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Kia Peyvan, David Danley

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CombiMatrix Corporation
6500 Harbour Heights Pkwy
Suite 301
Mukilteo, WA 98275 -

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14. ABSTRACT

This contract effort focused on the development of an automated, cartridge-based genotyping system that integrates a multiplex polymerase chain reaction (PCR) assay with a semiconductor microarray that uses electrochemical detection to identify biothreat agents. The DX-100 Genotyping Cartridge System has three subcomponents: a disposable cartridge, the cartridge processing instrument, and a personal computer. The alpha prototype is compact and solid state (no optics) and has a simple three step operation: 1. Load the cartridge with a DNA sample; 2. Load the cartridge in the instrument; 3. Move the operating handle to the front. A personal computer with integrated software controls the instrument's operation, collects

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Integrated Semiconductor-based Diagnostics System for Multiplexed Genomic Amplification and Electrochemical Detection of Biothreat Agents

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This contract effort focused on the development of an automated, cartridge-based genotyping system that integrates a multiplex polymerase chain reaction (PCR) assay with a semiconductor microarray that uses electrochemical detection to identify biothreat agents. The DX-100 Genotyping Cartridge System has three subcomponents: a disposable cartridge, the cartridge processing instrument, and a personal computer. The alpha prototype is compact and solid state (no optics) and has a simple three step operation: 1. Load the cartridge with a DNA sample; 2. Load the cartridge in the instrument; 3. Move the operating handle to the front. A personal computer with integrated software controls the instrument's operation, collects electrical readings from each electrode on the microarray, analyses the data, and provides a graphic output for agent identification. The fluidic cartridge is sealed and contains all of the reagents and a microarray to test for Bacillus anthracis, Clostridium botulinum, Yersinia pestis, Francisella tularensis, Burkholderia pseudomallei, Brucella suis, Vibrio cholera, and Yersinia enterocolitica.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Liu, R.H., T. Nugyen, K. Schwarzkopf, H.S. Fuji, A. Petrova, T. Siuda, K. Peyvean, M. Bizak, D. Danley and A. McShea. 2006 A fully integrated microfluidic CustomArray device fo bacterial genotyping and idenification. Anal. Chem. 78, 1980-1986

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NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

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Total Number:

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NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

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Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
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NAME

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1 b. 1511 6th Ave
4th Floor
Seattle WA 98101

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U.S. ARMY CONTRACTOR FINAL REPORT

INTEGRATED SEMICONDUCTOR-BASED DIAGNOSTICS SYSTEM FOR MULTIPLEXED GENOMIC AMPLIFICATION AND ELECTROCHEMICAL DETECTION OF BIOTHREAT AGENTS

Kia Peyvan and David L. Danley, Ph.D.

Prepared for
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JANUARY 2009

COMBIMATRIX
6500 Harbour Heights Parkway
Mukilteo, WA 98275

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algorithm will attempt to place amplicons that have significant interactions into separate multiplex pools.

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O. **Figure 15**. Photographs of the DX-100 cartridge from the top (**A**) and the bottom (**B**).

P. **Figure 16**. **A**) Melt curves for PCR primers for *B. pseudomallei B. suis V. cholera Y. enterocolitica, and C. botulinum*. The *C. botulinum* primers failed to amplify and the reverse primer was redesigned. **B**) Melt curve for the revised *C. botulinum* primers.

Q. **Figure 17**. Thermocycler test bed for evaluating the performance of the multiplex PCR chamber.

R. **Figure 18**. Results from mixing genomic DNA samples in dry PCR reagents and thermocycling in the serpentine PCR chamber on the breadboard thermocycler, shown in Figure 17. Detection was made using an ElectraSense microarray and reader. **A**) *B. anthracis*, **B**) *B. pseudomallei*, **C**) *F. tularensis*, **D**) *Y. pestis*, **E**) *C. botulinum*, **F**) *V. cholera.*

S. **Figure 19**. **A**) Bar graph showing the average signal from probes for different BTA on an ElectraSense microarray following the amplification, hybridization, and detection of 10 pg of *B. anthracis* genomic DNA in the DX-100 instrument. **B**) Results from the same experiment shown as ROC curves.

STATEMENT OF PROBLEM STUDIED

The Department of Defense, through the Joint Program Executive Office for Chemical and Biological Defense, has fielded a number of biological threat agent (BTA) identification, detection, and diagnostic systems. They range in complexity, sensitivity, and form factor from the individual handheld immunoassay to integrate vehicle-mounted field monitory systems. This contract effort focused on the development of a cartridge-based genotyping system that integrates a multiplexed polymerase chain reaction (PCR) assay with a semiconductor microarray that uses electrochemical detection (ECD) to identify BTAs based upon their unique genetic signatures.

The goals of this effort are:

1) To integrate a large number of individual PCR assays into a sealed, disposable cartridge so as to reduce the risk of laboratory contamination with PCR amplicons and allow multiplex genotyping using an integrated semiconductor microarray.
2) To significantly reduce the current logistics burden required to support PCR by using a small instrument to process and read the cartridge.
3) To significantly reduce the current level of user training through instrument automation and computer analysis

SUMMARY OF RESULTS

The DX-100 Genotyping Cartridge System has three subcomponents: the disposable cartridge, the cartridge processing instrument, and a personal computer. As illustrated in **Figure 1**, the alpha prototype delivered under this contract is a compact instrument and cartridge that offer a simple three step operation:

1. Load the cartridge with a DNA sample
2. Load the cartridge in the instrument.
3. Move the operating handle to the front and close the light tight door.

Figure 1. Photograph of the DX-100 Genotyping Cartridge System comprised of a disposable cartridge and a cartridge processing instrument.

Supporting the operation of this instrument is a personal computer (PC) with integrated software (not shown) that controls the system's operation, collects electrical readings from each electrode on the microarray, analyses the data, and provides a graphic output for agent identification. Details on these subsystems follows.

Fluidic Cartridge

The fluidic cartridge (**Figure 2**) is designed to be a sealed consumable that contains all of the reagents and a microarray to conduct a multiplex PCR genotyping assay for the following biothreat agents.

Bacillus anthracis
Clostridium botulinum
Yersinia pestis
Francisella tularensis
Burkholderia pseudomallei
Brucella suis
Vibrio cholera
Yersinia enterocolitica

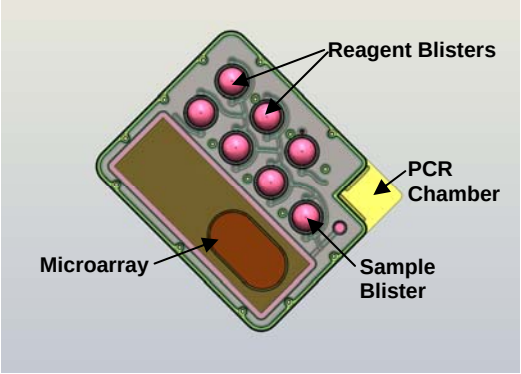


Figure 2. Illustration of the DX-100 cartridge for PCR amplification and microarray analysis.

As illustrated in **Figure 3**, the cartridge is comprised of three polymer layers. The top layer is rigid polycarbonate, the middle layer is silicone elastomer and the bottom layer is polypropylene. Once these layers are sandwiched together with a 12K ElectraSense® microarray, they form reagent blisters, fluidics channels and valves, a multiplex PCR chamber, a hybridization chamber, and a waste chamber.

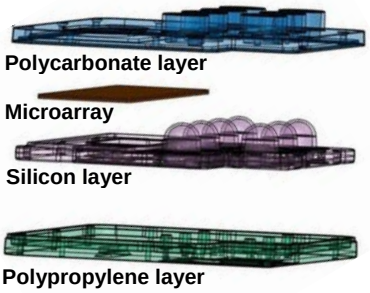


Figure 3. Graphic illustration showing the three layer of the fluidic cartridge.

Central to the design of this cartridge is the use of a silicone layer that has molded into it the reagent blisters, fluidics channels and duckbill valves (**Figure 4A).** For this design to operate correctly, two duckbill valves are required for each blister (**Figure 4B**). A high pressure valve on the outlet channel keeps fluid in the blister until it is depressed by the actuator and a low pressure valve keeps the blister from pushing its contents out the return channel. Reagent is expressed from a blister using a mechanical actuator; and the fluid can flow to a desired location (i.e., the hybridization chamber), oscillated for mixing, and returned to its original blister or sent to the waste chamber.

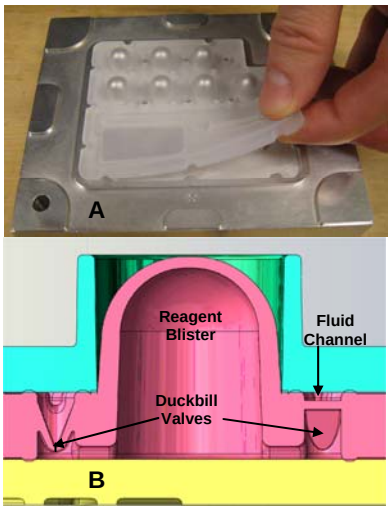


Figure 4. A) Silicone layer being removed from its mold. B) Tangential illustration of a reagent blister molded in the silicone layer.

The only active valve on the cartridge is used to seal the PCR chamber during amplification. This is required because of the pressure differentials that are created during thermocycling. The trade off in this design over those systems that use pneumatic pressure to control fluid flow is the requirement for each blister to have a dedicated actuator to express its contents. This approach provides exquisite control of fluid flow that makes it a viable alternative to a pneumatic system.

To test the integrity of reagent filled cartridges, colored solutions were loaded into each blister and the cartridges were shaken and dropped to determine whether the fluids would leak from the blisters. Leaks were addressed by changing the configuration of the silicone layer. For instance, raised shoulders were added along the fluid channels in the silicon layer to ensure even compression between the rigid top and bottom layers. The final test was to subject the cartridge to reduced air pressure equivalent to an airplane flying at 10,000 feet. Filled cartridges were placed in a vacuum chamber and the air pressure was lowered from 14,696 psi (29.92 inHg) to 10.1 psi (20.58 inHg). Leaks were observed at the duckbill valves, and the problem was corrected by sealing filling and vent ports with tape and placing the cartridges in a sealed bag inside a rigid can for shipping (**Figure 5**).

Figure 5. Photograph of a fluidics cartridge in a plastic bag and positioned in a rigid metal box for shipping. The box holds three cartridges.

The sides and bottom of the PCR chamber is molded into the bottom layer of the cartridge, which is polypropylene (**Figure 6**). The chamber is serpentine and has four legs, each of which can be loaded with a different primer set for multiplex PCR. For this study, two primer sets (A1 and A2) were created.

A1 to amplify sequences for

B. anthracis: flag, lef, CapA
F. tularensis: mviN

Y. pestis: tonB, lcrF, murine toxin, and plasminogen activator

A2 to amplify sequences for
B. pseudomallei
B. suis
V. cholera
Y. enterocolitica
C. botulinum

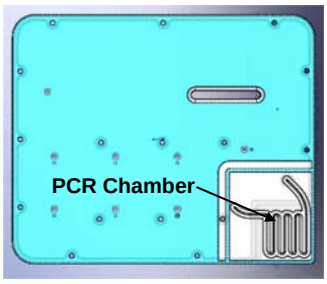


Figure 6. Illustration of the bottom polypropylene layer with the PCR chamber.

The following steps are used to assembly the cartridge

1. After manufacture, the three layers are cleaned. The rigid top and bottom layers are washed in RNase ZAP, rinsed and dried. The silicone middle layer is washed with lab detergent, rinsed and dried.
2. For the bottom polypropylene layer, 0.5µl of each primer pool is pipetted into separate legs of the PCR chamber and the allowed to dry. To prevent premature mixing of the primer pools upon addition of the PCR reagents, the dried primers are coated with a small amount of PCR-grade wax (DyNawax).
3. Following application of the primer pools, the PCR chamber is sealed by heat stacking two layers of film over the bottom layer. Silicone film (5µ thick) is placed on the board and a layer of 3M 9967 heat seal film is placed over it. Heat stacking is done at 385ºF for 8sec under adequate pressure to ensure sealing but not distort the bottom layer.
4. The hyb chamber and sample blister, which are molded in the middle silicone layer, are treated to prevent non-specific sample binding to the silicone. For treatment, the chambers are filled with a 100nM random 10-mer oligonucleotide in 2xPBST for 1h. The solution is removed, and the chambers are allowed to dry without rinsing. The chambers are filled with a casein block solution (BioFX Labs) for 1h, rinsed in distilled water, and dried.
5. For final assembly the three parts are stacked with an ElectraSense microarray inserted between the middle and bottom layers such that the hyb chamber in the former is aligned with the electrode array to form a seal between the two. For prototype cartridges, small screws are used to fasten the bottom and top layers and create the proper pressure between them and ensure proper sealing of the fluidic pathways in the silicone layer.
6. Following assembly, the cartridges are filled with liquid reagents through ports on the bottom layer, which are sealed using SealPlate™ adhesive sheets for 96-well plates. For transportation, the vent port on the waste chamber is sealed with a small piece of adhesive plastic to reduce effects on changes in atmospheric pressure. Prior to use, the plastic is removed and replaced with a Porex® microporous disc with adhesive backing.

DX-100 Instrument

The DX-100 was designed, developed, and fabricated by a subcontractor, Synapse, to interface with the microfluidic cartridge and provide computer-controlled operation. **Figure 7** is a CAD drawing illustrating the components and subsystems within the DX-100. **Figures 8A** and **B** are pictures of the finished instrument with and without covers respectively.

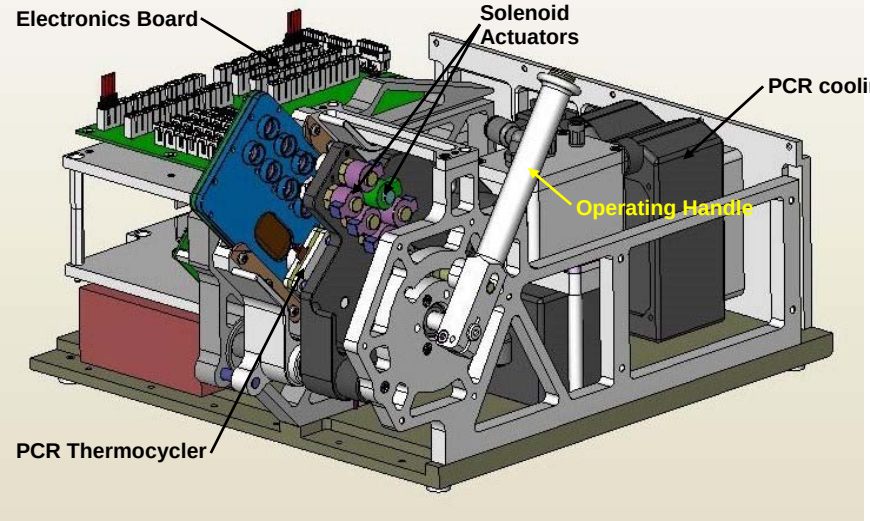


Figure 7. Graphic illustration of the components and subsystems in the DX-100 instrument.

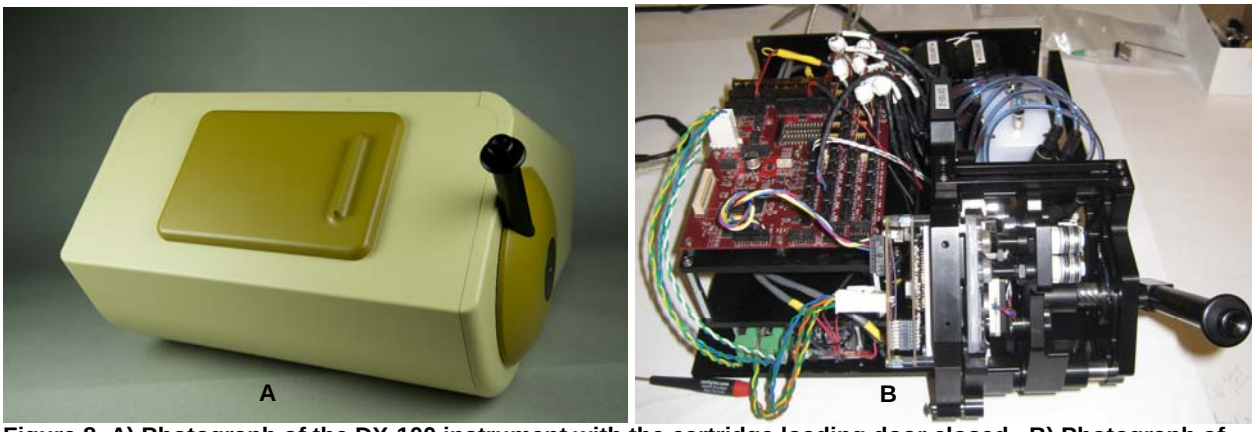


Figure 8. A) Photograph of the DX-100 instrument with the cartridge loading door closed. B) Photograph of the internal components and subsystems in the DX-100.

Firmware and software for the DX-100 were completed. This package includes protocols that use a standard PC running Windows XP to serve as the instrument controller and data analysis platform for genotyping BTAs. General operation of the software is described below, and more detailed information can be found in the User Manual (see Appendix). **Figures 9A-D** illustrate the opening screens for instrument operation. The software initially prompts the user to set up the assay by asking for a cartridge identification number and for a name that can be assigned to the data file (**Figure 9A**). The user is instructed to insert the cartridge into the instrument (**Figure 9B**) and continue the process by closing the handle and light tight door (**Figure 9C**). The instrument will run an electronic check to ensure that each electrode on the array is being

addressed. The software will instruct the user to reinstall the cartridge if it detects a problem (**Figure 9D**).

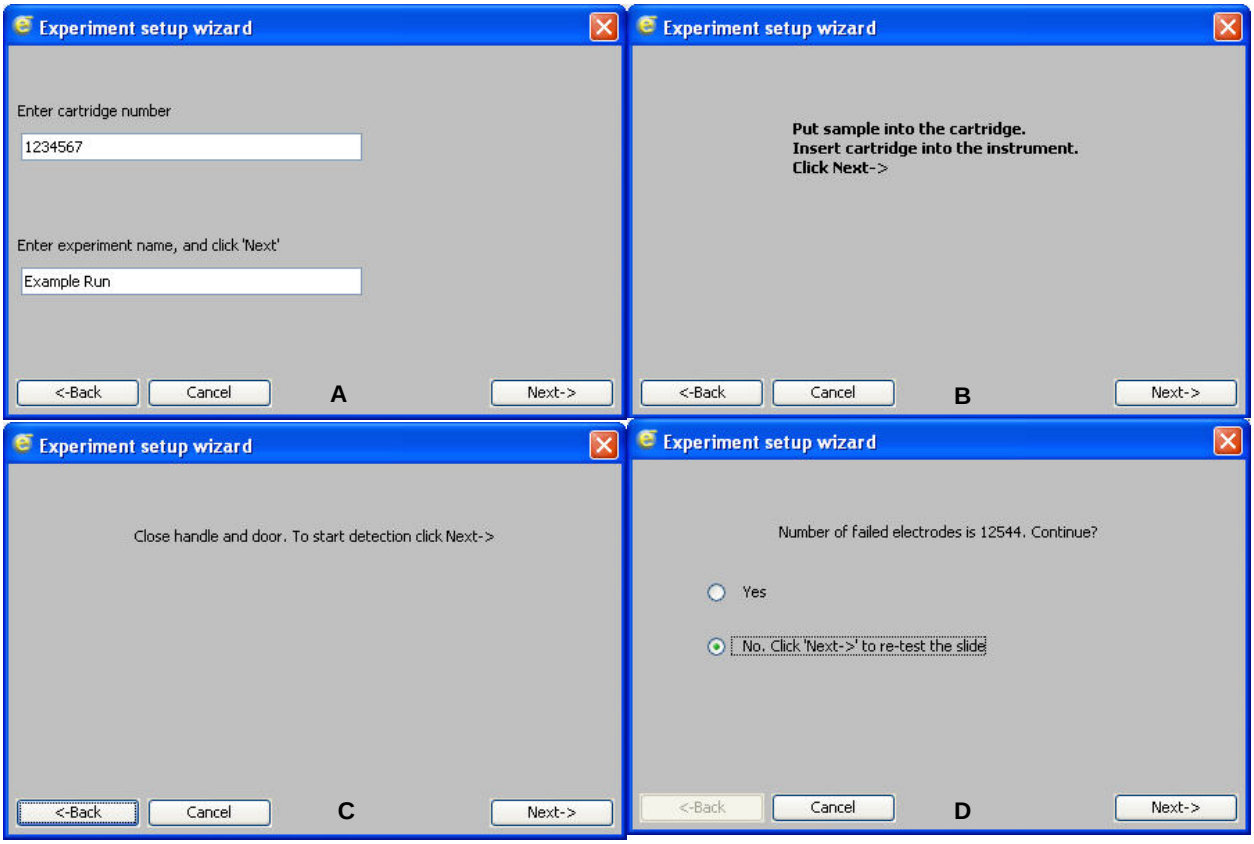


Figure 9. Screen shots of the sequential opening windows from the DX-100 Experiment Setup Wizard showing : A) request for experiment input data, B) instructions to insert cartridge, C) instructions to close the handle and door to initiate detection, and D) results from the electronic check.

