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Hotelling Test for Highly Correlated Data

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This paper is motivated by the analysis of gene expression sets, especially by finding differentially expressed gene sets between two phenotypes. Gene $\log_2$ expression levels are highly correlated and, very likely, have approximately normal distribution. Therefore, it seems reasonable to use two-sample Hotelling's test for such data. We discover some unexpected properties of the test making it different from the majority of tests previously used for such data. It appears that the Hotelling's test does not always reach maximal power when all marginal distributions are different. For highly correlated data its maximal power is attained when about a half of marginal distributions are essentially different. For the case when the correlation coefficient is greater than 0.5 this test is more powerful if only one marginal distribution is shifted, comparing to the case when all marginal distributions are equally shifted. Moreover, when the correlation coefficient increases the power of Hotelling's test increases as well.

1. Introduction

In many situations statisticians need to test multidimensional hypotheses. In a lot of cases components of observed random vectors are highly dependent, which may change the properties of the tests used. One of the examples of such data is provided by gene expression levels. Gene expressions are highly correlated between genes (see for example [5]). Moreover, often the genes are investigated not just separately, but also as a set of dependent genes. The most popular tests for gene sets are Hotelling's test, N-test and tests derived from marginal t-statistics. In the papers [1], [4], an

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approach to comparing these test in various situations was made. Our goal is not to make another comparison, but rather to describe some interesting properties of the Hotelling's test which seems to be unexpected.

2. Hotelling's test

One of the most well known tests is t-test. Hotelling's test is an multidimensional extension of t-test. Similar to t-test, we can consider both one-sample and two-sample Hotelling's test. One-sample case deals with the hypothesis that the expected value of a sample from multidimensional normal distribution is equal to some given vector. In the two-sample case it deals with the hypothesis of the equality of expected values of two samples from multidimensional normal distributions (with the equal covariance structure). In this paper we will focus on the two-sample Hotelling's test.

Suppose we have two independent samples (of sizes n_x and n_y, respectively) from two n-dimensional normal distributions with identical covariance matrices equal to Σ. In other words, we consider $X_1, \ldots, X_{n_x}$ as i.i.d random vectors having $N_n(\mu_x, \Sigma)$ and $Y_1, \ldots, Y_{n_y}$ as i.i.d random vectors having $N_n(\mu_y, \Sigma)$ (X_i and Y_j are independent for all $i = 1, \ldots, n_x$; $j = 1, \ldots, n_y$). For simplicity we assume that $n < n_x + n_y - 1$. Our goal is to test the hypothesis $H : \mu_x = \mu_y$ against alternative $A : \mu_x \neq \mu_y$. For this we use Hotelling's test based on the statistic

$$T^2 = \frac{n_x n_y}{n_x + n_y}(\bar{X} - \bar{Y})^T S^{-1}(\bar{X} - \bar{Y}), \tag{1}$$

where $\bar{X} = \frac{1}{n_x}\sum_{i=1}^{n_x} X_i$; $\bar{Y} = \frac{1}{n_y}\sum_{i=1}^{n_y} Y_i$ and

$$S = \frac{\sum_{i=1}^{n_x}(X_i - \bar{X})(X_i - \bar{X})^T + \sum_{i=1}^{n_y}(Y_i - \bar{Y})(Y_i - \bar{Y})^T}{n_x + n_y - 2}.$$

T^2 is related to the F-distribution by

$$\frac{n_x + n_y - n - 1}{n(n_x + n_y - 2)} T^2 \sim F(n, n_x + n_y - n - 1). \tag{2}$$

For more details about Hotelling's test see, for example, [3]. We made the assumption $n < n_x + n_y - 1$ for two reasons. For $n \geq n_x + n_y - 1$ the estimate S of Σ results in an irregular matrix, so that S^{-1} does not exist and moreover numerator of (2) is non-positive as well as the degree of freedom of the F-distribution. In such situations it is possible to use some pseudo-inversion of S and in order to estimate p-value of H, we can use permutations of $(X_1, \ldots, X_{n_x}, Y_1, \ldots, Y_{n_y})$.

