# Evaluation of Biochemical Biomarkers in Brain Tumors with Different Grade of Malignancy

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## Cooperation

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# THE ROLE OF METALS IN CANCEROUS PROCESSES

***Fe, Cu, Mn – participation in carcinogenesis for example by oxidative stress***

***Cu - promotion of angiogenesis***

***free ionic Zn - a potent killer of neurons and glia (by oxidative stress)***

***Potential influence of the tumor on the surrounding tissue promoted by metals:***

***Ca - excitotoxicity***

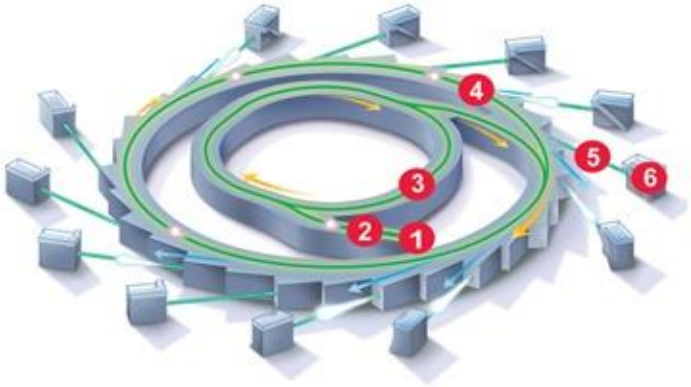
***Mn, Cu, Zn - disfunction of metalo-dependent enzymes like Mn-SOD (Mn) or Cu/Zn-SOD***

***Se, Zn, Cu - an important role in the protection of the organism against the mechanisms that can initiate or accelerate the carcinogenesis;***

# Types of tumors used in studies

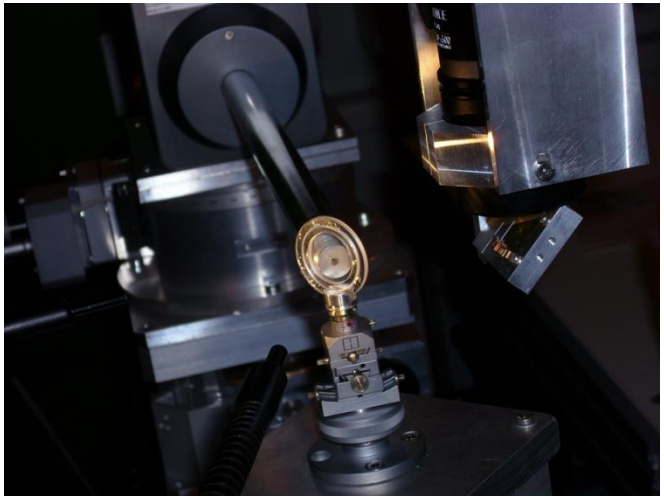
| TUMOR TYPE | ABBREVIATION | Grade of malignancy |
|---|---|---|
| glioblastoma multiforme | GM | IV |
| gemistocytic astrocytoma | AG | III |
| oligodendroglioma | O | II |
| anaplastic oligodendroglioma | OA | III |
| ganglioglioma | G | III |
| fibrillary astrocytoma | AF | II |
| atypical transitional meningioma | M | I |
| control | C | |

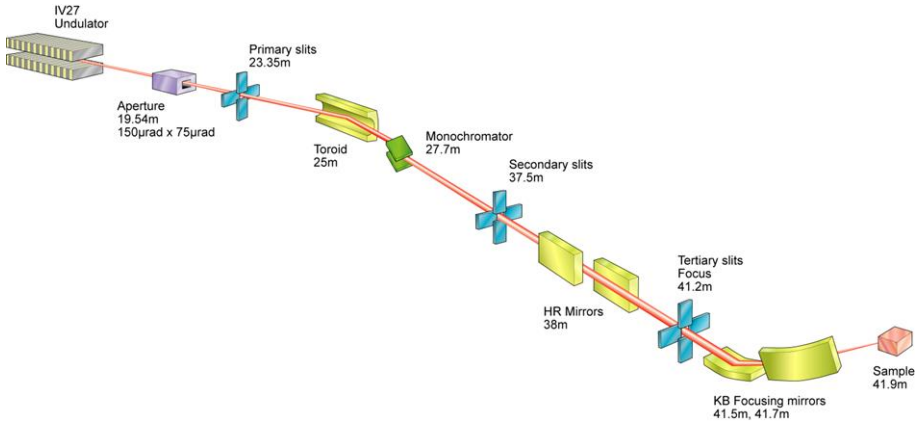
# CHEMICAL ELEMENTAL IMAGING WITH THE USE OF X-RAY FLUORESCENCE MICROSPECTROSCOPY




**Synchrotron**

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

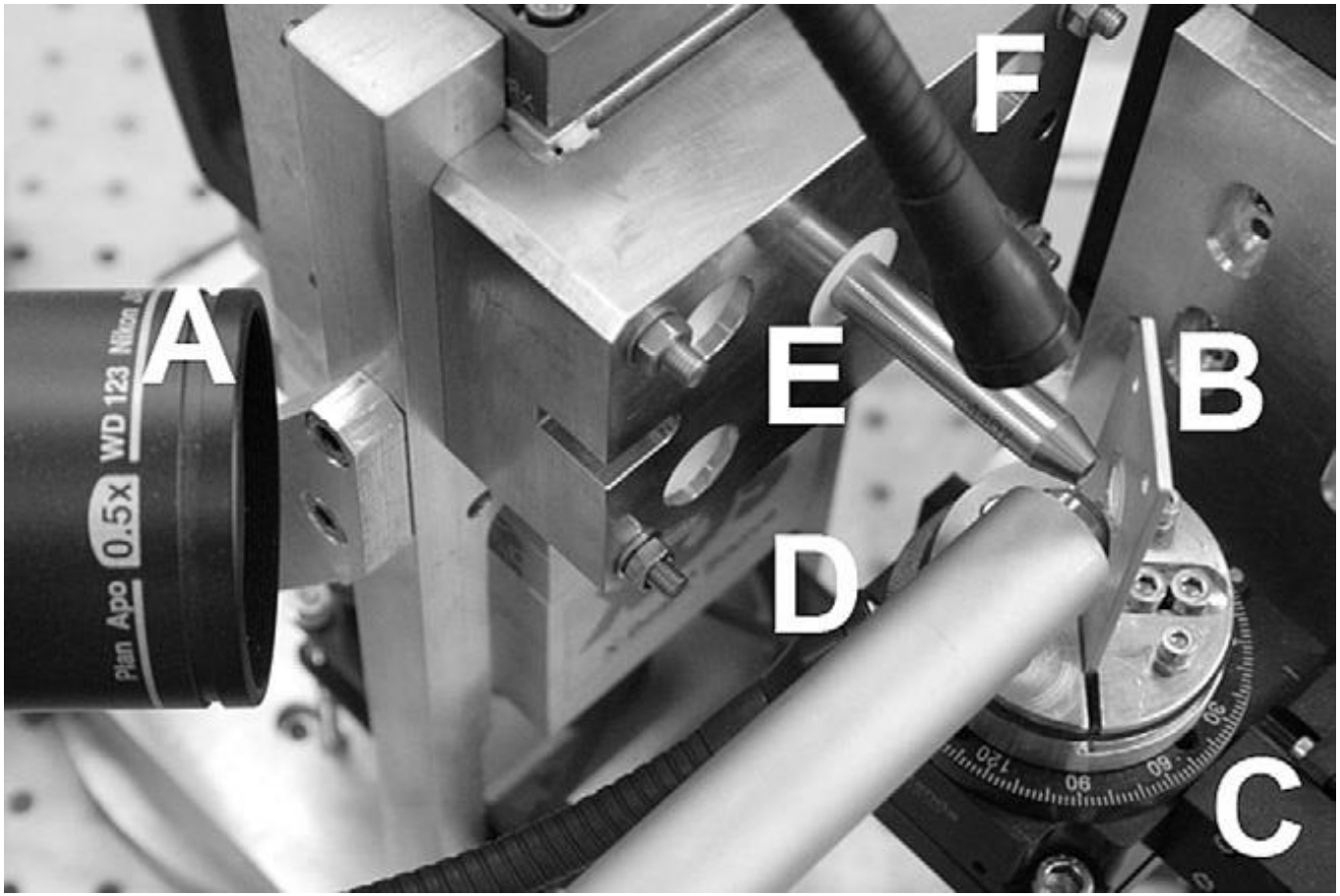





XRF equipment at the P06 beamline at Petra III
17.0 keV, 700 nm, 2 s/point

AGH

# Table-top 2D/3D X-ray spectrometer



# SAMPLE PREPARATION for biochemical micro-imaging

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Brain tissue

↓

cutting into 10 or 20 μm thick sections

(cryomicrotome)

