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Effect of ionizing radiation on prostate cancer cells studied by vibrational spectroscopy

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1. Introduction

- influence of ionizing radiation on living organisms
- data analysis
- Raman spectroscopy for analysis of radiation-induced damage / response

2. Results

2.1. Published data

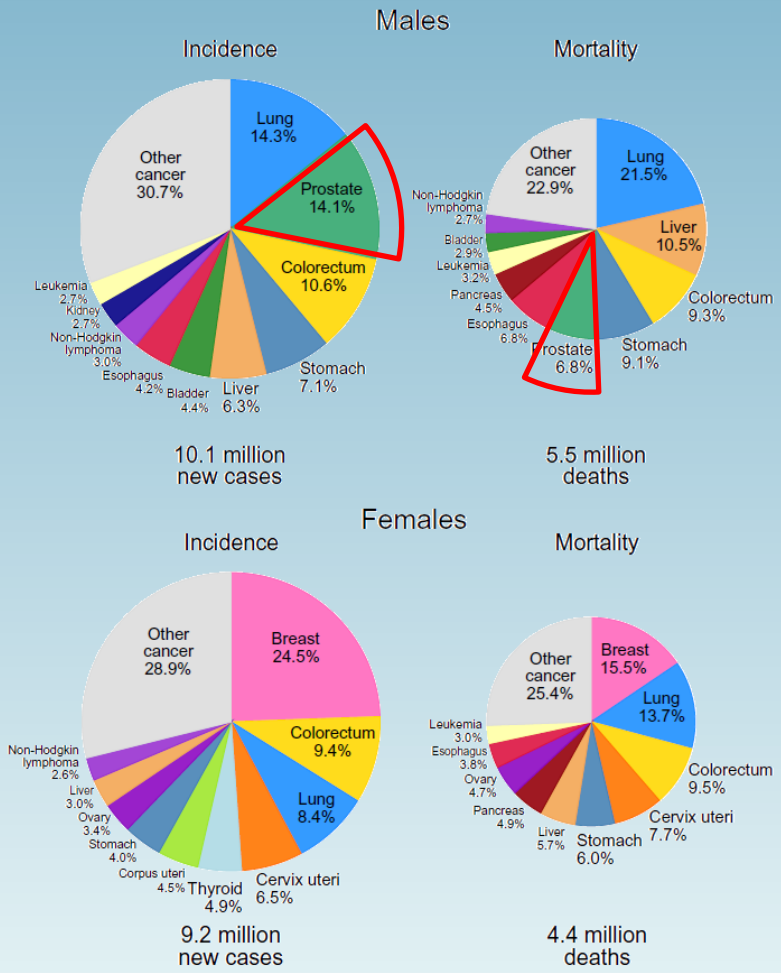
- influence of ionizing radiation on cytoplasm and nucleus
- response to clinical doses
- physicochemical damage vs. early-stage biological response
- lipid droplets in prostate cancer cells and effect of radiation
- nanoscale imaging of lipids

2.2. Current and future tasks

3. Conclusions

Neoplastic diseases and methods of their treatment

→ probably the most common cause of death in the 21st century!!!



Cancer treatment methods:

- surgery
- chemotherapy
- radiotherapy
- combination therapy (chemotherapy + radiotherapy, etc.)

Cyclotron Centre Bronowice

H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, *CA-Cancer J. Clin.*, **0**, 2021, 1.

Ionizing radiation and living matter

→ ionization
→ excitation
→ emission

} breaking / formation of chemical bonds → vibrational spectroscopy

Types of damage:

1. primary - caused directly by radiation

2. secondary - caused by knocked-out electrons, emitted photons or generated free radicals (water radiolysis)

a) lipid peroxidation

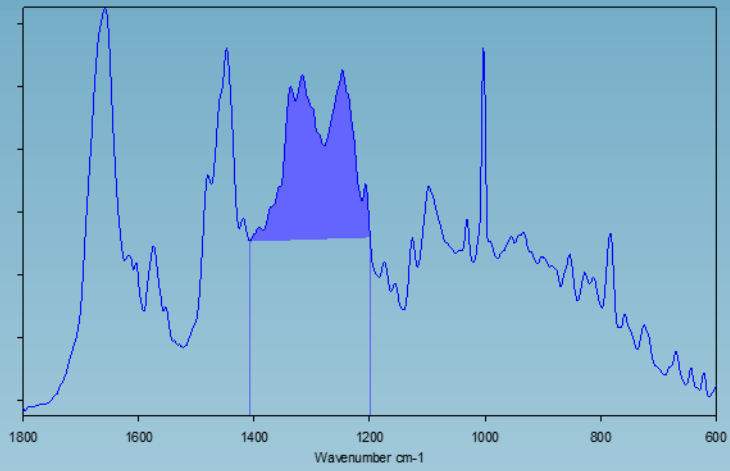
b) damage to the structure of proteins

c) DNA damage (DNA strands cross-linking, DNA strands cross-linking with proteins, single strand breaks, base damage, sugar damage and the most dangerous DNA double strand breaks)

•) fatal – apoptosis

•) repairable - repair processes (biological response of the cell)

Data analysis



Analysis of characteristic bands

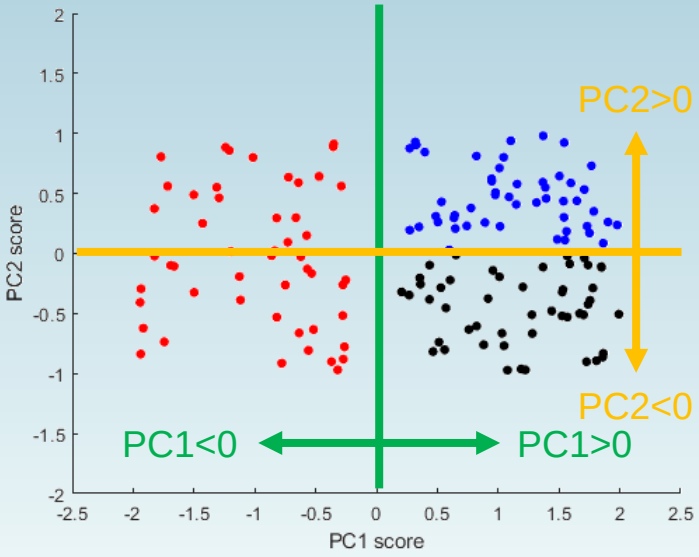
→ integral intensity of the bands

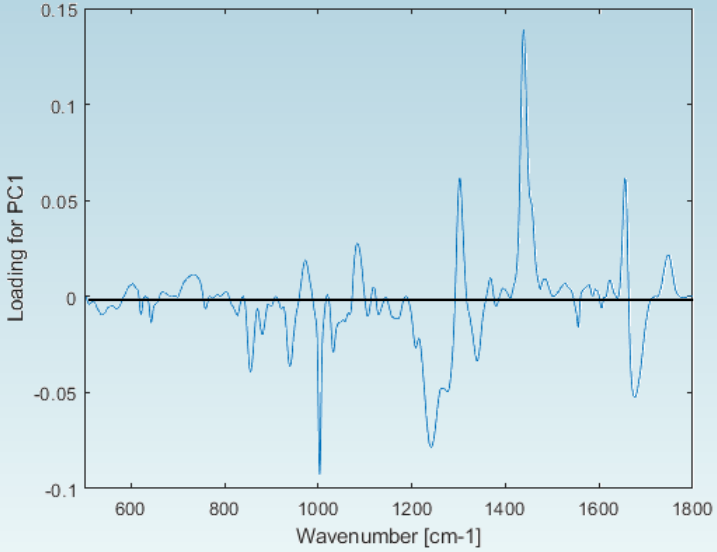
Chemometrics (multivariate analysis)

→ Principal Component Analysis (PCA)

- reduction of the data dimension (correlations between variables)

- finding principal components that are linear combinations of the input variables (taking into account the variability in the data)

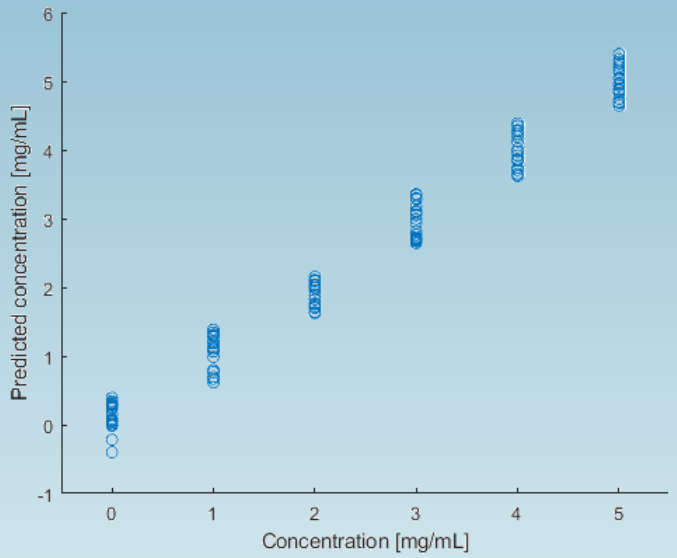


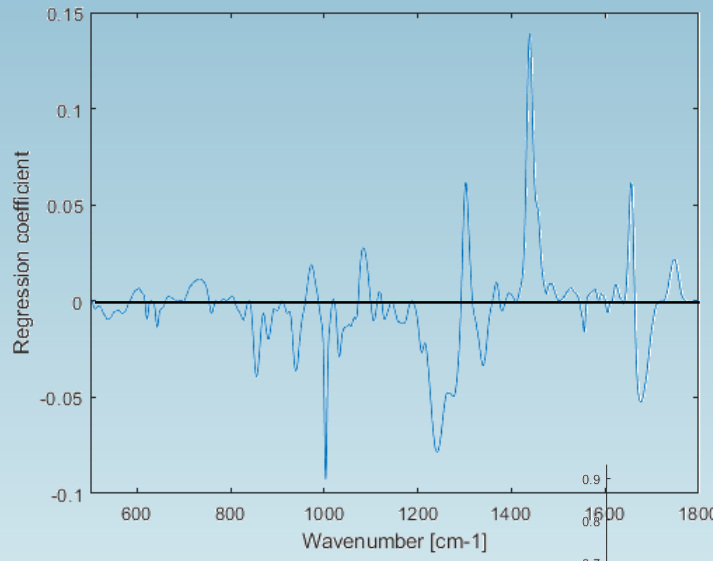


→ regression methods (e.g. Partial Least-Squares Regression, PLSR)

- observable variables (e.g. spectra) and predictor variables (e.g. concentration, radiation dose))

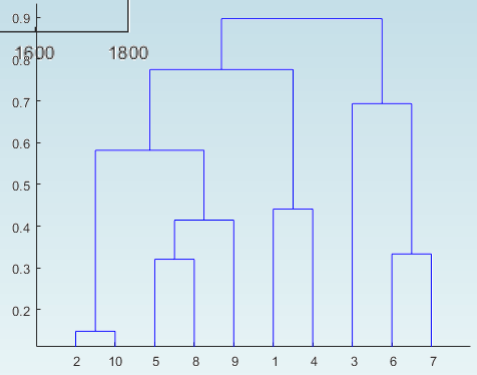
- PLSR finds a linear regression model by projecting the predicted variables and the observable variables to a new space





→ Cluster Analysis (CA)

- division of a set of objects into groups (similarity of objects)



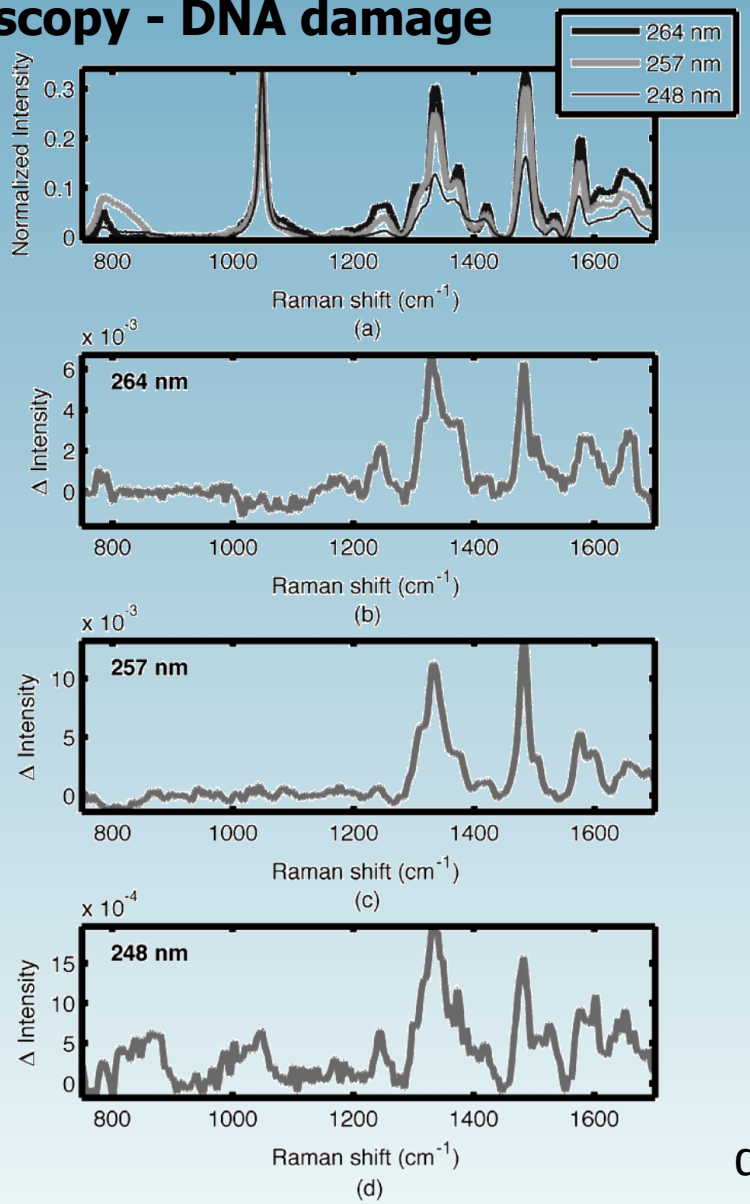
Application of Raman spectroscopy - DNA damage

unirradiated

irradiated – unirradiated (264 nm)

irradiated – unirradiated (257 nm)

irradiated – unirradiated (248 nm)



dose ~2000 Gy

C.P. Shaw, A. Jirasek, *Applied Spectroscopy,* **63**, 2009, 412.

