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J-PET

Molecular characterization of nanostructures of normal and cancer cells in vitro using Positron Annihilation Lifetime Spectroscopy (PALS)

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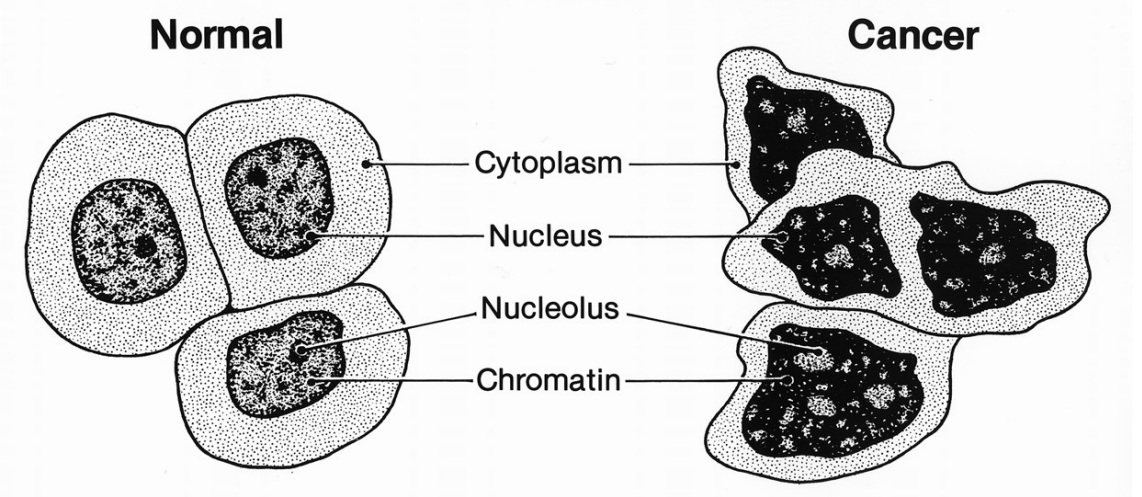
Outline

- 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

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	→ Small cytoplasm
→ Single nucleus and nucleolus	→ Multiple and large nucleus and nucleolus
→ Fine chromatin	→ Coarse chromatin
→ Smaller number of dividing cells	→ Large number of dividing cells
→ Similar in shape and size	→ Variation in cells shape and size
→ Organized arrangement of cells	→ Disorganized arrangement of cells
→ Apoptosis	→ Immortal

https://visualsonline.cancer.gov/details.cfm?imageid=2512

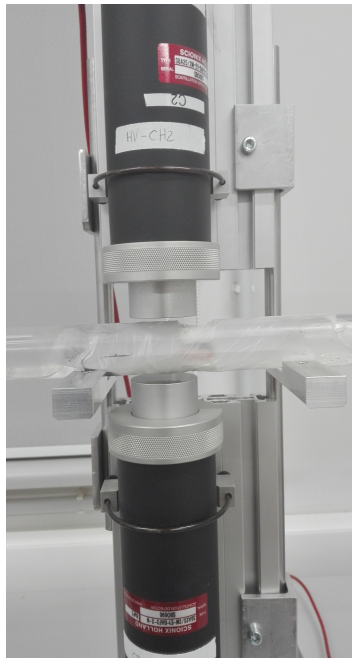
PALS setup

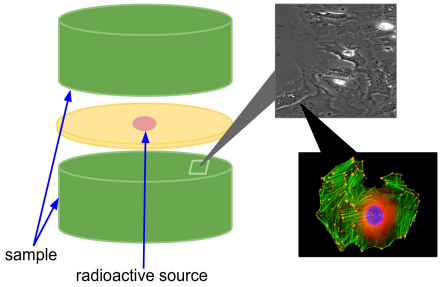
→ Two BaF_2 detectors with resolution ~250 ps

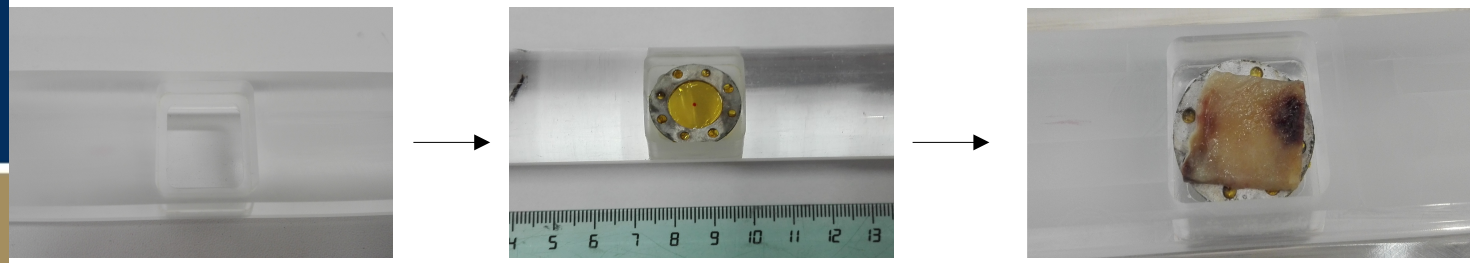
→ ^{22}Na source in Kapton foil with activity ~ 1 MBq sandwich between sample

