

Design, Generation, and Use of cDNA Microarrays on Glass

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SOT2000: Continuing Education Course PM-16

Toxicogenomics in the Trenches

Sunday, 19 March, 2000

Types of DNA Microarrays

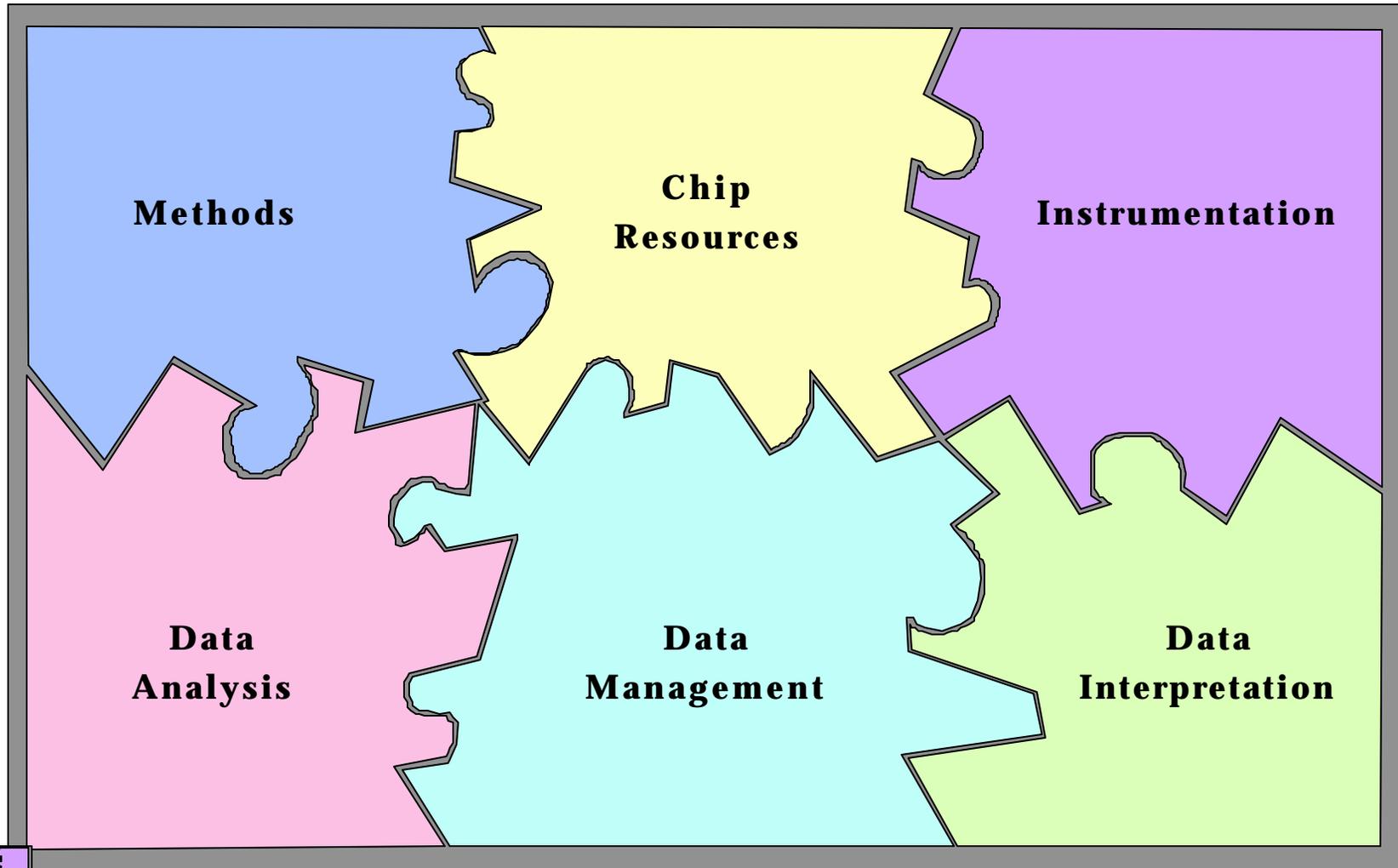
Oligonucleotide Arrays:

1. Photolithographic Synthesis
 - a. With Masks
 - b. Maskless
2. Ink-Jet Technology

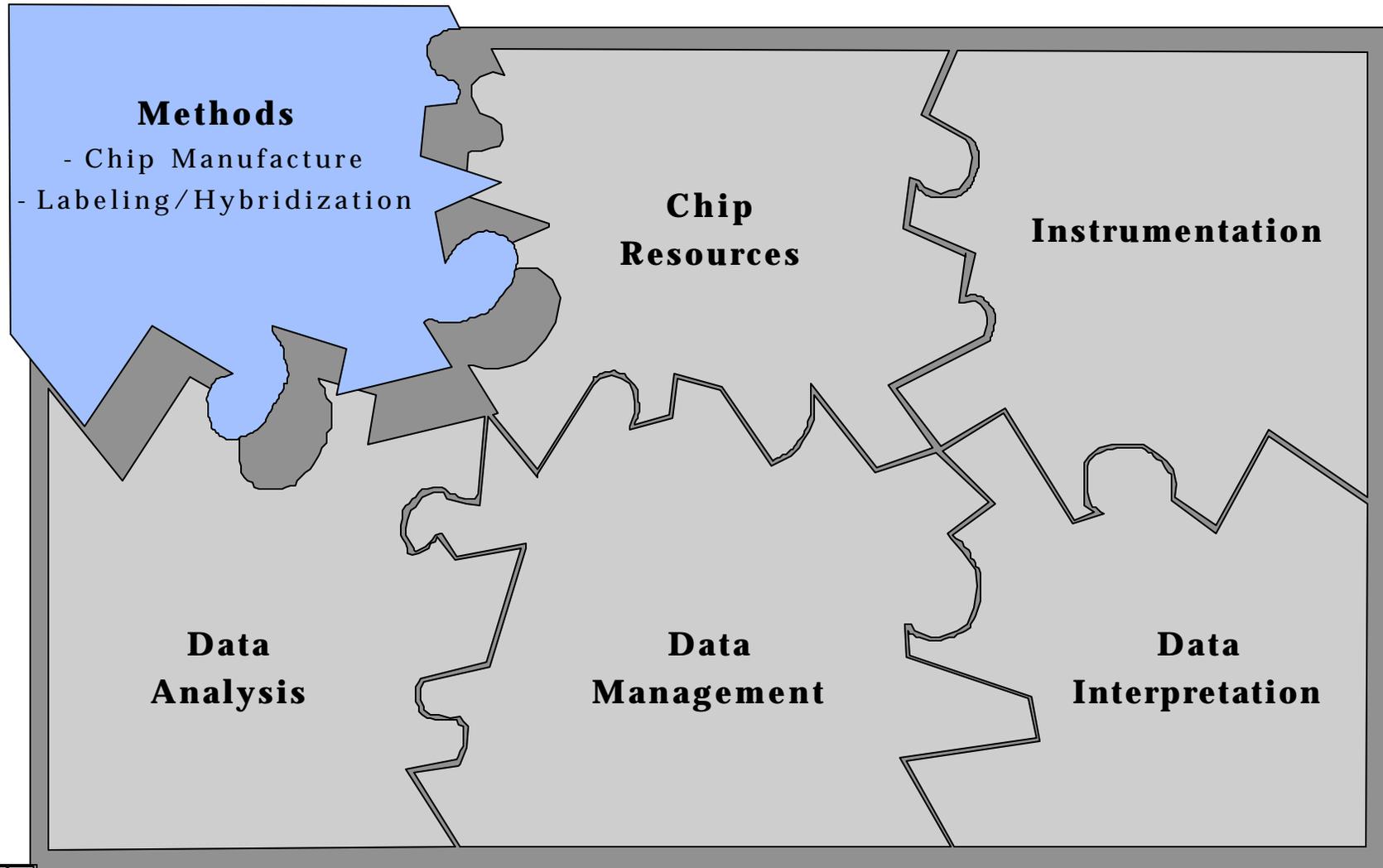
cDNA Arrays:

1. Contact Printing
2. Non-Contact Printing

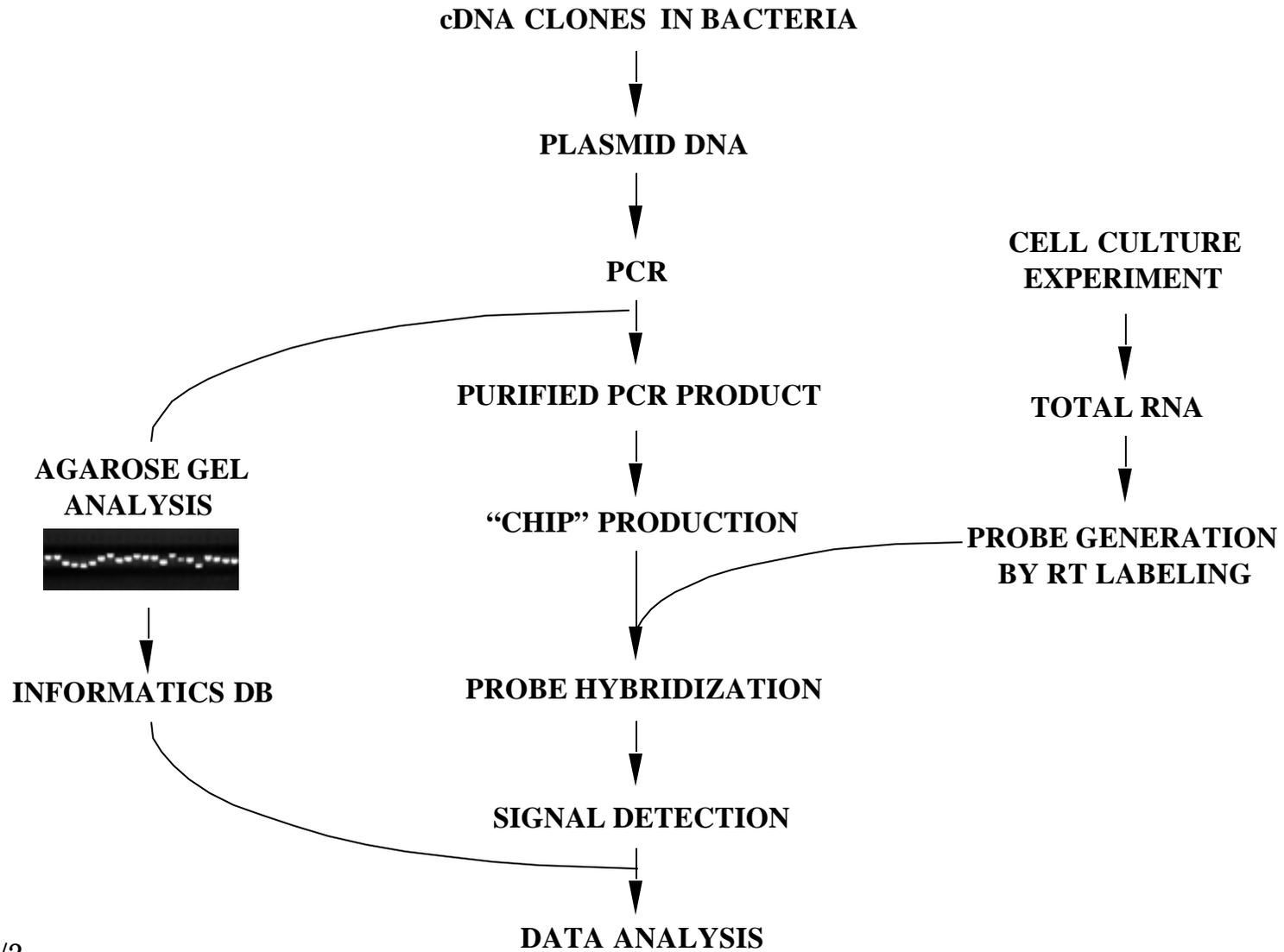
The cDNA Microarray Puzzle



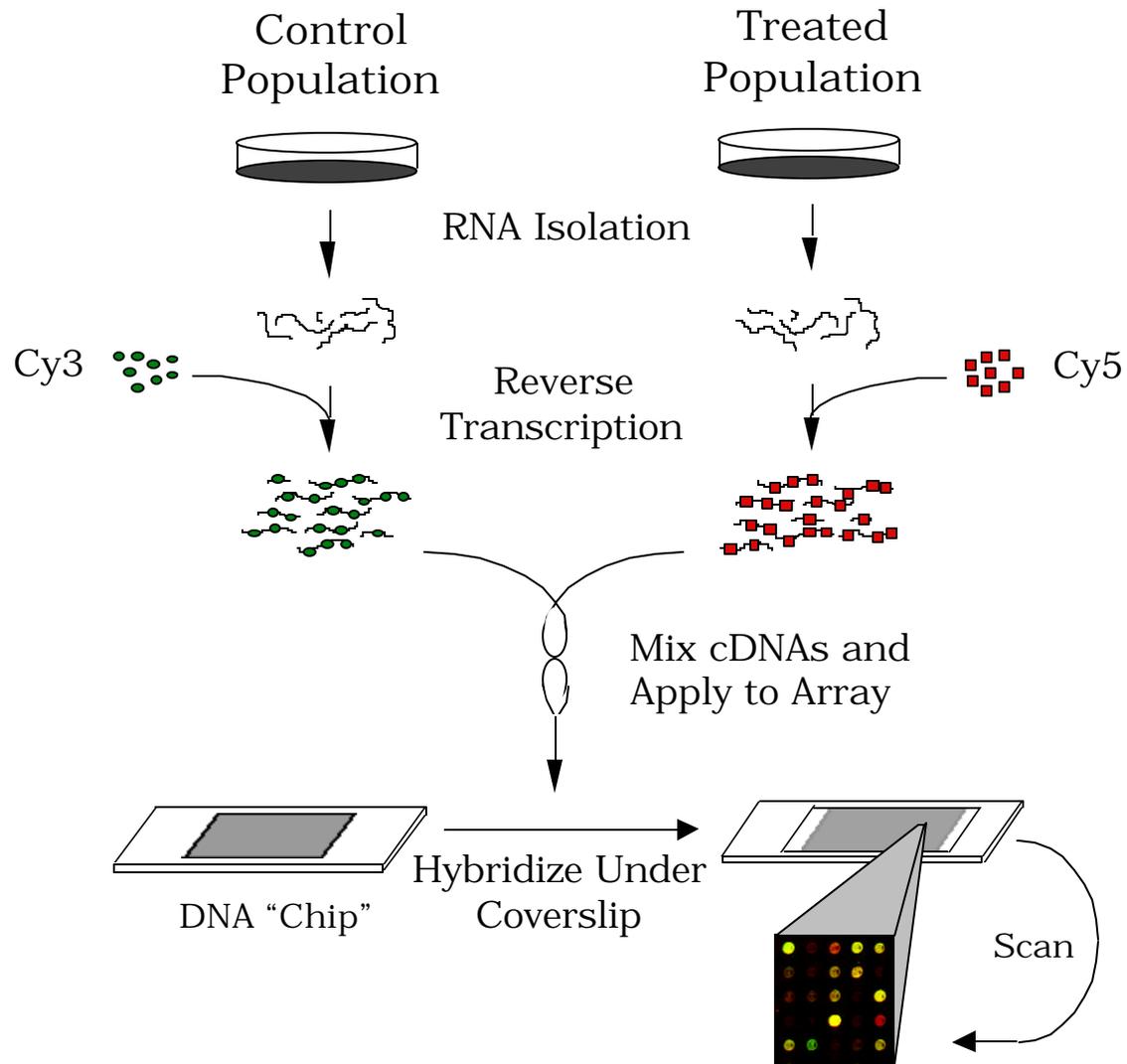
The cDNA Microarray Puzzle



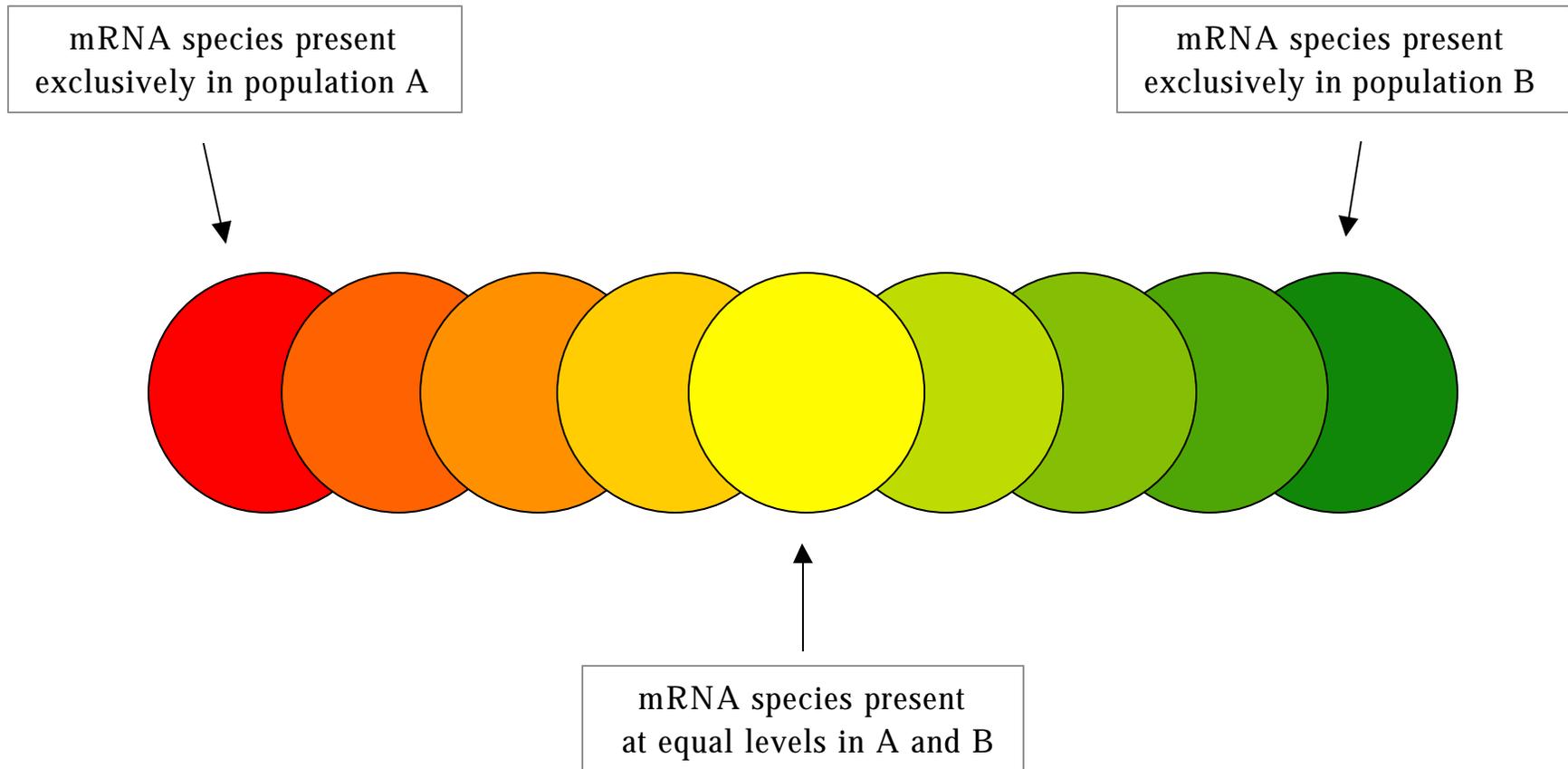
Procedure Overview for Manufacture and Use of cDNA Arrays



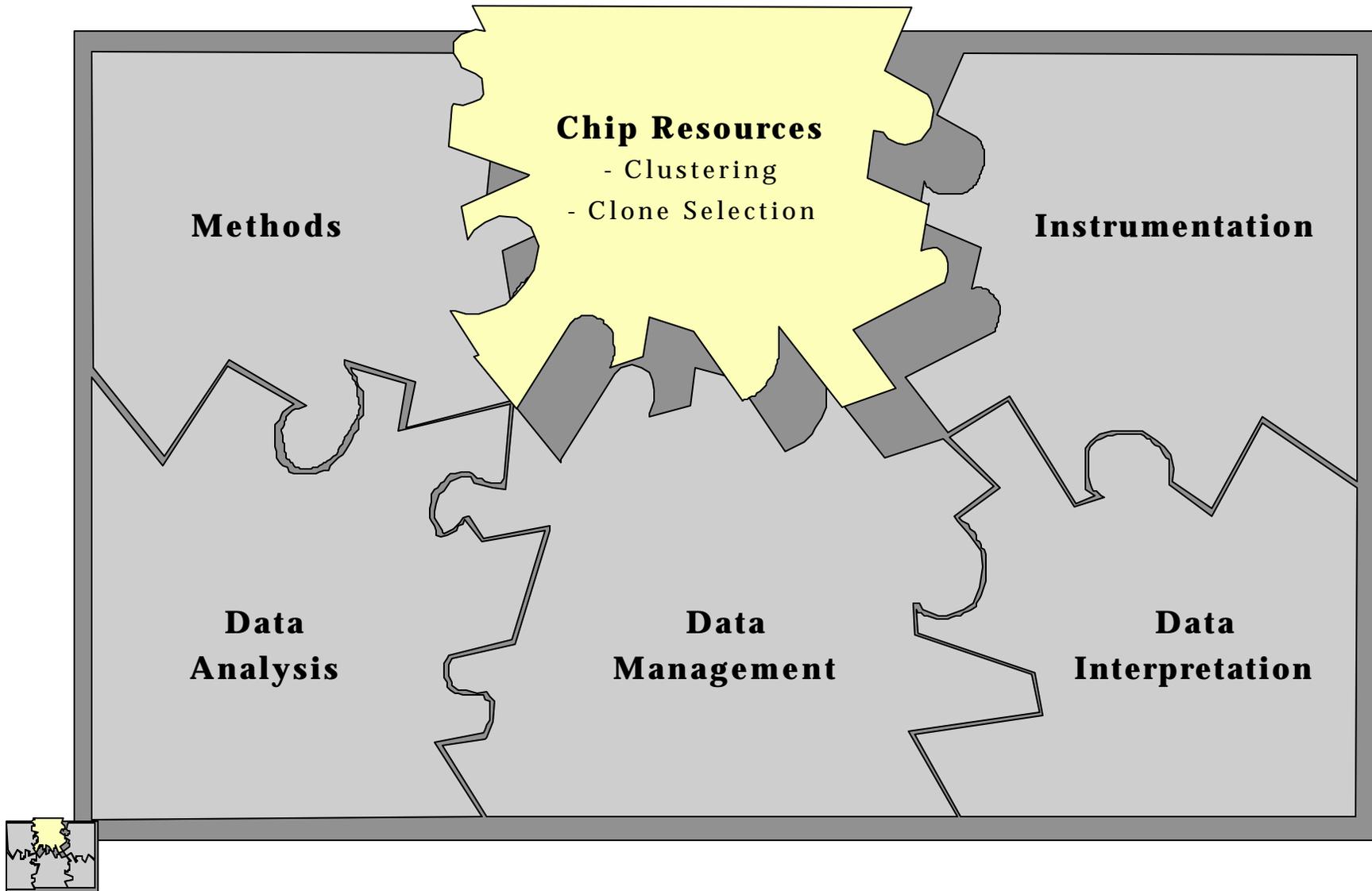
Overview of Gene Expression Analysis Using cDNA Microarrays



Data Collected from Two-Color Hybridizations



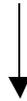
The cDNA Microarray Puzzle



The Process of Clustering Filters Redundancy

Individual GenBank Sequences

(All Sequences from a Given Species)



UniGene Database

(Nonredundant Gene "Clusters")

UniGene Statistics (11/15/99)

Human (build 100):

Total sequences - 1,350,216

Total clusters - 90,310

Known genes - 10,020

Mouse (build 69):

Total sequences - 602,525

Total clusters - 49,008

Known genes - 4,998

Rat (build 65):

Total sequences - 120,002

Total clusters - 28,207

Known genes - 3,220



Important Considerations for cDNA Clone Selection

1. Redundancy

(e.g. Serum albumin = 1972 sequences in human database),

2. Public Repositories Have Many Errors!

b. Incorrect identities (up to 30%!)
a. Contamination with multiple clones (5-10%)

c. Poor growth characteristics (variable)

d. Bacteriophage contamination! (< 10%)

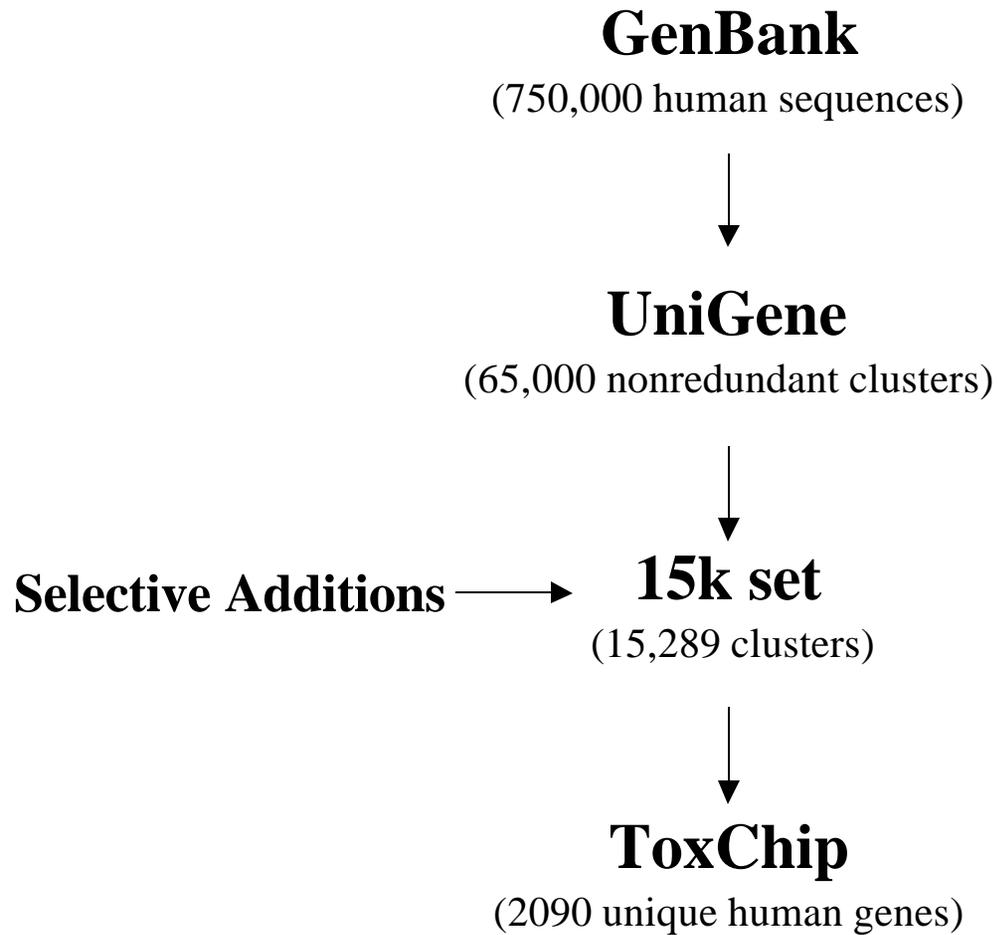
3. Location within ORF

(e.g. 3' anchoring required?)

4. Sequence Similarities Within Gene Families



Development of ToxChip: A Custom Human cDNA Microarray Chip



ToxChip v1.0: A Human cDNA Microarray **Designed for Toxicology Studies**

<u>Gene Category</u>	<u># Genes</u>
Apoptosis	72
Oxidative Stress/Redox Homeostasis	90
Peroxisome Proliferator Responsive	22
[Ah] Receptor Battery	12
Estrogen Responsive	63
Housekeeping	84
Oncogenes and Tumor Suppressor Genes	76
Cell Cycle Control	51
Transcription Factors	131
Kinases	276
Phosphatases	88
Heat Shock Proteins	23
Cytochrome P450	30
Receptors	349



Custom cDNA Arrays are Possible: The NIEHS Chip Inventory

Human

- UG/LO/TF (1320 genes)
- ToxChip v1.0, v2.0 (2090, 6501 genes)
- Discovery Chip (11057 genes)
- Senescence Chip

Yeast Whole-Genome (6218 genes)

Mouse (8734 genes, in preparation)

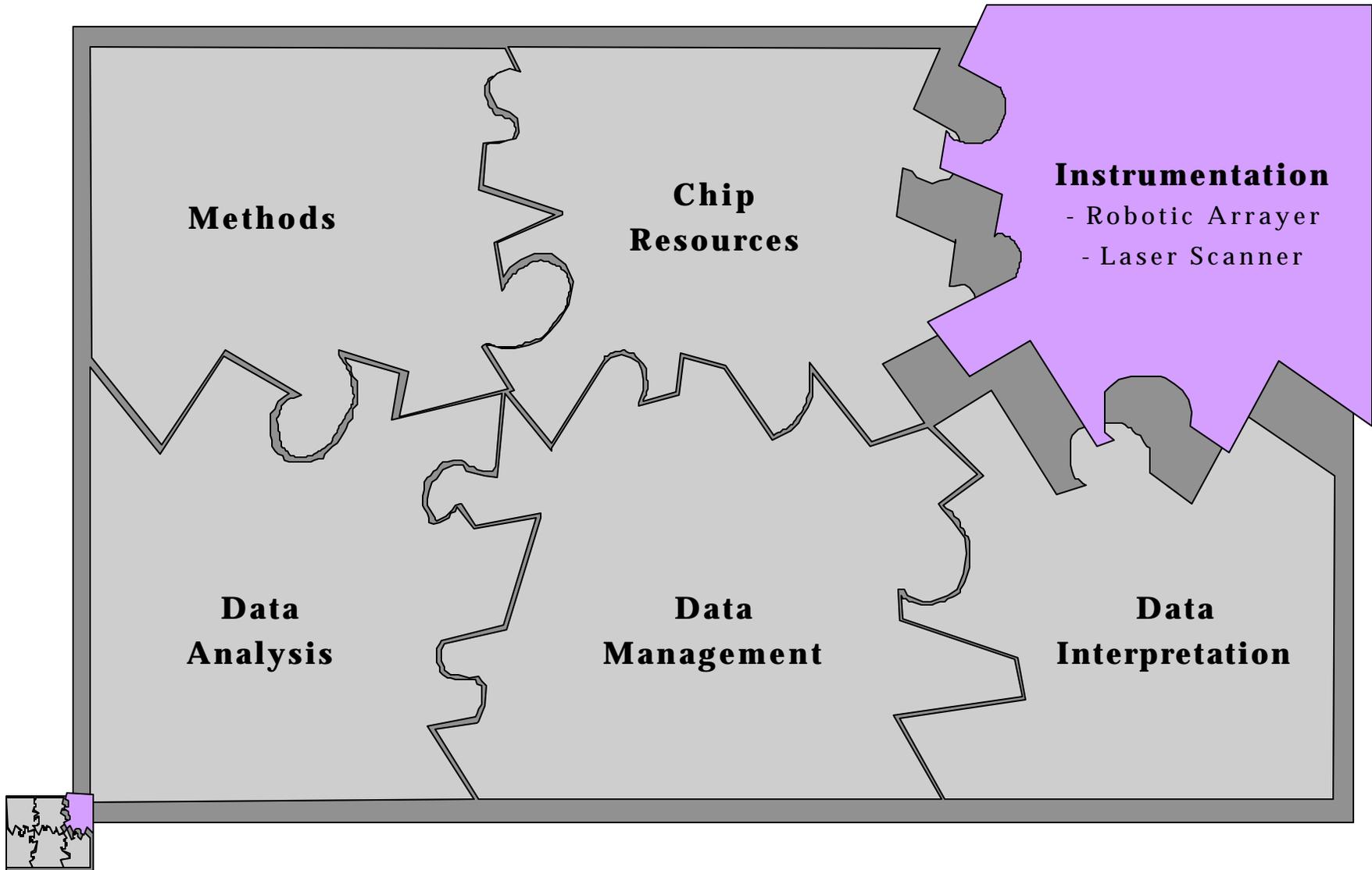
Rat

- Known Genes Set (1728 genes)
- Rat 17K (17090 genes, in preparation)

