**A study of non-linear feature extraction in spike sorting**

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# Introduction

## Spike Sorting [TBRewritten]

Extracellular recordings capture the neural activity as voltage fluctuations from multiple nearby neurons (1), producing a continuous signal. Each individual activity of a single neuron is called a spike and in the case of extracellular recordings the neuron that generated such an activity is unknown. Spike sorting is the process of assigning each detected spike waveform (2) from an extracellular recording to its source neuron, based on the assumption that each neuron produces spikes of consistent shape (3), while different neurons generate distinguishable shapes from each other (2). However, the shape of spikes can be distorted by noise, electrode drift (4) and biological variability which result in scattered clusters instead of pinpoints. Consequently, it is important to find a robust approach to reduce the impact of these phenomena. One approach is to extract a small set of discriminative features that preserve the information that differentiates between the shapes of spikes to improve clustering.

The cannonical approach to spike sorting (2) is a pipeline of four sequantials steps: filtering, spike detection and clustering. Filtering is done in a band-pass manner and is applied to the raw recorded signal to isolate the 300 and 3000Hz (5) frequency band where spiking occurs. Spikes are detected typically through a simple amplitude thresholding based on the standard deviation of the signal multiplied by a scalar value (typically, from 3 to 5) to identify the events that could represent the neuronal activity. The choice of this threshold is a compromise between identifying noise segments as spikes and losing spikes of lower amplitude. Feature extraction is utilised to both generate a more auspicious space and to improve efficiency for clustering. The newly generated feature space should be robust as in invariant to small changes in shape such that it provides an easily separable space for clustering while simultaneously it should reduce the dimensionality to improve the execution time of clustering. As the final step of the spike sorting pipeline, the spikes should be grouped to represent all instances of activity of each individual neuron. This final assignment of spikes by source neurons is done by the clustering algorithm.

The spike sorting pipeline has seen many forms over the years. Initially, a supervised manual approach was taken where an expert would classify spikes based on a visual interpretation of similarity in a reduce space. The reduced space was generated by simple features such as amplitude, width, or even principal components. Sinces the 1950s, the number of recorded neurons has increased exponentially (6) and recent developments in recording hardware (4,7) are following this trend, rendering manual approaches unfeasible. The choice of approach is also depedent upon the nature of the analysis, offline or online. Offline spike sorting allows for the use of more complex algorithms as there is no time constraint as the analysis is done after the recording has finished. However, in an online setting, the algorithms employed must have to ability to process the data during the recording, thus requiring low execution times. A common approach taken lately is template matching (8) on subsets of data, usually this approach substitutes the steps of spike detetion, feature extraction and even clustering in the canonical spike sorting pipeline. Due to it being applied on subsets rather than the whole data, it is an efficient approach from a computational perspective.

In this work, we attempt to examine the impact of feature extraction in spike sorting. Although, it is the clustering that outputs the final result and separation of the space into clusters, it is actually the feature extraction which must obtain a separable space for the clustering. Similarly to clustering algorithms, a golden standard (9,10) does not exist for feature extraction algorithms (2,5) either. Their performance depends on the particular set of characteristics of the input data. Here, we employ a number of non-linear feature extraction algorithms in the pursuit to identify the most adequate algorithm for the spike sorting problem.

## Non-linear feature extraction

The classical techniques for dimensinality reduction, such as PCA and MDS, are computationally eficient and perfectly able to find the structure of linear spaces (11). However, they encounter difficulties when non-linear structures are present (12). Non-linear manifold learning algorithms seek to discover a low-dimensional embedding (or a manifold) within the high-dimensional input data. These methods are capable of preserving the intristic geometry (including local neighbourshood and data topology) through their approximation of the underlying manifold, rather than relying on global linear projections such as PCA (13) (14).

In spike sorting, each detected spike waveform can be viewed as a high-dimensional vector. We can consider that the shapes of spikes vary from their ‘true shape’ due to recording artifacts. Therefore, non-linear manifold feature extraction techniques may be able to disentangle these factors by yielding embeddings that are robust to perturbations (15) and offer separability in overlapping clusters (generated by linear techniques). Moreover, modern manifold techniques have been designed to handle large volumes of data by employing sparse neighbourhoods graps and optimisation for scalability (16,17). This makes them a viable candidate for the spike sorting of high-density probes.

