Evaluation of High-Resolution Scanners for the Imaging of Microarray Experiments and Subsequent Feature Extraction

Dedeepya Vaka, Hung-Chung Huang
Functional Genomics Shared Resource, Vanderbilt University Medical Center
Nashville, USA

William P. Tansey
Functional Genomics Shared Resource, Vanderbilt University Medical Center
Nashville, USA

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine
Nashville, USA

Zhongming Zhao
Functional Genomics Shared Resource, Vanderbilt University Medical Center
Nashville, USA

Department of Biomedical Informatics, Vanderbilt University School of Medicine
Nashville, USA

1 Introduction

With the continuous and daily advancement in the fields of biological and biomedical research, many technologies have emerged to uncover the mysteries of biology, disease, and novel therapies and targets. One of the most powerful technologies is microarrays, which are typically used to detect expression levels of many genes or up to a few millions of markers in single experiment (NCBI, 2007). In a two-color microarray experiment, two samples are labeled with different fluorescent molecules and co-hybridized to an array. The fluorescence intensities are measured by imaging the array in an optical scanner (Lyng et al., 2004). The principle behind the quantification of expression levels is that the amount of fluorescence measured at each sequence specific location is directly proportional to the amount of expressed mRNA in the sample with complementary target sequence to the one on the array probe (Tarca et al., 2006). The similar principle applies to one-color microarray experiment too.

Scanners play an important role in the microarray processing workflow by collecting reliable information from the hybridized microarray chips. High throughput gene expression data from microarray experiments are collected by scanning the signal intensities of the corresponding spots on the array by dedicated fluorescence scanners. For the recent high-density microarray chips, the scanners used for
imaging need to have high resolution lasers for dye excitation at different wavelengths and photomultiplier tubes (PMT) for signal detection (Schermer, 1999).

Many improvements have been made in the next generation high resolution scanners. In this report we describe the specifications of three most popular scanners and how they have been improved from their previous models. We have performed systematic evaluation in the FGSR (Functional Genomics Shared Resource) core at the Vanderbilt University Medical Center on three different scanners (as demonstration purposes), which are well known throughout the world. They are Agilent Sure Scan („G2565CA‘), Molecular Devices GenePix Axon Scanner (4400A) and Roche NimbleGen Scanner (MS 200).

2 Scanner Specifications

2.1 Agilent Scanner

The high-resolution scanner released by Agilent is the model with ID number G2565CA which can scan high-density microarrays up to 2 um resolution (Figure 1).

![DNA Microarray Scanner (Agilent G25265CA) with Sure Scan High Resolution Technology.](image)

2.1.1 Autoloader

The Agilent DNA microarray scanner has a 48-slide autoloader scanning system enabled by Sure Scan High-Resolution Technology which can scan high and low density arrays at 2-, 3-, 5- or 10-micron resolution (Agilent, 2008).

2.1.2 Dynamic Range

Dynamic range is up to $10^6$ with 16-bit or 20-bit scanning possible for high sensitivity scanning.

2.1.3 Scan Time

Scans go from the backside of the slide, through the glass. It continually adjusts scanner's focus, keeping features in focus at all times. Fast 5 micron scans are in about 8 minutes by simultaneous two-color scanning, or less than 20 minutes for 2- or 3-micron scans (Corson et al., 2007).

2.1.4 Ozone Barrier

The Agilent Scanner allows the usage of Ozone-Barrier slide cover for a low-cost, easily-implemented solution to protect against Ozone-induced dye degradation while in the scanner’s slide holder. The Ozone slide cover can be reused.
2.1.5 Integrated Barcode Reader
The Agilent Scanner can read Code 128 (A, B, C), Code 39, Code 93, and CODABAR (only for Agilent arrays).

2.1.6 PMT Adjustment
PMT signal levels can be adjusted from 100% (default) to 1% (Agilent).

2.1.7 Lasers
1 - SHG-YAG laser, 532 nm.
Power: 20 mW at 532 nm and 23 mW at 633 nm both controlled to 13 mW (Agilent).

2.1.8 Platform Compatibility
The Agilent can scan slides of different platforms (e.g., Agilent, Exiqon, Nimblegen, CodeLink arrays). One advantage of this instrument is that there is no need for direct supervision of the instrument while scanning. The user can load the arrays for scanning and then walk away. This is an important feature since scanning of a slide takes some time and it will be a long time to scan many slides in a day, as typically seen in a core facility.

2.1.9 Scanning Procedure
Scanning is controlled with the Agilent Scan Control program. It uses a simultaneous scan procedure (on two-color array experiment) to scan the entire region. No image or histogram is available during the scan time and one can view at the .tiff files only after the scan completed.

