Using Recurrent Neural Networks for Automatic Chromosome Classification*

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Abstract. Partial recurrent connectionist models can be used for classification of objects of variable length. In this work, an Elman network has been used for chromosome classification. Experiments were carried out using the *Copenhagen* data set. Local features over normal slides to the axis of the chromosomes were calculated, which produced a type of time-varying input pattern. Results showed an overall error rate of 5.7%, which is a good perfomance in a task which does not take into account cell context (isolated chromosome classification).

1 Introduction

The genetic constitution of individuals at cell level is the focus of cytogenetics, where genetic material can be viewed as a number of distinct bodies –the *chromosomes*–. In computer-aided imaging systems, which are now widely used in cytogenetic laboratories, the automatic chromosome classification is an essential component of such systems, since it helps to reduce the tedium and labour-intensiveness of traditional methods of chromosome analysis.

In a normal, nucleated human cell, there are 46 chromosomes represented in the clinical routine by a structure called *the karyotype*, which shows the complete set of chromosomes organized into 22 classes (each of which consists of a matching pair of two *homologous* chromosomes) and two sex chromosomes, XX in females or XY in males.

Producing a karyotype of a cell is of practical importance since it greatly facilitates the detection of abnormalities in the chromosome structure. Suitable chromosome staining techniques produce dark bands which are perpendicular to the longitudinal axis and are characteristic of the biological class thereby allowing its recognition. Automatic classification is based on this band pattern and other kinds of information (e.g., width) along the chromosomes. This work presents a pattern recognition application to the classification of microscopic greyscale images of chromosomes by connectionist systems, the Recurrent Neural Networks (RNN).

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2 Background

2.1 Introduction to the Pattern Recognition Problem

In the early days, chromosomes were stained uniformly and as a result they could be distinguished only on the basis of size and shape, the so-called *Denver groups*. However, nowadays most routine karyotyping is carried out on Giemsa-stained chromosomes. The chromosomes appear as dark images on a light background and have a characteristic pattern of light and dark bands which is unique to each class, and which is referred to as *G-banding*. Fig. 1 shows the karyogram of a normal metaphase cell, where the characteristic band pattern can be observed. It serves as a basis for the image processing and pattern recognition algorithms.

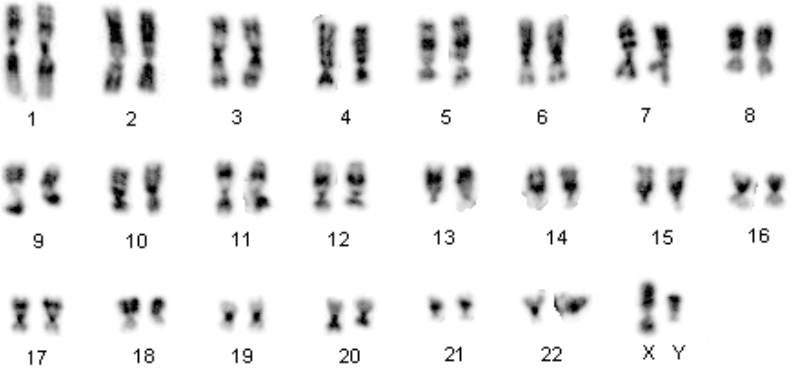

Fig. 1. Normal metaphase cell karyogram (images extracted from the Copenhagen data set).

2.2 Parameter Estimation

In 1989, Piper and Granum established a set of 30 features that were implemented in the chromosome analysis system MRC Edinburgh [1]. The features were classified according to how much a priori information is needed to measure them. Four feature *levels* were distinguished:

1. Direct measures on the image: area, density and convex hull perimeter.
2. Requirement of the axis: length, profiles of density, gradient and shape.
3. Requirement of axis plus polarity: asymetrical weight features.
4. Requirement of axis, polarity and centromere location: centromeric indices.

In this work, the parameters obtained from the images require the calculation of the longitudinal axis. The input patterns are built from measures over normal slices at unitary distance over the axis (details of the preprocessing methods are given in Section 3).

2.3 Recurrent Neural Networks

In many applications, time is inextricably bound up with many behaviors (such as language) and object representations. The question of how to represent time in connectionist models is very important, since it might seem to arise as a special problem which is unique to parallel processing models. This is because as the parallel nature of computation appears to be at odds with the serial nature of temporal events.

