Do my clusters make sense? Some statistical and biological clues to help..

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DIGIT-BIO seminar @ Ecully

Outline

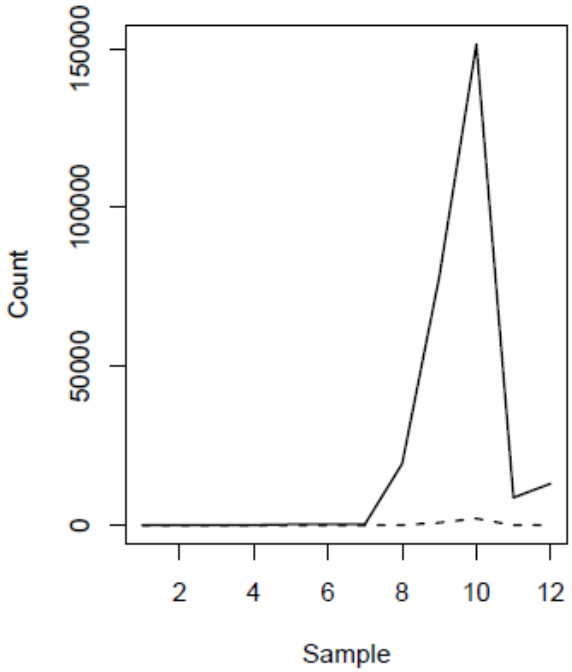
Gene co-expression is...

- Simultaneous expression[1] or co-transcription[2] of several genes
- Similarity[3] (correlation, mutual information, ...) of expression patterns over a range of different experiments[4]

Related to shared regulatory inputs,
functional pathways, and biological process(es)[5]
+ a tool to study genes without known or predicted function

[1] https://en.wiktionary.org/wiki/coexpression
[2] http://bioinfow.dep.usal.es/coexpression
[3] http://coxpresdb.jp/overview.shtml
[4] Yeung *et al.* (2001)
[5] Eisen *et al.* (1998)

RNA-seq co-expression: counts, transformed counts, or profiles?



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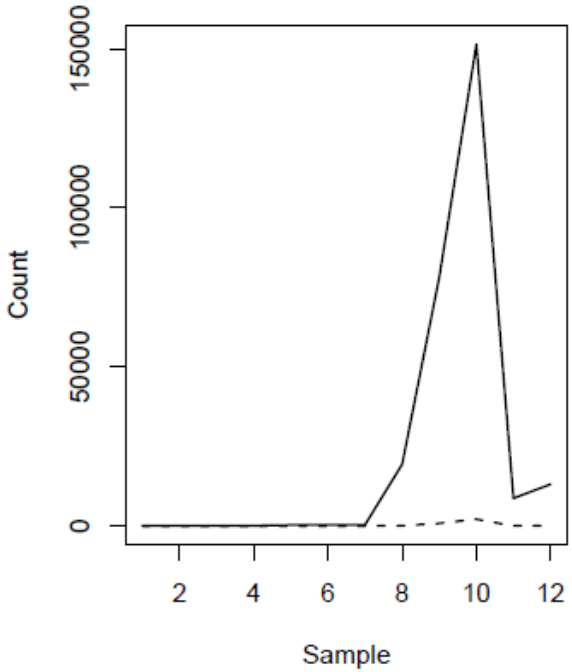

