

A Reliability Study in Resting-state EEG Network Characteristics: Frequency of Interest, Number of Oscillatory Cycles and Thresholding

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Abstract—Understanding how interactions between functional components in the brain form a network organization is a fundamental question in neuroscience. Graph theory has been applied to neural data to characterize these networks. However, multiple methodological decisions during network derivation raise questions about the reliability and reproducibility of such studies. In this study, we systematically investigated how frequency of interest, epoch length, and thresholding steps influence the stability and reliability of three key network measures derived from resting-state EEG: clustering coefficient, global efficiency, and Small-World Propensity. To ensure fair comparisons between different bands, we proposed using band-specific epoch lengths determined by the number of effective cycles at the frequency of interest, instead of a fixed length in seconds. Our findings reveal that clustering coefficient requires a specific threshold to achieve reliable estimates, while global efficiency benefits from fewer and stronger connections. Small-World Propensity showed less satisfactory reliability and may require more data for accurate estimation. These results emphasize the importance of examining reliability across different network measures before applying them in studies. The reliability varies across frequency bands, with higher-frequency oscillations needing more effective cycles to ensure accurate estimation. Our findings provide valuable insights for researchers in optimizing their choices of epoch length and thresholds in future EEG network studies, enhancing the reliability and reproducibility of these analyses.

I. INTRODUCTION

The brain is composed of numerous neuronal elements both microscopically and macroscopically. Understanding how the interactions between these functional components form a specific network organization that empower our brains is a fundamental challenge in neuroscience [1], [2], [3], [4]. Graph theory, a branch of mathematics that analyzes the structure and characteristics of graphs, provides mathematical tools for examining the network properties of the brain, where the sources of neural activities are treated as nodes, and the interactions between these sources as edges [2]. In electroencephalography (EEG) studies, the nodes are typically the scalp channels, and the edges are the functional connectivity measures computed between signals acquired from different channels. Various network measures can then be computed to characterize the constructed graphs in terms of network properties such as functional integration, functional segregation and small-worldness, etc. [2], [5]. As graph theoretical analyses has been gaining attention

in early twentieth century, there has been an increasing number of studies that study brain functions with network measures. For instance, the characteristic path length was shown to decreased significantly as we age both normally and pathologically [6], [7]. Significant differences in small-worldness were found between normal older adults, amnesic mild cognitive impairment and Alzheimer's disease groups [8]. Network properties were also found to correlate with cognitive abilities such as intelligence [9] and working memory function [10].

Despite a large body of literature supporting the usefulness of graph theoretical analyses of EEG networks in revealing changes in brain organization, questions remain about their reliability and reproducibility. The uncertainty arises from the multiple methodological decisions involved in the derivation of network measures from the raw signals, starting from the initial selection of connectivity measures to network construction. This increasing diversity has made it difficult to compare findings across studies, harmed the reproducibility of individual differences studies, and led to the calls for a standardized research pipeline [11].

A number of methodological studies have been conducted to examine how various factors, including the number of channels [12], [13], the choice of connectivity measures [14] and the network construction approaches [15], [16], affect the derived network measures and statistical significance between contrasts. While the choice of connectivity measures can be motivated by the type of relationship one wants to capture, the network construction approach and the optimal epoch length are still relatively arbitrary and remains a heavily debated topic.

Thresholding is a crucial procedure for removing connections from a connectivity matrix before network construction. It aims to improve signal-to-noise ratio and reduce spurious relationships. One common method is weight-based proportional thresholding, where a desired network density is achieved by preserving the top $x\%$ of strongest connections [5], [15]. However, it should be noted that the thresholding procedure can significantly impact the outcome of network analysis. Studies have shown that the chosen threshold alone can explain a substantial portion of the variance in measures like clustering coefficient and characteristic path length [17]. While thresholding was initially believed to be essential for revealing clear small-world topologies, the utility of weak connections has been increasingly recognized as they reflect unique individual differences [15], [18].

Nevertheless, both thresholded and unthresholded networks have offered valuable insights. Thresholded networks

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have been successful in highlighting changes in small-worldness associated with diseases like Alzheimer’s disease and frontotemporal dementia [19], [20]. They also appear sensitive to distinct emotional states [21]. Unthresholded networks, on the other hand, have revealed group differences related to aging and cognitive impairment as well [6], [7], [10].

