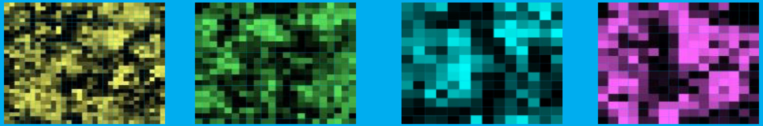
Gene Set Enrichment Analysis (GSEA) Part I



Network Analysis in Systems Biology

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Introduction to GSEA

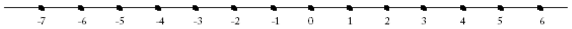
- Microarray experiments give the expression level of many genes.
- We would like to evaluate the difference in expression between two conditions, e.g., diseased and normal
- Traditionally methods look for individual genes which are differentially expressed between the two conditions but this has problems:
  - No single gene may stand out in the noise
  - Many genes may stand out but without any unifying biological theme
- GSEA looks for sets of genes which are differentially expressed, the advantages being:
  - A set of genes may be more likely to stand out (larger signal-noise ratio)
  - Biological theme is integral, and aids understanding and further investigation.

Elements of GSEA

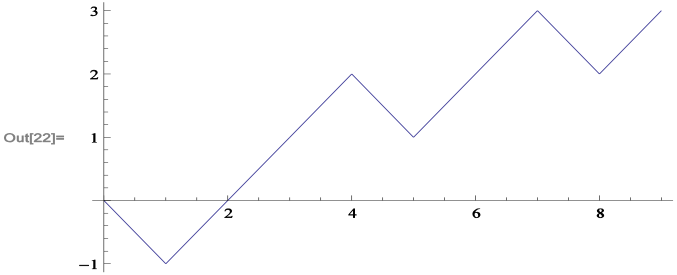
- One dimensional random walks
    - The Weiner process
    - The Brownian bridge
- The Kolmogorov-Smirnov test
    - Probability distributions
    - Statistical test of ‘goodness of fit’
- The GSEA test
    - The statistical test and evaluation of significance
    - An example from the literature

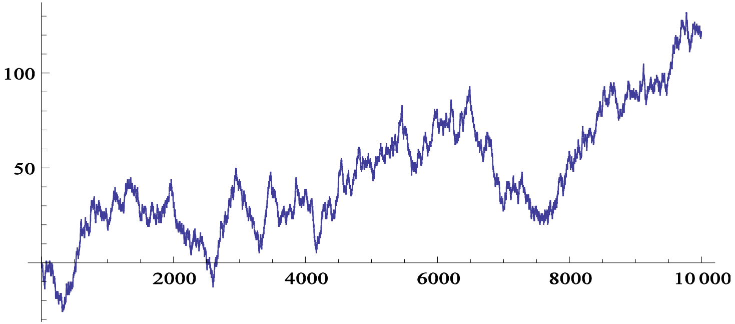
One-Dimensional Random Walks

Random walk on a one dimensional lattice



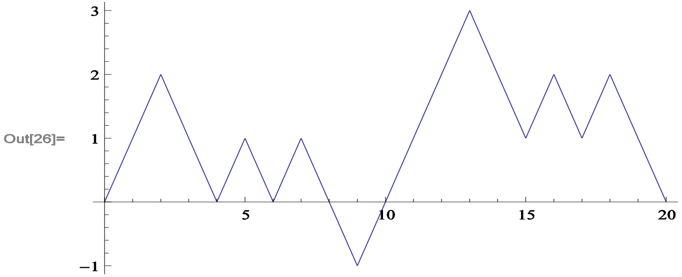
- Start at 0 and take discrete steps, left or right with equal probability.
- Can plot a random walk on a graph (upper right)
- It is possible to show that after *n* steps the mean distance from the starting point is proportional to $\sqrt{n}$
- With more steps comes more fluctuations (lower right)
- As the number of steps tends to infinity while the length of each step tends to zero the random walk tends to a ‘Weiner process’.

