Advances in medical imaging: Will positron annihilation spectroscopy be the promise of preventive diagnostics?

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Abstract: Positron annihilation spectroscopy (PAS) offers a transformative approach to medical imaging, providing detailed insights into molecular structures. Although PAS has been extensively applied in studying defects in semiconductors and synthetic materials, yielding quantitative data on their microscopic properties its potential in medical imaging could significantly enhance diagnostic methodologies. The application of positrons and other forms of radiation in analyzing living tissues necessitates careful consideration of potential damage. In this work, a method designed to determine the optimal dose for experimental measurements is introduced. While Positron Emission Tomography (PET) has been instrumental in clinical diagnostics using radiopharmaceuticals to visualize metabolic processes, PAS presents a cutting-edge tool for improving the specificity and accuracy of biological imaging. Its capability to non-destructively explore structural transformations and micro-environmental changes in biological samples represents a promising innovation in diagnostics, paving the way for enhanced healthcare outcomes globally.

Introduction

The ongoing advancement of medical imaging technologies has played a crucial role in modern healthcare, revolutionizing the diagnosis and treatment of diseases. From the initial discovery of X-rays by Wilhelm Conrad Roentgen in 1895 to the modern imaging techniques available today, every advancement contributed to more accurate diagnoses and a more profound understanding of the anatomy and pathology of the human body. Amidst the large variety of medical imaging techniques, Magnetic Resonance Imaging (MRI) (Fig. 1a), Ultrasound, Endoscopy, Computed Tomography (CT) (Fig. 1b), Positron Emission Tomography (PET) (Fig. 1c) and coupled techniques such as PET-MRI and PET-CT [1] played a crucial role in the diagnosis of neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's, Huntington's, and amyotrophic lateral sclerosis [2, 3]. These techniques, also coupled for simultaneous imaging acquisitions, can increase their diagnostic throughput in oncology [4], for example, mapping of gliomas [5], lymphoma [6], and lung cancer [7]. MRI is an imaging method based on nuclear magnetic resonance, a phenomenon discovered in the mid-20th century in which nuclei in atoms absorb and emit electromagnetic radiation when placed in a magnetic field. This phenomenon is especially crucial for hydrogen nuclei (protons) in water molecules, which are abundant in biological tissues. When subjected to a strong magnetic field, these protons align with this one, then a radiofrequency pulse is applied and temporarily disrupts this alignment. As the protons return to their original orientation, they release energy, which is detected and transformed into images by the MRI system. In clinical practice, MRI is used to generate tomography of soft tissues in a mini-invasive way. In the neurological field MRI is the gold standard for detecting structural abnormalities in the brain, such as tumors, stroke, and degenerative diseases [8]. The identification of cortical and hippocampal atrophy, commonly associated with AD, serves as

