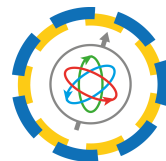




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J-PET



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EST 1364

Studies of positronium atoms in cardiac myxoma tumors and cultured cell lines - biomedical application of PALS

Ewelina Kubicz
13.09.2018

3rd Symposium on Positron Emission Tomography
Kraków



Republic
of Poland



Foundation for
Polish Science

European Union
European Regional
Development Fund



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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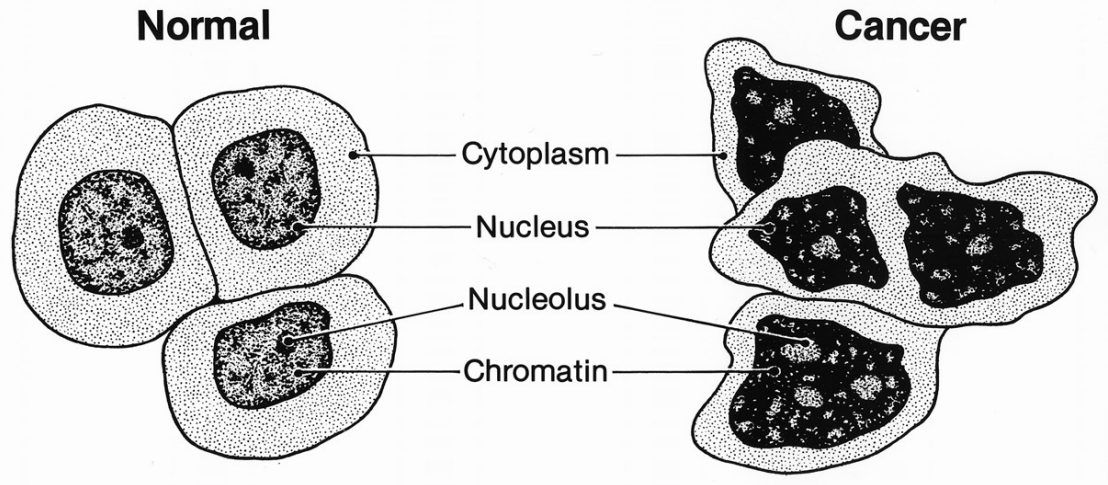
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Motivation

