Badania wybranych nowotworów z zastosowaniem technik analitycznych opartych na promieniowaniu synchrotronowym

Marek Lankosz

Klasyfikacja pierwiastków pod względem fizjologicznym

Makroelementy	od 0.01%
Mikroelementy	0.01%-0.0001%
Ultraelementy	poniżej 0,0001%

- pierwiastki konieczne do życia tzw. biopierwiastki
- pierwiastki obojętne, bez których przemiany metaboliczne mogą normalnie przebiegać
- pierwiastki toksyczne, wywierające szkodliwe działanie na organizm

W. Opoka i inni, Właściwości fizykochemiczne i biologiczne wybranych pierwiastków

Pierwiastki toksyczne

Pb- kumuluje się w kościach, zakłóca przemiany metaboliczne, **niedorozwój umysłowy**, trudności w uczeniu, anemia

Hg- **działa na ośrodkowy układ nerwowy**, zaburzenie wzroku, uszkadza nerki, powoduje trudności w uczeniu się, jest kancerogenny, kumulacja w rybach i skorupiakach**, amalgamat do uzupełniania ubytków w zębach, konserwacja niektórych szczepionek**.

Cd-deponowany w płucach, nerkach, wątrobie, rakotwórczy, **uszkadza komórki nerwowe**, zniekształcenia kości, zaburzenia wzrostu, **występuje w tworzywach sztucznych (pigmenty)**

As-kumuluje się we włosach, paznokciach, skórze, kościach, uszkodzenia nerek, **zaburzenia układu nerwowego,** skurcz mięśni, **kancerogenny**, **tritlenek diarsenu-leczenie ostrej białaczki i innych nowotworów.**

Al. Nie pełni funkcji biologicznej. **Zaburza funkcjonowanie układu nerwowego. Ma związek z chorobą Alzheimera i Parkinsona**, **Stosowany w medycynie w terapii.**

Biopierwiastki

Mn- wpływa na funkcjonowanie OUN, składnik metaloenzymów (dysmutaza ponadtlenkowa, polimeraza RNA i DNA), aktywuje enzymy dla wytwarzania energii, metabolizm węglowodanów, białek, lipidów, naturalny antyutleniacz,
Hipomanganemia- zaburzenie koordynacji ruchowej, zmęczenie, stany niepokoju
Zatrucia- neurodegeneracyjne zmiany dopaminergiczne, szaleństwo manganowe (impulsywność, pobudliwość, gadatliwość, schizofrenia)

Se- **pierwiastek życia**, wchodzi w skład aminokwasów, jest antyoksydantem, zapobiega proliferacji i wzrostowi komórek nowotworowych,, **stymuluje układ immunologiczny**
Niedobór-podatność na choroby,
Nadmiar- **depresje**, nerwowość, uszkodzenie wątroby, nerek i śledziony, czerwone zabarwienie paznokci, **zaburzenie metabolizmu siarki.**

Biopierwiastki

Cu- **składnik i aktywator enzymów, składnik dysmutazy ponadtlenkowej, bierze udział w syntezie dopaminy,**
Niedobór- niedokrwistość, osteoporoza, zmniejszenie liczby białych krwinek, zaburzenie gospodarki lipidowej, **zwyrodnienie wątrobowo-soczewkowe (Ch Wilsona), neurodegeneracja (Ch Menkesa)**
Nadmiar- zmiany metaboliczne, uszkodzenia nerek, mózgu, naczyń wieńcowych,

Zn- przemiany metaboliczne kwasów nukleinowych, lipidów, białek, cukrów, prawidłowe funkcjonowanie układu immunologicznego, **budulec dysmutazy ponadtlenkowej**, synteza i magazynowanie insuliny, składnik enzymów,
Niedobór- zaburzenia depresyjne

Biopierwiastki

Fe- katalizator reakcji enzymatycznych, składnik hemoprotein, białek, **generowanie wolnych rodników**, układ odpornościowy, procesy krwiotwórcze, **produkcja neuroprzekaźników**,
Nadmiar-zakłóca metabolizm innych metali śladowych,
Niedobór-niedokrwistość

Ni- występuje w enzymach (uerazach), erytropoeza, siarczek niklu (II) **działanie kancerogennie**

Cr (III)-niezbędny do metabolizmu glukozy, stymuluje syntezę kwasów tłuszczowych,
Niedobór- depresje, stany lękowe, uszkodzenia nerwów, **zaburzenia w gospodarce białek i lipidów**
Nadmiar-biegunka, skaza krwotoczna, martwica wątroby

Mg- Bierze udział w procesie skurczu mięśni (serce), **jest aktywatorem wielu enzymów.**
Brak- wzmożone napięcie mięśniowe, drżenie mięśni, skurcz.
Nadmiar-zawroty głowy, biegunka, nudności

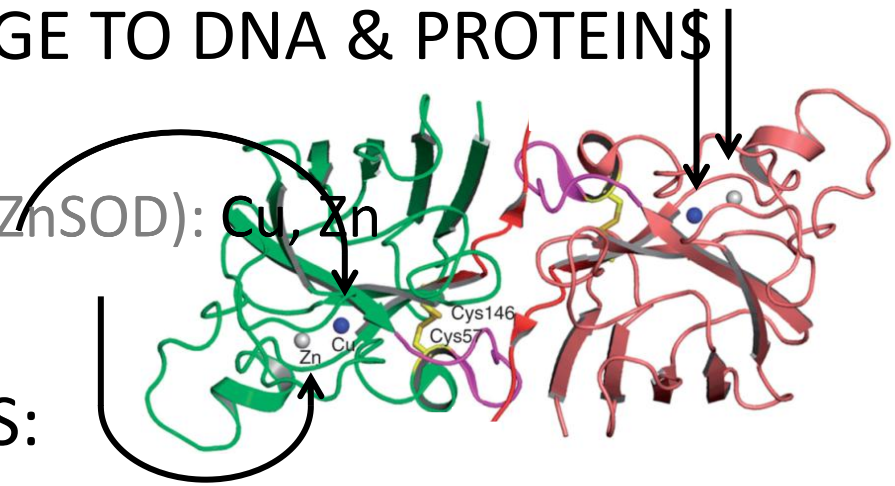
Metals in brain

- 1867 (Perls) histochemical visualization of iron in brain tissue
- 1886 (Zaleski) distinction of heme” and “non heme” iron in brain
- 1955 (Diezel) discovery of ferritin, main iron carrying protein
- 1985 (Bloch) discovery of transferrin main transporter of iron
- 1986 (Drayer) magnetic resonance imaging of brain iron

- Wilson’s disease – defective copper metabolism
- Menkes’ disease – copper deficiency
- Parkinsonian syndrome – chronic manganese toxicity
- Mental confusion – lead toxicity
- Minamata disease – methyl mercury toxicity
- Alzcheimer’s disease- aluminum and zinc toxicity
- Parkinson’s disease – iron deposition in brain

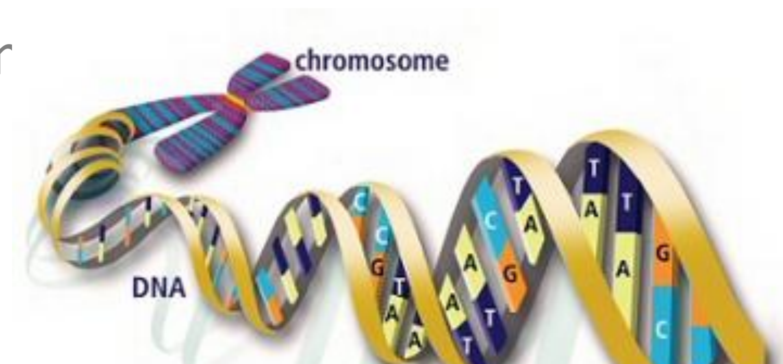
CHEMICAL ELEMENTS STUDIED – MARKERS?

- OXIDATIVE STRESS → DAMAGE TO DNA & PROTEINS
  - ROS (FENTON REACTION): Fe
  - PROTECTION AGAINST ROS (CuZnSOD): Cu, Zn



- MORE ABUNDANT ELEMENTS:
  - P (nucleic acids, energy carriers, phospholipides)
  - S (enzymes, cell breathing)
  - Na, K, Cl (electrolyte equilibrium

TISSUE ENRICHED OR DEPLETED



A.Wandzilak,
Selected chemical elements as potential indicators of cancerous brain tissue, doctoral thesis

MOTIVATION

LOOKING FOR THE RELATIONSHIP BETWEEN THE BRAIN TUMOUR MALIGNANCY GRADE AND LEVELS OF SELECTED ELEMENTS

→ **BIOCHEMICAL PROCESSES**

→ **DIAGNOSTICS: DETERMINING MALIGNANCY GRADE OF TUMOURS**

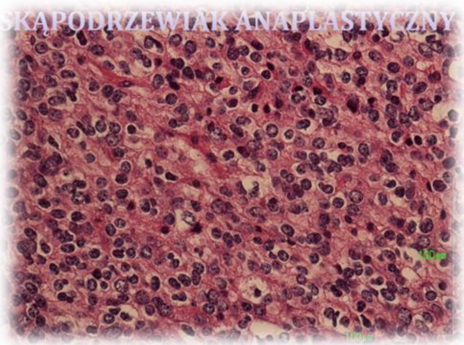
A.Wandzilak,
Selected chemical elements as potential indicators of cancerous brain tissue, doctoral thesis

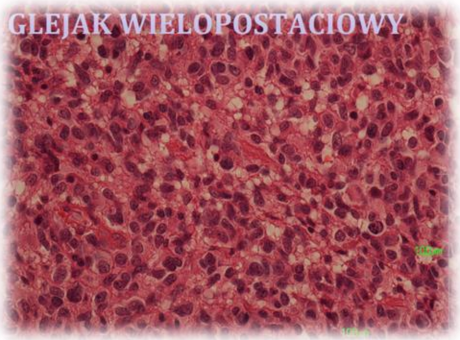
GLIAL BRAIN TUMOURS

CANCER OF GLIAL CELLS
(SUPPORT, NOURISHMENT AND PROTECTION FOR NEURONS)

MALIGNANCY GRADE ACCORDING TO WHO

(FOUR GRADES OF MALIGNANCY)





- **LOW MALIGNANCY GRADE:**

VERY MATURE CELLS, WELL DIFFERENCIATED

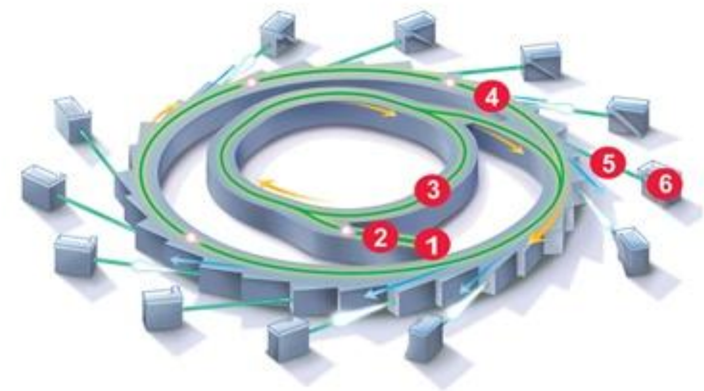
- **HIGH MALIGNANCY GRADE:**

FAST GROWING CELLS WHICH INFILTRATE TO NEIGHBOURING TISSUES, EXCESSIVE PROLIFERATION OF BLOOD VESSELS

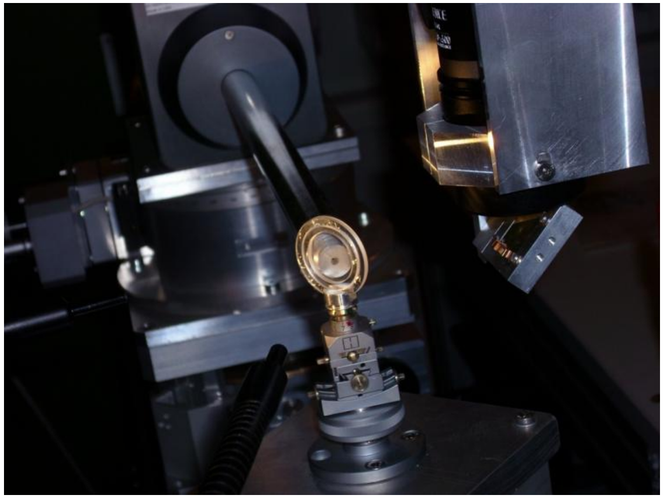
A.Wandzilak,
Selected chemical elements as potential indicators of cancerous brain tissue, doctoral thesis

