# Methodological analysis of dual voltage-calcium whole-heart optical signals during restitution pacing under different thermal states

Anna Crispino[1], Alessandro Loppini[1], Ilija Uzelac[2], Flavio H. Fenton[2], Alessio Gizzi[1,*], Simonetta Filippi[1,3,4]

2022 12th Conference of the European Study Group on Cardiovascular Oscillations (ESGCO) | 978-1-6654-8512-8/22/$31.00 ©2022 IEEE | DOI: 10.1109/ESGCO55423.2022.9931366

***Abstract*— Dual voltage-calcium fluorescence optical recordings are increasingly appealing to characterize complex spatiotemporal cardiac dynamics within ex-vivo whole-heart experimental preparations. Synchrony among voltage and calcium signals allows us to unveil novel multi-scale and multi-physics couplings at the ventricular scale and quantify features that define the intrinsic nonlinearities of the observed phenomena. Within such a complex scenario, we propose a rigorous methodological analysis comparing and contrasting multiple cardiac alternans onset and evolution indicators for rabbit pacing-down restitution protocols. We introduce a novel integral index quantified upon voltage and calcium signals, validated against well-accepted post-processing analyses, and generalized in terms of statistical restitution curves obtained under four different thermal states. Our study suggests that such a novel indicator can further advance our predictability on alternans onset, linking the concurrent evolution to an innovative quantification of the characteristic length obtained for both voltage and calcium at different thermal states.**

## I. INTRODUCTION

Understanding the effects of temperature on nonlinear cardiac dynamics has been of great clinical interest for over 70 years. It is well known that temperature changes have a critical role in developing irregular rhythms such as tachycardia and fibrillation [1], [2] in mammalian hearts compared to ectotherms [3]. Macroscopic non-negligible effects appear on action potential (AP) amplitude, morphology, and duration (APD), as well as on conduction velocity (CV) features [4], [5]. Spatiotemporal dispersion of APD has been further shown to increase at lower temperatures resulting in early period-doubling bifurcations and beat-to-beat AP alternans dynamics associated with complex spatial patterns both on endocardium and epicardium surfaces [6], [7]. Though such pro-arrhythmogenic abnormalities have been mainly characterized for transmembrane voltage dynamics, they are critically linked to alterations in intracellular calcium concentration ($[Ca^{2+}]_i$) due to the complex voltage-calcium couplings in cardiac tissue. Accordingly, innovative techniques that can simultaneously record calcium and voltage signals are becoming more accessible for the discovery of novel multi-scale and multi-physics couplings in cardiac electrophysiology [8].

The present study aims to fill this gap partially by using synchronous calcium (Ca) and voltage ($V_m$) fluorescence optical signals, recording and analyzing multiple sequences of restitution curves at different thermal states. It is worth noticing that during a pacing-down restitution protocol, i.e., applying a gradual reduction in pacing cycle length (PCL) that accounts for dynamic adaptations, a non-homogeneous accumulation of $[Ca^{2+}]_i$ occurs, such that the identification of an equivalent calcium duration (CaD) alternans index results challenging [9]. Here, we introduce and compare alternative methods based on integral features (e.g., the area under the curve), identifying robust and reliable parameters for the detection and classification of alternans dynamics for $V_m$ and Ca signals. We demonstrate how the proposed method reduces signal processing complexity, further connecting the results of our analysis with the length-scale transition obtained through spatio-temporal correlation measures. Introducing a characteristic length for Ca can critically advance our predictive power for period-doubling bifurcation currently based on the sole voltage CV features [10], [11].

## II. METHODS

Fluorescence optical mappings were performed on one ex-vivo rabbit under approved IACUC from Georgia Tech. Four thermal states, from 37°C down to 25°C, with a step of 4°C were considered. The full pacing down restitution protocol was applied at each temperature. Due to the intrinsic noise, we compared a low-pass spatial Gaussian filter (window = 7, variance = 2) with the stacking procedure proposed in [12], where no spatial or temporal filter is required. As a novel contribution, we introduce the *area method* defining the alternans ratio (*AR*), as:

$$\begin{cases} |AR(x,y) = 1 - \frac{B}{A}| \geq thr & \text{alternans} \\ |AR(x,y) = 1 - \frac{B}{A}| < thr & \text{no alternans} \end{cases} \quad (1)$$

