

# Comparison of data generated from Packard ScanArray 5000, ScanArray 4000 and Agilent microarray slide scanners.

The PMCI microarray facility presently offers users a choice of scanning their array using either a Packard or Agilent microarray scanner. A major difference between these two methods of array scanning is the method of focusing on the array surface. The Agilent method is a dynamic focusing method where the scanner adjusts the focus of the lasers for each individual array feature. The ScanArray scanners use a single point method whereby the focus is maintained across the surface of each slide scanned.

Details and specifications for these can be found in the CCGPM Bioinformatics area and also at: Agilent: <http://www.ccgpm.org/microarray/protocols/agilent.pdf>, ScanArray 5000:



## Dataset for comparison of scanners

A dataset of human 10.5k cDNA arrays hybridised with RNA extracted from a bank of ovarian cancer (OvCa) specimens was used for this analysis. Reference RNA was either pooled OvCa or a pool of RNA from 11 cell lines. 70 arrays were scanned on the Agilent machine, 29 on the ScanArray 4000 and 47 on the ScanArray 5000.

It is important to note that no normalisation or other statistical manipulation (other than log transformation) was carried out on data used in this comparison to avoid adding further sources of variation.

## Correlation of data between scanners

A practical question for array users is whether data generated from different scanners can be used in the same experiment. To investigate this the Pearson correlation co-efficient was calculated for all arrays scanned on >1 scanner.

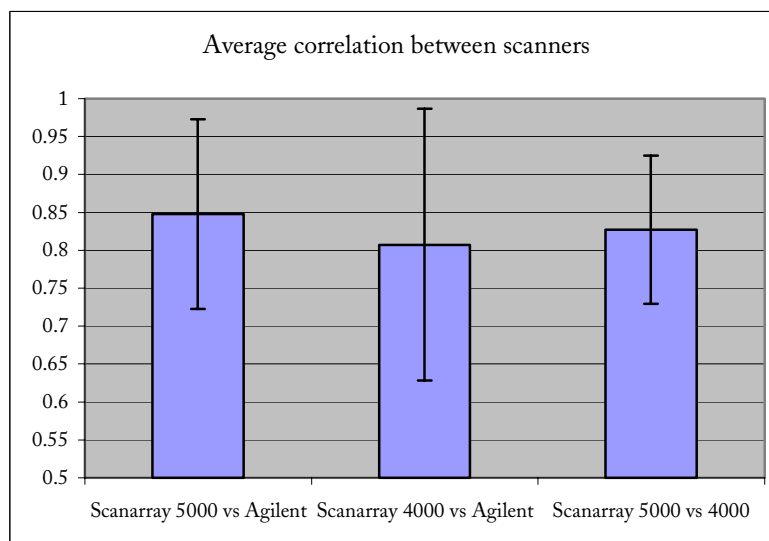


Figure 1: Mean Pearson correlation co-efficient between array scanners

The overall correlation revealed the ScanArray 5000 and Agilent had the highest mean correlation coefficient, however a large variance was also noted (see Figure 1). The ScanArray 4000 and 5000 had the next highest overall correlation and had a smaller range of coefficients than the Agilent and 5000. The poorest correlation and widest range of values was between the ScanArray 4000 and the Agilent. The upper range of the variances noted in the mean correlation scores indicates that different microarray scanners can generate highly similar data. However not at a consistent level that would be required to use data from different machines in the one study.

## Reproducibility of Scorecard QC genes across multiple arrays

An important assumption of array analysis is the consistency of expression values obtained from identical RNA samples hybridized to the same array features.

Data for QC genes on a series of arrays was extracted and analysed using a general linear model to investigate whether significant variation exists between measurements of these features using different scanners.