- 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



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



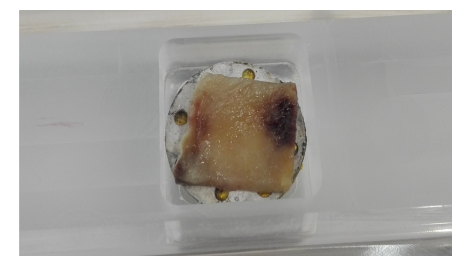
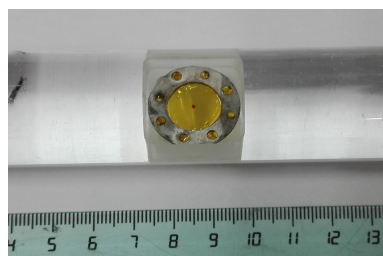
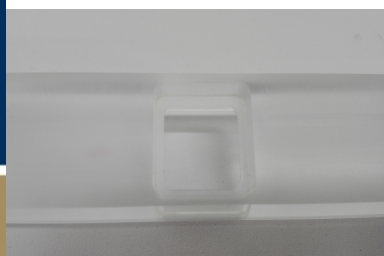
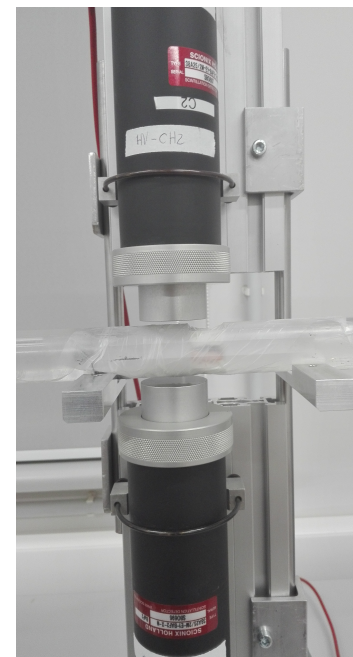
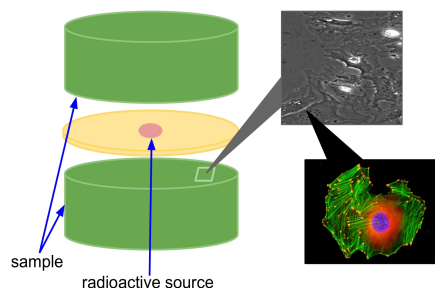
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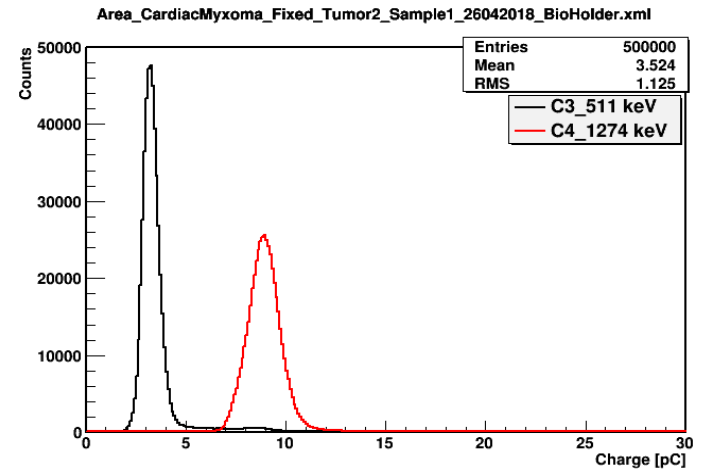
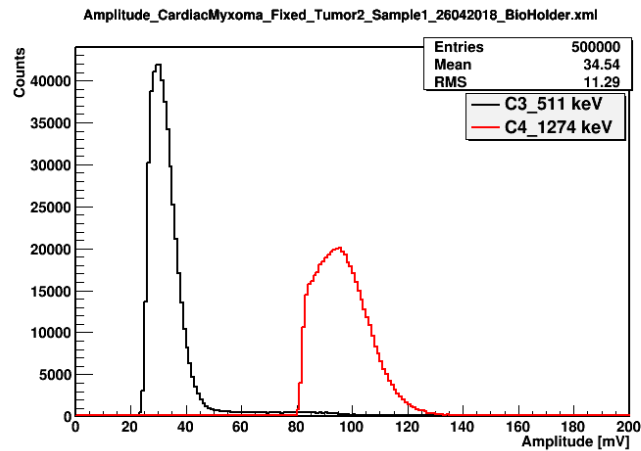
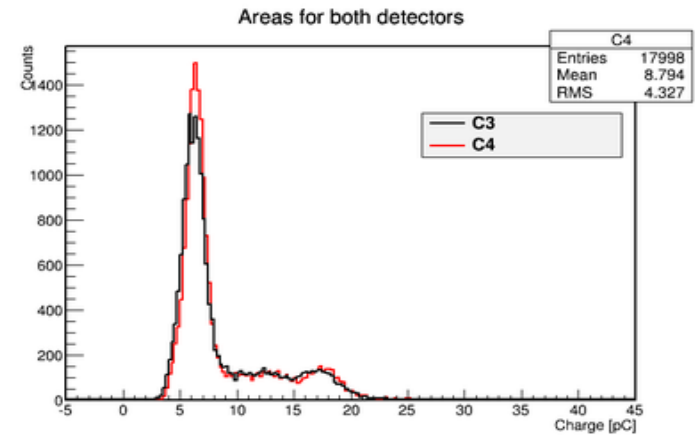
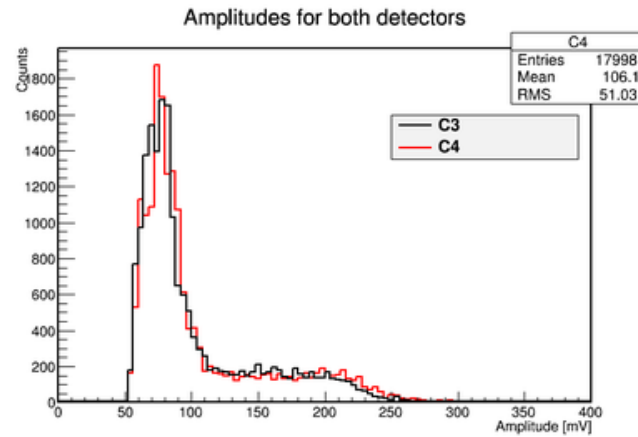
PALS setup

- 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

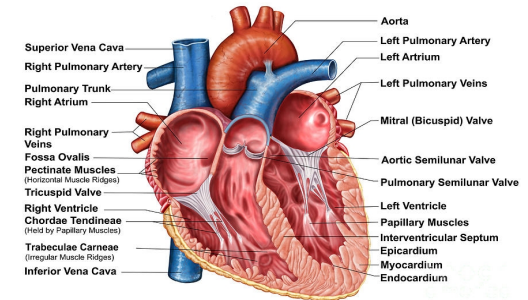
K. Dulski et. al., *Analysis procedure of the positronium lifetime spectra for the J-PET detector*, *Acta Phys. Polon. B48* no. 10, 1611 (2017)