Xenopus (960 clones)

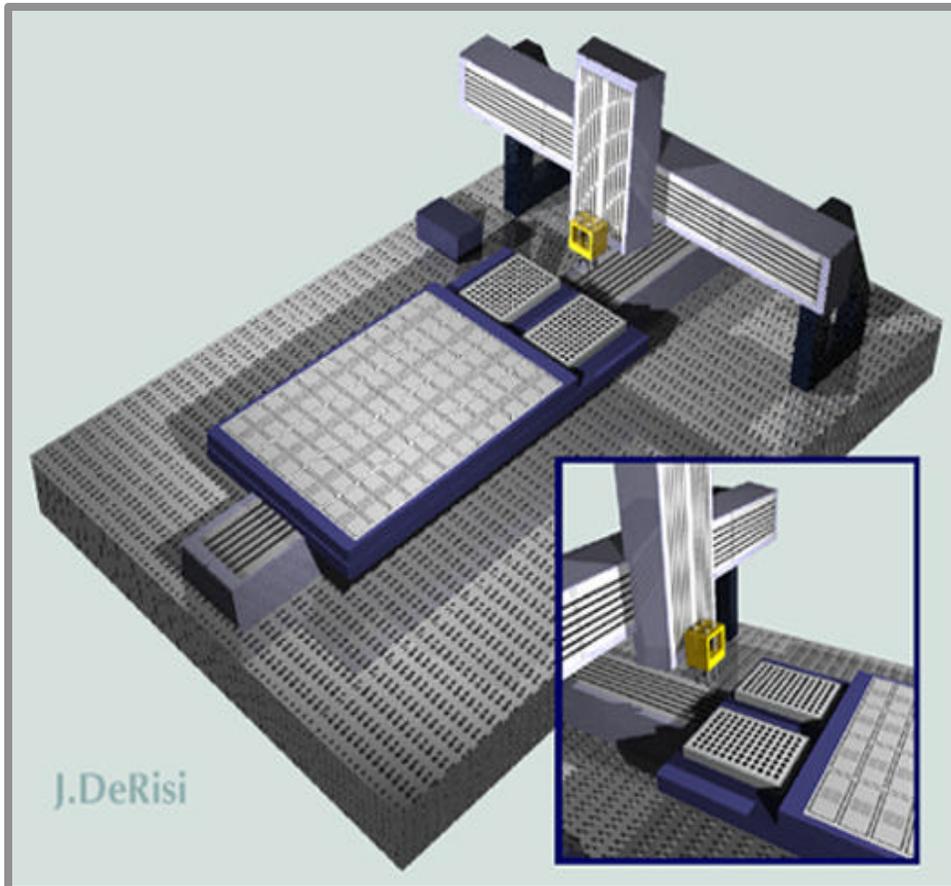


The cDNA Microarray Puzzle

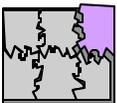
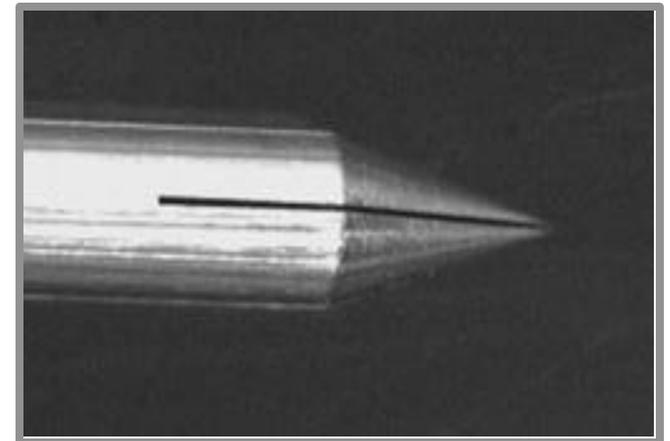


The Arrayer: Robotic Spotting Apparatus

X-Y-Z Robot



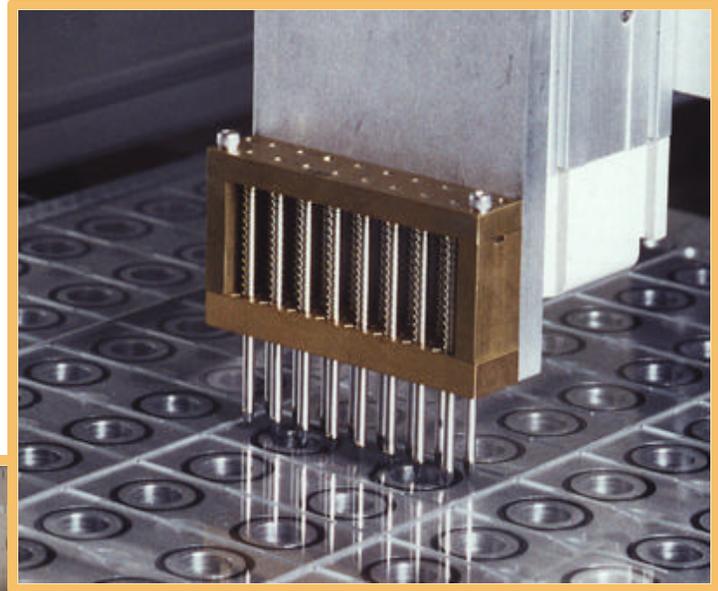
Quill-pen Spotting Tip ("fountain-pen" design)



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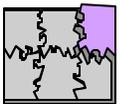
Image source: <http://cmgm.Stanford.edu/pbrown/>

The NIEHS Arrayer



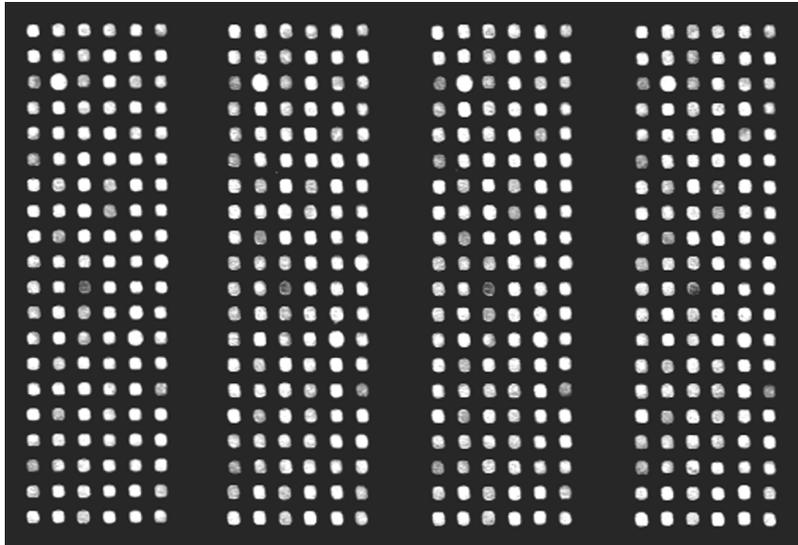
Desirable Features of a Robotic Spotter

- Flexible, user-friendly software
- High speed robotics (>1 slide per second)
- External cabinet enclosure
- Humidity and dust control devices
- Pretapping capabilities
- Slide capacity > 100
- 96- and 384- well format capabilities
- Plate stacker
- Rigorous wash/dry cycles
- Reproducible pin quality
- Spot size smaller than 150 um (for high density arrays)
- High precision machining (low tolerances)
- Emergency stop button with restart capabilities



Replicate Spotting Produces Replicate Data

Stain for Resident DNA



Two-color Hybridization

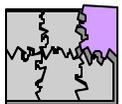
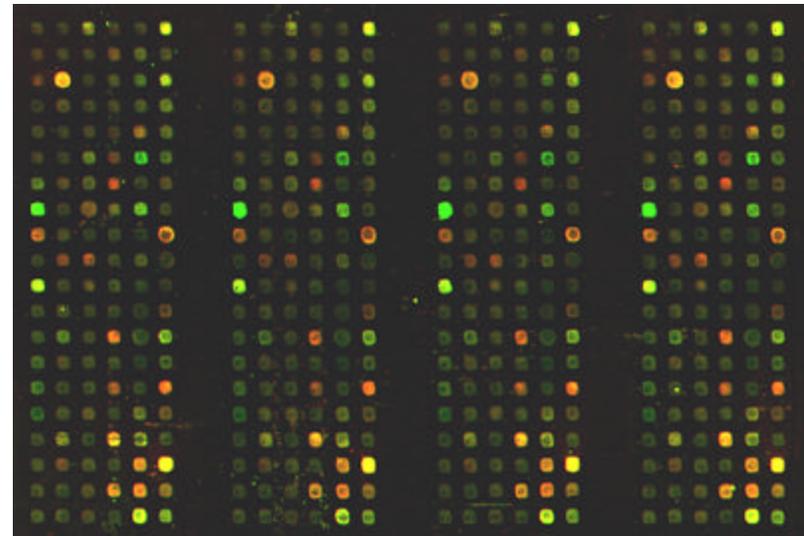
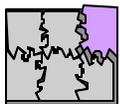
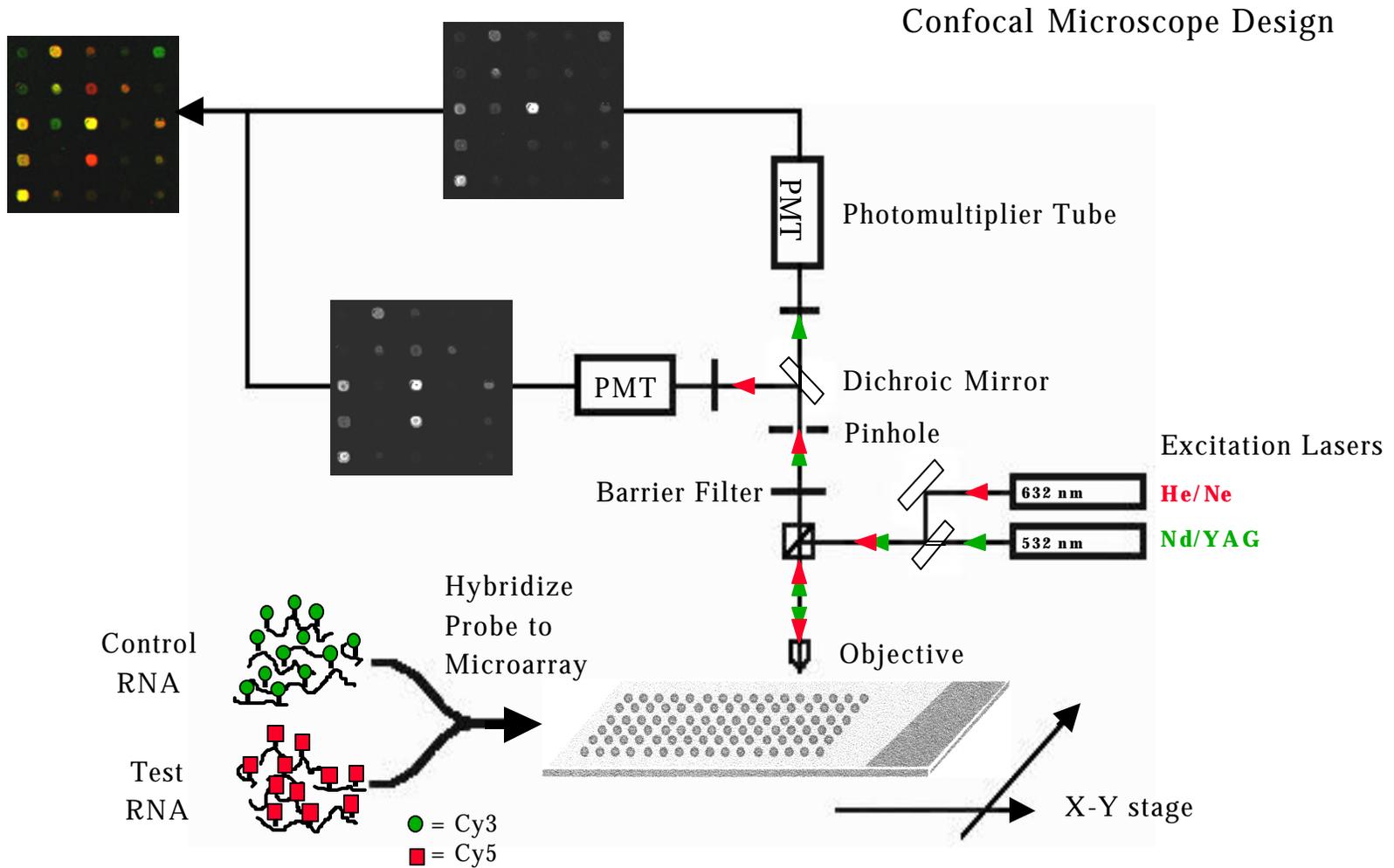
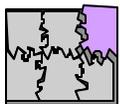
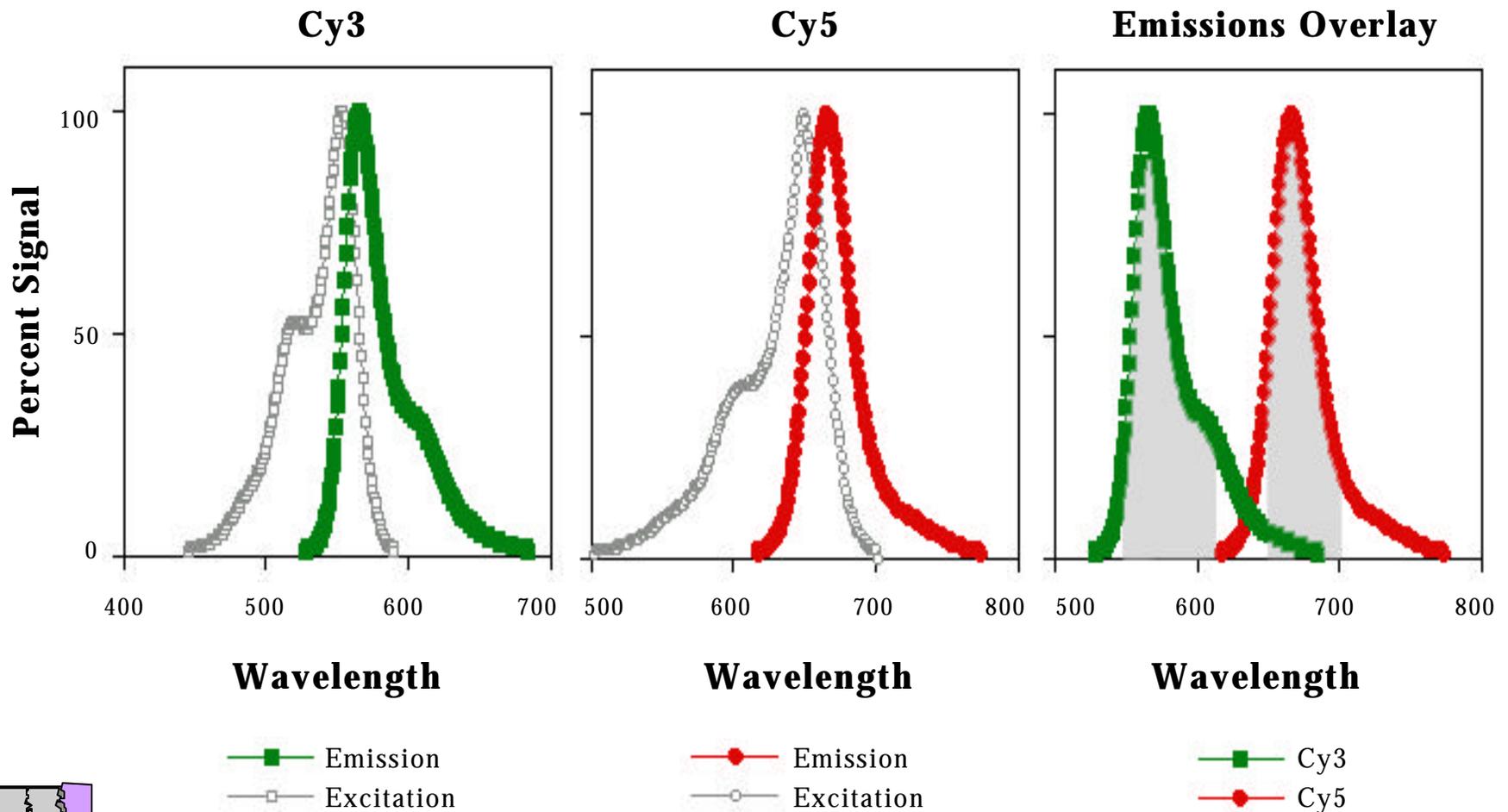