Effect of ionizing radiation – radiosensitivity

Studies on commercially available cell lines

- metastatic prostate cancer cell lines:

DU145 cell line – bone metastasis

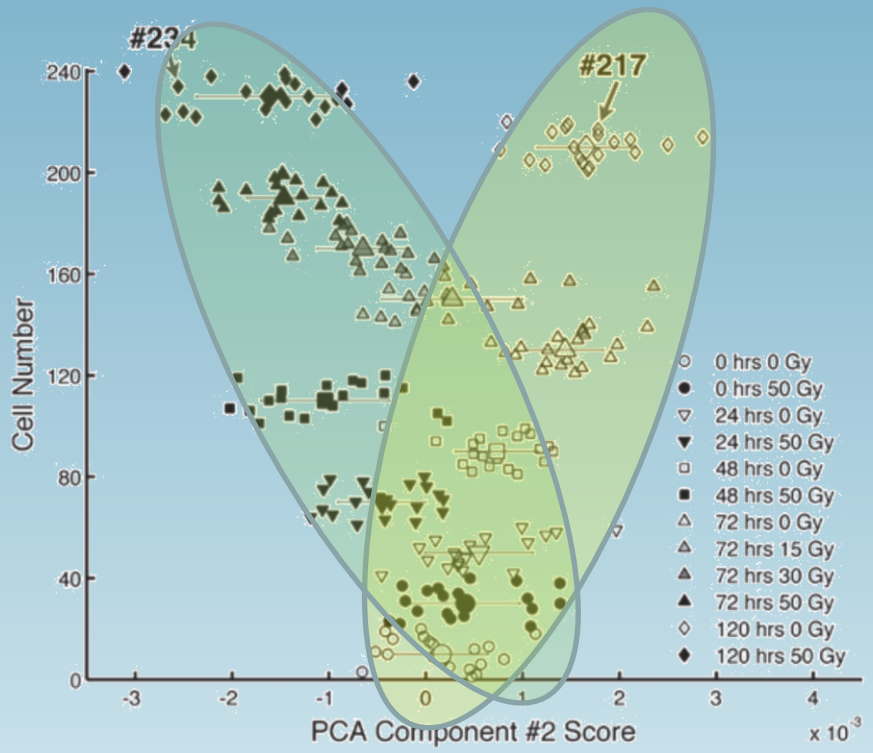
LNCaP cell line – lymph node metastasis

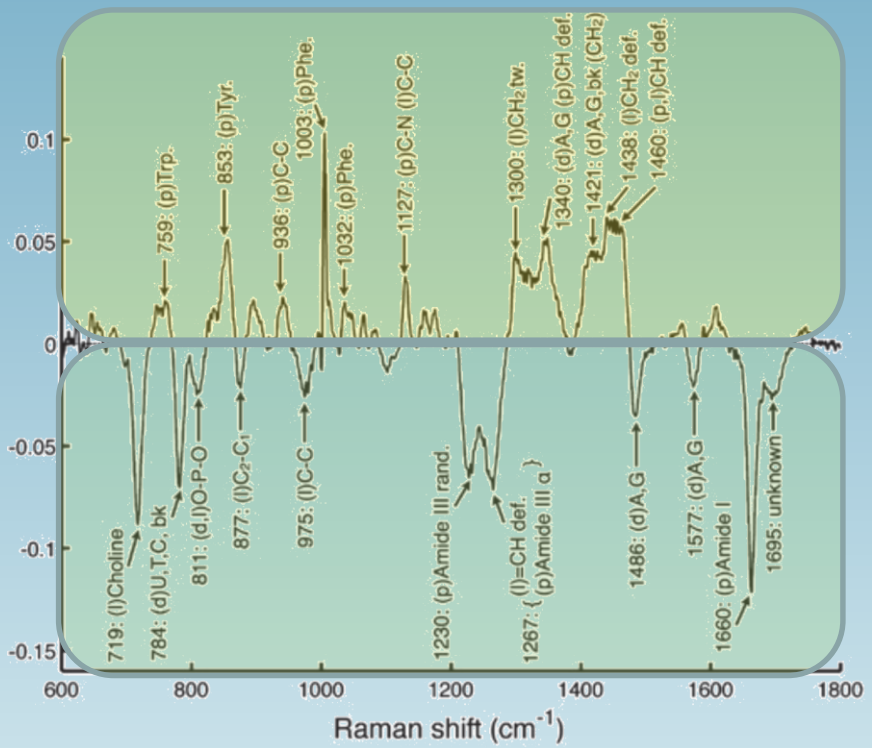
→ radiosensitive

PC-3 cell line – bone metastasis → radioresistant

Q. Matthews, A. Jirasek, J.J. Lum, A.G. Brolo, *Phys. Med. Biol.*, **56,** 2011, 6839.

Effect of ionizing radiation on prostate cancer cells (DU145 – radiosensitive)



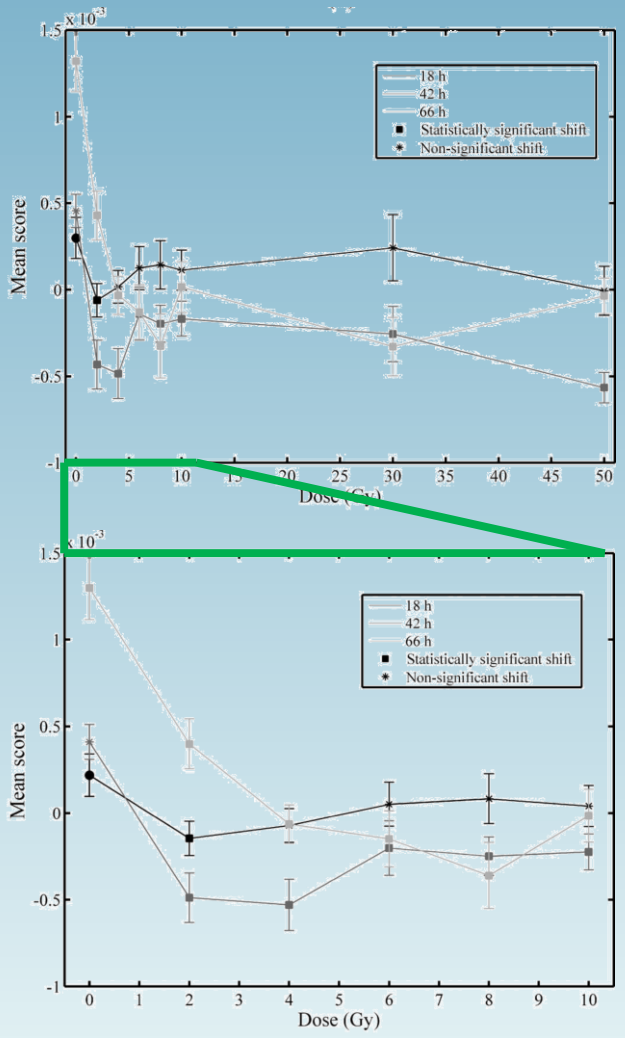


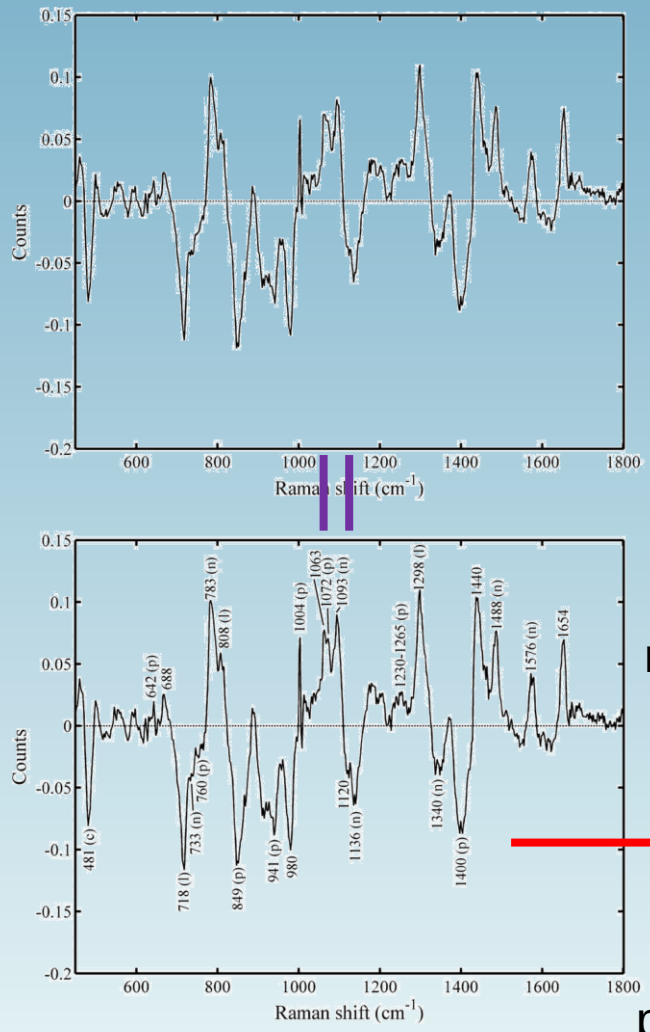
PC2 → DNA/RNA, proteins, lipids
(effect of ionizing radiation)

Q. Matthews, A.G. Brolo, J. Lum, X. Duan, A. Jirasek, *Phys. Med. Biol.*, **56,** 2011, 19.

photons 6 MV (5.9 Gy/min)

Effect of ionizing radiation – clinical doses (2 – 10 Gy)





LNCaP cell line
(radiosensitive)

membrane phospholipids

photons 6 MV (5.9 Gy/min)

S.J. Harder, Q. Matthews, M. Isabelle, A.G. Brolo, J.J. Lum, A. Jirasek, *Applied Spectroscopy,* **69**, 2015, 193.

RESULTS

Motivation:

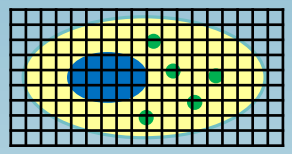
► radioresistance of cell lines → radioresistant PC-3 cell line

► single point measurements (one spectrum per cell) – cell heterogeneity → Raman mapping

► two effects of ionizing radiation → radiation-induced damage and biological response

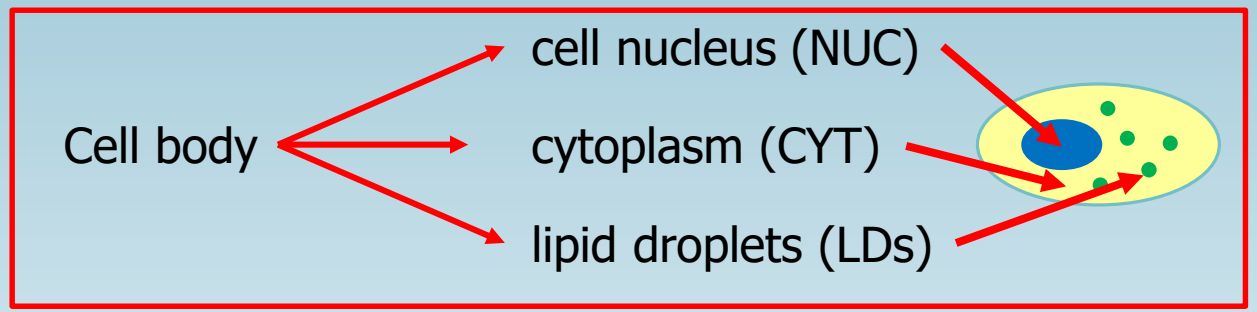
► weakness of PCA in analysis of radiation-induced effects → PLSR

- Sample preparation
- PC-3 cells (prostate cancer, bone metastasis) on the CaF_2 windows
- ionizing radiation (X-ray, doses: 2, 4, 6, 8, 10, 30, 50 Gy)
- fixation (3.7% PFA) just after irradiation (0h)
- fixation (3.7% PFA) 24h and 48h after exposure
- rinsing/drying



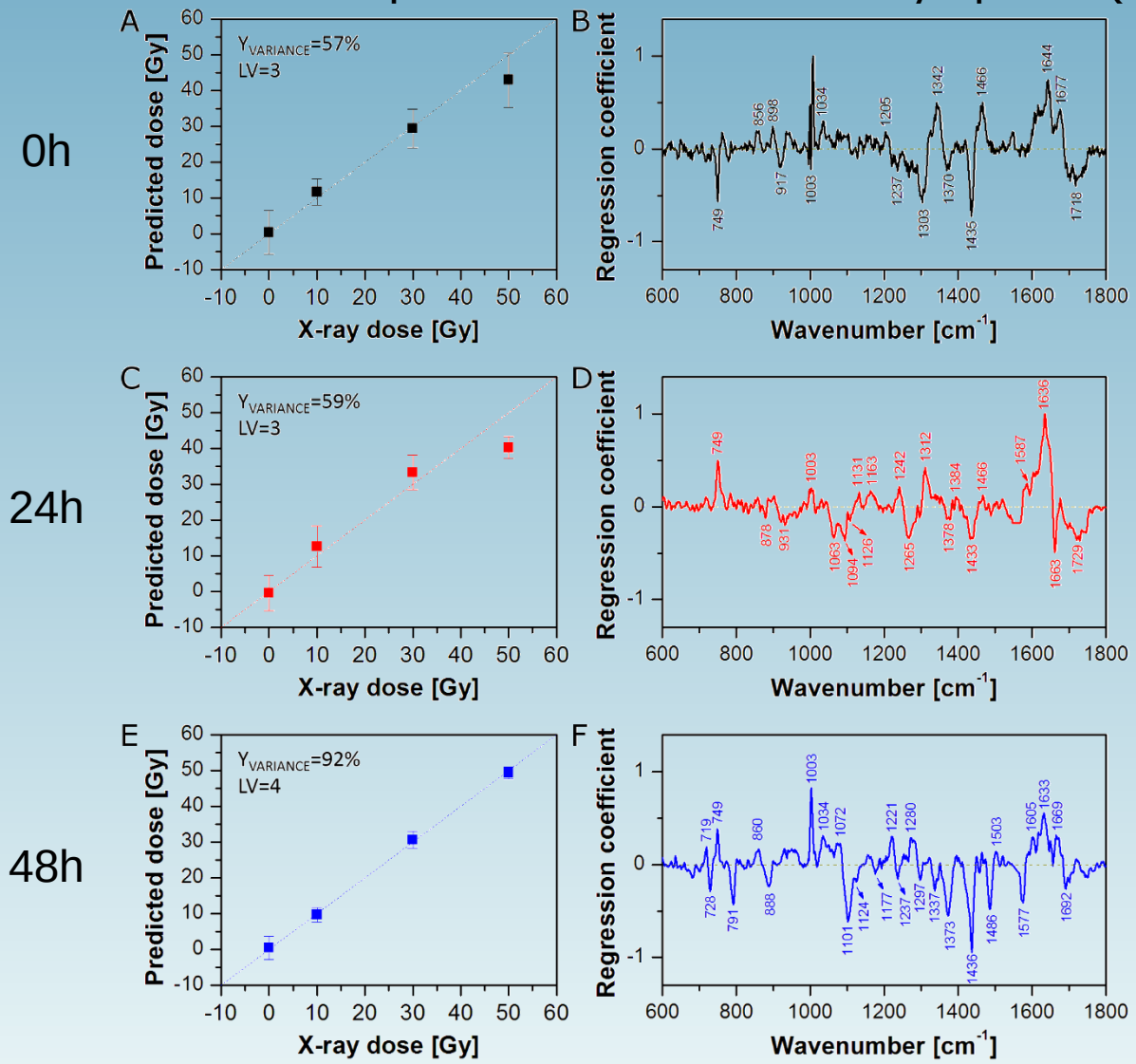
2 μm step size

- Raman mapping
- whole cell area
- 532 nm, ~ 7.5 mW
- 20 s, 3 scans



- AFM-IR imaging
- contact mode
- laser power 1%/0.4% (0.12μJ/0.05μJ per cell)
- images (scan rate of 0.06 Hz, 520 pixel resolution (0.04Hz/780 pixels for HR))
- Data analysis
- MatLab (cosmic ray removal, baseline correction, smoothing, normalization, chemometrics (PLSR))

