Image analysis

Source: Halliday D. and Resnick, R. (1988) Fundamentals of Physics, Third Edition. Johm Wiley & Sons, New York, page 844.

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Image analysis

The raw data from a microarray experiment is a series of scanned images.

- Images must be converted into quantitative data
- Steps to preprocess and transform the image into a format suitable for analysis are under the realm of "image analysis."

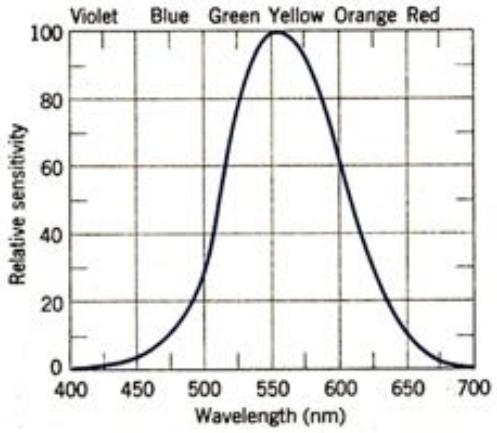


Figure 2 The relative sensitivity of the human eye at different wavelengths.

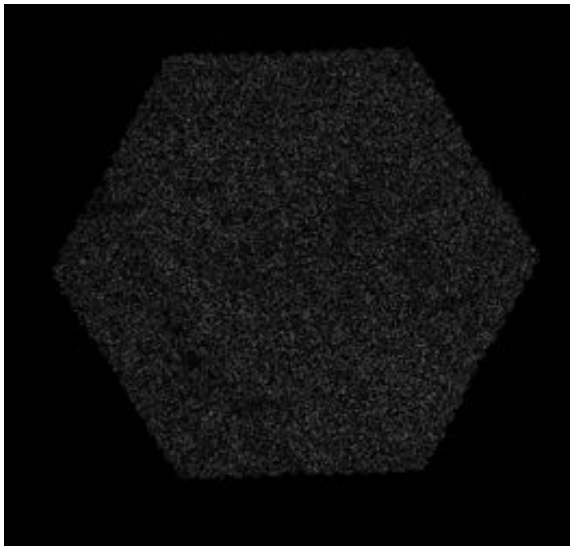
Custom spotted arrays

- One array has two images

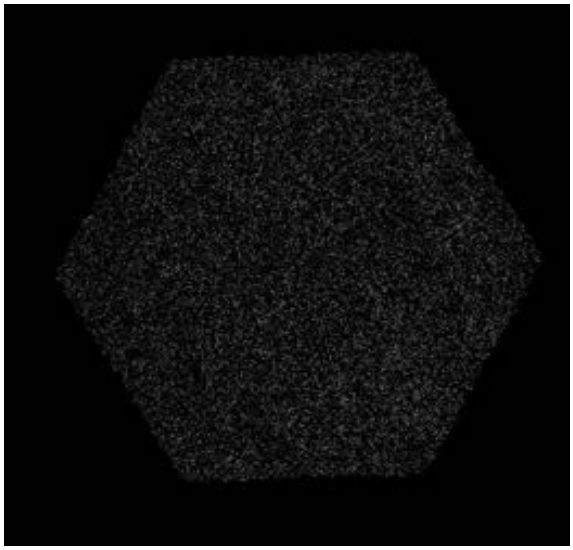
1. Cy3 dye, green channel, 510 - 550 nm
2. Cy5 dye, red channel, 630 - 660 nm

- These channels are distinguished by a scanning instrument
- Data for each channel are stored as monochromatic images

