

**GS01 0163**

# **Analysis of Microarray Data**

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## Lecture 24: Frozen Barcodes

- How big are batches?
- Are there questions or measures that are robust to batches?
- Can we account for them using other info?
- Barcode, Zilliox and Irizarry, Nat Meth 4:911-3, 2007
- fRMA – McCall et al, JHSPH Working Paper 189, 2009
- Barcode 2 – McCall et al, JHSPH WP 200, 2009
- TCGA

## Batches and Databases

A big reason we've been harping on batch effects is that (a) they're big, and (b) as more data gets assembled, dealing with them becomes more important (e.g., TCGA).

That said, batches are only a problem as long as we don't understand them. If we understand how bad they can be and what their characteristics are, we can either account for them or declare the whole exercise a waste of time.

For some types of arrays, we now have a *lot* of data. Can we use this to learn about batches?

## Channelling...



Most of the stuff I'll be talking about today comes from Rafa Irizarry's lab at Johns Hopkins. So just imagine I'm him for the remainder of the lecture.

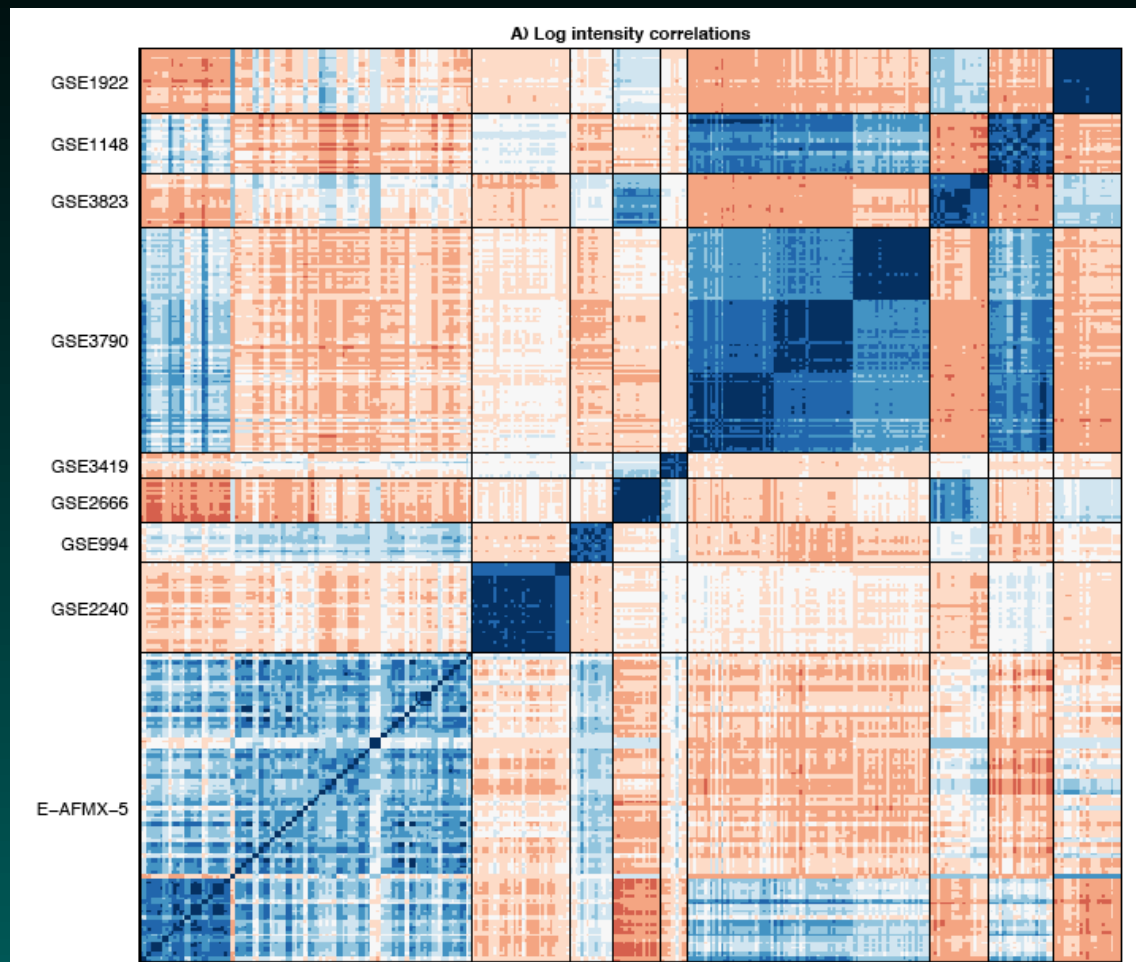
## GEO and ArrayExpress

For the past several years, the Nature, Science and Cell journal families have all adhered to the MIAME standards, and required that array data be deposited in a public database: either GEO or ArrayExpress.

For Affy U133A and U133+2, there are now literally hundreds of CEL files available from several labs and tissue types.

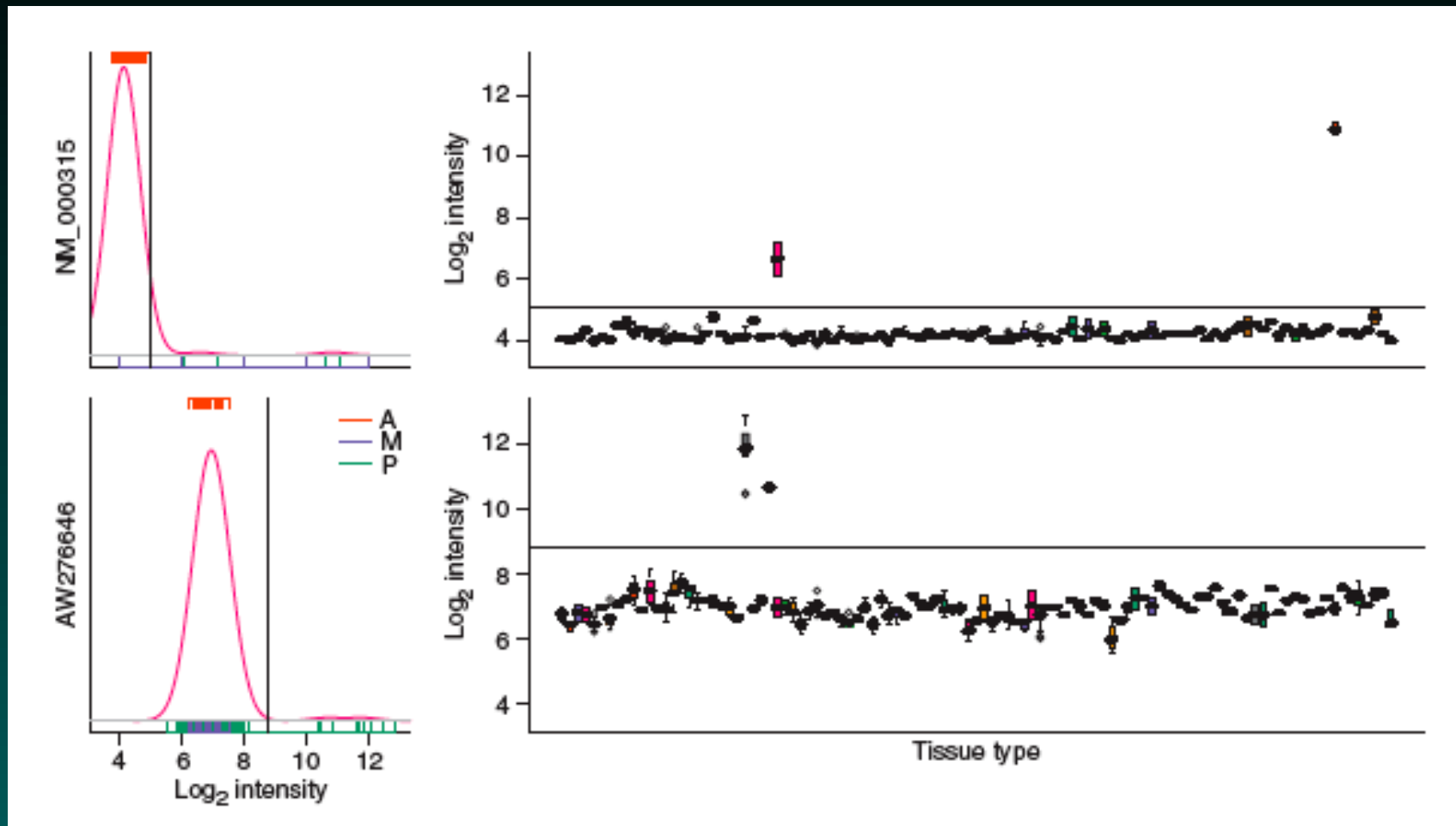
If we process all of these arrays in the same way, we may be able to get a better feel for the extent of batch effects.

