Brief Technical Note

UHN Microarray Centre

Definition of a high quality microarray experiment (scanned array image)

- The UHNMAC has a general set of criteria for scanned array images that constitutes a high quality microarray experiment.
- The set of criteria is used during Quality Control Testing and as part of the Expression Services.

Background

Experienced microarray users often just "eyeball" a microarray image in order to determine its quality. However this method is highly subjective and as such, what constitutes a high quality microarray array can vary greatly, depending on whom you ask. To ensure that a consistent definition is used among a group of users (core facility with many technicians, for example), it is useful to have some quantitative criteria. Although there can be exceptions, this set of criteria serves as a general definition of a high quality microarray image.

For criteria such as normalisation value and saturated/flagged spots, the actual values differ depending on the labelled RNA samples and which scanner was used to acquire the image. For example, if one set of cells is treated with a compound that severely alters RNA expression and its RNA is compared with a set of cells that was not treated, one should expect a large (or larger-than-average) normalisation value. Since some scanners have higher sensitivity than others, the background values (and hence the number of flagged spots) for the same slide will differ depending on which scanner is used to acquire the image. We have provided criteria using the Perkin Elmer (PE) ScanArray 4000 (manual scanning) and the Agilent DNA Microarray scanner, two commonly used scanners at the UHNMAC. One of the major differences between the scanners is that the Agilent scanner has a dynamic autofocus that enhances sensitivity, and precision optics with minimal spectral cross talk that allows users to detect weak features. As a result, the signal (and background) intensities for images scanned on the Agilent scanner are generally lower than if scanned on the PE scanner but with greatly reduced noise. Other criteria, such as spot morphology, spot size, and high-localised background are the same regardless of the scanner used to acquire the image.

UHNMAC general criteria that defines a "good image"

Spot Morphology and Spot Size

Contrary to many peoples' instincts, "doughnut" spots (spots with a high intensity outer ring around a less intense centre) are acceptable particularly when using a ratiometric measurement system (ie Cy3 vs Cy5 intensity on the same array). The intensity ratio will likely be unaffected by such printing artifacts as both Cy5-labelled cDNA and Cy3-labelled cDNA are hybridising to the same spot. Most of the latest versions of image quantitation software are able to segment the pixels in such spots effectively removing the doughnut hole that further prevents this morphology from interfering with analysis.

"Comet tails" and merged (dumb-bell) spots are unacceptable, as the spot data cannot be used. There should be less than 0.5% of spots with merged or "comet tail" spots.

With any physical deposition technology such as that used by the UHNMAC, the size of the spots will vary somewhat. For the most part small variances in spot size are acceptable as long as the spots are at not touching each other. Array quantitation software packages like ArrayVision (Imaging Research) allow users to specify the range of spot sizes that are acceptable. The UHNMAC flags spots that are not between 80% and 120% of the theoretical size (the nominal size of the spots on the array is 100 µm in diameter).

High-localised background

There should be no localised background that covers more than 0.5% of spots.

Scatterplot

When performing QC, the UHNMAC visually inspects the scatterplot generated using normalised data in GeneTraffic® (Stratagene). A high quality array in which both channels use the same RNA will have approximate 1:1 normality between the channels for the majority of spots. The amount of scatter from the 1:1 line and the distribution of data points within the linear range of signal intensities will give a qualitative estimate of the image quality.

Normalisation Value

For images acquired by the PE ScanArray 4000 scanner
After lowess (sub-grid) normalisation, the normalisation factor should be between 0.6 and 1.4. The normalisation factor can be used to assess whether the channels were scanned at appropriate scanner settings. An aberrantly high or low normalisation value can be a sign of a significant channel based bias (most commonly due to fluorophore signal inequality).

For images acquired by the Agilent DNA Microarray scanner
After lowess (sub-grid) normalisation, the normalisation factor should be between 0.5 and 4.0. Through experience with this scanner, it has been found that a broader range of normalisation values is acceptable due to the extremely low inherent noise.

Saturated/Low intensity Spots

An unusually high number of saturated or low intensity spots can indicate that the array

was scanned at inappropriate laser power and/or PMT settings. The recommendations below are based on the majority of microarray experiments performed at the UHNMAC.

For images acquired by the PE ScanArray 4000 scanner
For saturated spots, a maximum of 2% is accepted. For low intensity spots (generally less than twice the background intensity), a maximum of 2% is also accepted.

For images acquired by the Agilent DNA Microarray scanner
Since the Agilent scanner uses a dynamic autofocus that enhances sensitivity and intensity range, the number of low intensity spots will increase and the number of saturated spots decreases, compared to the PE ScanArray 4000 scanner. Similar to other scanners, a maximum of 2% for low intensity spots (less than twice the background intensity) is accepted. It is rare to have any saturated spots using the Agilent DNA microarray scanner, so a maximum of 0.25% is accepted.