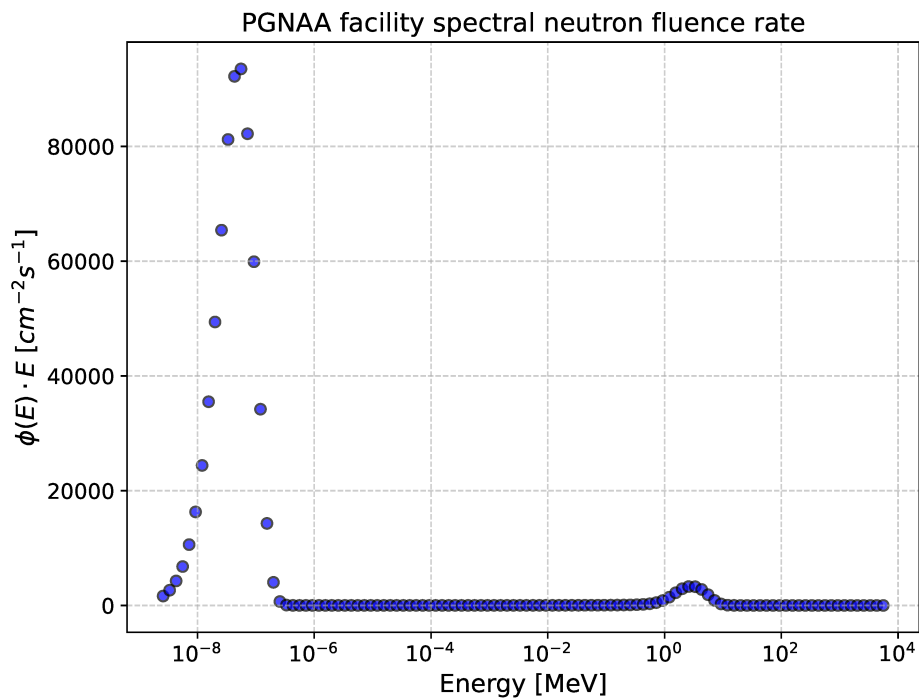


Figure 2.6: Spectral neutron fluence rate in the PGNAA facility. Data acquired with a DIAMON neutron spectrometer by RAYLAB s.r.l. [26] The measurements were performed with the reactor power at 10 kW. Courtesy of Dr. D.Rastelli, CTO RAYLAB.

The facility, in particular, provides a thermal neutron beam with a maximum in-

tensity of $\sim 6.6 \times 10^6 \text{ n cm}^{-2}\text{s}^{-1}$ at the reactor power of 250 kW. For this set of measurements, the maximum reactor power exploited was 200 kW. Since the neutron flux varies linearly with the power of the reactor, these measurements were taken at a flux of $\sim 5.28 \times 10^6 \text{ n cm}^{-2}\text{s}^{-1}$.

2.2.2 Thermal Column

In order to have a higher neutron flux, and thus a higher reaction rate, it was necessary to exploit the thermal column of the reactor to carry out a second measuring campaign. In the LENA laboratory, the thermal column represented in Figure 2.5 was modified with respect to the original reactor design for the BNCT TAORMINA project. In particular, much of the graphite wall was removed, making an actual room that is accessible by authorized personnel and an irradiation room where the samples are placed.

At the edge of the irradiation room, next to the Boral wall, the neutron flux that can be reached is $\sim 2 \times 10^9 \text{ n cm}^{-2}\text{s}^{-1}$ at the reactor power of 250 kW.

Considering that the maximum transfer speed of the QUAD-TimePix3 Kathrine readout module is 15 MHits/s per chip using the ethernet connection, and given that a transfer rate of 0.28 MHits/s was observed for the QUAD-TimePix3 in the PGNAa, it can be calculated that the detector can be exposed to a flux of up to $\sim 6.6 \times 10^7 \text{ n cm}^{-2}\text{s}^{-1}$.

2.3 Experimental Setup

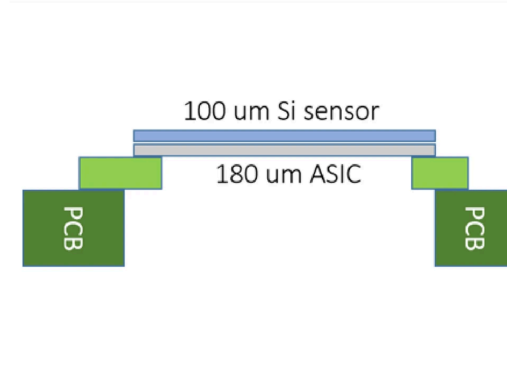
2.3.1 QUAD-TimePix3 and Sample-Holder Integration

For the measurements that were performed for this thesis work, a QUAD-TimePix3 chip was (Figure 2.7a). This consists of a 2×2 array of TimePix3 chips with a $100 \mu\text{m}$ silicon layer as sensitive material and the same readout electronics. In particular, the host PCB has an opening at the QUAD location in order to have the least amount possible of material behind the chips, to minimize the background due to the interaction of thermal neutrons with the elements of the PCB (see Figure 2.7b). This solution was developed for operation in the PGNAa facility, where the collimation of the beam made the amount of activation behind the detector relevant. In the thermal column, this is still a useful feature since it reduces the background hitting the sensitive area for a reason of geometric efficiency, but the non collimated radiation field makes it less effective.

The chip is read using an electronic module called Katherine via a VHDCI connector. The Katherine module is then connected to the PC via Ethernet.



(a)



(b)

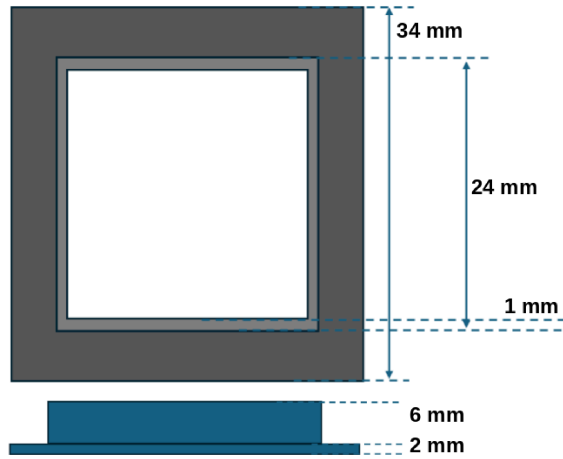
Figure 2.7: (a) Picture of the QUAD TimePix3 detector. The size active volume of the detector is marked by arrows. (b) shows the schematic layout of the PCB modified in order to keep the back of the detector hollow.

In order to hold the samples in front of the sensitive area of the detector, an aluminum support was mounted in front of it, held by four bolts. On the bolts, four nuts were mounted to set the distance between the sample holder and the detector and other four to keep it in place (see Figure 2.8b). This distance was estimated to be $1 \pm 0.5 \text{ mm}$, in order to minimize the air attenuation of the alpha particles and lithium ions that we wanted to measure, but also enough to avoid the risk of touching the sensitive area with the biological samples. Moreover, this was also a safety measure to reduce the risk of touching and damaging the micron-scale *wire-bonds* that are on the side of the chips (left and right) and allow the readout of the chips.

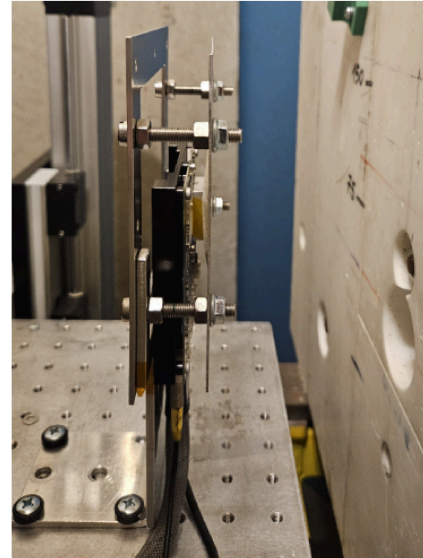
The choice of aluminum has been made considering that, despite its activation in a neutron field, it has a short decay time ($\sim 2.2 \text{ min}$) and it emits very high energy gamma rays, which are quite easy to filter using the cluster analysis (see later, in section 2.4).

A custom two-piece aluminum sample holder was used to position the samples in front of the detector. The primary component consists of an aluminum mounting plate with a central aperture, which was originally designed to accommodate the sample. To minimize the distance between the sample and the sensitive area of the detector without causing mechanical interference with the surrounding front-end electronics, a custom aluminum extension was machined (Figure 2.8a). This protruding insert was designed to fit the central hole of the main plate, allowing the sample to be safely housed in closer proximity to the active volume.

Since the experiment needed to be conducted maintaining the board vertically, Kapton tape was used to fix the samples on the sample holder. The choice to



(a)



(b)

Figure 2.8: (a) technical drawing of the protruding insert designed to bring closer the sample to the sensitive area of the detector. (b) picture of the detector with the sample holder mounted in front of the detector. It was possible to minimize the distance between the sample and the detector thanks to the aluminum extension.

use Kapton tape is due to its chemical composition, which makes it particularly suitable to be exposed to a neutron beam (Table 2.2).

Element	Fraction by weight
H	0.026362
C	0.691133
N	0.073270
O	0.209235

Table 2.2: Chemical composition of Kapton. Adapted from [23].

The detector was mounted using a stainless steel support, that could be fixed to a breadboard that is installed in the PGNAA facility that allows to manually adjust the x,y,z positioning of the detector in front of the beam port with a tolerance of the order of the mm (Figure 2.9). However, when the experiment migrated, a breadboard was unavailable in the thermal column and it was not possible to install one onsite, so a Teflon support was used to hold the detector at the beam port.

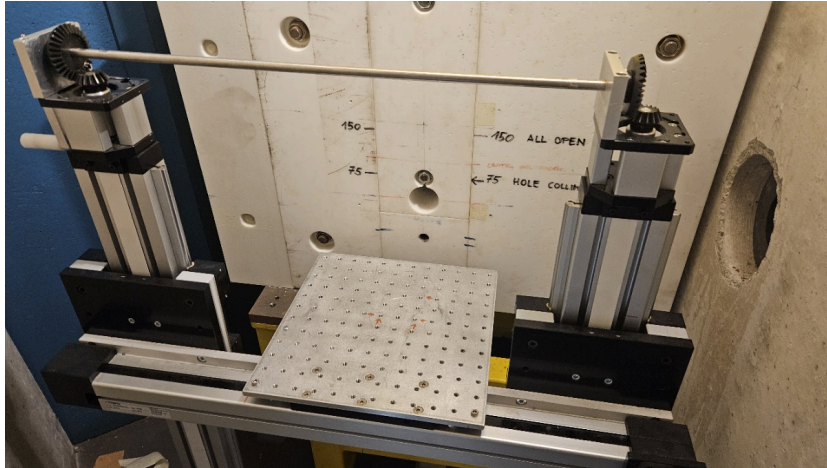


Figure 2.9: Picture of the PGNAA facility irradiation position. It can be seen the adjustable breadboard where the detector was fixed. On the shutter, in its closed position, it is possible to see the beam exit and the red line that corresponds to the height which is going to reach when the shutter is opened.

2.3.2 Samples

The samples used came from two preparations and the concentrations available where: 100 ppm, 70 ppm, 50 ppm, 30 ppm, 25 ppm, 20 ppm, 10 ppm, 5 ppm and control (0 ppm of added ^{10}B).

