Positive Probability Ltd

Note A1: Autoradiography – Quantifying Noisy Data

Introduction

Quantified errors are dependent on the quality of the data and the quality of the model. Errors are smallest when all the peaks have the same width and shape, the model fits the peak profiles within the noise and the S/N is high. These conditions are rare because there is usually some variation in peak width and shape and any designed model is therefore a compromise. Very often the S/N is poor and this increases the errors because there is less certainty about the peak positions and intensities. Errors also increase with increasing peak overlap, again reflecting the increased uncertainty of positions and intensities for overlapped peaks.

In this example, we compare the results from the PPL ***ReSpect™*** data reconstruction methodology with those obtained from traditional peak integration methods.

Data

The data are the autoradiograph of a radioactive metabolite extracted from dog blood. In this trial, a dog was dosed with a radioactively labelled drug and blood taken at hourly intervals (1 to 8 hours) following dosing. Following extraction, a plate was spotted and the film exposed for 16 hours. The film was developed and scanned to produce the data in Figure 1. Each peak represents a spot with time increasing from left to right. These data were selected because they are inexplicably noisy and ideally suited to showing the benefit of data reconstruction methods.

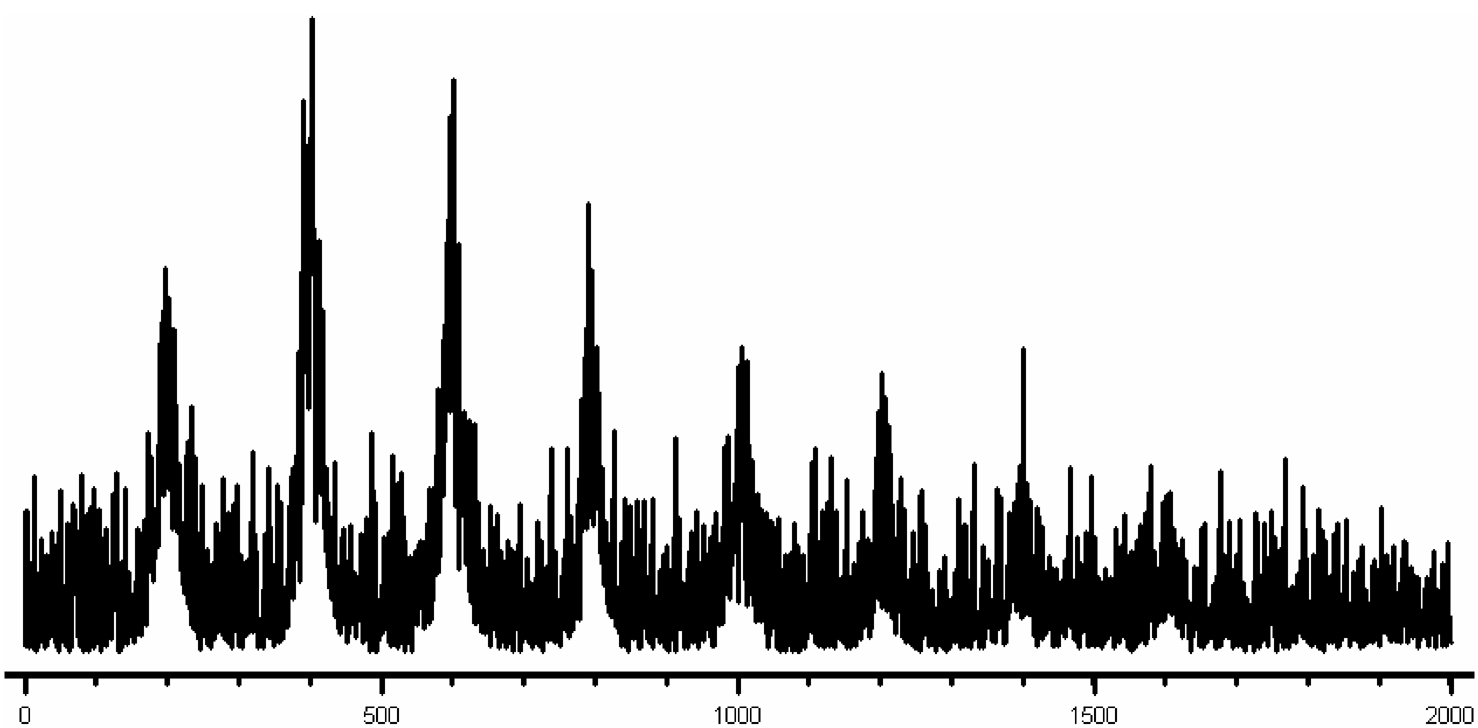

Figure 1. Raw data.