The impact of epoch length on network measures has been explored in some previous studies [22], [23]. For instance, network measures derived from 6-second epochs were found to effectively distinguish emotional states [21]. Another comprehensive methodological study found excellent reliability for different connectivity measures using both smaller number of longer epochs (for the phase lag index) and higher number of shorter epochs (1 – 2s, for the debiased weighted phase lag index) [23]. These findings suggest that the epoch length might be specific to connectivity measure.

However, these studies are not without limitations. They either focused on a wide range of frequencies, spanning several canonical bands [22] or just on one band [23]. Fraschini et al. examined the impact of epoch length network measures derived from EEG signals bandpass-filtered from 1 to 20 Hz [22]. This wide range of frequency may have biased their results towards measures that are influenced more by higher-frequency oscillations. Within a 16-second epoch, a 1 Hz oscillation completes only 16 cycles while a 20 Hz oscillation completes 320 cycles, potentially biasing the derived network dynamics. These studies neglected the potential interaction between different frequencies and epoch length [11]. While it was recommended to have at least 50 trials for epochs less than 2 seconds or using epochs spanning 4-6 seconds for phase-based connectivity measures [11], the ideal epoch length and number should be determined by a careful consideration of factors like the underlying brain process, frequency of interest, and specific connectivity measure being utilized [24], [11]. This highlights the need of an exploration of the combined effects of frequency and epoch length.

The present study attempts to study the stability and reliability of network measures and how they are modulated by the frequency of interest, epoch length and thresholding. Instead of selecting epoch length based on a fixed period of time, we propose an improved way to determine the epoch length that is based on the frequency of interest. Specifically, we will determine the epoch length by the number of cycles spanned by the oscillations of frequency of interest. As such, fair comparisons and evaluation of measures between different canonical bands can be achieved. Details of our methods are illustrated in the following section.

II. METHODS

A. Data Collection

As part of a separate ongoing study, 62 (30F) Cantonese speakers with no known neurological disorders were recruited. These individuals were between the ages of 60 and 70 (mean = 65.2(3.03)). Each participant completed the Montreal Cognitive Assessment Hong Kong Version [25],

and they were classified as cognitively normal according to the 7th percentile cutoff of the normative data, which were adjusted for education years [26].

Each participant underwent a four-minute resting-state EEG recording with their eyes closed. EEG data were acquired via a 64-channel BioSemi ActiveTwo System with Ag/AgCl electrodes, digitized at a 2048Hz sampling rate. To monitor eye movements, horizontal and vertical electrooculograms (HEOG and VEOG) were collected using two pairs of electrodes placed near the outer canthi and above or below the left eye, respectively. Participants were seated comfortably approximately 70 cm from a monitor. They were instructed to relax and minimize movement during the recording, which took place in a dimly lit room. Written informed consent was obtained from all participants. All experimental procedures were approved by the Ethical Review Committee at Hong Kong Polytechnic University.

B. EEG Preprocessing

EEG preprocessing were conducted with custom Python scripts with the *MNE-Python* package [27]. Before any preprocessing steps, the raw EEG signals were visually inspected to exclude channels that are contaminated by body movements which are several orders of magnitude larger than the EEG signal. The signals were then downsampled to 512 Hz and average referenced. Independent component analysis (ICA) was applied to remove eye artifacts. Specifically, Spearman correlation was computed between each extracted independent component and the VEOG/HEOG. The correlation values were transformed to z-scores, and the components z-score values higher than 3.0 were excluded. After eyes artifacts removal, the excluded channels during the visual inspection step were then interpolated. The signals were then filtered into the five canonical bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-45 Hz).

C. Epoching

The filtered signals were then segmented into epochs in a band-specific way. As we have described in the introduction, we adopted a different approach to examine the amount of data needed to achieve reliable network measures. Specifically, instead of varying the length of epoch in terms of seconds, we looked at the number of effective cycles within a period of time based on our frequency of interest. We varied the effective cycles from 6 to 30 in increments of 2. The epoch length would then be band-specific, and is determined by the lowest frequency within a band. For instance, the epoch length for delta band at effective cycles equals to 6 would be $(1/1\text{Hz} \times 6) = 6$ seconds, while that for the alpha band would be $(1/8\text{Hz} \times 6) = 0.75$ seconds. The longest epoch was 30-second, correspond to 30 effective cycles of delta band oscillations.