↙ biochemical analyses

↓

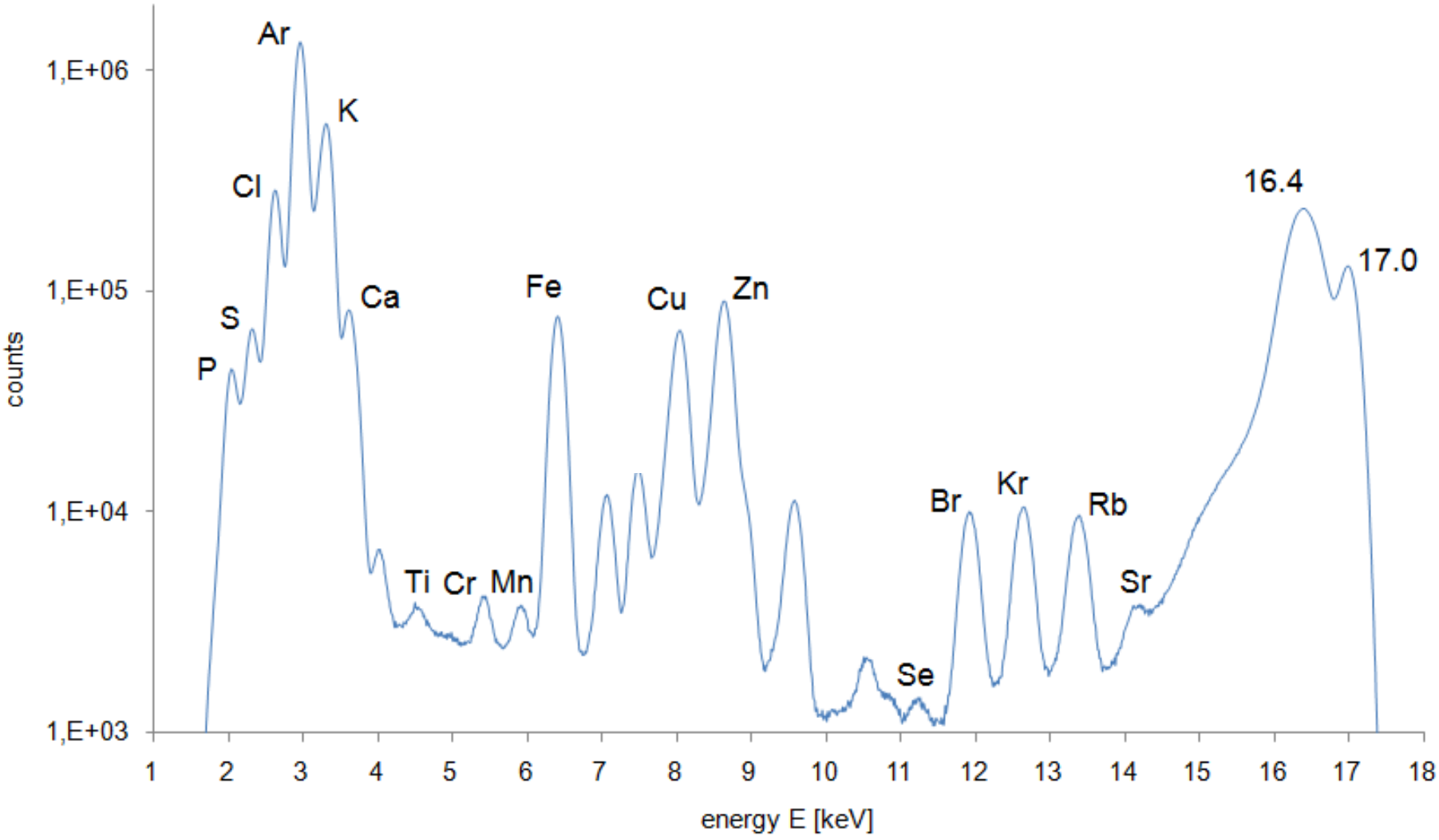
AP1 or ultralene foil

↓

freeze-drying at -80 °C

↘ histopathology

# XRF spectrum

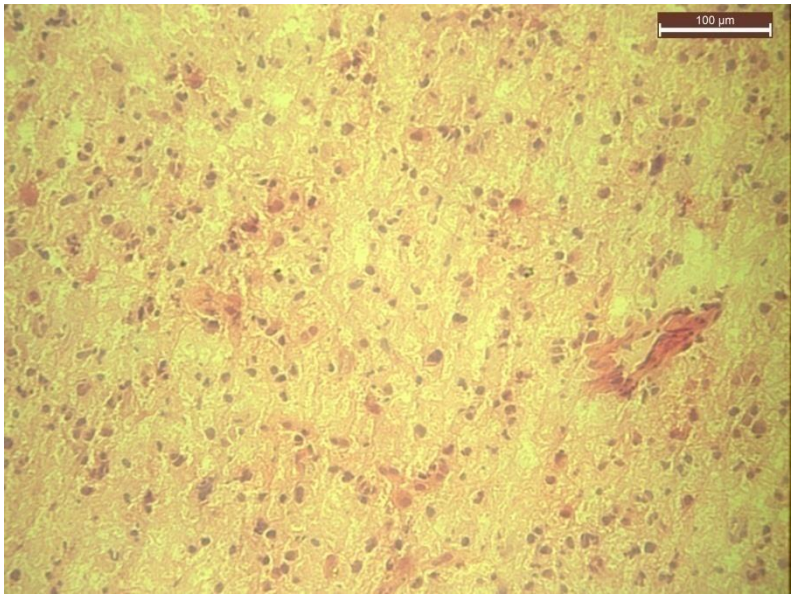


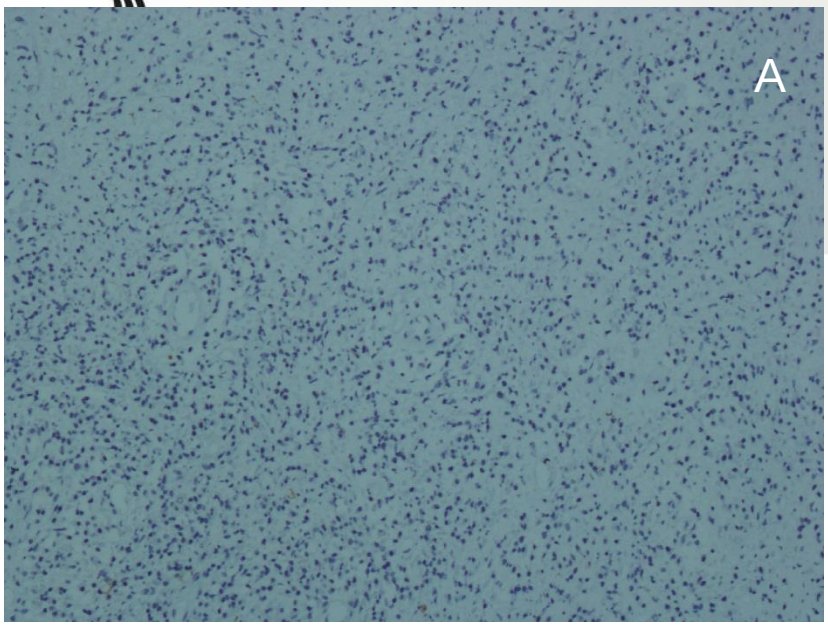

**Astrocytoma diffusum XRF sum spectrum probed from 15 352 points**

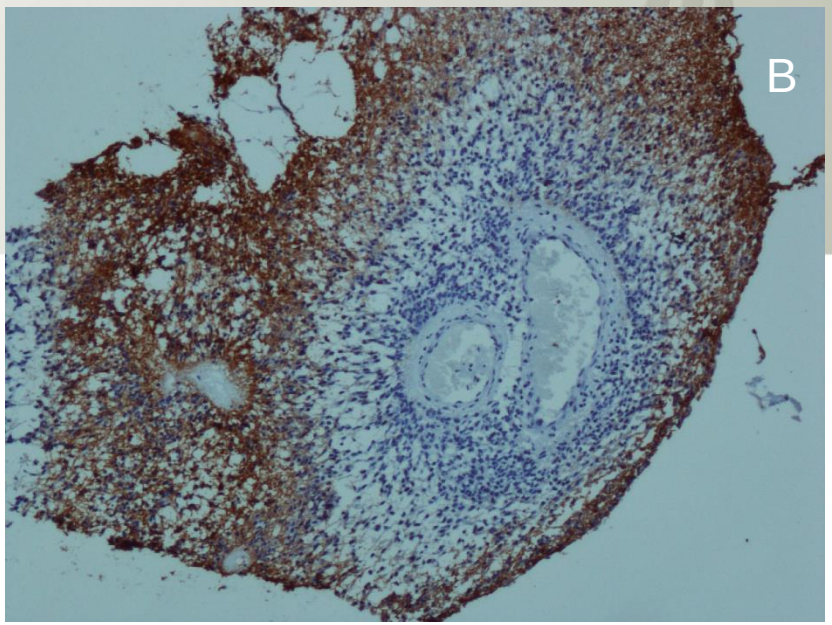
