Efficiency of two sampling designs for SELDI-TOF mass spectrometry

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Abstract

The analysis of some complex protein samples has been possible after the development and the use of the surface-enhanced laser desorption time of flight or so called SELDI-TOF. Furthermore, in dept research found out that SELDI-TOF is a powerful method which is useful in biology such that it helps to measure biomarkers for early detection of some diseases in other words it helps to spot biomarkers that enable doctors to make earlier diagnosis for their patients for instance diagnosis for different kinds of cancers.

The idea of the essay is to compare the two sampling designs: “multiple shots per position” and “only one shot per position”. We use bootstrap method to analyze the SELDI-TOF mass spectrometry data, and have the result: there is no clear advantage of only one shot per position.

Key words: SELDI-TOF, sampling designs, mass spectrometry, bootstrap test
Introduction

Time to flight mass spectrometry (TOF-MS) has become a powerful tool that is particularly used in diagnoses of specific diseases. TOF-MS is known also for its low cost, high speed and low mass range. Used mainly in the determination of the mass to charge ratio of particular molecules of interest using a mass spectrometer.

In the project our data consist of the number of laser beam shots that are focused on our sample mixture that is put on a chip array that consists of 16 spots. Hence, we will use this data and run a regression, our variables are \( Y_i \) which measures the intensity in single shot \( i \) at time of flight (TOF) \( t \) and \( \chi_i \) which is the sum of all the values in the specific spectra \( i \) and it also represent the area under the curve denoted by (AUC)

In this project our problem is to compare the two sampling designs: “multiple shots per position” and “only one shot per position”. And we use naïve and hierarchical bootstrap to compute the power function. In theory, a “narrow” power function correspond an efficient test, and the power function similar or not related to the different sampling designs.

Then by maximizing Poisson likelihood in other word by considering the assumption that \( Y_{it} \sim P(\alpha \mid \chi_i) \) and by comparing the simulated data from \( Y_{it} \sim P(\alpha \mid \chi_i) \) with the data then we actually have we come to conclude that data fit.

After that, we use naïve bootstrap test and hierarchical bootstrap test to draw two power functions, and then we can see the power functions are similar. And if the power functions for two designs are similar means it doesn’t matter which sampling designs we are using.

Then we come to result and conclusion, there we particular analyze the two power functions and have the result.

For our comparison of two bootstrap power functions, we can have the conclusion: there is no clear advantage of moving the laser between each shot, so it doesn’t matter which sampling designs we are using.
**Background**

The data that is obtained from spectroscopy is called a spectrum. A spectrum is a plot of the intensity of energy detected versus the wavelength (or mass or momentum or frequency, etc.) of the energy. Or Spectroscopy is a technique that uses the interaction of energy with a sample to perform an analysis.

There are some types of Spectroscopy in the following below, but we are going to identified our topic **Mass Spectrometry**.

- X-ray Spectroscopy
- Raman Spectroscopy
- Multiplex or Frequency-Modulated Spectroscopy
- Laser Spectroscopy
- Infrared Spectroscopy
- Gamma-ray Spectroscopy
- Fourier Transform Spectroscopy
- Electron Spectroscopy
- Electron Paramagnetic Spectroscopy
- Attenuated Total Reflectance Spectroscopy
- Atomic Absorption Spectroscopy
- Astronomical Spectroscopy
- **Mass Spectrometry**

The mass spectrometer consists of four major parts namely the inlet where the sample enter as a gas to be analysed, even solid samples can be analysed if they are volatile enough to spread some gaseous molecules that can be analysed. The second part is called ionization chamber where the samples molecules are ionized in other words they are given positive charge, molecules are ionized because it makes them easier to analyse than neutral molecules, the third part is the mass analyser where the molecules are separated into group by their mass, then the fourth part is the detector that converts the ion energy into electrical signals, it monitors the ion, then amplify it the send a signal to the system data The type of detector is supplied to suit the type
of analyser; the more common ones are the photomultiplier, the electron multiplier and the micro-channel plate detectors and finally the last part consists of a recorder for the data obtained.

