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CHRONOBIOMETRY WITH POCKET CALCULATORS AND COMPUTER SYSTEMS •

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I. *Introduction:* statu quo, *strategy and tactics*

Biologic time series usually exhibit predictable temporal variations, including so-called rhythms. These components of a biologic time structure can be analyzed from the viewpoint of chronobiology, a fledgling branch of biologic science employing methods of biorhythmometry or, briefly, rhythmometry. These methods are based on conventional mathematical procedures, the execution of which has been rendered more practical with the advent of the electronic computer. It is not fully realized, however, that procedures of rhythmometry depend not only upon a given computing routine but also critically upon 1. the availability of *physiologic* information and 2. corresponding *inferential statistical* considerations.

Key-words: Ambulatory monitoring; Chronobiologic serial section; Cosinor; Rhythmometry; Sampling.

• This paper is dedicated to the memory of Professor Eduard Batschelet just deceased, whose unpublished notes prepared in 1979 for the first course given in chronobiology at the University of L'Aquila, Italy (Halberg F.: Regular courses in chronobiology at the University of L'Aquila, Italy and the Université René Descartes, Paris - Physiologist *22*, 41, 1979) were used for the development of the program here appended to compute a population-mean cosinor on the Texas Instruments calculator. An abbreviated earlier version had been prepared by some of the authors for Documenta Geigy and by one of us (FH) for an Advanced Institute in Chronobiology.

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Chronobiometry dealing with all assessable temporal biologic signals must be distinguished from endeavors toward an automatic 'computerized' data analysis of certain conventional recordings of classical physiologic rhythms, such as those in the electroencephalogram or electrocardiogram. When analyses are restricted to one or a few frequency regions of a much broader spectrum, the conventional (electrocardiographer or) electroencephalographer accumulates in a relatively short time data over dozens, hundreds and even many thousands of cycles of special interest to him, e.g., in the so-called Berger range extending from 30 cycles to 1 cycle/sec. These data accumulate speedily, as compared to those of the chronobiologist dealing with the more complete spectrum of multiple frequencies. Within a year or two the chronobiologist may procure only a meager quantity of data for the identification of a circannual one, in an electroencephalographic, electrocardiographic, hormonal, metabolic or other variable. In a year one covers but a single circannual cycle. If the analysis of a circannual rhythm or of a circadian one is restricted to relatively short time series (approximately one year or one day in length, respectively), the chronobiologist must derive much more solid biologic background as an inferential statistical base in hypothesis formulation. It is much easier to validate and quantify the characteristics of a rhythm with a predicted period, than it is to search for unknown periodicities! Herein we sketch a few of the approaches that any practitioner in biology and medicine might use under conditions when he must deal with some limited data on rhythmic variables in a given individual or population investigated (in its own right or for comparative purposes).

For instance, an epidemiologist compares rhythmic variables in two populations. He faces the task of considering the collection of multiple samples, *in lieu* of the approach commonly followed by many biologists and physicians, in which a single sample is obtained at specified and conventional times and without any consideration of rhythms.

During the past few decades the fact has become apparent that usually several rhythms, each with a different frequency, characterize one and the same physiologic variable in any one subject. As yet, hardly any use is made of this information, for example in epidemiology. The conventional approaches derive from the circumstance that to most biologists the study of rhythms appears to be too complex or laborious. One therefore often compromises by taking a single sample or multiple ones — at a fixed time of day, time of the week and time of the year — without realizing that rhythms are not necessarily 'controlled' in this fashion.

By the same token, most physicians administer most treatments at times fixed by considerations other than chronobiologic. This approach is not only misleading but in certain contexts it is potentially harmful. Ample evidence, as yet mostly on experimental animals, documents this point for cancer chronotherapy [19]. One can assess, among others, not only the circadian acrophase or, preferably, the orthophase but also changes in response as a function of the stage of both circatrigintan (e.g., menstrual) and circannual cycles [16]. Unless one does so, a clock-hour qualified treatment remains a rhythm-unqualified 'potshot on a roller coaster' — hardly sufficient to 'control' various rhythms.

As more and more facts indicate that rhythmometry is essential, its indications and its premises have to be clarified; editors and referees of journals and eventually the biomedical community may realize that more is involved in rhythmometry than indiscriminately collecting data as a function of time of day or season — for coding and 'pushing them through the computer'. Just as the acquisition of a microscope does not take the place of training in histology and pathology, the acquisition of a set of computer programs does not take the place of training in chronobiology. Such training remains beyond our scope herein. Some quite common pitfalls must however be emphasized. The chronobiologist has as his *motto* the *primum nil nocere*. When the wrong time of administration can increase the patient's chance of dying from a given treatment, just as the right time can double the rate of cure, it is important that precautions in interpreting the results of rhythmometry should be taken on a broad scale.

Biologic facts and their implications

A first contribution that the biologist (e.g. epidemiologist) can make to a meaningful rhythmometry is the initial decision not only concerning the pertinent variables but also concerning the anticipated spectral structure of these same variables — at the site and in the fashion in which they are measured. One must specify at the outset the spectral regions to be explored in one or even in several broad domains — in order to gather pertinent information on the given biologic problem to be investigated. In each domain the biologist reviews the extent of direct or indirect information on rhythms and/or trends available for certain variables *a priori*. It should also be realized that trends other than traditional, developmental or age-dependent ones may need to be measured; every rhythm with a period longer than the total observation span will contribute an (unevaluated) trend. By the same token, rhythms with a period shorter than the interval between consecutive observations will also introduce artifacts, such as aliasing when the data are equidistant.

Sometimes special precautions may be required when animals have to be handled for the study of rhythms or for studies of interactions of rhythms with the effect of aging. Recently, core temperature of male inbred stroke-prone Okamoto rats was studied at about 12 months and again at about 14 months of age by comparison to that of rats of the same strain 2 months of age in the first session and about 4 months of age in the second measurement session [after standardization for several weeks at a room temperature of 24 $\pm$ 1 °C, first in L 06^{00}-18^{00} D 18^{00}-06^{00} ($LD_{12:12}$), and next on $LD_{8:16}$]. During these studies Purina Rat Chow and deionized water were freely available. Rectal temperature was measured with a thermistor-bridge circuit on each rat at 4-h intervals for 24 h or longer. A statistically significant rhythm was demonstrated (with the fit of a 24-h cosine curve) for the young rats (2- to 4-month-old). A prominent ultradian variation, with no demonstrable circadian component in rectal temperature was seen in 1-year-old Okamoto rats (fig. 1). By comparison, when from the same animals for several weeks intraperitoneal temperatures were monitored without handling, a statistically significant rhythm could be constantly demonstrated. Quite clearly, the circadian rhythm so prominent in animals up to four months of age (when blood pressures reach over 200 Torr on the average) persists during the continuing mesor-hypertension [16] in these animals beyond one year of age, but it is readily altered simply by the handling for measurement, to the point of a circadian-to-ultradian variance transposition.

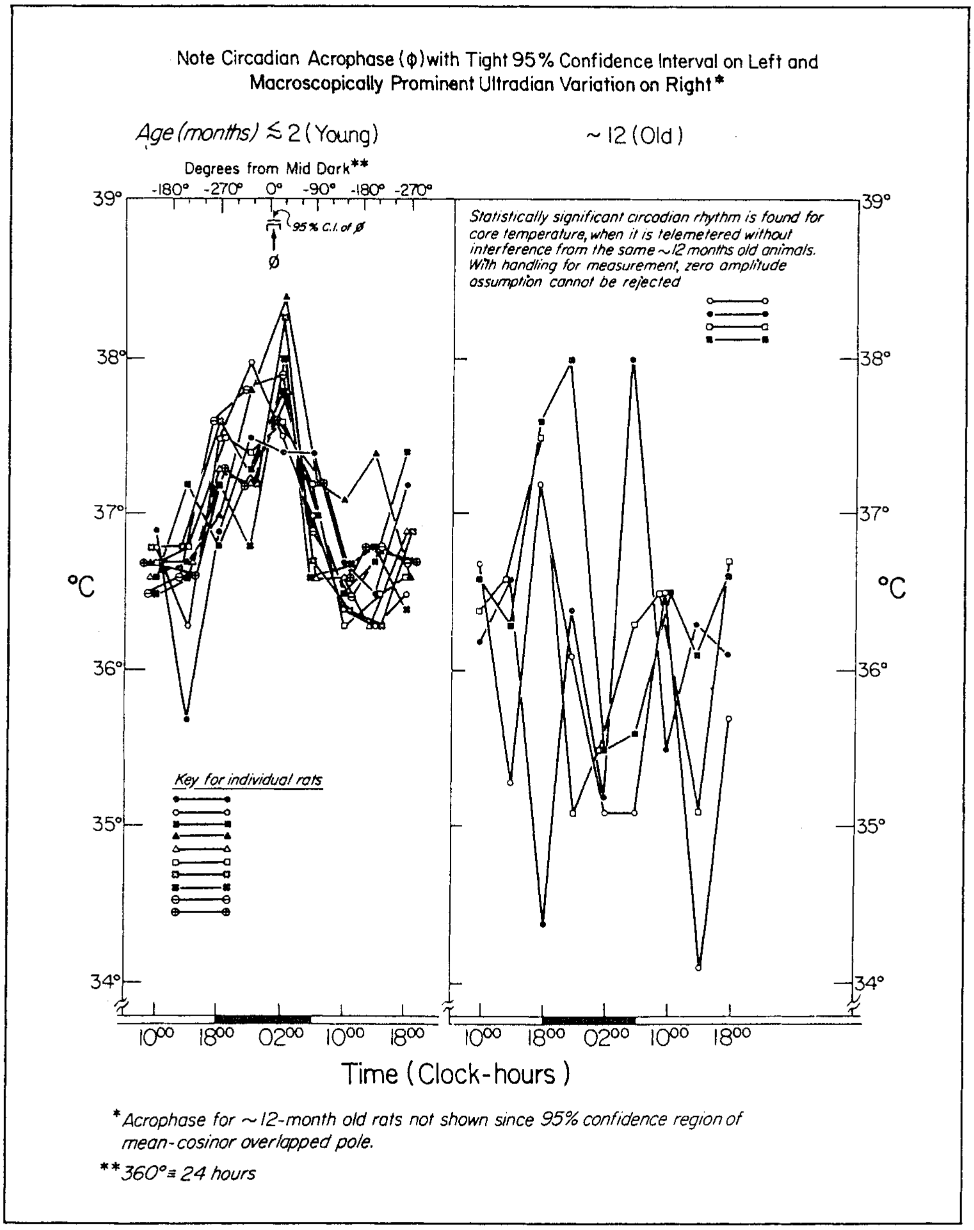


Fig. 1 - Uncertainties of measurement, basic and additive (see text). Age and circadian rectal temperature variation in stroke-prone Okamoto rats (handled for measurement).

In planning data collection the biologist must realize that the sampling requirements will be more drastic for demonstrating a heretofore unknown bioperiodicity (i.e., a given rhythm with an unknown frequency) than for assessing the characteristics of a known rhythm. Data collection may then also be related to the prominence (or, conversely, the uncertainties of the occurrence and/or the behavior) of such an unknown bioperiodicity. The uncertainty is somewhat reduced for a frequency near a known environmental counterpart even if it is desynchronized from a precise environmental cycle; and the uncertainty becomes considerably less once one can anticipate that a rhythm is synchronized with a precise environmental frequency.

Without prior information, the length of the time series necessary to analyze 'hidden periods' (and thus the sampling span, T) must be many times longer than the (unidentified) period length of the rhythm with the lowest frequency. From this viewpoint biologic time series may be empirically grouped as short, intermediate or long, as a function of whether they cover up to 2 periods, between 2 and 30 periods, or more than 30 periods of the rhythm investigated. Furthermore, the interval between consecutive observations, Δt, must be short enough to allow for a reliable resolution of the rhythm with the highest frequency. From the viewpoint of economy, one should scrutinize prior to data collection any evidence on hand concerning components expected to characterize all variables in all pertinent spectral regions. Since undue cost can be associated with unnecessarily decreasing the Δt or increasing the T, practicality forces one to ignore some components that may be present. In order to do so without introducing spurious effects, 1. non-equidistant sampling may be used to avoid aliasing from high frequency components insufficiently sampled (Δt too large); 2. filtering procedures can also be applied to eliminate effects from lower frequencies that may introduce artifactual trends when the observation span T is too short.

In a chronoepidemiologic study focusing upon hormonal aspects of human time structure, one may well anticipate some components with periods of about 7 days. Such circaseptan rhythms have now been demonstrated for a number of variables [33]. It should be kept in mind, however, that under ordinary conditions (without the added stimulation, e.g., of a kidney transplantation [9]) most known circaseptan components are not as prominent as rhythms with other frequencies in the same variable [18]. Great expense in funds and, what is more limiting, in the total amount of blood to be withdrawn would have to be incurred in sampling for many hormones with a view to quantifying with circadian and ultradian variability any as yet unknown circaseptan rhythms together with other components in the infradian range of frequencies. Hence, in a first chronoepidemiologic approach one may ignore possible circaseptan rhythms and take the calculated risk of missing valuable information. In so doing one realizes and emphasizes at the outset that the sampling scheme is incomplete, from the standpoint of covering all probable components that are likely to contribute to predictable variability, and that circaseptan variability remains to be assessed in subsequent explorations. If, in turn, the studies are carried out after a kidney transplant or after some other stimulation of the kidney, in particular, the assessment of circaseptan components, perhaps more broadly in proliferative phenomena, seems to be desirable, if not indispensable, at the outset. Again conditions and prior information will determine the placement of limited and costly samples.

Next, and again on biologic grounds, a decision must be made with regard to the sampling environment, such as a given subject's routine of living for a reasonable time span before as well as during sampling. Cost-benefit considerations may prompt one to study a rhythm with a given frequency after anticipated and preferably validated synchronization with some periodic environmental factor. There can be a large difference between the cost of sampling on the one hand for a 24-h synchronized circadian component (with a thus predictable period) and that of sampling, on the other hand, for a desynchronized (free-running) component (for semantics see [16]), unless on the basis of earlier information the likely period of the desynchronized component also is known.

Prior external information and concomitant data collection on reference variables, notably rhythmic ones

Great economies in both cost and effort can result from the use of 1. available external information, notably in the form of time-qualified reference intervals, and 2. concomitantly obtained reference or marker rhythm information. Both of these points can be illustrated by the series of studies on murine rectal temperature (fig. 2) that led to the coining of the term circadian to describe all rhythms with periods of about 24 h ($\pm$ 4 h). One hundred and twenty-eight animals were divided into two groups, some blinded and some sham-operated. The rectal temperature of each mouse was measured (with a thermistor-bridge circuit) every 4 h, around the clock, without interruption (except for short spans when the equipment had to be repaired). For a few days the 24-h pattern of changes in average temperature seemed to be nearly identical for the blinded and the control animals. As the days of round-the-clock measurements progressed, the temperature peak for the blinded animals occurred earlier and earlier in the day. The temperature rhythm of the blinded mice was running steadily faster than one cycle every 24 h! By day 22 (right part of fig. 2) the maximum of temperature in blinded animals occurred at the time of temperature minimum in the control animals and vice versa. We proceeded to measure the mice every 4 h, around the clock, with few interruptions

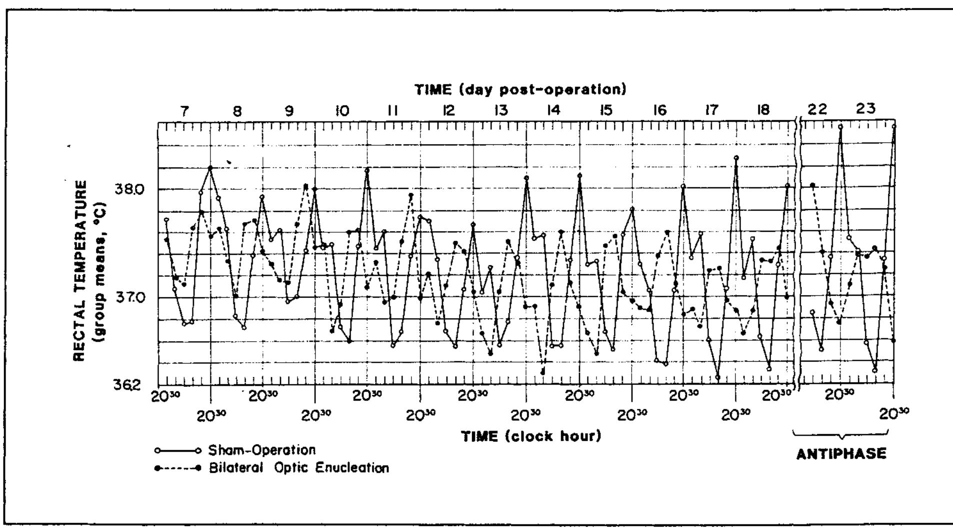


Fig. 2 - Circadian desynchronization after bilateral optic enucleation: free-running circadian rhythm of rectal temperature in blinded mice. Although for the first few days the 24-h pattern of changes in average temperature was nearly identical for the blinded animals and the control animals (sham-operated), by day 22 (right) the maximum of temperature in blinded animals occurred at the time of temperature minimum in the control animals and vice versa, i.e., the two rhythms are in antiphase.

for the next two years, and with longer interruptions for the life-times of other groups of mice throughout the fifties.

The data thus accumulated showed that mammalian core temperature can constitute a marker for some but not all other body functions. Since the 1960's, when surgically implanted radiotransmitters were introduced that monitor intraperitoneal temperature automatically every 10 min on hundreds of animals and for generations, it is difficult to appreciate the labor of measuring temperatures by hand every 4 h around the clock. It is by hand also that the possible use of tumor temperatures as a marker rhythm in the treatment of human peri-oral cancer was first checked [19]. The effect of radiation upon any human tumor temperature rhythms as yet is unevaluated. Nonetheless, in two studies, there is a statistically significant circadian stage-dependent difference in therapeutic response. The rhythm-dependent difference is between 30 % and 70 % regression during a 5-week course of radiotherapy. Modern technology is now a ready substitute for the clinical thermometer placed upon the tumor, or for a probe inserted into the rectum. Instrumentation is already available for experimental animals and clinical research. To follow validated marker rhythms on a large scale easily and cost-effectively as a function of treatment remains a task yet before us, and an urgent one.

Instrumentation

The study of biologic rhythms requires a system of instrumentation — for data collection, transfer, storage, analysis, and, again, storage of results and updating, with provisions for such cycles of data handling from appropriate bases, ideally from womb to tomb. We are far from meeting such goals for human beings but can achieve some of them, at least for telemetered temperature, in the rodent. Major reliance for human rhythmometry can be based on self-measurements initiated in the family and/or free of cost in secondary schools and analyzed on site (as an initial step only) by as simple a model as a cosine function, fitted by a pocket calculator [21] (see *Appendix*). Experiments involving laboratory animals are also feasible, relying primarily upon such relatively simple means as control of environmental lighting and feeding. Special instrumentation for rhythmometry, with telemetry of temperature and motor activity of rodents under various environmental conditions, is, however, extensively used [43].

For many life forms, appropriate lighting regimens such as the alternation of light and darkness at 12-h intervals, briefly $LD_{12:12}$, constitute a cheaply applied synchronizer, under conditions of *ad libitum* feeding. Manipulation of feeding versus lighting schedules may serve to achieve a desired interaction of the two synchronizers. With respect to one or several aspects of time structure, the documented results include for the case of the telemetered intraperitoneal temperature an advance or delay in acrophase of rhythm with or without an alteration of amplitude, mesor or waveform, or the dispersion indices of any of these parameters. Meal timing may thus also serve to manipulate and alter relations among rhythms as well as the relation of a rhythm to $LD_{12:12}$. Depending on the number of available separate rooms or controlled environmental units, different synchronizer schedules can be concomitantly imposed to facilitate work 'around the clock' [12, 26, 32] whenever the rhythm investigated is amenable to a manipulation of its timing by the synchronizer regimen. Thus, at a convenient time during the usual working hours, one may approximate several different circadian stages for certain variables (although not necessarily all of them).

