POIMs: positional oligomer importance matrices—understanding support vector machine-based signal detectors

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ABSTRACT

Motivation: At the heart of many important bioinformatics problems, such as gene finding and function prediction, is the classification of biological sequences. Frequently the most accurate classifiers are obtained by training support vector machines (SVMs) with complex sequence kernels. However, a cumbersome shortcoming of SVMs is that their learned decision rules are very hard to understand for humans and cannot easily be related to biological facts.

Results: To make SVM-based sequence classifiers more accessible and profitable, we introduce the concept of positional oligomer importance matrices (POIMs) and propose an efficient algorithm for their computation. In contrast to the raw SVM feature weighting, POIMs take the underlying correlation structure of k-mer features induced by overlaps of related k-mers into account. POIMs can be seen as a powerful generalization of sequence logos: they allow to capture complex relationships (e.g. binding site motifs) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Availability: All source code, datasets, tables and figures are available at http://www.fml.tuebingen.mpg.de/raetsch/projects/POIM.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

For many sequence classification problems, support vector machines (SVMs) (Schölkopf and Smola, 2002; Vapnik, 1995) with the right choice of sequence kernels perform better than other state-of-the-art methods, as exemplified in Table 1. While this success can in part be attributed to the SVM algorithm and the statistical learning theory that underlies it (Vapnik, 1995), it is essential to use appropriate kernels and features. In order to achieve the best prediction results, it typically pays off to rather include many, potentially weak features than to manually pre-select a small set of discriminative features. For instance, the SVM-based translation initiation start (TIS) signal detector StartScan (Saegs et al., 2007), which relies on a relatively small set of carefully designed features, shows a considerably higher error rate than an SVM with a standard kernel that implies a very high-dimensional feature space (cf. Table 1). The best methods in Table 1 are all based on SVMs that work in feature spaces that exhaustively represent the incidences of all k-mers up to a certain maximum length K. There are two cases: the k-mers are either (i) summarized over all positions or (ii) considered separately for each position. For (i) there are the popular spectrum kernel without (Leslie et al., 2002) and with mismatches (Leslie et al., 2003); for (ii) we use the weighted degree kernel without (WD; Rätsch and Sonnenburg, 2004) and with shift (WDS; Rätsch et al., 2005).

Nowadays, SVMs with string kernels can be trained efficiently on millions of DNA sequences even for large orders K (e.g. Sonnenburg et al., 2007a), thereby inducing enormous feature spaces (for instance, K = 30 gives rise to more than 4200 k-mers). Such feature spaces supply a solid basis for accurate predictions as they allow to capture complex relationships (e.g. binding site motifs), however, the importance of features in the induced feature space remains unclear.

Partial relief is offered by multiple kernel learning (MKL; e.g. Lanckriet et al., 2004; Rätsch et al., 2006). In MKL, convex combinations of M kernels are considered, i.e. k(x1, x2) = \sum_{m=1}^{M} \beta_m k_m(x_1, x_2) with \sum_{m=1}^{M} \beta_m = 1. For appropriately designed sub-kernels k_m, the optimized combination coefficients \beta can then be used to highlight which parts of an input sequence are important for discrimination (Rätsch et al., 2006). The use of the \ell_1-norm constraint (\sum_{m=1}^{M} \beta_m = 1) causes the resulting \beta to be sparse, however at the price of discarding relevant features, which may lead to inferior performance.

An alternative approach is to keep the SVM decision function unaltered, and to find adequate ways to ‘mine’ the decision boundary for good explanations of its high accuracy. A natural way is to compute and analyze the normal vector of the separation in feature space, w = \sum_{i=1}^{N} \alpha_i y_i \Phi(x_i), where \Phi is the feature mapping associated to the kernel k. This has been done, for instance, in cognitive sciences to understand the differences in human perception of pictures showing male and female faces. The resulting normal vector w was relatively easy to understand for humans since it can...
Table 1. Comparison of SVM performance (second column) versus competing state-of-the-art classifiers (third column) on six different DNA signal detection problems