## The challenges of spike sorting [TBRewritten]

Spike sorting is inherently complex for several reasons. Brain recordings are inherently subject to the distortion of the spike waveforms due to the reasons specified above, these phenomena affecting spike shape generate clusters that do not have a well-defined separation boundary. This overlap of clusters is a struggle for most clustering algorithms, especially if coupled with data imbalance. Cluster imbalance in neuronal data appears from the variability in firing rate of neurons. Neuronal activity is dynamically modulated by neural circuits, causing individual cells to fire at widely varying rates (18) (19); this variability generates clusters of disparate sizes and yields an intrinsic imbalance in the dataset. Moreover, neuronal activity takes place on a millisecond timescale, thus even relatively short brain recording sessions can produce a vast quantity of data (3). In this context, single-unit activity refers to the spikes of one neuron that can be isolated as a single cluster, whereas spikes from more distant neurons typically appear with lower amplitudes (poor signal-to-noise ratio), and cannot be reliably separated (resulting in a single cluster being identified)—these are generally referred to as multiunit activity (5).

These non-linear manifold feature extraction methods often outperform linear feature spaces (20) and may be able to simultaneously denoise waveforms which can create dense clusters and increase the variability between the spikes of different neurons which can create separable clusters. In this study, we therefore evaluate a suite of representative non-linear feature extractors (e.g., Isomap, LLE, Spectral Embedding, Diffusion Maps, UMAP, TriMap) in comparison with traditional feature extraction methods and other non-linear feature extracton methods, to systematically compare how each manifold embedding influences cluster separability and spike-sorting performance across datasets.

The paper is structured as follows. Section 2 reviews traditional feature‐extraction techniques and their performance in spike sorting, outlines the methods proposed for spike sorting, and describes the datasets and evaluation metrics. In Section 3, we assess the methods across multiple metrics and offer a critical interpretation of their performance. Finally, Section 4 examines the limitations of the methods proposed for spike sorting and presents our concluding findings.

# Materials and Methods

## Feature Extraction [TBRewritten]

As stated above, a crucial step in spike sorting is the description of spikes with a compact set of informative features. The aim of dimensionality reduction is to transform a dataset with a dimensionality of X into a dataset with Y dimensions, where Y<<X. Another important aim is to retain as much of the data geometry as possible, such that relations in the original space are retained in the reduced space, which is especially useful for spike sorting. Dimensionality reduction techniques can be divided by several criteria, such as: convexity or linearity (21). From the point of view of convexity, PCA is a convex algorithm, while Isomap is a non-convex approach. Among the first features used in the spike sorting were the spike amplitude and its width (22). Afterward, methods based on probabilistic models, created through empirical analysis, that used the entire waveform were developed (23). These could process a low number of electrodes. Shortly thereafter, transforms started being used to project the high-dimensional space of the waveform into a low-dimensional space through the use of principal components (24), the wavelet transform (25) and various combinations of them. Manual sorting of spikes is usually performed on a low dimensional space, containing features such as the amplitude, the peak-to-trough ratio, etc (26). The peak-to-trough ratio was found to be representative of the neuron type, inhibitory neurons produce narrow spikes and thus have a small peak-to-trough ratio, while excitatory have a large ratio (27).

In (28), the authors propose M-Sorter, an automatic method for spike detection and classification based on coefficients obtained through the wavelet transform and template matching. The proposed method separates spike sorting into two steps, the spike detection by multiple correlation of wavelet coefficients on the band pass filtered waveforms of the recorded signal and template matching for the classification of spikes to the neurons that generated it. The multiple correlation of wavelet coefficients is also used in the generation of templates through the application of K-Means. Each spike is assigned to the cluster to which it has the smallest distance.

### Linear feature extraction methods

Principal Component Analysis (PCA) (13) is the most frequently used algorithm for feature extraction, including spike sorting (14). PCA projects the spikes onto new characteristics called Principal Components that are a new set of orthogonal axes formed by linear combinations of the input features. The reduction of dimensionality of the feature space is performed by solving a problem of eigenvalues and eigenvectors. By retaining the most prominent principal components, PCA preserves the variance as much as possible while being able to reduce the number of features. It is common to keep only the first two or three principal components resulted from PCA (29) (30). These frequently retain more than 70% of the variance from the original space. However, variance does not necessarily offer the best separation (2) (5). To put it in another way, information required for separability may be encoded in those low-variance features that are discarded. Finally, PCA and its variations have been used in spike sorting for a long time (5) and it is still used in recently developed spike sorting pipelines (31).

