AXION BIOSYSTEMS

Neural Metric Tool

Metric Definitions

Version 4
July 2023

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2. Activity Metrics

Metric	Definition
Number of Spikes	Total number of spikes over the duration of the analysis.
Mean Firing Rate	Total number of spikes divided by the duration of the analysis, in Hz.
ISI Coefficient of Variation	The coefficient of variation (standard deviation/mean) of the inter-spike interval, the time between spikes, for electrodes with activity greater than the minimum spike rate ("active electrodes"). This metric is a measure of spike regularity and captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates bursting; as bursting becomes clearly distinguished from quiescence, the standard deviation of the ISI increases because spikes inside a burst have small ISIs, while the ISIs between the last spike in one burst and the first spike in the next are large.
Network ISI Coefficient of Variation	Coefficient of variation (standard deviation/mean) of the inter-spike interval for all spikes on all electrodes in a well. This is a measure of spike regularity across the network. This metric captures the distribution of spiking such that 0 indicates spikes perfectly distributed and > 1 indicates network bursting.
Fano Factor	Fano factor is a measure of spike count variability over time. Fano Factor is calculated as the variance of spike counts across 6 second bins divided by the mean of the spike counts in 6 second bins (*Becchetti et al 2012*). The Fano Factor is computed for each active electrode or unit independently, and then averaged across active electrodes or units to compute a well-wide Fano Factor. Active electrodes or units are defined as those with activity greater than the minimum spike rate. An evoked fano factor is also provided.
Number of Active Electrodes	Number of electrodes with activity greater than the minimum spike rate set in the Analysis Parameters.
Weighted Mean Firing Rate	The mean firing rate based on only electrodes with activity greater than minimum spike rate ("active electrodes").

3. Electrode Burst Metrics

The "Measurement" values describe single-electrode bursting on each electrode. Where applicable, a given metric is pooled across bursts according to the statistic after the dash. For example:

Burst Duration – Avg is the average burst duration across bursts on that electrode
Burst Duration – Std is the standard deviation of burst durations across bursts on that electrode
Burst Duration – Median is the median burst duration across all bursts on that electrode
Burst Duration – MAD is the median absolute deviation of burst durations across bursts on that electrode

The "Well Averages" are well-wide statistics where values are first computed as an average for each electrode and then pooled across electrodes according to the statistic after the dash. For example:

Burst Duration – Avg is the average across electrode average burst durations
Burst Duration – Std is the standard deviation across electrode average burst durations
Burst Duration (Median) – Avg is the average across electrode median burst durations
Burst Duration (Median) – Std is the standard deviation across electrode median burst durations

Metric	Definition
Number of Bursts	Total number of single-electrode bursts over the duration of the analysis. For a well, the total number of electrode bursts across all electrodes in a well is reported.
Number of Bursting Electrodes	Total number of electrodes within the well with bursts/minute greater than the burst electrode criterion.
Burst Duration	Average time from the first spike to last spike in a single-electrode burst. For an electrode, the average across bursts is reported. For a well, the average across electrode averages is reported. Longer bursts indicate more excitation, less inhibition, as it takes longer to shut down a burst.
Number of Spikes per Burst	Average number of spikes in a single-electrode burst. For an electrode, the average across bursts is reported. For a well, the average across electrode averages is reported.
Mean ISI within Burst	Mean inter-spike interval, time between spikes, for spikes in a single-electrode burst. For an electrode, the average across burst means is reported. For a well, the average across electrode averages is reported. This is a measure of burst intensity; smaller values mean more intense bursts.
Median ISI within Burst	Median inter-spike interval, time between spikes, for spikes in a single-electrode burst. For an electrode, the average across burst median ISIs is reported. For a well, the average across electrode averages is reported.
Median/Mean ISI within Burst	The median/mean inter-spike interval (ISI) within single-electrode electrode bursts. Values close to 1 indicate the distribution of ISIs within bursts is symmetric. For an electrode, the average across burst median/mean ISIs is reported. For a well, the average across electrode average median/mean ISIs is reported.
Inter-Burst Interval	Average time between the start of single-electrode bursts. For an electrode, the average across bursts is reported. For a well, the average across electrode averages is reported.
Burst Frequency	Total number of single-electrode bursts divided by the duration of the analysis, in Hz. For a well, the average across electrode burst frequencies is reported.
Normalized Duration IQR	Interquartile range of single-electrode burst durations. This metric provides a measure of single-electrode burst duration regularity. If the middle 50% of single-electrode bursts are close to the same duration, this value will be small, whereas, if the single-electrode bursts vary widely in duration, this range will be large. For a well, the average across electrode normalized duration IQRs is reported.
IBI Coefficient of Variation	The coefficient of variation (standard deviation/mean) of the inter-burst interval, the time between single-electrode bursts. This is a measure of single-electrode burst regularity. For a well, the average across electrode IBI CoVs is reported.

Burst Percentage	The number of spikes in single-electrode bursts divided by the total number of spikes, multiplied by 100. For a well, the average across electrode burst percentages is reported.