a prominent diagnostic tool. However, this approach has limitations in terms of early detection, as brain atrophy is not considered a definitive early marker of AD. Additionally, it lacks specificity for distinguishing between different neurodegenerative diseases, as several conditions can exhibit similar patterns of tissue loss. Thus, while valuable for diagnosis, it does not effectively offer preventative insight or specificity for Alzheimer's disease [9]. Among nuclear imaging techniques, tau-PET and FDG-PET provide the two most compelling alternatives to MRI. They provide complementary biomarkers regarding brain functionality: FDG-PET makes use of a ^{18}F radiotracer that, bonded to specific molecules like deoxyglucose, may accumulate in presence of enhanced metabolic activities, whereas tau-PET uses flortaucipir (AV-1451) tracer to recognize regions with higher chemical retention, usually associated with a higher concentration of accumulated twisted-fibers of Tau protein [10]. Furthermore, Fluoromisonidazole (FMISO) offers the opportunity to evaluate hypoxia levels intratumorally by binding to metabolic proteins [11]. These radionuclides emit positrons through β^+ decay, a type of radioactive decay during which a proton transforms into a neutron, releasing a positron, a neutrino and energy. After losing most of their kinetics energy via a process called thermalization, positrons annihilate with matter electrons producing as a result 2 or 3 photons (γ-rays). These photons are then revealed by using a system of two or more detectors. A functional image is then reconstructed from the detection of 2 γ photons resulting from the same annihilation event. The images will have different signal levels depending on the tracer presence in different regions, indicating higher cellular activity, such as presence of tumor cells [12]. Despite PET capability of providing functional imaging, it currently cannot distinguish between the various ways positrons annihilate with electrons. Recent evidence highlights that 30% of positions inside a patient's body produce positronium Ps, a positron-electron bound state, which means that a possible functional signal is overlooked [13]. Ps, in particular ortho-Ps (o-Ps), has the potential to infer properties of materials, such as voids, pores, oxygen concentration [14]. A technique able to take advantage of this signal is Positron Annihilation Spectroscopy (PAS), which can be integrated into PET scanners [15]. PAS may represent a transformative approach in medical imaging, with its unique capability of providing detailed insights into molecular details. Even though PAS has been widely used for studying defects in metals alloys and semiconductors [16-18] and synthetic materials [19-21] providing quantitative data about the microscopic structure and properties of these substances, its potential application in medical imaging could mark a significant turning point, promising to revolutionize diagnostic methodologies. Moreover, PAS may offer a distinct advantage with respect to conventional PET, by providing quantitative data about molecular interactions and structural changes, rather than just the radiopharmaceuticals localization. The last two decades demonstrated the capability of PAS to provide precise data on the microscopic structure of investigated samples, by detecting the lifetime of annihilated positrons (i.e., Positron Annihilation Lifetime Spectroscopy - PALS) or the shift in energy or angular momentum of the produced gamma rays through Coincidence Doppler Broadening (CDB), or Angular Correlation of Annihilation Radiation (ACAR), respectively. These approaches attracted the scientific community's attention to their potential in medical applications and in our vision as a breakthrough for the early detection of mental disorders and cancer.

Advances in positron annihilation spectroscopy for medical imaging

PAS has been widely used for the inspection of mesoporous materials, metal alloys, semiconductors, and solar cells. These inspections allowed us to assess the material presence of vacancies, defects, and pores [22]; however, over the last 20 years, notable new studies have been made in the fields of biology, bioengineering, and nuclear medicine. Biological samples having a millimetric thickness or above derived from mediastinal adipose tissue [23], liver and muscles [24], and metastatic melanoma cells [25] were investigated by means of PAS with a ^{22}Na radioactive source between two samples ("sandwich configuration"), spontaneously emitting positrons in a broad energy spectrum. PALS has been employed for the examination of cancer cells in 3D culture [26], cardiac myxoma and adipose tissue [27], and melanoma spheroids [25]. These works demonstrated the presence of three different annihilation modes: short-lived (~125 ps) singlet state Ps called para-Ps

(p-Ps), the component related to the annihilation of e^+ in the sample (free e^+ component), and the "pick-off" annihilation between the long-lived triplet-state ortho-Ps (o-Ps) and an electron of the sample (~2 ns). Specifically, o-Ps has been proposed as a possible biomarker for the recognition of altered or pathological tissue conditions, like hypoxia (i.e., reduction of the oxygen level below a physiological condition) [14, 25] and it can be used to estimate the nanoscale porosity of the sample on the base of numerical modeling based on the Tau-Eldrup model [26].