Data collection by human self-measurements of several physiologic variables, already noted as practicable, serves to demonstrate the occurrence of a whole spectrum of rhythms. As an example, data on oral temperature, pulse and blood pressure self-measured several times a day during ~5 years could not only be displayed in a chronogram [16] but also analyzed and resolved for the circannual variation. In a given subject, certain variables were characterized by circannual rhythms with a stable acrophase from year to year (biostationarity), whereas others were drifting and/or jumping from year to year. Reproducibility of circannual acrophases among subjects (bioergodicity) could be observed for two of the subjects in peak expiratory flow, although a difference of about 90° (~3 months) was observed for their mother.

When self-measurements are taken rather regularly at approximately equidistant timepoints, bioaliasing [16] may occur, i.e., relatively high frequencies may be interpreted as lower frequencies. Inappropriate sampling may also lead to other spurious results. An example of bioaliasing has been documented by SIMPSON and HALBERG [45]. Data of a kind frequently used in biology and medicine can contain both circadian and menstrual components. A woman, 39 years of age, measured her oral temperature over a 7-month span at various times of day. By linear least-squares rhythmometry [16] a 26-day cycle was found. Data from eight such 'menstrual cycles' of 26 days were superimposed and then folded again for consecutive 3-day spans. A change in the thus folded data is usually associated with the menstrual cycle, when body temperature is taken only once a day. But it is important to note that according to the time of day the measurements were taken, the resolved menstrual cycle is different. It can thus be seen that as a function of the 'clock hour' chosen for describing temperature behavior in the cycle, one may be on the average 3 or even 6 days off in the assessment of the start of the 'ovulation-associated' rise in temperature. To avoid such artifacts, including bioaliasing, special consideration must be given to sampling schedules. For example, denser and/or randomly picked sampling times may be desirable. Even if self-measurements are taken randomly in time, there will always be a gap corresponding to the daily sleep span. The desirability of automatic recording devices seems obvious and when these are unavailable 'family' rhythmometry may be carried out, different members of a unit measuring all others at different times during a habitual rest span.

Technological advances made in electronic data collection allow rapid manual or automatic recording of data from ambulatory subjects pursuing their daily activities. Several devices for measurement of rhythmic functions can be used:

1. the Yellow Springs Instrument (YSI) tele-thermometer [51], manufactured by the YSI Company of Ohio, can be used to monitor temperature. A Wheatstone bridge serves as the basic measurement device and temperature is sensed by thermistor probes, each containing a semi-conductor in which a slight temperature change causes a large change in electrical resistance. Single or multiple channel instruments used in our laboratory have a temperature range from 20 °C to 42 °C and a reported accuracy of 0.2 °C. Their batteries have an approximate 1,000-h life;

2. the chrono-thermograph, designed by K. Lange, consists of a miniature electromechanical chart-recorder with a temperature sensing probe. The prototype instrument recorded temperature in the range from 35 °C to 40 °C, with a precision better than 0.1 °C. Relative precision in time is about 1.4×10^{-3} over a span of

10 days when identified at the start only. Paper needed to be changed at least every 10 days; batteries had to be replaced every three weeks. It provided an easily visible record and it was smaller and lighter than devices now in use;

3. the Medilog, manufactured by Ambulatory Monitoring Inc., Ardsley, New York, is a cassette tape device capable of monitoring on 3 channels. With instruments used in our laboratory, a 4th channel must be reserved for time. Thus, one may record automatically temperature from 3 sites for 1 day, but the batteries and recording tape must then be changed. The Medilog also serves for monitoring EEG, ECG, respiration and motor activity for a 24-h span. Data are recorded in an analog form and need to be digitized before analysis. Recording speed stability is better than 2 % overall and the signal-to-noise ratio is better than 30 dB;

4. the Solicorder, manufactured by the Solicorder Company, Ardsley, New York, is capable of monitoring temperature in the range from 34 °C to 40.3 °C, from one site for up to 8 days, with a 6-min sampling interval and reasonable reliability in a water bath. With respect to data handling the Solicorder has two advantages over the Medilog: it does not require digitization of collected data and can be interfaced with a minicomputer for data analysis;

5. the Thermolog, manufactured by the Vitalog Company, Palo Alto, California, is capable of monitoring concomitantly temperature and motor activity; it also does not require digitization of collected data and is sold with an Apple II microcomputer and a Sanyo T.V. monitor for data display and minimal analysis.

The probes used with the Medilog, Solicorder and Vitalog are those produced by YSI and have the same characteristics. Usually, accuracy of recording does not seem to constitute the major problem; problems may arise with defective probes, their calibration, contact with the skin when so applied, and the battery.

Numerical methods

Figure 3 shows the large variety of intermediate results and endpoints of interest to chronobiologists and the methods for obtaining them. Any sequence of data in time will here be called a time series. The values may represent the same variable measured repeatedly on the same individual during a given span (longitudinal series) or each value may represent a single sample from an individual or a mean of such samples obtained from a group of individuals (transverse series). A new individual or group may be associated with each new timepoint not only because the endpoint under study is death (one can kill an animal only once) but also because a given sampling will alter the variable being sampled, as has been repeatedly documented for variables at different levels of organization [12]. The sampling uncertainty thus derived from a single measurement may be referred to as a basic uncertainty. In addition the uncertainties of repeated measurements on the same individual must be considered. The latter uncertainties may be cumulative but in a complex fashion. The complexity stems from the fact that the response to the measurement itself is often bioperiodic and if it is not identifiable and quantifiable as such, it forms a part of the variance and may result in a bioperiodic change of the variance that can be in phase with the rhythm investigated or out of phase with it.

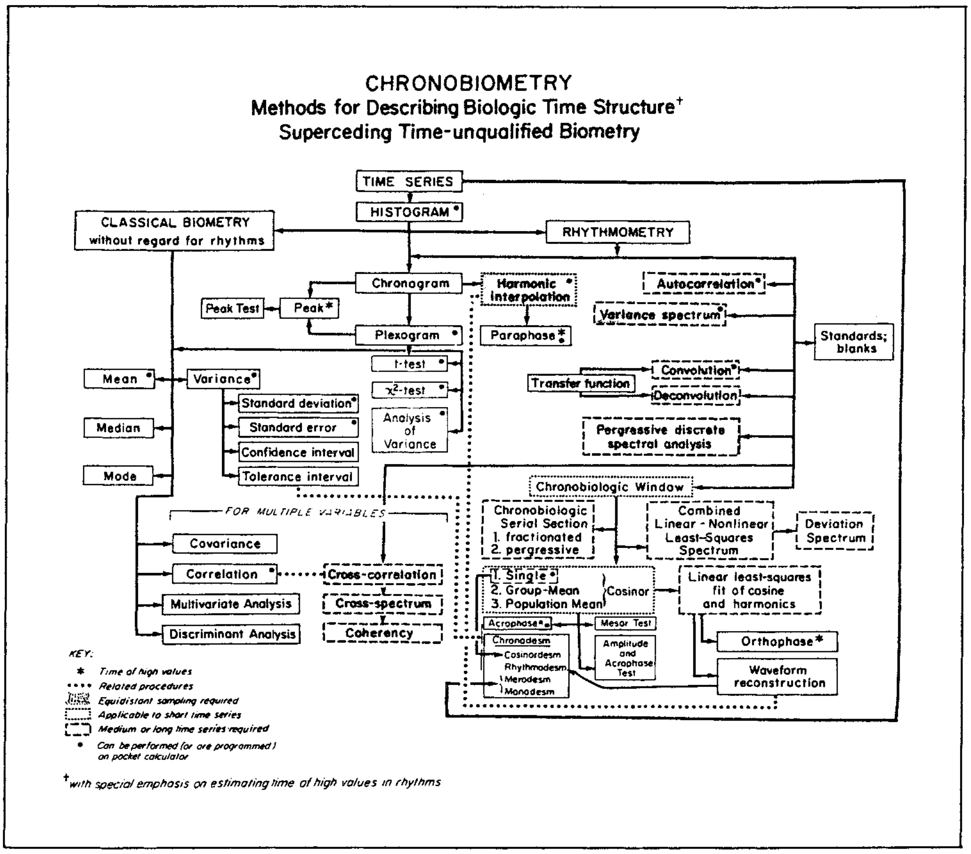


Fig. 3 - Flowchart of methods relevant to chronobiology.

A plot of the raw data (chronogram) is invariably useful but usually not sufficient. It can be readily complemented by the least–squares fit of the model chosen to account for the variability in the data. A chronogram of the residuals may in turn be obtained to allow better visualizing of the appropriateness of the fitted model. A histogram of the residuals, along with a χ^2-test, can help a decision whether to accept the model or to reject it. A histogram of the raw data can be considered as well and is sometimes used for a quick estimation of parameters such as the mean and the variance. In the case of a normal distribution, one can also derive, for a given parameter (such as the mean), a confidence interval at a certain given probability level.

If one wishes to gain a better impression of the shape of the rhythm and if the series length covers several cycles, one can fold the data within one cycle to obtain a plexogram. For each interval along the time scale covering a single period of the rhythm investigated, a mean and standard deviation or standard error are displayed to show the likely individual or group variabilities.

For comparison of two or more groups, classical tests such as the t-test, χ^2-test and analysis of variance can be applied, even by means of small calculators. If the data base available is large enough or if we can rely on previous biologic information, rhythmometric methods are preferred [16].

Classical methods of time series analysis, such as autocorrelation and spectral analysis, are well documented elsewhere and therefore will not be emphasized in this paper [2-4, 10, 24, 31, 38, 39]. From variance spectral analysis, a circadian quotient may be derived. The circadian quotient, or briefly CQ, can be the sum of the smooth spectral estimate of the period equal to 24 h plus the two adjacent such estimates, divided by the total variance. The latter is obtained, of course, by adding all the spectral estimates [24].

Pergressive discrete spectral analysis allows one to follow any changes with time in characteristics such as amplitude and frequency of statistically significant rhythms; non-stationary time series also may be analyzed by this method [5].

Classical time series analysis procedures usually require equidistant sampling, although approaches based on random sampling (with sampling intervals following a Poisson distribution) are being investigated [1, 30, 35, 36, 44, 49].

When only a few equidistant samples are available and the time series is known to contain a bioperiodicity and to cover an integral multiple of the period(s) characterizing the data, it is possible to reconstruct a continuous function from the samples by harmonic interpolation [8]. This method provides the detailed shape of the curve, the timing of high values (paraphases) on the reconstructed curve and interpolated values at times other than the sampling times.

Whether or not the data are equidistant, least-squares procedures can be used, i.e., procedures minimizing the residual sum of squares about a fitted function. A cosine function is usually fitted to the data (single cosinor method [21]):

$$f(t) = M + A \cos(\omega t + \varnothing)$$

Three parameters characterize this model: the mesor (M, or rhythm-adjusted mean); the amplitude (A = half the extent of rhythmic change in a cycle); and the acrophase ($\varnothing$ = lag from a defined reference timepoint of the crest time in the cosine function fitted to the data). Provided that the residuals around the fitted curve are normally distributed, confidence intervals may be given for $\hat{M}$, $\hat{A}$ and $\hat{\varnothing}$, the symbol ($\hat{}$) indicating estimates. It can then be shown that ($\hat{\beta}$, $\hat{\gamma}$), where $\beta = A \cos \varnothing$ and $\gamma = -A \sin \varnothing$, follow a bivariate normal distribution characterized by an $F(2, N-3)$-statistic, where N is the total number of data in the time series. The F-statistic can also be used to test the zero amplitude hypothesis for determining whether or not the data can be described by a cosine function, at a given probability level.

Three kinds of cosinor [37] have been designed in an integrated routine, each appropriate to a different analytical situation:

1. *single cosinor*: a cosinor procedure applicable to single biologic time series (from an individual or group);

2. *group mean-cosinor*: a cosinor procedure applicable to data series from 2 or more individuals for characterizing a rhythm in that particular group only;

3. *population mean-cosinor*: the original cosinor procedure applicable to parameter estimates from 3 or more series for assessing the rhythm characteristics of a population (figs 4a and 4b).

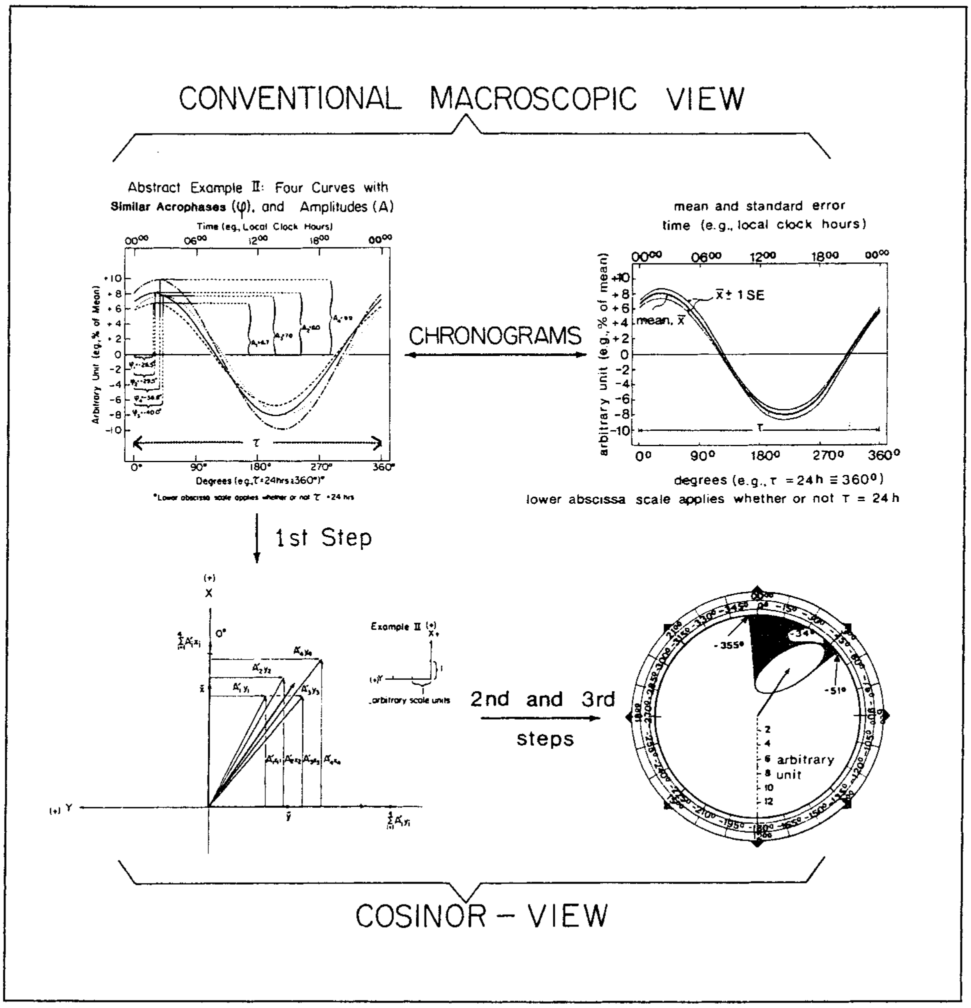


Fig. 4a - Illustration of population-mean-cosinor computations to get point and interval estimates for the population from individual circadian rhythms of similar amplitudes and acrophases.

When two or more rhythms with the same period need to be compared, a mesor test and/or a rhythm test can be applied. Both tests are described in the Glossary of Chronobiology [16].

More complex models can also be fitted to the data, including the fit of a cosine and its harmonics [50]. Since each additional harmonic included in the model requires 2 degrees of freedom, such models should only be used when the total number of data (or timepoints) is sufficiently large. Such models serve to provide information on the waveform of a rhythm. Moreover, an orthophase may now be defined as the lag from zero time of the crest time in a more complex model fitted to the data, consisting of all statistically significant harmonic (or other, biologically as well as statistically significant) components. Information provided by an orthophase has to be considered against the information provided by an acrophase (defined as the

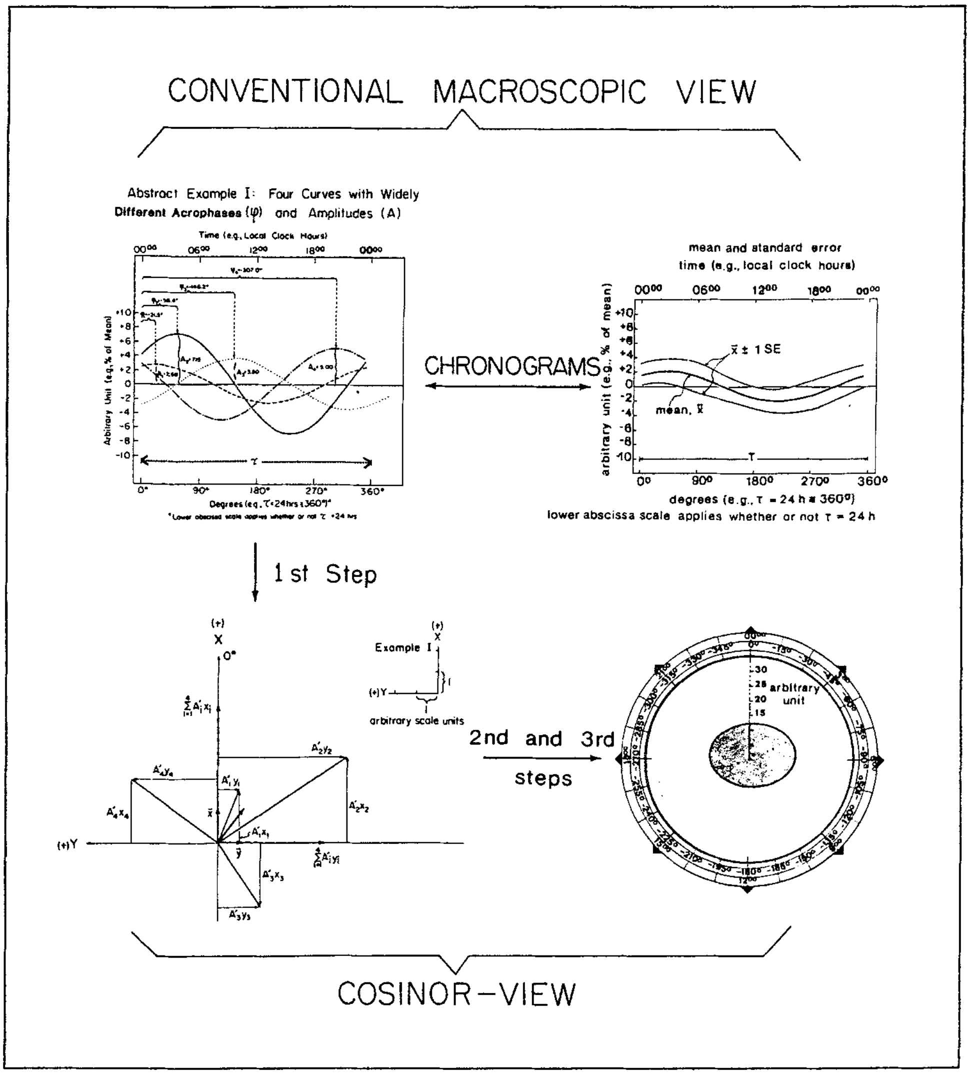


Fig. 4b - Illustration of population-mean-cosinor computations to get point and interval estimates for the population from individual circadian rhythms of very different amplitudes and acrophases.

lag from zero time of the crest in the cosine function fitted to the data) and the paraphase (defined as the corresponding lag from zero time of the crest time in the function reconstructed by harmonic interpolation).

One expects any rhythm characteristics to undergo changes spontaneously, following a change in routine, e.g., after a transmeridian flight or after treatment. A chronobiologic serial section can be applied when the time series is relatively long. This procedure consists of fitting a cosine function, with a fixed period, to consecutive overlapping or non-overlapping data sections, displaced in increments throughout the whole observation span. Parameter estimates are obtained for each section and displayed against time (position of the section's interval with respect to the entire series). This procedure is particularly useful in showing the occurrence of desynchronization, as is the case for instance in cave studies, when volunteers spend several weeks in a cave without any time clue [26].

Another procedure that can be applied to investigate the length of the desynchronized circadian period is called the 'chronobiologic window'. Since the actual waveform of the bioperiodicity under investigation may bias the estimate of period length when data are available only for a few cycles [17], this procedure is to be used preferably when the data cover a sufficiently large number of periods and/or against a biologic background. The method consists of applying the cosinor procedure not only at a single fixed period, but to a set of trial periods in a given range. In doing so, one is usually interested in determining the period length within that range, for which the residual sum of squares is minimal. To render this approach meaningful, the precision at which the period length can be determined should be considered.