→ 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)*

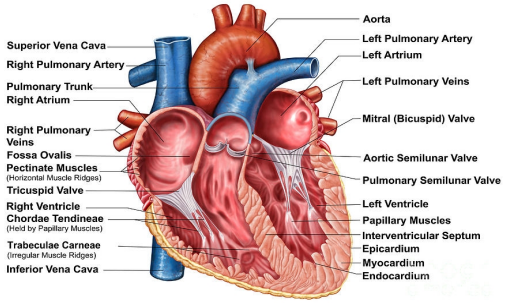






Cardiac Myxoma

- → 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

https://healthjade.com/human-heart-health/

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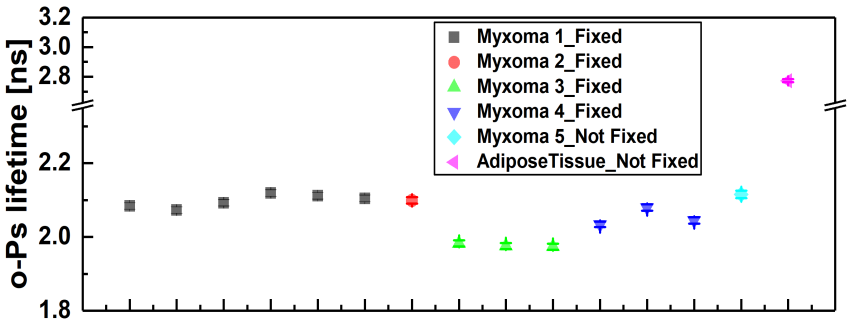
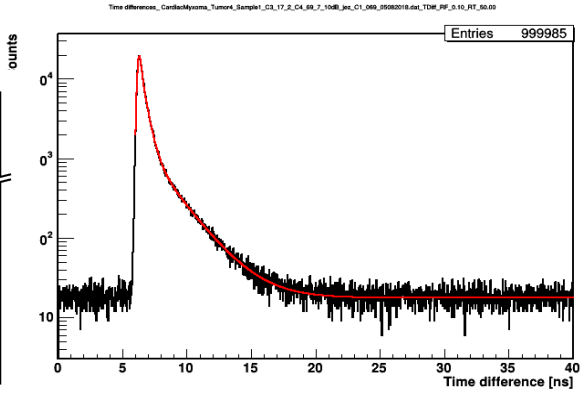
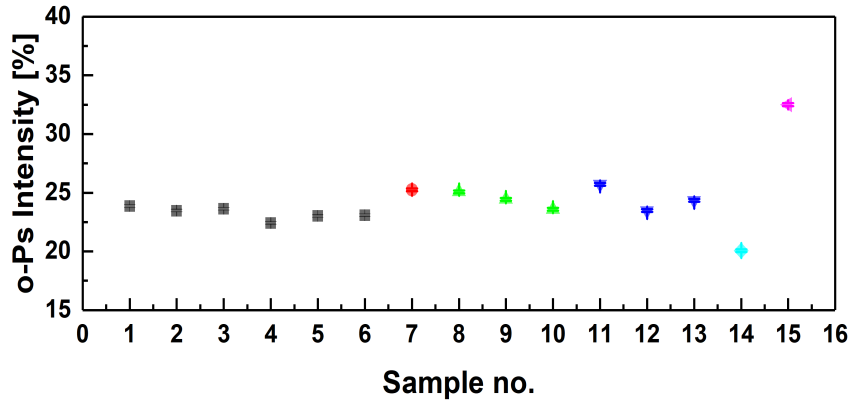
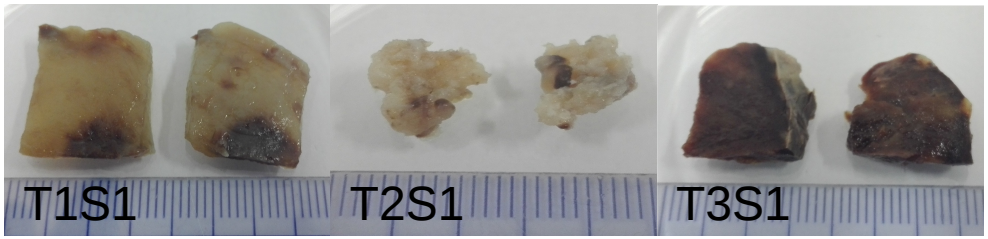
Cardiac Myxoma - PALS

