1. **Introduction.**

Autonomic nervous system [ANS] function is widely studied via heart-rate variability [HRV] analyses, and yet there remain critical methodological concerns regarding the handling of high-frequency signal artifacts—such as frequent motion or cardiac arrhythmia—bringing the validity of autonomic inferences derived from HRV into question [(Quigley et al., 2024)](https://sciwheel.com/work/citation?ids=16580473&pre=&suf=&sa=0&dbf=0). HRV analyses are usually performed on electrocardiogram [ECG] data, but photoplethysmogram [PPG] data is also commonly used [(Burma et al., 2024)](https://sciwheel.com/work/citation?ids=16580399&pre=&suf=&sa=0&dbf=0), with PPG P-P peak intervals showing a near-perfect correlation with ECG R-R intervals (r > 0.97) [(Gil et al., 2010)](https://sciwheel.com/work/citation?ids=2114364&pre=&suf=&sa=0&dbf=0).

Automated HRV data cleaning methods often use filtering (e.g., median, moving average), outlier interpolation (e.g., linear, cubic spline), and data censoring [(Quigley et al., 2024)](https://sciwheel.com/work/citation?ids=16580473&pre=&suf=&sa=0&dbf=0) applied to the interval tachogram derived from identified peaks in ECG or PPG data, with accurate inference depending on correct automated peak identification, which is often inaccurate under “noisy” conditions [(Kazemi et al., 2022)](https://sciwheel.com/work/citation?ids=16580469&pre=&suf=&sa=0&dbf=0). Existing automated computational methods can identify and process high-frequency signal artifacts—to some extent—but comprehensive PPG processing tools are especially lacking [(Bota et al., 2024; Makowski et al., 2021; Yamane et al., 2024)](https://sciwheel.com/work/citation?ids=11447243,16497555,16580390&pre=&pre=&pre=&suf=&suf=&suf=&sa=0,0,0&dbf=0&dbf=0&dbf=0). Visibility of the source signal (e.g., the PPG time-series) enables informed manual correction of mislabeled peaks and artifact identification, which are crucial for accurate tachogram derivation in complex cases and yet not accessible via automated tools alone. Consequentially, without an additional layer of manual oversight and intervention, complex artifact-contaminated cases either yield erroneous HRV estimates (e.g., overestimated parasympathetic function) or must be discarded as unusable data.

As a solution to improve on the limitations of automated approaches, we present an open-source prototype application that leverages interactive processing of the PPG source signal for salvaging complex PPG datasets [(McConnell, 2024)](https://sciwheel.com/work/citation?ids=16478695&pre=&suf=&sa=0&dbf=0). Our tool builds on the Neurokit2 Python toolbox—which offers a comprehensive suite of functions for ANS data processing [(Makowski et al., 2021)](https://sciwheel.com/work/citation?ids=11447243&pre=&suf=&sa=0&dbf=0)—but lacks interactive methods for handling complex PPG datasets. Our application enables manual correction of misidentified PPG peaks and identification of valid peak boundaries around observed artifact windows and then derives a local-average beat template by automatically identifying zero-crossings of the first derivative to mark pulse-wave start and endpoints for segmentation. This method handles larger gaps and multiple consecutive artifacts reasonably well and preserves more physiological signal than conventional automatic approaches (see an example artifact window dataset here as Supplemental Data 1: [figshare.com/s/569155ff8bd3d3144263](https://figshare.com/s/569155ff8bd3d3144263); and Supplemental Figures S1: [figshare.com/s/d579b95b8c4c2d349141](https://figshare.com/s/d579b95b8c4c2d349141); S2: [figshare.com/s/881dc506c62d836d7680](https://figshare.com/s/881dc506c62d836d7680); S3: [figshare.com/s/43f19d0db3d795ff8962](https://figshare.com/s/43f19d0db3d795ff8962); and S4: [figshare.com/s/1f02ebc332a354c0db23](https://figshare.com/s/1f02ebc332a354c0db23)).

Therefore, in this short report, we quantitatively illustrate the benefits of our manual correction tool vs. automated methods by presenting case examples where automated processing was moderately effective for individuals with infrequent arrhythmias but failed in cases of frequent arrhythmias, and also present a case where automated methods resulted in “overcleaning” of clean data. Initial processing of these complex datasets shows our approach can effectively improve parasympathetic HRV metric plausibility and salvage data that might otherwise need to be excluded as excessively 'noisy.' These preliminary results underscore our tool's potential to enhance HRV analysis accuracy in clinical populations. Although not fully deployment-ready, these findings validate the core concept and justify further development. We present the application “as is” and outline areas for future improvement to expand its impact and utility.

