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Review Article

Applications of X-ray fluorescence analysis (XRF) to dental and medical specimens

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Summary Human tissues contain many kinds of minerals and trace essential elements that act as catalytic or structural components of large biochemical molecules. In addition, various metallic and inorganic materials are used in dental and medical materials and devices. In the dental and medical fields, specimens that are wet and/or have low heat resistance are often requested for elemental analysis. Therefore, a rapid and non-destructive method of elemental analysis is required. X-ray fluorescence analysis (XRF) provides useful elemental information about specimens without causing specimen damage or requiring extra specimen preparations.

In this paper, an outline of the XRF apparatus and applications of XRF to hard and soft dental and medical specimen tissues are presented, and dental materials are reviewed.

Contents

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1. Introduction to the trace element analysis using X-ray

Human tissues contain many kinds of minerals and trace essential elements that as catalytic or structural components of large biochemical molecules. Therefore, analysis of the quantification, distribution, and chemical state of trace essential elements could provide useful information, for example, in metabolism analysis. In addition, skin, respiratory, and digestive mucosa are sometimes exposed to various foreign objects. Especially, the oral mucosa comes into contact and is exposed to dietary and various restorative materials, for example, eroded ions or debris generated from metallic restorations. Additionally, the respiratory mucosa comes into contact with inhaled and entrapped airborne debris. These foreign objects sometimes result in various lesions; therefore, the analysis of foreign objects in tissues is important in determining the diagnosis.

Qualitative and quantitative analyses of the heavy elements in biological, medical, and environmental specimens are performed using various methods, and are tabulated in Table 1. Atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (or mass spectroscopy) (ICP-AES, MS) are the most popular methods for trace element analysis. These methods have high sensitivity (ppm—ppb); however, they require a liquid specimen. Therefore, solid specimens (e.g., biological and medical tissues) should be solubilized, for example, with an acid treatment. The solubilization process decreases the concentrations of the target elements; thus, the detection of trace elements becomes more difficult. In addition, information about the distribution and chemical state of trace elements is lost during the solubilization process. Furthermore, biomedical specimens are rare and restricted in amount; therefore, elemental analysis should be performed in a non-destructive manner.

Microanalysis using an electron probe microanalysis (EPMA) and energy-dispersed spectroscopy (EDS) are also commonly used to analyze elemental information (elemental composition and distribution information). These methods provide both microscopic imaging and elemental information using emitted characteristic X-rays from the observed area. Fig. 1 shows the mechanism of characteristic X-ray generation. The bombardment of high-energy electrons and high-energy X-rays strikes a bound electron in a target atom. After the electron has been ejected, an outer shell electron falls into the vacant inner shell and then emits a characteristic X-ray with energy equal to the energy difference between the outer and inner shell energy levels. Characteristic X-ray generated with high energy X-ray irradiation is called as ''fluorescent X-ray''. Each element has unique energy level sets of electrons; therefore, emitted X-ray energies are characteristic of each element. Table 2 shows examples of characteristic X-rays energies emitted from various elements [1]. Characteristic X-rays can be used to perform an elemental analysis by electron or X-ray irradiation.

EPMA and SEM/EDS are popularly used for micro-elemental analysis because they simultaneously provide electron microscopic images and elemental distribution images. However, there are some requirements for specimens in electron microscopy observation. The specimen should have electroconductivity (or an electroconductive coating) and kept under a high vacuum during observation. Therefore, wet specimens (e.g., cells or wet tissue)

Table 1 Methods for trace element analysis.

	Name of analysis methods
Destructive analysis	AAS (atomic absorption spectroscopy) ICP-AES (inductively coupled plasma-atomic emission spectroscopy) ICP-MS (inductively coupled plasma-mass spectroscopy)
Semi-destructive	LA-ICP-MS (laser abrasion ICP-MS) SIMS (secondary ion mass spectroscopy)
Non-destructive	EDS (energy dispersive X-ray spectroscopy) WDS (wavelength dispersive X-ray spectroscopy) XRF (X-ray fluorescence spectroscopy) NAA (neutron activation analysis) PIXE (particle induced X-ray emission spectroscopy)

Table 2 Example of characteristic X-ray energies of various elements.