Through these steps useful information about the sample that is analysed can be obtained such as to monitor an organic chemistry synthesis or to get specific information about the molecule weight or some information about the molecules compounds so this technique can be used to find components that we are aiming for in a mixed sample without having to examine each individual mass spectrum. Hence, mass spectrometry can provide important and useful information to a huge number of specialists and professional for instance biologists, pharmacists, physicians, astronomers and so on…

**Basics for Mass Spectrometry**

Mass spectrometry can be defined as a sophisticated analytical method that helps to determine the molecular nature of the sample being analysed, some of the aims of this method are the determination of the molecular weight of the ionized molecules of interest, the characterization of the structure of the molecules, qualitative and quantitative analysis of components in a mixture. Nowadays, mass spectrometry has known outstanding technological improvements allowing its application to proteins, peptides, carbohydrates, DNA, drugs and many other biological molecules. Hence, due to its different useful functions it has become a powerful and irreplaceable tool in the biological sciences.

As cited above mass spectrometry is an irreplaceable and powerful tool in biological sciences that enables a huge number of professional to identify unknown compounds, quantify some known materials, furthermore, it help those specialist to determine the molecular structure and the chemical composition of different substances.
Time of flight (TOF) is the process where the ionized molecules enter the mass spectrometer, exactly in the mass analyzer part, are accelerated by an electric field coming from the acceleration grid, till they hit a specific place called the detector, the lighter the ion is the faster it will travel because its velocity depends on the kinetic energy and its mass. Hence, the time difference (T) between when the molecule was ionized ($t_1$) and the time when the ionized molecule touches the detector ($t_2$) help to determine the mass to charge ratio.

In fact, it is a used method in different fields, let’s mention some major fields such as biology in such a way that it helps biologists to analyse proteins, peptides and other, it is also used in pharmacy such that it helps in drugs discovery, combinatorial chemistry, pharmacokinetics, drug metabolism and in clinical field such that it helps in neonatal screening, haemoglobin analysis, drug testing, in the environment field such that it enable the analysis of water hence the determination of water quality. In Geology it enables the determination of oil composition.

**What is Mass Spectrometry used for?**

- Determine how drugs are used in the body
- Detect and identify illegitimate steroids in athletes
- Determine damage of human or animal genes due to environmental causes
- Determine the age and origins of specimens in geochemistry and archaeology
- Detect dioxins in food and humans
- Locate petroleum deposits by testing rock samples
- Determine country of origin of wine, rice or diamonds
- Test the purity of semi-conductor materials used in making microchips for computers
- Monitor and track air pollutants
- The identification and characterization of proteins involved in biological processes
Find novel proteins in biological samples for use as therapeutic or diagnostic targets

**Mass Spectrometry Data Analysis**

There are several tools that enable the analysis of the mass spectrometry data, they allow the preprocessing and the classification of the data obtained from the analysis.

The data obtained from the sample analysis or the so called spectrum can be smoothed, aligned then normalized then classification tools are used in order to create classifiers and hence identify potential biomarkers.

**Surface-enhanced laser desorption/ionization- time of flight (SELDI-TOF)**

The SELDI technology, developed by the Ciphergen Biosystems, is increasingly used to spot different kinds of diseases from complex mixture proteins that have been obtained from a tissue sample or from some biological fluids such as serum or urine.

It can be applied in biomarker analysis, where the biologists can make difference between proteins from diseased tissue sample and a normal one. It is also used in toxicology, detection of toxicity biomarkers.

Brief explanations of the different steps that SELDI-TOF consists of are presented as follows.

It start with the application of the tissue sample or the biological fluid over some stainless steel or aluminium based supports, these supports contain some chemicals that will help to bound the proteins of interest, these proteins are then stuck into arrays by specific groups based on their characteristics or their properties.

