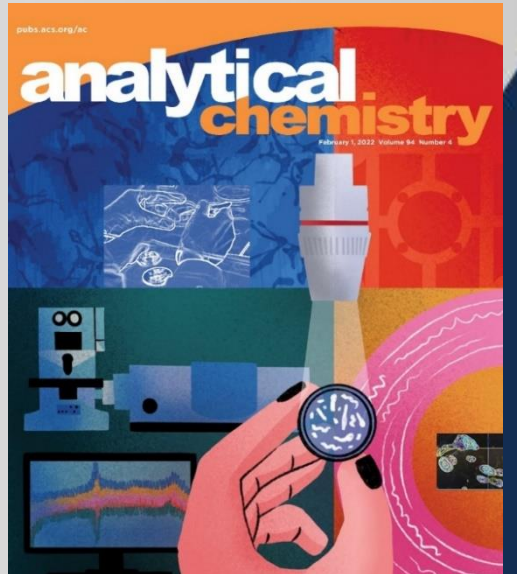
Live-cell synchrotron-based FTIR round out the high-resolution X-ray, electron, and visible light imaging of glioblastoma primary cells

Tanja Ducic

Beamline scientist @ MIRAS



Oviedo 4th/September/2024

ALBA SYNCHROTRON

I) Characterization of glioblastoma cells

Imaging of different primary cells of glioblastoma brain cancer

II) Evaluation of riluzole drug:

Live-Cell Synchrotron-Based FTIR Evaluation of Metabolic Compounds after Riluzole Treatment

III) Improving the treatment:

Carbon dots as a nanocarrier for riluzole

UICC Fellowship no. ICR/2014/339966

I) Characterization of glioblastoma cells

Imaging of different primary cells of glioblastoma brain cancer

University Medical Center Göttingen, Göttingen, Germany
APS, Argonne National Laboratory, Argonne, IL 60439, USA
Dept. Radiation Oncology, Northwestern University, Chicago, IL USA


G. Woloschak


T. Paunesku


S. Chen


M. Ninkovic

II) Evaluation of riluzole drug:

Live-Cell Synchrotron-Based FTIR Evaluation of Metabolic Compounds after Riluzole Treatment

Alba Cells and Elettra - Sincrotrone Trieste Trieste, Italia

ALBA In-house grant: “Synergetic multimodal FTIR and X-ray spectro-microscopical approach for 3D cell culture evaluation”.

III) Improving the treatment:

Carbon dots as a nanocarrier for riluzole

INAMAT² - Institute for Advanced Materials and Mathematics. Public University of Navarra. Pamplona, Spain
Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina-IBIMA Plataforma BIONAND, Málaga;
Dept. Cell Biology, Genetics and Physiology, Universidad de Málaga, Málaga, Spain