2.1.10 Software
The Agilent provides Feature Extraction Image Analysis Software (version 10.7), which reads and processes up to 100 raw microarray image files in an automated, “walk away” mode. The software automatically finds and places microarray grids, rejects outlier pixels, accurately determines feature intensities and ratios, flags outlier pixels, and calculates statistical confidences. Application-specific QC reports summarize results and provide an at-a-glance quality assessment (Agilent, 2008). This software is compatible with other platforms (e.g., Exiqon, CodeLink, TIGR) only if GAL (Gene Annotation List) file is provided for the gridding process and the related protocol is available or can be created.

2.2 Molecular Devices Axon Gene Pix Scanner

2.2.1 Axon Scanner
The high-resolution scanner released by Molecular Devices is the 4400A model which is the standard for High Resolution, High Uniformity, and Sample Type Flexibility (Figure 2) (Molecular Devices, 2009).
2.2.2 Scanning Resolution and Time
Scanning can be performed at a user-specified resolution between 2.5 and 100 um per pixel. Initially a preview can be done at 40 um and accordingly the PMT setting can be adjusted for the real scan. This scanner directly images the array surface, rather than passing lasers through the supporting substrate media, which enables imaging of the arrays with any type of microarray substrate, whether transparent, translucent, or even opaque. Scanning procedure is a 2-pass scan, where it initially performs a red scan and then a green scan. Scan on each color channel takes about 8 minutes and for the entire complete scan it will be around 20 minutes at the 2.5-um resolution.

2.2.3 Scanning
While scanning different sub arrays at multiple different PMT settings, multiple PMT scanning is possible with this scanner. All of the sub-scans can be saved and analyzed either separately or together. During scanning, the scan region for each sub-array is locked for fixed PMT setting and they can be adjusted only after the scan is completed. This may sometimes take longer time to get a perfect scan since adjustment may be needed after first or even several initial scans. Experienced hands may finish quicker with a single scan (Molecular Devices, 2009).

2.2.4 Dynamic Range
Dynamic range is up to 16 bit for sensitivity scanning with some saturation.

2.2.5 Ozone Protection
There is no ozone free environment to protect against ozone-induced dye degradation, but this can be achieved by passing nitrogen gas to the system.

2.2.6 Platform Compatibility
The Axon scans slides of different platforms (e.g., Agilent, Exiqon, NimbleGen, and CodeLink arrays). This instrument, however, requires the dedicated presence of a technician during the scan, as one needs to monitor the histogram and the scans during the preview to adjust the PMT’s for the real optimal scan later (Molecular Devices, 2009).

2.2.7 Unparalleled Signal-To-Noise Performance
Axon GenePix scanners have low-noise digitization technology. This technology allows using lower PMT settings, avoiding saturated pixels, and resulting in greater SNR values. The unparalleled sensitivity also

Figure 2: Axon GenePix® 4400A microarray scanner.
enables the use of lower laser power settings, which significantly reduces any perceived degradation of the dyes (Molecular Devices, 2009).

2.2.8 Laser Power Attenuation
A gradient neutral density filter allows attenuating laser transmission between 5-100% in 1% increments, providing maximum flexibility in optimizing channel intensities. This laser attenuation also helps reduce photo bleaching of sensitive samples (Molecular Devices, 2009).

2.2.9 Software
The software used for scanning and feature extraction is GenePix 7.0. For feature extraction it requires GAL file information to align the features to the spots on the arrays. The software sometimes may not align the grids properly, and this has to be corrected manually to adjust the grids to the right positions. Also the bad spots and outliers have to be flagged manually.

2.3 Roche NimbleGen Scanner
NimbleGen MS 200 scanner has high resolution, high sensitivity, and high throughput features and is designed for high-density genomics platforms (Figure 3). This is the first release version from Roche.

Figure 3: High-throughput, high-resolution NimbleGen scanner with the ability to load and automatically scan up to 48 slides for fast scans, high quality data, and confident results.

2.3.1 Autoloader
Autoloader for automated batch can process up to 48 slides for 24-hour unattended operation.

2.3.2 Scanning Resolution and Time
Scanning can be performed with a user-selected resolution between 2- to 40-um pixel resolutions with 2 μm for optimal scanning of high-density NimbleGen DNA microarrays. Dynamic range is up to $10^4$ (16-bit data format). Time taken for 2-μm scan is around 30 minutes and about 20 minutes for a 5-μm scan (NimbleGen, 2009).

2.3.3 Scanning Procedure
Initially a small area is selected and auto PMT is set for that region whose subsequent PMT setting is applied to the whole scan area. During autofocus, auto-adjustment is used for optimal focus position and angle to ensure features stay in focus while scanning light sources. It is a walk-away procedure – the user simply loads the slides and selects a small scan area for auto PMT, then the software takes care of everything. The auto PMT area can be saved for each sub-array respectively in a multi-array slide and the
saved same auto PMT areas can be used for multiple slides of same platform. For two-color scanning, this instrument performs a simultaneous scan, similar to the Agilent scanner (NimbleGen, 2009).