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):

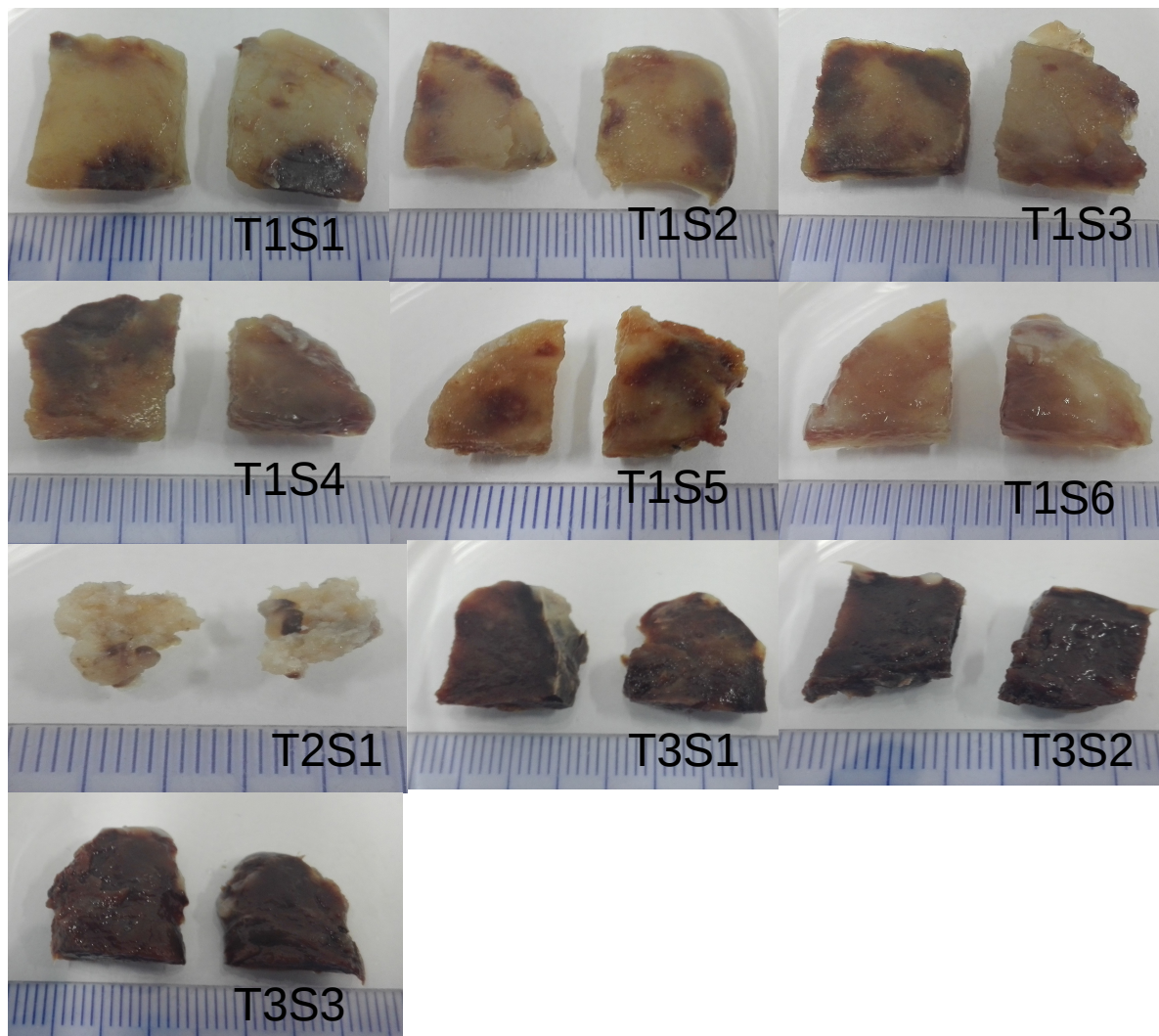
- 5) Myxoma V (1 sample) 77 year old men – measured within 4 hours after the surgery



