Design of an Automated Purification System for Biologically-Active Macromolecules

by

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B.S., Mechanical Engineering
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Abstract

All biologically active macromolecules (BAMs) including pharmaceutical drugs need purification as part of their production process to ascribe therapeutic properties. Therefore, the purification of BAMs has proven to be one of the fundamental challenges in drug production and discovery. There are different types of techniques presently being used to purify BAMs: manual, mechanical, or a combination of the two. Although purification techniques have improved dramatically in the last few years, the labor hours, chemicals used, delays and imprecision are still limiting factors.

This thesis first presents a stain-free ultraviolet absorption detection method that can be used to detect a variety of biologically active macromolecules in an electrophoresis gel. Next, it describes the design of a system that will automate the recovery and storage of samples from an electrophoresis gel.

The following components, which were designed and built for this system, are described in this thesis. An ultraviolet light source outputs a wide, collimated monochromatic beam of light to detect specific molecules within the gel. A mechanical cutting device excises bands of various shapes and sizes from an electrophoresis gel. A transportation system was developed to rapidly move the cutting device between the different stations. A cleaning station was implemented to clean and store the cutting tips that are used to excise the gel. Finally, a temperature-controlled storage station was developed to store the excised samples until needed for further analysis.

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Chapter 1

Introduction

1.1 Background Information

The development of new pharmaceuticals requires the use of pure biological samples, which allow for a thorough analysis of the therapeutic properties of newly developed drugs. Different methods are used to purify these samples such as chromatography, gel electrophoresis, capillary electrophoresis (CE), and high performance liquid chromatography (HPLC).

Each method has its advantages and disadvantages. Chromatography techniques are time-consuming, typically requiring five to seven working days, and are also imprecise, thus prone to errors. Although they are routine procedure, gel electrophoresis methods also require intensive labor—at least two or three working days and they result in a 30-40% product loss. The recovery of the sample is also problematic and imprecise. Currently, both these processes are carried out manually, which creates some repeatability and contamination issues.

This thesis develops the design of a system that will automate the recovery and storage of biological samples in an electrophoresis gel. It will also address the issues of increasing the efficiency and repeatability of the electrophoresis process, and eliminate possible sample contamination.

This first chapter provides a background and explanation of the electrophoresis process. It also states, in further detail, the objectives of the robotic system.

Chapter 2 describes and compares different possible visualization techniques, and presents the selected detection method.

Chapter 3 presents the design of each component, and also shows the performance and possible improvements for each.
1.2 Gel Electrophoresis Principle

Electrophoresis is a method used for the separation of biological macromolecules (such as proteins, nucleic acids, carbohydrates, and lipids) based on their size, charge, and conformation. Most biological molecules have an electrical charge, which is dependent on the molecule itself and on the pH of the solution surrounding it. A molecule that is placed in an electric field (see Figure 1) moves toward the cathode if negatively charged or toward the anode if positively charged. Molecules of different charges migrate at different rates.

If the distance between the plates is $d$ and the potential difference is $E$, then the force, $F$, exerted on a molecule of charge, $q$, moving in an electric field is

$$ F = \left(\frac{E}{d}\right) q $$  \hspace{1cm} (1.1)

Since the molecule of radius, $r$ is moving in a medium of viscosity $\mu$, this force is equal to the frictional force, $F_f$ (assuming that the molecule is moving at a constant velocity, $V$).

$$ F_f = 6\pi r \mu V = F $$  \hspace{1cm} (1.2)

$$ V = \frac{Eq}{6\pi r \mu d} $$  \hspace{1cm} (1.3)

Most electrophoresis techniques use a gel (polyacrylamide or agarose), a paper, or cellulose acetate as the supporting media. The matrix of the gel creates a sieving effect that interferes with the molecule’s motion and therefore increases the separation gap between molecules of different sizes. As a result, larger molecules migrate slower. The velocity equation above does not account for the sieving effect due to the pores in the gel. It is more accurate to use the mobility of the molecules to describe their motion. The mobility of the molecule in a gel matrix exhibits the characteristic profile shown in Figure 1.2. The gel concentration should be adjusted so that the molecule mobility lies in the linear region of the plot. This will ensure a more uniform spread of the molecule bands along the gel lane as seen in Figure 1.3. [1]
Once separated, the molecules are invisible to the naked eye. A staining process is usually done to detect them in the gel. Several staining methods are available. Molecules can be stained with an intercalating dye such as ethidium bromide. This stain is commonly used but is hazardous. Silver stains may also be used. They are the most sensitive but molecules cannot be recovered afterwards. The gel can also be blotted onto a nitrocellulose filter by electrophoresis and visualized using an x-ray film or other staining procedures. This method requires radioactive labels and is therefore highly hazardous. Another method used does not require any stains, and is known as UV shadowing. However, it requires a larger concentration of the sample than other methods, and is applicable specifically to DNA molecules. [1]
1.3 Purpose of the Research
As stated previously, current gel electrophoresis methods have several drawbacks. Namely, stains need to be used to detect the molecules in the gel. Most intercalating stains bond physically to the molecule, so de-staining is necessary if further analysis of the molecule is desired. De-staining is a long, inefficient, costly and hazardous process. Afterwards, the desired bands need to be excised from the gel. Since this is done manually by an operator, it creates problems. The operator is exposed to hazardous chemicals and must exercise a large amount of caution in his work. Also, due to low precision, the operation is not repeatable, and the sample might be lost. Furthermore, during the process, an unclean blade or a handling mistake could contaminate the sample.

In summary, six problems exist in current electrophoresis methods:
1. De-staining of desired bio-molecules is a costly and difficult process.
2. Electrophoresis is a slow process.
3. Large product losses occur during the operation.
4. Health concerns arise since the operator handles hazardous chemicals.
5. Procedure is prone to human error.
6. Method has low repeatability.

1.4 Functional Requirements and Scope of Research
The aim of this project is to design a robotic purification system for biologically active macromolecules. This machine is intended to combine the efficiency of a gel electrophoresis system with the precision of a mini-robot, and thus automate the whole electrophoresis process.

1.4.1 Functional Requirements
From a functional standpoint, this machine will have four-fold applications:

First, it will automate the manual electrophoretic procedures, which are routinely used all over the world by numerous scientific personnel. Specifically, the mini-robot system will speed up the purification of Biologically Active Macromolecules (BAM) by:

a) reducing the number of steps and automating repetitive steps,
b) increasing the amount of end product by reducing the waste of the BAM,
c) eliminating contact contamination.
Second, it should have the capability to:

a) determine molecular weights of BAMs,

b) quantify concentrations of BAMs,

c) record/store data for photo-imaging.

Third, the built-in artificial intelligence of the machine should enable it to adjust to various situations. It should also allow a scientist to operate an experiment from a remote position, access data in real-time, and change experimental parameters at his/her will.

Fourth, it should have the capability to provide 24-hour, real-time, on-line access to a repository of molecular data (protein/DNA sequences, structural homologues, physico-chemical properties etc.) once an experimental run is complete.

1.4.2 Scope of Research

This research is composed of two main parts. The first part addresses the development of a stain free detection system that can be used for a variety of BAMs. The second part involves of the design of the main components of the robotic system, namely:

- Variable ultraviolet light source
- Mechanical cutting device to excise desired bands
- Transportation system for the cutting device
- Washing station to clean and store cutting-tip ends
- Temperature controlled sample storage station
Chapter 2

Different Visualization Techniques

2.1 Overview

This chapter introduces the different techniques to visualize bio-molecules in a gel. The two most applicable to our set-up are reviewed, then after comparing them, the selection is made.

There are several detection methods for gel electrophoresis: absorbance, amperometry, conductometry, fluorescence, and indirect methods. The selection of an appropriate method is dependent on the application. The specifications for the detection system are:

- No stains should be used, so that further analysis of the sample is possible. This will also increase the efficiency of the process.
- The method should be applicable to a variety of BAMs: nucleic acids, proteins, and carbohydrates.
- The visualization of the bands in the gel should be fast enough to avoid photo bleaching and gel shrinkage. Photo bleaching is a change in the chemical structure of a molecule due to an intense or prolonged ultraviolet light exposure. Gel shrinkage occurs as the gel dries. This is caused by either by gel dehydration or by an increase in temperature due to UV light exposure. Both these phenomena should be avoided since they cause a change in the shape of the gel, and thus result in an inaccurate band location.
- The method should have enough resolution to be able to detect sample amounts less than 500 nanograms.
<table>
<thead>
<tr>
<th>Detection method</th>
<th>Applications</th>
<th>Characteristics</th>
<th>Equipment needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV Absorbance</td>
<td>Nucleic acids, proteins, peptides, small ions</td>
<td>Universal and easy to use but relatively low sensitivity</td>
<td>Variable UV lamp, light sensor</td>
</tr>
<tr>
<td>Amperometry</td>
<td>Only electroactive compounds in complex matrices</td>
<td>High sensitivity and selectivity, difficult to establish</td>
<td>Platinum electrodes, power supply</td>
</tr>
<tr>
<td>Conductometry</td>
<td>Ion analysis</td>
<td>Low sensitivity, not universal</td>
<td>Conductivity cell, conductometer</td>
</tr>
<tr>
<td>Fluorescence (LIF)</td>
<td>Amino acids, nucleic acids, peptides, proteins</td>
<td>High sensitivity, selective, expensive, not universal</td>
<td>Several lasers, Photomultiplier tube, spectroscopy method</td>
</tr>
<tr>
<td>Indirect absorbance</td>
<td>Ion analysis, carbohydrates</td>
<td>Universal, low sensitivity, restriction of buffer choice</td>
<td>Stable UV lamp, light sensor, dye screen</td>
</tr>
<tr>
<td>Indirect fluorescence</td>
<td>Only non-fluorescent compounds</td>
<td>Universal, high sensitivity, restriction of buffer</td>
<td>Stable arc lamp, laser, filter</td>
</tr>
</tbody>
</table>

Table 1.1: Comparison table for the different visualization techniques [2]

A comparison table of the different methods is shown in Table 1.1. Amperometry and conductometry methods are usually applied in conjunction with capillary electrophoresis techniques for the detection of specific compounds that other methods cannot detect. The indirect detection methods are primarily used for detecting substances with low absorptivity, that cannot be detected by their direct detection counterparts. They are restricted to specific substances and require the use of a low concentration background electrolyte to work well. They also need a highly stable light source to ensure a uniform background signal [2]. The last two methods (absorbance and LIF) satisfy most of the specifications. They could both be applied to our system and will now be presented. However, it is to be noted that this is only an overview of the methods. An in-depth analysis is outside the scope of this thesis, and one should consult appropriate literature for more information.
2.2 Laser Induced Fluorescence (LIF)

Laser induced fluorescence is one of the most sensitive detection methods of bio-molecules. It consists of exposing a sample to electromagnetic radiation (usually with a laser). The molecules in the gel become excited to higher energy levels, and fluoresce at different wavelengths as they decay to lower levels. The higher sensitivity is due to the low background noise of the fluorescent signal.

A typical set-up is shown in Figure 2.1. The laser is focused on the sample. A Photo-Multiplier Tube (PMT) senses the fluorescence emission collected by the lenses. A high power laser is necessary to obtain high resolution. This is because the fluorescence and background signal increase linearly with the laser intensity. However, the background noise increases proportionally to the square root of the laser intensity. Hence, a higher resolution is achieved by increasing the intensity. Since the laser used as the exciting beam has a small aperture, the LIF method is primarily used with capillary electrophoresis.
A Capillary Electrophoresis (CE) set-up is shown in Figure 2.2. A capillary tube is filled with gel and buffer. Then, using a high voltage power supply (up to 30 kV), an electric field is applied between the two buffer reservoirs. The sample is inserted into the capillary by replacing one of the buffers with the sample vial. In the detection section of the capillary, the capillary is usually replaced with a rectangular cuvette. This is necessary, because if the laser were passed through a round capillary, scattering and image distortion would occur. This would result in larger background noise and therefore a much lower sensitivity of the system.

The LIF technique allows for great sensitivity, but only at the cost of being expensive and not universal. Its strength lies in its ability to analyze the electronic structure of the bio-molecules, and to determine the analyte’s concentration, which is proportional to the area under the output signal curve. Also, LIF can only analyze one sample at a time, and since the capillary has an internal diameter on the order of 100-micrometers (equal to the diameter of a human hair), this results in a very low throughput. Systems that can process several capillaries at a time are being developed, but they are complex and expensive.
2.3 UV Absorption

UV absorption is one of the most popular detection methods in use today, even though it has a lower sensitivity than other techniques. This is due to the fact that it can be applied to a wide range of bio-molecules, and is easy to use. It consists of exposing a sample to electromagnetic radiation (usually with a UV lamp). At the location of the sample, the intensity of the UV signal drops because the molecules absorb UV light. This variation in signal can be detected. The molecule can also be identified because each bio-molecule has a characteristic absorption spectrum. The absorption peaks of different molecules are located at different wavelengths. The absorption peak is around 214nm and 280nm for proteins depending on their type, 230nm for carbohydrates and peptides, and 260nm for nucleic acids.

The Beer-Lambert Law describes the light absorption principle for a non-opaque sample.

\[ A = \varepsilon \times b \times c \]  

(2.1)

where

- \( A \) is the measured absorbance,
- \( b \) is the path length [cm],
- \( c \) is the analyte concentration [M],
- \( \varepsilon \) is the wavelength-dependent molar absorptivity coefficient [M\(^{-1}\) cm\(^{-1}\)].

Figure 2.3 presents a schematic of the absorption principle. The transmittance, \( T \), of a sample is the quantity usually measured by instruments.

\[ T = \frac{I}{I_0} \]  

(2.2)

where

- \( I \) is the light intensity after absorption,
- \( I_0 \) is the initial light intensity.
The relation between the absorbance and the transmittance is:

\[ A = -\log T = -\log \frac{I}{I_0} \]  

Typical absorption techniques use stains to increase the signal to noise ratio, and photo paper to record the band location. This is hazardous, costly, and time consuming. There is another set of absorption methods that does not use stains. They fall under the umbrella of UV shadowing techniques. The basic method for DNA molecules was described almost 50 years ago [5]. It consists of placing the gel above a 254nm light source. Atop the gel, a transparent UV-fluorescent material such as a standard, 1mm thick, minigel glass plate is placed. The plate will fluoresce everywhere except where the DNA has absorbed the UV light. The bands appear as dark regions on a light background. Quantities of unstained DNA in a gel of as low as 0.25 \( \mu \)g/bands have been detected in recent experiments [6]. In another application of UV shadowing, even smaller quantities were detected. The process involves transferring unstained nucleic acid to a nylon membrane and then visualizing the bands under UV light. The nylon membrane has a small UV induced fluorescence. Sensitivity down to 10ng has been achieved [7].

All the methods presented above record the band location on special photographic paper. The paper is then scanned to obtain an electronic version. This process is time-consuming and therefore it is not desirable. Two other methods have been previously developed that can visualize the gel directly. They have been developed for visualizing DNA molecules only and are not applicable to other bio-molecules. The first one is a variation of UV shadowing. It uses a phosphor storage screen (that fluoresces under UV light) to record the location of the bands in the gel. The lowest sensitivity obtained with this first method to date is around 400ng [8]. The second method uses direct absorption to detect the migration of DNA molecules through a gel. As seen in Figure 2.4, UV light is shone through a gel using a set of fiber optics. On the other side, a set of fiber optics collects the light and brings it to a Charged Coupled Device (CCD). Since the CCD camera used is not sensitive in the UV region, it needs to be coated with a phosphor lumogen coating that absorbs UV light (specifically at 260nm) and fluoresces in the visible range. The CCD then has a Quantum Efficiency (QE) of 12% at 260nm. With this system, the lowest sensitivity achieved was 1.25ng [9]. This method is far more sensitive than the previous one, but it only scans the gel, therefore it has a smaller throughput, even though it uses multiple fiber optics.
2.4 Comparison

After describing laser-induced fluorescence and UV absorption, the following conclusion can be made. The LIF method has better resolution capabilities than absorption, however it needs stains to achieve the high sensitivity. On the other hand, UV absorption can be used without stains, but has lower sensitivity. It can have a higher sensitivity, as explained in the CCD method, but only at the cost of losing its universality. Since the LIF technique is only applied in conjunction with capillary electrophoresis, it can sense one or a few samples at once, whereas the absorption technique allows for a snapshot picture of the entire gel at once.

For our purpose, UV absorption arises as the better solution. However, a new approach to UV absorption will be taken to increase its efficiency, without losing its universality.
2.5 Proposed Technique

Of all the UV absorption methods mentioned previously that use unstained samples, none can be universally applied to several bio-molecules. This is due to the lack of a detector with high quantum efficiency in the UV region. However, a new technology, known as back-thinned CCD, has been developed, and it allows for a dramatically improved UV response of the detector. Figure 2.5 shows the quantum efficiency of different CCD cameras. The back-thinned CCD has a minimum QE of 45% between 200 and 400 with a maximum of 83 % around 230nm. Front-sided (UV coated) cameras only have efficiencies of around 8% in the UV region.

![Figure 2.5: Quantum Efficiency of several CCD Arrays.](http://example.com/figure.png)

Using this CCD camera, the gel is visualized directly, and the band location is found by direct UV absorption measurement. A variable light source (200-400nm with 15nm bandwidth) was developed to allow for a precise selection of the target molecules to be visualized. This system can take pictures of a gel at any wavelength in the UV region, and has the potential to have a higher sensitivity than previous ones. The scanning approach is also investigated since it can produce information about the structure of the sample.
Chapter 3

Presentation of the Main Components

3.1 Overall Overview

In this chapter, the main components of the automated purification system are presented. Figure 3.1 shows the main components of the machine. These are the detection system (CCD camera and spectrometer), the precision XY stage, the variable light source, the cutting device, the sample transportation system, and the temperature controlled storage station.
The operation of the system is as follows. The gel is placed in a tray on top of the UV light source, which is attached to the XY stage. Once the CCD camera visualizes the gel and the desired bands to be cut are identified, the stage moves to place the first band to be excised under the cutting tool. The cutter excises the band and carries it to the temperature controlled storage area. The sample is expelled into a storage vial using compressed air. Then, the cutting tool moves to the cleaning station to exchange the used cutting tip with a clean one. Next, the cutter tool performs the next band excision, while the contaminated cutting tip is cleaned using an ultrasonic cleaner. This cycle is repeated until all the desired bands have been extracted from the gel. Now that the general operation of the machine has been explained, each individual component will be presented.

