Multiple Testing Embedded in an Aggregation Tree to Identify where Two Distributions Differ

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Abstract

A key goal of flow cytometry data analysis is to identify the subpopulation of cells whose attributes are responsive to the treatment. These cells are supposed to be sparse among the entire cell population. To identify them, we propose a novel multiple TEsting on the Aggregation tree Method (TEAM) to locate where the treated and the control distributions differ. TEAM has a bottom-up hierarchical structure. On the bottom layer, we search for the short-range spiky distributional differences; while on the higher layers, we search for the long-range weak distributional differences. Starting from layer 2, on each layer nested hypotheses are formed based on the testing results from the previous layers, and the rejection rule will also depend on the previous layer. Under the mild conditions, we proved that TEAM will yield consistent layer-specific and overall false discovery proportion (FDP). We also showed that when there are sufficient long-range weak distributional differences, TEAM will yield better power compared with the signal-layer multiple testing methods. The simulations under different settings verified our theoretical results. As an illustration, we applied TEAM to a flow cytometry study where we successfully identified the cell subpopulation that are responsive to the cytomegalovirus antigen.

Keywords: Multiple testing, aggregation tree, false discovery proportion (FDP), distribution difference, flow cytometry (FCM)

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1. Introduction

An key problem in statistics is that based on the collected samples from two distributions to identify where their probability density functions (PDFs) differ. In many applications, these two PDFs only differ in very small regions. The goal is to identify those regions as accurately as possible.

A motivating example is the flow cytometry (FCM) analysis. FCM is the standard assay for single cell analysis in solution, and used ubiquitously in biology and medicine, especially for characterizing the immune system. In the assay, single cells tagged with fluorochrome-labeled monoclonal antibodies against specific cell surface or intra-cellular proteins, give off light signals with wavelengths (“colors”) characteristic for particular fluorochrome when activated by lasers. The intensity of each color is proportional to the number of fluorochrome-labeled monoclonal antibodies bound to the cell, and hence to the concentration of marker protein that the antibody is targeting. In general, the number of cells $N$ could range from $10^5$ to $10^7$ or even larger, and the number of protein markers range from fewer than 10 up to 50. The profile of colors emitted by each cell is then used to identify the cell type it is a member of. The ability quantify the distribution of cell types is useful since it provides a profile of the immune response, important in applications from evaluation of autoimmunity to monitoring of cancer immunotherapy.

One challenge of characterizing the immune response is that the cell types are heterogeneous, and many important subsets such as antigen-specific memory CD4 and CD8 T cells are often present in very low frequencies ($\ll 1\%$), making their identification and quantification a challenging problem. Framing it statistically, the goal is to identify where the distributions of the protein marker expressions differ across the treated and control sample of cells. In practice, only a small proportion of cells (usually $< 1\%$) are expected to exhibit the difference. Thus, we expect the treated and control distributions would differ in a few local regions.

The problem of locating distributional difference has raised much attention in the statistical field over the past decade. Roederer and Hardy (2001) proposed the frequency difference gating method, which partitioned the sample space to subregions with equal treated
cells, and then use chi-square test statistics to identify regions with significant differential densities. Duong (2013) used Kernel density estimators to estimate the density, and then proposed a chi-square test statistic to identify local distributional difference at any given location. Antoniadis et al. (2015) divided the samples into equal length subintervals, and then modeled the number of case samples and control samples falling into each subinterval to follow Poisson distributions. After that, they used the mean-matching root transformation to Gaussianize the distributions, and applied the existing multiple testing methods to identify the differential density subintervals.

Recently, Soriano and Ma (2017) proposed a multi-resolution scanning (MRS) method. The method can be viewed as a testing method embedded in the Pólya tree process (Lavine, 1992; Holmes et al., 2015; Ma and Wong, 2011), which has a top-down hierarchical structure. The MRS method sequentially partitions the common support of two distributions into finer and finer windows. On each resolution level, each window is coupled with a null hypothesis. MRS extended the Pólya tree structure by allowing each hypothesis to be true or false by itself, not depending on the “true or false” of its parent hypothesis on the coarser resolution levels, even though the finer resolution level hypotheses are nested in its parent hypothesis. By including all these (possibly nested) hypotheses, MRS can asymptotically control the false discovery rate (FDR) in this multiple testing problem.

In contrast, we propose a novel TEsting on the Aggregation tree Method (TEAM), which has a bottom-up hierarchical structure. On the bottom layer, we partition the common support of two distributions into equal-sample-size leaf bins with the finest resolution level. These bins are the finest unit where the distribution differences can be identified. Each leaf bin is coupled with a leaf null hypothesis assuming no distributional difference exists in that bin. On higher layers, we aggregate the nearby non-rejected child bins into larger parent bins, and couple each parent bin with a parent hypothesis assuming no distributional difference in that parent bin. If a parent hypothesis is rejected, then all of its bottom-layer descendant hypotheses are rejected. In other words, although on higher layers parent hypotheses will be generated for the convenience of testing, only the leaf hypotheses (on the bottom layer) are referred as the hypotheses of interest. With respect to those leaf hypotheses, the TEAM method will asymptotically control the false discovery. We will also show that TEAM is
asymptotically optimal.

A major difference between the methods embedded in the Pólya tree structure and the aggregation tree structure is that, methods with the Pólya tree structure tests the hypotheses coupled with the coarsest resolution windows first while methods with the aggregation tree structure prioritizes testing the hypotheses coupled with the finest resolution windows. Thus, in the setting of testing distribution differences, the aggregation tree methods can pinpoint in finer resolution where the differences are. Another major difference between these two frameworks is that, the Pólya tree framework treats the hypotheses coupled with all resolution windows as hypotheses of interest, even if many hypotheses are nested and thus overlapping. The aggregation tree only treats the leaf hypotheses on the finest resolution level as the hypotheses of interest. For general large-scale multiple testing problems, this feature is important because the aggregation tree framework does not add additional hypotheses to the original problem. Thus, methods with the aggregation tree framework can nest any existing single-layer testing method, and probably increase their power because of the additional rejections on coarser resolution levels.

The rest of the paper is organized as follows. In Section 2, we introduce how we partition the bins and generate hypotheses coupled with them. In Section 3, we elaborate the algorithm of TEAM. In Section 4, we prove that under the mild conditions, TEAM will have false discover proportion (FDP) converging to the desired level $\alpha$ in probability. We compared the numerical performance of TEAM with the single-layer method and MRS in Section 5, and showed that TEAM asymptotically controls FDR and has much higher power. In Section 6, we applied TEAM on a flow cytometry data and identified the cells responsive to the cytomegalovirus antigen. The proportion of cells identified by TEAM makes more biological sense compared with those identified by MRS. A general discussion on TEAM is provided in Section 7.

2. Model

Let $f_1$ and $f_0$ be the treated and control PDFs of a particular cell marker. Denote the common support of $f_1$ and $f_0$ by $\Omega$. Clearly, $\int f_s(y)dy = 1$ for both $s \in \{0, 1\}$. A key goal of the FCM analysis is to identify the treated cell abundance region $\Omega^{+,*} = \{y \in \Omega : f_1(y) >$
The reference cell abundance region \( \Omega^{-*} = \{ y \in \Omega : f_1(y) < f_0(y) \} \) is of less interest because it is usually fairly large. Therefore, we focus on developing a method to identify \( \Omega^{+*} \). If both \( \Omega^{+*} \cup \Omega^{-*} \) are of interest, we can first identify \( \Omega^{+*} \), then flip the case and control labels to identify \( \Omega^{-*} \).

In the FCM analysis, \( f_s \) is unknown. Instead, \( N_s \) independent samples following \( f_s \) are collected, \( s \in \{0, 1\} \). Each sample represents a cell in the FCM data. We partition the \( \Omega \) into \( m \) disjoint, consecutive, almost equal-sample-size bins \( \Omega_1, \ldots, \Omega_m \). Each bin contains \( n_i \) samples with \( n_i = \lfloor N/m \rfloor \) or \( n_i = \lceil N/m \rceil \), where \( N = N_1 + N_0 = \sum_{i=1}^m n_i \) is the total sample size. Based on the partition, we set the true signal bin set

\[
\Omega^+ = \cup_{i \in B} \Omega_i, \text{ where } B = \{ i : f_1(y) > f_0(y) \text{ for some } y \in \Omega_i \}.
\]

We relax our goal to identify \( \Omega^+ \) because it is more achievable with the partition. The larger the \( m \) is (or equivalently, the smaller the \( n_i \) is), the better \( \Omega^+ \) can approximate \( \Omega^{+*} \). On the other hand, \( n_i \) has to be large enough to make the asymptotic properties hold. The proper choice of \( n_i \) will be discussed in Section 4.

Let \( X_i \) be the count of case cells in bin \( i \). Assume \( X_i \) independently follows the distribution Bin\((n_i, \theta_i)\), where

\[
\theta_i = \frac{N_1 \int_{\Omega_i} f_1(y)dy}{N_1 \int_{\Omega_i} f_1(y)dy + N_0 \int_{\Omega_i} f_0(y)dy}.
\]

If \( f_1(y) \leq f_0(y) \) for all \( y \in \Omega_i \), then \( \theta_i \leq \theta_0 = N_1/N \). Therefore, we set up the hypotheses:

\[
H_{\text{null},i} : \theta_i \leq \theta_0 \text{ versus } H_{\text{alt},i} : \theta_i > \theta_0.
\]

Because the pooled sample size are almost the same across all bins, when the population density \( f = \frac{N_1}{N} f_1 + \frac{N_0}{N} f_0 \) is non-uniform, the length of each bin also varies. The bins in the high density regions (where \( f \) is large) are shorter. If a hypothesis coupled with such a bin is identified, we can more specifically pinpoint the location of the difference. In other words, higher density regions can yield higher resolution findings.

3. TEAM: Multiple Testing on the Aggregation Tree

We propose the method TEAM to perform large scale multiple testing on an aggregation tree. The aggregation tree has multiple layers. The bottom layer consists of leaves, each of
them representing a bin coupled with a null and an alternative hypothesis in the form of (2).

