FREQUENTLY ASKED QUESTIONS

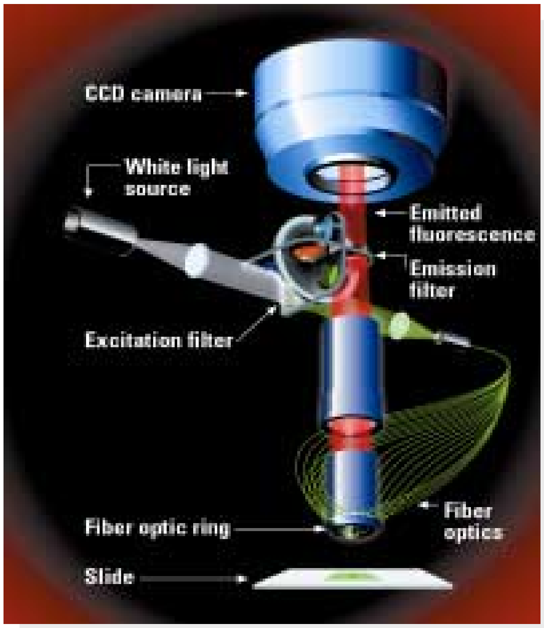
Section 1: Scanner Technology

The *arrayWoRx*e™ scanner uses white light, CCD-based technology – what does this mean?

Microarray scanning is based on the principle of fluorescence imaging. This imaging technique requires light of a specific wavelength to excite the fluorophores in a sample. Two types of technologies may be used: 1) a laser and photo-multiplier tube or 2) a white light source and a CCD camera. The ***arrayWoRx***e system utilizes a white light arc lamp for its illumination source. Since white light contains light from the entire spectrum, it is possible to excite virtually any fluorescent probe by using a filter to select the appropriate wavelength. In the ***arrayWoRx***e scanner, light from a metal halide bulb is guided via fiber optic cables to illuminate the sample. Emitted fluorescent light from the sample is collected and imaged using a high-performance CCD camera.

How does the system acquire images?

The ***arrayWoRx***e Biochip Reader's optical design is based on our experience with the ***DeltaVision***® Image Restoration Microscopy System and the original ***arrayWoRx*** Microarray Scanner. The microarray slide is illuminated one 1.4 mm square portion, or panel, at a time. The acquired images are then stitched together based on the position of each panel using Applied Precision's patented ***Stitch by Position***™ technology. A highly accurate stage positions the slide during image acquisition and provides for accurate and repeatable image reconstruction based each panel position. Application specific illumination optics greatly enhance signal sensitivity and a rotating filterwheel changes the excitation and emission filters for each fluorescent probe. This filterwheel design ensures that the slide does not move during the filter change and that no image misregistration occurs between the channels. With this unique opto-mechanical design, the ***arrayWoRx***e Biochip Reader can achieve 3.25 micron per pixel image resolution – unmatched in the industry.



Does stitching the panels together change the integrity of the image data?

Stitching has no affect on the integrity of the data. For more than 14 years, Applied Precision has been in the business of producing high-resolution imaging and positioning technology for the semiconductor industry and 3-D microscopy applications. The ***arrayWoRx***^e scanner is built around a high precision stage that allows image panels to be stitched together from precise positioning information. Illumination differences across panel edges are not seen due to a robust calibration routine that performs a linear regression on every pixel in the camera.

What type of white light source does the *arrayWoRx*^e scanner use? How much does it cost to replace?

The ***arrayWoRx***^e scanner uses a metal halide bulb, which lasts for 1,000 hours of scanning time. The system automatically shuts the bulb off when the scanner is not in use. The software tracks the amount of time it is illuminated. The bulb is inexpensive and easily replaceable - no service call is necessary.

What is photobleaching? Why is it a concern with other systems, but not with the *arrayWoRx*^e scanner?

Photobleaching is the effect of a fluorescent probe losing its ability to fluoresce due to an excessive amount of illumination applied to the sample. Although laser systems have a per-pixel exposure time, which is much shorter than a CCD scanner, the laser illumination has much higher peak intensity per unit of time. This energy spike can be high enough to photobleach the sample. ***Laser photobleaching not only reduces the ability to image the slide more than once, it results in data, which has been substantially altered during image acquisition.*** Non-linear photobleaching of each fluorescent probe also significantly reduces the integrity of ratio calculations. In contrast, the ***arrayWoRx***^e scanner has a much lower photon flux density during illumination, greatly reducing the risk of photobleaching.