| Signal detection problem to be solved | SVM performance | SVM-based approach and string kernel names | Performance of competitor | Competing approach |
|--------------------------------------|-----------------|-------------------------------------------|--------------------------|--------------------|
| Transcription start                   | 26.2% auPRC     | WDS and spectrum (ARTS, Sonnenburg et al., 2006b) | 11.8% auPRC | RVM (Eponine, Down and Hubbard, 2002) |
| Acceptor splice site                  | 54.4% auPRC     | WDS (Sonnenburg et al., 2007b)            | 16.2% auPRC  | IMC (Sonnenburg et al., 2007b) |
| Donor splice site                     | 56.5% auPRC     | WDS (Sonnenburg et al., 2007b)            | 25.0% auPRC  | IMC (Sonnenburg et al., 2007b) |
| Alternative splicing                  | 89.7% auROC     | WDS (Rätsch et al., 2005)                 | -             | -                  |
| Trans-splicing                       | 95.2% auROC     | WD (mGene, Schweikert et al., manuscript in preparation) | -  | -                  |
| Translation initiation                | 10.7% Se80      | WD (mGene, Schweikert et al., manuscript in preparation) | 12.5% Se80 | PWM, ICM (Saeys et al., 2007) |

The chosen best SVMs employ spectrum, WD or WDS kernels. Note that performance measures differ: AuROC denotes the area under the receiver-operator characteristic curve and auPRC the area under the precision recall curve; for both, larger values correspond to better performance. Se80 is the false positive rate at a true positive rate of 80% (lower values are better). Transcription start site (TSS) among the best TSS recognizers is the relevance vector machine (RVM) based Eponine, which is clearly outperformed by our ARTS TSS detector. Acceptor and donor splice site: the best existing splice site detectors are SVM based (Sonnenburg et al., 2007b). We further demonstrate our approach in the prediction of alternative splicing, using the popular inhomogeneous Markov chains (IMC), which achieve less than half of the auPRC on human splice sites. Alternative and trans-splice sites in Carnevalibotulina elegans: for the best of our knowledge no other ab initio approaches are available. Translation initiation site: for TSS recognition our method can be compared with Startscan (ISMB, 2007) which is based on positional weight matrices (PWM) and interpolated context models (ICM). Our WD-kernel SVM, trained using default settings $\epsilon=1, d=20$ (no model selection) on the dataset from Saeys et al. (2007), already performs favorably.

2 METHODS

First we introduce the necessary background, then define POIMs, provide recursions for efficient computation, and finally describe visualization and analysis.

2.1 Linear positional oligomer scoring systems

Given an alphabet $\Sigma$, here the DNA nucleotides $\Sigma = \{A, C, G, T\}$, let $x \in \Sigma^L$ be a sequence of length $L$. A sequence $y \in \sum^k$ is called a $k$-mer or oligomer of length $k$. A positional oligomer (PO) is defined by a pair $(y, i) \in I := \bigcup_{k=1}^{\infty} \sum^k \times \{1, \ldots, L - k + 1\}$, where $y$ is the subsequence of length $k$ and $i$ is the position at which it begins within the sequence of length $L$. We consider scoring systems of order $K$ that are based on POS of lengths $k \leq K$ defined by a weighting function $w: I \rightarrow R$. Let the score $s(x)$ be defined as a sum of PO weights:

$$s(x) = \sum_{i=1}^{L-K+1} \sum_{k=1}^{K} \sum_{w=1}^{W} w \left( k(x, i) \right) + b,$$

(2)

where $b$ is a constant offset (bias), and we write $k(x, i) \left( = k_{i-1, \ldots, i-k+1} \right)$ to denote the substring of $x$ that starts at position $i$ and has length $k$. Many classifiers implement such a scoring system as will be shown below.

2.1.1 The WD kernel

The WD kernel (Rätsch and Sonnenburg, 2004) of order $K$ compares two sequences $x$ and $x'$ of equal length $L$ by counting $k$-mer matches of lengths $k \in \{1, \ldots, K\}$ with predefined weights $\gamma_k$:

$$K(x, x') = \sum_{k=1}^{K} \sum_{i=1}^{L-k+1} \left[ k(x, i) = k(x', i) \right],$$

where $[\cdot]$ is the indicator function (see also Fig. 1 for illustration). A feature mapping $\Phi(x)$ is defined by a vector representing each PO of length $\leq K$: if it is present in $x$ then the vector entry is $\sqrt{\gamma_k}$ and 0 otherwise (Rätsch and Sonnenburg, 2004). It can be easily seen that $\Phi(x)$ is an explicit representation of the WD kernel, i.e. $k(x, x') = \Phi(x) \cdot \Phi(x')$.