Let $\mathcal{H}^{(\ell)}$ be index set of the non-rejected null hypotheses at the beginning of layer $\ell$, $\ell \in \{1, \ldots, L\}$. The bottom layer index set is $\mathcal{H}^{(1)} = \mathcal{H}^{(0)} = \{1, \ldots, m^{(1)}\}$, where $m^{(1)} = m$. On layer $\ell$, node $i$ is coupled with the index set $S_i^{(\ell)} = \{i_1, \ldots, i_d\}$, where $i_d$ is the $(i - 1)2^{\ell - 1} + d$th element in $\mathcal{H}^{(\ell)}$, for $d = 1, \ldots, 2^{\ell - 1}$. In total, there are $m^{(\ell)}$ index sets on layer $\ell$. The hypotheses coupled with $S_i^{(\ell)}$ is

$$H_{\text{null}, i}^{(\ell)} : \forall j \in S_i^{(\ell)}, \theta_j \leq \theta_0.$$ 

On layer $\ell$, to test $H_{\text{null}, i}^{(\ell)}$, we first denote the region coupled with $S_i^{(\ell)}$ by $\Omega_i^{(\ell)} = \bigcup_{j \in S_i^{(\ell)}} \Omega_j$. The pooled sample size in $\Omega_i^{(\ell)}$ is $n_i^{(\ell)} = \sum_{j \in S_i^{(\ell)}} n_j$. Among them, $X_i^{(\ell)} = \sum_{j \notin S_i^{(\ell)}} X_j$ are cases. On the bottom layer, $X_i^{(1)} = X_i$ for $i = 1, \ldots, m^{(1)}$, which are mutually independent. It is easy to see that for any $\ell \geq 2$, $\Omega_i^{(\ell)} = \Omega_i^{(\ell - 1)} \cup \Omega_i^{(\ell - 1)}$, $n_i^{(\ell)} = n_i^{(\ell - 1)} + n_i^{(\ell - 1)}$, and $X_i^{(\ell)} = X_i^{(\ell - 1)} + X_i^{(\ell - 1)}$, with $i_1, i_2$ are two child nodes on layer $\ell - 1$ of node $i$ on layer $\ell$.

We will show in Section 4 that the optimal testing rule is to reject $H_{\text{null}, i}^{(\ell)}$ if $X_i^{(\ell)} > t^{(\ell)}$, where $t^{(\ell)}$ depends on the FDR level $\alpha$. To sum up, TEAM has an aggregation tree structure. See Figure 1 for an illustrating example. On layer $\ell$, suppose TEAM will generate the rejection set

$$\mathcal{R}^{(\ell)} = \{j : j \in S_i^{(\ell)}, H_{\text{null}, i}^{(\ell)} \text{ is rejected}\}.$$ 

To start layer $\ell + 1$, set the index set of non-rejected nulls $\mathcal{H}^{(\ell + 1)} = \mathcal{H}^{(\ell)} \setminus \mathcal{R}^{(\ell)}$.

Now we discuss how to determine the threshold $\hat{c}^{(\ell)}$. Let

$$G_0^{(\ell)}(c^{(\ell)}, c^{(\ell - 1)}) = \mathbb{P}(X_i^{(\ell)} > c^{(\ell)} \mid X_i^{(\ell - 1)} \leq c^{(\ell - 1)}, X_i^{(\ell - 1)} \leq c^{(\ell - 1)})$$

assuming $X_i^{(\ell - 1)}$ and $X_i^{(\ell - 1)}$ independently follow $\text{Bin}(n^{(\ell)}, \theta_0)$ distribution. Then marginally, $X_i^{(\ell)} = X_i^{(\ell - 1)} + X_i^{(\ell - 1)}$ follows $\text{Bin}(n^{(\ell)}, \theta_0)$. To simplify the expression, we let $\hat{c}^{(0)} = \infty$ on layer 0, so that on layer 1, $G_0^{(1)}(::\hat{c}^{(0)}) = G_0^{(1)}(::+\infty)$ is the marginal complement CDF of $\text{Binom}(n^{(1)}, \theta_0)$. On layer $\ell$, TEAM sets the threshold $\hat{c}^{(\ell)}$ as

$$\hat{c}^{(\ell)}(\alpha) = \inf \left\{ n^{(\ell)} \theta_0 \leq c \leq \min \{a_N, 2\hat{c}^{(\ell - 1)} - 1\} : \frac{m^{(\ell)} G_0^{(\ell)}(c; \hat{c}^{(\ell - 1)})}{\max \left\{ \sum_{i \leq i \leq m^{(\ell)}} I(X_i^{(\ell)} > c), 1 \right\}} \leq \alpha \right\},$$

(3)
4. Theoretical Properties of TEAM

4.1. Conditions

To show the theoretical properties of TEAM, we need the following conditions.

C1 Assume \( n^{(1)}_2 \leq r_1 \{ m^{(1)} \} r_1 \) for some \( r_1 < 4(\sqrt{2} - 1)^2 \theta_0 (1 - \theta_0) \), and \( r_2 > 0 \). Also assume \( N \{ m_3 \} \leq N \{ m^{(1)} \} \leq N \{ m_4 \} \) for some constants \( \frac{r_1}{1 + r_1} < r_3 \leq r_4 < 1/2 \), where \( N = N_1 + N_0 \) is total number of samples.
Algorithm 1: TEAM Procedure.

Input: $X_1, \ldots, X_m$, $N_0$, $N_1$, $n$, $\alpha$.

Output: The rejection set $\mathcal{R}$.

Set $N = N_0 + N_1$, $\theta_0 = (N_1 + 1/2)/N$, $n^{(1)} = n$, $\mathcal{H}^{(0)} = \{1, \ldots, m\}$, $\mathcal{R} = \mathcal{R}^{(0)} = \emptyset$

Set $\hat{c} = (\hat{c}^{(1)}, \ldots, \hat{c}^{(L)}) = 0_L$ with $0_L$ is a zero vector with length $L$

For $\ell = 1, \ldots, L$:

Set $\mathcal{H}^{(\ell)} = \mathcal{H}^{(\ell-1)} \setminus \mathcal{R}^{(\ell-1)}$, $\mathcal{R}^{(\ell)} = \emptyset$

Derive $m^{(\ell)}$, $(X_1^{(\ell)}, \ldots, X_{m^{(\ell)}}^{(\ell)})$, $n^{(\ell)}$, $\hat{c}^{(\ell)} = a_N^{(\ell)}$

Rank $X_i^{(\ell)}$ so that $X_{h^{(\ell)}(1)}^{(\ell)} \geq \ldots \geq X_{h^{(\ell)}(m^{(\ell)})}^{(\ell)}$, where $h^{(\ell)} : \mathcal{H}^{(\ell)} \to \mathcal{H}^{(\ell)}$ is the ranking index mapping

Set $i = 1$, $r_0 = 0$

While $X_{h^{(\ell)}(i)}^{(\ell)} > a_N^{(\ell)}$:

Set $r_0 = r_0 + 1$, $i = i + 1$

End

Set $i = r_0$, $\text{state} = 0$

While $\text{state} = 0$:

Set $\text{fdr}^{(\ell)} = m^{(\ell)} G_0^{(\ell)} \left( X_{h^{(\ell)}(i)}^{(\ell)} ; \hat{c}^{(\ell-1)} \right) / i$

If $\text{fdr}^{(\ell)} > \alpha$:

Set $i^* = i$, $\hat{c}^{(\ell)} = X_{h^{(\ell)}(i^*)}^{(\ell)}$, $\text{state} = 1$

End

else:

If $X_{h^{(\ell)}(i)}^{(\ell)} < n\theta_0$:

Set $i^* = i$, $\hat{c}^{(\ell)} = n\theta_0$, $\text{state} = 1$

End
else:

Set $i = i + 1$

End

End

If $i^* \geq 1$:

Set $\mathcal{R}^{(\ell)} = \mathcal{R}^{(\ell)} \cup \{y_{i^*} S_{h^{(\ell)}(i^*)}^{(\ell)} \}$

End

Set $\mathcal{R} = \mathcal{R} \cup \mathcal{R}^{(\ell)}$

End
C2 The total number of layers \( L \) is a constant. For all \( i \in \{1, \ldots, m\} \), assume \( r_5 \leq \theta_i \leq 1 - r_5 \) for some constants \( r_5 \) satisfying \( 0 < r_5 < 0.5 \).

C3 Let \( \alpha^{(0)} = +\infty \). For any \( 1 \leq \ell \leq L \), let

\[
\alpha^{(\ell)} = 2^{-(\ell-1)/2} \left\{ n^{(1)} \theta_0 (1 - \theta_0) \left( 2 \log \frac{m^{(1)}}{2^{\ell-1}} - 2 \log \log \frac{m^{(1)}}{2^{\ell-1}} \right) \right\}^{1/2}. 
\]

Define

\[
\mathcal{U}^{(\ell)} = \left\{ j' \in \{1, \ldots, m\} : \forall j \in \{j' - 2^{\ell-1} + 1, \ldots, j' - 1, j', j' + 1, \ldots, j' + 2^{\ell-1} - 1\}, \right. \\
\alpha^{(\ell)} + \frac{1}{2(\ell-1)/2} \left[ n^{(1)} \theta_j (1 - \theta_j) (2r_1 \log \frac{m^{(1)}}{2^{\ell-1}}) \right]^{1/2} < n^{(1)} (\theta_{j'} - \theta_0) \leq \alpha^{(\ell-1)} \left. \right\}.
\]

Assume for some constant \( r_6 > 0 \), \( s_U^{(\ell)} = \text{Card} \{ \mathcal{U}^{(\ell)} \} \) satisfies

\[
s_U^{(\ell)} \geq r_6 \log m^{(1)}.
\]

C4 For any \( j \in \{2, \ldots, m^{(1)}\} \),

\[
|\theta_j - \theta_{j-1}| = o \left\{ \left( \frac{\log m^{(1)}}{n^{(1)}} \right)^{1/2} \right\}.
\]

Condition C1 assumes that the true alternative bins are sparse. The upper bound of \( r_1 \) is determined by the inequality in Condition C3 so that

\[
\alpha^{(\ell)} + \frac{1}{2(\ell-1)/2} \left[ n^{(1)} \theta_j (1 - \theta_j) (2r_1 \log \frac{m^{(1)}}{2^{\ell-1}}) \right]^{1/2} \leq \alpha^{(\ell-1)}.
\]

Because \( \theta_j (1 - \theta_j) \leq 1/4 \), we know that \( r_1 < (\sqrt{2} - 1)^2 \approx 0.17 \). Condition C1 also specifies a lower bound for the number of the pooled cells in each bin. This number cannot be too small to affect the asymptotic convergence in the individual bin test. Also, because \( m^{(1)} = N/n^{(1)} \), it also imposes an upper bound for the number of hypotheses on the bottom layer. The condition \( N^{r_3} \leq n^{(1)} \leq N^{r_4} \) is equivalent to the condition \( (n^{(1)})^{\frac{1-r_4}{r_3}} \leq m^{(1)} \leq (n^{(1)})^{\frac{1-r_3}{r_4}} \), where \( m^{(1)} \) is the number of hypotheses on the bottom layer.

Condition C2 assumes the TEAM will proceed finite layers. It also assumes all \( \theta_i \) is bounded away from 0 and 1.
Condition C3 divided the alternative signals into different strength levels. The corresponding signal sets are labelled by $\mathcal{U}^{(1)}, \ldots, \mathcal{U}^{(L)}$. Those $\mathcal{U}^{(i)}$ with smaller $\ell$ contain signal segments where the individual signal is strong ($\hat{\theta}_i$ is large) but the segment is short; Those $\mathcal{U}^{(i)}$ with larger $\ell$ contain signal segments where the individual signal is weak ($\hat{\theta}_i$ is close to $\hat{\theta}_0$) but the segment is long. The signal level and segment length in $\mathcal{U}^{(\ell)}$ is designed in a way such that at least a sub-segment with length $2^{\ell-1}$ will stay after $\ell - 1$ layers with a non-neglecting probability; and on layer $\ell$, this sub-segment will be identified with a probability converging to 1. The cardinality condition $s_U^{(\ell)} \geq r_0 \log m^{(1)}$ is for the simplicity of presentation in proof. In fact, we only require $\lim_{N \to +\infty} s_U^{(\ell)} = +\infty$. Compared with the allowed total number of alternatives $r_2 (m^{(1)})^{r_1}$, the minimum bound on the cardinality of $\mathcal{U}^{(\ell)}$ is very small.