### 3.2 Detection System

The detection system was explained in the previous chapter. There are two different set-ups used in our machine. The first one takes snapshot pictures of the gel to locate the band shape and position inside the gel, while the second one scans the gel to obtain the absorption spectrum of a specific band. The two systems are shown in Figure 3.2.

![Figure 3.2: Detection system by: a) taking a picture, b) scanning the gel.](image)

The CCD camera, spectrometer, fiber optic, and controlling software were purchased from Acton research. The light source was developed and is presented in a section 3.4. The CCD camera uses a back-illuminated and UV-coated Hamamatsu CCD with a 1024 x 256 pixel format. As mentioned in the previous chapter, this CCD camera was chosen because it has a high quantum efficiency (43%-85%) in the UV region (200-400nm). The system comes standard with a 100-kHz, 16-bit analog-to-digital (ADC) converter and a 12-bit, 1-MHz ADC for rapid kinetics and fast system alignment. The spectrometer is an Acton Research SpectraPro150. Two
1200 l/mm gratings are included, a 300nm blazed for the UV range and a 500nm blazed for the visible range. The software used to control the CCD camera and the spectrometer is Princeton Instrument WinView for image acquisition and WinSpec for scanning. To visualize the gel using the CCD camera, a model UV8040B lens (78mm, F/3.8, UV imaging lens) from Universe Kogaku Inc is used. The technical sheets of the CCD detector and the UV lens are shown in Appendix B1.

3.3 Precision XY stage
The XY stage is used for both placement of the gel under the CCD camera and for a precise placement of the band to be excised under the cutting tool. The stage is a model 4020006 XY table from Daedal. See Figure 3.3. It is designed for repeatable precision positioning of light payloads over short travels, and can be utilized in applications requiring horizontal, inverted, or vertical translation. The stage has 150-mm travel in both X and Y directions. It has a step size of .1 µm, a positional accuracy of 75 µm and a positional repeatability of 12 µm. Since the smallest expected width of the band to be excised is around 1 mm, this accuracy of .075mm is sufficient to achieve the desired positional accuracy. The technical specification and the drawings of the stage are presented in Appendix B2.

The stepper motors to drive the stages are from Compumotor. A digital I/O card is used to generate and send pulses to the power amplifier, which is connected to the stepper motors.

Figure 3.3: 402 LN series XY table from Daedal. [4]
3.4 Variable Light Source

As explained previously, each bio-molecule has a different absorption spectrum, and thus a different absorption peak. Since a direct UV absorption method is used, there is a need for a compact variable light source capable of focusing on specific wavelengths.

3.4.1 Specifications

The specifications for the light source are summarized first, and then are explained in more detail. They are:

- Variable over 200-400nm range.
- 5nm-15nm bandwidth.
- 20 x 40mm light beam area (similar to detector’s shape).
- Uniform spatially and temporally.
- Compact to fit on the XY stage.

Most of the bio-molecules of interest have their absorption peak in the UV region of the spectrum. Specifically, the peak is around 214nm and 280nm for proteins depending on their type, 230nm for carbohydrates and peptides, and 260nm for nucleic acids. The absorption spectrum might also be needed to fully characterize a molecule; therefore a range of 200-400nm is desired for the light source. The absorption spectrum of a double stranded DNA sample is shown in Figure 3.4. The molecule absorbs strongly at 260nm and below 215nm. The lower wavelengths are not often used to measure absorption because the agarose gel and the buffer absorb also below 230nm.

![Figure 3.4: Double stranded DNA absorbance spectrum.](http://www.cbs.dtu.dk/dave/roanoke/genetics980211.html)
The bandwidth is defined as the width of the peak at half its height. See Figure 3.5. The bandwidth of the light source is dependent on its application. For locating the band using absorption measurement, a bandwidth of half or less than half of the molecule absorption bandwidth is necessary. However, if the absorption spectrum is desired, then the bandwidth of the light source needs to be set to one-tenth the absorption bandwidth of the molecule. This is to ensure that no spectral details are lost. The reason for the larger bandwidth when locating the band is that the medium surrounding the molecules (buffer or gel) can cause a shift in the molecule absorption peak anywhere from 1-5nm. Therefore, a larger bandwidth will ensure that the band is detected. Another factor to take into account is the signal to noise ratio. The intensity of the signal increases as the bandwidth increases. Since the noise level is constant in the system, the signal to noise ratio increases too. Consequently, it is necessary to have the largest possible bandwidth that is within the specification to increase the imaging system resolution. The bandwidth of some of the bio-molecules of interest is about 30-45nm for nucleic acids, and 25nm for proteins. Therefore, our system should have a bandwidth of around 15nm for the imaging part and around 3-5nm for the absorption spectrum analysis.

![Figure 3.5: Absorbance and light source spectra.](image)

The light source output needs to be stable over time in order for the measurement to be repeatable. This also allows for good time based measurement, and for baseline correction methods to be used when visualizing the gel. Moreover, the noise level should be as small as possible to ensure a good resolution. Finally, the light source needs to be uniform spatially. Spatial uniformity means that the intensity is uniform across the beam, and that the spectrum peak shape is be the same throughout the beam area. Errors in the latter point are introduced by aberrations in the optical system, which will be discussed in Section 3.4.6.
3.4.2 Existing Designs

There are a variety of ultraviolet light sources available, however none fit our specifications. They can be categorized into two groups: spectroscopic and transilluminator. The spectroscopic systems are made of standard modular components - a light source, optics, and a monochromator- as seen in Figure 3.6.

![Power supply](Lamp) (Optics) (Motor drive) (Fiber optic)

Figure 3.6: Spectroscopic light system.

The spectroscopic systems allow for a chromatic tuning of the light, however they are bulky and expensive. More importantly, they have a small exit aperture (0.1 to 1mm) since they are primarily used to focus the light into a fiber optic bundle. They can have bandwidths as small as 1nm, which results in low output power. Their purpose is to direct a high intensity UV-light onto a small sample for spectroscopic analysis.

Transilluminators are used for imaging gels as shown in Figure 3.7. They have a large output area and are compact (one unit), however they have a fixed wavelength light output. Furthermore, the illumination is not uniform since the transilluminator uses a series of parallel tube lamps, and its stability is marginal. Figure 3.7 shows a transilluminator from Ultralum that has a 254nm light output. This type of light source is typically used for illuminating a gel to visually locate its bands. Fixed wavelength transilluminators usually cost 4-5 times less than the least expensive spectroscopic system.
These UV light systems cannot be used in our application since they do not fulfill some important specifications. The spectroscopic system is large and has a small output beam, whereas the transilluminator has a fixed wavelength and a non-uniform output. Therefore, the design of a custom variable light source that meets the aforementioned specification is needed. Figure 3.8 shows a drawing of the proposed light source.

The variable light source is composed of three main components: the lamp, the filtering system (concave grating and motorized system), and the optics to focus and collimate the light.
3.4.3 Lamp Choice

A lamp that can provide a stable continuous output in the UV range is needed for the light system. There are four types of UV lamps available: deuterium, xenon, xenon-mercury, and hollow cathode. Table 3.1 shows a summary of their properties. The deuterium lamp is the ideal choice for the desired application since it provides a highly stable continuous ultraviolet output with little visible and infrared emission.

<table>
<thead>
<tr>
<th>Lamp Type</th>
<th>Wavelength (nm)</th>
<th>Spectrum</th>
<th>Stability (% p-p)</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deuterium (L2D2)</td>
<td>185 - 400</td>
<td>Continuous broad</td>
<td>0.05</td>
<td>Cheapest</td>
</tr>
<tr>
<td>Xenon</td>
<td>185 - 2000</td>
<td>Continuous broad</td>
<td>1</td>
<td>Expensive</td>
</tr>
<tr>
<td>Xenon-mercury</td>
<td>185 - 2000</td>
<td>Continuous broad</td>
<td>2</td>
<td>Expensive</td>
</tr>
<tr>
<td>Hollow cathode</td>
<td>193 - 852</td>
<td>Line</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3.1: Different available UV lamps. (Courtesy of Hamamatsu Inc)

A L2D2 deuterium lamp and a C4545 power supply from Hamamatsu are used. L2D2 lamps have a lifetime twice as long as conventional deuterium lamps, around 2000 hours. Moreover, they are 1.3 times brighter and have a higher stability and lower drift as seen in Figure 3.9.

Fluctuation: 0.05% p-p

Figure 3.9: Comparison of lamp output stability (Courtesy of Hamamatsu Inc)

There are a variety of lamp sizes and shapes. Types L6311-50 (0.5mm aperture) and L6312-50 (1mm aperture) are selected because they have the smallest size and are already mounted to a base. This facilitates mounting and more importantly, alignment. The 0.5mm aperture has a high brightness arc, whereas the 1mm aperture provides a more uniform distribution. The selection between the two types is made through experiments.

To obtain the performances highlighted above, the lamp power supply needs to provide a constant current for the main power supply section and a constant voltage for the filament power supply. The Hamamatsu C4545 power supply is specifically designed for these lamps, and
therefore is used in the system. Technical information for these components is presented in Appendix C3.

To select the best lamp for our system, each was characterized by looking at its actual spectrum and its light distribution. Figure 3.10 shows the spectrum of the L6312 lamp taken using the spectroscopy system. L6311 spectrum is the same as the L6312 except for a slightly larger amplitude, therefore it is not shown.

Figure 3.10: Spectrum of the L2D2 deuterium lamp using the CCD detector.

The spectrum shown in Figure 3.10 was obtained at a distance of 100mm directly in front of the lamp. It exhibits some variation from the spectrum advertised by Hamamatsu. The curve from 210-400nm should have a smooth decrease. The spectrum taken with the CCD camera in Figure 3.10 shows bumps around 240nm, 300nm, and 370nm. The CCD detector causes these bumps. The CCD quantum efficiency is wavelength dependent. As seen in Figure 2.5, the QE curve has sharp variations in the UV region. The bump locations correlate perfectly with the sharp changes in the QE of the CCD. One should be aware that each optical element that interacts with the light changes the shape of the spectrum because of its wavelength dependent quantum efficiency. Therefore, these components should be chosen to ensure a uniform intensity distribution in the wavelength range of interest (200-400nm in the proposed design).
Deuterium lamp is rotated around its center axis.

Figure 3.11: Deuterium lamp test: a) apparatus, b) and c) test results.
Next, the light distribution of the bulb was determined by measuring the intensity variation at different angles relative to the lamp center as shown in Figure 3.11a. The spectrum of the lamp was recorded every 2.5 degrees from −15 to 15 degrees and analyzed using Matlab.

From the results shown in Figure 3.11 (b) and (c), two observations can be made. First, the separation distance between each spectra is proportional to their intensity, and this ratio does not change with different wavelengths. Therefore, in this case, the intensity distribution is wavelength independent. Second, the spectra from −12.5 to 0 degrees are close to each other. As the angle is increased outside this region, the intensity decreases quickly. Hence, there exist a range of angles where the light intensity is more uniform thereby exhibiting Gaussian distribution. However, this range is not centered at the origin (0-degree). To check this assumption, the intensity at the specific wavelength of 254nm versus the angle is plotted and presented in Figure 3.12.

![Figure 3.12: Lamp intensity at 254nm versus the angle.](image)

As seen in Figure 3.12, the maximum intensity is at −5 degrees, therefore in the system, the lamp should be rotated counterclockwise 5 degrees. Also, a section of 15 degrees centered at −5 degrees will ensure 95% uniformity of the lamp output.
Figure 3.13 shows the comparison between the two different lamps. Using the same set-up as Figure 3.11a, the maximum intensity between 200nm and 220nm versus the angle was recorded for each lamp at three different distances (50mm, 70mm, and 100mm).

![Lamp Comparison Diagram](image)

**Figure 3.13: Lamp intensity versus the angle for each bulb.**

This graph shows that both lamps have an off center peak around –5 degrees. As expected, the 0.5mm aperture lamp is brighter and has a sharper peak than its 1mm counterpart. This means that the 0.5mm aperture lamp should be used if light throughput is important, whereas the 1mm aperture should be used to provide a more uniform beam when using the 15-degree bandwidth. For the first prototype, the 1mm aperture is used since beam uniformity is more important for molecule visualization.
### 3.4.4 Different Approaches to Filtering

The deuterium lamp that was selected emits a broadband spectrum. Its output needs to be filtered so that the user can select a specific wavelength. Two different filtering methods, rotating filter wheel and diffraction grating, were identified and will now be presented.

Filter wheels are commonly used to select a specific wavelength beam. Band-pass filters that only transmit light of a specific wavelength are attached to a wheel as seen in Figure 3.14. The wheel is rotated about “axis 1” to bring the desired filter in the light path.

![Filter Wheel Diagram](image)

*Figure 3.14: Filter wheel approach.*

Band-pass filters are simpler in design and have a higher throughput than most other filtering methods. However, this design’s disadvantage is that only a finite number of wavelengths can be selected. One way to expand the application of this design is by taking advantage of the fact that the transmitted wavelength through a filter varies with the incidence angle of the light. Therefore by rotating the filter wheel around “axis 2”, the transmitted wavelength shifts towards shorter wavelengths with increasing angle. The relation between the angle and the transmitted wavelength is [10]:

$$\lambda = \lambda_0 \sqrt{1 - \left(\frac{n_0}{n_{\text{eff}}} \right)^2 \sin^2 \theta}$$  \hspace{1cm} (3.1)

Where

- $\lambda$ is the wavelength at angle of incidence,
- $\lambda_0$ is the wavelength at normal incidence,
- $\theta$ is the angle of incidence,
- $n_0$ is the refractive index of external medium,
- $n_{\text{eff}}$ is the effective refractive index of filter.
Figure 3.15 shows the variation of wavelength with the incidence angle for the UV region (260nm) and for the IR region (2350nm). The transmitted light intensity decreases sharply after approximately 25 degrees; therefore the maximum variation in the UV region is only 10nm. This means that about 20 filters would be required to cover the 200-400nm range desired. In the infrared region however, a variation of 95nm is possible at 25 degrees. This difference between the two regions of the light spectrum is because the variation at a given angle is proportional to the wavelength. In summary, this method could only be applied to an application in the infrared but not in the ultraviolet range. Another method is therefore necessary.

The second filtering method consists of using a diffraction grating to spatially separate the light into its monochromatic components. A schematic of a possible design is shown in Figure 3.16.
A brief overview of the diffraction grating concepts is presented below. For a detailed explanation, one should consult appropriate literature such as reference [11].

A typical diffraction grating consists of a substrate with a large number of grooves (>600 lines/mm) ruled on its surface as seen in Figure 3.17.

The incident light on the grating surface is diffracted into discrete directions. The grating equation, which governs this diffraction, can be written as:

\[ m\lambda = d \left( \sin \alpha + \sin \beta \right) \]  
\[ (3.2) \]

where

- \( m \) is the order of diffraction (an integer),
- \( \lambda \) is the wavelength,
- \( d \) is the groove spacing,
- \( \alpha \) is the incidence angle,
- \( \beta \) is the diffraction angle.

A common application of diffraction grating consists of changing the wavelength by rotating the grating about its axis, with the incidence and diffraction light direction remaining constant. The angles \( \alpha \) and \( \beta \) change as the grating is rotated, but the difference between them remains constant. This angle is called the deviation angle, \( 2K \):

\[ 2K = \alpha - \beta = \text{constant} . \]  
\[ (3.3) \]

The grating equation can be rewritten as

\[ m\lambda = 2d \cos K \sin \phi , \]  
\[ (3.4) \]

where

\[ \phi = (\alpha + \beta)/2 = \text{scan angle} , \]  
\[ (3.5) \]
Equation (3.4) is required to design the mount for the grating. It shows that the diffracted wavelength at the output slit is directly proportional to the sine of the angle $\phi$, which is the grating rotation angle. This relation is the basis for the grating driving mechanism seen in Figure 3.18.

![Figure 3.18: Grating driving mechanism. [11]](image)

The relation between the arm length $L$, the distance $X$, and the angle $\phi$ is:

$$\sin \phi = \frac{X}{L}. \quad (3.6)$$

Finally, the grating equation can be written as

$$m\lambda = 2d\frac{X}{L}\cos K. \quad (3.7)$$

Equation (3.7) shows that as the distance $X$ increases, the diffracted wavelength $\lambda$ increases linearly with $X$.

It is important to note that more than one combination of $m$ and $\lambda$ will satisfy the equality of Equation (3.7). For example both $m=1$, $\lambda=\lambda_f$ and $m=2$, $\lambda=\lambda_f/2$ are valid solutions. This means that at a given angle, there is overlapping of diffracted spectra as seen in Figure 3.19.

![Figure 3.19: Overlapping of spectral orders [11]](image)
Since usually only the first spectral order is desired, the higher orders may need to be blocked by using some order-sorting filters.

Even though the diffraction grating system has a smaller light throughput than the filter wheel, its ability to scan the entire spectrum makes it the first choice for the variable light source. Since the deuterium lamp only produces a useful spectrum from 200-400nm, no order overlapping will occur.

### 3.4.5 Different Diffraction Gratings

There are different ways of mounting diffraction gratings. They can be grouped into two categories: plane grating mounts, and concave grating mounts.

Plane gratings need a collimated incident light to disperse the light by wavelength. Optics such as mirrors or lenses are needed to collect and focus the beam. Figure 3.20 shows the four different types:

![Figure 3.20: Plane grating mounts.](image)

- **a) Czerny-Turner mount**
- **b) The Ebert-Fastie mount**
- **c) The Monk-Gillieson mount**
- **d) The Littrow monochromator mount**
The Czerny-Turner (Figure 3.20a) is the most common type of plane grating mount. The incident light is collimated with a mirror, and the diffracted light is focused with another mirror. Since the light reflecting off the grating is collimated, there is no aberration introduced by the grating, and the spectrum is always in focus at the exit slit. The mirrors however, introduce astigmatism and spherical aberration. The Ebert-Fastie mount (Figure 3.20b) is similar to the Czerny-Turner, with the difference that it uses one large mirror for both collimating and focusing the beam. This mount is not often used because aberration and stray light are difficult to control.

