Optimization of digital filters for the detection of trace elements in EELS. III – Gaussian, homomorphic and adaptive filters

J. Michel\textsuperscript{a,*}, N. Bonnet\textsuperscript{b}

\textsuperscript{a}Laboratory de Microscopie Electronique Analytique, Université de Reims, EA 2068 Biomatiériaux (IFR 53), 21 rue Clément Ader, 51685 Reims Cedex 2, France

\textsuperscript{b}INSERM U 514 (IFR 53) and LERI, Université de Reims, 45 rue Cognacq Jay, 51092 Reims Cedex, France

Received 28 July 2000; received in revised form 16 February 2001

Abstract

This paper is the third part of a series of papers dealing with the development of digital filters for the extraction of information from electron energy loss spectra, when the signal-to-background is very weak, as it is the case for elements in weak concentration. Three new filtering approaches are investigated: the use of high-order derivatives (typically, the fourth order derivative of a Gaussian), the use of homomorphic filtering (working on logarithmically-transformed spectra) and the use of adaptive filtering, taking into account the local background shape. These signal processing methods are applied to real spectra to demonstrate and comment their effects in three different cases of signal and background shapes related to calcium, phosphorus and boron detection in definite thickness organic matrix. © 2001 Elsevier Science B.V. All rights reserved.

PACS: 82.80.p; 82.80.e

Keywords: Electron energy loss spectroscopy (EELS); Data processing

1. Introduction

In this series of papers, we intend to investigate different ways of lowering the detection threshold in electron energy loss spectroscopy (EELS), by exploiting the possibilities offered by digital signal processing, which still remains largely unexplored in this application.

In the first paper [1], we have shown that the behavior of simple digital filters (first and second order difference filters) already proposed by Statham [2], Shuman and Kruit [3] and Zaluzec [4,5] and included in commercial spectroscopy packages can be explained easily through the consideration of their transfer function, defined in the frequency domain. We have shown that the three different parts which constitute a spectrum (namely, non-characteristic background, characteristic peak and statistical or instrumental noise) have to be considered simultaneously and that any filtering operation has to be considered as a...
compromise between three conflicting tasks: removing the largest part of the background, amplifying the characteristic signal and avoiding noise amplification as far as possible. In the case of trace element detection, the increase of the peak to background ratio prior to quantification is of prime importance since otherwise a small uncertainty in the background estimation would induce a large error in the evaluation of the net signal. We have shown that a better compromise can be obtained by using optimized filters, which have a simultaneous smoothing and differentiating, i.e. low- and high-pass, behavior. We have also shown that the characteristics of a digital filter can be optimized in order to meet given specifications, such as eliminating the background slope completely, for instance.

In the second part of the series [6], we concentrated on the experimental verification of the ideas proposed in the first paper. Taking the example of the detection (and quantification) of Ca and Fe as trace elements (<1%) in standard specimens, we investigated the improvement of digital filters compared to simple first and second difference filters from different points of view: background removal, characteristic peak amplification and correction of diode-to-diode fluctuation response in parallel detection. We estimated that a significant improvement can be brought about by these new implementations of difference filters.

In this paper, we introduce new ideas which could lead to further improvements and, hopefully, help in lowering the detection threshold of trace elements.

The first idea concerns digital filtering in general, with the general tasks described in the two previous papers of the series: how to optimize digital filters in order to get the best compromise among the three conflicting requirements listed above? Several considerations, to be developed in Section 2, led us to the idea of implementing Gaussian filters.

The second idea is more specifically devoted to the problem of background removal. For chemical elements with a low characteristic energy loss value whose characteristic peak is superimposed onto the foot of the carbon peak (e.g. calcium in an organic matrix) or of the plasmon losses (e.g. phosphorus or boron), the trace detection is mainly impaired by this background with steep slope. As it is known by many spectroscopists, this background cannot be eliminated completely by simple first or second difference filters. The idea is then to combine methods which are used for the spectrum modeling and for digital filtering. This led to the concept of adaptive filtering which will be the subject of Sections 3 and 4. In Section 3, the adaptation is made automatically through the homomorphic transformation: when the power-law model holds, a logarithmic transformation leads to a linear background, which can be eliminated through simple filters in the transformed space. In Section 4, the adaptation is made more general: the background is estimated locally and then eliminated through a filter specifically designed to suit the estimated local background.