# Examined material

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

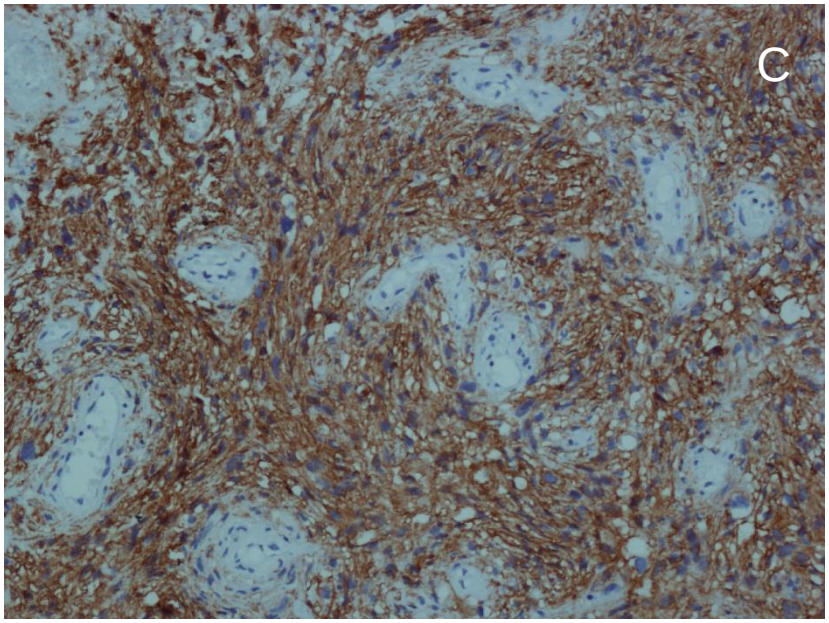
Astrocytoma diffusum



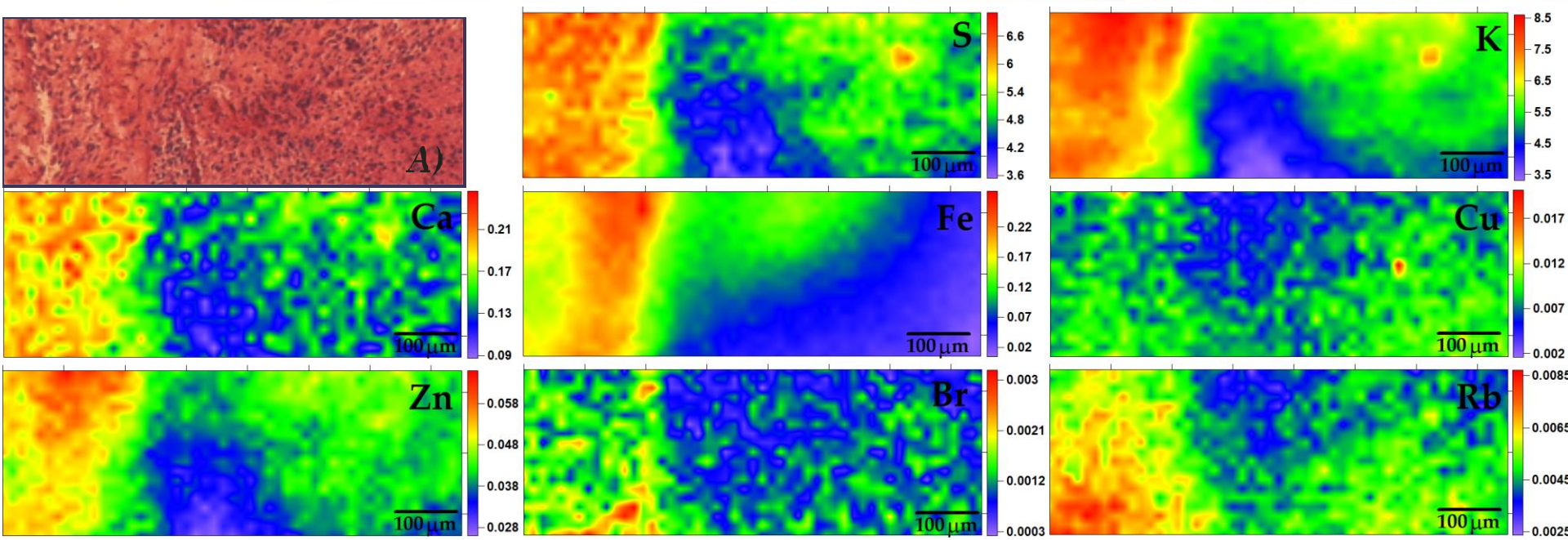



Tumour tissue with no hypoxia (A),
moderate hypoxia (B)
and high level of hypoxia (C).

