MCI5004: Molecular Biomarkers in Clinical Research

Principal Component Analysis in Biomarker Discovery

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Plan

PCA

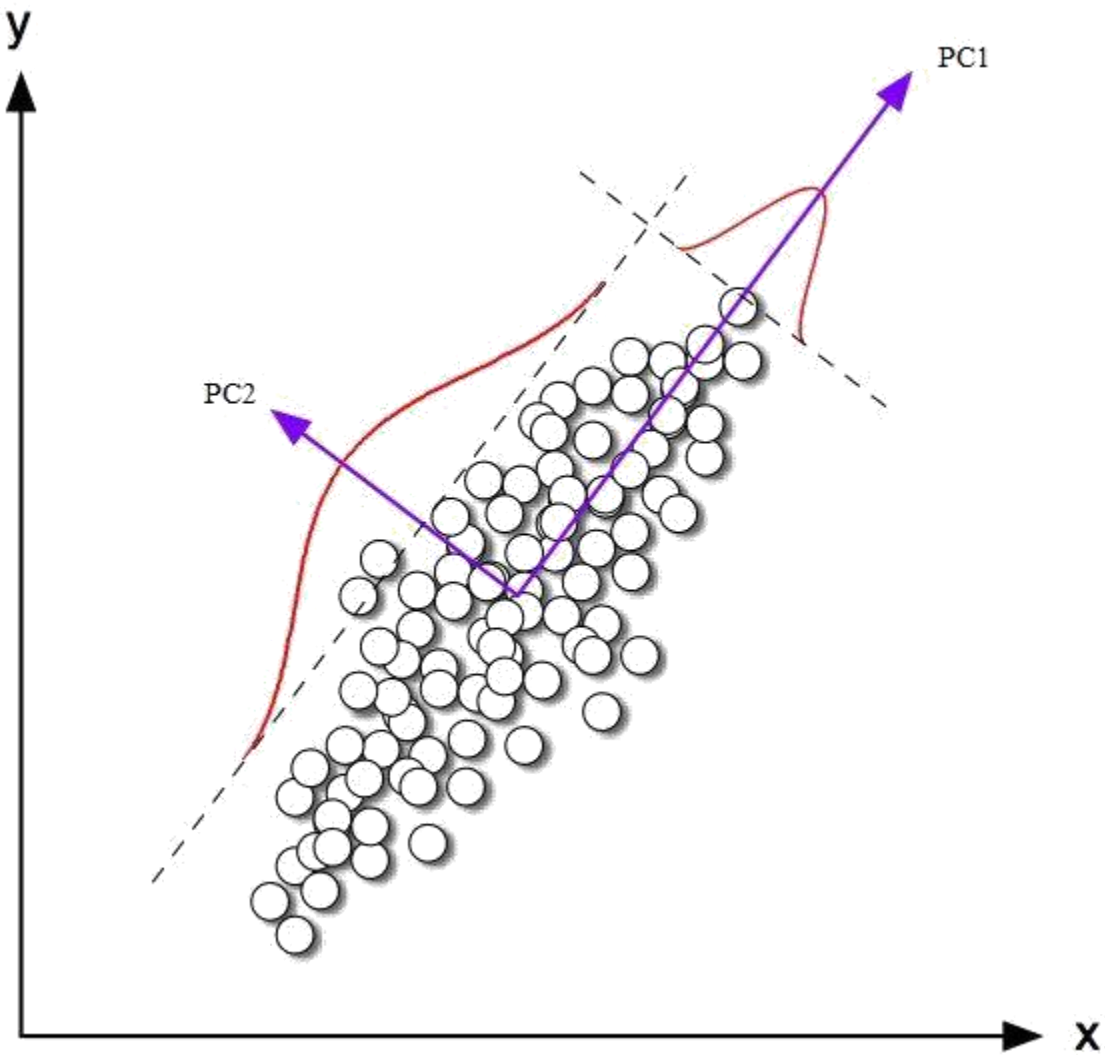
PCA in biomarker selection

Batch effects

PCA for isolating batch effects

PCA at the level of protein complexes / biological pathway subnetworks

PRINCIPAL COMPONENT ANALYSIS (PCA)



PCA, intuitively

Credit: Alessandro Giuliani

PCA, a la Pearson (1901)

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SULLE FUNZIONI BILINEARI

DI

E. BELTRAMI

LIII. *On Lines and Planes of Closest Fit to Systems of Points in Space.* By KARL PEARSON, *F.R.S., University College, London* *.

(1) IN many physical, statistical, and biological investigations it is desirable to represent a system of points in plane, three, or higher dimensioned space by the "best-fitting" straight line or plane. Analytically this consists in taking

$$y = a_0 + a_1 x, \quad \text{or} \quad z = a_0 + a_1 x + b_1 y,$$

$$\text{or} \quad z = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + \ldots + a_n x_n,$$

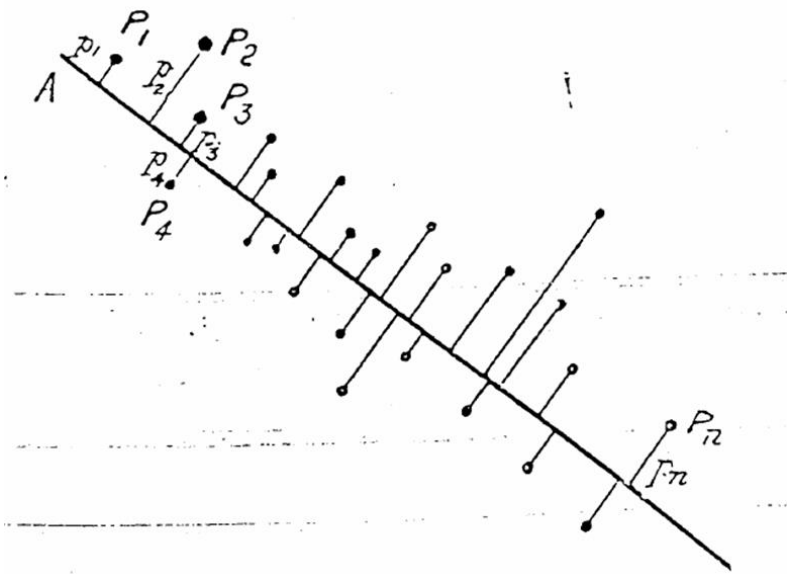
where $y, x, z, x_1, x_2, \ldots x_n$ are variables, and determining the "best" values for the constants $a_0, a_1, b_1, a_0, a_1, a_2, a_3, \ldots a_n$

For example:—Let $P_1, P_2, \ldots P_n$ be the system of points with coordinates $x_1, y_1; x_2, y_2; \ldots x_n\ y_n$, and perpendicular distances $p_1, p_2, \ldots p_n$ from a line A B. Then we shall make

$$U = S(p^2) = \text{a minimum.}$$

If y were the dependent variable, we should have made

$$S(y' - y)^2 = \text{a minimum}$$



Credit: Alessandro Giuliani

Credit: Marloes Maathuis

PCA, in modern English ☺

Introduction

- Technique quite old: Pearson (1901) and Hotelling (1933), but still one of the most used multivariate techniques today
- Main idea:
  - Start with variables $X_1, \ldots, X_p$
  - Find a *rotation* of these variables, say $Y_1, \ldots, Y_p$ (called principal components), so that:
    - $Y_1, \ldots, Y_p$ are uncorrelated. Idea: they measure different dimensions of the data.
    - $\text{Var}(Y_1) \geq \text{Var}(Y_2) \geq \ldots \text{Var}(Y_p)$. Idea: Y_1 is most important, then Y_2, etc.

9 / 33

Definition of PCA

- Given $X = (X_1, \ldots, X_p)'$
- We call $a'X$ a standard linear combination (SLC) if $\sum a_i^2 = 1$
- Find the SLC $a'_{(1)} = (a_{11}, \ldots, a_{p1})$ so that $Y_1 = a'_{(1)}X$ has maximal variance
- Find the SLC $a'_{(2)} = (a_{12}, \ldots, a_{p2})$ so that $Y_2 = a'_{(2)}X$ has maximal variance, subject to the constraint that Y_2 is uncorrelated to Y_1.
- Find the SLC $a'_{(3)} = (a_{13}, \ldots, a_{p3})$ so that $Y_3 = a'_{(3)}X$ has maximal variance, subject to the constraint that Y_3 is uncorrelated to Y_1 and Y_2
- Etc...

10 / 33

PCA, a nice tutorial for dummies

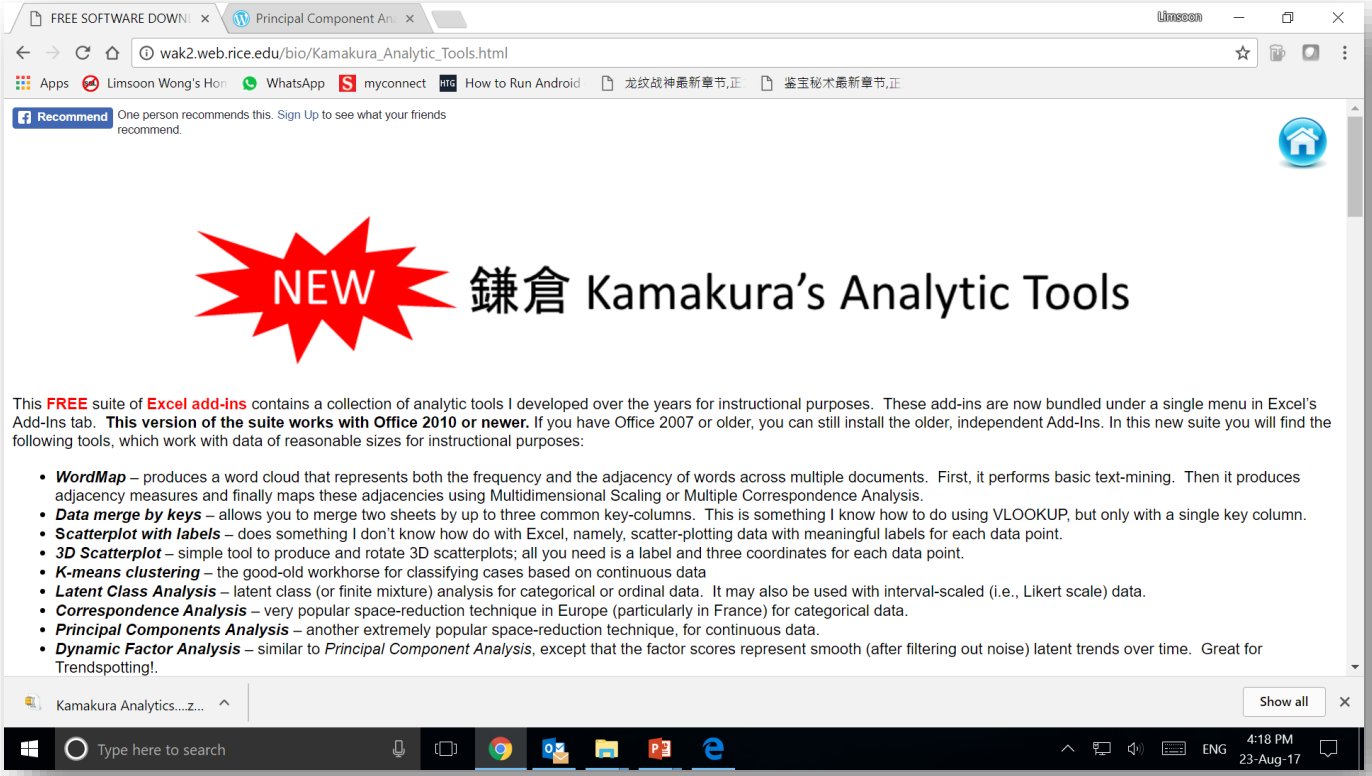
https://georgemdallas.wordpress.com/2013/10/30/principal-component-analysis-4-dummies-eigenvectors-eigenvalues-and-dimension-reduction

Principal Component Analysis 4 Dummies: Eigenvectors, Eigenvalues and Dimension Reduction

Having been in the social sciences for a couple of weeks it seems like a large amount of quantitative analysis relies on Principal Component Analysis (PCA). This is usually referred to in tandem with eigenvalues, eigenvectors and lots of numbers. So what's going on? Is this just mathematical jargon to get the non-maths scholars to stop asking questions? Maybe, but it's also a useful tool to use when you have to look at data. This post will give a very broad overview of PCA, describing eigenvectors and eigenvalues (which you need to know about to understand it) and showing how you can reduce the

Nice free Excel add-on

http://wak2.web.rice.edu/bio/Kamakura_Analytic_Tools.html



Growth, 1960, **24**, 339-354.

