Microarray Data Analysis

Table of Contents

In this tutorial, we will analyze microarray data available from GEO repository hosted at NCBI (http://www.ncbi.nlm.nih.gov/geo/). We will rely on Matlab's Bioinformatics Toolbox for some of the file parsing and data analysis functionality.

We'll analyze the experimental data that was used in the following study: "A stromal gene signature associated with inflammatory breast cancer.", Boersma et.al., 2008. https://www.ncbi.nlm.nih.gov/pubmed/17999412

Abstract: The factors that determine whether a breast carcinoma will develop into inflammatory breast cancer (IBC) remain poorly understood. Recent evidence indicates that the tumor stroma influences cancer phenotypes. We tested the hypotheses that the gene expression signature of the tumor stroma is a distinctive feature of IBC. We used laser capture microdissection to obtain enriched populations of tumor epithelial cells and adjacent stromal cells from 15 patients with IBC and 35 patients with invasive, noninflammatory breast cancer (non-IBC). Their mRNA expression profiles were assessed using Affymetrix GeneChips. In addition, a previously established classifier for IBC was evaluated for the resulting data sets. The gene expression profile of the tumor stroma distinguished IBC from non-IBC, and a previously established IBC prediction signature performed better in classifying IBC using the gene expression profile of the tumor stroma than it did using the profile of the tumor epithelium. In a pathway analysis, the genes differentially expressed between IBC and non-IBC tumors clustered in distinct pathways. We identified multiple pathways related to the endoplasmic stress response that could be functionally significant in IBC. Our findings suggest that the gene expression in the tumor stroma may play a role in determining the IBC phenotype.

The data is available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5847

GEO data types

- GSM: An individual microarray Sample (e.g., patient, or tissue).
- GSE: A Series, representiing an experimental study. A GSE contains one or more GSM entries.

- GPL: Platform data, containing information about microarray probes. Each GSM sample is associated with a GPL. E.g., to find out the gene symbols for a GSM, you would need to consult with the GPL used in that study.

Variable names and terms

- gse: The struct returned from Matlab's geoseriesread(). Contains all the information contained in a GSE.
- d: The DataMatrix object representing the GSE data. We'll also use the prefix d to denote other DataMatrix objects, e.g., dpvals.
- m: A numerical matrix (e.g., converting d into a double).
- MAP_GSE_GPL: used to map gse probes to gpl indices, so we can get e.g., gene symbol information for a probe. IBC: inflammatory breast cancer Epithelial: the type of cells forming the breast cancer Stroma: fatty tissue (adipocytes) and fibroblasts

```
if true
```

Download GSE file and use Matlab's geoseriesread() to parse it.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5847 See the bmes_downloadandparsegse() function for details.

```
gse=bmes_downloadandparsegse('GSE5847')

% gse.Data is a DataMatrix object, similar to a Matlab table. In
 addition
% to the numerical matrix, it internally stores the row & column
 names.
get(gse.Data)

d = gse.Data; %Let's use d for short, because we'll use this often.
```

```
gse =

  struct with fields:

    Header: [1×1 struct]
      Data: [22283×95 bioma.data.DataMatrix]

            Name: ''
        RowNames: {22283×1 cell}
        ColNames: {1×95 cell}
           NRows: 22283
           NCols: 95
           NDims: 2
    ElementClass: 'double'
```

Print first 6 Probes and first 5 samples

```
d(1:6,1:5)
```

```
ans =

                 GSM136326    GSM136327    GSM136328    GSM136329
 GSM136330
    1007_s_at     10.45        9.3995       9.4248       9.4729
 9.2788
    1053_at      5.7195       4.8493       4.7321       4.7289
 5.3264
    117_at       5.9387       6.0833        6.448       6.1769
 6.5446
    121_at       8.0231       7.8947        8.345       8.1632
 8.2338
    1255_g_at    3.9548       3.9632       3.9641       4.0878
 3.9989
    1294_at       7.909        8.364       8.2719       8.3582
  7.7
```

GSE Header information

```
gse.Header
```

```
ans =

  struct with fields:

     Series: [1×1 struct]
    Samples: [1×1 struct]
```

```
gse.Header.Series
```

```
ans =

  struct with fields:

                          title: 'Tumor and stroma from breast by LCM'
                  geo_accession: 'GSE5847'
                         status: 'Public on Sep 30 2007'
                submission_date: 'Sep 15 2006'
               last_update_date: 'Aug 10 2018'
                      pubmed_id: '17999412#19225562'
                        summary: 'Tumor epithelium and surrounding
 stromal cells were isolated using laser capture microdissection
 of human breast cancer to examine differences in gene expression
 based on tissue types from inflammatory and non-inflammatory breast
 cancer#Keywords: LCM'
                 overall_design: 'We applied LCM to obtain samples
 enriched in tumor epithelium and stroma from 15 IBC and 35 non-IBC
```

```
 cases to study the relative contribution of each component to the IBC
 phenotype and to patient survival. '
                          type: 'Expression profiling by array'
                   contributor:
 'Stefan,,Ambs#Brenda,,Boersma#Mark,,Reimers'
                     sample_id: 'GSM136326 GSM136327 GSM136328
 GSM136329 GSM136330 GSM136331 GSM136332 GSM136333 GSM136334 GSM136335
 GSM136336 GSM136337 GSM136338 GSM136339 GSM136340 GSM136341 GSM136342
 GSM136343 GSM136344 GSM136345 GSM136346 GSM136347 GSM136348 GSM136349
 GSM136350 GSM136351 GSM136352 GSM136353 GSM136354 GSM136355 GSM136356
 GSM136357 GSM136358 GSM136359 GSM136360 GSM136361 GSM136362 GSM136363
 GSM136364 GSM136365 GSM136366 GSM136367 GSM136368 GSM136369 GSM136370
 GSM136371 GSM136372 GSM136373 GSM136374 GSM136375 GSM136376 GSM136377
 GSM136378 GSM136379 GSM136380 GSM136381 GSM136382 GSM136383 GSM136384
 GSM136385 GSM136386 GSM136387 GSM136388 GSM136389 GSM136390 GSM136391
 GSM136392 GSM136393 GSM136394 GSM136395 GSM136396 GSM136397 GSM136398
 GSM136399 GSM136400 GSM136401 GSM136402 GSM136403 GSM136404 GSM136405
 GSM136406 GSM136407 GSM136408 GSM136409 GSM136410 GSM136411 GSM136412
 GSM136413 GSM136414 GSM136415 GSM136416 GSM136417 GSM136418 GSM136419
 GSM136420 '
                  contact_name: 'Stefan,,Ambs'
            contact_laboratory: 'LHC'
             contact_institute: 'NCI'
               contact_address: '37 Convent Dr Bldg 37 Room 3050'
                  contact_city: 'Bethesda'
                 contact_state: 'MD'
    contact_zip0x2Fpostal_code: '20892'
               contact_country: 'USA'
            supplementary_file: 'ftp://ftp.ncbi.nlm.nih.gov/geo/
series/GSE5nnn/GSE5847/suppl/GSE5847_RAW.tar'
                   platform_id: 'GPL96'
               platform_taxid: '9606'
                  sample_taxid: '9606'
                      relation: 'BioProject: https://
www.ncbi.nlm.nih.gov/bioproject/PRJNA97251'
```

Get the title of the first sample

```
gse.Header.Samples.title{1}
```

```
ans =

    'LCM stroma sample from patient #37'
```

Print all of the available characteristics of the 20th sample

```
gse.Header.Samples.characteristics_ch1(:,20)
```

```
ans =

  7×1 cell array
```

```
    {'diagnosis: non-IBC'     }
    {'status: Alive'          }
    {'patient_id: 8'          }
    {'race: European American'}
    {'tnm_stage: IIA'         }
    {'er_status: POS'         }
    {'tissue: Stroma'         }
```

