

Application of synchrotron radiation in otolaryngology: from atomic morphology to translational studies of laryngeal cancer

Małgorzata Toroń^{1,2}, Antoni Wołoszyn¹, Monika Szymańska-Czerwińska³, Krzysztof Niemczuk³, Krzysztof P. Jasik⁴, Jerzy Kubacki⁵, Marcin Zając⁶, Jarosław Paluch²

¹Doctoral School of Medical, University of Silesia, Katowice, Poland

²Department of Laryngology, Faculty of Medical Sciences in Katowice, Medical University of Silesia in Katowice, Poland

³Department of Biotechnology and Nutrigenomics, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Jastrzębiec, Poland

⁴Department of Medical Sciences, Władysław Bieganski Collegium Medicum Jan Długosz University, Częstochowa, Poland

⁵Faculty of Science and Technology, A. Chełkowski Institute of Physics in Chorzów, University of Silesia in Katowice, Poland

⁶National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland

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Abstract

Introduction: Synchrotron radiation enables precise imaging of biological structures at the subcellular level, providing insight into both elemental composition and chemical properties. Increasing evidence suggests that its application in otolaryngology – particularly in the diagnosis of laryngeal cancer – may offer valuable support to existing diagnostic methods and deserves further development and implementation in translational research.

Aim of the research: To evaluate the potential of synchrotron X-ray radiation in otolaryngology based on a literature review and to present findings from a pilot study using X-ray absorption spectroscopy (XAS) in laryngeal cancer samples.

Material and methods: A literature review was conducted following PRISMA guidelines using databases such as PubMed, Medline, and Google Scholar. Experimental studies were carried out at the SOLARIS National Synchrotron Radiation Centre on samples of laryngeal squamous cell carcinoma using XAS (TEY and TFY modes) and scanning transmission X-ray microscopy (STXM) for elemental mapping.

Results: The review confirmed the effectiveness of synchrotron-based techniques (XAS, STXM, μ XRF) in subcellular imaging. In the examined cancer samples, a predominance of Fe³⁺ was observed, indicating oxidative stress. STXM imaging revealed heterogeneous distribution of elements (C, N, O, Fe), reflecting the biochemical complexity of the tumour. Synchrotron techniques enable the assessment of iron redox alterations as potential cancer biomarkers. They support early diagnosis and expand our understanding of tumour pathophysiology.

Conclusions: The presented findings highlight the need for further research to facilitate the clinical translation of these methods.

Introduction

Advances in medical imaging techniques play a crucial role in deepening the understanding of the structure and function of tissues and cells in disease development mechanisms. Since the time of Hippocrates, observation has been a fundamental tool in medicine, and Aristotle's philosophical approach, based on empiricism and deduction, laid the foundations for the development of analytical methods [1, 2].

A breakthrough occurred in the 16th and 17th centuries with the invention of the microscope. The work of Jan and Zacharias Janssen, as well as Robert Hooke – who introduced the concept of the “cell” – enabled the visualisation of microstructures previously invisible to the human eye [3]. The following centuries brought advancements in optics and light theory,

which significantly increased the resolution of microscopy, although the first limitations also emerged, such as Abbe's diffraction limit [4].

The 20th century marked the beginning of high-resolution techniques: electron microscopy, X-ray spectroscopy, and particle physics. The electron microscope, constructed by Ernst Ruska in 1931, along with the development of X-ray-based research, allowed for the investigation of biological structures at the molecular and atomic levels [5]. Figure 1 schematically presents the evolution of imaging methods from macroscopic studies to atomic techniques such as synchrotron radiation, showing a systematic increase in resolution and diagnostic precision, culminating in modern experimental physics methods.

