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Master's Thesis



Automation in a Bioscience Research Centre

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Abstract

Title	Automation in a Bioscience Research Centre
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Tutors	Gunnar Bolmsjö, Professor, Division of Robotics, Department of Mechanical Engineering, Lund University Peter James, Professor, Swegene, Proteomics Platform in Lund
Key words	Automation, Offline Programming, Robotics, Protein 2D Gels
Problem description	Automate a scanning process of 2D protein gels using a robot
Objective	Create a robust automated system, which is able to handle a batch of 24 2D gel samples. The system primary includes robot, scanner, bar code reader, PC and storage for the samples. A user friendly interface to operate the process should also be made
Methods	3D robot modelling and simulations. Purchase and manufacturing. Programming. Component implementation
Conclusions	The automated system is a prototype that fulfils the desires, but can be further developed in order to achieve a more optimised solution

Preface

This Master's Thesis is made as a part of the completion in the program Master of Mechanical Engineering and the work corresponds to 20 credit points. The work was made at the Proteomics Research and Development Centre in Lund in cooperation with Division of Robotics at Lund University.

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Mr Roger McIntosh, Mr Lance Kasari and other employees at Amersham Bioscience, for standing by with their skills and resources and for providing us with great consultation and help.

Lund, October 2003



Peter Bäck



Fredrik Någård

Summary

Today there is a great interest in the use of proteomics, the study of the proteins produced by cell type and organism, as a clinical tool to help in diagnostics and prognostics. Swegene has decided to take an approach 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, since Swegene wish to be able to detect the protein leakage out into blood using the protein chips. The Proteomics Research and Development Centre is a part of Swegene and make protein analysis and identification for fingerprinting. A technique to achieve fingerprints is to separate the proteins. The separation is made by pH and by size, and then the proteins are scanned. The scanning is made with the scanner, Typhoon 9410, from Amersham. The scanning process is time demanding and is desired to be automated, so the research people can focus on other parts of their work. An automated system will also speed up the scanning process and larger volumes of proteins can be scanned. Today, the research centre has a volume of 3 000 samples from breast cancer waiting to be scanned and analysed.

The logistics in the automated system basically is the scanner, a robot and storage for the gels which should be scanned. To achieve this kind of system, which in this case is a prototype, a process with a well defined workflow was carried out. The workflow includes offline simulations, purchasing, manufacturing and assembling of robot and other equipment; programming the scanner, the robot, but also the communication between them.

The offline simulation was carried out for the purpose of achieving a good layout of the robot cell, optimising the logistic flow and for path planning. With offline simulations changes can easily be done, because of the non presence of physical devices. With that, concepts can be done in order to get a functionally good and robust solution.

The system is designed to manage 24 sample scans per batch and the batch is loaded in a hotel before the scan. The robot then picks one sample at a time, with a suction cup and goes to a bar code reader for sample identification. Then the robot puts the sample in the scanner and after the scanning, the robot picks the sample and puts it in another hotel.

A program is made to communicate with the robot, the scanner and the bar code reader. The robot itself has own programs for path movement, grip and release. Data is send to the scanner with parameters, which is used for each batch of samples.

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1 Introduction

1.1 Background

Recently a lot of attention has been focussed on the use of proteomics, the protein technology, as a clinical tool to help in diagnostics and prognostics. The demands on proteomics techniques in a clinical setting are quite different to those in the academic laboratory. Ease of use and reproducibility are major demands as is throughout. The approach Swegene has 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, since Swegene wish to be able to detect the protein leakage out into blood using the protein chips.¹

After initial evaluations, Swegene decided that the approach best suited to this type of study is two dimension gel electrophoresis. One small-scale preliminary study has involved profiling of 80 histologically and pathologically well-defined ovarian tumours. Using an approach called DIGE, Swegene ran each sample in duplicate of three with a control, generating 240 gel images from the tumours. This was run in two batches over two weeks. After image analysis, the gels can be placed in a robotic gel handling station, the Spot Handling Station from Amersham, which allows twelve gels to be loaded in a batch and in total 1500 spots to be cut, destained, digested and spotted onto a mass spectrometry 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 (about 1.500 samples per 24-hour period). The remaining unidentified protein can be scheduled for automated MS/MS using an Advion Nanomate robot interfaced to a Waters QTOF Ultima. MS/MS means that mass spectrometry is run twice. This allows 300 samples and 5 peptides in each sample, which means 1500 MS/MS, can be analysed in an 8-hour period.

¹ James, P. *Swegene*. Personal communication. (2003-10-16).

The bottleneck in this workflow has now become the acquisition of gel images by scanning. Since Cy dyes are used there is no staining, instead the gel cassettes are placed in a large box through which fixing solution can be pumped.

Two 24 cm x 20 cm 2D gels can be loaded onto a 9410 Typhoon scanner from Amersham. Thus six images, each gel contain 3 samples labelled with Cy2-, Cy3- and Cy5 dyes, in a half hour period. The scanning of 50 gels becomes a very labour demanding and extremely tedious undertaking. Therefore the procedure was decided to be automated.²

1.1.1 Swegene³

Swegene is a consortium established by the Chalmers University of technology, Göteborg University, Lund Institute of Technology and Lund University. It is funded primarily from Knut and Alice Wallenberg Foundation, but also with support from participating universities. The consortium is within the field of Functional Genomics, which is the science of the function of genes and gene products and their interaction in biological systems. Two high-technology platforms have been established in Göteborg and Lund and offer service to all researchers in Sweden.

1.1.2 The Proteomics Research and Development Centre in Lund⁴

The Proteomics Research and Development Centre in Lund is a Proteomic Platform, and established by Swegene and Lund University. The aim for the Proteomics Research and Development Centre is to provide access for the large medical, natural science and technology research communities to resource centres for medium-throughput protein analysis and identification. A technique for protein analysis and identification is two dimension electrophoresis.

1.1.3 Two Dimensional Electrophoresis⁵

2D electrophoresis is a method for separating proteins. Before the separation method, the proteins have to be released from the cell.

The first step in the 2D electrophoresis method is isoelectric focusing, which is the separation in the first dimension. Isoelectric focusing means that the proteins are separated on the basis of their contents of acidic and basic residues. Each protein has an isoelectric point, which is the pH when the proteins have no net charge. In isoelectric focusing each protein migrates to their isoelectric pH when the sample is

² James, P. *Swegene*. Personal communication. (2003-10-16).

³ <http://www.swegene.org/index.php>. *Swegene*. (2003-05-12).

⁴ <http://proteomics.swegene.lu.se/>. *Lund Proteomics Platform*. (2003-05-12).

⁵ Berg, J., Tymoczko, J., Stryer, L., *Biochemistry*, p. 78-86. (2002).

loaded and voltage applied in a strip gel with a prepared pH gradient, see figure 1.1 and figure 1.2.

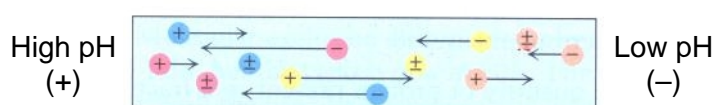


Figure 1.1 The proteins are migrating to their isoelectric pH.

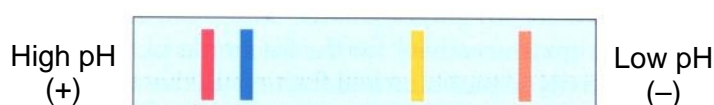


Figure 1.2 Bands are formed for further analysis.

The strip gel is then placed horizontally on a polyacrylamide gel for separation on the mass basis. The proteins are first dissolved in a solution of sodium dodecyl sulphate (SDS). Thereby the name of the technique, SDS-polyacrylamide gel electrophoresis, SDS-PAGE. When electrophoresis is performed in the second dimension, the proteins are separated vertically, where the small proteins move faster than the larger proteins, see figure 1.3. This result in a 2D protein gel for further image analysis, see figure 1.4.

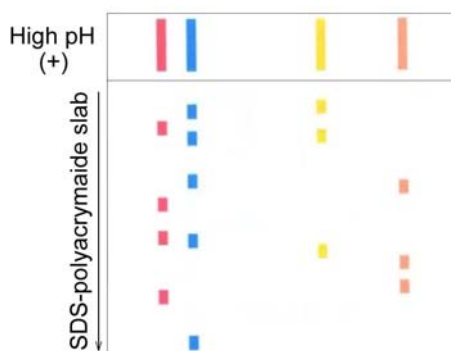


Figure 1.3 The gel separated in the second dimension by mass.

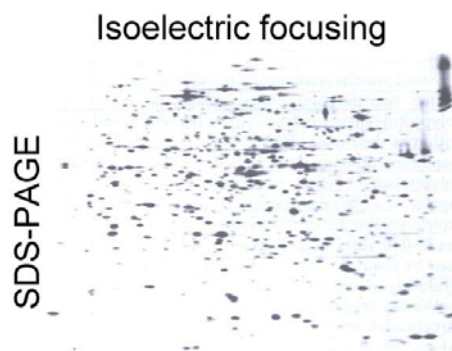


Figure 1.4 The image from gel separated in the second dimension, by mass.

