A Pragmatic Introduction to Signal Processing

with applications in scientific measurement

An illustrated essay with software available for free download

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Introduction

The interfacing of measurement instrumentation to computers for the purpose of online data acquisition has now become standard practice in the modern laboratory for the purposes of performing signal processing and data analysis and storage, using a large number of digital computer-based numerical methods that are used to transform signals into more useful forms, detect and measure peaks, reduce noise, improve the resolution of overlapping peaks, compensate for instrumental artifacts, test hypotheses, optimize measurement strategies, diagnose measurement difficulties, and decompose complex signals into their component parts. Many of these techniques are based on laborious mathematical procedures that were not even practical before the advent of computerized instrumentation. But in recent decades, computer storage and digital processing have become literally *millions* of times cheaper and more capable, reducing the cost of raw data and making complex computer-based signal processing techniques more practical and necessary. It is important to appreciate the abilities, *as well as the limitations,* of these techniques. As Erik Brynjolfsson and Andrew McAfee wrote in *The Second Machine Age* (W. W. Norton, 2014): "...many types of raw data are getting dramatically cheaper, and as data get cheaper, the bottleneck increasingly is the ability to interpret and use data".

In the science curriculum, signal processing may be covered as part of a course on measurement instrumentation[1, 2], electronics[3], laboratory interfacing[4], or statistical and mathematical methods[5]. The purpose of this essay is to give a *practical* introduction to some of the most widely used signal processing techniques and to give illustrations of their applications in scientific applications. Some of the examples come from my own field of research (analytical chemistry), but these techniques have been used in a wide range of application areas and my software has been cited in over 145 papers, theses, and patents, covering fields from industrial, environmental, medical, engineering, earth science, space, military, financial, agriculture, and even music and linguistics. Data sent by readers from their own work has helped shape my writing and software. Much effort has gone into making this document concise and understandable; it has been highly praised by many readers.

This essay covers only basic topics and is limited to mathematics through elementary calculus and simple matrix math. (If math is not your strong point, know that this essay contains more than twice as many figures as equations). It's true that math is *essential*, just as it is for cell phones, GPS, digital photography, and computer games, but you can get started using these tools without understanding all the underlying math and programming details. *Seeing* it work makes it more likely that you'll want to *understand how* it works.

At the present time, this work does not yet cover 2D and image processing, wavelet transforms, pattern recognition, or factor analysis. For these topics or for a more rigorous treatment of the underlying mathematics, refer to the literature on signal processing, statistics, and chemometrics (such as the ones listed in the references, pages 125-126).

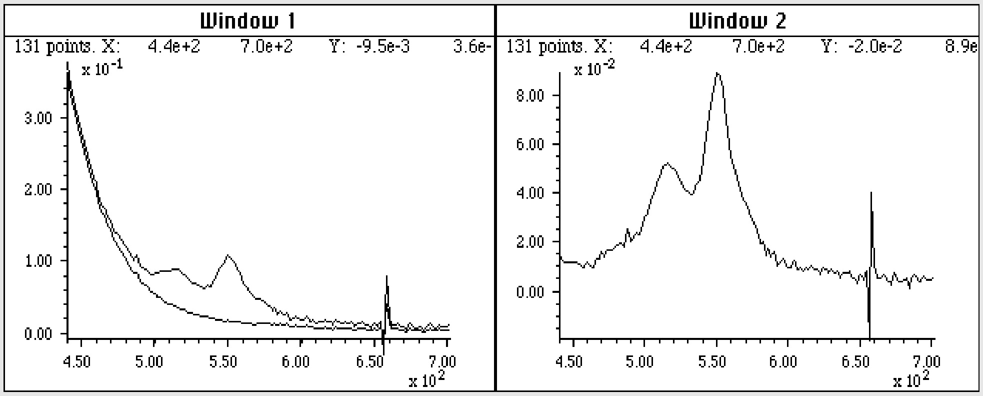
This tutorial makes extensive use of ***Matlab***, a high-performance commercial numerical computing environment and programming language that is widely used by scientists, researchers, and engineers, and ***Octave***, a free Matlab alternative that runs all of the Matlab scripts and command-line functions in this document without change (see page 73). There are Windows, Mac, and Unix versions of Octave; the Windows version can be downloaded from Octave Forge. Installation of Octave is somewhat more laborious than installing a commercial package like Matlab; be sure to install all the Octave Forge "packages" that add essential functions. Octave is also slower than Matlab - about half as fast for many computations and about 5 times slower for 2D graphics; see TimeTrial.txt for a speed comparison. Most of the techniques are also available for **spreadsheets** such as *Excel* and *OpenOffice Calc* . Some are illustrated by an old freeware Macintosh signal-processing program called **SPECTRUM** (see page 70).

Paragraphs in gray at the end of each section in this essay describe the related capabilities of each of these programs, including my own signal-processing modules written for Matlab, Octave, Excel, or Calc that you can download for your own use. For descriptions and download links to the latest versions of my downloadable spreadsheets and Matlab/Octave scripts and functions, see http://tinyurl.com/cey8rwh**.** Pages 70 to 111 of this document contain instructions for the operation of these software modules and many examples of their applications. Descriptions of my downloadable *interactive* signal processing tools (for Matlab only) are described on SignalProcessingTools.html. None of my Matlab scripts and functions require Matlab's *Signal Processing Toolbox*. My software has received extraordinarily positive feedback from users,

This document and its associated software are undated quite regularly. If you are reading this on paper or off-line, there is almost certainly a newer version available already. For the latest online version, in printed and online formats, go to http://terpconnect.umd.edu/~toh/spectrum/.

Signal arithmetic

The most basic signal processing functions are those that involve simple signal arithmetic: point-by-point addition, subtraction, multiplication, or division of two signals or of one signal and a constant. Despite their mathematical simplicity, these functions can be very useful. For example, in the left part of the figure below, the top curve is the optical spectrum of an extract of a sample of oil shale, a kind of rock that is a source of petroleum.



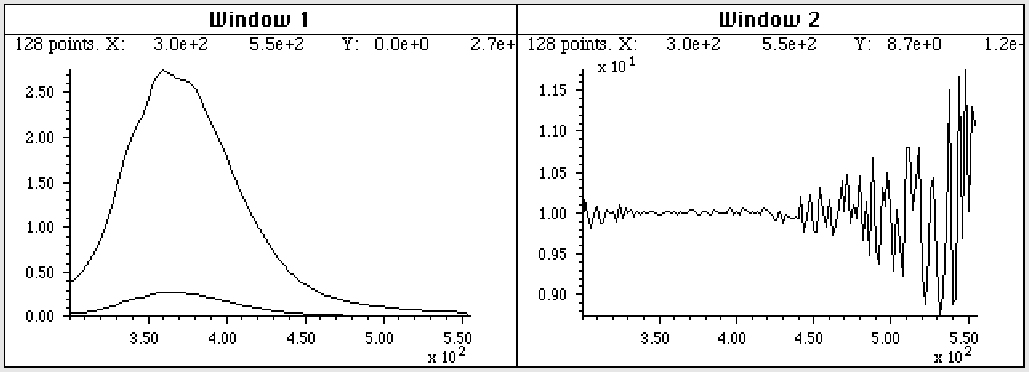
A simple point-by-point subtraction of two signals allows the background (bottom curve on the left) to be subtracted from a complex sample (top curve on the left), resulting in a clearer picture of what is really in the sample (right).

This optical spectrum exhibits two bands, at about 515 nm and 550 nm, that are due to a class of molecular fossils of chlorophyll called *porphyrins.* (Porphyrins are used as geomarkers in oil exploration). These bands are superimposed on a background signal caused by the extracting solvents and by other compounds extracted from the shale. The bottom curve is the spectrum of an extract of a shale that does *not* contain porphyrins, showing only the background signal. Here, the *independent* variable (sometimes referred to as “x”) is wavelength and the *dependent* variable (“y”) might be light intensity or absorbance, depending on the type of spectroscopy.

To obtain the spectrum of the shale extract without the background, the background (bottom curve) is simply subtracted from the sample spectrum (top curve). The difference is shown in the right in Window 2 (note the change in Y-axis scale). In this case the removal of the background is not perfect, because the background spectrum is measured on a separate shale sample. However, it works well enough that the two bands are now seen more clearly and it is easier to measure precisely the intensity of the peaks and their wavelengths.

In this example and in the one below, I am making the assumption that the two signals in Window 1 have the same x-axis values, in other words, that both spectra are digitized at the same set of wavelengths. Otherwise this subtraction operation would not be valid; the x-axis values must match up point for point. In practice, this is very often the case with data sets acquired within one experiment on one instrument, but the experimenter must take care if the instruments settings are changed or if data from two experiments or two instrument are combined. (Note: you can use the mathematical technique of *interpolation* to change the number of points or the x-axis intervals of signals; the results are only approximate but often close enough in practice. My multipurpose Matlab program *iSignal*, page 85, includes an interpolation function, activated by the **I** key.

Sometimes you might like to know whether two signals have the same shape, for example in comparing the optical spectrum of an unknown to a stored reference spectrum. Most likely the concentrations of the unknown and reference, and thus the amplitudes of the spectra, will be different, and so a direct overlay or subtraction of the two spectra will not be useful. One simple possibility is to compute the point-by-point *ratio* of the two signals; if they have the same shape, the ratio will be a constant. For example, examine the figure at the top of the next page:



Do the two spectra on the left have the same shape? They certainly do not look the same, but that may simply be due to the fact that one is much weaker than the other. The ratio of the two spectra, shown in the right part (Window 2), is relatively constant from 300 to 440 nm, with a value of 10 +/- 0.2. This means that the shape of these two signals is very similar over this wavelength range.

The left part (Window 1) shows two superimposed spectra, one of which is much weaker than the other. But do they have the same shape? The *ratio* of the two spectra, shown in the right part (Window 2), is relatively constant from 300 to 440 nm, with a value of 10 +/- 0.2. This means that the shape of these two signals is the same, within about +/-2 %, over this wavelength range, and that the top curve is about 10 times more intense than the bottom one. Above 440 nm the ratio is not even approximately constant, because of *random noise,* which is the topic of the next section (page 6). A similar calculation is done in absorption spectroscopy, where the "absorbance" *A* is defined as the base-10 logarithm of the ratio of the incident intensity, I_o, to the transmitted intensity, I, which compensates for the wavelength variation of the light source intensity and of the detector sensitivity.

Computational methods. Simple signal arithmetic operations such as these are easily done in any spreadsheet (e.g. *Excel* or the freely downloadable *OpenOffice Calc*), any general-purpose programming language, in a dedicated signal-processing program such as SPECTRUM (Page 70), or (most easily) in a vector-matrix programming language such as *Matlab* or *Octave* (Page 73).

Popular spreadsheet programs. *Excel* and *Open Office Calc* have built-in functions for all common math operations, and they support named variables, x,y plotting, text formatting, basic matrix math, etc. Cells can contain numerical values, text, mathematical expressions (formulas), or references to other cells. A vector of values such as an optical spectrum can be represented as a row or column of cells; a rectangular array of values, such as a set of spectra, can be represented as a rectangular block of cells. User-created names can be assigned to individual cells or to ranges of cells, then referred to in formulas by name, which makes the formulas easier to understand. Formulas can be easily copied across a range of cells, with the cell references changing or not as desired. Plots of various types can be created by menu selection. See http://www.youtube.com/watch?v=nTlkkbQWpVk for a nice video demonstration.

The latest versions of both ***Excel*** (*Excel 2013*) and ***OpenOffice Calc*** (4.0) can open and save spreadsheet file formats of the other (.xls and .ods, respectively). Simple spreadsheets in either format are compatible with the other program. However, there are small differences in the way that certain functions are interpreted, and for that reason I supply my spreadsheets in both .xls (for *Excel*) and in .ods (for *Calc*) formats. Basically, *Calc 4.0* can do most everything *Excel* can do, but *Calc* is free to download and is more Windows-standard in terms of look-and-feel. (Not every science worker who needs a spreadsheet can afford to buy, or has access to a site license for, expensive Microsoft products).

If you are working on a tablet or smartphone, you could use the Excel mobile app, Numbers for iPad, or several other mobile spreadsheets. These can do basic tasks but do not have the advanced capabilities of the desktop computer versions like Excel or Calc. By saving their data in the "cloud" (e.g. iCloud, Dropbox, or OneDrive), these apps automatically sync changes in both directions between mobile devices and desktop computers, making them useful for field data entry on a portable device.

In **Matlab** and in **Octave**, the variables can be either *scalar* (single values), *vector* (like a row or a column in a spreadsheet), representing one entire signal, optical spectrum or chromatogram, or *matrix* (like a rectangular block of cells in a spreadsheet), representing a *set* of signals. For example, define two vectors by typing `a=[1 2 5 2 1]` and `b=[4 3 2 1 0]`. Then to subtract `b` from `a` you would just type `a-b`, which gives the result `[-3 -1 3 1 1]`. To multiply `a` times `b` point by point, you would type `a.*b`, which gives the result `[4 6 10 2 0]`. If you have an optical spectrum in the variable `a`, you can plot it just by typing `plot(a)`. And if you also had a vector `w` of x-axis values (such as wavelengths), you can plot `a` vs `w` by typing `plot(w,a)`. You can place multiple smaller plots in one figure window by placing `subplot(m,n,p)` before the `plot` command to plot in the *p*th section of a *m*-by-*n* grid of plots.

Individual elements in a vector are referred to by *index number*; for example, **`w(10)`** is the 10th element in vector **w.** A colon indicates a range, like “to”, so **`w(10:20)`** is the vector of values of **w** from the 10th to the 20th entries. You can also find the index number of the entry closest to a given value in a vector by using my downloadable val2ind.m function; for example, **`w(val2ind(a,max(a)))`** returns the x value of the maximum of **`a`**, and **`w(val2ind(w,550):val2ind(w,560.5)`** is the vector of values of **w** between 550 and 560.5 (if **w** contains values within that range).

A Matlab variable can also be a *matrix*, a set of vectors of the same length combined into a rectangular array. For example, intensity readings of 10 different optical spectra, each taken at the same set of 100 wavelengths, could be combined into the 10 × 100 matrix **`S`**. So **`S(3,:)`** would be the third of those spectra and **`S(5,40)`** would be the intensity at the 40th wavelength of the 5th optical spectrum. The Matlab/Octave scripts plotting.m and plotting2.m show how to plot multiple signals using matrices and subplots.

The subtraction of two spectra **`a`** and **`b`**, if they have the same wavelengths, as in the figure on page 3, can be performed simply by writing **`a-b`**. To plot the difference, you would write **`plot(w,a-b)`**. To plot the ratio of two spectra, as in the figure on page 4, you would write **`plot(w,a./b)`**. So, "`./`" means divide point-by-point and ".*" means multiply point-by-point. The * by itself means *matrix multiplication*, which performs repeated multiplications without using loops. For example, if **`x`** is a vector, **`A=[1:1000]'*x;`** creates a matrix **`A`** in which each column is multiplied by the numbers 1, 2,...1000 respectively. It's shorter to write and *faster to compute* than using a loop: **`for n=1:1000;A(:,n)=n.*x;end;`**. Try it and see.

What's cool is that *Matlab and Octave don't force you to deal with vectors and matrices as collections of numbers*; it “knows” when you are dealing with those and adjusts your calculations accordingly.

Probably the *most common error* you'll make in learning Matlab/Octave is getting the rows and the columns switched, which often generates errors: here's a text file that gives examples of common vector and matrix operations and the kinds of error messages that you are likely to get in Matlab and Octave.

Both Matlab and Octave can be used to automate complex sequences of operations by saving them as scripts and functions (text files saved with a “.m” file name extension). Matlab and Octave are also considerably faster in computations and in graphing than spreadsheets.

Getting data into Matlab/Octave. You can easily import your own data into Matlab or Octave by using the **load** command. Data can be imported from plain text files, CSV (comma separated values), several image and sound formats, and spreadsheets. Matlab has a convenient Import Wizard (click **File > Import Data**). It is also possible to import data from graphical line plots or *printed* graphs by using the built-in "ginput" function that obtains numerical data from the coordinates of mouse clicks (as in DataTheif or Figure Digitizer). Matlab R2013a or newer can even read the sensors on your iPhone or Android phone via Wi-Fi. To read the outputs of older analog instruments, use an analog-to-digital converter or a USB voltmeter.

Spreadsheet or Matlab/Octave? For signal processing, Matlab/Octave is faster and more powerful than spreadsheets, but spreadsheets have their advantages: they are easier for novices to learn and they offer very flexible presentation and user interface design. Spreadsheets are better for data entry and are easily deployed on portable devices (e.g. using iCloud Numbers or the Excel app). Spreadsheets are concrete and more low-level, showing every single value explicitly in a cell. In contrast, Matlab/Octave is more high level and abstract, because a single variable or function can do so much. Also, user-defined functions can call other built-in or user-defined functions, which in turn can call other functions, and so on, allowing you to build up *very complex high-level functions in layers*. Fortunately, Matlab can easily read Excel .xls and .xlsx files and import the rows and columns into matrix variables.

The bottom line is that spreadsheets are easier at first, but in my experience the Matlab/Octave approach is more productive for many applications. This point is demonstrated by comparing both approaches to multilinear regression in multicomponent spectroscopy (page 51-52), and especially by the dramatic difference between the spreadsheet and Matlab/Octave approaches to finding and measuring peaks in signals (page 83), i.e. a 250 Kbyte spreadsheet vs a 7 Kbyte Matlab/Octave script that is 50 times faster (in Matlab).

Both spreadsheets and Matlab/Octave programs have an advantage over commercial end-user programs and self-contained programs such as SPECTRUM (page 70); they can be *inspected and modified by the user* to customize the routines for specific needs. Simple changes are easy to make with little or no knowledge of programming. For example, you could easily change the labels, titles, colors, or line style of the graphs - in Matlab or Octave programs, search for "`title(`", "`label(`" or "`plot(`". My code tells you where specific useful changes can be made by the user: just use the editor to run a search for the word "change".

Online calculations and plotting. One of my absolute favorites is Wolfram Alpha, a Web site and a smartphone app that is a very neat computational tool and information source, including capabilities for mathematics, plotting data and functions, vector and matrix manipulations, statistics and data analysis, and many other topics. Statpages.org can perform a huge range of statistical calculations and tests. SageMath is a free open-source mathematics software system. There are several Web sites that specialize in plotting data, including Plotly, Grapher, and Plotter. All of these require a reliable Internet connection and they are useful when working on a mobile device or on a computer that does not have math software installed.

Signals and noise

Experimental measurements are never perfect, even with sophisticated modern instruments. Two main types or measurement errors are recognized:

(a) *systematic error,* in which every measurement is consistently less than or greater than the correct value by a certain amount or relative percentage, and

(b) *random error,* in which there are unpredictable variations in the measured signal from moment to moment or from measurement to measurement.

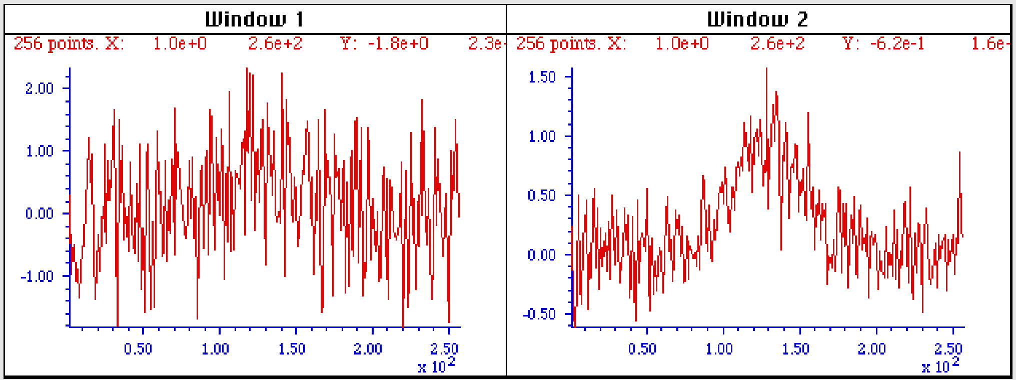
Systematic error can in principle be recognized and corrected, but random error is harder to eliminate. Random error is often called *noise*, by analogy to acoustic noise. Sources of noise in measurements might include such things as building vibrations, air currents, electric power fluctuations, stray radiation from nearby power lines or electrical apparatus, static electricity, interference from radio and TV transmissions, electrical storms, turbulence in the flow of gases or liquids, random thermal motion of electrons or molecules, background radiation from naturally occurring radio-active elements in the environment, "cosmic rays" from outer space (seriously), the basic quantum nature of matter and energy itself, and "digitization noise" (the rounding of numbers to a fixed number of digits; see page 122). Then of course there is the ever-present "human error", which can be a major factor anytime people are involved in operating, adjusting, recording, calibrating, or controlling instruments and in preparing samples for measurement.

One of the basic problems in signal measurement is distinguishing the noise from the signal. It's not always easy. The signal is the "important" part of the data that you want to measure - it might be the average of the signal over a certain time period or the height of a peak or the area under a peak that occurs in the data. For example, in the optical absorption spectrum in the right-hand half of the figure on page 3 in the previous section, the "important" parts of the data are probably the absorption peaks located at 520 and 550 nm. The height of either of those peaks might be considered the signal, depending on the application. In this example, the height of the largest peak is about 0.08 absorbance units. The noise would be the standard deviation of that peak height from spectrum to spectrum (if you could do repeat measurements of the same optical spectrum).

But what if you had only one recording of that spectrum? In that case, you'd be forced to estimate the noise in that single recording, based on the visible short-term fluctuations in the signal (the little random wiggles superimposed on the smooth signal), which we assume are noise, based on previous observations that no samples of that type ever exhibit reproducible short-term "fine structure" in their signals. In this case, those fluctuations amount to about 0.005 units peak-to-peak or a standard deviation of 0.001. For random fluctuations, the rule of thumb is that the peak-to-peak variation between the highest and the lowest readings is approximately 5 times the standard deviation, as demonstrated by this line of Matlab code: `rn=randn(1,100);(max(rn)-min(rn))/std(rn).` As another example, the data on the right half of the figure on the next page has a peak in the center with a height of about 1.0. The peak-to-peak noise on the baseline is also about 1.0, so the standard deviation of the noise is about 1/5th of that, or 0.2.

The *quality* of a signal is often expressed quantitatively as the *signal-to-noise ratio* (SNR), which is the ratio of the true signal amplitude (e.g. the average amplitude or the peak height) to the standard deviation of the noise. Thus the signal-to-noise ratio of the optical spectrum on page 3 is about 0.08/0.001 = 80, and the signal on page 7 has a signal-to-noise ratio of 1.0/0.2 = 5. So we would say that the signal-to-noise ratio of the signal on page 3 is better than the one on page 7. Signal-to-noise ratio is inversely proportional to the relative standard deviation of the signal amplitude. Measuring the signal-to-noise ratio is much easier if the noise can be measured separately, in the absence of signal. The relationship between signal-to-noise ratio and the relative standard deviation of the signal amplitude depends on how the signal amplitude is measured, specifically how may data points can be averaged or otherwise used; the relative standard deviation often varies with the *square root* of the number of noisy data points averaged.

Depending on the type of experiment, it may be possible to acquire readings of the noise alone, for example on a segment of the baseline before or after the occurrence of the signal. However, if the magnitude of the noise *depends on the level of the signal*, then the experimenter must try to produce a constant signal level to allow measurement of the noise on the signal. In some cases, where you can model the shape of the signal exactly by means of a mathematical function, the noise may be estimated by subtracting the model signal from the experimental signal, for example by looking at the *residuals* in *least-squares curve fitting* (see sections starting on pages 37 and 54). If practical, it's always better to determine the standard deviation of repeated measurements of the quantity that you want to measure, rather than trying to estimate the noise from a single recording of the data.



Window 1 (left) is a single measurement of a very noisy signal. There is actually a broad peak at the center of this signal, but it is not possible to measure its position, width, and height accurately because the signal-to-noise ratio is very poor (less than 1). Window 2 (right) is the average of 9 repeated measurements of this signal, clearly showing the reduction in the amplitude of the noise. The expected improvement in signal-to-noise ratio is 3 (the square root of 9). In some cases you may be able to average hundreds of measurements, resulting in more substantial improvement.

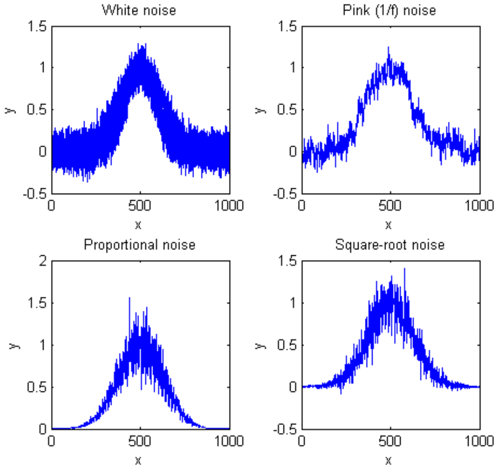
One thing that really distinguishes signal from noise is that random noise is not the same from one measurement of the signal to the next, whereas the genuine signal is at least partially reproducible. You can make use of this fact by measuring the signal over and over again, as fast as is practical, and computing the *average* of all the measurements point-by-point. This is called ***ensemble averaging***, and it is one of the most powerful methods for improving signals, when it can be applied. For this to work properly, the noise must be random and the signal must occur at the same time in each repeat. Examples are shown in the figure above and on pages 79 and 117. The signal-to-noise ratio usually improves with the square root of the number of independent signals added, if the noise is truly random and uncorrelated and if the repeats are synchronized. Digitization noise can also be reduced this way, but only if some random noise is already present in the signal *or is artificially added to it* (see Appendix I, page 122), a rare case where it may be *beneficial* to add noise to a signal.

Sometimes signal and noise can be partly distinguished on the basis of frequency components (page 28), that is, how rapidly it changes with time: for example, the signal may contain mostly low-frequency components and most of the noise may be located at higher frequencies. This is the basis of filtering and smoothing (page 11). In the figures above, the peaks contain mostly low-frequency components, whereas the noise is distributed over a much wider frequency range. The frequency characteristic of noise is described by its frequency spectrum (page 28, not to be confused with an *optical* spectrum). It is often described in terms of *color*. *White* noise has equal power at all frequencies; it derives its name from white light, which has equal brightness at all wavelengths in the visible region. The noise in the example above, and in the upper left quadrant of the figure on page 8, is white. In the acoustical domain, white noise sounds like a “hiss”. This is a common type of noise in measurement science; for example, digitization noise, photon noise and Johnson noise are white.

Another common type of noise has more power at *low* frequencies than at high frequencies. This is often called “pink noise”. In the acoustical domain, it sounds more like a “roar”. A sub-species of that type of noise is “1/f noise”, where the noise power in inversely proportional to frequency, shown in the upper right quadrant of the figure on the left, next page. Pink noise is more troublesome than white noise, because *a given standard deviation of pink noise has a greater effect on the accuracy of most measurements than the same standard deviation of white noise* (as demonstrated by the

Matlab/Octave function **noisetest.m** mentioned on page 10). Moreover, the application of smoothing (page 11) and low-pass filtering to reduce noise is more effective for white noise than for pink noise. When pink noise is present, it is sometimes beneficial to apply modulation techniques, such as optical chopping or wavelength modulation, to convert a direct-current (DC) signal into an alternating current (AC) signal, thereby increasing the frequency of the signal to a frequency region where the noise is lower. In such cases it is common to use a lock-in amplifier, or the digital equivalent thereof, to measure the amplitude of the signal. (You can download a Matlab/Octave function that demonstrates the appearance of white, pink, proportional, and square-root noise, and their effect on signal measurement, from noisetest.m).

Conversely, noise that has more power at *high* frequencies would be called “blue” noise. This type of noise is less commonly encountered in experimental work, but it can occur in processed signals that have been subjected to some sort of differentiation process (17) or that have been deconvoluted from some blurring process (pages 32 and 121). Blue noise is *easier* to reduce by smoothing, and it has less effect on least-squares fits than the equivalent amount (same standard deviation) of white noise.



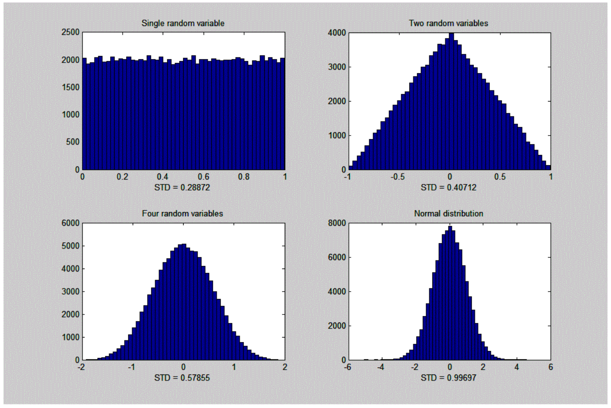
Noise can also be characterized by the way it varies with the signal amplitude. It may be a constant “background” noise that is independent of the signal amplitude. Or the noise may increase with signal amplitude; this is often observed in mass spectroscopy and in the frequency spectra of signals. One way to observe this is to select a segment of signal over which the signal amplitude varies widely, fit the signal to a polynomial or multiple peak model (pages 37, 54), and observe how the residuals vary with signal amplitude.

Often, there is a mix of noises with different behaviors. For example, in *optical spectroscopy*, three fundamental types of noise are contribute to the total noise, based on their origin and on how they vary with light intensity: photon noise, detector noise, and flicker (fluctuation) noise. *Photon noise* is *white* and is proportional to the square root of light intensity (illustrated in the lower right quadrant of the figure above), and therefore the SNR is proportional to the square root of light intensity. *Detector noise* is independent of the light intensity and therefore the detector SNR is directly proportional to the light intensity. *Flicker noise,* caused by light source instability, vibration, sample cell positioning errors, sample turbulence, light scattering by suspended particles, dust, bubbles, etc, is directly proportional to the light intensity (illustrated in the lower left quadrant of the figure above). Flicker noise is usually *pink* rather than white. In practice, the *total noise* observed is likely to exhibit a combination of amplitude dependence, as well as a mixture of white and pink noises.

Only in a few special cases is it possible to eliminate noise completely, so usually you must be satisfied by increasing the signal-to-noise ratio as much as possible. The key in any experimental system is to understand the possible sources of noise, break down the system into its parts and measure the noise generated by each part separately, then seek to reduce or compensate for as much of each noise source as possible. For example, in optical spectroscopy, source flicker noise can be reduced by feedback stabilization, choosing a better light source, using an internal standard, or using specialized instrument designs such as the double-beam, dual wavelength, derivative, and wavelength modulation designs that are employed in optical spectroscopy. The effect of photon noise and detector noise can be reduced by increasing the light intensity at the detector, and electronics noise can sometimes be reduced by cooling or upgrading the detector and/or electronics.

Another property of noise is its amplitude probability distribution, the function that describes the probability of a random variable falling within a certain range of values. In physical measurements, the most common distribution is the “Gaussian curve” (also called a “bell” or “haystack” curve) and is described by **y = e^(-(x-μ)^2/(2 σ^2))/(sqrt(2 π) σ)**, where **μ** is the average value and **σ** is the standard deviation. In this type of distribution, the most common noise errors are small and the errors become less common the greater their deviation. This is such a commonly-encountered type of distribution that it is called a "*normal"* distribution.

Why is this "normal" distribution so common? The noise observed in physical measurements is often the sum total of many unobserved random events, each of which has some unknown probability distribution related to, for example, the kinetic properties of gases or liquids or to the quantum mechanical description of fundamental particles such as photons or electrons. But when many such events combine to form the overall variability of an observed quantity, the resulting probability distribution is very often *normal*. This common observation is summed up in the *Central Limit Theorem*, and it is easily demonstrated by the following simulation.



CentralLimitDemo.m

In the figure on the left, we start with a set of 100,000 *uniformly distributed* random numbers that have an equal chance of having any value between certain limits - between 0 and +1 in this case (like the "rand" function in spreadsheets and Matlab/Octave). The graph in the upper left of the figure shows the probability distribution, called a "histogram", of that set of numbers. Next, we combine *two* sets of such independent, uniformly-distributed numbers (subtracting them so that the average is centered at zero). The result (shown in the graph in the upper right in the figure) has a *triangular* distribution between -1 and +1, with the highest point at zero, because there are *many* ways for the difference between two random numbers to be *small*, but only *one* way for the difference to be 1 or to -1 (that happens only if one number is exactly zero *and* the other is exactly 1).

Next, we combine *four* such sets of random numbers (lower left); the resulting distribution now has a total range of -2 to +2, but it is even *less* likely that the result be near 2 or -2 and many *more* ways for the result to be small, so the distribution is narrower and more rounded and is already starting to be visually close to a Gaussian distribution (shown for reference in the lower right). If we combine more and more independent uniform random variables, the probability distribution becomes closer to Gaussian. See **CentralLimitDemo.m** on http://tinyurl.com/cey8rwh.

Remarkably, *the distributions of the individual sets of numbers in this simulation hardly matter at all*. You could modify the individual distributions in this simulation by changing the "rand" function in CentralLimitDemo.m to sqrt(rand), sin(rand), rand^2, or log(rand), etc, to obtain other radically non-normal individual distributions. It seems that no matter what the distribution of the *original* random variable might be, by the time you combine even as few as four of them, the resulting distribution is already visually close to normal. Real world laboratory observations may be the result of *millions* of individual microscopic events, so whatever the probability distributions of the *individual* events, the *combined* macroscopic observations almost always approach a normal distribution *nearly perfectly*. It is on this common observance of normal distributions that the usual statistical procedures are based; the mean, standard deviation, least-squares fits, confidence limits, etc, are all based on the assumption of a *normal* Gaussian distribution.

It's important to understand that the three characteristics of noise just discussed in the paragraphs above - frequency distribution, signal dependence, and amplitude distribution - are mutually independent; a noise may in principle have any combination of those properties.

SPECTRUM (page 70) includes functions for measuring signals and noise, plus a signal generator for creating artificial signals with Gaussian and Lorentzian bands, sine waves, and normal random noise.

Spreadsheet programs, such as *Excel* or *Open Office Calc*, have built-in functions that can be used for calculating, measuring and plotting signals and noise. For example, the cell formula for one point on a **Gaussian** peak is `amplitude*EXP(-1*((x-position)/(0.6005615*width))^2)`, where 'amplitude' is the maximum peak height, 'position' is the location of the maximum on the x-axis, 'width' is the full width and half-maximum (FWHM) of the peak, and 'x' is the value of the independent variable x at that point. The cell formula for a **Lorentzian** peak is `amplitude/(1+((x-position)/(0.5*width))^2)`. Useful built-in functions include AVERAGE, MAX, MIN, ABS, STDEV, RAND, and QUARTILE. Some spreadsheets have only a *uniformly-distributed* random number function (rand) and not a *normally-distributed* random number function (randn), but you can create an approximately normal distribution by combining several uniformly-distributed RAND functions. For example, the expression 1.73*(RAND()-RAND()+RAND()-RAND()) creates approximately normal random numbers with a mean of zero, a standard deviation very close to 1, but with a numerical range limited to ±4. (The alternating + and – signs simply insures that the result averages to zero, and the empirical factor of 1.73 makes the average standard deviation equal to 1.00, as is the case for the normally-distributed RANDN function). The spreadsheets **RandomNumbers.xls/.ods** and the Matlab/Octave script **RANDtoRANDN.m** demonstrate how this works. The same technique is used in the spreadsheet SimulatedSignal6Gaussian.xlsx, which computes and plots a simulated signal consisting of up to 6 overlapping Gaussian bands plus random white noise. Download from http://tinyurl.com/cey8rwh**.**

Matlab and **Octave** have built-in functions that are used for measuring and plotting signals and noise, such as *plot, mean, max, min, std, log, log10, hist, rand,* and *randn*. Just type “help” and the function name at the command prompt, e.g. “help mean”. *Most of these Matlab and Octave functions apply to vectors and matrices as well as scalar variables*. You can subtract a scalar number from a vector (for example, `v = v-min(v)` sets the lowest value of vector v to zero). If you have a set of signals in the rows of a matrix **S,** where each column represents the value of each signal at the same value of the independent variable (for example, time), you can compute the *ensemble average* of all the columns of **S** just by typing “mean(**S**)”.

In the Matlab/Octave statements `[N,X]=hist(randn(size(1:100)));peakfit([X;N]);` the "randn" function generates 100 normally-distributed random numbers, then the "peakfit" function (page 90) graphs the histogram (probability distribution) as blue dots and compares that distribution to a Gaussian (the red line). Change the 100 to 1000 or a higher number to see how much closer to Gaussian the distribution becomes. The "randn" function is useful in signal processing for predicting the uncertainty of measurements in the presence of random noise, for example by using the Monte Carlo or the bootstrap methods (page 40).

You can also create user-defined functions in Matlab or Octave to automate commonly-used algorithms. For an explanation and a simple worked example, type “help function” at the command prompt. I have created many Matlab/Octave functions related to signal processing which are listed on **http://tinyurl.com/cey8rwh**: **plotit.m**, a simple function for plotting and fitting x,y data in matrices or in separate vectors; a set of functions for commonly encountered peak shapes (Gaussian, Lorentzian, etc.) and functions for different types of random noise (white noise, pink noise, blue noise, proportional noise, and square root noise). **ExpBroaden.m** applies exponential broadening to a signal; **Iqrange.m** computes the interquartile range ; **rmnan.m** removes “not-a-number” entries from vectors; **val2ind.m** returns the index and the value of the element of vector x that is closest to a particular value. These functions can be downloaded from http://tinyurl.com/cey8rwh. Once you have downloaded those functions into a folder in the Matlab/Octave path, you can use them just like any other built-in function. For example, you can get help for any function by typing “help <name>”. You can plot a simulated noisy peak Gaussian such as that on page 7:

```
x=[1:256];y=gaussian(x,128,64)+0.2*whitenoise(x);plotit(x,y)
```

The script SignalGenerator.m calls several of these downloadable functions to create and plot a realistic computer-generated signal with multiple peaks on a variable baseline plus variable random noise; you might try to modify the variables in the indicated places to make it look like your type of data.

noisetest.m is a self-contained Matlab/Octave function that demonstrates different noise types and their effects. It creates a set of Gaussian peaks with different types of added noise: constant white noise, constant pink (1/f) noise, proportional white noise, and square-root white noise. It then fits a Gaussian to each noisy data set and computes the average and the standard deviation of repeated measurements of best-fit peak height, position, width, and area for each noise type.

iSignal (page 85) can plot signals with pan and zoom controls, measure signal, noise amplitudes, and noise frequency distributions in selected regions of the signal, compute the signal-to-noise ratio of peaks, perform variable smoothing, differentiation, interpolation, peak sharpening, and measurement of the positions, heights, widths, and areas or noisy peaks. It's operated by simple keypresses. (Press **K** for a list)

iPeak (page 78) is an interactive peak detector that has an *ensemble averaging* function (**Shift-E**) capable of computing the average pattern of a repeating waveform. See page 79 and 117 for details.

For a complete list of my downloadable Matlab and Octave functions, demonstration scripts, and spreadsheets, see **http://tinyurl.com/cey8rwh**. None of these require Matlab's *Signal Processing Toolbox*.

Smoothing

In many experiments in physical science, the true signal amplitudes (the dependent variable or “y-axis” values) change rather smoothly as a function of the independent (“x-axis”) values, whereas many kinds of noise are seen as rapid, random changes in amplitude from point to point within the signal. In the latter situation it is common practice to attempt to reduce the noise by a process called *smoothing*. In smoothing, the data points of a signal are modified so that individual points that are higher than the immediately adjacent points (presumably because of noise) are reduced, and points that are lower than the adjacent points are increased. This naturally leads to a smoother signal (and a slower step response to signal changes). As long as the true underlying signal is actually smooth, then the true signal will not be much distorted by smoothing, but the noise will be reduced.

Smoothing algorithms. Most smoothing algorithms are based on the "shift and multiply" technique, in which a group of adjacent points in the original data are multiplied point-by-point by a set of numbers (coefficients) that defines the smooth shape, the products are added up to become one point of smoothed data, then the set of coefficients is shifted one point down the original data and the process is repeated. The simplest smoothing algorithm is the *rectangular* or *unweighted sliding-average smooth*; it simply replaces each point in the signal with the average of *m* adjacent points, where *m* is a positive integer called the *smooth width*. For example, for a 3-point smooth (*m*=3):

$$S_j = \frac{Y_{j-1} + Y_j + Y_{j+1}}{3}$$

for j = 2 to *n*-1, where S_j the j^th point in the smoothed signal, Y_j the j^th point in the original signal, and *n* is the total number of points in the signal. Similar smooth operations can be constructed for any desired smooth width, *m*. Usually *m* is an odd number. If the noise in the data is “white” (that is, evenly distributed over all frequencies) and its standard deviation is *s*, then the standard deviation of the noise remaining in the signal after the first pass of an unweighted sliding-average smooth will be approximately *s*/sqrt(*m*), where *m* is the smooth width. Despite its simplicity, this smooth is actually optimum for the problem of reducing white noise while keeping the *sharpest linear step response.*

The *triangular smooth* is like the rectangular smooth, above, except that it implements a weighted smoothing function. For a 5-point smooth (*m*=5):

$$S_j = \frac{Y_{j-2} + 2Y_{j-1} + 3Y_j + 2Y_{j+1} + Y_{j+2}}{9}$$

for j = 3 to *n*-2, and similarly for other smooth widths. In both of these cases, the denominator is the *sum of the coefficients* in the numerator, which results in a “unit-gain” smooth that has no effect on straight line regions the signal and which preserves the area under peaks (see page 35).

It is often useful to apply a smoothing operation more than once, that is, to smooth an already smoothed signal, in order to build longer and more complicated smooths. For example, the 5-point triangular smooth above is equivalent to *two* passes of a 3-point rectangular smooth. *Three* passes of a 3-point rectangular smooth result in a 7-point "pseudo-Gaussian" or haystack smooth, for which the coefficients are in the ratio 1:3:6:7:6:3:1. The general rule is that *p* passes of a *m*-width smooth results in a combined smooth width of *p***m*-*p*+1. For example, 3 passes of a 17-point smooth results in a 49-point smooth. These multi-pass smooths are more effective at reducing high-frequency noise in the signal than a single rectangular smooth of the same width but exhibit slower step response.

In all of these smooths, the width of the smooth *m* is usually chosen to be a odd integer, so that the smooth coefficients are symmetrically balanced around the central point. This is important because it preserves the x-axis position the features on the signal, which is especially critical in spectroscopic and chromatographic applications because the peak positions are important measurement objectives.

I am assuming here that the x-axis intervals of the signal is uniform, that is, that the difference between the x-axis values of adjacent points is the same throughout the signal. This is also assumed in some (but not all) of the other signal processing techniques described in this essay, and it is a very common (but not necessary) characteristic of signals that are acquired by computerized equipment.

The *Savitzky-Golay* smooth is based on the least-squares fitting of polynomials to segments of the data. Compared to the sliding-average smooths, the Savitzky-Golay smooth is less effective at reducing noise, but more effective at retaining the shape of the original signal. The algorithm is more complex and the computational times are greater than the smooth types discussed above, but with modern computers the difference is usually not significant (see page 110). It is capable of differentiation as well as smoothing. Code in various languages is widely available online.

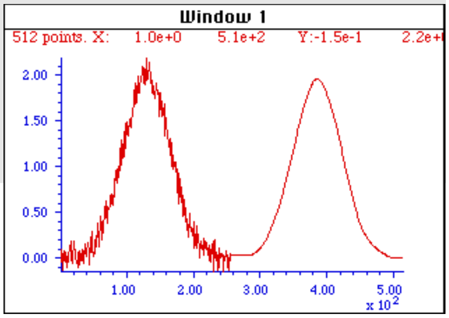
The shape of a smoothing algorithm can be determined by applying that smooth to a *delta function,* a signal consisting of all zeros except for one point, as demonstrated by the script DeltaTest.m.

Noise reduction. Smoothing can reduce the apparent noise in a signal. If the noise is "white" (that is, evenly distributed over all frequencies) and its standard deviation is *s*, then the standard deviation of the noise remaining in the signal after one pass of a triangular smooth will be approximately *s**0.8/sqrt(*m*), where *m* is the smooth width. Smoothing operations can be applied more than once: that is, a previously smoothed signal can be smoothed again. In some cases this can be useful if there is a great deal of high-frequency noise in the signal. However, the noise reduction for white noise is less less in each successive smooth; three passes of a rectangular smooth reduces white noise by a factor of *s**0.7/sqrt(*m*), a slight improvement over two passes.

Edge effects and the lost points problem. Note in the equations above that the 3-point rectangular smooth is defined only for j = 2 to *n*-1. There is not enough data in the signal to define a complete 3-point smooth for the first point in the signal (*j* = 1) or for the last point (*j* = *n*) , because there are no data points before the first point or after the last point. Similarly, a 5-point smooth is defined only for *j* = 3 to *n*-2, and therefore a smooth can not be calculated for the first two points or for the last two points. In general, for an *m*-width smooth, there will be (*m*-1)/2 points at the beginning of the signal and (*m*-1)/2 points at the end of the signal for which a complete *m*-width smooth can not be calculated like the other points.

What to do? There are two ways to go. One is to accept the loss of points and trim off those points or replace them with zeros in the smooth signal. The other way is to use progressively smaller smooths at the ends of the signal, for example to use 2, 3, 5, 7... point smooths for signal points 1, 2, 3,and 4.., and for points *n*, *n*-1, *n*-2, *n*-3.., respectively. The later may be preferable if the edges of the signal contain critical information, but it increases execution time.

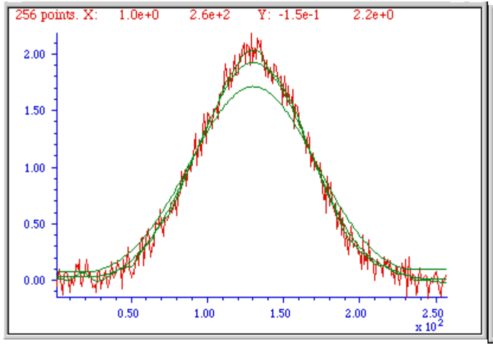
Examples of smoothing. A simple example of smoothing is shown in the figure below. The left half of this signal is a noisy peak, with constant white noise. The right half is the same peak after undergoing a triangular smoothing algorithm. The noise is greatly reduced while the peak itself is hardly changed. Smoothing increases the visual signal-to-noise ratio. The larger the smooth width, the greater the noise reduction, but also the greater the signal distortion by the smoothing operation.

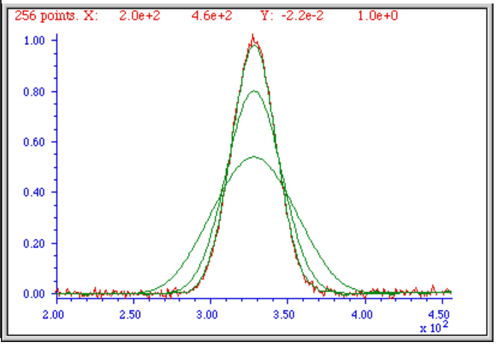


The left half of this signal is a noisy peak. The right half is the same peak after undergoing a ***smoothing*** *algorithm. The noise is reduced while the peak itself is hardly changed, resulting in a nicer looking signal and making it easier to estimate the peak position, height, and width directly by graphical or visual inspection (but it doesn't improve measurements of peak parameters made by least-squares curve-fitting.*

The optimum choice of smooth width depends upon the width and shape of the signal and the digitization interval. For peak-type signals, the critical factor is the *smoothing ratio*, the ratio between the smooth width *m* and the number of points in the half-width of the peak. In general, smoothing improves the signal-to-noise ratio but causes a reduction in amplitude and in increase in the bandwidth of the peak. If the peak shape is known, it's better to measure the peak by curve-fitting the *unsmoothed* signal (see page 69).

The figures at the top of the next page show examples of the effect of three different smooth widths on noisy Gaussian shaped peaks. In the figure on the left, below, the peak has a (true) height of 2.0 and there are 80 points in the half-width of the peak. The red line is the original unsmoothed peak. The three superimposed green lines are the results of smoothing this peak with a triangular smooth of width (from top to bottom) 7, 25, and 51 points. The peak width is 80 points, so the *smooth ratios* are 7/80 = 0.09, 25/80 = 0.31, and 51/80 = 0.64, respectively.

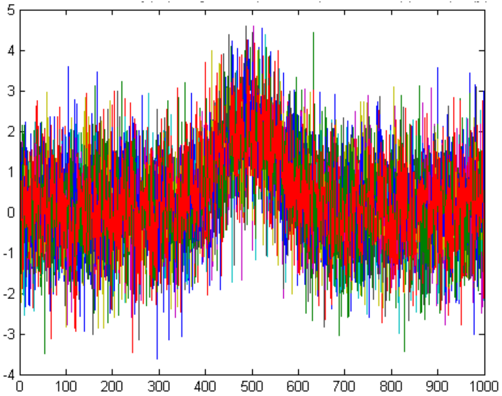


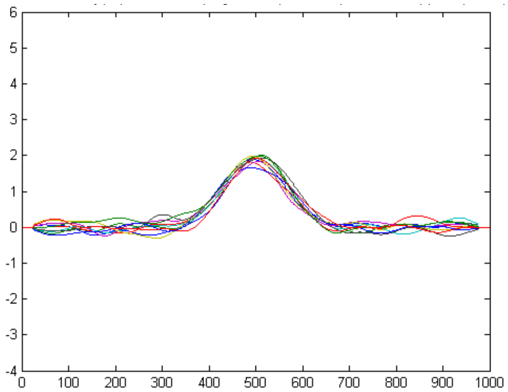


As the smooth width increases, the noise is progressively reduced but the peak height is reduced and the peak width is increased. In the figure on the right, the original peak (in red) has a true height of 1.0 and a half-width of 33 points. (It is also less noisy than the example on the left.) The three superimposed green lines are the results of the same three triangular smooths of width (from top to bottom) 7, 25, and 51 points. But because the peak width in this case is only 33 points, the *smooth ratios* of these three smooths are larger: 0.21, 0.76, and 1.55, respectively. You can see that the peak distortion effect (reduction of peak height and increase in peak width) is greater for the narrower peak because the smooth ratios are higher. The total *area* under the peak remains unchanged.

It should be clear that smoothing can never completely eliminate noise, because most noise is spread out over a wide range of frequencies, and smoothing simply reduces the noise in *part* of its frequency range. Only for very specific types of noise (e.g. discrete frequency noise or single-point spikes) is there hope of anything close to complete noise elimination (see page 15).

Limits of smoothing. It's very important to point out that smoothing results such as illustrated in the figure above may be *deceptively impressive* because they employ a *single sample* of a noisy signal that is smoothed to different degrees. This causes the viewer to *underestimate the contribution of low-frequency noise*, which is hard to estimate visually because there are so few low-frequency cycles in the signal record. This problem can visualized by recording a number of independent samples of a noisy signal, as illustrated in the two figures below. These figures show ten super-imposed plots with the same underlying peak but with independent white noise samples, each in a different color, unsmoothed on the left and smoothed on the right. Inspection of the smoothed signals on the right clearly reveals the variation in peak position, height, and width between the ten samples caused by the low frequency noise remaining in the smoothed signals. *Just because a signal looks smooth does not mean there is no noise.* Low-frequency noise remaining in the signals after smoothing will still interfere with precise measurement of peak position, height, and width.



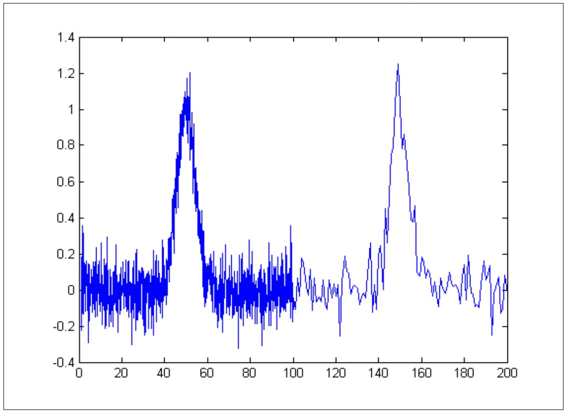


```
x=1:1000;
for n=1:10,
  y(n,:)=2.*gaussian(x,500,150)+whitenoise(x);
end
plot(x,y)
```

```
x=1:1000;
for n=1:10,;
  y(n,:)=2.*gaussian(x,500,150)+whitenoise(x);
  y(n,:)=fastsmooth(y(n,:),50,3);
end
plot(x,y)
```

The generating scripts below each figure require functions downloaded from http://tinyurl.com/cey8rwh.

An alternative to smoothing to reduce noise in the above set of unsmoothed signals is *ensemble averaging* (page 7) which can be performed in this case very simply by the Matlab/Octave statement `mean(y)`. The result shows a reduction in white noise by about sqrt(10) ≈ 3, good enough to judge that there is a single peak with Gaussian shape, best measured by curve fitting (page 54) using `peakfit([x;mean(y)],0,0,1)`, with the result showing excellent agreement within 1% of the position, height, and width of the Gaussian peak created in the third line of the generating script.



The figure on the right illustrates another aspect of smoothing. It consists of two Gaussian peaks, one located at x=50 and the second at x=150. Both peaks have a peak height of 1.0, a peak half-width of 10, and with normally-distributed random white noise with a standard deviation of 0.1 added to the entire signal. *The x-axis sampling interval, however, is different for the two peaks*; it's 0.1 for the first peaks and 1.0 for the second peak. This means that the first peak is characterized by *ten times more points* that the second peak. It may *look* like the first peak is noisier than the second, but that's just an illusion; the signal-to-noise ratio for both peaks is 10. The second peak *looks* less noisy only because there are fewer noise samples there and *people tend to underestimate the deviation of small samples*. When this signal is smoothed, the second peak is much more likely to be distorted by the smooth (it becomes shorter and wider) than the first peak. The first peak can tolerate a much wider smooth width, resulting in a greater degree of noise reduction. More data are almost always better. Similarly, if both peaks are measured by least squares methods (pages 37-69), the results on the first peak will be about 3 times more accurate than the second peak, because there are 10 times more data points in that peak, and the measurement precision improves roughly with the square root of the number of data points *if the noise is white*. (You can download this data file (named "udx") in TXT format or in Matlab MAT format from http://tinyurl.com/cey8rwh).

Optimization of smoothing. Which is the best smooth ratio? It depends on the purpose of the peak measurement. If the objective is to measure the true peak height and width, then smooth ratios below 0.2 should be used. (In the example on the left above, the original peak (red line) has a peak height greater than the true value 2.0 because of the noise, whereas the smoothed peak with a smooth ratio of 0.09 has a peak height that is much closer to the correct value). *Measuring the height of noisy peaks of known shape is much better done by curve fitting the unsmoothed data* rather than by taking the maximum of the smoothed data (page 69). But if the objective of the measurement is to count the peaks or to measure their peak position (x-axis value at the peak), much larger smooth ratios can be employed if desired, because smoothing has little effect on the peak position of a symmetrical peak (unless the increase in peak width is so large that it causes adjacent peaks to overlap).

In quantitative analysis applications where the system is calibrated with standards, the peak height reduction caused by smoothing is not so important, because if the same signal processing operations are applied to the samples and to the standards, the peak height reduction of the standard signals will be exactly the same as that of the sample signals and the effect will cancel out exactly. In such cases smooth widths from 0.5 to 1.0 can be used if necessary to further improve the signal-to-noise ratio, which is reduced by approximately the square root of the smooth width. In practical analytical chemistry, absolute peak height measurements are seldom required; calibration against standard solutions is far more common. See page 110 for supporting data.

When should you smooth a signal? There are two main reasons to smooth a signal:

a. for cosmetic reasons, to prepare a nicer or more impressive looking graphic for visual presentation or publication, specifically to emphasize *long-term* behavior over *short-term*, or

b. when the signal will be subsequently processed by a method that would be degraded by the presence of too much high-frequency noise, for example if the heights of peaks are determined graphically or by using the MAX function, or if peaks, valleys, or inflection points in the signal are to be automatically determined by detecting zero-crossings in derivatives of the signal. But generally smoothing will *not* significantly improve quantitative measurements of peak height, position, and width of peak-type signals when performed by least-squares methods; see page 69.

Smoothing in peak detection. Care must be used in the design of algorithms that employ smoothing. For example, in one popular technique for finding and measurement peaks in signals (page 74), the peaks are located by detecting downward zero-crossings in the *smoothed* first derivative (page 17), but the position, height, and width of each peak is determined by least-squares curve-fitting (page 37) of a model peak (e.g. Gaussian) to a segment of original *unsmoothed* data in the vicinity of the zero-crossing. Thus, even if heavy smoothing is necessary to provide reliable discrimination against noise peaks, the peak parameters extracted by curve fitting are not distorted.

When should you NOT smooth a signal? One common situation where you should usually *not* smooth signals (reference 43) is prior to least-squares curve fitting (page 37), for four reasons:

a. Smoothing will not usually improve the accuracy of parameter measurement by least-squares;

b. All smoothing algorithms are at least slightly “lossy”, distorting the signal to some extent;

c. It's harder to evaluate the fit by inspecting the residuals (page 38) if the data are smoothed, because *smoothed noise may be mistaken for an actual signal* (page 110); and

d. Smoothing will cause serious underestimation of the errors predicted by propagation-of-errors calculations and the “bootstrap method” (see page 41-42).

Dealing with spikes. Sometimes signals are contaminated with very tall, narrow “spikes” occurring at random intervals and with random amplitudes, but with widths of only one or a few points. It not only looks ugly, but it also upset the assumptions of least-squares computations because it is not normally-distributed random noise. This type of interference is difficult to eliminate using the above smoothing methods without distorting the signal. However, a “median” filter, which replaces each point in the signal with the *median* (rather than the *average*) of *m* adjacent points, can eliminate narrow spikes with very little change in the signal, if the width of the spikes is only one or a few points and less than *m*. The killspikes.m function is another spike-removing function that uses a different approach, based on linear interpolation. Unlike conventional smooths, these functions can be profitably applied *prior* to least-squares fitting functions. (Of course, if the spikes *are* the signal and you want to count or measure them, a different method is used, such as described on page 119).

Condensing oversampled signals. Sometimes signals are recorded more densely (that is, with a higher sampling rate or smaller x-axis interval) than really necessary to capture all the features of the signal. This results in larger-than-necessary data sizes, which slows down signal processing procedures and may tax storage capacity. To correct this, oversampled signals can be reduced in size either by eliminating data points (say, dropping every other point or every third point) or by replacing groups of adjacent points by their averages. The later approach has the advantage of *using* rather than *discarding* data points, and it provides some measure of noise reduction. (If the noise in the original signal is white, it is reduced in the condensed signal by the square root of *n*, with no change in frequency distribution of the noise).

SPECTRUM (page 70) includes simple rectangular and triangular smoothing functions.

Spreadsheets. Smoothing can be done in spreadsheets using the "shift and multiply" technique described above. In the spreadsheets **smoothing.xls/.ods** the set of multiplying coefficients is contained in the formulas that calculate the values of each cell of the smoothed data in columns **C** and **E**. Column **C** performs a 7-point rectangular smooth (1 1 1 1 1 1 1) and column **E** does a 7-point triangular smooth (1 2 3 4 3 2 1), applied to the data in column **A**. You can type in (or Copy and Paste) any data you like into column **A**, and you can extend the spreadsheet to longer columns of data by dragging the last row of columns **A**, **C**, and **E** down as needed. However, to change the smooth width, you would have to change the equations in columns **C** or **E** and copy the changes down the entire column.

The spreadsheets **UnitGainSmooths.xls/.ods** contain a collection of unit-gain convolution coefficients for rectangular, triangular, and Gaussian smooths of width 3 to 29 points, in both column and row format, that you can Copy and Paste into your own spreadsheets. The spreadsheets **MultipleSmoothing.xls/.ods** demonstrate a more flexible method in which the coefficients are contained in a group of 17 adjacent cells (in row 5, columns **I** through **Y**), making it easier to change the smooth shape and width (up to a maximum of 17). In this spreadsheet, the smooth is applied three times in succession, resulting in an effective smooth width of 49 points applied to column **G**. Download these spreadsheets from http://tinyurl.com/cey8rwh**.** Compared to Matlab/Octave, spreadsheets are slower, less flexible, and less easily automated. For example, in these spreadsheets, to change the signal or the number of points in the signal, or to change the smooth width or type, you have to modify the spreadsheet in several places, whereas to do the same using the Matlab/Octave "fastsmooth" function (below), you need only change in input arguments of a single line of code. Combining several different techniques into one spreadsheet is more complicated than Matlab/Octave.

Smoothing in Matlab and Octave. The user-defined function “fastsmooth” implements most of the types of smooths discussed above. (If you are viewing this document online, you can ctrl-click on this link to inspect the code). Fastsmooth is a function of the form **`s=fastsmooth(a, w, type, edge)`** The argument “**a**” is the input signal vector; “**w**” is the smooth width; “**type**” determines the smooth type: **type=1** gives a rectangular (sliding-average or boxcar); **type=2** gives a triangular (equivalent to 2 passes of a sliding average); **type=3** gives a pseudo-Gaussian (equivalent to 3 passes of a sliding average). See page 110 for a comparison of these smooth types. Fastsmooth uses a kind of recursive algorithm that computes each point based on the one before it. The argument “**edge**” controls how the “edges” of the signal (the first w/2 points and the last w/2 points) are handled. If **edge=0**, the edges are zero. (In this mode the elapsed time is independent of the smooth width. This gives the fastest execution time). If **edge=1**, the edges are smoothed with progressively smaller smooths the closer to the end. In this mode the execution time increases with smooth width. The smoothed signal is returned as the vector “**s**”. (You can leave off the last two input arguments: **`fastsmooth(Y,w,type)`** smooths with edge=0 and **`fastsmooth(Y,w)`** smooths with type=1 and edge=0). Compared to convolution-based smooths, fastsmooth gives faster execution times, especially for large smooth widths; it can smooth a 10^6 point signal with a 10^3 point sliding average in 0.1 sec. Here's a simple example of fastsmooth demonstrating the effect on white noise (graphic).

```
x=1:100;y=randn(size(x));
plot(x,y,x,fastsmooth(y,5,3,1),'r')
xlabel('Blue: white noise.    Red: smoothed white noise.')
```

Here's another experiment in Matlab or Octave that creates a Gaussian peak, smooths it with “fastsmooth”, compares the smoothed and unsmoothed version, then uses a peak-fitting function (**peakfit.m** , which will be covered on page 90) to show that smoothing *reduces the peak height* (from 1 to 0.786), *increases the peak width* (from 1.66 to 2.12), but has *no effect on the total peak area* (as long as you measure the *total* area under the broadened peak). Actually, there is no need to smooth the data if the peak parameters will be measured by least-squares fitting methods, because *the results obtained on the unsmoothed data will be more accurate*. See page 69.

```
>> x=[0:.1:10]';y=exp(-(x-5).^2);
>> ysmoothed=fastsmooth(y,11,3,1);
>> plot(x,y,x,ysmoothed,'r')
>> [FitResults,FitError]=peakfit([x y])
            Peak      Position      Height    Width       Area
FitResults = 1           5            1       1.6651      1.7725
FitError = 3.817e-005
>> [FitResults,FitError]=peakfit([x ysmoothed])
            Peak      Position      Height    Width       Area
FitResults = 1           5      0.78608       2.1224      1.7759
FitError = 0.13409
```

Diederick has published a Savitzky-Golay smooth function in Matlab, which you can download from the Matlab File Exchange. It's included in the interactive iSignal function. Greg Pittam has published a useful modification of the fastsmooth function that tolerates NaNs (Not a Number) in the data file (nanfastsmooth(Y,w,type,tol)) and a version for smoothing angle data (nanfastsmoothAngle(Y,w,type,tol)).

The Matlab/Octave user-defined function **condense.m**, `condense(y,n)`, returns a condensed version of the vector y in which each group of *n* points is replaced by its average, reducing the length of *y* by the factor *n*. (Use this function on *both x and y* variables so that the features of *y* will appear at the same *x* values). The function medianfilter.m performs a median filter operation that replaces each value of y with the median of w adjacent values. It is useful for removing spike artifacts; it's also included in iSignal.m.

iSignal (page 85) performs interactive smoothing for time-series signals using *all* the smoothing algorithms discussed above. It has keystrokes that allow you to adjust the smoothing parameters continuously while observing the effect on your signal dynamically, making it easy to observe how different types and amounts of smoothing effect noise and signal (such as the height, width, and areas of peaks). It has a frequency spectrum mode that displays the frequency components of any portion of the signal (page 28). It also condenses oversampled signals, interpolates signals to change their sampling intervals, and it has a median filter for removing spikes. The simple script “iSignalDeltaTest” demonstrates the frequency response of iSignal's smoothing functions by applying them to a delta function, allowing you to change the smooth type (**S** key) and the smooth width (**A** and **Z** keys) to see how the the frequency response changes.

Download any of the functions above from **http://tinyurl.com/cey8rwh**.

Differentiation

The symbolic differentiation of functions is a topic that is introduced in all elementary Calculus courses. The numerical differentiation of digitized signals is an application of this concept that has many uses in analytical signal processing. The first derivative of a signal is the rate of change of y with x, that is, dy/dx, which is interpreted as the slope of the tangent to the signal at each point. Assuming that the x-interval between adjacent points is constant, the simplest algorithm for computing a first derivative is:

$$Y_j' = \frac{Y_{j+1} - Y_j}{X_{j+1} - X_j} = \frac{Y_{j+1} - Y_j}{\Delta X} \qquad X_j' = \frac{X_{j+1} + X_j}{2} \quad \text{(for } 1 < j < n\text{-}1\text{).}$$

where X'_j and Y'_j are the X and Y values of the jth point of the derivative, n = number of points in the signal, and ΔX is the difference between the X values of adjacent data points. A commonly used variation of this algorithm computes the average slope between three adjacent points:

$$Y_j' = \frac{Y_{j+1} - Y_{j-1}}{2\Delta X} \qquad X_j' = X_j \quad \text{(for } 2 < j < n\text{-}1\text{).}$$

This is called a central-difference formula; it has the advantage that the X values are not changed.

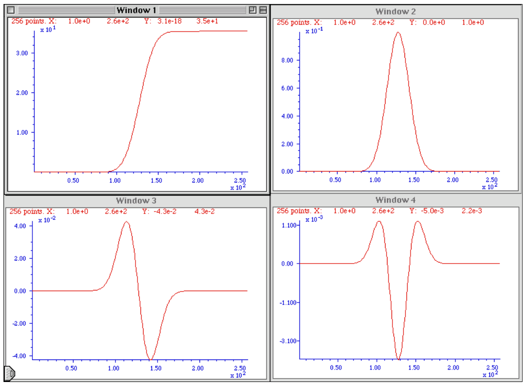
The *second derivative* is the derivative of the derivative: it is a measure of the *curvature* of the signal, that is, the rate of change of the slope of the signal. It can be calculated by applying the first derivative calculation twice in succession. The simplest algorithm for direct computation of the second derivative in one step is

$$Y_j'' = \frac{Y_{j+1} - 2Y_j + Y_{j-1}}{\Delta X^2} \qquad X_j' = X_j \quad \text{(for } 2 < j < n\text{-}1\text{).}$$

Similarly, higher derivative orders can be computed using the appropriate sequence of coefficients: for example +1, -2, +2, -1 for the third derivative and +1, -4, +6, -4, +1 for the 4th derivative. *Any* derivative of order *m* can also be computed simply by taking *m* successive first-order derivatives.