Print the diagnostic information of all samples

```
gse.Header.Samples.characteristics_ch1(1,:)
```

```
ans =

  1×95 cell array

  Columns 1 through 3

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 4 through 6

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 7 through 9

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 10 through 12

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 13 through 15

    {'diagnosis: IBC'}    {'diagnosis: non-…'}    {'diagnosis: non-…'}

  Columns 16 through 18

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 19 through 21

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 22 through 24

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 25 through 27
```

```
    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 28 through 30

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 31 through 33

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 34 through 36

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 37 through 39

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 40 through 42

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 43 through 45

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-
…'}

  Columns 46 through 48

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: IBC'}

  Columns 49 through 51

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 52 through 54

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 55 through 57

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}

  Columns 58 through 60

    {'diagnosis: IBC'}    {'diagnosis: IBC'}    {'diagnosis: IBC'}
```

```
  Columns 61 through 63

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-…'}

  Columns 64 through 66

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-…'}

  Columns 67 through 69

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-…'}

  Columns 70 through 72

    {'diagnosis: non-…'}    {'diagnosis: non-…'}    {'diagnosis: non-…'}
...
```

GPL Platform used in this study

```
gse.Header.Series.platform_id
```

```
ans =

    'GPL96'
```

GPL information is contained in a separate file. Download and parse it. See bmes_downloadandparsegpl().

```
gpl = bmes_downloadandparsegpl('GPL96')
```

```
gpl =

  struct with fields:

                 Scope: 'PLATFORM'
             Accession: 'GPL96'
                Header: [1×1 struct]
    ColumnDescriptions: {16×1 cell}
           ColumnNames: {16×1 cell}
                  Data: {22283×16 cell}
```

Here's the type of information we get for each microarray Probe.

```
gpl.ColumnNames
```

```
ans =
```

```
  16×1 cell array

    {'ID'                                 }
    {'GB_ACC'                             }
    {'SPOT_ID'                            }
    {'Species Scientific Name'            }
    {'Annotation Date'                    }
    {'Sequence Type'                      }
    {'Sequence Source'                    }
    {'Target Description'                 }
    {'Representative Public ID'           }
    {'Gene Title'                         }
    {'Gene Symbol'                        }
    {'ENTREZ_GENE_ID'                     }
    {'RefSeq Transcript ID'               }
    {'Gene Ontology Biological Process'}
    {'Gene Ontology Cellular Component'}
    {'Gene Ontology Molecular Function'}
```

```
%Let's look at the information for the first probe:
[gpl.ColumnNames gpl.Data(1,:)']
```

```
ans =

  16×2 cell array

    {'ID'                                }    {'1007_s_at'
       }
    {'GB_ACC'                            }    {'U48705'
       }
    {'SPOT_ID'                           }    {1×0 char
       }
    {'Species Scientific Name'           }    {'Homo sapiens'
       }
    {'Annotation Date'                   }    {'Oct 6, 2014'
       }
    {'Sequence Type'                     }    {'Exemplar sequence'
       }
    {'Sequence Source'                   }    {'Affymetrix Proprietary
 Database'}
    {'Target Description'                }    {'U48705 /FEATURE=mRNA /
DEFINIT…'}
    {'Representative Public ID'          }    {'U48705'
       }
    {'Gene Title'                        }    {'discoidin domain receptor
 tyr…'}
    {'Gene Symbol'                       }    {'DDR1 /// MIR4640'
       }
    {'ENTREZ_GENE_ID'                    }    {'780 /// 100616237'
       }
    {'RefSeq Transcript ID'              }    {'NM_001202521 ///
 NM_001202522…'}
```

```
   {'Gene Ontology Biological Proc…'}    {'0001558 // regulation of
cell…'}
   {'Gene Ontology Cellular Compon…'}    {'0005576 // extracellular
regi…'}
   {'Gene Ontology Molecular Funct…'}    {'0000166 // nucleotide
binding…'}
```

Translate probesets to gene symbols

```
gplprobes = gpl.Data(:, strcmp(gpl.ColumnNames, 'ID'));
gplgenes  = gpl.Data(:, strcmp(gpl.ColumnNames, 'Gene Symbol'));
gseprobes = d.rownames;
MAP_GSE_GPL = zeros(numel(gseprobes),1);

% For each gseprobe, we need to search gplprobes and use the
 corresponding
% gene. Doing string comparison for each of them will be too slow.
 Let's
% use a Map container to speed this up.
map = containers.Map(gplprobes,1:numel(gplprobes));
for i=1:numel(gseprobes)
 if map.isKey(gseprobes{i}); MAP_GSE_GPL(i)=map(gseprobes{i}); end
end

gsegenes = gseprobes; %make a copy, so entries not found will keep the
 probe name.
gsegenes(find(MAP_GSE_GPL)) =
 gplgenes(MAP_GSE_GPL(find(MAP_GSE_GPL)));

% Print first 5 probes and the gene symbols we found for them. We
 convert
% to table() just because we like how it prints.
table(gseprobes(1:5), gsegenes(1:5),'VariableNames',
{'gseprobe','gsegene'})


ans =

  5×2 table

     gseprobe            gsegene
    ___________    __________________

    '1007_s_at'    'DDR1 /// MIR4640'
    '1053_at'      'RFC2'
    '117_at'       'HSPA6'
    '121_at'       'PAX8'
    '1255_g_at'    'GUCA1A'
```

TODO: the above code is useful. Think about generalizing it to a function that takes gpl structure and gseprobes and returns any GPL column (e.g., genesymbol, refseq id, etc.) you need.

Let's replace the DataMatrix object so it uses genes as rownames. we would've liked to say "d.rownames=gsegenes", but datamatrix doesn't support that.

```
d = d.rownames(':',gsegenes);
```

print a random selection of rows and columns.

```
d( randi(d.size(1),1,6), randi(d.size(2),1,4))


ans =

                         GSM136406    GSM136341    GSM136406
 GSM136411
   EPN2 /// EPN2-IT1     6.9187       6.9944       6.9187       6.8776

   TPRKB                 7.4003       6.0686       7.4003       7.4459

   ATPAF2                5.1673       4.9552       5.1673       5.2271

   LGALS9                6.5531       6.6602       6.5531       6.8161

   LRIT1                 4.4198       4.2597       4.4198       4.6669

   ZNF444                7.0293       6.1526       7.0293       7.0737
```

Data Analysis: Determine sample groups we'll work with

We are often interested in comparing groups of samples. We need to look at the header information and decide which information for samples we can use to group them. For this experiment, we'll work with four groups, using IBC vs. non-IBC and stroma vs. epithelial characteristics. Where one finds the sample information is experiment-specific, but the Header.Samples structure usually contains what we need.

first characteristics_ch1 contains diagnosis info (IBC vs. non-IBC)

```
samplegroups = gse.Header.Samples.characteristics_ch1(1,:);
unique(samplegroups)'


ans =

  2×1 cell array

    {'diagnosis: IBC'    }
    {'diagnosis: non-IBC'}
```

source_name_ch1 contains tissue source info (stroma vs. epithelium)

```
samplesources = gse.Header.Samples.source_name_ch1;
unique(samplesources)'
```

```
ans =

  2×1 cell array

    {'human breast cancer stroma'         }
    {'human breast cancer tumor epithelium'}
```

create logical vectors to record which samples are IBC and which are stroma.

```
Iibc = strcmp(samplegroups,'diagnosis: IBC');
Istroma = strcmpi(samplesources, 'human breast cancer stroma');
Istroma_ibc = Istroma & Iibc;
Istroma_nonibc = Istroma & ~Iibc;
Iepi_ibc = ~Istroma & Iibc;
Iepi_nonibc = ~Istroma & ~Iibc;

% create a numerical vector to assign each sample to a group 1-4.
Igroups=zeros(1,numel(samplegroups));
Igroups(Istroma_ibc) = 1;
Igroups(Istroma_nonibc) = 2;
Igroups(Iepi_ibc) = 3;
Igroups(Iepi_nonibc) = 4;
groupnames={'sIBC' 's~IBC' 'eIBC' 'e~IBC'};

% let's change the columnnames in the datamatrix.
colnames=d.colnames;
for i=1:4; colnames(Igroups==i) = groupnames(i); end
d=d.colnames(':',colnames); %this really means: "d.colnames=colnames;"
```

print a random selection of rows and columns.

```
d( randi(d.size(1),1,6), randi(d.size(2),1,5))

%-------------------------------------------------------------------------


ans =

                sIBC      eIBC      eIBC      eIBC      sIBC
    TOR1AIP1    4.3469    4.5923    4.2612    4.5647    4.0404
    NAGA        7.6311    7.0032    8.4971    7.4084     6.917
    SMARCE1     9.3071    8.8959    8.6445    9.7433    8.0707
    EID1        8.2163    6.3046    8.2479    7.7487    7.3583
    TRIM27      7.9769    8.4402    8.1486    7.9938    7.8324
    RBPJ        7.5769    8.2995    9.1816    7.0899    7.9115
```

Data Analysis: Find differentially expressed genes between groups of samples.

