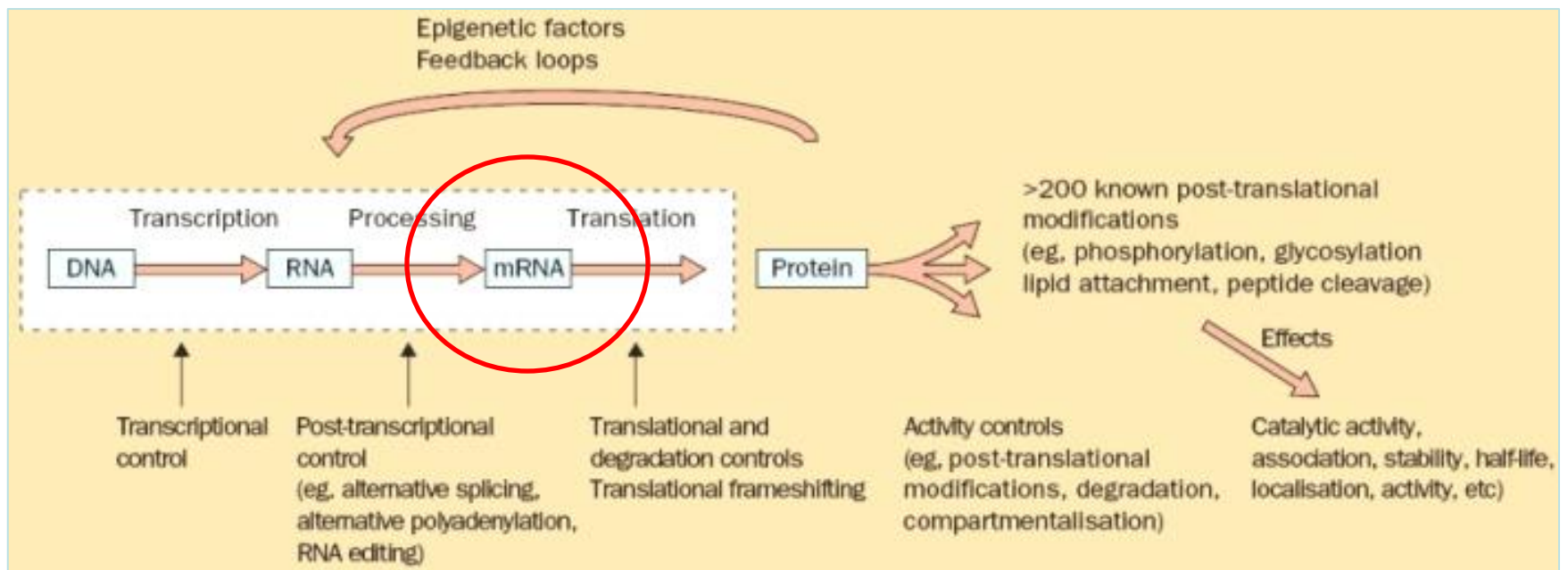


# Microarray

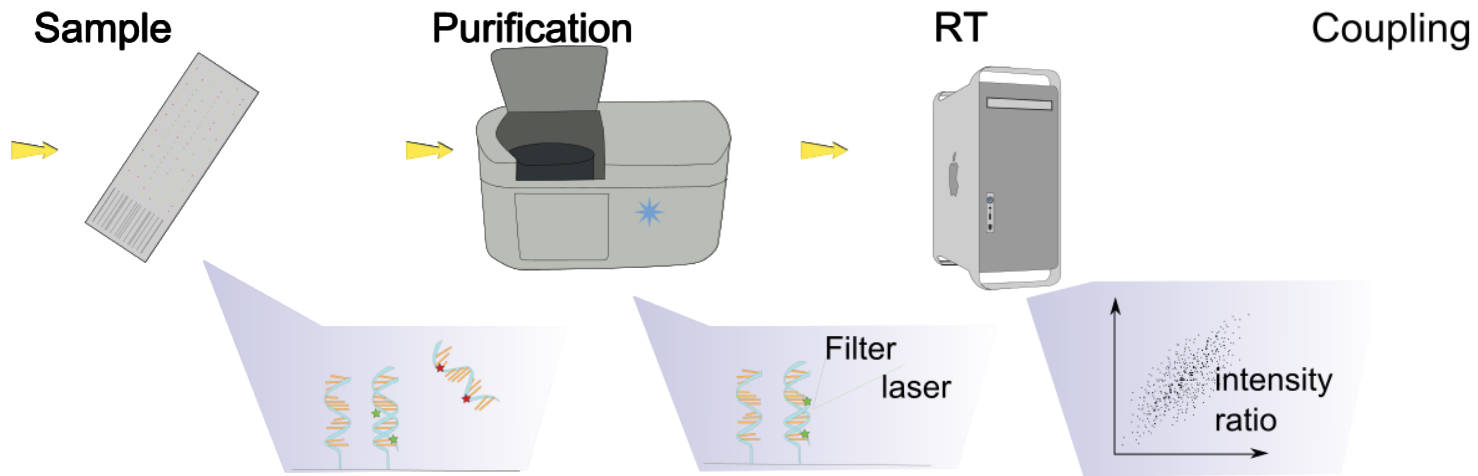
