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Detecting Space-Time Alternating Biological Signals Close to the Bifurcation Point

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Abstract

Time-alternating biological signals, i.e., alternans, arise in variety of physiological states marked by dynamic instabilities, e.g., period doubling. Normally, a sequence of large–small–large transients, they can exhibit variable patterns over time and space, including spatial discordance. Capture of the early formation of such alternating regions is challenging because of the spatiotemporal similarities between noise and the small-amplitude alternating signals close to the bifurcation point. We present a new approach for automatic detection of alternating signals in large noisy spatiotemporal datasets by exploiting quantitative measures of alternans evolution, e.g., temporal persistence, and by preserving phase information. The technique specifically targets low amplitude, relatively short alternating sequences and is validated by combinatorics-derived probabilities and empirical datasets with white noise. Using high-resolution optical mapping in live cardiomyocyte networks, exhibiting calcium alternans, we reveal for the first time early fine-scale alternans, close to the noise level, which are linked to the later formation of larger regions and evolution of spatially discordant alternans. This robust method aims at quantification and better understanding of the onset of cardiac arrhythmias and can be applied to general analysis of space-time alternating signals, including the vicinity of the bifurcation point.

Index Terms

Alternans detection; bifurcation point; space-time; temporal persistence

I. Introduction

Feedback regulated systems often exhibit damped oscillations in response to perturbations until the limit of control is reached at which critical juncture the amplitudes can rapidly

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This paper has supplementary downloadable material available at <http://ieeexplore.ieee.org> provided by the author. The material includes two movies of which one is split into three parts, namely, phase1.wmv, phase2.wmv, and phase3.wmv. The three parts represent consecutive time portions of the original data. The other movie, alt.wmv, represents the evolution of the alternans movie over different frequencies. The size of the movie is not specified. Contact emilia.entcheva@sunysb.edu for further questions about this work.

progress to wildly fluctuating values. At the brink of departure from stable conditions, (subtle) oscillations may persist, serving as a potential predictor of dynamic instability. Many biological processes feature negative feedback control and behave similarly. In spatially extended systems, persistent time-alternating signals, i.e., alternans, can appear as a sequence of large–small–large transients, and can display variable amplitude and variable patterns over time and space, including spatial discordance (alternation with opposite phase). Upon entry in period doubling regime, i.e., in the immediate vicinity of the bifurcation point, the alternans amplitude is typically very small [1], and thus, alternans detection is extremely challenging due to spatiotemporal similarities with white noise. Fig. 1 illustrates such uncertainty zones expected at the bifurcation point and/or at the border of phase reversal over space. In both cases, very small amplitude alternation (comparable to noise) would be expected. No previously published detection methods have tackled these uncertainty regions in space-time, particularly for limited duration signals.

For example, excitable tissues (heart, brain, and muscle) are biological systems susceptible to dynamic instabilities, e.g., period doubling. In the heart, capturing early bifurcation events (frequency being the control parameter) may translate in early diagnosis of life-threatening events. The onset of some arrhythmias, such as ventricular tachycardia (VT) and the more malignant ventricular fibrillation (VF), has been linked to the development of alternans at the cellular level [2]–[4] and alternans in the clinical records (ECG). Computerized algorithms based on spectral and nonlinear methods made possible the detection of microvolt-level T-wave alternans, barely discernable in ECG records [5], [6]. These subtle T-wave alternans were found to correlate with future arrhythmia occurrence in some disease conditions, e.g., postmyocardial infarction [7]. While the detection of microvolt alternans has been a major accomplishment and cannot be undermined, there are several facilitating factors, including typically long records and no need for preservation of spatial/phase information. At the cellular level, such instabilities of small amplitude may develop even earlier and may be buried in noise and have easily dismissible speckled spatial appearance due to inherent biological variability and lack of spatial synchronization close to the bifurcation point.

