Biomedical application of Positron Annihilation Lifetime Spectroscopy - in vitro studies of alive normal and cancer cell lines and tissues

Ewelina Kubicz

27.06.2019

3rd Jagiellonian Symposium on Fundamental and Applied Subatomic Physics
Kraków

European Funds Smart Growth
Republic of Poland
FNP Foundation for Polish Science
European Union European Regional Development Fund

Outline

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

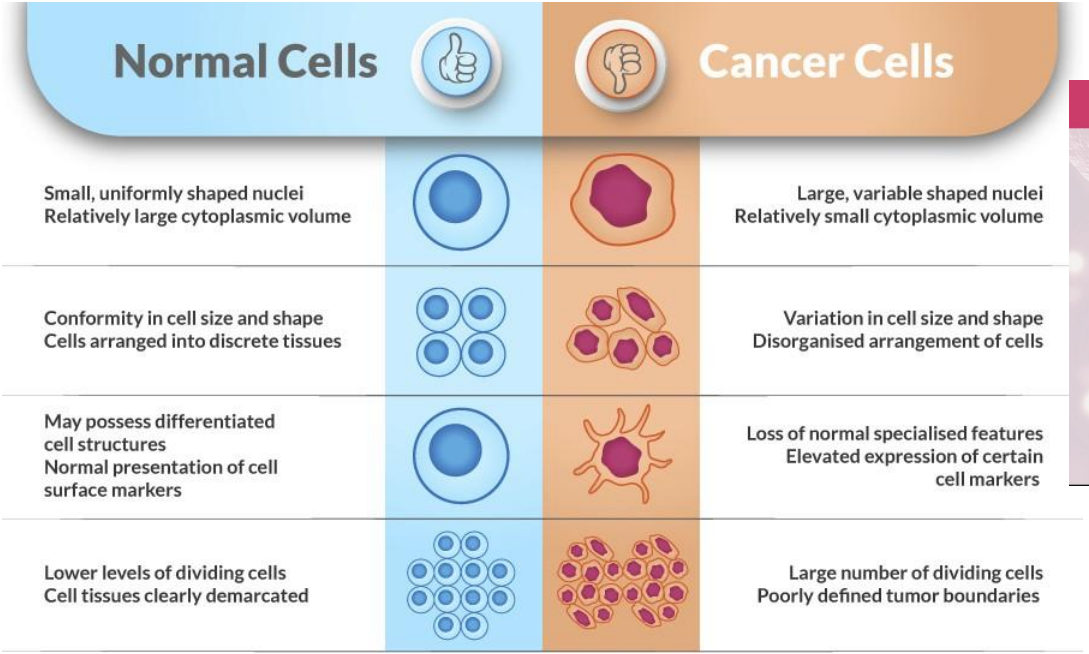
1. Motivation

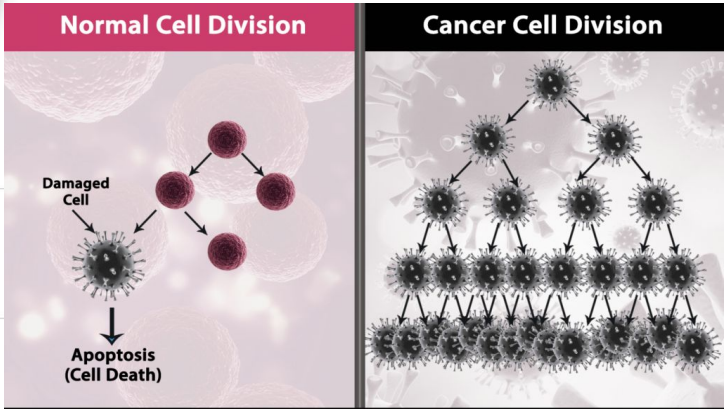
→ Positronium lifetime and intensity are related with temporal dynamics of nanostructures in cells and tissues

→ Possibility to determine early and advanced stages of carcinogenesis by observing changes in biomechanical parameters between normal and cancer cells

→ Combining J-PET scanner with PALS technique – new biomarker in cancer diagnosis