Currently, one of the most advanced research platforms is synchrotron radiation sources. This radi-

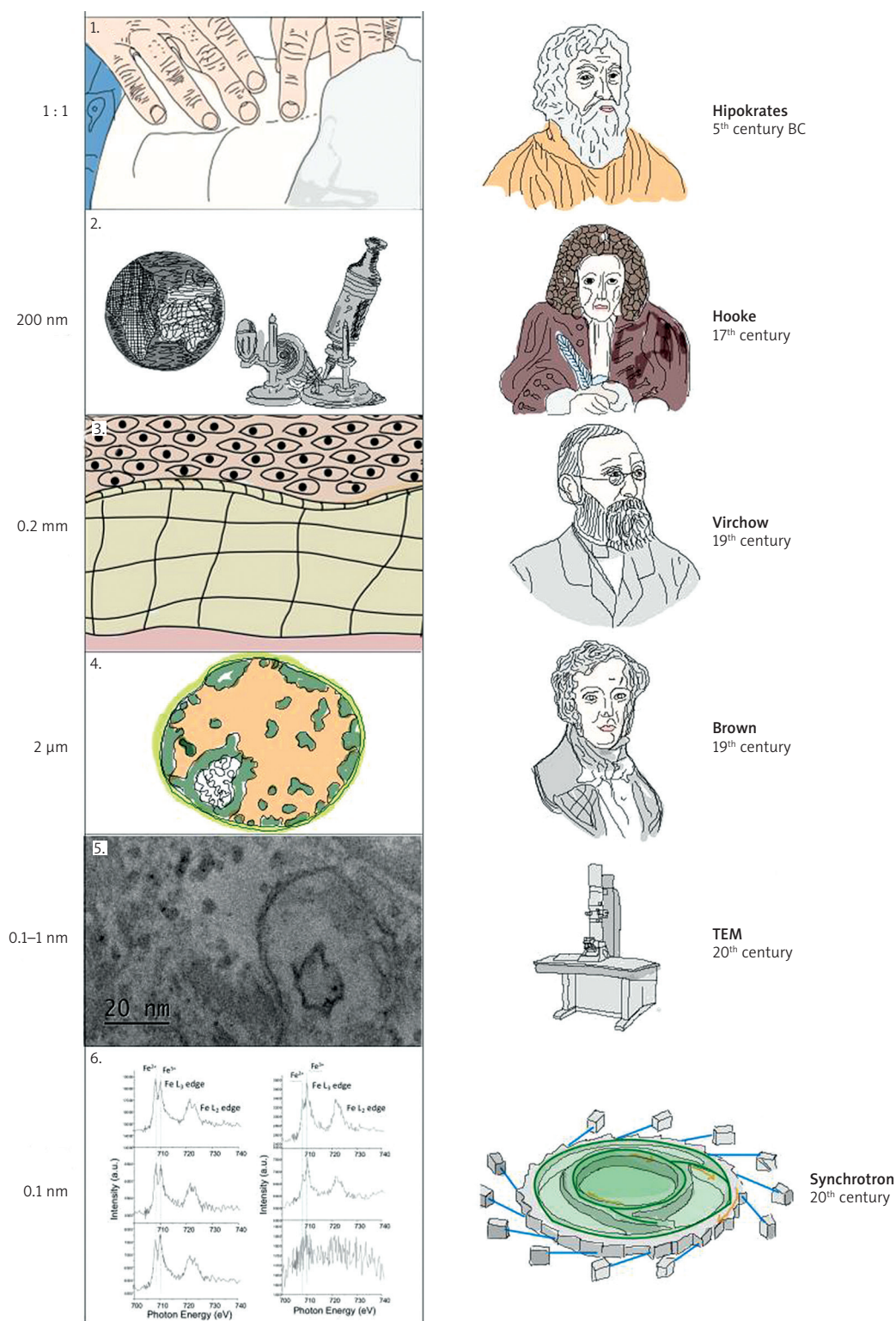


Figure 1. The development of morphology in medicine – from manual examination and macroscopic observation to light and electron microscopy, and finally to imaging using synchrotron radiation. The included images comprise, among others, transmission electron microscopy (TEM) of squamous cell carcinoma (own data) and XAS spectra obtained from synchrotron studies (also own data), enabling analysis at the sub-nanometre level

tion is characterised by exceptional brightness, collimation, and a broad spectral range – from infrared to X-rays. It enables precise structural, chemical, and spectroscopic analyses, including studies of biological tissues without significant interference with their structure.