As future applications develop and the need for flexibility in fluorescence channel selection becomes more important, how many wavelengths are available for the *arrayWoRx*^e scanner?

The ***arrayWoRx***^e scanner can collect information from up to four wavelengths per slide. The end user has the ability to choose wavelengths between 350-700 nm for excitation and between 400-750 for emission. Changing wavelengths requires only the purchase of optical filters that can be purchased at relatively low cost. With the included filter sets on the Standard model, the system supports up to 89 different fluorescent dyes. Details are available on our website.

What is the cost to add a set of filters to the *arrayWoRx*^e scanner? What is the cost to add additional lasers to a laser based system?

A filter set for the ***arrayWoRx***^e system costs is very cost effective - approximately $500 - $1,000 per channel. Additional lasers can cost upward of $2,000 - $10,000 per channel.

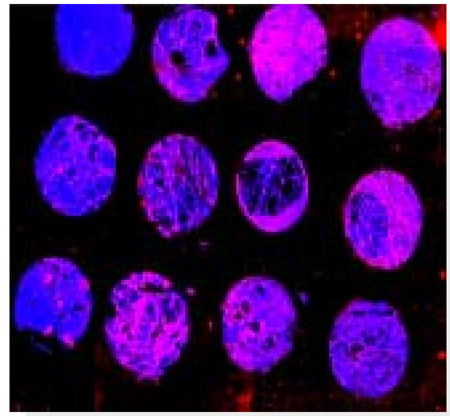
What kinds of filters are used with the *arrayWoRx*^e scanner? Where can I obtain filters?

Applied Precision currently supports Cy3, Cy5, FITC (Alexa 488) and DAPI (Alexa 350) filter sets. Additional filters may be obtained from Chroma Technology Corporation. The filters are standard 25 mm diameter excitation and emission filters, commonly used in fluorescence microscopy. The actual (as opposed to nominal) filter diameter should be 24.95 mm. **See http://www.chroma.com.**
The range of filters that can be used in the ***arrayWoRx***^e system is from UV (350nm) to Far Red (750nm).

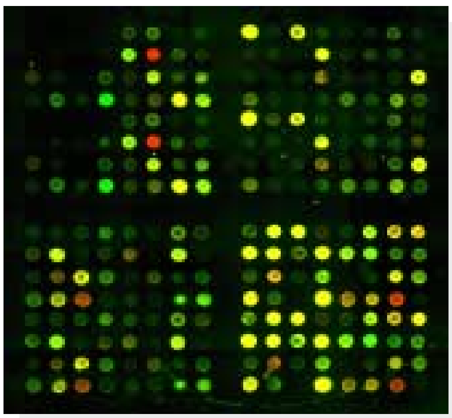
What applications does the *arrayWoRx* technology support?

The ***arrayWoRx*e** scanner has been used in applications such as:

- Alternative dyes
- Proteomics
- SNP's
- Microbeads
- Tissue arrays



First Generation Prostate Tissue Microarrays. Photo courtesy of: Dr. Rahul Mitra, Baylor College of Medicine



Direct Labeling with Alexa Dyes. Red = Alexa 594, Green = Alexa 546. Photo courtesy of: Dr. Rahul Mitra, Baylor College of Medicine

Can the excitation power of the scanner be adjusted?

The lamp illumination is maximized to provide the strongest signal from the sample. There is no need to reduce the light intensity, since the ***arrayWoRx*e** scanner does not suffer from the same extent of photobleaching as other systems. Signal intensity is controlled using exposure time. Exposure times are separate for each wavelength and are easily optimized using control spots on the slide. The scanner software suggests optimal exposure readings to maximize the signal intensity settings.

Can the detector gain be adjusted?

Detector gain does not apply to the ***arrayWoRx*e** scanner. Exposure time is used to control signal intensity; an amplified gain setting like that of a PMT is unnecessary. The exposure time can be specified via the user interface, and different times can be programmed for different fluors.

How sensitive is the CCD camera in the *arrayWoRx*e scanner? What is the dynamic range of the image file?

The CCD camera in the ***arrayWoRx*e** scanner has a quantum efficiency of 50-60%. Image files have a dynamic range of 16 bits, i.e., the maximum intensity value is 2^{16} (65,535). Intensity changes in the sample can be detected at this level of sensitivity – 1 in 2^{16}. In contrast, PMTs have a quantum efficiency of approximately 10-15%. This means that only 10-15% of the photons striking the PMT are converted into electrical signals.

