Introduction to DNA Microarray Data

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Outline

1) Principle of DNA Microarray Techniques

2) Pre-processing an affymetrix data related to prostate cancer with `Bioconductor` tools

3) A Simple Example of Using Expression Data:

   Finding differential genes related to a phenotype variable using univariate screening.

Part I

Principle of DNA Microarray Techniques

Central Dogma of Molecular Biology

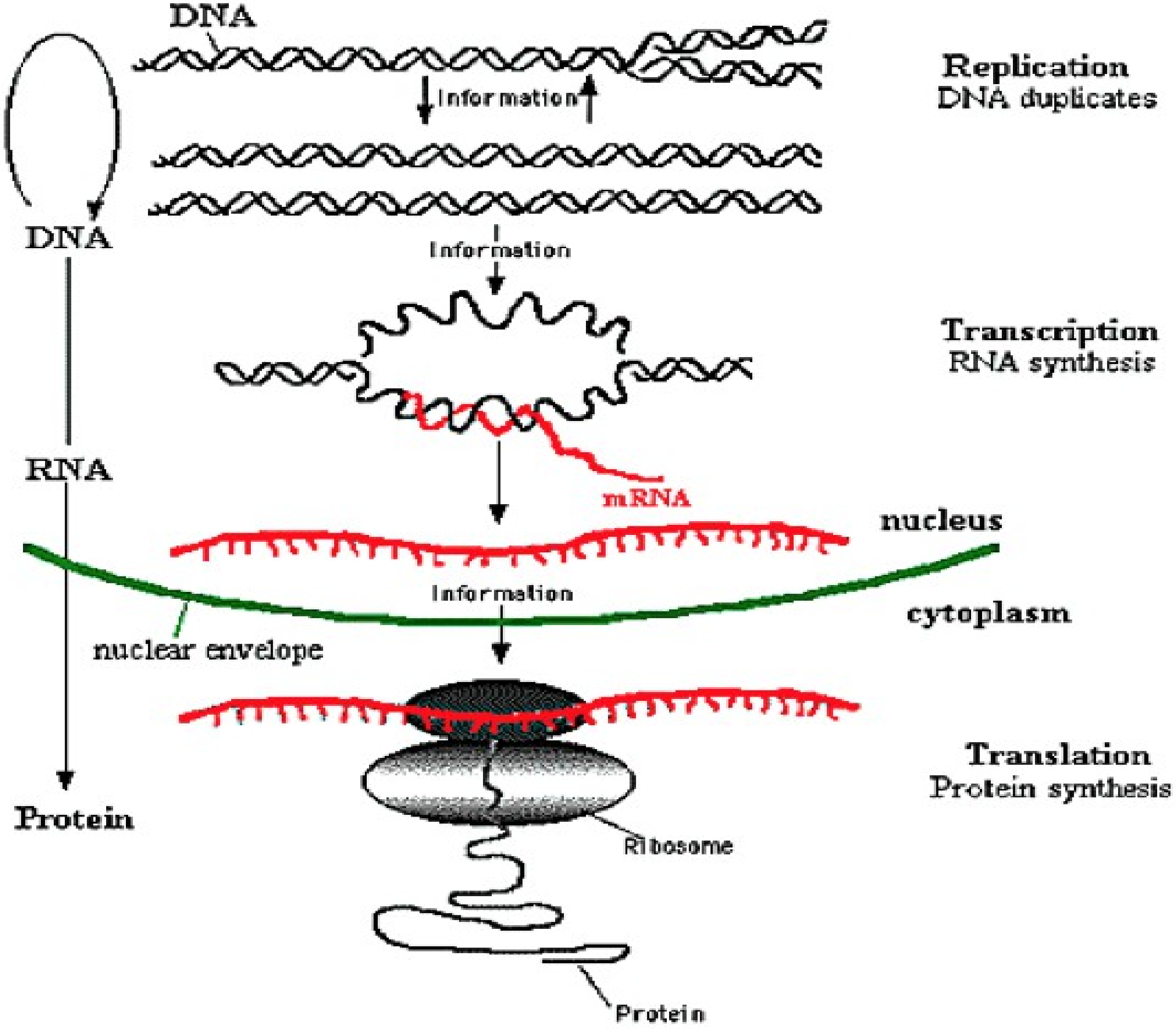
The genetic information is stored in the DNA molecules. When the cells are producing proteins, the expression of genetic information occurs in two stages:

1) transcription, during which DNA is transcribed into mRNA
2) translation, during which mRNA is translated to produce proteins.

DNA -> mRNA -> protein

During this process, there are other important aspects of regulation, such as methylation, alternative splicing, which controls which genes are transcribed in different cells.

Central Dogma of Molecular Biology

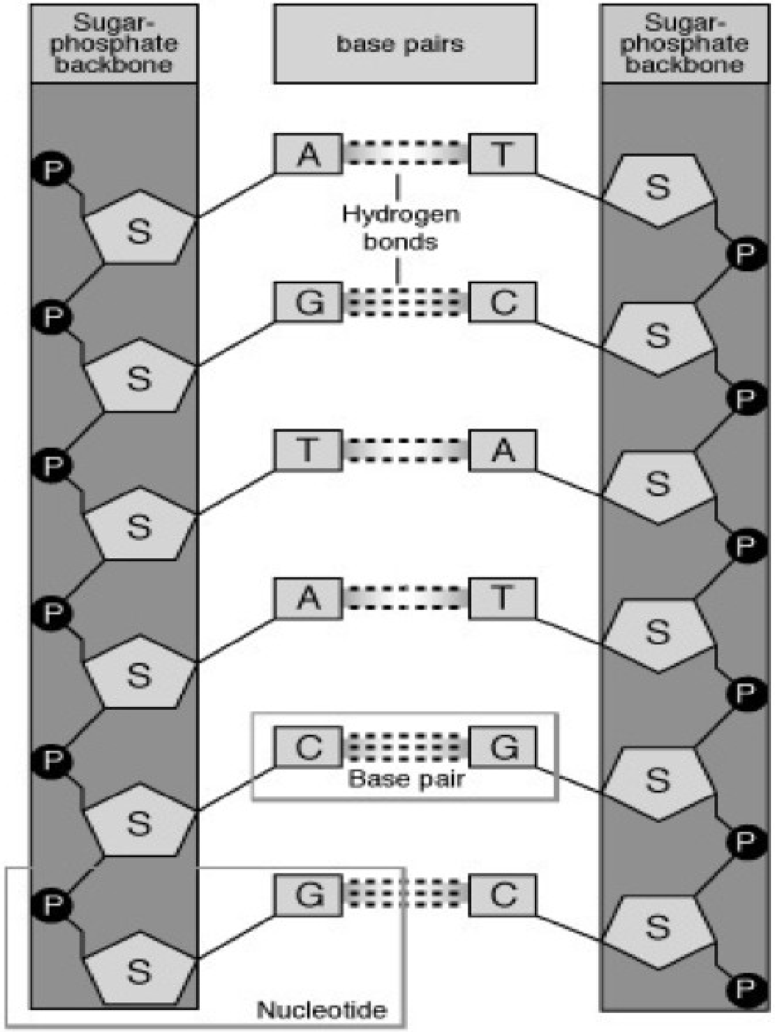


Transcriptome

- To investigate activities in different cells, we could measure protein levels. However, this is still very difficult.
- Alternatively, we can measure the abundance of all mRNAs (transcriptome) in cells. mRNA or transcript abundance sensitively reflect the state of a cell:
  - Tissue source: cell type, organ.
  - Tissue activity and state:
    - Stage of cell development, growth, death.
    - Cell cycle.
    - Disease or normal.
    - Response to therapy, stress.

Base-paring Rules in DNA and RNA

DNA Microarray is based on the base-paring rules, which are used in DNA replication and transcription of DNA to mRNA.