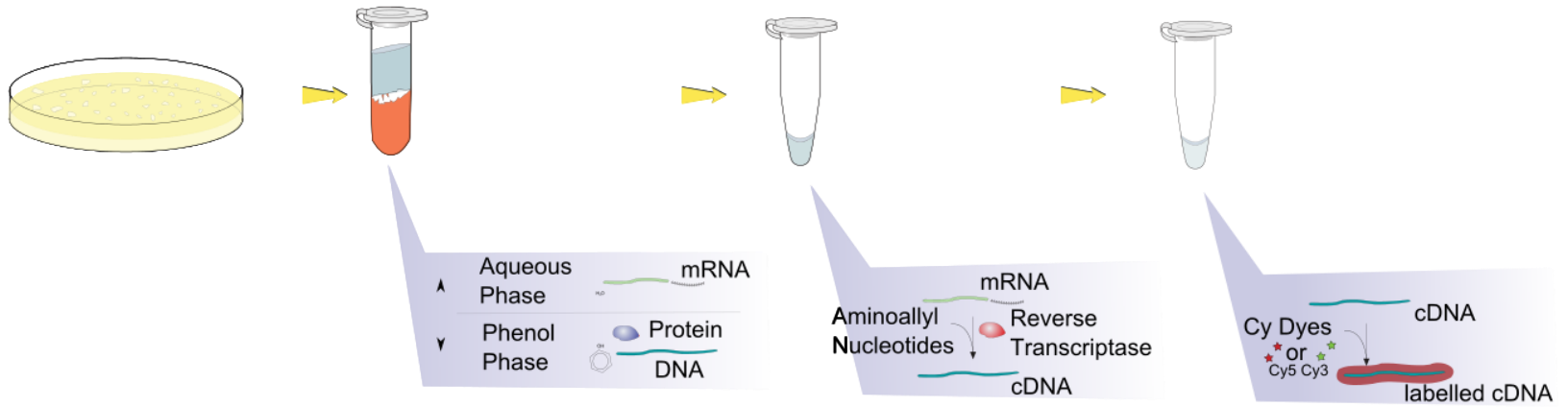
## Technology



