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Technical Note

Performance Evaluation of the GeneChip® Fluidics Station 450

Designed for use with the new GeneChip® Scanner 3000, the new GeneChip Fluidics Station 450 (FS 450) has been developed as the next-generation model to follow the GeneChip Fluidics Station 400 (FS 400). The FS 450 incorporates walk away freedom and modular design to provide increased efficiency and improved ease of use. For customers who wish to upgrade to the FS 450 from the FS 400, Affymetrix provides an effortless upgrade path, which involves minor modifications to the FS 400 base, replacement of the FS 400 modules, a download of FS 450 scripts onto the workstation, and a final field check.

This Technical Note describes controlled-comparison gene expression experiments between the FS 400 and FS 450 to determine whether data generated on the FS 450 is consistent with data generated on the FS 400. The results of these tests conclusively demonstrate equivalent performances in array washing and staining between both models.

Introduction

The continual evolution of Affymetrix' manufacturing processes has enabled breakthrough innovations in array technology, including greater consistency and higher overall signal intensity values. In order to leverage these advantages, Affymetrix devoted equal efforts towards evolving its state-of-the-art instrumentation, making the GeneChip® Instrument System the most advanced microarray analysis platform available. Used in conjunction with GeneChip brand microarrays, reagents, software, and the NetAffx™ Analysis Center, the GeneChip Instrument System reduces the time and cost of discovery research.

Designed for use with the GeneChip Scanner 3000, the GeneChip Fluidics Station 450 (FS 450) has been developed as the next-generation model. Its walk away freedom and modular design provide increased efficiency and improved ease-of-use. After loading the GeneChip arrays and all tubes required by the selected script, the FS 450 runs unattended until completion, freeing the operator to attend to other responsibilities, thereby helping to improve workflow in the laboratory. Further insight into design improvements, including enhanced fluid detection and cartridge loading, is provided in the *Affymetrix GeneChip® Fluidics Station 450 Data Sheet* and the *Fluidics Station 450 Design Improvements Product Bulletin*.

Affymetrix provides an easy upgrade path for existing GeneChip Fluidics Station 400 (FS 400) users to adopt the FS 450. The upgrade process involves minor modifications to the FS 400 base, replacement of FS 400 modules with FS 450 modules, a download of FS 450 scripts onto the workstation, and a final field check. All work is completed on-site by Affymetrix personnel with minimal disruption to the lab.

To ensure the smooth transition from the FS 400 to the FS 450, it is critical that data generated using these instruments are comparable. This Technical Note details the results of controlled-comparison experiments between the FS 400 and the new FS 450. The primary experiment describes multiple comparisons of FS 400 models with FS 450 models. All comparisons involved the processing of GeneChip gene expression arrays. The results of these tests conclusively demonstrated equivalent performances in array washing and staining between both models. The following data strongly suggest a seamless transition from the FS 400 to the new FS 450.

Experiments comparing the different stain-handling conditions showed equivalent performances between the FS 400 and FS 450. These results provide support for the walk away operation of the FS 450.

Experimental Design – Comparison of Multiple Systems

OVERVIEW

The new FS 450 was developed to perform to equivalent specifications as the FS 400. To evaluate the new FS 450, internal experiments were conducted to compare its performance to that of the FS 400. The

goals of these experiments were to:

- identify differences in global GeneChip microarray metrics, such as percent Present calls, False Change between replicates, and correlation coefficients from Signal values.
- identify differences in the Detection call accuracy of exogenous spiked transcripts in a complex cRNA target background.

In all experiments, the arrays were processed in a controlled manner such that any differences observed were due exclusively to the fluidics stations. All scans were performed on the same scanner using Affymetrix® Microarray Suite 5.0 (MAS) software. All analyses were performed using MAS software version 5.1.

Table 1. The Beta Test Plan compared the performance of three pairs of fluidics stations. Each pair consisted of one FS 450 and one FS 400. For each of the three comparisons, 24 HG-U133A probe arrays were hybridized with the complex cRNA target containing 53 spiked transcripts at four concentrations: 0.00 pM, 0.75 pM, 1.50 pM, and 3.00 pM. Twelve arrays were processed on the FS 400 and twelve arrays were processed on the FS 450.