3. Hotelling's test for strongly dependent data

Consider that we have two multidimensional samples and need to test the hypothesis suggesting the equality of expected values in these two samples. Assume for simplicity that all elements on the main diagonal of the covariance matrix Σ for both samples are equal to 1 and all other elements are equal to $\rho > 0$, i.e.

$$\Sigma = \begin{pmatrix} 1 & \rho & \rho & \dots & \rho \\ \rho & 1 & \rho & \dots & \rho \\ \dots & \dots & \dots & \dots & \dots \\ \rho & \dots & \dots & \rho & 1 \end{pmatrix}.$$

Further on, we assume that $\mu_x = (0, \dots, 0)^T$, but μ_y has first m elements equal to 1 and the others equal to 0, i.e.

$$\mu_y = (\underbrace{1, \dots, 1}_{m}, \underbrace{0, \dots, 0}_{n-m})^T.$$

For large n_x and n_y the matrix Σ and its estimate S are approximately the same as well as the differences between the expected values $(\mu_x - \mu_y)$ and between the mean values $(\bar{X} - \bar{Y})$. When dealing with real data, n_x and n_y might not be large enough, but for easier insight to the problem we use the approximations $S \approx \Sigma$ and $\bar{X} - \bar{Y} \approx \mu_x - \mu_y$. In this case $S^{-1} \approx \Sigma^{-1}$, that is

$$S^{-1} \approx \Sigma^{-1} = \begin{pmatrix} \alpha & -\beta & -\beta & \dots & -\beta \\ -\beta & \alpha & -\beta & \dots & -\beta \\ \dots & \dots & \dots & \dots & \dots \\ -\beta & \dots & \dots & -\beta & \alpha \end{pmatrix},$$

where $\alpha = \frac{(1+(n-2)\rho)}{(1-\rho)(1+(n-1)\rho)}$ and $\beta = \frac{\rho}{(1-\rho)(1+(n-1)\rho)}$. For fixed n_x and n_y we can consider the fraction $\frac{n_x n_y}{n_x + n_y} = k$ of Hotelling's statistic (1) as a normalizing constant. Let us denote by T^{*2} Hotelling's statistic with Σ^{-1} instead of S^{-1} and $\mu_x - \mu_y$ instead of $\bar{X} - \bar{Y}$ divided by the constant k. Then T^{*2} is squared Mahalanobis distance of μ_x and μ_y and it is given by

$$T^2/k \approx T^{*2} = (\mu_x - \mu_y)^T \Sigma^{-1} (\mu_x - \mu_y)$$

$$= (\underbrace{1, \dots, 1}_{m}, \underbrace{0, \dots, 0}_{n-m}) \begin{pmatrix} \alpha & -\beta & -\beta & \dots & -\beta \\ -\beta & \alpha & -\beta & \dots & -\beta \\ \dots & \dots & \dots & \dots & \dots \\ -\beta & \dots & \dots & -\beta & \alpha \end{pmatrix} \begin{pmatrix} 1 \\ \dots \\ 1 \\ 0 \\ \dots \\ 0 \end{pmatrix}$$

$$= m\alpha - (m^2 - m)\beta = \frac{m(1 + (n-2)\rho) - m(m-1)\rho}{(1-\rho)(1 + (n-1)\rho)} = \frac{m(1 + (n-m-1)\rho)}{(1-\rho)(1 + (n-1)\rho)}. \quad (3)$$

Let us note that it does not matter if μ_y consists of ones and zeros or equals to a constant a and zeros. In the latter case, squared distance T^{*2} would be multiplied by a^2.

Now we will work with T^{*2} and investigate its behavior.

If we changed m to $m+1$ (meaning that we add one more different marginal distribution) we would expect that T^{*2} increases and that so does the power of Hotelling's test. We need to check if it is indeed the case. For better understanding let the number of ones in μ_y be the index of T^{*2} (we will write it only when it is needed). Now we change m to $m + 1 = h$ and we have

$$T^{*2}_{m+1} = T^{*2}_m + \alpha - 2m\beta.$$

If we expected that T^{*2} is an increasing function of m then $\alpha - 2m\beta$ should be greater then zero. But we have

$$\alpha - 2m\beta = \frac{1 + (n-2)\rho}{(1-\rho)(1+(n-1)\rho)} - \frac{2m\rho}{(1-\rho)(1+(n-1)\rho)} = \frac{1 + (n-2m-2)\rho}{(1-\rho)(1+(n-1)\rho)}.$$

Since the denominator is greater than zero, then $\alpha - 2m\beta > 0$ only if $\frac{1}{2m+2-n} = \frac{1}{2h-n} > \rho$. It means that for not very small values of ρ's and $m > \frac{n}{2} - 1$ the square Mahalanobis distance T^{*2} is a decreasing function of m. This means that maximal power of Hotelling's test (as a function of m) is not always attained for $m = n$ but for ρ's which are not very small we have maximal power for m near $\frac{n}{2}$. Some examples of the behavior of T^{*2} as a function of m are illustrated in figure 1.

