Some practical considerations about the effects of radiation damage on hydrated cells imaged by X-ray fluorescence microscopy

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X-ray fluorescence (XRF) microscopy features unique capabilities which make it well suited for biological investigations. Its high sensitivity together with high spatial resolution and penetration depth provide a unique tool for trace elements analysis in heterogeneous samples. Like most of the X-ray based techniques, radiation damage sets hard limits on the ultimate performance. Although the interactions between matter and photons are well described from a physics point-of-view, there is a lack of experimental data, in particular for XRF imaging mode. In this context, this work proposes a practical approach in addressing the limits set by radiation damage to X-ray fluorescence imaging in the case of hydrated and unfixed cells at room temperature. We find that the maximum dose tolerated by ascidian blood cells is 10 Gy. A simple theoretical model allowed the minimal doses required for a good image contrast to be determined for various experimental schemes. The results are consistent with the experimental observation on ascidian blood cells which exemplifies the peculiar case of highly concentrated samples (>10,000 ppm) at room temperature. The same simple model predicts that in the case of the detection of high Z trace elements in cryo-preserved cells, the relative detection limit set by radiation damage is below 0.1 ppm at a spatial resolution of 100 nm.

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1. Introduction

The development of high brilliance, high energy X-ray sources coupled with advances in manufacturing technologies of focusing optics has led to significant improvements in sub-micrometer probes for spectroscopy, diffraction and imaging applications in the multi-keV X-ray energy range. The main fields of application are driven by the unique attributes of X-ray microscopy in this spectral domain: (i) access to K-absorption edges and fluorescence emission lines of medium-light elements and L-, M-edges of heavier materials for micro-spectroscopy, chemical or trace element mapping. (ii) Higher penetration depths compared to soft X-rays allowing imaging of thicker samples or in-situ experiments. (iii) Relatively long focal lengths and depths of focus which are advantageous for the use of specific sample environments (e.g. wet conditions, controlled temperatures,…). The synchrotron-based X-ray fluorescence (SR-XRF) microprobe enables elemental mapping without staining, and often allows quantification of the elements present in a sample.

Although the basic method is very similar to the one used with non-synchrotron techniques such as scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDXA) and particle-induced X-ray emission (PIXE), the advantage of SR-XRF lies in its sensitivity (0.1–10 µg/g), due to the high photon flux available, the possibility of beam tunability and weak scattering [1]. Quantification is comparatively straightforward because the physics of photon interaction with matter is simpler and well understood. Furthermore, by selecting an appropriate energy of the incoming X-ray photons, it is possible to generate chemical maps of an element in relation to its oxidation state. In comparison, ion microbeams can provide quantitative elemental mappings but the sensitivity is lower by two orders of magnitude. Electron microscopes coupled with electron energy loss spectrometry (EELS) have a resolution better than 1 nm but the probed depth is restricted to the surface and plural inelastic scattering dominates the signal, resulting in a poor signal-to-background ratio and consequently in a low sensitivity. Secondary ion mass spectrometry (SIMS) achieves a spatial resolution of 50 nm and is extremely sensitive but, again the depth of analysis is less than 100 nm. Finally, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is also extremely sensitive and can probe thick samples but the spatial resolution is not better than 5 μm. Through an incremental evolution, X-ray fluorescence microscopy is becoming nowadays a powerful tool...
that can provide unique information to elucidate for instance the mechanisms of metal accumulation, detoxification or toxicity at the single cell and subcellular levels [2–8].

Biology appeared from the start as one of the prime driving cases of the synchrotron-based X-ray microscopy technique [9–11]. Logically pioneered in the water window spectral region, where the contrast between organic material and water is naturally high, full-field and scanning microscopes aimed at providing high resolution images in the meso-scale complementary to electron and visible light microscopes, with expected advantages in terms of sample preparation labelling and probed thickness [12,13]. With the evolution towards X-ray tomography, where multi-exposures are necessary, radiation damage became a real limiting issue. [14,15].

