mvGST: TOOLS FOR MULTIVARIATE AND DIRECTIONAL GENE SET TESTING

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M.S. Statistics Defense

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Motivating Example

- An experiment was performed to understand how obatoclax mesylate treats leukemia.
- A 3x2 full factorial design was used with 2 replicates.
- The 3 levels of obatoclax mesylate were:
  - High dose (HIGH)
  - Low dose (LOW)
  - Control
- There were 2 blood cell lines treated:
  - RS4:11
  - SEM-K2
- Our objective is to determine how thousands of biological processes' activity levels are affected by obatoclax dose level
  - after accounting for cell line differences [non-stratified]
  - in each cell line separately [stratified]

Idea of Gene Sets

- Gene Ontology groups genes into sets that perform the same biological processes.
- Only genes that contribute to the process are included in each group.
- Genes that inhibit a process are not included in the gene set for that process.
- If genes in set are active, then the process proceeds.
- If even a single gene is not active, then a process may be “disturbed”.

Statistical Model Used

- Expression level of genes in set is used as a proxy for activity level of corresponding biological process
- Per-gene model:

$$Y_{jkl} = \mu + D_j + L_k + DL_{jk} + \varepsilon_{jkl}$$

  - Y_{jkl} is the log (base 2) of the expression level in replicate l of dosage level (D) j, for leukemia cell line (L) k
  - D_j is the dosage level: high, low, or control.
  - L_k is the leukemia cell line: RS4:11 or SEM-K2
  - ε_{jkl} follows a normal distribution with gene-specific variance σ^2
- Model fit using limma package in R

- Suppose a gene is significantly more active for HIGH vs. CTL in the RS4:11 line, less active for LOW vs. CTL in the RS4:11 line, and not significantly different for either dose vs. CTL in the SEM-K2 line.
- The profile could be summarized as:

  1, -1, 0, 0

- or the profile could be stratified and summarized as:

  1, -1 for RS4:11

  0, 0 for SEM-K2

Multivariate and Directional Differential Expression

- The term “multivariate” is used because of simultaneous interest in multiple contrasts
- The term “directional” is used because of interest in one-sided alternatives
- Four contrasts were tested for each gene (HIGH/LOW vs CTL at each cell line) with:
  - H_o: HIGH/LOW = CTL
  - H_a: HIGH/LOW > CTL
- These 4 tests can be summarized in 1 profile with 4 dimensions, or 2 profiles with 2 dimensions.

Summarizing Profiles: Non-Stratified

HIGH/RS4:11	LOW/RS4:11	HIGH/SEM-K2	LOW/SEM-K2	Biological Processes
0	0	0	0	#
0	0	0	1	#
0	0	0	-1	#
0	0	1	0	#
0	0	1	1	#
0	0	1	-1	#
0	0	-1	0	#
0	0	-1	1	#
0	0	-1	-1	#
0	1	0	0	#
…	…	…	…	…

Summarizing Profiles: Stratified

HIGH	LOW	RS4:11	SEM-K2
0	0	#	#
0	1	#	#
0	-1	#	#
1	0	#	#
1	1	#	#
1	-1	#	#
-1	0	#	#
-1	1	#	#
-1	-1	#	#

P-value Combination

- For each contrast, p-values of individual genes are combined to obtain a single p-value for each gene set.
- Fisher's Method
  - Alternative Hypothesis: at least one gene is significant
  - Not symmetric
- Stouffer's Method
  - Alternative Hypothesis: consensus of significance
  - Symmetric
- Whitlock (2005) showed that Stouffer's method is more powerful for the more meaningful alternative of consensus (see slide 3; since even 1 inactive gene may "disturb" a biological process, consensus is most appropriate)
- Symmetry preserves interest in directionality
  - i.e. if the result of combining p_1 and p_2 is p_{12}, then the result of combining $(1- p_1)$ and $(1- p_2)$ should be $(1- p_{12})$