When training any kernel method (e.g. an SVM or a kernel regression method) with this kernel, the resulting function is a weighted sum of kernel evaluations (1). If there exist an explicit feature map, the function $f(x)$ can
This is the central equation of this article. When evaluated for all positions $z$ and all the POs in overlap with each other and thus necessarily are dependent. To assess the likelihood of $x$ in a Markov model of order $T$.

2.1.3 Markov model of order $T$. In a Markov model of order $T$, it is assumed that each symbol in a sequence $x$ is independent of all other symbols in $x$ that have distance greater than $T$. Thus, the likelihood of $x$ can be computed as a product of conditional probabilities $P(x)=\prod_{i=1}^{n} P(x[i]|x[i-1],...,x[i-T])$. Note that a positional weight matrix (PWM) is essentially a Markov model of order $T=0$.

The log likelihood for $T=1$ is easily expressed as a linear PO scoring system for $k$-mers as follows: $\log P(x[i]|x[i-1])=\sum_{j=1}^{k} w(x[i]-k+1,...,i-1)+w(x[i]-k+1,i-1)+w(x[i]-k+1,i-k+1)=\log Pr(x[i]|x[i]-k+1)^{k-1}$. A log likelihood ratio is modeled by the difference of two such scoring systems. This derivation applies to both homogeneous (position-independent) and inhomogeneous (position-dependent) Markov models. It further extends to the decision rules of mixed order models.

2.2 POIMs

The goal of POIMs is to characterize the importance of each PO $(x,j)$ for the classification. On first sight, looking at the scoring function (2), it might seem that the weighting $w$ does exactly this: $w(x,j)$ seems to quantify the contribution of $(x,j)$ to the score of a sequence $x$. In fact this is true when the POs are statistically independent. However, already for $K \geq 2$ many POs overlap with each other and thus necessarily are dependent. To assess the influence of a PO on the classification, the impact of all dependent POs has to be taken into account. For example, whenever $x$ occurs at position $j$, the weights of all its positional substrings are also added to the score (2).

To take dependencies into account, we define the importance of $(x,j)$ as the expected increase (or decrease) of its induced score. We quantify this through the conditional expectation of $(x,j)$ conditioned on the occurrence of the PO in $x$ (i.e. on $x[j]=z$):

$$Q(x,j) = E(x[i]|x[j]=z) - E(x[i])$$

(3)

This is the central equation of this article. When evaluated for all positions $j$ and all $k$-mers $x$, it results in a POIM.

The relevance of (3) derives from the meaning of the score $s(x)$. Higher absolute values $|s(x)|$ of scores $s(x)$ indicate higher confidence about the classifiers decision. Consequently, high $|Q(x,j)|$ show that the presence of a $k$-mer at position $j$ in an input sequence is highly relevant for class separation.

Fig. 1. Example of WD kernel of order $K=3$. In the shown case, $k(x,y) = 2\phi_1 + \phi_2 + 3\phi_3$.

be equivalently computed as $f(x) = w^T \phi(x) + b$, where $w = \sum_{i=1}^{n} \phi(x[i])$ (Vapnik, 1995). Given the feature map of the WD kernel, it becomes apparent that the resulting $w$ is a weighting over all possible POs and, hence, $f(x)$ can be written in the form of (2).

2.1.2 Other kernels. The above findings extend to the oligomer kernel (Menonick et al., 2004) and the related WD kernel with shift (Rätsch et al., 2005), which additionally include displaced matches. The spectrum kernel (Leslie et al., 2002) counts pairs of identical $k$-mers independent of their position and can be modeled with (2) by using equal weights at each position $z \in \{1, \ldots, L\}$ (analogously for the spectrum kernel with mismatches, Leslie et al., 2002).

For example, with an SVM trained for splice sites a high-positive score $s(x)$ suggests that $x$ contains a splice site at its central location while a very negative score suggests that there were none. Thus a PO $(x,j)$ of high absolute importance $|Q(x,j)|$ might be part of a splice consensus sequence, or a regulatory element (an enhancer for positive importance $Q(x,j)>0$), or a silencer for $Q(x,j)<0$.

The computation of the expectations in (3) requires a probability distribution for the union of both classes. In this article, we use a zeroth-order Markov model, i.e. an independent single-symbol distribution at each position. Although this is quite simplistic, the approximation that it provides is sufficient for our applications. A generalization to Markov models of order $D \geq 0$ can be found in Zien et al. (2007).