# Correlating GEO



Zilliox and Irizarry, Supp Fig 3A. Correlating GEO data. This is disturbing. Note E-AFMX-5.

# Probes Across Many Arrays



Zilliox and Irizarry, Fig 1. Which genes are Present? Absent?

## When is a Gene Really Present?

Assembling the barcode: 1092 samples from 118 tissue types from 40 different studies. Of the samples, 500 are breast tumors, 498 are normal tissue, and 94 are other disease.

Fit a density estimate to the data across experiments. Record all local maxima. Declare the lowest maximum to correspond to “no expression”.

Note variation in “null” levels.



## Setting Cutoffs

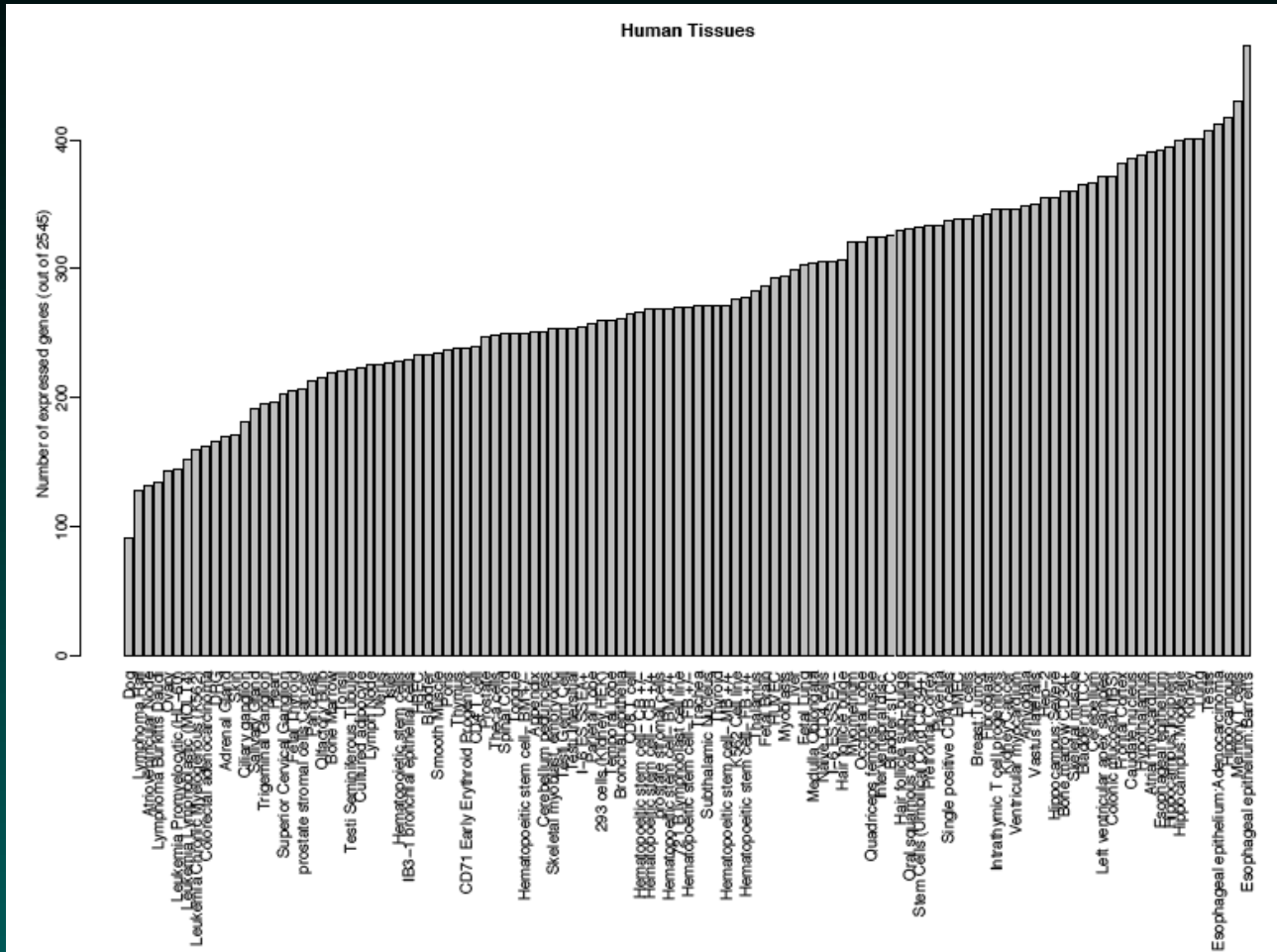
Use the lower half of the bottom mode to estimate the standard error, and flag a gene as “high” if it is more than  $K$  standard deviations above the no expression level (they used  $K = 6$ ).

Keep only bimodal genes for classification (2519 for U133A).

What are most of the barcode genes? About 3/4 encode membrane or extracellular proteins.

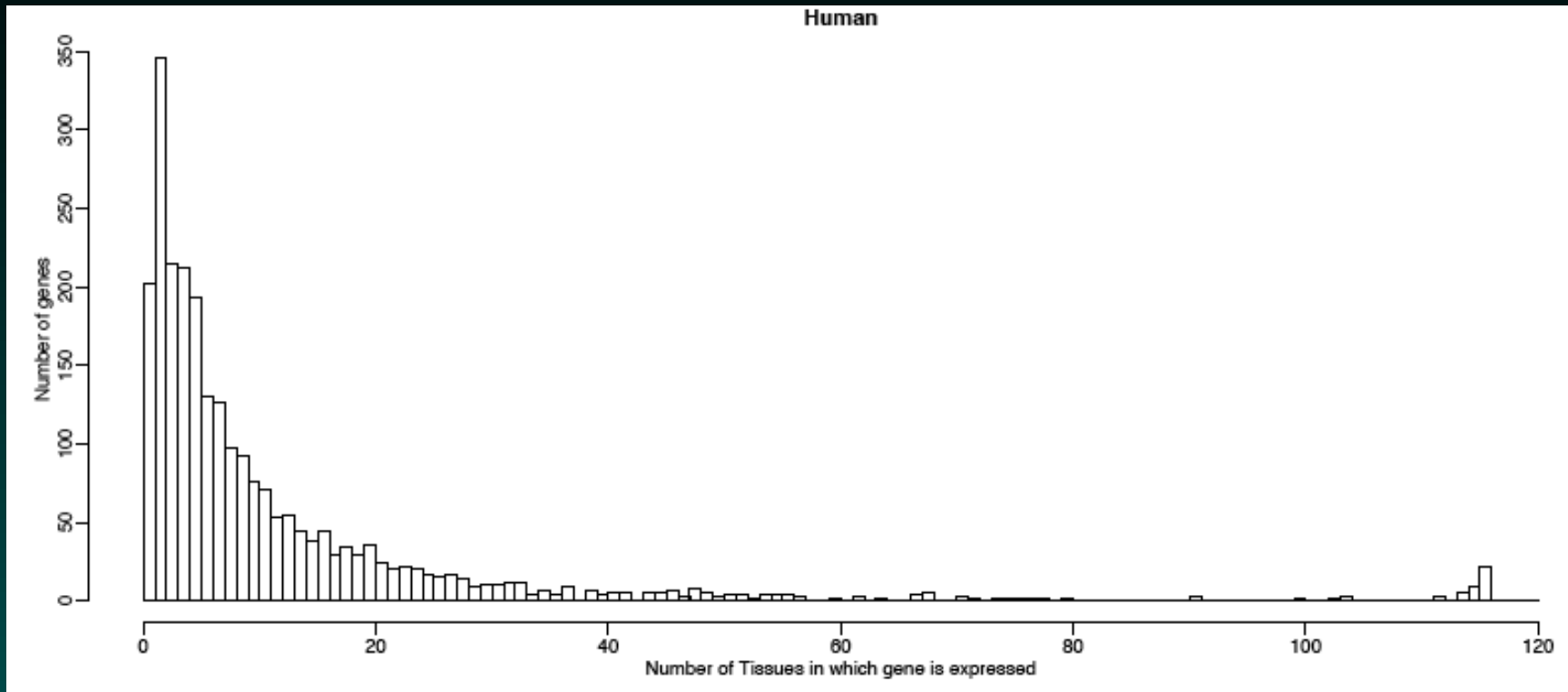
Store mean, standard deviation on a probeset by probeset basis, now that we've assembled them.

