Report on Independent Hypothesis Weighting

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1. Introduction

Numerous techniques have been devised for the analysis of high-throughput data to accurately quantify biological features, including genes and proteins. To ensure the reliability of discoveries, the false discovery rate (FDR) has become the dominant approach for setting thresholds. The methods for controlling FDR primarily rely on p-values, among which the Benjamini-Hochberg (BH) procedure (Benjamini and Hochberg 1995) and Storey's q-value(Storey 2002) are popular.

However, Ignatiadis, Klaus, Zaugg, and Huber suggest that FDR methods based solely on p-values exhibit suboptimal power when the individual tests vary in their statistical properties. (Ignatiadis et al. 2016). When these methods focus exclusively on p-values, they overlook potentially relevant covariates. For example, in RNA-seq differential expression analysis, one such covariate could be the normalized mean counts of genes. Intuitively, genes with higher counts are likely to have greater power in detection compared to those with lower counts, and it would be optimal to include this relationship in the analysis.

To address this limitation, a novel approach known as independent hypothesis weighting (IHW) has been introduced. IHW improves the power of multiple hypothesis testing while controlling the FDR. The key innovation of IHW is that it recognizes that not all tests have the same power or the same prior probability of being true. It allows for the assignment of different weights to different hypotheses based on covariates that are predictive of the test's power or its probability of being a true discovery. These covariates can be anything from the biological characteristics of the genes being tested to the technical aspects of the measurements. More explorations will be done in the following sections on this method.

2. Methodology and Simulation

There have been existing attempts to increase power by using covariates and this sections starts off by introducing some known methods. Their advantages and disadvantages are discussed, leading to the formation of IHW.

2.1 Weighted and Group weighted BH procedure

The weighted BH procedure has m hypotheses $H_1, H_2, \dots, H_m$ and m weights $w_1, w_2, \dots, w_m \geq 0$ that satisfy $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$. After the weights are obtained, the BH procedure is applied on the modified p-values: $\frac{p_i}{w_i}$. In this scenario, selecting the weights before observing the p-values is essential, relying on prior knowledge or information indicating the likelihood of some hypotheses being true over others. This requirement presents a significant challenge, one that IHW seeks to address, and it utilizes the grouped weighted BH procedure (GBH). In this method, there are G groups with covariate $X = (X_1, X_2, \dots, X_m)$, where each X_i takes the same value within each group. GBH first estimates the proportion of null hypotheses by $\hat{\pi}_0(g)$, then weights the hypotheses proportionally to $\frac{1-\hat{\pi}_0(g)}{\hat{\pi}_0(g)}$, and finally applies the BH procedure. However, the asymptotic theories in this method doesn't function well when the number of hypotheses $\frac{m}{G}$ is finite (Ignatiadis and Huber 2021). To resolve this issue, cross-weighting is used, where the idea is analogous to cross-fitting in regression settings, and this gives rise to the naive version of IHW.

2.2 Naive IHW and two-groups model

This version, also abbreviated as IHW-GBH since it is based off GBH, first divides the hypothesis tests into G groups based on the values of covariate X, with m_g number of hypotheses in the g-th group. It is also assumed that we have access to the p-values $P = (P_1, P_2, \dots, P_m)$, which is independent of X under the null. With this setup, $\sum_{g=1}^{G} m_g = m$. Then the weighted BH procedure is applied with each possible weight vector $w = (w_1, w_2, \dots, w_G)$, while the optimal w^* is the vector that leads to the most rejections. This method extends the BH procedure, making it pertinent to discuss the associated maximization problem. This problem stems from the two groups model (Efron 2008), which is a Bayesian framework that explains the BH procedure. Formally, assume that H_i takes values 0 or 1, and $\pi_0 = \mathbb{P}(H_i = 0)$. The distributions are as follows:

$$\begin{aligned} H_i &\sim \text{ Bernoulli } (1 - \pi_0) \\ P_i \mid H_i = 0 &\sim U[0, 1] \\ P_i \mid H_i = 1 &\sim F_1 \end{aligned}$$

The marginal distribution for p-value P_i is then

$$P_i \sim F(t) = \pi_0 t + (1 - \pi_0) F_1(t),$$

where F_1 is the distribution for the alternative hypothesis.