Multiple Hypothesis Testing

- Multiple hypothesis tests are performed on each gene set
  - Each test is another chance to make an error
  - Motivating example has 12,260 gene sets (x 4 contrasts)
  - Some adjustment must be made
- What to control?
  - Methods that control family wise error rate are often too conservative (but more confirmatory)
  - Controlling False Discovery Rate (FDR) is more powerful (but more exploratory)
- Benjamini – Yekutieli adjustment is used to control FDR
  - P-values of gene sets are dependent because genes are in multiple sets (all genes in child sets are also in the parent sets)
  - Dependency structure is unknown
  - Benjamini – Yekutieli adjustment allows for any dependency structure

profileTable

- Takes matrix of p-values, vector of gene names, and vector of contrasts and produces desired profile summaries (slides 7 and 8)
- Necessary arguments:
  - *gene.names*: a vector of gene names where the i^{th} gene name corresponds to the i^{th} row of *pvals*
  - *contrasts*: a character vector of contrasts tested; must be in one of the forms: *Var1* or *Var1.Var2* (stratified). The j^{th} contrast corresponds to the j^{th} column of *pvals*
  - *pvals*: a matrix of p-values (row = gene, column = contrast)

Non-Stratified Output

RS4Low	RS4High	SEMK2Low	SEMK2High	BP
0	0	0	0	10714
0	0	0	1	340
1	0	0	1	191
0	0	1	1	159
1	0	0	0	149
1	1	0	1	114
1	1	1	1	106
1	0	1	1	92
0	0	1	0	71
0	0	0	-1	68

Stratified Output

Low	High	RS4	SEMK2
0	0	11398	10970
0	1	18	647
1	1	251	370
1	0	432	88
0	-1	53	130
-1	-1	75	48
-1	0	33	7

Gene Name Translation

- Depending on naming system used, gene names may need to be translated to Entrez
- Differences in naming systems cause one-to-many and many-to-one problems
- Options in *profileTable:*
  - method 1: ignore problem
  - method 2: Stouffer combine p-values in many-to-one
  - method 3: arbitrarily ignore all but one of one-to-many
  - method 4: use method 2, then method 3

pickOut

- Returns a vector containing the ID's of gene sets that fit a specified profile
- Necessary Arguments:
  - *mvgst*: a mvGST object as returned by *profileTable*
  - *row:* the row number of the desired profile in the table returned by *profileTable*

Non-Stratified Output

```
> gene.sets <- pickOut(example2a, 15, 1)

 [1] "GO:0001510" "GO:0006400" "GO:0006417" "GO:0006431" "GO:0006437"
 [6] "GO:0006564" "GO:0006839" "GO:0007005" "GO:0008614" "GO:0008615"
[11] "GO:0009225" "GO:0009396" "GO:0009451" "GO:0031247" "GO:0032543"
[16] "GO:0034975" "GO:0042819" "GO:0055129" "GO:0071301" "GO:0071494"
```

Stratified Output

```
> gene.sets <- pickOut(example2b, 7, 2)

[1] "GO:0006813" "GO:0043266" "GO:0043268" "GO:0048745" "GO:0051481"
[6] "GO:0071526" "GO:0090075"
```

go2Profile

- Given a specific gene set(s), find its profile
- Returns a table, or list of tables, that are similar to the table from *profileTable* except that only one gene set is included
- Necessary Arguments:
  - *names*: a character vector with the names, or ID's, of the gene sets of interest
  - *object*: a mvGST object as returned by *profileTable*

Non-stratified Output

```
> profiles <- go2Profile(c("GO:0001510", "GO:0006171"), example2a)

  $`GO:0001510`
   RS4Low  RS4High  SEMK2Low  SEMK2High  BP
   -1      -1        0         0          1

  $`GO:0006171`
   RS4Low  RS4High  SEMK2Low  SEMK2High  BP
    1       1        0         0          1
```

