Signal detection and manipulation

Signal
- A response obtained from a stimuli
- It can arise from many sources
  - Classical Methods
  - Instruments
- Either qualitative or quantitative.

Signals

A signal is actually composed of several responses.

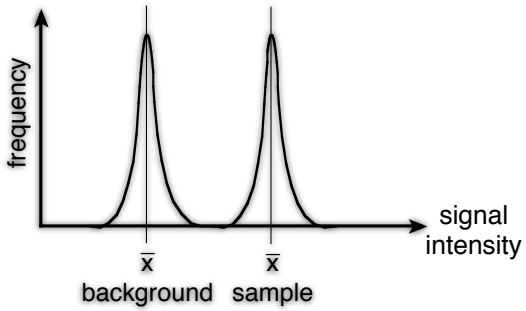
Sample response
Background response
Interference responses

Variation of signal

$\sigma^2_{signal} = \sigma^2_{sample} + \sigma^2_{background} + \sigma^2_{interferences}$

We'll deal with sample and background signals.

Signal detection



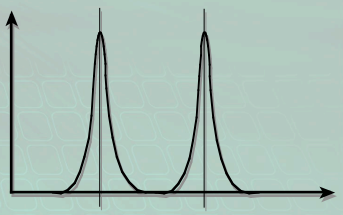
We can treat background and sample responses as normal distributions.

Signal detection

If you made a large number of background and sample measurements, you can construct a curve.

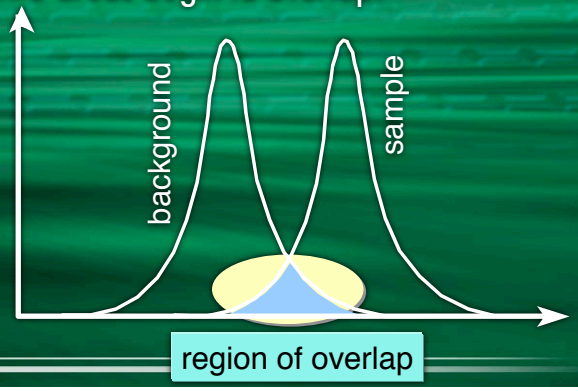
Average sample response is proportional to the amount of your analyte.

$\overline{X}_{sample} - \overline{X}_{background}$ decreases as the amount of sample is reduced.



Signal detection

As the sample signal approaches background, the two areas begin to overlap.



Limit of detection

IUPAC definition of detection limit

The amount of sample that gives a signal centered about $\mu_{bkg} + 3\ \sigma_{bkg}$

This definition assures that you will have an error and must make a decision:

"Is the signal truly above the background?"

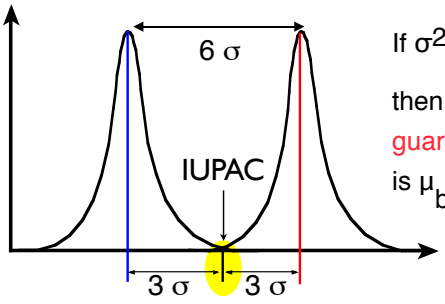
Limit of detection

The smallest amount of an analyte that can be detected with "absolute certainty."

- A sample that produces a response what a mean value of $\mu_{bkg} + 6\,\sigma$
- Detection limit - often expressed as a concentration but is actually based on signal domain (ex. volts, amps, ...)

Signal Detection

The amount of overlap is a measure of the uncertainty associated with the detection.



0.13% overlap

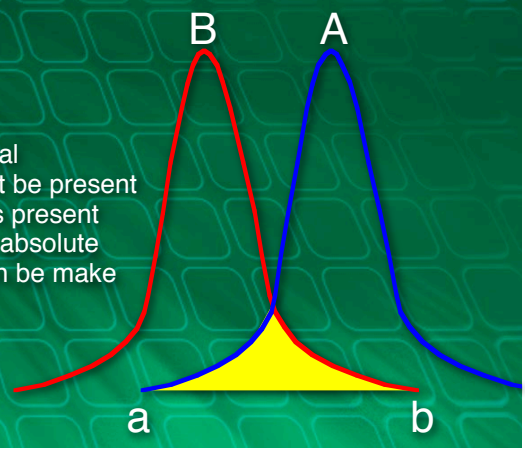
If $\sigma^2_{bkg} = \sigma^2_{sample}$ then the minimum for guaranteed detection is $\mu_{bkg} + 6\sigma$

Limit of detection

A - analyte
B - background

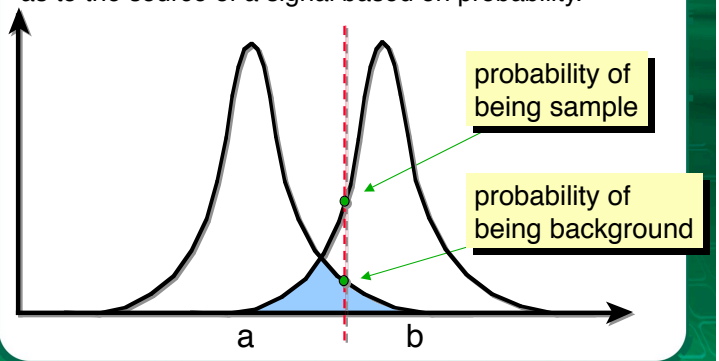
$a = \mu_A - 3\,\sigma_A$
$b = \mu_B + 3\,\sigma_B$