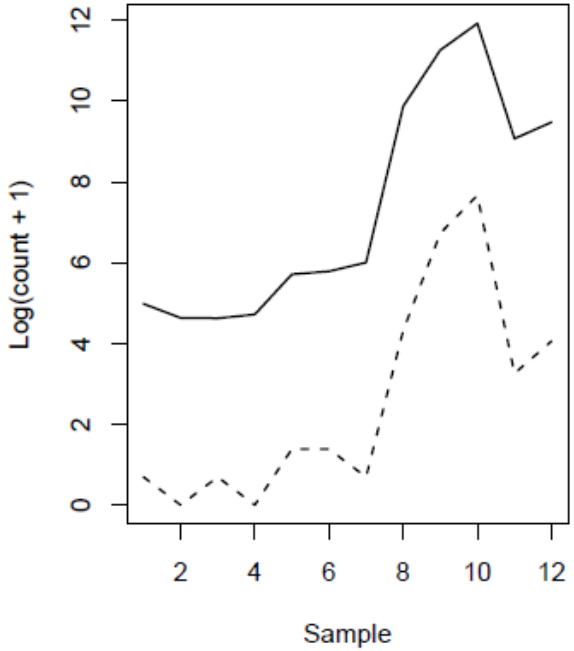

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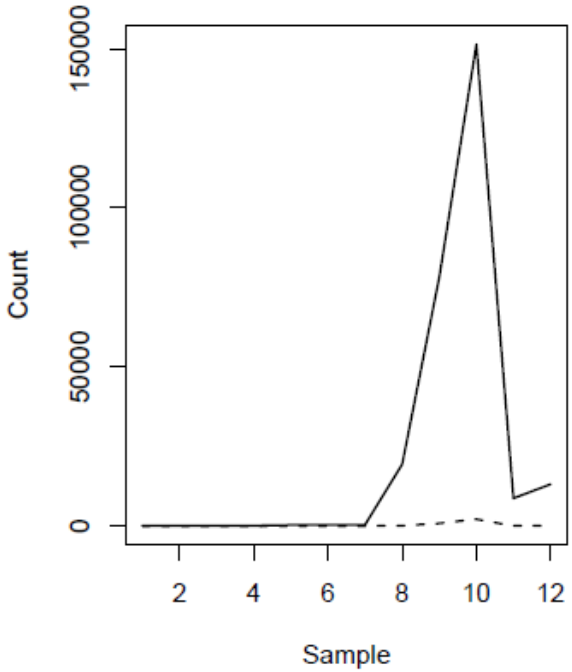
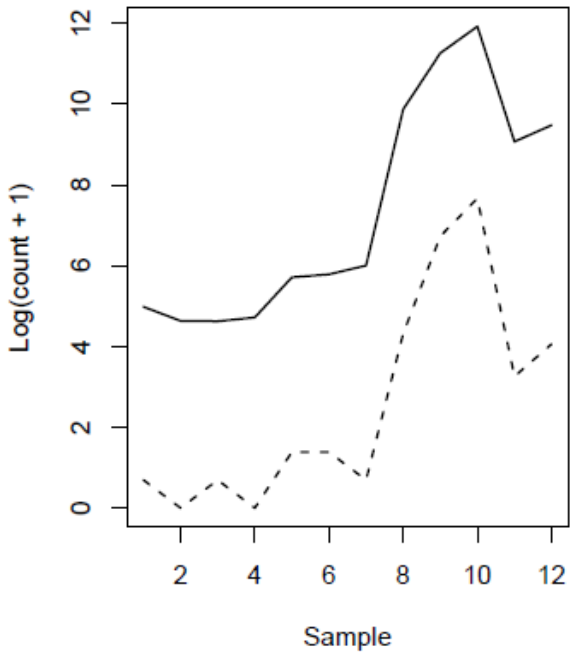
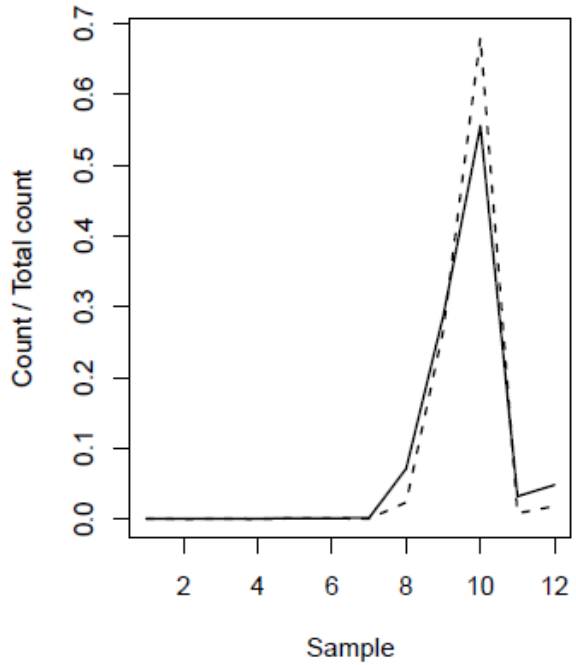






- y_{ij} = raw count for gene i in sample j, with library size normalization factor s_j
- Normalized profile for gene i: $p_{ij} = \frac{y_{ij}/s_j}{\sum_\ell y_{i\ell}/s_j}$

Unsupervised classification (aka clustering)

Objective

Define homogeneous and well-separated groups of genes from transcriptomic data

What does it mean for a pair of genes to be close?
Given this, how do we define groups?