1.2 Automation at the Proteomics Research and Development Centre

Automation within medical science and technology research centres means in general a relief for the employees and the employees can spend more time on research. It is often suitable to automate when processes are monotonous and with relative high frequency throughout.

The impacts of automation at the Proteomics Research and Development centre in Lund are less working hours spend on scanning gels and consequently more time for other important work. In the end the automation will fasten up the scanning process and more gels can be scanned per day. In economic terms, the automation investment will have a payback in roughly one year, with a base in size of an employee. At the time, when this report is written, a batch of 3 000 samples are going to be analysed for breast cancer research. In the future there will be a larger volume of samples to be analysed.

1.3 Problem Formulation

In the analyse process of proteins, first 2D gels are created by separating the proteins in two dimensions. When the proteins are separated and 2D gels are created, they are placed in-between two non florescence glass plates and then they are scanned for image analysis. The glass is non florescence because they should have no impact on the image. The scanning process is time demanding for the research people, this since the scanner needs to be loaded and unloaded before and after the scanning. The scanner also needs continuously supervision to obtain time efficiency. Therefore an automated system, which can handle the 2D gels in the scanning process, is desirable. The system includes principally a robot, the exciting scanner and periphery equipment.

1.4 Objective

To maintain automated handling of the 2D gels a complete robot working cell has to be set up. This is the main objective. Beside from that it is taken for granted that it is a stable and robust working system. This means that the system should work without any disturbance. Another objective is to adapt the robot system to the people who will be working with the scanning process in the future. The scanner should also retain to be able to be manually operated. In addition, a bar code is attached in the 2D gels for sample identification and a bar code reader has to be implemented in the system. An important objective is that no impact on the scanning are allowed. That means that the images that are created with the automated system should not be different to the images when the gels are manually scanned.

1.5 Delimitations

It is desired that the system render it possible to plug in more smart devices. In consulting with experts, this would require advanced programming in a time frame of six month. Since the project is scheduled for 20 weeks, it is considered impossible and therefore the system is reduced in the number of smart devices.

The automated system is required to handle 24 gels per batch, which means that the design of the robot cell has to manage 24 gels.

1.6 Target Groups

The primary target group is the employed research people at The Proteomics Research and Development Centre, who use and will use the scanner. Other target groups are people with interest of automation in medical science and technology centres and university students, but also anyone with knowledge and a great interest in robotics and automation.

2 Methodology and Workflow

2.1 Approach

The first stage in the project was to gather information and get an understanding about the problems and the background of the problems. The second stage in the project was to carry out possible solutions. Initially a preliminary work flow was carried out:

- Basic proteomics course
- Visit at Amersham, the scanner manufacturer
- Robot simulations
- Purchase and manufacturing of robot and equipment
- Assembling the work cell
- Communications and programming

The blocks in the work flow do not follow each other strictly, but some of the blocks work parallel to achieve time efficiency.

When the work flow was set up, it was taken into consideration that it would take time to order the robot. This since it is a large investment and it is important to make a good decision about which manufacturer and which model that should be chosen. The price is also another important aspect, and it takes time to deal with different suppliers.

2.1.1 Understanding

To obtain a better understanding of the project problem, a part of the course “Basic Proteomics Course” was carried out. The course includes creation and analyse of 2D gels. The project focus was from a process perspective. The achievement was to gain understanding behind the project. The course is run by the research centre in Lund and last for two weeks.

A better understanding about the scanner was also necessary. Therefore two weeks at Amersham Bioscience in the USA, the manufacturer of the scanner, was set up. The aim at Amersham was to be shown how the scanner works and how to control

the scanner. Amersham offered not only support with competent people within the area, but also equipment resources.

2.1.2 Solution Discussion

In the initial stage a preliminary solution had to be constructed in cooperation with Amersham. This solution was based on all the desires from the Research Centre. The discussion included questions about:

- how to handle the 2D gels with the robot
- how to establish a communication between the robot and the scanner, but also with other devices, such as a bar code reader
- how to perform a scan of 2D gels without the scanner lid
- how to make an interface for the future user and how to make it so the user easy can identify the settings of the parameters of the scanner
- level of safety

The answers of those questions will be further discussed in the report. Since this is a prototype and as the project goes on, more questions arise with the time. The new questions are mainly tradeoffs and they will be discussed in report as they appear in the project.

2.2 Simulations in UltraArc

UltraArc is a 3D simulation program for robot simulations, also called offline programming. The idea with offline programming is that it is easier to work without the presence of physical device and changes can easily be done.⁶ In the simulation program, design and placement with great accuracy can be achieved. The program, UltraArc made by Delmia⁷, is mainly made for arc welding, but for other applications like in this project the simulation tool could be really helpful. With earlier experience of UltraArc and the availability of the program, it was decided to make conceptual model design for the robot cell in UltraArc. UltraArc makes it possible to import certain robot model from famous robot manufacturers, but also importing 3D models from some 3D CAD programs. Modelling in 3D makes it possible to achieve a simulation model really close to the reality. The need for simulation was mainly for the design of the robot cell layout, but also for robot motion planning.

2.3 Purchase and Manufacturing of Equipment

The most important purchase is the robot, since this is the most expensive investment. The other investments stand for about 10 to 15 % of the robot investment. One important factor when purchasing major investment is to invite

⁶ Craig, J., *Introduction to Robotics*, p. 415. (1989).

⁷ <http://www.delmia.com/>. *Delmia*. (2003-05-19).

tenders from different suppliers to achieve a better price. Other factors for the purchase are guarantee and service.

Since the work cell is a prototype, it is expensive to outsource small manufacturing. Therefore in-house-manufacturing in a great extent is an advantage to keep the cost down. Another aspect of in-house-manufacturing is to fast and easy make changes over time.

Some of the parts are not possible to make in-house, because of the limited possibilities in the in-house work shop. For that reason some of the work had to be outsourced.

2.4 Assembling the Work Cell

After the design of the work cell is carried out and the equipment that is needed is purchased or manufactured, assembling is made. The main part of the assembling work is mechanical, but also some electrical work. The assembling mainly includes putting a gripper on the robot, assembling the robot table, put batch hotels together, and put all the parts and devices together.

2.5 Communications and Programming

There are some programs that have to be designed. A program for the communication with the scanner and with the robot has to be created. It is necessary to set up a communication between the robot and the scanner, so they can operate in the sequence order. The scanner is sophisticated and several of parameters to the scanner can be set. Therefore an interface for the user has to be created, so the users can set the parameters as desired.

2.5.1 Scanner Communication

To control the scanner, a program mainly made for testing the scanner, is used. The software is MS-DOS based, and basically a command line is send to the scanner with the parameter that is needed individually for each batch of gels.

2.5.2 Robot Communication and Programming

The robot is controlled with a program via an ActiveX instead of Mitsubishis own software. This because it easier to extend the software with custom programming. The programming is made in Microsoft's programming language, Visual Basic.

The robot programming is made in Melfa Basic from Mitsubishi. The programming includes creating paths, grip and release the 2D gels, but also other control commands.

2.5.3 Bar Code Reader Programming

Bar Code Readers can be connected to a computer in different ways depending on how the user wants to handle the data. The most common connections are via

keyboard wedge, RS232 serial interface and USB ports where the serial RS232 and USB connectors give the user a greater ability to make a tailor made data handling solution.

2.6 Safety

Safety in and around a robot cell is an important issue. It is important to have a well visualised safety system but even more important is to create a safety system that does not disturb the production or deteriorate the quality of the work.⁸

The robot movements can seem irrational to untrained persons which makes it hard to predict the next step for the robot move. 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.⁹

To increase the safety even more, fences can be used. The fence does not only prevent people to come in range of the robot it is also protection against outside disturbance.

A common way to make a cell with fences accessible is to use a gate. In cases of larger cells more than one gate may be installed. To keep the safety of the cell on a high level a safety switch can be attached to the gate. The functionality of the switch, production stop or emergency stop, can be adjusted to the demands set on the cell.

Sometimes it is desirable and necessary to get in to the cell to be able to inspect the work in progress, quality of the work and to calibrate the movements of the robot. To make this without affecting the process a production stop switch can be installed. A production stop switch has a great difference from an emergency stop. When a production stop is triggered the robot does not immediately stop but moves to a position that is suitable for resuming the program. Another big difference is that the connection between the control box and robot remains during a production stop.¹⁰

There is a lot of other safety equipment to use, i.e. pressure contact rug, Hold-To-Run control, light bar/gate and light curtains.¹¹ Since none of these are a part of the cell, they will not be further described.

