Expression Ratio

MBI401-High throughput Data Generation & analysis

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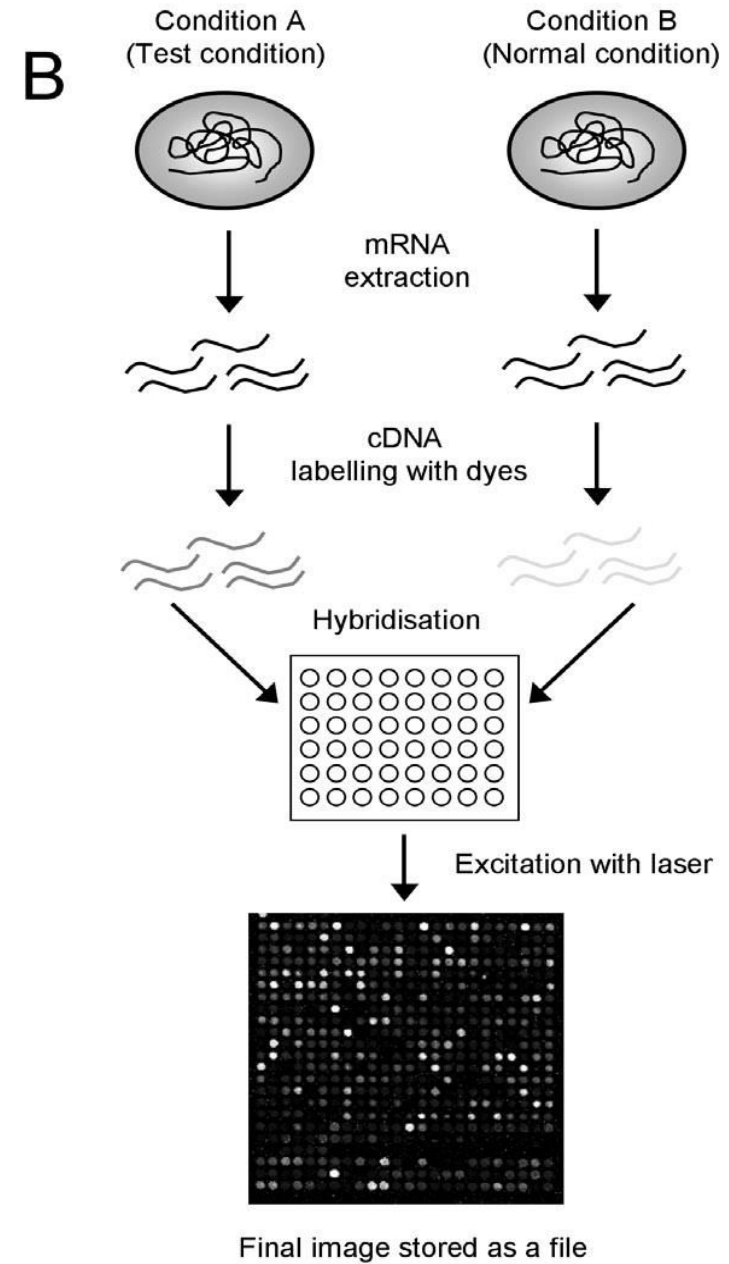
A

Cell

Genomic DNA

Each spot contains oligonucleotide sequence or genomic DNA that "uniquely" represents a gene

Spot (feature)

Pen-group (sub-array)

B

Condition A (Test condition)

Condition B (Normal condition)