Another linear method is Independent Component Analysis (ICA) (32) mainly designed for source separation. ICA is a linear unsupervised technique for dimensionality reduction that searches for independent components by relying on the statistical properties of the data. ICA has been previously applied to spike sorting with promising results (33) (34).

Linear Discriminant Analysis (LDA) (35) is a supervised linear learning technique with the goal of increasing the inter-cluster distance and decreasing intra-cluster distance. LDA assumes that the data has a Gaussian distribution. However, for our problem LDA is not a fit candidate due to several considerations. First, it is a supervised learning technique which cannot be applied to unlabelled data, as is the case in spike sorting. Second, the Gaussian distribution assumption is often violated in spike sorting due to: electrode drift, shape variation from bursts, simultaneous firing, multi-unit activity, and non-stationary background noise (2).

### Non-linear feature extraction methods

Kernel PCA (36)

In the category of unsupervised non-linear dimensionally reduction techniques

Isomap (12) uses Isometric Mapping to learn the low-dimensional projection in a manifold space while retaining the distances of the original space. It uses the geodesic distance, which can be thought of as the shortest path along the curved surface of the manifold space.

Isomap (12)

The overall complexity of Isomap is O[Dlog(k)Nlog(N)]+O[N2(k+log(N))]+O[dN2].

* N : number of training data points
* D : input dimension
* k : number of nearest neighbors
* d : output dimension

T-distributed Stochastic Neighbor Embedding (t-SNE) (37) is a non-linear dimensionality reduction method that minimizes the divergence between input features and the reduced feature space by using pairwise probability similarities. The divergence of two distributions is calculated using KL divergence, which is minimized by applying gradient descent. Due to its high time complexity, several orders of magnitude higher than PCA, and its main function being visualization, t-SNE was not considered a suitable candidate. A computation of a few seconds for PCA can become tens of minutes for t-SNE. Furthermore, from empirical observations, the separation offered by t-SNE for the datasets used here was small to non-existent.

Locally Linear Embedding (38) and Modified Locally Linear Embedding (39) and Hessian-based LLE/HLLE (40) and LTSA/Local Tangent Space Alignment (41)

The overall complexity of standard LLE/MLLE is O[Dlog(k)Nlog(N)]+O[DNk3]+O[dN2]. For HLLE: + O[Nd6] / For LTSA: + O[k2d]

* N : number of training data points
* D : input dimension
* k : number of nearest neighbors
* d : output dimension

Spectral embedding (15)

The overall complexity of spectral embedding is O[Dlog(k)Nlog(N)]+O[DNk3]+O[dN2].

* N : number of training data points
* D : input dimension
* k : number of nearest neighbors
* d : output dimension

MDS (42) linear transformation in its traditional form / it is non-linear for non-metric version. It is **not** a manifold.

UMAP (17)

Diffusion Map (43)

Self-Organizing Map (44)

PHATE (45)

TriMap (16)

KepplerMapper (46)

## State of the art Clustering algorithms [TBRewritten]

### Traditional clustering algorithms

## Performance metrics

Six metrics were used to evaluate the outcomes, the first three are external measures while the latter three serve as internal measures (48). They are: Adjusted Rand Index (ARI), Adjusted Mutual Information (AMI), V-Measure (VM), Calinski-Harabasz Score (CHS), Davies-Bouldin Score (DBS), and Silhouette Score (SS). Since spike sorting ends in clustering, these clustering metrics are suitable for evaluating the quality of feature extraction. External metrics measure how accurately the clustering algorithm recovers known class labels; they reflect how distinctly features separate the data—if features are perfectly separated then typically high external scores are achieved by the clustering algorithms. Internal metrics measure cluster compactness, separation, and shape as independent of ground truth; therefore they are appropriate for judging feature extraction when synthetic datasets provide true labels. Internal metrics describe cluster structure; external metrics measure agreement with true labels.

Using multiple measures instead of one index helps us take different aspects of clustering performance into account. Table 1 gives the intuitive meaning for each metric and its scoring range. A technique that does well across these varied measures suggests a more sound and even approach to feature extraction, lessening the chance of bias in the assessment.

