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SUMMARY. In this paper we propose a new procedure to select differentially expressed genes between several conditions in microarray experiments. Asymptotic properties for the false discovery rate are proved under mild conditions. We compare by simulations and on a pseudo-real data set our procedure to the Benjamini and Hochberg's procedure and a procedure based on mixture models.

KEY WORDS: Differential expression; Microarray data; Multiple testing procedure; Ordered statistics; Partial sums.

1 Introduction

The aim of this paper is to propose a new procedure to determine differentially expressed genes in microarray experiments. DNA microarrays are a new class of technology that enables molecular biologists to simultaneously measure the expression level of thousands of genes (Brown and Bolstein, 1999). Thousand of genes probes made of cDNA or oligonucleotides are spotted on a small glass slide or a nylon membrane in a known regular matrix pattern. Then some mARN is taken away from the biological samples to compare and is labeled with different fluorophores. The samples are mixed and hybridized to each microarray that is then scanned in a microarray scanner to visualize fluorescence of the different fluorophores. Then we can compare the concentration of the corresponding mRNA in the biological sample, and therefore compare the level of expression of the corresponding gene between several conditions. A basic experiment consists in comparing the expression levels of each gene in two different types of conditions (e.g. diseased tissue versus healthy tissue). More generally we can study several conditions with one or several repetitions. The detection of differentially expressed genes in DNA microarray experiments is an important question asked by biologists to statisticians (Quackenbush, 2001). At this stage, we suppose that intensity levels of genes are correctly normalized and we study microarray data from an experiment including n genes, J conditions and R repetitions for each gene in each condition (Figure 1).

The object of the paper is to separate differentially from non-differentially expressed genes. This corresponds to a multiple testing procedure. For all gene i, we want to test the null hypothesis $H_{0,i}$: "The expression of gene i obeys to the same law under the J conditions". To address this problem the simplest procedure would be the Bonferroni correction. This procedure controls the familywise error rate (FWER) which is the probability of accumulating one or more false-positives among all the tests. This crite-

rion is very stringent and may affect the power when the number of tests is large. An alternative procedure consists in controlling the false discovery rate (FDR). Benjamini and Hochberg (1995) introduced a procedure which controls the FDR for independent test statistics. Other properties on this procedure have been proved by Ferreira and Zwinderman (2006). Adaptive FDR controlling procedures have been proposed to increase the power while controlling the FDR (Benjamini and Hochberg, 2000; Benjamini et al, 2006; Storey, 2002, 2003; Storey et al., 2004). Genovese and Wasserman (2002, 2004) have proposed a method to minimize the false negative rate (FNR) while controlling the FDR. Mixture models have been studied to separate the central observations from the others (Bordes et al., 2007; Storey, 2002, 2003; Ghosh, 2006). On another way, some procedures based on model selection (Huet, 2006) or some procedures based on the partial sums of the absolute or squared ordered observations (Hoh et al., 2001; Lavielle and Ludeña, 2006) have been proposed. Zaykin et al. (2002) and Dudbridge and Koeleman (2003, 2004) have developed methods based on the partial products of the ordered p-values. Meinshausen and Rice (2006) have proposed a method to estimate the proportion of false null hypotheses among a large number of independently tested hypotheses. This method is based on the distribution of the p-values of the hypothesis tests, which are uniform on $[0, 1]$ under the null hypothesis. However, all these procedures are not generally easy to implement.

The aim of our paper is to propose a simple procedure based on partial sums of ordered statistics. The procedure can also be applied to the ordered p-values of the tests of null hypothesis $H_{0,i}$. We show that our method offers good asymptotic properties and performances on a simulated and a pseudo-real data sets. We prove under suitable conditions that the FDR and the FNR converge towards 0 as n tends to infinity. For the Benjamini and Hochberg's procedure, the FDR is controlled at a fixed level α for all n, but under our assumptions, it does not tend to 0 as n tends to infinity.

The paper is organized as follows. In Section 2 we introduce the models that we consider

and our procedures. In Sections 3 and 4, we compare by simulations and on a pseudo-real data set our procedures with different methods: the Benjamini and Hochberg's procedure and a method based on mixture models. The asymptotic properties for our procedure based on partial sums of ordered statistics are stated in Appendix and the proofs in Web Appendix.

2 The models and our procedures

2.1 The models

We denote by Y_{ijr} the rth repetition of the expression level of gene i in condition j with $r = 1, \ldots, R$; $i = 1, \ldots, n$ and $j = 1, \ldots, J$. We assume that for all i, j, r, $Y_{ijr} \sim \mathcal{N}(m_{ij}, \sigma_i^2)$ where $m_{ij} \in \mathbb{R}$, and $\sigma_i \in \mathbb{R}_+$ are unknown and we suppose that the Y_{ijr}'s are independent. We set $Y_{ij.} = \sum_{r=1}^{R} Y_{ijr}/\mathrm{R}$, and $Y_{i..} = \sum_{j=1}^{J} \sum_{r=1}^{R} Y_{ijr}/(JR)$.
If R is large enough, we can estimate σ_i^2 by $\hat{\sigma}_i^2 = \sum_{j=1}^{J} \sum_{r=1}^{R} (Y_{ijr} - Y_{ij.})^2/(\mathrm{J(R\text{-}1)})$. In that case, let

$$\forall \quad 1 \leq i \leq n, \quad X_i = \frac{1}{(J-1)\hat{\sigma}_i^2} \sum_{j=1}^{J} R(Y_{ij.} - Y_{i..})^2 \sim F(\eta_i; J-1, J(R-1)) \qquad (1)$$

where $\eta_i = 0$ if gene i is non-differentially expressed between the J conditions and $\eta_i > 0$ if not ; $F(\eta; n_1, n_2)$ denotes the non-central Fisher distribution with non-centrality parameter η and (n_1, n_2) degrees of freedom.

In some applications, R may be very small (1 or 2), hence we cannot estimate σ_i^2 by the estimator $\hat{\sigma}_i^2$. In this situation, we assume that $\sigma_i = \sigma$ for all the genes which are non-differentially expressed and we define

$$\forall \quad 1 \leq i \leq n, \quad X_i = \frac{1}{\sigma^2} \sum_{j=1}^{J} R(Y_{ij.} - Y_{i..})^2 \qquad (2)$$

If σ is assumed to be known and if gene i is not differentially expressed between the J conditions, then $X_i \sim \chi^2(J-1)$. If gene i is differentially expressed then $\sigma^2 X_i/\sigma_i^2 \sim \chi^2(\eta_i; J-1)$, that is to say $\sigma^2 X_i/\sigma_i^2$ is a noncentral chi-squared distribution variable with $(J-1)$ degrees of freedom and with non-centrality parameter $\eta_i > 0$. In the particular case where we compare only two conditions, $X_i = R(Y_{i1.} - Y_{i2.})^2/(2\sigma^2)$. If gene i is non differentially expressed between the two conditions: $X_i \sim \chi^2(1)$, else $\sigma^2 X_i/\sigma_i^2 \sim \chi^2(\eta_i; 1)$. The object of the paper is to separate differentially from non-differentially expressed genes, that is to say identify the set $\mathcal{J}_n = \{i : \eta_i > 0\}$ in the case of model (1) or (2).

2.2 The procedures

Assume we observe the sample defined by (1) or (2). We note k_n the number of differentially expressed genes.

DDLR procedure

Our first procedure to separate the differentially from the non-differentially expressed genes can be stated as follows:

(i) We order the X_i's: $X_{\sigma(1)} \geq X_{\sigma(2)} \geq \cdots \geq X_{\sigma(n)}$
We define for $0 \leq k < n$, $\hat{\tau}_k = \frac{1}{n-k} \sum_{i=k+1}^{n} X_{\sigma(i)}$.

(ii) We estimate k_n by

$$\hat{k}_n = \min_{0 \leq k < n} \{k : \hat{\tau}_k \leq \tau\} \tag{3}$$

where $\tau = E[X_i]$ under the assumption that the non-centrality parameter η_i is equal to 0, which means that gene i is non-differentially expressed. In the model (1), $\tau = J(R-1)/(J(R-1)-2)$ and in the model (2), $\tau = J-1$.
If for $0 \leq k < n$, $\hat{\tau}_k > \tau$, then we set $\hat{k}_n = n$.

(iii) We decide that $\sigma(1), \cdots, \sigma(\hat{k}_n)$ are the differentially expressed genes.

