NUKLEONIKA 2015;60(4):749–753
doi: 10.1515/nuka-2015-0135

ORIGINAL PAPER

DE GRUYTER
OPEN

Studies of unicellular microorganisms *Saccharomyces cerevisiae* by means of positron annihilation lifetime spectroscopy

Ewelina Kubicz, Bożena Jasińska, Bożena Zgardzińska, Tomasz Bednarski, Piotr Białas, Eryk Czerwiński, Aleksander Gajos, Marek Gorgol, Daria Kamińska, Łukasz Kapłon, Andrzej Kochanowski, Grzegorz Korcyl, Paweł Kowalski, Tomasz Kozik, Wojciech Krzemień, Szymon Niedźwiecki, Marek Pałka, Lech Raczyński, Zenon Rajfur, Zbigniew Rudy, Oleksandr Rundel, Neha Gupta Sharma, Michał Silarski, Artur Słomski, Adam Strzelecki, Anna Wieczorek, Wojciech Wiślicki, Marcin Zieliński, Paweł Moskal

Abstract. Results of positron annihilation lifetime spectroscopy (PALS) and microscopic studies on simple microorganisms, brewing yeasts, are presented. Lifetime of *ortho*-positronium (*o*-Ps) were found to change from 2.4 to 2.9 ns (longer-lived component) for lyophilized and aqueous yeasts, respectively. Also hygroscopicity of yeasts in time was examined, allowing to check how water – the main component of the cell – affects PALS parameters, thus lifetime of *o*-Ps were found to change from 1.2 to 1.4 ns (shorter-lived component) for the dried yeasts. The time sufficient to hydrate the cells was found below 10 hours. In the presence of liquid water, an indication of reorganization of yeast in the molecular scale was observed. Microscopic images of the lyophilized, dried, and wet yeasts with best possible resolution were obtained using inverted microscopy (IM) and environmental scanning electron microscopy (ESEM) methods. As a result, visible changes to the surface of the cell membrane were observed in ESEM images.

Key words: environmental scanning electron microscopy (ESEM) • free volume • inverted microscopy • positron annihilation • positronium • yeasts

E. Kubicz✉, T. Bednarski, P. Białas, E. Czerwiński, A. Gajos, D. Kamińska, G. Korcyl, T. Kozik, P. Moskal, Sz. Niedźwiecki, M. Pałka, Z. Rajfur, Z. Rudy, O. Rundel, N. G. Sharma, M. Silarski, A. Słomski, A. Strzelecki, M. Zieliński
Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University,
11 S. Łojasiewicza Str., 30-348 Kraków, Poland,
E-mail: ewelina.kubicz@uj.edu.pl

Ł. Kapłon, A. Wieczorek
Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University,
11 S. Łojasiewicza Str., 30-348 Kraków, Poland
and Institute of Metallurgy and Materials Science PAS,
25 W. Reymonta Str., 30-059 Kraków, Poland

B. Jasińska, B. Zgardzińska, M. Gorgol
Department of Nuclear Methods, Institute of Physics,
Maria Curie-Sklodowska University,
1 M. Curie-Sklodowskiej Sq., 20-031 Lublin, Poland

A. Kochanowski
Faculty of Chemistry, Department of Chemical Technology,
Jagiellonian University,
3 R. Ingardena Str., 30-059 Kraków, Poland

P. Kowalski, L. Raczyński, W. Wiślicki
Świerk Computing Centre,
National Centre for Nuclear Research,
7 Andrzeja Soltana Str., 05-400 Otwock/Świerk, Poland

W. Krzemień
High Energy Physics Division,
National Centre for Nuclear Research,
7 Andrzeja Soltana Str., 05-400 Otwock/Świerk, Poland

Received: 1 July 2015, Accepted: 30 August 2015

Introduction

Positron and its bound state positronium are applied since a few decades as a tool in material investigations. Especially, positron annihilation lifetime spectroscopy (PALS) is widely used, as it gives a possibility to correlate the mean *o*-Ps lifetime value with cavity size in which annihilation undergoes. The relationship called Tao-Eldrup model [1, 2], describing *o*-Ps pick-off annihilation, assumes free space as a spherical potential well in which positronium is trapped. It allows to find the dependence of the mean *o*-Ps lifetime value in the trap and its radius. Some modifications concerning other shapes and larger sizes [3–7] widespread the application area for new classes of media such as porous materials containing elongated pores, sometime opened to the surface.

