AFFYMETRIX®

Technical Note

The Affymetrix GeneChip® Scanner 3000

New Advances in Scanner Design for Superior Performance, Reliability, and Dynamic Range

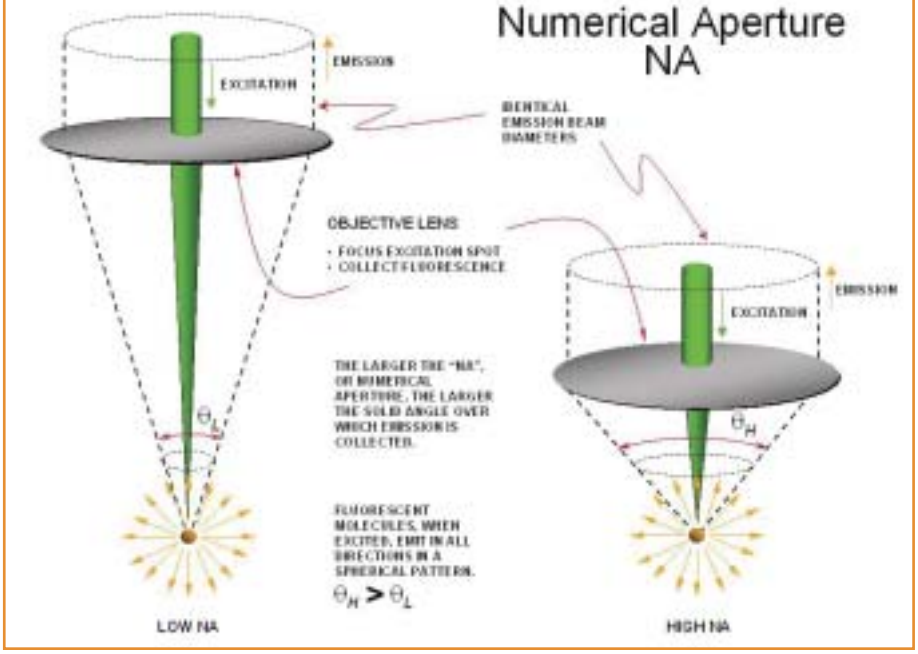
The Affymetrix GeneChip® Scanner 3000 design lays the foundation for continued evolution of the GeneChip® platform as an integrated genetic analysis system, supporting continued feature size reduction for higher resolution and greater information content of microarray-based products. The Affymetrix GeneChip Scanner 3000 will seamlessly incorporate future developments as GeneChip® technology continues to evolve. In order to address the requirements of new and emerging applications, advancements within the Affymetrix GeneChip system will build upon the GeneChip Scanner 3000. This document will describe the technical aspects of the GeneChip Scanner 3000 and provide details on the innovative technologies used to incorporate advanced design improvements. These advances have resulted in dramatic space savings, increased scan speeds, increased signal resolution, reduced noise, wider dynamic range, and real-time laser monitoring and adjustment.

Previous Scanner System

PRE-OBJECTIVE LINE SCANNING SYSTEM

This technology employs a mirror mounted on the end of a galvanometer—a limited rotation, oscillating motor. The term "pre-objective scanning" is derived from the fact that the scanning is accomplished by moving (scanning) the laser beam before a fixed objective lens. The galvanometer enables a laser beam to scan back and forth along a straight-line path, referred to as the excitation line-scan. The excitation line-scan, in turn, is directed to, and focused on, the array through a multi-element objective lens located several inches away from the array. Emission is collected by another lens/filter/lens system positioned at an angle to the excitation beam. This optical structure has three main limitations. First, imaging performance is characterized by geometric distortion and non-uniform emission collection. The visible manifestations of these effects are seen as inaccurate gridding and non-uniform imaging intensity. Non-ideal optics and open-loop, scan-motion control are the principle causes of geometric distortion. Lens aberrations and optical misalignment lead to non-uniform emission collection. Second, the low numerical aperture (NA) of the objective lens places a constraint on achievable scan speed. NA, which is related to distance from the objective to the sample (Figure 1),

Figure 1: Illustration of High and Low Numerical Aperture.