Illumina Green channel



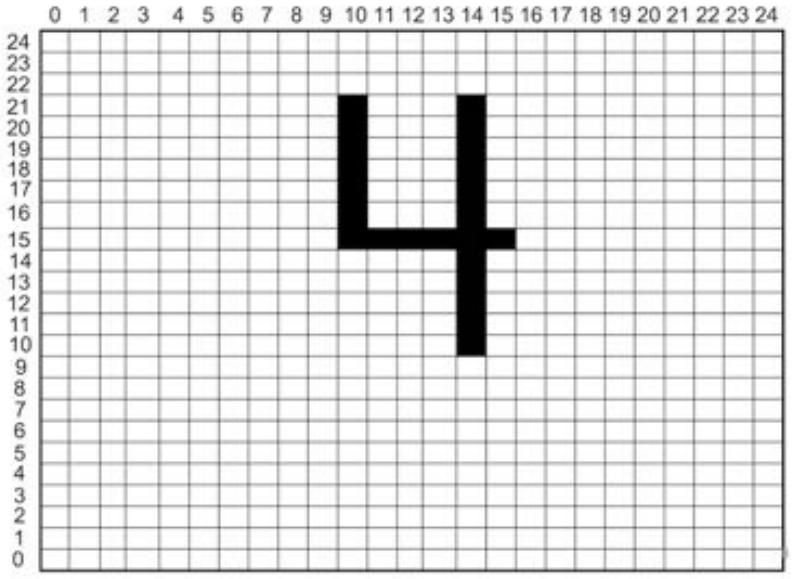
Illumina Red channel



Computer representation of images

- Consider the computer image to be a two dimensional array (matrix) of numbers.
- The smallest element of the image is a pixel.
- For an image with M x N pixels, each pixel has location (x, y).
- Each pixel has an intensity value $f(x, y)$, and the size of the pixel is $\Delta x * \Delta y$.

Computer representation of images



Computer representation of images

- For a monochromatic image, $f(x, y)$ is an integer called a *grayscale value* where $f = f(x, y) : x = 0, 1, \ldots, M - 1; y = 0, 1, \ldots, N - 1$
- Therefore, each $f(x, y)$ represents the brightness of a small picture element, called pixel, at location (x, y).
- The number of pixels contained in a digital image is called *resolution*

Computer representation of images

- Pixel intensity values are stored as binary numbers.
- Binary numbers are sequences of 0's and 1's.
- A binary digit (usually abbreviated 'bit') can hold one binary digit, a 0 or a 1.

Computer representation of images

- For a one-bit digital image, the computer uses one bit to represent a pixel value. The pixel is either 0 (black) or 1 (white).
- In a two-bit digital image, the computer uses two bits to represent a pixel value. The pixel may be 00 (black), 11 (white), or one of two shades of gray (10 or 01).

Computer representation of images

- A sequence of eight binary digits is called a byte.
- For example, using 8 bits, write the number 87.

Computer representation of images

- A sequence of eight binary digits is called a byte.
- For example, using 8 bits, write the number 87.

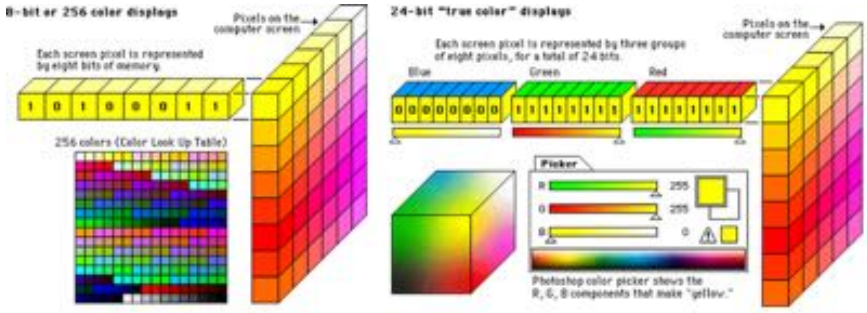
2^7=128	2^6=64	2^5=32	2^4=16	2^3=8	2^2=4	2^1=2	2^0=1
0	1	0	1	0	1	1	1

$$128 * 0 + 64 * 1 + 32 * 0 + 16 * 1 + 8 * 0 + 4 * 1 + 2 * 1 + 1 * 1$$

Computer representation of images

- Most images are stored using n-bits; there are 2^n possible binary sequences of length n.
- For example, an 8 bit (one byte) image has 2^8 possible values ranging from 0 to $2^8 - 1$; 0 (black), 255 (white), and 254 gray levels.
- The radix is 2 so each number is represented as linear combination of powers of 2.