When more than one rhythm is present in the time series, a concomitant fit of all components in each frequency range of interest should complement the fitting of separate components successively at each single frequency. A linear-nonlinear least-squares rhythmometry [16, 42] based on a combination of computer programs prepared by our laboratory staff and by MARQUARDT [34] can be used to solve such a problem. This computational procedure consists of 2 separate steps. First, one applies linear least-squares rhythmometry (LLS) followed by nonlinear least-squares rhythmometry (NLLS) (fig. 5a). The LLS method provides a set of initial values for the mesor and (amplitude, acrophase) pairs for each component. Using such initial values, the NLLS, after an appropriate number of iterations, yields final estimates. Table 1 illustrates the steps of the procedure in the case of a computer-

	mesor	periods	amplitudes	acrophases
set of initial values from the linear least-squares method	$M = 100$	$\tau_1 = 7$	$A_1 = 2.054$	$\varnothing_1 = -91°$
		$\tau_2 = 17$	$A_2 = 2.818$	$\varnothing_2 = -92°$
		$\tau_3 = 24$	$A_3 = 10.075$	$\varnothing_3 = -90°$
		$\tau_4 = 27$	$A_4 = 2.181$	$\varnothing_4 = -346°$
		$\tau_5 = 50$	$A_5 = 5.323$	$\varnothing_5 = -86°$
final estimates from the nonlinear method	M = 100 (0.000)	$\tau_1 = 7$ (0.000)	$A_1 = 2$	$\varnothing_1 = -90°$
		$\tau_2 = 17$ (0.000)	$A_2 = 3$	$\varnothing_2 = -90°$
		$\tau_3 = 24$ (0.000)	$A_3 = 10$	$\varnothing_3 = -90°$
		$\tau_4 =$ el *	$A_4 =$ el *	$\varnothing_4 =$ el *
		$\tau_5 = 50$ (0.000)	$A_5 = 5$	$\varnothing_5 = -90°$

* Eliminated by the procedure.

Tab. 1 - Resolution of function by combined linear-nonlinear least-squares spectral analysis. *Input*: computer-generated time series (at 1-h intervals) from combination of cosine functions without noise; 336 data points. $Y_i = 100 + 2\cos[-\pi/2 + (2\pi/7)t_i] + 3\cos[-\pi/2 + (2\pi/17)t_i] + 10\cos[-\pi/2 + (2\pi/24)t_i] + 5\cos[-\pi/2 + (2\pi/50)t_i]$.

generated time series containing 336 data points at 1-h intervals and characterized by the following equation:

$$y(t) = 100 + 2 \cos\left(\frac{2\pi t}{7} - \frac{\pi}{2}\right) + 3 \cos\left(\frac{2\pi t}{17} - \frac{\pi}{2}\right)$$
$$+ 10 \cos\left(\frac{2\pi t}{24} - \frac{\pi}{2}\right) + 5 \cos\left(\frac{2\pi t}{50} - \frac{\pi}{2}\right)$$

A further resolution can be obtained by iterative L-NLLS leading to a deviation spectrum, with the following steps (figs 5a, 5b and 5c):

step 1 - Data analysis by linear least-squares (LLS). This method provides imputations for rhythm mesor (M) as well as amplitude (A) and acrophase (∅) for each component with period τ, minimizing residual sum of squares;

step 2 - Using parameter imputations for all statistically significant components ($p \leqslant 0.05$) in step 1, nonlinear least-squares analysis (NLLS) provides estimates of rhythm characteristics A, ∅ and τ for all components fitted concomitantly;

step 3 - If one or several components in ranges indicated by LLS are not statistically significant, a concomitant fit (by NLLS) of all statistically significant components is computed to provide new estimates for rhythm characteristics;

step 4 - Computation of deviations or residuals by subtracting model (obtained in step 3) from original data;

step 5 - Perform NLLS analysis on deviations using components that were statistically significant in step 1 but not in step 2;

step 6 - If additional components are resolved by applying NLLS to the deviations, repeat steps 2 and 3 after changing the imputations for all parameters.

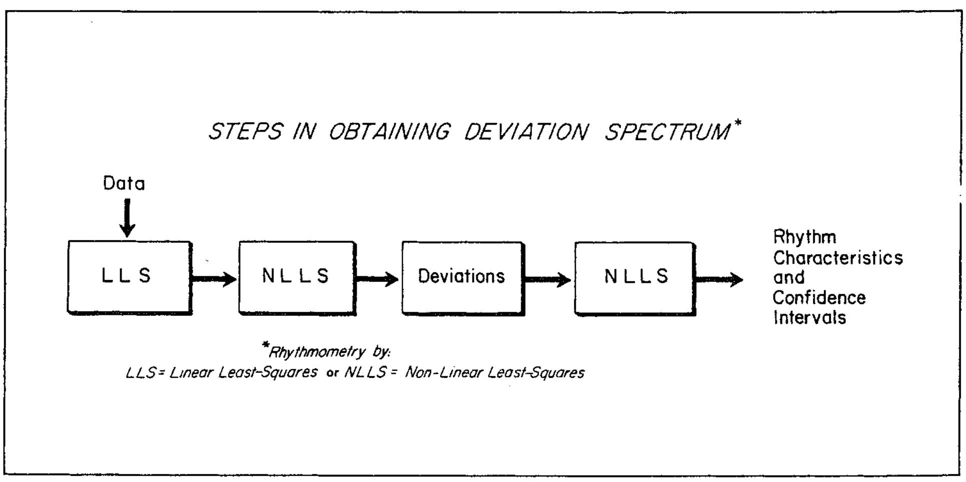


Fig. 5a - Flowchart of the different steps involved in the linear-nonlinear least-squares procedure.

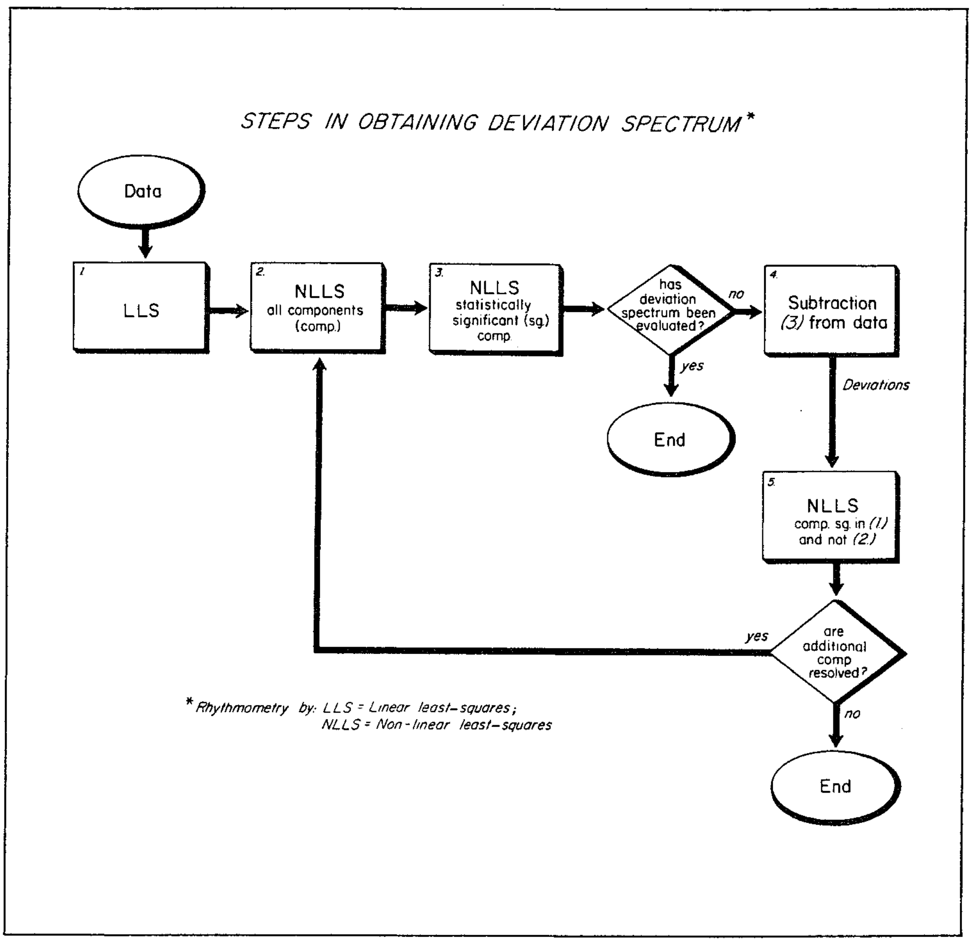
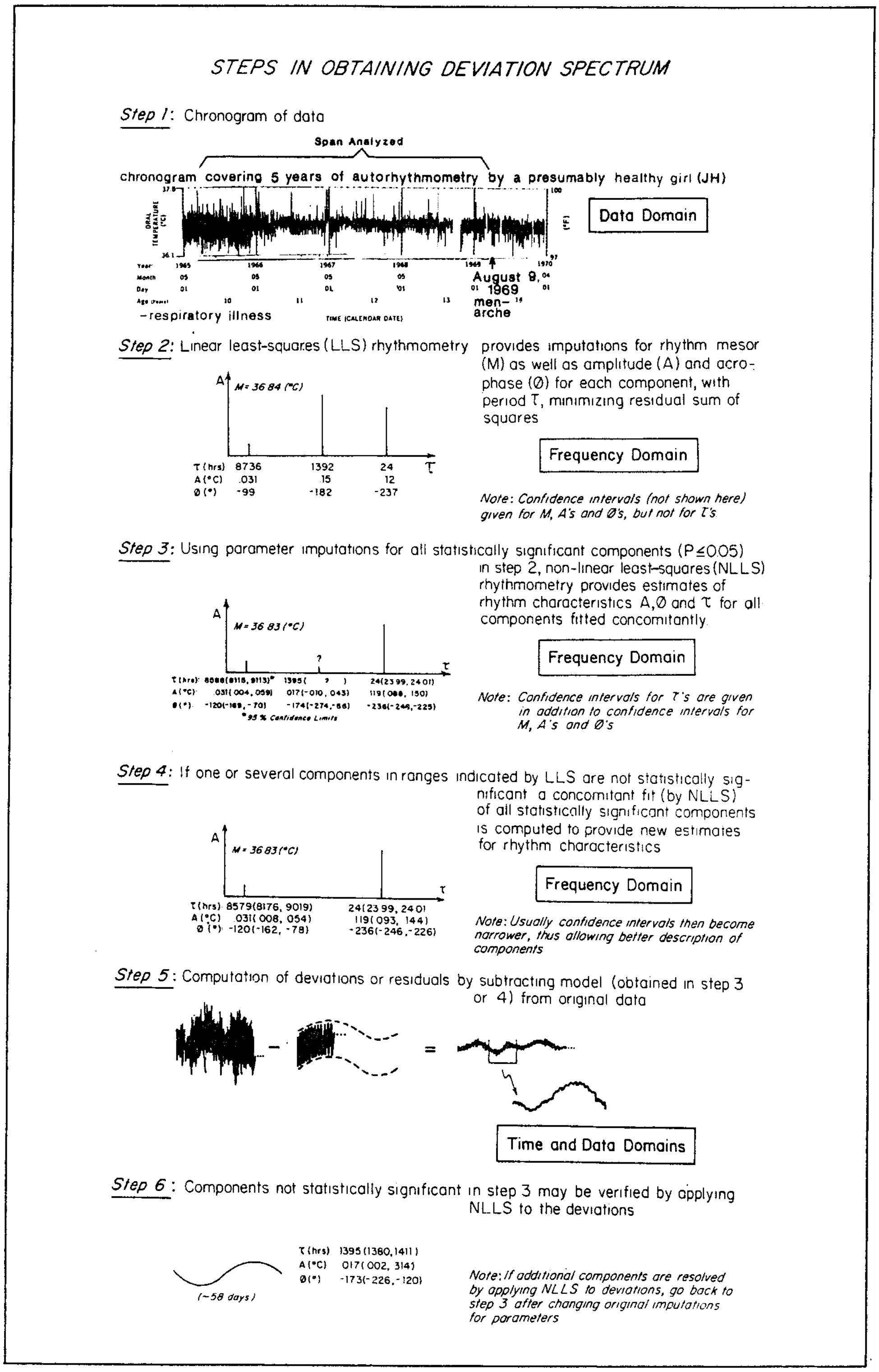


Fig. 5b - Flowchart of the different steps involved in the linear-nonlinear least-squares procedure.

All procedures described above were initially tested by using 'standards and blanks' [13] and proved to be adequate to analyze data containing bioperiodicities. For rhythmometric standards one can utilize certain functions that on the basis of past experience best approximate the data. As a start only, one can take certain pure sinusoids with known period, amplitude and acrophase. One then assigns the values of such functions to the timepoints at which a given time series to be analyzed is available and carries out the analyses on these artificially generated numbers concomitantly with the analysis of the original series. Any component yielded by the analysis of such standards, preferably with graded amounts of noise, can serve for comparisons with components resolved in the original time series. The equivalent of a biochemical blank for rhythmometry is some random process.

Fig. 5c - Illustrative example of the different steps involved in the linear-nonlinear least-squares procedure. ▶


STEPS IN OBTAINING DEVIATION SPECTRUM
Step 1: Chronogram of data
Span Analyzed
chronogram covering 5 years of autorhythmometry by a presumably healthy girl (JH)
Data Domain
August 9, 1969
men-arche
-respiratory illness
Step 2: Linear least-squares (LLS) rhythmometry provides imputations for rhythm mesor (M) as well as amplitude (A) and acrophase (Ø) for each component, with period τ, minimizing residual sum of squares
M = 36 84 (°C)
τ (hrs) 8736 1392 24
A (°C) .031 15 12
Ø (°) -99 -182 -237
Frequency Domain
Note: Confidence intervals (not shown here) given for M, A's and Ø's, but not for τ's
Step 3: Using parameter imputations for all statistically significant components (P≤0.05) in step 2, non-linear least-squares (NLLS) rhythmometry provides estimates of rhythm characteristics A, Ø and τ for all components fitted concomitantly
M = 36 83 (°C)
A (°C) .031(004, 059) 017(-010, 043) 119(088, 150)
Ø (°) -120(-169, -70) -174(-274, -66) -236(-249, -225)
*95 % Confidence Limits
Frequency Domain
Note: Confidence intervals for τ's are given in addition to confidence intervals for M, A's and Ø's
Step 4: If one or several components in ranges indicated by LLS are not statistically significant a concomitant fit (by NLLS) of all statistically significant components is computed to provide new estimates for rhythm characteristics
M = 36 83 (°C)
τ (hrs) 8579(8176, 9019) 24(23 99, 24 01)
A (°C) 031(008, 054) 119(093, 144)
Ø (°) -120(-162, -78) -236(-246, -226)
Frequency Domain
Note: Usually confidence intervals then become narrower, thus allowing better description of components
Step 5: Computation of deviations or residuals by subtracting model (obtained in step 3 or 4) from original data
Time and Data Domains
Step 6: Components not statistically significant in step 3 may be verified by applying NLLS to the deviations
τ (hrs) 1395 (1380, 1411)
A (°C) 017 (002, 314)
Ø (°) -173(-226, -120)
(~58 days)
Note: If additional components are resolved by applying NLLS to deviations, go back to step 3 after changing original imputations for parameters

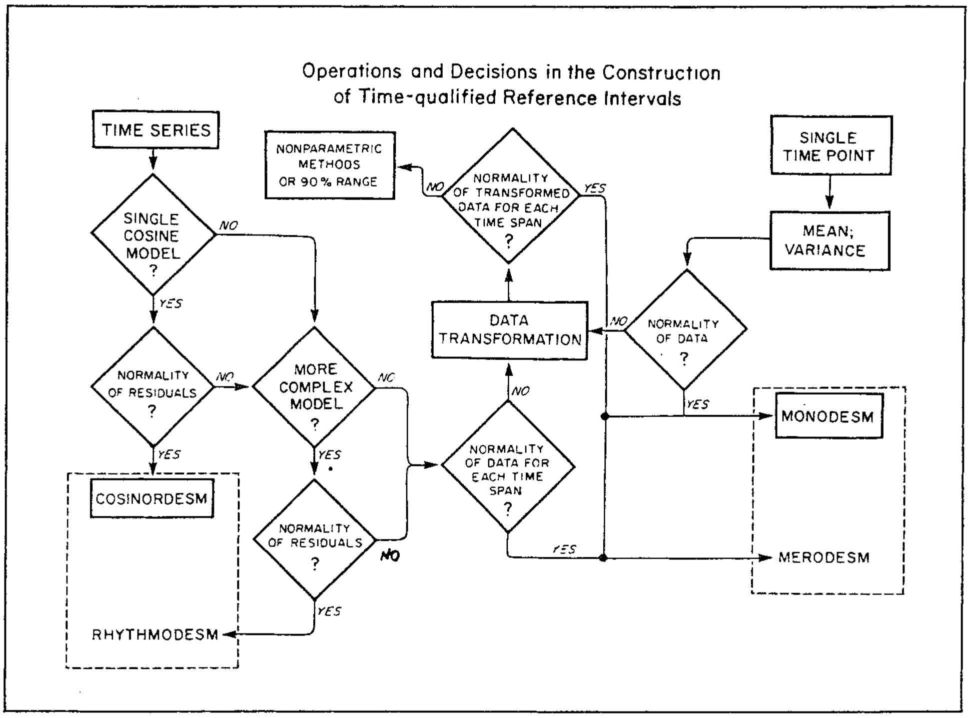


Fig. 6 - Tentative flowchart indicating a few of the many paths towards the establishment of chronodesms.

One may generate, for instance, at the intervals at which original data are available, 100 series of gaussian noise and 100 series of rectangular noise. These artificial (blank) series are then analyzed concomitantly with the raw (original) data and the standards. The continued use of certain standards and blanks and other test series for each new kind of analysis, if not with each analysis, is strongly recommended. As is the case in classical biometry, the results obtained by chronobiologic methods can be used to derive confidence and tolerance intervals, with the additional important feature that such reference intervals can now be time-specified.

First a distinction has to be made between data collected at a single timepoint and those collected at multiple timepoints. Let us start with the simple case of samples taken at a single timepoint chosen by necessity (in an emergency) or by pertinence (conditions permitting). Tolerance limits are defined as limiting values within which a specified proportion of a distribution will lie with a fixed probability. Tolerance intervals qualified in time are called chronodesms. When multiple timepoints are available, one may either apply the same procedure (if multiple samples are obtained at each timepoint) or one can derive such tolerance limits on the basis of a model [23] (fig. 6). If the model is a cosine function, the chronodesm is called a cosinordesm; if a more complex waveform has been considered, one uses the term rhythmodesm, and if no model applies, the chronodesm is called a merodesm (for several separate timepoints or timespans throughout the rhythm) or a monodesm (for a single timepoint or timespan). The importance of chronodesms lies in the validation of a diagnosis on the basis of time-specified single samples. Once a time-qualified reference interval has been obtained for a group or preferably for an individual, future single samples can be usefully compared for clinical evaluation.

As an example, an individualized merodesm was constructed for cortisol, measured every 20 min during 24 h in winter. To validate this chronodesm, values measured in spring in the same fashion for the same person were plotted against the winter chronodesm. A good agreement is found (fig. 7) from 02^{00} to 18^{00}, whereas from 18^{00} to 02^{00} larger tolerance limits characterize the spring data, perhaps because of circannual variation.

Applications

1. *Marker rhythms* - Among many other potential uses, a marker rhythm can serve to monitor the timing of treatment and/or to assess therapeutic response. The marker rhythm monitors a phenomenon by one or more of its quantified characteristics. If the characteristic involved is an acrophase, paraphase or orthophase, the marker rhythm is a useful reference rhythm. For example, one may find that radiation therapy yields faster remission of a tumor in the oral cavity when applied at the acrophase of the circadian rhythm in tumor temperature than if it is applied 4 or 8 h before or after that time. The circadian rhythm in tumor temperature might be used subsequently as a marker rhythm for timing treatment in that particular disease [19].