In spatially resolved measurements, the detection of alternans has to meet additional challenges: it is informative to track not only their existence (as in T-wave analysis), but also their magnitude and phase for each beat at each spatial location. Preserving phase information is necessary for identification of spatially discordant alternans (SDA) [Fig. 1(b)]. SDAs can precipitate or coexist with reentrant waves [8] and are more closely associated with the development of reentrant ventricular arrhythmias, irregularities in the ECG, and sudden cardiac death [2], [9], and [10].

In this paper, we tackle the general question of uncovering subtly alternating signals over time and space in conditions of noise and/or low-amplitude alternation (as illustrated in Fig. 1). We present a new approach for automatic detection of alternating signals in large spatiotemporal datasets by quantifying temporal persistence (TP) and preserving phase information. The technique is validated by combinatorics-derived probabilities and empirical tests with white noise. This new robust method can be useful in quantification and better understanding of the onset of cardiac arrhythmias and in general analysis of space-time alternating signals, including response to perturbations in the vicinity of bifurcation points or high noise conditions.

A. Identification of Alternating Signals

For a perfect period-2 rhythm, the amplitude of all transients during even beats should be consistently smaller (or larger) than transients during odd beats. However, in the presence of

noise, due to local instability dynamics or due to spatial interactions, the small–large–small sequence may be interrupted at different time points for different spatial locations.

Random noise can display surprisingly long runs of perfect alternation over time. In experiments with unbiased coin tossing, actual head–tail patterns have been known to “trick” human subjects—we generally tend to underestimate the longest run of heads, tails, or perfect alternation of the two that can arise in a purely random signal [11], [12]. This observation underscores the need for an objective quantitative criterion of distinguishing a “true” alternating signal from alternations by chance (noise). From statistical point of view, to confirm a (binary) pattern in a given signal with certain confidence, one needs to “see” the pattern a sufficient number of times. Hence, an index of TP of alternating signals seems like a natural choice for such a criterion, simple and without any assumptions about the underlying signal. We adopt TP here as the basis of our algorithm for automatic detection of alternans. TP is defined as the ratio between the longest length of a segment of uninterrupted alternans and the total signal length (number of beats).

As already emphasized, TP of alternation in random binary signals can be surprisingly high, especially for short sequences [see Fig. 1(c)]. We find the maximum-length alternating pattern m in a random signal of length n to obey a logarithmic relationship [see (1)], i.e., longer random signals exhibit lower TP [Fig. 1(c)]

$$m = a \ln(n) + b; \quad a = 2.1453; \quad b = -0.093. \quad (1)$$

For example, for a random binary sequence of length 30, $TP \approx 23\%$. Therefore, to distinguish with sufficient confidence alternans from noise, it is essential to select a threshold level (minimum TP of detection) sufficiently higher than TP seen in white noise sequence with the same temporal length. For longer records, a lower TP threshold can be comfortably chosen.

B. Theoretical Basis of the Algorithm

Automatic detection of alternating sequences in noise-containing nonstationary signals has to address the possibility for false identification due to random noise-related and/or short-lived alternations. For simplicity, we examine the case of binary alternating signals, since the peak calcium transient sequences can be binarized.

From combinatorics, one can derive the probability of a given binary dataset with length n to exhibit uninterrupted alternating pattern with length m . Intuitively, there are a total of 2^n possible binary strings of length n . Further, we consider all possible positions and bit assignments of the m -bit alternating sequence within a binary string of length n , if m can fully fit into the n -sequence only once. A given alternating sequence of length m can be positioned $(n-m+1)$ different ways within a binary string n , depending on where it begins.