⁸ http://www.robotics.lu.se/ARCHIVE/movies/general/safety/IVF_safety_1_large.mpg.
Division of Robotics. (2003-09-01).

⁹ Bolmsjö. G. S., *Industriell Robotteknik*, p. 278. (1992).

¹⁰ http://www.robotics.lu.se/ARCHIVE/movies/general/safety/IVF_safety_1_large.mpg.
Division of Robotics. (2003-09-01).

¹¹ Bolmsjö. G. S., *Industriell Robotteknik*, p. 291-297. (1992).

3 Simulations in UltraArc

The aim with the offline programming was robot placement, design of the layout and the environment in the robot cell, and path planning. Normally, the simulation program, UltraArc, contains the most common robot devices. But the Mitsubishi robots in the RV-series, which is used in this project, were not included in the standard module. The models of the robots could neither be supplied by the manufacturer of UltraArc or Mitsubishi Electric. Therefore the robot, the Mitsubishi RV-2A, had to be modelled up in a 3D CAD program and exported to UltraArc. In this case the modelling was made in ProEngineer, made by PTC¹², and then the 3D models were imported into UltraArc. After that, the robot kinematics was set up in UltraArc. Other parts of the system were also modelled in ProEngineer, such as the scanner, the tables, glass plate samples and the suction cup. Some experimental concepts were made to achieve a good solution. In each concept, different placements of the devices and design of the environment was done. After the placement and design, paths were created for the robot movement to confirm that all parts were placed in the range of the robot. Experimental paths were also created in the sense of receiving simple and fast path results. Two of the advantages of simple path programming are; it is easier to predict the robots motions and advanced paths increases failure to occur. The path programming languish in UltraArc is a line based programming language, similar to the language used for programming the real robot. Unfortunately could not the simulation path program be used in reality but it gave guidance in planning the movements. Some of the concepts that were created failed in the range aspect. Among the concepts that came through, the one with highest potential was chosen. The whole process was then simulated for this concept in the program. The choice of the final solution was based on if it was possible, justified and by robustness.

Figure 3.1 shows a circle of iteration used to come up with the final solution. All the steps must be passed, if not several of times, at least once to succeed. From step 3 to step 6 it is possible to go backwards in the circle without passing every step. If something does not work properly it is important to find the cause and also find out how to solve the problem in the best way.

¹² http://www.ptc.com/products/sw_landing.htm. PTC. (2003-05-12).

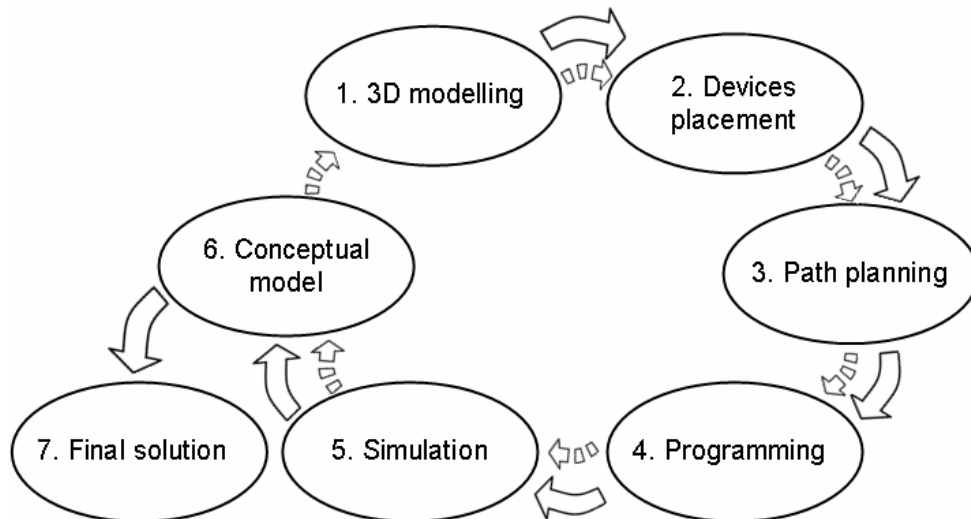


Figure 3.1 Circle of iteration used when performing a simulation.

The final simulation concept is shown in the pictures from figure 3.2 to figure 3.8, made in UltraArc.

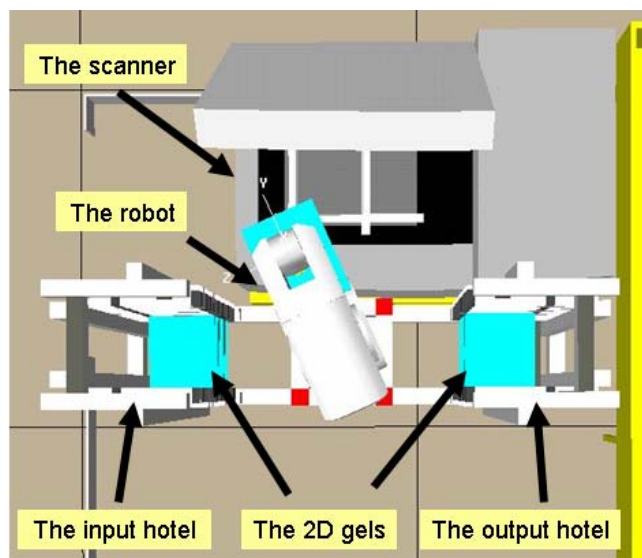


Figure 3.2 Robot cell overview, with main components displayed.

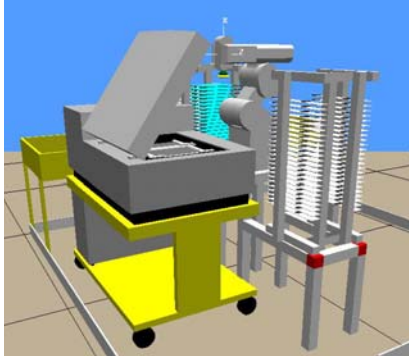


Figure 3.3 The robot work cell with the scanner to the left.

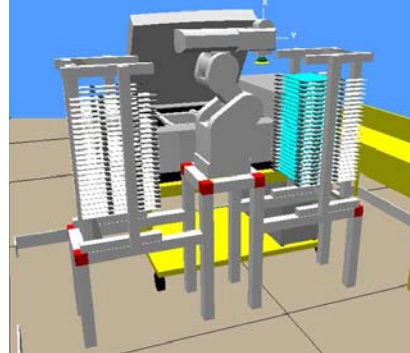


Figure 3.4 The robot work cell. The hotel on the left is filled with samples.

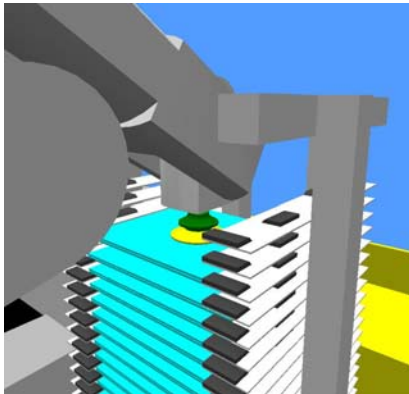


Figure 3.5 The robot is picking a 2D gel in a hotel.

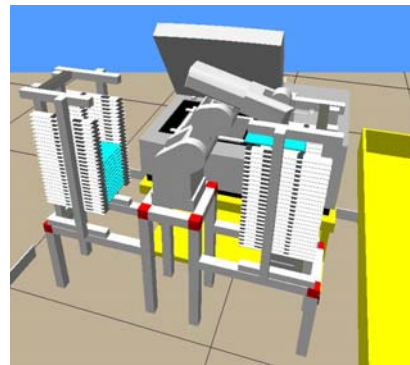


Figure 3.6 The robot in the output hotel.

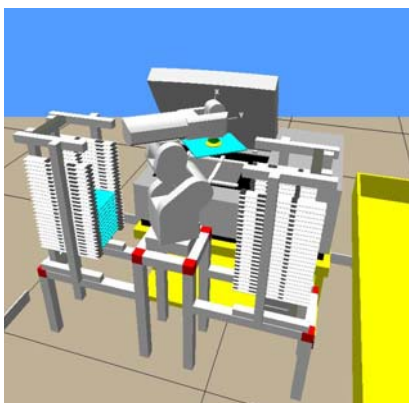


Figure 3.7 The robot is unloading the scanner.

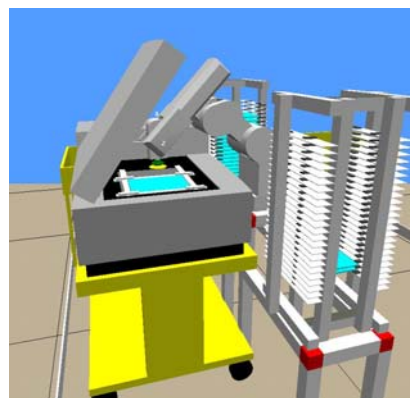


Figure 3.8 The robot has put a 2D gel in the scanner.