mRNA extraction

cDNA labelling with dyes

Hybridisation

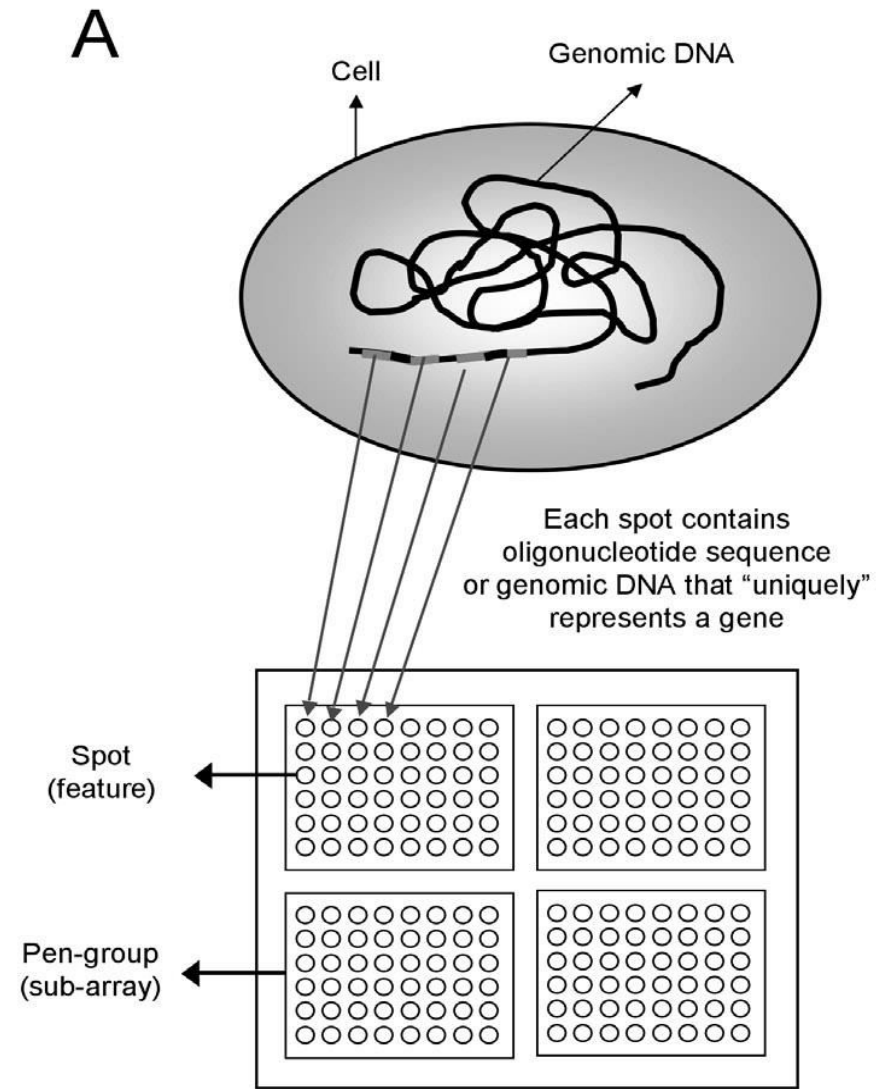Excitation with laser

Final image stored as a file

Figure 2. Zooming onto a spot on the microarray slide. The spot area and the background area are depicted by a blue circle and a white box, respectively. A pixel in the spot area is also shown. Any pixel within the blue circle will be treated as a signal from the spot. Pixels outside the blue circle but within the white box will be treated as a signal from the background. One can see that the images are not perfect, as it is often the case, which leads to many problems with spurious signals from dust particles, scratches, bright arrays, etc. This image was retrieved from Stanford Microarray Database. Colour figure at: http://www.mrc-lmb.cam.ac.uk/genomes/madanm/microarray/.
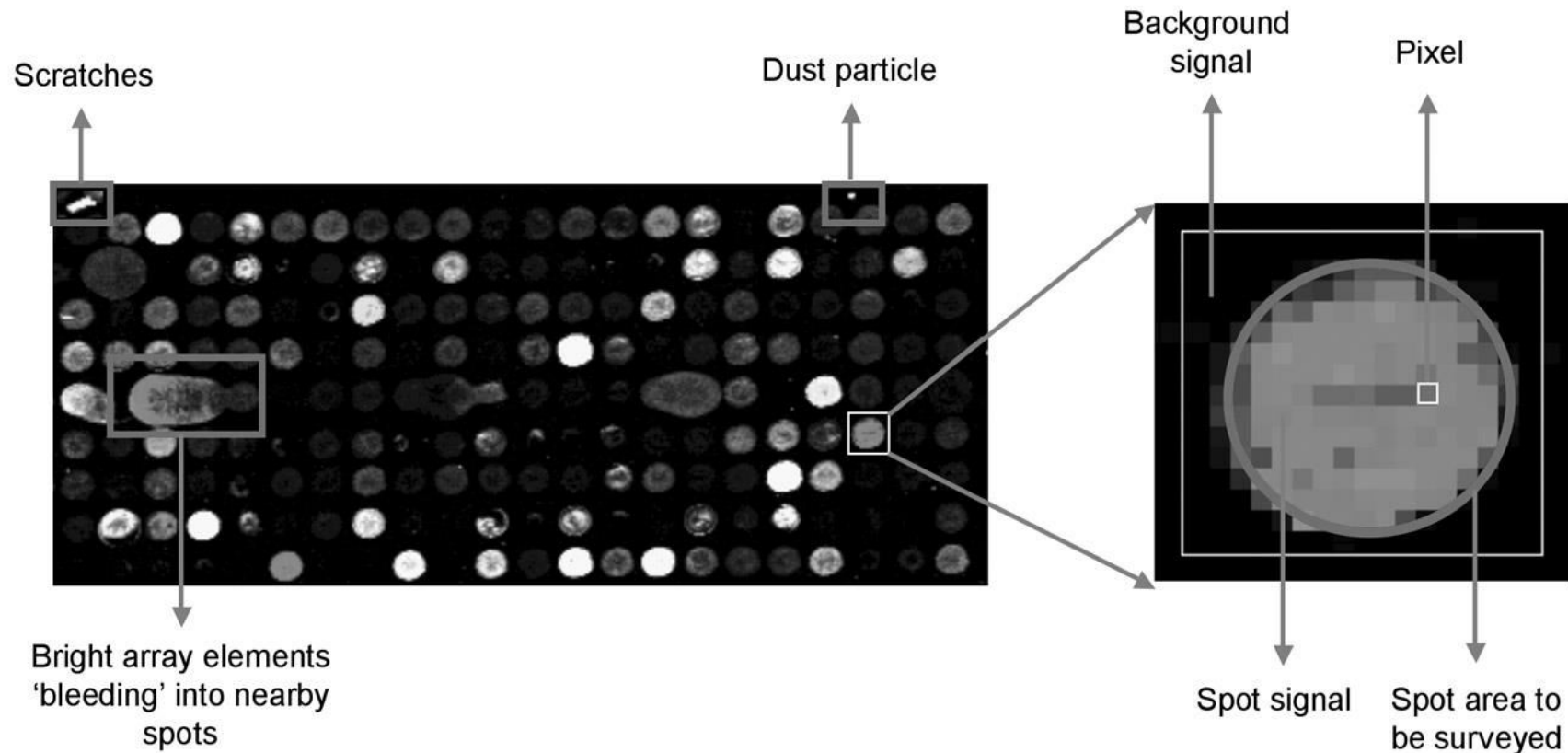
- If the gene was expressed to the same extent in both conditions, one would find the spot to be yellow, and if the gene was not expressed in both conditions, the spot would be black.

- Thus, what is seen at the end of the experimental stage is an image of the microarray, in which each spot that corresponds to a gene has an associated fluorescence value representing the relative expression level of that gene.

2.2 Expression ratios: the primary comparison