If measured signal
> b analyte must be present
< a no analyte is present
between no absolute decision can be make

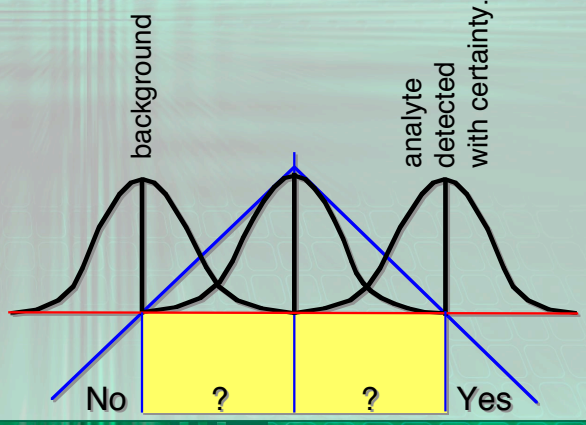


Measurement decision

Between a and b, you can give a 'best guess' as to the source of a signal based on probability.



Measurement decision



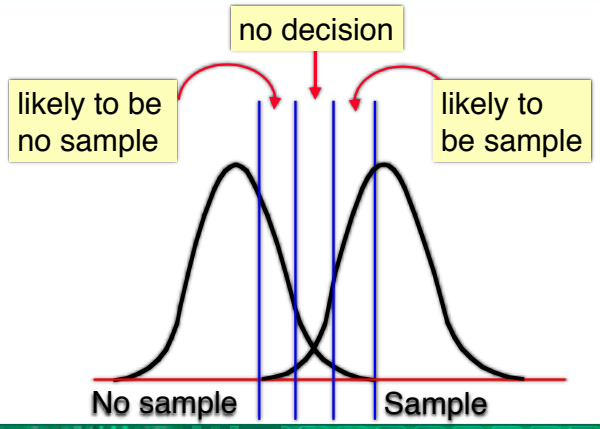
Measurement decision

It is common to be willing to accept 2-3% error. This corresponds to ~2.7 σ. This information can be found on a normal distribution table.

Now you end up with 5 regions

1. Sample must be present
2. No sample can be present
3. Confident that sample is present
4. Confident that sample is not present
5. Still not sure

Measurement decision



Improving detection limits

One way to improve detection limits is to make multiple measurements.

t tests can be used to estimate detection limits.

We'll review the basic steps involved in using this approach.

Improving detection limits

- Assume that several background measurements are made, N_B, with an average of $\bar{x}_B$ and s^2_B.
- Do the same for a sample containing the analyte and obtain N_A, $\bar{x}_A$ and s^2_A.
- If you can't run a large number of samples, assume that $s^2_A = s^2_B$
- Choose a confidence level, α, such as 0.01 - a one sided measurement so this is 99%

Improving detection limits

The number of degrees of freedom is

$$DF = N_A + N_B - 2$$

Now conduct a t test.

$$\frac{(x_{\bar{A}} - x_{\bar{B}})\sqrt{(N_A + N_B - 2)}}{\frac{1}{N_A + N_B}\sqrt{(N_A - 1)\,s_A^2 + (N_B - 1)\,s_B^2}}$$

If calculated value is ≥ t, then the mean sample is different from the background.

Improving detection limits

$$\frac{(x_{\bar{A}} - x_{\bar{B}})\sqrt{(N_A + N_B - 2)}}{\frac{1}{N_A + N_B}\sqrt{(N_A - 1)\,s_A^2 + (N_B - 1)\,s_B^2}}$$

This equation is just a modification of the t test to see if there is any significant overlap.

$$\bar{x}_{bkg} + \frac{t\,s_{x\,bkg}}{\sqrt{N}}$$

Our signal must exceed this value to be considered as coming from a sample.

Signal to noise ratio basis for calculating t

Let $D = (\bar{x}_A - \bar{x}_b)$

$$\sigma^2{}_D = \sigma^2{}_{\bar{x}A} + \sigma^2{}_{\bar{x}B} = \frac{\sigma^2{}_A}{N_A} + \frac{\sigma^2{}_B}{N_B}$$

If $\sigma^2{}_A = \sigma^2{}_B$ *then* $\sigma^2{}_D = \sigma^2\left(\frac{1}{N_A} + \frac{1}{N_B}\right)$

σ^2 can be estimated by

$$s^2 \simeq \sigma^2 \simeq \frac{(N_A - 1)\,s^2{}_A + (N_B - 1)\,s^2{}_B}{N_A + N_B - 2}$$

pooled statistics

Signal to noise ratio basis for calculating t

$$\frac{\bar{D}}{s_{\bar{D}}} = \frac{\text{Signal to noise difference}}{\text{standard deviation}}$$

$$\text{if } \frac{\bar{D}}{s_{\bar{D}}} > t, \text{ signal is detectable}$$

We're just looking to see if the difference between the two means is greater that the standard deviation.

Signal to noise

- At the 95% confidence level, the difference between the blank and sample must be at least 2.875 times larger that $s_{background}$.
- This assumes that $N_A = N_B = 10$.
- As the number of measurements increases, the minimum difference in S/N will decrease.