## The way of a protein:



# Microarray

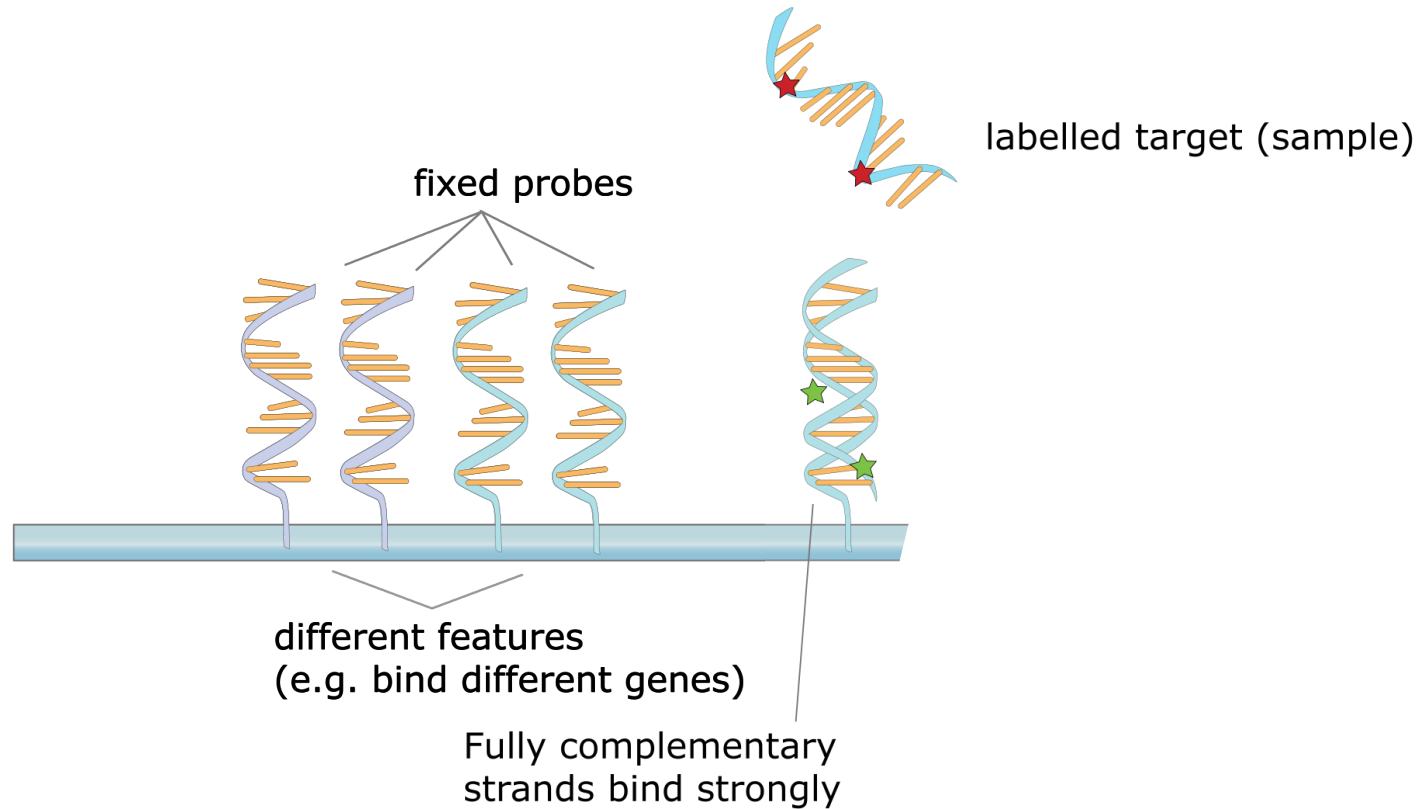


**Hybridization and washes**

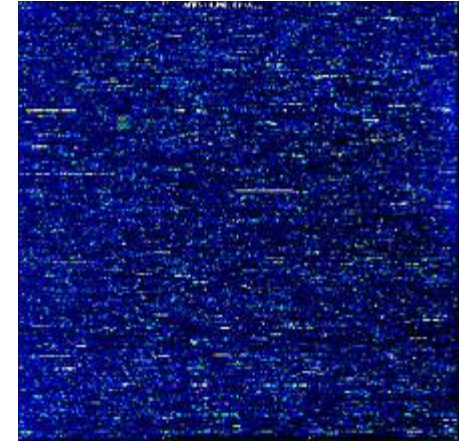
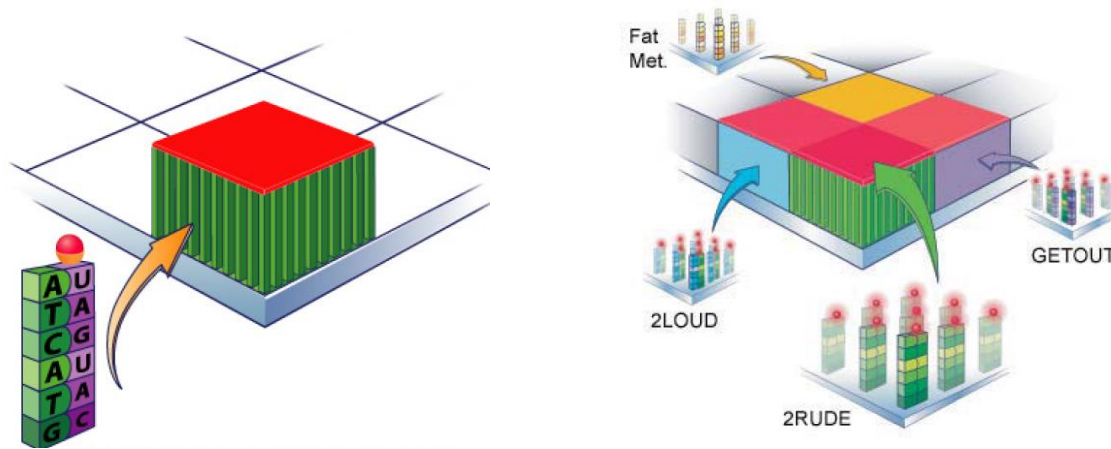
**Scanning**

**Normalization and analysis**

# Principle



# Principle



**TECHNOLOGY HOW IT WORKS**

## The Microarray Scanner

By Jeffrey M. Perkel

Five years into the microarray revolution, biochip images—rows upon rows of red and green spots on a field of black—have become an ubiquitous DNA data source.

But how are these pictures generated? Arrays are imaged using one of two classes of equipment: array imagers and array scanners. Both use lasers to excite the fluorophores on the chip, but array imagers capture a snapshot of the glowing array using a charge-coupled device (CCD) camera, whereas array scanners read the chip point by point using a photomultiplier tube.

The first microarray scanner, built in 1989 by Stephen Fodor and colleagues at Affymax, was a table-sized, home-built affair that included a Zeiss confocal microscope, a lens, and several mirrors. Fodor would go on to head Affymetrix, a Santa Clara, Calif., Affymax spin-off that now holds the lion's share of the microarray market.

Unlike some of its competitors, whose glass microscope objective-based arrays can be read in any array mode, Affymetrix's GeneChip microarrays are imaged using a proprietary instrument. Shown here are the major components of the newest version of that device, the GeneChip Scanner 3000.

DNA features on Affymetrix's GeneChip microarrays are built using a photolithographic process adapted from the semiconductor industry. The DNA building blocks (nucleic acid phosphoramidites) are photoresistive, meaning that they become "negative" or "opaque" to light. Photoreactive bearing substrates are mounted on a slide, deposited only those regions of the chip that are to couple to the next added nucleotide. In this way, thousands of chemically unique polymers can be assembled in parallel.