As Fig. 2(a) shows, if we first consider only central positioning of m , excluding the two border locations, the number of possible positions becomes $(n-m+1-2)$, i.e., $(n-m-1)$. In order to ensure that the alternating sequence does not extend beyond the specified length m , the two flanking bits must duplicate the first and last value of the alternating sequence in order to break the pattern, hence instead of $(n-m)$ bits, we have $(n-m-2)$ unassigned bits, resulting in $2^{(n-m-2)}$ possible bit assignments. Also there are two alternative forms for the m bits, i.e., phases of the alternating sequence: (“1, 0, 1, 0, ...” or “0, 1, 0, 1, ...”). Thus, the possibilities for centrally positioned m -sequence within n are

$$p_c = 2(n - m - 1)2^{(n-m-2)}. \quad (2)$$

If we consider now any of the two border locations of the m alternating sequence within the n bits, because of the one reserved flanking bit, there are $(n-m-1)$ bits left to assign values to, i.e., $2(n-m-1)$ possible bit assignments. Considering the two border position and the two phases of the m -sequence, we get the possibilities for the border positions of m

$$p_b = 2 \times 2 \times 2^{(n-m-1)}. \quad (3)$$

Adding (2) and (3), gives all possible cases of m -bit sequence positioned within an n -bit sequence

$$\begin{aligned} p &= p_c + p_b \\ &= 2(n - m - 1)2^{(n-m-2)} + 2 \times 2 \times 2^{(n-m-1)} \\ &= (n - m - 1 + 2^2)2^{(n-m-1)} \\ &= (n - m + 3)2^{(n-m-1)}. \end{aligned} \quad (4)$$

Finally, we obtain a closed-form expression for the probability of having an uninterrupted alternating set of strictly m bits in a random n -bit sequence

$$P(m, n) = \frac{(n - m + 3)2^{(n-m-1)}}{2^n} \left(n > m > \frac{n}{2} > 2 \right). \quad (5)$$

This is valid under some assumptions, e.g., if m has a minimum length exceeding the half-length of n . For short strings of m with respect to n , i.e., for $m < n/2$, (5) has to be modified to avoid miscounting of multiple occurrences of m within n .

In this paper, we illustrate the detection strategy using spatiotemporal records of intracellular calcium transients in cardiomyocytes. The original records can be binarized and Fig. 2(b) illustrates the typical steps in calculating TP; the theoretical curve (5) is plotted in Fig. 3(a). The prototype of this algorithm has been introduced in a conference proceedings form [13].

C. Spatial and Phase Considerations

Spatially-resolved measurements of alternans prompt considerations of relative phase over space, i.e., spatial concordance–discordance needs to be identified. SDAs can be recognized as neighboring regions alternating with opposite phase. The determination of phase is not trivial since the alternating sequence can be easily corrupted by noise. It becomes critical to determine a phase that can be representative and consistent between different spatial locations. Based on the TP concept, we introduce representative phase (RP) derived in time-overlapping segments for different spatial locations to help in identifying SDAs [Fig. 3(b)]; RP assumes values of -1 or $+1$.

II. Experimental Materials and Methods

A. Primary Cardiomyocyte Culture and Optical Mapping

The protocol for primary cardiomyocyte culture has been published previously [14], [15]. Briefly, neonatal rat cardiomyocytes were cultured on thin long silicone polymer (PDMS) surfaces (18×5 mm) to form confluent monolayers. Pacing was applied via a platinum line electrode on one of the sides of each sample. Optical mapping of intracellular calcium was performed at ultrahigh-resolution using an intensified CMOS camera [16].

B. TP and Alternans Quantification

Calculation of TP for each recorded trace is done as shown in Fig. 2(b). The original acquired traces of calcium transients are first filtered and preprocessed. From the original traces [synthetic signal is shown for simplicity in Fig. 2(b)], a representative signal parameter is extracted, e.g., peak height of calcium concentration. Then, derivative and sign are taken along the time dimension to accentuate the alternating patterns and the signal is binarized. Interruptions in alternating patterns are found by locating the zeros after another derivative. The optical mapping, the data preprocessing, and the computational efficiency of the algorithm are shown in Fig. 2(b).

In addition to TP, prior to conversion of the signal to binary, the magnitude of the alternans was quantified using the alternans ratio (AR), defined as $AR = |1 - A_o/A_e| 100\%$, where A_o and A_e are the amplitudes of odd and even calcium transients, respectively. For every pixel, the AR is the average AR in the longest continuous segment (used to calculate TP).