Diagram of a Confocal cDNA Microarray Scanner

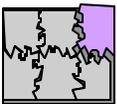


Excitation and Emission Spectra for Cy3 and Cy5



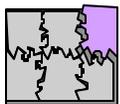
NIEHS Confocal Microarray Scanner

- Confocal Design
- Two Photomultiplier Tubes
- Excitation and Emission Filter-sets
- 3 Lasers on Vertical Breadboard
 - HeNe (633 nm, Cy5)
 - NdYag (532 nm, Cy3)
 - Ar (488 nm, FITC)
- Motorized X-Y Stage
- Pentium III 500 mHz Processor
- Windows NT Operating Environment
- Custom Image-Acquisition Software

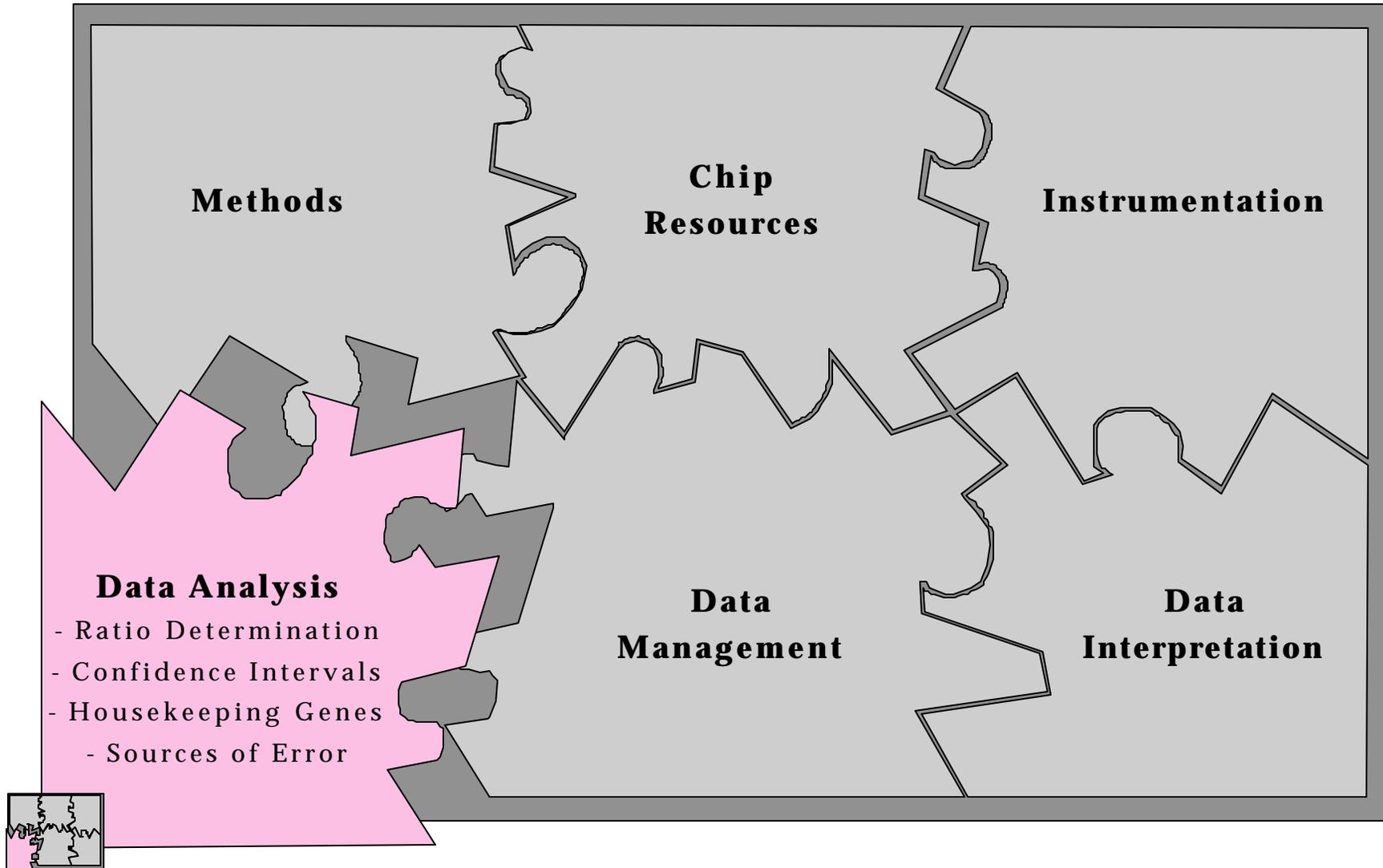


Desirable Features of a cDNA Chip Scanner

- High sensitivity
- Rapid scan time (<10 min per channel)
- Low crosstalk
- No spatial bias in signal collection
- Compatibility with existing software/hardware
- Low instrument noise
- Low background with no clipping
- User friendly software
- Autofocus
- At least two lasers
- Low photobleaching

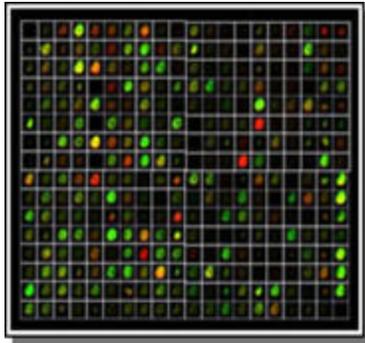


The cDNA Microarray Puzzle

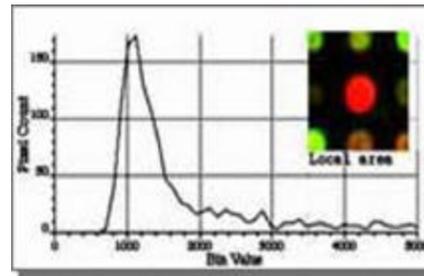


Stepwise Process of Data Analysis

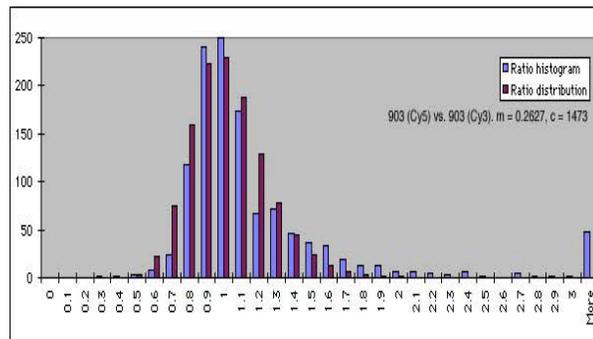
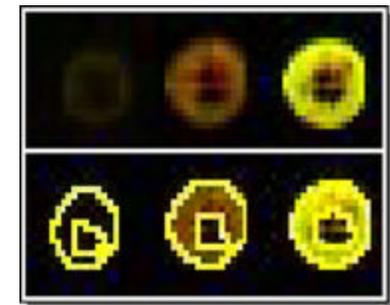
1. Target Segmentation