2. Cancer vs. normal cells





https://drjockers.com/cancer-cells/

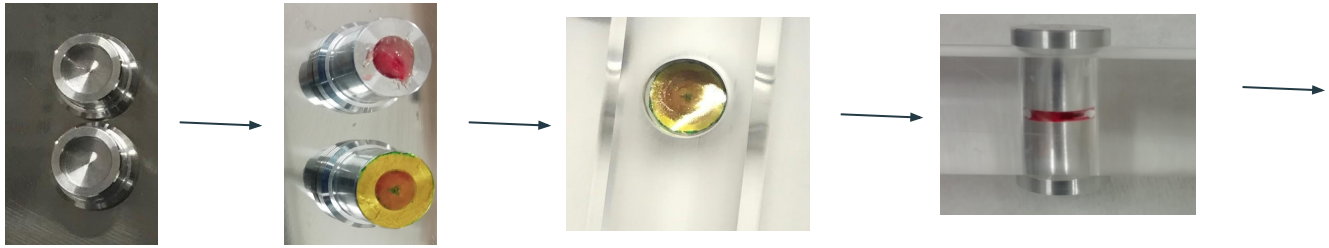
3. PALS setup

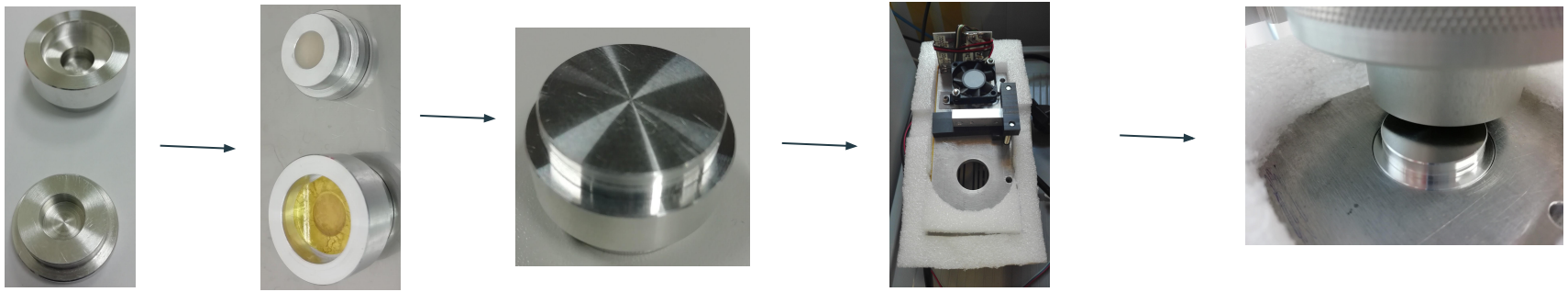
→ Two BaF_2 detectors with resolution ~250 ps

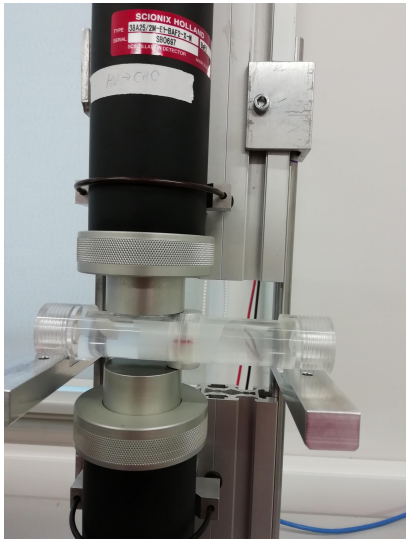
→ ^{22}Na source in Kapton foil with activity ~ 1 MBq sandwich between sample

→ 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)



