

New tips, tricks and techniques for users of Beckman Coulter laboratory automation systems

New robotics interface more powerful and easy to use

Rob Donoho ♦ Beckman Coulter, Inc.

Beckman Coulter has announced a new software interface for its SAGIAN™ Core Systems — integrated laboratory automation systems.

The new software, SAMI® NT, is a graphical method development interface coupled to the power of a true dynamic scheduler.

NEW PRODUCTS

SAMI NT is composed of all the best from the original SAMI software, which revolutionized high-throughput screening automation, and input from our many users. The result is a feature- and power-packed interface for automated systems that can produce sample prep methods for analytical divisions, microplate replication for compound repositories, and data output for screening laboratories.

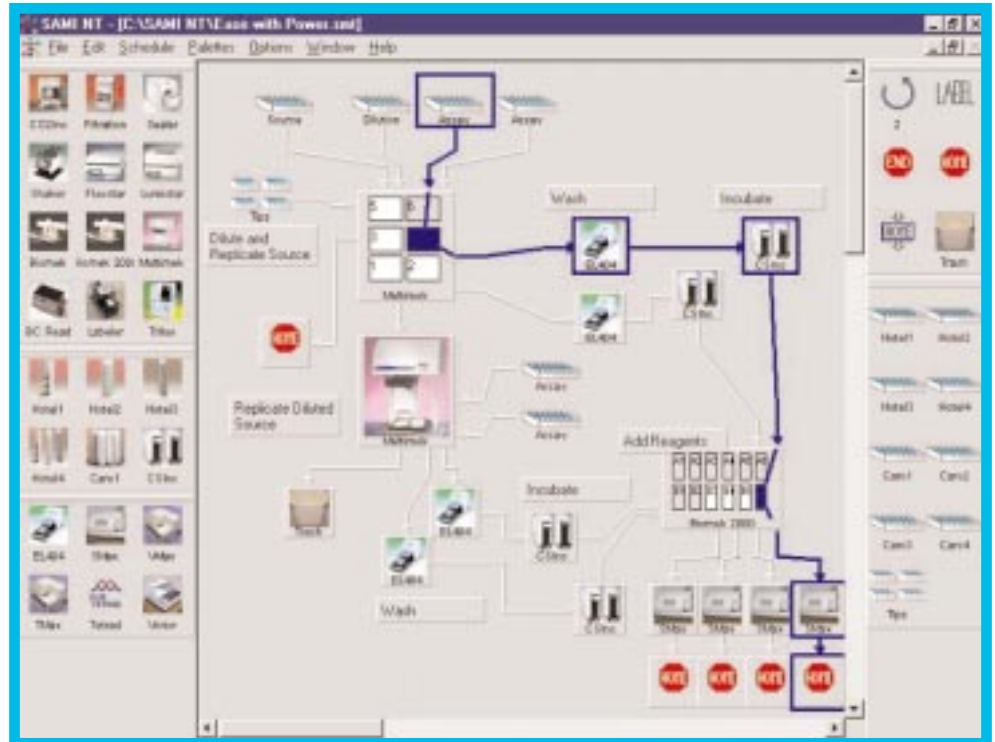


Figure 1. SAMI NT method development graphical interface showing OSH (One-look sample history).

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- SAMI[®] NT features include:
- Schedule optimization
 - Rigid and flexible scheduling
 - Dynamic rescheduling
 - Tip tracking
 - Resource tracking
 - Sample addition to currently running methods
 - Batching and pooling
 - OSH (One-look sample history)

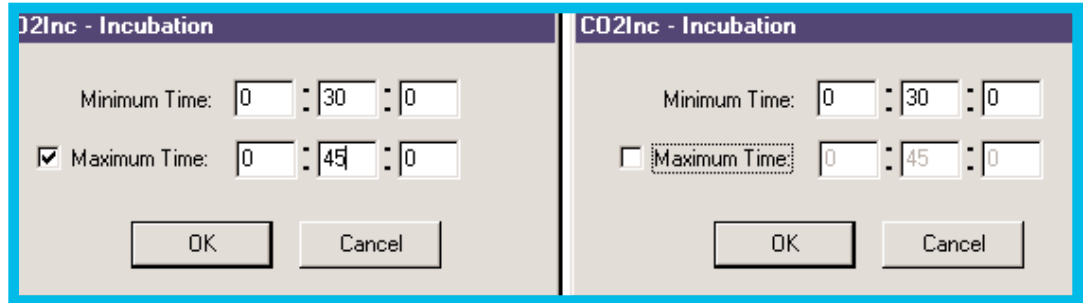


Figure 2. SAMI NT incubation pop-up interface.

SAMI[®] NT will provide the interface for all SAGIAN[™] Core Systems — from microplate replication to high-throughput screening.