2. Background Subtraction

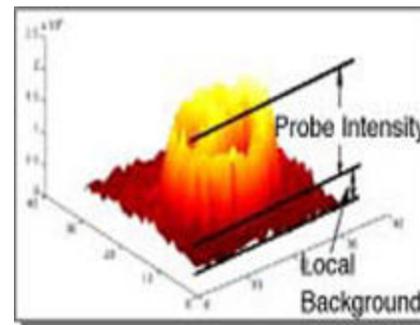


3. Target Detection



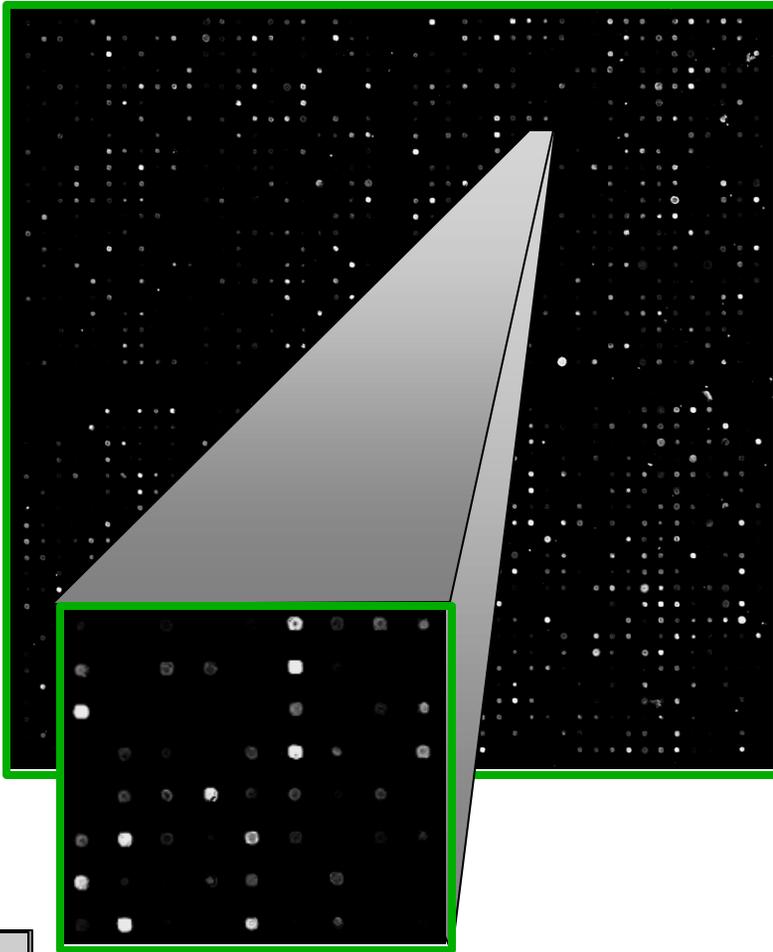
5. Ratio Analysis

4. Target Intensity Determination

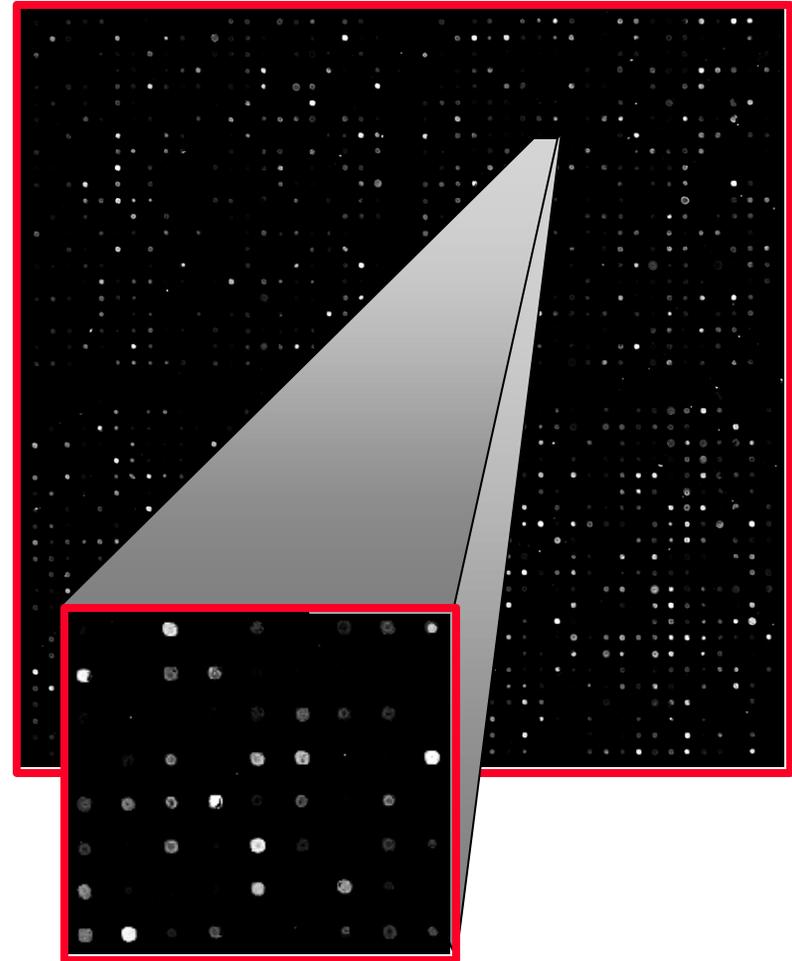


Raw Data in the Form of Two Grey-Scale Images

Sample A - Cy3



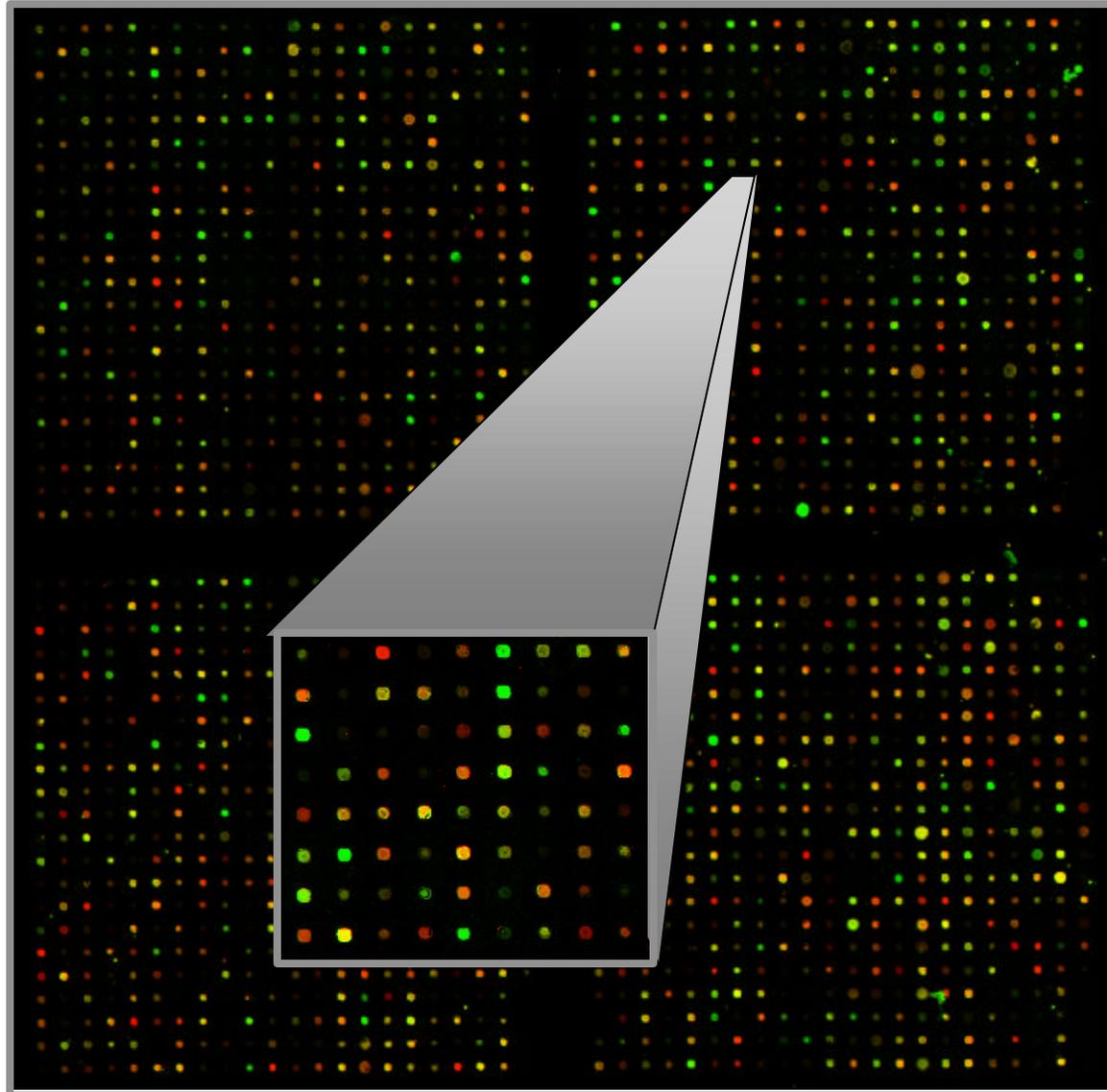
Sample B - Cy5



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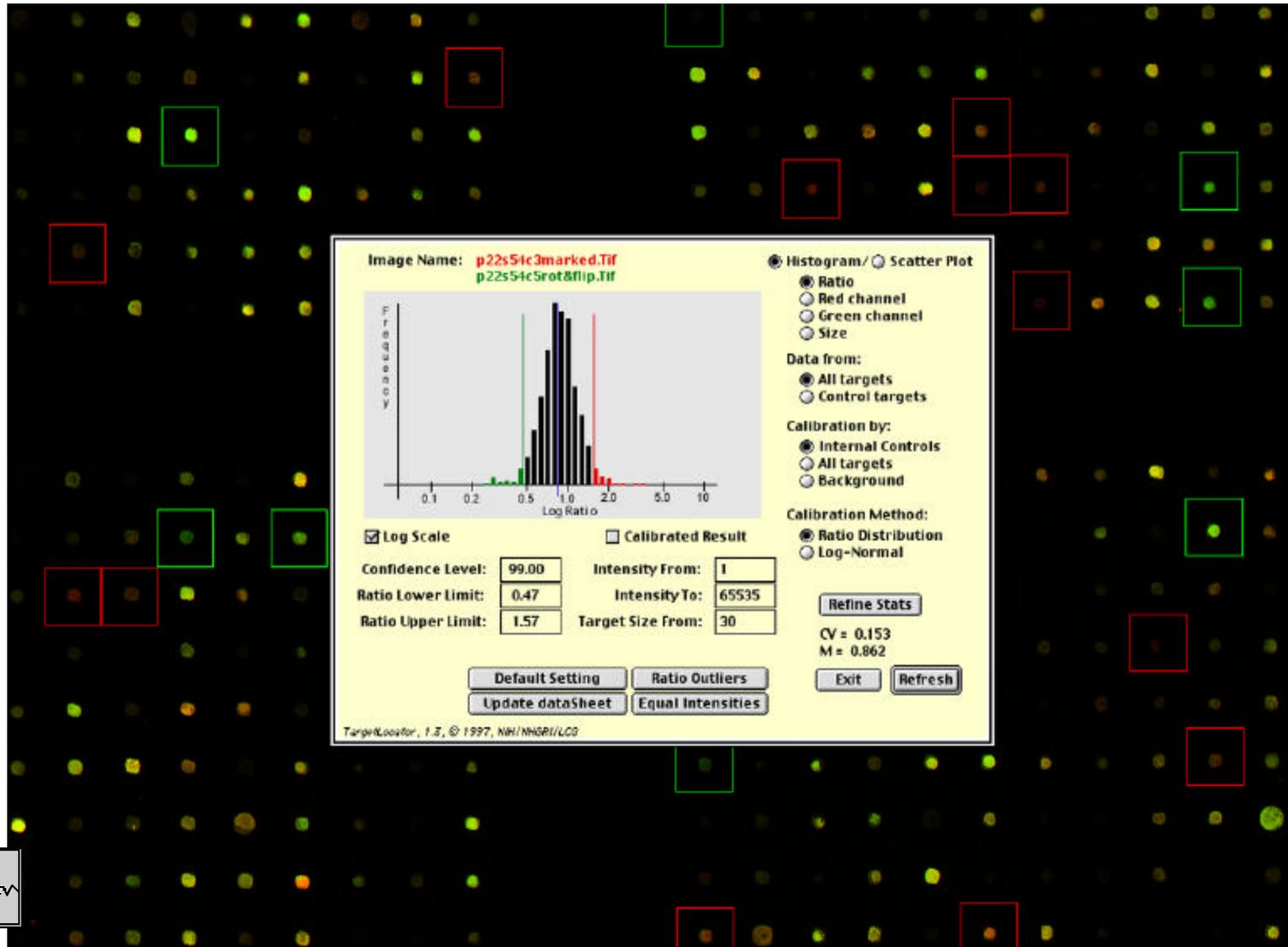


Combination of Two Grey-Scale Images to Create Pseudo-Color Ratio Image



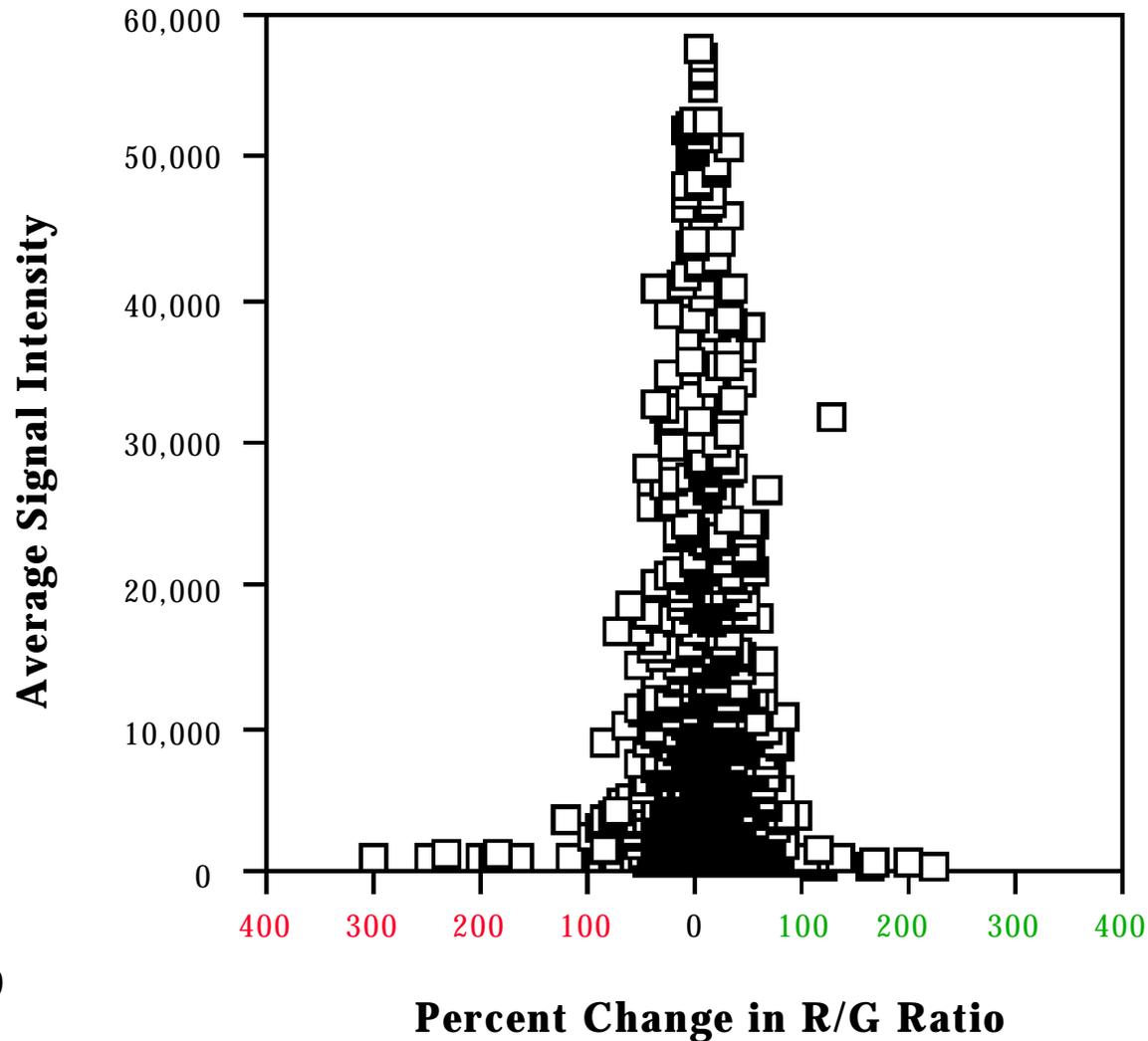
Data Visualization:

Using Population Distributions to Select Outliers



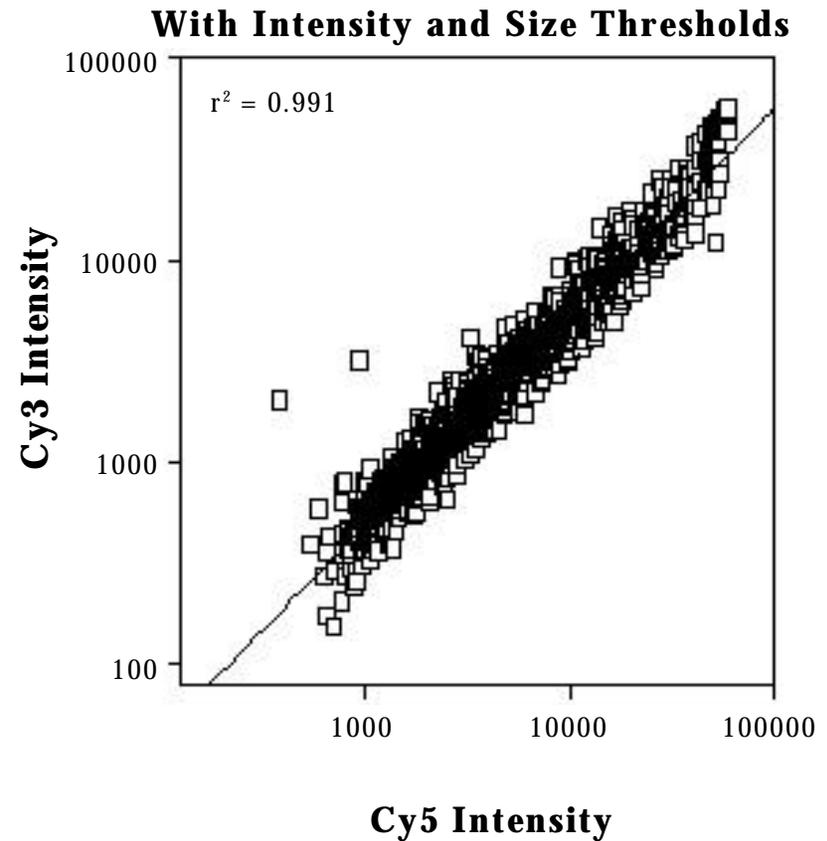
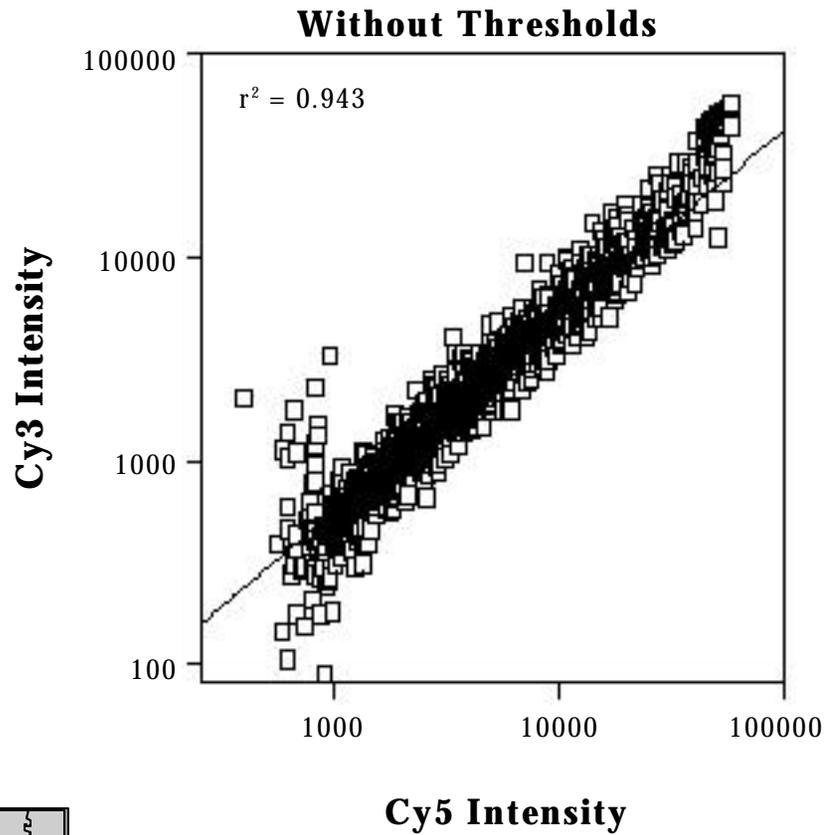
Data from Low Signal Intensities is Less Reliable