This work deals with the use of recurrent links in order to provide networks with a dynamic memory. In this approach, hidden unit patterns are fed back to themselves, acting as the context of prior internal states. Among the different networks proposed in the literature, the Elman network (EN) presents some advantages because of its internal time representation: the hidden units have the task of mapping both external input and the previous internal state (by means of the context units) to some desired output. This develops internal representations which are useful encodings of the temporal properties of the sequential input [7].

Fig. 2 shows an EN, where trainable connections are represented with dotted lines. Connections from the output of hidden units to the context layer are usually fixed to 1.0, and activations of hidden units are copied on a one-to-one basis. Basically, the training method involves activating the hidden units by means of input sequence segments plus prior activation of context units. Then, the output produced is compared with the teaching ouput and the backpropagation of the error algorithm is used to incrementally adjust connection strengths, where recurrent connections are not subject to adjustment [7].

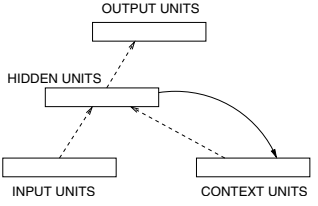

Fig. 2. A Recurrent Neural Network [7].

3 Materials and Methods

3.1 Corpus and Feature Extraction

The corpus used in the experiments was the *Cpa*, a corrected version of the large Copenhagen image data set *Cpr*. It consists of 2,804 karyotyped metaphase cells, 1,344 of which are female and 1,460 are male [4].

As a first step in the preprocessing stage, the histograms of the images are normalized and the holes are filled by means of contour chains to avoid problems in the axis calculation. Afterwards, mathematic morphology algorithms are applied (dilation-erosion) to smooth the chromosome outlines, which prevents the algorithm from producing an axis with more than two terminals.

Two skeleton techniques, the González-Woods algorithm [5] and the Hilditch algorithm [6], are successively applied to find the longitudinal medial axis. Finally, the transverse lines (slices), which are perpendicular to the axis are obtained.

A set of *local* features over the slices was calculated. The parameter set consisted of 9 bidimensional gaussian filtering points along the slices, all having an equidistant location between the slice bounds. At each point, a 5x5 pixel filter with the following weights was applied: 16 in the center, 2 at a in 1-pixel distance and 1 at a 2-pixel distance. Fig. 3 shows a chromosome along with an example of an input pattern resulting from 3 gaussian filtering points per frame (left, center and right points).

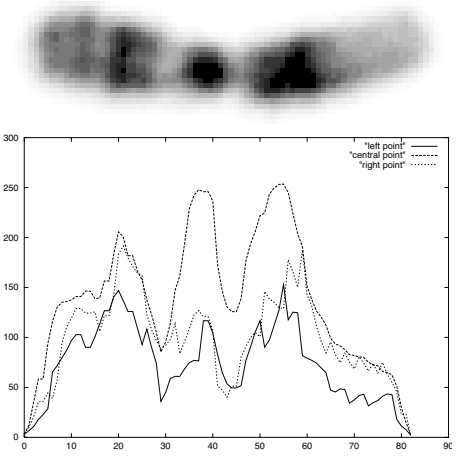

Fig. 3. Chromosome and 3-points gaussian profiles (chromosome image extracted from the Copenhagen data set).

3.2 Partial RNN

The SNNS toolkit provides the methods to implement different configurations of partial recurrent networks (Jordan and Elman networks) [2]. The EN implemented in this work had the following topology:

- 1 input layer of 45 units, in order to fit a 5-frame, moving window of 9 points each.
- 1 hidden layer, together with its context layer (variable number of units). The link weights from hidden units to context units were set to 1.0. There were no self-recurrent links for the context units.
- 1 output layer of 24 units, without context layer.

There is a special issue about testing the networks which should be highlighted. After the input frame presentation, the network produces a classification

in one of the 24 classes, so individual frames are classified based only on trained network knowledge. In order to test the EN perfomance, a special method was applied: a minimum number `MIN` of correct classified frames (in %) is established a priori. A chromosome is said to be correctly classified if more than `MIN` percent of frames are well-classified according to the desired output. For all the experiments, the value of `MIN` was fixed at 70%. This minimum was always surpassed in the slice voting scheme.