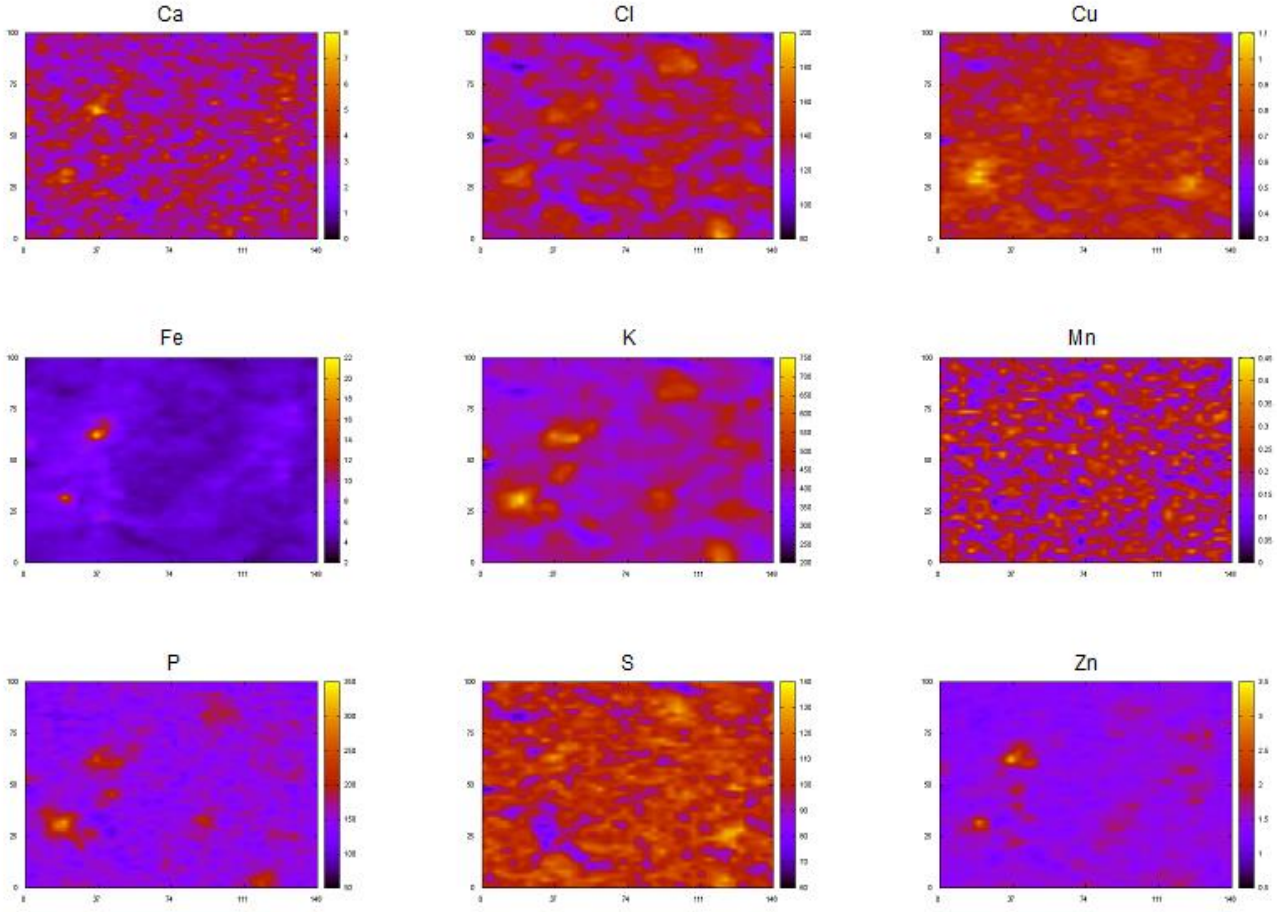



Metallomics, 2013, 5, 1547-1553



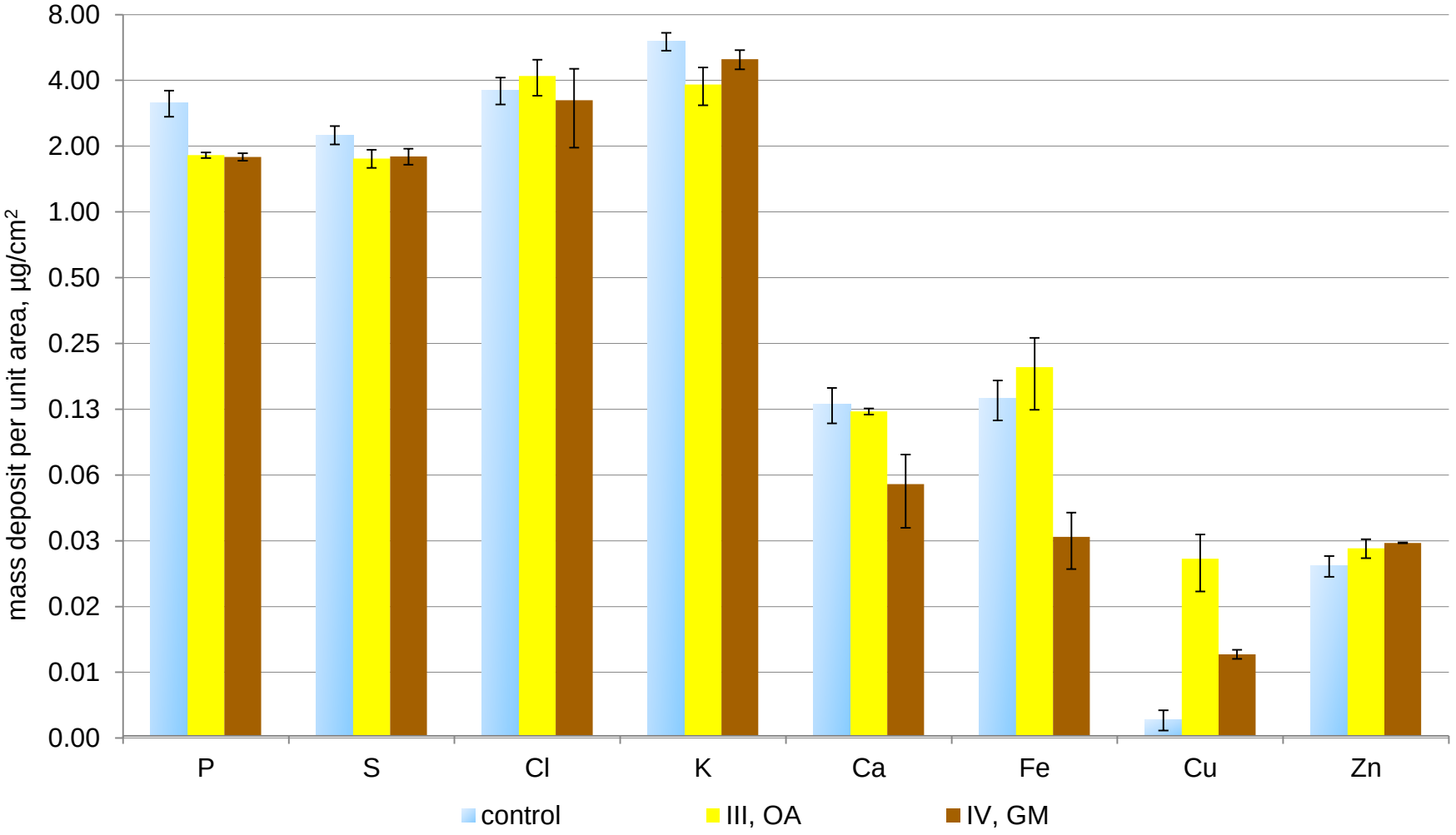

***Distribution of selected elements in glioblastoma multiforme tissue (IV WHO) in comparison with microscopis view of hematoxylin-eosin stained section (A). Data presented in [µg/cm$^2$].***