Atomic number	Element	Characteristic X-ray lines (keV)		
		$K\alpha_1$	$K\alpha_2$	$K\beta_1$
22	Ti	4.511	4.505	4.932
23	V	4.952	4.944	5.427
24	Cr	5.415	5.405	5.947
25	Mn	5.899	5.888	6.490
26	Fe	6.404	6.391	7.058
27	Co	6.930	6.915	7.649
28	Ni	7.478	7.461	8.265
29	Cu	8.048	8.028	8.905
30	Zn	8.639	8.616	9.572

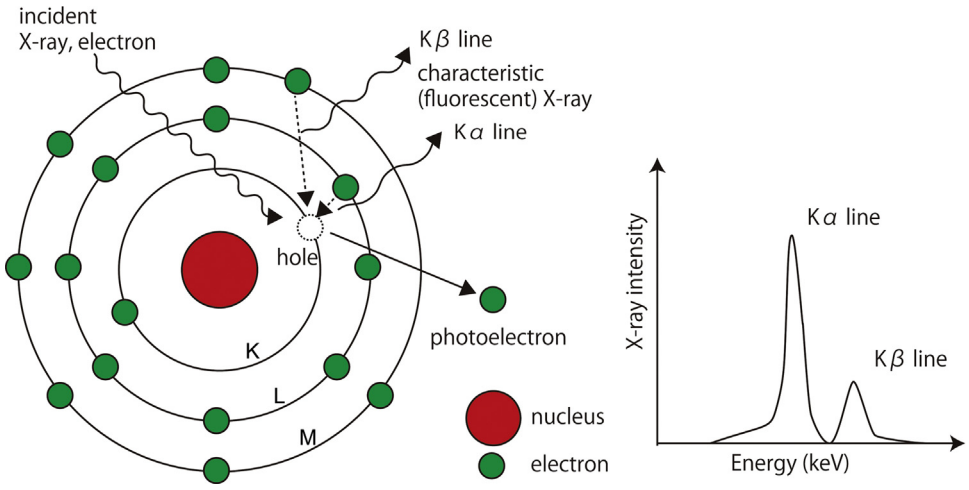

Figure 1 The mechanism of characteristic X-ray generation.

and specimens with a low heat resistance (e.g., paraffin-embedded tissue) are hard to analyze using EPMA or SEM/EDS methods. In addition, there is some possibility that electron irradiation can damage the specimens. Thus, high skills are required for the specimen preparation and observation for the EPMA or SEM/EDS analyses of scarce specimens [2].

2. X-ray fluorescence analysis (XRF): apparatus for elemental analysis and distribution imaging

X-ray fluorescence analysis (XRF) uses characteristic X-rays (called ''fluorescence X-rays'') emitted under high-energy X-ray irradiation. XRF has some advantages over EPMA and EDS as follows.

(1) X-ray irradiation and fluorescence X-ray detection can be carried out in air, because X-rays are easily transmitted in an air layer. Therefore, XRF analysis can be performed in air and evacuation of the specimen chamber is not necessary, as it is in electron microscopy methods.
(2) X-ray irradiation seldom damages specimens.
(3) There are no specimen requirements. Therefore, pretreatment of the specimen is not necessary, e.g., fixation, dehydration, or an electroconductive coating.
(4) A part of the irradiated X-ray transmits the specimen, and an X-ray transmission image can be obtained simultaneously.

In dental and medical analyses, specimens that are wet and/or have low heat resistance are often requested for elemental analysis. Additionally, scarce pathological specimens should be analyzed non-destructively. The features of XRF are quite appropriate for such specimens.