4 Experiments and Results

The same partition of Cpa corpus was used in all the experiments: the training set consisted of 33,000 chromosomes (It was not possible to use the full Cpa corpus size due to the high memory requirements of the feature set file). The validation set and the test set each consisted of 2,000 chromosomes. The number of units in the hidden layer was shifted from 50 to 250 (the same number of units was used in the context layer). The obtained error rate values are shown in Table 1.

Table 1. Test-set errors for different configurations of the hidden layer

Number of units	Classification error
50	22.8%
100	15.3%
150	9.5%
200	5.7%
250	6.7%

The best perfomance was achieved with 200 units in the hidden layer, which obtained a mean error rate for the 24 classes of 5.7%. The lowest individual error rate was 3.1% (for class 3), whereas the highest error rate was 40.0% (for class 24, which corresponds to sex chromosome Y).

5 Discussion and Conclusions

This work presents the application of partial recurrent neural networks (specifically Elman networks) to automatic chromosome classification. Local information from the length of the chromosome was extracted from the greyscale images. This information (gaussian filtering points sampled at equidistant distance over normal lines to the axis) constitute the local feature frame.

The best result achieved was a minimum error rate of 5.7% in isolated chromosome classification, using a test-set of 2,000 input patterns. This was achieved by means of a standard and well-known Neural Network technique. The use of

recurrent neural networks is supported given the fact that feedforward netowks, in the same conditions, achieved a minimum error rate of 12.88%.

This preliminary technique could be improved in further research studies. For example, a natural step for improving the error rate would be to restrict the system to doing classification by cell, exploiting the fact that a normal cell has only 46 chromosomes ordered in 24 classes. Ritter *et al* use this information and other improvements such as an enhanced profile extraction method to obtain an error rate of 0.61% using a Bayesian classifier [8]. The human error rate of an experienced cytogenetist lies between 0.1% (using good quality images) and 0.3% (clinical applications).

The higher individual error rate obtained for class 24 (sex chromosome Y, only present in normal male cells) was expected because of its lower a-priori probability: 1/92 compared to 3/92 for class 23 (sex chromosome X, present in both male and female cells) and 4/92 for classes 1 to 22.

The same partition of training-validation-test was used for all the experiments in this work due to high computational requirements. However, the application of a m-fold cross-validation method would be better to obtain statistically representative results. Experiments using the complete Cpa corpus should be done to improve the results.

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Chromosome Classification Using Continuous Hidden Markov Models*

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Abstract. Up-to-date results on the application of Markov models to chromosome analysis are presented. On the one hand, this means using *continuous Hidden Markov Models (HMMs)* instead of discrete models. On the other hand, this also means to conduct empirical tests on the same large chromosome datasets that are currently used to evaluate state-of-the-art classifiers. It is shown that the use of *continuous HMMs* allows to obtain error rates that are very close to those provided by the most accurate classifiers.

1 Introduction

A common task in cytogenetics is the *karyotye analysis of a cell*. It consists of labelling each chromosome of the cell with its class label, in order to have the genetic constitution of individuals. This analysis provides important information about number and shape of the chromosomes, which serves as a basis to study the possible abnormalities the individual could have.

In a normal, nucleated human cell there are 46 chromosomes. The *karyogram* is a standard format which shows the complete set organized into 22 classes (each one consisting of a matching pair of two *homologous* chromosomes), ordered by decreasing length, and two sex chromosomes, XX in females or XY in males.

The first attempts to automate this task were made in the early 1960s, motivated by the fact that manual analysis is very tedious and labour-intensive. Since then, many classification techniques have been tried, including both statistical and structural approaches. Most of them, however, are conventional, statistical

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classification techniques in the sense that they reduce each chromosome image to a point in some multi-dimensional feature space [6]. Instead, the use of structurally richer approaches has been rare and focused mainly on *discrete Markov models* [3, 7].