2. Gaussian filtering

2.1. Motivation

Several considerations led us to the idea of suggesting this kind of digital filters for the detection and quantification of trace elements by electron energy loss spectroscopy.

- The first consideration was already a motivation for the first paper of this series: in the course of digital filtering, with the aim of removing the non-characteristic background and enhancing the characteristic signal, one has to try to avoid excessive noise amplification. Otherwise, the characteristic signal of a trace element would be indistinguishable from the statistical and/or instrumental noise. This led us to the idea of avoiding step edges in the definition of the impulse response of the digital filters, because these step edges (see the top-hat filters, for instance) correspond to high frequency lobes of the transfer function and lead to high frequency (channel-to-channel) noise amplification. Therefore, we suggested the use of cosine functions for the shape of the different lobes of the digital impulse response. We
verified, both theoretically and experimentally, that the signal-to-noise ratio of the filtered spectra was fairly well improved by this method. However, it is known from other fields (image processing, for instance) that other shapes are even better from this point of view and offer at the same time a good localization in the real (experimental) space (here, the energy loss space) and in the reciprocal space. Gaussian functions in the real space have this property, since their equivalents in the frequency space are also Gaussian functions, whose standard deviation \( \sigma_E \) is simply related to the standard deviation in the real space \( \sigma_f \) by

\[
\begin{align*}
    h(E) = e^{-E^2/2\sigma_E^2} & \frac{\text{FT}}{\text{FT}^{-1}} H(f) \approx e^{-f^2/2\sigma_f^2} \\
    \text{with } \sigma_E \sigma_f = 1 \text{ where } E \text{ is the energy loss and } f \text{ its reciprocal } (f = E^{-1}).
\end{align*}
\]

The second motivation concerns the order of the derivative to be used in order to succeed in the tasks at hand. Concerning background removal, if one assumes that the classical model \( I(E) = A E^{-R} \) [7,8] holds, at least approximately, neither the first nor the second order derivatives are able to remove this background completely. They are only able to reduce the slope of the filtered spectrum, as shown in Fig. 1 for a spectrum background simulated according to the power-law model with \( R = 2.5 \) (the different derivatives were normalized at \( E = 125 \text{eV} \)). One can see on this figure, that the background slope is further decreased by using higher order derivatives (third order, fourth order, etc). This attempt of trying to compute higher order derivatives when the characteristic peak is so weak that second order derivatives are unable to provide a sufficient peak/background ratio led us also to Gaussian filters. The reason is that Gaussian functions are infinitely differentiable, as can be checked easily. Derivatives of Gaussian functions as impulse responses of digital filters have the properties expected for filters in the field of electron energy loss spectroscopy: they have a band-pass behavior, i.e. high-pass for background and low-pass for noise. Of course, the price to pay for going to higher order derivatives is that the signal-to-noise ratio is degraded, as it is when going from the first order to the second order derivative. Fortunately, the band-pass characteristics of digital filters can be easily tuned by selecting the order of the derivative and the standard deviation of the smoothing Gaussian function.

The third motivation for considering Gaussian filters is that they are easy to implement as discussed in Section 2.2 and capable of high-speed operation. One could argue that when working in the field of spectroscopy, which concerns a one-dimensional space, the running time is not of prime importance. We agree that this is true for spectroscopy itself, but the ultimate goal of these digital filtering techniques is to perform the quantitative mapping of an element from a given specimen and, for this, the processing of the spectrum has to be repeated for all the pixels of the spectrum-image, as described by Jeanguillaume and Colliex [9], Hunt and Williams [10], and Balossier et al. [11]. Thus, it is important that the digital filtering can be done in a short time, especially if the processing is to be done on-line.