People in the industry have said that CCD camera image quality is inferior when acquiring an image of the whole slide. Is this how the *arrayWoRx*e system acquires images?

For standard CCD camera systems, this is entirely correct. Applied Precision's ***Stitch by Position™*** technology overcomes this limitation by using a highly accurate stage to position the slide. Small, high-resolution images, or panels are acquired and combined into a single high-resolution image of the slide.

The *arrayWoRx*e scanner provides an image file of 16 bits. How much of the 16 bits is background noise and how much is signal? Why is this important?

The ***arrayWoRx*e** CCD is a highly linear, noise-free photon detector. Out of the 2^{16} counts possible in the image, there are about 6.8 counts of camera electronic noise. This is a dramatic improvement over other CCD camera systems. Less noise results in higher quality data (see below), which is critical for statistical validation of the experiment. The ***arrayWoRx*e** scanner provides an actual dynamic range of greater than 11 bits of information. Image files are saved in 16-bit format for direct comparison with laser images.

Section 2: Image Quality

What are the most important scientific parameters when determining scanner performance?

Four basic parameters determine scanner performance:

- Signal-to-Noise Ratio
- Spot Ratio Variability or CV_R
- Dynamic Range
- Data Reproducibility

Why is Signal-to-Noise more important than Signal-to-Background?

Signal-to-Noise Ratio (SNR) is the foundation of image quality as it quantifies how one resolves a true signal relative to system and sample noise. When a scanner has poor SNR, the variation in signal can prevent accurate quantification of each spot.
Signal-to-Background allows the researcher to look at the ratio between spot intensity and background intensity surrounding the spot. This usually does not take into account any measurement of signal variability, which makes such a calculation virtually meaningless.

Scanner manufacturers speak of Coefficient of Variation for the Ratios or Spot Ratio Variability. What is this measurement and why is it important?

The Spot Ratio Variability (SRV) or Coefficient of Variation for the Ratios (CV_R) is the statistical measure of the pixel ratio variability of a spot. It indicates the combined quality of both the slide chemistry *and* the imaging system.
The CV_R is the normalized variance for the spot ratio, expressed as percentage of the ratio.
Each pixel in a spot can be distributed by intensity and ratio, similar to scatter plot points in a gene expression array graph. With less variation in the ratio (i.e., a lower CV_R value), there is greater statistical resolving power to determine gene up- or down-regulation. Advantages of more accurate data include:

- Greater statistical resolving power for detecting changes in expression levels.
- The ability to statistically validate the data going into your bio-informatics database.
- The ability to "rescue" data, which would otherwise be eliminated due to an arbitrary 2-fold or 3-fold cutoff, and to reject poor quality data which would otherwise contaminate your database.

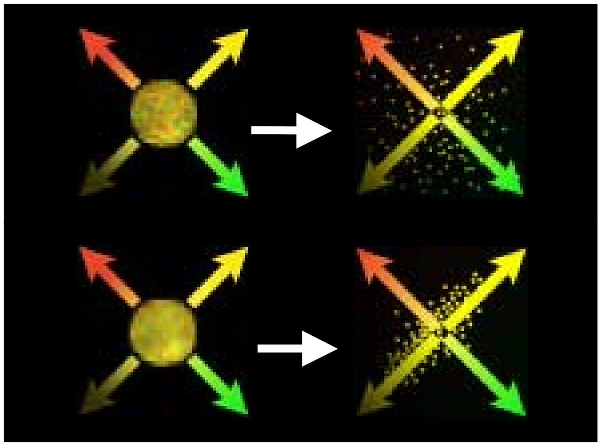


Figure 3. *CVR differences for two spots.*
The CVR of the upper spot is 24%, whereas the CVR of the lower spot is 8%. Intensity and ratio values can be plotted for each pixel in the spot, resulting in scatter around the average.
With lower CVR values, an image has higher statistical resolving power to detect significant ratio differences.
CVR = S.D. of Pixel ratios between the 2 channels / Mean Pixel ratio of the two channels.
Lower CVR = More Accurate Data. Higher CVR = Less Accurate Data. A high performance scanner delivers CVRs in the range of 2-10% with high quality spots.