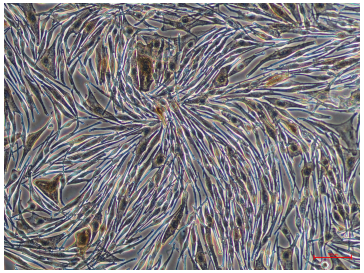


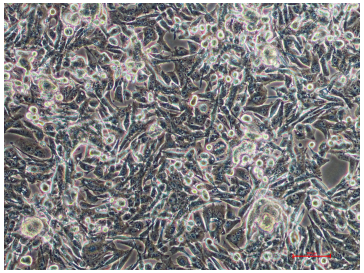


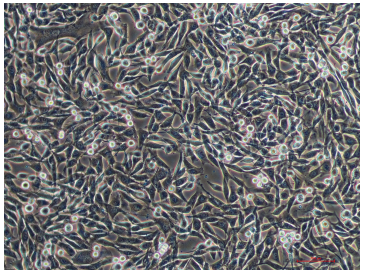
3. PALS studies of cells culture in vitro

Human cell lines:
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC

→ Cultured in M254/RPMI 1640 medium supplemented with HGMS-2/10% Fetal Bovine Serum, Penicillin 100U/ml and Streptomycin 100 ug/ml
→ Culture was incubated at 37°C in 5% CO_2 humidified atmosphere rinse

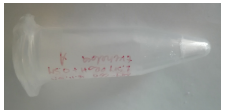

HEMa-LP


WM115

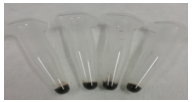

WM266

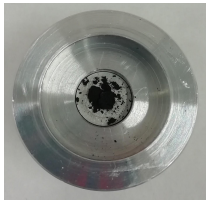
Each samples contains cells from 8 x 75cm^2 flasks, harvest upon 100% confluation (>10^8 cells).

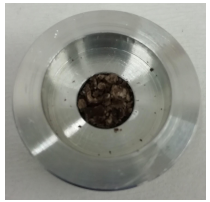
Freeze - dried (liophylized)

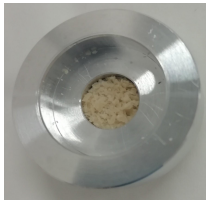


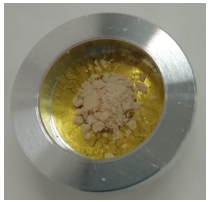
Alive Cells in 37 C deg.