There are two strong reasons for the subtractive normalization w.r.t. the (unconditionally) expected score. The first is conceptual: the magnitude of an expected score is hard to interpret by itself without knowing the cut-off value for the classification; it is more revealing to see how a feature would change the score. The second reason is about computational efficiency; this is shown next.

2.2.1 Efficient computation of $Q(x,j)$. Naive implementation of (3) would require a summation over all $\Sigma^L$ sequences $x$ of length $L$, which is clearly intractable. The following equalities show how the computational cost can be reduced in three steps; for proofs and details see our technical report (Zien et al., 2007).

$$Q(x,j) = \sum_{(y,j) \in x} w(y,j)Pr(x[j]=y|x[j]=z) - Pr(x[j]=z)$$

$$= \sum_{(y,j) \in x} w(y,j)Pr(x[j]=y|x[j]=z) - \sum_{(y,j) \in x} w(y,j)Pr(x[j]=z)$$

(4)

$$= u(x,j) - \sum_{x \in \Sigma^L} Pr(x[j]=z)u(x[j],j).$$

(5)

Here $u$ is defined by $u(x,j) := \sum_{(y,j) \in x} Pr(x[j]=y|x[j]=z)w(y,j).$

(6)

$\sum_{x \in \Sigma^L} Pr(x[j]=z)u(x[j],j)$

$\sum_{x \in \Sigma^L} Pr(x[j]=z)w(y,j).$

$\sum_{x \in \Sigma^L} Pr(x[j]=z)w(y,j).$

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Positional oligomer importance matrices

Fig. 2. Substrings, superstrings, left partial overlaps and right partial overlaps: definition and examples for the string AATACGTAC.

Fig. 3. The theorem which enables efficient POIM computation for zeroth-order Markov chains.

for all k-mers \( z \) up to length \( P \) at all positions \( 1, \ldots, L - p + 1 \), which takes advantage of shared intermediate terms. This results in a recursive algorithm operating on string prefix data structures and therefore is efficient enough to be applied to real biological analysis tasks.

The crucial idea is to treat the POs in \( I(z, j) \) separately according to their relative position to \((z, j)\). To do so, we subdivide the set \( I(z, j) \) into substrings, superstrings, left partial overlaps and right partial overlaps of \((z, j)\), as illustrated in Figure 2. The function \( u \) can be decomposed correspondingly; see Equation (8) in Figure 3. This figure also summarizes all the previous observations in the central POIM computation theorem. The complete derivation can be found in Zien et al. (2007).

Once \( w \) is available as a suffix trie (Sonnenburg et al., 2007a), the required amounts of memory and computation time for computing POIMs are dominated by the size of the output, i.e. \( O(|\Sigma|^kL) \). The recursions are implemented in the freely available toolbox SHOGUN (which also offers a non-positional version for the spectrum kernel) and applicable online.\(^2\)

2available from http://www.shogun-toolbox.org

2.3 Ranking features and condensing information for visualization

Given the POIMs, we can analyze POs for their contributions to the scoring function. Now we discuss several methods to visualize the information in POIMs and to find relevant POs. In the following, we will use the term motif either synonymously for PO or for a set of POs that are similar (e.g. that share the same oligomer at a small range of neighboring positions).

2.3.1 Ranking tables

A simple analysis of a POIM is to sort all POs by their importance \( Q(z, j) \) or absolute importance \( |Q(z, j)| \). As argued above, the highest ranking POs are likely to be related to relevant motifs. Examples of such tables can be found in the Supplementary Material.

2.3.2 POIM Plots

We can visualize an entire POIM of a fixed order \( k \leq 3 \) in the form of a heat map: each PO \((z, j)\) is represented by a cell, indexed by its position \( j \) on the x-axis and the \( k \)-mer \( z \) on the y-axis (e.g. in lexical ordering), and the cell is colored according to the importance value \( Q(z, j) \). This allows to quickly overview the importance of all possible POs at all positions. An example for \( k = 1 \) is found in Figure 6. There also the corresponding sequence logo is shown.