However, this issue is not the only one that is surprising about Hotelling's test. Now we look if T^{*2}_1 is always lower than T^{*2}_n. It is the case when one different marginal distribution influences more than all n different distributions. So we need to compare α with $n\alpha - n(n-1)\beta$. We have

$$T^{*2}_1 - T^{*2}_n = \alpha - n\alpha + n(n-1)\beta = (n-1)\frac{(1-2\rho)}{(1-\rho)(1+(n-1)\rho)}.$$

So $T^{*2}_1 - T^{*2}_n < 0$ only if $\rho < 0.5$. Therefore we can say that for $\rho > 0.5$ Hotelling's test has better power for alternative with only one marginal shift than for alternative that all marginal distributions are equally shifted. It can be seen from figure 1 as well. Moreover, T^{*2} is an increasing function of ρ, that may seem surprising as well.

4. Hotelling's test for two-dimensional data

Let generalize expected value μ_y to have components $(a_1, \ldots, a_n)$. We are interested in for which $\mu_y \in R^n$ the squared Mahalanobis distance has the same value. For some $d > 0$ we define the set

$$E_d = \{\mu_y = (a_1, \ldots, a_n); \mu_y^T \Sigma^{-1} \mu_y = d^2\}.$$

This set is created by iso-distance curves, i.e ellipsoids with center in $(0, \ldots, 0)$. Let denote the eigenvalues of matrix Σ^{-1} by $\lambda_1, \ldots, \lambda_n$ and the eigenvectors corresponding to these eigenvalues by $\gamma_1, \ldots, \gamma_n$. Then the principal axes of E_d are in the direction

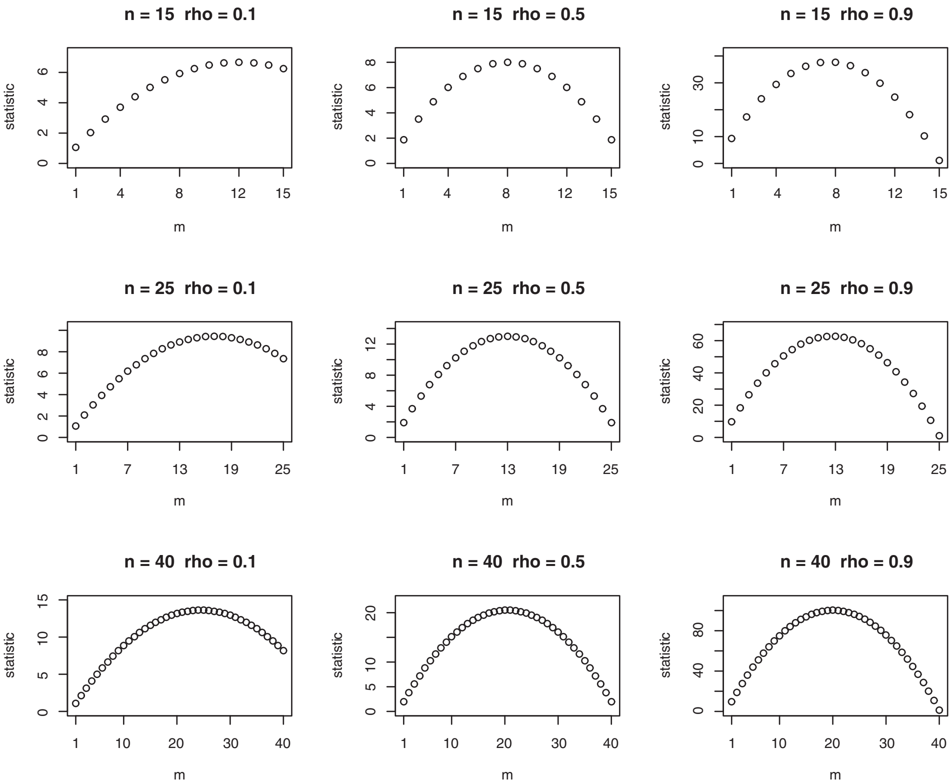

FIGURE 1. Plots of T^{*2} for $n = 15, 25, 40$; $\rho = 0.1, 0.5, 0.9$; and $m = 1, \dots, n$. Notice: each plot is differently scaled!

of γ_i; $i = 1, \dots, n$ and the half-lengths of the axes are given by $\sqrt{\frac{d^2}{\lambda_i}}$; $i = 1, \dots, n$. In our case with Σ^{-1}, the eigenvalues $\lambda_1 = \dots, \lambda_{n-1} = \frac{1}{1-\rho}$ and $\lambda_n = \frac{1}{1+(n-1)\rho}$. The eigenvector corresponding to the smallest eigenvalue λ_n is equal to $\gamma_n = (1, \dots, 1)$. Therefore squared Mahalanobis distance has the slowest increase in this direction.