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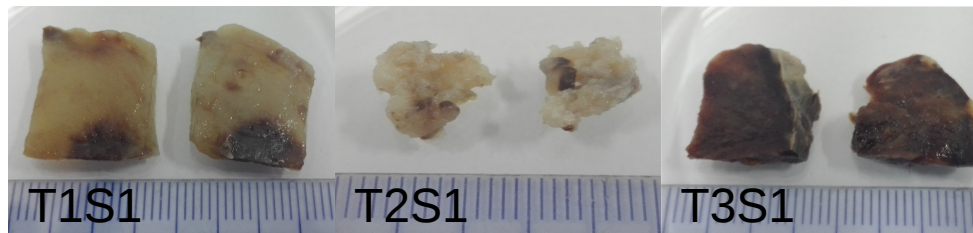
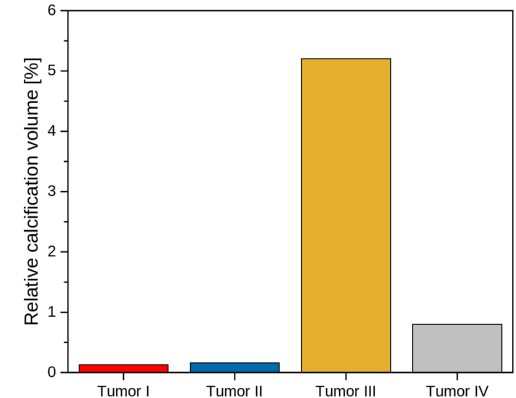
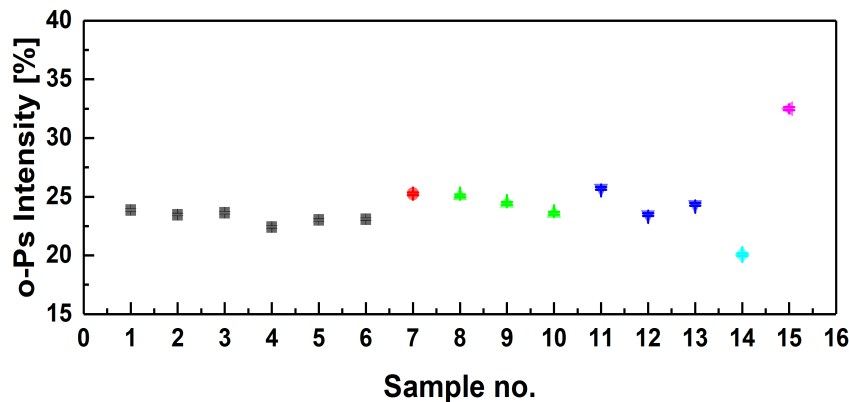
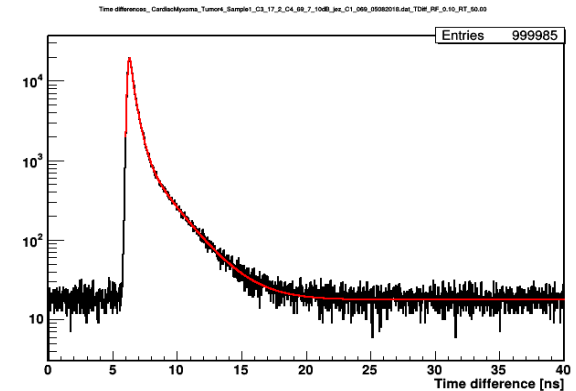
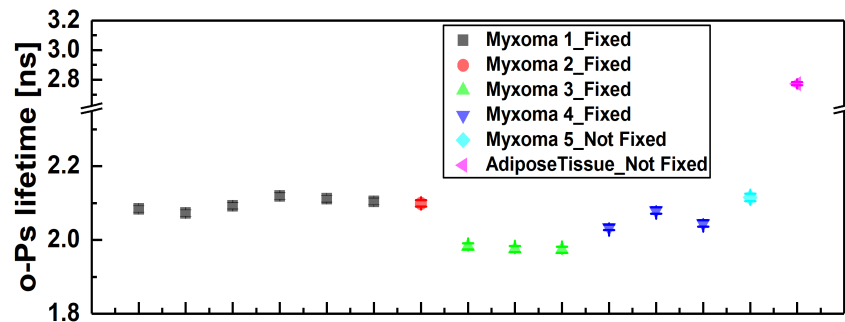
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Cardiac Myxoma



