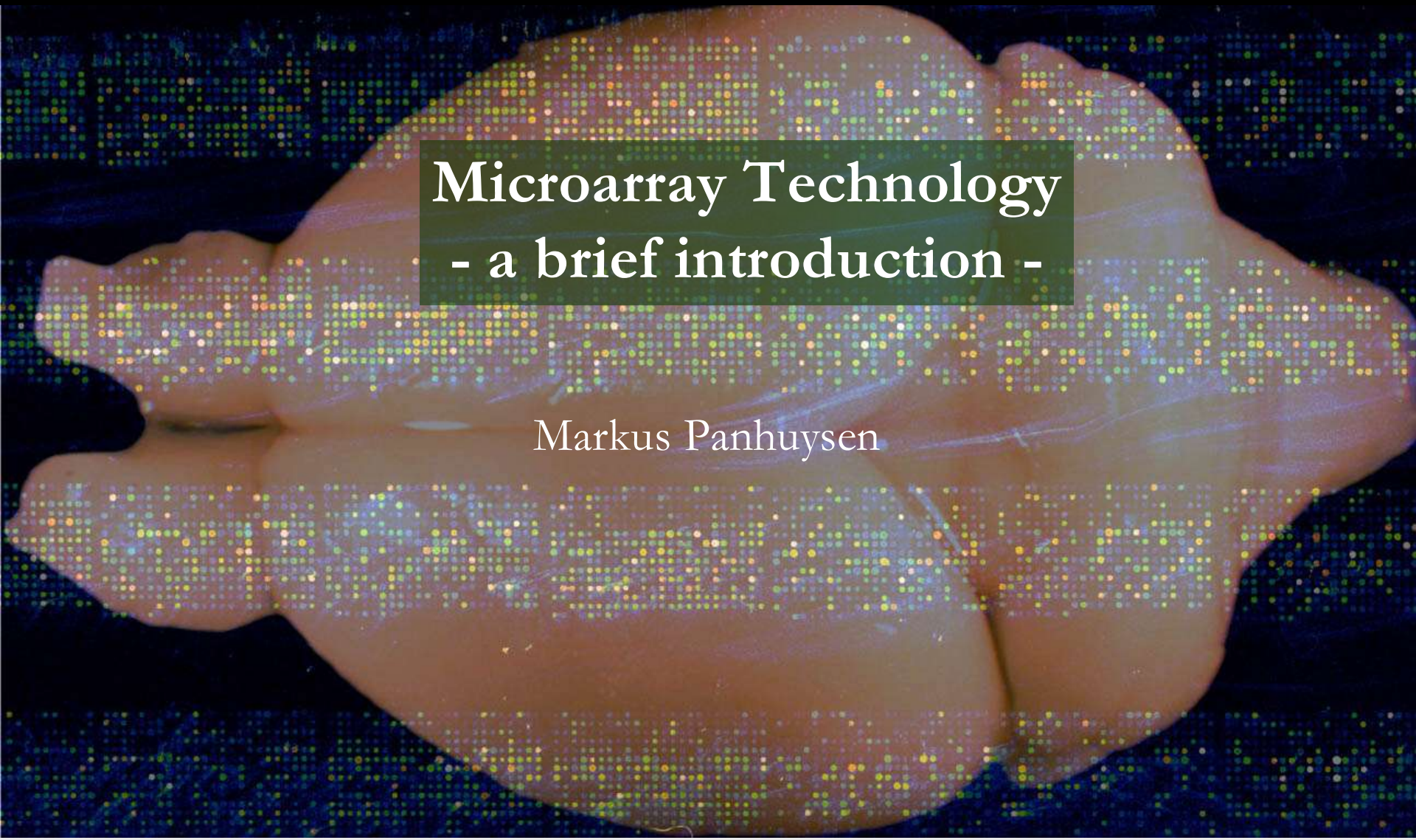

Microarray Technology
- a brief introduction -

Markus Panhuysen

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Microarray Technology is a powerful tool to monitor gene expression or gene expression changes of hundreds or thousands of genes in a single experiment.

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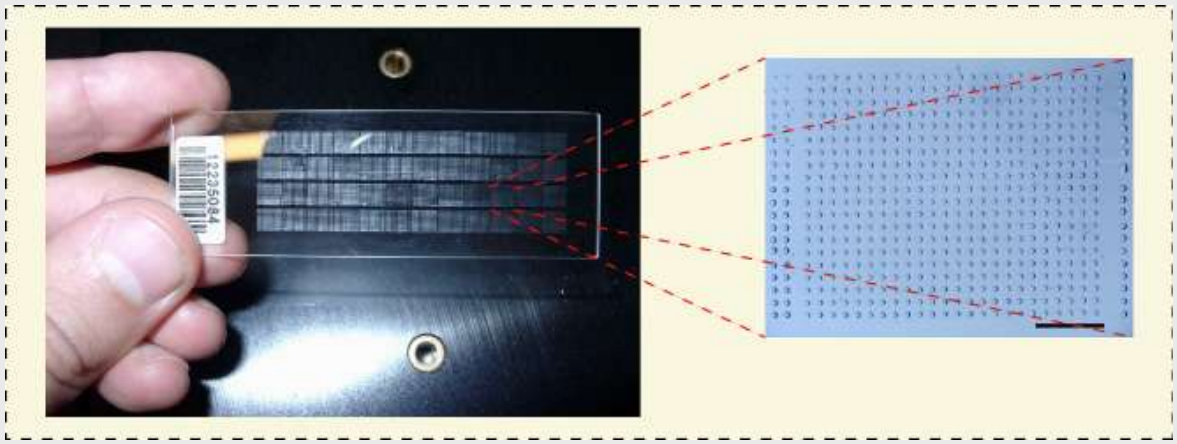
Microarrays

-> hundreds or thousands of gene specific probes (cDNA or Oligo-nucleotides) fixed on a solid support (usually glass or plastic slides or membranes)

-> self-made or commercially available (Agilent, Codelink, Clonetech, Stratagene, etc. ; or GeneChips from Affymetrix)



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Applications of Microarrays...

* Expression profiling

* Detection of changes in gene expression

(simple comparison or multiple conditions, time course experiments,...)

* Diagnostic tool

(tumor classification, genomic Microarrays: detection of chromosomal rearragements, deletions or duplications)

* Other applications

(SNP analysis, detection of methylation patterns,...)

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Term definitions...

PROBE: cDNA or oligonocleotide attached to the array surface

TARGET: cDNA or aRNA within the hybridization mix (which can hybridize to the complementary probe strand)

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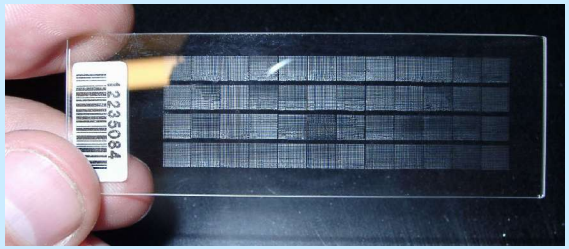
Experiment Overview

Array Production



Sample Production

cells / tissues

↓

RNA

↓

(amplification)

↓

Labeling

#1

#2

Hybridization
Stringency Washes

↓

Scanning
Quantification

↓

Normalization
Analysis
Annotation

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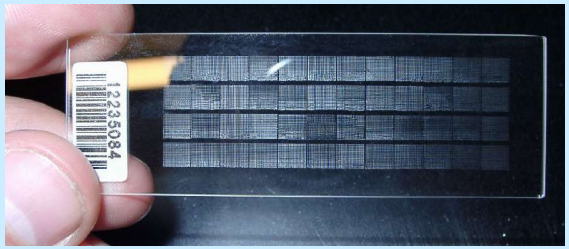

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Production of cDNA Microarrays

Bacterial Clone Library

↓

cDNA Amplification (PCR)

↓

PCR Product Purification/ transfer in Spotting Buffer

↓

Rearraying (96well → 384well)