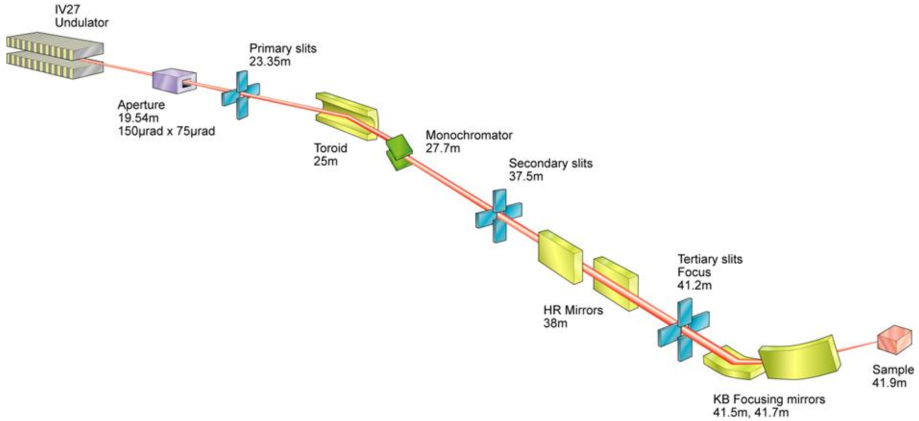
CHEMICAL ELEMENTAL IMAGING WITH THE USE OF X-RAY FLUORESCENCE MICROSPECTROSCOPY

Synchrotron radiation X-ray fluorescence microscopy



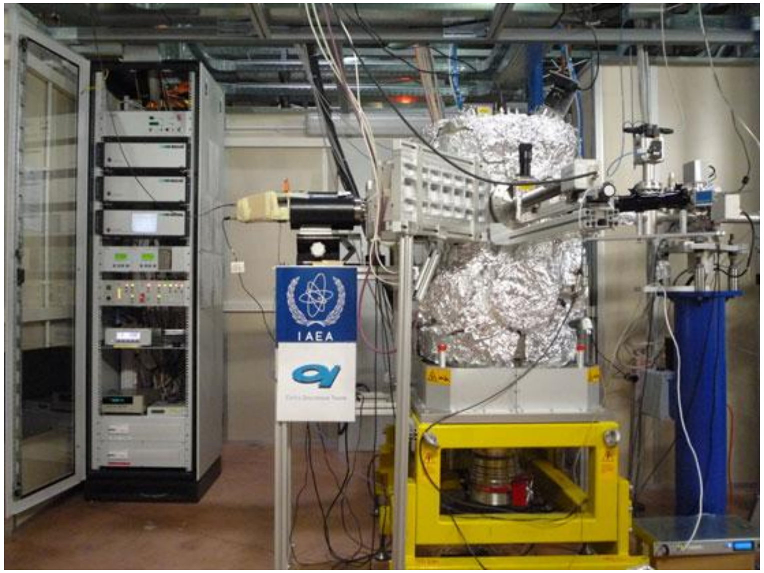
Synchrotron
1. Positron source
2. Linear accelerator
3. Booster
4. Accumulation ring
5. Beamline
6. Exprimental hutch

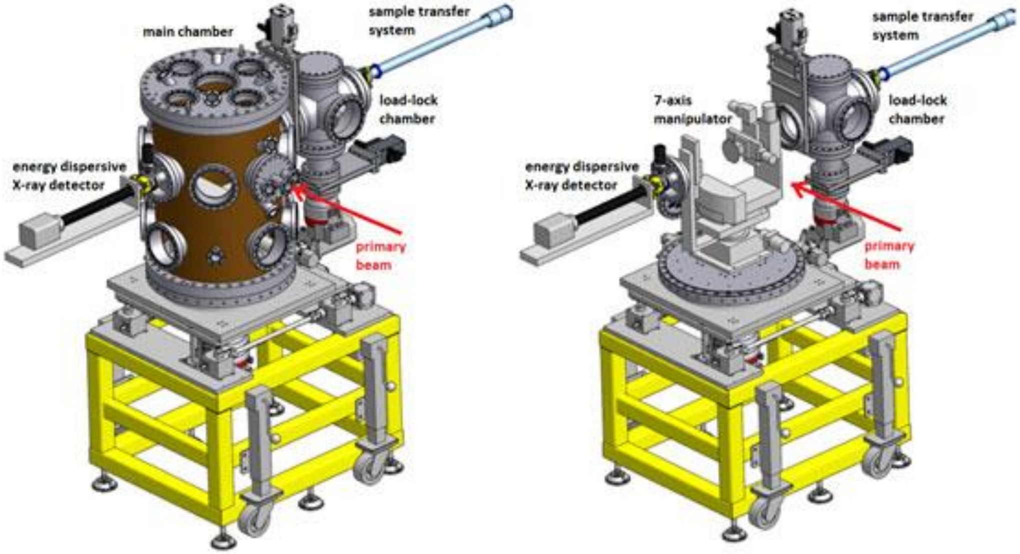




XRF equipment at the P06 beamline at Petra III
17.0 keV, 700 nm, 2 s/point

XRF BEAMLINE at ELETTRA

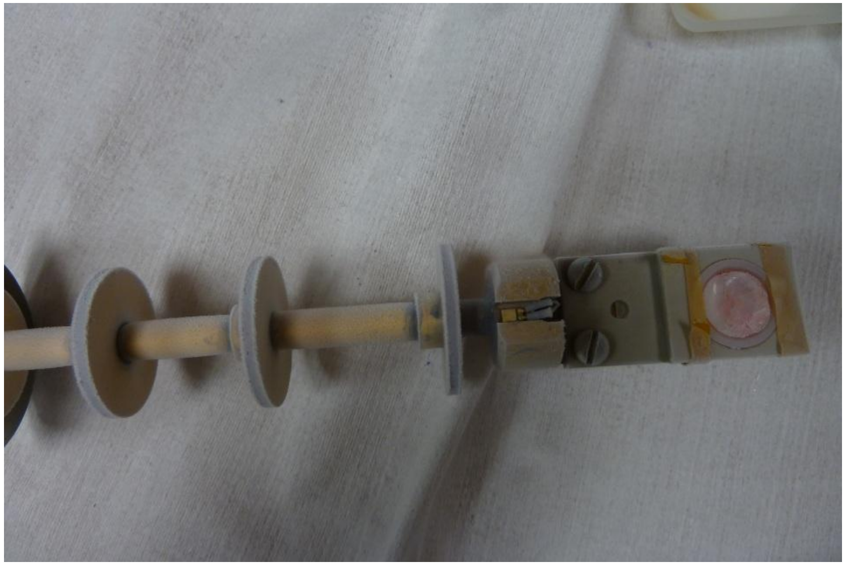




- RuB_4C monochromator
- pixel size: 120 x 250 μm^2
- 30 mm^2 SDD
- ulitra thin polymer window
- high vacuum ($2 \cdot 10^{-7}$ mbar)
- 45°/45° geometry
- 5 s / pixel
- PyMCA for data analysis

SAMPLE PREPARATION for bulk cryo analysis

- *freezing in cryomicrotome*
- *cutting into 1 mm thick slices*
- *placing in sealable polymer cup with ultralene window*
- *keeping frozen at -80ºC.*
- *transporting at LN temperature*



sample holder

LHe cryostat

Sample holder for cryo XRF microscopy

SAMPLE PREPARATION
for biochemical micro-imaging

Tissue - shock freezing

cutting into 5μm thick sections

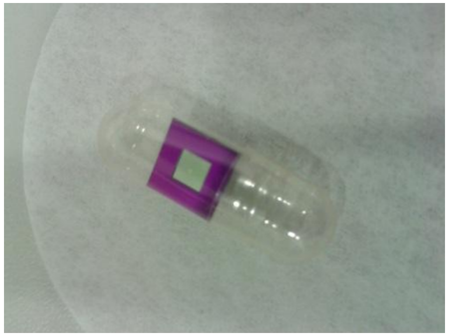
(cryomicrotome)

biochemical analyses

Silicon nitride membrane

freeze-drying at -80 °C

histopathology



Types of ovarian tumors used in elemental studies

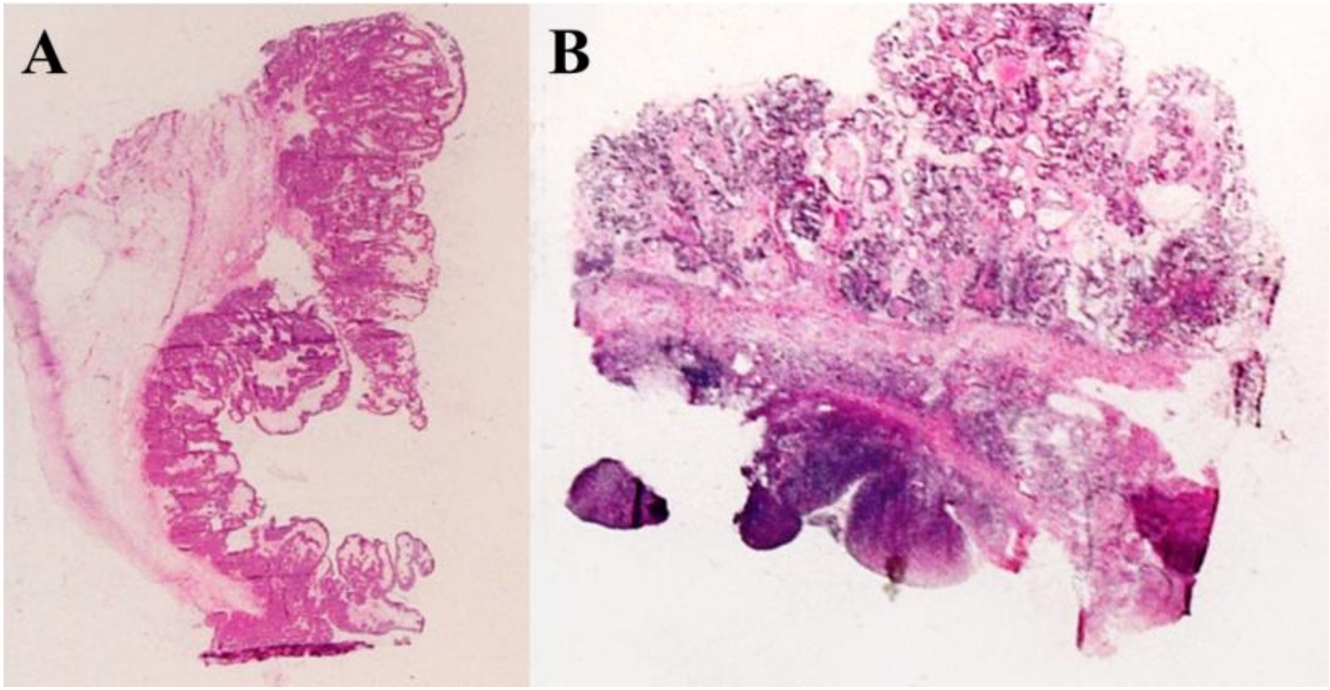
Sample labeling	Type of tumor	Number of analysed areas
1	Control	6
2	Bening tumor	4
3	Bordeline tumor	7
4	Cancer	12
5	Stroma	3

