Draft prepared for arXiv.
Manuscript information: 10 text pages, 2 figures, 3 tables.

Mining Mass Spectra: Metric Embeddings and Fast Near Neighbor Search

Debojyoti Dutta, Ting Chen

Molecular and Computational Biology Program
University of Southern California
Los Angeles, CA 90089-2910

February 9, 2008

\[1\text{To whom correspondence should be addressed. Molecular and Computational Biology Program, University of Southern California. MCB 201, 1050 Childs Way, Los Angeles, CA 90089-2910. E-mail: ddupta@usc.edu, tingchen@usc.edu Tel: (213)740-2416, (213)740-2415. Fax: (213)740-8631.}\]
Abstract

Mining large-scale high-throughput tandem mass spectrometry data sets is a very important problem in mass spectrometry based protein identification. One of the fundamental problems in large scale mining of spectra is to design appropriate metrics and algorithms to avoid all-pair-wise comparisons of spectra. In this paper, we present a general framework based on vector spaces to avoid pair-wise comparisons. We first robustly embed spectra in a high dimensional space in a novel fashion and then apply fast approximate near neighbor algorithms for tasks such as constructing filters for database search, indexing and similarity searching. We formally prove that our embedding has low distortion compared to the cosine similarity, and, along with locality sensitive hashing (LSH), we design filters for database search that can filter out more than 98.9% of peptides (118 times less) while missing at most 0.29% of the correct sequences. We then show how our framework can be used in similarity searching, which can then be used to detect tight clusters or replicates. On an average, for a cluster size of 16 spectra, LSH only misses 1 spectrum and admits only 1 false spectrum. In addition, our framework in conjunction with dimension reduction techniques allow us to visualize large datasets in 2D space. Our framework also has the potential to embed and compare datasets with post translation modifications (PTM).

1 Introduction

Proteomics aims to analyze proteins and peptides expressed by the dynamic biological processes within cells \[ [15, 1] \]. Proteins are responsible for many inter and intra-cellular activities such as metabolism and cell signaling where proteins are often modified after translation within cells \[ [13, 19] \]. In the post-genomic era, one of the most important problems is to characterize the proteome, i.e. the set of proteins within an organism.

Tandem mass spectrometry is one of the most promising and widely used high throughput techniques to analyze proteins and peptides \[ [15, 1] \]. It comprises of two stages. A protein mixture is enzymatically digested and separated by HPLC (High Performance Liquid Chromatography) before inserting into a mass spectrometer through a capillary. Then the peptides gets ionized and their precursor ion masses, or mass/charge ratios, are measured. This is the MS1 stage. The peaks (or ionized peptides) from the MS1 stage are selected and further fragmented in a second stage using techniques such as Collision Induced Dissociation (CID) to yield the MS2 fragment ions. Ideally, each peptide gets cleaved into two parts. The N-terminal ion (b-ion) represents the prefix while the C-terminal ion (y-ion) is the suffix. This stage is also known as the tandem MS or the MSMS stage. For more details beyond this oversimplified description, the reader is directed to the wonderful survey \[ 1 \].

There are two main approaches to analyzing tandem mass spectra data. First, and the most widely used, is the database search method \[ [10, 14, 20, 2] \]. Here, peptides from a sequence database are digested in-silico and the resultant virtual spectra are matched (or scored) with the real spectra. High scored peptides are typically chosen as the peptide candidates. This method leads to a combinatorial explosion when used to search for Post Translational Modifications (PTMs) \[ 19 \]. Second, the de-novo method \[ [4, 3, 12] \] reconstructs the sequence without the help of a database. Other approaches combine denovo sequencing and database search by first generating sequence tags, or subsequences, and then using these tags \[ 6 \] as filters for database search with and without PTMs \[ 17 \].

The promise of tandem mass spectrometry has led research groups to routinely use this method to probe the proteomes. A single run of a mass spectrometer can generate several thousands of spectra, and the sheer size as well as the number of real life mass spectra datasets is predicted to grow at an unprecedented rate with laboratories operating several spectrometers in parallel, round the clock. Thus, efficient mining of these large-scale mass spectra data to obtain useful clues for biological discovery is a very important problem.