The construction of the method graph is in your control. For example, if a method requires reading the assay plate immediately after

reagent addition on the Multitek[™] 96, then simply drag and drop the plate reader icon on the arrow below the plate. If, after reading, the plate needs to incubate for a minimum of 30 minutes but a maximum of 45 minutes, enter the times into the corresponding text boxes for the incubation times. If there is no maximum time, click off the check box and

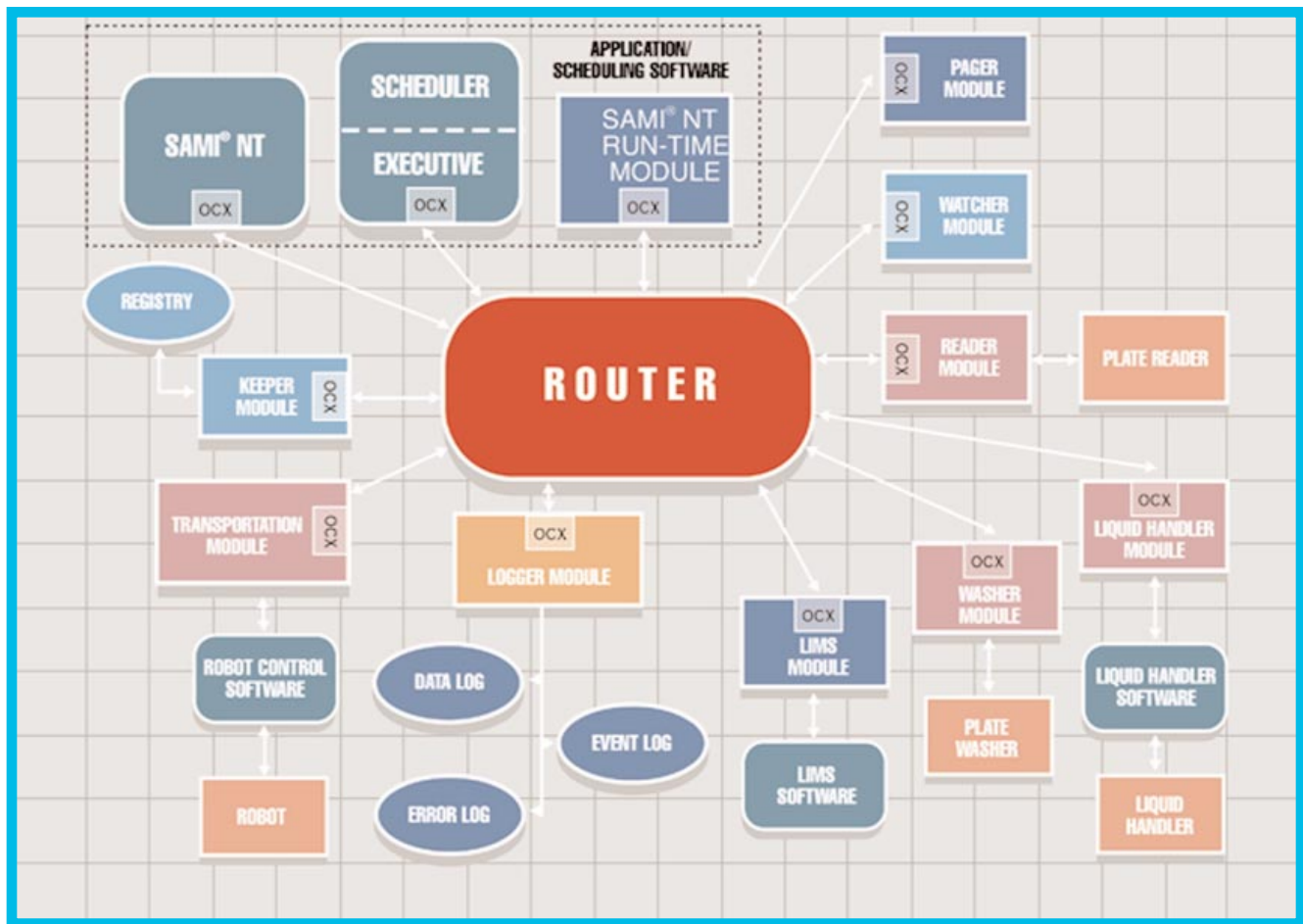


Figure 3. Sample integration software structure.



let the scheduler optimize the throughput of the method by flexing that maximum time.

This is just the beginning of the power of SAMI NT. A test drive is the best way to fully experience the ease with power. Think of the most challenging method or assay, and SAMI NT can complete it the way you want, when you want it.

For a test drag and drop of SAMI NT, contact your field representative.

Revolutionary integration software provides structure that's hardware-independent

Rob Donoho and Mac Williams ♦ Beckman Coulter, Inc.

Beckman Coulter unveiled its new landmark cross-platform integration software at Lab Automation '98 in January. This new router-optimized modular environment was developed under the direction of Dr. Carl Murray at Beckman Coulter.

The software is what provides the underlying system communication structure for method development software and schedulers such as the new SAMI NT. The power of this integration tool is that its use is not limited to SAMI NT, but provides a "universal" structure for a vast array of integration projects that are not manufacturer-dependent.

This new revolutionary software simplifies the integration of lab automation systems, so you can expand and reconfigure systems more easily. Its utility provides message sending between software modules that may be written in any language that supports Active X[®] controls. Some of this software's features include:

- Windows[®] NT based
- Modular environment is easy to create, integrate, modify and reuse software components
- Based on Active X[®], modules can be configured for compatibility with other industry standards, including COM, TCP/IP, and HTML
- Capable of supporting remote messaging, status monitoring, data collection, report generation and more.

APPLICATIONS

A solution for discontinuous sample splitting and dilution using the Biomek

Eleonora Hogan, Andrew Lipnik, Sarah Anstead and Robert Kennedy

♦ *Department of Chemistry, Parke-Davis/Warner-Lambert*

A software solution for the transfer of a set of discontinuous samples in 96-well storage plates to 80-well formatted plates has been designed and written. One thousand samples per day can be processed in this manner. The software is able to handle different sample volumes, dilution volumes, random sample locations and varying concentrations. This program was implemented on a Beckman Coulter Biomek 2000 laboratory automation workstation using Beckman Coulter's BioScript[™] software, BioWorks[™] 1.4A, tool command language (TCL), and Microsoft[®] Visual Basic. This system provides an effective means of selecting and transferring discrete samples.

