

# Using the GenePix 4400A Microarray Scanner to generate high-resolution images of CGH 2.1M feature arrays

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## Introduction

Genome-wide association studies are used to rapidly identify variations for a specific disease from a complete DNA set, or genome, and are a beneficial technique for researchers to study complex diseases, including asthma, cancer, diabetes or mental illness. To facilitate these large studies, high-density microarrays, with 2.1 million (2.1 M) probes on a single microarray slide, provide scientists a cost-effective solution to perform gene expression analysis, ChIP-Chip, and array-comparative genomic hybridization (aCGH) assays. The Molecular Devices GenePix® 4400A Microarray Scanner provides the resolution (2.5  $\mu\text{m}/\text{pixel}$ ) necessary to interrogate each probe within these high-density arrays, allowing for excellent probe signal-to-background ratio (SNR) levels and improved accuracy in the fluorescence calls that are made per probe.

## Materials and Methods

Copy number variant analysis using aCGH: Human genomic DNA was extracted from blood samples collected from affected patients and unaffected controls. A competitive hybridization was performed between equal amounts (34  $\mu\text{g}$ ) of cy5-labeled unaffected control gDNA and cy3-labeled affected patient gDNA. Using manufacture's recommended protocols (NimbleGen Arrays User's Guide: CGH Analysis v5.1), samples were hybridized for 2–3 days on Roche NimbleGen High Density 2.1M HD2 Arrays. The arrays were then washed, dried, and scanned on the GenePix 4400A Microarray Scanner and the fluorescence signal for each probe analyzed.

### Scanning an aCGH array

1. Under the "Laser Settings" window in the GenePix® Pro 7 Software, set "Pixel Size" to 2.5  $\mu\text{m}$ , and choose 635 for "Wavelength #1 (nm):" and 532 for "Wavelength #2 (nm):". Select "OK" when finished.
2. Under the "Hardware Settings" window, set "Power (%):" for each laser to 100%. Ensure that "Wavelength #1 – 635" is set to the Cy5 Filter, and "Wavelength #2 – 532" is set to the Cy3 Filter.
3. Place the microarray face down onto slide holder platform, with the barcode end of the slide closest to the front of the scanner, and close the lid.
4. Select the "Preview Scan" button to perform a fast, low-resolution (20  $\mu\text{m}/\text{pixel}$ ) scan of the entire slide, to determine the size and orientation of the array on the slide surface.
5. Designate the area of the slide to be scanned at the high resolution (2.5  $\mu\text{m}/\text{pixel}$ ) by choosing the "View Scan Area" button and adjust the white Scan Area box to cover the entire area of the array

6. The PMT Gain settings were manually optimized, prior to generating a final Data Scan. The slide was first scanned with the Red (635 nm) laser and the PMT gain was adjusted to match a goal of having maximal signal intensity and a minimum amount of background. The array was then scanned with the Green (532 nm) laser and the PMT gain was adjusted to match the signal intensity profile of the Red laser scan as shown in the Histogram tab. A final Data Scan was then performed of the entire Scan Area using the optimized PMT Gain settings.

Under an optimal PMT Gain setting, the Ratio (635/532) image of the array should look yellow for the majority of the probes, when viewed in the Image Tab. In the Histogram Tab, the Wavelength 635 and Wavelength 532 intensity profiles should show a similar distribution across the entire dynamic range of the instrument and have a Count Ratio between 0.9 and 1.1.

After the final Data Scan is complete, click the "File..." button icon located at the right-hand side of the GenePix Pro Software interface window and select the "Save Images-Selected Scan Area", to save the scanned images as a TIF file.

The saved TIF images are then further analyzed by importing the images into the NimbleScan software for processing and the resulting data files are viewed using the SignalMap software. These software packages are offered by Roche NimbleGen for analysis of Roche NimbleGen arrays.

## Results

Figure 1 shows a full length scan of the HD2 aCGH microarray slide separated between the red Cy5 image (left), the green Cy3 image (center), and the merged ratio image of Cy5/Cy3 (right).