Although X-rays are less destructive than electron beams or ion beams, imaging fully hydrated biological samples at room temperature remains a challenging task. Previous works carried out in the water window indicate that the radiation dose that is necessarily imparted for X-ray imaging at 50 nm or better resolution is in excess of 10^6 Gy [16]. Studies of initially living cells have shown that doses of 10^6 Gy are at the approximate threshold for producing immediate changes in bacteria and are well above the dose needed to affect more complex cells in X-ray microscopy investigations [9]. At this stage, it should be emphasized that doses as low as a few grays alter significantly the cell fate. A few hours or a few days after the irradiation, chromosomal aberrations, DNA mutations, apoptosis and loss of the ability to reproduce can be observed in the descendancy [17,18]. When ionizing radiations are used for imaging hydrated cells, the goal is not to probe individual cells non-destructively, but rather to collect the signal on a time scale that is much shorter than the time scale of the induced biochemical damages. For instance, the maximum tolerable dose by eukaryotic cells could be raised up to 10^6 Gy if the data acquisition were faster than the diffusion processes responsible for the observed damage (1–10 ms) [19]. In practice, only some robust specimens like human sperm have been imaged in their hydrated state with no observable damage, using soft X-ray microscopy [20].

Most hydrated biological specimens exhibit mass loss, shrinkage, and the loss of ultrastructural information when irradiated at doses required for high resolution images [19]. One of the main damage mechanisms is the creation and propagation of radiolysis free radicals in water. Directly inspired from the techniques developed by electron microscopists, cryomicroscopy with rapid specimen freezing was rapidly adapted for X-ray microscopy and X-ray microtomography. In frozen hydrated organic specimens, it is assumed that the ice matrix acts as a cage which blocks the diffusion of free radicals [16]. Frozen hydrated biological specimens exposed to radiation doses up to about 10^10 Gy remained well preserved and free of easily visible structural changes and mass loss [16,21]. Conversely and despite a lack of experimental studies, cryogenic methods seem to be less effective for spectroscopy applications and do not preserve XANES spectroscopic signatures [22].

The X-ray absorption cross-sections of biological matter rapidly drop when the X-ray energy increases. Furthermore, the mean free path of the secondary electrons also rapidly increases when their energy increases. Consequently, hard X-rays are expected to be less damaging than soft X-rays because they produce a lower density of ionizations at the molecular scale. It has even been suggested that XRF microscopy of single living cells was at hand [23,24]. Nevertheless, up to now no systematic studies have been carried out about the limits set by radiation damage to the X-ray fluorescence imaging of biological specimens. In this context, the purpose of this work is (i) to assess the feasibility of imaging unfixed hydrated cells by X-ray fluorescence microscopy at room temperature and (ii) to evaluate the limits set by radiation damage for analyzing single cells by X-ray fluorescence microscopy. The experiments were performed on blood cells of ascidians. These specimens have the property of accumulating vanadium from sea water at exceptionally high levels in the vanadocytes [25,26]. The rather high fluorescence signal from vanadium allows imaging of the cells even at low radiation doses. Furthermore, the exciting energy of the vanadium fluorescence K-line is high enough (5.5 keV) to make phase contrast practical in order to follow the possible morphological changes of the irradiated cells simultaneously.

2. Estimation of the radiation dose required for X-ray fluorescence imaging of hydrated cells

2.1. Multi-keV X-ray absorption of hydrated cells

According to the Beer–Lambert law, the fraction of X-rays of energy E absorbed by a thin sample of thickness t and density ρ is [1 − exp(−(μ(E)/ρ)t)] ∼(μ(E)/ρ)0t where μ(E)/ρ is the mass absorption coefficient of the sample. If the intensity of the incident beam is I0, the number of photons absorbed by the sample irradiated during a time δt is

\[
n_{abs}(E) = \left[ \frac{\mu(E)}{\rho} \right] I_0 t \delta t
\]

For a composite sample, [μ(E)/ρ] can be easily derived from the tabulated atomic mass absorption coefficients [μi(E)/ρi] by using the relationship [μ(E)/ρ] = Σi[μi(E)/ρi]xi, where xi is the mass fraction of the atoms i within the sample.

A typical cell is well described as a matrix of light atoms (H, C, N, O) that contains some heavier trace elements. Fig. 1 represents the mean mass absorption coefficient calculated for a typical hydrated cell (xH=10%, xC=11.4%, xN=3.2%, xO=74.3%, xP=0.2%, xS=0.15%, xCl=0.15%, xK=0.3%). The discontinuities correspond to the jumps in the absorption cross-sections when the X-rays have an energy higher than the K-edge energies of carbon, nitrogen and oxygen. Interestingly, no discontinuities appear near the K-edge energies of phosphorus, sulfur, chloride or potassium even though their atomic mass absorption coefficients are much greater than those of the lighter atoms. This fact illustrates why radiation damage sets a hard limit on the sensitivity of SR-XRF microscopy of hydrated cells: the XRF signal originates from the trace elements whose contribution to the total number of absorbed photons is negligible.