1. **Methods.**
   1. *Procedures.*

Written informed consent for participation was actively obtained before enrollment in the study. Protocol and all procedures were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Human Research Committee at the Medical University of South Carolina.

PPG signal was measured from the left-index toe—with a TSD200-MRI transducer Velcro strap using a Biopac PPG100C-MRI smart amplifier—concurrently with functional magnetic resonance imaging (fMRI) over four 6-minute runs. Care was taken to avoid overtightening the transducer strap, which could occlude blood flow. Transducer placement was verified to ensure clear signal acquisition with minimal noise or artifacts from the MRI environment. Hardware settings included a gain of 10 µS/V, a 3.0 Hz low-pass filter, and a 0.5 Hz high-pass filter.

* 1. *Participants.*

To demonstrate these findings of under- and over-cleaning via automated methods, we highlight three complex data sets from chronic stroke survivors (Table 1): (1) frequent-arrhythmia (sub-017); (2) infrequent-arrhythmia (sub-034); and (3) failed automatic peak detection (sub-026) (Supplemental Figures S5: [figshare.com/s/e711cf900f06db5b0ac4](https://figshare.com/s/e711cf900f06db5b0ac4), S6: [figshare.com/s/802d302f63727f7ba955](https://figshare.com/s/802d302f63727f7ba955), and S7: [figshare.com/s/a94e93398d6469fe9d40](https://figshare.com/s/a94e93398d6469fe9d40)) showing how failed automatic correction artificially inflates parasympathetic HRV estimates in both time- (i.e., root mean square of successive differences [RMSSD]) and frequency-domains (i.e., high-frequency [HF] power) (i.e., “under-cleaning).

Additionally, we present two clean datasets (sub-012, healthy; sub-021, stroke) that required no manual intervention, showing physiologically implausible yet statistically negligible alterations via automatic methods (i.e., “over-cleaning”) (Supplemental Figures S8: [figshare.com/s/2e89b254ff3b37803907](https://figshare.com/s/2e89b254ff3b37803907); and S9: [figshare.com/s/1749645c27a260368138](https://figshare.com/s/1749645c27a260368138)).

The effects of our interactive correction tool on these same examples are online for Supplemental Figures S10: [figshare.com/s/3fd2e96e4ba4c0213cc2](https://figshare.com/s/3fd2e96e4ba4c0213cc2); S11: [figshare.com/s/2e751cdf17ba9ca6526c](https://figshare.com/s/2e751cdf17ba9ca6526c); S12: [figshare.com/s/fc2ae62218c7638df880](https://figshare.com/s/fc2ae62218c7638df880); S13: [figshare.com/s/8bed16aaf820b1dfa5ac](https://figshare.com/s/8bed16aaf820b1dfa5ac); S14: [figshare.com/s/29184eaa94803ae1f3f7](https://figshare.com/s/29184eaa94803ae1f3f7); and the data can be explored in full via interactive html at Supplemental Data 3: [figshare.com/s/bd9f9d3b30a73e65a577](https://figshare.com/s/bd9f9d3b30a73e65a577).

* 1. *Data Processing.*

PPG signals were denoised ([pywt.wavedec](http://github.com/PyWavelets/), db4, periodic), down-sampled ([nk.signal\_resample](https://neuropsychology.github.io/NeuroKit/functions/signal.html" \l "neurokit2.signal_resample), 100 Hz, ‘pandas’), and cleaned ([nk.ppg\_clean](https://neuropsychology.github.io/NeuroKit/functions/ppg.html" \l "ppg-clean)) with a 3rd-order Butterworth filter (8 Hz low-pass, 0.5 Hz high-pass). Peak detection used the Elgendi method [(Elgendi et al., 2013)](https://sciwheel.com/work/citation?ids=2114357&pre=&suf=&sa=0&dbf=0). A beat interval (P-P) tachogram was generated for artifact inspection using cubic-spline interpolation. Time- and frequency-domain statistics were computed via Neurokit2 default settings ([nk.hrv](https://neuropsychology.github.io/NeuroKit/functions/hrv.html" \l "main)) before further processing [(Frasch, 2022; Pham et al., 2021)](https://sciwheel.com/work/citation?ids=13594739,13594736&pre=&pre=&suf=&suf=&sa=0,0&dbf=0&dbf=0).