Mining large spectra has several challenges, some of which are presented below. 1) Indexing huge databases of mass spectra is not standardized. Commonly used methods use precursion ion mass but this method has two main problems: i) there can be errors in precursor ion masses. ii) there may be many spectra (several thousands of them) that have masses close to each other. 2) It is difficult to search for similar spectra on a large scale quickly, or in sublinear time. This is a core function used by several data mining applications. 3) Clustering large databases of
spectra is a daunting task. Most similarity measures proposed in tandem mass spectrometry use pairwise metrics for similarity. Such pairwise methods lead to an explosion of similarity calculations, i.e. $O(n^2)$ for a set of $n$ spectra. Thus, a key open problem is to use methods that avoid the pairwise similarity calculations. If objects can be transformed into metric spaces, problems such as similarity searching and clustering becomes easier. Thus we need to find methods to robustly embed spectra in metric spaces.

4) Visualization of large groups of mass spectra is an important problem which can also be used to qualitatively identify outliers in the huge number of spectra produced.

In this paper, we present a general framework for large scale mining of tandem mass spectra. Our main contributions are the following: 1) We robustly embed spectra into a metric space, 2) We show, both formally and empirically that distances using our embedding areas good as those that use the well known cosine method. 3) Then we use apply a geometric fast near neighbor search technique, Locality Sensitive Hashing (LSH) \cite{5}, to solve several problems such as fast filters for database search, similarity searching of mass spectra, and visualization of large spectral database. 4) Our embedding in conjunction with PCA and manifold learning can be used to visualize large groups of spectra. 5) Our embedding holds promise for comparing spectra with Post Translational Modifications (PTM).

Our idea of robust embedding of vector spaces to mine mass spectra is novel. Previous work to embed spectra into vector spaces using vectors of amino acid counts to database search \cite{8, 9}. They focussed on clustering sequence databases based on this amino acid counts to search for mass spectra, given amino acid counts or sequence tags. However, getting an accurate estimate of amino acid composition is itself a hard problem, especially when the quality of spectra is not high. However, our method embeds ion fragments of spectra directly into a vector space and avoids estimating higher level features such as amino acid composition. Also our scheme is more general: using a single embedding, we can either compare spectra with each other or compare spectra with peptide sequences by generating their virtual, or in-silico digested, spectra. In addition, we demonstrate that our framework can be used in concrete mining applications. We first use our embedding along with Locality Sensitive Hashing to speed-up database search. We demonstrate that we can filter out more than 99.152% spectra with a false negative rate of 0.29%. The average query time for a spectra is 0.21s. Then, we answer similarity queries and find replicates or tight clusters. LSH misses an average of 1 spectrum per cluster, that have an average cluster size of 16 spectra, while admitting only 1 false spectrum.

To the best of our knowledge, we are not aware of any other work that robustly embeds spectra in metric spaces with provable guarantees and then uses fast approximate near neighbor techniques to solve mass spectrometry data mining problems.

2 Methods

Our approach is to use vector spaces which have been successful in numerous data mining applications including web search\cite{web mining}. Several fast mining algorithms become simpler to design in these spaces, compared to designing them in non metric spaces e.g. spaces where the only available measure is a pairwise similarity measure. Thus, the key problem in this approach is to robustly embed spectra into a high dimensional metric space and define appropriate distances. Also, these distances must be correlated with the well known cosine similarities. In other words, we desire an embedding with bounded distortion with respect to the cosine similarity.

2.1 Embedding Spectra

Noise Removal

The achilles heel of tandem mass spectra analysis is the amount of noise in the mass spectra. In fact, most peaks (around 80%) cannot be explained and are called ‘noise’ peaks. ‘Signal’ peaks (such as b, y ions) are useful for interpretation. As a first step, we remove noise peaks enriching the signal to noise ratio (SNR).

We use a statistical method to increase SNR. We first find the intensity distributions of signal and noise peaks in a set of annotated spectra. For this, we consider a set of good quality annotated spectra as
described in Section 3 and generate the virtual spectrum $v_p$ for each of the real spectra $r_p$ for a peptide $p$. For the virtual spectrum generation we consider the following ions: $b$, $b - H_2O$, $b - NH_3$, $y$, $y - H_2O$, $y - NH_3$. Then we divide the mass range of $r_p$ into $k = 10$ sections. For each section, and for each real peak, we consider its intensity rank i.e. the most intense peak has rank 0 and so on. We divide the peaks of $r_p$ into two sets $S_p$ and $N_p$. $S_p$ contains all those peaks, and their intensity ranks, which have a match in the virtual spectrum $v_p$. Thus, for each region, we can get a distribution of signal and noise intensity ranks for each region as shown in Figure 2.1.