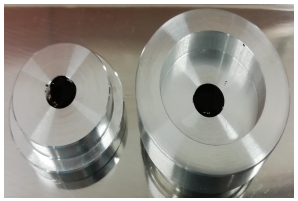


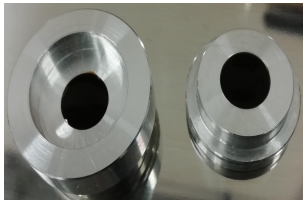

HEMa-LP

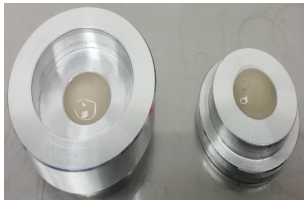

WM115


WM266




HEMa-LP

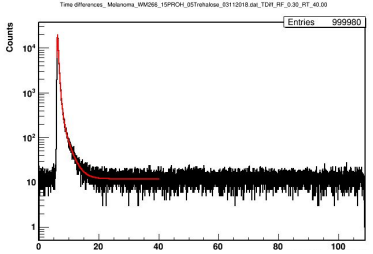

WM115

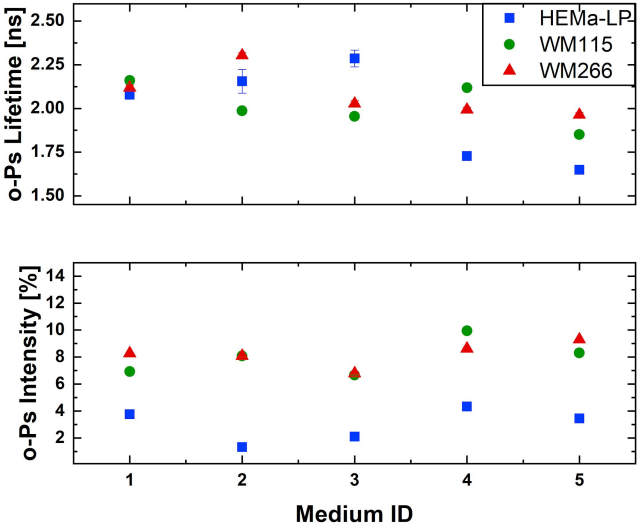

WM266

3. PALS - cells culture - freeze dried

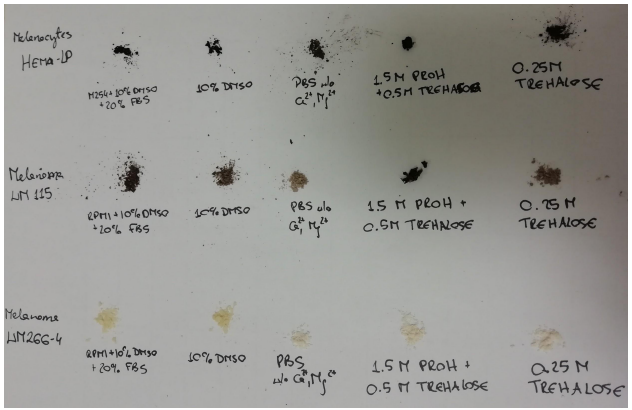
Human cell lines:

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





Cells were freeze - dried in -80 C deg., 0.0375 mbar for 24 h



Freeze Mediums:

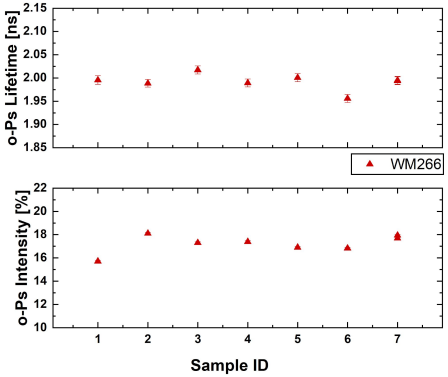
1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
2) 10% DMSO + PBS w/o $^{2+}$Ca, $^{2+}$Mg
3) PBS w/o $^{2+}$Ca, $^{2+}$Mg
4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg
5) 0.25 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg

3. PALS - cells culture -alive

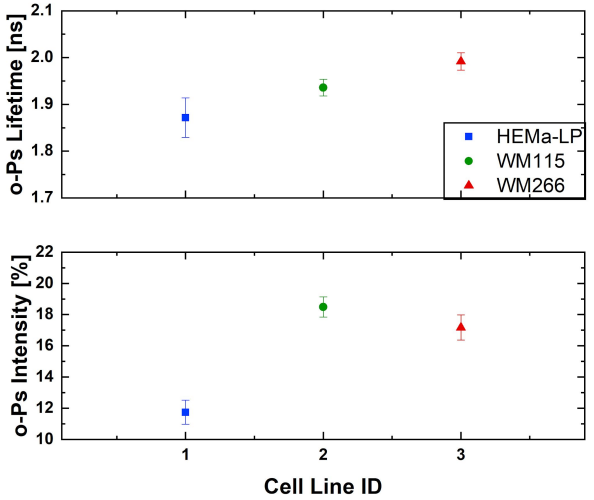
Human cell lines:

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

→ Cells were measured in 37 C deg. for 1 h



Cell Line	Viability before [%]	Viability after [%]
HEMa-LP	89.1	87.3
WM115	94.8	90.1
WM266	97.4	96.4



→ For WM266 cell line 7 repetitive measurements were done

→ Results from all are the in the line with each other within 2 sigma uncertainty

→ At least 2 repetition from each cell line were measured

→ Given results are calculated as an average value from all repetitive measurements

4. PALS studies of tumor and normal tissues in vitro

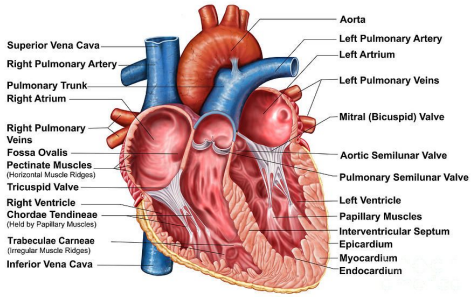
Cardiac Myxoma

→ 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

Fixed in formalin, tumor cut into ~2 mm thick samples

Patient ID	Sex	Age	Sample ID
1	woman	72	1-6
2	man	61	7
3	man	59	8-10
4	woman	54	11-13