In the Monk-Gillieson mount (Figure 3.20c), a mirror is used to converge the beam onto the grating. Since the incident light on the grating is not collimated, wavelength dependent aberrations are present and the beam is not always in focus at the exit slit. To counteract this problem, the grating needs to be rotated about an off-center axis and the angles of incidence and diffraction should be kept to a minimum. This type of grating is popular for low-resolution applications since it is the cheapest type of mount available.

Finally, the Littrow monochromator mount (Figure 3.20d) is used primarily in laser tuning applications. The incident and diffracted light beams have almost the same direction. The entrance and exit slits are slightly offset, which introduces out-of-plane aberrations.

On the other hand, concave gratings combine the function of optical imaging and wavelength diffraction in one component, such that external optics are not necessary. See Figure 3.21.

![Concave grating mount](image)

**Figure 3.21: Concave grating mount [11]**
The most common type of concave grating mount is the Seya-Namioka, which scans the light spectrum simply by rotating the grating about its axis. This grating offers two main advantages over plane gratings. It has a lower aberration at the designed wavelength, and a higher throughput since no other optic component is needed. It is the preferred type of grating mount for UV spectroscopy and therefore will be used in the design of the light source.

The Richardson grating laboratory offers a wide variety of high quality concave gratings. To maximize throughput, the grating needs to be as large as possible and have the largest number of grooves per millimeter. The grating coating and blazing wavelength affects its efficiency and consequently also influences the system throughput. For the proposed light source, two possible concave holographic gratings have been selected. Their characteristics are listed in Table 3.2:

<table>
<thead>
<tr>
<th>Grating</th>
<th>Grooves per mm</th>
<th>Imaging Range (nm)</th>
<th>Blaze wavelength (nm)</th>
<th>Dispersion (nm/mm)</th>
<th>Free Aperture (mm)</th>
<th>Input F/#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1200</td>
<td>200-800</td>
<td>250</td>
<td>8.5</td>
<td>30 * 30</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>1350</td>
<td>190-700</td>
<td>250</td>
<td>8.0</td>
<td>φ 40</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 3.2: Selected concave holographic grating from Richardson grating laboratory.

The Richardson grating laboratory only manufactures its concave grating on demand, and thus 8 weeks lead time is required for most of their gratings. To avoid the wait, an off-the-shelf monochromator model 9030 from Scincetech, Inc was purchased. This offers two advantages that simplify the light source design. First, the grating is similar to #1 in Table 3.2 except that it has a dispersion of 8nm/mm and an F/3.2. The F/# is the ratio of the lens focal length to the lens aperture diameter. Second, the grating is already mounted, and the sine drive to rotate it is included. Some major modifications of the monochromator were made to adapt it to the design and will be explained in Section 3.4.7.
3.4.6 Optical Components

Now that the deuterium lamp and the concave diffraction grating filtering method have been selected, the optics to focus the lamp beam into the entrance slit and to collimate the output from the exit slit are investigated. See Figure 3.22.

![Optics set-up to focus and collimate the light](image)

The collimator is described first since it is the simpler of the two systems. The diffracted light coming out of the exit slit of the grating mount has a F/3.2. It needs to be collimated and redirected at a 90° angle for the output beam to be vertical. An off-axis paraboloidal reflector with a diameter of 2.5 inches and a focal length of 119mm from Oriel Instruments was first selected, since it has a F/3.3 and can both collimate and redirect the light vertically. However, due to the high price and the required lead-time of 6 weeks, another approach was adopted. A concave reflector, model 7292, with a focal length of 125mm and a F/3.2 from Oriel instruments; and a square mirror model K45-340, 75mm, UV coated from Edmund Industrial Optics, are used instead. This system is composed of two elements but it has the advantage that each component is mounted on a separate minimount that allows for fine tilt adjustment, which is crucial for calibration of the system.

The incident light on the grating has to be focused to a F/3.2. Three different methods, using lenses, reflector, and direct illumination have been designed. After presenting each method, the test results for each are shown and explained.
The first method uses two fused silica plano-convex lenses from Coherent Inc. to match the F/# of the incident light with the F/# of the concave grating. See Figure 3.22. The first lens, with a focal length of 50.8mm, collimates the light coming from the deuterium lamp, whereas the second lens with a focal length of 76.2mm, focuses the light on the entrance slit with the correct F/#. This method has the advantage of maximizing the incoming light, however the lenses introduce chromatic and spherical aberration. Chromatic aberration is the chromatic spreading of light. Each wavelength is focused at a different point along the optical axis, because the index of refraction of all optical materials varies as a function of wavelength. Spherical aberration is the distortion of the image and the creation of a blur at the focal point because different radial sections of the lens focus at different points. To minimize spherical aberration in this set-up, lenses that are twice as large as the light beam diameter are used. Moving the lens slightly toward the focal point also reduces the aberration by averaging the error across the entire lens instead of just the outer rings.

![Figure 3.23: Light source set-up using a reflector.](image)

The second focusing method consists of using a square reflector model 7295 from Oriel Instruments with an 86mm focal length as shown in Figure 3.23. This system differs from the lens set-up, as a concave reflector replaces the lenses. This eliminates chromatic aberrations. However, off-axis aberrations are now introduced.
The third and last method is shown in Figure 3.24. It consists of eliminating all of the focusing components and placing the deuterium lamp at the entrance slit position. This approach might seem to be against all the good practices of an optics system design. However, due to the special characteristics of the designed system, this method is feasible and its advantages could exceed its disadvantages. As seen in the Section 3.4.3, the deuterium lamp has a small aperture (0.5mm for the L6311 and 1mm for the L6312). Therefore the lamp aperture can replace the slit. Moreover, the acceptance cone of the grating, with its base at the grating surface and its peak at the entrance slit, has an angle of 18°. As seen in Figure 3.12, the lamp has 95% illumination uniformity for an angle of 15°. Consequently, by placing the deuterium lamp at the entrance slit, the light incident on the grating is at least 92% uniform. This has been estimated from the data of Figure 3.12. By avoiding the focusing optics, chromatic and spherical aberrations should be minimized. However the light dispersion is expected to be larger.

Now that each of the three methods, using lenses, reflector, and direct illumination, has been presented, the experiments to compare them and their results will be presented. Three parameters are of importance in the comparison of the different systems:

- the uniformity of the illumination at different wavelengths,
- the bandwidth of the spectrum,
- the shift of the spectrum in the beam profile.
To test the uniformity of the illumination, the light output was visualized at eight wavelengths (200, 230, 260, 290, 320, 350, 380, and 400 nm) by taking a snap-shot picture using the CCD camera. See Figure 3.2a for the imaging set-up diagram. Since in the final application the camera will be taking a picture of a gel placed above the light source, the camera was focused on a plane just above the light to mimic its future application. A 3D-mesh plot of the results is shown in Figure 3.25, 3.26, and 3.27.

To find the bandwidth and the spectrum shift throughout the beam, a spectrum of the output beam was recorded at different locations in the beam and at different wavelengths by using the spectrometer. See Figure 3.2b for the spectrum analysis set-up diagram. The fiber optic was placed vertically above the light source to also mimic its future application. The results are shown in Figure 3.28.

The L6312 lamp (1 mm aperture) was used in this experiment for all three set-ups. The entrance slit for the lenses and reflector set-up had a 1 mm width and an 8 mm height. The slit width was selected to match the aperture of the L6312 lamp so that all three set-ups have the same slit entrance size, and therefore can be compared under the same conditions. The exit slit was first chosen to match the entrance slit for optimal throughput. However, after initial tests, it was observed that the output intensity was so large that the detector always saturated. To solve this problem, an exit slit of 0.5 mm width was placed to reduce the output signal intensity. The beam intensity was reduced by half, however the bandwidth of the output was slightly larger than the value of 4 nm/mm predicted by the grating specification. The grating dispersion and a convolution of the input and exit slit width determine the bandwidth. In the set-ups, the entrance slit was 1 mm wide, the exit was 0.5 mm wide, and the grating dispersion was 8 nm/mm. Therefore, the bandwidth for the lenses and the reflector systems should be around 6-7 nm.
Figure 3.25: Light source illumination at different wavelengths for set-up with lenses.
Set up with focusing mirror at 200nm

Set up with focusing mirror at 230nm

Set up with focusing mirror at 260nm

Set up with focusing mirror at 290nm

Set up with focusing mirror at 320nm

Set up with focusing mirror at 350nm

Set up with focusing mirror at 380nm

Set up with focusing mirror at 400nm

Figure 3.26: Light source illumination at different wavelengths for set-up with reflector.
Figure 3.27: Light source illumination at different wavelengths for set-up with direct illumination.
Figure 3.28: Light source spectrum analysis for three different focusing set-ups
a) set-up with lenses, b) set-up with reflector, c) set-up with direct illumination.
Figures 3.25, 3.26, and 3.27 show the illumination from the three set-ups. The picture of a ruler was taken to correlate the pixel number with the actual image size (mm). From the mesh plot, some conclusions were drawn about the uniformity of each set-up.

For all systems, the intensity is wavelength dependent. This is to be expected since the deuterium lamp intensity is wavelength dependent as seen in Figure 3.11. Besides the lamp, the optics and the detector efficiency also influence the final output intensity.

The set-ups can be arranged in order of increasing throughput: the lens system, the reflector system, and the direct illumination system. There are two reasons for this behavior. First, there are losses associated with each optical component that transmit or redirect the light. The more elements a system has, the higher its losses. The lens system has three elements, two lenses and a slit, to focus the beam; the reflecting system has two, the concave mirror and the slit; and the direct illumination system has none. This correlates with the result observed in the figures. The difference between the lens and the reflector system is further accentuated by the fact that lenses inherently have a lower efficiency than reflectors. Second, because of their larger size, reflectors can collect more light than lenses. The reason that the direct illumination system has a higher throughput lies in the fact that the deuterium output is highly directional. As seen previously, most of the beam intensity resides within a 20-25 degree cone. Since the acceptance cone of the grating is around 18 degrees, almost all of the incident light reaches the grating.

As seen in Figure 3.25, the illumination from the set-up with lenses is not uniform. This is mostly due to the spherical aberration introduced by the lenses, but is also due to misalignments between the lamp, lenses, slit, and grating. Even though spherical aberrations were minimized by using lenses twice as large as the beam diameter, they were still significant because of the low F/# of the lenses used.

Using a reflector instead of lenses in the focusing system greatly improves the illumination properties as shown in Figure 3.26. The light is more uniform and the profile of the illumination is larger than the lens system. The flat areas at the top of the profile at 260, 290, and 320nm are due to the detector saturation.

Figure 3.27 shows the results of the direct illumination system. It is observed that the beam profile is larger than the lens and reflector systems. Most of the plots show that the detector saturated due to intense light. However, some conclusions may be made. The part of the plot
that is not saturated suggests a uniform beam similar to the reflector system. The figures where the detector has not saturated confirm this hypothesis.

It should be noted that these 3D mesh plots (Figure 3.25, 3.26, and 3.27) only give an estimation of the illumination profile. The camera UV-lens system is focusing on a finite plane, trying to image the beam at that plane. However, since the beam is collimated, there is a superposition of imaging planes, which induces errors in the illumination. Nevertheless, this approach is sufficient for the purpose of comparing the different set-ups. For the final design, a scanning approach using fiber optics is implemented to give an accurate measurement of the output beam profile.

In conclusion, the illumination test illustrates that the profile of the lens system is not uniform, whereas the reflector and direct illumination systems have relatively uniform profiles. Also, the direct illumination set-up has a much higher throughput than the other two systems.

Another important characteristic of the light beam is needed to compare the different set-ups. Figure 3.28 shows the results of the spectrum shape and shift at three different locations in the beam. Position X0 is the center of the beam whereas position X1 and X2 are respectively the right and left side of the beam profile. The spectrum intensity values at X1 and X2 were multiplied by a factor so that their peak intensity matched the X0 spectrum peak intensity. The parameters of interest extracted from the data in Figure 3.28 are summarized in Table 3.3.

<table>
<thead>
<tr>
<th>Set-up system</th>
<th>Bandwidth (nm)</th>
<th>Shift in spectra (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Lenses</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>With reflector</td>
<td>7.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Direct Illumination</td>
<td>15</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 3.3: Results of the spectrum bandwidth and location test.

The results of this test confirm our predictions. The system with lenses has a small bandwidth of 7nm, but also the largest shift in the spectra location (6nm). This is mainly due to chromatic and spherical aberrations introduced by the lenses. The shift in spectra is of the same magnitude as the bandwidth. Therefore as seen in the figure, the spectrum at X1 and the spectrum at X2 are far enough from each other than there is almost no overlapping between them.

The reflector system exhibits the same characteristics as the lenses system. It has a small bandwidth of 7nm and also a shift of 4.6nm. The spectra locations do not vary as much since only spherical aberrations are present.
The direct illumination system on the other hand has only a 0.7nm shift in its spectra locations along the X direction. In the direction perpendicular to X, a shift of up to 2nm was observed. This large improvement comes at the cost of having a larger bandwidth of 15nm.

The shift in the spectra observed in the lens and reflector systems is not acceptable for this application because the light output will be of a different wavelength than expected and this could lead to large errors in measurements. Therefore, the direct illumination method will be used in the variable light source. The bandwidth of 15nm is within the specification for imaging the gel. However, the shape of the spectra for the direct illumination set-up in Figure 3.28 is not perfect and suggests that this method could be optimized. Possible optimizations are explained in Section 3.4.8.

3.4.7 Final Design and Lamp Characteristics

Now that the main components of the light source have been selected, namely the deuterium lamp, concave holographic diffraction grating, and collimating optics; the final design of the light source and its characteristics are presented.

![Diagram of Proposed light source](image-url)
The detail and assembly drawings for the light source are found in Appendix C1 and C2. As seen in Figure 3.29, the light source is separated into two compartments, the lamp and the collimating section. The lamp section contains the deuterium lamp, the diffraction grating and its motorized assembly, the cooling fan, and the electronic shutter. The collimating section includes the exit slit, the collimating reflector, and the 45-degree mirror.

The main optical component mounts, lamp, grating, and focusing optics, permit a full range of motion to enable the user to precisely adjust the component position and rotation.

The deuterium lamp has four degrees of freedom. It can be rotated through its vertical axis, and can be displaced horizontally and vertically. Also, the lamp holder allows for a change of the lamp without losing the positional settings. The deuterium lamp has a 30W output, and therefore a cooling system is needed to ensure that no overheating occurs. Overheating would result in poor performance and shorter life of the lamp. On the cover inside the enclosure, a small inverted heat sink is mounted above the lamp to prevent local heating of the cover. A small fan with a filter is mounted inside the frame to recirculate the air inside the lamp side of the light source. The fan and exit holes are placed in such a way that the filtered air passes by the lamp, the heat sink, the stepper motor, and then exits behind the motor.

The diffraction grating has five degrees of freedom. Its mount is based on the Sciencetech 9030 monochromator. Of the monochromator, only half of the base was kept because it contained the sine drive and an adjustment knob with a wavelength display. In the original monochromator, a mirror redirected the diffracted beam toward the exit slit. A clockwise rotation of the knob resulted in a clockwise rotation of the grating and an increase in the output beam wavelength. Since in our set-up there is no mirror between the grating and the exit slit, a clockwise rotation of knob would result in a decrease in the output beam wavelength, which is the reverse of the counter display. To correct this problem, the grating needs to rotate in the opposite direction when the knob is turned clockwise. This was accomplished by inverting the base, and mounting the grating and its holder on the opposite side of the shaft. In this set-up, a clockwise rotation of the knob results in a counterclockwise rotation of the grating, and as desired, an increase in wavelength.

A stepper motor and flexible coupling are mounted on the side of the diffraction grating base to automate the grating rotation via a computer. Turning the knob rotates the grating so that a different wavelength is focused on the exit slit. Each turn of the knob corresponds to a 100nm-
wavelength scan. The motor was selected to have 200 steps per revolution, resulting in a resolution of 0.5nm in the focused wavelength. A miniature switch is mounted under the base to allow for an automatic wavelength calibration when under computer control. A power switch on the back panel allows for switching between manual and motorized operation of the grating rotation.

When the light source is used for imaging a gel, an electronic shutter is needed to control the exposure time. The spectrometer contains a shutter. An identical one was purchased, a Prontor Magnetic 016 shutter from Schneider Optics, as to use the same controller for the light source shutter. However, using the same controller means that the light source shutter needs to be removed when using the spectrometer, since the controller can only operate one shutter at a time. There is a controller inside the CCD camera that could be used to control the light source shutter, but this would require the machining of some the CCD components to allow for the connection to be made.

The exit slit came with the monochromator and is mounted on the inside wall. It can be easily replaced with a different size slit. If the slit width needs to be adjusted automatically, motorized slits are widely available and can be adapted to the light source. The collimating reflector and the 45 degree mirror have five degrees of freedom for easy calibration.

To minimize stray light in the system, the inside wall needs to be coated with a matte black paint. However, optics researchers at Acton Research warned that even UV resistant paint would deteriorate quickly under the intense direct UV illumination of the deuterium lamp. The paint would chip away from the wall, and paint dust particles would start depositing all over the optics. This would seriously reduce the lamp performance and would therefore be unacceptable. Therefore, the walls in the lamp section of variable light source are left bare. The light reaching the collimating section is of much lesser intensity, and thus the walls in that section are painted with an ultra matte black paint.

The effects of leaving the walls bare in the lamp section were measured to check their importance. The experiment was performed in two parts. First, the reflectivity of an aluminum plate in the UV region was compared to a mirror and to a black wall. Second, the influence of the reflection in the light source was measured by blocking the acceptance cone of the diffraction grating, and measuring the output spectrum as seen in Figure 3.30.
Figure 3.30: Set-up to measure unwanted reflected light.

Concave grating
Baffle
Deuterium lamp
Exit Slit
Reflected light
Unwanted output
Collimating reflector

Table 3.4: Different surface reflectivity.