Under Conditions C1–C3, we can prove the layer-specific FDP is consistent. Condition C4 is needed to prove the overall FDP is consistent. It assumes that adjacent $\hat{\theta}_j$s will not change dramatically change. The following proposition shows when Condition C4 will hold with probability converging to 1. Here, the randomness comes from the partition of $[0, 1]$. The partition essentially depends on the samples. Different partition will lead to different $\hat{\theta}_i$s.

**Proposition 1.** Suppose that $f_1(y)$ and $f_0(y)$ have the same support on $[0, 1]$, where

$$0 < M_1 \leq \min\{f_1(y), f_0(y)\} \leq \max\{f_1(y), f_0(y)\} \leq M_2, \quad \forall y \in [0, 1].$$

Suppose their first derivatives are well-defined and satisfy

$$\max\{|f'_1(y)|, |f'_0(y)|\} \leq M_3, \quad \forall y \in [0, 1]$$

Then

$$P \left[ \sup_{j \in \{1, \ldots, m^{(1)}\}} |\hat{\theta}_j - \theta_{j-1}| = o \left\{ \frac{\log m^{(1)}}{n^{(1)}} \right\}^{1/2} \right] \geq 1 - \exp(-CN),$$

where $C$ is a constant only depending on $M_1$, $M_2$ and $M_3$.

In practice, the density functions of protein markers are very smooth. This indicates that Condition C4 will hold with probability converging to 1 for the FCM analysis.
Conditions C1 – C4 are imposed on the bottom layer, which reflects the characteristics of the original multiple testing problem. Because of the randomness in testing, we cannot directly impose conditions on higher layers. However, through the aggregation tree, the bottom layer conditions will naturally lead to the higher layer conditions.

4.2. Optimality in Thresholding $X_i^{(l)}$

The proposed test is based on thresholding $X_i^{(l)}$. This procedure has an internal connection with the local false discovery rate (lfdr) thresholding procedure proposed by Efron (2005) and Sun and Cai (2007). In particular, Sun and Cai (2007) and Xie et al. (2011) proved that thresholding lfdr is asymptotically optimal for multiple testing in the sense that this procedure will asymptotically control FDR and minimizes the false non-discovery rate (FNR).

On layer $\ell$, suppose $X_i^{(l)} = X_i^{(l-1)} + X_i^{(l-2)}$. Let the corresponding lfdr on layer $\ell - 1$ be $\text{lfdr}_i^{(l-1)}$ and $\text{lfdr}_i^{(l-2)}$. From a Bayesian perspective, lfdr is the posterior probability that the null hypothesis is true. Because the aggregation tree framework only aggregates those non-rejected hypotheses, on level $\ell$, the lfdr coupled with the $S_i^{(l)}$ is

$$\text{lfdr}_i^{(l)} = P(H_{null,i}^{(l)} \text{ is true} | X_i^{(l)} = x_i^{(l)}, \text{lfdr}_i^{(l-1)} \geq t^{(l-1)}, \text{lfdr}_i^{(l-2)} \geq t^{(l-1)})$$

$$= \frac{P(X_i^{(l)} = x_i^{(l)} | \text{lfdr}_i^{(l-1)} \geq t^{(l-1)}, \text{lfdr}_i^{(l-2)} \geq t^{(l-1)}, H_{null,i}^{(l)} \text{ is true})}{P(X_i^{(l)} = x_i^{(l)} | \text{lfdr}_i^{(l-1)} \geq t^{(l-1)}, \text{lfdr}_i^{(l-2)} \geq t^{(l-1)})} \times P(H_{null,i}^{(l)} \text{ is true} | \text{lfdr}_i^{(l-1)} \geq t^{(l-1)}, \text{lfdr}_i^{(l-2)} \geq t^{(l-1)}).$$

(4)

From an empirical Bayes perspective, each probability term in (4) can be estimated. However, deriving the estimators are not easy, especially now the conditional probabilities are involved. To simplify the computation, we propose to threshold $X_i^{(l)}$ instead of $\text{lfdr}_i^{(l)}$. Theorem 1 guarantees that thresholding $X_i^{(l)}$ is equivalent to thresholding $\text{lfdr}_i^{(l)}$, and therefore is optimal.

**Theorem 1.** $\text{lfdr}_i^{(l)}$ is monotonically decreasing in $X_i^{(l)}$. 


4.3. Asymptotic Properties

Define

\[ \mathcal{B}^{(l)}_{nul} = \{ i : S_i^{(l)} \subseteq \mathcal{H}^{(l)}, \forall j \in S_i^{(l)}, \theta_j \leq \theta_0 \}, \quad \mathcal{B}^{(l)}_{alt} = \{ i : S_i^{(l)} \subseteq \mathcal{H}^{(l)}, \exists j \in S_i^{(l)}, \theta_j > \theta_0 \} \]

\[ \mathcal{V}^{(l)} = \{ i \in \mathcal{B}^{(l)}_{nul} : S_i^{(l)} \subseteq \mathcal{R}^{(l)} \}, \quad \mathcal{W}^{(l)} = \{ i \in \mathcal{B}^{(l)}_{alt} : S_i^{(l)} \subseteq \mathcal{R}^{(l)} \}. \]

Also define \( \mathcal{U}^{(l)} = \mathcal{V}^{(l)} \cup \mathcal{W}^{(l)} \). Let

\[ U^{(l)} = \text{Card}(\mathcal{U}^{(l)}), \quad V^{(l)} = \text{Card}(\mathcal{V}^{(l)}), \quad W^{(l)} = \text{Card}(\mathcal{W}^{(l)}). \]

On layer \( \ell \), the layer-specific FDP is defined as \( \text{FDP}^{(l)} = V^{(l)}/U^{(l)} \).

Consider \( \text{FDP}^{(l)} \) as the product of four quantities,

\[
\text{FDP}^{(l)} = \frac{\sum_{i \in \mathcal{B}^{(l)}_{nul}} I(X_i^{(l)} > \hat{c}^{(l)})}{\sum_{i \in \mathcal{B}^{(l)}_{nul}} \tilde{G}_i^{(l)}(\hat{c}^{(l)}; \hat{c}^{(l-1)}/m_0^{(l)})} \times \frac{\sum_{i \in \mathcal{B}^{(l)}_{nul}} \tilde{G}_i^{(l)}(\hat{c}^{(l)}; \hat{c}^{(l-1)})}{\tilde{G}_0^{(l)}(\hat{c}^{(l)}; \hat{c}^{(l-1)})} \times \frac{\max(\sum_{1 \leq i \leq m^{(l)}} I(X_i^{(l)} > \hat{c}^{(l)}), 1)}{m^{(l)} \cdot \max(\sum_{1 \leq i \leq m^{(l)}} I(X_i^{(l)} > \hat{c}^{(l)}), 1)} \times \frac{m_0^{(l)}}{m^{(l)}}. \tag{5}
\]

In order to show convergence of \( \text{FDP}^{(l)} \) to \( \alpha \), we will need to accurately control the convergence of each of the quantities in (5). We will show that the first term and the second term converge to 1 in probability, the third term converges to \( \alpha \) in probability, and the fourth term converges to 1 in probability.

The following proposition is helpful in proving those consistencies.

**Proposition 2.** Define the set

\[ \mathcal{D}^{(1)}_{nul} = \{ i \in \mathcal{H}^{(1)}_{nul} : \theta_0 - (n^{(1)} \log m^{(1)})^{-1} < \theta_i \leq \theta_0 \} \tag{6} \]

Then under Condition \( C1 \),

\[ \lim_{N \to \infty} \frac{\text{Card}(\mathcal{D}^{(1)}_{nul})}{m_0^{(1)}} = 1. \]

Proposition 2 shows that under mild condition almost all the null \( \theta_i \)'s are very close to \( \theta_0 \).

The conclusion well approximates the real flow cytometry data, where the majority of case cells (usually more than 99%) share the similar protein marker expressions as the control cells.
Theorem 2. Under Conditions $C1$-$C3$, the multiple testing procedure (3) satisfies

$$
\lim_{N \to \infty} P(|FDP^{(\ell)} - \alpha| \leq \epsilon) = 1 \text{ for any } \epsilon > 0, \text{ and } \lim_{N \to \infty} FDR^{(\ell)} = \alpha.
$$

Theorem 2 is proved by induction. This is because the consistency of $FDP^{(\ell)}$ on higher layers depends on those on lower layers. The layer specific consistency does not necessarily lead to the overall consistency of $FDP$. This is because the alternative hypothesis on layer $\ell$ with $\ell \geq 2$ is that

$$
H^{(\ell)}_{alt, i} : \exists j \in S^{(\ell)}_i, \theta_j > \theta_0.
$$

Even if $S^{(\ell)}_i$ is a true rejection on layer $\ell$, it is likely that only one of its $\theta_j > \theta_0$, and the rest $\theta_j \leq \theta_0$. Based on the definition of null and hypothesis on the bottom layer, one true rejection set $S^{(\ell)}_i$ on layer $\ell$ do not necessarily turns into $2^{\ell-1}$ true rejection bins on the bottom layer. However, when the distributional difference have certain smoothness structures and other mild conditions, the probability that this will happen is converging to zero. Denote by $R^{(\ell)}_{null}$, $R^{(\ell)}_{alt}$, and $R^{(\ell)}$ the number of false discoveries, true discoveries, and total discoveries, respectively, on layer $\ell$. Also define the overall $FDP^{(1:L)}$ as the overall false discovery proportion,

$$
FDP^{(1:L)} = \frac{\sum_{\ell=1}^{L} R^{(\ell)}_{null}}{\sum_{\ell=1}^{L} R^{(\ell)}}.
$$

Theorem 3. Under Conditions $C1$–$C4$,

$$
FDP^{(1:L)} \xrightarrow{P} \alpha.
$$

The proof of Theorem 2 suggests that, with probability equal to 1, a significant amount of $\theta_j$s in $U^{(\ell)}$ cannot be identified on the previous $\ell - 1$ layers but will be identified on layer $\ell$. Therefore, the power of the TEAM method will be higher as the number of layers goes up.

Corollary 1. Under Conditions $C1$–$C4$, for any $1 \leq \ell_1 < \ell_2 \leq L$,

$$
P \left\{ \sum_{\ell_1 \leq \ell \leq \ell_2} S^{(\ell)} > 0 \right\} = 1.
$$
5. Numerical Experiments

To evaluate the performance of TEAM, we considered three settings: local shift (S1), local dispersion (S2), and local shift plus dispersion (S3). Each simulation setting consists of two univariate normal mixture distributions, whose PDFs are plotted at the first row of Figure 2. These patterns are commonly seen in FCM experiments.