Signal to noise

A statistical basis for determining a minimum S/N is important however it can be difficult to apply - based on method.

Example

Chromatographic methods

S/N based on peak heights or areas?

How many points do you use?

It can be confusing if the 'response' you use has been 'processed' prior to your seeing it.

Signal to noise

With some methods, the minimum detection limit is a defined value.

Atomic Absorbance Spectroscopy.

DL = concentration at 1% absorbance.

Gas Chromatography.

DL = amount that gives a peak with an S/N=10, compared to a blank or blank area near the peak.

Precision at the detection limit

We can define the precision as the relative standard deviation (RSD), so:

precision = RSD = 100 / (S/N)
(assuming you want a % value)

At the detection limit, S/N = $t_{\alpha,v}$, so
RSD = 100 / $t_{\alpha,v}$

Example

Assume you want 95% confidence with

N = 10 (DF = 9).

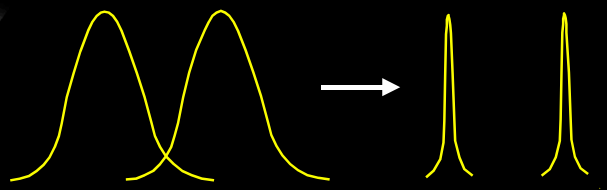
RSD = 100 / 3.25 = 30.8%

This is not very precise.

Experimentally determined detection limits may be so bad as to make quantitative analysis meaningless.

Optimization of a method

The key to improving a method is to improve the signal to noise ratio.



If we can reduce the variability of our signals, we can obtain improved S/N even though $\bar{x}_{sample} - \bar{x}_{background}$ is the same.

Optimization of a method

The first thing you should always do is:

- Assure that all experimental parameters except the analyte are invariant (constant).
- Examples - temperature, pH, solvent, matrix, instrumental conditions, steps in the procedure.
- This will result in your 'raw' data having the smallest σ^2

Optimization of a method

Prior to making each factor invariant, each must be evaluated for optimum response.

Example.

You might find that the highest response is at pH = 2 or at a λ of 356nm.

In UV/Vis, we use a λ max to two reasons - highest response AND more invariant do to minor λ variations.

You must be concerned with the response of both the blank and the sample.

This will result in the largest $(\bar{x}_{sample} - \bar{x}_{bkg})$.

Optimization of a method

After you have obtained the 'best' possible response, you may consider some type of signal treatment to improve S/N.

Types of signal treatment.

Signal averaging	Modulation
Boxcar integration	Curve fitting
Filtering	Smoothing

Signal averaging

$$Signal = \sum_{i=1}^{n} \frac{measurements}{n}$$

A very common approach which involves conducting replicate assays.

Assumptions

Response is repeatable so as to give several values that can be averaged.

Noise is random and its effect will cancel out with an average of 0.

Signal averaging

For n measurements, S/N improves by $N^{1/2}$. Requires a large number of measurements to get a significant improvement in S/N.

N	S/N improvement
2	1
4	2
9	3
16	4

Signal averaging

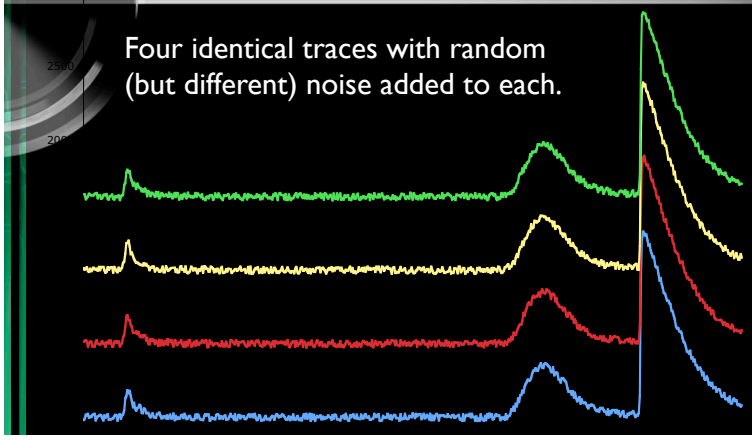
To get significant improvements, the method should be non-destructive and relatively fast.

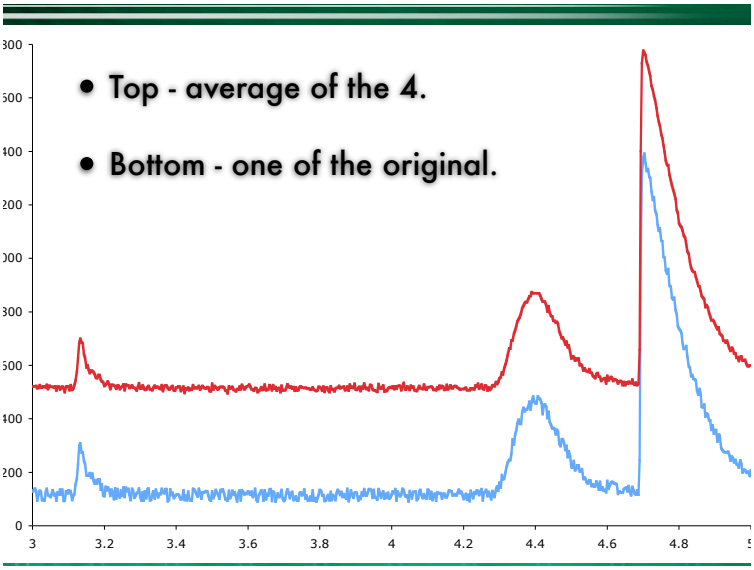
That way you will be able to collect many measurements in a short period of time.