Cardiac Myxoma - PALS

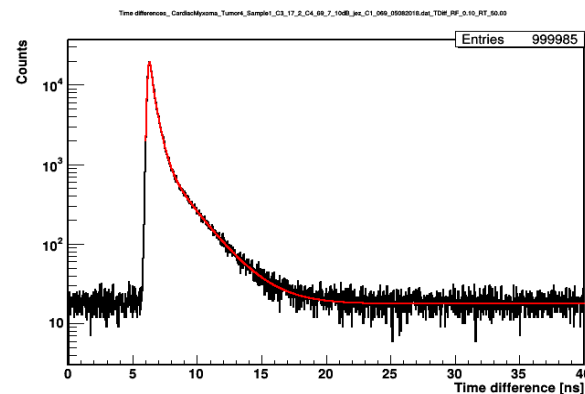
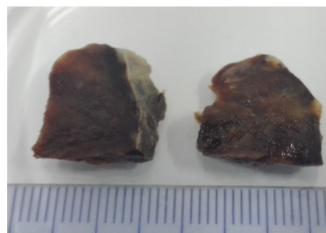
- Samples fixed in formaldehyde → not decomposing/changing in time
- Fresh sample measured within 4 hours after surgery
- Time of measurement ~70-80 min → 1 mln counts



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

Cardiac Myxoma – PALS – Kraków vs. Lublin

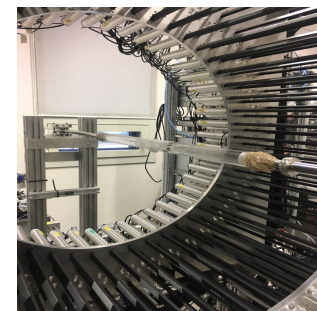
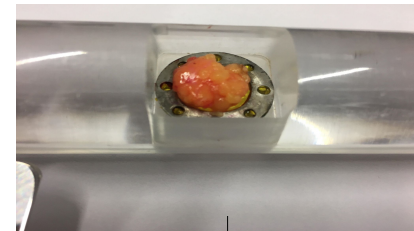
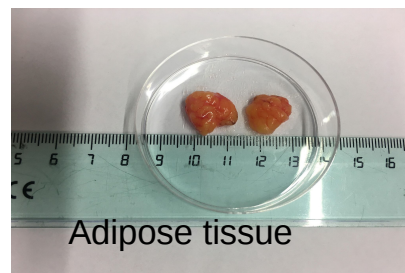
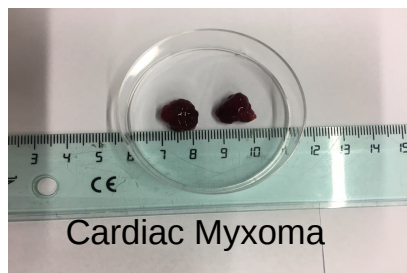
- Samples fixed in formaldehyde → not decomposing/changing in time
- Time of measurement ~70-80 min → 1 mln counts
- The same sample measured on PALS setups both in Lublin and in Kraków



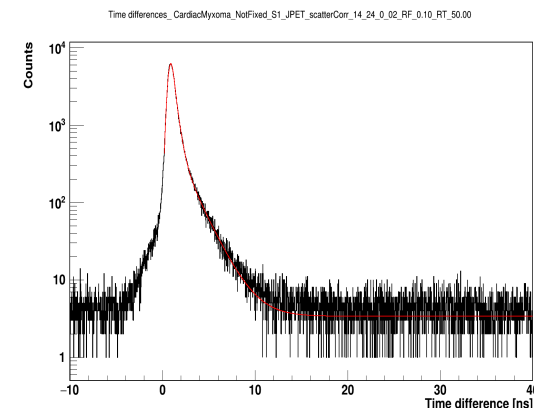
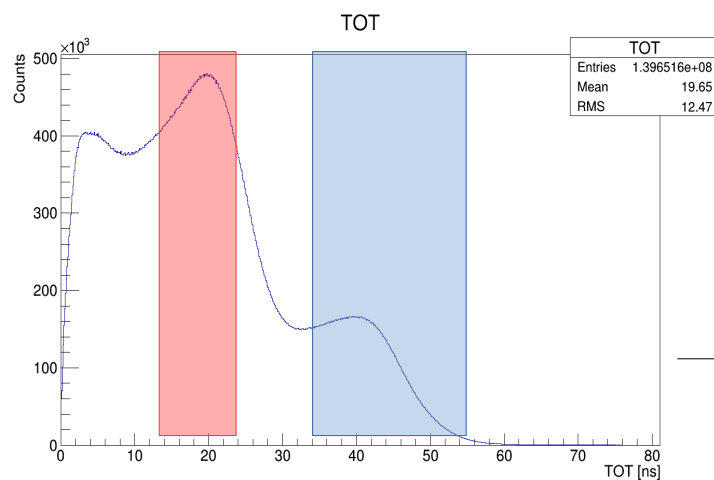
	In Lublin	In Kraków
Lifetime p-Ps [ns]	0.207(97)	0.123 (25)
Intensity p-Ps [%]	21.67(1.30)	18.55(97)
Lifetime free-Ps [ns]	0.428(85)	0.420(19)
Intensity free-Ps [%]	51.49(1.20)	55.73(67)
Lifetime o-Ps [ns]	2.03(08)	2.03(02)
Intensity o-Ps [%]	26.84(88)	25.72(79)
FitVariance/R2	0.9859	0.9997