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General Linear Model: Log2Ratio versus Gene, Scanner

Factor      Type Levels Values
Gene       fixed   11 1DR 1RC 2DR 2HG 2RC 3DR 3RC 4DR 4RC 5DR 6DR
Scanner    fixed     3 Agilent SA4000 SA5000

Analysis of Variance for Log2Rati, using Adjusted SS for Tests
Source      DF      Seq SS      Adj SS      Adj MS      F      P
Gene        10     33678.5     33678.5     3367.9     7672.73  0.000
Scanner     2       2597.5     2597.5     1298.7     2958.81  0.000
Error      ****     6135.9     6135.9         0.4
Total      ****     42411.9

Tukey Simultaneous Tests
Response Variable Log2Rati
All Pairwise Comparisons among Levels of Scanner

Scanner = Agilent subtracted from:
Level      Difference      SE of      Adjusted
Scanner    of Means      Difference    T-Value    P-Value
SA4000     0.8440         0.02088      40.42      0.0000
SA5000     0.8667         0.01172      73.97      0.0000

Scanner = SA4000 subtracted from:
Level      Difference      SE of      Adjusted
Scanner    of Means      Difference    T-Value    P-Value
SA5000     0.02265        0.02098      1.079      0.5269

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Log2Rati
All Pairwise Comparisons among Levels of Gene

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Figure 2: Output from general linear model testing of array QC genes

As shown in figure 2 the measurements of identical QC genes varied significantly ( $P < 0.001$ ) between the Agilent and both ScanArray models. No significant variation in QC gene expression values was detected between the ScanArray 4000 and 5000 machines.

## Irregular spatial distribution of expression data

Expression measurements can sometimes be closely correlated with the X,Y co-ordinates of an array. A method for quantifying this effect is Moods Median test (MMT), which describes the difference between the median expression values for each array subgrid. Arrays with high MMT chi-square scores tend to be those with obvious spatial irregularities, for example the majority of genes in one corner of the slide being highly up regulated and those in the opposite corner being down regulated.

From practical experience it has been observed that 10.5k human arrays with a MMT score over 200 have an irregular spatial distribution that can be observed by visual inspection of a false-array image or overlay created from scanned TIFF images.

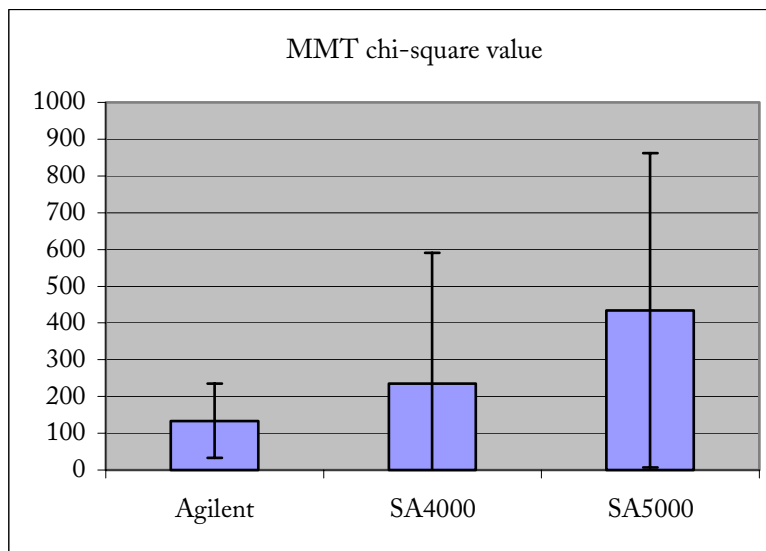


Figure 3: Mean MMT chi-square values for arrays scanned with three different scanners.

MMT scores were calculated for each array in the comparison. Data was grouped into scanner groups and the mean MMT score calculated, as shown in Figure 3. It can be seen that the Agilent scanner has the lowest mean MMT value and also the smallest standard deviation of the three scanners being investigated. Both the ScanArray 4000 and 5000 appear to produce array data with higher and more variable MMT scores, potentially reflecting a wider range and more severe irregularities in the spatial distribution of expression data.

### Sensitivity of array measurements

Another important assumption of microarray work is the reliable measurement of differentially expressed genes. The mean expression ratio of four ratio-control genes was calculated for arrays grouped by scanner model. The results are shown in Figure 4 and indicate that the Agilent scanner produced readings for these array features closer to their theoretical value than either of the ScanArray systems.