Example. With a single λ, UV/Vis method, it is possible to make many measurements by continuously reading. The same approach would not be valid for chromatographic methods.

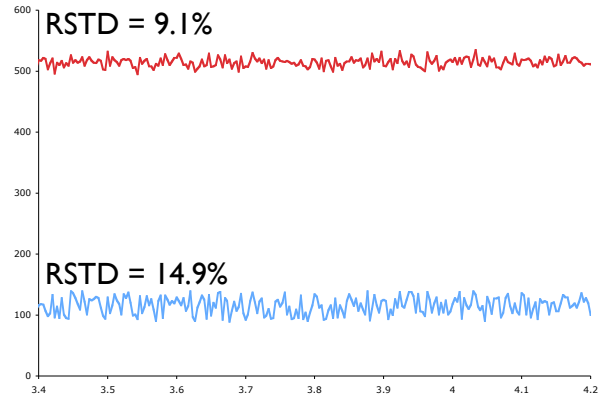
Example

Four identical traces with random (but different) noise added to each.





Another view.



Boxcar integration

A single-channel signal averager.

This method works by making multiple measurements at each point in a spectrum.

Steps

Turn detector on/off several times, storing the measured value when on.

Move to the next λ and repeat.

Boxcar integration

- This approach will increase n (the number of measurements) at each λ.
- The sample must be stable with respect to time and being analyzed.
- Method will make run times longer but not as much as taking multiple spectra.
- Goal is to cancel out noise by making several 'on-the-fly' measurements

Signal filtering and modulation

Filtering

Reduces noise by not allowing rapid changes in the signal.

Modulation

Applies a frequency to the signal that can be monitored and evaluated.

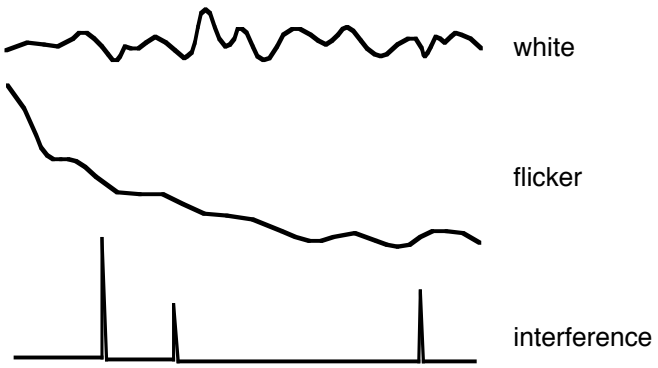
These are common approaches that can be used along with other methods. Typically are part of an instrument's electronics.

Signal filtering and modulation

Types of noise

- White
  - Random background.
- Flicker
  - Changes in response with changing operation or conditions.
- Interference
  - Noise spikes of random occurrence and intensity.

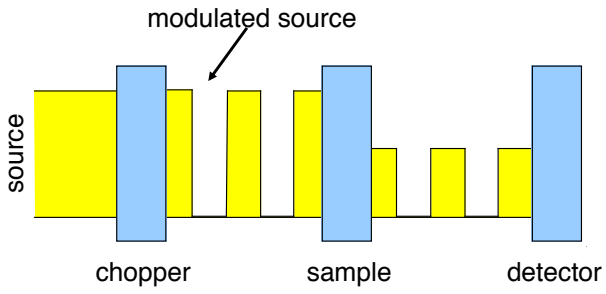
Signal filtering and modulation



Signal modulation

- Commonly done by 'chopping' the signal, usually at the source.
- Many other approaches can be used (Zeeman effect for AA).
- Detector (lock-in) will only be able to detect the modulated portion of the signal - from the sample.

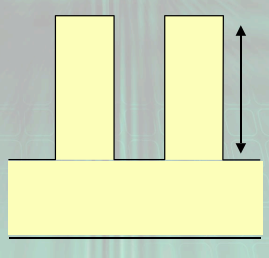
Signal modulation



Approach will reduce noise because it does not occur at the same frequency as the signal.

Signal modulation

The approach will also eliminate flicker and signal drift.



We can look at the ratio of background to sample.

This allows for the elimination of drift and flicker.

Rate must be significantly greater than sample changes

Post collection improvement of signal quality

Curve fitting.

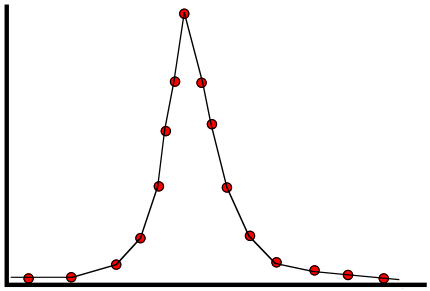
Estimate signal parameters such as maximum amplitude, area, general shape

Smoothing, deconvolution, differentiation.