With this, the Bayesian FDR becomes (Ignatiadis et al. 2016):

$$\mathrm{Fdr}(t) = \mathbb{P}\left[H_i = 0 \mid P_i \leq t\right] = \frac{\pi_0 t}{F(t)}.$$

A natural empirical estimator for the CDF would be the ECDF, and it can be written in terms of $R(t)$, which denotes the total number of rejections:

$$R(t) = m\widehat{F}(t) = \sum_{i=1}^{m} \mathbf{1}_{\{P_i \leq t\}}$$

Hence if $\widehat{\pi_0}$ is an estimator of π_0,

$$\widehat{\mathrm{Fdr}}(t) = \frac{\widehat{\pi_0} t}{\widehat{F}(t)} = \frac{\widehat{\pi_0} m t}{R(t)}$$

If a conservative estimate is made: $\widehat{\pi_0} = 1$, then

$$\widehat{\mathrm{Fdr}}(t) = \frac{mt}{R(t)} \tag{1}$$

With this, the optimization problem is:

$$\text{maximize}\, R(t), \text{ s.t. } \widehat{\text{Fdr}}(t) \leq \alpha, t \in [0,1]$$

The corresponding estimator in IHW-GBH is of the form

$$\widehat{\text{Fdr}}(t, \mathbf{w}) = \frac{mt}{R(t, \mathbf{w})} = \frac{\sum_{g=1}^{G} m_g w_g t}{R(t, \mathbf{w})}$$

where $R(t, \mathbf{w}) = \sum_{i=1}^{m} \mathbf{1}_{\{P_i \leq w_g t\}}$ is the number of rejections in bin g. Now the optimization problem is:

$$\text{maximize}\, R(t, \mathbf{w}), \text{ s.t. } \widehat{\text{Fdr}}(t, \mathbf{w}) \leq \alpha$$

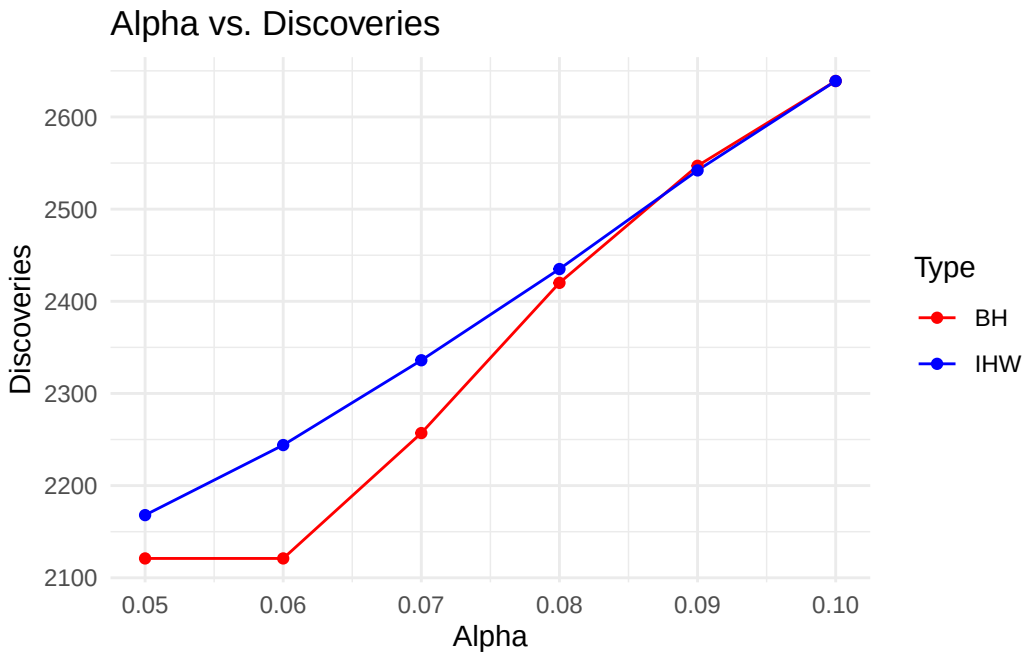
However, with this approach, there are also some disadvantages including potential loss of Type I error, complications in solving the maximization problem, and its inability to scale when large number of tests are present. That is why modifications are made, leading to the IHW method.