Stratified Output

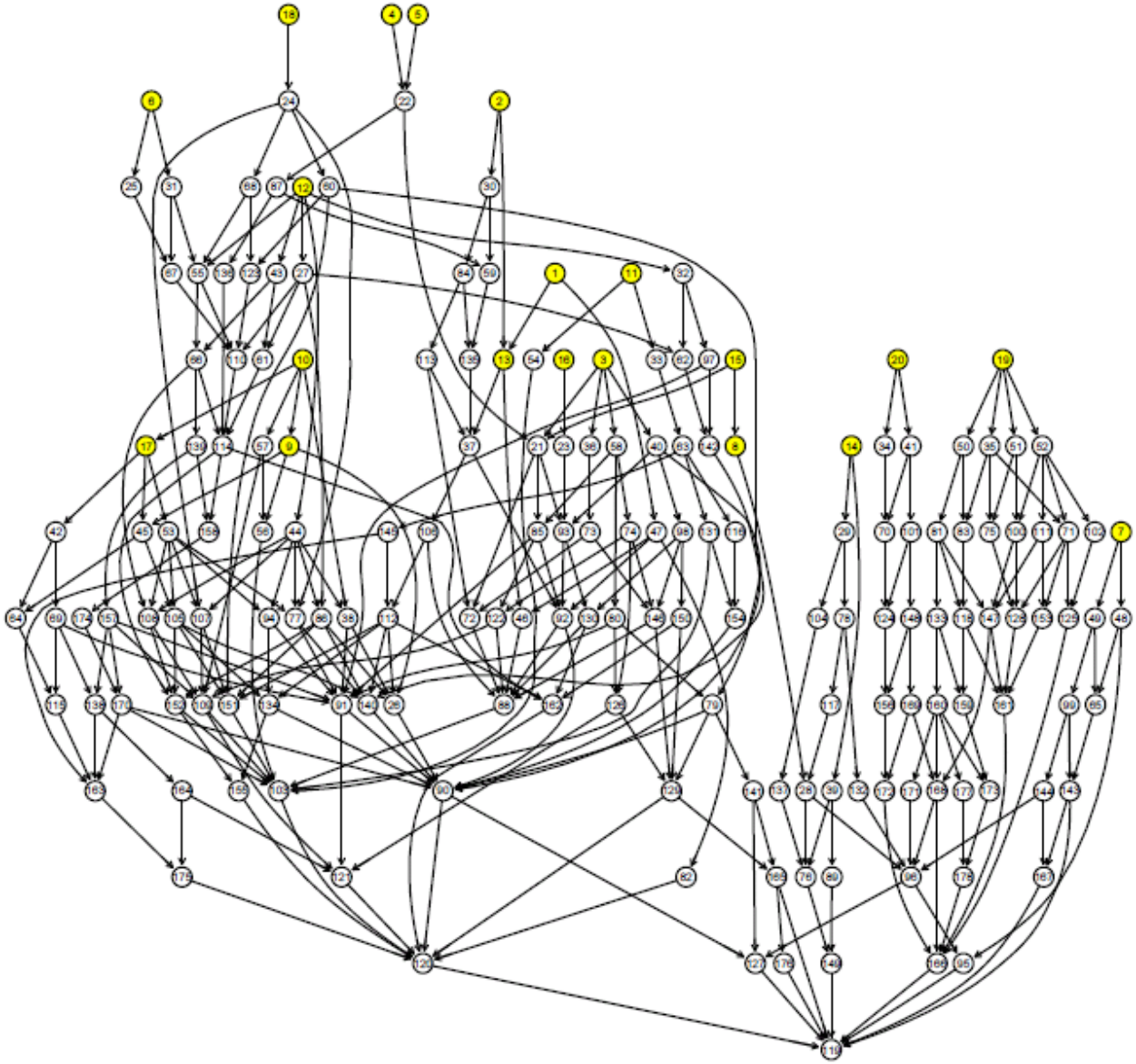
```
> profiles <- go2Profile(c("GO:0006813"), example2b)

  $`GO:0006813`
   Low  High  RS4    SEMK2
    0    0     1      0
   -1    0     0      1
```

graphCell

- Displays a GO graph of the gene sets that fit a specified profile and their parent sets
- Necessary Arguments:
  - *object*: a mvGST object as returned by *profileTable*
  - *row:* the row number of the desired profile in the table returned by *profileTable*

