from (15). The results of the deconvolution are sensitive to the values of $\{\mu_i\}$ and $\{\sigma_i\}$ and consequently they must be accurately transmitted.

CONCLUSIONS

Two signals which have been convolved can be separated by the technique outlined in this paper provided that one of the signals is modeled as a sample function from a cyclostationary random process with known or calculable statistics and the other signal is the impulse response of an unknown linear time-invariant system. Since stereotype ECG's can be modeled as sample functions from a CS process, it is conceivable that the technique can be applied to practical ECG's which have been linearly distorted provided an adequate test has been performed to elucidate the parameters required by (15).

Results presented show the power of the SDA and the speed with which substantial distortion, even that caused by non-minimum phase systems, can be removed.

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Computational Morphology: Three-Dimensional Computer Graphics for Electron Microscopy

M. J. SHANTZ AND G. D. MCCANN

***Abstract*—This paper describes a method for the computer reconstruction of surfaces from a sequence of electron micrographs, and a data structuring approach to the problem of representing and analyzing objects of physiological importance. The reconstruction technique involves the following stages: 1) object outlines are traced from each section, 2) the computer chain encodes these outlines, 3) the chain codes are reduced to the minimum number of boundary points which satisfactorily define the boundary, 4) polygons are mapped onto the boundary points between sections to approximate the surface, and 4) color coded, shaded surface views are computed of any subset of objects viewed and illuminated from arbitrary locations.**

This surface representation provides an ultrastructural data base from which quantitative morphological parameters as well as otherwise impossible visualizations can be computed. Important parameters include surface area, volume, areas of close proximity between surfaces, synaptic area, cross-sectional area, synaptic distribution, and others. This sort of analysis facilitates detailed quantitative correlation of ultrastructure and function in neuronal systems.

I. INTRODUCTION

One of the most spectacular and important developments in computer applications has been the emergence of algorithms for creating realistic shaded surface images of three-dimensional scenes represented in computer memory [1], [2], [3]. The shapes, surface properties, including light transmission and reflection properties, can be realistically rendered. Once such a "scene" has been created, algorithms exist for producing a particular image of the scene as viewed from any point and as illuminated from an arbitrary location. Particular objects in motion within the scene can also be handled.

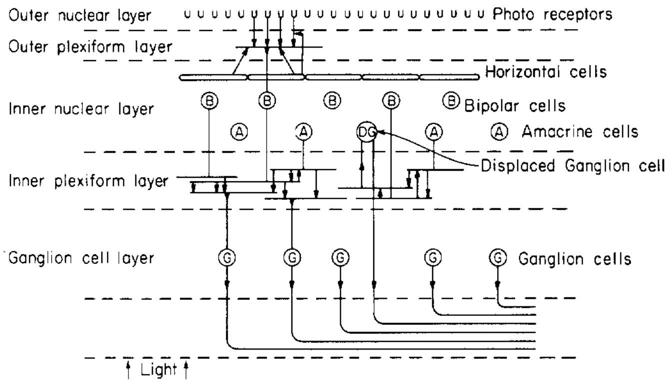

Fig. 1. Schematic of a vertebrate retina showing interconnections between the various neuron cell types.

There have, however, been two important missing elements in the effective application of this powerful capability to science and education. One of these has been the difficulty of getting the necessary three-dimensional information into the machine in appropriate form. The other has been the inability to perform quantitative structural analyses of scientific or engineering interest. These two problems are now being dealt with in our group in relation to a series of important scientific problems involving visual information.

Efforts to analyze anatomical structures frequently encounter difficulties associated with sectioning of tissues. Studies of brain morphology from slices, computerized axial tomography, optical or mechanical sectioning of stained cells for light microscope examination, and thin sectioning of tissue for transmission electron microscopy: all of these techniques involve piecing together three-dimensional structures from two-dimensional cross sections. The technique reported here utilizes polygonal surface approximations obtained from a series of outlines or contours to create color coded shaded surface renderings of complex anatomical structures.