SDA regions can be easily recognized as two alternating regions with opposite phase. In addition to opposite phases, SDAs imply spatial proximity—they are typically separated by nodal lines (no alternation regions). Here, we search the immediate neighborhood ($264 \times 264 \mu\text{m}$ in our case) of each pixel of identified alternans for pixel(s) exhibiting alternation with opposite phase. If such pixels are found, then the whole region is marked as an SDA region. The size of the neighborhood for identification of SDAs can vary, based on the system of interest and its spatial characteristics.

C. Sensitivity in Alternans Detection and SNR

Here, SNR is defined as the ratio between the average amplitude of the calcium transients to the amplitude of noise, estimated by the standard deviation of peak calcium at the lowest pacing frequency (assuming no alternans). For example, in our experiments SNR estimated as above was between 25 and 45 depending on pacing frequency.

III. Results

A. TP Criterion as Means of Improving Specificity and Sensitivity in Detection of Subtle Alternans

Previous studies of cardiac alternans in space-time have exclusively dealt with amplitude of alternans well above the noise level. Typically, *ad hoc* signal duration is chosen and 100% compliance within that signal is required for positive detection of alternans [2], [10], and [17]. In contrast, the focus of this report is on formalizing the criteria for alternans detection and on uncovering subtle alternating signals, close to the noise level, as in Fig. 1. These are of special interest as potential early indicators of destabilization.

Empirical testing of the algorithm was performed using uniformly distributed pseudorandom numbers, generated in MATLAB. To be comparable to the experiments (considering total number of observed pixels), 40 000 random datasets were created as noise samples, having 4

to 40 beats. After processing the noise data with our alternans identification algorithm, we perfectly obtained results matching the theoretical predictions. We plot in Fig. 3(a) the percent of false positive detections versus signals' total length n for $TP \geq 60\%$, 75% and $TP \geq 90\%$ as thresholds. Theoretical (5) and empirical results (random datasets with white noise) are shown to match perfectly. For example, if a line is drawn parallel to the x -axis at $p = 0.05$, one can determine that there is only 5% chance for random noise to be falsely identified as alternans for signal length $n = 13$ beats, of which $m \geq 8$ beats ($TP \geq 60\%$) form an uninterrupted alternating sequence. Conversely, such temporal sequence would be classified as alternans at $TP = 60\%$ with 95% confidence. Overall, theoretical and empirical data from Fig. 3(a) can be used for rationally deriving a TP threshold in alternans identification.

If a total signal length of at least $n = 25$ – 30 beats can be guaranteed, then identification of alternans can be done at $TP \geq 60\%$ with $>99\%$ confidence [Fig. 3(a)]. In fact, for such long records even $TP \geq 40\%$ would suffice for $>95\%$ confidence of detection [Fig. 3(a)]. Thus, lowering the TP threshold (from the conservative 100%) can be done without compromising the specificity of detection.

Even if the calculated signal TP is below some preset threshold for confirming alternans, the TP value itself is still a powerful measure of the level of organization and probability of consistent alternation compared to that seen in a random signal. Table I shows side-by-side TP calculated for white noise traces and TP from actual calcium records of the same length. At low pacing frequencies, where no alternation is expected, the TP for the calcium records is undistinguishable from noise. Signal length predictably lowers the TP in noise traces [as per Fig. 1(c)]. Increasing pacing frequency gradually increases the TP index in the calcium traces and makes it statistically higher than that seen in noise records with comparable length. Thus, even TP levels below threshold are informative for the physiological response of the system and the gradual recruitment of alternating spatial sites.

For analysis of alternans at multiple spatial locations, we apply the concept of RP, illustrated in Fig. 3(b), using $TP = 60\%$ for simplicity. Pixels A and B are selected to belong to different spatial regions. Pixel A exhibits uninterrupted alternation in the $(t_1 - t_2)$ time interval, while pixel B alternates continuously in the $(T_1 - t_2)$ interval. For $TP \geq 60\%$, there will be at least 20% temporal overlap for different spatial locations, allowing the determination of a RP in the $[t_1, t_2]$ interval; RP is used for identification of SDAs.

