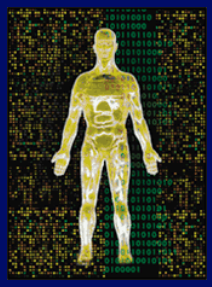
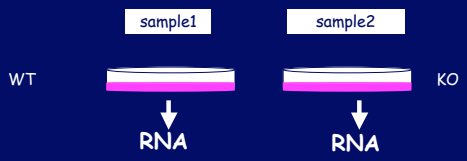
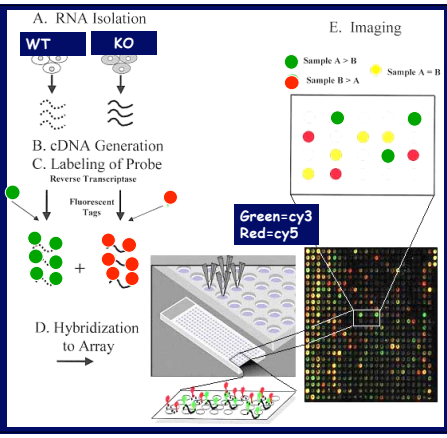
Data Analysis of DNA Microarrays



Can we detect siRNA-induced knockdown of gene expression using DNA microarrays?

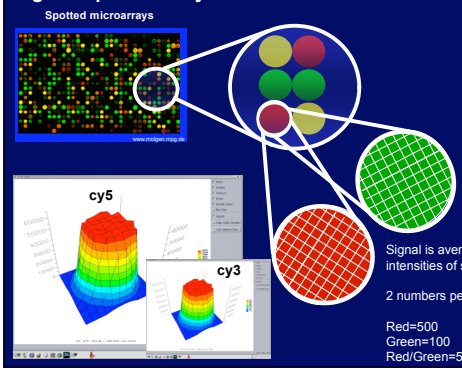
Starting with two biological samples





Microarray Measurements

Signal: Spotted arrays

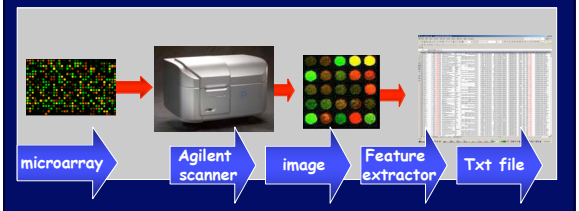


Signal is average of pixel intensities of spot

2 numbers per spot

Red=500
Green=100
Red/Green=5 (5 Fold Greater)

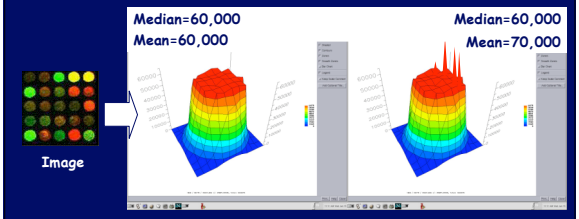
Processing microarrays: Scanning and Image analysis



File is large
Need to truncate

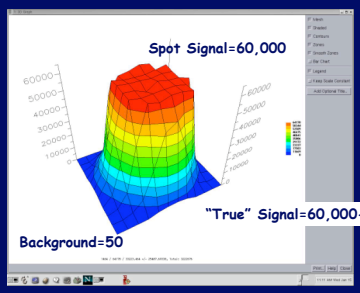
Spot Intensity: Mean or Median?

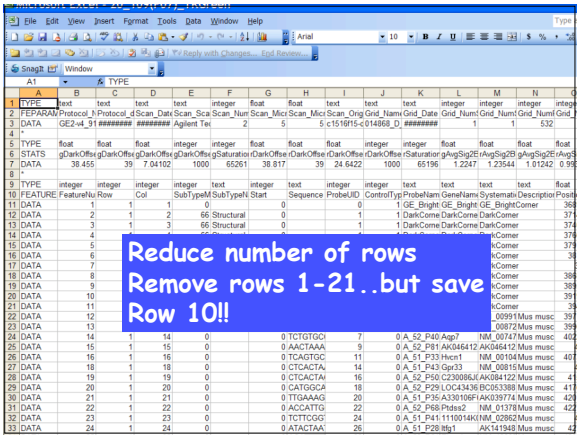
- Which is more affected by extremes?
- Which is better estimate of spot intensity?