PALS allows to study many properties of investigated materials such as the presence of defects, thermal expansion, temperature of phase transition in polymers, processes of gases, or steams sorption in pores. However, it was applied in a very limited number of papers concerning living biological material [8, 9]. Some applications in biological system were described by Jean and Ache in 1977 [10] and were further studied in Jean's group. They focused on the studies of healthy and abnormal skin samples [11, 12] and reported that S parameter from Doppler broadening (DB) technique is correlated with broadly defined level of skin damage. Over past few years biological systems appeared in interest

of annihilation techniques again [13]. The precise investigations seem to be very complex because of the presence of water in which positronium also undergoes annihilation. However, hydrated solid materials were studied using PALS [14] successfully. In the paper by Hugenschmidt *et al*. [15], some experiments concerning behavior of the free volume on H_2O loading and uniaxial pressure on glucose-gelatin compounds were performed.

In this study, we have performed a test experiment showing the possibility to observe dynamics of the water sorption by hydrophilic material – lyophilized yeast cells.

Yeasts are eukaryotic microorganisms, a spherical unicellular microscopic fungus. They are living microorganisms in both the presence or absence of oxygen in the environment. For presented studies, *Saccharomyces cerevisiae* were used. These are most commonly used for brewing, as they ferment by converting carbohydrates to carbon dioxide and alcohols. The diameter of a single yeast cell is on an average 4–6 μm. These species of yeasts is also commonly used as a model organism in cell biology research [16, 17].

Experimental

Sample preparation

Lyophilized *S. cerevisiae* cells used in these measurements are a commercial product Instaferm Instant Yeasts manufactured by Lallemand Iberia, SA, used in the distilling industry. Product 'Instaferm' has the form of fine granules, color beige with size of about 1.5 × 0.5 mm with less than 7% of liquids and 1 g of lyophilized yeasts containing minimum 3×10^9 live cells. In 10% solution, pH of yeasts is of about 6.

For microscopic and PALS studies, yeasts were used in three forms: (1) lyophilized cells (as originally produced by manufacturer), (2) dried, after addition of water to lyophilized cells, rinsing and evaporating in standard conditions, and (3) wet with added drop of water.

Experimental techniques

A standard 'fast-slow' delayed coincidence positron annihilation lifetime (PAL) spectrometer was used to conduct measurements. The ^{22}Na positron source of the activity of about 0.4 MBq, surrounded by two layers of sample, was placed inside a chamber. The γ quanta were registered using two detectors equipped with cylindrical BaF_2 scintillators of sizes ϕ 1.5" × 1.5" each. All measurements were conducted in room temperature in four stages: (1) at the beginning, material was investigated in vacuum in order to remove small amounts of water possibly present in the yeast; (2) next dried air was introduced to the sample chamber, as sorption experiment was planned to perform under normal pressure; (3) with the presence of water vapor (wet filter paper was placed in the chamber); and (4) with drop of water placed directly to the yeast. Spectra with a total statistics of ~10^6 counts for each experimental point were analyzed using LT [18] and MELT programs (for integrated ones with statistics of ~10^7).

Environmental scanning electron microscopy (ESEM) images were obtained using 'Quanta 3D FEG' microscope, produced by FEI company working in environmental mode, dedicated as gaseous secondary electron detector (GSED). Measurements were conducted in the room temperature, under pressure of water vapor of 130 Pa and a beam with energy of about 10 keV.