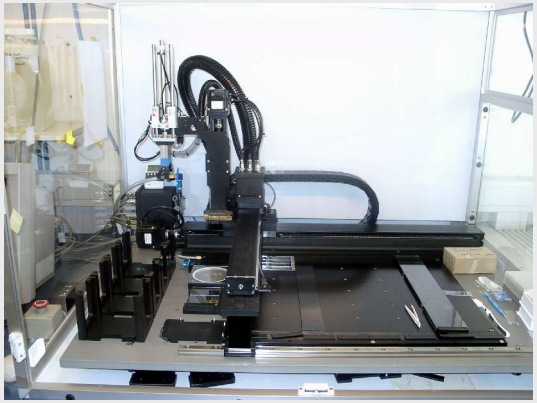
Spotting / Printing

↓

Probe Crosslinking

↓

Blocking / Prehybridization



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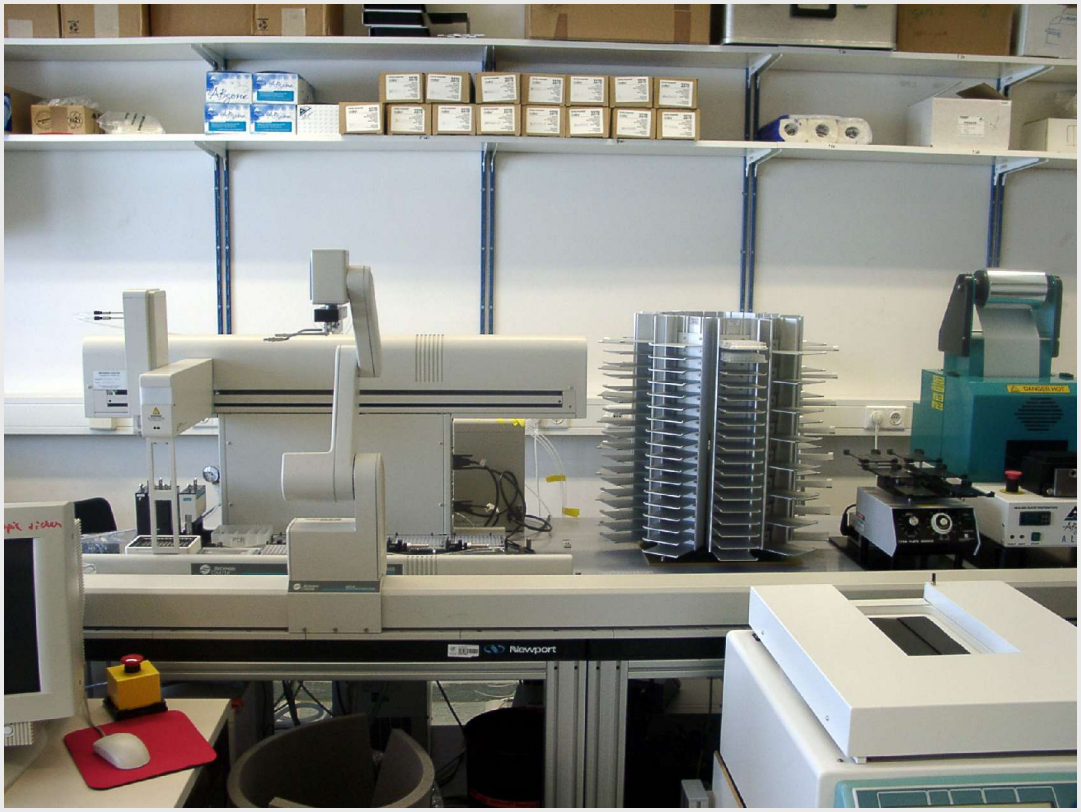
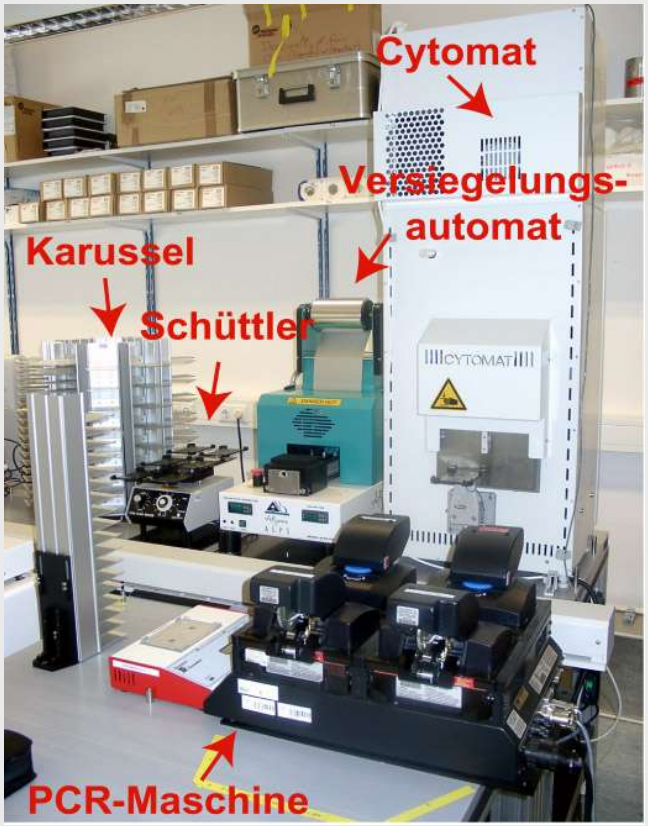
High Throughput System for cDNA Amplification





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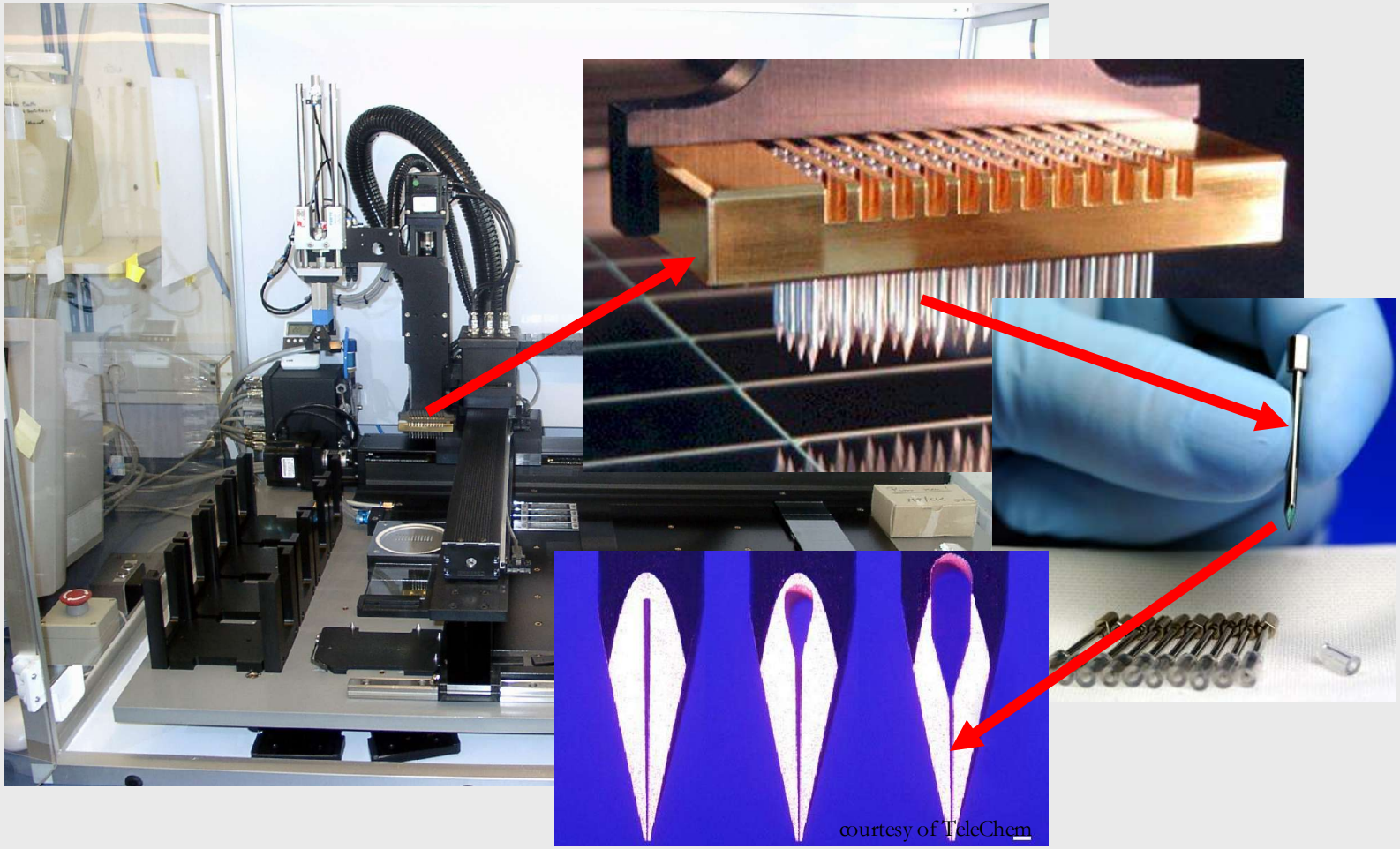
The Spotter



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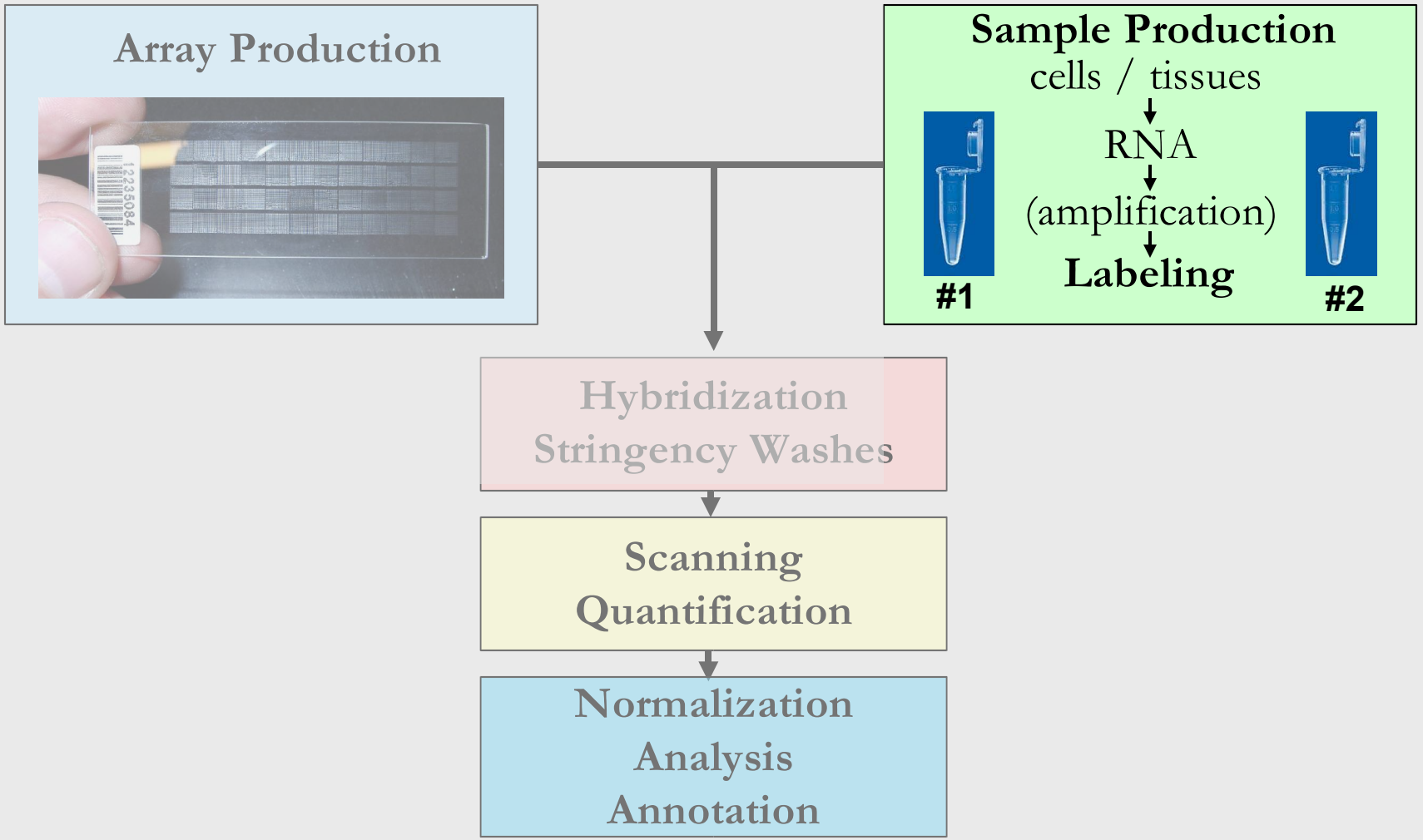

