

# A Comparison of Methods for Clustering Electrophysiological Multineuron Recordings

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**Abstract**—Techniques for the automatic clustering of extracellular multineuron recordings from the nervous system are compared for efficiency and accuracy. Selected waveforms were combined with noise to form test data with known classifications. After identical preprocessing using a Schmitt trigger threshold detector, the K-means, template matching and ART2 algorithms were applied to the same data. Measurements of the efficiency and utility of the three algorithms are presented using both the raw waveforms and the weightings of the first two principal components. Additionally, all three algorithms were tested with data obtained from electrophysiological experiments.

**Index terms**—Multi-unit spike train, Computer spike discrimination, neural network

## I. INTRODUCTION

Extracellular recordings of neuronal spiking activity are becoming increasingly important in studies of multi-neuronal activity in physiological laboratories. Such recordings allow the activities of multiple closely spaced neurons to be recorded from a single electrode as opposed to the usual case where only a single neuron can be monitored. This extra information may be an important tool for gaining improved understanding of the interactions between neurons when processing information.

A key problem in using multineuron extracellular recordings is that of demultiplexing individual neuronal activities from the spike trains recorded. This clustering procedure can be accomplished by sorting the different spikes by shape, each different shape assumed to be associated with a different neuron.

Ideally a solution to this spike-sorting problem should have the following features:

- Real-time - use an efficient online computation algorithm to facilitate operation in a real time environment
- Accurate - discard the occasional corrupted spike but form new templates for genuine spikes of a new class
- Unsupervised - operate with minimal human intervention
- Adaptive - adapt reference patterns (templates) dynamically to track changes in the shape of a spike

over time due to movement of the electrodes or changes in the membrane potential.

- Cost and convenience - should run on standard personal computers so no special hardware is required.

Many different techniques have been proposed for the automatic waveform classification of such signals (see Schmidt [1] for a review). The earliest techniques discriminated between waveforms based on the amplitude of several selected points [1]. They were hardware based so they met the real-time constraint, however their accuracy was limited and they did not adapt to changing spike patterns. Computer based methods have focussed on template matching in which templates which are representative of single neuron spikes are constructed and spikes classified by comparison with the templates [2]. Both online and batch (offline), adaptive and non-adaptive methods have been proposed for producing templates. The algorithms used have included generating new templates when no good match with existing templates are found [2], the K-means clustering algorithm [3,4], linear filters [5] and artificial neural networks [6]. Many of these algorithms employ principal component analysis [1] as a preprocessing step, serving to reduce the dimensionality of the patterns to be clustered as well as extract only the most salient features of the waveform.

Online computer based methods for spike sorting have typically relied on high-speed processors to meet the strict real-time requirements of the task. A state of the art real time system [7] uses two AT&T DSP32C floating point digital signal processing chips per channel to demultiplex up to 8 spikes in real-time. A standard modern desktop computer (e.g. a 400 MHz Pentium II processor) has much more computing power than a DSP32C.

Artificial neural networks (ANNs) have been successfully applied to many difficult pattern recognition problems such as optical character recognition, time series prediction and speech recognition [8]. In many cases ANN based solutions were found to be more accurate and robust than even the best conventional techniques. Work on using a backpropagation network for spike sorting [6] has been reported. However, this technique was an offline method and training of the ANN was performed prior to spike

sorting. This means that the network is unable to learn new spikes unseen presented during the training process. In this paper, we introduce an online, unsupervised software based spike recognition algorithm using the ART2 artificial neural network model [9]. This model can learn new templates when previously unseen spikes are encountered. The ART2 method is compared with two standard techniques: a simple template matching technique, and the K-means algorithm.

## II. CLUSTERING ALGORITHMS

In this section an overview of the three algorithms that will be compared is presented. Due to space limitations, a complete description of each algorithm cannot be presented although references are provided.

### A. Online Template Matching (TM)

The template matching algorithm used is an online modification of an algorithm proposed by Millecchia and McIntyre [10]. If an incoming spike is less than a user defined distance  $D$  (in Euclidian distance) from one or more templates, that spike is classified as belonging to the same class as the closest matching template. The template is then adapted using a weighted average between the template and the new spike. A new template is generated when a spike occurs which is greater than  $D$  from all of the other templates. After a maximum number of new templates (10) is generated, no further templates can be made.