What happens to spot CV_R when scanning artifacts such as channel misregistration occur?

Scanning artifacts often appear as misregistration between the two channels in an image. This occurs when separate scans for each channel are taken, resulting in differential errors of slide motion during acquisition. This is shown in Figure 4. These random motion errors cause offsets in the two channels, as shown by red and green pixels at spot edges.

The channel offset causes large increases in variability of the ratio calculation for the spots. This problem usually cannot be fixed by globally shifting one image channel – individual spots have to be adjusted, which is difficult with thousands of spots per image.

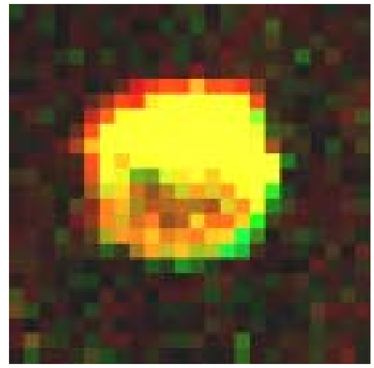
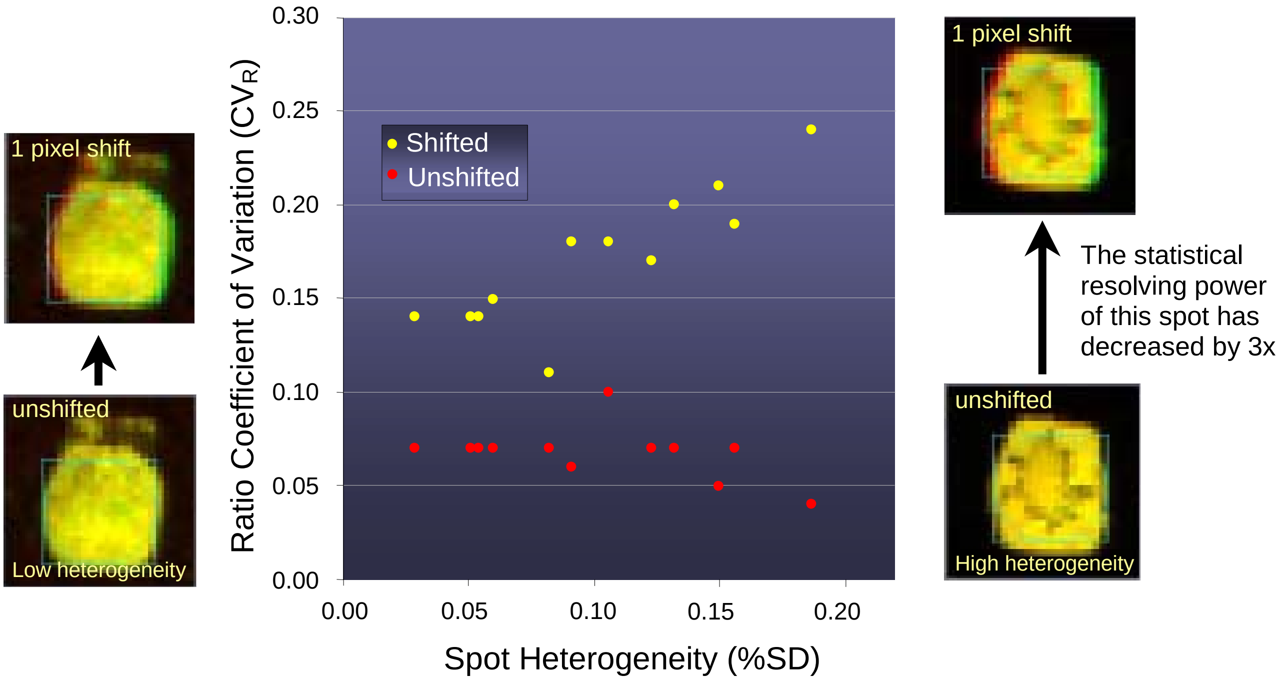


Figure 4. Scanning artifact caused by differential errors of motion during acquisition of the image.

Channel offset typically causes at least a 2-fold increase in the CV_R value for spots. This effect is dependent of spot morphology. With relatively smooth, uniform spots the CV_R increases about 2X. For spots with greater variation in their intensities (usually due to printing conditions or the hybridization), the CV_R can increase at least 3X, as shown in **Figure 5**:



What is Dynamic Range? Why is it important?