Another important field of application for biologic time series analysis involves the monitoring of physiologic variables such as the peak expiratory flow as gauges for effects as varied as those of drugs, on the one hand, and of environmental pollutants, on the other hand. REINBERG et al. [41] showed that this variable of physiologic and pathologic interest — as a gauge of the extent of airway patency or, rather, obstruction — reveals changes in asthmatic boys: mesor and acrophase of peak expiratory flow depend apparently upon the timing of methylprednisolone

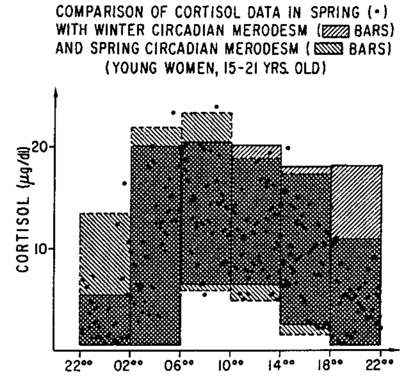


Fig. 7 - Extent of agreement of chronodesms constructed for 2 seasons.

injection. In the same line of thought, HALBERG et al. [27] found that the peak expiratory flow mesor of a peak flow self-measuring girl was statistically significantly higher while she stayed in suburban Minnesota than in metropolitan Paris. This finding suggests that peak expiratory flow could perhaps be monitored within the school system by autorhythmometry with a view of monitoring environmental integrity [22]. The suggestion that some human subjects may be tested as potential sentinel organisms or biologic detectors of overall pollution was supported by the increase of peak expiratory flow mesor in certain patients with exogenous asthma when they were introduced into a hypoallergenic room [40].

Another aspect of chronobiologic application in the field of environmental protection is discussed by STUPFEL et al. [47]. These authors documented rhythms in different frequency ranges of interest from the standpoint of environmental pollution. This approach allows a more accurate view of environmental factors in the production of air pollution and a clarification of their interaction, as well as of the physiopathological reactions of human beings to pollution. The authors underlined the need for the combination of appropriate statistical and physiologic methodologies in determining both short-term and long-term periodicities that have to be taken into account when considering the merits or demerits of air quality standards and other means of environmental protection. STEBBINGS and HALBERG [46] studied results provided by panel studies relating illness (such as attacks of asthma, cardiopulmonary symptoms and acute respiratory symptoms) to daily air pollution and weather. After evaluating amplitudes and acrophases of circannual ($\sim$ 1 year), circatrigintan ($\sim$ 1 month) and circaseptan ($\sim$ 1 week) rhythms, they found that circannual rhythms are prominent. Results from linear-nonlinear rhythmometry in this study are reported by TESLOW et al. [48]. Against this background it will be mandatory to determine rhythm-based chronodesms [7]. The daily checking of reported new cases of attacks against a population chronodesm provides an approach complementary to non-biologic environmental monitoring and should be complemented, in its turn, by longitudinal monitoring of physiologic functions, such as peak flow, among other potential marker variables.

2. *Sampling qualifications* - As explained above, chronodesms validate spot-checks and single samples. They usually are built, however, from a large data base, either on a group of individuals or on the individual himself. Minimal sampling requirements for estimation of parameters are also worth investigating. To illustrate an empirical approach to answering this question, cosine curves were fitted to data on an individual's blood pressure, measured every 10 min during 24 h. The best fitting 24-h cosine curve when all collected data were considered was compared to the results when data collected during the rest span are omitted. The procedure was repeated with the difference that only hourly data were taken. The mesor was the least affected by the sampling conditions whereas the amplitude was most affected. The acrophase also remained quite stable, although for the hourly intervals, the p-value was above the 0.05 probability level. Rhythm characteristics, in this case, can be taken into consideration, at least as imputations (when the zero amplitude hypothesis cannot be rejected).

3. *Manipulation of rhythms* - Certain rhythms can be manipulated, e.g., by changes in lighting or feeding regimens. This possibility is very attractive in view of the potential optimization of host tolerance and tumor susceptibility, to

cite but one example. Frequency demultiplication is another approach in attempting to improve the yield of anti-cancer therapy. If one can assume that two subsystems (e.g., one neural, another endocrine) affect the tolerance of a critical target or link (for instance, a circadian or other cell cycle in the bone marrow) in opposite ways (one augmenting, the other reducing it), frequency demultiplication (obtained, e.g., by desynchronization with both lighting and meals) could improve the therapeutic index of carcinostatic drugs by inducing proper relations between the hypothesized subsystems to achieve the highest % survivors, the largest safe span or other desiderata of treatment [6].

SUMMARY

Selected methods for the study of biologic time series are reviewed and their relative merits are discussed in the light of underlying assumptions. Their potential applications are exemplified in several fields of biology and medicine. The monitoring of environmental integrity, notably of pollution, is investigated. The need for specifying optimal sampling requirements is underlined. An individualized and time-qualified definition of health by the establishment of reference intervals is required for increasingly rational individualized program for the prevention and/or treatment of disease. With these reference intervals and rhythm characteristics available, one can better interpret with single samples or time series an increased risk of a certain disease or the inception of the disease. For all of these aims the monitoring of environmental and/or personal marker rhythms is essential — to obtain large data bases from which information can be more easily derived for monitoring personal health, to recognize risk as well as to diagnose disease early and to optimize treatment by timing according to rhythms.

REFERENCES

1. BEUTLER F. J.: Alias-free randomly timed sampling of stochastic processes - IEEE Trans. Information Theory *IT-16*, 147, 1970.
2. BLACKMAN R. B., TUKEY J. W.: The measurement of power spectra - Dover, New York, 1958.
3. BLOOMFIELD P.: Fourier analysis of time series: an introduction - Wiley Interscience, New York, 1976.
4. BOX G. E. P., JENKINS G. M.: Time series analysis: forecasting and control - Holden Day, San Francisco, 1970.
5. CORNÉLISSEN G.: Analyse de signaux et application aux problèmes de définition de la stabilité de fréquence - Ph.D. Thesis, University of Brussels, Belgium, 1976.
6. CORNÉLISSEN G., HALBERG F., HAUS E., NESBIT M., SCHEVING L. E.: Modeling partial frequency-demultiplication of mammalian circadian system (PFD-CS) to improve by marker rhythm monitoring anticancer therapy - Proc. Int. Symp. Clin. Chronopharmacol., Chronother. and Chronopharmacy. Tallahassee, Florida, February 9-12, 1978; p. 36.
7. CORNÉLISSEN G., HALBERG F., LEE J. K., TESLOW T., STEBBINGS J. E.: Biologic monitoring for environmental protection with rhythm-based chronodesms (tolerance intervals) - Proc. Minn. Acad. Sci. 1978; p. 21.
8. DE PRINS J., CORNÉLISSEN G., HALBERG F.: Harmonic interpolation on equispaced series covering integral periods of anticipated circadian rhythm in adriamycin tolerance - Chronobiologia *4*, 173, 1977.
9. DE VECCHI A., HALBERG F., SOTHERN R. B., CANTALUPPI A., PONTICELLI C.: Circaseptan rhythmic aspects of rejection in treated patients with kidney transplants - Proc. Int. Symp. Clin. Chronopharmacol., Chronother. and Chronopharmacy. Tallahassee, Florida, February 9-12, 1978; p. 23.
10. GRANGER C. W. J.: Spectral analysis of economic time series - Princeton University Press, 1964.
11. HALBERG F.: Beobachtungen über 24 Stunden-Periodik in standardisierter Versuchsanordnung vor und nach Epinephrektomie und bilateraler optischer Enukleation - In: 20th Meeting of the German Physiologic Society. Homburg/Saar, September 1953 - Ber. ges. Physiol. *162*, 354, 1954.

12. HALBERG F.: Physiologic 24-hour periodicity; general and procedural considerations with reference to the adrenal cycle - Z. Vitamin-, Hormon- u. Fermentforsch. *10*, 225, 1959.

13. HALBERG F.: Physiologic considerations underlying rhythmometry, with special reference to emotional illness - In: Symposium on biological cycles and psychiatry. Symposium Bel-Air III. Masson et Cie., Genève, 1968; p. 73.

14. HALBERG F.: Body temperature, circadian rhythms and the eye - In: BENOIT J., ASSENMACHER I. (Eds): La photorégulation de la reproduction chez les oiseaux et les mammifères. Centre National de la Recherche Scientifique, Paris, no. 172, 1970; p. 497. Discussion remarks pp. 520-528. (See also additional discussion remarks pp. 47, 51, 67, 69, 90, 113-116, 164-165, 187, 209, 380, 381, 382, 383, 384, 406, 407, 408, 541, 542, 546).

15. HALBERG F.: Biological as well as physical parameters relate to radiology - Guest Lecture, Proc. 30th Ann. Congr. Rad., Jan. 1977. Postgraduate Institute of Medical Education and Research, Chandigarh, India.

16. HALBERG F., CARANDENTE F., CORNÉLISSEN G., KATINAS G. S.: Glossary of chronobiology - Chronobiologia *4* (Suppl. 1), 1977; pp. 190.

17. HALBERG F., CORNÉLISSEN G., SOTHERN R. B., WALLACH L. A., HALBERG E., AHLGREN A., KUZEL M., RADKE A., BARBOSA J., GOETZ F., BUCKLEY J., MANDEL J., SCHUMAN L., HAUS E., LAKATUA D., SACKETT L., BERG H., KAWASAKI T., UENO M., UEZONO K., MATSUOKA M., OMAE T., TARQUINI B., CAGNONI M., GARCIA SAINZ M., GRIFFITHS K., WILSON D., DONATI L., TATTI P., VASTA M., LOCATELLI I., CAMAGNA A., LAURO R., KRIPKE D., TRITSCH G., WENDT H.: International studies of human host and tumor rhythms with multiple frequencies lead toward cost-effective sampling - In: KAISER H. (Ed.): Neoplasms - Comparative pathology of growth in animals, plants and man - Williams and Wilkins Co., Baltimore.

18. HALBERG F., ENGELI M., HAMBURGER C., HILLMAN D.: Spectral resolution of low-frequency, small-amplitude rhythms in excreted 17-ketosteroid; probable androgen-induced circaseptan desynchronization - Acta endocr. (Kbh.) *100* (Suppl.), 170, 1965.

19. HALBERG F., GUPTA B. D., HAUS E., HALBERG E., DEKA A. C., NELSON W., SOTHERN R. B., CORNÉLISSEN G., LEE J. K., LAKATUA D. J., SCHEVING L. E., BURNS E. R.: Steps toward a cancer chronopolytherapy - In: Proc. XIVth Int. Congr. of Therapeutics. Montpellier, France. L'Expansion Scientifique Française, 1977; p. 151.

20. HALBERG F., HALBERG E., CARANDENTE F.: Chronobiology and metabolism in the broader context of timely intervention and timed treatment - In: Diabetes Research Today. Meeting of the Minkowski Prize Winners. Symposia Medica Hoechst 12 (Capri). F. K. Schattauer Verlag, Stuttgart/New York, 1976; p. 45.

21. HALBERG F., JOHNSON E. A., NELSON W., RUNGE W., SOTHERN R.: Autorhythmometry - Procedures for physiologic self-measurements and their analysis - Physiology Teacher *1*, 1, 1972.

22. HALBERG F., LAURO R., CARANDENTE F.: Autorhythmometry leads from single-sample medical check-ups toward a health science of time series - La Ricerca Clin. Lab. *6*, 207, 1976.

23. HALBERG F., LEE J. K., NELSON W.: Time-qualified reference intervals – chronodesms - Experientia (Basel) *34*, 713, 1978.

24. HALBERG F., PANOFSKY H.: I. Thermo-variance spectra; method and clinical illustrations - Exp. Med. Surg. *19*, 284, 1961.

25. HALBERG F., RATTE J., KÜHL J. F. W., NAJARIAN J. S., POPOVICH V., SHIOTSUKA R., CHIBA Y., CUTKOMP L. K., HAUS E.: Rythmes circaseptidiens – environ 7 jours – synchronisés ou non avec la semaine sociale - C.R. Acad. Sci. (Paris) *278*, 2675, 1974.

26. HALBERG F., REINBERG A., HAUS E., GHATA J., SIFFRE M.: Human biological rhythms during and after several months of isolation underground in natural caves - Nat. speleolog. Soc. Bull. *32*, 89, 1970.

27. HALBERG F., REINBERG Alain, REINBERG Agnès: Chronobiological serial sections gauge circadian rhythm adjustments following transmeridian flights and life in novel environment - Waking and Sleeping *1*, 259, 1977.

28. HALBERG J., HALBERG E., HALBERG F., HALBERG Francine, LEVINE H., HAUS E., CARANDENTE F., BARTTER F. C., DELEA C.: Human and murine (SHRSP) blood pressure and telemetered core temperature variation and genetic disposition to mesor-hypertension - (In press).

29. HÜBNER K.: Kompensatorische Hypertrophie, Wachstum und Regeneration der Rattenniere - Ergebn. allg. Path. path. Anat. *100*, 1, 1967.

30. JONES R. H.: Spectrum estimation with unequally spaced observations - AFOSR 70-2747T8, AD7 6291, 1971.

31. KOOPMANS L. H.: Spectral analysis of time series - Academic Press, New York, 1974.

32. KOUKKARI W. L., DUKE S. H., HALBERG F., LEE J. K.: Circadian rhythmic leaflet movements: student exercise in chronobiology - Chronobiologia *1*, 281, 1974.

33. LEVI F., HALBERG F., NESBIT M., HAUS E., LEVINE H.: Chrono-oncology - In: KAISER H. (Ed.): Neoplasms - Comparative pathology of growth in animals, plants and man - Williams and Wilkins Co., Baltimore.

34. MARQUARDT D. W.: An algorithm for least-squares estimation of nonlinear parameters - J. Soc. indust. appl. Math. *11*, 431, 1963.

35. MASRY E.: Random sampling and reconstruction of spectra - Information and Control *19*, 275, 1971.

36. MASRY E., LUI M. C. C.: A consistent estimate of the spectrum by random sampling of the time series - J. Soc. indust. appl. Math. *28*, 793, 1975.

37. NELSON W., TONG Y. L., LEE J.-K., HALBERG F.: Methods for cosinor-rhythmometry - Chronobiologia *6*, 305, 1979.

38. OPPENHEIM A. V., SCHAFER R. W.: Digital signal processing - Prentice-Hall, New Jersey, 1975.

39. PANOFSKY H., HALBERG F.: II. Thermo-variance spectra; simplified computational example and other methodology - Exp. Med. Surg. *19*, 323, 1961.

40. REINBERG A., GERVAIS P., HALBERG Francine, HALBERG F.: Trisentinel monitoring of air pollution by autorhythmometry of peak expiratory flow - In: ENGLUND H. M., BERRY W. T. (Eds): Proc. 2nd Int. Clean Air Congress, Dec. 1970. Academic Press, New York/London, 1971; p. 217.

41. REINBERG A., HALBERG F., FALLIERS C. J.: Circadian timing of methylprednisolone effects in asthmatic boys - Chronobiologia *1*, 333, 1974.

42. RUMMEL J. A., LEE J. K., HALBERG F.: Combined linear-nonlinear chronobiologic windows by least squares revolve neighboring components in a physiologic rhythm spectrum - In: FERIN M., HALBERG F., RICHART R. M., VANDE WIELE R. (Eds): Biorhythms and human reproduction. John Wiley and Sons, Inc., New York, 1974; p. 53.

43. RUNGE W., LANGE K., HALBERG F.: Some instruments for chronobiologists developed or used at the University of Minnesota - Int. J. Chronobiol. *2*, 327, 1974.

44. SHAPIRO H. S., SILVERMAN R. A.: Alias-free sampling of random noise - J. Soc. indust. appl. Math. *8*, 225, 1960.

45. SIMPSON H. W., HALBERG E.: Menstrual changes of the circadian temperature rhythm in women - In: FERIN M., HALBERG F., RICHART R. M., VANDE WIELE R. (Eds): Biorhythms and human reproduction. John Wiley and Sons, Inc., New York, 1974.

46. STEBBINGS J. H., HALBERG F.: Strategies for relating daily illness to ambient air pollution - In: Proc. of the 8th Int. Sci. Meeting of the International Epidemiological Association. San Juan, Puerto Rico, September 17-23, 1977; p. 350.

47. STUPFEL M., HALBERG F., MORDELET-DAMBRINE M., MAGNIER M.: Perspectives in chronobiology of air pollution - Chronobiologia *4*, 333, 1977.

48. TESLOW T. N., STEBBINGS J., CORNÉLISSEN G., HALBERG F.: Asthma attack panel data as sentinels of environmental protection resolved by chronobiologic computer methods - Proc. Minn. Acad. Sci. 1978; p. 20.

49. THOMPSON R. O. R. Y.: Spectral estimation from irregularly spaced data - IEEE Trans. Geoscience Electronics *GE-9*, 107, 1971.

50. TONG Y. L., NELSON W. L., SOTHERN R. B., HALBERG F.: Estimation of the orthophase (timing of high values) on a non-sinusoidal rhythm, illustrated by the best timing for experimental cancer chronotherapy - In: Proceedings of the XIIth International Conference on Chronobiology. Washington, D.C., 1975. Publishing House Il Ponte, Milano, 1977; p. 765.

51. TROLANDER H. W.: The current state of electrical thermometry for biological applications - Presented at the 5th Symposium on Temperature. Washington, D.C., June 21-24, 1977 - Electrical Thermometry *187*, 2035, 1977.

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APPENDIX

Since rhythmometry of short series uses largely the least-squares fit of a cosine model, the single cosinor and the (population) mean cosinor procedures [6,7] have been programmed on a pocket calculator. It was desirable to use magnetic cards for storing data as well as programs, so that if several procedures needed to be applied, the data would not have to be entered manually each time. Library modules that can be plugged into the calculator, providing built-in (hardware) programs for other procedures, are also attractive. Because the Texas Instruments TI-59 pocket calculator has the above-mentioned features, it was chosen for implementing the single — and mean — cosinor programs. The programs can be used with or without the PC 100A printer. The development of programs such as the single — or mean — cosinor for pocket calculators is very attractive for achieving chronobiologic goals at the bedside or in the field, e.g., whenever data on potential marker rhythms can be self-measured or collected by staff.

The single cosinor program here proposed applies to non-equidistant as well as equidistant data. There is no restriction on the number of data involved (other than that dictated by the capabilities of the calculator itself such as the required precision, overflow, . . .), but obviously the time required for entering data ($\sim$6.5 sec/conventional datum and time code) should be considered. These 6.5 sec/datum include not only the time required for entry of the datum itself but also the computing time needed by the calculator to process the information before it is ready to receive the next datum. Once the data are entered, the computing time required to get the results is only $\sim$35 sec. In addition to the estimation of the mesor, double amplitude and acrophase with their respective standard errors, the program provides the percent rhythm and the associated p-value obtained in the zero-amplitude test. There is also an option (in the case of a 24-h cosine) of expressing the acrophase and its standard error in clock hours and minutes in addition to expressing them in degrees.

Again, there is no restriction on the number of time series involved when computing the mean cosinor. The procedure uses the rhythm parameter imputations computed via the single cosinor program and hence presupposes that the single cosinor procedures have already been applied. Data entry requires $\sim$ 10 sec per imputation set. Results are obtained in less than 1 min. If the coordinates of some of the points on the boundary of the confidence region (ellipse) are desired, an additional $\sim$ 5 sec per pair of (β, γ) and (A, $\varnothing$) coordinates is required. The program provides point estimates for (mean) percent rhythm, mesor, amplitude and acrophase, the p-value associated with the zero-amplitude test, the length of the semi-axes of the 95 % error ellipse, the angle between the major axis and the positive abscissa as well as the slopes and associated angles of the tangents drawn from the pole to the error ellipse [2,3].

A program combining the single — and mean — cosinor methods also is available. In this program, data on each of several series are entered, and a mean cosinor summary is directly obtained. This program provides point estimates of M, A and Ø for each series as well as polar coordinates of points on the boundary of the 95 % confidence region for the mean cosinor.

The user of these programs may desire other kinds of output. In such a case, one must consider the limits imposed by the capacity of the pocket calculator and may have to trade one output for another.

OUTLINE OF THE PROGRAMS

A. *Single cosinor*

Let us suppose that N data, y_i, are collected at times t_i, not necessarily equidistant. The following model is fitted to the data by the method of least-squares

$$y_i = y(t_i) = M + A \cos(\omega t_i + \varnothing) + e_i \qquad i = 1, \ldots, N$$

where M is the mesor, A is the amplitude and Ø is the acrophase. The e_i are assumed to be independent random normal deviates with mean zero and unknown variance σ^2. The model can be rewritten as

$$y_i = M + \beta \cos \omega t_i + \gamma \sin \omega t_i + e_i$$

where

$$\beta = A \cos \varnothing$$
$$\gamma = -A \sin \varnothing$$

to yield an equation linear in the parameters M, β and γ.