# Elements distribution




Glioblastoma multiforme Area of 100 μm × 148 μm

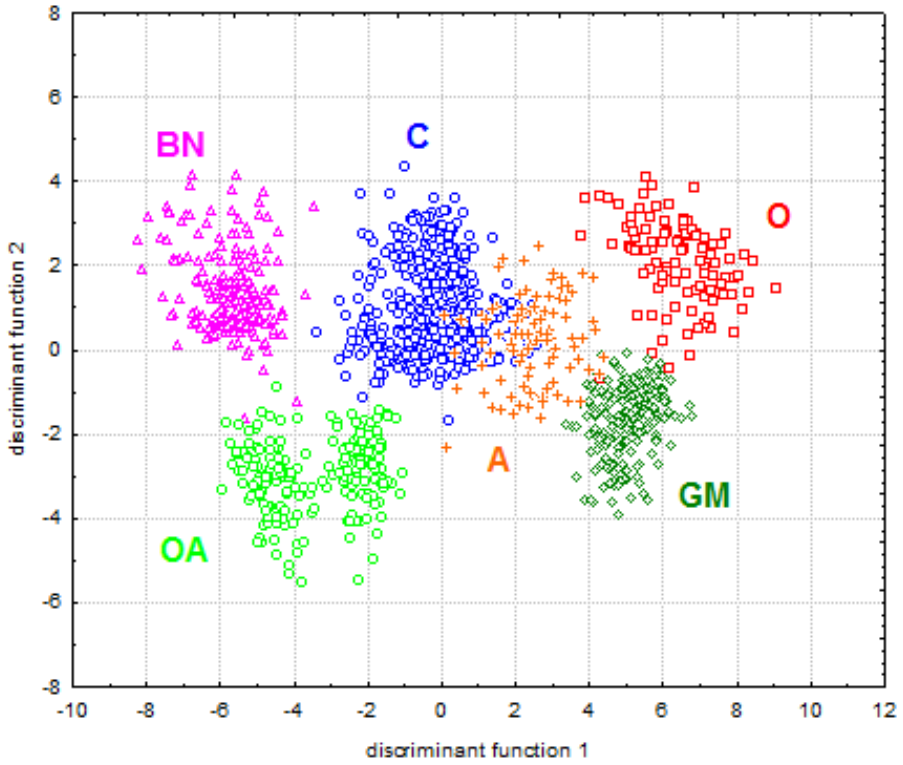
# Cancerous vs. healthy tissue




**Mean content of elements in healthy and cancerous tissues**

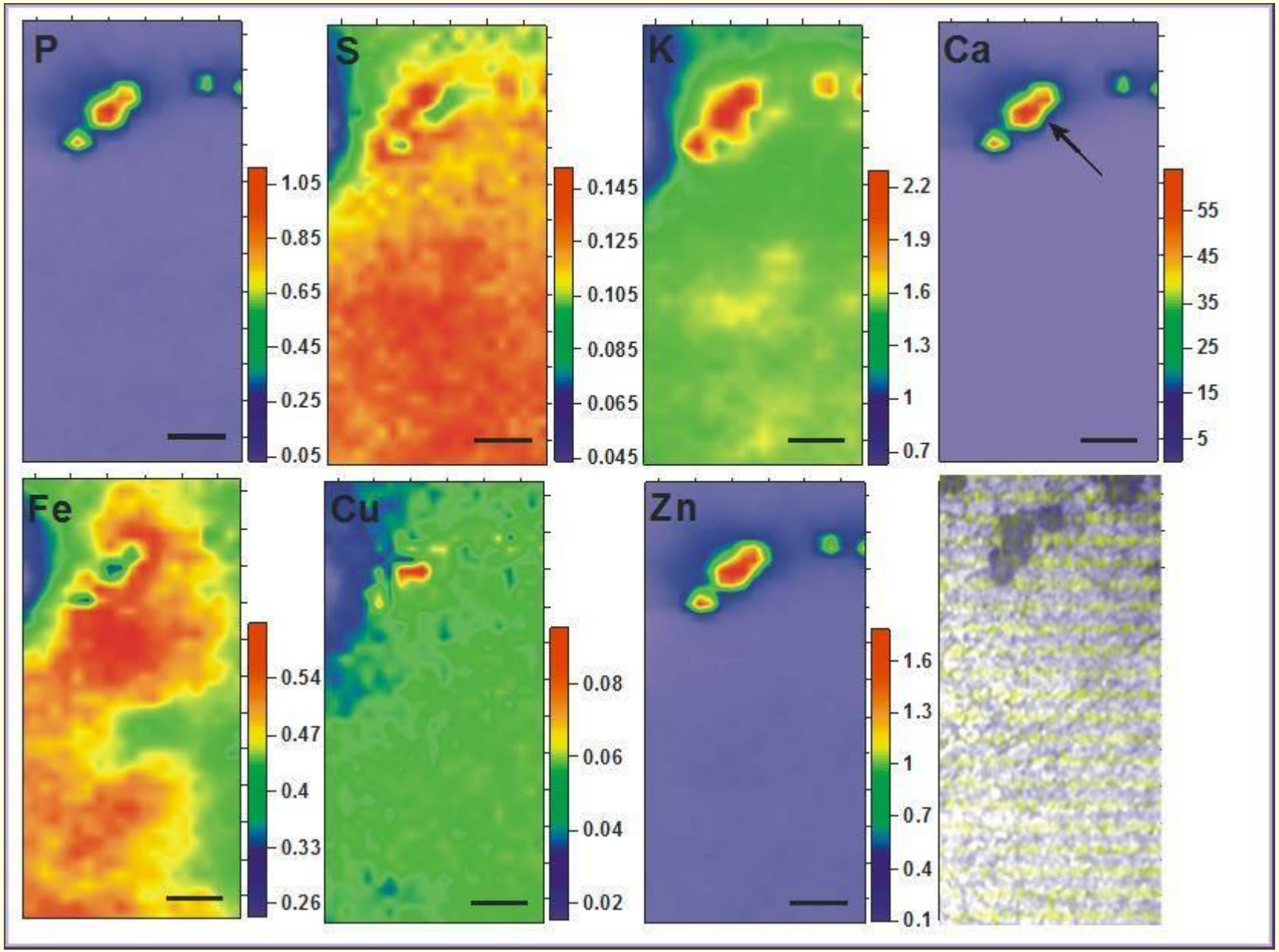
# Classifier

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)




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

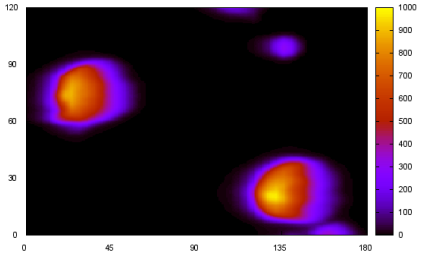
$$D_2 = 1.53 \cdot \mathrm{P} + 1.24 \cdot \mathrm{S} - 1.39 \cdot \mathrm{Cl} - 1.27 \cdot \mathrm{K} + 0.21 \cdot \mathrm{Ca} + 0.71 \cdot \mathrm{Fe} - 1.98 \cdot \mathrm{Cu} + 0.74 \cdot \mathrm{Zn}$$



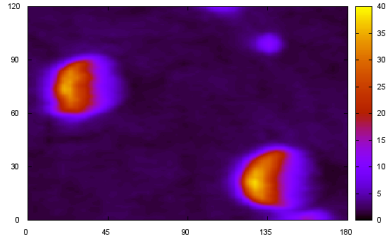

***μ-XRF maps of elemental distribution in brain glioma tissue with areas of <u>calcification</u> (see arrow) . Data presented in arbitrary units. Scale bars: 50 μm***

# Calcification in brain gliomas SRXRF

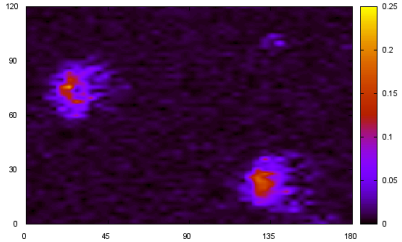
Ca



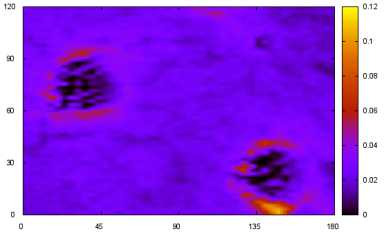
Cl



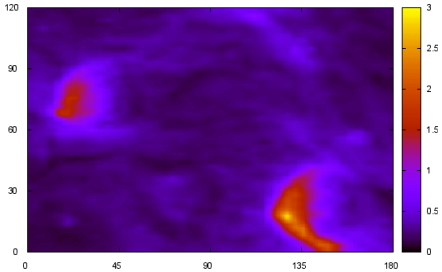
Cr



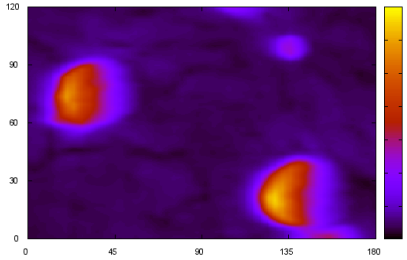
Cu



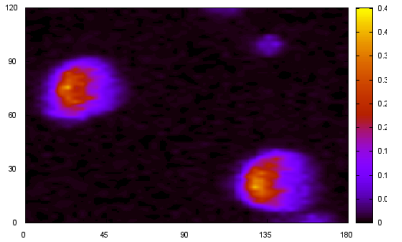
Fe



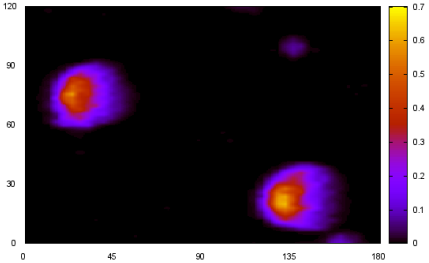
K



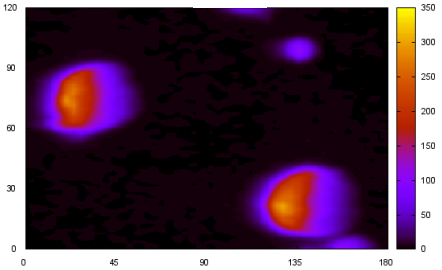
Mn



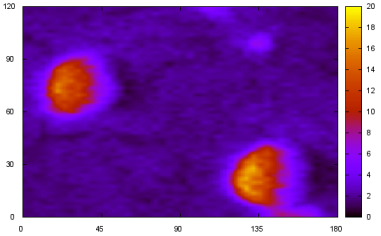
Ni



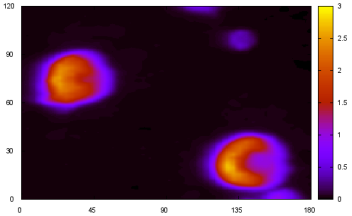
P



S



Zn



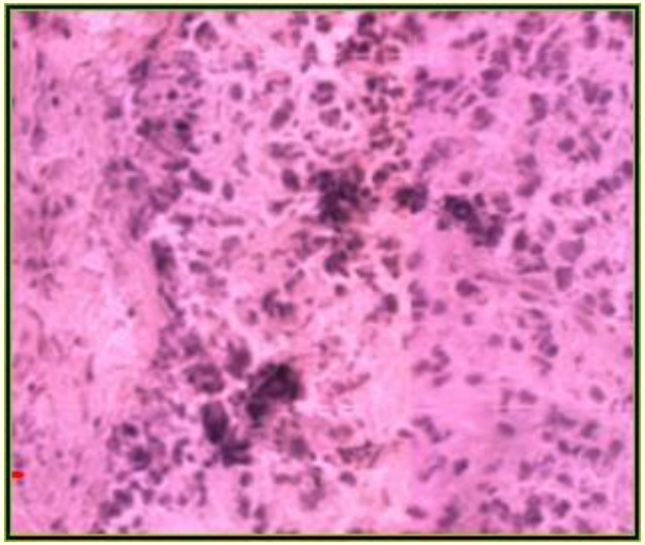
Scanned area: 180 μm / 120 μm.