Two broad classes of methods typically used:

1. Centroid-based clustering (K-means and hierarchical clustering)
2. Model-based clustering (mixture models)

Model-based clustering for co-expression

Assume data $\mathbf{y}$ come from K subpopulations, each modeled separately:

$$f(\mathbf{y}|K, \boldsymbol{\Psi}_K) = \prod_{i=1}^{n} \sum_{k=1}^{K} \pi_k f_k(\mathbf{y}_i; \boldsymbol{\theta}_k)$$

- $\boldsymbol{\pi} = (\pi_1, \ldots, \pi_K)'$ are the mixing proportions, where $\sum_{k=1}^{K} \pi_k = 1$
- f_k are the densities of each of the components

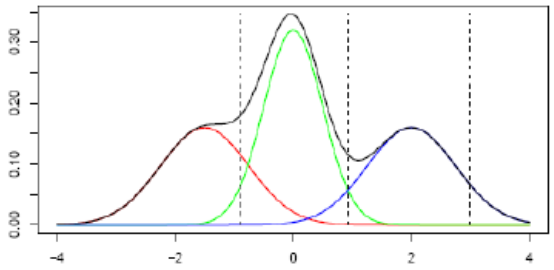
- Microarrays: typically assume $\mathbf{y}_i|k \sim \text{MVN}(\boldsymbol{\mu}_k, \Sigma_k)$
- RNA-seq: Poisson distribution for counts (`HTSCluster`), Gaussian distribution for arcsine or CLR-transformed normalized profiles (`coseq`)

$\rightarrow$ Estimation (EM algorithm), model selection (BIC/ICL/slope heuristics), ...
See DIGIT-BIO *Concepts en IA* talk by Cathy Maugis-Rabusseau for more!

From finite mixtures to clusters

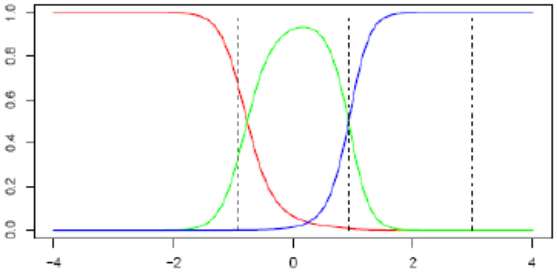
Distributions:

$$g(x) = \pi_1 f_1(x) + \pi_2 f_2(x) + \pi_3 f_3(x)$$



Conditional probabilities:

$$\tau_{ik} = \frac{\pi_k f_k(x_i)}{g(x_i)}$$



Maximum a posteriori (MAP) rule: Assign genes to the component with highest conditional probability τ_{ik}:

τ_{ik} (%)	$k = 1$	$k = 2$	$k = 3$
gene 1	65.8	34.2	0.0
gene 2	0.7	47.8	51.5
gene 3	0.0	0.0	100
...	...	...	...

Outline

Why validate co-expression clusters?

- Avoid finding patterns in noise: does non-random structure actually exist in the data?
- Evaluate fit of clustering on data, compare different algorithms or results ⇒ Internal
- Determine "correct" number of clusters

- Determine robustness of clustering results ⇒ Stability

- Compare clusters to externally known labels
- Characterize clusters with respect to externally known information ⇒ External

Internal cluster validation

Evaluate goodness of clustering structure using data alone ⇒ similarity of genes within cluster *vs* distinctness of genes between clusters

- Compactness, or within-cluster variation
- Connectivity, or extent to which genes clustered together are also neighbors in the data space
- Separation between clusters