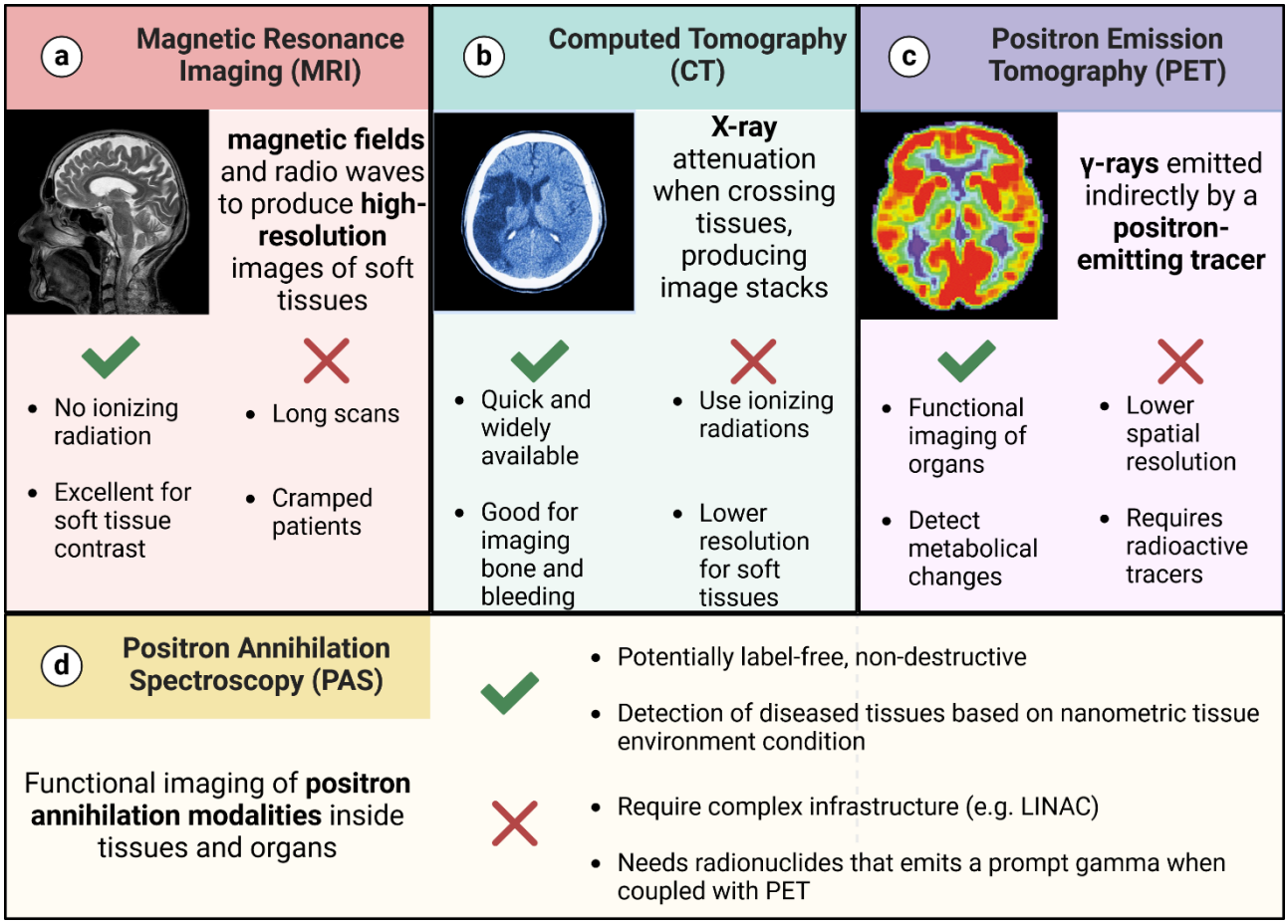

Figure 1: Overview of the principal medical imaging techniques currently used for diagnostic of neurodegenerative diseases and oncology. (a) Magnetic Resonance Imaging (MRI) leverages magnetic fields, currently it is the gold standard for brain diseases due to its high spatial resolution without the use of ionizing radiation. (b) Computed Tomography (CT) is a powerful and widely available anatomical imaging technique with strong capabilities in discriminating different densities of tissues. (c) Positron Emission Tomography (PET) is a functional imaging technique based on the detection of γ-rays emitted from the annihilation of positrons generated from radioactive tracers. A biodistribution image is formed with higher signal where the presence of the tracer is higher. (d) Positron Annihilation Spectroscopy is a technique capable of discriminating the different annihilation modes inside tissue and organs, potentially without any tracer involved.