Control v.s. Control Hybridization



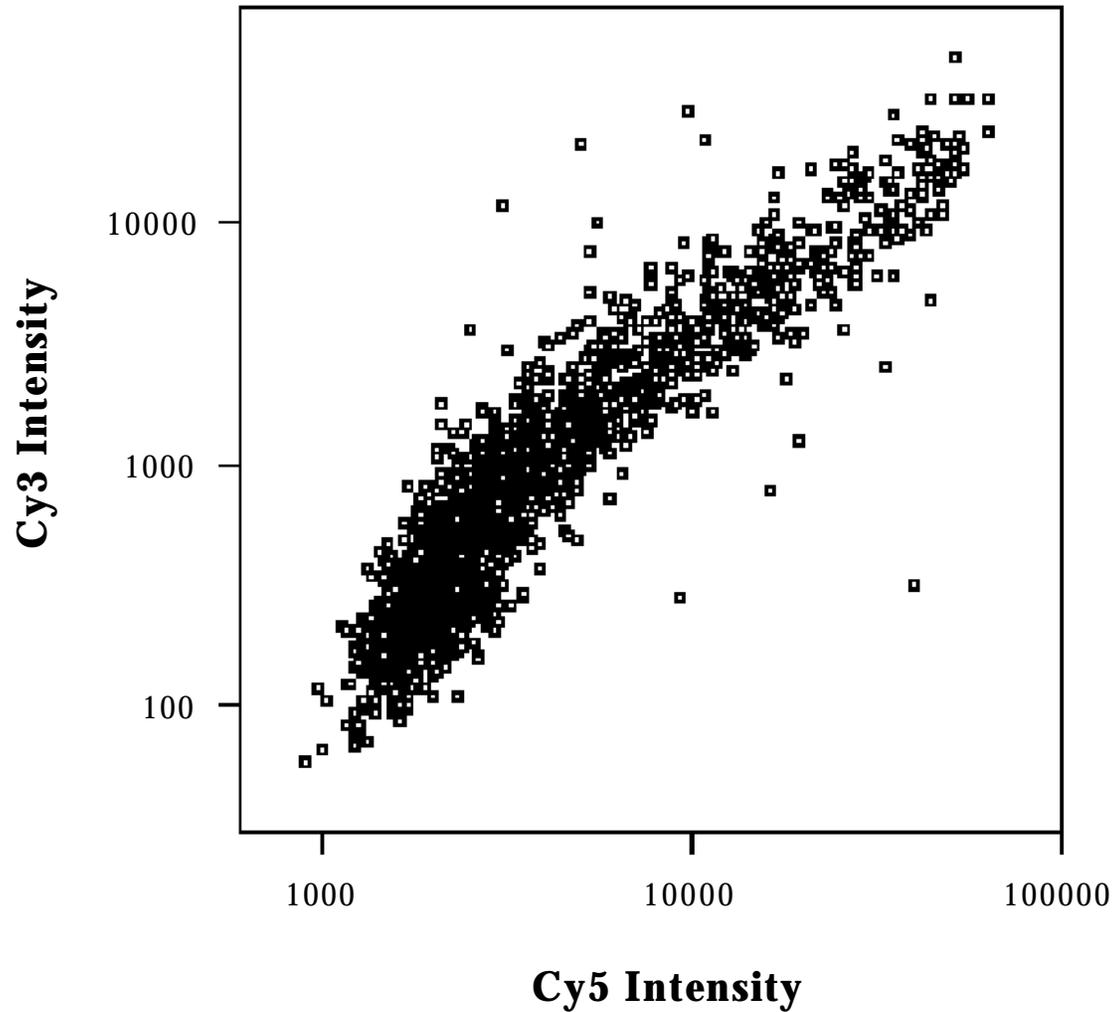
Error Associated with Low Signal Intensities Can be Filtered

Control v.s. Control Hybridization



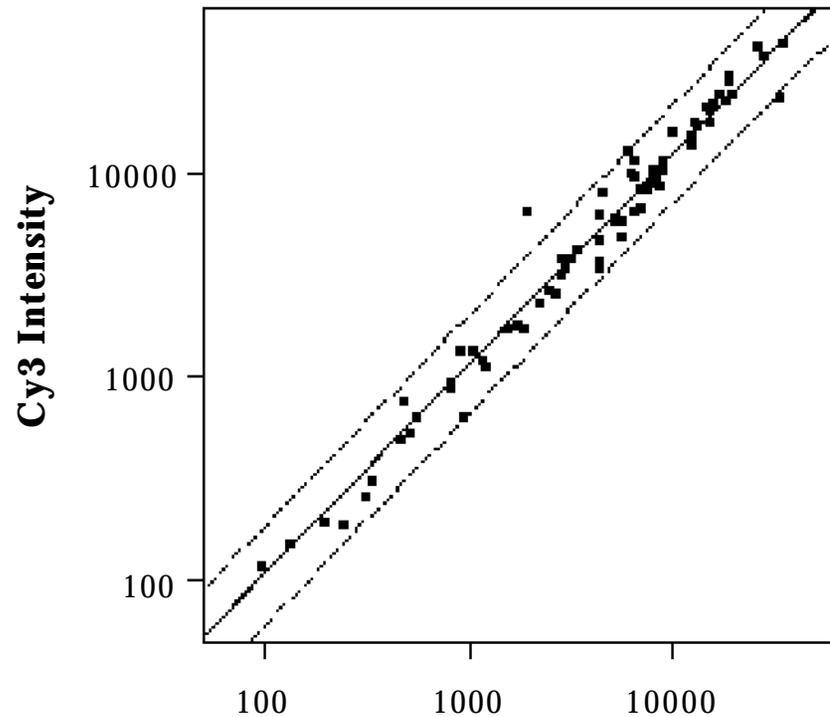
Inaccurate Background Estimation

Leads to Bias Datasets

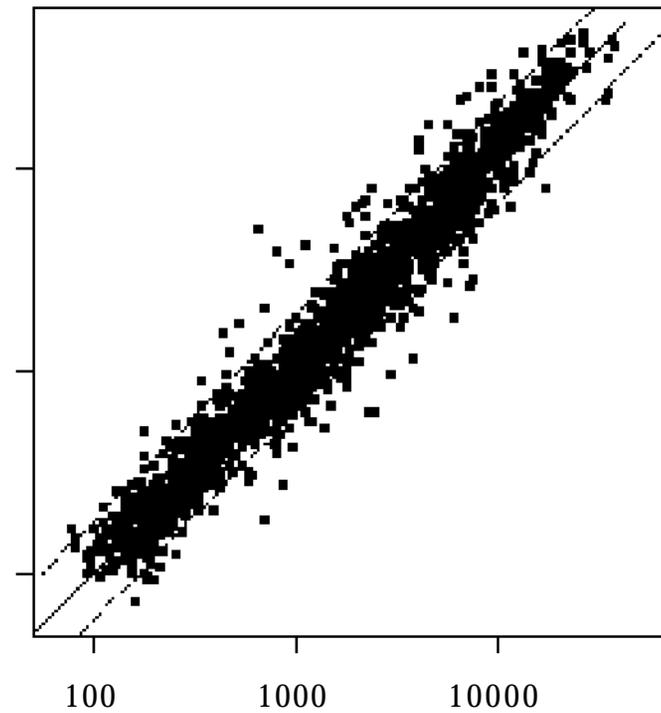


There is no *Single* Housekeeping Gene, But there is a Housekeeping *Population*

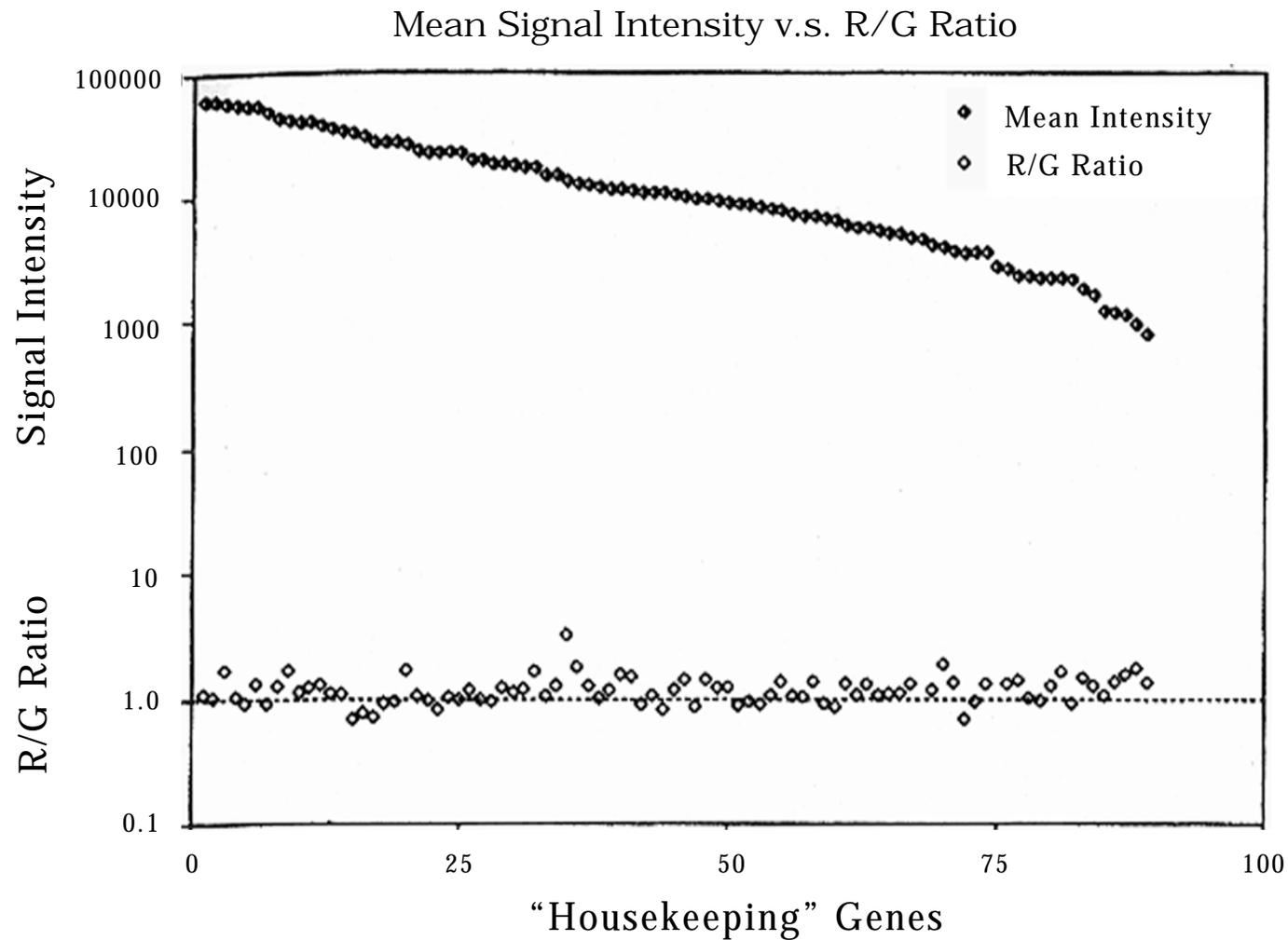
Housekeeping Set (n=84)



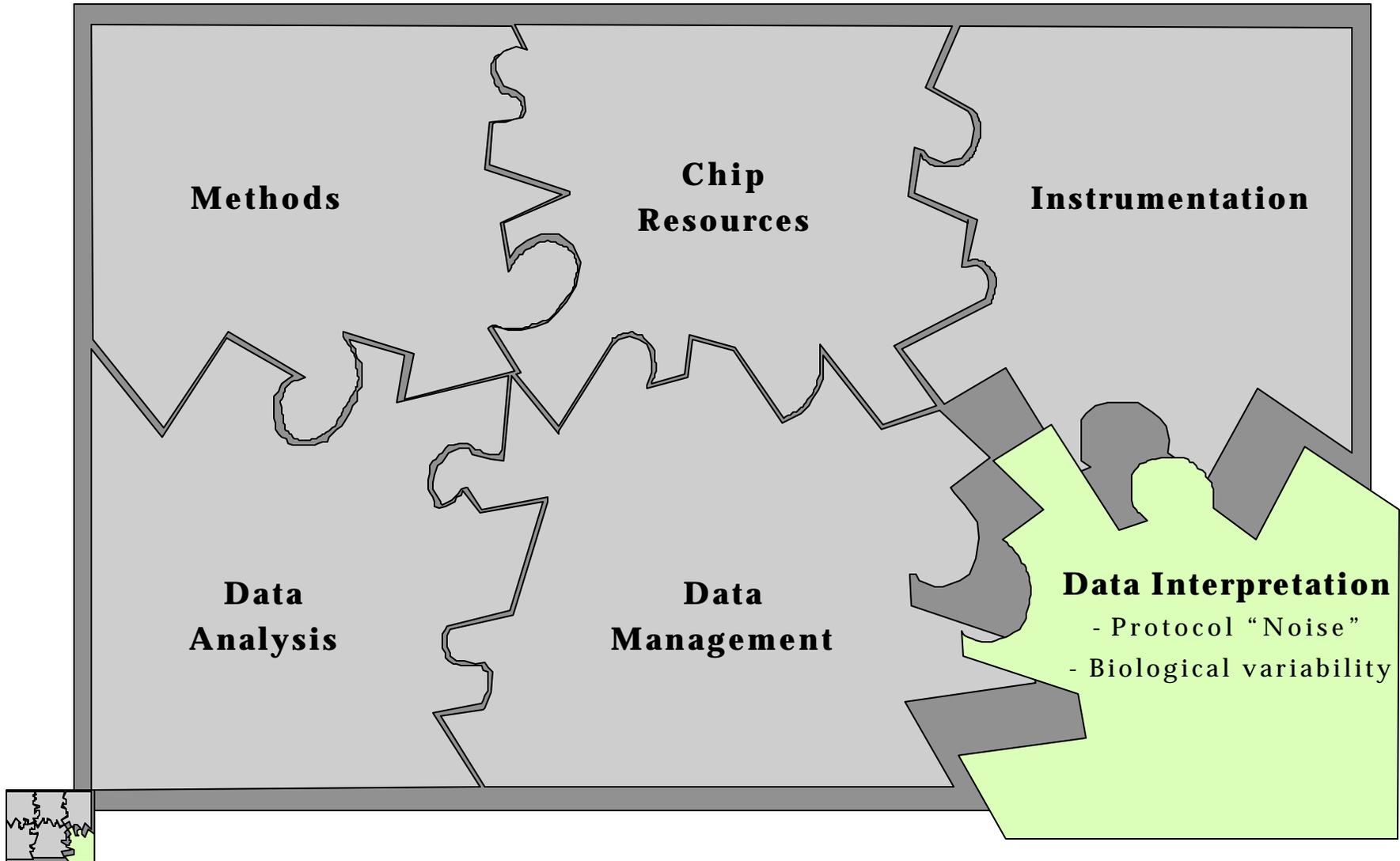
All Genes (n=2090)



