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Automating Gel Image Acquisition

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Abstract: We describe the design and implementation of a robotic solution to automate the acquisition of gel images. The soft- and hardware aspects are outlined together with the various safety aspects that need to be addressed.

Keywords: automation • image acquisition • throughput • robotics

Introduction

Recently, a great deal of attention has been focused on the use of proteomics as a clinical tool to aid in diagnostics and prognostics.[1] The demands on proteomics techniques in a clinical setting are quite different from those in the academic laboratory. Ease of use and reproducibility are major demands, as is throughput. The approach we have decided to take is to develop a "high" throughput academic environment to define protein markers for various diseases and states thereof that can then be used to define content for antibody-based protein arrays for use in a clinical setting. The proteins should be expressed at a fairly high level because we wish to be able to detect their leakage out into blood using the protein chips.

After initial evaluations, we decided that the approach best suited to this type of study is 2D gel electrophoresis. One small-scale preliminary study has involved the profiling of 80 histologically and pathologically well-defined ovarian tumors. Using the DIGE[2] approach, we ran each sample in duplicate with a control, generating 240 gel images. This was run in two batches over two weeks. After image analysis, the gels can be placed into a robotic gel-handling station, the Spot Handling Workstation from Amersham, which allows twelve gels to be loaded in a batch and 1500 spots to be cut, destained, digested, and spotted onto a MALDI target in a 24-hour period. The 96-target position MALDI plates (up to 50) can be loaded into the Waters MALDI TOF mass spectrometer for unattended protein fingerprinting (ca. 1500 samples per 24-h period). The remaining unidentified proteins can be scheduled for automated MS/MS using an Advion Nanomate robot interfaced to a Waters QTOF Ultima. This allows 300 samples to be analyzed (1500 MS/MS) in an 8-hour period.

The bottleneck in this workflow[3] has now become the acquisition of gel images by scanning (because Cydyes are used, there is no staining; instead, the gel cassettes are placed into a large box through which fixing solution can be pumped). Two 24 × 20 cm 2D gels can be loaded onto a 9410 Typhoon scanner (Amersham). Six images (each gel contains 3 samples labeled with Cy2, 3, and 5) can be acquired in a half-hour period. Hence, the scanning of 50 gels (150 images) becomes a very labor intensive and extremely boring undertaking. Thus, we decided to automate the procedure.

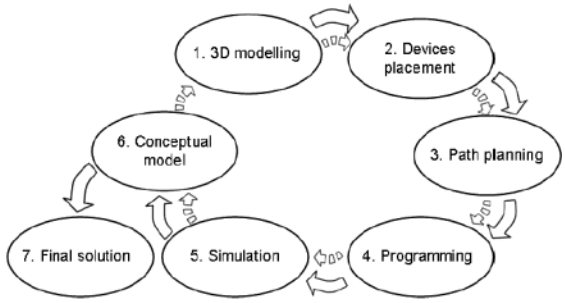

Figure 1. Workflow definition. The figure schematically represent the circle of iteration that is used in the design and implementation of a robotic solution.

Workflow Definition

The first stage in automating a process is to define a preliminary workflow. The number of gels to be scanned per batch was set at 25 per day (corresponding to 75 images for triple-labeled fluorescent gels). The logistics involved are as follows: managing gel storage during a batch, moving the gels, simulating the robots movements, manufacturing the parts required, assembling the work cell, and finally, defining communications between the various devices and programming the environment and interface. A diagram illustrating the workflow during a project definition is given in Figure 1. The simulation of the robots movements (also called offline programming) was done using UltraArc, a program developed by Delmia (http://www.delmia.com) for arc welding. The idea is that it is easier to work without the presence of physical devices and that changes can be easily made. The need for simulation was mainly for the design of the robot cell layout and the workflow therein, as well as the robot motion planning.