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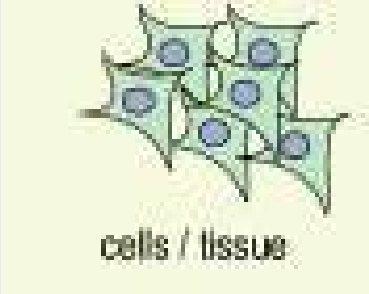
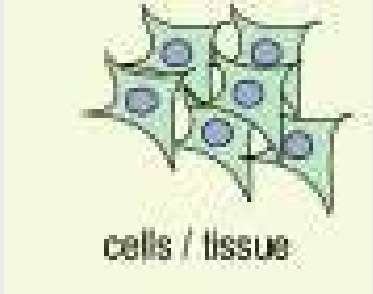
RNA

isolated from cells or tissues of interest
e.g., from RA treated cells and control cells



8 hrs retinoic acid treatment



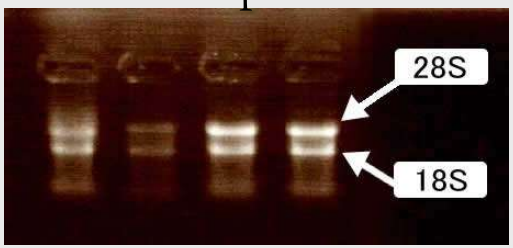
8 hrs control treatment

RNA extraction

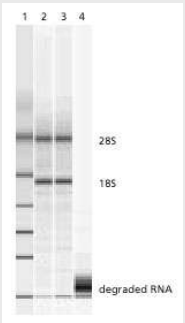
RNA_{RA}

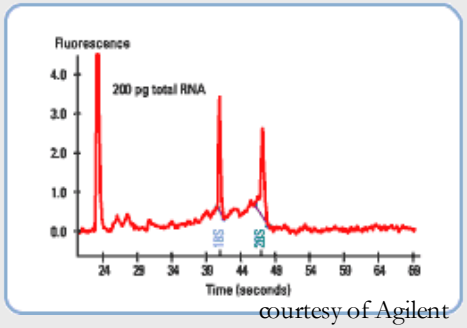
RNA_{Ctrl}

RNA Quality Check:
by denaturing gel electrophoresis



or RNA LabChip





courtesy of Agilent

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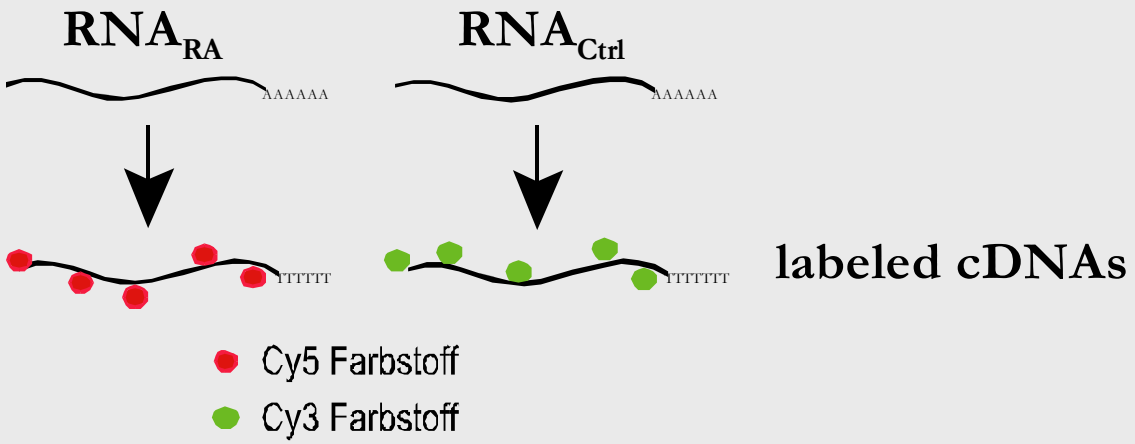
RNA Labeling

usually involves a reverse transcription step
optional: RNA amplification

→ direct or indirect incorporation of fluorescent dyes, radioactivity or epitope tags (e.g, biotin); single color or dual color experiments



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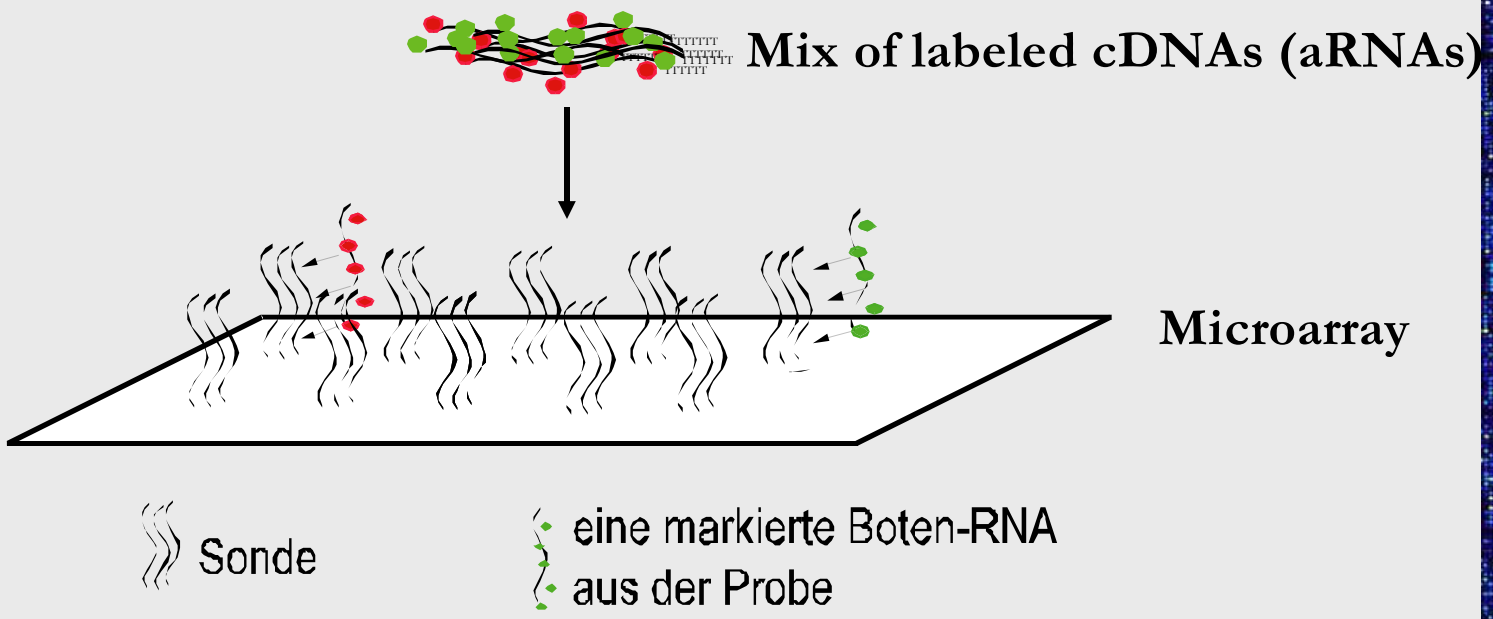
Hybridization

incubation of Microarrays for 16 hrs or more with a hybe-mix containing the labeled cDNAs



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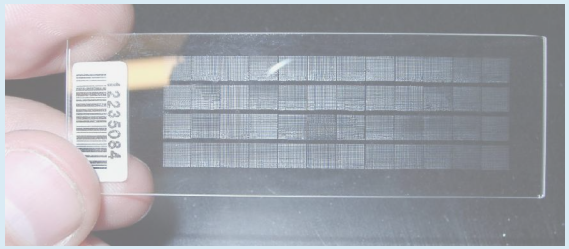
Experiment Overview

Array Production



Sample Production

cells / tissues

↓

RNA

↓

(amplification)