PLSR – mean RS spectra from nucleus and cytoplasm (NUC+CYT)

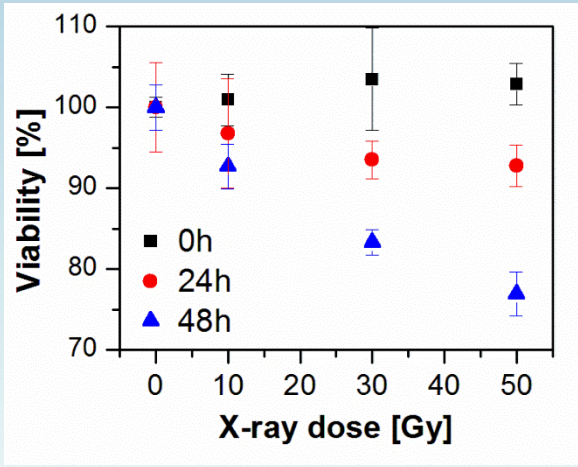


proteins ↑
- repair processes

DNA/RNA ↓
- damage to genetic material

lipids ↓
- stress (lipid release)

MTT – metabolic activity test

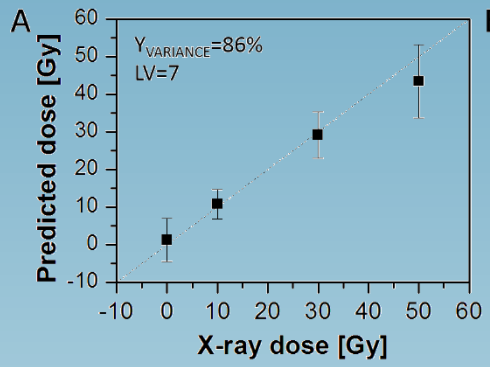


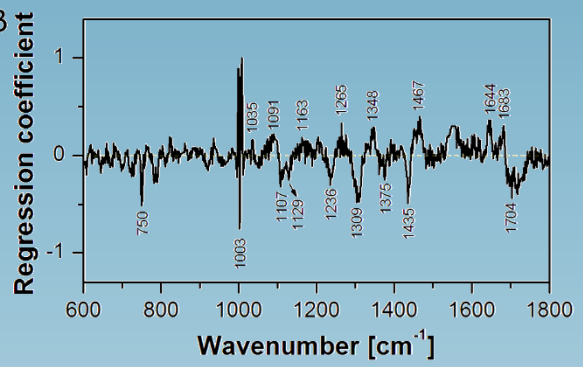
M. Roman *et al.*, *Sci. Rep.*, **9**, 2019, 8715.

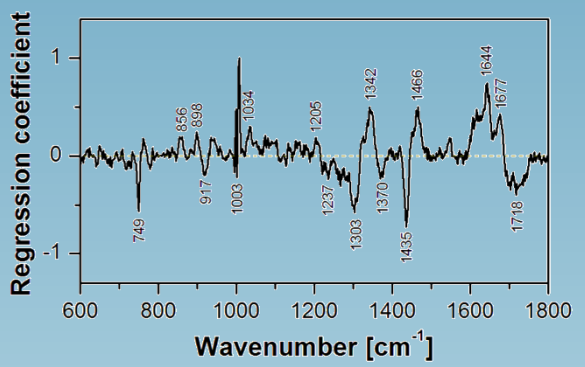
PLSR – mean RS spectra from cytoplasm (CYT)

NUC+CYT

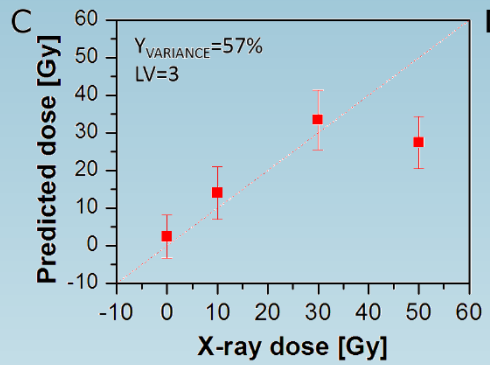
0h

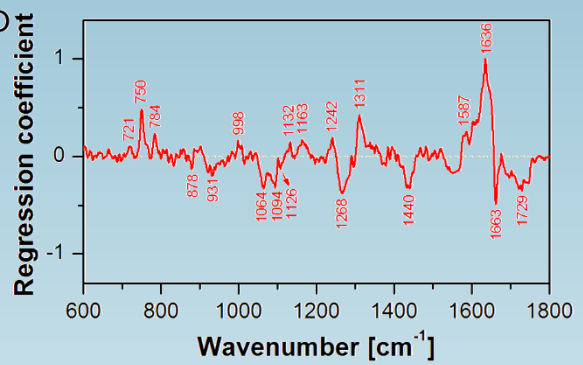


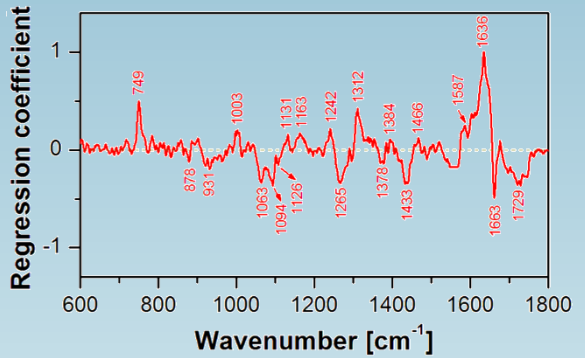




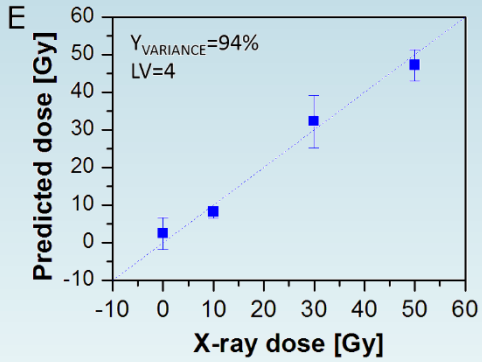
24h

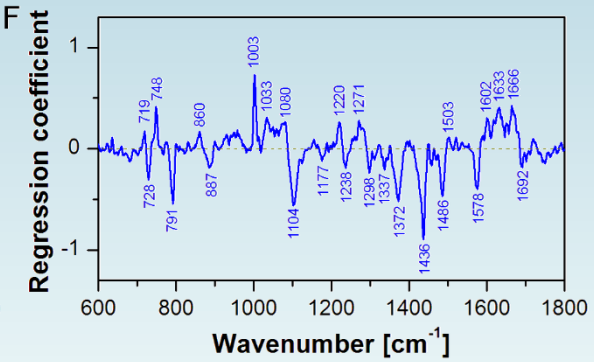


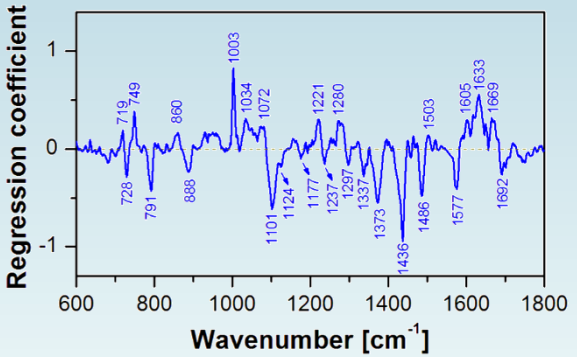




48h





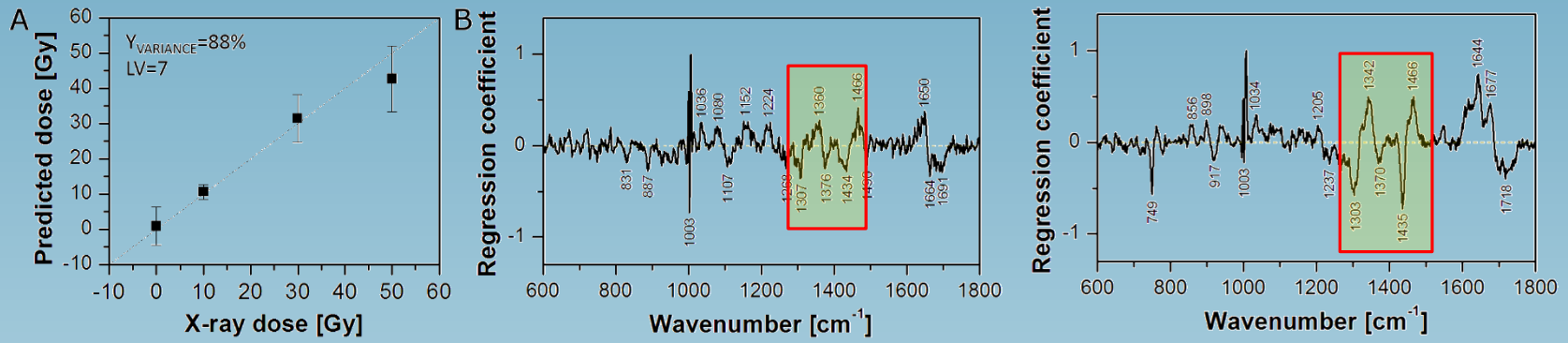


M. Roman *et al.*, *Sci. Rep.*, **9**, 2019, 8715.

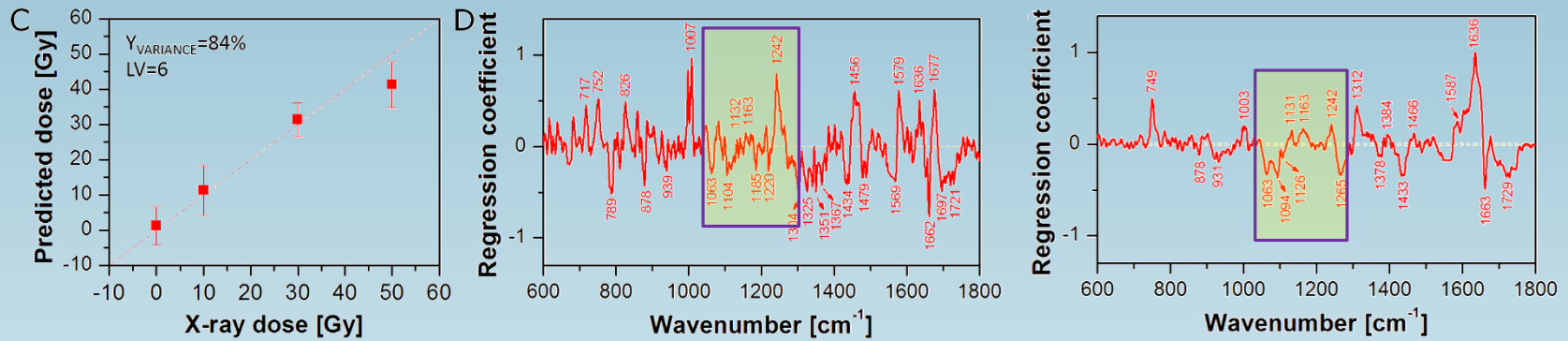
PLSR – mean RS spectra from nucleus (NUC)