The samples were made of rat liver extracted from sacrificed rats, and then homogenized using a pot homogenizer and curated with known concentrations of ^{10}B in the form of boronophenylalanine-HCl (BPA-HCl) solution at 500 ppm [12]. The tissue samples were then frozen in the form of small cylindrical rods of diameter ~ 1 cm at -80°C . The samples were then cut into $60\ \mu\text{m}$ slices using a Leica cryostat and deposited on Mylar $100\ \mu\text{m}$ thick disks.

The samples were conserved under non-controlled conditions since their production and stored within a sample holder.

2.4 Cluster Analysis

As mentioned earlier, the first step of data processing with the QUAD TimePix3 is Cluster Analysis. This technique allows for the identification of interesting events from the intense radiation background that is produced in a nuclear reactor along the neutron beam.

2.4.1 Radiation Background

As is often the case with neutron sources, the background of produced particles is substantial and, in this case, is associated with the source itself and with the reactions of neutrons with the materials surrounding the sensitive area of the detector. A qualitative description of the main background sources will be provided here. First of all, it is important to understand the contamination that is always present in a nuclear reactor:

- **γ -rays:** along any neutron source, it is unavoidable to have a certain contamination from γ -rays. In a nuclear reactor these are produced due to the fission reaction.
 - **Prompt γ :** They are emitted almost instantly ($\leq 10^{-14}$ s) during the fission of ^{235}U with a continuous spectrum up to ~ 10 MeV.
 - **Delayed γ :** These are due to the decay of unstable fission products, mainly through β decay. The characteristic time of production of these γ s depends on the nuclide and can span from seconds to years.
- **Fast neutrons:** Since the neutrons produced in the fission reaction are high energy (see Figure 1.2) some of these fast neutrons survive the dedicated moderation and filtration structures and contaminate the beam. The main contribution to background is the recoil proton from the $^1\text{H}(n,n)^1\text{H}$ reaction that carries, on average, half of the impinging neutron energy. An example of such fast neutron contamination can be seen in Figure 2.6.

The other background component is due to the interaction of the neutron beam with the components of the detection apparatus and the activation of the irradiation rooms. Regarding the apparatus, the main components are:

- **Aluminum holder:** Aluminum gets activated in a neutron field and emits characteristic high energy gamma rays (1.78 MeV).
- **PCB elements:** The exact composition of the circuit board used to hold the TimePix3 chips is unknown. As a reference the material commonly used for PCBs known as FR-4 is considered:
 - **Epoxy resin:** its chemical formula is $\text{C}_{18}\text{H}_{24}\text{O}_3\text{Br}_4$. The main reactions of these elements in a thermal neutron field is the production of gamma rays, especially for hydrogen following the $^1\text{H}(n,\gamma)^2\text{H}$ capture reaction. It is around 40% in weight of the board [24].

- **Glass fiber** (E-Glass): This can be found in different compositions, specifically distinguished by the boron concentration (see Table 2.3). The possible presence of boron, even in low percentages, is critical for this application, since its presence produces a signal that cannot be distinguished from the one relevant for the measurement. For this reason, the choice of having a personalized PCB design is fundamental to keep this noise limited to the edges of the detector.

	Low-boron	High-boron	Boron-free
B_2O_3	5	10	0
SiO_2	54	54	56
CaO	22	17	24
Al_2O_3	14	14	15
Fe_2O_3	0.2	0.2	0.2
MgO	3	3	3
Na_2O	0.8	0.8	0.8
F_2	0.5	0.5	0.5
TiO_2	0.5	0.5	0.5

Table 2.3: E-Glass chemical composition. Percentages by weight. Adapted from [24].

- **Kapton:** The main source of background in a neutron field is going to come from nitrogen's $^{14}N(n, p)^{14}C$ reaction, which is present in low concentration in this material (see Table 2.2). The proton from this reaction has an energy of 584 keV. The proton from this reaction is one of the main sources of concern, since it is a heavy charged particle like the ones we are interested in measuring, and the tracks it leaves in the detector are difficult to distinguish from a morphological point of view, from the α and 7Li . The reaction product is a radioactive isotope of carbon that decays through pure β decay and has a half-life of 5730 years.
- **Mylar:** the biological samples are deposited on a Mylar disc. This material has the composition reported in Table 2.4. All these elements can be activated in a thermal neutron field with a very low cross section [11], mainly leading to a characteristic γ background.

Finally, since it was not possible to operate the measurements under vacuum, it is important to keep in mind the interaction of the beam with air. In particular, it can interact with nitrogen (which constitutes $\sim 78\%$ of air) with the reaction examined earlier.

Element	Fraction by weight
H	0.041959
C	0.625017
O	0.333025

Table 2.4: Chemical composition of Mylar. Adapted from [23].

2.4.2 Cluster Cuts

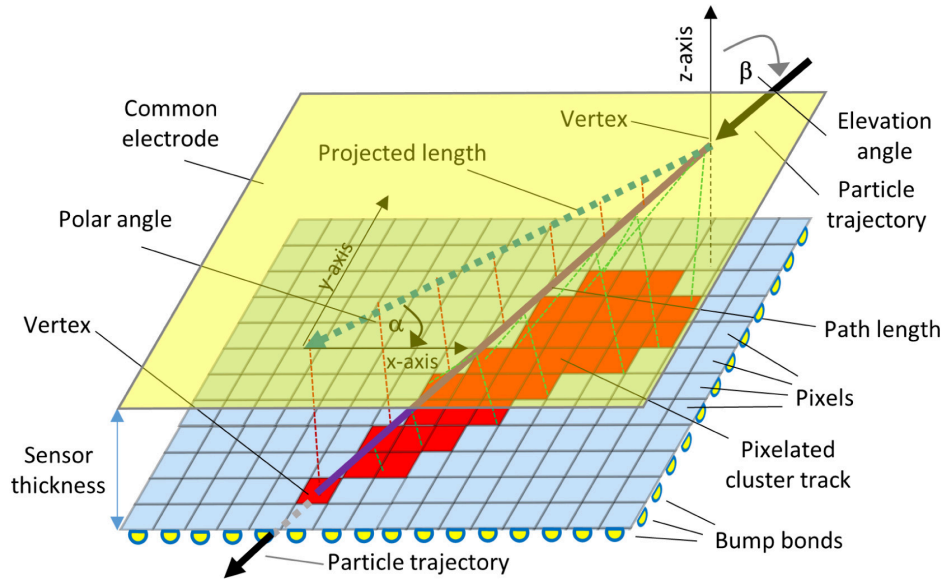


Figure 2.10: Schematics of the particle tracking capabilities of the TimePix detectors. From [13].

The TimePix itself is a 2D detector with high spatial resolution, but it is often used as a particle tracker, as in the present case. The interaction of a given particle activates a set of pixels with intensities proportional to the deposited energy and spatial distribution characteristic of the particle type.

As TimePix3 allows to measure simultaneously ToT and ToA for each event. Once a maximum time is defined within which the event must end, it is possible to reconstruct the single tracks left by a particle. It is defined the maximum time within which the event ends, contiguous pixels that are lit up within that time-frame are considered a single *cluster*.

The shape of a cluster is influenced by two main factors:

- **Particle type and energy:** having different types of interactions in the materials, different particles will deposit their energy in a different way. For

example, charged particles are going to have different tracks depending on their LET, which in turn depends on particle type and energy

- **Lateral spread:** the charges separated by the ionizing radiation are collected by different pixels of the matrix. This depends on two factors: the charge diffusion during transport within the semiconductor (and thus the interaction depth) and the Coulomb repulsion of the charge, especially for highly ionizing particles.

Figure 2.10 gives a schematic representation of how a cluster is formed.

The morphological analysis of the clusters allows to get information on the particle depositing the energy.

According to [33], there are six known shapes that can be identified by cluster analysis (Figure 2.11:

- **Dots:** these clusters are caused by particles that hit the detector perpendicularly. Since a particle can hit at the boundary between two pixel the maximum size of this kind of tracks is 2×2 . They are usually left by electrons and photons
- **Small blobs:** these kind of blobs are left by low energy photons and electrons that do not hit the detector close to a 90° angle. The difference with the dots is thus the amount of pixels lit
- **Curly tracks:** these clusters are produced by high energy electrons that undergo scattering along their path, thus forming a winding path in the detector. They can either be independent (primary electron) or connected to a heavy particle cluster, meaning that it is caused by a δ -electron.
- **Heavy tracks:** these tracks are caused by heavy charged particles that hit the detector at an angle. They are wide straight tracks that show the halo only at one end.
- **Heavy blobs:** these clusters are left when a heavy charged particle hits the detector almost perpendicularly. The pixels toward the center of the *blob* register the highest energy, while the least activated ones in the surrounding form a sort of halo.
- **Straight tracks:** these are caused by minimum ionizing particles such as cosmic muons. Their characteristic is that they are long and usually less than 4 pixel wide, with a energy distribution quite even among all the pixels.

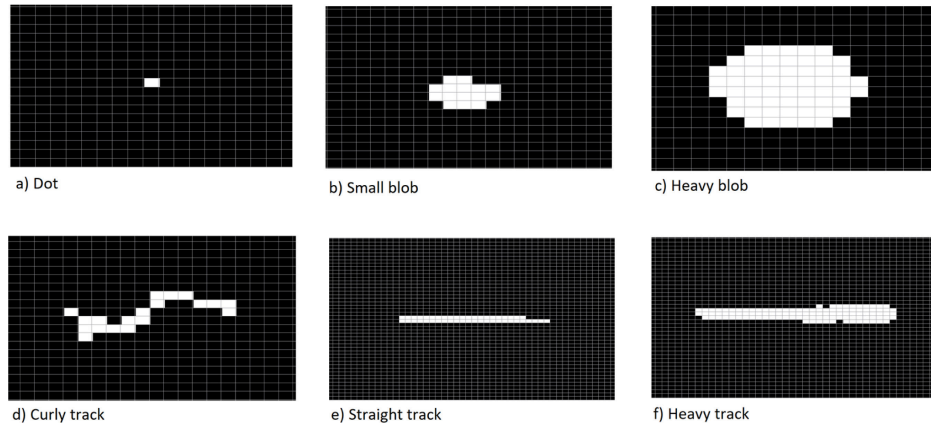


Figure 2.11: Representation of the six categories of clusters. From [33].

Furthermore, it is possible to impose other filters to discriminate interesting clusters from background. In particular, it is important for this work the *roundness*. This is a morphological classification based on the ratio of the minor over major axis of the ellipse that includes the cluster. In other cases it is also defined as the ratio between the area and the perimeter of the cluster.

2.5 Software Tools

In order to acquire data with the TimePix3, it has been necessary to use a specific acquisition software, developed by the Timepix Analysis Group of the Institute of Experimental and Applied Physics of the Czech Technical University in Prague [16].

2.5.1 TrackLab

TrackLab is a software for data acquisition designed to work with all TimePix devices. This software allows to read the Katherine output and register the events. Its user interface (UI) is a drag-and-drop type, so that it is possible to easily configure the data acquisition pipeline that better suits the user needs.

TrackLab allows to save the data in multiple formats. The most notable are

- **Native File:** it is a binary format that can only be read by TrackLab. It is the lighter format available.
- **Root:** it is an object-oriented binary data storage architecture developed by CERN to save high energy physics and nuclear data. This format is

optimized for I/O operations due to its columnar structure (TTree, TBranch, TLeaf) and memory management.

- **MM file:** it is a text data format that allows one to easily access the data, but it is not optimized for memory since it uses ASCII encoding.