Fig. 1. Mass absorption coefficient calculated using XOP database [27] for a typical cell composition: xH=10%; xC=11.4%; xN=3.2%; xO=74.3%; xP=0.2%; xS=0.15%; xCl=0.15%; xK=0.3% with no other trace elements and with 2000 ppm and 5% of vanadium.
while radiation damage originates from the massive absorption of X-rays by the light atoms of the matrix.

An ascidian blood cell can be simulated by a typical cell in which a variable fraction of vanadium is added. Again, Fig. 1 shows that the mass absorption coefficient of the cell depends on its vanadium content only if the latter represents more than 0.2% (i.e. 2 mg/g) of the cellular content. Only in this case, the contribution of vanadium atoms to X-ray absorption is not negligible.

2.2. Estimation of the radiation dose as a function of the imaging parameters

The dose $D$ is defined as the amount of energy absorbed per unit mass by an object subjected to an irradiation (1 Gy = 1 J/kg). When a sample is analyzed by SR-XRF microscopy, each pixel of size $\delta S$ is irradiated during a time $\delta t$. Hence, the imaging dose can be expressed as

$$D(E) = \frac{n_{\text{abs}}(E)E}{(\rho E \delta S)} = \frac{[\mu(E)/\rho]E_0 \delta t}{\delta S} \quad (2)$$

Once the excitation energy is set, the imaging dose mainly depends on the scanning parameters: the integration time per pixel and the pixel size. The above equality Eq. (2) implicitly assumes that the whole energy of the absorbed photons is deposited within the sample via secondary electrons and photons. In our case, the secondary electrons have a maximum energy of 5 keV. The mean path length of a 5 keV electron in water – about 600 nm [28] – is much shorter than the cell dimensions and therefore the electron escape is negligible. The fluorescence escape can be estimated as follows: the ratio $\gamma_i$ between the number of fluorescence photons emitted by deexcitation of a hole in the shell $L_i$ of an atom $i$, and the total number of photons absorbed by the same atom is maximal for an incident X-ray energy just above the energy threshold of $L_i$ and is given by:

$$\gamma_i = \frac{\omega_i(L_i)[I_i(L_i) - 1]}{I_i(L_i)} \quad (3)$$

where $\omega_i(L_i)$ is the total fluorescence yield [29] and $I_i(L_i)$ is the edge jump ratio of the atomic shell $L_i$ of atom $i$. For light atoms, the fluorescence yields are below $10^{-2}$ and most of the low-energy fluorescence photons are absorbed by the sample, therefore the fluorescence escape is negligible. For higher Z atoms, the fluorescence yields rapidly increase up to 1 but as long as their concentration is in the ppm range, they do not contribute significantly to X-ray absorption, as already mentioned. Therefore, a correction factor taking into account the fluorescence escape is only required for high concentration of high Z elements.

2.3. Required dose to fulfill the Rose criterion

The signal-to-noise ratio of an X-ray image is defined as

$$\text{SNR} = \frac{(n_{\text{max}} - n_{\text{min}})}{(n_{\text{max}} + n_{\text{min}})^{1/2}} \quad (4)$$

where $n_{\text{max}}$ and $n_{\text{min}}$ are the maximum and minimum number of counts detected per pixel, respectively. In XRF imaging, they correspond to the number of counts integrated over the bandwidth of the detected fluorescence line when the microbeam interacts with the object of interest and with the surrounding medium, respectively. Parasitic fluorescence background originating from both contamination and X-ray scattering falling into the detector collection aperture contributes to the overall background and related SNR degradation. The former contribution can be lowered with appropriate sample preparation and the latter is almost negligible in the case of X-rays of a few keV and appropriate detector geometry. As a first approximation, the signal-to-noise ratio of an X-ray image obtained from the XRF deexcitation of a hole in the shell $L_i$ of a given atom $i$ can thus be written as

$$\text{SNR} = (n_{\text{max}})^{1/2} = \left(\frac{n(E)}{\gamma_i(L_i)\phi_{\text{det}}}\right)^{1/2} \quad (5)$$

where $n(E) = x_i[\mu(E)/\rho]E_0 \delta t$ is the number of photons absorbed by the atoms $i$, and $\phi_{\text{det}}$ is the detection efficiency: $\phi_{\text{det}} = \eta_{\text{det}} \Omega_{\text{det}}/4\pi$ where $\eta_{\text{det}}$ is the detector efficiency and $\Omega_{\text{det}}$ is the solid angle of detection. According to the Rose criterion, an object is made visible into an image when $\text{SNR} \geq 3–5$ [30]. By combining Eqs. (2), (4) and (5) we can calculate the minimum dose $D_{\text{min}}$ required to fulfill the Rose criterion:

$$D_{\text{min}}(E, i, L_i) \geq 3^2 [\mu(E)/\rho] E/\phi_{\text{det}} \gamma_i(L_i) [\mu_i(E)/\rho] [x_i \delta S \rho e] \quad (6)$$