### 2.2. Implementation

Gaussian filters can be implemented along different lines, as large-support non-recursive
filters, as recursive filters, or as a multi-pass implementation of short-term non-recursive filters. We chose this last implementation and describe it below. Gaussian smoothing filters and their derivatives can be implemented as iterative convolutions with very simple impulse responses as \([+1 \quad 1]\) and \([+1 \quad -1]\). The Z-transform \([12]\) can be obtained easily (without heavy mathematics, see Hashimoto and Sklansky \([13]\)) by considering that the transfer function of an ideal derivative (complex gain: \([H(\omega) = j\omega]\) has a discrete equivalent through the bilinear transformation \((H_D(Z) = (1 - Z^{-1})/(1 + Z^{-1}))\) and that the simpler discrete smoothing function can be obtained by computing the signal mean on two adjacent channels \((H_S(Z) = (1 + Z^{-1})Z^{1/2})\).

Thus, \(K\) successive derivations and \(N\) successive smoothing passes lead to the following Z-transfer function:

\[
H(Z) = H^K_S(Z)H^N_D(Z) = \frac{(1 - Z^{-1})^K}{(1 + Z^{-1})^K}(1 + Z^{-1})^NZ^{N/2} = (1 - Z^{-1})^K(1 + Z^{-1})^{N-K}Z^{N/2}. \tag{2}
\]

This transfer function corresponds to

(a) \(K\) iterated first order derivatives, with the kernel \([+1 \quad 0 \quad -1]\). The energy shift can be related to the size of the signal that we want to reveal by padding the kernel with zero values, which thus becomes \([+1 \quad 0 \quad \ldots \quad 0 \quad -1]\).

(b) \((N - K)\) iterated first order smoothing passes, with the kernel \([+1 \quad +1]\).

(c) A shift of \(N/2\) channels in order to compensate for the shift of \(1/2\) pixel introduced by each smoothing operation.

Some impulse responses, and their equivalent transfer functions in the frequency domain, are displayed in Fig. 2, for illustration. It should be stressed again that the filters can be implemented through several passes on the data with the small kernels (involving additions only, and no multiplications) rather than by using the complete (equivalent) impulse responses displayed in Fig. 2.

### 2.3. Practical applications

All the experimental spectra presented in this paper were recorded with a CM30 Philips STEM coupled with a Gatan 666 PEELS. The cryosections were transferred into the microscope with a Gatan specimen cryoholder. The cryosections were then freeze-dried and analyzed at a temperature below 110 K. Preliminary classical treatments such as dark-current subtraction or linearity correction of the intensity scale \([14]\) are performed on each acquired spectrum. In order to correct, at least partially, the short-range fluctuations in gain between photodiode elements, raw spectra correspond to the sum of several equivalent and successive spectra recorded with different energy offsets and then superimposed using maximum correlation coefficient computation.

The first example concerns phosphorus detection. Phosphorus quantification in an organic matrix has still been studied in details \([15]\). Our aim in the present paper is limited to the proposition of new abilities for the preliminary step of quantification i.e digital filtering. Fig. 3a displays the raw spectrum recorded on a cell cryosection of 80 nm nominal thickness with an energy dispersion of 0.5 eV/channel and using 250-keV electrons. Spectrum acquisition corresponds to an electron dose of approximately \(3.8 \times 10^{10}\) electrons. Phosphorus is revealed by its characteristic \(L_{23}\) edge located at an energy loss of 132 eV and superimposed onto the plasmon tail. In biological specimens, phosphorus is associated with phosphate groups \((PO_4)\) leading to a typical fine structure signal in the spectrum with two peaks of approximately 8 eV width at the threshold. Fig. 3b displays the spectrum after Gaussian filtering with four iterated first order derivatives \((K = 4)\), four iterated first order smoothing passes \((N = 8)\) and an energy shift of 4 eV. These parameters lead to a band-pass behavior around 8 eV (16 channels corresponding to a reduced frequency of approximately 0.06) of the transfer function (see Fig. 2). Fig 3c displays the spectrum after second-difference filtering with an energy shift of 4 eV and six iterated first order smoothing passes. The
Fig. 2. Impulse response and transfer functions of Gaussian derivative filters (a–c) Impulse responses of first derivative Gaussian filters (shift = 1, 4 and 8 channels, respectively). (d) Transfer functions corresponding to the three impulse responses. (e–g) Impulse responses of second derivative Gaussian filters (shift = 1, 4 and 8 channels, respectively). (h) Transfer functions corresponding to the three impulse responses. (i–k) Impulse responses of fourth derivative Gaussian filters (shift = 1, 4 and 8 channels, respectively). (l) Transfer functions corresponding to the three impulse responses.
efficiency of Gaussian filtering for background removal is obvious since the background is almost suppressed in Fig. 3b.