Heuristic: Let us remind you that the differentially (non-differentially) expressed genes correspond to the central (non-central) observations X_i's. This procedure is based on the idea that if the two populations of the central and non-central observations are well separated then $\hat{\tau}_k$ is a good estimator of τ only for $k = k_n$. For $k < k_n$ the expression of $\hat{\tau}_k$ includes non-central variables and hence $\hat{\tau}_k$ tends to over estimate τ. For $k > k_n$ the expression of $\hat{\tau}_k$ does not include the largest observations of a sample of independent identically distributed variables with mean τ then $\hat{\tau}_k$ tends to under estimate τ.

pDDLR procedure

Instead of applying our procedure to the variables X_i's, one can also apply it to the p-values. Let p_i denote the p-value corresponding to the test of null hypothesis $H_{0,i}$ equivalent to "$H_{0,i} : \eta_i = 0$".

(i) We order the p_i's: $p_{\eta(1)} \leq p_{\eta(2)} \leq \cdots \leq p_{\eta(n)}$

We define for $0 \leq k < n$, $\tilde{\tau}_k = \frac{1}{n-k} \sum_{i=k+1}^{n} p_{\eta(i)}$.

(ii) We estimate k_n by

$$\tilde{k}_n = \min_{0 \leq k < n} \{k : \tilde{\tau}_k \geq \frac{1}{2}\} \tag{4}$$

(iii) We decide that $\eta(1), \cdots, \eta(\tilde{k}_n)$ are the differentially expressed genes.

Note that the smallest p-values correspond to the differentially expressed genes. The distribution of the p-values corresponding to the non-differentially expressed genes is the uniform $\mathcal{U}_{[0;1]}$ distribution. Its expectation is $1/2$, which explains the definition of $\tilde{k}_n$.

3 Simulations

We present several simulations results to compare our procedures DDLR and pDDLR with two methods recalled below: the procedure proposed by Benjamini and Hochberg (1995) and the procedure proposed by Storey (2003). We want to determine the number k_n of genes differentially expressed between two conditions, with only one observation (R=1) for each gene and each condition. We suppose that all the genes have the same variance σ^2. Let the variable $Y_i = Y_{i11} - Y_{i21}$ which represents the difference of expression of gene i between the two conditions. We simulated n independent observations Y_i as follows: k_n from a normal distribution $\mathcal{N}(m_{i1} - m_{i2}, 2\sigma^2)$ and $(n - k_n)$ from a normal distribution $\mathcal{N}(0, 2\sigma^2)$. We note $X_i = Y_i^2/(2\sigma^2)$. Generally in practice, the standard deviation σ is unknown. We note $s^2 = \text{var}(Y_i) = 2\sigma^2$ and we propose to use the estimator $\hat{s}$ of s presented by Haaland and O'Connell (1995).

3.1 Estimation of the variance

This estimator is defined as follows:

$$\hat{s} = 1.5 * median\{|Y_i|,\ |Y_i| \leq 2.5 s_0\} \tag{5}$$

where $s_0 = 1.5 * median\{|Y_i|, i = 1, ..., n\}$.

Intuitively, this estimator may be a consistent estimator if the proportion of variables Y_i with non-null expectation is quite small. Let us see some heuristic ideas about the construction of this estimator.

By considering the variables Y_i such that $|Y_i| \leq 2.5 s_0$, one removes from the sample the variables corresponding to the differentially expressed genes. Then for a centered Gaussian variable Y, the standard deviation is approximatively equal to $1.5 * F^{-1}_{|Y|}(1/2)$, where $F^{-1}_{|Y|}(1/2)$ denotes the median of $|Y|$, this explains the formula (5).

3.2 The other procedures

The Benjamini and Hochberg's procedure (1995)

The first method is a test method that we denote *BH*. It controls the expected proportion of errors among the rejected hypotheses, named the false discovery rate (FDR). Let V denote the number of genes that are falsely declared differentially expressed (false positives) and T be the number of genes that are falsely declared non-differentially expressed (false negatives) (see Table 1).

The false discovery number is connected with the proportion of the rejected null hypotheses which are erroneously rejected $V/\hat{k}_n$. Then Benjamini and Hochberg (1995) have introduced the false discovery rate (FDR) defined as $FDR = E[V/max(1, \hat{k}_n)]$ and the false negative rate: $FNR = E[T/max(1, n - \hat{k}_n)]$.

BH procedure is defined as follows:

Let $(X_i)_{i=1,...,n}$ obey to the model (1) or (2). For all $i \in \{1, ..., n\}$, we consider the test of null hypothesis $H_{0,i}$: "$\eta_i = 0$" against the alternative "$\eta_i > 0$".

- Let $p_1,\ p_2,\ ...,\ p_n$ be the n p-values corresponding to the n tests. These ones are sorted in an increasing order: $p_{\sigma(1)} \leq p_{\sigma(2)} \leq ... \leq p_{\sigma(n)}$.
- Let $H_{0,\sigma(1)},\ H_{0,\sigma(2)},\ ...,\ H_{0,\sigma(n)}$ be the corresponding null hypotheses.
- $\hat{k}_n$ denotes the largest integer $k \in \{1, ..., n\}$ such as $p_{\sigma(k)} \leq \frac{k}{n}\alpha$ where $\alpha \in]0, 1[$.
- The null hypothesis $H_{0,\sigma(i)}$ is rejected for $i = 1, ..., \hat{k}_n$.

Benjamini and Hochberg proved that for this procedure, $FDR \leq (n - k_n)\alpha/n$. In the application, we fixed the coefficient $\alpha = 0.05$. Then BH procedure controls the FDR at level 5%.

The mixture model method (Storey, 2003)

The second method, denoted by *MIXT*, is based on the mixture of two normal distribu-

tions $p\mathcal{N}(\mu, s^2)+(1-p)\mathcal{N}(0, s^2)$, where p and μ are unknown parameters, s is known. Let α_i be a variable corresponding to the conditional probability that gene i is differentially expressed, given that the observations Y_i, μ and p are known.
Y_i is a centered Gaussian variable with variance s^2 if gene i is non-differentially expressed. The estimations of p, μ and α_i can be obtained by the EM algorithm (Titterington et al., 1985). Then we note *iter* the number of iterations in the EM algorithm. We estimate k_n by $\hat{k}_n = \sum_{i=1}^{n} 1_{\alpha_i^{[iter]}>0.5}$

All these methods assume that the standard deviation s is known. In practice, we estimate s with the threshold estimator $\hat{s}$ defined by (5) presented in Section 3.1.

3.3 Results and discussion

For the simulations, we have considered different values for n, k_n and μ, where $\mu = (m_{i1} - m_{i2})/s$ for all $i \in J_n$. For each value of $n = 5000$ and $n = 10000$, we have considered: $k_n = n/10$ and $\mu \in \{3, 5, 8\}$. We recall that $X_i = Y_i^2/s^2 \sim \chi^2(\mu; 1)$ for all $i \in \mathcal{J}_n$, and $X_i \sim \chi^2(1)$ otherwise. In the expression of X_i, we replace s by $\hat{s}$ and we apply the procedures presented in Section 2.2 and 3.2 to the observations $(X_i)_{i=1,\dots,n}$ with $\tau = 1$. The following notations are used:
1. $\hat{k}_n$ denotes the estimation of the number of differentially expressed genes;
2. $F\hat{D}R = \hat{V}/\hat{k}_n$ denotes the estimation (in percentage) of the false discovery rate;
3. $F\hat{N}R = \hat{T}/(n-\hat{k}_n)$ denotes the estimation (in percentage) of the false negative rate;
4. $R\hat{D}R = \hat{S}/k_n$ denotes the estimation (in percentage) of the rate RDR (right discovery rate) defined as: $RDR = E[S/k_n]$.

All the results are obtained with an empirical mean based on 1000 simulations. When we compare the results obtained with $n = 10000$ and $n = 5000$, the proportions $R\hat{D}R$, $F\hat{N}R$, and $F\hat{D}R$, are almost the same. That is to say that the four methods may

depend only on the proportion $\frac{k_n}{n}$, which is an important parameter in the estimator of the variance s^2. Therefore we only discuss results for $n = 10000$ presented in Table 2. For example, in the case $\mu = 8$, DDLR method estimates k_n by $\hat{k}_n = 997.6$. Among these $\hat{k}_n = 997.6$ genes, $F\hat{D}R = 0.3\%$ of genes were not simulated differentially expressed. Among these $n - \hat{k}_n = 9002.4$ genes found non-differentially expressed, $F\hat{N}R = 0.1\%$ of genes were simulated differentially expressed. With this method, $R\hat{D}R = 99.5\%$ of the genes which were simulated differentially expressed are found.

