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INDIANA UNIV AT BLOOMINGTON DEPT OF CHEMISTRY
CORRELATION METHODS IN THE CHEMISTRY LABORATORY. (U)
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REPORT DOCUMENTATION PAGE

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AD A094384

1. REPORT NUMBER THIRTY-SIX		2. GOVT ACCESSION NO. AD-A094384		3. RECIPIENT'S CATALOG NUMBER 384	
4. TITLE (and Subtitle) Correlation Methods in the Chemistry Laboratory.				5. TYPE OF REPORT & PERIOD COVERED 9 Interim Technical Report.	
7. AUTHOR(s) 10 Gary M. Hieftje and Gary Horlick				8. CONTRACT OR GRANT NUMBER(s) 15 N 14 C 0838 N00014-76-C-0838	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry Indiana University Bloomington, Indiana 47405				10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 51-622 (13) 2	
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research Washington, D.C.				12. REPORT DATE January 30, 1981	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)				13. NUMBER OF PAGES	
				15. SECURITY CLASS. (of this report) UNCLASSIFIED	
				15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
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18. SUPPLEMENTARY NOTES
Prepared for publication in AMERICAN LABORATORIES B

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
convolution
cross correlation
autocorrelation
signal processing
correlation review

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
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Contract ~~NA~~-76-C-0838

Task No. NR 051-622

TECHNICAL REPORT NO. 36

CORRELATION METHODS IN THE CHEMISTRY LABORATORY

by

Gary M. Hieftje and Gary Horlick

Prepared for Publication

in

AMERICAN LABORATORIES

Indiana University

Department of Chemistry

Bloomington, Indiana 47405

January 30, 1981

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Correlation is the most ubiquitous of all signal processing and data treatment methods and is inherent in many of the procedures which are routinely followed in the chemistry laboratory. Yet, many chemists do not understand the fundamentals of correlation and therefore overlook many potentially useful data treatment options. In its many guises, correlation can be used to indicate the similarity of two recorded wave forms or spectra, help to smooth or average noisy data, improve the fidelity of a measurement system, separate overlapping or unresolved peaks in gas chromatography or spectroscopy, extract signals which seem to be hopelessly buried in noise, or make possible the measurement of such diverse things as the diffusion of macromolecules in solution, the electrophoretic mobility of various chemical compounds, or the sub-nanosecond kinetics of fluorescent species. In this brief paper, the basic operations inherent in the correlation process will be reviewed and the range of application of correlation methods examined. Specific examples of how correlation can be used in the chemical laboratory will be provided, and references will be cited where additional information on correlation can be found.

In general, correlation analysis simply enables one to determine the similarity between two wave forms. Ordinarily, the wave forms are considered to be time-varying functions, but need not be. Any wave form (or function) which could be recorded in any way or stored in a computer can be correlated. As a homely example of the correlation process one can cite a situation which occurred several years ago, when an extremely strong correlation was found between the birth rate in New York City and the blackout which occurred some nine months earlier. This example illustrates

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one of the important characteristics of the correlation process--a displacement of some kind, often a time-displaced delay.

The correlation process can be described mathematically in the following way:

$$C_{1,1}(\tau) = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^{+T} f_1(t) f(t-\tau) dt \quad (1)$$

$$C_{1,2}(\tau) = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^{+T} f_1(t) f_2(t-\tau) dt \quad (2)$$

These equations indicate that there are two kinds of correlation operations; Equation 1 illustrates the type called *autocorrelation* whereas Equation 2 illustrates *cross-correlation*. To understand the nature of these processes, let us examine what these equations physically represent. Equation 1 will serve as an example. In Equation 1, a time-varying wave form [$f_1(t)$] is multiplied by itself. The product is then integrated over all time and divided by the integration interval. In other words, the product is time-averaged. This time-averaged product constitutes one point of the *autocorrelation function*; in particular, that point which occurs at a selected value of τ . In this context, τ can be thought of as simply a time delay. That is, it indicates the fact that the two time-varying wave forms are not coincident in time but are displaced from each other by an amount equal to τ . Because the autocorrelation function must be expressed in terms of τ , this multiplication and averaging process must be carried out over a broad range of τ values and the results plotted versus τ . The cross-correlation process is similar to the above description,

but requires that two different wave forms [$f_1(t)$ and $f_2(t)$] must be multiplied and averaged.

It is often more intuitively satisfying to examine how the correlation process can be implemented experimentally. An instrument useful for autocorrelation is shown in Figure 1. In Figure 1, the exact steps required by Equation 1 are carried out. A wave form to be correlated is simply multiplied by a time-delayed version of itself. The resulting product is then averaged and indicates the value of the autocorrelation process at that particular delay. To trace out the entire autocorrelation function, it is only necessary to slowly sweep the delay between the original wave form and its replicate.