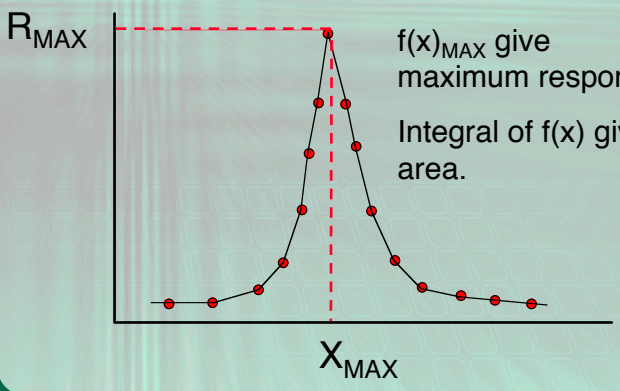
Enhance quality of data.

Curve fitting

Application of a function that approximates the data. Common in chromatography and spectral signals.



Curve fitting



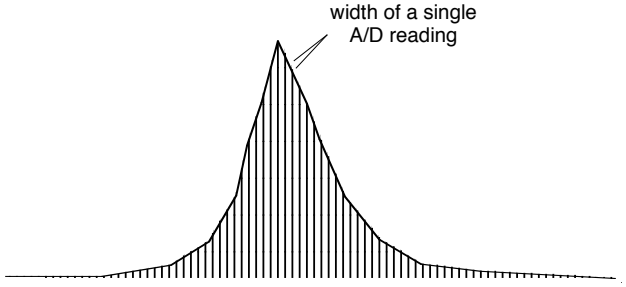
Curve fitting

You don't need to actually generate a function (or fit one) to get the desired results.

Lets look at how software for chromatographic integration works for detecting peaks.

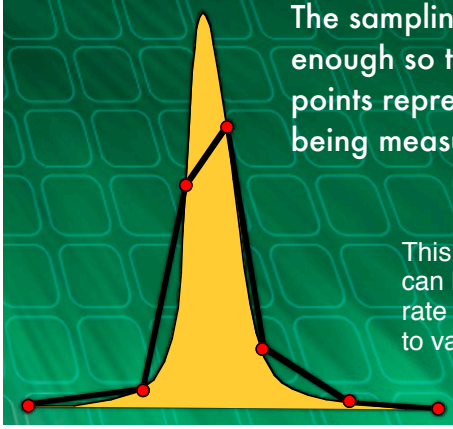
The same approaches can be applied to many other types of signals.

Peak recognition



A peak is initially subjected to A/D conversion. This results in a series of discrete measurements at known time internals. The instrument usually handles this.

Peak recognition



The sampling rate must be high enough so that the number of points represents the signal being measured.

This example shows what can happen if the sampling rate is too low compared to variations in the signal.

Peak recognition

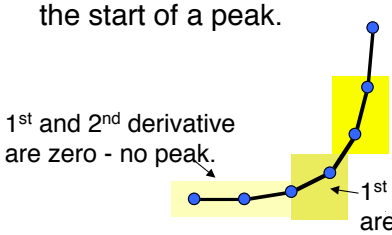
Let's assume that

- ✓ The sampling rate is high enough to give a good measure of the peak.
- ✓ Sampling is conducted at regular internals.
- ✓ The sensitivity is good enough that we can adequately see the start and end of a peak.
- ✓ Now, lets find the start, top and end of our peak and determine its area.

Peak recognition

Start of peak.

We can evaluate the change in our data (first and second derivative) as a way of detecting the start of a peak.



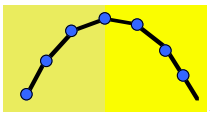
Prior to this starting, the background variation is tracked and counting won't start until you exceed a set S/N threshold.

Peak recognition

Top of peak.

We need to know the point of R_{MAX}.

positive slope



negative slope

We can look for a change in slope as a way of detecting the top of a peak.

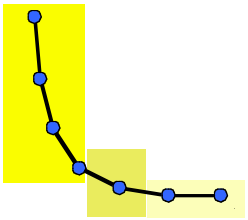
The 'true' apex can be calculated by using a quadratic fit of the surrounding points.

Peak recognition

End of peak.

Essentially the reverse of detecting the start of a peak.

Typically, a system will look for a minimum slope for termination of a peak.



The maximum peak width can also be used as a factor for ending a peak.

Peak recognition

Peak area

Determined by summing responses over the determined peak region. The baseline is typically estimated and subtracted.

Other integration options are usually available to improve the 'fit' of the model.

Examples

peak width	tangent skimming
threshold	'non-peak' baseline shifts.

Data smoothing

These methods can be used to remove small variations in your data.

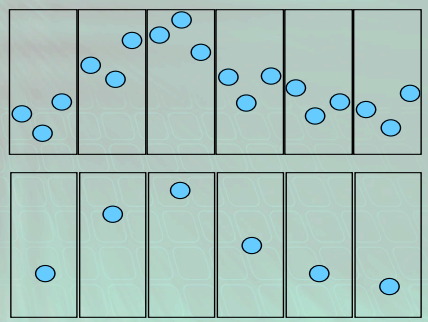
It can actually enhance larger ones. If overused, it can 'trash' all of your data.