This step is followed by a number of washes in objective of removing those proteins, particles of non interest or weak ones, those arrays containing bound proteins are inserted into a vacuum chamber in order to be treated by some light pulses of a nitrogen laser, resulting in an cloud of ionized protein molecules in the shape of a normal gas, then the resulting protein molecules are accelerated in a vacuum tube where they fly to land on an electrode that has negative charge.
The mass of these ionized proteins is calculated based on the time they fly till they touch the electrode the length of the tube and the used voltage are taken into consideration for this measure. This phase is so-called Time of Flight (TOF).

The data that results from this experiment is actually the intensity or the number of ionized proteins that touch the electrode taking into consideration their mass.
**Materials and method**

After we know some basic knowledge of spectra and SELDI-TOF, we provide some statistical properties of our project. In our experiment we have 64 positions, for each position laser irradiated 12 times, with fixed TOF 501, we have the raw data, it is a 501*768 matrix.

Before analyzing the statistical properties, we make a Base-line subtraction. The constant baseline was estimated as the most abundant value in the row data and subtracted from data. This value turned out to be identical for all single-shots in our data (i.e. 86). So remove 86 from all the spectra.

**Figure 1**: All spectra in our raw data after remove the base-line.

![Figure 1](image.png)

For next analysis we need introduce a new concept, it is the area under the curve (AUC). According to the calculus knowledge, the area under the cure equals to the sum of the measured intensity in single-shot i at time of flight (TOF) t. It will be used in the regression analyze part.

Figure 1 gives us some information of our raw data, for our analysis needed, we want to know weather the spectra within the same position are the similar, so we draw the planform of our raw data.
Figure 2: Planform of the all spectra.

The warm colour means the “peak” in the figure 1, and the cold colour means that is not a “peak” or it is not a high point in the figure 1.

Since the spectra within the same positions are similar, we consider that can we make a sampling design with only one shot per position? We choose some spectra from different positions to analysis the properties of spectra and get some more information of different positions.

Figure 3: Plot of five spectra from different positions (the first 5 positions).
Form figure 3 we can see that, some spectra have obvious peak, and some spectra are not.

To avoid ambiguities in determining the measurement of time to flight the time between each successive pulse must be longer than the time necessary for the heaviest ion before it reaches the detector. We choose a particular “peak” to analyze.

In our project we choose 295, because we think it is representative, and the problem of analysis is very computationally demanding. We plot of the measured intensity in single-shot at this time of flight (TOF) as following:

**Figure 4:** This is a plot of measured intensity against AUC

![Graph](image)

After we know some materials about our project, then we come to the statistic part. Before that we should mention our aim and how we plan to solve it.

Firstly our aim is to compare two sampling designs: “multiple shots per position” and “only one shot per position”.

Secondly, we use naïve and hierarchical bootstrap tests to draw the power functions. Naïve bootstrap is the standard bootstrap and the hierarchical bootstrap is the structure one. We use these two bootstrap tests to draw their power functions. In theory, a “narrow” power function correspond an efficient test, and the power function similar or not related to the different sampling designs, so next step we compare these two power functions.
Thirdly, we plan to compare efficiency by compare power functions. Something important is the relationship between the sampling designs and the two ways bootstrap power functions, it is: if the null-hypothesis is false, an efficient sampling design in our project should lead a rejection of the null-hypothesis with a high probability. In another word, a “narrow” power function corresponds to an efficient bootstrap test. And if these two power functions are similar in the certain sample size, it means it doesn’t matter which sampling designs we are using, but if not similar, the “narrow” one corresponds to the more efficient sampling design.

**Regression model**

Look at Figure 4, this is a plot of measured intensity against AUC, we choose a particular “peak” and plot it. X-axis is $X_i$ and Y-axis is $Y_{it}$ where $t$ is 295. It looks like a straight line passing through the origin.