M. Algarra

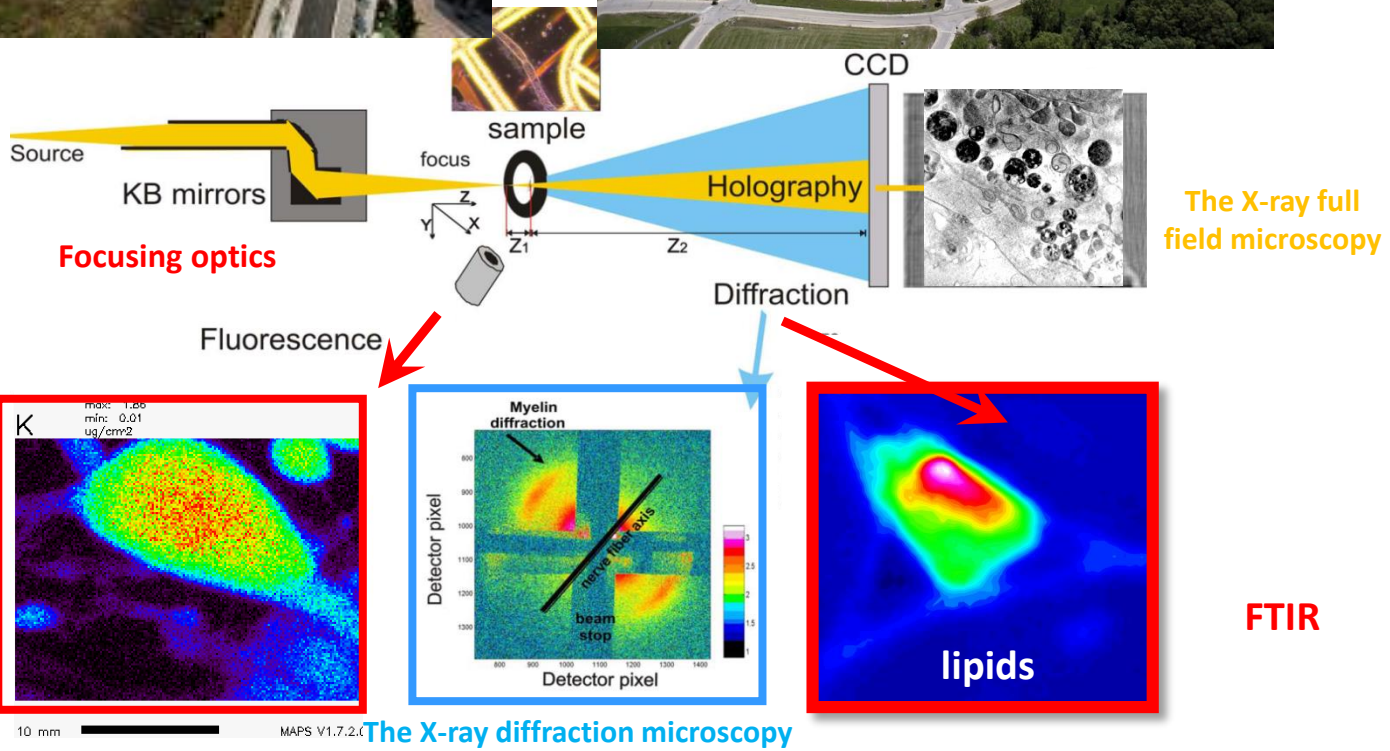

E. González-Muñoz

Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) project PID2021-122613OB-I00

Synchrotron Light Sources

ALBA Barcelona

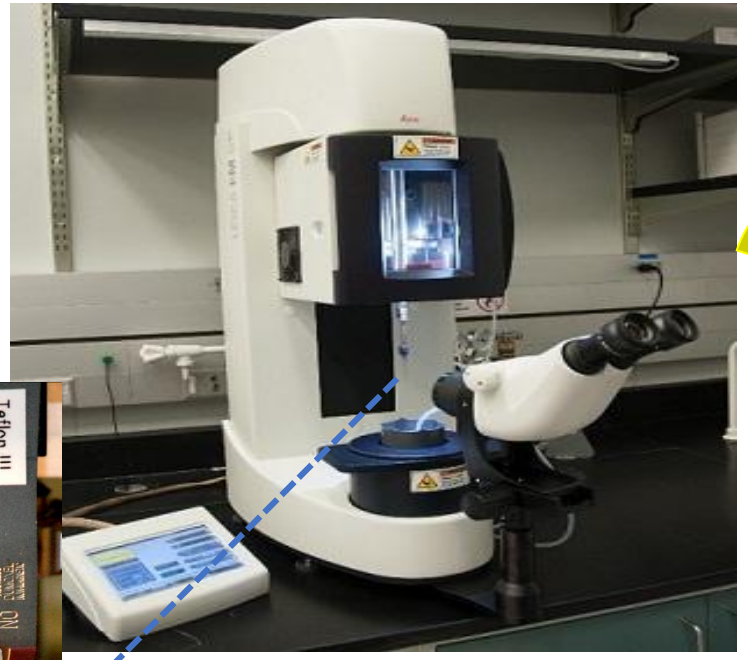
APS Argonne



Adapted from **Saldit & Dučić 2014:** *Super-Resolution Microscopy Techniques in the Neurosciences. Springer*

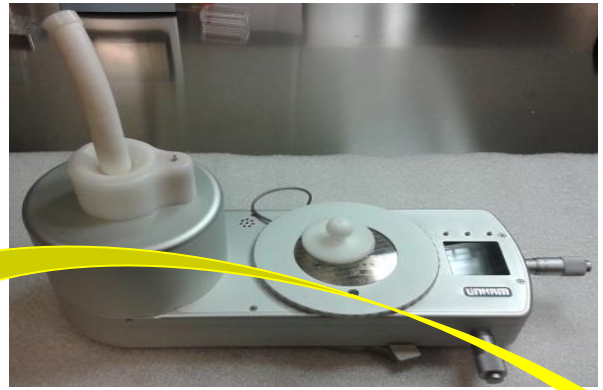
Sample preparation:

Vitrification of cells with a cryo plunger



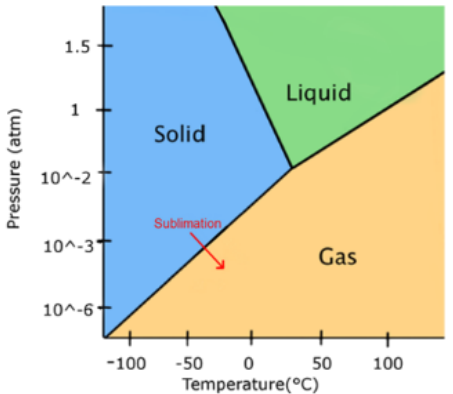
Sample holders:
CaF_2 or Si_3N_4

cryo VIS microcopy



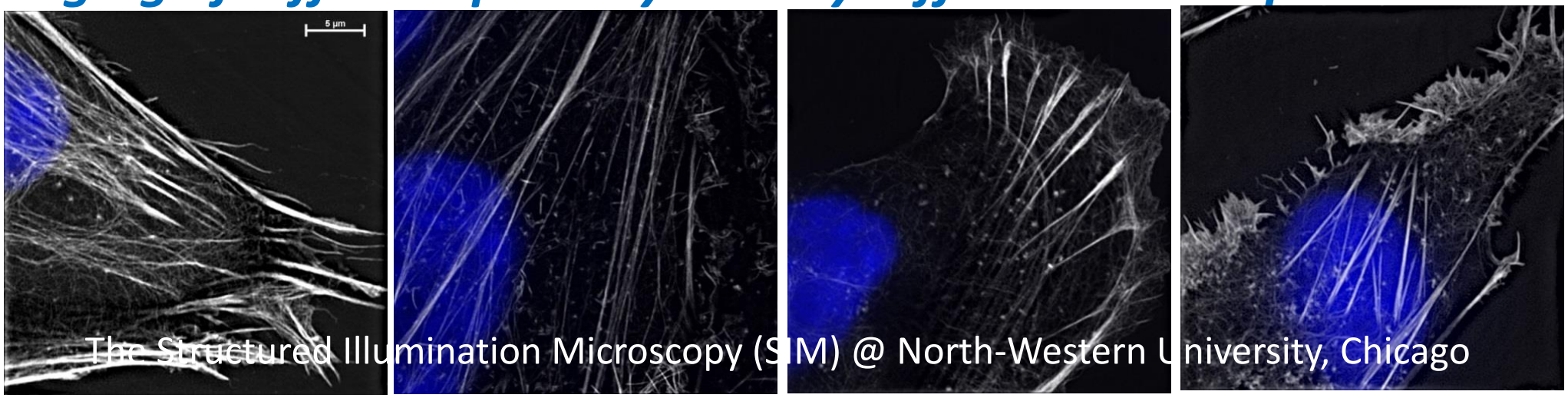
Freeze drying

(in-house developed @ -120°C)

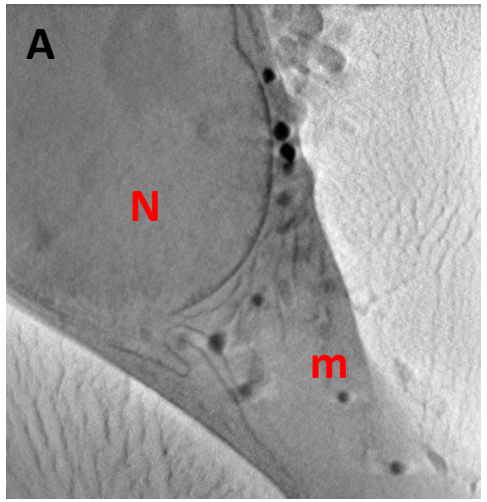


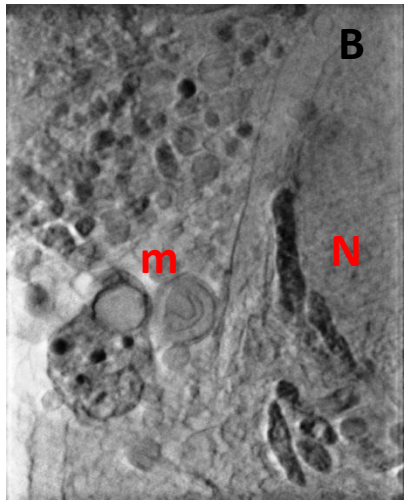
I) Characterization of glioblastoma cells

Imaging of different primary cells by different techniques

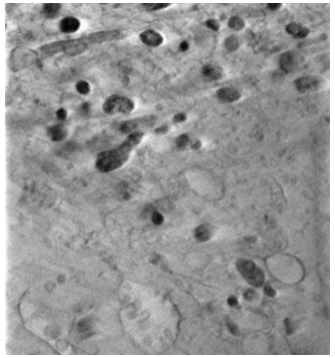


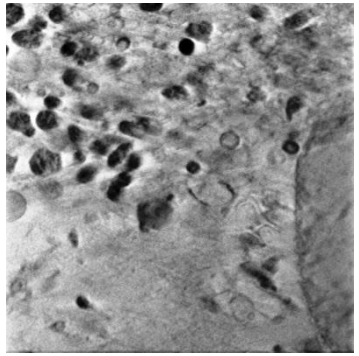
The structured illumination microscopy of glioblastoma cells isolated from 3 different patients (A, B, C) and glioma cell line U87 (D). White structure refers to cytoskeleton and blue to nucleus of cells.

