Journal of Chromatography A, 1216 (2009) 2548–2553

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Suppressing the charged coupled device noise in univariate thin-layer videoscans: A comparison of several algorithms

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ARTICLE INFO

Article history:
Received 24 November 2008
Received in revised form 12 January 2009
Accepted 14 January 2009
Available online 20 January 2009

Keywords:
Thin-layer chromatography
Videodensitometry
Videoscanning
Denoising
Fourier transform
Digital filters
Wavelet shrinkage

ABSTRACT

The digital processing of chromatographic thin-layer plate images has increasing popularity among last years. When using a camera instead of flatbed scanner, the charged coupled device (CCD) noise is a well-known problem—especially when scanning dark plates with weakly fluorescing spots. Various techniques are proposed to denoise (smooth) univariate signals in chemometric processing, but the choice could be difficult. In the current paper the classical filters (Savitzky–Golay, adaptive degree polynomial filter, Fourier denoising, Butterworth and Chebyshev infinite impulse response filters) were compared with the wavelet shrinkage (31 mother wavelets, 3 thresholding techniques and 8 decomposition levels). The signal obtained from 256 averaged videoscans was treated as the reference signal (with noise naturally suppressed, which was found to be almost white one). The best choice for denoising was the Haar mother wavelet with soft denoising and any decomposition level larger than 1. Satisfying similarity to reference signal was also observed in the case of Butterworth filter, Savitzky–Golay smoothing, ADPF filter, Fourier denoising and soft-thresholded wavelet shrinkage with any mother wavelet and middle to high decomposition level. The Chebyshev filters, Whittaker smoother and wavelet shrinkage with hard thresholding were found to be less efficient. The results obtained can be used as general recommendations for univariate denoising of such signals.

1. Introduction

Thin-layer chromatography is now a well established technique and one of its major applications is quantitative analysis. The densitometric scanning offers the possibility to evaluate the peak areas or heights in analogous way to high performance liquid chromatography [1,2]. It is generally preferred to the classical videoscanning due to higher sensitivity, low stability of the spot evaluating algorithms and possibility of recording of full spectra. Moreover, the inhomogeneous illumination of the plate can be a main source of quantitative error [3,4].

Despite of these drawbacks, a new trend appears in the literature. The videoscanning of TLC plates by a flatbed scanner or camera can be connected with image processing techniques to extract univariate, densitogram-like signal from the images. Soponar et al. [5] used this technique successfully for quantitative determination of some food dyes. It was done using the flatbed scanner, because the analyzed solutes are dyes, easily visible in the daylight. However, most samples needs to be visualized under ultraviolet light and it is then done by grabbing a frame from charged coupled device (CCD) camera [4]. A significant problem here is the CCD noise, especially when the plate is not fluorescent and when chromatographed ingredients show only a weak fluorescence. There is only one way to suppress it—grabbing many frames from camera and averaging them. Such technique cannot be used in routine practice, because there is a need to grab many frames—for example 256 frames suppresses the noise 16 times ($\sqrt{n}$). When there is one frame grabbed during a second, this prolonges the analysis from seconds to minutes. Moreover, in some applications the quick scanning is a critical issue, because after several minutes the spots may be quenched or invisible.

Despite of the quantitation purposes, the correct denoising of the chromatograms is a critical part of the fingerprinting, because the noise significantly affects the results [6]. When advanced chemometric processing is applied, the noise can make serious problems during further processing, for example warping [7–9] and filtering from baseline drifts [10]. Even when these techniques are applied successfully, a low signal-to-noise ratio of the resulting signals can affect significantly the final chemometric comparison and projection, making the real similarity of the samples disturbed. Therefore the proper denoising of signals obtained from videoscans is very important.

As there is no literature regarding comparing different techniques of univariate smoothing and denoising of such signals, the aim of the paper is to investigate and compare performance of common approaches for the removal of the noise from videoscanned

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doi:10.1016/j.chroma.2009.01.042

CCD signals. Several similar approaches for other techniques are already present. Felinger and Káré discussed the denoising of HPLC baseline with wavelets [11]. Daszykowski and Walczak [10] also discussed the denoising techniques of HPLC chromatograms. Liu et al. [12] tested several wavelet approaches to microchip capillary electropherogram denoising, whereas denoising of capillary electropherograms made with electrochemiluminescence detection was investigated by Cao et al. [13]. Shao et al. [14] cite in their review some older papers regarding wavelet denoising in HPLC. Finally Člupek et al. [15] performed analogous study in spectrometric area, comparing finite response filters and Savitzky–Golay smoothing in removal of noise in Raman spectra. The conclusions of above approaches cannot be directly used in the CCD noise case, because each technique is affected by noise of different properties.

2. Theory

There are three main groups of denoising approaches. The simplest works in the signal domain—moving average, Savitzky–Golay filter or regression with penalties. The second one is related to the digital filter theory and works in frequency domain—for example denoising by Fourier transform or different kind of digital filters. The last and newest promising one is the wavelet shrinkage, eliminating the noise in the wavelet domain.