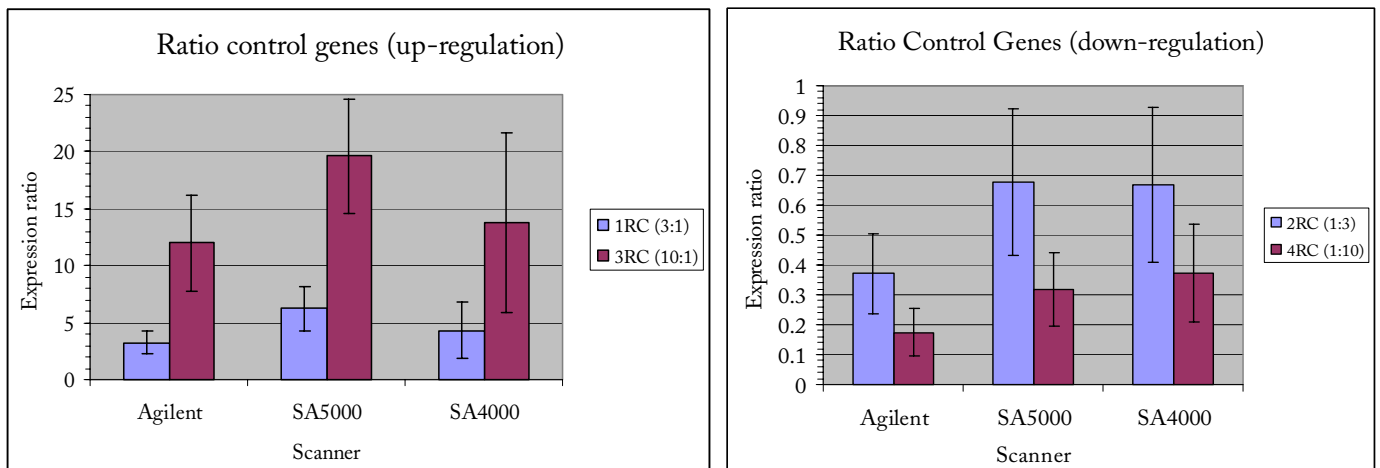


Figure 4: Ratio control genes across different scanners

In all cases the Agilent model had a smaller range of measurements for these QC ratio control genes and in all four cases this interval included the exact theoretical value for the specific array element.

### Measurement of background or non-specific hybridisation

All scanners record a spot intensity and background reading for each microarray feature. Accurate measurement of background hybridisation is crucial for downstream analysis as the background measurement is subtracted from the spot intensity value to calculate the intensity value due to specific probe hybridisation.