The minimum dose required to detect an element of interest is obtained when the X-ray energy is optimally tuned right above the K-threshold of the element. Fig. 2 displays the doses required to detect the various elements in a typical cell, as a function of their concentration and for a pixel size of 1 $\mu$m x 1 $\mu$m. We show the minimum imaging doses required in optimized conditions – the X-ray energy is right above the K-edge of the element of interest – and in our experimental conditions, the energy is set at 5.5 keV. The required imaging dose is inversely proportional to the image pixel size and thus to the square of the spatial resolution: if a resolution of a 100 nm was necessary, the required doses would be increased by a factor of 100 but the slope of the straight lines presented in Fig. 2 would not change. In fact, by taking into account the fluorescence escape, we find that the minimum required dose for imaging the distribution of a given element is inversely proportional to its concentration. Therefore, the minimum dose required for imaging the distribution of a given element whose concentration is 1 ppm in a biological matrix can be used as a reference for the sensitivity of the XRF set-up for detecting the element. The lower the minimum dose required for the imaging of one ppm of a given element, the higher the sensitivity of XRF do detect the element. It should be noted that the above-mentioned quantity is pertinent only if the spatial distribution of the trace elements.

![Fig. 2](image_url) Required dose for mapping the distribution of an element of interest – here vanadium (V), potassium (K) or sulfur (S) – in XRF contrast, with SNR > 3, as a function of its mass fraction in a typical biological cell. The calculation was performed according to Eq. (6) for a pixel size of 1 $\mu$m x 1 $\mu$m and for a typical fluorescence detector efficiency, including solid angle, of 10$^{-3}$. The plain plots correspond to optimized conditions with an excitation energy set right above the K-edge of the targeted elements. The dashed plots correspond to our experimental conditions with an excitation energy of 5.5 keV, i.e. optimized for the detection of vanadium.
element is relatively even, which is the case for ionic trace elements in solution but not necessarily for metallic trace elements bounded to proteins. In the case of a highly inhomogeneous distribution, the concentration that should be taken into account to calculate the minimum required dose for imaging is the “local” concentration i.e. the concentration in the volume irradiated per pixel.

3. Experimental method

3.1. X-ray microscope set-up at ID21

The ID21 scanning X-ray microscope [31] was operated at 5.5 keV, above the vanadium K-edge threshold. The monochromatic beam had an energy bandwidth of 1.3 eV. The 69.6 μm diameter Fresnel zone plate provided by the TASC-INFM has a 200 nm outermost zone width. The beam spot size, measured by scanning a knife-edge, was 0.5 μm x 1 μm (V x H). The photon intensity in the microprobe was ranging between $2 \times 10^7$ and $7 \times 10^7$ ph/s. A 30 ms time response fast shutter was inserted between the monochromator and the sample to limit the exposure of the sample to X-rays while maintaining the thermal stability of the monochromator. A 20 mm$^2$ silicon photodiode (International Radiation Detector Inc.) was used to detect the transmitted intensity. Alternatively, a 50 μm diameter aperture could be aligned in front of the photodiode to enhance phase sensitive measurement [32]. The image acquisition was performed by scanning the sample mounted on a piezoelectric driven stage. For each pixel, the full XRF spectrum was recorded simultaneously with the transmitted (absorption or phase) intensity.

We imaged about 70 cells with pixel sizes chosen between 0.1 μm$^2$ and 1 μm$^2$ and dwell times per pixel between 0.1 s and 1 s. In total, the radiation dose per image ranged from $10^3$ Gy to $10^6$ Gy.