\(^3\)via GALAXY at http://galaxy.fmi.tuebingen.mpg.de/
As a first step we demonstrate our method on two simulations with artificial data. Figure 7C shows the POIM plot for 1mer versus sequence logos for this level of mutation. As one can see, 1mer POIMs can capture the whole sequence logos and identify two clusters of important oligomers at and after positions 7 are important, but also that exactly 7mers at position 10 and 30 are most important.

Moving through the columns to the left, the mutation level increases. Simultaneously the performance deteriorates, as expected. In the second column (2/7 mutations), differential POIMs still manage to identify the exact length of the embedded POs. They do not only display that 7mers up to \( k = 7 \) are important, but also that exactly 7mers at position 10 and 30 are most important.

In the second column (2/7 mutations), differential POIMs still manage to identify the exact length of the embedded motifs, unlike the other approaches. For higher mutation rates even the POIMs assign more importance to shorter POs, but they continue to be closer to the truth than the two other approaches. In Figure 6 we show the POIM plot for 1mer versus sequence logos for this level of mutation. As one can see, 1mer POIMs can capture the whole information contained in sequence logos.
Positional oligomer importance matrices

Fig. 5. Comparison of different visualization techniques for the fixed-position-motifs experiment. Motifs GATTACA and AGTAGTG were inserted at positions 10 and 30 respectively with growing level of mutation (i.e. number of nucleotides randomly substituted in the motifs) from left to right. SVMs classifiers were trained to distinguish random sequences from sequences with the (mutated) motifs GATTACA, and AGTAGTG inserted. (A–D) We computed Differential POIMs [Equation (9)] for up to 8mers, from a WD-kernel SVM of order 20. Here each figure displays the importance of k-mer lengths (y-axis) for k = 1 … 8 at each position (x-axis) i = 1 … 50 as a heat map. Red and yellow color denotes relevant motifs, dark blue corresponds to motifs not conveying information about the problem. 1mers are at the bottom of the plot, 8mers at the top. (E–H) K-mer scoring overview (SVM-w) was computed using the same setup as for differential POIMs. The SVM-w is again displayed as a heat map. (I–L) It was obtained using MKL (averaged weighting obtained using 100 bootstrap runs, Rätsch et al., 2006). Again the result is displayed as a heat map, but for 1-to 7mers only. For a more detailed discussion see text.

3.2 Analysis of biological data

We now demonstrate the power of our technique on three real biological tasks, namely the recognition of splice sites, TSSs and TRSSs. Note that the SVMs investigated here are among the most accurate existing sensors for these signals (cf. Table 1). It is thus of highest interest which clues the SVM uses to distinguish true sites from decoys: the most important POs can be suspected to be the biological traits that determine the cellular events. However, the number of relevant POs may not be small, because (unlike common motif finders) we do not yet pool them. Due to space constraints we have to omit many detailed figures and lists of the extracted

Fig. 6. 1mer POIM plot (A) versus sequence logo (B) for motifs GATTACA and AGTAGTG at positions 10 and 30, respectively, with 4-out-of-7 mutations in the motifs.

Fig. 7. Comparison of different visualization techniques for the varying-positions-motif experiment. The mutated motif GATTACA was inserted at positions 0 ± 13 in uniformly distributed sequences. (A–C) shows the Differential POIM matrices [cf. Equation (9)] as a heat map, the POIM k-mer diversity for k = 3 as a heat map; (D–F) shows the SVM-w overview plot as a heat map, the SVM-w weight mass also for k = 1 … 8 and the k-mer diversity for k = 3 as a heat map; (G) sequence logo.
motifs for the following analyses, which however can be found in the supplementary material.

3.2.1 Splice site analysis First, we apply our techniques to a acceptor splice site recognition task derived from mRNA and genomic sequences of C. elegans. The data set contains 262,421 DNA sequences of length 141 nucleotides, each anchored at a AG, which is the acceptor splice site consensus [see Rätsch et al. (2006) for details]. We train an SVM (C = 1) with a WD kernel (K = 20) on the first 100,000 examples; the resulting classifier achieves 99.7% auROC on the remaining 162,421 examples. POIM results are depicted in Figure 8.