Furthermore, TrackLab allows to monitor the online data acquisition with a series of visualization aids such as the 2D Pixel Matrix, which shows which pixels are lit, the Timeline, which shows the count rate at every given moment and 1D and 2D histograms that allow to visualize different parameters.

TrackLab can also read the data saved in the native binary format and allows to process it for a preliminary analysis.

The most useful tool for this is the *Clustering* tool that groups the data from single pixels into cluster events (see section 2.4). Down the line, it is also possible to apply cuts to select interesting events and then save the data, already clustered and partially elaborated, in a different format.

This software allows in particular to work with the QUAD configuration such as the one used for this work.

Chapter 3

Experimental Results

In this chapter, the results obtained for the concentration calibration of the detector will be discussed. Previous attempts [31] already showed the linearity in the response of the detector. The first aim of this work is to test the ability of the TimePix3 to detect low concentrations of ^{10}B in biological samples, comparable to the ones actually found in BNCT clinical settings.

3.1 Measurements in the PGNAA Facility

The first series of measurements to calibrate the detector was carried out in the PGNAA facility, where previous attempts to calibrate the QUAD TimePix3 for BNCT applications had been carried out.

The detector was placed inside the PGNAA facility fastened on a breadboard in front of the beam port. The table can be adjusted vertically and horizontally to align the detector with the beam port once the vertical shutter opens. It is also possible to adjust the distance from the beam port, but due to the bulk of the external collimator of the PGNAA facility, the minimum distance that was possible between the beam port and the sensitive area of the detector was $19 \pm 1 \text{ cm}$.

Despite the presence of another shutter (horizontal), for safety reasons it is not possible to enter the facility when the shutter is open, so it had to be approximately aligned with the beam port when the reactor is off and actually corrected after a first irradiation that served to check if the detector was misaligned with the beam. Indeed, the collimated beam (that is produced when the vertical shutter is in its proper position to ensure a collimated neutron beam) is circular with diameter of 4.9 cm and a lateral spread of less than 1° . So a slight misplacement could mean not irradiating the samples properly.

After this step, the irradiation of the samples started. Each sample was put on the sample holder and irradiated for a different amount of time reported in Table 3.1

Sample	Irradiation time (h)
50 ppm	2
30 ppm	2
25 ppm	2.5
10 ppm	2
5 ppm	5

Table 3.1: Samples measured in the PGNAA facility and their irradiation time.

Due to the high number of events registered by the detector, the amount of data transferred to the PC was exceptionally large. For this reason it was chosen to save the raw data in the native binary format in order to be further processed after the end of the acquisition. Even with this solution it was necessary to divide the acquisitions into smaller files of 15 minutes each, because each ended up being ~ 20 GB. This phenomenon at the moment is unavoidable due to the fact that applying online cuts would slow down the acquisition since it would require the PC to elaborate data fluxes of the order of the MHits/s.

The TrackLab pipeline chosen for data acquisition is sketched in Figure 3.1

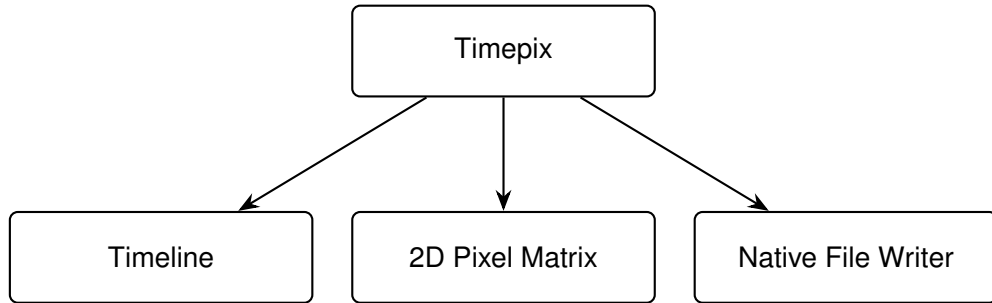


Figure 3.1: TrackLab data acquisition pipeline. Each block represent one of the drag-and-drop tools of TrackLab that were used. The data from the TimePix is split to be displayed on the screen of the PC as an online check and to be saved in native format for further analysis. Timeline is a tool shows the hit rate as a function of time, while the 2D Pixel Matrix shows which pixels are lit within a given timeframe.

Another reason for choosing to segment the acquisition was the problem of noisy pixels. These are pixels that exceed the overall count rate at a statistically significant level.

Noisy pixels can randomly appear during an acquisition and can be identified as a sharp and permanent increase in the count rate (which is displayed by the Timeline tool). They can then be identified using the visualization aid of the 2D

Pixel Matrix tool as pixels permanently (or almost permanently) lit. This tool allows the user to disable (mask) noisy pixels after the acquisition has ended.

This procedure can be performed only after the acquisition has ended, so it is good practice to divide the total acquisition into shorter acquisitions, in order to have the chance to periodically identify and mask the noisy pixels.

Due to the high number of pixels of the detector ($512 \times 512 = 262144$) masking even a few hundred pixels is not going to significantly affect the results of the measurement.

The emergence of noisy pixels, on the other hand, gives rise to two main problems:

- The amount of data transferred to the PC for storage increases, possibly causing loss of events and considerable inflation of the output file (even up to ~ 100 GB).
- Introducing a bias in the measurement by introducing an erroneous energy measurement.

The data acquired were then elaborated using TrackLab. In order to do so, another pipeline was used Figure 3.2

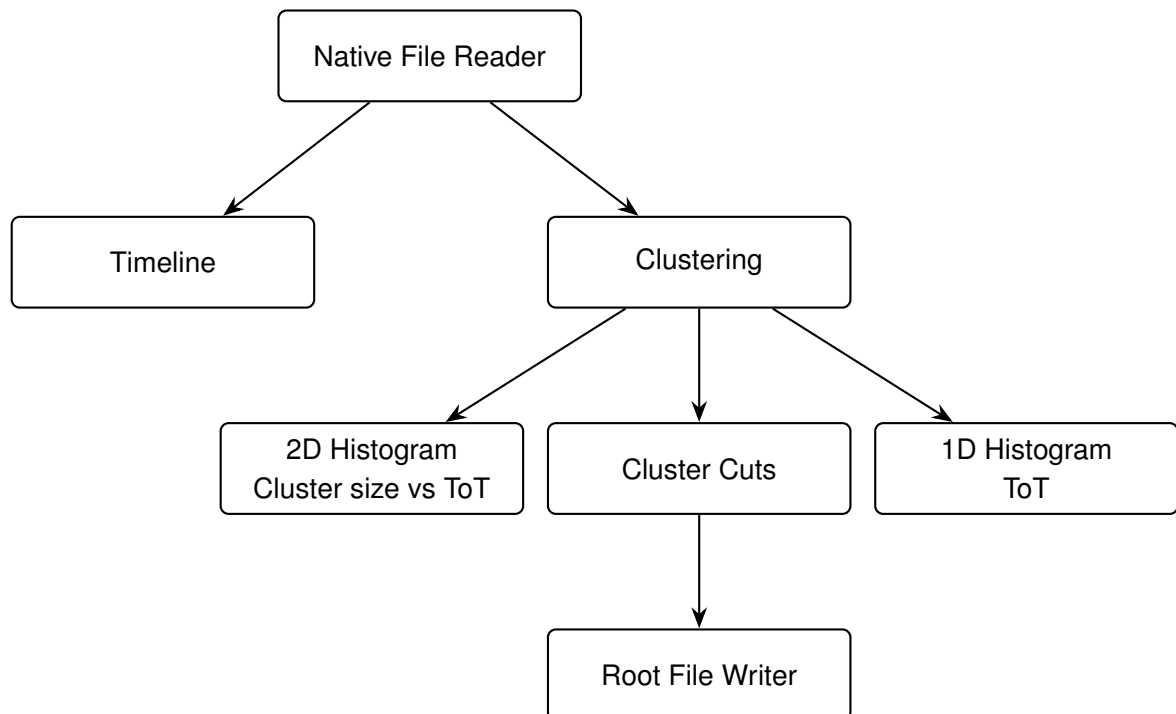


Figure 3.2: TrackLab data analysis pipeline.

The timeline was put in place to verify that the file was not corrupted (in case something was amiss it would show drops in the reading or stop advancing altogether).

Clustering is the tool that groups individual pixels hit into clusters. This procedure allows to work directly on the clusters and extract data from them.

Initially, it is necessary to check if it is possible to distinguish, albeit roughly, the particle types. By defining ToTv as the sum of all ToT values of the clusters and N as the cluster size, this was done by looking at the 2D histogram of Nvs ToTv (Figure 3.3a).

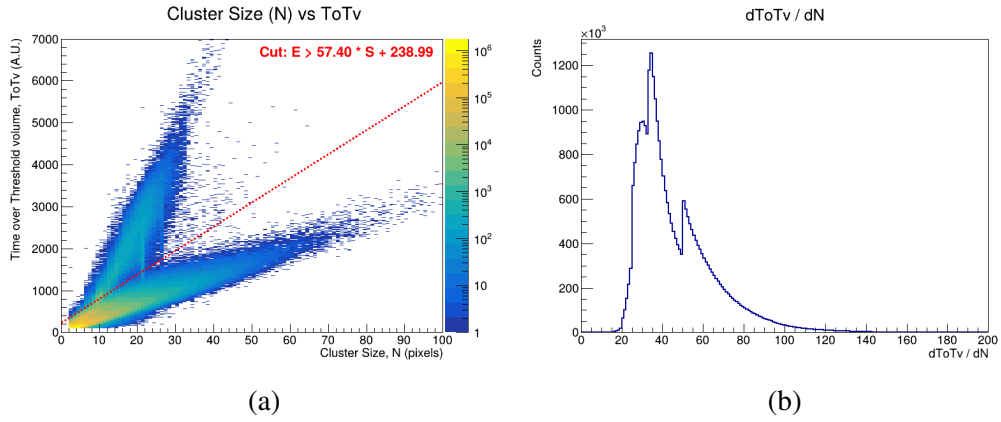


Figure 3.3: Relationship between Cluster Size (N) and ToTv used for particle identification (PID). The linear cut was defined by maximizing the separation margin between the photon/electron background and the high-LET particle distributions in the parameter space. (a) shows the bi-parametric histogram displaying the relationship between Cluster Size and ToT of the events registered on the TimePix3. The red line represents the energy relationship chosen to filter the interesting events from background. (b) shows the histogram of the ratio between ToT and Cluster Size of the events acquired.

The different populations that can be seen in the 2D histogram represent different particles types. This can be seen looking at the $d\text{ToTv} / dN$. This is a particle identification (PID) technique that can be employed when using pixel detectors. This technique is based on the different Stopping Power (dE/dx) of different particles. In this context ToT and E can be considered the same, since ToT can be calibrated into energy. On the other hand, N can be considered an approximation of path length (x), since the size of a pixel is constant.