The results presented in Table 2 suggest the following remarks:

- Concerning $F\hat{D}R$, our procedures DDLR and pDDLR behave quite similarly. Nevertheless pDDLR is less powerfull as showned by the estimations of RDR. Hence we recommand to use DDLR rather than pDDLR procedure.

- When $\mu = 8$, all the methods give a good estimation of k_n, and all the genes which were simulated differentially expressed are found: $R\hat{D}R = 100\%$ for almost all the methods. Nevertheless BH method tends to over estimate k_n. MIXT and DDLR methods give good results for the four criteria $\hat{k}_n$, $F\hat{D}R$, $F\hat{N}R$ and $R\hat{D}R$.

- Consider the case $\mu = 5$. All the methods find more than 96% of the genes simulated differentially expressed. Among the genes found differentially expressed, 1.4% ($F\hat{D}R$) of these genes were not simulated differentially expressed for DDLR method, against 1.5% for MIXT method and 4.2% for BH method. The choice of the method depends on the objective of the user. If we prefer to find more differentially expressed genes in spite of a high $F\hat{D}R$ ($F\hat{D}R \geq 4.17\%$) but a better $R\hat{D}R$ ($R\hat{D}R > 98\%$), we can choose BH method. If we prefer to control the error level $F\hat{D}R$, we may choose among the methods DDLR or MIXT.

- In the case $\mu = 5$, MIXT method seems to be the best method in terms of $F\hat{D}R$ (1.5%) and $R\hat{D}R$ (98%). However, in the case $\mu = 3$, the $F\hat{D}R$ of MIXT method is higher (10%) essentially because the mean μ of the genes differentially expressed is very weak, so the method cannot easily seperate the genes which are not differentially expressed from the others. Concerning BH method, the $F\hat{D}R$ is weak: 2.4% of genes, but the $R\hat{D}R$ is also weak comparing to the two other methods. On the other hand, in the case $\mu = 3$, DDLR method finds 57% of differentially expressed genes among the genes which are simulated differentially expressed. Moreover the $F\hat{D}R$ is quite weak: 7% of genes.

In real microarray data analysis, the mean of the differences of genes levels between two conditions, is generally small ($\mu \leq 3$). So DDLR method seems to be well adapted for transcriptomic data.

To conclude, for any level of μ, our method finds a high proportion of genes simulated differentially expressed ($R\hat{D}R \geq 57\%$) and the $F\hat{D}R$ ($\leq 7\%$) stands quite low. The other methods tend to privilege only one of the criteria $F\hat{D}R$, $F\hat{N}R$ or $R\hat{D}R$. Moreover, DDLR method is very easy to implement.

4 Application on a pseudo-real data set

The application presents an analysis of simulated data obtained from a workshop organised in the context of the EC-funded network excellence (NoE) EADGENE (European Animal Disease Genetics Network of Excellence for animal Health and Food Safety, www.EADGENE.org) in November 2006. Prior to the workshop both real and simulated microarray data are distributed to interested EADGENE participants. The results on simulated data of this workshop are presented in details in Watson et al (2007). The microarray data were simulated using SIMAGE package (Albers et al,

2006) which takes a number of parameters produced by using a slide from a real data set (Jaffrezic et al, 2007). The simulated data consists of ten microarrays each of which represent a direct comparison between biological samples from situation A and B with a dye balance. Each slide had 2400 genes in duplicate. The data has been simulated with a lot of noise factors and the aim of the challenge proposed to the EADGENE participants was to get a list of differentially expressed genes and compared them to the list of genes that were set to be differentially expressed. In the simulated data 624 genes were differentially expressed: 264 were up regulated from A to B while 360 were down regulated. But this information was only provided to the participants at the end of the workshop.

The first step of this study consists in normalizing and standardizing the data by classical approaches. The second step consists in detecting which genes are differentially expressed between conditions A and B.

4.1 Normalisation process

First of all in a pre-normalisation step, we choose to analyse the raw data without taking into account the background information to minimize the variance of the data. We split the two repetitions by slide into two different slides to improve the power of the test statistic. We remove from the analysis spots for which raw or net intensity is equal to zero. They are considered as "bad spots". So we have between 14 and 20 repetitions per gene.

For the normalisation step, we use a lowess regression by slide to correct the data for the intensity dependent bias. We note Y_{ijr} the raw intensity of gene i on slide r in

condition j. We define M_{ir} and A_{ir} as

$$M_{ir} = \log\left(\frac{Y_{i1r}}{Y_{i2r}}\right) \qquad\qquad A_{ir} = \frac{\log(Y_{i1r}) + \log(Y_{i2r})}{2}$$

The logarithmic transformation not only converts ratios into differences between the two channels at each spot but also stabilizes the variance of high intensity spots. Ratios provide an intuitive measure of expression changes and by performing a logarithmic transformation, up and down regulation can be treated on a symmetric and continuous scale and error variances become less dependent on the mean of the light intensities.

Moreover, intensity dependent bias is most easily detected in MA plots, which are scatter plot of logratios (M) versus intensity (A). In the case of intensity dependent bias, there will be curvature in the MA plot. So we construct the MA-plots and the lowess regression functions $\hat{f}_r$ by slide. Figure 2 gives the MA-plots and the lowess regression functions for the two repetitions of slide 1 and slide 2.

Then we obtain the corrected log-ratio $\hat{M}_{ir}$ first by substracting the lowess regression function to the log-ratio by slide (6) and then by centering and standardizing by slide (7):

$$\tilde{M}_{ir} = M_{ir} - \hat{f}_r(A_{ir}) \tag{6}$$

$$\hat{M}_{ir} = \frac{\tilde{M}_{ir} - \tilde{M}_{\cdot r}}{\sqrt{\frac{\sum_{i=1}^{I_r}(\tilde{M}_{ir} - \tilde{M}_{\cdot r})^2}{I_r - 1}}} \tag{7}$$

where I_r denotes the number of genes on slide r and $\tilde{M}_{\cdot r} = \frac{1}{I_r}\sum_{i=1}^{I_r}\tilde{M}_{ir}$. Figure 3 gives the corrected MA-plots for the two repetitions of slide 1 and slide 2.

Note that we obtain analogous results with a lowess regression by block. In addition, we have checked the homogeneity of the variance of genes and this assumption is valid.

4.2 Methods to find differentially expressed genes

We note $\hat{M}_{ir}$ the rth repetition of the difference of expression of gene i obtained in the normalisation step and n_i the number of repetitions of the difference of expression of gene i. $i = 1, \cdots, I = 2400$; $r = 1, \cdots, n_i$ and $n_i \in \{14, \cdots, 20\}$. We assume that $\hat{M}_{ir} \sim \mathcal{N}(\mu_i, \sigma_i^2)$. We construct the classical Fisher statistic X_i to test the null hypothesis $H_{0,i}$.

$$X_i = \frac{(\sum_{r=1}^{n_i} \hat{M}_{ir})^2/n_i}{\sum_{r=1}^{n_i}(\hat{M}_{ir} - \hat{M}_{i.})^2/(n_i - 1)}$$

Under $H_{0,i}$, $X_i \sim F(1, n_i - 1)$. Figure 3 gives the histogram of the p-values. The two populations of the null and non-null hypotheses seem to be well separated.

Then we have applied the three following procedures to decide which genes are differentially expressed : the Benjamini and Hochberg's procedure, our procedure DDLR and a two-component semi-parametric model proposed by Bordes et al (2007) and noted (Mixture model). This last method is a semi-parametric mixture model defined by (8) where the first component f_0 is known and corresponds to the null hypotheses distribution and the second component f is an unknown distribution corresponding to the non-null hypotheses distribution. Taking into account the number of repetitions for each gene, we obtain that f_0 is a mixture of Student distributions $\frac{1184}{2400}T(19) + \frac{813}{2400}T(18) + \frac{309}{2400}T(17) + \frac{75}{2400}T(16) + \frac{13}{2400}T(15) + \frac{5}{2400}T(14) + \frac{1}{2400}T(13)$ and $S_i^2 = X_i$. This mixture is well approximated by a $T(19)$ distribution so we choose f_0 to be a $T(19)$ probability density function (pdf).

$$S_i \sim (1 - p)f_0 + pf \tag{8}$$

The weight p corresponding to the proportion of non-null hypotheses is unknown. We propose to estimate p and f by an EM-type algorithm. Then the hypotheses corresponding to the pI smallest p-values are rejected.