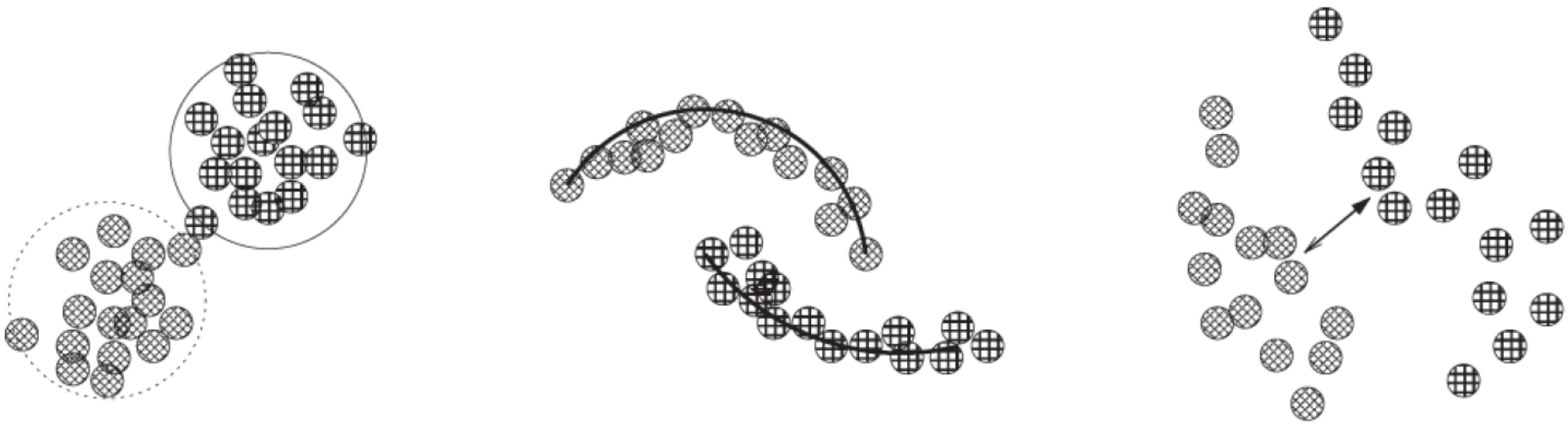


Image: 10.1093/bioinformatics/bti517

Examples of internal validation metrics

- Silhouette statistic: measure of how closely data within a cluster is matched (compactness) and how loosely it is matched to neighboring clusters (separation)

$$a(i) = \frac{1}{|C_I| - 1} \sum_{j \in C_I, i \neq j} d(i,j) \text{ and } b(i) = \min_{J \neq I} \frac{1}{|C_J|} \sum_{j \in C_J} d(i,j), \text{ for } i \in C_I$$

$$s(i) = \frac{b(i) - a(i)}{\max\{a(i), b(i)\}}, \qquad -1 \leq s(i) \leq 1$$

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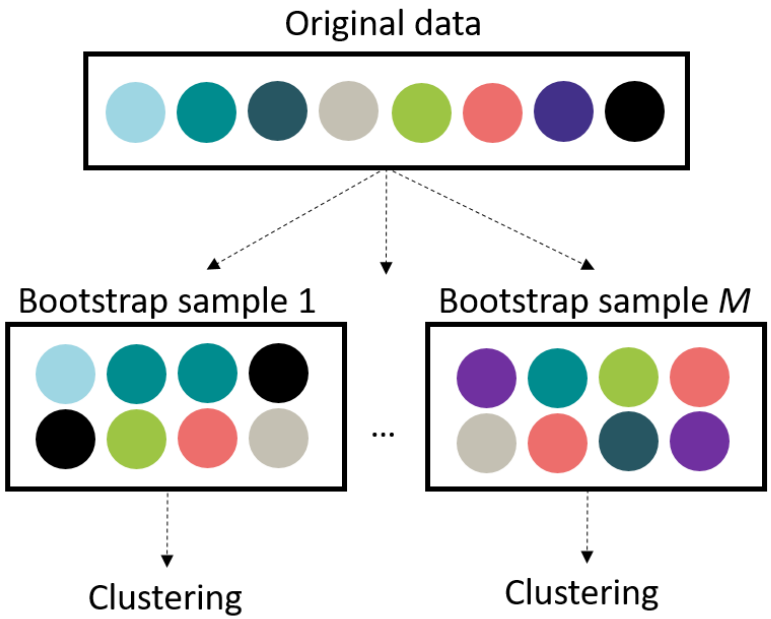
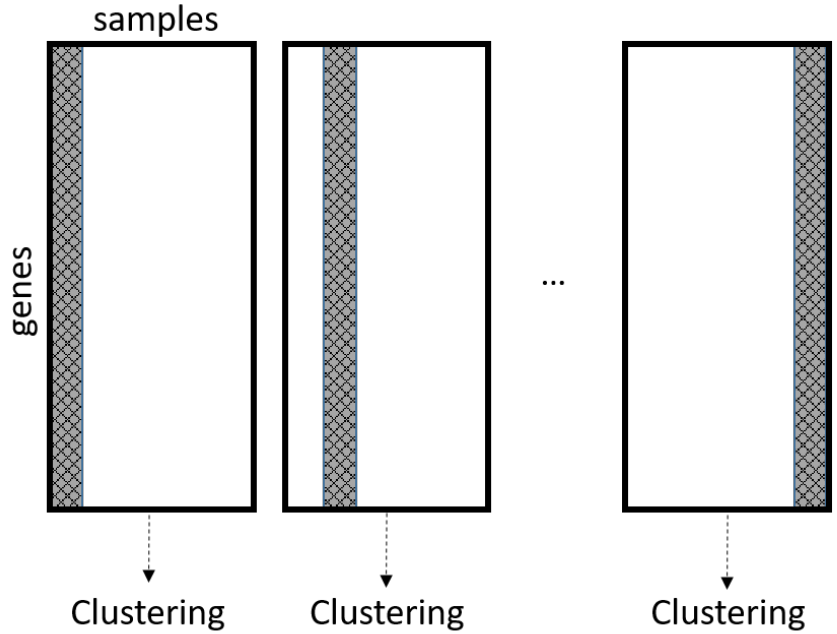
- Dunn index: ratio of smallest distance between clusters and their diameter (largest intra-cluster distance)