→ 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



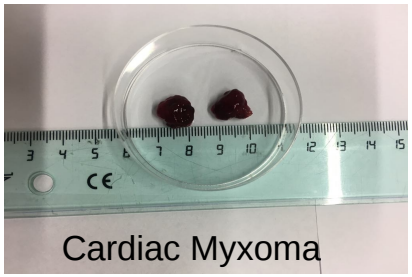


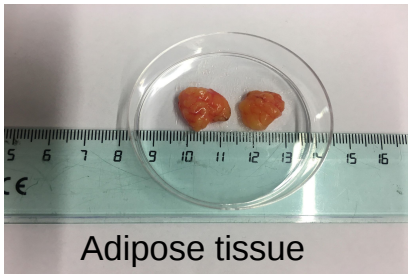


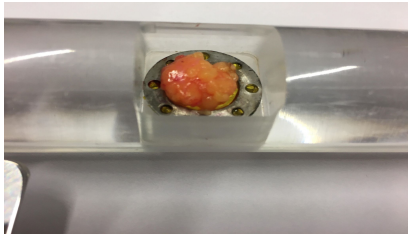


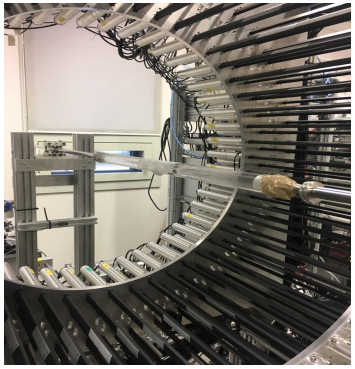
Cardiac Myxoma – JPET vs PALS

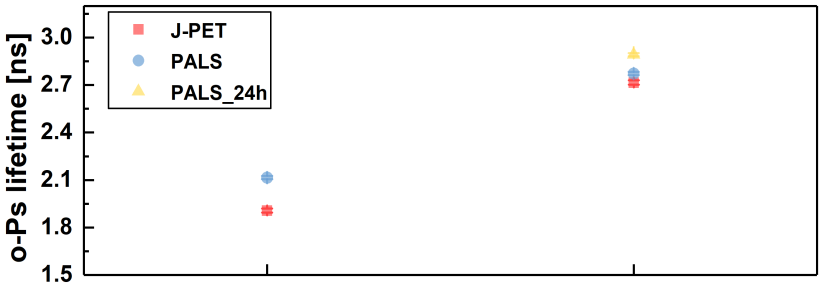
→ Samples fixed in formaldehyde → not decomposing/changing in time
→ Fresh sample measured within 4 hours after surgery
→ Time of measurement ~70-80 min → 1 mln counts

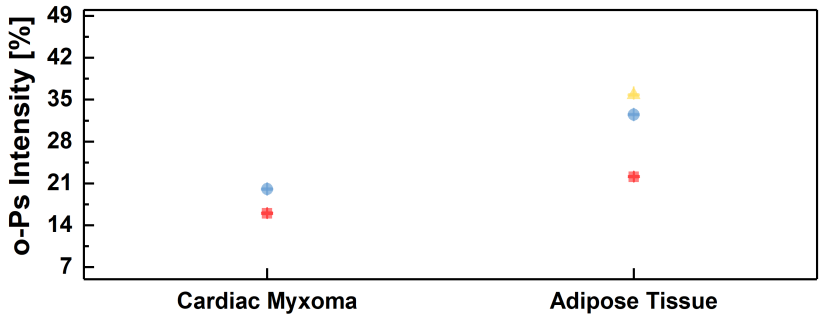

Cardiac Myxoma


Adipose tissue

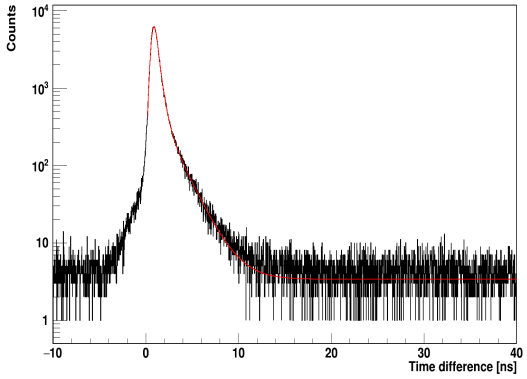




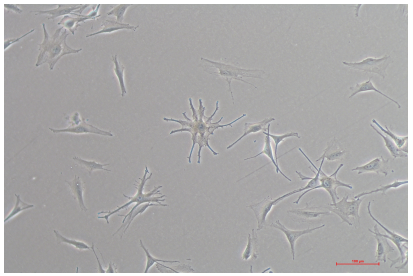




Time differences_ CardiacMyxoma_NotFixed_S1_JPET_scatterCorr_14_24_0_02_RF_0.10_RT_50.00