NUC+CYT

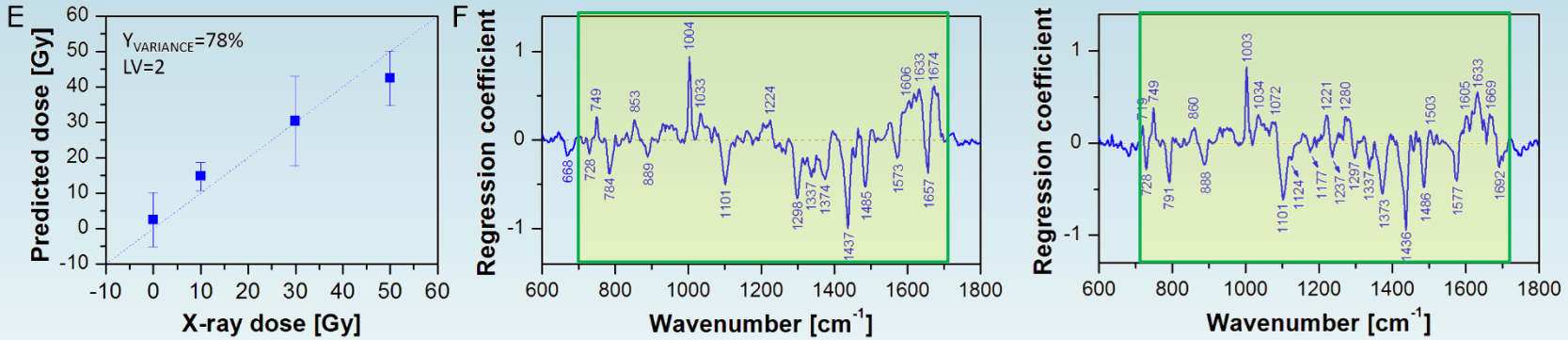
0h



24h

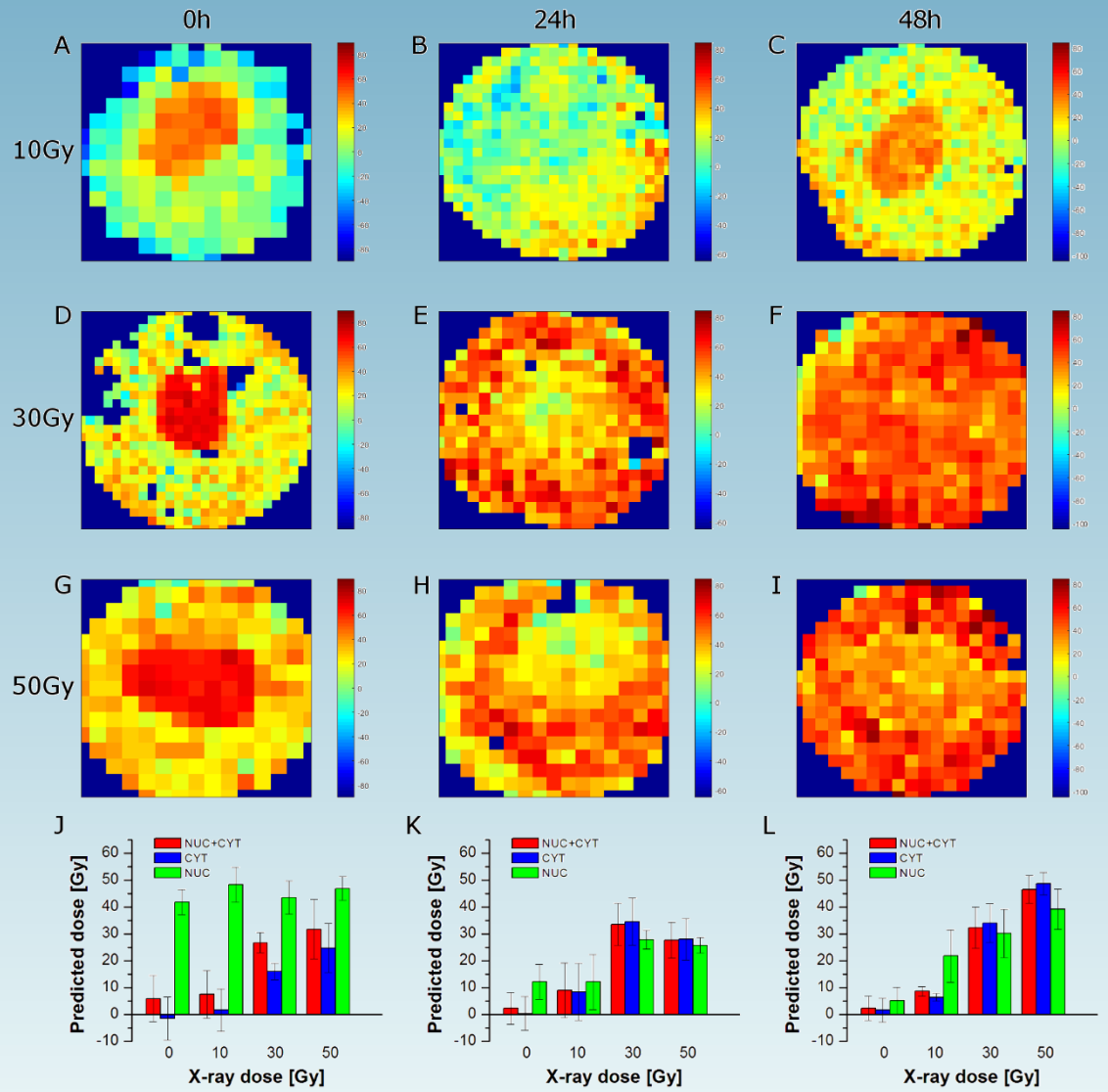


48h



M. Roman *et al.*, *Sci. Rep.*, **9**, 2019, 8715.

PLSR – doses predicted on the basis of mean spectra models (NUC+CYT)



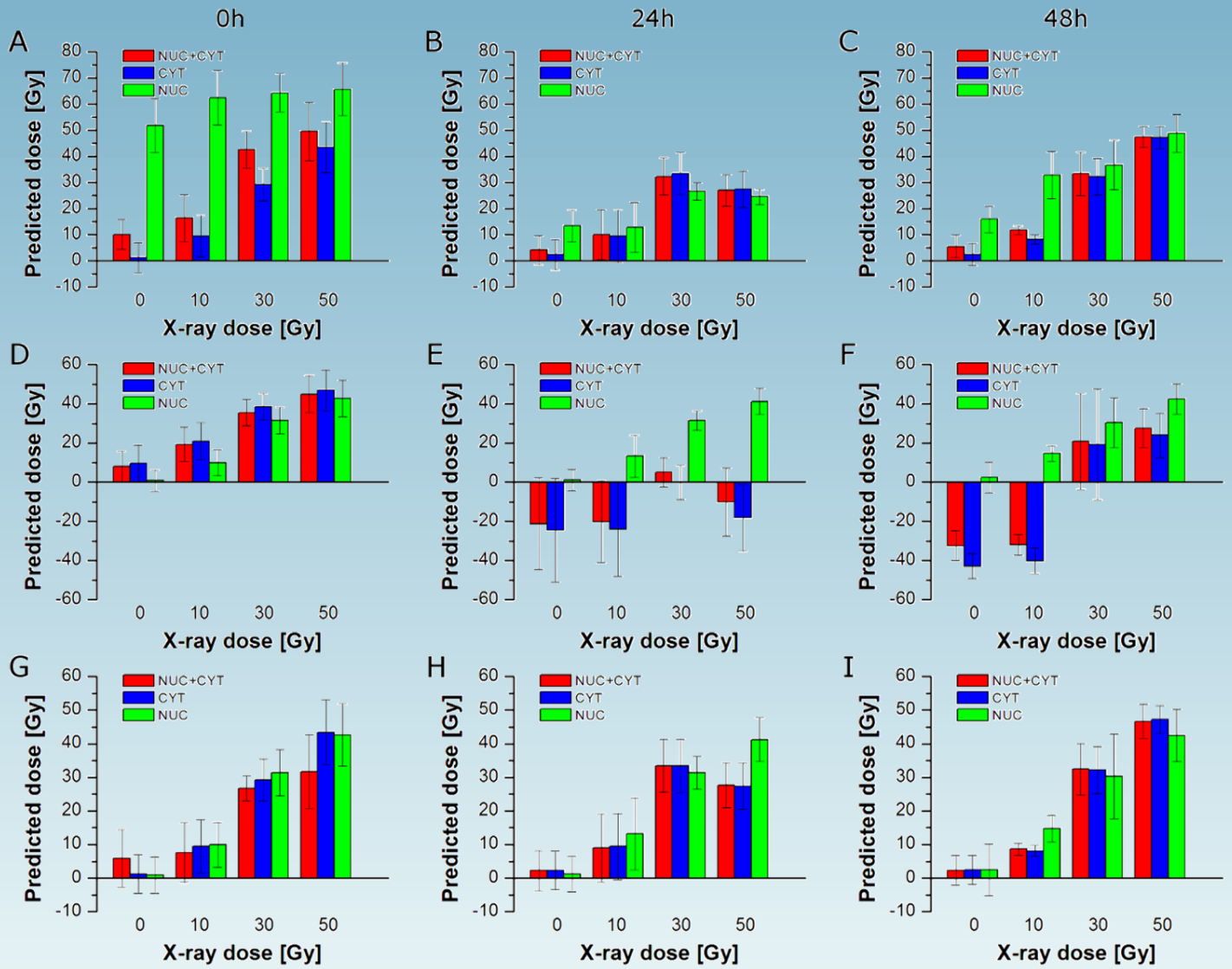
M. Roman *et al.*, *Sci. Rep.*, **9**, 2019, 8715.