Let's find the significantly differentially expressed genes between IBC and non-IBC samples, in stroma samples. (We are not using the epithelial samples in this analysis.)

```
[~,pvals]=ttest2(d(:,Istroma_ibc)', d(:, Istroma_nonibc)');
fpvals = mafdr(pvals);
% TODO: create dpvals from fpvals.
```

Matlab also offers mattest() function, which can use permutation tests for false discovery rate correction. It takes a longer time to compute..

```
[dpvals]=mattest(d(:,Istroma_ibc), d(:,
 Istroma_nonibc), 'permute',10);
```

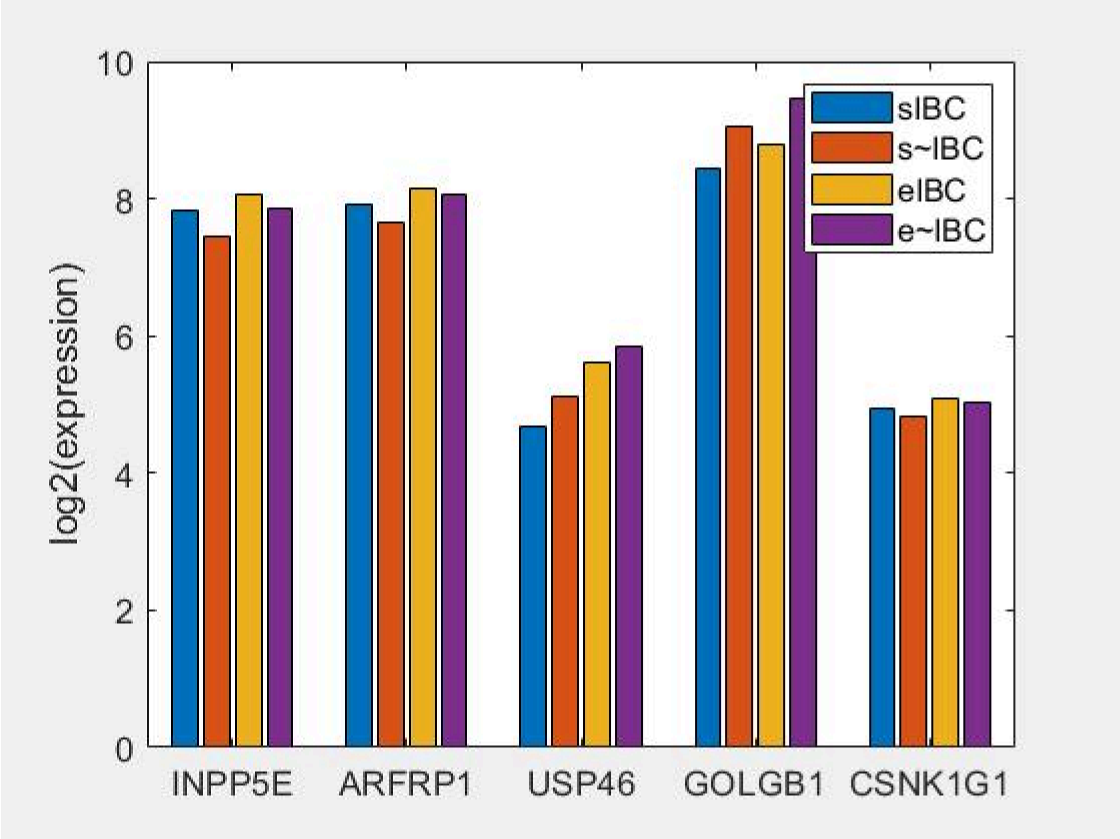
Print the list of top 5 most significantly different genes. (Note that dpvals is a DataMatrix object, so we'll just have it print itself.

```
dpvals_sorted = dpvals.sortrows('p-values');
fprintf('Found %d genes with pvalue<=0.01\n',nnz(dpvals(:,1) <=
 0.01));
fprintf('Top 5 most significantly different genes between IBC and non-
IBC stroma samples:\n');
disp(dpvals_sorted(1:5,:))
```

```
Found 449 genes with pvalue<=0.01
Top 5 most significantly different genes between IBC and non-IBC
 stroma samples:

              p-values
    INPP5E    5.8504e-06
    ARFRP1    7.1949e-06
    USP46     1.4278e-05
    GOLGB1    2.2338e-05
    CSNK1G1   7.4187e-05
```

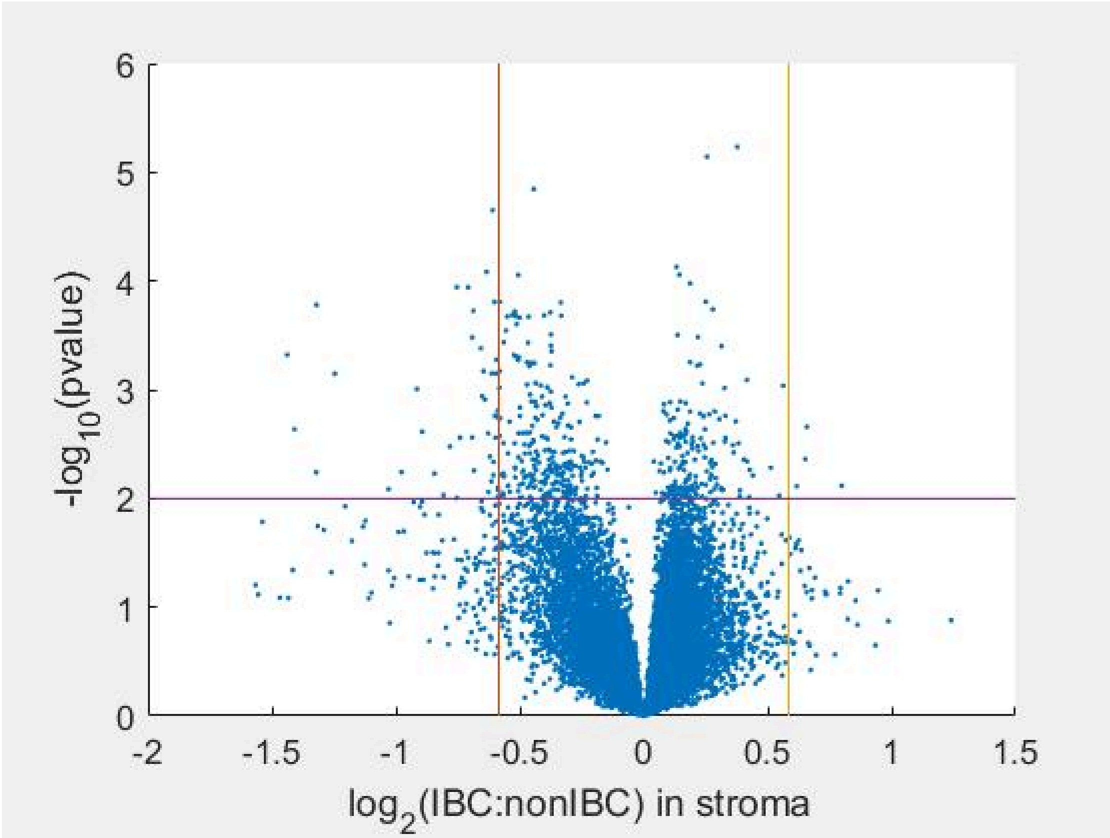
Plot the expression levels of top 5 genes in each group

```
[~,Itop5]=sort(dpvals.double(:,1));
Itop5 = Itop5(1:5);
top5avg = zeros(5,4);
top5std = zeros(5,4);
for gi=1:4
 top5avg(:,gi) = mean(d.double(Itop5, Igroups==gi),2);
 top5std(:,gi) = std(d.double(Itop5, Igroups==gi),[],2);
end
%bmes_fig top5; clf
bar(top5avg);
ylabel('log2(expression)')
h=gca; h.XTickLabel=d.rownames(Itop5);
legend(groupnames);
%bmes_setniceylim;
```



pvalue is good to know, but we usually also want to know the fold changes (How different the average expression is between groups).

```
% Note that this GSE data is already log2-transformed. So, calculation
 of
% fold change should involve a subtraction, not a division!
%log2fc = log2( mean(d(:,Istroma_ibc),2) ./ mean(d(:,
 Istroma_nonibc),2) );
log2fc = mean(d(:,Istroma_ibc),2) - mean(d(:, Istroma_nonibc),2);
scatter(log2fc, -log10(dpvals(:,1)), '.');
xlabel('log_2(IBC:nonIBC) in stroma'), ylabel('-log_{10}(pvalue)');

%mark fc>=1.5 and pvalue<=0.01
hold on;
plot(log2([2/3 2/3]), ylim, log2([1.5 1.5]), ylim)
plot(xlim, -log10([.01 .01]))
```



Print the top 5 genes, now with fold change. log2fc is not easy to interpret, let's convert it to negative fold change. So, a negfc=2 will mean IBC is 2-fold compared to nonIBC and a negfc=-2 will mean IBC is half of nonIBC.