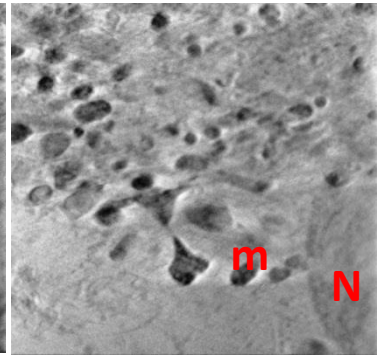


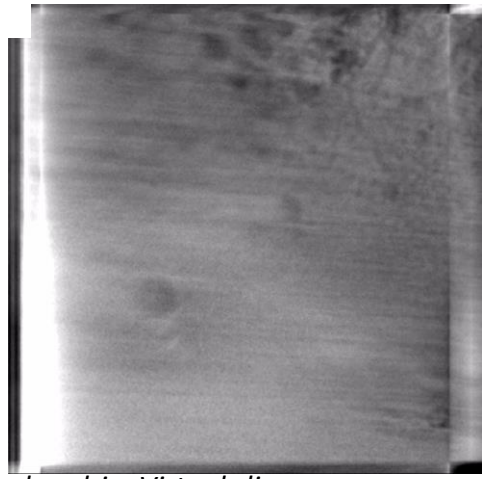


Soft X-ray tomography @ Mistral





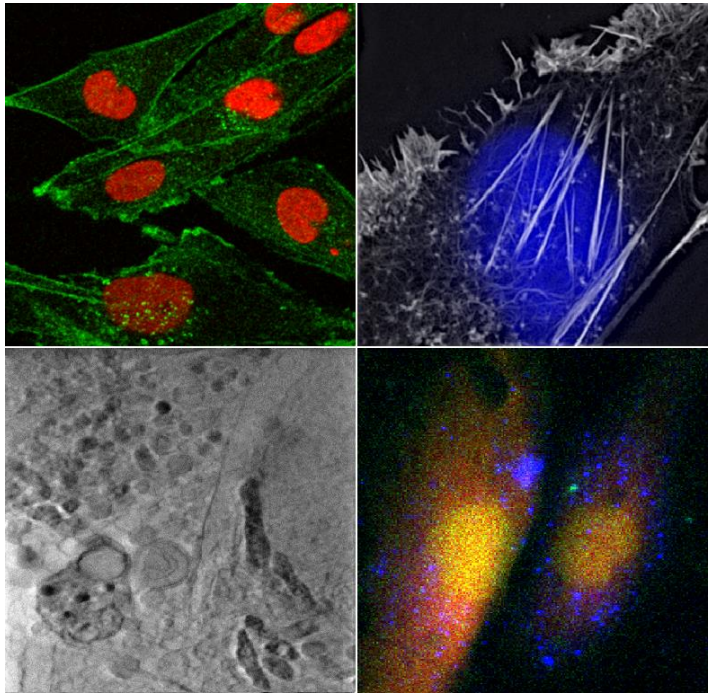




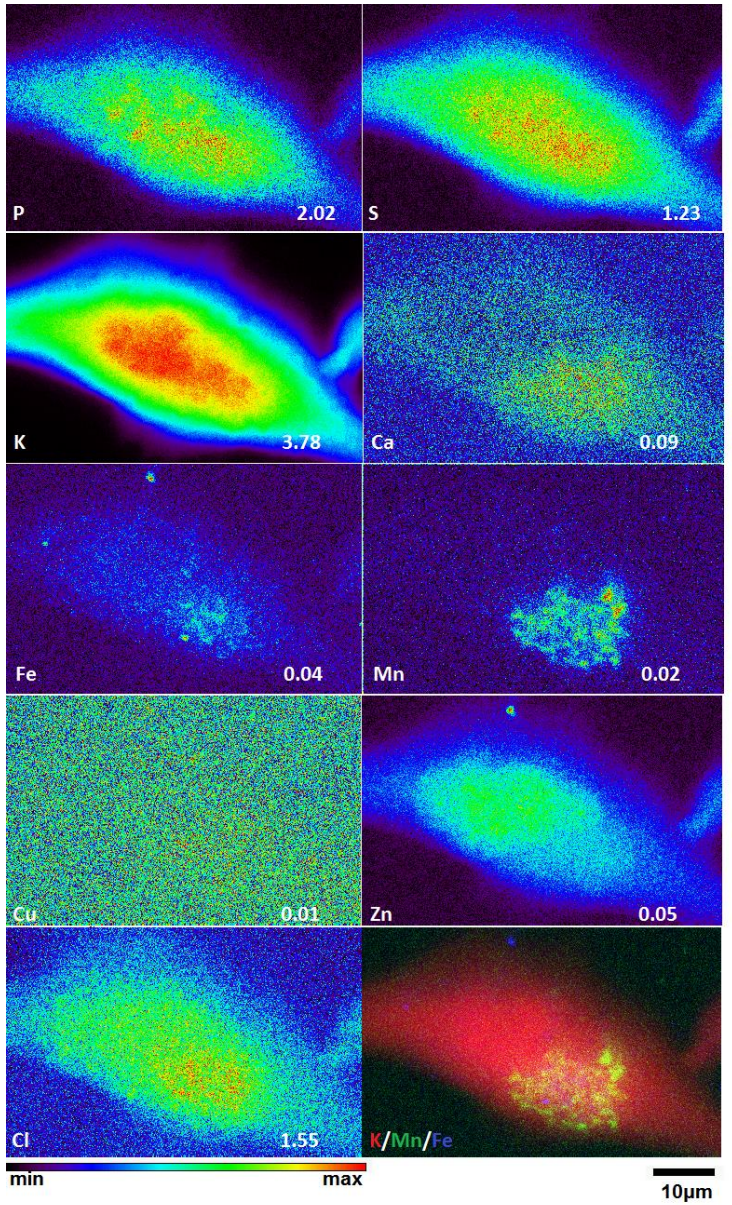
Soft X-ray microscopy: comparison of two different gliomas cells: (A) glioma cell line U87 and cell isolated from the patient #11 (B). N-nucleus of cell, m- mitochondria. Virtual slices.

Hard X-ray fluorescence microscopy:

- The bio-imaging station at the APS provides the cryo sample environment and handling capabilities for hard X-ray imaging in biomedical applications.