To appreciate the utility of the correlation method, let us examine the effect of autocorrelation on the simplest of all wave forms--a sine wave. To begin, let us compute the autocorrelation function when the delay (τ) is zero (see Figure 2a). At this setting, the sine wave will be multiplied by itself *in phase*, so that the product wave form will be a squared sine wave which is everywhere greater than zero. The average value of this sine-squared wave will then be the mean *square* value of the original sine wave and is plotted in Figure 2a as a dashed line and in Figure 2e as the corresponding point on the autocorrelation function. Let us now imagine that the time delay has been increased by an amount exactly equal to one-half period of the input sinusoidal signal (see Figure 2c). In this case, the replica wave will be exactly 180° out of phase with the original, so that its negative lobes will be multiplied by positive lobes

from the original and vice versa. The result will clearly be another sine squared wave, but which will be everywhere negative. Accordingly, the average value of this product will simply be the negative mean square of the original sine wave. This point has also been plotted on Figure 2e. Finally, if we increase the time delay by an amount exactly equal to the period of the input wave form, the replica wave will once more be in phase with the input, to produce a result similar to the first point we considered. Intermediate points between these selected values of delay will vary smoothly; moreover, the process will continue indefinitely, and repetitively, because of the continuous nature of the input wave form. The result, shown in Figure 2e is another sinusoidal wave, but one which has its maximum value at the horizontal axis (delay) point of zero. In other words, the autocorrelation function of a sine wave of arbitrary phase is a *cosine* wave of period equal to the original wave form and of amplitude equal to the mean square value of that wave. Thus, we can determine from the autocorrelation function both the frequency and the amplitude of the original wave.

Notice that the foregoing treatment imposes no constraints on the frequency or amplitude of the input sinusoid. Therefore, for a more complex input signal, which can itself be broken into a large number of individual sine waves or frequency components, a similar process will take place. In such a case, each of the sinusoidal frequency components of the complex wave form will undergo the same phase-relating process and become a cosine wave with its maximum value at the zero delay point. Four such cosine

autocorrelation images are shown in Figure 3a. Because all the frequency components will then be phase related, and will add at the zero delay point, the amplitude of that point will increase as the number and amplitude of the composite sine waves of the complex signal increase. However, beyond the zero delay point, the cosine waves will fall out of phase, being of different frequency, and might destructively interfere. For an extremely complex wave form, such as one might expect from a randomly varying pen trace on a recorder (Figure 3b), essentially all frequencies will exist, to produce a destructive interference pattern such as illustrated in Figure 3c. In such a situation, the autocorrelation function will consist ultimately of a single large point, located at $\tau = 0$, with values at all other τ being zero.

This example illustrates one of the powers of autocorrelation; that of signal-to-noise enhancement (1). When a periodic signal is difficult to measure because it varies in an apparently random fashion or is largely obscured by unwanted fluctuations (such as the wandering of a pen on a chart recorder), autocorrelation can often help extract the signal from the obscuring noise. If the signal is coherent in time, as essentially all signals are, it will yield an autocorrelation function which, like the sine wave, will contribute to the autocorrelation function even at very large values of delay. However, because noise is incoherent (that is, random), it will contribute strongly to the autocorrelation function only at small delay values. For a sinusoidal signal, originally buried in noise, the resulting autocorrelation function might look something like that in Figure 4. To extract the signal

from this *autocorrelogram*, one need only examine the trace at large values of delay.

Cross-correlation is essentially the same as autocorrelation except that the original and delayed wave forms do not emanate from the same source. Therefore, one would not expect the simple squaring behavior occurring in autocorrelation to be duplicated. However, if the two inputs (see Figure 5) happen to contain any common frequency, that frequency (consisting of a single sine wave) will behave much the same as described in autocorrelation. Of course, because the sine waves from the two different sources might not be in phase, the maximum value in the cross-correlation plot might not occur at a delay (τ) of zero. Importantly, the cross-correlation function between any two wave forms will contain all frequencies which are common to the two wave forms but will exclude all others. Moreover, the relative phases of the two input wave forms will be indicated; in other words, the cross-correlation function will indicate the temporal displacement between the two waves. To repeat the example cited earlier, a cross-correlation plot between birth rate and the illumination of the city of New York would produce a very strong peak with a delay of nine months.

In many ways, cross-correlation is even more powerful for signal extraction than is autocorrelation. Because noise present in the two inputs during cross-correlation would probably not originate from the same source, they will be incoherent and produce no output. Simply stated, each source of noise will appear random, and when two random waveforms are multiplied, their product

will be a third random pattern which will have an average value of zero. Obviously, this zero value contributes nothing to the cross-correlation function. In a typical application of cross-correlation, a signal to be measured (see Figure 5) might be cross-correlated with a reference wave form derived from the same source as the signal and which is synchronous with the signal. The resulting plot will be a noise-free indication of the signal amplitude. In the special case when the cross-correlation function is measured only at the single value of delay corresponding to the maximum overlap between signal and reference, a process called *lock-in amplification* (2) occurs. Thus, a lock-in amplifier is nothing but a cross-correlator configured for measurement at a specific delay.