D. corrected imaginary Phase Locking Value (ciPLV)

To compute the connectivity matrix from each epoch, we selected the corrected imaginary Phase Locking Value

(ciPLV) as the connectivity measure due to its robust properties [28]. It is an improvement over its predecessors PLV and iPLV, addressing the volume conduction issue in EEG connectivity studies. It was shown to be insensitive to zero-lag synchronization while being accurate in detecting true non-zero lag connectivity. The ciPLV is defined as in equation 1 [28]:

$$\text{ciPLV}_{i,j,t} = \frac{\frac{1}{T} \Im \{ \dot{x}_{i,t} \cdot \dot{x}_{j,t}^T \}}{\sqrt{1 - (\frac{1}{T} \Re \{ \dot{x}_{i,t} \cdot \dot{x}_{j,t}^T \})^2}} \quad (1)$$

where $\dot{x}_{i,t}$ and $\dot{x}_{j,t}$ represent the normalized analytic signals (computed via Hilbert transform) of channels i and j at time t , respectively. The operator $\Im \cdot$ extracts the imaginary part of the complex value, while $\Re \cdot$ extracts the real part. The ciPLV value ranges from 0 to 1.

E. Network construction

After computing the connectivity matrix from each epoch, we randomly sampled 6 epochs from all epochs to compute an averaged connectivity matrix. This number of epochs is chosen based on a previous study to facilitate better comparison [22]. Band-specific brain networks were then constructed with weight-based proportional thresholding on the averaged connectivity matrix. The threshold was varied from preserving 10% strongest connections to fully weighted, with an increment of 5%. The sampling and network construction procedure was repeated 29 times for each band, threshold and participant.

F. Graph theoretical measurements

After we have constructed 29 networks for each band, threshold and participant, three representative network measures were computed from each network: (1) clustering coefficient, (2) global efficiency and (3) Small-World Propensity; characterizing the functional integration, function segregation and small-worldness of the network. A brief description of these measures is listed as follows:

- 1) Global efficiency: A measure of functional integration, defined as the average inverse shortest path length [5]. It reflects the global capacity of a graph to pass information via short paths [29].
- 2) Clustering coefficient: A measure of functional segregation. The clustering coefficient quantifies the fraction of triangles around the node, reflecting its prevalence of clustered connectivity [5].
- 3) Small-World Propensity (SWP): A measure of small-worldness defined for both binary and weighted networks [30].

G. Stability and reliability analysis

Two major statistical analyses were conducted to evaluate the stability and the reliability of the derived network measures respectively. Our first analysis examined how different number of effective cycles and thresholds would affect the stability of the network measures. Linear mixed effect models were fitted with the network measure being the

dependent variable, the number of effective cycles being the independent variable with subject-specific random intercepts for modeling between-subject variability. After fitting the models, post-hoc comparisons were conducted with planned contrast between all successive pairs of effective cycles (8 vs. 6, 10 vs. 8, etc.). Through these comparisons, it is expected to observe the non-significant results as the number of cycles increases, indicating the stabilization of the measure.

The second analysis aimed to examine the reliability of network measures derived from each combination of band, number of effective cycles and threshold. Two-way random intercept models were fitted with network measure being the dependent variable, accounting for the random effects of both participants and the repeated measurements. We then compute the intra-class correlation from the fitted models. Specifically we computed the version ICC(2,1) [31], defined as in Equation 2:

$$\text{ICC}(2,1) = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2} \quad (2)$$

where σ_b^2 represents the between-subjects variance and σ_w^2 represents the within-subjects variance. The ICC values and their confidence intervals were estimated using parametric bootstrapping with 500 replicates using the *lme4* [32] package in *R*. The interpretations of our results were based on the lower bound of the confidence interval (ICC refers to the lower bound of the confidence interval hereafter) to facilitate a conservative reliability estimate [33]. We adopt the interpretation of the ICC following previous studies: poor ($\text{ICC} < 0.40$), fair ($0.40 < \text{ICC} \leq 0.59$), good ($0.60 < \text{ICC} \leq 0.74$) and excellent ($\text{ICC} \geq 0.75$) [23], [34].