However, since the number of studies regarding the different annihilation contributions in biological samples and tissues are still scarce, the mechanisms involved are yet to be fully understood. Recent literature highlights that 3D cell and organoid models effectively replicate in vivo conditions, making intravital imaging a crucial factor in the development of therapies [28]. In this regard, simplified in vitro cellular models, like organoids, spheroids, cell sheets, investigated by using monoenergetic positron beams may provide an advance in the field of particle physics and bioengineering. The energies reaching the samples would be in the range 1-20 keV, different from the broad spectrum typical of a ^{22}Na source (mean energy ~215 keV, end-point energy of ~545 keV), implying that implantation depth of positrons can be finely controlled at the nanometric and micrometric scale. By selecting different implantation energies, in-depth analysis of thin cellular substrates can be performed in a non-destructive way, reducing the contribution of the surroundings. In this regard, skin samples, derived from human biopsies were investigated with a positron beam,

registering different o-Ps lifetimes depending on implantation energy [29]. PAS has the potential to provide a label-free alternative to PET. While PET uses radiopharmaceuticals to create a functional image, PAS can distinguish annihilation modes of positrons without using them. Furthermore, by using an external positron source, like a positron beam, the ionizing radiation only affects the region of interest. On the other hand, integrating the technique with next-generation PET scanners could enhance their imaging capabilities. However, for lifetime studies to be feasible, the radioisotope annihilation must produce both a positron and a prompt gamma to provide a "start" signal. For this reason, only certain β+ emitters can be compatible. For instance, recently, the J-PET scanner, a modified PET system using plastic scintillators, captured the first in vivo positronium image of the brain. A patient affected by brain glioma was administered with a ^{68}Ga tracer. Then, o-Ps lifetime signals were registered by collecting around a hundred annihilation events, finally producing an image based on lifetime differences [30]. It is notable that differences in lifetime between healthy and pathological tissues were highlighted despite the reduced number of collected events due to the capability of ^{68}Ga in producing a prompt gamma in less than 2% of the occurred decays. More recently, o-Ps lifetime employed as a biomarker for oxygen concentration has been investigated by using a clinical brain-dedicated time-of-flight (TOF) PET equipped with novel silicon photomultipliers (SiPM). The samples were ^{22}Na–NaCl solutions prepared using distilled water with different oxygen partial pressure values (pO_2). The o-Ps lifetime measured varied by ~10 ps between the solution with pO_2 10 mmHg and the one with 40 pO_2 mmHg, which are representative values of oxygen concentration found in hypoxic and normoxic tissues [31]. While these differences may not fully represent a real biological sample, the findings indicate that oxygen plays a role in the variations in positron annihilation lifetimes. Additionally, the results suggest that PET with SiPM technology could be employed for PALS measurements in a clinical setting.

Feasibility studies of Dose Rate estimations

As described before, PAS uses radioactive substances emitting ionizing radiation (i.e. positrons) which may damage cells by their interaction with matter. Radiation induces the formation of free radicals and ions leading to lipid and DNA damages other than protein oxidation [32]. The widely used ^{22}Na positron source in bulk-PAS experiments emits particles in a wide energy spectrum between 0-542 keV, whereas monoenergetic positron beams work within a range of 1-20 keV, which means that different approaches for dose rate calculations are needed for the future application of the technique to patients. Currently, the published literature highlights limited research on the use of positrons in biological matter with positron beams, making it necessary to conduct a thorough evaluation of the impact these particles may have in PAS. Potential damage to a patient could lead to adverse effects or alter the acquired signal, thereby influencing the results. To address this, we outlined a framework for dosimetry calculations on biological samples in positron beam studies. This analysis requires consideration of the following factors:

A) Stopping power

The stopping power describes the loss of mean particle kinetic energy per distance traveled when passing through matter. Bethe and Bloch proposed the stopping power ($S = -\frac{dE}{dx}$) definition accounting both for relativistic and quantum effects, reaching the following result [33]:

$$\left(-\frac{dE}{\rho dx}\right)_{ion} = 4\pi r_e^2 m_e c^2 N_A \frac{Z}{A}\frac{z^2}{\beta^2}\left[\ln\left(\frac{2m_e c^2\beta^2\gamma^2}{<I>}\right) - \beta^2 - \frac{\delta(\gamma)}{2}\right]. \quad (1)$$

Which holds also for light particles except for electrons, where a further contribution coming from indistinguishability should be included. Here m_e is the electron or positron mass, r_e is the classical electron radius, ρ is the mass density, N_A is the Avogadro number, c is the speed of light, Z is the atomic number of absorber, z is the charge number of incident particle, A is the mass number, β is

the relativistic relative velocity, $<I>$ is the average ionization potential and γ is the relativistic Lorentz factor. In this relationship, the last term, δ(γ), accounts for the so called "density effect" due to a screening effect by the polarization of the medium against the electric field created by the incident particle, which spreads over greater distances at increasing particle's kinetic energy. Due to their small mass, positrons tend to be accelerated in the electric field of nuclei (much more massive), causing the phenomenon of Bremsstrahlung (braking radiation): so, a further contribution to energy loss must be added:

$$\left(-\frac{dE}{\rho dx}\right)_{rad} \cong \frac{E}{X_0} = 4\alpha r_e^2 \frac{N_A Z^2}{A} \ln\left(183 Z^{-\frac{1}{3}}\right) E. \quad (2)$$

Where α is the fine-structure constant (~1/137) and X_0 a given penetration depth. Eq. 2 becomes important for positron energies above 1 MeV, which is outside the energy range used in variable energy positron beam experiments (1-20 keV).
To account for annihilation radiation, the Bethe-Bloch formula is modified, with specific terms provided by Berger and Saltzer [34].

$$\left(-\frac{dE}{\rho dx}\right)_{ion} = 2\pi r_e^2 m_e c^2 N_A \frac{Z}{A}\frac{1}{\beta^2}\left[\ln\frac{\tau^2(\tau+2)}{2\left(\frac{<I>}{m_e c^2}\right)^2} + F(\tau) - \delta(\gamma)\right]. \quad (3)$$

Where

$$F(\tau) = 2\ln 2 - \frac{\beta^2}{12}\left[23 + \frac{14}{\tau+2} + \frac{10}{(\tau+2)^2} + \frac{4}{(\tau+2)^3}\right]. \quad (4a)$$

$$\tau = \frac{E}{m_e c^2}. \quad (4b)$$

$$\beta = \frac{(\tau(\tau+2))^{\frac{1}{2}}}{\tau+1}. \quad (4c)$$

Where τ is the relative positron energy as a function of the rest energy $m_e c^2$ (see Eq. 4b). This relationship has been employed by Gumus et al. for the computation of the stopping powers of positrons with biological nitrogenous based compounds and water [35]. Moreover, when energies comparable with that of the positrons are involved, atomic electrons are emitted at high kinetic energies, moving far away from the ionization site. The result of the stopping power in the scheme of Byakov & Stepanov [36], based on a numerical calculation derived from published analytical models, is in good agreement with Monte Carlo simulations with energies higher than 150 eV based on simulations made with the software "PENELOPE" [37].

B) Parameters of the material

For the definition of the sample, it is important to consider the geometrical parameters, for example the mean cell size of diameter (d≅10 μm) and a tissue replicate with dimensions comparable to the beam spot for example 2.3×2.5 mm^2, meaning 8×10^4 cells on beam area. Cells can be considered for this estimation with the same density as water ($\rho \cong 1$ g/cm^3).