The second example is about boron detection. Boron is involved in a promising therapy of brain tumors called boron neutron capture therapy [16]. The efficiency of such therapy depends directly on the subcellular boron distribution and EELS can be useful to obtain this information [17]. Fig. 4a displays the raw spectrum recorded on a melanoma cell cryosection of 80 nm nominal thickness. The cultured melanoma cells were exposed to sodium borocaptate (Na₂B₁₂H₁₁(SH)). The energy dispersion was 0.5 eV/channel and using 250-keV electrons. Spectrum acquisition corresponds to an electron dose of approximately 1.5 × 10¹⁰ electrons. Boron is revealed by its characteristic K edge located at an energy loss of 188 eV. As for the phosphorus, the edge is superimposed onto the plasmon tail. The boron K edge shape in this experiment is rather similar to the carbon K edge. We experimentally deduce that the optimum energy shift was 4 eV in order to extract the “sawtooth-shaped” signal at the threshold. Fig. 4b displays the spectrum after Gaussian filtering with four iterated first order derivatives (K = 4) and four iterated first order smoothing passes (N = 8). Fig. 4c displays the spectrum after second-difference filtering with an energy shift of 4 eV and six iterated first order smoothing passes (N = 8). Even if both digital filters raise the peak to background ratio appreciably, the Gaussian filtering is always more efficient for this purpose. However, it may be noted that the output of the Gaussian filtering seems slightly more noisy probably due to the use of high order derivatives.

Our last example deals with the detection of calcium. As for phosphorus, calcium quantification has still been largely investigated (see [18] for example). Fig. 5a displays the raw spectrum recorded on a 100 mmol/kg calcium concentration standard for X-ray microanalysis of biological specimens (Agar Scientific Ltd, England). The
nominal thickness of the standard section was 100 nm, the energy dispersion was 0.2 eV/channel using 300-keV electrons. Spectrum acquisition corresponds to an electron dose of approximately $7.7 \times 10^{10}$ electrons. Calcium is revealed by its characteristic $L_{23}$ edge located at an energy loss of 347 eV. This edge occurs just above the carbon K edge at 284 eV so the background is then essentially composed of the foot of the carbon edge and of its convolution with the plasmon peak. The calcium $L_{23}$ edge is dominated by the white lines (strong peaks at the threshold) and we demonstrated in the previous papers that an energy shift of 6 eV was optimal for their detection. Fig. 5b displays the spectrum after Gaussian filtering with four iterated first order derivatives ($K = 4$), 2 iterated first order smoothing passes ($N = 6$) and an energy shift of 6 eV. Fig. 5c displays the spectrum after second-difference filtering with an energy shift of 6 eV and four iterated first order smoothing passes. In both...
cases, a relevant increase of the peak to background ratio is apparent but even in the case of the Gaussian filtering the background is not totally suppressed. For further quantification, by MLS methods for instance, it must be taken into account.

3. Homomorphic filtering

3.1. Motivations

We have shown above that high order derivatives are able to remove the background from a composite signal better than low order derivatives and that the high-pass behavior of these filters can be efficiently counter-balanced by adding low-pass components. However, in situations characterized by a low signal-to-noise ratio, high-order derivatives remain more noisy than low-order ones. Thus, there is also some interest in developing alternative methods that are able to remove the background efficiently while keeping the signal-to-noise ratio at an acceptable level.

We first illustrate the principle of such methods by considering the specific case of the power law model. Then, in Section 4, we will consider the more general case of an arbitrarily-shaped background.