In our particular problem it can be shown that the unknown pdf f is a mixture of

non-central Student pdfs.

$$f \sim \sum_{j=1}^{J} \beta_j T(\delta_j, 19) \text{ with } \sum_{j=1}^{J} \beta_j = 1$$

Then

$$S_i \sim (1-p)T(19) + p\left(\sum_{j=1}^{J} \beta_j T(\delta_j, 19)\right)$$

By a BIC criterion we estimate the number of components.

4.3 Results

Table 3 presents the results obtained with the three procedures in terms of the total number of errors made (false positives and false negatives), the number of genes identified by each method as differentially expressed and the number of correct genes.

BH method gives 649 differential genes for a 5% FDR with 623 genes correctly detected. With DDLR method, 629 genes are found differentially expressed and 618 genes are correctly identified. With the semi-parametric mixture model, we obtain $J = 2$ components, $\delta_1 = -6.8$, $\delta_2 = 5.69$, $p_1 = p\beta_1 = 0.1099$, $p_2 = p\beta_2 = 0.1494$ and we obtain 622 differential genes and 617 correct genes. The 622 differential genes obtained by the mixture approach are also found by BH and DDLR procedures. DDLR and mixture methods only found 17 and 12 errors respectively against 27 errors with BH procedure. The number of false negatives is the lowest in BH procedure while the number of false positives is the biggest in this same procedure. Results obtained in terms of the total number of errors made with DDLR method are similar to the mixture method but in practice, DDLR method is easier to implement compared to the mixture method.

Similar results are obtained with a lowess normalisation by block and without splitting the two repetitions up into two different slides. Others approaches with different

normalisation steps are presented in the paper of Watson et al. (2007) and the results in terms of number of differentially expressed genes can be very different but DDLR and mixture procedures have given the best results.

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6 References

Albers, C.J., Jansen, R.C., Kok, J., Kuipers, O.P. and van Hijum, S.A. (2006). SIMAGE: simulation of DNA-microarray gene expression data, *BMC Bioinformatics* **7**, 205.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B* **57**, 289-300.

Benjamini, Y. and Hochberg, Y. (2000). The adaptive control of the false discovery rate in multiple comparison problems. *The Journal of Educational and Behavioral Statistics* **25**, 1, 60-83.

Benjamini, Y., Krieger A. M. and Yekutieli, D. (2006). Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **93**, 491-507.

Bordes, L., Delmas, C. and Vandekerkhove, P. (2007). Semiparametric Estimation of a two-component mixture model when a component is known. *Scandinavian Journal of Statistics* **33**, 733-752.

Brown, P. and Bolstein, D. 1999. Exploring the new world of the genome with DNA microarray. *Nature Genetics Supplements* **21**, 33-37.

Dudbridge, F. and Koeleman, B. P. (2003). Rank truncated product of P-values, with application to genomewide association scans. *Genetic Epidemiology* **25**, 360-366.

Dudbridge, F. and Koeleman, B. P. (2004). Efficient computation of significance levels for multiple associations in large studies of correlated data, including genomewide association studies. *The American Journal of Human Genetics* **75**, 424-435.

Ferreira, J. A. and Zwinderman, A. H. (2006). On the Benjamini-Hochberg Method. *The annals of statistics* **34**, 4, 1827-1849.

Genovese, C. R. and Wasserman, L. (2002). Operating characteristics and extensions of the FDR procedure. *Journal of the Royal Statistical Society. Series B* **64**, 499-518.

Genovese, C. R. and Wasserman, L. (2004). A stochastic process approach to false discovery control. *The Annals of Statistics* **32**, 1035-1061.

Ghosh, D. (2006). Shrunken p-values for assessing differential expression with applications to Genomic Data Analysis. *Biometrics* **62**, 1099-1106.

Haaland, D. and O'Connell, M.A. (1995). Inference for effect Satured Fractionnal. *Factorials. Technometrics* **137**, 1.

Hoh, J.,Wille, A. and Ott, J. (2001). Trimming, weighting, and grouping SNPs in human case-control association studies. *Genome Research* **11**, 2115-2119.

Huet, S. (2006). Model selection for estimating the non zero components of a Gaussian vector. *ESAIM. Probability and Statistics* **10**, 164.

Jaffrezic, F., de Koning, D. J., Sando Lund, M., Watson, M., Zerbe, H., Petzl, W., Yang, W., Schuberth, H. J., Jensen, K., Waddington, D., Jiang, L., Buitenhuis, B., Sorensen, P., Hornshoj, H., Hedegaard, J., L Cao, K. A., San Cristobal, M., Tosser-Klopp, G., Bonnet, A., Déjean, S., Delmas, C., Duval, M., Robert-Granié, C., Pool, M. C., Hulsegge, B., Detilleux, J. C., Lavric, M., Dovc, P., Closset, R., Nie, H., van Schothorst, E. M., Malinverni, R., Boettcher, P. J., Stella, A., Marot, G., Foulley, J. L. and Seyfert, H. M. (2007). Analysis of the real EADGENE data set: Comparison of methods and guidelines for data normalization and selection of differentially expressed

genes. *Accepted paper in Genetics Selection Evolution.*

Lavielle, M. and Ludeña, C. (2006). Random thresholds for linear model selection. *Submitted paper.*

Meinshausen, N. and Rice, J. (2006). Estimating the proportion of false null hypotheses among a large number of independently tested hypotheses. *The Annals of Statistics* **34**, 1, 373-393.

Quackenbush, J. (2001). Computational genetics: computational analysis of microarray data. *Nature Reviews Genetics* **2**, 418-427.

Resnick, S. I. (1987). *Extreme values, Regular Variation, and Point Processes.* Springer-Verlag: Applied Probability Trust.

Storey, J. D. (2002). A direct approach to false discovery rates. *Journal of the Royal Statistical Society. Series B* **64**, 479-498.

Storey, J. D. (2003). The positive false discovery rate: a bayesian interpretation and the q-value. *The Annals of Statistics* **31**, 6, 2013-2035.

Storey, J. D., Taylor, J. E. and Siegmund D. (2004). Strong control, conservative point estimation, and simultaneaous conservative consistency of false discovery rates: A unified approach. *Journal of the Royal Statistical Society. Series B* **66**, 187-205.

Titterington, D., Smith, M. and Markov, U. (1985). *Statistical analysis of finite mixture distributions.* Chichester, UK: John Wiley and Sons.

Watson, M., Pérez Alegre, M., Baron, M. D., Delmas, C., Dovc, P., Duval, M., Foulley, J. L., Garrido-Pavón, J. J., Hulsegge, B., Jaffrezic, F., Jiménez-Marn, A., Lavric, M., Le Cao, K. A., Marot, G., Pool, M. H., Robert-Granié, C., San Cristobal, M., Tosser-Klopp, G., Waddington, D. and De Koning, D. J. (2007). Analysis of a simulated microarray dataset: Comparison of methods for data normalization and detection of differential expression. *Accepted paper in Genetics Selection Evolution.*

Zaykin, D. V., Zhivotovsky, L. A., Westfall, P. H. and Weir, B. S. (2002). Truncated product method for combining P-values. *Genetic Epidemiology* **22**, 170-185.

7 Appendix: theoretical results

In this section, we consider the following model which is more general than the models defined by (1) or (2).

Model M : Let $X_1, \ldots, X_n$ be n independent random variables such that

$$X_i = \sum_{k=1}^{\nu_1} (\gamma_i N_{ik} + \delta_{ik})^2 \text{ for } 1 \leq i \leq k_n$$

and

$$X_i = \sum_{k=1}^{\nu_1} (N_{ik})^2 \text{ for } k_n + 1 \leq i \leq n$$

where for all $1 \leq i \leq k_n$, $\eta_i = \sum_{k=1}^{\nu_1} \delta_{ik}^2 > 0$ and $\gamma_i \geq 1$ are unknown parameters; $N_{ik}, 1 \leq i \leq n, 1 \leq k \leq \nu_1$ are independent identically distributed centered variables with unit variance.

Note that this model corresponds to the situation defined by (2) when the variables N_{ik} are Gaussian variables since in this case the variables X_i, $i = 1, ..., k_n$ are non-central $\chi^2(\eta_i, \nu_1)$ and for $i > k_n$, $X_i \sim \chi^2(\nu_1)$.