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

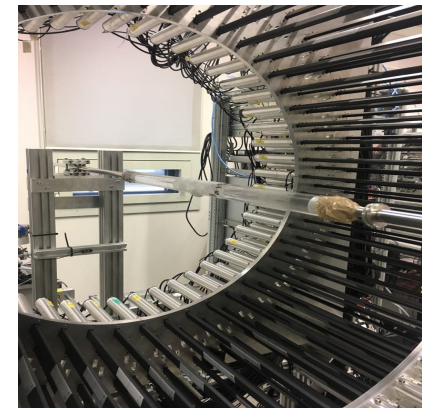
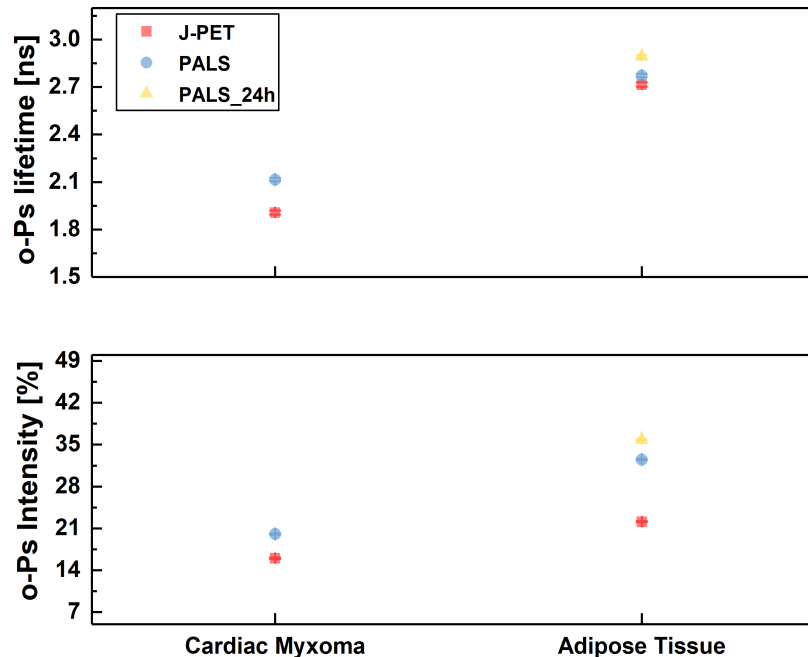
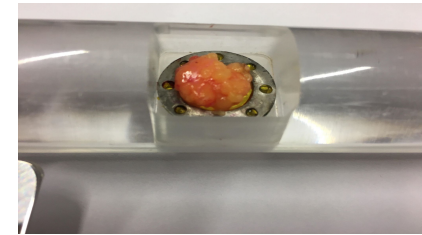
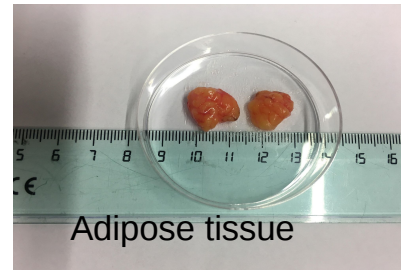
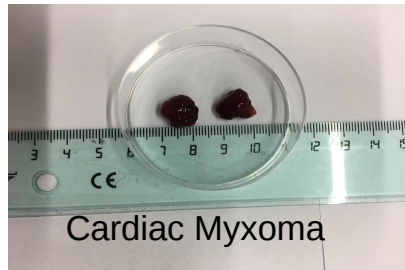


- Cut on TOT 14-24 ns (511 keV) and 35-55 ns (1274 keV)



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- 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:

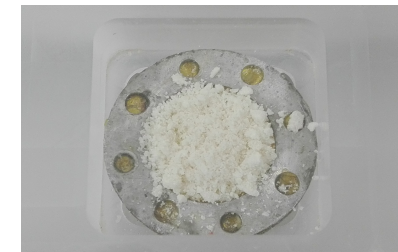
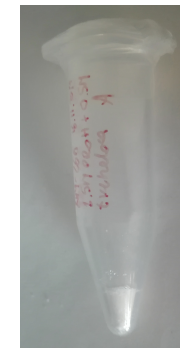
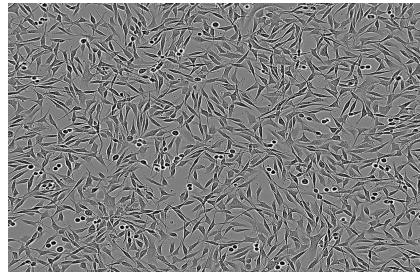
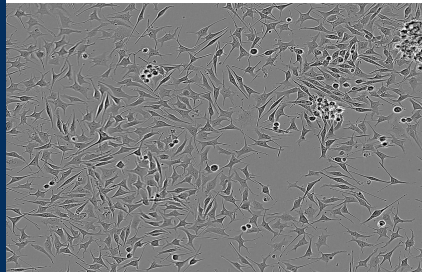
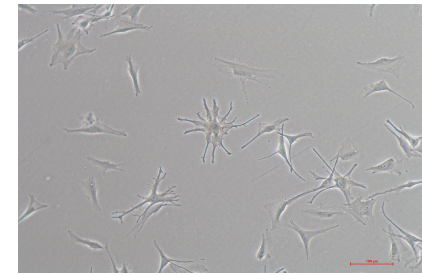
- 1) Melanocytes HEMa-LP from ThermoFisher
- 2) Melanoma WM115 from ATCC
- 3) Melanoma WM266 from ATCC

→ 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.

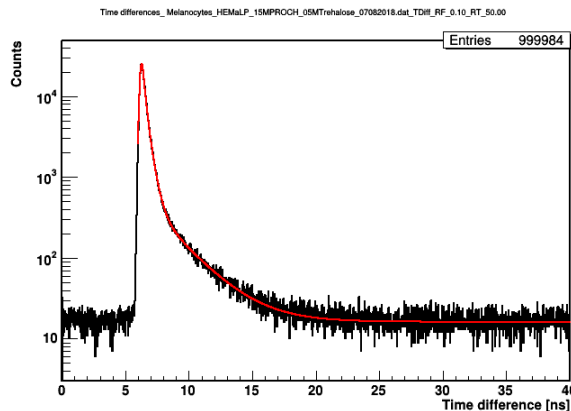
→ Culture was incubated at 37°C in 5% CO₂ humidified atmosphere rinse with PBS w/o Ca²⁺, Mg²⁺ and passage with 0.25% Trypsin every 3-4 days.

→ Each samples contains cells from 8 T75 flasks, harvest upon 100% confluence and freeze – dried (lyophilized).



Freeze Mediums:

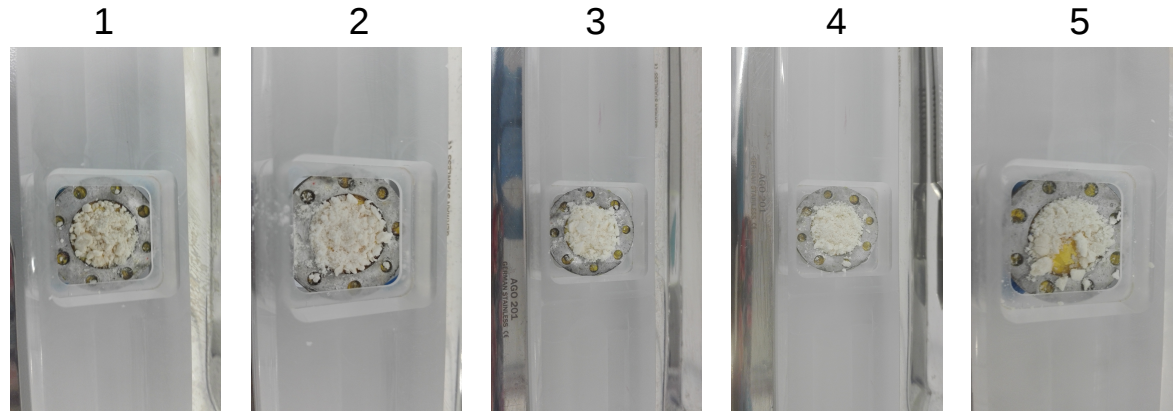
- 1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
- 2) 10% DMSO + PBS w/o ²⁺Ca, ²⁺Mg
- 3) PBS w/o ²⁺Ca, ²⁺Mg
- 4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o ²⁺Ca, ²⁺Mg
- 5) 0.25 M D-trehalose in PBS w/o ²⁺Ca, ²⁺Mg



