

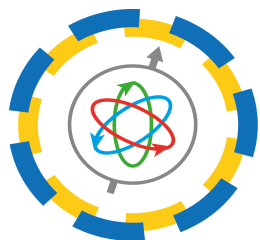


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Studies of positronium atoms in normal and cancer tissues and cultured cell lines - biomedical application

Ewelina Kubicz
15.11.2018

New Quantum Horizons: From Foundations To Biology
Symposium
Frascati



J-PET



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en.uj.edu.pl

- 1) Motivation
- 2) Cancer vs. normal cells
- 3) PALS studies of tumor and normal tissues in vitro
- 4) First PALS studies of human tissues in vitro with J-PET
- 5) PALS studies of cells cultures in vitro
- 6) Summary and future plans



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Motivation

- Determination of early and advanced stages of carcinogenesis by observing changes in biomechanical parameters between normal and cancer cells
- PALS parameters (lifetime, intensity, radius) are related with temporal dynamics of nanostructures in whole cells and tissues
- Combining J-PET scanner with PALS technique – better diagnostic tool
- Positronium imaging in the human body in vivo !
- Over 50 publication and 15 patents on J-PET and positronium imaging

P. Moskal et al., Feasibility study of the positronium imaging with the J-PET tomograph, submitted to Physics in Med. And Bio.

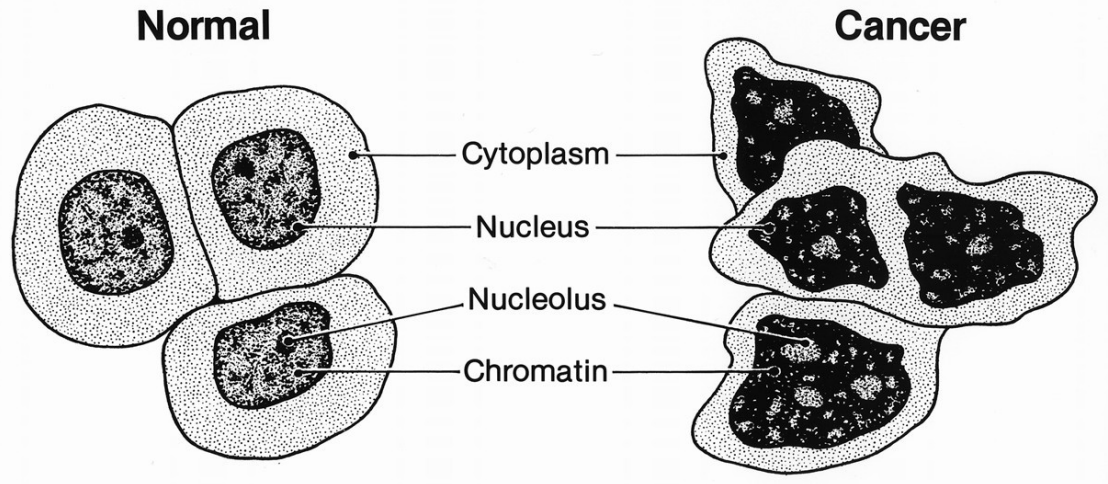
P. Moskal, TOF-PET tomograph and a method of imaging using a TOF-PET tomograph, based on a probability of production and lifetime of a positronium, patent no. P405185, PCT/EP2014/068374



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Cancer vs normal cells



→ Large cytoplasm

→ Single nucleus and nucleolus

→ Fine chromatin

→ Smaller number of dividing cells

→ Similar in shape and size

→ Organized arrangement of cells

→ Apoptosis

→ Small cytoplasm

→ Multiple and large nucleus and nucleolus

→ Coarse chromatin

→ Large number of dividing cells

→ Variation in cells shape and size

→ Disorganized arrangement of cells

→ Immortal



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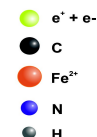
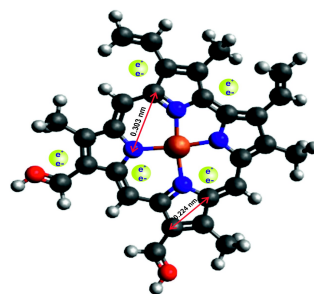
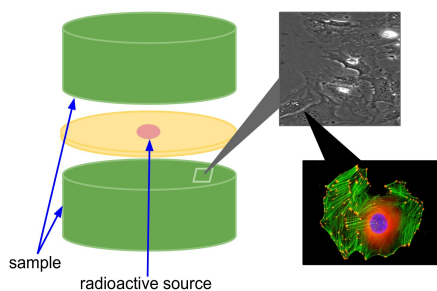
PALS setup