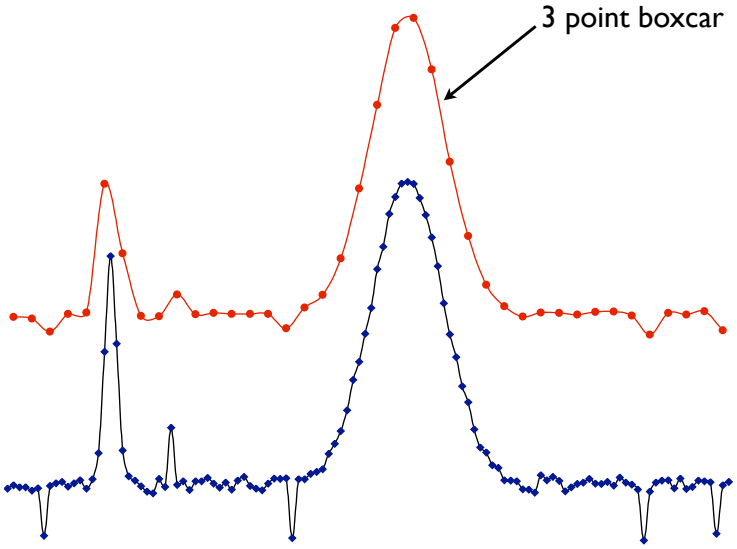
Boxcar averaging
Moving window
Golay smoothing
Least-squares polynomial smooth
Fourier transform smooth
Signal differentiation

Boxcar averaging

Takes the average or sum of a set of points as specific ranges. This is best when you must be fast and can tolerate a loss in resolution.

This approach is very useful for GC/MS



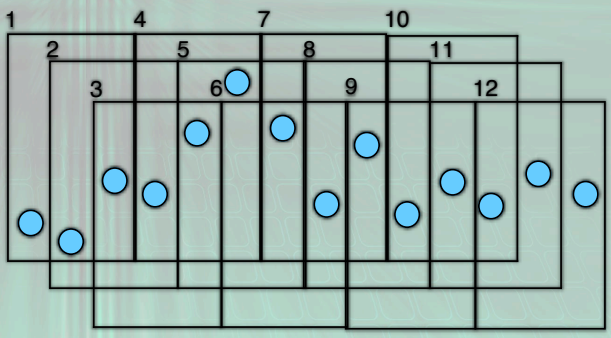


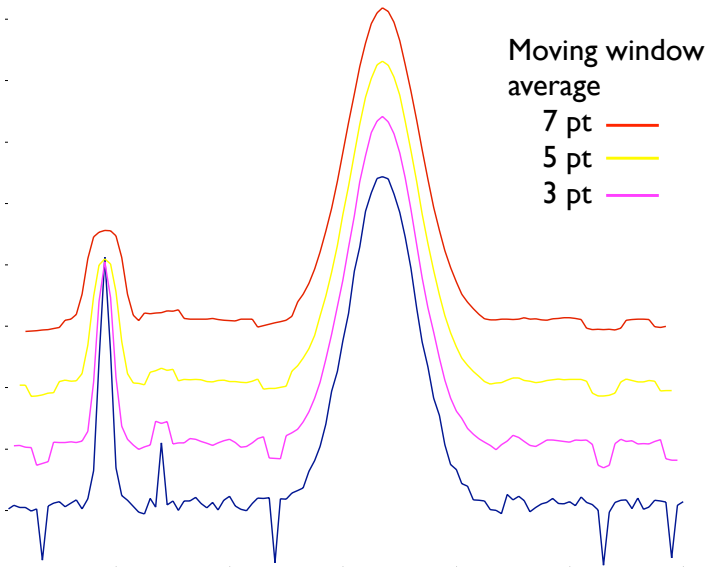
Moving window

- Pick a number of points and calculate an average.
- Store the value on a new array.
- Move over one point and repeat the process.
- You lose the first and last points for a three point smooth but the resolution of your data is maintained.

Moving window

Windows





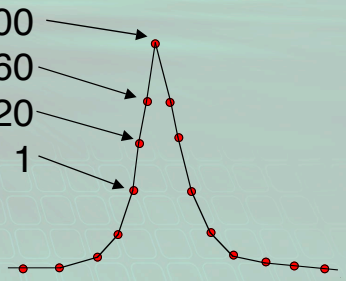
Golay smooth

- Similar to a moving window smooth but applies Gaussian weight to the points.
- Method assumes that each point is actually the center of a Gaussian peak.
- The application of the weight will enhance the peak shape if the shape is truly Gaussian.