- We saw that the relative expression level for a gene can be measured as the amount of red or green light emitted after excitation. The most common metric used to relate this information is called expression ratio. It is denoted here as *Tk and defined as:*

$$T_k = \frac{R_k}{G_k}$$

- For each gene *k on the array, where Rk represents the spot intensity metric for the test* sample and *Gk represents the spot intensity metric for the reference sample. As mentioned* above, the spot intensity metric for each gene can be represented as a total intensity value or a background subtracted median value. If we choose the median pixel value, then the median expression ratio for a given spot is:

$$T_{median} = \frac{R_{median}^{spot} - R_{median}^{background}}{G_{median}^{spot} - G_{median}^{background}}$$

- where R_{median}^{spot} *and* $R_{median}^{background}$

  are the median intensity values for the spot and background respectively, for the test sample.

References

Lecture was prepared using following study material

- Roger Bumgarner, DNA microarrays: Types, Applications and their future Curr Protoc Mol Biol. 2013 January ; 0 22: Unit–22.1.. doi:10.1002/0471142727.mb2201s101.

- Madan Babu, M., 2015. *An Introduction to Microarray Data Analysis*. [online] Mrc-lmb.cam.ac.uk. Available at: <http://www.mrc-lmb.cam.ac.uk/genomes/madanm/microarray/> [Accessed 3 December 2015].