is a measure of the optical collection efficiency of the objective lens, i.e., the ability to collect fluorescent photons. Simply put, due to the low NA, the detector needs to "dwell" on the biological sample site for a relatively long time in order to acquire sufficient photons to ensure acceptable signal-to-noise performance. The previous GeneChip® array scanner addressed collection efficiency by averaging two or more successive lines, or successive images, to reduce the effects of signal noise. Third, emission path alignment is difficult. Photons from the fluorescent emission are collected along a line that coincides with the excitation line-scan. The returning emission beam is collected by the objective lens, passed through an emission filter, and re-focused to a small spot—actually a line since the beam is scanning. The beam then passes through a slit that serves a function analogous to the pinhole in a conventional confocal microscope, i.e., rejection of light emitted or scattered into the objective from planes other than the focus plane. Precise alignment of the emission line (a result of the scanned emission beam) with the slit over the entire width of the scan is difficult, leading to another source of non-uniform collection efficiency across the image field. It is not uncommon to have systematic intensity gradients of 15% or more across the image field because of misalignment. On any given scanner, the fluorescent intensity gradient can vary from edge to edge. The gradient may have opposite signs and/or unequal slopes. The implication of this characteristic is that a probe-by-probe "gain matching" of two (or more) scanners is almost impossible. Gain calibration procedures can only match the average intensity of reference fluorescence material to an average target value. Even if the gain matching procedure was perfect, comparison of specific features from images of the same array scanned on different scanners can exhibit absolute intensity mismatches of 30% or more.

It should be noted that biological performance is not adversely affected, due to the "Perfect Match/Mis-Match" strategy (referred to as PM/MM) of the array design, coupled with robust Affymetrix® Microarray Suite expression algorithms.

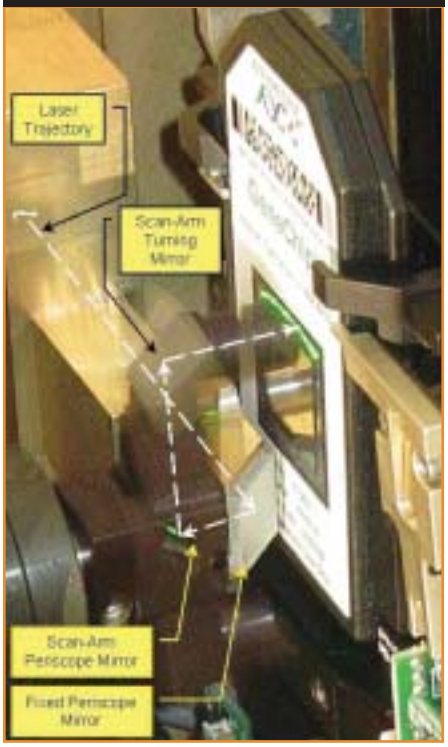
Affymetrix GeneChip® Scanner 3000 System

FLYING OBJECTIVE™ LENS

The Affymetrix patented "flying objective" technology represents a radical departure from the previous GeneChip array scanner that employed "pre-objective line-scan" technology (Figure 2). Affymetrix' patented Flying Objective™ Lens (FOL) technology addresses all of the above issues with a simple, yet elegant, opto-mechanical design. Scan speed is increased, optical distortion is decreased, and emission path alignment robustness is ensured by simultaneously moving the scanning mirrors and the objective lens. The scan motion, corresponding to the "fast," or X-axis, is achieved by rotating a scan-arm (objective lens and mirror assembly) around a section of the optical path located between two mirrors, one fixed to the internal optics bench, the other in the center of the scan-arm. This optical structure creates a periscope, whose optical path is co-axial with the axis of rotation of the galvanometer, and carries both excitation (forward path) and emission (return path) beams (Figure 2). As a result of this geometry, optical path length and alignment are independent of scan-arm rotation. Thus, optical performance (i.e., excitation spot size and emission focus) is quite uniform across the width of the scan field. These relationships hold throughout the slow, Y-axis translation that completes the array-scan operation. The beneficial result is that excitation/emission path lengths and optical alignments are always optimal over the entire two-dimensional field of view.

FOL technology addresses limitations observed in prior scanners in several ways:

Figure 2: A probe array being scanned by the green laser through the flying objective.