An important question is how different SNR conditions affect the performance of the proposed strategy, especially when the alternans are very subtle (low AR, see Section II). We address this question in Fig. 4, where variable SNR was created by adding white noise to four perfect alternans sequences ($TP = 100\%$, $n = 30$) having $AR = 6\%$, 10% , 20% , and 50% . As noise level increases and SNR decreases (right-to-left on the x -axis), the detected TP drops following a sigmoid curve. If the alternans detection threshold is set at $TP \geq 60\%$, all signals with SNR below the crossing point with the dark horizontal line will be misclassified (false negative); as expected, signals with very subtle alternans (low AR) are more sensitive to noise resulting in more false negatives. While Fig. 3(a) illustrates the specificity of the algorithm as function of TP and signal length, Fig. 4 helps assess its sensitivity as function of AR and SNR. Setting TP threshold lower than 100% increases sensitivity, i.e., allows detection of alternans at lower SNR; for example, at $SNR = 30$ is possible to detect alternans with $AR = 6\%$, while requiring $TP = 100\%$ would not allow that.

B. Revealing Fine Spatial Heterogeneities of the Bifurcation Point in Cardiac Cell Networks Under Periodic Electrical Stimulation

Strong cell-to-cell coupling in cardiac tissue does not guarantee spatial synchronization at the bifurcation point. Due to inherent cellular variations in electrophysiological properties, different spatial locations may enter a period-doubling regime at different values of the bifurcation parameter (frequency, in this case), thus, creating intricate spatiotemporal patterns, which can be revealed only at very high-resolution mapping. To test this, we applied our algorithm to high-resolution optical mapping of intracellular calcium in thin long strips of cultured cardiomyocytes, subjected to electrical pacing at progressively increasing frequency starting from 1 Hz (Fig. 5(a), see also Section II). For consistent results, in the alternans analysis, 30 beats were used for each pacing frequency, so that at $TP \geq 60\%$, the confidence level in alternans detection (specificity) was $>99\%$ [see Fig. 3(a)].

With increasing pacing frequency, the number of locations (pixels) exhibiting alternans gradually increases [Fig. 5(b) and (c)]. The gray scale in Panel B represents the degree of alternation, AR; white areas are without alternans. The images in the left column show the spatial localization of alternans identified by the current algorithm over 30 beats using $TP \geq 60\%$. For comparison, the middle and the right column present maps of alternans, if only the first eight or the last eight beats are considered and 100% compliance (8/8) is imposed, as done in a recent paper [17]. Despite similarities in structure, the alternans regions uncovered by the different approaches are not identical. The spatial alternans map for the final 8/8 beats (right column) bears more similarity to the results from our approach compared to the first 8/8 beats (middle column). This is most likely due to the more transient response during the first eight beats after change in pacing frequency and achievement of steady state toward the end. The different spatial maps for the first and the last eight beats highlight the dynamic nature of alternans and their dependence on the particular pacing protocol (in [17] only eight (transient) beats were analyzed). Interestingly, by (5), the longer examined sequence by our approach (30 beats) yields higher specificity of detection than the 8/8 method despite the lower chosen TP threshold and regardless whether the signal is at steady state or not.

Frequency dependent increase in alternans is further illustrated in the time domain for two selected spatial locations—A and B [Fig. 5(c)]. For these two locations, the algorithm reports no alternans ($TP < 60\%$) for pacing frequencies up to 2.78 Hz. Then, at 2.78 Hz, only point B shows persistent alternans, while at 3.12 Hz, the two neighboring locations start alternating out-of-phase, i.e., they form a small-scale (microscopic) SDA region. In the original traces (bottom row) these small-magnitude SDAs for points A and B are discernable.