2.3 IHW

The formal IHW method derives from the naive approach with three modifications (Ignatiadis et al. 2016). In the first modification, the ECDF $\hat{F}_g$ of the p-values in group g is replaced by the least concave majorant version called the Grenander estimator $\tilde{F}_g$. With this the maximization problem can be efficiently solved. The second modification involves randomly splitting the hypotheses into K folds, where K is usually taken to be 5. Then the maximization problem with the previous modification is applied to the remaining folds, leading to a weight $\widetilde{w} = (\widetilde{w}_1, \ldots, \widetilde{w}_G)$. The independence criterion between the hypotheses would guarantee the p-value P_i to be independent of the assigned weight w_i when the null hypothesis is true. The third modification ensures that the weights learned with $K-1$ folds can be generalized to the held-out fold by adding a regularization parameter λ. The specific constraints are customized to whether the covariates are ordered or not.

2.4 Simulation

Wanting to see how this packages work exactly, I used one of the datasets suggested by the paper using RNA-seq data with read counts for genes as a covariate (Bottomly et al. 2011). It is worth noting that in the original dataset provided in the paper by Bottomly, the column of read counts is only binary, providing information of whether the counts are considered low or not based on a threshold after a log transformation. Without knowing the specific transformation applied, it is hard to achieve the original column, hence I self-generated a column of count reads using the negative binomial distribution. After trying some combinations, I chose the dispersion parameter to be 0.2 while the mean read counts is 1,000. The plot generated is as follows. There is a discrepancy between this plot and that generated in the paper possibly because the difference in the read counts, however, it can still be observed that the number of discoveries of IHW is no fewer than that of BH in most cases. This was an initial attempt in understanding the package and the dataset. More explorations and better results will be achieved in the following sections.



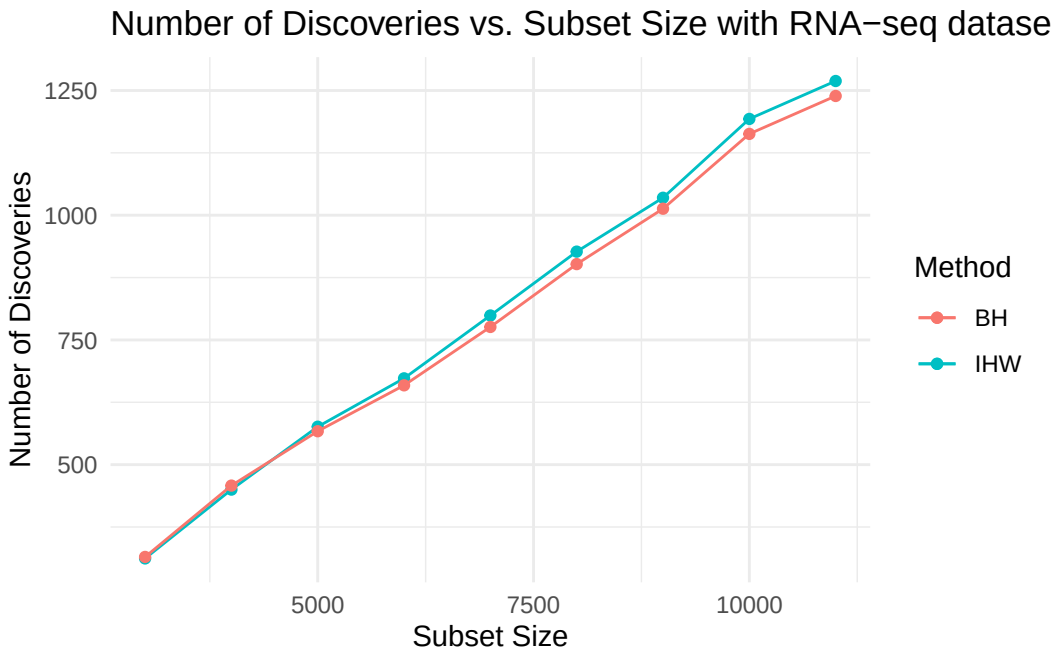
2.5 Discussions

One challenge that this method faces is that it largely depends on selecting an appropriate covariate that influences the power of each hypothesis test but is independent of the p-values under the null hypothesis. Identifying such a covariate is challenging and crucial; an unsuitable choice can diminish IHW's benefits or even degrade performance compared to traditional correction methods. This is a reason of why the plot above doesn't show great improvements. Another possible limitation is when there are datasets with high heterogeneity, where the covariate's relationship to test power varies significantly. In such cases, IHW's ability to accurately weight hypotheses could be compromised, potentially leading to less effective multiple testing correction.