- High-accuracy position detectors, used in the X and Y axes, accurately locate each fluorescent pixel. Optic aberrations and galvanometer imperfections no longer dominate scan linearity; thus, consistently accurate scan linearity and excellent gridding performance are ensured.
- The scan arm is physically small, enabling the objective, a low-mass, high-Numerical Aperture (NA) lens, to be located within a millimeter of the probe array. The large NA (0.62) objective provides a four-fold improvement in collection efficiency, which eliminates the need for multiple pass scans; only a single pass is required to deliver consistently high performance. Since line-scan speeds greater than fifty lines per second are easily achieved, Affymetrix' largest format chips scan in just about half the time of that of the previous scanner.

- The confocal optics structure results in an emission beam (a point instead of a line) detection path. This configuration allows emission detection to be implemented with a fixed-collection lens and pinhole that greatly facilitate simple and robust optical alignment. Taken as a whole, FOL technology consistently delivers outstandingly crisp, uniform, and geometrically accurate images.

A SOLID STATE GREEN LASER

Regardless of scanning technology, the laser is one of the most critical components in the system. Laser selection criteria include physical size, cost, reliability, lifespan, output power stability, noise, spot fidelity, pointing accuracy, etc. Gas lasers offer excellent beam characteristics and unit cost, but have size, power supply, and cooling requirements that create cost and complexity for system packaging design. On the other hand, solid-state lasers are highly reliable and provide stable, low-noise, high-quality beam characteristics, but at a higher cost. Because of the aggressive data quality and system reliability goals that were set for the GeneChip Scanner 3000, Affymetrix engineers ultimately chose a high-quality, solid state 532nm Diode-Pumped, Frequency Doubled Nd:YAG (Neodymium-doped Yttrium Aluminum Garnet) Green Laser (Figure 3).

Additionally, because it uses a solid-state laser, there is no need for an external laser power supply under the bench or for a special cooling system. The scanner optics are designed to condition the laser beam to deliver an excitation spot size of 3.5µm (measured at the $1/e^2$ points), resulting in a two-fold improvement in optical resolution, as compared to the previous generation of array scanners.

INCREASED DYNAMIC RANGE AND SIGNAL RESOLUTION

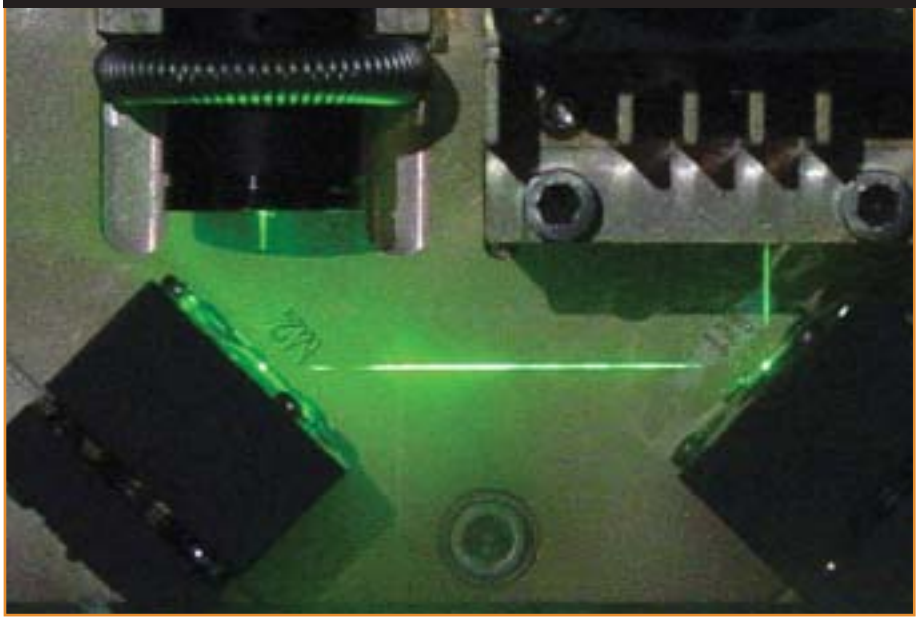
High-performance instrumentation must be able to accurately measure both very small and very large signals. This performance metric is referred to as dynamic range, and is usually specified as a ratio of the largest value of data that can be expressed divided by the smallest increment into which the data can be resolved or digitized.

