Supplement to:

Detection of patient subgroups with differential expression in omics data: a comprehensive comparison of univariate measures

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1 Figures

1.1 Simulation results (composite null situation)

In the following we provide the simulation results that complement the results in Figure 1 in the main paper. The study includes all combinations of sample sizes $n = 20, 30, 50, 70, 100$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$, and the null situation is comprised of $H_{0a}$ and $H_{0b}$ in equal shares (i.e. $p_{H_{0a}} = 0.5$).

Table S1-S3 give summary of the best tests for each combination of sample size $n$ and subgroup proportion $q$ for the different scenarios for the composite null situation with $p_{H_{0a}} = 0.5$. In general, $t$-test performs best, if the majority of the disease is upregulated, i.e. for subgroups with $q \geq 0.5$, in scenario I and II. For smaller subgroup sizes, the best test depends on the degree of deviation. Whereas the $t$-test is able to detect very small deviations, our FisherSum is best for rather moderate deviations, and for larger deviations Bartlett’s test yields best results. Please note that for large deviations several tests gain AUC values virtually equal to 1, see following pages. Even in scenario III (bidirectional deregulation), FisherSum yields slightly better results than Bartlett’s test for small deviations and subgroup proportions.
Table S1: Best tests for all combinations of sample size $n$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$ in scenario I, $p_{H_{0a}} = 0.5$.

Color code: □ FisherSum, □ Bartlett’s test, □ $t$-test, □ ORT, □ Kurtosis, □ OS, □ P ADGE.
**Table S2:** Best tests for all combinations of sample size $n$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$ in scenario II, $pH_{0a} = 0.5$.

**Color code:**
- FisherSum
- Bartlett’s test
- t-test
- ORT
- Kurtosis
- OS
- PADGE.
| $\sigma$ | n = 20 | n = 30 | n = 50 | n = 70 | n = 100 |
|---------|---------|---------|--------|--------|---------|
| 0.10    |         |         |        |        |         |
| 0.20    |         |         |        |        |         |
| 0.30    |         |         |        |        |         |
| 0.50    |         |         |        |        |         |
| 0.75    |         |         |        |        |         |
| 1.00    |         |         |        |        |         |

**Table S3:** Best tests for all combinations of sample size $n$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$ in scenario III, $p_{H_0} = 0.5$.

Color code:  ■ FisherSum,  □ Bartlett’s test,  ■ $t$-test,  ■ ORT,  ■ Kurtosis,  ■ OS,  ■ PADGE.
Scenario I, n = 20, q = 0.1, p_H0a = 0.5

Scenario I, n = 20, q = 0.2, p_H0a = 0.5

Scenario I, n = 20, q = 0.3, p_H0a = 0.5

Scenario I, n = 20, q = 0.5, p_H0a = 0.5

Scenario I, n = 20, q = 0.75, p_H0a = 0.5

Scenario I, n = 20, q = 1, p_H0a = 0.5
| Scenario I, n = 70, q = 0.1, p_{H0a} = 0.5 | Scenario I, n = 70, q = 0.2, p_{H0a} = 0.5 |
|-------------------------------------------|-------------------------------------------|
| delta                                    | delta                                    |
| 0 1 2 3 4 5 6 7                          | 0 1 2 3 4 5 6 7                          |
| 0.2 0.4 0.6 0.8 1.0                       | 0.2 0.4 0.6 0.8 1.0                       |

| Scenario I, n = 70, q = 0.3, p_{H0a} = 0.5 | Scenario I, n = 70, q = 0.5, p_{H0a} = 0.5 |
|-------------------------------------------|-------------------------------------------|
| delta                                    | delta                                    |
| 0 1 2 3 4 5 6 7                          | 0 1 2 3 4 5 6 7                          |
| 0.2 0.4 0.6 0.8 1.0                       | 0.2 0.4 0.6 0.8 1.0                       |

| Scenario I, n = 70, q = 0.75, p_{H0a} = 0.5 | Scenario I, n = 70, q = 1, p_{H0a} = 0.5 |
|-------------------------------------------|-------------------------------------------|
| delta                                    | delta                                    |
| 0 1 2 3 4 5 6 7                          | 0 1 2 3 4 5 6 7                          |
| 0.2 0.4 0.6 0.8 1.0                       | 0.2 0.4 0.6 0.8 1.0                       |
Scenario I, n = 100, q = 0.1, p_H0a = 0.5