Here, $(x,y)$ stands for the position of the selected pixel within the field of view, $B$ and $A$ represent the integral area values of odd and even beats, respectively, sorted such that odd beats values are greater than even ones. We then built global *AR* restitution curves for both $V_m$ and Ca by averaging the odd and even beats over time and space, respectively. Alternans onset (at the bifurcation points of the computed curves) was defined as the pacing value whose alternans ratio falls within a given threshold (*thr*), according to (1), thus characterizing the appearance of alternans at the four thermal states. A dedicated parametric analysis was carried out to find the optimal value of threshold respecting the known trends of alternans onset as temperature changes. Finally, a spatio-temporal correlation analysis was performed for both

[1]Department of Engineering, University of Rome Campus Bio-Medico, via A. del Portillo 21, 00128 Rome, IT a.gizzi@unicampus.it
[2]School of Physics, Georgia Institute of Technology, Atlanta, GA, USA.
[3]ICRANet, Piazza delle Repubblica 10, I-65122 Pescara, Italy.
[4]CNR-INO, I-50019 Sesto Fiorentino, Italy.

978-1-6654-8512-8/22/$31.00 ©2022 IEEE

Authorized licensed use limited to: Georgia Institute of Technology. Downloaded on September 11,2023 at 06:26:25 UTC from IEEE Xplore. Restrictions apply.

signals evaluating the Pearson product-moment correlation index $R(\vec{r})$ as a function of the distance between pixels ($\vec{r}$) and extracting the associated characteristic length $L_0$ from the exponential decay tract, i.e., $R(\vec{r}) \propto \exp(||\vec{r}||/L_0)$ [10].

## III. RESULTS

The proposed analyses allowed us to recover the expected trends from other indices, e.g., APD alternans, further extending the predictive power to Ca traces. Figure 1 shows the averaged restitution curves obtained for both $V_m$ and Ca signals at two selected temperatures, i.e., 37 and 25°C. The curves highlight that: i) at basal temperature, rabbit hearts do not present $V_m$ alternans while a clear bifurcation appears for Ca; ii) the integral area index increases at lower temperatures, mimicking what is known for APD; iii) alternans onset is obtained at higher pacing cycle length for lower temperatures as known from APD analyses. Furthermore, we quantified several other features based on the area method (not shown for the sake of space), revealing that concordant and discordant alternans patterns appear within the field of view with different phenomenologies varying temperature. For example, the percentage of alternating pixels linked to AR threshold values is higher for Ca (at 37°C, PCL = 160 ms, and $thr = 0.15$ we get 76,84% for Ca and 0.93% for $V_m$). Interestingly, we discovered that the bifurcation point dependents on the chosen threshold value in a similar fashion as the classical APD-based methods are affected by such a parameter. A dedicated sensitivity analysis indicated that the optimal threshold values range within $10 \div 12\%$ of the alternans ratio index.

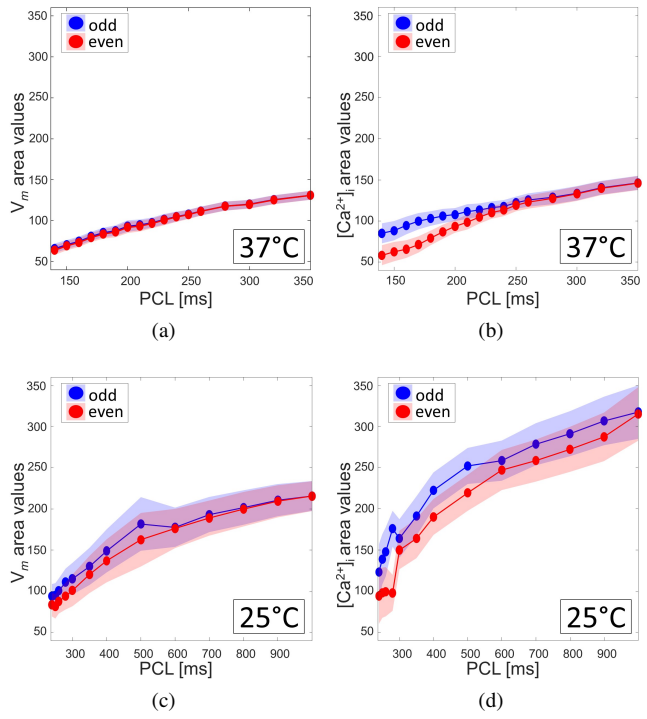


Fig. 1: Voltage and Calcium restitution curves at 37°C (a,b) and 25°C (c,d). Mean and standard deviation are provided for odd (blue) and even (red) beats.