Conventional XRF irradiates an unfocused, wide beam onto the specimen. Therefore, a large specimen surface was required for analysis. Recently, a micro-focused X-ray source has been developed; thus, micro-sample analysis and elemental distribution analysis have become available. The optics for visible light are created by transparent materials with refractive indices greater than 1. However, for X-rays, materials have a refractive index almost equal to 1; therefore, different optics are required for X-ray focusing. Capillary focusing is widely used for XRF focusing optics. The inner surface of the capillary is designed to be the paraboloid of revolution, and the total reflection from the inner surface guides the X-ray to the focus. XRF analysis while scanning the specimen additionally provides elemental distribution images. A schematic diagram of micro-focused XRF equipment is shown in Fig. 2. Spatial resolution, which depends on the focus size, is 10—100 μm. Transmission X-ray intensity can also be monitored by a scintillation detector behind the specimen; then, a high-resolution X-ray transmission image can also be provided while the specimen is scanned.

In the following sections, an outline of the XRF apparatus and examples of various applications of XRF for dental and medical specimens are described.

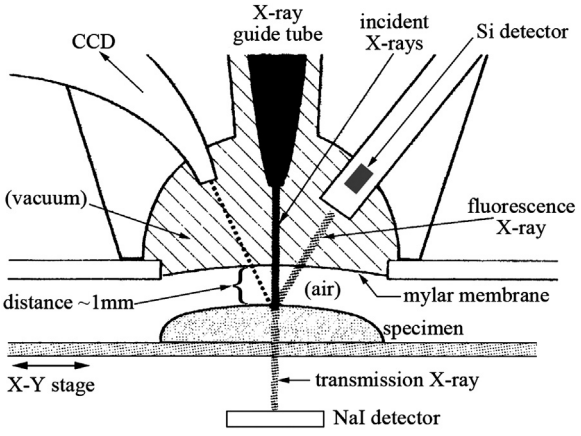

Figure 2 A schematic diagram of micro-focused XRF equipment.

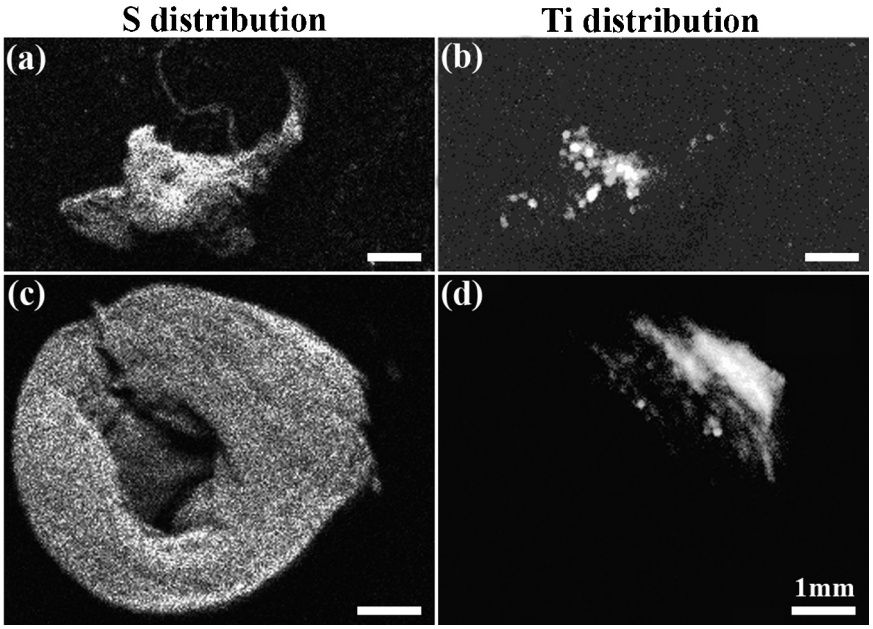

Figure 3 S and Ti distribution images from oral mucosa in contact with a pure titanium cover screw of a dental implant.

3. Applications of XRF

3.1. Tooth analysis with XRF: trace element detection and estimation of caries

''Minimal intervention,'' which is a recent treatment technique for dental caries, requires the minimal removal of tooth volume and retaining as much sound tooth as possible. For this technique, accurate recognition of the carious region is necessary. Conventional caries diagnosis is based mainly on visual and probing inspections and X-ray transparency. Decrease in Ca caused by demineralization is one clear index of caries. Hiraishi et al. applied scanning XRF microscopy to a Ca content evaluation of demineralized tooth surfaces. Contact microradiography is used as the standard in the evaluation of demineralization. However, the transmission of X-rays is strongly affected not only by Ca concentration, but also by other factors, e.g., other mineral content and organic material content. Direct evaluation of Ca content with XRF microscopy is feasible for more accurate estimations of tooth demineralization [3,4].