SIZE AND SHAPE VARIATION IN THE PAINTED TURTLE.[1] A PRINCIPAL COMPONENT ANALYSIS

PIERRE JOLICOEUR AND JAMES E. MOSIMANN[2]

Walker Museum, University of Chicago
and
Institut de Biologie, Université de Montréal

(Received for publication July 11, 1960)

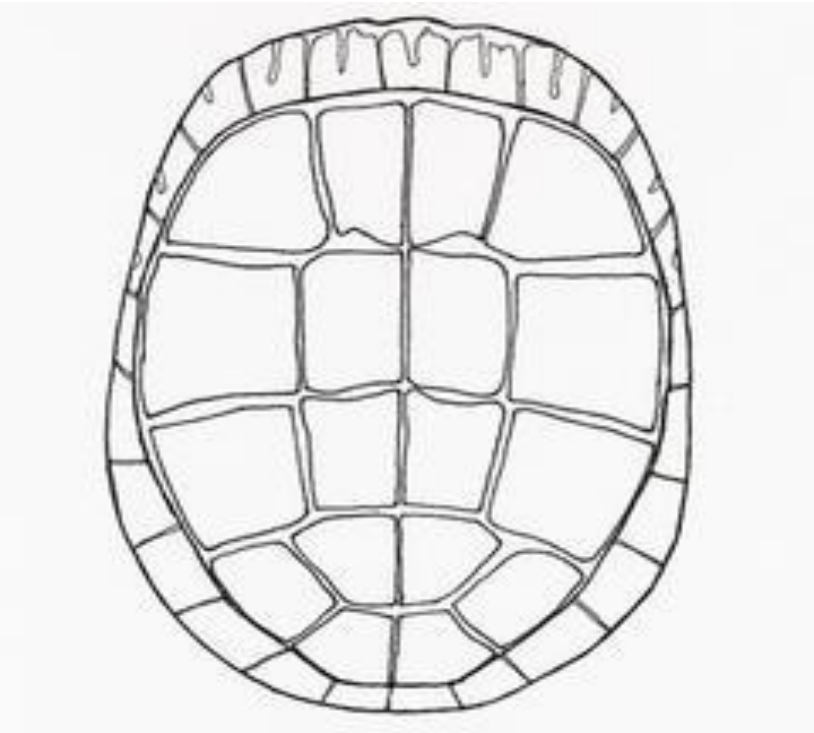
Credit: Alessandro Giuliani

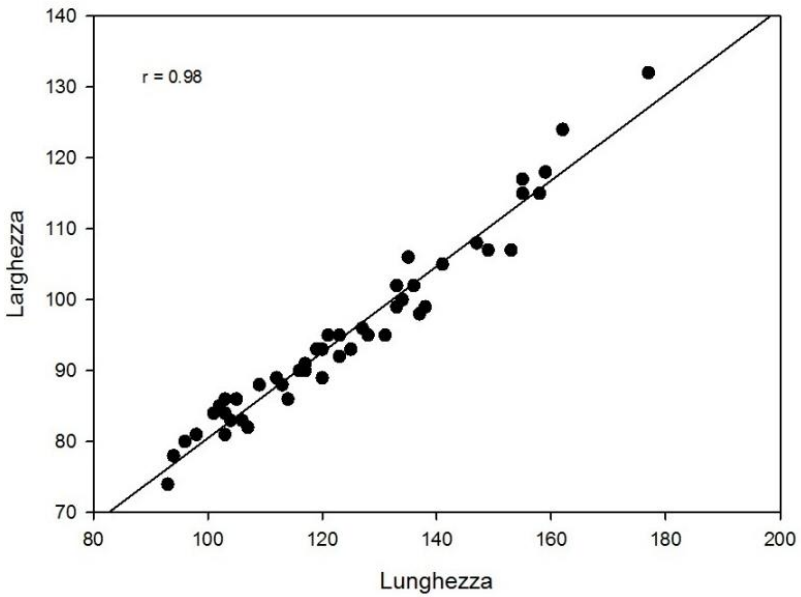
TABLE 1

CARAPACE DIMENSIONS OF PAINTED TURTLES (*Chrysemys picta marginata*) IN MM.

24 Males			24 Females		
length	width	height	length	width	height
93	74	37	98	81	38
94	78	35	103	84	38
96	80	35	103	86	42
101	84	39	105	86	40
102	85	38	109	88	44
103	81	37	123	92	50
104	83	39	123	95	46
106	83	39	133	99	51
107	82	38	133	102	51
112	89	40	133	102	51
113	88	40	134	100	48
114	86	40	136	102	49
116	90	43	137	98	51
117	90	41	138	99	51
117	91	41	141	105	53
119	93	41	147	108	57
120	89	40	149	107	55
120	93	44	153	107	56
121	95	42	155	115	63
125	93	45	155	117	60
127	96	45	158	115	62
128	95	45	159	118	63
131	95	46	162	124	61
135	106	47	177	132	67

Credit: Alessandro Giuliani





Pearson Correlation Coefficients,

	length	width	height
length	1.00000	0.97831	0.96469
width	0.97831	1.00000	0.96057
height	0.96469	0.96057	1.00000

Width = 19,94 + 0,605*Length

Credit: Alessandro Giuliani

Credit: Alessandro Giuliani

Principal components

Variance of PC1

	PC1 (98%)	PC2 (1.4%)
Length	0,992	-0,067
Width	0,990	-0,100
Height	0,986	0,168

Loading / correlation of Length to PC2

PC1= 33.78*Length +33.73*Width + 33.57*Height

PC2 = -1.57*Length – 2.33*Width + 3.93*Height

Presence of an overwhelming size component explaining system variance comes from the presence of a ‘typical’ common shape

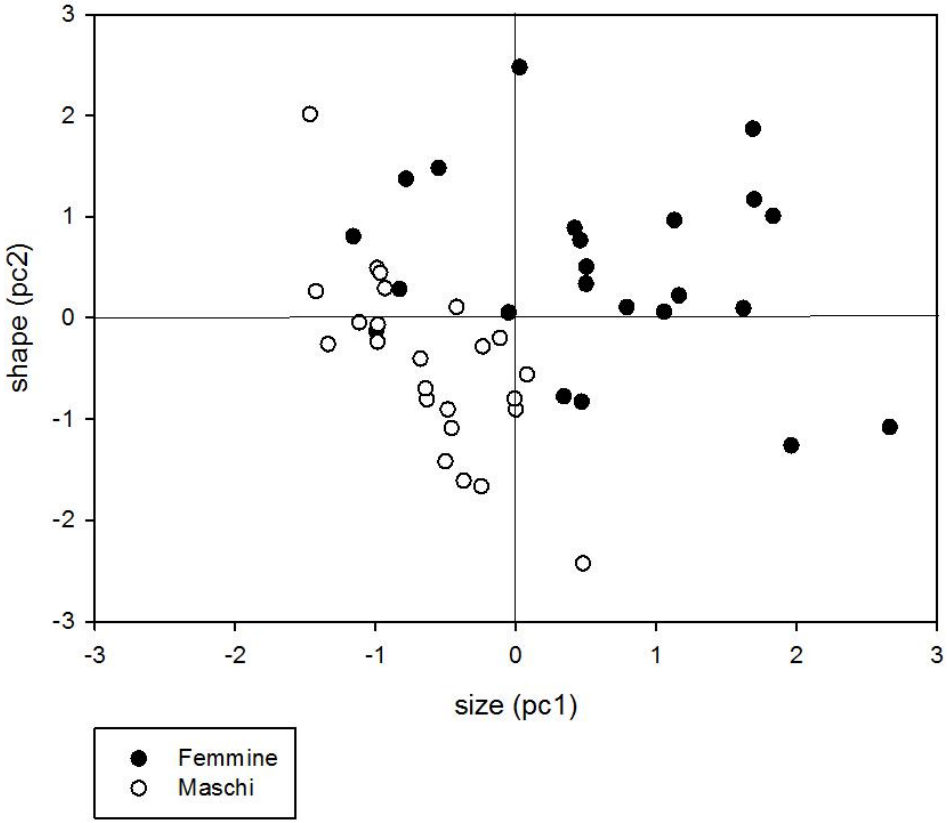
Displacement along pc1 = size variation (all positive terms)

Displacement along pc2 = shape deformation (both positive and negative terms)

unit	sex	Length	Width	Height	PC1(size)	PC2(shape)
T25	F	98	81	38	-1,15774	0,80754832
T26	F	103	84	38	-0,99544	-0,1285916
T27	F	103	86	42	-0,7822	1,37433475
T28	F	105	86	40	-0,82922	0,28526912
T29	F	109	88	44	-0,55001	1,4815252
T30	F	123	92	50	0,027368	2,47830153
T31	F	123	95	46	-0,05281	0,05403839
T32	F	133	99	51	0,418589	0,88961967
T33	F	133	102	51	0,498425	0,33681756
T34	F	133	102	51	0,498425	0,33681756
T35	F	134	100	48	0,341684	-0,774911
T36	F	136	102	49	0,467898	-0,8289156
T37	F	137	98	51	0,457949	0,76721682
T38	F	138	99	51	0,501055	0,50628189
T39	F	141	105	53	0,790215	0,10640554
T40	F	147	108	57	1,129025	0,96505915
T41	F	149	107	55	1,055392	0,06026089
T42	F	153	107	56	1,161368	0,22145593
T43	F	155	115	63	1,687277	1,86903869
T44	F	158	115	62	1,696753	1,17117077
T45	F	159	118	63	1,833086	1,00956637
T46	F	162	124	61	1,962232	-1,261771
T47	F	177	132	67	2,662548	-1,0787317
T48	F	155	117	60	1,620491	0,09690818
T1	M	93	74	37	-1,46649	2,01289241
T2	M	94	78	35	-1,42356	0,26342486
T3	M	96	80	35	-1,33735	-0,258445
T4	M	101	84	39	-0,98842	0,49260881
T5	M	102	85	38	-0,98532	-0,2361914
T6	M	103	81	37	-1,11528	-0,0436547
T7	M	104	83	39	-0,96555	0,44687352
T8	M	106	83	39	-0,93257	0,29353841
T9	M	107	82	38	-0,98269	-0,066727
T10	M	112	89	40	-0,63393	-0,8042059
T11	M	113	88	40	-0,64405	-0,6966061
T12	M	114	86	40	-0,68078	-0,4047389
T13	M	116	90	43	-0,42133	0,10845233
T14	M	117	90	41	-0,48485	-0,9039457
T15	M	117	91	41	-0,45824	-1,0882131
T16	M	119	93	41	-0,37202	-1,610083
T17	M	120	89	40	-0,50198	-1,4175463
T18	M	120	93	44	-0,23552	-0,2831547
T19	M	121	95	42	-0,24581	-1,6640875
T20	M	125	93	45	-0,11305	-0,1986272
T21	M	127	96	45	-0,00023	-0,9047645
T22	M	128	95	45	-0,01035	-0,7971646
T23	M	131	95	46	0,079136	-0,559302
T24	M	135	106	47	0,477846	-2,4250481

Credit: Alessandro Giuliani

Female turtles are larger and have more exaggerated height ☺



Exercise

Madrid and Warsaw are at almost the same distance to Latium cities

Are Madrid and Warsaw near each other?