S1. Local shift difference
Treated: $Y_1 \sim 0.97\mathcal{N}(0.2, 0.04^2) + 0.03\mathcal{N}(0.89, 0.01^2)$
Control: $Y_0 \sim 0.97\mathcal{N}(0.2, 0.04^2) + 0.03\mathcal{N}(0.88, 0.01^2)$

S2. Local dispersion difference
Treated: $Y_1 \sim 0.97\mathcal{N}(0.4, 0.04^2) + 0.03\mathcal{N}(0.8, 0.03^2)$
Control: $Y_0 \sim 0.97\mathcal{N}(0.4, 0.04^2) + 0.03\mathcal{N}(0.8, 0.02^2)$

S3. Local shift + dispersion difference
Treated: $Y_1 \sim 0.97\mathcal{N}(0.4, 0.04^2) + 0.03\mathcal{N}(0.82, 0.05^2)$
Control: $Y_0 \sim 0.98\mathcal{N}(0.4, 0.04^2) + 0.02\mathcal{N}(0.8, 0.04^2)$

For each setting, we generated $N_1 = 1,474,560$ cells from the treated distribution and $N_0 = 1,474,560$ cells from the reference distribution. On the bottom layer, we specified $m = 2^{14}$ bins, such that each bin contains $n = 180$ pooled samples. The number $n$ is set such that $n \approx \{2(N_0 + N_1)\}^{1/3}$. Our numerical experiments show that the choice of $n$ will not impose large impact on the analysis. We also tried numbers between 120 and 200, the results are similar. The number of layers $L$ is chosen so that $1000 \leq m^{(L)} < 2000$. In these simulation settings, the bottom layer has $m = 2^{14}$ bins; assuming only a small proportion of hypotheses will be rejected, at layer 5, $m^{(5)} \approx 2^{10} = 1024$. Thus, we run TEAM up to five layers. We also tried six layers in practice, but many times TEAM stopped to rejecting more null hypotheses on layer 6, indicating that running five layers is sufficient. The experiment is repeated 1000 times. TEAM is very computationally efficient. One repetition only takes less than 5 seconds on a 2.6 GHz Intel Core i5 processor with 16Gb memory. The code for running TEAM is available at https://github.com/jbp7/TEAM.
Figure 2 shows the empirical FDR (average realized FDP), the average FN (number of false negatives), and the average total discoveries for the three simulated scenarios. Clearly, TEAM method can control FDR under the designed level. As the number of layers goes up, some regions with contingent signals are identified, resulting in more and more true discoveries (See the fourth row of Figure 2). In Setting 3, as layers went up, TEAM identified many true alternatives so that the empirical FDP goes down.

Figure 3 shows the average sensitivities of the TEAM stratified by different values of alternative $\theta_i$s. The null value in the simulation is $\theta_0 = 0.5$. When $\theta_i$ is close to 0.5, the signal is weak; otherwise, the signal is strong. The average sensitivity should depend on the distribution of alternative signals, the desired FDR level, and the number of layers. In practice, we found that the number of layers has the most impact on the average sensitivity.

Figure 4 compares the performance of our method with the single-layer multiple testing method (SLM) and the multi-resolution scanning method (MRS). SLM is the same as the first layer of TEAM. MRS is a testing method proposed by Soriano and Ma (2017). It is embedded in the Pólya tree process. It starts from the coarsest resolution and each time bisection the region to perform multiple testing on finer resolution levels. To make the results from MRS and TEAM comparable, we took the following three procedures. First, we ran MRS to 14 layers with $2^{14}$ hypotheses on that level, and summarize its results on the finest five layers. We then map all the rejected hypotheses to the finest resolution level with $2^{14}$ hypotheses. Second, the MRS is a two-sided testing method, and TEAM is a one-sided testing method. To match the results, we first ran TEAM on the original sample, and then flip the treated and control label of the samples and ran TEAM again. Then we can identify those regions with differential densities of protein markers in the treated and control samples. Third, it turns out that if we consider the original multiple testing problem with each hypothesis coupled to a bottom layer bin, MRS often yields very high false discovery rates, around or over 50%. This is because Soriano and Ma (2017) treat the hypotheses on different resolution levels as different hypotheses. In total, MRS consider $2^{14} + 2^{13} + 2^{12} + 2^{11} + 2^{10}$ hypotheses. Many of their hypotheses are nested. MRS is trying to control FDR among those hypotheses. This FDR is different from what our method is trying to control. To compare TEAM and MRS fairly, we mapped their FDP to the same
level and compare their average false negatives. In the first two settings, TEAM uniformly outperformed SLM and MRS, in the sense that when these methods have the same level of empirical FDR, TEAM has much fewer average false negatives. In Setting 3, neither SLM or TEAM works well. This is because after we flipped the “treated” and “control” labels, almost all hypotheses are alternatives. The assumption of sparse alternatives is violated here and therefore both SLM and TEAM fail.

6. Data Analysis of a Multi-color Flow Cytometry Study

We illustrate the proposed method using data from the External Quality Assurance Program Oversight Laboratory (EQAPOL) proficiency program by the Duke Immune Profiling Core (DIPC) (Staats et al., 2014). This data set comes from 11 healthy volunteers who provided blood samples for flow cytometric intra-cellular cytokine staining (ICS) experiments. Blood samples from each individual was used as a negative control (“Costim”) or treated with a peptide mixture from the immunodominant cytomegalovirus (CMV) pp65 protein. Each sample contains approximately 200,000 cells for which 11 attributes, protein markers for discriminating between T cell basic, maturational and functional subsets, have been measured. The expectation is that T cells specific for the CMV pp65 would be activated and show elevated intra-cellular levels of some or all of the functional markers, which are the cytokines TNF-α, IL-2, and IFN-γ, and CD107 (a protein associated with cytotoxic activity). We also expect that CMV-specific T cells are a small fraction of total T cell (<1%) and hence generate a weak signal for detection in the data. To this end, statistically, we compare the density of functional marker intensity levels, and identify those regions where the densities differ in the treated and control samples. Cells with functional markers fall into this range are the candidates of the stimulated cells.

ICS data was preprocessed using manual gating in FlowJo software (v9.9.6) to remove monocytes, debris, doublet, and aggregate cells. There are 11 individuals with paired treated and control samples. To apply our procedure, we partitioned the common range of the 401,294 pooled case and control cells into $2^{11}$ bins, such that each bin contained approximately 200 cells. The desired FDR is set at 0.05. As an illustration, we mainly display the
Figure 2: Two-sample problems and the performance of our algorithm based on averaged FDP, FN and total discoveries. (First row) Univariate densities corresponding to each of the three simulation scenarios. The case density is in solid red, while the control density is in dotted black. (Second row) Average FDP, (third row) Average FN and (last row) Average TD (total discoveries) versus nominal FDR, $\alpha$. In the local shift, dispersion, and shift+dispersion models, there were 246, 160, and 329 non-null bins, respectively.
Figure 3: Sensitivity for quartiles of nonnull signal effect sizes, $\theta_i > \theta_0$ across varying nominal FDR, $\alpha$, and simulation settings. Discoveries were taken at the last layer ($L = 5$) of our procedure.
Figure 4: Comparison of three methods - MRS, Single Layer, and TEAM for two univariate two-sample settings. For both settings, there were 492 nonnull bins containing two-sided density differences.

Analysis results for one individual. The analysis results of the other 10 individuals are displayed in the Supplementary Material. As an illustration, we discuss the results for a single individual. The Kernel density estimates of TNF-α, IL-2, IFN-γ, and CD107 are shown in Figure 5. The results for the remaining 10 subjects can be found in the Supplementary Material.

To apply our procedure, we partitioned the common range of the 401,294 pooled case and control cells into $2^{11}$ bins, such that each bin contained approximately 200 cells. Using an FDR of 0.05, we applied our procedure to identify the cell subsets associated with an increase in CMV/case density for each of the four protein markers. We then located the case cells that are in the identified region, and plotted the upset chart (the left panel of Figure 6) of these cells, labeling the number of cells with one, two, three, or four protein markers in the identified regions. Most cells with only one or two protein markers located in the density differential regions could still be non-stimulated cells. We target those case cells with three or four protein markers that are located in the differential regions. The TEAM method identified 3631 such cells, comprising about 1.89% of the total case cells.

We also applied the MRS method to the same data set. MRS is a top-down method which starts from the low-resolution level. It is also a two-sided method. MRS stopped after six layers. We also marked the number of cells with one, two, three, or four protein
markers located in the density differential regions (the right panel of Figure 6). It identified 49325 case cells that have three or four protein makers in the differential regions, comprising 25.64% of the total case cells. In most situations, only 1% of the cells that are expected to be stimulated by the CMV antigen. Compared with TEAM, MRS does not perform as well in narrowing down those cells.

![Figure 5: Kernel density estimates of the four protein markers, TNF-α, IL-2, IFN-γ, and CD107, in case samples (solid red) and control samples (dotted black).](image)

7. Discussion

We presented TEAM, a new framework of multiple testing embedded in the aggregation tree. Although in this paper TEAM focused on identifying those region where two distributions differ, the same idea can be extended to other multiple testing problems. Almost all existing multiple testing procedures are single-layer methods. They can be easily adapted to the first layer of TEAM. Compared to these single-layer methods, TEAM will have at least the same power as long as the first layer testing procedure is the same. Any additional layers of TEAM will likely to increase the power. TEAM is in favor of those situations where the true alternatives are adjoining. Compared to the single-layer methods, the con
The performance of TEAM will be affected by some preset numbers, such as the number of cell samples included in each bin on the bottom layer $n$, and the total number of layers $L$. Based on the results in Section 4 and Section 5, we suggest choosing $n \approx (2N)^{1/3}$, where $N$ is the number of cells. The theoretical justification implies that the power of TEAM will go up when there exists many long segments of weak signals. Heuristically, if TEAM stop to reject additional null hypotheses on a specific layer, this can be viewed a stop sign. Also, when $L$ is too large, the asymptotic convergence of FDP could be affected so that the overall FDR might be inflated. To make sure the asymptotic validity of TEAM, the maximum (top) layer should have enough number of nested hypotheses to be tested. Based on our experience, we suggest having at least 500 nested hypotheses on the maximum layer.

In terms of the FCM analysis, researchers found that it is more powerful to compare the joint distributions of two or three protein markers among the treated and the control cell samples. The TEAM method proposed in the paper can be extended to identify the joint distribution differences. We can sequentially binning along each direction to make sure each multi-dimensional rectangle bin contains approximately same number of cells. The testing
procedure is similar. The discussion is beyond the scope of this paper so we will not describe the details.

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Appendix A. Proofs of the Main Results

For simplicity of the proof of the asymptotic properties, let \( n_i = n \) for \( i = 1, \ldots, m \). Then \( n^{(\ell)} = 2^{\ell-1}n \). Recall that \( S_i^{(\ell)} \) is the index set coupled with the \( i \)th node on layer \( \ell \). Further, denote by \( \tilde{G}_i^{(\ell)}(x; c) \) the conditional complementary CDF (ccCDF) of \( X_i^{(\ell)} \) conditioning on \( X_i^{(\ell-1)} \leq c, X_{i_2}^{(\ell-1)} \leq c \). When \( \ell = 1 \), \( \tilde{G}_i^{(1)}(x; c) \) decays to the marginal complementary CDF of Bin\((n, \theta_j)\).