The method of least-squares, minimizing $\Sigma_i e_i^2$ solves the normal equations:

$$\begin{cases} \Sigma y_i = MN + \beta \Sigma \cos \omega t_i + \gamma \Sigma \sin \omega t_i \\ \Sigma y_i \cos \omega t_i = M \Sigma \cos \omega t_i + \beta \Sigma \cos^2 \omega t_i + \gamma \Sigma \sin \omega t_i \cos \omega t_i \\ \Sigma y_i \sin \omega t_i = M \Sigma \sin \omega t_i + \beta \Sigma \sin \omega t_i \cos \omega t_i + \gamma \Sigma \sin^2 \omega t_i \end{cases}$$

This system can also be written as the matrix equation

$$b = S \cdot x$$

or

$$\begin{bmatrix} \Sigma y_i \\ \Sigma y_i \cos \omega t_i \\ \Sigma y_i \sin \omega t_i \end{bmatrix} = \begin{bmatrix} N & \Sigma \cos \omega t_i & \Sigma \sin \omega t_i \\ \Sigma \cos \omega t_i & \Sigma \cos^2 \omega t_i & \Sigma \sin \omega t_i \cos \omega t_i \\ \Sigma \sin \omega t_i & \Sigma \sin \omega t_i \cos \omega t_i & \Sigma \sin^2 \omega t_i \end{bmatrix} \cdot \begin{bmatrix} M \\ \beta \\ \gamma \end{bmatrix}$$

The estimates $\hat{M}$, $\hat{\beta}$ and $\hat{\gamma}$ are obtained by inverting the S matrix:

$$x = S^{-1} \cdot b$$

where the matrix elements $s_{ji}^{-1} = \frac{1}{|S|} \text{cof}(s_{ij})$ with $|S|$ being the determinant of S and $\text{cof}(s_{ij})$ being the cofactor of s_{ij}.

The amplitude and acrophase are then obtained by

$$\hat{A} = (\hat{\beta}^2 + \hat{\gamma}^2)^{1/2}; \ \hat{\varnothing} \ \arctan(-\hat{\gamma}/\hat{\beta}) + K\pi \text{ (where K is an integer)}$$

The correct value of $\hat{\varnothing}$ is determined by taking account of the signs of $\hat{\beta}$ and $\hat{\gamma}$.

The variability ratio (VR) or proportion of the variability accounted for by the cosine model can be computed as

$$VR = \frac{\hat{\beta}\Sigma (y_i - \bar{y}) \cos \omega t_i + \hat{\gamma}\Sigma (y_i - \bar{y}) \sin \omega t_i}{\Sigma (y_i - \bar{y})^2} \quad \text{with } \bar{y} = \frac{1}{N} \Sigma y_i$$

It can be shown that the p-value associated with the zero-amplitude test can be obtained by:

$$P = (1 - VR)^{(N-3)/2}$$

Percent rhythm (PR) and percent error (PE) are then defined by

$$PR = 100 \ VR; \quad PE = 100 \ (1 - VR)$$

Standard errors of the parameters M, β and γ can be derived from the inverse matrix (S^{-1}) and from the estimation of the variance.

$$\hat{\sigma}^2 = (1 - VR) \ \Sigma (y_i - \bar{y})^2 / (N - 3)$$

To evaluate the standard error for the amplitude and the acrophase, their function in terms of β and γ is expanded in a Taylor series about ($\hat{\beta}$, $\hat{\gamma}$) and the development is limited to the first term (linear approximation [8]). Standard errors for the mesor, the amplitude and the acrophase are then computed respectively as:

$$\delta M = \hat{\sigma} \sqrt{s_{11}^{-1}}$$

$$\delta A = \hat{\sigma} \, [s_{22}^{-1} \cos^2 \hat{\varnothing} + 2 \, s_{23}^{-1} \sin \hat{\varnothing} \cos \hat{\varnothing} + s_{33}^{-1} \sin^2 \hat{\varnothing}]^{1/2}$$

$$\delta \varnothing = \frac{\hat{\sigma}}{\hat{A}} \, [s_{22}^{-1} \sin^2 \hat{\varnothing} - 2 s_{23}^{-1} \sin \hat{\varnothing} \cos \hat{\varnothing} + s_{33}^{-1} \cos^2 \hat{\varnothing}]^{1/2}$$

B. *Population mean cosinor*

Let us suppose that N time series were analyzed by the single cosinor with the following results for each:

— the percent rhythm PR_i;
— the mesor M_i;
— the amplitude A_i;
— the acrophase $\varnothing_i$ (expressed as negative degrees) for $i = 1, \ldots, N$.

Let us define

$$\beta_i = A_i \cos \varnothing_i$$
$$\gamma_i = -A_i \sin \varnothing_i$$

When all point estimates have been entered for all N series, the mean parameter estimates are computed as:

$$\hat{PR} = \frac{1}{N} \Sigma PR_i; \quad \hat{M} = \frac{1}{N} \Sigma M_i$$

$$\hat{\beta} = \frac{1}{N} \Sigma \beta_i; \quad \hat{\gamma} = \frac{1}{N} \Sigma \gamma_i$$

The estimation of $\hat{\beta}$ and $\hat{\gamma}$ yields

$$\hat{A} = (\hat{\beta}^2 + \hat{\gamma}^2)^{1/2} \quad \text{and}$$

$$\hat{\varnothing} = \arctan(-\hat{\gamma}/\hat{\beta}) + K\pi \text{ (where K is an integer).}$$

The uncertainty of $K\pi$ is resolved by taking account of the signs of $\hat{\beta}$ and $\hat{\gamma}$.

To test the statistical significance of the (population) rhythm investigated, the F-statistic with 2 and (N — 2) degrees of freedom is used:

$$F_{1-\alpha}(2, N-2) = \frac{N-2}{2}(\alpha^{-2/(N-2)} - 1)$$

The p-value associated with the zero-amplitude test is computed in the following way:

$$csb2n = \Sigma\beta_i^2/N - \hat{\beta}^2; \; csbgn = \Sigma\beta_i\gamma_i/N - \hat{\beta}\hat{\gamma}; \; csg2n = \Sigma\gamma_i^2/N - \hat{\gamma}^2$$

$$\Delta = csb2n \cdot csg2n - (csbgn)^2$$

$$a_{11} = csg2n/\Delta; \; a_{12} = -2csbgn/\Delta; \; a_{22} = csb2n/\Delta$$

$$p = [1 + a_{11}\hat{\beta}^2 + a_{12}\hat{\beta}\hat{\gamma} + a_{22}\hat{\gamma}^2]^{-(N-2)/2}$$

In order to determine the error ellipse, given a level of significance, α (e.g., $\alpha = 0.05$ for the 95 % confidence region), estimates for the variance and covariance terms are calculated:

$$s_\beta^2 = (\Sigma\beta_i^2 - N\hat{\beta}^2) / (N-1)$$

$$s_\gamma^2 = (\Sigma\gamma_i^2 - N\hat{\gamma}^2) / (N-1)$$

$$\hat{r}_{\beta\gamma} = (\Sigma\beta_i\gamma_i - N\hat{\beta}\hat{\gamma}) / [(N-1)\, \hat{s}_\beta\hat{s}_\gamma]$$

The equation of the error ellipse may then be written as:

$$E = A(\gamma - \hat{\gamma})^2 + 2B(\beta - \hat{\beta})(\gamma - \hat{\gamma}) + C(\beta - \hat{\beta})^2 = D$$

with

$$A = 1/\hat{s}_\gamma^2$$

$$B = -\hat{r}_{\beta\gamma}/(\hat{s}_\beta\hat{s}_\gamma)$$

$$C = 1/\hat{s}_\beta^2$$

and

$$D = \frac{2(N-1)}{(N-2)N}(1 - \hat{r}_{\beta\gamma}^2)\, F_{1-\alpha}(2, N-2)$$

The lengths of the semi-axes are estimated as follows:

— major axis: $\hat{a} = [2D / (A + C — R)]^{1/2}$

— minor axis: $\hat{b} = [2D / (A + C — R)]^{1/2}$

where $R = [(A — C)^2 + 4B^2]^{1/2}$.

The angle of the major axis versus the positive abscissa is then given by

$$\hat{\theta} = \arctan \frac{2B}{A - C - R}$$

In order to determine a conservative confidence interval [1] for the acrophase, tangents may be drawn from the pole to the ellipse. The slopes (m_1 and m_2) and corresponding angles [$\theta(m_1)$ and $\theta(m_2)$] are derived as

$$m_1 = U(W + V) \qquad \theta(m_1) = \arctan(m_1)$$

$$m_2 = U(W - V) \qquad \theta(m_2) = \arctan(m_2)$$

where $U = [(AC - B^2)\hat{\gamma}^2 - CD]^{-1}$

$$V = \{(AC - B^2) D [A\hat{\gamma}^2 + 2B\hat{\beta}\hat{\gamma} + C\hat{\beta}^2 - D]\}^{1/2}$$

$$W = (AC - B^2)\hat{\beta}\hat{\gamma} + BD.$$

If one desires to draw the error ellipse, the coordinates of a few points on the boundary of the confidence region may be computed. The maximum and minimum values taken by β are first computed and displayed (or printed).

$$\beta_{\substack{max \\ min}} = \hat{\beta} \pm \sqrt{\frac{2(N-1)}{(N-2)N} \hat{s}^2_\beta F_{1-\alpha}(2, N-2)}$$

Values lying between β_{max} and β_{min} may then be entered. For each β value entered (β_0), two corresponding γ values (γ_1 and γ_2) are calculated, leading to coordinates of two points on the error ellipse:

$$\gamma_{1,2} = \hat{\gamma} + \frac{\hat{s}_\gamma}{\sqrt{N}} \left\{ \hat{r}_{\beta\gamma} \frac{\sqrt{N}(\beta_0 - \hat{\beta})}{\hat{s}_\beta} \pm \left[(1 - \hat{r}^2_{\beta\gamma}) \left(\frac{2(N-1)}{(N-2)} F_{1-\alpha}(2, N-2) - \frac{N(\beta_0 - \hat{\beta})^2}{\hat{s}^2_\beta} \right) \right]^{1/2} \right\}$$

The cartesian coordinates (β_0, γ_1) and (β_0, γ_2) are also converted to polar coordinates (A_1, $\varnothing_1$) and (A_2, $\varnothing_2$).

To get ellipses of a different size [i.e., corresponding to a different level of significance (α)], just replace '20' by $1/\alpha$ on steps 250-251 of the program.

APPLICATION OF SINGLE COSINOR

The systolic blood pressure of a 13-year-old boy and of a post-menopausal woman were automatically recorded with a Roche Arteriosonde about every 10 min during 24 h. For each subject, four series, each with $\Delta t \cong 40$ min, were derived from the original data and the single cosinor program was applied to each series on the TI-59 calculator. The results obtained are tabulated below:

systolic blood pressure: circadian rhythm characteristics

series	PR(%)	p	N	M (± SE)	DA * (± SE)	Ø ** (± SE)
— *13-year-old boy*				(mm Hg)		
1	59	< 0.01	29	106.57 ± 1.16	18.95 ± 3.32	— 256 ± 10
2	66	< 0.01	27	105.40 ± 1.07	20.20 ± 3.13	— 229 ± 8
3	65	< 0.01	25	104.85 ± 1.04	18.32 ± 3.03	— 245 ± 9
4	54	< 0.01	31	106.53 ± 1.13	17.70 ± 3.32	— 230 ± 10
— *post-menopausal woman*						
1	50	< 0.01	30	124.37 ± 2.43	36.94 ± 6.69	— 238 ± 11
2	55	< 0.01	30	126.01 ± 2.02	32.43 ± 5.71	— 250 ± 10
3	49	< 0.01	29	124.69 ± 2.33	33.68 ± 6.36	— 242 ± 11
4	43	< 0.01	30	124.97 ± 2.59	33.09 ± 7.42	— 248 ± 12

* DA = double amplitude or total extent of predictable change.
** Reference: local midnight; 360° ≡ 24 h.

These examples demonstrate the ease of quantifying rhythms on important physiologic variables.

When one has to deal with incidence data (e.g., the number of events observed on successive days), rather than with data that are not restricted to integer values, the test developed first by EDWARDS [5] and further extended by ROGER [9] is applicable. Let us suppose that a sample of N events is taken (e.g., 29 cases of human kidney transplant rejection [4]) and that each event is classified into one of k possible categories in a particular cycle of interest (e.g., a 7-day cycle of rejection following surgery. In this case, category 1 includes events occurring on days 1, 8, 15, . . . ; category 2 events on days 2, 9, 16, . . . ; category 7 events on days 7, 14, 21, . . . ; i.e., the events are 'folded' into a comprehensive 7-day period). The model considered is

$$P_i = q\,(1 + \alpha S_i + \beta C_i)$$

where α and β are parameters of the model, $q = 1/k$, $C_i = \cos(2\pi_i/k)$, and $S_i = \sin(2\pi_i/k)$ $(i = 1, \ldots, k)$.

Under the null hypothesis H_0: $\alpha = \beta = 0$, the individuals are equally likely to be allocated to each of the k categories. Under the alternative hypothesis H_1: $\alpha \neq 0$ or $\beta \neq 0$, the frequencies in the categories have some sinusoidal variation over their cyclic order.

The test statistic

$$R = 2\,[\{\Sigma\, N_i \sin(2\pi i/k)\}^2 + \{\Sigma\, N_i \cos(2\pi i/k)\}^2]/N$$

where N_i represents the number of events observed in the ith category, is derived by considering the partial derivatives of the log likelihood function evaluated at $\alpha = \beta = 0$, the null hypothesis. This test statistic is approximately chi-squared distributed with two degrees of freedom on the null hypothesis.

The test statistic in effect allocates different weights to each category according to the number of events observed in each category. The test may then be viewed as testing the departure of the center of gravity from the center of the circle used to represent the data (origin). The position of the center of gravity is indicated by the phase $\varnothing$ on the trigonometric circle and its distance from the origin is given by d, where

$$\varnothing = \arctan \frac{\bar{x}}{\bar{y}}; \quad d = (\bar{x}^2 + \bar{y}^2)^{1/2}$$

with

$$\bar{x} = \frac{1}{k} \sum_{1}^{k} N_i \sin(2\pi i/k); \quad \bar{y} = \frac{1}{k} \sum_{1}^{k} N_i \cos(2\pi i/k).$$

REFERENCES

1. Anderson T. W.: The statistical analysis of time series - J. Wiley and Sons, Inc., New York, 1971; p. 200.
2. Batschelet E.: On Hotelling's T^2 test: an expository paper designed for the needs of biologists - (Unpublished manuscript).
3. Batschelet E.: Statistical analysis of data related to biological rhythms - Handout presented at the First Course of Chronobiology. L'Aquila, Italy, February 1979.
4. De Vecchi A., Carandente F., Fryd D. S., Halberg F., Sutherland D. E., Howard R. J., Simmons R. L., Najarian J. S.: Circaseptan (about 7-day) rhythms in human kidney allograft rejection in different geographic locations - In: Reinberg A., Halberg F. (Eds): Chronopharmacology - Advanc. Biosci. *19*, 193, 1979.
5. Edwards J. H.: The recognition and estimation of cyclic trends - Ann. hum. Genet. *25*, 83, 1961.
6. Halberg F., Johnson E. A., Nelson W., Runge W., Sothern R.: Autorhythmometry - Procedures for physiologic self-measurements and their analysis - Physiology Teacher *1*, 1, 1972.
7. Halberg F., Tong Y. L., Johnson E. A.: Circadian system phase: an aspect of temporal morphology; procedures and illustrative examples - In: The cellular aspects of biorhythms. Proc. Int. Congr. of Anatomists. Symposium on Biorhythms. Springer-Verlag, 1967; p. 20.
8. Ostle B.: Statistics in research - Iowa State University Press, 1963; p. 585.
9. Roger J. H.: A significance test for cyclic trends in incidence data - Biometrika *64*, 152, 1977.

Note: The reading of program cards is more easily and more safely done by entering each program card (n) into the slot after pressing CLR for each n, instead of using the INV 2nd write command. Indeed, if this command is wrongly executed, the program card could be erased by mistake.

TITLE SINGLE COSINOR PAGE ____ OF ____ **TI Programmable Program Record**

PROGRAMMER G. CORNÉLISSEN DATE ____

Partitioning (Op 17) 7.19.29 Library Module ____ Printer ____ Cards ____

PROGRAM DESCRIPTION

LEAST SQUARES FIT OF A COSINE FUNCTION TO DATA, NOT NECESSARILY EQUIDISTANT.

MODEL : $y_i = M + A\cos(\omega t_i + \phi) + e_i \qquad i = 1, \ldots, N$

$= M + \beta \cos \omega t_i + \gamma \sin \omega t_i + e_i \qquad (\omega = 2\pi/\tau)$

USER INSTRUCTIONS

STEP	PROCEDURE	ENTER	PRESS			DISPLAY
1	Turn calculator ON					0
2	Partition	3	2nd	Op	17	719.29
3	Read program cards (n = 1, 2, 3, 4): for each n, press CLR and enter card (n) into slot	CLR	2nd	fix	0	
4	Initialize		CLR	RST	R/S	0.0000 [TAU = ?]
5	Enter value of period tested (τ)	τ			R/S	τ [N = ?]
6	Enter number of data points (N)	N			R/S	N [DATA]
7	Enter data (y_i, t_i)	y_i			R/S	
	Repeat step 7 for i = 1, ..., N	t_i			R/S	
8	Get percent rhythm PR (in %)					
	P-value p					
	Mesor (M) and S.E.					
	Double amplitude (DA) and S.E.					
	Acrophase (ϕ) and S.E. (in degrees)				R/S	ϕ ± S.E. (hr.min.)
	Go back to step 4 to analyze new series					

USER DEFINED KEYS	DATA REGISTERS (INV List)		LABELS (Op 08)
none	0 N (counter)	10	
DATA REG.	1 τ	11 $\Sigma \sin\omega t_i$	
20 s_{22}^{-1}	2 N	12 $\Sigma \sin^2\omega t_i$	
21 s_{23}^{-1}	3	13 $\Sigma \sin\omega t_i \cos\omega t_i$	
22 s_{33}^{-1}	4 Σy_i	14 $\Sigma y_i \cos\omega t_i$	
23 $\hat{M}$	5 Σy_i^2	15 $\Sigma y_i \sin\omega t_i$	
24 $\hat{\beta}$	6	16 |S|	
25 $\hat{\gamma}$	7	17 s_{11}^{-1}	
26 PE	8 $\Sigma \cos\omega t_i$	18 s_{12}^{-1}	
	9 $\Sigma \cos^2\omega t_i$	19 s_{13}^{-1}	