Distances of European cities (km) from the main cities of Latium

	Rome	Latina	Frosinone	Viterbo	Rieti
Amsterdam	430	447	449	415	409
Athens	347	321	331	346	364
Barcelona	283	305	293	292	271
Beograd	227	222	236	220	238
Berlin	393	400	409	374	373
Bern	227	249	247	220	205
Bonn	353	370	372	339	330
Bruselles	388	406	406	371	365
Bucharest	364	355	368	359	378
Budapest	268	261	274	246	259
Calais	418	448	446	418	405
Copenhagen	510	522	527	492	491
Dublin	622	645	641	615	600
Edinburgh	637	655	655	625	615
Frankfurt	318	333	336	302	295
Hamburg	435	448	453	417	414
Helsinki	727	729	739	706	713
Istanbul	452	430	443	443	464
Lisbon	615	637	622	624	604
London	474	494	493	464	456
Luxembourg	325	346	346	315	307
Madrid	449	470	458	460	440
Marseille	200	223	213	202	183
Moscow	782	773	785	759	774
Munich	230	245	250	216	213
Oslo	664	675	682	646	645
Paris	365	386	383	357	343
Prague	305	313	320	286	290
Sofia	294	273	286	280	301
Stockholm	653	658	668	632	636
Warsaw	435	433	444	413	421
Vienna	255	254	265	233	240
Zurich	227	246	246	214	205

Giuliani et al., Physics Letters A, 247:47-52, 1998

PCA of distance matrix of European cities to Latium cities

Factor loadings and proportions of explained variance

Variables	Components				
	PC1	PC2	PC3	PC4	PC5
Rome	0.9997	0.0137	−0.0184	−0.0120	0.0001
Frosinone	0.9973	−0.0715	0.0132	0.0011	0.0029
Latina	0.9987	−0.0420	−0.0272	0.0058	−0.0024
Rieti	0.9909	0.0162	0.0393	−0.0009	−0.0023
Viterbo	0.9964	0.0837	−0.0070	0.0060	0.0017
Explained variance	0.9965	0.0029	0.000569	0.000043	0.000005

PC1 accounts for >99% of variance

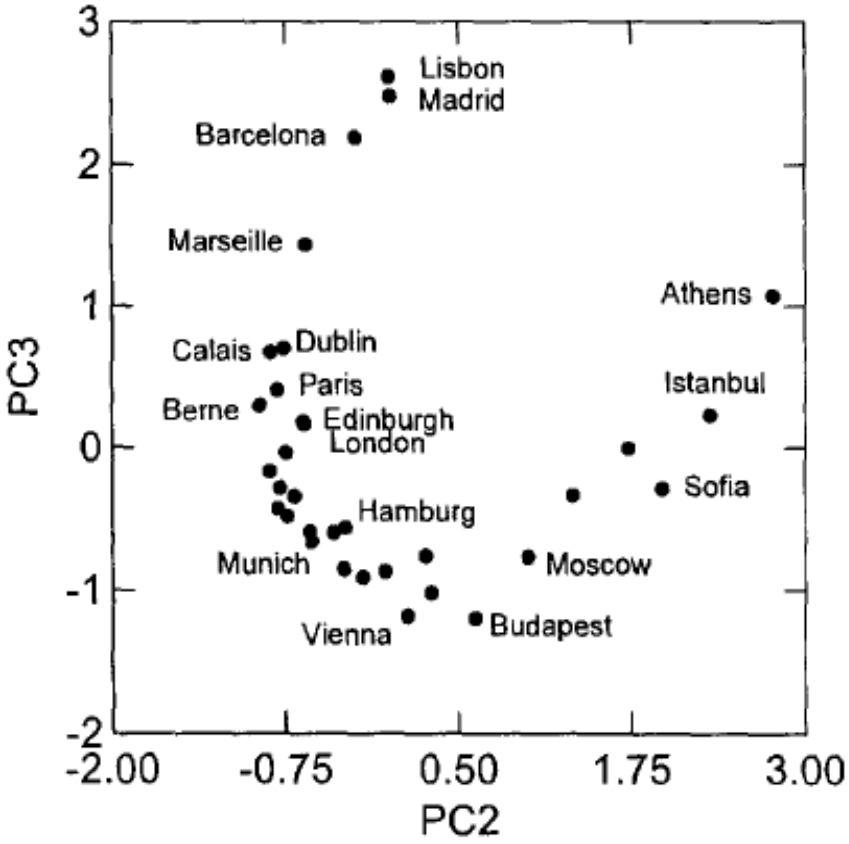
PC1 correlates with distance of European cities to Latium cities

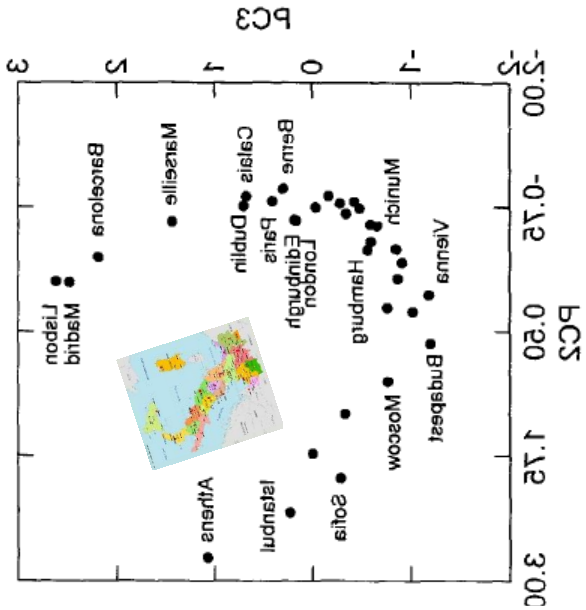
PC2, PC3, … account for < 1% of variance

Are PC2, PC3, … useless / non-informative?

PC2 & PC3 are the angular orientation of European cities centered on Latium

So you can tell Madrid is not near Warsaw





Intuitive points

PCA gives the axes that orthogonally account for variance in the data

PCs correspond to explanations / factors giving rise to the variance

Coefficient of a variable in a PC suggests how relevant that variable is for that PC

Surprising point

PCs accounting for a very small portion of the variance can also be informative, if you know how to find these

Caution: PCA is not scale invariant

Suppose we have measurements in kg and meters, and we want to have principal components expressed in grams and hectometers

Option 1: multiply measurements in kg by 1000, multiply measurements in meters by 1/100, and then apply PCA

Option 2: apply PCA on original measurements, and then re-scale to the appropriate units

These two options generally give different results!

Credit: Marloes Maathuis

Re-scaling in PCA

When to re-scale

Variables in different units should be re-scaled

Variables in same units but have very different variances should be re-scaled

How to re-scale

Divide each variable by its deviation

Simple linear interpolation to e.g. [0, 1]

Take log

PCA IN BIOMARKER SELECTION

PCA in biomarker selection

When PCA is applied e.g. on gene expression data,

PCs w/ large variance ≈ diff expressed pathways

Variables w/ large coefficient/loading in a PC ≈ key genes in the pathway associated with that PC

PCA can be a useful biomarker-selection approach

E.g., biomarkers ≈ genes w/ high loading

Loading of gene x = $\Sigma_j \mid \alpha_{xj} * \sigma_j^2 \mid$, where α_{xj} is coefficient of x in PC_j, and σ_j^2 is variance of PC_j

Example

Major subtypes: T-ALL, E2A-PBX, TEL-AML, BCR-ABL, MLL genome rearrangements, Hyperdiploid>50

Diff subtypes respond differently to same Tx

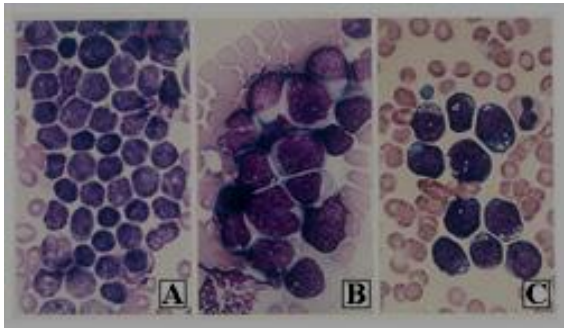
Over-intensive Tx

- Development of secondary cancers
- Reduction of IQ

Under-intensiveTx

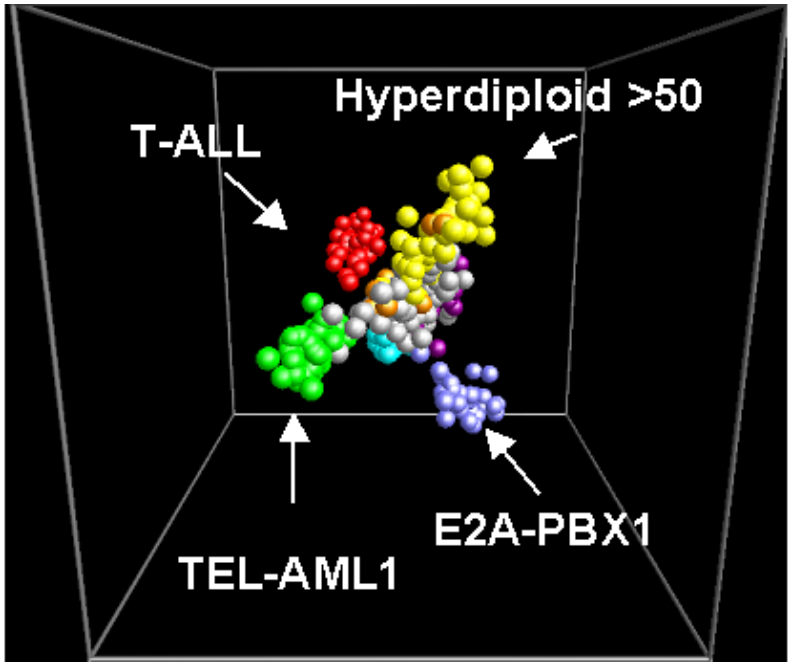
- Relapse

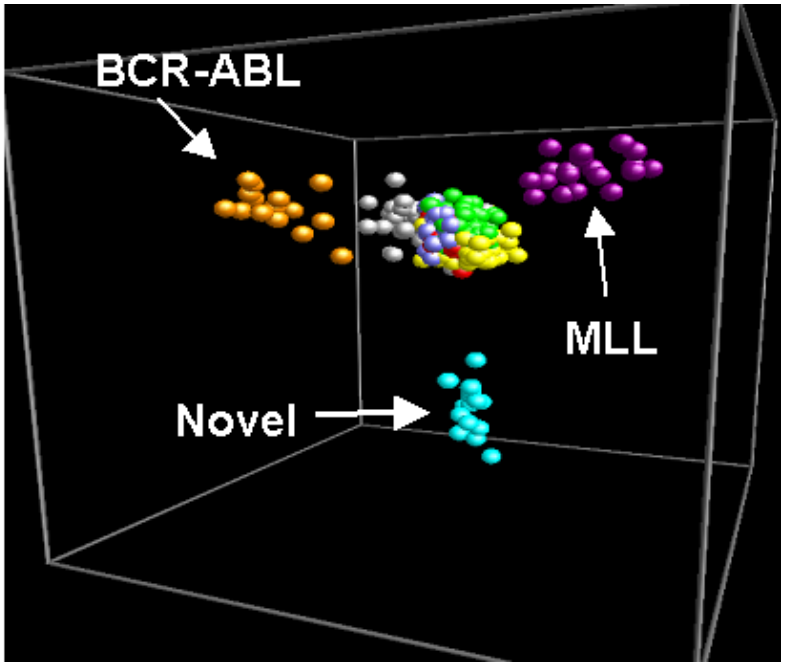
The subtypes look similar



Can we diagnosis the subtypes based on gene expression profiling?

PCA in ALL subtype diagnosis





Steps:

Identify genes with high variance

Perform PCA on them

Plot using PC1 to 3

Induction of hypothesis

The PCs capture different biological pathways. The values of PCs capture different states of these pathways

Hypothesis: If patient X has ALL subtype T, X's biological pathways are in state S_T

… and abduction during diagnosis

Observation: John's biological pathways are in state S_T

Abduction: John has ALL subtype T

BATCH EFFECTS

What are batch effects?

Batch effects are unwanted sources of variation caused by different processing date, handling personnel, reagent lots, equipment/machines, etc.