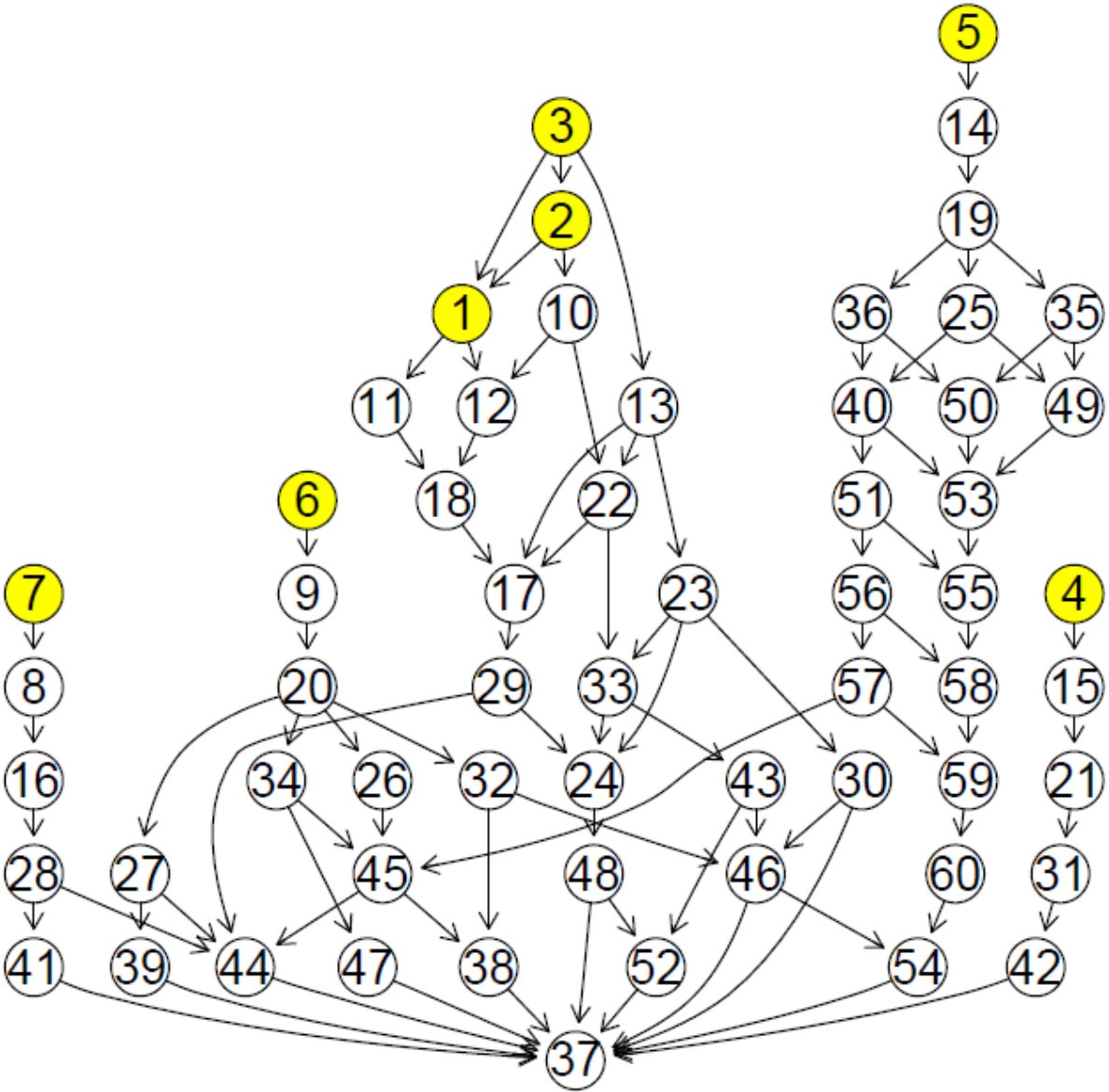
Non-Stratified Output

```
> graphCell(example2a, 15, 1, interact = FALSE, print.legend = FALSE)
```



Stratified Output

```
> graphCell(example2b, 7, 2, interact = FALSE, print.legend = FALSE)
```



Demonstration on Motivating Example

```
library(mvGST)
data2 <- read.csv("C:/examples/second.csv")
gene.names2 <- as.character(data2[, 1])
contrasts2a <- c(“RS4Low”, “RS4High”, “SEMK2Low”,
  “SEMK2High”)
contrasts2b <- c(“Low.RS4”, “High.RS4”, “Low.SEMK2”,
  “High.SEMK2”)
pvals2 <- as.matrix(data2[, 2:5])
chip <- "hgu133plus2“

example2a <- profileTable(gene.names2, contrasts2a,
  pvals2, gene.ID ="affy", organism = "hsapiens",
  ontology = "BP", affy.chip = "hgu133plus2")
gene.sets <- pickOut(example2a, 15, 1)
```