Introduction

In the Parke-Davis combinatorial chemistry group, samples are synthesized as single entities, and all are subjected to chemical analysis. To facilitate ease of sample retrieval and to conserve space, samples are stored in 96-well deep plates (Beckman Coulter, Inc., Fullerton, Calif.) Decisions are made to screen compounds on the basis of analytical

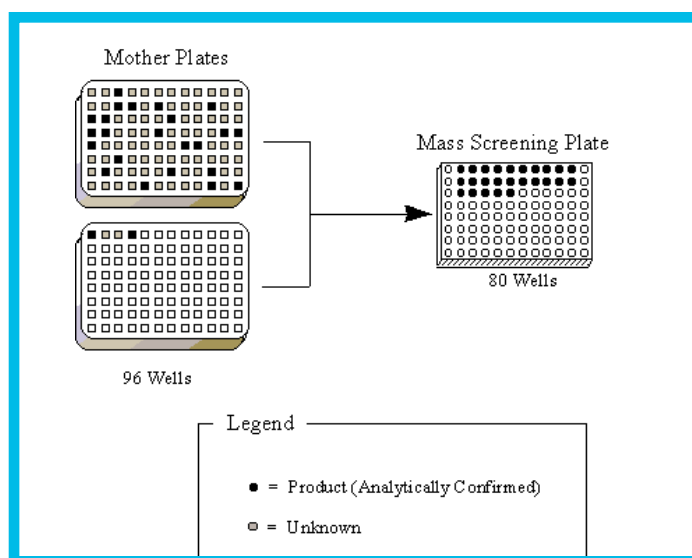


Figure 4. Schematic of discontinuous sample splitting.

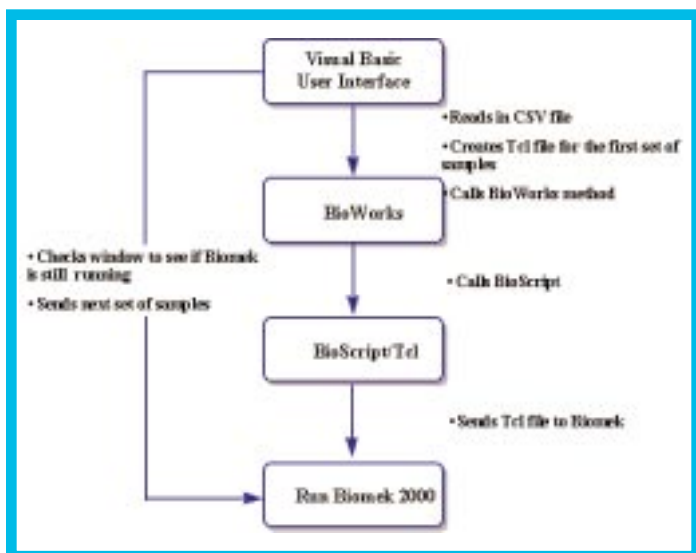


Figure 5. Flowchart illustrating software interaction.

results. Identity, based on mass spectrometry (MS), and a level of purity, based on high-performance liquid chromatography (HPLC), are required for a sample to be screened. Dry samples to be screened are diluted to a known concentration in dimethyl sulfoxide (DMSO) and individually transferred to a separate set of consolidated screening plates.

The challenge

There is a need to transfer samples from random locations in 96-well plates to consecutive locations in 80-well formatted plates. Samples are at varying concentrations in the source plates but must have a uniform concentration in the destination plates. The process should be accomplished with a minimum amount of manual operation.

The solution

The solution included a combination of BioWorks™, BioScript™, Tcl, and Visual Basic programs which allow a comma-delimited file of sample locations and concentrations to be read and processed by the Biomek® 2000.

Key features

Disposable tip management

1. Disposable tips are used to minimize sample contamination.
2. The Visual Basic program sends a set of 144 (number of tips on

the deck at one time) samples to the Biomek.

3. It then uses a Windows API function, *FindWindow ()*, to check the status of the Biomek window. If the window status shows that the Biomek has finished processing the first set of 144 samples, the user is prompted to change tip boxes, and the program waits until this is done.

4. The program sends the next set of 144 samples.

Plate management

1. The program keeps track of how many source and destination plates can be used on the Biomek and ensures that a user file does not exceed the allotted number of plates.

2. When the Visual Basic program encounters a source or destination plate for the first time, it loads it into its respective source or destination plate array. Each subsequent plate is checked against the array of plates already referenced in the file. When either array contains more than four plates, an error message is generated warning the user that he or she is trying to use more plates than the deck will hold.

DMSO dilution

1. The user file contains sample number, source plate and well location, destination plate and well location, volume to transfer and DMSO volume for dilution.