Batch effects is a big challenge faced in biological research, especially towards translational research and precision medicine

Visualizing batch effects

Rank variables / genes by variance

Keep those with high variance (e.g. top 30-50%)

Perform PCA on them

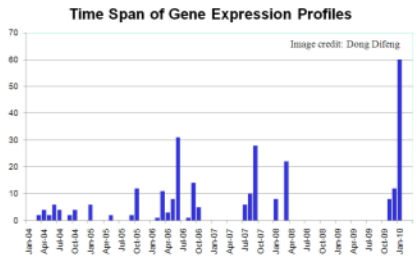
Make scatter plot of the first 2-3 PCs

Do the subjects clusters by batch?

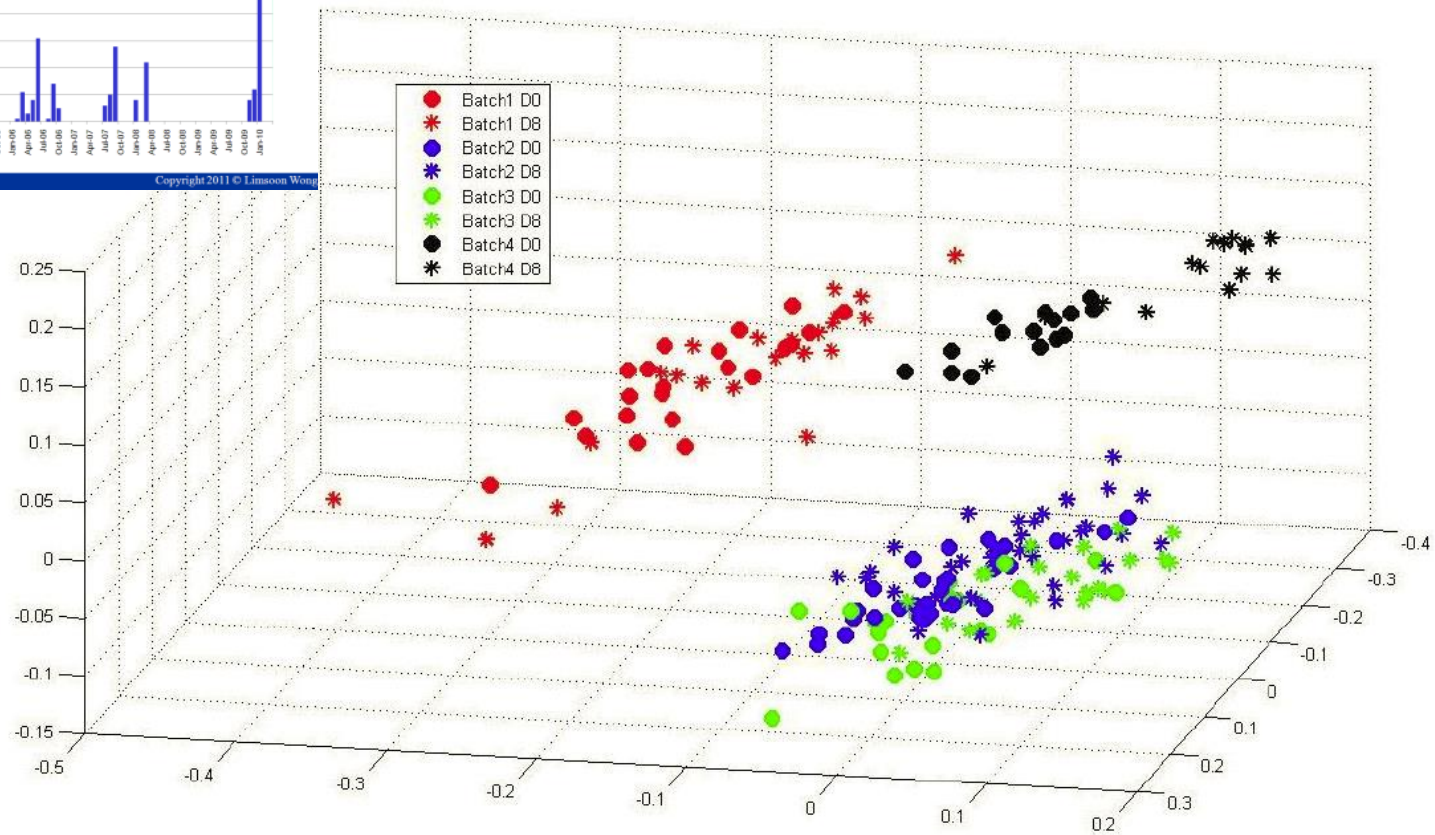
Make paired boxplot of each PC wrt class and batch variables

Is PC more correlated with batch?

Sometimes, a gene expression study may involve batches of data collected over a long period of time…



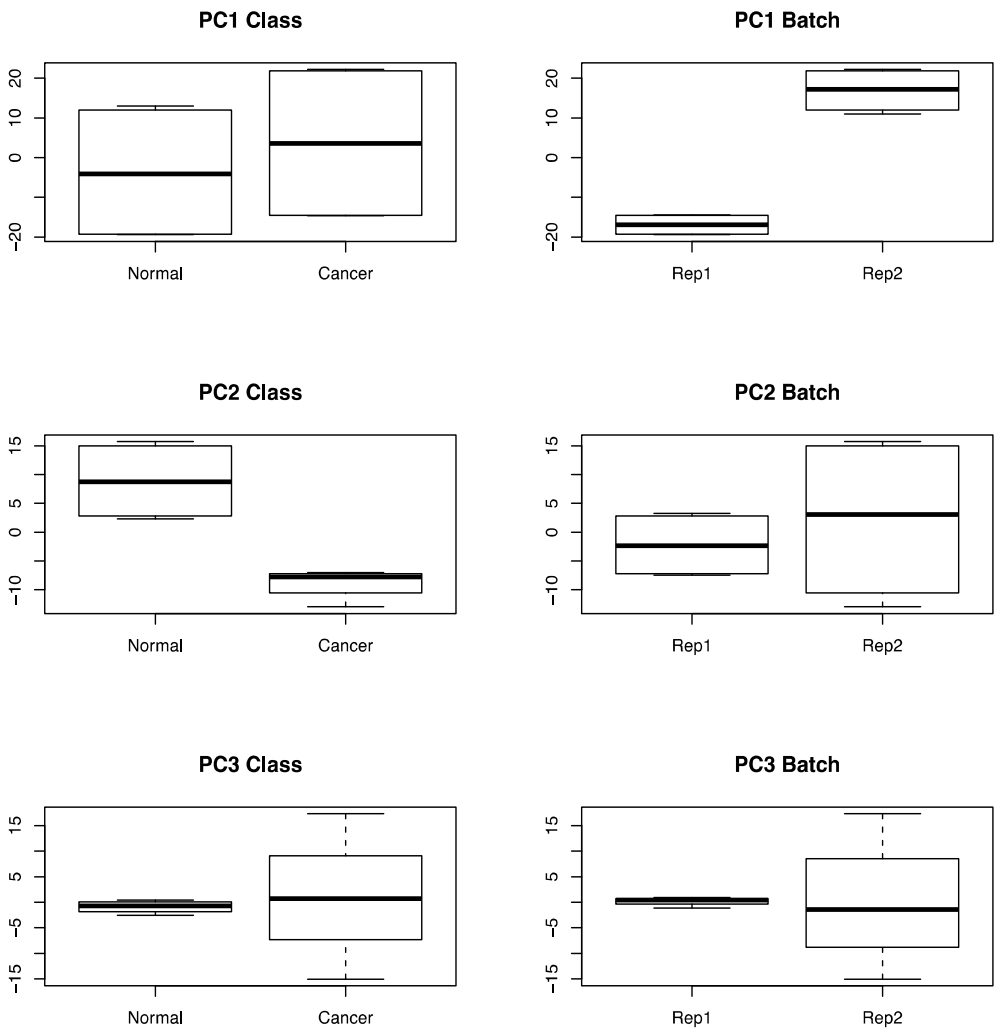
PCA scatter plot



Samples from diff batches are grouped together, regardless of subtypes and treatment response

Image credit: Difeng Dong's PhD dissertation, 2011

Paired boxplots of PCs



It is easier to see which PC is enriched in batch effects by showing, side by side, the distribution of values of each PC stratified by class and suspected batch variables

Normalization

Aim of normalization:

Reduce variance w/o increasing bias

Xform data so that probe intensity distribution is same on all arrays

E.g., $(x - \mu) / \sigma$

Scaling method

Intensities are scaled so that each array has same average value

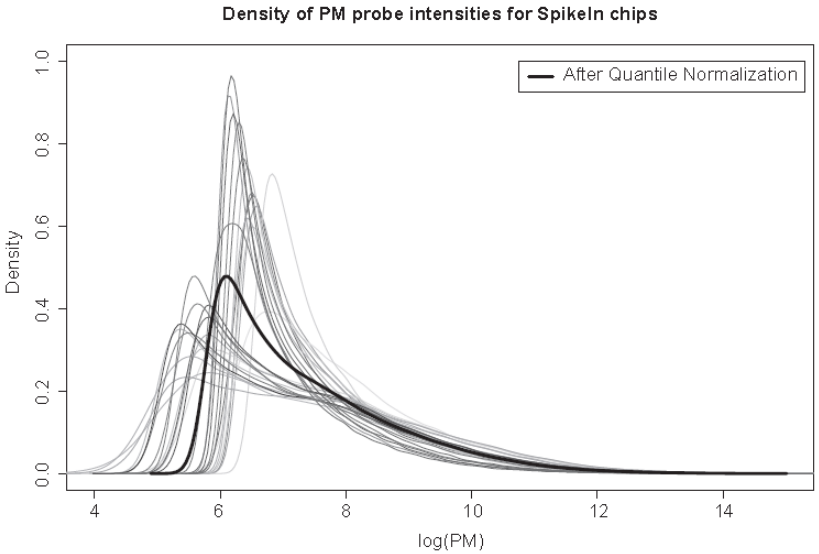
E.g., Affymetrix's

Quantile normalization

Gene fuzzy score, GFS

Quantile normalization

- **Given *n arrays of length p, form X of size p × n where each array is a column***
- **Sort each column of *X to give* X_{sort}**
- **Take means across rows of X_{sort} *and assign this* mean to each elem in the row to get X'_{sort}**
- **Get $X_{normalized}$ *by arranging each column of* X'_{sort} to have same ordering as *X***



- Implemented in some microarray s/w, e.g., EXPANDER

48

In such a case, batch effect may be severe… to the extent that you can predict the batch that each sample comes!