Results

PAL spectra of yeasts samples were measured as a function of hydration time. The intensity of *o*-Ps component is commonly accepted as the source of information about concentration of free volumes in the medium, where the *o*-Ps lifetime is measured as a value of free volumes sizes. In the PAL spectra measured in lyophilized material, two components related to *o*-Ps annihilation in the voids were found. The first one with mean lifetime of about $\tau_3 \approx 1.3$ ns and the second one $\tau_4 \approx 2.9$ ns. Free volumes radii determined from Tao-Eldrup model assuming spherical shape of void reach about $r_3 \approx 0.2$ nm and $r_4 \approx 0.35$ nm, respectively. In Fig. 1, the changes in the *o*-Ps lifetimes (τ_3, τ_4) and intensities (I_3, I_4) as a

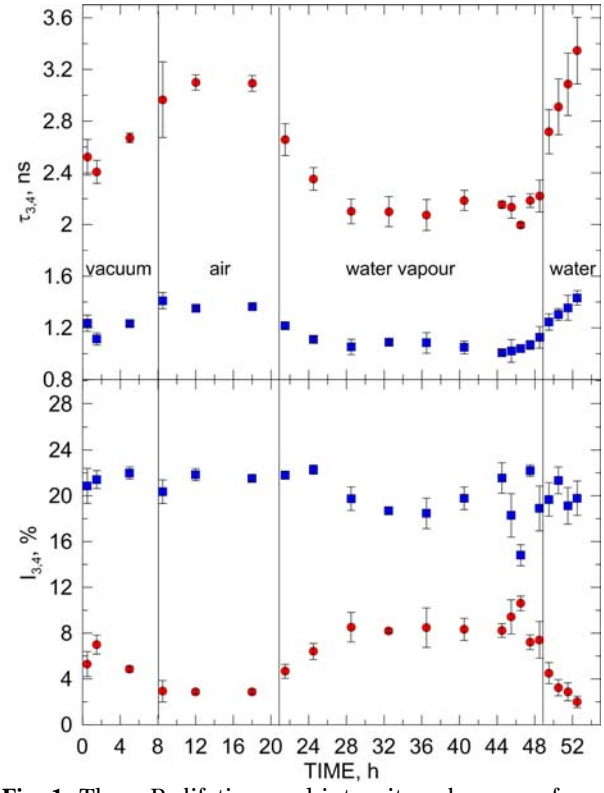

Fig. 1. The *o*-Ps lifetime and intensity values as a function of the water vapor sorption time, index 3 denotes shorter-lived component (squares) while 4 longer-lived components (circles). Measurement were conducted in four stages: (1) in vacuum, (2) in dried air, (3) with the presence of water vapor, and (4) with drop of water placed in the chamber containing yeast.

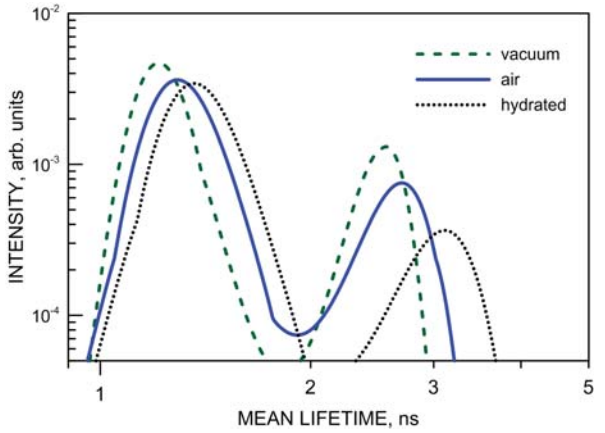

Fig. 2. Distributions of the *o*-Ps lifetimes in lyophilized yeasts in vacuum, then with the chamber open (air), and after adding drop of water to the chamber (hydrated).