→ Two BaF_2 detectors with resolution ~ 250 ps

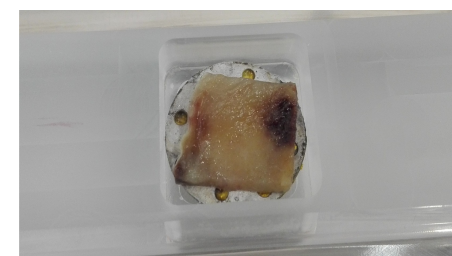
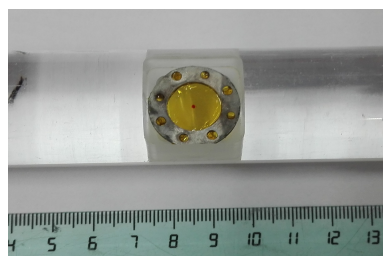
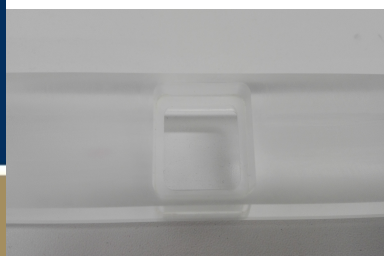
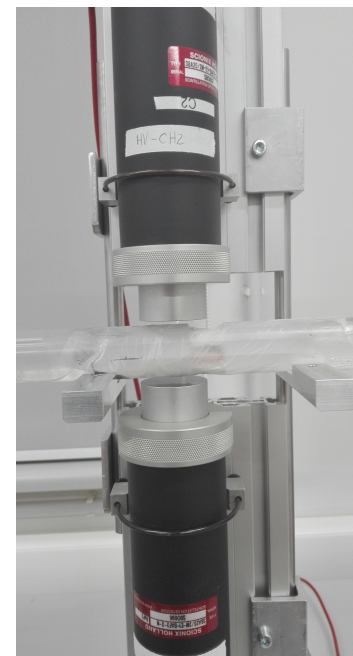
→ ^{22}Na source in Kapton foil with activity ~ 1 MBq sandwich between sample

→ PALS spectra analysis with PALS_Avalanche program developed by K. Dulski – J-PET collaboration

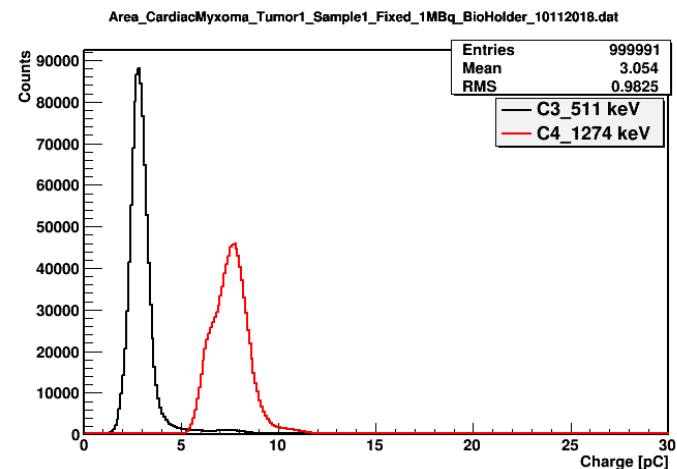
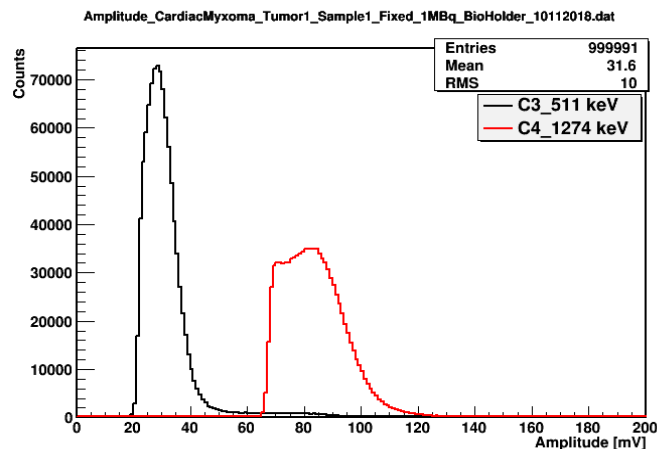
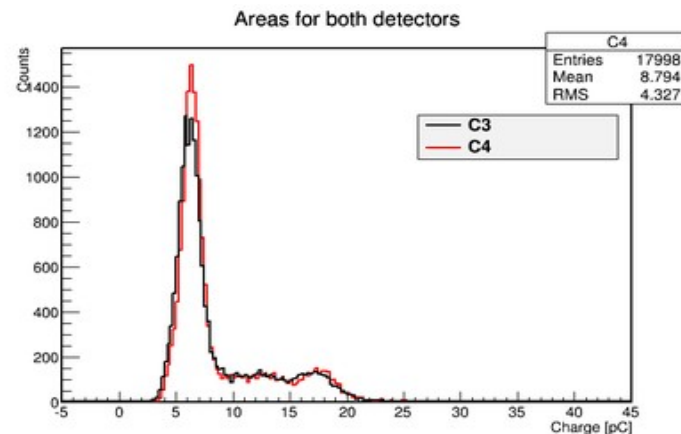
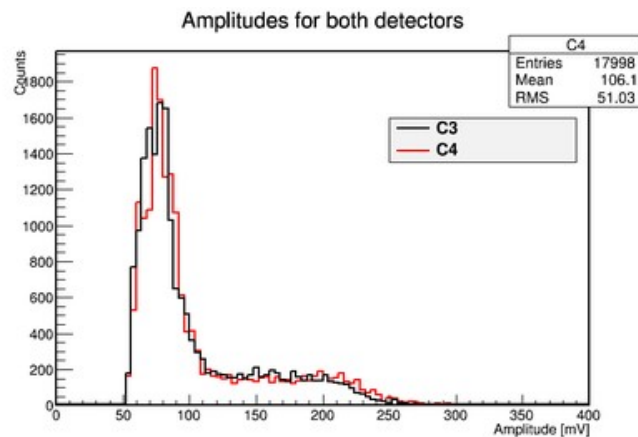
K. Dulski et. al., *Analysis procedure of the positronium lifetime spectra for the J-PET detector*, *Acta Phys. Polon. B48 no. 10*, 1611 (2017)