4. Network Burst Metrics

Network bursts are detected as near synchronous spiking across electrodes in a well. All metrics apply to bursts across the well (aka the network). For "Well Averages," averages, standard deviations, medians, and median absolute deviations (MADs) values for the metrics are computed across network bursts.

Metric	Definition
Network Bursts Ignored Flag	A flag to indicate whether network bursts were ignored in this well. Network bursts ignored = 1, network bursts included = 0.
Number of Network Bursts	Total number of network bursts over the duration of the analysis.
Network Burst Frequency	Total number of network bursts divided by the duration of the analysis, in Hz.
Network Burst Duration	Average time from the first spike to last spike in a network burst. Longer bursts indicate more excitation, less inhibition, as it takes longer to shut down a burst.
Number of Spikes per Network Burst	Average number of spikes in a network burst.
Mean ISI within Network Burst	Average of the mean ISIs within network bursts.
Median ISI within Network Burst	Average of the median ISIs within network bursts.
Median/Mean ISI within Network Burst	Average of the median/mean ISI within network bursts. Values close to 1 indicate the distribution of ISIs within bursts is symmetric.
ISI CoV within Network Burst	Average across network bursts of the ISI CoV (standard deviation/mean of the inter-spike interval) within network bursts.
Number of Electrodes Participating in Burst	Average number of electrodes with activity during a network burst.
Number of Spikes per Network Burst per Channel	Average across network bursts of the number of spikes in the network burst divided by the number of electrodes participating in that burst.
Network Burst Percentage	The number of spikes in network bursts divided by the total number of spikes, multiplied by 100.
Network IBI Coefficient of Variation	The coefficient of variation (standard deviation/mean) for the inter-network burst interval, the time between network bursts. This is a measure of network burst rhythmicity; bursts occurring at regular intervals have a small coefficient of variation, whereas sporadic bursting has a larger coefficient of variation
Network Normalized Duration IQR	Interquartile range of network burst durations. This metric provides a measure of network burst duration regularity. If the middle 50% of network bursts are close to the same duration, this value will be small; if the network bursts vary widely in duration, this range will be large.

5. Average Network Burst Metrics

Metric	Definition

Start Electrode	The electrode that most commonly contributes the first spike to the network burst(s).
Percent Bursts with Start Electrode	The percent of network bursts that start with a spike from the Start Electrode.
Burst Peak (Max Spikes per sec)	The maximum number of spikes per second in the average network burst. This value is equal to the peak of the Average Network Burst Histogram divided by the histogram bin size to yield spikes per sec (Hz).
Time to Burst Peak (ms)	The time from the start to the peak of the average network burst. This value is equal to the time to the peak of the Average Network Burst Histogram.
Avg Network Burst CoV^2	The square of the coefficient of variation (standard deviation/mean) of the inter-spike interval within the average network burst time window. This metric is a measure of spike timing regularity during network bursts.
Avg Network Burst Fano Factor	Fano Factor is calculated as the variance of spike count across network bursts divided by the mean of the spike count across network bursts (*Becchetti et al 2012*) and is calculated for each active electrode (or active unit). Spikes are counted for the average network burst time window entered in the Average Network Burst Parameters. Fano Factor is a measure of spike count variability across network bursts.

6. Synchrony Metrics

The Neural Metric Tool computes synchrony by first finding the cross-correlation between spiking on pairs of electrodes and then pooling across all pairwise combinations in a well (*Halliday et al 2006*). Cross-correlation assesses the probability of a spike on one electrode relative to a spike on the reference electrode. Phase lags of zero (x-axis on correlogram) indicate synchronous spiking between electrodes. When more electrodes have a similar phase relationship, the probability (y-axis height on the correlogram) of that phase-lag (zero for synchrony) increases. The normalized cross-correlation normalizes the cross-correlation to the auto-correlation, removing the effects of spiking regularity that can increase the probability of identifying spikes on one electrode near spikes on other electrodes due simply to high MFR and/or high spiking regularity. Tall, sharp cross-correlograms indicate high synchrony. Short, wide cross-correlograms indicate low synchrony. DC offset typically represents high MFR which is removed by normalization. Downward dip in the cross-correlogram indicates quiescent period or burst refractory period.

$$\frac{Cxy}{\sqrt{Cxx * Cyy}} \sim \frac{synchrony}{regularity}$$

Metric	Definition
Area Under Normalized Cross-Correlation	Area under the well-wide pooled inter-electrode cross-correlation normalized to the auto-correlations. Higher areas indicate greater synchrony.
Area Under Cross-Correlation	Area under the well-wide pooled inter-electrode cross-correlation.
Full Width at Half Height of Normalized Cross-Correlation	Distance along the x-axis (phase lag) from left half height to right half height (probability) of the normalized cross-correlogram. This is a measure of network