III. RESULTS

A. Stability of derived network measures

Our first analysis investigated how the number of effective cycles and threshold influence the stability of network measures. Figure 1 provides a general overview of the variation of the network measures across the number of effective cycles at a specific band and threshold. Figure 2 presents the planned comparisons of estimated marginal means between consecutive effective cycles. The figure shows the t -ratio for each comparison, with non-significant results marked with a cross. Regarding clustering coefficient and global efficiency, most comparisons were statistically significant (see Figure 2), indicating limited stability across different number of effective cycles. However, it should be noted that the absolute value of the t -ratio decreased as the number of effective cycles increased. This suggests that while differences between consecutive cycles remained statistically significant, their magnitude diminished. Conversely, most comparisons for SWP were non-significant, implying relative stability in SWP values across various number of effective cycles. Importantly, these patterns of stability and instability held consistent across all five bands analyzed.

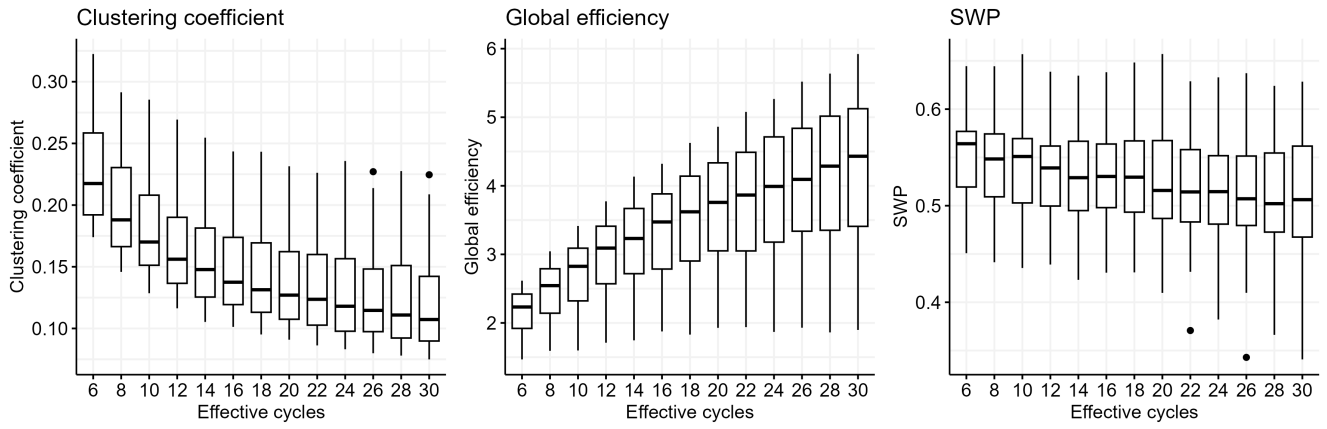


Fig. 1. Network measures derived from alpha band at every number of effective cycles, threshold at 50% strongest connections. The measures were averaged within subject. Left: clustering coefficient; middle: global efficiency; right: SWP.