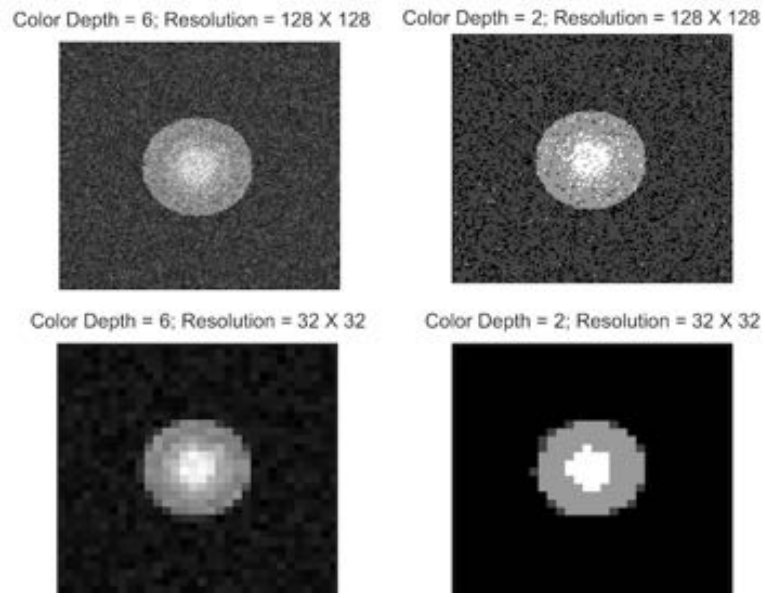
Pixel intensity (color depth)



What do we finally get

- *Digital image*: rectangular array of intensity values
- Each intensity value corresponds to a *pixel*
- *Color Depth*: is the number of bits used to store the intensity value of one pixel

Color depth of 16 bits/pixel (common for microarray scanners) means the intensity values of each pixel is an integer between 0 and $65,535 (= 2^{16} - 1)$



Steps in image analysis

- **Addressing**: locate the spots
- **Segmentation**: categorize each spot as foreground (signal), background, or other
- **Intensity extraction**: assign signal and background values to each spot
- **Spot quality assessment**: compute measures of spot quality for each spot

These steps use specialized software and can involve varying degrees of human intervention.

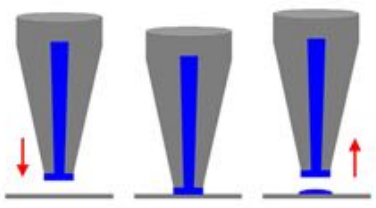
ImageJ

http://imagej.nih.gov/ij/

http://image.bio.methods.free.fr/ImageJ/?Protein-Array-Analyzer-for-ImageJ.html&artpage=5-6

Addressing: Custom Spotted Arrays

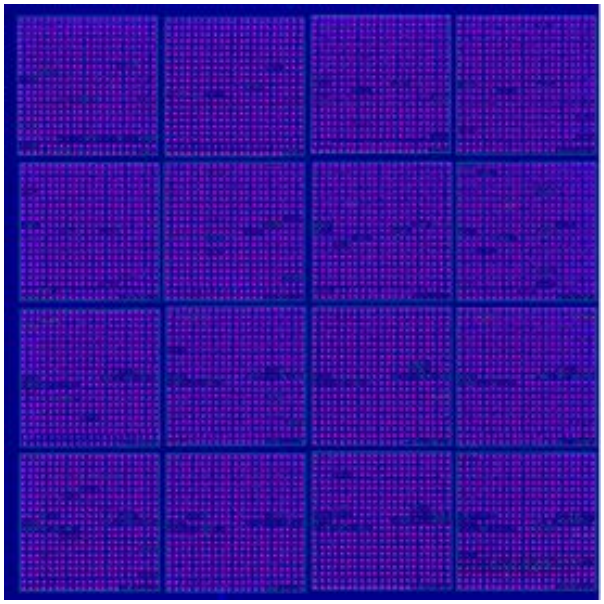
- Custom spotted arrays are manufactured by a robotic system that uses several print tips (pins, pinheads) to deposit the cDNA fragments on each of the spots.
- Typically each of the *n* print tip spots in a regular sized sub-grid, such that the entire microarray is composed of *n* matrices with the same number of rows and columns.
- Ideally, the spots are of the same size, the same shape and are equally spaced throughout the array.