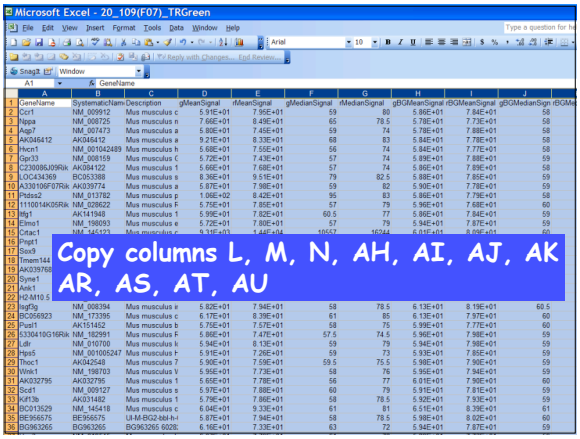


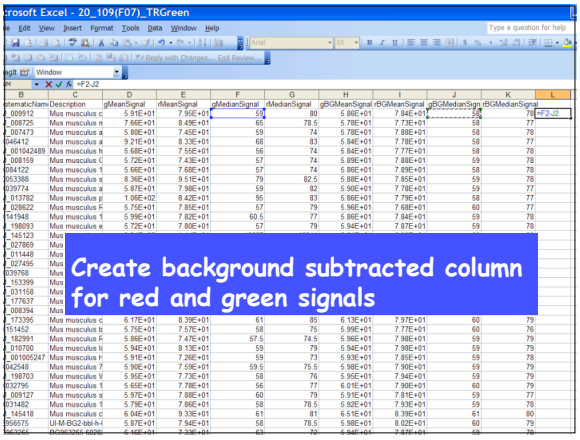
All pixels of a spot are used to calculate a Mean or Median Intensity

Subtracting Background





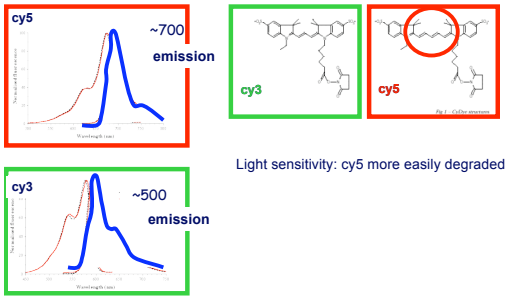




Within-Slide Normalization

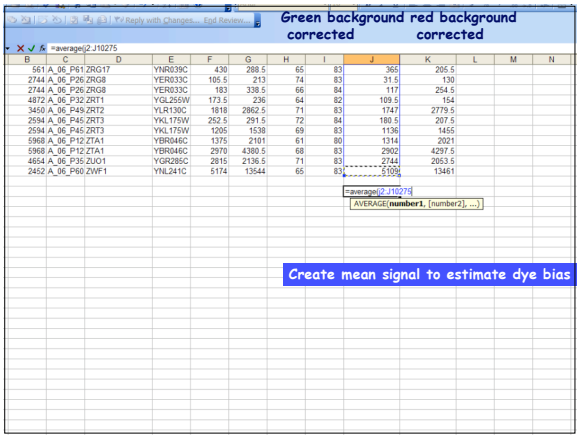
- Normalization balances red and green intensities.
- Imbalances can be caused by
  - Different incorporation of dyes
  - Different degradation of dye
- In practice, we usually need to increase the red intensity a bit to balance the green