PLSR – doses predicted on the basis of different models

cytoplasm models

cell nucleus models

separate models:
whole cell
cytoplasm
cell nucleus

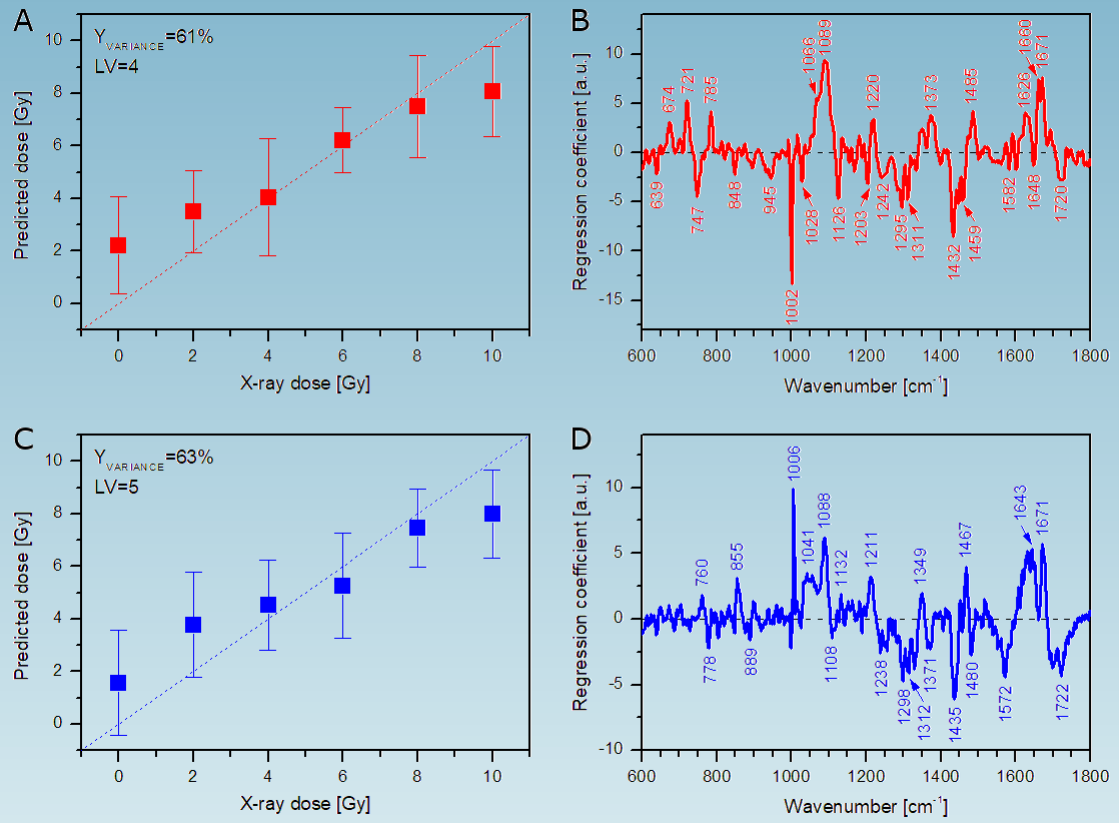


M. Roman *et al.*, *Sci. Rep.*, **9**, 2019, 8715.

PLSR – mean RS spectra for 0h – low doses

cytoplasm

cell nucleus

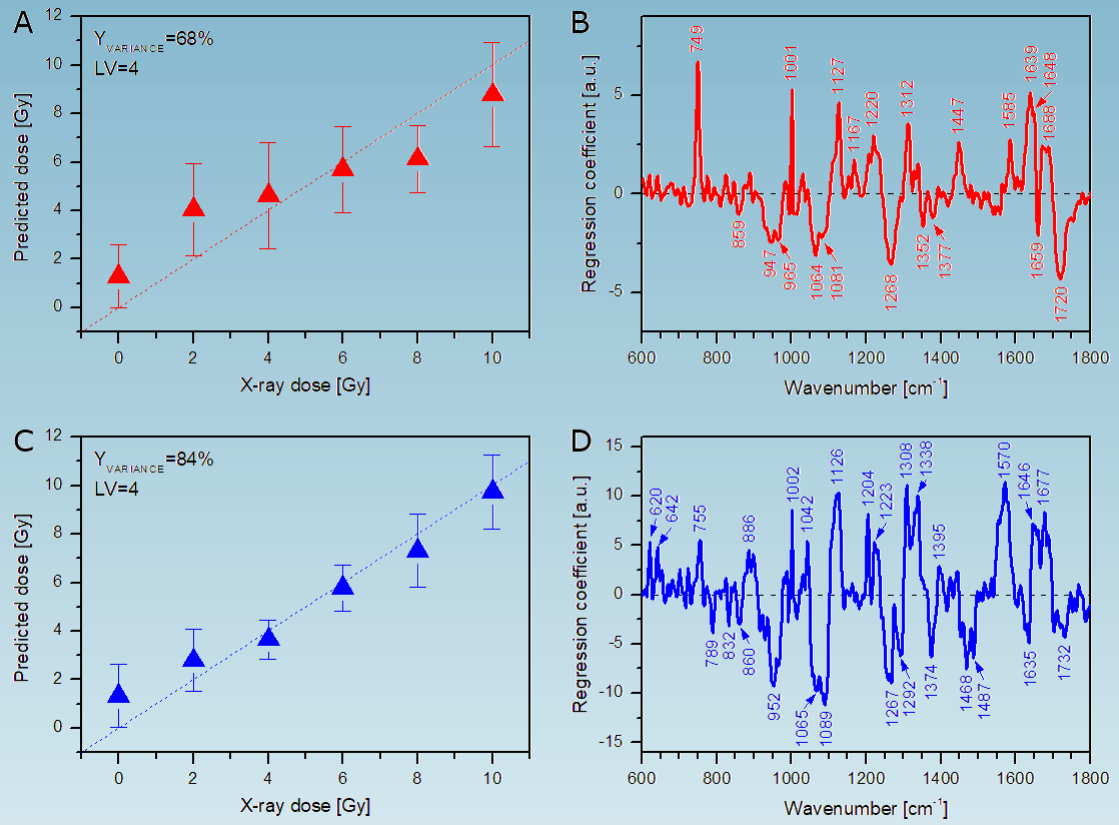


M. Roman *et al.*, *Spectrochim. Acta A*, 2021, in press.

PLSR – mean RS spectra for 24h – low doses

cytoplasm

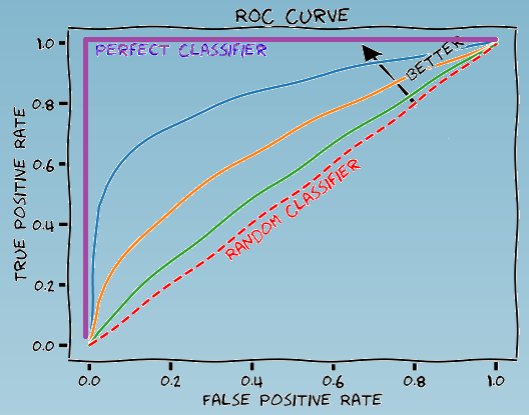
cell nucleus



M. Roman *et al.*, *Spectrochim. Acta A*, 2021, in press.

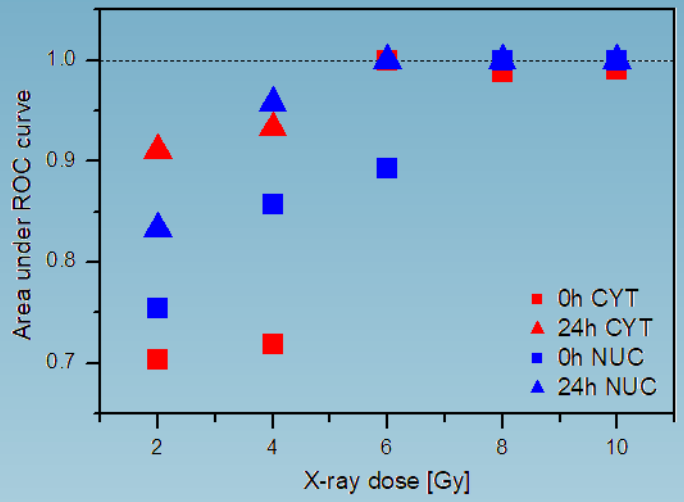
PLSR – classification – low doses

Receiver operating characteristic



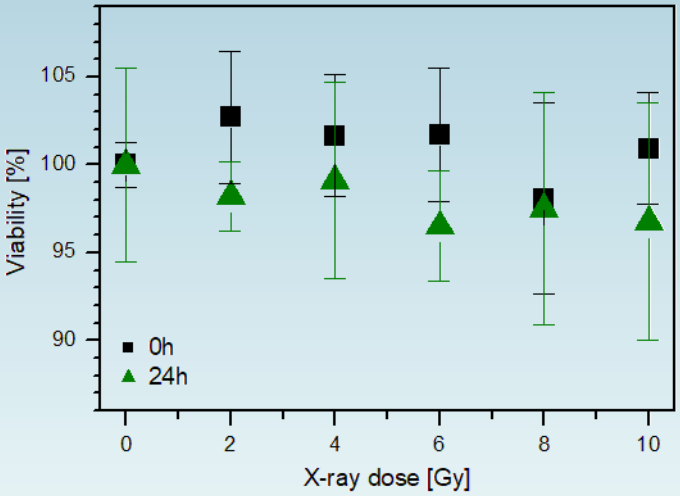
$$TPR = \frac{TP}{TP + FN}$$

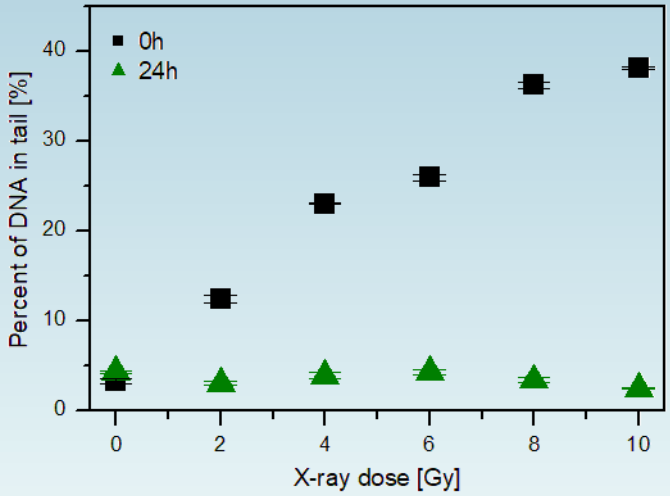
$$FPR = \frac{FP}{FP + TN}$$

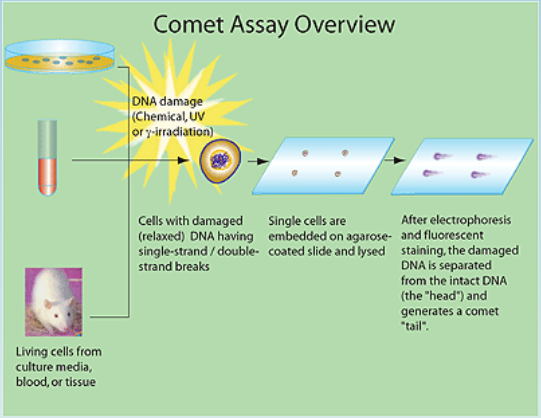


https://glassboxmedicine.com/2019/02/23/measuring-performance-auc-auroc/

Biological assays (MTT and comet)



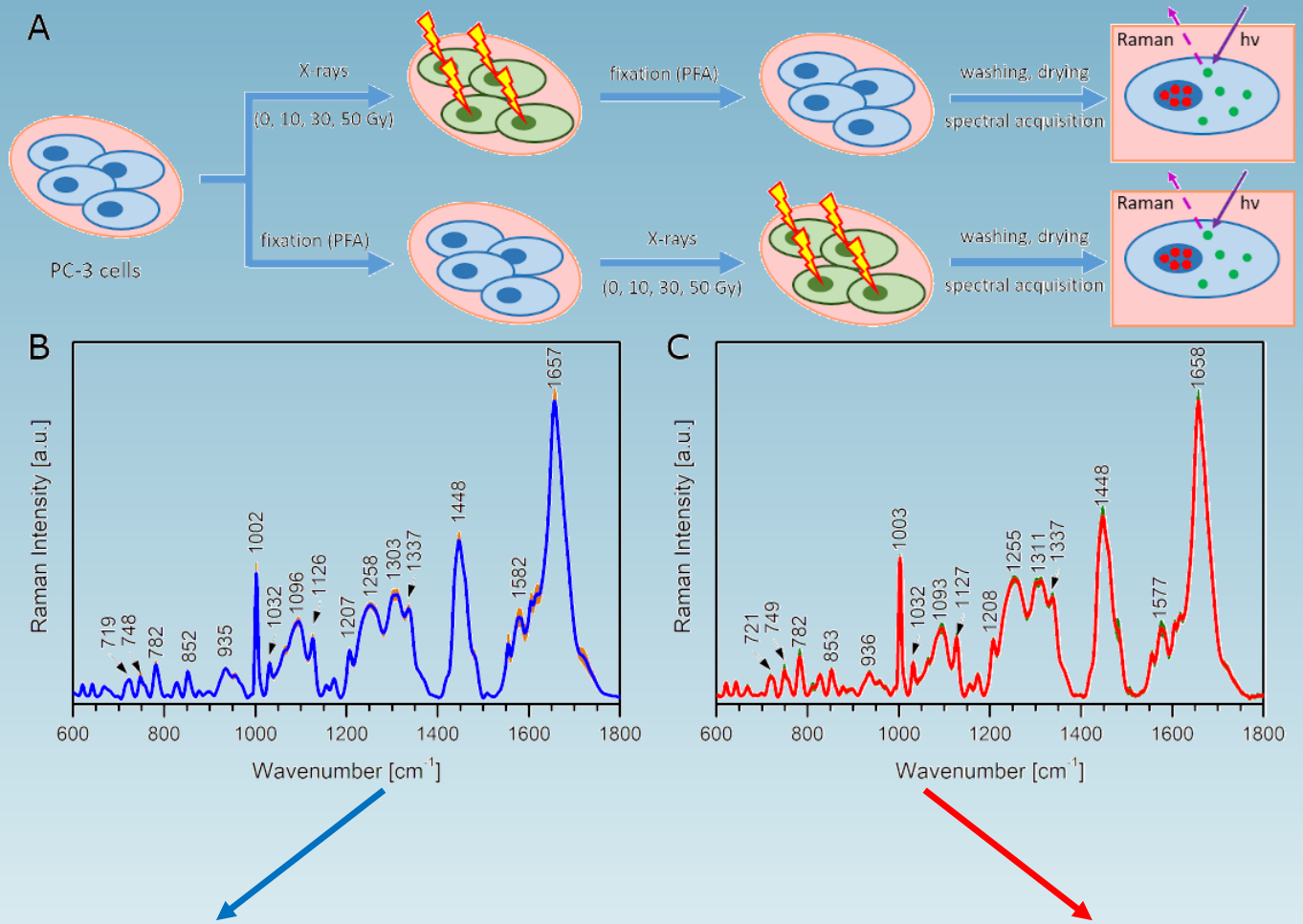




https://www.sigmaaldrich.com/life-science/cell-biology/cancer-research/learning-center/cancer-research-protocols/comet-assay.html

M. Roman *et al.*, *Spectrochim. Acta A*, 2021, in press.

Physicochemical damage vs. early-stage biological response



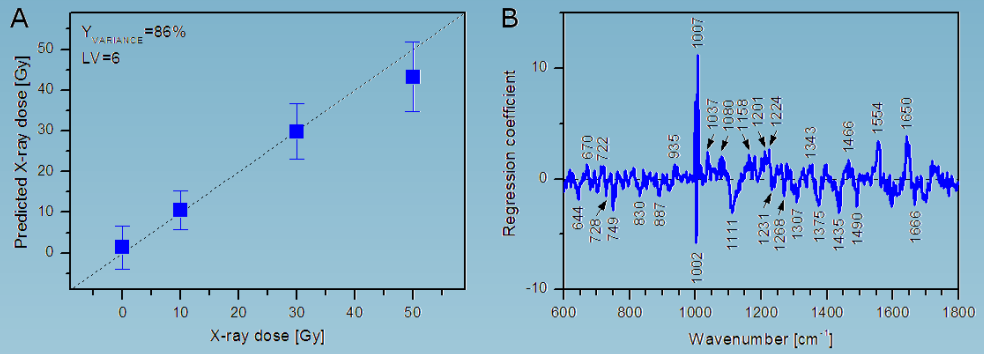
firstly irradiated and then fixed
(early-stage biological response)

firstly fixed and then irradiated
(physicochemical damage)

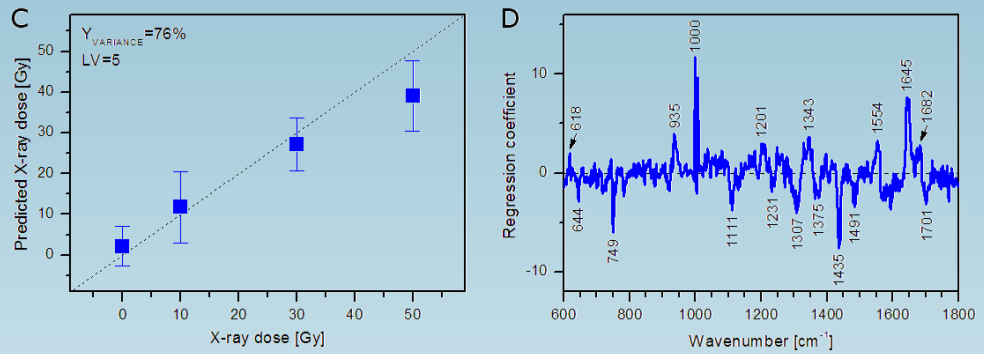
M. Roman *et al.*, *J. Biophotonics*, **13**, 2020, e202000252.

Physicochemical damage vs. **early-stage biological response**

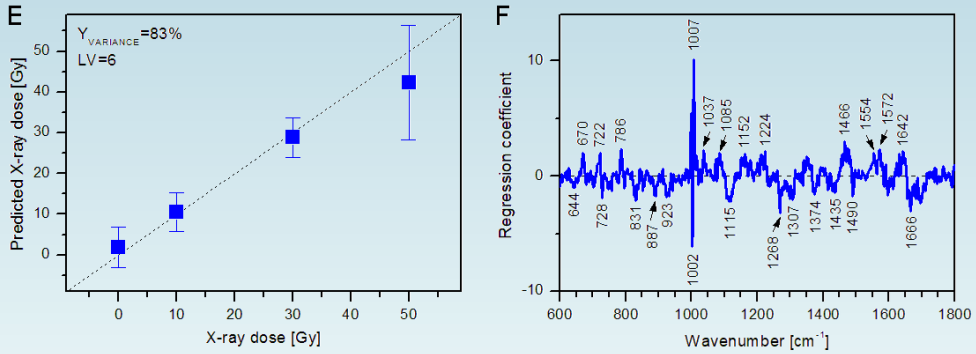
whole cell



cytoplasm



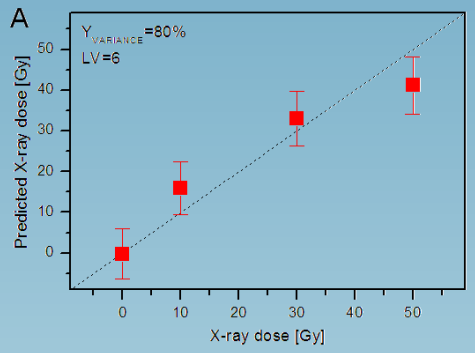
cell nucleus

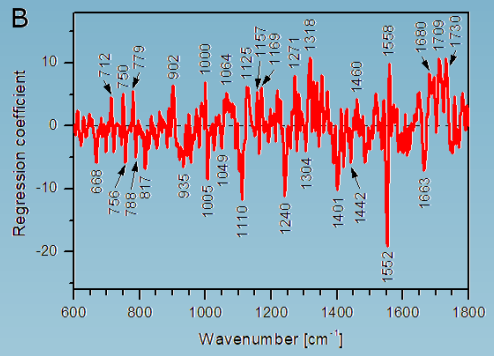


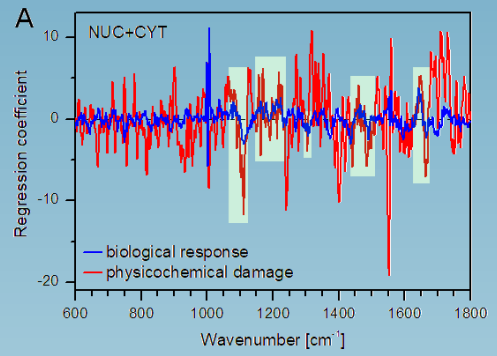
M. Roman *et al.*, *J. Biophotonics*, **13**, 2020, e202000252.

Physicochemical damage vs. early-stage biological response

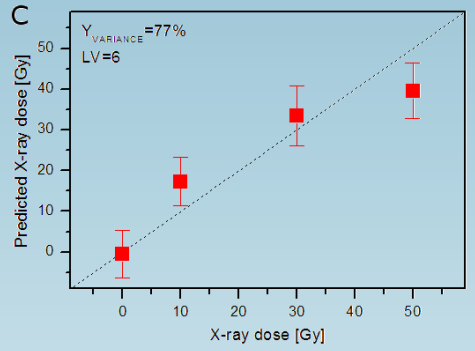
whole cell

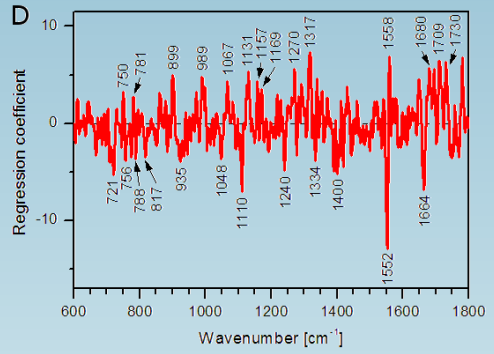


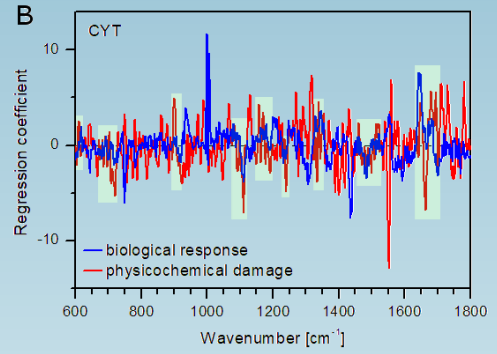




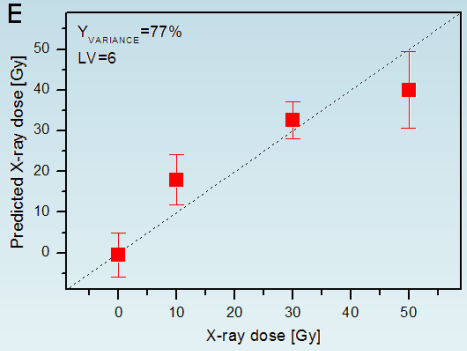
cytoplasm

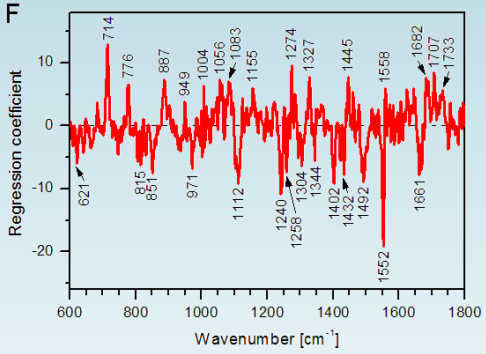


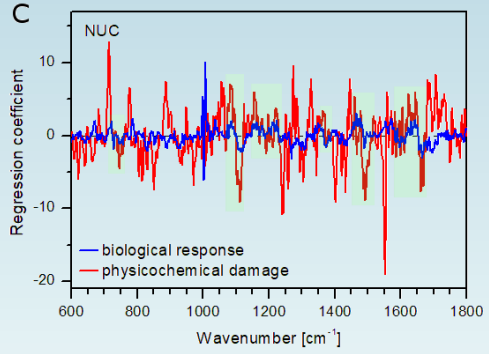




cell nucleus







M. Roman *et al.*, *J. Biophotonics*, **13**, 2020, e202000252.

Physicochemical damage vs. early-stage biological response

Correlation coefficients (R^2) and polynomial coefficients calculated for linear and quadratic fits