Teeth and hair may accumulate heavy elements from environmental pollution. In addition, teeth may accumulate elements derived from nutrition, cigarettes, and dental restorations. Therefore, trace element analysis of teeth would be an appropriate index of the influence of various heavy element environmental pollutants [5,6]. Baranowska et al. [5] reported XRF quantification of trace elements in teeth derived from inhabitants of the most polluted and less polluted areas in Poland. In this report, a positive correlation between Zn, S, and Pb concentrations in teeth and the level of pollution in the environment was observed. Additionally, Zn and Pb concentrations in teeth from smokers were significantly higher than those from nonsmokers.

3.2. XRF applications for soft tissue and pathological specimens

Some pathological specimens contain calcified or precipitated solid objects, and rarely contain foreign objects. The identification of these unknown objects is important for diagnosis. Pathological specimens are specific to each case and patient. Therefore, the analysis should be carried out non-destructively. XRF analysis can be performed without damage to or pre-treatment of pathological specimens; therefore, it is suitable for this purpose.

Fig. 3 shows elemental distribution images of oral mucosa in contact with a pure titanium cover screw from a dental implant [7,8]. Sulfur distribution images (Fig. 3(a) and (c)) show the outer shape of the specimens. Ti distribution images (Fig. 3(b) and (d)) show the localization of Ti in these specimens. In Fig. 3(b), Ti was localized in areas, which suggests the existence of particle-like materials consisting of Ti. These Ti particles were determined to be metallic Ti using X-ray absorption fine structure (XAFS) analysis, then suggested to be abrasion-generated debris resulting from implant surgery. In contrast, in Fig. 3(d), Ti was homogeneously distributed in parts of the specimen. The chemical state of the Ti was determined to be TiO_2 (anatase) by the XAFS method. As for the origin of the TiO_2, it is assumed that Ti eroded and dissolved into the surrounding tissue and might have oxidized and localized.

Pathological specimens are commonly in a paraffin-embedded form. Paraffin has a low melting temperature and high volatility; therefore, EPMA or SEM/EDS cannot be applied to paraffin-embedded pathological specimens without a deparaffinization process. XRF analysis can be applied to paraffin-embedded specimens without causing radiation damage. Fig. 4 shows the XRF spectrum of a paraffin-embedded lung biopsy specimen derived from tungsten carbide pneumoconiosis. Fine particle dust from cemented

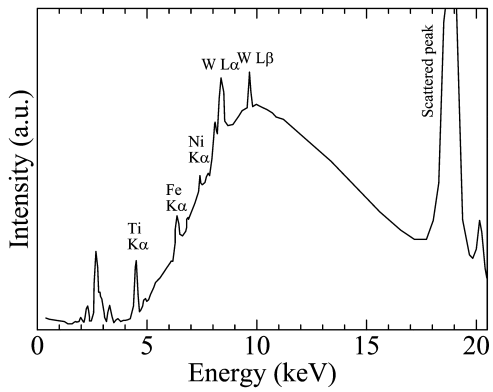

Figure 4 The XRF spectrum of a paraffin-embedded lung biopsy specimen derived from tungsten carbide pneumoconiosis (measurement conditions: 50 kV, 1 mA with Rh target, 600 s/point).

tungsten carbide (WC) cutting tools can cause severe pneumoconiosis, called ''tungsten carbide pneumoconiosis.'' For the diagnosis of this disease, not only a histologic estimation, but also the detection of tungsten in lung tissue is necessary. In Fig. 4, peaks assigned to tungsten L lines are clearly found in the lung biopsy specimen derived from inhaled WC, which suggested the existence of tungsten or a tungsten compound in the lung tissue. Thus, elemental information from XRF was useful in the identification of the source material in pneumoconiosis [9].