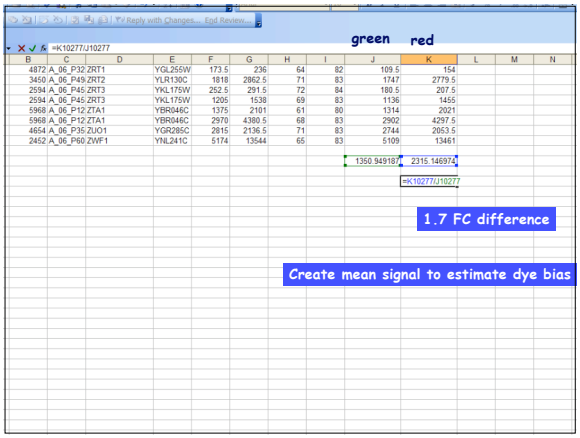
cy3 and cy5: Commonly used dyes

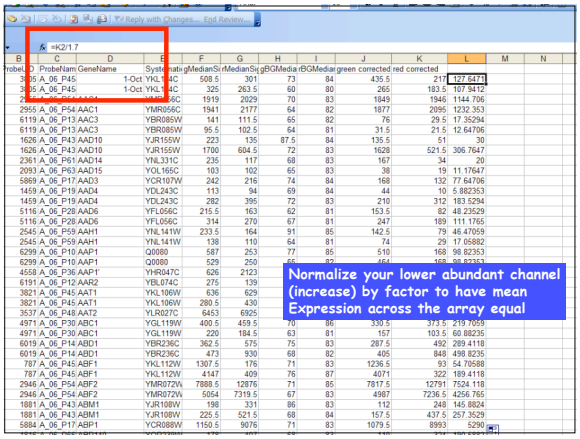


Light sensitivity: cy5 more easily degraded

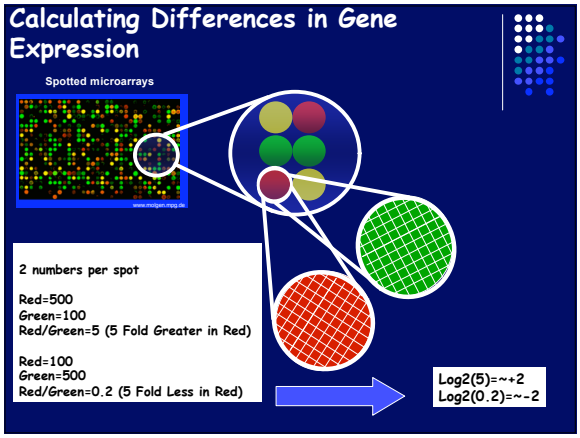
Let's begin the normalization process:

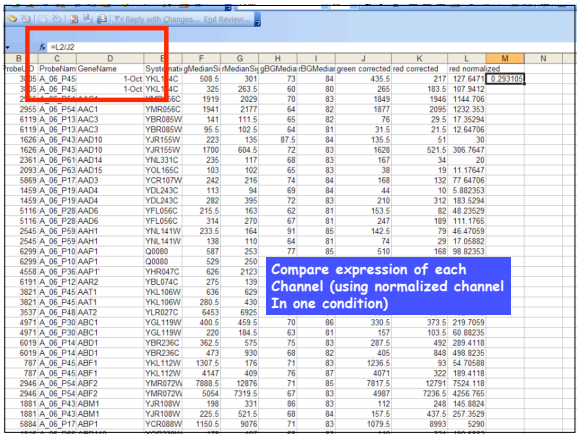


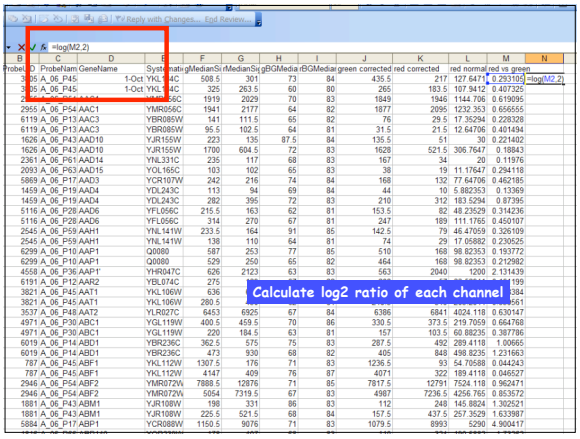


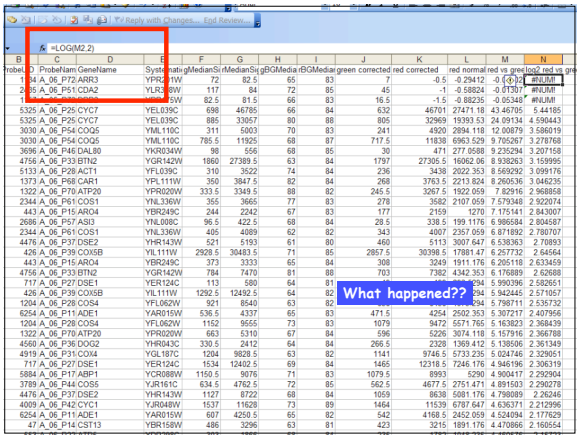


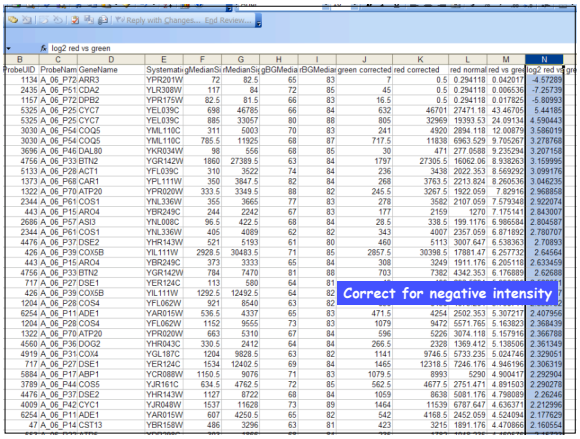
Calculating Differences in Gene Expression





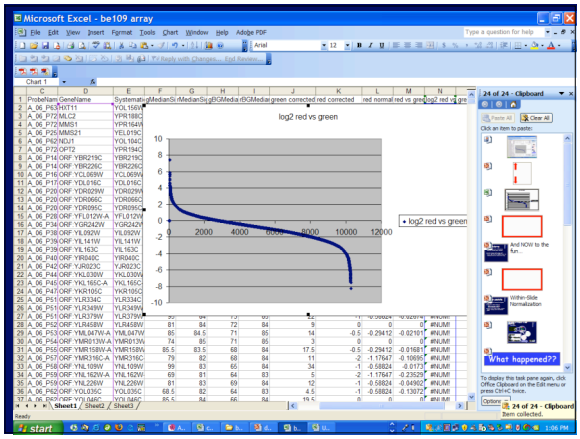




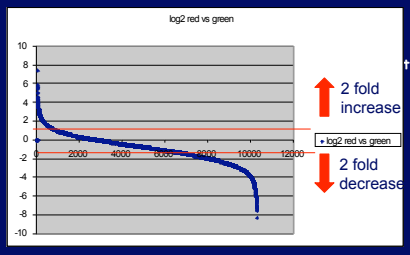


And NOW to the fun…

- How many genes were differentially expressed between your 2 samples?
- Was the expression of your gene of interest significantly changed between the two samples?…can we assess this directly



Create scatter plot of log2 ratios (green versus red)



tter Plot

Distribution of log2 ratios

- What are we expecting????
- What color would all of these spots be??

Trends in Data

- How many changes do you see?
- What could these changes mean?
- How can we find out more about these genes and their functions?
- Which biological processes are up-regulated, down-regulated, no change?

Good luck!!