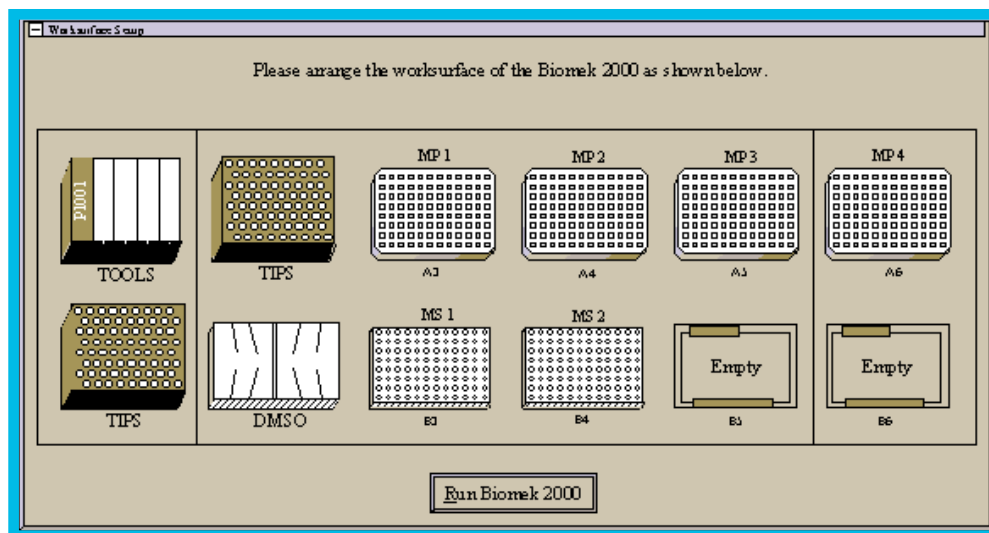
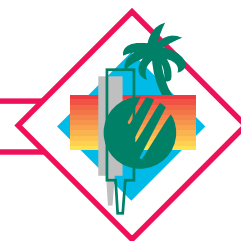


Figure 6. Representative layout of the Biomek workstation, generated by the Visual Basic program before executing Tcl script.

2. The Biomek aspirates DMSO first to avoid contaminating the DMSO reservoir with trace amounts of sample.
3. An airgap is aspirated between sample and DMSO to prevent dripping or contamination and to facilitate accurate volumetric measurements.



Volumetric accuracy

1. We have calibrated the Biomek[®] 2000 using our software and introduced a calibration curve, which has improved accuracy. Prior to calibration, sample volumes varied by as much as 36 percent from the expected value. With the calibration curve, the variation from expected value has dropped to less than 5 percent.

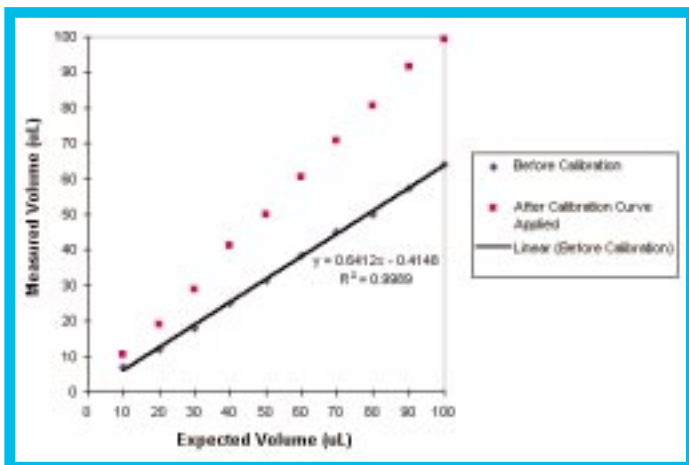


Figure 7: Sample split calibration curve.

Conclusions

With the advent of this software, a user is able to split discontinuous samples by merely supplying a file containing sample information. The file is of a comma-delimited format and can be easily exported from Excel. This information is interpreted by the Visual Basic program to transfer discrete samples from multiple plates to consolidated screening plates on the Biomek 2000. The robot prompts the user for all necessary plates at the start of a run, allowing for minimal user intervention during the run. This software has proven to be a significant labor-saving device.

Some of the Biomek's built-in functionality — such as liquid level detection — is not incorporated into the BioWorks™ 1.4A version of the Tcl interpreter. However, in the next release of BioWorks™ (2.x), Beckman Coulter provides a new developer's environment that will support more advanced functions with Tcl [BioScript™ Pro]. We are currently working to implement this upgrade into our system.

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Automated, solid-phase DNA isolation

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Dynabeads* DNA DIRECT is a solid-phase DNA isolation technique that yields PCR-ready DNA in just 10 minutes. Successful isolations have been performed from a variety of tissues and organisms, including both eukaryotes and prokaryotes. To take advantage of the obvious automation potential of DNA DIRECT, we have developed an automatic magnet station that can be run directly from any software that can address a serial port. Using the magnet station on a Biomek[®] 2000, we have achieved processing times of approximately one minute per sample for DNA DIRECT-based DNA isolation from blood and mouth-scrape.

Magnet station

To take advantage of the paramagnetic properties of the Dynabeads, we used a magnet station consisting of a rack for microtiter plates and a lift shifting the magnets (a modified Dynal MPC-96) between an upper and a lower position.

In the upper position the magnets come up between the wells of microtiter plates, and any paramagnetic particles in the wells are attracted to the magnets.



Figure 8: Magnet station, controller unit and power supply, above. Figure 9: Magnet station on the Biomek worksurface, below.