- The spatial resolution >40 nm allow us to perform X-ray fluorescence imaging on high energy combined with high spatial resolution.
- Using this instrument we were able to examine *in situ* microelements like Zn, Cu, Fe, Mn, and Ca in the intact cells with high spatial and spectral resolution.



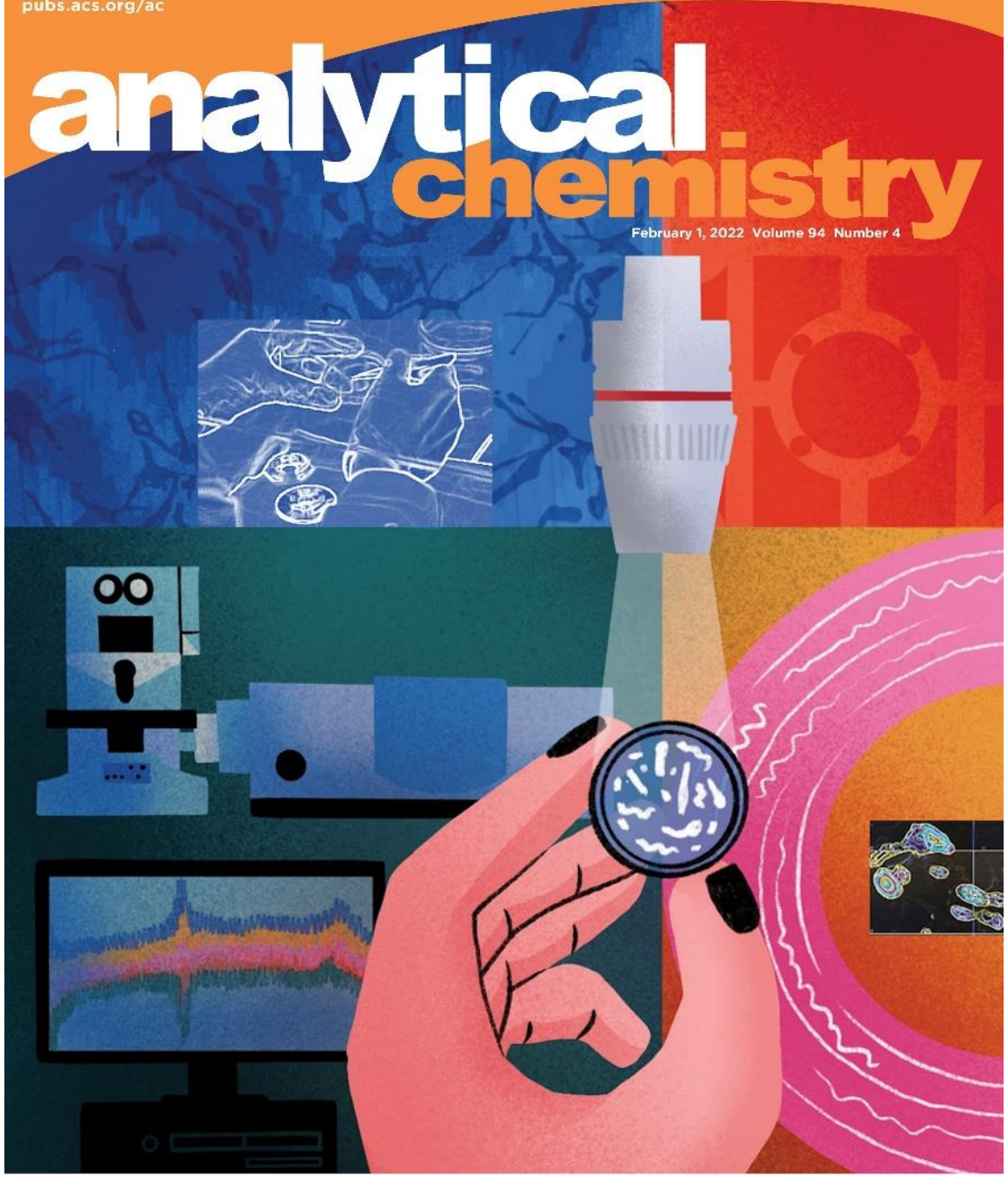
Visible, TXM and X-ray fluorescence maps of P and Fe of cryo-preserved glioma cell line U87. Image 105x50µm, pixel size 250nm and dwell time 300ms. The numbers above the images show concentration in µg/cm^2.