4 System Layout and Robot Components

The system was made with different components. Some of the components are called smart devices, whether they can send and receive data or not. Basically the system includes:

- The robot with control unit
- Suction cup for handling the glass plates
- The scanner
- The bar code reader
- The hotel for the 2D gels
- Tables for the robot, the scanner and the hotel
- Safety and emergency stops

4.1 The Robot

4.1.1 The Choice of Robot

Initially, it was desired to work with a Mitsubishi robot. The reasons were the knowledge that Mitsubishi makes small robots for similar applications like in this project and that they have high-quality products within this area. Another reason was previous experience of the Mitsubishi robots during the visit at Amersham. Mitsubishi has different series of robots¹³. Their main series are vertically and horizontally articulated. In this case it is obvious that a vertically articulated robot should be used, since the horizontally articulated series have more limited work space. The vertically articulated series are divided into models based on load capacity. Typically, with increased load capacity, the models have also increased working space. For each load capacity interval Mitsubishi has 5- or 6-axis robots. In an early stage it was obvious to make a decision of which robot that should be used in this project. Following factors were taken in consideration when deciding robot; load capacity, flexibility, working space and price. The work load is approximately 1 kg, which all the vertically models from Mitsubishi can handle. The demand of flexibility is fairly high, and therefore a 6-axis robot was chosen.

¹³ http://www.nagoya.melco.co.jp/english/robot/details/series_e.htm. *Mitsubishi Electric*. (2003-04-16).

Regarding to the working space, the RV-1A is too small, but the RV-2A is suitable, see figure 4.1. Of course the price is important and the chosen robot is the smallest suitable, since the price raises for the bigger robots.



Figure 4.1 The Mitsubishi RV-2A, which is used in the project. Here with a gripper attached.¹⁴

4.1.2 Equipment and Functions

The robot comes with a controller and a teaching pendant. The controller is connected to the robot with two cables, one to provide the electrical motors and sensors with power supply and one for data transfer. The controller also has a serial connector, which makes it possible to connect the controller to a computer. The teaching pendant; see figure 4.2 and figure 4.3, is connected to the controller and has several of functions. Initially it is used to set parameters, which makes it possible to connect a computer to the controller. Other functions are to operate and control the robot such as by speed, limitation for each axis and gripper device. Since programming the robot with a computer is easier and more time efficient, the teaching pendant is not used more than as an emergency stop when online programming is carried out. The robot also has internal air channels and input channels for external sensors.

¹⁴ http://www.nagoya.melco.co.jp/english/robot/details/work/rv2a/rv2a_01.htm. *Mitsubishi Electric*. (2003-04-16).



Figure 4.2 The teaching pendant for the controller.¹⁵

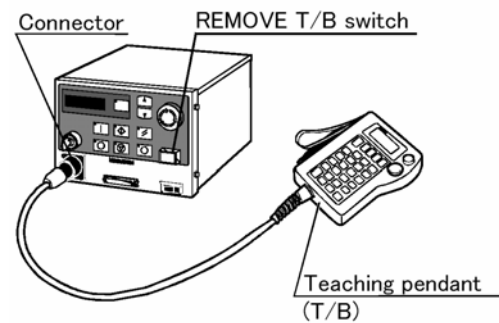


Figure 4.3 The connection between the teaching pendant and the controller.¹⁶

4.2 The Suction Cup

4.2.1 Selection of Tool Gripper

There were different suggestions for choice of gripper, such as a mechanical gripper. Since the glass plates have quite large dimensions it was considered hard to have a mechanical gripper for this application, because of the size of that kind of gripper. It would also be hard for the gripper to grip the glass plates from the scanner, since the glass plates are put directly on the scanner glass and the scanner glass is high sensitive for scratches. Therefore the gripper was out of the question. Instead there were some thoughts about vacuum types. The vacuum types are suitable for flat objects.¹⁷ There are different types of vacuum tools, but the most common and the most appropriate in the application is the suction cup, see figure 4.4 and 4.5. The vacuum can be maintained with air pressure and an ejector, see figure 4.6. The principle to maintain vacuum is when compressed air passes through the nozzles; air is pulled through with the stream of compressed air see figure 4.7.¹⁸ The vacuum ejector is connected to one of the output air channels.

¹⁵ http://www.meau.com/eprise/main/Web_Site_Pages/Public/Products/Robots/P-Robots. *Mitsubishi Electric*. (2003-06-18)

¹⁶ *ibid.*

¹⁷ Bolmsjö, G. S., *Industriell robotteknik*, p. 239. (1992).

¹⁸ <http://www.piab.com/com/>. *PIAB*. (2003-05-08).



Figure 4.4 The suction cup from PIAB.¹⁹



Figure 4.5 The suction cup mounted on the robot with an adapter attached in-between the robot and the suction cup.



Figure 4.6 An ejector from PIAB.²⁰



Figure 4.7 The ejector principle.²¹

The suction cup must fulfil the requirements of the lift force, but the suction cup must also be mark free, since marks can affect the scanned sample. Some of the suction cups are made of materials that are marketed as mark free. To be sure, tests were made to confirm this. The safety factor is based on the formula below in figure 4.8. In this case it seems over dimensioned, but a larger diameter of the suction makes the handling more stable, since the vacuum is scattered over a larger area. This was also laboratory tested for confirmation.

$$D = 113 \cdot \sqrt{\frac{m \cdot n}{U \cdot s}} \Rightarrow n = \left(\frac{D}{113}\right)^2 \cdot \frac{U \cdot s}{m} =$$

$$= \left(\frac{80}{113}\right)^2 \cdot \frac{300 \cdot 1}{1} \approx 150$$

D – diameter of suction cup (mm)
 m – mass (kg)
 U – vacuum (-kPa)
 N – safety factor
 S – number of suction cups

Figure 4.8 Formula for the suction cup, used to calculate the safety factor.²²

¹⁹ <http://www.piab.com/com/>. PIAB. (2003-05-12).

²⁰ *ibid.*

²¹ *ibid.*

²² *ibid.*

4.2.2 Reliability and the Clips Function

The reliability is high of the actual lifting of the 2D gels, in the aspects of the robot's load capacity and the suction cup. All together the plates and the gel weight about 1 kilogram and this robot model has a load capacity of 2 kilograms, which results in a safety factor of two. Another point of view is if the glass plates might separate during the handling with the robot. The gel between the glass plates works as a force to hold the glass plates together, but there have been cases when the glass plates have been separated. Consequently, this had to be taken into consideration. The only functional proposal that have come up is to hold the glass plates together with clips. The idea came from benchmarking the bill clips, which are made in some kind of flexible stainless steel. This kind of clips have to be dimensioned regarding to the glass sandwich and the used area of the scanned image. The clips also meet another requirement, which is that the glass plates are not allowed to lie directly on the scanner glass and the clips work as a 0.1 mm spacer between the scanner glass and the glass plates. In case of direct contact, optical refraction artefacts and interference pattern might appear. The clips also protect the glass plates from scratches, when they are placed in the hotels.

4.2.3 Solenoid Valve

As previous mentioned the Mitsubishi robot has internal air piping. It is obvious that internal piping makes easier than external piping. One advantage with the robot is that it is possible to install a solenoid valve set. The solenoid valve set is an electromagnetic valve set, which can control the output air channels. In this case it is desired to be able to set the output air channel, in order to control the air channel connected to the suction cup.

4.3 The Scanner

The scanner, Typhoon 9410, see figure 4.9, is made by Amersham and is a sophisticated scanner with a moving scanner head. The Typhoon is provided with three lasers; green, red and blue, where the blue laser can be set to two different wave lengths. The scanner instrument can scan three types of samples:²³

- A storage phosphor screen that has been exposed to a radioactive sample
- A fluorescent sample
- A chemiluminescent sample

Since the fluorescent scanning is the only one used at the laboratory, the other two will not be further described.

²³ Molecular Dynamics., *Typhoon Instrument Guide*, Ch. 1 p. 1-5. (2001).



Figure 4.9 The Typhoon 9410 scanner from Amersham.²⁴

4.3.1 How Fluorescence Scanning works²⁵

All fluorescence imaging system require the following key elements:

- Excitation source
- Light delivery optics
- Light collection optics
- Filtration of the emitted light
- Detection, amplification and digitisation

A basic design and components is illustrated in figure 4.10.

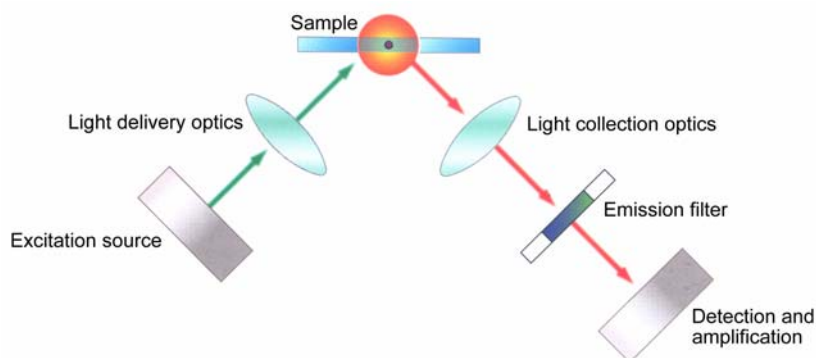


Figure 4.10 The figure shows a basic structure of a fluorescence imaging system.