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



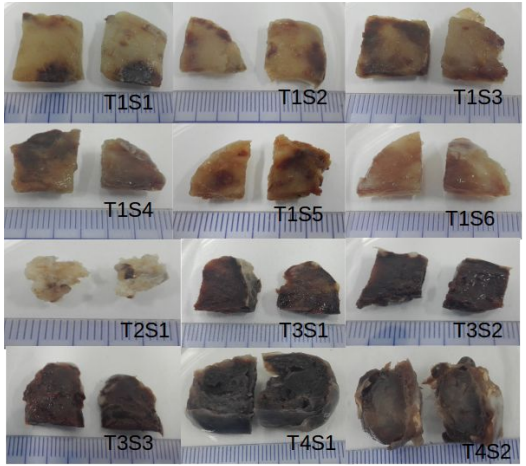
Not fixed - tumor sample measured with mediastinal adipose tissue for comparison

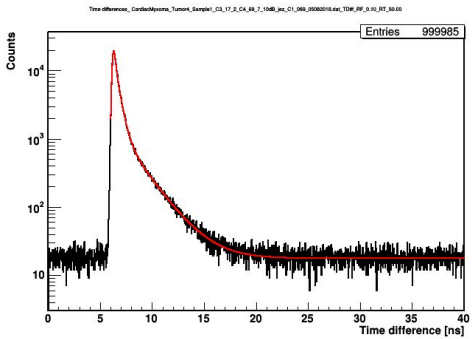
Patient ID	Sex	Age
1	man	70
2	man	58
3	woman	59
4	woman	85
5	woman	60
6	man	64

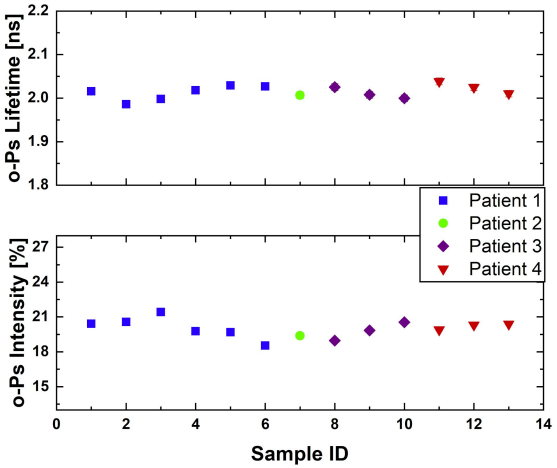
4. PALS - tissues in vitro - fixed

Cardiac Myxoma - fixed

Fixed in formalin, samples ~2 mm thick







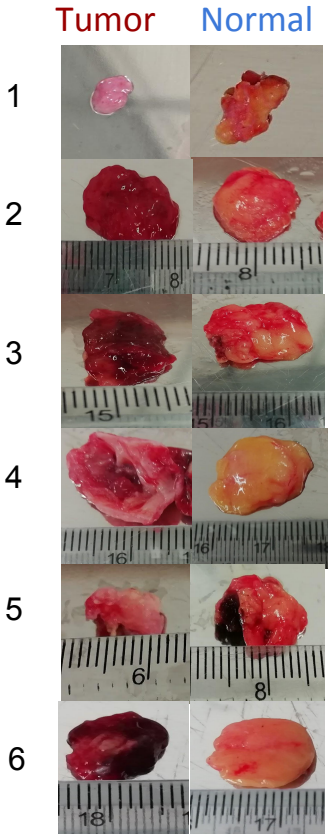
Patient ID	Sex	Age	Sample ID
1	woman	72	1-6
2	man	61	7
3	man	59	8-10
4	woman	54	11-13