Beta Test Experimental Design

Fluidics Station 450

Run #	Module 1	Module 2	Module 3	Module 4
1	0.00 pM	0.75 pM	1.50 pM	3.00 pM
2	3.00 pM	0.00 pM	0.75 pM	1.50 pM
3	1.50 pM	3.00 pM	0.00 pM	0.75 pM

Fluidics Station 400

Run #	Module 1	Module 2	Module 3	Module 4
1	0.00 pM	0.75 pM	1.50 pM	3.00 pM
2	3.00 pM	0.00 pM	0.75 pM	1.50 pM
3	1.50 pM	3.00 pM	0.00 pM	0.75 pM

BETA TEST PLAN

The FS 450 Beta Test Plan compared the performance of three pairs of fluidics stations. Each pair consisted of one Beta FS 450 and one FS 400. Affymetrix GeneChip Human Genome U133A microarrays (HG-U133A) were used in each experiment. Standard preventive maintenance was performed on all comparison FS 400 units prior to the test, ensuring all units were within specifications.

Complex cRNA targets (labeled and fragmented cRNA ready for hybridization) were prepared from HeLa cell total RNA following procedures outlined in the *GeneChip® Expression Analysis Technical Manual*.

For each of the three comparison experiments, 24 HG-U133A probe arrays were hybridized with the complex cRNA target, containing 53 spiked transcripts at four concentrations: 0.00 picoMolar (pM), 0.75 pM, 1.50 pM, and 3.00 pM. For each paired comparison, twelve arrays were processed using the FS 450 and 12 arrays were processed on the FS 400. All fluidics processing for paired comparisons was completed in the same day and in the same lab. Assay performance of the FS 450 was evaluated, in part, on the Detection call accuracy of cRNA transcripts spiked into the target sample. These 53 transcripts were selected on the basis of their apparent lack of expression in the source RNA, as determined by their Absent calls on HG-U133A arrays. The spiked transcripts were generated by *in vitro* transcription from vectors containing the cloned DNA sequence. Labeled cRNA transcripts were then quantified, fragmented, and spiked back into labeled, fragmented, complex background cRNA. All 53 transcripts were spiked in at one of three concentrations: 0.75 pM, 1.50 pM, or 3.00 pM. A target sample created from background cRNA without spiked transcripts (0.00 pM) was also assayed.

Large pools of complex sample containing the 53 transcripts at each of the four concentrations were created and split among the probe arrays; thus, identical samples were run on both the FS 450 and the FS 400.

A single lot of HG-U133A arrays were used for all testing. Samples were run once on each array. Twenty-four arrays were processed on each pair of fluidics stations, with twelve arrays on the FS 400 and twelve arrays on the FS 450. Arrays hybridized with different target sample concentrations were distributed throughout the four modules of the fluidics stations (see Table 1). The placement of these arrays removed the possibility of module bias from the experiment. All arrays were washed and stained according to procedures outlined in the *GeneChip® Expression Analysis Technical Manual* and scanned on a single GeneArray® 2500 Scanner.

PERFORMANCE DATA & ANALYSIS

In these experiments, the performance of each fluidics station was assessed on the basis of both single array and comparison analyses generated within the MAS 5.1 software. Analysis output files (.chp files) were created at Affymetrix by globally scaling all probe sets on image data files (.dat files) to a target intensity of 250. Data sets were examined to assess overall Signal correlation, global percent Present calls, False Change between replicates, and Detection call accuracy of exogenous spiked transcripts.

Data from each pair of fluidics stations were tested for statistical significance, where appropriate, by applying paired Student's *t*-tests. The null hypothesis was defined as follows: The FS 450 performance is significantly better than that of the FS 400 at a $p>0.95$ confidence level. Thus, *p*-values greater than or equal to 0.95 were interpreted as supporting the null hypothesis (indicating significantly better performance for the FS 450), *p*-values approaching 0.5 were interpreted as equivalent fluidics station performance, and *p*-values less than or equal to 0.05 were interpreted as contradicting the null hypothesis (indicating significantly better performance for the FS 400).