Dynamic range is the maximum range of low to high intensity signals that provides a *linear* data relationship (Figure 1). The criterion for linearity failure is a deviation or progressive loss in the regression slope which exceeds 5%.

The importance of extended dynamic range lies in the ability to accurately measure all signal intensities in the experiment from background at low levels to the maximum or saturated signal intensities.

How does the *arrayWoRx*[e] scanner utilize the full dynamic range?

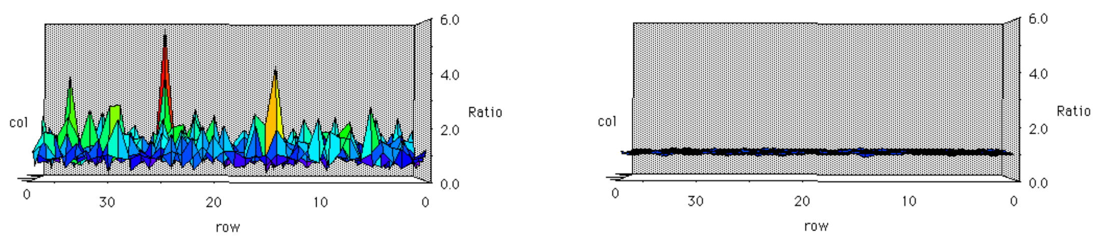
The ***arrayWoRx***[e] scanner has the ability to extend the actual dynamic range *as measured in photons* compared to a PMT-based scanner. This is due to its ability to integrate the signal for a much longer period of time using the highly efficient photon detection of its scientific-grade cooled CCD camera. Combining these advantages, the ***arrayWoRx***[e] scanner provides the researcher the ability to obtain accurate low signal intensities and maximum signals to the full range of 65,536 counts.

PMT based scanners are more limited in Dynamic Range due to limitations in the number of photons they can detect during the short pixel dwell time of the laser. “Turning up the gain” of the PMT amplifies both signal *and* noise. This can increase the values measured, but the variation also increases and the applied gain does not improve the ability to differentiate between low-level spot intensities and the background.

The ability to obtain repeatable results is important for validating data. How reproducible is the *arrayWoRx*[e] system? How does it compare to other scanners?

The ability to take the same slide and scan it forward or backward with identical results is a test easily passed by the ***arrayWoRx***[e] scanner. The ***arrayWoRx***[e] scanner has scanned large arrays (>4,000 spots) on the same scanner on different days, using identically printed slides and different scanning exposure times. The data from these experiments have a high degree of correlation, with an R^2 value greater than 0.95.

PMT Scanner | ***arrayWoRx***[e]



Ratio of Cy3/Cy3 - .998±.4569 (CV = 0.46) | *Ratio of Cy3/Cy3 = .987± 0.0321 (CV = 0.03)*

Figure 6: *Figure 6 is a comparison of measurement accuracy for a PMT scanner and the* **arrayWoRx**[e] *scanner. This test reveals scanner & statistical noise independent of intensity differences on the slide, and there is good agreement between the predicted and actual measurement accuracy of both scanners. The same slide scanned 2x on each scanner. The same area compared from each scan (background, no spots). Each replicate wavelength is overlaid; the pixel ratio coefficient of variation (CV_R) is measured.*

Are there any other factors that should be included with image quality?

Our customers often mention these factors as associated with scanner performance and image quality:

- Sensitivity
- Resolution

How is sensitivity measured for the *arrayWoRx*[e] system?

Spotting known dye concentrations in a sample and measuring the data with the scanner to determine its lower limits of detection will measure the sensitivity of a system.

What is the sensitivity of the scanner for Cy3 and Cy5?

The ***arrayWoRx***^e scanner has a measured sensitivity of better than 0.1 molecules for Cy3/μm^2 and Cy5/μm^2.

What is the highest resolution of the *arrayWoRx*^e system? Are different resolutions available? What resolutions are available with other systems?

The ***arrayWoRx***^e system can acquire images at resolutions of 3.25, 6.5, 9.75, 13.0, 16.25, 19.5, 22.75 or 26.0 µm per pixel, easily selected via software control. Lower resolution (and higher sensitivity) is obtained by "binning" the read-out from the CCD chip. When 2 × 2 camera pixels are binned (i.e., read and summed as a single data point), the resolution becomes 6.5 µm/pixel and the signal increases by a factor of 4. Binning 3 × 3, 4 × 4, etc. (up to 8 × 8) yields image pixels that increment by 3.25 µm to a maximum of 26 µm/pixel. Signal intensities increase with pixel size. Most laser scanners scan at 10 µm/pixel, and several offer 5 µm/pixel size.