For two sequences of real numbers \( \{a_n\} \) and \( \{b_n\} \), write \( a_n = O(b_n) \) if there exists a constant \( C \) such that \( a_n \leq Cb_n \) holds for all sufficiently large \( n \). Write \( a_n = o(b_n) \) if \( \lim_{n \to \infty} a_n/b_n = 0 \). If \( a_n = O(b_n) \) and \( b_n = O(a_n) \), then \( a_n \asymp b_n \). If \( \lim_{n \to \infty} a_n/b_n = 1 \), write \( a_n \sim b_n \).

To prove the asymptotic properties of TEAM, we need the following lemmas. Lemma provide a binomial local limit theorem and moderate deviation result for the binomial tail probability. The proofs of Lemmas 1 and 2 can be found in Chapter 8 of Lesigne (2005). The proofs of Lemmas 3 – 6 can be found in the supplementary materials.
Lemma 1. For a Bin\( (n, \theta) \) random variable \( X \), \( 0 \leq k \leq n \), for any \( k \) satisfies \( |k - n\theta| < c_n n^{2/3} \) with \( \lim_{n \to \infty} c_n = 0 \), we have

\[
P(X = k) \sim \frac{1}{\sqrt{2\pi n\theta(1 - \theta)}} \exp \left( -\frac{(k - n\theta)^2}{2n\theta(1 - \theta)} \right).
\]

Lemma 2. Let \( X \) be a Bin\( (n, \theta) \) random variable. Suppose that \( \{\tau_n\} \) is a sequence of real numbers such that \( \lim_{n \to \infty} \tau_n = +\infty \) and \( \lim_{n \to \infty} \tau_n n^{-1/6} = 0 \). Then

\[
P(X \geq n\theta + \tau_n n\theta(1 - \theta)) \sim \frac{\varphi(\tau_n)}{\tau_n}.
\]

Here, \( \varphi(\cdot) \) is the standard normal density function.

Lemma 3. Consider the \( D_{\text{mul}}^{(1)} \) defined in (6). For \( c^{(\ell-1)} \geq n^{(\ell-1)} \theta_0 \) and \( c^{(\ell)} \geq n^{(\ell)} \theta_0 \),

\[
\lim_{n,m \to \infty} \sup_{S_n^{(\ell)} \leq D_{\text{mul}}^{(1)}} \left| \tilde{G}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) - G_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) \right| = 0.
\]

Lemma 4. For \( c^{(\ell-1)} \geq n^{(\ell-1)} \theta_0 \) and \( c^{(\ell)} \geq n^{(\ell)} \theta_0 \),

\[
\lim_{n,m \to \infty} \sup_{\ell \in \{1, \ldots, L\}} \sup_{i \in B_{\text{mul}}^{(\ell)}} \left( \tilde{G}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) - G_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) \right) \leq 0. \tag{A.1}
\]

\[
\lim_{n,m \to \infty} \inf_{\ell \in \{1, \ldots, L\}} \inf_{i \in B_{\text{mul}}^{(\ell)}} \left( \tilde{G}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) - G_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) \right) \geq 0. \tag{A.2}
\]

Also, define

\[
\tilde{g}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) = P(X_i^{(\ell)} = c^{(\ell)} | X_i \leq c^{(\ell-1)}, X_{ij} \leq c^{(\ell-1)})
\]

\[
g_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) = P(X_i^{(\ell)} = c^{(\ell)} | X_i \leq c^{(\ell-1)}, X_{ij} \leq c^{(\ell-1)}, \theta_j = \theta_0, \forall j \in S_i^{(\ell)}).
\]

Then

\[
\lim_{n,m \to \infty} \sup_{\ell \in \{1, \ldots, L\}} \sup_{i \in B_{\text{mul}}^{(\ell)}} \left( \tilde{g}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) - g_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) \right) \leq 0. \tag{A.3}
\]

\[
\lim_{n,m \to \infty} \inf_{\ell \in \{1, \ldots, L\}} \inf_{i \in B_{\text{mul}}^{(\ell)}} \left( \tilde{g}_i^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) - g_0^{(\ell)}(c^{(\ell)}; c^{(\ell-1)}) \right) \geq 0. \tag{A.4}
\]

Lemma 5. Under Conditions \( C1 \) and \( C2 \), the cutoff \( \hat{c}^{(\ell)} \) defined in (3) satisfies

\[
\lim_{N \to +\infty} \sup_{1 \leq \ell \leq L} P \left[ \hat{c}^{(\ell)} \leq n^{(\ell)} \theta_0 + \left( n^{(\ell)} \theta_0 (1 - \theta_0) (2 \log m^{(\ell)} - 2 \log \log m^{(\ell)}) \right)^{1/2} (1 + o(1)) \right] = 0. \tag{A.5}
\]
Lemma 6. Under Conditions C1 and C2, we have

\[
\lim_{N \to +\infty} \inf_{1 \leq \ell \leq L} \mathbb{P}\left[ m_{0}(\ell) G_{0}(\hat{c}(\ell); \hat{c}(\ell-1)) \leq \alpha \max \left\{ I(X_{i}^{(\ell)} > \hat{c}(\ell)), 1 \right\} \right.
\leq m_{0}(\ell) G_{0}(\hat{c}(\ell); \hat{c}(\ell-1)) + C \frac{\log m(\ell)}{n^{1/2}} \right] = 1.
\]

Proof of Proposition 1. Note that \( \theta_{i} = \frac{N_{1} \int_{\Omega_{i}} f_{1}(y)dy}{N_{1} \int_{\Omega_{i}} f_{1}(y)dy + N_{0} \int_{\Omega_{i}} f_{0}(y)dy} \). For any \( j \in \{2, \ldots, m^{(1)}\} \), it suffices to show that

\[
\mathbb{P}\left[ \frac{N_{1} \int_{\Omega_{i}} f_{1}(y)dy}{N_{0} \int_{\Omega_{i}} f_{0}(y)dy} - \frac{N_{1} \int_{\Omega_{j}} f_{1}(y)dy}{N_{0} \int_{\Omega_{j}} f_{0}(y)dy} \right] = o \left( \left( \frac{\log m^{(1)}}{n^{(1)}} \right)^{1/2} \right) \geq 1 - C \exp[-\{n^{(1)}\}^{1/2}].
\]

Let \( f(y) = \{N_{1}f_{1}(y) + N_{0}f_{0}(y)\}/(N_{1} + N_{0}). \) It is easy to see that \( M_{1} \leq f(y) \leq M_{2}. \) Let \( \text{Span}(\Omega_{i}) = \sup_{y \in \Omega_{i}} y - \inf_{y \in \Omega_{i}} y. \)

\[
\mathbb{P}\left\{ \sup_{i} \text{Span}(\Omega_{i}) \geq \frac{2n^{(1)}}{M_{1}N} \right\} \leq \mathbb{P}\left\{ \exists \Omega^{*}, \text{ such that } \text{Span}(\Omega^{*}) = \frac{2n^{(1)}}{M_{1}N} \text{ and } \sum_{k=1}^{N} I(y_{k} \in \Omega^{*}) \leq n^{(1)} \right\}
\]

Because

\[
\frac{2n^{(1)}}{N} \leq P(y_{k} \in \Omega^{*}) = \int_{\Omega^{*}} f(y)dy \leq \frac{2M_{2}n^{(1)}}{M_{1}N},
\]

by Azuma’s inequality, we have

\[
\mathbb{P}\left[ \sum_{k=1}^{N} I(y_{k} \in \Omega^{*}) - N \int_{\Omega_{i}} f(y)dy < -n^{(1)} \right] \leq \exp \left\{ - \frac{M_{1}^{2}N}{4M_{2}^{2}} \right\}.
\]

Thus,

\[
\mathbb{P}\left\{ \sup_{i} \text{Span}(\Omega_{i}) \geq \frac{2n^{(1)}}{M_{1}N} \right\} \leq \mathbb{P}\left\{ \sum_{k=1}^{N} I(y_{k} \in \Omega^{*}) \leq n^{(1)} \right\} \leq \exp \left\{ - \frac{M_{1}^{2}N}{4M_{2}^{2}} \right\}.
\]

Now consider the space \( X = \{x : \sup_{i} \text{Span}(\Omega_{i}) \leq \frac{2n^{(1)}}{M_{1}N}\}. \) Let \( y_{r,i,\max} = \arg \max_{\Omega_{i}} f_{s}(y) \)
and \( y_{s,i,min} = \arg \min_{\Omega_i} f_s(y) \), for \( s \in \{0, 1\} \), and any \( i \in \{1, \ldots, m^{(1)}\} \). Then

\[
\frac{N_1 f_{\Omega_j} f_1(y)dy}{N_0 f_{\Omega_j} f_0(y)dy} - \frac{N_1 f_{\Omega_{j-1}} f_1(y)dy}{N_0 f_{\Omega_{j-1}} f_0(y)dy} \leq \frac{f_1(y_{0,j,max})}{f_0(y_{0,j,min})} - \frac{f_1(y_{0,j-1,min})}{f_0(y_{0,j-1,max})} \leq \frac{f_1(y_{0,j,min}) + f'_1(y_{1,j})(y_{1,j,max} - y_{0,j,min})}{f_0(y_{0,j,min})} - \frac{f_1(y_{0,j-1,max} + f'_1(y_{1,j})(y_{1,j-1,min} - y_{0,j-1,max})}{f_0(y_{0,j-1,max})} \leq \frac{f_1(y_{0,j,min}) + |f'_1(y_{1,j})|\text{Span}(\Omega_j)}{f_0(y_{0,j,min})} - \frac{f_1(y_{0,j-1,max}) - |f'_1(y_{1,j})|\text{Span}(\Omega_{j-1})}{f_0(y_{0,j-1,max})} \leq h(y_{0,j,min}) - h(y_{0,j-1,max}) + M_4 \frac{n^{(1)}}{N},
\]

where \( h(y) = \frac{h(y)}{f_0(y)} \). It is easy to see that \( M_5 \leq |h'(y)| \leq M_6 \). Here \( M_4, M_5, \) and \( M_6 \) all depend on \( M_1, M_2, \) and \( M_3 \). Therefore,

\[
\frac{N_1 f_{\Omega_j} f_1(y)dy}{N_0 f_{\Omega_j} f_0(y)dy} - \frac{N_1 f_{\Omega_{j-1}} f_1(y)dy}{N_0 f_{\Omega_{j-1}} f_0(y)dy} \leq M_6 \cdot 2 \sup_i \text{Span}(\Omega_i) + M_4 \frac{n^{(1)}}{N} \leq M_7 \frac{n^{(1)}}{N}.
\]

Similarly, we can prove that

\[
\frac{N_1 f_{\Omega_{j-1}} f_1(y)dy}{N_0 f_{\Omega_{j-1}} f_0(y)dy} - \frac{N_1 f_{\Omega_j} f_1(y)dy}{N_0 f_{\Omega_j} f_0(y)dy} \leq M_8 \frac{n^{(1)}}{N}.
\]

It is easy to see that \( \frac{n^{(1)}}{N} \leq N^{r_4 - 1} \), where \( r_4 - 1 < -1/2 \). Therefore, \( \frac{n^{(1)}}{N} = o\left(\frac{1}{m^{(1)}+1}\right) \).