Affymetrix's microarrays are built in parallel on large wafers and then cut into individual chips, with one wafer yielding anywhere from 40 to 400 chips, depending just on the size of a slide. The GeneChip Scanner 3000 Plus Plus 2.0 array contains 1,400,000 distinct sequence features. The chip covers more than 40 mm (individual transcripts, each represented by 10 genes, of 45,000 oligonucleotides).

**1.** The flying objective performs fast horizontal scans, the actual trajectory in an arc with a radius of 100 mm. The scanner acquires fluorescence data only during the right-to-left scan (left to right in the Figure above), and the array is scanned from the top to bottom, so the array is fully covered. The chip transport moves the array by a distance equal to one pixel and then stops, allowing the objective lens to scan one line (row) of data.

**2.** The objective separates excitation and emission beams and allows a small sample of the beam to be recombined.

**3.** The system auto-focuses using laser light reflected from the chip's surface.

**4.** The fluorescence signal is measured using a photomultiplier tube (PMT). Photons strike a photocathode surface, which emits electrons due to the photoelectric effect. These electrons are accelerated through a series of dynodes (called dynodes, each of which generates additional electrons). This cascading effect creates 10<sup>6</sup> or more electrons for each photon hitting the first cathode, depending on the number of dynodes and the accelerating voltage. This amplified signal is finally collected at the anode, where it can be measured.