Ł. Chmura, et al. Journal of Physiology and Pathomorphology, submitted for publication

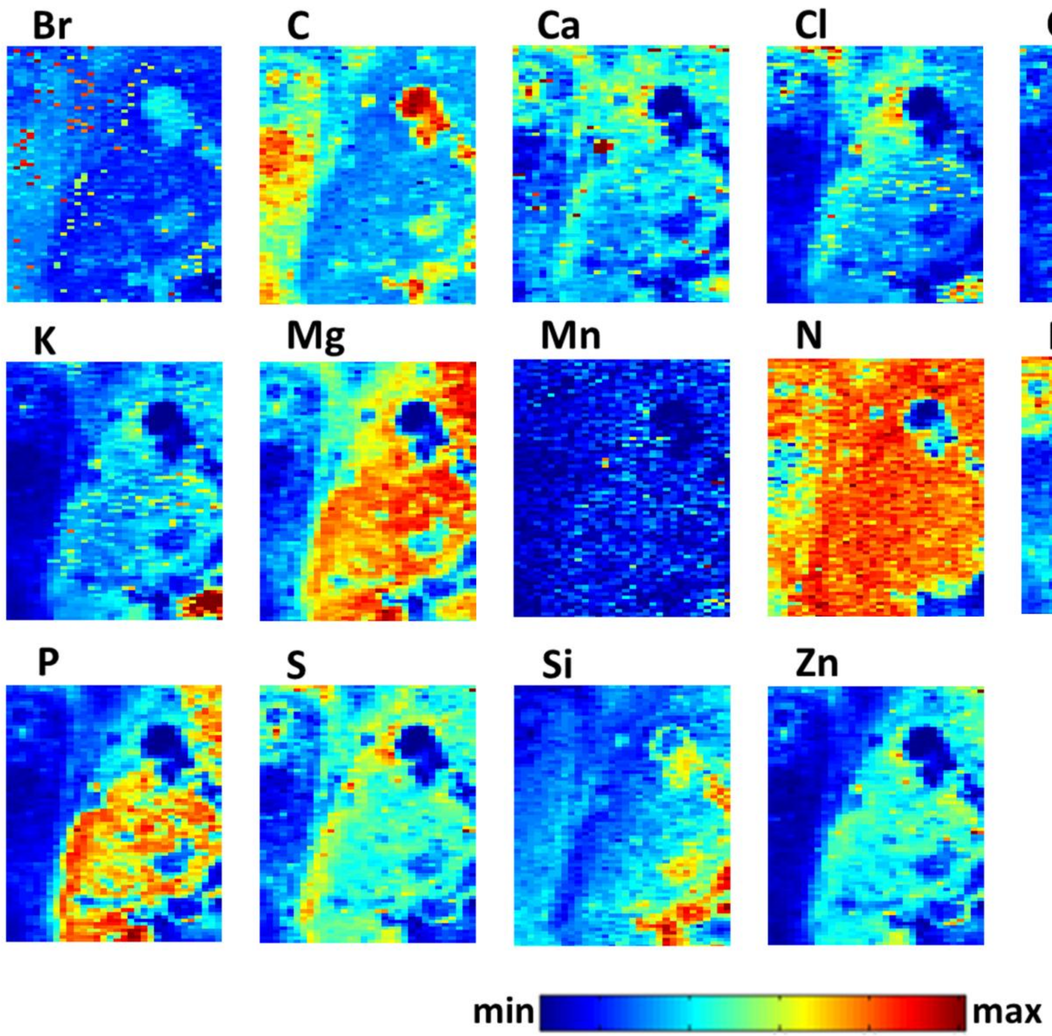
ARCHITECTURE OF OVARIAN CANCER TISSUES

A) **Solid tumours – solid sheets of epithelial cells**

B) **Borderline tumours – single layer of epithelial cells grown into stromal cells**