# How Many Genes Characterize Each Tissue?



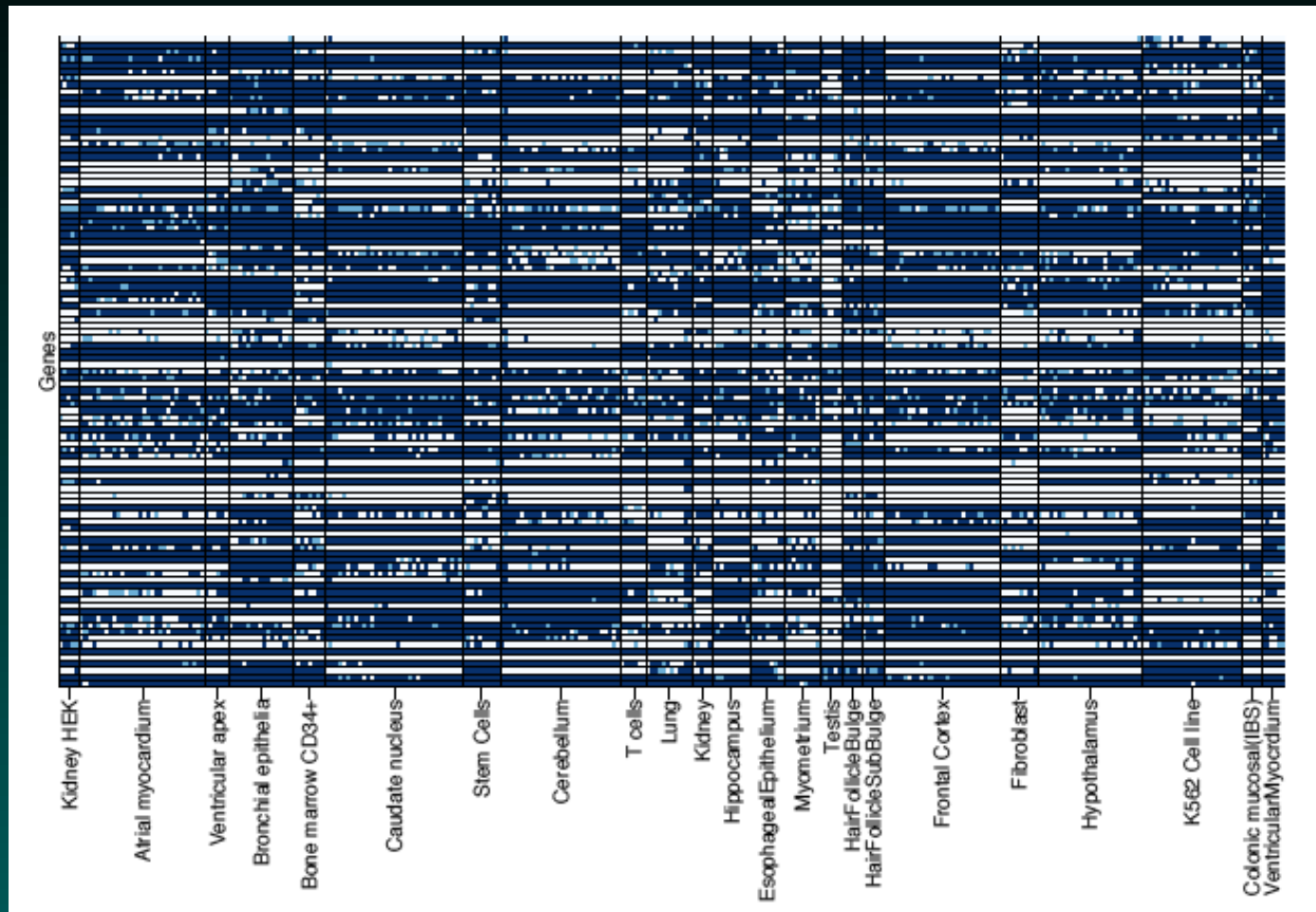
Number of high genes by tissue

# How Many Tissues Is a Gene High In?



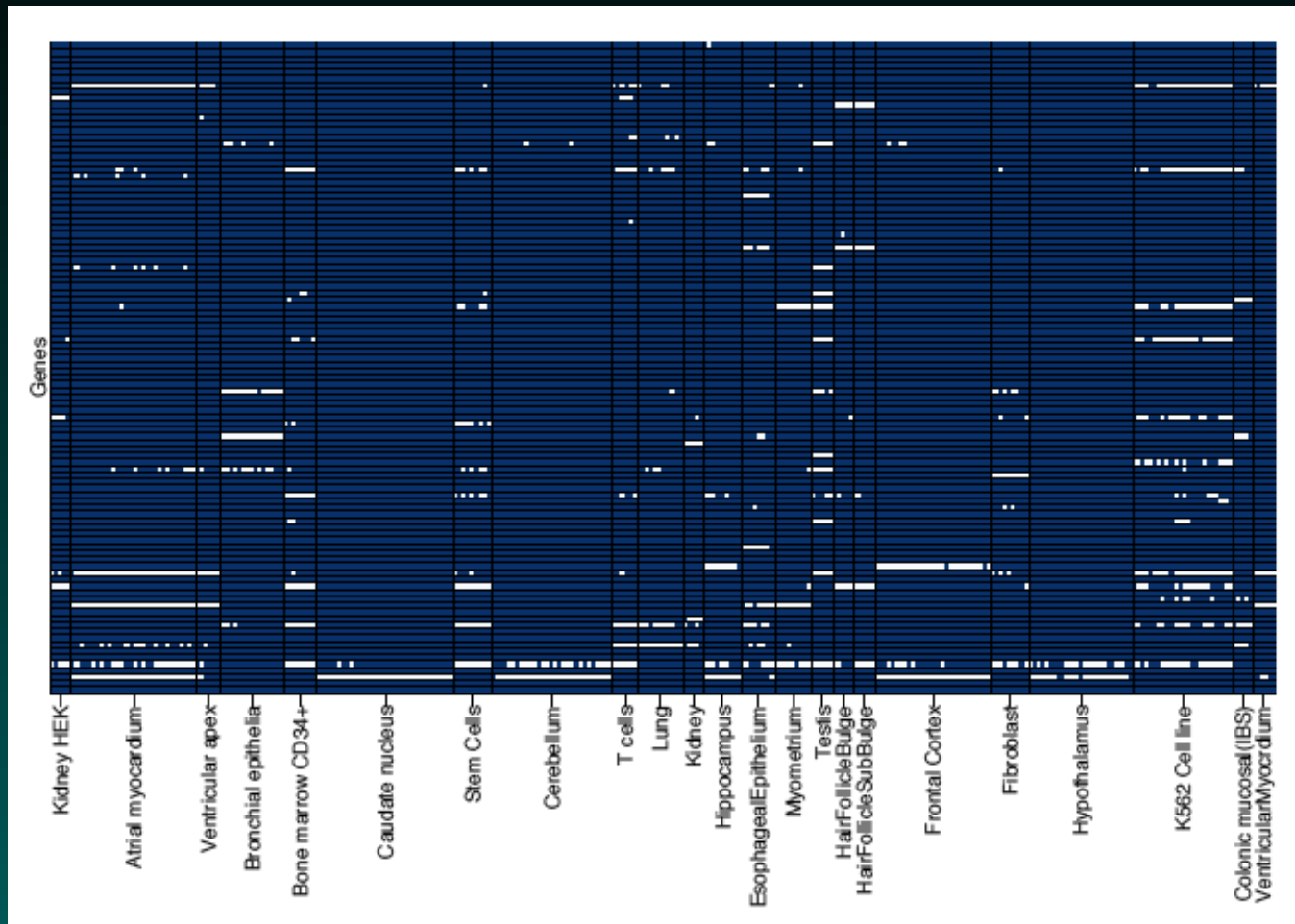
Number of high tissues by gene

