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# X-ray fluorescence microscopy artefacts in elemental maps of topologically complex samples: Analytical observations, simulation and a map correction method

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ABSTRACT

XRF spectroscopy is among the most widely used non-destructive techniques for elemental analysis. Despite the known angular dependence of X-ray fluorescence (XRF), topological artefacts remain an unresolved issue when using X-ray micro- or nano-probes. In this work we investigate the origin of the artefacts in XRF imaging of topologically complex samples, which are unresolved problems in studies of organic matter due to the limited travel distances of low energy XRF emission from the light elements. In particular we mapped Human Embryonic Kidney (HEK293T) cells. The exemplary results with biological samples, obtained with a soft X-ray scanning microscope installed at a synchrotron facility were used for testing a mathematical model based on detector response simulations, and for proposing an artefact correction method based on directional derivatives. Despite the peculiar and specific application, the methodology can be easily extended to hard X-rays and to set-ups with multi-array detector systems when the dimensions of surface reliefs are in the order of the probing beam size.


## 1. Introduction

X-ray fluorescence (XRF) spectroscopy is among the most requested tools for qualitative and quantitative elemental analyses of all types of materials. In the past decades, laboratory and portable instruments have been developed and applied in various fields, including cultural heritage [1,2] and forensic, material science, geology [3] and biology [4–6]. Compared to traditional XRF systems using conventional X-ray sources, the synchrotron-based ones combine the advantages of high photon flux, X-ray tunability and focusing to submicrometre spot, opening the opportunity for analysis of multicomponent and morphologically complex systems, monitoring the spatial distribution of sample constituents as well [7,8].

Despite the isotropic nature of XRF emission, a distinct feature of the XRF collection is its strong angular dependence [9,10], so for quantitative analysis one has to take into account the significant variations of the X-ray penetration depth with changing the incidence angle of the beam and the dependence of the collected signal on the take-off angle of the detector (defined as the angle between the sample surface and the detector axis) [11]. This strong angular dependence of the XRF signal is naturally related to each element present in the scanned sample. Recent developments of angular dependence X-ray fluorescence, based on the use of portable XRF systems, have opened excellent opportunities for non-destructive characterization of e.g. layer-structured materials [12] analysing the elemental distribution on Mona Lisa and other paintings [13].

Although the angular dependence can be considered as an advantage, the sample surface roughness or topography becomes a problem in XRF microscopy where the elemental maps are obtained by raster scanning the sample with respect to the X-ray microprobe. The main reason is that the topographic features may have dimensions comparable to the probing beam size. Therefore they should be taken into account since they affect the signal level that, if not carefully considered, may lead to wrong interpretations. For correcting the effect of the surface relieves in archaeological samples Smilgies et al. used a dual detector system [14] and simply summed the response of the two XRF detectors, located symmetrically in respect to normal of the average sample surface plane. Although no mathematical model was suggested for a quantitative map correction the proposed method gives satisfactory results for the selected dual detector laboratory set-up. Obtaining more detailed information requires indeed the installation of detectors at other take-off angles. On the other hand, for retrieving elemental concentration and local take off angle, Trojek [15] recently proposed to use the Fundamental Parameter Methods equation. This mathematical approach was successfully employed to obtain the relief reconstruction of metallic samples by using a single XRF detector. The method was

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applied to samples with ideal composition (metallic and bi-alloy) assuming that all elements were detected by XRF, which is not always the case. However, by using a single detector it is not an easy task to determine the surface landscape for complex systems with variable inclinations with respect to the incident and detection angles.

Following a more analytical approach, Chukalina et al. [16] carefully simulated and studied the signal formation in scanning fluorescent X-ray microscopy using focused beams for the analysis of the surface relief. The authors quantitatively investigated the problem of surface microrelief determination from an XRF signal. They deeply dealt with the inverse problem, which is the reconstruction of the surface shape from the XRF signal. They successfully solved the inverse problem by providing three-dimensional simulations of the surface relief. Moreover their simulations can permit one to evaluate the value of a signal and to choose an optimal detector position. Despite the success of their theoretical approach it was not adequately tested on real samples.

In the recent years with the advent of efficient and fast XRF multi-array detectors, handling the surface artefacts have become an important issue in XRF microscopy experiments at Synchrotron facilities, where the beam size may have dimension of the order of possible surface reliefs.

In this paper we report on the specific case of the XRF microscopy set-up in the scanning X-ray microscopy station at the TwinMic beamline at Elettra, used in numerous studies across Life Science [17–20], Environmental Science [21–23], Material Science [24–27] and Cultural Heritage. Despite focusing on a specific case we propose a method that in principle can be applied and extended to any multi-array detector system, based on our experience during investigations of various types of specimen and critical evaluations of the collected data. In particular, using the XRF maps acquired on a topographically complex biological sample, we performed simulations to highlight possible artefacts that should be taken into account when processing the raw XRF images. Based on this information we propose a novel correction approach for reducing the entity of the observed artefacts — after their acquisition, as a post-processing algorithm.