In otolaryngology, where precise evaluation of the anatomical structures of the head and neck is of critical importance, there is an increasing demand for techniques capable of analysing tissues at the subcellular level. Laryngeal cancer – one of the most common malignancies in this region – is still primarily diagnosed using histopathology and immunohistochemistry [6, 7]. However, these methods have limited ability to detect early molecular and biochemical changes.

The application of synchrotron-based techniques, such as X-ray absorption spectroscopy (XAS), may significantly enhance early diagnostic capabilities and allow for more precise metabolic and structural characterisation of cancerous lesions [8].

Aim of the research

The aim of this article is to review the current potential applications of synchrotron radiation in otolaryngology, with particular emphasis on its use in research on laryngeal cancer. Pilot results from our own studies conducted at the National Synchrotron Radiation Centre SOLARIS, using XAS spectroscopy to analyse cancerous tissue samples, are also presented.

Material and methods and Results

Imaging techniques and morphological analysis

Traditional imaging methods such as light and electron microscopy allow for the analysis of cellular structures and tissues within the physical resolution limits of light (~200 nm) or electrons (as low as 0.1 nm) [9]. However, limitations in 3D imaging, the complexity of sample preparation, and the inability to perform *in situ* chemical analysis present significant research barriers. Synchrotron-based techniques, including XAS, scanning transmission X-ray microscopy (STXM), X-ray fluorescence (XRF), and X-ray diffraction (XRD), enable simultaneous investigation of structure, chemical composition, and oxidation states of elements in biological samples without the need for staining or chemical modification of tissues [10].

Synchrotron radiation in biological and medical research

In molecular biology, synchrotron-based techniques have played a fundamental role in understanding life's structure at the atomic level. They have enabled, among others, the determination of the three-

dimensional structure of DNA [11], the study of ribosome architecture [12], and structural analysis of proteins, contributing to the development of modern targeted therapies [13, 14]. Synchrotron crystallography has been key in antiviral drug design (e.g. against HIV and SARS-CoV-2) [15] and in developing kinase inhibitors used in cancer therapy [16].

In clinical practice, synchrotrons are used for imaging bone microarchitecture (e.g. in osteoporosis and fracture healing) [17], diagnosing neurodegenerative changes (e.g. Parkinson's and Alzheimer's diseases) [18], and analysing the structure of soft tissues such as muscle or brain [19, 20]. Especially valuable is the capability for non-invasive biochemical analyses and spatial mapping of elemental distribution within the cellular environment.

In oncology, synchrotron techniques are applied to the analysis of protein mutations (e.g. p53), studies on redox process dynamics, and mapping of reactive oxygen species (ROS) in cancer cells [21]. These capabilities support the advancement of personalised medicine by enabling therapies tailored to the tumour's molecular profile.

Although most available data concern brain, breast, and lung cancers, an increasing number of studies focus on head and neck tumours, including laryngeal, pharyngeal, and oral cancers. Synchrotron-based spectroscopic techniques offer new diagnostic and prognostic possibilities in these malignancies. Their advantages – high spatial resolution, chemical selectivity, and the ability to monitor dynamic changes in real time – are aligned with the needs of precision oncologic diagnostics in the head and neck region.

Oncogenesis and tumour metabolism in light of synchrotron imaging

Understanding the mechanisms of oncogenesis, including mutations in genetic material, is a key goal of modern molecular biology. Special attention is paid to DNA stability and its potential disruption due to physicochemical processes. One compelling hypothesis involves proton tunnelling along hydrogen bonds, particularly in guanine–cytosine base pairs [22]. Synchrotron crystallography has shown that mispairing of cytosine with adenine may adopt a Watson–Crick-like configuration through proton transfer, disrupting classical bonding patterns and potentially leading to mutations [23]. While the impact of quantum effects on mutagenesis remains under investigation, synchrotron techniques allow for analysis with a resolution unattainable by traditional methods.