Upstream: A relatively weak, but surprising signal can be seen around 43 nt upstream of the acceptor splice site. Looking at the dinucleotide weighting w (Fig. 8F), one can see an increased weighting for the GT dinucleotide. This leads to the discovery of the upstream donor splice site. In C. elegans this site is indeed often located 40–50 nt upstream of the acceptor splice site. Zooming into the −55 nt to −35 nt window in the differential POIM figure (Fig. 8C and p. 1 in Supplementary Material) one may notice strong signals for up to 7mers. Extracting the three highest scoring 7mers one detect the motifs GGTAAAG, AGTAAAG, GGGTAAAG which have high importances in this whole region, especially around −43 nt. This perfectly matches parts of the known donor consensus AGTAAUG. Upstream in the interval −18 nt to −14 nt we find in the 5mer POIMs that GGGGGGG has a strong negative score, which makes it a potential silencer. Furthermore in the same region one also finds TAAT which is one of the known branch site signals (Harris and Senapathy, 1990).

Central: one can observe a very strong and localized signal in front of the acceptor splice site, which is located at position 0 and followed by the AG consensus at positions 1, 2 [see Rätsch et al. (2006) for more details]. Extracting the highest scoring 7mers in the window from −9 nt to +5 nt one detects several sequences ending on …TTTC directly in front of the splice site. Following this known strong T-rich region are the motifs TTTAGG and TTTTGA scoring highest at −3 nt, recovering the known acceptor consensus TTTTGA (A/G).

Downstream: finally, one also recognizes the strong penalty against T’s downstream of the splice site (+6 nt to +20 nt), where the motif TTTTTTT scores most negative (Supplementary Material).

3.2.2 Promoter regulatory elements In the following we apply these techniques to the search for promoter regulatory elements. We train a classifier with the WDS kernel—the core ingredient of our SVM-based human TSS finder (ARTS; Sonnenburg et al., 2006b) —Drosophila melanogaster TSS detection. We use the same data that have been used for training and evaluation McPromoter (Ohler et al., 2002) and follow the same cross-validation procedure for training and evaluation (optimal SVM parameters after model selection: C = 10, K = 10, shift = 40). Our cross-validation performance is auROC = 96.2%, and comparable to the one achieved by McPromoter (version 2: 95.8% and version 3: 98.1%, (Ohler, 2006). The POIM analysis is displayed in Figure 9. Inspection of POIM tables in the discriminative regions from −33 to −21 upstream, around position 0, and downstream +15 to +35 retrieve the following known motifs:

Upstream: extracting the highest scoring 7mers from the POIM tables in −70 nt to −16 nt let us find several variants of the TATA-box’s core motif TATTTAA (e.g. Burke and Kadonaga, 1997): TATATAA, GTATAAA, ATATATAA, TATATATA, TATAAAG,
AGT and signal 8mers in the one to three Cs within a poly-at−next highest scoring in the 8mer POIM at −scoring 4mer, peaking at findings using POIMs with recent work by Graber CAGTCAGT window 69 808 sequences (4970 positive, 64 838 negative) sites. We used a positive examples. Negative sites were generated from the remaining and by mapping annotated TRSSs to known, i.e. EST/cDNA set has been constructed using the Wormbase WS170 annotation classifier (K [110x273]be distinguished well from other splice site acceptors. Figure10 et al apply our methods to the 3.2.3 Detection of trans-splicing control elements We finally of POs may help to identify such occluded motifs. C/G DPE is hidden by the and its mostly GC-rich variations GTATATA for [110x673]=1, CGT CGTCGCG region have a negative effect. A specific search for the downstream promoter element (DPE) of [110x673]=2 and POIM [110x673]=3, [110x673]=4 are identical to the ones found in these in Graber (2007). In Graber et al. (2007), two classes of TRSS leader sequences were separately analyzed: the SL1 class (trans-spliced to individual or operon-contained genes) and the SL2 class (nearly exclusively found attached to the downstream genes in operons). We do not split the data into two sets, and therefore compute the overlap for both parts (Gramer et al., 2007); part 3 – SL1, part 4 – SL2, table 2). For N=40, the agreement is 15 of 25 and 18 of 25 in SL1 and SL2, respectively. For N=10 it is 9 of 25 and 14 of 25. The agreeing motifs are AAATG, AGAAT, AGATG, CTTTT, GAATG, GATGA, GATGG, GATGT, TAAA, TCTTT, TCTTC, TTTTC, TTTCT, TTTTC, and TTTTTT (made unique). Please note that such large overlaps are very unlikely to happen by chance. The remaining differences can be attributed to the fact that in Graber et al. (2007) SL1 and SL2 were separately compared to vanilla acceptor sites, whereas we do compare SL1 + SL2 with acceptor sites simultaneously, and thus we expect weaker (overlapping) motifs to be harder to detect. And indeed, when looking at the [−60, −50] region, we find 7/8 of the SL1 motifs but only 3/8 of the motifs in the SL2 category; similarly we find 5/5 motifs in the [0, 10] interval for SL2 but only 4/8 for SL1. This suggests that one motif class is more dominant in a certain region. Central: one can clearly observe the high importance of positions around the TRSS and the region ~−50 nt upstream (UR). The POIM plots of order 2 display the dominance for TT, CT and TCs in the UR, and the dominance of AAAs around the TRSS. By extracting 3mers around the TRSS, we find the high ranking ATG start codon. This finding again perfectly matches the prior reported fact that the distance between TRSSs and the TIS is often very short (often 0 nt) (Graber et al., 2007). 4 DISCUSSION Modern kernel methods with complex, oligomer-based sequence kernels are very powerful for biological sequence classification. However, until now no satisfactory tool for visualization was available that helps to understand their complex decision surfaces. In this work we close this gap by introducing a method which efficiently computes the importance of POs defined as their expected contribution to the score. Different from the discrimination normal vector w, the importance takes into account the correlation structure of all features. We illustrated on simulated data how the GTATATA etc., with a peak score at −29 (see Supplemental Files at http://www.fml.tuebingen.mpg.de/raetsch/projects/POIM). Central: one finds the motif CAGT at positions −1 to +3 (and it’s repeated version CAGTCAGT as a high scoring 8mer); as the next highest scoring in the 8mer POIM at −15 to +9 CAGTTGT at −1, CAGTTTTT at −3, GTTTTTT at −2, GGTCTTTT at −3 and AGTTCTTTT at 0 are detected. These all match the known signal CAGTTT from the initiator (Inr) element consensus TCA $>$ +7 (cf. Arkhipova, 1995; Burke and Kadonaga, 1997). Downstream: many CG-rich motifs score high for 2-, 4-, 6-, 8mers and peak at ≈+23 nt, which is consistent with CpG-overrepresentation downstream of the TSS. At positions +15 to +22 nt, the oligomer CAGTTTTT and its mostly GC-rich variations score highest while ATATTAT (and AT-dominated variants) in this region have a negative effect. A specific search for the downstream promoter element (DPE) C^{2+23nt}−CAGTTTTT reported in Arkhipova (1995) and Burke and Kadonaga (1997)—in the POIM 8mer rankings finds AGACGTG ranked 1024 (top 8%) with an importance weight half of the highest scoring CAGTTTTT. This shows that the DPE is hidden by the C/G-richness as dominating effect. Clustering of POs may help to identify such occluded motifs. 3.2.3 Detection of trans-splicing control elements We finally apply our methods to the trans-splice-detective power used in the mGene finding (Schweikert et al., manuscript in preparation). The data set has been constructed using the Wormbase WS170 annotation and by mapping annotated TRSSs to known, i.e. EST/cDNA confirmed genes (if possible). The remaining TRSSs were used as positive examples. Negative sites were generated from the remaining successfully mapped interior acceptor sites, leading to a total of 69 808 sequences (4970 positive, 64 838 negative) sites. We used a window −190 to +70 nt around the splice site and train an SVM classifier (K = 30) on 60% of the data. The randomization test that this classifier achieves auROC of 95.2%, indicating that TRSSs can be distinguished well from other splice site acceptors. Figure 10 displays the POIMs computed based on this classifier. Upstream: in the region −150 nt to −71 nt TTTTT is the highest scoring 4mer, peaking at −78 nt. We then extract the top 40 scoring 8mers in the −70 nt to −21 nt window and find that they all contain one to three Cs within a poly-C motif. Finally, for comparison of our findings using POIMs with recent work by Graber et al. (2007), we extract the top N scoring 5mers separately for each of the windows [−60, −50], [−50, −40], [−40, −30], [−30, −20], [−10, 0], [0, 10]; we then verify how many of the motifs we determined are identical to the ones found in these in Graber et al. (2007). In Graber et al. (2007), two classes of TRSS leader sequences were separately analyzed: the SL1 class (trans-spliced to individual or operon-contained genes) and the SL2 class (nearly exclusively found attached to the downstream genes in operons). We do not split the data into two sets, and therefore compute the overlap for both parts (Graber et al., 2007); part 3 – SL1, part 4 – SL2, table 2). For N=40, the agreement is 15 of 25 and 18 of 25 in SL1 and SL2, respectively. For N=10 it is 9 of 25 and 14 of 25. The agreeing motifs are AAATG, AGAAT, AGATG, CTTTT, GAATG, GATGA, GATGG, GATGT, TAAA, TCTTT, TCTTC, TTTTC, TTTCT, TTTTC, and TTTTTT (made unique). Please note that such large overlaps are very unlikely to happen by chance. The remaining differences can be attributed to the fact that in Graber et al. (2007) SL1 and SL2 were separately compared to vanilla acceptor sites, whereas we do compare SL1 + SL2 with acceptor sites simultaneously, and thus we expect weaker (overlapping) motifs to be harder to detect. And indeed, when looking at the [−60, −50] region, we find 7/8 of the SL1 motifs but only 3/8 of the motifs in the SL2 category; similarly we find 5/5 motifs in the [0, 10] interval for SL2 but only 4/8 for SL1. This suggests that one motif class is more dominant in a certain region. Central: one can clearly observe the high importance of positions around the TRSS and the region ~−50 nt upstream (UR). The POIM plots of order 2 display the dominance for TT, CT and TCs in the UR, and the dominance of AAAs around the TRSS. By extracting 3mers around the TRSS, we find the high ranking ATG start codon. This finding again perfectly matches the prior reported fact that the distance between TRSSs and the TIS is often very short (often 0 nt) (Graber et al., 2007).
visualization of POIMs can help to identify even vaguely localized motifs where pure sequence logos will fail. On three genomic signal detection applications we demonstrated that many previously known regulatory patterns can be recovered with POIM-based ranking and visualization tools.