3.2. Sample preparation

Ascidia *Phallusia mammillata* samples were collected from the Gulf of Naples, Italy. The extraction of the blood cells was performed as previously described [33]. Blood was mixed with F12 medium and blood cells were isolated by centrifugation at 300 x g for 10 min, re-suspended in the same medium and stored at 4 °C until analysis. Some batches of blood cell suspension were mixed with low-melting-point agarose gel at a final concentration of 0.5% and kept at 4 °C until analysis. The cell lifetime in these conditions has been measured to be at least one week. For the analysis, a drop of cell suspension was placed between two 23 μm thick PET films separated by a 10 μm polyimide spacer sealed by silicon grease. This preparation prevents the sample from dehydration and ensures the conservation of the 10 μm round shape blood cells throughout the entire duration of the experiment.

4. Results and discussion

4.1. Determination of the cellular concentration of vanadium

The mean content in vanadium was estimated from measurements of the cellular absorption of X-rays above vanadium K-edge. No absorption contrast was detected compared to surrounding agarose for cells containing no or little vanadium. For the other ones, the mean cellular absorption was $0.022 \pm 0.06$. Assuming that the cell density is 1.05 g/cm$^3$, we have calculated an average concentration of vanadium of $(520 \pm 160)$ mM per vanadocyte. As expected, the concentration in vanadium is extremely high and represents about $(2.5 \pm 0.8)$ mg/g of the vanadocytes. This value concurs with previous measurements performed on total blood cell populations: in the case of ascidia *P. mammillata*, the total vanadium concentration for the blood cells is 19.3 mM [34]. Vanadocytes are expected to represent about 5% of the total blood cell population [35] so the expected vanadium concentration in *P. mammillata* vanadocytes would be about 400 mM. High variations of vanadium content and speciation are expected even within the same species [36] and they can fully account for the difference observed between the approximate expected value and our measurement.

4.2. Experimental determination of the maximum tolerated dose for vanadium imaging

The dose absorbed by each cell was calculated according to Eq. (3) with $x_a = 2.5%$ and a correcting factor of 0.94 to take into account the fluorescence escape for the vanadocytes and with $x_a = 0%$ and no correction factor for all the other cells. For a single image, the ratio between the radiation doses in the vanadocytes and in the other types of cells is 1.31.

Imaging single hydrated cells was possible at doses up to $10^5$ Gy. The imaging parameters were typically a pixel size of 1 μm x 1 μm and an integration time of 0.5 s. As shown in Figs. 3(a) and (b), the cell morphology is preserved and the vanadium distribution is uniform in the vanadocytes. Nevertheless for doses closed to $10^5$ Gy the imaging of living cells was not reproducible. For instance, Figs. 3(c) and (d) present the same cells imaged twice consecutively. In the second image, the cell in the center has disappeared. It was a vanadocyte imaged at a dose of $8.4 \times 10^4$ Gy while the imaging dose of its neighbor was about $6.4 \times 10^4$ Gy. Above $2 \times 10^5$ Gy, imaging a whole single cell was not possible. The cells were morphologically damaged during imaging. Generally they were physically cut after several line scans as shown in Figs. 3(e) and (f).

The fraction of a cell that can be imaged as a function of the radiation dose is presented in Fig. 4. The large dispersion observed in the region around $2 \times 10^5$ Gy could reflect the fact that the imaging dose is not the most pertinent parameter to describe the effects of radiation: the response of a cell depends on the initial distribution of clusters of ionizations at the nanometer scale. Therefore, we can expect a different cellular response to the same radiation dose if different imaging parameters are used. A high resolution image performed with a small integration time versus a low resolution image performed with a long integration time can require the same dose but they induce a very different spatio-temporal profile of initial interactions. In spite of these considerations, Fig. 4 tends to confirm that the maximum dose that a hydrated blood cell can tolerate is about $10^5$ Gy. Our result can be compared to similar experiments performed on CHO cells imaged by soft X-ray transmission microscopy [9] that showed that doses of $10^4$ Gy could severely damage hydrated biological samples, at room temperature.