Because cross-correlation yields a result which contains all the frequency components common to the two correlated wave forms, it indicates the similarity of the two wave forms. This property makes the method useful in such laboratory applications as spectral file searching (3), where the identity of an unknown substance is ascertained by comparison of its infrared, mass, or elemental emission spectrum with those of known compounds.

In addition, this property of correlation enables one to extract from a complex wave form or spectrum only those features which are of interest. This procedure, known as *matched filtering*, is useful not only to extract complex signals from others which interfere, but aids in the highlighting of specific features from such things as mass spectra or elemental emission spectra (4,5). One merely has to record a trace of the kind of features desired (for example, a spectrum of the pure compound) and then to cross-correlate that trace with one which contains potentially inter-

fering features. The resulting cross-correlation function will then be free of interference and indicate only the amplitude of the desired component.

Besides signal detection, cross-correlation operations are utilized in the chemical laboratory to smooth, differentiate, resolution enhance and deconvolve all kinds of chemical signals. In fact the classic data processing procedures presented by Savitzky and Golay (6,7) for smoothing and differentiation of wave forms are based on cross-correlation operations.

One interesting application of the correlation method is in the realm of *linear response theory*. Briefly, linear response theory enables one to characterize the temporal behavior of any desired system or network merely by applying an appropriate perturbation to the system. The simplest example of such a procedure is shown in Figure 6. In this figure, a very brief input perturbation is sent into the device to be tested. In the limit, this abrupt input perturbation resembles a true *impulse* or Dirac delta function as shown on the left hand side of Figure 6. In response to this perturbation, the system will elicit an output which indicates its time response. According to linear response theory, this specific output is termed, appropriately, the *impulse response function*. It should be recognized that such measurements are extremely common in chemistry. For example, rapid kinetics are usually determined by perturbing the chemical system which is to be examined with a very brief pulse of exciting energy. Usually, the energy is in the form of an electrical, temperature, or pressure jump, or pulse which exerts a stress upon the chemical system. In turn, the chemical system either

relaxes back to its old equilibrium point or approaches a new equilibrium situation at a rate which reflects the chemical kinetics. Similarly, in Fourier transform NMR spectroscopy, a pulse of radio-frequency energy is sent into the nuclei of interest, which then exhibit a free induction decay characteristic of the species being probed. Although not part of the perturbation process, Fourier transformation of the free induction decay then yields the desired nuclear magnetic resonance spectrum of the molecule.

Time-resolved fluorimetric measurements also employ an impulsive kind of perturbation. In this application, the perturbation is a brief, intense flash of light, which elicits from the molecule of interest a measurable fluorescence whose decay rate indicates the molecule's excited-state kinetics. Finally, even gas chromatography employs the principles embodied in linear response theory. In GC techniques, an impulse of sample is directed onto the column, so that the output of the gas chromatograph reveals the response of the instrument to that particular sample. In other words, a gas chromatogram is simply an impulse response function of the GC instrument to that particular chemical sample.

Clearly, impulse response measurements have broad applications. Moreover, from the foregoing examples it can be seen that the impulse need not be electrical in nature, but can consist of any rapidly changing energy source, from electrical to mechanical to temperature, even to the chemical sample itself. Because of this broad application and the importance of impulse-type measurements to chemistry, various alternative schemes to obtain the same information have been sought. Those scientists involved in linear response theory have explored these alternative schemes in some detail and we can benefit from their experience.

To understand these alternative approaches, let us examine the measurement portrayed in Figure 6 in more detail. First, let us recognize that the delta function or impulse used to perturb the system under study contains all frequencies; that is, its Fourier transform is flat. Consequently, sending a delta function or impulse into a system is equivalent to perturbing the system with all frequencies *at the same time*. Therefore, the impulse response function is analogous to the frequency response of the device under test, but is a time-domain representation. As a result, phase shifts, distortions, and other phenomena easily perceived in the time-domain can readily be studied.

From these simple considerations, one can imagine that different perturbation wave forms than an impulse can be used, as long as those waveforms contain a broad range of frequencies. The most common and most useful such waveform that has been studied is one which is stochastic, i.e. noisy. Like an impulse, white noise contains all frequencies, although the frequencies have random phases and amplitudes. Therefore, from the concepts embodied in linear response theory, it should be possible to obtain time-response characteristics of a system by perturbing it with white noise and observing the response of the system to that perturbation. However, one intuitively realizes that the response of the system under these conditions would also appear to be noisy. Of course, this noisy response would exhibit the same attenuations, distortions, and changes of frequency composition as would the impulse response. The trick, then, is to extract from the "noisy" response the same information that is readily discerned from the impulse response.

This "trick" is readily accomplished through use of cross-correlation. It will be recalled that the autocorrelation function of white noise is simply a delta function. Therefore, it can readily be understood that cross-correlation of white noise with a changed representation of the noise will produce no longer a delta function, but a delta function which is distorted, skewed, or changed in a way which reflects those distortions. This approach is represented schematically in Figure 7.

