COMP 755 Final Project: Adapting Target-Decoy Competition For Real Time Experiments

Trent Stohrer

November 30, 2018

1 Abstract

One of the key computational tasks of proteomics is matching data from mass spectrometers (mass spectra) to a database of theoretical mass spectra of peptides based on known protein amino acid (AA) sequences. My main project in the lab has been based on doing these database matches in real-time to more efficiently decide when the mass spectometer (MS) should perform various types of scans during our experiments. Currently, these decisions are based on a static threshold; only if a candidate database match is above a pre-defined cross correlation threshold will the MS perform a scan dedicated to quantifying the peptide. However, using a static threshold leads to many quantification scans for peptides whose AA sequences are not reliably identified, and these false positives waste the MS's limited resources. Many of the advancements in improving database search identification rates have been based on machine learning methods that incorporate multiple features. The general method for training these models is a per-experiment semi-supervised binary classification model that is trained after all the data has been acquired. In this model, "target" peptides from a database are considered to be "possibly" positive examples, and decoy peptides, created by reversing or shuffling real peptide sequences, are used as negative examples on which to train. In this project, I evaluate the effectiveness of various classification schemes that do not use the full data set, including training on previous experiments, training on the first n scans, and classification using

online learning, the goal of all of these being to better identify peptides in real-time, with an emphasis on reducing false positives, and therefore improve decision making with regards to which scans the MS should perform next.

2 Introduction

Semi-supervised methods for predicting peptide-spectrum matches (PSMs) are based on the concept of target-decoy competition, which uses the aforementioned shuffled peptide sequences as definitive negative examples for training, and controls the false discovery rate using the number of decoys that are misclassified as correct PSMs. On the other hand, PSMs that map to target peptides are an unknown mixture of positive and negative matches. The simplifying assumption of the target-decoy competition is that incorrect matches are equally likely to be assigned to either a decoy or target peptide. Therefore, for every decoy sequence which is misclassified as a correct match, we expect there is also a target peptide that is misidentified as a correct match. I will further expound on the repercussions of semi-supervision in the methods section.

Without going too far in depth, the features we use are all meant to be indicative of a good peptide-spectrum-match or to otherwise inform how the other features should be interpreted. For an example of the first, IonFrac is a fraction of how many of the ion peaks we expect based on the theoretical spectrum are actually present in the experimental spectrum. The general idea here is that in a good match we will see most, or almost all of the ion peaks we expect to see. For the other type of feature, charge1-5 is a good example. These are five "one hot" features that represent the charge state of the ion in the experimental spectra. It is common to require higher cross correlation scores for peptides with higher charge, so by including these features, the hope is the classifier will learn this aspect of the boundary. [1]

[1]For a full description of each feature used see: https://media.nature.com/original/nature-assets/nmeth/journal/v4/n11/extref/nmeth1113-S1.pdf. Of those features the ones we actually used were XCorr, δC_n, δC_n^L, Sp, ln(rSp), δM $abs(\delta M)$, ionFrac, ln(NumSp), enzInt, pepLen, charge1-3 (expanded to 1-5). We had access to Mass, enzN, and enzC as well, but due to some processing errors not noticed until very late, these were not included in the data import.

3 Methods

The model used by the algorithm we wanted to approximate (Percolator) is SVM, so we decided to go with the sklearn python packages SGDClassifier using hinge loss and L2 regularization to build a similar model that would allow for online learning, though this did limit us to linear SVM. We also standardized our data, based on the understanding that SGD classifiers can be sensitive to data scaling issues. For all the schemes we used, we used the means and standard deviations of the training set to scale all of the data, as in practice we would not have access to these overall values for a live experiment. To generate the input data we ran our own experimental data (mzML mass spec file) through the tide database search as part of the crux analysis toolkit, stripped out the non-feature columns, and sorted by scan number to simulate the arrival time of an actual experiment.