During the run, an Experiment Window is shown (**Figure 10A**) to inform the user about the progress of the assay. At completion, the results are displayed in a Data Window (**Figure 10B)**, which compiles the information from the array layout file with the measurements taken off each electrode. This window also displays a grey scale image of the array based upon the amplitude of the current at each electrode – the higher the current, the brighter the electrode on the display. The user can highlight specific electrodes on the image and find immediate reference to probes in the layout file.

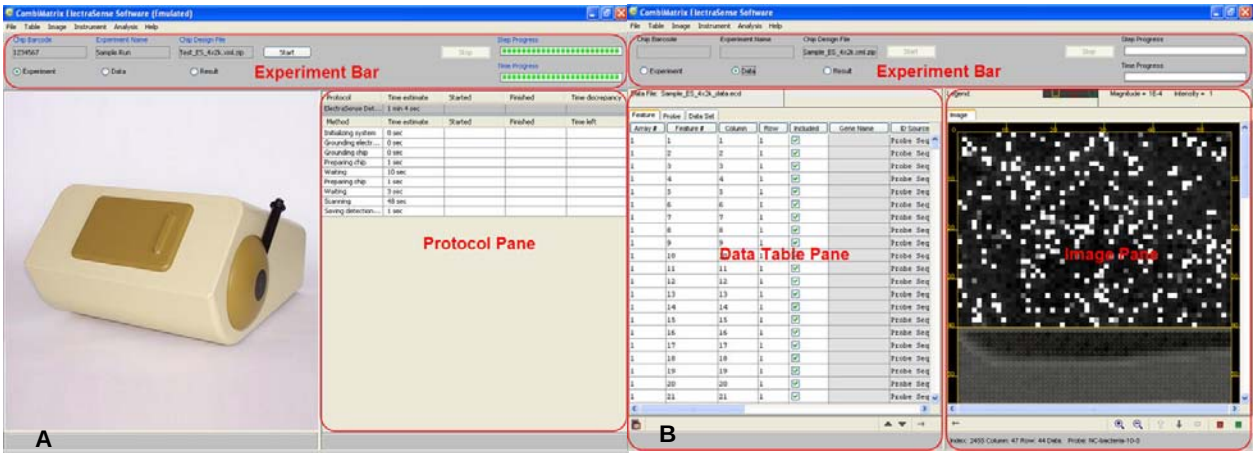


Figure 10. Screen shots from the DX-100 Experiment Bar software showing: A) the Protocol Pane with the status of the instrument's progress, and B) the Data Table results from the analysis.

Data from an experiment can be analyzed further using two subroutines that are illustrated in **Figures 11** and **12**. In the first (**Figure 11)**, the results for the probes that are unique to an organism or virulence factor are simply averaged and compared to the average results from probes that define other BTAs. The user can define a limit (e.g., three standard deviations above background) above which results are considered positive. This simple approach has proven very effective in identifying both bacteria and viruses in complex samples where unanticipated cross reactivities at a single probe are minimized by averaging with un-hybridized probes belonging to the same set.

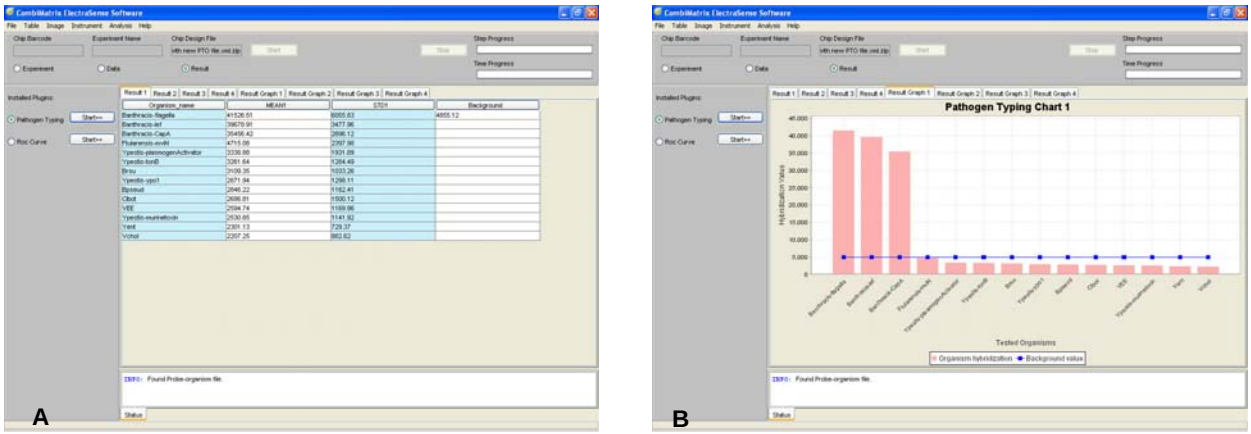


Figure 11. Data analysis screens showing an averaging method for BTA identification. A) Readings from probe sets for each organism or virulence factor are averaged and B) displayed on bar graph. The blue line represents a user-defined limit (e.g., three standard deviations above background) above which results are considered positive.

The second method applies ROC analysis to each set of BTA probes, which provides probability statistics to agent identification (**Figure 12**). In this subroutine, the user is provided with three levels of false positive rates (1%, 3%, and 5%), and the probability that a set of results is positive is listed for each rate (**Figure 12A**). As the false positive rate increases the probability of a set of results being positive also increases, which demonstrates the inverse relationship between specificity and sensitivity. Results can also be represented as ROC curves (**Figure 12B**).

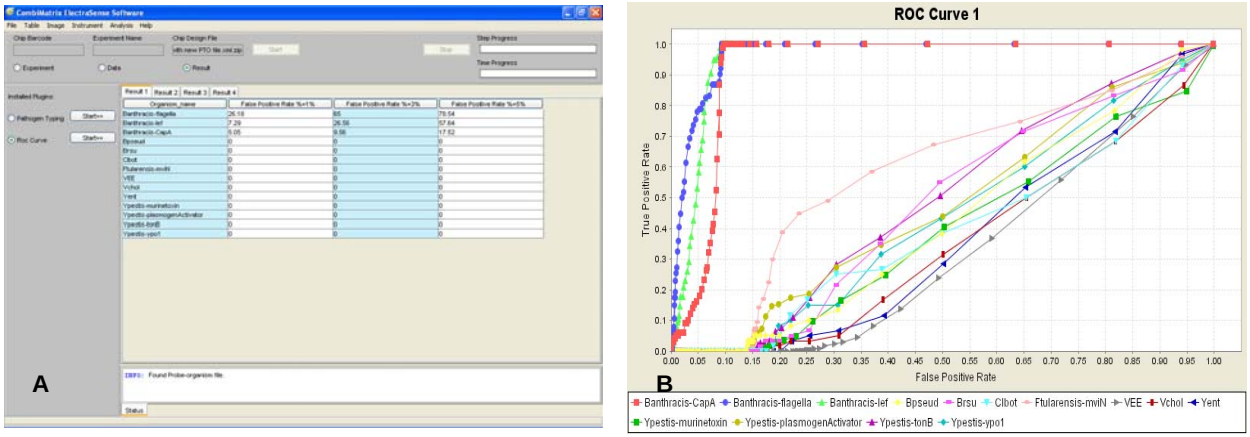


Figure 12. Data analysis screens showing BTA identification using ROC analysis. A) The program provides the user with three false positive rates and the probability that a set of results is positive for each rate. B) Results for all data sets can be represented as ROC curves (at right).

Biothreat Agent Assay

As reported above, the multiplex PCR amplification and microarray assay were designed to identify the following:

B. anthracis: flag, lef, CapA
F. tularensis: mviN
Y. pestis: tonB, lcrF, murine toxin, and plasminogen activator
B. pseudomallei
B. suis
V. cholera
Y. enterocolitica
C. botulinum

Sequence Source: For each organism its genus and species were resolved to a taxonomic ID, as given by NCBI. Tax browser from NCBI was used to identify all related GenBank records that are associated with that organism. The full GenBank record of each organism's genome and plasmids were downloaded, and the gene sequences were extracted. Typically, there are ~2000 genes in each bacterial genome and a few hundred genes in the larger plasmids. For each of these genes, a labeling scheme was generated that allows the designer to know the origin of the gene whether it comes from a plasmid or genome.

The specificity of each gene or gene region was determined so that each amplicon unambiguously identified a specific agent or plasmid. An appropriate list of genomes was used to create a context database that contained the entire phylogenetic neighborhood of the target organism. For example, for *B. cereus* (taxid: 1396), the *Bacilli* context database (taxid: 186817) would be used. This phylogenetic neighborhood would correspond to a database of ~134 genomes. Alternatively, if amplicons for multiple organisms were being designed, then the entire database of bacterial genomes would be used as context. Unique amplicons were identified by using the Basic Local Alignment Search Tool (BLAST) in which each gene from the genome of interest was run against the context database. Each hit was parsed from one gene to a given genome. Each set of contiguous hits were mapped to the gene sequence. Each gene to

genome hit was defined from the perspective of the gene. Each gene to genome hit was calculated by computing the average similarity across each gene sequence. Multiple hits from one gene to a genome were allowed by the algorithm. Therefore, the algorithm was designed to detect orthologs as well as paralogs in neighboring and self genomes. Hits greater than 55% were considered significant. After characterization of each gene, amplicons were chosen within genes that were unique to the genome of interest. Amplicons represented unique gene regions and were designed to be $\leq$500bp long for effective PCR amplification.

To develop probes for the microarray, they were first designed across the entire unique gene region, regardless of size. Tiling was used to rendered gene regions into shorter oligomer probes. These probes were designed to have a melting temperature (Tm) of 72ºC. The following formula was used to calculate the melting temperature of a given probe (SantaLucia, J. and Hicks, D. 2004).

Tm = dH/(dS + R * Ln(Ct/4)) + salt,

Where dH, dS Enthalpy and Entropy, respectively are determined by means of a nearest neighbor look-up table in Fig 900,

R is the universal gas constant,

Ct is the DNA strand concentration, in this case 1 uM

Salt = salt correction = 16.6 log10(1.7) + 16.6*log10(totalSalt/(1.0+0.7*totalSalt))

TotalSalt = Na Concentration + 4 * sqrt(Mg concentration)

Where Na Concentration is set to 0.33M

And Mg Concentration is set to 0.0 M

Once the probes were designed, they were evaluated against a proprietary quality criteria algorithm. A probe was rejected if any of the following criteria were met.

- The GC-content was outside a range of 35-65 %
- The length was outside the range of 15-40 bases
- There were five or more repeats of a single base [TTTTTT]
- There were four or more dual repeats of two different bases[TATATATATA]
- There were palindromes (the antisense of the sequence region is the same as the sense: *ie* ttatctGCCCCCGGGGCtatta) of 7 or more bases
- There was a secondary structure, such as a hairpin, having a melting temperature of approximately 60°C or more (note that local concentration is set to 1 molar for calculation of melting temperature of secondary structures)
- There is a duplicate of the same probe

Usually, a 500 bp region produced approximately 30 useable probes. Each probe was "BLASTed" against the same context database as before. The specificity for each probe was reported as the number of hits to other genomes. A hit was considered significant if the calculated Tm for the aligned region corresponded to a Tm that was within 15ºC of the Tm of the probe itself. For a completely unique probe, having a single hit would indicate a completely unique probe. However, as this was not always the case, runs of probes that had the lowest

number of hits were selected. These regions became the start and end of each amplicon. Forward and reverse primers were designed from the first and last probes from each contiguous set of probes. The forward primer was designed to have a Tm of 55ºC. The reverse primer was designed to have a Tm of 70ºC. This allows amplification to be performed in two stages. The first generates the dsDNA amplicon, while the second stage, performed at a higher temperature, generates the antisense single-stranded DNA.

For each organism of interest, at least 5 potential amplicons were chosen and tested individually to determine amplification specificity and efficiency. From this evaluation, sets of compatible amplicons were chosen based on whether their primers could engage in primer-dimer formation. Primer-dimers form when compatible 3' ends appear in a mix of primers. This is a major contributor to poor performance in multiplex PCR, and it becomes progressively more difficult to choose completely compatible 3' primer ends within multiplex pools that contain more than 15 amplicons.

To identify the 8 BTAs and select virulence factors with our cartridge, a "choice" algorithm was used to calculate all possible interactions for all primers in a multiplex set (**Figure 13**). Once all interactions were calculated, the algorithm created two non-interacting clusters by a process similar to k-means clustering

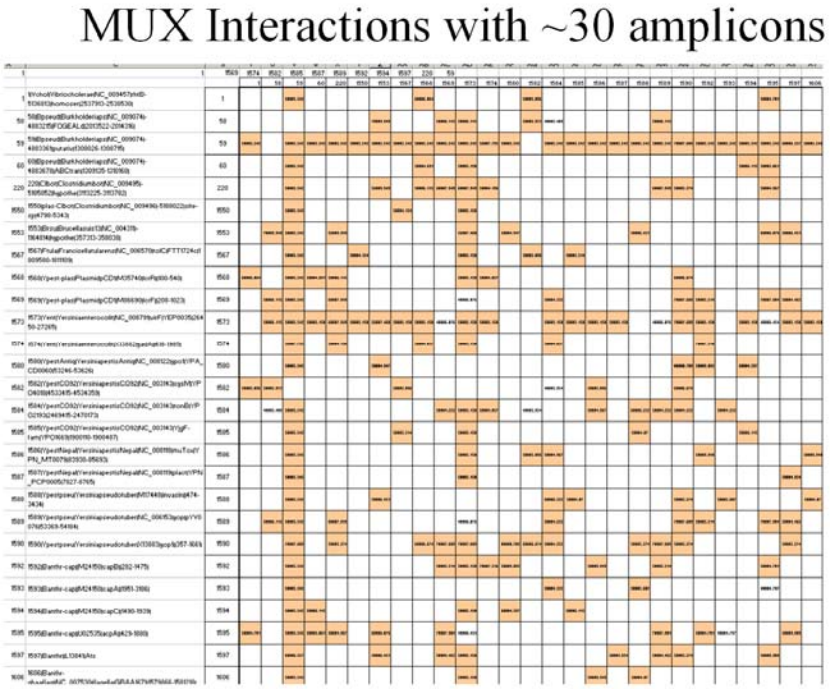


Figure 13: Primer dimer pairs calculated for a set of 30 amplicons. Orange numbers in the grid are interactions that are considered significant and that should be avoided. The algorithm will attempt to place amplicons that have significant interactions into separate multiplex pools.

Based upon the results of these analyses, two primer pools were developed. The A1 pool includes primers for *B. anthracis* - flag, lef(pXO1), and CapA (pXO2); *F. tularensis* - mviN; and *Y. pestis* - tonB, lcrF, murine toxin, and plasminogen activator. The A2 pool includes primers for *B. pseudomallei, B. suis, V. cholera, Y. enterocolitica, and C. botulinum.*

Amplification

To generate biotinylated amplicons for ECD on the microarray, a two stage PCR amplification reaction was used as illustrated in **Figure 14**. The forward primers in each primer pool were designed with a Tm of 55ºC. The reverse primers in each primer pool were designed with a Tm of 70ºC, and the 3' end was biotinylated.

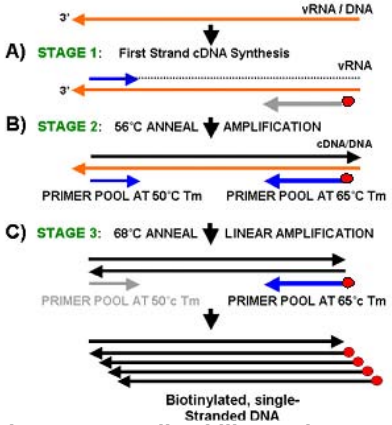


Figure 14. Stylized illustration showing the two-stage PCR reaction using biotinylated reverse primers to generate labeled amplicons.

Figure 15 illustrates the different chambers and features of the DX-100 cartridge from top and bottom views. As shipped, the blisters were filled with the following reagents:

Blister	Reagent
0	350 µL TMB Conductivity 1 Component HRP Microwell Substrate
1	350 µL 2xPBST (2x phosphate buffered saline with Tween 20)
2	4 pellets of Clontech Sprint Advantage® Dry PCR reagent
3	Empty
4	350 µL 5xPBSC (5x phosphate buffered saline with casein)
5	350 µL 0.5xSSPE buffer
6	350 µL 1000:1 BSA Peroxidase Stabilizing Solution: Poly HRP 80 Streptavidin

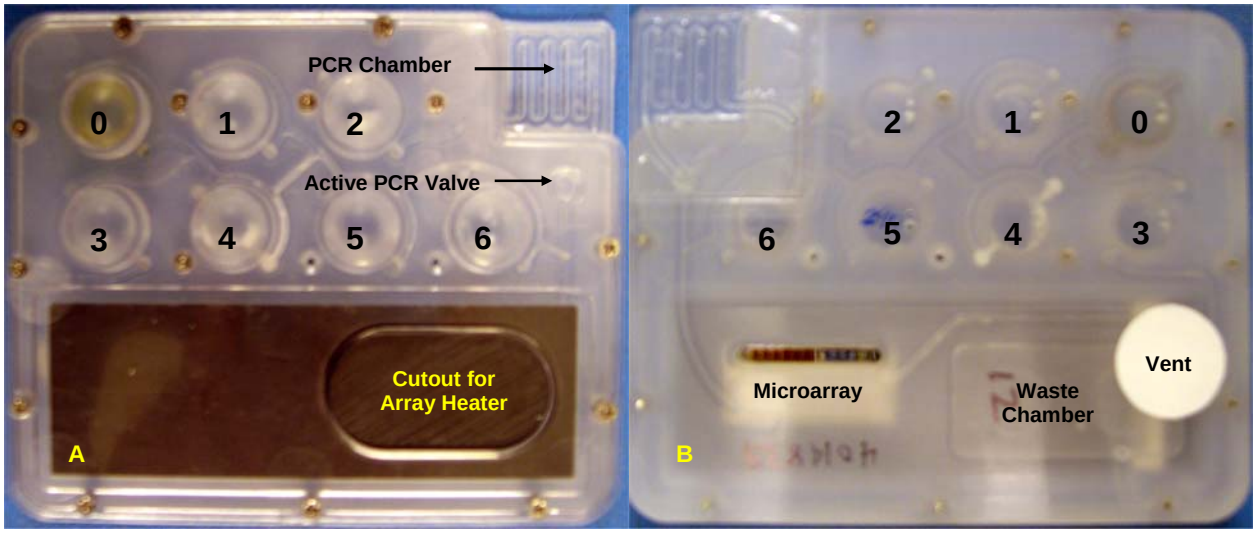


Figure 15. Photographs of the DX-100 cartridge from the top (A) and the bottom (B).