²⁴

<http://www4.amershambiosciences.com/aptrix/upp01077.nsf/Content/Products?OpenDocument&ModuleId=165431&hometitle=search>. Amersham Bioscience. (2003-07-14).

²⁵ Amersham Pharmacia Biotech., *Fluorescence Image principles and methods*, p. 9-20. (2000).

The excitation source, here using a laser, sends out a light beam at a specific wave length. The light delivery optics, consisting of lenses and mirrors, creates an optical path to direct the excitation beam to the sample. If the molecules in sample are fluorescence at this spot the molecules absorb light. The light absorption raises their energy level to a brief unstable excited state. As they decay from this excited state, they emit fluorescent light. Since the emitted fluorescent light radiates from the molecules in all direction the light collection optics must be as efficient as possible. All reflecting laser light must be rejected from the collection pathway by a series of optical filters. Even the emitted light can be filtered to select only the range or band of wavelength that is of interest to the user.

To detect and quantify the emitted light a photomultiplier tube (PMT) is used. It works by converting the photon energy from the emitted fluorescent light into electrical energy. This produces a measurable analogue signal that is proportional to the number of photons detected. The analogue signal is then converted into a digital signal, which creates an image file that can be visualised by software created by Amersham.

In figure 4.11 and figure 4.12, a more detailed structure over how the Typhoon works is showed.

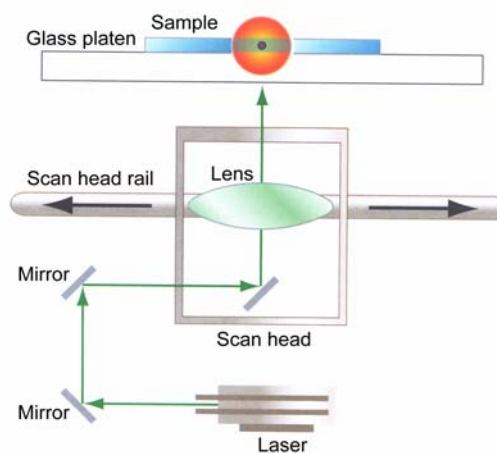


Figure 4.11 Excited light from the laser hits the sample via mirrors and the lens.

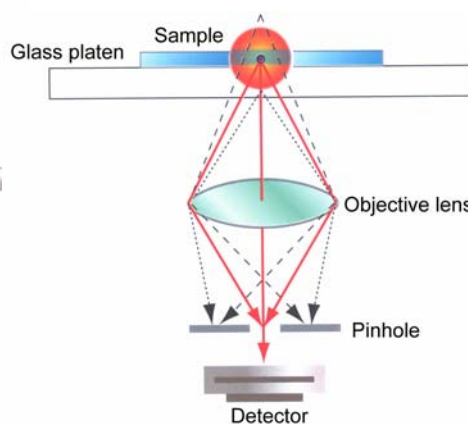


Figure 4.12 The emitted light from the sample is detected.

4.3.2 The Scanner Lid

There are some reasons why the scanner has a lid. Primary, the scan has to be done in a dark environment. Secondary, since the laser travels very fast back and forth, it causes vibrations. To reduce the affects on the scanned image, the 2D samples are pressed against the scanner glass with an electrical driven press device in the lid. To make the system solution simple, the scanner was put in a dark room. This conclusion was made in consulting with the manufacturer of the scanner. To reduce the affects of the vibrations, a fixture was made, so the 2D gel could not move in

the vibration directions. The scanner has a magnetic sensor, which let the scanner know if the lid is opened or closed. When the lid is opened, the scanner will not allow any start of a scanning. To come around this a bypass is used. It is simply a jumper that is put on the sensor cable and gives the scanner the signal when the lid is closed. The lid could easily be removed, but since manual scans is desired, it was decided to keep the lid on, but opened when the batch scanning is operating.

The reasons mentioned above is from a technical point of view, of course the lid also works as a protection to prevent the user to be exposed by the laser.

4.3.3 Scanner Frame

The scanner lid is retained opened during the scanning; the 2D gel samples are not fixed in the horizontal plane. Since the scanner vibrates, the scanned image would not be proper. To prevent this, a scanner frame was made in order to fix the 2D gel samples in the horizontal plane. The inner dimensions of the scanner frame are the same as the dimensions of the glass plates. The inner sides of the scanner frame are chamfered, so the 2D gel sample will be self centred while it is put in the scanner by the robot. The scanner frame and its placement are shown in figure 4.13.

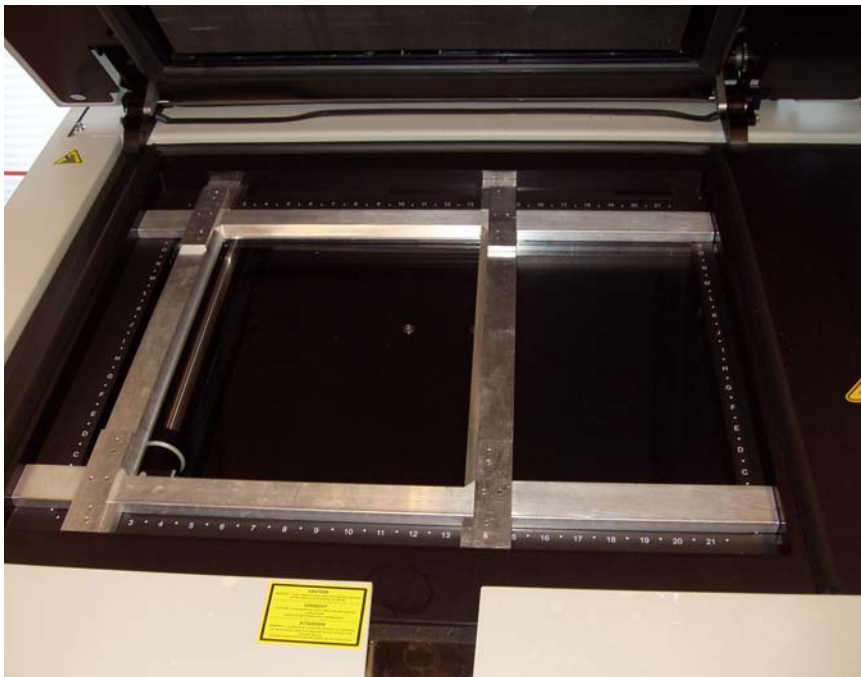


Figure 4.13 The frame put in the scanner.

4.3.4 Fluorescent Dyes²⁶

The proteins themselves are not fluorescent enough to generate a good scan image. To improve the ability to spot the proteins a fluorescent marker substance, a dye that binds to the proteins, is attached to the gels. As free molecules, the dyes are not very fluorescent either, but when they bind to proteins the fluorescence enhances to a sufficient level. The choice of dye is depending on what laser is being used; different dyes get excited at different wave lengths. If a gel is to be scanned with more than one laser it is possible to add more than one dye to the gel at the same time.

4.3.5 Parameter Settings for the Typhoon

When performing a scan with the Typhoon several parameters can be set by the user.²⁷ The parameters to set are described below; both functionality and setting values are described.

- Emission filters – As mentioned before, emission filters are used for filtering the emitted light. The Typhoon can contain up to 16 different filters to suit the user's requirements. Choice of emission filter is based both on the choice of laser and dye. The filters can be divided into three different main types:²⁸
 - Long-pass filters - pass light that is longer than specific wavelength and reject shorter wavelengths.
 - Short-pass filters - pass light that is shorter than specific wavelength and reject longer wavelengths.
 - Band-pass filters – allow a band of selected wavelengths to pass through, while rejecting all shorter and longer wavelengths.
- Laser – The Typhoon holds three different lasers. The three consisting lasers to chose from are:²⁹
 - Green – Neodymium: Yttrium Aluminium Garnet with a wavelength of 532 nm.
 - Red – Helium neon with a wavelength of 633 nm.
 - Blue – Argon ion laser with a wavelength of 488 nm or 457 nm.

²⁶ Amersham Pharmacia Biotech, *Fluorescence Imaging Principles and Methods*, p. 63. (2000).

²⁷ Molecular Dynamics, *Typhoon Instrument Guide*, Ch. 9 p. 2. (2001).

²⁸ Amersham Pharmacia Biotech, *Fluorescence Imaging Principles and Methods*, p. 25-26. (2000).

²⁹ Amersham Pharmacia Biotech, *Fluorescence Imaging Principles and Methods*, p. 12. (2000).