Traditional Methodology and Results

The aim is to obtain the intensity of each sample (spot). For noisy data, traditional methods involve first filtering the data and then either:

a) Removing an arbitrary offset and then measuring the peak areas between two points considered to represent the baseline. This is often called the perpendicular method.

b) Applying a linear, sloping offset between minima in the wings of each peak and then measuring the area between the minima. This is often called tangential skimming.

The perpendicular method will generally produce low peak intensities because wing intensity masked by noise is ignored. Tangential skimming will generally give high intensities because the selected minima represent low points in the noise and not the noise centre. A common "trick" is to use both methods and average the results. Even so, both methods are arbitrary, as is any offset whether linear or sloping and it is not possible to assess the error on the measurements.

In this experiment, it is expected that the drug will be eliminated exponentially after an initial rise. Figure 2 below shows the found peak areas using the perpendicular method (red), tangential skimming (blue) and their average (black). The expected low and high bias for the methods is clear and neither shows a clear logarithmic decay after the maximum.

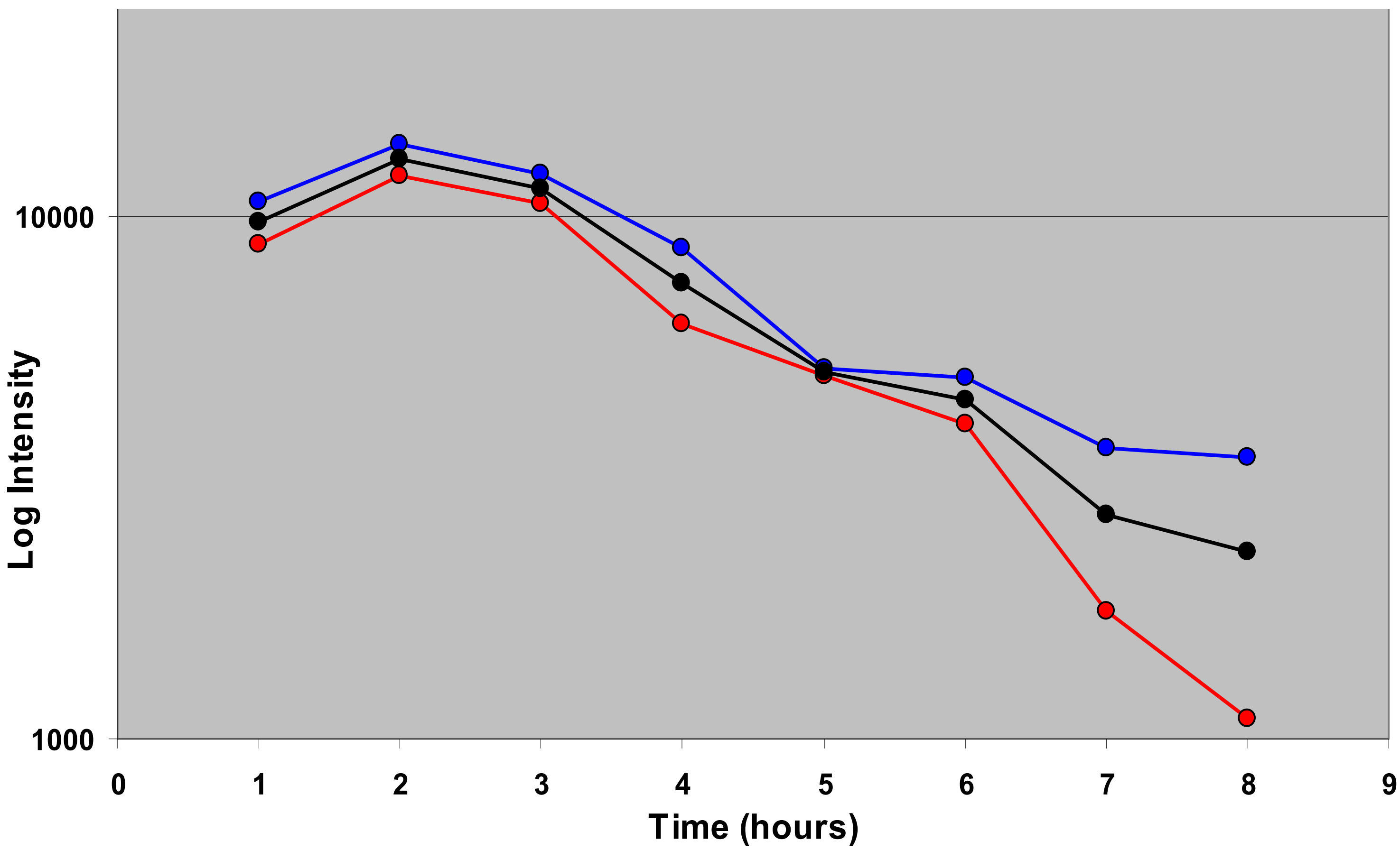

Figure 2. Drug elimination with time. Perpendicular method (red); Tangential skimming (blue); Average of both methods (black).

Data Reconstruction Methodology and Results

The data were first baseline corrected using the ***Nadir™*** program. The peaks all have a similar width and shape and the four most intense peaks were modelled. The baseline corrected data were deconvolved using the ***ReSpect™*** data reconstruction methodology and the result displayed as a spike plot at 99% confidence. The result is shown in Figure 3 below, along with spot positions and intensities.

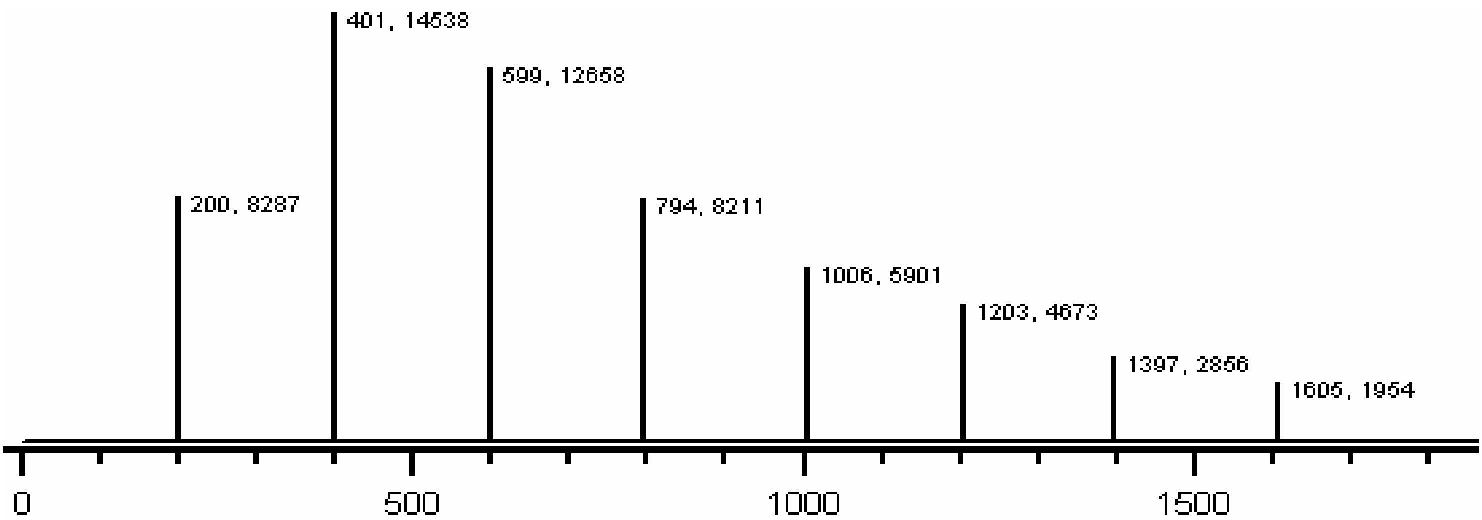

Figure 3. Data reconstruction spike plot.

The corresponding plot of log intensity against time is shown in Figure 4 below.