We define a metric SNR of a peak $(mz_j, I_j)$ as follows

$$SNR(j) = \frac{P[\text{rank}(j) | (mz_j, I_j) \in S_p]}{P[\text{rank}(j) | (mz_j, I_j) \in N_p]}$$

If larger SNR, the peak is likely to be a useful peak, else its a noise peak. From Figure 2.1 we can conclude that the noise is very poor at the ends of the spectra, i.e. at low mass regions and high mass regions. This statistical observation reinforces the mass spectrometry folklore that the middle region is the most suitable for finding signal peaks.

Features and Distances

There are several possible ways to embed tandem mass spectra into a vector space that support the most common operation of comparing two spectra and find similarities. For example, the cosine similarity metric [10] and their different variants have been very popular in the recent papers. Unfortunately the cosine metric does not yield a metric embedding because the triangle inequality is violated. Also the cosine similarity metric implies algorithms that consider pairs of spectra. Clearly such algorithms are difficult to scale due to the $O(n^2)$ number of similarity calculations.

For metric embeddings, the design space is quite large. A simple idea is to directly bin the peaks and use the intensities to form a vector space. However spectra from different datasets have different intensities and we would like to have a single embedding that could potentially integrate multiple spectral databases.

We first clean spectra as mentioned in the previous subsection. Then we divide the entire mass range (from 0 to some maximum range) into discrete intervals of $2da$. For each interval of $2da$, a bit is set to 1 if the cleaned spectrum contains a peak in that interval, else it is 0. This embeds each spectra into the vertices of a n-dimensional hypercube. A 3D version is shown in Figure 2.1. Our feature vectors are defined to be the unit vectors in the direction of the corresponding vertices of the n-dimensional hypercube. Thus the space of our embedding is a n-dimensional
unit hyper-sphere.

We define the spectral similarity or distance between spectra \( x, y \), as \( ||x - y|| \). If the angle between two similar spectra \( x, y \) is \( \theta \), \( \cos \theta \) will be close to 1, or \( 1 - \cos \theta \) will be very small. Since \( x, y \) are unit vectors, their Euclidean distance will also be small. Thus, for small angles, \( 1 - \cos \theta \approx D(x, y) \), where \( D \) is the Euclidean distance. It is easy to show that as \( n \) or the number of dimensions increases, the minimum angle for pairs of very similar spectra \( x, y \) becomes smaller. Thus, instead of calculating the \( 1 - \cos \theta \), we calculate \( D(x, y) \). The natural question that arises is the distortion of our embedding. We will now show that it is has bounded accuracy in theory, and we will later show that the accuracy is empirically quite high in comparison with the cosine similarity.

We prove some properties of the embeddings. It is easy to show the following theorem:

**Theorem 2.1.** The embedding discussed above defines a metric space.

**Proof.** The proof is very simple. To show that our embedding defines a metric space, we need to prove three things: 1) \( ||x - y|| = 0 \) iff \( x = y \), 2) \( ||x - y|| = ||y - x|| \) and 3) the distance measure obeys the triangle inequality. These properties are trivial to prove in our case as our embedding uses Euclidean distances.

We then show that the maximum euclidean distance is bounded by \( \sqrt{2} \).

**Lemma 2.1.** The distance between the feature vectors of any two mass spectra is bounded above by \( \sqrt{2} \).

**Proof.** Suppose there are two spectra \( x, y \) respectively. We shall use the names of the spectra and their feature vectors interchangeably. According to our scheme we first filter the noisy peaks and generate the binary vector after binning. Now assume \( x \) has \( k \) bits set to 1 and \( y \) has \( k' \) bits set to 1. Also assume that \( c \) of the common bits are 1. Then \( ||x|| = \frac{1}{k} \) and \( ||y|| = \frac{1}{k'} \). Since \( c \) bits are common, the number of dissimilar bits between \( x \) and \( y \) are \( (k - c) + (k' - c) \). We have

\[
||x - y|| = \sqrt{(k - c) \cdot \left( \frac{1}{k} \right)^2 + (k' - c) \cdot \left( \frac{1}{k'} \right)^2}
\]

Next we show that our embedding has bounded distortion when we compare with the well known cosine similarity. We have the following theorem:

**Theorem 2.2.** If \( \theta \) is the angle made by the feature vectors of spectra \( x, y \), and the number of ones in each of the vectors after binning is the same we must have \( 0 < \frac{1 - \cos \theta}{||x - y||} < \frac{1}{\sqrt{2}} \). Or in other words, the distortion between our Euclidean embedding and the cosine similarity is bounded.