Base on the article “A statistical framework for preprocessing SELDI-TOF mass spectrometry data”, we have the regression model as:

$$Y_{it} = p_i X + e_{it}$$

$Y_{it}$: After subtraction of base-line, $y_{it}$ is the measured intensity in single-shot $i$ at time of flight (TOF) $t$.

$X_i$: After subtraction of base-line, they are obtained as the sum of all the values in spectra $i$, and often referred to as the AUC.

And we can calculate $\hat{p}_i$ with following formula.

$$\hat{p}_i = \arg \max_p \sum_{t = 0}^{n} \{ y_{it} \log(p_i x_i) - p_i \log(y_{it}) \} = \frac{\sum_{i=1}^{n} y_{it}}{\sum_{i=1}^{n} x_i}$$

(*More information and the proof of this formula we can consult in the article “A statistical framework for preprocessing SELDI-TOF mass spectrometry data”, Martin Sköld et. al 2007)

In our designing of the test, we assumed $Y_{it} \sim P(\mu x_i)$ which is suggested by the above article. We compare the simulate data form $Y_{it} \sim P(\mu x_i)$ with the data
we already have. The figure as following:

**Figure 5**: the comparison of Poisson plot and raw data

(Red points are simulate value and blue points are from our data set)

Form figure 5 we can see it fit well with the assumption of $Y_{it} \sim Po(\bar{\mu} \times x)$. So in the next hypothesis test part, we assume Poisson distribution in the test.
Hypothesis test

Which we are interested in is we want to test the hypothesis \( H_0 : p = q \) or \( H_0 : p \neq q \), where \( p \) is unknown concentration (of proteins with TOF \( t \)) of the observed sample. \( q \) is the known concentration, they are the different values in the neighborhood of \( \hat{p}_0 \).

With the model \( \hat{p}_0 = \frac{\sum_{i=1}^{n} y_i}{\sum_{i=1}^{n} x_i} \), we can compute \( \hat{p}_0 \) form whole sample size, \( \hat{p}_0 = 0.0032 \). In our essay, we choose 30 values in \((0.002, 0.005)\). After these, we choose different sample sizes \((192, 384, \text{ and } 768)\), we approximate the power function for this interval.

The power function of a hypothesis test defined by \( B(p) = P(\hat{p} \in R) \) which with rejection region \( R \) is function of \( p \). The ideal power function is 0 for all \( p \in \Theta_0 \) and 1 for all \( p \in \Theta_0^c \). Except trivial situations, this ideal cannot be attained. Qualitatively, a good test has power function near 1 for most \( p \in \Theta_0^c \) and near 0 for most \( p \in \Theta_0 \).

From the knowledge of power function the larger sample size which we choose we can explain it like this: if the \( H_0 \) is “true” the \( P \)-value will be smaller, but if \( H_0 \) is “false” the \( P \)-value will be bigger.

For our case, we don’t know \( p \), but we know \( \hat{p}_0 \) which computed base on the whole sample, we can use it to draw a figure of power function \( B(p) = P(\hat{p} \in R) \).

Typically, the power function depends on the sample size. If we can choose \( n \), consideration of the power function might help us determine which sampling design is appropriate in the certain sample size. So we choose different sample sizes in the two ways bootstrap tests to find which sampling design is our expected.

The procedure of bootstrap test as follows:
1. We draw \( y_i, K, y_n \) form Poisson distribution \( Y_i \sim P \alpha(p_i x_i) \). We choose different
n (192,384,768)

2. Compute \( \hat{p} = \frac{1}{N} \sum_{i=1}^{n} \mathbb{1}_{T(y_i) > T(y)} \) where

\[
\mathbb{1}_{T(y_i) > T(y)} = \begin{cases} 
1 & \text{if } T(y_i) > T(y) \\
0 & \text{else}
\end{cases}
\]

3. Reject \( H_0 \) if \( \hat{p} < \alpha \) (\( \alpha \) level of significance)

What we really need are \( T(y_i) \) and \( T(y) \), and we have two way to compute this test-statistic.