C) Characteristics of positron source

It is necessary to know the performance of the positron beam in terms of beam spot (Full Width at Half Maximum - FWHM) and positron flux. In a realistic scenario, FWHM could be around 2 mm and the positron rate (*dN/dt*) proposed of the order of 10^3 positrons/s.

D) Evaluation of the dose

Starting from the stopping power as described above, we can now evaluate the dose and the dose rate for a given example. The dose is defined as the energy absorbed per unit mass:

$$D = \frac{d\varepsilon}{dm} = -\frac{dEdN}{\rho dx da}. \quad (7)$$

where *da* is the surface area of the sample, *dx* the depth, and ρ the mass density, considering *-dE* the energy released by a positron. The dose rate is then defined as the energy absorbed per unit mass and per unit time.

$$\dot{D} = \frac{dD}{dt} = \left(-\frac{1}{\rho}\frac{dE}{dx}\right)\Phi = \Phi\, S/\rho\,. \quad (8)$$

Where S/ρ is the mass stopping power and Φ is the flux or positron rate by unit area. From the presented parameters, a first estimation of the dose rate impinging on the sample is represented in Fig. 2a. In view of the measurement to be performed with our experimental apparatus, which means a positron energy around 10-20 keV, the dose rate runs between 7×10^{-5} and 10^{-4} Gy/s. Since a reasonable spectrum should contain around 10^6 counts, corresponding to 10^3 s with a realistic positron flux, and accounting also for the acceptance of the detectors covering approximatively 1/3 of the solid angle, we need an hour to collect enough events: multiplying this time by the dose rate we get ~0.3 Gy. Survival curves, which represent the fraction of survived cells as a function of dose (Fig. 2b), demonstrate that for low linear energy transfer -LET- radiation (γ-rays, X-rays, electrons and positrons), we still are in a "safe" region [38]. Therefore, Fig. 2b shows in fact survival as a function of dose and for low LET radiation we can have ~100% survival rate. This is pivotal to obtaining reliable data of living biological samples analyzed with positron beams as recently proposed [39. 40].