Results & Discussion

RESULTS SUMMARY

Array data comparing the GeneChip® Fluidics Station 450 (FS 450) to the Fluidics Station 400 (FS 400) from beta testing indicated that there is ***no significant performance difference between the two fluidics station models.*** Both the FS 400 and the FS 450 produced arrays yielding high-quality data for Affymetrix expression analysis. ***Array data from all three pairs of fluidics stations yielded statistical equivalence in most cases. Student's t-test p-values obtained for all metrics analyzed tended to distribute at about 0.7, indicating statistical equivalence.***

SIGNAL CORRELATION BETWEEN FLUIDICS TYPES

For all three pairs of fluidics stations in this experiment, the Signal values of probe sets on arrays processed by the FS 450 were highly correlated with those of replicate arrays processed by the FS 400. For this analysis, a data set from a hybridized target sample was selected as representative of all GeneChip Human Genome U133A (HG-U133A) probe arrays evaluated in this experiment. Signal values for all probe sets from this data set were directly compared to the Signal values from the same probe sets in the corresponding data set from each fluidics station in the experiment. Correlation coefficients were calculated between all six fluidics stations using Signal values from all 22,283 probe sets present on the HG-U133A probe array. Table 2 shows the correlation coefficients for all pair-wise comparisons of FS 400 and FS 450. It should be noted that there is a high degree of similarity of Signal values among all nine samples in addition to the similarity between replicates.

Table 2. Correlation coefficients calculated for all pair-wise comparisons of fluidics stations used in the experiment are summarized in Table 2. The high degree of similarity of Signal values between the FS 400 and the FS 450 is indicated by correlation coefficients approaching 1.0. Correlation coefficients for other Signal comparisons (all possible pair-wise comparisons of the three FS 400 units and all possible pair-wise comparisons of the three FS 450 units) were also calculated and yielded similarly high values (data not shown). Values listed represent comparisons of Signal values for all probe sets on the HG-U133A Array.

Correlation Coefficients of Signal Values between FS 400 and FS 450 Using GeneChip® Human Genome U133A Arrays.

HG-U133A	Beta FS 450 Unit 1	Beta FS 450 Unit 2	Beta FS 450 Unit 3
FS 400 Unit 1	0.9948	0.9708	0.9707
FS 400 Unit 2	0.9744	0.9892	0.9790
FS 400 Unit 3	0.9742	0.9852	0.9903

PERCENT PRESENT COMPARISONS

The percent Present metric is often a useful measure of assay and instrument performance. It represents the total number of transcripts detected (called Present) out of the total number of probe sets on an array.

Figure 1 illustrates the average percent Present for twelve HG-U133A arrays processed on each FS 400 and FS 450 in the experiment. Error bars represent one standard deviation. In two of three comparisons, the average percent Present of

Figure 1. Comparison of average percent Present between FS 400 and FS 450. Percent Present values represent the total number of probe sets called Present in Affymetrix® Microarray Suite analysis software out of the total number of probe sets on the GeneChip® Human Genome U133A Array. Each histogram represents the average percent Present for 12 target samples. Error bars represent one standard deviation. Based on Student's *t*-tests, instances where FS 450 performance significantly exceeded FS 400 performance are indicated by (*). All other instances were statistically equivalent.

Percent Present Comparisons All Genes

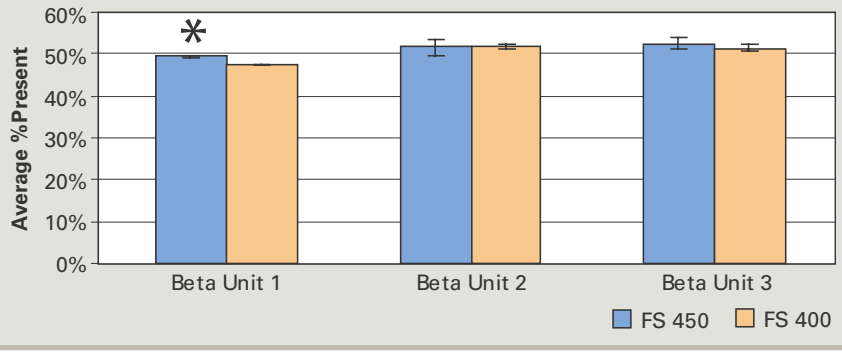
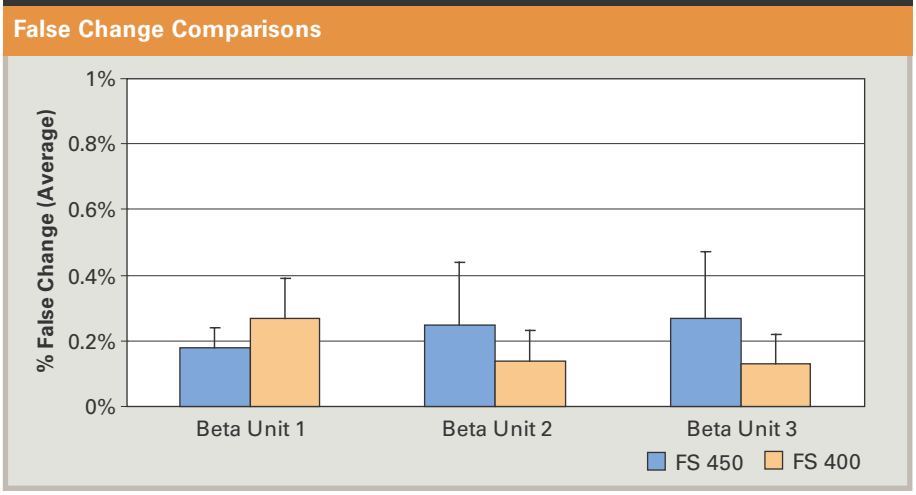