In our project, we want to draw the power function \( P \) in the two ways bootstrap tests. So we compute the \( \hat{p} = \frac{1}{N} \sum_{i=1}^{n} \mathbb{1}_{T(y_i) \geq T(y)} \) and let \( \alpha = 0.05 \), we choose 30 values around \( \hat{p}_0 \), and for each value we have a hypothesis test. For each hypothesis test, we reject \( H_0 \) if \( \hat{p} \leq \alpha \), with the bootstrap theory, we repeat large number times (100 times in our project) to compute the probability of \( \hat{p} \leq \alpha \), it is \( \text{prob} = \frac{1}{N} \sum_{i=1}^{n} \mathbb{1}_{\hat{p} \leq \alpha} \), after this, we can have 30 \( \text{prob} \), then we use these \( \text{prob} \) to draw our power functions.

For our case, in theory of power function, if we choose a larger sample size, the figure of power function will be narrow and the lowest point should be 0.05 according to theory. In our project, we use two kinds of bootstrap tests, they are naïve bootstrap and hierarchical bootstrap, for each bootstrap test, they have their own power functions in the different sample sizes. We use bootstrap test because the Poisson distribution which is not entirely correct is only assumed in our test, we want to avoid making distributional assumption, and we want to approximate the power function of this test we applied to mass-spectrometry data. The approximation is done by the bootstrap, which is convenient.

With the Poisson distribution assumption we want to approximate its power function for different alternative and sample size. A good approach is to use \( P \)-value
(it is prob in our essay) based bootstrap test. The Bootstrap idea is to estimate \( \hat{p}_0 \) by \( \hat{p} \) and then proceed as if the latter was the correct distribution. Hence we want to find an approximation of \( P \)-value. If \( H_0 \) is true and \( \hat{p} \) is a good estimate, it should provide sensible results.

After the data analyzed, we now need to write an algorithm that draws bootstrap samples. It seems reasonable to takes \( n \) (number of samples) from data (our 12*16*4 pairs \( (y_j, \chi) \)) and returns \( n \) pairs "randomly" drawn from data.

1) Naive way.
This is the standard bootstrap that draws pairs randomly with replacement.

**Test-statistic** \( T(y) \): We want to test \( p = p_0 \) for different \( 30 \) \( p_0 \)'s, we need first to define a test-statistic \( T(y) \) for example \( T(y) = \left| \hat{p}(y) - p_0 \right| \), and we must compute \( \hat{p}(y) \). In order to compute \( \hat{p}(y) \), we draw a sample size 192 from the full sample for example, with the random dram of this sample, we got 192 \( y_j \) and \( \chi \), with the method \( \hat{p}(y) = \frac{\sum_{i=1}^{n} y_i}{\sum_{i=1}^{n} x_i} \), which we have used before, and then we can get \( T(y) \).

**Test-statistic** \( T(y_j) \), firstly, we draw \( y^{(i)} \) from \( p_0(\chi^i \cdot p) \), \( i = 1, K, n \), draw \( \chi^i \) randomly. Secondly, compute \( \hat{p}(y^{(i)}) = \frac{\sum_{i=1}^{n} y_i^{(i)}}{\sum_{i=1}^{n} x_i^{(i)}} \). Thirdly repeat these two steps for a large number time. (We repeat 100 times in our project). Finally compute \( T(y_j) = \left| \hat{p}(y^{(i)}) - p_0 \right|, \quad j = 1, K, 100 \).

With The procedure of bootstrap test, we can compute \( \hat{p} = \frac{1}{N} \sum_{i=1}^{n} \mathbb{1}_{T(y_j) \geq T(y)} \).
The figure of with sample size 192 use the naïve bootstrap method is following:

**Figure 6**: power function of naïve bootstrap test with sample size 192

The figure with different sample sizes use naïve bootstrap method (from left to right sample size n=192, 384, 768)

**Figure 7**: comparison of different sample sizes of the naïve bootstrap test’s power functions.

2) Structured way: The hierarchical bootstrap.