Scenario I, n = 100, q = 0.2, p_H0a = 0.5

Scenario I, n = 100, q = 0.3, p_H0a = 0.5

Scenario I, n = 100, q = 0.5, p_H0a = 0.5

Scenario I, n = 100, q = 0.75, p_H0a = 0.5

Scenario I, n = 100, q = 1, p_H0a = 0.5

Scenario I, n = 100, q = 1.1, p_H0a = 0.5

Scenario I, n = 100, q = 1.2, p_H0a = 0.5
Scenario III, n = 50, q = 0.1, p_H0a = 0.5

Scenario III, n = 50, q = 0.2, p_H0a = 0.5

Scenario III, n = 50, q = 0.3, p_H0a = 0.5

Scenario III, n = 50, q = 0.5, p_H0a = 0.5

Scenario III, n = 50, q = 0.75, p_H0a = 0.5

Scenario III, n = 50, q = 1, p_H0a = 0.5
Figure S1. In-depth comparison of different methods’ AUC values for the composite null situation. Included are all combinations of sample sizes $n$, subgroup proportions $q$, and scenarios $s$ with $p_{H_{0a}} = 0.5$. 
1.2 Simulation results (simple null situation)

Analogously to the study presented in section 1.1, we here show the results from a simulation with $p_{H_0} = 1$. In this case, the different methods have to cope with distinctly smaller numbers of false positives among the null situation. Compared to $p_{H_0} = 0.5$, ORT seems to benefit the most from this simplification. For more details, please refer to the main paper.

Table S4–S6 give summary of the best tests for each combination of sample size $n$ and subgroup proportion $q$ for the different scenarios, when the simple null situation is used. According to the simulation results for scenario I and II, FisherSum performs best for small to moderate sample sizes with subgroups up to 30–50 percent. In case of larger samples, FisherSum yields best results for moderate deviations. Again, $t$-test performs best, for large values of $q \geq 0.5$ and for small deviations from the null situation resp., in scenario I and II. Results scenario III (bidirectional deregulation) suggest that generally, Kurtosis is appropriate in case of small subgroups and Bartlett’s test for larger subgroup proportions.
Table S4: Best tests for all combinations of sample size \( n \) and subgroup proportions \( q = 0.1, 0.2, 0.3, 0.5, 0.75, 1 \) in scenario I, \( \rho_{H_0} = 1 \).

Color code: ■ FisherSum, □ Bartlett’s test, ■ \( t \)-test, ■ ORT, ■ Kurtosis, ■ OS, ■ PADGE.
| $b$ | $n = 20$ | $n = 30$ | $n = 50$ | $n = 70$ | $n = 100$ |
|-----|-----------|-----------|-----------|-----------|-----------|
| 0.3 |   |   |   |   |   |
| 0.7 |   |   |   |   |   |
| 1.3 |   |   |   |   |   |
| 2.0 |   |   |   |   |   |
| 2.6 |   |   |   |   |   |
| 3.0 |   |   |   |   |   |
| 3.6 |   |   |   |   |   |
| 4.0 |   |   |   |   |   |
| 4.3 |   |   |   |   |   |
| 4.6 |   |   |   |   |   |
| 5.0 |   |   |   |   |   |
| 5.3 |   |   |   |   |   |
| 5.6 |   |   |   |   |   |
| 6.0 |   |   |   |   |   |
| 6.3 |   |   |   |   |   |
| 6.6 |   |   |   |   |   |
| 7.0 |   |   |   |   |   |
| 7.3 |   |   |   |   |   |
| 7.6 |   |   |   |   |   |
| 7.9 |   |   |   |   |   |
| 8.3 |   |   |   |   |   |
| 8.6 |   |   |   |   |   |
| 8.9 |   |   |   |   |   |
| 9.2 |   |   |   |   |   |
| 9.5 |   |   |   |   |   |
| 9.9 |   |   |   |   |   |

**Table S5:** Best tests for all combinations of sample size $n$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$ in scenario II, $p_{H_0} = 1$.