The nominal resolution can be significantly degraded by noise. The real determinant of resolution is not the specification sheet but the smallest details a scanner can distinguish. For evaluation, the same slide must be scanned on different systems and compared. Because resolution is a function of high sensitivity and low noise *as well as* pixel size, the ***arrayWoRx***^e scanner provides significantly better resolution compared to similar or higher nominal specifications for other scanners.

Does the *arrayWoRx*^e scanner have a calibration procedure?

Each system is calibrated before it leaves the factory. The camera is calibrated for each wavelength using a process that measures the response of each individual pixel in the camera. A linear regression for each pixel on the CCD is determined for each wavelength to provide flat-field illumination correction. In addition, Applied Precision measures the intensity of the system at installation.

How often should I calibrate the system?

We recommend that you measure the lamp intensity each week and perform a flat-field calibration once every 200 hours of use. To do this, load the flat-field calibration slide and use the scanner software to perform calibration. The calibration procedure takes about one minute per wavelength to complete.

Other systems utilizing different technologies claim that overall scanning speed is an essential need for researchers. Is faster scan time more important or is image quality more important, and why?

The bottleneck for most microarray labs is slide production. Considered within the entire context of the microarray experiment, scanning time usually has a negligible impact on productivity. The most important requirement for researchers is high quality data from each scan. Rapid scans do not derive the maximum benefit from the efforts spent creating the slide. A fast scan limits a scanner's ability to detect the fluorescent signal. With any scanner, the faster the scan, the fewer the number of photons that can be detected. "Blazingly fast scans" are either photon or resolution-limited, with significant detrimental effects on data quality.

I have heard that the *arrayWoRx*^e system allows slides to be loaded either array-side up or down. You recommend imaging the slides array-side down. Why?

Inverting the slide, i.e. illuminating and imaging the spot through the slide, allows for optimal collection of light. The spot-to-glass pathway refracts the emitted signal less than the spot-to-air pathway. The signal is almost doubled when scanning through the slide. This signal optimization can be easily demonstrated by comparing the results of scanning the same slide with the array face up and then again face down**.** Because the ***arrayWoRx***^e scanner can acquire images from either side of a slide, it can scan different slide types such as mirrored or opaque slides.

Section 3: Acquisition & Analysis Software

How easy is it to acquire an image and perform the image analysis?

The ***arrayWoRx*e** scanner was designed to be the easiest to use and best performing scanner in the market.

Obtaining an image and analysis of a slide, the requires the following simple steps:

1. Place slide into scanner, define the boundaries of the array, and click Preview Scan.
2. Set the integration time for each channel and the pixel size (or use the default settings to save time and effort).
3. Click Scan, assign a name, and the image is scanned and automatically saved as a TIFF file.
4. Open the ***softWoRx™* Tracker** analysis software. Select a robot project file and merge it with the TIFF image files.
5. During the process of merging files the data is automatically analyzed in less than a minute. You can then review spot intensities, generate data queries, create data plots, or export information to other databases for further downstream processing (Spotfire, GeneSpring, etc.).

How many slides can the *arrayWoRx*e system scan at a time? Could you describe how the *arrayWoRx*e scanner allows simultaneous scanning, viewing, and analysis of images?

Two models are available: The ***arrayWoRx*e** system for manual single-slide operation and the ***arrayWoRx*e *Auto*** for automated 25-slide operation. In manual loading you insert one slide per scan. In automated loading you load 25 slides into a cassette, which you then insert into the slide tray. Slides are loaded horizontally. The scanner performs batch analysis by allowing acquisition of the image while simultaneously performing image analysis. View a movie of the auto system on our website.

What software comes with the system?

The ***arrayWoRx*e Standard** and ***Auto*** scanners come with one license of ***softWoRx™* Tracker** analysis software on board. The ***arrayWoRx*e Basic** scanner comes with one license of ***softWoRx™* Tracker** analysis software for use offline.

What is the image analysis software and what is included in the package?

The ***softWoRx™* Tracker** analysis package emphasizes data integration and automated image analysis. We achieve complete data integration by combining true sample tracking with data generated upstream from the printing robot, all within a single software environment. The result is that at all of the pixel intensities that you acquire are linked to the protocols used to generate the slide.