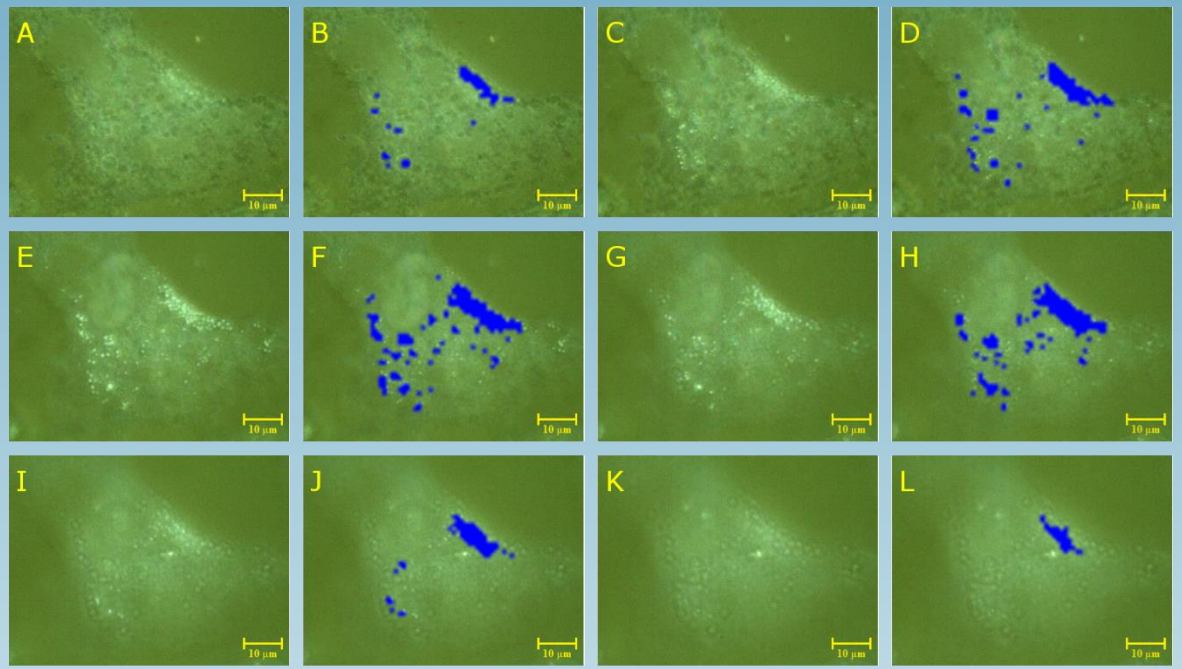
		biological response		physicochemical damage	
		R^2	polynomial coefficients (b_1 or b_1/b_2)	R^2	polynomial coefficients (b_1 or b_1/b_2)
linear fit	NUC+CYT	0.856	0.919	0.777	0.918
	CYT	0.724	0.853	0.729	0.903
	NUC	0.817	0.898	0.728	0.903
quadratic fit	NUC+CYT	0.866	1.138/-0.005	0.857	1.607/-0.016
	CYT	0.751	1.203/-0.009	0.848	1.727/-0.019
	NUC	0.827	1.117/-0.005	0.836	1.684/-0.018

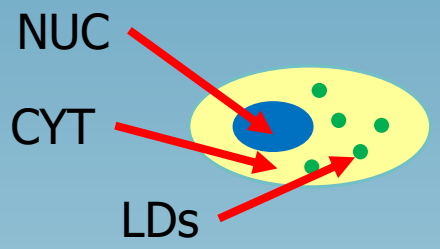
Predicted X-ray doses calculated using the physicochemical damage PLSR models (biological response part) and the biological response PLSR models (physicochemical damage part).

biological response						
applied dose [Gy]	predicted dose (NUC+CYT)		predicted dose (CYT)		predicted dose (NUC)	
	mean	SD	mean	SD	mean	SD
0	54.9	24.8	48.5	18.3	163.2	37.4
10	67.9	15.7	60.9	11.3	188.9	23.8
30	70.1	14.0	56.4	2.7	219.6	23.4
50	55.9	20.8	55.9	14.7	155.3	40.0
physicochemical damage						
applied dose [Gy]	predicted dose (NUC+CYT)		predicted dose (CYT)		predicted dose (NUC)	
	mean	SD	mean	SD	mean	SD
0	31.2	7.1	-10.6	8.8	41.4	9.6
10	37.3	6.3	-7.3	8.9	47.9	9.5
30	36.8	7.1	-6.6	8.8	43.9	6.9
50	39.4	6.5	-4.6	6.7	45.6	7.6

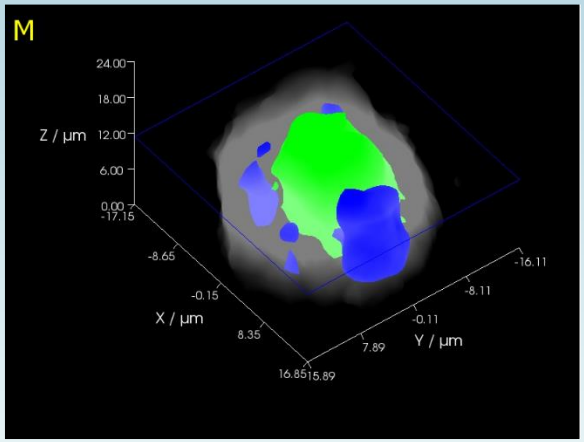
M. Roman *et al.*, *J. Biophotonics*, **13**, 2020, e202000252.

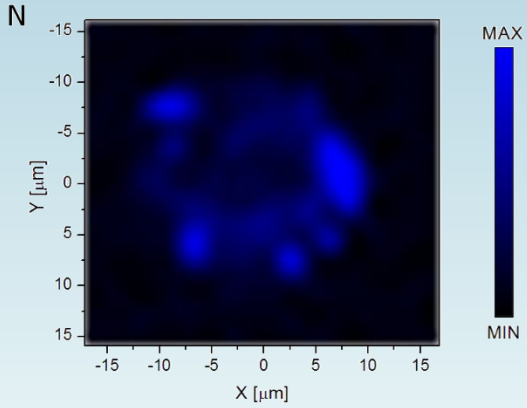
Lipid droplets (LDs) in PC-3 prostate cancer cells





Height above the substrate:
A,B – 0 μm
C,D – 2 μm
E,F – 4 μm
G,H – 6 μm
I,J – 8 μm
K,L – 10 μm





N – cross-section of the 3D map (M) at a height of 12 μm

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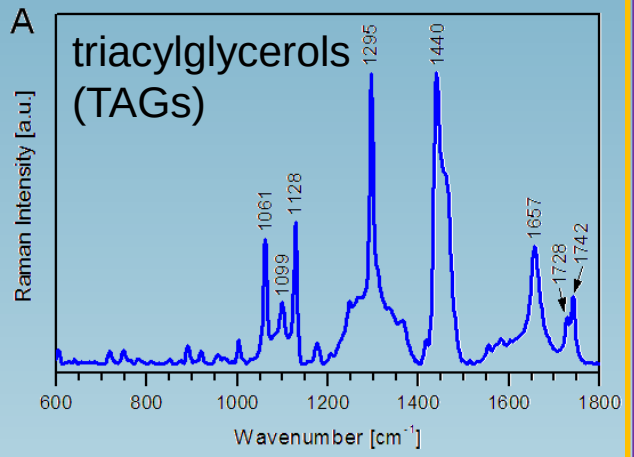
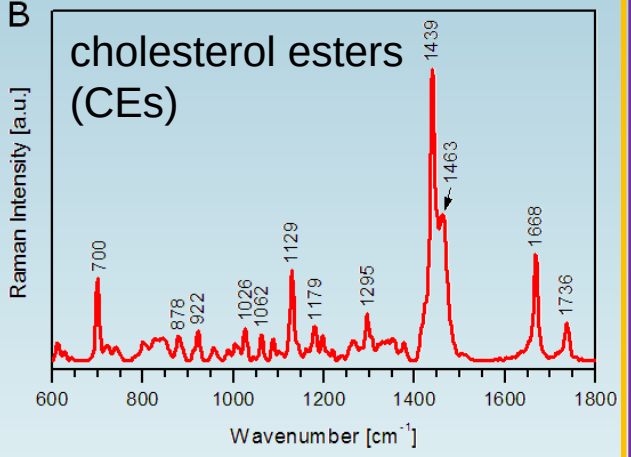
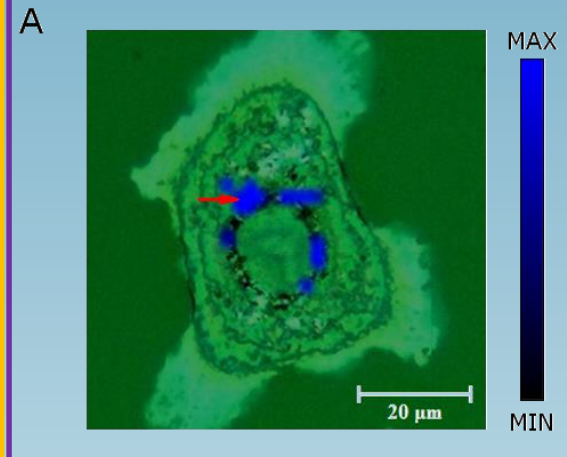
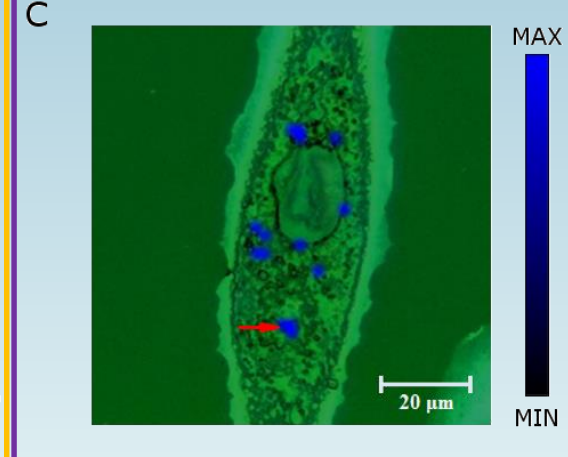
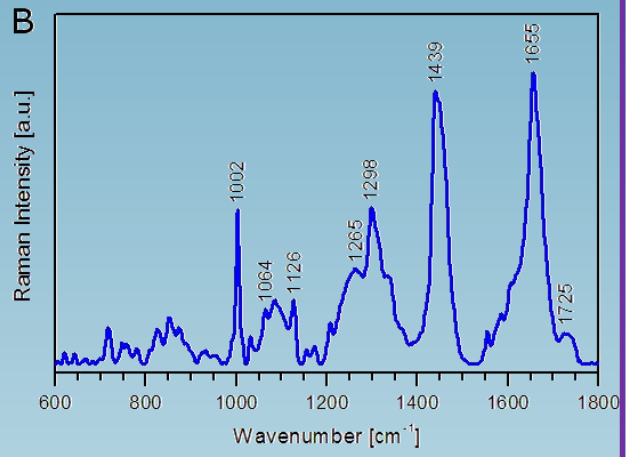
Lipid droplets (LDs) in PC-3 prostate cancer cells – chemical composition

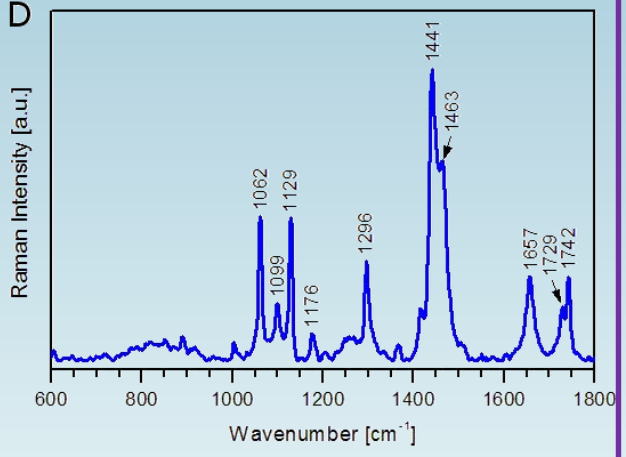






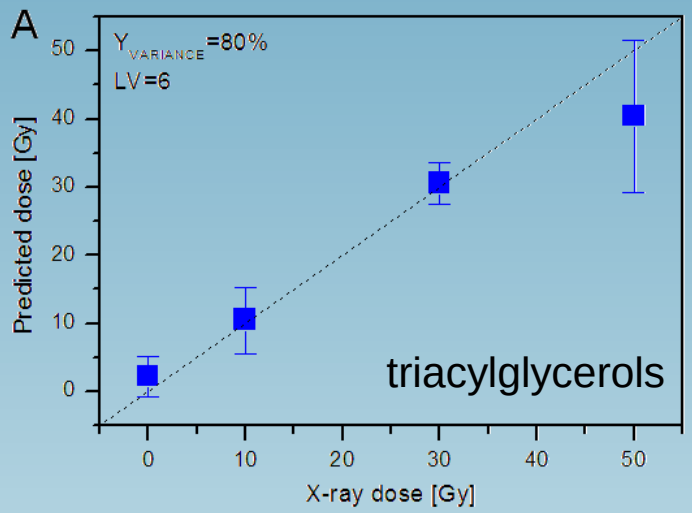


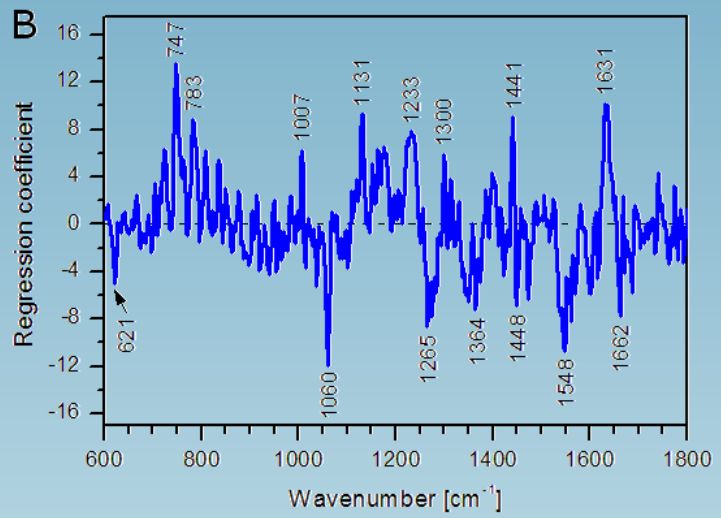




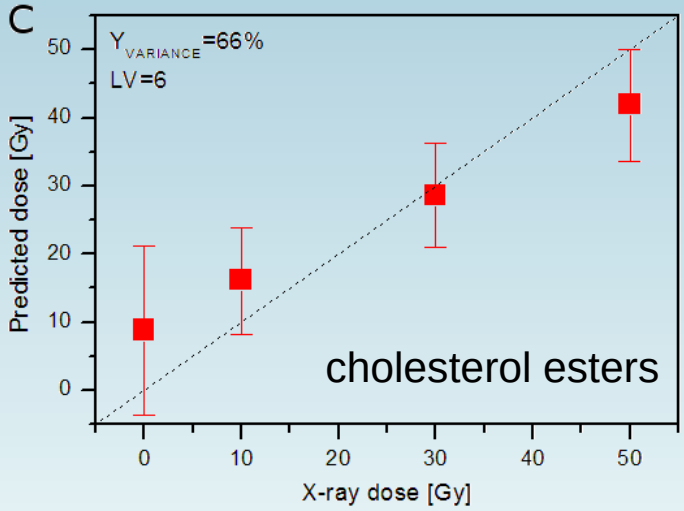
M. Roman *et al.*, *BBA-Mol. Cell Biol. L.*, **1865**, 2020, 158753.