Four nucleotide bases:
purines: A, G
pyrimidine: T, C

A pairs with T, 2 H bonds
C pairs with G, 3 H bonds

In transcribing DNA to mRNA,
A pairs with Uracil in mRNA

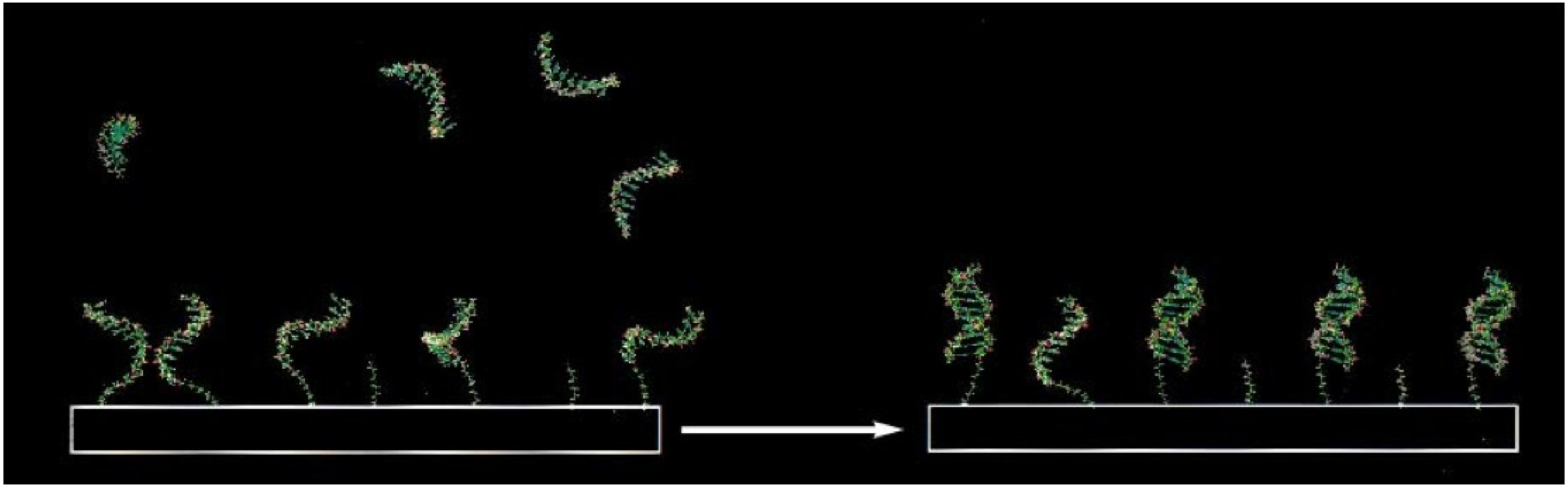
Hybridization

- We can use DNA single strands to make probes representing different genes.

- In principle, the mRNA that complements a probe sequence by the base-paring rules will be more likely to bind (or hybridize) to the probe.

- We measure mRNA levels of a sample by looking at the hybridization levels to different probes.

Hybridization



Types of Gene Expression Assays

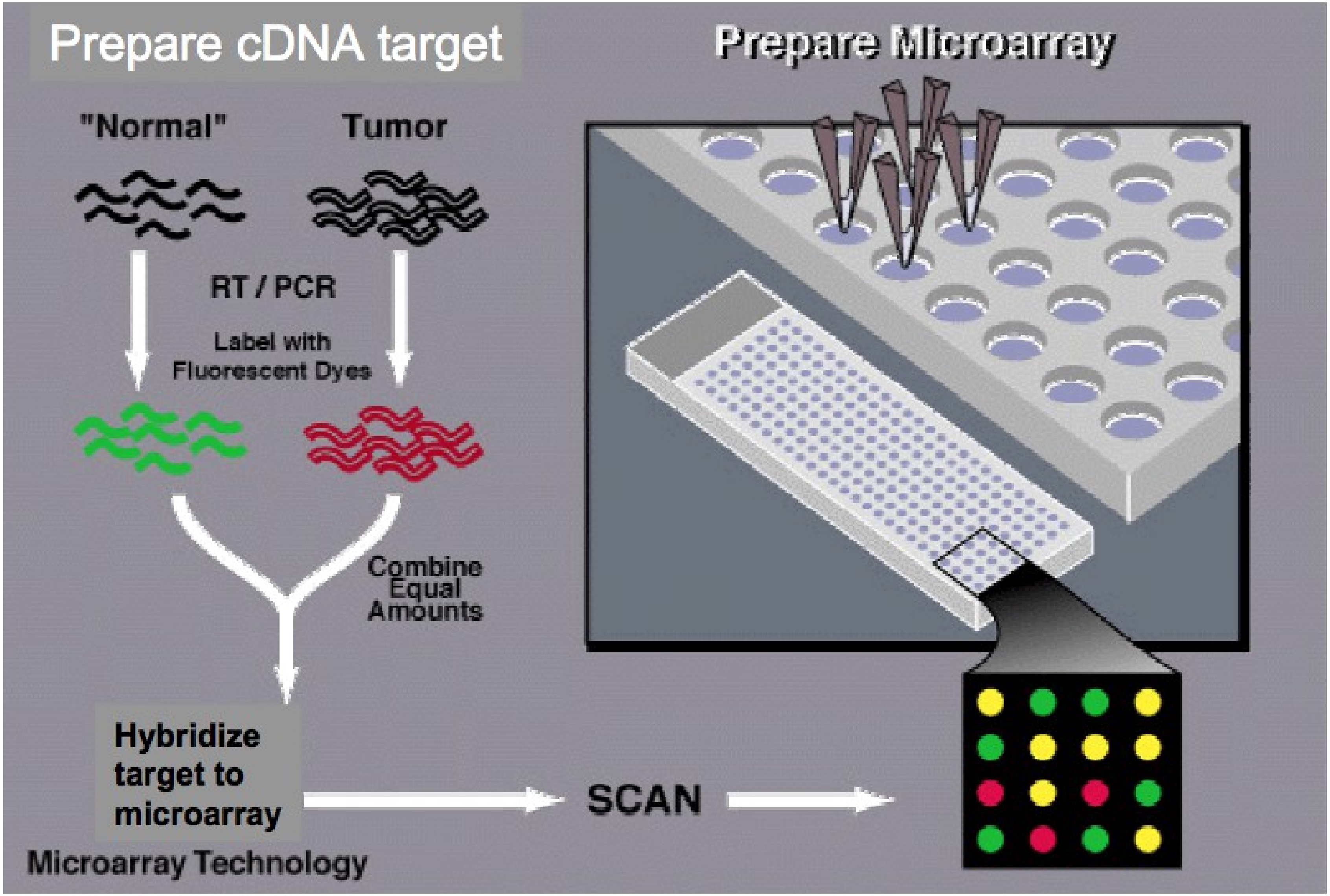
The main types of gene expression assays:

- Serial analysis of gene expression (SAGE);
- Short oligonucleotide arrays (Affymetrix);
- Long oligonucleotide arrays (Agilent Inkjet);
- Fibre optic arrays (Illumina);
- Spotted cDNA arrays (Brown/Botstein).
- RNA-seq.

Spotted DNA Microarrays

- Probes: DNA sequences spotted on the array
- Targets: Fluorescent cDNA samples synthesized from mRNA samples following base-paring rules.
- The ratio of the red and green fluorescence intensities for each spot is indicative of the relative abundance of the corresponding DNA probe in the two nucleic acid target samples.

Spotted DNA Microarrays



Oligonucleotide chips (Affymetrix)

- Each gene or portion of a gene is represented by 16 to 20 oligonucleotides of 25 base-pairs.
- Probe: an oligonucleotide of 25 base-pairs, i.e., a 25-mer.
  - Perfect match (PM): A 25-mer complementary to a reference sequence of interest (e.g., part of a gene).
  - Mismatch (MM): same as PM but with a single homomeric base change for the middle (13th) base (transversion purine <-> pyrimidine, G <->C, A <->T) .
- Probe-pair: a (PM,MM) pair.
- The purpose of the MM probe design is to measure non-specific binding and background noise.
- Affy ID: an identifier for a probe-pair set.