The mechanisms that lead to cell destruction are very different from the biological mechanisms responsible for the cell death at lower radiation doses and on longer time scale. Synchrotron microprobes are so intense that the cell can be destroyed within a few seconds of irradiation due to charge accumulation and/or oxidative attack by the many free radicals produced upon water irradiation which generates an accumulation of biochemical alterations.
4.3. Limits set by radiation to the imaging of ascidian blood cells at room temperature

In our experimental configuration, the limit of detection set by radiation damage is represented by the horizontal dashed line in Fig. 2. For vanadium, it corresponds to $x_v = 0.2\%$ or 2000 ppm. This finding confirms that under our experimental conditions, imaging the vanadium distribution is possible at a spatial resolution of 1 $\mu$m while it is impossible at a spatial resolution of 100 nm. By calculating the signal-to-noise ratio of the vanadium fluorescence images acquired during our experiment with a pixel size of 1 $\mu$m$^2$, we found that the minimum required dose for vanadium fluorescence imaging of an ascidian vanadocyte is about $2 \times 10^4$ Gy. This is in reasonable agreement with our calculations which predict a required dose of $10^4$ Gy for imaging the distribution of vanadium at a concentration of 25,000 ppm (see Fig. 2). According to Fig. 2, imaging sulfur and potassium at a dose of $10^5$ Gy in our experimental conditions is possible if the concentrations are higher than 15,000 ppm and 5000 ppm, respectively. Given the estimated concentrations of these elements (see caption of Fig. 1) it confirms that imaging was not possible in our conditions. Finally, based on our calculations and our experimental results, we can conclude that XRF imaging of trace elements in the ppm range is impossible at room temperature in hydrated ascidian blood cells. Given the orders of magnitude, this conclusion should hold for most biological single cells. Only some extremely radioresistant cells could face the doses required for trace elements mapping without the need for any cryo-fixation technique. Further experiments are required to characterize in greater detail the cellular response to irradiation when the cells are not isolated but a part of a tissue section.

4.4. Practical implications for X-ray fluorescence imaging of trace elements in hydrated cells

The fast development of ultra-high resolution SR-XRF microscopy inevitably raises the question of the ultimate performance of such a technique when cryo-fixed cells are expected to tolerate a maximum dose of $10^{10}$ Gy [21]. We have used Eq. (6) to calculate the minimum required dose for imaging the distribution of high Z atoms at a spatial resolution of 100 nm. The incident X-ray energy was chosen for each element 30 eV above their K-threshold. The results are presented in Fig. 5. They do not aim at giving an absolute value for the limit of detection but rather to show trends. The minimum required dose for one ppm at a given resolution decreases with increasing Z and reaches a lower limit for Z > 30. It means that in an optimized configuration the required dose to map the distribution of an heavy trace element is about 10 times lower than the minimum required dose to detect the same amount of sulfur or potassium at the same spatial resolution.

By plotting the maximum dose tolerated by cryo-fixed cells, we can see that the detection limit of XRF microscopy for high Z elements could be pushed below 0.1 ppm with a resolution of 100 nm. In fact the figures here are calculated for a detector efficiency of $10^{-3}$ that can be increased by at least one order of magnitude if multi-element XRF detectors or any other means of
Fig. 5. Required dose for imaging a given element of atomic number Z in optimized conditions at a spatial resolution of 100 nm. The element concentration was set to 1 ppm, the cell thickness to 10 μm and the detection efficiency to 10⁻³.

collecting a higher fraction of the fluorescence signal are used. Furthermore, temporal considerations were not included in our study but we can anticipate that ultrafast imaging would allow the maximum tolerable dose to be raised. The new X-ray microprobes are expected to reach 10-nm resolution and the ultimate detection limit is not only set-up dependent but also sample dependent. For instance, if the distribution of the element of interest was very inhomogeneous at the nanometer scale, the best dose-fluorescence signal compromise would be achieved for a spot size of the same dimension as the heterogeneity [24]. This is simply because locally, the apparent concentration of the element would be much higher.

5. Conclusions

This work aimed at exploring the limits set by radiation damage to XRF microscopy of individual hydrated cells. The first question we addressed was the possibility of imaging single hydrated cells at room temperature. Our experimental results show that the maximum tolerable dose for a typical hydrated ascidian blood cell is about 10⁵ Gy. We used a simple model to estimate the minimal dose required for a good image contrast according to the Rose criterion. Our calculations and our experimental results show that it is possible to map the vanadium distribution of highly concentrated cells (>10,000 ppm) at room temperature. Nevertheless, the maximum tolerable dose is too low to consider imaging trace elements in the ppm range in hydrated samples at room temperature.

We used the same simple model to estimate that in the case of cryo-preserved cells the detection limit for high Z trace elements set by radiation damage is below 0.1 ppm at a spatial resolution of 100 nm. This estimated limit represents in itself a challenge for the development of the future nanoprobes and associated management of irradiation conditions. In particular, development of fast detectors with high collection angle and dynamical range is a prerequisite to the successful deployment of the nano-XRF towards biology.

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