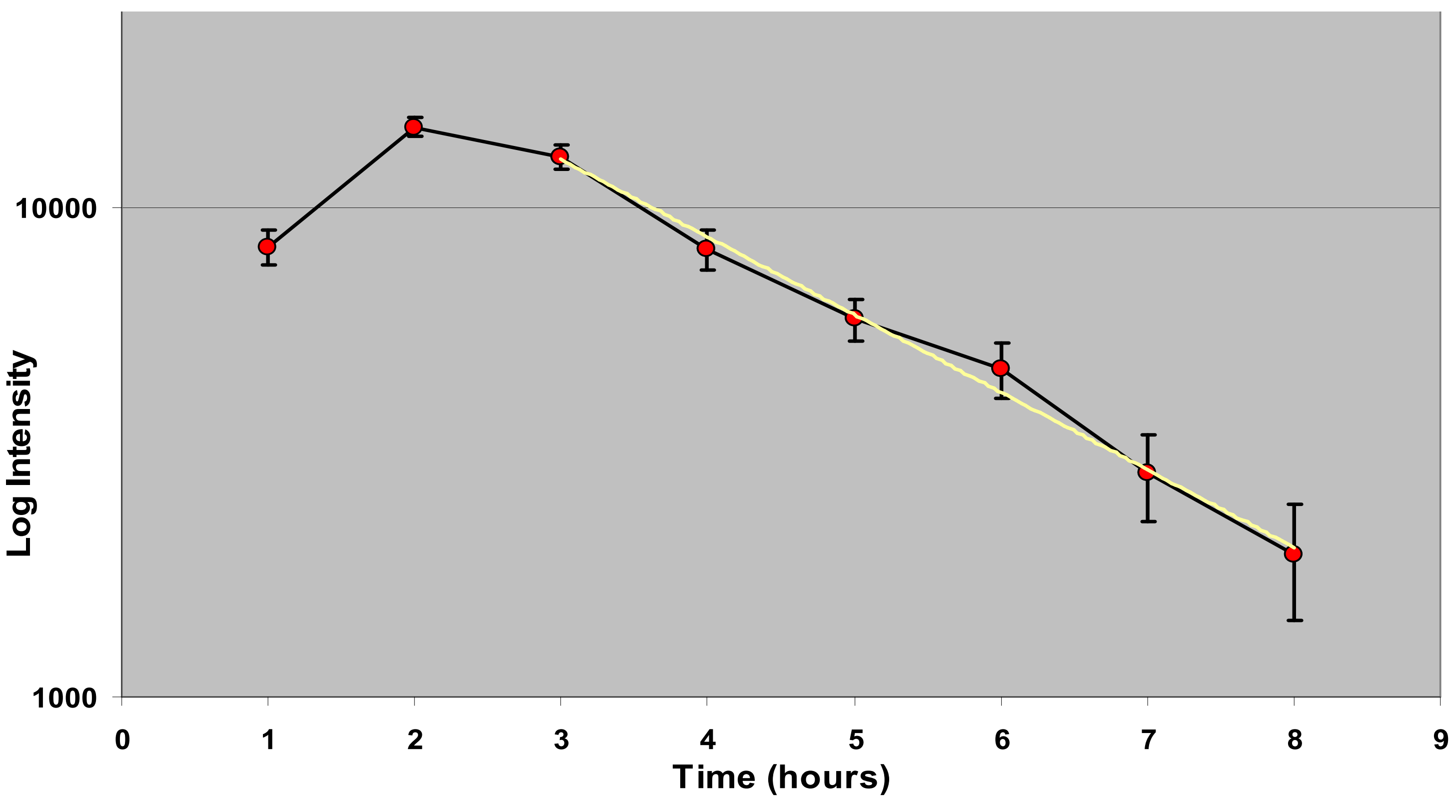

Figure 4. Drug elimination with time. ***ReSpect™*** *data reconstruction methodology.*

In Figure 4 the displayed errors are for 1 standard deviation and the pale yellow line is the trend line for the points following the maximum. The expected logarithmic elimination is clear and all points are within the expected error bars. The full results are shown in the table below.

Time (hr)	Perp.	Tan skim	ReSpect	1 sd error
1	8832	10695	8287	688
2	11935	13726	14538	679
3	10565	12087	12658	716
4	6221	8676	8211	782
5	4965	5099	5901	590
6	3995	4931	4673	604
7	1753	3608	2856	581
8	1098	3469	1954	519

Conclusions

This example has served to illustrate how reliable results may be obtained from noisy data using the data reconstruction methodology compared with traditional methods.