Jeffrey M. Perkel can be contacted at [jperkel@affymetrix.com](mailto:jperkel@affymetrix.com).

July 5, 2004

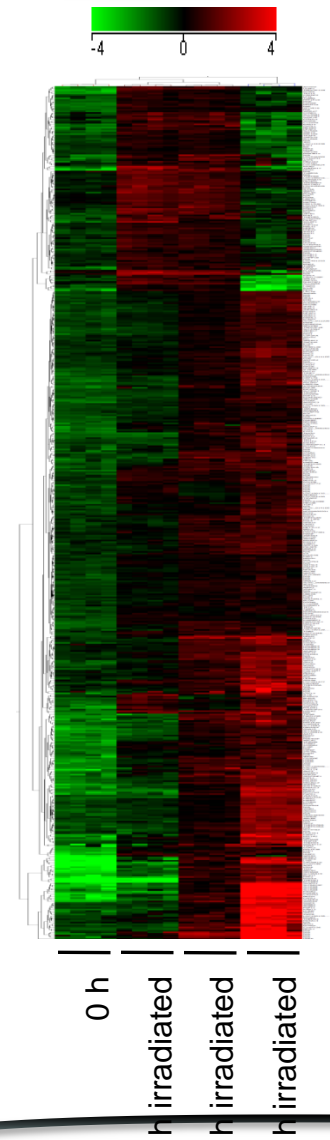
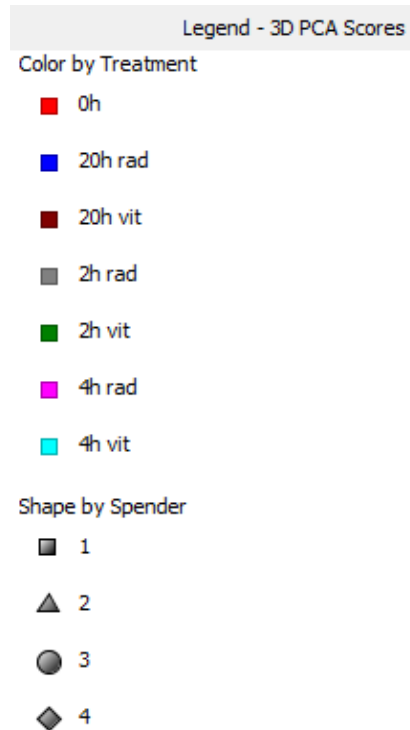
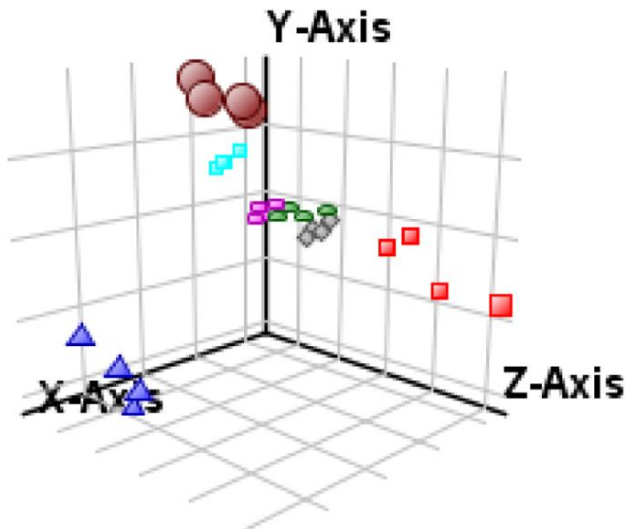
<http://www.the-scientist.com/?articles.view/articleNo/15785/title/The-Microarray-Scanner/>