We introduce some notations. We define

$$\mathcal{J}_n = \{i, \eta_i > 0\} = \{1, 2, \ldots k_n\}$$

and

$$\hat{\mathcal{J}}_n = \left\{\sigma(1), \sigma(2), \ldots, \sigma(\hat{k}_n)\right\}$$

where $X_{\sigma(1)} \geq X_{\sigma(2)} \geq \ldots \geq X_{\sigma(n)}$ and $\hat{k}_n$ is defined by (3).

Let V and T respectively denote the number of false positives and the number of false negatives defined by

$$V = \text{Card}(\hat{\mathcal{J}}_n \cap \bar{\mathcal{J}}_n) \quad (9)$$

$$T = \text{Card}(\bar{\hat{\mathcal{J}}}_n \cap \mathcal{J}_n) \quad (10)$$

where, for all set J, $\bar{J}$ denotes the complementary set of J.

Before giving our main theorem, we state a lemma, to show that, under suitable assumptions, with high probability, the variables satisfying $\eta_i > 0$ are the largest variables of the sample $\{X_1, \ldots, X_n\}$. We first introduce an assumption.

Assumption H:

Let $\phi_{(1)}$ be the cumulative distribution function of $L_{(1)} = \max_{i=1,..,n} \sum_{k=1}^{\nu_1} N_{ik}^2$. We assume that there is a sequence $(a_n, b_n)_{n\geq 0}$ with $b_n > 0$ for all $n \in \mathbb{N}$ such that $\phi_{(1)}(a_n + b_n x) \xrightarrow[n\to\infty]{} F(x)$, where F is a cumulative distribution function. We also assume that,

$$\min_{i\in\mathcal{J}_n} \left(\frac{\eta_i}{2\gamma_i^2} \right) \geq \alpha_n$$

where the sequence $(\alpha_n)_{n\in\mathbb{N}}$ satisfies

$$\frac{\alpha_n/2 - a_n}{b_n} \xrightarrow[n\to\infty]{} +\infty.$$

Example:

Assume that the N_{ik}'s are independent identically distributed centered Gaussian random variables with unit variance. Then for $1 \leq i \leq n$, $\sum_{k=1}^{\nu_1} N_{ik}^2 \sim \chi^2(\nu_1)$. In that case we can prove that F is the cumulative distribution function of a Gumbel variable, with

$$a_n = 2\log(n) + (\nu_1 - 2)\log(\log(n)) - 2\log(2^{\nu_1/2}\Gamma(\nu_1/2)) + \nu_1 \log(2) \quad \forall n \geq 1$$

and

$$b_n = 2 \quad \forall n \geq 1$$

(see Resnick (1987) Section 1.5 for similar calculations).

Then we have to choose α_n such that $\frac{\alpha_n/2-a_n}{b_n} \xrightarrow[n\to\infty]{} +\infty$, for example $\alpha_n = 2a_n + \gamma_n$ for all n where $\gamma_n \xrightarrow[n\to\infty]{} +\infty$.

Lemma 1 *Let $X_1, \ldots, X_n$ be defined by* **Model M**. *We assume that Assumption* **H** *holds. We define*

$$\Omega_n = \left\{ \min_{1 \leq i \leq k_n} X_i \geq \max_{k_n+1 \leq i \leq n} X_i \right\}.$$

Then

$$\mathbb{P}(\Omega_n) \xrightarrow[n \to \infty]{} 1.$$

This lemma shows that, under the assumption **H**, the probability that the variables X_i corresponding to differentially expressed genes are the largests of the sample tends to 1 as n tends to infinity.

The aim of the following Theorem is to control the False Discovery Rate (FDR) and False Negative Rate (FNR) for our procedure.

Theorem 1 *Let $X_1, \ldots, X_n$ be defined by* **Model M**. *Let $\hat{k}_n$ be defined by (3). We assume that the cardinality of $\mathcal{J}_n$ equals $k_n = \lambda n$ with $0 < \lambda < 1$. Let $(u_n)_{n \geq 0}$ be a sequence of positive numbers such that $u_n \longrightarrow 0$ and $\sqrt{n} u_n \longrightarrow +\infty$ as n tends to infinity. Let V and T be defined by (9) and (10).*
Then, under Assumption **H**,

$$\mathbb{P}\left[\frac{V}{\max(\hat{k}_n, 1)} > u_n \right] \xrightarrow[n \to \infty]{} 0 \tag{11}$$

$$\mathbb{P}\left[\frac{T}{\max(n - \hat{k}_n, 1)} > u_n \right] \xrightarrow[n \to \infty]{} 0. \tag{12}$$

Moreover,

$$\mathbb{E}\left[\frac{V}{\max(\hat{k}_n, 1)} \right] \xrightarrow[n \to \infty]{} 0 \tag{13}$$

$$\mathbb{E}\left[\frac{T}{\max(n - \hat{k}_n, 1)} \right] \xrightarrow[n \to \infty]{} 0. \tag{14}$$

Comments.

1. Results (11) and (12) show that the proportion of false positive among the genes that are declared differentially expressed tends to 0 in probability at the rate $1/\sqrt{n}$ as n tends to infinity, as well as the proportion of false negative among the genes that are not declared differentially expressed. Results (13) and (14) show respectively that the FDR and FNR for our procedure converge towards 0 as n tends to infinity.

2. For the Benjamini and Hochberg's procedure at level α, Ferreira and Zwinderman (2006) have shown that $\frac{V}{\max(\hat{k}_n,1)} \xrightarrow[n\to\infty]{P} \alpha(1-\lambda)$ if and only if $\hat{k}_n \xrightarrow[n\to\infty]{P} +\infty$. Under our hypothesis that the two populations are well separated (Assumption H), it is easy to see that $\hat{k}_n \xrightarrow[n\to\infty]{P} +\infty$ for the Benjamini and Hochberg's procedure. Then $\frac{V}{\max(\hat{k}_n,1)} \xrightarrow[n\to\infty]{P} \alpha(1-\lambda)$ and $E[\frac{V}{\max(\hat{k}_n,1)}] \xrightarrow[n\to\infty]{} \alpha(1-\lambda)$. Thus our result (13) is not satisfied by the Benjamini and Hochberg's procedure.

3. Let us now comment Assumption **H**. This assumption can seem to be restrictive, but the results stated in Theorem 1 cannot be achieved if $\min_{i\in\mathcal{J}_n}\left(\frac{\eta_i}{2\gamma_i^2}\right)$ does not tend to infinity as n tends to infinity. Let us explain this in the simple case where the Model M is defined as follows : $N_1,\ldots,N_n$ are i.i.d. $\mathcal{N}(0,1)$; for $1 \le i \le k_n = \lambda n$, $X_i = (N_i+\mu)^2$ with $\mu > 0$ and for $k_n+1 \le i \le n$, $X_i = N_i^2$. Let us denote by Z the number of variables among $X_{k_n+1},\ldots,X_n$ that are greater than μ^2. The distribution of Z is a Binomial distribution, with parameters $(n-k_n, p_\mu)$ where $p_\mu = P(N^2 > \mu^2) > 0$. This implies that $E(Z/n) = (1-\lambda)p_\mu$. Let us denote by $\tilde{Z}$ the number of variables among $X_1,\ldots,X_{k_n}$ that are smaller than μ^2. The distribution of $\tilde{Z}$ is a Binomial distribution, with parameters (k_n, p'_μ) where $p'_\mu = P(-2\mu < N < 0) > 0$. Hence, $E(\tilde{Z}/n) = \lambda p'_\mu$. Any procedure to separe the non-centered from the centered observations will consider as non-centered the variables that exceed some threshold. If the threshold is smaller than μ^2, then $V/n \ge Z/n$, otherwise, $T/n \ge \tilde{Z}/n$. Hence, if μ is fixed, the result of

Theorem 1 cannot be obtained since either $E(V/n) \geq (1-\lambda)p_\mu$ or $E(T/n) \geq \lambda p'_\mu$. Assumption **H** in this case is satisfied if μ is greater than $C\sqrt{\log(n)}$ for some $C > 2$.