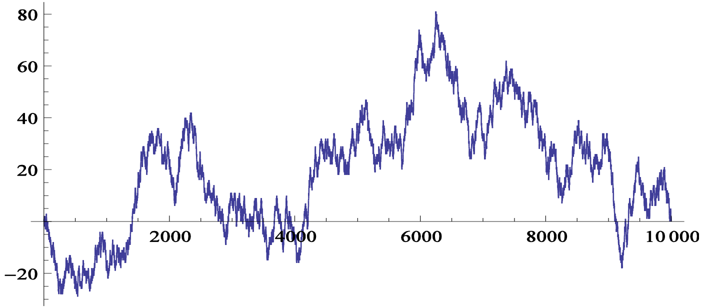




The Brownian Bridge

- A random walk with the end points fixed at zero is shown in the upper right figure
- As before we can plot the same kind of walk but with more steps, and see a broader range of fluctuations
- Let the number of steps go to infinity while letting the step size tend to zero, and this becomes the 'Brownian Bridge', *B(t)*
- May ask how far from the fixed end points is the walk likely to travel in the course of the Brownian Bridge.
- This maximum displacement is called the 'Supremum' of the Bridge, and has a cumulative probability distribution:





$$cdf(x) = 1 - 2\sum_{i}^{\infty}(-1)^{i-1}e^{-2i^2x^2}$$

Probability Distributions

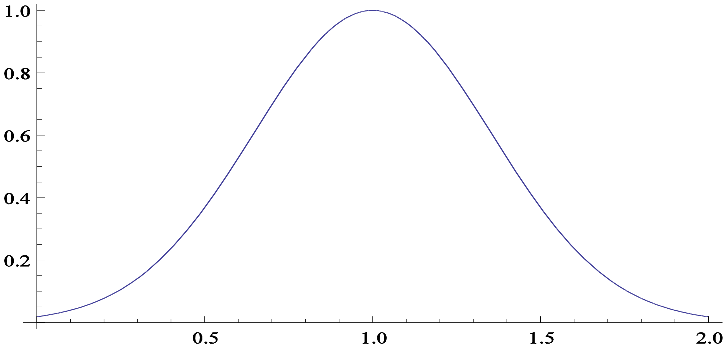
- The probability density function of the random variable *X*, gives the probability of measuring *X* in a given range by integration,

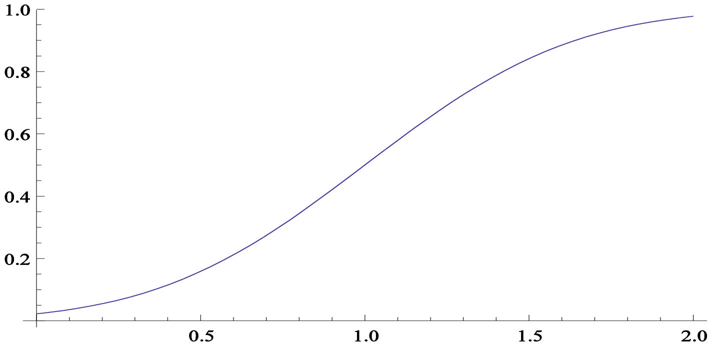
$$\int_a^b p(x)dx$$

- The upper right figure shows the probability density function of a Gaussian variable with mean 1, and variance 0.5
- The cumulative distribution function cdf(x), gives the probability of measuring *X* to have the value of *x* or lower,

$$cdf(x) = \int_{-\infty}^{x} p(x')dx$$

- The lower right figure shows the cumulative distribution function of the Gaussian variable described above





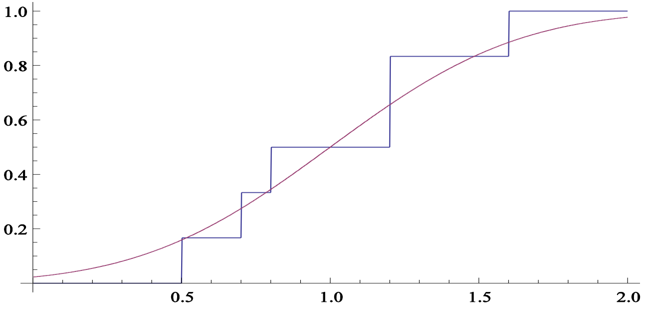
The Kolmogorov-Smirnov Test

- A statistical test of ‘Goodness of fit’
- Tests whether the data is consistent with a hypothesized cumulative distribution function
- Good when the sample size is small as there is no binning of the data