In Figure 7, a randomly varying signal (i.e. noise) is used to perturb the system under test. Because of the system's finite frequency response, its response to the perturbation is no longer identical in appearance to the white noise, but has lost some of its high frequency content because of the limited frequency response. Therefore, cross-correlation of the random input with the elicited response produces not a delta function, but a pulse which has attenuated high-frequency composition. In turn, this loss in high-frequency content is reflected in the finite rise and decay times of the cross-correlation function.

How do these apparently esoteric concepts apply to chemistry? In the case of nuclear magnetic resonance spectrometry (8), the random input perturbation consists of the application to the nuclei of interest not a single radio frequency pulse, but a train of such pulses which are spaced randomly in time. In this example, it is important to recognize that the amplitude of the pulses need not be random (i.e. stochastic), but only their repetition rate. Therefore, application of such a random sequence of pulses to the nuclei and cross-correlating the sequence with the apparently noisy response will produce a function identical to the free induction decay. The advantage derived from this

approach is that the rf energy can be applied to the nuclei continuously but at low amplitude, to avoid saturating the nuclei but still yield a high-quality free induction decay signal.

In a similar fashion, gas chromatography can be pursued by injecting onto a column not a single increment (delta function) of sample, but either a random sequence of sample aliquots or a randomly varying sample concentration (9). This latter kind of injection can be implemented by means of several switching schemes involving solvent and sample. Obviously, such an injection approach would produce a noisy, apparently meaningless chromatogram. However, cross-correlation of this noisy chromatogram with the input injection pattern will yield a conventional-appearing chromatogram, with the advantage that the column was used more efficiently and that continuous monitoring becomes possible. In this particular application, it is important to recognize that sample concentration fluctuations must be small to prevent the column from being overloaded and inducing non-linearity into its operation. Also, it must be recognized that no increase in the speed of separation is derived from this approach; although continuous monitoring becomes possible, the cross-correlation chromatogram which is obtained from any particular sample aliquot is only available after a length of time equal to the elution time of the slowest-moving sample component.

In time-resolved fluorimetry, use of the correlation approach poses several difficult problems, because of the short time scale on which such measurements must be made. In particular, most fluorescence decay times are on the order of nanoseconds, requiring a perturbing light source which fluctuates at extremely high (gigahertz) frequencies and a cross-correlation device which can respond to nanosecond-duration signals. Fortunately, these experimental difficulties can be overcome through use of novel measurement techniques which involve the use of laser mode noise as a perturbing source and high-frequency microwave components in the correlation instrument. The interested reader is referred to the original literature for discussions of these components (10,11).

Finally, for the measurement of chemical kinetics, random temperature, pressure, or chemical concentration fluctuations can be used to perturb the system. Perhaps the most elegant of the resulting procedures arises from the realization that chemical substances are intrinsically perturbed by the random events occurring in any medium of any temperature above absolute zero. That is, chemical reactions at equilibrium are actually dynamic systems, with forward and reverse reactions going on at all times. Therefore, if one would observe the concentration of any of the components in the equilibrium, one would observe not just an equilibrium concentration, but minute fluctuations about that concentration, because of small but fine perturbations (e.g. collisions) which occur in the medium to produce the measured substance and the random events which cause its consumption. Presuming these perturbations to be truly random, one can obtain a measure of the chemical kinetics affecting that substance by autocorrelating the fluctuations it exhibits. Although such a procedure requires one

to essentially observe single molecular events, it has been found possible to do so, using highly sensitive detection techniques such as fluorescence (12). The elegance of this procedure is obvious; not only does it enable one to observe the kinetic behavior of reactions truly at equilibrium, but it employs the natural stochastic events experienced by a molecule to probe its behavior.

From this overview, it should be evident that correlation techniques are indeed ubiquitous in the chemical laboratory. Not only are they inherently involved in many measurements which chemists take, but they can be used to advantage to extract chemical data from apparently meaningless signals, to improve the quality of chemical data, to isolate desired signal features from interfering wave forms, or they can be used to develop entirely new techniques for chemical measurements which employ the fundamental but unavoidable stochastic events which each chemical substance experiences. Hopefully, this introductory overview will stimulate the reader to explore correlation methods in more detail, to employ them in his own laboratory, and to perhaps develop new procedures based on correlation analysis.