FLAGS 0 1 2 3 4 5 6 7 8 9

 1014966-1

TITLE *SINGLE COSINOR* PAGE *1* OF ____ **TI Programmable Coding Form**

PROGRAMMER *G. CORNÉLISSEN* DATE ____

LOC	CODE	KEY	COMMENTS
000	60	DEG	
001	47	CMS	
002	58	FIX	
003	04	04	
004	25	CLR	
005	69	OP	
006	00	00	
007	03	3	
008	07	7	
009	01	1	
010	03	3	TAU = ?
011	04	4	
012	01	1	
013	06	6	
014	04	4	
015	07	7	
016	01	1	
017	69	OP	
018	01	01	
019	69	OP	
020	05	05	
021	25	CLR	
022	91	R/S	$\leftarrow \tau$
023	99	PRT	
024	42	STO	
025	01	01	
026	25	CLR	
027	69	OP	
028	00	00	
029	03	3	
030	01	1	
031	00	0	
032	00	0	N = ?
033	06	6	
034	04	4	
035	00	0	
036	00	0	
037	07	7	
038	01	1	
039	69	OP	
040	01	01	
041	69	OP	
042	05	05	
043	25	CLR	
044	91	R/S	← N
045	99	PRT	
046	42	STO	
047	02	02	
048	42	STO	
049	00	00	
050	98	ADV	
051	25	CLR	
052	69	OP	
053	00	00	
054	01	1	
055	06	6	
056	01	1	
057	03	3	DATA
058	03	3	
059	07	7	
060	01	1	
061	03	3	
062	69	OP	
063	01	01	
064	69	OP	
065	05	05	
066	25	CLR	
067	76	LBL	
068	87	IFF	
069	00	0	
070	91	R/S	$\leftarrow \{y_i\}$
071	99	PRT	
072	42	STO	
073	03	03	
074	44	SUM	
075	04	04	
076	33	X²	
077	44	SUM	
078	05	05	
079	00	0	
080	91	R/S	$\leftarrow \{t_i\}$
081	99	PRT	
082	65	×	
083	03	3	
084	06	6	
085	00	0	
086	55	÷	
087	43	RCL	
088	01	01	
089	95	=	
090	42	STO	
091	06	06	
092	39	COS	
093	42	STO	
094	07	07	
095	44	SUM	
096	08	08	
097	33	X²	
098	44	SUM	
099	09	09	
100	43	RCL	
101	06	06	
102	38	SIN	
103	42	STO	
104	10	10	
105	44	SUM	
106	11	11	
107	33	X²	
108	44	SUM	
109	12	12	
110	43	RCL	
111	07	07	
112	65	×	
113	43	RCL	
114	10	10	
115	95	=	
116	44	SUM	
117	13	13	
118	43	RCL	
119	07	07	
120	65	×	
121	43	RCL	
122	03	03	
123	95	=	
124	44	SUM	
125	14	14	
126	43	RCL	
127	10	10	
128	65	×	
129	43	RCL	
130	03	03	
131	95	=	
132	44	SUM	
133	15	15	
134	97	DSZ	
135	00	00	
136	87	IFF	
137	53	(	
138	43	RCL	
139	09	09	
140	65	×	
141	43	RCL	
142	12	12	
143	75	-	
144	43	RCL	
145	13	13	
146	33	X²	
147	54	)	
148	42	STO	
149	03	03	
150	65	×	
151	43	RCL	
152	02	02	
153	95	=	
154	42	STO	
155	16	16	
156	53	(	
157	43	RCL	
158	11	11	
159	65	×	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE Single Cosinor PAGE 2 OF ____ **TI Programmable Coding Form**

PROGRAMMER G. Cornélissen DATE ____

LOC	CODE	KEY	COMMENTS
160	43	RCL	
161	13	13	
162	75	-	
163	43	RCL	
164	08	08	
165	65	×	
166	43	RCL	
167	12	12	
168	54	)	
169	42	STO	
170	06	06	
171	65	×	
172	43	RCL	
173	08	08	
174	95	=	
175	44	SUM	
176	16	16	
177	53	(	
178	43	RCL	
179	08	08	
180	65	×	
181	43	RCL	
182	13	13	
183	75	-	
184	43	RCL	
185	11	11	
186	65	×	
187	43	RCL	
188	09	09	
189	54	)	
190	42	STO	
191	07	07	
192	65	×	
193	43	RCL	
194	11	11	
195	95	=	
196	44	SUM	$\lvert S \rvert$
197	16	16	
198	43	RCL	
199	03	03	
200	55	÷	
201	43	RCL	
202	16	16	
203	95	=	
204	42	STO	S_{11}^{-1}
205	17	17	
206	43	RCL	
207	06	06	
208	55	÷	
209	43	RCL	
210	16	16	
211	95	=	
212	42	STO	
213	18	18	S_{12}^{-1}
214	43	RCL	

LOC	CODE	KEY	COMMENTS
215	07	07	
216	55	÷	
217	43	RCL	
218	16	16	
219	95	=	
220	42	STO	S_{13}^{-1}
221	19	19	
222	43	RCL	
223	02	02	
224	65	×	
225	43	RCL	
226	12	12	
227	75	-	
228	43	RCL	
229	11	11	
230	33	X²	
231	95	=	
232	55	÷	
233	43	RCL	
234	16	16	
235	95	=	
236	42	STO	S_{22}^{-1}
237	20	20	
238	43	RCL	
239	08	08	
240	65	×	
241	43	RCL	
242	11	11	
243	75	-	
244	43	RCL	
245	02	02	
246	65	×	
247	43	RCL	
248	13	13	
249	95	=	
250	55	÷	
251	43	RCL	
252	16	16	
253	95	=	
254	42	STO	S_{23}^{-1}
255	21	21	
256	43	RCL	
257	02	02	
258	65	×	
259	43	RCL	
260	09	09	
261	75	-	
262	43	RCL	
263	08	08	
264	33	X²	
265	95	=	
266	55	÷	
267	43	RCL	
268	16	16	
269	95	=	

LOC	CODE	KEY	COMMENTS
270	42	STO	
271	22	22	S_{33}^{-1}
272	43	RCL	
273	17	17	
274	65	×	
275	43	RCL	
276	04	04	
277	85	+	
278	43	RCL	
279	18	18	
280	65	×	
281	43	RCL	
282	14	14	
283	85	+	
284	43	RCL	
285	19	19	
286	65	×	
287	43	RCL	
288	15	15	
289	95	=	
290	42	STO	$\hat{M}$
291	23	23	
292	43	RCL	
293	18	18	
294	65	×	
295	43	RCL	
296	04	04	
297	85	+	
298	43	RCL	
299	20	20	
300	65	×	
301	43	RCL	
302	14	14	
303	85	+	
304	43	RCL	
305	21	21	
306	65	×	
307	43	RCL	
308	15	15	
309	95	=	
310	42	STO	$\hat{\beta}$
311	24	24	
312	43	RCL	
313	19	19	
314	65	×	
315	43	RCL	
316	04	04	
317	85	+	
318	43	RCL	
319	21	21	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE *SINGLE COSINOR* PAGE *3* OF ____ **TI Programmable Coding Form**

PROGRAMMER *G. CORNÉLISSEN* DATE ____

LOC	CODE	KEY	COMMENTS
320	65	×	
321	43	RCL	
322	14	14	
323	85	+	
324	43	RCL	
325	22	22	
326	65	×	
327	43	RCL	
328	15	15	
329	95	=	
330	42	STO	$\hat{\gamma}$
331	25	25	
332	43	RCL	
333	24	24	
334	32	X⇌T	
335	43	RCL	
336	25	25	
337	22	INV	
338	37	P/R	
339	42	STO	
340	26	26	
341	32	X⇌T	
342	42	STO	
343	27	27	
344	29	CP	
345	43	RCL	
346	26	26	
347	77	GE	
348	88	DMS	
349	85	+	
350	03	3	
351	06	6	
352	00	0	
353	95	=	
354	76	LBL	
355	88	DMS	
356	94	+/-	
357	98	ADV	
358	42	STO	
359	28	28	
360	25	CLR	
361	69	OP	
362	00	00	
363	03	3	
364	03	3	
365	03	3	
366	05	5	PR =
367	00	0	
368	00	0	
369	06	6	
370	04	4	
371	00	0	
372	00	0	
373	69	OP	
374	01	01	

LOC	CODE	KEY	COMMENTS
375	69	OP	
376	05	05	
377	25	CLR	
378	01	1	
379	75	-	
380	53	(	
381	43	RCL	
382	24	24	
383	65	×	
384	53	(	
385	43	RCL	
386	14	14	
387	75	-	
388	43	RCL	
389	04	04	
390	65	×	
391	43	RCL	
392	08	08	
393	55	÷	
394	43	RCL	
395	02	02	
396	54	)	
397	85	+	
398	43	RCL	
399	25	25	
400	65	×	
401	53	(	
402	43	RCL	
403	15	15	
404	75	-	
405	43	RCL	
406	04	04	
407	65	×	
408	43	RCL	
409	11	11	
410	55	÷	
411	43	RCL	
412	02	02	
413	54	)	
414	54	)	
415	55	÷	
416	53	(	
417	43	RCL	
418	05	05	
419	75	-	
420	43	RCL	
421	04	04	
422	33	X²	
423	55	÷	
424	43	RCL	
425	02	02	
426	54	)	
427	42	STO	
428	10	10	
429	95	=	

LOC	CODE	KEY	COMMENTS
430	42	STO	
431	26	26	PE
432	94	+/-	
433	85	+	
434	01	1	
435	95	=	
436	65	×	
437	01	1	
438	00	0	
439	00	0	
440	95	=	
441	58	FIX	
442	00	00	
443	99	PRT	PR (%)
444	58	FIX	
445	04	04	
446	25	CLR	
447	69	OP	
448	00	00	
449	03	3	
450	03	3	
451	00	0	
452	00	0	ρ =
453	06	6	
454	04	4	
455	00	0	
456	00	0	
457	00	0	
458	00	0	
459	69	OP	
460	01	01	
461	69	OP	
462	05	05	
463	25	CLR	
464	43	RCL	
465	26	26	
466	45	Y^X	
467	53	(	
468	43	RCL	
469	02	02	
470	75	-	
471	03	3	
472	54	)	
473	95	=	
474	34	√X	
475	99	PRT	ρ
476	98	ADV	
477	25	CLR	
478	69	OP	
479	00	00	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE SINGLE COSINOR PAGE 4 OF ____

TI Programmable Coding Form

PROGRAMMER G. CORNÉLISSEN DATE ____

LOC	CODE	KEY	COMMENTS
480	03	3	
481	00	0	
482	04	4	
483	07	7	
484	03	3	M + SE
485	06	6	
486	01	1	
487	07	7	
488	00	0	
489	00	0	
490	69	OP	
491	01	01	
492	69	OP	
493	05	05	
494	25	CLR	
495	43	RCL	
496	23	23	
497	99	PRT	M
498	43	RCL	
499	26	26	
500	65	×	
501	43	RCL	
502	10	10	
503	55	÷	
504	53	(	
505	43	RCL	
506	02	02	
507	75	-	
508	03	3	
509	54	)	
510	95	=	
511	42	STO	
512	29	29	
513	65	×	
514	43	RCL	
515	17	17	
516	95	=	
517	34	√X	
518	99	PRT	δM
519	98	ADV	
520	25	CLR	
521	69	OP	
522	00	00	
523	01	1	
524	06	6	
525	01	1	
526	03	3	DA + SE
527	04	4	
528	07	7	
529	03	3	
530	06	6	
531	01	1	
532	07	7	
533	69	OP	
534	01	01	
535	69	OP	
536	05	05	
537	25	CLR	
538	43	RCL	
539	27	27	
540	65	×	
541	02	2	
542	95	=	
543	99	PRT	2A
544	43	RCL	
545	28	28	
546	39	COS	
547	33	X²	
548	65	×	
549	43	RCL	
550	20	20	
551	95	=	
552	42	STO	
553	10	10	
554	43	RCL	
555	28	28	
556	39	COS	
557	65	×	
558	43	RCL	
559	28	28	
560	38	SIN	
561	65	×	
562	43	RCL	
563	21	21	
564	65	×	
565	02	2	
566	95	=	
567	44	SUM	
568	10	10	
569	43	RCL	
570	28	28	
571	38	SIN	
572	33	X²	
573	65	×	
574	43	RCL	
575	22	22	
576	85	+	
577	43	RCL	
578	10	10	
579	95	=	
580	65	×	
581	43	RCL	
582	29	29	
583	95	=	
584	34	√X	
585	65	×	
586	02	2	
587	95	=	
588	99	PRT	2δA
589	98	ADV	
590	25	CLR	
591	69	OP	
592	00	00	
593	03	3	
594	03	3	
595	02	2	
596	03	3	PHI + SE
597	02	2	
598	04	4	
599	04	4	
600	07	7	
601	03	3	
602	06	6	
603	69	OP	
604	01	01	
605	01	1	
606	07	7	
607	00	0	
608	00	0	
609	00	0	
610	00	0	
611	00	0	
612	00	0	
613	00	0	
614	00	0	
615	69	OP	
616	02	02	
617	69	OP	
618	05	05	
619	25	CLR	
620	43	RCL	
621	28	28	
622	99	PRT	φ
623	43	RCL	
624	28	28	
625	38	SIN	
626	33	X²	
627	65	×	
628	43	RCL	
629	20	20	
630	95	=	
631	42	STO	
632	10	10	
633	43	RCL	
634	28	28	
635	39	COS	
636	65	×	
637	43	RCL	
638	28	28	
639	38	SIN	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
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64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE SINGLE COSINOR PAGE 5 OF ____

PROGRAMMER G. CORNÉLISSEN DATE ____

TI Programmable Coding Form

LOC	CODE	KEY	COMMENTS
640	65	×	
641	43	RCL	
642	21	21	
643	65	×	
644	02	2	
645	94	+/-	
646	95	=	
647	44	SUM	
648	10	10	
649	43	RCL	
650	28	28	
651	39	COS	
652	33	X²	
653	65	×	
654	43	RCL	
655	22	22	
656	85	+	
657	43	RCL	
658	10	10	
659	95	=	
660	65	×	
661	43	RCL	
662	29	29	
663	95	=	
664	34	ΓX	
665	65	×	
666	05	5	
667	07	7	
668	93	.	
669	02	2	
670	09	9	
671	05	5	
672	07	7	
673	08	8	
674	55	÷	
675	43	RCL	
676	27	27	
677	95	=	
678	99	PRT	Dψ
679	42	STO	
680	07	07	
681	91	R/S	
682	98	ADV	
683	43	RCL	
684	28	28	
685	94	+/-	
686	71	SBR	
687	89	π	
688	43	RCL	
689	07	07	
690	71	SBR	
691	89	π	
692	91	R/S	
693	76	LBL	
694	89	π	

LOC	CODE	KEY	COMMENTS
695	55	÷	
696	01	1	
697	05	5	
698	95	=	
699	42	STO	
700	03	03	
701	59	INT	
702	42	STO	
703	06	06	
704	43	RCL	
705	03	03	
706	22	INV	
707	59	INT	
708	65	×	
709	93	.	
710	06	6	
711	95	=	
712	44	SUM	
713	06	06	
714	43	RCL	
715	06	06	
716	58	FIX	
717	02	02	
718	99	PRT	φ (hr. min)
719	92	RTN	SE

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

<u>Test series</u> (single cosinor)

CLR
RST
R/S

```
TAU=?
     24.0000            R/S
N = ?
      6.0000            R/S

DATA                    R/S
     50.0000
      2.0000             .
     40.0000             .
      6.0000             .
     46.6667
     10.0000
     33.3333
     14.0000
     70.0000
     18.0000
     60.0000
     22.0000            R/S

PR =
         55.
P =
      0.2977

M+SE
     50.0000
      4.6922

DA+SE
     25.6279
     13.2715

PHI+SE
   -312.5199
     29.6708            R/S

       20.50
        1.59
```

TITLE MEAN COSINOR PAGE ____ OF ____ **TI Programmable Program Record**

PROGRAMMER G. CORNÉLISSEN DATE ____________

Partitioning (Op 17) 7.9.9.1.9 Library Module ____________ Printer ______ Cards ______

PROGRAM DESCRIPTION

ASSESSMENT OF RHYTHM CHARACTERISTICS OF A POPULATION USING AS IMPUTATIONS THE PARAMETER ESTIMATES FROM 3 OR MORE BIOLOGIC SERIES (OBTAINED BY SINGLE COSINOR).

N.B. * To analyze A as % of M, enter A_i/M_i instead of A_i in step 7
** All acrophases ϕ_i should be referred to the same reference time.

USER INSTRUCTIONS

STEP	PROCEDURE	ENTER	PRESS			DISPLAY
1	Turn calculator ON					0
2	Partition	2	2nd	Op	17	799.19
3	Read program cards (n = 1, 2, 3, 4): for each n, press CLR and enter card (n) into slot	CLR	2nd	fix	0	
4	Initialize		CLR	E		0.0000
5	Enter percent rhythm (PR_i)	PR_i	A			PR_i
6	Enter mesor (M_i)	M_i	B			1
7	Enter amplitude (A_i)	A_i	C			A_i
8	Enter acrophase (ϕ_i) - negative degrees	ϕ_i	D			
	Repeat steps 5-8 for each series $i = 1, \ldots, N$					
9	Get $\bar{\gamma}, \bar{\beta}, \bar{M}, \overline{PR}$; $\bar{A}, \bar{\phi}$, P-value; a, b, θ; $m_1, \theta(m_1)$; $m_2, \theta(m_2)$.		2nd	A'		
10	"Draw" ellipse:					
a	Get β_{max} and β_{min}		2nd	B'		β_{max}; β_{min}
b	Enter $\beta_{min} < \beta_0 < \beta_{max}$ and get γ_1 and γ_2; (A_1, ϕ_1) and (A_2, ϕ_2).	β_0	R/S			γ_1, γ_2 (A_1, ϕ_1); (A_2, ϕ_2)
	Repeat step 10b as often as desired.					

USER DEFINED KEYS	
A	PR_i
B	M_i
C	A_i
D	ϕ_i
E	Initialize
A'	Get results
B'	"Draw" ellipse
C'	
D'	$(\beta, \gamma) \to (A, \phi)$
E'	1. Rcl 19

DATA REGISTERS (INV List)

0	4	10	$\Sigma\gamma_i^2$
1	ΣPR_i; $\overline{PR}$; T^2	11	$\Sigma\beta_i\gamma_i$
2	ΣM_i; $\bar{M}$; R; β_0	12	$\bar{A}$; a; K; W
3	$\Sigma\beta_i$; $\bar{\beta}$	13	$\bar{\phi}$; b; U
4	$\Sigma\gamma_i$; $\bar{\gamma}$	14	csb2n; a_{22}; $A\sqrt{\frac{T}{INC}}$; $\frac{\sqrt{N}}{s_\beta}(\beta_0-\bar{\beta})$
5	N	15	csbgn; a_{12}; B; radical
6	A_i; $\hat{s}_\gamma$	16	csg2n; a_{11}; C; $\frac{r\sqrt{N}}{s_\beta}(\beta_0-\bar{\beta})$
7	ϕ_i; $\hat{r}_{\beta\gamma}$	17	Δ; D
8	β_i; $\hat{s}_\beta$	18	θ; V; $\phi_{1,2}$
9	$\Sigma\beta_i^2$	19	N; Δ; (N-1); γ_1; γ_2

LABELS (Op 08)

FLAGS 0 1 2 3 4 5 6 7 8 9

 1014966-1

TITLE MEAN COSINOR PAGE 1 OF ____ **TI Programmable Coding Form**

PROGRAMMER G. CORNÉLISSEN DATE ____

LOC	CODE	KEY	COMMENTS
000	76	LBL	
001	15	E	
002	70	RAD	
003	47	CMS	
004	58	FIX	
005	04	04	
006	92	RTN	
007	76	LBL	
008	11	A	
009	99	PRT	PR_i
010	44	SUM	
011	01	01	
012	92	RTN	
013	76	LBL	
014	12	B	
015	99	PRT	M_i
016	44	SUM	
017	02	02	
018	01	1	
019	44	SUM	
020	05	05	
021	92	RTN	
022	76	LBL	
023	13	C	
024	99	PRT	A_i
025	42	STO	
026	06	06	
027	92	RTN	
028	76	LBL	
029	14	D	
030	99	PRT	ϕ_i
031	98	ADV	
032	65	×	
033	89	π	
034	55	÷	
035	01	1	
036	08	8	
037	00	0	
038	95	=	
039	42	STO	
040	07	07	
041	94	+/-	
042	39	COS	
043	65	×	
044	43	RCL	
045	06	06	
046	95	=	β_i
047	42	STO	
048	08	08	
049	44	SUM	
050	03	03	
051	33	X²	
052	44	SUM	
053	09	09	
054	43	RCL	

LOC	CODE	KEY	COMMENTS
055	07	07	
056	94	+/-	
057	38	SIN	
058	65	×	
059	43	RCL	
060	06	06	
061	95	=	γ_i
062	49	PRD	
063	08	08	
064	44	SUM	
065	04	04	
066	33	X²	
067	44	SUM	
068	10	10	
069	43	RCL	
070	08	08	
071	44	SUM	
072	11	11	
073	25	CLR	
074	92	RTN	
075	76	LBL	
076	10	E'	
077	55	÷	
078	43	RCL	
079	19	19	
080	95	=	
081	92	RTN	
082	76	LBL	
083	16	A'	
084	43	RCL	
085	05	05	
086	42	STO	
087	19	19	
088	04	4	
089	42	STO	
090	00	00	
091	29	CP	
092	76	LBL	
093	87	IFF	
094	73	RC*	
095	00	00	
096	10	E'	
097	72	ST*	
098	00	00	
099	99	PRT	$\bar{\gamma}; \bar{\beta}; \bar{M}; \overline{PR}$
100	97	DSZ	
101	00	00	
102	87	IFF	
103	98	ADV	
104	43	RCL	
105	03	03	
106	32	X⇌T	
107	43	RCL	
108	04	04	
109	22	INV	