# How do Present/Absent Calls Do?



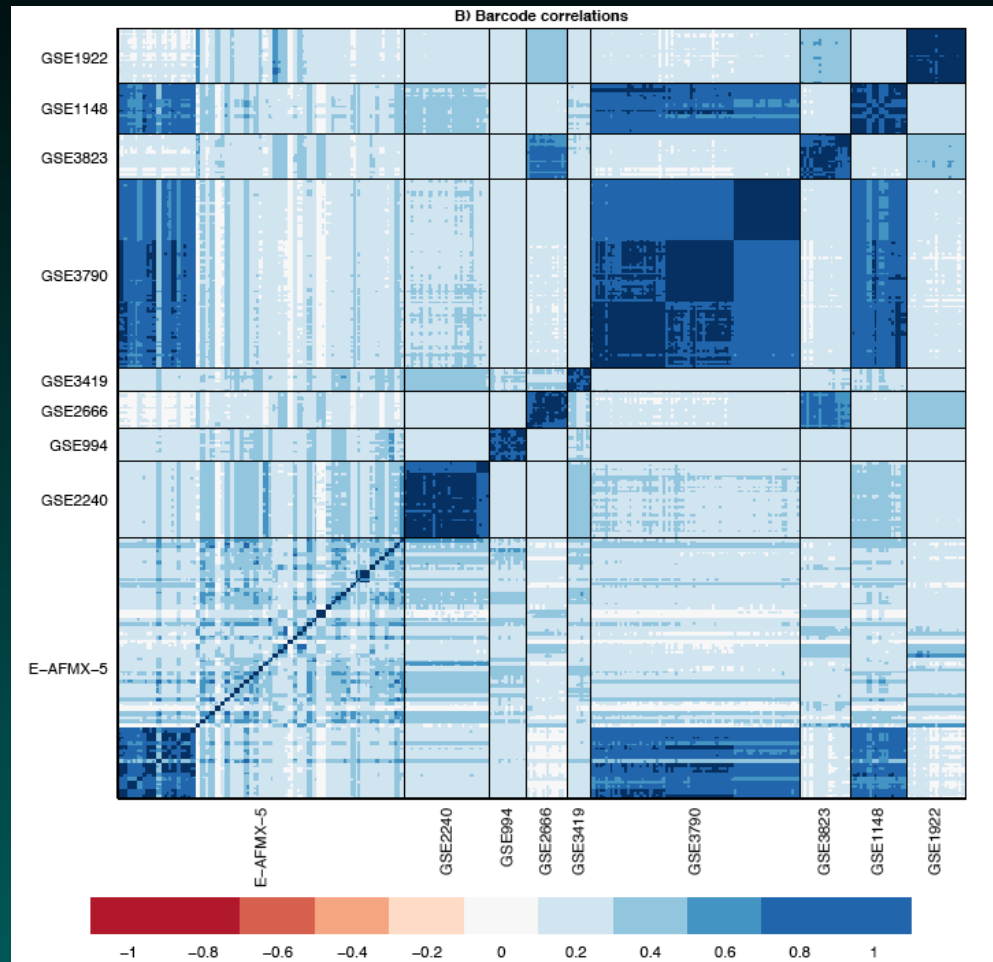
Defined one array at a time, largely driven by intensity (100 random genes).

# How do Barcode Present/Absent Calls Do?



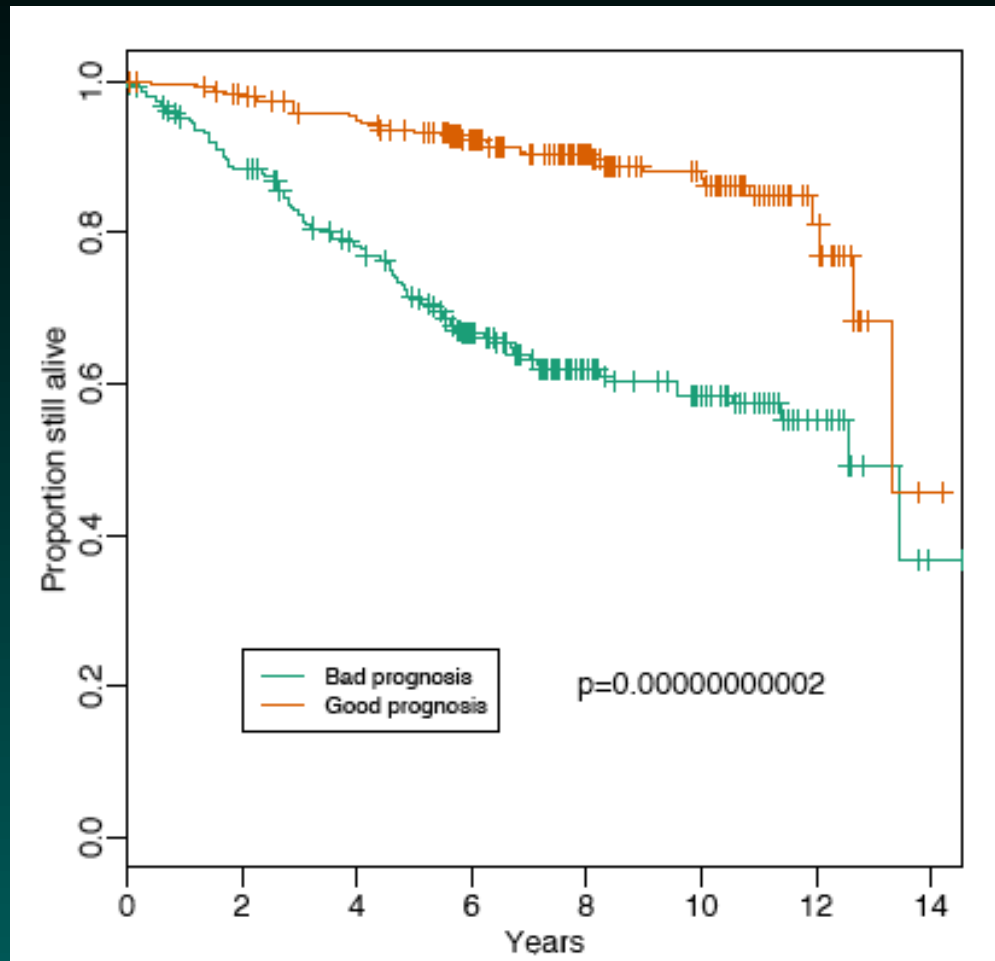
Defined cross-study. Note broad blueness.