Figure 2. Comparison of average percent False Change between the FS 400 and the FS 450. False Change is described by the frequency of Increase calls greater than or equal to a Signal Log Ratio of 1 and Decrease calls less than or equal to a Signal Log Ratio of -1 for a comparison analysis between identical replicate samples hybridized to two probe arrays of the same design. Each histogram represents the average percent False Change for 12 target samples. Error bars represent one standard deviation. Based on Student's *t*-tests, percent False Change values for the FS 400 and FS 450 were statistically equivalent in all three comparisons.



the FS 450 was statistically equivalent to that of the FS 400. In one pair, the FS 450 showed a small, but statistically significant, increase in percent Present. (Figure 1)

FALSE CHANGE COMPARISONS

False Change is an assay performance metric useful in measuring the reproducibility of results from different probe arrays hybridized with identical target sample. False Change is defined as the frequency of the sum of Increase calls greater than or equal to a Signal Log Ratio of 1 and Decrease calls less than or equal to a Signal Log Ratio of -1 (as determined by Affymetrix Microarray Suite analysis software) for a comparison analysis between identical replicate arrays. Replicate arrays in this experiment were hybridized with identical target sample and scanned on the same scanner. Thus, any significant differences in False Change values could be attributed to fluidics station performance.

Figure 2 illustrates the average False Change for sample targets hybridized to three replicate HG-U133A arrays across the different spiked target concentrations for each fluidics station. For each fluidics station, the False Change listed is an average from a total of twelve samples. Error bars represent one standard deviation.

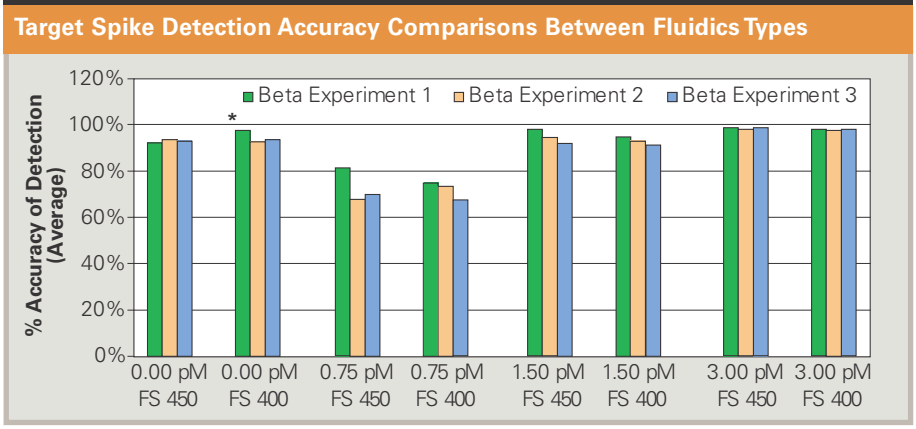
The specification for HG-U133A probe arrays is a False Change of less than one percent. The average False Change across all units was well below one percent and was statistically equivalent between the FS 400 and the FS 450. The slight variations in False Change observed between fluidics stations could have been due to differences in probe array processing, such as hybridization of probe arrays on different days or with different operators.

ACCURACY OF TARGET SPIKE DETECTION COMPARISONS

The results discussed above utilized the entire probe set population and provided global analysis of instrument performance. For further comparisons, additional analyses utilized the cRNA spikes described in the Beta Test Plan.