II. RECONSTRUCTION TECHNIQUES

Graphics for Electron Microscopy

Computer graphics and data structuring techniques have first been applied to the problem of representing and analyzing the ultrastructure of neuronal tissue in the vertebrate retina. This type of data is obtained through a series of histological techniques. The retinal tissue is fixed, embedded in plastic and then sectioned (sliced) very thinly (about .05 microns thick). This produces a host of two-dimensional cross sections through the three-dimensional tissue. These sections are placed in the electron microscope and a photographic representation of the two-dimensional electron density of the tissue section emerges.

These data from the transmission electron microscope are difficult to analyze without the aid of some form of three-dimensional reconstruction. Previously, investigators have used the tedious and time-consuming method of manually constructing wax or plexiglass models of intricate ultrastructural objects only to find them inflexible as visualization aids and inadequate for quantitative morphological studies.

An appreciation for the complexity of the neuronal structures in the outer plexiform layer of the vertebrate retina can be gained by viewing the simplified schematic diagram of Fig. 1.

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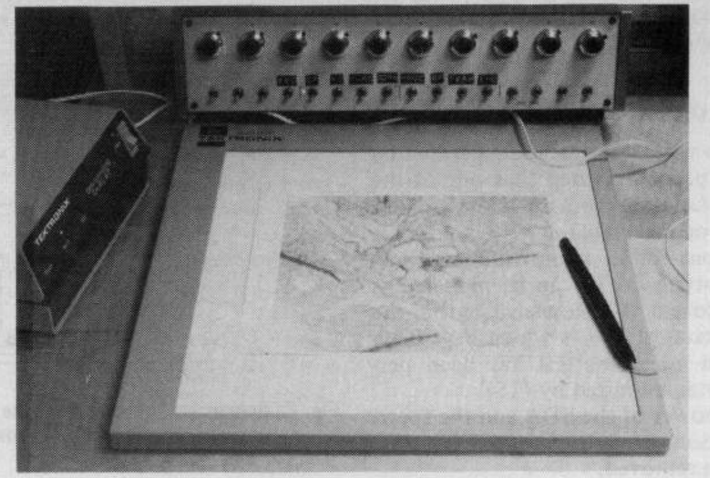

Fig. 2. Magnetic pen for tracing, aligning and digitizing membrane boundaries in a sequence of EM section images. In actual use section images are on transparencies and are projected from above.

The rod cell, one kind of photoreceptor cell, has embedded in its "foot" long flat structures called ribbon synapses. Dendrites from horizontal cells and bipolar cells nestle up to this ribbon and entwine about each other. Visual signals are transmitted via the ribbon synapses and numerous other synaptic contacts between these three types of cells. These contacts allow molecules to pass through the membranes effecting information transfer. The reconstructions shown here (Fig. 4) are only for the very small region where this transfer is occurring.

In order to conveniently visualize these three-dimensional structures of intricate intertwining neuron systems, to compute parameters such as surface area, and to "dissect" cell surfaces out to view separately, some form of reconstruction is necessary.

There are two central problems in attempts to analyze cell structures from EM sections. Problem one is to reconstruct the surface of a cell or set of cells from a sequence of electron micrograph cross sections. This involves 1) identifying the membrane of a cell (in this case a neuron of the vertebrate retina) in each cross section, 2) digitizing the coordinates of this membrane or section boundary, and 3) mapping polygons between boundaries in adjacent sections to form the cell membrane surface approximation. Problem two is to structure this cell surface data base in a manner which facilitates three-dimensional visualization and quantitative interrogation. This involves 1) setting up a semantic net (or other appropriate data structure) to represent objects and relations on objects, 2) representing in this net such information as synaptic surface areas, spatial relationships between surfaces, surface properties possibly including functional properties, 3) generating three-dimensional color coded shaded surface images of objects or sets of objects for visualization purposes, and 4) developing semantic net manipulation procedures for inserting and extracting structural information.