3. Extensions

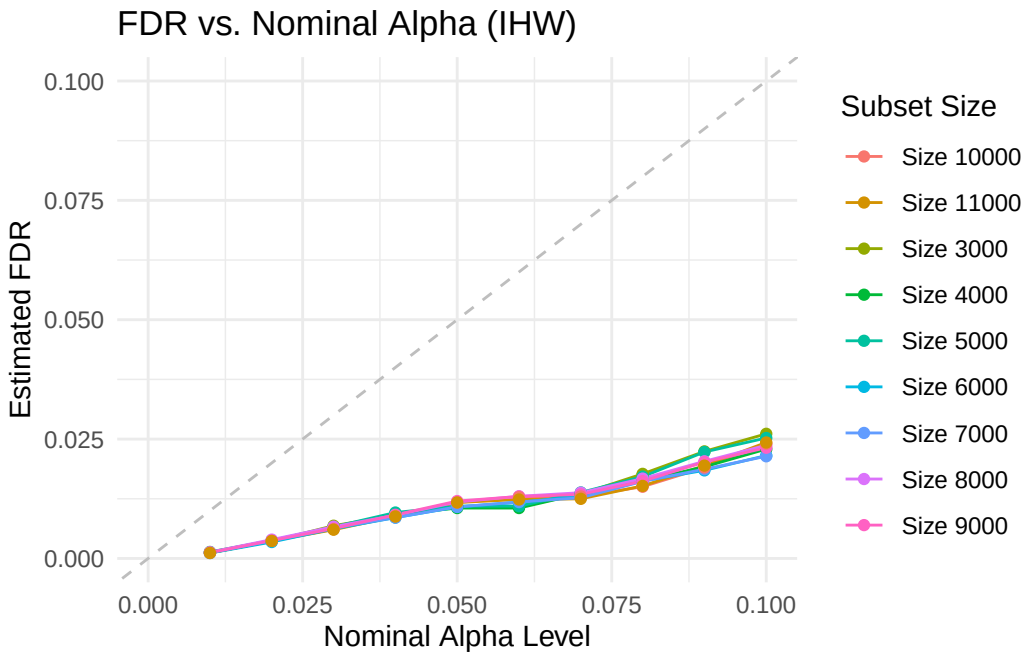
3.1 IHW performance through dataset

In statistical analysis, especially with multiple hypothesis testing, model performance is influenced by the number of tests. The variations observed across different numbers of tests can shed light on the efficiency and reliability of the model. In this section, these variations are looked into, while seeking to understand their underlying causes and proposing potential improvements. The number of tests in the analysis directly correlates with the number of genes under examination, as each gene represents a unique hypothesis to be tested. Given this relationship, analyzing subsets of the gene dataset becomes a viable strategy to examine the impact of test quantity on model performance. The RNA-seq dataset is used again here, with different sizes of subsets taken to firstly explore how the number of discoveries fluctuates. The plot is shown as follows:



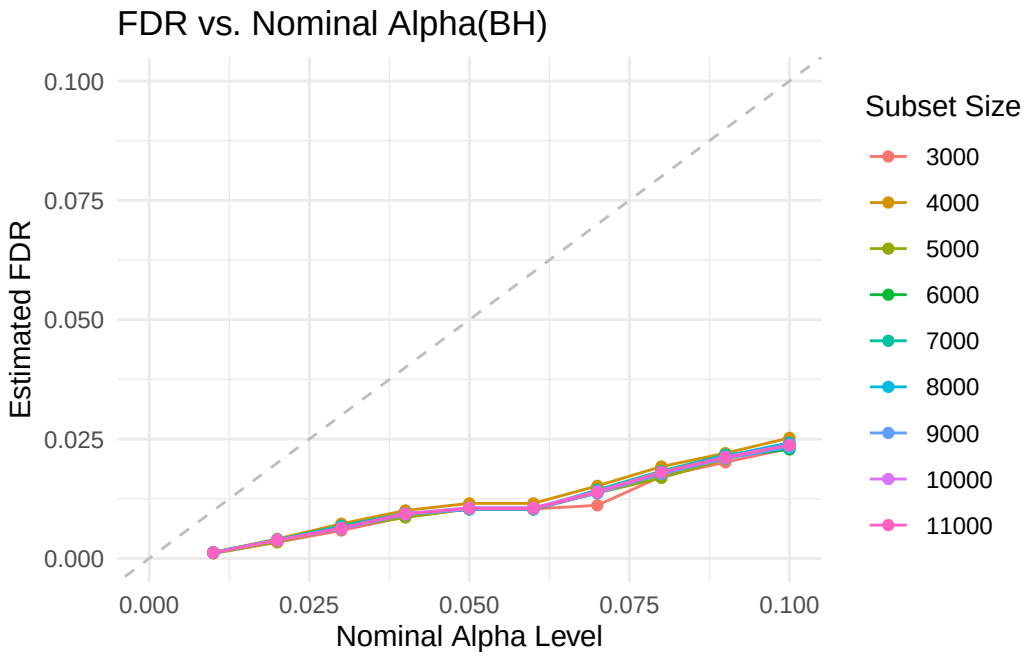
The nominal α level is set to be 0.01 here. I have tried various values ranging from 0.01 to 0.10 while the plot doesn't differ much. First off, it can be observed that as subset size grows, there are more discoveries for both IHW and BH. This can be due to several reasons. As the size grows, the statistical power is increased, leading to more rejections. It is also possible that larger subsets may enable the detection of smaller effect sizes that would be indiscernible in smaller subsets due to insufficient power. The ability to identify these smaller effects contributes to the overall increase in the number of discoveries as subset size grows. However, the number of discoveries for BH and IHW seem similar in general, with that of IHW to be higher at some points. This doesn't seem desired, but I wanted to explore and interpret through more plots.

I thought it would also be interesting to generate a plot of nominal α and FDR for comparisons. I applied it usin the IHW method first, and obtained the following:



Initially, I found it quite surprising that the curves were well below the $y = x$ line, suggesting that IHW is more conservative than expected. This observation was unexpected, especially since the plots in the paper indicated that for IHW, the curves should be closer to the diagonal line. The main reason why I think this is happening is because of the fact that I generated the covariate column myself through negative binomial distributions. In the context of IHW, the covariate should be informative about the power of each test, while randomly generating covariates might violate this condition. As for the effect of the subset sizes, it seems that the curves don't differ much as the size grows. One likely explanation is that all subsets are large enough to have adequate power. Then the increase in subset size may not lead to a proportionate increase in the number of rejections because the power of the test is already high in the smaller subsets.

It then occurred to me then that it could be beneficial to also have a similar plot but with the BH procedure. Hence I also produced the following plot. Since there are multiple sizes I tried, I placed this as a separate plot.