Displaying alternans regions by their respective phase ($RP \times AR\%$) reveals the nature of local organization as different spatial locations start alternating [Fig. 6(a)]. As frequency increases, left column displays all confirmed locations of alternans in peak calcium ($TP \geq 60\%$), while the right column shows only SDA regions identified based on proximity criterion (see Section II). Upon increasing pacing frequency, both peak calcium concentration and the duration of calcium transients change in the same direction, hence the product of the two can be used to improve SNR and the detection of alternans; Fig. 6(b) shows this enhanced version of the data from Fig. 6(a). It is apparent that higher frequency not only recruits more alternating regions, but also increases the yield of SDA regions. At 2.78 Hz, the tissue is largely alternating in-phase as a contiguous region, but the presence of a small number of pixels mildly alternating out-of-phase (blue) leads to the identification of SDAs. At even higher pacing frequency—3.12 Hz, the tissue clearly breaks in local SDA regions with complex shape.

Alternans specifically targeted by our algorithm fall in the uncertainty zones depicted in Fig. 1, and are shown in Fig. 6(a) and (b); they are subtle ($AR < 20\%$), and can easily be missed if insufficient imaging resolution is used. Speckled spatial maps of alternans as shown here have not been reported even for similar experimental systems. For comparison, in Fig. 6(c), we show a different sample where more familiar contiguous regions of large amplitude alternans ($AR > 50\%$) with sharp nodal lines of no alternans between them exist. Spatial maps like the one in Fig. 6(c) are more typical of previous reports [10], [17]; such large AR alternans can be easily detected by a variety of methods. In contrast, subtle alternans with low-amplitude and high-spatial frequency normally are dismissed by conventional imaging and analysis tools due to spatial averaging (insufficient spatial resolution) and/or insufficient sensitivity of the analysis algorithms.

IV. Discussion and Conclusion

An important transition in the system's dynamics to a qualitatively different state occurs at the bifurcation point. Capturing this transition is highly desirable, as illustrated, for example, by recent interest in defining the type of bifurcation in cardiac tissue [18]. Knowing if a border collision versus pitchfork period doubling bifurcation takes place may yield a better understanding of the inherent structure of the system, continuous versus piece-wise continuous (hybrid).

Quantification of properties in the immediate vicinity of the bifurcation point, however, is extremely challenging due to noise-like behavior over time (small amplitude alternations may be buried in noise) and space (bifurcation threshold may vary between cells and yield a speckled spatial appearance) (Fig. 1). Previous papers have dealt with more prominent high AR alternans away from the bifurcation point, and have employed mostly *ad hoc* selection of the temporal sequence length needed to confirm alternans by imposing 100% compliance within that length. The limits of detection from statistics point of view (confidence in detection) have not been determined earlier. This requirement may be too conservative and lead to inferior sensitivity in the presence of noise compared to the method proposed here [see Fig. 4]. This deficiency in sensing subtle alternans does not necessarily come with superior specificity (depending on signal length). For example, specificity for the (8/8) method of identifying alternans [17] is 99.41%, while a TP-based strategy, requiring 18 out of 30 beats yields 99.997% specificity. Furthermore, if sufficient knowledge exists about the acquisition system (SNR and expected AR) and TP index (even $< 60\%$) can be selected to obtain optimal specificity and sensitivity, guided by Figs. 3 and 4. The detection limit (lowest possible AR) of the algorithm will depend on the SNR, which may also be affected by the bifurcation parameter. The approximate limit of our system in detecting calcium alternans in large cell monolayers ($2.5\text{ cm} \times 2.5\text{ cm}$) with spatial resolution of $44\text{ }\mu\text{m}$ is $AR = 4\text{--}7\%$ depending on frequency. In comparison, for T-wave alternans detection in 12-lead ECG, the equivalent AR varies between 1–65% [19]–[21]. However, in such analysis much longer event sequences are employed, typically > 100 to 2000 or more beats [19], [21], and [22]. Such long sequences combined with advanced spectral analysis make possible the detection of alternans for lower AR. Our algorithm targets cases, where relatively short signals (< 30 beats) can be used and phase information is of interest.