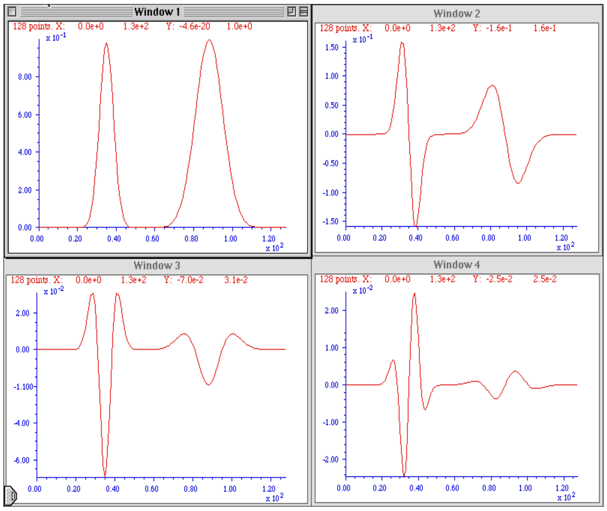
The *Savitzky-Golay* smooth can also be used for differentiation with the appropriate choice of input arguments; it combines differentiation and smoothing (page 11) into one algorithm, which is sensible because smoothing is always required with differentiation.

Basic Properties of Derivative Signals



The figure on the left shows the results of the successive differentiation of a computer-generated signal. The signal in each of the four windows is the first derivative of the one before it; that is, Window 2 is the first derivative of Window 1, Window 3 is the first derivative of Window 2, Window 3 is the *second* derivative of Window 1, and so on. You can predict the shape of each signal by recalling that the derivative is simply the slope of the original signal: where a signal slopes up, its derivative is positive; where a signal slopes down, its derivative is negative; and where a signal has zero slope, its derivative is zero. The sigmoidal signal shown in Window 1 has an *inflection point* (point where the slope is maximum) at the center of the x axis range. This corresponds to the *maximum* in its first derivative (Window 2) and to the *zero-crossing* (point where the signal crosses the x-axis going either from positive to negative or *vice versa*) in the second derivative in Window 3. This behavior can be useful for locating the inflection point in a sigmoid signal, by computing the location of the zero-crossing in its second derivative. Similarly, or for locating the maximum in a peak-type signal by computing the location of the zero-crossing in its first derivative. Other signal shapes have different derivatives shapes: the Matlab/Octave function DerivativeShapeDemo.m demonstrates the first derivative forms of 16 different model signal shapes (graphic).

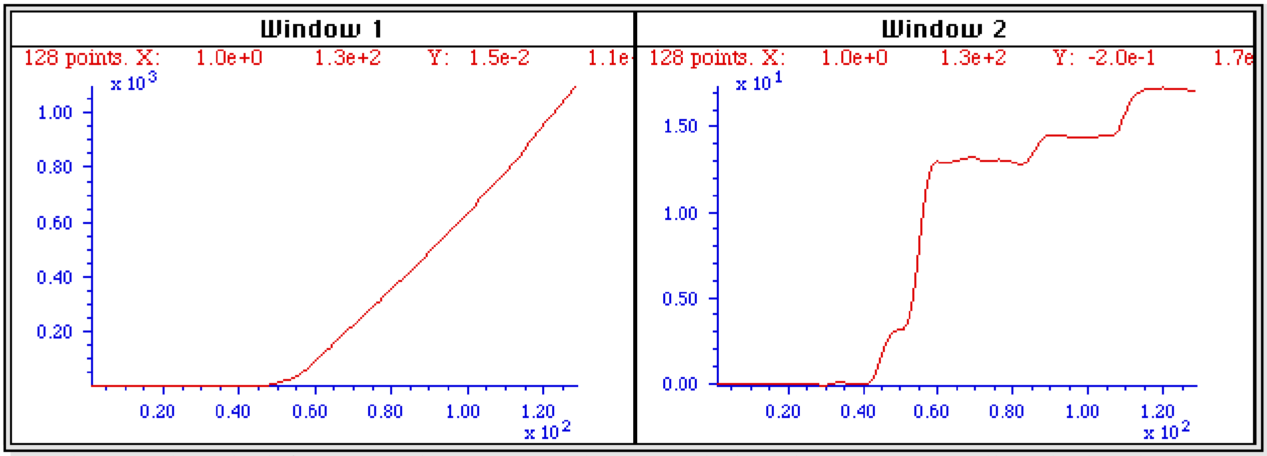
Another important property of the differentiation of peak-type signals is the effect of the peak width on the amplitude of derivatives. The four windows in the figure on the left shows the results of four successive differentiations of two computer-generated Gaussian peaks. The two peaks have the same amplitude (peak height) but second peak has exactly *twice* the width of the first. As you can see, the *wider* peak, the *smaller* derivative amplitude, and this effect becomes more noticeable at higher derivative orders. *In general, the amplitude of the* n^{th} *derivative of a peak is inversely proportional to the* n^{th} *power of its width, for signals having the same shape and amplitude.* Thus differentiation discriminates against wider peaks; and the higher the order of differentiation, the greater the discrimination. This behavior very is useful in quantitative analytical applications for detecting peaks that are superimposed on and obscured by stronger but broader background peaks. The amplitude of a derivative of a peak also depends on the *shape* of the peak and is directly proportional to its peak *height*.



Although differentiation changes the shape of *peak-type signals* drastically, a *periodic signal,* like a sine wave, behaves very differently. The derivative of a sine wave of frequency *f* is a phase-shifted sine wave of the *same frequency* and with an amplitude that is proportional to *f*, as can be shown by Wolfram Alpha. For this reason, when a music or speech signal is differentiated, the music or speech is still completely recognizable, but the low frequencies are attenuated and the high frequencies amplified (that is, it sounds "thin" or "tinny"). See page 89 for a demonstration.

Applications of Differentiation

A simple example of the application of differentiation of experimental signals is shown below.

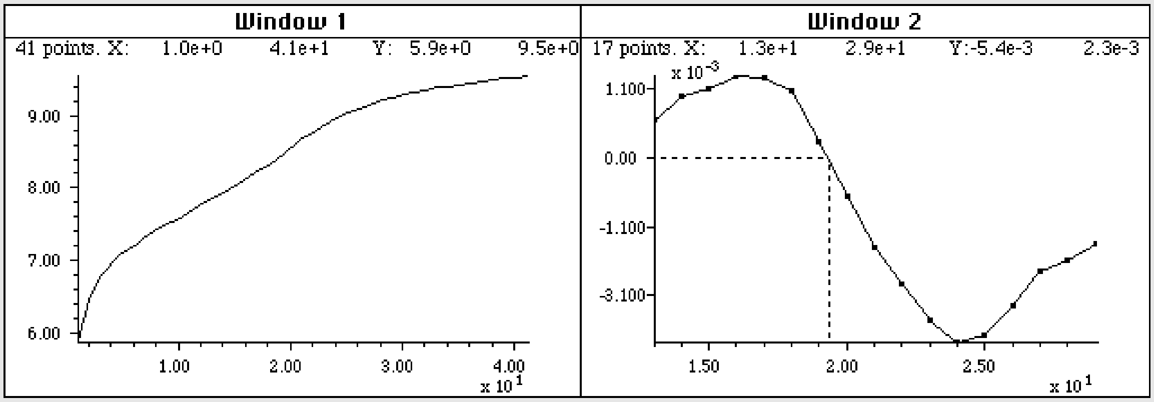


The signal on the left seems to be a more-or-less straight line, but its numerically calculated ***derivative*** *(dx/dy), plotted on the right, shows that the line actually has several approximately straight-line segments with distinctly different slopes and with well-defined breaks between each segment.*

This signal is typical of the type of signal recorded in amperometric titrations and some kinds of thermal analysis and kinetic experiments: a series of straight line segments of different slope. The objective is to determine how many segments there are, where the breaks between then fall, and the slopes of each segment. This is difficult to do from the raw data, because the slope differences are small and the pixel resolution of the computer screen display is limiting. The task is much simpler if the first derivative (slope) of the signal is calculated (Window 2). Each segment is now clearly seen as a separate step whose height (y-axis value) is the slope. The y-axis now takes on the units of dy/dx. Note that in this example the steps in the derivative signal are not completely flat, indicating that the line segments in the original signal were not perfectly straight. This is most likely due to random noise in the original signal. Although this noise was not particularly evident in the original signal, it is much typically more noticeable in the derivatives.

It is commonly observed that differentiation degrades signal-to-noise ratio, unless the differentiation algorithm includes smoothing (page 11) that is carefully optimized for each application. Numerical algorithms for differentiation are as numerous as for smoothing and must be carefully chosen to control signal-to-noise degradation.

A classic use of second differentiation in chemical analysis is in the location of endpoints in potentiometric titration. In most titrations, the titration curve has a sigmoidal shape and the endpoint is indicated by the *inflection point*, the point where the slope is maximum and the curvature is zero. The first derivative of the titration curve will therefore exhibit a *maximum* at the inflection point, and the second derivative will exhibit a *zero-crossing* at that point. Maxima and zero crossings are easier to locate precisely than inflection points.



The signal on the left is the pH titration curve of a very weak acid with a strong base, with volume in mL on the X-axis and pH on the Y-axis. The endpoint is the point of greatest slope; this is also an inflection point, where the curvature of the signal is zero. With a weak acid such as this, it is difficult to locate this point precisely from the original titration curve. The endpoint is much more easily located in the ***second derivative****, shown on the right, as the zero crossing, located in this case by graphical interpolation.*

This figure shows a pH titration curve of a very weak acid with a strong base, with volume in mL on the X-axis and pH on the Y-axis. The volumetric equivalence point (the “theoretical” endpoint) is 20 mL. The endpoint is the point of greatest slope; this is also an inflection point, where the curvature of the signal is zero. With a weak acid such as this, it is difficult to locate this point precisely from the original titration curve. The second derivative of the curve is shown on the right. The zero crossing of the second derivative corresponds to the endpoint and is much more precisely measurable. Note that in the second derivative plot, both the x-axis and the y-axis scales have been expanded to show the zero crossing point more clearly. The dotted lines show that the zero crossing is about 19.4 mL, fairly close to the theoretical value of 20 mL.

Peak Detection

Another common use of differentiation is in the detection of peaks in a signal. It's clear from the basic properties described in the previous section that the first derivative of a peak has a downward-going zero-crossing at the peak maximum, which you can use to locate the x-value of the peak. If there is *no noise* in the signal, than any data point that has lower values on both sides of it will be a peak maximum. But there is always at least a little noise in real experimental signals, and that will cause many false zero-crossings simply due to the noise. To avoid this problem, one popular technique (page 74) smooths the first derivative of the signal first, before looking for downward-going zero-crossings, and then takes only those zero crossings whose slope exceeds a certain predetermined minimum (called the "slope threshold") at a point where the original signal amplitude exceeds a certain minimum (called the "amplitude threshold"). By carefully adjusting the smooth width, slope threshold, and amplitude threshold, you try to count only the desired peaks and ignore peaks that are too small, too wide, or too narrow. Moreover, because smoothing can distort peak signals, reducing peak heights, and increasing peak widths (see page 11), this technique determines the position, height, and width of each peak by least-squares curve-fitting (page 37) of a segment of *original unsmoothed signal* in the vicinity of the zero-crossing. Even if heavy smoothing is necessary to provide reliable discrimination against noise peaks, the peak parameters extracted by curve fitting are not distorted.

Derivative Spectroscopy

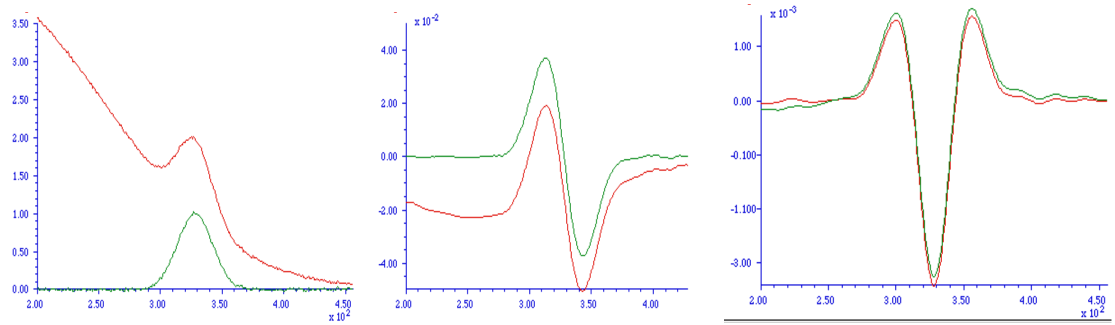
In spectroscopy, the differentiation of spectra is a widely used technique, particularly in infra-red, u.v.-visible absorption, fluorescence, and reflectance spectrophotometry, referred to as *derivative spectroscopy* (references 29-32). Derivative methods have been used in analytical spectroscopy for three main purposes:

(a) spectral discrimination, as a qualitative fingerprinting technique to accentuate small structural differences between nearly identical spectra;

(b) spectral resolution enhancement, as a technique for increasing the apparent resolution of overlapping spectral bands in order to more easily determine the number of bands and their wavelengths;

(c) quantitative analysis, as a technique for the correction for irrelevant background absorption and as a way to facilitate multicomponent analysis. (Because differentiation is a linear technique, the amplitude of a derivative is proportional to the amplitude of the original signal, which allows quantitative analysis applications employing any of the standard calibration techniques). Most commercial spectrophotometers now have built-in derivative capability. Some instruments are designed to measure the spectral derivatives optically, by means of dual wavelength or wavelength modulation designs.

Because of the fact that the amplitude of the nth derivative of a peak-shaped signal is inversely proportional to the nth power of the width of the peak, differentiation may be employed as a general way to discriminate against broad spectral features in favor of narrow components. This is the basis for the application of differentiation as a method of correction for background signals in quantitative spectrophotometric analysis. Very often in the practical applications of spectrophotometry to the analysis of complex samples, the spectral bands of the analyte (i.e. the compound to be measured) are superimposed on a broad, gradually curved background caused by the sides of off-scale peaks originating from other components or by light scattering.



This is illustrated by the figure above, which shows a simulated optical spectrum (absorbance vs wavelength in nm), with the *green curve* representing the spectrum of the pure analyte and the *red line* representing the spectrum of a mixture containing the analyte plus other compounds that give rise to the large sloping background absorption. The first derivatives of these two signals are shown in the center; you can see that the difference between the pure analyte spectrum (green) and the mixture spectrum (red) is reduced. This effect is considerably enhanced in the *second* derivative, shown on the right. In this case the spectra of the pure analyte and of the mixture are almost identical. In order for this technique to work, it is necessary that the background absorption be broader (that is, have lower curvature) than the analyte spectral peak, but this turns out to be a rather common situation. Because of their greater discrimination against broad background, second and higher-order derivatives are often used for such purposes.

It is sometimes (mistakenly) said that differentiation "increases the sensitivity" of analysis. You can see how it would be tempting to say something like that by inspecting the three figures above; it does seems that the signal amplitude of the derivatives is greater (at least graphically) than that of the original analyte signal. However, it is not valid to compare the amplitudes of signals and their derivatives because they have different units. The units of the original optical spectrum are *absorbance*; the units of the first derivative are *absorbance per nm*, and the units of the second derivative are *absorbance per nm^2*. You can't compare *absorbance* to *absorbance per nm* any more than you can compare *miles* to *miles per hour*. (It's meaningless, for instance, to say that *30 miles per hour* is greater than *20 miles*.) You can, however, compare the *signal-to-background ratio* and the *signal-to-noise ratio*. For instance, in the above example, it would be valid to say that the signal-to-background ratio is increased in the derivatives.

Loosely speaking, the opposite of differentiation is integration, so if you are given a first derivative of a signal, you might expect to regenerate the original (zeroth derivative) by integration. However, there is a catch; the constant term in original signal (like a flat baseline) is completely lost in differentiation; integration can not restore it. So strictly speaking, differentiation represents a net loss of information, and therefore differentiation is used only in situations where the constant term in the original signal is not of interest.

It is also often said that "differentiation increases the noise". Strictly speaking, that is true, but *it is not the main problem*. In fact, computing the unsmoothed first derivative of any set of random numbers increases its standard deviation merely by the square root of 2, simply due to the usual propagation of errors in the difference of two numbers. But even the *slightest* degree of smoothing applied to that derivative will reduce this standard deviation greatly. More important is that the signal-to-noise ratio of an *unsmoothed* derivative is almost always much lower (poorer) than that of the original signal, but smoothing is *always* used in any practical application to control this problem (See "The Importance of Smoothing Derivatives", page 23). Differentiation does not actually add noise to the signal; if there were no noise at all in the original signal, then the derivatives would also have no noise (except for the numerical floating point precision of the computer, usually negligible).

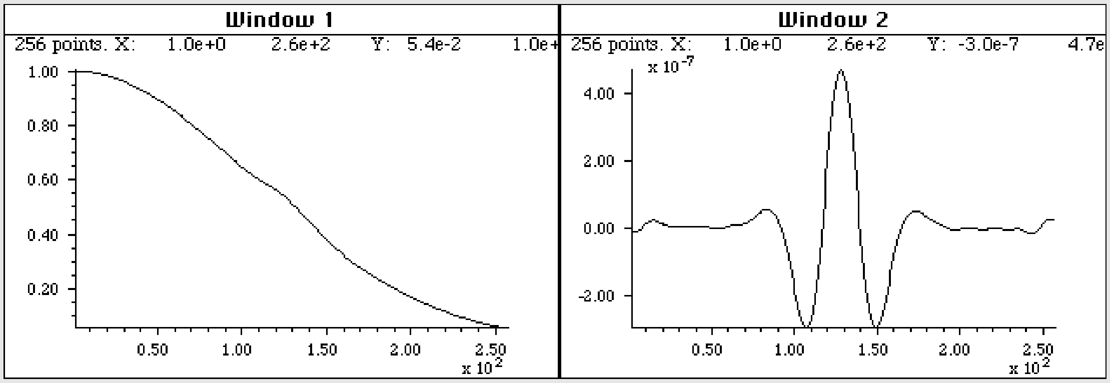
There are several ways to measure the amplitude of a derivative spectrum for quantitative analysis: the absolute value of the derivative at a specific wavelength, the value of a specific feature (such as a maximum), or the difference between a maximum and a minimum. Another widely-used technique is the zero-crossing measurement - taking readings of derivative amplitude at the wavelength where an interfering peak crosses the zero on the y (amplitude) axis. In all cases, it's important to measure the standards and the unknown samples in exactly the same way. Also, because the amplitude of a derivative of a peak depends strongly on its width, it's important to control factors that might change the spectral peak width, such as temperature, solvent properties, and spectrometer resolution.

Trace Analysis

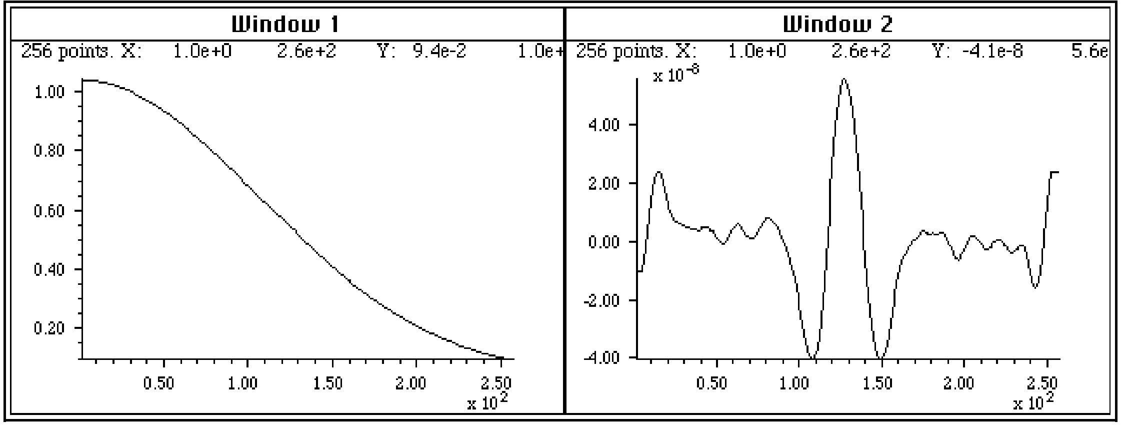
One of the widest uses of the derivative signal processing technique in practical analytical work is in the measurement of small amounts of substances in the presence of large amounts of potentially interfering materials. In such applications it is common that the analytical signals are weak, noisy, and superimposed on large background signals.

Measurement precision is often degraded by sample-to-sample baseline shifts due to non-specific broadband interfering absorption, non-reproducible sample cell positioning in the light beam, dirt or fingerprints on the cell walls, imperfect cell transmission matching, and solution turbidity. Baseline shifts from these sources are usually either wavelength-independent (light blockage caused by bubbles or large suspended particles) or exhibit a gradual wavelength dependence (small-particle turbidity). Therefore differentiation is useful to help to discriminate relevant absorption from these sources of baseline shift.

An obvious benefit of the suppression of broad background by differentiation is that *variations* in the background amplitude from sample to sample are also reduced. This can result in improved precision or measurement in many instances, especially when the analyte signal is small compared to the background and if there is a lot of uncontrolled variability in the background, which is useful when trying to detect a trace component in the presence of a strong background. For example, the spectrum on the left shows a weak shoulder near the center due to the analyte. The signal-to-noise ratio is very good in this spectrum, but in spite of that the broad, sloping background obscures the peak and makes quantitative measurement very difficult. The smoothed fourth derivative of this spectrum is shown on the right. The background has been almost completely suppressed and the analyte peak now stands out clearly, facilitating measurement.



*The optical spectrum on the left shows a weak shoulder near the center due to a small concentration of the substance that is to be measured (e.g. the active ingredient in a pharmaceutical preparation). It is difficult to measure the intensity of this peak because it is obscured by the strong background caused by other substances in the sample. The smoothed **fourth derivative** of this spectrum is shown on the right. The background has been almost completely suppressed and the analyte peak now stands out clearly.*

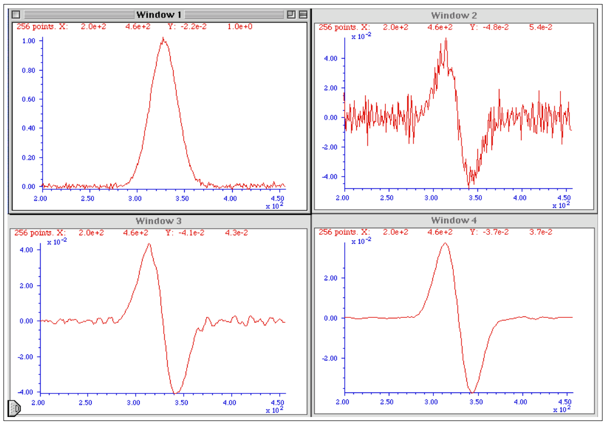


*Similar to the figure above, but in the case the peak is **10 times smaller** than previously, so that it can not even be seen in the spectrum on the left. The fourth derivative (right) shows that a peak is still there, but reduced in amplitude (note the smaller y-axis scale) and with poorer signal-to-noise ratio.*

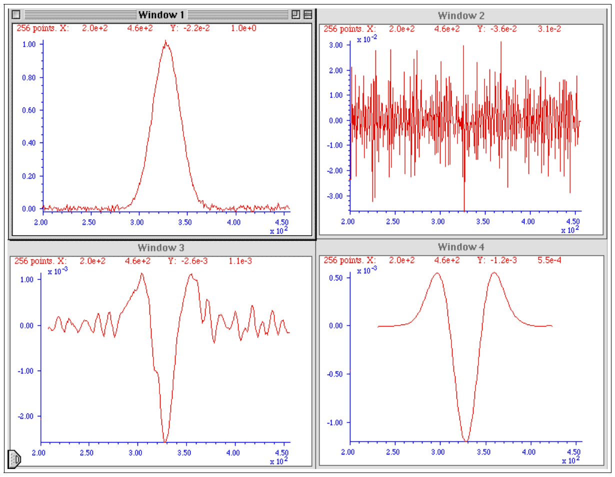
An more dramatic case is shown above. This is essentially the same optical spectrum as before, except that the concentration of the analyte is even lower. Is there even a detectable amount of analyte in this spectrum? This is quite impossible to say from the *normal* optical spectrum, but inspection of the fourth derivative (right) shows that the answer is *yes*. Some noise is clearly evident here, but even so the signal-to-noise ratio is good enough for a reasonable quantitative measurement.

This use of signal differentiation has become widely used in quantitative spectroscopy, particularly for quality control in the pharmaceutical industry. In that application the analyte would typically be the active ingredient in a pharmaceutical preparation and the background interference might arise from the presence of fillers, emulsifiers, flavoring or coloring agents, buffers, stabilizers, or other excipients (“inactive ingredients”). Of course, in trace analysis applications, care must be taken to optimize signal-to-noise ratio of the instrument as much as possible, possibly including differentiation and smoothing.

The Importance of Smoothing Derivatives



With most data, smoothing is more a matter of cosmetics (see page 14). However, for the successful application of differentiation in quantitative analytical applications, it is *essential* to use differentiation in combination with sufficient smoothing, in order to optimize the signal-to-noise ratio. This is illustrated in the figure on the left. Window 1 shows a Gaussian band with a small amount of added noise. Windows 2, 3, and 4, show the first derivative of that signal with increasing smooth widths. As you can see, without sufficient smoothing, the signal-to-noise ratio of the derivative can be substantially poorer than the original signal. However, with adequate amounts of smoothing, the signal-to-noise ratio of the smoothed derivative *can be better* than that of the unsmoothed original.



This effect is even more evident in the second derivative, as shown on the right. In this case, the signal-to-noise ratio of the unsmoothed second derivative (Window 2) is so poor you can not even see the signal visually. What is particularly interesting about the noise in these derivative signals, however, is their "*color*". This noise is not *white;* rather, it is *blue* - that is, it has much more power at high frequencies than white noise, and because of that, it is especially subject to reduction by *smoothing*.

It makes no difference mathematically whether the smooth operation is applied before or after the differentiation. What is important, however, is the nature of the smooth, its smooth ratio (ratio of the smooth width to the width of the original peak), and the number of times the signal is smoothed. When a noisy signal is differentiated, the noise in that signal is also differentiated. If the noise was *white* in the original signal (upper left quadrant), it becomes '*blue*' (page 8) in the derivative (upper right quadrant). The optimum values of smooth ratio for derivative signals is approximately 0.5 to 1.0. For a first derivative, two applications of a simple rectangular smooth or one application of a triangular smooth is adequate. For a second derivative, three applications of a simple rectangular smooth or two applications of a triangular smooth is adequate. The additional passes of smooth operators are required to provide the rapid high-frequency cut-off needed to reduce the excess blue noise. The general rule is: for the n^{th} derivative, use at least n+1 applications of rectangular smooth (or half that number of triangular smooths). Such heavy amounts of smoothing result in substantial attenuation of the derivative amplitude; in the figure above, the amplitude of the most heavily smoothed derivative (in Window 4, above) is much less than its less-smoothed version (Window 3). However, this won't be a problem, as long as the standard (analytical) curve is prepared using the exact same derivative, smoothing, and measurement procedure as is applied to the unknown samples. (Because differentiation and smoothing are both *linear* techniques, the amplitude of a smoothed derivative is proportional to the amplitude of the original signal and can be used as the basis for quantitative analysis employing any of the standard calibration techniques).

Because of the different kinds and degrees of smoothing that might be incorporated into the computation of digital differentiation of experimental signals, it's difficult to compare the results of different instruments and experiments unless the details of these computations are known. In commercial instruments and software packages, these details may well be hidden. However, if you can obtain both the original (zeroth derivative) signal, as well as the derivative and/or smoothed version of that signal from the same instrument or software package, then the technique of *Fourier deconvolution*, which will be discussed later on page 32, can be used to discover and duplicate the underlying hidden computations.

Interestingly, the failure to smooth a derivative was ultimately responsible for the crash of the first spacecraft of NASA's Mariner program on July 22, 1962, which was reported in InfoWorld's "11 infamous software bugs". In his 1968 book "The Promise of Space", Arthur C. Clarke described the mission as "...wrecked by the most expensive hyphen in history." The "hyphen" was actually *superscript bar* over a radius symbol, handwritten in a notebook, which was reportedly overlooked or misinterpreted. The overbar signifies a *smoothing or averaging function*, so the formula should have calculated the *smoothed value of the time derivative* of a radius. Without the smoothing function, even minor short-term variations in velocity could trigger the corrective boosters to kick in, causing the rocket's flight to become unstable.

SPECTRUM (page 70), for Macintosh OS 8, includes first and second derivative functions, which can be applied successively to compute derivatives of any order.

Differentiation in Spreadsheets. Differentiation operations such as described above can be performed in spreadsheets such as *Excel* or *OpenOffice Calc*. Both the derivative and the required smoothing operations can be performed by the shift-and-multiply method described in the section on smoothing. There are two approaches:

(a) You can compute each stage of differentiation and smoothing separately in successive columns, as illustrated by **DerivativeSmoothing.ods/.xls**), which computes the first derivative, smooths it, and then applies those two steps successively to compute the smoothed second and third derivatives. The variant **DerivativeSmoothingWithNoise.xlsx** shows how noise in the signal effects the result.

(b) You can combine any degree of differentiation and smoothing into one large set of shift-and-multiply coefficients, as illustrated in CombinedDerivativesAndSmooths.txt**.**

The first approach is more flexible and easier to adjust.

Another example of a derivative application is the spreadsheet **SecondDerivativeXY2.xlsx**, which locates and measures changes in the second derivative (a measure of curvature or acceleration) of a time-changing signal. This spreadsheet shows the apparent increase in noise caused by differentiation and the extent to which the noise can be reduced by smoothing (in this case by two passes of a 5-point triangular smooth). In this example, the smoothed second derivative shows a large peak the point at which the acceleration changes (at x=30) and plateaus on either side showing the magnitude of the acceleration before and after the change (y=2 and 4, respectively). Download any of these spreadsheets from **http://tinyurl.com/cey8rwh**.

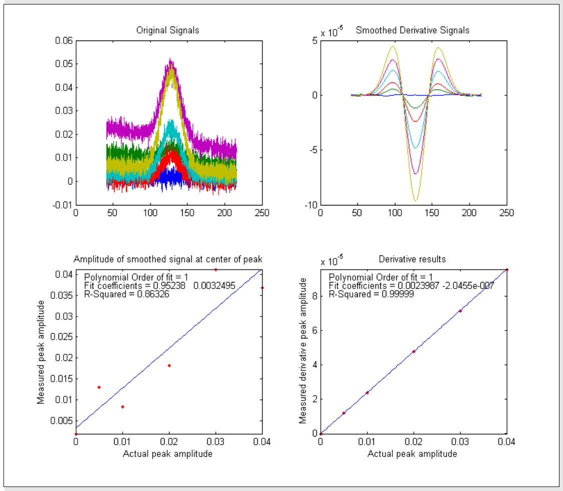
In **Matlab** or in **Octave**, differentiation functions such as described above can easily be created. Some simple examples that you can download include: deriv, a first derivative using the 2-point central-difference method, deriv2, a simple second derivative using the 3-point central-difference method, a third derivative deriv3 using a 4-point formula, and deriv4, a 4th derivative using a 5-point formula. Each of these is a simple function of the form `d=deriv(a)`; the input argument is a signal vector "**a**", and the differentiated signal is returned as the vector "**d**". There are versions of the first and second derivative functions, derivxy and secderivxy, that take *two* input arguments (x,y), where **x** and **y** are vectors containing the independent and dependent variables; use these for data that are *not* equally-spaced on the independent variable (x) axis.

The following code shows how to use the first derivative to find all the maxima in a smooth x,y data set by locating the points of *zero-crossing*, that is, the points at which the first derivative **d** (computed by `derivxy.m`) passes from positive to negative. In this example, the "sign" function is a built-in Matlab/Octave function that returns 1 if the element is greater than zero, 0 if it equals zero and -1 if it is less than zero. The “disp” function in the 4th line prints out the value of **x** and **y** at each zero-crossing:

```
d=derivxy(x,y);
 for j=1:length(x)-1
    if sign(d(j))>sign(d(j+1))
      disp([x(j) y(j)])
    end
 end
```

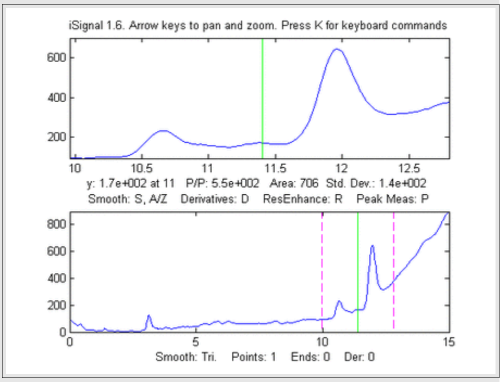
If the data are noisy, many false zero crossings will be reported; smoothing the data will reduce that. If the data are sparsely sampled, a more accurate value for the peak position (x-axis value at the zero crossing) can be obtained by interpolating between the two points before and after the zero-crossing using the Matlab/Octave “interp1” function: `interp1([d(j) d(j+1)],[x(j) x(j+1)],0);`

Derivative and smoothing functions are easily combined by “nesting” the basic functions. For example, `deriv(fastsmooth(y,w,type,edge))` computes the smoothed first derivative of the vector **y**, with a smooth of width “w” and type “type”. The value of “type” should be *one more than the derivative order* to insure that high-frequency noise is reduced, so that if you want to compute smoothed derivatives of higher order, you must nest multiple smooth operations so the sum of the “type” values is at least one more than the derivative order. Alternatively, you can use **ProcessSignal.m**, a Matlab/Octave command-line function that performs variable smoothing and differentiation on the time-series data set x,y (column or row vectors). Type "help ProcessSignal". It returns the processed signal as a vector that has the same shape as x, regardless of the shape of y. The syntax is `Processed=ProcessSignal(x,y,DerivativeMode,w,type,ends,Sharpen,factor1,factor2,SlewRate,MedianWidth)`.



DerivativeDemo.m (shown on the left) is a self-contained Matlab/Octave demo function that demonstrates an application of differentiation to the quantitative measurement of a peak buried in an *unstable background* that shifts up and down (e.g. as in various forms of spectroscopy). The objective is to derive a measure that varies *linearly* with the peak amplitude but that is *minimally effected* by the background and the noise. To run it, just type DerivativeDemo at the command prompt. You can change several of the internal variables (e.g. Noise, BackgroundAmplitude) to make the problem harder or easier. Note that, despite the fact that the *magnitude* of the derivative is numerically smaller than the original signal (because it has *different units*), the *signal-to-noise ratio* of the derivative is better because it is much less effected by the background instability.

iSignal (page 85, screen image shown on the right) performs differentiation and smoothing for time-series signals, up to the 5th derivative, using the fastsmooth and *Savitzky-Golay* algorithms, plus several other signal-processing functions described in this essay. It has simple keystrokes that allow you to adjust the differentiation and smoothing parameters continuously while observing the effect on your signal (Press **K** for a list).


iSignal 1.6. Arrow keys to pan and zoom. Press K for keyboard commands

y: 1.7e+002 at 11   P/P: 5.5e+002   Area: 706   Std. Dev.: 1.4e+002

Smooth: S, A/Z   Derivatives: D   ResEnhance: R   Peak Meas: P

Smooth: Tri.   Points: 1   Ends: 0   Der: 0

Derivative-based Peak Finding and Measurement

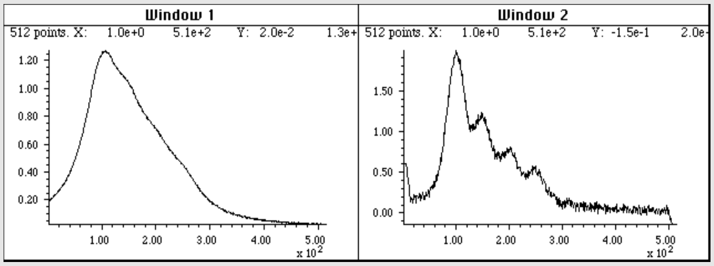
Page 74 describes a Matlab/Octave algorithm for locating and measuring the peaks in noisy time-series data sets which detects peaks by looking for downward zero-crossings in the smoothed first derivative, and determines the position, height, and width of each peak by least-squares curve-fitting. (This is useful primarily for signals that have several data points in each peak, not for spikes that have only one or two points). It can find and measure 1000 peaks in a 1,000,000 point signal in 8 seconds. The routine is available in several different versions: including command-line functions (page 74); an interactive keypress-operated function **(ipeak.m)** for adjusting the peak detection criteria in real-time to optimize for any particular peak type (page 78); and a spreadsheet (page 83) in Excel and OpenOffice formats. See Software Details 3, page 74-84. You may download any of these functions or spreadsheets from **http://tinyurl.com/cey8rwh**.

Peak Sharpening (Resolution enhancement)

The figure below shows a signal that consists of several poorly-resolved bands. The extensive overlap of the bands makes the accurate measurement of their intensities and positions impossible, even though the signal-to-noise ratio is very good. Things would be easier if the bands were more completely resolved, that is, if the bands were narrower. Here use can be made of *peak sharpening* algorithms to artificially improve the apparent resolution of the peaks. One of the simplest such algorithms is based on the weighted sum of the original signal and the *negative* of its *2nd derivative*:

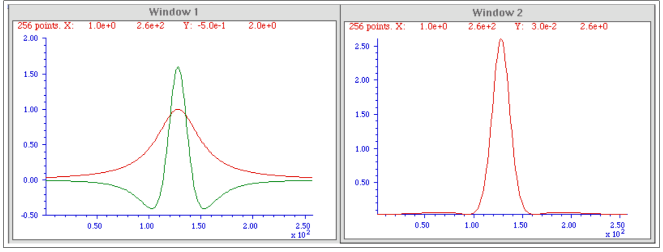
$$R_j = Y_j - kY_j''$$

where $\mathbf{R_j}$ is the peak sharpened signal, **Y** is the original signal, **Y''** is the second derivative of **Y**, and **k** is a user-selected weighting factor. The key is selecting the weighting factor **k** that gives the best trade-off between peak sharpening, signal-to-noise degradation, and baseline undershoot. The optimum choice depends upon the width, shape, and digitization interval of the signal. A reasonable srarting value for **k** is $w^2/25$ for peaks of Gaussian shape, or $\boldsymbol{w}^2/6$ for peaks of Lorentzian shape, where ***w*** is the number of data points in the half-width of the component peaks.



*A **peak sharpening** algorithm has been applied to the signal on the left to artificially improve the apparent resolution of the peaks. In the resulting signal, right, the component bands are narrowed so that the intensities and positions can be measured.*

The result of the application of this algorithm is shown on the right in the figure above. The component bands have been artificially narrowed so that the intensities and positions can be measured. However, the signal-to-noise ratio is visibly degraded.



Here's how it works. The figure above shows, in Window 1, a computer-generated peak (with a Lorentzian shape) in red, superimposed on the *negative* of its second derivative in green). The second derivative is amplified (by multiplying it by an adjustable constant) so that the negative sides of the inverted second derivative (from approximately X = 0 to 100 and from X = 150 to 250) are a mirror image of the sides of the original peak over those regions. In this way, when the original peak is added to the inverted second derivative, the two signals will approximately cancel out in the two side regions but will reinforce each other in the central region (from X = 100 to 150). The result, shown in Window 2, is a substantial (about 50%) reduction in the width, and an increase in height, of the peak. This works best with Lorentzian shaped peaks; with Gaussian-shaped peaks, the effect is less dramatic (only about 20%). An interesting property of this procedure is that *it does not change the total peak area* (that is, the area under the peak) because the total area under the curve of the derivative of a peak-shaped signal is zero - the area under the negatives lobes cancels the area under the positive lobes. As a result, this technique can be useful in measuring the areas under overlapped peaks (page 35). However, this technique is not perfect because the baseline on either side of the sharpened peak is not completely flat, leaving some interference from nearby peaks. For a Lorentzian peak, about 80% of the area of the peak is contained in the central maximum. For a Gaussian peak, over 99% of the area of the peak is contained in the central maximum.

Because differentiation and smoothing are both linear techniques, the amplitude of a sharpened signal is therefore proportional to (but usually greater than) the amplitude of the original signal, which allows quantitative analysis applications employing any of the standard calibration techniques). *As long as you apply the same signal-processing techniques to the standards as well as to the samples*, everything works.

Note: Another technique that can increase the resolution of overlapping peaks is Fourier deconvolution (page 32), which is applicable when the broadening function responsible for the overlap of the peaks is known. Deconvolution of the broadening function from the broadened peaks is in principle capable of extracting the underlying peaks shapes, whereas this resolution enhancement technique can not be expected to do that.

SPECTRUM (page 70) includes this simple peak sharpening algorithm, with adjustable weighting factor and derivative smoothing width.

Peak sharpening in Matlab and Octave. The user-defined **Matlab/Octave** function **enhance.m** uses a slightly more advanced algorithm that extends the above approach by adding in a small amount of the 4th derivative of the signal:

$$R = Y - k_2Y'' + k_4Y''''$$

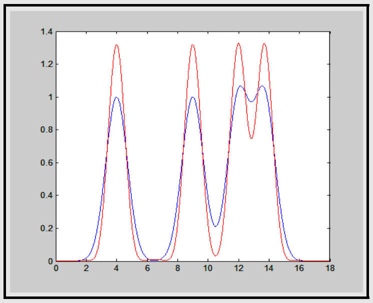
This function has the form:

```
  Enhancedsignal=enhance(signal,k2,k4,SmoothWidth)
```

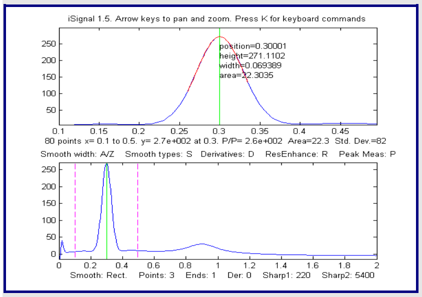
where `signal` is the original signal vector, the arguments `k2` and `k4` are 2nd and 4th derivative weighting factors, and `SmoothWidth` is the width of the built-in smooth. The peak sharpened signal is returned in the vector `Enhancedsignal`.

Here's a simple example for Matlab or Octave that creates a signal consisting of four Gaussian peaks, applies the enhance function, and compares a plot (shown on the right) of the original signal (in blue) to the sharpened version (in red):

```
  x=[0:.01:18];
  y=exp(-(x-4).^2)+exp(-(x-9).^2)+exp(-(x-12).^2)+exp(-(x-13.7).^2);
  SharpenedSignal=enhance(y,1000,1000000,3)
  plot(x,y,x,SharpenedSignal,'r')
```



Peak sharpening can be a useful pre-process before measuring the areas under overlapping peaks (page 35), because it's easier and more accurate to measure the areas of peaks that are more completely separated, and because in principle this method of sharpening does not change the total area under each peak.


iSignal 1.5. Arrow keys to pan and zoom. Press K for keyboard commands

position=0.30001
height=271.1102
width=0.069389
area=22.3035

80 points x= 0.1 to 0.5. y= 2.7e+002 at 0.3. P/P= 2.6e+002 Area=22.3 Std. Dev.=82

Smooth width: A/Z Smooth types: S Derivatives: D ResEnhance: R Peak Meas: P

Smooth: Rect. Points: 3 Ends: 1 Der: 0 Sharp1: 220 Sharp2: 5400

iSignal (page 85) can perform peak sharpening for time-series signals, using the above **enhance** function, with keystrokes that allow you to adjust the 2nd and 4th derivative weighting factors and the smoothing continuously while observing the effect on your signal dynamically. The **E** key turns the peak sharpening function on and off. View the code here or download the ZIP file with sample data for testing. *iSignal* calculates the sharpening and smoothing settings for Gaussian and for Lorentzian peak shapes using the **Y** and **U** keys, respectively. Just isolate a single typical peak in the upper window using the pan and zoom keys, then press **Y** for Gaussian or **U** for Lorentzian peaks. (The optimum settings depends on the width of the peak, so if your signal has peaks of widely different widths, one setting will not be optimum for all the peaks). You can fine tune the sharpening with the **F/V** and **G/B** keys and the smoothing with the **A/Z** keys. ***iPeak*** (page 78) also has a peak sharpening mode.

Harmonic analysis and the Fourier Transform

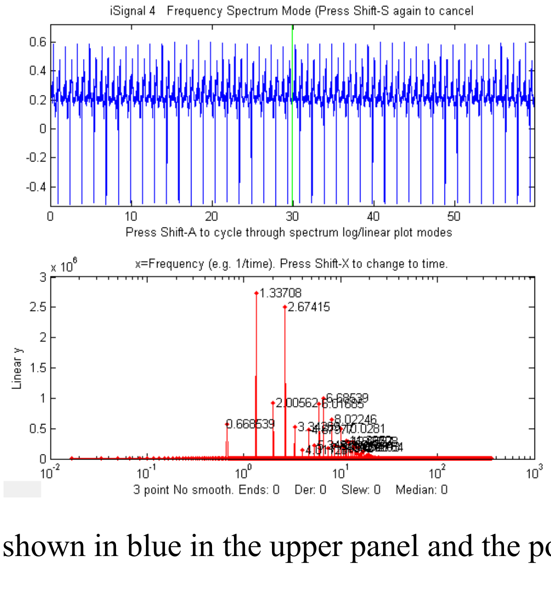
Some signals exhibit periodic components that repeat at fixed intervals throughout the signal, like a sine wave. It is often useful to describe the amplitude and frequency of such periodic components exactly. Actually, you can analyze *any* arbitrary set of data into periodic components.

Harmonic analysis is conventionally based on the *Fourier transform*, which is a way of expressing a signal as a sum of sine and cosine waves. It can be shown that any arbitrary discretely sampled signal can be described completely by the sum of a finite number of sine and cosine components whose frequencies are 0,1,2,3 ... **n**/2 times the fundamental frequency **f**=1/**n**Δx, where Δx is the interval between adjacent x-axis values and **n** is the total number of points. The Fourier transform is the set of coefficients of those sine and cosine components. You could calculate those coefficients yourself just by multiplying the signal point-by-point with each of those sine and cosine components and adding up the products. The famous "Fast Fourier Transform" (FFT) is just a faster and more efficient algorithm that makes use of the symmetry of the sine and cosine functions and other math shortcuts to get the same result much more quickly. The *inverse* Fourier transform (IFT) is a similar algorithm that converts a Fourier transform back into the original signal. The "power spectrum" (not to be confused with an *optical* spectrum) combines the absolute value of the sine and cosine components at each frequency. This is a convenient way to display the total power at each frequency, but it discards the phase information (the relative contribution of sine and cosine components), so for that reason it's impossible to reconstruct the waveform completely from the power spectrum alone.

Two important instrumental methods of chemical analysis are based on the concept of the Fourier transform (FT). In Fourier transform infrared spectroscopy (FTIR), the Fourier transform of the infrared spectrum is measured directly by the instrument, as the interferogram formed by recording the detector signal vs mirror displacement in a scanning Michelson interferometer. In Fourier transform nuclear magnetic resonance spectroscopy (FTNMR), excitation of the sample by a short pulse of radio frequency energy produces a free induction decay signal that is the Fourier transform of the resonance spectrum. In both cases the spectrum is the *inverse* FT of the measured signal.

A time-series signal with *n* points gives a power spectrum with only (*n*/2)+1 points. The first point is the zero-frequency (constant) component. The second point corresponds to a frequency of 1/*n*Δx (which has a single cycle over the signal's duration), the next point to 2/*n*Δx, etc., where Δx is the interval between the x-axis values and *n* is the total number of points. The last (highest frequency) point in the power spectrum (*n*/2)/*n*Δx=1/2Δx, which is one-half the sampling rate. The *highest* frequency that can be represented in a discretely-sampled waveform is o*ne-half the sampling frequency*, which is called the *Nyquist frequency*; attempts to sample frequencies *above* the Nyquist frequency are "folded back" to lower frequencies, severely distorting the signal.

A pure sine or cosine wave with an exactly integral number of cycles within the recorded signal will have a *single non-zero Fourier component* corresponding to its frequency. Conversely, a signal consisting of zeros everywhere except at a single point, called a delta function, has *equal* Fourier components at all frequencies. *Random noise* also has a power spectrum that is spread out over a wide frequency range according to its *noise color* (page 8), with *pink* noise having more power at *low* frequencies, *blue* noise having more power at *high* frequencies, and *white* noise having roughly the *same* power at all frequencies.



iSignal 4   Frequency Spectrum Mode (Press Shift-S again to cancel

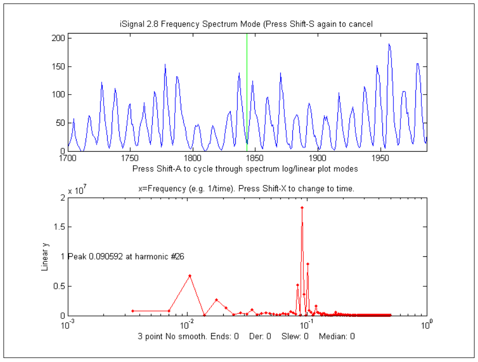
Press Shift-A to cycle through spectrum log/linear plot modes

x=Frequency (e.g. 1/time). Press Shift-X to change to time.

3 point No smooth. Ends: 0   Der: 0   Slew: 0   Median: 0

For periodic waveforms that repeat over time, a single period is the smallest repeating unit of the signal, and the reciprocal of that period is called the fundamental frequency. Complex periodic waveforms usually exhibit a series of frequency components that are *multiples* of the fundamental frequency; these are called "*harmonics*". A familiar example is the electrical recording of a heartbeat, called an electrocardiograph (ECG), which consists of a highly repeatable series of waveforms. In the real data example on the left, the ECG waveform is shown in blue in the upper panel and the power spectrum is shown in red in the lower panel. The fundamental frequency is 0.6685 Hz and the other peaks are the *harmonics*. Recorded vocal sounds, especially vowels, also have a periodic waveform with harmonics. Another example of an extremely regular variation is the seasonal change in average daily temperature, which has a sharp peak at exactly one year, plus random white noise throughout, which is distributed evenly over the frequency spectrum. *The sharpness of the peaks in these spectra shows that the amplitude and frequency are nearly constant over the recording interval in this example.* Changes in amplitude or frequency over the recording interval will produce clusters or bands of Fourier components rather than sharp peaks, such as in the sunspot data below and in the sound of a passing car horn shown on page 118.

The figures below show a historic example of harmonic analysis: the annual variation in the number of observed sunspots, which have been recorded *since the year 1700!* In this case the time axis is in *years*. A plot of the power spectrum (bottom window) shows a strong peak at 0.09 cycles/year. If the data are plotted (on the bottom right) with *time* on the x-axis, the plot more clearly shows the well-known *11-year sunspot cycle* (plus some evidence of a weaker cycle at around a 100-year period). These power spectra were plotted by pressing **Shift-S** in ***iSignal*** (see page 85). The zeroth harmonic (the DC component) is not shown. In this case the peaks in the frequency spectrum are *not* sharp single peak at 11, but rather form a *cluster* of Fourier components, because the amplitude and frequency are not perfectly constant over the 300-year interval of the data.



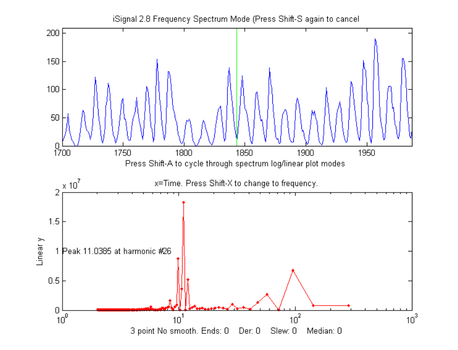
iSignal 2.8 Frequency Spectrum Mode (Press Shift-S again to cancel

Press Shift-A to cycle through spectrum log/linear plot modes

x=Frequency (e.g. 1/time). Press Shift-X to change to time.

Peak 0.090592 at harmonic #26

3 point No smooth. Ends: 0 Der: 0 Slew: 0 Median: 0



iSignal 2.8 Frequency Spectrum Mode (Press Shift-S again to cancel

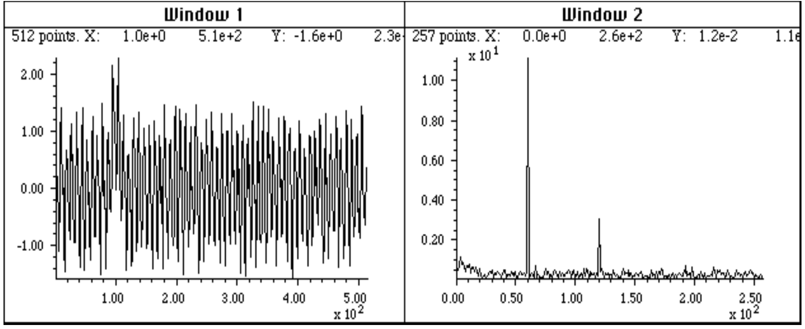
Press Shift-A to cycle through spectrum log/linear plot modes

x=Time. Press Shift-X to change to frequency.

Peak 11.0385 at harmonic #26

3 point No smooth. Ends: 0 Der: 0 Slew: 0 Median: 0

A common application of the power spectrum is as a diagnostic tool to distinguish signal and noise. Peak-type signals have power spectra that are concentrated in a range of low frequencies, whereas noise is often spread out over a much wider frequency range.



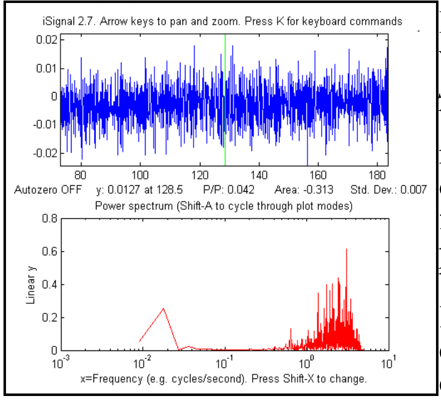
Window 1

512 points. X: 1.0e+0 5.1e+2 Y: -1.6e+0 2.3e

Window 2

257 points. X: 0.0e+0 2.6e+2 Y: 1.2e-2 1.1e

The signal on the right (x = time; y = voltage), which was expected to contain a single peak near x=100, is clearly very noisy. The ***power spectrum*** *of this signal (x = frequency in Hz) shows the signal peak at low frequencies (0-20 Hz) and a strong narrow component at 60 Hz, suggesting that much of the noise is caused by stray pick-up from the 60 Hz power line frequency used in the USA (50 Hz in some countries). The peak at 120 Hz (the second harmonic of 60 Hz) comes from the same source. A smaller amount of white noise is distributed evenly over the spectrum.(Again, the sharpness of the peaks in the spectrum shows that the amplitude and the frequency are very constant; power companies keep the frequency of the AC power constant to avoid problems between sections of the power grid).*



iSignal 2.7. Arrow keys to pan and zoom. Press K for keyboard commands

Autozero OFF y: 0.0127 at 128.5 P/P: 0.042 Area: -0.313 Std. Dev.: 0.007

Power spectrum (Shift-A to cycle through plot modes)

x=Frequency (e.g. cycles/second). Press Shift-X to change.

In the example illustrated on the left, the signal (in the top window) contains no visually evident periodic components; it *seems* to be only random noise. But the frequency spectrum (in the bottom window) shows that there is *much more to this signal* than meets the eye. There are in fact *two* major frequency components: one at *low* frequencies around 0.02 and a stronger one at *high* frequencies between 0.5 and 5. (If the x-axis units of the signal plot is in *seconds*, the units of the frequency spectrum plot would be *cycles per second*; note that the x-axis in this example is logarithmic). In this particular case, the *lower* frequency component is in fact the signal, and the *higher* frequency component is residual “blue” noise left over from previous signal processing. The two components are well separated on the frequency axis, suggesting that low-pass filtering (i.e. smoothing) will able to reduce the noise without distorting the signal.

You can compute the Fourier transform and power spectrum only of time series, but of *any* signal, such as an optical spectrum, where the independent variable might be *wavelength* or *wavenumber*, or an electrochemical signal, where the independent variable might be *volts*. The units of the x-axis of the power spectrum are simply the *reciprocal* of the units of the x-axis of the original signal.

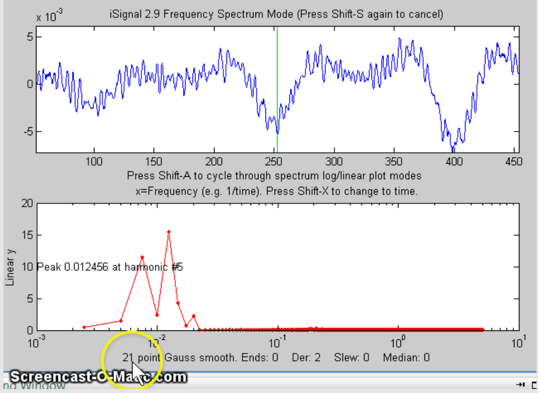
Harmonic analysis provides another way to understand signal-to-noise ratio, filtering, smoothing, and differentiation. Smoothing (page 11) is a form of l*ow-pass filtering,* which reduces the high-frequency components of a signal. If a signal consists of smooth features, such as Gaussian peaks, then its power spectrum will be concentrated at *low* frequencies. If that signal is contaminated with white noise (which is spread out evenly over all frequencies), then smoothing will make the signal *look* better, because it reduces the *high-frequency* components of the noise. However, the *low*-frequency noise will remain in the signal after smoothing (page 13), where it will continue to interfere with the measurement of signal parameters such as peak heights, positions, widths, and areas, as can be demonstrated by least-squares measurement (page 69).

Conversely, differentiation (page 17) is a form of *high-pass* filtering, which reduces the *low* frequency components of a signal and emphasizes any *high*-frequency components in the signal. As successive orders of differentiation are applied, the frequency spectrum shifts progressively to higher frequencies. So the optimum range for signal information of a *differentiated signal* is restricted to an intermediate frequency range, with little useful information above and below that range. Real experimental signals are often contaminated with drift and baseline shift, which are essentially *low-frequency* effects, and with random noise, which is usually spread out over a*ll frequencies*. For these reasons, differentiation is *always* used in conjunction with smoothing. Working together, they act as a kind of frequency-selective *bandpass* filter that optimally passes the band of frequencies containing the signal information but reduces both the *lower-frequency* drift and background, as well as the *high-frequency* noise. DerivativeDemo on page 25 is an example. See page 119 for another example.

SPECTRUM (page 70) includes a power spectrum function and forward and reverse Fourier transforms. The freeware program **Audacity** has frequency spectrum plotting and visualization for audio signals.

Matlab and **Octave** have built-in functions for computing the Fast Fourier Transform and its inverse (FFT and IFFT). A "Slow Fourier Transform" function has also been published. Or you can use my downloadable function **PlotFrequencySpectrum.m** that can plot frequency spectra and periodograms on linear or log coordinates. Type "help PlotFrequencySpectrum" and try the example there.

iSignal (page 86, 89-90) has a **Frequency Spectrum** mode, toggled on and off by **Shift-S**, that computes the frequency spectrum of the segment of the signal isolated in the upper window and displays it in the lower window. (**iSignal** created the three figures above). The pan and zoom keys adjust the region of the signal to be viewed. **Shift-A** cycles through four plot modes (linear, semilog X, semilog Y, or log-log) and **Shift-X** toggles between *frequency* on the x axis and *time* on the x-axis. **Shift-Z** locates the peaks in the spectrum and labels them with their frequency or time. *All signal processing functions remain active in the frequency spectrum mode* (smooth, derivative, etc) so you can observe the effect of these functions on the frequency spectrum of the signal. Press **Shift-S** again to return to the normal mode. See pages 118-120 for some examples. The script "**iSignalDeltaTest**" demonstrates the power spectrum of the smoothing and differentation functions of **iSignal** applied to a delta function, whose power spectrum is *flat*. Change the smooth type, smooth width, and derivative order and see how the power spectrum changes. The power spectrum of a *processed* delta function is the frequency response of the *process itself*. An experiment on page 89 uses **iSignal** to demonstrate the effect of smoothing and differentiation on digitized speech.


iSignal 2.9 Frequency Spectrum Mode (Press Shift-S again to cancel)

Press Shift-A to cycle through spectrum log/linear plot modes
x=Frequency (e.g. 1/time). Press Shift-X to change to time.

Peak 0.012456 at harmonic #5

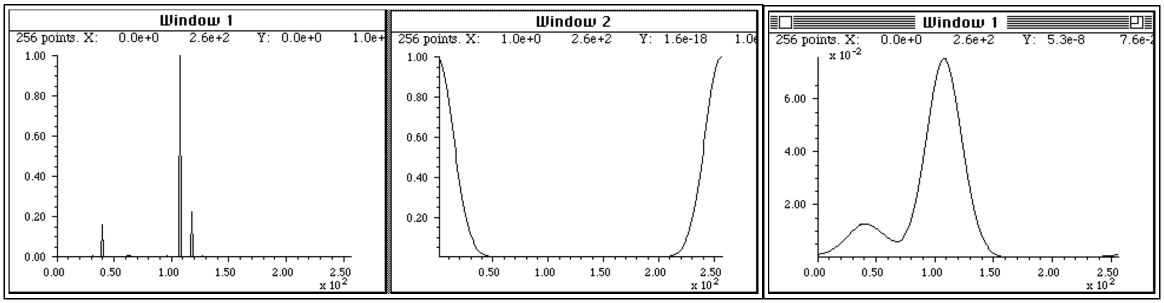
21 point Gauss smooth. Ends: 0 Der: 2 Slew: 0 Median: 0

iPower is a keyboard-controlled Matlab interactive power spectrum demonstrator for teaching and learning about the power spectra of different types of signals and the effect of *signal duration* and *sampling rate*. Single keystrokes allow you to select the type of signal, the total duration of the signal, the sampling rate, and the characteristics of the 12 different signals. Press **K** to see a list of all the keyboard commands. See **http://tinyurl.com/cey8rwh**.

Convolution

Convolution, in mathematics and signal processing, is an operation performed on two signals the “shift-and-multiply” technique, which involves multiplying one signal point-by-point by a delayed version of another signal, integrating or averaging the product, and repeating the process for different delays. Convolution is a useful process because it accurately describes some effects that occur widely in scientific measurements, such as the influence of a low-pass filter on an electrical signal or of the resolution of an optical spectrometer on the shape of the recorded optical spectrum.

The calculation is often performed by multiplying the two signals point-by-point in the Fourier domain. First, the Fourier transform of each signal is obtained. Then the two Fourier transforms are multiplied by the rules for complex multiplication, and the result is then inverse Fourier transformed. This is faster then the shift-and-multiply algorithm when the number of points in the signal is large.



The figure above shows how Fourier convolution can be used to determine how the optical spectrum on the left will appear when scanned with a spectrometer whose slit function (spectral resolution) is described by the Gaussian function in the center figure (which has been shifted so that its maximum falls at x=0). The resulting convoluted signal (right) shows that the two lines near x=110 and 120 will not be resolved but the line at x=40 will be partly resolved. (Fourier convolution is used in this way to correct the analytical curve non-linearity caused by spectrometer resolution, in the “Tfit” method for absorption spectroscopy, described on page 103).

Fourier convolution is the basis for a very general algorithm for the smoothing and differentiation of digital signals, by convoluting the signal with a (usually) small set of numbers representing the *convolution vector*. Smoothing is performed by convolution with sets of positive numbers, e.g. [1 1 1] for a 3-point boxcar. Convolution with [–1 1] computes a first derivative; [1 -2 1] computes a second derivative. Successive convolutions by two convolution vectors *Conv1* and then *Conv2* is equivalent to *one* convolution with the *convolution of Conv1 and Conv2*. First differentiation with smoothing is done by using a convolution vector in which the first half of the coefficients are negative and the second half are positive (e.g.[-1 -2 0 2 1]). Further examples are given in the text file “Convolution.txt” (download from http://tinyurl.com/cey8rwh).

SPECTRUM (page 70) includes convolution and auto-correlation (self-convolution) functions.

Spreadsheets can perform convolution by the shift-and-multiply technique. For some examples of the application of shift-and-multiply convolution to smoothing and differentiation, see “**MultipleConvolution.xls/.ods** (download from **http://tinyurl.com/cey8rwh**). For large data sets, Fourier convolution is faster than the shift-and-multiply technique.

Matlab and **Octave** have built-in Fourier convolution function: conv, which can be used to create very general type of filters and smoothing operations applied to a signal vector *y*. (The keyword 'same' returns the central part of the convolution that is the same size as y):

`ysmoothed=conv(y,[1 1 1 1 1],'same')/5` for a 5-point sliding-average smooth or
`ysmoothed=conv(y,[1 2 3 2 1],'same')/9` for a 5-point triangular smooth.

A simple RC low-pass filter applied to the signal in vector *y* can be simulated by:

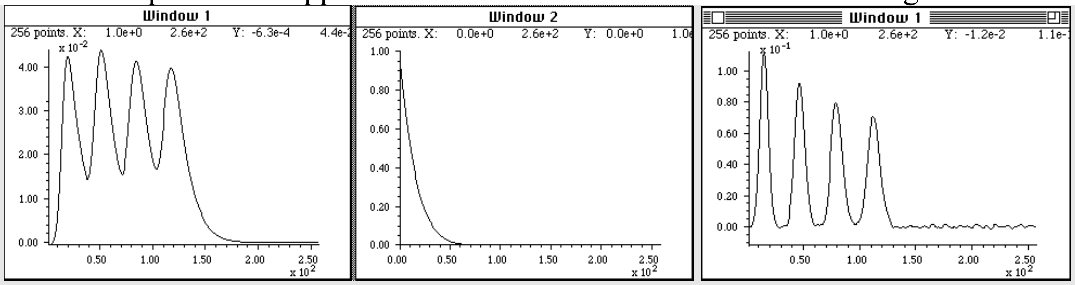
```
c=exp(-(1:length(y))./30); yc=conv(y,c,'full')./sum(c);
```

(In these examples, division by the sum of the convolution transfer function *c* insures that the convolution does not change the area under the curve of the signal). Alternatively, you could perform the convolution yourself *without* using the built-in Matlab/Octave "conv" function by multiplying the Fourier transforms of *y* and *c* using the "fft.m" function, and then inverse transform the result with the "ifft.m" function: `yc=ifft(fft(y).*fft(c))./sum(c);`. The results are essentially the same (except for the numerical floating point precision of the computer, which is usually negligible) and the elapsed time is actually less than using the conv function.

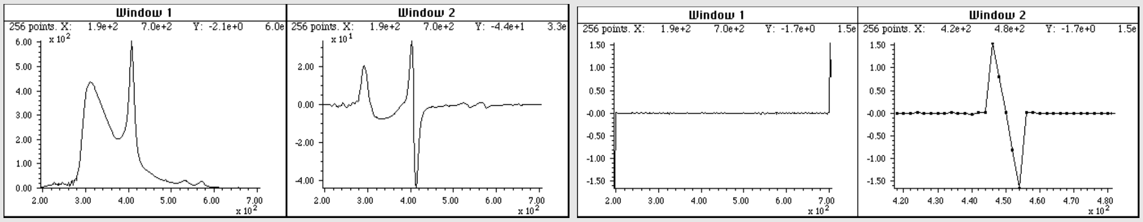
Deconvolution

Deconvolution is the converse of convolution in the sense that division is the converse of multiplication. If you know that ***m*x*** equals ***n***, where ***m*** and ***n*** are known but ***x*** is unknown, then ***x*** equals ***n/m***. Similarly, if you know that ***m*** convoluted with ***x*** equals ***n***, where ***m*** and ***n*** are known but ***x*** is unknown, then ***x*** equals ***m*** deconvoluted from ***n***. In practice, the deconvolution of one signal from another is usually performed by *point-by-point division* of the two signals in the Fourier domain - dividing the Fourier transforms of the two signals and then inverse-transforming the result.