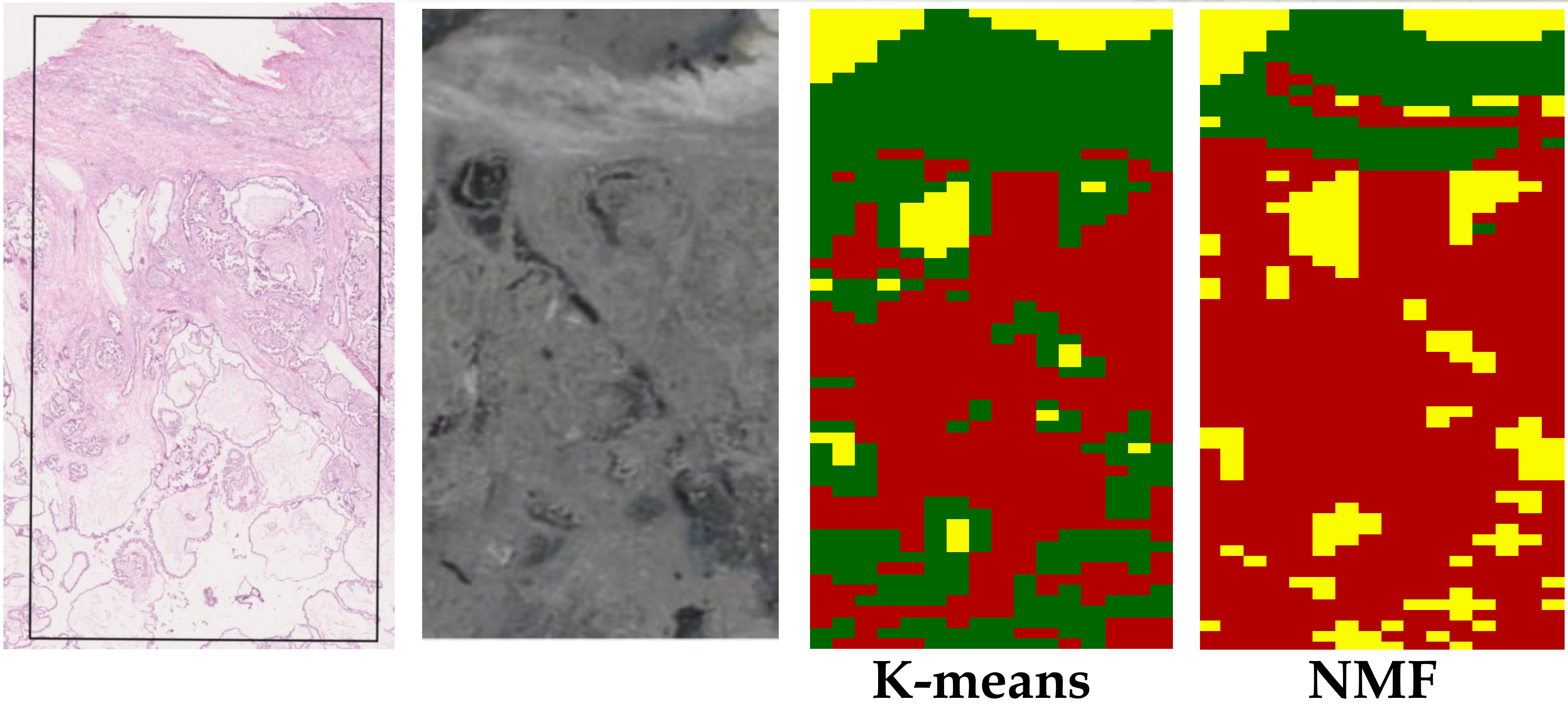
EXAMPLE of XRF IMAGING



endometroid adenocarcinoma 8 x 9.4 mm

NMF: Ca, Cu, Fe, Mn, Si, Zn excluded

BORDERLINE SEROUS TUMOUR

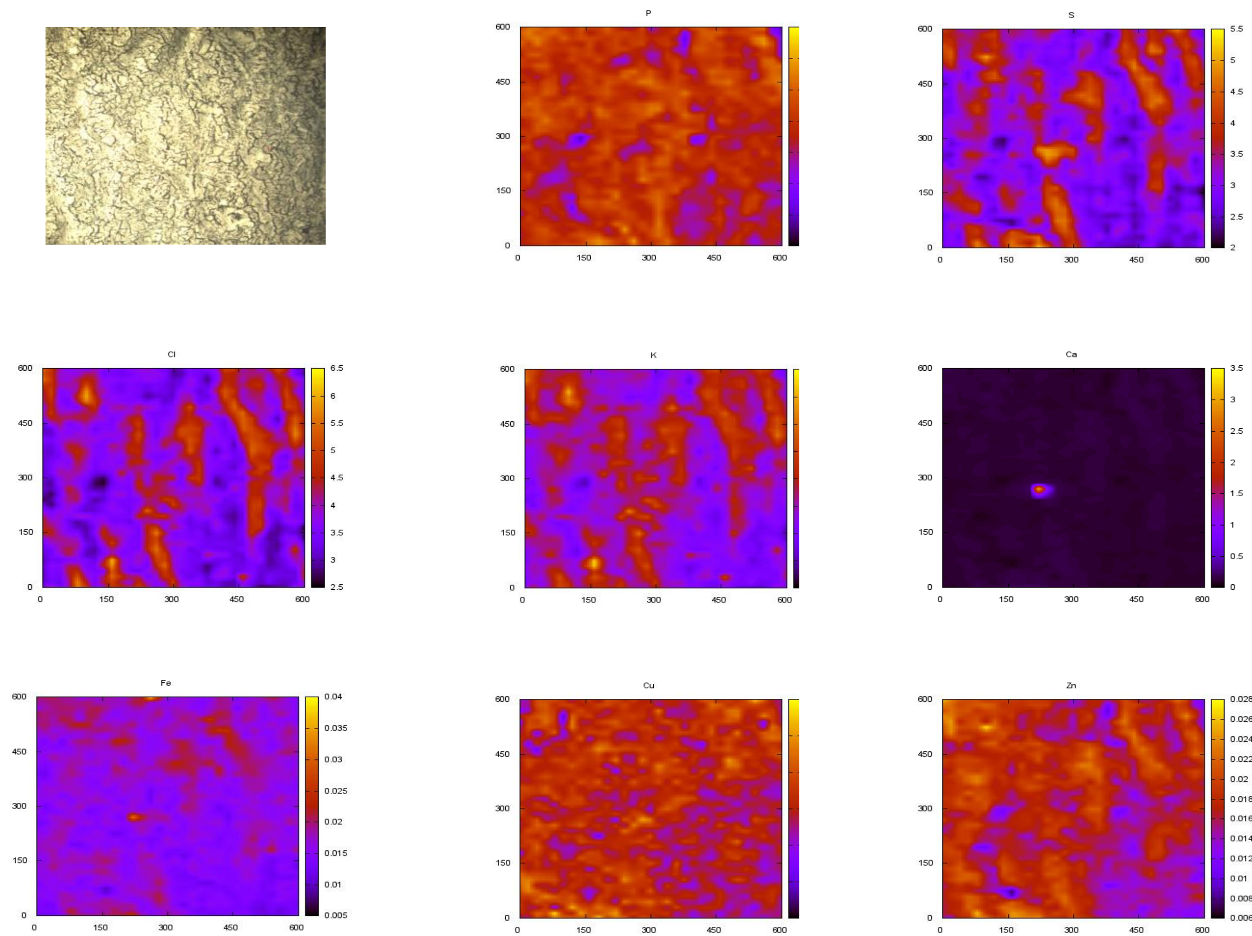


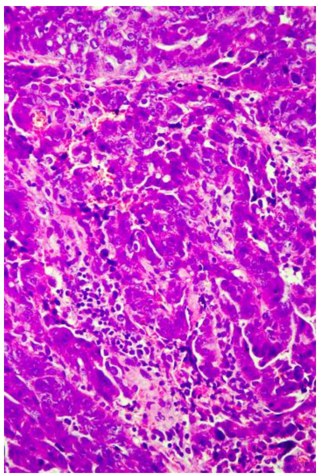
Tumour

Stroma

AGH

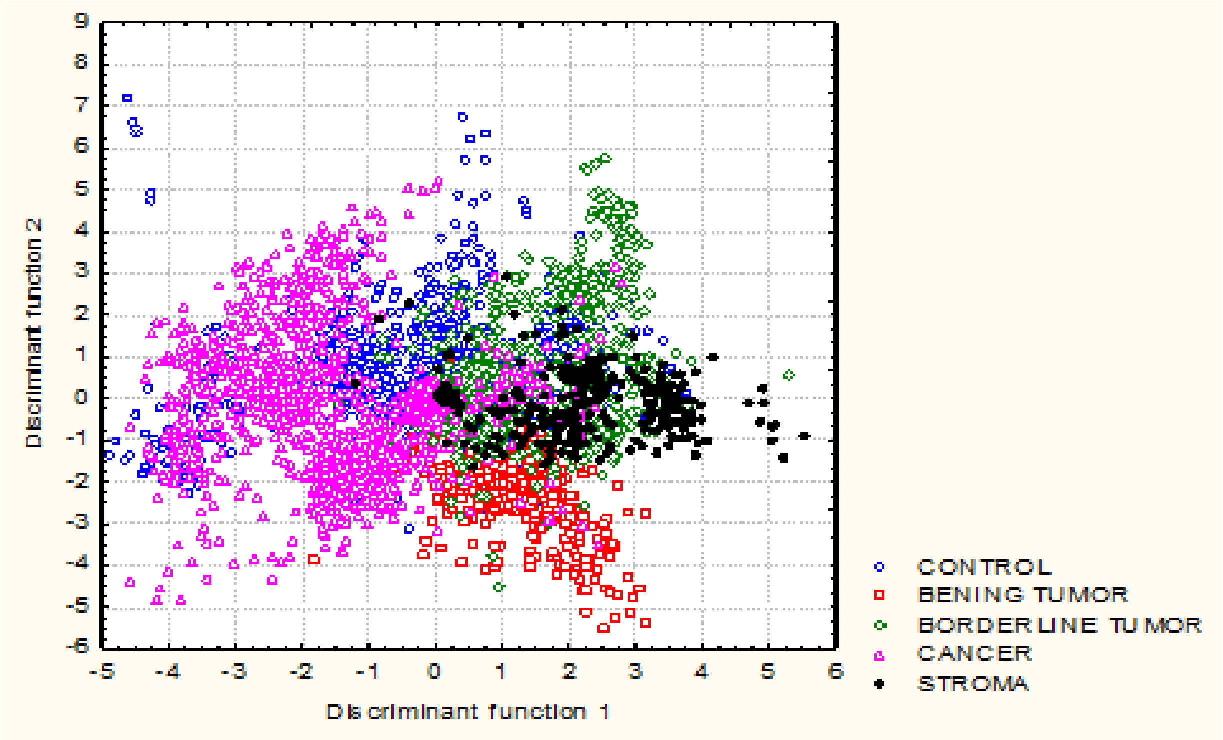
Maps of elemental distribution in malignant tissue and optical microscope image of tissue. Data presented in µg/cm^2. X-Y coordinates in µm





Optical microscope image of malignant tissue stained with the use of hematoxylin-eosin

Multivariate Discriminant Analysis



$$DF1 = -2,498K + 1,501S + 0,824Cl - 0,596Fe$$
$$DF2 = 2,673S - 2,952Cl + 1,191Br + 0,904Rb - 0,690Zn$$

The scatter plot of observations in the space of discriminant variables for different types of ovarian cancer (two factors)

Chemical elemental analysis of mean concentrations of elements in brain cancers

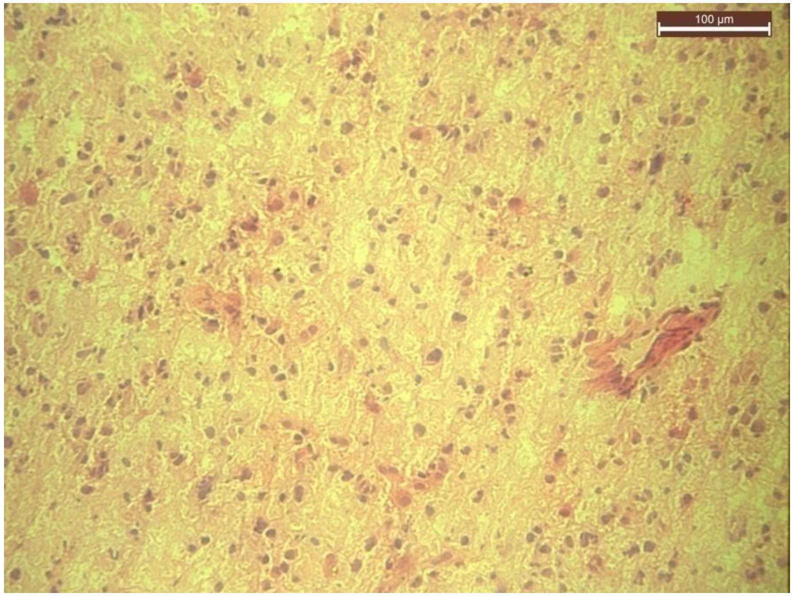
A.Wandzilak et al.
Metallomics, 5 (2013) 1547-1553

M.Lankosz, et al
Spectrochimica Acta Part B, 101 (2014) 98–105

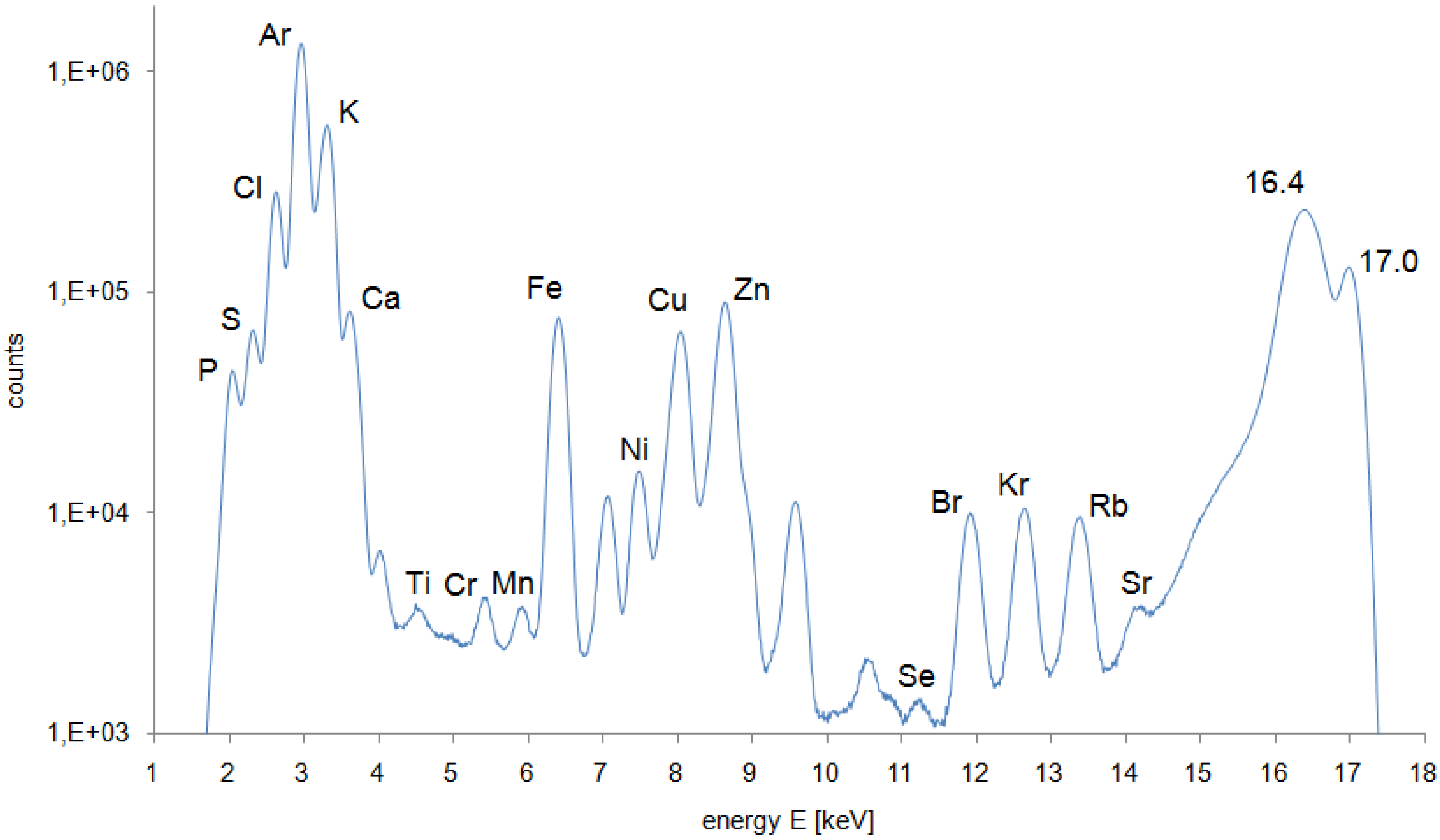
Examined material

- Neoplasma benignum
- Oligodendroglioma, II grade WHO
- Astrocytoma diffusum, II-III grade WHO
- Oligodendroglioma anaplasticum, III grade WHO
- Glioblastoma multiforme, IV grade WHO
- Control tissues