Probe-pair Set

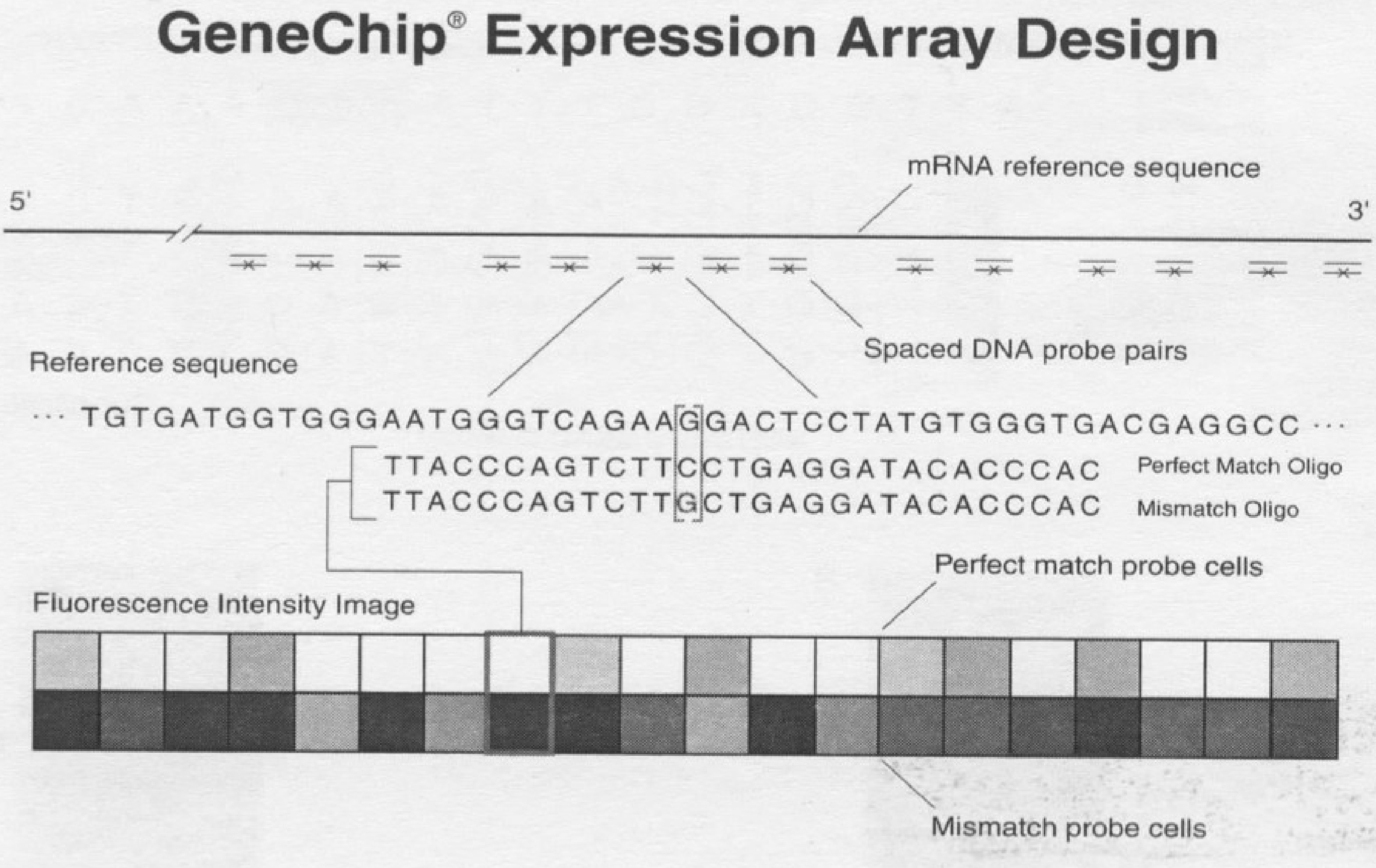

GeneChip® Expression Array Design
mRNA reference sequence
5'
3'
Spaced DNA probe pairs
Reference sequence
··· TGTGATGGTGGGAATGGGTCAGAAGGACTCCTATGTGGGTGACGAGGCC ···
TTACCCAGTCTTCCTGAGGATACACCCAC
Perfect Match Oligo
TTACCCAGTCTTGCTGAGGATACACCCAC
Mismatch Oligo
Perfect match probe cells
Fluorescence Intensity Image
Mismatch probe cells

Figure 1-3 Expression tiling strategy

Part II

Pre-processing an affymetrix data related to prostate cancer with Bioconductor tools

Preliminary:

Install bioconductor and packages:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite ("affy") ## install affy package
> biocLite ("oligo") ## install oligo package
```

Import and Access Probe-level Data

- Place raw data (CEL files) of all arrays in a directory
- Import CEL Data

```
> library ("affy")
> Prostate <- ReadAffy()
# Prostate is an affyBatch class object
```

- Access Meta information

```
> probeNames(Prostate)
> featureNames(Prostate)
> pData (Prostate) # access phenotype data
> annotation (Prostate)
```

- Access Probe-level PM Data

```
> pm (Prostate, "1001_at")
```

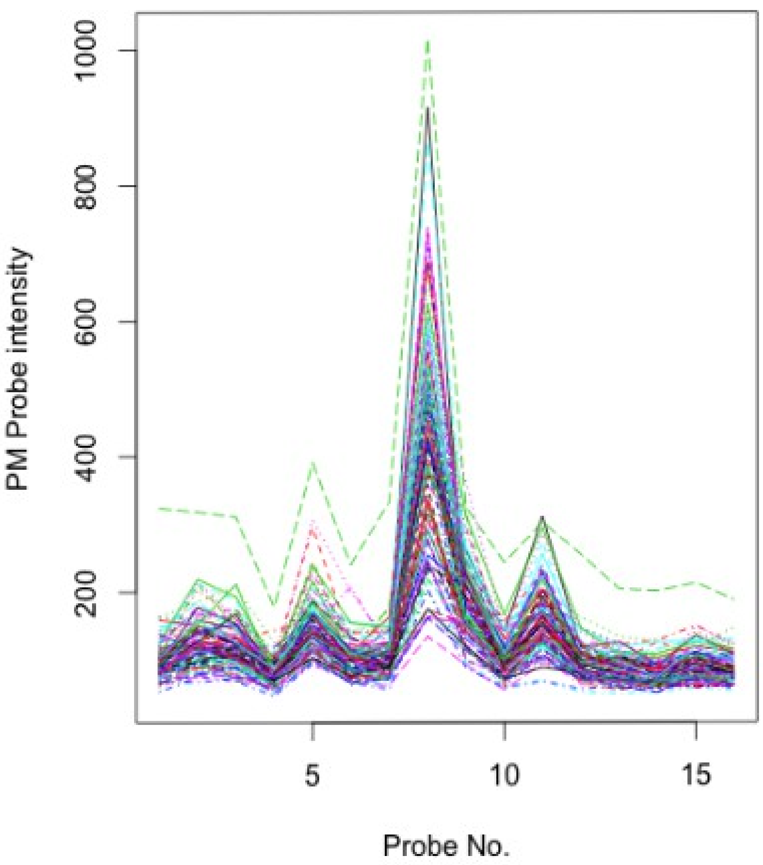
Visualize Raw Probe-level Data

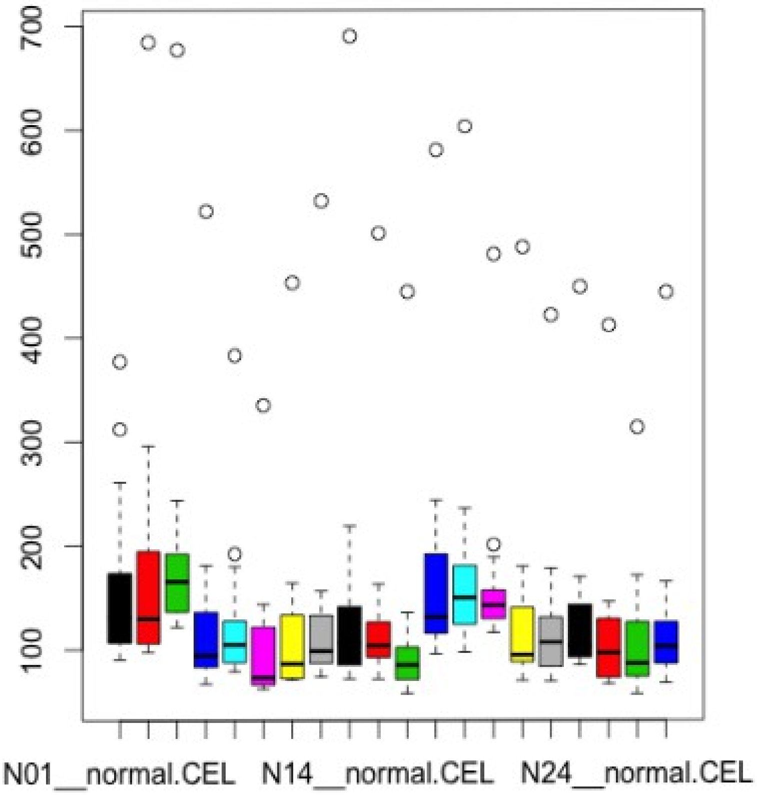
- Display intensity of probeset (gene) "1001_at"

```
> matplot(t(pm(Prostate, "1001_at")),  type = "l”)
```

- Show boxplots of 20 arrays on probeset “1001_at”