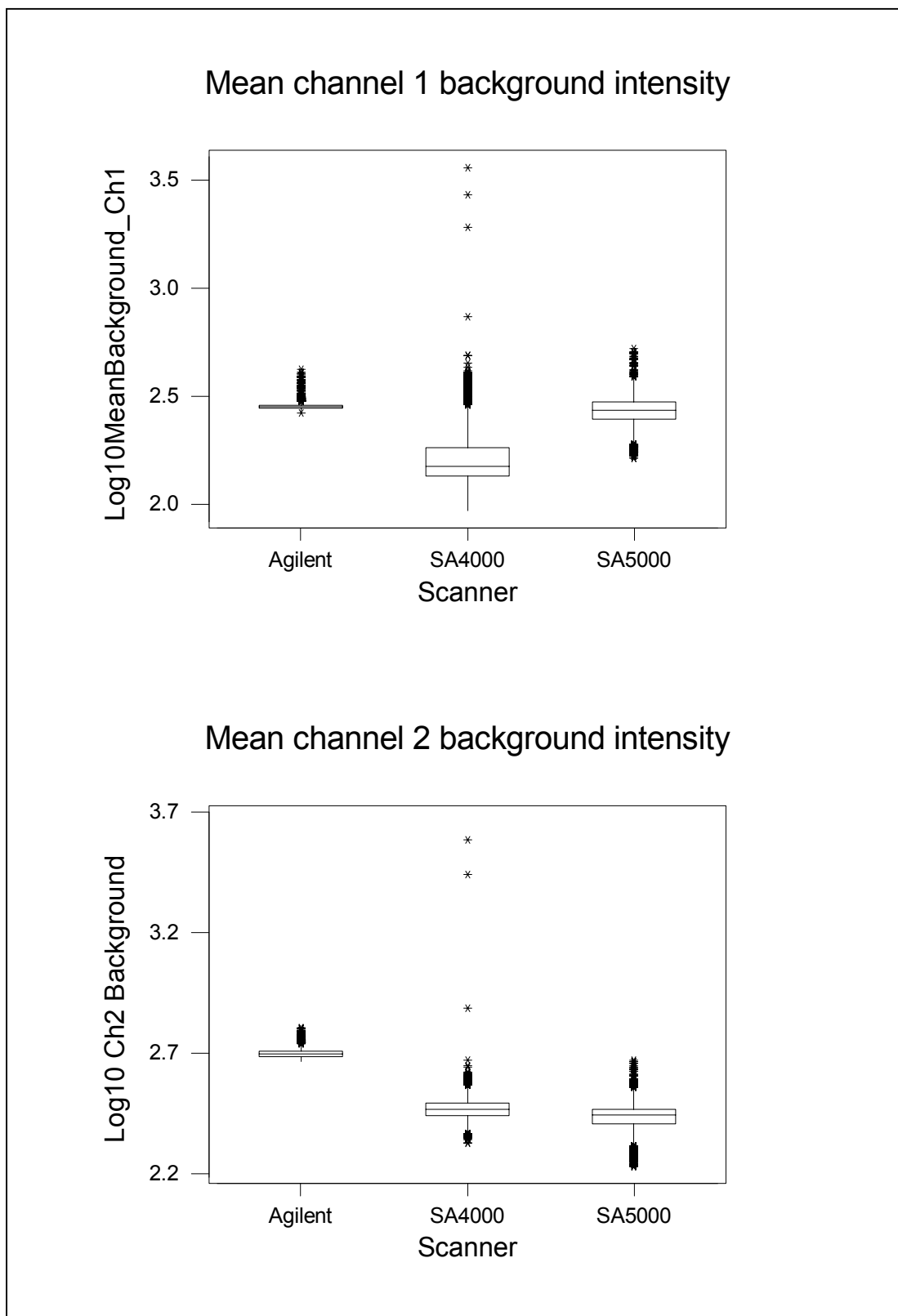


Figure 5: Box plots of mean log-transformed background measurements.  
Channel 1 = cy5, Channel 2 – Cy3

From comparing the mean background intensity values recorded by each of the three scanners across the entire dataset it can be seen that the Agilent machine has the least variation in its measurement of background hybridisation. In the Cy5 channel the ScanArray models appear to differ substantially in range of background intensity values whereas they are quite similar in the Cy3 channel.

## Dynamic range of scanner – measuring genes at varying concentrations

Accurate measurement of genes ratios across a wide spectrum of RNA concentrations is important for validity of results. Dynamic range genes (shown in Table 1) can be used to assess how accurately the microarray platform being used is measuring genes of varying abundances.

Table 1: Details of Scorecard Dynamic Range genes

Sample	Cy3:cy5 ratio	Cy3 Conc. (pg.5uL)	Cy5 Conc. (pg.5uL)	Expected expression value	Relative abundance
1DR	1:1	33 0000	33 000	1.0	3.3%
2DR	1:1	10 000	10 000	1.0	1%
3DR	1:1	1 000	1 000	1.0	0.1%
4DR	1:1	330	330	1.0	0.033%
5DR	1:1	100	100	1.0	0.01%
6DR	1:1	33	33	1.0	0.0033%