**Proof.** As in the previous lemma, \( ||x - y|| = \sqrt{2 - c \cdot \left( \frac{1}{k} + \frac{1}{k'} \right)} \). Now the cosine of the angle \( \theta \) between \( x, y \) can be written as \( \cos \theta = \frac{c}{\sqrt{kk'}} \). Assume \( k = k' \) and note that \( 0 \leq \frac{c}{k} \leq 1 \). Thus, we must have

\[
\frac{1 - \cos \theta}{||x - y||} = \frac{1 - \frac{c}{\sqrt{kk'}}}{\sqrt{2 - c \cdot \left( \frac{1}{k} + \frac{1}{k'} \right)}} \leq \frac{1}{\sqrt{2}} \cdot \frac{1 - \frac{c}{k}}{\sqrt{2 - 2 \cdot \frac{c}{k}}} \leq \frac{1}{\sqrt{2}} \cdot \frac{1 - \frac{c}{k}}{\sqrt{2 - 2 \cdot \frac{c}{k}}}
\]

We note that since, \( 0 \leq \frac{c}{k} \leq 1 \), we must also have \( 0 \leq 1 - \frac{c}{k} \leq 1 \) and the theorem follows.

Thus our embedding will perform almost as good as the standard cosine metric. We show in the next section that this is indeed the case, empirically. Also, since the points are in a Euclidean space, we can elegant geometric techniques that yield fast approximate algorithms for mining the data.

### 2.2 Similarity Searching

The ability to calculate distances as opposed to cosines is an important feature of our framework. Now, we apply elegant near neighbor algorithms to answer queries quickly but approximately, as we show in the paper. The basic query primitive we use is the following:
Primitive 1: Given a spectrum $x$ and a set of spectra $S$, we want to find all the spectra $S_y$ that are similar to $x$, i.e. spectrum $y \in S_y$, iff $D(x, y) < r_q$, where $D$ is the Euclidean distance and the $r_q$ is a query radius.

A very simple approach would be to do a linear scan on the database and output every spectrum $y$ such that $D(x, y) < r_q$. This takes $O(n)$ time. However, if $S$ becomes very large and so do the number of queries say $O(n)$, then we have a $O(n^2)$ algorithm. This is clearly unacceptable for our problem. Thus, we design methods that will yield results near neighbor queries in sub-linear time. For this we are willing to tradeoff some accuracy for speedup. Several sub-linear near neighbor methods exist but we leverage Locality Sensitive Hashing [5] since, unlike others, it promises bounded guarantees and is also easy to implement. We briefly present the idea below.

### Locality Sensitive Hashing

The basic idea behind random projections is a class of hash functions that are locality sensitive i.e. if two points $(p, q)$ are close they will have small $|p - q|$ and they will hash to the same value with high probability. If they are far they should collide with small probability.

**Definition 1:** A family $\{H = f : S \rightarrow U\}$ is called locality-sensitive, if for any point $q$, the function

$$p(t) = Pr_H[h(q) = h(v) : |q - v| = t]$$

is strictly decreasing in $t$. That is, the probability of collision of points $q$ and $v$ is decreasing with the distance between them.

**Definition 2:** A family $H = \{h : S \rightarrow U\}$ is called $(r_1, r_2, p_1, p_2)$ sensitive for distribution $D$ if for any $v, q \in S$, we have

- if $v \in B(q, r_1)$ then $Pr[h(q) = h(v)] \geq p_1$
- if $v \notin B(q, r_2)$ then $Pr[h(q) = h(v)] \leq p_2$

Here $B(q, r)$ represents a ball around point $q$ with a radius $r$. Thus a good family of hash functions will try to amplify the gap between $p_1$ and $p_2$.

Indyk et. al. [5] showed that s-stable distributions can be used to construct such families of locality sensitive hash functions. An s-stable distribution is defined as follows.

**Definition 3:** A distribution $D$ over $R$ is called s-stable, if there exists $s$ such that for any $n$ real numbers $v_1...v_n$ and i.i.d. variables $X_1...X_n$ with distribution $D$, the random variable $\sum_i v_i X_i$ has the same distribution as the variable $(\sum_i v_i^2)^{\frac{1}{2}} X$, where $X$ is a random variable with distribution $D$.

Consider a random vector $a$ of $n$ dimensions. For any two $n$-dimensional vectors $(p, q)$ the distance between their projections $(a.p - a.q)$ is distributed as $|p - q|_a X$ where $X$ is a s-stable distribution. We chop the real line into equal width segments of appropriate size and assign hash values to vectors based on which segment they project onto. The above can be shown to be locality preserving.