Assay

A. Tab 3 on the back of the cartridge (not shown in **Figure 15**) is peeled back to expose the fill hole on blister 2. A 200 µl of DNA sample is loaded into the chamber, the tab is replaced, and the cartridge is gently shaken before insertion into the DX-100.

B. Following assay initiation, the DX-100 depresses blister 2 to push the PCR mix into PCR chamber. The instrument closes the active valve to contain the mixture in the chamber during thermocycling.

C. The PCR chamber is heated to 95˚C for 120 seconds to denature HotStart antibodies and activate the Taq polymerase

D. For the first PCR stage 30 three-step PCR cycles are performed starting with a cycle of 95˚C for 37 secs, 41˚C for 37 secs, and 72˚C for 52 secs. For each subsequent cycle, the annealing temperature (41˚C on the first cycle) is increased by 1˚C until the thirtieth cycle is reached.

E. For the second state PCR. 20 two-step PCR cycles are performed at 95˚C for 37 seconds and at 65˚C for 70 seconds.

F. The instrument opens the PCR valve and depresses blister 2 again, which pushes the amplified sample into the hybridization chamber.

G. The amplified material is heated to 48˚C on the microarray for 1h.

H. To clear the hyb chamber, blister 3 (air) is depressed, released, and depressed, which withdraws the sample from chamber into the blister and expresses it into the waste chamber.

I. Blister 5 is depressed to add 0.5xSSPE to the hyb chamber for washing. The blister is repeatedly oscillated for 1.5 minutes to create agitation and it then withdraws the SSPE back into blister 5.

J. Blister 4 is depressed to add 5xPBS with casein to the hyb chamber to blocked non specific binding by the ECD reagents. After 15min, the PBS-casein is withdrawn back into blister 4.

K. Blister 6 is depressed to add BSA Peroxidase Stabilizing Solution: Poly HRP 80 Streptavidin. After 15min, the solution is withdrawn back into blister 6.

L. Blister 4 is depressed to add 5xPBS with casein to the hyb chamber and wash the hyb chamber 1min. The solution is withdrawn back into blister 4.

M. Blister 1 is depressed to wash the hyb chamber with 2xPBST for 1 minute. The solution is withdrawn back into blister 1.

N. Blister 0 is depressed to add TMB Conductivity 1 Component HRP Microwell Substrate is pushed into hybridization chamber to react with bound HRP and create a current for ECD. After reading, the substrate is withdrawn into blister 0.

Findings

To evaluate cartridge performance and make appropriate changes, its functions were divided into two efforts: PCR amplification and microarray hybridization and detection. Each primer pair was tested against a complementary DNA sample using a commercial thermocycler, and melt curves were measured to ensure primer performance. As illustrated in **Figure 16A**, all of the primer pairs in the A2 pool amplified their respective DNA samples except for *C. botulinum.* The reverse primer (biotin-labeled) for the *C. botulinum* was redesigned and subsequently demonstrated excellent amplification (**Figure 16B**).

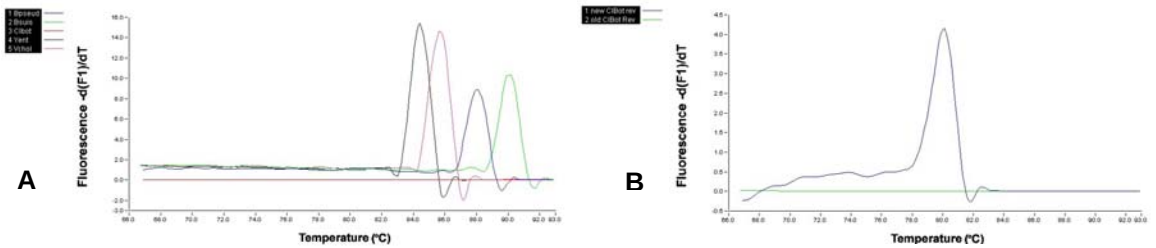


Figure 16. A) Melt curves for PCR primers for *B. pseudomallei B. suis V. cholera Y. enterocolitica, and C. botulinum*. The *C. botulinum* primers failed to amplify and the reverse primer was redesigned. B) Melt curve for the revised *C. botulinum* primers.

After the performance of the individual primers was established, their performance in a multiplex PCR reaction was evaluated using a breadboard instrument shown in **Figure 17**. This breadboard included a sealed PCR chamber with the serpentine design (described previously) and a thermocycler designed specifically for the PCR chamber. This test bed provided critical information on the performance of the PCR chamber and Peltier thermocyclers. Successful PCR amplification was achieved by using matched Peltier heaters with liquid cooling to rapidly dissipate heat. Because the heaters reside on either side of the PCR chamber, temperature sensors also had to be matched and strategically placed in the thermocycler so that the system could be tuned to achieve even, consistent heating in the PCR chamber. Using insulation and a modified commercial temperature sensor, consistent thermal cycling was achieved with this design, which was adapted to the DX-100 instrument.

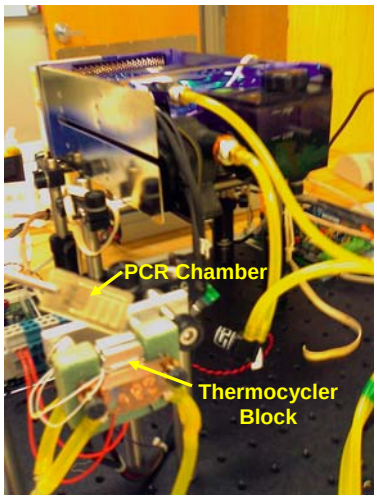


Figure 17. Thermocycler test bed for evaluating the performance of the multiplex PCR chamber.

Leakage from the PCR chamber proved to be a problem during thermocycling. Because of the broad temperature swings that occur repeatedly during PCR, the cartridge is subjected to physical stresses from expanding liquids as well as bonding issues from temperatures that cause adhesives to soften and fail. To eliminate leaking during thermocycling, springs were added behind each Peltier to apply even pressure on both sides of the PCR chamber. In addition, the polypropylene surface of the PCR chamber was milled to ensure its flatness prior to heat stacking the silicon film to enclose the PCR chamber.

Figure 18 illustrates the results from six experiments in which PCR chambers were prepared with both A1 and A2 pools and 10pg of genomic DNA were mixed with dry PCR reagents and added to the chamber for thermocycling. After the reaction, the mixture was aspirated and incubated on a separate ElectraSense microarray, which was read using ECD in an ElectraSense reader.

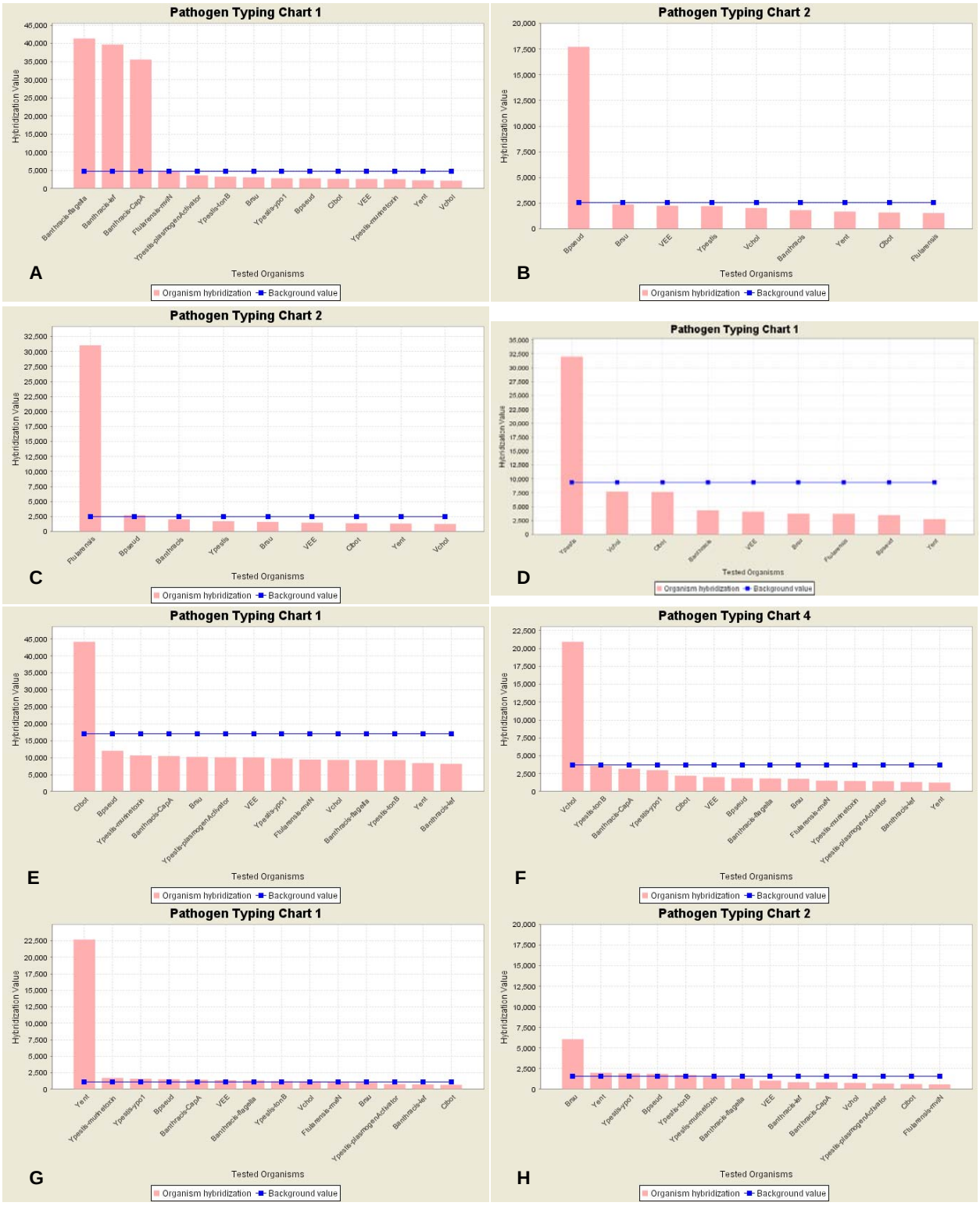


Figure 18. Results from mixing genomic DNA samples in dry PCR reagents and thermocycling in the serpentine PCR chamber on the breadboard thermocycler, shown in Figure 17. Detection was made using an ElectraSense microarray and reader. A) *B. anthracis*, B) *B. pseudomallei,* C) *F. tularensis,* D) *Y. pestis,* E) *C. botulinum,* F) *V. cholera,* G) *Y. enterocolitica,* H) *B. suis.*

Figure 19 illustrates initial results from integrating the primer pools into the PCR chamber on the cartridge, loading the sample blister with 10 pg of *B. anthracis* DNA, and running the entire amplification/hybridization/detection process on the DX-100. As can be seen from the bar chart (**Figure 19A**), PCR amplification in the cartridge on the DX-100 was not as robust as that observed in the breadboard instrument. Nevertheless, the bar chart and ROC curves (**Figure 19B**) clearly show that *B. anthracis* DNA was detected above background.

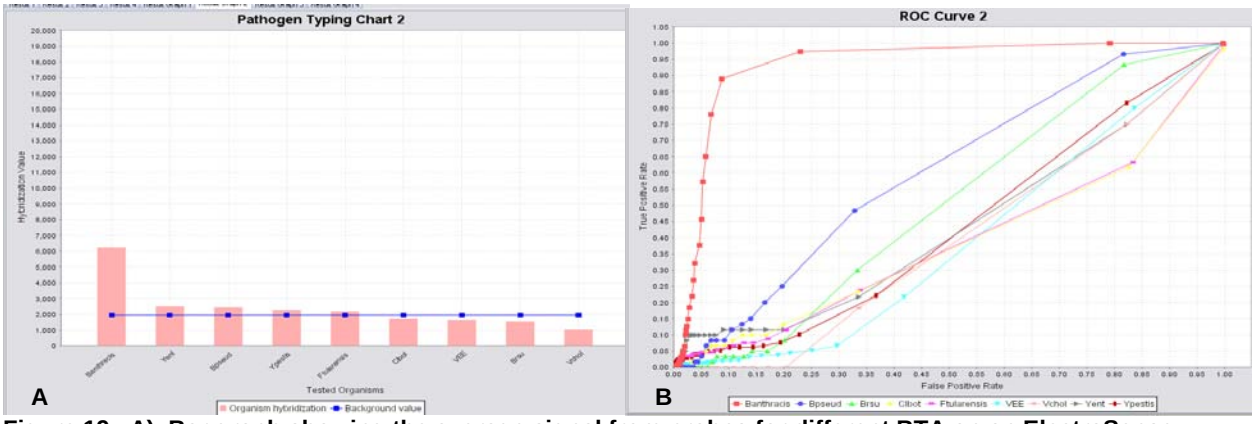


Figure 19. A) Bar graph showing the average signal from probes for different BTA on an ElectraSense microarray following the amplification, hybridization, and detection of 10 pg of *B. anthracis* genomic DNA in the DX-100 instrument. B) Results from the same experiment shown as ROC curves.

Further testing at CombiMatrix and USAMRIID on the DX-100 has been delayed because of hardware issues. The connection between the PC and the DX-100 was dropping at random, which caused the assay to fail. Following extensive testing, the source of this problem appears to be related to a subroutine running in the microprocessor. New code has been written and is being tested. CombiMatrix investigators will continue to resolve all mechanical and software issues with the DX-100 and collaborate with investigators at the US Army Research Institute of Infectious Diseases to ensure that the instrument, delivered 13 November 2008, is fully functional.

BIBLIOGRAPHY

SantaLucia, J. and Hicks, D. 2004. The thermodynamics of DNA structural motifs. *Ann Rev Biophys Biomol Struct,* **33:** 415-40.

APPENDIX A

OPERATOR'S MANUAL

DX-100 GENOTYPING CARTRIDGE SYSTEM

COMBIMATRIX

6500 Harbour Heights Parkway
Mukilteo, WA 98275

Table of Contents

CombiMatrix DX100

Introduction

The **CombiMatrix DX100** system integrates software and hardware to automate sample handling for amplification, hybridization and reading microarray results with ElectraSense® microarrays. ElectraSense microarrays are read electronically by addressing the individual electrodes on the microarray after hybridization.

The ElectraSense Software allows data to be displayed visually and allows some basic processing of the signal data from the DX100 to produce data files more useful for further analysis.

The ElectraSense Software takes as input a chip design file, which gives detailed information about the placement of probes on the microarray, and data from the DX100. The program will produce two types of files, data from the instrument in files ending in (.ecd) and tab-delimited text files with data based on this information combined with information from the chip design file in table form.

Optional plug-in packages for analysis can also produce tables and charts based on the read data.

System Specifications

Microarray Format

ElectraSense® 12K Microarray

Footprint

12.5" w X 9.0" h X 16.5" d (31.75 x 22.86 x 41.91 cm)

Allow 12.0" (30.48 cm) vertical clearance to operate chamber clamp lever

Weight

30.5 lb. (13.84 Kg)

Electrical Input

100-240VAC

50/60Hz

400Watts max,

Fuse=6.3A (250v rating)

Temperature Requirements

Room temperature. (20-28 degrees C)

Minimum Computer Requirements

Minimum Computer Requirements

Intel Pentium IV processor 2 GHz or higher

512 MB RAM

USB Port

Serial Port or USB to Serial adaptor

Operating System

Microsoft® Windows XP Pro or Windows 2000 Pro

Software Requirements

ElectraSense® Software

Report Output Types

Data (ECD or Text Format)

Image (BMP, PNG, or TIFF)

Installation

Instructions for connecting the DX100 to a controller PC and installing the necessary software.

Connect USB, Serial, and Power Cables

The back of the DX100 has a connection for power, a USB port for connection to the PC controller, and two serial ports.

1. Connect the USB cable provided to the back of the reader.
2. Connect the serial cable to the serial port marked Thermal Controller.
3. Connect the power cable provided to the back of the reader and plug the other end into a power source. A surge protector is recommended.
4. Turn the power switch to 1.

Install ElectraSense® Software

Installation of the ElectraSense Software application requires the user to have Administrator privileges on the PC being used. One indication that the user does not have correct privileges to install the software would be the following message.

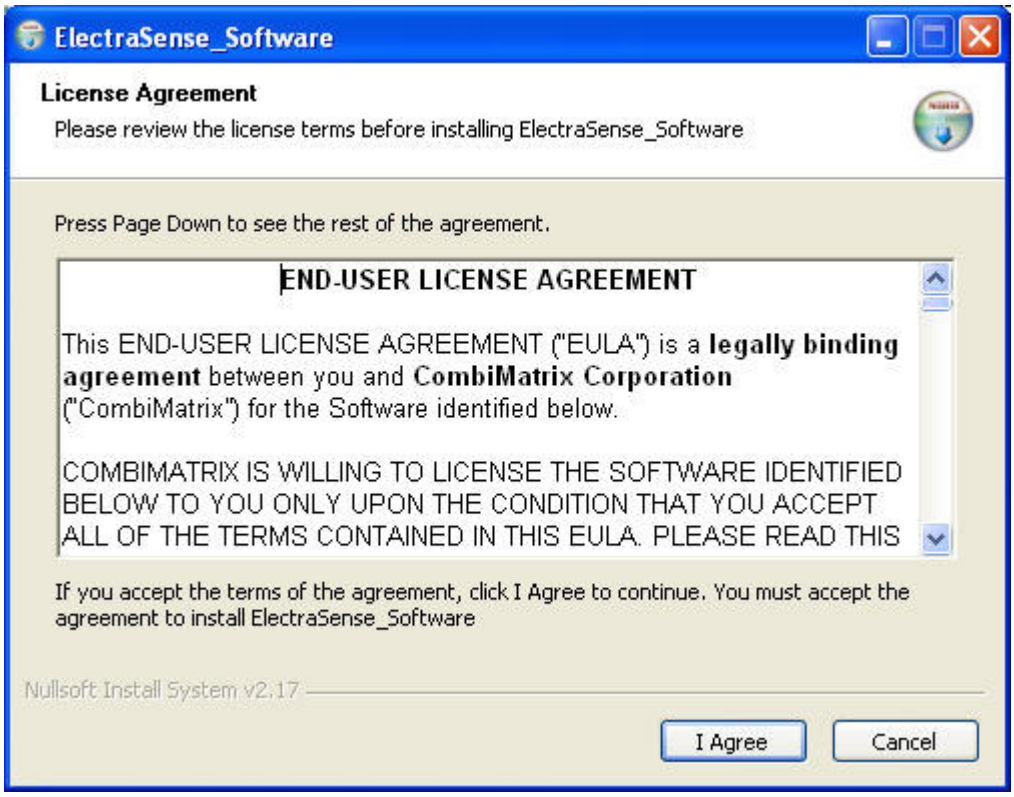
The software can be used by regular users after installation since running the software requires no special privileges.

Connect the serial cable from the DX100 to the serial port of the PC controller.