The aim of this paper is to provide up-to-date results on the application of Markov models to chromosome analysis. On the one hand, this means using *continuous Hidden Markov Models (HMMs)* instead of discrete models. On the other hand, this also means to conduct empirical tests on the same large chromosome datasets that are currently used to evaluate state-of-the-art classifiers [6]. It is worth noting that this is a new application of standard Speech Recognition technology and, in particular, a new application of the well-known and widely available standard *HMM Tool Kit (HTK)* [8]. However, this is not a straightforward application of HTK since we also have to take care of preprocessing, feature extraction and HMM topology design. These aspects are covered in the next section. Empirical results and the main conclusions drawn are given in sections 3 and 4, respectively.

2 The Approach

The basic steps of our HTK-based approach are illustrated in Figure 1. They are described in what follows.

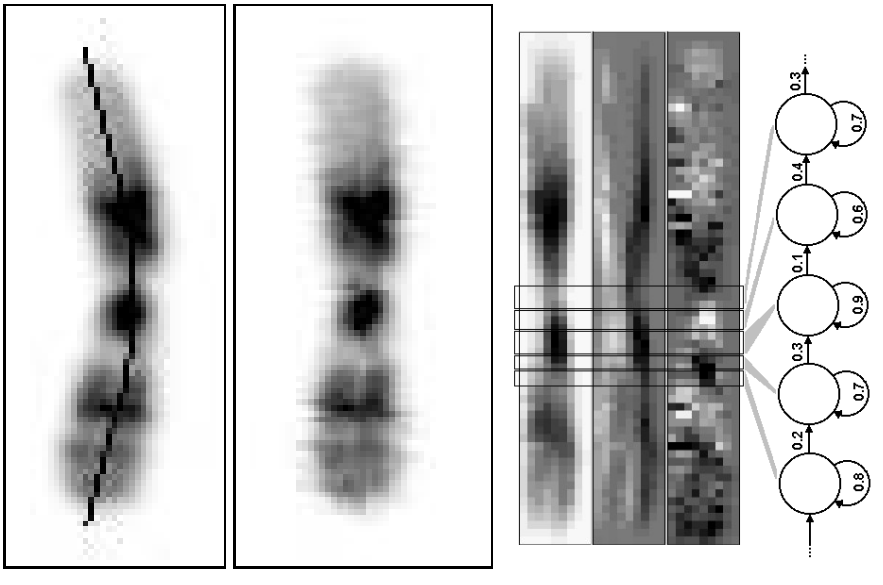

Fig. 1. Basic steps of our HTK-based approach. From left to right: computation of the longitudinal axis, unfolding, feature extraction and HMM modelling

Computation of the Longitudinal Axis and Unfolding

The computation of the longitudinal axis of a chromosome is a standard preprocessing step in chromosome analysis. However, no precise definition has been widely accepted and, in fact, it is a matter of current research [6]. In our case, we have used a rather standard procedure that includes the classical Hilditch's thinning algorithm for *medial axis* computation and some refinements [2].

Once the longitudinal axis has been computed, it is traversed at unit speed and a perpendicular slice is cut at each sampled axis point to obtain an unfolded, straight version of the chromosome. After this *chromosome unfolding*, feature extraction reduces to compute an appropriate set of features from each image row.

Feature Extraction

Feature extraction for *local* characterization of chromosomes is an interesting, open problem. Based on our previous experience [5] and some informal tests, we have considered the following four types of features:

- **9p:** grey densities
- **D:** horizontal derivative
- **A:** horizontal acceleration
- **V:** vertical derivative

The set of features referred to as 9p corresponds to 9 equidistant Gaussian-filtered points. Concretely, each point was filtered by convolution with a 5×5 filter mask with weights: 16 in the center, 2 at a 1-pixel distance and 1 at a 2-pixel distance. Derivatives and acceleration were computed from successive 9p vectors. As an example, the sequence of feature vectors shown Fig. 1 comprises grey densities plus horizontal and vertical derivatives (9p+D+V).

It must be noted that these types of features come from previous work on HTK-based handwriting recognition. Please see [1, 4] for more details on the computation of these types of features.

HMM Chromosome Modeling

As illustrated in Fig. 1, chromosomes are modelled by *continuous left-to-right HMMs*. Basically, an HMM for a chromosome class is a stochastic finite-state device aimed at modelling the succession, along the longitudinal axis, of feature vectors extracted from instances of the chromosome class. Each HMM state generates feature vectors following an adequate parametric probabilistic law; typically a *mixture of Gaussian densities*. The number of states and number of densities per state that are appropriate to model each chromosome class depend on the class variability and the amount of training data available. So, these numbers need some empirical tuning. The training process is carried out with the HTK toolkit, using conventional re-estimation formulas [8].