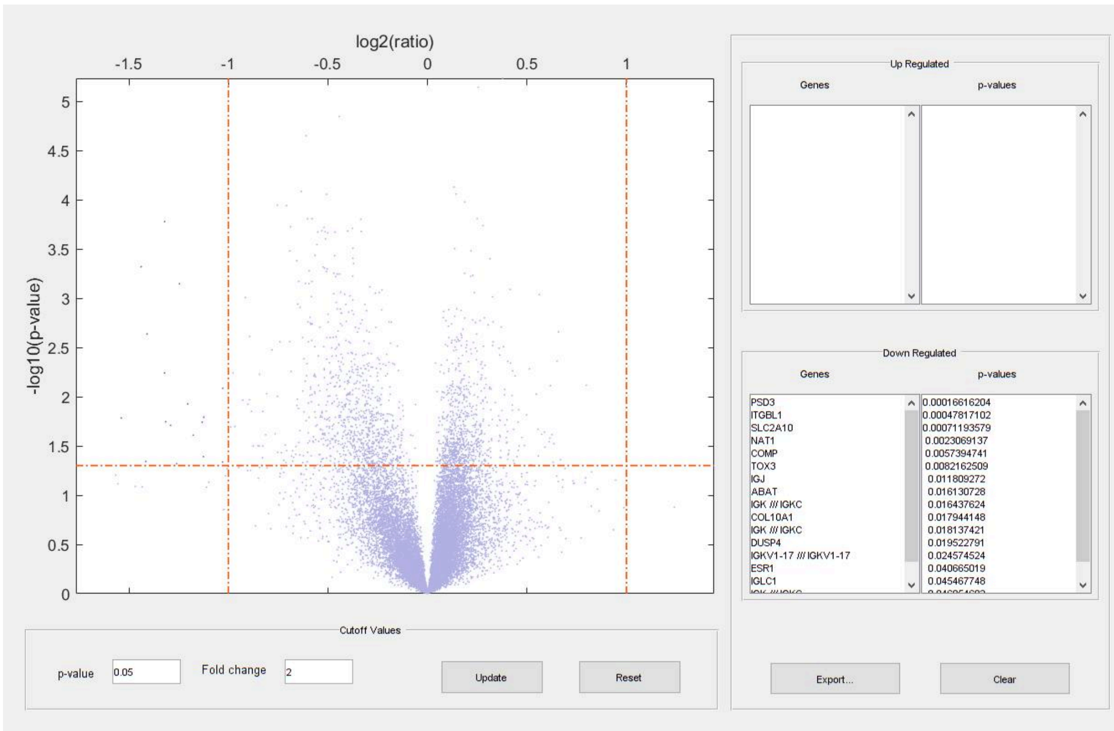
```
negfc = 2.^log2fc;
negfc(negfc<1) = - 1./negfc(negfc<1);

% Add the foldchange information to the dpvals object:
dpvals=[dpvals bioma.data.DataMatrix(negfc,'ColNames',{'negfc'})];
% Select the genes with pvalue<=0.01 and FC>=1.5.
I = dpvals(:,'p-values')<=0.01 & abs(dpvals(:,'negfc'))>=1.5;
dsigfc = dpvals(I,:);
dsigfc = dsigfc.sortrows('p-values');
fprintf('Found %d genes with pvalue<=0.01 and FC>=1.5. Showing top 5:
\n',size(dsigfc,1));
disp(dsigfc(1:5,:))
```

Found 47 genes with pvalue<=0.01 and FC>=1.5. Showing top 5:

	p-values	*negfc*
GOLGB1	*2.2338e-05*	*-1.526*
TTC3 /// TTC3P1	*8.2384e-05*	*-1.5519*
MAGED2	*0.00011381*	*-1.6866*
DNAJB9	*0.00011448*	*-1.6335*
DZIP3	*0.00015583*	*-1.5177*

Matlab has a mavolcanoplot() tool that helps you explore significantly different genes for different pvalue and fold change cutoffs.

```
mavolcanoplot(d.double(:,Istroma_ibc), d.double(:,Istroma_nonibc),
 dpvals.double(:,1),'Labels',d.rownames)
```



Writing data into Excel File

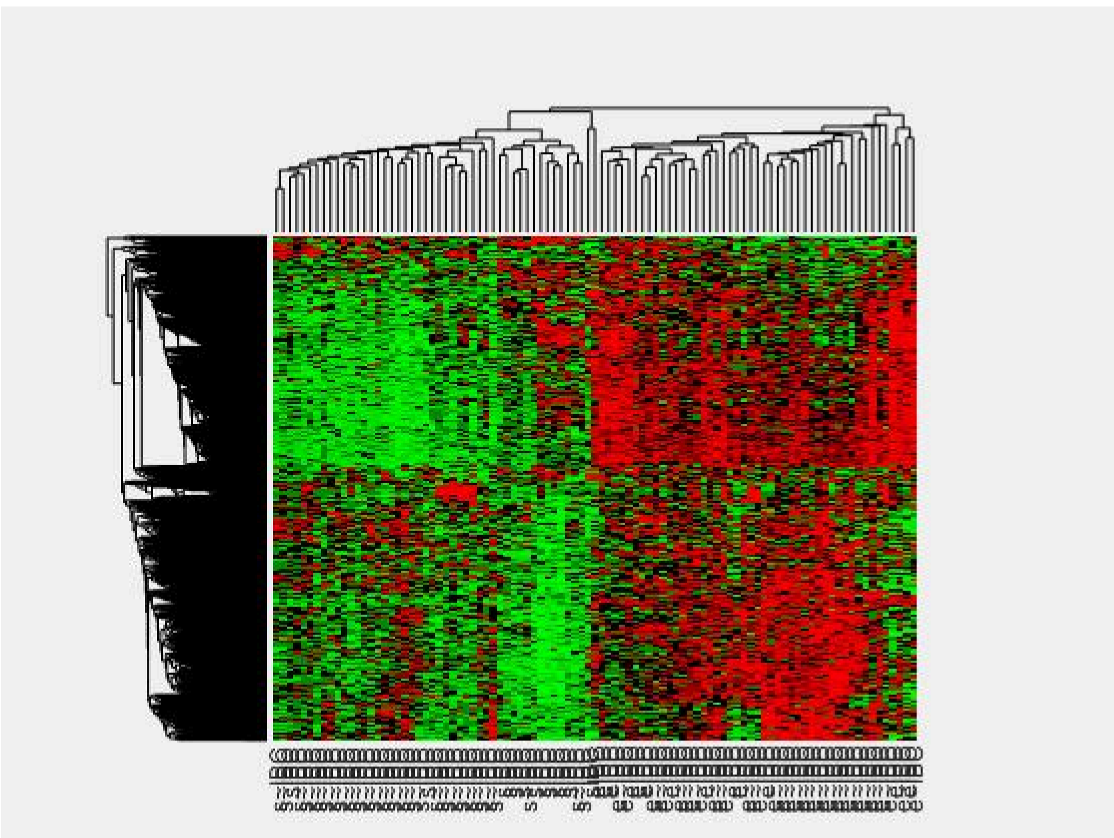
Create a cell array containing the data that you want appear in an Excel sheet.

```
I=find(dpvals(:,1)<=0.01);
nsig=numel(I);
xlsdata = cell(nsig, 3); %each row will contain
 genesymbol,pvalue,negfc
for i=1:nsig
 gene=dpvals.rownames{I(i)};
 p=dpvals.double(I(i), 1);
 nfc=dpvals.double(I(i), 2);
 xlsdata(i,:) = {gene p nfc};
end

xlsdata=[ {'genesymbol' 'pvalue' 'negfc'}; xlsdata]; %add the header
 row.
xlswrite('stromaibc.xlsx',xlsdata,'siggenesIBC_nonIBC');


%-------------------------------------------------------------------------
```

Data Analysis: Hierarchical Clustering

Let's cluster genes and samples using hierarchical clustering. Clustering can be very time consuming, so let's only do it for a subset of the genes.