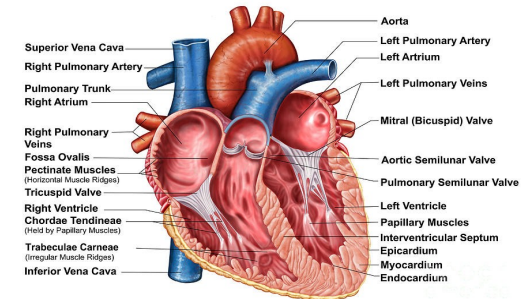
Heme group in hemoglobin molecule



PALS setup



- primitive connective tissue tumor (benign), very rare in comparison to metastatic tumors
- 75 % of them are located in the left atrium
- occur mainly in people over the age of 50



Types	Solid	Papillary
Surface	smooth	irregular
Mass	firm	soft, gelatinous
Calcification	+	-
Embolism	-	+

Fixed in formaldehyde:

- 1) Myxoma I (6 samples for study, around 2 mm thick) 72 years old women
- 2) Myxoma II (1 sample) 61 year old men
- 3) Myxoma III (3 samples) 59 year old men
- 4) Myxoma IV (3 samples) 54 year old woman

Not fixed (fresh):

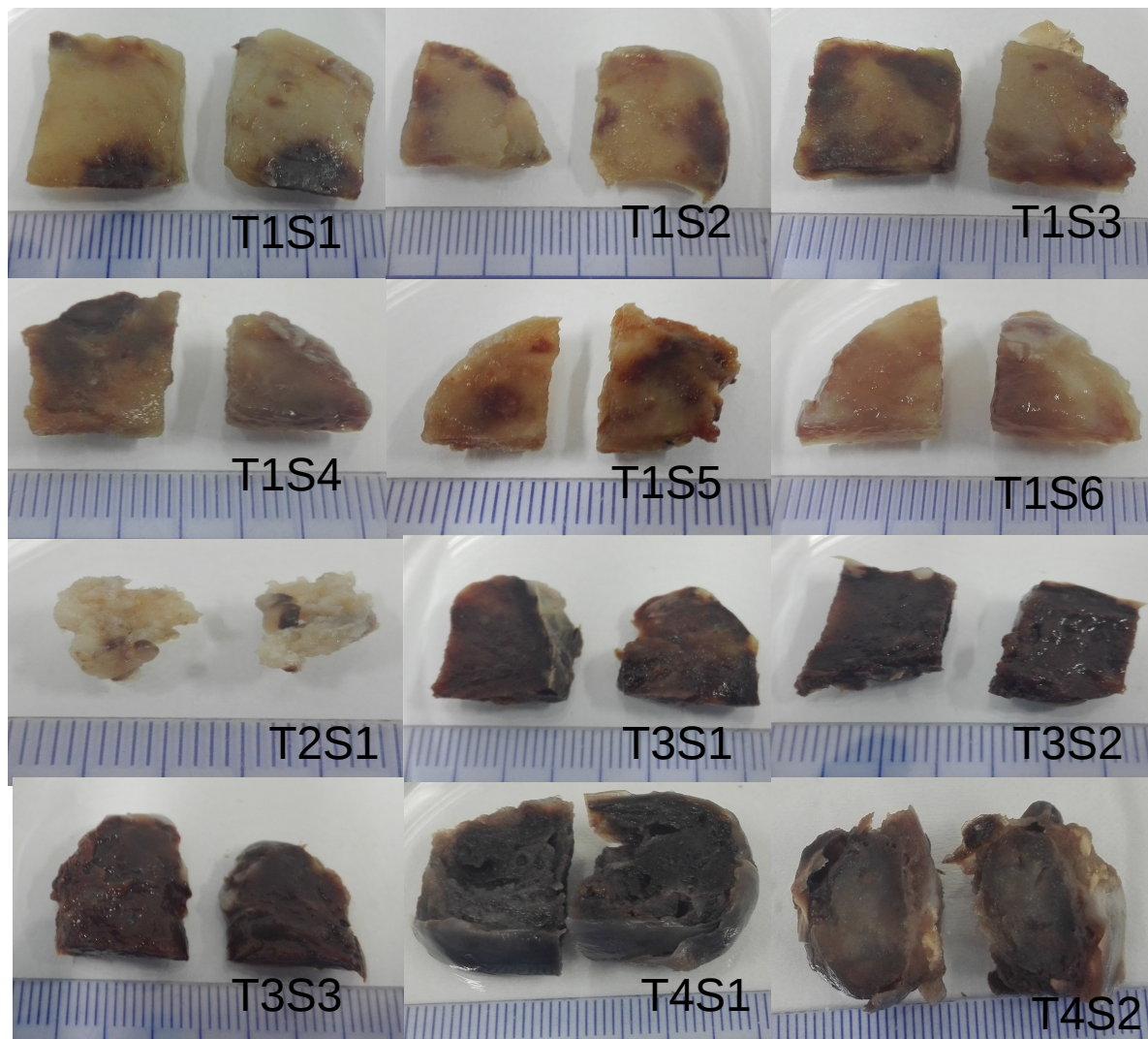
- 5) Myxoma V (1 sample) 77 year old men – measured within 4 hours after the surgery