There are two parameters to tune LSH. Given a family $H$ of hash functions as defined above, the LSH algorithm chooses $k$ of them and concatenates them to amplify the gap between $p_1$ and $p_2$. Thus, for a point $v$, $g(v) = (h_1(v)...h_k(v))$. Also, $L$ such groups of hash functions are chosen, independently and uniformly at random, (i.e. $g_1...g_L$) to reduce the error. During pre-processing, each point $v$ is hashed by the $L$ functions buckets and stored in the bucket given by each of $g_i(v)$. For any query point $q$, all the buckets $g_1(q)...g_L(q)$ are searched. For each point $x$ in the buckets, if the distance between $q$ and $x$ is within the query distance, we output this as the near-neighbor. Thus, the parameters $k$ and $L$ are crucial. It has been shown [4] that $k = \log_{1/p_2} n$ and $L = n^\rho$, where $\rho = \frac{\log 1/p_1}{\log 1/p_2}$, ensures locality sensitive properties. In Ref. [5], the authors consider L2 spaces and bound $\rho$ above empirically by $\frac{1}{2} \cdot c$ being the approximation guarantee, i.e. for a given radius $R$, the algorithm returns points whose distance is within $c \times R$. The time complexity of LSH has been shown to be $O(dn^\rho \log n)$, where $d$ is the number of dimensions and $\rho$ is as defined above. Thus, if we desire a coarse level of approximation, LSH can guarantee sub-linear run times for geometric queries.

### 2.3 Similarity Searching

Using our embedding and a fast near neighbor algorithm, we can find spectra similar to a given query spectrum. The key is to use the correct query radius. We show in the next section how this can be chosen. If we give too high a radius, it might yield a
large dataset and if the radius is too low, it might not yield any neighbor.

If an appropriate query radius is chosen, it is easy to find tight clusters using the following heuristic: ANN-cluster: 1) Embed spectra into a Euclidean space and form the set \( S \). 2) Hash the feature vectors, \( S \), using LSH. 3) Choose some \( k \) random spectra, find their near neighbors (tight clusters). For each random spectra add their neighbors to set \( S \). 4) \( S = S - C \). 5) Go to step 3 till \( S \) is empty.

Another immediate consequence of our framework is to find outliers. To check for outlier, we need to determine whether a spectrum has at most 1 or 2 neighbors. If the neighbors remain unchanged even on increasing the query radius by \( \delta \), a spectrum is indeed an outlier. Since near neighbors take sub-linear time with LSH, outliers can be detected in sub-quadratic time.

2.4 Speedup Database search

In this section, we discuss a sample application using our mining framework. Database search is the primary tandem mass spectrometry data mining applications. Given a query spectrum \( x \), and a mass spectra database \( MSDB \) (described in Section 3) the problem is to find out which peptide \( p \in MSDB \) corresponds to \( x \).

Database search is a well explored topic, see [18] for example. Most tools index the the MSDB by the peptide mass. Then for a spectrum \( x \), the precursor mass \( m_x \) is found. Then all the spectra \( S_p = \{ y | y \in MSDB \} \) are compared with \( x \) such that \( |m_y - m_x| < \delta \), where \( \delta \) is some pre-defined mass tolerance. Each comparison operation between the query spectrum and the candidate spectrum takes a while depending on the scoring function used. We reduce the size of \( S_p \) by filtering the unrelated spectra, speeding up the search. We ensure that we do not filter out the true peptide for a given spectrum while we discard most of the unrelated peptide.

We generate the virtual spectra from each peptide sequence in the database, and then embed those virtual spectra in the Euclidean space, as mentioned. Then for filtering, we choose an appropriate threshold radius \( r \) and query the LSH algorithm to yield all the candidates within a ball of radius \( r \). The ratio of the total number of peptides within a mass tolerance divided by the number of candidates returned is our speedup.

2.5 Visualization and Dimension Reduction

As mentioned earlier, visualizing thousands of spectra is a very hard problem. We are not aware of any previous work that allows us to visualize large mass spectrometry data sets. Our embedding followed by dimension reduction allows to view spectra on a two or three dimensional space. As a bonus, it qualitatively allows us to identify outliers in the data set.

Once we have embedded the spectra in a Euclidean space, we can use some of the common techniques to visualize high dimensional data by dimensionality reduction. The most common linear method is to use PCA [16]. Recently, several non-linear methods for dimensionality reduction have been discovered, the majority of them exploiting the low dimensional manifold structure of the dataset. In this paper, we leverage one of these techniques, the isomap method, to project the high dimensional data on a 2D plane. Due to lack of space we do not provide a description of the method.