2.3.4 Ozone Protection
There is a built-in ozone free environment to prevent the slides from photo bleaching. This feature gives some benefit for this scanner’s hardware features as compared to other microarray scanners.

2.3.5 Barcode Reader
There is an integrated barcode reader for sample identification and tracking.

2.3.6 Compatibility and Upgradability
This scanner can scan arrays from different vendors (e.g., Exiqon, TIGR, NimbleGen, and Agilent). It is upgradeable to ensure compatibility with high-density NimbleGen arrays of today and ultra-high density arrays of the future.

2.3.7 Lasers and Dyes
Recommended dyes are Cyanine 3 (Cy3) and Cyanine 5 (Cy5). It has two solid-state lasers (532 nm and 635 nm) (NimbleGen, 2009).

2.3.8 Software
The software provided by Roche NimbleGen is Nimble Scan. It can only be used for NimbleGen arrays. For other platform arrays (e.g., Exiqon, TIGR, CodeLink, and Agilent), the files have to be imported to third party software for feature extraction.

3 Discussion
In this report, we systematically compared the performance of three powerful scanners designed for obtaining high resolution and high quality data from microarray experiments. A summary of the features and differences is shown in Table 1.

3.1 Time
This factor is very important when there are multiple slides to scan, as is common in a core facility. The Agilent G2565CA scanner takes approximately about 20 minutes to scan (simultaneously) any kind of microarray slide at 2um. Dynamic autofocus continually adjusts to keep the features in focus, and allows PMT adjustments of signal levels from 100% to 1%. These features make the effective scan time for the Agilent to be fast and very efficient, as multiple scans are not needed. The NimbleGen scanner, in contrast, needs to first scan a small area for auto PMT at 40um and this settings area can be saved and used for the complete (simultaneous) scan region, making the effective scan time 20-25 minutes at 2um. For the Molecular Devices Axon scanners, a preview scan is also required for setting the PMT values for real scan. As the scan procedure used here is double-pass (Cy5 in first pass and Cy3 in second pass) this scan takes approximately about 30 minutes to perform the complete scan at 2.5um. There is auto PMT option available for the Axon scanner but this may take more time than manual PMT settings.
3.2 Autoloader
All three scanners can hold a maximum of 48 slides. The NimbleGen and Agilent scanners have built-in autoloaders whereas the Molecular Devices Axon scanner can hold just one slide in the machine. The Axon can work with an external hotel autoloader, which is an advantage if the autoloader breaks as scans can still be run.

3.3 Dynamic Range
For the NimbleGen and Molecular Devices Axon scanners, the dynamic range is up to $10^4$ (16-bit), whereas for the Agilent instrument the dynamic ranges are: $10^4$ (16-bit), $10^5$ (20-bit), and $10^6$ (with XDR scanning).

<table>
<thead>
<tr>
<th>Features</th>
<th>Agilent Scanner</th>
<th>Axon Gene Pix Scanner</th>
<th>NimbleGen Scanner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>GC26265</td>
<td>4400A</td>
<td>MS 200</td>
</tr>
<tr>
<td>Resolution</td>
<td>2 μm</td>
<td>2.5 μm</td>
<td>2 μm</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>20 bit</td>
<td>16 bit</td>
<td>16 bit</td>
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<tr>
<td>Time</td>
<td>&lt; 20 min</td>
<td>30 min</td>
<td>25 min</td>
</tr>
<tr>
<td>Ozone protection</td>
<td>Ozone covers are provided</td>
<td>No ozone protection</td>
<td>Built in ozone free environment</td>
</tr>
<tr>
<td>Scanning software</td>
<td>Agilent Scan Control</td>
<td>GenePix Pro 7</td>
<td>MS200 Instrument software</td>
</tr>
<tr>
<td>Hardware</td>
<td>Scans slides from all platforms</td>
<td>Scans slides from all platforms</td>
<td>Scans slides from all platforms</td>
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<tr>
<td>Software compatibility</td>
<td>Feature Extraction software</td>
<td>Gene Pix works in all platforms if Gal file is provided</td>
<td>Nimble Scan works only for Only NimbleGen slides</td>
</tr>
<tr>
<td>Man power</td>
<td>Walk away</td>
<td>Monitor</td>
<td>Walk away</td>
</tr>
<tr>
<td>Scan procedure</td>
<td>Simultaneous</td>
<td>Dual pass</td>
<td>Simultaneous</td>
</tr>
</tbody>
</table>

Table 1: Comparison of specifications among three scanners

3.4 Ozone Barrier
The NimbleGen scanner has been built in ozone free environment, while Agilent provides ozone covers which can protect the slides from photo bleaching. The Axon scanner has provision for pumping Nitrogen gas into the scanner to overcome the photo bleaching.