# Data processing

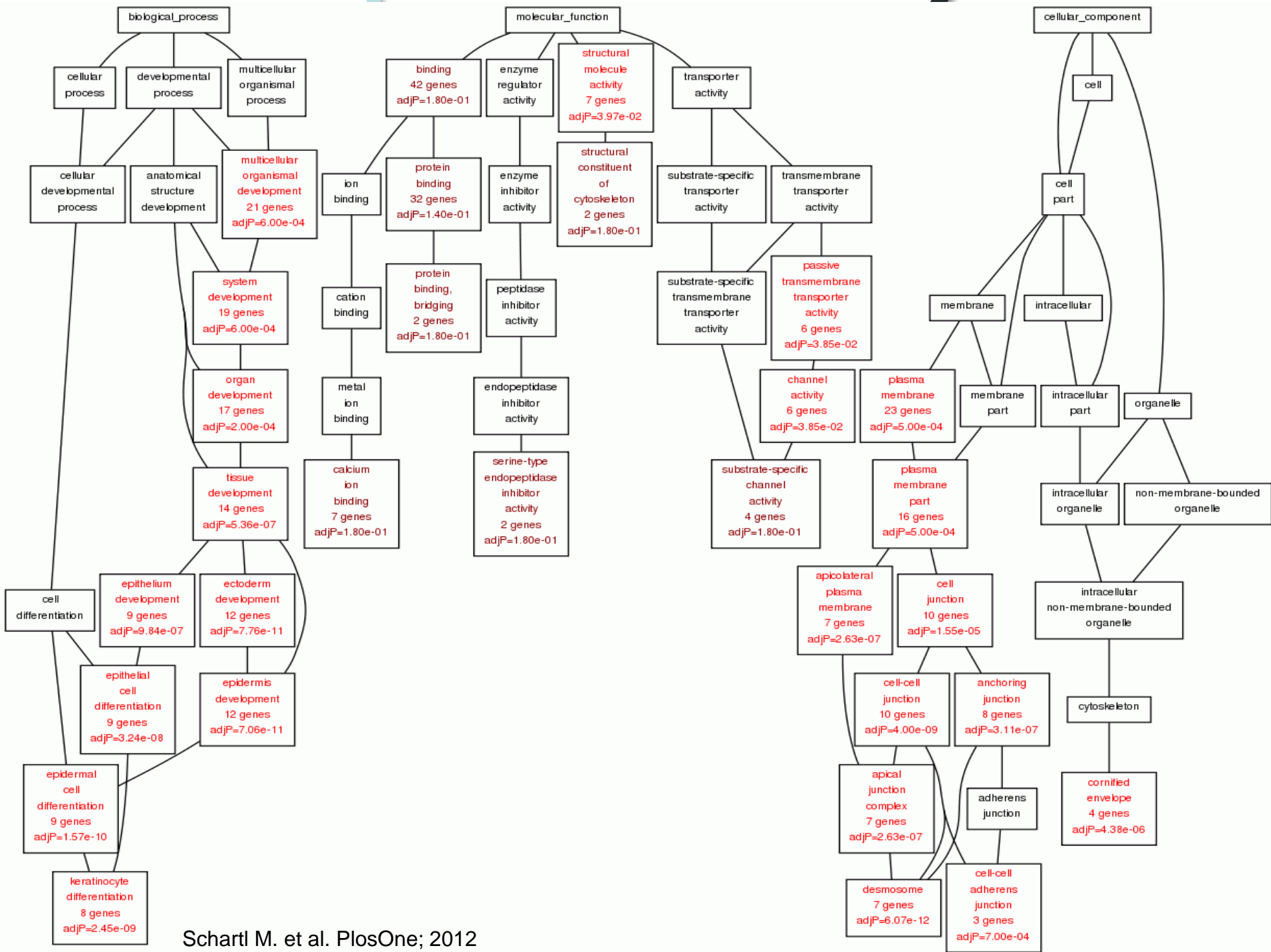
Experimental design

# Clustering

## Principal Component Analysis







## Take Home Messages for Microarrays:

- suitable technique to get an overview of cellular processes
- easy to use; fast results
- good screening tool
- imprecise data of low expressed genes
- need follow up validation (RT-PCR)
- problem of data analysis (e.g. normalization; >70.000 data points per chip, alpha bias...)

# Thank you for your attention