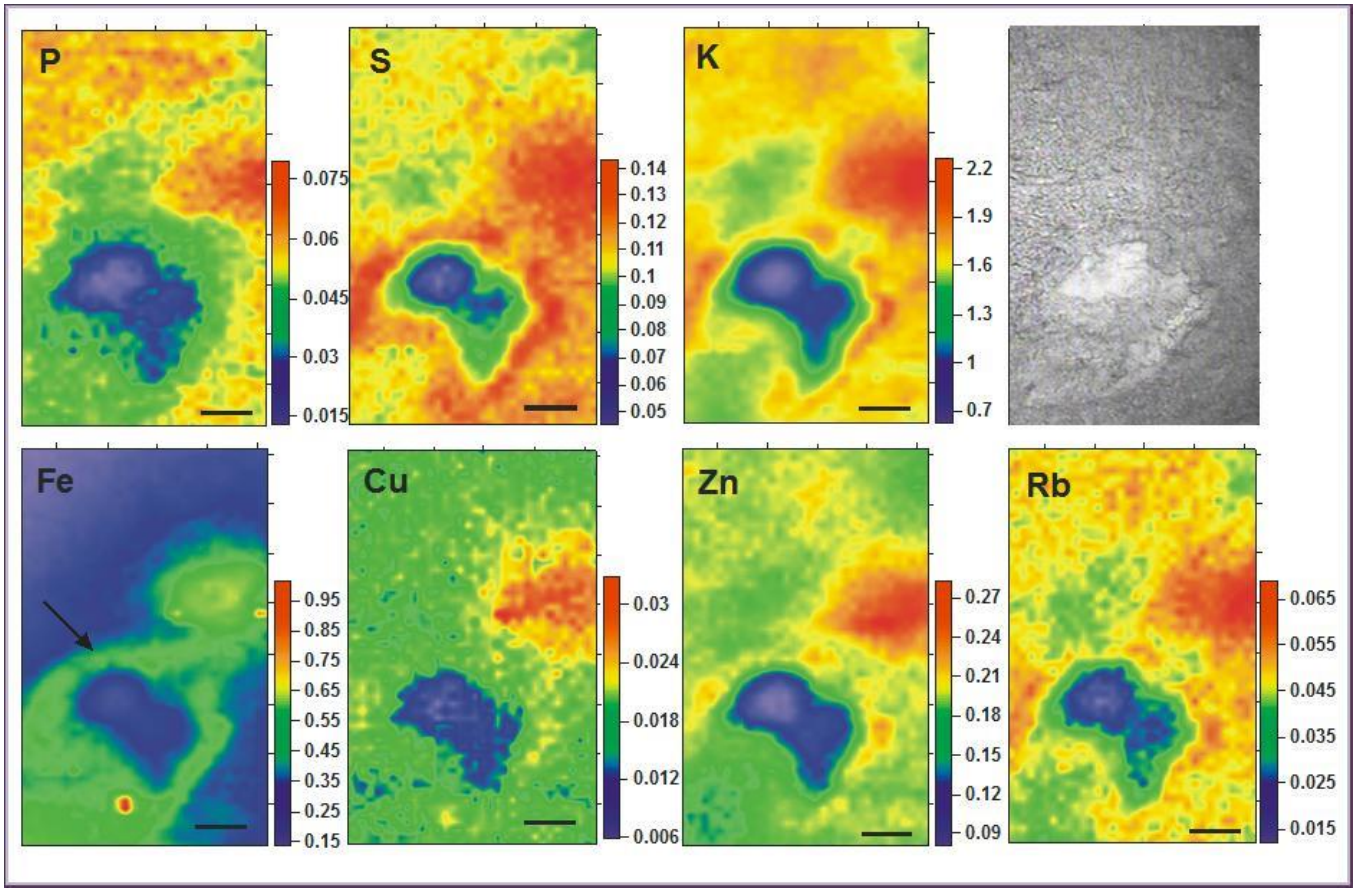
# Calcification ↔ neoplastic tissue

***Parameters describing the significance of the primary variables for the model.***

| Element | Partial Wilk's Λ | F – Fisher's statistics | p-value |
|---|---|---|---|
| S | 0,525 | 54,2 | 0,000 |
| Zn | 0,850 | 10,5 | 0,001 |
| Ca | 0,875 | 8,53 | 0,004 |
| Rb | 0,897 | 6,81 | 0,011 |
| Br | 0,921 | 5,13 | 0,027 |
| Fe | 0,923 | 4,95 | 0,029 |
| Cu | 0,945 | 3,44 | 0,068 |
| P | 0,947 | 3,34 | 0,072 |
| Cl | 0,974 | 1,56 | 0,215 |
| K | 0,991 | 0,523 | 0,472 |

***S, Zn, Ca, Rb, Br, Fe***
***the elements of the highest importance for the discrimination between cancerous tissue and calcification***






***μ-XRF maps of elemental distribution in brain glioma tissue with blood vessel (see arrow) . Data presented in arbitrary units. Scale bars: 100 μm***

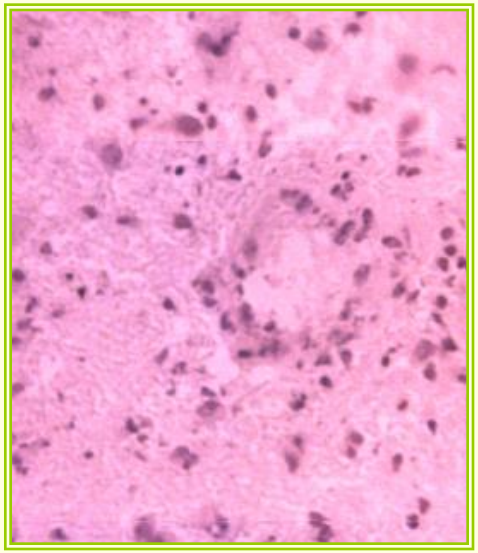
# Blood vessel ↔ neoplastic tissue

***Parameters describing the significance of the primary variables for the model.***

| Element | Partial Wilk's Λ | F – Fisher's statistics | p-value |
|---|---|---|---|
| Fe | 0,613 | 40,8 | 0,000 |
| K | 0,695 | 28,4 | 0,000 |
| Br | 0,913 | 6,11 | 0,015 |
| S | 0,931 | 4,76 | 0,032 |
| Zn | 0,956 | 2,94 | 0,090 |
| Cu | 0,961 | 2,56 | 0,113 |