Color code: ▢ FisherSum, □ Bartlett’s test, ▣ $t$-test, ▦ ORT, ▧ Kurtosis, ▪ OS, ▲ P ADGE.
Table S6: Best tests for all combinations of sample size $n$ and subgroup proportions $q = 0.1, 0.2, 0.3, 0.5, 0.75, 1$ in scenario III, $p_{H_{0a}} = 1$.
Color code: ▢ FisherSum, □ Bartlett’s test, ■ $t$-test, ▲ ORT, ▪ Kurtosis, □ OS, ▲ PADDGE.
Scenario I, $n = 50$, $q = 0.1$, $p_{H0a} = 1$

Scenario I, $n = 50$, $q = 0.2$, $p_{H0a} = 1$

Scenario I, $n = 50$, $q = 0.3$, $p_{H0a} = 1$

Scenario I, $n = 50$, $q = 0.5$, $p_{H0a} = 1$

Scenario I, $n = 50$, $q = 0.75$, $p_{H0a} = 1$

Scenario I, $n = 50$, $q = 1$, $p_{H0a} = 1$
Scenario III, $n = 70$, $q = 0.1$, $p_{H0a} = 1$

![Graph](image1)

Scenario III, $n = 70$, $q = 0.2$, $p_{H0a} = 1$

![Graph](image2)

Scenario III, $n = 70$, $q = 0.3$, $p_{H0a} = 1$

![Graph](image3)

Scenario III, $n = 70$, $q = 0.5$, $p_{H0a} = 1$

![Graph](image4)

Scenario III, $n = 70$, $q = 0.75$, $p_{H0a} = 1$

![Graph](image5)

Scenario III, $n = 70$, $q = 1$, $p_{H0a} = 1$

![Graph](image6)
Figure S2. In-depth comparison of different methods’ AUC values for the simple null situation. Included are all combinations of sample sizes $n$, subgroup proportions $q$, and scenarios $s$ with $p_{H0a} = 1$. 
1.3 Top candidates of different measures for ParkCHIP data

On the following pages, we present \((\log_2)-\)intensity plots of the top candidates of each method. In the ParkCHIP project more than two groups were analysed. The data set used for the following analyses is available at [www.medizinisches-proteom-center.de/Ahrens_et_al](http://www.medizinisches-proteom-center.de/Ahrens_et_al).

Observations on the left-hand side of each plot represent the control group (healthy controls, HC), and the right-hand side corresponds to patients with Parkinson’s disease (PD). Observations per group are in random but fixed order across the variables. The header of each plot gives the Database ID corresponding to the depicted spot. For reasons of comparability of the characteristic distributional patterns of the top candidates, we used the same scale for all plots.

Here are some notes to summarize the distributional patterns of each method’s top candidates:

**FisherSum** most top candidates show desired distributional pattern

**ORT, OS** mainly non-disease-specific subgroups (esp. ORT), detect slight to large increases

**t-test** detects global shifts as expected and apparently subgroups with slight to moderate up-regulation (distinct shift in means while variance remains similar)

**PADGE** some good candidates, some candidates do not show obvious subgroup patterns

**Kurtosis** most top candidates show single outliers

**Bartlett** single outliers and few potential subgroup candidates

**Fisher10** 50 variables yield the minimal \(p\)-value of 0.014, among them some with pretty slight up-regulation that seem to be false positive results, but some promising ones as well

To avoid any differences in the ranking of ORT and OS due to simulated \(p\)-values, we ranked the variables according to the actual test statistics. This approach also reduces the number of ties. In case of Fisher10, ties are non-avoidable. For simplicity, we chose the first 15 variables in the data sheet corresponding to the microarray that yield the minimal \(p\)-value.
Top candidates: FisherSum
Top candidates: ORT Score

NM_030920.1

NM_199054.1

NM_199054.1

NM_012315.1

NM_012315.1

NM_001688.2

BC053600.1

NM_021728.2

NM_001688.2

BC016901.1

NM_018201.2

BC032307.1

BC030516.1

BC030516.1

NM_005142.2

NM_005142.2

NM_017830.1

BC030516.1

NM_021728.2

NM_01688.2

NM_01688.2

NM_0175907.3

NM_0175907.3

NM_0175907.3

NM_0175907.3

P3001

P3001

P3001

P3001

NM_012315.1

NM_017830.1

NM_0175907.3

NM_018201.2

NM_018201.2
Top candidates: t-test

- NM_177938.1
- NM_174903.2
- NM_144588.2
- PV3370
- NM_173843.1
- BC068456.1
- NM_006243.2
- NM_004169.2
- NM_021175.1
- NM_025231.1
- NM_002029.3
- NM_001011667.1
- BC062359.1
- BC016790.1
Top candidates: PADGE