The red and green laser can be linked together; the two lasers will run simultaneously, to speed up the scanning process. This might though affect the image result in a negative way, since the lasers can interfere but also the dyes in the samples can give misleading image by being excited twice in a short time.

The scanning process is performed with the scanner lid opened, which results in laser exposure. A special care should always be taken when laser light is exposed. All three lasers are classified as Class 3B.³⁰ This means that direct eye contact may cause eye injuries but diffuse reflections are considered harmless.³¹ Since the predefined scanning area is covered by a sample during the scanning process and no persons are allowed to be in the cell while operating the risk of harmful exposure is considered small.

- Beam splitter - When running a linked scanning process a beam splitter has to be included to split the emitted light into “red-emitted” and “green-emitted”. The beam splitter distinguishes light on the basis of wavelength. Wavelengths above the beam splitter range pass through and those below are reflected. The two channels that been created can then be independently filtered and detected.³²
- Scan area – The area to scan can be set in both x and y directions to only scan the area where the sample is placed.³³ In this case the area is represented by the area of the frame.
- Sample orientation – This parameter makes it possible to change how the image orientation will be displayed in the image analyze program.³⁴
- Press sample – The scanner lid has a built in press that is made primary to press sample that does not lie flat against the glass platen of the scanner but can also be used to fix samples during the scan process.³⁵
- Pixel size – The pixel size refers to the size of each individual picture element recorded. This setting can be chosen by a seven step scale in the range from 10µm to 1000µm. The smaller pixel size used the larger will the size of the image file be.³⁶

³⁰ Molecular Dynamics, *Typhoon Instrument Guide*, Ch. 2 p. 11. (2001).

³¹ <http://www.latronix.se/>. *Latronix AB*. (2003-09-19).

³² Amersham Pharmacia Biotech, *Fluorescence Imaging Principles and Methods*, p. 17. (2000).

³³ Molecular Dynamics, *Typhoon Instrument Guide*, Ch. 9 p. 3-11. (2001).

³⁴ *ibid.*

³⁵ *ibid.*

³⁶ *ibid.*

-
- Focal plane – The focal plane can be set to either Platen or +3mm. Platen is used when the gels are placed directly on scanner glass and +3mm is used for gels placed in a glass plate sandwich.³⁷
 - Photomultiplier Tube (PMT) voltage – The PMT voltage improves the signal collection capabilities of the scanner. The value can be set in a range from 0V to 1000V depending on what laser and kind of dye that is used. Guidelines to set a PMT value are very rough and must be tested for each combination to get a sufficient image, which is a very time consuming process. Normally the value is around 600-800V.³⁸
 - Sensitivity – This controls how long the scanner collects data from each pixel. There are three alternatives; Normal, Medium and High. Normal collects data only once while Medium and High collects data four respectively eight times and returns an average value. This setting does not affect the image file size but increases the scanning time.³⁹

4.4 The Bar Code Reader

For identifying each 2D gel sample, there is a bar code attached in each gel. It is desired to have a bar code reader in the automated system to be able to give each sample a unique identity. When choosing a bar code reader several of aspects have to be considered.

Decisions have to be made for following:⁴⁰

- Brand
- Handheld or mounted
- Working range
- Type of codes to read
- Raster or linear reader

Since The Proteomics Centre in Lund exclusively uses bar code readers from HHP, former Welch Allen, a decision was made to choose this brand. HHP only offers mounted bar code readers with the ability to control the bar code reader, i.e. when to turn power on and to collect the data in a desirable way, which is desirable in this project.⁴¹ The working range is not really critical aspect, since the distance between the sample and the reader can be adjusted by the robot. The codes that are being used are only standard types that can be read by almost all bar code readers.

³⁷ Molecular Dynamics, *Typhoon Instrument Guide*, Ch. 9 p. 3-11. (2001).

³⁸ *ibid.*

³⁹ *ibid.*

⁴⁰ <http://pos.e-newsletter.com.au/index.php?type=0&archive=1>. *POS e-news*. (2003-08-18)

⁴¹ www.hhp.com. *HHP*. (2003-07-08).

This together with the precise reading that can be accomplished with the robot arm makes it possible to use a linear bar code reader. A disadvantage the linear readers have towards the raster readers, are that they only allow a small angular deviation when reading a bar code. But on the other hand, the price for a linear bar code reader is about a tenth of a raster bar code reader. After consulting retailers, the ImageTeam™ 3900 from HHP, see figure 4.14, with RS232 connection interface was chosen.



Figure 4.14 The bar code reader from HHP, used in the application.

4.5 The Tables

The table functions are to set the robot and the hotels into right height and position. The design is made in 3D modelling program, ProEngineer, and exported to and used in the simulation program for analysis and adjustments. The table is assembled with modular profile system in aluminium, see figure 4.15. The profiles were cut in the dimensions based on the dimension from the simulations and then put together with brackets, see figure 4.16.



Figure 4.15 Modular profiles for table assembling.⁴²



Figure 4.16 Bracket for compounding the modular profiles.⁴³

⁴²http://app10.bosch.de/ElectronKat/FMScatalog/ProdStart_eng.asp?id=20&topic1=Basic%20Mechanic%20Elements&topic2=Strut%20Profiles. *Rexroth Bosch Group*. (2003-05-05).

⁴³ *ibid.*

The robot table is assembled as shown in figure 4.17.

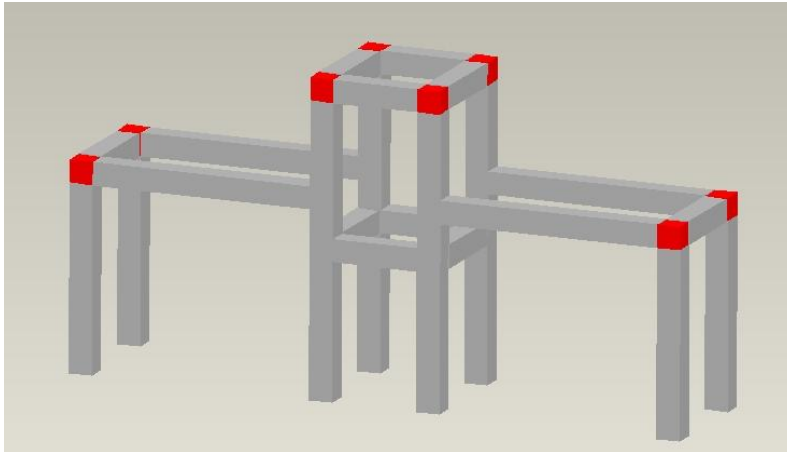


Figure 4.17 The table for the robot and hotels, made in ProEngineer.

The table for the robot is fixed to the scanner table. That is because the robot coordinates are connected to the scanner and the scanner should not move, since the coordinates will then get lost.

4.6 The Hotels

The purpose of the hotels is to be the incoming and outgoing buffers for the scanner. The incoming hotel is buffer of sandwiches which are not scanned and the outgoing hotel is the buffer of sandwiches which are scanned. Each hotel contains shelves for 24 sandwiches.

4.6.1 Design and placement of the hotels

The design and placement of the hotels are based on constrains from the robot. Since the robot has a finite working space and the hotels have shelves for 24 sandwiches in each hotel, there had to be more than one conceptual model to achieve the final solution. The conceptual modelling where made in the offline programming. It was required to find the best height in the work space, in order to be able to work with the optimal working space from the robot at the hotel. At this height, the robot path was planned, and the hotels placement was set in order to the path. The hotels are made flexible and shelves can be moved, removed or added. The hotels are also fixed in the way so they easily can be moved for trimming in height direction. The finished hotels are shown in figure 4.18 and figure 4.19.

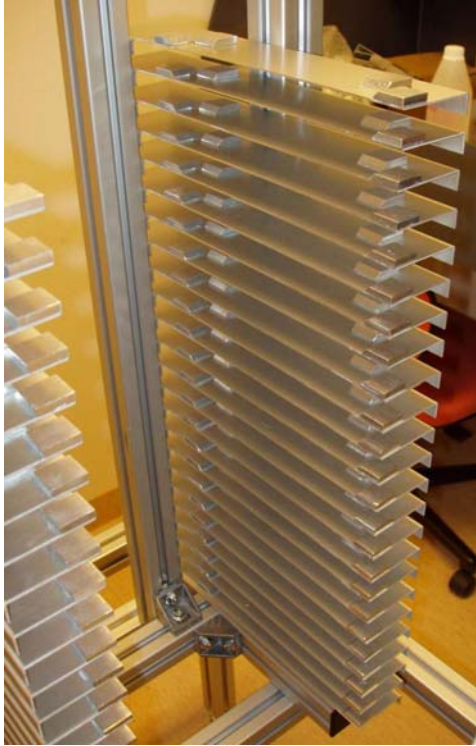


Figure 4.18 The hotel with 24 shelves and .
fixture blocks.