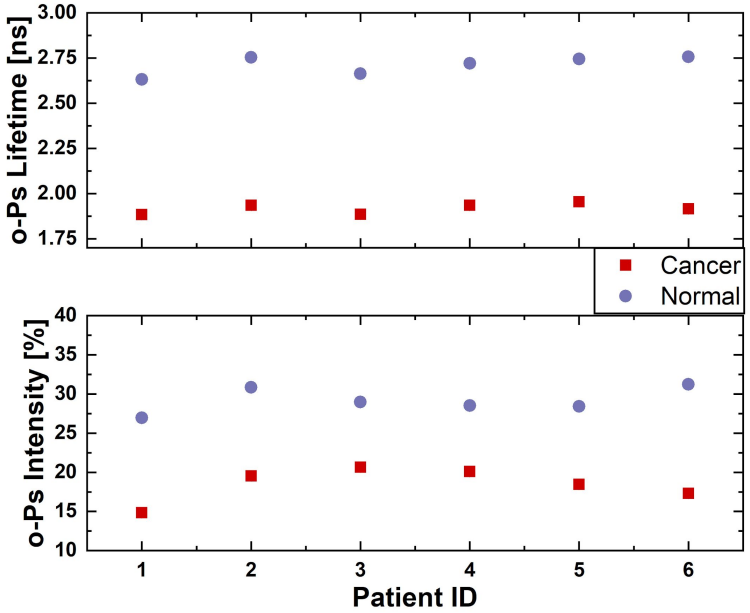
4. PALS - tissues in vitro - not fixed

Cardiac Myxoma - not fixed

Not fixed - tumor sample measured with mediastinal adipose tissue for comparison

Patient ID	Sex	Age
1	man	70
2	man	58
3	woman	59
4	woman	85
5	woman	60
6	man	64





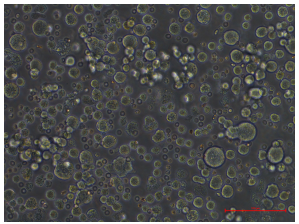
4. PALS - cell vs tissues in vitro - not fixed

Cardiac Myxoma

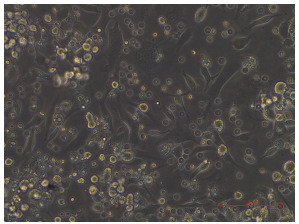
⟶ Tumor sample placed in Collagenase II 200U/ml soluble in DMEM+10% FBS + P/S for 48 h

⟶ Squeezed through 70 um nylon mesh to isolate cells from extracellular matrix

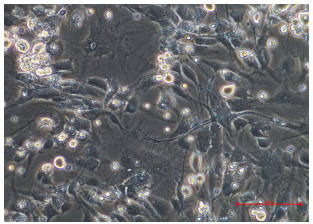
⟶ Cells were seeded on flasks and culture in DMEM + 10% FBS + P/S