**Table 1** – A short description of each performance evaluation metric, specifying its type and range.

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Type | Description | Range [worst, best] |
| ARI | External | Chance‐corrected score based on pairwise comparisons of objects, rewarding when pairs are either consistently grouped together or separated in both predicted and ground‐truth clusters | [-1, 1] |
| AMI | External | Chance‐corrected score based on entropy‐based mutual information between the predicted and actual labels, with an adjustment for the expected value under random assignments | [0, 1] |
| Purity | External | Fraction of correctly assigned points across all clusters by assigning each cluster to the majority true class within it. | [0, 1] |
| DBS | Internal | Average similarity ratio of each cluster with its most similar cluster, where similarity is defined as the sum of within‐cluster scatter relative to between‐cluster separation. | (Inf, 0] |
| CHS | Internal | Ratio of between‐cluster dispersion to within‐cluster dispersion, normalized by the number of clusters and total points. | [0, Inf) |
| SS | Internal | Average across all data points for the normalized difference between its mean intra‐cluster distance and lowest mean inter‐cluster distance | [-1, 1] |

External evaluation metrics require both the predicted cluster labels and the corresponding ground truth labels. As such, we employed K-Means (49) clustering to obtain the labels necessary for external metrics, applying it immediately after feature extraction. K-Means has long been used in clustering, with many adaptations developed over time. It was first utilized for spike sorting in 1988 (50) (51) and has remained the standard method for many years. Even recent spike sorting pipelines (8) (52) either rely on or are inspired by K-Means, and in a recent comparative study of 25 clustering methods, it demonstrated its continued competitive performance by ranking third (50).

K-Means (49)is a centroid-based clustering technique that achieves clustering by dividing the data space into *k* groups and allocating each point to the closest centroid according to Euclidean distance. But it has a number of drawbacks. First of all, it necessitates pre-specifying the number of clusters, which can be difficult for the real-world data. Nevertheless, there are preprocessing methods for finding the *k* parameter. Second, the method is non-deterministic in its most basic version, which means that different outcomes may be obtained from repeated executions. This problem has been resolved by more recent improvements that have increased its consistency. Thirdly, overlapping clusters are hard for K-Means to handle. However, this disadvantage is benefic for our analysis: the more performant feature extraction methods will improve cluster separation which will be shown by a higher K-Means performance.

[TBRewritten]

ARI (53) (54) (55) (3) is an adjustment of the Rand Index (RI) metric in order to handle chances. ARI is an external clustering metric; therefore, it requires a ground truth for the dataset. RI (56) (2) makes comparisons between pairs of points to determine if it is an agreement, when the two points are in the same cluster for both the predicted and the true labels, or a disagreement, when they belong to different clusters. The formulas used to calculate the metric are the following:

where *MaxRI* is the upper bound and *ExpectedRI* is theexpected placement of pairs in the same class using the permutation model and calculated based on the contingency table (53).

AMI (54) (57) (5) is an adjustment of the Mutual Information (MI) metric through the use of entropy, denoted as H. Moreover, AMI also contains the normalization (54) (58) (59) of Normalized Mutual Information. MI (4) is calculated between two clusters U and V, where N is the size of the dataset and |X| is the number of points in subset X.

V-Measure (60) (6) is the harmonic mean of Homogeneity and Completeness. A cluster is considered to be homogeneous (7) when all the points of that cluster are part of the same class. By switching the predicted and true labels, completeness is obtained. Completeness (8) is achieved when all the points of a class are part of the same cluster. We have chosen beta equal to 1 as given by the original formula (60).

where H(C|K) is the conditional entropy of the true cluster given the predicted cluster, H(C) is the entropy of the true cluster, while H(K|C) is the conditional entropy of the predicted cluster given the true cluster and H(K) is the entropy of the predicted cluster.

All the metrics presented until this point are external metrics and require a ground truth to compare with the predicted labels. Furthermore, all these metrics have bounded scores in the [0, 1] interval with higher values being more desirable.

The following three metrics are internal and therefore do not require a ground truth to be used. The internal metrics were used with the ground truth labels for the evaluation of the synthetic datasets. These metrics evaluate the intra-cluster and inter-cluster distances and the morphology of the clusters producing an adequate evaluation of the feature extraction capabilities.