Astrocytoma diffusum

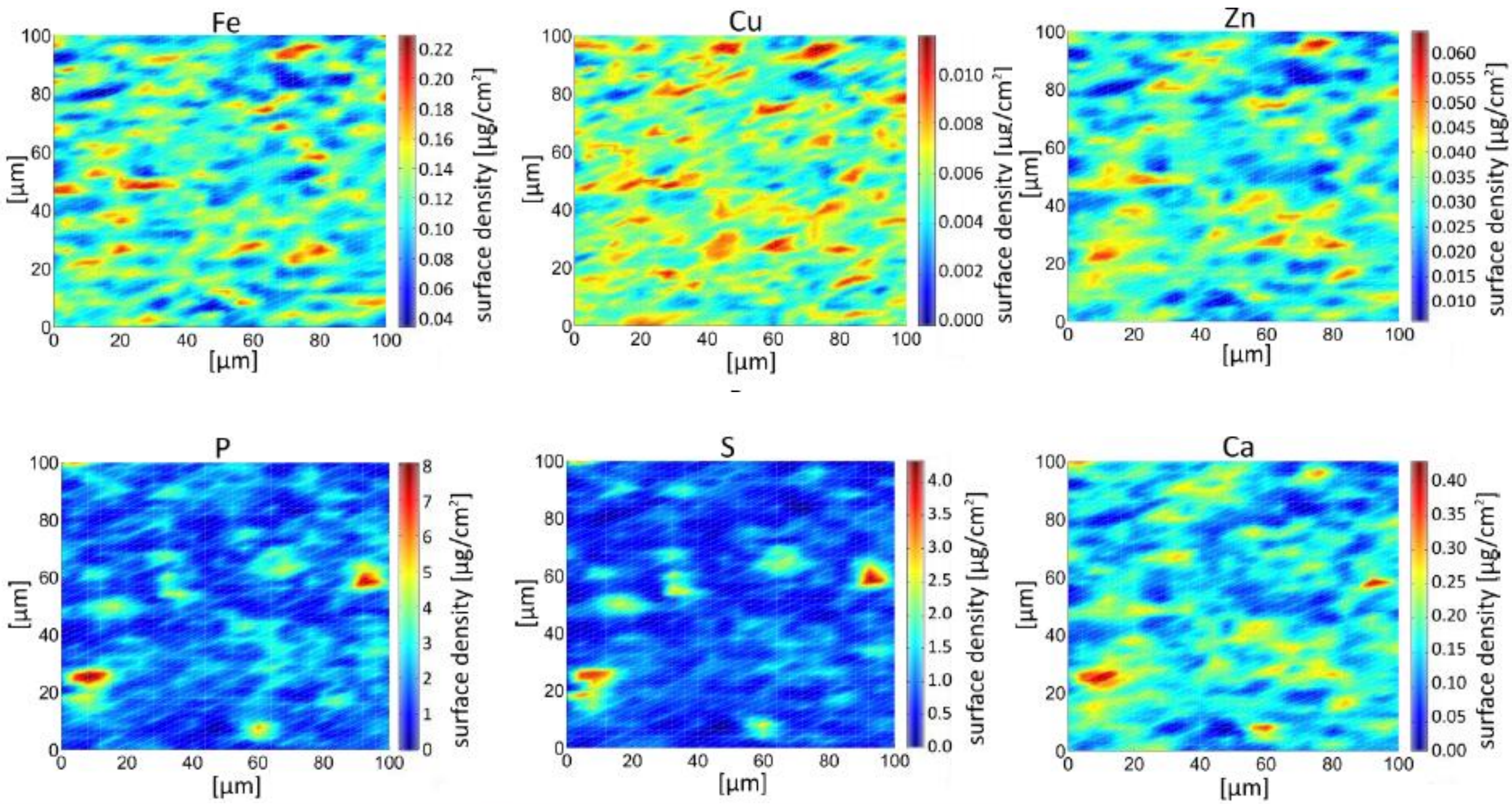


XRF spectrum

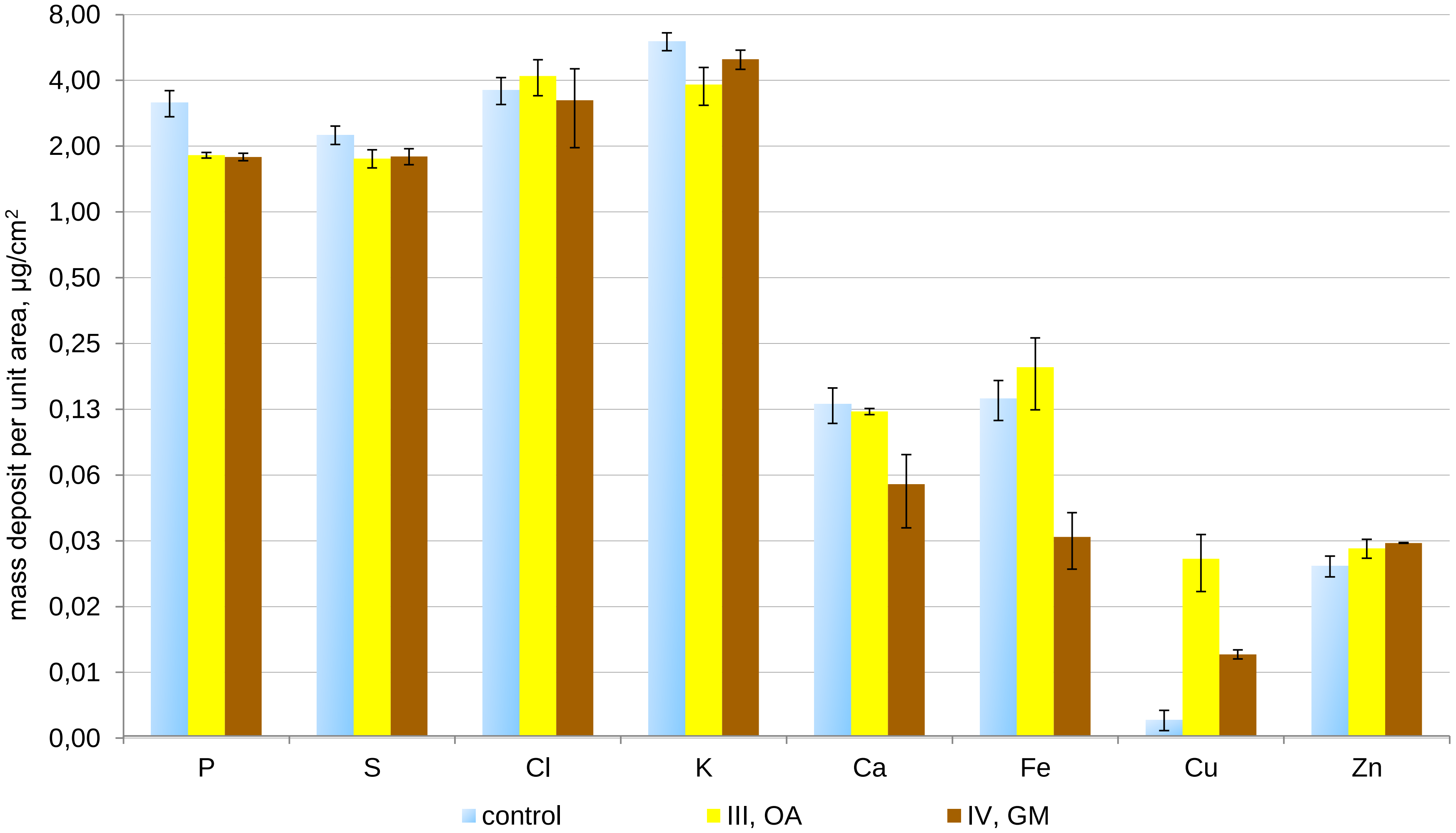


Astrocytoma diffusum XRF sum spectrum probed from 15 352 points

Distribution of Ca, P, S, Fe, Cu and Zn in a section of diffuse astrocytoma



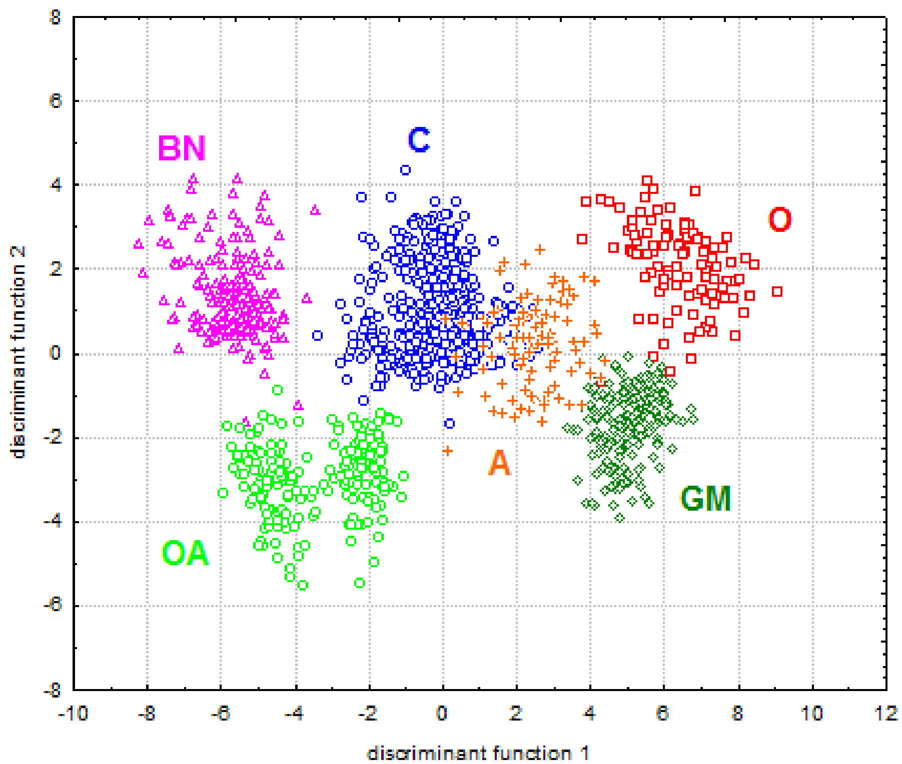
Cancerous vs. healthy tissue



Mean content of elements in healthy and cancerous tissues

Classifier

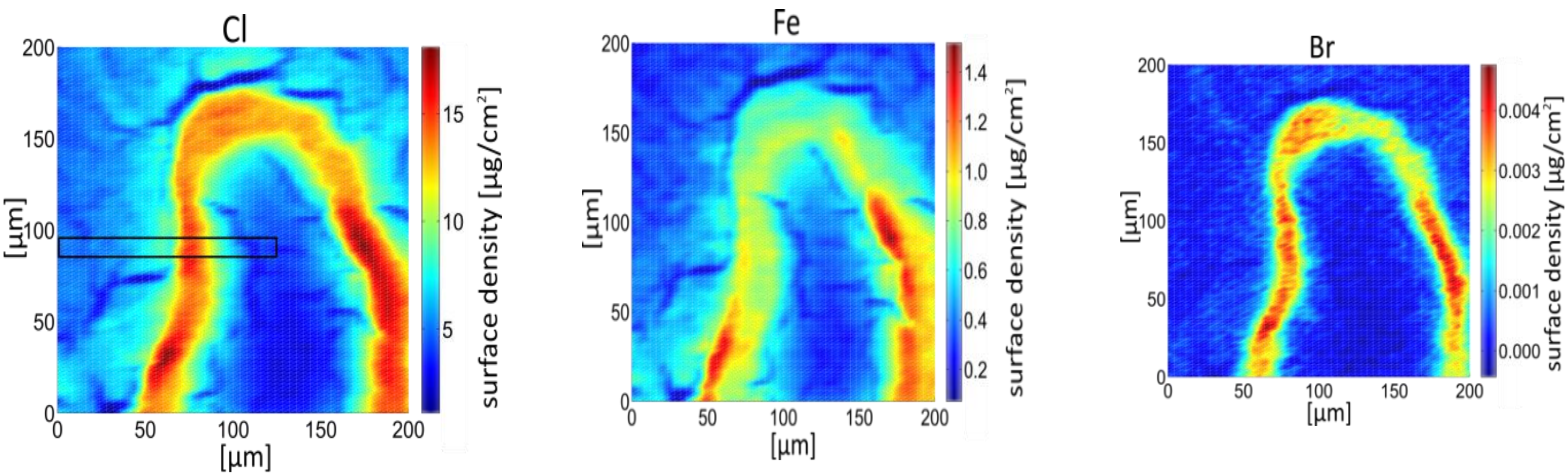
1. Differences in the chemical composition of tissues with different cancer type
2. Elements of the greatest importance in the differentiation of cancer type
3. Model to identify the cancerous case by its chemical composition (the fingerprint of cancer)