Each of the denoising algorithms requires some parameters to be optimized. The optimization is a critical issue, because aggressive denoising produces a distorted signal, whereas too slight one removes only the small part of noise.

2.1. The Savitzky–Golay filter

Savitzky and Golay proposed their famous filter (often abbreviated as SG) for smoothing and differentiation of the spectral signals in 1960s [16]. As it can be applied to any kind of the signal, it became very popular denoising method. The main advantages of this filter are: the simplicity of the computation (even on slow machines), the ability to processing the signals in real-time (with a small delay) and no shifts of the peaks. If the differentiation is needed, it can be performed at a single computational step.

The filter fits a polynomial to a windowed part of the signal and computes its fitted value. Therefore two parameters need to be optimized: a width of the filter (a length of the window) and degree of the fitted polynomial. An extension called adaptive degree polynomial filter (ADPF) [17] checks the optimal polynomial degree automatically, so the window length is the only one parameter to be set.

When a sample of pure noise cannot be collected, there is no general rule to optimize SG filter parameters. If such sample can be collected, the filter optimization may be done against its autocorrelation. The lag-one autocorrelation is computed for the noise extracted from the signal (difference between original and smoothed version) and the case when these autocorrelations are most similar is treated as optimal. This method, proposed by Vivo-Truylos and Schoenmakers [18] was invented for SG smoothing, but it can be also used with any denoising algorithm.

2.2. The Whittaker smoother

The Whittaker smoother, based on the least squares with penalties, has an interesting history. The first original proposal was done by Whittaker in 1923 [21]. Next this approach was forgotten for the years and bring into the world of chemometrics by Eilers in 2003 [22]. Transforming the signal $\mathbf{x}$ into the smoothed signal $\mathbf{y}$ requires solving the equation system:

$$(\mathbf{I} + \lambda \mathbf{D}^{\mathbf{T}}\mathbf{D})\mathbf{y} = \mathbf{x} \tag{1}$$

where $\mathbf{I}$ is the identity matrix, $\mathbf{D}$ is the "difference matrix", (such that $\mathbf{Dx} = \Delta\mathbf{x}$) and λ is smoothing parameter. Such operation can be understood as some kind of the least squares approach with added penalty for differences between subsequent signal samples. Increasing the λ parameter increases smoothing. Although there is a method for finding optimal λ parameter based on cross-validation, the autocorrelation approach can be also used.

2.3. The Fourier denoising

The Fourier denoising is based on the Fast Fourier Transform [19], converting the data from the signal domain to the frequency domain. The signal sequence $x_1 \ldots x_N$ is transformed to the sequence of $X_1 \ldots X_N$ complex numbers with the following formula:

$$X_k = \sum_{n=0}^{N-1} x_n e^{-(2\pi i/N)kn} \quad k = 0, \ldots, N-1 \tag{2}$$

Resulting coefficients are related to the components of increased frequency (real part to sinusoids, imaginary to cosinusoids). After considering some cutoff frequency and zeroing all the components above, the inverse transform is applied and the real part of the resulting x'_n sequence is the desired denoised signal:

$$x'_n = \frac{1}{N}\sum_{k=0}^{N-1} X_k e^{(2\pi i/N)kn} \quad n = 0, \ldots, N-1. \tag{3}$$

As the cutoff frequency is the only one parameter to be optimized, Lam and Isenhour [20] gave a comprehensive review on its choice. The approaches proposed till now are: taking the frequency at which the signal is below 0.1% of maximum (Rogers method), taking the frequency at which the signal is 5 times greater than last few points of the spectrum (Westeberg method) or taking the frequency at which standard deviation of the spectrum significantly increases (Bush method). Their own method, based on the equivalent width criterion of the peaks presented in signal is very smart, but it requires the manual analysis of the data. In the current paper, the optimization is done also against the noise autocorrelation.

2.4. Digital filters

The digital filters are applied to many tasks in digital signal processing (DSP) and the reader could find the review books regarding them in almost any language. They are based on general ARMA model (auto-regressive moving average) [19], given by following formula:

$$y[n] = \sum_{i=0}^{P} b_i x[n-i] - \sum_{j=1}^{Q} a_j y[n-j] \tag{4}$$

where P is the feedforward filter order (number of coefficients), b_i are the feedforward filter coefficients, Q is the feedback filter order, a_i are the feedback filter coefficients, $x[n]$ is the n-th sample of the input signal, and $y[n]$ is the n-th sample of the output signal.

There are two main kinds of such digital filters. When there is no feedback in the filter (all a_j coefficients are zero), the filter is called Finite Impulse Response (FIR) filter, in the other case it is Infinite Impulse Response (IIR) filter. The IIR filters are generally more efficient, but their design is more difficult due to the risk of instability. Therefore in most cases only predefined ones (Butterworth, Chebyshev, Elliptic, Bessel) are used. The Butterworth filter is designed to have a frequency response as flat as mathematically possible in the passband. The Chebyshev filter minimizes the error between the idealized filter characteristic and the actual over the range of the filter (very high slope at the cutoff), but it has ripples (inequalities) in the passband or stopband. The important disadvantage of these