DBS (61) (62) (63) (10) finds the mean similarity between clusters, where similarity, denoted as *R* (9), is defined by the distance between clusters and their sizes. The minimum value of this index is 0. The closer the result is to 0, the better separation exists between clusters. This may come as counterintuitive as it is the only metric where lower values represent a higher performance. The DBS metric is given by the following equations:

where *si* is the mean of all distances between the points of cluster *i* and its centroid, *di,j* is the distance between clusters *i* and *j* given by their centroids, and *max(Ri,j)* is the maximum similarity of clusters *i* and *j*.

CHS (48) (60) (11), also known as Variance Ratio Criterion, calculates the ratio between the intra-cluster and inter-cluster dispersion. Where *tr(X)* denotes the trace of between cluster *Bk* or within-cluster *Wk* dispersion matrix, *n* denotes the size of the dataset and *k* the number of clusters. The dispersion is defined as the sum of squared distances. For this metric, a higher value indicates a better result.

SS (60) (64) (12) is calculated by measuring the mean distance between a point and the rest of the points of that cluster and the mean distance between the point and all the points of the nearest cluster. The score is bound between [-1, 1] where -1 represents an incorrect clustering, 0 overlapping clusters, and 1 a dense clustering. SS aims for the standard concept of a cluster, dense and well separated, therefore such cases will give a higher score. The equation of SS is the following:

where *b* denotes the average of all distances between a point in cluster *i* and all points of the nearest cluster *j*, and *a* the average of all distances between a point in cluster *i* and all other points in the same cluster.

It is important to mention that although used in evaluation of spike sorting techniques (65) (66), accuracy is not a suitable performance metric. First, because spike sorting is unsupervised and accuracy requires labels. Second, neuronal data is imbalanced because of the various firing rates of individual neurons, and it is has been extensively shown that accuracy is not appropriate for evaluating tasks on imbalanced data (67) (68) (69) (70). Nevertheless, through the use of the chosen metrics, we are able to evaluate the separation and shapes of cluster using the internal metrics and the correctness of clustering using the created features using the external metrics.

## Data [TBRewritten]

### Synthetic datasets

The validation of deep clustering methods was made by comparing the different methods with [\*\*\*PCA, ICA and Isomap\*\*\*]. The chosen datasets, 95 in number and denominated as simulations, originate from the Department of Engineering, University of Leicester UK and are publicly available. Each simulation is a dataset. The creation of these simulations was based on recordings from the neocortex of a monkey. They were generated using 594 different spike shapes (9). The original study that introduces the simulations (9) also reviews different clustering algorithms and their results. Out of 20 different units, these algorithms were able to detect 10 in the best case.

The datasets were generated based on a real dataset recorded “in vivo”. The waveform contains 316 points originally sampled at 96 KHz; afterwards this frequency was reduced to 24KHz, therefore 79 samples describe a spike. Being synthetic datasets, each of these spikes has a label, which allows for the use of external metrics to evaluate performance. Each simulation contains a multi-unit cluster, which is the noise, and a number of clusters that varies between 2 and 20. Each unique number of clusters has 5 simulations. Thus, there are 5 simulations with 2 clusters, 5 simulations with 3 clusters, and so on.

All but one of the clusters are single-units between 0 and 50μm away from the electrode. The firing rate follows a Poisson distribution with a mean between 0.1 and 2Hz. The amplitudes follow a normal distribution and have been scaled to values between 0.9 and 2 to simulate real data. No spikes with temporal overlapping are present in the data, such that spikes have at least 0.3ms between them.

The generated multi-unit cluster was added in order to increase the complexity of clustering for the tested algorithms. The simulated multi-unit contains 20 spike shapes, each of the 20 neurons firing being between 50-140μm away from the electrode. The amplitude of the spikes was fixed to 0.5, with an overall composite firing rate of 5Hz, with each of the 20 individual composing neurons having a firing rate mean of 0.25Hz following an independent Poisson distribution. Here, in order to increase clarity, the multi-unit cluster is always color-coded in white in all figures.

To evaluate the proposed approach in comparison with other state-of-the-art methods we have chosen the following 4 simulations out of the 95 available as they are representative of the issues that are present in feature extraction methods and allow for the evaluation of the methods on varying numbers of clusters covering a wide range and enabling a comprehensive evaluation of performance:

* Simulation 1 (Sim1 - Fig 3a), containing 16 single-unit clusters and a multi-unit cluster (in total 17) with 12012 samples.
* Simulation 4 (Sim4 - Fig 3b), containing 4 single-unit clusters and a multi-unit cluster (in total 5) with 5127 samples.
* Simulation 16 (Sim16 - Fig 3c), containing 8 single-unit clusters and a multi-unit cluster (in total 9) with 7556 samples.
* Simulation 35 (Sim35 - Fig 3d), containing 12 single-unit clusters and a multi-unit cluster (in total 13) with 9481 samples.
* Simulation 14, containing 3 single-unit clusters and a multi-unit cluster (in total 4) with 4507 samples. This dataset was used for the visualization of the impact of alignment on feature extraction in section 3.1.