```
> boxplot (pm(Prostate, "1001_at")[,1:20])
```

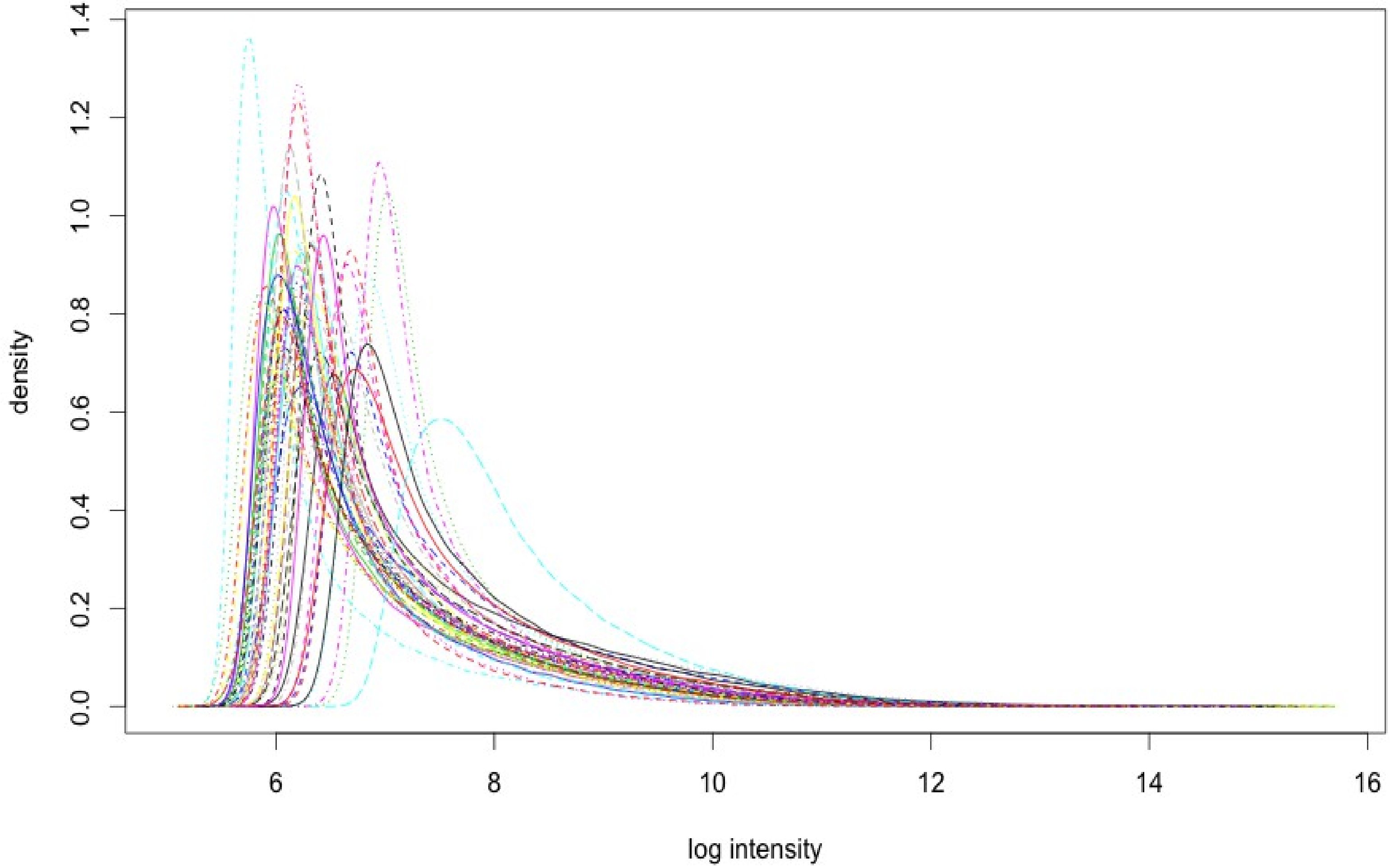




Visualize Raw Probe-level Data

Draw smoothed histograms of all probes of 50 arrays

```
> hist (Prostate[,1:50], col = 1:50)
```



A Generic Error Model

- A generic model for the value of the intensity Y of a single probe on a microarray is given by

$$Y = B + \alpha S$$

where *B* is background noise, usually composed of optical effects and non-specific binding, α is a gain factor, and *S* is the amount of measured specific binding.

- The signal *S* is considered a random variable as well and accounts for measurement error and probe effects:

$$\log(S) = \theta + \varphi + \epsilon$$

Here θ represents the logarithm of the true abundance of a gene, φ is a probe-specific effect, and ε accounts for measurement error.

Background Correction

Many background correction methods have been proposed in the microarray literature. Two examples:

- **MAS 5.0**: The chip is divided into a grid of k (default k = 16) rectangular regions. For each region, the lowest 2% of probe intensities are used to compute a background value for that grid.

- **RMA convolution:** The observed PM probes are modelled as the sum of a Gaussian noise component, B, with mean μ and variance σ^2 and an exponential signal component, *S*. Based on this model, adjust *Y* with:

$$E\left(S|Y=y\right)=a+b\frac{\phi\left(\frac{a}{b}\right)-\phi\left(\frac{y-a}{b}\right)}{\Phi\left(\frac{a}{b}\right)+\Phi\left(\frac{y-a}{b}\right)-1}$$

Background Correction

- Find available methods for background correction

```
> bgcorrect.methods()
```

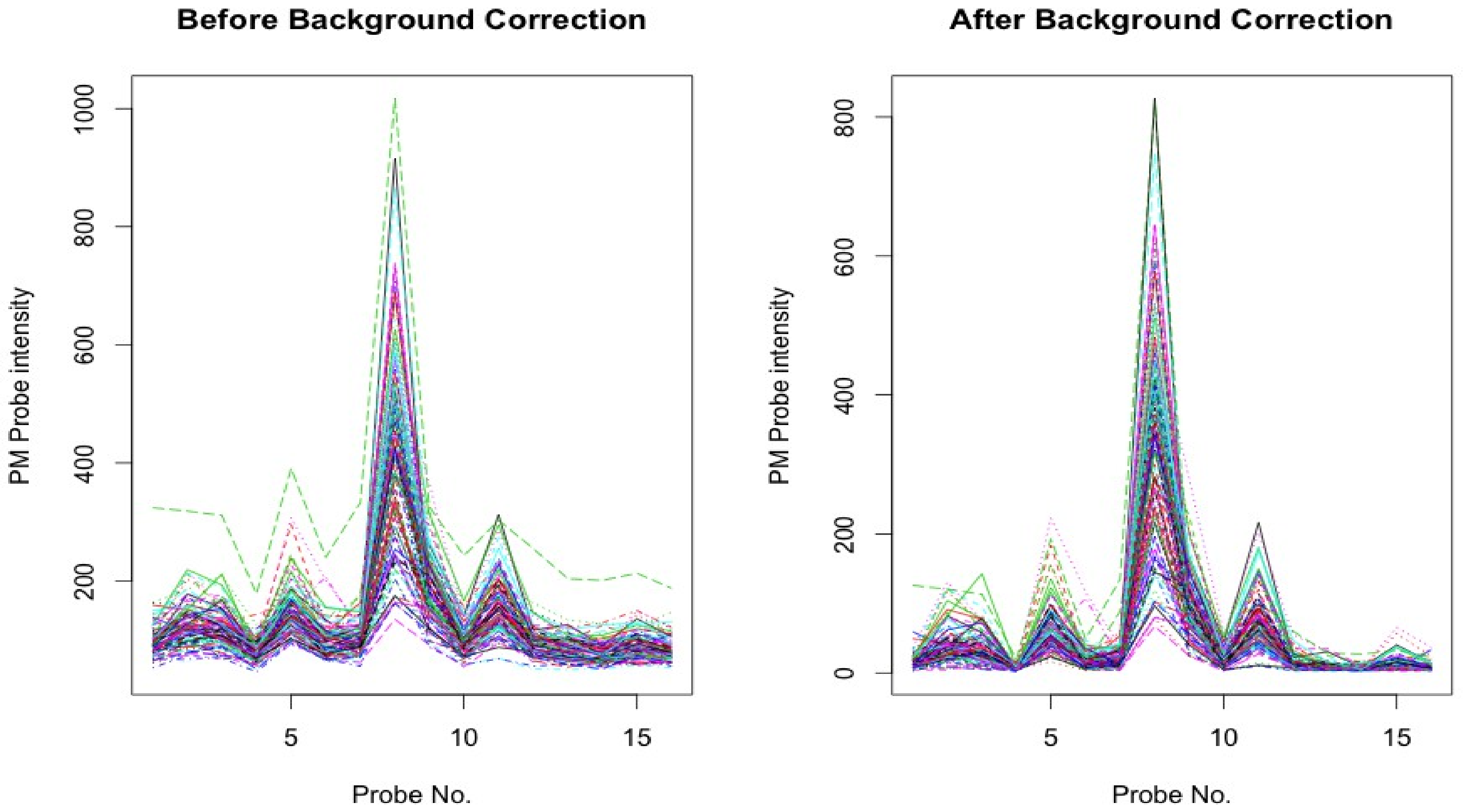
```
[1] "bg.correct" "mas"         "none"        "rma"
```

- Correct for background with rma convolution method