Digitization of EM Data

The reconstruction procedure developed starts with a sequence of section images from the electron microscope. By means of the light projection system and computer transcription tablet shown in Fig. 2, each transparency can be enlarged and a trained observer can trace those discrete features of each two-dimensional image which are to form the three-dimensional data. In this case the outer boundaries of all interacting dendrites would be traced and those surface elements where interactions are seen to be present can be identified separately. These data are quickly transcribed into digital form by the magnetic "pen" shown in Fig. 2 which produces digital data that can be redisplayed from the computer as illustrated in Fig. 3.

This boundary encoding step involves the use of high level knowledge about what these EM objects are like and about what kind of objects are expected and thus, must be carried out interactively by an experienced neuroanatomist. Once these boundaries are encoded the machine can build the surface descriptions.

Computer algorithms to trace objects such as membrane boundaries have been developed for simple cases. It has been found, however, that the decisions required for most accurately accomplishing this can best be made by a competent histologist. For this reason the tracing tablet concept shown in Fig. 2 has been developed. Here successive EM transparencies are projected on a reference tablet and the defining boundaries drawn in with a sharp pencil. Each boundary is associated with an object, a section and some identification. For example, an object may represent a region where vesicle transmission is occurring between two dendrites.

Once such a reference drawing has been finished to the satisfaction of the histologist it is rapidly transferred to the computer with the Tektronix tracing pen shown in Fig. 2.

In order to align successive sections properly additional fiducial marks may be copied onto this reference drawing but not necessarily transmitted to the computer. The alignment of the next section is then made by adjusting either the tablet or transparency position so that its projected image is correctly aligned with the first drawing and the process is repeated. It has been found that this procedure can be carried out quite rapidly and provides the most accurate data base in the computer.

Construction of Shaded Surfaces

Next, the computer chain encodes each boundary ($b_{i,j}$) at each section level (S_i) and reduces these chain codes to "keychains". Keychains include only important or key boundary points. On those segments of the boundary where the curvature is small, few boundary points are saved in the keychain whereas for higher curvature segments more points are saved. This reduces the number of polygons required for mapping between $b_{i,j}$ and $b_{i+1,j}$.

Once the keychains for each boundary $b_{i,j}$ at each level i

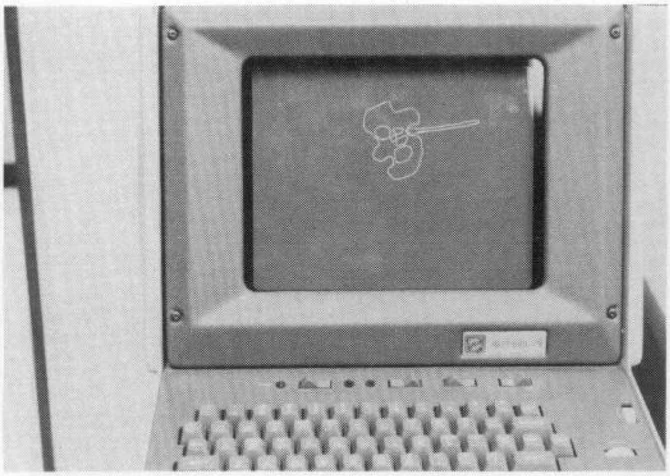

Fig. 3. Computer (DEC, PDP-11/45) terminal CRT. Boundaries are replotted from memory to assure satisfactory tracing.

are obtained, polygons are mapped between corresponding boundaries of adjacent levels. For this an optimal surface mapping algorithm is used which was described by Fuchs [4]. This algorithm maps triangular patches onto boundary points in such a way as to achieve a minimal surface area mapping.

The set of polygons mapped between b_{ij} and $b_{i+1,k}$ is referred to as a surface segment (SS_{ijk}) where i specifies the ith section, j specifies the boundary number in the ith section and k specifies the boundary number in $i + 1$ section. An object is composed of one or more surface segments and has a color attached. Fig. 4 shows shaded surface views of several objects reconstructed from EM sections.