The process of hierarchical bootstrap test is the as the naive bootstrap test, the difference is the method of draw the sub sample size.

The problem with the method of naive bootstrap is that it assumes the different spectra to be independent. The reason why we use the hierarchical way is the laser shoot several times at the same position when the experiment was performed, there exist dependence in data. In reality, they are not since spectra recorded at the same
position are similar to each other (a position is a group of 12 spectra).

The bootstrap method samples are drawing (e.g. 192) individual spectra (with replacement).

The hierarchical method first draws a position and then draws 12 individual spectra from this position (this was repeated 16 times to get 192 spectra).

We also choose 3 different sample sizes \( n \) (192, 384, 768), and we take \( n=192 \) for example.

**Test-statistic** \( T(y) \): Firstly, we draw a position form all positions, secondly we draw 12 individual spectra, and then repeat these two steps for 16 times, we get 192 spectra. If we repeat 32 and 64 times, we will get 384 and 768 spectra., with these spectra we can get \( y_i \) and \( \chi_i \), then we can compute \( T(y) = \left| \hat{p}(y) - p_i \right| \), with \( \hat{p}(y) = \frac{\sum_{i=1}^{n} y_i}{n} \) and \( \sum_{i=1}^{n} \chi_i \)

**Test-statistic** \( T(y_i) \): We draw \( y_i^{(j)} \) from \( p(x|y, p), i=1, K, n \), draw \( \chi_i \) like previous step. Compute \( \hat{p}(y_i^{(j)}) = \frac{\sum_{i=1}^{n} y_i^{(j)}}{\sum_{i=1}^{n} \chi_i} \) and repeat 100 times to get \( T(y_i) = \left| \hat{p}(y_i^{(j)}) - p_i \right| \) \( j=1, K, 100 \).

The figure of \( \hat{p} \) with sample size 192 use the hierarchical bootstrap method is following:

**Figure 8**: power function of hierarchical bootstrap test with sample size 192
The figure with different sample sizes use hierarchical bootstrap (from left to right sample size n=192, 384, 768)

**Figure 9**: comparison of different sample sizes of the hierarchical bootstrap test’s power functions.

The both two kinds of power functions don’t reach 0.05 is probably of a Poisson distribution is not entirely correct. Hence, the tests we are performing do not have the correct level of significance, perhaps they are conservative. But this is not a major problem though since we are not interested in the test itself, we really interested in is just want to compare the power functions.
**Result**

With the result of naïve and hierarchical bootstrap power function, we can compare their figures, and we can easily to see that the change of power function of both bootstrap tests.

**Figure 10:** comparison of two bootstrap power functions.

![Comparison of two bootstrap power functions](image)

We can see the naïve bootstrap power function looks more narrow than the hierarchical bootstrap power function for all different sample sizes. But for smaller sample sizes this is not obvious for the hierarchical one. So we can say the naïve one is better than the hierarchical one for a certain sample size.

According to theory, the hierarchical one should be get narrow, but is looks the widest for the largest sample size. It is difficult for us to explain, perhaps it just an effect of us using a bootstrap approximation.

Our aim is compare the two sampling designs: “multiple shots per position” and “only one shot per position”. And the key question is weather the two bootstrap power function are similar. If they are similar, we can say there is no clear advantage of moving the laser between each shot, if not similar, the sampling design which the narrower one correspond to is better.

For our comparison of two bootstrap power functions, they are more or less similar. So there is no clear advantage of moving the laser between each shot, and it doesn’t matter which sampling designs we are using in the smaller sample size.
Conclusions

By studying the single-shot spectra, we choose a representative peak from the mass-spectra, made a regression and estimate a p value, and then we made a hypothesis test we draw the power function of both naïve bootstrap and hierarchical bootstrap.