In particular, referring to Figure 3.3, it is possible to recognize two main families of particles, one below the value of $d\text{ToTv} / dN$ of 50 and one above. The former can be identified to be the photons and electrons family, while the latter is the

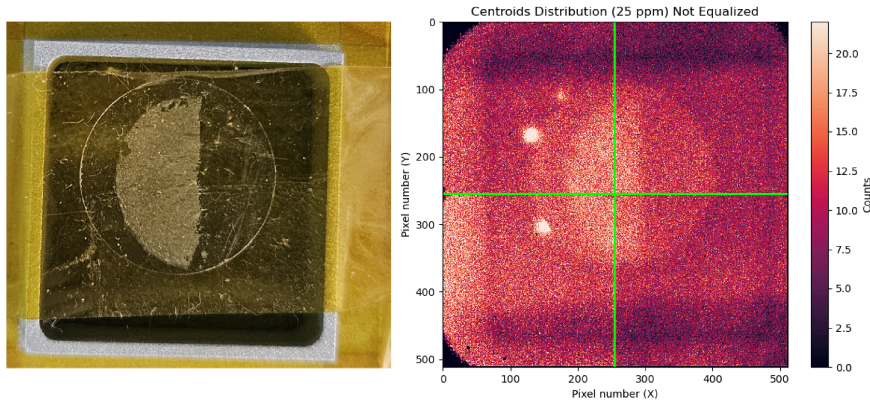


Figure 3.4: Comparison between the 25 ppm sample already positioned on the Kapton tape before the measure (left) and the image reconstructed using the QUAD TimePix3 (right). The green cross covers an artifact¹ that arises at the boundaries between the four chips of the QUAD TimePix3. The bright spots that can be observed in the reconstructed image are due to contamination that has been left on the Kapton tape by the tweezers used to handle the samples.

heavy charged particle family, such as protons from nitrogen and alpha particles and recoil Lithium nuclei from the BNCT reaction.

After having separated these families, it is possible to look at further ways to isolate the useful signal from the background. One such method is the morphological classification that was introduced in subsection 2.4.2.

Although this approach is theoretically optimal, it did not yield significant results during testing. One possible explanation is that in the PGNAA facility the flux is not high enough, so the number of events from ^{10}B is too low. This, coupled with the fact that the experiment has been carried out in air, thus attenuating the particles reaching the detector, has led to a very low count rate that makes it impossible to distinguish the interesting events from background.

An alternative to the built-in morphological classification is to rely on other morphological parameters, in particular the roundness of the cluster.

As the name suggests, roundness is a parameter that tells how *round* a cluster is. It ranges from 0 to 100% and it is calculated as the ratio between the major and minor axis of the ellipse that includes the cluster.

¹At the boundary between the four chips there is an effect that looks like the superposition of the signal between the different chips. For this reason this area cannot be considered during the analysis and it has been removed in order not to affect the quality of the image. Moreover having the "cross" as a reference helps to identify the quadrant of the detector on which the analysis is being carried out.

By selecting the roundness parameter between 60 and 100% and keeping the cut in energy as the one specified in Figure 3.3a, it was possible to visualize the sample over the background, as reported in Figure 3.4.

The plot that is used to reconstruct the image of the sample is the 2D histogram of the cluster centroid position. One of the images that has been reconstructed with this technique is shown in Figure 3.4.

In Figure 3.4 it is possible to notice inhomogeneities in the response between the four chips. In particular, the bottom-left one is giving a higher read than the others. In order to correct this, an equalization of the chips has been carried out. In the absence of a background acquisition without a sample, an acquisition featuring an empty Mylar disk, like the one used to hold the biological samples, was utilized. Since its image falls on all four chips, it has been possible to select a rectangle of the same size in all four to calculate the average and use it to normalize the measurement matrix M using the formula

$$M_{n,m}^{j,(norm)} = \frac{\min(\mu_i)}{\mu_j} \cdot M_{n,m}^j \quad (3.1)$$

where μ_i with $i \in [1, 4]$ is the average calculated in the four rectangles shown in Figure 3.5

The results are shown in Figure 3.6 and are shown to solve at least partially the inhomogeneities observed. The remaining inhomogeneities are more apparent on the border, due to the fact that the area chosen for the equalization were toward the center of the image. This does not affect the analysis, since areas used for the calibration were chosen at the center of the image where the sample is placed.

The data from these images has been analyzed visually by selecting a rectangle within which the counts for each pixel were added and then normalized by the area selected, such as shown in Figure 3.6

This led to the first calibration curve, shown in Figure 3.7.

The error for the number of counts was calculated as the standard error on the mean (SEM)

$$\sigma_{SEM} = \frac{\sigma_{std}}{\sqrt{N}} \quad (3.2)$$

where N is the number of pixels in the Region of Interest (ROI) and σ_{std} the measured standard deviation in the ROI.

It can be seen that the reduced chi-square (χ^2_v) of the fit is relatively high, one of the reasons being that the error appears to be underestimated. One explanation for this is the statistics, since the number of counts per pixel is rather low. This problem cannot be solved in the PGNA facility, since the neutron flux in this position cannot increase by much. Indeed the measurements were carried out at

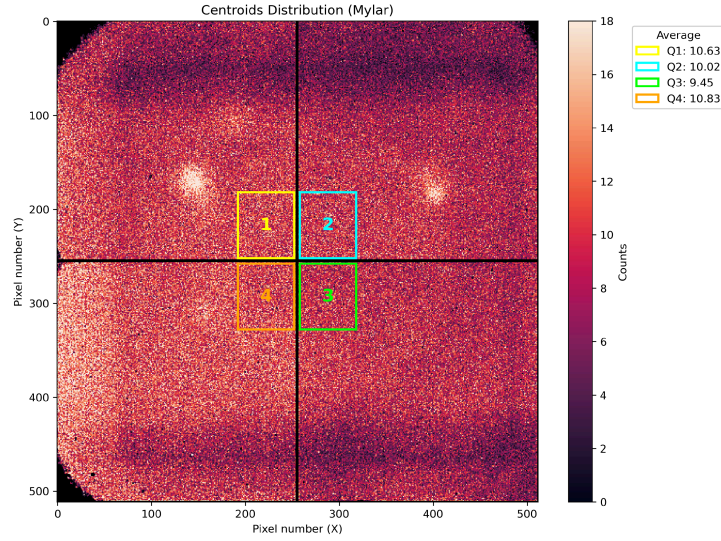


Figure 3.5: Centroids distribution of the Mylar only sample. The shape of the Mylar disk can be distinguished above the background. The four rectangles depicted are the ones within which the averages to carry out the equalization were calculated. The positioning of the four rectangles was chosen to fall completely on the Mylar disk and to be the same area on each quadrant. The borders of the rectangles were chosen so that they were 1 pixel away from the cross of the QUAD TimePix3.

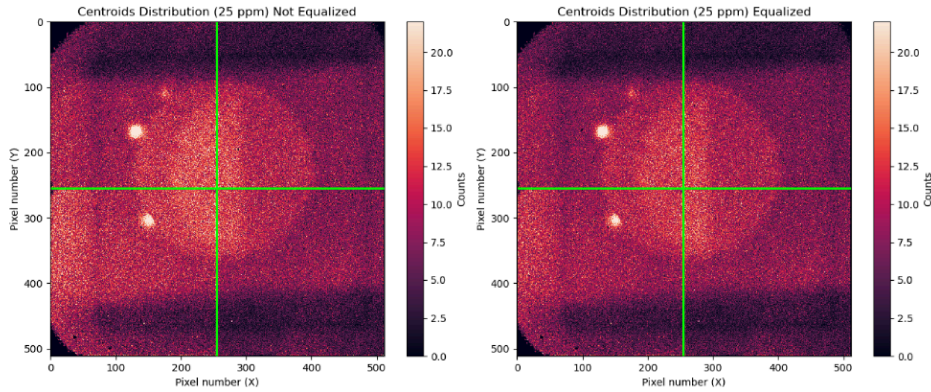


Figure 3.6: Comparison between the raw data acquired for the 25ppm sample and the newly equalized data. It can be seen that the inhomogeneities of the image have been partially corrected.

reactor power of 200 kW, and, although it can be increased until 250 kW, the neutron flux scales linearly with reactor power, meaning a gain factor of just 1.25.

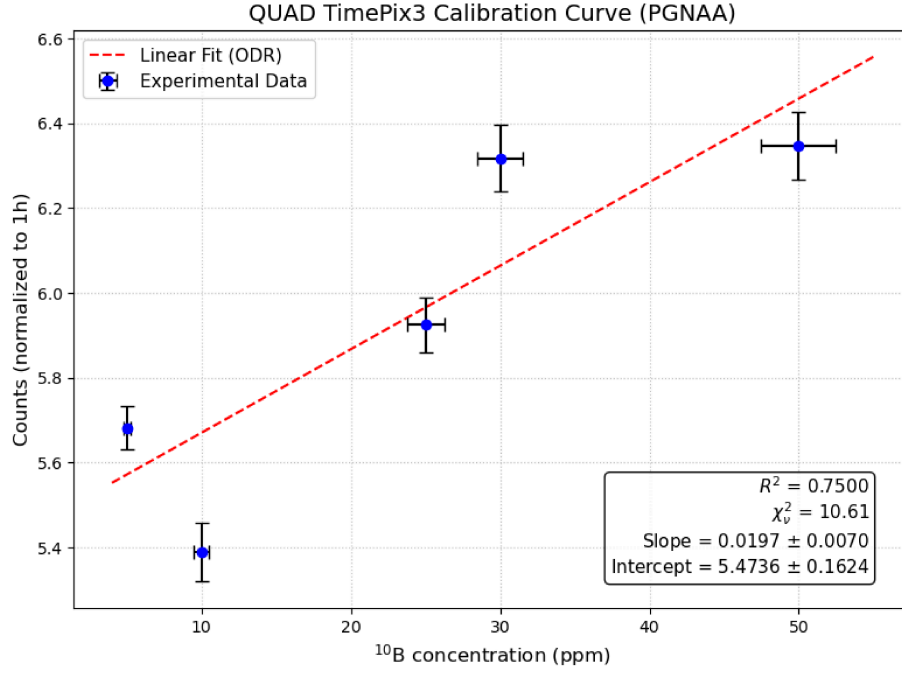


Figure 3.7: QUAD TimePix3 calibration curve acquired in the Prompt Neutron Gamma Activation Analysis (PGNAA) facility. The experimental counts, normalized to 1 hour of acquisition, are plotted as a function of the nominal ^{10}B concentration (ppm). A linear regression model (red dashed line) was applied to the experimental data (blue circles). The statistical metrics of the fit ($R^2 = 0.7500$, $\chi^2_v = 10.61$) indicate a significant deviation from the expected linear trend. This large discrepancy is primarily driven by the data points at low concentrations (5 and 10 ppm). The high χ^2_v value suggests the presence of unaccounted variance in the system, which may arise from uncharacterized systematic uncertainties, background fluctuations dominating the low signal-to-noise ratio regime. A conservative relative uncertainty of 5% was assigned to the nominal ^{10}B concentrations after further analysis described in section 3.3.

3.2 Second Calibration Campaign

In order to solve the low neutron flux problem, it has been decided to transfer the experiment inside the thermal column of the reactor to carry out a new calibration campaign.

This facility allows very high fluxes to be reached, up to $\phi_{tc} \sim 2 \times 10^9 \text{ n cm}^{-2}\text{s}^{-1}$, but there are several downsides.

- The neutron beam is not collimated

- There is higher ambient background, due to the higher neutron fluxes that activate the elements of the facility
- Due to the length of the available VHDCI connector, specifically tailored for the detector readout, the Katherine electronic module had to be placed outside of the actual irradiation room where the flux is maximum, but still inside of the modified thermal column. This readout module was not built to be exposed directly to the radiation field, and this could compromise its functionality. As a mitigating measure, a shielding was arranged around it using a cadmium sheet to reduce its activation.
- Every intervention on the detector, after the reactor had been turned off, required a 15 minutes cool-down time to let the short-lived activation to decay (in particular, the 15 minute rule had been set due to the characteristic decay time of aluminum, which is present in both the sample holder and the boral walls, a composite material made of two aluminum sheets that contain wall of boron carbide B_4C). This also complicated the sample substitution process and thus it was decided to try to fully use the QUAD configuration of the detector by placing at least two samples at the same time.