As a note for terminology, from here I will refer to PSMs which appear to be correct matches to target peptides as target matches. Semi-supervision means that the labels for potential target peptides will change as the classifier is better able to differentiate decoy and incorrect targets from the correct target matches. This leads to a programmatic structure where we go through multiple rounds of training, finding a new false-discovery-rate-controlled threshold for each round, re-classifying based on that threshold and the current classifier, randomly selecting new false examples, and re-training in each round.

Semi-supervision also means that we have to bias our initial positive examples or we will be learning the boundary between targets and decoys rather than the boundary between target matches and all non-matches (both decoys and targets). We actually end up using the same feature as the static threshold, cross correlation, controlled for false discovery in the training set, as the initial direction for classification before subsequent rounds classified based on the SGDClassifier for that round. All of the methods we tried use the same general training sequence: pick an FDR-controlled cross correlation threshold, do nine additional rounds of training with their own FDR-controlled SVM score thresholds then test on the test set. Since we have access to all the classifier scores as we go, we can write these out to a file and select a false discovery after the fact. In an actual experiment we would need to select our false discovery rate before hand, but in this setting we can build a ROC curve to evaluate the selectivity/sensitivity tradeoff for a variety of FDRs. The difference between the methods is how much additional training we do,

how we do the additional training, what we use as the training set, and to a smaller degree how we use the threshold. I will go into more detail on the difference between methods in the results section. I did not find the time to tweak the parameters of the SGDClassifier, but I did tweak and test some other parameters important to the semi-supervised method. For all of the methods I changed the initial direction false discovery rate as well as a separate false discovery rate for the learning thresholds and the final threshold. For the"first N" methods I changed the FDR's as well as the N. There was not sufficient time to fully test different values for these parameters, and I did not get to change batch size for the online learning models at all, but my results have included a few different results where relevant. The results are based on two distinct experiments, a whole cell lysate experiment with no labeling and no MS3 scans, MA1923, and a MIBMS experiment with labeling and interleaved MS3 scans, MA1750.

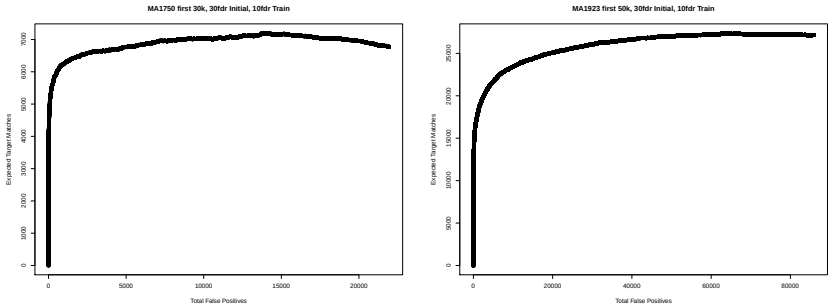
4 Results

The first method we tried was to use a previous, similar experiment as the training set and to test on a subsequent experiment. This limited us to only testing on MA1750, as getting data for an experiment similar to MA1923 was not time-feasible. Before I present the results, I need to explain the columns of the chart, which will apply to subsequent charts. I have included a row for static in each chart, though this will be the same regardless of the method were looking at. All columns between "Static or ML" and "TM Found" are the parameters we tweaked. "TM Found" is the number of True Matches found in the test set, here we are using the results of percolator run on the test set using a false discovery rate cutoff of 1% to define which matches are "True Matches." "TM Missed" is the number of True Matches found by percolator that we did not find with the given parameters and classifier and "False TM" is the number of targets that the classifier mistakenly classifies as True Matches (mistakenly, as in Percolator does not identify them as such). Finally, "Test Set FDR" is the false discovery rate of the classifier over the test set, here calculated as (2 * decoys as targets) / (decoys as targets + targets as targets), where we double the numerator from the assumption that each matched decoy has an equivalent mistakenly matched target. The results for various parameters can be seen in Table 1.