Is *softWoRx™* Tracker Windows 2000 compatible?

Yes, as a matter of fact ***softWoRx™* Tracker** runs on Windows 2000 or Windows NT 4.0 with Service Pack 6 or later.

What are the capabilities of *softWoRx™* Tracker?

The capabilities of ***softWoRx™* Tracker** are as follows:

- Automatic image analysis created from the robot printer file
- Fully automated spot finding
- Import & display robot project files
- Track plate replication & reformatting data (e.g., 96-well plate consolidation to 384-well plates)
- Auto-Load printing information from arrayer
- Barcode, Clone, Sample-tracking info
- Supports the following Robot Arrayer project files: Affymetrix, BioRobotics, Cartesian, Gene Machines, Pat Brown (home-made), Packard
- Open framework for capturing user defined annotations, microarray QA notes, scanner acquisition parameters
- Interfaces with image analysis
- Automatic spot confidence/process control flagging

- Three different background subtraction methods
- Open Database Schema for integration into existing or new databases
- Three intensity normalization methods
- Gene Expression scatter plots, with the ability to select points by extensive queries.
- Intensity information automatically linked to upstream slide production information
- Scanner & robot output data integration & management
- User defined annotations.

How many software licenses of *softWoRx™* Tracker do I receive when I purchase an *arrayWoRx*^e System?

The ***arrayWoRx***^e **Standard** and ***Auto*** scanners come with one license. The **Basic** system also comes with one license for loading on an offline workstation

What are the requirements for the offline workstation to run *softWoRx™* Tracker software?

The following are recommended requirements:

- Intel Pentium III or higher, > 1.5 GHz
- 768 GB RAM or more
- 40 GB hard disk
- 100 MB of available hard disk space
- 8X CD or CDRW
- Monitor/video card capable of 1024x768 pixel resolution or higher
- Windows 2000 Professional or Windows Professional XP.

Can *softWoRx™* Tracker track genes by name? From specific databases, Internet, existing downstream analysis packages, such as Biodiscovery GeneSight, Silicon Genetics GeneSpring or Spotfire DecisionSite?

Yes ***softWoRx™* Tracker** is fully compatible with these packages. This software package allows you to obtain the clone information from the robot arrayer and match it with the TIFF file obtained from the scanner. These results can be linked and integrated with local genetic databases, or with databases accessible via either a local intranet or the global Internet. It also has an open database schema that allows you to place the analyzed information into further downstream software analysis packages, such as those listed above. In addition, ***softWoRx*™ Tracker** eliminates the need to cut and paste from Excel files when integrating data from the clones with image analysis. Merging the robot project file with the TIFF images from the scanner is automatic.

You say the software performs automated image analysis. How does it lay out the grid and find spots?

The ***softWoRx™* Tracker** package offers two ways of generating an analysis:

- Automated – To perform an analysis in about 30 seconds (depending on file size), the robot file from the printer is imported into the software. The information obtained includes clones, array size, format, spot size, etc. and any other user defined annotations. This is combined with the 16-bit TIFF image generated by the scanner. The robot files automatically recognize the same image file name types for associating the merge. In 30 seconds the spot image analysis is complete. *This requires no grid laying, typing in numbers, measuring spots moving grids, or any type of grid manipulation normally needed for an analysis.*
- Grid Wizard – Provides a guided template to generate a grid overlay for analysis. This process takes only a few minutes to complete and eliminates all of the measuring and manipulations usually associated with manual grid generation.

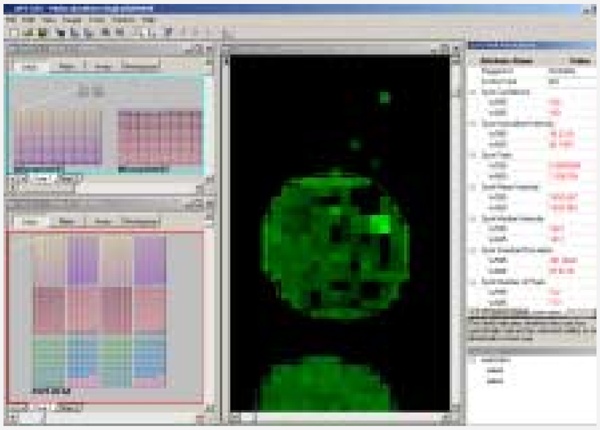


Figure 7. Software screen shot of *softWoRx™* Tracker. The window depicts microtiter-plate information, grid location, image view, measurements, and user defined annotation.