## IV. DISCUSSION & CONCLUSIONS

We discuss a novel methodological approach for analyzing optical fluorescence signals affected by noise and accumulation effects under four thermal states. Our analysis reveals that the *area method* is a robust, fast, and reliable indicator for characterizing alternans onset and evolution, as this measure accounts for changes in both duration and amplitude of intracellular calcium. The features obtained are validated against classical analyses performed on APD and further generalized, for the first time, in terms of Ca characteristic lengths at different thermal states. The main limitation of the present study relies on the number of experiments performed that we are currently expanding, also considering other species. We foresee the broad usage of such a methodology to extract experimental features that can be easily integrated into complex electrophysiological cardiac models.

## V. ACKNOWLEDGMENT

Authors acknowledge the support of the Italian National Group for Mathematical Physics (GNFM-INdAM), NSF-FDA-203789 and 1R01HL143450-01

## REFERENCES

[1] W. G. Bigelow, J. C. Callaghan, and J. A. Hopps, "General Hypothermia for Experimental Intracardiac Surgery", *Ann Surg*, vol. 132, pp. 531–537, Sept. 1950.

[2] F. H. Fenton, A. Gizzi, C. Cherubini, N. Pomella, and S. Filippi. "Role of temperature on nonlinear cardiac dynamics", *Physical Review E*, vol. 87, 042717, April 2013.

[3] C. Herndon, H. Astley, T. Owerkowicz, and F.H. Fenton, "Defibrillate you later, alligator: Q10 scaling and refractoriness keeps alligators from fibrillation", *Int Org Bio*, vol 3, pp. obaa047, 2021.

[4] T. Kiyosue, M. Arita, H. Muramatsu, A. J. Spindler, and D. Noble, "Ionic mechanisms of action potential prolongation at low temperature in guinea-pig ventricular myocytes", *The Journal of Physiology*, vol. 468, pp. 85-106, Aug. 1993.

[5] H. Bjørnstad, P. M. Tande, D. A. Lathrop, H. Refsum, "Effects of temperature on cycle length dependent changes and restitution of action potential duration in guinea pig ventricular muscle", *Cardiovascular Research*, vol. 27, June 1993, pp. 946–950.

[6] A. Gizzi, E. M. Cherry, R. F. Gilmour Jr., S. Luther, S. Filippi and F. H. Fenton, "Effects of pacing site and stimulation history on alternans dynamics and the development of complex spatiotemporal patterns in cardiac tissue", *Frontiers in Physiology*, vol. 4, 71, April 2013.

[7] A. Gizzi, A. Loppini, E. M. Cherry, C. Cherubini, F. H. Fenton, and S. Filippi, "Multi-band decomposition analysis: application to cardiac alternans as a function of temperature", *Physiological Measurement*, vol. 38, pp. 833-847, April 2017.

[8] I. Uzelac, J. C. Crowley, S. Iravanian, T. Y. Kim, H. C. Cho, F. H. Fenton. "Methodology for Cross-Talk Elimination in Simultaneous Voltage and Calcium Optical Mapping Measurements With Semasbestic Wavelengths" *Frontiers in Physiology*, vol. 13, Feb. 2022.

[9] V. Kappadan, S. Telele, I. Uzelac, F. H. Fenton, U. Parlitz, S. Luther and J. Christoph, "High-resolution optical measurement of cardiac restitution, contraction, and fibrillation dynamics in beating vs. blebbistatin-uncoupled isolated rabbit hearts", *Frontiers in Physiology*, vol. 11, pp. 464, May 2020.

[10] A. Loppini, A. Gizzi, C. Cherubini, E. M. Cherry, F. H. Fenton, and S. Filippi, "Spatiotemporal correlation uncovers characteristic lengths in cardiac tissue", *Physical Review E*, vol. 100, 020201, 2019.

[11] A. Loppini, A. Barone, A. Gizzi, C. Cherubini, F. H. Fenton, and S. Filippi, "Thermal effects on cardiac alternans onset and development: A spatiotemporal correlation analysis", *Physical Review E*, vol. 103, L040201, 2021.

[12] I. Uzelac and F. H. Fenton, "Robust framework for quantitative analysis of optical mapping signals without filtering" *2015 Computing in Cardiology Conference*, pp. 461-464, 2015.

Authorized licensed use limited to: Georgia Institute of Technology. Downloaded on September 11,2023 at 06:26:25 UTC from IEEE Xplore. Restrictions apply.