The GeneChip® Scanner 3000 is able to distinguish very low levels of fluorescence, due to its powerful new high-speed, 16-bit data acquisition system. A full 16 bits of resolution means that 65,535 different levels of fluorescence can now be resolved—a 31% improvement in data precision over the 50,000 level capability delivered by the previous scanner. The data acquisition electronics have been implemented using the latest generation of high-speed sampling analog-to-digital converters. The use of high-quality electronic components is critical; however, it is only part of the story. The best components in the world cannot differentiate between "real" signal from fluorescent emissions, and "noise" signal caused by radiated or conducted "pick-up" resulting from poor circuit implementation. Electrical pollution of signals can be caused by proximity to nearby conductors carrying "dirty" signals, local radio or television stations, or certain types of equipment plugged into the same power source. Sampled data are used to create a snapshot in time of the signal that is presented to the system regardless of its origin. Considerable design effort was put into the optimization of the data acquisition electronics packaging and printed circuit layout to ensure exceptional immunity from external noise sources. Affymetrix specifically custom-designed and continues to build this key sub-system in the manufacturing facility to ensure complete control of the design and manufacturing quality.

REDUCED MEASUREMENT NOISE

One of the biggest hurdles to getting accurate, high-quality, biological data is measurement noise. There are two main sources of system noise—Instrument and Process. Instrument-related sources include data acquisition electronics (previously discussed), laser output noise, optical non-uniformity, auto-fluorescence of optical components, etc. Process-related sources (that, in general, are not mitigated by scanner design elements) include sample contamination, non-specific binding of fluorophores to array features, and non-uniform hybridization. System noise, or total noise

Figure 3: Photograph of the green laser light path.



from all sources, causes signal measurement uncertainty, i.e., any given data sample may be inaccurate within the system noise band. Noise becomes a significant issue when measuring very small signals. Generally, when comparing instruments, there is little, if any, difference in data quality when analyzing high-level (highly expressed) signals. Most of the differentiation in data quality is seen when comparing results obtained from analyzing low-level signals. The specification used to quantify this property is called Signal-to-Noise Ratio, sometimes abbreviated as S/N Ratio or SNR. Since noise in a practical instrument can approach, but never achieve, zero, there is a finite limit to the lowest level of signal that can, to a reasonable degree of certainty, be measured.

SNR EXAMPLE

The following example is a good way to understand these principles. How accurately can you measure the depth (i.e., Signal) of the ocean at two different locations? The first location is near the shore where the depth is about two feet; the second location is further out where the depth is about one hundred feet. Let us further assume that the water is choppy (Noise) with disturbances of two feet, peak to trough. Exactly how deep is the water when you are standing in two feet of water with two-foot, peak-to-trough waves rolling by? At any instant in time, two-foot waves add about a ±50% uncertainty to your measurement accuracy.

Now sail out to water that is 100 feet deep. The same two-foot waves only introduce a ±1% uncertainty, relative to the much deeper water. Likewise, the instrument noise-floor is significantly more dominant when measuring small signals versus large signals. The instrumentation noise floor in the GeneChip Scanner 3000 is below the expected biological noise-floor of the array itself.

Reduced Measurement Offset

PREVIOUS SYSTEMS

While noise limits the ability of an electro-optical measurement system to accurately quantify low-level signals, offset reduces the available dynamic range of the system. Offset is the average value returned by the acquisition system with no optical signal (i.e., "dark") input. Prior systems added a small, positive input signal to the fluorescence data in order to ensure that the digitizing system would not "see" negative or zero data values. When presented to the digitizer, negative values are "clipped" to zero, resulting in loss of the ability to see small signals. If a sufficient number of zeros cluster together, average value and noise calculations of probes will be corrupted. This positive signal, referred to as offset, is subject to drift with time and temperature. The offset value must, therefore, be set high enough to guarantee that the system returns positive, non-zero values over all time and environmental conditions. Since the offset signal is not signal related it provides no useful data value; however, it uses up some of the available range of data values. Prior scanner systems exhibited system offsets of 30 to 50 counts but, in some cases, could range up to 100 counts.