The practical significance of deconvolution is that it can be used as an artificial (i.e. computational) way to reverse the result of a convolution occurring in the physical domain, for example, to reverse the signal distortion effect of an electrical filter or of the finite resolution of an optical spectrometer. Two different examples of the application of deconvolution are shown in the figures below.



***Deconvolution** is used here to remove the distorting influence of an exponential tailing transfer function from a recorded signal (Window 1, left) that is the result of an unavoidable RC low-pass filter action in the electronics. The transfer function (Window 2, center) must be known and is usually either calculated on the basis of some theoretical model or is measured experimentally as the output signal produced by applying an impulse (delta) function to the input of the system. The response function, with its maximum at x=0, is deconvoluted from the original signal . The result (right) shows a closer approximation to the real shape of the peaks; however, the signal-to-noise ratio is unavoidably degraded. (cf. page 121).*



A different application of the deconvolution function reveals the nature of a hidden data transformation function that has been applied to a data set by the measurement instrument itself. In this example, the first signal (from left to right) is a uv-visible absorption spectrum recorded on a commercial photodiode array spectrometer (X-axis: nanometers; Y-axis: milliabsorbance). The second signal is the first derivative of this spectrum produced by an (unknown) algorithm in the software supplied with the spectrometer. The objective here is to understand the nature of the differentiation/ smoothing algorithm that the instrument's software uses. The third signal is the result of deconvoluting the derivative spectrum (second from left) from the original spectrum (left). This therefore must be the convolution function used by that differentiation algorithm. Shifting and expanding it on the x-axis makes the function easier to see (last signal). Expressed in terms of the smallest whole numbers, the convolution series is seen to be +2, +1, 0, -1, -2. This simple example of "reverse engineering" makes it possible to compare results from other instruments or to duplicate these result on other equipment.

When applying deconvolution to experimental data, to remove the effect of a known broadening or low-pass filter operator caused by the experimental system, a serious signal-to-noise degradation commonly occurs. Any noise added to the signal by the system *after* the convolution by the broadening or low-pass filter operator will be greatly amplified when the Fourier transform of the signal is divided by the Fourier transform of the broadening operator, because the high frequency components of the broadening operator (the denominator in the division of the Fourier transforms) are typically very small, resulting in a great amplification of *high frequency* ('blue') noise in the resulting deconvoluted signal (page 8). See the code example at the bottom of page 33 and on page 121. This can be controlled but not completely eliminated by smoothing and by constraining the deconvolution to a frequency region where the signal has a sufficiently high signal-to-noise ratio. You can see this noise amplification happening in the example in the first example above. However, this is not observed in the example in the second example, because in that case the noise is in the original signal, *before* the convolution by the spectrometer's differentiation algorithm – the high frequency components of the denominator in the division of the Fourier transforms are typically much larger than in the previous example - and the only *post*-convolution noise comes from numerical round-off errors in the computations, which is almost always insignificant compared to the noise in the experimental signal.

Note: The word "deconvolution" appears in the Oxford English dictionary, where its meaning is "A process of resolving something into its constituent elements or removing complication in order to clarify it". The *same* word is also sometimes used for *another* process: resolving or decomposing a set of overlapping peaks into separate additive components, usually by the technique of multiple regression (page 49) or iterative least-squares curve fitting of a peak model to the data set (page 54). That process is actually conceptually and mathematically distinct from Fourier deconvolution discussed here, because in Fourier deconvolution the underlying peak shape is *unknown* but the broadening function is assumed to be known, whereas in iterative least-squares curve fitting the underlying peak shape is assumed to be *known*. *It's confusing to use the same word for both!* When I write "deconvolution", I mean *Fourier deconvolution*, not regression or curve fitting.

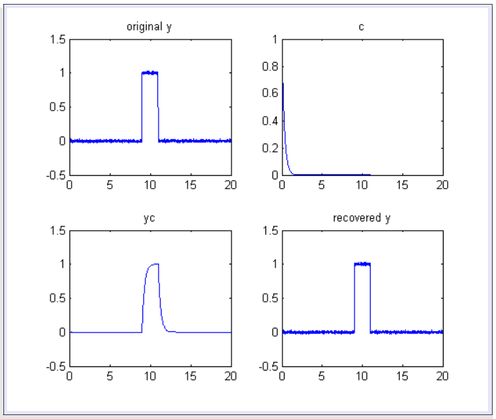
* Fourier transforms are usually expressed in terms of complex numbers, with real and imaginary parts. If the Fourier transform of the first signal is $a + ib$, and the Fourier transform of the second signal is $c + id$, then the ratio of the two Fourier transforms is

$$\frac{a + ib}{c + id} = \frac{ac + bd}{c^2 + d^2} + i\,\frac{bc - ad}{c^2 + d^2}$$

by the rules for the division of complex numbers.

SPECTRUM (page 70) has a Fourier deconvolution function.

Matlab and **Octave** have a built-in function for Fourier deconvolution: deconv. A simple example of its application is shown here. The vector *yc* (line 6) represents a noisy signal (*y*) that has been previously convoluted with a known "transfer function" *c* before being measured as *yc*. In line 7, *c* is *deconvoluted* from *yc*, to try to recover the original signal *y* before the convolution (as *ydc*). This works only if the transfer function *c* is known. Noise added to the original signal (line 4) is *recovered unchanged* by the deconvolution; in contrast, noise added *after* the convolution by the transfer function (line 7) will be *significantly amplified* in the recovered signal *ydc*.



```
X=0:.01:20;
y=zeros(size(x));              % Start with 2000 zeros
y(900:1100)=1;                 % Add a rectangular pulse 200 points wide
y=y+.01.*randn(size(y));       % Add some random noise before the convolution
c=exp(-(1:length(y))./30);     % exponential trailing convolution function
yc=conv(y,c,'full')./sum(c);   % Create exponential rectangular function yc
% yc=yc+.01.*randn(size(yc)); % Optionally add some random noise after the convolution
ydc=deconv(yc,c).*sum(c);      % Deconvolute exponential function c from yc
subplot(2,2,1);plot(x,y);title('original y');subplot(2,2,2);plot(x,c);title('c')
subplot(2,2,3);plot(x,yc(1:2001));title('yc');
subplot(2,2,4);plot(x,ydc);title('recovered y')
```

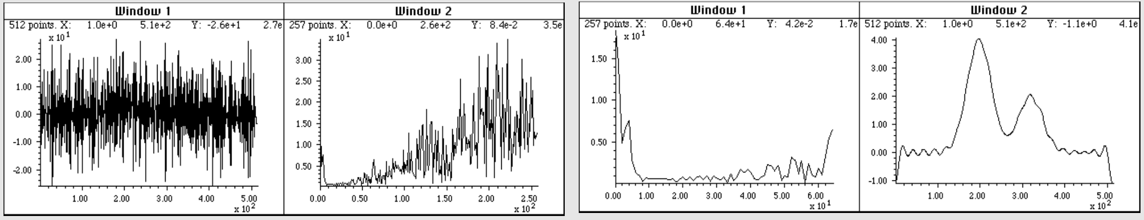
Alternatively, you could perform the deconvolution yourself *without* using the built-in Matlab/Octave "deconv.m" function by using the "fft.m" function to compute the Fourier transforms of *yc* and of *c*, dividing them point-by-point, then inverse transforming the result with the "ifft.m" function. Note that *c* must be zero-filled to match the size of *yc*: `ydc=ifft(fft(yc)./fft([c zeros(1,2000)])).*sum(c);`

The results are essentially the same (except for the numerical floating point precision of the computer, which is usually negligible), and the elapsed time is actually less that using the deconv function.

Appendix H, page 121, shows another example with multiple Gaussian peaks (similar to the signal at the top of page 32) in which the peak parameters of the underlying signal are measured by curve fitting (page 54) *after* the broadening convolution is removed by Fourier deconvolution.

Fourier filter

The Fourier filter is a type of filtering or smoothing function that is based on the frequency components of a signal. It works by taking the Fourier transform of the signal, manipulating the amplitudes of the frequency components, then inverse transforming the result. Care must be taken to use both the sine *and* cosine (or frequency *and* phase, or real *and* imaginary) components of the Fourier transform. A crude example of the application of the Fourier filter is given in the figure below. The assumption is made here that the frequency components of the signal fall mostly at low frequencies and those of the noise fall mostly at high frequencies, so all the frequency components above a certain limit are simply cut off (multiplied by zero). The cut-off frequency is adjusted so that it will allow most of the noise to be eliminated while not distorting the signal significantly.



The signal at the far left seems to be only random noise, but its ***power spectrum*** *(Window 2, second from left) shows that high-frequency components dominate the signal. The power spectrum is expanded in the X and Y directions to show more clearly the low-frequency region (Window 1, right). Working on the hypothesis that the components above the 20th harmonic are noise, simple* ***Fourier cut-off*** *function can be used to delete the higher harmonics and to reconstruct the signal from the first 20 harmonics. The result (far right) reveals two bands at about x=200 and x=300 that were totally obscured by noise.*

Other types of filters, such as low-pass, high-pass, band-pass, or band-reject (notch), can be constructed simply by manipulating the amplitudes of the Fourier transform.

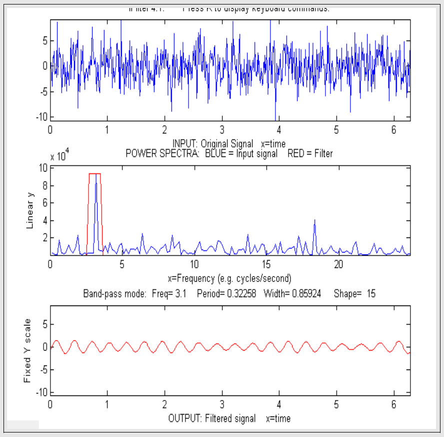
SPECTRUM includes a crude Fourier low-pass filter function with adjustable harmonic cut-off.

Matlab and **Octave: FouFilter.m** is a more flexible Fourier filter that can serve as a low-pass, high-pass, band-pass, or band-reject (notch) filter with variable cut-off rate. It has the form:

```
ry=FouFilter(y,samplingtime,centerfrequency,frequencywidth,shape,mode)
```

where y is the time-series signal vector, 'samplingtime' is the total duration of sampled signal in seconds, milliseconds, or microseconds; 'centerfrequency' and 'frequencywidth' are the center frequency and width of the filter in Hz, KHz, or MHz, respectively; 'Shape' determines the sharpness of the cut-off. If shape = 1, the filter is Gaussian; as shape increases the filter shape becomes more and more rectangular. Set mode = 0 for bandpass filter, mode = 1 for band-reject (notch) filter. FouFilter returns the filtered signal in ry. It can handle signals of virtually any length, limited only by the memory in your computer. Example:

```
clf;subplot(211);y=randn(size(1:1000));plot(y);
title('White noise')
subplot(212);plot(FouFilter(y,1,19,2,2,0))
title('narrow band of frequencies')
```



iFilter 4.1:   Press K to display keyboard commands.
5
0
-5
-10
0
1
2
3
4
5
6
INPUT: Original Signal   x=time
POWER SPECTRA: BLUE = Input signal   RED = Filter
x 10^4
10
8
6
4
2
Linear y
0
5
10
15
20
x=Frequency (e.g. cycles/second)
Band-pass mode: Freq= 3.1   Period= 0.32258   Width= 0.85924   Shape= 15
Fixed Y scale
5
0
-5
-10
0
1
2
3
4
5
6
OUTPUT: Filtered signal   x=time

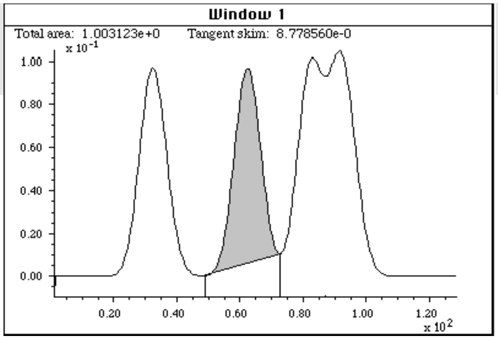
iFilter 4.1, an Interactive Fourier Filter for Matlab shown on the right, allows you to adjust the Fourier filter parameters (center frequency, filter width, and cut-off rate) while observing the effect on the signal output dynamically, with keyboard controls that allow you to adjust the filter parameters continuously while observing the effect on your signal dynamically: center frequency, filter width, shape, plotmode (1=linear; 2=semilog frequency; 3=semilog amplitude; 4=log-log) and filter mode ('band-pass', 'low-pass', 'high-pass', 'band-reject (notch), 'comb pass', and 'comb notch'). In the comb modes, the filter has multiple bands located at frequencies 1, 2, 3, 4... times the center frequency, each with the same (controlable) width and shape. This self-contained Matlab function does not require any toolboxes or add-on functions. Download any of these functions from **http://tinyurl.com/cey8rwh**. For instructions, see http://terpconnect.umd.edu/~toh/spectrum/InteractiveFourierFilter.htm

Integration and peak area measurement

The numerical integration of digitized signals finds application in analytical signal processing mainly as a method for measuring the *areas under the curves* of peak-type signals, especially in chromatography, a class of chemical measurement techniques in which a mixture of components is made to flow through a chemically-prepared tube or layer that allows some of the components in the mixture to travel faster than others, followed by a device called a *detector* that measures and records the quantity of each component after separation. Ideally, the components are sufficiently separated so that each forms a distinct *peak* in the detector signal. Quantitative measurement is customarily carried out on by measuring the *area* under the detector peaks rather than the *height* of the peaks. That's because peak area is less sensitive to the influence of peak broadening (dispersion) mechanisms that cause the molecules of a specific substance to be be diluted and spread out rather than being concentrated on one "plug" of material as it travels. These dispersion effects, which arise from many sources, cause chromatographic peaks to become shorter, broader, and more unsymmetrical, but they have little effect on the total area under the peak, as long as the total number of molecules remains the same. If the detector response is linear with respect to the concentration of the material, the peak *area* remains proportional to the total quantity of substance passing into the detector, even though the peak *height* is smaller. In such situations peak area measurements are often found to be more reliable than peak height measurements. Peak height will be proportional to the quantity of material only if the peak width and shape are constant.

Before computers, it was common to measure peak areas by using a ball-and disk analog integrator, or by counting the grid squares under a curve recorded on gridded graph paper, or by cutting and weighing peaks on a paper recording, or by figuring the area under a triangle drawn with its sides tangent to the sides of the peak, or by integrating the signal and measuring the heights of the resulting steps. But now that computing power is built into or connected to most measuring instruments, more accurate and convenient digital methods can be employed.

In chromatographic analysis there is often the problem of measuring the area under the curve of the peaks when they are not well resolved or are superimposed on a background. For example, the figure below shows a series of four computer-synthesized Gaussian peaks that all have the *same height, width, and area*, but the overlap between the last three peaks makes it harder to measure their areas.



Total area: 1.003123e+0 Tangent skim: 8.778560e-0

Left: Peak area measurement for overlapping peaks, using the perpendicular drop method (vertical lines at the bottom) and tangent skim method (shaded area).

The classical way to handle this problem is to draw two vertical lines from the left and right bounds of the peak down to the x-axis and then to measure the total area bounded by the signal curve, the x-axis (y=0 line), and the two vertical lines. This is often called the *perpendicular drop* method; it's an easy task for a computer but tedious to do by hand. The idea is illustrated for the second peak from the left in the figure . The left and right bounds of the peak are usually taken as the valleys (minima) between the peaks. Using this method, you can estimate the area of the second peak to an accuracy of about 0.3% and the third and fourth peaks to an accuracy of better than 4%. However, this simple method is not accurate if the peaks are superimposed on a non-zero baseline or if the peaks are asymmetrical, very different in height, or too highly overlapped, as is the case for the last two peaks in this example. In some cases it's helpful to apply a peak sharpening (page 26) to narrow the peaks before the measurement. Highly overlapped peaks can be measured by curve fitting (page 54). In the case where a peak is superimposed on a much broader curved baseline, you can use the *tangent skim* method, which measures the area between the curve and a linear baseline drawn across the bottom of the peak (e.g. the shaded area in the figure above). On the other hand, peak height measurements are less interfered with by neighboring, slightly overlapping peaks. Moreover, peak area measurements are difficult if the peaks overlap. Iterative least-squares curve fitting (pages 54-69) can measure the separate areas of overlapping peaks, but it requires that the underlying peak shape be known; the exponential-broadened Gaussian peak shape is the most commonly used for that application, especially in chromatography.

Software details.

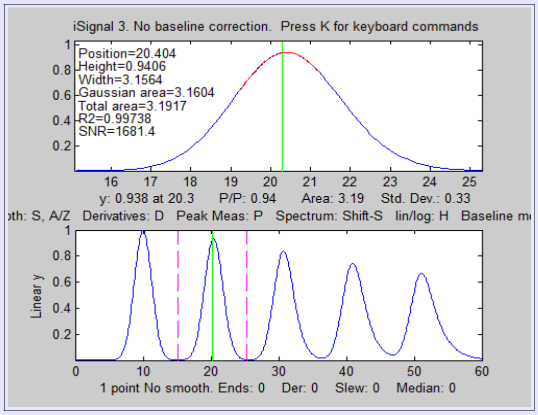
CumulativeSum.xls is a spreadsheet template that illustrates the integration of a peak-type signal by normalized cumulative sum; you can paste your own data into columns A and B. CumulativeSumExample.xls is the same with example data.

Matlab and Octave have built-in commands for the sum of elements ("sum", "cumsum"), trapezoidal numerical integration ("trapz"), and adaptive Simpson quadrature ("quad"). For example:

```
>> x=-5:.1:5;  y=exp(-(x).^2);trapz(x,y)
```

accurately computes the area under the curve of an isolated Gaussian, which is theoretically predicted to be the square root of π (that is, about 1.7725).

The Matlab peak-finding functions discussed on page 74 (e.g. **iPeak**) include code that measures peak area by several methods. ***iSignal*** (page 85) performs several of the signal processing functions described in this tutorial, including measurement of peak area using Simpson's Rule, the *perpendicular drop* and t*angent skim* methods, and baseline subtraction from a series of peaks using a manual piecewise-linear approximation.



```
Position=20.404
Height=0.9406
Width=3.1564
Gaussian area=3.1604
Total area=3.1917
R2=0.99738
SNR=1681.4
```

iSignal 3. No baseline correction. Press K for keyboard commands
1
0.8
0.6
0.4
0.2
16 17 18 19 20 21 22 23 24 25
y: 0.938 at 20.3   P/P: 0.94   Area: 3.19   Std. Dev.: 0.33
oth: S, A/Z   Derivatives: D   Peak Meas: P   Spectrum: Shift-S   lin/log: H   Baseline m
Linear y
0 10 20 30 40 50 60
1 point No smooth. Ends: 0   Der: 0   Slew: 0   Median: 0

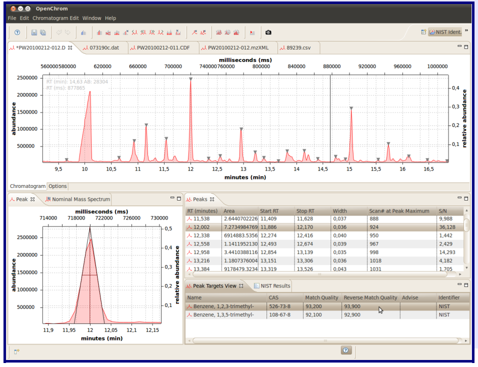
As a test case, the figure on the left shows a signal containing five peaks with the *same peak area* (3.1938) but broadened by increasing degrees of exponential broadening, similar to the peak broadening commonly seen in chromatography. To use ***iSignal*** to measure the areas of each of these peaks by the perpendicular drop method, use the pan and zoom keys to position the two outer cursor lines (dotted magenta lines) in the valley on either side of each peak. The area is displayed below the upper window.: 3.193, 3.194, 3.187, 3.178, and 3.231, a mean of 3.1966 and standard deviation of only 0.02 (0.63%). Alternatively, integrating the signal (`cumsum(y).*dx`) and measuring the heights of the resulting steps gives similar results: 3.19, 3.19, 3.18, 3.17, 3.23. By either method, the peak *heights* are very different but the *areas* are more nearly equal, which shows why peak area is used in chromatography.

Peakfit. If the peaks are much more overlapped that this, however, *curve fitting* (introduced on page 54) works better than perpendicular drop or integration/step height, for example using **iSignal** (page 85), ***peakfit.m*** (page 90) or ***ipf.m*** (page 95). The following code shown this with a signal like the figure on page 35, where all the underlying peaks have the same height, width, and area, fitting it with a four Gaussians:

```
>> x=[0:.01:18];
>> y=exp(-(x-4).^2)+exp(-(x-9).^2)+exp(-(x-12).^2)+exp(-(x-13.7).^2);
>> peakfit([x;y],9,18,4,1,0,10,0,0,0)
```

Sometimes a curved baseline can be corrected by fitting it using one of the basic peak shapes (e.g. Gaussian, Lorentzian) or a polynomial (see **Example 12b** on page 92 and **Example 20** on page 94). Matlab/Octave experiments demonstrate methods of baseline correction and the application to overlapping exponentially broadened peaks. A practical chromatography example is given on page 96.

iPeak (see page 74) can also be used to estimate peak areas. It uses the same Gaussian curve fitting method as ***iSignal*,** and it has the advantage that it can detect and measure all the peaks in a signal in one operation, but the areas are accurate only for well-separated Gaussian or Lorentzian peaks. In general, the most accurate peak area measurements can be made with iterative least-squares peak fitting (page 54), for example using ***peakfit.m*** or ***ipf.m*** (page 90, 95, 96), provided that the *shape* of the peaks is known. In all of these methods, the presence of a *background* signal on which the peaks are superimposed will greatly influence the measured peak area if not corrected or compensated. ***iSignal***, ***iPeak***, and ***peakfit*** all have four automatic baseline correction modes (page 62), and ***iSignal*** and ***iPeak*** have a multipoint piecewise linear background subtraction function (page 86). See **http://tinyurl.com/cey8rwh**.



For gas chromatography and mass spectrometry specifically, I recommend **Philip Wenig's OpenChrom** software (left), an open source data system that can import binary and textual chromatographic data files directly in several common data formats. It includes methods to detect baselines and peaks and to integrate peaks in a chromatogram. Extensive documentation is available. It runs on Windows, Linux, Solaris and Mac OS X. The program and its documentation is regularly updated by its author.

Curve fitting A: Linear Least Squares

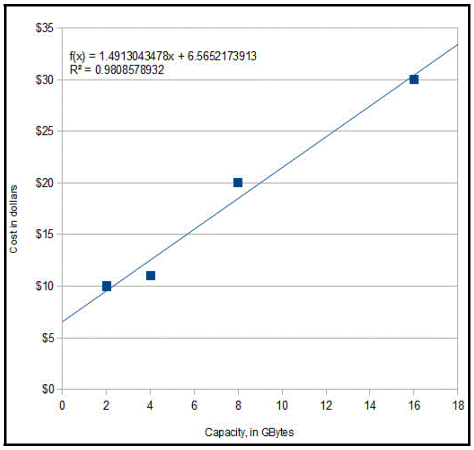
The objective of curve fitting is to find the parameters of a mathematical model that describes a set of (usually noisy) data in a way that minimizes the difference between the model and the data. The most common approach is the "linear least squares" method, also called "polynomial least squares", a well-known mathematical procedure for finding the coefficients of polynomial equations that are a "best fit" to a set of X,Y data. A polynomial equation expresses the dependent variable Y as a polynomial in the independent variable X, for example as a straight line (Y = **a** + **b**X, where **a** is the *intercept* and **b** is the *slope*), or a quadratic (Y = **a** + **b**X + **c**X^2), or a cubic (Y = **a** + **b**X + **c**X^2 + **d**X^3), or higher-order polynomial. Those coefficients (**a**, **b**, **c**, etc) can be used to predict values of Y for each X. "Least squares" simply means that the squares of the differences between the actual measured Y values and the Y values predicted by that equation are *minimized*. It does *not* mean a "perfect" fit; in most cases, a least-squares best fit *does not go through all the points* in the data set. Above all, a least-squares fit *must conform to the selected model* - for example, a straight line or a quadratic parabola - and there will almost always be some data points that do not fall exactly on the best-fit line, either because of random error in the data or because the model is not capable of describing the data exactly.

In all these cases, Y is a *linear function* of the parameters **a**,**b**,**c**, etc. ***This** is why we call it a "linear" least-squares fit, **not** because the plot of X vs Y is linear.* Only for the *first*-order polynomial is the plot of X vs Y linear.

Least-squares fits can be calculated by some hand-held calculators and smartphones, by spreadsheets, and by dedicated computer programs (see page 46 for details). Although you could draw the best-fit straight line by visual estimation and a straightedge, the least-squares method is more objective and easier to automate. (If you were to give a set of data to five different people and ask them to estimate the best-fit line visually, you'd get five slightly different answers, but if you gave that data set to five different computer programs, you'd get the same answer every time).

Here's a very simple example: the historical prices of different sizes of **SD memory cards** advertised in the February 19, 2012, issue of the *New York Times*.

Memory Capacity in GBytes	Price in US dollars
2	$9.99
4	$10.99
8	$19.99
16	$29.99



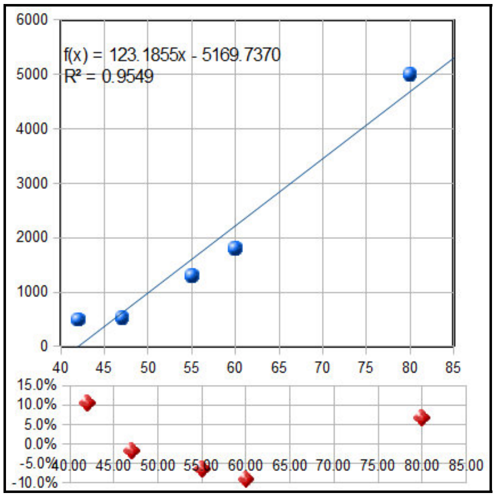
What's the relationship between memory capacity and cost? Of course, we expect that the larger-capacity cards should cost more than the smaller-capacity ones, and if we plot cost vs capacity (graph on the right), we can see a rough straight-line relationship. Using a linear least-squares calculation, where **X = capacity** and **Y = cost**, the straight-line equation that most simply describes these data (rounding to the nearest penny) is:

Cost = $6.56 + Capacity * $1.49

So, 1.49 is the *slope* (**b**) and 6.56 is the *intercept* (**a**). (The equation is plotted as the solid line that passes among the data points in the figure). Basically, this is saying that the cost of a memory card consists of a fixed cost of $6.56 plus $1.49 for each Gbyte of capacity. How can we interpret this? The $6.56 represents the costs that are the same regardless of the memory capacity: a reasonable guess is that it includes things like packaging (the different cards are the same physical size and are packaged the same way), shipping, marketing, advertising, and retail shop shelf space. The $1.49 (1.49 dollars/Gbyte) represents the increasing retail price of the larger integrated circuit chips inside the larger capacity cards, primarily because they *have more value for the consumer* but also may cost more to make because they use more silicon, are more complex, and have a higher chip-testing rejection rate in production. So in this case the slope and intercept have real physical and economic meanings.

What can we do with this information? First, we can see how closely the actual prices conform to this equation: pretty well *but not perfectly*. The line of the equation passes *among* the data points but does not go exactly *through* each one. That's because actual retail prices are also influenced by several factors that are *unpredictable* and *random*: local competition, supply, demand, and even rounding to the nearest "neat" number; all those factors constitute the "noise" in these data. The least squares procedure also calculates R^2, called the *coefficient of determination* or the *correlation coefficient*, which is an indicator of the "goodness of fit". R^2 is exactly 1.0000 when the fit is perfect, less than that when the fit is imperfect. The closer to 1.0000 the better. An R^2 value of 0.99 means a "fairly good" fit; 0.999 is a "very good" fit.

The second way we can use these data is to predict the likely prices of other card capacities, if they were available, by putting in the capacity into the equation and evaluating the cost. For example, a 12 Gbyte card would be expected to cost $24.44 according to this model. And a 32 Gbyte card would be predicted to cost $54.29, but beware, *that would be predicting beyond the range of the available data.* That's called "*extrapolation"- and it's very risky* because you don't really know what other factors may influence the data beyond the last data point. You could also solve the equation for capacity as a function of cost and use it to predict how much capacity could be expected to be bought for a given amount of money (if such a product were available).

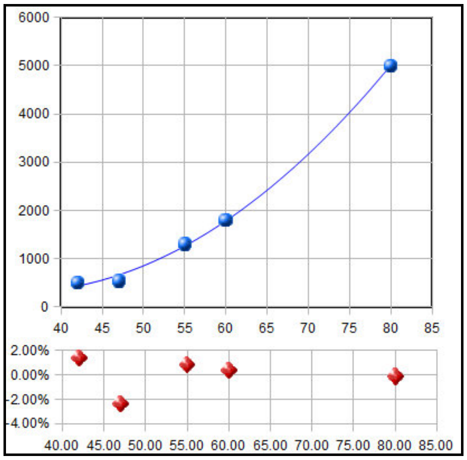


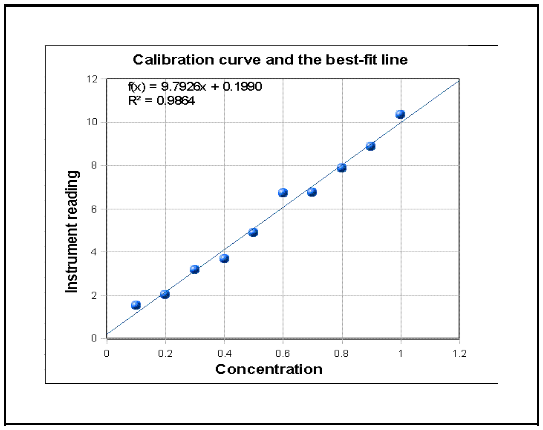
Here's another related example: the historical prices of **flat-screen LCD TVs** as a function of screen size, as they were advertised on the Web in 2012 (yes, those sets really did cost that much back then). The prices of five selected models, similar except for screen size, are plotted against the screen size in inches, in the figure on the left, and are fit to a first-order (straight-line) model. As for the previous example, the fit is not perfect. The equation of the best-fit model is shown at the top of the graph, along with the R^2 value (0.9649) indicating that the fit is not particularly good. Worse, you can see from the best-fit line that a 40 inch set would be predicted to have a *negative cost!* They would *pay* you to take these sets? I don't *think* so.

The goodness of fit is shown even more clearly in the little graph at the bottom of this figure, with the red dots, which shows the "residuals" - the differences between each data point and the least-squares fit at that point. You can see that the deviations from zero are fairly large (±10%), but more important, *they are not completely random*; they form a clearly visible U-shaped curve. This is a tip-off that the straight-line model we have used here may not be ideal and that we might get a better fit with another model. (Or it might be just *chance*: the first and last points might be higher than expected because those were unusually expensive TVs for those sizes. How would you really know for sure, unless your data collection was very careful?)

Linear least-squares calculations can fit not only straight-line data, but *any set of data that can be described by a polynomial*, for example a second-order (quadratic) equation ($Y = \mathbf{a} + \mathbf{b}X + \mathbf{c}X^2$). Applying a second-order ("quadratic") fit to these data, we get the graph on the right. Now the R^2 value is higher, 0.9985, indicating that the fit is much better (but again not perfect), and also the residuals (the red dots at the bottom) are smaller and more random.

The observation that a quadratic fits the data better is not really surprising, because the size of a TV screen is quoted as the *length* of the diagonal (from one corner of the screen to its opposite corner), but the quantity of material, the difficulty of manufacture, the weight, and the power supply requirements of the screen all scale with the *screen area*. Area is proportional to the *square* of the linear measure, so the inclusion of an X^2 term in the model is quite reasonable in this case. With this quadratic fit, the 40 inch set would be predicted to cost under $500, which is more sensible than the linear fit. In this case a quadratic (rather than linear) model is justified not simply because it fits the data better, but because it is *expected in principal* based on the relationship between length and area.

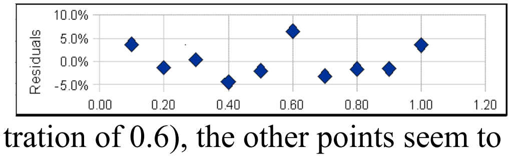




The graph on the left shows a third example, taken from analytical chemistry: a straight-line calibration data set where X = concentration and Y = instrument reading (Y = **a** + **b**X). (If you are viewing this online, click to download that data). The blue dots are the data points. They don't all fall in a perfect straight line because of random noise and measurement error in the instrument readings and possibly also volumetric errors in the concentrations of the standards (which are usually prepared in the laboratory by diluting a stock solution). For this set of data, the measured slope is 9.7926, the intercept is 0.199 and R^2=0.9864.

The slope of the calibration curve is often called the "sensitivity". The intercept indicates the instrument reading that would be expected if the concentration were zero. Ordinarily instruments are adjusted ("zeroed") by the operator to give a reading of zero for a concentration of zero, but random noise and instrument drift can cause the intercept to be non-zero for any particular calibration set. In fact, *this data set is computer-generated*; the "true" value of the slope was exactly 10 and of the intercept was exactly zero before noise was added, and the noise was added by a random-number generator with zero mean. So in this case the presence of the noise caused this particular measurement of slope to be off by about 2%. Had there been a larger number of standard solutions over the same concentration range, the calculated values of slope and intercept would almost certainly have been better. (On average, the accuracy of measurements of slope and intercept improve with the *square root of the number of points in the data set*).

Once the calibration curve is established, it can be used to determine the concentrations of unknown samples that are measured on the same instrument, for example by solving the equation for concentration as a function of instrument reading. The concentration and the instrument readings can be recorded in any convenient units, as long as the same units are used for calibration and for the measurement of unknowns.



A plot of the "residuals" for the calibration data (left) raises a question. Except for the 6th data point (at a concentration of 0.6), the other points seem to form a rough U-shaped curve, indicating that a quadratic equation might be a better model for those points than a straight line. Can we reject the 6th point as being an "outlier", perhaps caused by a mistake in preparing that solution standard or in reading the instrument for that point? Discarding that point would improve the quality of fit (R^2=0.992 instead of 0.986), especially if a quadratic fit were used (R^2=0.998). *The only way to know for sure is to repeat* that standard solution preparation and calibration and see if that U shape persists in the residuals. Many instruments *do* give a very linear calibration response, while others show a slightly non-linear response under some circumstances. But *in fact*, the calibration data used for this particular example were *computer-generated to be perfectly linear*, with normally-distributed random numbers added to simulate noise. So actually that 6th point is really *not* an outlier and the underlying data are *not* curved, *but you would not know that in a real application*. *It would have been a mistake to discard that 6th point* and use a quadratic fit in this case. Moral: *don't throw out data points* just because they seem a little off, unless you have good reason, and don't use higher-order polynomial fits just to get better fits if the instrument is known to give linear response under those circumstances. *Even perfectly normally-distributed random errors can occasionally give individual deviations* that are quite far from the average and might tempt you into thinking that they are outliers. Don't be fooled. (Full disclosure: I obtained the above example by "cherry-picking" from among dozens of randomly generated data sets, in order to find one that, although actually random, *seemed* to have an outlier).

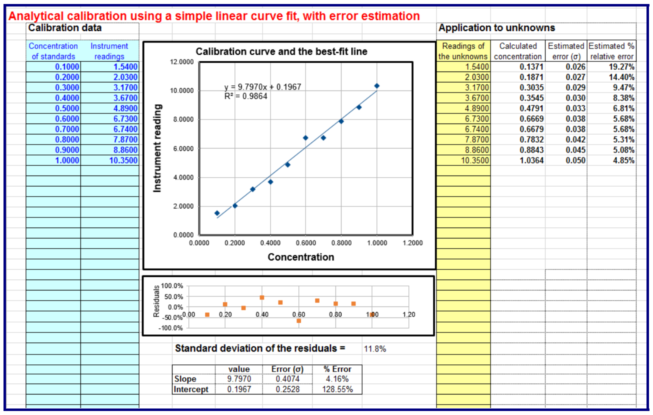
Reliability of curve fitting results

How reliable are the slope, intercept and other polynomial coefficients obtained from least-squares calculations on experimental data? The single most important factor is the appropriateness of the model chosen; it's critical that the model (e.g. linear, quadratic, whatever) be a good match to the actual underlying shape of the data. You can choose a model based on the known and expected behavior of that system (like using a linear calibration model for an instrument that is known to give linear response under those conditions) or you can choose a model that always gives randomly-scattered residuals that do not exhibit a regular shape. But even with a perfect model, the least-squares procedure applied to repetitive sets of measurements will not give the same results every time because of random error ("noise") in the data. If you were to repeat the entire set of measurements many times and do least-squares calculations on each data set, the standard deviations of the coefficients would vary directly with the standard deviation of the noise and inversely with the square root of the number of data points in each fit, all else being equal. The problem, obviously, is that it is not always possible to repeat the entire set of measurements many times. You may have only one set of measurements and each experiment may be very expensive to repeat. So, it would be good to have some sort of short-cut method that would *predict* the standard deviations of the coefficients *without* actually repeating the measurements. Here I will describe three general ways to predict the standard deviations of the polynomial coefficients.

Algebraic Propagation of errors. The classical way is based on the rules for mathematical error propagation. The propagation of errors of the entire curve-fitting method can be described in closed-form algebra by breaking down the method into a series of simple differences, sums, products, and ratios, and applying those rules to each step. The result of this procedure for a first-order (straight line) least-squares fit are shown in the last two lines of the set of equations on page 46. Essentially, these equations make use of the deviations from the least-squares line (the "residuals") to estimate the standard deviations of the slope and intercept, based on the assumption that the deviations in that single data set are random and representative of the deviations that would be obtained upon repeated measurements. *Because these predictions are based only on a single data set, they are good only insofar as that data set is typical of others that might be obtained in repeated measurements.* If your random deviations happen to be *small* when you acquire your data set, you'll get a deceptively *good*-looking fit, but then your estimates of the standard deviation of the slope and intercept will be *too low*, on average. If your random deviations happen to be *large* in that data set, you'll get a deceptively *bad*-looking fit, but then your estimates of the standard deviation will be *too high*, on average. The problem becomes worse with a small number of data points. It's still worth the trouble to calculate the predicted standard deviations of slope and intercept, but keep in mind that these predictions are accurate only if the number of data points is large and if the errors are random and normally distributed. A larger number of data points is always better, but the problem is that, in laboratory work, getting more data may not be possible or cost effective. In analytical chemistry calibration, for example, the labor and cost of preparing and running large numbers of standard solutions, and safely disposing of them afterwards, often limits the number of standards to a rather small set, by statistical standards, so these estimates of standard deviation are often fairly rough.

In the application to *analytical calibration*, the concentration of the sample Cx is given by Cx = (Sx - *intercept*)/*slope*, where Sx is the signal given by the sample solution. The uncertainty of all three terms contribute to the uncertainty of Cx. The standard deviation of Cx can be estimated from the standard deviations of slope, intercept, and Sx using the rules for mathematical error propagation.

A spreadsheet template (CalibrationLinear.xls) that performs these error-propagation calculations for your own first-order (linear) analytical calibration data can be downloaded from **http://tinyurl.com/cey8rwh**. For example, the linear calibration example just given in the previous section, where the "true" value of the slope was 10 and the intercept was zero, this spreadsheet (shown on the right) predicts that the slope is 9.8 with a standard deviation 0.407 (4.2%) and that the intercept is 0.197 with a standard deviation 0.25. So *the measured slope and intercept are both well within one standard deviation of the true values*. This spreadsheet also performs the propagation of error calculations for the calculated concentrations of each unknown in the last two columns on the right. In the example in this figure, the instrument readings of the standards are taken as the unknowns, showing that the predicted percent concentration errors range from about 5% to 19% of the true values of those standards. (Note that the standard deviation of the concentration is greater at high concentrations than the standard deviation of the slope, and considerably greater at low concentrations because of the greater influence of the uncertainly in the intercept). For a further discussion and some examples, see Bracket.html#Cal_curve_linear. The downloadable Matlab/Octave plotit.m function also uses the algebraic method to compute the standard deviations of least-squares coefficients for any order.



Analytical calibration using a simple linear curve fit, with error estimation

Calibration data

Concentration of standards	Instrument readings
0.1000	1.5400
0.2000	2.0300
0.3000	3.1700
0.4000	3.6700
0.5000	4.8900
0.6000	6.7300
0.7000	6.7400
0.8000	7.8700
0.9000	8.8600
1.0000	10.3500

Standard deviation of the residuals = 11.8%

	value	Error (σ)	% Error
Slope	9.7970	0.4074	4.16%
Intercept	0.1967	0.2528	128.55%

Application to unknowns

Readings of the unknowns	Calculated concentration	Estimated error (σ)	Estimated % relative error
1.5400	0.1371	0.026	19.27%
2.0300	0.1871	0.027	14.40%
3.1700	0.3035	0.029	9.47%
3.6700	0.3545	0.030	8.38%
4.8900	0.4791	0.033	6.81%
6.7300	0.6669	0.038	5.68%
6.7400	0.6679	0.038	5.68%
7.8700	0.7832	0.042	5.31%
8.8600	0.8843	0.045	5.08%
10.3500	1.0364	0.050	4.85%

Monte Carlo simulation. Another way of estimating the standard deviations of the least-squares coefficients is to perform a random-number simulation (a type of Monte Carlo simulation). This requires that you know (by previous measurements) the average standard deviation of the random noise in the data. Using a computer, you construct a model of your data over the normal range of X and Y values (for example Y = **intercept** + **slope***X + **noise**, where **noise** is the noise in the data), compute the slope and intercept of each simulated noisy data set, then repeat that process many times (usually a few thousand) with different sets of random noise, and finally compute the standard deviation of all the resulting slopes and intercepts. This is ordinarily done with normally-distributed random white noise, using the RANDN function that many programming languages have. If the model is good and the noise is well-characterized, the results will be a very good estimate of the expected standard deviations of the least-squares coefficients. (If the noise is not white or is not constant, but rather varies with the X or Y values, or if the data have been smoothed, then that must be included in the simulation).

Obviously this method requires a computer and prior knowledge of the noise, and it is not so convenient as evaluating a simple algebraic expression. But there are two important advantages to this method: (1) is has great generality; it can be applied to curve fitting methods that are too complicated for the classical closed-form algebraic propagation of error calculations, even iterative non-linear methods; and (2) its predictions are based on the average noise in the data, not the noise in just a single data set. For that reason, it gives more reliable estimations, particularly when the number of data points in each data set is small. Nevertheless, you can not always apply this method because you don't always know the average standard deviation or the frequency distribution of the noise.

LinearFiMC.m, from http://tinyurl.com/cey8rwh, is a Matlab/Octave script that compares the Monte Carlo simulation to the algebraic method above. By running this script with different sizes of data sets (*NumPoints* in line 10), you can see that the standard deviation predicted by the algebraic method fluctuates from run to run when *NumPoints* is small (e.g. 10), but the Monte Carlo predictions are much more steady. When *NumPoints* is large (e.g. 1000), both methods agree very well.

The Bootstrap. The third method is the "bootstrap", a procedure that involves choosing random samples with replacement from a single data set and analyzing each sub-sample the same way (e.g.

by a least-squares fit). Every data point is returned to the data set after sampling, so that (a) a particular data point from the original data set could appear multiple times in a given sub-sample, and (b) the number of elements in each bootstrap sub-sample equals the number of elements in the original data set. As a simple example, consider a data set with 10 x,y pairs assigned the letters "a" through "j". The original data set is represented as [a b c d e f g h i j], and some typical bootstrap sub-samples might be [a b b d e f f h i i] or [a a c c e f g g i j], each bootstrap sample containing the same number of data points, but with about half of the data pairs skipped and the others duplicated.

You would use a computer to generate hundreds or thousands of bootstrap samples like that and apply the calculation procedure under investigation (in this case a linear least-squares, but it could be any calculation) to each set. If there were *no noise* in the data set, and if the model were perfectly chosen, then all the points in the original data set and in all the bootstrap sub-samples would fall *exactly on the model line*, and the least-squares results would be the *same* for every sub-sample. But if there *is noise* in the data set, most bootstrap samples would give a *slightly different result* for the least-squares polynomial coefficients, because each sample has a different subset of the random noise. The greater the amount of random noise in the data set, the greater would be the range of results from the bootstrap sub-samples. This enables you to estimate the uncertainty of the quantity you are estimating, just as in the Monte-Carlo method above. The difference is that the Monte-Carlo method is based on the assumption that the noise is can be accurately simulated by a random-number generator on a computer (i.e. is random, normally distributed, and has a known standard deviation), whereas the bootstrap method uses the *actual noise in the data set at hand*, just like the algebraic method, except that it *does not need an algebraic solution* of error propagation. The bootstrap method thus shares its generality with the Monte Carlo approach, but like the algebraic method is limited by the assumption that the noise in that (possibly small) single data set is unsmoothed and is representative of the noise that would be obtained upon repeated measurements. Bootstrap computations can be done in Matlab/Octave (page 48) or in spreadsheets.

The Matlab/Octave script **TestLinearFit.m** (download from **http://tinyurl.com/cey8rwh**) compares all three of these methods (the algebraic method, Monte Carlo simulation, and the bootstrap method) for a 100-point first-order linear least-squares fit. Each method is repeated on different simulated data sets with the same average slope, intercept, and selected noise model, then the standard deviations (SD) of the slopes (`SDslope`) and intercepts (`SDint`) were compiled:

```
NumPoints = 100     SD Noise = 9.236    x-range = 30
            Simulation    Algebraic equation  Bootstrap method
           SDslope SDint    SDslope SDint       SDslope SDint
Mean SD:   0.1140  4.1158   0.1133  4.4821      0.1096  4.0203
SD of SDs: 0.0026  0.0927   0.0081  0.3185      0.0122  0.4552
```

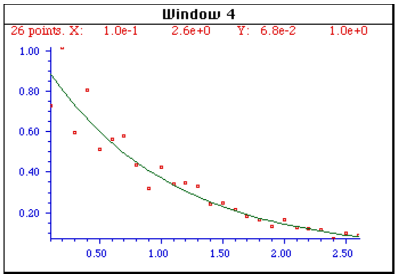
If the noise is *white*, the mean standard deviations ("Mean SD") of the three methods agree very well, but the algebraic and bootstrap methods fluctuate more than the Monte Carlo simulation each time this script is run (the "SD of the SDs" is higher), because those methods are based on the noise in *one single* 100-point data set, whereas the Monte Carlo simulation reports the average of *many* data sets. If the noise is *pink* rather than white (page 8), the bootstrap error estimates will also be *low*. Conversely, if the noise is *blue* (as occurs in processed signals that have been subjected to differentiation or that have been deconvoluted from some blurring process), then the errors predicted by the algebraic propagation-of-errors and the bootstrap methods will be *high*. All three methods show that the standard deviations are inversely proportional to the square root of the number of data points (see EffectOfSampleSize.ods).

In simple cases the algebraic method is faster to compute than the other methods, and its validity is more readily determined by inspection. On the other hand, an algebraic solution is not always possible to obtain (it's quite complicated even for a cubic polynomial fit), whereas the Monte Carlo and bootstrap methods, which do not depend on algebraic solutions, can be applied readily to *any* curve-fitting situation, even non-linear iterative least squares (page 54). However, *the validity of computer programs is less easy to verify than algebraic solutions* and so the Monte Carlo and bootstrap error estimates may not be as well trusted as an algebraic derivation, especially by those with less computer experience.

Effect of the number of data points on least-squares fit precision. The spreadsheets EffectOfSampleSize.ods or EffectOfSampleSize.xlxs, which collect the results of many runs of TestLinearFit.m with different numbers of data points ("NumPoints"), demonstrates that the standard deviation of the slope and the intercept *decrease* if the number of data points is *increased*; specifically, the *standard deviations are inversely proportional to the square root of the number of data points*. These plots really dramatize the problem of small sample sizes, but this must be balanced against the cost of obtaining more data points. For example, in analytical chemistry calibration, a larger number of calibration points could be obtained either by preparing and measuring more standard solutions or by reading each of a smaller number of standards repeatedly. The former approach accounts for both the volumetric errors in preparing solutions and the random noise in the instrument readings, but the labor and cost of preparing and running large numbers of standard solutions, and safely disposing of them afterwards, is limiting. The latter approach is less expensive but is less reliable because it accounts only for the random noise in the instrument readings. Overall, it better to refine the laboratory techniques and instrument settings to minimize error that to attempts to compensate by taking lots of readings.

Transforming non-linear relationships

In some cases, a fundamentally non-linear relationship can be transformed into a form that is amenable to polynomial curve fitting by means of a coordinate transformation (e.g. taking the log or the reciprocal of the data) and then applying the least-squares method to the transformed data. For example, the signal in the figure below is from a computer simulation of an exponential decay (X=time, Y=signal intensity) that has the mathematical form Y = **a** exp(**b**X), where **a** is the Y-value at X=0 and **b** is the decay constant. This is a fundamentally non-linear problem because Y is a non-linear function of the parameter **b**. However, by taking the natural log of both sides of the equation, we obtain ln(Y)=ln(**a**) +**b**X. In this equation, Y is a linear function of both parameters ln(**a**) and **b**, so it can be fit by the least squares method in order to estimate ln(**a**) and **b**, from which you get a by computing exp(ln(**a**)). In this particular example, the "true" values of the coefficients are **a** =1 and **b** = -0.9, but random noise has been added to each data point, with a standard deviation equal to 10% of the value of that data point, in order to simulate a typical experimental measurement in the laboratory. An estimate of the values of ln(**a**) and b, given only the noisy data points, can be determined by least-squares curve fitting of ln(Y) vs X.



Left: An exponential least-squares fit (solid line) applied to a noisy data set (points) in order to estimate the decay constant.

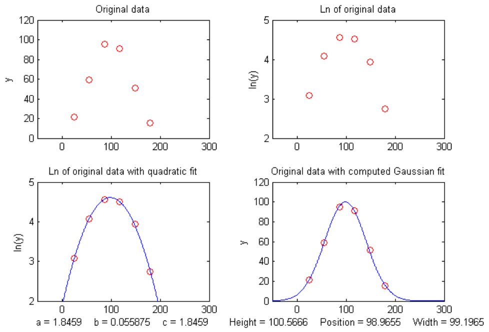
The best fit equation, shown by the green solid line in the figure, is Y =0.959 exp(- 0.905 X), that is, **a** = 0.959 and **b** = -0.905, which are reasonably close to the expected values of 1 and -0.9, respectively. Thus, even in the presence of substantial random noise (10% relative standard deviation), you can get reasonable estimates of the parameters of the underlying equation (to within about 5%). The most important requirement is that the model be good, that is, that the equation selected for the model accurately describes the underlying behavior of the system (except for noise). Often that is the most difficult aspect, because the underlying models are not always known with certainty. In Matlab and in Octave, the fit can be performed in one line: `polyfit(x,log(y),1)`, which returns [b log(a)]. (Note that in Matlab and Octave, "log" is the natural log; "log10" is the base-10 log).

Other examples of non-linear relationships that can be linearized by coordinate trans-formation include the logarithmic (Y = **a** ln(**b**X)) and power (Y=**a**$X^{\mathbf{b}}$) relationships. Methods of this type were once very common, back in the days before computers, when fitting anything other than a straight line was difficult. It is still used today to extend the range of functional relationships that can be handled by common linear least-squares routines and to display relationships in an easily-verified way. Only a few non-linear relationships can be handled this way, however; to fit any arbitrary function, use the Non-linear Iterative Curve Fitting method discussed on page 54).

Fitting Gaussian and Lorentzian peaks. A useful example of transformation to convert a non-linear relationship into a form that is amenable to polynomial curve fitting is the use of the natural log (ln) transformation to convert a **Gaussian** peak (y = **h***exp(-((x-**p**)/(0.6005612***w**)) ^2), where **h** is peak height, **p** is peak maximum position, and **w** is the full width at half maximum) into a quadratic (y = **a** + **bx** + **c**x^2) that can be fit to the data by quadratic least squares. All three parameters of the Gaussian (**h**, **p**, and **w**) can be calculated from the three quadratic coefficients **a**, **b**, and **c** by solving 3 equations in 3 unknowns (for example, using Wolfram Alpha), resulting in **h** = exp(**a-c***(**b**/(2***c**))^2), **p** = -**b**/(2***c**), and **w** = 2.35703/(sqrt(2)*sqrt(-**c**)). This is called "Caruana's algorithm"; see reference 46 on page 126.

One advantage of this type of Gaussian curve fitting, as opposed to simple visual estimation, is shown in the figure on the next page. The signal is a Gaussian peak with a true peak height of 100 units, a true peak position of 100 units, and a true half-width of 100 units, but it is sparsely sampled only every 31 units on the x-axis. The resulting data set, shown by the red points in the upper left quadrant, has only 6 data points on the peak itself. If we were to take the maximum of those 6 points (the 3rd point from the left, with x=87, y=95) as the peak maximum, that would not be very close to the true values of peak position (100) and peak height (100). If we were to take the distance between the 2nd the 5th data points as the peak width, we'd get 3*31=93, compared to the true value of 100.

On page 25, we learned that you can locate the x-axis position of a peak by finding the zero-crossing of the first derivative. However, because the data are sparsely sampled in this example, the actual peak falls *between two points*, making it hard to measure the peak height and position accurately.



One solution to this is to use curve fitting, taking the natural log of the data (upper right) to produce a *parabola* that can be fit with a quadratic least-squares fit (shown by the blue line in the lower left). From the three coefficients of the quadratic fit you can calculate much more accurate values of the Gaussian peak parameters, shown at the bottom of the figure (height=100.57; position=98.96; width=99.2). The plot in the lower right shows the resulting Gaussian fit (in blue) displayed with the original data (red points). The accuracy of those calculated peak parameters (about 1% in this example) is far better than the previous estimates and is limited only by the noise in the data. (This figure was generated in Matlab/Octave, using the script "**QuadFitToGaussian.m**"). Note: in order for this method to work properly, the data set must not contain any zeros or negative points; if the signal-to-noise ratio is very poor, it may be useful to smooth the data slightly to prevent this problem. Moreover, the original Gaussian peak signal must have a zero baseline, that is, must tend to zero far from the peak center. In practice this means that any non-zero baseline must be subtracted from the data set before using this method.

A similar method can be derived for a **Lorentzian** peak, which has the form y=**h**/(1+((x-**p**)/(0.5***w**))^2), by fitting a quadratic to the *reciprocal* of the y values. Just as for the Gaussian peak, the peak height **h**, maximum position **p**, and width **w** can be calculated from the three quadratic coefficients **a**, **b**, and **c** of the quadratic fit: **h** = 4***a**/((4***a*****c**)-**b**^2), **p** = -**b**/(2***a**), and **w** = sqrt(((4***a*****c**)-**b**^2)/**a**)/sqrt(**a**). Again, the data set must not contain any zero or negative y values.

In order to apply the above methods to signals containing *two or more* Gaussian or Lorentzian peaks, it's necessary to locate all the peak maxima first, so that the proper groups of points centered on each peak can be processed with the algorithms just discussed. That is discussed in the section on Peak Finding and Measurement on page 74. (A more general approach to fitting peaks, which works for data sets with zeros and negative numbers and also for data with strongly overlapping peaks, is the non-linear iterative curve fitting method, discussed on Page 54).

But there is a downside to using coordinate transformation methods to convert non-linear relationships into simple polynomial form: the noise is *also* effected by the transformation, with the result that the propagation of error from the original data to the final results is often difficult to predict. In the method just described for measuring the peak height, position, and width of Gaussian peaks, the results depends not only on the amplitude of noise in the signal, but also on how many points across the peak are taken for fitting. In particular, as you take more points far from the peak center, where the y-values approach zero, the natural log of those points approaches negative infinity as y approaches zero. The result is that the noise of those low-magnitude points is unduly magnified and has a disproportional effect on the curve fitting. This runs counter the usual expectation that the quality of the curve fitting results improves with the square root of the number of data points.

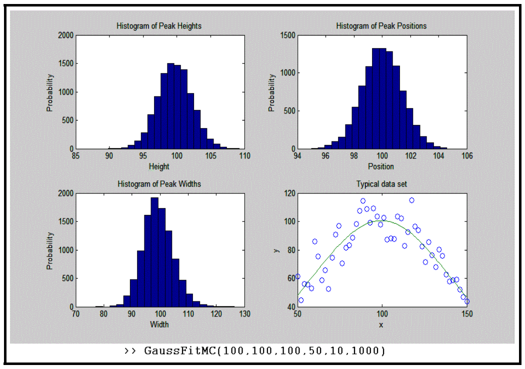
If you take only the points in the *top half of the peak*, with Y-values down to one-half of the peak maximum, the error propagation (predicted by a Monte Carlo simulation with constant normally-distributed random noise) shows that the relative standard deviations of the measured peak parameters are predicted by these empirical expressions:

Relative standard deviation of the peak position =*noise*/sqrt(*N*),

Relative standard deviation of the peak height = 1.73**noise*/sqrt(*N*),

Relative standard deviation of the peak width = 3.62**noise*/sqrt(*N*).

where *noise* is the standard deviation of the noise in the data and *N* in the number of data points taken for the least-squares fit. You can see from these results that the measurement of peak *position* is most precise, followed by the peak *height*, with the peak *width* being the least precise.



```
>> GaussFitMC(100,100,100,50,10,1000)
```

If one were to include points far from the peak maximum, where the signal-to-noise ratio is very low, the results would be poorer than predicted. These predictions depend on knowledge of the noise in the signal; if only a single sample of that noise is available for measurement, there is no guarantee that sample is a representative sample, especially if the total number of points in the measured signal is small; the standard deviation of small samples is notoriously variable. Moreover, these predictions are based on a simulation with *constant normally-distributed white* noise; had the actual noise varied with signal level or with x-axis value, or if the probability distribution had been non-normal, those predictions would not necessarily have been accurate. The bootstrap method has the advantage that it samples the actual noise in the signal. You can download the Matlab/Octave code for this Monte Carlo simulation from http://tinyurl.com/cey8rwh. A similar simulation (**GaussFitMC2.m**) compares this method to fitting the entire Gaussian peak with the iterative method on pages 54 - 69, finding that the precision of the results is slightly better with the slower iterative method.

Note 1: If you are viewing this document online, you can right-click on any of the m-file links above and select **Save Link As...** to download them for use within Matlab/Octave.

Note 2: In the curve fitting techniques described here and in the next two sections, there is no requirement that the x-axis interval between data points be uniform, as is the assumption in many of the other signal processing techniques previously covered. Curve fitting algorithms typically accept a set of arbitrarily-spaced x-axis values and a corresponding set of y-axis values.

Note 3: It's important that the noisy signal not be smoothed before the least-squares calculations, because doing so will not improve the reliability of the least-squares results, but it will cause both the algebraic propagation-of-errors and the bootstrap calculations to seriously underestimate the standard deviation of the least-squares results. You can demonstrate using the script **TestLinearFit.m** by setting SmoothWidth in line 10 to something higher than 1, which will smooth the data before the least-squares calculations. This has no significant effect on the *actual* standard deviation as calculated by the Monte Carlo method, but causes the *predicted* standard deviation calculated by both the algebraic and the bootstrap method (page 41) to be much too low. (If the data are contaminated with large narrow spikes, it may be best to apply a median filter, page 15, *before* least-squares computations).

Math details

The least-squares best fit for an x,y data set can be computed using only basic arithmetic and square roots. Here are the relevant equations for computing the slope and intercept of the first-order best-fit equation, y = intercept + slope*x, as well as the predicted standard deviation (SD) of the slope and intercept, and the coefficient of determination, “R2”, which is an indicator of the “goodness of fit”. (R2 is 1.0000 if the fit is perfect and less than that if the fit is imperfect):

```
n = number of x,y data points
sumx = Σx
sumy = Σy
sumxy = Σ(x*y)
sumx2 = Σ(x*x)
meanx = sumx / n
meany = sumy / n
slope = (n*sumxy - sumx*sumy) / (n*sumx2 - sumx*sumx
intercept = meany-(slope*meanx)
ssy = Σ(y-meany)^2
ssr = Σ(y-intercept-slope*x)^2
R2 = 1-(ssr/ssy)
SD of slope = SQRT(ssr/(n-2))*SQRT(n/(n*sumx2 - sumx*sumx))
SD of intercept = SQRT(ssr/(n-2))*SQRT(sumx2/(n*sumx2 - sumx*sumx))
```

(In these equations, Σ represents summation; for example, Σx means the sum of all the x values, and Σ(x*y) means the sum of all the x*y products, etc). A slightly more complex set of equations can be written to fit a second-order (quadratic or parabolic) equations to a set of data.

The last two lines predict the standard deviation of the slope and intercept, based only on that data sample, assuming that the noise is normally distributed. These are estimates of the variability of slopes and intercepts you are likely to get if you repeated the data measurements over and over multiple times under the same conditions, *assuming that the deviations from the straight line are due to random variability and not systematic error caused by non-linearity*. If the deviations are random, they will be slightly *different* from time to time, causing the slope and intercept to vary from measurement to measurement, with a standard deviation predicted by these last two equations. However, if the deviations are caused by systematic non-linearity, the deviations will be the *same* from from measurement to measurement, in which case the prediction of these last two equations will not be relevant, and you might be better off using a polynomial fit such as a quadratic or cubic. The reliability of these standard deviation estimates depends also on the number of data points *n* in the curve fit; they improve with the square root of *n*, assuming the deviations are random.

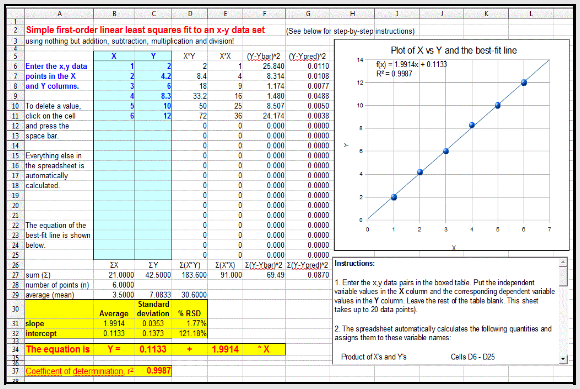
These calculations could be performed step-by-step by hand, but most people use a calculator, a spreadsheet, a program written in any programming language, a math Web page such as *Wolfram Alpha* (using the “linear fit” command), or a Matlab/Octave script.

The minimum number of data points required for a polynomial least-squares fit depends on the polynomial order: you need a minimum of *two* points for a first-order (straight-line) fit, a minimum of *three* points for a second-order (quadratic or parabolic) fit, a minimum of *four* points for a third-order (cubic) fit, etc, *always one more than the polynomial order*. With that minimum number of points, the fit will always be artificially perfect, no matter how large the errors in the data might be; so you get no hint of possible errors in the data because the best-fit line will always go right though all the points. The greater the number of points the better, because (a) you can see when there are errors in the data, and (b) the errors have a greater changes of partially “canceling out” and resulting in fit coefficients that are closer to the true long-term average.

Web sites: Zunzun can curve fit and surface fit 2D and 3D data online with a rich set of error histograms, error plots, curve plots, surface plots, contour plots, VRML, auto-generated source code, and PDF file output. Wolfram Alpha includes capabilities for least-squares regression analysis, including linear, polynomial, exponential, and logarithmic fits. Statpages.org can perform a huge range of statistical calculations and tests, and there are several Web sites that specialize in plotting and analyzing data that have curve-fitting capabilities, including Plotly and Plotter.

Spreadsheets can perform the math described above easily. The two spreadsheets shown below, LeastSquares.xls and LeastSquares.odt for linear fits, and QuadraticLeastSquares.xls and QuadraticLeastSquares.ods for quadratic fits, utilize the expressions given above to compute and plot linear and quadratic (parabolic) least-squares fit, respectively. Download from http://tinyurl.com/cey8rwh.

Modern spreadsheets also have built-in facilities for computing polynomial least-squares curve fits of any order. For example, the LINEST function in both Excel and OpenOffice Calc can be used to compute polynomial and other curvilinear least-squares fits. (In addition to the best-fit polynomial coefficients, the LINEST function also calculates at the same time the standard error values, the determination coefficient



Simple first-order linear least squares fit to an x-y data set (See below for step-by-step instructions)

using nothing but addition, subtraction, multiplication and division!

	A	B	C	D	E	F	G
5		X	Y	X*Y	X*X	(Y-Ybar)^2	(Y-Ypred)^2
6	Enter the x,y data	1	2	2	1	25.840	0.0110
7	points in the X	2	4.2	8.4	4	8.314	0.0108
8	and Y columns.	3	6	18	9	1.174	0.0077
9		4	8.3	33.2	16	1.480	0.0488
10	To delete a value,	5	10	50	25	8.507	0.0050
11	click on the cell	6	12	72	36	24.174	0.0038
12	and press the			0	0	0.000	0.0000
13	space bar.			0	0	0.000	0.0000
14				0	0	0.000	0.0000
15	Everything else in			0	0	0.000	0.0000
16	the spreadsheet is			0	0	0.000	0.0000
17	automatically			0	0	0.000	0.0000
18	calculated.			0	0	0.000	0.0000
19				0	0	0.000	0.0000
20				0	0	0.000	0.0000
21				0	0	0.000	0.0000
22	The equation of the			0	0	0.000	0.0000
23	best-fit line is shown			0	0	0.000	0.0000
24	below.			0	0	0.000	0.0000
25				0	0	0.000	0.0000
26		ΣX	ΣY	Σ(X*Y)	Σ(X*X)	Σ(Y-Ybar)^2	Σ(Y-Ypred)^2
27	sum (Σ)	21.0000	42.5000	183.600	91.000	69.49	0.0870
28	number of points (n)	6.0000					
29	average (mean)	3.5000	7.0833	30.6000			
30		Average	Standard deviation	% RSD			
31	slope	1.9914	0.0353	1.77%			
32	intercept	0.1133	0.1373	121.18%			
34	The equation is	Y =	0.1133	+	1.9914	* X	
37	Coefficent of determiniation, r²		0.9987				

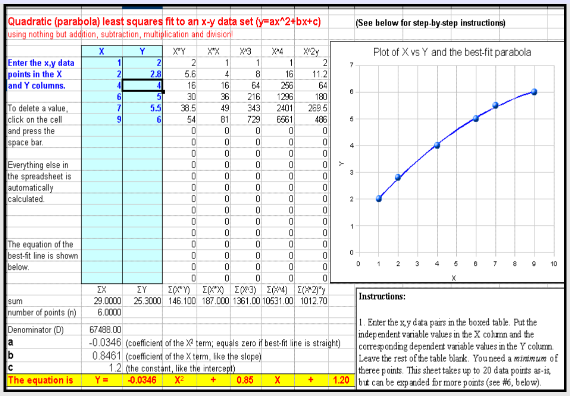
Instructions:

1. Enter the x,y data pairs in the boxed table. Put the independent variable values in the **X** column and the corresponding dependent variable values in the **Y** column. Leave the rest of the table blank. This sheet takes up to 20 data points).

2. The spreadsheet automatically calculates the following quantities and assigns them to these variable names:

Product of X's and Y's Cells D6 - D25

LeastSquares



Quadratic (parabola) least squares fit to an x-y data set (y=ax^2+bx+c) (See below for step-by-step instructions)

using nothing but addition, subtraction, multiplication and division!