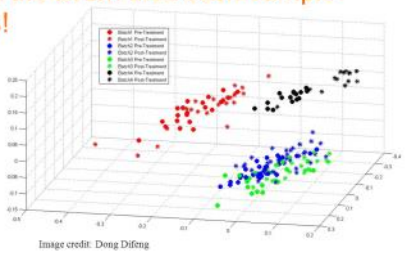


Image credit: Dong Difeng

⇒ Need normalization to correct for batch effect

After quantile normalization

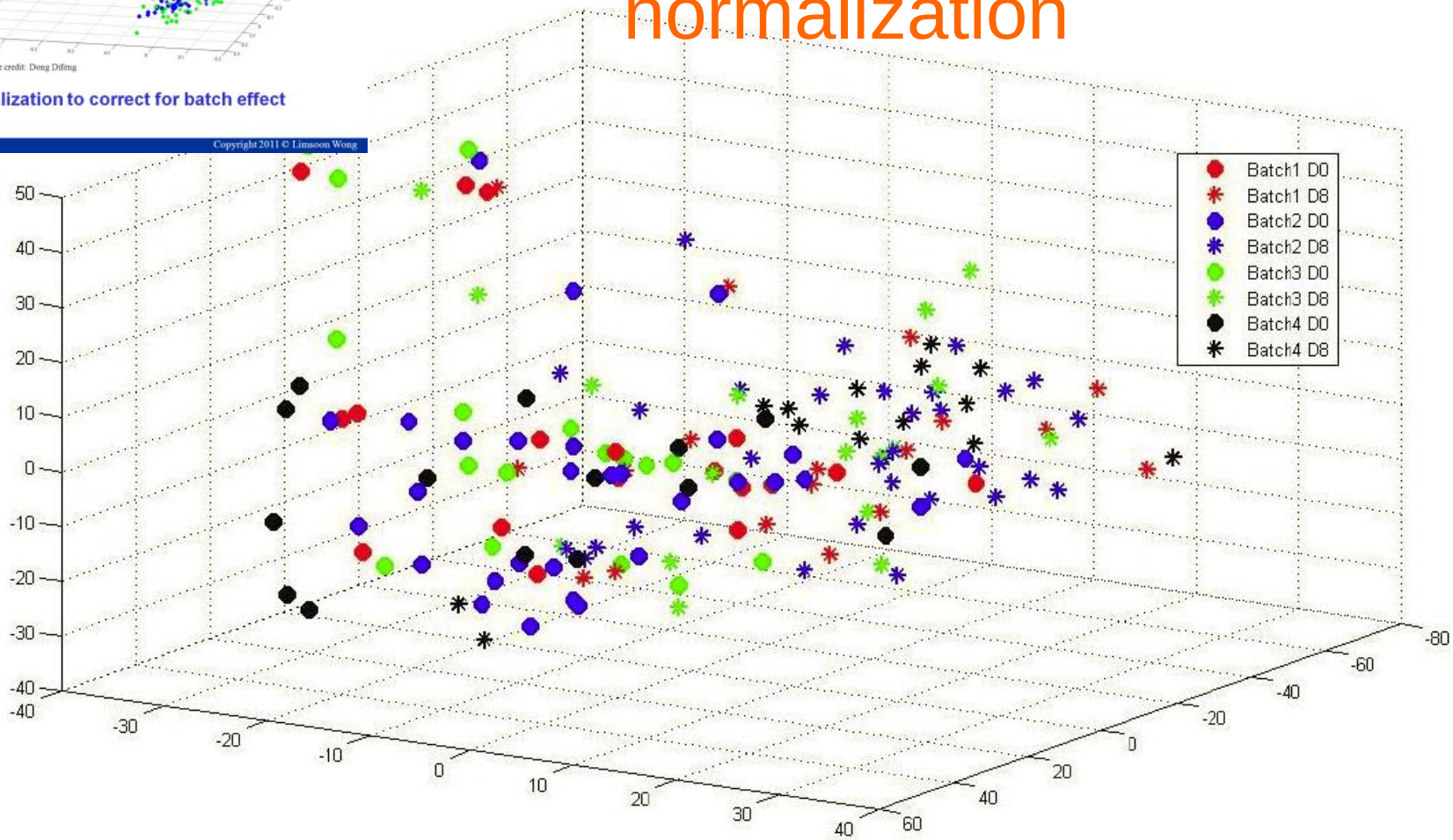
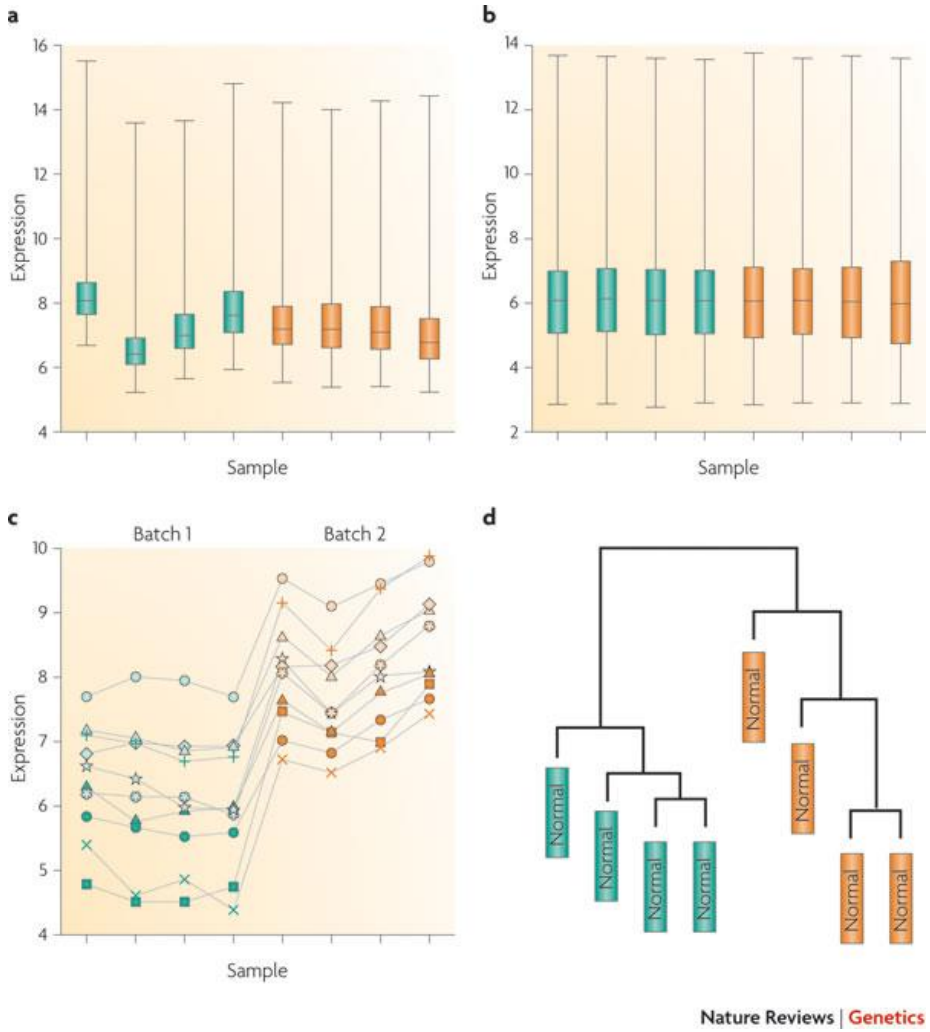


Image credit: Difeng Dong's PhD dissertation, 2011

Caution: It is difficult to eliminate batch effects effectively



Leek et al, Nature Reviews Genetics, 11:733-739, 2010

Green and orange are normal samples differing in processing date

a: Before normalization

b: Post normalization

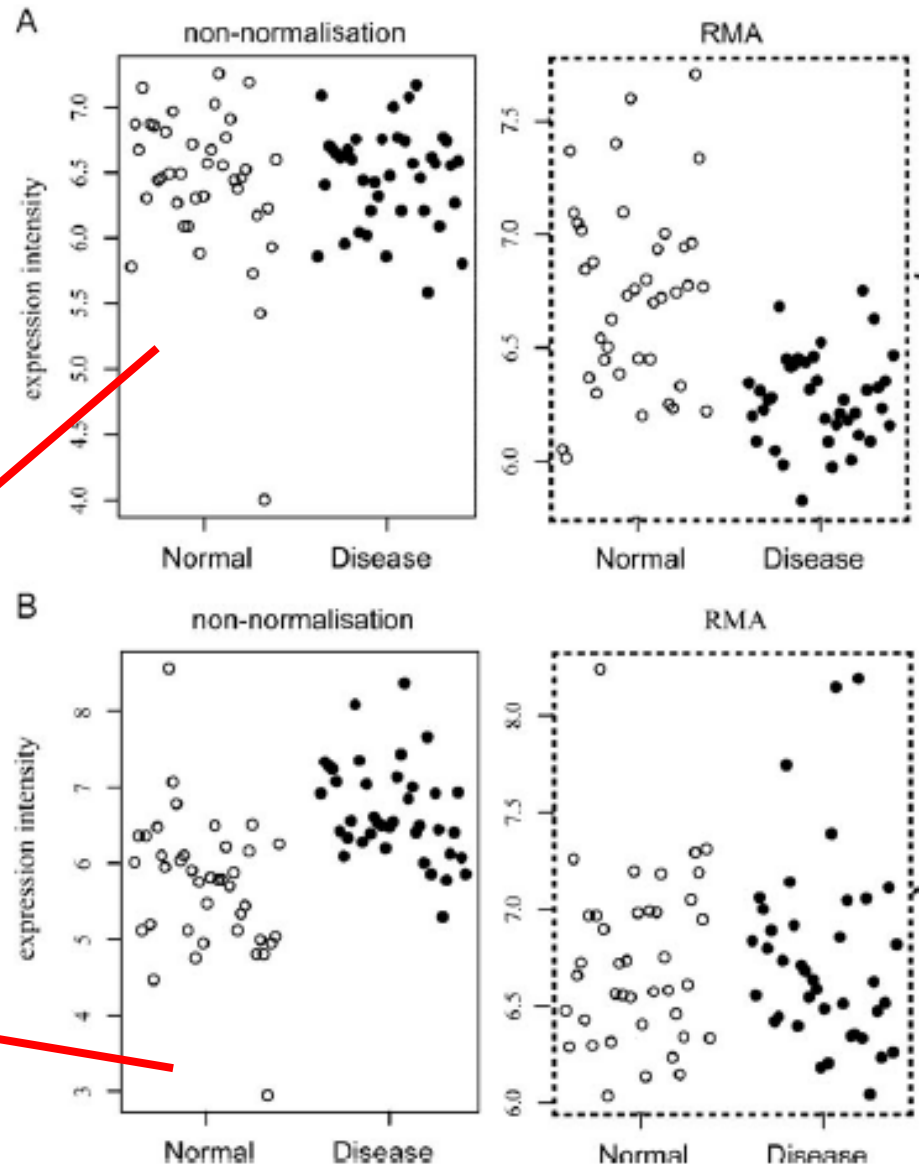
c: Checks on individual genes susceptible to batch effects

d: Clustering after normalization (samples still cluster by processing date)

Caution: “Over normalized” signals in cancer samples

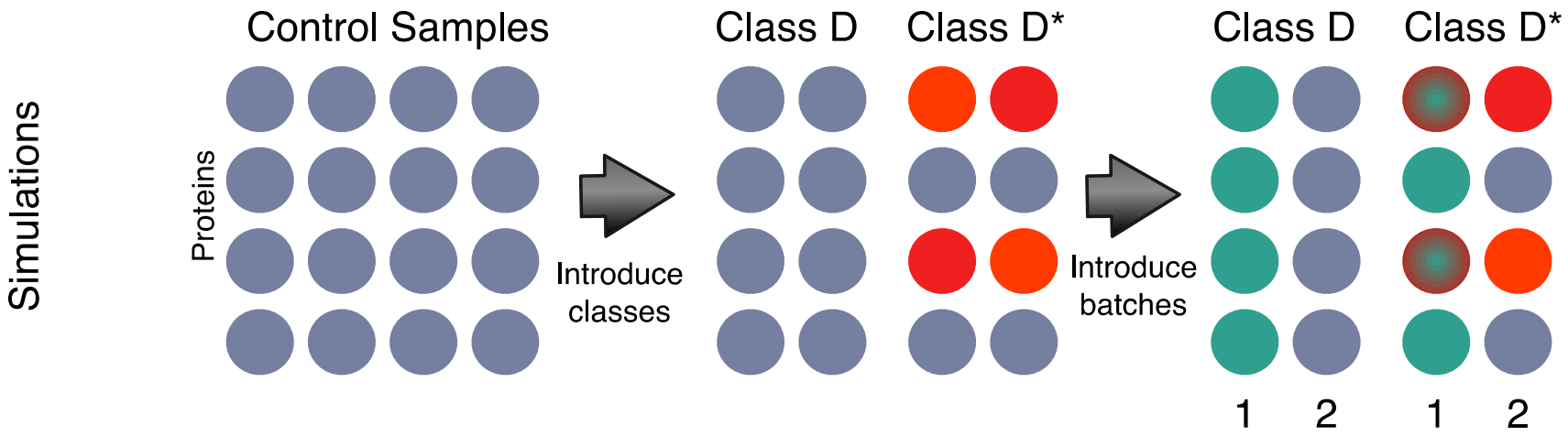
A gene normalized by quantile normalization (RMA) was detected as down-regulated DE gene, but the original probe intensities in cancer samples were not diff from those in normal samples

A gene was detected as an up-regulated DE gene in the non-normalized data, but was not identified as a DE gene in the quantile-normalized data