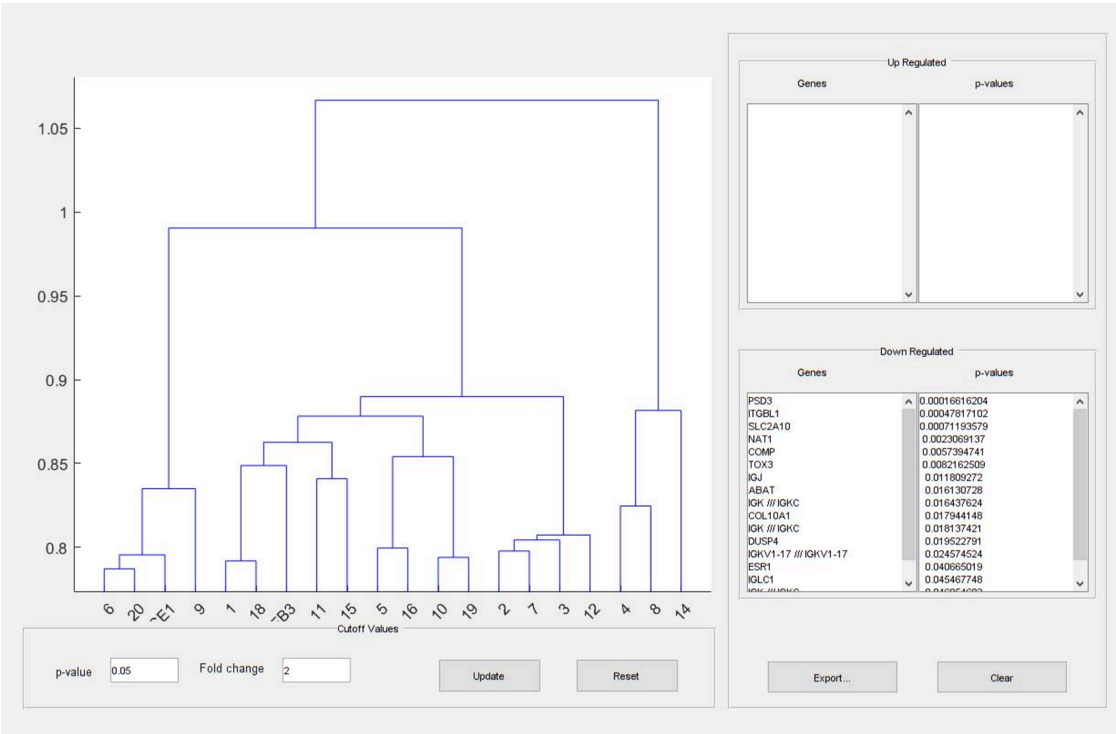
```
% One idea is to only keep the genes that vary most across samples
% (ingoring sample groups.) This can be done using:
I=genevarfilter(d, 'Percentile',99); %remove 99% of genes
d2 = d( I, :);

% Another option is to keep the genes that vary across sample groups. Since
% we already did differential expression analysis above, let's use the genes
% resulting from it. (Note that we only compared IBC vs. nonIBC in stroma;
% one would really need to repeat differential expression analysis for
% other pairs of groups of samples, and use the union of all significant
% genes).
Isig=dpvals(:,'p-values')<=0.05; %let's use 0.05 threshold to get some more genes.
d2 = d(Isig,:);
cg = clustergram(d2,'Standardize','Row');
```



Data Analysis: "Flat" clustering

You can cluster genes (or samples) without a hierarchy. There are different methods for that. You can start off from a hierarchical clustering and "cut" the tree at a certain level to get a flat level of groupings.

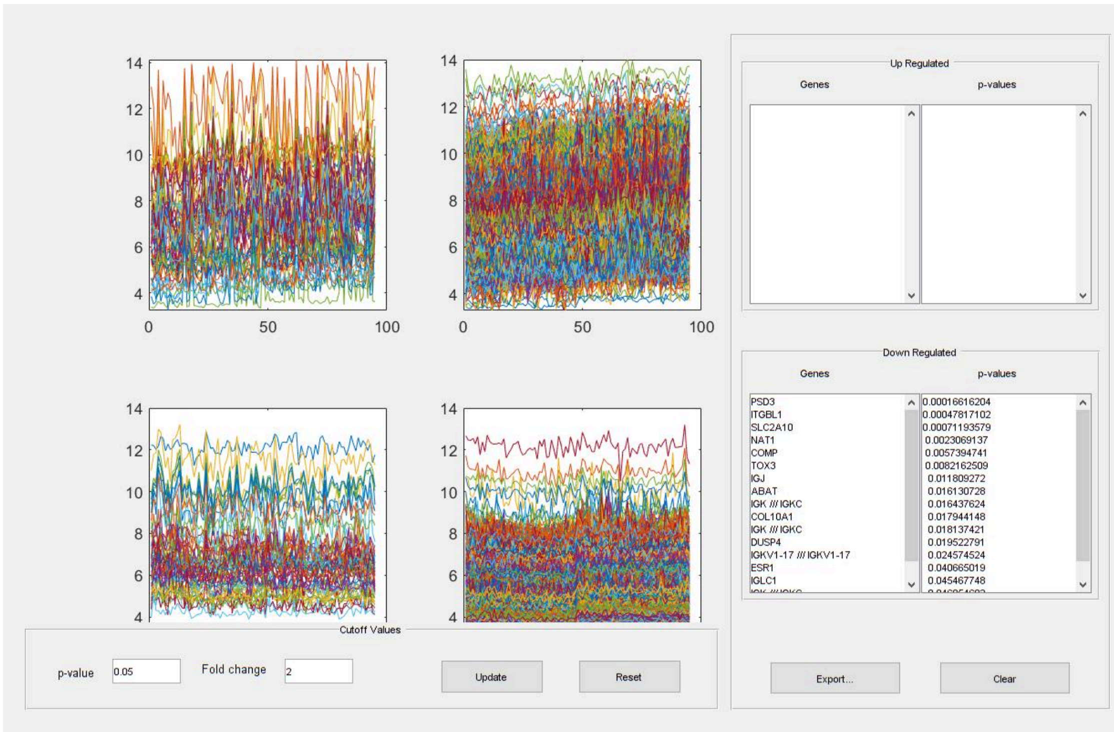
```
% Let's create a distance matrix between pairs of genes.
% pdist() gives a vector (to save space). If you want the symmetric
 matrix,
% just pass the result through squareform().
genedist = pdist(d2,'corr');

% linkage() hierarchically groups the genes. The result contains
% information about which two groups are combined at each branch.
tree = linkage(genedist,'average');

% visualize the tree, show only 20 nodes.
% Groups of genes will have a numerical id for labels.
%bmes_fig geneclust; clf
dendrogram(tree,20,'Labels',d2.rownames);
h=gca;
h.XTickLabelRotation=45;
```



let's now cut the tree to generate 6 clusters. The result is a membership vector, assigning each gene to one of the 6 clusters.