3 Experimental Results

In this section, we describe the empirical evaluation of our embedding followed by some representative data mining tasks. Unless otherwise stated we use the following dataset from Keller et. al. [11]. For calculating statistics, we used 80% of the 1618 spectra from this annotation at random. The statistics were independent of the exact choices of the spectra. Note that our techniques are unsupervised except for the selection of query radii. Out of this, 1014 spectra were digested with trypsin and were used for database search filter.

For database search filters, a non-redundant protein sequence database called MSDB, which is maintained by the Imperial College, London. The release (20042301) has 1,454,651 protein sequences (around 550M amino acids) from multiple organisms. Peptide sequences were generated by in-silico digestion and the list of peptides were grouped into different files by their precursor ion mass, a different file for 10da.
3.1 Empirical evaluation of the embedding

In this section, we critically analyze our embedding and different distance metric. For these analyzes, we chose a set of 1014 curated spectra of proteins digested with trypsin and reported by Keller et. al. We then cleaned the spectra picked the most likely to be the signal peaks. Then we constructed the binary bit vector as discussed earlier. For the set of spectra, we knew that there were 100 odd clusters with 15 spectra per cluster on an average. We calculate the pairwise distances between spectra within the same cluster and we term this the similar set $SS$. We then choose a representative from each cluster at random and calculate the distances and we call this set the dissimilar set $DS$. Then we plot the frequency distribution of $DS$ and $SS$ as they both have similar number of pairwise distances in Figure 3.1 for three metrics: hamming, 1-cosine and euclidean. Its very clear that hamming is unsuitable as a metric as it has low discriminability. As expected, 1-cosine and euclidean looks almost similar with low overlaps between the sets $DS$ and $SS$. Also note that the cosine metric used here is not exactly the same used by others. We do not take the intensities into consideration after we have selected the peaks.

Now, we consider the database of tryptic peptides, $MSDB$. For each peptide, we generate its virtual spectrum and then construct the feature vector as above. For each real spectrum, we calculate the distance with the correct virtual spectra and we call this set of scores to be $SS$. Then we choose, from the database, 100 random peptides having almost the same mass as the precursion ion mass of the given spectrum. We then add the set of scores to the dissimilar set $DS$. We then plot the probability distribution of $SS$ and $DS$ in Figure 3.1. Again we can see the clear separation between the two sets of distances (with $<1\%$ overlap). This indicates that the efficacy of euclidean distance in our embedded space is a good metric to design filters for database search. Note the sharp impulse at 1.414 corresponding to distances between real spectra and completely dissimilar peptides within a mass tolerance of 2 da, providing empirical evidence for Lemma 2.2.

![Figure 3: Distribution of scores with real spectra using different metrics (hamming, 1-cosine, euclidean). The dotted curve plots the inter-cluster distances while the solid line represents the intra-cluster distribution.](image)

3.2 Post Translational Modifications

Now we present some very preliminary results on a set of spectra from the PFTau protein. We picked 8 good quality spectra with known Phosphorylations. We wanted to study whether our metric can help design filters that might work for PTM studies. From the Figure 3.2, we note that distances between spectra and their PTM variants have a higher likelihood of being classified as similar than dissimilar. This is evident from Figure 3.1.

3.3 Query processing using LSH

In this section, we quantify the accuracy of our framework for similarity searching and clustering. As mentioned earlier, we use LSH to answer queries with bounded errors in expected sub-linear time.

We first indexed the 1014 spectra using our em-
Figure 4: Distribution of distance between real and virtual spectra using different metric. The dotted curve represents the distance between real spectra and distances to virtual spectra from 100 different peptides of similar precursor masses. The sequences are from MSDB. The other curve shows the distribution of distances between spectra and the virtual spectra from the true peptides.

Figure 5: Some sample distances between spectra and their PTM variants. Note the low scores between the pairs. Distances between spectra of different peptides had a mean $\mu = 1.388$ and $\sigma = 0.017$.

Figure 6: The average number of spectra that are present in the cluster containing the query spectrum but are missed by LSH

bedding followed by LSH. For each of the 1014 spectra, we queried LSH with a radius $r$. We varied $r$. We plot the number of missed spectra that were actually present in the cluster of the query spectrum in Figure 3.3 and the number of false positives in Figure 3.3. As we increased the radius, we the number of misses decreased. This is expected as the radius of the query ball increases the number of possible data points that can be considered. As expected, the number of false positives also increased as $r$ increased. This indirectly demonstrates the accuracy of any clustering algorithm based on LSH. We miss an average of 1 spectrum within each cluster while admitting only 1 false spectrum.