	X	Y	X*Y	X*X	X^3	X^4	X^2y
Enter the x,y data	1	2	2	1	1	1	2
points in the X	2	2.8	5.6	4	8	16	11.2
and Y columns.	4	4	16	16	64	256	64
	6	5	30	36	216	1296	180
To delete a value,	7	5.5	38.5	49	343	2401	269.5
click on the cell	9	6	54	81	729	6561	486
and press the			0	0	0	0	0
space bar.			0	0	0	0	0
			0	0	0	0	0
Everything else in			0	0	0	0	0
the spreadsheet is			0	0	0	0	0
automatically			0	0	0	0	0
calculated.			0	0	0	0	0
			0	0	0	0	0
			0	0	0	0	0
			0	0	0	0	0
The equation of the			0	0	0	0	0
best-fit line is shown			0	0	0	0	0
below.			0	0	0	0	0
			0	0	0	0	0
	ΣX	ΣY	Σ(X*Y)	Σ(X*X)	Σ(X^3)	Σ(X^4)	Σ(X^2)*y
sum	29.0000	25.3000	146.100	187.000	1361.00	10531.00	1012.70
number of points (n)	6.0000						
Denominator (D)	67488.00						
a	-0.0346	(coefficient of the X² term; equals zero if best-fit line is straight)					
b	0.8461	(coefficient of the X term, like the slope)					
c	1.2	(the constant, like the intercept)					

The equation is Y = -0.0346 X² + 0.85 X + 1.20

Instructions:

1. Enter the x,y data pairs in the boxed table. Put the independent variable values in the X column and the corresponding dependent variable values in the Y column. Leave the rest of the table blank. You need a *minimum* of theree points. This sheet takes up to 20 data points as-is, but can be expanded for more points (see #6, below).

QuadraticLeastSquares

(R²), the standard error value for the *y* estimate, the F statistic, the number of degrees of freedom, the regression sum of squares, and the residual sum of squares). The disadvantage of LINEST, compared to working out the math using the math described above, is that it is more difficult to adjust to a variable number of data points and to remove suspect data points.

For some examples of applications to calibration in chemical analysis, see "Calibration Curve Fitting Methods in Absorption Spectroscopy" and "Error propagation in Analytical Calibration".

You can also download the spreadsheets pictured above, in *Excel* or *OpenOffice Calc* format, that automate the computation of those equations, plot the data and the best-fit line, and compute the expected standard deviation of the slope and intercept, requiring only that you type in (or paste in) the x-y data. There is one for linear fits (**LeastSquares.xls/ods**) and one for quadratic (parabolic) fits (**QuadraticLeastSquares.xls/ods**). Download from http://tinyurl.com/cey8rwh**.**

For the application to measurement calibration, there are specific versions of these spreadsheets that also use the best-fit equation to calculate x (e.g. the concentrations) given the instrument responses of the unknowns. For more on this, see http://terpconnect.umd.edu/~toh/models/CalibrationCurve.html.

GaussianLeastSquares.odt/.xls are spreadsheets that fit a quadratic function to the natural log of y(x) and computes the height, position, and width of the best-fit Gaussian. LorentzianLeastSquares.ods/.xls fits a quadratic function to the reciprocal of y(x) and computes the height, position, and width of the best-fit Lorentzian. Note that the data may *not* contain zeros or negative points, and the baseline (value that y approaches far from the peak center) must be zero. See page 43.

SPECTRUM (page 70) includes least-squares curve fitting for polynomials of order 1 through 5, plus exponential, logarithmic, and power relationships.

Matlab and Octave have simple built-in functions for polynomial least-squares curve fitting: polyfit and polyval. For example, if you have a set of x,y data points in the vectors "x" and "y", then the vector of coefficients for the least-squares fit, in decreasing powers of x, are given by `coef=polyfit(x,y,n)`, where "n" is the order of the polynomial fit: n = 1 for a straight-line fit, 2 for a quadratic (parabola) fit, etc. For a straight-line fit (n=1), coef(1) is the slope ("b") and coef(2) is the intercept ("a"). For a quadratic fit (n=2), coef(1) is the x^2 term ("c"), coef(2) is the x term ("b") and coef(3) is the constant term ("a"). The fit equation can be evaluated using the function polyval, for example `y= polyval(coef,x)`. This works for any order of polynomial fit ("n"). Writing `[coef,S] = polyfit(x,y,n)` also returns a structure 'S' for use with polyval to obtain coefficient error estimates (page 40); the vector of standard deviations can be computed directly from S by: `sqrt(diag(inv(S.R)*inv(S.R')).*S.normr.^2./S.df)'`, in the same order as the coefficients. You can plot the data in blue and the fitted equation in red together in one graph this way: `xx=linspace(min(x),max(x)); plot(x,y,'ob',xx,polyval(coef,xx),'-r')`.

You may also perform the polynomial least squares calculations for the row vectors x,y *without* using the Matlab/Octave built-in polyfit function by using the matrix method. The coefficients of a first order fit are given by `y/[x;ones(size(y))]` and a second order (quadratic) fit by `y/[x.^2;x;ones(size(y))]`. For higher-order polynomials, just add additional rows to the denominator matrix, e.g. `y/[x.^3;x.^2;x;ones(size(y))]`. The coefficients are returned in the same order as polyfit.

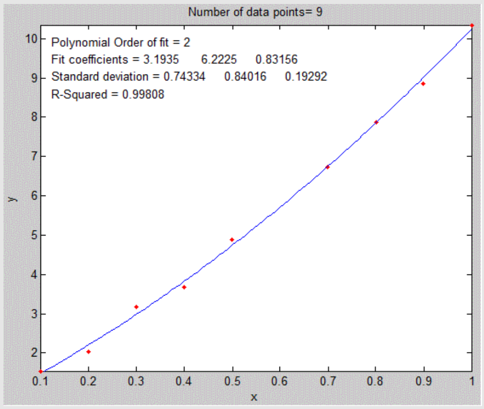
Fitting peaks. The function **gaussfit.m** performs a least-squares fit of a single Gaussian function to an x,y data set, returning the height, position, and width of the best-fit Gaussian. The syntax is `[Height,Position,Width] = gaussfit(x,y)`. See page 43. **Plotgaussfit.m** does the same thing but also plots the data and the fit. The similar function **lorentzfit.m** performs an analogous calculation for a Lorentzian peak shape. An expanded variant of gaussfit.m, is **bootgaussfit.m**, which does the same thing but also optionally plots the data and the fit and computes estimates of the random error in the height, width, and position of the fitted Gaussian function by the bootstrap sampling method (page 41-42). For example:

```
>> x=50:150;y=100.*gaussian(x,100,100)+10.*randn(size(x));
>> [Height,Position,Width,BootResults]=bootgaussfit(x,y,1);
```

This code additionally returns the error estimates of the height, position, and width, which are displayed in a table and returned in the 3×5 matrix "BootResults". Type "help bootgaussfit" for help.

```
                  Height      Position       Width
Bootstrap Mean:  100.84       101.325       98.341
Bootstrap STD:   1.3458       0.63091       2.0686
Bootstrap IQR:   1.7692       0.86874       2.9735
Percent RSD:     1.3346       0.62266       2.1035
Percent IQR:     1.7543       0.85737       3.0237
```

Plotit. The graph on the right was generated by my downloadable function **plotit(x,y,polyorder)**. It uses *all the techniques mentioned in the previous paragraph*. It accepts data in the form of a single vector, or a pair of vectors "x" and "y", or a 2×n or n×2 matrix with x in the first row or column and y in the second, and plots the data points as small red dots. If the optional input argument "polyorder" is provided, it fits a polynomial of order "polyorder" to the data and plots the fit as a green line and displays the fit coefficients, their standard deviations, and the goodness-of-fit measure R2 (R-squared) in the upper left corner of the graph. Some examples, assuming x=[1 2 3 4 5] and y=[0 2 3 2 0]:



For plotting only: plotit(y) or plotit(x,y) or plotit([x;y]) or plotit([x;y]');

Including an integer as the third input argument triggers the polynomial fitting routine:

`plotit(y,1);` plots y vs its index and fits to first order (linear) equation.
`plotit(x,y,2);` plots x vs y and fits to second order (quadratic) polynomial
`coef,RSquared]=plotit([x;y],2)` returns fit coefficients (coef) and R-squared (RSquared)
`[coef,RSquared,stdev]=plotit([x;y],2)` returns standard deviations of coefficients 'stdev'
`plotit(x,y,2,datastyle,fitstyle)` where datastyle and fitstyle are optional strings specifying the line and symbol style and color, in standard Matlab convention (in version 6 and later).

You can use plotit.m to linearize and plot other nonlinear relationships (page 43) such as:

`y = a exp(bx): [coeff,R2]=plotit(x,log(y),1);a=exp(coeff(2));b=coeff(1);`
`y = a ln(bx) : [coeff,R2]=plotit(log(x),y,1);a=coeff(1);b=log(coeff(2));`
y=a x^b : `[coeff,R2]=plotit(log(x),log(y),1);a=exp(coeff(2));b=coeff(1);`

Don't forget that in Matlab/Octave, "log" means *natural log*; the *log to base 10* is "log10".

Plotit.m also has a built-in bootstrap routine (page 41-42) that gives another estimate of the coefficient standard deviations and returns the results in the matrix "BootResults" (of size 5 × polyorder+1). The bootstrap calculation is triggered by including a third output argument, e.g.[coef, Rsquared, BootResults]= plotit(x,y,polyorder). This works for any polynomial order. You can change the number of bootstrap samples in line 93 (higher = slower but more accurate error estimates). There are two variations: plotfita animates the bootstrap process for instructional purposes, and logplotfit plots and fits log(x) vs log(y), for data that follows a power law relationship or that covers a very wide numerical range. Type "help plotit" for more.

Other functions employing curve fitting. My Matlab/Octave function **trypolyplot(x,y)** fits the data in x,y with a series of polynomials of degree 1 through length(x)-1 and returns the coefficients of determination (R^2) of each fit as a vector, and plots order vs R^2, showing that, for any data, R^2 approaches 1 as the polynomial order approaches length(x)-1. The related function **trydatatrans(x,y,polyorder)** tries 8 different simple data transformations on the x,y data, fits a polynomial of order 'polyorder' to the transformed data, displays results graphically in 3 x 3 array of small plots, and returns the R^2 values in a vector.

The latest versions of my downloadable interactive Matlab functions **iSignal.m** (page 85) and **ipf.m** (page 90) have a built-in polynomial fitting function: press the **Shift-o** key, then enter the desired polynomial order.

You may download any of these functions or spreadsheets from http://tinyurl.com/cey8rwh.

Note: recent versions of Matlab have a convenient tool for interactive manually-controlled (rather than programmed) polynomial curve fitting in the Figure window. Also, the add-on Matlab Curve Fitting Toolbox includes a very flexible curve fit function. If you do not have the Matlab Curve Fitting Toolbox, you may still use any of my curve fitting functions described here.

Curve fitting B: Multicomponent Optical Spectroscopy

The optical spectroscopic analysis of mixtures, when the optical spectra of the individual components overlap considerably, requires special calibration methods based on a type of linear least squares called *multiple linear regression*. This method is widely used in multiwavelength techniques such as diode-array, Fourier transform, and automated scanning spectrometers. In this case the math involves the application of a little basic matrix algebra (a.k.a., "linear algebra"), which is just a shorthand notation for dealing with signals expressed as equations with one term for each data point.

Definitions:

A = analytical signal	*n* = number of distinct chemical components in the mixture.
ε = analytical sensitivity	c1, c2 = component 1, component 2, etc. (up to *n*)
c = molar concentration	λ1, λ2 = wavelength 1, wavelength 2, etc. (up to *w*)
s = number of samples	s1, s2 = sample 1, sample 2, etc. (up to *s*)

w = number of wavelengths at which signal is measured

Assumptions:

a. The measured analytical signal, A (such as absorbance in absorption spectroscopy, fluorescence intensity in fluorescence spectroscopy, and reflectance in reflectance spectroscopy) is directly proportional to concentration, c. The proportionality constant (the slope of a plot of A vs c) is ε.

$$A = \varepsilon c$$

b. The total signal observed for the mixture is the sum of the signals for each component in a mixture, which is at least approximately true for many forms of spectroscopy:

$A_{total} = A_{c1} + A_{c2} + ...$ for all *n* components, where A_{c1} is the signal for component 1, etc.

c. The wavelength registration of all the optical spectra is perfect (no uncertainty in the x-axis)

Classical Least Squares (CLS) calibration. This method is applicable to the quantitative analysis of a mixture of components when the *optical spectra of the individual components are known*. Measurement of the spectra of known concentrations of the separate components allows their analytical sensitivity ε at each wavelength to be determined. Then it follows that:

$A_{\lambda 1}=\varepsilon_{c1,\lambda 1}\, c_{c1} + \varepsilon_{c2,\lambda 1}\, c_{c2} + \varepsilon_{c3,\lambda 1}\, c_{c3} + \ldots$ for all *n* components.

$A_{\lambda 2}=\varepsilon_{c1,\lambda 2}\, c_{c1} + \varepsilon_{c2,\lambda 2}\, c_{c2} + \varepsilon_{c3,\lambda 2}\, c_{c3} + ...$

and so on for all *w* wavelengths - λ3, λ4, etc. It's too tedious to write out *all* these individual terms every time, especially because there may be *hundreds* of wavelengths in modern array-detector spectrometers. And despite the large number of terms, these are really nothing more than long linear equations, and the calculations required are actually very simple - certainly trivial for a computer to do. So it would be nice to have a *correspondingly simple notation* that would save us from writing out all those terms. That's what "matrix notation" does. We can write this big set of linear equations:

$$\mathbf{A} = \boldsymbol{\varepsilon}\mathbf{C}$$

where bold-face **A** represents the *w*-length vector of measured signals of the mixture at each wavelength (i.e. the optical spectrum), boldface **ε** is the *n* x *w* rectangular matrix of the known ε-values for each of the *n* components at each of the *w* wavelengths, and boldface **C** is the *n*-length vector of concentrations of all the components. **εC** means that **ε** "pre-multiplies" **C**, which means that each column of **ε** is multiplied point-by-point by the vector **C.** The beauty of this notation is that *it's the same no matter how many wavelengths (w) or components (n) you have*. That's a big advantage, especially as you might easily have spectra with *many hundreds of wavelengths*.

If you have a sample solution containing a mixture of unknown concentrations of those *n* components, then you measure its spectrum **A** and seek to calculate the *n*-length concentration vector **C**. In order to solve the above matrix equation for **C**, the number of wavelengths *w* must be equal to or greater than the number of components *n*. If *w* = *n*, then we have a system of *n* equations in *n* unknowns which can be solved by pre-multiplying both sides of the equation by $\boldsymbol{\varepsilon}^{-1}$, the *matrix inverse* of **ε**, and using the fact that any matrix times its inverse is unity:

$$\mathbf{C} = \varepsilon^{-1}\mathbf{A}$$

Because real experimental spectra are subject to random noise (e.g. photon noise and detector noise), the solution will be more precise if signals at a larger number of wavelengths are used, that is if $w > n$. In general, the more wavelengths are used, the more effectively the random noise will be “averaged out” - although it won’t help to use wavelengths where none of the components produce analytical signals. The optimum region is usually determined empirically. But then the equation can not be solved by matrix inversion, because the ε matrix is a w x n matrix and a matrix inverse exists only for square matrices. But a solution can still be obtained in this case by pre-multiplying both sides of the equation by the expression $(\varepsilon^T\varepsilon)^{-1}\varepsilon^T$, where ε^T means the transpose of ε:

$$(\varepsilon^T\varepsilon)^{-1}\varepsilon^T\mathbf{A} = (\varepsilon^T\varepsilon)^{-1}\varepsilon^T\varepsilon\mathbf{C} = (\varepsilon^T\varepsilon)^{-1}(\varepsilon^T\varepsilon)\mathbf{C}$$

But the quantity $(\varepsilon^T\varepsilon)^{-1}(\varepsilon^T\varepsilon)$ is a matrix times its inverse and is therefore unity. Thus:

$$\mathbf{C} = (\varepsilon^T\varepsilon)^{-1}\varepsilon^T\mathbf{A}$$

Once the quantity $(\varepsilon^T\varepsilon)^{-1}\varepsilon^T$ is computed, you can measure multiple samples containing different unknown amounts of the components by measuring the spectrum **A** of each sample and pre-multiplying it by that quantity.

Two extensions of the CLS method are commonly made. First, in order to account for baseline shift caused by drift, background, and light scattering, a column of 1s is added to the ε matrix. This has the effect of introducing into the solution an additional component with a flat spectrum; this is referred to as “background correction”. Second, in order to account for the fact that the precision of measurement may vary with wavelength, it is common to perform a *weighted* least squares solution that de-emphasizes wavelength regions where precision is poor:

$$\mathbf{C} = (\varepsilon^T\,\mathbf{V}^{-1}\varepsilon)^{-1}\,\varepsilon^T\,\mathbf{V}^{-1}\,\mathbf{A}$$

where **V** is an w x w diagonal matrix of variances at each wavelength. In *absorption* spectroscopy, where the precision of measurement is poor in spectral regions where the absorbance is very high (and the light level and signal-to-noise ratio therefore low), it is common to use the transmittance T or its square T^2 as weighting factors.

The classical least-squares method is in principle applicable to any number of overlapping components. Its accuracy is limited by how accurately the spectra of the individual components are known, the amount of noise in the signal, the extent of overlap of the spectra, the x-axis (wavelength) registration, and the linearity of the analytical curves of each component (the extent to which the signal amplitudes are proportional to concentration). The method is widely applied in absorption spectrophotometry, especially using array detectors or Fourier transform instruments. The well-known deviations from analytical curve linearity set a limit to the performance to this method, but they can be circumvented by applying curve fitting to the transmission spectra (see page 103).

Inverse Least Squares (ILS) calibration. Inverse Least Squares is a method that can be used to measure the concentrations of components in samples in which *the optical spectrum of the components in the sample is not known beforehand*. Whereas the classical least squares (CLS) method models the signal at each wavelength as the sum of the concentrations of the components times the analytical sensitivity, *inverse* least squares methods use the *reverse* approach and models the components concentration c in each sample as the sum of the signals A at each wavelength times calibration coefficients m that express how the concentration of that component is related to the signal at each wavelength:

$c_{s1} = m_{\lambda1}A_{s1,\lambda1} + m_{\lambda2}A_{s1,\lambda2} + m_{\lambda2}A_{s1,\lambda2} + \ldots$ for all w wavelengths.

$c_{s2} = m_{\lambda1}A_{s2,\lambda1} + m_{\lambda2}A_{s2,\lambda2} + m_{\lambda2}A_{s2,\lambda2} + \ldots$

and so on for all s samples. In matrix form:

$$\mathbf{C} = \mathbf{AM}$$

where **C** is the *s*-length vector of concentrations of the components in the *s* samples, **A** is the *w* x *s* matrix of measured signals at the *w* wavelengths in the *s* samples, and **M** is the *w*-length vector of calibration coefficients.

Now, suppose that you have a set of standard samples that are typical of the type of sample that you wish to be able to measure and which contain a range of components concentrations that span the range of concentrations expected to be found in other samples of that type. This will serve as the *calibration set.* You measure the optical spectrum of each of the samples in this calibration set and put these data into a *w* x *s* matrix of measured signals **A**. You then measure the component concentrations in each of the samples by some *reliable and independent analytical method* and put those data into a *s*-length vector of concentrations **C.** Together these data allow you to calculate the calibration vector **M** by solving the above equation. *This only works if the number of samples in the calibration set is greater than the number of wavelengths at which the samples are measured.* The least-squares solution is:

$$\mathbf{M} = (\mathbf{A}^{\mathrm{T}}\mathbf{A})^{-1}\mathbf{A}^{\mathrm{T}}\mathbf{C}$$

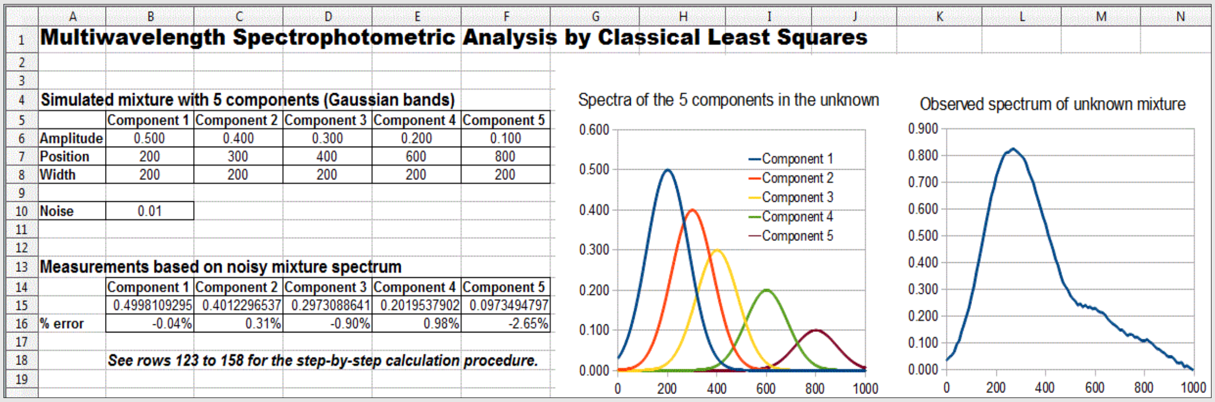
(Note that $\mathbf{A}^{\mathrm{T}}\mathbf{A}$ is a square matrix of size *w*, the number of wavelengths, which *must be less* than *s*). This calibration vector can be used to compute the components concentrations of other samples, which are similar to but not in the calibration set, from the measured spectra of the samples:

$$\mathbf{C} = \mathbf{AM}$$

Clearly this will work well only if the analytical samples are similar to the calibration set. However, this is a very common analytical situation in commerce, for example in industrial quality control and in agricultural foodstuffs analysis, where *large numbers of samples of a similar predictable type* must be analyzed quickly and cheaply.

Software details. Most modern spreadsheets have basic matrix manipulation capabilities and can be used for multicomponent calibration, for example *Excel*, *OpenOffice* Calc, or WingZ. The spreadsheets RegressionDemo.xls/**.ods** demonstrate the classical least squares procedure for a simulated optical spectrum of a 5-component mixture measured at 100 wavelengths. (Download from **http://tinyurl.com/cey8rwh**). The matrix calculations described above solves for the concentration of the components on the unknown mixture:

$$\mathbf{C} = (\boldsymbol{\varepsilon}^{\mathrm{T}}\boldsymbol{\varepsilon})^{-1}\boldsymbol{\varepsilon}^{\mathrm{T}}\mathbf{A}$$



	A	B	C	D	E	F
1	**Multiwavelength Spectrophotometric Analysis by Classical Least Squares**					
2						
3						
4	**Simulated mixture with 5 components (Gaussian bands)**					
5		Component 1	Component 2	Component 3	Component 4	Component 5
6	Amplitude	0.500	0.400	0.300	0.200	0.100
7	Position	200	300	400	600	800
8	Width	200	200	200	200	200
9						
10	Noise	0.01				
11						
12						
13	**Measurements based on noisy mixture spectrum**					
14		Component 1	Component 2	Component 3	Component 4	Component 5
15		0.4998109295	0.4012296537	0.2973088641	0.2019537902	0.0973494797
16	% error	-0.04%	0.31%	-0.90%	0.98%	-2.65%
17						
18		***See rows 123 to 158 for the step-by-step calculation procedure.***				
19						

This calculation is performed in these spreadsheets by the TRANSPOSE (matrix transpose), MMULT (matrix multiplication), and MINVERSE (matrix inverse) array functions, laid out step-by-step in rows 123 to 158 of this spreadsheet. Alternatively, these array operations may be combined into one cell equation:

$$\mathbf{C} = \mathrm{MMULT(MMULT(MINVERSE(MMULT(TRANSPOSE(}\mathbf{e}\mathrm{);}\mathbf{e}\mathrm{));TRANSPOSE(}\mathbf{e}\mathrm{));}\mathbf{A}\mathrm{)}$$

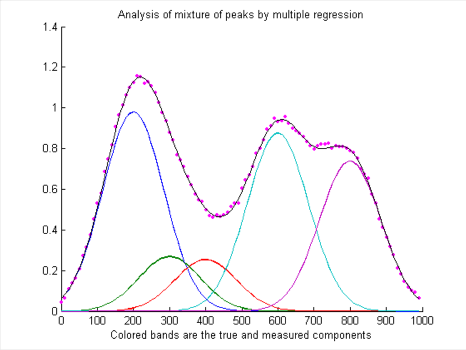
where **C** is the vector of the 5 concentrations of all the components in the mixture, **e** is the 5 x 100 rectangular matrix of the known sensitivities (e.g. absorptivities) for each of the 5 components at each of the 100 wavelengths, and **A** is the vector of measured signals at each of the 100 wavelengths (i.e. the signal spectrum) of the unknown mixture. (Note: spreadsheet array functions like this must be entered by typing

Ctrl-Shift-Enter, not just **Enter** as usual, See http://www.openofficetips.com/blog/archives/2004/10/array_formulas.html)

Using the LINEST function. Alternatively, you can skip over all the math above and use the **LINEST** function, in *Excel* or *OpenOffice Calc*, which performs this type of calculation in a single function statement. This is illustrated in **RegressionDemo.xls**, in cell **P23**. A big advantage of the LINEST function is that it can compute the standard errors of the coefficients and the R^2 value in the same operation; using Matlab or Octave, that would require some extra work. (LINEST is also an array function that must also be entered by typing **Ctrl-Shift-Enter**, not just **Enter**). Note that this is the same LINEST function that was used for the polynomial least-squares on page 46; the difference is that in polynomial least-squares, the multiple columns of x values are *computed*, for example by taking the powers (squares, cubes, etc) of the first column of x values, whereas in the multicomponent CLS method, the multiple columns of x values are *experimental* values of the different standard solutions. The *math* is the same, but the *origin of the x data* is different.

A template for performing a 100-point 5-component analysis on your own data, with step-by-step instructions, in available as RegressionTemplate.xls and RegressionTemplate.ods (Graphic with example data). Replace the data in columns B - G with your own data and adjust the formulas if your number of data points or of components is different from this example (100 and 5, respectively).

Matlab and **Octave** are really the natural computer approach to multicomponent analysis because they handle all types of matrix math so easily, compactly, and quickly, adapting *automatically* to any number of components and wavelengths. In these languages, the notation is very compact but a little different: the transpose of a matrix **A** is **`A'`**, the inverse of **A** is **`inv(A)`**, and matrix multiplication is designated by *. Thus the solution to the classical least squares method above is written **`C = inv(E'*E)*E'*A`,** where **E** is the rectangular matrix of sensitivities at each wavelength for each component and **A** is the observed optical spectrum of the mixture. Note that the Matlab/Octave notation is not only shorter than the spreadsheet notation, it's also closer to the traditional mathematical notation, and it's the same no matter the number of components and wavelengths. Alternatively, you can write **C = A * pinv(E')'** or **C = A/E**, which use different internal mathematics to yield essentially the same results in the same execution time (except for the numerical floating point precision of the computer, which is usually negligible).



The script **RegressionDemo.m** (for Matlab or Octave) demonstrates the same classical least squares procedure for a simulated absorption spectrum of a 5-component mixture, illustrated on the left. In this example the dots represent the observed spectrum of the mixture (with noise) and the five colored bands represent the five components in the mixture, whose spectra are known but whose concentrations in the mixture are unknown. The black line represents the "best fit" to the observed spectrum calculated by the program. In this example the concentrations of the five components are measured to an accuracy of about 1% relative (limited by the noise in the observed spectrum). Comparing **RegressionDemo.m** to its spreadsheet equivalent, **RegressionDemo.xls**, both running the same computer, you can see that the Matlab/Octave code computes and plots the results quicker than the spreadsheet, although neither takes no more than a fraction of a second for this example.

The extension to "background correction" is easily accomplished in Matlab/Octave by adding a column of 1s to the **A** matrix containing the absorption spectrum of each of the components:

```
background=ones(size(ObservedSpectrum));
 A=[background A1 A2 A3];
```

where A1, A2, A3... are the absorption spectra of the individual components. Performing a T-weighted regression is also readily performed:

```
weight=T;
MeasuredAmp=([weight weight] .* A)\(ObservedSpectrum .* weight);
```

where T is the transmission spectrum. Here, the matrix division backslash "\" is used as a shortcut to the classical least-squares matrix solution (See http://www.mathworks.com/help/techdoc/ref/mldivide.html).

Using a computer-generated calibration matrix. Ordinarily, the **calibration** matrix **M** is assembled from the *experimentally measured* signals (e.g. spectra) of the individual components of the mixture, but it is also possible to fit a *computer-generated model* of basic peak shapes (e.g. Gaussians, Lorentzians, etc) to a signal to determine if that signal can be represented as the weighted sum of overlapping basic peak shapes. The function **cls.m** computes such a model consisting of the sum of any number of peaks of known shape, width, and position, but of unknown height, and fit it to noisy x,y data sets. The syntax is

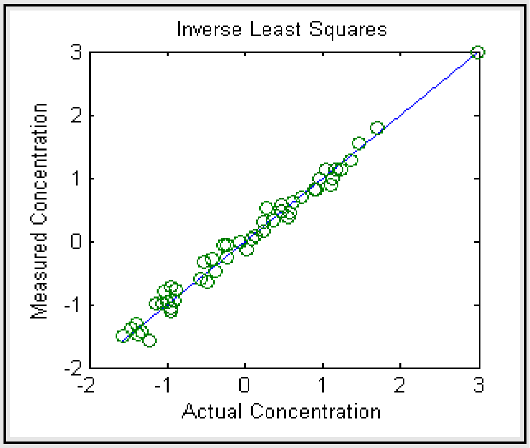
```
heights=cls(x,y,NumPeaks,PeakShape,Positions,Widths,extra)
```

where x and y are the vectors of measured data (e.g. x might be wavelength and y might be the absorbance at each wavelength), '**NumPeaks**' is the number of peaks, '**PeakShape**' is the peak shape number (1=Gaussian, 2=Lorentzian, 3=logistic distribution, 4=Pearson, 5=exponentially broadened Gaussian; 6=equal-width Gaussians; 7=Equal-width Lorentzians; 8=exponentially broadened equal-width Gaussian, 9=exponential pulse, 10=sigmoid, 11=Fixed-width Gaussian, 12=Fixed-width Lorentzian; 13=Gaussian/Lorentzian blend; 14=BiGaussian, 15=BiLorentzian), '**Positions**' is the vector of peak positions on the x axis (one entry per peak), '**Widths**' is the vector of peak widths in x units (one entry per peak), and '**extra**' is the additional shape parameter required by the exponentially broadened, Pearson, Gaussian/Lorentzian blend, BiGaussian and BiLorentzian shapes. **Cls.m** returns a vector of measured peak heights for each peak.

The downloadable Matlab/Octave function "**clsdemo.m**" creates some noisy model data, fits it with **cls.m**, computes the accuracy of the measured heights, then repeats the calculation using *iterative non-linear least squares peak fitting* (INLS, covered in on page 54) with the downloadable peakfit.m function, making use of the known peak positions and widths only as starting guesses ("start"). You can see that CLS is faster and (usually) more accurate, especially if the peaks are highly overlapped. (This script requires cls.m, modelpeaks.m, and peakfit.m in the Matlab/Octave path). A related function, cls2.m, also computes the baseline, using the extension described on page 52, and returns it as the first element of the heights vector.

Weighted regression. Another example of the classical least squares procedure applied to a mixture measurement problem is contained in the demo function "**tfit.m**", which simulates the measurement measurement of the absorption spectrum of a mixture of three components by weighted linear regression (on line 61), demonstrates the effect of the amount of noise in the signal, the extent of overlap of the spectra, and the linearity of the analytical curves of each component. (This demo also compares the results to another calibration method that applies convolution and curve fitting to the *transmission* spectra rather than to the *absorbance* spectra, treated on page 103). The idea of weighting is also applied to polynomial regression (page 37), for example when applied to measurement calibration.

You may download any of these functions from **http://tinyurl.com/cey8rwh**.



The **Inverse Least Squares** (ILS) technique is demonstrated by the Matlab/Octave example wheat.m, (located at http://terpconnect.umd.edu/~toh/spectrum/wheatILS.zip), shown in the graph on the right. The math, described on page 50, is similar to the Classical Least Squares method, and can be done by any of the methods on page 52. This example is based on a real data set derived from the near infrared (NIR) reflectance spectroscopy of agricultural wheat samples analyzed for protein content. In this example there are 50 calibration samples measured at 6 carefully chosen wavelengths. The samples had already been analyzed by a reliable, but laborious and time consuming, wet chemical reference method.

The purpose of this calibration is to establish whether near-infrared reflectance spectroscopy, which can be measured much more quickly on wheat paste preparations, correlates to their protein content. These results indicate that it does, at least for this set of 50 wheat samples, and therefore is it likely that near-infrared spectroscopy should do a pretty good job of estimating the protein content of similar unknown samples. The key is that the unknown samples *must be similar* to the calibration samples, except for the protein content. However, this is a very common analytical situation in quality control, where large numbers of samples of products of a similar predictable type must often be tested quickly and cheaply. Cf. http://en.wikipedia.org/wiki/Near-infrared_spectroscopy

Curve fitting C: Non-linear Iterative Curve Fitting ("spectral deconvolution" or "peak deconvolution")

The linear least squares curve fitting described above in "Curve Fitting A" is simple and fast, but it is limited to situations where the dependent variable can be modeled as a polynomial with *linear* coefficients. We saw on page 43 that in some cases a non-linear situation can be converted into a linear one by a coordinate transformation, but this is possible only in some special cases and, in any case, the resulting transformation of the noise in the data can result in inaccuracies in the parameters measured in that way.

The most general way of fitting any model to a set of data is the iterative method, a kind of "trial and error" procedure in which the parameters of the model are adjusted in a systematic fashion until the equation fits the data as close as required. This is basically a brute-force approach. In fact, in the days before computers, this method was only grudgingly applied. But its great generality, coupled with huge advances in computer speed and algorithm efficiency in recent decades, means that iterative methods are now more widely used now than ever before.

Iterative methods proceed in the following general way:

(1) You select a model for the data (e.g, a straight line, parabola, Gaussian, Lorentzian, etc);

(2) You (or a computer program) make *first guesses* of all the variable parameters (e.g. slopes, intercepts, positions, widths);

(3) A computer program computes the model and compares it to the data set, calculating a fitting error;

(4) If the fitting error is greater than the required fitting accuracy, the program systematically changes one or more of the parameters and loops back around to step **3**. This continues until the fitting error is less than the specified acceptable error. One popular technique for doing this is called the *Nelder-Mead Modified Simplex*. This is essentially a way of organizing and optimizing the changes in parameters (step 4, above) to shorten the time required to fit the function to the required degree of accuracy.

With modern personal computers, *the entire process typically takes only a fraction of a second*. The first guess (step **2**) can usually be supplied automatically by software, using various approximate methods that give rough but quick estimates, but in difficult cases you can provide your own starting first guess.

The reliability of iterative fitting, like classical least-squares fitting (page 37), depends strongly on the suitability of the model, the signal-to-noise ratio of the data, and the number of independent non-linear variables that must be adjusted. It is not possible to predict the standard deviations of the measured model parameters using the algebraic approach, but both the Monte Carlo simulation and bootstrap method (page 41-42) are applicable. (See #15 on page 93 for a specific example of a bootstrap statistics function in an iterative curve fitting program).

The main difficulty of the iterative methods is that they sometime fail to converge at an optimum solution in difficult cases. The standard approach to handle this is to restart the algorithm with a slightly different set of first guesses; software can automate that process, trying different starting points until the best fit is obtained. Iterative curve fitting also takes longer than linear regression - with typical modern personal computers, an iterative fit might take fractions of a second where a multilinear regression might take fractions of a millisecond. Still, this is already fast enough for many purposes, and computers will only continue to get faster and faster in the future.

Note: the term "spectral deconvolution" or "band deconvolution" or "curve deconvolution" is often used to refer to this technique, but in this essay, "deconvolution" specifically means ***Fourier** deconvolution*, an independent concept that is treated on page 32.

It's instructive to compare this iterative method with *classical least-squares curve fitting*, discussed on page 49, which can also fit peaks in a signal. The difference is that in the classical least squares method, the positions, widths, and shapes of all the individual components are all known beforehand; the *only* unknowns are the amplitudes (e.g. peak heights) of the components in the mixture. In non-linear iterative curve fitting, on the other hand, the positions, widths, and heights of the peaks *are all unknown* beforehand; the *only* thing that is known is the fundamental underlying *shape* of the peaks. So, because it is determining more unknown variables, the non-linear iterative curve fitting is more difficult to do computationally and more prone to error, but it's necessary if you need to track shifts in peak position or widths or to fit peaks in a signal knowing only their shape. (See "**CLSvsINLS.m**" on page 56 for an example).

Spreadsheets and stand-alone programs. Both *Excel* and *OpenOffice Calc* has a "Solver" capability that will automatically change specified cells in an attempt to produce a specified goal, such as minimizing a value like the RMS fitting error between a set of data and a proposed calculated model, for example a set of overlapping Gaussian bands. A set of Excel spreadsheet templates demonstrates the application to multiple Gaussian curve fitting; instructions for using and modifying these templates are given on the Web.

There are also a number of downloadable non-linear iterative curve fitting adds-ons and macros for *Excel* and *OpenOffice*, as well as some stand-alone freeware and commercial programs that perform this type of optimization. Code for Nelder-Mead optimization in the *C* language and in *Fortran* is available from Mike Hutt. Dr. Roger Nix of Queen Mary University of London has developed a very nice Excel/VBA spreadsheet for curve fitting X-ray photoelectron spectroscopy (XPS) data, but it could be used to fit other types of spectroscopic data; a 4-page instruction sheet is provided.

The disadvantage of using a spreadsheet for this type of curve fitting is that you have to make a custom spreadsheet for each problem, with the right number of rows for the data and with the desired number of components. The template CurveFitter.xlsx is only for a 100-point signal and a 5-component Gaussian model; you would have to edit it to handle other number of components or data points or model shapes. In contrast, my Matlab/Octave peakfit functions automatically adapt to any number of data points and is easily set to different model shapes, numbers of peaks, and baseline correction methods. But a significant *advantage* of spreadsheets is that it is relatively easy to add your own shape functions and constraints, even complicated ones, using standard spreadsheet cell formula construction.

Matlab and **Octave** have a function called "fminsearch" that uses the Nelder-Mead method. It was originally designed for finding the minimum values of functions, but it can be applied to least-squares curve fitting by creating an anonymous "fitting function" that computes the model, compares it to the data, and returns the fitting error to the fminsearch function, which attempts to minimize that error by adjusting the parameters of the model. For example, writing

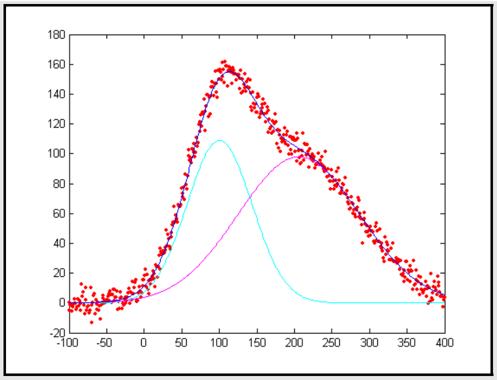
```
parameters = fminsearch(@(lambda)(fitfunction(lambda,x,y)),start)
```

performs an iterative fit of the data in the vectors x,y to a model described in a previously-created function called `fitfunction`, using the first guesses in the vector `start`. The parameters of the best-fit model are returned in the vector "parameters", in the same order as they appear in "start". Note: Octave users must install the latest version of "Optim" and other packages from http://octave.sourceforge.net/packages.php; follow the directions on that site.

A simple example is fitting the blackbody equation to the optical spectrum of an incandescent body for the purpose of estimating its color temperature. In this case there is only one nonlinear parameter (temperature) and one linear parameter (emissivity). **BlackbodyDataFit.m** demonstrates the technique, placing the experimentally measured optical spectrum in the vectors "wavelength" and "radiance" and then calling fminsearch with the fitting function **fitblackbody.m.**

Another application is demonstrated by Matlab's built-in demo **fitdemo.m** and its fitting function **fitfun.m,** which models the sum of two exponential decays. (Type "fitdemo" in the command window).

Fitting peaks. Many experiments produce signals in the form of peaks of various types; a common requirement is to measure the positions, heights, widths, and/or areas of those peaks, even when they are noisy or overlapped with one another. This cannot be done by linear least-squares methods, because such signals can not be modeled as polynomials with linear coefficients (the positions and widths of the peaks are not linear functions), so iterative curve fitting techniques are used instead, often using Gaussian, Lorentzian, or some other basic peak shape as a model.



The Matlab/Octave demonstration script **Demofitgauss.m** demonstrates fitting a Gaussian function to a set of data, using the fitting function **fitgauss2.m**. In this case there are two iterated non-linear parameters: the peak position and the peak width. The peak height is a *linear* parameter and is determined by linear regression in line 9 of the fitting function **fitgauss2.m** and returned in the global variable “c”. To accommodate the possibility that the baseline may shift, we can add a column of 1s to the **A** matrix, as was done in the CLS method on page 53, and the baseline amplitude is returned with the peak heights in the vector “c”; **Demofitgaussb.m** and **fitgauss2b.m** illustrates this. This is easily extended to *two or more* overlapping peaks of the same type in **Demofitgauss2.m** (shown in the figure on the left) using the *same* fitting function, which adapts to any number of peaks, depending on the length of the first-guess “start” vector. All these functions can call any of the user-defined peak type functions such as **gaussian.m**, **lorentzian.m**, and others that might be similarly designed (see functions.html). A more detailed explanation of the Matlab code is available online.

fitshape.m (`[FitResults,Error]=fitshape(x,y,start)`) pulls all of this together into a simplified Matlab/Octave function for fitting a peak model (the expression in line 47) to x,y data contained in the vector variables *x* and *y*. You must provide *x* and *y* and the first-guess starting vector 'start', in the form [position1 width1 position2 width2 ...etc], which specifies the first-guess position and width of each component (one pair of position and width for each peak in the model). The function returns best-fit positions and widths in FitResults (in the same order as 'start'), the peak height in the global variable PEAKHEIGHTS, and the percent error between the data and the model in Error. It also plots the data as dots and the fitted model as a line. The interesting thing about this function is that the *only part that defines the shape of the model is the last line*. As it stands, that line contains the expression for a Gaussian peak, but you could change that to *any other expression* that computes *g* as a function of *x* with two unknown parameters 'pos' and 'wid' (position and width, respectively, for peak-type shapes); everything else can remain the same and it will still work.

Variable shapes, such as the Voigt profile, Pearson, and the exponentially-broadened types, are defined not only by a peak position, height, and width, but also by an additional parameter that fine tunes the shape of the peak. If that parameter is *equal* for all peaks in a group, it can be passed as an additional input argument to the peak function, as shown in **VoigtFixedAlpha.m**. If the shape parameter is allowed to be *different* for each peak in the group and is to be determined by iteration (just as is position and width), then the routine must be modified to accommodate three, rather than two, iterated variables for each peak, as shown in **VoigtVariableAlpha.m**. Although the fitting error is *lower* with variable alphas, the execution time is longer and the alphas values so determined are not very stable, especially for multiple peaks. Version 7 of the downloadable Matlab/Octave function **peakfit.m** includes independently variable shape types for the Pearson, ExpGaussian, Voigt, and Gaussian/Lorentzian blend.

Signals with peaks of *different* shape in one signal can be fit by the fitting function **fitmultiple.m**, which takes as input a vector of peak types and a vector of shape variables (See **Demofitmultiple.m**).

For the quantitative measurement of peaks, it's instructive to compare the iterative least-squares method with simpler, less computationally-intensive, methods. For example, the measurement of the peak height of a single peak of uncertain width and position could be done simply by taking the maximum of the signal in that region. If the signal is noisy, a more accurate peak height will be obtained if the signal is smoothed beforehand. But smoothing can distort the signal and reduce peak heights. Using an iterative peak fitting method, assuming only that the peak shape is known, can give the best possible accuracy and precision, without requiring smoothing even under high noise conditions, e.g. when the signal-to-noise ratio is 1, as in the demo script **SmoothVsFit.m**:

```
   True peak height = 1    NumTrials = 100    SmoothWidth = 50
         Method          Maximum y    Max Smoothed y     Peakfit
   Average peak height    3.65          0.96625          1.0165
   Standard deviation     0.36395       0.10364          0.11571
```

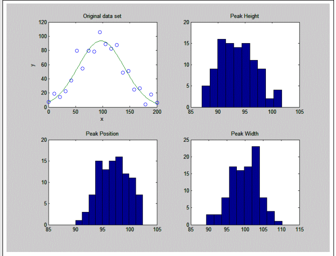
If peak area is measured rather than peak height, smoothing is unnecessary (unless to locate the peak beginning and end) but peak fitting still yields the best precision. See **SmoothVsFitArea.m**.

The Matlab/Octave script "**CLSvsINLS.m**" compares the classical least-squares (CLS) method with three different variations of the iterative method (INLS) method for measuring the peak heights of three Gaussian peaks in a noisy test signal, demonstrating that the *fewer the number of unknown parameters, the faster and more accurate is the peak height calculation*.

```
 Method     Positions   Widths          Execution time    Accuracy
  CLS        known      known            0.00133          0.30831
  INLS       unknown    unknown          0.61289          0.6693
  INLS       known      unknown          0.16385          0.67824
  INLS       unknown    known            0.24631          0.33026
  INLS       unknown    known (equal)    0.15883          0.31131
```

The script **clsdemo.m** also demonstrates under what conditions INLS is better than CLS.

The uncertainty of the peak parameters determined by iterative least-squares fitting is readily determined by the bootstrap sampling method (p. 40-41), as long as the data are unsmoothed. A demonstration of this method is given by the Matlab/Octave function "**BootstrapIterativeFit.m**", which creates a single x,y data set consisting of a single noisy Gaussian peak, extracts bootstrap samples from that data set, performs an iterative fit to the peak on each of the bootstrap samples, and plots the histograms of peak height, position, and width of the bootstrap samples. The syntax is `BootstrapIterativeFit (TrueHeight,TruePosition,TrueWidth, NumPoints,Noise,NumTrials)` where `TrueHeight` is the true peak height of the Gaussian peak, `TruePosition` is the true x-axis value at the peak maximum, `TrueWidth` is the true half-width (FWHM) of the peak, `NumPoints` is the number of points taken for the least-squares fit, Noise is the standard deviation of (normally-distributed) random noise, and `NumTrials` is the number of bootstrap samples. A typical example for `BootstrapIterativeFit (100,100,100,20,10,100);` is displayed on the right.

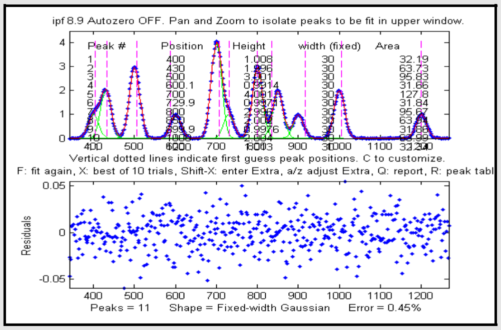


```
     Peak Height  Peak Position Peak Width
mean:   93.543       97.0227     99.8805
STD:    4.7441       3.8025      5.6621
```

If you were to run this simulation again, you'd get different results, but the mean peak parameters will almost always be within two standard deviations of the true values (100). A similar demonstration function, "**BootstrapIterativeFit2.m**", is expanded to *two* overlapping Gaussian peaks.

You can create your own fitting functions for any purpose; they are not limited to single algebraic expressions, but can be *any* complex multistep algorithm. For example, in the *Tfit method* in optical absorption spectroscopy (page 103) a model of the instrumentally-broadened transmission spectrum is fit to the observed transmission data, using a fitting function that performs Fourier convolution of the transmission spectrum with the slit function of the spectrometer, resulting in an extension of the dynamic range and calibration linearity beyond the normal limits. The bootstrap sampling method can be used to predict the precision of the measured model parameters in complicated methods such as this where the algebraic method is impossible. **Note:** You can download any of these m-files from http://tinyurl.com/cey8rwh.

Peak Fitter functions for Matlab and Octave


ipf 8.9 Autozero OFF. Pan and Zoom to isolate peaks to be fit in upper window.

```
Peak #     Position     Height      width (fixed)     Area
 1          400          1.008          30            32.19
 2          430          1.996          30            63.73
 3          500          3.001          30            95.83
 4          600.1        0.9914         30            31.66
 5          700          4.001          30            127.8
 6          729.9        0.9971         30            31.84
 7          800          2.996          30            95.67
 8          850          2.002          30            63.74
 9          899.9        0.9976         30            31.86
10          1000         2.004          30            63.99
11          1200         1.003          30            32.24
```

Vertical dotted lines indicate first guess peak positions. C to customize.

F: fit again, X: best of 10 trials, Shift-X: enter Extra, a/z adjust Extra, Q: report, R: peak tabl

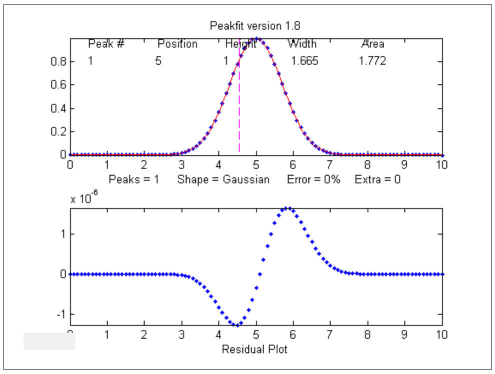
Peaks = 11   Shape = Fixed-width Gaussian   Error = 0.45%

These are Matlab or Octave peak fitting programs for time-series signals, which uses an unconstrained non-linear optimization algorithm to decompose a complex, overlapping-peak signal into its component parts. The objective is to determine whether your signal can be represented as the sum of any combination of 23 fundamental underlying peaks shapes. They accept signals of any length, including those with non-integer and non-uniform x-values, and can fits groups of peaks with many different peak shape models. There are two different versions, **peakfit.m**, a command line version for Matlab and Octave (page 90), and **ipf.m**, a keypress operated interactive version for Matlab only (page 95). The latest versions of these functions can optionally estimate the expected standard deviation and interquartile range of the peak parameters using the bootstrap sampling method (Page 41 – 42). The peakfit.m function is also an keypress-selected internal function of the **iPeak** (page 78) and **iSignal** (page 85).

Accuracy and precision of peak parameter measurement

This section describes the major sources of error in measuring the “peak parameters” (peak positions, heights, widths, and areas) by iterative curve fitting.

a. Model errors.



Peak shape. If you have the wrong model for your peaks, the results can't be expected to be accurate; for instance, if your actual peaks are Lorentzian in shape, but you fit them with a Gaussian model, or *vice versa*. For example, a single isolated Gaussian peak at x=5, with a height of 1.000 fits a Gaussian model virtually perfectly, using the Matlab/Octave user-defined **peakfit.m** function, as shown on the right. The 5th input argument for the peakfit function specifies the shape of peaks to be used in the fit; “1” means Gaussian.

```
>> x=[0:.1:10];y=exp(-(x-5).^2);
>> [FitResults,FitError]=peakfit([x' y'],5,10,1,1)
           Peak #    Position  Height        Width     Area
FitResults =  1       5          1          .6649      1.7724
FitError = 0.001679
```

The “FitResults” are, from left to right, peak number, peak position, peak height, peak width, and peak area. The fitting error “FitError” is the root mean square difference between the data and the best-fit model, as a percentage of the maximum signal in the fitted region. Note the good agreement of the area measurement, 1.7724, with the theoretical area under the curve of exp(-x^2), which is the square root of π, or 1.7725.

But this same peak, when fit with a Logistic distribution (peak shape number 3), gives a fitting error of 1.4% and height and width errors of 3% and 6%, respectively. When fit with a Lorentzian model (peak shape 2), this peak gives a 6% fitting error and height and width errors of 8% and 20%, respectively. So clearly the larger the fitting errors, the larger are the parameter errors, but the parameter errors are of course not *equal* to the fitting error (that would just be *too* easy). Also, clearly the peak *width* and *area* are the parameters most susceptible to errors. The peak *positions*, as you can see here, are measured accurately (and will be, even if the model is way wrong, as long as the peak is symmetrical and not highly overlapping with other peaks).

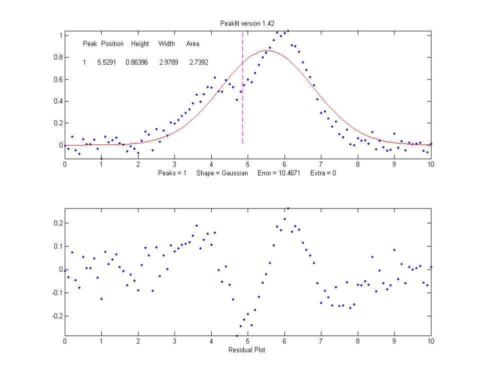
A good fit is not by itself proof that the shape function you have chose is the correct one; in some cases the wrong function can give a fit that looks perfect. As an example, a data set consisting of peaks with a Voigt profile peak shape can be fit with a weighted sum of a Gaussian and a Lorentzian almost as well as an with an actual Voigt model, even though those models are not the same mathematically; the difference in fitting error is so small that it would likely be obscured by the random noise if it were a real experimental signal. For another example, this fit of a real data set to a 5-peak Gaussian model has a low percent fitting error and the residuals look random - usually an indicator of a good fit. But in fact in this case the model is wrong; that data came from an experimental domain where the underlying shape is fundamentally non-Gaussian but in some cases can look very like a Gaussian. In such cases *you can not depend solely on the goodness of fit*.

Number of peaks. Another source of model error occurs if you have the wrong *number of peaks* in your model, for example if the signal actually has *n* peaks but you try to fit it with only *n-1* peaks. In the example below, a bit of Matlab/Octave code generates a simulated signal with of two Gaussian peaks at x=4 and x=6 with peaks heights of 1.000 and 0.5000 respectively and widths of 1.665, plus random noise with a standard deviation 5% of the height of the largest peak (an SNR of 20):

```
>> x=[0:.1:10];y=exp(-(x-6).^2)+.5*exp(-(x-4).^2) +.05*randn(size(x));
```

In a real experiment you would not usually know the peak positions, heights, and widths; you would be using curve fitting to measure those parameters. Let's assume that, on the basis of previous experience or some preliminary trial fits, you have established that the optimum peak *shape* model is Gaussian, but you don't know for sure how many peaks are in this group. If you fit this signal with a *single*-peak Gaussian model, you get these results:

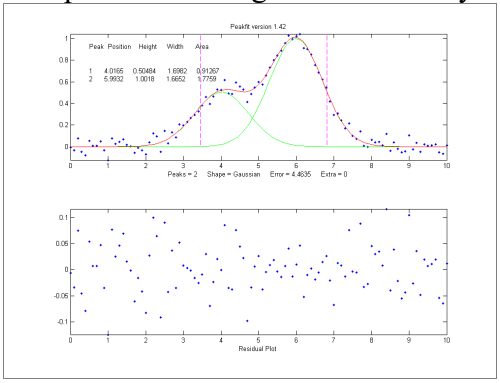
```
>> [FitResults,FitError]=peakfit([x' y'],5,10,1,1)
                Peak #    Position   Height      Width      Area
FitResults = 1            5.5291     0.86396     2.9789     2.7392
FitError = 10.467
```



The residual plot shown in the bottom panel on the right exhibits a “wavy” structure rather than a completely random scatter of points due to the random noise in the signal. This means that the fitting error is not limited by the random noise; that is a clue that the model is not quite right.

But a fit with *two* peaks yields much better results (The 4th input argument for the peakfit function specifies the number of peaks to be used in the fit).

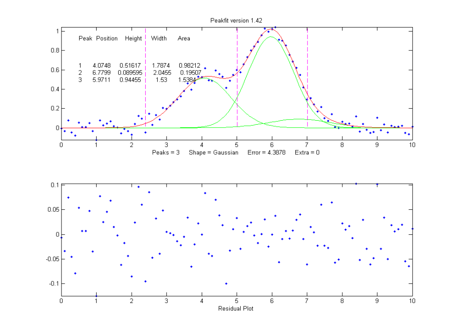
```
[FitResults,FitError]=peakfit([x' y'],5,10,2,1)

Peak #    Position   Height      Width      Area
   1      4.0165     0.50484     1.6982     0.91267
   2      5.9932     1.0018      1.6652     1.7759
FitError = 4.4635
```



Now the residuals have a random scatter of points, as would be expected if the signal is accurately fit except for the random noise. Moreover, the fitting error is much lower (less than half) of the error with only one peak. In fact, the fitting error is just about what we would expect in this case based on the 5% random noise in the signal (estimating the relative standard deviation of the points in the baseline visible at the edges of the signal).

Because this is a simulation, and we know beforehand the true values of the peak parameters (peaks at x=4 and x=6 with peaks heights of 1.0 and 0.50 respectively and widths of 1.665), we can actually calculate the parameter errors (the difference between the real peak positions, heights, and widths and the measured values). We see that they are quite accurate (in this case within about 1% relative on the peak height and 2% on the widths), which is actually better than the 5% random noise in this signal because of the averaging effect of fitting to multiple data points in the signal.

But if going from one peak to two peaks gave us a better fit, why not go to *three* peaks? Changing the number of peaks to three (the fourth argument) gives these results:



```
>> [FitResults,FitError]=peakfit([x' y'],5,10,3,1)

Peak #    Position   Height       Width      Area
FitResults =
   1      4.0748     0.51617      1.7874     0.98212
   2      6.7799     0.089595     2.0455     0.19507
   3      5.9711     0.94455      1.53       1.5384
FitError = 4.3878
```

The fitting algorithm has now tried to fit an additional low-amplitude peak (numbered peak 2 in this case) located at x=6.78. The fitting error is actually *lower* than for the 2-peak fit, but only slightly lower, and the residuals are no less visually random that with a 2-peak fit. So, knowing nothing else, a 3-peak fit might be rejected on that basis alone. In fact, *there is a serious downside to fitting more peaks than are present in the signal: it increases the parameter measurement errors of the peaks that are actually present*. Again, we can prove this because we know beforehand the true values of the peak parameters: clearly the peak positions, heights, and widths of the two real peaks than are actually in the signal (peaks 1 and 3) are significantly less accurate than the 2-peak fit. This can be verified by performing a bootstrap test (pages 41-42 and #16 on page 98).

If we repeat that fit with the *same signal* but with a *different* sample of random noise (simulating a repeat measurement of a stable experimental signal in the presence of random noise), the third peak in the 3-peak fit will vary from fit to fit, because the third peak is actually fitting the random *noise*, not an actual peak in the signal). This is called “fitting the noise”.

```
>> x=[0:.1:10];
>> y=exp(-(x-6).^2)+.5*exp(-(x-4).^2)+.05*randn(size(x));
>> [FitResults,FitError]=peakfit([x' y'],5,10,3,1
Peak #   Position  Height      Width     Area)
FitResults =
  1     4.115    0.44767     1.8768    0.89442
  2     5.3118   0.093402    2.6986    0.26832
  3     6.0681   0.91085     1.5116    1.4657
FitError = 4.4089
```

With this new set of data, two of the peaks (numbers 1 and 3) have roughly the same position, height, and width, but peak number 2 has changed substantially compared to the previous run. Now we have an even more compelling reason to reject the 3-peak model: *the 3-peak solution is not stable*. And because this is a simulation in which we know the right answers, we can also verify that the *accuracy* of the known peak heights is substantially poorer (about 10% error) than expected with this level of random noise in the signal (5%). If we were to run a 2-peak fit on the same new data, we get much better measurements of the peak heights.

```
>> [FitResults,FitError]=peakfit([x' y'],5,10,2,1)
Peak #   Position  Height      Width     Area
FitResults =
  1     4.1601    0.49981     1.9108    1.0167
  2     6.0585    0.97557     1.548     1.6076
FitError = 4.4113
```

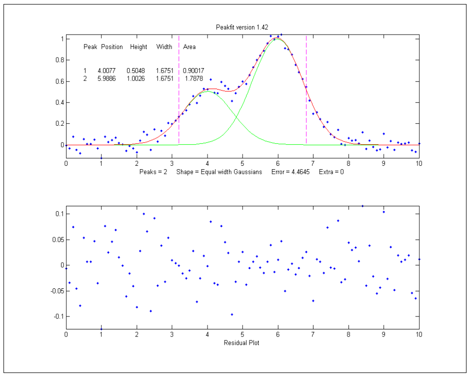
If this is repeated several times, the peak parameters of the peaks at x=4 and x=6 are, on average, *more accurately measured by the 2-peak fit*. In practice, the best way to evaluate a proposed fitting model is to fit several repeat measurements of the same signal (if that is practical experimentally) and to compute the standard deviation of the peak parameter values. Or, you can use the “bootstrap method” (pages 42, 93, 100) to evaluate the robustness of the model with respect to noise in the data; *superfluous peaks will reveal themselves as unstable*.

In real experimental work, of course, you usually don't *know* the right answers beforehand, so that's why it's important to use methods that work well when you *do* know. Here's a *real data* example in a spreadsheet, fit with 2, 3, 4 and 5 Gaussians, until the residuals become random. Another way to find the minimum number of models peaks is to fit the data with increasing numbers of model peaks until the fitting error stops decreasing or reaches a minimum; see Matlab/Octave script NumPeaksTest.m.

Peak width constraints. Finally, there is one more thing that we can do that might improve the peak parameter measurement accuracy, and it concerns the peak widths. In all the above simulations, the basic assumption that all the peak parameters were unknown and independent of one another. In some types of measurements, however, the peak widths of each group of adjacent peaks can be expected to be equal to each other, on the basis of first principles or previous experiments. This is a common situation in analytical chemistry, especially in atomic spectroscopy and in chromatography, where the peak widths are determined largely by instrumental factors. In the current simulation, the *true peaks widths are in fact equal*, but all the results above show that the *measured* peak widths are close but not quite equal, due to random noise in the signal. But we can introduce an equal-width *constraint* into the fit by using peak shape 6 (Equal width Gaussians) or peak shape 7 (Equal width Lorentzians). Using peak shape 6 on the same set of data as the previous example:

```
>> [FitResults,FitError]=peakfit([x' y'],5,10,2,6)
Peak #   Position  Height      Width     Area
FitResults =
       1     4.0435    0.4942     1.6559    0.87113
       2     6.0039    0.99218    1.6559    1.7489
FitError = 4.5076
```

This “equal width” fit forces all the peaks within one group to have exactly the same width, but that width is adjusted by the program to fit the data. The result is a slightly higher fitting error (in this case 4.5 rather than 4.4), but - perhaps surprisingly - the peak parameter measurements are usually more accurate and more reproducible (Specifically, the relative standard deviations are on average lower for the equal-width fit than for an unconstrained-width fit to the same data, assuming of course that the true underlying peak widths are really equal). This is an exception to the general expectation that lower fitting errors result in lower peak parameter errors. The more general rule is that t*he more you know about the nature of your signals, and the closer your chosen model adheres to that knowledge, the better the results*. In this case we knew that the peak shape was Gaussian (although we could have verified that choice by trying other candidate peaks shapes), we determined that the number of peaks was two by inspecting the residuals and fitting errors for 1, 2, and 3 peak models, and then we introduced the constraint of equal peak widths within each group of peaks (based on prior knowledge of the experiment rather than on inspection of residuals and fitting errors). Not every experiment yields peaks of equal width, but when it does, it's better to make use of that constraint.



```
Peak  Position    Height    Width    Area
1     4.0077      0.5048    1.6751   0.90017
2     5.9886      1.0026    1.6751    1.7878
```

Going one step beyond *equal* widths, you can also specify *fixed*-width Gaussian or Lorentzian shapes (shape numbers 11, 12, 34-37), in which the width of the peaks are not only equal to each other but are *known beforehand* and are specified in a *vector* as input argument 10, rather than being determined from the data as in the equal-width fit above. Introducing this constraint onto the previous example, and supplying an (almost-accurate) width as the 10th input argument:

```
>> [FitResults,FitError]=peakfit([x' y'],0,0,2,11,0,0,0,0,[1.666 1.666])
          Peak #   Position  Height       Width      Area
FitResults =
             1       3.9943      0.49537         1.666       0.87849
             2       5.9924      0.98612         1.666        1.7488
FitError = 4.8128
```

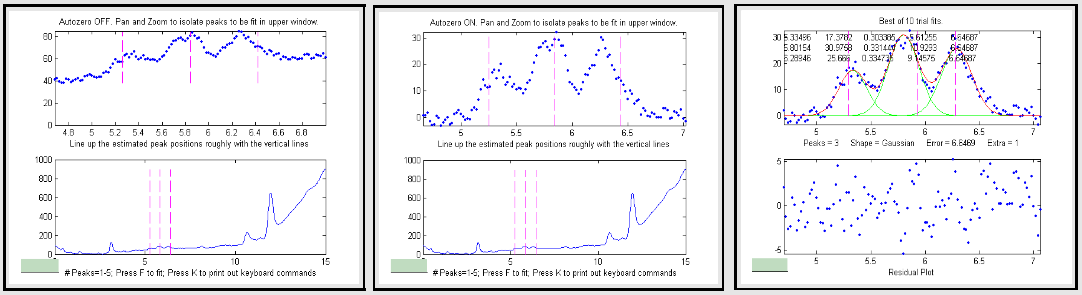
Comparing to the previous equal-width fit, the fitting error is slightly larger here (because there are fewer degrees of freedom to minimize the error), but the parameter errors, particularly the peaks heights, are *more accurate* because the width information provided in the input argument was more accurate (1.666) than the width determined by the equal-width fit (1.5666). Again, not every experiment yields peaks of known width, but when it does, it's better to make use of that constraint. (For a more complex example of model selection with real data, see this link).

Note that if the peak *positions* are also known, and *only* the peak heights are unknown, you don't even need to use the iterative fitting method at all; you can use the much easier and faster multilinear regression technique ("classical least squares") described on pages 49 – 53.

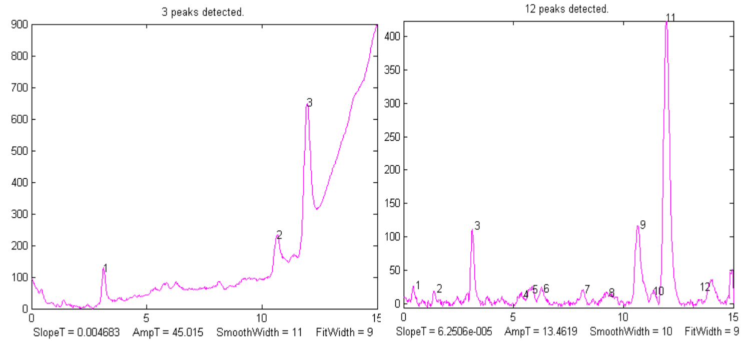
b. Background correction.

The peaks that are measured in many scientific instruments are often superimposed on a non-specific background. Ordinarily the experiment protocol is designed to minimize the background or to compensate for the background, for example by subtracting a "blank" signal from the signal of an actual specimen. But even so there is often a residual background that can not be eliminated completely experimentally. The origin and shape of that background depends on saturthe specific measurement method, but often this background is a flat, tilted, or curved shape, and the peaks of interest are comparatively narrow features superimposed on that background. The presence of the background has little effect on the peak positions, but it is impossible to measure the peak heights, width, and areas accurately unless the background is corrected or subtracted.