function of time and sample environment are shown. In the course of water vapor sorption, the lifetime value of the fourth component decreases to about 2.0 ns and its intensity value rises to about 8%, whereas changes of parameters describing τ_3 component are much smaller. It is a result of water loaded to the material via larger voids having probably the shape of elongated channels open to the surface. The *o*-Ps lifetime value (in the presence of water vapor) of a range of 2.0 ns is known from literature as *o*-Ps lifetime value in aqueous solutions. In the presence of liquid water (4th stage), both *o*-Ps lifetime values rapidly rise. This may be a result of water molecules entering the cell by osmosis process and possible changes in cell's wall proteins conformation, but because yeasts cells have never before been studied at such small scale (below 1 nm), explanation of these results needs additional investigation. One can only say that it suggest reorganization of material in the molecular scale. Additionally, in Fig. 2, distributions of the *o*-Ps lifetimes in lyophilized and hydrated yeast are presented, as one can observe mean lifetimes of both *o*-Ps components are longer for cells with the addition of water; these, probably, are the result of channels in the cell wall being elongated because of the present of water, but further investigation is needed on this subject.

In order to see how surface of yeasts cells changes in the presence of water, studies with IM (Fig. 3) and ESEM (Fig. 4) were performed. In Fig. 3, it is well visible that after adding drop of water (upper right), cells starts to grow and move on the glass filling all voids. In Fig. 4, results from ESEM are presented. As one can observe, this method shows that cell surface structure is clearly changed after the addition of water, not only cells growths but its surface is smother and shape of cells are also changed. This is in line with the result inferred from PALS analysis, that some changes on molecular level are occurring in yeasts during hydration process.

Conclusions

In this study, an experiment showing the possibility to observe dynamics of the water sorption by a

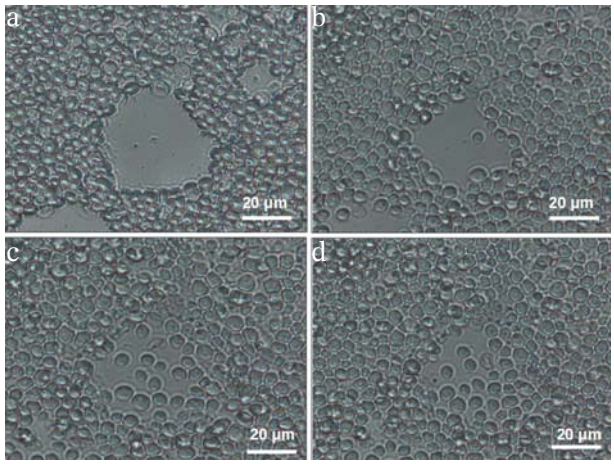

Fig. 3. Inverted microscopy (IM) images for dried yeasts (a), 1 s after adding drop of water (b), 1 min after (c), and 5 min after (d).

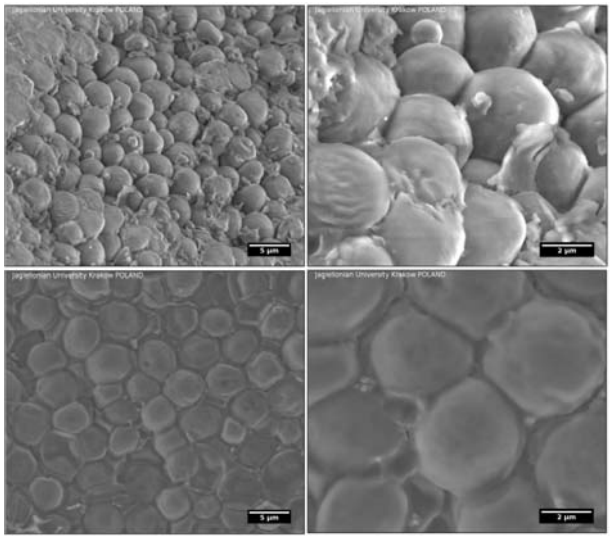

Fig. 4. Environmental scanning electron microscopy (ESEM) images of lyophilized yeasts (upper) and dried under normal conditions after addition of water (bottom).

hydrophilic material such as lyophilized yeast cells was performed. As a result, we proved that PALS can be successfully used for the studies of biological materials in the nanoscale. Knowing the mean lifetimes of both *o*-Ps components, we were able to evaluate sizes of free volumes in yeasts, which in presented results were smaller than 1 nm.

