Power and Sample Size Estimation for Microarray Studies

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UseR! 2011
What is the appropriate sample size when testing many features simultaneously?

For example, measuring gene expression differences between groups using microarray or RNAseq.

Appropriate means: *When desired power is reached.*

**Power** does not only depend on **sample size** but also on **effect size, sample variability** and **significance level**.

Sample size determination either simulation or **pilot-data** based.
Single hypothesis vs multiple hypotheses testing

- not a *single* rejection region but *many* (multiple testing problem)
- not a *single* effect size but *distribution* of effect sizes
- only a proportion will be rejected

**average power**: the proportion of correctly rejected observations
Histograms of observed test statistics (A) and p-values (B).

Figure: Parametric null distribution (solid) and estimated alternative distribution (dashed).
A mixture model for the probability distribution

\[ m(t) = \pi_0 f_0(t) + (1 - \pi_0) \int f_1(t, \theta; N) \lambda(\theta) d\theta. \quad (1) \]

- \( m(t) \): observed test statistics (given)
A mixture model for the probability distribution

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- \( m(t) \): observed test statistics (given)
- \( \pi_0 \): indicates the proportion of non-differentially expressed genes (unknown)
- \( f_0(t) \): Normal or Student's t distribution (known)
- \( f_1(t, \theta; N) \): Normal with mean \( \neq 0 \) or non-central t (known)
- \( \lambda(\theta) \): density of effect sizes (unknown)
- \( N \): represents the effective sample size; \( \left( \frac{1}{n_A} + \frac{1}{n_B} \right) - 1 \) (given)
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Estimation of the density of effect sizes (analytically)

\[ f_1(t, \theta; N) \text{ normally distributed leads to the following convolution} \]

\[ \int \Phi(t - \theta \sqrt{N}) \lambda(\theta) d\theta \]  \hspace{1cm} (2)

which can be solved using a kernel deconvolution estimator\(^1\)

\[ \lambda(\theta) = \frac{1}{2\pi} \int e^{-is\theta \sqrt{N}} \frac{\psi_w(s) \psi_m(s)}{\psi_f(s)} ds \]  \hspace{1cm} (3)

- numerical approximation to the real-part (very time-consuming)
- using \texttt{fft}-function like implementation of the density-function (really fast)

\(^1\)Ferreira and Zwinderman, SAGMB, (2006).
Generalization to any kind of statistics

approximate the integral by a summation:

\[ m_n(t_i) = \pi_0 f_0(t_i) + (1 - \pi_0) \sum_{j=1}^{M} f_1(t_i, \theta_j) \lambda(\theta_j) \Delta \theta. \] (4)

express the density of effect sizes as a sum of B-splines:

\[ m_n(t_i) = \pi_0 f_0(t_i) + (1 - \pi_0) \sum_{j=1}^{M} f_1(t_i, \theta_j) \sum_{k=1}^{K} \alpha_k b_k(\theta_j) \Delta \theta. \] (5)
Estimation of the density of effect sizes

the discretization transforms the integral equation to matrix equation: $y = X\beta$

$X$ ill-conditioned - **no OLS-solution**
need regularization e.g. minimize $||y - X\beta||^2 + \lambda W(\beta)$

- constrained optimization$^{2,3}$ ($\int \lambda(\theta)d\theta = 1$ and $\lambda(\theta) > 0$).

- **ridge regression**

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$^3$van de Wiel and In Kim, Biometrics, (2007)
Estimation of the proportion of non-differentially expressed genes

Figure: Boxplots of $\pi_0$ estimates with method of Langaas (JRSS, 2005), Storey (JRSS, 2002) or as part of ridge regression estimation of $\lambda(\theta)$ on 250 simulated datasets.
Estimation of the average power using Bisection method

Figure: Ferreira and Zwinderman, Int. J Biostat, (2006) showed that, $u^*$, the solution to $\hat{G}_1(u; N) = \int H_1(u, \theta; N)\hat{\lambda}(\theta)d\theta = u\frac{\alpha(1-\hat{\pi}_0)}{\hat{\pi}_0(1-\alpha)}$ gives the average power, where $\alpha$ is the desired False Discovery Rate.
Sample size determination

- given pilot-data
- calculate test statistics and p-values
- assume parametric form for the null and alternative
- estimate \( \pi_0 \) and density of effect sizes, \( \lambda(\theta) \)
- estimate the power of the pilot-data
- or predict power at sample sizes larger than the pilot-data

\[
\hat{G}_1(u^*; N') = \int H_1(u^*, \theta; N') \hat{\lambda}(\theta) d\theta = u^* \frac{\alpha(1 - \hat{\pi}_0)}{\hat{\pi}_0(1 - \alpha)}
\] (6)
Nutrigenomics example

- PPAR-α activation in small intestine
- wild-type and PPAR-α knock out mice
- different PPAR-α agonist: high (Wy14,643), intermediate (trilinolenin or C18:3) and low (fenofibrate) potency
- different exposure times (6 hours and 5 days)
- Affymetrix GeneChip Mouse 430 2.0 arrays

<table>
<thead>
<tr>
<th>probe-sets</th>
<th>group A</th>
<th>group B</th>
<th>experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16539 4 (wild-type)</td>
<td>4 (knock-out)</td>
<td>high, 6 hours</td>
</tr>
<tr>
<td>2</td>
<td>16539 4 (wild-type)</td>
<td>5 (knock-out)</td>
<td>intermediate, 6 hours</td>
</tr>
<tr>
<td>3</td>
<td>16539 5 (wild-type)</td>
<td>5 (knock-out)</td>
<td>low, 6 hours</td>
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<tr>
<td>4</td>
<td>16539 4 (wild-type)</td>
<td>4 (knock-out)</td>
<td>high, 5 days</td>
</tr>
<tr>
<td>5</td>
<td>16539 4 (wild-type)</td>
<td>4 (knock-out)</td>
<td>low, 5 days</td>
</tr>
</tbody>
</table>

van Iterson et al. BMC Genomics (2009).
Nutrigenomics example: density of effect sizes

![Graph showing density of effect sizes over 6 Hours and 5 Days for fenofibrate, trilinolenin (C18:3), and Wy14,643.](image)
Nutrigenomics example: power curves

fenofibrate  trilinolenin (C18:3)  Wy14,643

sample size (per group)

5 Days

6 Hours

5 Days

6 Hours

fenofibrate  trilinolenin (C18:3)  Wy14,643
Conclusion/Future Plans

General method for sample size determination for high-dimensional data with control of the FDR.

- likelihood ratio statistics ($\chi^2$ and non-central $\chi^2$) or F-statistics
- nonparametric null and assume location-model for the alternative
Relative power and sample size analysis on gene expression profiling data.

J.A. Ferreia, A. Zwinderman.
Approximate sample size calculations with microarray data: an illustration.
*Statistical application in genetics and molecular biology*, 5, 1, 2006.

SSPA:
Other cran and BioConductor packages: OCplus, sizepower, ssize, ssize.fdr