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EXTRANEOUS BACKGROUND-CORRECTION PROGRAM FOR MATRIX BOUND
MULTIPLE POINT X-RAY MICROANALYSIS

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Abstract

A program is described that allows on-line determination of extraneous background in multiple point X-ray microanalytical matrices. The program is based upon the calculations of the extraneous background for the film (when present), the standard and the unknown by (100 sec.) point analysis. The program searches for a peak-free part of the spectrum in which the calculated value for the extraneous background is about equal to the value in this region of the spectrum ($=b_e$). On-line the contents of this b_e -region is subtracted from an unmanipulated continuum region in the vicinity of the element present in the unknown and standard (Pt).

During the subsequently performed matrix analysis two arrays are acquired (P-b) and (b- b_e). From these two arrays, the $R_{X,ST}$ and subsequently the $R_{X,SP}$ are calculated per pixel, which are converted to (b_e corrected) concentration arrays.

In addition Z^2/A -differences between standard and the analyzed specimen are corrected off-line. For each pixel the program judges whether the calculated concentration deviates from the value introduced for the standard. Once differences are registered, adequate corrections are made.

Introduction

Matrix-bound multiple point X-ray microanalysis allows the analysis of cross-sectioned heteromorphic cell organelles in which several elements may be inhomogeneously distributed [1,4,5,7,10]. In net-intensity arrays ($=$ peak P - background b), the topographical distribution of co-localized elements can be demonstrated [3,8]. The relative elemental concentrations can be expressed in R_X -arrays ($R_X = P-b/b$).

Relative elemental concentration arrays of cross-sectioned organelles, obtained by multiple point matrix analyses, can be compared to those from co-embedded cross-sectioned standards [2,3,11,12]. Recently, it was confirmed that such analyses were independent of the beam intensity and variations in section thickness [1,7]. This allowed us to compare the fate of iron and cerium in human liver biopsy material, before and after phlebotomy treatment of one patient [1]. The procedure used was criticized, because the acquired R_X -arrays for both the standard and unknown used continuum arrays, that were not corrected for extraneous background contributions. Moreover, the results were not corrected for differences in Z^2/A between standard and specimen, calculated from the relative elemental concentrations in the "unknown" [1,7].

In this paper a program is described that addresses these aspects. The equations previously proposed and described by Roomans for point analyses are used in this program [12, see also 8 and 9]. In the presently used program, the contents of the main and satellite regions are acquired by the DBL-method, as described before [8]. However, an additional region is now introduced around the peak of the grid material (copper in this case) that allows the extraneous background to be estimated.

Materials and Methods

The instrumental conditions and cytochemical procedures have been described previously [1,5,6,7]. Untreated ultrathin sections on carbon-coated 70 mesh copper grids were used. In addition, some sections, collected on 100 mesh copper grids without film, were analyzed, to exclude the contribution of the supporting film to the extraneous background.

Results

Point analysis

In Fig. 1, the position of the four regions,

Key words: Image analysis, X-ray microanalysis, cytochemistry, morphometry, Scanning transmission electron microscopy, signal integration, ultrathin sections, biological tissue, bio-standards, background corrections.

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needed for the calculation procedure to obtain net-intensity values for copper (K_{α}) and platinum (L_{α}) by the DBL-method is shown [6].

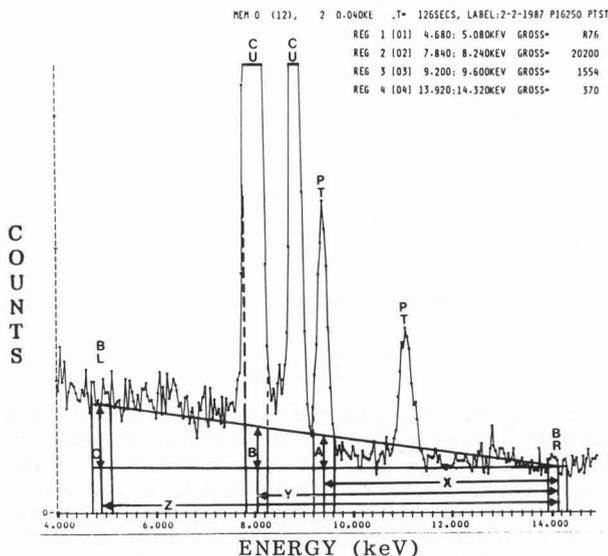


Fig.1 Spectrum of the cross-sectioned Pt-standard, containing the regions for the calculation.