At $r = 1.0 - 1.1$ the false positives are not very high. This might be important when we want to query for similar spectra in order to generate the consensus spectra. In such situations, it might be fine to miss out some bad quality spectra (distances to bad quality spectra are usually higher). Also, consider situations where we would like to coarsely partition the data set (e.g. for clustering). Then, we can afford to have a few false positives but we cannot miss any true positives. In such cases we increase the radius to at most 1.25 as the likelihood of an intra-cluster distance being greater than 1.25 is low, from Figure 3.1.

3.4 Speeding up Database Search

To test the efficacy of our framework on speeding up database search, we first use our metric to filter out candidate spectra. Since our distance calculation is much faster than the detailed scoring of two
spectra, we define speedup by the ratio of total number of candidate peptides with a mass tolerance of 2 daltons and the total number of peptides that have a distance of $\Delta$ with the query spectrum and have the same mass tolerance. Then we increase $\Delta$ and calculate the number of true peptides missed in this filtering process. In Figure 3.4, we plot the speedup on a logarithmic scale against the miss percentage. This gives us the speedup (or quality of filtering) versus accuracy tradeoff of using our framework. For a 2 dalton range the number of peptides are around 100-200K. For around a 100K peptide set, LSH takes 0.21s on an average to answer queries. As we see from Figure 3.4, we can get an average speedup of 118 if we allow 0.19% misses. This may be reasonable for many applications. In fact, we found that our errors were due to low quality spectra in our test dataset.

### 3.5 Visualization and Dimension Reduction

Consider the training dataset of mass spectra. We first generate Euclidean feature vectors for each spectra. Then we used PCA and plotted the first two components on the x-axis and the y-axis as shown in Figure 3.5(i). The clusters are visible and so are the outliers. But the visualization is coarse grained.

Then we use Isomaps on the same dataset. Recall that in Isomaps, one first needs to calculate the near neighbors. Thus in our plot, we also show the near neighbor graph along with the projected points as shown in Figure 3.5(ii). The cluster structure seem to be qualitatively clearer than with PCA.

### 4 Discussion

The results in the previous section look promising. The clear separation between the DS and SS set during the metrics comparision was a surprise to us, initially. One of the reasons for the good result is the quality of the dataset. We first wanted to validate our simple assumptions and claims on a dataset which had reliable interpretations. Since we first transform the spectra into binary bit strings we avoided the huge variations of density in spectra. The signal to noise ratio pilot study also underscored the fact that we need to study spectra by segmenting them. Note that one reason why we obtained clear separations between the DS and SS in all cases with our embedding is that we avoided using precursor ion mass as a feature. Even though its fine to use the precursor mass as a coarser grain filter, it will lead to less ro-
bust embeddings as such masses are prone to errors due to isotope effects. Also our theoretical results will not hold.

For LSH, the speed and the accuracy is quite satisfying. However, there are two implementation issues. Our current indexing is memory bound. This means we need lots of memory to index millions of mass spectra. Even though this is possible with the current 64 bit machines, we need to design disk based LSH schemes. We are working on a large scale implementation of our framework based on such techniques. Another issue is the choice of the number of bins and the mass coverage. Increasing the number of bins leads us to the curse of dimensionality which would slow down LSH and reduce the filtering speedup. If we choose fine grained bins with a lower maximum mass, our embedding will result in a pseudo-metric space as several different spectra will now satisfy assumption one in Theorem 2.1.

5 Conclusions and Future Work

In this paper, we showed that our embedding with geometric algorithms provides a good framework for mining mass spectra. In particular, we have demonstrated both theoretically as well as empirically, that our embedding coupled with Euclidean distance performs as well as the well known cosine similarity while providing us with the benefits of a metric space and enabling us to use approximate sub-linear time near neighbor techniques for data mining. Using this framework, we showed how we can do similarity searches and find tight clusters. Also, we demonstrated that we can get 2 order of magnitude filtering for database search. As an aside, we are also able to visualize large datasets in two dimensions qualitatively identifying the outliers.