The microscopic data allows to observe the cell and its membrane in the scale of micrometers, while from PALS measurements, we received information in the scale below 1 nm. We observed changes in free volumes between molecules in cell membrane. As the single yeasts cells are of range of 2–6 μm, the space between the cells is of the same range and is probably seen by PALS as a vacuum level.

In the future, these studies of model biological structures such as yeasts will enable the determination of early and advanced stages of carcinogenesis by observing changes in biomechanical parameters between normal and tumor cells using PALS method combined with J-PET system [19], which is currently being developed at the Jagiellonian University [20–25].

Acknowledgments. We acknowledge the financial support by the Polish National Center for Development and Research through grant INNOTECH-K1/1N1/64/159174/NCBR/12, the Foundation for Polish Science through MPD program and the EU and MSHE Grant no. POIG.02.03.00-161 00-013/09. Microscopy research was carried out with the equipment purchased, thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation–Economy Operational Program (contract no. POIG.02.01.00-12-023/08) and supported by 1.1.2 POIG EU project POMOST FNP: "Elasticity parameter and strength of cell to cell interaction as a new marker of endothelial cell dysfunction in hyperglycemia/hypoglycemia". We acknowledge Dr M. Targosz-Korecka and Dr B. R. Jany for help with microscopic measurements and images interpretation. We would also like to acknowledge technical and administrative support by T. Gucwa-Ryś, A. Heczko, M. Kajetanowicz, G. Konopka-Cupiał, W. Migdał.