The proofs of lemma 1 and theorem 1 are stated in Web Appendix (see Section 5).

	decision		
	H_0 accepted	H_0 rejected	total
true null hypotheses	U	V	$n - k_n$
non-true null hypotheses	T	S	k_n
total	$n - \hat{k}_n$	$\hat{k}_n$	n

Table 1: Number of errors when testing n hypotheses.

method	μ	$\hat{k}_n$	$F\hat{D}R$	$F\hat{N}R$	$R\hat{D}R$
BH	3	396.6	2.4	6.4	38.7
	5	1028.1	4.2	0.2	98.5
	8	1044.6	4.3	0.0	100.0
MIXT	3	820.2	10.0	2.9	73.8
	5	994.9	1.5	0.2	98.0
	8	999.9	0.0	0.0	100.0
DDLR	3	613.2	7.0	4.6	57.0
	5	973.5	1.4	0.4	96.0
	8	997.6	0.3	0.1	99.5
pDDLR	3	578.2	6.1	4.8	54.2
	5	949.8	1.0	0.7	94.0
	8	960.4	0.3	0.5	95.7

Table 2: Comparison between BH, MIXT, DDLR and pDDLR methods for different values of μ, in the case $n = 10000$ genes and $k_n = 1000$ genes simulated differentially expressed.

	Number of identified genes ($\hat{k}_{2400}$)	Number of correct genes (S)	V	T
BH method	649	623	26	1
DDLR method	629	618	11	6
Mixture model	622	617	5	7

Table 3: Results on the pseudo-real data set with BH, DDLR and mixture model procedures.

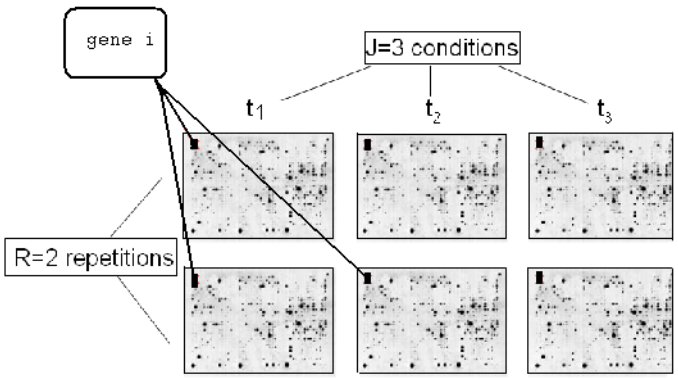

Figure 1: A microarray experiment where we compare the expression of gene i in 3 conditions with 2 repetitions.

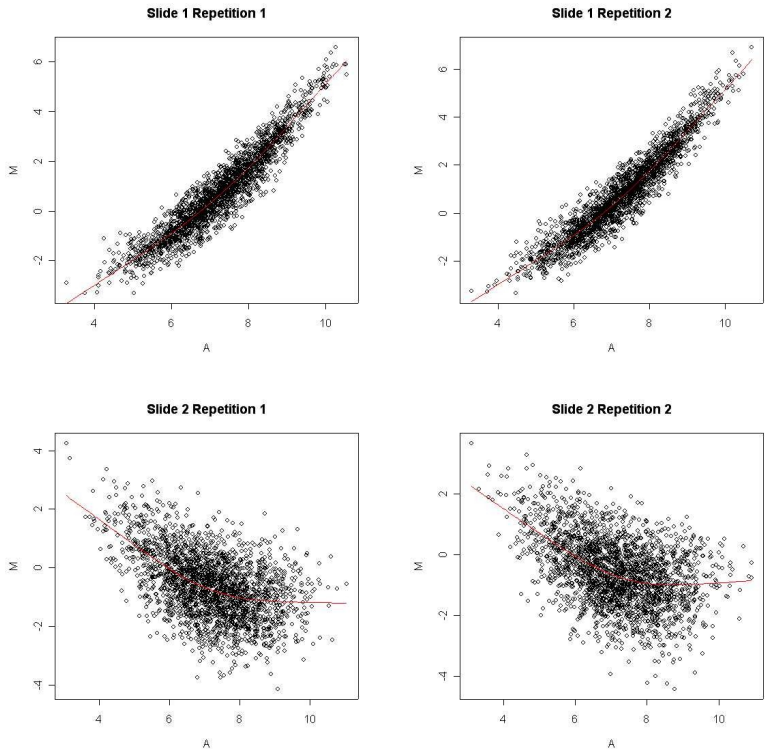

Figure 2 : MA-plots and lowess regression functions for the two repetitions of slide 1 and slide 2.

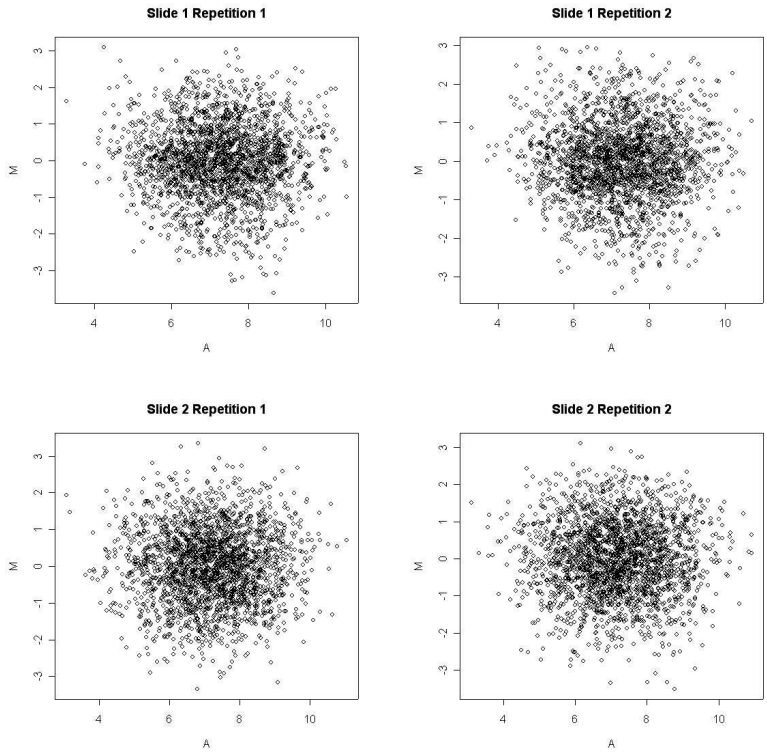

Figure 3 : Corrected MA-plots for the two repetitions of slide 1 and slide 2.

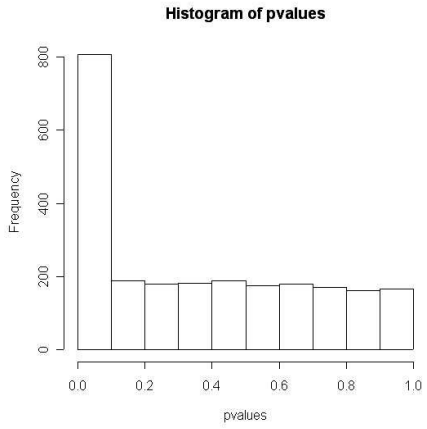

Figure 4

8 Supplementary materials

1. *Proof of Lemma 1*

We define for $i = 1, ..., n - k_n$ $V_i = X_{k_n+i}$.

We write $X_{(1)} \geq X_{(2)} \geq \dots X_{(k_n)}$ and $V_{(1)} \geq V_{(2)} \geq \dots V_{(n-k_n)}$. The complementary set of Ω_n is the event $\left\{V_{(1)} > X_{(k_n)}\right\}$. Since $X_i = \sum_{k=1}^{\nu_1} (\gamma_i N_{ik} + \delta_{ik})^2$, we use the inequality $2ab \geq -a^2/2 - 2b^2$ which holds for all $a, b \in \mathbb{R}$ to obtain that for all $1 \leq i \leq k_n$,

$$\frac{X_i}{\gamma_i^2} \geq -\sum_{k=1}^{\nu_1} N_{ik}^2 + \frac{\eta_i}{2\gamma_i^2}.$$

Setting $L_{(1)} = \max_{1 \leq i \leq n} \sum_{k=1}^{\nu_1} N_{ik}^2$, this implies that

$$\frac{X_{(k_n)}}{\gamma_{(k_n)}^2} \geq \min_{1 \leq i \leq k_n} \frac{\eta_i}{2\gamma_i^2} - L_{(1)}.$$

Then, since for $1 \leq i \leq k_n$, $\gamma_i \geq 1$,

$$\begin{aligned}
\mathbb{P}(\Omega_n^c) &= \mathbb{P}(V_{(1)} > X_{(k_n)}) \\
&\leq \mathbb{P}\left(\frac{V_{(1)}}{\gamma_{(k_n)}^2} + L_{(1)} > \min_{1 \leq i \leq k_n} \frac{\eta_i}{2\gamma_i^2} \right) \\
&\leq \mathbb{P}\left(V_{(1)} + L_{(1)} > \min_{1 \leq i \leq k_n} \frac{\eta_i}{2\gamma_i^2} \right).
\end{aligned}$$

$V_{(1)} \leq L_{(1)}$, hence $\mathbb{P}(\Omega_n^c) \leq \mathbb{P}(2L_{(1)} > \alpha_n)$. Since $\left(L_{(1)} - a_n\right)/b_n \xrightarrow{\mathcal{L}} F$ and $(\alpha_n/2 - a_n)/b_n \xrightarrow[n\to\infty]{} +\infty$ with **Assumption H**, we obtain that $\mathbb{P}(\Omega_n^c) \xrightarrow[n\to\infty]{} 0$. This concludes the proof of Lemma 1.