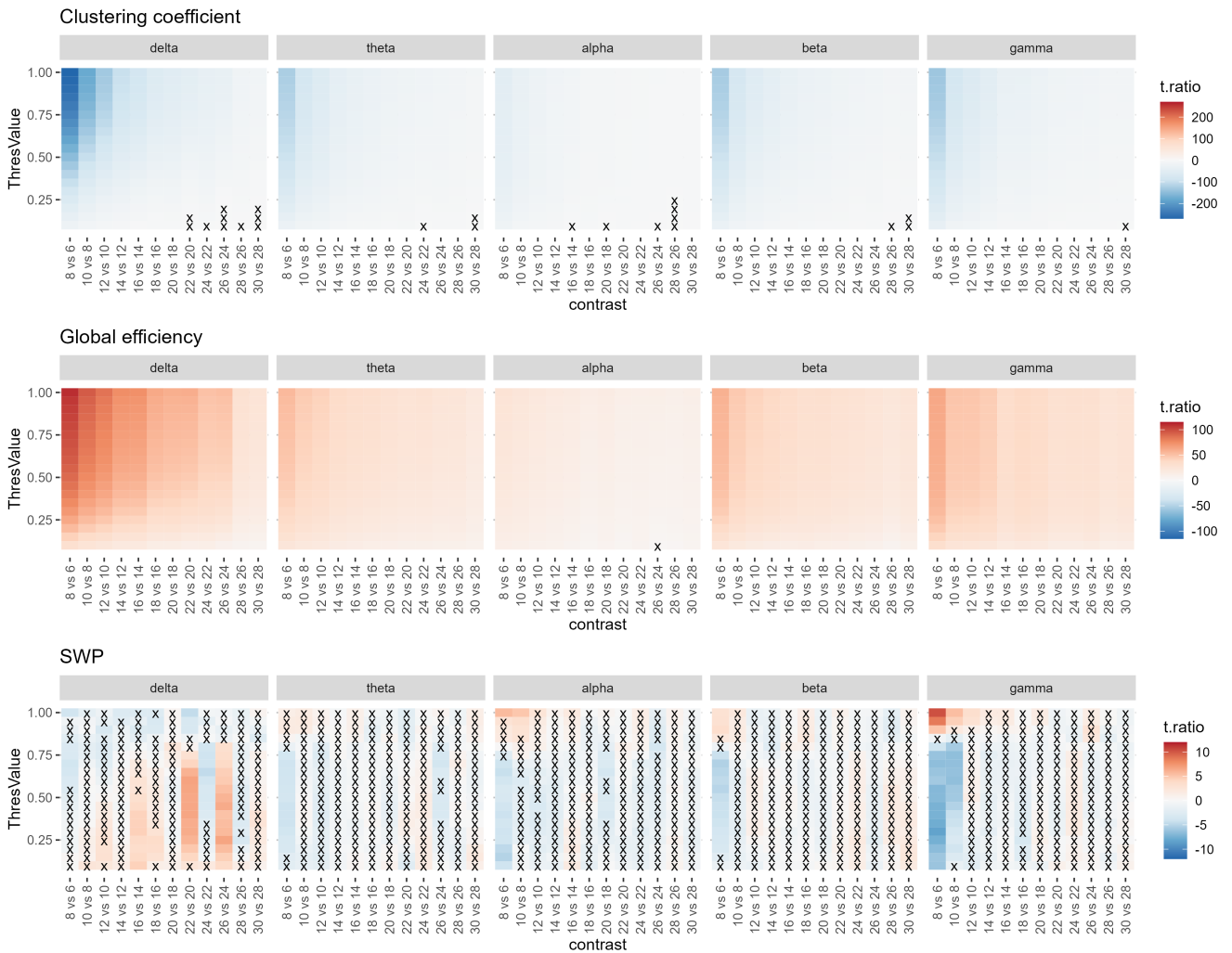


Fig. 2. The t -ratio of the pairwise comparisons between the measures derived from consecutive number of effective cycles. Non-significant results are marked with a cross. Top: clustering coefficient; mid: global efficiency; bottom: SWP.

B. Reliability of derived network measures

Figure 3 illustrates how the reliability of network measures increases with the number of effective cycles, but reveals

nuanced effects of thresholding across different measures. For clustering coefficient, increasing thresholds generally