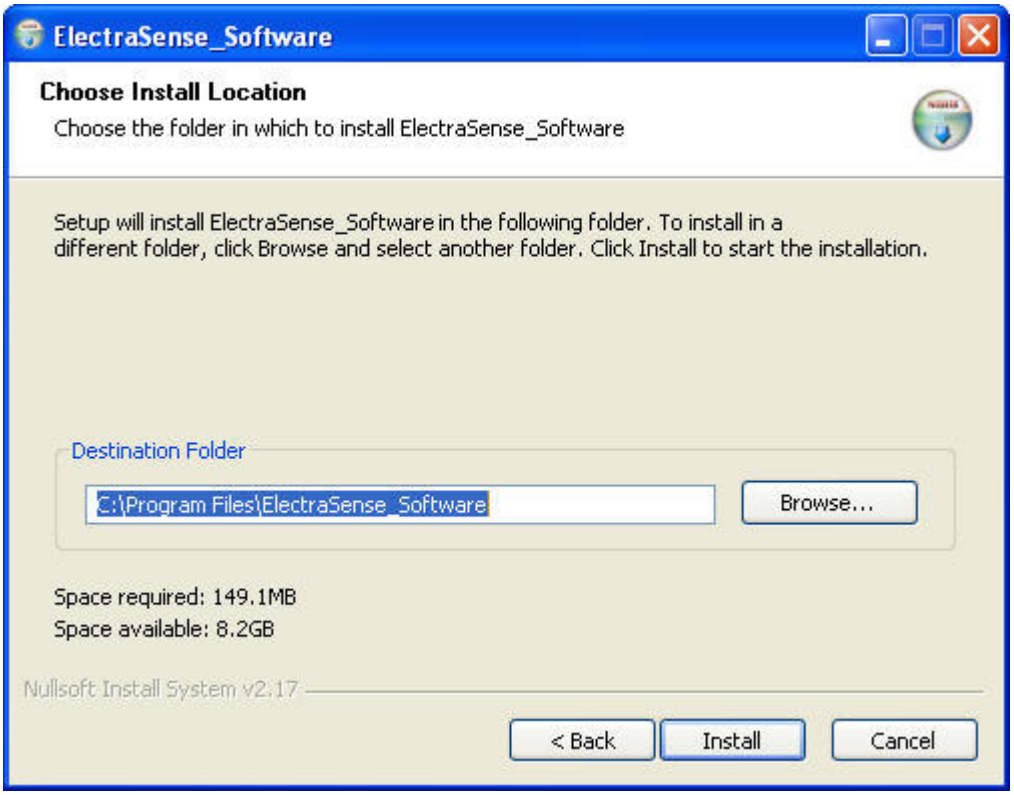
Transfer the installer for the ElectraSense Software to the PC controller and then connect the USB cable from the DX100 to a USB port on the PC.

Windows will recognize that a new device has been connected and display the New Hardware Wizard. The required software will be installed as part of the ElectraSense Software installation. **Click the Cancel button.**

Browse to the ElectraSense Software installer and click the icon to run it. Agree to the software license.

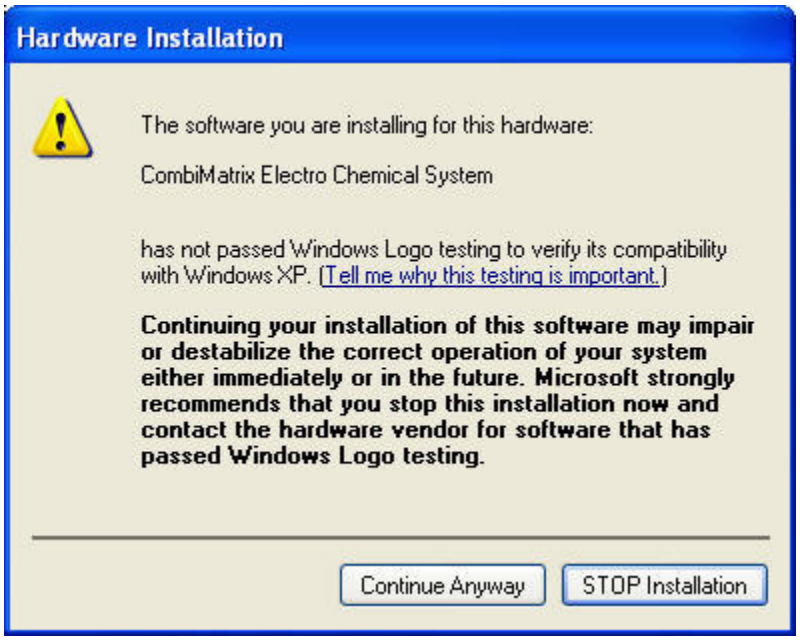


Click the Install button to install the software to the default directory.



The installer will give a warning that the DX100 should be connected and turned on. Click OK to continue.

After the ElectraSense software has been installed, the USB drivers for the hardware will be installed. Windows will display a warning. Please select **Continue Anyway**.



Operation

Common steps for running a sample on the DX100.

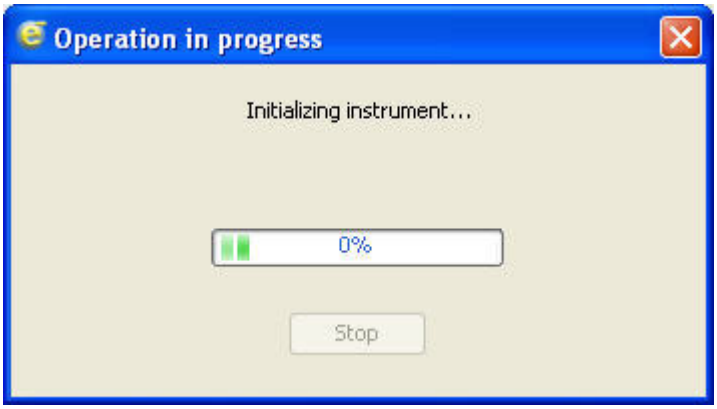
Launch Software

The installer for the ElectraSense® Software application creates an icon on the Desktop. Click this icon to start the application.

Click Start Button

Click the Start button in the Experiment Bar.

The software will initialize the system and start the experiment setup wizard. If a new version of the software has been installed, part of the initialization may include updates to the firmware of the DX100.

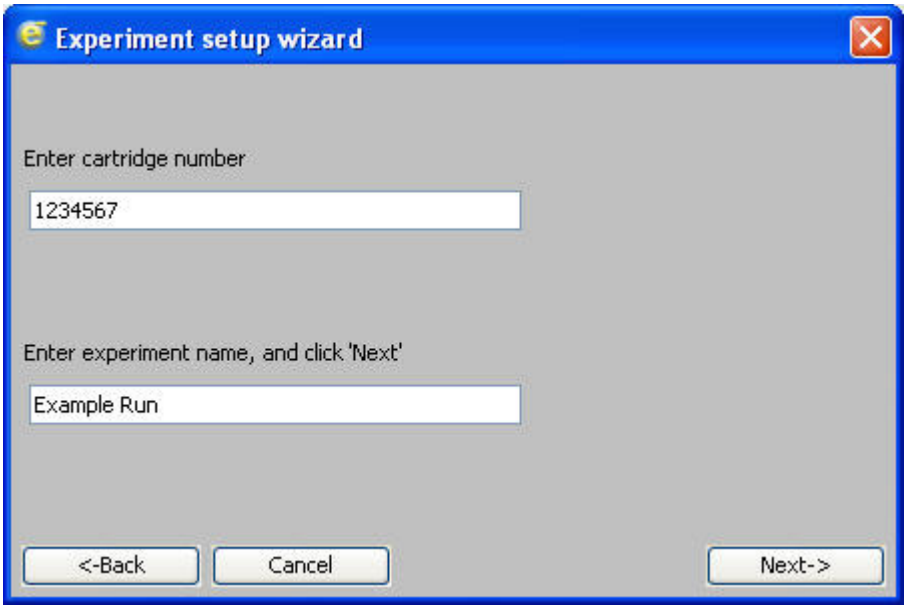


Experiment Setup Wizard

The Setup Wizard will go through the steps required to prepare for a run of the DX100.

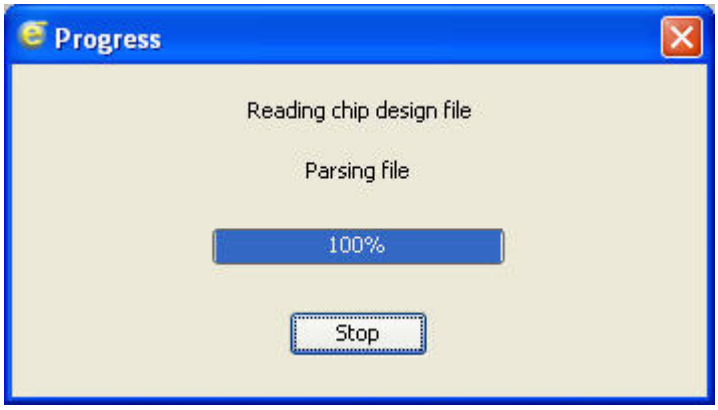
Enter Cartridge and Experiment Information

This window prompts for the number of the cartridge to be used and a name for the run. This information will appear as part of the default name for data files saved.



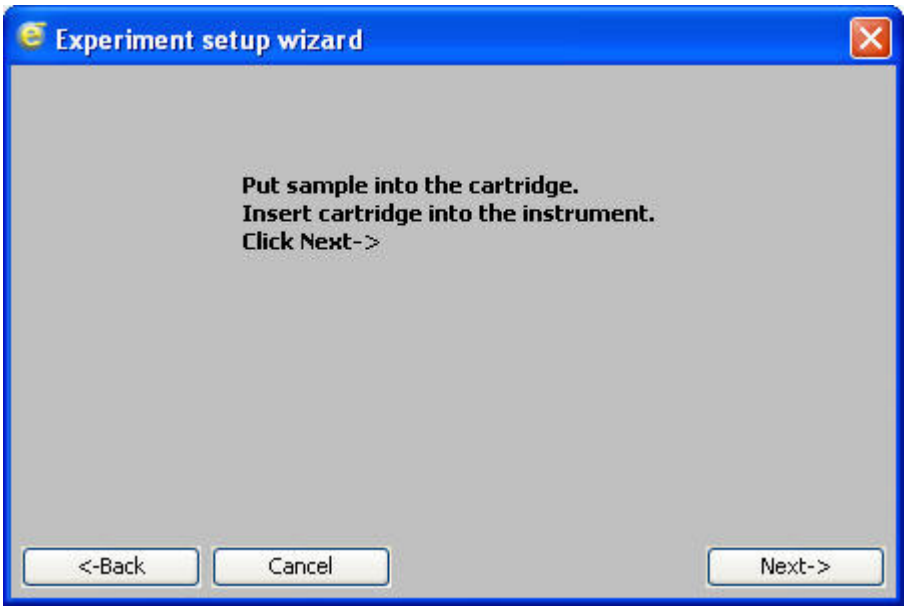
Enter or scan the cartridge barcode number.

Enter an experiment name and click the Next button. The wizard will load and check the protocol file and chip design file before continuing to the next step.



Errors involving the chip design file or protocol file are not expected since these will usually be pre-programmed. Any problems encountered should be referred to support. Please see the section of the manual covering support and service contacts.

Put Sample Into Cartridge



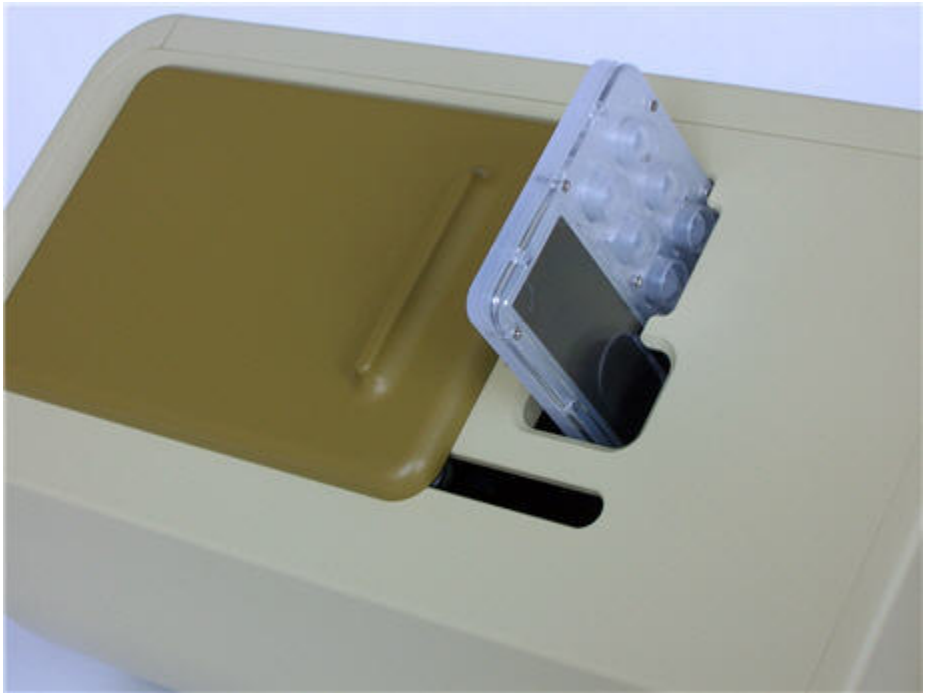
Sample Preparation

1. Dry Sample: add 150 uL of nuclease free water to sample tube.
   Liquid Sample: add nuclease free water to make 150 uL total.
2. Shake it, then shake or spin droplets down.

Cartridge Preparation

1. Remove tab (1)
2. Remove tab (2) and put Porex sticker on the waste port
3. Pull back tab (3) until sample port is uncovered, add 150 ul of sample. Put the tab back, and press down on transparent tape and seal it well. Remove the tab on dashed line.
4. Shake the cartridge gently until you see no white clumps in the sample blister.
5. Hold the cartridge the way it's inserted into the DX100 and tap it several times in the table to collect liquid down.

Insert the cartridge into the DX100 as pictured below.



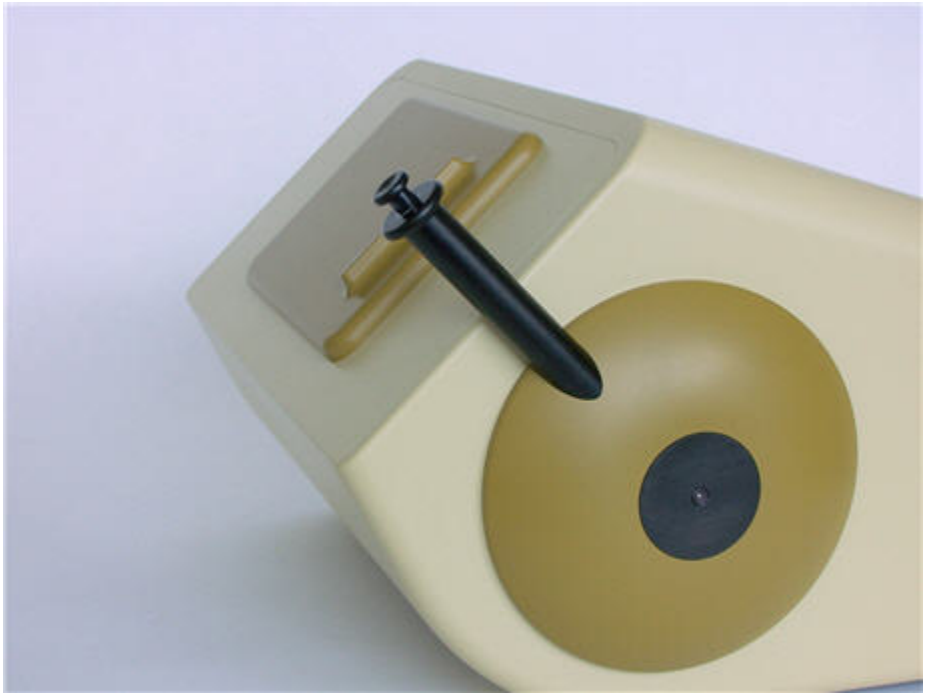
Click the Next button to continue. If the internal sensors show that the cartridge is not inserted correctly, an error message will be displayed. Adjust the cartridge in the chamber and click the Next button again.

Close Cartridge Door And Set Chamber Clamp

Slide the cartridge chamber door to the right to close.

Push the lock button on the cartridge chamber clamp handle.

Pull the handle forward slowly until it stops and the lock button clicks out.



When the Next button is clicked, the system will check that the cartridge chamber door is closed and that electrical connections to the microarray in the cartridge are good. If internal checks pass, the processing protocol will start immediately.

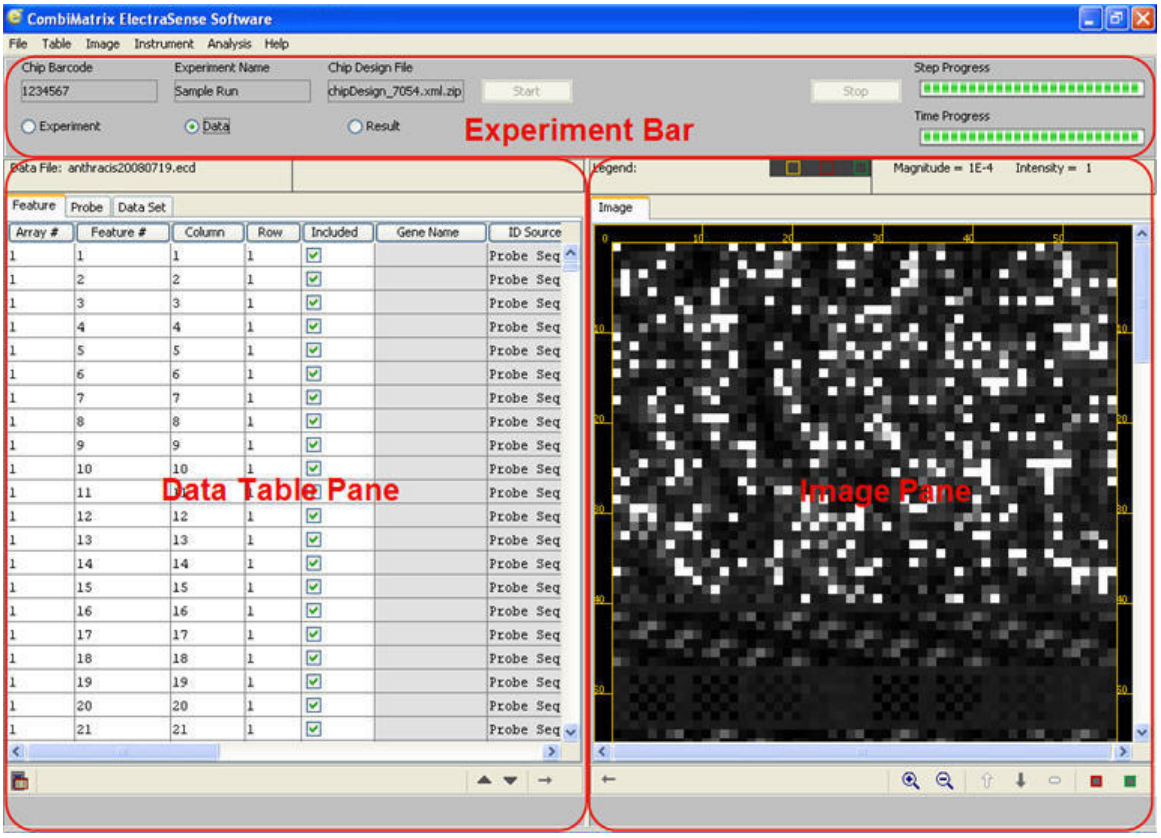
If this message occurs, make sure the the door has been pushed all the way to the right.

If the cartridge chamber clamp has not been closed properly, a message will display showing all electrodes have failed. Select No and click Next to return to the previous wizard screen.