The lowest detection limit with XRF analysis was suggested as few ppm for most of the transition metals and more than 10 ppm for light elements (e.g. Na, Mg, Si) and a part of heavy elements [10]. The lowest detection limit strongly depends on equipments and specimen compositions. Then, actual detection limit would be more higher than above concentrations.

For the quantitative analysis, ''fundamental parameter method (FPM)'' is widely used. FPM is estimating the concentrations the theoretical calculation using incident X-ray spectrum, mass absorption coefficient and fluorescent yield of each element. FPM is useful method for the quantitative analysis of metals and inorganic materials which consist of heavy elements. However, in case of the biological specimens, light element (H, C, N and O) is the major component. The detection of X-ray fluorescence from those light elements is impossible or quite difficult. Therefore, the quantification of the target element contained in the biological tissue should be carried using the standard specimens [11].

Thin-sliced pathological specimens, which are ordinarily used in pathological diagnosis, are usually not suitable for XRF analysis because of the very small specimen volumes. However, the synchrotron radiation XRF (SR-XRF) makes possible to analyze sliced pathological specimens, and is described in a later section.

3.3. Rapid analysis of metallic restorations

Metal allergies related to metallic dental restorations have been a cause for concern [12—14]. The fundamental treatment for a metal allergy is the removal of the metallic

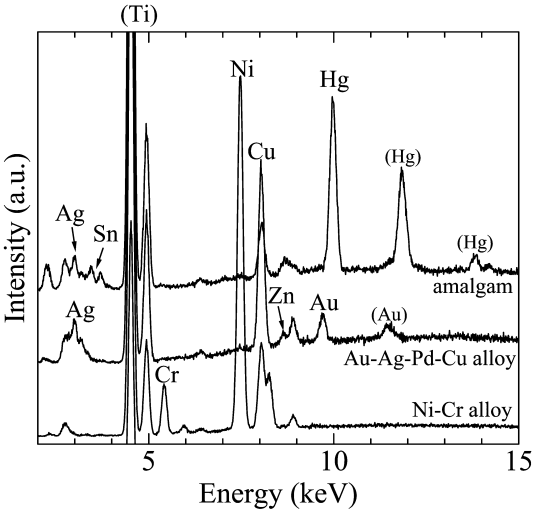

Figure 5 XRF spectra of typical dental alloys (50 kV, 1 mA with Rh target, 100 s/point).

accessories or dental restorations that contain the allergens. However, an elemental analysis of fixed restorations is not as easy as an analysis of removable restorations or accessories. Therefore, an analysis method is required for fixed metal restorations without removing them or causing damage to them. The microsampling method uses a silicone point and disk, and the compositional analysis method uses X-ray photoelectron spectrometry [15—17]. This method also makes it possible to analyze metallic restorations with little damage.

The authors used silicone points as microsampling tools. Polishing the target metallic restorations for a few seconds under ordinary polishing conditions captures a sufficient amount of metal debris [18,19]. A silicone point consists of the oxides and carbides of aluminum, silicone, and titanium, but does not contain the major elements found in dental alloys. Therefore, an elemental analysis of polished metallic restorations can be performed by XRF spectrum analysis. Fig. 5 shows an example of the XRF spectra of typical dental alloys. The strong peaks at 4.5 and 5.0 keV assigned as Ti were derived from the metal sampling tool

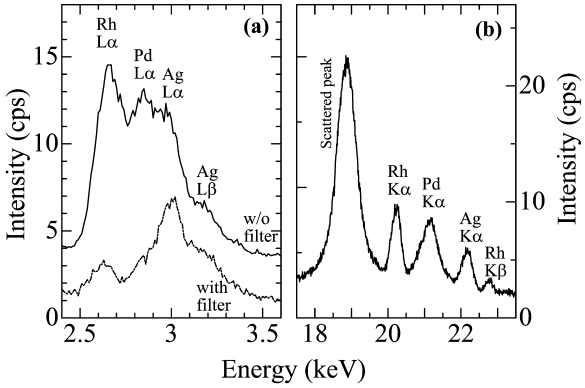

Figure 6 XRF spectra of Au—Ag—Pd—Cu alloy attached silicone point (50 kV, 1 mA with Rh target, 100 s/point). (a) L line region with and without incident X-ray filter, (b) K line region without filter.