<table>
<thead>
<tr>
<th>Wall type</th>
<th>Reflectivity relative to straight light (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirror</td>
<td>76.9</td>
</tr>
<tr>
<td>Bare aluminum</td>
<td>9.7</td>
</tr>
<tr>
<td>Painted Aluminum (black)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The reflectivity at 260nm of different surfaces relative to direct light is shown in Table 3.4. The reflectivity of bare aluminum is 9.7% and is significant. However as seen in Figure 3.31, the test measuring the influence of the bare aluminum walls in the light source reveals that they have no effect on the output signal.

Figure 3.31: Influence of bare aluminum walls on the output signal.
Now that the final design has been presented, the light beam output power and profile will be precisely characterized. By using the spectrometer with the fiber optic, the output power throughout the spectral range was recorded and is shown in Figure 3.32. In the figure, some characteristic spectra are shown at selected wavelengths of 210, 260, 310, 360, and 400nm.

![Variable light source Power output](image)

**Figure 3.32: Output power of the variable light source.**

A program was used to control the XY stages and record the spectrum of the light at 260nm. The spectrum was recorded every 2mm throughout a square area of 40mm length. The resulting 400 spectra were analyzed to extract the wavelength and the intensity of the maximum peak. Mesh plots of the results were created and are shown in Figures 3.33 and 3.34. The light source output beam has an area of around 32mm x 32mm. Figure 3.34 shows that there is a small shift in the peak location. On the side of the picture, the surface is rough. At those locations, the data was just background noise because there was no illumination. Consequently, the Matlab program used to extract the maximum peak wavelength generated random values at these locations.
Mesh plot of Light illumination at 260nm obtained using scanning.

Figure 3.33: Illumination profile of light source at 260nm.

Wavelength at the peak throughout the illuminated profile.

Figure 3.34: Peak location throughout the beam.
3.4.8 Recommendations and Future Work

In this section, three possible improvements of the light source are described. The first improvement consists of increasing both the throughput of the system and the output beam size. The deuterium lamp has large directivity and using a larger grating would be more effective. This should be combined with characterizing the effect of using different slit widths and heights. In all the experiments, the slits had a height of 8mm. A longer slit would increase both the light throughput and the beam size, if the output beam in the exit slit plane was long.

The second improvement consists of reducing the bandwidth of the output beam. As mentioned previously, the shape of the spectrum in the final design shown in Figure 3.28c is not a true Gaussian, and therefore, could be improved. The shape of the spectrum is possibly the addition of three different spectra as shown in Figure 3.35.

![Figure 3.35: Possible Gaussian addition to explain the spectrum shape](image)

The light path helps us to understand the source for the extra peaks on the side of the spectrum. Figure 3.36 shows a schematic of the light bulb and its light path.

![Figure 3.36: Deuterium lamp light path](image)
As was explained previously, the incident light on the grating needs to come from one point source for optimum performance of the system. The deuterium lamp has a small aperture, and ideally, its light output path should be a cone. However, the lamp is enclosed in a cylindrical glass bulb, and as seen in the drawing of Figure 3.36, this induces some of the light to be refracted toward the grating. Since this light is coming from a different point than the desired light, it will diffract on the grating at a different angle and appear in the output beam with a different wavelength.

A simple experiment was performed to check this assumption. The experiment consists of placing a baffle between the deuterium lamp and the grating on either side as shown in Figure 3.37. This would eliminate some of the undesired light and reduce the bandwidth of the spectrum.

![Figure 3.37: Direct illumination with a baffle to restrict the light reaching the grating.](image)

The results of this experiment are shown in Figure 3.38. Placing the baffle on the left side of the lamp results in the thinning of the right side of the spectra with no effect on the location of the center peak. See Figure 3.38a. This is similar to removing the peak corresponding to $\lambda 2$ in Figure 3.35. Repeating this experiment with the right side yields the same result except that the left side gets thinner. See Figure 3.38b. It may be concluded that the spectrum for the direct illumination set-up is possibly an addition of three spectra as seen in Figure 3.35 and is caused by the deuterium lamp.
Baffle test on direct Illumination Set-up

Figure 3.38: Results from testing the influence of the baffles on the direct illumination set-up.
This experiment suggests that adding a baffle on each side of the light source reduces the bandwidth of the direct illumination method. A direct illumination set-up using baffles was developed and tested. As seen in Figure 3.38c, the bandwidth was reduced from 15nm to 9nm, however the illumination profile was much smaller and the spectrum location shifted at different locations in the beam. See Figure 3.38d. Due to the obstruction by the baffles, the grating was not entirely illuminated. This resulted in the smaller profile obtained. The shift of the peak location is much larger than the one measured without baffles, $\pm 6.5\text{nm}$ versus $\pm 0.35\text{nm}$, and is possibly paused by the interference patterns introduced by the baffles. Despite this spatial wavelength shift, the spectrum shape was consistent for different wavelengths. See Figure 3.38e. Now that it has been demonstrated that the lamp is most likely the origin of the problem, two different approaches could be taken to solve it and should be investigated. The first one consists of conducting more research on the effect of baffles and could result in an improved performance of the current light source. The second approach consists of eliminating the problem by using a different lamp. Hamamatsu offers another type of deuterium lamp called projecting type lamp as shown in Figure 3.39.

![Figure 3.39: Projecting type deuterium lamp. (Courtesy of Hamamatsu Photonics)](image)

The projecting type lamp has a more uniform transmittance due to the plane glass and a smaller directivity. Using this lamp could reduce both the spectrum bandwidth and the shift in the peak location. However, the projecting type lamp requires a different power supply and a different, more complex, lamp mount.

The third improvement consists of increasing the wavelength range of the light source into the visible region of the spectrum by adding an halogen lamp to the system. The halogen lamp would be placed behind a see-through Hamamatsu deuterium lamp. The light of the halogen lamp would pass directly through the deuterium lamp. Some order sorting filters need to be used in this design to eliminate spectra overlapping.
3.5 Cutting Tool

Once the bands are identified in the gel using the variable light source and the CCD camera, the desired bands need to be excised and stored. An automated cutting tool designed specifically for this application is presented in this section.

3.5.1 Specifications

The following are the requirements for the excising device:

- Excision of a band from an agarose or polyacrylamide gel.
- Expulsion of the band in a storage vial.
- Extraction of band of varying rectangular shape, 5-10mm width, .5-5mm length, and 1-10mm thickness.
- No cross contamination of samples between different cuts.

A device that can meet all these requirements is difficult to design. The fact that the gel and bio-molecules it contains are fragile and temperature sensitive makes this task even more challenging.

3.5.2 Existing Designs

The process of excising the desired band out of the gel is currently performed manually. The operator locates the desired band by looking at the gel on a transilluminator. Then using a razor blade, the band is cut and extracted. This process is neither repeatable nor accurate. Since the operator is exposed to UV light and is handling hazardous chemicals with a sharp object, health concerns are also a major issue.

An automated process can solve these problems and is clearly needed. At the time of the design of the excising system, no automated system was available commercially. However, Bio-Rad Laboratories has recently developed an automated spot cutter. The Bio-Rad system has similar features to the one proposed in this thesis, namely it excises protein spots from a gel using a mechanical cutter and deposits them on a microtiter plate. The specifications for the Bio-Rad machine are listed in Appendix D4. There are three main differences between the two systems:
1. The Bio-Rad system was specifically designed for excising protein spots from a 2-D electrophoresis gel. It employs a rotating circular blade to cut the gel. The cutting tip has a round shape because in two-dimensional gels, the samples' shape is round. The gels used in the purification of bio-molecules are usually one-dimensional, and the bands have a rectangular shape. In contrast, the proposed system utilizes rectangular cutting tips. Cutting tips of any shape can be mounted on the holder of the designed machine, whereas the Bio-Rad machine has to utilize round tips because of the rotating cutting motion.

2. The Bio-Rad system uses a 1mm-diameter cutting tip that needs to be manually mounted on the machine by the operator. If a large spot needs to be excised, then multiple cuts are necessary. Since a round cutter is used and as specified in the instructions, overlapping the cuts is not recommended, the samples between the cut circles will not be extracted. On the other hand, the proposed system automatically loads different sized cutting tips depending on the size of the band to be excised. Since the cutter shape is rectangular, multiple cuts next to each other will fully extract the desired sample.

3. In the Bio-Rad system, the cutting tip height needs to be calibrated manually by the operator to ensure that the gel is cut to the full depth. This calibration is not necessary in the proposed design because, as explained in Section 3.5.4, a feedback system senses when the cutter touches a hard surface. This acts also as a safety feature since the cutter will stop if an obstacle is encountered.

3.5.3 Possible Designs

In the design of the automated cutting system, the cutting method was first selected because the rest of the design is dependent on it. The cutting device has to excise a band out of an agarose or polyacrylamide gel. The gel has the consistency of gelatin. It is flexible yet brittle. Figure 3.40 shows different possible methods to cut the gel:
Figure 3.40: Potential design of the cutting device using a:
a) stamp, b) scoop, c) cutting blade, d) roller, e) wire, f) vibrating wire, g) air or water jet, h) laser

The first method consists of using a stamping device as shown in Figure 3.40a. This device can excise the gel in one step, but has a fixed size, so different cutting tips of various sizes are needed. The scooping device shown in Figure 3.40b can excise a band of fixed width and varying length. A different cutter is needed to cut the end pieces of the band. All the other designs can cut a band of any size, however a secondary operation is required to extract the band from the gel. Figures 3.40(c) and (d) show respectively a blade cutter and a cutting roller. The roller uses a serial process to cut the gel whereas the blade uses a parallel one. This means that less force is required to cut the gel for the roller system than the blade. However, the roller will make a longer cut than the desired band size because of its round external shape. Another possible design consists of a thin wire passing between two very thin tubes as shown in Figure 3.40e. This design has several problems. It is difficult to obtain a high tension in the wire because the tubes holding the wire cannot support a large bending moment. The tube size will cause indentations at the corners of the cut that could result in an incorrect band shape. The subsequent design shown in Figure 3.40f consists of a sharp wire vibrating in the ultrasonic range. The high frequency vibration will create a crack that will propagate in front of the sharp edge of the wire. It has the advantage that the wire stays clean because the high vibration will prevent contaminants from sticking to the blade. All the methods stated previously involve
direct contact between the cutter and the gel. Two other methods, laser and air/water jet cut the
gel without being directly in contact with it. See Figure 3.40g,h. These have the advantage of
eliminating cross contamination between different cuts. The laser cuts a material by sublimating
parts of it, and consequently the gel near the cutting point is heated during the process. The gel
is heat sensitive; it shrinks significantly when heated. Moreover, this heat could damage the
biological sample contained in the gel. These issues are especially important for thicker gels.
Therefore, a cooling method is necessary if a laser is used to cut the gel. The water and air jet
methods involve sending high-pressure jets onto the gel to cut it. Since the gel is placed on a flat
surface, the cutting fluid is trapped under the gel. This could cause the gel to move and some of
the biological sample to be washed away from the gel.

From the methods presented, the mechanical stamping arises as the method of choice. It has
the advantage of excising (cutting and extracting) the desired band in one step. It is also the
simplest method, which means that it is more reliable and less expensive.

3.5.4 Design of the Cutting device

Figure 3.41 shows the final design of the cutting device. Assembly and detail drawings are
presented in Appendix D1 and D2 respectively.

The cutting device is composed of four subassemblies, main base, motor base, cutting tip
holder, and the cutting tip. The operation of the cutting device is as follows. During the excision
part, a band that needs to be excised is placed under the cutter. The motor base and the cutting
holder start moving downward. Once the band has been cut and the end of the cutting tip has
reached the gel tray surface, the cutting tip and its holder stop moving. However, the motor base
keeps moving down until the cutting tip holder activates the limit switch attached to the motor
base. Then, the motor base stops and moves upward to its original position. To deposit the
sample into a storage vial, the cutting device is moved to the storage area and is placed above an
empty vial. The motor base and the cutting tip move downwards until the bottom limit switch on
the main frame is activated. Then, the assembly stops and pressurized air expels the sample out
of the cutting tip.
The cutting device’s specifications are as follows:

- Vertical motion of 1.5-inches
- Limit switches for sensing end positions, and sensing if cutting tip is touching gel tray
- Sample expulsion by pressurized air
- Different cutting tips for different band shapes

A vertical motion of 1.5-inches is sufficient since all the devices that the cutting tool is interacting with are within this distance range. A voice coil was first selected as the vertical actuator because of its simplicity and accuracy of motion. However, due to the high price of a voice coil and controller system, this design was discarded. Instead, the proposed design uses a Digital Linear Actuator (DLA) from Thomson Airpax Mechatronics as shown in the figure. DLA is a modified rotary stepper motor. The rotor is internally threaded so that it mates to a fixed lead screw shaft. When the rotor turns, the motor moves along the shaft. This design has the advantage that it uses a minimum number of components since the shaft and motor are
included in one package. The selected DLA is a L92121-P12. It has a 1.875-inch travel distance with a 0.002-inch resolution and a 26-ounce maximum force. The complete specification of the L92100 motor and its driver is presented in Appendix D3. The lead screw shaft is connected to the main base by a spring metal plate as shown in Figure 3.41. This provides flexibility to the shaft to compensate for any misalignment between the shaft and the rotor.

Two linear slides from IKO, model BSP 10-45 SL and BSP 10-25 SL, are used to hold respectively the motor base and the cutting tip base. The BSP series slide is a lightweight and compact stainless steel rolling glide. It has an accuracy of 0.004mm in the vertical direction and 0.008mm in the lateral direction.

Three limit switches are necessary for the proper operation of the cutting device. Two limit switches on the main base initialize and sense the maximum positions of the motor base. The third limit switch, which is not shown in the figure, is attached to the motor base and sense when the cutting tip base is touching the gel tray. This limit switch adds two important features to the designed cutting tool. No calibration of the vertical position of the cutting tip relative to the gel tray is needed since the cutting tip will automatically stop once it has reached the gel tray surface. Furthermore, this is also a safety feature because if the cutting tip hits an unexpected obstacle while moving downward, it will stop automatically. The force required to move the cutting tip holder relative to the motor base is determined by a spring located between the two frames as seen in Figure 3.41. The spring has to be stiff enough so that the cutting tip is able to cut the gel, but not too stiff to avoid scratching the gel tray.

The expulsion of the sample from the cutting tip is done using pressurized air. A flexible tube is connected to the back of the cutting tip holder and a small hole passes the air through the cutting tip and its holder to the inside of the tip.

The cutting tip shown in Figure 3.42 is magnetically attached to its holder. A precise alignment of the cutting tip on its holder is made possible by two guide pins on the holder that fits in the position holes of the cutting tip. In order to accommodate the different sizes and shapes of the bands to be excised, cutting tips with different blade sizes are used.
The tip of the cutter is made of extra sharp stainless steel blades of 0.009-inch thickness. The blade cover was removed and the blades were cut into strips to form the walls of the tip. The strips were then glued together using epoxy. These tips were made only to prove the concept. For the final design, a different process is needed to make the rectangular tips. This issue is discussed in more detail in the next section.

3.5.5 Results and Recommendations

An experiment was conducted to check the ability of the cutter to excise and expulse a band from an electrophoresis gel. For this experiment, the cutting tip had an internal dimension of 1mm x 5mm. Two agarose gels of 1mm and 6mm respectively were tested to check the influence of gel thickness on the cutting process. To expel the band from the cutting tip, a syringe was connected to the air tube. In this test, the cutter was moved manually onto the gel. The results of this experiment are as follows.

- The cutter was able to cut a band for both the 1mm and the 6mm gel. However, it was observed that when the cutter came in contact with the gel surface, the gel surface compressed first for a short vertical distance vertical of around 0.1-0.2mm, and then the blade cut through the gel. This phenomenon did not affect either the shape or integrity of the gel and the extracted band. If necessary, this behavior could be avoided by inducing small ultrasonic vibration in the blade with a piezoelectric actuator. The ultrasonic vibrations would facilitate the cutting process and also prevent small unwanted-particles to stick to the cutting tip.
The cutter extracted the 6mm thick bands, whereas the 1mm bands were not always pickup from the gel. An examination of the razor blade can provide some insight into this observation. The razor blade is sharpened on both sizes as seen in Figure 3.43a. The height of the edge is 1mm, which is the same height as the gel. Therefore, the band is being pushed outward by the walls. To eliminate this problem, a cutter that is only sharpened on the outside is necessary as shown in Figure 3.43b. Such a component can be made – either of plastic or metal – by the extrusion of a hollow rectangular bar and the sharpening of the outside edges.

![Figure 3.43: Cutting tips cross section](image)

To expel the sample out of the cutting tip, the syringe was used to send air inside the tip. It was observed that the bands came out, but remained stuck on one side as shown in Figure 3.44. The location of the air hole causes the observed phenomena. The orifice was placed on the side of the cutter and therefore the air only pushed one side of the band.

![Figure 3.44: Band expulsion test result](image)
To improve this design, the air path needs to be changed so that the air jet is pointed towards the center of the band. The location of the air orifice on the top cannot be changed because the magnet has to be close to the center to ensure a good connection between the cutting tip and its holder. The two possible modifications to the orifice location are shown in Figure 3.45. The first one is shown in Figure 3.45a, and consists of inclining the air orifice so that the air jet is pointing toward the center of the cutting tip. In the second modification, as seen in Figure 3.45b, the cutting tip is moved to the side so that it is centered on the air orifice. The orifice diameter should also be larger to increase the volumetric flow rate of the air entering the cutting tip.

![Diagram showing two modifications: (a) Inclined air orifice and (b) Off-center cutting tip.]

Figure 3.45: Possible modifications of the cutting tip expulsion system

- It was also observed that, when pressurized, some air leaked from two places in the device: the sharp end of the cutting tip and the interface between the cutting tip and its holder. The blade being sharpened on both sides causes the leak at the cutting tip end. This problem will be resolved by using the new type of cutting presented in Figure 3.43b. Improving the joint design between the cutting tip base and its holder can minimize the leak at the interface. This could be accomplished with a gasket.

In conclusion, the mechanical stamping approach is effective in excising a band out of an electrophoresis gel. Some of the proposed changes need to be reviewed and implemented to the current design to improve its effectiveness and reliability.
3.6 Design of the Cutter Transportation System

Once a desired band is excised from the gel, the cutter system needs to be moved to the storage area in order for the band to be deposited there. An automated transportation system for the cutting tool is presented in this section.

