**Overview**

- Microarray data analysis
- M@cbeth webservice
- Ovarian cancer study

**Benchmarking study**

State-of-the-art when estimating the generalization performance of a prediction model generated using microarray data:

1) Independent test set performance (on one setting of the data)

2) Varied test set with leave-one-out cross-validation (LOO-CV) performance

Training & validation set

Test set

Test set

Test set

Training & validation set

Training & validation set

Training & validation set

# repeats: Number of randomizations

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http://www.esat.kuleuven.be/scd/

Datasets:
- 9 binary cancer classification problems
- 20 randomizations of each problem

Methods:
- Randomized independent test set performance
- 9 classification methods
  (based on LS-SVMlab - http://www.esat.kuleuven.be/sista/lssvmlab/)
  - LS-SVM with linear kernel
  - LS-SVM with RBF kernel
  - Fisher Discriminant Analysis (FDA)
  - PCA + FDA (unsupervised PC selection)
  - Kernel PCA with linear kernel + FDA (unsupervised PC selection)
  - Kernel PCA with RBF kernel + FDA (unsupervised PC selection)
- Performing regularization is very important

Observations:
- LS-SVM with linear kernel versus LS-SVM with RBF kernel
  1. Using well-tuned RBF kernels for LS-SVM can be applied without risking overfitting
  2. Performing regularization is very important
Observations:

Kernel PCA with RBF versus linear kernel + FDA (supervised PC selection)

3. Using an RBF kernel for kernel PCA tends to result in overfitting

M@CBETH webservice

Optimal classifier can differ for each dataset

⇒ M@CBETH web service:
- MicroArray Classification Benchmarking Tool on a Host server
- Can easily be used by clinicians for making optimal two-class predictions
- Finding a good prediction among different classification methods by using randomizations of a benchmarking dataset


M@CBETH: a microarray classification benchmarking tool.

Bioinformatics, 21(14), 3185-3186.

M@CBETH webservice

Algorithm

- All selected classification methods
- Best generalizing method

Overview

- Microarray benchmarking study
- M@cbeth webservice
- Ovarian cancer study
Clinical case: Ovarian cancer

- Leading cause of death from gynecological malignancies in women
- 4% of new cases of cancer and 6% of cancer deaths in women
- Prognosis is generally poor:
  - The highest fatality to case ratio of all gynecologic malignancies
  - Overall five year survival of 30%
  - Most women (70%) present with advanced disease
  - Early disease is usually asymptomatic and not detected
  - It requires intensive and often complex therapies

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Microarray benchmarking
M@CBETH: benchmarking service
Ovarian cancer study

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Stage at diagnosis is the most important prognostic factor:
International Federation of Gynecology and Obstetrics (FIGO 1998)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Area reached</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>The cancer is confined to one or both ovaries. Tumor may be found on the surface of the ovary.</td>
<td>70%</td>
</tr>
<tr>
<td>II</td>
<td>The cancer invades one or both ovaries with extension into the pelvic region, e.g., it is found in the uterus, fallopian tubes, bladder, sigmoid colon or rectum. Few cases are diagnosed at this stage.</td>
<td>45%</td>
</tr>
<tr>
<td>III</td>
<td>The cancer has spread beyond the pelvis to the abdominal wall or diaphragm. Metastasis to regional lymph nodes or liver surface.</td>
<td>21%</td>
</tr>
<tr>
<td>IV</td>
<td>This is the most advanced form of ovarian cancer. Stage IV cancers have spread to distant organs such as the liver (spread beyond just the surface of the liver), spleen or lung.</td>
<td>5%</td>
</tr>
</tbody>
</table>

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Reliably estimating the generalization performance of a prediction model generated using microarray data:

1) Independent test set performance
   (in case of a large prospective data set)
   M@CBETH: prediction service

2) Varied test set with 5-fold performance
   (while repeating gene selection
   within each iteration)

3) Randomized test set performance
   M@CBETH: benchmarking service
Clinical case: Ovarian cancer

Early-stage (FIGO stage I) versus advanced-stage (stage III/IV)

- FIGO staging: localization of the tumor
- Reflected in expression patterns of primary tumor?