# Barcode Correlations?



Zilliox and Irizarry, Supp Fig 3B. Barcoding GEO data. Much better.

# Barcode Survival



Zilliox and Irizarry, Supp Fig 5A. Barcoding normal and tumor breast, then coding tumors.

## Does it Work?

In general, this is pretty robust, and pretty simple.

That said, we've had several ovarian samples classified as breast. This may reflect the preponderance of breast samples used in assembling the classifier, or that we didn't score our samples in the same way.



## How Did They Score Samples?

They like RMA. But.

They have over 1000 CEL files to work with. What problem do you think they encountered?

What are other problems with RMA?

How can we use RMA for a single sample (eventually required for clinical applications)?

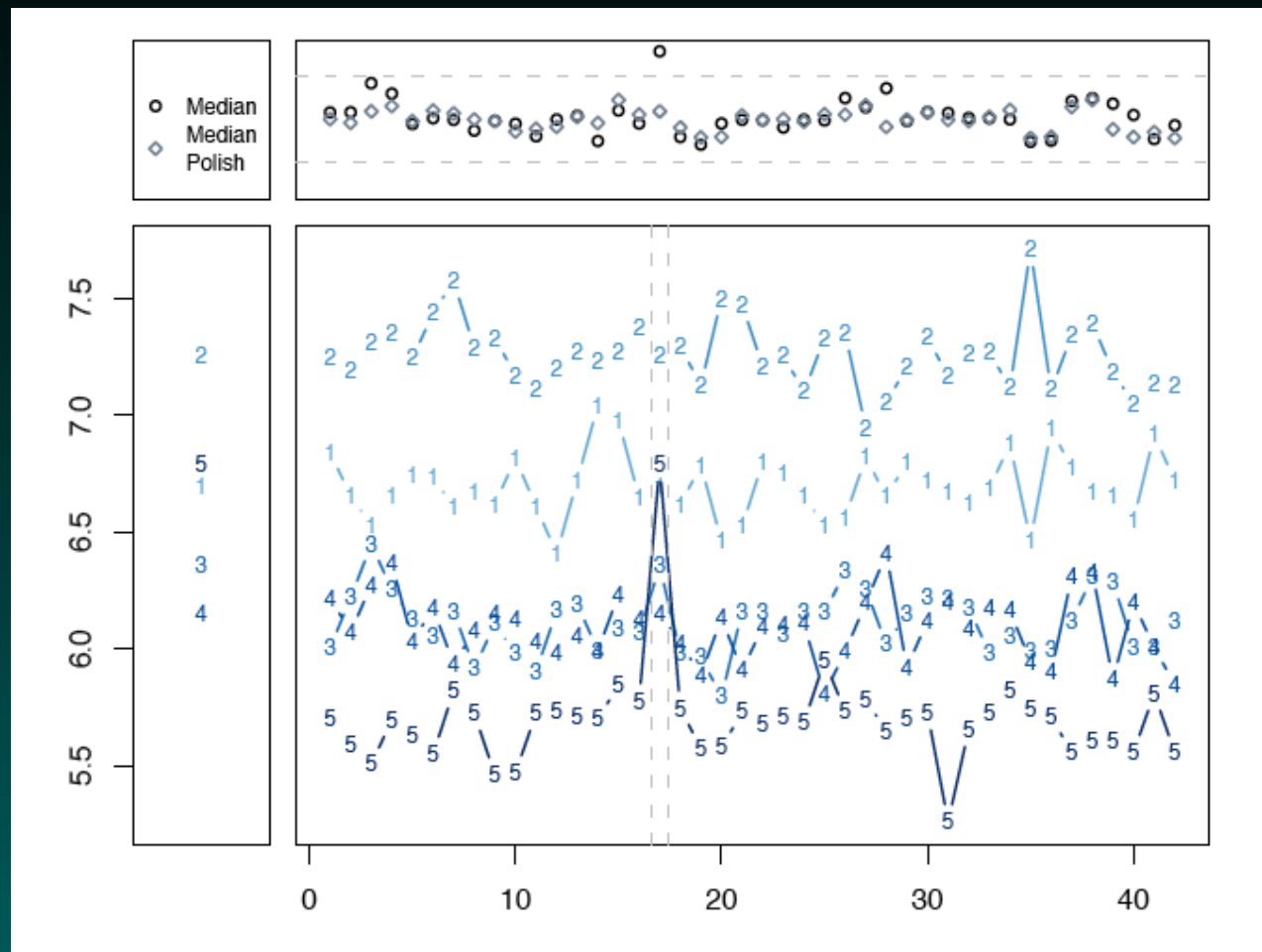
## What's Good About RMA?

The model is simple, and it learns.

$$Y_{ijn} = \theta_{in} + \phi_{jn} + \epsilon_{ijn},$$

for probe  $j$  in probeset  $n$  on array  $i$ . We're interested in the sample-specific intensities  $\theta$ , but we have to deal with the probe effects  $\phi$  and probeset errors  $\epsilon_{ijn}$ .

# Picturing Benefits



McCall et al, Fig 1. Robust learning. But how much learning do we need?

## Start With a Reference Set

Katz et al., BMC Bioinformatics, 7:464, 2006.

Run RMA on a single large database, and record (a) the overall quantile vector, and (b) the estimated probe effects  $\hat{\phi}$ . These values are now fixed, or *frozen*.

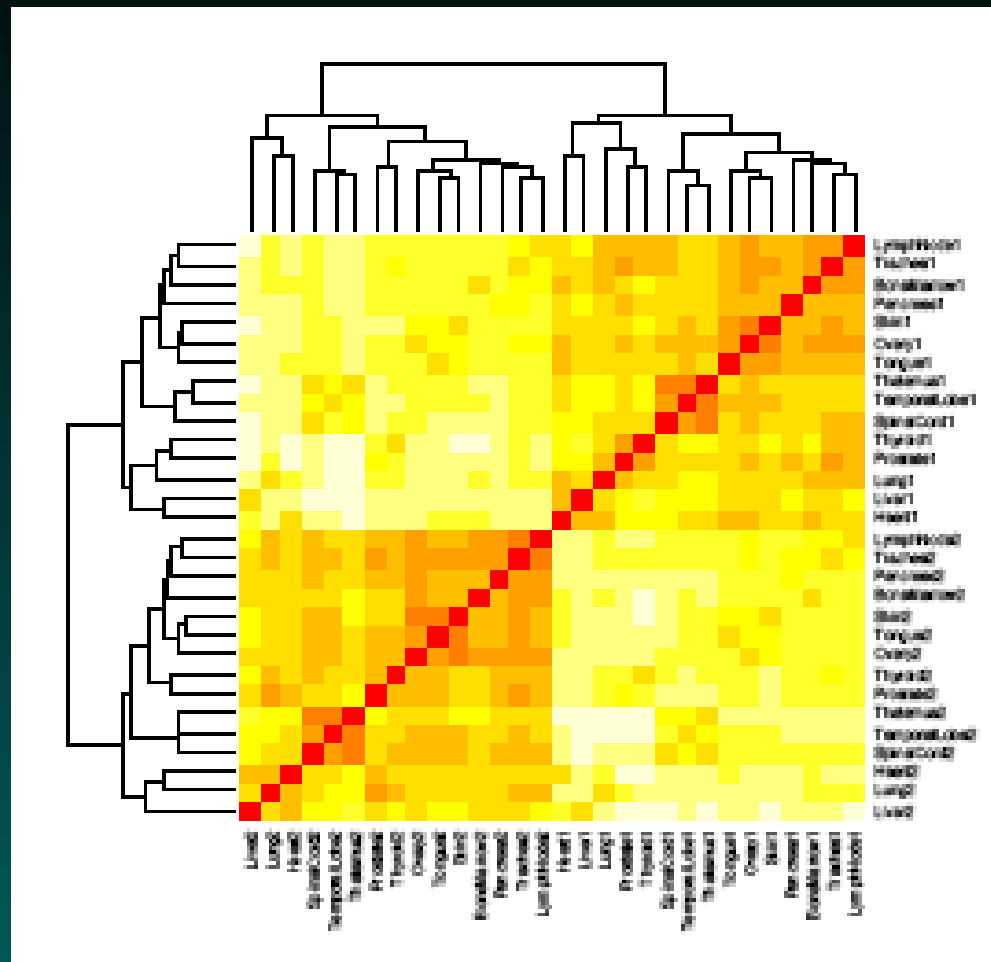
For a given new array, report the median of