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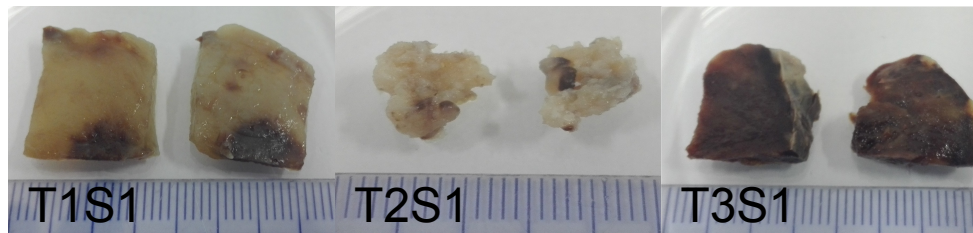
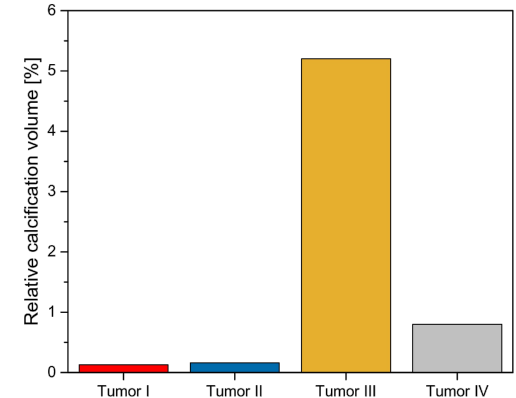
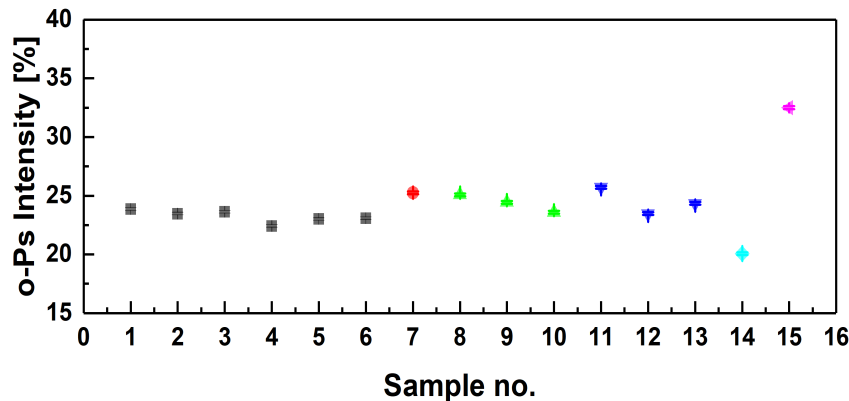
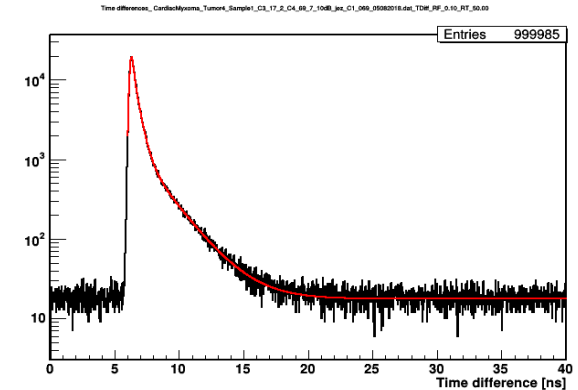
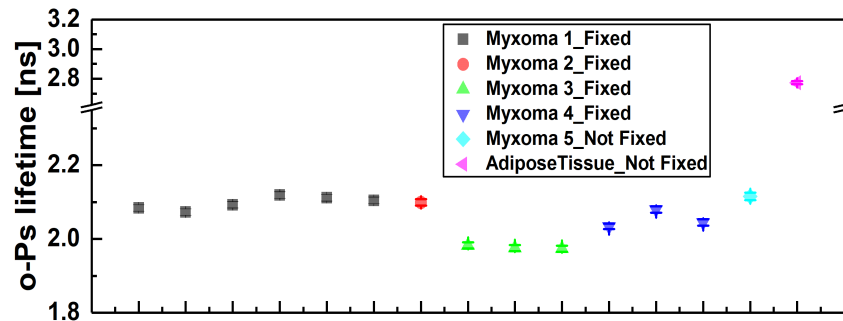
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Cardiac Myxoma



Cardiac Myxoma - PALS

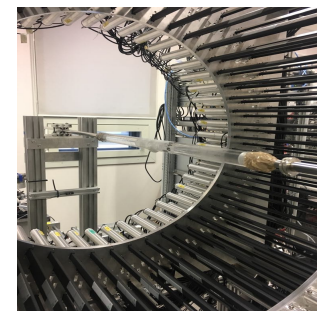
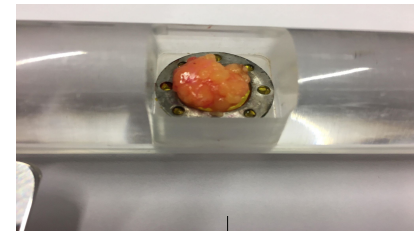
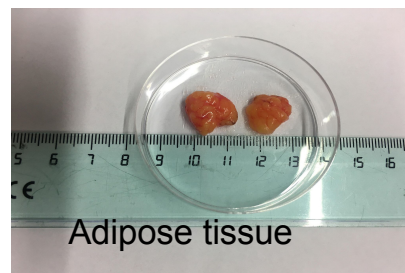
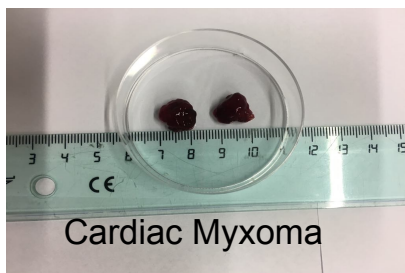
- Samples fixed in formaldehyde → not decomposing/changing in time
- Fresh sample measured within 4 hours after surgery
- Time of measurement ~60 min → 1 mln counts