3.6.1 Requirements

The purpose of the transportation system is to quickly move the cutter between the different stations. The following are its requirements:

- Able to carry the cutting tool between stations.
- Capable of reaching a maximum speed of 6-inch per second.
- Having a linear resolution of 0.5-millimeter.

The only process in the entire machine that need high precision placement is the band excision process. The XY stage provides a positional accuracy of 0.075mm, which is ample for that process. Therefore, the transportation system can have a coarser precision. An accuracy of 0.5mm is selected since that will allow a repeatable placement of the cutter at each station. Having a lower precision has the advantages of being able to reach higher speeds.

3.6.2 Possible Designs

There are three major designs for transportation systems, pulley and belt, lead screw, and rack and pinion. Figure 3.46 illustrates them.

![Figure 3.46: Transportation systems: a) pulley-belt, b) lead screw, c) rack-pinion](image)

The pulley and belt system is used in application where it is needed to move a light object rapidly between two points. See Figure 3.46a. It is an efficient way of moving an object for short travel distance but not for long travel distance. A long belt results in an increase in backlash, and a decrease in positioning accuracy due to the belt flexibility. On the other hand,
the lead screw system, shown in Figure 3.46b, can have high resolution, but then moves slowly. It is also a more complex system since the lead screw has to be properly supported. The rack and pinion system is shown in Figure 3.46c. Its performance lies in between the two previous systems. It has a good balance of traveling speed and positioning accuracy that makes it the ideal choice for the proposed automated system.

3.6.3 Design of the Transportation System

The final design of the transportation system is shown in Figure 3.47. The assembly and the detail drawings are shown in Appendix E2. The transportation system is composed of four subassemblies: frame, sliding mechanism, rack and pinion, motor and its holder.

![Diagram of the cutter transportation system](image)

**Figure 3.47: The cutter transportation system**

The cutting device transportation system specifications are as follow:

- Maximum speed of 150 mm/s at 300 pulses per second.
- Linear resolution of 0.5 millimeter.
- Starting acceleration of 150 mm/s².
- Linear distance range of 850 millimeter.
Two types of sliding mechanism can be used in our automated system, a shaft-bushing system, and a rail bearing system. The rail system was chosen because it is a smaller and a more easily implemented system. An AccuGlide miniature linear ball bearing from Thomson Industries, Inc was selected. It provides a smooth, quiet linear motion and is designed for stand-alone application.

The rack, gear, and motor were selected together because they have interdependent characteristics. The pitch for the rack and pinion system needs to be as large as possible to obtain a smooth motion. However, as the pitch increases, the lesser the load that can be applied to the teeth of the rack and gear. A 48 pitch was selected because it has a good balance of gear tooth strength and linear pitch distance. The linear pitch is 1.7mm and the gear has a tooth strength of 122N. The diameter of the pinion gear determines the linear resolution and the speed of the system. No gear trains are used between the motor shaft and the pinion because that would result in a large backlash and a lower efficiency. Instead, the pinion gear is directly connected to the motor shaft. The stepper motor needs to have small step angle to achieve the desired resolution. A 4SQ-120BA34S stepper motor for Thomson airpax mechatronics was purchased. It provides 37mN-mm of torque at 300-pulse-per-second, and has 200-steps-per-revolution. An anti-backlash pinion gear with a pitch diameter of 1.25-inch from Berg, Inc was then purchased to achieve the desired linear resolution of 0.5mm and a speed of 150mm/s. The derivation for the required stepper motor torque and the system specifications are shown Appendix E1.

The motor driver and electronics were designed and are shown in Appendix A4. The motor is controlled through a program developed in C++ builder 4 by a project member. An example of the interface screen is shown in Appendix H1. The program controls the direction and the linear speed of the cutter transportation assembly. Each pulse sent through the D/A card to the motor driver results in a step of 0.5mm. For proper operation of the motor, a ramping scheme was implemented in the software for the pulse rate. The assembly accelerates to cruising speed in about 1.5-second. The pulse rate starts at 60-Hz, and then increases linearly until the desired final speed is reached. The same ramping scheme is also implemented for the deceleration section of the motion. A magnetic reed switch is used to calibrate the system.
3.6.4 Results and Recommendations

The transportation system was built and tested. Two tests were performed to check the repeatability of the transportation system. The first test consisted in calibrating the stage to the home position and then sending it 600mm away. This test was performed 20 times. The stage repeatedly calibrated itself correctly for all the runs. For the position test, 1200 pulses were sent to the driver, and the final location was measured. Out of the 20 runs, 19 times the stage moved exactly 60cm, and once it moved 59.5cm. Therefore, the motor missed one step out of twelve thousands once. The second test consisted of making the transportation system perform a series of movement and checking its position at every location. This test was also successful and it is concluded that transportation system is working properly. In the final design, a position encoder needs to be added to the system to ensure an accurate positioning of the carriage along the track.

During the system assembly, it was observed that the alignment between the pinion gear and the racks is critical for a smooth operation. The system was designed with four racks of 9-inch to allow the user to easily increase or reduce the traveling range of the transportation assembly. However, this greatly complicated the racks alignment process. In the next generation, a one-piece rack should be used instead.
3.7 Cutting Tips Changing Station

Once a desired band is excised from the gel and is deposited in the storage area, the used cutting tip needs to be exchanged with a clean one before the next excision. A cutting tip changing station is presented in this section.

3.7.1 Requirements

The purpose of the cutting tips changing station is to grab the cutting tip from the cutting tool and store it. The main requirements are to:

- Remove the cutting tip from the cutting tool.
- Store at least four cutting tips.
- Store different cutting tip sizes.

The station needs to store different cutting tip sizes so that a wide range of band shapes can be excised. Two cutting tips of each size need to be stored so that if the same size is necessary in successive cuts, one tip is being cleaned while the other is used.

3.7.2 Design of the Tool Changing Station

Two possible mechanisms to remove the cutting tips from the cutter and to store them have been developed: an electromagnetic design and a mechanical clamp.

The electromagnetic design consists of using a strong electromagnet to attract and hold the cutting tip onto the changing station. The electromagnet needs to be placed below the platform that holds the cutting tips. The tool station magnet power has to be large enough to overcome the attractive force between the cutting tip magnet and the cutting tip holder. This design has the advantage of requiring only a few components. However, holding the cutting tip accurately at its stored location might be difficult to achieve when the magnet is not energized. A self-centering platform needs to be used.

The mechanical clamp design consists of physically holding the cutting tip by using a clamp as shown in Figure 3.48. This design has the advantage of ensuring reliable storage of the cutting tip and therefore will be used in the design of the tool changing station.

The final design of the tool storage station is shown in Figure 3.48. The assembly and the detail drawings are shown in Appendices F1. The tool station is composed of four subassemblies: frame, sliding mechanism, solenoid actuator, and locking mechanism.
The operation of the tool changing station is as follows. To drop off a cutting tip, the solenoid is first energized to open the clamping mechanism. Then, the cutting device lowers the cutting tip to be cleaned in an open slot. Once in position, the solenoid is deactivated, thus releasing the sliding arm and allowing it to clamp on the cutting tip. Finally, the cutting device moves back up leaving the cutting tip behind. The sequence to pick-up a cutting tip starts with the cutting device moving down on the desired tool. Then, the sliding arm moves back to release the cutting tip. Finally, the excising device moves upward with the tip attached to its holder.

The cutting tool changing station specifications are as follows:

- Holds five cutting tips.
- Actuated by a pull-type, 12-Oz force solenoid.
- Soft closure motion to ensure a reliable tool grabbing.
- Cutting tips are secured in placed in the default/empowered position.
In the final design, the tool changing station is incorporated with the cutting tip cleaning station. Therefore, the blades of the cutting tip need to protrude under the changing station. For this reason, the base frame is made out of a sheet of clear plexiglass. The transparency of the base will allow the user to see the cleaning process from above. As seen in Figure 3.48, five tools can be stored at the same time. The sliding arm is holding them in place. The arm is made out of delrin material. Delrin is ideal for our application because no bushings are necessary if used to slide on stainless steel and it provides some viscous damping that will slow down the arm motion. When the solenoid is not energized the sliding arm position is controlled by two sets of springs. The back springs push the sliding arm onto the cutting tips to hold them at their respective position and the front springs act as kinematic dampers to slow down the locking motion. The shaft holder position can be changed to adjust the force exerted on the cutting tips by the sliding arms. The solenoid can also be moved to vary the sliding arm traveling distance. As seen in the figure, setscrews with plastic heads are mounted on the sliding arm. Their purpose is to securely hold the cutting tip during its removal from the cutting device. There are also centering grooves both on the base plate and on the fixed part of the clamping mechanism. They ensure that the cutting tip is correctly positioned during the clamping process. The computer controls the solenoid. A transistor type circuit is used for the power switching. A schematic of the electronics is shown in Appendix A4.

3.7.3 Results and Recommendations

The tool changing station was built and tested. During experimental tests, the station repeatedly centered and locked the cutting tips in place. The input voltage of the solenoid for opening and closing the clamping mechanism is shown Figure 3.49. The solenoid can open and release the clamp mechanism quickly. However, this would cause the sliding mechanism to collide with the cutting tip when closing and the solenoid frame when opening. Springs are used to slow down the motion. Consequently, the sliding mechanism impacts are significantly reduced. In the final the clamps opens in 1 second, and closes in around 3 seconds. The closing is intentionally slower. It smoothes the motion ensuring that the cutting tips are properly locked in place.

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The designed tool changing station can hold five cutting tips. That number can be changed by reconfiguring the holes' position. As seen in Figure 3.50, a multiple layer or even a circular pattern could be easily implemented.

![Figure 3.49: Tool changing station solenoid input voltage versus time](image1)

![Figure 3.50: Other designs to increase number of stored cutting tips](image2)
3.8 Cutting Tip Cleaning Station

The cutting tips need to be cleaned between excisions. They are removed from the cutting device and stored at the tool storage station. The blades of the cutting tip extrude by 9mm under the base plate of the tool station. This allows the blades to be cleaned by the cleaning station. A cutting tip cleaning station is presented in this section.

3.8.1 Requirements

The requirements of the cleaning station are simple, yet very difficult to achieve. They are:

- To completely remove contaminants from the cutting tip blades,
- To leave the cutting tip free of cleaning chemicals,
- To have a short cleaning cycle.

Each band in the gel contains different molecules. Therefore, it is critical to avoid sample cross contamination between excision of different bands. Since the cutting tips are used repeatedly, they need to be thoroughly cleaned. The cleaning process has to remove all traces of gel from the cutting blade as well as removing any chemicals such as the gel buffer. To reduce the excision cycle time, the cleaning process should be as short as possible.

3.8.2 Possible Cleaning Methods

Five different cleaning methods have been reviewed for the cleaning station: external spray, spray in cutting tool, liquid jet, ultrasonic wave, vibrating tool. Figure 3.51 illustrates them.

The spray system is shown in Figure 3.51a. It consists of three nozzles to disperse the cleaning fluid on the cutting blade. The contaminated fluid is then collected at the drain and is recycled by passing it through a filter for reuse. In this system, the nozzles are located in the cleaning station. They could also be placed in the cutting tip itself as shown in Figure 3.51b. Two nozzles spray on the outside of the tip, and one sprays in the inside. The advantage of this method is that the inside nozzle can more effectively clean the inside of the cutting tip than the external nozzle of the previous design. This is because with the internal nozzle, the liquid enters from the top and exits at the bottom, whereas with the external nozzle, the liquid enters and exits at the bottom. However, placing three nozzles on the cutting tip is difficult due to its small size. An alternative approach would be to combine the two methods by using one nozzle on the cutting tip spraying the inside and two other external nozzles for the outside of the tip.
Figure 3.51: Different cutting tip cleaning methods

The next three methods involve the immersion of the tip in the cleaning fluid. The liquid jet system is shown in Figure 3.51c. A nozzle creates a turbulent flow that cleans the blade. This method is simple to implement. However, the jet power might not be sufficient to fully clean the cutting tip. The liquid jet coming out of the nozzle is dispersed quickly because it is passing through a liquid with the same viscosity. An alternative would be to create a turbulent flow around the cutting blade by vibrating the cutting tip itself as shown in Figure 3.51d. The cutter’s stepper motor could be used for this purpose. For this method to be effective, the cutter needs to be vibrated both horizontally to clean the outside of the tip and vertically to clean the inside walls. The final method, ultrasonic cleaning, is shown in Figure 3.51e. Ultrasonic cleaning can remove contaminants such as buffing compounds, dried blood, oil, greases, and surface debris. This method can be used on a variety of materials such as metals, glass, plastic, and ceramics. It can penetrate and thoroughly clean microscopic crevices. Of all the presented methods, ultrasonic cleaning is the most appropriate method for this tool because of its cleaning efficiency and effectiveness. Therefore it is the selected method in the design of the cleaning station.
3.8.3 Design of the Cleaning Station

An understanding of the ultrasonic cleaning process is necessary to properly implement such a system. Therefore a brief explanation is now presented. For more details, please consult appropriate literature such as References [12] and [13].

A typical ultrasonic cleaner is shown in Figure 3.52. A piezo-electric transducer is attached to the bottom of the tank and is used to send high-frequency sound waves in the 20-40 kHz range through the liquid. The pressure fluctuation causes micro-sized vapor bubbles to form in the liquid. Since there is insufficient energy in the liquid to sustain the vapor state of the bubbles, they collapse violently. This phenomenon is called “cavitation”. When collapsing, the pressure in the micro-bubble is on the order of 500atm and the temperature ranges from 5000°C to 10,000°C. When the micro-bubble collapses, it creates a jet of about one-tenth the bubble size with speeds of up to 110m/s. When this happens next to a contaminated surface, the jet removes the unwanted molecules from the surface. Because of the small jet size and high energy released, the cleaning solution can easily penetrate and clean small crevices and tightly spaced parts. [12]

Three main parameters affect the effectiveness of the ultrasonic cleaning process: the cleaning chemical, solution temperature, and solution degassing.

The selection of the cleaning chemical is essential to the cleaning process. The chemical has to be capable of removing the contaminants and also be compatible with the material being cleaned. Most cleaning chemicals can be used with ultrasonic cleaning.

The temperature of the solution is an important factor in optimizing the cavitation process. In general, a rise in temperature will increase the cavitation intensity, and consequently will result in better cleaning. However, the temperature should always be below the boiling point of the solution because the cavitation intensity will be significantly reduced as the solution starts boiling. For instance, the cavitation effect is maximized at around 71°C for a water solution.

The cleaning solution should be degassed to increase the cavitation intensity. When a cavitation bubble forms, any dissolved gas in the solution will diffuse into the bubble. As the bubble collapses, these gases will create a visible bubble since they cannot diffuse fast enough into the liquid. This effect reduces the cavitation intensity. However, as the sonification continues, these bubbles of gas will coalesce and rise out of the liquid thus degassing the
solution. Therefore, the solution should be degassed prior to the cleaning process by operating the ultrasonic cleaner until no bubbles are seen rising to the surface of the solution. [13]

In summary, ultrasonic energy can effectively and efficiently clean the cutting tips. A model UC-1 ultrasonic cleaner from Electrowave Corporation was purchased and is shown in Figure 3.53. This unit was selected because it has the smallest tank capacity found. It has a capacity of 270-mL and a peak power of 100-Watts.

![Figure 3.52: The UC-1 ultrasonic cleaner](image)

### 3.8.4 Results and Recommendations

The ultrasonic cleaner was tested to check its cleaning effectiveness. The goal of this experiment was to mimic its future application. Two different solutions were tested: distilled water and a general-purpose aquasonic cleaning solution from VWR Scientific. The cleaning solution was selected because it is compatible with a variety of substrates, especially stainless steel and aluminum, and because it is environmentally friendly. The same stainless steel razor blades that are used to make the cutting tip are used for the substrate. The project sponsor suggested using writing inks as contaminant samples since they can be easily visualized. Five different contaminants were tested on the blades:

- Black ink from a ball pen
- Black ink from a permanent marker
- Red ink from a dry erase marker
- Graphite particles from a #2 pencil
- General purpose oil

The first three were selected to test different types of ink. Graphite left by a pencil mark is made up of many small particles, which provides a fourth test contaminant. Oil is used as a test
contaminants to check the ability of the system to remove external contaminants such as fingerprints.

Two identical groups of five blades were prepared: one for the solvent test and one for the water test. Four spots of the same contaminant were deposited on each blade in a group. The blades were then allowed to dry for 3 hours. The five blades were mounted on a plastic support and placed in the tank of the ultrasonic cleaner with the correct amount of cleaning solution as shown in Figure 3.52.

The blades cleaned with the solvent and water solutions for each contaminants are shown at various time throughout the cleaning process in Table 3.5 and 3.6

<table>
<thead>
<tr>
<th>Black ink from a ball pen</th>
<th>Solvent Cleaning Solution</th>
<th>Water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>1 minute</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>3 minutes</td>
<td>6 minutes</td>
</tr>
<tr>
<td></td>
<td>3.5 minutes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Black ink from a permanent marker</th>
<th>Solvent Cleaning Solution</th>
<th>Water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>1 minute</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>3 minutes</td>
<td>6 minutes</td>
</tr>
<tr>
<td></td>
<td>6 minutes</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.5: Results of the ultrasonic test for different solution
<table>
<thead>
<tr>
<th>Red ink from a dry erase marker</th>
<th>Solvent Cleaning Solution</th>
<th>Water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>1 minute</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>3 minutes</td>
<td>6 minutes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Graphite particles from a #2 pencil</th>
<th>Solvent Cleaning Solution</th>
<th>Water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>1 minute</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>3 minutes</td>
<td>6 minutes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>General purpose oil</th>
<th>Solvent Cleaning Solution</th>
<th>Water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>&lt;30 seconds</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 minutes</td>
</tr>
</tbody>
</table>

Table 3.6: Results of the ultrasonic test for different solution
Two observations can be made from this experiment.

First, the solvent solution unlike the water solution was able to remove all the contaminants from the blades. The water did however have a limited effect on the blade with the oil contaminant. This confirms the importance of the solution in the cleaning process. The cavitation effect can be pictured as a microscopic scrubbing effect. Its purpose is to accelerate the cleaning cycle and to increase the cleaning capabilities.