Without the optical breadboard to fix the detector in place, it was necessary to find a new solution to hold it. It was decided to modify a pre-existing Teflon support, to fix the detector on its side (Figure 3.8) in order to fit at the entrance side of the thermal column irradiation room. Due to the cable length problem previously mentioned, it was also necessary to put the detector at the far end of the irradiation facility

The TimePix3 cannot sustain the highest flux available in this position of the thermal column. In particular, it has a data transfer limit of 15 MHits/s. For this reason, the first measurement that was carried out was to put the detector in position and start irradiating with the reactor at low power. The power chosen was 125 W, the power at which the neutron flux in the thermal column is of the order of magnitude of the one used in the PGNA facility ($10^6 \text{ n cm}^{-2}\text{s}^{-1}$).

$$\phi = \frac{125 \text{ W}}{250 \text{ kW}} \cdot 2 \times 10^9 \text{ n cm}^{-2}\text{s}^{-1} = 10^6 \text{ n cm}^{-2}\text{s}^{-1}$$

From this value, the reactor power was gradually increased until 10 kW. At this power of the reactor, the flux at the detector position can be estimated to be $\sim 8 \times 10^7 \text{ n cm}^{-2}\text{s}^{-1}$, which is higher respect to the one that was estimated to be the theoretical limit (see subsection 2.2.2). The reason for this can be attributed to the fact that the neutron flux that was used to calculate the flux in the position of irradiation (ϕ_{tc}) was measured at a slightly different position and the detector assembly could perturbate the neutron field in the area. The data transfer reached



Figure 3.8: Position of the detector in the thermal column. In the image it is possible to see the detector, mounted on the Teflon support, at the edge of the thermal column irradiation room. It is also possible to see where the Katherine readout module was placed due to the cable length issue (the cadmium shielding was removed to take the picture).

by the TimePix3 at this position with the reactor power of 10 kW was around 4 MHits/s. This is below the maximum limit for the detector, but two facts had to be taken into account:

- **Safety margin:** in case a noisy pixel were to fire up, it would add to the data transfer, so in order not to risk hitting the transfer limit it is recommended to work at a lower hit rate
- **File size:** In order to have manageable files, it was necessary to split up the acquisition in 2 minute sessions. Nonetheless, the average output files after each session was ~ 30 GB. This was a constraint also because of loading issues. Indeed, in order to read a file of that size the PC needs to load it into the RAM and the actual limit encountered with the PC used was ~ 38 GB. It could have been possible to split the acquisition in even shorter sessions, but since each acquisition had to be started manually while also changing the file name, this was deemed to be the optimal solution.

With these measures in place, it was possible to start the data acquisition.

The samples were placed in front of the detector in the same way as it was done in the PGNA facility by gluing them to a piece of Kapton tape held by the aluminum support and put in front of the sensitive area of the detector. Because of the intrinsic positional uncertainty introduced by the bolted support system, it was not possible to exactly reproduce the sample-to-detector placement, but still it was kept at a distance of 1 ± 0.5 mm.

To acquire and analyze the data it was used the same TrackLab pipelines described in Figure 3.1 and Figure 3.2. The cluster cuts were changed with respect to the ones used in PGNA: to better handle the ROOT files in output from the data acquisition pipeline, a cluster cut that keeps the events with $\text{ToT} > 200$ and $1 < \text{Pixel Size} < 31$ was applied, since it was shown that no interesting events fall outside of these ranges. With this in place, the family of heavy charged particles was identified in Figure 3.9

The samples used and the acquisition times are summarized in Table 3.2.

Due to the raw amount of data, the analysis process that led to the development of the images could not be done while the measurements were being carried out. For this reason it was not noticed at first that there were some artifacts in the images. As it can be seen in Figure 3.10b one of the chips appear to light up more than the others and some stripes can be seen across the other chips.

Since this artifact shows across all the images of the samples that were measured in the thermal column, at first, it was supposed that some contamination could have been on the detector. For this reason it was also carried out a measurement with only the detector, without the sample holder, to see what would be the detector response (Figure 3.10d).

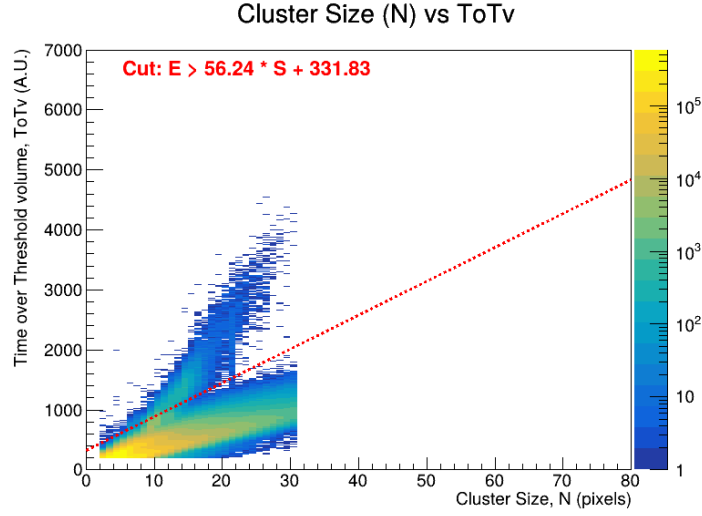


Figure 3.9: Bi-parametric histogram displaying the relationship between Cluster Size (N) and ToTv of the events registered on the TimePix3 during an acquisition in the thermal column. The red line represents the energy relationship chosen to filter the interesting events from background. The linear cut was defined by maximizing the separation margin between the photon/electron background and the high-LET particle distributions in the parameter space.

This irradiation was carried out at a reactor power of 5 kW, since it was expected that, without the sample holder acting as shielding, all the protons coming from the $^{14}\text{N}(n,p)^{14}\text{C}$ reaction would not be attenuated, thus, leading to a hit rate too high and an output file too large for processing.

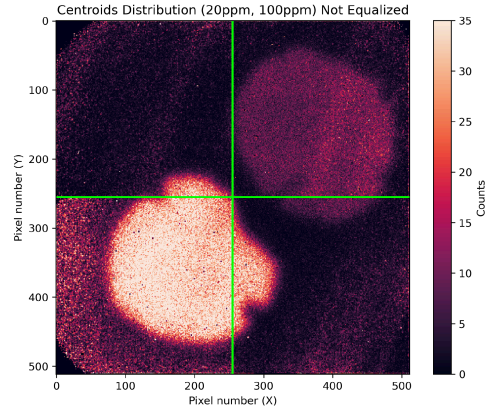
The irradiation was carried out for 30 minutes, and the results can be seen in Figure 3.10d. This image was obtained without applying any filters regarding the morphology of the clusters, just a filter on cluster size > 1 px in order to exclude noisy pixels and $\text{ToT} > 100$ in order to reduce a bit the dimension of the root file to be processed. This choice was taken in order to check that the detector response would be uniform when exposed in a uniform radiation field. However, the image show that the artifact is still present.

The explanation of this phenomenon is something different than what previously expected. There are two main effects:

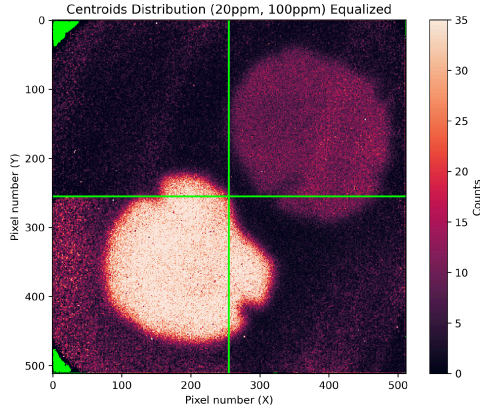
- The higher intensity near the borders can be explained as the effect of the PCB that holds the chips.
- The stripes are caused by inhomogeneities in the levels of Silicon doping and can appear when working with electric fields that are either low or null. The reason for their appearance in this case can be that the intense flux to



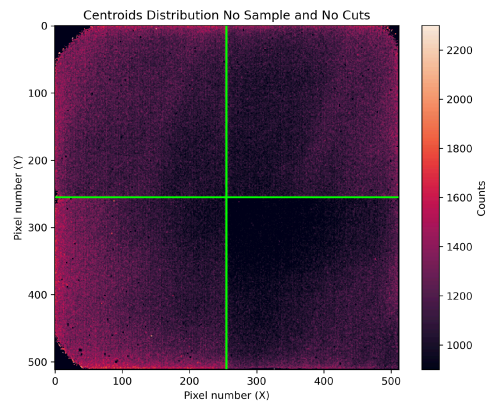
(a) Picture of the 20 ppm (top-right) and 100 ppm (bottom-left) samples positioned on the Kapton tape.



(b) Raw image obtained by applying the cuts obtained in the bi-parametric space of Figure 3.9 and plotting the centroids positions. It can be seen that the bottom-left chip is more lit than the rest. Moreover 'stripes' can be seen in the top-left and bottom-right chips.



(c) Image obtained by applying a correction pixel by pixel using the data obtained by irradiating the detector without sample holder.



(d) Image of the detector response by being irradiated without mounting the sample holder. The inhomogeneities that show correspond to the artifacts that can be observed in the images with the samples.

Figure 3.10: Comparison of the images obtained for 20 ppm and 100 ppm samples ((b) not equalized, (c) equalized) with the actual samples (a). (d) instead shows the image used to equalize the response of the detector pixel-by-pixel.

which the detector was subjected generated enough charge to distort and degrade the drift field, leading to these spatial non-uniformities over the chip's surface.

A possible solution to this phenomenon is to study the behavior of the detector exposed to comparable hit rates, for examples using a x-ray tube, and correcting the bias voltage as a consequence. Since however it was not possible to do this kind of test and repeat all the measurements, it was chosen to try to use the image obtained without the mask to correct the results. In particular, letting M be the pixel matrix of the images to correct, N the one of the mask-less image and μ the average of the elements of N , equation 3.3 can be applied:

$$M_{ij}^{(norm)} = \frac{\mu}{N_{ij}} \cdot M_{ij} \quad (3.3)$$

The result of this equalization can be seen in Figure 3.10c. Although it is not sufficient to remove completely the artifacts, mainly because many pixels that are not in the samples are actually zeros, it shows a net improvement, both in the image quality and the measurements of samples that fall on different chips.

After this equalization, the systematic analysis of the images of the irradiated samples was carried out. To do this it was used the software ImageJ, which allows for easier selection of the areas to be analyzed and faster measurement, especially in the presence of multiple samples in a single image.

ppm	Mean counts	StdDev	Acquisition time [min]	Area [px]	Chip
0 (ctrl)	2.7	1.8	90	625	2
5	7.0	3.1	90	625	4
10	9.9	3.4	90	625	1
20	11.4	4.0	90	625	3
25	16.0	4.7	90	625	1
30	19.1	5.4	90	625	4
50	12.0	4.2	60	625	4
70	31.6	6.6	90	625	3
100	38.5	7.7	90	625	3

Table 3.2: Average number of counts used for the calibration. The area has been chosen to be the same for each measurement in order not to introduce a bias. Due to the previously observed inhomogeneities between the chips, it has been kept track of the chip on which the measurement has been carried out. All the areas has been chosen to be on a single chip.