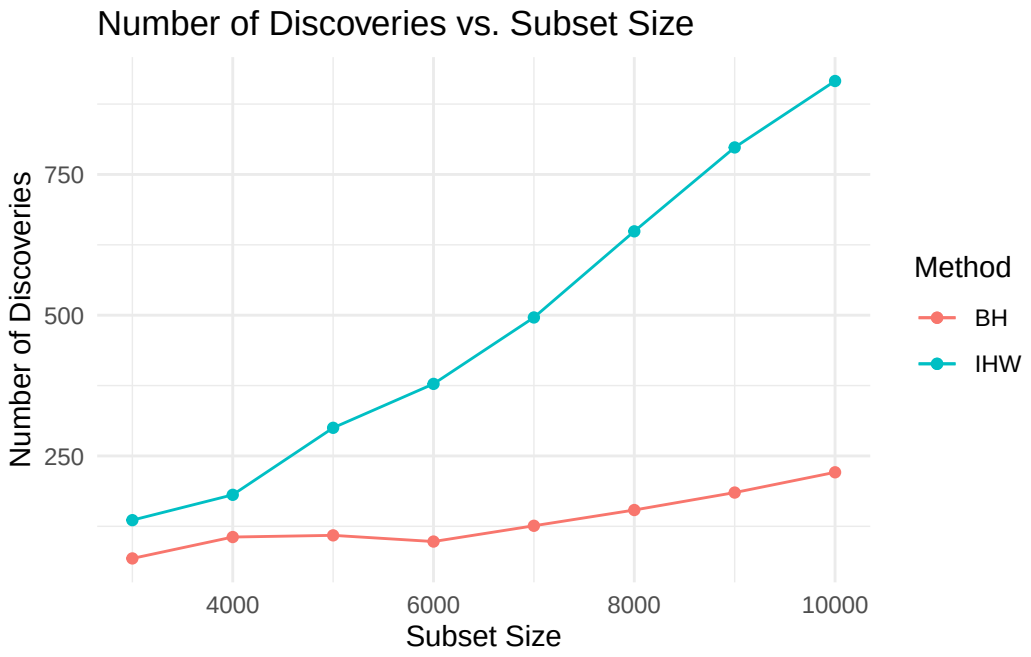


This plot with nominal α and estimated FDR using the BH procedure looks similar to that with IHW, which also matches what the paper suggested with one of the datasets in figure 2(f) (Ignatiadis et al. 2016).

Although the results are not desired since the number of discoveries for IHW doesn't exceed BH by much, indicating that there's not an improvement by using IHW, I wanted to keep these plots and analyses because they are a great indication that this happens when the covariate is not informative. This is further explored through simulations of my own, while intentionally enforcing the covariates to be informative.

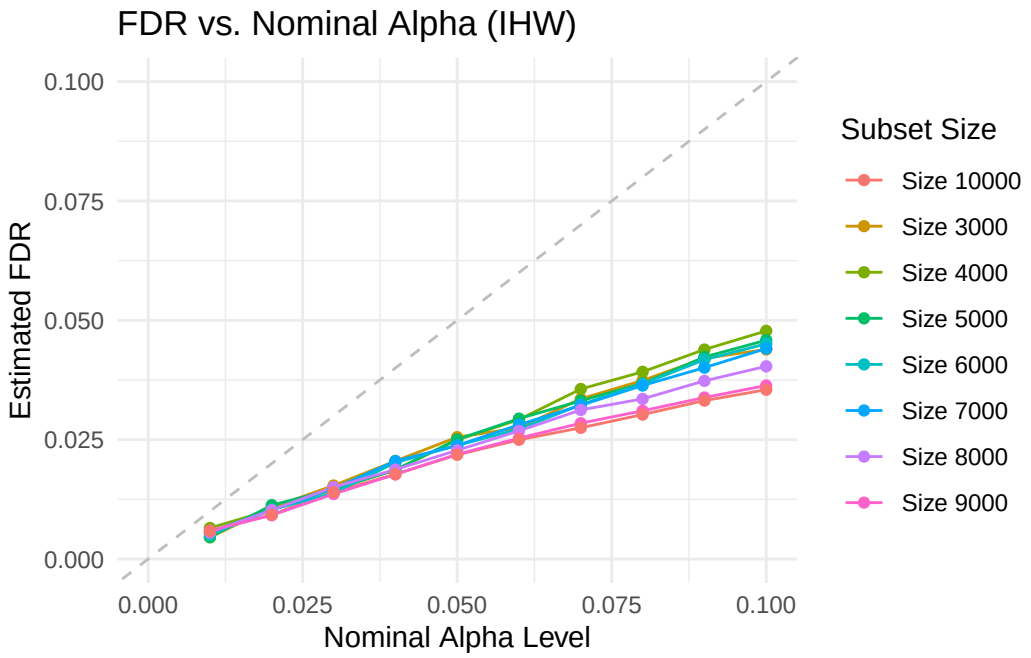
3.2 IHW performance through simulations

In this section I simulated p-values and covariates myself, specifically requiring both of them to relate to the effect size, which was generated through a normal distribution. The following procedures are similar to the previous subsection, however, the results are drastically different. The number of discoveries is shown as:

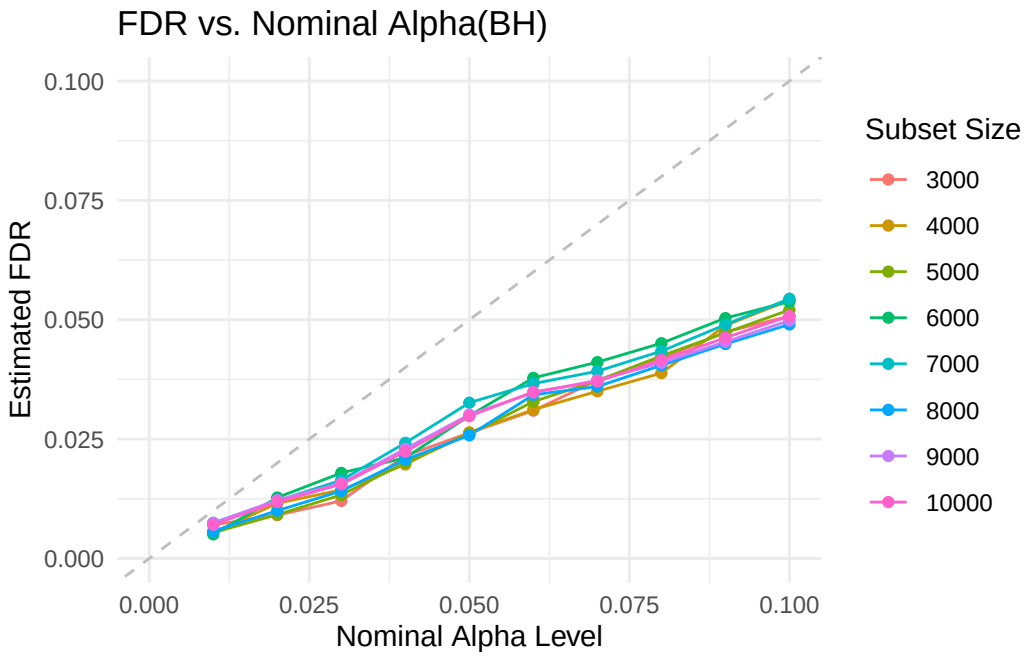


The nominal α is chosen to be 0.05 here. As it can be seen, the number of discoveries for IHW grows more than BH as the subset size increases. When the size is 10000, there are many more discoveries by IHW than BH compared with when the size is 3000. The discrepancy between the discoveries is now due to the fact that the covariate is informative.

Again, plots of nominal α and estimated FDR are plotted for IHW:



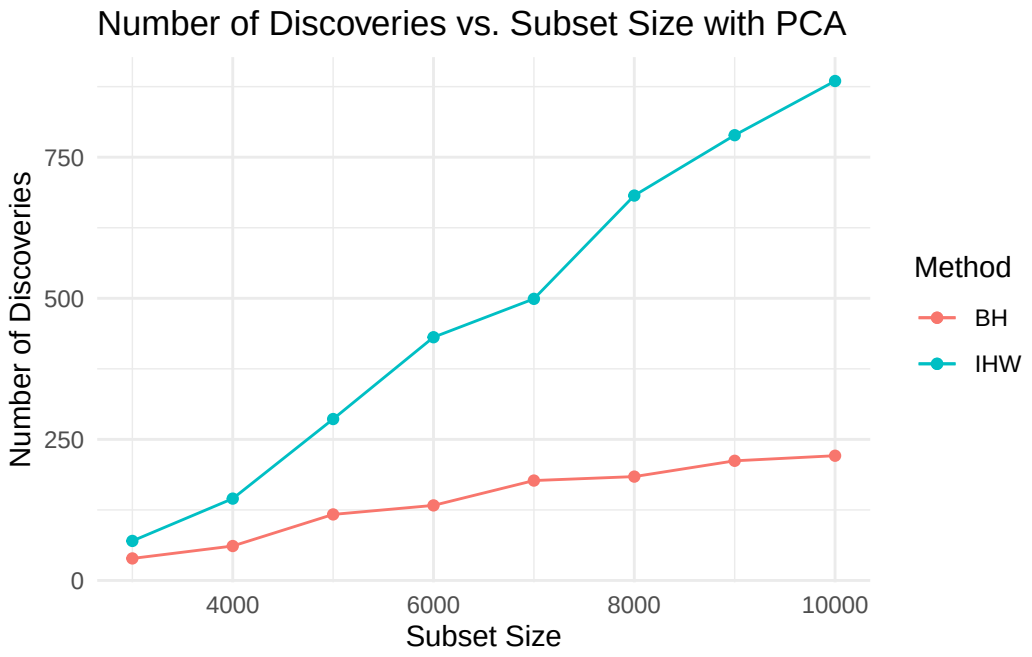
This is still similar to that by using the BH procedure:



As it can been seen, the informativeness of the covariate in using IHW is essential in increasing the number of discoveries. Improvements can be made by selecting covariates that are more predictive of the power of each test. If possible, transformations could potentially be applied to the covariate vector to better capture information that affects test power. As shown in the first plot of the section, as the sample sizes increases, it helps IHW to find more discoveries than methods like BH procedure while both have FDR under control.