↓

Labeling

#1

#2

Hybridization
Stringency Washes

↓

Scanning
Quantification

↓

Normalization
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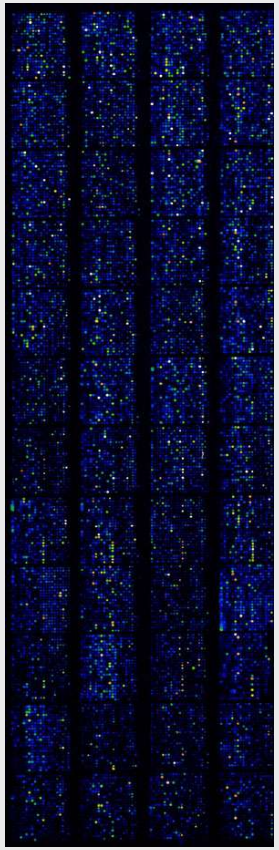
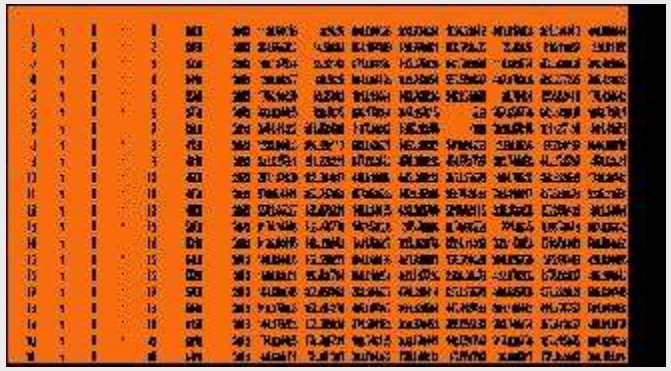
Stringency Washes, Drying, Scanning and Quantification





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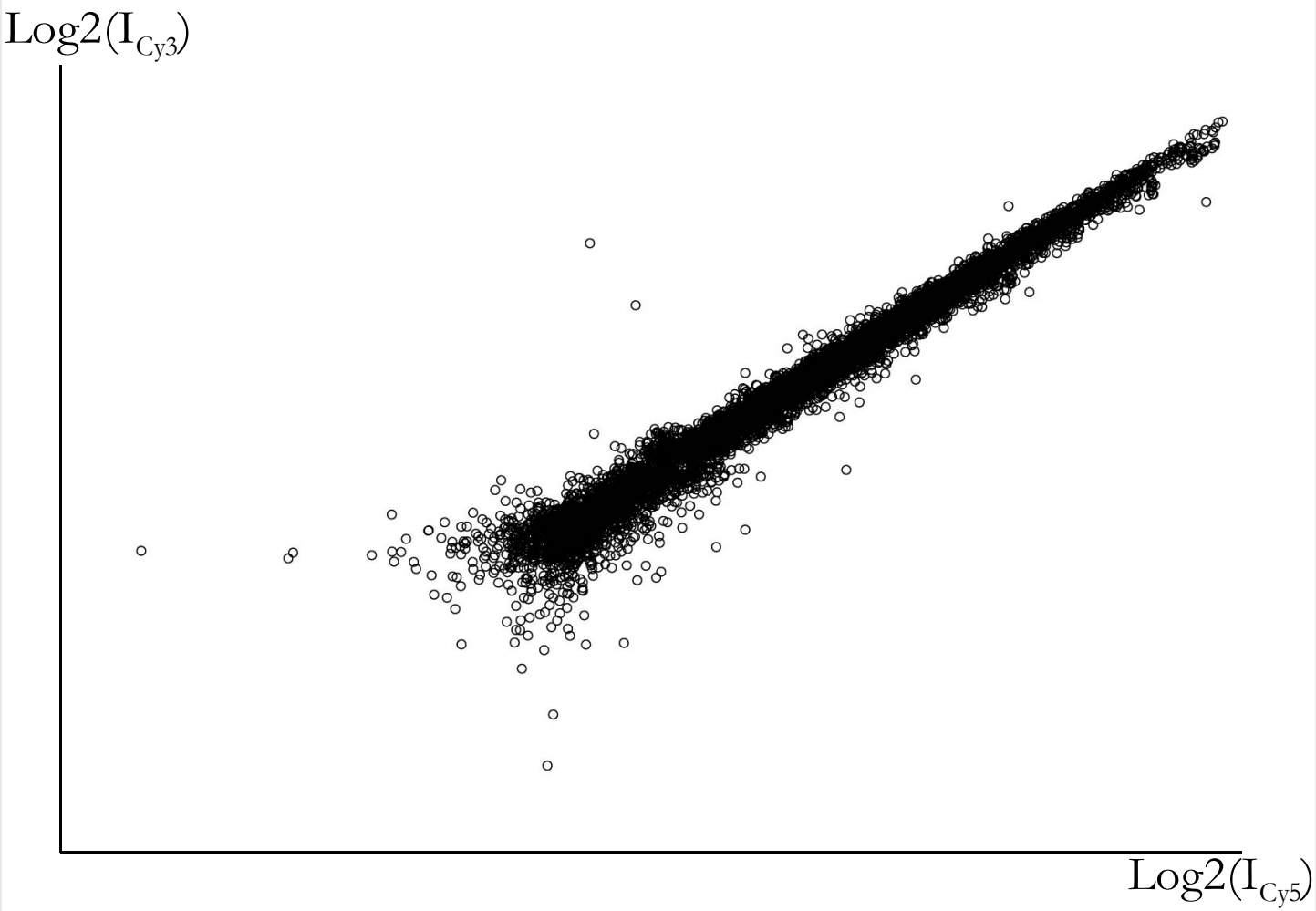
Log-Plot



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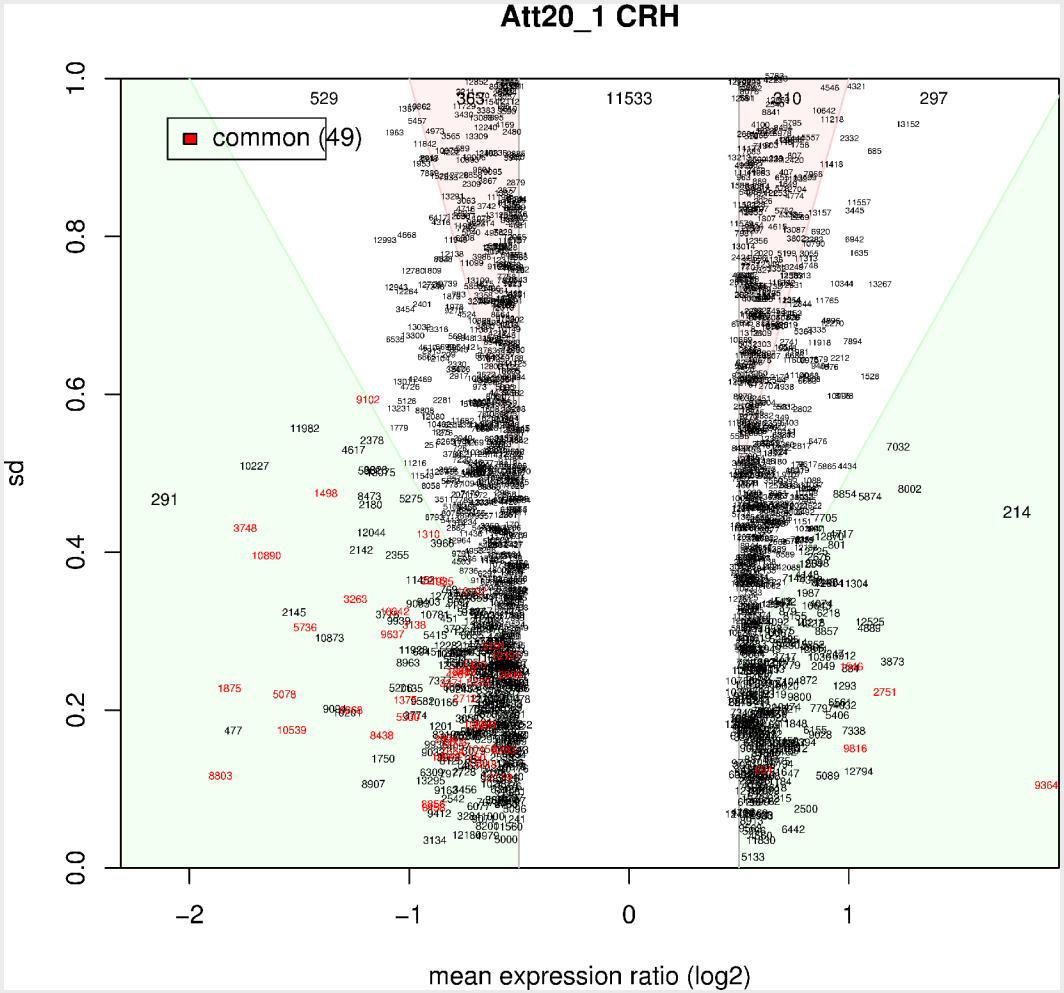
Data Analysis



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Experiment Design

* single color/labeling or dual color experiment

* direct sample labeling or indirect sample labeling

* with or without Dye Swap

* sample by sample or sample pools

* technical replicates or biological replicates

* with or without reference RNA

* in case of multiple conditions: Which samples should be directly compared?

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Sample complexity affects the efficiency of microarray experiments...

Gene expression changes in a subset of cells within a sample are diluted by unaltered expression in other cells, resulting in reduced measurable expression changes.

From the technical point of view, samples should be as little complex as possible.