These simulations can also be viewed in Fig 3 through the use of PCA to reduce the dimensionality from 79 to 2. The overlapping clusters produced by PCA can be clearly seen in Fig 3, in none of the datasets is it able to perfectly separate all clusters.

Scatter chart

Description automatically generated

**Fig 3. PCA projection of the synthetic datasets.** PCA projection of 4 different simulations with distinct numbers of clusters; the colors represent the cluster assignment given in the ground truth.

### Real datasets [TBRewritten]

The electrophysiological *“in vivo”* data was recorded from the brain of anaesthetized adult mice of the C57/B16 strain with A32-tet probes (NeuroNexus Technologies, Inc) at 32 kSamples /s (Multi Channel Systems MCS GmbH) during a visual stimulation. The stimuli were presented monocularly on a Beetronics 12VG3 12-inch monitor with a resolution of 1440x900, at 60fps and consisted of full-field drifting gratings (0.11 cycles/deg; 1.75 cycles/s; variable contrast 25–100%; 8 directions in steps of 45°). The animals, on which the extracellular activity was recorded, were placed in the stereotaxic holder (Stoelting Co, Illinois, United States) and anaesthetized. Anesthesia was induced and maintained with isoflurane (ISO) in oxygen (5% for induction, 1-3% for maintenance). The heart rate, respiration rate, core body temperature, and pedal reflex were constantly monitored. A circular craniotomy (1x1 mm) was performed over the left visual cortex of the animal centred on 0-0.5 mm anterior to lambda, 2-2.5 mm lateral to midline. To obtain multiunit activity (MUA) containing signals, the extracellular data was digitally filtered using a band-pass filter with a range of 300Hz-7000Hz using a bidirectional Butterworth IIR filter of order 3. An amplitude threshold, most commonly chosen between 3 and 5 (2) standard deviations of the recorded signal, was used to detect spike, which were then fed into the feature extraction algorithms. Spikes were identified as threshold crossings and subsequently used as input for the feature extraction algorithm.

Multiple datasets were accumulated from each animal over a period of 4 to 6h in order to minimise animal use. All experiments were performed in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and approved by the Local Ethics Committee (3/CE/02.11.2018) and the National Veterinary Authority (147/04.12.2018).

## Data preprocessing [TBRewritten]

Alignment has to be applied as a first step of preprocessing before the execution of the feature extraction method. We have used the following formula, for multiple types of alignment at a chosen index:

Naturally, the point of start of a portion of the samples has to be changed; this is indicated in formula (1) through the *new\_start* and *old\_start* terms. The *index* in equation (1) represents the point to which all spikes will be shifted. Thus, we can choose to align the maximum peak of all spikes to the average index of the maximum peak across all samples, as shown in Fig 4c. Another, better option, is to align the amplitudes to the middle of the sample as it provides information about the spike from the perspective of both pre- and post-amplitude. The *peak* in equation (1) represents the index at which the desired point of reference (typically, the peak) is found. For the alignment of the amplitude, it is the index of the maximum peak of each sample. The formula permits the alignment of any point of reference, such as the minimum peak (47).

In addition, we have applied two other preprocessing steps: scaling and shuffling.

Chart, scatter chart

Description automatically generated

**Fig 4. Impact of alignment.** PCA applied on Sim14 with and without alignment. The white cluster is kept together but the overlap with the blue cluster remains.

# Results [TBRewritten]

## Performance evaluation

### Performance evaluation of synthetic data [TBRewritten]

The 95 synthetic datasets (9) contain varying numbers of clusters and spike shapes providing the complexity required for a comprehensive evaluation of the methods. In Fig 5, we present the results obtained for each metric across all 95 datasets for each method presented. A statistical analysis using t-tests with a Bonferroni correction can be examined in the S4 Fig and a ranking of the methods based on their performance for each metric using Borda rank aggregation (71) in S2 Table.

### Performance evaluation of real data

# Discussion

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