From a diagnostic perspective, the identification of molecular tumour markers is particularly important. Synchrotron spectroscopy enables precise analysis of biomolecules such as mutated p53 protein [24], HER2 receptor [25], or metal-dependent enzymes. Its usefulness has also been demonstrated in studying

the mechanisms of anticancer drugs (e.g. cisplatin), which may aid in therapy optimisation [26].

In otolaryngology, where endoscopy supported by artificial intelligence algorithms remains the primary imaging tool [27], cancer diagnostics still relies largely on histopathological and immunohistochemical studies. However, molecular techniques are gaining importance in tumour classification, grading, and treatment personalisation [28]. Synchrotron-based techniques may provide a valuable complement by enabling highly precise chemical and structural analysis of tissues – particularly relevant for subclinical diagnostics.

Research interest is also growing regarding the role of transition metals such as iron (Fe) and copper (Cu) in cancer metabolism. These metals act as cofactors in enzymatic and redox systems; however, their disrupted homeostasis may promote ROS generation, DNA damage, angiogenesis, and tumour progression. XAS techniques allow for mapping oxidation states of Fe and Cu and determining their local concentrations in tumour tissues.

In the context of tumour bioenergetics, synchrotron radiation can support research into the Warburg effect – preferential glycolysis by cancer cells even in the presence of oxygen – and its reverse variant, in which tumours retain the ability for oxidative phosphorylation [29, 30]. Analysis of local redox parameters and their influence on tumour metabolism may have future prognostic and therapeutic significance.

Synchrotron application in otolaryngology: pilot study on laryngeal cancer tissue

Our study was a pilot project conducted as part of an interdisciplinary initiative by students and clinicians from the Medical University of Silesia, in collaboration with researchers from the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences. The experiment was performed at the National Synchrotron Radiation Centre SOLARIS in Kraków, using X-ray absorption spectroscopy (XAS; Figure 2). This project represents a practical application of advanced experimental physics in the diagnostics of head and neck cancers. The results support the rationale for further use of synchrotron radiation in otolaryngology.

The research material consisted of squamous cell carcinoma (SCC) samples of the larynx, prepared according to XAS requirements. The analysed material included thin sections (15 µm) obtained using a cryostat, tissue homogenates, and monolayers of cells applied to silicon plates. Sample preparation followed biological standards to preserve molecular structure integrity. Measurements were performed at room temperature under adiabatic conditions.

The primary objective was to analyse local redox changes of iron (Fe) and copper (Cu) – elements that

serve as enzymatic cofactors in both physiological and pathological metabolic pathways, including those associated with oncogenesis. Under oxidative stress, ROS such as superoxide anion or hydrogen peroxide can damage DNA, proteins, and cell membranes [31]. Iron ions, particularly, are key participants in the Fenton reaction. Copper, being a component of cytochromes, also plays a role in redox pathways [32].

The experiment involved Fe $L_{3,2}$ -edge absorption analysis (~705 eV), which provides insights into iron oxidation states in tissues. Measurements were conducted on the SOLARIS beamline using two detection modes: total electron yield (TEY) for surface signals, and total fluorescence yield (TFY) for deeper tissue analysis and trace element detection [33]. TFY mode allowed for more accurate assessment of Fe and Cu distribution and chemical speciation in cancerous tissues. Figure 3 shows typical XAS spectra for healthy and cancerous laryngeal tissue. A shift in peak intensity from Fe²⁺ to Fe³⁺ in cancer samples suggests increased oxidative stress and changes in the local redox microenvironment.

Point analyses in various tissue regions allowed for the identification of local chemical differences. XAS spectra revealed the presence of sulphur (S), phosphorus (P), carbon (C), copper (Cu), and iron (Fe). Special attention was given to iron analysis, where a clear shift in redox balance toward Fe³⁺ was observed. This may indicate increased oxidative stress in tumour tissue, associated with disrupted mitochondrial metabolism and molecular changes such as spontaneous mutations or chromatin remodeling.