**Full length scan (Figure 1)**

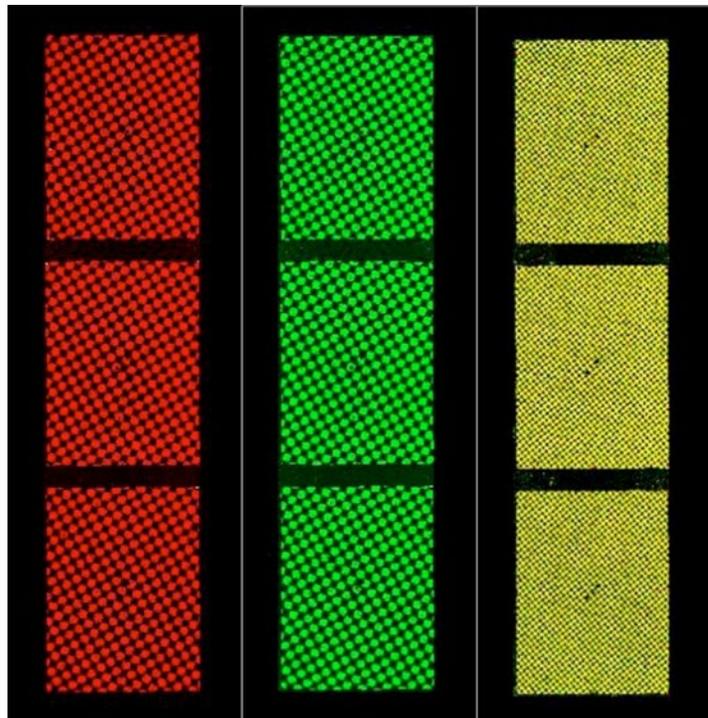


Figure 2 highlights the difference in image quality between the same HD2 aCGH microarray scanned at the 5  $\mu\text{m}/\text{pixel}$  resolution of more traditional microarray scanners (top row) and the 2.5  $\mu\text{m}/\text{pixel}$  high resolution of the GenePix 4400A Microarray Scanner (bottom row).

Image quality (Figure 2)

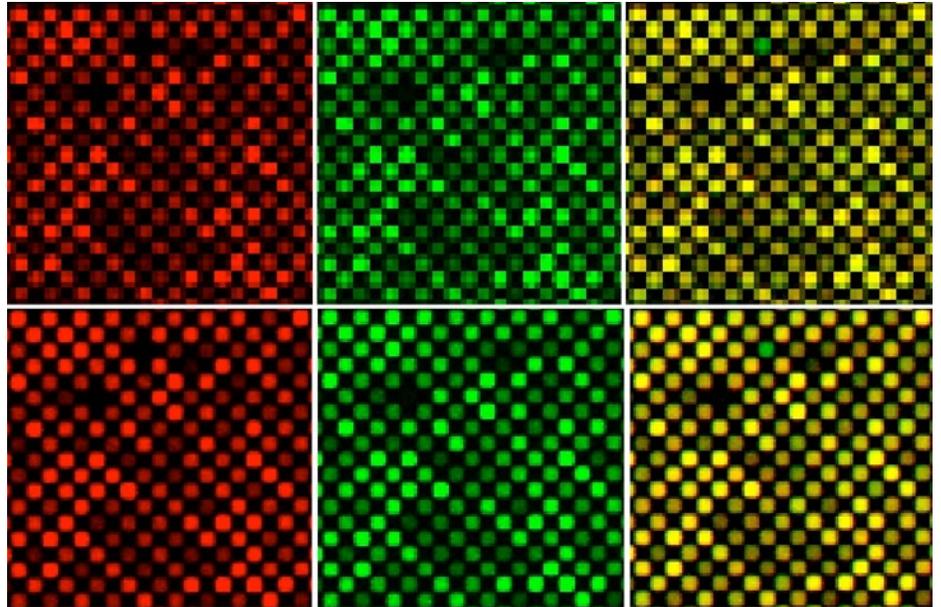
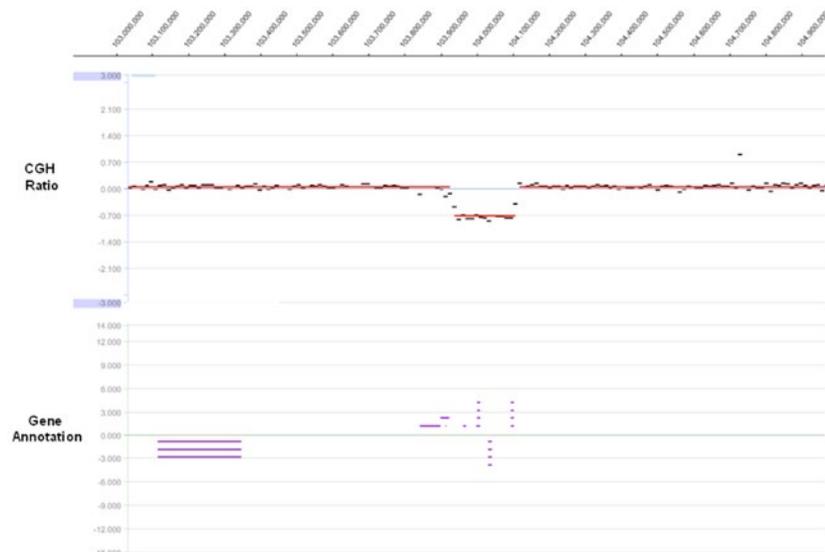


Figure 3 shows an aCGH microarray fully processed through the NimbleScan software and then viewed in the SignalMap genome browser. The averaged segmentation GFF file (using default Roche NimbleGen CGH analysis settings) identifies a heterozygous deletion in the patient sample. The gene annotation track identifies a group of genes in the human genome affected by this deletion.

aCGH microarray data analysis (Figure 3)



## Conclusion

The GenePix 4400A Microarray Scanner is a robust tool that differentiates probe boundaries and accurately analyzes probe fluorescence within high-density microarrays using the 2.5  $\mu\text{m}/\text{pixel}$  resolution setting. When combined with the high-density array of your choice, researchers are able to expand their capabilities in a cost-effective manner to complete genome-wide associate studies in a timely manner.

## Acknowledgements

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## References

NimbleGen Arrays User's Guide: CGH Analysis v5.1, Roche NimbleGen, Inc.

GenePix Pro Software v7 User Guide, Molecular Devices, LLC

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