The program then creates a new satellite region, of which the contents match the continuum value under the Pt (L_{α}) peak.

In Fig. 2 a flow chart of the program is given. The parameters are acquired in the following way:

Grid material is irradiated for 100 sec., and the (mean) r-factor (background/net-Cu) is established ($n = 5$) [12, equation 30]

Subsequently, (100 sec.) point analyses are acquired:

- a) over an empty square, when grids without a film are used, (this step is omitted when a film is present, see Fig.2), W_u , or
- a') over an empty part of the support film, W_{film} ,
- b) over the co-embedded standard, and
- c) over the section containing the "unknown".

From these values, the $W_{b,st}$ (=extraneous background from the standard) is calculated, using equation 1 (Roomans' formulae [12, equations 31 and 32] and symbols):

$$W_{b,st} = W_{film} + r (\text{net Me}_{st} - \text{net Me}_{film}) \quad (1)$$

$W_{b,sp}$ is calculated according to:

$$W_{b,sp} = (\text{net Me}_{sp} / \text{net Me}_{st}) \cdot W_{b,st} \quad (2)$$

in which net Me_{st} and Me_{sp} are the net counts from the grid metal peaks from the standard and specimen. When no film is present:

$$W_{b,st} = W_u + r (\text{net Me}_{st} - \text{net Me}_h) \quad (3)$$

and subsequently $W_{b,sp}$ is calculated according to equation 2.

Once $W_{b,sp}$ is calculated (either via the with or without film pathway), the program creates a new satellite region around 14.0 keV, of which the contents match the value of $W_{b,sp}$.

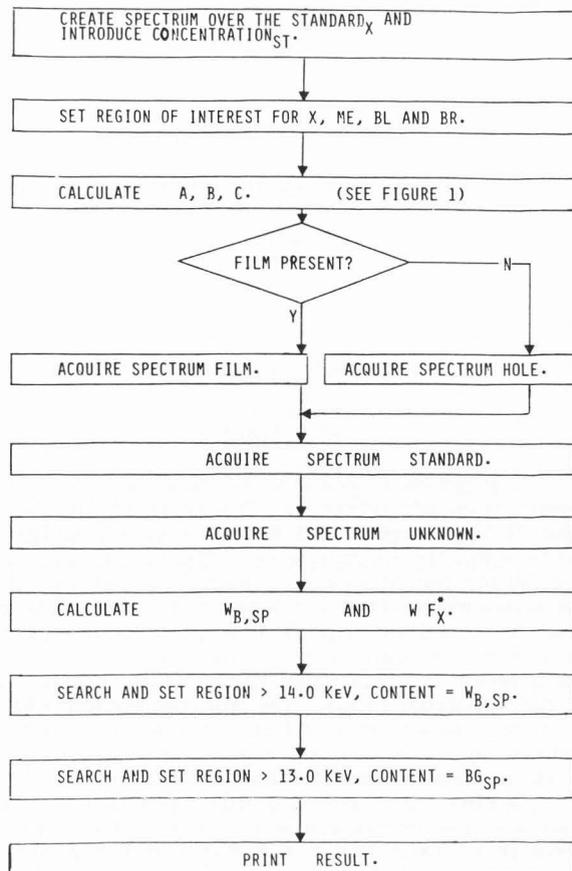


Fig. 2. Flow chart extraneous background.

The values acquired from the standard are converted into a factor F_X^* , according to:

$$F_X^* = C_{X,st} / R_{X,st} \quad (4)$$

in which $R_{X,st} = (P-b)_X / (W - W_{b,st})$ and $C_{X,st}$ the concentration of x in the standard. Finally $R_{X,sp}$ is calculated according to:

$$R_{X,sp} = (P-b)_X / (W - W_{b,sp}) \quad (5).$$

The concentration in the unknown is obtained according to:

$$C_{X,sp} = F_X^* \cdot R_{X,sp} \quad (6).$$

Measurements were made to test the functioning of the program. Simultaneous point analyses were performed over items in the specimen, in the standard, and the film (by the program) and manually over similar sections on a grid without a film present. In Table 1, the mean Pt-concentration values are given, calculated from regions indicated in Figure 1.

From these measurements, one can conclude that the results calculated manually were not significantly different from those calculated by the program (p greater than 0.05).