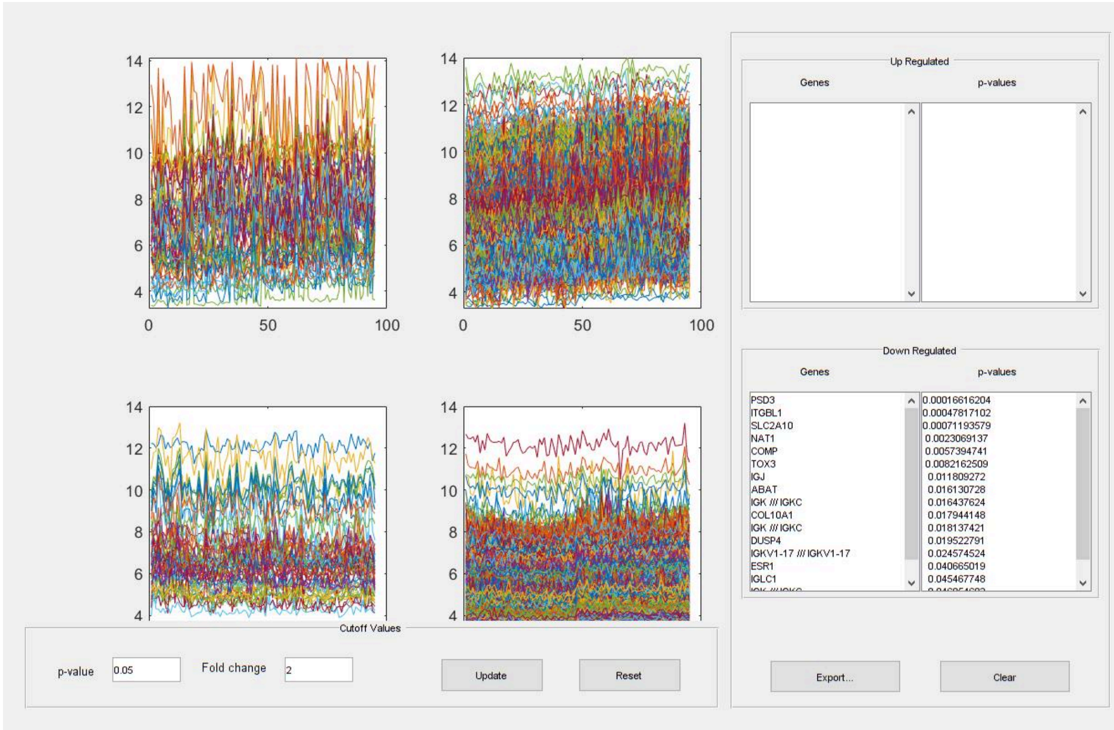
```
Iclust = cluster(tree, 'maxclust', 6);

% Let's plot each group of genes separately.
%bmes_fig geneclust; clf
```

```
for i=1:6
 subplot(2,3, i);
 plot( d2(Iclust==i, :)' );
end
```

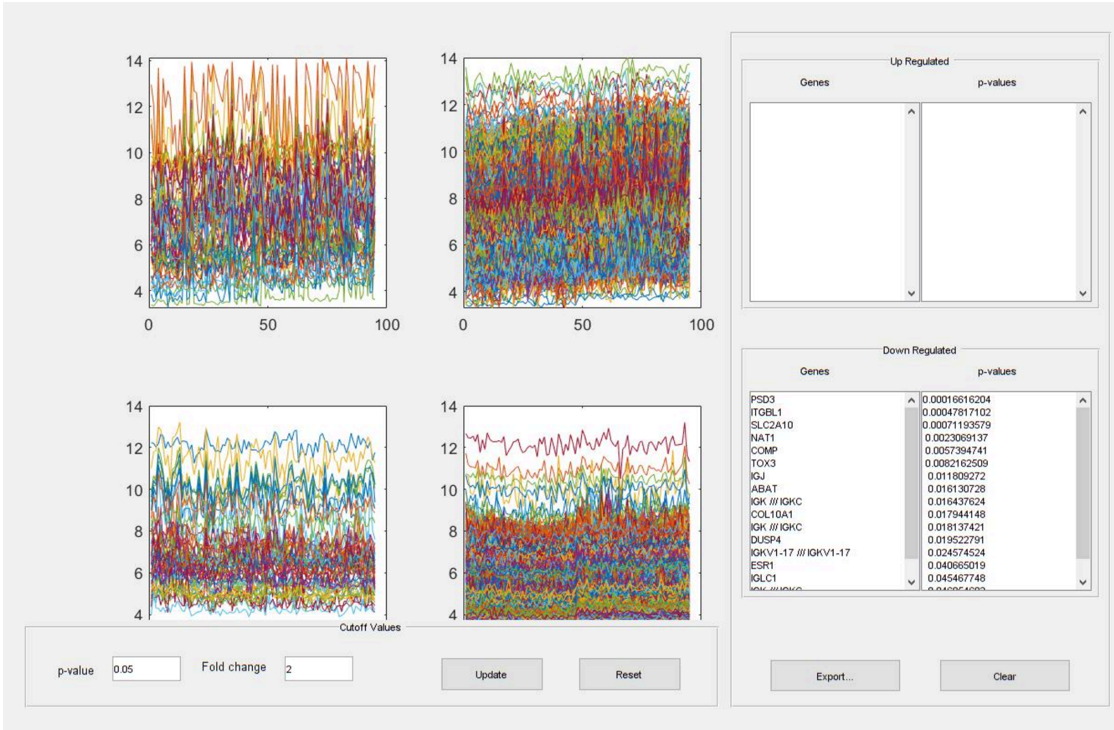


The figure is too crowded. Let's show the average expression for the genes in each cluster.

```
d2avg=zeros(6,size(d2,2));
for i=1:6
 d2avg(i,:) = mean(d2(Iclust==i,:),1);
end
%bmes_fig geneclust; clf
plot(d2avg');
```



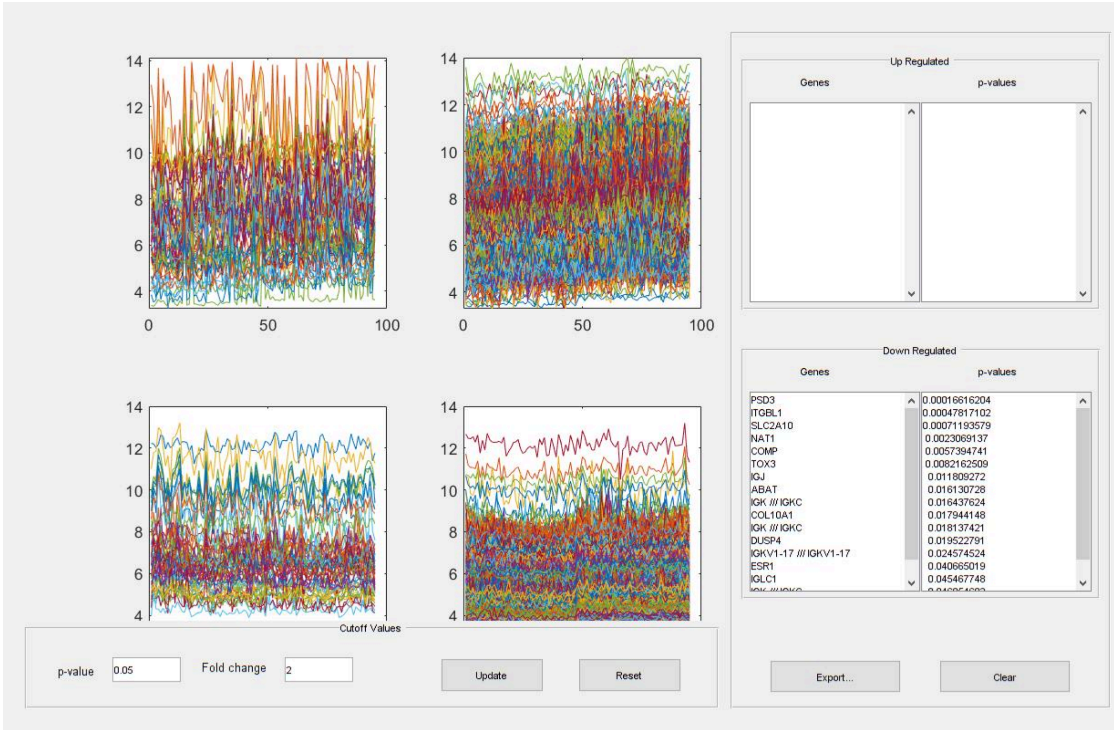

```
end

% If this were a time-series experiment, plotting with x axis
 representing
% time would be informative. However, in this experiment, The x axis
% represents samples, whose ordering doesn't mean much.. Let's instead
% show average values for different groups.
d2avgavg=zeros(6,4);
for i=1:6; for j=1:4
  d2avgavg(i,j) = mean(mean(d2(Iclust==i,Igroups==j)));
end; end
%bmes_fig geneclust; clf
bar(d2avgavg); legend(groupnames);
xlabel('gene clusters');
%bmes_setniceylim;
```



Data-Analysis: k-means clustering

k-means is another method to generate groups of genes.