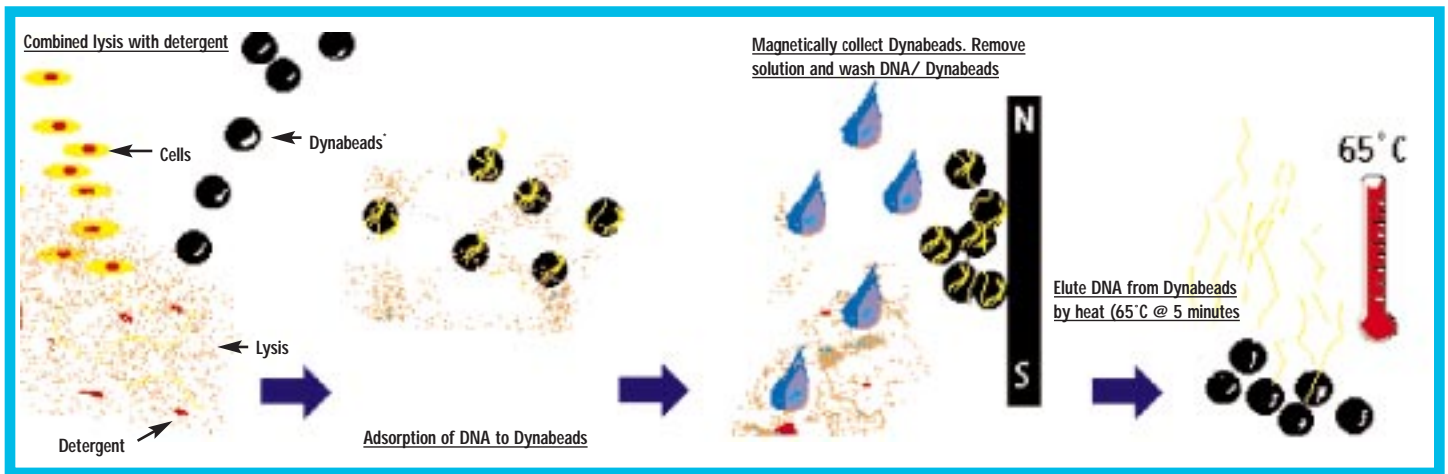


Figure 10. The principle for DNA isolation with DNA DIRECT.

In the lower position, the distance between the wells and the magnets is too great for any such interaction to take place. The device is controlled from within BioWorks™

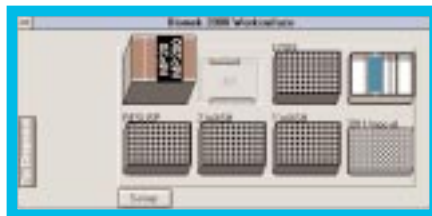


Figure 11: Biomek® workspace.

through one of the serial ports of the Biomek and an external electronic controller. It can alternatively be operated by two buttons located at the side of the station. Both Biomek 1000 and Biomek 2000 can be programmed to press the buttons of the station with any single channel tool. The magnet station is designed to fit the standard labware holders of either of the two Biomek robots, and will be marketed by Dynal as Dynal MPC-auto96.

Biomek 2000 setup and programming

The Biomek 2000 was in a configuration without Sideloader, and with seven rack positions in addition to the one that held the tool rack. The work-surface layout is shown in Figure 12. Whenever possible, tips were reused. All pipetting operations were performed with MP200 (eight channel 5-200 µl pipetting tool).

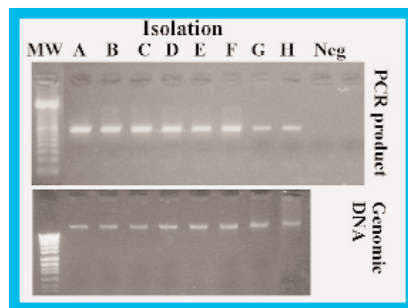


Figure 12: DNA isolation from blood.

sists of a series of pre-programmed modules and usually covers the needs of most applications. To make a method in BioWorks, one must first define the layout of the worksurface, then select the appropriate modules and fill in variables, such as the number of wells that should be processed and whether or not the tips should be reused. However, using BioWorks, the Biomek cannot dispense/aspirate off-center in the wells of a microtiter plate. This action, necessary for implementation of DNA DIRECT, had to be programmed in BioScript™, a tool for direct programming of absolute or relative movement of the four stepmotors. Bioscript routines must be written in relatively small fragments that are subsequently run as subroutines from BioWorks.

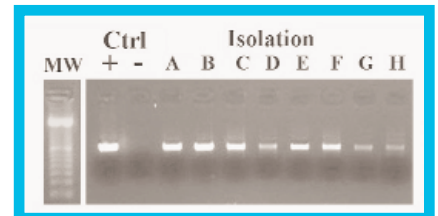


Figure 13: DNA isolated from Buccal scrapes. CD19 PCR from 10 percent of isolated material.

Summary

- Based on proven technology (Dynabeads DNA DIRECT)
- 20 µl of blood yields enough DNA for 20 PCR reactions
- Works with blood and buccal scrapes
- Time consumed is approximately one minute per sample
- Magnet station can be run from within BioWorks



Automation of human molecular diagnostics in the lab of Patrick Merel

Michael W. Clark, Ph.D. ♦ Beckman Coulter, Inc.

Amplification of the diagnostic target is necessary in many human molecular diagnostic procedures because the analysis is being done on genomic DNA samples from blood. Specificity must be maintained, while requiring the highest possible discrimination of nucleotide variabilities for the tissues and/or individual typing. Although other methods of nucleic acid amplifications are available — such as NASBA for RNA detection — the most commonly used technique is polymerase chain reaction (PCR*). All nucleic acid amplification procedures are susceptible to contamination from external sources of nucleic acid and, considering the increasing volume of samples that require molecular diagnostic analysis, automation of these protocols is imperative.