LOC	CODE	KEY	COMMENTS
110	37	P/R	
111	55	÷	
112	89	π	
113	65	×	
114	01	1	
115	08	8	
116	00	0	
117	95	=	
118	42	STO	
119	13	13	
120	32	X⇌T	
121	42	STO	
122	12	12	
123	99	PRT	$\bar{A}$
124	00	0	
125	32	X⇌T	
126	43	RCL	
127	13	13	
128	77	GE	
129	88	DMS	
130	85	+	
131	03	3	
132	06	6	
133	00	0	
134	95	=	
135	76	LBL	
136	88	DMS	
137	94	+/-	
138	42	STO	
139	13	13	
140	99	PRT	$\bar{\phi}$
141	43	RCL	
142	09	09	
143	10	E'	
144	75	-	
145	43	RCL	
146	03	03	
147	33	X²	
148	95	=	csbzm =
149	42	STO	$\Sigma\beta_i^2/N - \bar{\beta}^2$
150	14	14	
151	43	RCL	
152	11	11	
153	10	E'	
154	75	-	
155	43	RCL	
156	03	03	
157	65	×	
158	43	RCL	
159	04	04	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
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TEXAS INSTRUMENTS INCORPORATED

TITLE MEAN COSINOR PAGE 2 OF

PROGRAMMER G. CORNÉLISSEN DATE

TI Programmable Coding Form

LOC	CODE	KEY	COMMENTS
160	95	=	$csbgn = \Sigma\beta_i\gamma_i/N - \bar\beta\bar\gamma$
161	42	STO	
162	15	15	
163	43	RCL	
164	10	10	
165	10	E'	
166	75	-	
167	43	RCL	
168	04	04	
169	33	X²	
170	95	=	$csg2n = \Sigma\gamma_i^2/N - \bar\gamma^2$
171	42	STO	
172	16	16	
173	43	RCL	
174	14	14	
175	65	×	
176	43	RCL	
177	16	16	
178	75	-	
179	43	RCL	
180	15	15	
181	33	X²	
182	95	=	
183	42	STO	Δ
184	17	17	
185	42	STO	
186	19	19	
187	43	RCL	
188	16	16	
189	10	E'	
190	42	STO	$a_{11} = \frac{csg2n}{\Delta}$
191	16	16	
192	43	RCL	
193	15	15	
194	10	E'	
195	65	×	
196	02	2	
197	94	+/-	
198	95	=	
199	42	STO	$a_{12} = \frac{-2}{\Delta} csbgn$
200	15	15	
201	43	RCL	
202	14	14	
203	10	E'	
204	42	STO	$a_{22} = \frac{csb2n}{\Delta}$
205	14	14	
206	53	(	
207	01	1	
208	85	+	
209	43	RCL	
210	16	16	
211	65	×	
212	43	RCL	
213	03	03	
214	33	X²	
215	85	+	
216	43	RCL	
217	15	15	
218	65	×	
219	43	RCL	
220	03	03	
221	65	×	
222	43	RCL	
223	04	04	
224	85	+	
225	43	RCL	
226	14	14	
227	65	×	
228	43	RCL	
229	04	04	
230	33	X²	
231	54	)	
232	45	Yˣ	
233	53	(	
234	02	2	
235	75	-	
236	43	RCL	
237	05	05	
238	54	)	
239	95	=	
240	34	√X	
241	99	PRT	P
242	43	RCL	
243	05	05	
244	75	-	
245	01	1	
246	95	=	
247	42	STO	
248	19	19	
249	53	(	
250	02	2	
251	00	0	
252	45	Yˣ	
253	53	(	
254	02	2	
255	55	÷	
256	53	(	
257	43	RCL	
258	05	05	
259	75	-	
260	02	2	
261	54	)	
262	54	)	
263	75	-	
264	01	1	
265	54	)	
266	65	×	
267	43	RCL	
268	19	19	
269	95	=	
270	42	STO	$T^2 = \frac{2(N-1)}{N-2} F_{1-\alpha}(2,N-2)$
271	01	01	
272	43	RCL	
273	10	10	
274	75	-	
275	43	RCL	
276	05	05	
277	65	×	
278	43	RCL	
279	04	04	
280	33	X²	
281	95	=	
282	55	÷	
283	43	RCL	
284	19	19	
285	95	=	
286	34	√X	
287	42	STO	$\hat{s}_\gamma$
288	06	06	
289	43	RCL	
290	09	09	
291	75	-	
292	43	RCL	
293	05	05	
294	65	×	
295	43	RCL	
296	03	03	
297	33	X²	
298	95	=	
299	55	÷	
300	43	RCL	
301	19	19	
302	95	=	
303	34	√X	
304	42	STO	$\hat{s}_\beta$
305	08	08	
306	43	RCL	
307	11	11	
308	75	-	
309	43	RCL	
310	05	05	
311	65	×	
312	43	RCL	
313	03	03	
314	65	×	
315	43	RCL	
316	04	04	
317	95	=	
318	55	÷	
319	43	RCL	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE MEAN COSINOR PAGE 3 OF ____ **TI Programmable Coding Form**

PROGRAMMER G. CORNÉLISSEN DATE ____

LOC	CODE	KEY	COMMENTS
320	19	19	
321	55	÷	
322	43	RCL	
323	06	06	
324	55	÷	
325	43	RCL	
326	08	08	
327	95	=	
328	42	STO	$\hat{r}_{p\gamma}$
329	07	07	
330	43	RCL	
331	06	06	
332	33	X²	
333	35	1/X	
334	42	STO	A
335	14	14	
336	43	RCL	
337	07	07	
338	94	+/-	
339	55	÷	
340	43	RCL	
341	06	06	
342	55	÷	
343	43	RCL	
344	08	08	
345	95	=	
346	42	STO	B
347	15	15	
348	43	RCL	
349	08	08	
350	33	X²	
351	35	1/X	
352	42	STO	C
353	16	16	
354	53	(	
355	01	1	
356	75	-	
357	43	RCL	
358	07	07	
359	33	X²	
360	54	)	
361	55	÷	
362	43	RCL	
363	05	05	
364	65	×	
365	43	RCL	
366	01	01	
367	95	=	
368	42	STO	D
369	17	17	
370	98	ADV	
371	53	(	
372	43	RCL	
373	14	14	
374	75	-	

LOC	CODE	KEY	COMMENTS
375	43	RCL	
376	16	16	
377	54	)	
378	33	X²	
379	85	+	
380	04	4	
381	65	×	
382	43	RCL	
383	15	15	
384	33	X²	
385	95	=	
386	34	√X	
387	42	STO	R
388	02	02	
389	02	2	
390	65	×	
391	43	RCL	
392	17	17	
393	55	÷	
394	53	(	
395	43	RCL	
396	14	14	
397	85	+	
398	43	RCL	
399	16	16	
400	75	-	
401	43	RCL	
402	02	02	
403	54	)	
404	95	=	
405	34	√X	
406	99	PRT	a
407	42	STO	
408	12	12	
409	02	2	
410	65	×	
411	43	RCL	
412	17	17	
413	55	÷	
414	53	(	
415	43	RCL	
416	14	14	
417	85	+	
418	43	RCL	
419	16	16	
420	85	+	
421	43	RCL	
422	02	02	
423	54	)	
424	95	=	
425	34	√X	
426	99	PRT	b
427	42	STO	
428	13	13	
429	02	2	

LOC	CODE	KEY	COMMENTS
430	65	×	
431	43	RCL	
432	15	15	
433	55	÷	
434	53	(	
435	43	RCL	
436	14	14	
437	75	-	
438	43	RCL	
439	16	16	
440	75	-	
441	43	RCL	
442	02	02	
443	54	)	
444	95	=	
445	22	INV	
446	30	TAN	
447	42	STO	
448	18	18	
449	65	×	
450	01	1	
451	08	8	
452	00	0	
453	55	÷	
454	89	π	
455	95	=	
456	99	PRT	θ
457	98	ADV	
458	43	RCL	
459	14	14	
460	65	×	
461	43	RCL	
462	16	16	
463	75	-	
464	43	RCL	
465	15	15	
466	33	X²	
467	95	=	
468	42	STO	K=(AC-B²)
469	12	12	
470	65	×	
471	43	RCL	
472	04	04	
473	33	X²	
474	75	-	
475	43	RCL	
476	16	16	
477	65	×	
478	43	RCL	
479	17	17	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE MEAN COSINOR PAGE 4 OF ____ **TI Programmable Coding Form**

PROGRAMMER G. CORNÉLISSEN DATE ____

LOC	CODE	KEY	COMMENTS
480	95	=	
481	35	1/X	
482	42	STO	U
483	13	13	
484	43	RCL	
485	14	14	
486	65	×	
487	43	RCL	
488	04	04	
489	33	X²	
490	85	+	
491	02	2	
492	65	×	
493	43	RCL	
494	15	15	
495	65	×	
496	43	RCL	
497	03	03	
498	65	×	
499	43	RCL	
500	04	04	
501	85	+	
502	43	RCL	
503	16	16	
504	65	×	
505	43	RCL	
506	03	03	
507	33	X²	
508	75	-	
509	43	RCL	
510	17	17	
511	95	=	
512	65	×	
513	43	RCL	
514	17	17	
515	65	×	
516	43	RCL	
517	12	12	
518	95	=	
519	34	√X	
520	42	STO	V
521	18	18	
522	43	RCL	
523	12	12	
524	65	×	
525	43	RCL	
526	03	03	
527	65	×	
528	43	RCL	
529	04	04	
530	85	+	
531	43	RCL	
532	15	15	
533	65	×	
534	43	RCL	
535	17	17	
536	95	=	
537	42	STO	W
538	12	12	
539	85	+	
540	43	RCL	
541	18	18	
542	95	=	
543	65	×	
544	43	RCL	
545	13	13	
546	95	=	
547	99	PRT	m_1
548	22	INV	
549	30	TAN	
550	65	×	
551	01	1	
552	08	8	
553	00	0	
554	55	÷	
555	89	π	
556	95	=	
557	99	PRT	$\theta(m_1)$
558	98	ADV	
559	43	RCL	
560	13	13	
561	65	×	
562	53	(	
563	43	RCL	
564	12	12	
565	75	-	
566	43	RCL	
567	18	18	
568	54	)	
569	95	=	
570	99	PRT	m_2
571	22	INV	
572	30	TAN	
573	65	×	
574	01	1	
575	08	8	
576	00	0	
577	55	÷	
578	89	π	
579	95	=	
580	99	PRT	$\theta(m_2)$
581	98	ADV	
582	92	RTN	
583	76	LBL	
584	17	B'	
585	43	RCL	
586	01	01	
587	55	÷	
588	43	RCL	
589	05	05	
590	55	÷	
591	43	RCL	
592	16	16	
593	95	=	
594	34	√X	
595	42	STO	
596	14	14	
597	85	+	
598	43	RCL	
599	03	03	
600	95	=	
601	99	PRT	β_{max}
602	43	RCL	
603	03	03	
604	75	-	
605	43	RCL	
606	14	14	
607	95	=	
608	99	PRT	β_{min}
609	76	LBL	
610	89	π	
611	98	ADV	
612	91	R/S	
613	99	PRT	β_0
614	98	ADV	
615	42	STO	
616	02	02	
617	75	-	
618	43	RCL	
619	03	03	
620	95	=	
621	65	×	
622	43	RCL	
623	05	05	
624	34	√X	
625	55	÷	
626	43	RCL	
627	08	08	
628	95	=	
629	42	STO	$\sqrt{N}(\beta_0-\bar{\beta})/s_\beta$
630	14	14	
631	53	(	
632	01	1	
633	75	-	
634	43	RCL	
635	07	07	
636	33	X²	
637	54	)	
638	65	×	
639	53	(	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
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64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE MEAN COSINOR PAGE 5 OF ____

PROGRAMMER G. CORNÉLISSEN DATE ____

TI Programmable Coding Form

LOC	CODE	KEY	COMMENTS
640	43	RCL	
641	01	01	
642	75	-	
643	43	RCL	
644	14	14	
645	33	X²	
646	54	)	
647	95	=	
648	34	√X	
649	42	STO	
650	15	15	radical
651	43	RCL	
652	07	07	
653	65	×	
654	43	RCL	
655	14	14	
656	95	=	
657	42	STO	$\hat{r}_{\beta\gamma} \frac{\sqrt{N}}{\hat{s}_\beta}(\beta_0-\bar{\beta})$
658	16	16	
659	85	+	
660	43	RCL	
661	15	15	
662	95	=	
663	65	×	
664	43	RCL	
665	06	06	
666	55	÷	
667	43	RCL	
668	05	05	
669	34	√X	
670	85	+	
671	43	RCL	
672	04	04	
673	95	=	
674	99	PRT	γ_1
675	42	STO	
676	19	19	
677	19	D'	
678	98	ADV	
679	43	RCL	
680	16	16	
681	75	-	
682	43	RCL	
683	15	15	
684	95	=	
685	65	×	
686	43	RCL	
687	06	06	
688	55	÷	
689	43	RCL	
690	05	05	
691	34	√X	
692	85	+	
693	43	RCL	
694	04	04	

LOC	CODE	KEY	COMMENTS
695	95	=	
696	99	PRT	γ_2
697	42	STO	
698	19	19	
699	19	D'	
700	98	ADV	
701	98	ADV	
702	61	GTO	
703	89	π	
704	92	RTN	
705	76	LBL	
706	19	D'	
707	43	RCL	(β_0)
708	02	02	
709	32	X⇌T	
710	43	RCL	
711	19	19	$(\gamma_i); i=1,2$
712	22	INV	
713	37	P/R	
714	55	÷	
715	89	π	
716	65	×	
717	01	1	
718	08	8	
719	00	0	
720	95	=	
721	42	STO	
722	18	18	
723	32	X⇌T	
724	99	PRT	$A_i ; i=1,2$
725	00	0	
726	32	X⇌T	
727	43	RCL	
728	18	18	
729	77	GE	
730	77	GE	
731	85	+	
732	03	3	
733	06	6	
734	00	0	
735	95	=	
736	76	LBL	
737	77	GE	
738	94	+/-	
739	99	PRT	$\phi_i ; i=1,2$
740	92	RTN	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
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64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

Test series (Population mean cosinor)

1. Data entry

E

1	90.0000	(PR) A
	99.9800	(M) B
	75.5000	(A) C
	-296.0000	(Ø) D
2	84.0000	
	100.1000	
	39.3300	
	-247.0000	
3	58.0000	
	100.2300	
	36.2400	
	-267.0000	
4	20.0000	
	99.7500	
	31.3000	
	-299.0000	
5	50.0000	
	99.5700	
	62.5500	
	-160.0000	
6	67.0000	
	99.9800	
	50.8700	
	-322.0000	
7	91.0000	
	100.3700	
	40.7600	
	-266.0000	
8	49.0000	
	99.9800	
	67.4900	
	-142.0000	

2. Results

2nd A'

-22.0829	$\bar{\gamma}$
-5.4638	$\bar{\beta}$
99.9950	$\bar{M}$
63.6250	$\overline{PR}$
22.7488	$\bar{A}$
-256.1029	$\bar{\phi}$
0.0309	P-value
59.7676	a
17.6143	b
-45.6269	θ
-1.6253	m_1
-58.3973	$\theta(m_1)$
-0.8449	m_2
-40.1945	$\theta(m_2)$

3. Ellipse

2nd B'

	38.9986	β_{max}
	-49.9262	β_{min}
	30.0000	β_0 R/S
1	-37.0520	γ_1
	47.6744	A_1
	-308.9961	ϕ_1
	-65.6153	δ
	72.1482	A_2
	-294.5704	ϕ_2
	10.0000	
2	-12.6381	
	16.1158	
	-308.3532	
	-57.0370	
	57.9070	
	-279.9443	
	-20.0000	
3	12.2831	
	23.4707	
	-148.4436	
	-32.4699	
	38.1352	
	-238.3688	

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TITLE SINGLE & MEAN COSINORS PAGE ____ OF ____ **TI Programmable Program Record**

PROGRAMMER B. HSI DATE ________

Partitioning (Op 17) 7.9.9.1.9 Library Module ________ Printer ________ Cards ________

PROGRAM DESCRIPTION

This program uses the least squares method to estimate the mesor, M, the amplitude, A, and the acrophase, ϕ, for each single cosinor series with data correction feature. It also accumulates the estimates $\hat{M}$, $\hat{A}$ and $\hat{\phi}$ for multiple series and computes the mean cosinor, giving the coordinates of A and ϕ of points on the boundary of the 95% confidence ellipse.

NB. 1) Error correction procedure will only work after both t_i and y_i are entered. So, if t_i is entered erroneously, complete y_i, R/S, then correct;

2) To start a new mean cosinor, start from step 4.

USER INSTRUCTIONS

STEP	PROCEDURE	ENTER	PRESS			DISPLAY
1	Turn calculator ON					0
2	Partition	2	2nd	Op	17	799.19
3	Read program cards (n = 1, 2, 3): for each n, press CLR and enter card (n) into slot	CLR	2nd	fix	0	0
4	Initialize		CLR	RST	R/S	0
5.	Enter unit interval length (e.g., $\omega = 15°/h$)	ω			R/S	0
6.	Enter data (t_i in dec. hrs)	t_i			R/S	0
	Repeat step 6 for $i = 1, \ldots, N$	y_i			R/S	0
7.	In case of data input error, (t'_i, y'_i) may be taken out by pressing E and reenter (t'_i, y'_i) correctly				E	0
		t'_i			R/S	0
		y'_i			R/S	0
8.	After completion of each series				B	$\hat{M}$
9.	Get $\hat{A}$ and $\hat{\phi}$ (optional)		2nd	D'	R/S	$\hat{A}$, $\hat{\phi}$
10.	To start a new series: press A, return to 6				A	0
11.	To start mean cosinor, enter k (# of series)	k			D	$\bar{M}$
	to get $\bar{M}$ and v_{max}				R/S	v_{max}
12.	Compute values of the 95% confidence ellipse, enter value of $v \leq \lvert v_{max} \rvert$ (as many as desired)	$v \leq \lvert v_{max} \rvert$		C	R/S	A_i, ϕ_i
			2nd	C'	R/S	A'_i, ϕ'_i

USER DEFINED KEYS	DATA REGISTERS (INV [list])		LABELS (Op 08)
A Start new series	0 ω ; k	10 $\Sigma y_i \cos \omega t$	INV lnx CE CLR x⇄t x²
B Complete 1 series	1 $\Sigma \sin \omega t$; $\bar{\beta}$	11 $\Sigma \hat{M}$	√x 1/x STO RCL SUM yˣ
C Confidence ellipse	2 $\Sigma \cos \omega t$; $\bar{\gamma}$	12 $\Sigma \hat{\beta}$	EE ( ) ÷ GTO ×
D Mean cosinor	3 Σy_i ; s_β	13 $\Sigma -\hat{\gamma}$	SBR (−) RST + R/S .
E Error correction	4 $\Sigma \sin^2 \omega t$; s_γ	14 $\Sigma \hat{M}^2$	+/- (=) CLR INV
A'	5 $\Sigma \cos^2 \omega t$; r	15 $\Sigma \hat{\beta}^2$	
B'	6 Σy_i^2 ; H	16 $\Sigma \hat{\gamma}^2$	
C' Confidence ellipse	7 $\Sigma y_i \sin \omega t$	17 $\Sigma \hat{\beta}\hat{\gamma}$	
D' Estimate A & ϕ	8 $\Sigma \sin \omega t \cos \omega t$	18 misc.	
E'	9 N (counter)	19 "	

