Quality Checks for Microarray Data

Utah State University – Spring 2014
STAT 5570: Statistical Bioinformatics
Notes 2.1

References & Reminder

- Chapter 3 of Bioconductor Monograph (course text)
- (Same issues here recur in other technologies)

Recall Background Correction and Normalization

- Why background correction? -
  to remove "noise" and "local artifacts"
- Why normalization? -
  to make arrays more comparable
- What is the intended effect of both steps? -
  to make spot-level data comparable -
  so differences are biologically meaningful

Is it enough?

- Even after background correction and normalization-
  some arrays are "beyond correction"
- How to assess this?
  - Sometimes obvious -
    array image has glaring problem
  - Sometimes more subtle -
    a "local" problem on one array
- Rely on – graphical checks

Example data: ALL MLL

- A large acute lymphoblastic leukemia (ALL) study using the HGU133A and HGU133B (human) arrays
- A subset of the ALL data is provided in the MLL.B AffyBatch object
- Let's just look at 8 arrays

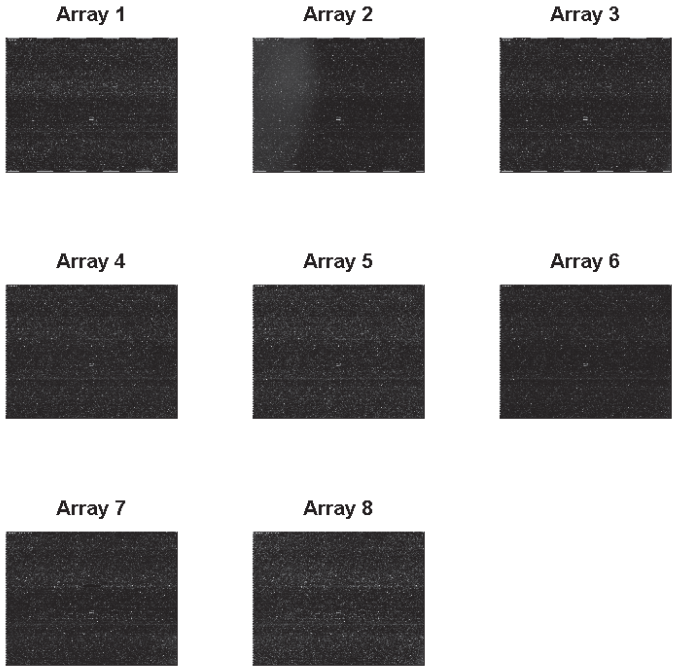
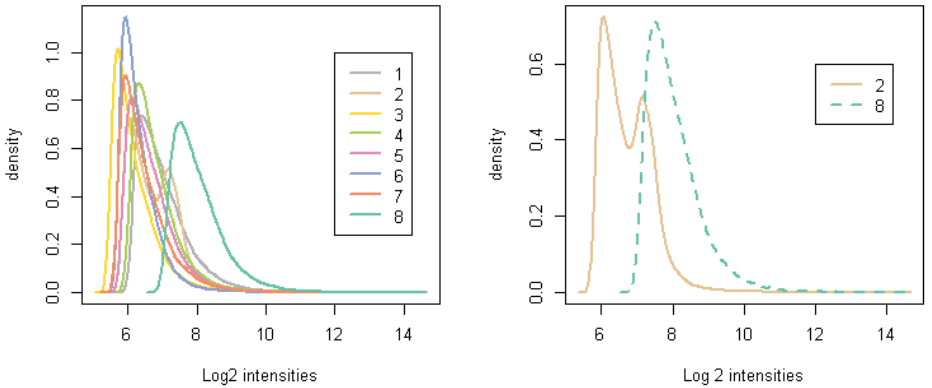


Image – compare arrays (#2?)

```
library(affy)
library(ALLMLL)
data(MLL.B)
Data <- MLL.B[,c(1:6,13,14)]

par(mfrow=c(3,3))
for(i in 1:8)
  {
   image(Data[,i],  main=paste('Array',i),
   cex.main=2)
  }
```


[Smoothed] Histograms



Bimodality suggests - a spatial artifact

Histograms – compare arrays (#2!)

```
library(RColorBrewer)
par(mfrow=c(2,2))
cols <- rev(brewer.pal(8, "Set2"))

hist(Data,col=cols, lty=1,
  xlab="Log2 intensities",lwd=2)
legend(12,1,1:8,lty=1,col=cols,lwd=2)

hist(Data[,c(2,8)],col=cols[c(2,8)],
  lty=c(1,2),xlab="Log 2 intensities",lwd=2)
legend(12,0.6,c(2,8),lty=c(1,2),
  col=cols[c(2,8)],lwd=2)
```