Stage I:
- 10-50% of patients will recur after initial surgery
- Might benefit from adjuvant therapy

- Presence of subclinical metastases?

Stage III/IV:
- A subset of patients will prove to be resistant to platinum-based chemotherapy
- Develop experimental therapeutic alternatives for these patients

- Determination of chemoresistance?
**Clinical case: Ovarian cancer**

- Stage III/IV: Resistance to platin-based chemotherapy?
- Sample A: chemosensitive tumors
- Sample B: reference pool
- Sample A: chemoresistant tumors
- Sample B: reference pool
- Differential expression?

**Microarray data analysis**

- Large number of gene expression levels and small number of patients
- Most methods have problems with the high-dimensional nature of the data
- Larger number of microarray experiments in the future
- Most methods rely on linear functions and are not capable to find nonlinear relationships in the data
Ovarian cancer study

Pilot study: 20 microarray experiments
- Early-stage (FIGO stage I) versus advanced-stage (stage III/IV)
- Platin-sensitive (Class A) versus platin-resistant (Class A) advanced-stage

<table>
<thead>
<tr>
<th>FIGO Stage, n</th>
<th>Class A (n = 6)</th>
<th>Class A (n = 7)</th>
<th>Class A (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Time to progression, n
- < 6 months after first-line platin-based chemotherapy: 6 - -
- > 12 months after first-line platin-based chemotherapy: 5 - -
- no recurrence: 2 2 2

Is prognostic information reflected in expression patterns?

Ovarian cancer study

Pilot study: Detection of differential expression
- Selection of the individual genes with the highest differential expression between 2 of the 3 or the 3 classes:
  - Wilcoxon rank sum test
  - Kruskal-Wallis test
  - p-values
- Rejection level α (e.g., 0.05) or selection of the N (e.g., 500) genes with the lowest p-value

Ovarian cancer study

Most extreme cases
No real differential expression
All differential expression is accidental

Non-accidental differential expression
Superposition of two distributions

TP + FN = 5000
TP = 4943
FP = 57
TN = 1409
A_1 = 210
A_2 = 2028
Ovarian cancer study

Pilot study: PCA (all 21372 genes)

Unsupervised

Pilot study: Classification models

- Classifier: LS-SVM with a linear kernel
- Varied test set with LOO-CV performance while repeating gene selection within each iteration (Simon et al., 2003)

Caution: possibility of overfitting!

Results:
- Stage I versus advanced-stage: 100%
- Platin-sensitive versus platin-resistant advanced-stage: 76.92%
- Single classifier build to distinguish between both advanced-stage classes: assigns all stage I samples to platin-sensitive advanced-stage class.

Observations:
- Possible to discriminate between early-stage, platin-sensitive and platin-resistant advanced-stage ovarian tumors with reasonable accuracy


Ovarian cancer study

Pilot study: Classification with M@CBETH
- Randomized independent test set performance
- Results:
  - Stage I versus advanced-stage:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Bivariate</th>
<th>Multivariate</th>
<th>Logistic ROC</th>
<th>Partial ROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.5474</td>
<td>0.5411</td>
<td>0.5402</td>
<td>0.5407</td>
</tr>
<tr>
<td>II</td>
<td>0.5474</td>
<td>0.5411</td>
<td>0.5402</td>
<td>0.5407</td>
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<td>III</td>
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</tbody>
</table>

Prospective study: 50 microarray experiments
- Evaluation of models generated in pilot study
- Independent test set performance
- Early-stage versus advanced-stage
- Platin-sensitive and platin-resistant advanced-stage
- Early-stage (FIGO stage I) with and without recurrence

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