```
> Prostate.bg.rma <- bg.correct (Prostate, method = 
"rma")
```

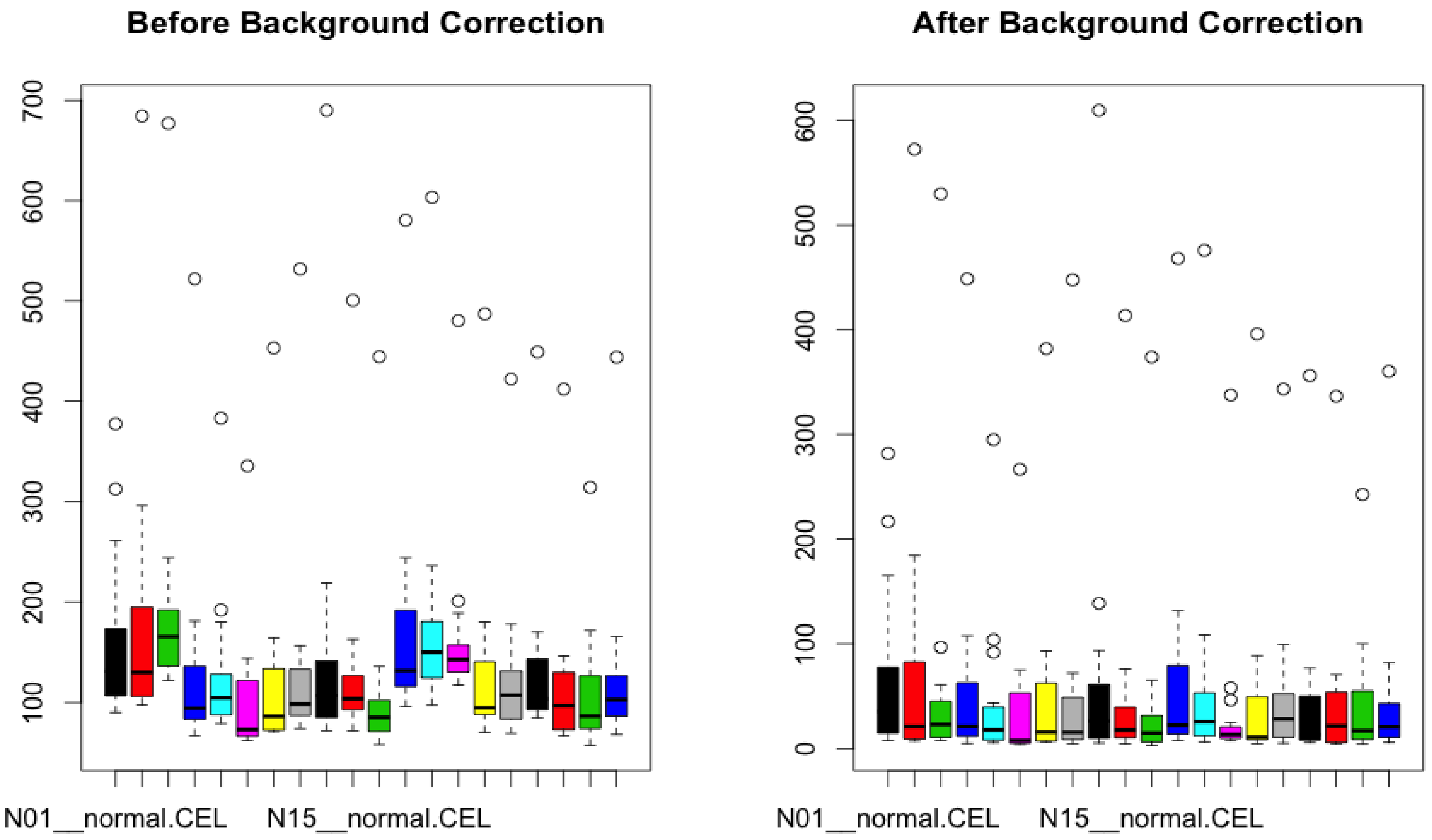
Background Correction

Matplot of intensities of probeset “1001_at” of 20 normal tissues:



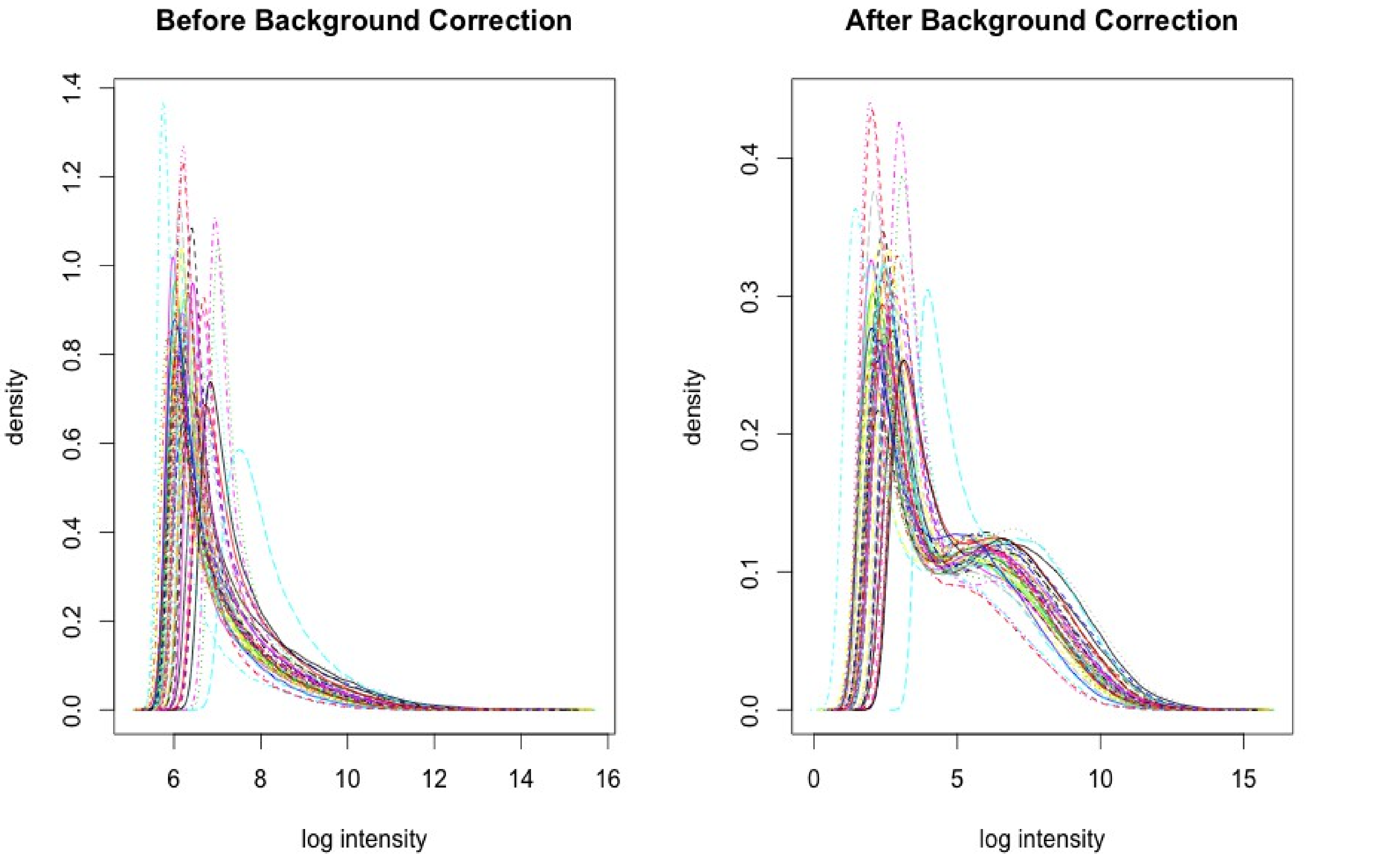
Background Correction

boxplot of intensities of probeset “1001_at” on 20 normal tissues:



Background Correction

Smoothed histogram of all probe intensities of 50 arrays (tissues)



Normalization

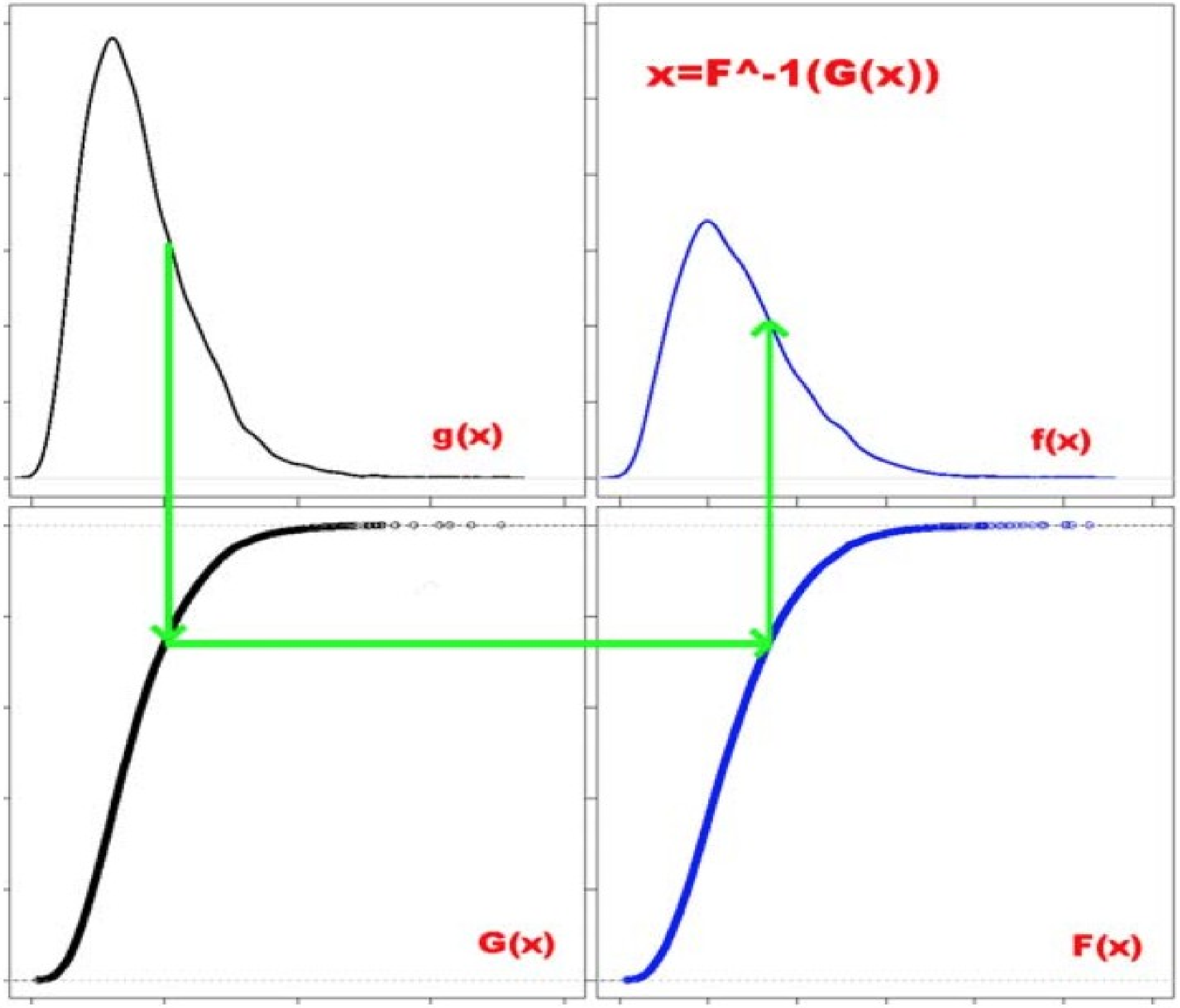
Normalization refers to the task of manipulating data to make measurements from different arrays comparable. One characterization is that the gain factor α varies for different arrays. Many methods are proposed to normalize microarray data. Two examples:

- **Scaling:** A baseline array is chosen and all the other arrays are scaled to have the same mean intensity as this array.
- **Quantile normalization:** Impose the same empirical distribution of intensities to all arrays.Transform each value with

  $$x_i = F^{-1}\,[G(x_i)],$$

  where *G* is estimated by the empirical distribution of each array and *F* is the empirical distribution of the averaged sample quantiles.

Quantile Normalization



Normalization

- Check available methods for normalizing

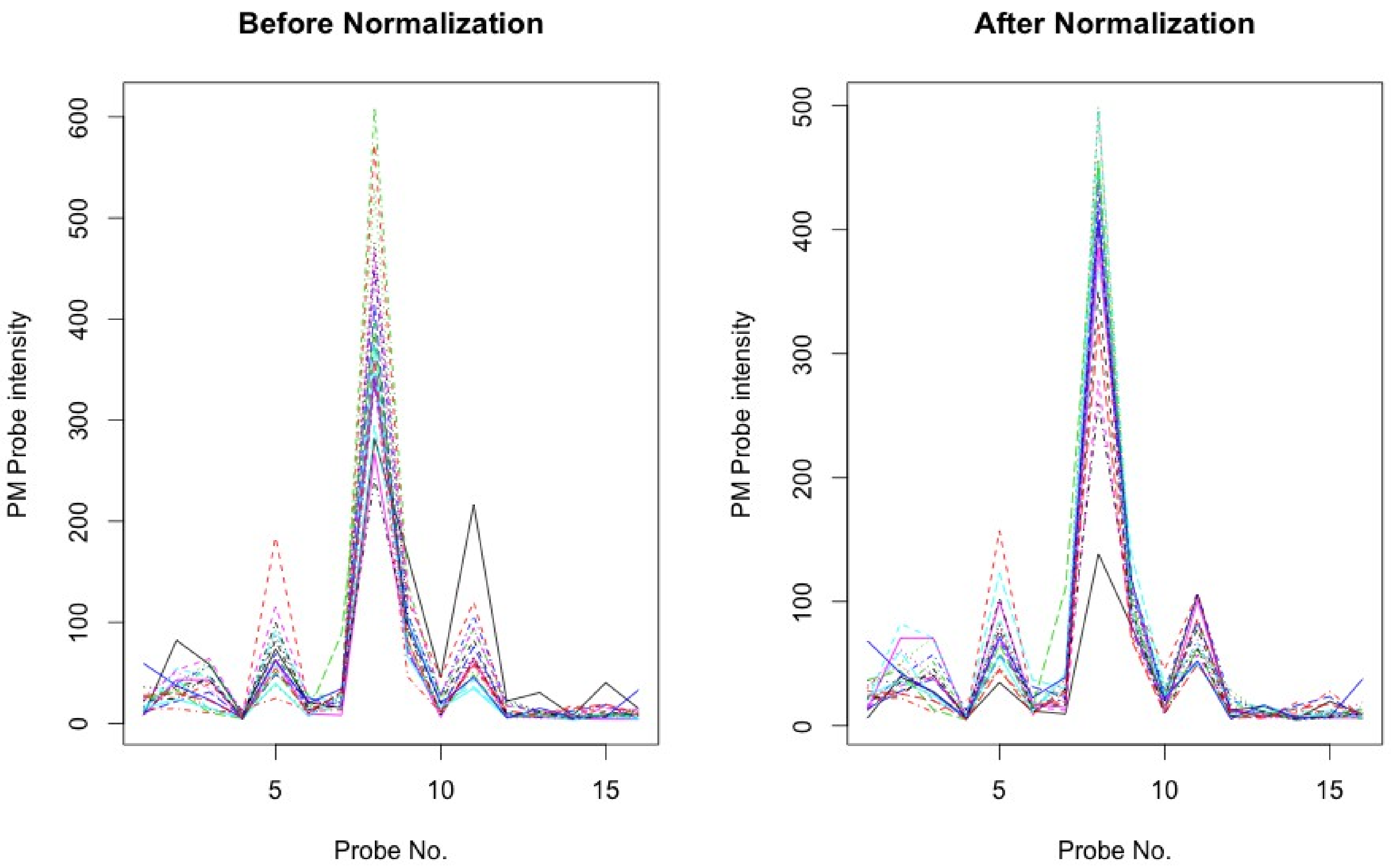
```
> normalize.methods (Prostate)
[1] "constant"           "contrasts"          "invariantset"
[4] "loess"              "methods"            "qspline"
[7] "quantiles"          "quantiles.robust"   "vsn"
[10] "quantiles.probeset" "scaling"
```

- Normalize with quantiles method