Next we tried using the first N scans of the experiment, each having

Data Set	Static or ML	Initial FDR	Threshold FDR	TM Found	TM Missed	False TM	Test Set FDR
MA1750	Static	N/A	N/A	6836	107	4760	41.4%
MA1750	ML	10%	10%	4178	2765	26	0.332%
MA1750	ML	20%	10%	5567	1367	104	1.44%
MA1750	ML	30%	10%	6191	752	184	2.23%
MA1750	ML	20%	20%	6512	431	267	2.88%
MA1750	ML	30%	20%	6752	191	907	9.31%

Table 1: Previous Scan Learning



(a) MA1750 ROC Curve (b) MA1923 ROC Curve

around 5 targets and 5 decoys, as the training set, and the remaining E-N scans as the test set (Table 2). Here we also made ROC curves for our various attempts, for reasons I will explain when we get to our first attempt at online learning. These are a modified version of the typical ROC curve, and can be found above as figures a) and b). Here both axes are raw counts rather than percentages, with the x-axis being decoys identified as targets for the given threshold, and the y axis being targets identified as targets minus the number of decoys identified as targets, using the target-decoy competition assumption. Note that in these ROC curves we do not use the results of percolator as our ground truth.

In our next model we do the same initial training as the first N training, but do subsequent additional training using SGDClassifiers partial_fit method on additional batches of scans to further refine the classifier. One of the issues with this method is that each additional fit changes what FDR rate any given score threshold will represent. You can see this in Table 3 below as a 10% FDR cutoff learned right before testing can lead to drastically

Data Set	Static or ML	N	Initial FDR	Threshold FDR	TM Found	TM Missed	False TM	Test Set FDR
MA1750	Static	30,000	N/A	N/A	5349	83	3475	41.0%
MA1750	ML	30,000	1%	1%	4544	888	149	4.34%
MA1750	ML	30,000	10%	10%	5322	110	869	8.98%
MA1750	ML	30,000	20%	10%	5312	120	921	13.1%
MA1750	ML	30,000	20%	20%	5349	83	1533	18.9%
MA1750	ML	30,000	30%	10%	5352	80	944	9.56%
MA1750	Static	20,000	N/A	N/A	6236	91	4169	40.6%
MA1750	ML	20,000	30%	10%	5974	353	332	2.66%
MA1923	Static	50,000	N/A	N/A	18363	349	7464	27.6%
MA1923	ML	50,000	30%	10%	18286	426	2144	8.86%
MA1923	Static	40,000	N/A	N/A	19471	367	8158	27.7%
MA1923	ML	40,000	30%	10%	18965	873	1916	8.14%
MA1923	Static	30,000	N/A	N/A	20501	400	8896	28.1%
MA1923	ML	30,000	30%	10%	20255	646	2281	8.33%

Table 2: First N Scan Learning

different results. Since finding the FDR constantly in small batches takes too much time, we chose to use the results from the ROC curves of the first N method and statically use that threshold to test the online learning method, doing multiple runs at each threshold to test the stability. Each of these experiments uses batch size 5.

The online learning results were not where we wanted them to be, and from here we could have gone two different ways: we could have seen how adjusting the threshold on each partial fit works, or we could do a full retrain using the first N+x*batch_size scans as the train set. We decided to go with this second method (Table 4). The results in this table both use batch size 1000.

5 Discussion

With just parameter tweaking all of our models are capable of having nearly as much sensitivity as the static threshold, or more sensitivity in some cases, while having considerably more specificity, as evidenced by the large decreases in both False TM and Test Set FDR across all of the methods and