Golay smooth

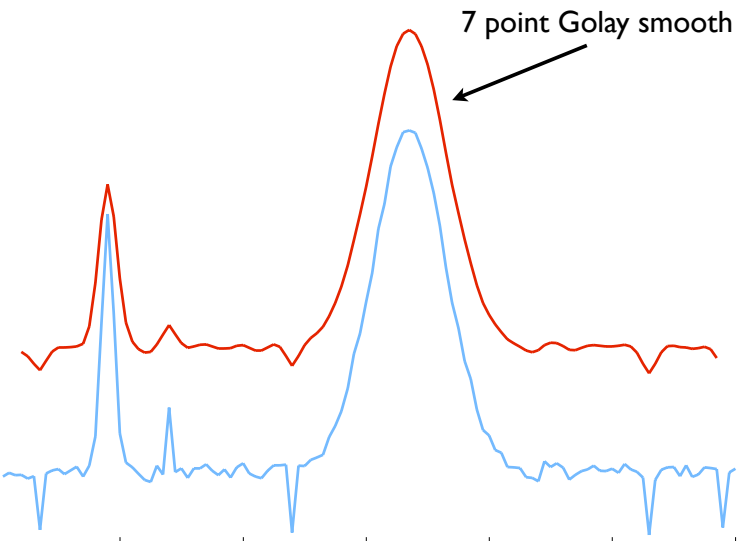
weights

100
60
20
1



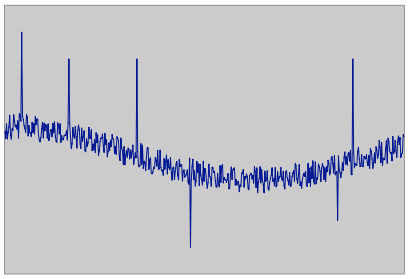
For a 7 point Golay smooth, X_{NEW} would be calculated as:

$$X_{NEW} = \frac{100\,x + 60\,(x_{-1} + x_{+1}) + 20\,(x_{-2} + x_{+2}) + 1\,(x_{-3} + x_{+3})}{262}$$

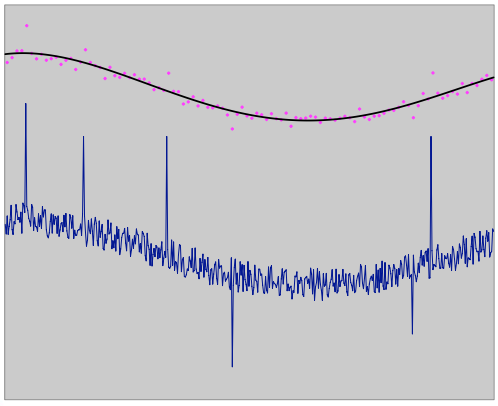


Smoothing noise.

- Here is a data set that contains 'white', 'flicker' and 'spike/interference' types of noise.



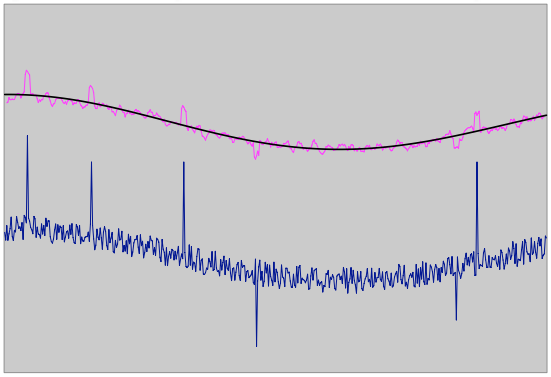
Boxcar



- Reduced white and spike noise.

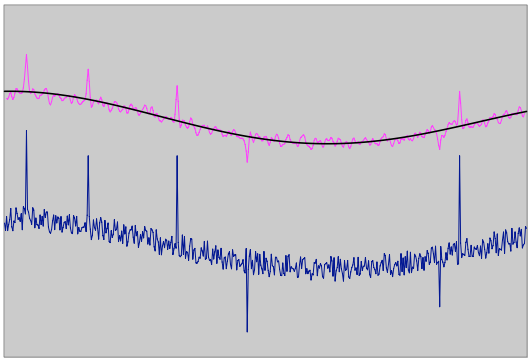
Moving window.

- Again, some improvement in white and spike.



Golay

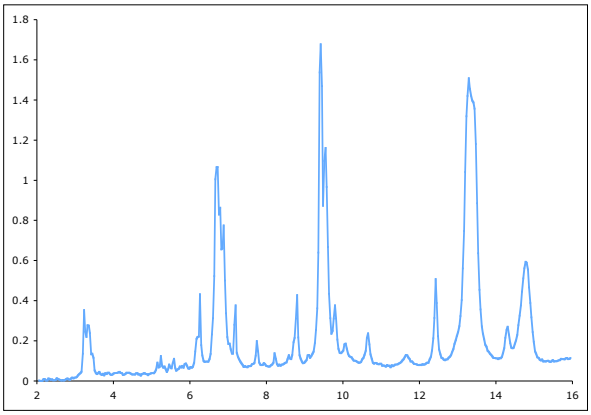
- Did the poorest job on spikes but they approximated peaks.



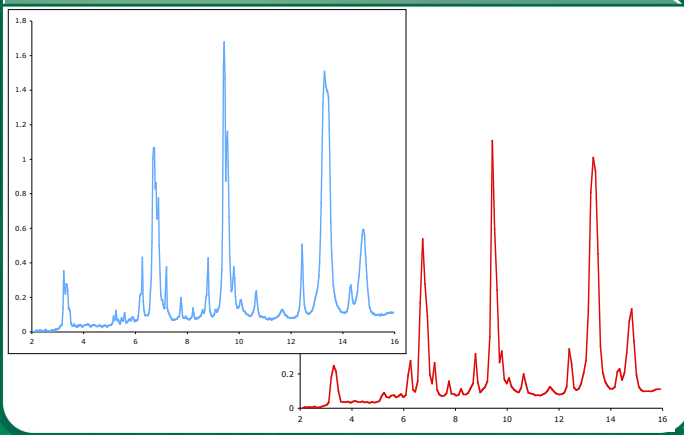
Smoothing is not always a good idea

- Let's look at a different data set.
- FT-IR of 2-chlorotoluene, condensed phase.
- Displayed in microns vs absorbance.
- Standard dataset taken from NIST databook.