If this message or one showing fewer failed electrodes persists, open the chamber clamp and chamber door and re-insert the cartridge before re-testing.

Switch to Data Window

To view the results of the run, select the Data radio button in the Experiment Bar to switch to the Data Window. This window will display a graphic of the intensity values read from the microarray and tables of this data related to the probes used and their locations.

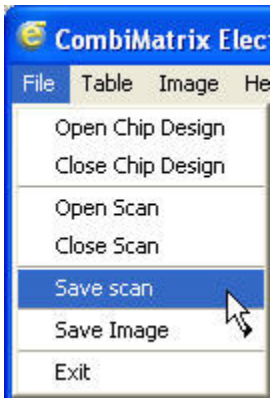


Save Scan Data

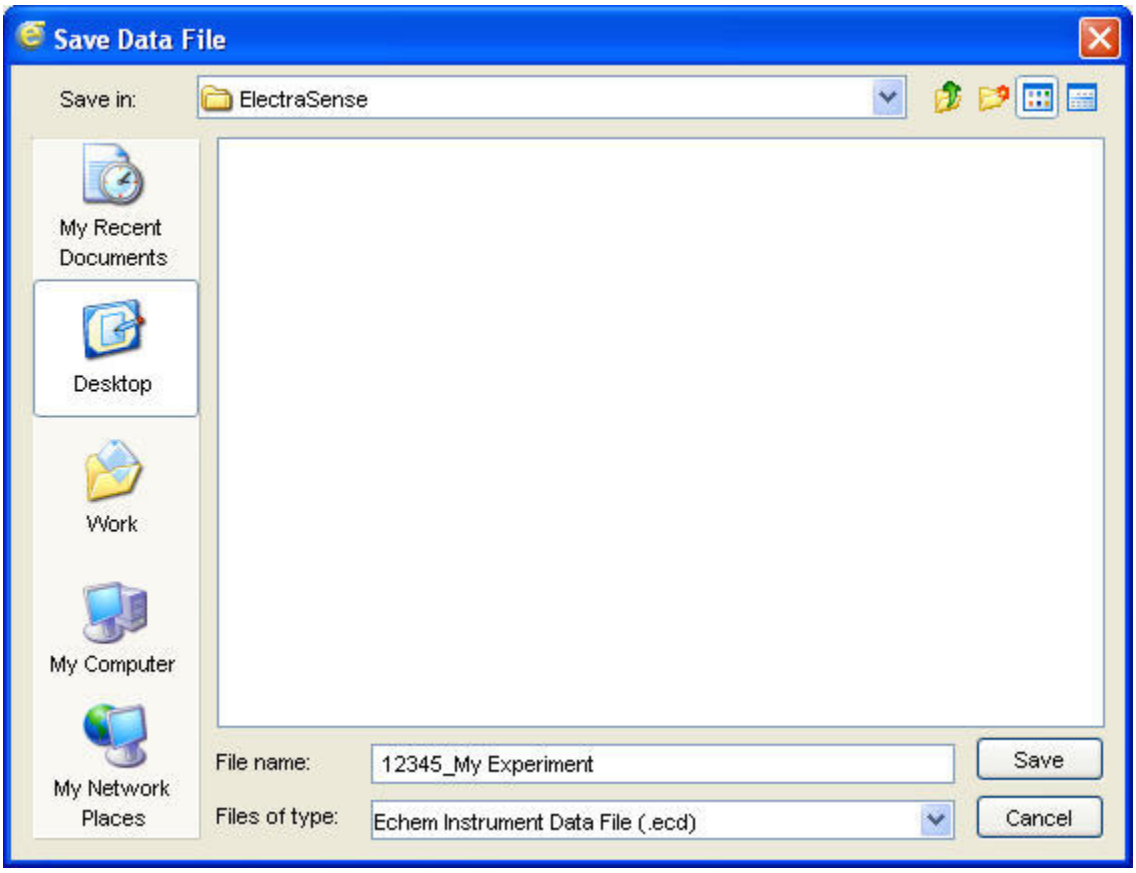
Two types of data from a scan can be saved. Data from the scan and data associated with probe information.

The values from the scan will be saved to a scan file which will usually end in (.ecd). These files contain just the feature locations and the values read for those locations. These files can be opened in the ElectraSense® Software at anytime along with their matching chip design file.

To save the scan data, select Save scan from the File menu.



The file dialogue that appears will display the default name for the .ecd data file which is composed of the cartridge number and the name entered for the experiment.



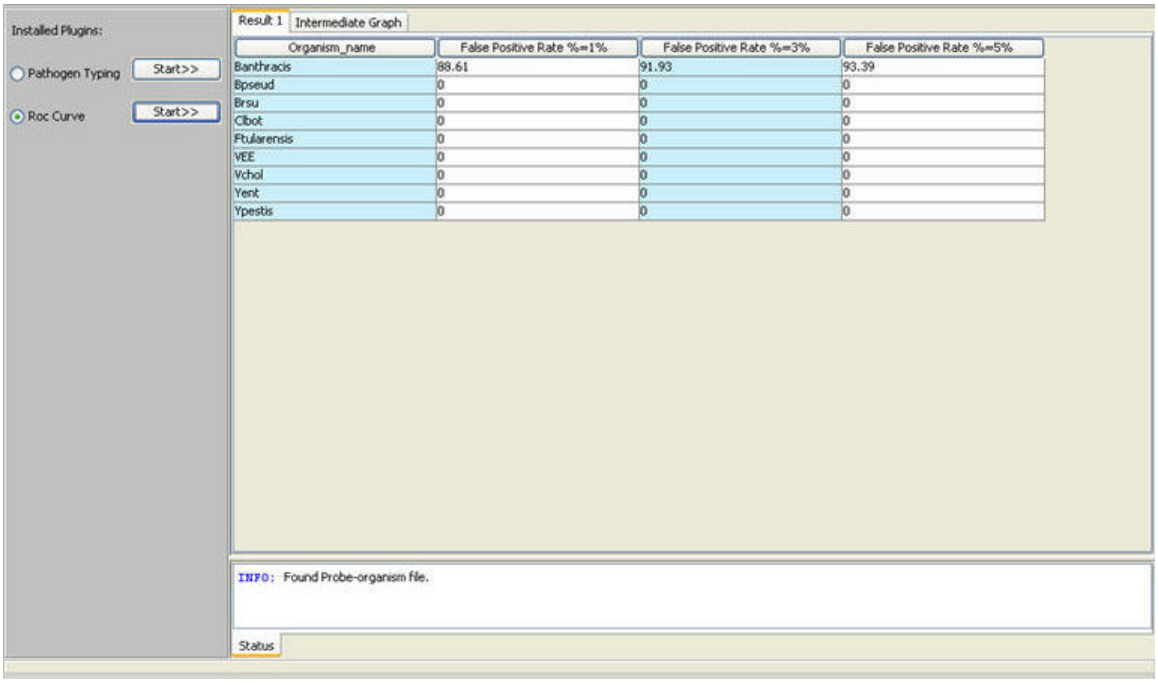
Save Table Data

The data tables combine the scanned values with the information about all probes read from the chip design file selected. **Export feature table** and **Export probe table** will export data for all features and probes on the microarray.

View Analysis

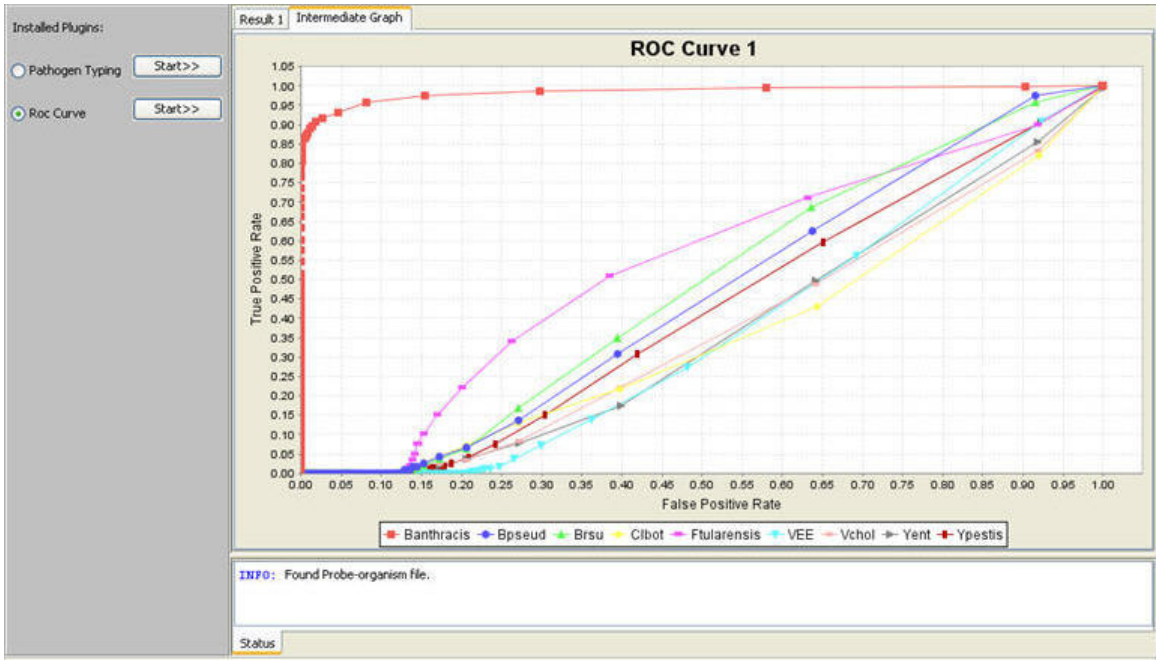
To view an analysis of the data, select the Result radio button in the Experiment Bar. Click the Start button for the desired analysis method in the Installed Plugins area to run the plug-in.

Tables displayed can be saved as tab-delimited text files using the **Analysis | Export** sub-menu.



It is expected that the result table data is the primary data to be saved from the Result window. Other tables of intermediate data and graphs can be displayed as well using the **Analysis | View** sub-menu.

Any graphs displayed can be saved by moving the cursor over the graph, clicking the right-mouse button, and selecting **Save as...** from the pop-up menu.

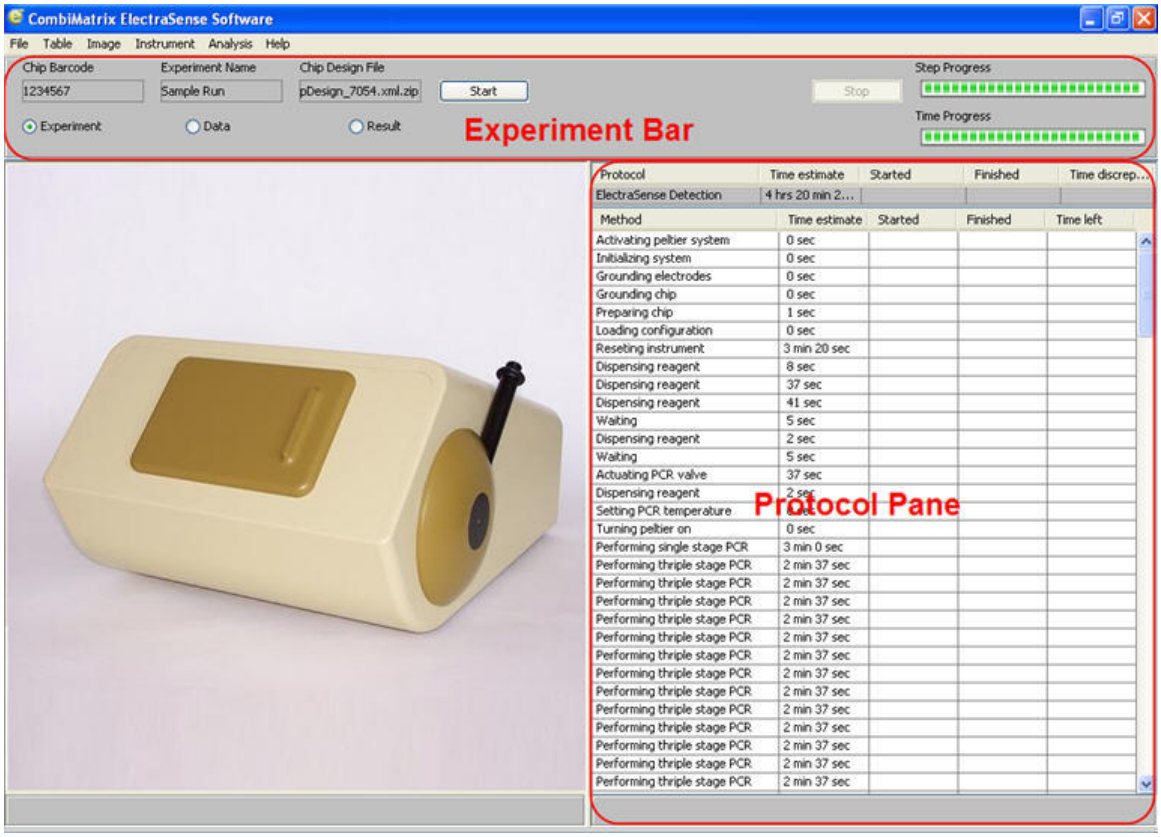

Installed Plugins:
Pathogen Typing
Start>>
Roc Curve
Start>>
Result 1
Intermediate Graph
ROC Curve 1
True Positive Rate
False Positive Rate
Banthracis
Bpseud
Brsu
Clbot
Ftularensis
VEE
Vchol
Yent
Ypestis
INFO: Found Probe-organism file.
Status

Software

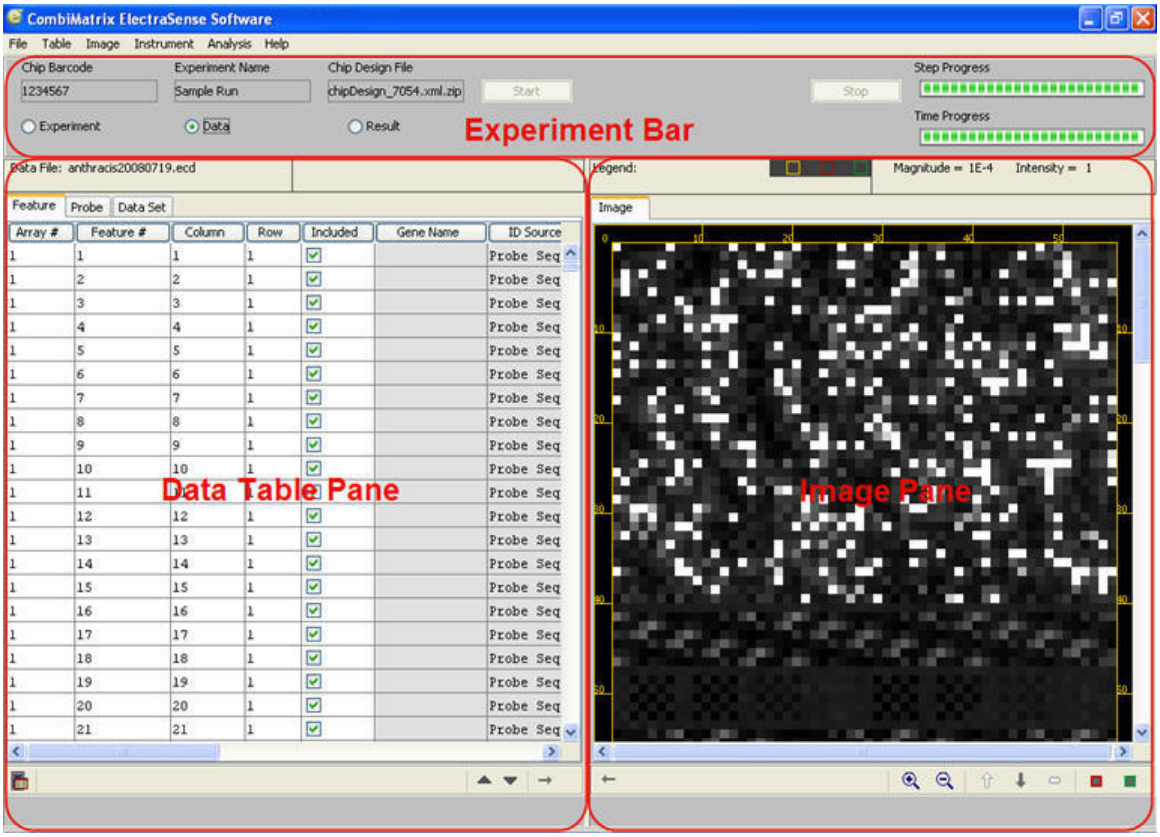
The **Experiment Bar** displays the serial number of the cartridge being processed, the name of the experiment it relates to, and the name of the pre-installed chip design file associated with the ElectraSense® microarray in the cartridges. The Experiment Bar also contains the buttons to start and stop the protocol being run by the DX100 and progress indication for this process.

The radio buttons Experiment, Data, and Result switch between different windows in the software.

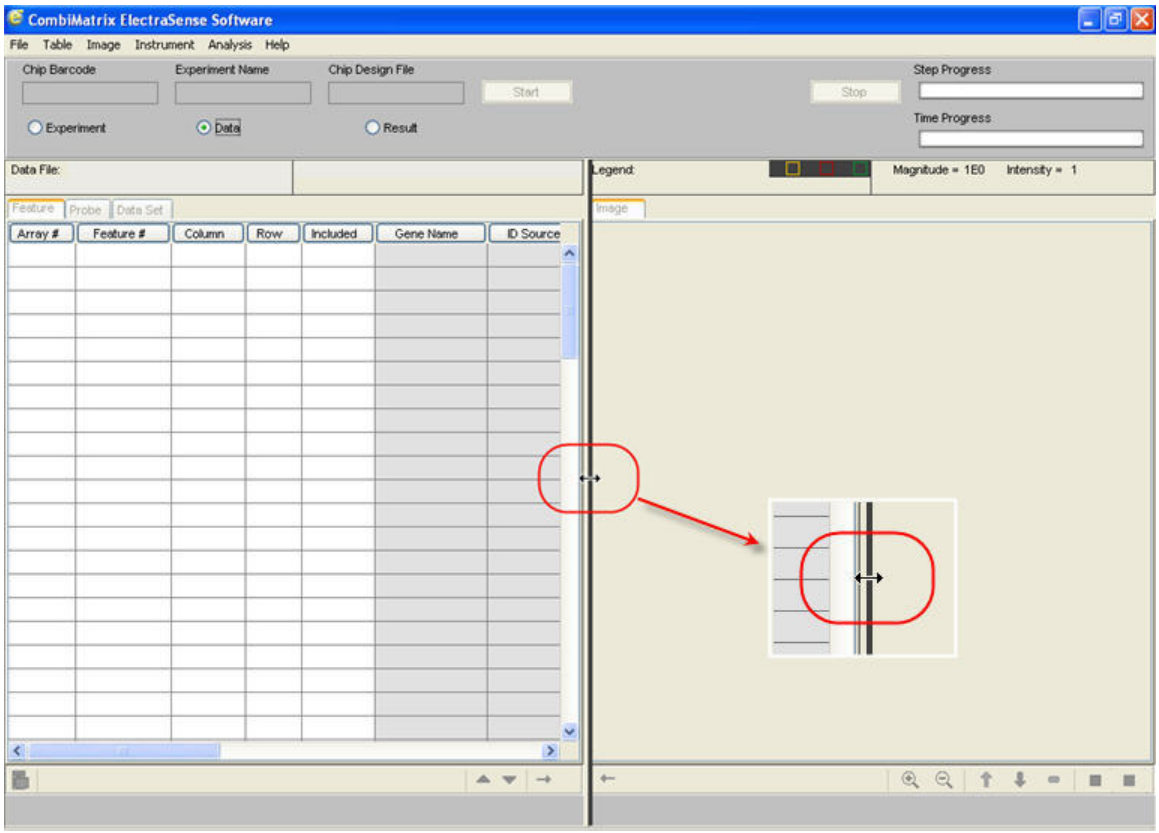
The **Experiment Window** will be displayed when the ElectraSense Software is started or when the Experiment radio button is selected in the Experiment Bar. This window will display the details and times associated with running the protocol for processing a cartridge.