Wang et al. *Molecular Biosystems*, 8:818-827, 2012

Simulated data



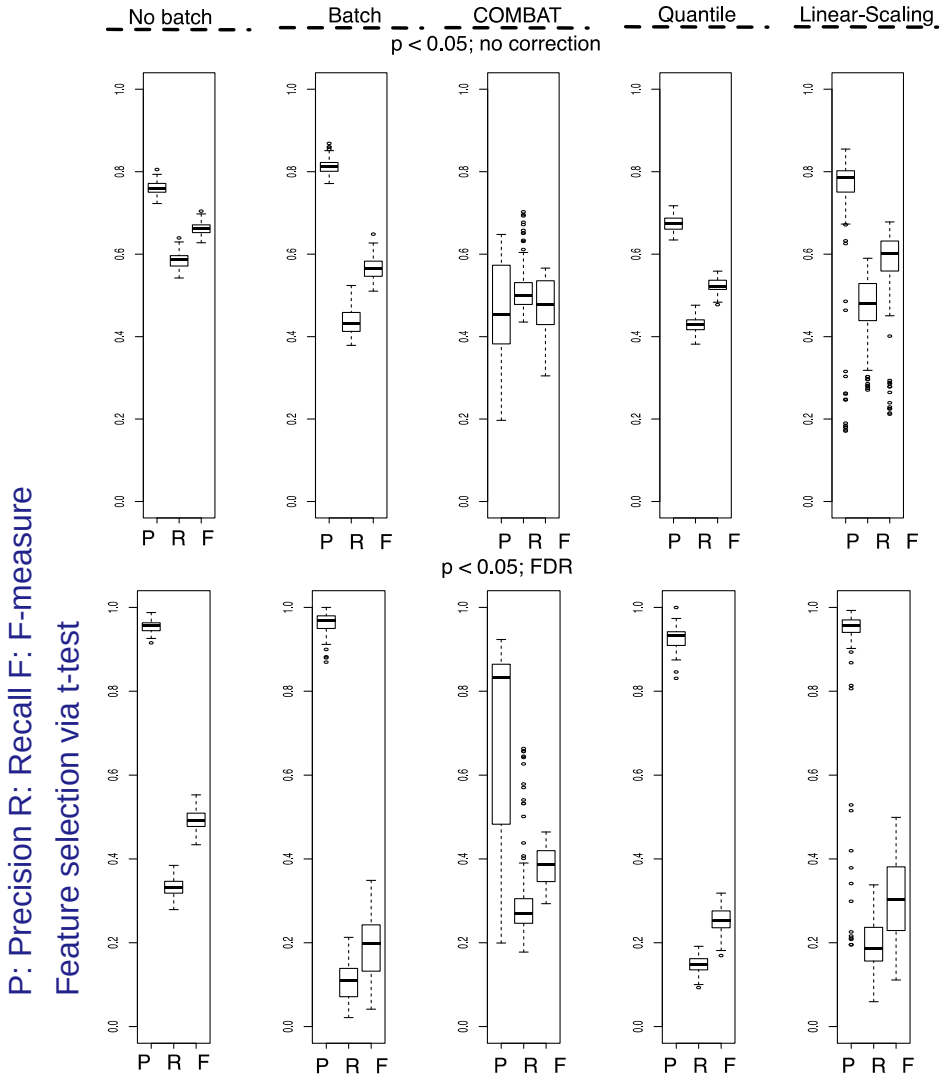
Real one-class data from a multiplex experiment (no batches); n = 8

Randomly assigned into two phenotype classes D and D*, 100x

20% biological features are assigned as differential, and a randomly selected effect size (20%, 50%, 80%, 100% and 200%) added to D*

Half of D and D* are assigned to batch 1, and the other half assigned to batch 2. A randomly selected batch effect (20%, 50%, 80%, 100% and 200%) is added to all features in batch 1

Batch-effect correction can introduce false positives



Precision is strongly affected by batch correction via COMBAT

⇒ False +ve are added post-batch correction. Data integrity is affected

Post-batch correction does not restore performance to where no batch is present

Exercise

Why normalization methods like mean scaling, z-score, and quantile normalization sometimes do not work well?

Suppose you have two batches of gene expression data, and two phenotypes: { (A_1, B_1), (A_2, B_2) }. How should you do quantile normalization?

- $Q(A_1, A_2, B_1, B_2)$
- $Q(A_1, A_2)$, $Q(B_1, B_2)$
- $Q(A_1, B_1)$, $Q(A_2, B_2)$
- $Q(A_1)$. $Q(A_2)$, $Q(B_1)$ $Q(B_2)$

Interesting homework for you

Answer

Mean-scaling

- based on absolute gene expression value
- linearity assumption
- sensitivity to outliers

Z-score normalization

- based on absolute gene expression value
- assumption of gaussian distribution

Quantile normalization

- assumption of identical distribution across samples
- affected by rank instability of low expression genes

These assumptions may not hold

E.g. disease and normal samples are likely to have different gene-expression distributions

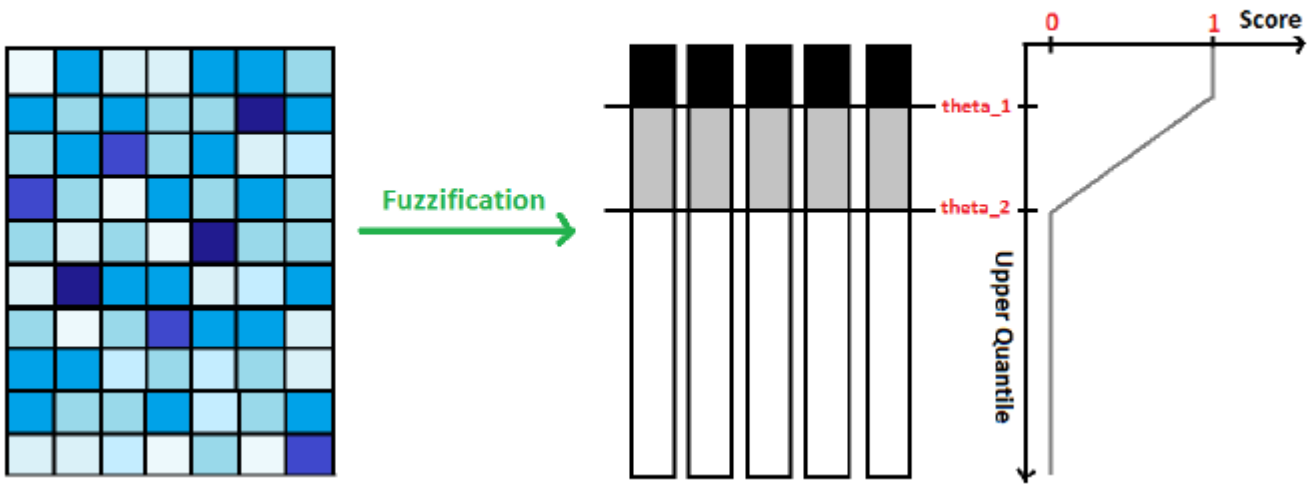
Preprocessing w/ these methods reduces quality of subsequent predictive models in ~25% of the cases

Luo et al. *Pharmacogenomics Journal*, 10(4):278-291, 2010

Belorkar & Wong, “GFS: Fuzzy preprocessing for effective gene expression analysis”, BMC Bioinformatics, 17(S17):1327, 2016

Gene fuzzy score (GFS)

Raw gene expression → gene ranks within microarrays → fuzzified scores



Ranks rather than absolute values

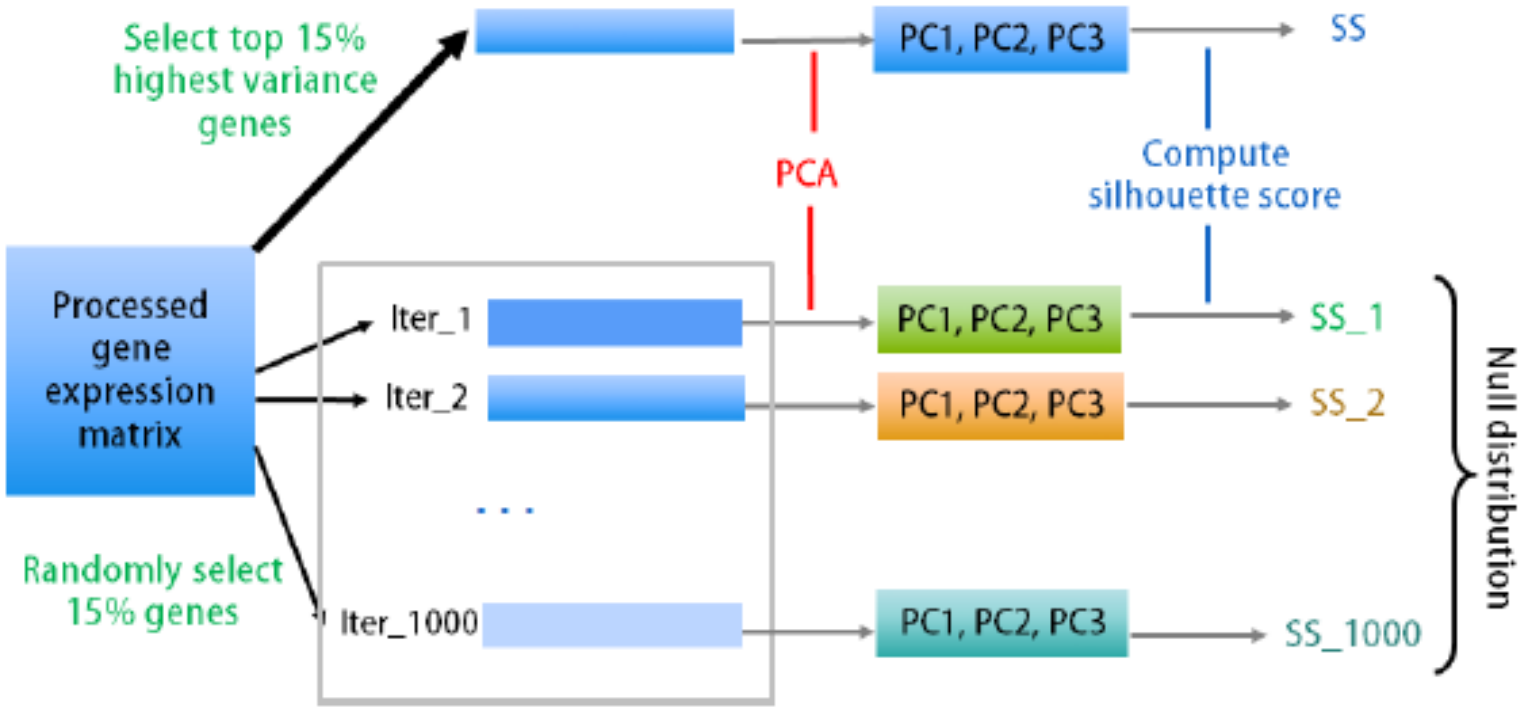
No assumption on identical expression distribution

Fuzzification

Reduced fluctuations from minor rank differences

Noise from rank variation in low-expression genes discarded

Evaluating quality

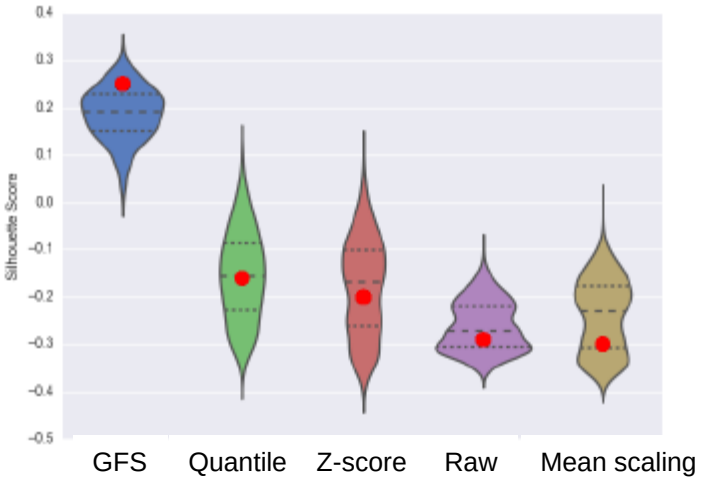


An ideal normalization method should produce a silhoutte score distribution that is high and stable