3 Experiments

The data set used in the experiments was the Cpa, a corrected version of the Cpr corpus (the complete *Copenhagen* data set) [6]. The corpus contains the segmented chromosome images of 2804 human cells, 1344 of which are female and 1460 are male.

3.1 Context-Free Classification

The usual method for classifying a test chromosome is to find the HMM with the highest probability in the Viterbi decoding. This is the so-called *context-free* classification, because each chromosome is classified with independence of each other. The experiments reported in this subsection were done under this context-free framework.

As discussed before, one of the two basic parameters characterising continuous left-to-right HMMs is the number of states chosen for each class-conditional HMM M_i. This number has been computed as $s_i = \frac{f_i}{k}$, where f_i is the average length of the sequence of vectors used to train M_i, and k is a design parameter measuring the average number of feature vectors modelled per state. This rule of setting up s_i attempts to balance modelling effort across states and also captures important discriminative information about the typical length of each chromosome class. Following this rule, a first series of experiments was carried out on a partition involving $2400 + 400$ training and testing cells. We used the 9p feature set and the values of k: $1.5, 2, 2.5, 3$ and 4. For each value of k, the number of Gaussian densities per state was varied as $1, 2, 4, ldots$ until reaching a minimum classification error rate. The results obtained, which are omitted here for brevity, showed a degradation of the error rate as the value of k increases. So, in accordance with these results, a value of $k = 1.5$ was fixed for the remaining experiments.

After deciding on the value of k, a second series of experiments was conducted on the same data partition to study the classifier performance as a function of the number of Gaussian densities per state, and for several feature sets. The results are shown in Fig. 2. From these results, it is clear that an appropriate feature set consist of using grey densities plus horizontal and vertical derivatives (9p+D+V). Also, 64 seems to be an adequate number of Gaussian densities per state.

Although the results obtained up to this point were satisfactory, we decided to do further experiments using windows of feature vectors instead of single vectors. This was done as as an attempt to reproduce the behaviour of the input layer of Recurrent Neural Networks, in which a small moving-window of feature frames is processed at each time and this seems to be very effective in improving classification results [5]. Fig. 3 shows the classification error rate (estimated as before) for varying Gaussian densities per state and several window sizes. As expected, the use of windows of feature vectors helps in improving classification error and, in fact, the best result (5.6%) was obtained with a window of 3 feature vectors (and 64 Gaussian densities per state).

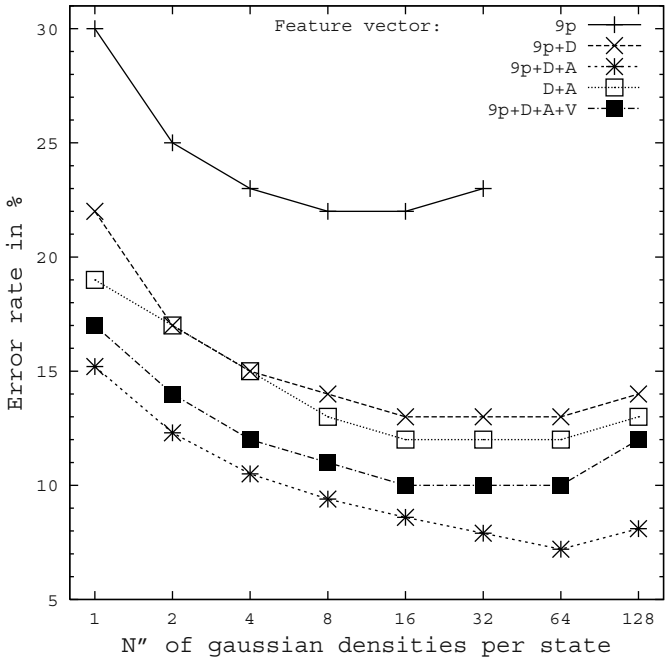

Fig. 2. Classification error rate as a function of the number of Gaussian densities per state, and for several feature sets

All the experiments reported so far were carried out using a single partition of the complete corpus. In order to obtain more precise results, the classification error rate was also estimated using a 7-fold cross-validation procedure in which the blocks were chosen to have 400 cells each. It is given in Table 3.1 as a function of the number of Gaussian densities per state (the remaining parameters were set to the values that provided a 5.6% of error). The best result for the cross-validation method, again obtained with 64 Gaussian densities, is 7.5%.