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Additional Reading

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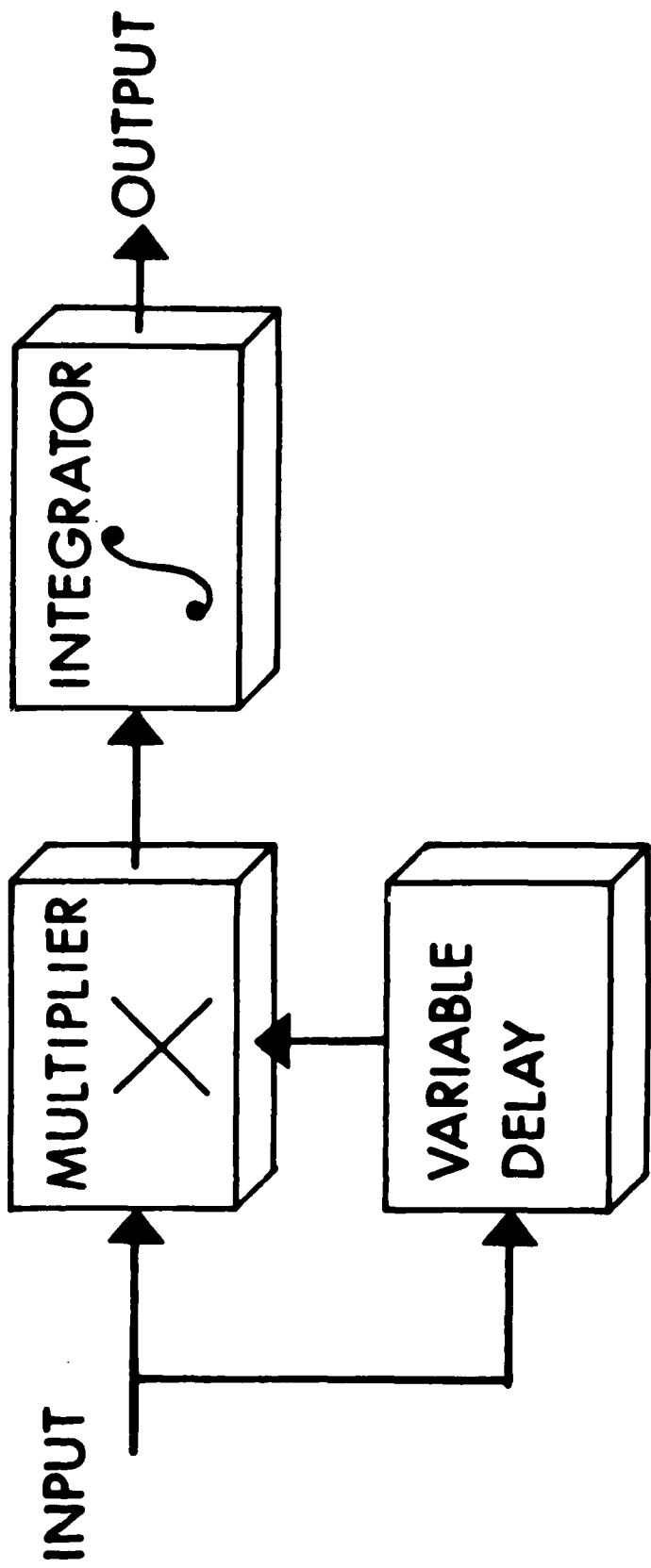
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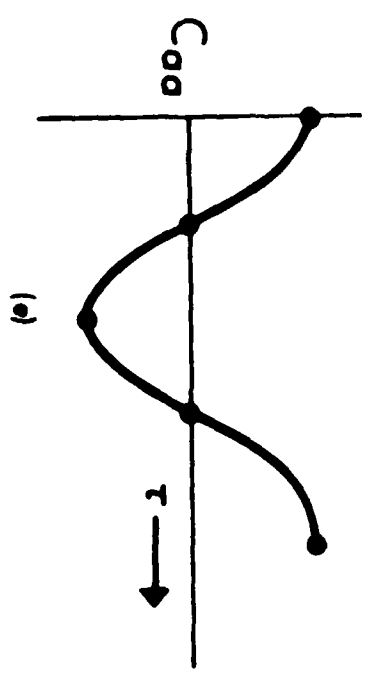
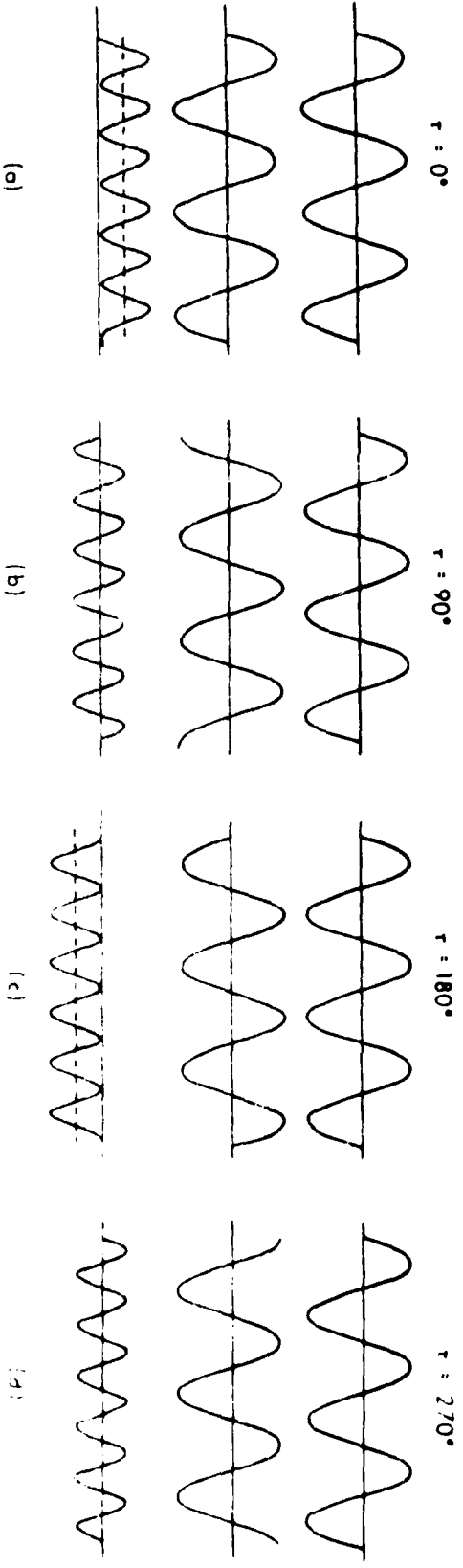
- Figure 1. Block diagram of the auto-correlation operation.
- Figure 2. Autocorrelation of a sine wave. Schematic for auto-correlation process at $\tau = 0^\circ$ (a), $\tau = 90^\circ$ (b), $\tau = 180^\circ$ (c), $\tau = 270^\circ$ (d) and the resulting auto-correlation function (e). (Figure used with permission of Plenum Publishing Corp.)
- Figure 3. (a) Cosine autocorrelation images.
(b) Random waveform.
(c) Ideal autocorrelation function of random waveform.
(Figures used with the permission of Plenum Publishing Corp.)
- Figure 4. Autocorrelation function of a noisy sine wave. (Figure used with permission of Plenum Publishing Corp.)
- Figure 5. Cross-correlation of a sine and square wave (lock-in amplifier) at $\tau = 0$. (Figure used with permission of Plenum Publishing Corp.)