As previously done with the PGNA data the areas were selected in a way to

always be of the same size and in order to avoid inhomogeneities effects between chips, the area was selected to fall on a single chip, which was also recorded. The data are reported in Table 3.2 and it can be seen the calibration curve in Figure 3.11

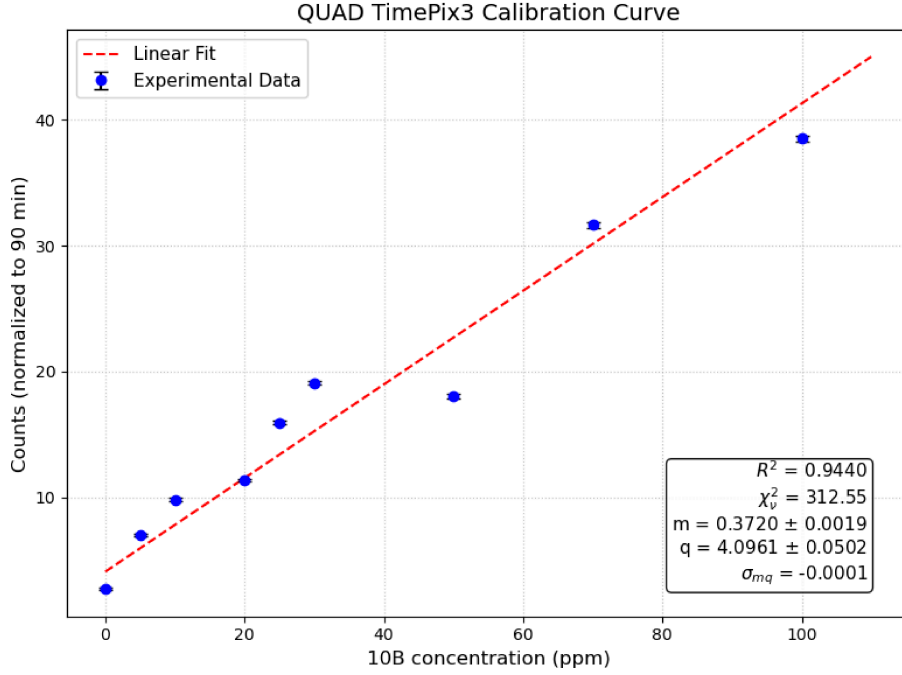


Figure 3.11: First calibration curve for the QUAD TimePix3 detector obtained in the thermal column. Experimental data (blue circles) show the counting data normalized to 90 minutes as a function of the nominal ^{10}B concentration (ppm). The linear fit (red dashed line) yields a high correlation coefficient ($R^2 = 0.9440$). The reduced chi-squared coefficient however is too high ($\chi^2_v = 312.55$), meaning that the errors have been underestimated

The error in Figure 3.11 has been calculated as the SEM using Equation 3.2. As is apparent, also looking at the reduced chi-squared test to check the goodness of fit, the error is underestimated. This can be explained by multiple reasons:

- The number of events on one pixel is not independent from one another: the readings of neighboring pixels are correlated, meaning the number of reconstructed events on a single pixel is not independent of its surroundings. This lack of independence is driven by transverse charge diffusion within the sensor (charge sharing), capacitive cross-talk between adjacent read-out channels, and the centroid reconstruction algorithm itself, which calculates

the center of gravity of an event by actively integrating the correlated ToT (Time-over-Threshold) signals from a cluster of adjacent pixels.

- The calibration samples that were used were considered without uncertainty. The reason for this is that they were prepared some years back with the methodology described in subsection 2.3.2. These samples were produced as calibration samples in the radiobiology laboratory of LENA, but do not constitute a standard for calibration. Moreover, the samples themselves could have degraded over time, meaning that the concentration of boron could be non-uniform and thus the choice of the area to analyze could have affected the reading.

The first can be solved by introducing an error that takes into account the standard deviation of the pixels in the sampled area.

The second required further investigation on the samples that were used for calibration. The technique that was chosen to carry out this check was Neutron Autoradiography.

3.3 Neutron Autoradiography

In order to assess the status of the samples it was chosen to perform a neutron autoradiography on the samples used. This technique is well-established for measuring ^{10}B concentrations and can therefore be used as a supporting method to attempt to interpret the non-ideal trend of the TimePix3 calibration.

In particular, it was chosen to perform a quantitative autoradiography on the samples that were actually used for calibration and a qualitative autoradiography on other samples that were available.

Quantitative autoradiography allows to link the number of tracks per unit of area left on the SSNTD (a piece of CR39) to the actual concentration of the sample.

On the other hand, qualitative autoradiography allows to check for spatial inhomogeneities of the sample, although it is not possible to measure the concentration of ^{10}B .

3.3.1 Methodology

The samples were placed on CR-39 detectors (SSNTDs) and placed in the thermal column for irradiation.

The two different techniques, qualitative and quantitative, require different irradiation times. The parameters are reported in Table 3.3

After irradiation, the samples were developed by putting the CR-39 in a thermostatic bath of NaOH. At the end of the etching process the CR-39 detectors were

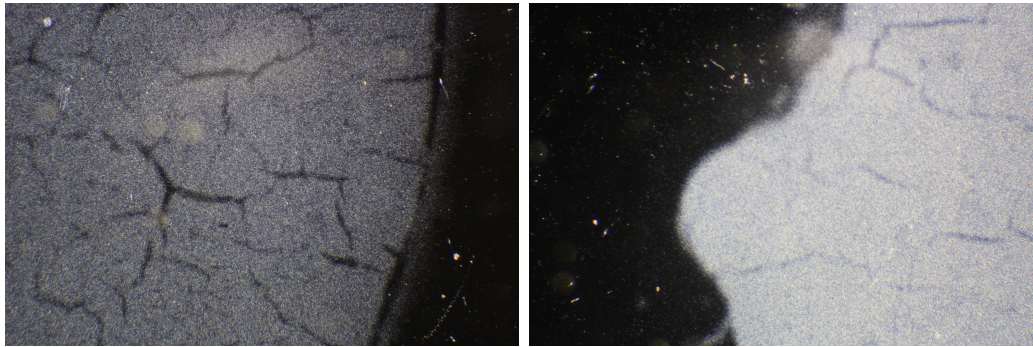
	Quantitative	Qualitative
Irradiation Time	30 minutes	2 hours
Reactor Power	5 kW	250 kW
Etching Time	45 minutes	15 minutes
Etching Solution	NaOH 6.25N at 70°C	

Table 3.3: Comparison of the parameters used for quantitative and qualitative neutron autoradiography. These parameters were previously optimized with the methods described in [1] [12].

thoughtfully rinsed with deionized water in order to remove the etchant from the detector surface and stop the etching process.

3.3.2 Data Analysis

The CR-39 were then analyzed using optical microscopes. Qualitative autoradiographies were analyzed using a stereoscopic microscope with grazing incidence illumination (Olympus IX83 equipped with 10X, 20X and 60X objectives and motorized Märzhäuser stage) of the Istituto Nazionale di Fisica Nucleare (INFN) section of Pavia located in the Physics Department of the University of Pavia. Some of the images obtained can be seen in Figure 3.12.



(a) Qualitative autoradiography of a sample with a concentration of boron of 10 ppm. (b) Qualitative autoradiography of a sample with a concentration of boron of 70 ppm.

Figure 3.12: Images obtained via qualitative autoradiography of selected samples. The analysis was performed using a stereoscopic microscope with grazing incidence illumination (horizontal to the tracks). This setup causes the light to scatter upon encountering the pits, making the tracks appear white against a dark background. Both samples show cracks across the surface.

The samples show cracks that can be attributed their conservation status. For this

reason it is necessary to take into account that the spatial distribution of boron could have been modified over time. This fact was also proven by the quantitative measurements.

Quantitative autoradiographies were analyzed using a Leica widefield microscope at the Optical Microscopy Laboratory, part of the Centro Grandi Strumenti (CGS) of the University of Pavia. For each sample, 15 regions of interest were randomly selected for image acquisition. The resulting proprietary .lif (Leica Image File) datasets were imported and analyzed using Fiji, an image processing package based on ImageJ, utilizing the Bio-Formats importer plugin. An automated micro-track counting algorithm was developed and applied, consisting of the following steps:

- **Channel splitting:** Separation of the multi-channel image into its constituent components (RGB), retaining only the channel that exhibited the optimal signal-to-noise ratio for micro-track identification (Red).
- **Grayscale conversion:** Conversion of the selected channel into an 8-bit grayscale format.
- **Background subtraction:** Correction of uneven illumination across the field of view by applying a rolling-ball background subtraction algorithm.
- **Image binarization:** Conversion of the grayscale image into a binary mask through the application of a fixed global intensity threshold (0 - 206).
- **Watershed segmentation:** Application of a watershed transform algorithm to physically separate and divide spatially adjacent or overlapping micro-tracks.
- **Morphological filtering and counting:** Quantitative particle analysis to count the tracks, applying explicit morphological filters based on spatial area (0 - 60) and roundness (0.6 - 1) to exclude artifacts and partial structures on the image edges.
- **Data averaging:** Calculation of the mean track count across the 15 independent acquisitions for each respective sample.

The microscope used for the analysis was not calibrated for quantitative neutron autoradiography, so it was not possible to measure the absolute concentration of ^{10}B , but it was only possible to check if there was correlation between the dataset obtained by the TimePix3 detector and the neutron autoradiography.

To do so, a Pearson correlation test was carried out between the data obtained using the TimePix3 and the neutron autoradiography. The results are reported in

Figure 3.13. These results show a strong correlation between the data obtained with TimePix3 and the qualitative neutron autoradiography with high statistical significance, which shows that the samples might have a different ^{10}B concentration than the nominal value that was reported.

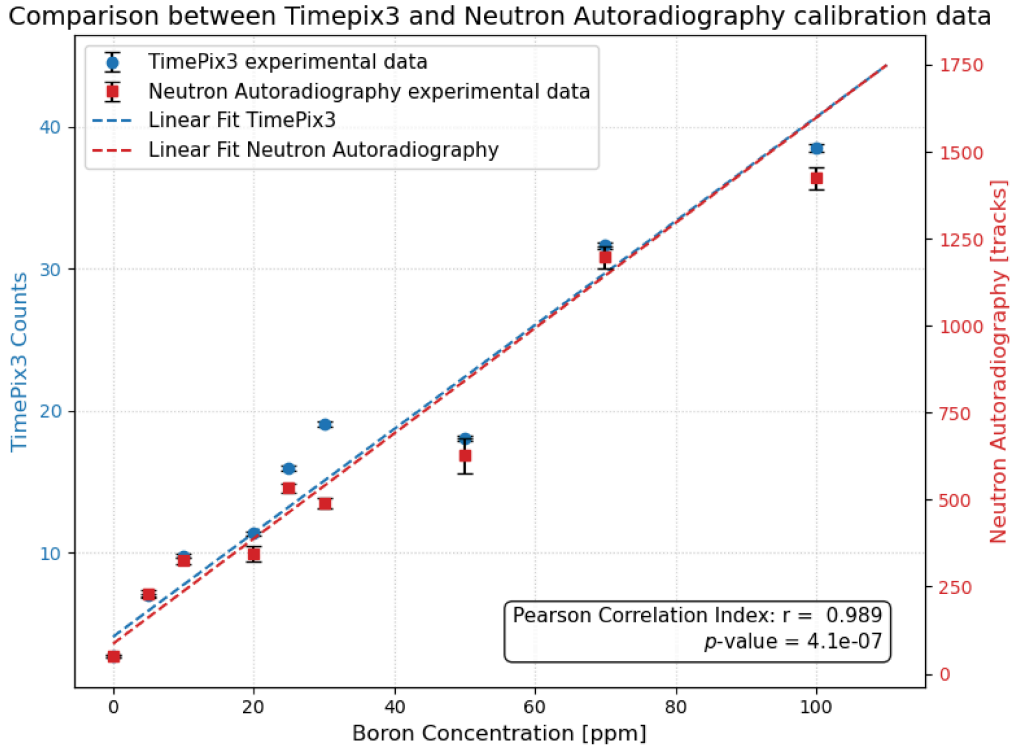


Figure 3.13: Comparison between the TimePix3 calibration data and the Quantitative Neutron Autoradiography data. The two datasets exhibit a very strong positive linear correlation (Pearson's $r = 0.989$) with a highly statistical significance ($p = 4.1 \times 10^{-7}$).