3.2 Context-Dependent Classification

The classification error rate can be reduced by taking into account the fact that the normal karyotype consists of 22 pairs of autosomes and a pair of sex chromosomes. This knowledge imposes a constraint that penalizes, e.g., the allocation of more than two chromosomes to one class and less than two chromosomes to other class. This is the called *context-dependent* classification.

An iterative algorithm was formulated to restrict the isolated chromosome classification by including the contextual cell information. The algorithm receives as input, for each chromosome of a cell, the output probabilities of each HMM. Then, in successive iterations, the algorithm classifies pairs of chromosomes for each class using *solved classes*; i.e., classes with only two chromosomes having

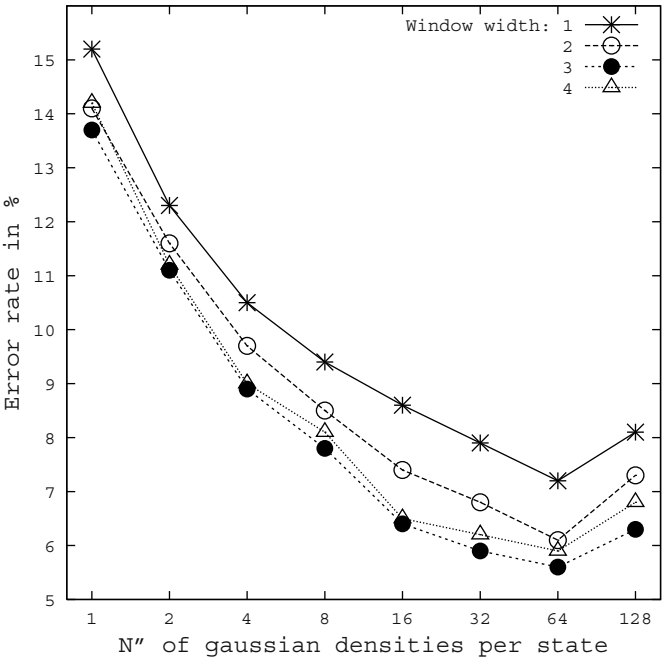

Fig. 3. Classification error rate as a function of the number of Gaussian densities per state, and for window widths

the highest probability for that class, and both probabilities greater than a lower bound. After that, the probabilities of solved classes are crossed-out for the remaining chromosomes, whose probabilities are renormalized and the process is repeated until the complete cell is classified.

In order to complete the experiments reported in the preceding subsection, the context-dependent classification algorithm discussed above was applied to the best classifier found under the context-free framework. The classification error rate was again estimated using the 7-fold cross-validation procedure and, as expected, it was reduced from 7.5% up to 4.6%. This figure is close to those provided by the most accurate classifiers [6].

4 Conclusions

We have provided up-to-date results on the application of Markov models to chromosome analysis. It has been shown that the use of *continuous Hidden Markov Models (HMMs)* allows to obtain error rates that are very close to those provided by the most accurate classifiers. As in the case of handwriting recognition, the main advantage of using HMMs is that they can be easily integrated into systems based on finite-state technology [4].

Table 1. Classification error rate estimated using a 7-fold cross-validation procedure

N° of G. D.	error rate
1	15.7
2	12.5
4	10.4
8	9.3
16	8.4
32	7.9
64	7.5
128	7.7

A number of improvements can be applied to the entire system. The skeleton technique used to obtain the longitudinal axis has the disadvantage of being non-parametric, so approximations using eigenvectors have to be used to calculate the slices. A parametric axis could help to reduce some errors introduced by the current method due to morphological filtering and the skeletonization algorithm (especially in short chromosomes). In this line, other methods are being studied: polynomial curve-fitting, implicit polynomials, etc. The iterative algorithm that implements the *context-dependent* classification could be improved by dynamic programming, allowing a more detailed analysis for finding the *solved classes*.

Acknowledgements

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