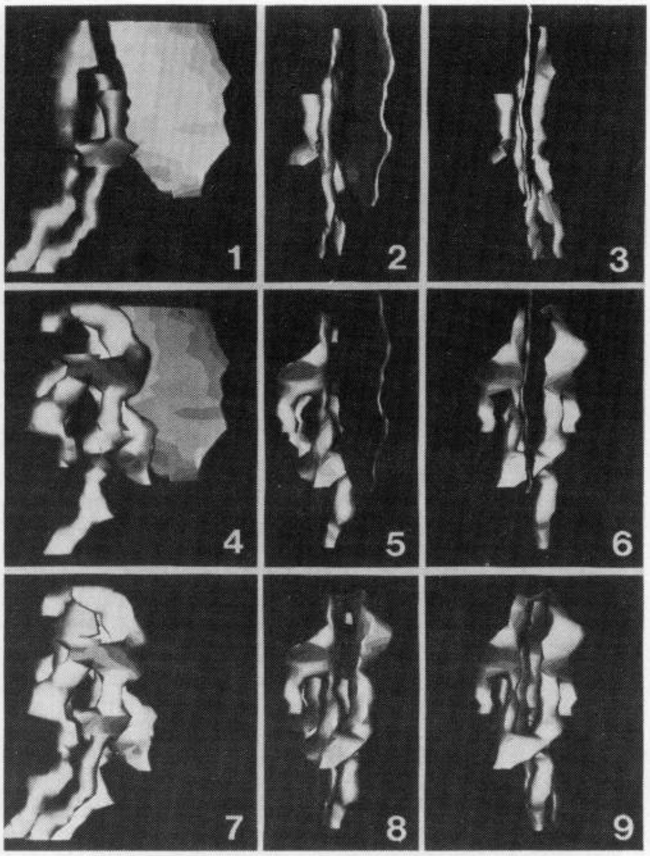

Fig. 4. After polygons are mapped between boundaries in 3-space, color coded, shaded surface views are computed of various combinations of objects in the scene. 1–3 show views of the spatial relationships between the thin flat ribbon synapse and the two bipolar cell processes. 4–6 show the ribbon and the convoluted horizontal cell "claw." Note the slot for the ribbon synapse in the back of the claw. 7–9 show the horizontal and the two bipolar dendrites showing how the claw wraps around the entire structure.

III. APPLICATIONS

What good is such a surface approximation of ultrastructural objects? At present two applications are being considered: 1) enhanced visualizations, and 2) quantitative interrogation of a structural data base.

The difficulty of visualizing EM structure from section images alone makes reconstruction and visualization aids essential tools in many ultrastructural studies. The ability to generate stereo shaded surface views and to separate the objects for individual viewing facilitates the interpretation of electron micrographs, especially in the vertebrate retina application where the torturous spatial interrelations of the neurons encumber interpretation.

Much of the value from three-dimensional reconstruction techniques in computational morphology derives from its quantitative or objective character. Parameters such as surface area, cross-sectional area, and synaptic area can be extracted from the structural data base as well as relations between objects such as the surface area of object A which lies within x microns of object B.

A fundamental deficiency in previous computational morphology efforts is the inflexibility of the data structures for representing objects. It is proposed to use a semantic net structure to represent objects and relations on objects. Semantic nets have been used to advantage in computational linguistics for representing the meaning of natural language sentences [5]. They have been used in artificial intelligence and cognitive science efforts to represent knowledge about a restricted domain of interest [6]. In the case of ultrastructural analysis a semantic net representation will facilitate 1) the addition of details to a description of an object, and 2) the representation of quantitative relations between parts of objects, both structural and functional relationships. The details of this net structure for object representation will be outlined in a forthcoming paper.

The extension of this application of graphics and data structuring techniques will hopefully lead to computerized electron microscopes which provide reconstruction and analysis tools as accessories to the microscope.

