Positive Probability Ltd

Note P3: S/N Enhancement – Fluorescence DNA Sequencing

Introduction

This example illustrates the value of the data reconstruction methodology compared with a conventional filter. It should be remembered that, unlike filter methods, **ReSpect™** is capable of providing substantial gains in S/N without broadening peaks.

Methodology

Although there may be some variation in peak width, refining a model is generally unimportant for generating a reconstruction. Depending on the model parameters, the fast reconstruction program, **Enhance™**, automatically modifies any input model to ensure that the data can be fitted adequately within the noise level.

Data & Data Processing

In this example, the data are from the fluorescence detection of the T bases for part of a DNA strand. The data are particularly noisy and are therefore useful for comparing the quality of the results obtained from traditional smoothing with those obtained from the fast **ReSpect™**-based data reconstruction method - **Enhance™**.

The isolated peak near the centre of the data was assumed to be representative of all the peaks in the data and was used to obtain an estimate of the peak width for baseline correction using the **Nadir™** program (Baseline3). The Model was also obtained directly from this peak using the **Profile™** program. **Enhance™** was then run which converges very rapidly.

Results

The figure below compares the smoothed result and the reconstruction with the data.

The reconstruction is shown at the bottom of the figure and it clearly provides a dramatic gain in S/N compared with that in the data. The "calling" of the individual bases is unambiguous. The clusters of peaks are regions where the T base repeats and, within expected small experimental variations, the spacing between the peaks is identical. "Cross-talk" between the fluorescence channels is also apparent and weak peaks are seen for some of the A, C and G bases at the same separation or multiples of it. Negative-going cross-talk signals are correctly treated as noise and so they are no longer present.

The result of triangular smoothing is shown below the data. Although this simple method provides a substantial gain in S/N, the improvement is not so dramatic as that observed in the reconstruction. Peak broadening is also very marked and further smoothing would have removed detail and potentially introduced ambiguities. Furthermore, because filters can only provide damaged results, the cross-talk between the fluorescence channels is less obvious.
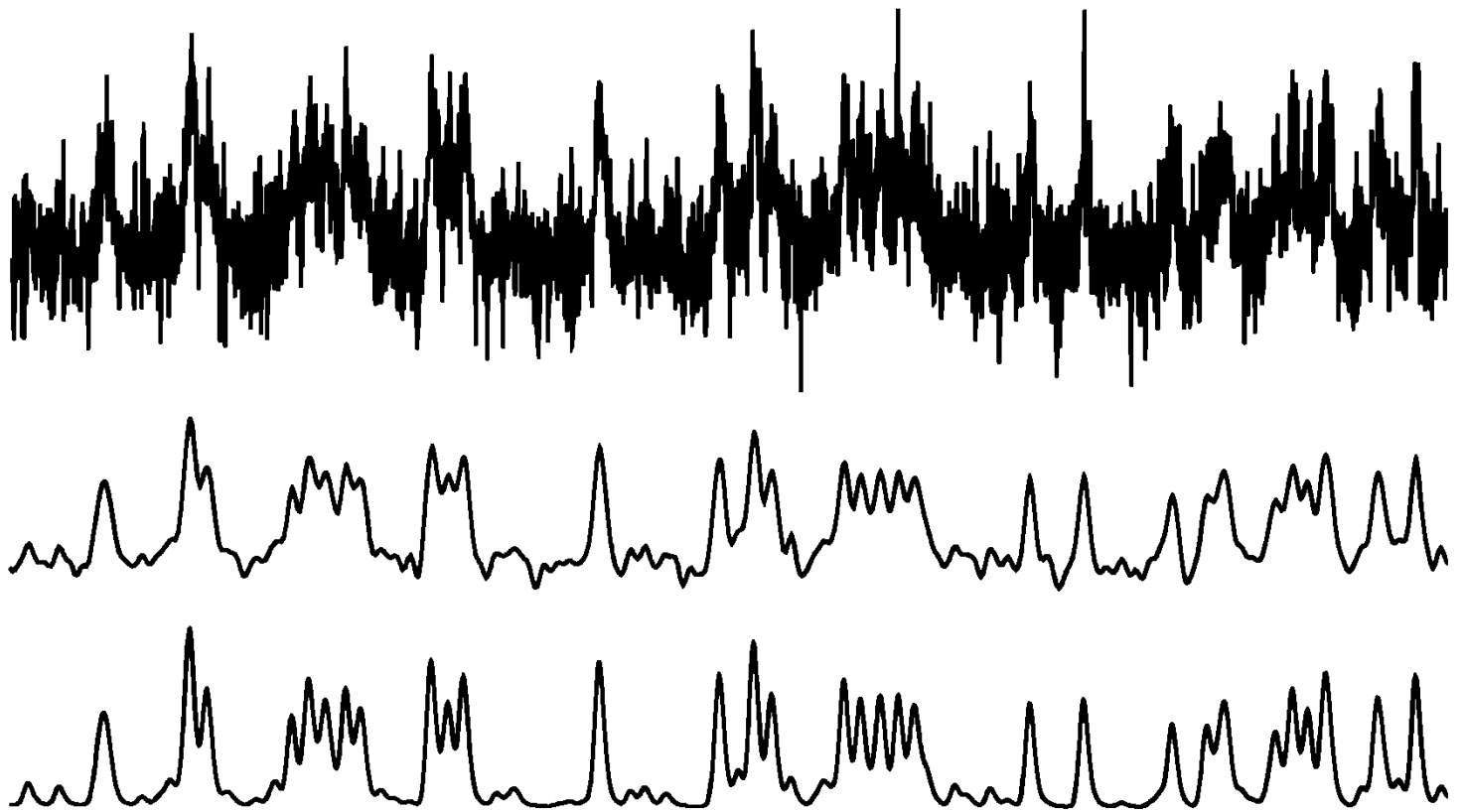
Top: Data; Centre: Triangular smoothing; Bottom: Enhance™ reconstruction.

Conclusions

A reconstruction provides the superior gains in S/N compared with traditional triangular smoothing. In addition, peaks are not broadened and detail is retained in the result.