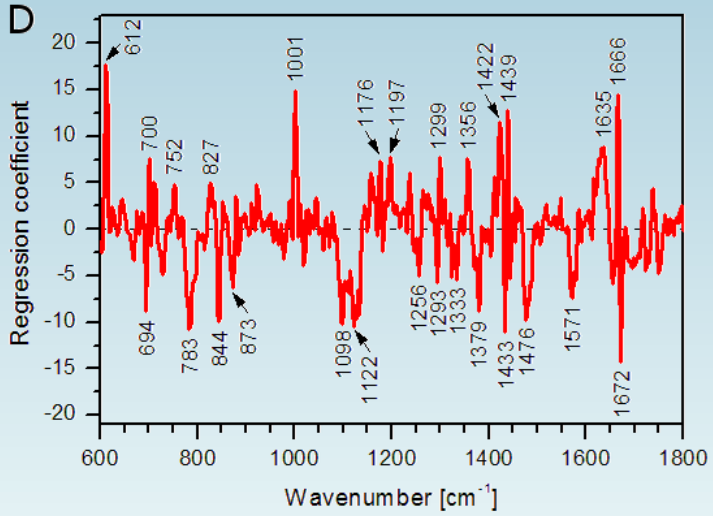
Lipid droplets (LDs) in PC-3 prostate cancer cells – effect of radiation (24h)





+ Cyt c, DNA/RNA, proteins
– lipids

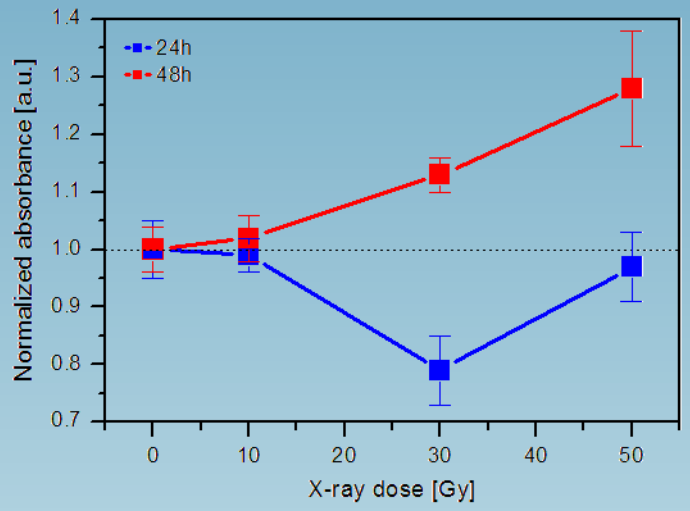




+ proteins, cholesterol
– DNA/RNA, lipids

M. Roman *et al.*, *BBA-Mol. Cell Biol. L.*, **1865**, 2020, 158753.

Lipid droplets (LDs) in PC-3 prostate cancer cells – effect of radiation



decrease – peroxidation, lipid decomposition (lipolysis)

increase – apoptosis, endoplasmic reticulum stress

Conclusions:

- slight effect on the chemical composition
- significant influence on the amount of lipids

→ lipid metabolism as a target of radiotherapy

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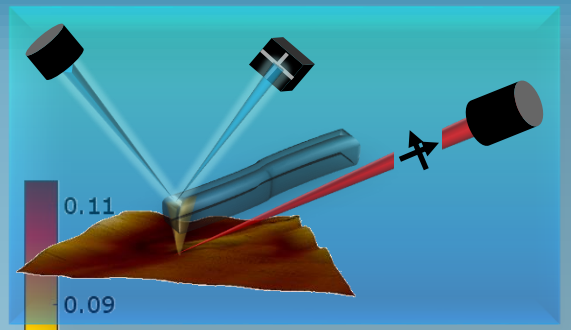
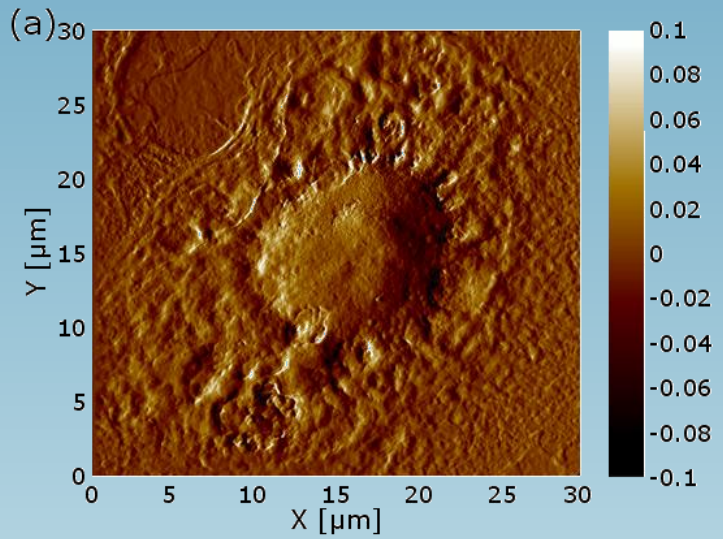
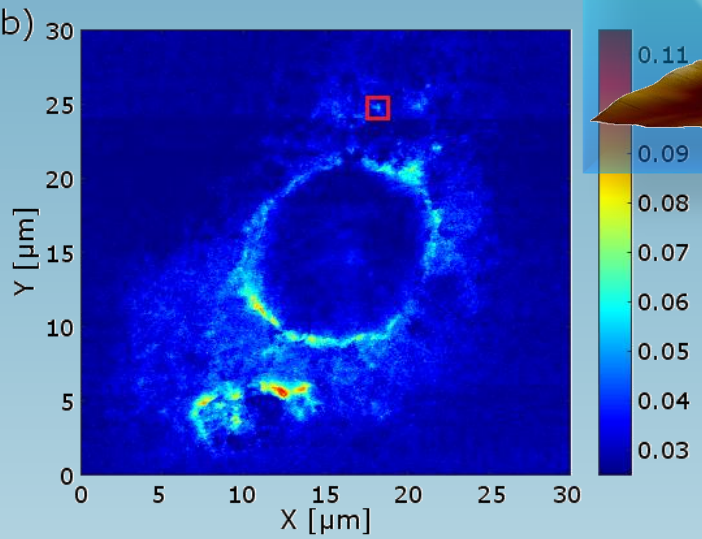
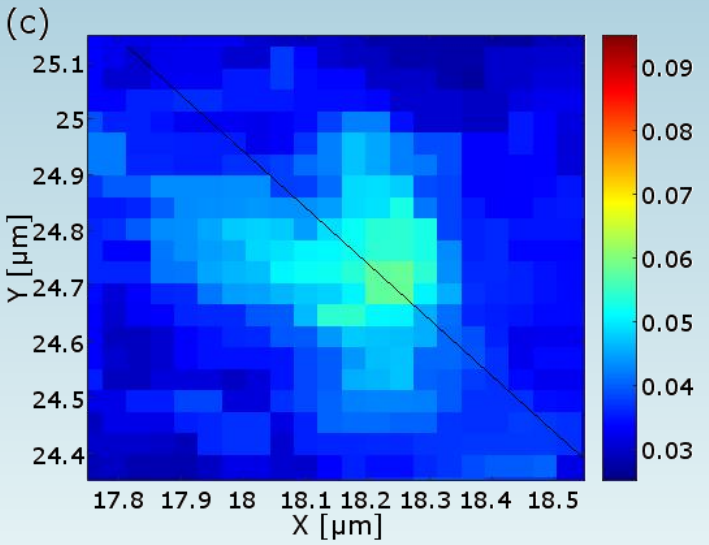
Lipid droplets (LDs) in PC-3 prostate cancer cells – AFM-IR

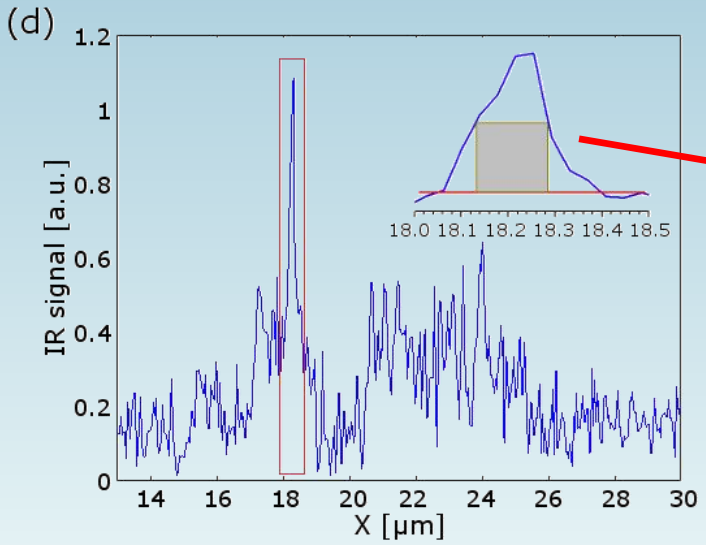






unirradiated cell

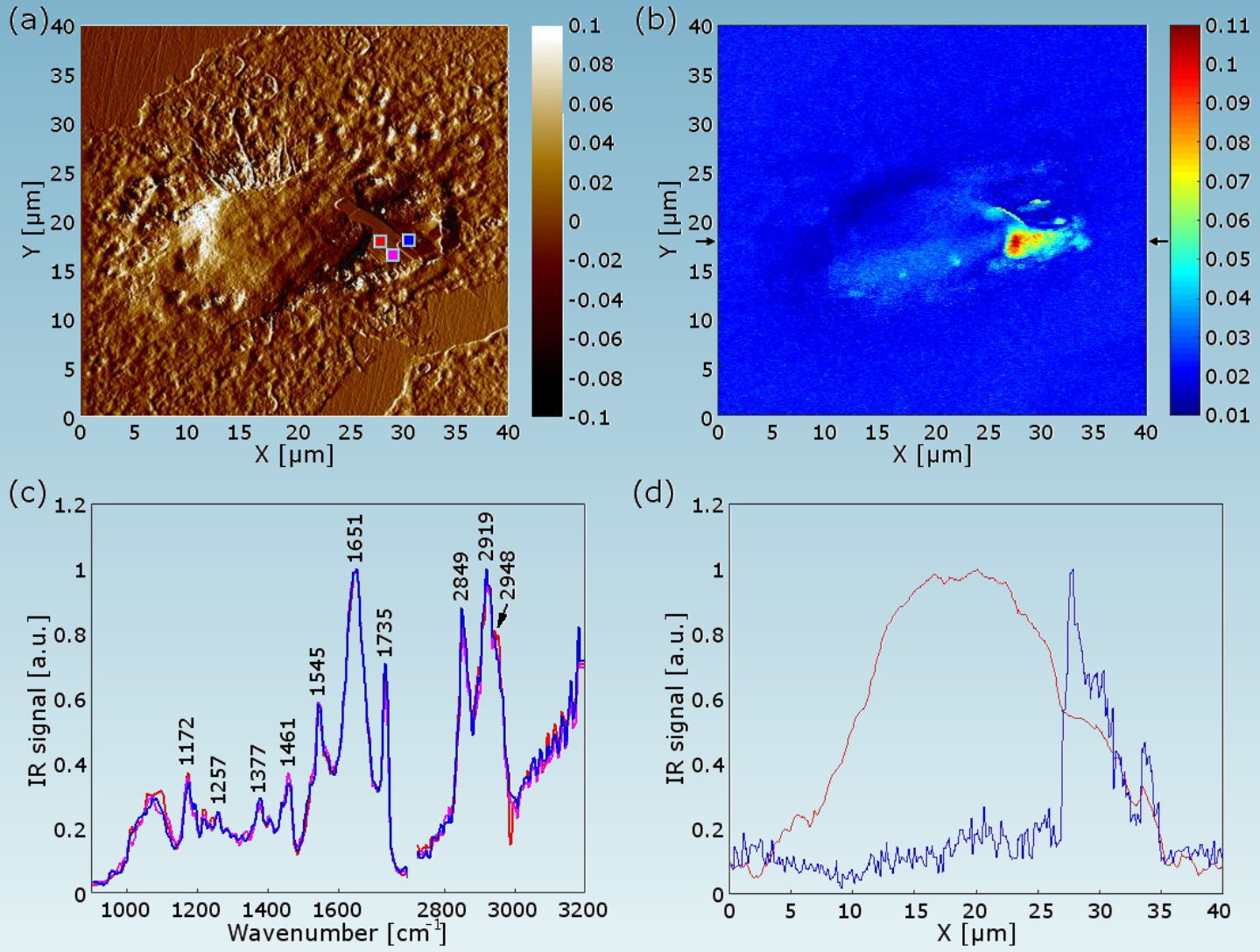




~150 nm !!!