$$\text{DI} = \frac{\min\limits_{C_I, C_J, I \neq J} \left(\min\limits_{i \in C_I, j \in C_J} d(i,j) \right)}{\max\limits_{C_M} \text{diam}(C_M)}$$

where $\text{diam}(C_M)$ is maximum distance among $i \in C_M$, $0 < \text{DI}$ (maximize)

Validation of cluster stability





- `fpc`: resampling of data using bootstrap $\rightarrow$ calculate Jaccard similarities to original clusters
- `clValid`: remove columns one by one, compare to clustering from full data (several criteria proposed)

External cluster validation

Evaluate ability of clustering algorithm to produce biologically meaningful clusters with respect to:

- Gene ontology (GO) term annotations
- A priori functional categorizations of genes
- Pathway membership
- ...

$\Rightarrow$ Assume set of F known biological classes (not necessarily disjoint)

Examples of external validation metrics: *comparison*

- Adjusted Rand Index (ARI): corrected-for-chance Rand index

$$R = \frac{\mathrm{TP} + \mathrm{TN}}{\mathrm{TP} + \mathrm{FP} + \mathrm{FN} + \mathrm{TN}} \in [0, 1]$$

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- Biological Homogeneity Index (BHI): if $B(i)$ is the known biological class of gene i,

$$\text{BHI} = \frac{1}{K} \sum_{k=1}^{K} \frac{1}{n_k(n_k - 1)} \sum_{i \neq j \in C_k} I(B(i) = B(j)) \in [0, 1]$$

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- Biological Stability Index (BSI):

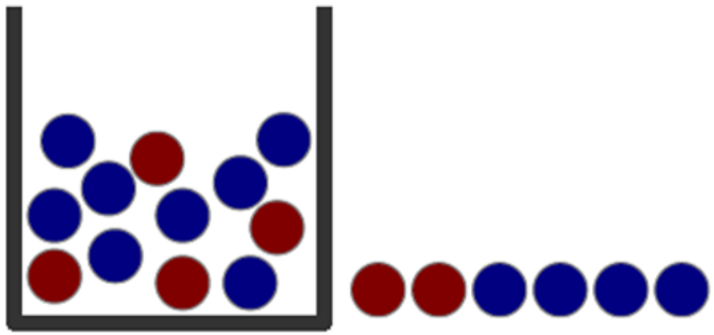
$$\text{BSI} = \frac{1}{F} \sum_{k=1}^{F} \frac{1}{|B_k|(|B_k| - 1)M} \sum_{\ell=1}^{M} \sum_{i \neq j \in B_k} \frac{|C^{i,0} \cap C^{j,\ell}|}{|C^{i,0}|} \in [0, 1]$$

based on removing each of the M data columns one at a time

Examples of external validation metrics: *characterization*

What biological processus are over-represented in each cluster?