References

1. Eldrup, M., Lightbody, D., & Sherwood, J. N. (1981). The temperature dependence of positron lifetimes in solid pivalic acid. *Chem. Phys*., *63*, 51–58. DOI: 10.1016/0301-0104(81)80307-2.
2. Tao, S. (1972). Positronium annihilation in molecular substances. *J. Chem. Phys*., *56*, 5499–5510.
3. Gidley, D. W., Frieze, W. E., Dull, T. L., Yee, A. F., Ryan, E. T., & Ho, H. -M. (1999). Positronium annihilation in mesoporous thin films. *Phys. Rev. B*, *60*, R5157(R). DOI: 10.1103/PhysRevB.60.R5157.
4. Goworek, T., Ciesielski, K., Jasinska, B., & Wawryszczuk, J. (1997). Positronium in large voids Silicagel. *Chem. Phys. Lett*., *272*(1/2), 91–95.
5. Goworek, T., Ciesielski, K., Jasinska, B., & Wawryszczuk, J. (1998). Positronium states in the pores of silicagel. *Chem. Phys*., *230*(2/3), 305–315.
6. Jasińska, B., Kozioł, A. E., & Goworek, T. (1996). Ortho-positronium lifetimes in nanospherical voids. *J. Radioanal. Chem*., *210*(2), 617–623. DOI: 10.1007/BF02056403.
7. Jasińska, B., Kozioł, A. E., & Goworek, T. (1999). Void shapes and o-Ps lifetime in molecular crystals. *Acta Phys. Pol. A*, *95*, 557–561.
8. Ismail, R. A., Haburi, N. F., & Ali, A. M. (2012). Structural and electrical properties of CdO/porous-Si heterojunction. *Iraqi Journal of Physics*, *10*, 76–85.
9. Pietrzak, R., Borbulak, S., & Szatanik, R. (2013). Influence of neoplastic therapy on the investigated blood using positron annihilation lifetime spectroscope. *Nukleonika*, *58*(1), 199–202.
10. Jean, Y. C., & Ache, H. J. (1977). Positronium reactions in micellar systems. *J. Am. Chem. Soc*., *99*(23), 7504–7509. DOI: 10.1021/ja00465a018.
11. Jean, Y. C., Li, Y., Liu, G., Chen, H. M., Zhang, J. J., & Gadzia, J. E. (2006). Applications of slow positrons to cancer research: Search for selectivity of positron annihilation to skin cancer. *Appl. Surf. Sci*., *252*, 3166–3171. DOI: 10.1016/j.apsusc.2005.08.101.
12. Liu, G., Chen, H., Chakka, L., Gadzia, J. E., & Jean, Y. C. (2007). Applications of positron annihilation to dermatology and skin cancer. *Phys. Status Solidi C*, *4*(10), 3912–3915. DOI: 10.1002/pssc.200675736.
13. Axpe, E., Lopez-Euba, T., Castellanos-Rubio, A., Merida, D., Garcia, J. A., Plaza-Izurieta, L., Fernandez-Jimenez, N., Plazaola, F., & Bilbao, J. R. (2014). Detection of atomic scale changes in the free volume void size of three-dimensional colorectal cancer cell culture using positron annihilation lifetime spectroscopy. PLoS One 2, *9*(1). DOI: 10.1371/journal.pone.0083838.
14. Salgueiro, W., Somoza, A., Cabrera, O., & Consolati, G. (2004). Porosity study on free mineral addition cement paste. *Cement Concrete Res*., *34*(1), 91–97. DOI: 10.1016/S0008-8846(03)00258-8.
15. Hugenschmidt, C., & Ceeh, H. (2014). The free volume in dried and H_2O-loaded biopolymers studied by positron lifetime measurements. *J. Phys. Chem. B*, *118*, 9356–9360. DOI: 10.1021/jp504504p.
16. Barnett, J. A., Payne, R. W., & Yarrow, D. (1983). *Yeasts: characteristics and identification*. Cambridge University Press.
17. Hohmann, S. (2002). Osmotic stress signaling and osmoadaptation in yeasts. *Microbiol. Mol. Biol. Rev.*, *66*(2), 300–372. DOI: 10.1128/MMBR.66.2.300-372.2002.
18. Kansy, J. (1996). Microcomputer program for analysis of positron annihilation lifetime spectra. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.*, *374*, 235–244. DOI: 10.1016/0168-9002(96)00075-7.
19. Moskal, P. (2014). Patent Application No.: PCT/EP2014/068374; WO2015028604.
20. Moskal, P., Niedźwiecki, Sz., Bednarski, T., Czerwiński, E., Kapłon, Ł., Kubicz, E., Moskal, I., Pawlik-Niedźwiecka, M., Sharma, N. G., Silarski, M., Zieliński, M., Zoń, N., Białas, P., Gajos, A., Kochanowski, A., Korcyl, G., Kowal, J., Kowalski, P., Kozik, T., Krzemień, W., Molenda, M., Pałka, M., Raczyński, L., Rudy, Z., Salabura, P., Słomski, A., Smyrski, J., Strzelecki, A., Wieczorek, A., & Wiślicki, W. (2014). Test of a single module of the J-PET scanner based on plastic scintillators. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.*, *764*, 317–321. DOI: 10.1016/j.nima.2014.07.052. [arXiv:1407.7395].
21. Moskal, P., Zoń, N., Bednarski, T., Białas, P., Czerwiński, E., Gajos, A., Kamińska, D., Kapłon, Ł., Kochanowski, A., Korcyl, G., Kowal, J., Kowalski, P., Kozik, T., Krzemień, W., Kubicz, E., Niedźwiecki, Sz., Pałka, M., Raczyński, L., Rudy, Z., Rundel, O., Salabura, P., Sharma, N. G., Silarski, M., Słomski, A., Smyrski, J., Strzelecki, A., Wieczorek, A., Wiślicki, W., & Zieliński, M. (2015). A novel method for the line-of-response and time-of-flight reconstruction in TOF-PET detectors based on a library of synchronized model signals. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.*, *775*, 54–62. DOI: 10.1016/j.nima.2014.12.005. [arXiv:1412.6963].
22. Moskal, P., Sharma, N. G., Silarski, M., Bednarski, T., Białas, P., Bulka, J., Czerwiński, E., Gajos, A., Kamińska, D., Kapłon, Ł., Kochanowski, A., Korcyl, G., Kowal, J., Kowalski, P., Kozik, T., Krzemień, W., Kubicz, E., Niedźwiecki, Sz., Pałka, M., Raczyński, L., Rudy, Z., Rundel, O., Salabura, P., Słomski, A., Smyrski, J., Strzelecki, A., Wieczorek, A., Wiślicki, W., Wochlik, I., Zieliński, M., & Zoń, N. (2015). Hit time and hit position reconstruction in the J-PET detector based on a library of averaged model signals. *Acta Phys. Pol. A*, *127*(5), 1495–1499. DOI: 10.12693/APhysPolA.127.149. [arXiv:1502.07886].
23. Raczyński, L., Moskal, P., Kowalski, P., Wiślicki, W., Bednarski, T., Białas, P., Czerwiński, E., Kapłon, Ł., Kochanowski, A., Korcyl, G., Kowal, J., Kozik, T.,