### B. K-means Clustering

The K-means clustering algorithm [3] minimizes the sum of square errors with  $K$  clusters (which is user-defined). Each cluster contains a set of spikes that are similar in shape. The templates are derived during a learning phase in which the best partition of a set of training data (we use all of the data available) is found by minimizing the sum of the Euclidian distances between each spike and their cluster mean. The algorithm starts by performing a rough initial clustering based on the sum of the data points in the spike. This sum is used to group the training spikes into one of the  $K$  initial clusters. A test is performed on each spike by moving it from its current cluster to each other clusters and calculating the total error. The cluster is moved (if necessary) to the cluster which gives the smallest total error. This local optimization procedure is repeated for each spike until the algorithm iterates over the entire training set without changing the cluster assigned to any spike. After the clusters have been assigned, fast classification can be accomplished by assigning the spike to the closest cluster.

### C. ART2

Adaptive resonance theory (ART2) [9] is a neural computational algorithm designed for real time self-organizing stable pattern recognition codes in response to arbitrary sequences of analog input patterns. In an ART2

network, an input pattern is applied to the F1 layer (see Figure 1). A feedback structure in the F1 layer encodes the input in an amplitude independent resonant state. During this resonant period, learning or adaptation can occur via interaction with the F2 layer.

ART2 adopts a competitive learning model in which a stream of input patterns to a network F1 can adapt the Long Term Memory (LTM) values that multiply the signals in the pathways between F1 and F2. Layer F2 is a competitive network capable of choosing the node which receives the largest total input (this corresponds to the closest matching template stored in the LTM).

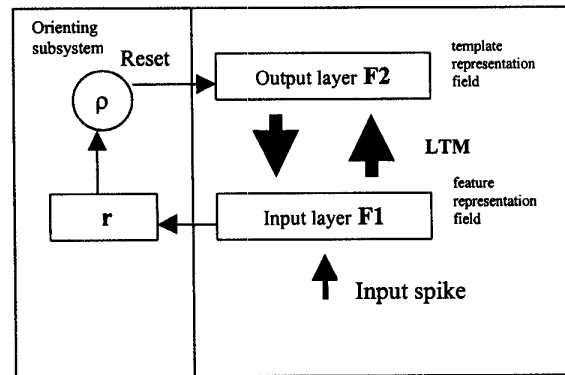


Figure 1. Block diagram of the ART2 network.

The criterion for an adequate match between an input pattern and a chosen template is adjustable by the vigilance parameter  $\rho$ . An auxiliary orienting attentional subsystem becomes active when a bottom-up input to F1 fails to match the learned top-down expectation read-out by the active template representation at F2. The orientation subsystem is activated and causes a reset of the active template. Alternative templates are tested in the hope of finding an adequate match. If there is no successful match, the cycle will end by selecting an uncommitted node OF F2, then the bottom-up and top-down adaptive filters linked to this node learn the F1 activation pattern generated directly by the input. If the full capacity of the system is used, the system will not be able to accommodate new input pattern.

## III. METHODS

The data used in our experiments were taken from the lateral geniculate nucleus, part of the visual pathway between the retina and the visual cortex of the common marmoset, *Callithrix jacchus*, a small South American monkey which has a visual system similar to that of humans and other primates.

The signals from the extracellular electrodes are amplified by a factor of 50000, passed through an anti-aliasing filter and digitized with a 12 bit National Instruments NI-MIO-16H data acquisition card at 22.5 kHz and saved to disk. All experiments described in this paper were performed off-line from recorded data.

A software implemented Schmitt trigger is used to identify spikes. When the waveform amplitude exceeds a user-defined trigger point  $\delta_1$  a spike is identified. The spike waveform consists of 16 samples, 4 from prior to the trigger and the 12 subsequent samples. Before a new trigger can occur, the signal must pass below the second threshold  $\delta_2$ , implementing a hysteresis effect to reduce false triggering in the presence of noise.

Principal component analysis (PCA) [1] was used as an optional preprocessor to reduce the dimensionality of spike data. One thousand spike waveforms obtained using the Schmitt trigger are chosen as the training set and a set of orthogonal basis functions computed using PCA. Each spike is then represented as the weighted sum of the eigenvectors corresponding to the largest eigenvalues of the training set's covariance matrix. These two eigenvectors form a basis which corresponds to the directions of greatest data variance. This technique is the optimal linear compression of the data, PCA serving to maintain most of the information about the waveform while achieving an eightfold compression.

The Schmitt trigger, PCA, clustering and data generation software were all implemented using the Mathworks Inc, MATLAB Version 5.0 language running on a Sun Sparc Ultra 5 machine. MATLAB provides a fast prototyping language for expressing computations, good data plotting facilities and an interactive environment for testing different algorithms.