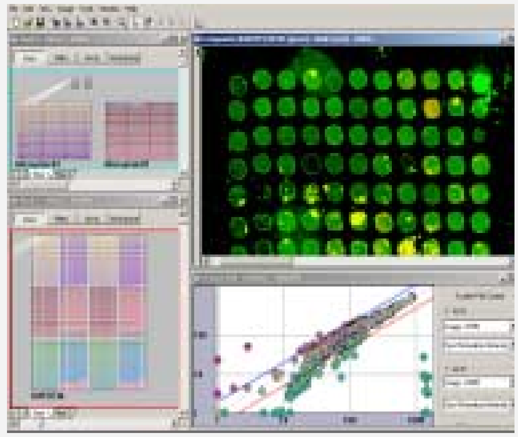


Figure 8. Screenshot depicts the complete process of *softWoRx™* Tracker. Shown are microtiter plate, grid & image, and scatter plot windows.

I have noticed different spot morphologies on my slides. Does the *arrayWoRx*e software have the ability to identify problem spots?

Process control is a key feature provided in ***softWoRx™* Tracker.** Our analysis software package allows the researcher to increase the quality and yield of slides in microarray production. The software performs multiple analyses on every spot in the microarray and compares specific measures with operator-defined values that can indicate potential problems in array fabrication. Dye separation, drying, fading, no spot, etc, are all problems of microarray printing and hybridization, which can significantly affect the results from the sample. This feature allows rapid refinement of printing and hybridization parameters to increase data yield, as well as providing multiple quality metrics for each spot in the dataset.

What types of file formats are used for the image files?

The images are stored in ***arrayWoRx*e** image format, and are normally exported automatically as separate 16-bit grayscale images (one image for each channel).

Can TIFF images from another scanner be imported into *softWoRx™* Tracker?

Yes, as long as the images comply with standard TIFF specifications.

What robot project files do you support to allow automated image analysis?

Currently the software supports:

- Affymetrix
- BioRobotics
- Cartesian Technologies
- Gene Machine
- Pat Brown printer
- Packard
- Virtek

Section 4: System Specifications and Other Common Questions

What are the power requirements for the scanner?

The ***arrayWoRx***e system has an auto-switching power supply for worldwide use. The system requires a grounded, 15 A power supply and either 120 V or 220 V of dedicated (i.e., non-shared) current.

How precise is the positioning used to move the slide during image acquisition?

Stage positioning currently has accuracy within 10 µm (i.e., 3 pixels or less at the highest imaging resolution).

What type of CCD camera is used?

The camera uses a CCD chip with $^1/_4$ million pixels, acquires 14 bits of information, and is cooled to –5 degrees C.

What type of operating system does the *arrayWoRx*e system use?

A PC running the Linux Red Hat 7.0 controls the ***arrayWoRx***e **Basic** scanner. The Linux environment is very similar to Windows or Macintosh in ease-of-use. The processing architecture of the workstation and video card has been specifically designed for large graphics files and is particularly beneficial when working with large scans (which can be up to 400MB for some studies). Finally, the Linux operating system enables the ***arrayWoRx***e system to be connected to any *network (most network servers in the world are running Linux or Unix today). The **arrayWoRx**e **Basic** and **Auto*** systems use a Tower workstation running Windows 2000 operating system for basic PC utilities and as the host for ***softWoRx*** *TM* **Tracker**. Linux Red Hat 7.0 runs the scanner operations as in the **Basic** system.

Is the *arrayWoRx*e *Auto* system compatible with lab automation systems?

The design of the cassette and its door provide possibilities for robotic slide loading.

Is the *arrayWoRx*e system able to read bar codes? If so, what bar-coding systems are compatible?

Yes — the system has an internal bar-code reader that is part of the automation process. The reader recognizes the following formats:

- Code 39
- Interleaved 2 of 5
- Codabar
- EAN-8
- EAN-13/UPC-A
- Code 128
- Standard 2 of 5
- Matrix 2 of 5
- ISBN/ISSN
- EAN/UPC ADDON 2/5
- Code 93
- Chinese 2 of 5

The narrowest bar width the scanner can read is 0.25 mm.