The findings align with hypotheses regarding iron's role in oncogenesis and with observations in other research, such as in Parkinson's disease, where Fe ions contribute to localised cytotoxicity [18]. Iron is increasingly recognised as a potential prognostic marker and therapeutic target in both oncology and neurodegenerative diseases. Our results support further investigation, including with STXM (scanning transmission X-ray microscopy). Figure 4 presents sample STXM images of laryngeal cancer tissue, showing elemental mapping (C, N, O, Fe) at their characteristic absorption edges. The visible heterogeneity suggests that high-resolution chemical imaging is feasible in cancer research.

The data indicate that synchrotron-based XAS spectroscopy may be an effective tool for assessing metabolic changes in tumour tissues. A predominance of Fe³⁺ may reflect activation of carcinogenic pathways and intensified oxidative stress. Changes in Cu distribution may reflect angiogenesis processes and activity of Cu-dependent enzymes. Although this was a pilot study, the results highlight the potential of this technology in identifying molecular biomarkers in laryngeal cancer.



Figure 2. **A** – Aerial view of the “SOLARIS” Centre. **B** – Samples mounted for scanning transmission microscopy. **C** – Scanning transmission X-ray microscope at a synchrotron beamline. **D** – Research hall at the SOLARIS synchrotron with various beamline stations

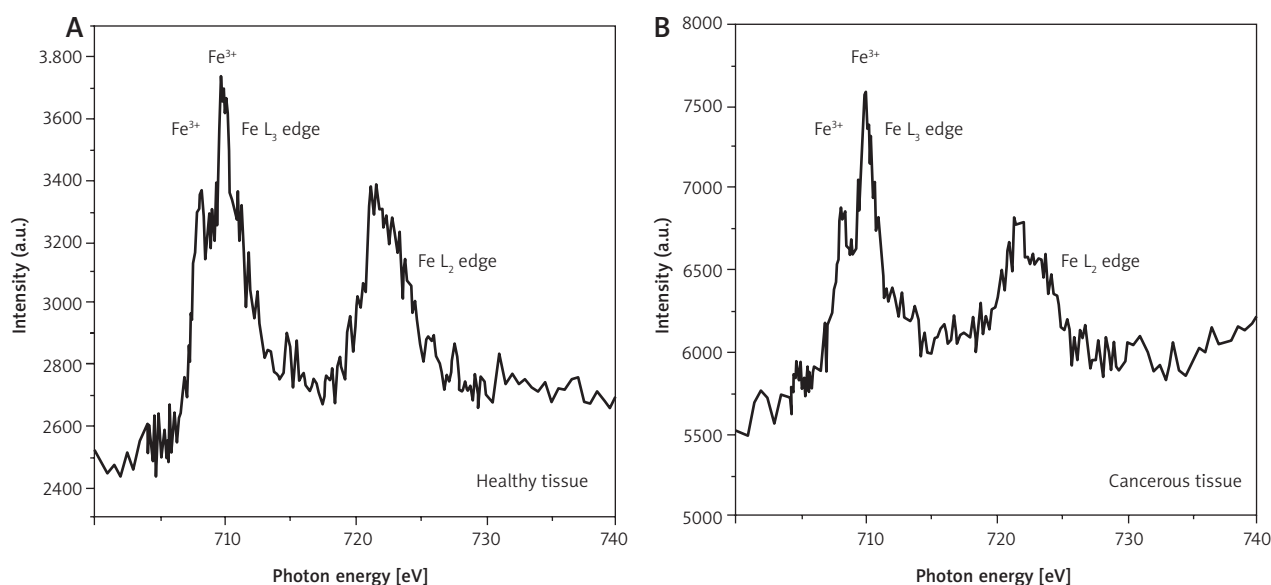


Figure 3. XAS spectra ($\text{Fe L}_{2,3}$ absorption edge) obtained in TFY (total fluorescence yield) mode for healthy (A) and cancerous (B) laryngeal tissue

This study confirms the usefulness of synchrotron XAS in assessing Fe and Cu redox states in laryngeal carcinoma. Differences between healthy and tumour tissue suggest that metal speciation may serve as a potential diagnostic and prognostic biomarker. In the future, this method may be useful in tumour classification, treatment response evaluation, and disease course prediction. Despite limited synchrotron

availability, technological progress may enable clinical translation of this method, particularly within the framework of personalised medicine.