3. *Proof of Theorem 1*

Let us first prove that the fonction $k \to \hat{\tau}_k$ is non increasing.

For $1 \leq k \leq n-1$:

$$\begin{aligned}\hat{\tau}_{k-1} &= \frac{1}{(n-k+1)} \sum_{i=k}^{n} X_{(i)} \\ &= \frac{X_{(k)}}{(n-k+1)} + \frac{1}{(n-k+1)} \sum_{i=k+1}^{n} X_{(i)} \\ &= \frac{X_{(k)}}{(n-k+1)} + \frac{n-k}{(n-k+1)} \hat{\tau}_k\end{aligned}$$

Since for $i \geq k \quad X_{(k)} \geq X_{(i)}$, we get $X_{(k)} \geq \frac{1}{n-k} \sum_{i=k+1}^{n} X_{(i)}$. This implies that

$$\hat{\tau}_{k-1} \geq \frac{1}{(n-k+1)} \hat{\tau}_k + \frac{n-k}{(n-k+1)} \hat{\tau}_k = \hat{\tau}_k.$$

Hence, the function $k \mapsto \hat{\tau}_k$ is non increasing.

Let us now prove the two following results, under the assumptions of Theorem 1:

$$\mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} < -u_n \right) \overset{n\to\infty}{\longrightarrow} 0 \tag{15}$$

$$\mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} > u_n \right) \overset{n\to\infty}{\longrightarrow} 0. \tag{16}$$

Proof of (15) :

$$\mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} < -u_n \right) \leq \mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} < -u_n \cap \Omega_n \right) + \mathbb{P}(\Omega_n^c).$$

Since $\mathbb{P}(\Omega_n^c)$ tends to 0 as n tends to infinity, we have to prove that

$$\mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} < -u_n \cap \Omega_n \right) \overset{n\to\infty}{\longrightarrow} 0.$$

Using the fact that $k \to \hat{\tau}_k$ is non increasing, we obtain

$$\mathbb{P}\big(\{\hat{k}_n < k_n - nu_n\} \cap \Omega_n \big) \leq \mathbb{P}\big(\{\hat{\tau}_{k_n - nu_n} \leq \tau\} \cap \Omega_n \big) = P1$$

where

$$P1 = \mathbb{P}\Big(\Big\{ \frac{1}{n - k_n + nu_n} \sum_{i=k_n - nu_n + 1}^{k_n} \frac{X_{(i)}}{\tau} + \frac{1}{n - k_n + nu_n} \sum_{i=k_n+1}^{n} \frac{X_{(i)}}{\tau} \leq 1 \Big\} \cap \Omega_n \Big).$$

We recall that, on the event Ω_n, the variables $\{X_{(i)}, i > k_n\}$ are the variables $\{X_{k_n+1}, \dots, X_n\}$. Let $\epsilon > 0$.

$$\begin{aligned} P_1 &\leq \mathbb{P}\Big(\Big\{\frac{1}{n-k_n+nu_n}\sum_{i=k_n-nu_n+1}^{k_n}\frac{X_{(i)}}{\tau} \leq \frac{(1+\epsilon)nu_n}{n-k_n+nu_n}\Big\}\cap\Omega_n\Big) \\ &+ \mathbb{P}\Big(\Big\{\frac{1}{n-k_n+nu_n}\sum_{i=k_n+1}^{n}\frac{X_i}{\tau} \leq 1-\frac{(1+\epsilon)nu_n}{n-k_n+nu_n}\Big\}\Big) \end{aligned}$$

We set for $i = 1, \dots n-k_n$, $Z_i = X_{k_n+i}/\tau$. The variables $(Z_i)_{i=1,..,n-k_n}$ are i.i.d., $E(Z_i) = 1$,moreover, under the definition of **Model M**, Z_i cannot be constant equal to 1, hence, there exists $\epsilon_0 > 0$ such that $\mathbb{P}(Z_i \leq 1+\epsilon_0) < 1$. We denote by v the standard deviation of the Z_i's.

$$\mathbb{P}\left(\frac{1}{n-k_n+nu_n}\sum_{i=1}^{n-k_n}Z_i \leq 1-\frac{(1+\epsilon_0)nu_n}{n-k_n+nu_n}\right) = \mathbb{P}\left(\frac{\sum_{i=1}^{n-k_n}Z_i-(n-k_n)}{v\sqrt{n-k_n}} \leq \frac{-nu_n\epsilon_0}{v\sqrt{n-k_n}}\right).$$

The central limit theorem implies that

$$\frac{\sum_{i=1}^{n-k_n}Z_i-(n-k_n)}{v\sqrt{n-k_n}} \xrightarrow{\mathcal{L}} \mathcal{N}(0,1),$$

and since $k_n = \lambda n$ and $\sqrt{n}u_n \xrightarrow[n\to\infty]{} +\infty$, we obtain

$$\frac{-nu_n\epsilon_0}{v\sqrt{(n-k_n)}} \xrightarrow[n\to\infty]{} -\infty.$$

This implies that

$$\mathbb{P}\left(\frac{1}{n-k_n+nu_n}\sum_{i=1}^{n-k_n}Z_i \leq 1-\frac{(1+\epsilon_0)nu_n}{n-k_n+nu_n}\right) \xrightarrow[n\to\infty]{} 0.$$

Let us now control the other term appearing in the upper bound for P_1.

$$\begin{aligned} &\mathbb{P}\left(\left\{\frac{1}{n-k_n+nu_n}\sum_{i=k_n-nu_n+1}^{k_n}\frac{X_{(i)}}{\tau} \leq \frac{(1+\epsilon_0)nu_n}{n-k_n+nu_n}\right\}\cap\Omega_n\right) \\ &= \mathbb{P}\left(\left\{\frac{1}{nu_n}\sum_{i=k_n-nu_n+1}^{k_n}\frac{X_{(i)}}{\tau} \leq (1+\epsilon_0)\right\}\cap\Omega_n\right). \end{aligned}$$

On the event Ω_n, $\dfrac{1}{nu_n}\displaystyle\sum_{i=k_n-nu_n+1}^{k_n}\dfrac{X_{(i)}}{\tau} \geq Z_{(1)}$, where $Z_{(1)} = \max\{Z_i, 1 \leq i \leq n-k_n\}$.

Hence

$$\mathbb{P}\left(\left\{\frac{1}{nu_n}\sum_{i=k_n-nu_n+1}^{k_n}\frac{X_{(i)}}{\tau} \leq 1+\epsilon_0\right\}\cap\Omega_n\right) \leq \mathbb{P}\left(Z_{(1)} \leq (1+\epsilon_0)\right) = \left(\mathbb{P}\left(Z_1 \leq (1+\epsilon_0)\right)\right)^{n-k_n}.$$

This tends to 0 as n tends to infinity since $\mathbb{P}\left(Z_1 \leq (1+\epsilon_0)\right) < 1$.

We have proved that $P_1 \xrightarrow[n\to\infty]{} 0$, this concludes the proof of (15).