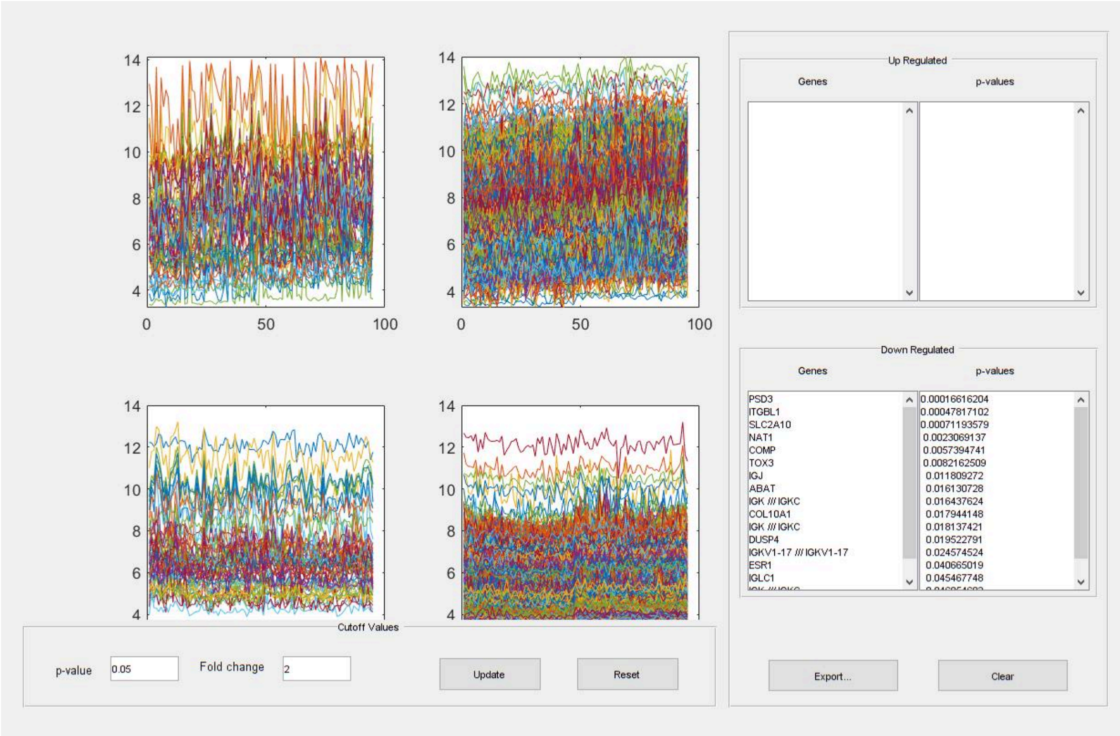
```
Iclust = kmeans(d2, 6, 'dist','corr');

% Once you have the Iclust membership values, you can again generate
% similar plots as above.
d2avgavg=zeros(6,4);
for i=1:6; for j=1:4
  d2avgavg(i,j) = mean(mean(d2(Iclust==i,Igroups==j)));
end; end
%bmes_fig geneclust; clf
bar(d2avgavg); legend(groupnames);
xlabel('gene clusters');
%bmes_setniceylim;
```



Data Analysis: Principal Component Analysis (PCA)

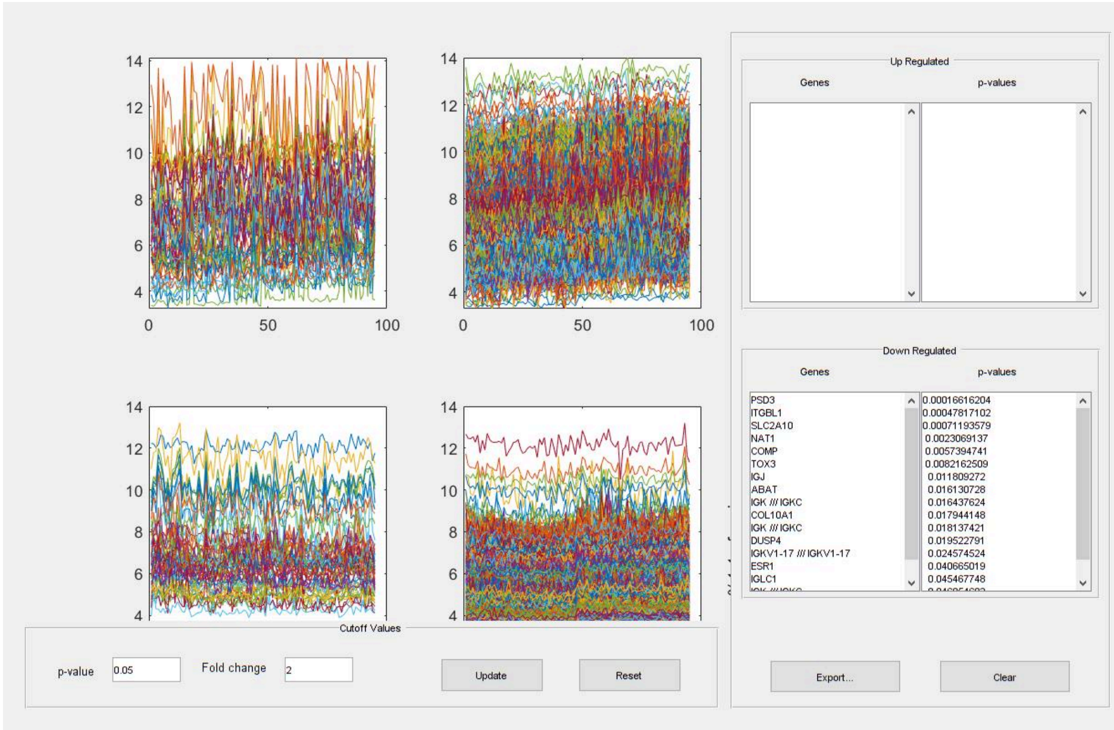
Each gene is a multi-dimensional vector (as many dimensions as the number of samples). We often want to visualize genes in a lower (2D) space. Let's use PCA to reduce data dimensionality while preserving as much of the original information as possible.

```
[pc, reduced, pcvars] = pca(d2);

% reduced has as many columns as the original data. But the first
 column
% now contains the most information, the second column contains the
 second
% most, etc. Let's just use the first two columns to show genes on a
% figure.
%bmes_fig pca; clf
scatter(reduced(:,1), reduced(:,2));
```



We can combine the clustering and PCA and show the genes in different clusters with a different color.

```
%bmes_fig pca; clf
gscatter(reduced(:,1), reduced(:,2), Iclust);

% we can label the points using the text() function. Let's do that for
 the
% first 5 genes.
for i=1:5; text(reduced(i,1),reduced(i,2),
 d2.rownames{i}, 'FontWeight','bold', 'FontSize',13); end

% Whenever you show results of PCA, you should report the amount of
% variance captured by the reduced data.
pcvars = pcvars / sum(pcvars);
xlabel(sprintf('%%%.1f of variance', pcvars(1)*100));
ylabel(sprintf('%%%.1f of variance', pcvars(2)*100));



%-------------------------------------------------------------------------
```