Matrix-bound multiple point analysis

In the set-up menu of the reduced scan mode [4,7], the satellite region that has a value equal to

Extraneous Background-Correction Program

the contents of the continuum under the elemental peak (measured by point analyses) is introduced (flow chart, Figure 2). This satellite (BG_{SP}) region is subtracted on-line from the main region installed over the peak of the element Pt [(P-b)_X]. The newly created satellite region representing the extraneous background (W_{b,sp}) is subtracted on-line from an unmanipulated continuum region (=BL in Figure 1).

These two arrays can be divided point by point or be divided by the mean value of the extraneous background-corrected continuum array, and multiplied, per point by the factor F_X^{*}, to give concentration arrays, as outlined before [1].

Z²/A - correction

The calculated C_{X,sp}-values per pixel (above the installed threshold value) are compared with the calculated concentration of element (Pt) in the standard. The program judges whether a Z²/A-correction should be applied or not.

When Z²/A-corrections have to be applied, the parameters of equation (7), (equivalent to equation 34 proposed by Roomans [12]) are calculated and introduced in the computer. For Z²/A_m the value 3.20 is used and Z²/A_{st} and Z²/A_x are calculated.

$$C_{X,sp} = \frac{C_{X,st} \cdot R_{X,sp} \cdot Z^2/A_m}{R_{X,st} \cdot \overline{Z^2/A_{st}}} \cdot \frac{(1 - C_{X,st}) \cdot R_{X,sp} \cdot (Z^2/A_x - Z^2/A_m)}{R_{X,st} \cdot \overline{Z^2/A_{st}}} \quad (7).$$

From these values a correction factor is calculated that converts the uncorrected C_{X,sp} into corrected value (per pixel above the threshold). A new concentration array can be printed, in which the values introduced below the threshold are set to zero and the zero values inside are set to one. In Figure 3, a flow chart shows the decisions taken in this sub-routine, per pixel.

Discussion

The program described is based upon the assumption:

$$(W/W_e)_{p.a.} = (W/W_e)_{p.p.} \quad (8)$$

in which W is the unmanipulated continuum value and W_e the extraneous-background contribution, obtained by point analysis (p.a.) or per pixel (p.p.).

A second assumption used is: because the standard and unknown are on the same grid, the contribution of the film to the extraneous background is assumed to be equal for both positions. The remaining influences: the concentration differences (st vs sp), the relative position of the two (st, sp) measuring sites with respect to the grid bars, are assumed to be reflected by the respective net-Me values. When no film is present the calculation is not essentially changed.

In this study we used a linear interpolation method to calculate the contents of various main and satellite regions.

Previously, it was shown that the values obtained by this method of background subtraction were

directly related to values obtained by digital filter method [9]. Values obtained by digital filtering can also be used to find a satellite region, that can be subtracted on-line, and that represents these two values best.

FLOW-CHART:

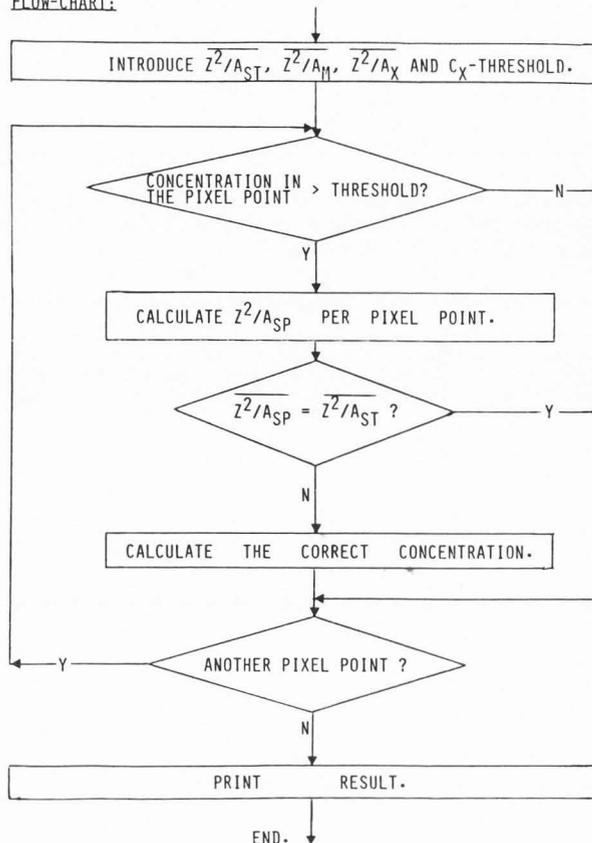


Fig. 3. Flow chart Z²/A correction.

Table 1: Platinum concentrations in eosinophil granules. The results from the program compared with those from the calculations by hand.