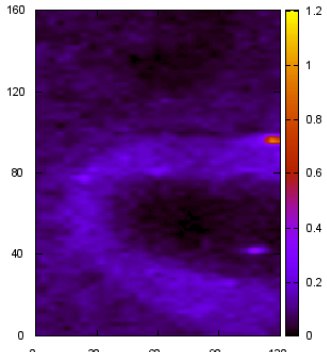
***Fe, K, Br, S***

***the elements of the highest importance for the discrimination between cancerous tissue and blood vessel***

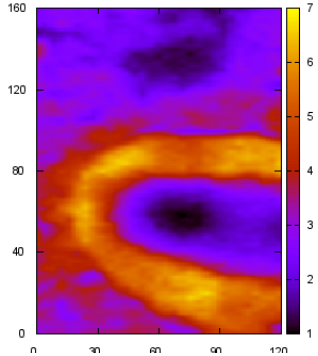


# Blood vessel in brain cancer SRXRF

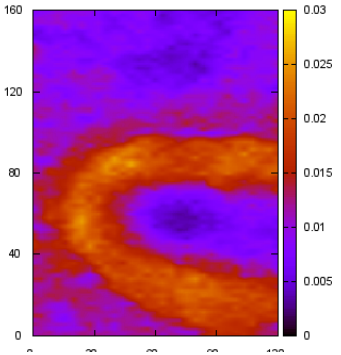
Ca



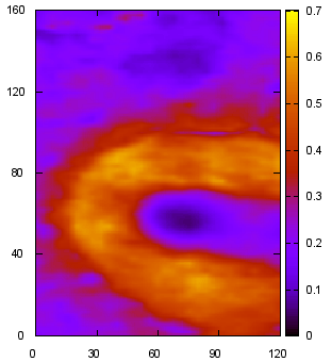
Cl



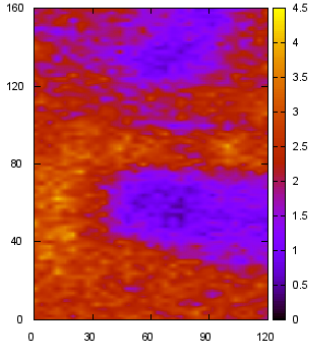
Cu



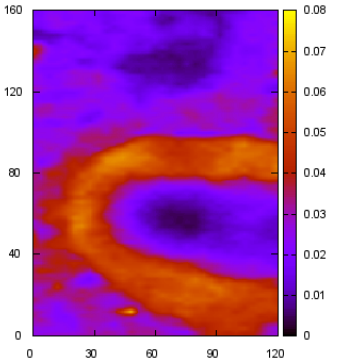
Fe



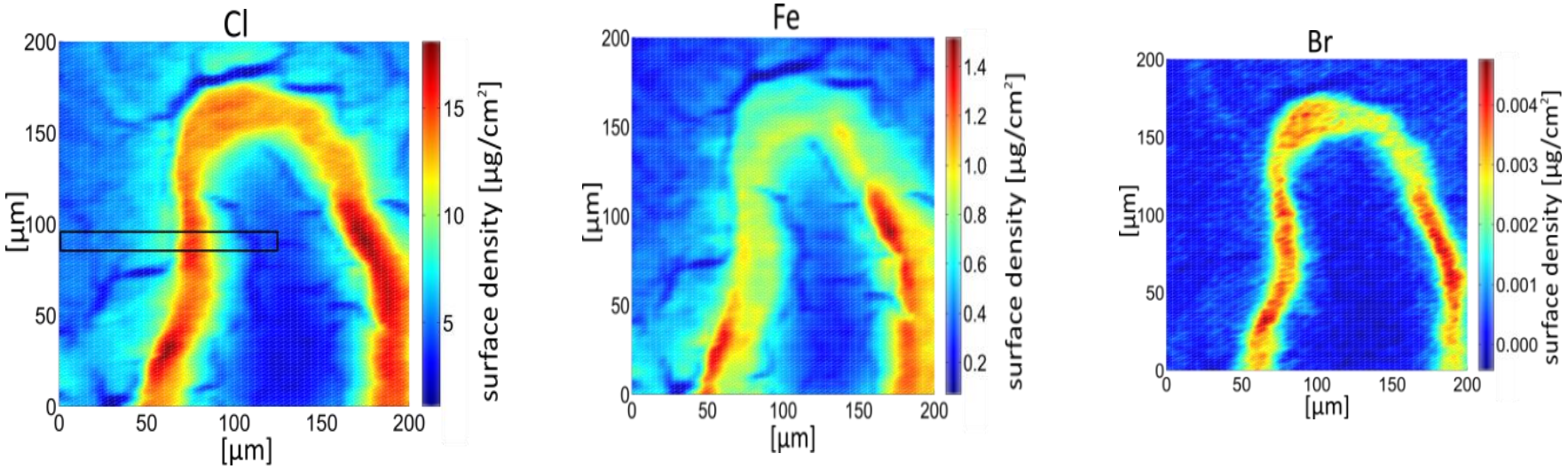
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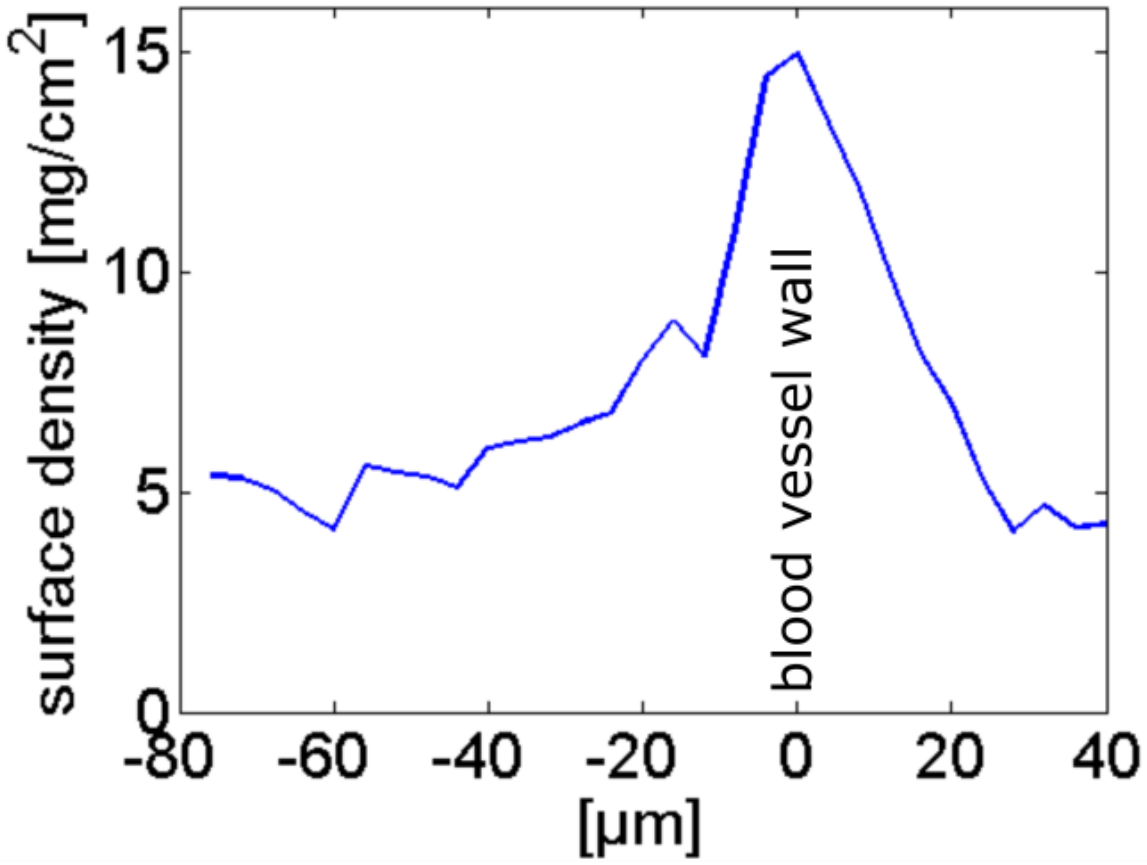
Zn



Scanned area: 180 μm / 120 μm.