Data Analysis: Gene Set Enrichment

We have found the significantly different genes between two groups. But what do these genes do? Are there significant differences in biological functions between two groups? To answer these questions, we'll make use of the Gene Ontology terms, which annotate each gene to one or more Biological Processes, Cellular Components, and Molecular Functions.

```
gplgobio  = gpl.Data(:, strcmp(gpl.ColumnNames, 'Gene Ontology
 Biological Process'));
% The biological process of the first microarray probe:
gplgobio{1}
```

```
ans =

    '0001558 // regulation of cell growth // inferred from
 electronic annotation /// 0001952 // regulation of cell-matrix
 adhesion // inferred from electronic annotation /// 0006468 //
 protein phosphorylation // inferred from electronic annotation ///
 0007155 // cell adhesion // traceable author statement ///
 0007169 // transmembrane receptor protein tyrosine kinase signaling
 pathway // inferred from electronic annotation /// 0007565 // female
 pregnancy // inferred from electronic annotation /// 0007566 //
 embryo implantation // inferred from electronic annotation ///
 0007595 // lactation // inferred from electronic annotation ///
 0008285 // negative regulation of cell proliferation // inferred
 from electronic annotation /// 0010715 // regulation of extracellular
 matrix disassembly // inferred from mutant phenotype /// 0014909 //
 smooth muscle cell migration // inferred from mutant phenotype ///
```

```
 0016310 // phosphorylation // inferred from electronic annotation ///
 0018108 // peptidyl-tyrosine phosphorylation // inferred from
 electronic annotation /// 0030198 // extracellular matrix
 organization // traceable author statement /// 0038063 // collagen-
activated tyrosine kinase receptor signaling pathway // inferred
 from direct assay /// 0038063 // collagen-activated tyrosine kinase
 receptor signaling pathway // inferred from mutant phenotype ///
 0038083 // peptidyl-tyrosine autophosphorylation // inferred
 from direct assay /// 0043583 // ear development // inferred from
 electronic annotation /// 0044319 // wound healing, spreading of
 cells // inferred from mutant phenotype /// 0046777 // protein
 autophosphorylation // inferred from direct assay /// 0060444 //
 branching involved in mammary gland duct morphogenesis // inferred
 from electronic annotation /// 0060749 // mammary gland alveolus
 development // inferred from electronic annotation /// 0061302 //
 smooth muscle cell-matrix adhesion // inferred from mutant phenotype'
```

The Gene Ontology terms are given as a list separated by '///'. Let's convert each into a cell array, to make programming with them easier.

```
for i=1:numel(gplgobio)
 %handle empty separately, b/c strsplit doesn't work as we want for
 empty strings.
 if isempty(gplgobio{i}); gplgobio{i}={};
 else gplgobio{i} = strsplit(gplgobio{i},' /// '); end
end

% Let's reduce amount of memory by keeping a master list of Biological
% Processes and have each probe have indices to that master list.
GOBIO = unique( [gplgobio{:}] );

% And use the "map" technique to search for each term in the GOBIO
 list.
map = containers.Map(GOBIO,1:numel(GOBIO));
for i=1:numel(gplgobio)
 for j=1:numel(gplgobio{i})
  gplgobio{i}{j} = map(gplgobio{i}{j});
 end
 gplgobio{i} = cell2mat(gplgobio{i});
end
```

Now each gplgobio is a numeric array.

```
gplgobio{1}
```

```
ans =

  Columns 1 through 6

         502          937         2722         4113         4181
 4747

  Columns 7 through 12
```

```
       4754         4795         5065         6042         6595
 7036

  Columns 13 through 18

       7312         8359        11533        11534        11537
 12928

  Columns 19 through 23

      13127        14563        17378        17637        17941
```

To get the actual terms, use these numbers as indices to the GOBIO list.

```
GOBIO( gplgobio{1} )'
```

```
ans =

  23×1 cell array

    {'0001558 // regulation of cell growth // inferred from electronic
 annotation'                                        }
    {'0001952 // regulation of cell-matrix adhesion // inferred from
 electronic annotation'                               }
    {'0006468 // protein phosphorylation // inferred from electronic
 annotation'                                          }
    {'0007155 // cell adhesion // traceable author statement'
                                                    }
    {'0007169 // transmembrane receptor protein tyrosine kinase
 signaling pathway // inferred from electronic annotation'}
    {'0007565 // female pregnancy // inferred from electronic
 annotation'                                                }
    {'0007566 // embryo implantation // inferred from electronic
 annotation'                                             }
    {'0007595 // lactation // inferred from electronic annotation'
                                                    }
    {'0008285 // negative regulation of cell proliferation // inferred
 from electronic annotation'                        }
    {'0010715 // regulation of extracellular matrix disassembly //
 inferred from mutant phenotype'                        }
    {'0014909 // smooth muscle cell migration // inferred from mutant
 phenotype'                                          }
    {'0016310 // phosphorylation // inferred from electronic
 annotation'                                                 }
    {'0018108 // peptidyl-tyrosine phosphorylation // inferred from
 electronic annotation'                                 }
    {'0030198 // extracellular matrix organization // traceable author
 statement'                                         }
    {'0038063 // collagen-activated tyrosine kinase receptor signaling
 pathway // inferred from direct assay'             }
    {'0038063 // collagen-activated tyrosine kinase receptor signaling
 pathway // inferred from mutant phenotype'         }
```

```
   {'0038083 // peptidyl-tyrosine autophosphorylation // inferred
 from direct assay'                                        }
   {'0043583 // ear development // inferred from electronic
 annotation'                                                     }
   {'0044319 // wound healing, spreading of cells // inferred from
 mutant phenotype'                                         }
   {'0046777 // protein autophosphorylation // inferred from direct
 assay'                                                    }
   {'0060444 // branching involved in mammary gland duct
 morphogenesis // inferred from electronic annotation'          }
   {'0060749 // mammary gland alveolus development // inferred from
 electronic annotation'                                    }
   {'0061302 // smooth muscle cell-matrix adhesion // inferred from
 mutant phenotype'                                         }
```

We have made the gplgobio more convenient to work with, but we really need to get the gobio information for the gse probes. We need to map take the GSE probes and search them in the GPL probes again. We have previously created the MAP_GSE_GPL, which we can use again.

```
gsegobio = cell(size(d,1),1); %any probe not found in gpl will remain
 an empty vector.
gsegobio(find(MAP_GSE_GPL)) =
 gplgobio(MAP_GSE_GPL(find(MAP_GSE_GPL)));
```

Now we can go through each GOBIO term and see if our list of significant genes is enriched for that term (ie. if that Biological Process is significantly different between the groups we are comparing). We only need to do this for the GOBIO terms that have at least one significant gene for it. If it has no significant genes for it at all, we already know that Biological Process is not affected, so no need to run a statistical test for it.

Find the terms that have at least one significant gene for it.

```
Isig=dpvals(:,'p-values')<=0.01;
dsig = d(Isig,:);
dpvalssig = dpvals(Isig,:);
gsegobiosig = gsegobio(Isig);
candidategobio = unique( [gsegobiosig{:}] );
```

Let's just analyze the first candidate GO Biological process.

```
gobioid = candidategobio(1);
GOBIO{ gobioid }


ans =

    '0000038 // very long-chain fatty acid metabolic process //
 inferred from direct assay'
```

Hypergeometric Test

To calculate the significance of a Biological Process, we'll use the hypergeometric test. We need the following four numbers:

x: number of samples drawn, with the desired characteristic. K: number of items with the desired characteristic in the population N: number of samples drawn M: size of the population

```
% x: The number of significant genes that are in this term.
x = nnz([gsegobiosig{:}] == gobioid)
```

```
x =

     1
```

K: Number of genes (significant or not) that are in this term.

```
K = nnz([gsegobio{:}] == gobioid)
```

```
K =

     9
```

- N: Total number of significant genes. (ignore those with no GO term).

```
N = nnz( ~cellfun(@isempty,gsegobiosig) )
```

```
N =

   397
```

M: Total number of genes. (ignore those with no GO term).

```
M = nnz( ~cellfun(@isempty,gsegobio) )
```

```
M =

       19017
```

Calculate and print the pvalue for this Biological Process.

```
pval = hygecdf(x,M,K,N,'upper');
fprintf('pvalue=%f for the GO Biological Process
 [%s]\n',pval,GOBIO{gobioid});

% We calculated the pvalue for only one of the candidate terms. You
 would
% of course want to calculate a pvalue for all candidate terms, apply
 FDR
% correction, and report only the most significant ones.
```

```
pvalue=0.014202 for the GO Biological Process [0000038 // very long-
chain fatty acid metabolic process // inferred from direct assay]
```

Published with MATLAB® R2018b