BC007894.2

BC056673.1

NM_080659.1

BC051716.1

NM_002093.2

BC053509.1

PHR5001

BC029267.1

NM_021912.2

BC047720.1

NM_177938.1

NM_003588.2

NM_001037293.1

NM_005324.3

NM_005324.2

NM_080659.1

BC047720.1
Top candidates: Kurtosis

- NM_007256.1
- BC002656.1
- BC013172.1
- NM_000284.1
- NM_018129.1
- NM_005750.2
- BC015947.1
- NM_000562.1
- NM_005750.2
- NM_014697.1
- BC009819.1
- NM_002093.2
- BC016828.1
Top candidates: Bartlett
Figure S3. To compare the considered methods, the respective top 15 candidates of each method are shown in (log2-)intensity plots. We used controls and Parkinson patients from the ParkCHIP project.
2 Pseudocode

2.1 FisherSum and Fisher10

Simple version with weights $w_C = w_D = 1$.

```r
fishXp.score <- function(control, disease, q = 0.1, 
  cut.in='sg', # c('sg', 'both')
  plot=FALSE){
  n.c <- length(control)
  n.d <- length(disease)
  
  # define cut.off
  if(cut.in=='both'){
    ord <- round( (n.c + n.d) * q/2)
    cut <- sort(c(disease, control), decreasing=TRUE)[ord]
    rows <- c( paste('>', pooled['', ord, ''], sep=''),
               paste('<= pooled[', ord, ''], sep=''))
  }else{
    ord <- round( n.d * q)
    cut <- sort(disease, decreasing=TRUE)[ord]
    rows <- c( paste('>', disease['', ord, ''], sep=''),
               paste('<= disease[', ord, ''], sep=''))
  }
  
  # fill in contingency table
  n.sg.d <- sum(disease > cut)
  which.up <- which(disease > cut)
  n.sg.c <- sum(control > cut)
  
  tab <- matrix(c( n.sg.d, n.sg.c, 
                  n.d - n.sg.d, n.c - n.sg.c ),2,2,
    byrow=TRUE,
    dimnames=list(rows, c('disease', 'control')))
  p <- fisher.test(tab, alternative= 'greater')$p.value
  
  # calculate FisherSum
  FS <- 0
  if(n.sg.d > 0){
    location.sg <- median(disease[which(disease > cut)])
    location.rest <- median(disease[which(disease <= cut)])
    FS <- (sum( disease[which.up] - median(control))-
           (sum( control[control > cut ] - median(control))))
  }
  return(list(row = rows, cut = cut, FS = FS, p = p))
}
```

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2.2 PADGE

padge <- function(C, D, test= c('wilcox', 't'),
props = c(0.1,.15,.2)
){
  props <- sort(props, decreasing=TRUE)
test <- as.character(test)

  n.C <- length(C); n.D <- length(D)
  r1 <- mean(D)/ mean(C)
  if(! (1 %in% props)) props <- c(1, props)

  ratios <- rel.ratios <- scores <- p.values <- numeric(length(props))

  for(i in 1:length(props)){
    q.C <- quantile(C, 1-props[i])
    q.D <- quantile(D, 1-props[i])
    subset.C <- C[which(C >= q.C)]
    subset.D <- D[which(D >= q.D)]
    # 'expression ratio'
    ratios[i] <- rn <- mean(subset.D)/ mean(subset.C)
    # 'relative variability of overexpression'
    rel.ratios[i] <- rn/r1

    # calculate p-values
    if(length(subset.C) > 0 & length(subset.D) > 0){
      my.call <- call(paste(test, '.test', sep=''),
                      subset.C, subset.D, alt='less')
      p <- try(eval(my.call)$p.value, silent=TRUE)
    }
  }

  return( list(p.value = p, S= FS, table = tab,
               SG.cand = which.up, cutoff= cut )
)}
p.values[i] <- as.numeric(p)
} else{
  p.values[i] <- NA
}
}
p.values.adj <- p.adjust(p.values, method='bonferroni')

for(i in 1:length(scores)){
  scores[i] <- -rel.ratios[i]*log(p.values[i])
}
max.score <- max(scores, na.rm=TRUE)
# return(...)
}

