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Applications Note

Gene expression

DiscoRhythm: an easy-to-use web application and R package for discovering rhythmicity

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Abstract

Motivation: Biological rhythmicity is fundamental to almost all organisms on Earth and plays a key role in health and disease. Identification of oscillating signals could lead to novel biological insights, yet its investigation is impeded by the extensive computational and statistical knowledge required to perform such analysis.

Results: To address this issue, we present *DiscoRhythm* (*Disco*vering *Rhythm*icity), a user-friendly application for characterizing rhythmicity in temporal biological data. *DiscoRhythm* is available as a web application or an R/Bioconductor package for estimating phase, amplitude and statistical significance using four popular approaches to rhythm detection (Cosinor, JTK Cycle, ARSER and Lomb-Scargle). We optimized these algorithms for speed, improving their execution times up to 30-fold to enable rapid analysis of -omic-scale datasets in real-time. Informative visualizations, interactive modules for quality control, dimensionality reduction, periodicity profiling and incorporation of experimental replicates make *DiscoRhythm* a thorough toolkit for analyzing rhythmicity.

Availability and implementation: The *DiscoRhythm* R package is available on Bioconductor (https://bioconductor.org/packages/DiscoRhythm), with source code available on GitHub (https://github.com/matthewcarlucci/DiscoRhythm) under a GPL-3 license. The web application is securely deployed over HTTPS (https://disco.camh.ca) and is freely available for use worldwide. Local instances of the *DiscoRhythm* web application can be created using the R package or by deploying the publicly available Docker container (https://hub.docker.com/r/mcarlucci/discorhythm).

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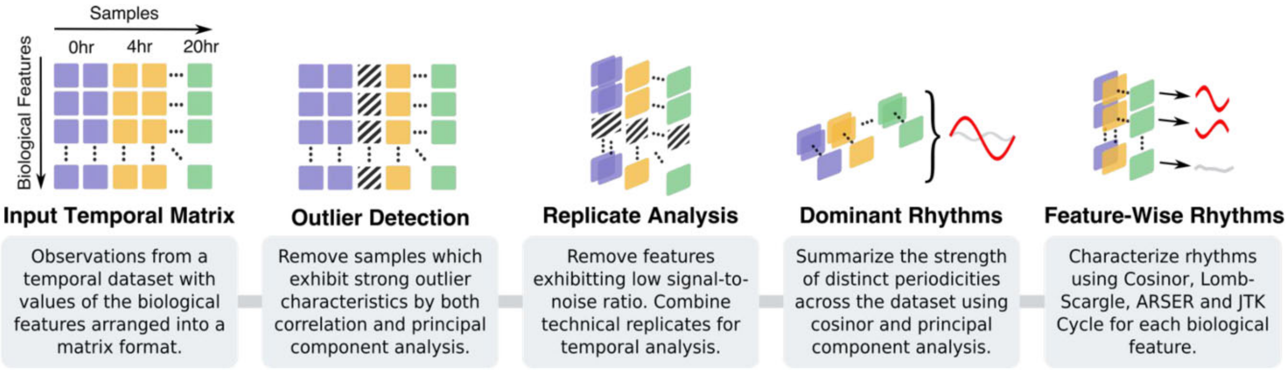
Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Biological rhythmicity, including circadian and other cycles (Schibler and Naef, 2005), are fundamentally important to life on Earth (Bell-Pedersen *et al.*, 2005). Numerous lines of evidence have indicated that disturbance of biological rhythms is a risk factor for human morbidities, including psychiatric, metabolic and malignant diseases (Roenneberg and Merrow, 2016). Several approaches can be used to estimate the phase, amplitude and statistical significance of these rhythms in time-series data, where each methodology has strengths and weaknesses under various conditions (Deckard *et al.*, 2013). Current implementations of these algorithms in R (e.g. JTK Cycle, ARSER and Lomb-Scargle) tend to be slow and difficult to use. Additionally, no unified toolkit exists for performing pre-processing, dimensionality reduction, period detection and visualization of oscillation statistics, all of which require specialized knowledge and expertise. To address these challenges, we developed *DiscoRhythm* (*Disco*vering *Rhythm*icity), a web application and accompanying R/Bioconductor package for analyzing rhythmicity in temporal biological datasets. *DiscoRhythm* provides a unified interface to execute four methods of rhythm estimation, and heuristically selects suitable approaches for the data being analyzed. By providing interactive modules for outlier detection, analysis of replicates and periodicity profiling, *DiscoRhythm* offers a framework for accessible analysis of periodic datasets in a web browser or in R.

2 Results

DiscoRhythm is implemented as a package in the R programming language (ver. 3.6+) with the web interface based on the R Shiny platform (Chang *et al.*, 2018), capable of reproducing findings in transcriptomic (Li *et al.*, 2013), epigenomic (Oh *et al.*, 2019), metabolomic (Krishnaiah *et al.*, 2017), proteomic (Hurley *et al.*, 2018)



Observations from a temporal dataset with values of the biological features arranged into a matrix format.

Remove samples which exhibit strong outlier characteristics by both correlation and principal component analysis.

Remove features exhibitting low signal-to-noise ratio. Combine technical replicates for temporal analysis.

