

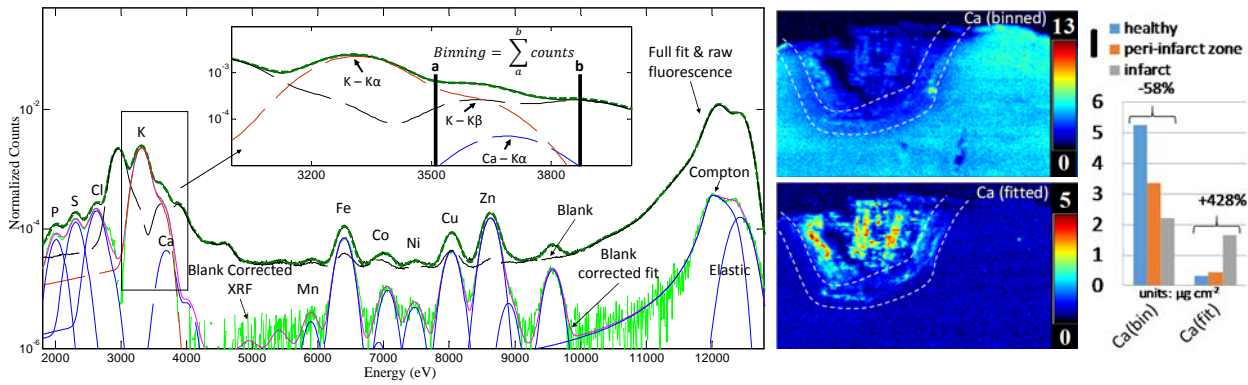
## Cross and Internal Comparisons: Integrative and Parametric Approaches to X-ray Fluorescence Data Analysis and Processing

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Synchrotron X-ray fluorescence (XRF) imaging (XFI) has matured to the point where it is used in a wide range of research fields to quantitate the levels of different elements in the image on a pixel-by-pixel basis. Two approaches to X-ray fluorescence image analysis are commonly used, namely, 1) integrative or window binning which simply sums the numbers of all photons detected within a specific energy region of interest and 2) parametric analysis or fitting in which emission spectra are represented by the sum of parameters representing a series of peaks and other contributing factors. As an introductory review and reminder, we present a quantitative comparison between these two methods of image analysis using XFI of mouse brain tissue sections to show that substantial errors can result when data from overlapping emission lines are binned rather than fitted. These differences are explored using two different digital signal processing data acquisition systems with different count-rate and emission line resolution characteristics. We show that, irrespective of the digital signal processing electronics, there are substantial differences in quantitation between the two approaches. Thus, binning analyses are thus shown to contain significant errors that not only distort the data but in some cases result in complete reversal of trends between different tissue regions. As such, the X-ray fluorescence data from X-ray microprobe or nanoprobe measurements must be fitted to obtain reliable elemental maps.



However, not all fitting schemes are equal. The most common approach to fitting is to initially remove a per-pixel baseline. Alternatively to baseline subtraction, one can typically take advantage of the fact that most XRF images (e.g., cells deposited on  $\text{Si}_3\text{N}_4$  wafers) inevitably contain some sample-free regions; and these regions can thus be used to define a blank. Such a blank can thus be used to correct the x-ray fluorescence data instead of a baseline. If there are no “blank pixels” in an image, one may use a blank calculated from non-sample pixels in XFI measurements from a related image if said template material is the same. By comparison, we demonstrate that per pixel baselines can result in significant, systematic errors in quantitation and that significantly improved data can be obtained by calculating an average blank spectrum and subtracting this from each pixel.