Housekeeping Genes are Stable Across the Dynamic Range of Signal Intensities

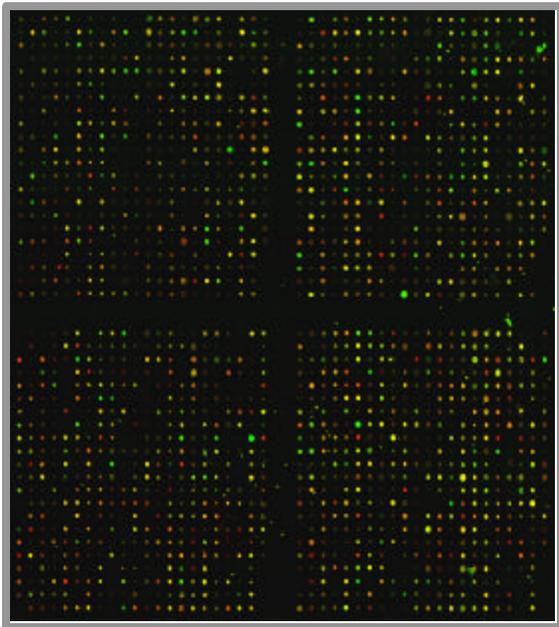


The cDNA Microarray Puzzle

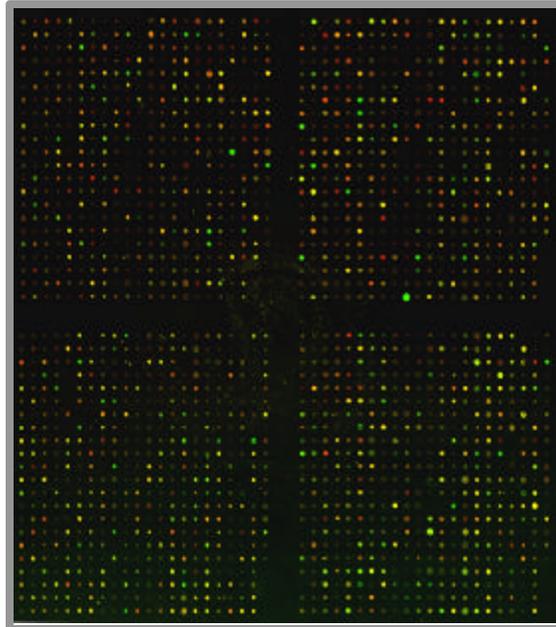


Replicate Hybridizations can Eliminate Error

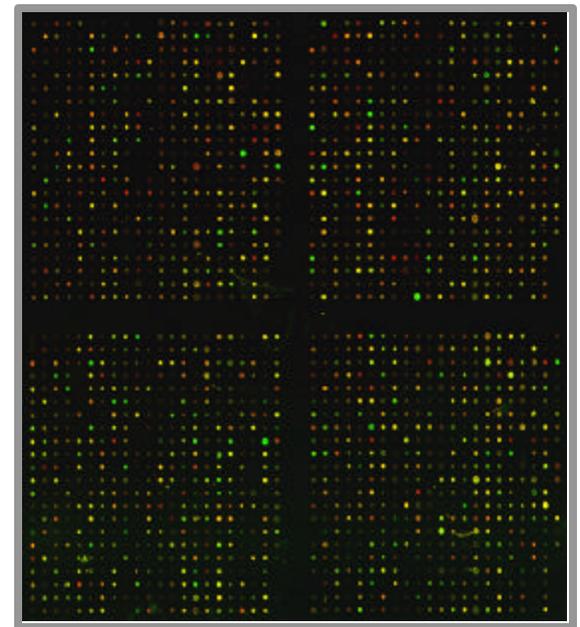
HYBE #1



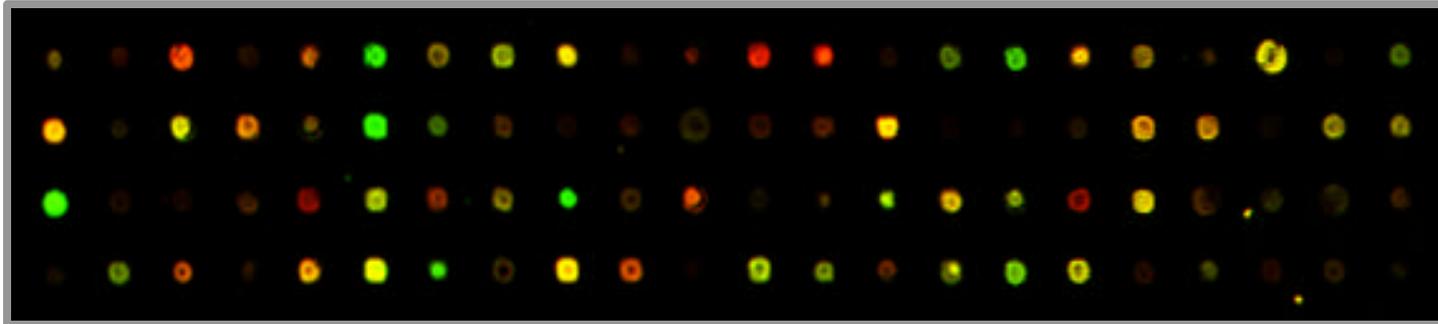
HYBE #2



HYBE #3

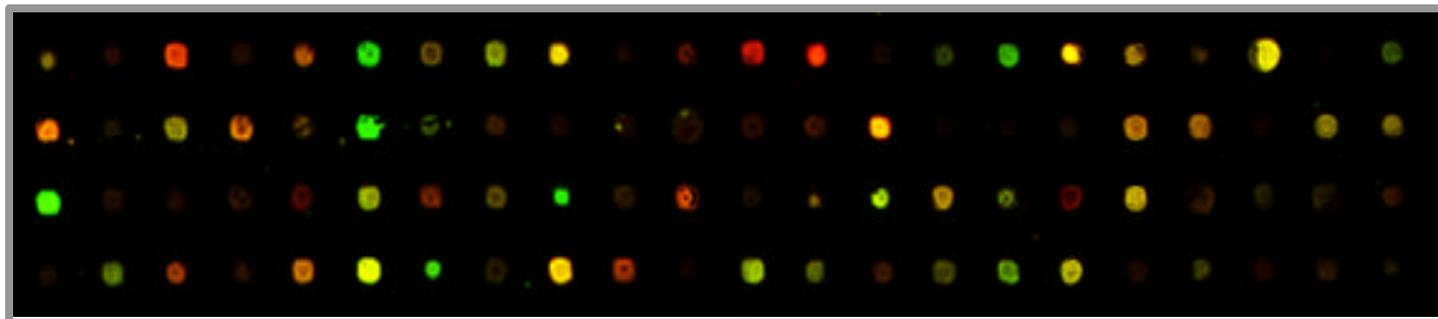


Replicate Hybridizations can Eliminate Error

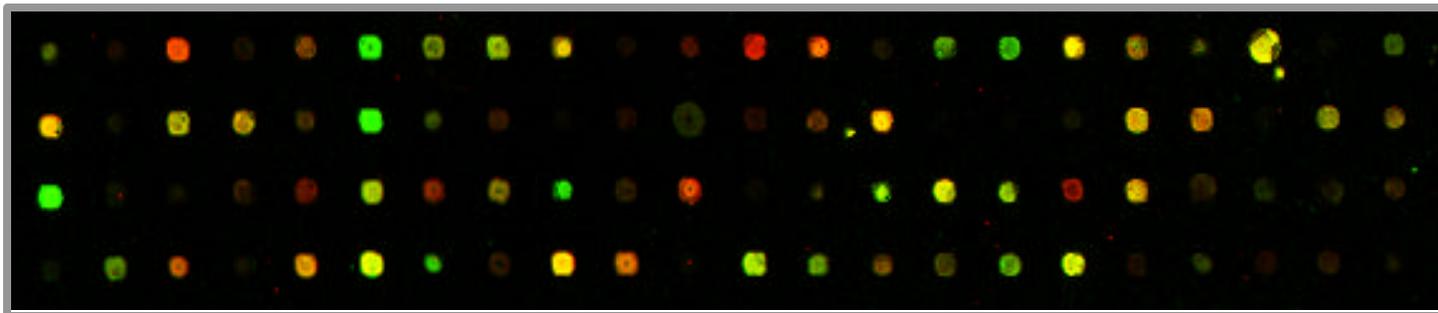


Hybe

1



2

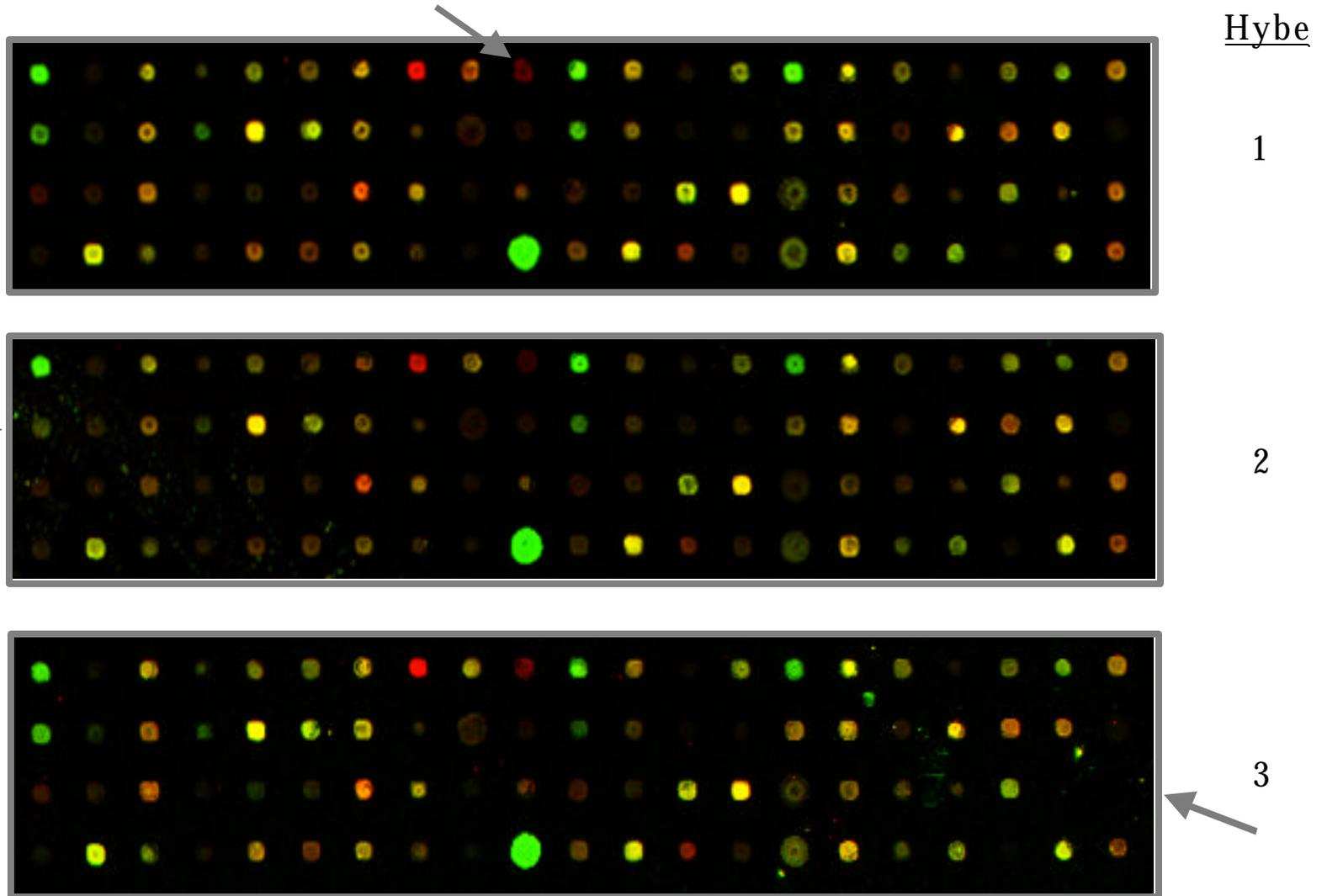


3

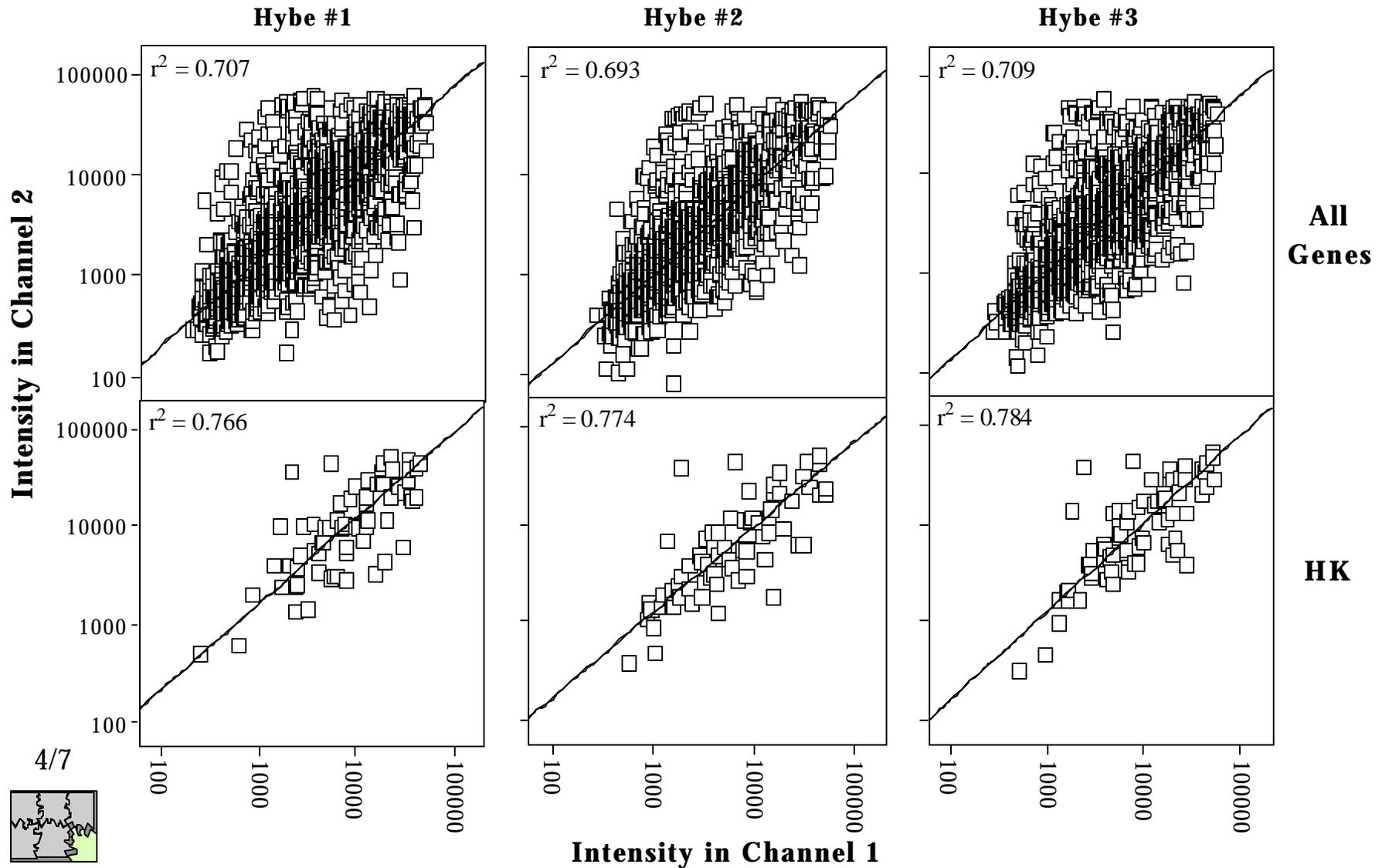
2/7



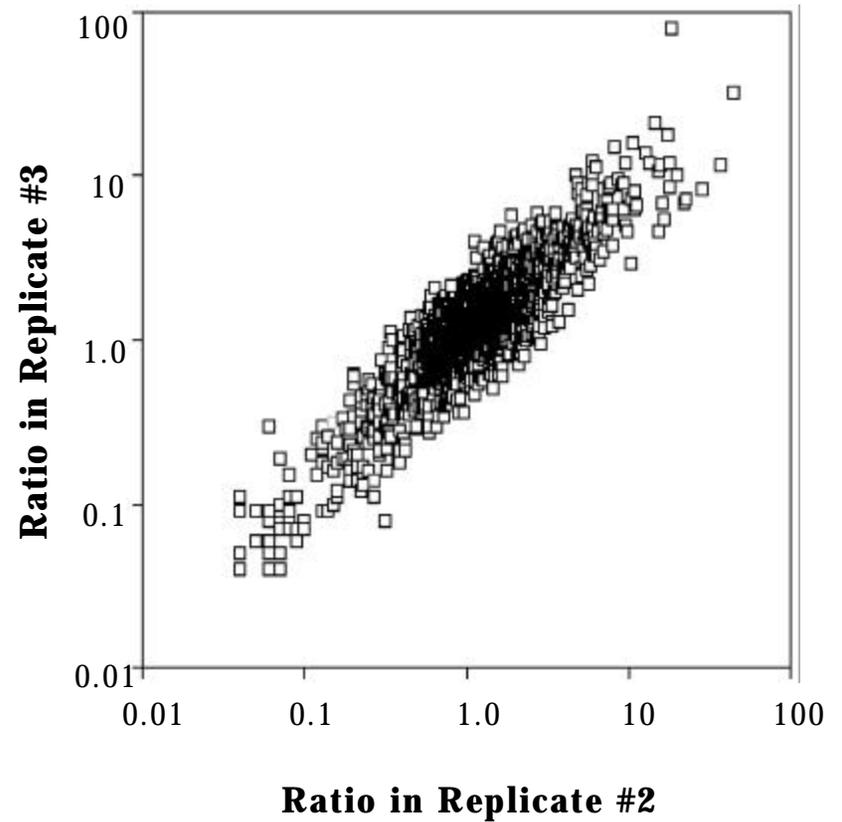
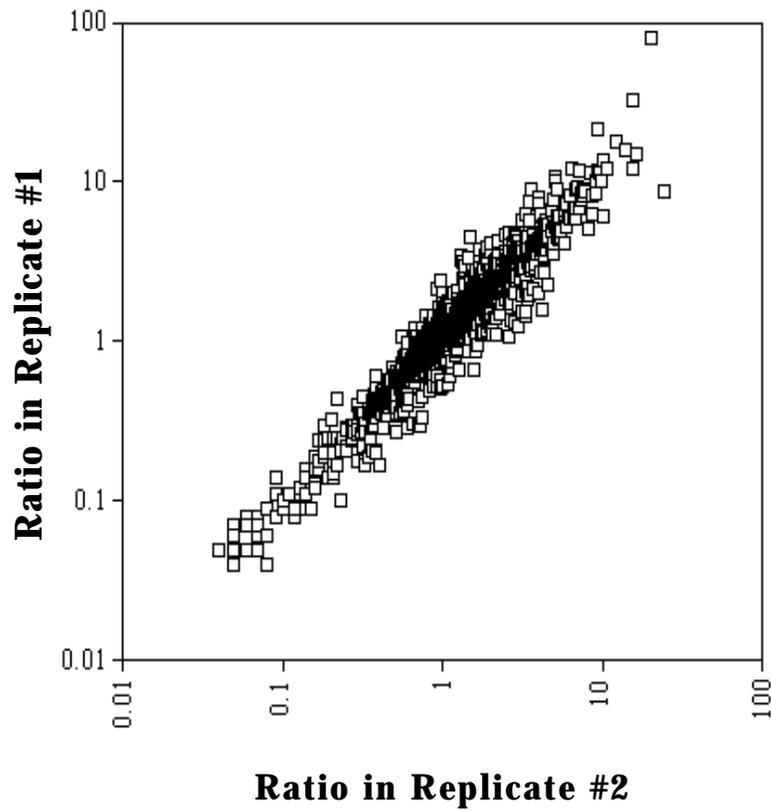
Replicate Hybridizations can Eliminate Error



Replicate Hybridizations: Scatter-plot distributions

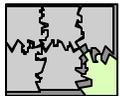
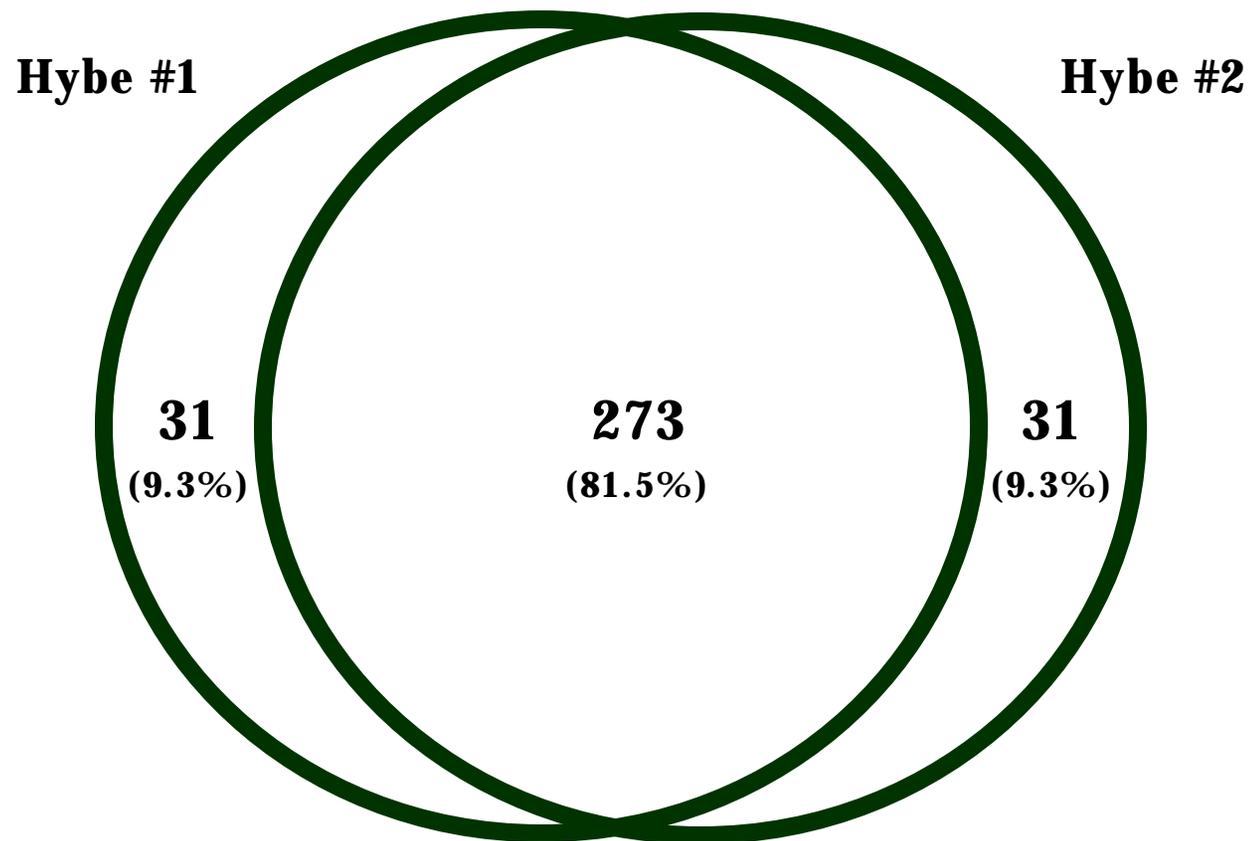


Replicate Hybridizations can Eliminate Error



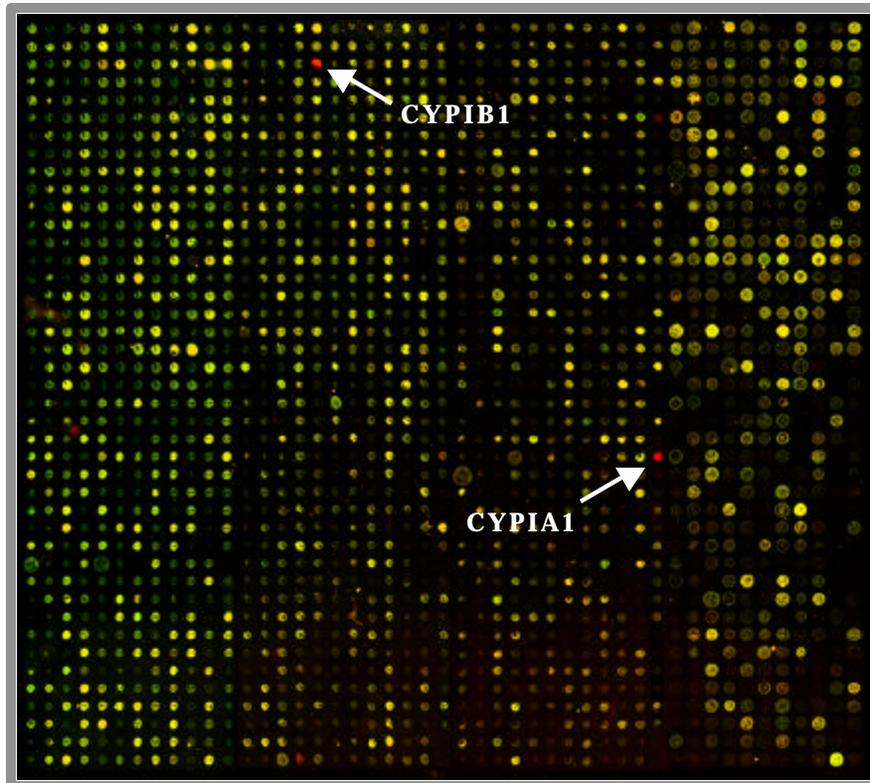