Let us look at Hotelling's test in the two-dimensional case. Some plots of 2-dimensional ellipsoids for different values of the correlation coefficient ρ are given on figure 2. The squared Mahalanobis distance has the weakest increase in the direction of $a_1 = a_2$, while the fastest increases is observed towards the direction of $a_1 = -a_2$. For example, for $\rho = 0.9$ and $d = \alpha$ the principal axes are equal to 3.162 and 0.725. It means that for $a_1 = a_2 = \sqrt{\frac{3.162^2}{2}} = 2.236$ squared Mahalanobis distance is the same as for $a_1 = 1$, $a_2 = 0$ (or for $a_1 = -a_2 = \sqrt{\frac{0.725^2}{2}} = 0.513$ as well). Hence, if there is only one marginal distribution shifted by one unit, then the power of Hotelling's test is expected to be the same as if both marginal distribution were equally shifted (in the same direction) by 2.236 units (for the shift in opposite

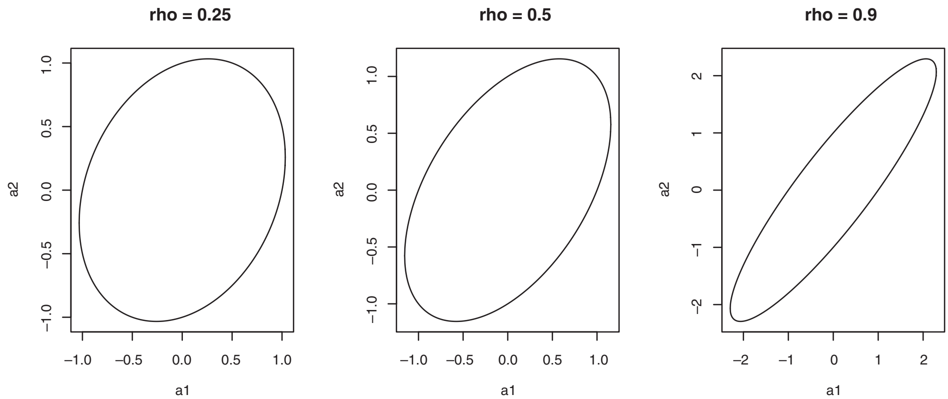

FIGURE 2. Plots of solutions of equation (**??**) for two-dimensional case for $\rho = 0.25;0.5;0.9$. Notice: each plot is differently scaled!

direction it should be only 0.513 unit). These results are in contradiction with other multidimensional tests. For example, consider the test based on marginal t-statistics. The power of this test is higher if both distributions are shifted by the same amount (both t-statistics are "large", not depending on direction of shift) than if there was only one marginal distribution shifted (one t-statistic is "near" zero).

5. Theory and reality

The analytical results obtained above should be verified by checking if actual Hotelling's test outcomes correspond to the analytical results regarding real data. In this section we will compare the behavior of squared Mahalanobis distance T^{*2} with Hotelling's statistic T^2. For large n_x and n_y we assumed that $T^{*2} \approx T^2/k$, where $k = \frac{n_x n_y}{n_x+n_y}$. Constant k changes as n_x and n_y change. It is reasonable to divide Hotelling's statistic T^2 by k instead of multiplying T^{*2} by k in order to be able to compare how do T^2 and T^{*2} differ for various n_x and n_y.

In order to compare the actual results with the analytical ones, we did the following simulations. All data were simulated from n-dimensional normal distributions. We set the dimension n to be 10, 15 and $n = 25$. All simulations were performed for three different values of the correlation coefficient ρ: $\rho = 0.1$, $\rho = 0.5$ and $\rho = 0.9$. In order to compare the behavior of Hotelling's test for various sizes of samples we took three choices of n_x and n_y: $n_x = n_y = n, n_x = n_y = 1.4n$ and $n_x = n_y = 2.4n$. The value m which is the number of false marginal distributions varies from one to n. The shift value for each of the different marginal distributions is set to one. The squared Mahalanobis distance is calculated according to (3). Hotelling's statistic is estimated from 1000 simulations for each case (as the mean of T^2/k obtained from the simulations).