Patrick Merel, Ph.D., has utilized the existing laboratory technologies and the Biomek® 2000 from Beckman Coulter to implement the automation of many of the routine processes in his molecular diagnostic laboratory. The specific points that his group has been able to address are:

- Reduced reagent usage and assay costs by reducing sample sizes to 10-50 microliters
- Nuclease-free DNA/RNA extraction
- Contamination-free amplification reaction setup
- Rapid loading and running of agarose gels for PCR product analysis

Automation of DNA extraction and reduced sample size

Using a low-cost magnetic plate (XS-96T) located on the worksurface of a Biomek 2000, Merel's group utilized a magnetic bead (DynaBeads DNA Direct from Dynal, France) approach to genomic DNA purification from human blood. Starting with only 30 microliters of whole blood, this procedure yields 20-30 nanograms of DNA per 50 microliters final volume. This quantity is sufficient for running 10 amplification reactions. The totally automated process is complete in approximately 30 minutes. For more details regarding Dr. Merel's magnetic bead DNA separation, see his article reprinted on our newly designed website at <http://www.beckman-coulter.com>. Simply click on "Research and Drug Discovery," then select "Drug Discovery Systems" from the "Area of Interest" pull-down menu, select "Go There," then "User Resources," then click on the Merel article.

Contamination-free amplification reaction setup

The Biomek 2000 has been integrated with the MJ Research PTC-200 thermocycler with the automated lid. A 10-microliter reaction can thus

be set up into the thermocycler directly by the Biomek 2000. As a result, 96 amplification reactions can be distributed in 12 minutes. After the reactions are distributed, the PTC-200 closes its heated lid, runs the temperature regime, then opens the lid to allow the Biomek 2000 to remove the amplified products. Since human "hands" are not involved, no contamination of patient samples by technician DNA occurs. HLA tissue typing at the DPB1, DRB1/3-5, and DQB1 loci are done regularly using this apparatus.

Loading and running of agarose gels for PCR product analysis

To maintain the contamination-free manipulation of the patient samples, Merel's group modified the Biomek 2000 by the addition of the Micro SSP gel electrophoresis apparatus (One Lambda Inc.) onto the worksurface. The amplification products can be automatically loaded into the Micro SSP Gel System, the Biomek can activate the electrophoresis and a run is completed in four minutes. The gel is then ready for examination and analysis.

The Gripper tool

Merel envisions a further exploitation of the Biomek 2000 capabilities in regard to the Gripper tool. He sees the ability of the Biomek/Gripper — in conjunction with a thermocycler and a fluorimeter — to set up and run fluorescence-based amplification reactions, followed by dispersal into a microplate, and the Gripper moving that plate into the fluorimeter for quantitation.

High-throughput, contamination-free automated systems for molecular diagnostics can be constructed from existing devices to assist the diagnostic laboratory in meeting the increasing demands for contamination-free, reliable and consistent services.

For more information, contact Patrick Merel, Molecular Biology Laboratory, Blood Transfusion Center, Bordeaux, France: patrick.merel@crt.s.u-bordeaux2.fr

Interactive applications, methods developed on the Biomek 2000

Dennis Patrick O'Brien ♦ Beckman Coulter, Inc.

Introduction

The Beckman Biomek 2000 can now be part of interactive applications and methods. These include work listing, smart pipetting (normalization), integration with other software packages and user applications. These are developed with a scripting language called BioScript™ and Tcl.

Dynamic Data Exchange (DDE) client capability is now part of BioScript with BioWorks™ version 2.0. This new feature makes it possible

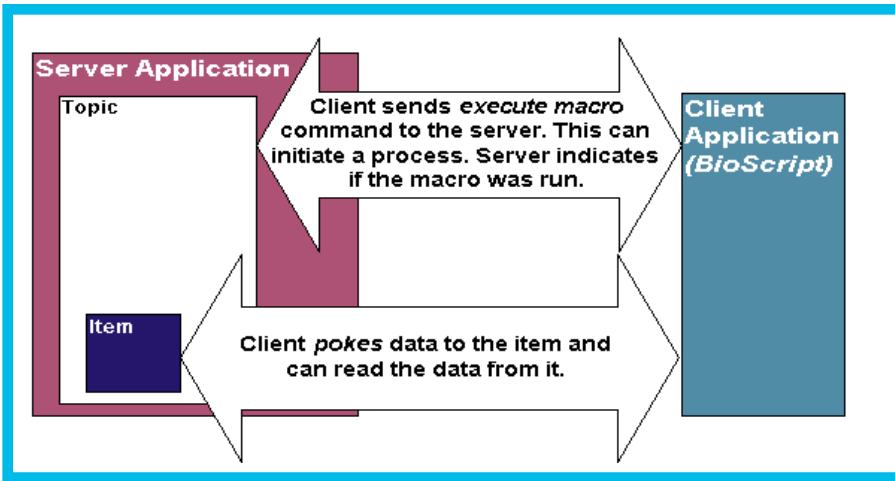


Figure 14: DDE conversation.

to create truly interactive methods where applications running on the controller (PC) and the Biomek 2000 can transfer data directly. The Biomek can even react to this data. User applications, databases, spreadsheets and other applications can now be part of a method.

DDE allows applications to share data by engaging in a DDE conversation. In this conversation, one application is the DDE client and the other the DDE server. The server provides a topic and a place called the item for data to be placed (poked) or read. The server can react to changes in the data via its on-poke event (Figure 14).

For example, BioScript™ can engage in a DDE conversation with

Microsoft Excel* to request the data in a specific cell of a spreadsheet.

The request is poked by BioScript to Excel and Excel responds by placing the contents of the cell on the DDE Item. All DDE data is transferred as a string (character data).

The client, in this case BioScript, monitors the item to see if the data has changed. This is usually done through iteration or looping, where the client repeats the process of checking the item until its value changes.

