♠ Principal Components and Singular Factors

- $p \times n$ data matrix $\mathbf{X}$ (tall, skinny): rows are genes, columns are samples/microarrays
- Correlations, relationships, patterns among genes:
  - clustering, similar expression patterns
  - co-regulated genes, (up/down), genetic pathways
- Correlations, relationships, patterns among samples:
  - different tumor types, clinical outcomes, cell cycle positions, tumor or normal, ...
- Correlation is a global measure: decomposes into constituent sources using
  - principal components analysis (PCA), or (equivalently)
  - singular value decomposition (SVD) for singular factor analysis

♠ Motivating PCA and Factors: Two genes

- Two genes: gene $1, 2$, sample j, expression levels $x_{1,j}, x_{2,j}$ for $j = 1, ..., n$
- Imagine that there are numbers b_1, b_2 and $f_1, \ldots, f_n$ such that

$$x_{1,j} \approx b_1 f_j \quad \text{and} \quad x_{2,j} \approx b_2 f_j$$

  or, better,

$$x_{1,j} = b_1 f_j + \epsilon_{1,j} \quad \text{and} \quad x_{2,j} = b_2 f_j + \epsilon_{2,j}$$

  for "small" ϵs and the ϵs are uncorrelated.
- f_j is the value of a *factor* on sample j, and the factor determines all of the correlation (relationship) between levels of expression on genes 1 and 2
- e.g., $b_1 = b_2$, so $x_{1,j} \approx x_{2,j}$ or at least highly correlated
- e.g., $b_1 = 1, b_2 = -1$, so $x_{1,j} \approx -x_{2,j}$, highly negatively correlated
- e.g., $b_1 = 0$, so $x_{1,j}$ and $x_{1,j}$ are uncorrelated
- Linear regression format: the factor variable is a predictor of each x in the two linear regression models.

- Now suppose the ϵs are not so small, and are themselves related between gene 1 and 2
- Apply the same idea to the ϵs – introduces a second factor
- Then

$$x_{1,j} = b_{1,1} f_{1,j} + b_{1,2} f_{2,j} + \epsilon^*_{1,j} \quad \text{and} \quad x_{2,j} \approx b_{2,1} f_{1,j} + b_{2,2} f_{2,j} + \epsilon^*_{2,j}$$

  (relabelling $b_1 \to b_{1,1}, b_2 \to b_{2,1}$ and $f_j \to f_{1,j}$)
- If the $f_{1,j}$ are uncorrelated with the $f_{2,j}$, this describes patterns of dependence between xs 'driven' by the two separate, unrelated factors
- Linear regression format: the 2 factor variables are predictors of each x in the two linear regression models.

♠ PCA and Factor Decompositions

- $p \times n$ data matrix $\mathbf{X}$ – p genes, n samples
- Take row (gene) i and sample (microarray) j
- Singular value decomposition (SVD) of $\mathbf{X}$ can be expressed *exactly* as

$$x_{i,j} = b_{i,1} f_{1,j} + b_{i,2} f_{2,j} + \cdots + b_{i,n-1} f_{n-1,j} + b_{i,n} f_{n,j}$$

for some numbers $b_{\cdot\cdot}$ and $f_{\cdot\cdot}$
(this is just linear algebra; no statistics, and no magic).

- Generally, higher order $b_{i,n}$ terms are small, so

$$x_{i,j} = b_{i,1}f_{1,j} + b_{i,2}f_{2,j} + \cdots + b_{i,k}f_{k,j} + \epsilon_{i,j}$$

for some $k < n$ and some 'small' terms $\epsilon_{i,j}$ that are uncorrelated across genes i and arrays j (i.e., they are small, residual 'noise' terms)
- Linear regression format: the k factor variables are predictors of each of the p x response variables in p separate, parallel linear regression models.
- The k factor variables explain variability in the expression patterns of the many genes and represent k different aspects of the correlations, structure, patterns exhibited among the genes and across samples
- The regression parameters $b_{i,r}$ for gene i represent different weightings, or loadings, on factor r for this gene – the factors influence/explain the variation in genes differently due to differing values of these loadings.
- The factors have various alternative names: principal components, principal factors, singular factors, among others

