# Element distribution imaging in rat kidney using a 2D rapid scan EDXRF device

R.G. Figueroa[a], E. Lozano[b], and G. Bongiovanni[c]
[a]*Deparatmento de Ciencias Físicas, Universidad of La Frontera*
*Av. Francisco Salazar 01145, C.P. 4811230, Temuco, Chile.*
[b]*Instituto Nacional del Cáncer, Unidad de Física Médica,*
*Av. Profesor Zañartu 1010, Santiago, Chile,*
[c]*IDEPA-CONICET, Instituto Multidisciplinario de Investigación y Desarrollo de la Patagonia Norte,*
*Buenos Aires 1400, C.P. 8300, Neuquén, Argentina.*
*Universidad Nacional del Comahue, Neuquén, Argentina,*
*e-mail: figueror@ufro.cl*

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Visualization of elemental distributions of biological tissue is gaining importance in many disciplines of biological, forensic, and medical research. Furthermore, the maps of elements have wide application in archeology for the understanding of the pigments, modes of preservation and environmental context. Since major advances in relation to collimators and detectors have yielded micro scale images, the chemical mapping via synchrotron scanning micro-X-ray fluorescence spectrometry (SR-$\mu$XRF) is widely used as microanalytical techniques. However, the acquisition time is a limitation of current SR-$\mu$XRF imaging protocols, doing tedious micro analysis of samples of more than 1 cm and very difficult to study of larger samples such as animal organ, whole organisms, work of art, etc.
Recently we have developed a robotic system to image the chemistry of large specimens rapidly at concentration levels of parts per million. Multiple images of distribution of elements can be obtained on surfaces of 100x100 mm and a spatial resolution of up to 0.2 mm$^2$ per pixel, with a spectral capture time up to 1 ms per point. This system has proven to be highly efficient for the XRF mapping of elements in large biological samples, achieving comparables results to those obtained by SR-$\mu$XRF. Thus, images of As and Cu accumulation in renal cortex of arsenic-exposed rats were obtained by both methodologies. However, the new imaging system enables the XRF scanning in few minutes, whereas SR-$\mu$XRF required several hours. These and other advantages as well as the potential applications of this system, will be discussed.

*Keywords:* XRF-Imaging; biological samples; multi-elemental mapping

La visualización de distribuciones elementales espaciales de tejido biológico está adquiriendo importancia en muchas disciplinas de la investigación biológica, forense y médica. Por otro lado, los mapas de elementos tienen una aplicación amplia en la arqueológica para el entendimiento de los pigmentos, modos de conservación y el contexto del medio ambiente. Dado que los principales avances con relación a colimadores y detectores han dado imágenes de micro escala, la cartografía química a través de análisis de espectrometría de microfluorescencia de rayos X (SR-$\mu$XRF) mediante radiación sincrotrón es ampliamente utilizada como técnica de microanálisis. Sin embargo, el tiempo de adquisición es una limitación común en el protocolo de imagen SR-$\mu$XRF, haciendo tedioso el análisis micro de las muestras de más de 1 cm y es muy difícil el estudio de muestras más grandes, como órganos de un animal, organismos completos, obras de arte, etc. Recientemente hemos desarrollado un dispositivo robótico de bajo costo para una obtención rápida de una imagen química en muestras de gran tamaño con niveles de concentración de partes por millón. Las imágenes múltiples de distribución de los elementos pueden obtenerse en superficies de hasta 100 mm por 100 mm y con una resolución espacial de hasta 0,2 mm, con un tiempo de captura espectral de hasta 1 ms por punto. Este sistema ha demostrado ser altamente eficaz para el mapeo FRX de elementos en muestras biológicas de gran tamaño, los resultados son comparables a los obtenidos por SR-$\mu$XRF. De este modo, fueron obtenidas imágenes de acumulación de As y Cu en la corteza renal de ratas expuestas a arsénico por ambas metodologías. El nuevo sistema de imágenes XRF permite el escaneo en pocos minutos, mientras que SR-$\mu$XRF requiere más de una hora. Se discuten estas y otras ventajas, así como las aplicaciones potenciales de este sistema.

*Descriptores:* Imágenes EDXRF; muestras biológicas; mapeo multi elemental.