MA plot – compare arrays

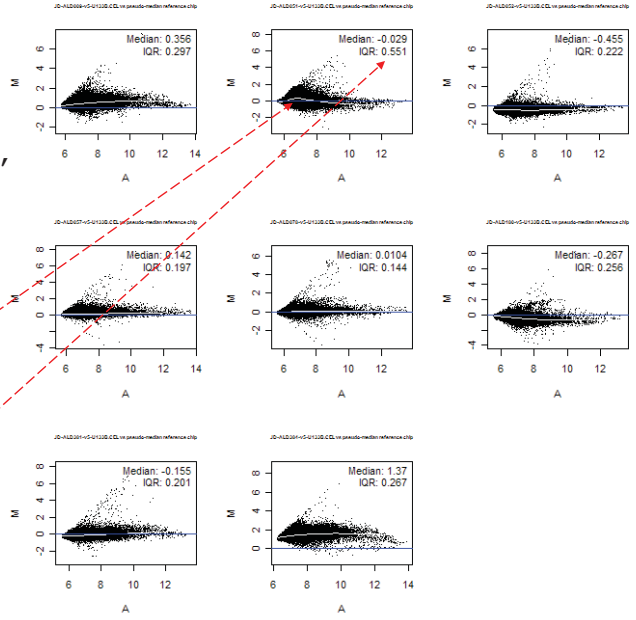
- MA plot: M=Y-X vs. A=0.5(Y+X)
  - Rotate and scale Y vs. X scatterplot
  - For log-scale expression on arrays Y and X:
    - M = log fold-change (Y vs. X)
    - A = average expression
- For comparing multiple arrays, create a "pseudo-array" reference by taking the median for each probe across all arrays
- Loess curve:
  locally weighted polynomial regression

MA plot

```
par(mfrow=c(3,3))
MAplot(Data,
 loess.col='white',
 cex=1,
 cex.main=0.5)
# this can take
# a few minutes
```

Quality problems most apparent when:

- Loess line oscillates much
- M-variability is much greater than other arrays

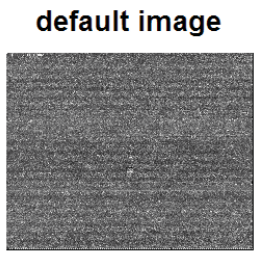


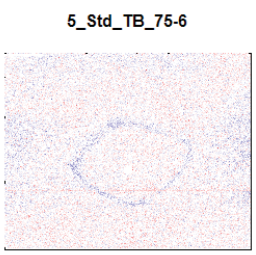
PLM Image

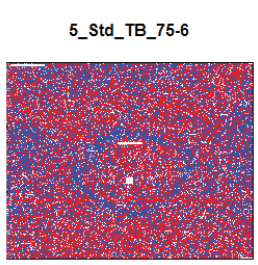
- Probe Level Model
- Recall RMA model (Notes 1.4):

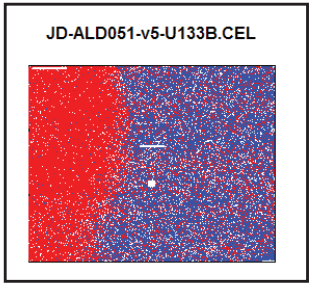
$$Y_{ijk} = \underbrace{\mu_{ik}}_{\text{Log-scale expression level for gene k on array i}} + \underbrace{\alpha_{jk}}_{\text{Probe affinity effect}} + \varepsilon_{ijk}$$

- Use robust measure to estimate model parameters
- To identify quality problems, look at residuals









Look for:

- Substantial artifacts

- Systematic patterns

PLM Image – look at residuals

```
library(affyPLM)
library(AmpAffyExample)
data(AmpData)
par(mfrow=c(2,2))

# Fit RMA PLM to data; could also use fitPLM function
Pset1 <- rmaPLM(AmpData)
image(AmpData[,3],main='default image', cex.main=2)
image(Pset1, type="resids", which=3)
image(Pset1, type="sign.resids",which=3)

# Fit RMA PLM to other data; look at problem array
Pset2 <- rmaPLM(Data)
image(Pset2, type="sign.resids",which=2)

# Could also consider type="pos.resids"
# or type="neg.resids"
```


Summary

- Use graphical checks to look at microarray data quality
  - Image
  - Histogram
  - MA plot
  - PLM (residual) image
- Also consider:
  - Boxplots
  - RNA degradation (3'/5' ratios)
  - Normalized Unscaled Standard Error (NUSE) plot