Replication of the Hybridization will Eliminate Protocol-Associated Noise

Total Outliers: 335

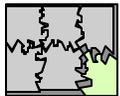
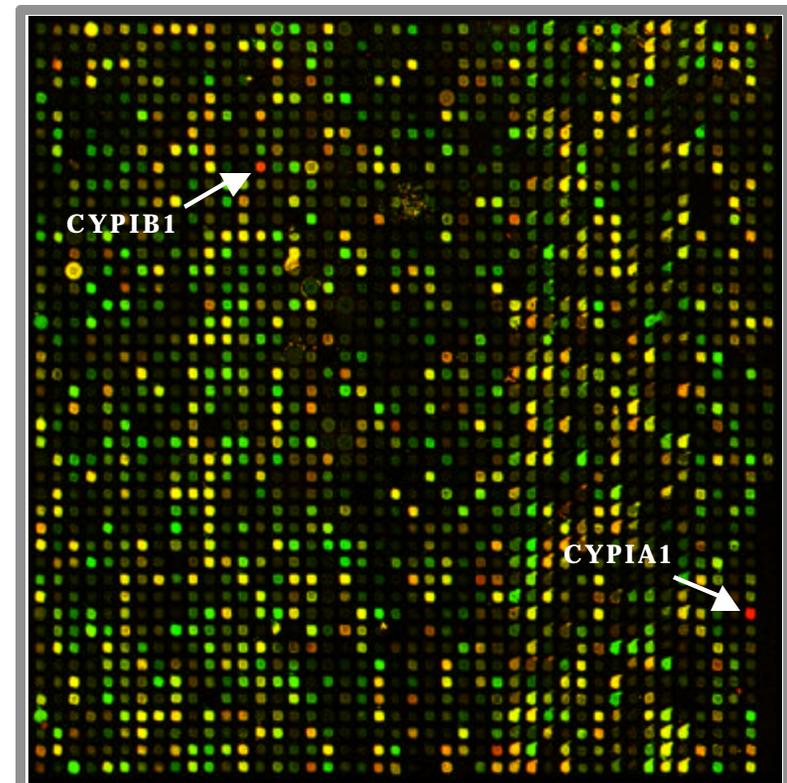


Biological Replicates are Not Always the Same

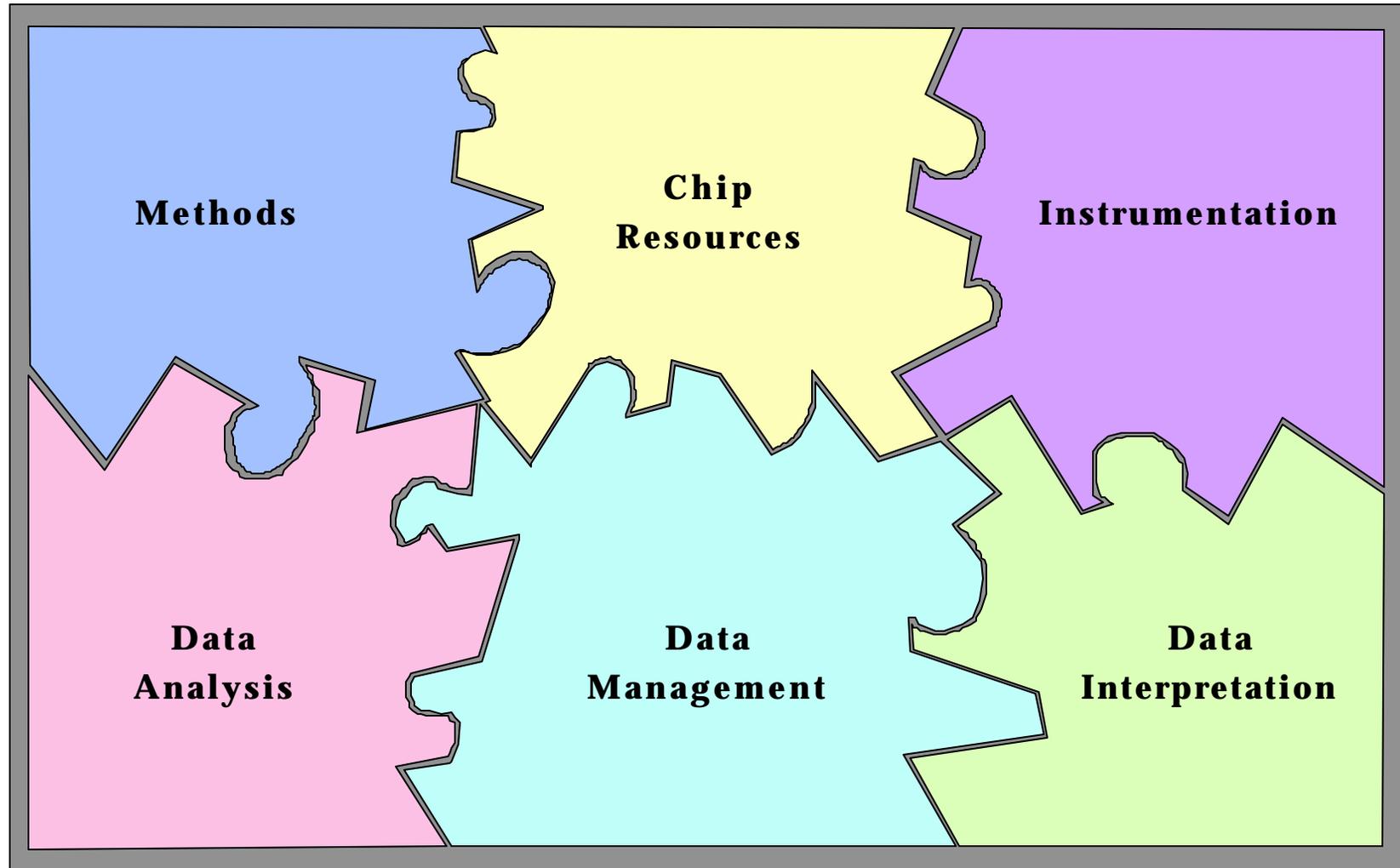
Biological Experiment #1



Biological Experiment #2



The cDNA Microarray Puzzle



Disadvantages of cDNA Microarrays on Glass Substrate

- Relatively Large Amount of RNA Required
- Cross-Hybridization Between Family Members
- Chip Manufacture is Labor Intensive
- Laboratory Set-Up is Expensive
- Highly Specialized Equipment is Required
- High Reagent Costs (Taq, Fluors)

Advantages of cDNA Microarrays on Glass Substrate

- Thousands of Genes Analyzed Per Experiment
- Simultaneous Two-Color Hybridization
- Clones Available for Signal Validation
- Large Linear Range (4-5 orders)
- Highly Flexible
- Low Cost per Chip
- Replicate Experiments are Feasible