Krzemień, W., Kubicz, E., Molenda, M., Moskal, I., Niedźwiecki, Sz., Pałka, M., Pawlik-Niedźwiecka, M., Rudy, Z., Salabura, P., Sharma, N. G., Silarski, M., Słomski, A., Smyrski, J., Strzelecki, A., Wieczorek, A., Zieliński, M., & Zoń, N. (2014). Novel method for hit-position reconstruction using voltage signals in plastic scintillators and its application to Positron Emission Tomography. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.*, *764*, 186–192. DOI: 10.1016/j.nima.2014.07.032. [arXiv:1311.6127].

24. Raczyński, L., Moskal, P., Kowalski, P., Wiślicki, W., Bednarski, T., Białas, P., Czerwiński, E., Gajos, A., Kapłon, Ł., Kochanowski, A., Korcyl, G., Kowal, J., Kozik, T., Krzemień, W., Kubicz, E., Niedźwiecki, Sz., Pałka, M., Rudy, Z., Rundel, O., Salabura, P., Sharma, N. G., Silarski, M., Słomski, A., Smyrski, J., Strzelecki, A., Wieczorek, A., Zieliński, M., & Zoń, N. (2015). Compressive sensing of signals generated in plastic scintillators in a novel J-PET instrument. *Nucl. Instrum. Methods Phys. Res. Sect. A-Accel. Spectrom. Dect. Assoc. Equip.*, *786*, 105–112. DOI: 10.1016/j.nima.2015.03.032. [arXiv:1503.05188].
25. Wieczorek, A., Moskal, P., Niedźwiecki, Sz., Bednarski, T., Białas, P., Czerwiński, E., Danel, A., Gajos, A., Gruntowski, A., Kamińska, D., Kapłon, Ł., Kochanowski, A., Korcyl, G., Kowal, J., Kowalski, P., Kozik, T., Krzemień, W., Kubicz, E., Molenda, M., Pałka, M., Raczyński, L., Rudy, Z., Rundel, O., Salabura, P., Sharma, N. G., Silarski, M., Słomski, A., Smyrski, J., Strzelecki, A., Uchacz, T., Wiślicki, W., Zieliński, M., & Zoń, N. (2015). A pilot study of the novel J-PET plastic scintillator with 2-(4-styrylphenyl)benzoxazole as a wavelength shifter. *Acta Phys. Pol. A*, *127*(5), 1487–1490. DOI: 10.12693/APhysPolA.127.1487. [arXiv:1502.02901].