- Gene Ontology (GO) terms = group genes into categories by a common biological property
- Assumptions: under the null hypothesis, genes are independent and equally likely to be grouped together in the list of interest (= cluster)
- Test for over-representation using a hypergeometric distribution (Fisher's exact test)



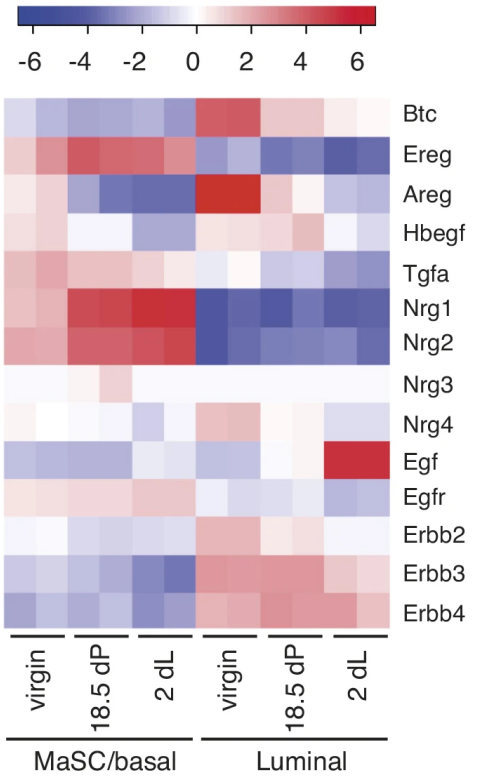
	Red	Blue	Total
Chosen	2	4	6
Remaining	4	8	12
Total	6	12	18

Outline

Data description: Fu *et al.* (2015)

- RNA-seq experiment to study lineage of luminal cells in mouse mammary gland and changes in expression upon pregnancy & lactation

- 2 cell types {basal stem-cell enriched cells, committed luminal cells} × 3 statuses {virgin, pregnant, lactating} × 2 biological replicates
  - Illumina HiSeq → 30 million 100bp SE reads
  - *Pre-processing*: Subread to align reads to mm10 genome + featureCounts for quantification of Entrez genes (RefSeq)
  - *Initial analysis*: filter genes with weak expression or unambiguous/missing IDs + `DESeq2` normalization/differential analysis



Supp Fig 4: 10.1038/ncb3117

Outline

Validating clustering approaches in practice

Clustering results can be evaluated based on internal and stability criteria (e.g., statistical properties of clusters) or external criteria (e.g., functional annotations)

- Preprocessing steps will affect clustering outcome
- Methods that give different results depending on the initialization should be rerun multiple times to check for stability
- Repeated subsampling to identify consensus clusters (`ConsensusClusterPlus`)

- Most methods will find clusters even when no structure is present ⇒ good idea to compare to results with randomized data

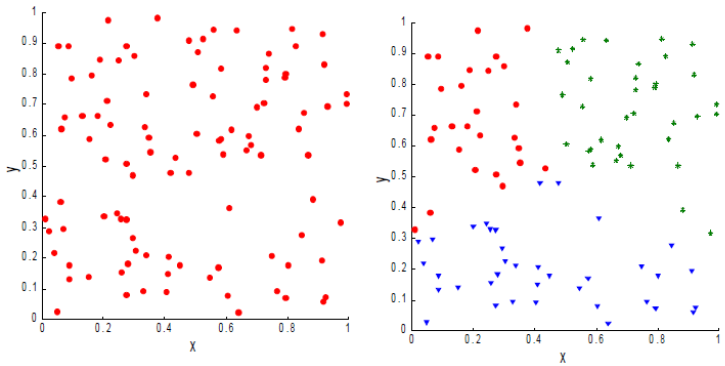


Image: Rumoing Jin, *Cluster validation*

Final thoughts[6]

> There is no single best criterion for obtaining a partition because no precise and workable definition of *cluster* exists. Clusters can be of any arbitrary shapes and sizes in a multidimensional pattern space. Each clustering criterion imposes a certain structure on the data, and if the data happen to conform to the requirements of a particular criterion, the true clusters are recovered.

[6] Jain & Dubes, 1988

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Some useful references

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