There are various methods described in the literature for estimating and subtracting the background in such cases. The simplest assumption is that the background can be approximated as a simple function in the local region of group of peaks being fit together, for example as a constant (flat), straight line (linear), or curved line (quadratic). This is the basis of the "autozero" modes in the **ipf.m**, **iSignal.m**, and **iPeak.m** functions, which are selected by the **T** key to cycle thorough *none*, *linear*, *quadratic*, and *flat* modes. In the *flat* mode, a constant baseline is included in the curve fitting calculation, as described on page 55. In *linear* mode, a straight-line baseline connecting the two ends of the signal segment in the upper panel will be automatically subtracted. In *quadratic* mode, a parabolic baseline is subtracted. (In the last two modes, you must adjust the pan and zoom controls to isolate the group of overlapping peaks to be fit, so that the signal returns to the local background at the left and right ends of the window).

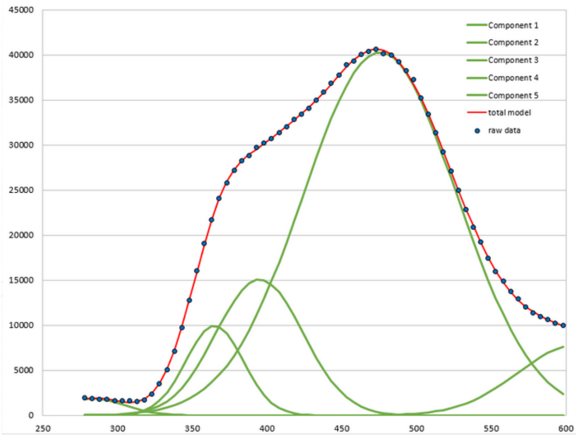

Autozero OFF. Pan and Zoom to isolate peaks to be fit in upper window.
Line up the estimated peak positions roughly with the vertical lines
# Peaks=1-5; Press F to fit; Press K to print out keyboard commands
Autozero ON. Pan and Zoom to isolate peaks to be fit in upper window.
Line up the estimated peak positions roughly with the vertical lines
# Peaks=1-5; Press F to fit; Press K to print out keyboard commands
Best of 10 trial fits.
Peaks = 3
Shape = Gaussian
Error = 6.6469
Extra = 1
Residual Plot

*Above: Example of an experimental chromatographic signal. From left to right, (1) Raw data with peaks superimposed on a sloping baseline. One group of peaks is selected using the pan and zoom controls, adjusted so that the signal returns to the local background at the edges of the segment displayed in the upper window; (2) The linear baseline is subtracted when the "autozero" mode 1 in **ipf.m**; (3) the signal in this region is fit with a three-peak Gaussian model, by pressing the keys: **3**, **G**, **F** (3 peaks, **G**aussian, **F**it).*



*Left: Raw data with peaks superimposed on baseline. Right: Background subtracted from the entire signal using the multi-point background subtraction function in **iPeak.m** (**ipf.m** and **iSignal.m** have the same function).*

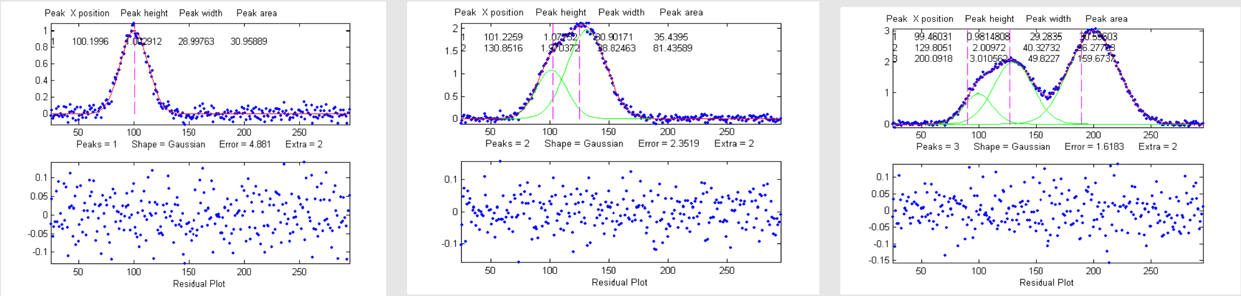
Another possibility is to subtract the background from the *entire signal* first, before further operations are performed. The simplest assumption is that the background is piece-wise *linear* and can be approximated as a series of small straight line segments. This is the basis of the multipoint background subtraction mode in ***ipf.m*** (page 95), ***iPeak.m*** (page 76), and ***iSignal.m*** (page 85). The user enters the number of points that is thought to be sufficient to define the baseline, then clicks where the baseline is thought to be along the entire length of the signal in the lower whole-signal display (e.g. on the valleys between the peaks), and the program interpolates between the clicked points and subtracts the piece-wise linear background from the original signal.



In some cases the background may be able to be modeled as one or more peaks whose maxima may fall outside of the range of data acquired, and you can fit it simply by including extra peaks in the model to account for the baseline. (Don't use the equal-width shapes for this, because it's likely that measured and background peaks have different widths). For some examples, see **Example 12b** on page 92 , **Example 20** on page 94, and the top of page 101. In the real data example on the left, which uses a spreadsheet Solver function (page 55), the peaks in the data whose maxima are *within* the x-axis range are fit with three Gaussians, but the fit is better if two additional Gaussian components are included *outside* the x-axis range as the background.

c. Random noise in the signal.
Any experimental signal has a certain amount of random noise, which means that the individual data points scatter randomly above and below their mean values. Ordinarily one assumes that the scatter is equally above and below the true signal, so that the long-term average approaches the true mean value; the noise "averages to zero", as it is often said. The practical problem is that any given recording of the signal contains only one finite sample of the noise. If another recording of the signal is made, it will contain another independent sample of the noise. These noise samples are not infinitely long and therefore do not represent the true long-term nature of the noise. This presents two problems: (1) an individual sample of the noise will not "average to zero" and thus the parameters of the best-fit model will not necessarily equal the true values, and (2) the magnitude of the noise during one sample might not be typical; the noise might have been randomly greater or smaller than average during that time. This means that the mathematical "propagation of error" methods, which seek to estimate the likely error in the model parameters based on the noise in the signal, will *underestimate* the error if the noise in that sample happens to be *lower* than average and *overestimate* the error if the noise happens to be *larger* than average in that sample.

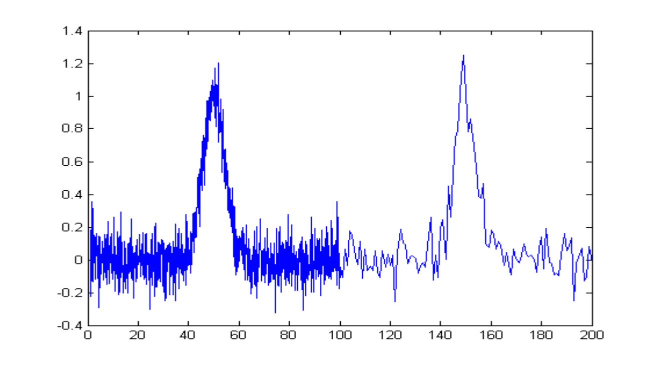
A better way to estimate the parameter errors is to record multiple samples of the signal, fit each of those separately, compute the models parameters from each fit, and calculate the standard error of each parameter. This is exactly what the script **DemoPeakfit.m** does (which requires the **peakfit.m** function) for simulated noisy peak signals such as those illustrated in the figure below. It's easy to demonstrate that, as expected, the average fitting error precision and the relative standard deviation (RSD) of the parameters increases directly with the random noise level in the signal. But the precision and the accuracy of the measured parameters *also* depend on which parameter it is (peak positions are always measured more accurately than their heights, widths, or areas) and on the peak height and extent of peak overlap (the two left-most peaks in this example are not only weaker but also more overlapped than the right-most peak, and thus exhibit poorer parameter measurements). In this example, the fitting error is 1.6% and the percent RSD of the parameters ranges from 0.05% for the peak position of the largest peak to 12% for the peak area of the smallest peak.



The errors in the values of peak parameters measured by curve fitting depend not only on the characteristics of the peaks in question and the signal-to-noise ratio, but also upon other peaks that are overlapping it. From left to right: (1) a single peak at x=100 with a peak height of 1.0 and width of 30 is fit with a Gaussian model, yielding a relative fit error of 4.9% and relative standard deviation (RSD) of peak position, height, and width of 0.2%, 0.95%, and 1.5% , respectively. (2) The same peak, with the same noise level but with another peak overlapping it, reduces the relative fit error to 2.4% (because the addition of the second peak increases overall signal amplitude), but increases the RSD of peak position, height, and width to 0.84%, 5%, and 4% - a seemingly better fit, but with poorer precision for the first peak. (3) The addition of a third peak further reduces the fit error to 1.6% , but the RSD of peak position, height, and width of the first peak are still 0.8%, 5.8%, and 3.64%, about the same as with two peaks, as the third peak does not overlap the first.

If it is not possible to record multiple samples of the signal, if the average noise in the signal is not known, or if its probability distribution is uncertain, it is still possible to use the *bootstrap sampling method* to estimate the uncertainty of the peak heights, positions, and widths, as described on page 41 - 42, as long as the data are unsmoothed. The latest versions of **peakfit.m** (page 93, example 15) and of **ipf.m** (page 95) have a function that estimates the expected standard deviation of the peak parameters from a single signal, using the "bootstrap method" (#16 on page 98). Don't smooth the data before curve fitting; it will not actually reduce the accuracy of peak parameter measurement and it will cause the bootstrap method to seriously underestimate the parameter errors. The same thing occurs if the noise is "pink" (page 8) ; the errors will be underestimated by the bootstrap.

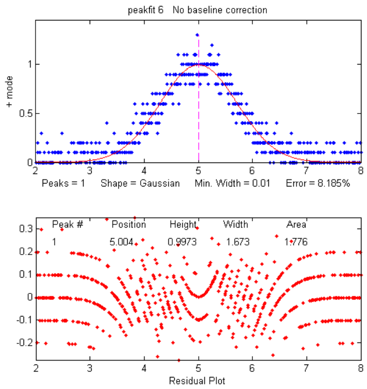
One way to reduce the effect of noise is to take more data per signal. If the experiment makes it possible to reduce the x-axis interval between points, or to take multiple readings at each x-axis value, then the resulting increase in the number of data points in each peak should help reduce the effect of noise. As a demonstration, using the script **DemoPeakfit.m** to create a simulated overlapping peak signal like that shown above right, it's possible to change the interval between x values and thus the total number of data points in the signal. With a noise level of 1% and 75 points in the signal, the fitting error is 0.35 and the average parameter error is 0.8%. With 300 points in the signal and the same noise level, the fitting error is essentially the same, but the average parameter error drops to 0.4%, suggesting that the accuracy of the measured parameters varies inversely with the square root of the number of data points in the peaks.



The figure on the right below illustrates the importance of sampling rate and data density. The signal consists of two Gaussian peaks, one located at x=50 and the second at x=150. Both peaks have a peak height of 1.0 and a peak half-width of 10 units, and normally-distributed random white noise with a standard deviation of 0.1 has been added to the entire signal. The x-axis sampling interval, however, is different for the two peaks; it's 0.1 for the first peaks and 1.0 for the second peak. This means that the first peak is characterized by ten times more data points than the second peak. When you fit these peaks to a Gaussian model (e.g. using **peakfit.m** or **ipf.m**), you will find that *the parameters of the first peak are measured more accurately* than the second, even though the fitting error is not much different (because the noise is the same for both peaks):

```
First peak:                        Second peak:
  Percent Fitting Error = 7.6434%    Percent Fitting Error = 8.8827%
  Position    Height    Width        Position    Height    Width
  49.95      1.005     10.11         149.64      1.0313    9.94
```

So far this discussion has applied to *white* noise. But other noise colors (page 8) have different effects. Low-frequency weighted ("pink") noise has a *greater* effect on the accuracy of peak parameters measured by curve fitting, and, in a nice symmetry, high-frequency "blue" noise has a *smaller* effect on the accuracy of peak parameters that would be expected on the basis of its standard deviation, because the information in a smooth peak signal is concentrated at low frequencies (page 7, 29). An example of this occurs when curve fitting is applied to a signal that has been deconvoluted to remove a broadening effect (Appendix H: page 121).

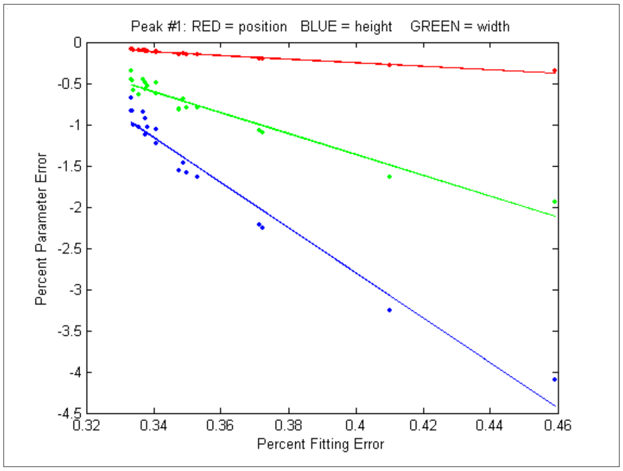


```
Peak #    Position    Height    Width    Area
1         5.004       0.9973    1.673    1.776
```

Sometime you may notice that the signals and the residuals in a curve fitting operation are weirdly structured into bands or lines rather than being completely random and unstructured. This can occur if either the independent variable or the dependent variable is *quantized* into discrete steps. This is called *quantization noise* or *digitization noise* and is discussed on page 122. It may look strange, but it usually has little effect on the results, which remain limited by the random noise in the signal.

d. Iterative fitting errors.

Unlike multiple linear regression curve fitting, iterative methods may not converge on the exact same model parameters each time the fit is repeated with slightly different starting values (first guesses). The Interactive Peak Fitter (**ipf.m**, page 95) makes it easy to test this, because it uses slightly different starting values each time the signal is fit (by pressing the **F** key in **ipf.m**, for example). Even better, by pressing the **X** key, the ipf.m function silently computes 10 fits with different starting values and *takes the one with the lowest fitting error*. It is a basic assumption of any curve fitting operation is that if the fitting error (the RMS difference between the model and the data) is minimized, the parameter errors (the difference between the actual parameters and the parameters of the best-fit model) will also be minimized. This is *usually* a good assumption. For example, the graph on the right shows typical percent parameters errors as a function of fitting error for the left-most peak in one sample of the simulated signal generated by the script **DemoPeakfit.m** (shown in the previous section). The variability of the fitting error here is caused by random small variations in the first guesses, rather than by random noise in the signal. In many practical cases there is enough random noise in the signals that the iterative fitting errors within one sample of the signal are small compared to the random noise errors between samples.

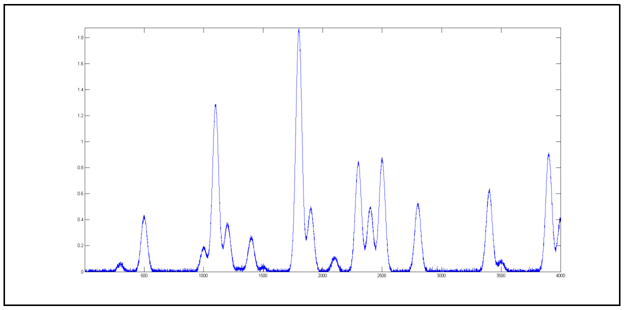


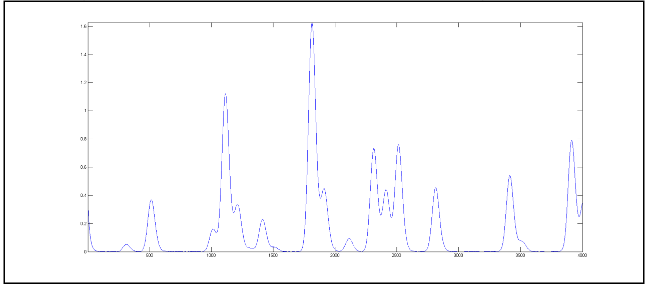
Remember that the variability in measured peak parameters from fit to fit of a single sample of the signal is *not* a good estimate of the precision or accuracy of those parameters, for the simple reason that those results represent only *one sample* of the signal, noise, and background. The sample-to-sample variations are likely to be much greater than the within-sample variations due to the iterative curve fitting. (In this case, a "sample" is a single recording of signal). To estimate the contribution of random noise to the variability in measured peak parameters when only a single sample if the signal is available, use the "bootstrap method" (page 41 – 42).

So, to sum up, we can make the following observations about the accuracy of peak parameters:

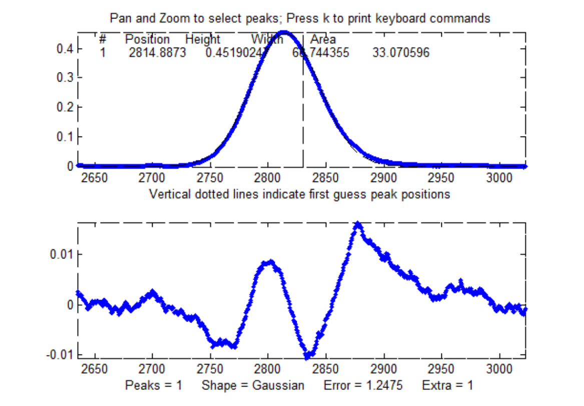
1. Parameter errors depend on the accuracy of the model and the number of overlapping peaks;
2. All else being equal, the parameter errors are directly proportional to the noise in the data (and worse for low-frequency or pink noise);
3. All else being equal, parameter errors are proportional to the fitting error, but a constrained model that fits the underlying reality better, e.g. equal or fixed widths or shapes, often gives lower parameter errors *even if the fitting error is larger*;
4. The errors are typically least for peak position and worse for peak width and area;
5. The errors depend on the *data density* (number of independent data points in the width of each peak) and on the *extent of peak overlap* (the parameters of isolated peaks are easier to measure than highly overlapped peaks);
6. If only a single signal is available, the effect of noise on the standard deviation of the peak parameters in many cases can be predicted approximately by the bootstrap method (page 40), but if the overlap of the peaks is too great, the actual errors of the parameter measurements can be much greater than predicted.

Fitting signals that are subject to exponential broadening.



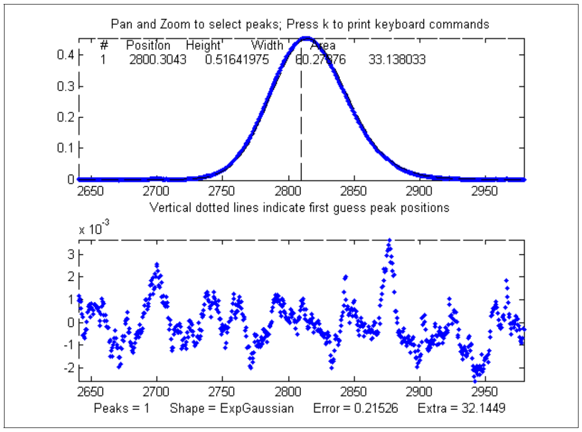


DataMatrix2 (right) is a computer-generated test signal consisting of 16 symmetrical Gaussian peaks with random white noise added. The peaks occur in groups of 1, 2, or 3 overlapping peaks, and the peak maxima are located at *exactly integer values of x* from 300 to 3900 (on the 100's) and the peak widths are always *exactly 60 units*. The peak heights vary from 0.06 to 1.85. The standard deviation of the noise is 0.01. (You can use this signal to test curve-fitting programs and to determine the accuracy of their measurements of peak parameters. Download these mat files from the bottom of **http://tinyurl.com/cey8rwh**, put it in the Matlab/Octave path, then type "load DataMatrix2" to load it into the workspace). DataMatrix3 (below left) is an exponentially broadened version of DataMatrix2, with a "time constant" of 33 points on the x-axis. The result of the exponential broadening is that all the peaks in this signal are asymmetrical, their peak maxima are shifted slightly to longer x values, and their peak heights are smaller and their peak widths are larger than the corresponding peaks in DataMatrix2. Also, the random noise is damped in this signal compared to the original and is no longer "white", as a consequence of the broadening. This type of effect is common in physical measurements and often arises from some physical or electrical effect in the measurement system that is apart from the fundamental peak characteristics. In such cases it is usually desirable to compensate for the effect of the broadening, either by Fourier deconvolution (page 32) or by curve fitting, in an attempt to measure what the peak parameters would have been *before* the broadening (and also to measure the broadening itself). This can be done for Gaussian peaks that are exponentially broadened by using the "ExpGaussian" peak shape in **peakfit.m** and **ipf.m**. The example illustrated on the right above focuses on the single isolated peak whose "true" peak position, height, width, and area in the original unbroadened signal, are 2800, 0.52, 60, and 33.2 respectively. (The relative standard deviation of the noise is 0.01/0.52=2%.) In the broadened signal, the peak is visibly asymmetrical, the peak maximum is shifted to larger x values, and it has a shorter height and larger width, as demonstrated by the attempt to fit a normal (symmetrical) Gaussian to the broadened peak. (Note that the peak *area*, in contrast, is not much effected).



```
>> load DataMatrix3
>> (DataMatrix3);

Peak Shape = Gaussian
Autozero ON
Number of peaks = 1
Fitted range = 2640 - 2979.5 (339.5) (2809.75)
Percent Error = 1.2084
Peak #    Position  Height    Width      Area
1         2814.832  0.4510    68.4412    32.8594
```

The large "wavy" residual plot is a tip-off that the model is not quite right. Moreover, the fitting error (1.2%) is larger than expected for a peak with a half-width of 60 points and a 2% noise RSD (which should have been roughly 2%/sqrt(60)=0.25%).



```
Pan and Zoom to select peaks; Press k to print keyboard commands
  #    Position     Height        Width        Area
  1    2800.3043    0.51641975    60.27876     33.138033
Vertical dotted lines indicate first guess peak positions
Peaks = 1    Shape = ExpGaussian    Error = 0.21526    Extra = 32.1449
```

Fitting to an exponentially-broadened Gaussian (pictured on the right) gives a much lower fitting error ("Percent error") and a more random residual plot. But the interesting thing is that it also recovers the *original* peak position, height, and width to an accuracy of a fraction of 1%. In performing this fit, the time constant ("extra") was experimentally determined from the broadened signal by adjusting it with the A and Z keys to give the lowest fitting error; that also gives a pretty good measurement of the broadening factor (32.6, vs the actual value of 33). Note: When using peakshape 5 (exponentially broadened Gaussian) you have to give it a reasonably good value for the time constant ('extra'), the input argument right after the peakshape number. If the value is too far off, the fit may fail completely, returning all zeros. A little trial and error suffice. (Or use peakfit.m version 7, shape number 31, to measure the time constant as an iterated variable).

```
Peak Shape = Exponentially-broadened Gaussian
Autozero ON
Number of peaks = 1
Extra = 32.6327
Percent Error = 0.21696
Peak #    Position    Height      Width      Area
1         2800.13     0.5183     60.086      33.152
```

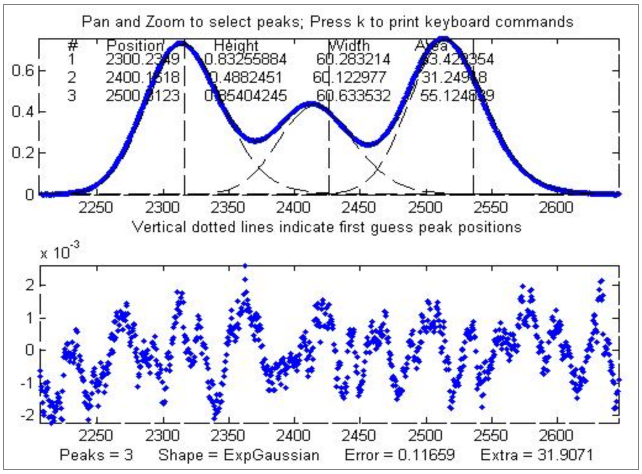
Comparing the two methods, the exponentially-broadened Gaussian fit recovers all the underlying peak parameters quite accurately:

	Position	Height	Width	Area
Actual peak parameters	2800	0.52	60	33.2155
Gaussian fit to broadened signal	2814.832	0.45100549	68.441262	32.859436
ExpGaussian fit to broadened signal	2800.1302	0.51829906	60.086295	33.152429

Other peaks in the same signal, if they are under the broadening influence of the same time constant, can be fit with similar settings, for example the set of three overlapping peaks near x=2400. The peak positions are recovered almost exactly and even the width measurements are reasonably accurate (1% or better). (The smaller fitting error evident here is just a reflection of the larger peak heights in this group of peaks - the noise is the same everywhere in this signal).

```
Peak Shape = Exponentially-broadened Gaussian
Autozero OFF
Number of peaks = 3
Extra = 31.9071
Fitted range = 2206 - 2646.5 (440.5) (2426.25)
Percent Error = 0.11659
Peak #    Position      Height          Width          Area
1       2300.2349    0.83255884     60.283214     53.422354
2       2400.1618    0.4882451      60.122977     31.24918
3       2500.3123    0.85404245     60.633532     55.124839
```

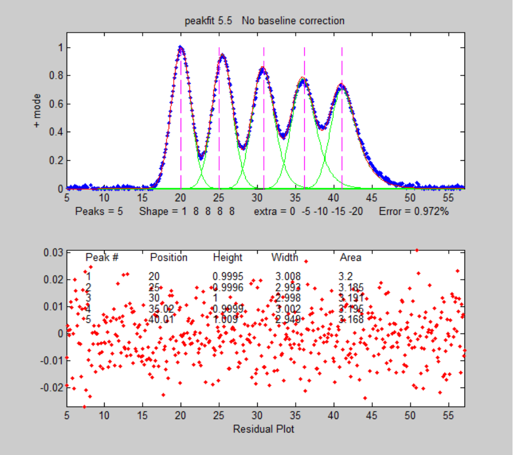
The residual plots in both of these examples still have some "wavy" character, rather than being completely random and "white". The exponential broadening smooths out any white noise in the original signal that was introduced *before* the exponential effect, acting as a low-pass filter in the time domain and resulting in a low-frequency dominated "pink" noise, which is what remains in the residuals after the broadened peaks have been fit as well as possible. On the other hand, white noise that is introduced *after* the exponential effect would remain white and random in the residuals. In real experimental data, both types of noise may be present in varying amounts.



```
#   Position    Height       Width       Area
1   2300.2349   0.83255884   60.283214   53.422354
2   2400.1518   0.4882451    60.122977   31.24918
3   2500.3123   0.85404245   60.633532   55.124639
```

One warning: peak asymmetry similar to exponential broadening can in principle be the result a pair of closely-spaced peaks of different peak heights. In fact, a single exponential broadened Gaussian peak can be fit with two or three symmetrical Gaussians to a fitting error at least as low as a single exponential broadened Gaussian fit. This makes it hard to distinguish between these two models on the basis of fitting error alone. However, this can usually be decided by inspecting the other peaks in the signal: in many experiments, the same exponential broadening applies to *every* peak in the signal, and the broadening is either constant or changes gradually over the length of the signal. On the other hand, it is less likely that every peak in the signal will be accompanied by a smaller side peak that varies in exactly this way. So, if a only one or a few peaks exhibit asymmetry, and the others are symmetrical, it's most likely that the asymmetry is due to closely-spaced peaks of different peak heights. If *all* peaks have the same or similar asymmetry, it's more likely to be caused by a broadening factor that applies to the entire signal. Human judgment, based on knowledge of the experimental system and the types of signals it generates, is always valuable and is often essential in such cases.

More generally, it is technically possible to fit *any* arbitrary peak, symmetrical or not, with the sum of a number of Gaussians; and the greater the number of Gaussians, the lower will be the fitting error. But the Gaussian peak parameters so determined will usually not be reproducible with respect to small changes in starting values or to variations in the noise in the data.



```
Peak #   Position   Height   Width   Area
1        20         0.9995   3.008   3.2
2        25         0.9996   2.993   3.185
3        30         1        2.998   3.191
4        35.02      0.9999   3.002   3.195
5        40.01      1.009    2.949   3.168
```

Here's another illustrative simulation: the original signal here consists of five overlapping Gaussian peaks with the same initial peak height (1.0) and width (FWHM=3) that have been subjected to *increasing degrees of exponential broadening* (similar to the broadening of peaks encountered in chromatography), and white noise is added *after* the broadening. Each peak has a *different* degree of broadening, so we use peakfit (page 90) with *vectors* of peak shapes *and 'extra' values*. It works best if we supply a vector of 'start' values [position1 width1 position2 width2 …] obtained from ***findpeaksG*** (page74) or by preliminary fitting with 5 plain Gaussians. The measured peak parameters of the original signal (bottom panel) are accurate to 0.3%.

```
x=5:.1:65;;
y=modelpeaks2(x,[1 5 5 5 5],[1 1 1 1 1],[20 25 30 35 40],[3 3 3 3 3],[0 -5 -10
-15 -20])+.01*randn(size(x));
```

Alternatively, you could try peak shape 31 to measure the time constants directly, but that can be unstable with multiple peaks (because there are too many interacting variables); you'll need a good 'start' value, the 8th input argument, as shown in this peakfit.m example:

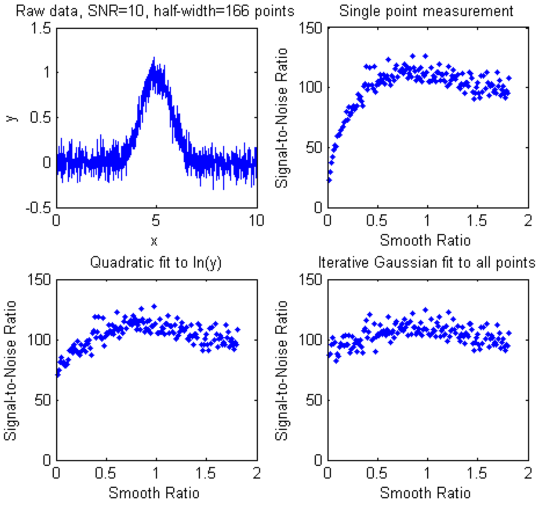
```
FitResults,FittingError]=peakfit([x;y], 30, 54, 5,[1 8 8 8 8],[0 -5 -10 -15
-20],10, [20 3.5 25 3.5 31 3.5 36 3.5 41 3.5],0)
```

Effect of smoothing before curve fitting: To Smooth or not to Smooth

In general, it is *not* advisable to smooth a signal before applying least-squares fitting (reference 43), because doing so might distort the signal, make it hard to evaluate the residuals properly, and bias the results of bootstrap sampling estimations of precision, causing it to underestimate the between-signal variations in peak parameters.

The Matlab/Octave script SmoothOptimization.m (download from **http://tinyurl.com/cey8rwh**) compares the effect of smoothing on the measurements of peak height of a Gaussian peak with a half-width of 166 points, plus white noise with a signal-to-noise ratio (SNR) of 10, using three different methods:

(a) simply taking the single point at the center of the peak as the peak height;
(b) using the *gaussfit* method to fit the top half of the peak (page 43), and
(c) fitting the entire signal with a Gaussian using the iterative method (page 54).



The results of 150 trials with independent white noise samples are shown on the left: a typical raw signal is shown in the upper left. The other three plots show the effect of the SNR of the measured peak height vs the smooth ratio (the ratio of the smooth width to the half-width of the peak) for those three measurement methods.

The results show that the simple single-point measurement is indeed much improved by smoothing, as would be expected; however, the optimum SNR is achieved only when the smooth ratio approaches 1.0 (which improves the SNR by roughly the square root of the peak width of 166 points), *but that much smoothing distorts the peak shape significantly*, reducing the peak height by about 40%. The curve-fitting methods are much less effected by smoothing and the iterative method hardly at all. In terms of *harmonic analysis* (page 28), smoothing removes only the high-frequency noise, leaving the low-frequency noise where most of the signal information is concentrated, resulting in no real improvement. Smoothing just makes things *look* better.

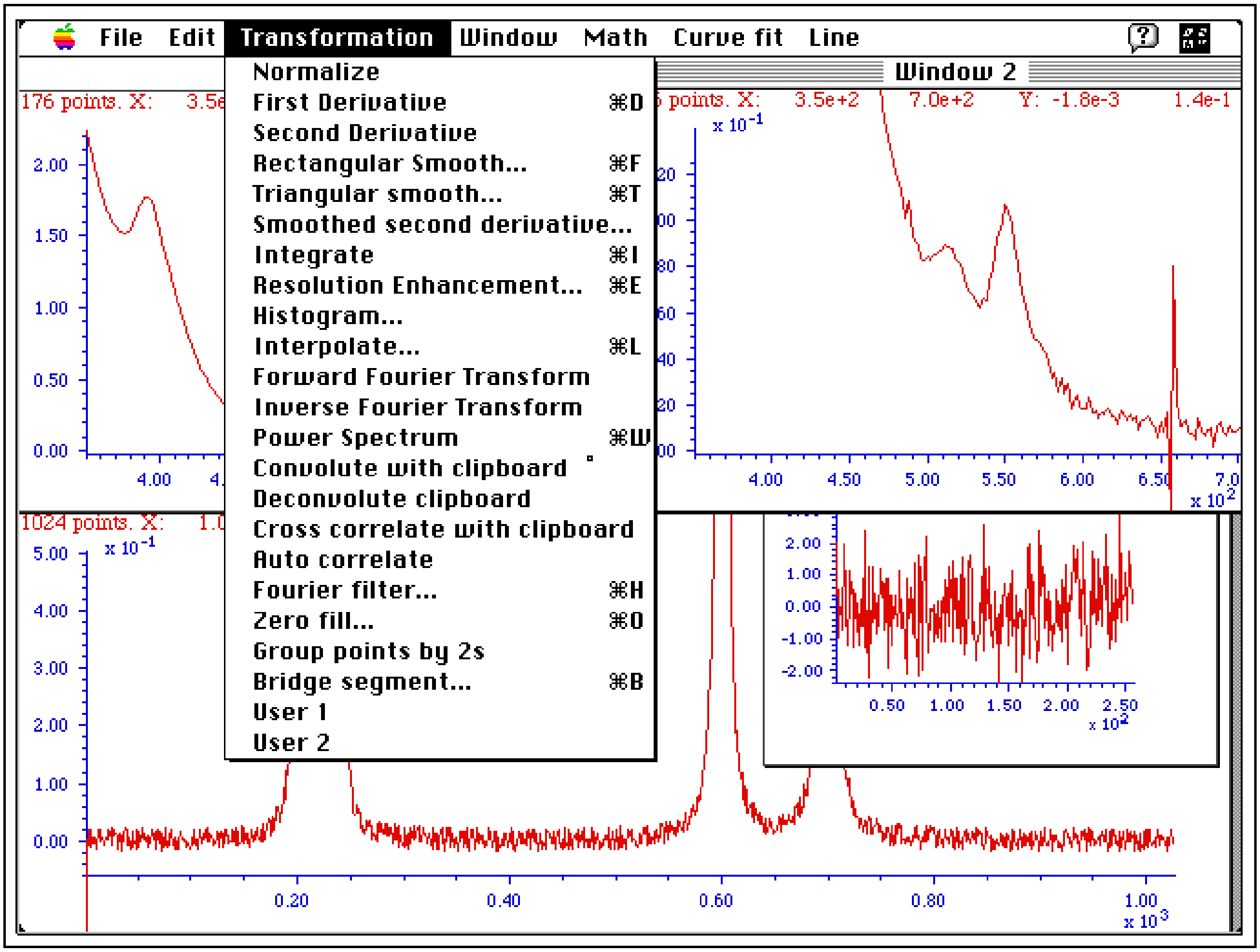
So the conclusion is that you should *not* smooth prior to curve-fitting, because it will distort the peak and will not gain any significant SNR advantage. The only situation where it might be advantageous so smooth is when the noise in the signal is high-frequency weighted (page 7) or if the signal is contaminated with high-amplitude narrow spike artifacts, in which case a median-based pre-filter is useful (page 14).

Unfortunately in some cases the signal source itself may be filtered internally (either inherently or by design, to make the output look better), and in those cases the usual methods of error prediction (page 40) will not be accurate.

Software details

1. SPECTRUM for Macintosh OS 7 or 8

Some of the figures in this essay are screen images from S.P.E.C.T.R.U.M. (**S**ignal **P**rocessing for **E**xperimental **C**hemistry **T**eaching and **R**esearch/ **U**niversity of **M**aryland), a simple Macintosh program that I wrote in 1989 for teaching signal processing to chemistry students. Unfortunately it runs only in Mac OS 8.1 and earlier, but it can be made to run on Windows 7 PCs and various specific Linux distributions using the Executor emulator.



SPECTRUM is designed for post-run (rather than real-time) processing of "spectral" or time-series data (y values at equally-spaced x intervals), such as spectra, chromatograms, electrochemical signals, etc. The program enhances the information content of instrument signals, for example by reducing noise, improving resolution, compensating for instrumental artifacts, and testing hypotheses.

SPECTRUM was the winner of two EDUCOM/NCRIPTAL national higher education software awards in 1990, in two categories: ***Best Chemistry*** *software and* ***Best Design****.*

Features

- Reads one- or two- column (y-only or x-y) text data tables with either tab or space separators
- Displays fast, labeled plots in standard re-sizable windows with full x- and y-axis scale expansion and a mouse-controlled measurement cursor

- Addition, subtraction, multiplication, and division of two signals
- Two kinds of smoothing.
- Three kinds of differentiation
- Integration
- Resolution enhancement
- Interpolation
- Fused peak area measurement by perpendicular drop or tangent skim methods, with mouse-controlled setting of start and end points
- Fourier transformation
- Power spectra
- Fourier filtering
- Convolution and Deconvolution
- Cross- and auto-correlation
- Built-in signal simulator with Gaussian and Lorentzian bands, sine wave and normally-distributed random noise
- A number of other useful functions, including: inspect and edit individual data points, normalize, histogram, interpolate, zero fill, group points by 2s, bridge segment, superimpose, extract subset of points, concatenate, reverse X-axis, rotate, set X axis values, reciprocal, log, ln, antilog, antiln, standard deviation, absolute value, square root

SPECTRUM can be used both as a simple research tool and as an instructional aid in teaching signal processing techniques. The program and its associated tutorial was originally developed for students of analytical chemistry, but the program could be used in any field in which instrumental measurements are used: e.g. chemistry, biochemistry, physics, engineering, medical research, clinical psychology, biology, environmental and earth sciences, agricultural sciences, or materials testing.

SPRECTUM performs only polynomial curve fitting and does not include non-linear iterative curve fitting.

Machine Requirements: SPECTRUM runs on older Macintosh models running OS 7 or 8, minimum 1 MByte RAM, any standard printer. Color screen desirable. SPECTRUM has been tested on most Macintosh models and on all versions of the operating system through OS 8.1. No PC version or more recent Mac version is available or planned, but if you have some older model Macs laying around, you might find this program useful.

SPECTRUM was written in Borland's *Turbo Pascal* in 1989 (yes, it's that old). Borland has long been out of business, neither Turbo Pascal nor the executable code generated by that compiler runs on current Macs, and therefore there is no way for me to update SPECTRUM without completely rewriting it in another language.

SPECTRUM also runs on Windows 7 PCs using the Executor emulator, which since 2008 has been made available as open source software.

The full version of SPECTRUM 1.1 is available as freeware, and can be downloaded from http://terpconnect.umd.edu/~toh/spectrum/. There are two versions:

> **SPECTRUM 1.1e**: Signals are stored internally as *extended-precision* real variables and there is a limit of 1024 points per signal. This version performs all its calculations in extended precision and thus has the best dynamic range and the smallest numeric round-off errors. The download address of this version in HQX format is http://terpconnect.umd.edu/~toh/spectrum/SPECTRUM11e.hqx.
>
> **SPECTRUM 1.1b**: Signals are stored internally as *single-precision* real variables and there is a limit of 4000 points per signal. This version is less precise in its calculations (has more

numerical round-off error) than the other version, but allows signals with data more points. The download address of this version in HQX format is http://terpconnect.umd.edu/~toh/spectrum/SPECTRUM11b.hqx.

The two versions are otherwise identical.

There is also a documentation package (located at http://terpconnect.umd.edu/~toh/spectrum/SPECTRUMdemo.hqx) consisting of:

a. Reference manual. Macwrite format (Can be opened from within MacWrite, Microsoft Word, ClarisWorks, WriteNow, and most other full-featured Macintosh word processors). Explains each menu selection and describes the algorithms and mathematical formulas for each operation. The SPECTRUM Reference Manual is also available separately in PDF format at http://terpconnect.umd.edu/~toh/spectrum/SPECTRUMReferenceManual.pdf.

b. Signal processing tutorial. Macwrite format (Can be opened from within MacWrite, Microsoft Word, ClarisWorks, WriteNow, and most other full-featured Macintosh word processors). Self-guided tutorial on the applications of signal processing in analytical chemistry. This tutorial is also available in PDF format and in Web format (http://terpconnect.umd.edu/~toh/Chem498C/SignalProcessing.html)

c. Tutorial signals: A library of prerecorded data files for use with the signal processing tutorial. These are plain decimal ASCII (tab-delimited) data files.

These files are binhex encoded: use Stuffit Expander to decode and decompress as usual. If you are downloading on a Macintosh, all this should happen completely automatically. If you are viewing this online, shift-click on the download links above to begin the download. If you are using the ARDI Executor Mac simulator, download the “HQX” files to your C drive, launch Executor, then open the downloaded HQX files with Stuffit Expander, which is pre-loaded into the Executor Macintosh environment. Stuffit Expander will automatically decode and decompress the downloaded files.

Note: Because it was developed for academic teaching application where the most modern and powerful models of computers may not be available, SPECTRUM was designed to be “lean and mean” - that is, it has a simple Macintosh-type user interface and very small memory and disk space requirements. It will work quite well on Macintosh models as old as the Macintosh II, and will even run on older monochrome models (with some cramping of screen space). It does not require a math co-processor.

What SPECTRUM does *not* do: this program does not have a peak detector, multiple linear regression, or an iterative non-linear curve fitter.

(c) 1989 T. C. O'Haver. This program is free and may be freely distributed. It may be included on CD-ROM collections or other archives.

2. Matlab and Octave

Matlab is a high-performance commercial numerical computing environment and programming language that is *very widely used* in research and education. It may be harder to learn *at first*, but there is a good reason why this language is so massively popular in science and engineering; it's *powerful, fast,* and there are thousands of useful free downloadable user-contributed functions. Bite the bullet and go for it. See http://en.wikipedia.org/wiki/MATLAB for a general description. There are *many* good online tutorials, YouTube videos, and collections of sample code:

a. Video Tutorials for New MATLAB Users (http://www.youtube.com/results?search_query=matlab+tutorial&aq=f).

b. A Brief Introductory Guide to MATLAB. (http://www.cs.unc.edu/~snoeyink/c/c205/matlab.htm)

c. Matlab Summary and Tutorial. (http://www.math.ufl.edu/help/matlab-tutorial/)

d. A Practical Introduction to Matlab (http://www.math.mtu.edu/~msgocken/intro/intro.html)

e. Matlab Chemometrics Index http://www.mathworks.com/matlabcentral/linkexchange/?term=chemometrics

f. Introduction to Matlab: http://homepages.math.uic.edu/~jan/mcs320s07/

g. Practical Statistical Signal Processing using MATLAB: http://www.aticourses.com/sampler/Practical%20Signal%20Processing%20using%20MATLAB.pdf**.**

h. Multivariate Curve Resolution. http://www.mcrals.info/

i. MATLAB Tutorials and Learning Resources: http://www.mathworks.com/academia/student_center/tutorials/launchpad.html

A *Google* or *YouTube* search for “signal processing” or “matlab” will often prove useful in turning up recently available tutorial materials. Several experienced Matlab users, as well as the *MathWorks* company itself, have produced excellent tutorial YouTube videos.

A collection of downloadable Matlab modules that I have written is freely available on http://terpconnect.umd.edu/~toh/spectrum/SignalProcessingTools.html. These are described in the following sections of this document.

Octave. There are less expensive alternatives to the relatively costly Matlab that operate in the same way. I recommend *Octave* (http://www.gnu.org/software/octave/); it's free and *almost* completely compatible with Matlab; DspGURU says that Octave is “...a mature high-quality Matlab clone. It has the highest degree of Matlab compatibility of all the clones.” In fact, all of my *command-line* functions, scripts, demos, and examples work in the latest version of Octave without change. However, the keyboard-operated interactive functions of ***iPeak***, ***iSignal***, ***iFilter***, and ***ipf*** do not currently work in Octave (but that might change in the future if I can figure out how to get my keypress-reading code working in Octave). The latest version is 4.0.0, as of June, 2015, which has a screen layout similar to Matlab. There are Windows, Mac, and Unix versions of Octave; download Windows versions from Octave Forge (http://sourceforge.net/projects/octave/). Be sure to install all the “packages”. Installation of Octave is somewhat more laborious than installing a commercial package like Matlab. More seriously, *Octave is slower than Matlab* computationally - see TimeTrial.txt for specific execution time comparisons for typical signal processing tasks. There is a lot of help available online: Google “GNU Octave” or see the YouTube videos for help. For signal processing applications specifically, Google "signal processing octave" to find the latest stuff.

There are also other alternatives to MATLAB, in particular Scilab, FreeMat, Julia, and Sage which are intended to be mostly compatible with the MATLAB language. For a discussion of other possibilities, see http://www.dspguru.com/dsp/links/matlab-clones.

An additional advantage of Matlab and Octave is that their function and script files (“m-files”) are just *plan text files* with a “.m” extension, so *those files can be opened and inspected even on devices that do not have Matlab or Octave installed*.

3. Peak Finding and Measurement

A common requirement in signal processing is to detect peaks in signals and to measure their positions, heights, and widths. A common way to do this is to make use of the fact that the first derivative (page 17) of a peak has a downward-going zero-crossing at the peak maximum. But the presence of random noise in real experimental signal will cause many false zero-crossing simply due to the noise. To avoid this problem, the technique described here first smooths (page 11) the first derivative of the signal before looking for the zero-crossings, and then it takes only those zero crossings whose slope exceeds a predetermined minimum, called the "slope threshold", at a point where the original signal exceeds a certain minimum, called the "amplitude threshold". The idea is to adjust the smooth width and the slope and amplitude thresholds to detect only the "real" peaks and to ignore peaks that are too small, too wide, or too narrow. Moreover, because smoothing can distort peak signals, reducing peak heights and increasing peak widths (page 11), this technique estimates the position, height, and width of each peak by least-squares curve-fitting (page 37) of a segment of the *original unsmoothed signal* in the vicinity of the zero-crossing. As a result, even if heavy smoothing of the first derivative is necessary to provide reliable discrimination against noise peaks, the peak parameters extracted by curve fitting are not distorted. (This is useful primarily for signals that have several data points in each peak, not for spikes that have only one or two points). The technique is capable of measuring peak positions and heights quite accurately, but the measurements of peak widths and areas is accurate only if the peak shapes are approximately Gaussian (or Lorentzian, using **findpeaksL.m**). The routine is available in two different versions:

(a) a series of command-line functions, available in several different variations;

(b) an interactive keypress-operated function (**ipeak**.m) for adjusting the peak detection criteria in real-time to optimize for any particular peak type, described on page 78. (For the most accurate measurement of highly overlapped peaks of any shape, iPeak is capable of utilizing non-linear iterative curve fitting, page 54). If you are viewing this online, click here to download the ZIP file "Peakfinder.zip" that includes all the **findpeaksG** variants and supporting functions and several self-contained demos to show how it works. Download from http://tinyurl.com/cey8rwh.

a. The findpeaksG and findvalleys functions (**findpeaksG.m** and **findvalleys.m**)

```
P=findpeaksG(x,y,SlopeThreshold,AmpThreshold,SmoothWidth,FitWidth,smoothtype)
```

This is the basic command-line function for Matlab or Octave to locate and measure the positive peaks in a noisy data sets. It's an alternative to the findpeaks function in the *Signal Processing Toolkit*. It detects peaks by looking for downward zero-crossings in the smoothed first derivative that exceed SlopeThreshold and peak amplitudes that exceed AmpThreshold, and determines the position, height, and approximate width of each peak by least-squares curve fitting the top part of the peak. The function returns a peak table matrix containing the peak number and the estimated position, height, width, and area of each peak. The signal is passed to the function in the vectors x and y (independent and dependent variables); other parameters are user-adjustable:

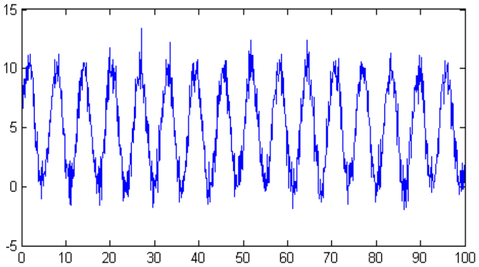
SlopeThreshold - Slope of the smoothed first-derivative that is taken to indicate a peak. This discriminates on the basis of peak width. Larger values of this parameter will neglect broad features of the signal. A reasonable value for Gaussian peaks is 0.7*WidthPoints^-2, where WidthPoints is the number of data points in the half-width of the peak.
AmpThreshold - Discriminates on the basis of peak height. Smaller peaks than this are ignored.
SmoothWidth - Width of the smooth function that is applied to data before the slope is measured. Larger values of SmoothWidth will neglect small, sharp features. A reasonable value is about equal to 1/2 of *the number of data points* in the half-width of the peaks.
FitWidth - The number of points around the "top part" of the (unsmoothed) peak that are taken to estimate the peak heights, positions, and widths. A reasonable value is typically about equal to 1/2 of *the number of data points* in the half-width of the peaks. The minimum value is 3.
Smoothtype determines the smoothing algorithm (see page 11)
If smoothtype=1, rectangular (sliding-average or boxcar)
If smoothtype=2, triangular (2 passes of sliding-average)
If smoothtype=3, pseudo-Gaussian (3 passes of sliding-average)
Basically, higher values yield greater reduction in high-frequency noise, at the expense of slower execution. See page 110 for a comparison of smooth types.

Example: `>> x=[0:.01:50];y=cos(x);P=findpeaksG(x,y,0,-1,5,5)`

```
ans =
    1.0000    6.2832    1.0000    2.3547    2.5068
    2.0000   12.5664    1.0000    2.3547    2.5068
    3.0000   18.8496    1.0000    2.3547    2.5068 . . . etc
```

How is 'findpeaksG' different from 'findpeaks' in the Signal Processing Toolkit?

The function 'findpeaks.m' in Matlab's *Signal Processing Toolbox* (SPT) can be used to find the values and indexes of all the peaks in a vector that are higher than a specified peak height and are separated from their neighbors by a specified minimum distance. My version of findpeaks (**findpeaksG.m**) accepts both an independent variable (x) and dependent variable (y) vectors, finds the places where the average curvature over a specified region is concave down, fits that region with a least-squares fit, and returns the peak position (in x units), height, width, and area, of any peak that exceeds a specified height. For example, let's create a noisy series of peaks (plotted on the right) and apply each of the functions to the resulting data.



```
>> x=[0:.1:100];
>> y=5+5.*sin(x)+randn(size(x));
>> plot(x,y)
```

Now, anyone looking at this plot of data would count 16 peaks, with peak heights averaging about 10 units. Every time these three statements are run, the random noise is different, but you would still count the 16 peaks. But the findpeaks function in the SPT

```
>> {PKS,LOCS]=findpeaks(y,'MINPEAKHEIGHT',5,'MINPEAKDISTANCE',11)
```

counts anywhere from 11 to 20 peaks, with an average height (PKS) of 11.5. In contrast, my findpeaksG function returns 16 peaks every time, with a mean height of 10 ±0.3.

```
>> findpeaksG(x,y,0.001,5,11,11,3)
```

It also measures the width and area, if the peaks are Gaussian (or Lorentzian, in the variant findpeaksL). To be fair, findpeaks in the Signal Processing Toolbox works better for peaks that have only 1-3 data points on the peak; my findpeaksG is better for peaks with many points.

Findvalleys. There is also a similar function for finding *valleys* (minima) called findvalleys.m, which works the same way as **findpeaksG.m**, except that it locates *minima* instead of *maxima*. Only valleys above (that is, more positive or less negative than) the AmpThreshold are detected; if you wish to detect valleys with *negative* minima, AmpThreshold must be set more negative than that.

```
>> x=[0:.01:50];y=cos(x);P=findvalleys(x,y,0,-1,5,5)
ans =
        1     3.1416      -1     2.3571  0
        2     9.4248      -1     2.3571  0
        3     15.708      -1     2.3571  0 ...
```

The accuracy of the measurements of peak position, height, width, and area by findpeaksG depends on the shape of the peaks, the extent of peak overlap, the baseline, and signal-to-noise ratio. The width and area measurements particularly are strongly influenced by peak overlap, noise, and the choice of FitWidth. Isolated peaks of Gaussian shape are measured most accurately. For Lorentzian peaks, use findpeaksL.m instead (the only difference is that the reported peak heights, widths, and areas will be more accurate if the peak are actually Lorentzian). See "ipeakdemo.m" (below) for an accuracy trial and peakfitVSfindpeaks.m for a comparison of findpeaks to peakfit.

findpeaksb.m is a variant of findpeaksG.m that more accurately measures peak parameters by using iterative least-square curve fitting based on peakfit.m. This yields better peak parameter values that findpeaksG alone, because it can be set for 30 different peak shapes, it fits the entire peak, not just the top part, and it has provision for background correction (linear, quadratic, or flat). This function works best with isolated peaks that do not overlap. The syntax is `P=findpeaksb(x,y, SlopeThreshold,AmpThreshold,smoothwidth,peakgroup,smoothtype,windowspan, PeakShape,extra,autozero)`. The first seven input arguments are exactly the same as for the findpeaksG.m function; if you have been using findpeaksG or iPeak to find and measure peaks in your signals, you can use those same input argument values for findpeaksb.m. The remaining four input arguments of are for the peakfit function: "windowspan" specifies the number of data points over which each peak is fit to the model shape (if the peaks are superimposed on a background, 'windowspan' must be large enough to cover the entire single peak and get down to the background on both sides of the peak. Some trial and error may be needed to get this setting right.); "PeakShape" specifies the model peak shape (1=Gaussian, 2=Lorentzian, etc), "extra" is the shape modifier variable for the Voigt, Pearson, exponentially broadened Gaussian and Lorentzian, Gaussian/Lorentzian blend, and bifurcated Gaussian and Lorentzian shapes to fine-tune the peak shape; “autozero” is 0, 1, 2, or 3 for no, linear, quadratic, or flat background subtraction. The peak table returned by the function has a 6th column listing the percent fitting errors for each peak. This example, of three Gaussian peaks on a linear background, illustrates the accuracy advantages of this function over findpeaksG.m:

```
x=1:.2:100;Heights=[1 2 3];Positions=[20 50 80];Widths=[3 3 3];
y=2-(x./50)+modelpeaks(x,3,1,Heights,Positions,Widths)+.02*randn(size(x));plot(x,y);
disp('        Peak      Position      Height      Width        Area         % error')
PlainFindpeaks=findpeaksG(x,y,.00005,.3,15,15,3)
NoBackgroundSubtraction=findpeaksb(x,y,.00005,.5,30,20,3,150,1,0)
LinearBackgroundSubtraction=findpeaksb(x,y,.00005,.5,30,20,3,150,1,1)
```

findpeaksb3.m is a variant of findpeaksb.m that fits each detected peak *along with the previous and following peaks* found by findpeaksG.m, so as to deal better with overlapping peaks. Type “help findpeaksb3.m” for syntax and examples. See this example graphic.

findpeaksplot.m plots the x,y data and *numbers the peaks on the graph* (if any are found).

findpeaksE.m is a variant of findpeaksG.m that returns the percent relative fitting error of each peak (assuming a Gaussian peak shape) in the 6th column of the peak table. Example:

```
x=[0:.01:5];findpeaksnr(x,x.*sin(x.^2).^2+.1*whitenoise(x),.001,1,15,10)
```

Peak start and end. Defining the "start" and "end" of the peak (the x-values where the peak begins and ends), is a bit arbitrary because typical peak shapes approach the baseline gradually rather than abruptly. You might define the peak start and end points as where the *y* value is low, say, 1% of the peak height. But then the random noise on the baseline will often be a large fraction of the signal amplitude at that point. Smoothing to reduce noise is known to distort and broaden peaks, effectively changing their start and end points. Overlap of peaks also greatly complicates the issue. One solution is to fit each peak to a model shape, then calculate the peak start and end from the model expression. That minimizes the noise problem by fitting the data over the entire peak, and it can handle overlapping peaks, but it works only if the peaks can be modeled by available fitting programs. For example, Gaussian peaks reach a fraction *a* of the peak height of x=**p**±sqrt(**w**^2 log(1/*a*))/(2 sqrt (log(2))) where **p** is the peak position and **w** is the peak width. So, for example if *a*=.01 (that is, 1%), x=**p**±1.288784***w**. Lorentzian peaks reach a fraction *a* of the peak height of x=**p**±sqrt[(**w**^2 - *a* **w**^2)/*a*]/2. If *a*=.01, x=**p**±4.97493***w**. The findpeaksG variants findpeaksGSS.m and findpeaksLSS.m, for Gaussian and Lorentzian peaks respectively, compute the 1% peak start and end positions in this manner and return them in the 6th and 7th columns of the peak table.

findpeaksT.m is like findpeaksG, except that it measures the peak parameters by constructing a triangle with sides tangent to the sides of each peak. See triangulation.m for a demonstration.

findpeaksfit.m is a serial combination of findpeaksG.m and peakfit.m, using the number of peaks and the peak positions and widths determined by findpeaksG as input for the peakfit.m function, which then fits *the entire signal* with the specified peak model. This yields better values than findpeaks alone, because peakfit fits the entire peak, not just the top part, and it deals with non-Gaussian and overlapped peaks, but *it fits only those peaks that are found by findpeaks*. It can measure peak areas, even of overlapping peaks, without defining the peak start and stop times.

peakstats.m uses the same algorithm as findpeaksG.m, but it returns a table of summary statistics of the peak intervals (the x-axis interval between adjacent detected peaks), heights, widths, and areas, listing the max, min, average, median, mode, and percent standard deviation of each, and optionally plotting the x,y data with numbered peaks in figure window 1, displaying the table of peak statistics in the command window and histograms of the peak intervals, heights, widths, and areas in figure 2.

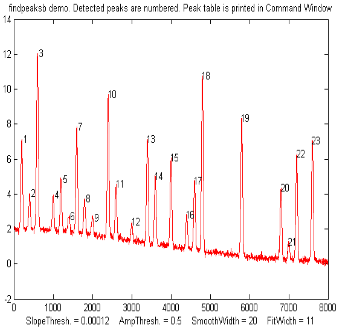
```
>> x=[0:.1:1000];y=5+5.*cos(x)+randn(size(x));PS=peakstats(x,y,0,-1,15,23,3,1);
Peak Summary Statistics: 15 peaks detected
          Interval      Height      Width         Area
Maximum    6.6552      10.7393       4.2968       47.1482
Minimum    5.8525       9.5884       2.2744       26.0028
Mean       6.272       10.1845       3.0942       33.4194
% STD      3.1852       3.4479      18.3062       16.5684
Median     6.2719       10.0262      3.2468       34.6473
Mode       6.0035       9.1217       2.5063       28.2559
```

(median and mode in version 2 only)

tablestats.m is similar to peakstats.m except that it takes as input a peak table **P** returned from any of my peak finding functions listed above whose name begins with “findpeaks...”.

findsteps.m `P=findpulses(x,y,SlopeThreshold,AmpThreshold,SmoothWidth,peakgroup)` has the same input arguments as findpeaksG.m, but it locates positive transient *steps* (a sharp rise followed by a flat plateau or slow drop). Returns list (P) with step number, x position, y position, and the step height of each step detected. "SlopeThreshold" and "AmpThreshold" control step sensitivity; higher values will neglect smaller features. Increasing "SmoothWidth" reduces small sharp false steps caused by random noise. See findsteps.png for a real example.

Demo scripts: DemoFindPeak.m and DemoFindPeaksb.m



These are demo scripts using the findpeaks and findpeaksb functions on noisy synthetic data. They number the peaks and print out the P matrix in the Matlab/Octave command window. The difference between them is that DemoFindPeaksb.m uses findpeaksb, which has the ability to correct for background. (The findpeaksG function can't give accurate measurements for this signal, because it does not correct for background). **DemoFindPeakSNR** is a variant of DemoFindPeak.m that computes the signal-to-noise ratio (SNR) of each peak and returns it in the 5th column.

Which to use: findpeaksG, findpeaksb, findpeaksb3, or findpeaksfit? The Matlab/Octave script "FindpeaksComparison.m" compares these four peak detection functions applied to an adjustable computer-generated signal with multiple peaks plus variable types and amounts of baseline and random noise. Discover which works best for your own type of peak data. Click here for details.

Using the peak table matrix. The above functions return the peak table as a matrix, in the order peak number, position, height, width, etc, which you can assign to a variable (e.g. **P**) and then use Matlab/Octave functions to extract specific information. For example: `[P(:,2) P(:,3)]` is the time series of peak heights); `mean(P(:,3))` returns the average peak height (because peak height is in the 3rd column); `max(P(:,3))` returns the maximum peak height; `hist(P(:,3))` displays the histogram of peak heights; `std(P(:,4))/mean(P(:,4))` returns the relative standard deviation of the peak widths (column 4); `P(:,3)./max(P(:,3))` returns the ratio of each peak height (column **3**) to the height of the highest peak detected. `100.*P(:,5)./sum(P(:,5))` returns the percentage of each peak area (column **5**) of the total area of all peaks detected. `for n=1:length(P)-1;d(n)=max(P(n+1,2)-P(n,2));end` creates "d" as the vector of x-axis distance between peaks. `sortrows(P,2)` sorts **P** by peak position. `sortrows(P,3)` sorts **P** by peak height.

Using my downloadable val2ind.m function: `val2ind(P(:,3),7.5)` returns the peak number of the peak whose height is closest to 7.5; `P(val2ind(P(:,2),18.5),3)` returns the peak height (3rd column) whose position (2nd column) is closest to 18.5; `P(val2ind(P(:,3),max(P(:,3))),:)` returns the row vector of peak parameters of the *highest* peak in peak table P.

Peak Identification

The command line function **idpeaks.m** is used for identifying peaks according to peak positions:

```
[IdentPeaks,AllPeaks]=idpeaks(M,AmpT,SlopeT,sw,fw,maxerror,Positions,Names)
```

It finds peaks in the signal "M" (x-values in column 1 and y-values in column 2), according to the peak detection parameters AmpT, SlopeT, sw, fw (see the "findpeaks" function above), then compares the found peak positions (x-values) to a database of known peaks, in the form of an array of known peak maximum positions ("Positions") and matching cell array of names ("Names"). If the position of a peak found in the signal is closer to one of the known peaks by less than the specified maximum error "maxerror", that peak is considered a match and its peak position, name, error, and peak amplitude (height) are entered into the output cell array "IdentPeaks". The full list of detected peaks, identified or not, is returned in "AllPeaks". You can use "cell2mat" to access numeric elements of IdentPeaks, e.g. `cell2mat(IdentPeaks(2,1))` returns the position of the first identified peak, `cell2mat(IdentPeaks(2,2))`returns its name, and so forth for the peak position error and peak height. The related function **idpeaktable.m** does the same thing for a peak table **P** returned by any of my peak finder (page 74) or peak fitting (page 90) functions, with **P** having one row for each peak and columns for peak number, position, and height as the first three columns. (The interactive iPeak function, discussed in the next section, also has the same peak identification feature; see page 81).

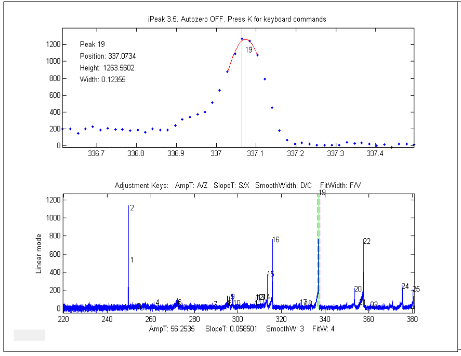
Example: Download idpeaks.zip, extract it, and place the extracted files in the Matlab or Octave path. This contains a high-resolution atomic emission spectrum of copper ('spectrum') and a data table of known Cu I and II atomic lines ('DataTable') containing their positions and names.

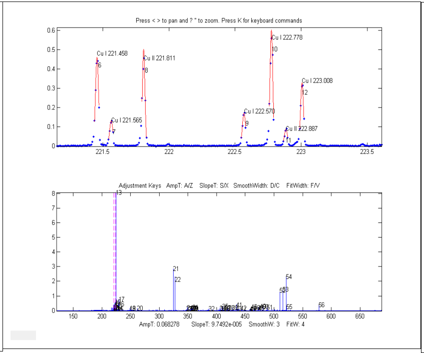
```
>> load DataTable
>> load spectrum
>> idpeaks(Cu,0.01,.001,5,5,.01,Positions,Names)
ans=
    'Position'    'Name'              'Error'           'Amplitude'
    [  221.02]    'Cu II 221.027'     [ -0.0025773]     [ 0.019536]
    [  221.46]    'Cu I 221.458'      [ -0.0014301]     [   0.4615]
    [  221.56]    'Cu I 221.565'      [-0.00093125]     [  0.13191]
```

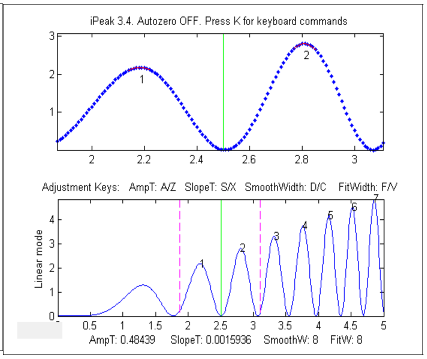
The lower the settings of the AmpThreshold, SlopeThreshold, and SmoothWidth, the more peaks will be detected; and the higher the setting of "MaxError", the more peaks will be close enough to the reference peaks to be considered identified. Of course, the accuracy of identification depends on the x-axis calibration of the measuring instrument (e.g. the wavelength accuracy of a spectrometer). For atomic emission or absorption spectroscopy, tables of known atomic lines can be obtained from NIST (the National Institute for Standards and Technology) and other sources.