Interactive data processing was handled through custom integration of Neurokit2 code into our new interactive application: [fix\_ppg\_peaks\_artifacts.py](https://github.com/PAmcconnell/physio_stats/blob/main/correct/fix_ppg_peaks_artifacts_rev22.0.py) [(McConnell, 2024)](https://sciwheel.com/work/citation?ids=16478695&pre=&suf=&sa=0&dbf=0) (Supplemental Code 1: [figshare.com/s/e9201079cd6b918f3a95](https://figshare.com/s/e9201079cd6b918f3a95)), referred to as “Dash-app” for brevity. The application reads in Neurokit2-cleaned PPG data and displays the automatic peak detection results, the interpolated beat interval tachogram, and the framewise displacement of the head (as an indicator of motion via FSL MCFLIRT) [(Jenkinson et al., 2002)](https://sciwheel.com/work/citation?ids=8414456&pre=&suf=&sa=0&dbf=0). While loading the PPG input data, our application performs default Neurokit2 artifact processing ([nk.signal\_fixpeaks](https://neuropsychology.github.io/NeuroKit/functions/signal.html" \l "signal-fixpeaks), with method=”Kubios”, iterative=“True”) [(Makowski et al. 2021)](https://sciwheel.com/work/citation?ids=11447243&pre=&suf=&sa=0&dbf=0), and quantifies the number of altered samples by comparing the resultant cleaned tachogram with the original. Next, the application allows for interactive misidentified peak labeling through plot-click interactions in the tool’s browser interface. When artifacts are encountered, deleting any peaks from within the artifact window, entering the x-axis values of the nearest valid boundary peaks, and then selecting the “correct artifact” button will perform local average beat template generation and artifact window interpolation. The number of samples altered through this approach is tracked by comparing the cleaned PPG signal against the original. After correction, the application saves out visualizations of all heartbeat templates, updates the dataframe, and recomputes HRV statistics via [nk.hrv](https://neuropsychology.github.io/NeuroKit/functions/hrv.html#main) for each of the four processing methods: uncorrected, NK2-Kubios, Dash-app, and censored [(Frasch, 2022; Pham et al., 2021)](https://sciwheel.com/work/citation?ids=13594739,13594736&pre=&pre=&suf=&suf=&sa=0,0&dbf=0&dbf=0). The censored method removes marked artifact data, concatenates the remaining clean data, and computes HRV estimates based on it.

* 1. *Descriptive Statistics for Processing Methods.*

HRV statistics for each participant and method were compiled into a csv file for each run and averaged to obtain mean and standard deviation. Nonlinear metrics (SD1 & SD2) were computed using [neurokit2.hrv.hrv\_nonlinear](https://neuropsychology.github.io/NeuroKit/functions/hrv.html#neurokit2.hrv.hrv_nonlinear) functions and plotted with code adapted from Neurokit2 in [poincare\_plot\_ppg.py](https://github.com/PAmcconnell/physio_stats/blob/main/visualize/poincare_plot_ppg_v3.0.py) [(McConnell, 2024)](https://sciwheel.com/work/citation?ids=16478695&pre=&suf=&sa=0&dbf=0).

1. **Results.**

The effects of each processing method on select HRV variables are reported below in Table 1; all HRV statistics are in Supplemental Data 2: [figshare.com/s/f0e5488bc5cabfdcea86](http://figshare.com/s/f0e5488bc5cabfdcea86).