Preservation of phase information (RP) at different spatial locations, offers automated identification of SDA. Large-scale SDAs have been more directly linked to complex local dynamics [8], [23], pronounced alternations in the ECG signal, e.g., alternating QRS complex [9], and identified as a possible path to induction of spiral waves in VT and VF [10], [24]. Calcium alternans and especially SDAs occurring over a small (subcellular) spatial scale have been only recently suggested by computational studies [25] and experimentally confirmed in a tissue setting [26]. The imaging and analysis strategy

presented here allow tracking of the early evolution of such subtle alternans over a large field of view (Figs. 5 and 6) essential in the study of reentrant arrhythmias occurring over centimeter scale. We found that initially sporadic small-AR spatially concordant alternans become more pronounced with pacing frequency and consolidate in larger regions, followed by complex-shaped SDAs at even higher pacing rates. A deterministic continuous model for alternans generation in a reaction-diffusion system [24], [27] would have predicted large well-defined regions of SDAs [as in Fig. 6(c)]. Instead, at this early stage we observe more speckled SDA patterns, which could be linked to inherent heterogeneities, revealed only when imaging at sufficient spatial resolution and using high-sensitivity analysis. Ability to identify alternating signals and their phase permits exploration of spatiotemporal patterns at the bifurcation point. Because of the general nature of the algorithm, it can be applied to identification of any potentially alternating dynamic signals (cardiac or not).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

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Fig. 1.

Uncertainty in detection of alternating signals. (a) Uncertainty in the bifurcation point: as a bifurcation parameter increases, a critical transition takes place, e.g., transition from a 1:1 response to 2:2 response to external perturbation. For a given system's dynamics and in the presence of noise (including natural variability), there exists an uncertainty zone, for which it is challenging to determine the exact state of the system. (b) Uncertainty in transition zones between opposite phases of oscillation: over space, it is possible for neighboring regions to exhibit opposite phase of alternation. Between them a "nodal line" or no-alternation zone must exist. In real experiments, the width of this zone will depend on the system's dynamics and the noise in the system. (c) TP of alternation in white noise: maximum-length alternating sequence, m , in a random binary signal of length n (left); TP of alternation in a random binary signal of length n (right). Presented empirical data (10 000 trials per point) show 95% confidence interval for m and TP. Based on these data, a threshold TP for alternans detection can safely be chosen above the curve (right).

	Possible positions	Unassigned bits	Possibilities
Central case	$(n-m-1)$	$(n-m-2)$	$2^{(n-m-1)} \cdot 2^{(n-m-2)}$
Border case	2	$(n-m-1)$	$2 \cdot 2^{(n-m-1)}$
Total			$(n-m+3) \cdot 2^{(n-m-1)}$

$$\text{Temporal Persistence (TP)} = \frac{\text{max length}}{\text{total length}} \cdot 100\%$$

(b)

Fig. 2.

Identification of time-alternating signals and derivation of (5). (a) Derivation of (5): the denominator is 2^n all possible combinations of a binary string of length n . For the numerator: a given alternating sequence of length m can be positioned in $(n-m-1)$ positions with $(n-m-2)$ unassigned bits (central case), or positioned in two positions with $(n-m-1)$ unassigned bits (border case). The sum of both cases gives the total number of possibilities. (b) Time-alternating signals are identified based on their TP. From the original traces (synthetic signal is shown for simplicity), a representative signal parameter is extracted, e.g., peak height of calcium concentration. Then derivative and sign are taken along the time dimension to accentuate the alternating patterns and the signal is binarized. Interruptions in alternating patterns are found by locating the zeros after another derivative.

Fig. 3.