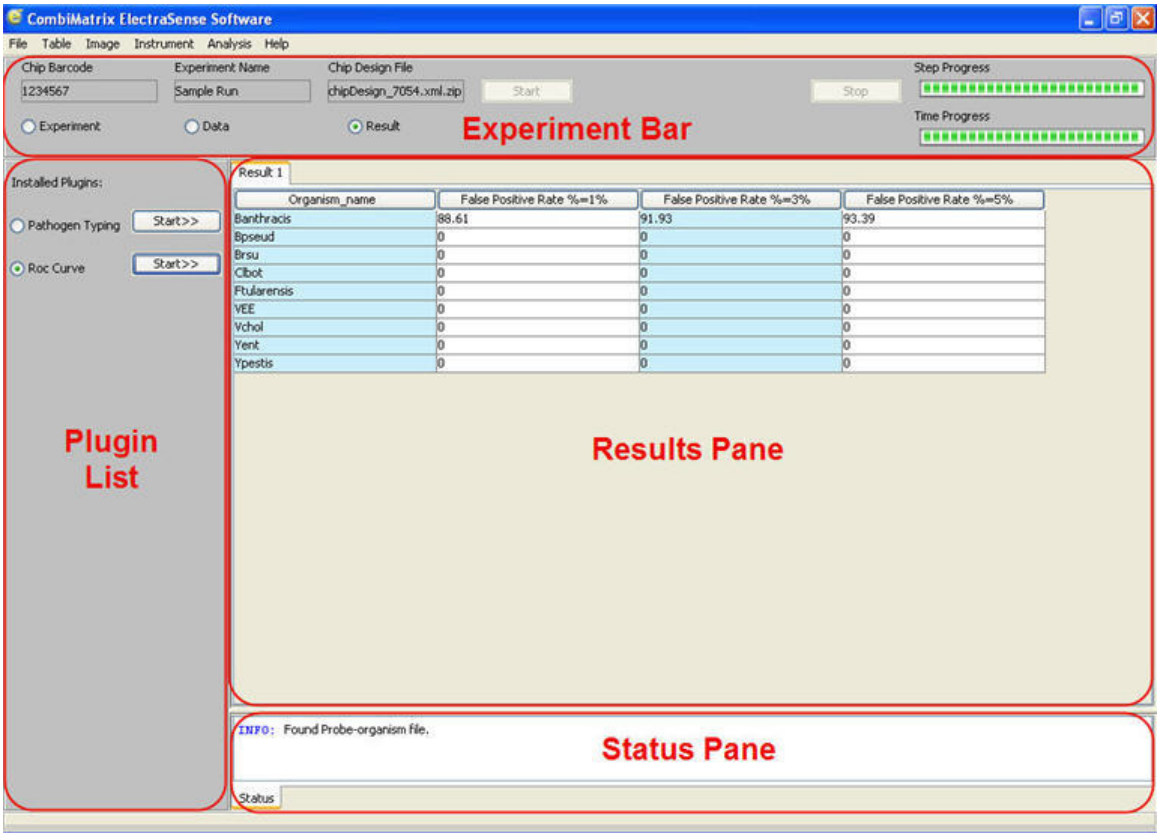
The **Data Window** of the ElectraSense software consists of the Menu Bar, the Experiment Bar, the Data Table Pane, and the Image Pane. This window will display the results of an ElectraSense read as data associated with probes in a table and as a pseudo-image generated from the read results.



The size of the panes can be changed by moving the cursor over the border dividing the panes, clicking the left mouse button, and dragging left or right.



The **Result Window** supports optional analysis methods for ElectraSense data. New methods can be installed as needed using plug-in packages from CombiMatrix. This window consists of The Experiment Bar, the Plugin List, the Results Pane, and the Status Pane.

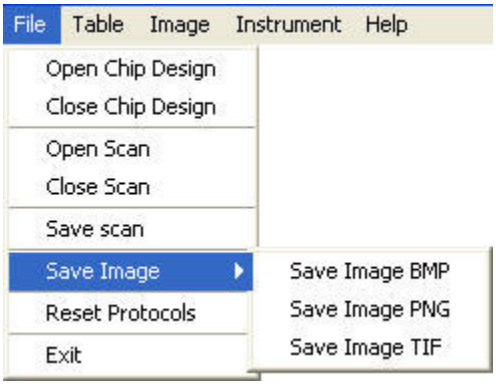


Menu Bar

The Menu bar provides access to functions of the ElectraSense® Software.

File Menu

The file menu provides access to functions for opening and saving files used by the ElectraSense® Software.



Chip Design File Selection

A chip design file provides information about the probes and their locations on an ElectraSense® microarray. The name of a chip design file will usually end in either **.xml** or **.xml.zip** . The software will read either format; it is not necessary to unzip the chip design file before using it.

Open Chip Design allows the operator to browse for the appropriate file.

Close Chip Design will clear the data table and image pane for a new chip design file and related image.

Currently the DX100 uses one chip design file for all cartridges. This file is located in the folder:

C:\Program Files\ElectraSense_Software\resources\Danley\chipdesign

Scan File Options

Data from previous readings of ElectraSense® microarrays can be opened and viewed at any time. This will usually require that the related chip design file be opened first.

Open Scan allows the operator to browse for scan data files. These files will usually end in the extension **.ecd** .

Close Scan will close the current image of the scan data but keep the previously selected chip design file open. This way scans of data from related arrays can be viewed in quick succession.

Save Scan will save the data from a scanned microarray to an **.ecd** file.

Save Image

The image displayed in the image pane is a grey scale representation of the current values read from an ElectraSense® microarray. This image can be saved in several formats for printing or use in publications.

Reset Protocols

This menu option will restore all protocols installed with the software to their original state.

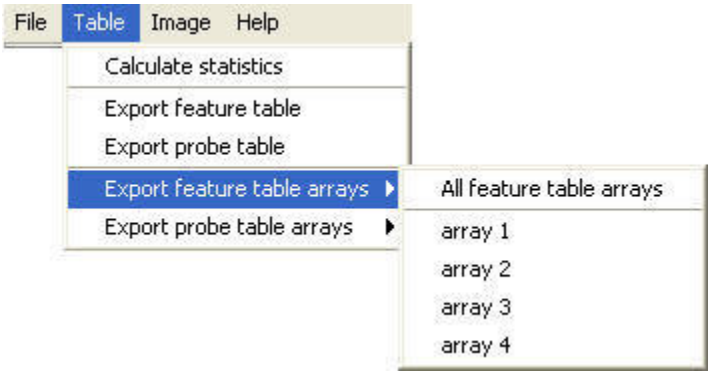
This operation requires administrator privileges for the computer.

Exit

Exit will close the ElectraSense® Software.

Table Menu

The Table menu has functions for changing and saving the contents of the Data Table Pane.



Calculate Statistics

Calculate Statistics will recalculate the values in the data tables. This should be done if features have been included or excluded.

For example, if the operator selects features in the Image Pane that should not be included in the final data using the controls to change included features, Calculate Statistics should be selected to recalculate the values in the data table.

Changes that can effect the values in the data tables will usually cause the figures in the tables to turn red along with a message that data values will need to be recalculated

There is also an icon at the lower left of the data table pane for this function.

See also:

Image Pane Controls

Data Table Pane Controls

Export Tables

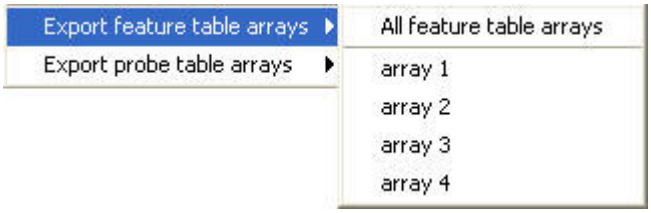
The data in the data table pane can be exported as tab-delimited files.

Export Table Feature will save the contents of the Feature table to a file.

Export Table Probe will save the contents of the Probe table to a file.

Export Tables By Array

When using ElectraSense® 4x2k CustomArrays® it is possible to export individual tables of data for each array. The file name will give the slide number, experiment name, and array number by default. All array data tables can be saved at one time automatically by using the **All feature table arrays** option, or individually by array.



While the complete feature table gives data for all locations, the probe table and individual array tables will contain only data for locations that are not located under the gasket.

In the pseudo-image generated from the scan data, the individual arrays start with array 1 at the top. The yellow banded regions represent the area under the gasket.

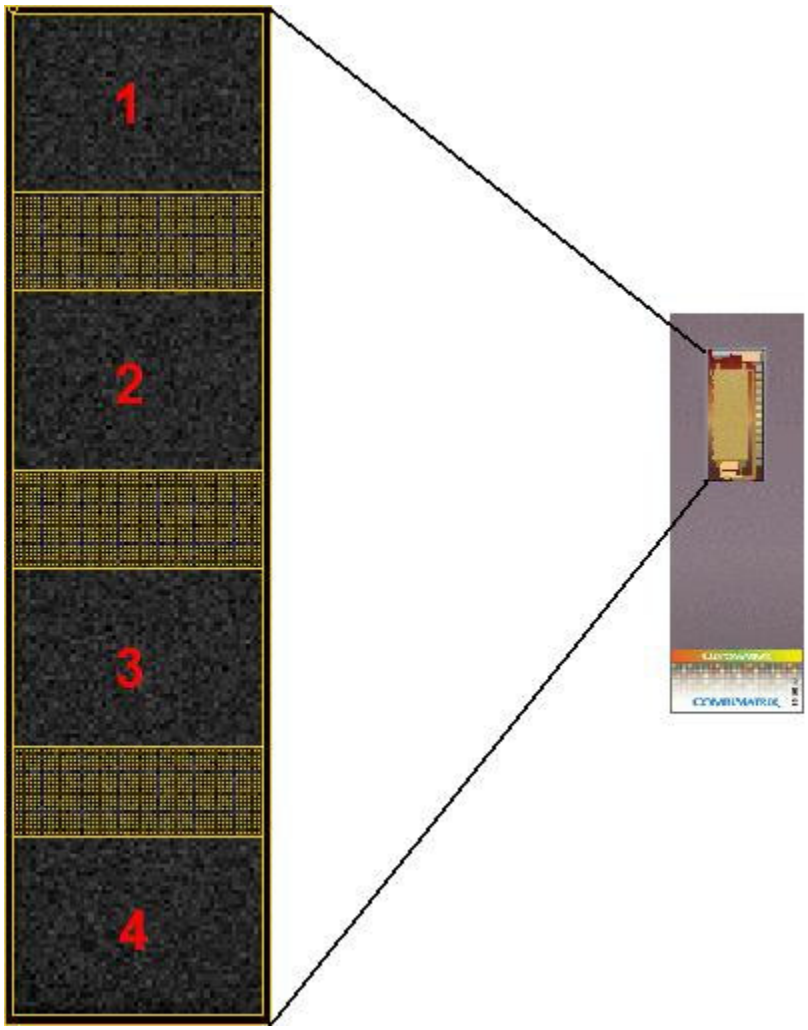
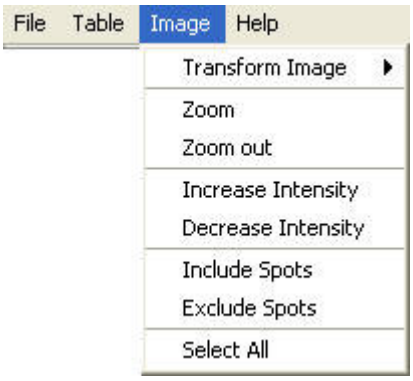


Image Menu

The Image menu provides functions for dealing with the image in the Image Pane.



Transform Image

Depending on the content of the ElectraSense® microarray in the cartridge used, features may be easier to see if the conversion of data from the .ecd file is done on a logarithmic scale rather than a linear scale.

This will not change the underlying data read by the DX100.

Zoom

Zoom will increase or decrease the size of the image in the image pane. This will not change the underlying data read by the DX100.

See also:

Image Pane Controls

Intensity

Intensity will cause the features in the Image Pane to be brighter or darker. This will not change the underlying data read by the DX100.

See also:

Image Pane Controls

Include and Exclude

Features in the image pane can be marked included or excluded. All features are usually included by default.

This will not change the underlying data read by the DX100, but it will change the displayed data tables. If a feature is excluded, that feature will be marked in the Feature Data Table and data from that location will not be used in calculating the various values in the Probe Data Table.

See also:

Image Pane Controls

Select All

Select All will highlight all features in the image pane. This can be used to reset all features previously set to excluded to included in one operation.

Instrument

Controls for the ElectraSense® reader.

Emulate Device

Selecting Emulate Device will allow the software to simulate some functions that would usually cause an error message to occur when not connected to hardware. This can be useful for demonstrating the software when a device is not available.

Service Screen

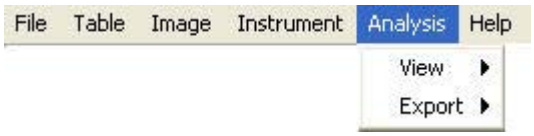
This screen will have no available options for most users. It is not needed for regular operation of the DX100.

Select Instrument

This menu option is not always displayed in the ElectraSense® Software application. The selection made in this menu should match the hardware connected to the PC.

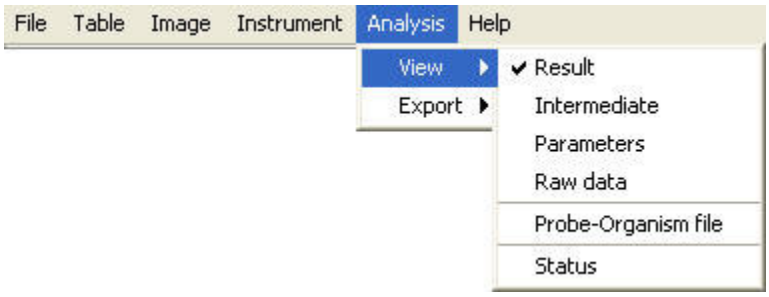
Analysis

The Analysis Menu provides controls for viewing and saving information in the Result Window.



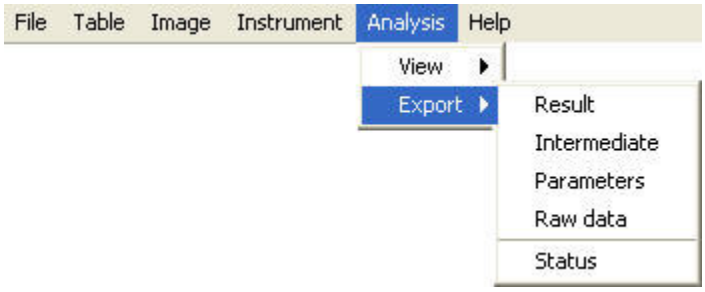
View

The Analysis View sub-menu controls which tables of data will be displayed in the Result Pane. The tables available for view will depend on the analysis plug-in selected.



Export

The Analysis Export sub-menu allows saving the tables of data in the Result Pane. A file dialog will appear when an item in the sub-menu is selected.



Help Menu

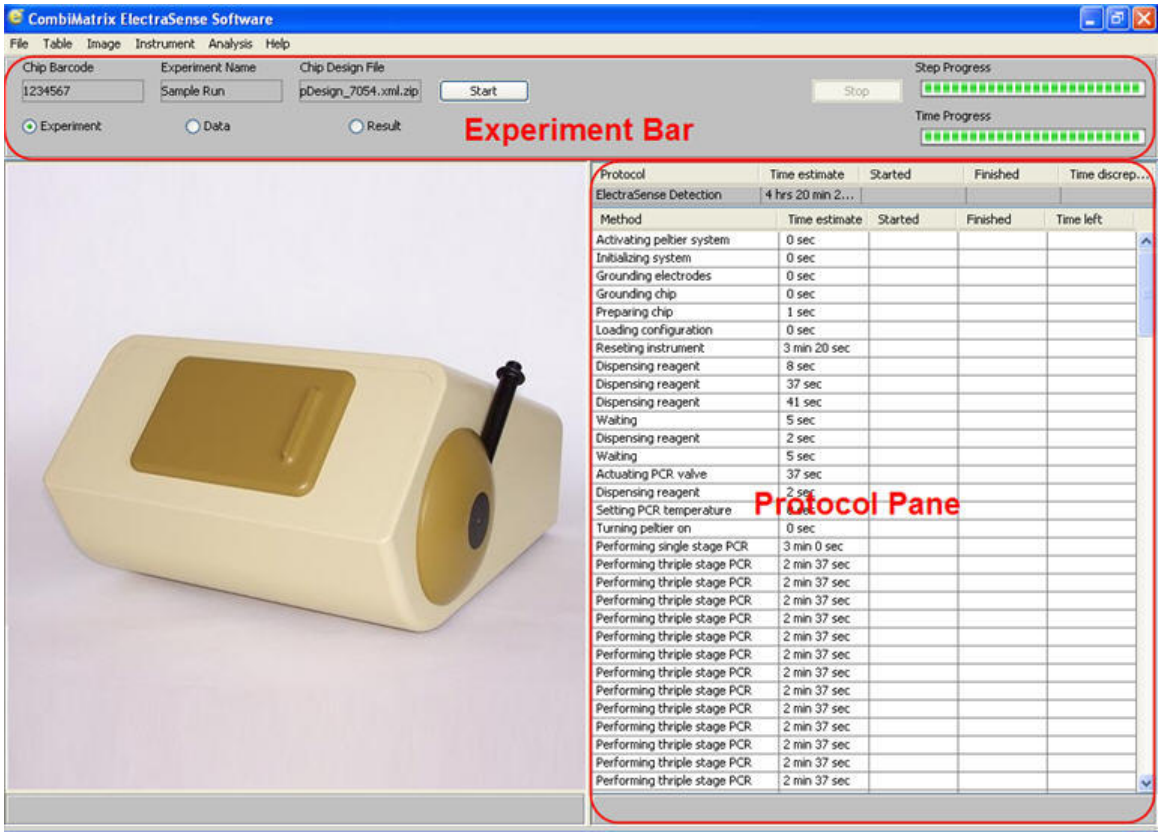
Help Topics

This function will display this manual.

About

About will report the details about the ElectraSense® Software currently running.