FLAGS	0	1	2	3	4	5	6	7	8	9

 1014966-1

TITLE SINGLE & MEAN COSINORS PAGE 1 OF ____

PROGRAMMER B. HSI DATE ____

TI Programmable Coding Form

LOC	CODE	KEY	COMMENTS
000	60	DEG	
001	47	CMS	
002	58	FIX	
003	04	04	
004	91	R/S	ω
005	42	STO	
006	00	00	< (prt)
007	76	LBL	
008	11	A	
009	25	CLR	
010	42	STO	
011	01	01	
012	42	STO	
013	02	02	
014	42	STO	
015	03	03	
016	42	STO	
017	04	04	
018	42	STO	
019	05	05	
020	42	STO	
021	06	06	
022	42	STO	
023	07	07	
024	42	STO	
025	08	08	
026	42	STO	
027	09	09	
028	42	STO	
029	10	10	
030	86	STF	
031	00	00	
032	76	LBL	
033	16	A'	
034	25	CLR	
035	91	R/S	t_i
036	65	×	<(prt)
037	43	RCL	
038	00	00	
039	95	=	
040	42	STO	$\omega t \to 18$
041	18	18	
042	38	SIN	
043	87	IFF	
044	00	00	
045	00	00	
046	48	48	
047	94	+/-	
048	44	SUM	$\Sigma \pm \sin \omega t$
049	01	01	
050	33	X²	
051	87	IFF	
052	00	00	
053	00	00	
054	56	56	
055	94	+/-	
056	44	SUM	$\Sigma \pm \sin^2 \omega t$
057	04	04	
058	43	RCL	
059	18	18	
060	39	COS	
061	87	IFF	
062	00	00	
063	00	00	
064	66	66	
065	94	+/-	
066	44	SUM	$\Sigma \pm \cos \omega t$
067	02	02	
068	42	STO	
069	19	19	
070	33	X²	
071	87	IFF	
072	00	00	
073	00	00	
074	76	76	
075	94	+/-	
076	44	SUM	$\Sigma \pm \cos^2 \omega t$
077	05	05	
078	43	RCL	
079	18	18	
080	38	SIN	
081	42	STO	$\pm \sin \omega t \to 18$
082	18	18	
083	65	×	
084	43	RCL	
085	19	19	
086	32	X:T	$\pm \cos \omega t \to t$
087	43	RCL	
088	19	19	
089	95	=	
090	44	SUM	$\Sigma \pm \sin \omega t \cos \omega t$
091	08	08	
092	25	CLR	
093	91	R/S	$y_i \to 19$
094	42	STO	
095	19	19	< (prt)
096	87	IFF	
097	00	00	
098	01	01	
099	01	01	
100	94	+/-	
101	44	SUM	
102	03	03	$\Sigma \pm y$
103	65	×	
104	43	RCL	
105	18	18	
106	95	=	
107	44	SUM	
108	07	07	$\Sigma \pm y \sin \omega t$
109	43	RCL	
110	19	19	
111	33	X²	
112	87	IFF	
113	00	00	
114	01	01	
115	17	17	
116	94	+/-	
117	44	SUM	$\Sigma \pm y^2$
118	06	06	
119	25	CLR	
120	32	X:T	
121	65	×	
122	43	RCL	
123	19	19	
124	95	=	
125	44	SUM	
126	10	10	$\Sigma \pm y \cos \omega t$
127	25	CLR	
128	01	1	
129	94	+/-	
130	87	IFF	
131	00	00	
132	01	01	
133	35	35	
134	94	+/-	
135	44	SUM	$-N = -(N+1)$
136	09	09	
137	86	STF	
138	00	00	
139	61	GTO	
140	16	A'	
141	76	LBL	
142	12	B	
143	43	RCL	
144	01	01	
145	33	X²	
146	55	÷	
147	43	RCL	
148	09	09	
149	95	=	$\Sigma x_2^2 \to 4$
150	44	SUM	
151	04	04	
152	43	RCL	
153	02	02	
154	33	X²	
155	55	÷	
156	43	RCL	
157	09	09	
158	95	=	
159	44	SUM	$\Sigma x_1^2 \to 5$

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE SINGLE & MEAN COSINORS PAGE 2 OF ____

PROGRAMMER B. HSI DATE ____

TI Programmable Coding Form

LOC	CODE	KEY	COMMENTS
160	05	05	
161	43	RCL	
162	03	03	
163	33	X^2	
164	55	÷	
165	43	RCL	
166	09	09	
167	95	=	
168	44	SUM	$\Sigma y^2 \to 6$
169	06	06	
170	43	RCL	
171	01	01	
172	65	×	
173	43	RCL	
174	02	02	
175	55	÷	
176	43	RCL	
177	09	09	
178	95	=	
179	44	SUM	$\Sigma x_1 x_2 \to 8$
180	08	08	
181	43	RCL	
182	01	01	
183	65	×	
184	43	RCL	
185	03	03	
186	55	÷	
187	43	RCL	
188	09	09	
189	95	=	
190	44	SUM	$\Sigma y x_2 \to 7$
191	07	07	
192	43	RCL	
193	02	02	
194	65	×	
195	43	RCL	
196	03	03	
197	55	÷	
198	43	RCL	
199	09	09	
200	95	=	
201	44	SUM	$\Sigma y x_1 \to 10$
202	10	10	
203	43	RCL	
204	04	04	
205	65	×	
206	43	RCL	
207	05	05	
208	75	-	
209	43	RCL	
210	08	08	
211	33	X^2	
212	95	=	
213	42	STO	
214	19	19	Det. $\to 19$

LOC	CODE	KEY	COMMENTS
215	43	RCL	
216	04	04	
217	65	×	
218	43	RCL	
219	10	10	
220	75	-	
221	43	RCL	
222	08	08	
223	65	×	
224	43	RCL	
225	07	07	
226	95	=	
227	55	÷	
228	43	RCL	
229	19	19	
230	95	=	
231	42	STO	$\hat{\beta} \to 18$
232	18	18	
233	44	SUM	$\Sigma \hat{\beta}$
234	12	12	
235	33	X^2	
236	44	SUM	$\Sigma \hat{\beta}^2$
237	15	15	
238	43	RCL	
239	05	05	
240	65	×	
241	43	RCL	
242	07	07	
243	75	-	
244	43	RCL	
245	08	08	
246	65	×	
247	43	RCL	
248	10	10	
249	95	=	
250	55	÷	
251	43	RCL	
252	19	19	
253	95	=	$\hat{\gamma}$
254	94	+/-	
255	42	STO	
256	19	19	$-\hat{\gamma} \to 19$
257	44	SUM	
258	13	13	$\Sigma -\hat{\gamma}$
259	65	×	
260	43	RCL	
261	18	18	
262	95	=	
263	44	SUM	$\Sigma -\hat{\beta}\hat{\gamma}$
264	17	17	
265	43	RCL	
266	19	19	
267	33	X^2	
268	44	SUM	$\Sigma \hat{\gamma}^2$
269	16	16	

LOC	CODE	KEY	COMMENTS
270	43	RCL	
271	18	18	
272	65	×	
273	43	RCL	
274	02	02	
275	75	-	
276	43	RCL	
277	19	19	
278	65	×	
279	43	RCL	
280	01	01	
281	75	-	
282	43	RCL	
283	03	03	
284	95	=	
285	55	÷	
286	43	RCL	
287	09	09	
288	95	=	$\hat{M}$
289	42	STO	< (prt)
290	09	09	
291	44	SUM	$\Sigma \hat{M}$
292	11	11	
293	33	X^2	
294	44	SUM	$\Sigma \hat{M}^2$
295	14	14	
296	43	RCL	
297	09	09	
298	91	R/S	k
299	76	LBL	< (prt)
300	14	D	
301	42	STO	
302	00	00	
303	35	1/X	
304	65	×	
305	43	RCL	
306	11	11	
307	95	=	$\bar{M}$
308	91	R/S	< (prt)
309	43	RCL	
310	12	12	
311	55	÷	
312	43	RCL	
313	00	00	
314	95	=	
315	42	STO	$\bar{\beta} \to 1$
316	01	01	
317	43	RCL	
318	13	13	
319	55	÷	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE Single & Mean Cosinors PAGE 3 OF ____ **TI Programmable Coding Form**

PROGRAMMER B Hsi DATE ____

LOC	CODE	KEY	COMMENTS
320	43	RCL	
321	00	00	
322	95	=	
323	42	STO	$-\bar{\gamma} \to 2$
324	02	02	
325	43	RCL	
326	15	15	
327	75	-	
328	43	RCL	
329	01	01	
330	65	×	
331	43	RCL	
332	12	12	
333	95	=	
334	42	STO	$\Sigma(\hat{\beta}-\bar{\beta})^2 \to 4$
335	04	04	
336	43	RCL	
337	16	16	
338	75	-	
339	43	RCL	
340	13	13	
341	65	×	
342	43	RCL	
343	02	02	
344	95	=	
345	42	STO	$\Sigma(\hat{\gamma}-\bar{\gamma})^2 \to 3$
346	03	03	
347	43	RCL	
348	17	17	
349	75	-	
350	43	RCL	
351	01	01	
352	65	×	
353	43	RCL	
354	13	13	
355	95	=	
356	55	÷	
357	43	RCL	
358	03	03	
359	34	√X	
360	55	÷	
361	43	RCL	
362	04	04	
363	34	√X	
364	95	=	
365	42	STO	$r \to 5$
366	05	05	
367	43	RCL	
368	03	03	
369	55	÷	
370	53	(	
371	43	RCL	
372	00	00	
373	33	X²	
374	75	-	

LOC	CODE	KEY	COMMENTS
375	43	RCL	
376	00	00	
377	54	)	
378	42	STO	
379	10	10	
380	95	=	
381	34	√X	
382	48	EXC	$s_\gamma \to 4$
383	04	04	
384	55	÷	
385	43	RCL	
386	10	10	
387	95	=	
388	34	√X	
389	42	STO	$s_\beta \to 3$
390	03	03	
391	43	RCL	
392	00	00	
393	55	÷	
394	02	2	
395	75	-	
396	01	1	
397	95	=	
398	35	1/X	
399	32	X:T	
400	02	2	
401	00	0	
402	45	Y^X	
403	32	X:T	
404	75	-	
405	01	1	
406	95	=	
407	65	×	
408	53	(	
409	43	RCL	
410	00	00	
411	75	-	
412	01	1	
413	54	)	
414	95	=	
415	42	STO	$H \to 6$ 2)
416	06	06	
417	34	√X	
418	65	×	
419	43	RCL	
420	04	04	
421	95	=	
422	91	R/S	v_{max} 2)
423	76	LBL	↖ (prt)
424	13	C	
425	42	STO	
426	19	19	
427	85	+	
428	43	RCL	
429	02	02	

LOC	CODE	KEY	COMMENTS
430	95	=	
431	48	EXC	$-\gamma \to 19$
432	19	19	
433	55	÷	
434	43	RCL	
435	04	04	
436	95	=	
437	42	STO	
438	07	07	
439	33	X²	
440	75	-	
441	43	RCL	
442	06	06	
443	95	=	
444	65	×	
445	53	(	
446	43	RCL	
447	05	05	
448	33	X²	
449	75	-	
450	01	1	
451	54	)	
452	95	=	
453	34	√X	
454	42	STO	
455	08	08	
456	76	LBL	
457	75	-	
458	85	+	
459	43	RCL	
460	05	05	
461	65	×	
462	43	RCL	
463	07	07	
464	95	=	
465	65	×	
466	43	RCL	
467	03	03	
468	85	+	
469	43	RCL	
470	01	01	
471	95	=	
472	42	STO	$\beta \to 18$
473	18	18	
474	76	LBL	
475	19	D'	
476	29	CP	
477	43	RCL	
478	18	18	
479	32	X:T	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

TITLE SINGLE & MEAN COSINORS PAGE 4 OF ____ **TI Programmable Coding Form**

PROGRAMMER B. HSI DATE ____

LOC	CODE	KEY	COMMENTS
480	43	RCL	
481	19	19	
482	22	INV	
483	37	P/R	
484	32	X⇌T	
485	91	R/S	← (prt) A
486	25	CLR	
487	32	X⇌T	
488	48	EXC	
489	18	18	
490	22	INV	
491	77	GE	
492	10	E'	
493	48	EXC	
494	18	18	
495	91	R/S	← (prt) ϕ
496	76	LBL	
497	10	E'	
498	48	EXC	
499	18	18	
500	75	-	
501	03	3	
502	06	6	
503	00	0	
504	95	=	
505	91	R/S	← (prt) ϕ
506	76	LBL	
507	15	E	
508	22	INV	
509	86	STF	
510	00	00	
511	61	GTO	
512	16	A'	3)
513	76	LBL	For 2nd root
514	18	C'	of u
515	43	RCL	
516	08	08	
517	94	+/-	
518	61	GTO	
519	75	-	

1) $x_1 = \cos\omega t_i - \frac{1}{N}\sum_1^N \cos\omega t_i$

$x_2 = \sin\omega t_i - \frac{1}{N}\sum_1^N \sin\omega t_i$

2) $H = (k-1)\left[20^{2/(k-2)} - 1\right]$: $|v_{max}| = b\sqrt{H}$

3) $\left(\frac{u}{a}\right)^2 - 2r\left(\frac{u}{a}\right)\left(\frac{v}{b}\right) + \left(\frac{v}{b}\right)^2 = (1-r^2)H \equiv$ Ellipse (95% confidence)

For each value of $v \le |v_{max}|$, two solutions for u, leading to (A_i, ϕ_i) and (A'_i, ϕ'_i).

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
63 Exc Ind	73 RCL Ind	84 Op Ind
64 Prd Ind	74 SUM Ind	92 INV SBR

TEXAS INSTRUMENTS INCORPORATED

Test series (single & mean cosinors)   2. **Results**

1. **Data entry**

CLR
RST
R/S 15.0000 R/S (w)

2.0000 R/S (t)
64.9000 R/S (y)
6.0000 .
46.0000 .
1 10.0000 .
62.1000
14.0000
46.9000
18.0000
91.9000
22.0000
71.9000

B 63.9500 M
2nd D' 16.0792 A
R/S 60.0594 ϕ

A
2.0000
66.7000
6.0000
38.2000
2 10.0000
40.3000
14.0000
43.7000
18.0000
54.6000
22.0000
40.8000

47.3833
6.9977
14.2007

A
2.0000
47.2000
6.0000
40.4000
3 10.0000
41.3000
14.0000
64.5000
18.0000
94.5000
22.0000
64.9000

58.8000
24.9165
85.8143

A
2.0000
74.7000
6.0000
40.2000
10.0000
59.7000
14.0000
61.2000 4
18.0000
89.7000
22.0000
87.9000

68.9000
22.4502
57.5748

A
2.0000
59.4000
6.0000
60.7000
10.0000
44.9000 5
14.0000
66.1000
18.0000
84.7000
22.0000
86.7000

67.0833
19.0090
57.7891

(k) 5.0000 D

61.2233 M
R/S 19.2623 V_{max}

v 19.2500 C
34.9339 A_1
R/S 79.5703 ϕ_1,
2nd C' 34.8279 A_1',
R/S 80.5637 ϕ_1'

v 15.0000
. 32.3320
. 68.6185
. 30.1238
88.0671

10.0000
28.8400
60.5227
25.1113
-268.8984

5.0000
25.4471
52.1982
20.1307
-267.2000

0.0000
22.2563
42.7467
15.1292
-266.8740

-5.0000
19.3515
31.4846
10.1070
-269.5568

-10.0000
16.7970
17.6993
5.2764
75.4265

-15.0000
14.5035
0.4214
3.7332
1.6373

-19.2500
10.6483
-22.8990
10.0855
-24.2564

TITLE Roger's Test PAGE ___ OF ___ **TI Programmable Program Record**

PROGRAMMER G. Cornélissen DATE ___

Partitioning (Op 17) 4.7.9.5.9 Library Module ___ Printer ___ Cards ___

PROGRAM DESCRIPTION

Method for testing cyclic trends in incidence rates, where a sample of (n) individuals are categorized, the categories having a cyclic order. The null hypothesis is that the individuals are equally likely to be allocated to each of the (k) categories. The alternative hypothesis is that the frequencies in the categories (N_i) have some sinusoidal variation over their cyclic order. The test statistic is $R = 2[\{\sum N_i \sin(2\pi i/k)\}^2 + \{\sum N_i \cos(2\pi i/k)\}^2]/n$, which is approximately chi-squared distributed with two degrees of freedom on the null hypothesis [J.H. ROGER: "A significance test for cyclic trends in incidence data", Biometrika (1977), 64, 1, 152-155].

USER INSTRUCTIONS

STEP	PROCEDURE	ENTER	PRESS			DISPLAY
1	Turn calculator ON					0
2	Partition	6	2nd	Op	17	479.59
3	Read program card (n = 1): press CLR and enter card into slot	CLR	2nd	fix	0	
4	Initialize		CLR		E	0.0000
5	Enter period (number of classes)	k			B	k
6	Enter number of events in class i	N_i			A	
7	Enter category number (i)	C_i			R/S	
	Repeat steps 6 and 7 for i = 1, ..., k					
8	Get R- and P- values				C	R ; P
9	Get phase (ϕ) and distance (d)				D	ϕ ; d

USER DEFINED KEYS		DATA REGISTERS (INV [key])			
A	N_i ; C_i	0		10	
B	k	1	N_i	11	
C	R ; P	2	C_i	12	
D	ϕ ; d	3	$\sum N_i \sin \omega t_i$	13	
E	Initialization	4	$\sum N_i \cos \omega t_i$	14	
A'		5	$\sum N_i$	15	
B'		6	R	16	
C'		7		17	
D'		8		18	ωt_i
E'		9		19	k

LABELS (Op 08)

FLAGS 0 1 2 3 4 5 6 7 8 9

 1014986-1

TITLE ROGER'S TEST PAGE 1 OF ____ TI Programmable **Coding Form**

PROGRAMMER G. CORNÉLISSEN DATE ____

LOC	CODE	KEY	COMMENTS
000	76	LBL	
001	15	E	
002	70	RAD	
003	25	CLR	
004	47	CMS	
005	58	FIX	
006	04	04	
007	92	RTN	
008	76	LBL	
009	12	B	
010	42	STO	(k)
011	19	19	
012	99	PRT	
013	98	ADV	
014	92	RTN	
015	76	LBL	
016	11	A	
017	42	STO	(N_i)
018	01	01	
019	99	PRT	
020	44	SUM	ΣN_i
021	05	05	
022	91	R/S	
023	42	STO	(C_i)
024	02	02	
025	99	PRT	
026	98	ADV	
027	65	×	
028	02	2	
029	65	×	
030	89	π	
031	55	÷	
032	43	RCL	
033	19	19	
034	95	=	
035	42	STO	ωt_i
036	18	18	
037	38	SIN	
038	65	×	
039	43	RCL	
040	01	01	
041	95	=	
042	44	SUM	$\Sigma N_i \sin \omega t_i$
043	03	03	
044	43	RCL	
045	18	18	
046	39	COS	
047	65	×	
048	43	RCL	
049	01	01	
050	95	=	
051	44	SUM	$\Sigma N_i \cos \omega t_i$
052	04	04	
053	25	CLR	
054	92	RTN	
055	76	LBL	
056	13	C	
057	43	RCL	
058	03	03	
059	33	X²	
060	85	+	
061	43	RCL	
062	04	04	
063	33	X²	
064	95	=	
065	65	×	
066	02	2	
067	55	÷	
068	43	RCL	
069	05	05	
070	95	=	
071	99	PRT	(R)
072	42	STO	
073	06	06	
074	55	÷	
075	02	2	
076	95	=	
077	94	+/-	
078	22	INV	
079	23	LNX	
080	99	PRT	(ℓ)
081	98	ADV	
082	92	RTN	
083	76	LBL	
084	14	D	
085	43	RCL	
086	04	04	
087	55	÷	
088	43	RCL	
089	19	19	
090	95	=	
091	32	X⇌T	
092	43	RCL	
093	03	03	
094	55	÷	
095	43	RCL	
096	19	19	
097	95	=	
098	22	INV	
099	37	P/R	
100	99	PRT	(ϕ)
101	32	X⇌T	
102	99	PRT	(d)
103	98	ADV	
104	98	ADV	
105	92	RTN	

MERGED CODES

62 Pgm Ind	72 STO Ind	83 GTO Ind
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TEXAS INSTRUMENTS INCORPORATED

Test series (Roger's test)

CLR E

	(k)	7.0000	B
i=1	(N_i)	1.0000	A
	(C_i)	1.0000	R/S
		2.0000	
2		2.0000	
		3.0000	
3		3.0000	
		2.0000	
4		4.0000	
		1.0000	
5		5.0000	
		3.0000	
6		6.0000	
		17.0000	
7	(k)	7.0000	
	C	14.1470	(R)
		0.0008	(P-value)
	D	-0.0108	(ϕ)
		2.0461	(d)