Hardware Implementation

After electrophoresis is finished, the gel sandwich is disassembled. The gel that is fixed on a glass support is placed in a specially designed container through which fixing solution can be pumped. The gel cassette is reassembled, and four specially designed clips are used to hold the sandwich together. The gels

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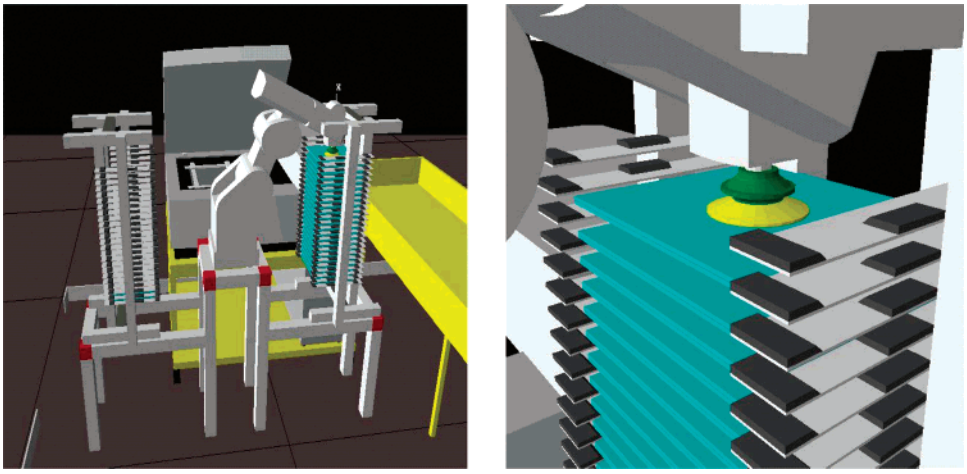

Figure 2. Computer animation shots of the design. The gel hotels loading and receiving shelves are located on either side of the robot and the scanner is bolted to the front of the workstation. The gels are picked up by the suction cups and moved by the arm from the top of the loading station to the scanner and then to the bottom of the receiving station.

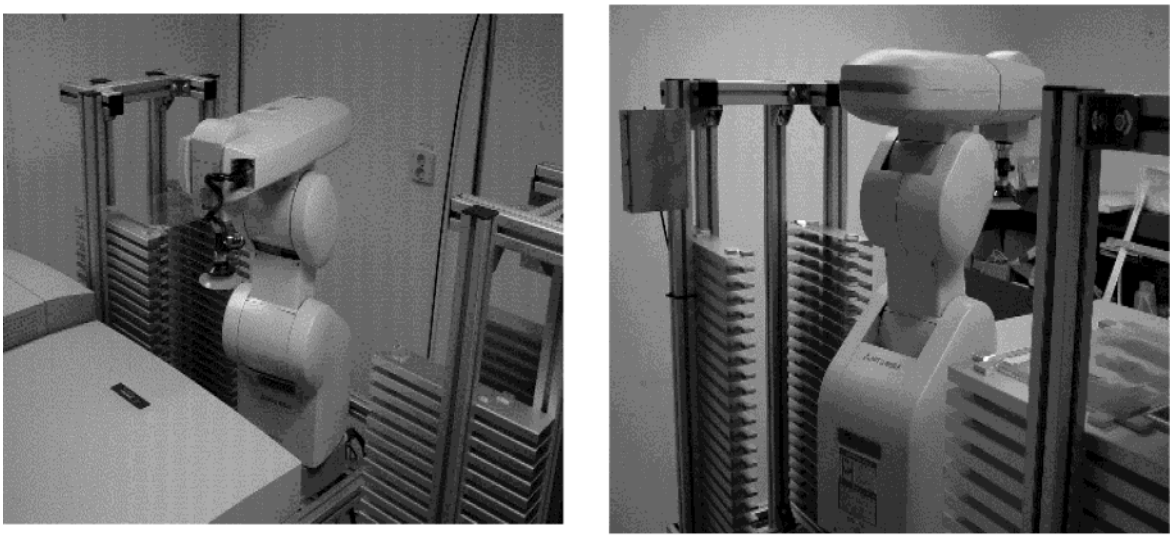

Figure 3. Actual setup of the robot with the scanner is shown in the left panel. The bar code reader is shown in the right panel and is contained in a metal box near the top of the receiving column.

are stored in a vertical rack (a gel hotel) on one side of the robot and are systematically removed starting from the top of the hotel and working downward via the scanner to the bottom of the rack on the opposite side and working upward (see Figures 2 and 3). The robot chosen for this task was a Mitsubishi RV-2A because the demand for flexibility is fairly high, and therefore, a 6-axis robot is a prerequisite. The scanner, a Typhoon 9410 from Amersham Biosciences was chosen because it can handle two large-format gels at a time and can carry out three-color laser fluorescence scanning. A suction cup system was chosen to pick up and move the gels because this gives the simplest set of movements.