PT-CONCENTRATION IN EOSINOPHIL GRANULES.				
HAND METHOD (WITHOUT FILM)			PROGRAM METHOD (WITH FILM)	
		C _{PT} (%)		C _{PT} (%)
CELL 1	GRAN. 1	37.5	GRAN. 1	31.8
	GRAN. 2	31.1	GRAN. 2	30.3
	GRAN. 3	38.5	GRAN. 3	39.1
CELL 2	GRAN. 1	26.9	GRAN. 4	55.6
	GRAN. 2	25.7	GRAN. 5	23.1
	GRAN. 3	25.7	GRAN. 6	24.8
	GRAN. 4	27.0	GRAN. 7	27.7
	GRAN. 5	25.4	GRAN. 8	28.9
CELL 3	GRAN. 1	31.5	GRAN. 9	31.7
	GRAN. 2	30.2	GRAN. 10	38.3
	GRAN. 3	29.0	GRAN. 11	27.3
MEAN C _{PT} = 29.8 %			MEAN C _{PT} = 31.7 %	
S.DEV. = 4.5			S.DEV. = 9.2	

The third assumption is, that the changes in the value of Z^2/A of the specimen are reflected also in these two satellite regions.

The incorporation of all changes in the F_x -factor of our ratio program finally creates the possibility to choose either the (extraneous-corrected) background per pixel or the mean value of the whole matrix. Changes in the Z^2/A_{sp} with respect to Z^2/A_{st} are treated in the same way as an additional contribution to this multiplication factor per pixel above the threshold.

The Z^2/A -correction factor calculation is only applied to the situation in which the differences in Z^2/A are caused by the element of interest (Pt). When the differences in Z^2/A are caused by a different element, a standard for that element must also be present in the same section.

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Discussion with Reviewer

G.M. Roomans: Equation 2 has a limited validity,

namely when no support film is present. A more general equation and a recalculated Table I should be given.

Authors: We agree. The general equations are:

$$W_{b,sp} = W_{film} + r (\text{net Me}_{sp} - \text{net Me}_{film}) \text{ and}$$

$$W_{b,st} = W_{film*} + r (\text{net Me}_{st} - \text{net Me}_{film*}) \quad (9).$$

Both equations have to be applied when the standard is present in a different section on a separate grid. However, in our case both standard and unknown are present in the same section on the same grid, and only occasionally in the same grid square. It was noticed that as a consequence: $W_{film} = W_{film*}$ and also: $r (\text{net Me}_{film}) = r (\text{net Me}_{film*})$.

Two additional factors can influence the extraneous background value: (a) differences in elemental concentrations between the two measuring positions (st and sp); and (b) differences in the position of the two measuring points with respect to the surrounding grid bars.

It is assumed that the (net Me) differences between the two points are the expression of both factors. When the two positions and elemental concentrations are about the same for the two measuring points ($\text{net Me}_{sp} / \text{net Me}_{st}$) is about unity. Hence, $W_{b,sp} = W_{b,st}$, other positions or elemental concentrations can give correction factors $\neq 1$. To judge whether these different approaches would lead to significant differences in the final results, the information collected in Table 1 (with film) was recalculated by hand. The results are given below. Student's t-tests showed that the results were not significantly different. So we maintained the original approach and equation 2.

Nr.	$W_{b,sp}^*$	$W_{b,sp}^{**}$	$W_{b,sp}^{***}$	$W_{b,st}$
1	1582	1656	1741	1287
2	1535	1584	1600	1419
3	1254	1305	1305	1096
4	940	1077	1035	825
5	386	484	424	815
6	756	974	999	928
7	815	827	846	754
8	737	745	755	705
9	1237	1282	1298	1147
10	1403	1417	1420	1035
11	850	871	853	980
12	1234	1250	1250	1054

X	1061	1121	1127	-
S.D.	367.4	354.1	378.3	-
t-test:				

$$W_{b,sp}^* \text{ vs } W_{b,sp}^{**} : p > 0.05$$

$$W_{b,sp}^* \text{ vs } W_{b,sp}^{***} : p > 0.05$$

$$W_{b,sp}^{**} \text{ vs } W_{b,sp}^{***} : p > 0.05$$

$W_{b,sp}^*$: represents results obtained by the computer program;

$W_{b,sp}^{**}$: the calculation according to equation 1 for $W_{b,st}$ and $W_{b,sp}$ respectively; and

$W_{b,sp}^{***}$: calculation according to equation 2.