M. Roman *et al.*, *Nanotechnology*, **30**, 2019, 425502.

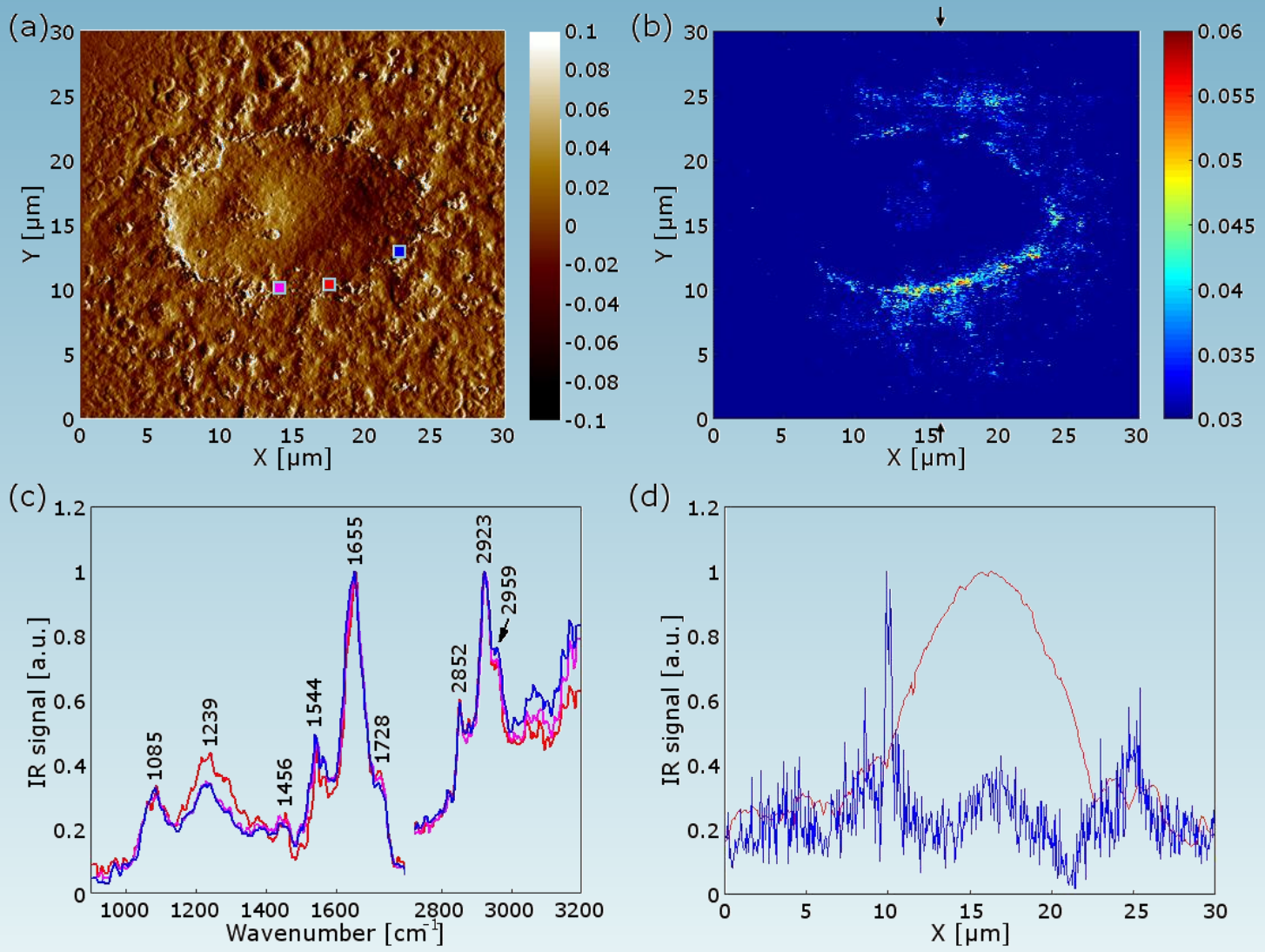
Lipid droplets (LDs) in PC-3 prostate cancer cells – AFM-IR



unirradiated cell
(CEs crystal)

M. Roman *et al.*, *Nanotechnology*, **30**, 2019, 425502.

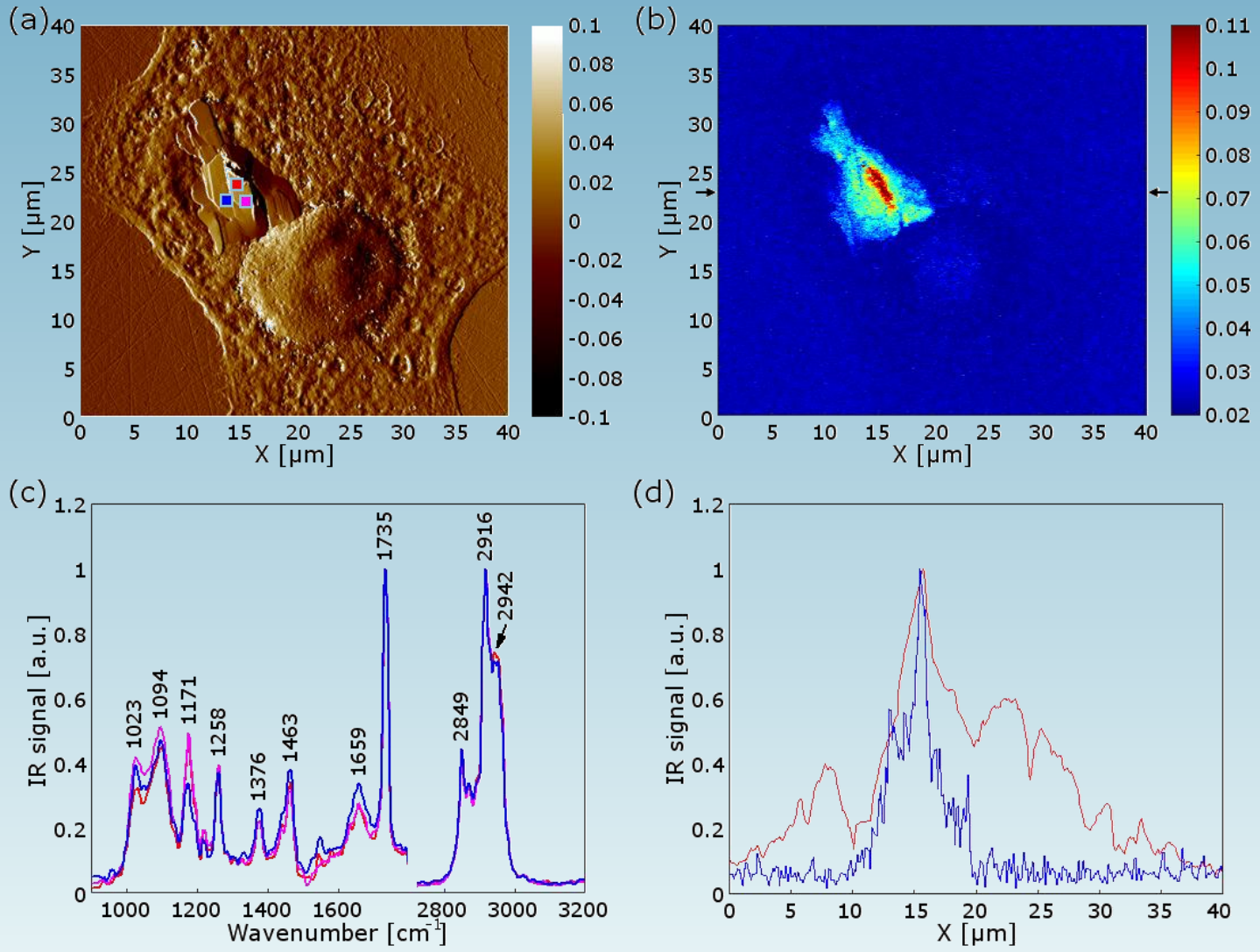
Lipid droplets (LDs) in PC-3 prostate cancer cells – AFM-IR



irradiated cell (30Gy)

M. Roman *et al.*, *Nanotechnology*, **30**, 2019, 425502.

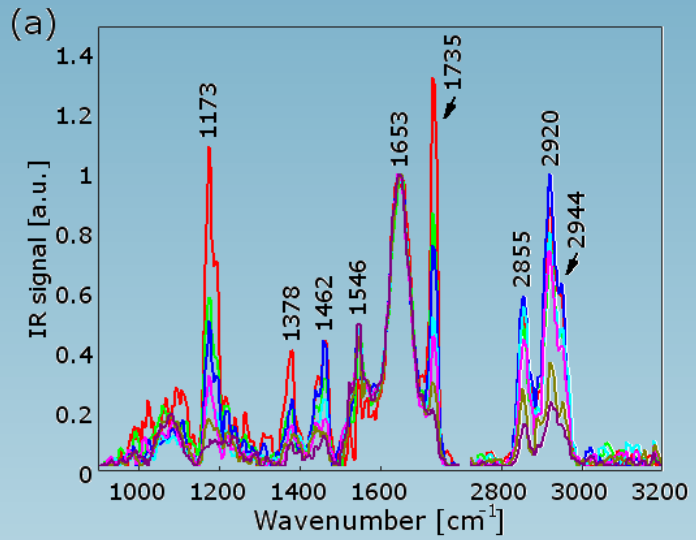
Lipid droplets (LDs) in PC-3 prostate cancer cells – AFM-IR

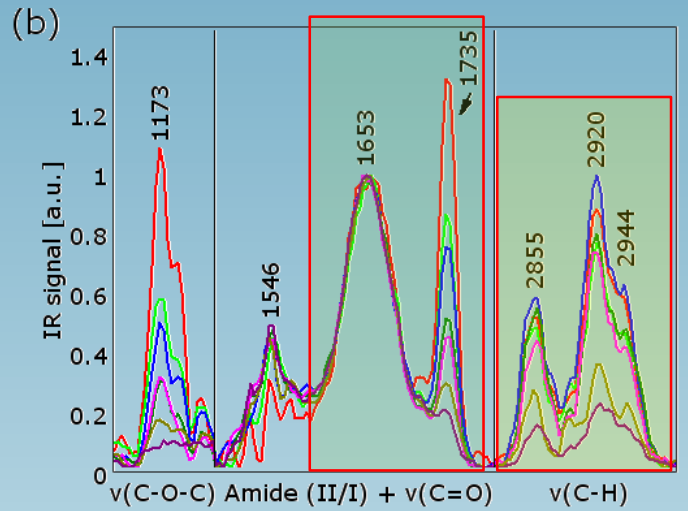


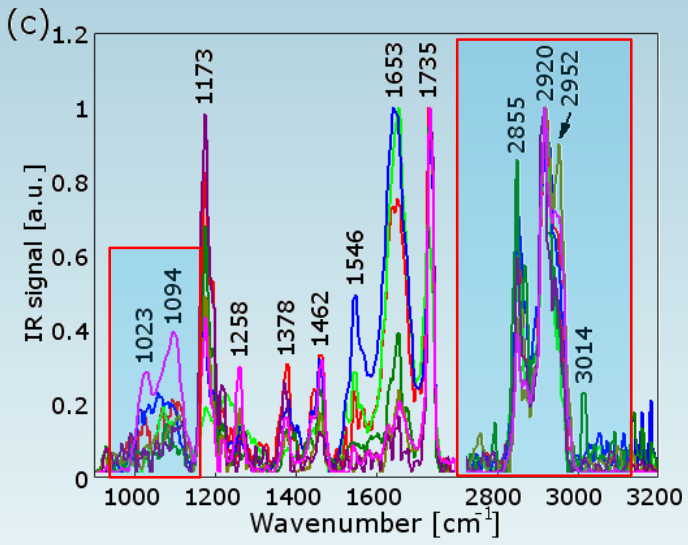
irradiated cell (30Gy, CEs crystal)

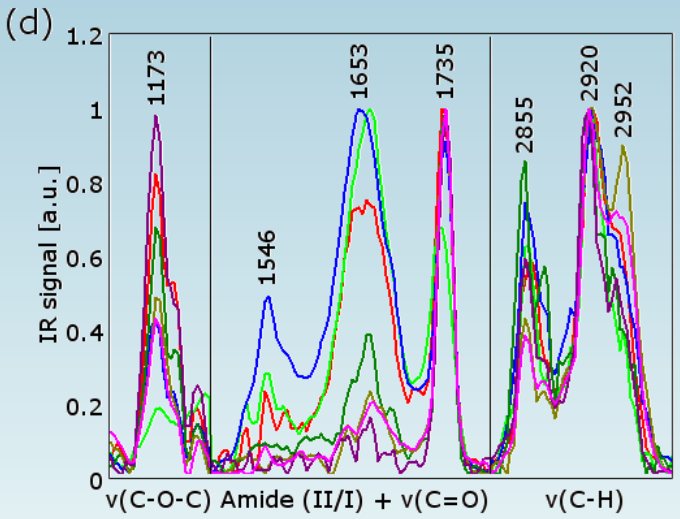
M. Roman *et al.*, *Nanotechnology*, **30**, 2019, 425502.

Lipid droplets (LDs) in PC-3 prostate cancer cells – AFM-IR









Conclusions:

» single cells
- constant lipid content
- different lipid/protein ratio

» whole population
- different CEs/CHL ratio

» no significant influence of irradiation on the chemical composition (→ RS)

M. Roman *et al.*, *Nanotechnology*, **30**, 2019, 425502.

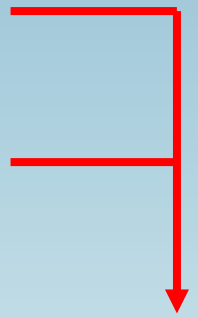
Current and future tasks

Cytochrom C release as response to ionizing radiation

Cell sensitizing to ionizing radiation using therapeutic agents

Effect of proton irradiation on prostate cancer cells

Radiation-induced damage to proteins studied by AFM-IR



carried out as part of two Masters theses
(students from AGH, supervisor: M. Roman)

CONCLUSIONS

Take-home messages

1. Raman spectroscopy as an efficient tool to study the effect of ionizing radiation on cancer cells (even for 0h!, even for low doses!)
2. Different response of the cytoplasm and the cell nucleus (necessity of separate analysis of cell organelles)
3. PLSR-based classification gives (almost) perfect differentiation between unirradiated and irradiated cells at 6 Gy
4. Differentiation between physicochemical damage and early-stage biological response – both can be successfully analyzed by RS
5. Important role of lipids in the metabolism of prostate cancer cells (accumulation of lipids in lipid droplets)
6. Influence of ionizing radiation on lipid droplets (slight on the chemical composition, significant on the amount) - lipid metabolism as a target of radiotherapy
7. Usefulness of AFM-IR spectroscopy in the study of lipid droplets in PC-3 cells (nanoscale imaging) - confirmation of the results from RS

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- dr Esen Efeoglu (FOCAS, TU, Dublin)
- prof. Hugh Byrne (FOCAS, TU, Dublin)

- prof. dr hab. Wojciech M. Kwiatek
- dr hab. inż. Czesława Paluszkiewicz, prof. IFJ PAN
- NZ52 staff

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THANK YOU FOR YOUR ATTENTION