Software Implementation

A program called tscan developed by Amersham for scanner testing was used as the base for the scanner. It is a simple command line DOS program allowing information and parameters to be entered, such as where the scanned images should be saved and the name of the file to be created. If any parameter is missing, then the command line will assume a default value. Because the parameters are the same for one batch, tscan is run repeatedly with just a change of the image name. This was achieved by programming a batch file with the command line and running the batch file repeatedly with the change of the image name. A bar code reader inputs the scanned code on the gel, which is sent to the communication port where it is read by the interface program.

A user interface was created that was designed to be similar to the program made by Amersham for controlling the scanner. The interface includes all of the parameters that the scanner needs, which are stored in the batch file. The parameters are set for all of the gels in a batch. A prescan is used to obtain an overall value of total intergrated fluorescence for the gel at low resolution, around 1000 microns. Then, for the main acquisition, a resolution of 100 microns is typically used, and the detector is set so that a preset number of spots can be allowed to go into saturation. This is highly sample dependent. For a bacterial sample such as *Escherichia coli*, where most of the spots are well-distributed and are of roughly even intensity over the gel, three to four spots may be allowed to saturate. The other extreme is a serum type sample where the major spots are of little interest, and the detector is allowed to saturate up

to 70% of the signal coming from the albumin, Ig subclasses, and so forth.

The interface programming is done in Microsoft Visual Basic. The robot is controlled via an ActiveX instead of the Mitsubishi-supplied software. This is because it easier to extend the software with custom programming. The robot programming involves creating movement trajectories, grip and release of the 2D gels, and other control commands. The program, in Visual Basic, communicates with the robot controller via an ActiveX in the program. The program calls a subprogram in the robot controller and the sequence decides which program in the control box that should be called.

Image Acquisition

To obtain reproducible images with approximately the same number of spots on each, the correct photomultiplier setting must be used. This is determined empirically using two gels picked randomly from a batch. The gels are scanned at several different PMT settings, and the number of spots detected and number of spots saturated are determined and plotted against the PMT value. Thus, for each type of sample (serum, bacterial extract, etc.) a constant can be determined which allows one to calculate the correct PMT setting after a quick low-resolution prescan is done to determine the extent of label incorporation. The prescan value is passed to the control program that then runs the real scan at the appropriate PMT setting. Thus, each gel can be scanned to give the same total fluorescence intensity hence the same total number of spots and same number of saturated spots. This greatly facilitates subsequent gel matching. The apparatus is being used in tumor analyses programs (for example, an analysis of 80 ovarian tumors run in duplicate with standards comprising a total of 240 images, a 1200 image set of hereditary breast cancer samples and a larger set of around 2000 images for a sporadic cancer set). The main problem now is cleaning the gel plates, so an automatic dish washer is next on the automation agenda.

Safety Considerations

Safety in and around a robot cell is an important issue. It is important to have a well-visualized safety system, but it is even more important to create a safety system that does not disturb production or deteriorate the quality of the work. The robot movements may seem irrational to untrained persons, which makes it hard to predict the next step for the robot. To avoid injuries, it is important to keep unauthorized people out of range of the robot and give the user a good comprehension and knowledge of the system. The instrumentation is located in a converted dark room with the computer terminal outside. Safety power off buttons are mounted both inside and outside the room, which is kept locked. A complete description of the programmng and construction will be made available at http://www.proteomics.swegene.lu.se and www.robotics.lu.se.

Abbreviations: PMT, photomultiplier tube

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References

(1) Krieg, R. C.; Paweletz, C. P.; Liotta, L. A., Petricoin, E. F. Clinical proteomics for cancer biomarker discovery and therapeutic targeting. *Technol. Cancer Res. Treat*. **2002**, *1*, 263−272.
(2) Unlu, M.; Morgan, M. E.; Minden, J. S. Difference gel electrophoresis: A single gel method for detecting changes in protein extracts. *Electrophoresis* **1997**, *18*, 2071−2077.
(3) Quadroni, M.; James, P. Proteomics and automation. *Electrophoresis* **1999**, *20*, 664−677.

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