With the knowledge of power function, we would like to have a narrower region for the larger sample sizes, compare the power functions for the two designs, the power function look more optimal when we use the naive bootstrap in the larger sample size, but it not obvious in the smaller sample size. So we can say there is no clear advantage of only one shot per position.

In our analysis, we choose a particular value of TOF because it was representative, but it was not the only one who was representative, and we can choose others “peaks” for analyze.
Appendix:

References:


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**Code in matlab:**

Code of figure 1:
load SELDI.dat;
SELDI= SELDI-86;
y=SELDI;
x=sum(y);
plot(SELDI)

Code of figure 2:
imagesc(SELDI)

Code of figure 3:
pos=1:5
spec=pos*12
plot(SELDI(:,spec))

Code of figure 4:
plot(x,y(295,:),'.')

Code of figure 5:
plot(x,y(295,:),'.')
hold
plot(x,poissrnd(x*p0),'r')

Code of figure 6:
load SELDI.dat;
SELDI= SELDI-86;
y=SELDI;
x=sum(y);
linspace(0.002,.005,30)

p0vec=linspace(0.002,.005,30);

\[ \text{for } k=1:30 \]
\[ \text{prob}(k)=\text{bootprop}(p0vec(k),x,y); \]
\[ \text{end} \]

\[ \text{function } \text{prob}=\text{bootprop}(p0,x,y) \]

\[ \text{for } i=1:100 \]
\[ I=\text{ceil}(768*\text{rand}(1,192)); \]
\[ \text{ystar}=y(295,I); \]
\[ \text{xstar}=x(I); \]

\[ p\text{star}=\frac{\text{sum(ystar)}}{\text{sum(xstar)}}; \]
\[ T=|p\text{star}-p0|; \]

\[ \text{for } j=1:100 \]
\[ x\text{star2}=x(\text{ceil}(768*\text{rand}(1,192))); \]
\[ y\text{star2}=\text{poissrnd}(x\text{star2}*p0); \]
\[ \text{phat}(j)=\frac{\text{sum(y\text{star2})}}{\text{sum(x\text{star2})}}; \]
\[ \text{end} \]
\[ T\text{star}=|\text{phat}(j)-p0|; \]

\[ \text{P}(i)=\text{mean}(T\text{star}>T); \]
\[ \text{end} \]
\[ \text{prob}=\text{mean}(\text{P}<.05) \]

If we change 192 to 384 and 768, we will get figure 7.

Code of figure 8:

\[ \text{load SELDI.dat;} \]
SELDI= SELDI-86;
y=SELDI;
x=sum(y);

linspace(0.002,.005,30)
p0vec=linspace(0.002,.005,30);

for k=1:30
    prob(k)=hiebootprop(p0vec(k),x,y)
end

function prob=hiebootprop(p0,x,y)
    for i=1:100
        for m=1:16
            pos=floor(16*rand);
            spec=pos*12+ceil(rand(1,12)*12) ;
            ystar(((m-1)*12+1):(m*12))=y(295,spec);
            xstar(((m-1)*12+1):(m*12))=x(spec);
        end
        pstar=sum(ystar)/sum(xstar);
        T=abs(pstar-p0);
        for j=1:100
            for n=1:16
                xstar2(((n-1)*12+1):(n*12))=x(pos*12+ceil(rand(1,12)*12));
            end
            ystar2=poissrnd(xstar2*p0);
phat(j) = \frac{\text{sum}(ystar2)}{\text{sum}(xstar2)};

\text{end}

Tstar = \text{abs}(\text{phat} - p0);

P(i) = \text{mean}(Tstar > T);

\text{end}

\text{prob} = \text{mean}(P < 0.05)

If we change \( m = 1:32 \) and \( m = 1:64 \), \( n = 1:32 \) and \( n = 1:64 \), we will get the figure 9