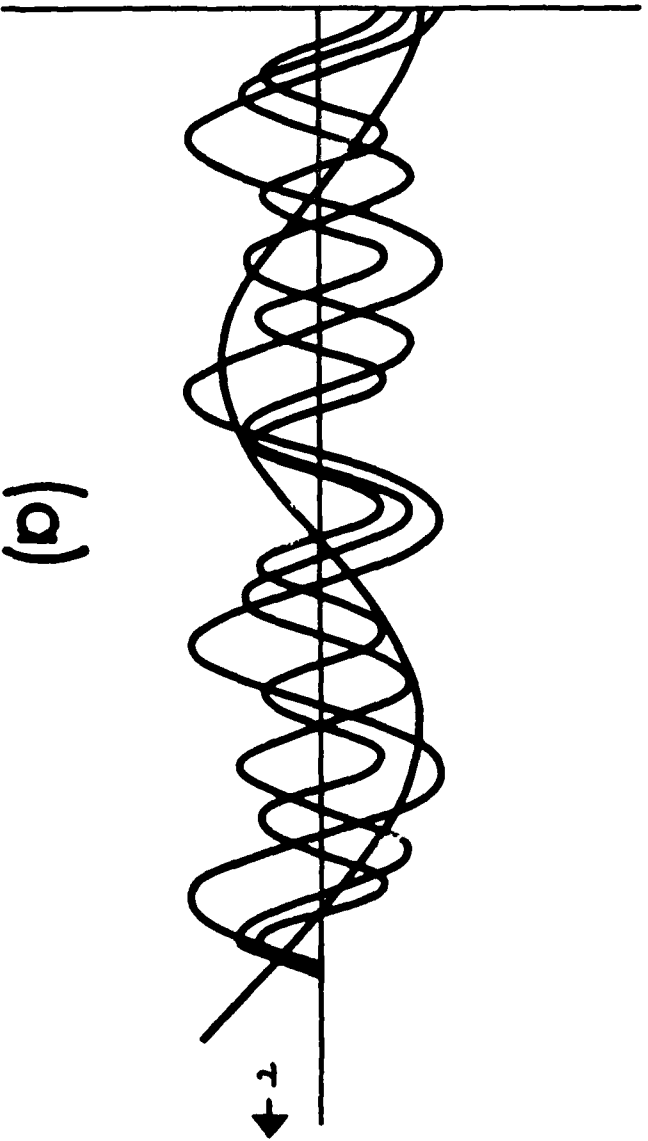
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Figure 6. Illustration of how an Impulse Response Function is obtained. The "Instrument" in this illustration might be any system to be tested, including an electronic network, a large instrumental array, or chemical species themselves. The input perturbation to the system, shown on the left, resembles mathematically a Dirac delta (δ) function and produces the impulse response shown on the right.