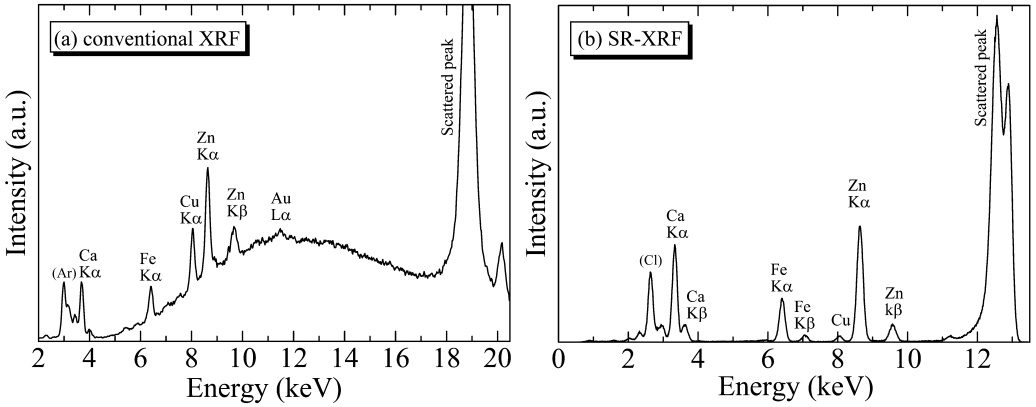

Figure 7 A comparison of XRF spectra obtained by conventional XRF (a) and SR-XRF (b).

(silicone point). All major components of these alloys could be successfully detected. The microsampling technique using a silicone point to dislodge a sufficient amount of specimen debris for XRF analysis could be performed; however, the dislodged and captured amount of debris for analysis were less than 10 μg samples. Placing the sample into the XRF chamber is quite simple because it is not necessary to evacuate the chamber. Therefore, the total required time from sample placement until the conclusion of the analysis is several minutes in duration.

Typical XRF spectrometer uses the X-ray tube with rhodium target. Rhodium is the adjacent element to palladium and silver, which are the major component of dental alloys. Therefore, the scattered characteristic X-rays (L lines) of rhodium, which contained in the incident X-ray from the X-ray source, overlapped on the fluorescent X-ray from palladium and silver (L lines) and interferred their detection. Recent XRF spectrometer provides the various filters to cut off the scattered incident X-rays. As shown in Fig. 6(a), an appropriate filter insertion would increase the signal/background ratio of XRF spectrum and improve the detection especially for palladium and silver. In the K line region, the peaks Rh, Pd and Ag were identified as individual peaks (Fig. 6(b)), then Pd and Ag could be easily detected.

3.4. Application of synchrotron radiation to XRF (SR-XRF)

Synchrotron radiation (SR) generates quite strong X-rays (and other electromagnetic waves, e.g., ultra violet, visible, and infrared light). Its intensity is higher than laboratory