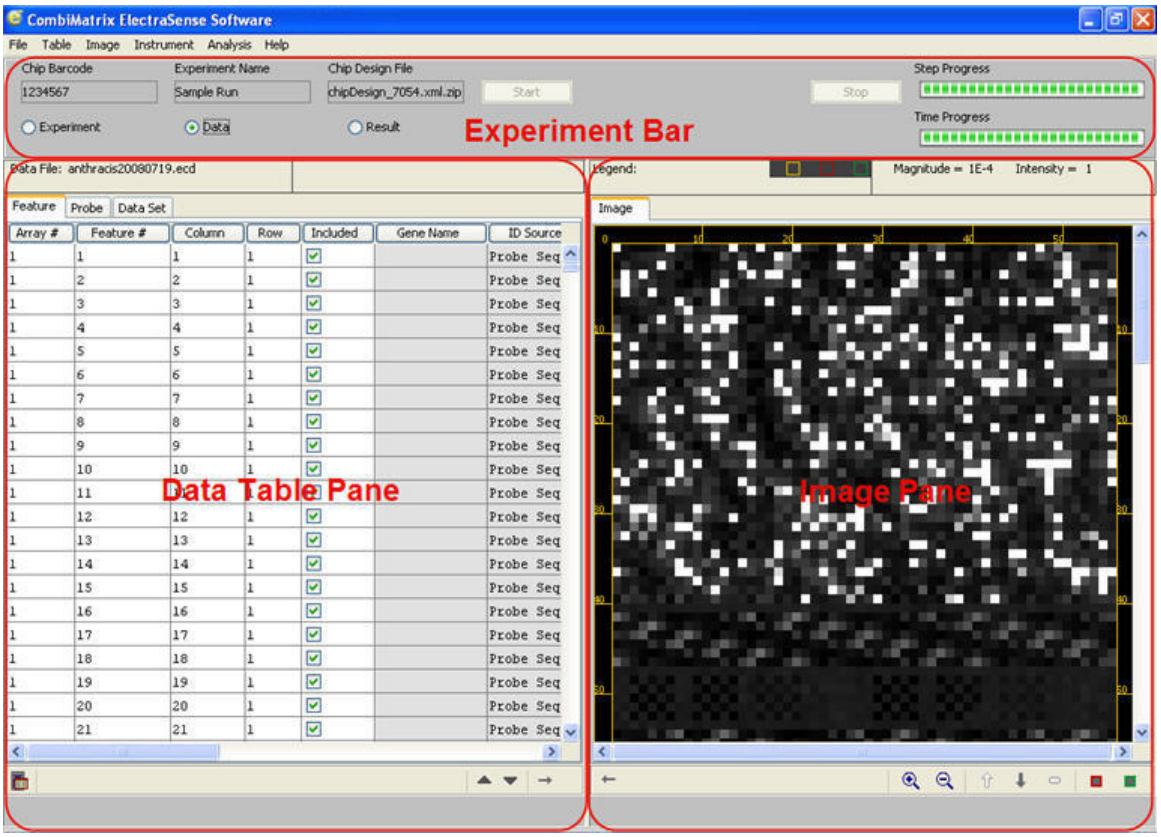
Experiment Window



Protocol Pane

The protocol pane shows the steps of a read operation and the time required.

Data Window



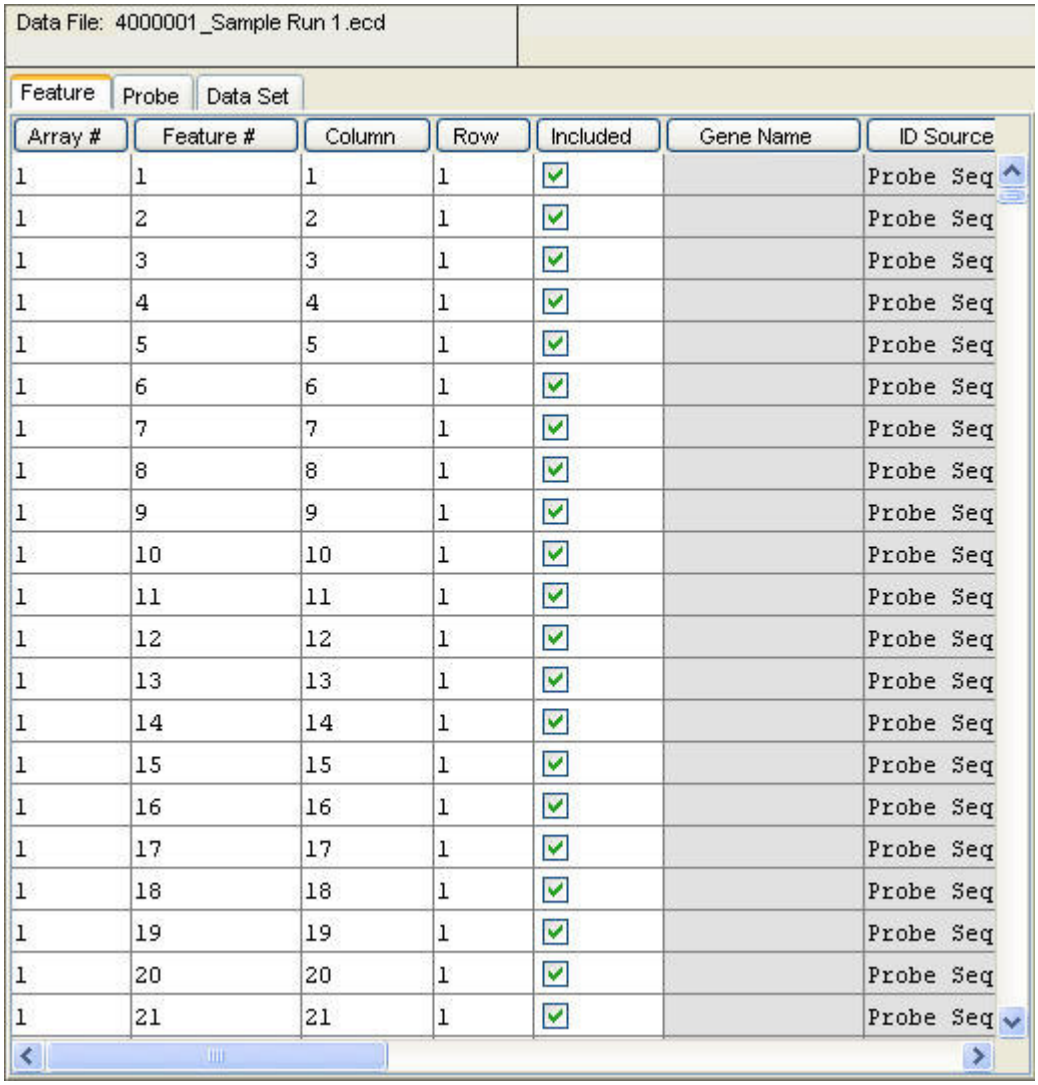
Data Table Pane

The Data Table Pane displays information about the data read from the DX100 and information about the probes on the microarray as specified by the chip design file loaded in the Experiment bar.

The **Feature** tab will display information about all features on the microarray.

The **Probe** tab will display information aggregated by probe name.

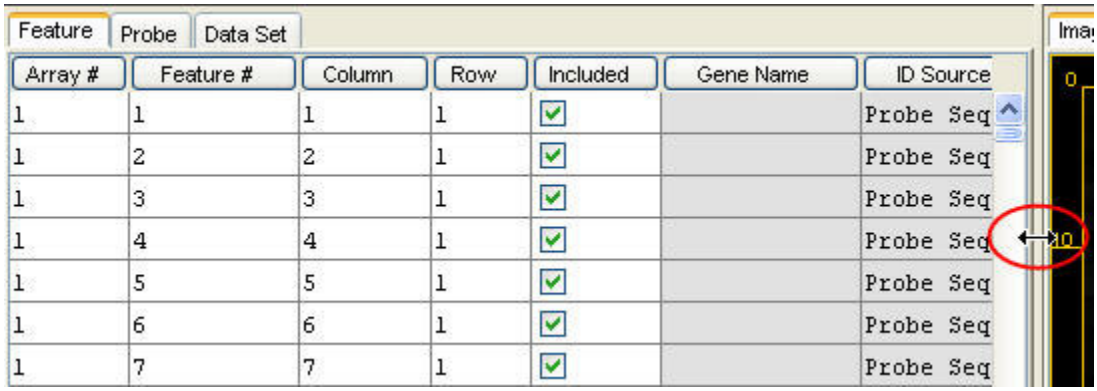
The **Data** Set tab will display information about intensity values for all features combined.


Data File: 4000001_Sample Run 1.ecd

Feature | Probe | Data Set

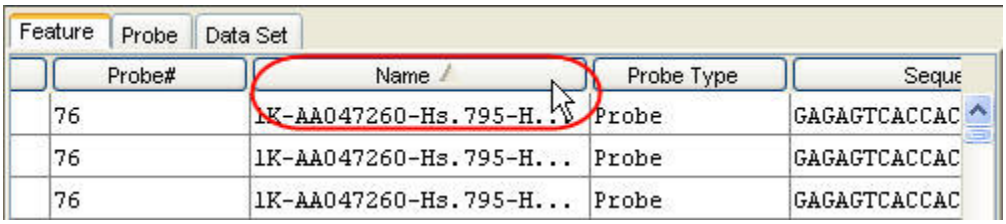
Array #	Feature #	Column	Row	Included	Gene Name	ID Source
1	1	1	1	☑		Probe Seq
1	2	2	1	☑		Probe Seq
1	3	3	1	☑		Probe Seq
1	4	4	1	☑		Probe Seq
1	5	5	1	☑		Probe Seq
1	6	6	1	☑		Probe Seq
1	7	7	1	☑		Probe Seq
1	8	8	1	☑		Probe Seq
1	9	9	1	☑		Probe Seq
1	10	10	1	☑		Probe Seq
1	11	11	1	☑		Probe Seq
1	12	12	1	☑		Probe Seq
1	13	13	1	☑		Probe Seq
1	14	14	1	☑		Probe Seq
1	15	15	1	☑		Probe Seq
1	16	16	1	☑		Probe Seq
1	17	17	1	☑		Probe Seq
1	18	18	1	☑		Probe Seq
1	19	19	1	☑		Probe Seq
1	20	20	1	☑		Probe Seq
1	21	21	1	☑		Probe Seq

The size of the Data Table Pane can be changed by moving the mouse over the border with the Image Pane until a double headed arrow appears, clicking and holding the left mouse button, and dragging left or right.

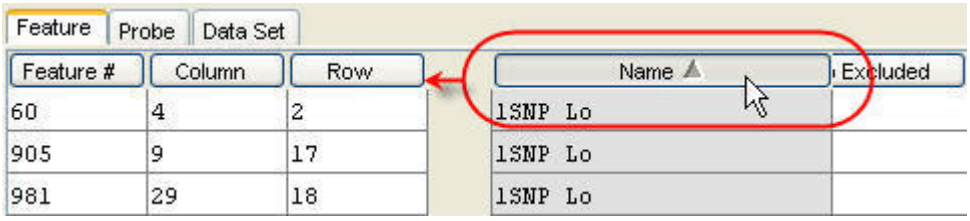

Feature | Probe | Data Set

Array #	Feature #	Column	Row	Included	Gene Name	ID Source
1	1	1	1	☑		Probe Seq
1	2	2	1	☑		Probe Seq
1	3	3	1	☑		Probe Seq
1	4	4	1	☑		Probe Seq
1	5	5	1	☑		Probe Seq
1	6	6	1	☑		Probe Seq
1	7	7	1	☑		Probe Seq

The data table displayed can be sorted by the contents of any column by clicking on the header of the column. A triangle will indicate which column is being used and the order of the sort.



Columns in the data table can be moved by clicking and holding the left mouse button on the header of the column and dragging the column to the desired location.



Data Table Pane Controls

There are three sets of controls found at the bottom of the Data Table Pane.

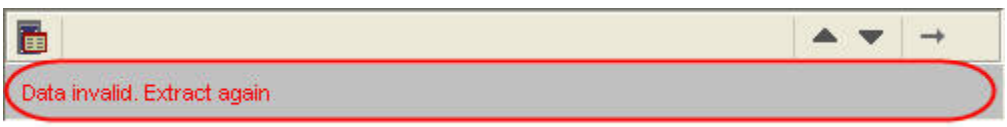
Calculate Statistics recalculates data read by the DX100 to be entered in the current table and associated with the probe information from the current chip design file. This would be used if features have recently been included or excluded or changes have been made to probe types in the Probe Data Table.

The arrows pointing up and down cause the table displayed to jump to rows that have been previously selected either by highlighting them in the Data Table or by highlighting the associated features in the Image Pane.

The arrow pointing toward the Image Pane causes the features associated with rows highlighted in the data table to be selected in the Image Pane.

Data Table Pane Information

Changes to the Data Table that require user attention are signaled by messages at the bottom of the Data Table Pane. The most common message will be that data needs to be re-extracted or recalculated after some change made in the Data Tables or Image Pane.



Data Table Tabs

The **Feature** tab will display information about all features on the microarray.

The **Probe** tab will display information aggregated by probe name.

The **Data** Set tab will display information about intensity values for all features combined.

Feature Table

The Feature table displays signal intensity values for all locations on the ElectraSense® microarray. These are the columns currently available and a brief description:

- Array # - For a 12k array, this column will show 1 for all features. For a 4x2k array this column will show either a number from 1-4 to indicate the array or "-" if the feature falls under a gasket area.
- Feature # - The location of a feature as given by a number starting at 1 in the upper left-hand corner of the microarray and continuing left to right and from top to bottom.
- Column - The column number of the feature
- Row - The row number of the feature
- Included - This will be checked if the intensity values for a feature are to be included in various other calculations in the tables. For example, the intensity values for an included probe will be included in calculations for the median values reported in the Probe Table. This can also be used as a flag in downstream analysis packages that the feature is special in some way.
- Gene Name - The name of the target associated with this probe, if the information is contained in the chip design file.
- ID Source - The source of information used to generate the value in the ID column
- ID - A unique code for the probe calculated from the sequence of the probe or information associated with the target.
- Gene Comment - Annotations for the target associated with this probe
- Probe # - The number associated with the probe. The number is determined by the position of the probe in the chip design file.
- Name - The unique name for the probe.
- Sequence - The full sequence for the probe. This may not be displayed for some designs.
- Length - The mer length of the probe
- Probe comment - Annotation for this probe
- Signal - The value for the signal read from the DX100

Probe Table

The Probe tab will display information aggregated by probe name.

- Index - An index that allows sorting the table to its original arrangement.
- Array # - For a 12k array, this column will show 1 for all probes. For a 4x2k array this column will show a number from 1-4 to indicate the array.
- Probe # - The number associated with the probe. The number is determined by the position of the probe in the chip design file.
- Name - A name for the probe unique to the current chip design file.
- Comment - Annotation for the probe
- Sequence - The full sequence for the probe
- Length - The mer length for the probe
- Tm - Calculated Tm of the probe as it appears in the chip design file.
- Config Level - Level of stringincy the probe passed when generated by probe design system
- Origin - Tells whether this probe was generated by the probe design system, submitted pre-designed, or part of the quality control probe set
- Gene Name - The name of the target associated with this probe, if the information is contained in the chip design file.
- ID Source - The source of information used to generate the value in the ID column
- ID - A unique code for the probe calculated from the sequence of the probe or information associated with the target.
- Gene Comment - Annotations for the target associated with this probe
- Included - The number of replicates of this probe marked as included in the feature table
- Excluded - The number of replicates of this probe marked as excluded in the feature table
- Total - Included + Excluded
- Median - The median value of all **included** intensity values for the probe
- Mean - The mean value of all **included** intensity values for the probe
- Stdev - The standard deviation of all **included** intensity values for the probe
- CV% - The standard deviation of all **included** intensity values for the probe divided by the mean
- Min - Minimum signal found for this probe in feature table
- Max - Maximum signal found for this probe in feature table

Data Set

The Data Set tab will display information about intensity values for all features combined.

- Array # - For a 12k array, this column will show 1 for all features. For a 4x2k array this column will show either a number from 1-4 to indicate the array or "-" if the feature falls under a gasket area.
- Mean - The mean of all feature intensity values
- Median - The median of all feature intensity values
- STD - The standard deviation of all feature intensity values
- CV (%) - The standard deviation of all feature intensity values divided by the mean.
- Min - The lowest feature intensity value
- Max - The highest feature intensity value

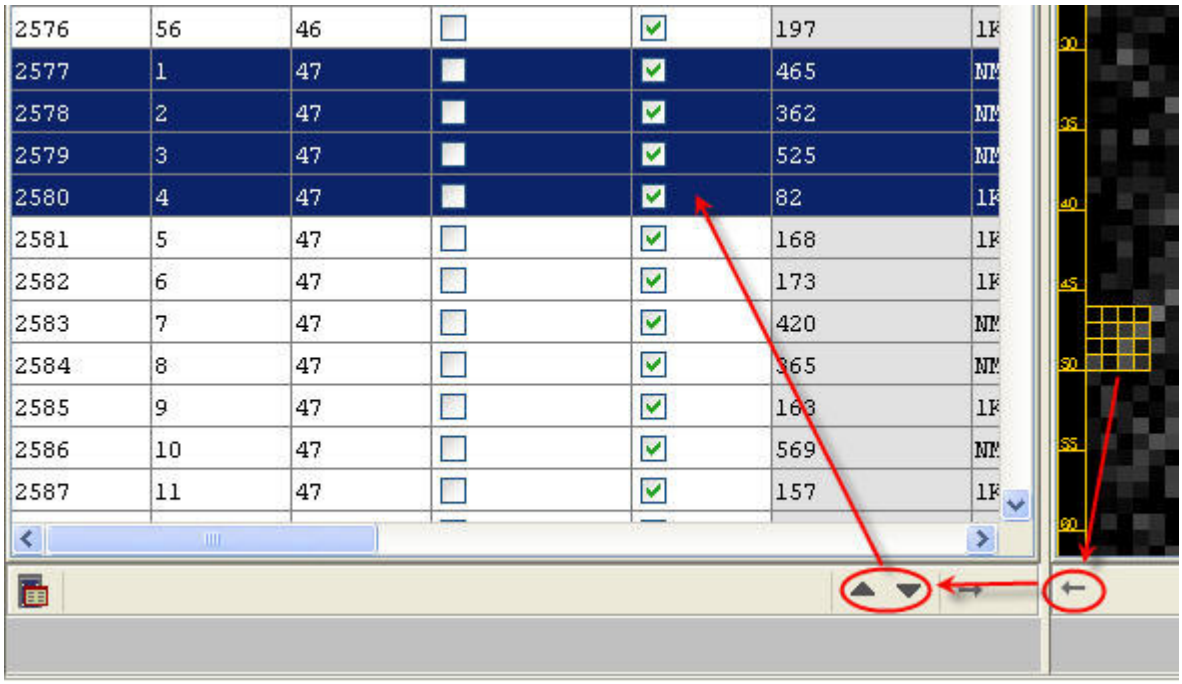
Image Pane

The Image Pane displays a graphic representation of the electrochemical detection signals read from the microarray during a scan.

Image Pane Controls

The Image Pane controls can change the displayed graphic representation of the intensity values for the microarray. Various controls also manage how these values are used and displayed in associated tables in the Table pane.

The arrow pointing to the right will cause entries related to selected features in the image to be highlighted in the currently displayed table. Features are selected by clicking and holding the left hand mouse button and dragging over features.