#### IV. RESULTS

In order to test the accuracy of the three different algorithms ART2, K-means and template matching (TM), 4 clearly differently shaped spikes were taken from a clustering of recorded electrophysiological data and varying amounts of Gaussian noise added to each waveform. For each of the 4 spike shapes, 30 corrupted versions were generated to form a test set of 120 spikes. This test set was generated at noise levels corresponding to 1%, 20%, 40%, 60% and 80% of the maximum amplitude of the data (see Figure 2). The performance of each algorithm for the test set described (with and without PCA preprocessing) are shown in Table 1. For each algorithm, the user-tunable parameters were adjusted to give the best results. The number of floating point operations (FLOPS) required to perform clustering was also recorded for the 1% noise level case and is shown in Table 2.

From Table 1, it can be seen that the K-means and the TM+PCA algorithms have the best performance. The TM algorithm's poor performance for noise levels  $>40\%$  was due to its simplistic method of creating new templates. The ART2 algorithm, did not have good overall performance.

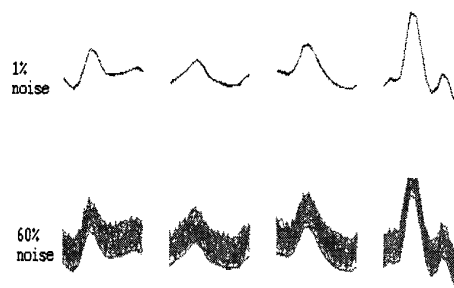


Figure 2. Example data sets with 1% noise and 60% noise.

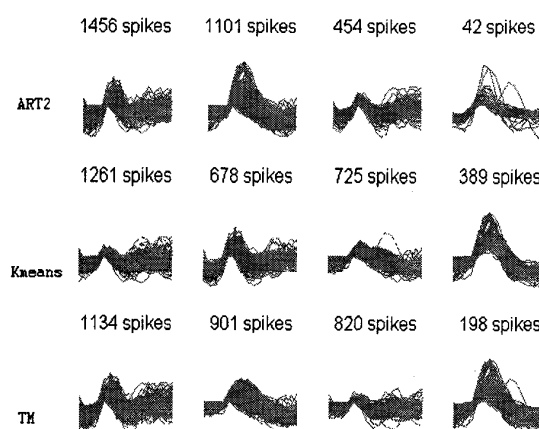


Figure 3. Clustering results comparing the performance of the three algorithms on recorded electrophysiological data.

Table 1. Results for randomly generated data. The entries correspond to the number of incorrectly classified data in a 120 point test set.

Noise Level	1%	20%	40%	60%	80%
ART2	0	1	21	29	31
ART2 + PCA	0	0	9	24	26
Kmeans	0	0	1	7	12
Kmeans + PCA	0	0	8	7	30
TM	0	0	90	90	90
TM + PCA	0	0	8	10	17

Table 2. Comparison of the efficiency of the algorithms for the 1% noise level case of Table 1.

<i>Algorithm</i>	<i>FLOPS</i>
ART2	537808
ART2 + PCA	79280
Kmeans	291936
Kmeans + PCA	64352
TM	47561
TM + PCA	16110

It can be seen from Tables 1 and 2 that PCA improves the accuracy and efficiency of clustering algorithms. The reduction in the dimensionality of the data means less computation is needed for clustering. PCA also serves as a form of feature extraction, removing unnecessary dimensions from the data so that the clustering algorithm deals only with the most salient features of the signal.

It is clear from Table 2 that the TM algorithm is clearly the most efficient, followed by K-means and then ART2. Using PCA analysis prior to clustering significantly reduces the amount of computation required. In this case, ART2 and K-means have approximately the same computational requirements. Template matching, however, is approximately four times faster.

The K-means algorithm does not have any parameters to tune (except the number of clusters) hence is the easiest to use. ART2 showed much less sensitivity to the value of the vigilance parameter than TM to the distance measure. For some of the cases, slightly changing the distance for TM would greatly change the results.

The three classification algorithms with PCA preprocessing were also tested on a set of real recordings taken from electrophysiological experiments. The clustering obtained from the 3 different algorithms are shown in Figure 3. Since it is not possible to associate spikes in the extracellular recordings with their respective neurons, it is not possible to make quantitative conclusions about the quality of the clustering. Qualitatively, the authors feel that none of the algorithms are clearly inferior or superior.

## V. CONCLUSION

Three different clustering techniques were tested on extracellular multineuron recordings. It was established that applying principal component analysis to the waveforms before clustering was beneficial both in terms of the accuracy of the results and the efficiency of the clustering. The three different algorithms, template matching, K-means and ART2 had similar qualitative clustering performance. K-means was found to be slightly more robust to large amounts of noise than template matching with PCA, and both methods were superior to ART2. Template matching was clearly the most efficient algorithm followed by K-means and PCA.

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