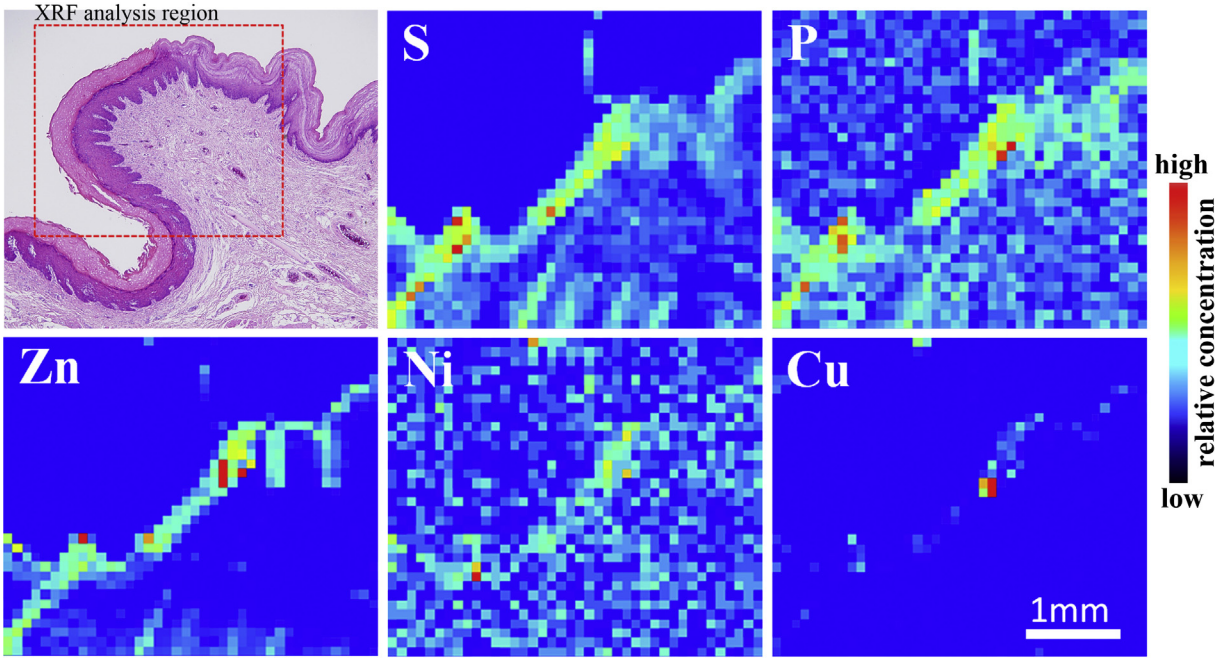

Figure 8 A histological image and elemental distribution images of a SCC specimen using SR-XRF.

X-ray sources by several orders of magnitude. SR X-rays make it possible to apply monochromatized X-rays as incident X-rays in XRF. Fig. 7 shows a comparison of the XRF spectra obtained by continuous X-ray (conventional XRF) and monochromatized X-ray (SR-XRF) irradiation. The XRF spectrum from continuous X-ray irradiation (Fig. 7(a)) shows a high background caused by the characteristic X-rays of the target material and of the X-ray source, and also a broad background caused by ''Bremsstrahlung.'' Therefore, the weak characteristic X-rays from the specimens were hidden by the background, and this creates difficulties in trace element analysis with conventional XRF. In contrast, the XRF spectrum from a monochromatized X-ray (Fig. 7(b)) shows a negligible background, except for the Compton scattering peak; then, trace elements (~ppm) could be easily detected. In recent years, SR X-rays can be focused into diameters of μm to nm; therefore, trace element distributions at the cellular or intracellular levels can be measured.

Wilson disease results in neurological or psychiatric symptoms and liver disease from copper accumulation in tissues. Early detection and treatment determine the following convalescence; therefore, copper concentration estimations in tissue (liver) should be carried out during the early stages. Matsuura et al. applied SR-XRF for the diagnosis of Wilson disease. Copper distribution in liver tissue was visualized, and information regarding copper concentrations in the liver could assist in treatment planning [20].

Moreover, changes in trace element concentrations in various cancer tissues have been reported [21,22]. The authors measured thin-sliced (10 μm in thickness) paraffin-embedded specimens of oral squamous cell carcinoma (SCC). Fig. 8 shows a pathological image of an SCC specimen and elemental distribution images obtained by SR-XRF. SR-XRF measurements were performed at BL-4A of the Photon Factory, High-Energy Accelerator Research Organization. Conventional XRF elemental imaging of such thin-sliced specimens is not applicable, because the generated fluorescence X-ray from thin specimen is quite weak. SR-XRF makes it possible to detect fluorescence X-rays from thin-sliced specimens with low backgrounds. In this method, elemental distribution images could be obtained from consecutive slices of the pathological specimen, then, a comparison of the pathological images and elemental distribution images becomes possible. In Fig. 7, Ni, Co, and Cu were localized in the epidermis layer of a region of SCC. The relationship between the localization of metallic elements and the cause of SCC was unknown; however, the visualization of metallic trace element distributions in such histological regions is expected to provide useful information for diagnosis.

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