Conclusions

Synchrotron techniques such as XAS, XRF, and STXM represent cutting-edge tools for precise structur-

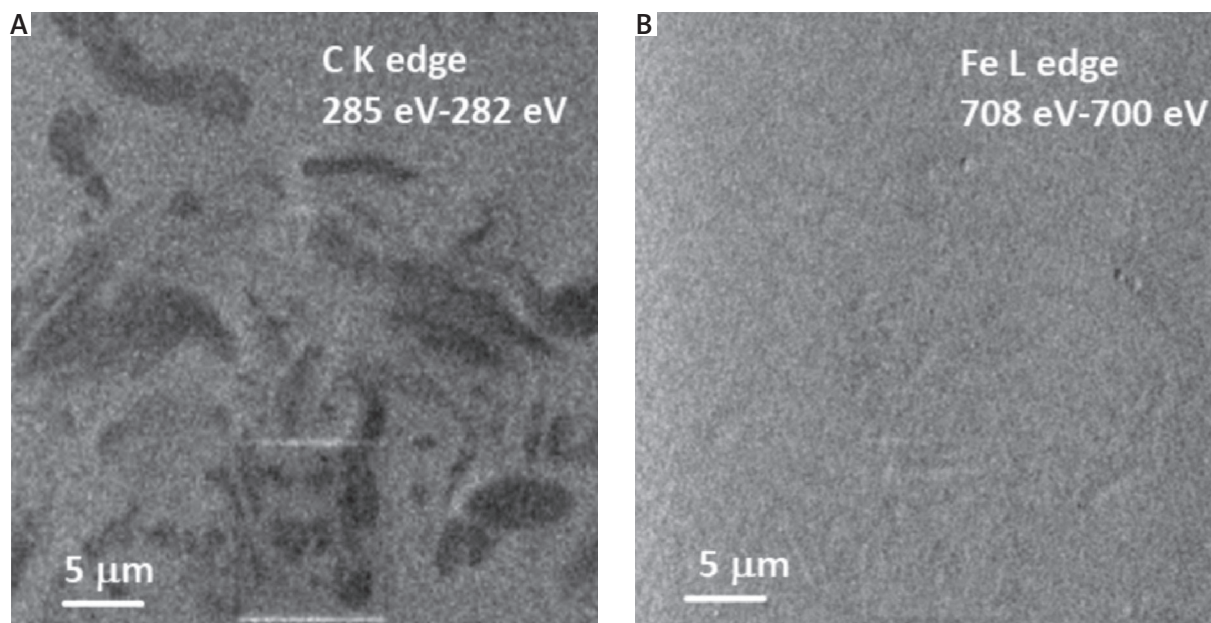


Figure 4. STXM (scanning transmission X-ray microscopy) images of laryngeal cancer tissue at selected absorption edges: A – carbon (C K edge), B – iron (Fe L edge);