ACKNOWLEDGMENT

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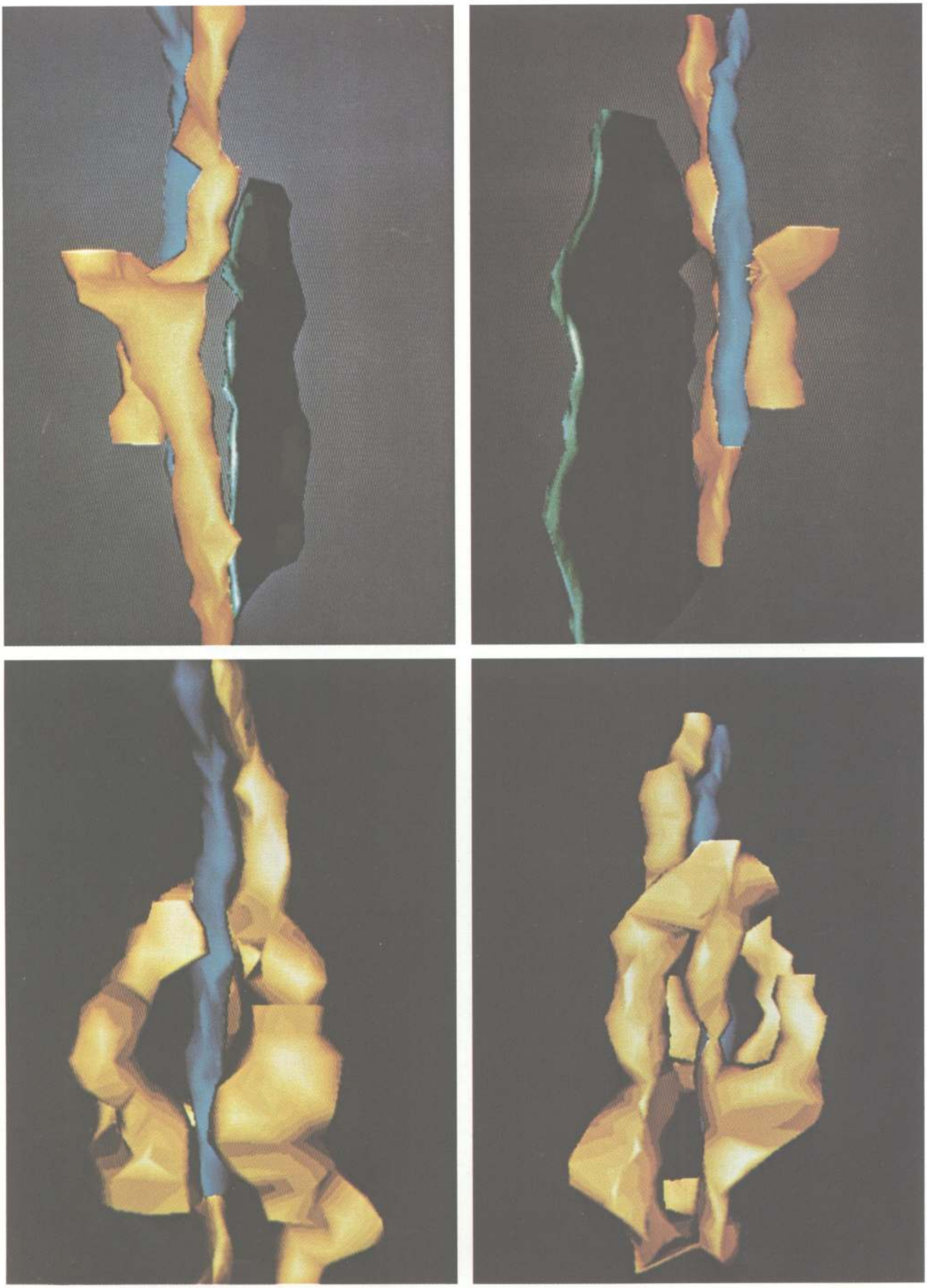

Fig. 5. Color coded, surface views using the Gouraud hidden surface algorithm with Phong's shading algorithm. The red and blue are the bipolar processes, the yellow is the horizontal cell and the green is the ribbon synapse.

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Use of Swept Sine Wave in Physiological Systems Analysis

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RAMANATH LAXMINARAYAN

Abstract—**The use of a swept sine wave as a transient forcing function for measuring the frequency response characteristics of a physiological system is suggested. The spectral characteristics of a linear frequency swept sine wave are illustrated and it is shown that, with relatively short sweep sine wave excitations, one can obtain almost rectangular modulus spectra with relatively good frequency selectivity. Such a transient method may be of potential use in physiological systems analysis.**

INTRODUCTION

The use of spectral analysis has become fundamental to many physiological problems which involve deriving the input-output relationships. For example, in the characterization of the arterial system, a parameter that is of mutual interest to many researchers is the frequency-dependent arterial input impedance (1).