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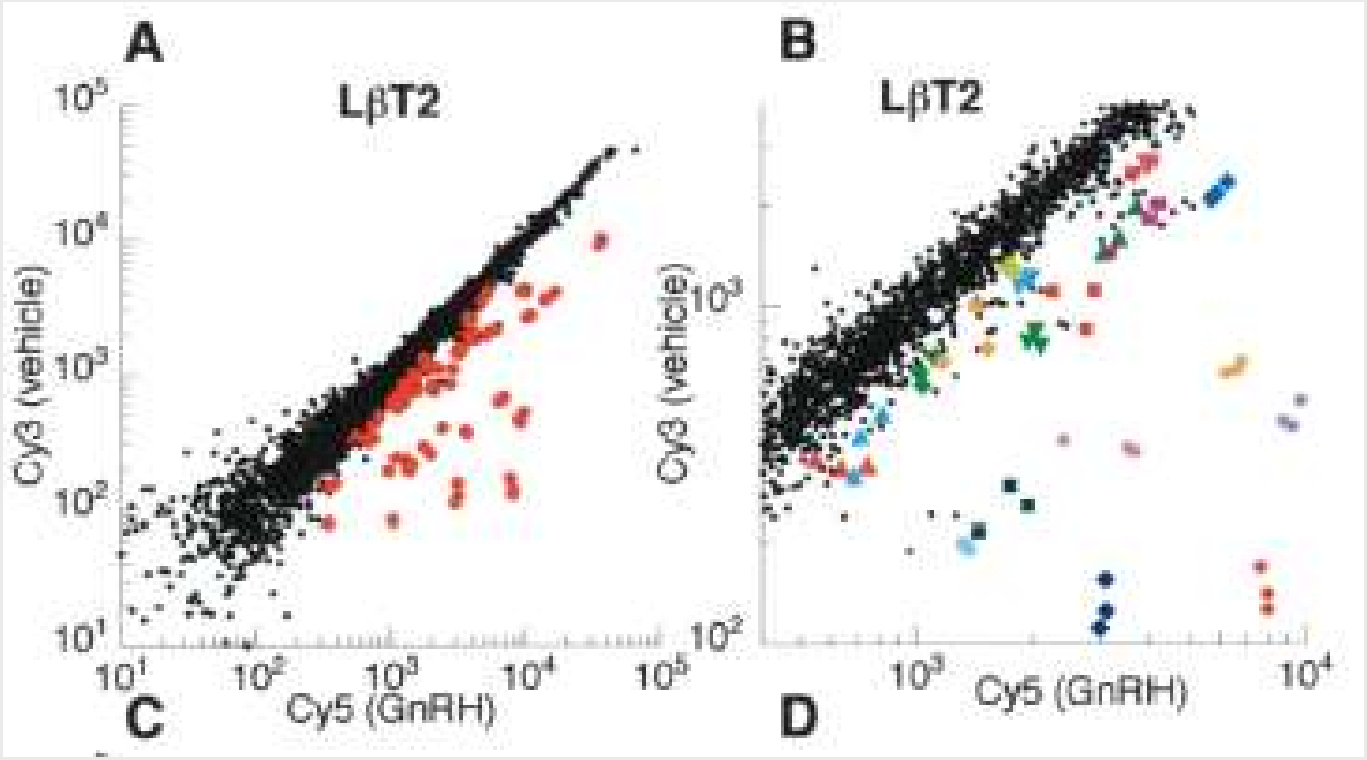
Differential expression in a cell line...



Wurmbach et al., 2002

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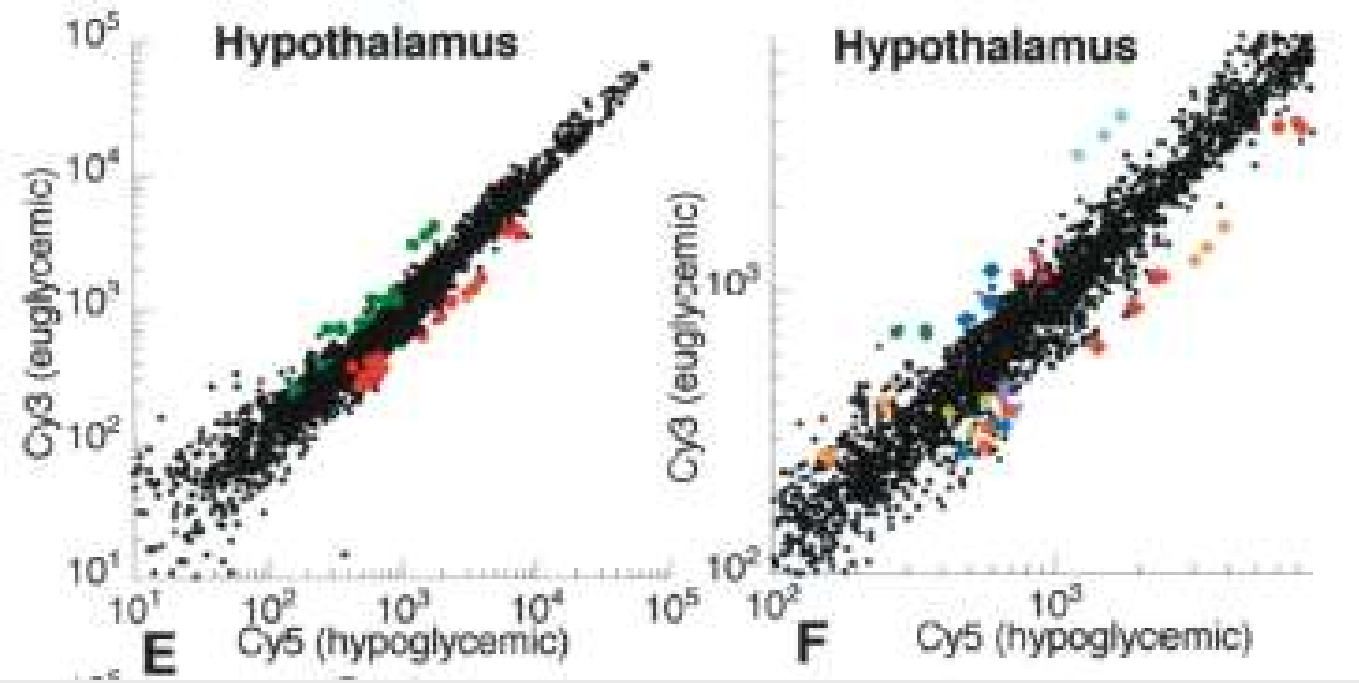
... in the hypothalamus...



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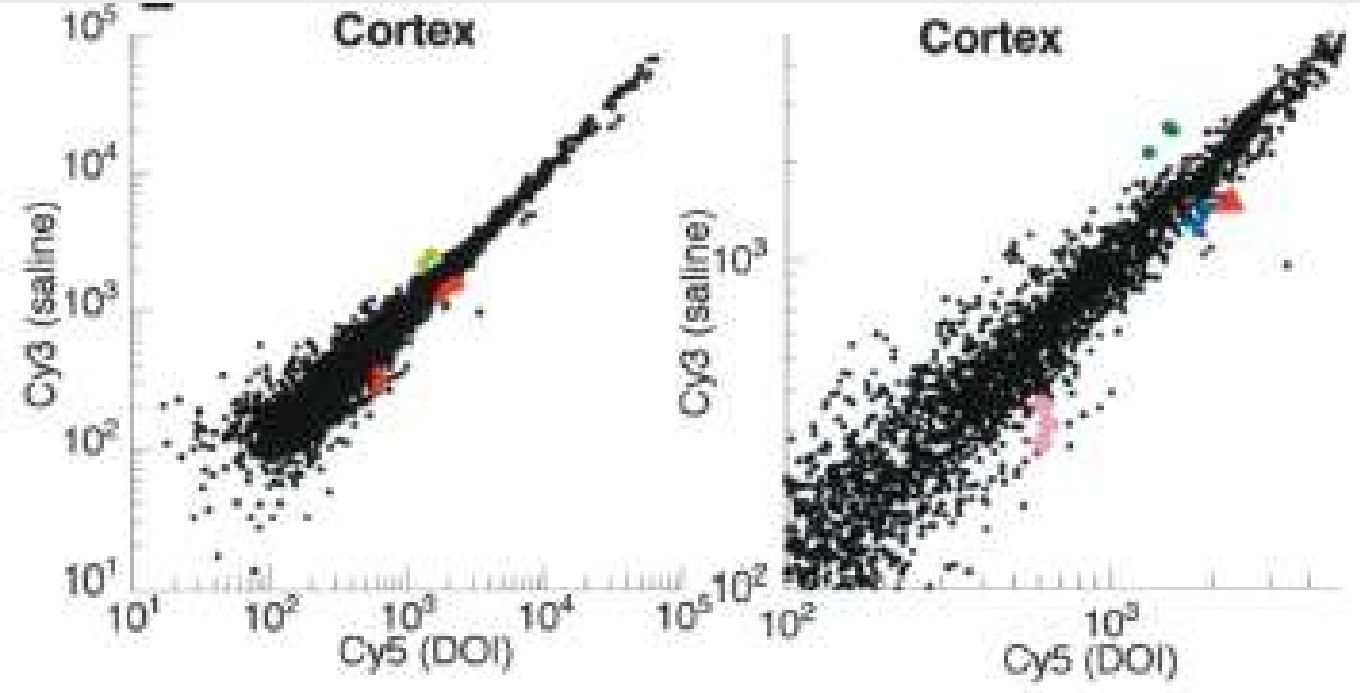
...and in the cerebral cortex...