♠ PCA and Factor Decompositions: Matrix/vector form

- Sample $j = 1, \ldots, n$, with $\mathbf{x}_j$ = column j of $\mathbf{X}$

$$\mathbf{x}_j = \mathbf{b}_1 f_{1,j} + \mathbf{b}_2 f_{2,j} + \cdots + \mathbf{b}_n f_{n,j}$$

or

$$\mathbf{x}_j = \sum_{r=1}^{n} \mathbf{b}_r f_{r,j}$$

where each $\mathbf{b}_r$ is a $p \times 1$ column vector of the loadings for all genes on factor r as $r = 1, \ldots, n$
- Or,

$$\mathbf{x}_j = \mathbf{B}\mathbf{f}_j$$

with $\mathbf{B} = [\mathbf{b}_1, \ldots, \mathbf{b}_n]$ and

$$\mathbf{f}_j = \begin{pmatrix} f_{1,j} \\ f_{2,j} \\ \vdots \\ f_{n,j} \end{pmatrix}$$

- Or,

$$\mathbf{X} = \mathbf{B}\mathbf{F}$$

where now $\mathbf{F} = [\mathbf{f}_1, \mathbf{f}_2, \ldots, \mathbf{f}_n]$

♠ Important Mathematical features

- The factor variables are uncorrelated, so represent different underlying sources of covariability in the $\mathbf{X}$ data
- Sample correlations between any two factor variables is zero
- Sample variance of each factor variable is 1
- Formally, $\mathbf{F}$ is an *orthogonal* matrix: $\mathbf{F}'\mathbf{F} = \mathbf{I}$ and $\mathbf{F}\mathbf{F}' = \mathbf{I}$, where $\mathbf{I}$ is the $p \times p$ identity matrix

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$$\mathbf{X} = \mathbf{BF} = \mathbf{ADF}$$

with
  - $n \times n$ diagonal $\mathbf{D} = \text{diag}(d_1, \ldots, d_n)$ of non-negative values in decreasing order
  - the *singular values of* $\mathbf{X}$
  - $\mathbf{A}$ is $p \times n$ matrix such that $\mathbf{B} = \mathbf{AD}$
  - $\mathbf{A}$ has *orthonormal columns*: $\mathbf{A}'\mathbf{A} = \mathbf{I}$ the $p \times p$ identity matrix
- In terms of $\mathbf{A}, \mathbf{D}$, $\mathbf{x}_j = \mathbf{ADf}_j$ or

$$x_{i,j} = a_{i,1}d_1 f_{1,j} + a_{i,2}d_2 f_{2,j} + \cdots + a_{i,n}d_n f_{n,j}$$

- Singular values describe relative importance of factors in describing relationships and variability in data matrix
- Percent "total variation explained" by factor j is $100d_j^2 / \sum_{i=1}^n d_i^2$
- Elements in column r of $\mathbf{A}$ describe relationships among genes due to factor r
- Elements in rows r of $\mathbf{F}$ describe relationships among samples/microarrays due to factor r

♠ Properties and More Interpretation

- The factors (principal components) are themselves linear combinations of the data variables, namely $\mathbf{f}_j = \mathbf{D}^{-1}\mathbf{A}'\mathbf{x}_j$ for each sample j, or

$$f_{i,j} = a_{1,i}d_1^{-1}x_{1,j} + a_{2,i}d_2^{-1}x_{2,j} + \cdots + a_{p,i}d_n^{-1}x_{p,j}$$

- In fact, among all possible linear combinations of the data variables, the factors are those that explain the most "variability" in the x data, in the sense that
  - The first factor is the linear combination of the data that has the largest sample variance. (For any vector $\mathbf{c}$, compute the n values $\mathbf{c}'\mathbf{x}_j$, $(j = 1, \ldots, n)$, and then find the sample variance of these n values; choose another vector $\mathbf{c}$, do it again; the largest variance arises when $\mathbf{c}$ is the first column of $\mathbf{AD}^{-1}$ so that the linear combination is the first factor.)
  - The second factor is the linear combination of the data that has the the largest sample variance once corrected for the first factor (and subject to being orthogonal)
  - Many other properties (e.g., see Ripley section 9.1)

♠ Plotting Data and Factors

- Often informative displays are achieved by plotting factors against sample number and scatter plotting data on pairs of factors
- Useful for discrimination of samples: finding patterns and structure in the n samples that may be related to a biological state or features (e.g., tumor versus normals)
- First factor often represents average levels of genes in each sample
- Clustering methods can be applied, often most usefully, to factors rather than the full data set – computational efficiencies
- Higher order factors can represent small, idiosyncratic features in data
- Factors can be most useful in regression models as predictors of outcomes

♠ Practical consideration

- PCA/SVD depends on scale of measurement of variables
- Gene expression on a standard scale – same for all genes
  - best on some kind of log scale
  - require normalisation off all arrays to a standard scale