$$y_{ij} - \hat{\phi}$$

as the sample estimate.

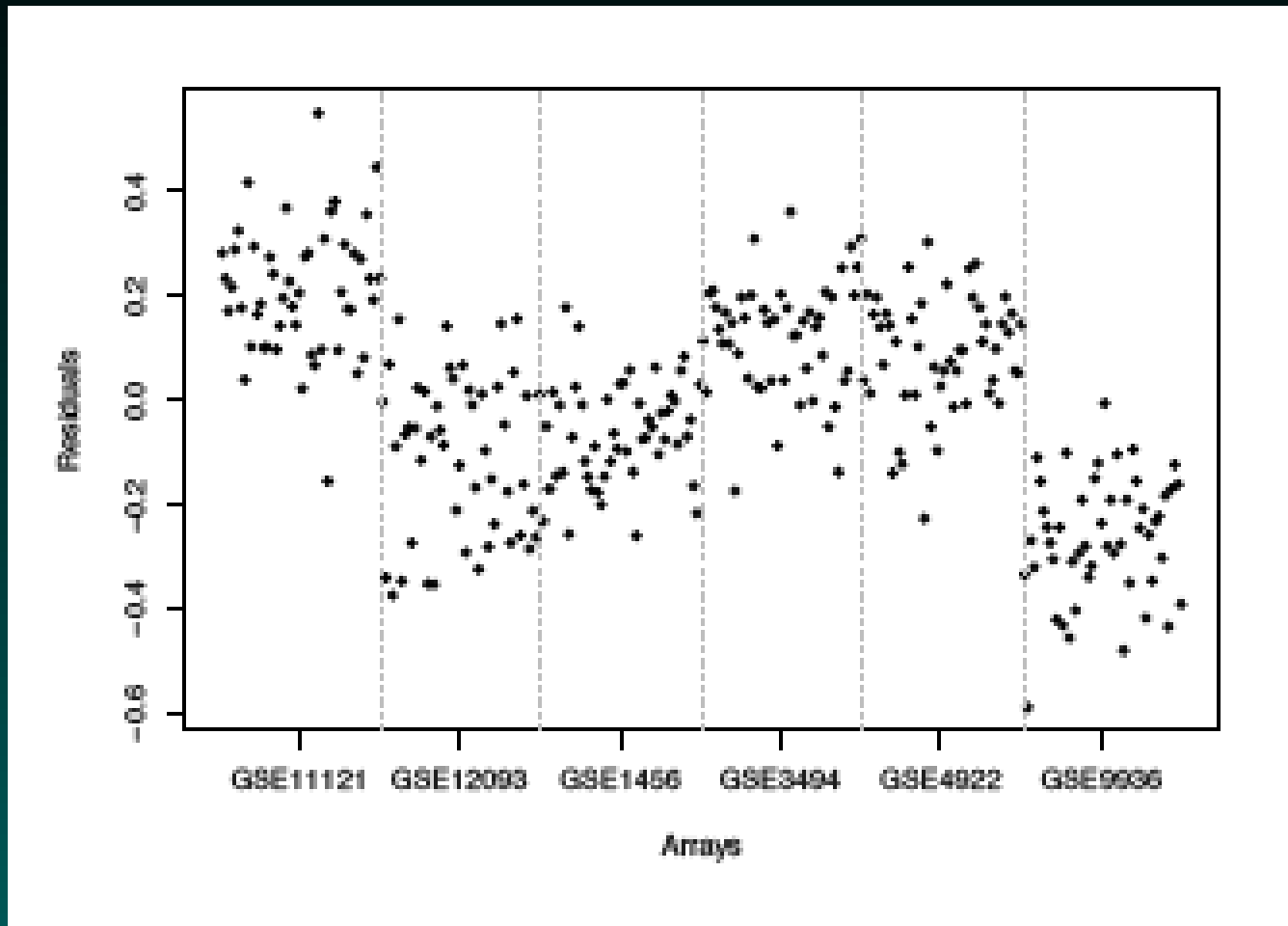
Does it work?

# Why Might it Fail?



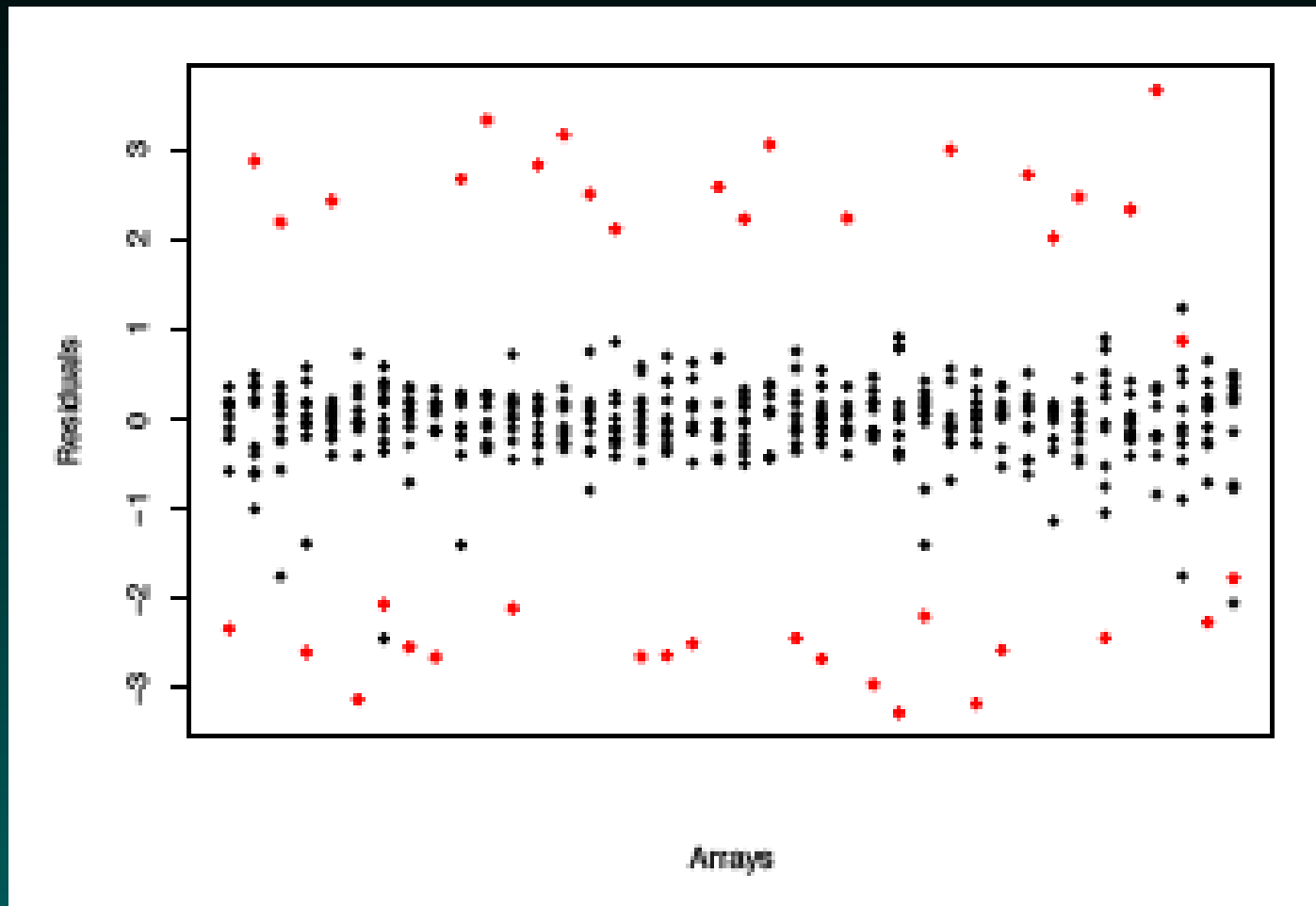
McCall et al, Fig 4A. What's driving the clustering?