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Sources of Variation within Microarray Experiments...

biological

animal to animal differences
in gene expression

technical

caused by the experimentator,
by equipment measuring errors,
by technical limitations,
by bad protocols,
...

systematic

unsystematic, "noise"

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Sources of errors / technical variation

Array production: Clone quality

...clone sequence incorrect

...clone contaminated (with other clones)

Nucleic Acids Research, 2001, Vol. 29, No. 2 **582-588**

Assessment of clone identity and sequence fidelity for 1189 IMAGE cDNA clones

Robert G. Halgren, Mark R. Fielden, Cora J. Fong and Timothy R. Zacharewski

Department of Biochemistry and Molecular Biology, National Food Safety and Toxicology Center, and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824-1319, USA

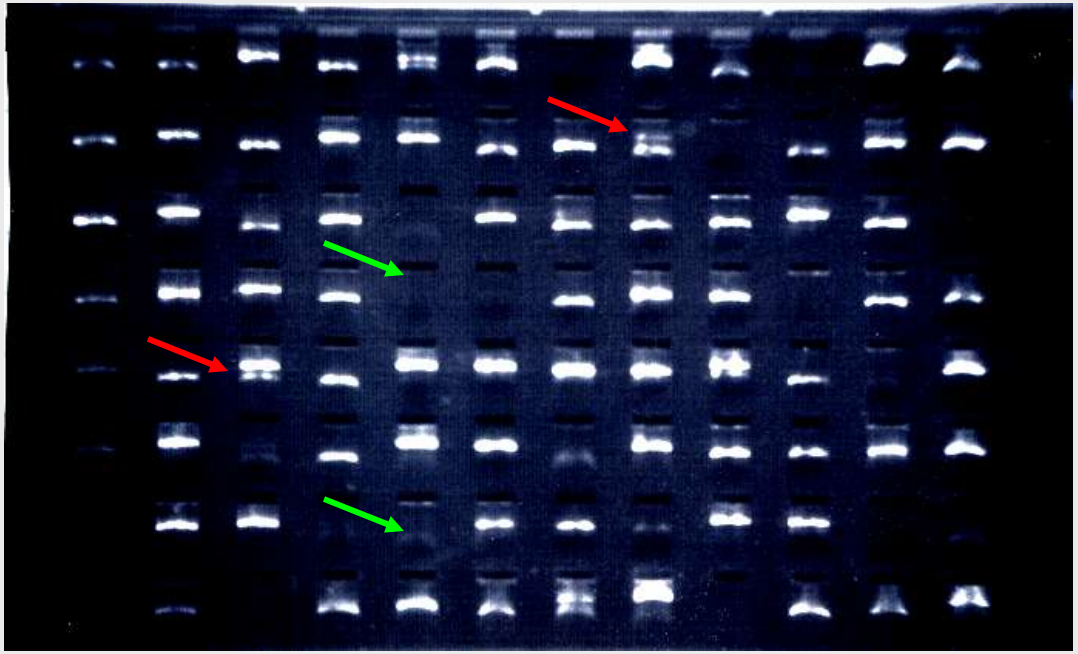
...After isolation of plasmid DNA from 1189 bacterial stock cultures, only 62.2% were uncontaminated and contained cDNA inserts that had significant sequence identity to published data for the ordered clones....

Sources of errors / technical variation

Array production: cDNA probe amplification

...does not work for some clones

...results in too low concentration of the PCR product



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Sources of errors / technical variation

Array production: Spotting process

pin printing failures

spot size varies too much, probes mix on the slides

bad spot morphology, tadpole effects, horseshoes etc.

pin-specific artefacts

Array production: Slides

scratches, dust or other contaminations

inhomogenous surface coating

surface chemistry and spotting buffer

Array production: Blocking / Prehybridization

array surface not completely inactivated

(results in unspecific probe binding)

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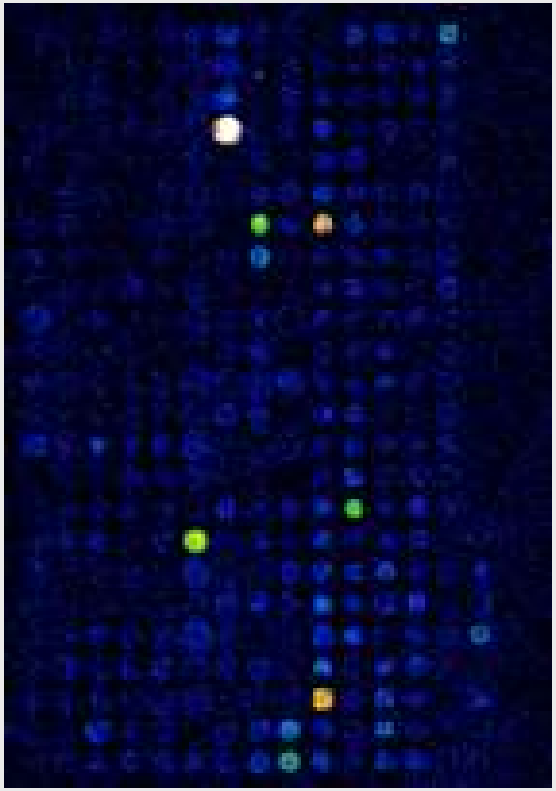
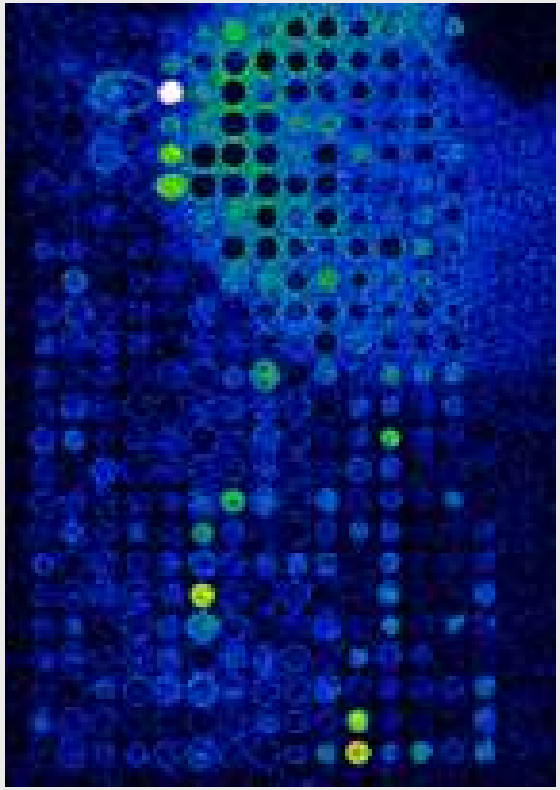
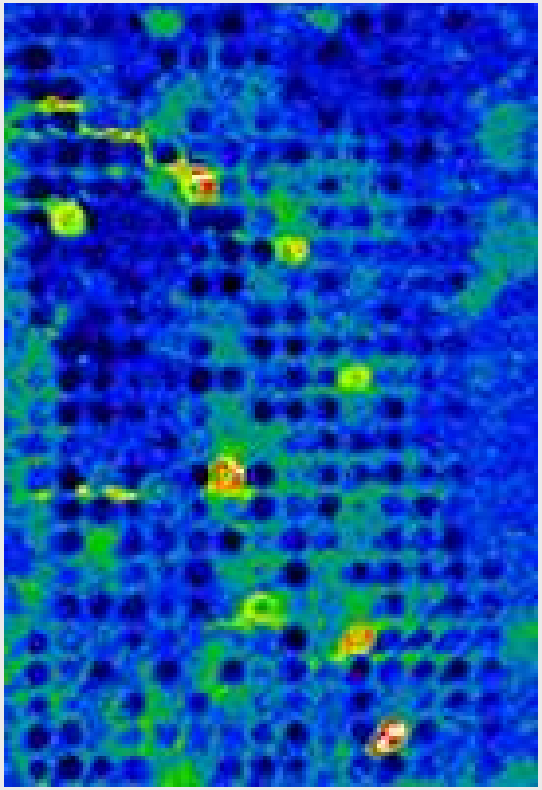
some examples...



the good...



...the bad...



...the ugly

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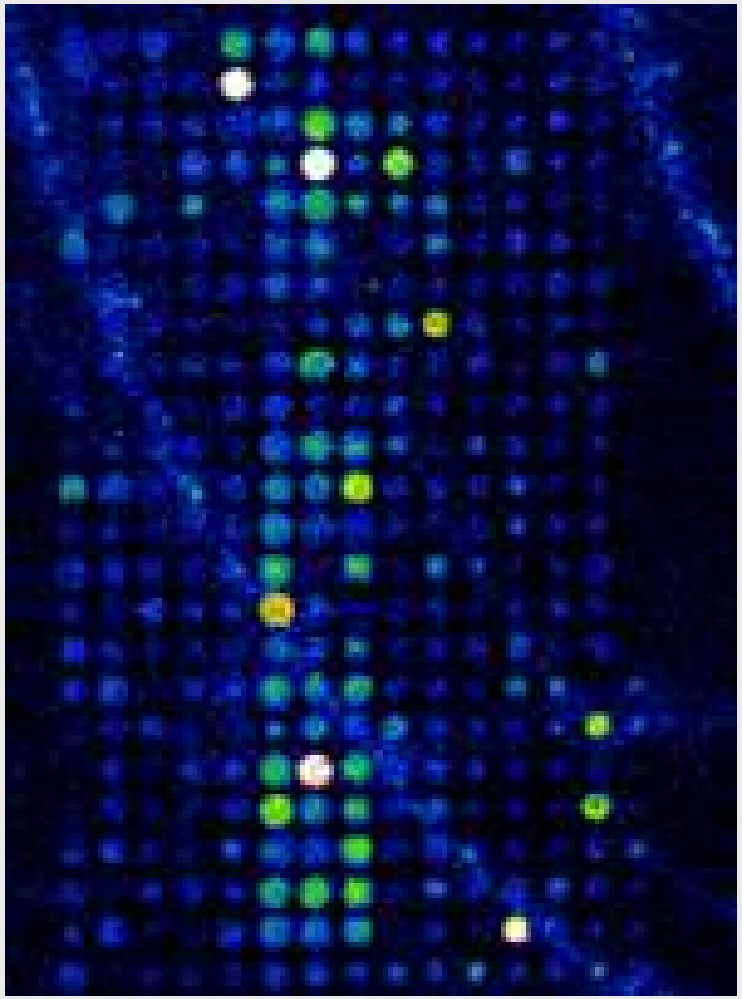