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



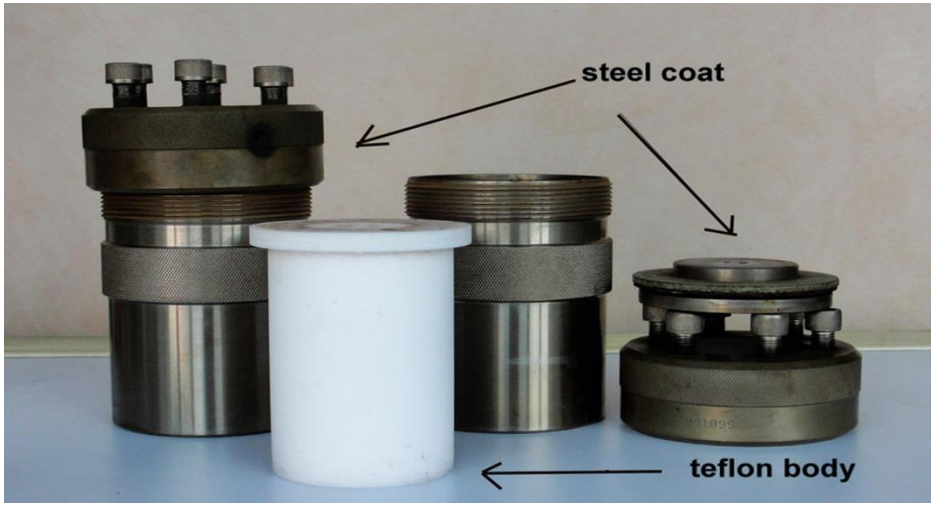

**Changes in the surface density of Cl as a function of distance from a blood vessel.**

# Classification vs. diagnosis

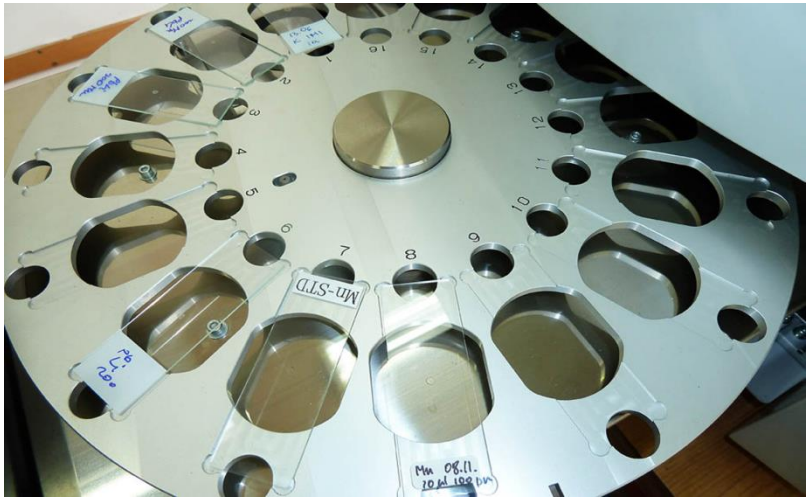
| Diagnosis by neuropathologist (type and grade) | Correctly classified (%) | |
|---|---|---|
| | Cases (12) used to the model construction | New cases (6) |
| Control case | 92.5 | 78.5 |
| BN | 100.0 | 100.0 |
| O, 2nd | 98.0 | 98.0 |
| AD, 2nd – 3rd | 99.0 | 99.0 |
| OA, 3rd | 100.0 | 80.0 |
| GM, 4th | 100.0 | 100.0 |
| **Average** | **97.3** | **91.9** |

Chemical elemental analysis of mean concentrations of elements in brain cancers
X-ray fluorescence analysis

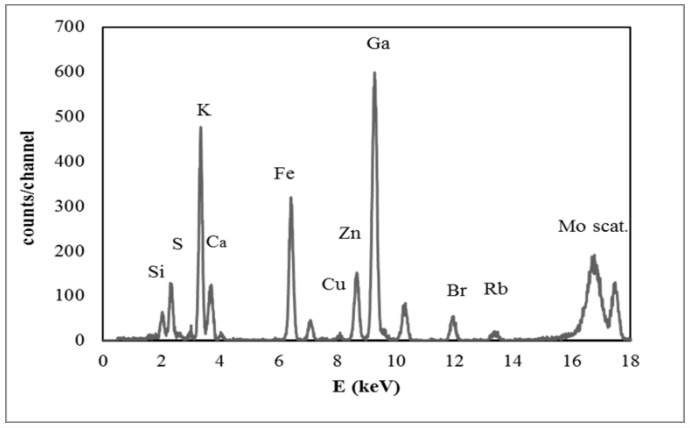
## Equipment for tissue digestion




## Samples changer with reflectors

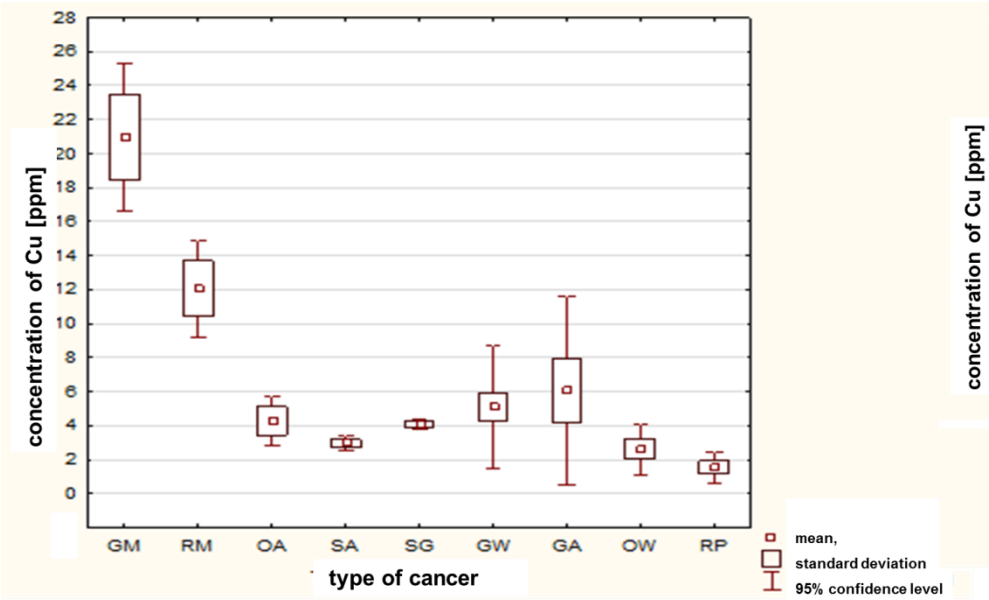


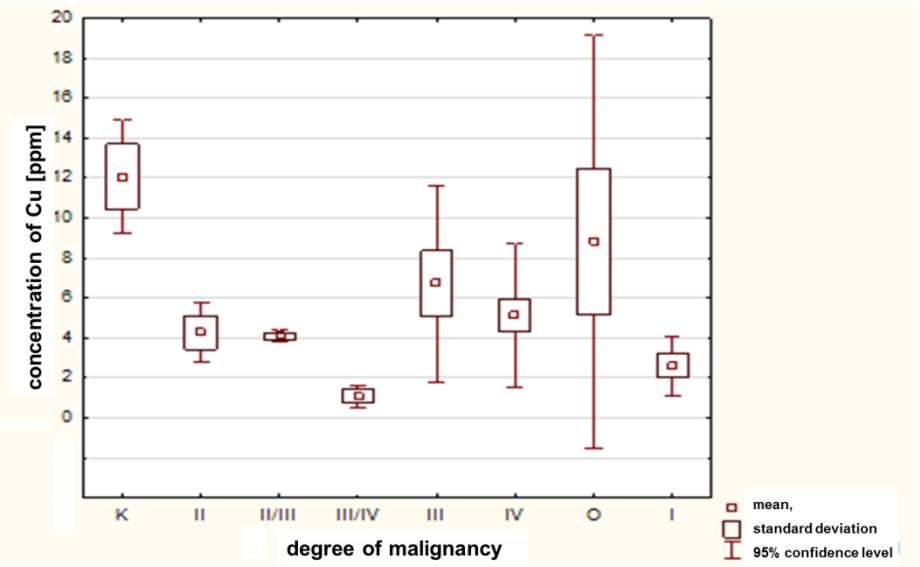
## XRF spectrum excited in digested brain glioma




## TXRF spectrometer






Concentrations of Cu for neoplastic tissues. (GM-brain tumor, RM-cerebral abscess, OA-atypical transitional meningioma, SA-anaplastic oligodendroglioma, SG-oligoastrocytoma, GW-gliobastoma multiforme, GA-anaplastic astrocytoma, OW-fibrous meningioma, RP-metastatic carcinoma

Concentrations of Cu in brain cancers with different WHO grade of malignancy. (0-control sample, I-IV degrees)

# Conclusions

✓*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 brain tissues including doubtful cases*

✓*SR XRF spectroscopy allowed to determine the distribution and amounts of trace elements in brain glioma tissues with a good detectability and a spatial resolution at sub-micron level.*

✓ *The results obtained showed that the elemental composition of a relatively small fragment of homogeneous tissue represents satisfactorily the biochemical ‘‘signature’’ of cancer. On the basis of the element levels determined in such a small sample by means of the TXRF technique it was possible to differentiate some types of brain tumors*

# Cooperation

1. P06 at Petra III:
   G. Falkenberg, M. Alfeld and U. Bösenberg
2. CEMO at DORIS III
   E. Welter, K. Apple
3. I18 at Diamond
   T. Geraki, F. Mosselmann
4. BM23 at ESRF
   Olivier Mathon, Sacura Pacarelli

## Acknowledgements:

The research leading to these results has received funding from:
The European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 226716,
Diamond Light Source Ltd,, Didcot Oxfordshire,
European Synchrotron Radiation Facility, Grenoble, France,
HASYLAB, Hamburg, Germany and the
Ministry of Science and Higher Education (Warsaw, Poland)
grant no N N518 377 537”

This presentation was supported by „Euro Health Care and Fitness Summit”

**Thank you for your attention!**