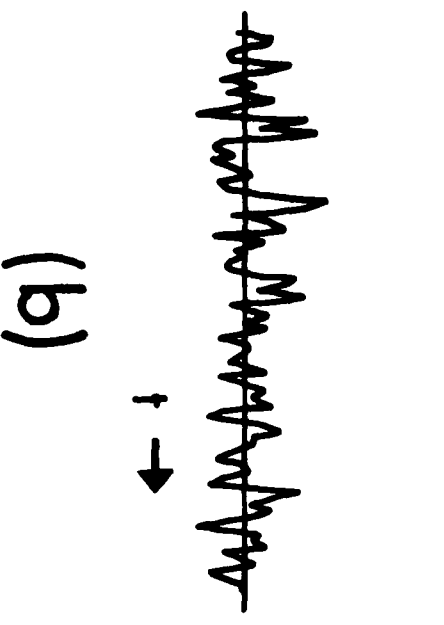
Figure 7. Use of cross-correlation and *white noise (random perturbation)* to test a system's response. The noisy perturbation (at left), when correlated with the response it elicits, generates the impulse response function of the system, shown on the right.







(a)

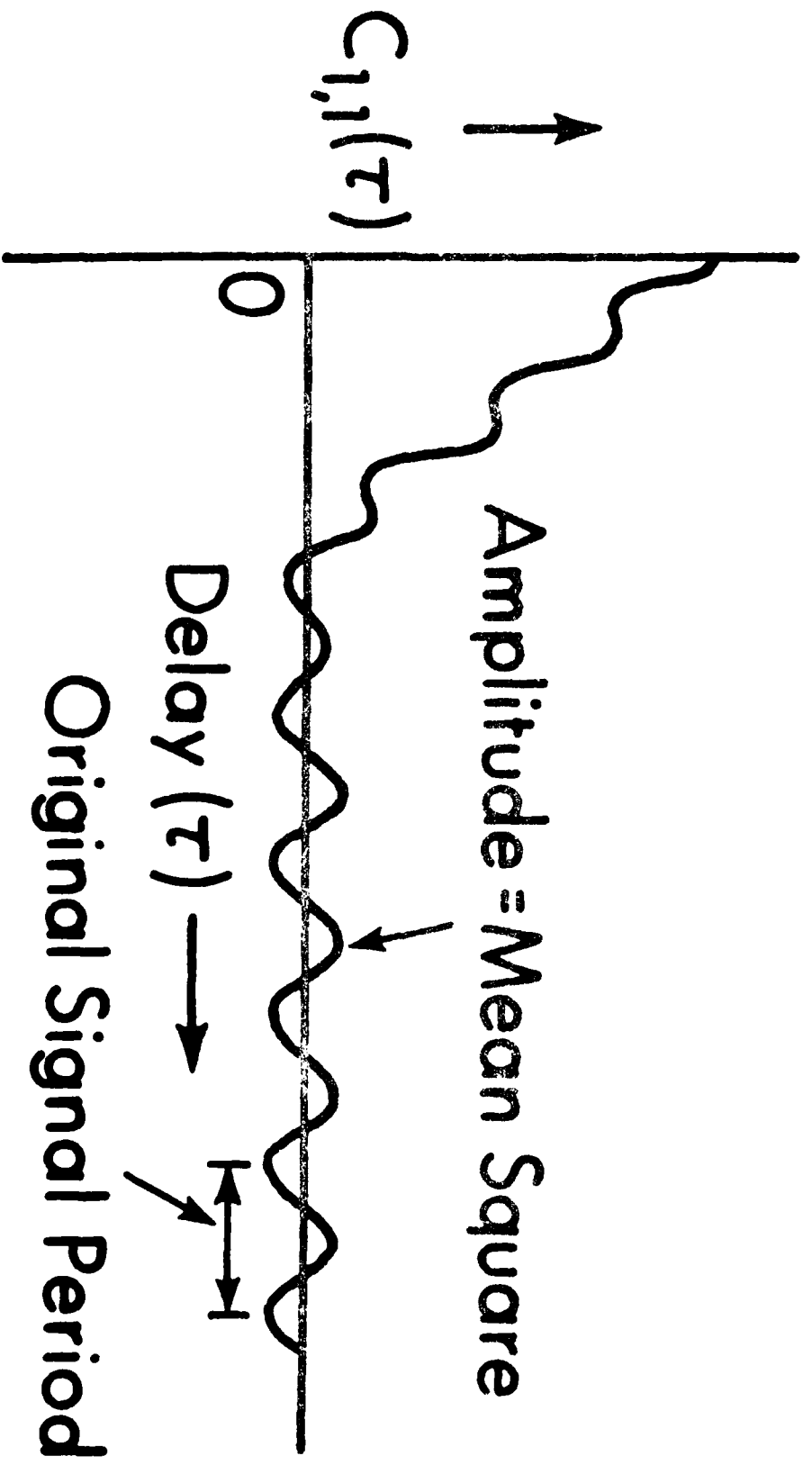