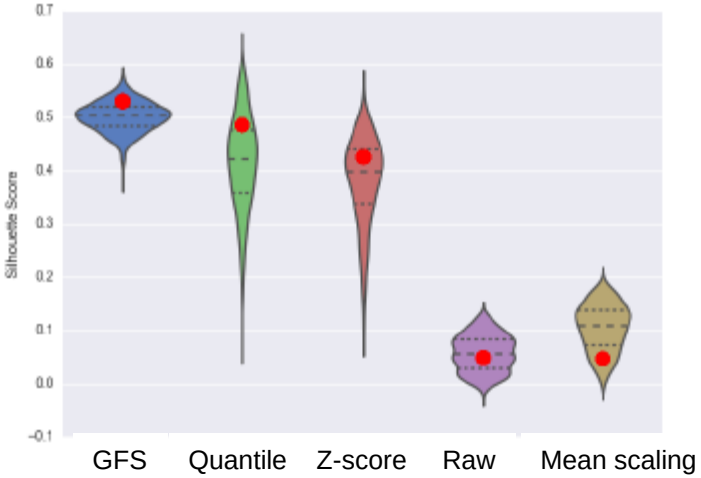
Observations

The GFS null distribution is stable, w/ high silhouette scores

For GFS, the score obtained from the top 15% highest variance genes is always in the top quartile of the null distribution



(a) Acute Lymphoblastic Leukemia (ALL)



(b) Duschenne Muscular Dystrophy (DMD)

PCA FOR ISOLATING BATCH EFFECTS

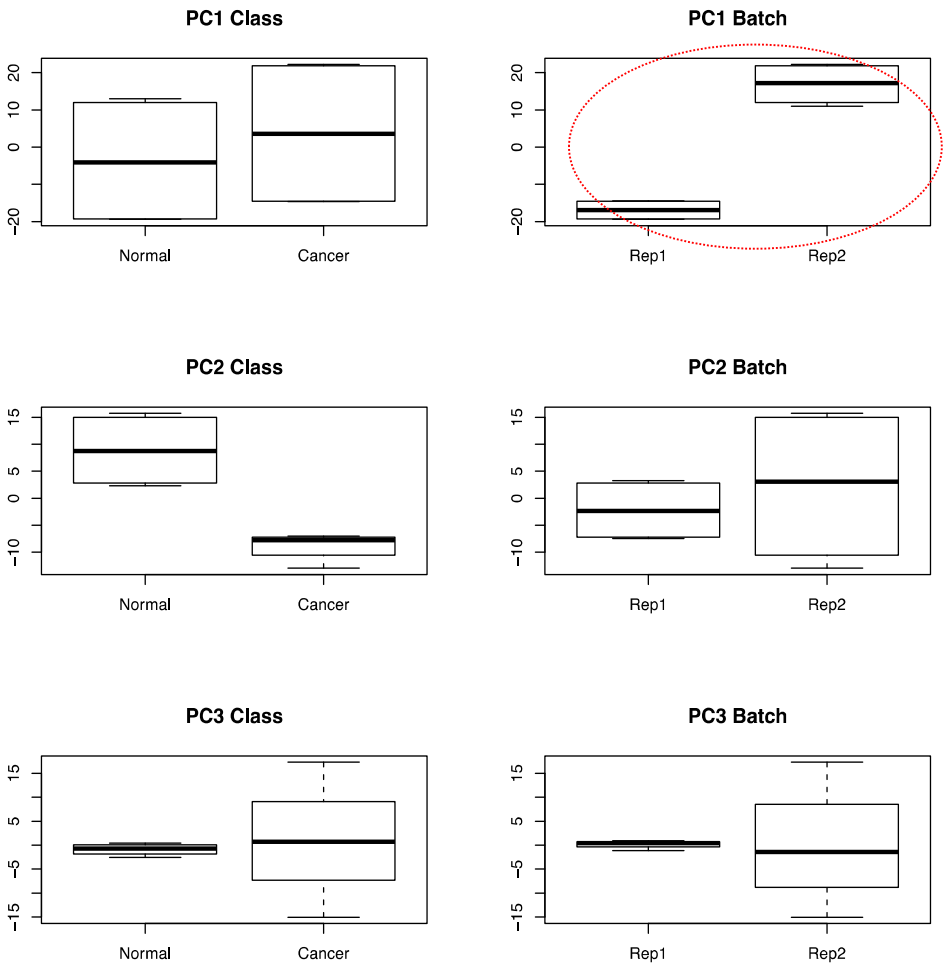
PCA for isolating batch effects

When a batch effect is observed, it is common practice to apply a batch-effect removal or correction method

But this does not necessarily work well in practice. Also, if the data does not fit the correction method's assumptions, it may lead to false positives

Instead, we may opt for a more direct strategy by simply removing PCs (usually PC1) enriched in batch effects, and deploying the remaining PCs as features for analysis

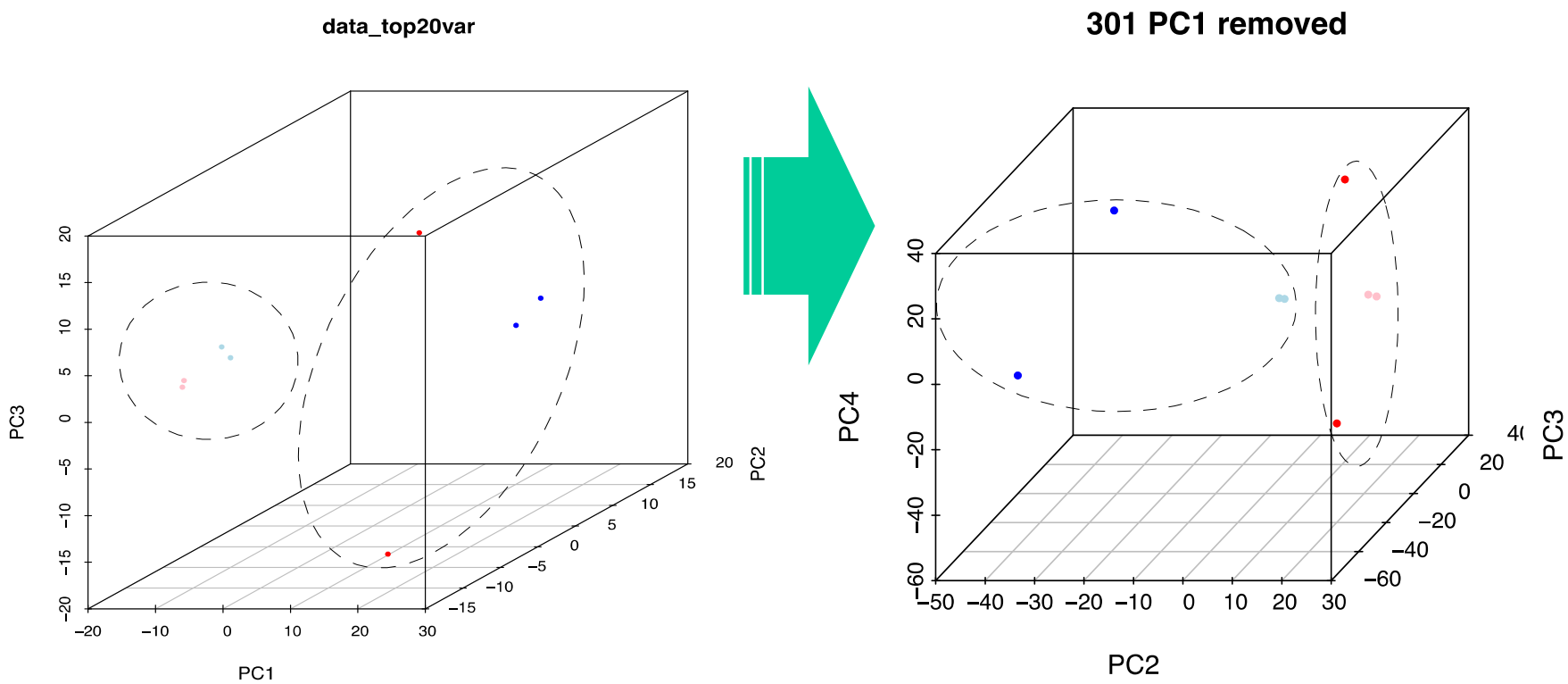
Goh & Wong, “Protein complex-based analysis is resistant to the obfuscating consequences of batch effects”, *BMC Genomics*18(Suppl2):142, 2017



Determine PCs associated with batch using paired boxplots of PCs

Batch effects dominate PC1

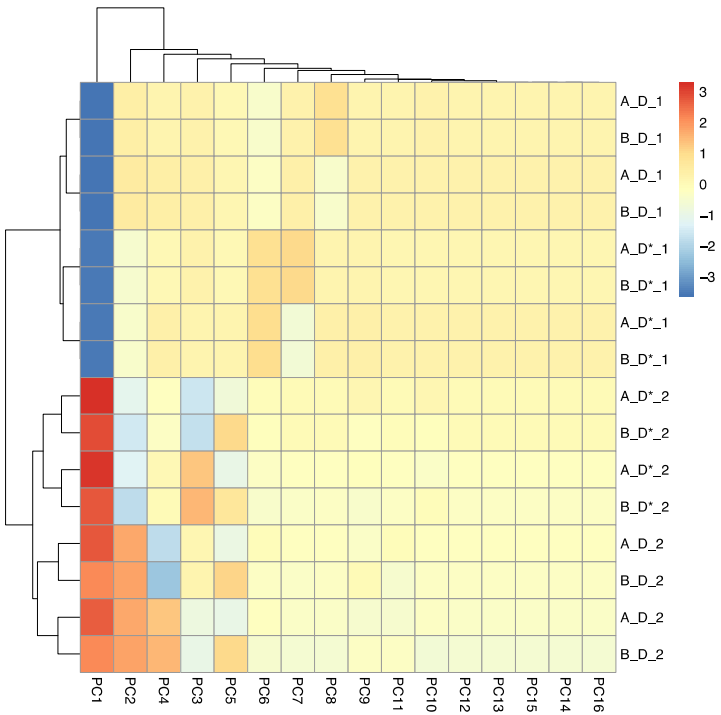
Removal of batch effect-laden PCs removes most batch effects



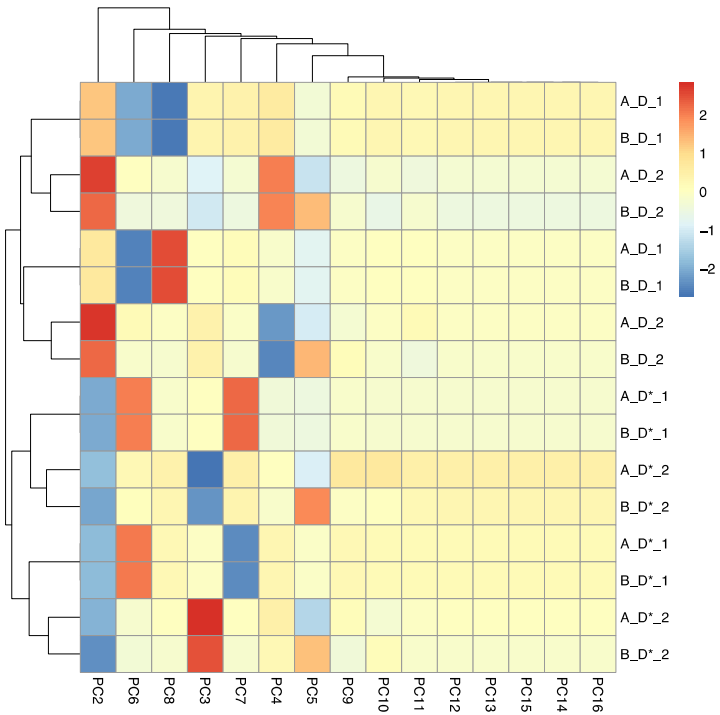
D, Rep 1 D*, Rep 1 D, Rep 2 D*, Rep 2

Samples separate by class post PC1 removal, no batch subgrouping

A and B are different datasets with different batch effects inserted



Batch effects dominate



Class-effect discrimination recovered

(Notation: A/B_D/D*_1/2 refers to the dataset, class and batches respectively)

Exercise

Suggest a modification to the formula below to avoid selecting genes laden with batch effects

PCA can be a useful biomarker-selection approach

- **E.g., biomarkers ≈ genes w/ high loading**
  - Loading of gene x = $\Sigma_j \mid \alpha_{xj} * \sigma_j^2 \mid$, where α_{xj} is coefficient of x in PC_j, and σ_j^2 is variance of PC_j

Answer

Suggest a modification to the formula below to avoid selecting genes laden with batch effects

PCA can be a useful biomarker-selection approach

- **E.g., biomarkers ≈ genes w/ high loading**
  - Loading of gene x = $\Sigma_j \mid \alpha_{xj} * \sigma_j^2 \mid$, where α_{xj} is coefficient of x in PC_j, and σ_j^2 is variance of PC_j

Restrict the summation to PCs that are not laden w/ batch effects

BATCH EFFECT-RESISTANT FEATURE SELECTION

What if class and batch effects are strongly confounded?