Second, in the solvent solution experiment, the time required to clean the blades is dependent on the type of contaminant used. The oil was removed under 30 seconds. The dry erase ink and the graphite were removed in about 1 minute. The permanent ink and the ball-point pen ink took about 3 minutes to be extracted from the blade surface.

A second experiment was developed and performed to check if the cleaning time could be reduced further. It consists of not using the plastic blade mounts and instead placing the blade directly on the bottom of the tank so that it touches the tank's vibrating surface. The results are shown in Table 3.7.

<table>
<thead>
<tr>
<th>Before sonification.</th>
<th><img src="image_url" alt="Image" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines are from left to right:</td>
<td>ball pen, permanent marker, dry erase marker, pencil, and oil</td>
</tr>
<tr>
<td>After 30 seconds of sonification</td>
<td><img src="image_url" alt="Image" /></td>
</tr>
<tr>
<td>After 2 minutes of sonification</td>
<td><img src="image_url" alt="Image" /></td>
</tr>
</tbody>
</table>

Table 3.7: Test result for blade cleaned while touching the bottom of the tank

As seen in Table 3.7, the cleaning cycle time in this set-up is about half as long as the set up using the mounts. One possible reason for this observation is that by touching the bottom surface, the blade vibrated thus accelerating the cleaning process.
In conclusion, the ultrasonic cleaner was successful in cleaning the cutting blades. However, a chemical based solution needs to be used to effectively remove chemical contaminants from the cutting tip. This means that the cutting tip needs to be rinsed after being cleaned to remove the cleaning solvent. Water can be used for this purpose. To accommodate for this new requirement, two beakers need to be placed in the tank of the ultrasonic cleaner, one filled with a solvent cleaning solution, and the other with a rinsing solution. Figure 3.53 shows such a set-up.

Figure 3.53: Ultrasonic cleaner assembly using one beaker for cleaning and one for rinsing
3.9 Temperature Controlled Sample Storage Station

Once a band is excised from the gel, it needs to be stored until it is needed for further analysis. A temperature controlled storage station for the excised samples is presented in this section.

3.9.1 Requirements

The main requirements of the sample storage station are:

- Storage for at least 20 different samples,
- Temperature controlled from 4-90°C,
- Minimal possible size.

The storage station has to be able to store different bands in individual container since the user might be interested in excising several bands from the gel. Depending on the type of post-analysis desired, the sample needs to be stored at a specific temperature.

3.9.2 Design of the Temperature Controlled Storage Station

The operating temperature of the storage station ranges from below to above the ambient temperature. The simplest and most efficient method of achieving this consists of using Thermoelectric Coolers (TEC). TEC or Peltier coolers are solid-state heat pumps. A schematic diagram is shown in Figure 3.54. Applying a DC voltage across its leads will cause the heat to move from one side of the TEC to the other. Changing the polarity will result in the heat moving in the opposite direction. Consequently, a TEC can be used for both cooling and heating.

![Figure 3.54: Schematic of a thermoelectric cooler](image)
A typical thermoelectric module consists of a series of P and N doped bismuth-telluride semiconductor material sandwiched between two ceramic plates. The N type material has an excess of electrons, and the P type material has a deficit of electrons. A pair of each makes a couple and there are between one and a few hundred couples in a TEC. When an electron moves from a P to an N type material on the cold side, it jumps to a higher energy state thus absorbing some thermal energy. On the hot side, the electron moves from an N to a P type material, dropping to a lower energy state. This results in a release of thermal energy to the heat sink.

The final design of the temperature controlled storage station is shown in Figure 3.55. The assembly and technical information for each component are presented in Appendix G2 and G3. The station is composed of three subassemblies: the thermoelectric coolers system, the storage enclosure, and the power supply.

![Figure 3.55: The temperature-controlled sample storage station](image)

Two 127-couple, 8.5-amp thermoelectric cooler modules from Ferrotec Inc. were purchased. A model 5C7-350A temperature controller from Oven Industries, Inc. is used to control both modules. It provides proportional and integral control of the temperature within a range of -20°C to 100°C. Two CPU cooling fans with heat sinks are used to dissipate the heat from the thermoelectric coolers. The hot side of the thermoelectric module is bonded to the heat sink with
a thermally conductive epoxy. The cold side is coated with thermal conductive grease and is clamped to the stainless steel pan. The DC power supply is from Power-one Inc. and can output up to 28-volts at 6-amps.

The enclosure is composed of a stainless steel pan insulated on the outside. A model TS67-178 thermistor from Oven Industries Inc. was bonded to the side of the pan. It provides the temperature feedback for the controller. Expanding polyurethane foam was applied around the sides and part of the bottom of the pan to insulate it. An aluminum rack that can hold up to 24 1.5-2.2 ml micro-centrifuge tubes is mounted inside the pan.

The calculations for the storage system parameters are shown in Appendix G1.

3.9.3 Results and Recommendations

The temperature controlled storage station was tested under various conditions to check its effectiveness. Only one of the two cooling modules was used for these tests.

The first test consisted of measuring the system response to a step input. While at room temperature, the temperature control knob was set to its minimum. The temperature measurements were made at the bottom of the pan directly above the module location. Once the system stabilized, the knob was set to its maximum. The results are shown in Figure 3.56.

As seen in Figure 3.56, the response has the characteristic of a second order system. The rise time was approximately 10 seconds and the settling time was around 50 seconds for both the
powering-up and powering-down of the module. The oscillations in the temperature are caused because the module was only in contact with ambient air and thus it had a small heat load. When powering-down, some of the heat from the heat sink is transferred by conduction to the cold side of the module. This results in a peak temperature that is above the room temperature, as seen in the powering-down response.

It should be noted that during the test, the minimum temperature the system achieved for a prolonged period of time was 0.5°C. However, theoretically, the module should be able to reach -20°C. This discrepancy is caused by the inability of the system to dissipate the heat generated by the module fast enough. There are two solutions to this problem. First, replacing the current heat sink and fan by higher performance components would increase the heat dissipation. This would result in a lower temperature at the hot side of the module and therefore a lower temperature at the cold side. Second, two modules could be stacked on top of each other so that one of the module is used to cool the other one. In this set up, the modules would be connected thermally in series and electrically in parallel.

The second test consisted of measuring the temperature at a height of 10mm from the bottom of the pan. The test was performed with three different mediums in the pan: still air, 20-mm of still water, and 20-mm of continuously mixed water. The results are shown in Figure 3.57.

![Cooling test for different heat transfer materials](image)

**Figure 3.57:** Temperature at a height of 10-mm with different heat transfer materials
As seen in Figure 3.57, the temperature of the mixed water medium dropped the fastest, followed by the still water medium and finally by the still air medium. This result is to be expected since water has a higher thermal conductivity than air. Mixing the medium also accelerates the cooling because it increases the heat transfer coefficient. However, for all three set-ups the rate of cooling is relatively slow. The mixed-water cooled from 22°C to 15.7°C in 12 minutes. This is not fast enough for the desired application. One possible approach to improve the performance is to look at the thermal properties of the materials used. A few selected material properties are shown in Table 3.8.

<table>
<thead>
<tr>
<th>Material</th>
<th>Density $ gm/cm^2 $</th>
<th>Thermal conductivity $ Watts/m-\circ C $</th>
<th>Specific heat $ cal/gm-\circ C $</th>
<th>Thermal expansion coefficient $ Cm/cm/\circ C $</th>
</tr>
</thead>
<tbody>
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Table 3.8: Selected material property at 21°C

For the temperature-controlled station, the heat transfer between the module and the stored sample needs to be maximized. Therefore the medium used to transfer the energy has to have a high thermal conductivity to increase the heat transfer. A low specific heat is also important to reduce the time required to change the temperature of the medium. As seen in Table 3.8, air is a thermal insulator and water needs a lot of energy to change its temperature. Aluminum is a better choice since its thermal conductivity is 330 times greater than water and its specific heat is one-fifth that of water.

A third test was run to check this hypothesis. An aluminum block was placed in the pan, and the temperature was measured at a 10mm height from the bottom. The system was first turned on to cool the block, then, once the temperature had settled, the module power was turned off. The results are shown in Figure 3.58. A heating test was also performed. The thermocouple power supply was turned on and the temperature was measured at the same location used for the cooling test. Once the system settled, the power supply was turned off and the temperature was recorded for 20 minutes. The results are shown in Figure 3.59.
Cooling test with an aluminum block
Temperature measurements at 10mm from bottom

Heating test with the aluminum block
Temperature measurements at 10mm from bottom

Figure 3.58: Cooling test, temperature at a height of 10-mm with an aluminum block

Figure 3.59: Heating test, temperature at a height of 10-mm with an aluminum block
As seen in Figure 3.58, the system performance significantly improved with the use of the aluminum block as the heat transfer medium. The set-up with mixed water reached 16°C in 12 minutes, whereas this set up reached 4°C in 4 minutes. The settling temperature was 3°C, which is close to the 2°C temperature at the surface of the pan.

For the heating test, the temperature reached 100°C. The rise time and settling time for heating the block were 2.5 minutes and 4.5 minutes respectively. These results are similar to the ones observed for the cooling test.

The results of these tests suggest that an aluminum block needs to be used to hold the storage tubes since it significantly improves the performance of the system.

In conclusion, the first prototype of the temperature controlled storage station is working well. In future work, the heat sink performance must be improved by implementing one of the two aforementioned solutions. The tank is not needed and should be replaced with an aluminum block that is machined to fit the micro-centrifuge tubes.
Chapter 4

Conclusion

The first part of this thesis presented a stain free detection system that can be used for the purification of a variety of biologically active macromolecules. The method is based on direct UV absorption and takes advantage of the latest development in CCD technology to obtain high detection sensitivity.

The second part of the thesis presented six main components of the robotic system: a variable ultraviolet light source, a gel cutting device, a cutter transportation system, a cutting tip changing station, a cutting tip cleaning station, and a temperature controlled sample storage station.

The variable light source was developed to allow for a precise selection of the target molecules to be visualized. It outputs a uniform, collimated beam with a 32mm cross-sectional diameter and a 15nm bandwidth over the 200-400nm light spectrum range. The light source can be used either as a monochromatic light source to visualize the location of a specific molecule, or as a scanning instrument to obtain the absorption spectrum of a bio-molecule. The light source meets the functional requirements, however its performance could be improved. The use of a larger grating could increase the throughput. The bandwidth of the light output beam could be reduced by either using baffles or by replacing the lamp with a projecting type lamp. Finally, the effects of varying the slit height and width on the output beam characteristics need to be investigated.

The mechanical cutting device can excise bands of various shapes and sizes from an electrophoresis gel. It has a 1.5 inch vertical travel and automatically senses when a cut has been made. The sample is expelled from the cutting tip using pressurized air. It is recommended to change the cutting tip blades by ones that are sharpened only on the outside to ensure the pick-up of the band from the gel. The sample expulsion needs to be improved by changing the location of the pressurized air orifice.

The transportation system was developed to move the cutting device between the different stations. The system has a 0.5mm linear resolution and an 850mm traveling distance. The
moving assembly can travel at speeds of up to 150mm/s. In the final design, a one-piece rack should be used to facilitate assembly. A position encoder should be added to the system to ensure an accurate positioning of the carriage.

The cutting tip changing station removes the excision tip from the cutting device and stores up to five tips. In the next prototype, the base platform needs to be redesigned to fit on top of the cleaning station.

The cutting tip changing station removes the excision tip from the cutting device and stores up to five tips. In the next prototype, the base platform needs to be redesigned to fit on top of the cleaning station.

The cleaning station uses ultrasonic energy to effectively and efficiently cleans the cutting tips. The cleaning is done in two stages. First, a solvent solution removes contaminants from the blades. Then, a rinsing solution is used to remove the solvent from the blades. In future work, a filtering system could be added to the station to recycle the cleaning solutions.

The temperature-controlled station uses solid-state air-cooled thermoelectric devices. It can store 24 micro-centrifuge tubes and has a temperature range of 4°C to 100°C. A lower temperature can be achieved by using a higher performance heat dissipation system.

In conclusion, the proposed system has been successfully designed and implemented. However, it is the first prototype. Additional features need to be incorporated prior to commercialization.
Bibliography

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Additional References


Appendix A
Figure A1: The designed automated purification system for biologically active macromolecules
Figure A2: Pictures of selected components
(See Appendix G3 for pictures of the temperature-controlled storage station)
## A2 Parts List

### Light Source

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<td>SS set-screws with nylon tip 4-40 thread, 3/8&quot; length</td>
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<td>Clamp- split type 1/8&quot; bore</td>
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<td>Precision ground shaft 1/8&quot; diam., 12&quot; long, stainless steel 303</td>
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<td>A1-120</td>
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<td>Holder base plate</td>
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<tr>
<td>Delrin arm</td>
<td>TH102</td>
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<td>Shaft holder 1</td>
<td>TH103</td>
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<td>Shaft holder 2</td>
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<td>Shaft</td>
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# Temperature Controlled Sample Storage System

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<td>Thermoelectric module</td>
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<td>6300/127/085A</td>
<td>Ferrotec America</td>
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<td>Module controller</td>
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<td>5C7-350A</td>
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<td>Temperature sensor</td>
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<td>TS67-178</td>
<td>Oven Industries</td>
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<td>Cooling fans + Heat sinks</td>
<td></td>
<td>2730246; 2730248</td>
<td>RadioShack</td>
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<td>Silicone grease</td>
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<td>2761372</td>
<td>RadioShack</td>
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<td>Stainless steel pan</td>
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<td>4191T13</td>
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<td>3/8-16,2&quot; hex head cap screw</td>
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<td>913009A632</td>
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<td>Polyurethane expanding foam</td>
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<td>8551K11</td>
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<td>Swivel leveling mounts</td>
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<td>6111K83</td>
<td>McMaster-Carr</td>
<td>4</td>
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A3 Vendors Information

Active Electronics,
73 first Street, Cambridge, MA
Tel: (617) 864-3588

Berg
499 Ocean Avenue, East Rockaway, NY 11518
Tel: (800) 232-BERG
http://www.wmberg.com

Digi-Key
Tel: (800) DIGI-KEY
http://www.digikey.com

Edmund Industrial Optics,
Tel: (800) 363-1992, Fax: (856) 573-6295
http://www.edmundoptics.com

Electron Microscopy Sciences,
321 Morris road Ft, Washington, PA 19034,
Tel: (215) 646-1566, Fax: (215) 646-8931

Electrowave Ultrasonic Corporation,
Tel: (715) 426-7378 Fax: (715) 426-7351
Email: info@inter-netco.com
http://www.inter-netco.com

Esco Products Inc.,
171 Oak Ridge Road, Oak Ridge, NJ 07438
Tel: (800) 922-3726, Fax (973) 697-3011
http://www.escoproducts.com

FerroTec America
40 Simon St. Nashua, NH 03060
Tel: (603) 598-7336, Fax: (603) 598-7272

Future Bearings,
15 Walkers Brook Dr, Reading, MA
Tel: (781) 942-9880,
http://www.ikont.co.jp/eg/product/product.htm
Hamamatsu,
360 Foothill road, P.O. Box 6910;
Bridgewater, NJ 08807-0910,
Tel:(908) 231-0960, Fax:(908) 231-1218
http://www.hamamatsu.com/

McMaster-Carr,
473 Ridge Road, Dayton, NJ 08810
Tel:(732) 329-3200, Fax:(732) 329-3772
http://www.mcmaster.com

Newark Electronics,
59 Composite Way, Lowell, MA 01851-5144,
Tel: 978-551-4300, Fax: 978-551-4329
http://www.newark.com

Oriel Instruments,
150 Long Beach Boulevard, Stratford, CT, USA, 06615-0872;
Tel: (203) 377-8282, Fax: (203) 378-2457,
http://www.oriel.com

PIC Design,
Tel: (800) 243-6125, Fax: (203) 758-8271
http://www.pic-design.com

RadioShack,
493 Massachusetts Ave, Cambridge, MA, 02139
Tel: (617) 547-7332

Schneider Optics,
285 Oser Ave, Hauppauge, NY 11788
Tel: (631) 761-5000, Fax: (631) 761-5090

Sciencetech Inc,
45 Meg drive, London, Ontario, Canada, N6E 2V2;
Tel: (519) 668-0131, Fax: (519) 668-0132,
http://www.sciencetech-inc.com/

Thomson Airpax,
Seven McKee Pl., Cheshire, CT 06410
Tel:(203) 271-6444, Fax:(203) 271-6400

VWR scientific,
Tel:(800) 932-5000
http://www.vwrsp.com
Figure A3: Schematic for the 4SQ-120BA34S stepper motor
Figure A4: Schematic for the L92121-P2 stepper motor

Figure A5: Schematic for the solenoid

Figure A6: Schematic for one of the feedback sensor
Appendix B
The SpectruMM:250B is a high-performance digital camera system featuring a Hamamatsu back-illuminated spectroscopic format CCD. The 1024 x 250 imaging array is ideal for general-purpose spectroscopy. Back-illumination and thermoelectric cooling to -35 °C give the SpectruMM:250B the sensitivity and low noise necessary for Raman or weak-fluorescence applications. Its 6-mm height and full 24-mm spectral coverage deliver multistripe capability as well.