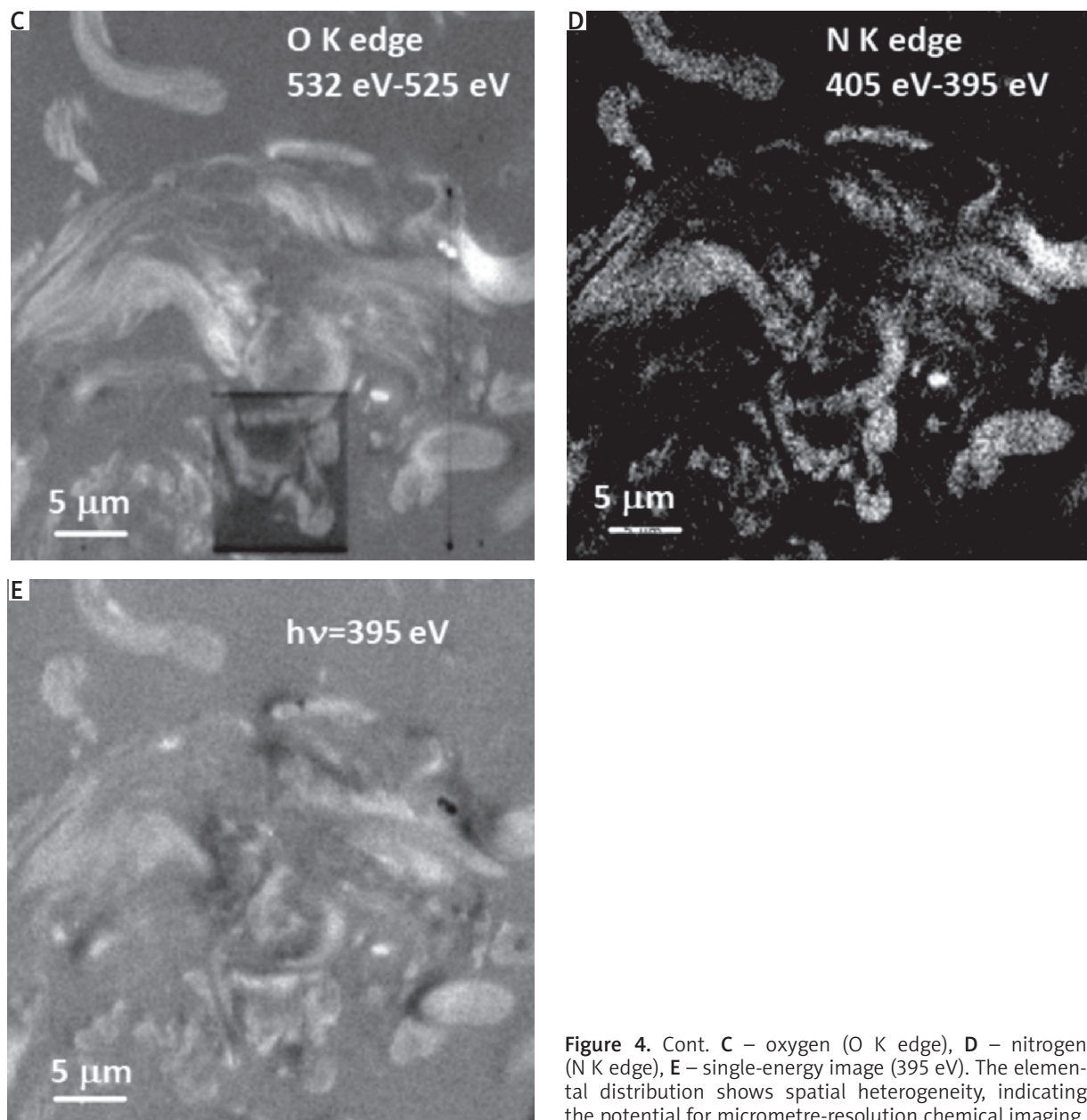


Figure 4. Cont. **C** – oxygen (O K edge), **D** – nitrogen (N K edge), **E** – single-energy image (395 eV). The elemental distribution shows spatial heterogeneity, indicating the potential for micrometre-resolution chemical imaging

al and chemical analysis of biological tissues. In otolaryngology, where early detection of malignant changes in the head and neck region is critical, their application opens new diagnostic and prognostic possibilities.

This article presents the current state of knowledge on the use of synchrotron radiation in biomedical research, with a particular focus on oncology and laryngeal cancer. Pilot study results are also presented, confirming the potential of XAS spectroscopy in analysing redox changes of transition metals in tumour tissues.

Although synchrotron imaging methods are not yet part of routine clinical practice, their development and increasing accessibility indicate a growing role in

translational medicine – including diagnostics of otolaryngological cancers and identification of molecular disease markers.

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Ethical approval

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Conflict of interest

The authors declare no conflict of interest.

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Address for correspondence

Antoni Wołoszyn

Doctoral School of Medical, University of Silesia
Katowice, Poland
E-mail: antoniwołoszyn@outlook.com

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