Neither batch-effect correction nor PCA work well

We also do not want to inadvertently lose information on disease subpopulations (which look like batch effects but are meaningful)

⇒ Consider using protein complexes / subnetworks of biological pathways as biomarkers / context for biomarker selection

FSNET

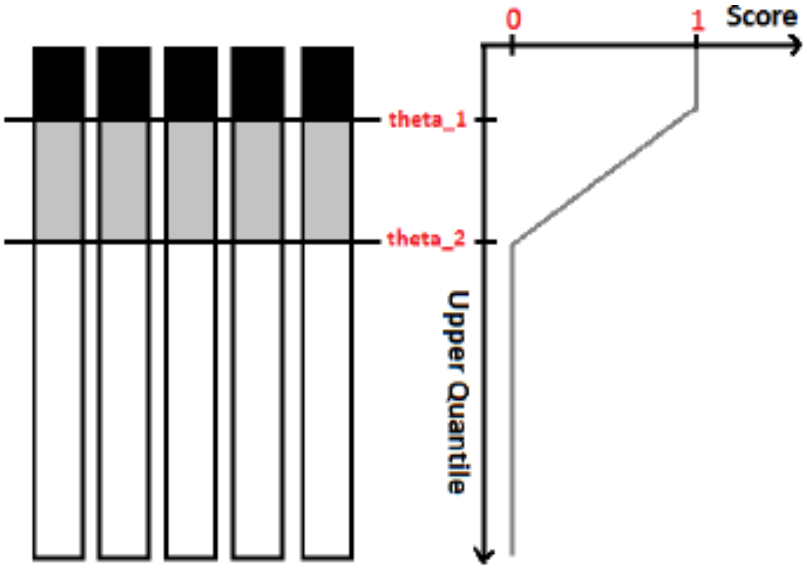
FSNET --- a protein complex-based feature-selection methods. Use expression rank-based weighting method (viz. GFS) on individual proteins, followed by intra-class-proportion weighting

And for comparison …

SP is the protein-based two-sample t-test

Goh & Wong, "Protein complex-based analysis is resistant to the obfuscating consequences of batch effects", *BMC Genomics*, 18(Suppl 2):142, 2017

FSNET



β(g,C)

Proportion of tissues in class C that have protein g among their most-abundant proteins

$$\beta(g_i, C_j) = \sum_{pk \epsilon c_j} \frac{fs(g_i, p_k)}{|C_j|}$$

Score(S,p,C)

Score of protein complex S and tissue p weighted based on class C

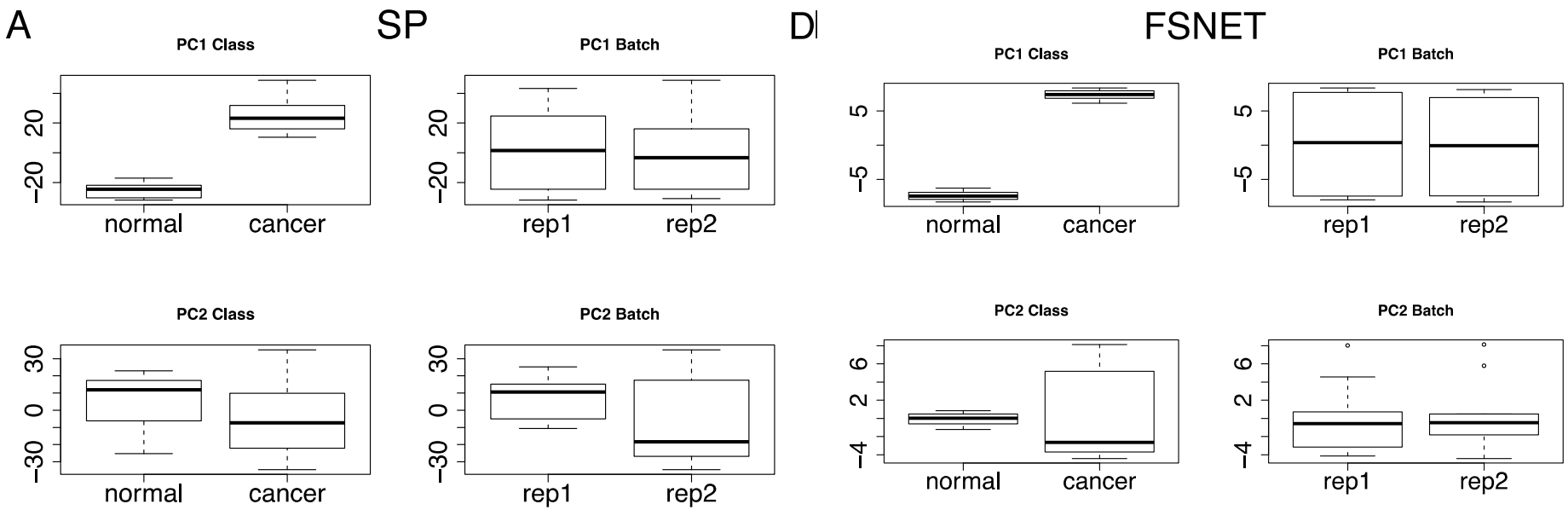
$$\text{score}(S, p_k, C_j) = \sum_{g_i \in S} fs(g_i, p_k) * \beta(g_i, C_j)$$

f_{SNET}(S,X,Y,C)

Complex S is differentially high in sample set X and low in sample set Y, weighted based on class C, when f_{SNET}(S,X,Y,C) is at largest 5% extreme of t-distribution

$$f_{\text{SNET}}(S, X, Y, C_j) = \frac{\text{mean}(S, X, C_j) - \text{mean}(S, Y, C_j)}{\sqrt{\frac{\text{var}(S,X,C_j)}{|X|} + \frac{\text{var}(S,Y,C_j)}{|Y|}}}$$

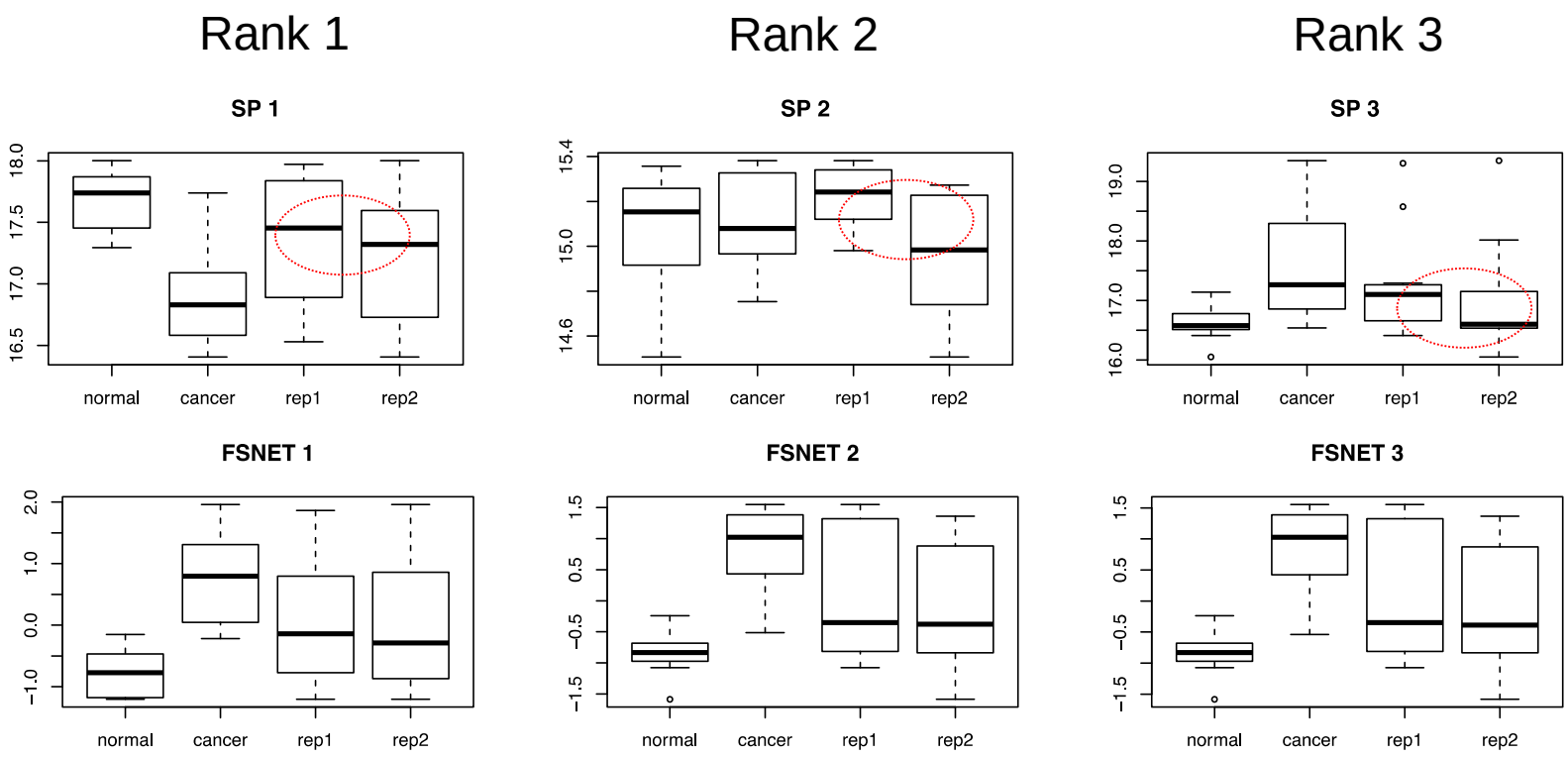
Network-based methods are enriched for class-related variation (Real data)



PCA on SP-selected genes: Class & batch effects are confounded; cf. PC2

PCA on FSNET-selected complexes: Class & batch effects are less confounded in top PCs

Top complex-based features are strongly associated with class, not batch



FSNET captures class effects & is robust against batch effects. In contrast, both class and batch variability are present in the top variables selected by SP

CONCLUDING REMARKS

What have we learned?

PCA is a useful paradigm for biomarker selection

PCA is not just a visualization tool; it can also be used for dealing with batch effects

When class & batch effects are deeply confounded at the level of proteins / genes, it is might be better to analyze at the level of protein complexes / pathway subnetworks

References

[PCA] Jolicoeur & Mosimann, *Growth*, 24:339-354, 1960

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[Batch effects] Leek et al., *Nature Reviews Genetics*, 11:733-739, 2010

[Batch effects] Wang et al., *Molecular Biosystems*, 8:818-827, 2012

[GFS] Belorkar & Wong. *BMC Bioinformatics*, 17(Suppl 17):540, 2016

[FSNET] Goh & Wong, *BMC Genomics*, 18(Suppl 2):142, 2017