3.2. Homomorphic filtering: the power-law model

Two different approaches are used for the processing of EELS spectra. When the concentration of the element to be studied is high enough, the non-characteristic background is modeled and then subtracted, giving the net signal which can then be integrated and quantified according to the cross-section. In order to perform the background modeling, a Log–Log conversion is often performed, based on the fact that the power-law model can thus be converted into a linear model \( \log I = \log A - R \log E \). When the concentration of the element is very low, as is often the case for biological applications, the tendency is rather to perform digital filtering and to quantify the amount of element by fitting the filtered spectrum to reference spectra filtered in the same way [19–21]. We have seen in the previous section that the background removal is very imperfect when its original slope is large therefore a background component must also be taken into account.

Thus, the idea is to combine the advantages of the two approaches, i.e. to apply digital filters to
quasi-linearized spectra, since it is well-known that
top-hat filters (or, more generally, symmetric
filters) are very efficient in removing linear signals.
This idea leads to a three-steps procedure:

(a) linearize the spectrum by taking the logarithm
    of the experimental intensities ($I' = \log I$),
(b) apply a simple first or second derivative digital
    filter to this linearized spectrum,
(c) smooth the result by applying a low-pass filter
    (average filter or Gaussian filter) to the result
    obtained after differentiation.

(Note that steps (b) and (c) can be performed
simultaneously, with a filter implementing the
derivative of a Gaussian, for instance).

In practice, the experimental data are most often
recorded with a constant energy loss increment,
and the differentiating filter has to be computed
with symmetrical values of the logarithm of the
energy shift:

$$\frac{dI'(\log E)}{d(\log E)} \approx I'(\log E + \frac{\Delta \log E}{2})$$
$$- I'(\log E - \frac{\Delta \log E}{2})$$

(3)

and

$$\frac{d^2 I'(\log E)}{d^2(\log E)} \approx 2I'(\log E) - I'(\log E + \Delta \log E)$$
$$- I'(\log E - \Delta \log E)$$

(4)

for first and second derivatives, respectively.

There are two potential solutions to this problem:

- either resample the complete spectrum according
to a logarithmic scale for the energy loss $E$,
- or recompute the shift value $\Delta \log E$ in terms of
  the experimental energy loss values:

$$\delta E_{1,2} = \exp(\log E_0 \pm \Delta \log E) - E_0,$$

(5)

where $E_0$ stands for the energy loss value at
which the filtered signal is computed.

We have chosen to implement the second
solution. Since the resultant values $\delta E$ do not
 correspond, in general, to experimentally sampled
values, one has to perform interpolation in order
to get a good approximation to $I(E - \delta E_1)$ and
$I(E + \delta E_2)$.

3.3. Practical applications

We applied the homomorphic filtering in the
two cases, phosphorus and boron, where the
power law model for background holds at least
approximately. Figs. 6a and b display the outputs
of homomorphic filtering applied to the raw
spectra presented in Section 2.3 (Figs. 3a and 4a).

Fig. 6. Outputs of homomorphic filters (see Section 3.3 for
details) (a) in the case of phosphorus (raw spectrum in Fig. 3a);
(b) in the case of boron (raw spectrum in Fig. 4a).
order to be adapted to the signals. These shifts were recomputed in terms of the experimental energy loss values according to Eq. (5). The shift values were interpolated between the two nearest recorded values.

- application of six iterated first order smoothing passes as the final step in both cases. The smoothing operation is then similar to the Gaussian filtering one.

The output spectra reveal that this approach is very efficient for background removal. Moreover, spectra appear, as expected, less noisy than after the fourth order Gaussian derivative filtering. In order to confirm this impression, we evaluated in each case the quadratic signal-to-noise ratio (QSNR) according to the method described by Frank [22] and Bonnet et al. [23]. The QSNR, defined as the ratio of the signal variance to the noise variance, was estimated from the correlation coefficient between two successive and equivalent recorded spectra. Use of this method requires considerable care since we have to check that no mass loss or contamination occurs during the acquisition. We performed the computation in the 170–215 eV area for boron signal and in the 110–160 eV area for phosphorus signal. For boron we obtained QSNR = 3.06 after Gaussian filtering and QSNR = 7.36 after homomorphic filtering. For phosphorus, the corresponding values were QSNR = 2.83 and 5.27, respectively.