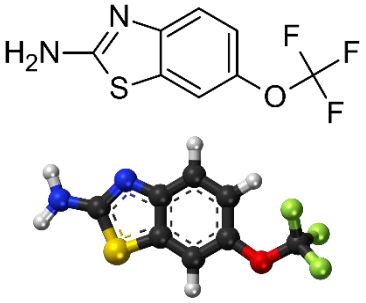
Dučić T., et al., Analyst, 2017

II) Evaluation of riluzole drug:

Live-Cell Synchrotron-Based FTIR Evaluation of Metabolic Compounds after Riluzole Treatment In Glioblastoma- brain cancer cells

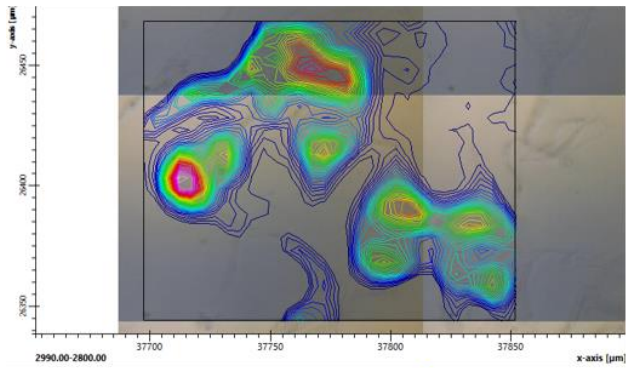


Live cells- synchrotron- based FTIR evaluation of metabolic compounds in brain glioblastoma cells after riluzole treatment