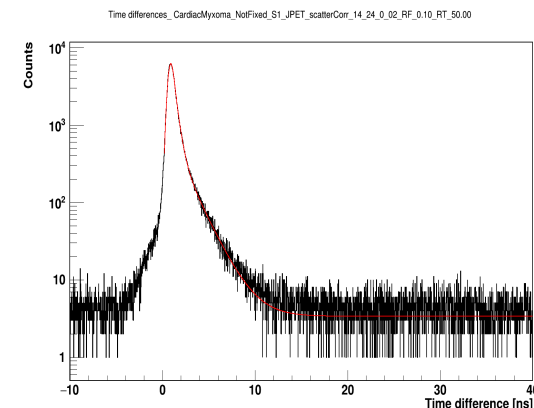
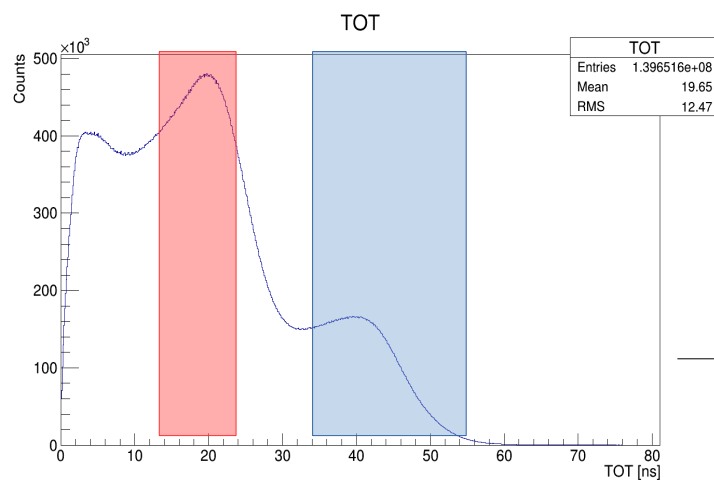
Courtesy of Dr Bartosz Leszczyński, Dr hab. Roman Pędrys, Dr Andrzej Wróbel

Cardiac Myxoma – JPET vs PALS

- Samples after extraction from patient were placed in sterile container with DMEM medium supplemented with 10% FBS, Penicillin/Streptomycin and HEPES buffer
- Fresh sample measured within 4 hours after surgery
- Time of measurement ~70-80 min → 1 mln counts

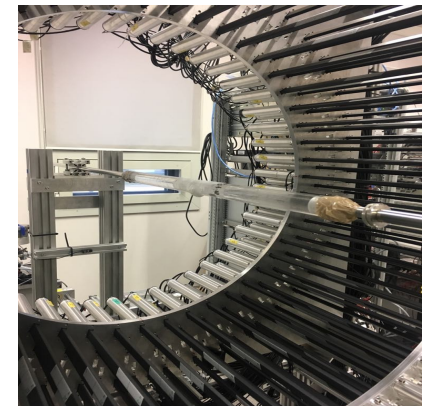
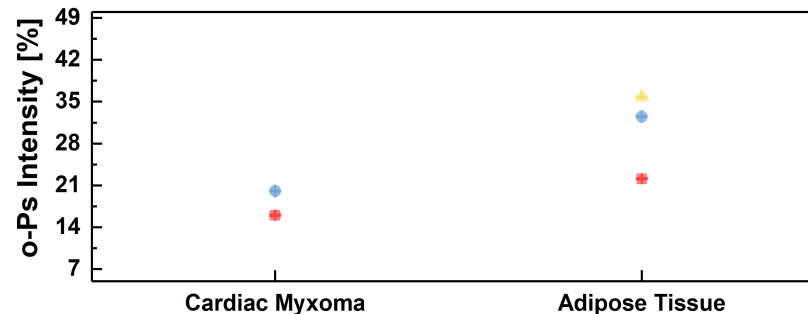
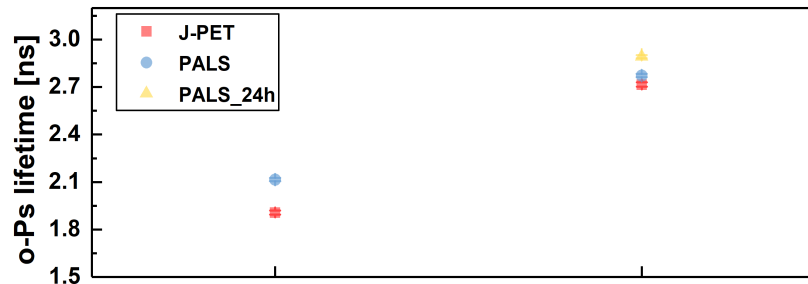
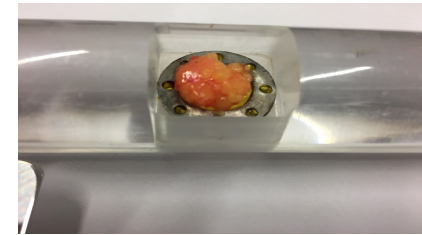
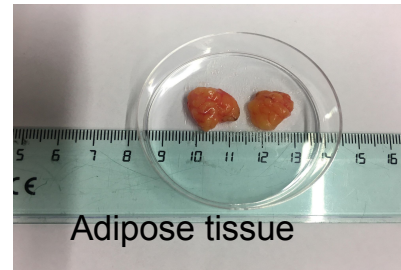
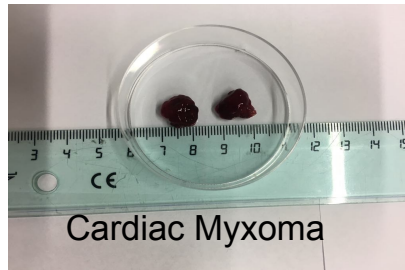