Specificity of alternans detection based on TP. (a) Specificity of alternans detection: the stars are the theoretical probabilities (5), while the solid lines represent empirical results, computed from white noise datasets. The theoretical and empirical data match perfectly for TP of 60%, 75%, and 90%. Signal length $n \geq 13$ is needed for $p < 0.05$ ($\geq 95\%$ specificity of detection) at TP $\geq 60\%$, while shorter signals will suffice when enforcing stricter TP thresholds. (b) Spatial locations are said to exhibit alternans, if their TP exceeds some critical value, e.g., TP $\geq 60\%$. Two neighboring pixels A and B satisfying this criterion in the interval from T_1 to T_2 , have their uninterrupted alternating sequences in the $[t_1, T_2]$ and $[T_1, t_2]$ intervals, respectively. There will be at least 20% temporal overlap for the different spatial locations, allowing the determination of RP, used for characterization of SDAs.

Fig. 4.

Sensitivity of alternans detection as a function of SNR and AR. (a) Variable SNR was generated by adding white noise to four perfect alternans sequences (TP = 100%) of length $n = 30$, having different AR = 6%, 10%, 20%, and 50%. Curves show 95% probability of detecting alternans of different AR at the corresponding SNRs and TP values. For TP $\geq 60\%$, all signals with SNR below the cross point with the dark horizontal line will be misclassified (false negative). Setting TP < 100% increases sensitivity, i.e., allows detection of alternans at lower SNR. (b) For low (left) and high (right) SNR, the 95% sensitivity limit of detection is shown at three different TP thresholds. Y-axis scale (ARs) is different for low and high SNR. Alternans in the area above the curves will be obscured by noise, while detecting alternans is safe with 95% sensitivity under the curves.

Fig. 5.

Frequency as control parameter for alternans development in space-time. (a) Experimental setup: cardiomyocytes are cultured on rectangular strips and paced during experiments with a line electrode on one side. A preprocessed Ca^{2+} fluorescence intensity signal from a single pixel is shown. (b) Spatial alternans patterns over different pacing frequencies. Gray scale represents AR with white areas not satisfying the TP criterion. Images on the left are for $\text{TP} \geq 60\%$ (18/30 beats); images in the middle and to the right are the result of imposing $\text{TP} = 100\%$ (8/8 beats), with the eight beats selected at the beginning or the end of the 30 beat record. (c) Time series—the original intensity traces and derivatives of peak height in calcium are shown for two selected points A and B over different pacing frequencies. Blue and red in the left column represent the original data for points A and B, respectively. Blue and red in the middle and right column refer to derivative values during odd and even beats, respectively. For a perfectly alternating segment, all odd and even beats should be evenly distributed above and under the zero line, e.g., derivatives for A and B at 3.12 Hz ($\text{TP} = 100\%$ for both locations).

Fig. 6.

Identification of SDAs. (a) Phase-based identification of SDA patterns over different pacing frequencies. Color shows the magnitude of alternans at different spatial locations, quantified by $RP \times AR\%$. Red and blue identify opposite RP; green areas are regions with no detected alternans. Images on the left display all points with confirmed alternans; images in the middle show confirmed SDAs based on proximity criterion. (b) Enhancements of alternans by using the product of peak height (PH) and calcium transient duration (CTD) are due to changes in the same direction for both in response to frequency, and lead to improved noise resistance. (c) Large-scale alternans ($AR \geq 50$) in solid contiguous in-phase regions are shown in contrast to previous examples with low AR and speckled spatial distribution. Two traces extracted from sites A and B illustrate spatial discordance.

TABLE ITP in Noise and in Ca^{2+} Recordings as Function of Length and Pacing Frequency

Freq	1.11 Hz (19 beats)	1.66Hz (29 beats)	2.20Hz (36 beats)	2.44Hz (37 beats)
TP				
White noise	32.65%	24.49%	21.04%	20.55%
Ca^{2+} data (all beats)	31.39%	26.38%	32.44%	34.09%
White noise (19 beats)	32.65%	32.65%	32.65%	32.65%
Ca^{2+} data (last 19 beats)	31.39%	35.60%	51.70%	44.28%

TP for Ca^{2+} reflects spatially averaged values over thousands of pixels.