3.3.3 Error Analysis

Since it is not experimentally feasible to assess the exact concentration of ^{10}B in the standard samples, a conservative relative uncertainty of 5% was assigned to the nominal ^{10}B concentration.

Furthermore, the uncertainty on the detector counts was refined to account for systematic effects that intrinsically affect the measurements. These include:

- **Residual Spatial Non-Uniformity:** Although partially mitigated by the background matrix correction, spatial variations in the pixel response and

readout electronics persist, as evidenced by the residual stripe patterns in the images (Figure 3.10c).

- **Sample Inhomogeneity:** The samples may present morphological irregularities or local density variations due to structural aging.
- **Radiation Field Asymmetry:** The presence of the sample holder and the Teflon support may introduce slight gradients or anisotropies in the incident radiation field across the sample area.

These combined effects introduce an extra spatial variance, denoted as σ_{sys}^2 . This systematic contribution can be quantified as the deviation of the measured sample variance (σ_{std}^2) from the purely statistical Poisson variance expected for ideal counting statistics ($\sigma_p^2 = \mu$):

$$\sigma_{sys}^2 = \sigma_{std}^2 - \mu \quad (3.4)$$

The total experimental uncertainty on the mean counts (σ_c) is then obtained by adding the statistical and systematic variances in quadrature:

$$\sigma_c = \sqrt{\frac{\mu}{N} + \sigma_{sys}^2} \quad (3.5)$$

where N is the number of pixels within the chosen Region of Interest (ROI).

It is important to note that while the Poisson standard error ($\sqrt{\mu/N}$) decreases with the square root of the number of pixels (as it represents the error on the mean), the systematic spatial uncertainty (σ_{sys}) is an intrinsic property of the measured area and is therefore independent of N .

The validity of this model is confirmed by the significant improvement in the reduced chi-squared coefficient ($\chi_\nu^2 = 1.42$) of the calibration curve, as shown in Figure 3.14.

3.4 Intercomparison With Other Measurement Techniques

The calibration curve obtained in the LENA thermal column was then used to measure the concentration of boron of samples with unknown ^{10}B .

The samples used come from two different experiments:

- **TAORMINA project:** The TAORMINA project was a BNCT project funded by INFN and based on the collaboration between Pavia University Physics Department and the Experimental Surgical Department of San Matteo University Hospital in Pavia. The project aimed to prove for the first time

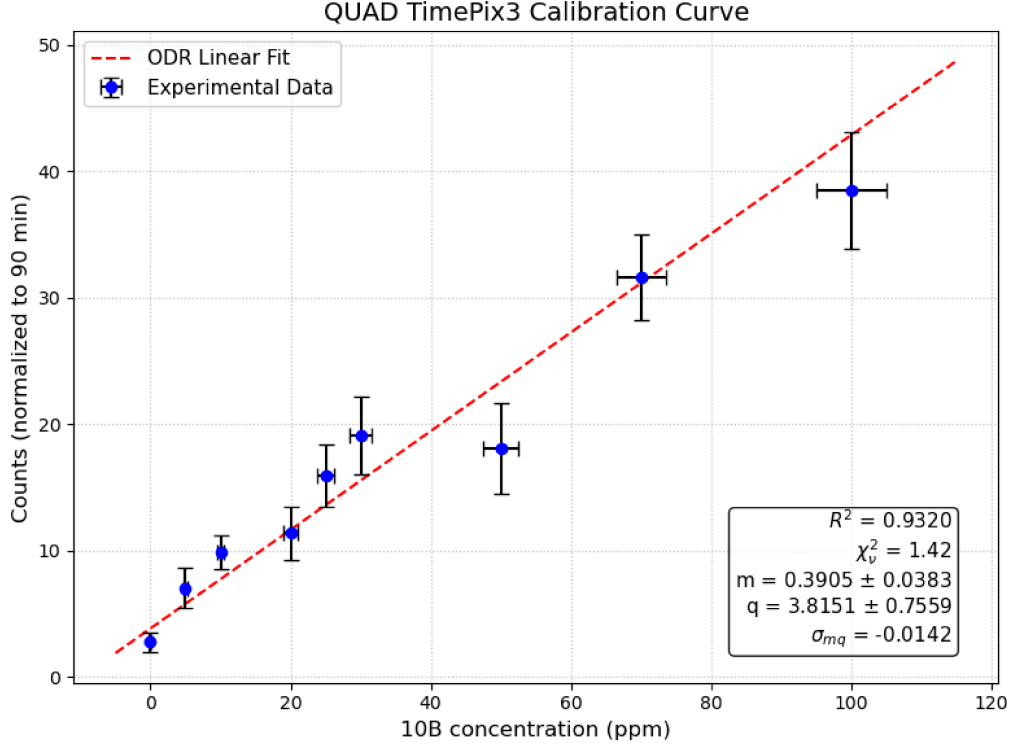


Figure 3.14: Calibration curve for the QUAD TimePix3 detector obtained in the thermal column. Experimental data (blue circles) show the counting data normalized to 90 minutes as a function of the nominal ^{10}B concentration (ppm). The linear fit (red dashed line) yields a high correlation coefficient ($R^2 = 0.9320$). The uncertainty calculated for the counts (y-axis) takes into account both the fact that it is an average calculation and systematic effect. Since specific uncertainties for the concentration values were unavailable, the error bars associated with the concentrations (x-axis) were conservatively estimated to be of the order of 5% of the nominal concentration to account for this lack of information. This methodological choice results in the observed reduced chi-squared value ($\chi^2_v = 1.42$). Specific fit parameters (slope m , intercept q) and their covariance (σ_{mq}) are detailed in the legend box.

worldwide the feasibility and effectiveness of BNCT performed on a living explanted organ affected by multiple cancer metastases. The designated pathology was liver metastases due to primary colon adenocarcinoma. The project indeed culminated with the irradiation of two patient's livers affected by multiple metastasis that were non-treatable with traditional techniques (surgery and radiotherapy) respectively in 2001 and 2003 [37]. Both liver irradiations were carried out at the modified thermal column of the LENA laboratory. At the time of the treatments, due to the multi-focal nature of the hepatic disease, several biopsies were taken for analysis just before the neutron irradiation to confirm the exposition duration; the analyses were carried out exploiting techniques such as neutron autoradiography and PANS. Thanks to the non destructive process associated with PANS, the thin tissue samples used at the time of the patient's treatments are still available. Two of these samples (one with tumor and one with normal tissue obtained from the first patient treated in 2001), were re-analyzed by the TimePix3.

- **PhD mobility at TAE Life Sciences:** The samples were produced during a PhD mobility program in the context of a collaboration between the US company TAE Life Sciences (TLS) [29] and the Physics Department of Pavia University. TLS is presently involved in the renovation program of CNAO foundation site in Pavia [6], in particular for the installation of a clinical BNCT facility exploiting an accelerator-based neutron source produced and installed by TLS at the CNAO center. The PhD project during which the mobility was performed is studying the combination of Carbon Ion Radiation Therapy and BNCT to evaluate on a preclinical level the possibility of treating radioresistant tumors (specifically, pancreatic cancer). The samples that were analyzed come from a head and neck cancer (cell line FaDu) implanted in immunodepressed mice. Once the tumor reached 347 mm³ the mice were treated with a solution of Ala-BPA (a molecule under investigation by TAE Life Sciences for BNCT applications [32]) of 900 mg/kg concentration for 2 hours. The mice were then sacrificed and the tumors extracted and frozen at -80°C. Part of these organs have been analyzed directly at TLS premises exploiting the ICP-OES technique. The second half has been shipped in dry ice to Italy, where the tumors were sectioned into contiguous 60 μ m slices. The sectioning was carried out using a Leica CM 1850 UV cryostat at the Istituto Mario Negri in Milan, which collaborates scientifically with the BNCT group hosting this thesis. These second samples have been analyzed in the present work by TimePix3.

The irradiations were carried out in the same environments and the same settings as the ones used for the calibration process described in the previous sessions.

Both group of samples were irradiated for a total of 90 minutes. The result can be seen in Figure 3.15.

From these images a series of areas were selected in which the number of counts per unit area was measured. With these measures, using the calibration curve reported in Figure 3.14, it was attempted a calculation of the boron concentration in these samples. Let B be the ^{10}B concentration and C the mean number of counts measured. The equations for the concentration and variance are the following:

$$B = \frac{C - q}{m} \quad (3.6)$$

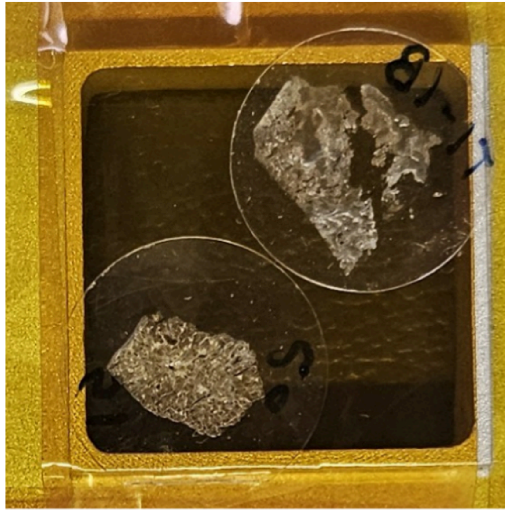
$$\sigma_B^2 = \frac{1}{m^2} [\sigma_C^2 + \sigma_q^2 + B^2 \sigma_m^2 + 2B\sigma_{mq}] \quad (3.7)$$

Region	Mean	StdDev	Area	Concentration [ppm]	Error [ppm]
1	8.3	3.2	625	11.41	4.21
2	8.8	3.4	625	12.87	4.52
3	10.3	3.6	625	16.50	4.77
4	4.3	2.5	625	1.13	4.04
5	5.2	2.7	625	3.57	4.31
6	4.5	2.5	625	1.81	3.95

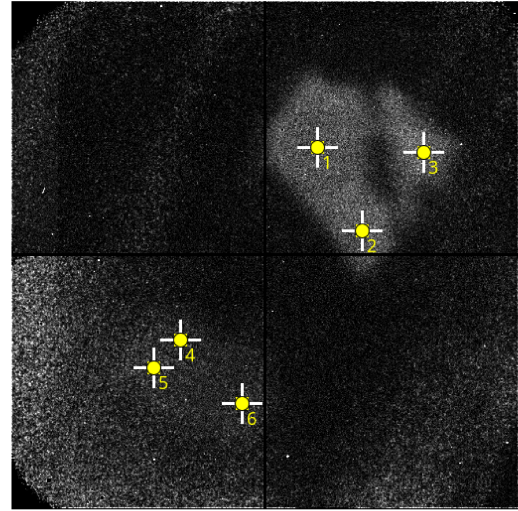
Table 3.4: Estimation of ^{10}B concentration for the TAORMINA project samples. The region number refers to the regions highlighted in Figure 3.15b. The Mean and StdDev were measured using ImageJ. Concentration and Error were extrapolated using the data from the calibration curve via Equation 3.6 and Equation 3.7.