Figure 4.19 The hotel from above.

4.7 Safety and emergency stops

The safety system is a basic system with one magnetic door switch, see figure 4.20, and two emergency brakes, see figure 4.21. One emergency brake is placed outside the cell and the other one inside the door. The room it self works like a safety fence and the door as a gate.

The emergency brakes create an immediate signal to the controller to turn servo off, release power from the engines and exit the running program. After an emergency stop, the user needs to restore the emergency button and reset the controller to be able to start a new procedure. The emergency brakes are connected to special emergency brake input connector. The event of emergency brake is predefined by the manufacturer and can not be changed.

The magnetic door switch works in a similar manner as a production stop switch. It does not create a stop in the program but a brake until the door is closed again. This is made to make it possible to accidentally open the door but also entering the robot cell for movement inspection, without losing connection to between robot and computer. A lost connection requires a rerun. The door switch is wired to an external input/output connector on the controller box and generates a one bit signal;

0 or 1. The signal is checked in the robot program and performs desired measures when state is changed.

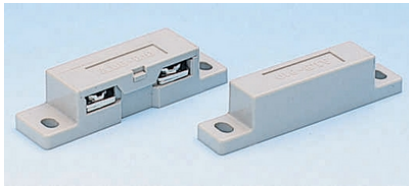


Figure 4.20 The magnetic door switch used in the work cell.⁴⁴

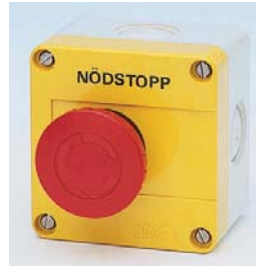


Figure 4.21 The emergency brake, which is used in the project.⁴⁵

The emergency brakes are placed close to the door both inside and outside the cell to make them easy to reach and see.

The door should, as a routine, be locked before starting the process to indicate that the process is running. This is done manually and might be forgotten and that is why the door switch is installed. It is of course also possible to see in user interface if the process is running or not.

⁴⁴ <http://www.elfa.se/en/>. ELFA. (2003-09-04).

⁴⁵ *ibid.*

4.8 Process steps

The whole process, from sample preparation to analysing the images, contains several of steps. In figure 4.22 a principle schedule for the steps is shown. The process is divided into three blocks; preparations, scanning and analysis. The O in top of block one and three indicates that the steps are manually performed by an operator and the second block, marked with an A, means that the steps are automated.

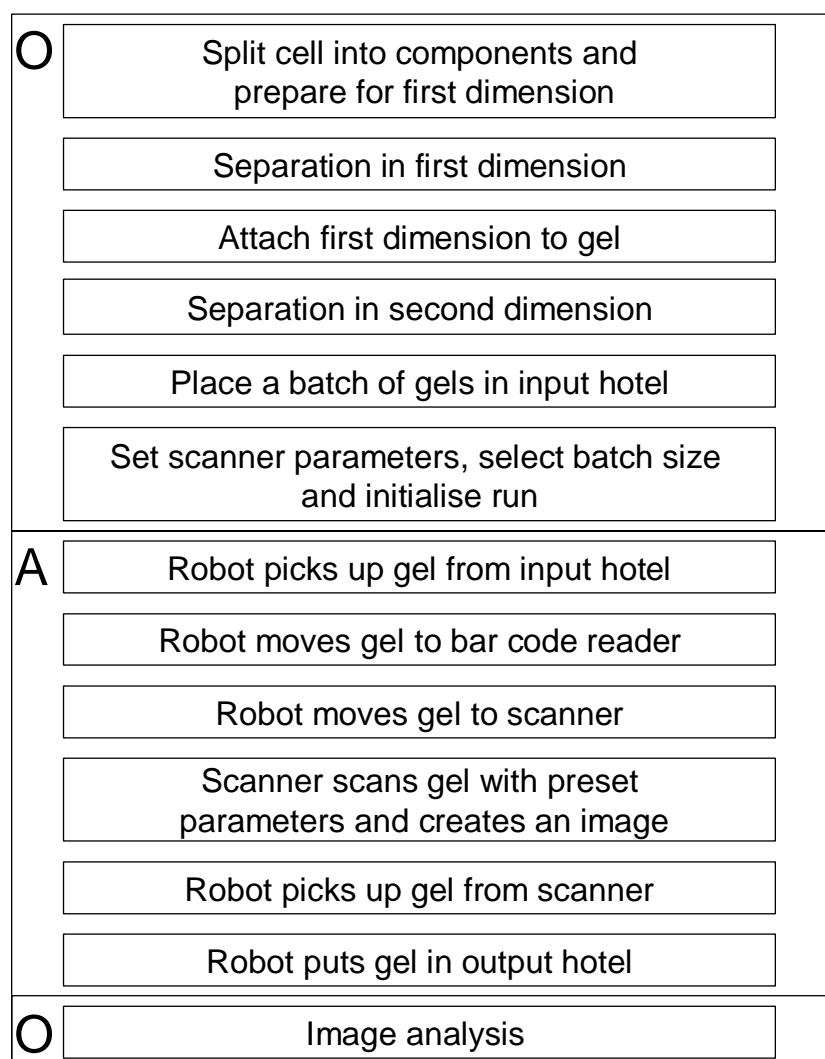


Figure 4.22 Principle schedule for the process from preparation of sample to image analysis.

5 Communications and Programming

The smart devices in the automated system include as earlier mentioned, a personal computer, the scanner, the robot and the bar code reader, see figure 5.1. A smart device means a device that is able to receive, send data, or both receive and send data. The scanner is connected via Ethernet and the robot and bar code reader are connected via RS-232.

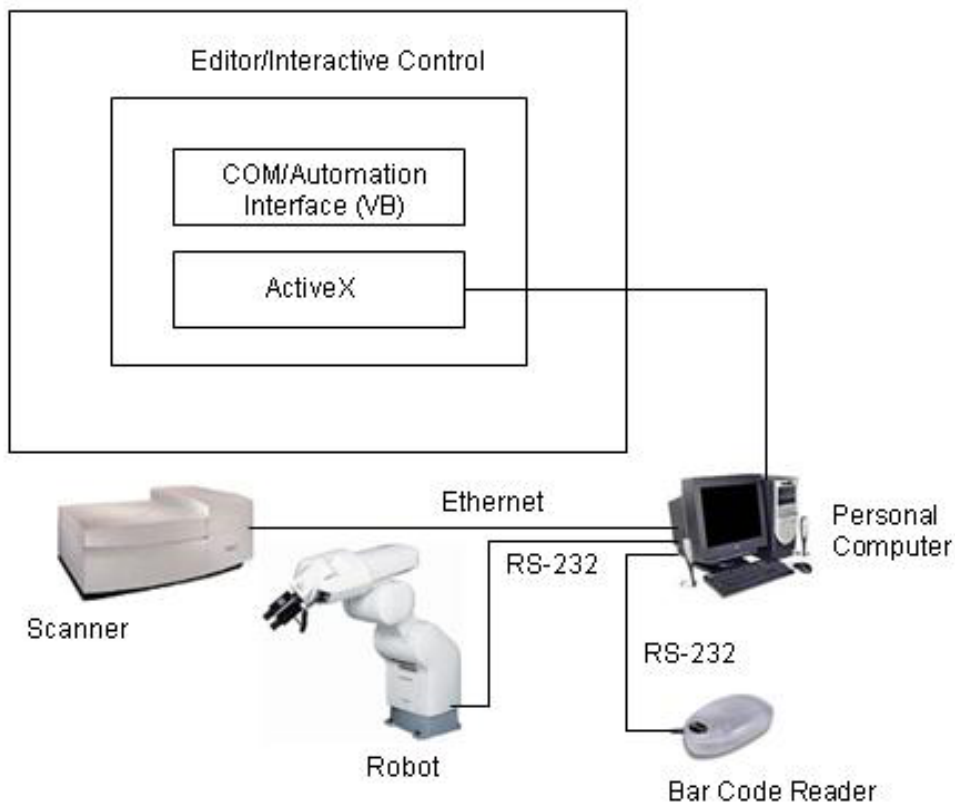


Figure 5.1 System structure with the smart devices used in the project.

5.1 Scanner Communications

As a base for the scanner communication an existing program for the scanner, which is used for testing at Amersham, is used. The program is called *tscan* and is MS-DOS compatible. To run the program some information and parameters are written in a command line. The information is where the scanned images should be saved and to the name of the file. Further, the parameters, described in chapter 4.3.5, for the scan should be written in the command line. If any parameter is missing the command line will assume a default value. Since the parameter settings are the same for one batch, *tscan* is run repeatedly with a change of the image name. This could be done by programming a batch file with the command line and run the batch file repeatedly with the change of the name of the image file. A batch file is a programmable file, which could be run in MS-DOS.