3.3 More covariates with PCA

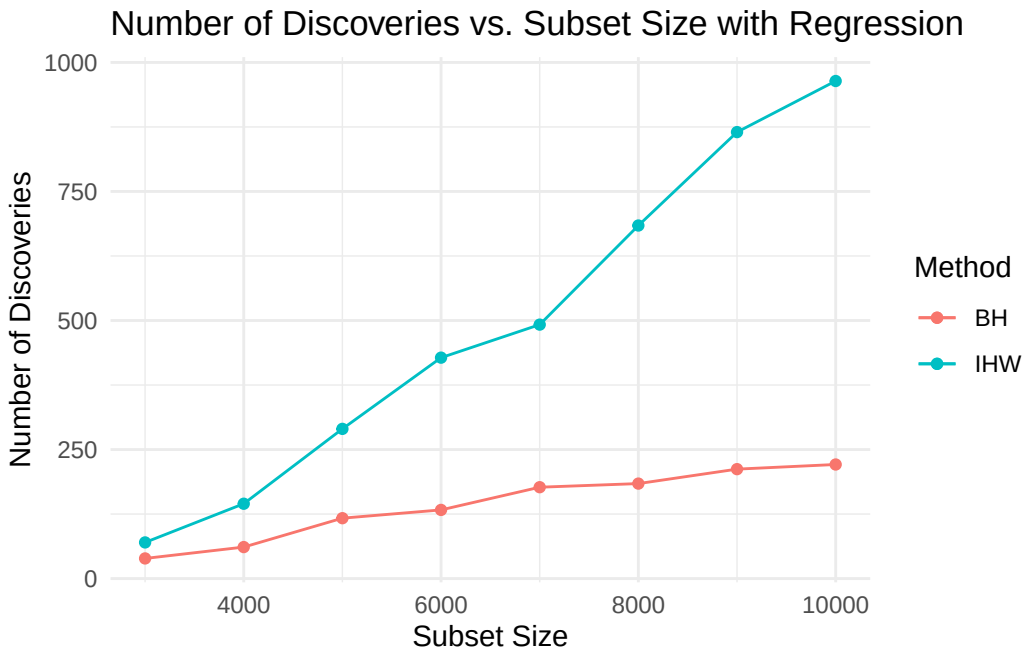
Given that the current implementation of IHW is designed to work with a single covariate, integrating multiple covariates into this framework presents a methodological challenge. However, several strategies could be employed to utilize multiple covariates effectively within the IHW. For instance, when there are multiple columns of informative covariates, Principal Component Analysis (PCA) can be utilized to reduce the dimensionality of covariates for IHW. Using my own simulated data again, in addition to the one column of covariate from before, I added another column that is related to the effect size with some slight parameter variations, while applying PCA. I used the first principal component, which explains the most variance, as the new covariate and applied the same procedure as before. The number of discoveries made compared with the BH procedure is again plotted:



There are some slight differences as before since now the covariate vector has been modified, however, the general trend still remains the same and similar interpretations can be made.

3.4 More covariates with regression

Another possible way is to incorporate the information of the multiple columns of covariates into one composite column, which is similiar compared with PCA in the sense that PCA decomposes the covariate matrix into principal components (PCs), each a linear combination of the original covariates. A regression-based approach to generate this composite covariate can be particularly informative, as it allows the use of a model to estimate an aspect of the tests from multiple covariates. I included effect size in the regression model while using the predicted covariate as the new column. I expected the plot to be similar and it did:



4. Conclusion

In summary, Independent Hypothesis Weighting (IHW) emerges as an enhancement to traditional multiple testing correction methods, notably through its incorporation of covariate information to refine hypothesis weighting. As evidenced by the simulations plots, the informativeness of the covariate is crucial in the efficacy of IHW. Extensions employing Principal Component Analysis (PCA) and regression models further augment IHW's adaptability, enabling the effective consolidation of multiple covariates into a singular, informative metric. Consequently, IHW offers a sophisticated framework for multiple testing correction, ensuring control over false discoveries while enhancing the discovery of true signals.

References

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