Work listing

In work listing (Figure 15), data on the PC — in this case in a Microsoft* Excel spreadsheet — is used to determine if a transfer will take place. A table of data associated with a 96-well source plate is

processed and the contents of wells associated with data above a specified threshold value are transferred to a destination 96-well plate. When filling the destination plate, selected samples are placed in consecutive wells.

The data in the spreadsheet represents absorbance data for the wells of a 96-well plate. The BioScript reads the data in the cells. If the value in a cell is greater than one, it will move the corresponding source plate well contents to the destination plate.

The script utilizes nested loops to increment the row and column counters. These counters are used for determining the spreadsheet cell

from which to retrieve data, and which row and column of the source plate from which to aspirate.

Another set of counters is used to determine the next tip and the destination well for dispense. These counters are incremented only when a value in the spreadsheet exceeds the threshold value in the script.

An example of this application is documented in the BioScript Pro manuals, and an example for BioScript can be found on the Beckman Coulter website (<http://www.beckmancoulter.com>). These can be tailored to meet other needs.

Smart pipetting

In smart pipetting, data on the PC — in this case in a Microsoft Excel spreadsheet — is used to determine what amount of buffer to add to samples to bring them to a uniform concentration. A table of data associated with a 96-well source plate is processed and used for determining the correct vol-

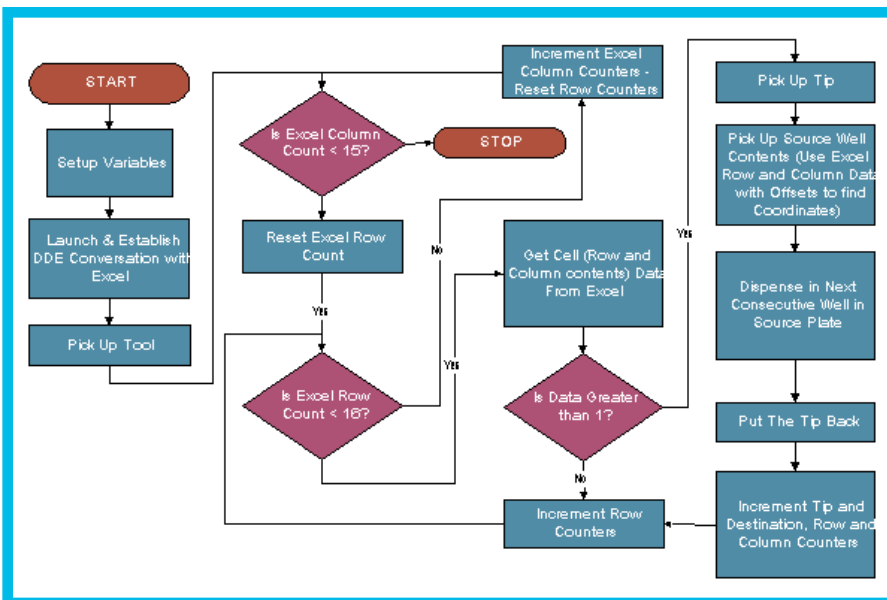
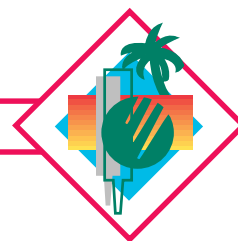


Figure 18: Work listing flow schematic.



umes of sample and diluent aspirated and transferred to the plate.

The data in the spreadsheet represents absorbance data for the wells of a 96-well plate. The BioScript™ reads the data in cells and uses this data to calculate the volumes needed to normalize the plate.

The BioScript would be similar to work listing, but aspiration volumes are controlled by the formula:

All wells are transferred, but the volumes are changed.

An example of this application is documented in the BioScript Pro manuals. This can be tailored to meet other needs.

User applications

DDE server applications can be created using other development tools. These include C/C++, C++ Builder, Delphi and Visual Basic.

The developer determines what the application does. It might convert plate reader data and provide it to the script. It might communicate with other devices through the PC comm ports, or an application could be written to respond to user input.

A DDE server will have a service name, topic and item. A server can have more than one topic, and each topic can have multiple items.

The server may have code that responds to the on-poke event when a

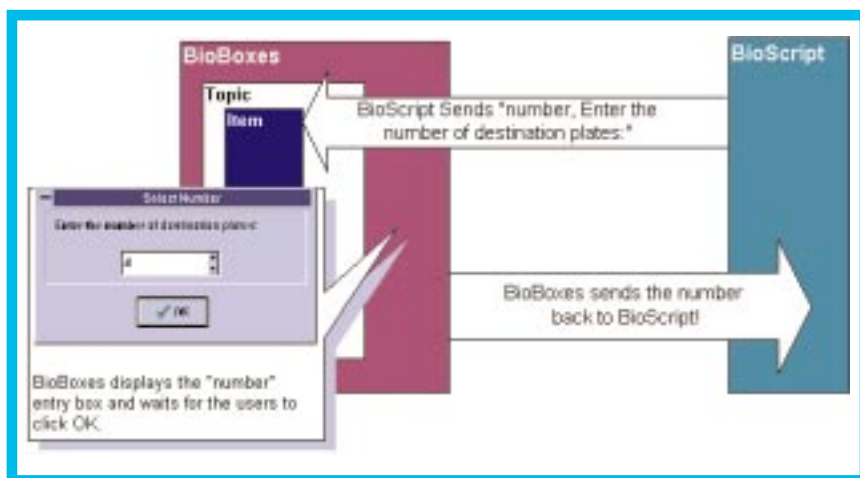


Figure 16: BioBoxes.

client changes the data on the item. This is where data is shared. Both the client and the server can change the data on the item. When the data is changed, the server can examine it and react accordingly.