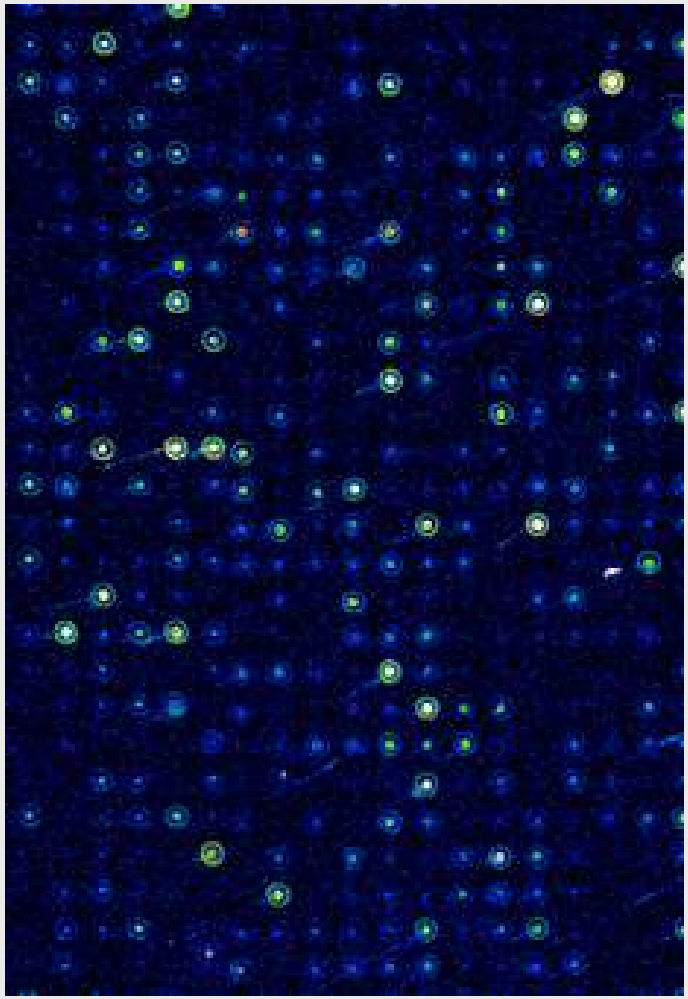

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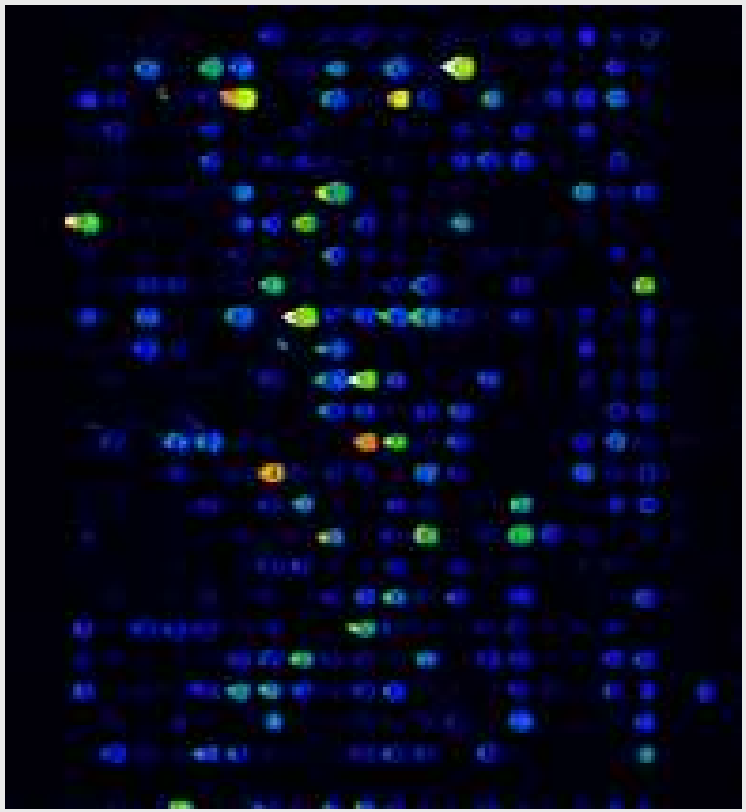

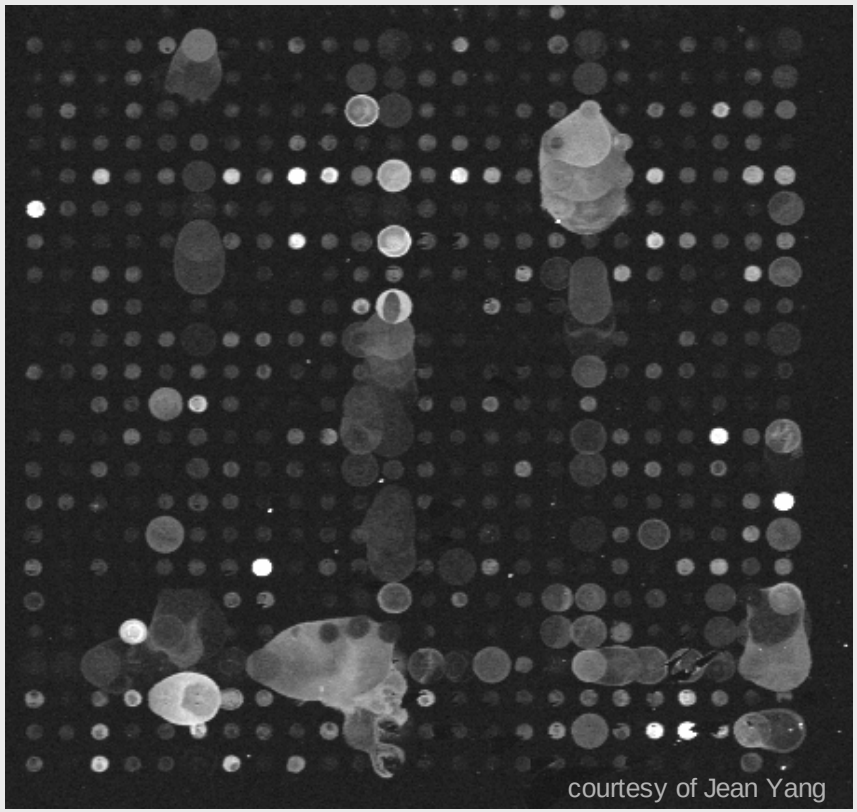

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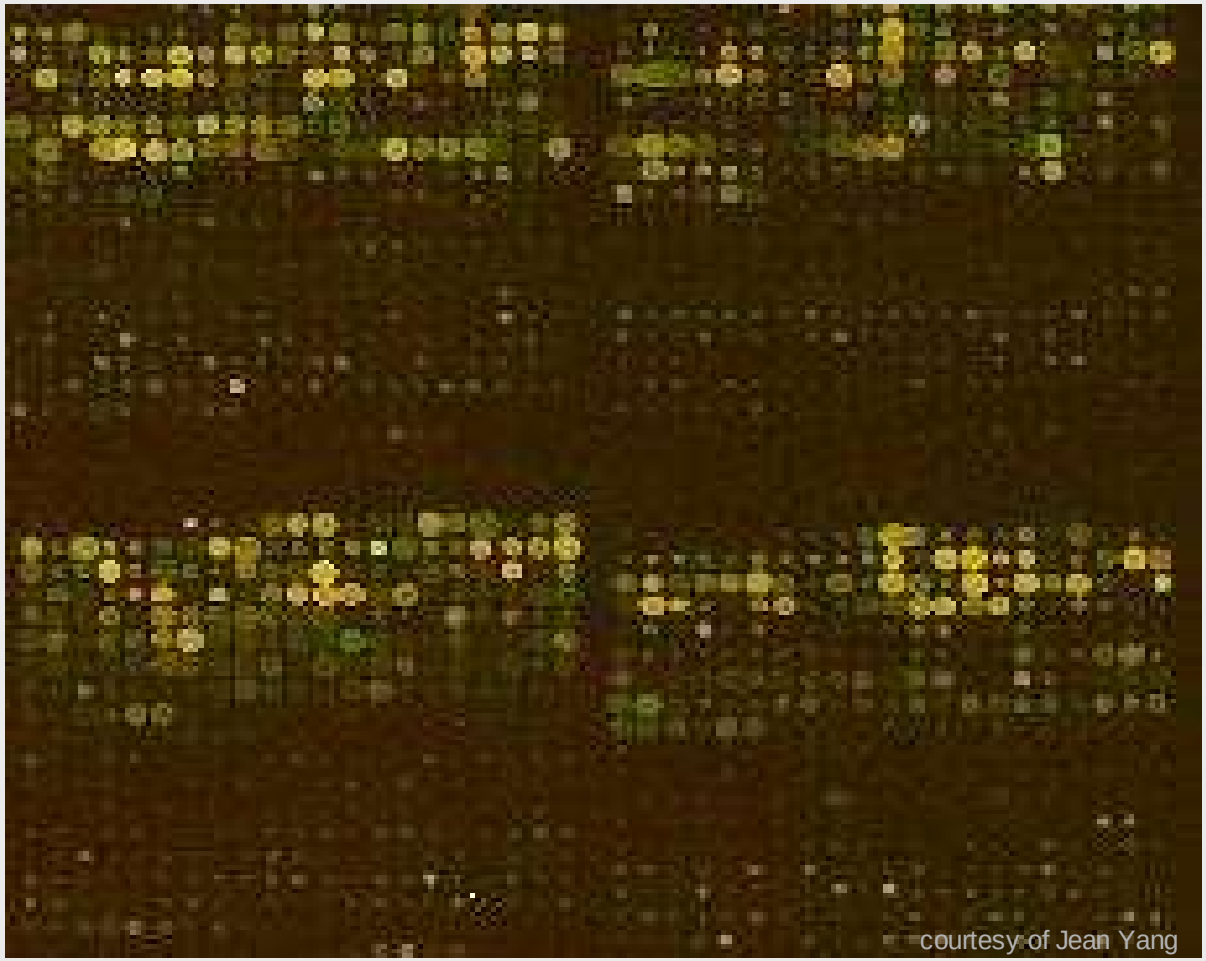
Kraepelinstr. 2
80804 Munich