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## 1. Introduction

Prevention and early diagnostic of diseases, including cancer, neurodegenerative and heavy metal poisoning, are major issue in medicine. Since disorders in Mg, Fe, Cu, As, Sr, I, Pb (among others) composition and/or distribution has been related to initiation and progression of various pathologies, visualization of elements in tissues becomes a necessary study target for a better health risk assessment, diagnostic and trail therapy. X-ray fluorescence (XRF) is one of the most widely used spectroscopic techniques in elemental identification and quantification [1]. From the non-invasive in vivo XRF measurements of iodine in the thyroid gland by Hoffer *et al*. [2] in 1968, several in vivo studies of cadmium, mercury, gold, platinum, uranium, zinc, arsenic, etc. have been published [3,4]. Synchrotron- based micro X-ray fluorescence (SR-$\mu$XRF) is the only available technique for quantitative elemental imaging of whole cells due to the high spatial resolution to the high sensitivity for most elements of biological interest [5]. In addition, these X-ray imaging/microanalysis techniques follow an evident trend in the development of nanoscience by pushing spatial resolution down towards the nanoscale. However,

the sample size and the sampled volume in a living human being are huge compared to, *e.g.* a laboratory sample studied in vitro. Thus, practical aspects of measurements need to be considered. In this regard, the acquisition time is a limitation of current SR-$\mu$XRF imaging protocols, doing tedious the analysis of samples of more than 1 cm and very difficult to study of larger samples such as animal organ, bone, whole organisms, etc. On the other hand, the building of medical centers close to the synchrotron facility could be required for diagnostic, dosimetric, and therapeutic purposed.

Hence, the main characteristics of a rapid robotic EDXRF imaging device is reported. In order to determine the feasibility of this System to rapid imaging of elements in medical relevant samples, multiple images of distribution of elements in human and animal bones as well rat kidney were obtained.

## 2. Experimental setup

### 2.1. XRF setup

Figure 1 shows our experimental setup for the XRF imaging system. The Spectrometer is composed by the mini X-ray tube (MXRT), the digital pulse processor with MCA and the detector SDD (Silicon Drift Detector). the SDD and the MXRT were positioned at 90° and 45° (respectively) respect to the X-Y sample stage by a robotic arm. The MTRX-sample distance was approximately 1.3 cm, while sample-SDD distance was approx 1.5 cm (Fig. 2).

### 2.2. Measurement and calibration

Each scan is defined as the area of interest, shape and size of the sample. A maximum 100×100 mm (100 cm$^2$) area can be scanned with variable spatial resolution that can reach according to the step and diameter collimation, with a minimum of 0.1 mm$^2$ per pixel. The step ranges from 0.2 mm to 50 mm with a minimum of XRF spectral capture time of 1 ms per point, with 256 energy channels.

### 2.3. Multi-elemental images of biological samples

Using this XRF device, multi-elemental images were obtained of animal and human bones [6] and rat kidneys samples. The Figs. 3 show representative images of rat kidney samples. The total imaging time can range from a few seconds to several minutes depending on concentrations, counting rate, step size and the scanning area. The multi-elemental images of rat kidney cut were acquired in 22 min, using 1.0 mm collimator an 0.6 mm step Significant fluorescence area-intensities were obtained for 14 elements. Major ones were for P, K, Ca, Fe, Cu and Zn in bones, while Mg, Al, Ti, Cr, Ni, Mn, Pb and Zn were in minor proportion (not showed spectrum).

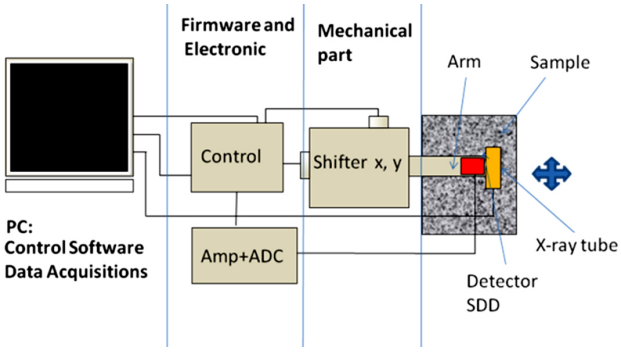


FIGURE 1. Experimental setup for the XRF Imaging System.

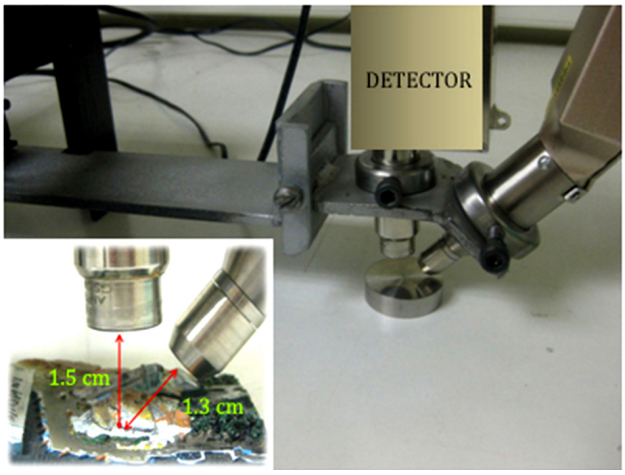


FIGURE 2. Robotic arm. It is shown MTRX-sample and sample-SDD distances.