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<th>Benefits</th>
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<tr>
<td>Hamamatsu CCD sensor</td>
<td>Delivers industry-standard performance</td>
</tr>
<tr>
<td>1024 x 250 imaging array</td>
<td>Ideal format for general-purpose spectroscopy</td>
</tr>
<tr>
<td>24 x 24-µm pixels</td>
<td>Provides good resolution and excellent full well capacity</td>
</tr>
<tr>
<td>6-mm tall imaging area</td>
<td>Ideal for rapid, multistripe spectroscopy</td>
</tr>
<tr>
<td>Back-illuminated CCD</td>
<td>Offers higher sensitivity for low-photon-flux applications</td>
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<tr>
<td>Optional dual digitizers</td>
<td>High speed provides rapid spectral acquisition</td>
</tr>
<tr>
<td></td>
<td>Low noise provides the best signal-to-noise ratio</td>
</tr>
<tr>
<td>WinSpec</td>
<td>Easy yet sophisticated Windows GUI controls and integrates camera with spectrometer</td>
</tr>
<tr>
<td></td>
<td>Automates data acquisition, analysis, and display</td>
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**Specifications**

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<th>Parameter</th>
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<td>CCD image sensor</td>
<td>Hamamatsu, scientific grade; MPP: back-illuminated; available with UV-enhancement coating</td>
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<tr>
<td>CCD format</td>
<td>1024 x 250 imaging pixels; 24 x 24 μm pixels; 100% fill factor; 24.5 x 6.9 mm imaging area</td>
</tr>
<tr>
<td>Spectrometric well capacity</td>
<td>550,000 e -1</td>
</tr>
<tr>
<td>CCD read noise</td>
<td>8 e- rms @ 150 kHz</td>
</tr>
<tr>
<td>System read noise</td>
<td>10 e- rms @ 100 kHz; 30 e- rms @ 1 MHz</td>
</tr>
<tr>
<td>Nonlinearity</td>
<td>&lt;2% for 16 bits</td>
</tr>
<tr>
<td>Nonuniformity</td>
<td>&lt;±3% over entire CCD area</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>16 bits @ 100 kHz; 12 bits @ 1 MHz</td>
</tr>
<tr>
<td>Scan rate</td>
<td>100 kHz or 1 MHz</td>
</tr>
<tr>
<td>Vertical shift time</td>
<td>10 μs</td>
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<tr>
<td>Spectral rate</td>
<td>50 Hz, full-vertical binning, 100 kHz digitization; 200 Hz, full-vertical binning, 1 MHz digitization</td>
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<tr>
<td>Dark current</td>
<td>&lt;15 e- /p/s @ 30 C</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>-35 °C with forced air circulation</td>
</tr>
<tr>
<td>Thermostating precision</td>
<td>±0.04 °C over entire temperature range; dark change stabilized to ±0.0%</td>
</tr>
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</table>

Note: Specifications are typical and subject to change.
Lens Specification

- **Lens construction:** 3 group 3 element (all QUARTZ LENS)
- **Wave length:** 250nm
- **Focal length:** 77.55mm ± 5%
- **Back focal:** 71.31mm ± 5% (in air)
- **Fringe back:** 17.526mm C-mount
- **Aperture ratio:** F/3.8 ± 5% ~ F/22
- **Image circle:** ø18.0

**Angle of view**

- Image to object distance = Infinity
- Diagonal: 6.64°
- OPT. Distortion: -0.27% (Diagonal)

**Magnification**

- Magnification: -0.3x

**Image to object distance**

- Working distance: 280.4 mm
- OPT. Distortion: -0.13% (Diagonal)

**Aperture of front lens:** ø23.0
**Aperture of rear lens:** ø210

**Coating**

- All surface are antireflection coated (MgF2 single coat)

**Mounting**

- C-mount & T-mount

**Filter thread:** ø49 mm P=0.75

---

**T-MOUNT Thread**

- M4.20 P=0.75
- (101.5) at Mag = -0.3x
- (138.9) at Mag = -0.3x

---

**UNIVERSE KOGAKU (AMERICA), INC.**

**TITLE**

78mm F/3.8 UV IMAGING LENS

**DATE**

9/2/99

**SCALE**

None

**NO.**

UV8040B

**DRAWN**

Approved
B2 XY Stage Specifications

Figure B1: 402 LN Series XY Table from Daedal. [4]

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<tr>
<td>Life @ rated specification X1 million</td>
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<tr>
<td>inches (mm)</td>
<td>10</td>
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<tr>
<td>Positional Accuracy x 0.001 in (mm)</td>
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</tr>
<tr>
<td>over table travel</td>
<td>2.9</td>
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<tr>
<td>Positional Repeatability x 0.001 in (mm)</td>
<td>±0.46</td>
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<tr>
<td>Std Grd</td>
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<tr>
<td>Prec. Grd</td>
<td>±0.076</td>
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<td>Straight Line Accuracy x 0.001 in (mm)</td>
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<td>over total table travel</td>
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<td>Flatness Accuracy x 0.001 in (mm)</td>
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<td>over total table travel</td>
<td>0.93</td>
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<td>Max screw speed (Rpm)</td>
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<td>Std Grd</td>
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<td>Prec. Grd</td>
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<td>Max acceleration (in/sec^2)</td>
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<tr>
<td>Inverted</td>
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<td>Load per bearing (lbs)</td>
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<tr>
<td>Both grades, Normal</td>
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<td>Inverted</td>
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<td>Axial Loading (lbs)</td>
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<td>Prec. Grd</td>
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<td>Input inertia** (lbs-in sec^2)</td>
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<td>Maximum running torque (oz-in N-m)</td>
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<td>Prec. Grd</td>
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<td>Truck Center</td>
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116
Miniature Linear Positioning Systems

402000LN Series Dimensions

---

40mm mount

M6 x 1.0
4 holes

NEMA 23 mount

45mm x 45mm
4 holes

NEMA 17 mount

50mm x 50mm
4 holes

M5 x 0.8
4 holes

NEMA 23 mount

516 mount

4 holes

---

See associated errata sheet for details.
Appendix C

The Light Source

C1 Assembly Drawings
1. Side walls attached to blocks by 4x 6-32, 1/4" button head screws
2. Front wall attached to blocks by 4x 6-32, 1/4" button head screws
3. Cover attached to blocks by 4x 6-32, 1/4" button head screws
4. Back wall attached to blocks by 4x 6-32, 1/4" button head screws
5. Wall block top (3 sides tapped)
5*. Wall block bottom (clearance hole vertical, attached to base by 4x 6.32, 11/16" pan head phillips screws)
6. Heat sink face down (attached to cover by 2x 2-56, 3/8" cap screws with a #2 washer between cover and heat sink)
7. Window (attached to cover with silicon sealant)
Figure C2: Assembly of the light source components #1

1. Grating assembly
   (bolted to base by 2x 1/4"-20, 1/2" screws + 1/4" washers)
2. Lamp assembly
   (bolted to base by 1x 1/4"-20, 1/4" screw)
3. Fan + Filter
   (bolted to base by 2x 6-62, 7/8" screws)
4. Inside wall
5. Collimating assembly
   (bolted to base by 1x 1/4"-20, 5/8" screw + 1/4" washer)
6. Mirror assembly
   (bolted to base by 1x 1/4"-20, 5/8" screw + 1/4" washer)
7. Brackets for inside wall
   (bolted by 2x 6-32, 3/8" screws + 6 washers)
8. Electronic shutter
9. Terminal block
   (bolted to base by 1x 6-32, 3/8" screw)
10. Shutter holder
    (bolted to inside wall by 2x 6-32, 3/8" screws)
11. Slit
    (held in place by 1x 4-40, 3/16" screw + 4 washer)
**45° Mirror Assembly**

1. Rail (2.5")
2. Rail Block (1")
3. Postholder (1" length)
   - (bolted to base with a 1/4"-20,3/8" bolt)
4. Post (1" length)
5. Lamp base
   - (bolted to post with a 8-32,3/8" bolt + #8 washer)
6. Deuterium lamp
   - (bolted to lamp base with 2x 4-40,3/8" bolt + #4 washers)
7. Base fork
8. Post (1/2" length)
9. Mirror + Minimount + 45° adaptor
   - (attached to post with a 8-32,5/8" set screw)
10. Post holder (1.5" length)
    - (bolted to base with a 1/4"-20,3/8" bolt)
11. Post (1" length)
12. Collimator (model 7292)
    - (attached to post with a 8-32,5/8" set screw)

**Collimator Assembly**

**Lamp Assembly**

---

**PARTS LIST**

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<th>PART OR IDENTIFYING NO.</th>
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<td>Aluminum 5052</td>
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**MIT Mechatronics Research Laboratory**

Light Source Assembly Components #2

**CAD GENERATED DRAWING**

- DO NOT MANUALLY UPDATE

**DESIGN**

- Eric Iloaau
- March 200

**SCALE**

- 1:1

**DRAWING NO.**

- LS003

---
1. Base
2. Miniature switch (bolted to base by 2x 0-80,3/8" cap screws)
3. Grating arm (bolted to grating shaft by 1x 8-32,3/8" cap screw and a plastic washer)
4. Counter
5. Base leg #1 (bolted to base by 1x 1/4-20, 1/4" cap screw)
6. Base leg #2 (bolted to base by 1x 1/4-20, 1/4" cap screw)
7. Motor holder bracket (bolted to base by 2x 4-40,3/8" cap screw)
8. Motor holder (bolted to motor bracket by 2x 4-40,3/8" cap screw)
9. Motor (attached motor holder by 2x M3 nut)
10. Flexible coupling
11. Grating holder
12. Grating shaft
13. Bearing
14. Spacer
15. Concave grating
Figure C5: Drawing of the light source cover plate

Plate has a 3/16" thickness

Top View

Bottom View

Material: Aluminum 5052

Light Source Cover

MIT Mechatronics Research Laboratory

Light Source Cover

Eric Horvay March 2001

Dimensions are in inches.

Tolerances are:

- Fractions: ±xxx ±0.005
- Decimals: ±xxx ±0.01
- Angles: ±xxx ±1°

Light Source Cover

Applications do not scale drawing.

Drawing generated by CAD software.
Once
Figure C7: Drawing of the light source sidewall

- Plate Thickness: 1/8"
- 2 Parts are needed

**Dimensions:**
- 4x Ø.150 THRU
- 4.224
- 4.724
- .375
- .250
- 11.300
- 10.550

**Material:** Aluminum 5052

**Specifications:**
- CAD GENERATED DRAWING
- DO NOT MANUALLY UPDATE

**Part List:**

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<th>DESCRIPTION OF PART</th>
<th>MATERIAL</th>
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<td>Light Source Side Wall</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>MIT Mechatronics Research Laboratory</td>
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**Drawn by:** Eric Hoarau
**Date:** March 2001

**Scale:** DESIGNED

**Scale:** 2003

**CAD File:** LS103
The information contained in this drawing is the sole property of MIT and Amana Products. No reproduction in part or whole without written permission is prohibited.

DETAIL A
SCALE 1:1.5

DETAIL B
SCALE 1:1

Plate Thickness: 1/8''

Figure C8: Drawing of the light source back wall

MIT Mechatronics Research Laboratory

Application: Light Source Back Wall

Material: Aluminum 5052

Approvals Date: Feb 2001

Check: Eric Weege

No. ASSY: 104

Sheet: 1

Scale: CAD FILE

Parts List:

<table>
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<tr>
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<th>DESCRIPTION</th>
<th>QUANTITY</th>
<th>MATERIAL</th>
<th>FINISH</th>
<th>NOTES</th>
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<td>LS104</td>
<td>Light Source Back Wall</td>
<td>1</td>
<td>Aluminum 5052</td>
<td>--</td>
<td>--</td>
</tr>
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</table>
Plate thickness: 3/16"
Plate thickness: 3/16"
8 SQUARE BLOCKS NEEDED
* 4 are 6-32 tapped on all sides
* 4 are 6-32 tapped on 2 sides and 6-32 clearance hole on third side

6-32 TAP THRU ON 2 SIDES
6-32 CLEARANCE THRU ON THIRD SIDE

Bottom Wall Block

6-32 TAP THRU ON ALL 3 SIDES

Top Wall Block

MIT Mechatronics Research Laboratory

Light Source Wall Blocks

Material: Aluminum 5052
Figure C15: Drawing of the light source shutter holder

**SECTION A-A**

**SCALE 1:1**

- **2x 8-32 TAP THRU**
- **Ø 1.300 THRU**
- **Ø 2.087 x .177**

Material: Aluminum 5052

Application: Light source shutter holder

**Technical Details**

- Dimensions are in inches
- Tolerances:
  - Fractions: ± 1/32
  - Angles: ± 1
  - Decimals: ± .005
  - Angles: ± 1

**Drawn by:** Eric Hoarau, Feb 2001

**Approved by:**

**MIT Mechatronics Research Laboratory**
Figure C16: Drawing of the light source heat sink.
Figure C17: Drawing of the light source base plate for the deuterium lamp

8-32 Clearance hole

3x 4-40 TAP THRU

Material: Aluminum 5052

Light Source Base for Deuterium Lamp

136
This part is from the 9030 monochromator:
- Base is cut to the specified dimension
- Pocket is machined on the top
- Four tapped holes are machined on the side
- Two tapped holes are machined at the bottom

**Detail A**
- Scale 1:1
- 2x 2-56 TAP \( \Psi = 0.500 \)

**Material**: Aluminum 5052

**Application**
- Do not scale drawing

**Dimensions**
- Tolerances for fractions, decimals, and angles:
  - Tolerance: \( \pm 0.005 \)
This part is from the 9030 monochromator. The arm at the base is cut from the original part to obtain the drawn part.

Figure C19. Drawing of the light source grating holder.
This part is the grating shaft from the 9030 monochromator. The shaft length is reduced from the threaded side of the shaft. The threaded hole is then retapped.

---

**Parts List**

<table>
<thead>
<tr>
<th>Item</th>
<th>Part or Name</th>
<th>Description</th>
<th>Application</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.150</td>
<td>8-32 TAP</td>
<td>( \phi , 0.600 )</td>
<td>Light Source Grating Shaft</td>
<td>Aluminum 5052</td>
</tr>
<tr>
<td>0.250</td>
<td></td>
<td>( \phi , 0.250 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Drawing Information**

- **Title:** Light Source Grating Shaft
- **Scale:** CAD
- **Drawing No.:** LS116
- **Date:** March 2001
- **File:** SHEET OF 1 3 1
- **Approval:** Eric Hoarau
This part is the grating shaft bearing spacer from the 9030 monochromator. The spacer length is reduced to .330".

<table>
<thead>
<tr>
<th>PART NO.</th>
<th>DESCRIPTION</th>
<th>MATERIAL</th>
<th>FINISH</th>
<th>NEXT ASSY USED ON</th>
<th>APPLICATION</th>
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</thead>
<tbody>
<tr>
<td>LS117</td>
<td>Light Source Grating shaft bearings spacer</td>
<td>Aluminum 5052</td>
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<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

DIMENSIONS ARE IN INCHES

TOLERANCES ARE:

- FRACTIONS DECIMALS ANGLES
  - XXX ± .01
  - .XXX ± .005

DO NOT SCALE DRAWING

SPECIFICATION

MIT Mechatronics Research Laboratory

Erik Haarau March 2001

REQ: —

ITEM PART OR IDENTIFYING NO. OR DESCRIPTION SPECIFICATION REV

MIT Mechatronics Research Laboratory

Grating shaft bearings spacer

MLT-117

SHEET 8/7

SCALE COND. 100

DATE

March 2001

DO NOT MANUALLY UPDATE

APPROVALS

RAW NO.

ERIK HAARAU

CHECKED

USP

-END-
Figure C22: Drawing of the light source diffraction grating shaft arm

**Light Source Grating Arm**

- **Material**: Aluminum 5052
- **Scale**: CAD FILE
- **Approval**: MIT Mechatronics Research Laboratory

**Dimensions**:
- \( \phi 0.175 \text{ THRU} \) \( \phi 0.290 \text{ THRU} 0.180 \)
- \( \phi 0.120 \text{ THRU} \)
- \( 4-40 \text{ TAP THRU} \)
- \( 2.300 \), \( 1.500 \), \( 0.250 \), \( 0.624 \), \( 0.312 \), \( 0.203 \), \( 0.062 \)

**Notes**:
- Do not scale drawing
- Do not manually update
- CAD generated
- MIT hoop March 2001
- Eric Haojiu

**Specifications**:
- Material: Aluminum 5052
- Scale: CAD file
- Approval: MIT Mechatronics Research Laboratory

**Parts List**:

<table>
<thead>
<tr>
<th>PART</th>
<th>IDENTIFYING NO.</th>
<th>MANUFACTURER</th>
<th>OF DESCRIPTION</th>
<th>MATERIAL</th>
<th>SPECIFICATION</th>
<th>QTY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Tolerances**:
- Fractions
- Decimals
- Angles

**Remarks**:
- The information contained in this drawing is the sole property of MIT and Alpine Pharmaceuticals. Any reproduction in part or whole without written permission is prohibited.
Figure C24: Drawing of the light source diffraction grating base leg #2

- Material: Aluminum 5052
- Notes: Do not scale drawing
- Scale: CAD file

<table>
<thead>
<tr>
<th>PART</th>
<th>IDENTIFYING NO.</th>
<th>DESCRIPTION</th>
<th>MATERIAL</th>
<th>SIZE</th>
<th>IDWG. NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light Source Grating base leg 2</td>
<td></td>
<td></td>
<td>LS120</td>
</tr>
</tbody>
</table>

DIMENSIONS ARE IN INCHES
TOLERANCES ARE:
- Fractions: ±0.01
- Decimals: ±0.005
- Angles: ±1°

Approvals:
- ERC Hoarau: Feb 2001

Overview:
- Scale CAD file: Sheet of 8
- Table:
  - Material: Aluminum 5052
  - Notes: Do not scale drawing
  - Scale: CAD file
  - IDWG. NO.: LS120
Figure C.26: Drawing of the light source diffraction grating motor holder.