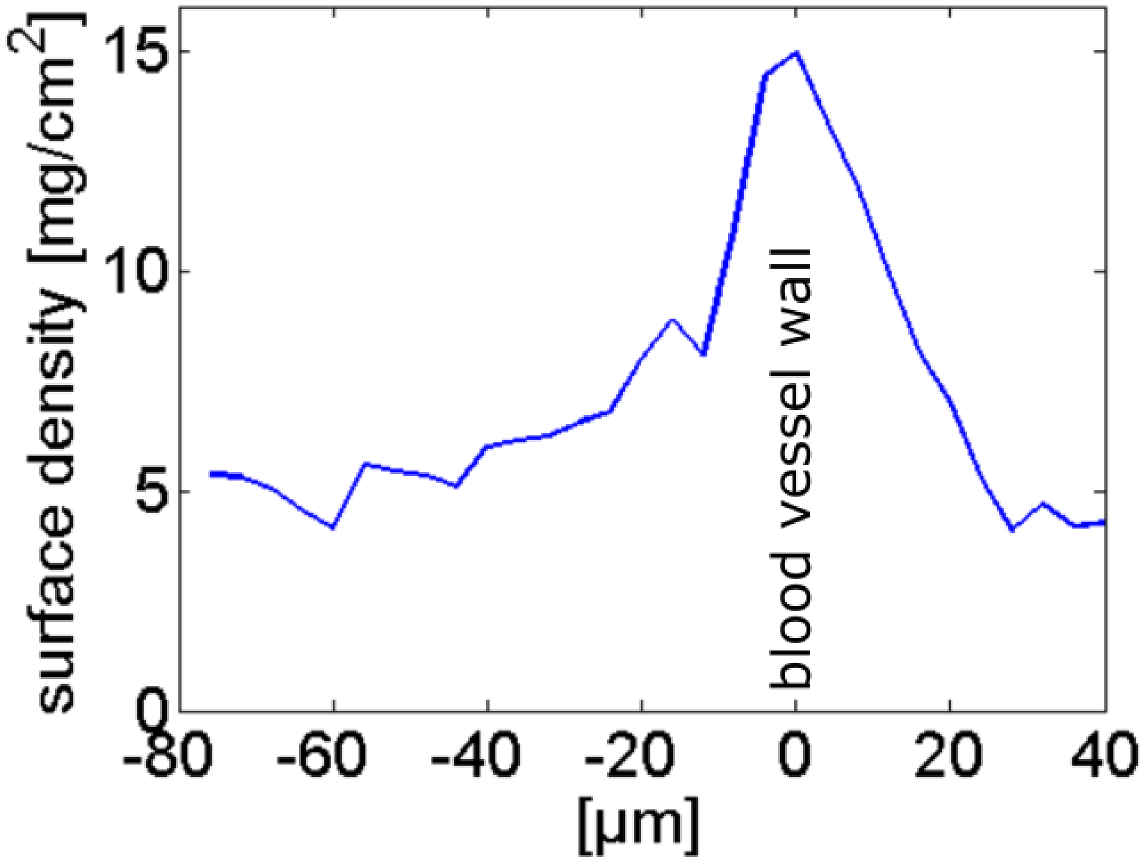


$$D_1 = -0.44 \cdot \mathrm{P} + 1.17 \cdot \mathrm{S} + 0.53 \cdot \mathrm{Cl} - 0.22 \cdot \mathrm{K} - 0.55 \cdot \mathrm{Ca} \boxed{-1.80 \cdot \mathrm{Fe} - 2.45 \cdot \mathrm{Cu} + 2.97 \cdot \mathrm{Zn}}$$

$$D_2 = \boxed{1.53 \cdot \mathrm{P} + 1.24 \cdot \mathrm{S} - 1.39 \cdot \mathrm{Cl} - 1.27 \cdot \mathrm{K}} + 0.21 \cdot \mathrm{Ca} + 0.71 \cdot \mathrm{Fe} \boxed{-1.98 \cdot \mathrm{Cu}} + 0.74 \cdot \mathrm{Zn}$$

Surface densities of Cl, Fe and Br within blood vessel area



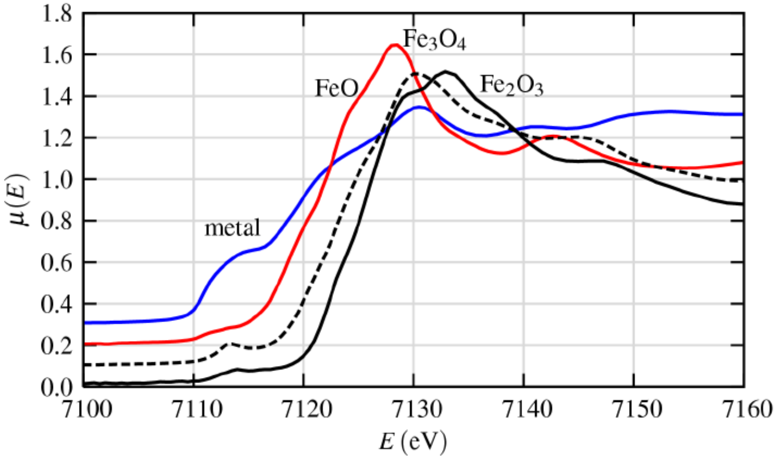


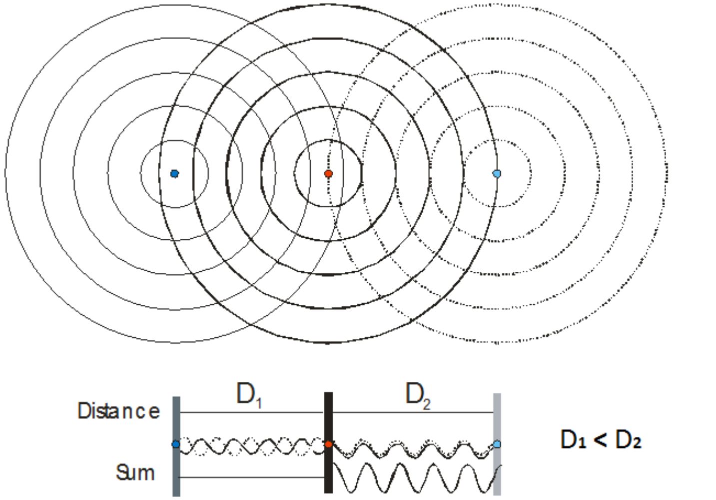
Changes in the surface density of Cl as a function of distance from a blood vessel.

Analysis of Fe, Cu and Zn chemical environment and oxidation states in brain cancers with the use of XANES and EXAFS microspectroscopies

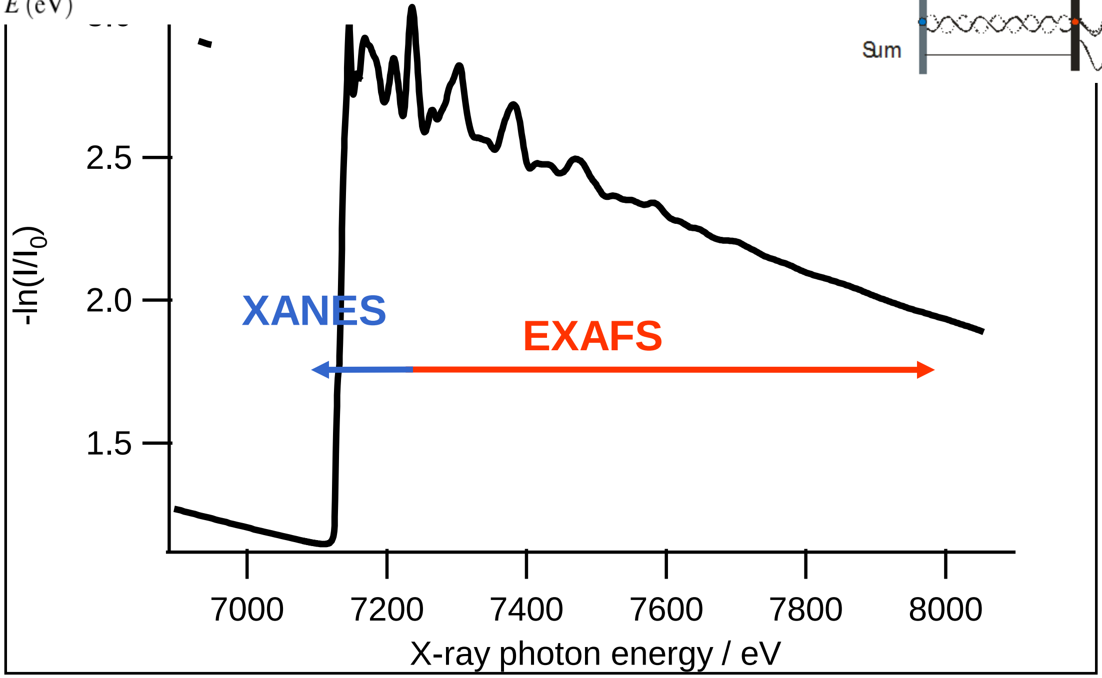
A.Wandzilak et al.
Metallomics, 5 (2013) 1547-1553
DLS Report 2012