\( \square \)

**Proof of Theorem 1.** Let

\[
\Lambda^{(t)}_i = \frac{\text{P}(X_i^{(t)} = x_i^{(t)}, X_{i1}^{(t-1)} \leq c^{(t-1)}, X_{i2}^{(t-1)} \leq c^{(t-1)} | H_{\text{null},i}^{(t)} \text{ is true})}{\text{P}(X_i^{(t)} = x_i^{(t)}, X_{i1}^{(t-1)} \leq c^{(t-1)}, X_{i2}^{(t-1)} \leq c^{(t-1)} | H_{\text{null},i}^{(t)} \text{ is not true})},
\]

\[
\tilde{\Lambda}^{(t)}_i = \frac{\text{P}(X_i^{(t)} = x_i^{(t)}, \lfdr_{i1}^{(t-1)} \geq t^{(t-1)}, \lfdr_{i2}^{(t-1)} \geq t^{(t-1)} | H_{\text{null},i}^{(t)} \text{ is true})}{\text{P}(X_i^{(t)} = x_i^{(t)}, \lfdr_{i1}^{(t-1)} \geq t^{(t-1)}, \lfdr_{i2}^{(t-1)} \geq t^{(t-1)} | H_{\text{null},i}^{(t)} \text{ is not true})},
\]

\[
r_0^{(t)}_{i,t} = \frac{\text{P}(H_{\text{null},i}^{(t)} \text{ is true})}{\text{P}(H_{\text{null},i}^{(t)} \text{ is not true})}.
\]

Please note that when \( \ell = 1 \), the parts \( X_{i1}^{(t-1)} \leq c^{(t-1)}, X_{i2}^{(t-1)} \leq c^{(t-1)}, \lfdr_{i1}^{(t-1)} \geq t^{(t-1)}, \) and \( \lfdr_{i2}^{(t-1)} \geq t^{(t-1)} \) can be removed from the above expressions.
We prove this proposition by induction.

a) Let $\ell = 1$. We know that

$$\text{lfdr}_i^{(1)} = \frac{\Lambda_i^{(1)} r_{0,i}^{(1)}}{\Lambda_i^{(1)} r_{0,i}^{(1)} + 1}.$$ 

Thus, $\text{lfdr}_i^{(1)}$ is monotonically increasing with $\Lambda_i^{(1)}$.

Because $X_i^{(1)} \sim \text{Bin}(n^{(1)}, \theta_i^*)$ under the null and $X_i^{(1)} \sim \text{Bin}(n^{(1)}, \theta_i)$ under the alternative, with $\theta_i^* \leq \theta_0 < \theta_i$,

$$\Lambda_i^{(1)} = \frac{\binom{n^{(1)}}{x_i^{(1)}}(\theta_i^*)^{x_i^{(1)}}(1 - \theta_i^*)^{n^{(1)} - x_i^{(1)}}}{\binom{n^{(1)}}{x_i^{(1)}}(\theta_i)^{x_i^{(1)}}(1 - \theta_i)^{n^{(1)} - x_i^{(1)}}} = \left(\frac{\theta_i^*}{\theta_i}\right)^{x_i^{(1)}} \left(\frac{1 - \theta_i^*}{1 - \theta_i}\right)^{n^{(1)} - x_i^{(1)}}$$

Thus $\Lambda_i^{(1)}$ is monotonically decreasing with $X_i^{(1)}$, and this leads to the conclusion.

b) Consider $\ell \geq 2$. Now assume that $\text{lfdr}_i^{(k)}$ is monotonically decreasing with $X_i^{(k)}$, for $k = 1, \ldots, \ell - 1$. Then for some $t^{(\ell - 1)} \in (0, 1)$, there exists some $c^{(\ell - 1)} \in [0, n^{(\ell - 1)}]$, such that the events $\{\text{lfdr}_i^{(\ell - 1)} \geq t^{(\ell - 1)}\}$ and $\{X_i^{(\ell - 1)} \leq c^{(\ell - 1)}\}$ are the same. Thus, $\Lambda_i^{(\ell - 1)} = \Lambda_i^{(\ell - 1)}$. It follows that

$$\text{lfdr}_i^{(\ell)} = \frac{\tilde{\Lambda}_i^{(\ell)} r_{0,i}^{(\ell)}}{\Lambda_i^{(\ell)} r_{0,i}^{(\ell)} + 1} = \frac{\Lambda_i^{(\ell)} r_{0,i}^{(\ell)}}{\Lambda_i^{(\ell)} r_{0,i}^{(\ell)} + 1}.$$ 

Clearly, $\text{lfdr}_i^{(\ell)}$ is monotonically increasing with $\Lambda_i^{(\ell)}$. Let the event

$$\mathcal{X}_i^{(\ell)} = \{X_j, j \in S_i^{(\ell)} : X_i^{(\ell)} = x_i^{(\ell)}, X_i^{(\ell - 1)} \leq c^{(\ell - 1)}, X_i^{(\ell - 1)} \leq c^{(\ell - 1)}\}.$$

Then

$$\Lambda_i^{(\ell)} = \frac{\sum_{j \in S_i^{(\ell)}} \prod_{j \in S_i^{(\ell)}} \binom{n^{(1)}}{x_j^{(1)}}(\theta_j^{*})^{x_j^{(1)}}(1 - \theta_j^{*})^{n^{(1)} - x_j^{(1)}}}{\sum_{j \in S_i^{(\ell)}} \prod_{j \in S_i^{(\ell)}} \binom{n^{(1)}}{x_j^{(1)}}(\theta_j)^{x_j^{(1)}}(1 - \theta_j)^{n^{(1)} - x_j^{(1)}}},$$

where $\theta_j^{*} \leq \theta_0 < \theta_j$, for all $j \in S_i^{(\ell)}$. Because for all $j \in S_i^{(\ell)}$,

$$\binom{n^{(1)}}{x_j^{(1)}}(\theta_j^{*})^{x_j^{(1)}}(1 - \theta_j^{*})^{n^{(1)} - x_j^{(1)}} < \binom{n^{(1)}}{x_j^{(1)}}(\theta_j)^{x_j^{(1)}}(1 - \theta_j)^{n^{(1)} - x_j^{(1)}},$$

$\Lambda_i^{(\ell)}$ is monotonically decreasing with $X_i^{(\ell)}$. 

Proof of Proposition 2. Based on the partition process, for any bin $\Omega_i$,

$$\sum_{k=1}^{N} I(\text{Cell } k \text{ falls into } \Omega_i) = n.$$ 

Taking expectation on both sides, we have

$$N \cdot P(\text{Cell } k \text{ falls into } \Omega_i) = \int_{\Omega_i} \{N_1 f_1(y) + N_0 f_0(y)\} \, dy = n.$$ 

Combined with the definition of $\theta_i$ in (1), we have

$$n^{(1)} \sum_{i=1}^{m_0^{(1)}} \theta_i = N_1 \sum_{i=1}^{n^{(1)}} \int_{\Omega_i} f_1(y) \, dy = N_1 = N \theta_0.$$ 

It leads to

$$\sum_{i=1}^{m_0^{(1)}} \theta_i = \sum_{i \in \mathcal{H}_{nul}^{(1)}} \theta_i + \sum_{i \in \mathcal{H}_{alt}^{(1)}} \theta_i = m_0^{(1)} \theta_0.$$ 

Now let $\delta = \sup_{i \in \mathcal{H}_{alt}^{(1)}} (\theta_i - \theta_0)$. Then

$$m_0^{(1)} \theta_0 - \sum_{i \in \mathcal{H}_{nul}^{(1)}} \theta_i = \sum_{i \in \mathcal{H}_{alt}^{(1)}} (\theta_i - \theta_0) \leq m_1^{(1)} \delta.$$ 

Because $\theta_i \leq \theta_0$ in $\mathcal{H}_{nul}^{(1)}$ and $\mathcal{E}_{nul}^{(1)} = \mathcal{H}_{nul}^{(1)} \setminus \mathcal{D}_{nul}^{(1)} \subseteq \mathcal{H}_{nul}^{(1)}$,

$$\sum_{i \in \mathcal{E}_{nul}^{(1)}} (\theta_i - \theta_0) \leq m_1^{(1)} \delta.$$ 

On $\mathcal{E}_{nul}^{(1)}$, $\theta_i - \theta_0 \geq (n^{(1)} \log m^{(1)})^{-1}$. Combined with Condition C1,

$$\text{Card}(\mathcal{E}_{nul}^{(1)}) \leq m_1^{(1)} \delta (n^{(1)} \log m^{(1)}) \leq r_2 \delta n^{(1)} \{m^{(1)}\}^{r_1 \log m^{(1)}}.$$ 

Thus,

$$\frac{\text{Card}(\mathcal{D}_{nul}^{(1)})}{m_0^{(1)}} = 1 - \frac{\text{Card}(\mathcal{E}_{nul}^{(1)})}{m_0^{(1)}} \geq 1 - \frac{r_2 \delta n^{(1)} \{m^{(1)}\}^{r_1 \log m^{(1)}}}{m_0^{(1)}} = 1 - o(1).$$ 

$\square$
Proof of Theorem 2. Based on (5), in order to prove
\[ \lim_{N \to +\infty} P(|FDP^{(\ell)} - \alpha| \leq \epsilon) = 1, \]
we only need prove
\[ P \left\{ \frac{\sum_{i \in B^{(\ell)}} \ell(X_i^{(\ell)} > \hat{c}^{(\ell)})}{\sum_{i \in B^{(\ell)}} \tilde{G}_i^{(\ell)}(\hat{c}^{(\ell)}; \hat{c}^{(\ell-1)})} - 1 > \epsilon \right\} \to 0, \quad \text{as } N \to \infty. \]  
\[ (A.7) \]
\[ \frac{\sum_{i \in \mathcal{B}^{(\ell)}} \tilde{G}_i^{(\ell)}(\hat{c}^{(\ell)}; \hat{c}^{(\ell-1)})}{m_0(\hat{c}^{(\ell)}; \hat{c}^{(\ell-1)})} - 1 > \epsilon \to 0, \quad \text{as } N \to \infty. \]  
\[ (A.8) \]
\[ \frac{\max(\sum_{1 \leq i \leq m^{(\ell)}} \ell(X_i^{(\ell)} > \hat{c}^{(\ell)}, 1)/m^{(\ell)})}{\alpha} > \epsilon \to 0, \quad \text{as } N \to \infty. \]  
\[ (A.9) \]
\[ \frac{m_0^{(\ell)}}{m^{(\ell)}} > \epsilon \to 0, \quad \text{as } N \to \infty. \]  
\[ (A.10) \]
We will prove (A.6) and equivalently (A.7)–(A.10) by induction.

a) First, we prove (A.6) on layer 1, or equivalently, (A.7) – (A.10) hold when \( \ell = 1, \hat{c}^{(0)} = +\infty \). We know that (A.10) holds by Condition C1. We now prove (A.7), (A.8), and (A.9), respectively.

a1) First we prove (A.9).