This work is the first step in the direction of an integrated framework for large scale mining of tandem mass spectra using simple techniques from embeddings, vector spaces and computational geometry. Several directions are being investigated at this point. The main areas of investigation are 1) Better embeddings that offer better resolution for PTM spectra 2) Faster external database searching algorithms that use embedding 3) More effective blind PTM searching using embeddings 4) Large scale clustering and visualization of mass spectrometry data and 5) Integrating data from different sources using our embeddings.

We should note that several sections in the paper could be of independent interest. For example, we need to explore the probabilistic cleaning of mass spectra in more details. Our embedding promises to work across datasets and this general method can be used to do integrated study of other biological datasets eg. microarray data sets.

References

[1] R. Aebersold and M. Mann. Mass spectrometry-based proteomics. Nature, 422,6928 (2003),198-207.
[2] V. Bafna and N. Edwards. Scope: a probabilistic model for scoring tandem mass spectra against a peptide database. Bioinformatics, 17, Suppl 1 (2001),S13-21.
[3] T. Chen, M.Y. Kao, M. Tepel, J. Rush, and G. Church. A dynamic programming approach to de novo peptide sequencing via tandem mass spectrometry. J Comput Biol, 8,(2001),325-37.
[4] V. Dancik, T.A. Addona, K.R. Clauser, J.E. Vath, and P.A. Pevzner. De novo peptide sequencing via tandem mass spectrometry. J Comput Biol, 6,3-4 (1999),327-42.
[5] M. Datar, N. Immorlica, P. Indyk, and V. S. Mirrokni. Locality-sensitive hashing scheme based on p-stable distributions. In SCG ’04: Proceedings of the twentieth annual symposium on Computational geometry, pages 253–262, New York, NY, USA, 2004. ACM Press.
[6] A. Frank, S. Tanner, V. Bafna, and P. Pevzner. Peptide sequence tags for fast database search in mass-spectrometry. J. Proteome Res., 2005; 4(4); 1287-1295.
[7] Aristides Gionis, Piotr Indyk, and Rajeev Motwani. Similarity search in high dimensions via hashing. In VLDB ’99: Proceedings of the 25th International Conference on Very Large
[8] B.D. Halligan, E.A. Dratz, X. Feng, S.N. Twigger, P.J. Tonellato, and A.S. Greene. Peptide identification using peptide amino acid attributed vectors. *J. Proteome Res.*, 2004,3,813–820.

[9] B.D. Halligan, V. Ruotti, S.N. Twigger, and A.S. Greene. Peptide identification using peptide amino acid attributed vectors. *Nucleic Acid Research*, 2005,Vol33,WebServer issue.

[10] A. Keller, A.I. Nesvizhskii, E. Kolker, and R. Aebersold. Empirical statistical model to estimate the accuracy of peptide identifications made by ms/ms and database search. *Anal. Chem.*, 74,20(2002),5383-92.

[11] et al. Keller, A. Experimental protein mixture for validating tandem mass spectral analysis. *OMICS*, 6,2 (2002),207-12.

[12] B. Ma, A. Doherty-Kirby, and G. Lajoie. Peaks: powerful software for peptide de novo sequencing by tandem mass spectrometry. *Rapid Commun. Mass. Spectrometry*, 17,20 (2003),2337-42.

[13] M. Mann and O. Jensen. Proteomic analysis of post-transaltional modifications. *Nature Biotechnology*, 21,255-261, 2003.

[14] A. Nesvizhskii, A. Keller, E. Kolker, and R. Aebersold. A statistical model for identifying proteins by tandem mass spectrometry. *Analytical Chemistry*, 75, 4646-4658, 2003.

[15] A. Pandey and M. Mann. Proteomics to study genes and genomes. *Nature*, 405, 837-846, 2003.

[16] G. Strang. *Linear Algebra*. 3rd edition, 2003.

[17] S. Tanner, H. Shu, A. Frank, L.C Wang, E. Zandi, M. Mumbi, P. Pevzner, and V. Bafna. INSPECT: Identification of posttranslationally modified peptides from tandem mass spectra. *Anal.Chem.*, 77(14) pp 4626 - 4639,2005.

[18] Y. Wan and T. Chen. A hidden markov model based scoring function for tandem mass spectrometry. In *RECOMB 2005*.

[19] J.R. Yates 3rd., J.K. Eng, A.L. McCormack, and D. Schieltz. Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database. *Anal. Chem.*, Apr 15;67(8):1426-36, 1995.

[20] N. Zhang et.al. Probid: a probabilistic algorithm to identify peptides through sequence database searching using tandem mass spectral data. *Proteomics*, 2,10 (2002),1406-12.