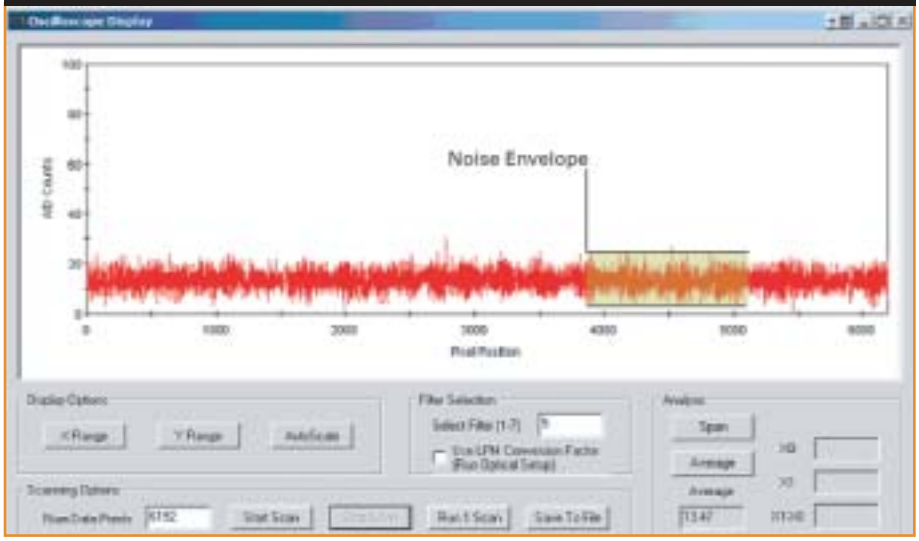
OFFSET EXAMPLE

Consider the implementation of a vertical measurement system where you only have access to a meter stick 1,000mm in length (dynamic range), divided into 1mm increments (resolution). A further requirement is that the system must be freestanding because both of your hands are required to hold the object to be measured. The simplest solution would be to poke the meter stick sufficiently into the ground to allow it to stand upright—say 50mm. The meter stick remains 1,000mm in length, but in this configuration only 950mm extends above ground available for use as a measurement tool. The resolution of your measurement system is unchanged at 1mm but the dynamic range of the system has been reduced to 950mm.

THE GENECHIP® SCANNER 3000

The GeneChip® Scanner 3000 Auto-Zero sub-system provides typical instrumentation system offset performance of around 20 counts. The new Auto-Zero sub-system is designed to measure, and compensate for, long-term offset effects in the scanner hardware. Immediately prior to each scan, the system shutters the photomultiplier tube in order to produce a "dark" scan. The "dark" signal is the cumulative result of all of the electronic noise and offsets in the

Figure 4: Scan line with the photomultiplier tube shuttered, resulting in a dark scan.



system at that time. The returned signal is measured and characterized as to offset (average value) and noise (peak-to-peak envelope). A proprietary algorithm is used to calculate an appropriate system control voltage. When properly adjusted, the lower boundary of the system noise envelope can be driven very close to zero; in fact, only one or two data samples per thousand will be zero or lower. This performance is sufficient to satisfy the requirement for positive, non-zero data values while leaving the largest possible range available for fluorescence signal acquisition. An infrequent, zero-valued pixel does not cause any adverse effect on the data analysis. Figure 4 shows a "dark" line-scan capture. The GeneChip Scanner 3000 delivers this performance consistently, scan after scan, month after month.

AUTO-SET LASER POWER

One challenge of dealing with lasers is compensating for changes in laser intensity. Variables, such as ambient temperature, dust accumulation on optical component surfaces, and aging of the laser, are among some of the factors that cause system gain drift, thereby affecting scanner vs. time and scanner-to-scanner consistency. The GeneChip Scanner 3000 employs an Auto-Set Laser Power feature. Excitation laser power is accurately sampled immediately prior to every scan and, if necessary, precisely adjusted to the proper value for that assay.

Conclusion

The advanced design improvements described in this technical note that are incorporated in the Affymetrix GeneChip® Scanner 3000 result in significant improvements in scan speed, signal resolution, noise, and dynamic range, as well as a significant reduction in lab benchtop space requirements. The GeneChip Scanner 3000 design will allow incorporation of future hardware and software advances as GeneChip technology continues to evolve to address new and emerging applications in genetic analysis and enable next-generation, whole-genome research.

Current GeneChip instrument users may easily transition to the GeneChip Scanner 3000 and confidently compare data sets and experiments from the previous-generation scanner for data concordance. For more in-depth information, see technical note, The New Affymetrix GeneChip® Scanner 3000: Seamless Performance Between the GeneChip® Scanner 3000 and the Affymetrix GeneArray® 2500 Scanner.

For additional information, please refer to the Affymetrix GeneChip Scanner 3000 Specifications Sheet, which can be found at www.affymetrix.com.

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