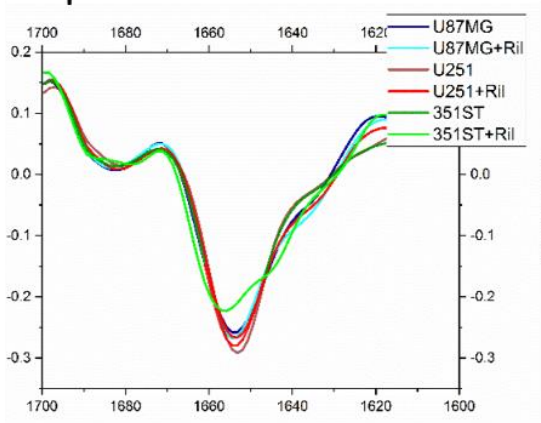


riluzole

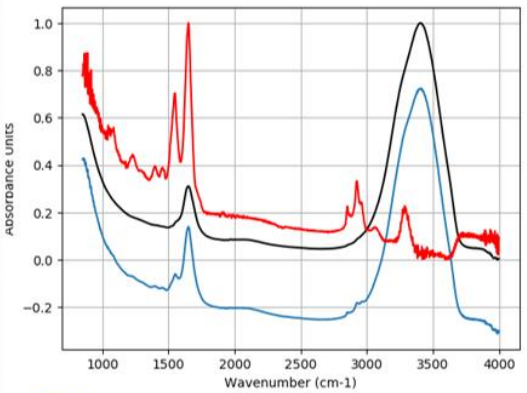
lipids image



protein conformation

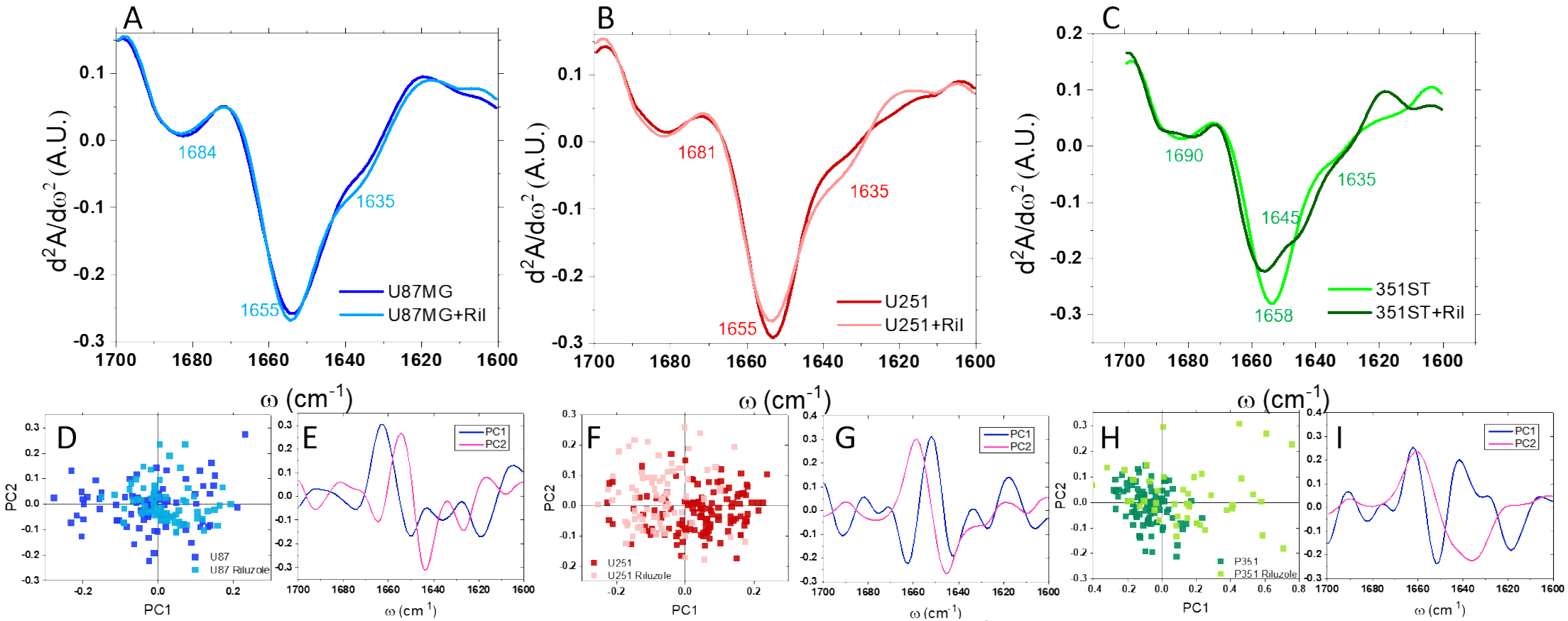


water correction



Dučić T., et al., Analytical Chemistry, 2022

Live cells synchrotron-based FTIR evaluation of metabolic compounds in brain glioblastoma cells after riluzole treatment