**Table 1.** Effects of data processing methods on selected HRV metrics.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Processing Method** | **sub-012**  **clean data** | **sub-017**  **frequent arrhythmia** | **sub-021**  **clean data** | **sub-026**  **failed peak** | **sub-034**  **infrequent arrhythmia** |
| **% Samples Altered** | NK2-Kubios | 29.86 (27.46) | 73.89 (13.26) | 34.07 (21.94) | 89.31 (14.73) | 91.12 (4.80) |
| Dash App | 0.00 (0.00) | 52.52 (7.68) | 0.00 (0.00) | 9.28 (7.02) | 18.78 (9.24) |
| **RMSSD (ms)** | Uncorrected | 25.93 (1.25) | 194.74 (15.61) | 35.99 (5.09) | 198.91 (93.00) | 212.08 (127.87) |
| NK2-Kubios | 25.53 (1.54) | 174.32 (18.13) | 35.08 (4.63) | 94.38 (23.98) | 119.17 (101.11) |
| Dash App | 25.92 (1.26) | 54.15 (5.68) | 35.99 (5.10) | 83.16 (19.93) | 68.43 (5.41) |
| Censored | 25.92 (1.26) | 38.79 (2.75) | 35.99 (5.10) | 76.91 (16.15) | 65.98 (6.61) |
| **High Frequency Power (n.u.)** | Uncorrected | 0.009 (0.004) | 0.023 (0.003) | 0.027 (0.008) | 0.047 (0.028) | 0.026 (0.023) |
| NK2-Kubios | 0.009 (0.004) | 0.021 (0.006) | 0.027 (0.008) | 0.031 (0.026) | 0.025 (0.030) |
| Dash App | 0.008 (0.003) | 0.013 (0.005) | 0.030 (0.011) | 0.021 (0.018) | 0.026 (0.019) |
| Censored | 0.008 (0.003) | 0.038 (0.014) | 0.030 (0.011) | 0.027 (0.028) | 0.049 (0.015) |
| **Low Frequency Power (n.u.)** | Uncorrected | 0.011 (0.006) | 0.008 (0.006) | 0.022 (0.014) | 0.023 (0.009) | 0.013 (0.012) |
| NK2-Kubios | 0.011 (0.006) | 0.007 (0.005) | 0.021 (0.014) | 0.021 (0.018) | 0.015 (0.019) |
| Dash App | 0.009 (0.004) | 0.017 (0.003) | 0.023 (0.014) | 0.015 (0.009) | 0.023 (0.013) |
| Censored | 0.009 (0.004) | 0.046 (0.011) | 0.023 (0.014) | 0.011 (0.012) | 0.023 (0.007) |
| **LF/HF** | Uncorrected | 1.24 (0.58) | 0.34 (0.25) | 0.80 (0.45) | 0.93 (1.09) | 0.56 (0.25) |
| NK2-Kubios | 1.25 (0.57) | 0.37 (0.29) | 0.80 (0.44) | 0.75 (0.25) | 0.71 (0.35) |
| Dash App | 1.16 (0.59) | 1.49 (0.60) | 0.80 (0.45) | 0.84 (0.23) | 0.98 (0.25) |
| Censored | 1.16 (0.59) | 1.33 (0.57) | 0.80 (0.45) | 0.55 (0.29) | 0.49 (0.16) |

*Note.* Table values are presented as mean (standard deviation) for each subject across four resting-state fMRI runs of equal length (six minutes each) for each of the four data processing methods presented. n.u. = normalized units (normalized by maximum power spectral density value; non-normalized units in ms2). LF/HF interpreted as metric of sympathovagal balance with values > 1.0 implying sympathetic dominance and < 1.0 parasympathetic dominance [(Montano et al., 2009)](https://sciwheel.com/work/citation?ids=356482&pre=&suf=&sa=0&dbf=0). Table 1 is online at [figshare.com/s/953895c96cc7d7f60dc6](https://figshare.com/s/953895c96cc7d7f60dc6).

The Poincaré plots in Figure 1 illustrate how incomplete automatic correction can bias HRV estimates, indicating artificially-inflated estimates of parasympathetic HRV [(Kamen et al., 1996)](https://sciwheel.com/work/citation?ids=5721420&pre=&suf=&sa=0&dbf=0). The plots demonstrate the effects of each of the four processing methods tested on both clean data and problematic data from stroke survivors and highlights the inadequacy of the default NK2-Kubios correction in handling frequent arrhythmia, which is better addressed by manual processing and censoring—as evidenced by increased data linearity and zero-centering of the marginal density plots. Enhanced effects of manual cleaning on data linearity and marginal density centering are also seen in cases of failed peak detection and infrequent arrhythmia.

**A screenshot of a graph

Description automatically generated**

**Figure 1.** The Poincaré plot depicts effects of processing methods on spread and linearity. Columns represent the four processing methods: **(1) Uncorrected** (automatic peak detection only); **(2) NK2-Kubios** (default NK2 correction); **(3) Dash-App** (interactive processing); **(4) Censored** (artifact-window free segments only). Rows represent exemplar cases: **(A) sub-012** (healthy, clean data); **(B) sub-021** (stroke, clean data); **(C) sub-017** (stroke, frequent arrhythmia); **(D) sub-026** (stroke, failed peak detection); **(E) sub-034** (stroke, infrequent arrhythmia). Figure 1 online at: [figshare.com/s/8918f9af38adf1c8633f](https://figshare.com/s/8918f9af38adf1c8633f)**. Supplemental caption:** [figshare.com/s/0d3dbf06154c7cc658fe](http://figshare.com/s/0d3dbf06154c7cc658fe)

The interactive processing method using our Dash-app (column 3) effectively enabled the correction of peak misidentifications and attenuation of inflated parasympathetic estimates resulting from under-cleaned automated data processing. With sufficient training to recognize specific abnormalities in the PPG signal (e.g., arrhythmia, artifacts, misidentified peaks), our interactive tool provides more physiologically plausible estimations of parasympathetic autonomic function, as indicated by increased data linearity and better-centered marginal densities compared to automatic corrections (Figure 1, subplots C3, D3, E3). All subject dataframes are available in Supplemental Data 4: [figshare.com/s/c7fc787344446dacf26d](https://figshare.com/s/c7fc787344446dacf26d).