XAS = XANES + EXAFS





Chemical shift→
Oxidation state

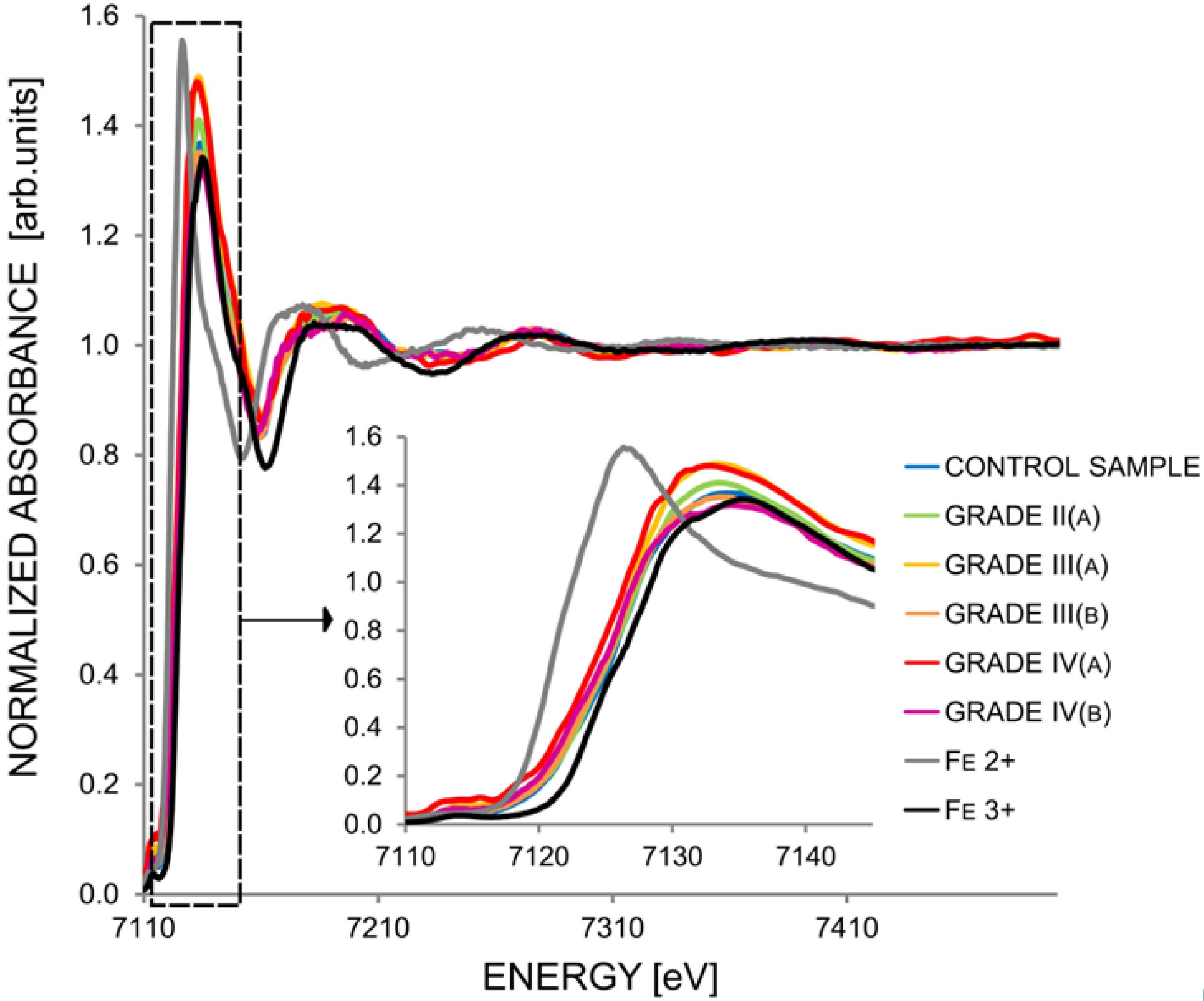


photoelectrons →
MATTER WAVES

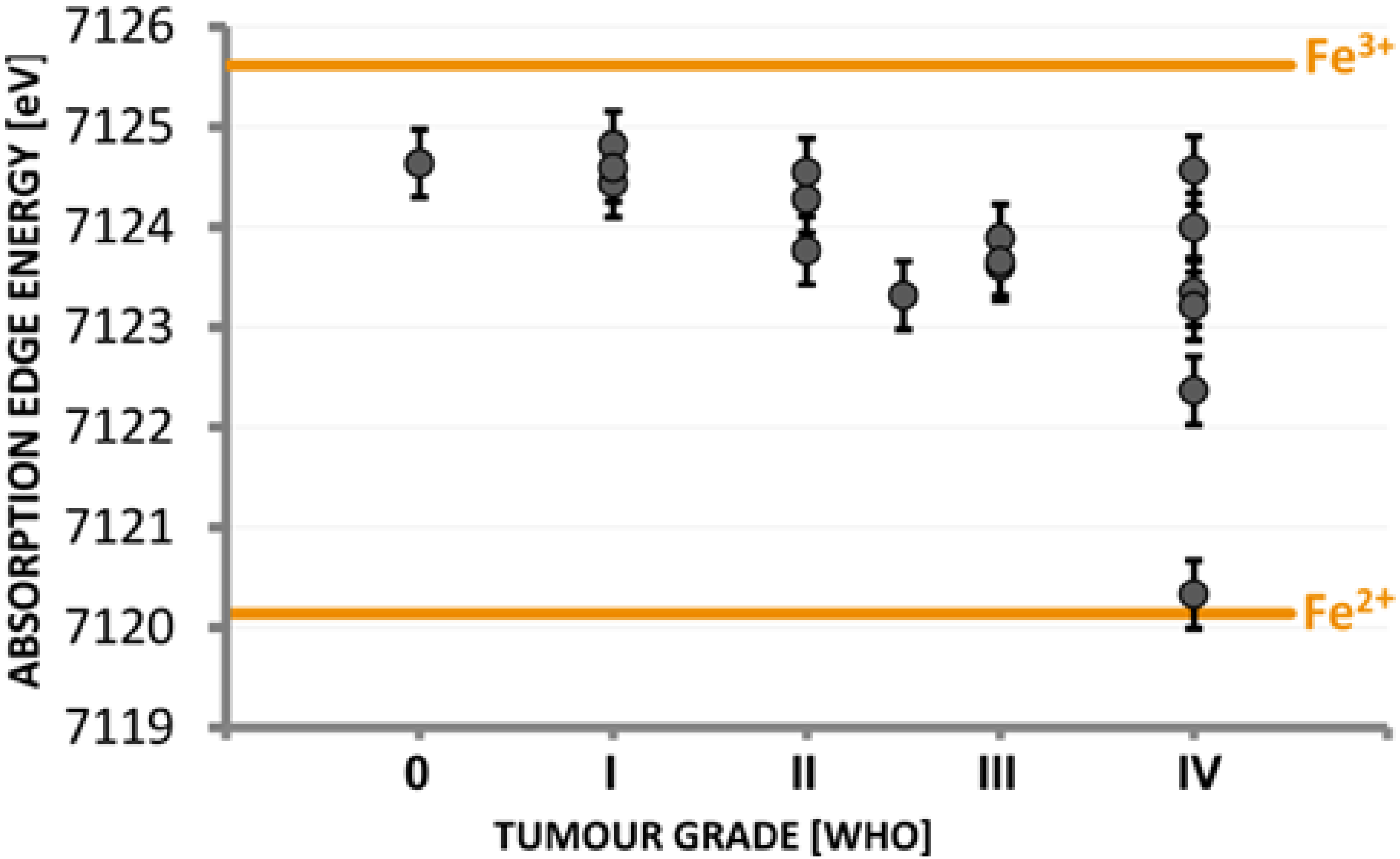
XANES - X-RAY ABSORPTION NEAR EDGE STRUCTURE

EXAFS - EXTENDED X-RAY ABSORPTION FINE STRUCTURE

The FeK XANES spectra (absorption edge regions) for reference materials (Fe2+ and Fe3+) and brain samples (tumours and control). Results from bulk analysis