These 53 transcripts were selected on the basis of their apparent lack of expession in the source RNA as determined by their Absent calls on HG-U133A arrays. Figure 3 illustrates average Detection call accuracy

Figure 3. Comparison of average percent accuracy of detection (Present or Absent calls) for spiked target samples between the FS 400 and the FS 450. The Detection call for each of the 53 spiked transcripts was determined using Affymetrix® Microarray Suite analysis software. For 0.00 pM spikes, Absent calls were scored as accurate. For 0.75, 1.50, and 3.00 pM spikes, Present calls were scored as accurate. An average percent accuracy call based on calls for all 53 transcripts was calculated for each spike concentration. Each histogram shown reflects the average percent accuracy of detection for three target samples hybridized to a single lot of HG-U133A arrays for each unit in the experiment. Based on Student's *t*-tests, instances where FS 400 performance significantly exceeded FS 450 performance are indicated by (*). All other instances were statistically equivalent.



for the 53 transcripts at each concentration of 0.75, 1.50, or 3.00 pM in the final complex cRNA target. The histogram value is the average frequency for which a Present call was made for the 53 transcripts at 0.75 pM, 1.50 pM, or 3.00 pM in the final complex cRNA target. An average of three hybridized target samples is shown. In general, the analysis demonstrates that accurate transcript detection was similar for both the FS 400 and FS 450. The 3.00 pM target spike was called Present with nearly 100 percent fidelity by all fluidics stations. The Detection accuracy of the 1.50 pM target spike was similarly high (91-98 percent). All results were within expectations and system specifications. With the exception of one pair at the 0.00 pM level, Student's *t*-tests comparing the FS 400 to the FS 450 indicated that overall Detection accuracy was statistically equivalent.

Additional Testing

OVERVIEW

A series of experiments was performed to assess the potential for photo-bleaching of stains, as well as any subsequent effects on assay performance caused by prolonged exposure of the stain tube to light while residing on the GeneChip® Fluidics Station 450 during the fluidics protocols.

These experiments all utilized complex cRNA targets (labeled and fragmented cRNA ready for hybridization) prepared from HeLa cell total RNA following procedures outlined in the *GeneChip® Expression Analysis Technical Manual*. For each comparison experiment, three replicate GeneChip Human Genome U133A arrays were hybridized with the complex cRNA target, containing 53 spiked transcripts at 1.50 pM concentration. Arrays were run on one FS 400 module and one FS 450 module with three replicate arrays for each experimental condition. All fluidics processing for a paired comparison was completed in the same day and in the same lab. All arrays were scanned on a GeneChip Scanner 3000.

TUBE TYPES

The first experiment compared the effect of tube type on assay performance. Clear, amber, and 'dark' Eppendorf tubes run on the FS 450 were compared against amber tubes run on the FS 400. 'Dark' tubes were created by wrapping Eppendorfs in aluminum foil to block all light. Results from this experiment showed that for all three tube types run on the FS 450, performance was equivalent to performance of the FS 400 when amber tubes were used. Additionally, the results of the comparison of three tube types on the FS 450 revealed no statistical differences in system performance (data not shown).

STAIN STABILITY

A second experiment tested the potential for photo-bleaching of the stain solution at three different light levels: 280 Lux, 839 Lux, and 4,000 Lux. Most lab lighting is expected to be in the 280-839 range, with 4,000 representing an extreme situation. Testing on both amber and clear tubes showed no significant impact of increased light levels on overall assay performance (data not shown).

PROBE ARRAY TYPES

Finally, to further test for a performance difference between the FS 450 and the FS 400, comparative testing was performed on the following probe array types and formats:

Array	Format (size)
Test 3	400
HG-Focus	100
RG-U34	169
E.coli Antisense	49
GenFlex®	169
Arabidopsis	49
YG-S98	49
Rat 230A	64
Mouse 430A	49

All comparisons showed statistically equivalent performance (data not shown).

Conclusion

The results described in this Technical Note demonstrate that the performance of the GeneChip® Fluidics Station 450 (FS 450) is equivalent to the performance of the Fluidics Station 400 (FS 400). This was shown in comparative studies using the GeneChip Human Genome U133A (HG-U133A) probe arrays, as well as other probe array formats. Results from testing different tube types and exposing the stain to different light levels demonstrated that leaving a tube of stain solution on the FS 450 for an extended time has no significant impact on assay performance. Users of the FS 450 can be confident that new data generated using the FS 450 can be compared to previously generated results with the FS 400 across a wide range of commercially available GeneChip probe arrays.

The design improvements and walk away freedom offered by the new FS 450 provide the potential for significant improvements in laboratory workflow. The easy upgrade path and demonstrated concordance with FS 400 make for a seamless transition for current Affymetrix GeneChip System users.

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