The server may have code that responds to the execute macro event. This can initiate a process such as opening a file or converting data.

Example user application — BioBoxes

BioBoxes is a DDE server that displays various dialog boxes on the PC (Figure 16). If a dialog box requests user input, it will return that data on its DDE item.

The BioScript can use the returned data to determine how many plates to process, as a threshold value, as a command or other purpose. In this way the same method and script can be used for different volumes, numbers of plates, configurations and more.

BioScript Pro expands the number and type of tasks for which this data can be used. It has access to system variables and can alter the method and labware configuration.

BioBoxes was created using Borland® Delphi* (Object PASCAL). It is available on the Beckman Coulter website with documentation in the help file.

DDE clients, BioScript in this case, can poke data to the BioBoxes item. This data contains the type of dialog box to display and any other parameters needed to display it such as the text to be placed in the dialog box.

BioBoxes responds to the on-poke event by reading the item, displaying the requested dialog box and waiting for the user to respond. The results of the response are returned to the script through the DDE item

BioBoxes supports the following dialog boxes:

- Yes/No — Returns if a user has answered yes or no to a question the script provides
 - Warning — A warning dialog box with text defined by the script
 - Abort — An abort/retry dialog box that returns the user's choice
 - Error — An error dialog box with text defined by the script
 - Number — Returns an integer value entered by the user
 - Text — Returns a text string entered by the user
 - PC Communication Port — Sends a text string out of the PC comm port requested by the script, waits for response and returns that response to the script
- Example DDE Command in Tcl and BioScript to BioBoxes (This would display a yes/no dialog box):

```
extref_init "BioBoxes|BioBoxes"
extref_set "BioBoxes|BioBoxes|DataIn" "yesno,Select Yes or No!"
```

Conclusion

BioScript and BioScript Pro DDE communications open the door for completely interactive methods on the Biomek 2000. Developers can control the Biomek™ 2000 from an external application and monitor the status of the method.

The method can interact with many standard applications on the PC, including Microsoft Office* and other DDE-enabled applications.



With BioScript™ Pro, these applications can create custom labware and operations. This simplifies the development of methods involving scripting and integration of other instruments and systems.

Because the developer can now create external applications that interact with the Biomek® 2000's software, the possibilities are endless for custom methods on the Biomek 2000.

More information and examples are available on the Beckman website.

EVENTS

Lab Automation '98 best attended to date

Lauren Eckert ♦ Beckman Coulter, Inc.

Beckman Coulter's exhibit was one of the most prominent and popular spaces at Lab Automation '98, held Jan. 18-20 in San Diego, Calif.

Large, colorful graphics and a fast-paced biorobotics video commanded attention as visitors entered the exhibit. The Bioresearch area featured the only fully operational robots with application at the show, including a core plate replicating and high-throughput screening systems, SAGIAN Sealer, Print & Apply and Filtration Stations, plus the Biomek 2000 integrated with Cartesian Engineering's Biodot for nanoliter volume dispensing. Booth traffic was consistently heavy during and after show hours.

Of particular interest was the computer demonstration introducing new SAMI® NT and groundbreaking ROME software. SAMI NT is the new drag-and-drop methods development interface and dynamic scheduler now available for use with all core systems. The much-anticipated ROME software transportation module eases integration of robotic systems by streamlining the addition process of new components. Both systems were premiered at the show to an extraordinary reception.

Beckman Coulter sponsored an award for pioneering research in biorobotics presented during Monday evening's Lab Automation dinner. This year's recipient was Dr. LeRoy Hood of the University of Washington, Seattle, Wash., for his pioneering and original research in the general field of automation.

Club Biomek Customer Appreciation Night 'under the stars' a success

Lauren Eckert ♦ Beckman Coulter, Inc.

The Ruben H. Fleet Space Theater and Science Center was the place to be Jan. 19 after a busy day at Lab Automation '98. "May the Fun be

with You" was an appropriate theme for more than 175 Beckman Coulter customers, sporting colorful glow necklaces as they spent a stellar evening listening to jazz and chatting over risotto, hors d'oeuvres and carved roast beef.

The Fleet Science Center was open for self-guided tours, which included hands-on interactive space exhibits and learning galleries. The evening culminated with a star-filled treat for the senses, "Cosmic Voyage," a mesmerizing IMAX film in the Space Theater. And then, a treat for the taste buds — chocolate-covered strawberries, fruit tarts, star cookies and a cappuccino bar.

Just before embarking on the bus back to the Sheraton Hotel, guests were gifted with a useful remembrance of their evening, a travel calendar/calculator/world-time clock to take with them on their next cosmic voyage, or trade show, whichever comes first.



ANNOUNCEMENTS

A fond farewell to Charles Powell

The staff of Beckman Coulter takes this opportunity to bid farewell to Charles Powell, Manager of Field Operations.

Charles and his family will be moving to Whitefish, Montana, where he will join the Paine Webber Corporation. But the primary reason for the move, Charles said, is to enable him and his wife, Shelby, to establish and operate a camp for children.

In his 12 years with Beckman and five-year association with Biorobotics, Charles established Club Biomek, founded the T3 newsletter, and invented the Core System concept, resulting in what has today become a strong support system for users of Biomek and other automated integration products.

If you'd like to send Charles a message, he welcomes your emails to crpowell@beckman.com. After May 15, send your emails to Charles c/o John Gerace, NAO-B Product Manager for Drug Discovery Systems, Beckman Coulter, at jdgerace@Beckman.com.

We wish Charles and Shelby all the best!



Charles Powell

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