1. **Discussion.**

Our data emphasizes the need for comprehensive PPG signal processing tools for complex cases, e.g., in populations with high arrhythmia prevalence. We show that automatic HRV analysis suffers from inflated parasympathetic estimates (e.g., RMSSD, LF/HF) resulting from uncorrected arrhythmia and misidentified peaks. For participants with arrhythmias (e.g., sub-017, sub-034), RMSSD values are implausibly high and LF/HF values are implausibly low. Healthy adult ranges (non-athlete) for these metrics are 19-75 ms for RMSSD and 1.1-11.6 for LF/HF [(Nunan et al., 2010)](https://sciwheel.com/work/citation?ids=1220096&pre=&suf=&sa=0&dbf=0). Table 1 shows the effects of processing methods on reducing mean RMSSD (ms) and LF/HF from 194.74, 0.34 (uncorrected), to 174.32, 0.37 (NK2-Kubios), to 54.15, 1.49 (Dash-app) for frequent arrhythmia, and from 212.08, 0.56 (uncorrected), to 119.17, 0.71 (NK2-Kubios), to 68.43, 0.98 (Dash-app) for infrequent arrhythmia. This demonstrates the varying efficacy of different data processing methods on HRV indices, establishing the feasibility of our interactive tool for reducing the impact of arrhythmias and other artifacts on HRV estimates.

Although censoring improved upon both uncorrected and NK2-Kubios methods in attenuating the impact of frequent arrhythmias on HRV, censoring removes entire artifact windows and computes HRV estimates from the remaining clean data—this reduces data quantity and thereby can compromise statistical power, HRV estimate robustness, and disrupt time-series continuity—and thus it is not optimal for integrating continuous HRV data with neural measures such as BOLD fMRI to map central autonomic networks (Valenza et al., 2019).

Our interactive processing tool, although potentially time-consuming and requiring training, offers a more tailored approach to handling complex cases that performed well in reducing inflated parasympathetic HRV estimates in the cases tested. We highlight that existing automatic methods neglect significant over- and under-cleaning issues, suggesting a need for combining automatic methods with visual inspection and manual processing in complex cases [(Quigley et al., 2024)](https://sciwheel.com/work/citation?ids=16580473&pre=&suf=&sa=0&dbf=0). This requires knowledge of artifacts or features in cardiovascular pathologies, and our interactive tool implements this preprocessing layer for PPG data. Notably, direct manipulation of the PPG signal reduces the total amount of altered data compared to tachogram-based approaches, avoiding over-correction of clean data.

1. **Conclusions.**

Our in-development application addresses significant limitations in current HRV analysis methods by offering a framework for robust, interactive processing of complex PPG data. By accurately reflecting underlying autonomic function, further development and large-scale validation of this tool could significantly improve HRV analyses, leading to better assessments of autonomic function and more accurate mapping of central autonomic brain networks in clinical populations.

1. **Future Directions.**

Future work should refine the application, improve automation, and conduct comprehensive validation studies in various clinical populations. We envision broader utility with additional features, such as non-linear fitting of average beat templates (e.g., matching local variability before and after artifact windows), flexible interactive control of processing options (e.g., filtering, smoothing, artifact window censoring), and advanced autonomic analyses based on pulse-wave morphology. Pulse wave morphology, which can provide insights into blood pressure variability, arterial blood oxygenation, respiratory activity, arterial stiffness, and cardiac output, should not be overlooked [(Almarshad et al., 2022)](https://sciwheel.com/work/citation?ids=14544695&pre=&suf=&sa=0&dbf=0). Traditional methods that solely focus on the interval tachogram may miss nuances such as these, leading to, at best, incomplete, and at worst, erroneous, assessments of autonomic function. Additionally, expanding the application to handle various signal types (e.g., ECG) and integrating advanced statistical features like entropy measures and smoothness will broaden its clinical research applicability. In severe cases, obtaining ground truth about autonomic dynamics from a single source like PPG may be challenging. Thus, developing methods that integrate information from multiple sources (e.g., electrodermal activity) and further enhancing the application for interactive cleaning of multiple autonomic function probes is warranted.

**Supplemental data sharing statement.**

Supplemental material is provided through figshare. shared with private links for the purpose of the review process and will be made publicly available with DOIs after manuscript acceptance. Please also see Supplemental References at: [figshare.com/s/dbc7cc3c066584563c0e](https://figshare.com/s/dbc7cc3c066584563c0e).

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**Declaration of interests.**  
The authors declare no conflict of interest.

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