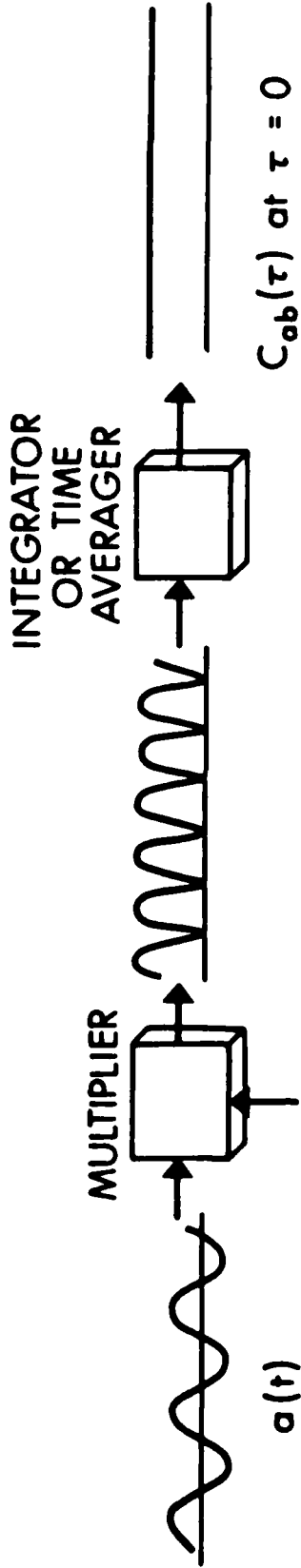
(b)

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(c)

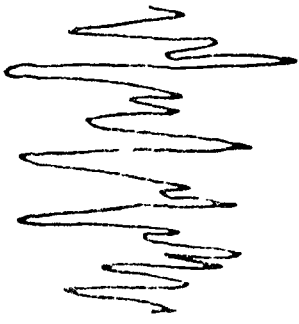




$a(t)$

$b(t)$

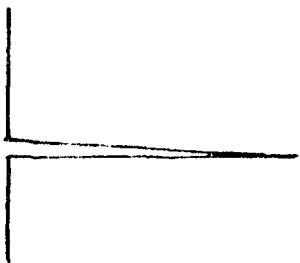
WHITE NOISE

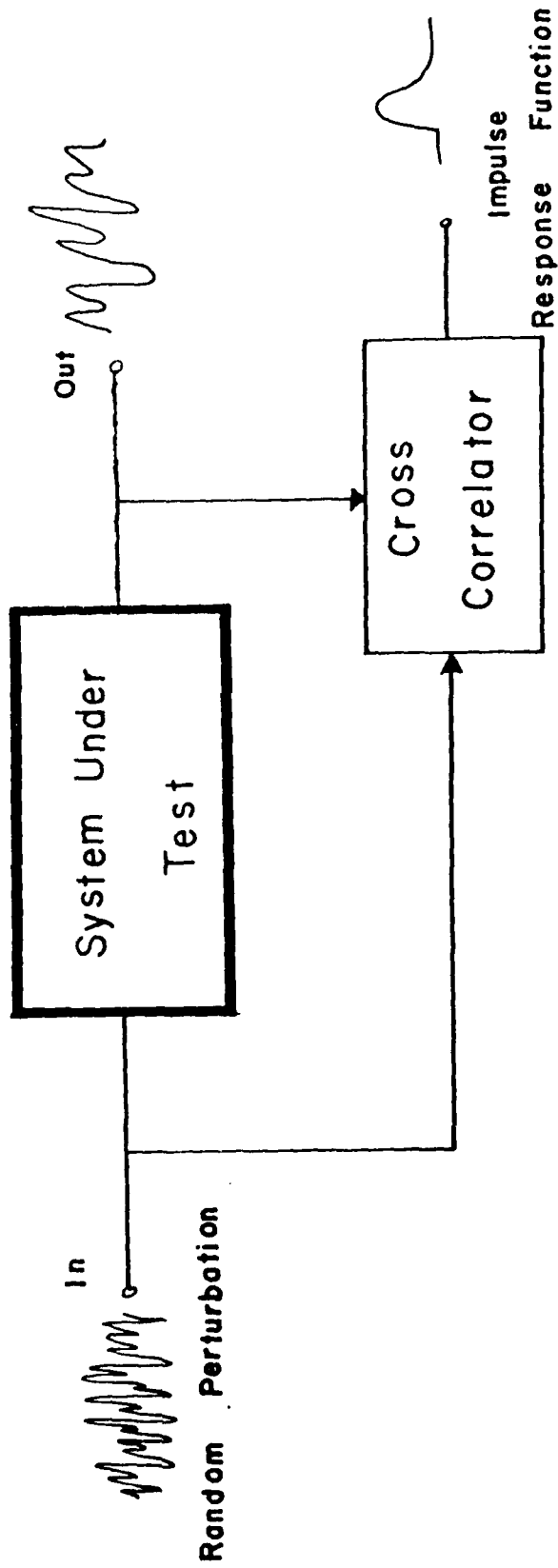


Autocorrelation



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