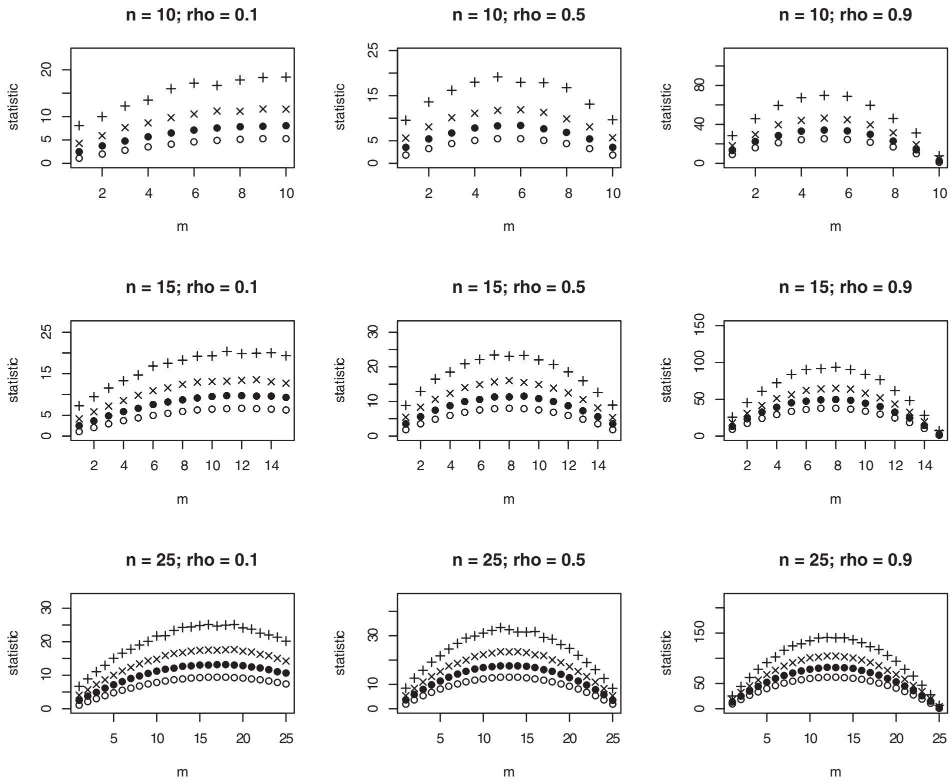

FIGURE 3. Comparisons of squared Mahalanobis distance T^{*2} and real Hotelling's statistic T^2/k for the dimension $n = 10$ 15, 25 (from the top to the bottom); for correlation coefficient $\rho = 0.1, 0.5, 0.9$ (from the left to the right) and number of observations in each sample $n_x = n_y = n$ (denoted by '+'), $n_x = n_y = 1.4n$ (denoted by 'x') and $n_x = n_y = 2.4n$ (denoted by '•'). Squared Mahalanobis distance T^{*2} is denoted by '∘'. Number of different marginal distribution m is set from one to n. Notice: each plot is differently scaled!

Plots of our simulated cases are shown on figure 3. We can see that for all simulated situations, the shapes squared Mahalanobis distance and Hotelling's statistics are similar. The only difference is in the heights of these curves. For small n_x and n_y statistic T^2 has higher values than for large n_x and n_y. The reason for that stems from the inaccurate estimates of the expected values and of the covariance matrix. However, we observe that with the increase of n_x and n_y, statistic T^2/k goes to T^{*2} relatively fast. Therefore, the behavior of Hotelling's test for real data is expected to be very similar to the behavior of squared Mahalanobis distance T^{*2}.

In previous section we saw that for the two-dimensional case the plotted shifts with equal values of the power of theoretical Hotelling's test form elliptic curves. Hotelling's statistics T^2 are random variables. Therefore, we can only estimate if their expected values form elliptic curves when plotted. To check this we did following simulations. Instead of calculating the shifts for which Hotelling's test has equal powers, we took some points with coordinates (a_1, a_2) from the elliptic curves observed for squared Mahalanobis distance. For each such point we did 1000 simulations and calculated Hotelling's statistic. We estimated the expected value $\mathrm{E}T^2/k$ as the mean for these 1000 repetitions. We divided Hotelling's statistics by k for better understanding how fast these statistics go to T^{*2}. We did this simulation for the values of the correlation coefficient $\rho = 0.3$ and $\rho = 0.9$ and as the number of observations in each sample we took $n_x = n_y = 5$, $n_x = n_y = 10$ and $n_x = n_y = 20$. Results of our simulation are given in Table 1. We observe that estimated mean values of $\overline{T^2/k}$ are not very different, that they go to T^{*2} and that their variance decreases with increasing number of observations. Clearly, these points form elliptic curves. Hence, we can claim that the real Hotelling's test behaves very similar to the theoretical one and the theory derived for the theoretical test holds for the real Hotelling's test as well.