Data Set	Iter -ation	N	Initial FDR	Threshold FDR	Classifier Threshold	TM Found	TM Missed	False TM	Test Set FDR
MA1750	1	30,000	30%	10%	-7.57	5357	75	3899	43.9%
MA1750	2	30,000	30%	10%	-7.57	5358	74	3326	39.4%
MA1750	1	30,000	30%	10%	-4.19	5345	87	2537	32.7%
MA1750	2	30,000	30%	10%	-4.19	5339	93	1652	21.4%
MA1750	1	30,000	30%	10%	-0.73	5294	138	1120	17.7%
MA1750	2	30,000	30%	10%	-0.73	5306	126	938	14.6%
MA1750	1	30,000	30%	10%	Runtime 10%	5168	264	504	5.19%
MA1750	2	30,000	30%	10%	Runtime 10%	4648	138	784	1.82%
MA1923	1	50,000	30%	10%	-1.63	18561	151	8712	27.6%
MA1923	2	50,000	30%	10%	-1.63	18557	155	8952	27.3%
MA1923	1	50,000	30%	10%	Runtime 10%	9442	9270	164	1.06%
MA1923	2	50,000	30%	10%	Runtime 10%	11046	7666	180	0.799%

Table 3: Simple Online Learning

Data Set	N	Initial FDR	Threshold FDR	TM Found	TM Missed	False TM	Test Set FDR
MA1750	30,000	30%	10%	5349	83	832	9.97%
MA1923	50,000	30%	10%	17170	1542	1074	10.0%

Table 4: Full Retrain Online Learning

parameter configurations when compared to the static method. All of these methods seem good enough to serve as replacements for the static threshold. The previous experiment method has the drawback that we would have to run a less efficient experiment every time we wanted to build a classifier for a new type of experiment, and we would have to manually retrain the model somewhat frequently, which would involve a lot of additional engineering to make it easy for biologists to use. The strength of the simple first N model is somewhat surprising, and very encouraging, and it alone could serve as a useful model going forward, though we would want to perform more tests on a wider variety of experiments and work on refining the ideal N and FDR rates. As an example of such tweaking, in table 2 it's clear that though a learning FDR of 1% does an excellent job of controlling False TMs, it is too restrictive of a rate and ends up missing about 800 of the 5300 TMs that the static threshold finds for MA1750.

The online learning model with static pre-picked thresholds has the same issue as the previous experiment training, that we need to run other experiments to find a good threshold, and the results of the small batch online learning with FDR-control after initial training are far too volatile for us to be comfortable using that method. Finally, the full retrain online learning has quite good results on the shorter MIBMS experiment but has issues with the longer full lysate experiment. My suspicion is that so many rounds of re-training leads to an overfit that has us losing candidates, leading to the weaker results, though I would like to investigate this more.

All of these small shortcomings and caveats of the various methods said, these are excellent results. We were able to not only dramatically decrease the number of false positives but also even capture some of the scant TMs not already picked up by the static threshold. The huge decrease in false positives will allow the mass spectrometer to waste less time performing unnecessary quantification scans on peptides that won't be identified. This in turn will allow the MS to dedicate more time to other scans that we can classify, and increase the number of identifications and quantifications we are able to get out of our experiments.

6 Future

Even in the results section it is clear there are some directions that should have been further explored. How well would online learning with just thresh-

old picking have worked? How feasible is the larger retraining method and what engineering would need to be done in terms of concurrency to use it? How big of a difference do the parameters I didnt test for (such as batch size) make? There is also a gap between how well my classifier performs and how well percolator performs that I would like to close, even when using the previous experiment method on the same experiment. I think part of the gap is likely from me not using cross validation methods in the training. I also only ever evaluate the top cross correlation candidate for each spectra, so that the comparison between the static threshold and the ML classifier is apples-to-apples, as thats the only candidate the static threshold ever considers. To get a better comparison with percolator I would want to use the best scoring candidate in terms of the classifier, not cross correlation. Finally, in terms of feasibility most of the features are trivially simple to generate or are already generated by the static methods, with two exceptions, both based on the same metric, sp. We do not currently calculate this metric so in the future I would like to be able to evaluate these classifiers without using it, or alternatively implement its calculation in our real time pipeline and time how long these additional calculations take. Once we have the features and the classifier in memory we should have ample time to classify in sufficient time for the MS to make real time decisions, but this is another aspect of feasibility that we did not quite get to.