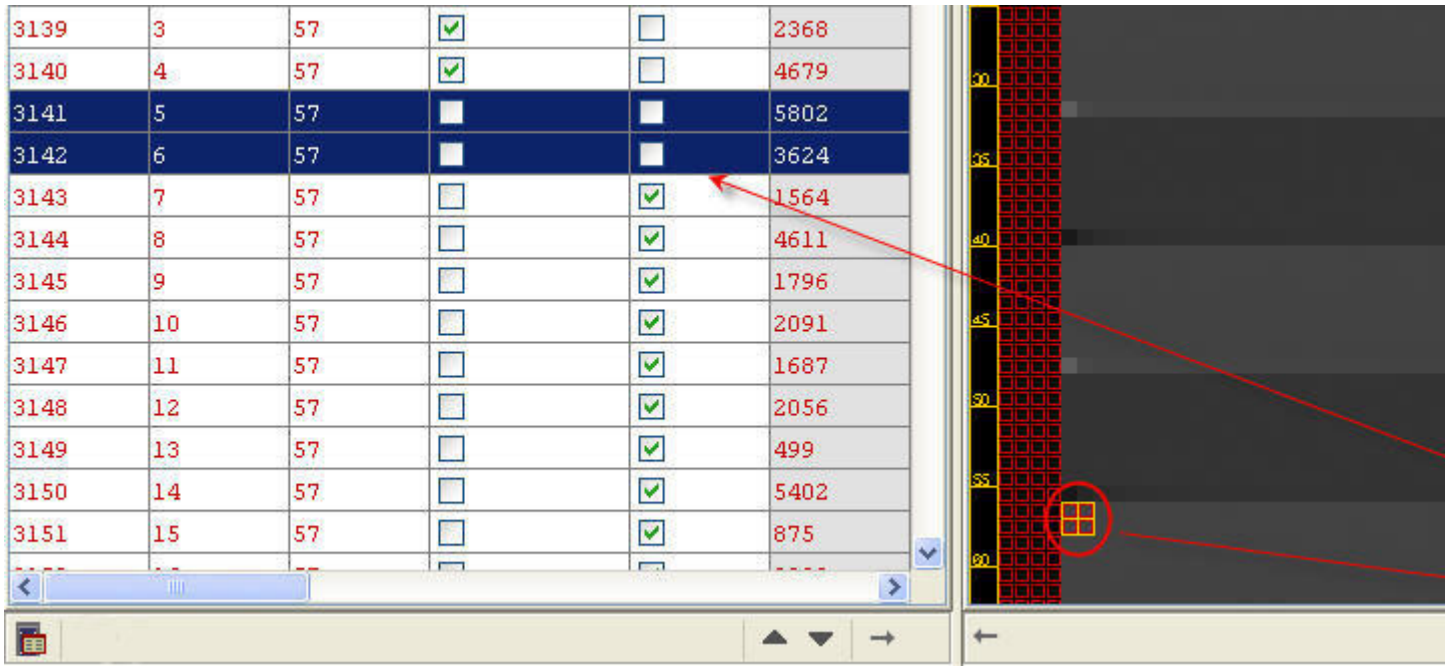


Change size will change the size of the displayed image.

Change displayed intensity will change the brightness of the displayed image.

Change included will cause selected features in the image to be listed as included or excluded in the feature table. In the probe table, intensity values for the probes at these locations will not be used for probe mean, median, and other statistics.

Note that the check boxes for these features marked in red are unchecked. Also, this causes the table entries to change to red, which indicates that a Calculate Statistics operation should be done to update all tables with this new information.



3139	3	57	☑	☐	2368
3140	4	57	☑	☐	4679
3141	5	57	☐	☐	5802
3142	6	57	☐	☐	3624
3143	7	57	☐	☑	1564
3144	8	57	☐	☑	4611
3145	9	57	☐	☑	1796
3146	10	57	☐	☑	2091
3147	11	57	☐	☑	1687
3148	12	57	☐	☑	2056
3149	13	57	☐	☑	499
3150	14	57	☐	☑	5402
3151	15	57	☐	☑	875

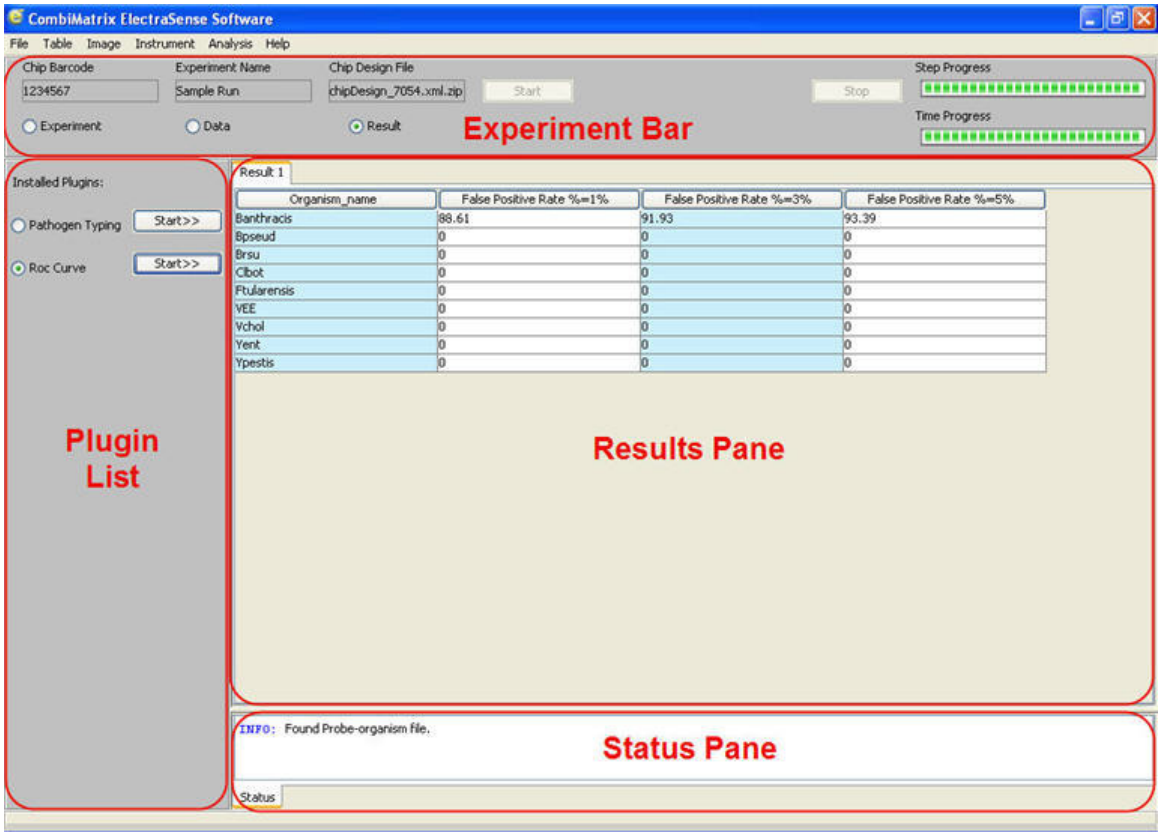
Image Pane Information

The Image Pane information section at the bottom of the image pane reports information about whatever feature is currently under the arrow cursor.

Image Pane Legend

Placing the cursor over each of the color coded boxes will tell what this color indicates for marked features.

Result Window



Plug In List

A plugin generates tables of analysis results from the current chip design file and read data file. Plug-ins are distributed by CombiMatrix as separate installable programs.

Plug-ins are installed to the current user directory, so if there are multiple users of the software on the same machine plug-ins will need to be installed separately for each user.

Results Pane

The results pane displays tables of results from the selected analysis plug-in.

Only certain tables generated by the analysis process are displayed by default, to control which tables are displayed, use the **Analysis | View** menu.

All tables displayed can be exported as tab delimited files using the controls in the **Analysis | Export** menu.

Status Pane

The status pane displays any errors or warnings generated by the analysis plug-in.

Possible Problems During Operation

Recovery from Power Failure

Interruption of a running protocol usually requires that the cartridge be replaced and the run started again.

If the protocol has finished running before a prolonged power failure the intensity data for the microarray last run can be recovered by locating the file **lastdata.ecd** located in the user directory C:\Documents and Settings\<username>\cbmx\ecdapplication\appdata.

Accidental Closure of Application

The ElectraSense® Software will warn the user if the current scan data has not been saved before closing or starting a new run. In case of accident, the data from the last scan can be found in the file **lastdata.ecd** in the user directory under C:\Documents and Settings\<username>\cbmx\ecdapplication\appdata.

Unexpected Error Message

If an error message occurs that prevents the normal operation of the DX100, it is best to close the software and restart.

To help diagnose the problem, it is useful to make copies of all log files found in the directory C:\Documents and Settings\<username>\cbmx\ecdapplication\appdata, especially ecdapp.log and ECDevice5.log .

Service and Maintenance

Preventive Maintenance

The CombiMatrix DX100 is calibrated and tested before being shipped. It is expected to work properly without special service or maintenance for some time.

Care should be taken to avoid damage to the mechanical and electrical contacts that connect a loaded cartridge to the machine. Open and close the chamber clamp carefully. Keep the cartridge chamber door of the DX100 closed when not in use.

Support and Service Contacts

For at least the period during which the first 50 cartridges specified by contract are being used, service and support can be obtained directly from the primary developer of the system, Kia Peyvan.

PeyvanSystems

206 734-3636

kia@peyvanSystems.com

Afterwards, or for other concerns, please contact:

CombiMatrix Support.

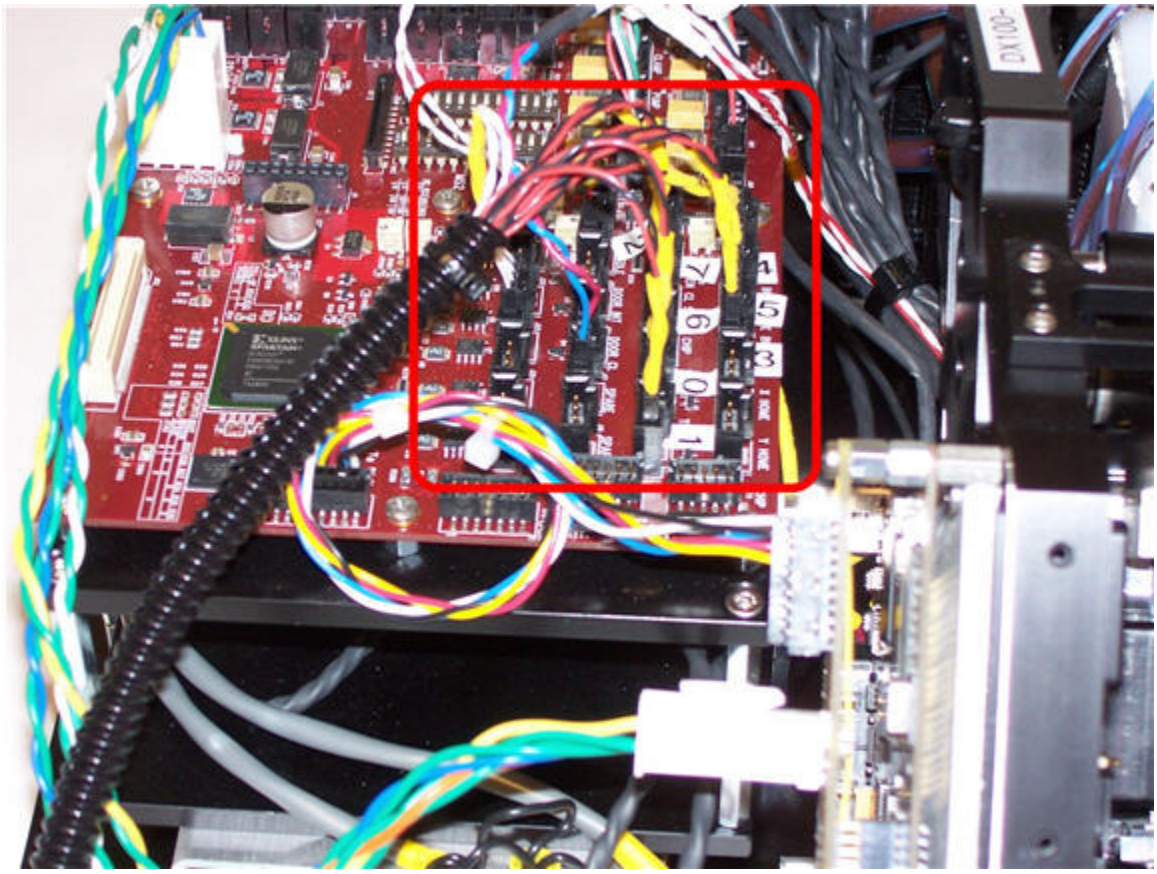
800 493-2000

support@combimatrix.com

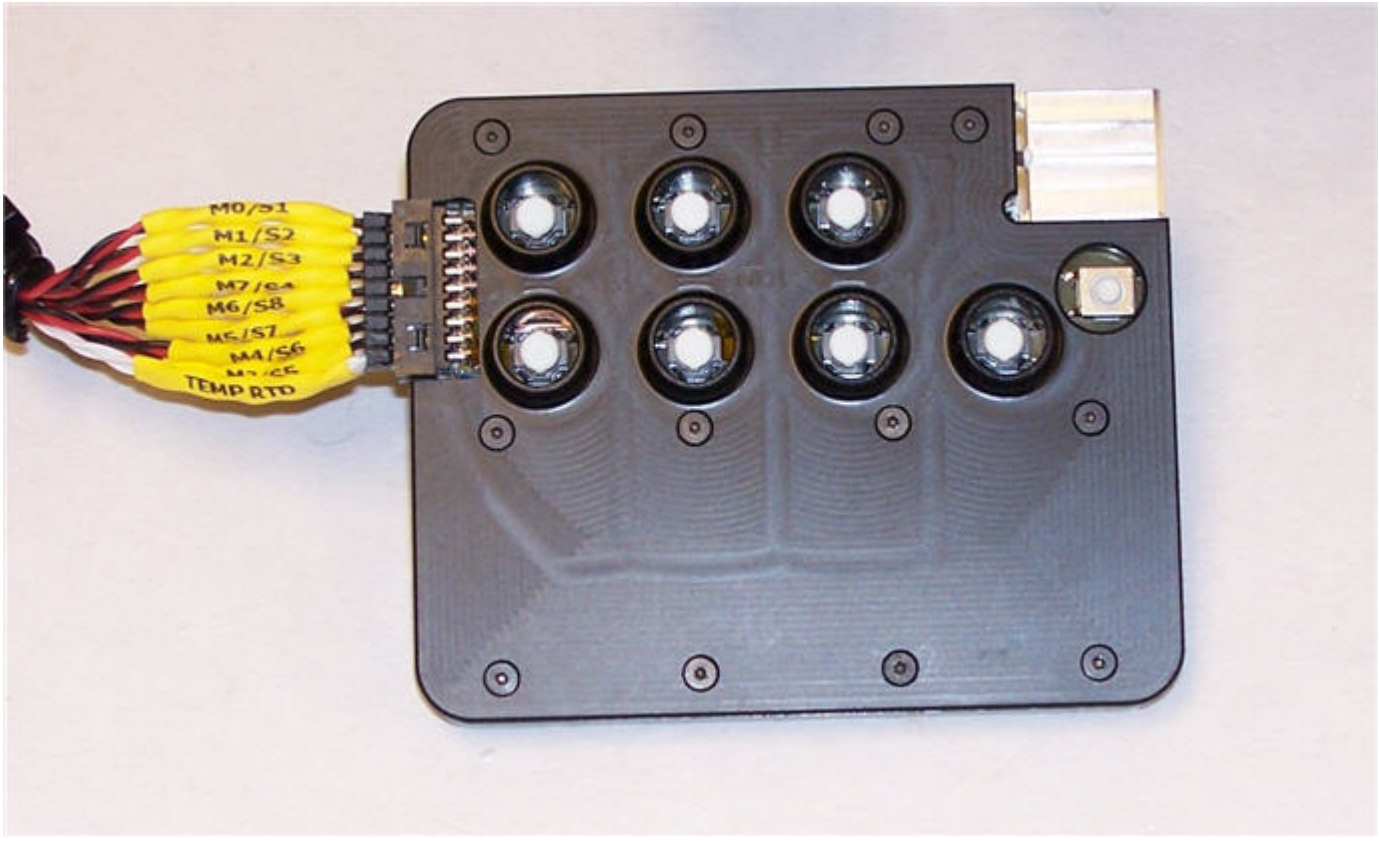
Calibration Cartridge

The DX100 ships with a test cartridge with sensors to check the operation of the actuators that pump fluids in cassettes, the operation of the PCR heating unit, and the operation of the circuits that read the microrrays in the cassettes.

To use the test cartridge, open the case of the DX100 and find the test cartridge cable. This should be stowed in the empty area to the front of the machine and already connected to the proper locations on on the interface board. The board is numbered to match the numbers on the separate leads in the cable in case the cable needs to be removed for other service work.

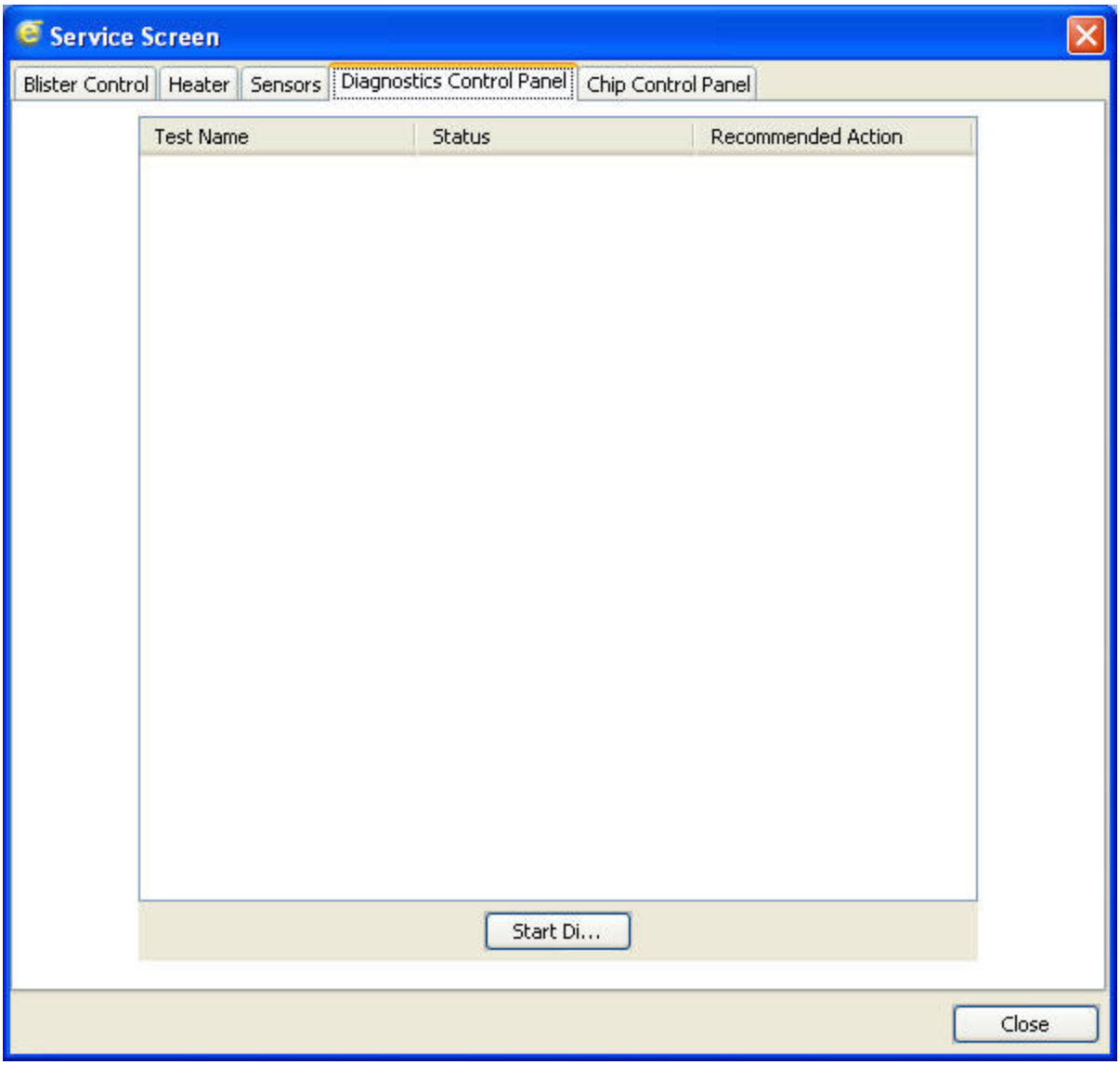


Connect the cable to the test cartridge.



Launch the DX100 software and select **Instrument | Service Screen.**

Select the tab for the Diagnostics Control Panel.



Click the **Start Diagnostics** button and follow the prompts to insert the cartridge and run the diagnostic protocol.