Note that the structure of the feature spaces of the considered string kernels allows us to accurately learn complex local correlations as opposed to long range correlations which are for instance modeled in Bayesian networks (Barath et al., 2003; Ben-Gal et al., 2005; Chen et al., 2005). It therefore seems surprising that Bayesian networks fail to achieve state-of-the-art results for tasks like the splice site recognition (Chen et al., 2005; Sonnenburg et al., 2007b). This suggests that for such tasks explicit modeling of long range relationships is not mandatory. Also, many other motif discovery algorithms and discrimination methods have been proposed before, but they typically come at the price of decreased classification accuracy as compared to SVMs with exhaustive kernels. It therefore seems promising to thoroughly analyze the SVM’s decision boundary to understand the nature of the differences between the classes. Different from our previous MKL approach (Rätsch et al., 2006), we propose here to leave the SVM untouched for classification to retain its high accuracy, and to defer motif extraction to subsequent steps. This way motif finding can take advantage of the SVMs power: it ensures that the truly most important POIs are identified.

Even though we have shown the usefulness of POIs and their visualization, for instance our analysis of promoter regulatory elements for D. melanogaster reveals several obstacles to systematic motif discovery with POIMs. First, we realize that the list of POIs can be prohibitively large for manual inspection. Hence, if several discriminative motifs occur in the same sequence region, we currently only find the strongest ones. Second, if a motif has positional variance exceeding its length, we also find its duplicate zz. This effect gets even stronger for self-overlapping motifs (like AAAG where appending a single further A already yields a second motif occurrence). We currently work on clustering approaches to summarize POIs scoring high in similar regions to obtain motifs like PWMs with positional preferences. We believe that such approaches will solve both problems.

Additional work in progress includes computational techniques to efficiently determine the highest scoring ‘consensus’ sequence x+=argmaxx 〈x|z〉 which can be of interest for sequence design applications. Finally, note that protein sequences are known to show dependencies of XOR type, e.g. a pair of a positively and a negatively charged amino acid that can swap their positions. As such dependencies escape sequence logos, but can be modeled by POIMs, an implementation of protein POIMs seems desirable.

We believe that our new POIM-based ranking and visualization algorithms are easy to use yet very helpful analysis tools. It is freely available as part of the SHOGUN toolbox (Sonnenburg et al., 2006a) at http://www.shogun-toolbox.org. Finally, note that it seems possible to transfer the underlying concept to other kernels and feature spaces to aid understanding of SVMs that are used for other biological tasks.

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