4. Adaptive filtering

4.1. Principle

The developments of the previous section rely on the assumption that a power-law model \( I = AE^{-k} \) holds for the background shape. It should be stressed here that this implementation of a symmetric filter impulse response in the \((\log I, \log E)\) space is completely equivalent to the implementation of asymmetric filters in the \((I, E)\) representation space, which was described in the first paper of this series. This implementation through a computation of the logarithm was developed in order to show the connection between digital filtering and background modeling.

Now, the question is: can this method be extended to parts of the spectrum where the power-law model no longer holds, behind the major carbon edge for instance?

We claim that the answer is yes, provided an analytical description of the background shape is available.

For this, we suggest the following procedure:

(a) Model the background, according to an analytical model, such as a polynomial model, for instance [24].

In the case of trace elements, this modeling is, in general, not precise enough to get a realistic quantification of the characteristic signal by simple background subtraction. Thus, the background description here is only used to devise an adaptive digital filter, in order to better remove the background and thus enhance the signal-to-background ratio.

(b) The coefficients of the filter should be derived from the local background shape in the vicinity of the characteristic peak, as modeled in step a)

Suppose, for instance, that we want to implement a second derivative filter with a lobe shift \( \Delta E \). This derivative filter is described by three coefficients: the amplitude of the central positive lobe (that we fix arbitrarily to 1), the amplitude of the negative side lobe at energy loss \( E_0 - \Delta E \), say \( C1(E_0) \), and the amplitude of the negative side lobe at energy loss \( E_0 + \Delta E \), say \( C2(E_0) \). If the local background shape has been modeled as \( B(E) \) in step (a), then a filter which is able to remove the largest part of the background intensity has to fulfill the following requirements:

\[
B(E_0) - C1(E_0)B(E_0 - \Delta E) + C2(E_0)B(E_0 + \Delta E) = 0
\]

\[
C1(E_0) + C2(E_0) = 1
\]

(6)
from which $C_1(E_0)$ and $C_2(E_0)$ can be deduced easily.

4.2. Practical application

We applied the adaptive filtering to the case of calcium in organic matrix. Fig. 7 displays the output of adaptive filtering applied to the raw spectra presented in Section 2.3 (Fig. 5a). Details of the signal processing are:

- Modeling of the background by a second order polynomial. The modeling was done for each energy loss with a surrounding fitting region of 15 eV.
- Computation of the second derivative filter coefficients $C_1$ and $C_2$ from Eqs. (6) for each value of the energy loss $E_0$. Since we want to detect calcium white lines, the energy shift for the second difference was 6 eV.
- Finally, application of four iterated first order smoothing passes.

From an inspection of Fig. 7, we can conclude that this approach allows complete removal of the background and leads to clear visibility of the signal which was not the case using a simple second difference filter or even Gaussian filtering (see Figs. 5b and c).

5. Conclusions

In this paper, we have introduced three new ideas concerning the digital filtering of electron energy loss spectra for the detection and quantification of trace elements. All ideas relate to the design of digital filters with the aim of finding a good compromise between the tasks of (a) removing the largest part of the background without removing small characteristic peaks and (b) not amplifying noise dramatically.

The first idea consists in working in the direction of higher order derivatives (typically the fourth order derivative) of Gaussians. High order derivatives help to improve the background subtraction while the Gaussian shape avoids high frequency noise amplification. One among several possible implementations of derivatives of Gaussians was described.

The second idea consists in moving from symmetric filters to asymmetric filters. For the homomorphic filtering, a preliminary double logarithmic transformation allows linearization of the background, at least when the power-law model holds. In this situation, simple filters allow suppression of the background almost completely. First or second derivative filters can be designed on the basis of an estimation of the local background shape. The third idea consists in combining modeling and filtering. First, a local modeling of the background is performed and then an asymmetric filter is designed in order to remove the background completely.

In all three cases, the filters remain linear, thus allowing quantification of the filtered spectra. Moreover, these procedures can be easily implemented in the field of chemical mapping, in the spectrum-image mode or even in the image-spectrum mode provided that a sufficient set of images is recorded.

References