```
> Prostate.norm.quantile <- normalize
(Prostate.bg.rma, method = "quantiles")
```

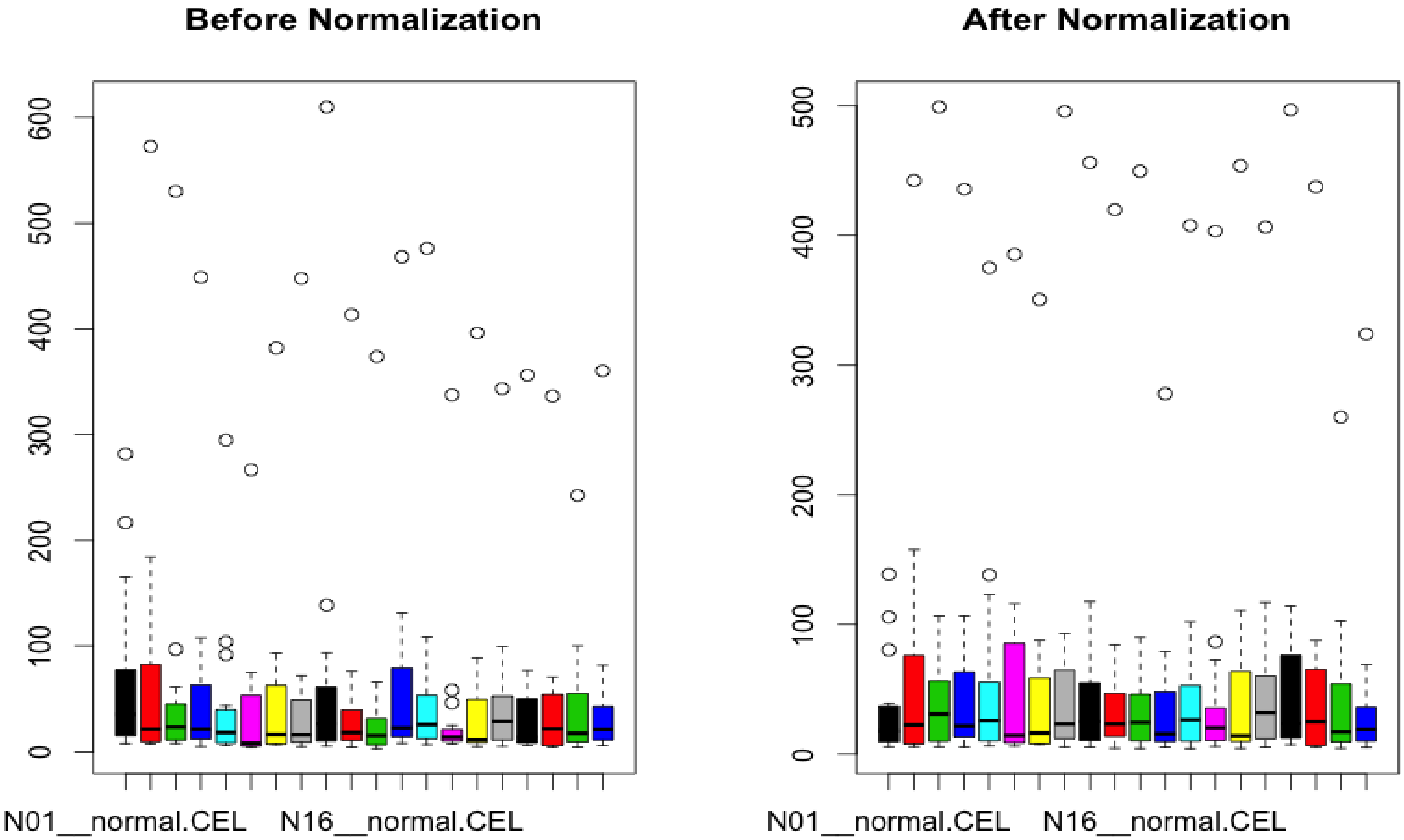
Normalization

Matplot of intensities of probeset “1001_at” of 20 normal tissues:



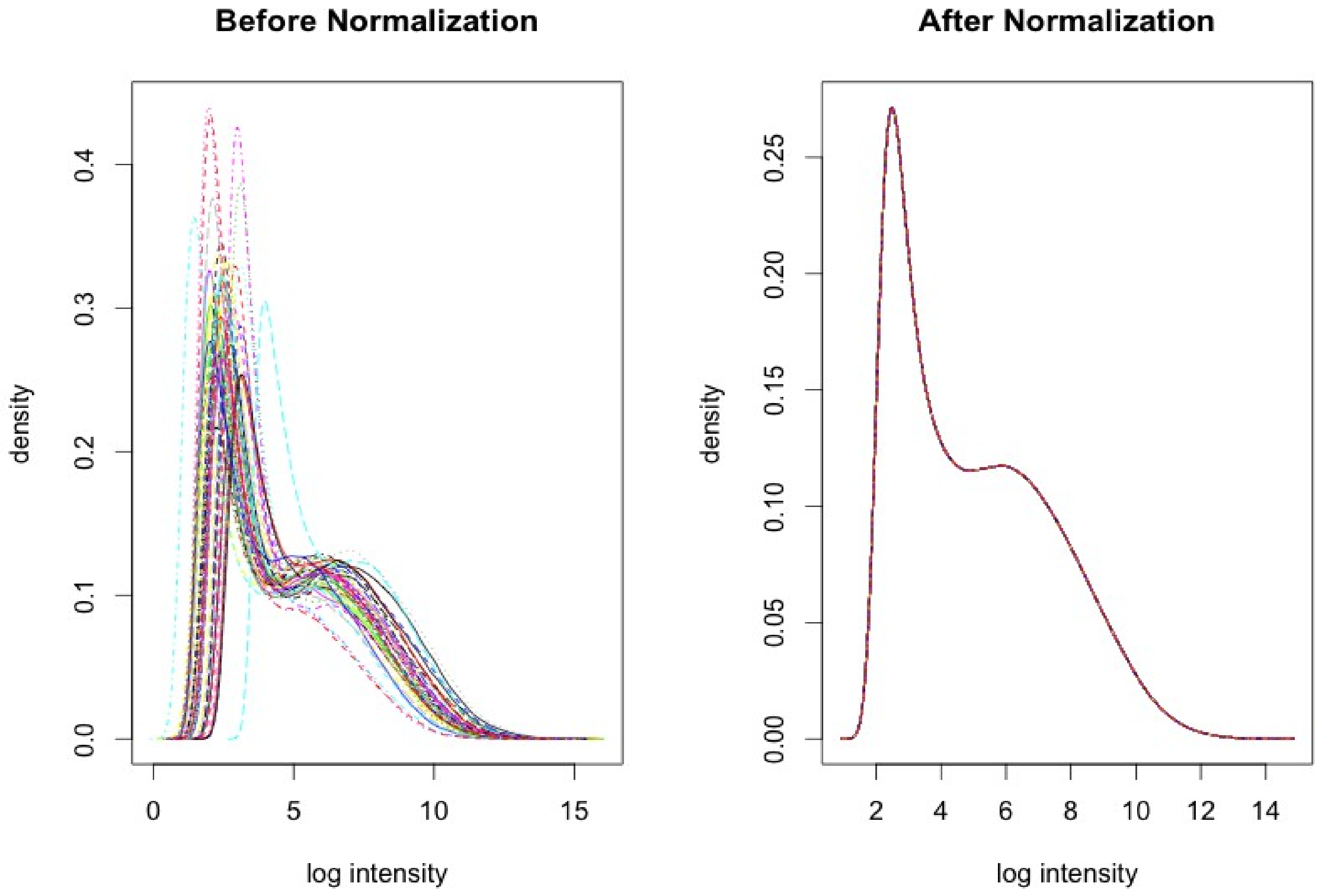
Normalization

boxplot of intensities of probeset “1001_at” on 20 normal tissues:



Normalization

Smoothed histogram of log intensities of all probes of 50 arrays (tissues)



Generate Expression Values

- Check out available methods for summarizing intensities a probeset into a single expression value:

```
> express.summary.stat.methods()
```

- Use a few 3-step generic functions, such as `expresso` and `threestep`, which also do background correction and normalization, as well as correction for PM values with MM values if desired. For example:

```
Prostate_eset_medpol <- expresso(Prostate,
                          normalize.method = "quantiles",
                          bgcorrect.method = "rma",
                          pmcorrect.method = "pmonly",
                          summary.method = "medianpolish")
```

RMA Summary of Probe-level Intensities

- To obtain an expression measure, assume that for each probe set *n*, the background-adjusted, normalized, and log-transformed PM intensities, denoted with Y_{ijn} , follow a linear additive model:

$$Y_{ijn} = \mu_{in} + \alpha_{jn} + \varepsilon_{ijn},\ i=1,\ldots,I,\ \ j=1,\ldots,J,\ n=1,\ldots,N$$

  with μ_i representing the log scale expression level for array i, α_j a probe affinity effect, and ε_{ij} representing an independent identically distributed error term with mean 0.

- The estimate of μ_{in} gives the expression measures for probe set n on array i.

Generate Expression Values

- There are also specialized functions that do all of the three steps, such as `rma` and `gcrma`. In `rma` function, RMA is used for background correction, quantile is used for normalization, and a robust multi-array method is used to summarize intensities of probesets.
  - Using rma

    ```
    > Prostate_eset_rma <- rma (Prostate)
    ```

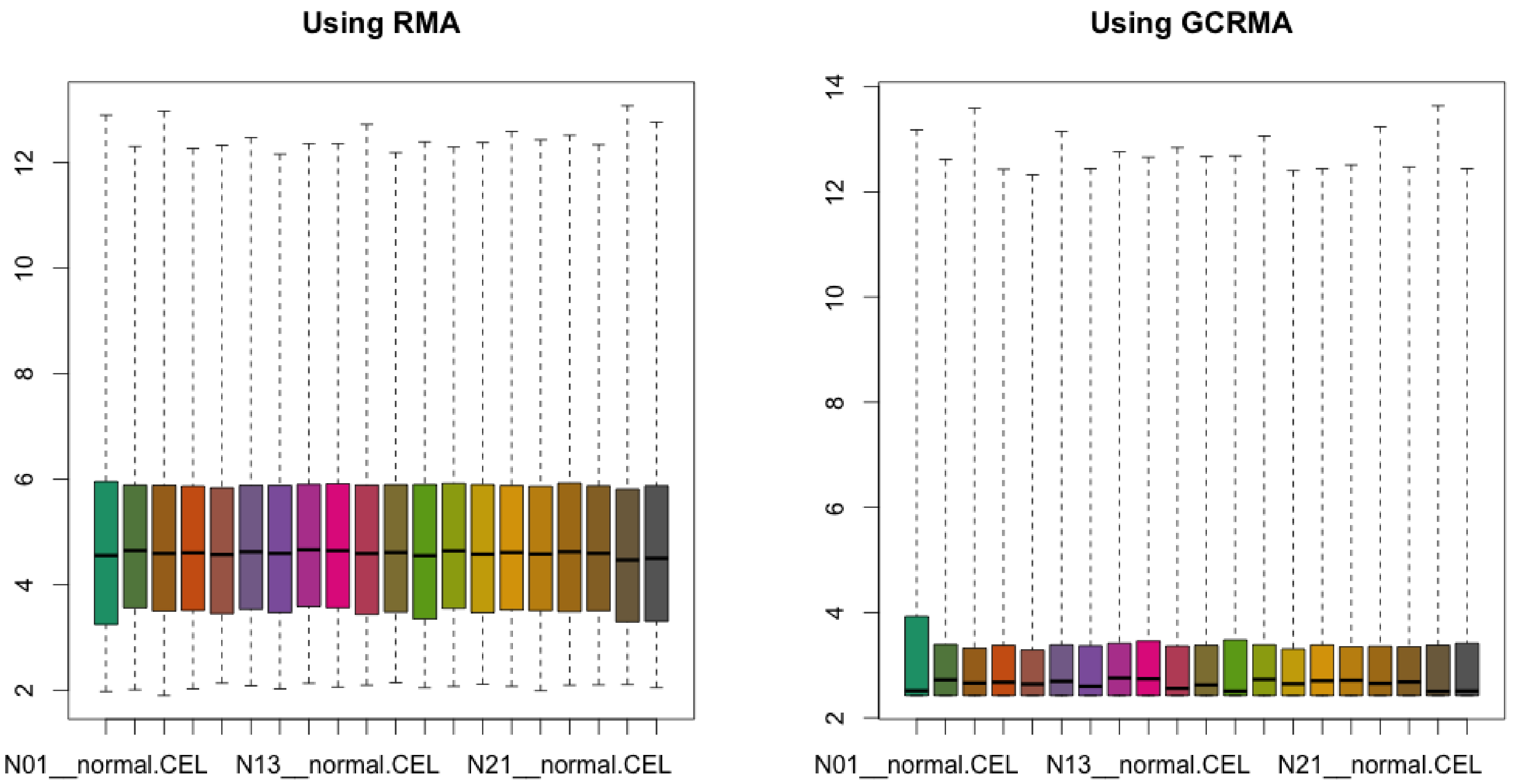
  - Using `gcrma`

    ```
    > Prostate_eset_gcrma <- gcrma (Prostate)
    ```

- The results, such as `Prostate_eset_rma`, are an *`ExpressionSet`* object.

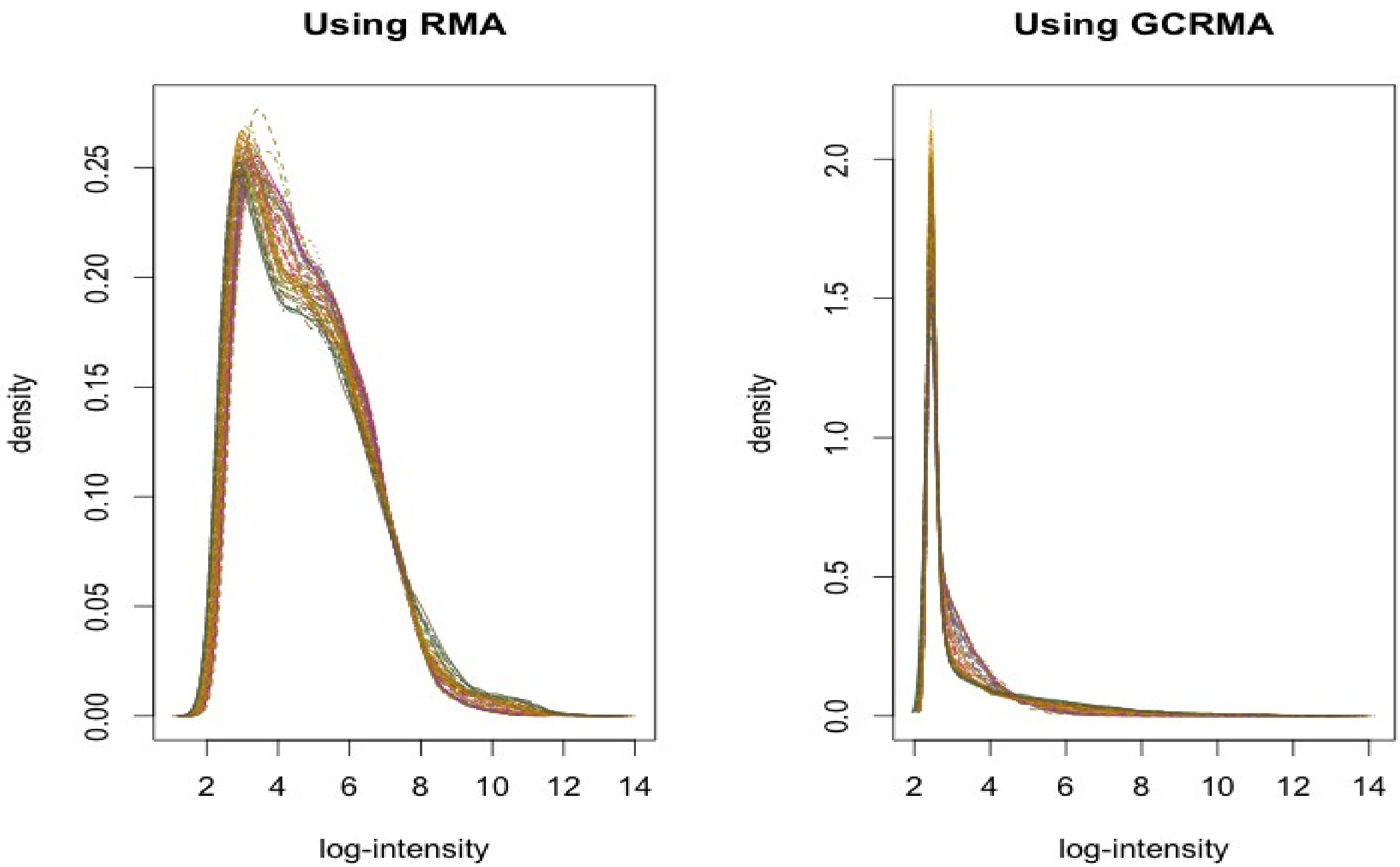
Generate Expression Values

Boxplots of log expression values of all 12625 genes of 20 arrays



Generate Expression Values

Smoothed histogram of log expression values of all 12625 of 50 arrays



A Quick Summary for Part II

We need only three commands to produce expression matrix from CEL data files:

- read CEL data into `affyBatch` object:

  ```
  > Prostate <- ReadAffy()
  ```

- Preprocess Probe-level data and generate `ExpressionSet` object:

  ```
  > Prostate_eset_rma <- rma (Prostate)
  ```

  In this step, one can choose other preprocessing functions too.

- Access expression values in matrix

  ```
  > exprs(Prostate_eset_rma)
  ```

Part III

A Simple Example of Using Expression Data:

Finding differential genes related to a phenotype variable using univariate screening

Generate Top Genes Table

- Specify phenotype and design data

```
> cancer <- c(rep (1, 50), rep (2, 52))
```

- Fit linear model for each gene as a response

```
> fit_rma <- lmFit (Prostate_eset_rma, cancer)
```

- Compute moderated t-statistics and others by empirical Bayes moderation of the standard errors.

```
> efit_rma <- eBayes (fit)
```

- Extract a table of the top-ranked genes

```
> topTable_rma <- topTable (efit_rma, number = 20)
```

- Find a list of top genes (Probe ID)

```
> topgenes_rma <- rownames (topTable_rma)
```

Generate Top Genes Table

A snapshot of top genes table:

```
> head (topTable_rma)
             logFC  AveExpr        t      P.Value    adj.P.Val        B
41468_at  4.356643 6.920753 40.79516 5.549054e-67 7.005680e-63 142.5652
37639_at  5.087711 8.324154 39.22109 2.864858e-65 1.260118e-61 138.6458
37366_at  4.175774 6.743498 39.20376 2.994341e-65 1.260118e-61 138.6019
41706_at  3.774081 6.132773 38.32262 2.896583e-64 9.142341e-61 136.3449
36491_at  3.503627 5.665337 37.30346 4.232732e-63 1.068765e-59 133.6760
1740_g_at 3.799499 6.088183 36.83541 1.481559e-62 3.117447e-59 132.4287
```

Access Annotation Information

A quick sample

```
library("GO.db") ## Go database
library("hgu95av2.db") ## gene chip (platform) database

## To list the kinds of things that can be retrieved
> columns(hgu95av2.db)

## list ENTREZID, GENENAMES with probe id in topgenes_rma
> select(hgu95av2.db, topgenes_rma, c("ENTREZID","GENENAME"),
"PROBEID")

## find and extract the GO ids associated with the first id
> GO_top <- select(hgu95av2.db, topgenes_rma[2], "GO", "PROBEID")

## use GO.db to find the Terms associated with GO_top
head(select(GO.db, GO_top$GO, "TERM", "GOID"))
```

Access Annotation Information

A Snapshot of GO terms related the top selected gene:

```
> head(select(GO.db, GO_top$GO, "TERM", "GOID"))
```

```
       GOID                                  TERM
1 GO:0004252    serine-type endopeptidase activity
2 GO:0005515                       protein binding
3 GO:0005789        endoplasmic reticulum membrane
4 GO:0005886                       plasma membrane
5 GO:0005887 integral component of plasma membrane
6 GO:0005911                    cell-cell junction
```

Conclusions and Discussions

- Today, it is very easy to generate and analyze micorarray expression matrix with `bioconductor` tools
- Microarray data have many limitations. The actual mRNA signals are contaminated by various noise, including background noise, varying gaining factor, and cross-hybridization noise. In addition, multiple probe sets represent the same gene.
- RNA-Seq is a powerful technology that is predicted to replace microarrays for transcriptome profiling. RNA-Seq avoids technical issues in microarray studies related to probe performance such as cross-hybridization. However, the cost of RNA-seq is still too high. Also, the tools for RNA-Seq data analysis are far from mature.

References

- Gentleman, Robert, Vincent J. Carey, Wolfgang Huber, Rafael A. Irizarry, and Sandrine Dudoit. *Bioinformatics and Computational Biology Solutions Using R and Bioconductor.* Springer, 2005.

  The book is free and comprehensive.

- http://www.bioconductor.org. The website contains a large archive of software documentations, workshop slides, and workflow examples for different tasks.