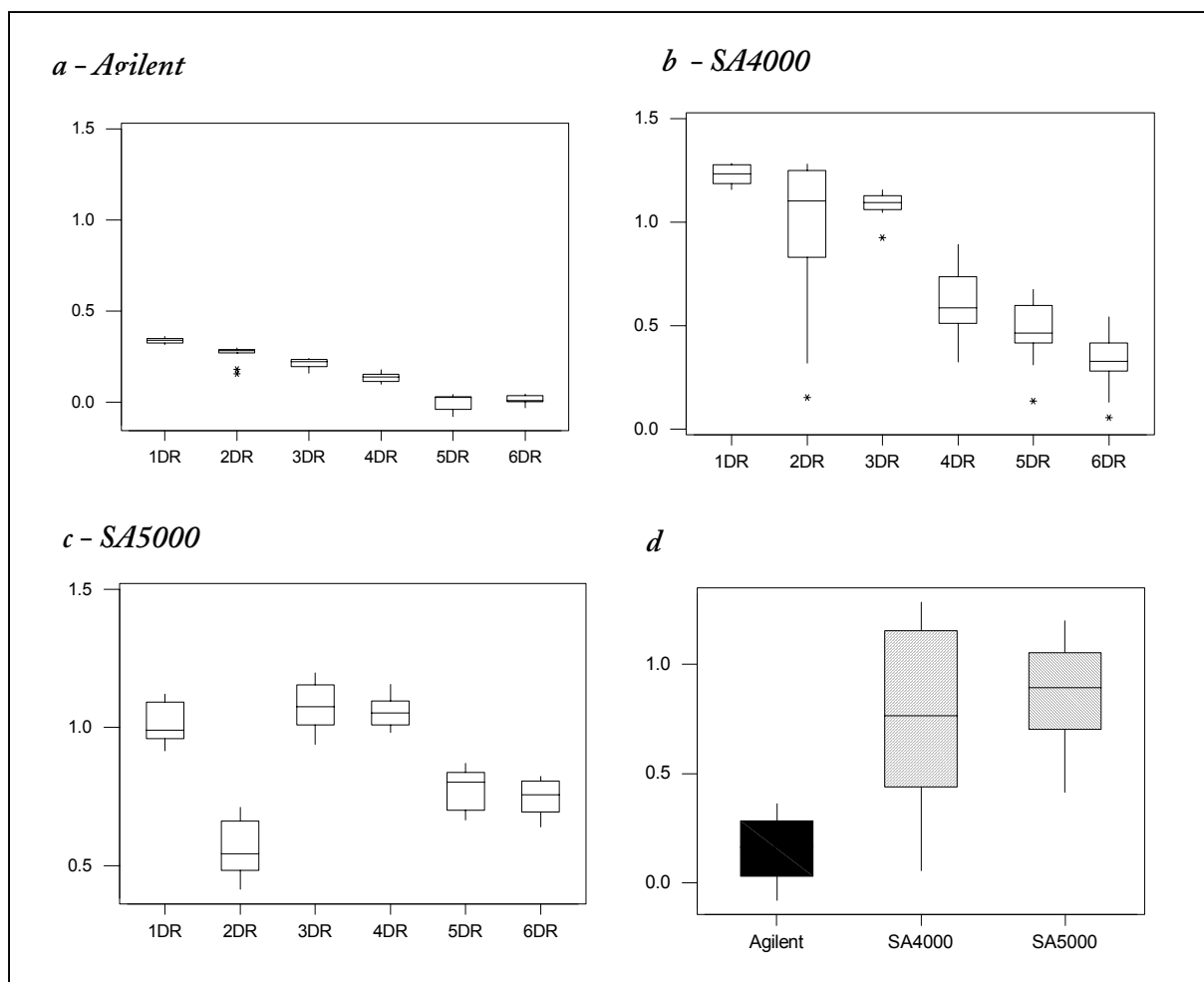


Figure 6: Comparison of dynamic range readings between scanner models

Comparison of array features hybridised with RNA at different concentrations revealed substantial differences between scanners (Figure 6). The Agilent machine exhibited the lowest variation in the measurements of these features. All scanners appear to measure RNA levels more accurately at lower relative abundances (Figure 6a-c). The Agilent scanner also produces readings closer to the expected ratio value (0 ie.  $\log_2(1)$ ).

## Summary

- Array experiments should be conducted using one scanner only.
- The ScanArray 4000 and 5000 models are more similar to each other than to the Agilent scanner, however significant differences still appear in data generated from by the ScanArray machines.
- The Agilent microarray scanner is superior to the ScanArray machines in terms of reproducibility and accuracy of results obtained.