## 2. Materials and methods

The XRF measurements were performed at the TwinMic beamline (Elettra — Sincrotrone Trieste, Trieste, Italy) that hosts a Scanning X-ray Microscope (SXM) [28], illustrated in Fig. 1. The sub-micrometre microprobe in SXM is provided by a zone plate (ZP)–order sorting aperture (OSA) system, and the sample is raster scanned perpendicularly to the X-ray microprobe. In particular the OSA allows selecting only the first diffraction order of the zone plate diffractive optics. The SXM is equipped with a Fast Readout CCD (FRCCD) for collecting the transmitted X-rays and a low energy XRF system consisting of 8 Silicon Drift Detectors (SDDs) located symmetrically and facing the sample [29–31]. The SXM is operated between 400 eV and 2200 eV, monitoring simultaneously the absorption, phase contrast and XRF images. Due to space constraint the 8 SDDs are positioned at an angle of 20° in respect to the sample surface. The XRF spectra are saved separately for each SDD and then can be selectively summed [30], providing elemental map distribution of the light elements till P and some transition metals such as Mn, Fe, Co, Ni, Cu and Zn [5]. The described set up has been used in a great number of Life [17,22,32–34] and Material Science [35–38] applications producing original and remarkable results.

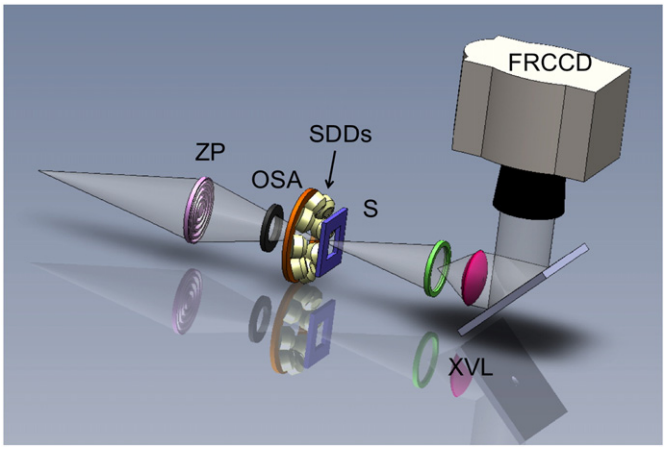


**Fig. 1.** Schematic view of the Scanning Transmission mode of TwinMic station. The microprobe is delivered on the sample (S) by a zone plate optics (ZP) together with a suitable OSA. The transmitted X-rays are collected by a Fast Readout CCD (FRCCD) camera through an X-ray to visible light converting system (XVL) consisting of a phosphor screen, a lens, a 45 degrees tilted mirror and an objective lens. The emitted XRF photons are collected by 8 SDDs located symmetrically around the sample.

In the present study focused on solving artefact effects in XRF imaging we used as a sample Human Embryonic Kidney (HEK293T) cells grown on 100 nm thick $Si_3N_4$ membranes and fixed with 4% paraformaldehyde [20]. Different groups of cells were mapped with a beam size and step size of 1 μm diameter using photon energy of 1.225 keV in order to detect C, N, O, Fe, Cu, Zn and Na. The XRF spectra were first fitted by using the PyMCA software package [39]. The implementation of the reported new algorithms, mathematical methods and image processing was done in Python.

## 3. Results and discussion

### 3.1. XRF artefacts in angular detection

When collecting XRF images with a multi-array detector system, the final displayed signal is usually the overall detector recorded signal, obtained by summing of the single cell response [40]. In the same way at TwinMic beamline we would normally sum the signal of all 8 SDDs in order to maximise the statistics and the solid angle of XRF collection. When the sample is perfectly flat at a length scale comparable with the microprobe, this method works very well, but, when using microprobes with sizes ≤1 $\mu m^2$ some types of samples do not obey the "flat" sample condition, in particular for grazing acceptance angles. We demonstrate here for the case of cell imaging, specimen that is very often studied by X-ray microscopy and whose topography profile cannot be considered flat and uniform. The first easy step to understand whether the sample can be considered flat is by examining the response of each of the 8 SDDs by considering them as 8 different XRF maps. The subsequent step of summing the 8 SDDs can also reveal asymmetries hinting on the presence of artefacts. Fig. 2 depicts the overall C, N, O and Na XRF maps, displayed together with the scattering peak intensity, collected on a group of Human Embryonic Kidney (HEK293T) cells. The elemental distributions of these light elements are accompanied by the corresponding X-ray absorption image acquired concurrently with the XRF map. Since the beam impinges normally the sample support, the absorption image displays the transmitted photons along the sample thickness crossed by the beam point by point in the raster scan. The absorption image intensity thus depends on the local specimen thickness and density. Considering that the cells would normally be mainly constituted by light elements such as C, N, O, H and Na, one would expect that the specimen density does not vary substantially along the cell. However by looking at C, N and O sum maps it seems that these chemical elements have a curious distribution, with some intensity enhancement on the borders. For instance assuming uniform oxygen distribution and 'flat' sample one can expect that the O XRF maps measured by each detector are identical and very similar to the absorption map trend, which contradicts the results shown in Fig. 2. Na and scattering peak distribution seems instead to be more similar to what one would expect. Therefore the next logical step is to investigate the response of the single SDDs. Fig. 3 shows the lateral distribution of oxygen (a) and carbon (c) content in the group of cells measured by each SDD. One can easily find some differences comparing the eight O XRF maps,