Flat-topped pulses require a different approach, based on a simple amplitude threshold rather than differentiation. The function "`findsquarepulse.m`" (syntax `S=findsquarepulse (t,y,threshold)`) locates the sections in the signal t,y that exceed a y-value of "threshold" and determines their start time, average height (relative to the baseline) and width. Returns the start time, height, and width of each pulse. `DemoFindsquare.m` demonstrates this function.

b. The iPeak Function (`ipeak.m`), for Matlab


iPeak 3.5. Autozero OFF. Press K for keyboard commands
Peak 19
Position: 337.0734
Height: 1263.5602
Width: 0.12355
Adjustment Keys: AmpT: A/Z SlopeT: S/X SmoothWidth: D/C FitWidth: F/V
Linear mode
AmpT: 56.2535 SlopeT: 0.058501 SmoothW: 3 FitW: 4


Press < > to pan and ? " to zoom. Press K for keyboard commands
Cu I 221.458
Cu II 221.811
Cu I 221.565
Cu I 222.778
Cu I 223.008
Cu I 222.570
Cu II 222.887
Adjustment Keys AmpT: A/Z SlopeT: S/X SmoothWidth: D/C FitWidth: F/V
AmpT: 0.068278 SlopeT: 9.7492e-005 SmoothW: 3 FitW: 4


iPeak 3.4. Autozero OFF. Press K for keyboard commands
Adjustment Keys: AmpT: A/Z SlopeT: S/X SmoothWidth: D/C FitWidth: F/V
Linear mode
AmpT: 0.48439 SlopeT: 0.0015936 SmoothW: 8 FitW: 8

ZIP file available at http://terpconnect.umd.edu/~toh/spectrum/ipeak7.zip

Animated step-by-step instructions at http://terpconnect.umd.edu/~toh/spectrum/ipeak.html

iPeak is a Matlab keyboard-operated interactive peak finder for time series data, based on the "findpeaksG.m" function. It displays the entire signal in the lower half of the Figure window and an adjustable zoomed-in section in the upper window. Simple keystrokes allow you to adjust the peak detection parameters AmpThreshold (**A**/**Z** keys), SlopeThreshold (**S**/**X** keys), SmoothWidth (**D**/**C** keys), FitWidth (**F**/**V** keys), and other controls. (See list of Keyboard Controls, below). This function can be used to determine experimentally what values of those parameters give the most reliable peak detection for a particular type of data, detecting the desired peaks and ignoring those that are too small, too broad, or too narrow to be of interest. Detected peaks are numbered from left to right. Returns the peak table in a matrix (columns: peak #, position, height, width, area, and percent fitting error; one row for each peak detected). Press **P** to display a labeled peak table of all the detected peaks in the command window. Press **Shift-P** to save peak table as disc file.

EXAMPLE 1: Two input arguments; data in separate x and y vectors

```
>> x=[0:.1:100];y=(x.*sin(x)).^2;ipeak(x,y);
```

EXAMPLE 2: One input argument; data in two columns of a matrix

```
>> x=[0:.01:5]';y=x.*sin(x.^2).^2;ipeak([x y])
```

EXAMPLE 3: One input argument; data in single vector

```
>> y=cos(.1:.1:100);ipeak(y)
```

The cursor keys pan and zoom the signal in the upper window, to inspect each peak in detail if desired. You can set the initial values of pan and zoom in optional input arguments 7 ('xcenter') and 8 ('xrange'). See example 6 below.

EXAMPLE 4: An additional scalar argument (shown in **bold face** below) controls peak sensitivity.

```
 >> x=[0:.1:100];y=5+5.*cos(x)+randn(size(x));ipeak(x,y,10);
 or  >> ipeak([x;y],10);
 or  >> ipeak(humps(0:.01:2),3)
 or  >> x=[0:.1:10];y=exp(-(x-5).^2);ipeak([x' y'],1)
```

This additional argument is an estimate of the ratio of the typical peak width to the length of the entire data record. Small values detect fewer peaks; larger values detect more peaks. It effects only the *starting* values for the peak detection parameters, and it's a quick way to set initial values for *all* the peak detection parameters, rather than specifying each one individually as in the next example).

EXAMPLE 5: Six input arguments. As above, but input arguments 3 to 6 directly specify the initial values of PeakD, AmpT, SlopeT, SmoothW, FitW. (PeakD is ignored in this case, so just type a '0' as the second argument after the data matrix).

```
>> ipeak(datamatrix,0,.5,.0001,20,20);
```

EXAMPLE 6: Eight input arguments. As above, but input arguments 7 and 8 specify the initial pan and zoom settings, 'xcenter' and 'xrange', respectively. In this example, the x-axis data are wavelengths in nanometers (nm), and the upper window zooms in on a very small 0.4 nm region centered on 249.7 nm. (These data are from a high-resolution atomic spectrum).

```
  >> load ipeakdata.mat
  >> ipeak(Sample1,0,110,0.06,3,4,249.7,0.4);
```

EXAMPLE 7: Nine input arguments. As example 6, but the 9th input argument controls the baseline correction mode (equivalent to pressing the **T** key), which can be 0 (none), 1 (linear), 2 (quadratic), or 3 (flat). If not specified, it is 0 (none).

```
>> ipeak(Sample1,0,110,0.06,3,4,249.7,0.4,1);
```

The **Spacebar**/**Tab** keys jump to the next/previous detected peak and displays it in the upper window at the current zoom setting (use the up and down cursor arrow keys to adjust the zoom range). Or you can press the **J** key to jump directly to a specified peak number. The **L** key turns off and on peak parameter labeling. The **Y** key toggles between linear and log y-axis scale in the lower window (good for inspecting signals with high dynamic range; it effects only the lower window display and has no effect on the peak detection or measurements). The **U** key switches between peak and valley mode. **Shift-G** switches between Gaussian and Lorentzian peak modes.

The **T** key cycles the baseline correction mode through four modes: *OFF, linear, quadratic*, and *flat*. When OFF, peak heights are measured relative to zero. (If the peaks are superimposed on a background, use the baseline subtract keys - **B** and **G** - first to subtract the background). In linear and quadratic modes, peak heights are automatically measured relative to a calculated baseline that is linearly or quadratically interpolated from the signal at the *edges of the signal in the upper window*; use the zoom controls to isolate the peaks so that the signal returns to the local baseline between the peaks as displayed in the upper window. (In those modes, the peak heights, widths, and areas in the peak table (**R** or **P** keys) will be automatically corrected for the baseline. OFF will give better results when the baseline is zero, or has been subtracted using the **B** key, even if the peaks are partly overlapped. Linear and quadratic will work best if the peaks are well separated so that the signal returns to the local baseline between the peaks. Flat mode applies only to the curve fitting operation (**N** and **M** keys); it corrects for a flat baseline shift even if the signal does not return to the baseline.

Peak summary statistics table. The **E** key command prints a table of summary statistics of the peak intervals (the x-axis interval between adjacent detected peaks), heights, widths, and areas, listing the maximum, minimum, average, and percent standard deviation, and displaying the histogram of each of these in figure window 2.

Ensemble averaging. For signals that contain repetitive waveform patterns occurring in one continuous signal, with nominally the same shape except for noise, the ensemble averaging function (**Shift-E**) can compute the average of all the repeating waveforms. It works by detecting a single peak in each repeat waveform in order to synchronize the repeats (and thus does not require that the repeats be equally spaced or synchronized to an external reference signal). To use this function, first adjust the peak detection controls to detect only one peak in each repeat pattern, then zoom in to isolate any one of those repeat patterns, and press **Shift-E**. The average waveform is displayed in Figure 2 and saved as “EnsembleAverage.mat” in the current directory so that other processing (e.g. curve fitting) may be applied. For an example, see page 117.

Normal and Multiple Curve fitting: If the peaks are highly overlapped, or if they are not Gaussian in shape, better results will be obtained by using the curve fitting function - the **N** or **M** keys. The **N** key applies iterative curve fitting only to the detected peaks that are displayed in the *upper* window (referred to here as "**N**ormal" curve fitting). The use of the iterative least-squares function can result in more accurate peak parameter measurements that the normal peak table (**R** or **P** keys), especially if the peaks are non-Gaussian in shape or are highly overlapped. If the peaks are superimposed on a background, use the **T** key to set the baseline correction mode to flat, linear, or quadratic. Then use the pan and zoom keys to select a peak or a group of overlapping peaks in the upper window, with the signal returning all the way to the local baseline at the ends of the upper window. Make sure that AmpThreshold, SlopeThreshold, SmoothWidth are adjusted so that each peak is numbered once. Then press the **N** key, type a number for the desired peak shape from the menu displayed in the Command window and press **Enter**, then type in a number of repeat trial fits and press **Enter** (the default is 1; start with that and then increase if necessary). If you have selected a variable-shape peak, the program will ask you to type in a number that controls the shape ("extra" in the peakfit input arguments). The program performs the fit and prints out the peakfit function with all its input arguments and shows the results in Figure window 2 and in the command window:

```
Peak shape (1-37): 1
Number of trials: 1
Least-squares fit to Gaussian peak model using the peakfit function:
>> peakfit(DataMatrix,355,292,3,1,1,1,[225 54 274 55 323 45],0);
Fitting Error 0.23243%
          Peak#     Position     Height       Width         Area       Extra
            2       224.63       214.31       60.949        10106
            3       276.35       278.66       47.409        14057
            4       324.67       403.88       50.321        21634
```

The use of this function gives more accurate peak parameter measurements that the normal peak table (**R** or **P** keys) if the peaks are non-Gaussian in shape or are highly overlapped.

There is also a "**M**ultiple" peak fit function (**M** key) that will apply iterative curve fitting to *all the detected peaks in the signal simultaneously*. Before using this function, it's best to set the baseline mode to 'none' (**T** key) and use the multi-segment baseline correction function (**B** key) to remove the background (because the autozero function will probably not be able to subtract the baseline from the entire signal). Then press **M** and proceed as for the normal curve fit. A multiple curve fit may take a minute or so to complete if the number of peaks is large, possibly longer than the Normal curve fitting function on each group of peaks separately. It will fit only those peaks that it finds.

The **N** and **M** key fitting functions perform non-linear iterative curve fitting (page 54) using the peakfit.m function (page 90). The number of peaks and the starting values of peak positions and widths for the curve fit function are automatically supplied by the findpeaks function, so *it is essential that the peak detection variables in iPeak be adjusted so that all the peaks in the selected region are detected and numbered once*. (For more flexible curve fitting, use **ipf.m**, page 95). Pressing the **H** key may help to detect overlapped peaks.

Note 1: If the peaks are too overlapped to be detected and numbered separately, try pressing the **H** key to activate the sharpen function before pressing **M**. If they are too overlapped even for that, use **ipf.m** instead of **iPeak.m**

Note 2: If you plan to use a variable-shape peak (numbers 4, 5, 8, 13, 14, 15, 30-33) for the Multiple peak fit, it's a good idea to obtain a reasonable value for the requested "extra" shape parameter by performing a Normal peak fit on an isolated single peak (or small group of partly-overlapping peaks) of the same shape, then use that value for the Multiple curve fit of the entire signal.

Note 3: If the peak shape varies across the signal, you can either use the Normal peak fit to fit each section with a different shape rather than the Multiple peak fit, or you can use the unconstrained shapes that fit the shape individually for each peak: Voigt (30), ExpGaussian (31), Pearson (32), or Gaussian/Lorentzian blend (33).

Note 4: If the density of data points on the peaks is *too low* - less than about 4 points - the peaks may not be reliably detected; you can improve reliability by using the interpolation command (**Shift-I**) to re-sample the data by linear interpolation to a *larger* number of points. Conversely, if the density of data points on the peaks is very high, then you can speed up the operation of iPeak by interpolating to a *smaller* number of points.

Peak identification: There is an optional "peak identification" function if optional input arguments 9 ("MaxError"), 10 ("Positions"), and 11 ("Names") are included. The "**i**" key toggles this function ON and OFF. This function compares the found peak positions (maximum x-values) to a database of known peaks, in the form of an array of known peak maximum positions ("Positions") and matching cell array of names ("Names"). If the position of a found peak in the signal is closer to one of the known peaks by less than the specified maximum error (“MaxError”), then that peak is considered a match and its name is displayed next to the peak in the upper window. When the “**o**” key is pressed, the peak positions, names, errors, and amplitudes are printed out in a table in the command window.

EXAMPLE 8: Eleven input arguments. As above, but also specifies “MaxError”, “Positions”, and “Names” in optional input arguments 9, 10, and 11, for peak identification function. Pressing the “**i**” key toggles off and on the peak identification labels in the upper window. Pressing “o” prints the peak positions, names, errors, and amplitudes in a table in the command window. These data (provided in the ZIP file mentioned above) are from an atomic spectrum (x-axis in nanometers).

```
 >> load ipeakdata.mat
 >> ipeak(Sample1,0,120,0.06,3,6,296,5,0.1,Positions,Names);
```

Note: The ZIP file contains the latest version of the iPeak function as well as some sample data to demonstrate peak identification (Example 7 and 8). Obviously for your own applications, it's up to you to provide your own array of known peak maximum positions ('Positions') and matching cell array of names ('Names') for your particular types of signals.

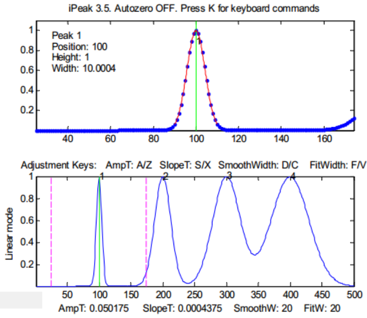
iPeak Keyboard Controls

```
 Pan signal left and right...Coarse pan: < and > keys
                             Fine pan: left and right cursor arrow keys
                             Nudge left and right: [ and ] keys
  Zoom in and out............Coarse zoom: / and ' keys
                             Fine zoom: up and down cursor arrow keys
 Resets pan and zoom.........ESC
 Select entire signal........Crtl-A  Zooms out to entire signal
 Change plot color...........Enter
 Adjust AmpThreshold.........A,Z  (Larger values ignore short peaks)
 Type in AmpThreshold........Shift-A
 Adjust SlopeThreshold.......S,X  (Larger values ignore broad peaks)
 Type in SlopeThreshold......Shift-S
 Adjust SmoothWidth..........D,C  (Larger values ignore sharp peaks}
 Adjust FitWidth.............F,V  (Adjust to cover just top part of peaks)
 Toggle sharpen mode ........H  Helps detect overlapped peaks.
 Baseline correction.........B, then click baseline at multiple points
 Restore original signal.....G  to cancel previous background subtraction
 Invert signal...............-  Invert (negate) the signal (flip + and -)
 Set minimum to zero.........0 (Zero) Sets minimum signal to zero
 Interpolate signal..........Shift-I Interpolate (re-sample) to N points
 Toggle log y mode OFF/ON....Y  Plot log Y axis on lower graph
 Cycles baseline modes.......T  0=none; 1=linear; 2=quadratic; 3=Flat.
 Toggle valley mode OFF/ON...U  Switch between peak and valley modes
 Gaussian/Lorentzian switch..Shift-G  Switch between Gaussian/Lorentzian
 Print peak table............P  Prints Peak #, Position, Height, Width
 Save peak table.............Shift-P  Saves peak table as disc file
 Step through peaks..........Space/Tab  Jumps to next/previous peak
 Jump to peak number.........J  Type peak number and press Enter.
 Normal peak fit.............N  Fit peaks in upper window with peakfit.m
 Multiple peak fit...........M  Fit all peaks in signal with peakfit.m
 Ensemble Average all peaks..Shift-E  Zoom to display single peak first
 Print keyboard commands.....K  Prints this list
 Print findpeaks arguments...Q  Prints findpeaks function with arguments.
 Print ipeak arguments.......W  Prints ipeak function with all arguments.
 Print report................R  Prints Peak table and parameters
 Print peak statistics.......E  prints mean, std of peak intervals, heights...
 Peak labels ON/OFF......... L  Label all peaks detected in upper window.
 Peak ID ON/OFF..............I  Identifies closest peaks in 'Names' database.
 Print peak IDs..............O  Prints table of peaks Ids
 Switch to ipf.m.............Shift-Ctrl-F  Transfer current signal to ipf.m
 Switch to iSignal...........Shift-Ctrl-S  Transfer current signal to iSignal.m
```

Which to use: iPeak or Peakfit? To help decide, download the ZIP file at http://terpconnect.umd.edu/~toh/spectrum/idemos.zip that contains some Matlab demo functions comparing iPeak.m with Peakfit.m for signals with a few peaks and signals with many peaks and that shows how to adjust iPeak to detect broad or narrow peaks. These are self-contained demos that include all required Matlab functions. Just place them in your path and click Run or type their name at the command prompt.

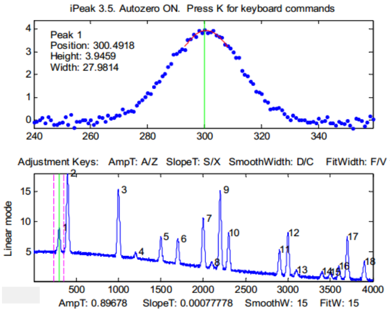
iPeak Demo functions

The ZIP file at http://terpconnect.umd.edu/~toh/spectrum/ipeak7.zip contains several demo functions (ipeakdemo.m, ipeakdemo1.m, etc) that illustrate various aspect of the iPeak function:



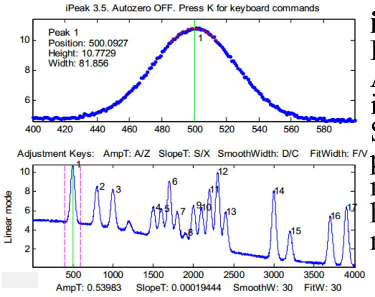
ipeakdemo: effect of the peak detection parameters

Four Gaussian peaks with the same heights but different widths (10, 30, 50 and 70 units). This demonstrates the effect of SlopeThreshold and SmoothWidth on peak detection. Increasing SlopeThreshold (**S** key) will discriminate against the broader peaks. Increasing SmoothWidth (**D** key) will discriminate against the narrower peaks and noise. FitWidth (**F/V** keys) controls the number of points around the “top part” of the (unsmoothed) peak that are taken to estimate the peak heights, positions, and widths. A reasonable value is about equal to 1/2 of *the number of data points* in the half-width of the peaks. In this case, where the peak widths are different, set it to about 1/2 of the number of data points in the narrowest peak.



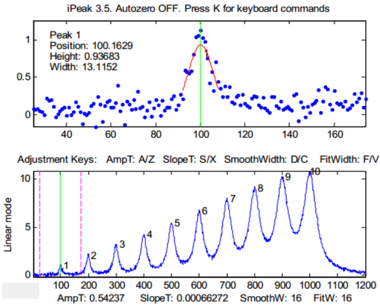
ipeakdemo1: the background correction modes

Demonstration of background correction, for separated, narrow peaks on a large baseline. A table of the actual peak positions, heights, widths, and areas is printed out in the command window. Jump to the next/previous peaks using the Spacebar/Tab keys. Hint: Use the **T** key to set the baseline correction mode to “Linear” or “Quadratic”, adjust the zoom setting so that the peaks are shown one at a time in the upper window, then press the **P** key to display the peak table. (Each time you run this demo, you will get a different set of peaks and noise).



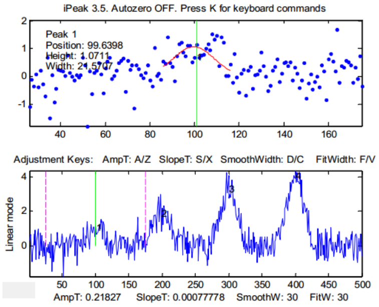
ipeakdemo2: peak overlap and the curve fitting functions.

Demonstration of error caused by overlapping peaks on a large offset baseline. A table of the actual peak positions, heights, widths, and areas is printed out in the command window. Jump to the next/previous peaks using the Spacebar/Tab keys. Hint: Use the **B** key and click on the baseline points, then press the **P** key to display the peak table. Or turn on the baseline correction mode (**T** key) and use the Normal curve fit (**N** key) or Multiple curve fit (**M** key). (Each time you run this demo, you will get a different set of peaks and noise).



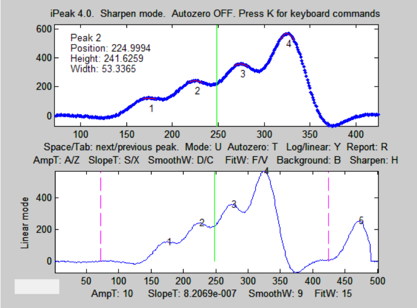
ipeakdemo3: Non-Gaussian peak shapes

Demonstration of overlapping Lorentzian peaks, without an added background. Overlap of peaks causes significant errors in peak height, width, and area. Jump to the next/previous peaks using the **Spacebar/Tab** keys. Each time you run this demo, you will get a different set of noise. A table of the actual peak positions, heights, widths, and areas is printed out in the command window. Hint: Press **Shift-G** to switch to Lorentzian mode; set the baseline correction mode to OFF (**T** key) and use the Normal curve fit (**N** key) with peak shape 2 (Lorentzian).



ipeakdemo4: dealing with very noisy signals

Detection and measurement of four peaks in a very noisy signal. The signal-to-noise ratio of first peak is 2. A table of the actual peak positions, heights, widths, and areas is printed out in the command window. Jump to the next/previous peaks using the **Spacebar/Tab** keys. The peak at x=100 is usually detected, but the accuracy of peak parameter measurement is poor because of the low signal-to-noise ratio. **Hint**: Increase SmoothWidth and FitWidth to help reduce the effect of the noise. Each time you run this demo, you will get a different noise.


iPeak 4.0. Sharpen mode. Autozero OFF. Press K for keyboard commands

Peak 2
Position: 224.9994
Height: 241.6259
Width: 53.3365

Space/Tab: next/previous peak. Mode: U Autozero: T Log/linear: Y Report: R
AmpT: A/Z SlopeT: S/X SmoothW: D/C FitW: F/V Background: B Sharpen: H

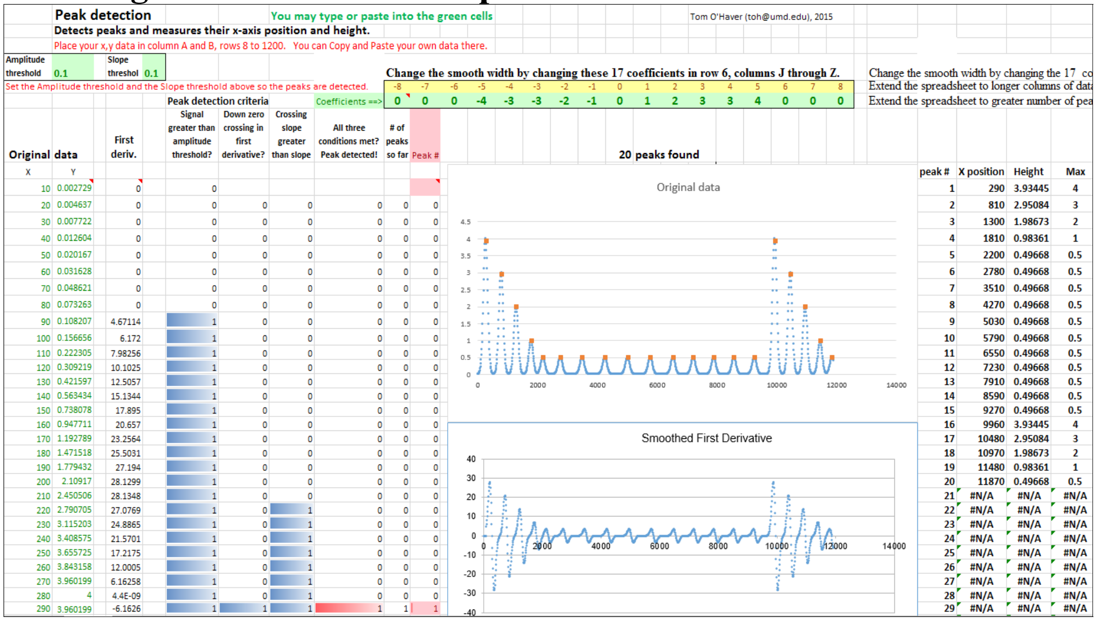
AmpT: 10 SlopeT: 8.2069e-007 SmoothW: 9 FitW: 15

ipeakdemo5: dealing with highly overlapped peaks

In this demo the peaks are so highly overlapped that only one or two of the highest peaks yield distinct maxima that are detected by iPeak. The height, width, and area estimates are highly inaccurate because of this overlap. The normal peak fit function ('**N**' key) would be useful in this case, but it depends on iPeak for the number of peaks and for the initial guesses, and so it would fit only the peaks that were found and numbered. To help in this case, pressing the '**H**' key will activate the *peak sharpen* function (page 26) that decreases peak width and increases peak height of all the peaks, making it easier for Findpeaks to detect and number them for use by the peakfit function. Note: peakfit fits the original unsharpened peaks; sharpening is used only to *locate* the peaks.

Note: The ZIP file available at **http://terpconnect.umd.edu/~toh/spectrum/ipeak7.zip** contains findpeaks, DemoFindPeaks, ipeak.m, all the ipeakdemos described above, and the ipeakdata.mat file with the high-resolution atomic spectrum used in example 8.

c. Peak Finding and Measurement Spreadsheet


Peak detection

You may type or paste into the green cells

Tom O'Haver (toh@umd.edu), 2015

Detects peaks and measures their x-axis position and height.

Place your x,y data in column A and B, rows 8 to 1200. You can Copy and Paste your own data there.

Amplitude threshold 0.1 Slope threshol 0.1

Set the Amplitude threshold and the Slope threshold above so the peaks are detected.

Change the smooth width by changing these 17 coefficients in row 6, columns J through Z.

	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8
Coefficients ==>	0	0	0	-4	-3	-3	-2	-1	0	1	2	3	3	4	0	0	0

Change the smooth width by changing the 17 co
Extend the spreadsheet to longer columns of data
Extend the spreadsheet to greater number of pea

Peak detection criteria

20 peaks found

Original	data	First deriv.	Signal greater than amplitude threshold?	Down zero crossing in first derivative?	Crossing slope greater than slope	All three conditions met? Peak detected!	# of peaks so far	Peak #
X	Y							
10	0.002729	0	0					
20	0.004637	0	0	0	0	0	0	0
30	0.007722	0	0	0	0	0	0	0
40	0.012604	0	0	0	0	0	0	0
50	0.020167	0	0	0	0	0	0	0
60	0.031628	0	0	0	0	0	0	0
70	0.048621	0	0	0	0	0	0	0
80	0.073263	0	0	0	0	0	0	0
90	0.108207	4.67114	1	0	0	0	0	0
100	0.156656	6.172	1	0	0	0	0	0
110	0.222305	7.98256	1	0	0	0	0	0
120	0.309219	10.1025	1	0	0	0	0	0
130	0.421597	12.5057	1	0	0	0	0	0
140	0.563434	15.1344	1	0	0	0	0	0
150	0.738078	17.895	1	0	0	0	0	0
160	0.947711	20.657	1	0	0	0	0	0
170	1.192789	23.2564	1	0	0	0	0	0
180	1.471518	25.5031	1	0	0	0	0	0
190	1.779432	27.194	1	0	0	0	0	0
200	2.10917	28.1299	1	0	0	0	0	0
210	2.450506	28.1348	1	0	0	0	0	0
220	2.790705	27.0769	1	0	1	0	0	0
230	3.115203	24.8865	1	0	1	0	0	0
240	3.408575	21.5701	1	0	1	0	0	0
250	3.655725	17.2175	1	0	1	0	0	0
260	3.843158	12.0005	1	0	1	0	0	0
270	3.960199	6.16258	1	0	1	0	0	0
280	4	4.4E-09	1	0	1	0	0	0
290	3.960199	-6.1626	1	1	1	1	1	1

peak #	X position	Height	Max
1	290	3.93445	4
2	810	2.95084	3
3	1300	1.98673	2
4	1810	0.98361	1
5	2200	0.49668	0.5
6	2780	0.49668	0.5
7	3510	0.49668	0.5
8	4270	0.49668	0.5
9	5030	0.49668	0.5
10	5790	0.49668	0.5
11	6550	0.49668	0.5
12	7230	0.49668	0.5
13	7910	0.49668	0.5
14	8590	0.49668	0.5
15	9270	0.49668	0.5
16	9960	3.93445	4
17	10480	2.95084	3
18	10970	1.98673	2
19	11480	0.98361	1
20	11870	0.49668	0.5
21	#N/A	#N/A	#N/A
22	#N/A	#N/A	#N/A
23	#N/A	#N/A	#N/A
24	#N/A	#N/A	#N/A
25	#N/A	#N/A	#N/A
26	#N/A	#N/A	#N/A
27	#N/A	#N/A	#N/A
28	#N/A	#N/A	#N/A
29	#N/A	#N/A	#N/A

The spreadsheets PeakDetection.xls/xlsx (pictured above with data) implements a simple derivative-based peak detection method described on page 19. The input x,y data are contained in columns **A** and **B**, rows 9 to 1200. You can Copy and Paste your own data there. The amplitude threshold and slope threshold are set in cells **B4** and **E4**, respectively. Smoothing and differentiation are performed by the convolution technique used by the DerivativeSmoothing.xls spreadsheet (page 24). The Smooth Width and the Fit Width are both controlled by the number of non-zero convolution coefficients in row 6, columns **J** through **Z**. (In order to compute a symmetrical first derivative, the coefficients in columns **J** to **Q** must be the negatives of the positive coefficients in columns **S** to **Z**). The original data and the smoothed derivative are shown in the two charts on Sheet1. To detect peaks in the data, a series of three conditions are tested for each data point in columns F, G, and H, corresponding to the three nested loops in findpeaksG.m:

1. Is the signal greater than Amplitude Threshold? (line 45 of findpeaksG.m; column **F**)
2. Is there a downward directed zero crossing in the smoothed first derivative? (line 43 of findpeaksG.m; column **G** in the spreadsheet)
3. Is the slope of the derivative at that point greater than the Slope Threshold? (line 44 of findpeaksG.m; column **H** in the spreadsheet)

If the answer to *all three* questions is *yes* (highlighted by blue cell coloring), a peak is registered at that point (column **I**), counted in column **J**, and assigned an index number in column **K**. The original data and the smoothed derivative are shown in the two charts. The peak index numbers, X-axis positions, and peak heights are listed on the right in columns **AC** to **AF**. Peak heights are computed *two ways*: "Height" is based on slightly smoothed Y values (which is more accurate if the data are noisy) and "Max" is the highest individual Y value near the peak (which is more accurate if the data are smooth or if the peaks are very narrow). You can extend the spreadsheet to longer columns of data by dragging down the last row of columns **A** through **K** as needed, and to greater number of peaks by dragging down the last row of columns **AC** - **AF** as needed and modifying cell **R7** to include the additional peaks. See **PeakDetectionExample**.xlsx/.xls) for an example with data already pasted in, and **PeakDetectionDemo2**.xls/xlsx is a demonstration with a user-controlled computer-generated series of noisy peaks.

An extension of that method is made in PeakDetectionAndMeasurement.xlsx, which makes the assumption that the peaks are *Gaussian* and measures their height, position, and width using a least-squares technique, just like "findpeaksG.m". For the first 10 peaks found, the x,y original unsmoothed data are copied to **Sheet2** through **Sheet11**, where that segment of data is subjected to a Gaussian least-squares fit, using the technique described on page 43. The best-fit Gaussian parameter results are copied back to **Sheet1**, in the table in columns **AH-AK**. (In its present form, the spreadsheet is limited to *measuring* 10 peaks, although it can *detect* any number of peaks. It's also limited in Smooth Width and Fit Width by the 17-point convolution coefficients).

This spreadsheet template is available in OpenOffice (.ods) and in Excel (.xls) and (.xlsx) formats. They are functionally equivalent and differ only in minor cosmetic aspects. An example spreadsheet, with data, is available. A demo version, with a calculated noisy waveform that you can modify, is also available. If the peaks in the data are too much overlapped, they may not make sufficiently distinct maxima to be detected reliably. If the noise level is low enough, the peaks can be artificially sharpened by the technique described on page 26. This idea is implemented by the spreadsheet template PeakDetectionAndMeasurementPS.xlsx and its calculated demo version PeakDetectionAndMeasurementDemoPS.xlsx. Download from http://tinyurl.com/cey8rwh.

To expand this spreadsheet to *larger numbers of data points*, simply drag down the last row of columns A through K, so that the formulas in those rows are replicated for the required number of additional rows, then adjust the charts to accommodate the extra rows. *However*, expanding the spreadsheet to *larger numbers of measured peaks* is more difficult: (1) drag down row 17, columns AC through AK, and adjust the formulas in those rows for the required number of additional peaks; (2) copy all of Sheet11 and paste it into a series of new sheets (Sheet12, Sheet13, etc), one for each additional peak; (3) adjust the formulas in columns B and C in each of these additional sheets to refer to the appropriate row in Sheet1; (4) carefully modify these additional equations in the added sheets, using the same pattern as those for peaks 1-10. Whew!

A comparison of this spreadsheet to its Matlab/Octave equivalent “findpeaksplot.m” is instructive. On the positive side, the spreadsheet literally "spreads out" the data and the calculations spatially over a large number of cells and sheets, breaking down the discrete steps in a very graphic way. In particular, the use of conditional formatting in columns **F** through **K** make the peak detection decision process more evident for each peak, and the least-squares sheets 2 through 11 lay out every detail of those calculations. Spreadsheet programs have many flexible formatting options to make displays more attractive. Data entry, by typing or pasting, is intuitive. On the down side, a spreadsheet as complicated as this one is far more difficult to construct than its Matlab/Octave equivalent. Much more serious, the spreadsheet is less flexible and harder to expand to larger signals and larger number of peaks. In contrast, the Matlab/Octave equivalent, while requiring some understanding of programming to *construct* initially, is more flexible, can easily handle signals and smooth/fit widths of any size, and can detect and measure any number of peaks, with *no additional effort* on your part. The Matlab equivalent is about 50 times faster than the Excel or Calc spreadsheet above. Moreover, because it is written as a *function*, it can readily be employed as an element in your own custom Matlab/Octave scripts to perform even larger tasks. See reference 50 on page 124.

4. *iSignal*: Interactive Smoothing, Differentiation, and Peak Sharpening

iSignal is a Matlab function, written as a single self-contained m-file, for performing smoothing (page 11), differentiation (page 16), peak sharpening (page 26), peak area measurement (page 35), signal and noise measurement (page 6), frequency spectra (page 28), least-squares fitting (page 37) and other useful functions on time-series data. Using simple keystrokes, you can select any region of the signal and adjust the signal processing parameters continuously while observing the effect on your signal dynamically. The ZIP file for the current version is available for download at http://terpconnect.umd.edu/~toh/spectrum/iSignal5.zip. These files, and the instructions for their use, are available online at http://terpconnect.umd.edu/~toh/spectrum/SignalProcessingTools.htm.

The syntax of iSignal is:

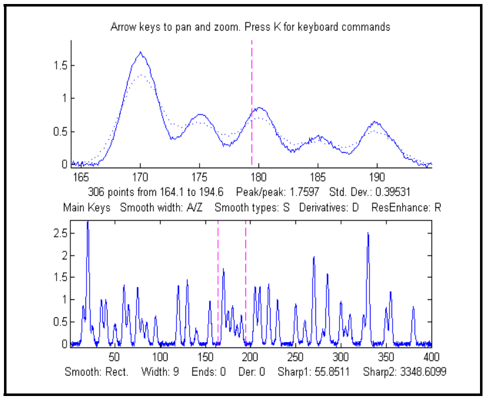
```
Y=isignal(Data);
```

or

```
[pY,SpectrumOut]=isignal(Data,xcenter,xrange,SmoothMode,SmoothWidth,ends,...
DerivativeMode,Sharpen,Sharp1,Sharp2,SlewRate,MedianWidth,SpectrumMode);
```

or `[pY,SpectrumOut,,maxy,miny,area,stdev]=isignal(Data,...`

“Data” may be a 2-column matrix with the independent variable (x-values) in the first column and dependent variable (y values) in the second column, or separate x and y vectors, or a single y-vector (in which case the data points are plotted against their index numbers on the x axis). Only the first argument is required; all the others are optional. Returns the processed dependent axis (Y) vector as the output argument. Plots the data in the figure window, the lower half of the window showing the entire signal, and the upper half showing a selected portion controlled by the pan and zoom keystrokes, with the initial pan and zoom settings optionally controlled by input arguments '**xcenter**' and '**xrange**', respectively. Other keystrokes allow you to control the smooth type, width, and ends treatment, the derivative order (0th through 5th), and peak sharpening. (The initial values of all these parameters can be passed to the function via the optional input arguments **SmoothMode**, **SmoothWidth**, **ends**, **DerivativeMode**, **Sharpen**, **Sharp1**, and **Sharp2**. See the examples below). Press **K** to see all the keyboard commands.



Arrow keys to pan and zoom. Press K for keyboard commands

306 points from 164.1 to 194.6 Peak/peak: 1.7597 Std. Dev.: 0.39531

Main Keys Smooth width: A/Z Smooth types: S Derivatives: D ResEnhance: R

Smooth: Rect. Width: 9 Ends: 0 Der: 0 Sharp1: 55.8511 Sharp2: 3348.6099

Smoothing (see page 11)

The **S** key (or input argument “**SmoothMode**”) cycles through four smoothing modes:
If **SmoothMode**=0, the signal is not smoothed.
If **SmoothMode**=1, rectangular (sliding-average or boxcar)
If **SmoothMode**=2, triangular (2 passes of sliding-average)
If **SmoothMode**=3, pseudo-Gaussian (3 passes of sliding-average)
If **SmoothMode**=4, Savitzky-Golay smooth.

The **A** and **Z** keys (or input argument **SmoothWidth**) control the **SmoothWidth**, *w*.
The **X** key toggles “**ends**” between 0 and 1. This determines how the “ends” of the signal (the first *w*/2 points and the last *w*/2 points) are handled when smoothing. If **ends**=0, the ends are zero. If **ends**=1, the ends are smoothed with progressively smaller smooths the closer to the end. Generally, **ends**=1 is best, except in some cases using the derivative mode when **ends**=0 result in better vertical centering of the signal.
Notes: (1) When smoothing peaks, you can easily measure the effect of smoothing on peak height and width by turning on peak measure mode (press **P**) and then press **S** to cycle through the smooth modes. (2) There are two functions for removing or reducing sharp spikes in signals: the **M** key, which implements a median filter (it asks you to enter the spike width, e.g. 1, 2, 3... points). The ~ key limits the maximum rate of change, which can reduce the amplitude of sharp spikes and steps.

Differentiation (see page 17)

The **D** / **Shift-D** keys (or optional input argument “**DerivativeMode**”) increase/decrease the derivative order. The default is 0. Careful optimization of smoothing of derivatives is critical for acceptable signal-to-noise ratio.

Peak sharpening or resolution enhancement (see page 26)

The **E** key (or optional input argument "**Sharpen**") turns off and on peak sharpening (resolution enhancement). The sharpening strength is controlled by the **F** and **V** keys (or optional input argument "**Sharp1**") and **B** and **G** keys (or optional argument "**Sharp2**"). The optimum values depend on the peak shape and width. For peaks of Gaussian shape, a reasonable value for **Sharp1** is PeakWidth^2/25 and for **Sharp2** is PeakWidth^4/800 (or PeakWidth^2/6 and PeakWidth^4/700 for Lorentzian peaks), where PeakWidth is the full-width at half maximum of the peaks *expressed in number of data points*. However, you don't need to do the math yourself; *iSignal 1.6* and later can calculate sharpening and smoothing settings for Gaussian and for Lorentzian peak shapes using the **Y** and **U** keys, respectively. Just isolate a single typical peak in the upper window using the pan and zoom keys, press **P** to turn on the peak measurement mode, then press **Y** for Gaussian or **U** for Lorentzian peaks. (The optimum settings depends on the width of the peak, so if your signal has peaks of widely different widths, one setting will not be optimum for all the peaks).

Fine-tune the sharpening with the **F/V** and **G/B** keys and the smoothing with the **A/Z** keys. Expect a decrease in peak width (and corresponding increase in peak height) of about 20% - 50%, depending on the shape of the peak (the peak area is largely unchanged). Excessive sharpening leads to baseline artifacts and increased noise. *iSignal* allows you to experimentally determine the values of these parameters that give the best trade-off between sharpening, noise, and baseline artifacts, for your purposes. To measure the effect of sharpening on peak width, turn on peak measure mode (press **P**) and then press **E** to toggle the sharpen mode off and on.

Signal measurement

The cursor keys control the position of the green cursor and the dotted red cursors that mark the selected range displayed in the upper graph window. The label under the top graph window shows the value of the signal (y) at the green cursor, the peak-to-peak (min and max) signal range, the area under the signal, and the standard deviation within the selected range (the dotted cursors). Pressing the **Q** key prints out a table of the signal information in the command window. If the optional output arguments **maxy**, **miny**, **area**, **stdev** are specified, iSignal returns the maximum and minimum values of y, the total area under the curve, and the standard deviation of y, in the selected range displayed in the upper panel. The demo script demoisignal.m illustrates some of these features.

Background correction

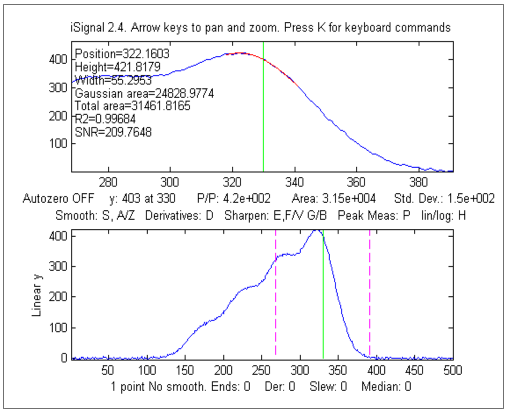
Background (or baseline) correction is important because the peak heights, widths, and areas measured by the Peak Measure (**P**) command are based on the assumption that the baseline under the peaks is zero. There are two methods: *manual* and *automatic*. The **Backspace** key starts *manual* background correction operation. In the command window, type in the number of background points to click and press the Enter key. The cursor changes to cross hairs; click along the presumed background, starting to the left of the x axis and placing the last click to the right of the x axis. When the last point is clicked, the linearly interpolated baseline between those points is subtracted from the signal. To restore the original background (i.e. to correct an error or to try again), press the '\' key (just below the backspace key).

To select the *automatic* baseline correction, press the **T** key repeatedly; it cycles thorough four modes: *No* baseline correction, *linear*, *quadratic*, and *flat* mode. In *linear mode*, a straight-line baseline connecting the two ends of the signal segment in the upper panel will be subtracted. In *quadratic mode*, a parabolic baseline connecting the two ends of the signal segment in the upper panel will be automatically subtracted. The baseline is calculated by computing a least-squares fit to the signal in the first 1/10th of the points and the signal last 1/10th of the points. Try to adjust the pan and zoom to include some of the baseline at the beginning and end of the segment in the upper window, allowing the automatic baseline subtract gets a good reading of the baseline. The calculation of the signal amplitude, peak-to-peak signal, and peak area are all based on the baseline-subtracted signal in the upper window. The *flat* baseline *mode* is used only for peak fitting (**Shift-P**).

Saving the results

To save the processed signal as a .mat file, press the '**o**' key, type in a file name, then press **Enter**.

Peak measurement



iSignal 2.4. Arrow keys to pan and zoom. Press K for keyboard commands

```
Position=322.1603
Height=421.8179
Width=55.2953
Gaussian area=24828.9774
Total area=31461.8165
R2=0.99684
SNR=209.7648
```

```
Autozero OFF    y: 403 at 330    P/P: 4.2e+002    Area: 3.15e+004    Std. Dev.: 1.5e+002
Smooth: S, A/Z   Derivatives: D   Sharpen: E,F/V G/B   Peak Meas: P   lin/log: H
```

```
1 point No smooth. Ends: 0    Der: 0    Slew: 0    Median: 0
```

The **P** key turns off and on the "peak" mode, which attempts to measure the one peak (or valley) that is centered in the upper window under the green cursor by superimposing a least-squares best-fit parabola, in red, on the center portion of the signal displayed in the upper window. (Zoom in so that the red overlays just the top of the peak or the bottom of the valley as closely as possible). The peak position, height, and "Gaussian width" are measured by least-squares curve fitting of a parabola to the isolated peak. The "RSquared" value is the coefficient of determination; the closer to 1.00 the better. "SNR" is the signal-to-noise-ratio of the peak under the green cursor; it's the ratio of the peak height to the standard deviation of the residuals between the data and the red best-fit line.

The peak widths will most accurate if the peaks are Gaussian; other shapes, and very noisy peaks of any shape, will give only approximate results. However, the position, height, and area values are pretty good for any peak shape as long as the "RSquared" value is at least 0.99. The "total area" is measured by the trapezoidal method over the entire selected segment displayed in the upper window. If the peaks are superimposed on a non-zero background, subtract the background before measuring peaks, either by using auto baseline correction (**T** key) or the multi-point background subtraction (backspace key). Press the **R** key to print out the peak parameters in the command window.

Note: iSignal measures peak area two ways: the "Gaussian area" and the "Total area". The "Gaussian area" is the area under the Gaussian that is a best fit to the center portion of the signal displayed in the upper window, marked in red. The "Total area" is the area by the trapezoidal method over the entire selected segment displayed in the upper window. (In version 4.3, the percent of total area is also calculated). If the portion of the signal displayed in the upper window is a pure Gaussian with no noise and a zero baseline, then the two measures should agree almost exactly. If the peak is not Gaussian in shape, then the total area is likely to be more accurate, as long as the peak is well separated from other peaks.

Peak fitting (version 5). To fit an overlapping peak model to the data in the upper panel, press Shift-F, type in the desired peak shape number from the menu, enter the number of peaks, enter the number of repeat trial fits, then click the mouse pointer on each proposed peak position. A graph of the fit is displayed in Figure window 2 and a table of results is printed out. See page 90 for details. Note: if you have a peak that is an exponentially-broadened Gaussian or Lorentzian, you can measure both the "after-broadening" height, position, and approximate width using the P key function, and the "before-broadening" height, position, and width by fitting the peak to an exponentially-broadened Gaussian or Lorentzian model (shapes 5, 8, 36, 31, or 18) using the Shift-F key function. The peak areas will be the same; broadening does not effect the total peak area.

Polynomial fitting. Shift-o fits a simple polynomial (linear, quadratic, cubic, etc) to the upper panel segment and displays the coefficients (in descending powers) and the R_2. See page 37.

Frequency Spectrum. The Shift-S key toggles on and off the Frequency Spectrum mode, which computes the Fourier frequency spectrum (page 28) of the segment of the signal displayed in the upper window and displays it in the lower window, temporarily replacing the full-signal display. Pan and zoom to adjust the region of the signal to be viewed. Shift-A cycles through four plot modes (linear, semilog X, semilog Y, or log-log) and Shift-X toggles between a frequency on the x axis and time on the x-axis (periodogram). Shift-Z toggles on and off peak detection and labeling on the frequency spectrum or periodogram. You can adjust the peak detection in lines 1970-1973. All signal processing functions remain active in the frequency spectrum mode (smooth, derivative, etc) so you can observe the effect of these functions on the frequency spectrum of the signal. Press Shift-Z to label the peaks in the frequency spectrum with their frequencies. Press Shift-S again to return to the normal mode. To save the frequency spectrum as a new variable, call iSignal with output arguments `[pY,Spectrum]` and set the 13th input argument 'SpectrumMode' to 1. **Shift-W** displays the 3D waterfall spectrum, by dividing up the signal into segments and computing the power spectrum of each; you choose the number of segments and the type of 3D display from a menu.

Other keystroke controls. The **L** key toggles off and on the Overlay mode, which overlays the current processed signal with the original signal as a dotted line, for the purposes of comparison. The tab key restores the original signal and cursor settings. The "-" (minus sign) key is used to negate the signal (flip + for -). The "+" key computes the absolute value of the entire signal. Press H to toggle the display between a linear y and semilog y plot in the lower window, which is useful for signals with very wide dynamic range (zero and negative points are ignored in the log plot). The 0 key (number 0) removes offset from the signal; sets minimum y value to zero. The semicolon (;) key sets the selected region (between the dotted red cursor lines) to zero; use it to remove uninteresting regions of the signal. The C key condenses the signal by the specified factor n, replacing each group of n points with their average, prompts the user to enter the value of n. (typically, 2, 3, 4, etc). The I key replaces the signal with a linearly interpolated version containing m data points, prompts the user to enter the value of m. This can be used to increase or decrease the sampling rate or to change an unevenly sampled signal to an evenly sampled one. You can press Shift-C, then click on the graph to print out the x,y coordinates of that point. This works on both the upper and lower panels, and on the frequency spectrum as well.

Spacebar or Shift-P plays the signal segment in the upper panel as a sound; Shift-R allows you to enter the sampling rate at which the signal will be played back.

EXAMPLE 1: Single input argument; data in two columns [x;y] or in a single y vector

```
   >> isignal(y);
   >> isignal([x;y]);
```

EXAMPLE 2: Two input arguments. Data in separate x and y vectors.

```
    >> isignal(x,y);
```

EXAMPLE 3: Three or four input arguments. The arguments two and three specify the initial values of pan (xcenter) and zoom (xrange) in the last two input arguments. Using data in the ZIP file:

```
      >> load data.mat
      >> isignal(DataMatrix,180,40); or
      >> isignal(x,y,180,40);
```

EXAMPLE 4: As above, but additionally specifies initial values of SmoothMode, SmoothWidth, ends, and DerivativeMode in input arguments 4 - 7.

```
    >> isignal(DataMatrix,180,40,2,9,0,1);
```

EXAMPLE 5: As above, but additionally specifies initial values of the peak sharpening parameters Sharpen, Sharp1, and Sharp2 (input arguments 8, 9 and 10). Press **E** to turn sharpening on and off.

```
    >> isignal(DataMatrix,180,40,2,9,0,0,1,51,6000);
```

EXAMPLE 6: Using the built-in "humps" function:

```
    >> x=[0:.005:2];y=humps(x);Data=[x;y];
```

4th derivative of the peak at x=0.9:

```
    >> isignal(Data,0.9,0.5,1,3,1,4);
```

Peak sharpening applied to the peak at x=0.3:

```
    >> isignal(Data,0.3,0.5,1,3,1,0,1,220,5400);
```

Press '**E**' key to toggle sharpening ON/OFF to compare)

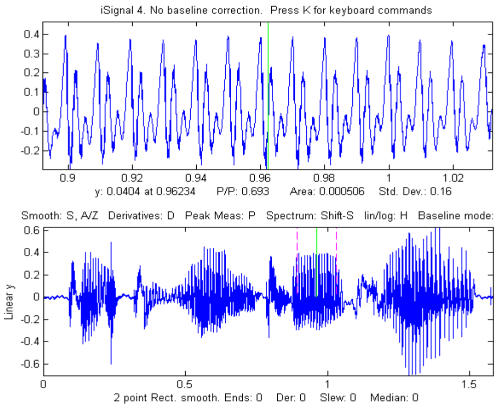
EXAMPLE 7: Measurement of peak area.

```
   >> x=[0:.01:20];
   >> y=exp(-(x-4).^2)+exp(-(x-9).^2)+exp(-(x-13).^2)+exp(-(x-15).^2);
   >> isignal(x,y);
```

This example generates four Gaussian peaks with the exact same peak height (1.00) and area (1.77). The first peak (at x=4) is isolated, the second peak (x=9) is slightly overlapped with the third one, and the last two peaks (at x= 13 and 15) are strongly overlapped. To measure the area under a peak using the perpendicular drop method, position the dotted red marker lines straddling the peak at the minimum of the valley between the overlapped peaks.

EXAMPLE 8: Measurement of single peak with random noise spikes. Compare smoothing vs spike filter (**M** key). Alternatively, use the slew rate limit (~ key) to reduce spike amplitude.

```
   >> x=-5:.01:5;
   >> y=exp(-(x).^2);for n=1:1000,if randn()>2,y(n)=rand()+y(n);end,end;
   >> isignal(x,y);
```


iSignal 4. No baseline correction. Press K for keyboard commands
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0.9
0.92
0.94
0.96
0.98
1
1.02
y: 0.0404 at 0.96234
P/P: 0.693
Area: 0.000506
Std. Dev.: 0.16
Smooth: S, A/Z   Derivatives: D   Peak Meas: P   Spectrum: Shift-S   lin/log: H   Baseline mode: T
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Linear y
0
0.5
1
1.5
2 point Rect. smooth. Ends: 0
Der: 0
Slew: 0
Median: 0

EXAMPLE 9: The example on the right shows a 1.58 second duration audio recording of the phrase "Testing, one, two, three" recorded at 44000 Hz, saved in WAV format, loaded into iSignal and zoomed in on the "oo" sound in the word "two". Press the **Spacebar** to play the selected sound; press **Shift-S** to display the frequency spectrum of the selected region. Press **Shift-R** and type 44000 to set the sampling rate.

```
>> v=wavread('TestingOneTwoThree.wav');
>> t=0:1/44001:1.5825;
>> isignal(t,v(:,2));
```

It's interesting to experiment with the effect of smoothing, differentiation, and interpolation on the sound of this speech, which will change timbre of the voice but has little *effect on the intelligibility*, because the frequency components of the sounds are not shifted in pitch or time but merely changed in amplitude by smoothing and differentiation. In fact, recorded speech can typically survive digitization, compression, truncation, transmission over long distances, and playback via tiny speakers without significant loss of intelligibility.

ISignal 5.3 keyboard controls

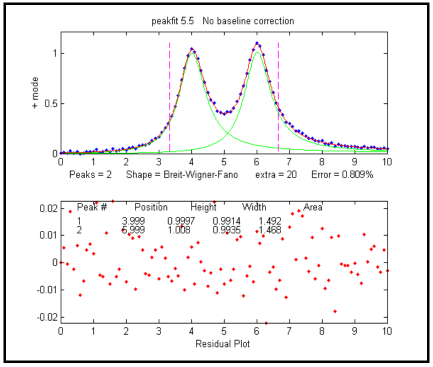
```
Pan left and right........... Coarse pan: < and >
                                Fine pan: left and right cursor arrows
                                Nudge one point left or right: [ and ]
Zoom in and out.............. Coarse zoom: / and "
                               Fine zoom: up and down cursor arrows
Reset pan and zoom........... ESC (resets to initial default values)
Select entire signal.........Ctrl-A  Zooms out to entire signal
Display Grid (on/off).........Shift-G Temporarily displays grid on plots
Adjust smooth width.......... A,Z (A=>more smoothing, Z=>less smoothing)
Adjust smooth type........... S (Cycles through None, Rectangular, Triangle,
                                 Gaussian, Savitzky-Golay)
Toggle smooth ends........... X Flips between 0=ends zeroed  1=ends smoothed
Adjust derivative order...... D/Shift-D (Increase/Decrease derivative order)
Toggle peak sharpening....... E (flips between 0=OFF 1=ON)
Sharpening for Gaussian...... Y  Set sharpen settings for Gaussians
Sharpening for Lorentzian.... U  Set sharpen settings for Lorentzians
Adjust sharp1................ F,V  F=>sharper, V=>less sharpening
Adjust sharp2................ G,B  G=>sharper, B=>less sharpening
Slew rate limit (0=OFF)...... ~  Largest allowed y change between points
Spike filter width (0=OFF)... M  spike filter eliminates sharp spikes
Toggle peak labeling......... P  Labels center peak in upper window
Fits peak in upper window.... Shift-F (Asks for shape, number of peaks, etc)
Fit polynomial...............Shift-o  Fits polynomial to data in upper panel
Toggle Spectrum mode on/off.. Shift-S (Shift-A and Shift-X to change axes)
Peak labels on spectrum.......Shift-Z (on frequency spectrum or periodogram)
Display Waterfall spectrum....Shift-W in mesh, surf, contour, or pcolor form
Click graph to print out x,y..Shift-C  Click graph to print coordinates
Toggle overlay mode.......... L  Overlays original signal in upper window
Toggle log y mode............ H  Display semilog y plot in lower window
Select baseline mode......... T  no, linear, quadratic, or flat baseline mode
Restores original signal..... Tab key resets to original signal and modes
Baseline subtraction......... Backspace, then click baseline at 8 points
Restore background........... \  to cancel previous background subtraction
Invert signal................ -  Invert (negate) the signal (flip + and -)
Remove offset................ 0  (zero) set minimum signal to zero
Trim region to zero.......... ;  Sets selected region to zero.
Absolute value................+  Computes absolute value of entire signal
Condense signal.............. C  Condense oversampled signal by factor of N
Interpolate signal........... i  Interpolate (re-sample) to N points
Print keyboard commands...... K  Prints this list
Print signal report.......... Q  Prints signal info and current settings
Print iSignal arguments...... W  Prints iSignal (with current arguments)
Save output to disk.......... O  Save .mat file containing processed signal and, in
                                 Spectrum Mode, the frequency spectrum.
Play signal as sound..........Spacebar or Shift-P Play through computer sound system.
Set sound sample rate.........Shift-R for the Shift-P command.
Switch to ipf.m...............Shift-Ctrl-F  Transfer current signal to ipf.m
Switch to iPeak...............Shift-Ctrl-P  Transfer current signal to ipeak.m
```

5. Peak Fitter

This section describes Matlab and Octave peak fitting program for time-series signals that uses an unconstrained non-linear optimization algorithm (Page 54). The objective is to determine whether your signal can be represented as the sum of fundamental underlying peaks shapes. The program accepts signals of any length, including those with non-integer and non-uniform x-values and fits any number of peaks at a time with 37 selectable peak shapes. (To add new peak shapes, see http://terpconnect.umd.edu/~toh/spectrum/InteractivePeakFitter.htm#NewShape).

a. Command line function: peakfit.m, for Matlab or Octave (Version 7.6)

Peakfit.m is a user-defined command-line window peak fitting function, usable from a remote terminal. It is written as a self-contained Matlab/Octave function in a single m-file. The screen display is shown on the right; the upper panel shows the data as blue dots, the combined model as a red line (ideally overlapping the blue dots), and the model components as green lines. The residuals are shown in the lower panel. Download peakfit.m and related files from **http://tinyurl.com/cey8rwh**. The peakfit.m function can also be accessed by the keypress-operated interactive functions **ipf** (page 95), **iPeak** (page 78) and **iSignal** (page 85).



```
Peak #      Position     Height      Width      Area
1           3.999        0.9997      0.9914     1.492
2           5.999        1.008       0.9935     1.468
```

As a command line function, peakfit has flexible input argument requirements:

```
peakfit(S);
```

Performs an iterative least-squares fit of a single Gaussian peak to the entire data matrix "**S**", which has X values in row 1 and Y values in row 2 (e.g. [x y]) or which may be a single signal vector.

```
peakfit(S,center,window);
```

Fits a single Gaussian peak to a portion of the matrix "**S**" centered on the x-value "center" and has width "window" (in x units). All input arguments (except the signal itself) can be replaced by zeros to use their default values.

```
peakfit(S, center, window, NumPeaks);
```

"NumPeaks" = number of peaks in the model (Any positive integer, 1 if not specified).

```
peakfit(S,center,window,NumPeaks,peakshape);
```

Specifies the peak shape of the model: "peakshape" = 1-37. (**1**=unconstrained Gaussian, **2**=unconstrained Lorentzian, **3**=logistic *distribution*, **4**=Pearson, **5**=exponentially broadened Gaussian; **6**=equal-width Gaussians, **7**=equal-width Lorentzians, **8**=exponentially broadened equal-width Gaussians, **9**=exponential pulse, **10**=up-sigmoid (logistic *function*), **11**=fixed-width Gaussians, **12**=fixed-width Lorentzians, **13**=Gaussian/Lorentzian blend; **14**=bifurcated Gaussian, **15**=Breit-Wigner-Fano; **16**=Fixed-position Gaussians; **17**=Fixed-position Lorentzians; **18**= exponentially broadened Lorentzian; **19**=alpha function; **20**=Voigt profile; **21**=triangle; **23**=down-sigmoid; **25**=lognormal; **26**=slope (see Example 12b, page 92); **28**=polynomial (Example 26, page 95); **29**=articulated linear segmented (see Example 26, page 95); **30**= unconstrained Voigt; **31**= unconstrained exponentially broadened Gaussian; **32**= unconstrained Pearson; **33**= unconstrained Gaussian/Lorentzian blend; **34**=fixed-width Voigt; **35**=fixed-width Gaussian/Lorentzian blend; **36**=fixed-width exponentially-broadened Gaussian; **37**=fixed-width Pearson.

Note 1: Shapes 11, 12, 16, 17 and 34 require the the fixed parameter be *specified* in the 10th input argument. **Note 2:** "peakshape" can be a *vector of different shapes for each peak*, e.g. [1 2 1] for a Gaussian, Lorentzian, Gaussian sequence. See Example 22 on page 94. **Note 3:** "unconstrained" simply means that the position, height, and width of each peak in the model can vary independently of the other peaks, as opposed to the equal-width, fixed-width, or fixed-position variants. Shapes 4, 5, 13, 14, 15, 18, 20, 34-37 are *constrained* to the same specified shape constant ('extra') specified in the 6th input argument; shapes 30-33 are *unconstrained* in position, width, *and* shape; their shape variables are automatically determined from the data by iterative fitting.

```
peakfit(S,center,window,NumPeaks,peakshape,extra)
```

The 'extra' variable is used in shapes 4, 5, 8, 13, 14,15, 18, and 20 to fine-tune the peak shape. It can be a vector of different values for each peak. See **Example 23** on page 94.

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials);
```

Restarts the fit “NumTrials” times and selects the best one (with lowest fitting error). NumTrials can be any positive integer (default is 1).

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials,start)
```

Specifies the first guesses vector “start” for the starting values of peak positions and widths, e.g. start=[position1 width1 position2 width2 …]. (If **start** is omitted, peakfit estimates its own start).

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials,...
start,autozero)
```

Specifies the *autozero mode* in the 9th argument; it can have 4 values: 0, 1, 2, or 3: mode **0** (default) does *not* subtract baseline from data segment; **1** interpolates a *linear* baseline from the edges of the data segment and subtracts it from the signal (assumes that the peak returns to the baseline at the edges of the signal); **2**, like mode 1 except that it computes a *quadratic* curved baseline; **3** corrects for a *flat* baseline without reference to the signal itself (works even if the peak does *not* return to the baseline at the edges). In modes **1** and **2**, the 3rd output argument 'baseline' returns the polynomial coefficients of the baseline; in mode **3**, the baseline value itself is returned as an output argument.

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials,start,...
autozero,fixedparameters)
```

Uses optional 10th input argument to set fixed width or positions in shapes 11, 12, 16, 17, 34-37.

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials,start,...
autozero,fixedparameters,0)
```

Uses optional 11th input argument set to 0 to suppress plotting and printing (default is 1).

```
peakfit(S,center,window,NumPeaks,peakshape,extra,NumTrials,start,...
autozero,fixedparameters,plot,bipolar)
```

'bipolar', optional 12th input argument, is set to 1 to allow negative as well as positive peak heights in the fit. The default is 0, which allows only positive peak heights.

```
peakfit(signal,center,window,NumPeaks,peakshape,extra,NumTrials,...
start,autozero,fixedparameters,plots,bipolar,minwidth)
```

'minwidth' (optional 13th input argument) sets the minimum allowed peak width. The default if not specified is equal to the x-axis interval. Must be a vector of minimum widths, one value for each peak, if the multiple peak shape is chosen. Version 6.2 and later only.

```
peakfit(signal,center,window,NumPeaks,peakshape,extra,NumTrials,...
start,autozero,fixedparameters,plots,bipolar,minwidth,DELTA)
```

'DELTA' (optional 14th input argument) controls the restart variance when NumTrials>1. Default value is 1.0. Larger values give more variance. Version 5.8 and later only.

```
peakfit(signal,center,window,NumPeaks,peakshape,extra,NumTrials,...
start,autozero,fixedparameters,plots,bipolar,minwidth,DELTA,SatPoint)
```

'`SatPoint`' (optional 15th input argument) skips any data points greater than this value, useful for flat top peaks that are clipped or saturated, e.g. by the detector or electronics. Version 6.2 and later.

Optional output arguments:

```
[FitResults,LowestError,Baseline,coeff,residuals,xi,yi,BootstrapErrors]=....
```

1. **FitResults**: a table of model peak parameters, one row for each peak, listing Peak number, Peak position, Height, Width, and Peak area (or, for shape 28, the polynomial coefficients, and for shape 29, the x-axis breakpoints).
2. **GOF (Goodness of Fit)**, a 2-element vector containing the percent fitting error of the best trial fit and the R-squared (R^2, coefficient of determination).
3. **baseline**: the polynomial coefficients of the baseline in linear and quadratic baseline modes **1** and **2** or the value of the constant baseline in flat baseline mode **3** (version 6.1 or later).

4. **coeff**: Coefficients for the polynomial fits (shapes 26, 27, 28).
5. **residual**: the difference between the data and the best fit.
6. **xi**: vector containing 600 interpolated x-values for the model peaks.
7. **yi**: matrix containing the y values of model peaks at each xi.
 Type `plot(xi,yi(1,:))` to plot peak 1 or `plot(xi,yi)` to plot all the peaks.
8. **BootstrapErrors:** a matrix containing bootstrap standard deviations and interquartile ranges for each peak parameter of each peak in the fit.

Example 1: Signal is a single vector: Create a small data set and fit Gaussian model to the data:

```
>> peakfit([1 4 9 14 17 14 9 4 1])
    Peak number  Peak position   Height    Width    Peak area
        1       5       16.75       4.151       72.3
```

Example 2: Signal is a matrix: Fits exp(-x)^2 with a single Gaussian peak model.

```
 >> x=[0:.1:10];y=exp(-(x-5).^2);peakfit([x' y'])
 ans =  1       5       1      1.665      1.7725
```

Example 3: Measurement of very noisy peak with signal-to-noise ratio = 1. (Try several times).

```
>> x=[0:.01:10];y=exp(-(x-5).^2)+randn(size(x));peakfit([x;y])
ans=   1       5.0951       1.0699       1.6668       1.8984
```

Example 4: Fits a noisy two-peak signal with a double Gaussian model (NumPeaks=2).

```
 >> x=[0:.1:10];y=exp(-(x-5).^2)+.5*exp(-(x-3).^2)+.1*randn(1,length(x));
 >> peakfit([x' y'],5,19,2,1,0,1)
  ans =   1     3.0001    0.4948       1.642    0.86504
          2     4.9927    1.0016       1.6597   1.7696
```

Example 5: Fits a portion of the “humps” function, 0.7 units wide, centered on x=0.3, with a single (NumPeaks=1) Pearson function (peakshape=4) with extra=3 (controls shape).

```
 >> x=[0:.005:1];y=humps(x);peakfit([x' y'],.3,.7,1,4,3);
```

Example 6: Creates a data matrix 'smatrix', fits a portion to a two-peak Gaussian model, takes the best of 10 trials. Returns FitResults and FitError.

```
 >> x=[0:.005:1];y=(humps(x)+humps(x-.13)).^3;smatrix=[x' y'];
 >> [FitResults,FitError]=peakfit(smatrix,.4,.7,2,1,0,10)
```

Example 7: As above, but specifies the first-guess position and width of the two peaks, in the order [position1 width1 position2 width2]

```
 >> peakfit([x' y'],.4,.7,2,1,0,10,[.3 .1 .5 .1]);
```

Example 8: As above, returns the vector xi containing 100 interpolated x-values for the model peaks and the matrix yi containing the y values of each model peak at each xi. Type `plot(xi,yi(1,:))` to plot peak 1 or `plot(xi,yi)` to plot all peaks.

```
>> [FitResults,LowestError,residuals,xi,yi]=peakfit(smatrix,.4,.7,2,1,0,10)
```

Example 9: Sets the baseline correction mode (0, 1, 2, or 3) in the last argument.

```
>> peakfit([x' y'],.4,.7,2,1,0,10,[.3 .1 .5 .1],mode);
```

Example 10: Fits a group of three peaks near x=2400 in DataMatrix3 with three equal-width exponentially-broadened Gaussians.

```
>> [FitResults,FitError]=peakfit(DataMatrix3,2400,440,3,8,31,1)
```

Example 11: Example of an unstable fit to a signal consisting of two Gaussian peaks of equal height (1.0). The peaks are too highly overlapped for a stable fit, even though the fit error is small and the residuals are unstructured. Each time you re-generate this signal, it gives a different fit, with the peaks heights varying about 15% from signal to signal.