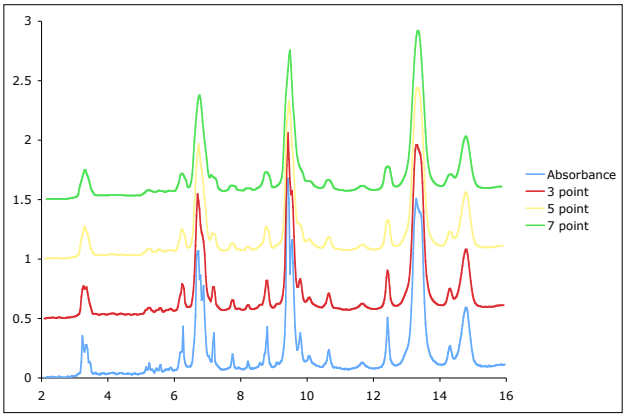
Unprocessed spectrum



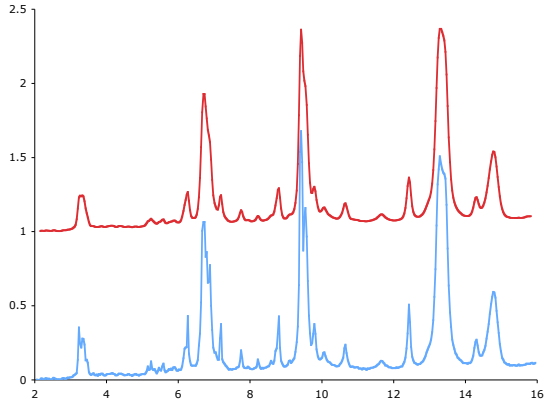
3 point boxcar (red)

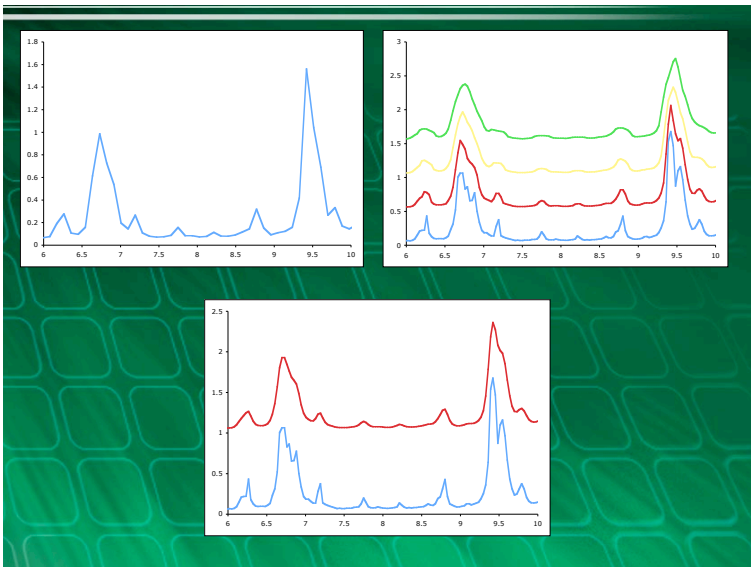


Moving average.



7-point Golay

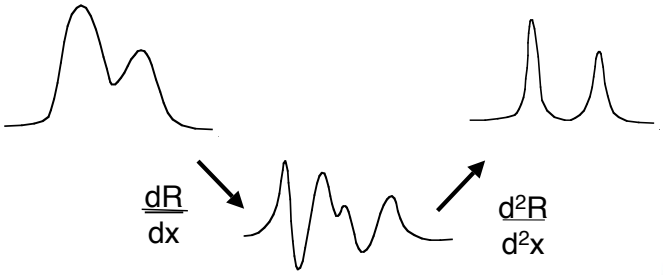




Signal differentiation

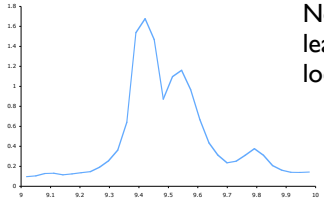
Used to make it easier to see small effects.

Examples. peak shoulder, peak resolution.



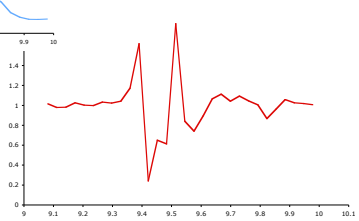
$\frac{dR}{dx}$ $\frac{d^2R}{d^2x}$

IR example again.



Not of much help in this case. At least it would help find the location of peak tops.

It turns out that you simply don't have good enough resolution for this type of filtering.



Some other methods

Least-squares polynomial smooth.

Fit a curve, linear or quadratic, to your data. Used to model a subset of the data.

Fourier transform smoothing.

Take IFT of data, apply a smooth function (typically a simple multiplier) then take FT to re-transform.