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Sources of errors / technical variation

RNA

RNA degradation

Sloppy sample isolation

(spatial variation of tissue punches from sections, variation in sample quality due to contaminations)

Labeling Efficiency too low or varying among samples

Labeling Bias (e.g., due to direct incorporation of CyeDyes)

Inhomogenous hybridization / washing conditions

Spatial bias caused by the Scanner

Quantification method not appropriate for specific spot characteristics

Inappropriate normalization methods (e.g, subtraction of nonadditive backgrounds)

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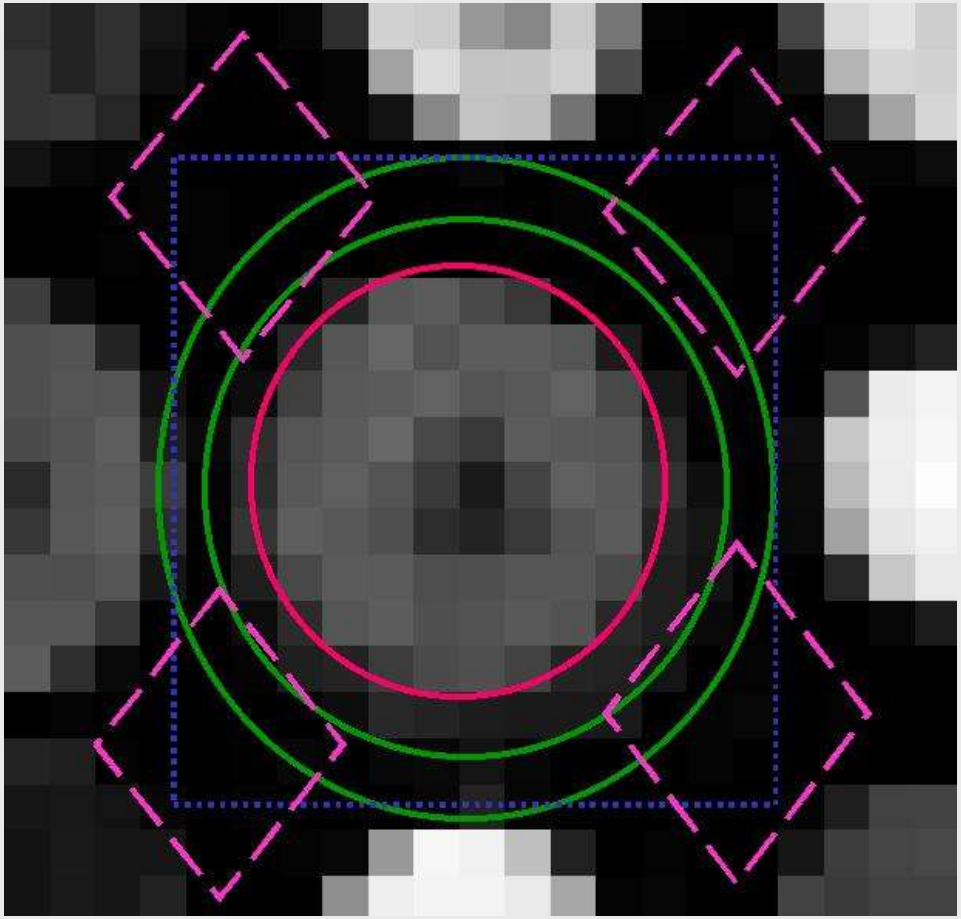
Quantification methods



courtesy of Jean Yang

Fixed circle

Adaptive Circle

Adaptive Shape
(Edge detection/ Seeded Region Growing)

Histogram
(Adaptive threshold based)

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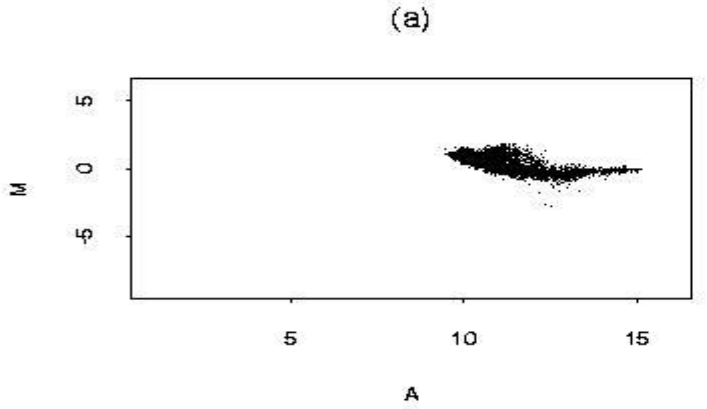
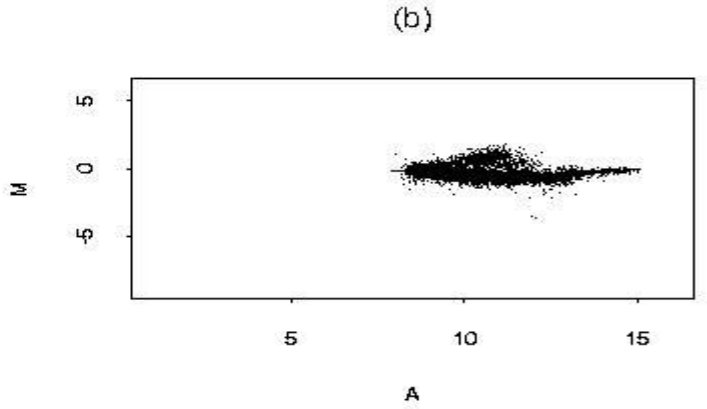
The Quantification method does matter...

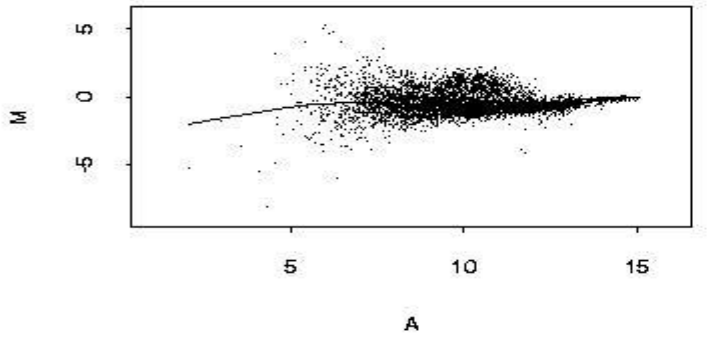
Spot.nbg



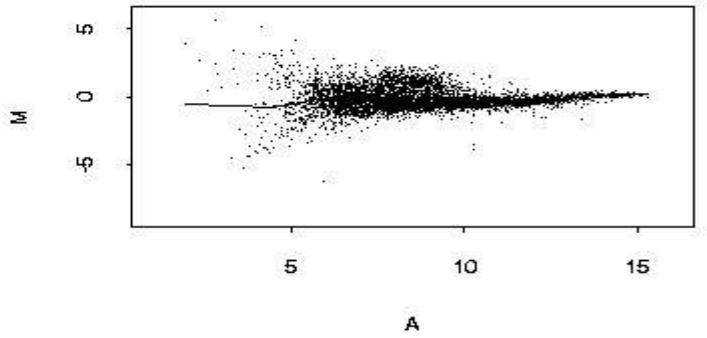
Spot.morph



Spot.valley



ScanAlyze



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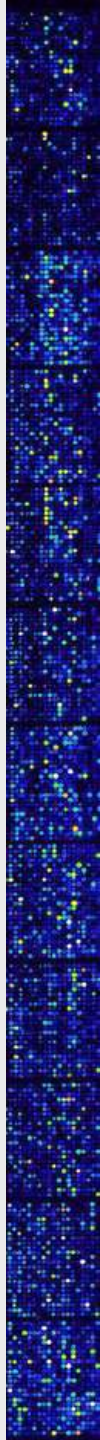

Thanks!

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Commercial Microarray Solutions...

... are standardized (array production and experimental protocols)

... are quality controlled

... usually provide tools for quantification, normalization, analysis, visualization, data warehousing, project management

... are expensive

... are either inflexible (only standard sets are available)

... or even more expensive (custom arrays)

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In-House Microarray Solutions...

... are cheaper than commercial solutions

→ if big amounts of experiments are to be performed

... provide maximum control of any parameter involved

... are very flexible

→ protocols can be adapted to individual demands,e.g, performing experiments using specific subsets of probes

... enable the use of special applications

such as suppressive subtractive library experiments, RNA Arrays, etc

.

... have to be established (protocols, analysis tools, ...)

There are as many different experimental protocols as there are different Microarray Laboratories

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