Synchrotron –based FTIR spectra of Amide I

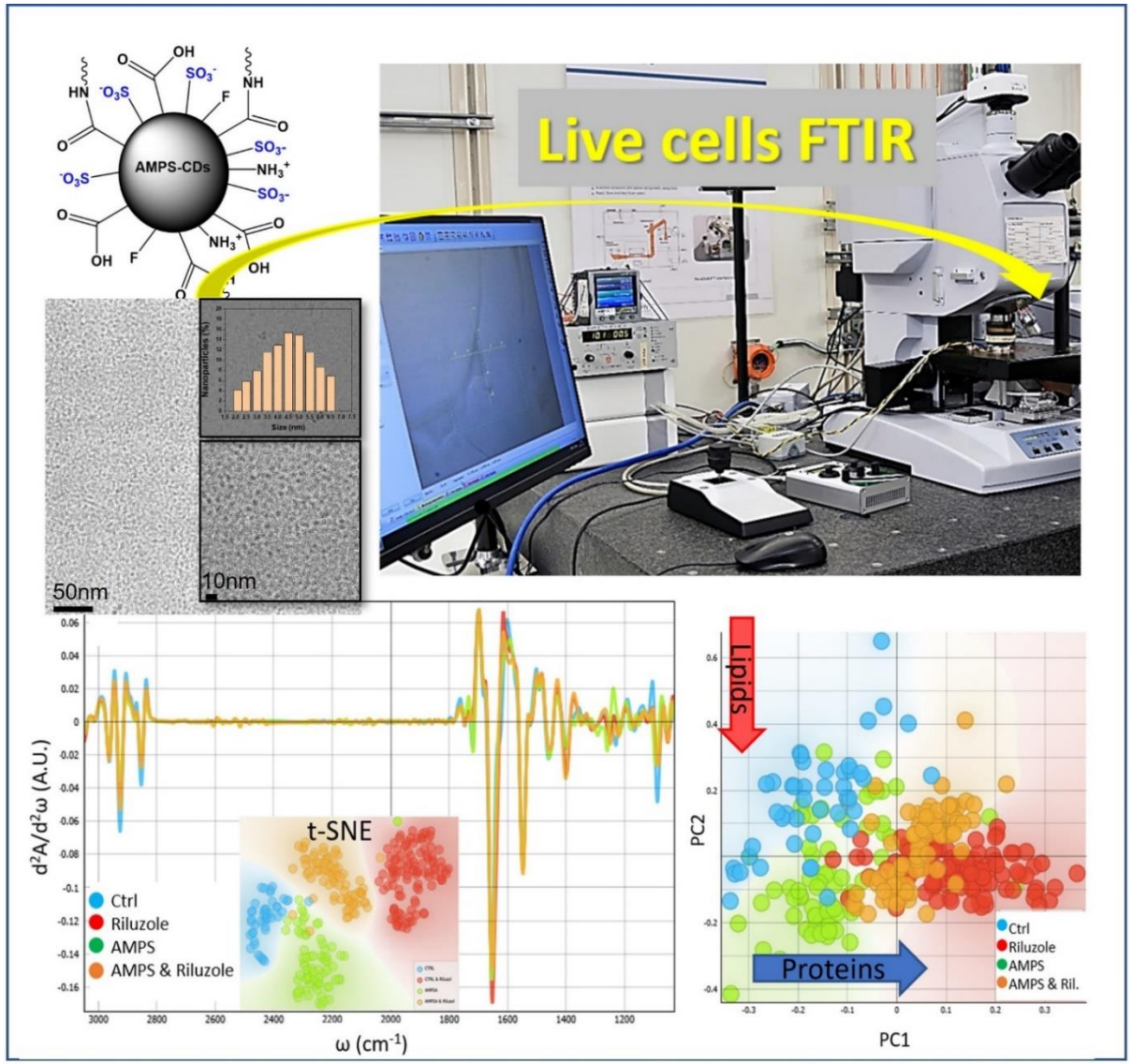
The second derivate and vector-normalized spectra of live glioblastoma cells grown in cell medium and after treatment of riluzole. PCA analysis of each region assigned above and loading values of the first two principal components PC1 (blue) and PC2 (red) showing the contribution of individual absorbance of the corresponding areas in the graphs above. N = 45–120 cells.

Dučić T., *et al., Analytical Chemistry 2022*

Synergistic effect of carbon dots as a nanocarrier for riluzole treatment on glioblastoma cells

III) Improving the treatment:

Carbon-based nanoparticles: 2-acrylamido-2-methylpropanesulfonic acid (AMPS-CD)



An overview of the experimental setup from the carbon dots synthesis, characterisation by cryo-TEM and liver cells FTIR test. The *t*-SNE and the PCA analysis show the contribution of individual absorbance of corresponding areas of lipids and proteins.

Algarra et al., ACS Omega 2024

Conclusions

- **The Spectro-microscopy imaging approach** allows for elucidation of subcellular structures in bio-medical samples and elemental specification at the nanometer scale which could be used for the investigation of different samples *in situ*.

- **The multimodal combination** of all techniques enables to get a deeper understanding of molecular changes after different treatments.

- **Live-cell FTIR** showed that riluzole treatment with the nanocarrier system extends beyond DNA to protein conformation in GBM: the nanoparticles induced fine changes in the protein secondary structure in tumor cells altering the presence of specific structures (glutamate and α-helix) suggest that *AMPS-CDs* nanoparticles could serve as an effective carrier for riluzole in glioblastoma cancer cells.

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Si Chen
Barry Lai

ALBA Synchrotron, Spain
MIRAS Beamline

Cover page of ASC Chemical Neuroscience 2015, Volume6, Issue 10