5.2 Robot Communication

The program made in Visual Basic has communication with the robot controller via an ActiveX in the program. The program calls a program in the robot controller with one variable, the self number. The sequence decides which program in the control box that should be called. When a program in the robot controller is called from the main program, the scanner is either loaded or unloaded.

The controller is connected to a serial port. To get the connection to work between the robot and the computer, with a proper data transfer, all communication parameters must match. These settings are for the bit transfer.

5.3 Robot Programming

The robot programming is made with Melfa Basic, which is one of Mitsubishi's own program languages. The programs include robot paths, grip and release commands to the suction cup. The programs are stored in the robot controller. The robot paths are based on the path planning made in the offline simulation. The paths are almost the same for each shelf in the hotel, but with the difference of a multiple offset for each shelf number sent from the main program. The grip and releases are made with controlling the solenoid valve set, where the valve switches between two air channels. The suction cup also has a vacuum guard to make sure a grip or release has been made. The vacuum guard is an input sensor and is attached to the ejector part at the suction cup.

5.4 Bar Code Reader Programming

All the bar code readers from HHP are factory programmed for most terminal and communications settings. These settings are easily changed by scanning factory defined bar codes to suite the customer's demands. Settings are connection type, bit transfer, delay between readings, trigger mode and symbologies. Programming in

the main program is carried out in order to get the data from the bar code reader via the com port. The data is used to name the images with the bar code.⁴⁶

5.5 User Interface

The process controlling program was originally developed by Amersham for controlling one of their automated systems. The program contained functions to control several of different smart devices and has been modified to suit this process. The workflow the user needs to perform for a batch run is to set the number of shelves, set the scanner parameters and initiate the run. The user also has possibilities to make tests. The user interface is shown figure 5.2.

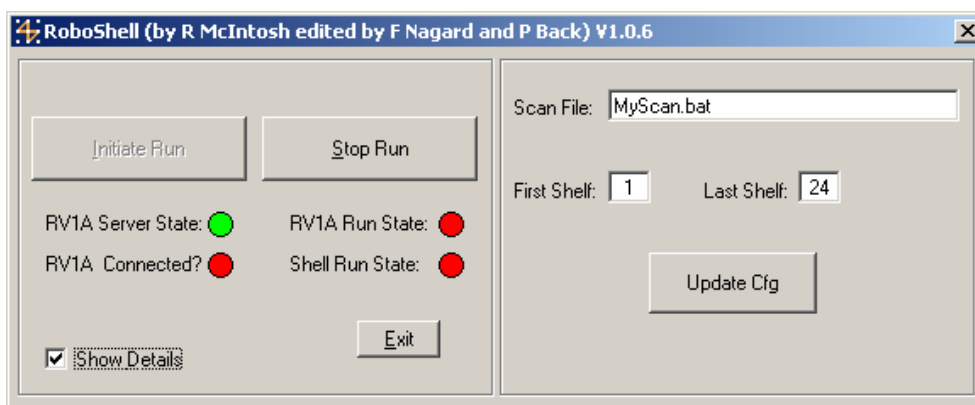


Figure 5.2 User interface for process control.

For the best user interface, it is desired to have a similar interface to the previous scanning program, which is made by Amersham. This since the research people are used to their interface. The interface includes all the parameters that the scanner needs, and the parameters are stored in the batch file, which mentioned earlier. The interface programming is made in Microsoft Visual Basic. The scanner parameter setting interface can be seen in figure 5.3.

⁴⁶ http://www.hhp.com/hhp/files/documentation/38-3900_UG_Rev%20E.pdf. *User's guide* Ch. 7 p. 1. HHP. (2003-07-22).

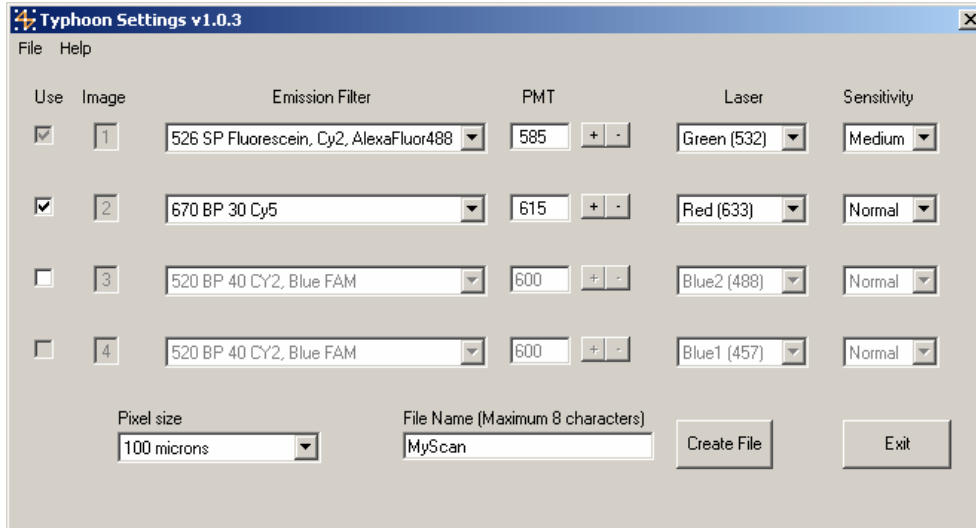


Figure 5.3 User interface for Typhoon parameter settings.

6 Results

6.1 Robustness

The robot cell has been set up with good physical accuracy. Since the robot handles the 2D gels twice, accuracy is important in order to give the robot the right positions of the 2D gels. Thanks to the fixture blocks in the hotels and the fixture in the scanner the robustness increases for placement accuracy, both when the 2D gels are placed manually in the input hotel and in the scanner and output hotel by the robot.

Tests made with the system verify that the robot, the scanner, the bar code reader and the other equipment behaves properly together, both physically and in software.

6.2 Usability

The scanner is retaining its ability to be manually operated, which was one criterion in the objectives. To achieve a user friendly interface, the interface with parameter settings was built up as Amersham's software for manual scanning. The interface for the main program is made to be easy to use and with as few functions as needed.

6.3 Safety

Safety aspects are really important issues. This has been considered and two emergency stops have been set up. The emergency stops shut down the robot immediately, which is desired. Though it is complicated to reset the robot, the emergency stop should only be used in case of an emergency. Otherwise the stop function in the program should be used.

6.4 Other aspects

Since the project has been built up as a prototype, it is hard to predict what the best and most optimal solution is. Especially, if parts should be bought or made in-house. Because of uncertainty many parts in the project was made in-house. This also reduced the cost essentially, but with the trade-off that it takes longer time.

The layout and design of the hotels are static in terms of extension of larger batches. The solution might be able to be more advanced and more flexible. If it is desired to run larger batches in the future, the hotels need to be rebuilt. The hotels are also made to be suitable for dimensions of the glass plates. If the manufacturer decides to change the dimensions of the glass plates or changes the material problems with the hotel will occur. Also the bar code reader may be static. If the laboratory would like to change to 2D bar codes, the present reader must be replaced.

7 Discussion and Conclusions

When this project was handed out the expectations were to work a lot with advanced robot programming and with a simple setup of the environment. During and after the project it was realized that this was a bit wrong. Creating a robot cell or an automated system from scratch involves much more than just robot programming.

When manufacturing, every containing part needs to be designed and often also redesigned. Creating one part that work on its own is rather simple but to get all parts to fit together takes time. Although simulation was carried out, a lot of time was also spent on trimming the placement of hotels, robot and scanner.

Of course the project contained a lot of programming and simulations but about half of the time was spent on dealing with suppliers, modelling, manufacturing and assembling the parts.

The project concerns many different areas and a lot of people have been involved in order to give professional advises to perform the best solution.

The importance in keeping a regular discussion with the end users was also discovered. This, not only for feedback of the work but also to get hold of their expectations and keep them updated in the working progress.

The authors can pleased state that this project ended up to be very instructive and has given a lot of experience that might be valuable in future working life. The experience includes purchasing, product- and programming advising. The project also reflected to a lot of the courses, both theoretical and practical, included in the Mechanical Engineering program at Lund Institute of Technology.

During the time we have been working with the robot cell, we have noticed interest from people working at other laboratories and we have realised that there is a great demand of automation in the bioscience and medical research area.

8 Future

8.1 Future Work

The system is now automated with a robot, which can handle up to 24 scans per batch. To achieve a good image, the parameters need to be optimised. This is possible to automate by using image analysis. The image analysis should be a program, and should be able to analyse the data in the image by pixel values and saturation spots. With analysis and algorithms, a prescan can optimise the following scan. This was although not in the scope of the project.

8.2 Future in automation at medical research centres

From experience, there are activities in processes in research within the medical research area, which are time demanding for well competent personal. When these activities are simpler or if there is no need from competent personal to make activities, there is a need of automation. Where automation is possible, there can be opportunities of time efficiency. An automated system can be faster than manually work, but it can also be able to work after office hours.

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