0 h

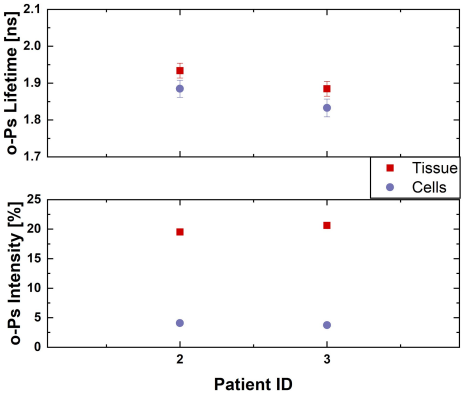


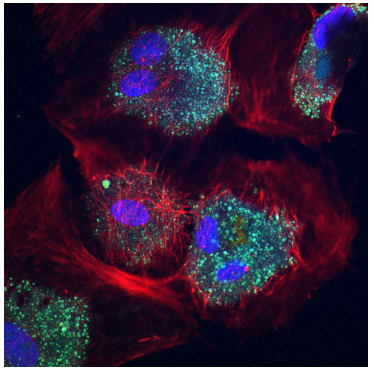
24 h

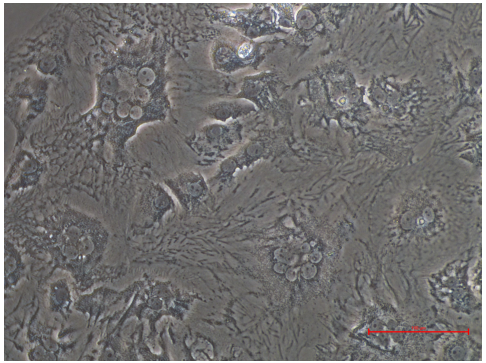


72 h

Patient ID	Sex	Age
2	man	58
3	woman	59

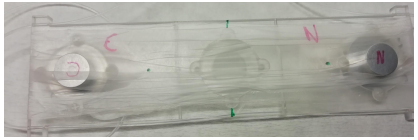


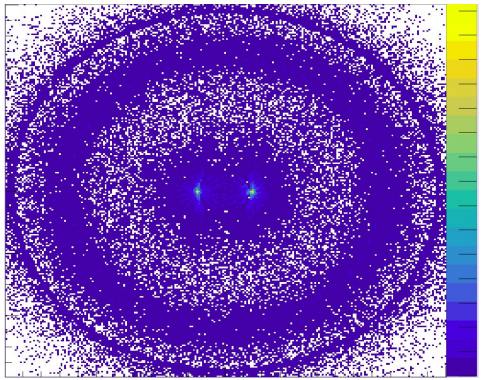


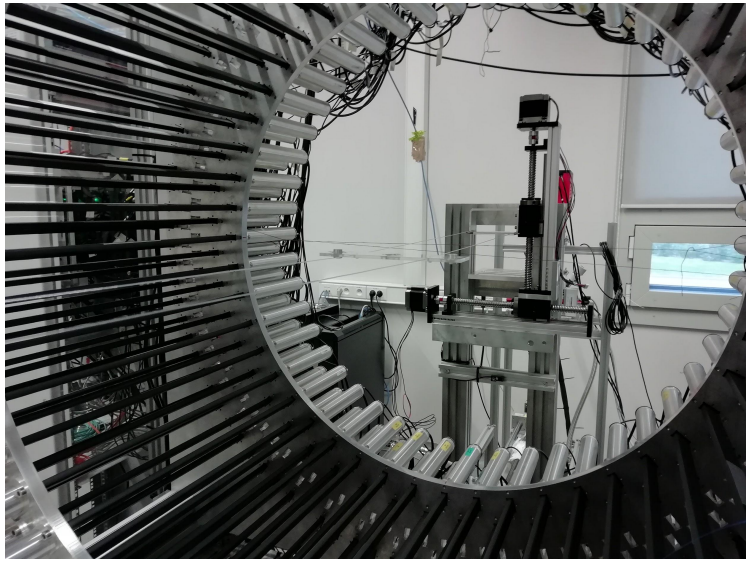


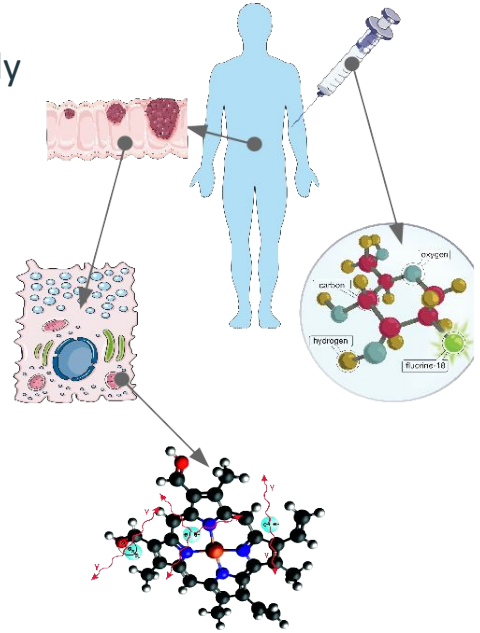
5. PALS on J-PET

→ Preliminary research proved PALS technique can be applied on J-PET
→ Studies with cardiac myxoma and colon cancer increasing statistic of the study
→ Cancer and normal tissue sample can be measured at the same time
→ o - Ps lifetime and intensity - new diagnostic biomarker for cancer









6. Summary and future plans

→ 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

→ Studies with alive cell cultures and tissues – comparing normal vs cancer

→ Checking for possible o-Ps formation model in living cells

Thank you for attention