Region	Mean	StdDev	Area	Concentration [ppm]	Error [ppm]
1	15.1	4.4	625	28.81	6.11
2	13.1	4.1	625	23.84	5.58
3	11.7	3.8	625	20.07	4.87
4	15.7	4.6	625	30.41	6.76
5	15.8	4.3	625	30.75	5.19
6	15.7	4.7	625	30.47	6.86
7	13.7	4.1	625	25.38	5.27

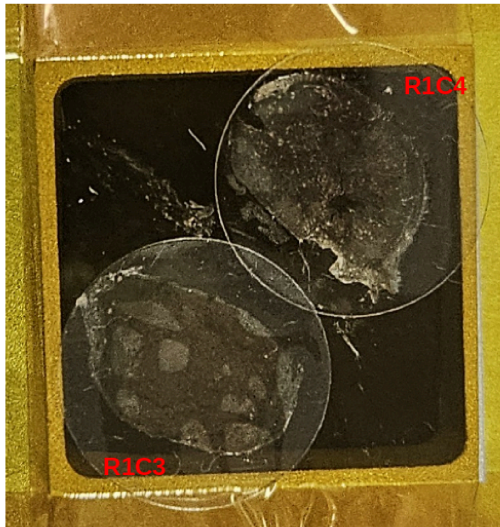
Table 3.5: Estimation of ^{10}B concentration for the TAE Life Sciences samples. The region number refers to the regions highlighted in Figure 3.15d. The Mean and StdDev were measured using ImageJ. Concentration and Error were extrapolated using the data from the calibration curve via Equation 3.6 and Equation 3.7.



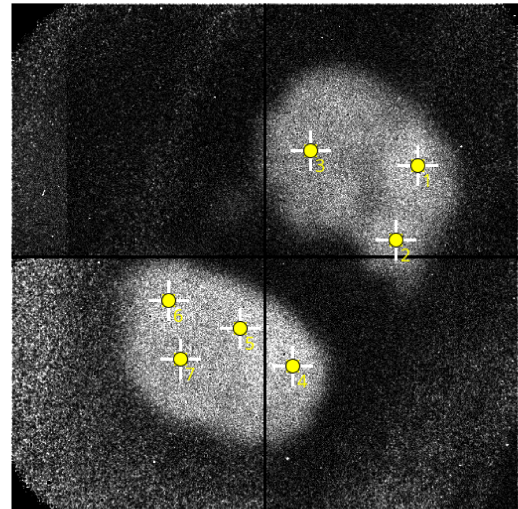
(a) Picture of the TAORMINA project samples. The top-right one is a tumour sample, while the bottom-left is sane tissue.



(b) Reconstructed image of the TAORMINA project samples using the software ImageJ. The yellow crosses represent the points selected where the ^{10}B concentration measurements were carried out exploiting the calibration curve reported in Figure 3.14.



(c) Picture of the samples developed during the PhD mobility at TAE Life Science.



(d) Reconstructed image of the TAE Life Sciences samples using the software ImageJ. The yellow crosses represent the points selected where the ^{10}B concentration measurements were carried out exploiting the calibration curve reported in Figure 3.14.

Figure 3.15: Comparison of the images obtained with the QUAD TimePix3 with a 90 minutes irradiation and the samples photographs. (a),(b) are the TAORMINA project samples images, while (c),(d) are the TAE Life Sciences samples images.

The concentration values determined for the TAE Life Sciences samples are consistent with the results obtained via ICP-OES on similarly prepared tissues. The ICP-OES average across four tumors was 40 ± 18 ppm. Although this value is higher than the overall TimePix3 measurements, several factors regarding sample preparation and variance must be considered. First, ICP-OES was performed on fresh samples, whereas the TimePix3 analysis utilized dried samples. This is a crucial distinction, as freezing and freeze-drying processes have been shown to alter the concentration and distribution of ^{10}B [36]. Second, the TimePix3 samples originated from a different biological specimen, and bulk ICP-OES measurements do not account for inter-animal variance. Finally, since ICP-OES lacks spatial resolution, the appropriate TimePix3 values for comparison are the spatial averages of each sample. These averaged concentrations correspond to 24.24 ± 3.20 ppm (for sample R1C4) and 29.25 ± 3.04 ppm (for sample R1C3). Considering the large uncertainties introduced by biological variability and sample processing, these results are in good agreement with the expected values within experimental uncertainties.

Regarding the TAORMINA project samples, the obtained results require further contextualization. The measured average concentrations are 13.59 ± 2.60 ppm for the tumor and 2.17 ± 2.37 ppm for the healthy tissue. These values show a substantial underestimation when compared to the historical PANS data, which established expected concentrations of 47 ± 2 ppm and 8 ± 1 ppm for tumor and healthy tissues, respectively [28]. A possible explanation for this signal loss lies in the sample preservation conditions. After having been freeze-dried and sectioned in 2001, the samples have been stored for 25 years without a controlled environment. Surface contamination, such as dust accumulation over time, along with potential biological degradation occurring over this timeframe, can form an attenuation layer for the short-range α and lithium particles before they reach the sensitive area of the detector. Although this attenuation mechanism is currently under investigation, the relative boron uptake information remains consistent: the tumor-to-healthy concentration ratio measured with the TimePix3 is 6.26, demonstrating a good agreement with the expected ratio of 5.88.

Chapter 4

Conclusions and Further Developments

In this thesis, the possibility to use a TimePix3 detector to measure the concentration and spatial distribution of ^{10}B in biological matrices at low concentrations has been investigated. The preliminary results obtained show the linearity of the response of the detector in biological samples with varying ^{10}B concentrations which compare well with the typical concentration values expected in BNCT clinics and experimental studies. The imaging capability of the detector has also been confirmed considering previous work [31], but it was not possible to actually verify the possibility to resolve inhomogeneities in the spatial distributions because none of the analyzed samples showed a significant heterogeneous spatial distribution of boron (which in addition would need to be confirmed by a histological analysis). In particular, the calibration that was carried out involved samples with concentrations ranging from 100 to 5 ppm. While a formal evaluation of the limit of detection (LOD) would require dedicated statistics, the data provide empirical evidence that the system is capable of distinguishing the 5 ppm sample from the control. This is remarkably significant given that the control sample is not devoid of boron, but naturally contains trace amounts (between 0.5 and 1 ppm) of endogenous boron. The obtained separation, even though of the order of 1σ , provides a confidence level that is highly viable for radiobiological applications, where the intrinsic variability of the organic matrix frequently overshadows purely instrumental uncertainties.

The performed calibration was tested against samples whose ^{10}B concentration had been measured previously using other techniques such as PANS and ICP-OES. The results of the test were promising, since the technique showed good agreement with the fresher samples analyzed with ICP-OES. On the other hand, the samples analyzed and coming from the TAORMINA project showed significantly lower boron concentrations than those measured with PANS. Presently,

further investigations are ongoing; nonetheless this result can be attributed at least partially to the conservation of the samples than to the technique itself. In conclusion, despite the promising results, it is necessary to further validate the QUAD TimePix3 technique with other samples that are presently being prepared and possibly through other intercomparison campaigns.

Finally, the possibility of working with neutron fluxes higher than previously attempted in this framework with this kind of detector was successfully demonstrated.

Despite being encouraging, these results were not achieved without technical difficulties. Specifically, it is necessary to improve three critical aspects of the experimental setup:

- **Detector positioning and noise reduction:** The transfer to the thermal column from the external channel in LENA laboratory initially exploited was dictated by technical constraints (low neutron flux that led to long acquisition times) and was not optimally prepared. In particular, it would be highly beneficial to design and implement a setup to collimate the neutron beam while simultaneously reducing the γ noise reaching the detector. This would enable operation at higher reactor power and simultaneously increase the S/N ratio.
- **Data handling:** The current data acquisition pipeline is severely inadequate to handle this high volume of data. It would be ideal to develop a dedicated software pipeline that allows for the application of online cuts on the acquired data to reduce the final dimension of the output files.
- **Sample preparation:** The samples available were not certified calibration standards, they are locally homemade and produced in order to calibrate another technique (i.e. neutron capture radiography [12]). Moreover, they were at least 13 years old and due to their storage condition it was not possible to guarantee that their state was not altered. Due to time restrictions, it was not possible to produce new samples during the work for this thesis, but the preparation of new calibration samples is underway.

Aside from these optimizations, it is possible to look forward to new developments in the TimePix family. Notably, the newly developed TimePix4 allows for a higher timing resolution (200 ps) and a higher data transfer rate of $3.6 \text{ Mhits mm}^{-2} \text{ s}^{-1}$ [21]. This detector is already manufactured as a 448×512 pixel matrix, eliminating the need for a quad configuration. Additionally, the new Through Silicon Vias (TSV) technology enables the detector to be tiled on four sides, providing greater flexibility in covering a larger area.

Another improvement currently underway is the implementation of a GEMPix configuration. In this setup, the TimePix ASIC is coupled with a GEM (Gas Electron Multiplier) chamber, that is a gas detector working in the proportional region [20]. Considering that the mean free path of charged particles increases in gas, it becomes easier to identify the tracks of charged products. Furthermore, by appropriately setting the GEM gain, the tracks from gammas and electrons/positrons drop below the threshold, and their contribution can be significantly reduced. At present, the latest GEMPix prototype features the TimePix4 as readout electronics, which will allow for 3D reconstruction of the tracks detected in the gas, thus the identification of the charged products.

For the BNCT application, the GEMPix detector would solve the problem of event losses and degradation due to the air present between the sample and the detector. Since it allows the sample to be positioned directly within the gas chamber, the charged particles produced by the boron neutron capture reaction could be detected as soon as they are generated.

The GEMPix detector with TimePix1 readout has already been tested in the N-TOF facility at CERN using a ^6Li target irradiated with neutrons produced by N-TOF. Despite the fact that the particles produced in this reaction, an α particle and a ^3H nucleus, are more energetic than those obtained by the capture reaction on ^{10}B , these results are promising for the application of this technology in BNCT. Finally, the possibility of performing the experiments under vacuum must also be discussed. This solution would enable the measurement of most of the charged particles produced by eliminating air moderation and absorption, but it introduces specific engineering challenges. First, it is necessary to design a dedicated vacuum chamber to house the detector and the sample, ensuring easy sample substitution and a reliable way to extract the cables. Second, the detector's capability to operate under vacuum must be tested. This would require designing a dedicated PCB that can withstand vacuum conditions: standard PCBs are typically made by pouring epoxy resin into a mold, which can trap microscopic air bubbles that would cause the board to crack under vacuum.

This solution would enable a different approach to concentration measurement. The current technique, counting the centroids per pixel, closely resembles the approach to ^{10}B concentration measurement characteristic of neutron autoradiography, which relies on counting the number of micro-tracks per unit area. By performing the measurement in a vacuum, it would be possible to attempt a *spectroscopy* approach, more akin to that of PANS, with the added advantage of a pixelated readout that enables the simultaneous detection of the spatial distribution of boron.

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