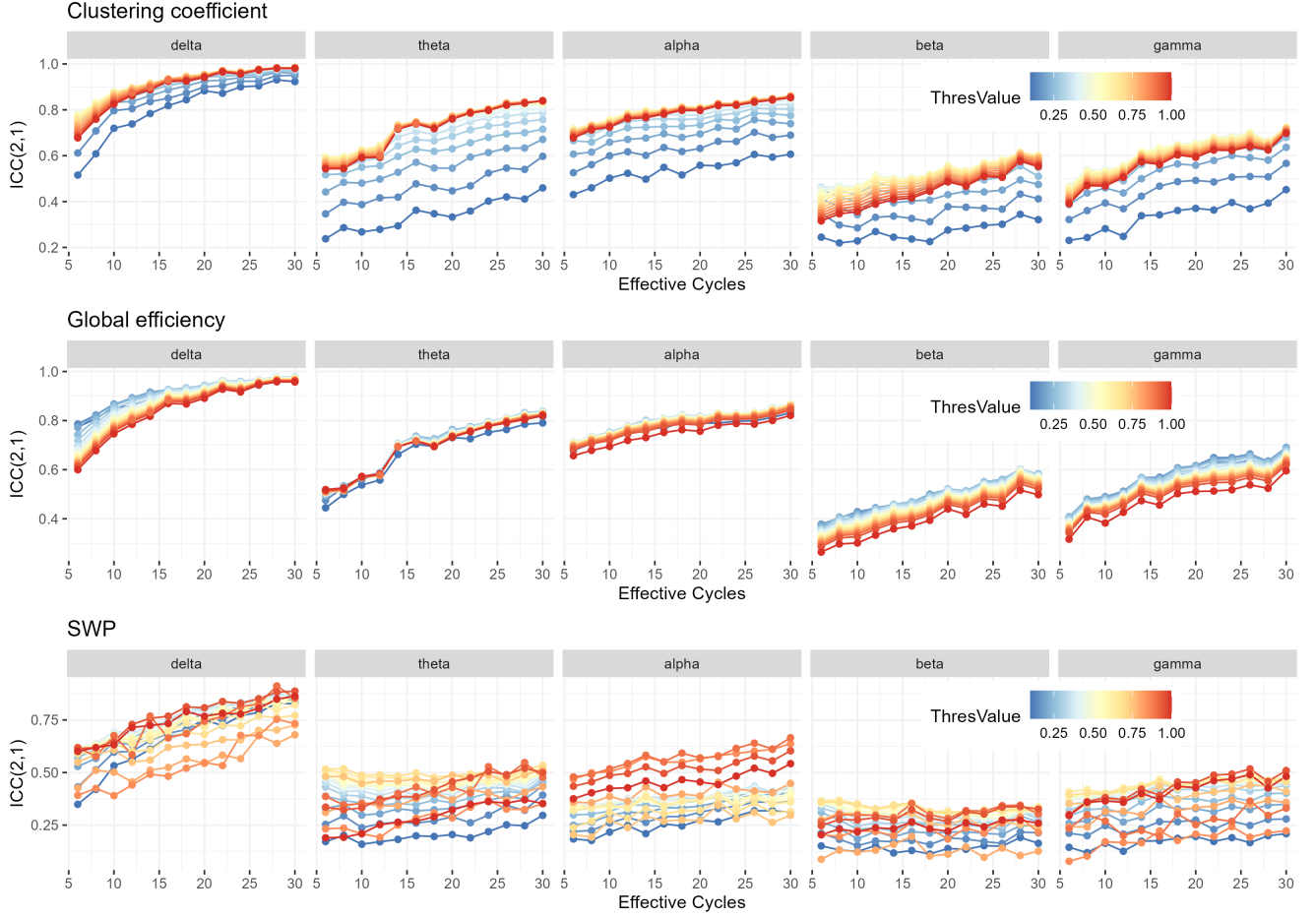


Fig. 3. The trend of intra-class correlation (ICC(2,1)) plotted against increasing effective cycles. ThresValue corresponds to the proportion of strongest connections preserved. Top: clustering coefficient; mid: global efficiency; bottom: SWP.

lead to higher reliability, reaching the highest reliability when threshold is around 0.5 before declining again. This pattern is observed across all five canonical bands. Conversely, global efficiency shows a decrease in reliability with increasing thresholds in most bands (except for theta in which the reliability is relatively stable). The effect of thresholding on SWP is the most complex. While reliability generally increases with higher thresholds, there are no consistent patterns. For instance, higher threshold seems to harm reliability in the theta band, but improves reliability in the alpha band. This inconsistent pattern highlights the need for careful consideration of threshold selection when analyzing SWP.

Our observations from Figure 3 are supported by Table I, which shows that highest ICC derived from different factor combinations. For clustering coefficient, it is evident that across all five bands, the highest ICC was achieved when the number of effective cycles is approximately 30 — the upper limit within this study’s scope. The corresponding thresholds were all greater than 0.5, but not necessarily at 1.0, indicating a potential U-shaped relationships between ICC and thresholds. The measures derived from delta, theta

and alpha band showed excellent reliability ($ICC \geq 0.75$). In contrast, those from beta and gamma band showed fair ($0.40 < ICC \leq 0.59$) and good ($0.60 < ICC \leq 0.74$) reliability respectively.

Similarly, higher number of effective cycles resulted in higher reliability for deriving global efficiency. The measures derived from delta, theta and alpha band showed excellent reliability while that from beta and gamma band showed fair and good reliability respectively. However, the corresponding thresholds were all below 0.5, in contrast to the results of clustering coefficient.

For SWP, the highest ICC was achieved with a higher number of effective cycles except for the beta band. However, the ICC from theta, alpha and gamma band showed that the reliability of SWP derived from them was just fair. Even worse, the beta band showed poor reliability ($ICC = 0.27$). The most reliable SWP was derived from the delta band, in which the lower bound of the ICC was 0.87, categorized as excellent reliability.

IV. DISCUSSIONS

In the present study, we systematically investigated the how frequency of interest, epoch length and the thresholding

TABLE I

THE HIGHEST ICC RESULTED FROM DIFFERENT NETWORK MEASURE,
BAND, EFFECTIVE CYCLES AND THRESHOLD.