Summarize the strength of distinct periodicities across the dataset using cosinor and principal component analysis.

Characterize rhythms using Cosinor, Lomb-Scargle, ARSER and JTK Cycle for each biological feature.

Fig. 1. Overview of the analysis procedures performed by *DiscoRhythm*. The illustration shows the step-wise operations being performed on the input circadian data matrix. All procedures are performed on a matrix with the biological features represented by rows and temporal samples by columns. Columns with the same color (e.g. purple and yellow) represent technical replicates, while stripped boxes indicate samples or features removed for downstream analysis. Red and gray oscillating lines show significant and non-significant rhythms, respectively. hr, hours

and other similar datasets. A workflow in *DiscoRhythm* begins with a matrix of temporal data (Fig. 1), where two metrics are computed to filter outlier samples, followed by a feature selection procedure based on the ratio of biological signal to technical noise. Dominant periods are determined using dataset-wide period evaluation procedures, and finally, multiple rhythm detection methods are executed on each feature to infer the presence of rhythms. Results of the web session may be emailed or downloaded upon completion as a zip file also containing the R data and code required for future reproducibility.

2.1 Input

Input for the web interface is a single table in a CSV (comma separated values) format. Columns contain samples named according to their time of collection, and rows contain values of observed features. Experimental design specifications regarding technical replicates, units of time and the main period of interest are also required. A circadian gene expression dataset simulated using simphony (Singer *et al.*, 2019) is provided in order to highlight the available features and demonstrate the sample naming scheme. In addition to the tabular input of the graphical interface, the *DiscoRhythm* R package also accepts SummarizedExperiment objects commonly returned by other R packages in Bioconductor (Gentleman *et al.*, 2004).

2.2 Outlier detection and feature selection

Sample quality is assessed using two commonly utilized metrics for outlier detection. The first metric is the average inter-sample correlation, computed as a mean pairwise correlation between a given sample and all other samples (Oldham *et al.*, 2008), while the second metric(s) is the sample score returned by principal component analysis (PCA). For both metrics, samples that deviate considerably from the rest (beyond a user-defined threshold) are flagged as outliers for removal from further analysis.

If present, technical replicates can be used to determine the signal-to-noise ratio for each feature (i.e. F statistic of biological versus technical variation). For further analysis, the user is able to only select the features exhibiting high signal-to-noise ratio, determined either by effect size or statistical significance. Technical replicates can then be combined by taking the mean, median or by choosing one replicate at random to prevent inflated sample size stemming from non-independent measurements.

2.3 Period detection

Two approaches are available for identifying the dominant period of rhythmicity. First, a goodness of fit can be evaluated for each period using a cosinor model across all selected features, returning the median coefficient of determination (R^2) of the fits. Alternatively, global rhythmic patterns may be investigated using PCA scores. If 'circular time' is used for sample collection [e.g. time of day, in hours, is recorded over multiple days as 2, 4, …, 24, 2, 4, …, where samples with the same collection times are assumed to be biological replicates (Hughes *et al.*, 2017)], *DiscoRhythm* will limit the candidate periods for rhythm detection to p/k where p is the length of the cycle and k is a positive integer (Cornelissen, 2014).

2.4 Estimating rhythm characteristics

Oscillations can be detected for each feature using a user-specified period. The period should be chosen by an *a priori* hypothesis or detected by the procedures in Section 2.3. An interface is provided to four commonly used approaches to oscillation detection [Cosinor (Cornelissen, 2014), ARSER (Yang and Su, 2010), JTK Cycle (Hughes *et al.*, 2010) and Lomb-Scargle (Glynn *et al.*, 2006)]. Each is heuristically made available if the input dataset satisfies algorithm-specific criteria, such as: the presence (or absence) of missing values, biological replicates, uneven sampling frequencies or non-integer intervals. To make *DiscoRhythm* suitable for -omic-scale and real-time analysis, high-performance implementations of each algorithm were developed, with runtime improvements of up to 30-fold [Supplementary Table S1 and Fig. S1; parallelized ARSER, JTK Cycle and Lomb-Scargle were contributed directly to MetaCycle version 1.2 (Wu *et al.*, 2016)]. Each method returns estimated phases, amplitudes, and P-values, both raw and adjusted for multiple testing (Benjamini and Hochberg, 1995). These feature-specific rhythm characteristics can be interactively visualized and downloaded for further exploration.

3 Discussion

Rhythmicity is a common topic of discussion for most biological researchers. Yet the quantitative analysis is difficult and, therefore, almost exclusively performed by researchers with specialization in statistics and computation. To democratize the field of chronobiology, we developed *DiscoRhythm*—a suite of standardized analytical procedures made approachable through interactivity, informative visualizations and key statistics for characterizing rhythmic patterns of temporal datasets. This new tool will enable even non-computational researchers to extract insights on the rhythmicity of biological data in a highly efficient manner. While our workflow is optimized for -omic-scale experiments, future versions of *DiscoRhythm* will also tailor to lower throughput datasets. Lastly, to maintain and extend accessibility to relevant periodic analysis approaches, we plan to adopt new methods as they become available in R/Bioconductor.

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