Recall that \( s_U^{(1)} = Card(\mathcal{U}^{(1)}) \).

\[ P \left[ \sum_{i \in \mathcal{U}^{(1)}} \ell(X_i^{(1)} > c^{(1)}) - \sum_{i \in \mathcal{U}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty) \geq \sqrt{2} \left\{ s_U^{(1)} \log(s_U^{(1)}) \right\}^{1/2} \right] \leq 2\{s_U^{(1)}\}^{-1}. \]

For \( i \in \mathcal{U}^{(1)} \),
\[ n^{(1)} \theta_i - \{n^{(1)} \theta_i(1 - \theta_i)(2r_1 \log m^{(1)})\}^{1/2} \geq n^{(1)} \theta_0 + \{n^{(1)} \theta_0(1 - \theta_0)(2 \log m^{(1)} - 2 \log \log m^{(1)})\}^{1/2}. \]

By Lemma 2 and Lemma 4, \( \tilde{G}_i^{(1)}(c^{(1)}; +\infty) > 1 - (m^{(1)})^{-r_1}(2r_1 \log m^{(1)})^{-1/2}(1 + o(1)) \). Because \( s_U^{(1)} \leq m_1^{(1)} \leq r_2(m^{(1)})^r_1, \sum_{i \in \mathcal{U}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty) > s_U^{(1)} - o(1) \). Then
\[ P \left\{ \sum_{i \in \mathcal{U}^{(1)}} \ell(X_i^{(1)} > c^{(1)}) > s_U^{(1)} - o(s_U^{(1)}) \right\} \leq 2\{s_U^{(1)}\}^{-1}. \]  
\[ (A.11) \]
It is easy to see that
\[ \sum_{1 \leq i \leq m^{(1)}} I(X_i^{(1)} > c^{(1)}) \leq \sum_{i \in d^{(1)}} I(X_i^{(1)} > c^{(1)}) \]

Combining Lemma 6 and (A.11), we have
\[ P \left\{ m_0^{(1)} G_0^{(1)}(\hat{c}^{(1)}; +\infty) = \alpha(1 + o(1)) \max \left\{ \sum_{1 \leq i \leq m^{(1)}} I(X_i^{(1)} > \hat{c}^{(1)}), 1 \right\} \right\} \to 1. \]

a2) Then we prove (A.8).

\[ \sum_{i \in H_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(\hat{c}^{(1)}; +\infty) - m_0^{(1)} G_0^{(1)}(\hat{c}^{(1)}; +\infty) = \]
\[ \sum_{i \in D_{\text{nul}}^{(1)}} \left\{ \tilde{G}_i^{(1)}(\hat{c}^{(1)}; +\infty) - G_0^{(1)}(\hat{c}^{(1)}; +\infty) \right\} + \sum_{i \in E_{\text{nul}}^{(1)}} \left\{ \tilde{G}_i^{(1)}(\hat{c}^{(1)}; +\infty) - G_0^{(1)}(\hat{c}^{(1)}; +\infty) \right\}, \quad (A.12) \]

where \( E_{\text{nul}}^{(1)} = H_{\text{nul}}^{(1)} \setminus D_{\text{nul}}^{(1)} \).

By Lemma 3 and Proposition 2, we have
\[ \sum_{i \in D_{\text{nul}}^{(1)}} \left\{ \tilde{G}_i^{(1)}(\hat{c}^{(1)}; +\infty) - G_0^{(1)}(\hat{c}^{(1)}; +\infty) \right\} = m_0^{(1)} G_0^{(1)}(\hat{c}^{(1)}; +\infty)(1 + o(1)). \]

By Proposition 2, we have
\[ \sum_{i \in E_{\text{nul}}^{(1)}} \left\{ \tilde{G}_i^{(1)}(\hat{c}^{(1)}; +\infty) - G_0^{(1)}(\hat{c}^{(1)}; +\infty) \right\} = o(m_0^{(1)}) \]

Combined with (A.12), (A.8) holds.

a3) Now we prove (A.7).

\[ P \left\{ \frac{\sum_{i \in H_{\text{nul}}^{(1)}} I(X_i^{(1)} > c^{(1)})}{\sum_{i \in H_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty)} - 1 > \epsilon \right\} \leq \frac{1}{\epsilon^2} \text{Var} \left\{ \frac{\sum_{i \in H_{\text{nul}}^{(1)}} I(X_i^{(1)} > c^{(1)})}{\sum_{i \in H_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty)} - 1 \right\} \]
\[ \leq \frac{1}{\epsilon^2} \sum_{i \in H_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty) \left\{ 1 - \tilde{G}_i^{(1)}(c^{(1)}; +\infty) \right\} \]
\[ \leq \frac{1}{\epsilon^2} \sum_{i \in H_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty) \]
\[ \leq \frac{1}{\epsilon^2} \sum_{i \in D_{\text{nul}}^{(1)}} \tilde{G}_i^{(1)}(c^{(1)}; +\infty). \]
Combining with Lemma 3, we have
\[
P \left\{ \frac{\sum_{i \in \mathcal{H}_\text{null}^{(1)}} I(X^{(1)}_i > c^{(1)})}{\sum_{i \in \mathcal{H}_\text{null}^{(1)}} \tilde{G}^{(i)}_1(c^{(1)}; +\infty)} - 1 \geq \epsilon \right\} \leq \frac{2}{\epsilon^2 m^{(1)}_1 G_0^{(1)}(c^{(1)}; +\infty)}. \tag{A.13}
\]

By Lemma 6 and (A.11),
\[
P \left\{ \frac{\sum_{i \in \mathcal{H}_\text{null}^{(1)}} I(X^{(1)}_i > \tilde{c}^{(1)})}{\sum_{i \in \mathcal{H}_\text{null}^{(1)}} \tilde{G}^{(i)}_1(\tilde{c}^{(1)}; +\infty)} - 1 \geq \epsilon \right\} \rightarrow 0.
\]

b) Now we assume (A.6) – (A.10) hold for layer 1, 2, \ldots, \ell - 1. We will prove (A.7) – (A.10) hold for layer \ell.

b1) First we prove (A.10) on layer \ell.

Denote by \(V^{(k)}\) and \(R^{(k)}\) the numbers of false discoveries and total rejections on layer \(k\), then the total number of true discoveries is \(R^{(k)} - V^{(k)}\).

Take any \(\bar{\alpha} > \alpha\), we have
\[
P (\cap_{k=1}^{\ell} \{ \mathbf{x} : \text{FDP}^{(k)} < \bar{\alpha} \}) \rightarrow 1. \tag{A.14}
\]

When \(\cap_{k=1}^{\ell-1} \text{FDP}^{(k)} < \bar{\alpha}\) holds, \(V^{(k)} \leq \bar{\alpha} R^{(k)}\), and it follows that
\[
\text{FDP}^{(k)} \leq \frac{\bar{\alpha}}{1 + \bar{\alpha}} (R^{(k)} - V^{(k)}) \leq \frac{\bar{\alpha}}{1 + \bar{\alpha}} m^{(1)}.
\]

Then, \(m^{(\ell)}_0 \geq m^{(\ell)} - \frac{\tilde{c}^{(\ell-1)} - 2^{\ell-1}}{2^{\ell+1}}\). Meanwhile, \(m^{(\ell)} \leq \frac{m^{(1)}_{\alpha}}{2^\ell}\). Thus, \(m^{(\ell)}_{0}/m^{(\ell)} \geq 1 - o(1)\) on the space \(\cap_{k=1}^{\ell} \{ \mathbf{x} : \text{FDP}^{(k)} < \bar{\alpha} \}\). Combined with (A.14), we can get (A.10).

b2) Next we prove (A.9) on layer \(\ell\).

For any set \(A\) with length \(2^{k-1}\), let \(X^{(k)}_A = \sum_{j \in A} X^{(1)}_j\).

Suppose \(j' \in \mathcal{U}^{(\ell)}\). For any two disjoint index sets \(A^{(k-1)}_1\) and \(A^{(k-1)}_2\) with length \(2^{k-2}\), suppose both of them are subsets of \(B^{(\ell)}_j = \{ j' - 2^{\ell-1} + 1, \ldots, j' - 1, j', j' + 1, \ldots, j' + 2^{\ell-1} - 1 \}\), let \(A^{(k)} = A^{(k-1)}_1 \cup A^{(k-1)}_2\), a index set of length \(2^{k-1}\). For any \(j \in A^{(k)}\), \(n^{(\ell)} \theta_j \leq n^{(\ell)} \theta_0 + \alpha^{(\ell)}\). Let \(\tilde{\theta}_0 = \theta_j + \alpha^{(\ell)}/n^{(\ell)}\), then \(n^{(\ell)} \theta_j \leq n^{(\ell)} \tilde{\theta}_0\). By Lemma 4, By Lemma 6, we have
\[
\sup_{1 \leq k \leq \ell-1} \sup_{j \in A^{(k)}} P \left( \hat{c}^{(k)} \geq n^{(\ell)} \tilde{\theta}_0 \right) \rightarrow 1. \tag{A.15}
\]
Thus,

\[
\begin{align*}
& \Pr\left( X_{A_{1j}}^{(k)} \leq \hat{c}^{(k)} \mid X_{A_{1j}}^{(k-1)} \leq \hat{c}^{(k-1)}, X_{A_{2j}}^{(k-1)} \leq \hat{c}^{(k-1)}, \forall j \in A^{(k)}, \theta_j \leq \tilde{\theta}_0 \right) \\
& \geq \Pr\left( X_{A_{1j}}^{(k)} \leq \hat{c}^{(k)} \mid X_{A_{1j}}^{(k-1)} \leq \hat{c}^{(k-1)}, X_{A_{2j}}^{(k-1)} \leq \hat{c}^{(k-1)}, \forall j \in A^{(k)}, \theta_j = \tilde{\theta}_0 \right) \\
& \geq \Pr\left( X_{A_{1j}}^{(k)} \leq \hat{c}^{(k)} \mid \forall j \in A^{(k)}, \theta_j = \tilde{\theta}_0 \right) \\
& \geq 1/2 - \varepsilon,
\end{align*}
\]

for some \( \varepsilon > 0 \). The first inequality is because the similar argument as Lemma 4. The third inequality is because of normal approximation of binomial distribution and (A.15).

Therefore, for any \( j' \in U^{(l)} \), the probability that some subset \( A^{(l)} \subset B_{j'}^{(l)} \) of length \( 2^{\ell-1} \) belongs to the testing sets is greater than \( (1/2 - \varepsilon)^{\ell-1} \).