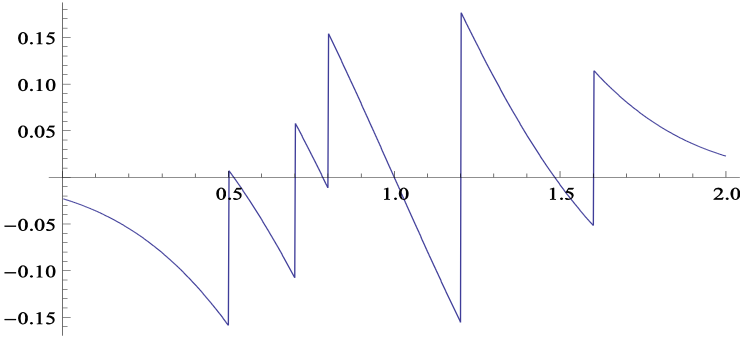
An example of the Kolmogorov-Smirnov test

- Take the data set {0.5, 0.7, 0.8, 1.2, 1.2, 1.6} and ask whether it is consistent with a Gaussian distribution of mean 1 and variance 0.5?
- The premise of the Kolmogorv-Smirnov test is that, if the data is consistent with the cdf, then the difference between them should be a random walk.
- The supremum of the difference should not be an outlier in the distribution for a Brownian Bridge.
- Use the cdf for the supremum of a Brownian bridge to estimate the significance of the test.

cdf of data (blue), and hypothesized distribution(red):



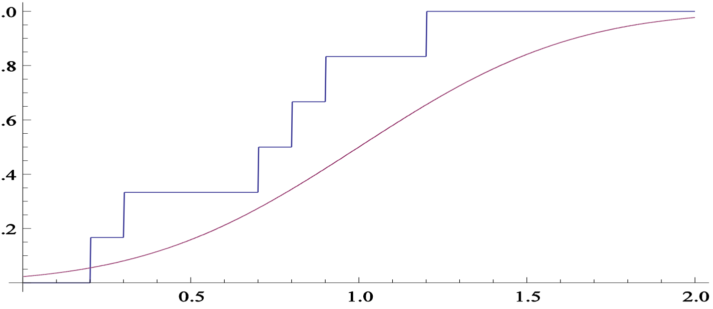
The difference between the two :



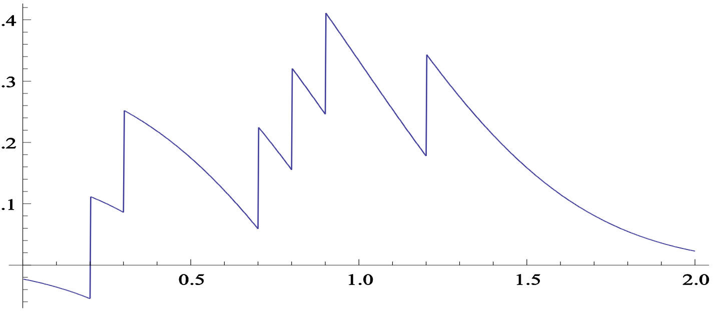
Another example of the Kolmogorov-Smirnov test

- Take the data set {0.2, 0.3, 0.7, 0.8, 0.9, 1.2} and repeat the test
- In this case we see the walk shown on the lower right: there is a clear bias.
- Comparing to the statistical tables, this data does not fit the hypothesized distribution so any significant degree.

cdf of data (blue), and hypothesized distribution(red):



Difference between the two



Overview of GSEA

- Take gene expression data from two different conditions and rank according to the differential expression across the conditions
- Take a test set of genes and determine whether they are collectively differentially expressed
- Randomly swap the class labels of the data and repeat the test many times as a gauge of significance