Proof of (16) : We shall prove that

$$\mathbb{P}\left(\left\{\frac{\hat{k}_n}{n} - \frac{k_n}{n} > u_n\right\}\cap\Omega_n\right) \xrightarrow[n\to\infty]{} 0.$$

$$\begin{aligned}\mathbb{P}\Big(\big\{\hat{k}_n - k_n > nu_n\big\}\cap\Omega_n\Big) &= \mathbb{P}\Big(\big\{\hat{\tau}_{k_n+nu_n} > \tau\big\}\cap\Omega_n\Big)\\ &= \mathbb{P}\left(\left\{\frac{1}{n-(k_n+nu_n)}\sum_{i=k_n+nu_n+1}^{n}X_{(i)} > \tau\right\}\cap\Omega_n\right).\end{aligned}$$

Let $Z_{(1)} \geq Z_{(2)} \geq .. \geq Z_{(n-k_n)}$.

$$\begin{aligned}\mathbb{P}(\hat{k}_n - k_n > nu_n \cap \Omega_n) &= \mathbb{P}\left(\left\{\frac{1}{n-(k_n+nu_n)}\sum_{i=nu_n+1}^{n-k_n}Z_{(i)} > 1\right\}\cap\Omega_n\right)\\ &\leq \mathbb{P}\Big(\sum_{i=1}^{n-k_n}Z_{(i)} - \sum_{i=1}^{nu_n}Z_{(i)} > n-(k_n+nu_n)\Big)\\ &\leq P_2 + P_3\end{aligned}$$

where

$$P_2 = \mathbb{P}\Big(\sum_{i=1}^{n-k_n}Z_{(i)} > n-k_n+\sqrt{n-k_n}(\sqrt{n}u_n)^{1/2}\Big)$$

$$P_3 = \mathbb{P}\Big(-\sum_{i=1}^{nu_n}Z_{(i)} > -nu_n - \sqrt{n-k_n}(\sqrt{n}u_n)^{1/2}\Big).$$

Since $\sum_{i=1}^{n-k_n}Z_{(i)} = \sum_{i=1}^{n-k_n}Z_i$,

$$P_2 = \mathbb{P}\Big(\frac{\sum_{i=1}^{n-k_n}Z_i - (n-k_n)}{v\sqrt{n-k_n}} > \frac{(\sqrt{n}u_n)^{1/2}}{v}\Big).$$

The central limit theorem implies that

$$\frac{\sum_{i=1}^{n-k_n} Z_i - (n-k_n)}{v\sqrt{n-k_n}} \xrightarrow{\mathcal{L}} \mathcal{N}(0,1)$$

since $\sqrt{n}u_n \xrightarrow[n\to\infty]{} +\infty$, we obtain $P_2 \xrightarrow[n\to\infty]{} 0$.

Moreover, since $k_n = \lambda n$,

$$P_3 = \mathbb{P}\left(\frac{1}{nu_n}\sum_{i=1}^{nu_n} Z_{(i)} < 1 + \frac{\sqrt{1-\lambda}}{(\sqrt{n}u_n)^{1/2}}\right).$$

We recall that $\epsilon_0 > 0$ satisfies $\mathbb{P}(Z_i \le 1+\epsilon_0) \in]0,1[$. For n large enough, $\sqrt{1-\lambda}/(\sqrt{n}u_n)^{1/2} \le \epsilon_0$, which implies that

$$\begin{aligned} P_3 &\le \mathbb{P}\Big(Z_{(nu_n)} \le 1+\epsilon_0\Big) \\ &\le \mathbb{P}\Big(Z_{(n-k_n)} \le .. \le Z_{(nu_n)} \le 1+\epsilon_0\Big) \\ &\le \mathbb{P}\Big(\sum_{i=1}^{n-k_n}(1_{Z_i\le 1+\epsilon_0} - p) \ge n - k_n - nu_n - (n-k_n)p\Big). \end{aligned}$$

where $p = P(Z_i \le 1+\epsilon_0) \in]0,1[$. We obtain that, for n large enough,

$$P_3 \le \mathbb{P}\Big(\sum_{i=1}^{n-k_n} \frac{(1_{Z_i\le 1+\epsilon_0} - p)}{\sqrt{n-k_n}} \ge \sqrt{n-k_n}(1-p) - \frac{nu_n}{\sqrt{n-k_n}}\Big).$$

Since $\sqrt{n-k_n}(1-p) - nu_n/\sqrt{n-k_n}$ tends to infinity as n tends to infinity, the central limit theorem implies that P_3 converges towards 0.

This concludes the proof of (16). Let us now prove Theorem 1.

Proof of (11):

$$\mathbb{P}\left(\frac{V}{\max(\hat{k}_n,1)} > u_n \cap \Omega_n\right) \le \mathbb{P}\left(\hat{k}_n = 0 \cap \Omega_n\right) + \mathbb{P}\left(V \ge u_n\hat{k}_n \cap \Omega_n\right).$$

$$\begin{aligned} \mathbb{P}\left(\hat{k}_n = 0 \cap \Omega_n\right) &= \mathbb{P}\left(\hat{k}_n - k_n = -k_n \cap \Omega_n\right) \\ &\le \mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} = -\lambda \cap \Omega_n\right) \end{aligned}$$

since $k_n = \lambda n$. For n large enough, $u_n < \lambda$ and

$$\mathbb{P}\left(\hat{k}_n = 0 \cap \Omega_n\right) \leq \mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} \leq -u_n \cap \Omega_n\right).$$

This probability tends to 0 as n tends to infinity thanks to (15).

$$\begin{aligned}
\mathbb{P}\left(V \geq u_n \hat{k}_n \cap \Omega_n\right) &= \mathbb{P}\left(\hat{k}_n - k_n \geq u_n \hat{k}_n \cap \Omega_n\right) \\
&= \mathbb{P}\left(\hat{k}_n \geq \frac{k_n}{1-u_n} \cap \Omega_n\right) \\
&\leq \mathbb{P}\left(\hat{k}_n \geq k_n(1+u_n) \cap \Omega_n\right) \\
&\leq \mathbb{P}\left(\frac{\hat{k}_n - k_n}{n} \geq \lambda u_n \cap \Omega_n\right).
\end{aligned}$$

Since (16) holds for any sequence (u_n) satisfying the assumptions of Theorem 1, it holds for the sequence (λu_n), hence the above probability tends to 0 as n tends to infinity.

Proof of (12) :

$$\mathbb{P}\left(\frac{T}{\max(n-\hat{k}_n, 1)} > u_n \cap \Omega_n\right) \leq \mathbb{P}\left(\hat{k}_n = n \cap \Omega_n\right) + \mathbb{P}\left(T > u_n(n-\hat{k}_n) \cap \Omega_n\right).$$

$$\mathbb{P}\left(\hat{k}_n = n \cap \Omega_n\right) = \mathbb{P}\left(\frac{\hat{k}_n}{n} - \frac{k_n}{n} = (1-\lambda) \cap \Omega_n\right)$$

which converges towards 0 thanks to (16).

$$\begin{aligned}
\mathbb{P}\left(T > u_n(n-\hat{k}_n) \cap \Omega_n\right) &= \mathbb{P}\left(k_n - \hat{k}_n > u_n(n\lambda + n(1-\lambda) - \hat{k}_n) \cap \Omega_n\right) \\
&= \mathbb{P}\left((k_n - \hat{k}_n)(1-u_n) > n(1-\lambda)u_n \cap \Omega_n\right) \\
&\leq \mathbb{P}\left(\frac{k_n - \hat{k}_n}{n} > \frac{(1-\lambda)}{2} u_n \cap \Omega_n\right)
\end{aligned}$$

as soon as $1 - u_n < 2$ which holds for n large enough. This last probability tends to 0 thanks to (16).

Proof of (13) :

$$\mathbb{E}\left(\frac{V}{\max(\hat{k}_n, 1)}\right) = \mathbb{E}\left(\frac{V}{\max(\hat{k}_n, 1)} \mathbb{1}_{\frac{V}{\max(\hat{k}_n,1)} > u_n}\right) + \mathbb{E}\left(\frac{V}{\max(\hat{k}_n, 1)} \mathbb{1}_{\frac{V}{\max(\hat{k}_n,1)} \leq u_n}\right)$$

Note that $V/\max(\hat{k}_n, 1) \leq 1$. Hence,

$$\mathbb{E}\left(\frac{V}{\max(\hat{k}_n, 1)}\ \mathbb{1}_{\frac{V}{\max(\hat{k}_n,1)}>u_n}\right) \leq \mathbb{P}\left(\frac{V}{\max(\hat{k}_n, 1)} > u_n\right)$$

which converges towards 0 as n tends to infinity.

$$\mathbb{E}\left(\frac{V}{\max(\hat{k}_n, 1)}\ \mathbb{1}_{\frac{V}{\max(\hat{k}_n,1)}\leq u_n}\right) \leq u_n$$

which also converges towards 0 as n tends to infinity. This concludes the proof of (13) and the proof of (14) is similar.