	synchrony; higher half widths indicate a wider correlogram (less synchrony) whereas lower half widths indicate a taller correlogram (greater synchrony).
Full Width at Half Height of Cross-Correlation	Distance along the x-axis (phase lag) from left half height to right half height (probability) of the cross-correlogram. Higher full widths indicate a wider correlogram (less synchrony) whereas lower full widths indicate a taller correlogram (greater synchrony).
Synchrony Index	A unitless measure of synchrony between 0 and 1 (*Paiva et al 2010*). Values closer to 1 indicate higher synchrony.
Kreuz SPIKE Distance	1-Kreuz SPIKE distance (*Kreuz et al 2013*) such that 1 is perfect synchrony and 0 is perfect asynchrony. It is computed with only one previous and one subsequent spike for each reference spike. The time window for each computation varies with local firing rate of each spike train. It tracks changes in instantaneous clustering without being skewed by individual electrode inter-spike interval.

7. LFP Metrics

Metric	Definition
Number of LFPs	Total number of LFPs over the duration of the analysis.
Number of LFPs Per Electrode	Total number of LFPs detected on a single electrode over the duration of the analysis. For a well, the average number of detected LFPs across all electrodes in a well is reported.
LFP Rate (Hz)	Total number of LFPs divided by the duration of the analysis, in Hz.
LFP Amplitude (μV)	Peak-to-peak amplitude of LFPs averaged across electrodes. Electrodes with no detected LFPs report an amplitude of 0.
LFP Rectified Area (μVs)	Area under the curve of rectified LFP waveforms averaged across electrodes. Electrodes with no detected LFPs do not contribute.
LFP Low (0-1 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 0 – 1 Hz.
LFP Delta (1-4 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 1– 4 Hz.
LFP Theta (4-8 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 4 – 8 Hz.
LFP Alpha (8-14 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 8 – 14 Hz.
LFP Beta (14-30 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density

	curve for each well. Power is computed through integration of the per-well power spectral density curve from 14 – 30 Hz.
LFP Gamma (30-50 Hz) Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 30 – 50 Hz.
LFP Total Power (μV^2)	Power spectral density is averaged across detected LFPs for each electrode and then averaged across electrodes for a representative power spectral density curve for each well. Power is computed through integration of the per-well power spectral density curve from 0 – 50 Hz.
LFP Low (0-1 Hz) Relative Power	Ratio of Low Power to Total Power
LFP Delta (1-4 Hz) Relative Power	Ratio of Delta Power to Total Power
LFP Theta (4-8 Hz) Relative Power	Ratio of Theta Power to Total Power
LFP Alpha (8-14 Hz) Relative Power	Ratio of Alpha Power to Total Power
LFP Beta (14-30 Hz) Relative Power	Ratio of Beta Power to Total Power
LFP Gamma (30-50 Hz) Relative Power	Ratio of Gamma Power to Total Power

8. Evoked Metrics

Metric	Definition
Number of Trials	Number of stimulation events used for analysis.
Evoked Spike Count	Average across trials of the number of spikes detected across active electrodes in the well during the time window specified by the Stimulation Parameters section. This is a measure of the magnitude of stimulus response.
Evoked Response Probability	Probability of finding at least one spike in the well during the time window specified by the Stimulation Parameters section. Computed as the number of stimulation events that evoked a response divided by the total number of stimulation events. A measure of the response to the stimulus.
Evoked First Spike Latency	The average across trials of the time between the stimulation event and the first post-stimulus spike detected in the well.
Evoked Jitter	The standard deviation across trials of the time between the stimulation event and the first post-stimulus spike detected in the well. This is a measure of response consistency; lower values indicate more consistent responses.
Evoked CoV^2	The square of the coefficient of variation (standard deviation/mean) of the inter-spike interval during the evoked response window, averaged across trials. This metric is a measure of spike timing regularity during the evoked response.
Evoked Fano Factor	Fano Factor is measure of spike count variability across trials, or, in other words, the variability of the evoked response magnitude. Fano Factor is calculated as the variance of spike count across trials divided by the mean of the spike count across trials (*Becchetti et al 2012*) and is calculated for each active electrode (or active unit). Spikes are counted for the evoked response time window entered in

	the Stimulation Parameters. For the well-wide evoked Fano Factor, the Fano Factor is computed based on the pooled spikes from all active electrodes (or active units) in the well during the evoked response time window.

9. Viability Metrics

Metric	Definition
Resistance (kOhms)	Resistance is a measure of viable cell coverage over the electrode. Higher values indicate more intact cells are attached to the electrode. For a well, the average across electrodes is reported. (See the MEA Viability chapter of the AxIS Navigator user guide for more details.)
Number of Covered Electrodes	Total number of covered electrodes within the well. Covered electrodes are defined as electrodes with resistance greater than the Covered Electrode Threshold (default 18 kOhms). Uncovered CytoView MEA microelectrodes in media exhibit a resistance of 8-12 kOhms. Following neuronal cell death, debris may leave a slightly higher uncovered resistance of 15-18 kOhms.
Weighted Mean Resistance (kOhms)	The mean resistance across covered electrodes only (resistance greater than the Covered Electrode Threshold).