In deriving a transfer function characteristic such as the arterial impedance, the approach to the problem of experimental measurement consists of varying the system input signal, say for example, flow, to some known excitation and then measure the response of the system in terms of pressure. The measured input and output time histories are subsequently Fourier transformed and the transfer function of the system is determined by division of the Fourier transforms. Recent advances in data analysis techniques enable these operations to be performed conveniently over a digital computer.

The traditional methods for exciting the system input have in the past, involved steady state testing which entails the observation of the response of the system to a known frequency sinusoidal excitation. These steady state methods however tend to be much too tedious and time-consuming, and the use of random excitation has proved to be a more potential tool in the study of input-output relationships (2).

In practice, it is well known that ideal random excitations are unobtainable. White noise generators usually produce band-limited noise, the spectrum of which may be idealized as indicated in Figure 1. If the excitation bandwidth, $\Delta\omega$, is sufficiently large, then bandwidth-limited random noise provides a suitable excitation function. Therefore bandwidth limited noise is often obtained deliberately by passing random noise through a band-pass filter.

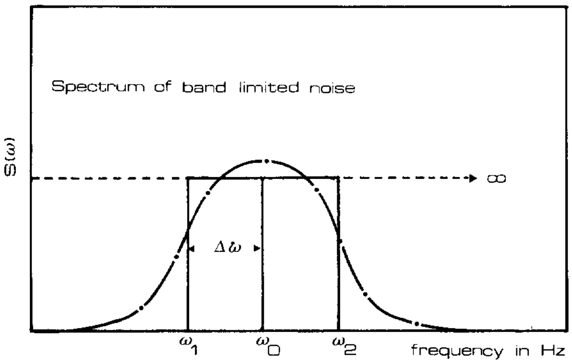

Figure 1. Spectrum of white noise: Fully drawn line represents idealized bandwidth limited noise. Broken line (horizontal) represents white noise and the broken line with dots represents filtered bandwidth limited noise.

In contrast to band-limited random noise, transient analysis methods are of considerable interest in measuring the frequency response of physical systems. Practical considerations in the past have often led to the use of a single pulse of simple geometric shape, such as for example a rectangular pulse. The use of the swept sine wave as a transient forcing function for measuring the frequency response characteristics has not received any attention so far in the study of physiological systems.

SWEPT SINE WAVE

Swept sine wave is defined as:

$$F(t) = F_0 \operatorname{Sin} \phi(t), \qquad 0 \leqslant t \leqslant T \tag{1}$$

from which two characteristics are evident, namely that (a) the function is of constant amplitude and (b) the frequency is time dependent. The instantaneous variation of frequency with time is given by

$$\omega(t) = \frac{d\phi(t)}{dt}. \tag{2}$$

The use of swept sine wave was first considered by Reed and Hall (3) in generating a function having a flat spectrum between two given frequency limits. It has been shown that a linear frequency swept sine wave gives such a desired spectrum. A linear swept sine wave is one in which the variation of frequency with time is linear. This is given by

$$F(t) = F_0 \cdot \operatorname{Sin}(at^2 + bt), 0 \leqslant t \leqslant T$$

for which the instantaneous frequency is:

$$= \frac{d}{dt}(at^2 + bt) = (2at + b)$$

and

$$a = \frac{\omega_2 - \omega_1}{2T} = \frac{\Delta\omega}{2T}$$

$$b = \omega_1, \qquad (\omega = 2\pi f)$$

where ω_1 and ω_2 are respectively the initial and final frequencies of the sweep and T is the duration.

SPECTRAL CHARACTERISTICS OF SWEPT SINE WAVE

In order to examine the spectral characteristics of the linear swept sine wave, we need to evaluate the Fourier transform of equation (2) i.e.:

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