Demo continued

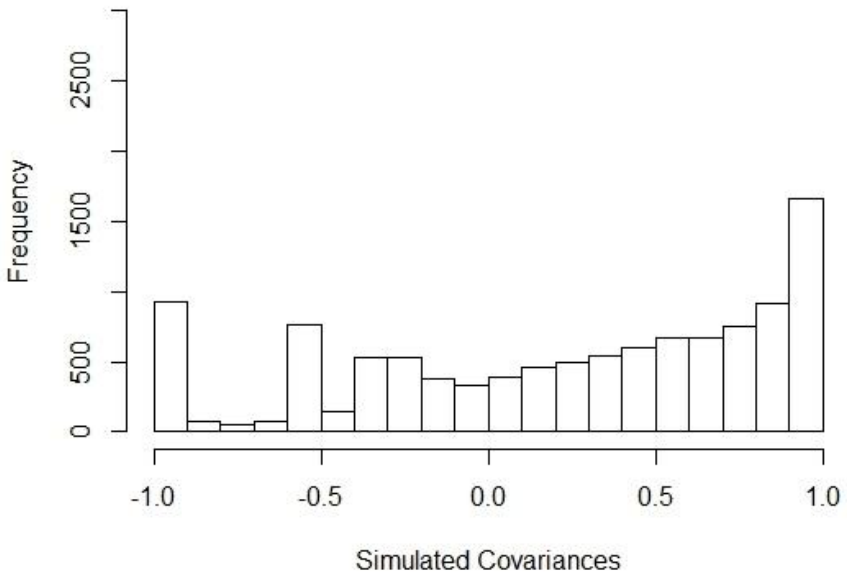
```
graphCell(example2a, 15, 1, interact = FALSE,
  print.legend = FALSE)
profiles <- go2Profile(c("GO:0001510",
  "GO:0006171"), example2a)

example2b <- profileTable(gene.names2,
  contrasts2b, pvals2, gene.ID ="affy",
  organism = "hsapiens", ontology = "BP",
  affy.chip = "hgu133plus2")
gene.sets <- pickOut(example2b, 7, 2)
graphCell(example2b, 7, 2, interact = FALSE,
  print.legend = FALSE)
profiles <- go2Profile(c("GO:0006813"),
  example2b)
```

Hartung versus Stouffer

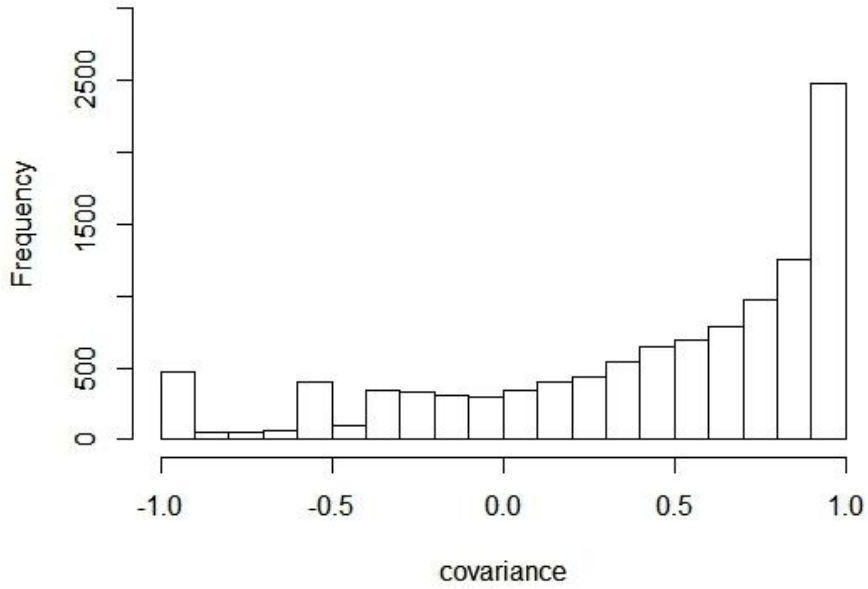
- When many gene names are translated to one gene name, the expression levels of the many genes may be biologically dependent.
  - If the “many” really are separate genes, they are expressed independently
  - If the “many” really are parts of the same gene, they may not be expressed independently
- Stouffer’s p-value combination method may overstate significance when p-values from biologically dependent “genes” are combined
- Hartung’s p-value combination method accounts for dependent p-values
  - Hartung’s assumes positive, constant covariance (between p-values), but is robust to non-constant covariance

- Covariance estimates come from the formula used in Hartung's method
- Observed covariance estimates do not differ greatly from covariance estimates of simulated independent p-values
- No clear evidence that Hartung's method is necessary

Covariances from Simulated Independent P-values



Covariances from Observed P-values



Recent Additions

- Add Short Focus Level adjustment as an option instead of Benjamini-Yekutieli adjustment
- Short Focus Level adjustment controls family-wise error rate while accounting for GO graph structure (Saunders 2014 dissertation)
- A few other minor modifications to improve usability (version 1.1)

Utility of mvGST Package

- Platform independent (Affymetrix, Next Gen Seq, …)
- Design independent
- Controls for other factors
- Multivariate Summary
- Directional interest preserved

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