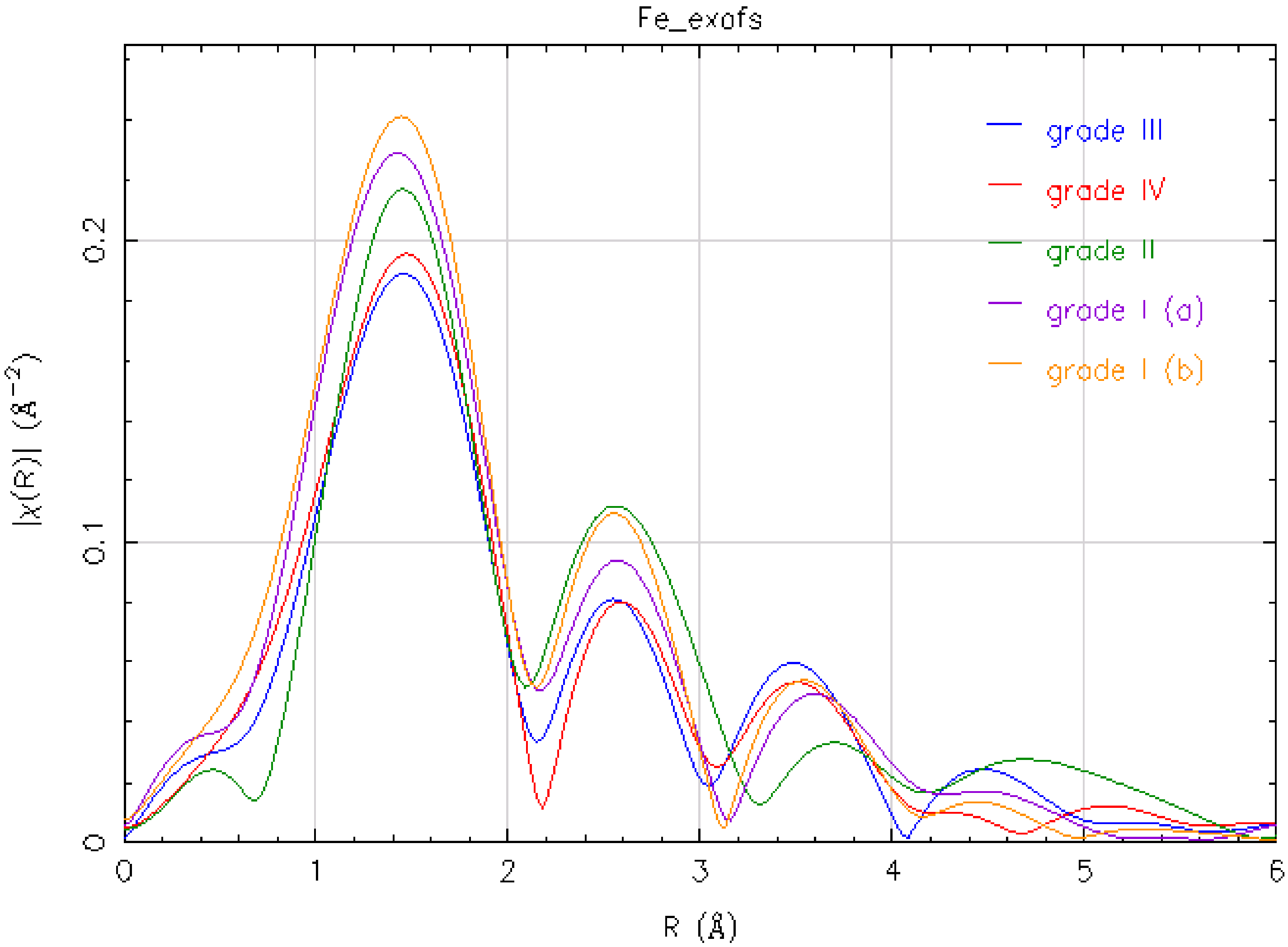


Fe average oxidation state in neoplastic tissues

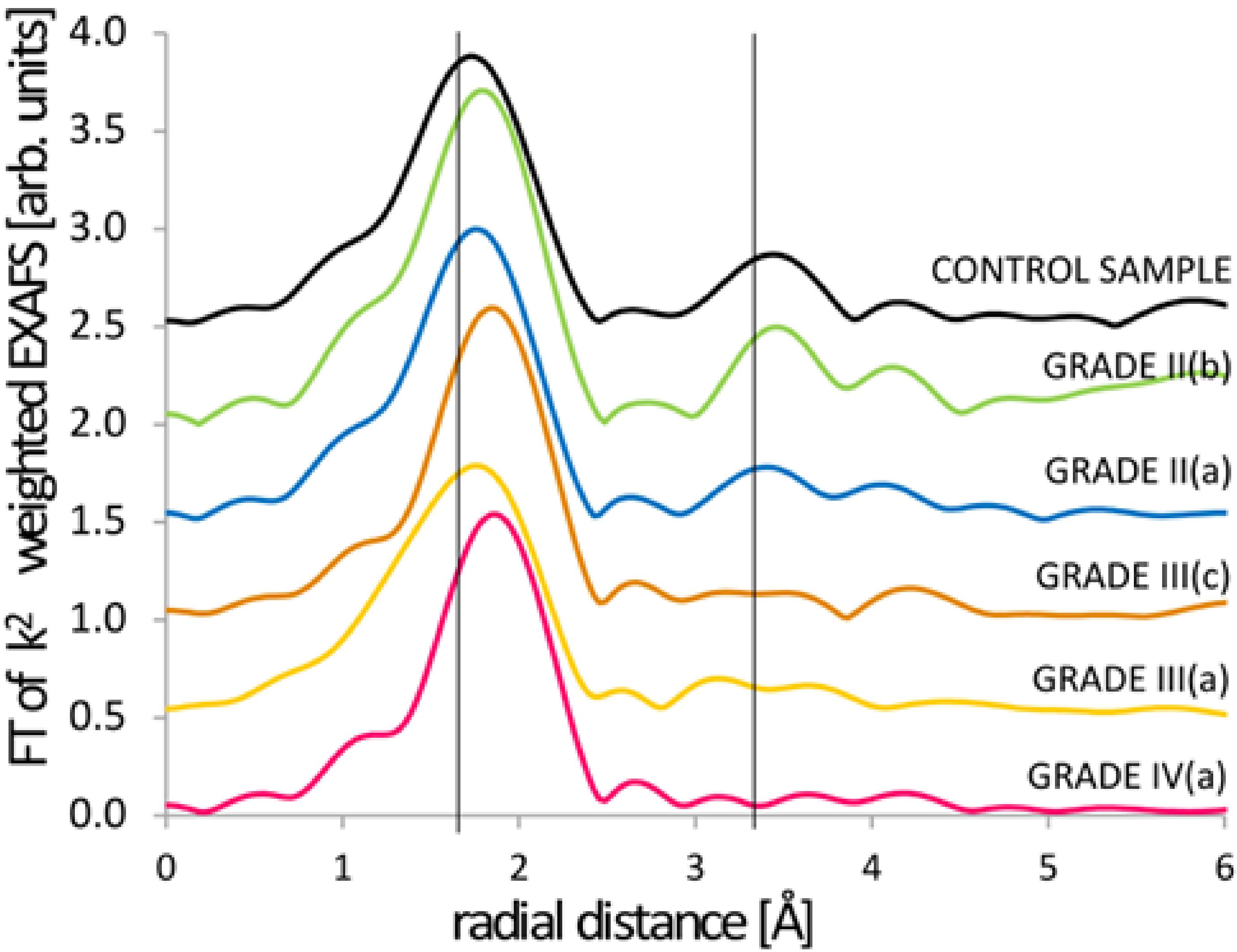


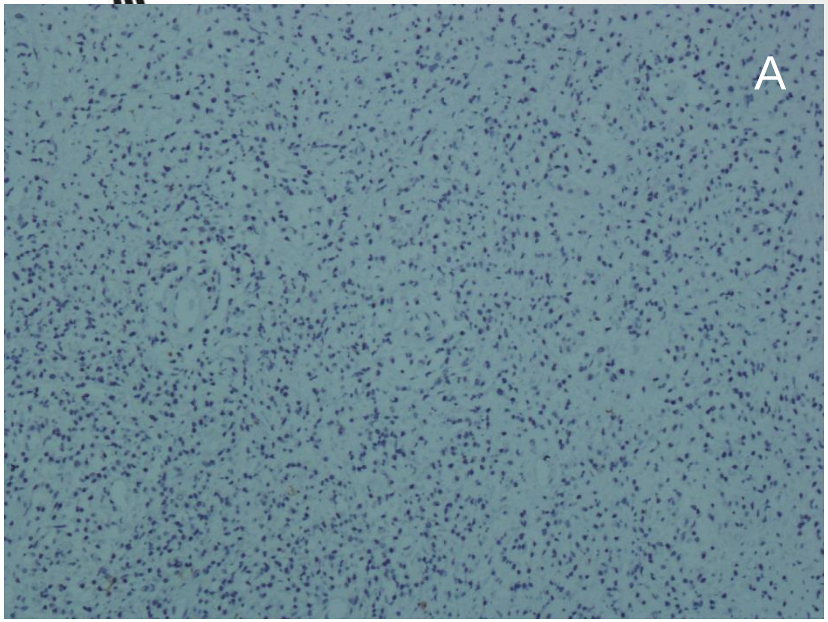
Absorption edge energies of Fe for various malignancy grade

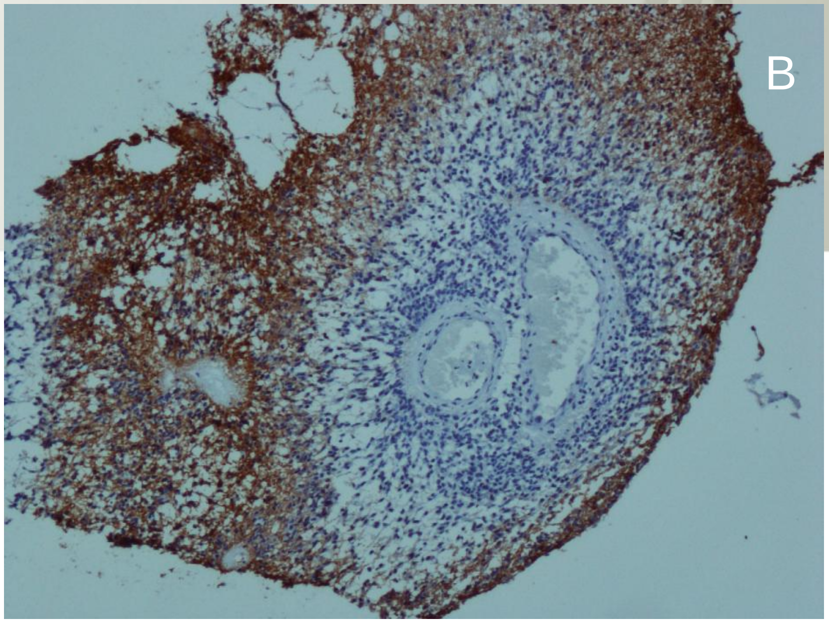
Fourier transform of Fe EXAFS data for brain tumor samples with various malignancy grades as a function of radial coordinate

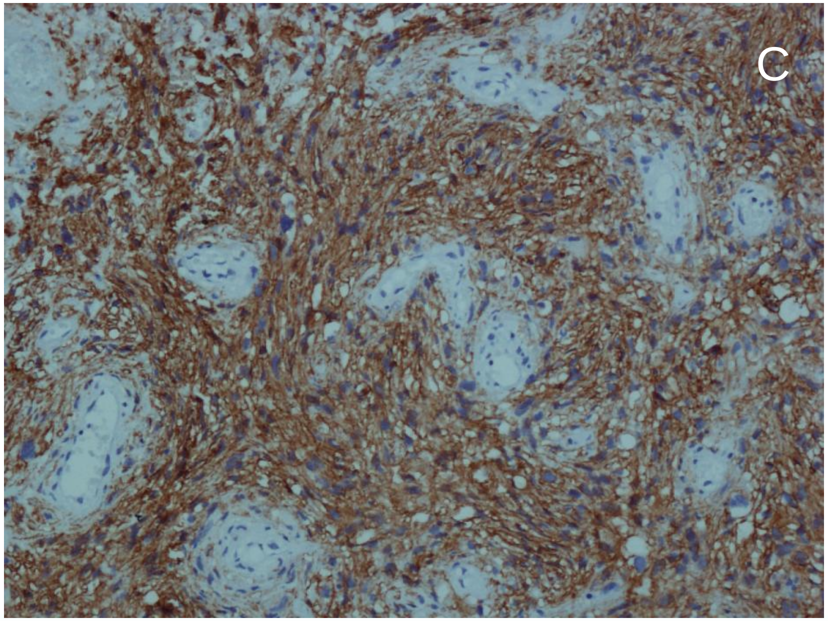


Fourier transform of Zn EXAFS data for brain tumor samples with various malignancy grades as a function of radial coordinate









Tumour tissue with no hypoxia (A),
moderate hypoxia (B)
and high level of hypoxia (C).

Metallomics, 2013, 5, 1547-1553

https://www.thebraintumourcharity.org/media-centre/news/latest-news/uk-based-scientists-study-trace-metal-elements-ide/

Scientists based at Diamond Light Source, the UK's national synchrotron science facility in Oxfordshire, have used a technique known as x-ray fluorescence to track microscopic trace metals and correctly identify malignant brain tumour cells.

A synchrotron is a type of particle accelerator used to study molecular levels of particles among other applications, and Diamond Light Source is used by thousands of researchers and scientists in the medical, structural biology, and nanoscience fields each year.

The scientists are trying to explore the link between such trace metals and the growth, and crucially, the malignancy of cancerous brain cells.

“This work is still in its early stages but, in time, the discovery of the link between certain trace metals and their role in the growth of cancer cells could help to redefine the way we identify brain tumours, allowing for earlier diagnosis and, ultimately, a better chance for patients," said Diamond's CEO, Andrew Harrison.

Professor Marek Lankosz from AGH University of Science and Technology and principal investigator on the research explained further: “When exposed to X-rays, elements fluoresce in certain ways: this allows us to determine what elements are present and where. The technique is commonly used in many fields, including space science, ecological and conservation work – but we have now shown that it could have hitherto unrecognised uses in the diagnosis of brain cancer and may provide a significant new clinical tool."

Dr Tina Geraki, senior support scientist summed the research up: “These findings can make an impact on our understanding of the changes in the brain associated with the mechanisms of malignancy."

Summary

- ✓ ***The MDA based on the elemental composition of tissue (SRXRF) may be a potentially valuable method in assisting the differentiation and/or classification (diagnosis) of ovarian and brain tumors including doubtful cases.***

- ✓ ***The external hybridization of images obtained from optical microscopy of stained tissue, SR XRF elemental microscopy and IR micro spectroscopy should be improved***

- ✓ ***The techniques based on SR for physicochemical characterization of tissue samples (XANES, EXAFS) should be performed in cryo conditions***

- ✓ ***XANES and EXAFS enable analysis of oxidation states and chemical environment of Fe, Cu and Zn in tumors cells. Methods for modelling of chemical environment and identification of proteins binding Fe, Cu and Zn in cancer cells should be improved***

Cooperation

1. Chair of Pathomorphology, CM UJ
   Dariusz Adamek, Edyta Radwańska, Łukasz Chmura
   Department of Gynecology and Oncology, CM UJ
   Robert Jach
2. P06 at Petra III:
   G. Falkenberg, M. Alfeld and U. Bösenberg
3. CEMO at DORIS III, P64 at Petra III
   E. Welter, K. Apple
4. I18 at Diamond
   T. Geraki, F. Mosselmann
5. BM23 at ESRF
   Olivier Mathon, Sacura Pacarelli
6. ID21 at ESRF M. Salome, H. Castillo-Michel, B. Hesse and G. Veronesi
7. FLUO at ANKA
   Rolf Simon

Faculty of Physics and Applied Computer Sciences

Aleksandra Wandzilak
Paweł Wróbel
Mateusz Czyżycki
Maria Grzelak
Daria Krauze
Beata Ostachowicz
Zdzisław Stęgowski
Magdalena Szczerbowska-Boruchowska

Proposals

DESY

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