- Cut on TOT 14-24 ns (511 keV) and 35-55 ns (1274 keV)



Cardiac Myxoma – JPET vs PALS

- Samples after extraction from patient were placed in sterile container with DMEM medium supplemented with 10% FBS, Penicillin/Streptomycin and HEPES buffer
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PALS – Cells culture in vitro

Human cell lines:

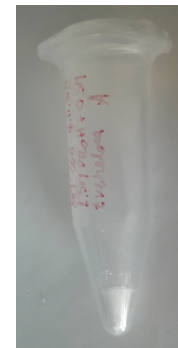
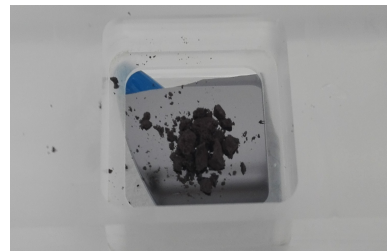
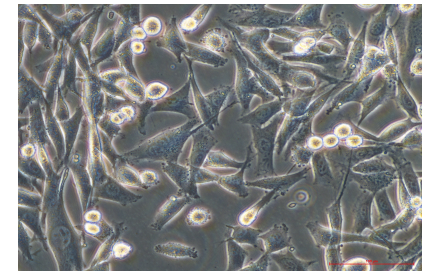
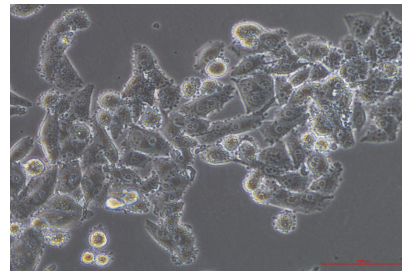
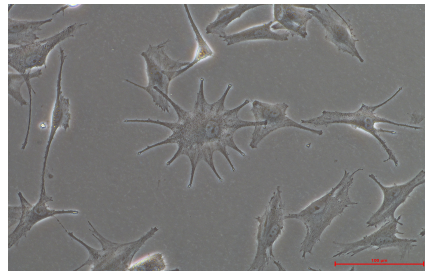
- 1) Melanocytes HEMa-LP from ThermoFisher
- 2) Melanoma WM115 from ATCC
- 3) Melanoma WM266 from ATCC

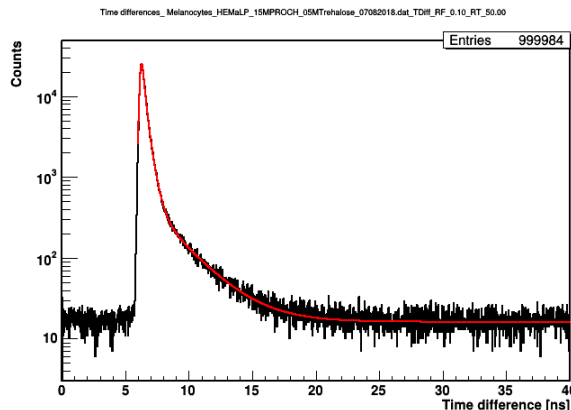
→ Cells were cultured in M254/RPMI 1640 medium supplemented with 4.5g/L glucose, 2 mM L-glutamine and HGMS-2/10% fetal bovine serum, additionally Penicillin 100U/ml and Streptomycin 100 ug/ml was added to the culture.

→ Medium was changed every 2 days.

→ Culture was incubated at 37°C in 5% CO₂ humidified atmosphere rinse with PBS w/o Ca²⁺, Mg²⁺ and passage with 0.25% Trypsin every 3-4 days.