## 3. Results

As shown in Fig. 3, it was possible to observe the As and Cu accumulation in renal cortex from As-exposed rats by drinking water. Major fluorescence area-intensities in kidney of As-exposed rats were for Cl, K, Fe, Cu and As (not showed spectrograms). Because the kidney slices were fixed in Ti-catalyzed acrylic disc according to Pérez *et al.* [6], this element is seen.

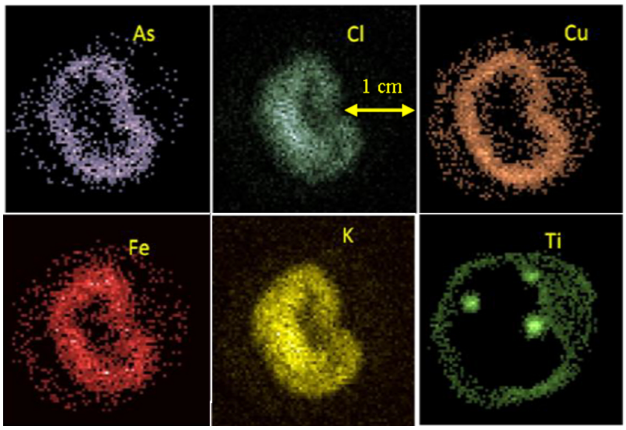


FIGURE 3. Multi-elemental XRF images of As-exposed rat kidney.

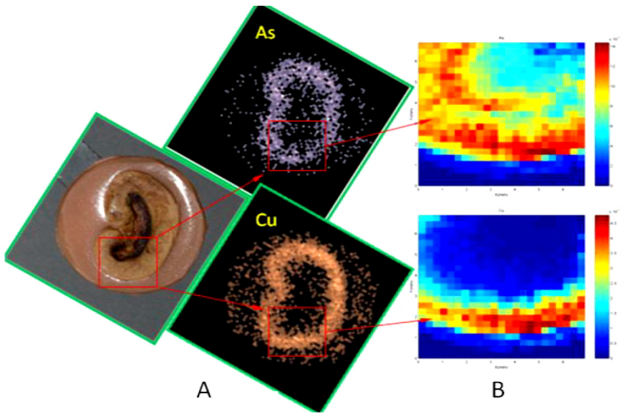


FIGURE 4. Multi-elemental XRF images of As-exposed rat kidney. Images obtained by 2D rapid EDXRF device, counting time 30 ms/pixel (200×200 $\mu$m), total acquisition time 22 min (A). Images obtained by SR-$\mu$XRF, counting time 10 s/pixel (200×200 $\mu$m), total acquisition time 1.47 h (B).

### 3.1. Comparison of muti-elemental imaging methodologies

Images of distribution of As y Cu in kidney of arsenic-exposed rat were compared with those previously obtained by using the method of SR-$\mu$XRF. (experimental and instrumental setting details can be reviewed in Ref. 7). It is clear that As and Cu distribution in kidney (Fig. 4) are positively correlated with those by SR-$\mu$XRF However, 1,47 hours would be required for similar imaging by SR-$\mu$XRF required.

## 4. Conclusions

Noninvasive and fast visualization of multi-elemental distribution in great biological samples is an important issue for physicians, biologists, physical-medics, etc. and this XRF-imaging System can be a powerful tool for them.

### 4.1. Significant benefits to using XRF System

The XRF system here presented is capable to acquire multi-element images by scan with imaging time which can range from a few seconds to several minutes, making them more suitable for in vivo studies. This System provides an alternative method for determining the concentration and distribution of major element directly in superficial tissues (skin, bone, adipose) in a living human. A biopsy presents the element concentration at the site of sampling and is not necessarily a representation of the level in the whole organ. The procedure is invasive and repetition of the sampling may not be possible. Finally, the XRF imaging system is faster and cheaper that SR-$\mu$XRF technology.

### 4.2. Approach in environmental researches

Arsenic is a widely-spread environmental contaminant, and the prolonged exposure to it has been related to several human health disorders, including cancer [8]. In Latin America, 15 millions of people are exposed to As contaminated drinking water [9]. However, only a minor percentage of As-exposed people actually develop arsenic-induced skin lesions that are considered to be the hallmark of arsenicosis [10]. Since symptoms of As toxicity may take 8-14 years to be manifested, an earlier diagnostic could be archived by skin scanning with the XRF-imaging System. On the other hand, images of As and Cu distribution in target organs as kidney [11] could provide complementary data for a better health risk assessment.

Within this perspective, we conclude that the HIGH SPEED XRF IMAGING SYSTEM as that here presented, will play an important role in the study of elemental disturbances-associated disease.

Within this perspective, we conclude that the 2D RAPID XRF IMAGING DEVICE as that here presented, will play an important role in the study of elemental disturbances-associated disease.

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