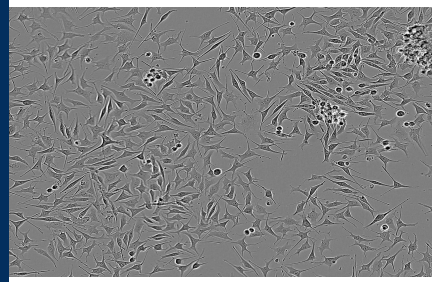
PALS – Cells culture in vitro

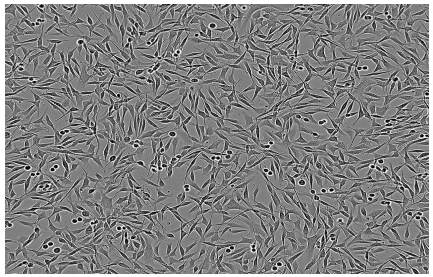


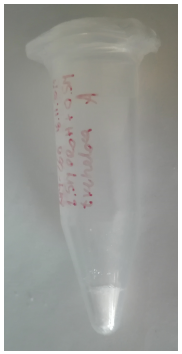
Human cell lines:

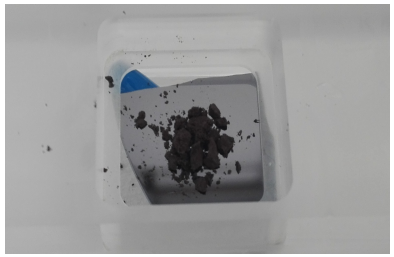
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC

- → 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% CO_2 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% confluation and freeze – dried (lyophilized).



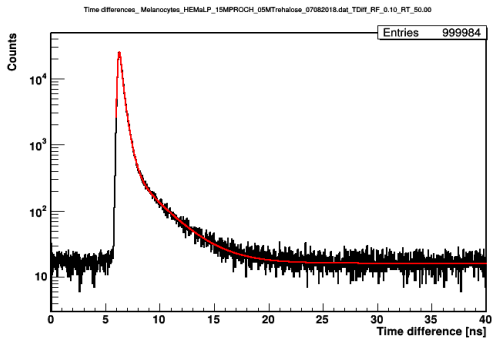






- Freeze Mediums:
- 1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
- 2) 10% DMSO + PBS w/o $^{2+}$Ca, $^{2+}$Mg
- 3) PBS w/o $^{2+}$Ca, $^{2+}$Mg
- 4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg
- 5) 0.25 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg

PALS – Cells culture in vitro



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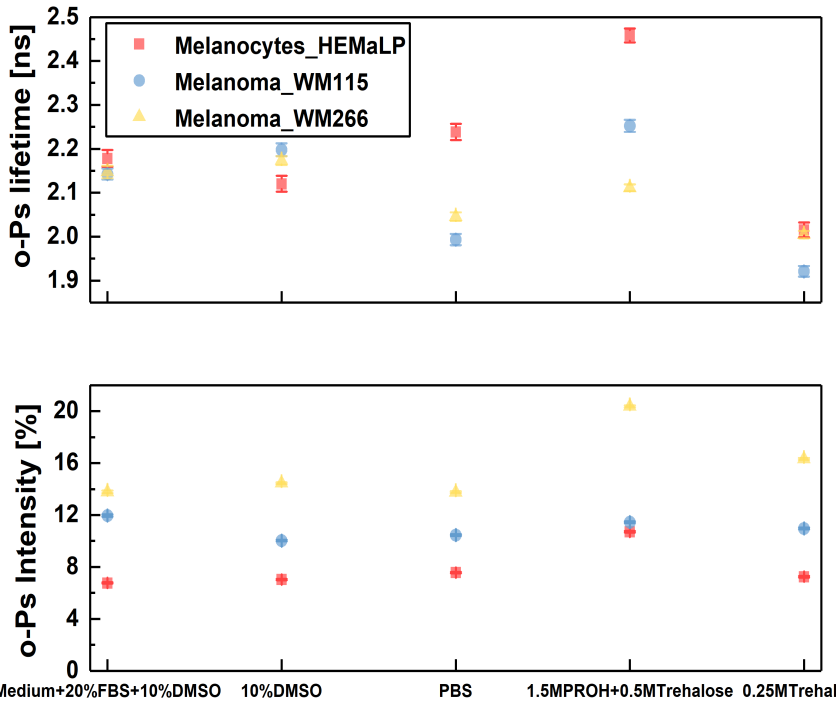
	Viability [%]	% of water	Remaining mass [%]
1	43.8	44	-
2	40.5	31	14.69%
3	5.4	22	14.24%
4	**49.7**	**49**	**46.25%**
5	10.7	16	24.62%



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Summary and future plan

→ 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