→ Each samples contains cells from 8 T75 flasks, harvest upon 100% confluence and freeze – dried (lyophilized) – In total ~ 20*10⁷ cells (~0.5 cm³)

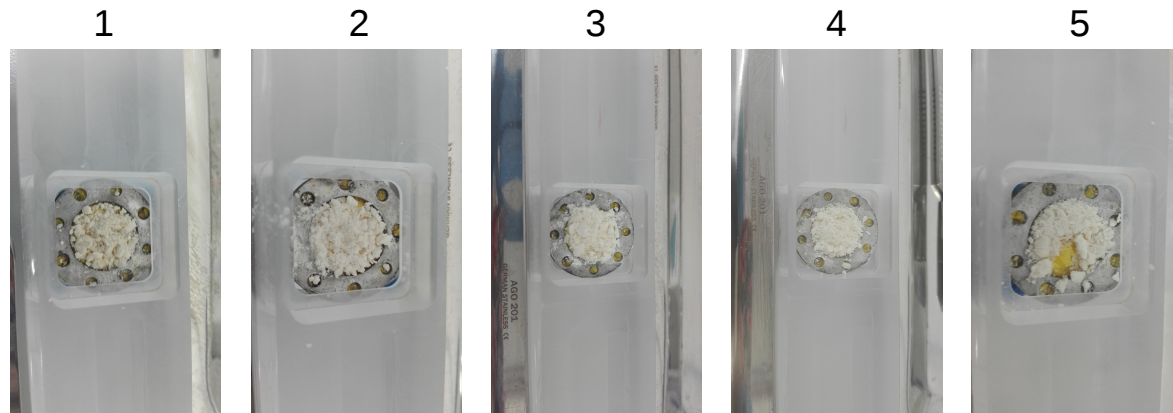




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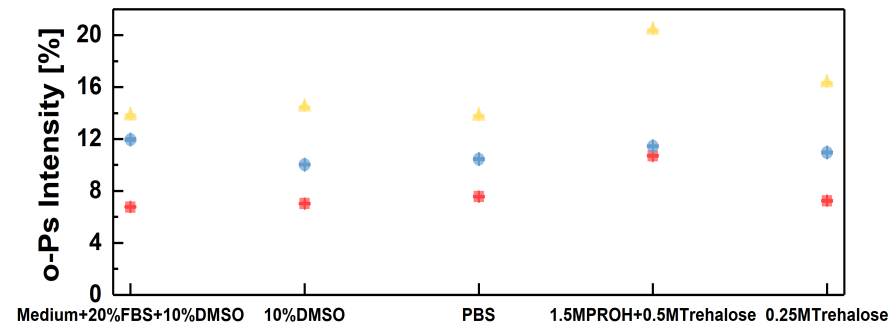
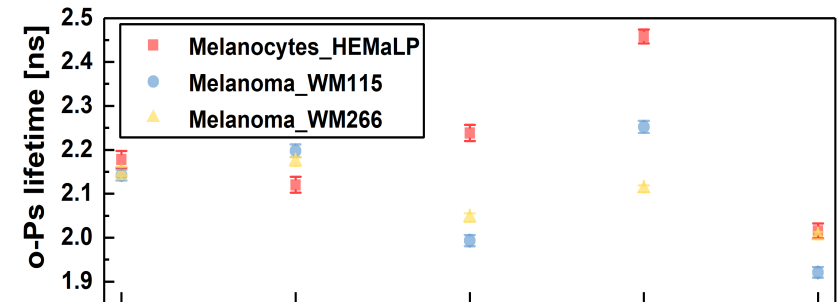
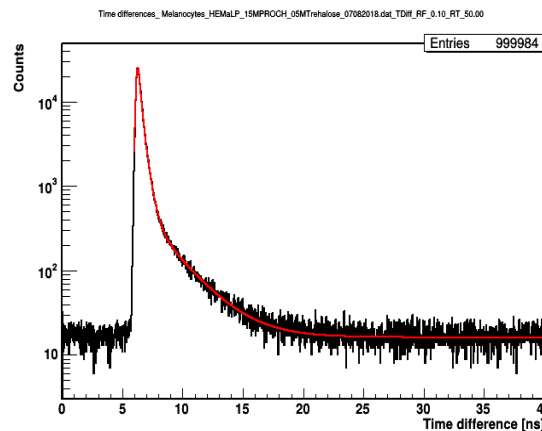


Freeze Mediums:

- 1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
- 2) 10% DMSO + PBS w/o 2^{+}Ca , 2^{+}Mg
- 3) PBS w/o 2^{+}Ca , 2^{+}Mg
- 4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o 2^{+}Ca , 2^{+}Mg
- 5) 0.25 M D-trehalose in PBS w/o 2^{+}Ca , 2^{+}Mg

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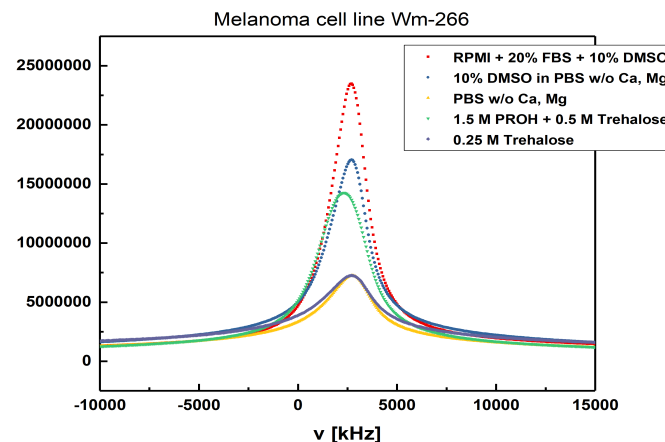
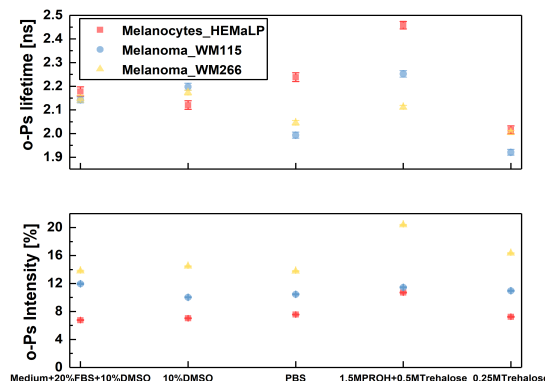
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	Viability [%]	% of water	Remaining mass [%]
1	43.8	44	-
2	40.5	31	14.69
3	5.4	22	14.24
4	49.7	49	46.25
5	10.7	16	24.62