Microarray Layout Parameters

- The ultimate goal of any image analysis technique should be the automation of the image analysis process.
- Although the layout of the cDNA array is known and can be used for addressing, the known model must be matched to the scanned image.
- Therefore, most software packages include both automatic and manual procedures for addressing.

Addressing



Microarray Layout Parameters

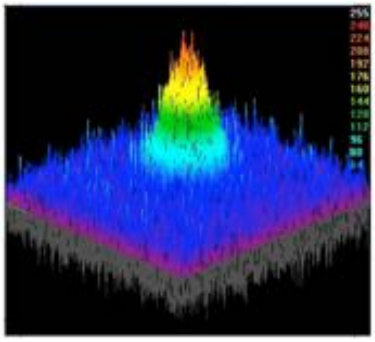
Microarray Layout Parameters	Value
Array Rows	4
Array Columns	4
Rows	21
Columns	21
Array Row Spacing	9000
Array Column Spacing	9000
Spot Row Spacing	425
Spot Column Spacing	425
Spot Diameter	300
Spots per Array	441
Total Spots	7056

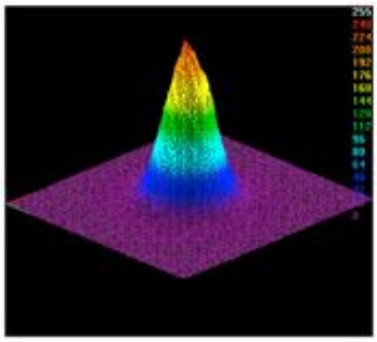
Segmentation

- Once the address of the spots has been identified, the pixels must be classified as signal versus background, a process called *segmentation*.
- Background represents a value of the measured signal intensity that is presumed to be due to non-specific binding of target to the probe
- Thought to be removed from the signal intensity measurement in order to accurately quantitate the amount of target RNA present in the sample.

Foreground vs. Background

Uneven hybridization, auto fluorescence, non-specific binding - measurements outsize the spot not at 0 intensity





Segmentation

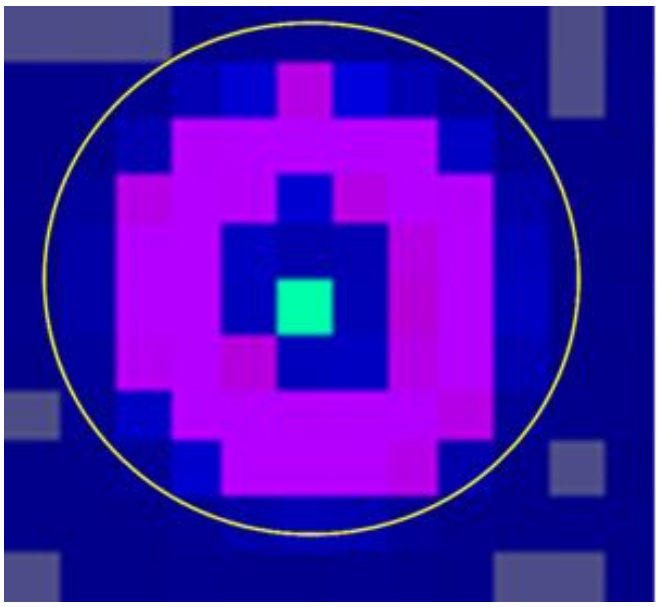
Spatial based segmentation:

- fixed circle
- adaptive circle
- adaptive shape

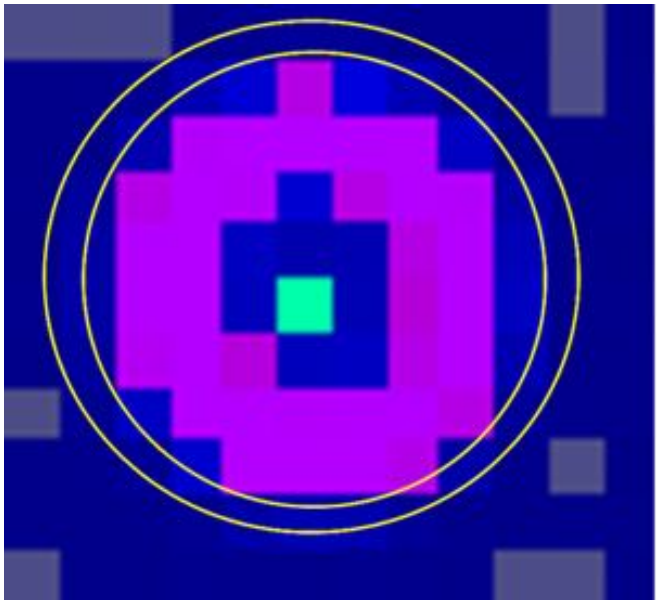
Intensity-based segmentation

- Ranked intensities
- Mann-Whitney method

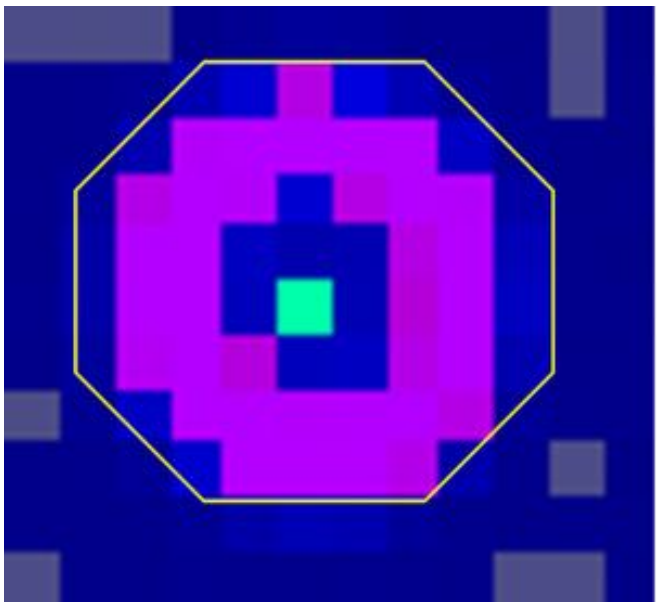
Segmentation



Segmentation



Segmentation



Ranked intensities

- Get intensities of all pixels in a rectangluar region
- Estimate the number of pixels in a circle fitted in this region
- Select this number of intensities from the ranked list of all intensities

Ranked intensities

- A rectangular region, $40x40$ pixels: a total of 1600 pixels
- Assume signal spot being 20 pixels in diameter: contains $\pi * 10^2 = 314$ pixels, or 20
- Select 20 top intensities from the ranked list of all 1600 intensities

Mann-Whitney method

- Chen et al (1997)

Chen Y, Dougherty ER, Bittner ML. "Ratio-based decisions and the quantitative analysis of cDNA microarray images." J Biomed Opt. 1997. PMID: 23014960

http://bcb.dfci.harvard.edu/~gp/teaching/688/chen1997.pdf

Issues with background subtraction

- Again, the purpose of segmentation is to partition pixels into one of two classes, foreground versus background.
- Most often, correcting for background takes on the form of subtracting the estimate for background from the estimate from signal.
- Subtracting background has been noted to increase the variability of genes, particularly at low levels of expression.