On layer \( \ell \), for any \( j \in A^{(l)} \),

\[
\begin{align*}
& n^{(l)} \theta_j - \{n^{(l)} \theta_i (1 - \theta_i)(2r_1 \log m^{(l)})\}^{1/2} \\
& \geq \{n^{(l)} \theta_0 + \{n^{(l)} \theta_0 (1 - \theta_0)(2 \log m^{(l)} - 2 \log \log m^{(l)})\}^{1/2}\}(1 + o(1)).
\end{align*}
\]

Therefore, for any \( j' \in U^{(l)} \)

\[
\begin{align*}
& \Pr\left( \text{There exists some } S_i^{(l)} \subset B_{j'}^{(l)}, X_i^{(l)} > \hat{c}^{(l)} \mid X_{i_1}^{(l-1)} \leq \hat{c}^{(l-1)}, X_{i_2}^{(l-1)} \leq \hat{c}^{(l-1)} \right) \\
& \geq \left\{1 - (m^{(l)})^{-r_1} (2r_1 \log m^{(l)})^{-1/2}(1 + o(1))\right\}^{1/2} > \frac{1}{3}.
\end{align*}
\]

Recall that \( s_U^{(l)} = \text{Card}(U^{(l)}) \).

\[
\begin{align*}
& \Pr\left( \sum_{S_i^{(l)} \subset U^{(l)}} I(X_i^{(l)} > c^{(l)}) - \sum_{S_i^{(l)} \subset U^{(l)}} \tilde{G}_i^{(l)}(c^{(l)}; c^{(l-1)}) \geq \sqrt{2} \left\{ s_U^{(l)} \log(s_U^{(l)}) \right\}^{1/2} \right) \leq 2\{s_U^{(l)}\}^{-1}.
\end{align*}
\]

Therefore

\[
\begin{align*}
& \Pr\left( \sum_{S_i^{(l)} \subset U^{(l)}} I(X_i^{(l)} > c^{(l)}) > \frac{1}{3} s_U^{(l)} - o(s_U^{(l)}) \right) \leq 2\{s_U^{(l)}\}^{-1}.
\end{align*}
\]

it is easy to see that

\[
\sum_{1 \leq i \leq m^{(l)}} I(X_i^{(l)} > c^{(l)}) \geq \sum_{S_i^{(l)} \subset U^{(l)}} I(X_i^{(l)} > c^{(l)})
\]

\( \square \)
Combined with Lemma 6, Condition C3, we have
\[ P \left\{ m_0^{(\ell)} G_0^{(\ell)}(\hat{c}^{(\ell)}; \hat{c}^{(\ell-1)}) = \alpha(1 + o(1)) \max \left\{ \sum_{1 \leq i \leq m^{(\ell)}} I(X_i^{(\ell)} > \hat{c}^{(\ell)}), 1 \right\} \right\} \to 1. \]

b3) Now we prove (A.8) on layer \( \ell \).
Let
\[ D_{\text{null}}^{(\ell)} = \left\{ i \in \{1, \ldots, m_0^{(\ell)}\} : \forall j \in S_i^{(\ell)}, \theta_0 - (n \log m^{(1)})^{-1} < \theta_j < \theta_0 \right\}. \]
By the proof of (A.10) in Part b1), we know that
\[ \text{Card}(D_{\text{null}}^{(\ell)}) \geq \frac{\text{Card}(D_{\text{null}}^{(1)}) - m_1^{(1)} - 2^{\ell-1}}{2^{\ell-1}}. \]
Combined with \( \text{Card}(D_{\text{null}}^{(1)}) \sim m^{(1)}, m_0^{(\ell)} \leq m^{(\ell)} \leq m^{(\ell)}, \) we have \( \text{Card}(D_{\text{null}}^{(\ell)}) \sim m_0^{(\ell)} \sim m^{(\ell)}. \)
Then following the similar argument in Part a2), we can prove (A.8).

b4) Finally, following the similar argument of Part a3), we can prove (A.7) in layer \( \ell \).

**Proof of Theorem 3.** Recall that \( \mathcal{H}^{(\ell)} \) is the set of remaining hypothesis index at the beginning of layer \( \ell \). Let \( \mathcal{H}^{(\ell)} = \mathcal{H}_{\text{null}}^{(\ell)} \cup \mathcal{H}_{\text{alt}}^{(\ell)} \), where \( \mathcal{H}_{\text{null}}^{(\ell)} = \{ j \in \mathcal{H}^{(\ell)} : \theta_j \leq \theta_0 \} \), and \( \mathcal{H}_{\text{alt}}^{(\ell)} = \mathcal{H}^{(\ell)} \setminus \mathcal{H}_{\text{null}}^{(\ell)} \).

First, we will prove that
\[ P \left\{ \inf_{i \in \mathcal{W}^{(\ell)}} \inf_{j \in S_i^{(\ell)}} \theta_j > \theta_0 \right\} \geq 1 - 2(m^{(\ell)})^{-1} - 2(\log m^{(1)})^{-1/2}. \tag{A.16} \]
By (S13), we know that
\[ P \left[ \inf_{i \in \mathcal{W}^{(\ell)}} \sum_{j \in S_i^{(\ell)}} X_j^{(1)} \geq n^{(1)} \theta_0 + \left\{ n^{(1)} \theta_0 (1 - \theta_0) (2 \log m^{(\ell)} - 2 \log \log m^{(\ell)}) \right\}^{1/2} (1 + o(1)) \right] \leq 1 - 2(m^{(\ell)})^{-1}. \]
Thus,
\[ P \left[ \inf_{i \in \mathcal{W}^{(\ell)}} \sup_{j \in S_i^{(\ell)}} X_j^{(1)} \geq n^{(1)} \theta_0 + 2^{-\ell-1} \left\{ n^{(1)} \theta_0 (1 - \theta_0) (2 \log m^{(\ell)} - 2 \log \log m^{(\ell)}) \right\}^{1/2} (1 + o(1)) \right] \leq 1 - 2(m^{(\ell)})^{-1}. \tag{A.17} \]
For any $j \in \mathcal{H}^{(\ell)}$, by Lemma 2,
\[
P \left\{ X_j - n^{(1)} \theta_j > 2 \left\{ m_1^{(1)} \right\}^{1/2} \left( \log m_1^{(1)} \right) \right\} \leq \left\{ m_1^{(1)} \right\}^{-1} \left\{ \log m_1^{(1)} \right\}^{-1/2}.
\]
Let $j_i^{(\ell)} = \arg \sup_{j \in S_i^{(\ell)}} X_j^{(1)}$. Because $W^{(\ell)} = \text{Card}(W^{(\ell)}) \leq m_1^{(1)}$,
\[
P \left\{ \sup_{i \in W^{(\ell)}} (X_{j_i^{(\ell)}} - n^{(1)} \theta_j) > 2 \left\{ m_1^{(1)} \right\}^{1/2} \left( \log m_1^{(1)} \right) \right\} \leq \left\{ \log m_1^{(1)} \right\}^{-1/2}. \quad (A.18)
\]
By (A.17) and (A.18),
\[
P \left[ \inf_{i \in W^{(\ell)}} n^{(1)} \theta_{j_i^{(\ell)}}, \theta_{j_i^{(\ell)}} / n^{(1)} \right] \geq n^{(1)} \theta_0 + 2^{-\frac{1}{2}} \left\{ n^{(1)} \theta_0 (1 - \theta_0) (2 \log m^{(\ell)} - 2 \log m^{(\ell)}) \right\}^{1/2} (1 + o(1))
- 2 \left\{ m_1^{(1)} \right\}^{1/2} \left\{ \log m_1^{(1)} \right\} \geq 1 - 2(m^{(\ell)})^{-1} - \left\{ \log m_1^{(1)} \right\}^{-1/2}.
\]
By Condition C1, we know that $r_3 > \frac{r_1}{r_1 + r_4}$. Thus,
\[
\left\{ m_1^{(1)} \right\}^{1/2} \log m_1^{(1)} = o \left\{ \left( n^{(1)} \theta_0 (1 - \theta_0) (2 \log m^{(\ell)} - 2 \log m^{(\ell)}) \right) \right\}^{1/2}.
\]
Also by Condition C4,
\[
|\theta_j - \theta_{j-1}| = o \left\{ \left( \frac{\log m_1^{(1)}}{n^{(1)}} \right) \right\}^{1/2},
\]
and $\text{Card}(S_i^{(\ell)}) = 2^{\ell-1}$, we know that
\[
P \left[ \inf_{i \in W^{(\ell)}} \inf_{j \in S_i^{(\ell)}} \theta_{j_i^{(\ell)}} \geq \theta_0 + 2^{-\frac{1}{2}} \left\{ 2 \theta_0 (1 - \theta_0) \log m^{(\ell)} \right\}^{1/2} (1 + o(1)) \right]
\geq 1 - 2(m^{(\ell)})^{-1} - \left\{ \log m_1^{(1)} \right\}^{-1/2}.
\]
Thus
\[
P \left\{ \bigcup_{i \in W^{(\ell)}} S_i^{(\ell)} \subseteq \mathcal{H}_{\text{alt}}^{(\ell)} \right\} \geq 1 - 2(m^{(\ell)})^{-1} - \left\{ \log m_1^{(1)} \right\}^{-1/2}.
\]
Subsequently,
\[
P \left[ \cap_{\ell=1}^L \{ R^{(\ell)}_{\text{alt}} = 2^{\ell-1} W^{(\ell)} \} \right] \geq 1 - \sum_{\ell=1}^L 2(m^{(\ell)})^{-1} - L \left\{ \log m_1^{(1)} \right\}^{-1/2} \rightarrow 1.
\]
For any $\varepsilon > 0$, by Theorem 2, for any $\varepsilon > 0$ and $\delta > 0$, as $N$ sufficiently large,
\[
P \left\{ \sup_{\ell=1}^L \left| \frac{V^{(\ell)}}{U^{(\ell)}} - a \right| > \varepsilon \right\} < \delta.
\]
It is easy to see that $R^{(l)} = 2^{l-1}U^{(l)}$. If $R^{(l)}_{\text{alt}} = 2^{l-1}W^{(l)}$, then $R^{(l)}_{\text{nul}} = 2^{l-1}V^{(l)}$. Thus

$$
\mathbb{P}\left( |\text{FDP}^{(1:L)} - \alpha| > \varepsilon \right) = \mathbb{P}\left( \frac{\sum_{l=1}^{L} R^{(l)}_{\text{nul}}}{\sum_{l=1}^{L} R^{(l)}} - \alpha \bigg| > \varepsilon \right) \leq \mathbb{P}\left\{ \sup_{l=1}^{L} \frac{R^{(l)}_{\text{nul}}}{R^{(l)}} - \alpha > \varepsilon \right\}
$$

$$
\leq \mathbb{P}\left\{ \frac{L}{\sup_{l=1}^{L}} \left| \frac{2^{l-1}V^{(l)}}{2^{l-1}U^{(l)}} \right| \right\} + \mathbb{P}\left[ \bigcup_{l=1}^{L} \{ R^{(l)}_{\text{alt}} \neq 2^{l-1}W^{(l)} \} \right] < \delta + \sum_{l=1}^{L} 2(m^{(l)})^{-1} + L\{ \log m^{(1)} \}^{-1/2}.
$$

\[ \square \]

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