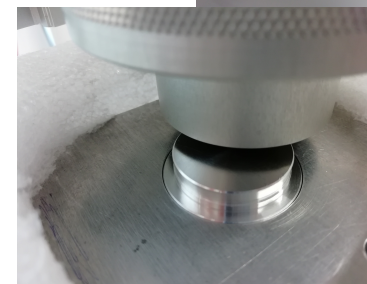
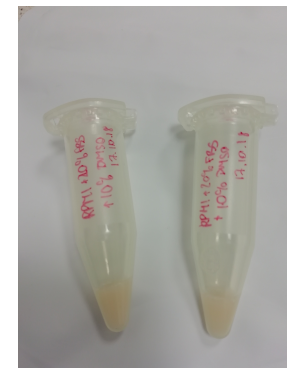
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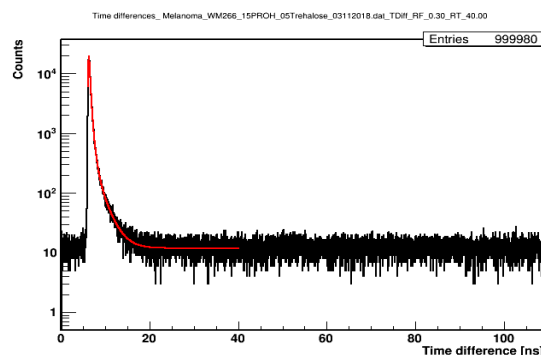
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Sample	o-Ps lifetime[ns]	o-Ps Intensity [%]
1	2.01(01)	15.77(09)
2	2.01(01)	18.16(09)
3	2.06(01)	17.23(09)
4	1.99(01)	17.87(09)
5	2.05(01)	16.94(09)
6	1.96(01)	17.17(10)
7	2.00(01)	18.23(10)
7_2h	2.01(01)	18.17(09)

	Viability before [%]	Viability after [%]
1	97.4	95.8
2	96.8	96.7
3	96.6	97.3
4	97.9	97.7
5	98.1	97.6
6	98.2	98.0
7	97.1	91.4



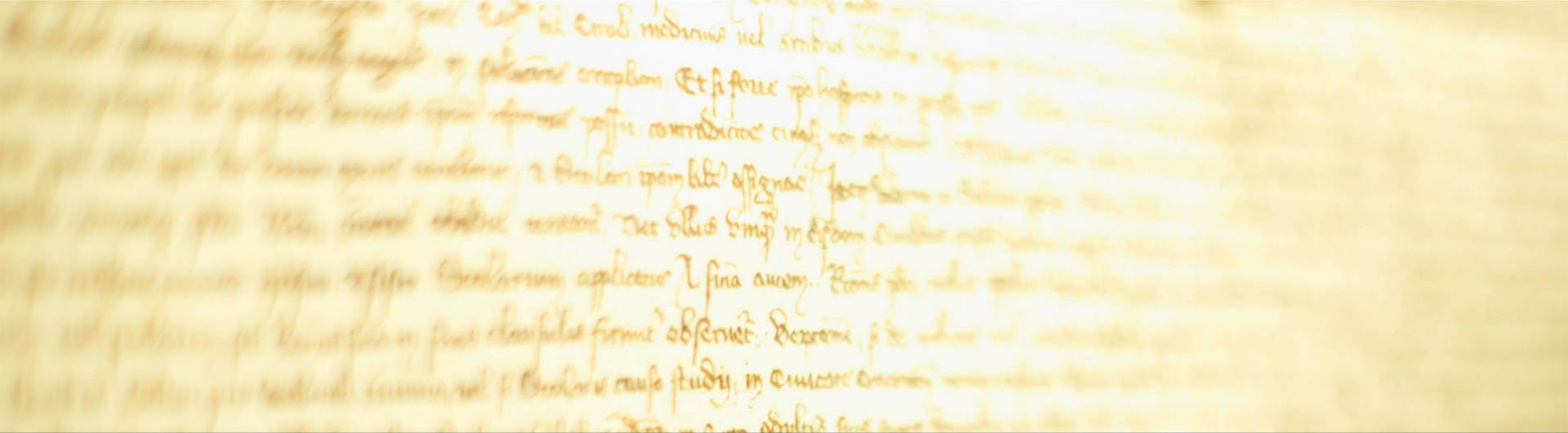
Summary and future plan



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- PALS is applicable to study biological structures
- Preliminary results shown that PALS parameters differ for normal and cancer cells and tissue
- First studies of human tissue on JPET scanner proves that o-Ps lifetime can be used as additional diagnostic parameter
- Development of the method for sample preparation in order to study alive cell cultures
- Studies with alive cell cultures and tissues – comparing normal vs cancer
- Primary cell culture derived from cardiac myxoma tumor
- Checking for possible o-Ps formation model in living cells



Thank you for your attention