# Residuals for One Probe



McCall et al, Fig 2B. Batch effects are large, and can corrupt further estimates.

## and On a Related Note



McCall et al, Fig 3B. Some probes are simply far more variable than others.

## Revising the Model

$$Y_{ijkn} = \theta_{in} + \phi_{jn} + \gamma_{jkn} + \epsilon_{ijkn}$$

where  $k$  denotes batch,  $\gamma$  is a random effect with variance

$Var(\gamma_{jkn}) = \tau_{jn}^2$ , and the random error is probe-specific:

$Var(\epsilon_{ijkn}) = \sigma_{jn}^2$ .

So, how do we estimate all of the terms?



## Start with the Familiar

Begin with standard RMA to get starting estimates of  $\hat{\theta}_i$  and  $\hat{\phi}_j$ . Then, use the residuals to estimate the between and within batch variation terms.

The reference set was chosen to balance tissue and experiment, with 5 arrays from 170 distinct combinations.

## Estimating One New Array

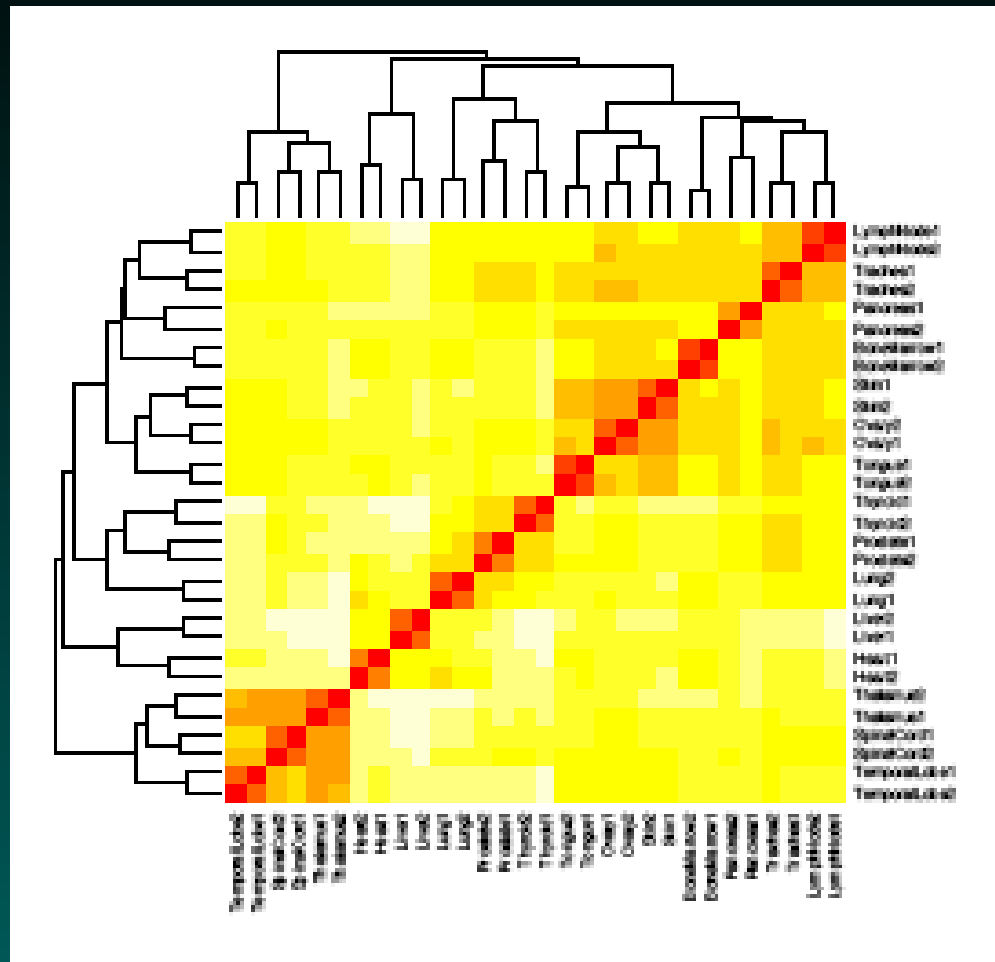
Given that we have probe-specific batch and variance terms,

$$\text{Var}(\hat{Y}_{jn}) = \tau_{jn}^2 + \sigma_{jn}^2$$

use these to produce a weighted mean from the individual probe values (with weights inversely proportional to the variance).

Does it work?

# Clustering Redux



McCall et al, Fig 4B. What's driving clustering now?

## Can We Do Better With More Arrays?

Sure. More information is (almost) always good.

If we have a new batch of arrays (and recognize the batch boundaries), then we can estimate the batch effect for all of the arrays and render our estimates more precise (effectively removing the  $\tau^2$  term).

Our estimates are correlated, but again it boils down to a weighted mean.

## Are These Easy to Use?

Good question. The packages are still in early release form, but they are R packages nonetheless.

Since the barcode weights were initially assembled using refRMA as opposed to fRMA, it would probably be good to go back and reweight things (which I suspect they're doing or have done).

## Revisiting the Barcode

It's been two years since the initial barcode paper. Since then, fRMA has been better locked down, and more extensive reference databases have come online.

Can we come up with a better definition?

Can we keep more genes?

Can we use barcodes for more than tissue type?