Intensity Extraction

Spot intensity: Some statistics representing intensities for all pixels in spot area; similarly for background intensity

- **Mean**: mean of pixel intensities
- **Median**: median of pixel intensities
- **Mode**: location of peak in histrogram of intensities
- **Area**: number of pixels
- **Total**: sum of pixel intensities

Still, no consensus what to use

Intensity Extraction

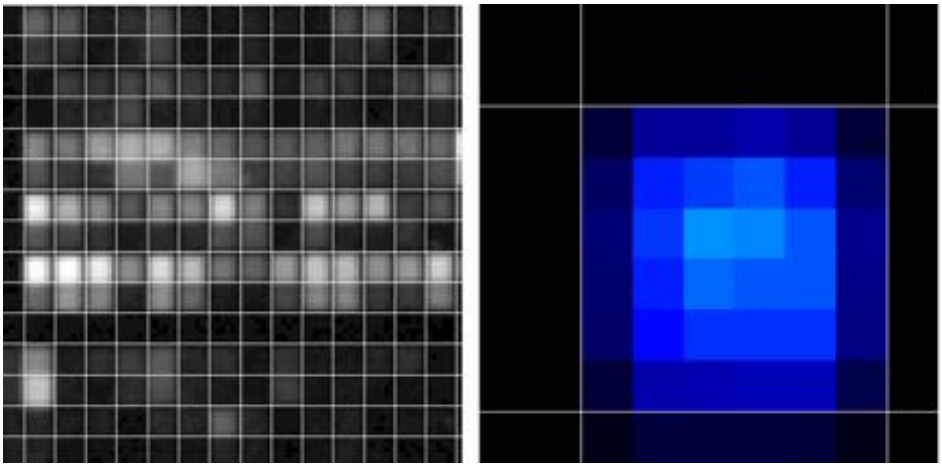
- The underlying principle that should be used to guide the selection of a method for data quantification is to select the statistical summary that best correlates with the amount of DNA target in the hybridized sample.
- **L-estimators** - linear combination of order statistics

Image processing for oligo arrays

- Affymetrix Genechips use propriety Affymetrix software
- Genechip surface covered with square shaped cells containing probes
- Probes are synthesized on the chip in precise locations
- Thus spot finding and image segmentation are not major issues

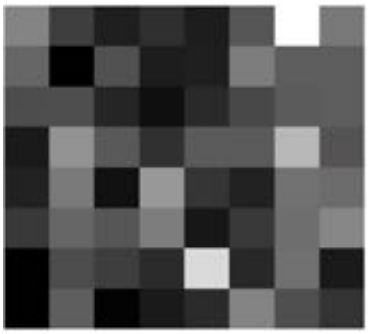
Image Analysis: Pixel Level Data

6 x 6 matrix of pixels for each PM and MM probe HG-U133A GeneChip



One Affymetrix probe

- $8x8 = 64$ pixels
- Border pixels excluded
- 75th percentile of the 36 pixel intensities corresponding to the center 36 pixels is used to quantify fluorescence intensity for each probe cell
- These values are called PM values for perfect-match probe cells and MM values for mismatch probe cells
- The PM and MM values are used to compute expression measures for each probe set



Intensities for one Affy PM cell

(X,Y)	Y=2433	Y=2434	Y=2435	Y=2436	Y=2437	Y=2438
X=2366	164	209	225	215	200	145
X=2365	294	438	511	562	432	238
X=2364	259	433	542	514	530	275
X=2363	374	597	595	621	672	358
X=2362	319	542	555	518	594	286
X=2361	267	372	369	356	378	190

Intensities for one Affy PM cell

(X,Y)	Y=2433	Y=2434	Y=2435	Y=2436	Y=2437	Y=2438
X=2366	**164**	**209**	**225**	**215**	**200**	**145**
X=2365	**294**	438	511	562	432	**238**
X=2364	**259**	433	542	514	530	**275**
X=2363	**374**	597	595	621	672	**358**
X=2362	**319**	542	555	518	594	**286**
X=2361	**267**	**372**	**369**	**356**	**378**	**190**