```
>> x=[0:.1:10]';y=exp(-(x-5.5).^2)+exp(-(x-4.5).^2)+.01*randn(size(x));
[FitResults,FitError]=peakfit([x y],5,19,2,6,0,10)
```

The equal-width Gaussian model (peak shape 6) yields more stable results, but that is justified only if the experiment is legitimately expected to yield peaks of equal width. See page 60-62.

Example 12a: Baseline correction mode 3 (9th input argument), which subtracts a flat baseline automatically without requiring that the signal return to the baseline at the edges. Flat baseline with single Gaussian: position=10, height=1, width=1.66,

```
>> x=8:.05:12;y=1+exp(-(x-10).^2);
>> [FitResults,FitError,Baseline]=peakfit([x;y],0,0,1,1,0,1,0,3)
  FitResults =  1          10      0.99999      1.6651      1.7641
  FitError = 0.0012156
  Baseline= 0.99985
```

Example 12b: Same signal, using a 2-peak fit with one Gaussian and one "slope" (shape 26).

```
>> peakfit([x;y],0,0,2,[1 26],[1 1],1,0)
```

Example 13: Same as example 4, with 2 fixed-width Gaussians (shape 11), width=1.666 for both.

```
>> x=[0:.1:10];y=exp(-(x-5).^2)+.5*exp(-(x-3).^2)+.1*randn(size(x));
>> [FitResults,FitError]=peakfit([x' y'],0,0,2,11,0,0,0,0,[1.666 1.666])
FitResults = 1       3.9943      0.49537        1.666      0.87849
             2       5.9924      0.98612        1.666       1.7488
```

Example 14: Peak area measurements. Same as the example in the figure on page 34. All four peaks have the same theoretical peak area (1.772) and can be fit together in one fitting operation using a 4-peak Gaussian model, with only rough estimates of the first-guess positions and widths. The measurements are much more accurate than the perpendicular drop method:

```
>> x=[0:.01:18];
>> y=exp(-(x-4).^2)+exp(-(x-9).^2)+exp(-(x-12).^2)+exp(-(x-13.7).^2);
>> peakfit([x;y],0,0,4,1,0,1,[4 2 9 2 12 2 14 2],0,0)
         Peak#    Position       Height       Width          Area
ans=       1           4            1       1.6651       1.7725
           2           9            1       1.6651       1.7725...
```

This works well even in the presence of substantial amounts of random noise:

```
>> x=[0:.01:18];y=exp(-(x-4).^2)+exp(-(x-9).^2)+exp(-(x-12).^2)+...
exp(-(x-13.7).^2)+.1.*randn(size(x));
>> peakfit([x;y],0,0,4,1,0,1,[4 2 9 2 12 2 14 2],0,0)
ans=       1       4.0086      0.98555       1.6693       1.7513
           2       9.0223       1.0007        1.669       1.7779...
```

Sometimes experimental peaks are effected by exponential broadening, which does not by itself change the true peak areas, but does shift peak positions and increases peak width, overlap, and asymmetry, making it harder to separate the peaks. **Peakfit.m** (and **ipf.m**) have an exponentially-broadened Gaussian peak shape (shape #5) that works well in those cases, recovering the original peak positions, heights, and widths:

```
>> y1=ExpBroaden(y',-50);
>> peakfit([x;y1'],0,0,4,5,50,1,[4 2 9 2 12 2 14 2],0,0)
ans=       1           4            1       1.6651       1.7725
           2           9            1       1.6651       1.7725...
```

Example 15: Prints out a table of peak parameter errors, determined by the bootstrap procedure (page 42). See DemoPeakfitBootstrap.m for a self-contained demo of this function.

```
x=0:.05:9;y=exp(-(x-5).^2)+.5*exp(-(x-3).^2)+.01*randn(1,length(x));
Results,FitError,Baseline,Start,xi,yi,PErrors]=peakfit([x;y],0,0,2,6,0,1,0,0,0);

Peak #1          Position    Height       Width       Area
Bootstrap Mean: 2.9987      0.49717     1.6657      0.88151
Percent RSD:    0.13175     0.37726     0.15769     0.37047
Percent IQR:    0.18271     0.55234     0.19502     0.50658

Peak #2  ... etc
```

Example 16:

```
 >> x=[0:.1:10];y=exp(-(x-4).^2)+.5*exp(-(x-5).^2)+.01*randn(size(x));
 >> [FitResults,FitError]=peakfit([x' y'],0,0,1,14,45,10,0,0,0)
 FitResults = 1       4.2028       1.2315        4.077       2.6723
 FitError = 0.84461
```

Fits a slightly asymmetrical peak with a bifurcated Gaussian (shape 14). The 'Extra' argument (=45) controls the peak asymmetry (50 is symmetrical).

Example 17:

```
 >> x=[0:.1:10]';y=exp(-(x-5).^2);FitResults=peakfit([x y],0,0,1,1,0,0,0,0,0,0)
```

Returns output arguments only, no plotting or command window printing (11th input argument = 0).

Example 18. Same as example 4, but with fixed-position Gaussian (shape 16), positions=[3 5].

```
>> x=[0:.1:10];y=exp(-(x-5).^2)+.5*exp(-(x-3).^2)+.1*randn(size(x));
>> [FitResults,FitError]=peakfit([x' y'],0,0,2,16,0,0,0,0,[3 5])
```

```
FitResults = 1             3      0.49153       1.6492      0.86285
             2             5      1.0114        1.6589      1.786
FitError = 8.2693
```

Example 19. **"**Humps" function fit with two Voigt profiles, flat baseline mode 3.

```
>> [FitResults,FitError]=peakfit(humps(0:.01:2),71,140,2,20,1.7,1,...
[31 4.7 90 8.8],3)
 FitResults = 1       31.047        96.762        4.6785        2550.1
              2        90.09        22.935        8.8253        1089.5
 FitError = 0.80501
```

Example 20. peakfitdemob.m Measures the heights of three weak Gaussian peaks (true heights 1, 2, and 3), buried in a very strong baseline, plus noise. The peakfit function actually fits *four* peaks, treating the baseline as an 4th peak whose first-guess peak position is negative. (You can "stress" this method by changing the peak parameters in lines 11, 12, and 13 and see if the peakfit function will successfully track those changes and give accurate results).

Example 21. 12th input argument (+/- mode) set to 1 (bipolar) to allow negative as well as positive peak heights. (Default is 0)

```
 >> x=[0:.1:10];y=exp(-(x-5).^2)-.5*exp(-(x-3).^2)+.1*randn(size(x));
 >> peakfit([x' y'],0,0,2,1,0,1,0,0,0,1,1)
 FitResults = 1   3.1636    -0.5433      1.62    -0.9369
              2   4.9487     0.96859     1.8456   1.9029
 FitError = 8.2757
```

Example 22. Fits humps function to a model consisting of one Lorentzian and one Gaussian peak (5th input argument is a vector = [1 2]).

```
 >> x=[0:.005:1.2];y=humps(x);
 >> [FitResults,FitError]=peakfit([x' y'],0,0,2,[2 1],[0 0])
```

Example 23. Five peaks, five different shapes, all heights = 1, all widths = 3, "extra" vector has values for peaks 4 and 5. Click for graphic.

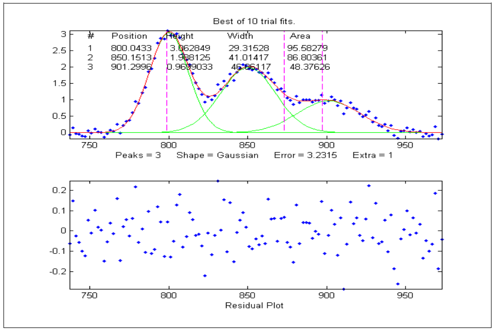
```
>> x=0:.1:60; y=modelpeaks2(x, [1 2 3 4 5], [1 1 1 1 1], [10 20 30 40 50], [3 3
3 3 3], [0 0 0 2 -20])+.01*randn(size(x));
>>  peakfit([x' y'],0,0,5, [1 2 3 4 5], [0 0 0 2 -20])
```

You can also use this technique to create models with all the same shapes but with different values of 'extra' using a vector of 'extra' values, or (in version 5.7) with different minimum width restrictions by using a vector of 'minwidth' values as input argument 13.

Example 24. Minimum width limit (13th input argument)

```
>> x=1:30;y=gaussian(x,15,8)+.05*randn(size(x));
```

No constraint (minwidth=0): `peakfit([x;y],0,0,5,1,0,10,0,0,0,1,0,`**`0`**`);`
Widths constrained to values 7 or above: `peakfit([x;y],0,0,5,1,0,10,0,0,0,1,0,`**`7`**`);`



```
#   Position    Height      Width      Area
1   800.0433    3.062849    29.31528   95.58279
2   850.1513    1.938125    41.01417   86.80361
3   901.2996    0.9699033   46.86117   48.37626
```

Example 25. DemoPeakFit.m generates an overlapping Gaussian peak signal, adds random noise, fits it with the peakfit.m function (in line 78), repeats this several times (*NumRepeats* in line 20), then compares the peak parameters (position, height, width, and area) of the measurements to their actual values and computes the accuracy and relative standard deviation). You can change any of the initial values in lines 13-30.

Example 26. Polynomial fit (shape 28); NumPeaks = number of linear segments.

```
>> x=[0.3:.005:1.7];y=humps(x);y=y+cumsum(y);peakfit([x' y'],0,0,1,28,6,10,0,0,0,1,1)
```

Example 27. Articulated segmented fit (shape 29); NumPeaks = number of linear segments.

```
>> x=[0.9:.005:1.7];y=humps(x);peakfit([x' y'],0,0,9,29,0,1,0,0,0,1,1)
```

Example 29: NumPeaksTest.m demonstrates one way to determine the minimum number of model peaks needed to fit a set of data, by plotting the fitting error vs the number of model peaks and looking for the point at which the fitting error reaches a minimum.

Example 30: Version 7 and later supports *unconstrained* variable shapes 30-33 that have *three* iterated variables per peak (position, width, *and* shape):

a. Voigt (shape 30): `x=1:.1:30;y=modelpeaks2(x,[13 13],[1 1],[10 20],[3 3],[20 80]);`

`[FitResults,FitError] = peakfit([x;y],0,0,2,30,0,10).`

b. ExpGaussian (shape 31): `load DataMatrix3;[FitResults,FitError] = peakfit(DataMatrix3,1860.5,364,2,31,32.9731,5,[1810 60 30 1910 60 30])`

c. Pearson (shape 32) `x=1:.1:30;y=modelpeaks2(x,[4 4],[1 1],[10 20],[5 5],[1 10]); [FitResults,FitError] = peakfit([x;y],0,0,2,32,0,5)`

d. Gaussian/Lorentzian blend (shape 33): `x=1:.1:30;y=modelpeaks2(x,[13 13],[1 1],[10 20],[3 3],[20 80]); [FitResults,FitError]=peakfit([x;y],0,0,2,`**`33`**`,0,5)`

Example 31: Version 7.4. Fixed-height Gaussians (heights vector specified in 10th input argument).

```
>> x=[0:.1:10];y=exp(-(x-5).^2)+.5*exp(-(x-3).^2)+.1*randn(size(x));
>> peakfit([x' y'],0,0,2,34,0,0,0,0,[.5 1])
```

Example 32: Version 7.6. Fixed-width 50% Gaussian/Lorentzian blend, shape 35.

```
>> x=0:.1:10;y=GL(x,4,3,50)+.5*GL(x,6,3,50)+.1*randn(size(x));
>> [FitResults,FitError]=peakfit([x;y],0,0,2,35,50,1,0,0,[3 3])
```

Compare to variable width shape 13: ...`peakfit([x;y],0,0,2,`**`13`**`,50,1)`

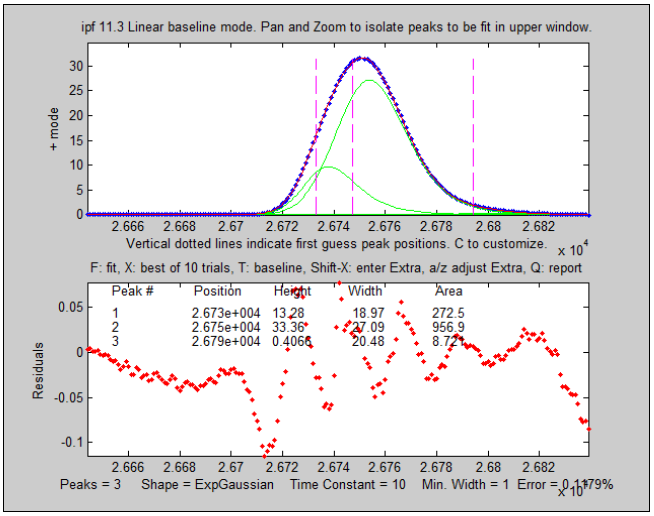
DemoPeakFitTime.m is a simple script that demonstrates how to apply multiple curve fits to a signal that is changing with time. The signal (x,y) contains two noisy Gaussian peaks (similar to the illustration above) in which the peak position of the *second* peak increases with time and the other parameters remain constant (except for the noise). The script creates a matrix of 100 noisy signals (on line 5) each containing two Gaussian peaks where the position of the second peak changes with time (from x=6 to 8) and the first peak remains the same. Then it fits a 2-Gaussian model to each of those signals (on line 8), displays the signals and fits graphically with time as a kind of animation, then plots the measured peak position of the two peaks vs time on line 12.

Which to use: Peakfit or iPeak? You can download some Matlab demos that compare Peakfit.m with iPeak.m for signals with a few peaks and signals with many peaks. DemoPeakfitBootstrap demonstrates the ability of peakfit to compute estimates of the errors in the measured peak parameters. These are self-contained demos that include all required Matlab functions. Just place them in your path and click Run or type their name at the command prompt. You can download all these demos together in http://terpconnect.umd.edu/~toh/spectrum/idemos.zip.

findpeaksfit.m (page 76) is combination of findpeaksG.m (page 74) and peakfit.m (page 90). It uses the number of peaks and the positions and widths determined by findpeaks as input for the peakfit.m function, which then fits the entire signal with the specified peak model. This yields better values than findpeaks alone, because peakfit fits the entire peak, not just the top part, and it handles non-Gaussian and overlapped peaks. It fits only those peaks that are found by findpeaks. See the demo.

b. Interactive version: ipf.m, for Matlab

ipf.m is an interactive peak fitter that uses keyboard commands and the mouse cursor. The syntax is **ipf(x,y)**, where x and y are the independent and dependent variables of your data set, or **ipf(M)** where "**M**" is a matrix that has x values in row 1 and y values in row 2. It shows the entire signal in the lower panel and the selected region in the upper panel (adjusted by the same cursor controls keys as in iPeak and iSignal). After performing a fit (figure on the right), the upper panel shows the data as **blue dots**, the total model as a **red line**, and the model components as **green lines**; the lower panel shows the *residuals* (the difference between the data and the total model).



ipf 11.3 Linear baseline mode. Pan and Zoom to isolate peaks to be fit in upper window.

Vertical dotted lines indicate first guess peak positions. C to customize.

F: fit, X: best of 10 trials, T: baseline, Shift-X: enter Extra, a/z adjust Extra, Q: report

```
Peak #    Position      Height    Width    Area
1         2.673e+004    13.28     18.97    272.5
2         2.675e+004    33.36     27.09    956.9
3         2.679e+004    0.4066    20.48    8.721
```

Peaks = 3   Shape = ExpGaussian   Time Constant = 10   Min. Width = 1   Error = 0.1179%

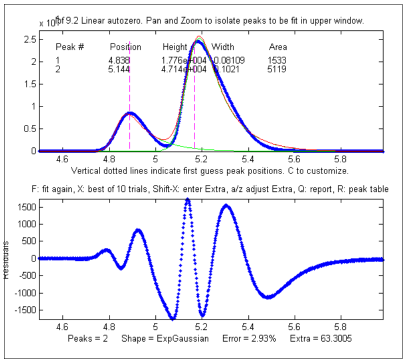
Example 1: Test with pure Gaussian function, default settings of all input arguments.

```
>> x=[0:.1:10];y=exp(-(x-5).^2);ipf(x,y)
```

Here the fit is essentially perfect. However, the peak area (the last fit result reported) includes only the area within the upper window, so it varies with the pan and zoom settings. (If there were noise in the data or if the model were imperfect, then *all* the fit results will depend on the pan and zoom settings).

Example 2: `x=[0:.005:1];y=humps(x).^3;ipf(x,y)` fits the entire signal; `ipf(x,y,0.335,0.39)` focuses on first peak; `ipf(x,y,0.91,0.18)` focuses on second peak.

Some examples with experimental data

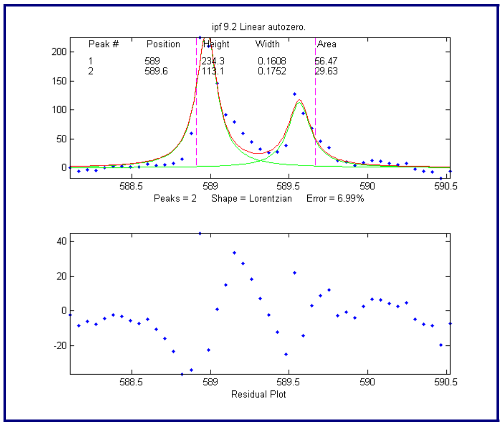


```
Peak #      Position      Height        Width       Area
1           4.838         1.776e+004    0.08109     1533
2           5.144         4.714e+004    0.1021      5119
```

Example 1: In the example shown on the right, a sample of room air is analyzed by gas chromatography (data source: reference 48). The resulting chromatogram shows two overlapping peaks, the first for oxygen and the second for nitrogen. The area under each peak is expected to be proportional to the gas composition. Because the peaks are visibly asymmetric, I chose an exponentially-broadened Gaussian model (a commonly encountered peak shape in chromatography) to fit the data, using **ipf.m** and adjusting the exponential term with the **A** and **Z** keys to get the best fit. The results, shown in the **ipf.m** screen on the left and in the **Q**-key report below, show that the peak areas are in a ratio of 23% and 77%. This is fairly close to the actual 21% and 78% composition, and the results would have been even more accurate if argon were included and if the areas were calibrated for the different detector responses of nitrogen and oxygen.

```
Percent Fitting Error = 2.9%    Elapsed time = 11.5 sec.
Peak#  Position     Height     Width       Area
  1      4.8385      17762    0.081094    1533.2
  2      5.1439      47142    0.10205     5119.2
```

Example 2. In this example, **ipf.m** is used to examine an experimental high-resolution atomic emission spectrum in the region of the well-known spectral lines of the element sodium. Two lines are found there (figure on the right), and when fit to a Lorentzian model, the peak wavelengths are determined to be 588.98 nm and 589.57 nm. Compare this to the ASTM recommended wavelengths for sodium (588.995 and 589.59 nm) and you can see that the error is no greater than 0.02 nm (*less than the interval between the data points*, 0.05 nm), despite the fact that the fit is not very good because the peaks shapes are rather distorted (perhaps by self-absorption).



```
Peak #     Position     Height     Width      Area
1          589          234.3      0.1608     56.47
2          589.6        113.1      0.1752     29.63
```

```
Percent Fitting Error 6.9922%
Peak#  Position    Height      Width       Area
1      588.98      234.34      0.16079     56.473
2      589.57      113.18      0.17509     29.63
```

These results show that the wavelength calibration of the instrument on which these experimental data were obtained is excellent. In general, *peak position is by far the most accurately measurable parameter in peak fitting*. The bootstrap standard deviation estimates for both wavelengths is 0.015 nm, so using the 2 x standard deviation rule-of-thumb would have predicted a probable error within 0.03 nm.

Operating instructions for ipf.m (Version 11.3)

1. Make sure you have the most recent version of **ipf.m**. At the command line, type **ipf(x, y)**, (x = independent variable, y = dependent variable) or **ipf(M)** where "**M**" is a 2 x n or n x 2 matrix that has x values the first row or column. Or if you have only one signal vector y, type **ipf(y)**. Optionally, you can specify the initial focus by adding "center" and "window" values as additional input arguments, where 'center' is the desired x-value in the center of the upper window and "window" is the desired width of that window: `ipf(x,y,center,window) or ipf(M,center,window).`
2. Use the four **cursor arrow keys** on the keyboard to pan and zoom the signal to isolate the peak or group of peaks that you want to fit in the upper window. (Use the **<** and **>** and **?** and **"** keys for coarse pan and zoom and the square bracket keys **[** and **]** to nudge one point left and right). *The curve fitting operation applies only to the segment of the signal shown in the top plot*. The bottom plot shows the entire signal. Try not to get any undesired peaks in the upper window or the program may try to fit them. Or press **Ctrl-A** to select the *entire* signal.
3. Press the number keys (**1**– **9**) to choose the number of model peaks, that is, the minimum number of peaks that you think will suffice to fit this segment of the signal. For more than 9 peaks, press **0** , type the number of peaks, and press **Enter**.

4. Select the desired model peak shape by pressing the '-' key and selecting the desired shape by number from the menu that is displayed. Or you can also type in a *vector* of shape numbers with a different shape for each peak, e.g. [1 2 1]. Or you can select single-shape models in one keystroke by pressing the following keys: unconstrained Gaussian (lower case **g**), equal-width Gaussians (lower case **h**), fixed-width Gaussians (**Shift-G**), fixed-position Gaussians (**Shift-P**), exponentially-broadened Gaussian (**e**), exponentially-broadened equal-width Gaussians (**j**); bifurcated Gaussian (**Shift-H**), unconstrained Lorentzian (lower case **L**), exponentially-broadened Lorentzian (**Shift-E**); fixed-width Lorentzians (**Shift-L**), fixed-position Lorentzians (**Shift [**), equal-width Lorentzians (lower case **;**), Breit-Wigner-Fano resonance (**Shift-B**), Voigt profile (**Shift-V**), triangular (**Shift-T**), logistic distribution (lower case **o**), Pearson (**p**), exponential pulse (**u**), alpha function (**Shift-U**), logistic function or up-sigmoid (**s**), down-sigmoid (**Shift-D**), and Gaussian/Lorentzian blend (`).

   If the peak widths of each group of peaks is expected to be the same, select the equal-width or fixed-width fits (available only for the Gaussian and Lorentzian shapes), which are faster, easier, and much more stable than regular variable-width fits, especially if the number of model peaks is greater than three, because there are fewer variable parameters for the program to adjust.
5. A set of vertical dashed lines are shown on the plot, one for each model peak. Try to fine-tune the **Pan** and **Zoom** keys so that the signal drops down to the baseline at both ends of the upper plot and so that the peaks (or humps) in the signal roughly line up with the vertical dashed lines. This does not have to be exact.
6. If you want to allow *negative* peaks as well as *positive* peaks, press the **+** key to flip to the +/- mode (indicated by the +/- sign in the y-axis label of the upper panel). Press it again to return to the + mode (positive peaks only). You can switch at any time.
7. Press **F** to initiate the curve-fitting calculation. Each time press **F**, another fit of the selected model to the data is performed with slightly different starting values, so that you can judge the stability of the fit *with respect to **starting guesses***. (To judge the stability of the fit *with respect to **noise** in the data*, press **N**. See #16). Keep your eye on the residuals plot and on the "Error" display. Do this several times, trying for the lowest error and the most unstructured random residuals plot. If the fit is unstable, try pressing **X**, which takes longer to compute but may give better results (see #13). At any time, you can refine the signal region to be fit (step 2), change the number or peaks (step 3), peak shape (step 4), or change the baseline correction mode (**T** key) to get a better fit.
8. The model parameters of the last fit are shown the lower window. For example, for a 3-peak fit:

   ```
   Peak#  Position    Height    Width      Area
    1    5.33329    14.8274    0.262253    4.13361
    2    5.80253    26.825     0.326065    9.31117
    3    6.27707    22.1461    0.249248    5.87425
   ```

   The columns are, left to right: the peak number, peak position, peak height, peak width, and the peak area. Press **R** to print this table out in the command window. Peaks are numbered from left to right. (The area of each component peak within the upper window is computed using the trapezoidal method).

   Pressing **Q** prints out a report of settings and results in the command window, like so:

   ```
   Peak Shape = Gaussian
   Positive peaks only
   Flat baseline mode
   Number of peaks = 3
   Fitted range = 5 - 6.64
   Percent Error = 7.4514
   Peak#  Position    Height     Width     Area
   1    5.33329    14.8274    0.262253    4.13361
   … etc
   ```
9. To select the baseline correction mode, press the **T** key repeatedly; it cycles thorough *none*, *linear*, *quadratic*, and *flat* background modes. In linear mode, a straight-line baseline connecting the two ends of the signal segment in the upper panel will be automatically subtracted. In quadratic mode, a parabolic baseline connecting the two ends of the signal segment in the upper panel will be automatically subtracted. (Use quadratic mode if the baseline is curved). Use the

flat mode if the signal does not return to the baseline at the ends.

10. If you prefer to set the baseline manually, press the **B** key, then click on the baseline to the LEFT of the peak(s), then click on the baseline to the RIGHT of the peak(s). The new baseline will be subtracted and the fit re-calculated. (The new baseline remains in effect until you use the pan or zoom controls). Alternatively, you may use the *multi-point background correction* for the entire signal: press the **Backspace** key, type in the desired number of background points and press the **Enter** key, then click on the baseline starting at the left of the lowest x-value and ending to the right of the highest x-value. Press \ to restore the previous background to start over.

11. If the peaks are not approximately lined up with the dotted magenta marker lines, it may help to manually ***customize*** the first-guess peak positions: press **C**, then click on your estimates of the peak positions in the upper graph, once for each peak. The fit is automatically performed after the last click. Peaks are numbered in the order clicked. Or type **Shift-C** to type in or paste in the start vector, e.g. "[pos1 wid1 pos2 wid2 ...]". (The custom start values remain in effect until you change the number of peaks or use the pan or zoom controls).

12. The **A** and **Z** keys control the "extra" parameter that is used only if you are using Pearson, exponentially-broadened Gaussian and Lorentzian (ExpGaussian and ExpLorentzian), bifurcated Gaussian, Breit-Wigner-Fano, Gaussian/Lorentzian blend, or Voigt models. For the Pearson shape, an "extra" value of 1.0 gives a Lorentzian shape, a value of 2.0 gives a shape roughly half-way between a Lorentzian and a Gaussian, and a larger values give a nearly Gaussian shape. For the exponentially broadened Gaussian shapes, "extra" controls the exponential "time constant" (expressed as the number of points). For the Gaussian/Lorentzian blend and the bifurcated Gaussian and Lorentzian shapes, "extra" controls the peak asymmetry (a values of 50 gives a symmetrical peak). You can also enter an initial value of "extra" directly by pressing **Shift-X** , typing in a value (or vector, for multiple shape models), and pressing the **Enter** key. Then you can adjust this value using the **A** and **Z** keys (hold down the **Shift** key to fine tune). Seek to minimize the Error % or set it to a previously-determined value. (Note: if fitting multiple over-lapping peaks with an "extra" parameter, it's better to fit a *single* peak first, to get a rough value for the "extra" parameter, then just fine-tune that parameter for the multi-peak fit if necessary).

13. For difficult fits, press **X,** which restarts the fit 10 times with slightly different first guesses and takes the one with the lowest fitting error. This also resets the starting points for subsequent fits, so pressing **X** repeatedly will usually converge on the best fit. (You can change the number of trials,"NumTrials", in or near line 224 in **ipf.m**; the factory default is 10). As always: equal-width Gaussian (**H** key) and Lorentzian (**;** key) shapes, and exponentially-broadened equal-width Gaussian (**J** key) peak shapes and fixed-width Gaussian (**Shift-G** key) shapes Lorentzian (**Shift-L** key) shapes, are faster, easier, and more stable than regular variable-width fits, so use equal-width fits whenever the peak widths are expected to be equal or nearly so, or fixed-width fits when the peak widths are known.

14. Press **Y** to display the entire signal full screen without cursors, with the last fit displayed in green. The residual is displayed in red, on the same y-axis scale.

15. Press **M** to switch back and forth between *log* and *linear* modes. In log mode, the y-axis of the upper plot switches to semilog-y, and log(model) is fit to log(y), which may be useful if the peaks vary greatly in amplitude. The first-guess values and 'extra' values do not change.

16. Press the **D** key to print out a table of model data in the command window (x, y1, y2, ..., where x is the column of x values of the fitted region and the y's are the y-values of each component, one for each peak in the model. You can then Copy and Paste this table into a spreadsheet or data plotting program of your choice.

17. Press **W** to print out the **ipf.m** function in the command window with the current values of 'center' and 'window' as input arguments. This is useful when you want to return to that specific data segment later. Also prints out **peakfit.m** with all input arguments, including the last best-fit values of the first guess vector. You can copy and Paste the displayed text into your own code.

18. ipf.m can estimate the expected variability of the peak position, height, width, and area from the signal, by using the *bootstrap sampling method* (see page 41 - 42). This involves extracting 100 bootstrap samples from the signal, fitting each of those samples with the model, then computing the percent relative standard deviation (RSD) and the interquartile range (IQR) of the parameters of each peak. Basically this method calculates weighted fits to a single data set, using a different set of different weights for each fit. (The process is computationally intensive can take several minutes to complete, especially if the number of peaks in the model is high or if you are using an exponentially broadened shape).

To activate this process, press the **V** key. It first asks you to type in the number of “best-of-x” trial fits per bootstrap sample (the default is 1, but you may use a higher number here if the fits are too unstable). The results are displayed in the command window. For example, for a three-peak fit (to the same 3 peaks used by the Demoipf demo script described in the next section):

```
>> Number of fit trials per bootstrap sample (0 to cancel): 10
  Computing bootstrap sampling statistics....May take several minutes.

  Peak #1         Position   Height      Width      Area
  Bootstrap Mean: 800.5387   2.969539    31.0374   98.10405
  Bootstrap STD:  0.20336    0.02848      0.5061    1.2732
  Bootstrap IQR:  0.21933    0.027387     0.5218    1.1555
  Percent RSD:    0.025402   0.95908      1.6309    1.2978
  Percent IQR:    0.027398   0.92226      1.6812    1.1778

 (Peak #2, etc..... for all other peaks)
  Elapsed time is 98.394381 seconds.
  Min/Max Fitting Error of the 100 bootstrap samples: 3.0971/2.5747
```

Observe that the percent RSD and IRQ of the peak positions are lowest, followed by heights and widths and areas. This is a typical pattern. Also, remember that these results depend on the assumption that the noise in the signal is unsmoothed and is representative of the average noise in repeated measurements. If the number of data points in the signal is small, these estimates can be very approximate. Don't smooth the data beforehand; that will cause the bootstrap to underestimate the variability drastically.

One pitfall with the bootstrap method when applied to iterative fits is the possibility that one (or more) of the bootstrap fits will go astray, that is, will result in peak parameters that are wildly different from the norm, causing the estimated variability of the parameters to be too high. For that reason, in ipf 8.7, the interquartile range (IQR) as well as the standard deviation (STD) is reported. The IQR is more robust to outliers. For a normal distribution, the interquartile range is equal to 1.34896 times the standard deviation. But if one or more of the bootstrap sample fits fails, resulting in a distribution of peak parameters with large outliers, the STD will be much greater than the IQR. In that case, a more realistic estimate of standard deviation without the outliers is IRQ/1.34896.

It's best to increase the fit stability by choosing a better model (for example, using an equal-width of fixed-width model, if appropriate), adjusting the fitted range (pan and zoom keys), the background subtraction (**T** or **B** keys), or the start positions (**C** key), and/or selecting a higher number of fit trials per bootstrap (which will increase the computation time). As a quick test of bootstrap fit stability, the **N** key will perform a single fit to a single random bootstrap sample and plot the result; do that several times to see whether the bootstrap fits are stable enough to be worth computing the statistics of 100 bootstrap samples.

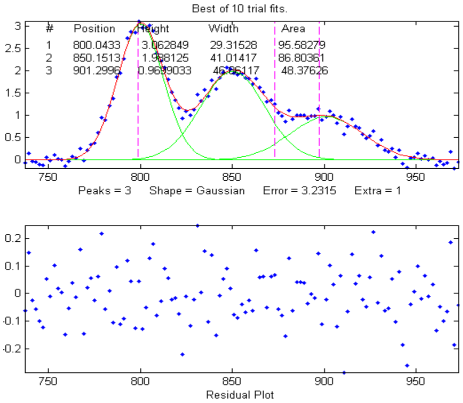
Note: it's normal for the stability of the bootstrap sample fits (**N** key) to be poorer than the full-sample fits (**F** key) because the latter includes only the variability caused by changing the starting positions for one set of data and noise, whereas the **N** and **V** keys aim to include the variability caused by the random noise in the sample by fitting bootstrap sub-samples. Moreover, the best estimates of the measured peak parameters are those obtained by the normal fits of the full signal (**F** and **X** keys), not the means reported for the bootstrap samples (**V** and **N** keys), because there are more independent data points in the full fits and because the bootstrap means are influenced by the outliers that occur more commonly in the bootstrap fits. Use the bootstrap results for estimating the variability of the peak parameters, not for estimating their mean values. The **N and V keys** are also very useful way to determine if you are using too many peaks in your model; *superfluous peaks will be very unstable when N is press repeatedly* and will have much higher standard deviation of its peak height when the **V key** is used.

19. Shift-o fits a simple *polynomial* (linear, quadratic, cubic, etc) to the upper panel segment and displays the polynomial coefficients (in descending powers) and R^2. See page 37.

20. If some peaks are saturated (clipped at a maximum height), you can make the program ignore the saturated points by pressing **Shift-M** and entering the maximum Y values to keep.

Demoipf.m

Demoipf.m is a demonstration script for ipf.m, with a built-in simulated signal generator. The true values of the simulated peak positions, heights, and widths are displayed in the Matlab command window, for comparison to the Fit Results obtained by peak fitting. The default simulated signal contains six independent groups of peaks that you can use for practice: a triplet near x = 150, a singlet at 400, a doublet near 600, a triplet near 850, and two broad single peaks at 1200 and 1700. Run this demo and see how close to the actual true peak parameters you get.



```
#   Position    Height      Width      Area
1   800.0433    3.062849    29.31528   95.58279
2   850.1513    1.988125    41.01417   86.80361
3   901.2996    0.9699033   46.86117   48.37626
```

The useful thing about a simulation like this is that you can get a feel for the accuracy of peak parameter measurements, that is, the difference between the true and measured values of peak parameters. To run it, place both **ipf.m** and **Demoipf.m** in the Matlab path, then type **Demoipf** at the Matlab command prompt. A ZIP file containing peakfit.m, DemoPeakFit.m, ipf.m, Demoipf.m, and some data for testing is available at from http://terpconnect.umd.edu/~toh/spectrum/ipf11.zip .

An example of the use of this script is shown above. Here we focus in on the 3 fused peaks located near x=850. The *true* peak parameters (before the addition of the noise) are:

```
    Position   Height     Width     Area
     800          3         30      95.808
     850          2         40      85.163
     900          1         50      53.227
```

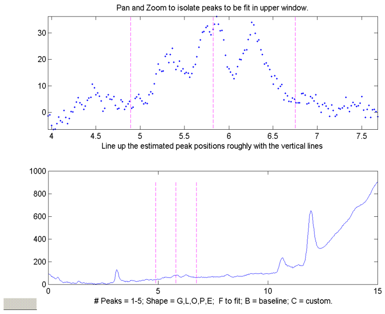
When these peaks are isolated in the upper window and fit with three Gaussians, the typical measured peak parameters are:

```
    Position   Height     Width       Area
    800.04      3.0628     29.315      95.583
    850.15      1.9881     41.014      86.804
    901.3       0.9699     46.861      48.376
```

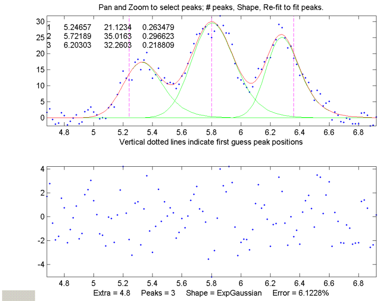
So you can see that the accuracy of the measurements are excellent for peak position, good for peak height, and least good for peak width and area. As expected, the least accurate measurements are for the smallest peak with the poorest signal-to-noise ratio.

Note: The expected standard deviations of these peak parameters can be determined by the bootstrap sampling method (page 41 - 42), as described in the previous section. We would expect that the measured values of the peak parameters (comparing the true to the measured values) would be within about 2 standard deviations of the true values listed above).

Peak identification. You can use the peak identifier function **idpeaktable.m** (page 77, 81) with the peak table **P** that is returned by either **peakfit.m** or **ipf.m**, for identifying peaks according to their peak positions.



In this example with experimental data, pan and zoom controls are used to isolate a segment of a chromatogram that contains three very weak peaks near 5.8 minutes The lower plot shows the whole chromatogram and the upper plot shows the segment. Only the peaks in that segment are subjected to the fit. Pan and zoom are adjusted so that the signal returns to the local baseline at the ends of the segment.



```
1     5.24657     21.1234     0.263479
2     5.72189     35.0163     0.296623
3     6.20303     32.2603     0.218809
```

*Pressing **T** sets the baseline mode to linear, causing the program to compute and subtract a linear baseline between the ends of the upper segment. Pressing **3, E, F** performs a 3-peak exponentially-broadened Gaussian fit. The time constant of the exponential is adjusted by the **A** and **Z** keys. The randomness and lack of obvious structure of the residuals indicates that the fit is as good as possible with this level of noise.*

Execution time

By "execution time" I mean the time it takes for one fit to be performed, exclusive of plotting or printing the results. The major factors that determine the execution time are the peak shape, the number of peaks, and the speed of the computer:

a) The execution time varies greatly (sometimes by a factor of 100 or more) with the *peak shape,* with the exponentially-broadened Gaussian being the *slowest* and the fixed-width Gaussian being the *fastest*. See PeakfitTimeTest2.m and PeakfitTimeTest2a.m. The equal-width and fixed-width shape variations are always faster than the corresponding variable-width models. Unconstrained variable shapes in peakfit 7 that have *three* iterated variables (30: variable-alpha Voigt; 31: variable time constant ExpGaussian; 32: variable shape Pearson; 33: variable Gaussian/ Lorentzian blend) are slower.

b) The execution time typically increases with the *square* of the number of peaks in the model.

c) The execution time can vary over a factor of 4 or 5 or more between different computers, for example, between a small laptop with 1.6 GHz, dual core Athlon CPU and 4 Gbytes RAM, compared to a big desktop with a 3.4 GHz i7 CPU and 16 Gbytes RAM). Run the Matlab "bench.m" benchmark test to see how your computer stacks up compared to other computers.

d) The execution time increases directly with NumTrials in peakfit.m; the "Best of 10 trials" function (**X** key in **ipf.m**) takes about 10 times longer than a single fit.

e) Other factors that influence execution time but are less important are (1) the number of data points in the fitted region (see PeakfitTimeTest3.m) and (2) the starting values (good starting values can reduce execution time slightly; PeakfitTimeTest2.m and PeakfitTimeTest2a.m have examples. (Some of these scripts need DataMatrix2.mat and DataMatrix3.mat).

Note: All these scripts (m-files) and data files (mat-files) can be downloaded from **http://tinyurl.com/cey8rwh**.

Notes concerning the interactive functions ipeak.m, isignal.m, and ipf.m:

(a) Make sure you don't click on the "Show Plot Tools" button in the toolbar above the figure; that will disable normal program functioning. If you do; close the Figure window and start again.

(b) To facilitate transfer of settings from one of these functions to another or to a command-line version, all these functions use the **W** key to print out the syntax of other related functions, with the pan and zoom settings and other numerical input arguments specified, ready for you to Copy, Paste and edit into your own scripts or back into the command window. For example, you can convert an curve fit from **ipf.m** into the command-line **peakfit.m** function; or you can convert a peak finding operation from **ipeak.m** into the command-line **findpeaksG.m** or **findpeaksb.m** or **findpeaksb3.m** functions.

(c) Recent versions of these three programs use the **Shift-Ctrl-S**, **Shift-Ctrl-F**, and **Shift-Ctrl-P** keys to transfer the current signal between i**S**ignal.m, ip**f**.m, and i**P**eak.m, respectively, if you have installed those functions in your Matlab path. Think **S**ignal, **F**it, and **P**eak. This is done via the global variables X and Y.

Ipf 11.2 Keyboard Controls

```
 Pan signal left and right...Coarse: < and >
                             Fine: left and right cursor arrow keys
                             Nudge: [ ]
 Zoom in and out.............Coarse zoom: ?/ and "'
                             Fine zoom: up and down cursor arrow keys
 Select entire signal........Crtl-A (Zoom all the way out)
 Resets pan and zoom.........ESC
 Select # of peaks...........Number keys 1-9, or press 0 key to enter number manually
 Select peak shape from menu _- (minus or hyphen), type number or shape vector, Enter
 Select peak shape by key....g Gaussian
                             h equal-width Gaussians
                             Shift-G fixed-width Gaussians
                             Shift-P fixed-position Gaussians
                             Shift-H bifurcated Gaussians (a,z keys adjust shape)
                             e Exponential-broadened Gaussian
                               (a,z keys adjust broadening)
                             j exponential-broadened equal-width Gaussians
                                 (a,z keys adjust broadening)
                             l Lorentzian
                             :; equal-width Lorentzians
                             Shift [ fixed-position Lorentzians
                             Shift-E Exponential-broadened Lorentzians
                                 (a,z keys adjust broadening)
                             Shift-L Fixed-width Lorentzians
                               (a,z keys adjust broadening)
                             o LOgistic distribution (Sigmoid = logistic function)
                             p Pearson (a,z keys adjust shape)
                             u exponential pUlse  y=exp(-tau1.*x).*(1-exp(-tau2.*x))
                             Shift-U Alpha: y=(x-tau2)./tau1.*exp(1-(x-tau2)./tau1)
                             s Up Sigmoid (logistic function):
                                y=.5+.5*erf((x-tau1)/sqrt(2*tau2))
                             Shift-D Down Sigmoid y=.5-.5*erf((x-tau1)/sqrt(2*tau2))
                             ~` Gauss/Lorentz blend (a/z adjust fraction of Gaussian
                             Shift-V Voigt profile (a/z adjusts shape)
                             Shift-B Breit-Wigner-Fano (a/z adjusts Fano factor)
 Fit.........................f  Perform single Fit from another start point.
 Select autozero mode........t  selects none, linear, quadratic, or flat baseline mode
 + or +/- peak mode..........+=  Flips between + peaks only and +/- peak mode
 Invert (negate) signal......Shift-N
 Fit polynomial..............Shift-o  Fits polynomial to data in upper panel
 Toggle log y mode OFF/ON....m  Log mode plots and fits log(model) to log(y).
 2-point Baseline............b, then click left and right baseline
 Set manual baseline.........Backspace, then click baseline at multiple points
 Restore original baseline...|\  to cancel previous background subtraction
 Click start positions.......c  Click on estimated peak position for each peak.
 Type in start vector........Shift-C Type or Paste start vector [p1 w1 p2 w2 ...]
 Print current start vector..Shift-Q
 Enter value of 'extra'......Shift-x, type value (or vector in brackets), press Enter.
 Adjust 'extra' up/down......a,z: 5% change; upper case A,Z: 0.5% change.
 Print parameters & results..q
 Print fit results only......r
 eValuate errors.............v  Estimates standard deviations of peak parameters.
 Test effect of Noise........n  by fitting a subset of data points.
 Plot signal in figure 2.....y
 Print model data table......d
 Refine fit..................x  Takes best of 10 trial fits (change number in line 219)
 Print peakfit function......w  Print peakfit function with all input arguments
 Enter minimum width.........Shift-W
 Enter saturation maximum....Shift-M  Ignores points above this magnitude
 Save Figure as png file.....Shift-S  Saves as Figure1.png, Figure2.png, etc.
 Display current settings....Shift-? displays list of current settings
 Switch to iPeak.m...........Shift-Ctrl-P  Transfer current signal to iPeak.m
 Switch to iSignal...........Shift-Ctrl-S  Transfer current signal to iSignal.m
```

* To specify a *multiple shape model*, press the '-' key, type a vector of "Shape" values, one for every peak, enclosed in square brackets, e.g. [1 1 3], and press **Enter**.

** The **a** and **z** keys adjust the "extra" variable that controls the *alpha* of the Voigt profile, the time constant of the exponentially broadened Gaussian and Lorentzian, the peak shape of the Pearson and bifurcated Gaussian, the Fano factor of the *Breit-Wigner-Fano* peak, and the % Gaussian of the Gaussian-Lorentzian blend.

Adding new peak shapes. It's easier than you think to add new peak shapes to **peakfit.m** or to **ipf.m**; see http://terpconnect.umd.edu/~toh/spectrum/InteractivePeakFitter.htm#NewShape

6. Hyperlinear analytical absorption spectroscopy

This example shows how knowledge of the specific measurement system can be used to design a custom signal processing procedure that expands the classical limits of measurement. This is a Matlab/Octave implementation of a computational method for quantitative analysis by multi-wavelength absorption spectroscopy, called the transmission-fitting or "TFit" method, based on fitting a model of the instrumentally-broadened transmission spectrum to the observed transmission data, rather than the conventional calculation of absorbance as log(Izero/I). The method is described in References 25, 26, and 27 on page 126. It is included here because it combines several important concepts that are covered in this essay: signal-to-noise ratio (pg 6), Fourier convolution (pg 31), multicomponent spectroscopy (pg 49), iterative least-squares fitting (pg 54), and calibration (pg 47).

Advantages of the TFit method compared to conventional absorbance-based methods are:

(a) much wider *dynamic range* (i.e., the concentration range over which one calibration curve can be expected to give good results) ;

(b) greatly improved calibration linearity;

(c) operates under conditions that are optimized for signal-to-noise ratio rather than for absorbance linearity (e.g. small spectrometers with low dispersion and large slit widths).

Just like the multilinear regression (classical least squares) methods conventionally used in absorption spectroscopy (Page 49), the Tfit method:

(a) requires an accurate reference spectrum of each component,

(b) utilizes accurately-registered multi-wavelength data such as would be acquired on diode-array, Fourier transform, or automated scanning spectrometers, and

(c) applies both to single-component and multi-component mixture analysis.

The disadvantages of the TFit method are:

(a) it makes the computer work harder than the multilinear regression methods (but, on a typical personal computer, calculations take only a fraction of a second, even for the analysis of a mixture of several components);

(b) it requires knowledge of the instrument function, i.e, the slit function or the resolution function of an optical spectrometer (but this is a fixed characteristic of the instrument and is easily measured beforehand by scanning the spectrum of a narrow atomic line source such as a hollow cathode lamp); and

(c) it is an iterative method that under unfavorable circumstances can converge on a local optimum (but this is handled by proper selection of the starting values, which are automatically supplied by the approximations calculated by conventional methods).

If you are viewing this online, click here to download a self-contained demo m-file that works in recent versions of Matlab. (You can also download the ZIP file "TFit.zip" ; you can also download it from the Matlab File Exchange.

a. Background

In absorption spectroscopy, the intensity I of light passing through an absorbing sample is given by the Beer-Lambert Law. In Matlab/Octave notation:

```
    I = Izero.*10^-(alpha*L*c)
```

where "**Izero**" is the intensity of the light incident on the sample, "**alpha**" is the absorption coefficient of the absorber, "**L**" is the distance that the light travels through the material (the path length), and "**c**" is the concentration of absorber in the sample. The variables **I**, **Izero**, and **alpha** are all functions of wavelength; **L** and **c** are scalar.

In conventional applications, measured values of **I** and **Izero** are used to compute the quantity called "absorbance", defined as

```
      A = log10(Izero./I)
```

Absorbance is defined in this way so that, when you combine this definition with the Beer-Lambert law, you get:

```
        A = alpha*L*c
```

So, absorbance is proportional to concentration, ideally, which simplifies analytical calibration. However, any real spectrometer has a finite spectral resolution, meaning that the light beam passing through the sample is not truly monochromatic, with the result that an intensity reading at one wavelength setting is actually an average over a small spectral interval. More exactly, what is actually measured is the convolution of the true spectrum of the absorber and the instrument function. If the absorption coefficient "alpha" varies over that interval, then the calculated absorbance will no longer be linearly proportional to concentration (this is called the "polychromicity" error). The effect is most noticeable at high absorbances. In practice, many instruments will become non-linear starting at an absorbance of 2 (~1% Transmission). As the absorbance increases, the effect of unabsorbed stray light and instrument noise also becomes more significant. Traditionally, there was no way to circumvent these problems, and the non-linearity so produced made calibration more complex and less certain.

The theoretical best signal-to-noise ratio and absorbance precision for a photon-noise limited optical absorption instrument can be shown to be close to an absorbance of 1.0 (see http://terpconnect.umd.edu/~toh/models/AbsSlitWidth.html#BestAbsorbance). However, if one attempts to arrange sample dilutions and absorption cell path lengths to obtain a working range centered on an absorbance of 1.0 , for example over the range .1 – 10, or 0.01 – 100, the measurements will obviously fail at the high end. (Clearly, the direct measurement of an absorbance of 100 is unthinkable, as it implies the measurement of light attenuation of 100 powers of ten - no real measurement system has a dynamic range remotely close to that). In practice, it is difficult to achieve an dynamic range even as high as 5 or 6 absorbance, so that much of the theoretically optimum absorbance range is actually unusable. (c.f. http://en.wikipedia.org/wiki/Absorbance). So, in conventional practice, greater sample dilutions and shorter path lengths are required to force the absorbance range to lower values, even if this means poorer signal-to-noise ratio and measurement precision at the low end.

It is true that the non-linearity caused by polychromicity can be reduced by operating the instrument at the highest resolution setting (reducing the instrumental slit width). However, this has a serious undesired side effect: in dispersive instruments, reducing the slit width to increase the spectral resolution degrades the signal-to-noise substantially. The optimum signal-to-noise ratio is often achieved when *the resolution of the instrument approximately matches the width of the analyte absorption*, but operating the instrument in that way would result in very substantial non-linearity over most of the absorbance range because of the "polychromicity" error. This non-linearity has its origin in the *spectral domain* (intensity vs wavelength), not in the *calibration domain* (absorbance vs concentration). Therefore it should be no surprise that curve fitting in the calibration domain, for example fitting the non-linear calibration data with a quadratic or cubic fit, might not be the best solution. Perhaps a better approach would be to perform the curve fitting in the spectral domain, where the problem originates. This is possible with modern absorption spectrometers that use *array detectors* with many tiny detector elements that slice up the spectrum of the transmitted beam into many small wavelength segments, rather than detecting the sum of all those segments with one big photo-tube detector as older instruments do.

The TFit method does exactly that by calculating the absorbance in a completely different way: it starts with the reference spectra (an accurate absorption spectrum for each analyte, recorded in the linear absorbance range, which is also required by the multilinear regression methods described on page 49), normalizes them to unit height, multiplies each by an adjustable coefficient (usually equal to the conventional absorbance measurement for that component in the mixture), adds them up, computes the transmission spectrum by taking the antilog, and convolutes it (page 31) with the previously-measured slit function. The result, representing the instrumentally broadened transmission spectrum, is compared to the observed transmission spectrum. The adjustable coefficients (one for each unknown component in the mixture) are adjusted by *Nelder-Mead Modified Simplex Optimization* (page 54) until the computed transmission model is a least-squares best fit to the observed transmission spectrum. The best-fit coefficients are then equal to the *absorbances that would have been observed under ideal optical conditions*. Provision is also made to compensate for unabsorbed stray light and changes in background intensity (background absorption). The Fit method gives measurements of absorbance that are much closer to the "true" peak absorbance that would have been measured in the absence of stray light and polychromatic light errors, and it allows linear and wide dynamic range measurements to be made even if the slit width of the instrument is increased to optimize the signal-to-noise ratio. (The calculations are performed by the function fitM, used as a fitting function for the iterative non-linear fitting function *fminsearch.m* in Matlab or Octave).

The TFit method does not guarantee a perfectly linear analytical curve under all conditions, despite the impression given by these simulations. It simply removes the non-linearity caused by unabsorbed stray light and the polychromatic light effect. Other sources of non-linearity remain - in particular, *chemical effects* such as photolysis, equilibrium shifts, temperature effects, pH effects, binding, dimerization, polymerization, molecular phototropism, fluorescence, etc.

Note: It's important to understand that the *TFit method is based on the Beer-Lambert Law*, but it calculates the absorbance in a different way that does not require the assumption that stray light and polychromatic radiation effects are zero, using the conventional log(Izero/I) absorbance only as a *starting point*. Because it allows larger slit widths to be used without calibration non-linearity, you get *greater signal-to-noise ratios* and *much wider linear dynamic range* than usual. Keep in mind that the log(Izero/I) absorbance is a 160-year-old simplification that dates from the time before electronics and computers, when the only computational tools were slide rules and mechanical gadgets, and when a method such as described here would have been unthinkably impractical.

b. The fitM.m function

```
function err = fitM(lam,yobsd,Spectra,InstFun,StrayLight)
```

fitM is a fitting function for the Tfit method, for use with the *fminsearch* function (page 55). The input arguments of fitM are:

lam = vector of adjustable parameters (in this case, peak absorbances) that are varied to obtained the best fit.
yobsd = observed transmission spectrum of the mixture sample over the spectral range (column vector)
Spectra = reference spectra for each component, over the same spectral range, one column/component, normalized to 1.00.
InstFun = Zero-centered instrument function or slit function (column vector)
StrayLight = fractional stray light (scalar or column vector, if it varies with wavelength)

Note: **yobsd**, **Spectra**, and **InstFun** must have the same number of rows (wavelengths). **Spectra** must have one column for each absorbing component.

Typical use: `absorbance=fminsearch(@(lambda)(fitM(lambda, yobsd, TrueSpectrum, InstFunction, straylight)), start);`

where `start` is the first guess (or guesses) of the absorbance(s) of the analyte(s); the Tfit method uses the conventional log10(Izero/I) estimate of absorbance(s) for its `start`. The other arguments (described above) are passed on to FitM. In this example, *fminsearch* returns the value of absorbance that would have been measured in the absence of stray light and polychromatic light errors (which is either a single value or a vector of absorbances, if it is a multicomponent analysis). The absorbance can then be converted into concentration by any of the usual calibration procedures (Beer's Law, external standards, standard addition, etc.)

Here is a very simple numerical example for a single absorbing component, using only 4-point spectra for simplicity (normally an array-detector system would acquire many more wavelengths than that). In this case the true monochromatic absorbance is 1.00, but the instrument width (InstFun) is twice the absorption width, and the stray light is 0.01 (1%), so the conventional single-wavelength estimate of absorbance, based on the minimum transmission, is far too low: `log10(1/.38696)=0.4123`. In contrast, the TFit method using fitM:

```
fminsearch(@(lambda)(fitM(lambda,[0.56529 0.38696 0.56529 0.73496]',[0.2 1 0.2
0.058824]',[1 0.5 0.0625 0.5]',.01)),.4)
```

returns the correct value of 1.0. (The "start" value, which is .4 in this case, is not critical and can be just about any value you like).

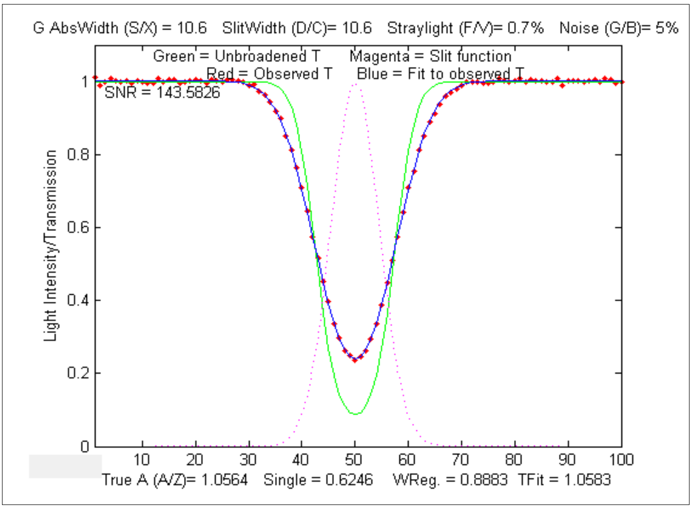
Iterative least-squares methods (page 54) are ordinarily considered to be more difficult and less reliable than multilinear regression methods (page 49), and this can be true if there are more than one nonlinear variable that must be iterated, especially if those variables are correlated. However, in the TFit method, there is only *one* iterated variable (absorbance) per measured component, and reasonable first guesses are readily available from the conventional single-wavelength absorbance calculation or multiwavelength regression methods. As a result, an iterative method works very well here. Comparing the expression for absorbance given above for the TFit method to that for the weighted regression method:

```
 absorbance=([weight weight].*[Background ReferenceSpectra])\(-
log10(yobsd).*weight)
```

You can see that, in addition to the ReferenceSpectra and observed transmission spectrum (yobsd), the TFit method additionally requires a measurement of the Instrument function (spectral bandpass) and the stray light (which the linear regression methods assume to be zero). However, these are characteristics of the spectrometer and need be measured only once for a given spectrometer. Finally, although the TFit method does make the computer work harder, the computation time on a typical laboratory personal computer is only a fraction of a second (roughly 25 µsec per spectral data point per component analyzed), using Matlab as the computational environment.

c. Basic demo of the Tfit method: TfitDemo.m (Matlab) and tfit.m (Octave)

The Matlab-only script **TfitDemo** is an interactive explorer for the Tfit method applied to the measurement of a single component with a Lorentzian or Gaussian absorption peak, with keystroke controls for adjusting the parameters while observing the effects graphically and numerically. The adjustable parameters are: the true absorbance, “True A” (adjusted with the **A**/**Z** keys), the spectral width of the absorption peak, “AbsWidth”, (adjusted with the **S**/**X** keys), the spectral width of the slit function, “SlitWidth” (adjusted with the **D**/**C** keys), the percent stray light, “Straylight” (adjusted with the **F**/**V** keys), and the noise level, “Noise” (adjusted with the **G**/**B** keys). (The x-axis, and the values of both AbsWidth and SlitWidth, are in arbitrary units).



The equivalent function for **Octave** users is **tfit.m**; the true absorbance is specified in the single input argument of that function, while the other parameters are set in lines 28-33.

The simulation includes the effect of photon noise, unabsorbed stray light, and random background intensity shifts (light source flicker), and it compares observed absorbances computed by the single-wavelength, “*Single*”, weighted multilinear regression, “*WReg*” (sometimes called Classical Least Squares in the chemometrics literature), and the *TFit* methods. If you are viewing this online, right-click TFitDemo.m click “Save link as...”, save it in a folder in the Matlab path, then type “TFitDemo” at the Matlab command prompt. With the figure window topmost, press **K** to get a list of the keypress functions.

In the example shown in the figure on the previous page, the true peak absorbance is exactly 1.000, the absorption widths and slit function widths are equal, the unabsorbed stray light is 0.5%, and the photon noise is 5%. The results below the graphs show that the TFit method gives a much more accurate measurement (1.0056) than the single-wavelength method (0.6246) or weighted multilinear regression method (0.8883).

TFitDemo KEYBOARD COMMANDS

```
Peak shape....Q.....Toggles between Gaussian and Lorentzian shapes
True peak A...A/Z...True absorbance of analyte at peak center, without
                    instrumental broadening, stray light, or noise.
AbsWidth......S/X...Width of absorption peak
SlitWidth.....D/C...Width of instrument function (spectral bandpass)
Straylight....F/V...Fractional unabsorbed stray light.
Noise.........G/B...Random noise level
Re-measure....Spacebar   Re-measure signal
Switch mode...W.....Switch between transmission and absorbance display
Statistics....Tab...Prints table of statistics of 50 repeats
Cal. Curve....M.....Displays analytical calibration curve in Figure 2
Keys..........K.....Print this list of keyboard commands
```

Why does the noise on the graph change if I change the instrument function (slit width or InstWidth)? In the most common type of absorption spectrometer, the spectrometer's spectral bandwidth (*InstWidth*) is changed by changing the slit width, which also effects the light intensity and thus the signal-to-noise ratio. Therefore, in all these programs, when you change *InstWidth*, the photon noise changes just as it would in a real spectrophotometer.

d. TFitStats.m: Statistics of methods compared (Matlab or Octave)

The Matlab/Octave script TFitStats computes the statistics of the TFit method compared to single-wavelength (SingleW), simple regression (SimpleR), and weighted regression (WeightR) methods. Simulates photon noise, unabsorbed stray light and random background intensity shifts. Estimates the precision and accuracy of the four methods by repeating the calculations 50 times with different random noise samples. Computes the mean, relative percent standard deviation, and relative percent deviation from true absorbance. Parameters are easily changed in lines 19 - 26. Results are displayed in the MATLAB command window. **Note:** This statistics function is included as a keypress command (**Tab** key) in the Matlab interactive demo TFitDemo.m.

Typical results are shown in the table on the next page, for a simulation with AbsWidth=10; SlitWidth=20; Straylight=0.5%, and Noise=5% of Izero). Results for true absorbances of 0.001. 1.00, and 100 are compared, demonstrating that the accuracy and the precision of these methods over a 10,000-fold concentration range. Notice how much closer the TFit method comes to the True A.

Statistical comparison of single-wavelength, weighted regression and TFit methods

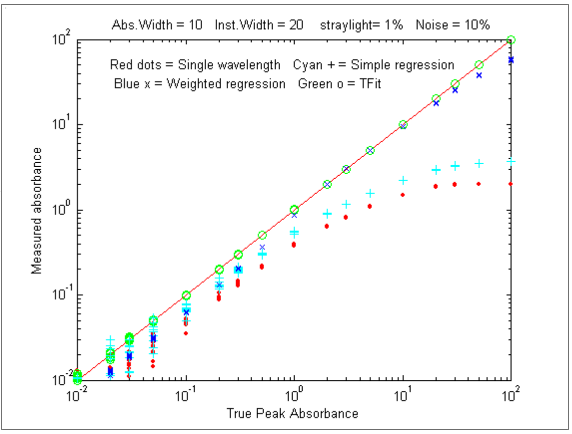
	True A	SingleW	SimpleR	WeightR	TFit
Mean result*	**.0010**	**.0003**	**.00057**	**.00070**	**.00097**
% RSD**		435%	275%	40%	38%
% Accuracy***		-70%	-40%	-30%	2.30%
Mean result*	**1.0000**	**0.599**	**0.656**	**0.841**	**1.001**
% RSD**		0.69%	0.33%	0.27%	0.32%
% Accuracy***		-40%	-34%	-16%	0.07%
Mean result*	**100**	**2.0038**	**3.7013**	**57.1530**	**99.9967**
% RSD**		22.00%	23.00%	78.00%	6.80%
% Accuracy***		-98.00%	-96.00%	-43.00%	0.33%

* Average value of the 50 measured absorbances

** Percent relative standard deviation of the 50 measured absorbances

*** Percent difference between the average of the 50 measured absorbances and the true absorbance

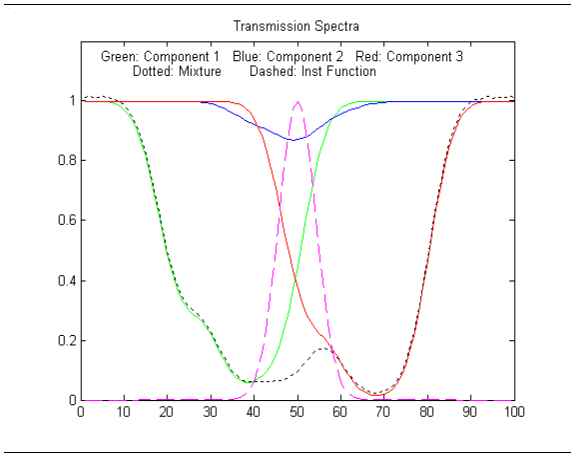
e. TFitCalDemo.m: Comparison of analytical curves (Matlab or Octave)



Matlab/Octave function that compares the analytical curves for single-wavelength, simple regression, weighted regression, and the TFit method over any specified absorbance range (specified by the vector "absorbancelist" in line 20). Simulates photon noise, unabsorbed stray light and random background intensity shifts. Plots a log-log scatter plot with each repeat measurement plotted as a separate point (so you can see the scatter of points at low absorbances). The parameters can be changed in lines 43 - 51.

In the sample result shown above, analytical curves for the four methods are computed over a 10,000-fold range, up to a peak absorbance of 100, demonstrating that the TFit method (shown by the green circles) is much more linear over the whole range than the single-wavelength, simple regression, or weighted regression methods. **Note:** This calibration curve function is included as a keypress command (**M** key) in the Matlab interactive demo function TFitDemo.m.

f. Application to a three-component mixture: TFit3Demo.m and Tfit3.m



Tfit3Demo is a Matlab interactive demonstration function of the T-Fit method applied to the multi-component absorption spectroscopy of a *mixture of three absorbers*, with keystrokes that allow you to adjust the parameters continuously while observing the effect dynamically. The adjustable parameters are: the absorbances of the three components (A1, A2, and A3, adjusted by the **A**/**Z**, **S**/**X**, and **D**/**C** keys respectively), spectral overlap between the component spectra ("Sepn", adjusted by the **F**/**V** keys), width of the instrument function ("InstWidth" adjusted by the **G**/**B** keys), and the noise level ("Noise", adjusted by the **H**/**N** keys). The equivalent function for Octave users is Tfit.m, which has the syntax `Tfit(AbsorbanceVector)`, where AbsorbanceVector is the vector of the three true absorbances in the mixture, for example: TFit3([3 .1 5]).

This demonstration compares quantitative measurement by *weighted regression* (page 50) and TFit methods. It simulates photon noise, unabsorbed stray light and random background intensity shifts. Note: After executing this m-file, slide the “Figure 1” and “Figure 2” windows side-by-side so that they don't overlap. Figure 1 shows a log-log scatter plot of the true vs. measured absorbances, with the three absorbers plotted in different colors and symbols. Figure 2 shows the transmission spectra of the three absorbers plotted in the corresponding colors. As you adjust the variable parameters in Figure No. 1, both graphs change accordingly.

In the sample calculation shown above, component 2 (shown in blue) is almost completely buried by the stronger absorption bands on either side, giving a much weaker absorbance (0.1) than the other two components (3 and 5, respectively). Even in this case the TFit method gives a result within 1 to 2% of the correct value (A2=0.1). In fact, over most combinations of the three concentrations, the TFit method works better, although of course nothing works if the spectral differences between the components is too small. (In this program, as in all of the above, when you change InstWidth, the photon noise is automatically changed accordingly just as it would in a real spectrophotometer).

TFitDemo3 KEYBOARD COMMANDS

```
A1..........A/Z  Increase/decrease true absorbance of component 1
A2..........S/X  Increase/decrease true absorbance of component 2
A3..........D/C  Increase/decrease true absorbance of component 3
Sepn........F/V  Increase/decrease spectral separation of the components
InstWidth...G/B  Increase/decrease width of spectral bandpass
Noise.......H/N  Increase/decrease random noise level when InstWidth = 1
Peak shape..Q    Toggles between Gaussian and Lorentzian absorption peak shape
Table.......Tab  Print table of results
Keys........K    Print this list of keyboard commands
```

Another run of the same simulation, showing the sample table of results obtained by pressing the **Tab** key:

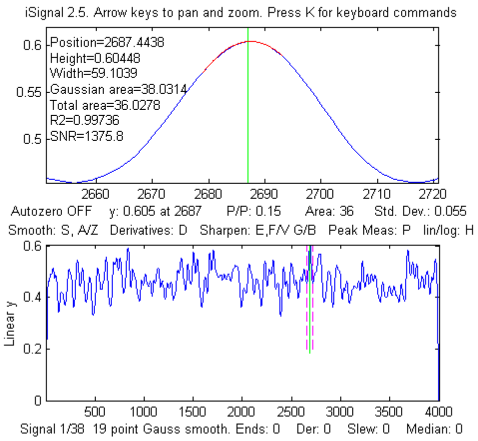
```
              True       Weighted    TFit
           absorbance   Regression  method
Component 1    3.00        2.06     3.001
Component 2    0.10        0.4316   0.098
Component 3    5.00        2.464    4.998
```

***Note for Octave users*:** the current versions of fitM.m, tfit.m, TfitStats.m and TfitCalDemo.m work in Octave as well as in Matlab. However, the interactive features of TfitDemo.m and Tfit3Demo.m work only in Matlab; Octave users should use the command-line functions tfit.m and Tfit3.m. See **http://tinyurl.com/cey8rwh** for a list of and links to these and other Matlab and Octave functions.

Appendix A: More on smoothing

This section presents additional supporting information related to *smoothing* (page 11).

1. Can smoothed noise may be mistaken for an actual signal? Here are two examples that show that the answer to this question is *yes*. The first example is shown on the left. This shows **iSignal** (page 85) displaying a computer-generated 4000-point signal consisting only of random white noise, smoothed with a 19-point Gaussian smooth. The upper window shows a tiny slice of this signal that looks like a Gaussian peak with a calculated SNR over 1000. Only by looking at the entire signal (bottom window) do you see the true picture; that "peak" is just part of the noise, smoothed to look nice. Don't fool yourself.



iSignal 2.5. Arrow keys to pan and zoom. Press K for keyboard commands

Position=2687.4438
Height=0.60448
Width=59.1039
Gaussian area=38.0314
Total area=36.0278
R2=0.99736
SNR=1375.8

Autozero OFF y: 0.605 at 2687 P/P: 0.15 Area: 36 Std. Dev.: 0.055
Smooth: S, A/Z Derivatives: D Sharpen: E,F/V G/B Peak Meas: P lin/log: H

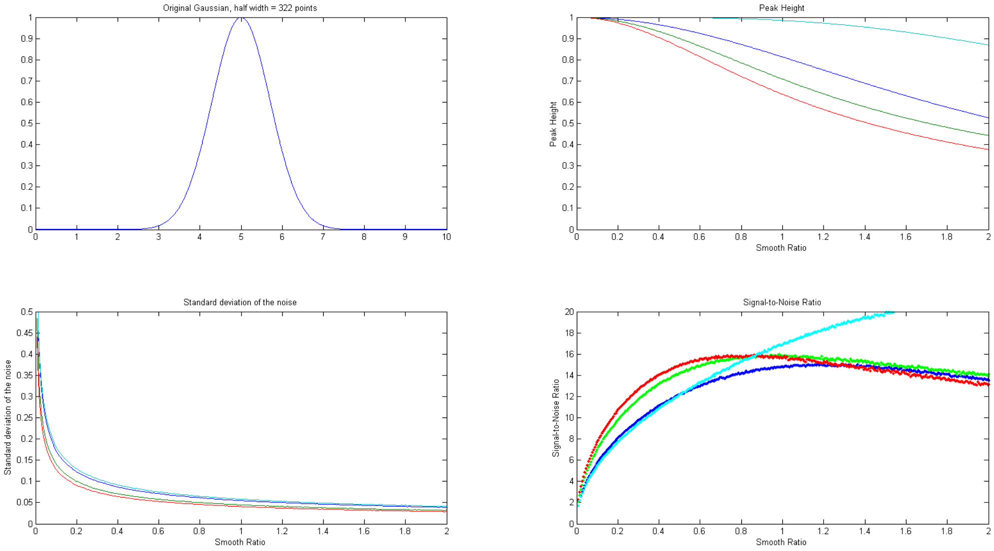
Signal 1/38 19 point Gauss smooth. Ends: 0 Der: 0 Slew: 0 Median: 0

The second example is a simple series of three Matlab commands that uses the 'randn' function to generate a 10000-point data set containing *only normally-distributed white noise*. Then it uses 'fastmooth' (page 15-16) to smooth that noise, resulting in a 'signal' with a standard deviation of about 0.3 and a maximum value around 1.0. That signal is then submitted to iPeak (page 78). If the peak detection criteria (e.g. AmpThreshold and SmoothWidth) are set too low, many peaks will be found. But *setting the AmpThreshold to 3 times the standard deviation* (3 x 0.3 = 0.9) will greatly reduce the incidence of these false peaks.