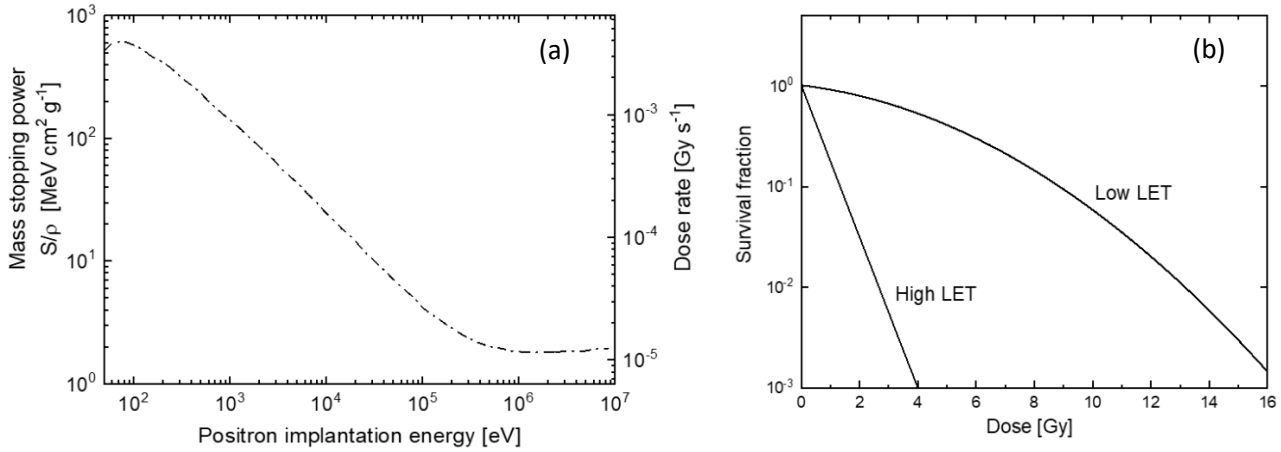

Figure 2: Qualitative representation of the (a) mass stopping power and dose rate evaluated as a function of the positron implantation energy based on the above presented parameters, see Eq.1. Qualitative representation of the (b) fraction of surviving cells as a function of dose for low LET (γ-rays, X-rays, electrons and positrons) and high LET (heavy particles, such as protons and others) -see e.g. Ref. [38]-.

Advantages, prospects and challenges of PAS in biology, medical imaging and bioengineering

As explained in previous paragraphs, PAS can offer a significant advantage with respect to PET regarding functional imaging, increasing the interest of translating the technique to clinics. While

the latter can result in a different annihilation signal caused by the tracer biodistribution in pathological regions with respect to health one, PAS can discriminate between different annihilation modes, offering a broader picture of the nanoscale and microscale environment. This can open to the detection of the Ps annihilation modes, which can be used to probe voids, different O_2 content and free radicals present in the tissues [29-31] which may be correlated to the presence of a cancerous environment [41]. Furthermore, PET requires the administration of a radiotracer to the patient that needs to be cleared: a radioactive nuclide with suitable decay time for minimizing the dose absorbed by the patient (^{18}F has a half time of 109 minutes) and a suitable molecule with the ability to have more retention in organs or tissues of interest with selectivity. On the other hand, PAS can be developed to be performed label-free as there is no need to have an associated molecule to induce bioaccumulation, since the signal registered depends on time or photon energy instead of position and tracer concentration in a specific body region. On the other hand, to perform PALS in a clinical scenario, where the use of particle accelerator requires specific patient needs, a "start" signal is mandatory. Moreover, if we consider the most employed radiotracer: ^{18}F-FDG, this is a pure β^+ emitter without the production of a prompt gamma, required for lifetime signal reconstruction. Alternative radionuclides should be investigated such as ^{44}Sc, ^{68}Ga, ^{86}Y [42]. In this scenario, it is possible to rule out the challenge related to the lack of high efficiency prompt gamma emitters with tracers in clinical settings by performing PALS and CDB, by using a variable energy positron beam. For its implementation in diagnostics, further investigations are needed to understand the effects of low energy (1-20 keV) positrons to tissue samples to have a proper calibration between the duration of the measures and the deposited dose. One of the limitations of this approach is the implantation depth of positrons: they cannot probe thick portions of the body limiting its potential application. Currently, there are more than 11000 medical facilities with the ability to perform radiotherapy [43] which means that PAS can potentially be performed in these centers, adding a novel diagnostic tool to hospitals. Moreover, PET became a standard medical imaging practice for oncology and the technology is widespread, meaning that there may be a strong interest in developing new methods for the integration of PAS with current PET scanners.

Conclusion

Positron Annihilation Spectroscopy (PAS) has the potential to revolutionize medical imaging by integrating positron annihilation information into clinical practice, particularly for neurodegenerative diseases and cancer. By analyzing positron lifetime and annihilation momentum, PAS can provide critical insights into the nanoscale structure of tissues, including aspects such as free volume, the presence of free radicals, reactive oxygen species (ROS), and hypoxic conditions. This technology could serve as a powerful tool for the early detection of various pathological conditions.

However, implementing PAS in clinical settings presents significant technological challenges. Advancements in PET detection systems equipped with PAS capabilities, as well as the development of alternative tracers, are essential for effective hospital use. Additionally, a deeper understanding of positron annihilation phenomena in both healthy and pathological biological samples is crucial for accurate result interpretation. This underscores the need for further research, including new experiments and models, to elucidate the interactions between antimatter and biological systems.

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