2.3 ORT

ORT <- function(data, col.C=NULL, col.D=NULL, pvalue=TRUE, L=10^4){
  # define observations in control group...
  if(is.null(col.C)){ ifelse( !(is.null(ncol(data))) ,
    col.C <- 1:(ncol(data)/2),
    col.C <- 1:(length(data)/2) )
  }
  # ... and diseased group
  if( is.null(col.D)){ ifelse( !(is.null(ncol(data))) ,
    col.D <- (ncol(data)/2) + (1: (ncol(data)/2) ),
    col.D <- (length(data)/2) + (1: (length(data)/2) ) )
  }
  # numbers of samples and variables
  ns <- length(c(col.C , col.D))
  ng <- nrow(data)
  # equation (2.8) in original publication
  expr.med1 <- data[,col.C] - apply(data[,col.C], 1, median) *
    matrix(1, nrow = ng, ncol = length(col.C))
  expr.med2 <- data[,col.D] - apply(data[,col.D] , 1, median)*
    matrix(1, nrow = ng, ncol = length(col.D))
  mads <- apply(cbind(expr.med1, expr.med2), 1, function(x){
    x <- abs(as.numeric(x)) ; median(x) })
  #
  expr.centr <- data - apply(data[,col.C], 1, median) *
    matrix(1, nrow = ng, ncol = ns)
  expr.std <- expr.centr/(mads*matrix(1, nrow = ng, ncol = ns))
  #

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q25 <- apply(data[, col.C], 1, function(x) quantile(x, 0.25))
q75 <- apply(data[, col.C], 1, function(x) quantile(x, 0.75))
iqr <- q75 - q25
# equation (2.9)
ind.pos <- data[,col.D] > q75 + iqr
s1 <- rowSums(ind.pos)
t <- rowSums(ind.pos * expr.std[,col.D])
#
### simulate p-values
if(pvalue==TRUE){
sim.data <- matrix(rnorm(ns*L), nrow=L)
sim.centr <- (sim.data - apply(sim.data[,1:round(ns/2)],
1, median))*matrix(1, nrow = L, ncol = ns))
sim.std <- sim.centr / ( apply(sim.data, 1, function(x){
x <- as.numeric(x)
median( c( abs( x[ 1:round(ns/2) ] -
median(x[ 1:round(ns/2) ] ) ),
abs( x[-(1:round(ns/2))] -
median(x[-(1:round(ns/2))]) ) ) )
}) * matrix(1, nrow = L, ncol = ns) )
sim.q25 <- apply(sim.data, 1, function(x) quantile(x, 0.25))
sim.q75 <- apply(sim.data, 1, function(x) quantile(x, 0.75))
sim.iqr <- sim.q75 - sim.q25
sim.ind.pos <- sim.data[,-(1:round(ns/2))] > sim.q75 + sim.iqr
sim.s1 <- rowSums(sim.ind.pos)
sim.t <- rowSums(sim.ind.pos * sim.std[,-(1:(ns/2))])
sim.p <- as.numeric( length(t) )
for(i in 1: length(t)) sim.p[i] <- sum(sim.t>t[i])/length(sim.t)
}
#### return()
ret <- t
if(pvalue == TRUE & ng == 1){
  ret <- c(ret, 'p' = sim.p )
if(pvalue == TRUE & ng > 1){
  ret <- list('t*'=ret, 'p' = sim.p )
return(noquote(ret) )
}

2.4 OS

OS <- function(data, col.c=NULL, col.d=NULL,
pvalue=TRUE, L=10^4){

# default: first half of columns corresponds to controls
# second half to diseased samples
if(is.null(col.c)) col.c <- 1:(ncol(data)/2)
if(is.null(col.d)) col.d <- (ncol(data)/2)+(1:(ncol(data)/2))

# calculate numbers of samples and genes
ns <- length(c(col.c, col.d))
ng <- nrow(data)

# equation (2.2) in original paper
exprs.mad <- (data - apply(data, 1, median) * 
  matrix(1, nrow = ng, ncol = ns)) / 
  (apply(data, 1, mad) * matrix(1, nrow = ng, ncol = ns))

# calculate quantiles
q25 <- apply(data, 1, function(x) quantile(x, 0.25))
q75 <- apply(data, 1, function(x) quantile(x, 0.75))
iqr <- q75 - q25

# identify up-regulated outliers in disease group
ind.pos <- exprs.mad[,col.d] > q75 + iqr

# equation (2.3)
w1 <- rowSums(ind.pos * exprs.mad[,col.d])

# simulate p-values if desired
if(pvalue==TRUE){
  sim.data <- matrix(rnorm(ns*L), nrow=L)
  # analogously to real data:
  # sim.exprs.mad, sim.q25, sim.q75, sim.iqr, sim.ind.pos, sim.w1

  # proportion of larger OS values
  sim.p <- as.numeric( length(w1) )
  for(i in 1:length(w1)){
    sim.p[i] <- sum( sim.w1 > w1[i])/length(sim.w1)
  }
}

# return(...)