```
>> noise=randn(1,10000);
>> signal=fastsmooth(noise,13);
>> ipeak([1:10000;signal],0,0.9,1e-006,17,17)
```

The peak *identification* function, described on page 77 and 80, which identifies peaks based on their exact x-axis peak position, is even *less* likely to be fooled by random noise, because in addition to the peak detection criteria of the findpeaks algorithm, any detected peak must also match closely to a peak position in the table of known peaks, in order for it to be reported as an identified peak.

2. Smoothing performance comparison. The Matlab/Octave function "**smoothdemo.m**" (on http://tinyurl.com/cey8rwh) is a self-contained function that compares the performance of four types of smooth operations: (1) sliding-average, (2) triangular, (3) pseudo-Gaussian (equivalent to three passes of a sliding-average), and (4) Savitzky-Golay. These are the four smooth types discussed on page 11, corresponding to the four values of the SmoothMode input argument of the iSignal function (page 85). These four smooth operations are applied to a 2000-point signal consisting of a Gaussian peak with a FWHM (full-width at half-maximum) of 322 points and to a noise array consisting of



10^7 samples of normally-distributed random white noise with a mean of zero and a standard deviation of 1.0. The peak height of the smoothed peak, the standard deviation of the smoothed noise, and the signal-to-noise ratio are all measured as a function of smooth width, for each smooth type. Smooth width is expressed in terms of "smooth ratio", the ratio of the width of the smooth to the width (FWHM) of the peak. You may download this from http://tinyurl.com/cey8rwh**.**

The results, when "**smoothdemo.m**" is run (with a noise array length of 10^7 to insure accurate sampling of the noise), are shown by the figure and text print-out below. The four quadrants of the graph are: (upper left) the original Gaussian peak before smoothing and without noise; (upper right) the peak height of the smoothed signal as a function of smooth ratio; (lower left) the standard deviation of the noise as a function of smooth ratio; the signal-to-noise ratio (SNR) as a function of smooth ratio (lower right). The different smooth types are indicated by color: **blue** - sliding-average; **green** - triangular; **red** - pseudo-Gaussian, and **cyan** - Savitzky-Golay. The function also calculates and prints out the elapsed time for a each smooth type and the maximum in the SNR plot.

```
1. Sliding-average:   Elapsed Time: 0.2615 sec  Optimum SNR: 15.1 at a smooth width of 1.26
2. Triangular:        Elapsed Time: 0.5956 sec  Optimum SNR: 15.8 at a smooth width of 1.11
3. Pseudo-Gaussian:   Elapsed Time: 0.8695 sec  Optimum SNR: 15.6 at a smooth width of 0.94
4. Savitzky-Golay:    Elapsed Time: 4.4995 sec  Optimum SNR: 20.3 at a smooth width of 1.74
```

These results clearly show that the Savitzky-Golay smooth gives the smallest peak distortion (smallest reduction in peak height), but, on the other hand, it gives the smallest reduction in noise amplitude and the longest computation time (by x5 or more). The pseudo-Gaussian smooth gives the greatest noise reduction and, below a smooth ratio of about 1.0, the highest signal-to-noise ratio, but the Savitzky-Golay smooth gives the highest SNR above a smooth ratio of 1.0.

For applications where speed is not an issue and where the shape of the signal must be preserved as much as possible, the Savitzky-Golay is clearly the method of choice. In the peak detection function described in page 74, on the other hand, the purpose of smoothing is to reduce the noise in the derivative signal and the retention of the shape of that derivative is less important. Therefore the triangular or pseudo-Gaussian smooth is well suited to this purpose and has the additional advantage of faster computation speed.

The conclusions are essentially the same for a Lorentzian peak, as demonstrated by a similar function "**smoothdemoL.m**", the main difference being that the peak height reduction is greater for the Lorentzian at a given smooth ratio.

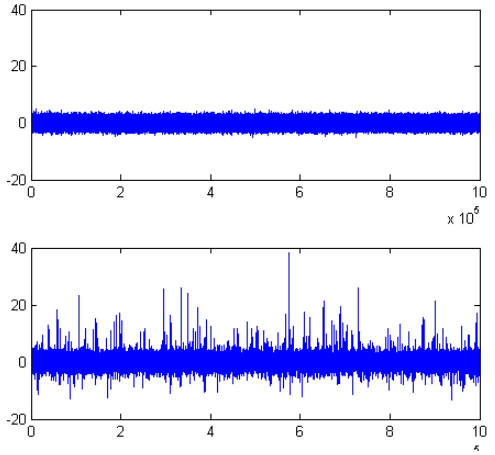
3. Effect of noise color. The frequency distribution of noise, designated by noise "color" (page 8), substantially effects the ability of smoothing to reduce noise. The Matlab/Octave function "**NoiseColorTest.m**" compares the effect of a 100-point boxcar (unweighted sliding average) smooth on the standard deviation of white, pink, and blue noise (page 8), all of which have an original unsmoothed standard deviation of 1.0. Because smoothing is a low-pass filter process, it effects low frequency (pink) noise *less*, and high-frequency (blue) noise *more*, than white noise.

Original unsmoothed noise	1
Smoothed white noise	0.1
Smoothed pink noise	0.55
Smoothed blue noise	0.010

4. It is possible to reverse the effect of smoothing? Not generally, because smoothing is essentially *averaging* and you can not recreate a set of numbers given only its average. There are *many* sets of numbers that could have the same average; there's no single right answer. However, you could apply a *high*-pass filter or peak-sharpening algorithm to partially compensate for a previous *low*-pass filtering (pages 26, 34). Alternatively, if you knew the response function of the smoothing operation that had been applied, you could deconvolute it from the smoothed data (page 32). But such operations are only approximate and invariably degrade the signal-to-noise ratio.

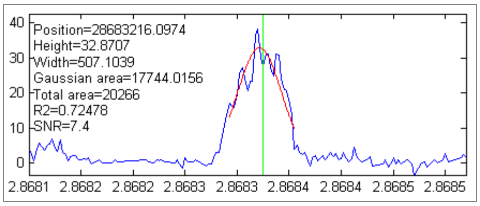
Appendix B. Case study of an unusual signal.

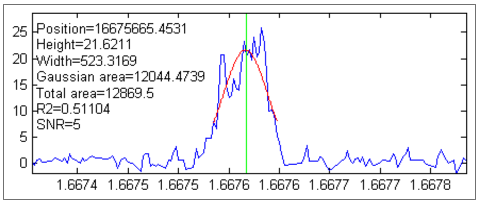
The experimental signal in this case was unusual in that it did not look like a typical signal when plotted. In fact, at first glance it looked a lot like noise. The figure on the left compares the raw signal (bottom) with the same number of points of normally-distributed white noise (top) with a mean of zero and a standard deviation of 1.0 (obtained from the Matlab/ Octave 'randn' function). As you can see, the main difference is that the experimental signal has more large 'spikes', especially in the positive direction. This difference is evident when you look at the descriptive statistics of the signal and the 'randn' function:



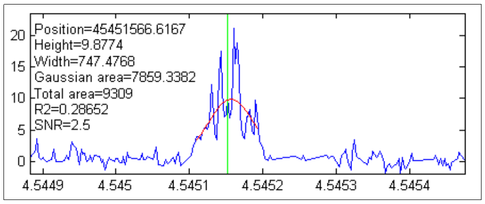
DESCRIPTIVE STATISTICS	Raw signal	random noise (randn function)
Mean	0.4	0
Maximum	38	about 5
Standard deviation (std)	1.05	1.0
Interquartile Range (iqr)	1.04	1.3489
Kurtosis	38	3
Skewness	1.64	0

Even though the standard deviation of these two are about the same, the other statistics (especially the kurtosis and skewness) indicate that the probability distribution of the signal is far from norm in particular, there are far more positive spikes in the signal than expected for pure noise. Most of these are actually the peaks of interest for this signal; they look like spikes only because the length of the signal (over 1 million points) causes the peaks to be compressed into one screen pixel or less when the entire signal is plotted on the screen. In the figures on the left, **iSignal** (page 85) is used to "zoom in" on some of the larger of these peaks. The peaks are very sparsely separated (by an average of 1000 half-widths between peaks) and are well above the level of background noise (which has a standard deviation of roughly 0.9 throughout the signal).





The researcher who obtained this signal said that a 'good' peak was 'bell shaped', with an amplitude above 5 and a width of 500-1000 x-axis units. So that means that we can expect the signal-to-background-noise ratio to be at least 5/0.9 = 5.5. You can see in the three example peaks on the left that the peak widths do indeed meet those expectations. The interval between adjacent x-axis points is 25, so we can expect the peaks to have about 20 to 40 points in their widths. Based on that, we can expect that the positions, heights and widths of the peaks should be able to be measured fairly accurately using least-squares methods (which reduce the uncertainty of measured parameters by about the square root of the number of points used, about a factor of 5 in this case). However, the noise



appears to be *signal-dependent* (page 8); that is, the noise on the top of the peaks is distinctly greater than the noise on the baseline. The result is that the actual signal-to-noise ratio of the peak parameter measurements for the larger peaks will not be as good as expected based on the ratio of the peak height to the noise on the background. Most likely, the total noise in this signal is the sum of at least two major components, one with a fixed standard deviation of 0.9 and the other equal to about 10% of the peak height.

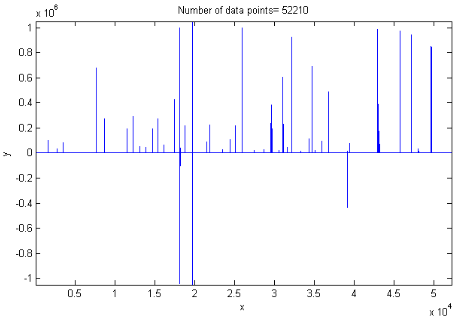
To automate the detection of large numbers of peaks, we can use the ***findpeaksG*** function (page 74). Reasonable initial values of the input arguments AmplitudeThreshold, SlopeThreshold, SmoothWidth, and FitWidth for those functions can be estimated based on the expected peak height (5) and width (20 to 40 data points) of the “good” peaks. For example, using AmplitudeThreshold=5, SlopeThreshold=.001, SmoothWidth=25, and FitWidth=25, findpeaks detects *76 peaks* above an amplitude of 5 and with an average peak width of 523. My Matlab interactive peak finder **iPeak** (page 76) is especially convenient for exploring the effect of these peak detection parameters and for graphically inspecting the peaks that it finds. Ideally the objective is to find a set of peak detection arguments that detect and accurately measure all the peaks that you would consider 'good' and skip all the 'bad' ones. But in reality the criteria for good and bad peaks is at least partly subjective, so it's usually best to err on the side of caution and avoid skipping 'good' peaks at the risk of including a few 'bad' peaks, which can be weeded out manually based on unusual position, height, width, or appearance by simple processing on the peak table P (e.g. page 77).

Of course it must be expected that the values of the peak position, height, and width given by the findpeaks or iPeak functions will only be approximate and will vary depending on the exact setting of the peak detection arguments; the noisier the data, the greater the uncertainty in the peak parameters. In this regard the peak-fitting functions **peakfit.m** and **ipf.m** (page 90, 95) may give slightly more accurate results, because they make use of *all* the data across the peak, not just the top of the peak as do findpeaks and iPeak. Also, the peak fitting functions are better for dealing with overlapping peaks, and they have the ability to estimate the uncertainty of the measured peak parameters, using the bootstrap options of those functions (pages 42 and 96). Using that method, the largest peak in the signal is found to have an x-axis position of 2.8683e+007, height of 32, and width of 500, with standard deviations of 4, 0.92, and 9.3, respectively.

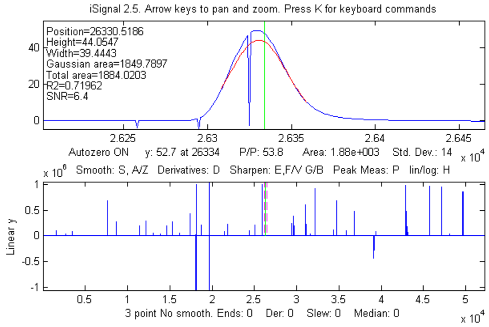
Because the signal in the case was so large (over 1,000,000 points), the interactive programs such as **iPeak** (page 74), **iSignal** (page 85), and **ipf** (page 95) may be sluggish in operation, especially if your computer is not fast computationally or graphically. If this is a serious problem, it may be best to break the signal up into two or more segments, deal with each segment separately, then combine the results. Alternatively, you can use the condense function to average the entire signal into a smaller number of points by a factor of 2 or 3, at the risk of slightly reducing peak heights and increasing peak widths, but then you should adjust “SmoothWidth” and “FitWidth” to compensate for the reduced number of data points across the peaks.

Appendix C. Buried treasure.

The experimental signal in this case, shown on the right, had a number of narrow spikes above a seemingly flat baseline.



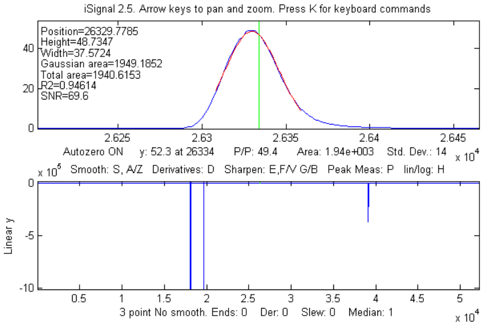
Using **iSignal** to investigate the signal, it was found that the positive spikes were *single points* of very large amplitude (up to 10^6), whereas the regions *between* the spikes contained bell-shaped peaks so small they can't be seen on this scale.


iSignal 2.5. Arrow keys to pan and zoom. Press K for keyboard commands
40
20
0
2.625
2.63
2.635
2.64
2.645
x 10^4
Smooth: S, A/Z   Derivatives: D   Sharpen: E,F/V G/B   Peak Meas: P   lin/log: H
x 10^6
1
0.5
0
-0.5
-1
Linear y
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
x 10^4

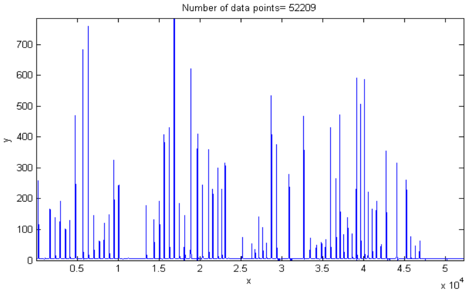
```
Position=26330.5186
Height=44.0547
Width=39.4443
Gaussian area=1849.7897
Total area=1884.0203
R2=0.71962
SNR=6.4
Autozero ON     y: 52.7 at 26334     P/P: 53.8     Area: 1.88e+003     Std. Dev.: 14
3 point No smooth. Ends: 0     Der: 0     Slew: 0     Median: 0
```

For example, using the cursor keys in **iSignal** to zoom in to the region around x=26300, I found one of those bell-shaped peaks, with a single-point negative spike near its peak, in the screen shot on the left.

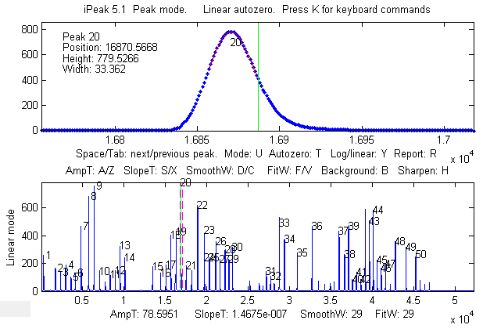
Very narrow spikes like this are common artifacts in some experimental signals; they are easy to eliminate by using a *median filter* (page 15). **iSignal** has such a filter (the "**M**" key). The result, in the plot on the right, shows that the single-point spike artifacts have been eliminated, with little effect on the character of the bell-shaped peak. Other filter types, like most forms of smoothing, would be far less effective than a median filter for this type of artifact and would distort the peaks. The negative spikes in this signal turned out to be negative-going steps, which can either be reduced by using iSignal's slew rate limit function (the ` key) or manually eliminated by using the semicolon key (**;**) to set the selected region between the dotted red cursor lines to zero. Using the latter approach, the entire cleaned-up signal is shown on the left. The remaining peaks are all positive, smooth, bell-shaped and have amplitudes from about 6 to about 750.


iSignal 2.5. Arrow keys to pan and zoom. Press K for keyboard commands
40
20
0
2.625
2.63
2.635
2.64
2.645
x 10^4
Smooth: S, A/Z   Derivatives: D   Sharpen: E,F/V G/B   Peak Meas: P   lin/log: H
x 10^5
0
-5
-10
Linear y
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
x 10^4

```
Position=26329.7785
Height=48.7347
Width=37.5724
Gaussian area=1949.1852
Total area=1940.6153
R2=0.94614
SNR=69.6
Autozero ON     y: 52.3 at 26334     P/P: 49.4     Area: 1.94e+003     Std. Dev.: 14
3 point No smooth. Ends: 0     Der: 0     Slew: 0     Median: 1
```

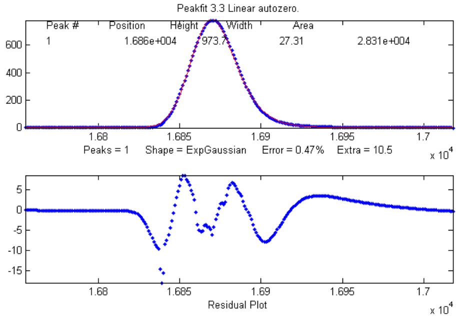


iPeak can automate the estimation of peak positions, heights, and widths for the entire signal, finding 50 peaks above the amplitude threshold (see figure on right).


iPeak 5.1 Peak mode.   Linear autozero. Press K for keyboard commands
800
600
400
200
0
20
1.68
1.685
1.69
1.695
1.7
x 10^4
Space/Tab: next/previous peak. Mode: U Autozero: T Log/linear: Y Report: R
AmpT: A/Z   SlopeT: S/X   SmoothW: D/C   FitW: F/V   Background: B   Sharpen: H
600
400
200
0
Linear mode
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
x 10^4

```
Peak 20
Position: 16870.5668
Height: 779.5266
Width: 33.362
AmpT: 78.5951     SlopeT: 1.4675e-007     SmoothW: 29     FitW: 29
```

Individual peaks can be measured more accurately by fitting the whole peak with iPeak's "**N**" key or with **peakfit.m** or **ipf.m**. The peaks are all slightly asymmetrical. Fitting an exponentially-broadened Gaussian model (page 66) to a fitting error less than about 0.5%, as shown on the left. The smooth shape of the residual plot suggests that the signal was smooth *before* the spikes were introduced.



```
Peak #      Position       Height      Width       Area
1           1.686e+004     973.7       27.31       2.831e+004
Peaks = 1     Shape = ExpGaussian     Error = 0.47%     Extra = 10.5
```

Appendix D. The Battle Rounds: a comparison of methods.

This example compares the application of several techniques described in this paper to the quantitative measurement of a weak peak buried in an noisy and unstable background, a common problem in the quantitative analysis applications of various forms of spectroscopy (ref 49) and remote sensing. The objective is to derive a measure of peak amplitude that varies linearly with the actual peak amplitude but that is not effected by the changes in the background and the random noise. In this example, the peak to be measured is located at a fixed location in the center of the recorded signal, at x=100, and has a fixed shape (Gaussian) and width (30). The background, on the other hand, is highly variable, both in amplitude and in shape. The simulation shows six superimposed recordings of the signal with six increasing peak amplitudes and with randomly varying background amplitudes and shapes (top row left in the figures below). The signal processing techniques that are used here include *smoothing* (page 10), *differentiation* (page 16), *classical least squares multicomponent method* (CLS, page 48), and *iterative non-linear curve fitting* (page 53).

This example is illustrated by **CaseStudyC.m,** a self-contained Matlab/Octave function (download from http://tinyurl.com/cey8rwh and place in the Matlab path). To run it, just type “CaseStudyC” at the command prompt. Each time you run it, you get the same series of true peak amplitudes (set by the vector “SignalAmplitudes”) but a different set of noise, background shapes and amplitudes. The background is modeled as a Gaussian peak of randomly varying amplitude, position, and width; you can control the average *amplitude* of the background by changing “BackgroundAmplitude” and the average *change* in the background by changing “BackgroundChange”.
The five methods compared here are:

1: Top row center. A simple zero-to-peak measurement of the smoothed signal, which will be accurate only if the background is *zero*.

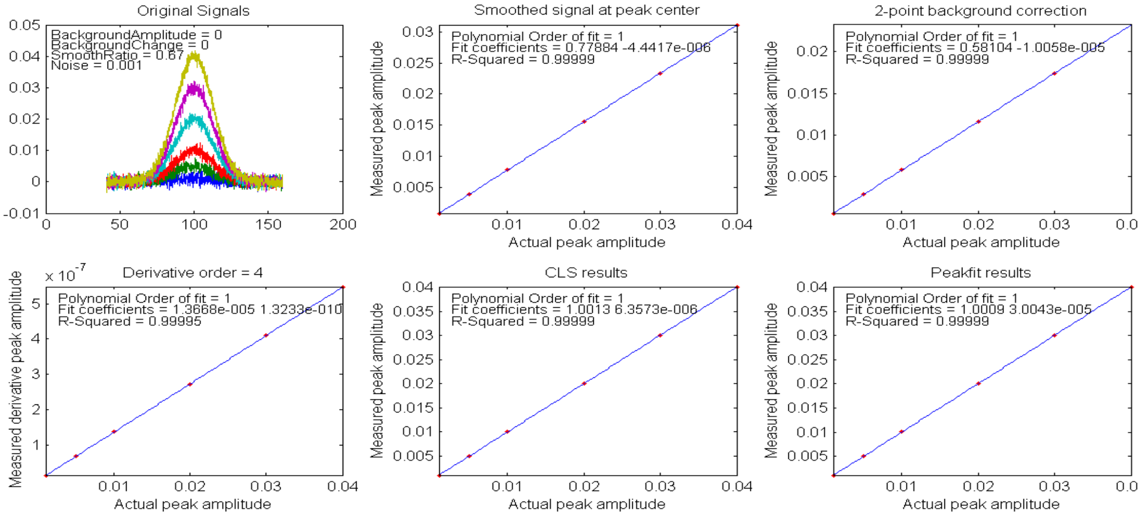
2: Top row right. The difference between the peak signal and the average background on both sides of the peak (both smoothed), which assumes that the background is *flat*.

3: Bottom row left. A smoothed derivative-based method, based on the assumption that the background is *very broad* compared to the measured peak.

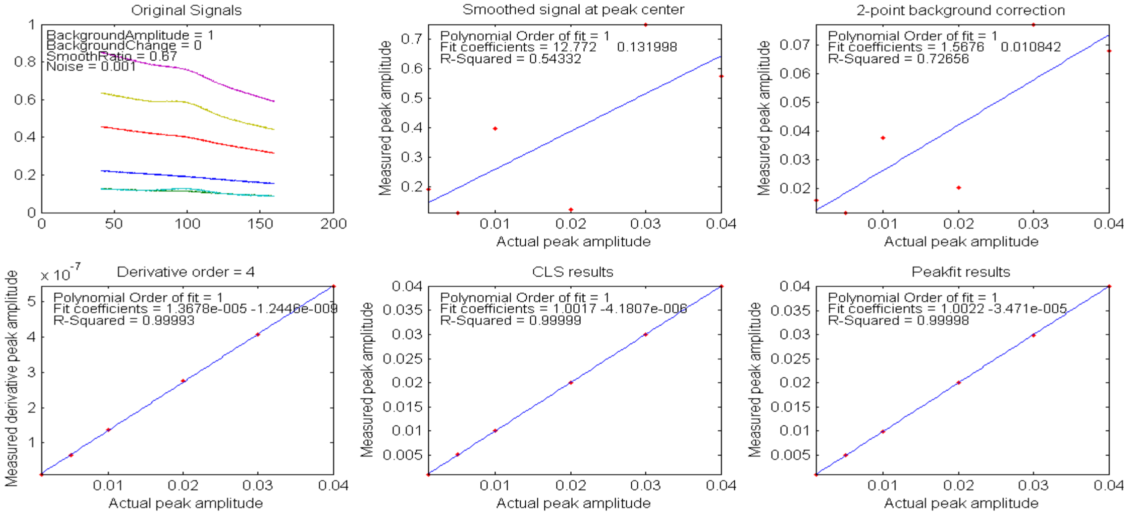
4: Bottom row center. Classical least squares (CLS, page 48) applied to the raw signal, which assumes that the background is a peak of *known shape, width, and position* (but not height).

5: Bottom row right. iterative non-linear curve fitting (INLS, page 53) applied to the raw signal, which assumes that the background is a peak of *known shape* but unknown width and position. This method can track changes in the background peak position and width (within limits), as long as the measured peak and the background *shapes* are known.

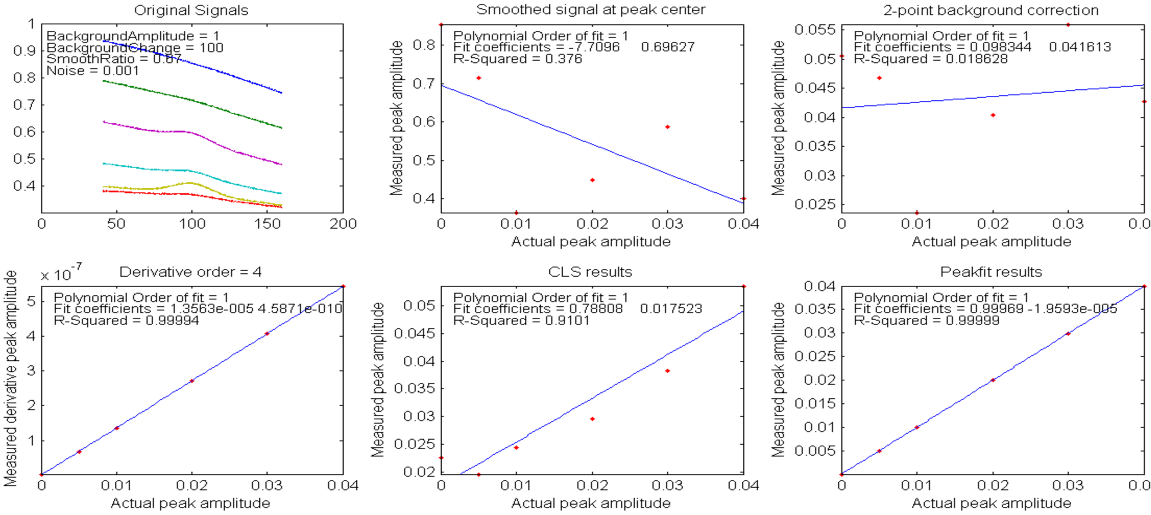
These five methods are compared by plotting the actual peak heights (defined by the vector “SignalAmplitudes”) vs the measure derived from that method, fitting the data to a straight line, and computing the *coefficient of determination,* R^2 which ideally is 1.0000.



For the first test (shown in the figure above on the previous page), both “BackgroundAmplitude” and “BackgroundChange” are set to zero, so that only the random noise is present. In that case *all* the methods work well, with R^2 values all very close to 1.0000. Too easy, right?



For the second test (shown in the figure immediately above), the background is allowed to have significant *amplitude* variation but a *fixed* shape, position, and width, so we set “BackgroundAmplitude”=1 and “BackgroundChange”=0. In that case, the first two methods fail completely, but the derivative and INLS methods still work well.



For the third test, shown in the figure above, “BackgroundAmplitude”=1 and “BackgroundChange”=100, so the background varies in position, width, and amplitude (but remains broad compared to the signal). In that case, the CLS methods fails as well, because that method assumes that the background varies only in amplitude.

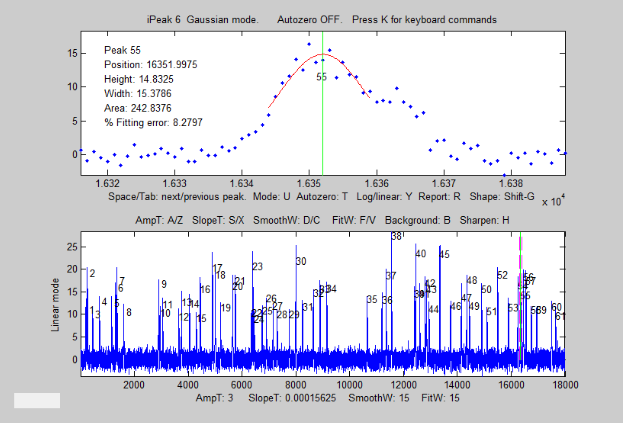
However, if we go one step further and set “BackgroundChange”=1000, the background shape is now so unstable that even the INLS method fails, but still the derivative method remains effective as long as the background is broader than the measured peak, whatever its shape. On the other hand, if the width and position of the *measured* peak changes from sample to sample, the derivative method will fail and the INLS method is more effective, as long as the basic shape of both measured peak and the background are accurately modeled. Bottom line: the best method depends on the situation.

Appendix E: Ensemble averaging patterns in a continuous signal.

Ensemble averaging (page 7) is a powerful method of reducing the effect of random noise in experimental signals, when it can be applied. The idea is that the signal is repeated, preferably a large number of times, and all the repeats are averaged. The signal builds up, and the noise gradually averages towards zero, as the number of repeats increases.

An important requirement is that the repeats be aligned or synchronized, so that in the absence of random noise, the repeated signals would line up exactly. There are two ways of managing this: (a) the signal repeats are triggered by some external event and the data acquisition uses that trigger to synchronize the acquisition of signals, or (b) the signal itself has some internal feature that can be used to detect each repeat, whenever it occurs.

The first method has the advantage that the signal-to-noise ratio can be arbitrarily low and the average signal will still gradually emerge from the noise if the number of repeats is large enough. However, not every experiment has a reliable external trigger.



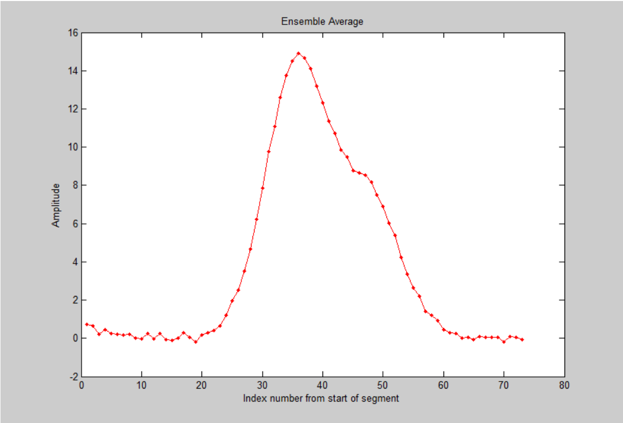
iPeak 6 Gaussian mode.   Autozero OFF.   Press K for keyboard commands

Peak 55
Position: 16351.9975
Height: 14.8325
Width: 15.3786
Area: 242.8376
% Fitting error: 8.2797

Space/Tab: next/previous peak. Mode: U Autozero: T Log/linear: Y Report: R Shape: Shift-G

AmpT: A/Z SlopeT: S/X SmoothW: D/C FitW: F/V Background: B Sharpen: H

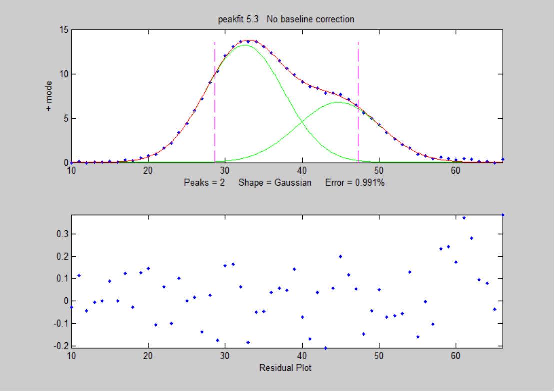
AmpT: 3 SlopeT: 0.00015625 SmoothW: 15 FitW: 15



The second method can be used to average repeated patterns in one signal *without* an external trigger, but then the signal must then contain some feature (for example, a peak) with a signal-to-noise ratio large enough to *detect reliably in each repeat*. This method can be used even when the signal patterns occur at random intervals. The interactive peak detector ***iPeak*** (page 78) has a built-in ensemble averaging function (**Shift-E**) can compute the average of all the repeating waveforms. It works by detecting a single peak in each repeat in order to synchronize the repeats.

The Matlab script **iPeakEnsembleAverageDemo.m** demonstrates this idea, with a signal that contains a repeated underlying pattern of two overlapping Gaussian peaks, 12 points apart, both of width 12, with a 2:1 height ratio. These patterns occur a random intervals, and the noise level is about 10% of the average peak height. Using **iPeak** (above left), you adjust the peak detection controls to detect only one peak in each repeat pattern, zoom in to isolate any one of those repeat patterns, and press **Shift-E**. In this case there are about 60 repeats, so the expected signal-to-noise ratio improvement is sqrt(60) = 7.7. You can save the averaged pattern (above right) into the Matlab workspace as "EA" by typing "`load EnsembleAverage; EA=EnsembleAverage;`", then curve-fit this averaged pattern to a 2-Gaussian model using the peakfit.m function (page 90):

```
peakfit([1:length(EA);EA],40,60,2,1,0,10)
   Position    height    width    area
   32.54       13.255    12.003   169.36
   44.722      6.7916    12.677   91.648
```



This is a significant improvement in the measurement of the peak separation, height ratio and width, compared to fitting a *single* pattern in the original x,y signal:

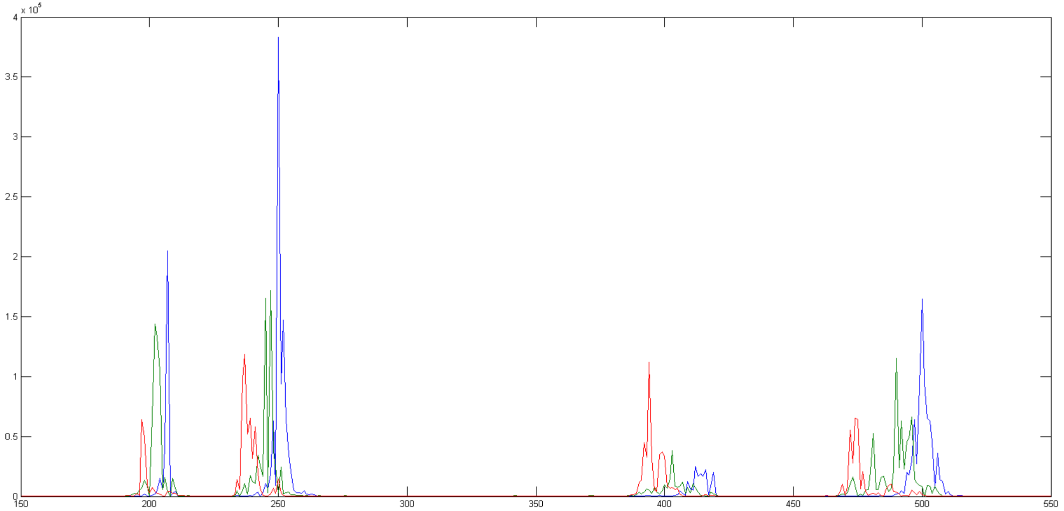
```
peakfit([x;y],16352,60,2,1,0,10)
```

Appendix F: Harmonic Analysis of the Doppler Effect

The wav file “horngoby.wav” (Ctrl-click to open) is a 2-second recording of the sound of a passing automobile horn, exhibiting the familiar Doppler effect. The sampling rate is 22000 Hz. You can load this into the Matlab workspace as the variable “doppler” using Matlab's 'wavread' function and display it using ***iSignal*** (page 85):

```
t=0:1/21920:2;
doppler=wavread('horngoby.wav');
isignal(t,doppler);
```

Press **Shift-S to** switch to frequency spectrum mode (page 28) and zoom in on different portions of the waveform to observe the downward frequency shift and measure it quantitatively. Actually, it's much easier to *hear* the frequency shift (**Shift-P**) than to *see* it graphically; the shift is rather small on a percentage basis, but human hearing is very sensitive to small pitch (frequency) changes. It helps to re-plot the data to stretch out the frequency region around the fundamental frequency or one of the harmonics. I used **iSignal** to zoom in on three time slices of this waveform and to plot the frequency spectrum near the *beginning* (plotted in blue), *middle* (green), and *end* (red) of the sound. A portion of those data are plotted in the figure below:



The group of peaks near 200 are the fundamental frequency of the lowest note of the horn and the group of peaks near 400 are its second harmonic. (Pitched sounds usually have a harmonic structure of 1, 2, 3... times a fundamental frequency). The group of peaks near 250 are the fundamental frequency of the *next higher note* of the horn, and the group of peaks near 500 are its second harmonic. (Car and train horns often have two or more harmonious notes sounded together). In each of these groups of harmonics, you can clearly see that the *blue* peak (the frequency spectrum measured at the *beginning* of the sound) has a *higher* frequency than the *red* peak (the spectrum measured at the *end* of the sound). The green peak is taken in the middle of the sound. The peaks are ragged because the amplitude and frequency varies somewhat over each slice of waveform, but despite that you can still get good quantitative measures of the frequency of each component by *curve fitting* (page 39, 54) using **peakfit.m** or **ipf.m** (page 74):

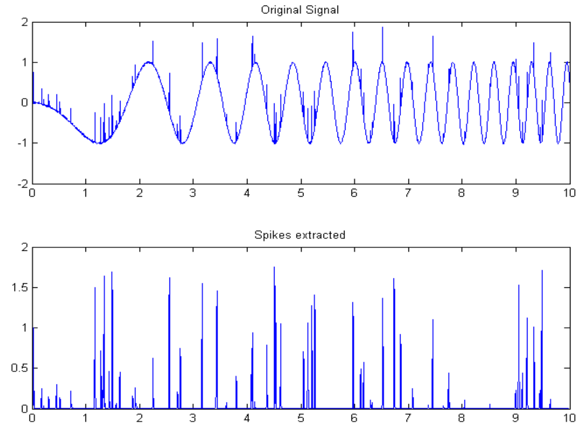
```
Peak       Position     Height       Width     Area
Beginning   206.69    3.0191e+005   0.81866   2.4636e+005
Middle      202.65    1.5481e+005   2.911     4.797e+005
End         197.42        81906     1.3785    1.1994e+005
```

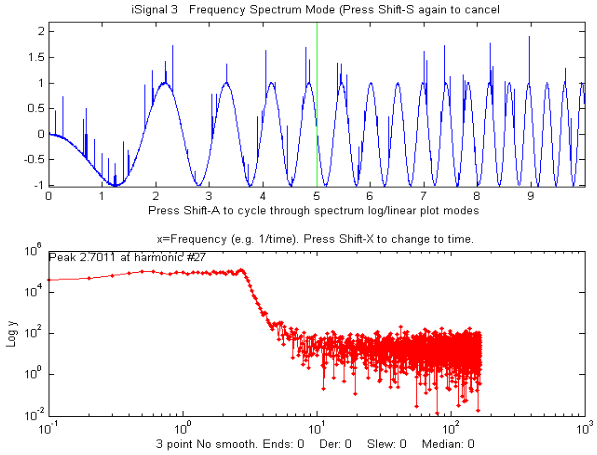
The precision of the peak position (i.e. frequency) is about 0.2% relative, by the “bootstrap method”, page 41), good enough to allow accurate calculation of the frequency shift and the speed of the vehicle. Also the ratio of the second harmonic to the fundamental for these data is 2.0023, which is *very* close to the expected theoretical value of 2.

Appendix G: Measuring spikes

Spikes, narrow pulses with a width of only one or two points, are sometimes encountered in signals as a result of an electronic “glitch” or stray pickup from nearby equipment. On page 15, we saw that narrow spikes can easily be eliminated by the use of a “median” filter. But it is possible that in some experiments the spikes *themselves* are important and that it is required to count or measure them in the presence of interfering signals. That opens up some interesting twists on the usual procedures.

For example, the Matlab/Octave simulation **SpikeDemo1.m** creates a waveform (top panel of figure below) in which a series of spikes are randomly distributed in time, contaminated by two types of noise: white noise (page 7) and a large-amplitude oscillatory interference simulated by a swept-frequency sine wave. Direct application of findpeaks or iPeak does not work well in this case because the baseline of the spikes is relatively large and highly variable.




Log y
x=Frequency (e.g. 1/time). Press Shift-X to change to time.
Peak 2.7011 at harmonic #27
10^{6}
10^{4}
10^{2}
10^{0}
10^{-2}
10^{-1}
10^{1}
10^{3}

iSignal 3   Frequency Spectrum Mode (Press Shift-S again to cancel

Press Shift-A to cycle through spectrum log/linear plot modes

3 point No smooth. Ends: 0   Der: 0   Slew: 0   Median: 0

A single-point spike, called a delta function in mathematics, has a power spectrum that is *flat*; that is, it has *equal power at all frequencies*, just like white noise, so there is not much hope of reducing the white noise by smoothing or low-pass filtering without broadening and shortening the spikes. But the oscillatory interference in this case is located in a specific range of frequencies, which leads to some interesting possibilities. One approach would be to use a Fourier filter (page 34), for example, a notch or band-reject filter to remove the troublesome oscillations selectively. But if the objective of the measurement is only to count the spikes and measure their times, a simpler approach would be to

(1) compute the second derivative (which greatly amplifies the spikes relative to the oscillations),

(2) smooth the result slightly (to limit the white noise amplification caused by differentiation),

(3) invert the result and count the positive peaks.

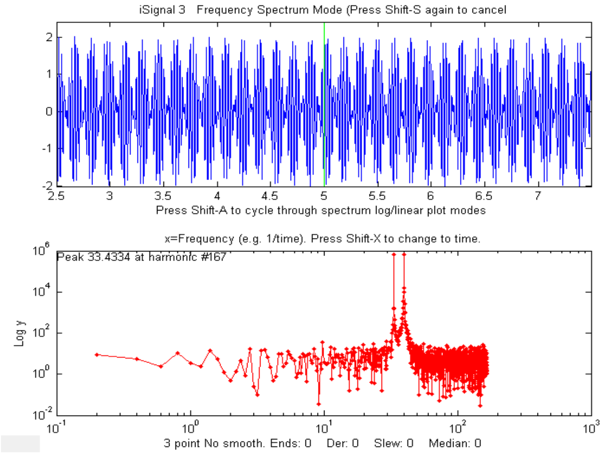
The first two steps can be done in a *single line* of Matlab/Octave code:

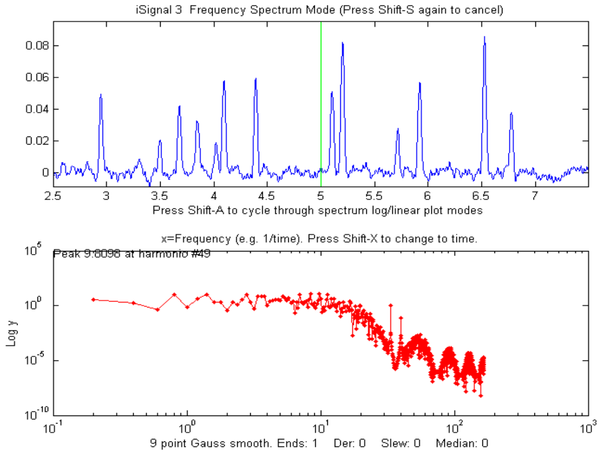
```
>> y1=-fastsmooth((deriv2(y)),3,2);plot(x,y1)
```

The result, shown the lower panel of the figure on the left above, is an almost complete extraction of the spikes, which can then be counted with **findpeaksG.m** or **peakstats.m** or **iPeak.m**, e.g.

```
P=ipeak([x;y1],0,0.1,2e-005,1,3,3,0.2,0);
```

The second simulation, **SpikeDemo2.m**, is similar except that in this case a very strong oscillatory interference is caused by *two* fixed-frequency sine waves at a higher frequency, which completely obscure the much weaker spikes in the raw signal (top panel of the left figure below). In the power spectrum (bottom panel, in red), the oscillatory interference shows as two sharp peaks that dominate the power spectrum and have a huge maximum intensity of 10^6, whereas the spikes show as the much lower broad flat plateau at about 10. In this case, use can be made of an interesting property of sliding-average smooths, such as the boxcar, triangular, and Gaussian smooths (page 11, 16); their frequency responses exhibit a series of deep dips or cusps at frequencies that are inversely proportional to their filter widths. So this suggests the possibility of suppressing specific frequencies of oscillatory interference by adjusting the filter widths appropriately. Since the signal in this cases are spikes that have a flat power spectrum, they are simply smoothed by this operation, which will reduce their heights and increase their widths, but will have little or no effect on their number, x-axis positions, or areas. In this particular case a 9 or 10-point pseudo-Gaussian is about optimum.





In the figure on the right, you can see the effect of applying this filter; the spikes, which were not even visible in the original signal, are now cleanly extracted (upper panel), and you can see in the power spectrum (right lower panel, in red) that the oscillatory interference is reduced by about a factor of about 10^6. This simple operation can be performed by a single command-line function, adjusting the smooth width (second input argument) by trial and error to minimize the oscillatory interference:

```
  y1=fastsmooth(y,9,3);
```

The extracted peaks can then be counted with any of the peak finding functions, such as:

```
 P=findpeaks(x,y1,2e-005,0.01,2,5,3);
```

or

```
 P=findpeaksplot(x,y1,2e-005,0.01,2,5,3);
```

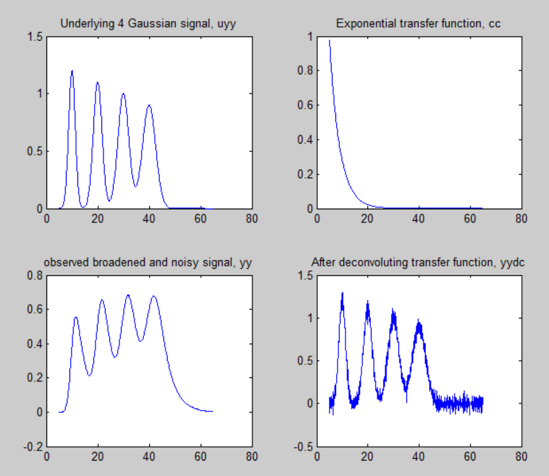
or

```
 PS=peakstats(x,y1,2e-005,0.01,2,5,3,1);
```

The simple script “iSignalDeltaTest” demonstrates the power spectrum of the smoothing and differentiation functions of **iSignal** by applying them to a delta function. Use the keypress controls of this program to change the smooth type (**S** key), smooth width **A** and **Z** keys), and derivative order (**D** key) and other functions in order to see how the power spectrum changes. Press **K** to display a list of keypress controls.

Appendix H: Fourier deconvolution vs curve fitting (they are not the same)

Some experiments produce peaks that are distorted by convolution by processes (in this example, exponential broadening, page 66) that make peaks less distinct and modify their position, height and width. Fourier deconvolution (page 32) and iterative curve fitting (page 54) are two methods that can help to measure the true underlying peak parameters, assuming that the exponential broadening time constant is known or can be estimated or measured. In the simulation below, the *underlying* signal (**uyy**) is a set of four Gaussians, but the *observed* signal (**yy**) is broadened exponentially by the function **cc**, resulting in shifted, shorter, and wider peaks, and then a little constant white noise is added *after* the broadening.



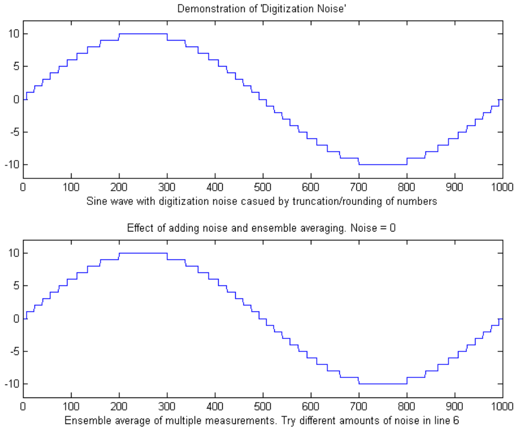
```
xx=5:.1:65;
% Underlying Gaussian peaks with unknown heights, positions, and widths.
uyy=modelpeaks2(xx,[1 1 1 1],[1.2 1.1 1 .9],[10 20 30 40 50],[3 4 5 6],...
[0 0 0 0]);
% Observed signal yy, with noise added AFTER the broadening convolution
Noise=.001; % Try larger amounts of noise to see how this method handles it.
yy=modelpeaks2(xx,[5 5 5 5],[1.2 1.1 1 .9],[10 20 30 40 50],[3 4 5 6],...
[-40 -40 -40 -40])+Noise.*randn(size(xx));
% Compute transfer function, cc,
cc=exp(-(1:length(yy))./40);
% Attempt to recover original signal uyy by deconvoluting cc from yy
% It's necessary to zero-pad the observed signal yy as shown here.
yydc=deconv([yy zeros(1,length(yy)-1)],cc).*sum(cc);
subplot(2,2,1);plot(xx,uyy);title('Underlying four Gaussian signal, uyy');
subplot(2,2,2);plot(xx,cc);title('Exponential transfer function, cc')
subplot(2,2,3);plot(xx,yy);title('observed broadened and noisy signal, yy');
subplot(2,2,4);plot(xx,yydc);title('Recovered underlying signal, yydc')
```

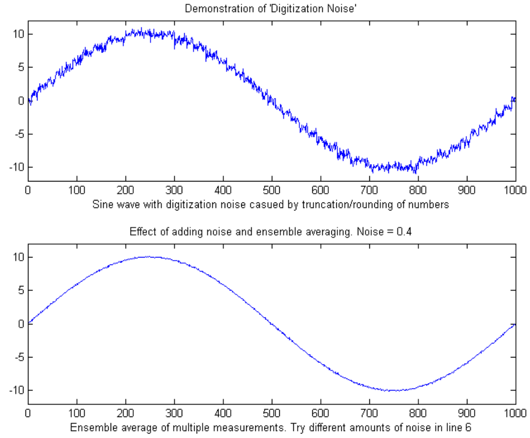
The deconvolution of **cc** from **yy** successfully removes the broadening (**yydc**), but at the expense of a substantial noise increase. However, the extra noise in the deconvoluted signal is *high-frequency weighted* ("blue", see page 8, 64) and so is easily reduced by smoothing and *has less effect on least-squares fits than does white noise*. To plot the recovered signal overlaid with the underlying signal: `plot(xx,uyy,xx,yydc)`. To plot the observed signal overlaid with the underlying signal: `plot(xx,uyy,xx,yy)`. Excellent values for the original underlying peak positions, heights, and widths can be obtained by curve-fitting the recovered signal to four Gaussians: `[FitResults, FitError]= peakfit([xx;yydc],26,42,4,1,0,10);` click for a graphic. But for a greater challenge, try more noise in line 6 or a bad estimate of time constant in line 10. With *ten times* the previous noise level (Noise=.01), the values of peak parameters determined by curve fitting are still quite good, and even with *100x more noise* (Noise=.1) the peak parameters are more accurate than you might expect for that amount of noise (That's because the noise is *blue*).

An alternative to the above deconvolution approach is to curve-fit the observed signal *directly* with an *exponentially broadened* Gaussian (shape number 5): `[FitResults,FitError]= peakfit([xx;yy],26,50,4,5,40,10)`. Both methods give good values of the peak parameters, but the deconvolution method is *faster*, because fitting with a simple Gaussian model is faster than fitting with a exponentially broadened peak model, especially if the number of peaks is large (page 101). But an advantage of the curve fitting method is that, if the exponential factor is not known, it can be measured by fitting one peak of the observed signal using peakfit.m version 7 with shape number 31, which *measures* the time constant as an iterated variable. If the time constant is expected to be the same for all peaks, you can use shape 31 to measure the time constant of *one* peak (preferably an isolated one with a good signal-to-noise ratio), then apply that fixed time constant with shape 5 to *all the other* groups of overlapping peaks.

Appendix I: Digitization noise - can adding noise really help?

Digitization noise, also called quantization noise, is an artifact caused by the rounding or truncation of numbers to a fixed number of figures. It can originate in the analog-to-digital converter that converts an analog signal to a digital one, or in the circuitry or software involved in transmitting the digital signal to a computer, or even in the process of transferring the data from one program to another, for example in copying and pasting data to and from a spreadsheet. The result is a series of non-random steps of equal height, as shown in the figure below. The frequency distribution is *white*, because of the sharpness of the steps, as you can see by observing the power spectrum.





The figure on the left, top panel, shows the effect of integer digitization on a sine wave with an amplitude of +/- 10. *Ensemble averaging* (page 7), which is usually a highly effective noise reduction technique, does not reduce this type of noise (bottom panel) because it is non-random.

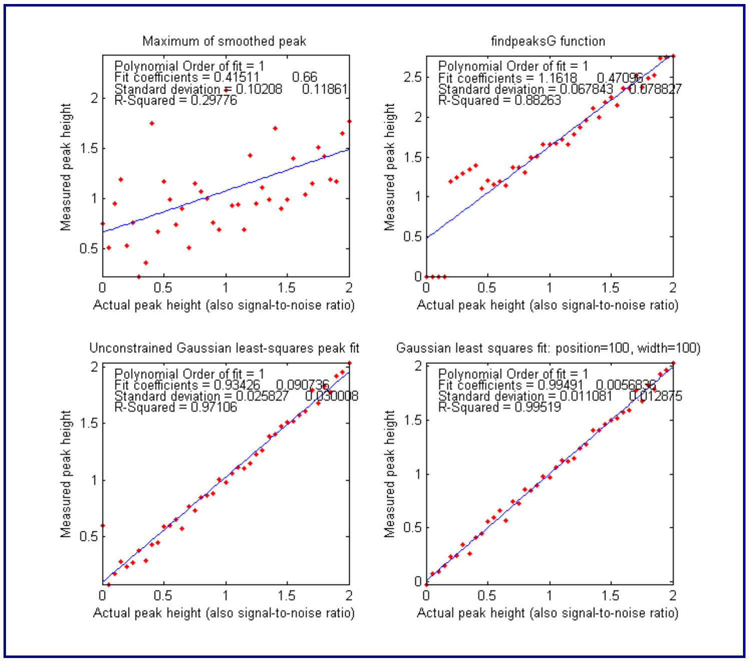
Interestingly, if additional *random* noise is present in the signal, then ensemble averaging becomes effective in reducing *both* the random noise *and* the digitization noise. In essence, the added noise randomizes the digitization, allowing it to be reduced by ensemble averaging. Moreover, if there is insufficient random noise already in the signal, *it can actually be beneficial to add additional noise* artificially! The Matlab/Octave script RoundingError.m illustrates this effect, as shown the figure above on the right. The top panel shows the sine wave with both digitization noise and added random noise (generated by the randn.m function), and the bottom panel shows an ensemble average of 100 repeats. The optimum standard deviation of random noise is about 0.36 times the quantization size, as you can demonstrate by adding lesser or greater amounts via the variable *Noise* in line 6 of this script. This technique is called "dithering"; it is also used in audio and in image processing.

Appendix J: How Low can you Go? Signals with very low signal-to-noise ratios.

This section simulates the application of several techniques described in this paper to the quantitative measurement of a peak that is buried in excess of random noise, where the signal-to-noise ratio is below 2, plus a flat non-zero baseline. The Matlab/Octave script LowSNRdemo.m performs the simulations and calculations and compares the results graphically, *focusing on the behavior of each method as the signal-to-noise ratio approaches zero*. Four methods are compared:

(1) simple peak-to-peak measurement of the smoothed signal and background (page 11);
(2) a peak finding method based on the findpeakG function (page 74);
(3) unconstrained iterative least-squares fitting (INLS) based on the peakfit.m function (page 54);
(4) constrained classical least squares fitting (CLS) based on the cls2.m function (page 49).

The measurements are carried out over a range of peak heights for which the signal-to-noise ratio varies from 0 to 2 (similar to the picture at the bottom of page 13). The noise is random, constant, and white. Each time you run the script, you get another sample of the random noise.



Results for the initial values in the script are show in the plots on the left and in the table printed below, both of which are created by the script LowSNRdemo.m. The graphs on the left show correlation plots of the measured peak height vs the real peak height, which should ideally be a straight line with a slope of 1, an intercept of zero, and an R of 1. As you can see, the simplest smoothed-peak method (upper left) is completely inadequate, with a low slope (because smoothing reduces peak height) and a high intercept (because even smoothed noise has a non-zero peak-to-peak value). The findpeaks function (upper right) works OK for higher peak heights but fails completely below a signal-to-noise ratio of 0.5 because the peak height falls below the amplitude threshold setting and because the baseline (set in line 7) is not corrected. In comparison, the two least-squares techniques work much better, reporting much better values of slope, intercept, and R^2. But if you look closely at the *low* end of the peak height range, near zero, you can see that the values reported by the *unconstrained* fit (lower left) occasionally stray far from the line, whereas the *constrained* fit (lower right) decrease gracefully all the way to zero every time you run the script. (The reason why it's even possible to make measurements at such low signal-to-noise ratios is that the data density is very high: that is, there are many data points in each signal: about 1000 points across the half-width of the peak in the initial script).

The results are summarized in the table below. The height errors are reported as a percentage of the maximum height (initially 2). You can see that the CLS method has a slight edge in accuracy, but you have to consider also that this method works well only if the peak shape, position, and width are *known*. The unconstrained iterative method *can track changes* in peak position and width. (For the first three methods, the peak position is also measured and its relative accuracy is reported. The constrained classical least squares fitting does not measure peak position but rather assumes that it remains fixed at the initial value of 100).

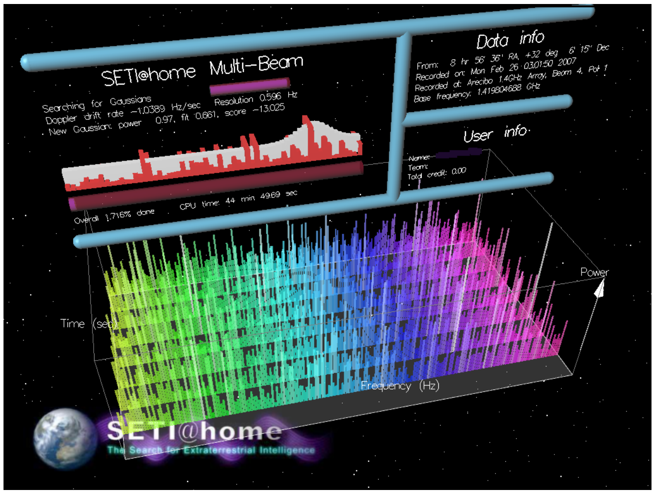
```
Number of points in half-width of peak: 1000
Method          Height Error      Position Error
Smoothed peak     21.2359%          120.688%
findpeaksG.m      32.3709%          33.363%
peakfit.m         2.7542%           4.6466%
cls2.m            1.6565%
```

You can change several of the factors in this simulation to test the robustness of these methods. Search for the word 'change' in the comments for values that can be changed. You can reduce MaxPeakHeight (line 8) to make the problem harder, or change peak position and/or width (lines 9 and 10) to show how the CLS method fails. As usual, the more you know, the better your results. Change the increment (line 4) to change the data density; more data is always better.

LowSNRdemo.m also computes the power spectrum of the signal and the amplitude (square root of the power) of the fundamental, where most of the power of a broad Gaussian peak falls, and plots it in Figure(2). The correlation to peak height is similar to the CLS method.

The 21st century is the era of "big data", where high-speed automated data acquisition can acquire, store, and process greater quantities of data than ever before. As this little example shows, greater quantities of data allow researchers to probe deeper and to measure smaller effects than ever before.

Appendix K: Signal processing and the search for extraterrestrial intelligence


SETI@home Multi-Beam

Searching for Gaussians Resolution 0.596 Hz
Doppler drift rate -1.0389 Hz/sec
New Gaussian: power 0.97, fit 0.661, score -13.025

Overall 1.716% done CPU time: 44 min 49.69 sec

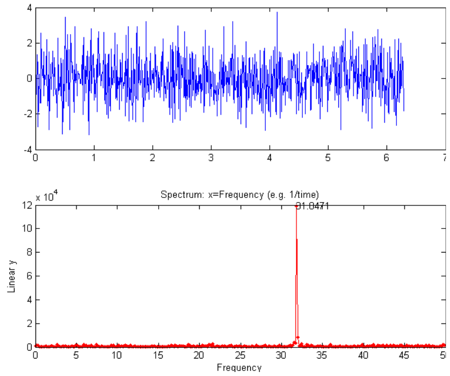
Data info

From: 8 hr 56' 36" RA, +32 deg 6' 15" Dec
Recorded on: Mon Feb 26 03:01:50 2007
Recorded at: Arecibo 1.4GHz Array, Beam 4, Pol 1
Base frequency: 1.419804688 GHz

User info

Name:
Team:
Total credit: 0.00

The signal detection problems facing those who search the sky for evidence of extraterrestrial civilizations or interesting natural phenomena are enormous. Among those problems are the fact that we don't know much about what to expect. In particular, we don't know exactly where to look, or what frequencies might be used, or the possible forms of the transmissions. Moreover, the many powerful sources of natural and terrestrial sources of interfering signals must not be confused for extraterrestrial ones. There is also the massive computer power required, which has driven the development of specialized hardware and software as well as distributed computation over thousands of Internet-connected personal computers across the world using the SETI@home computational screen-saver shown above. Although many of the computational techniques used in this search are far more sophisticated than those in this paper, they begin with the basic concepts covered in this paper.



One of the reoccurring themes of this essay has been that the more you know about your data, the more likely you are to obtain a reliable measurement. In the case of possible extraterrestrial signals, we don't know much, but we do know a few things. We know that electromagnetic radiation over a wide range of frequencies is used for long-distance transmission on earth and between earth and satellites and probes far from earth. Astronomers already use radio telescopes to receive natural radiations from vast distances. In order to look at different frequencies at once, *Fourier transforms* (page 28) of the raw telescope signals are computed over multiple time segments. The figure on the right is a simulation that shows how hard it is to see a periodic component in the presence of random noise (upper panel), and yet how easy it is to pick it out in the frequency spectrum (lower panel). Also, transmissions from extraterrestrial civilizations might be in the form of groups of spaced pulses, so their detection and verification is also part of SETI signal processing.

One thing that we know for sure that the earth rotates around its axis once a day and that it revolves around the sun once a year. So if we look at a fixed direction out from the earth, the distant stars will seem to move in a predictable pattern, whereas extraterrestrial sources will remain fixed on earth. Many terrestrial radio telescopes, such as the huge Arecibo Observatory in Puerto Rico, are fixed in position and are often used to look in one selected direction for extended periods of time. The field of view of this telescope is such that a point source at a distance takes 12 seconds to pass. As SETI says: “Radio signals from a distant transmitter should get stronger and then weaker as the telescope's focal point moves across that area of the sky. Specifically, the power should increase and then decrease with a bell shaped curve (a Gaussian curve). Gaussian curve-fitting... ” (page 55) “...is an excellent test to determine if a radio wave was generated 'out there' rather than a simple source of interference somewhere here on Earth, since signals originating from Earth will typically show constant power patterns rather than curves”. Any observed 12 second peaks are re-examined with another focal point shifted towards the west to see if it repeats at the expected time and duration.

We also know that there will be a Doppler shift in the frequencies observed if the source is moving relative to the receiver; this is observed with sound waves (page 118) as well as with electromagnetic waves like radio or light. Because the earth is rotating and revolving at a known and constant speed, we can accurately predict and compensate for the Doppler shift caused by earth's motion, which is called “de-chirping” the data. (For more on the details of SETI signal processing, see SETI@home).

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