Network measure	Band	Effective cycles	Threshold	ICC(2,1) [95% CI]
Clustering coefficient	delta	30	0.60	0.984 [0.977, 0.989]
	theta	30	1.00	0.840 [0.778, 0.879]
	alpha	30	0.70	0.861 [0.806, 0.894]
	beta	28	0.60	0.616 [0.513, 0.687]
	gamma	30	0.55	0.724 [0.638, 0.784]
Global efficiency	delta	30	0.30	0.979 [0.969, 0.984]
	theta	30	0.30	0.838 [0.776, 0.877]
	alpha	30	0.40	0.868 [0.815, 0.902]
	beta	28	0.30	0.603 [0.494, 0.683]
	gamma	30	0.20	0.691 [0.607, 0.759]
SWP	delta	28	0.90	0.912 [0.870, 0.936]
	theta	28	0.90	0.548 [0.444, 0.627]
	alpha	30	0.90	0.665 [0.569, 0.737]
	beta	8	0.65	0.370 [0.270, 0.450]
	gamma	26	0.95	0.517 [0.415, 0.604]

step affect the stability and the reliability of three representative network measures (clustering coefficient, global efficiency and small world propensity). Specifically, we adopted a different perspective from existing studies [22], [23] in selecting the epoch length, as we proposed to have band-specific epoch length by equating the number of effective oscillatory cycles. We believe that our approach would mitigate the bias introduced for selecting epoch length based on seconds, which favors higher-frequency oscillations.

The results from our stability analysis showed that for clustering coefficient and global efficiency, the differences between the measures in consecutive number of effective cycles decreased as the number of effective cycles increased. However, the comparisons were still significant within the scope of this study. The upper limit of the number of effective cycles in the present study was selected to align with previous studies in terms of recommended epoch length [11], [22] and to facilitate fair comparisons across all five bands. The epoch length of the delta band would be 30-second if the number of effective cycles is 30. Since our EEG recordings last for 240-second, we determined 30 effective cycles as our upper limit. In a future study, we will consider frequency of interest starting from theta band and increase the number of effective cycles. Based on the present findings, we expect to see the stabilization of measures as the number of effective cycles increases.

Although the stabilization of measures was not significant, the reliability of the network measures was encouraging. It should be noted that the stability and reliability are not necessarily related. A measure can be reliable as long as it reflects between-subject differences. From our results, the reliability of clustering coefficient and global efficiency measures derived from delta, theta and alpha band was excellent, although the optimal thresholds required were different. Reliable estimates of clustering coefficient can be achieved at a sweet spot when slightly more than 50% of the strongest connections were kept. A threshold that is too high or too

low would sabotage the reliability. For global efficiency, we only needed to keep 20% to 40% of strongest connections to have a reliable estimate or otherwise the reliability would decrease. These results indicate that clustering coefficient can only be estimated reliably when the network is properly thresholded. As such, global efficiency might be a noisier measures that can only be reliably estimated from a cleaner graph that provides higher signal-to-noise ratio. Reliability of measures from beta and gamma band was as good as those in the slower frequency bands. This prompts a question to be answered in the future study on whether we need more number of effective cycles to reliably estimate network measures from them.

For Small-World Propensity, the results were not satisfactory in general as the ICC values were just fair or even poor, except for the delta band. Still, we observed increased reliability as the number of effective cycles increased. That suggests that the number of cycles to reliably estimate small-worldness property might be a lot more compared to the other two measures. Also, the reliability increased as more connections were included in the network construction.

V. CONCLUSION

Our study systematically investigated how frequency of interest, epoch length, and thresholding steps affect the stability and reliability of three key network measures: clustering coefficient, global efficiency, and Small-World Propensity. We proposed the use of band-specific epoch length in terms of the number of effective cycles for fair comparisons between bands. Our findings suggest that clustering coefficient requires a specific threshold to ensure reliability, while global efficiency benefits from a cleaner graph with fewer connections. Small-world propensity showed less satisfactory reliability overall. These results suggest the need of examining reliability across different measures before applying them. Our findings also highlight the different reliability in different bands, suggesting that more cycles are needed while estimating network properties higher-frequency oscillations. We believe these results will guide researchers in making better empirical decisions regarding their choice of epoch length and thresholds in future EEG network studies.

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