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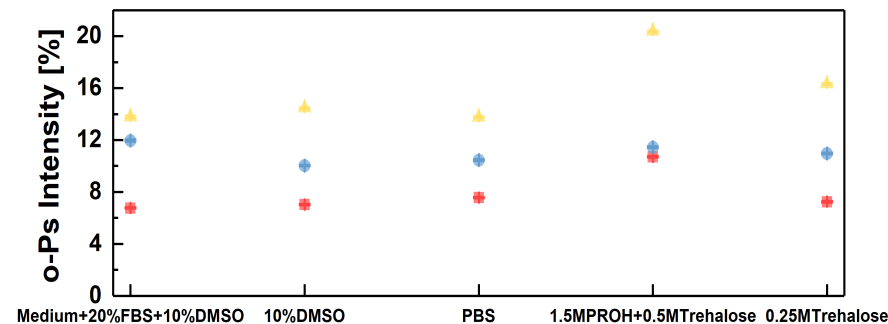
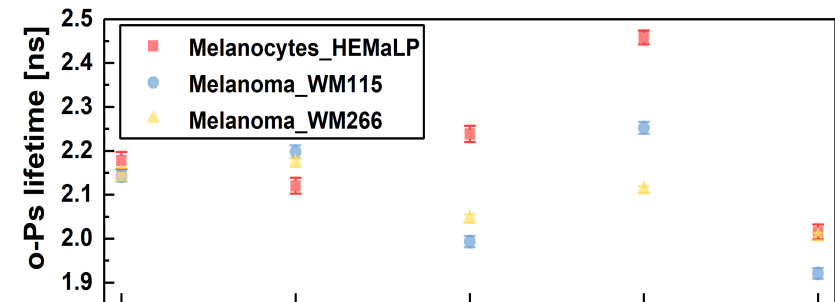
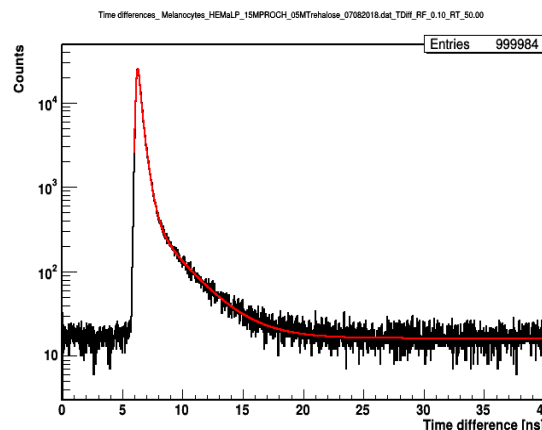


Freeze Mediums:

- 1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
- 2) 10% DMSO + PBS w/o 2^{+}Ca , 2^{+}Mg
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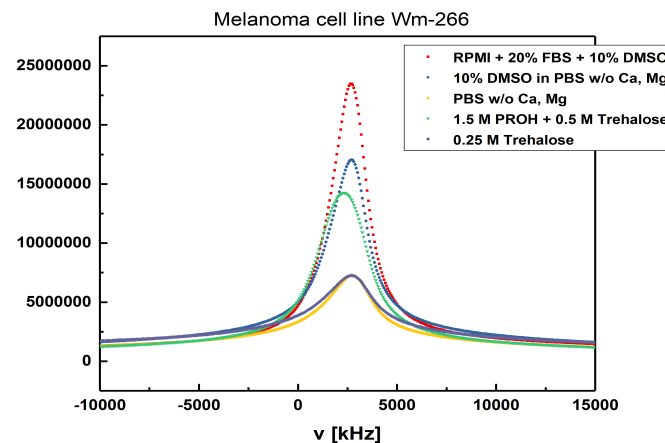
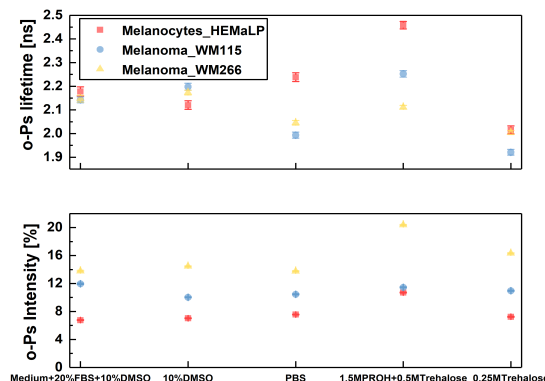
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- 3) Melanoma WM266 from ATCC

	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%



Freeze Mediums:

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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

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qui hanc sufficiam penam de p...
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