3.5 Software
The Agilent scanner comes with Feature Extraction software (version 10.7). This software is compatible with all non-Agilent platforms, providing the GAL file is provided. The Molecular Devices Axon Scanner comes with GenePix software which can be used for both scanning and feature extraction. This software is compatible with all platforms if a GAL file is provided. The NimbleGen scanner has its own scanning program - Nimble Scan software (for extraction), which is compatible only with NimbleGen arrays, but third party software may be used for extracting the data from features (spots) for other array platforms scanned with NimbleGen scanner.
3.6 High Density Microarrays for Copy Number Variations (CNVs)

In recent years, array-based Comparative Genomic Hybridization (aCGH) has been refined to determine chromosomal changes at progressively higher resolutions (Barrett et al, 2004). Here we discuss the new high density microarrays that were recently released for aCGH studies and need to be scanned on high resolution scanners to obtain high quality data. We specifically discuss the microarrays from NimbleGen and Agilent.

3.6.1 NimbleGen CGH Array

With up to 2.1 million probes on a single slide, NimbleGen CGH arrays enable genome-wide detection of CNVs at a resolution of ~5kb in size across the human genome, and analysis of targeted regions at exon-level resolution. NimbleGen CGH whole-genome designs consist of probes tiled through both genic and intergenic regions for comprehensive and unbiased detection of copy number gains and/or losses. So far, whole-genome designs are available for a variety of eukaryotic organisms including human, mouse, rat, cow, dog, monkey, chicken, zebrafish, fly, worm, and others.

For researchers interested in targeted CNV analysis of chromosomal regions or genes, NimbleGen offers an opportunity to easily and quickly design custom arrays for any eukaryotic organism. Custom CGH array designs consisting of either uniform or mixed-density probe spacing can be created for all available array formats (12x135K, 2.1M, 385K, 4x72K) and may include whole genomes, single chromosomal regions, or multiple loci of interest.

NimbleGen 2.1M CGH array experiments are recommended to be scanned in a high-resolution scanner as described in this report. The features of scanned images can be extracted and then data can be analyzed with CGH-DNACopy or CGH-segMNT algorithm implemented in the NimbleGen NimbleScan program. Some down-stream data analysis can be followed subsequently.

3.6.2 Agilent CGH Array

Agilent’s microarray-based comparative genomic hybridization (aCGH) technology is another powerful solution to the research such as in cancer and developmental disorders. Whole genome and zoom-in 60-mer oligo CGH microarrays for human, mouse and rat are available. Agilent’s CGH end-to-end solution consists of flexible microarray formats, optimized and easy-to-use protocol, high resolution microarray scanning and powerful analytics software. Using 60-mer oligonucleotide probes, the microarray provides very high sensitivity, enabling researchers to reliably identify both highly localized and broadly extended single copy deletions, homozygous gene deletions and amplicons. Available are human genome CGH arrays, custom arrays and arrays for other model organisms (i.e. mouse, chicken, fly, frog, bovine, rabbit, Rhesus monkey, yeast, zebrafish, and others).

Agilent's custom microarrays offer the flexibility and accuracy needed to detect CNVs. The platform offers high-quality long oligonucleotide catalog and custom arrays to interrogate any genome at high-resolution and accuracy. Agilent's custom arrays allow the users to easily select the regions of the genome (or the entire genome) that they would like to target. Custom arrays are available in all eight formats (1x1M, 2x400K, 4x180K, 8x60K, 1x244K, 2x105K, 4x44K, and 8x15K).

Agilent 1x1M SurePrint G3 CGH microarray experiments are also recommended to be scanned in a high-resolution scanners as described in this report. The scanned images can be analyzed and features can be extracted with Agilent Feature Extraction program followed by down-stream data analyses.

Compared to low resolution CNV arrays or Agilent arrays, NimbleGen 2.1M arrays provide more probes that allow the researchers to examine CNVs at high resolution. Thus, currently most of the customers...
decided to choose NimbleGen 2.1M arrays for their CNV studies, while NimbleGen 385K arrays were more popularly used 1-2 years ago.

4 Conclusion

High resolution scanners are required to scan high density microarrays. Although there have been several major scanners just available in the market, no perfect scanner can fit the requirements in various array platforms. Based on the specifications we tested, we recommended the users choose the scanner based on the specific needs and develop the workflow accordingly.

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References


Chapter 17 – Evaluation of High-Resolution Scanners for the Imaging