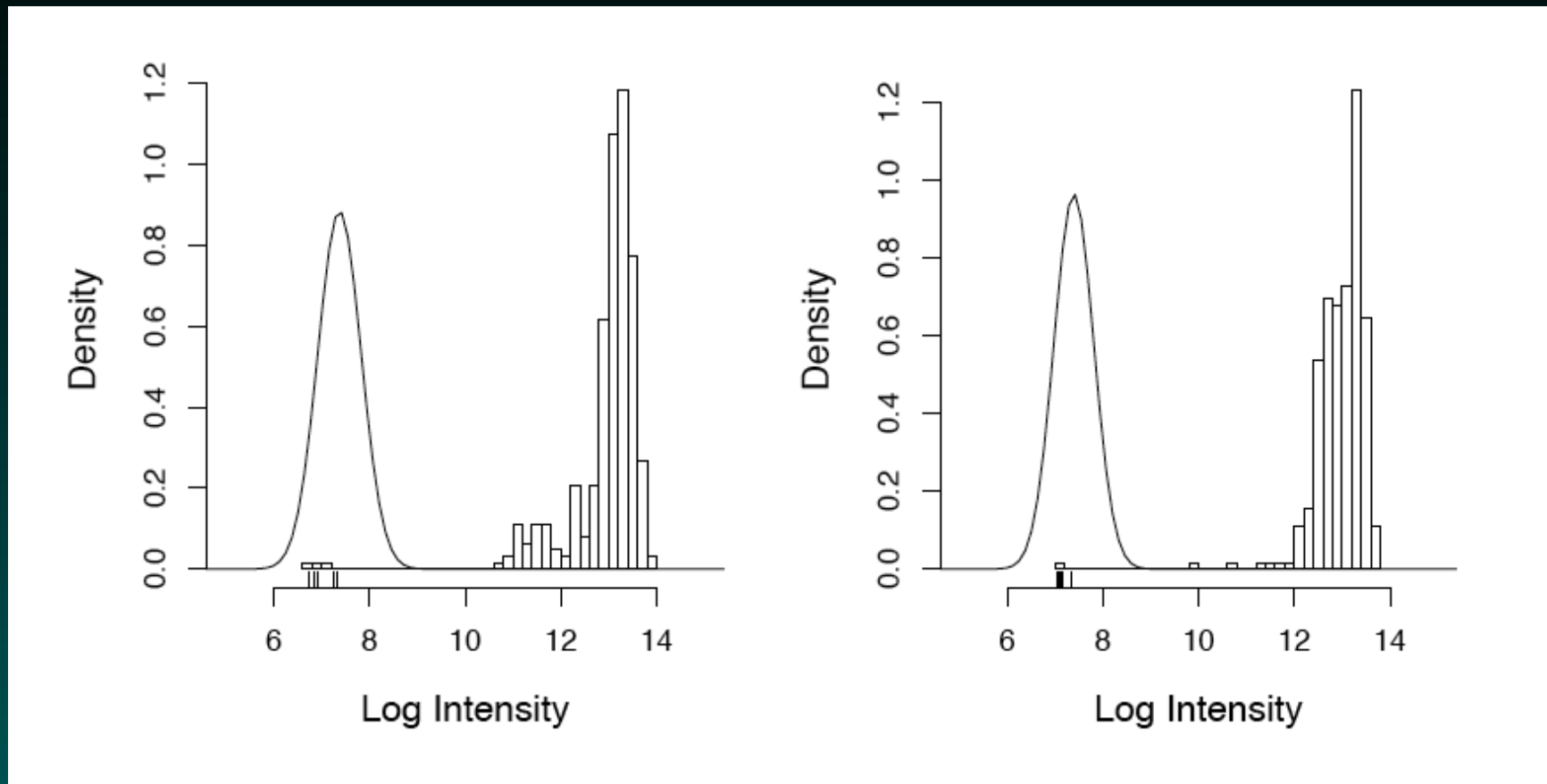
## New Data

316 tissue types profiled at least 5 times in GEO; 8277 U133A arrays in all.

All arrays were processed with fRMA to give expression values.

Also ran 5 yeast samples on human arrays to get a better idea of “null” expression.

# The Vital Yeast



McCall et al (WP 200), Fig 3. Yeast levels clearly identify null expression.



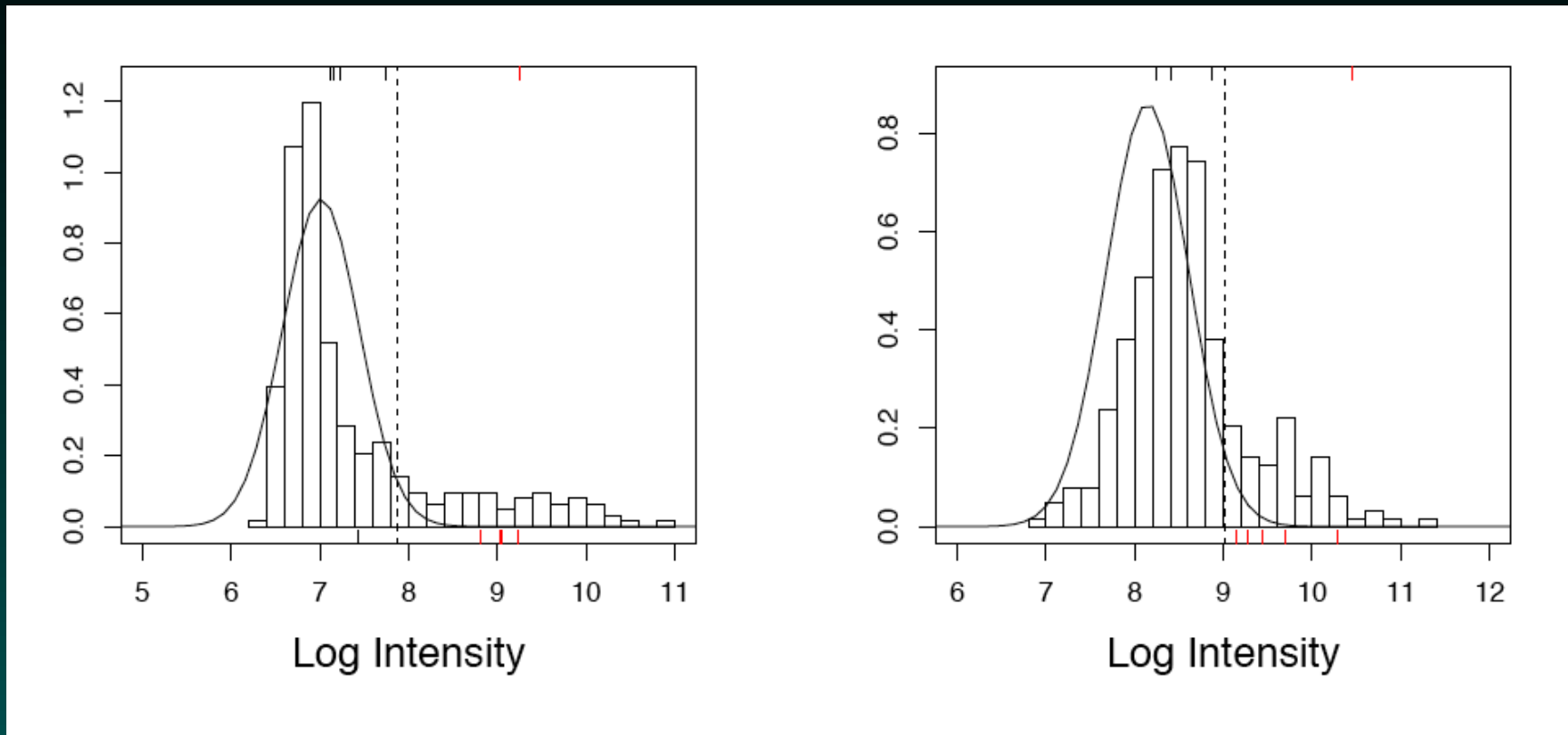
## Keeping More Genes

The new model:

$$\begin{aligned}(y_{ijg}|\theta_{jg}) &\sim N(\theta_{jg}, \sigma_g^2) \\ \theta_{jg}|\mu_g &\sim (1 - p_g) * N(\mu_g, \tau_g^2) + p_g * U(\mu_g, S) \\ \mu_g &\sim N(\epsilon, \lambda^2)\end{aligned}$$

For gene  $g$  in sample  $i$  from tissue  $j$ . Values are assumed to come from a mixture of the unexpressed and expressed intensity distributions.

# Revisiting Cancer Prediction



McCall et al (WP 200), Fig 6. Clear split between expressed and not, but not by 6 SD.