TABLE 1. Results of simulations of two-dimensional adjusted Hotelling's statistics T^2/k with $n_s = n_x = n_y$ observations for each sample and correlation coefficient ρ. T^{*2} stands for squared Mahalanobis distance and (a_1, a_2) is difference between expected values $\mu_x - \mu_y$ of these samples. On bottom line is the estimate of variance of each column.

$T^{*2} = 1.0989$		$\rho = 0.3$			$T^{*2} = 5.2632$		$\rho = 0.9$		
a_1	a_2	$n_s = 5$	$n_s = 10$	$n_s = 20$	a_1	a_2	$n_s = 5$	$n_s = 10$	$n_s = 20$
−0.84	0.35	3.12	1.74	1.35	−1.83	−1.05	9.58	6.72	5.96
−0.63	0.61	3.03	1.81	1.42	−1.38	−0.44	9.55	6.51	5.96
−0.42	0.79	3.04	1.82	1.39	−0.92	0.09	9.55	6.65	5.99
−0.21	0.92	3.00	1.75	1.42	−0.46	0.57	9.62	6.93	5.98
0.00	1.00	3.03	1.72	1.42	0.00	1.00	9.10	6.99	5.83
0.21	1.04	3.04	1.74	1.36	0.46	1.39	9.74	6.78	5.99
0.42	1.04	3.01	1.87	1.39	0.92	1.74	10.11	6.75	5.86
0.63	0.99	3.00	1.79	1.40	1.38	2.04	9.36	6.87	5.85
0.84	0.85	3.32	1.81	1.41	1.83	2.25	10.21	6.87	5.96
1.05	0.35	3.35	1.85	1.36	2.29	2.09	9.94	6.85	5.97
var:		0.0176	0.0025	0.0007	var:		0.1133	0.0202	0.0039

6. Discussion

In this paper we have discovered that two-sample Hotelling's test (for testing the equality of the expected values of two samples from multidimensional normal distribution with equal covariance structure) has some unexpected properties. At first sight, one could expect that with a larger number of false marginal distributions the power of this test increases. But we have discovered that this is not true in general. For highly correlated and high dimensional data (such as data sets of gene expressions) maximal power of Hotelling's test is reached when only about one half of the marginal distributions are shifted. We have found out that when the correlation inside the sample is greater than 0.5, then the Hotelling's test can have a better power if only one marginal distribution is different, as opposed to the case when all marginal hypotheses are false. Moreover, the power of Hotelling's test increases for higher correlations. That observation may seem somewhat unexpected as well. We have investigated Hotelling's test in detail in two-dimensional case. We have found that properties of this test are much different from ones of the tests based on marginal t-statistic. All reasonable tests based on marginal t-statistic do not depend on the direction of the shift. But the power of Hotelling's test increases very slowly if both of the marginal distributions are equally shifted and increases much faster if marginal distributions are shifted in opposite directions. Moreover, alternatives with equal values of the power form ellipsoids.

References

[1] Ackerman, M., Strimmer, K.: *A general modular framework for gene set enrichment analysis*, BMC Bioinformatics **10**, 47 (2009), 297–317.

[2] Barry, W. T., Nobel, A. B., Wright, F. A.: *A statistical framework for testing functional categories in microarray data*, Ann. Appl. Stat. **2**, No. 1 (2008), 286–315.

[3] Chatfirld, X., Collins, A. J.: Introduction To Multivariate Analysis, Chapman&Hall/CRC 1980.

[4] Glazko, G., Emmert-Streib, F.: *Unite and conquer: univariate and ultivariate approaches for finding differentially expressed gene sets*, Bioinformatics **25**, No. 18 (2009), 2348–2354.

[5] Klebanov, L., Yakovlev, A.: *Diverse correlation structures in gene expression data and their utility in improving statistical inference*, Ann. Appl. Stat. **1**, No. 2 (2007), 538–559.