Material: Aluminum 5052

Light source Motor holder

Scale 1.5:1

SECTION C-C

Dimension Tolerances:
- Fractions 
- Decimals 
- Angles

+ .01

邳州Shea
Feb 2001

MIT Mechatronics Research Laboratory

Drawing No.: LS122

Approved by: Eric Hoarau

Drawing Date: Feb 2001

Light source

Motor holder

4x φ.138

R.156

2x φ.125

φ.900

1.654

.610

.827

.610

.827

.063

φ.313

.063

.188
This part is purchased. The small center hole is rebored to a diameter of 5mm to fit on the motor shaft.
### Characteristics

<table>
<thead>
<tr>
<th>Type No</th>
<th>Aperture (mm)</th>
<th>Spectral Distribution (nm)</th>
<th>Required Discharge Starting Voltage Min (Vdc)</th>
<th>Anode Current (mA/dc)</th>
<th>Tube Drop Voltage Typ (Vdc)</th>
<th>Output Stability Drift Max (%/h)</th>
<th>Fluuctuation Max (%p-p)</th>
<th>Heater Ratings</th>
<th>Warm-up Voltage (Vdc, ac)</th>
<th>Current (A/dc, ac)</th>
<th>Time Min(s)</th>
<th>Operation Voltage (Vdc)</th>
<th>Current (A/dc)</th>
<th>Guaranteed Life * (h)</th>
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</thead>
<tbody>
<tr>
<td>L6565</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>2.5 +/- 0.25</td>
<td>4</td>
<td>20</td>
<td>1.0 +/- 0.1</td>
<td>1.8</td>
<td>4000</td>
<td></td>
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<tr>
<td>L6566</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>3.0 +/- 0.3</td>
<td>5</td>
<td>20</td>
<td>0 to 1</td>
<td>0 to 1.6</td>
<td>4000</td>
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<tr>
<td>L6301</td>
<td>0.5</td>
<td>185 to 400</td>
<td>400</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>2.5 +/- 0.25</td>
<td>4</td>
<td>20</td>
<td>1.0 +/- 0.1</td>
<td>1.8</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6302</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
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<td>0.05</td>
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<td>1.8</td>
<td>2000</td>
<td></td>
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<tr>
<td>L6303</td>
<td>0.5</td>
<td>185 to 400</td>
<td>400</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>2.5 +/- 0.25</td>
<td>4</td>
<td>20</td>
<td>1.7 +/- 0.2</td>
<td>3.3</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6304</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>2.5 +/- 0.25</td>
<td>4</td>
<td>20</td>
<td>1.7 +/- 0.2</td>
<td>3.3</td>
<td>2000</td>
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<tr>
<td>L6305</td>
<td>0.5</td>
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<td>400</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>3.0 +/- 0.3</td>
<td>5</td>
<td>20</td>
<td>0 to 1</td>
<td>0 to 1.6</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6306</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>3.0 +/- 0.3</td>
<td>5</td>
<td>20</td>
<td>0 to 1</td>
<td>0 to 1.6</td>
<td>2000</td>
<td></td>
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<tr>
<td>L6307</td>
<td>0.5</td>
<td>185 to 400</td>
<td>400</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>10 +/- 1</td>
<td>0.8</td>
<td>20</td>
<td>2.5 to 6.0</td>
<td>0.3 to 0.6</td>
<td>2000</td>
<td></td>
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<tr>
<td>L6308</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>10 +/- 1</td>
<td>0.8</td>
<td>20</td>
<td>2.5 to 6.0</td>
<td>0.3 to 0.6</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6309</td>
<td>0.5</td>
<td>185 to 400</td>
<td>400</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>10 +/- 1</td>
<td>1.2</td>
<td>20</td>
<td>7.0 +/- 0.5</td>
<td>1</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6310</td>
<td>1.0</td>
<td>185 to 400</td>
<td>350</td>
<td>300 +/- 30</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>10 +/- 1</td>
<td>1.2</td>
<td>20</td>
<td>7.0 +/- 0.5</td>
<td>1</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6311</td>
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<td>185 to 400</td>
<td>400</td>
<td>300 +/- 10</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>12 to 15</td>
<td>0.5 to 0.55</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>L6312</td>
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<td>350</td>
<td>300 +/- 10</td>
<td>80</td>
<td>+/- 0.3</td>
<td>0.05</td>
<td>12 to 15</td>
<td>0.5 to 0.55</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>2000</td>
<td></td>
</tr>
</tbody>
</table>

*: The life end is defined as the time when the radiant intensity falls to 50% of its initial value or when the output fluctuation exceeds 0.05% p-p.

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# Power Supply

## CHARACTERISTICS

### Anode output

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C1518</th>
<th>C4544</th>
<th>C4545</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output current</td>
<td>300</td>
<td>300</td>
<td></td>
<td>mAdc</td>
</tr>
<tr>
<td>Output voltage</td>
<td>80±20</td>
<td>80±10</td>
<td></td>
<td>Vdc</td>
</tr>
<tr>
<td>No load</td>
<td>160</td>
<td>160</td>
<td></td>
<td>Vdc Typ.</td>
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<tr>
<td>Trigger voltage</td>
<td>600±50</td>
<td>500</td>
<td></td>
<td>Vpeak</td>
</tr>
<tr>
<td>Load fluctuation (normal operation range)</td>
<td>±0.05</td>
<td>±0.05</td>
<td>% Max.</td>
<td></td>
</tr>
<tr>
<td>Drift</td>
<td>±0.1</td>
<td>±0.05</td>
<td>%h Max.</td>
<td></td>
</tr>
<tr>
<td>Ripple</td>
<td>0.1</td>
<td>0.1</td>
<td>%p-p Max.</td>
<td></td>
</tr>
<tr>
<td>Over-load protection</td>
<td>0.5A Fuse</td>
<td>0.5A Fuse</td>
<td>—</td>
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</tbody>
</table>

### Filament (heater) output

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C1518</th>
<th>C4544</th>
<th>C4545</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output voltage for warm-up</td>
<td>10±1</td>
<td>12±1</td>
<td></td>
<td>Vdc</td>
</tr>
<tr>
<td>Warm-up time(Approx.)</td>
<td>20</td>
<td>20</td>
<td></td>
<td>s</td>
</tr>
<tr>
<td>Output voltage for operation</td>
<td>0.5±0.5 / 3.5±0.5</td>
<td>0</td>
<td></td>
<td>Vdc</td>
</tr>
<tr>
<td>Input fluctuation (±10%)</td>
<td>±0.1</td>
<td></td>
<td></td>
<td>% Max.</td>
</tr>
<tr>
<td>Over-load protection</td>
<td>10V side : 2A slow-blow</td>
<td>2.5V side : Fuse (5A)</td>
<td>Fuse (1A)</td>
<td>—</td>
</tr>
<tr>
<td>Input voltage</td>
<td>100/118/230±10%</td>
<td>100/118/230±10%</td>
<td></td>
<td>Vac</td>
</tr>
<tr>
<td>Operating ambient temperature</td>
<td>0 to +40</td>
<td>0 to +40</td>
<td></td>
<td>°C</td>
</tr>
<tr>
<td>Performance guaranteed temperature</td>
<td>+5 to +35</td>
<td>+5 to +35</td>
<td></td>
<td>°C</td>
</tr>
<tr>
<td>Cooling method</td>
<td>Not required</td>
<td>Not required</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>External dimensions (W×H×D)</td>
<td>200×107×240</td>
<td>70×118×195</td>
<td>83×136×214</td>
<td>mm</td>
</tr>
<tr>
<td>Weight(Approx.)</td>
<td>6.7</td>
<td>2</td>
<td>3.4</td>
<td>kg</td>
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</table>

### Applicable lamps

<table>
<thead>
<tr>
<th>Applicable lamps</th>
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</tr>
</thead>
<tbody>
<tr>
<td>C4544</td>
<td>L6311, L6311-50, L6312, L6312-50</td>
</tr>
<tr>
<td>C4545</td>
<td>—</td>
</tr>
</tbody>
</table>
Stepper Motor 4SQ-120BA34S (for light source and transportation system)

Not available for sale in Europe

Series 4SQ Stepper Motors 1.8°

Specifications

<table>
<thead>
<tr>
<th>Part Number</th>
<th>DC Operating Voltage</th>
<th>Res. per Winding (Ω)</th>
<th>Ind. per Winding mH</th>
<th>Holding Torque mN/m oz-in</th>
<th>Motor Moment of Inertia g*cm²</th>
<th>Step Angle</th>
<th>Step Angle Tolerance</th>
<th>Steps per Rev.</th>
<th>Max. Radial Load kg/lb</th>
<th>Max. Axial Load kg/lb</th>
<th>Max. Temp. Rise</th>
<th>Ambient Temp Range Operating Storage</th>
<th>Bearing Type</th>
<th>Insulation Res. at 500Vdc</th>
<th>Dielectric Withstanding Voltage</th>
<th>Weight goz</th>
</tr>
</thead>
<tbody>
<tr>
<td>4SQ - 120BA34S</td>
<td>12</td>
<td>5</td>
<td>26</td>
<td>66/6.2</td>
<td>1.9 x 10³</td>
<td>1.5/0.2</td>
<td>±5%</td>
<td>200</td>
<td>4/8.8</td>
<td>8/17.6</td>
<td>55°C</td>
<td>-20°C to +50°C</td>
<td>-20°C to +60°C</td>
<td>BAI, Double Shielded</td>
<td>50 megohms</td>
<td>500 Vac for 60 Sec</td>
</tr>
</tbody>
</table>

NOTE: Unless otherwise indicated, all values shown are typical. Other windings available on special order. Consult Thomson Airpes for availability of motors with 3.6" step angle.

1 Measured with 2 phases energized. 2 Measured at 10mm from mounting plate surface.

For information or to place an order in North America: 1 (203) 271-6444 Asia: (65) 7474-8888

The specifications in this publication are believed to be accurate and reliable. However, it is the responsibility of the product user to determine the suitability of Thomson products for a specific application. While defective products will be replaced without charge if promptly returned, no liability is assumed beyond such replacement.
**Electronic Shutter: Prontor Magnetic 016 from Schneider Optics**

**PRONTOR magnetic**
- **Größe 0**
- **Size 0**
- **Taille 0**

![Prontor Magnetic 016 Diagram]

<table>
<thead>
<tr>
<th>Exposure times (s)</th>
<th>Temps de pose (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32s 16s 8s 4s 2s 1s</td>
<td>1/2s 1/4s 1/8s 1/15s 1/30s</td>
</tr>
</tbody>
</table>

- **Blende** (Diaphragm)
  - Größe Öffnung 23 Ø
  - Kleinste Öffnung 1,5 Ø

- **Gesamthöhe** (Total height): 20mm + 0,03mm
- **Abstand der Objektivrohr-Stirnflächen** (Distance between the front and the rear faces of the lens tube): 20mm + 0,03mm

- **Gehäusedurchmesser** (Housing diameter): 61mm
- **Fassungsgewinde vorn und hinten** (Front and rear lens mounting thread): M29.5x0.5
- **Anschraubgewinde** (Thread of retaining ring): M32.5x0.5

- **Kürzeste mögliche Belichtungzeit** (Shortest possible exposure time): 1/60s

- **Magnetischer Antrieb** (Magnetic drive (long life))
  - remote speed control with power pack, electronically controlled shutter speeds, manually controlled aperture continuously adjustable.

- **Magnetischer Antrieb** (Magnetic drive)
  - remote speed control with power pack, electronically controlled shutter speeds, manually controlled aperture continuously adjustable.

- **Belichtungszeiten** (Exposure times): 32s 16s 8s 4s 2s 1s
  - 1/2s 1/4s 1/8s 1/15s 1/30s

**For dimensions and other data: original factory drawing 1016 602 available.**

*For Maße und Daten sind nur Angaben der Originalzeichnung 1016 602 verbindlich.*

*Seules les dimensions et les données du dessin original d’atelier 1016 602 sont valables.*

150
Appendix D

The Excision Device

D1 Assembly Drawings
1. Cutting blades (glued to cutting tip with epoxy)
2. Cutting tip
3. Cutting tip holder
   (bolted to slide by M2.6, 3mm in front, 6mm in back)
4. Positioning pins (1/16" diameter, 5/16" length
   press fitted to cutting tip holder)
5. Magnet (glued to cutting tip with epoxy
   and #4 nut glued to cutting tip holder)
6. Stopper blocks
   (bolted with a 0-80, 5/16" cap screw)
7. Spring
8. Motor base
   (bolted to slide by 2x M2.6, 6mm with #4 washer)
9. Stopping pins (1/16"x5/16")
   (press fitted 3/32" deep)
10. Slide (BSP 10-25 SL)
    (bolted to motor base by 2x M2.6, 4mm cap screws)
11. Limit switches
    (bolted with 2x 0-80, 1/4" cap screws)
12. Digital linear actuator (L92121-P2)
    (bolted to its holder by 2x 6-32, 1/4" screws)
13. Main frame
14. Slide (BSP 10-45 SL)
    (bolted to main base by 2x M2.6, 4mm cap screws
    hold to the shaft plate by #4 nut and washer)
15. Motor Shaft (cut to 3" total length)
    (hold to the shaft plate by #4 nut and washer)
16. Shaft plate
    (bolted the main frame by a 4-40, 1/4" and #4 washer
    a #4 plastic washer is placed between the plate and frame)
D2  Parts Drawings
Figure D7: Drawing of the flexible shaft plate

This component is made from a spring metal sheet.
D3 Purchased Parts
Digital Linear Actuator L92121-P2

Specifications

<table>
<thead>
<tr>
<th>Part Number</th>
<th>DC Operating Voltage</th>
<th>Maximum Travel</th>
<th>Linear Travel Per Step</th>
<th>Maximum Force</th>
<th>Minimum Holding Force (Unenergized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K92111-P1</td>
<td>5</td>
<td>0.5&quot; (12.7mm)</td>
<td>.001&quot; (.025mm)</td>
<td>45 oz (12.5N)</td>
<td>60 oz (16.68N)</td>
</tr>
<tr>
<td>K92111-P2</td>
<td>12</td>
<td>0.8&quot; (20.3mm)</td>
<td>.002&quot; (.05mm)</td>
<td>28 oz (7.23N)</td>
<td>40 oz (11.13N)</td>
</tr>
<tr>
<td>L92111-P1</td>
<td>5</td>
<td>0.5&quot; (12.7mm)</td>
<td>.001&quot; (.025mm)</td>
<td>45 oz (12.5N)</td>
<td>60 oz (16.68N)</td>
</tr>
<tr>
<td>L92111-P2</td>
<td>12</td>
<td>0.8&quot; (20.3mm)</td>
<td>.002&quot; (.05mm)</td>
<td>28 oz (7.23N)</td>
<td>40 oz (11.13N)</td>
</tr>
<tr>
<td>L92121-P1</td>
<td>5</td>
<td>0.5&quot; (12.7mm)</td>
<td>.001&quot; (.025mm)</td>
<td>45 oz (12.5N)</td>
<td>60 oz (16.68N)</td>
</tr>
<tr>
<td>L92121-P2</td>
<td>12</td>
<td>0.8&quot; (20.3mm)</td>
<td>.002&quot; (.05mm)</td>
<td>28 oz (7.23N)</td>
<td>40 oz (11.13N)</td>
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<tr>
<td>L92141-P1</td>
<td>5</td>
<td>0.5&quot; (12.7mm)</td>
<td>.001&quot; (.025mm)</td>
<td>45 oz (12.5N)</td>
<td>60 oz (16.68N)</td>
</tr>
<tr>
<td>L92141-P2</td>
<td>12</td>
<td>0.8&quot; (20.3mm)</td>
<td>.002&quot; (.05mm)</td>
<td>28 oz (7.23N)</td>
<td>40 oz (11.13N)</td>
</tr>
</tbody>
</table>

Note: Shaft Options Series K92100
Add Suffix-S1 for #4-40 NC-2A Threaded Tip
Add Suffix-S2 for #2-56 NC-2A Threaded Tip

Unipolar Drive
Max Push Rate (Steps/Sec) 1000
Max Pull-out Rate (Steps/Sec) 2000

Typical Pull-in Force vs. Linear Rate at 20°C

Standard Switching Sequence for Linear Actuators – Unipolar Drive 5Vdc and 12Vdc

Lead Wire Color Codes

<table>
<thead>
<tr>
<th>Series</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
<th>Q7</th>
<th>Q8</th>
</tr>
</thead>
<tbody>
<tr>
<td>L92111</td>
<td>YEL</td>
<td>ORN</td>
<td>BLK</td>
<td>BRN</td>
<td>RED</td>
<td>Q5</td>
<td>Q6</td>
<td>Q7</td>
</tr>
<tr>
<td>L92121</td>
<td>YEL</td>
<td>ORN</td>
<td>BLK</td>
<td>BRN</td>
<td>RED</td>
<td>Q5</td>
<td>Q6</td>
<td>Q7</td>
</tr>
<tr>
<td>L92141</td>
<td>YEL</td>
<td>ORN</td>
<td>BLK</td>
<td>BRN</td>
<td>RED</td>
<td>Q5</td>
<td>Q6</td>
<td>Q7</td>
</tr>
</tbody>
</table>

Note: Chart sequence repeats after four pushes. For outward thrust, use switching from top of chart to bottom. For inward thrust, use switching from bottom of chart to top.

THOMSON
AIRPAK MECHATRONICS
## Specifications

### Accuracy

- ± 0.1 mm in 10 consecutive cuts.
- > 95% spot pickup on first cut.
- 0.01 mm increments for cutting head movement.
- Cuts 96 spots in 20 minutes.

### Sample Output

- Reduces levels of keratin contamination, as detected in mass spectrometry.
- No protein carryover detectable in mass spectrometry.

### Hardware

**Excision Tip**

- Internal diameter 1.0 mm; outer diameter 1.5 mm.
- Cuts acrylamide gels and PVDF membranes.

**Dimensions (without computer)**

- Size: 50 x 52 x 50 cm (W x H x D);
- weight: 30 kg (66 lb)

**Lighting**

- White light under cutting stage and microtiter plate.
- Flash in camera for overhead lighting.

**Regulatory**

- CE, EN61010-1

**Maximum Cutting Area**

- 11.8 x 8.8 cm (W x H)

**Accessories**

- 11.8 x 8.8 cm (W x H) large and 9.5 x 7 (W x H) cm mini gel sizers and frames, cutting mats, cutting tips, starter kit

**Imaging System**

- Picture resolution: 1280 x 960 pixels (W x H)

**Operation**

- Operating temperature: 10–30 °C.
- Operating humidity range: 0–95% RH non-condensing.

**Minimum Computer Requirements (computer not included)**

- PC — 300 MX (Pentium® II or equivalent), Windows® 95/98/NT, 64 MB RAM, PS2 mouse, 2 GB hard drive, 24 speed CD ROM drive, 15" XVGA monitor, 24-bit color, 1024 x 768 resolution

---

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Appendix E

The Excision Device Transportation System
E1 Derivations for the transportation system

E1.1 System Torque Calculation

![Figure E1: Schematic of the rack and pinion system](image)

The total torque required by the motor is

\[ T_{\text{total}} = T_{\text{rotational}} + T_{\text{linear}} + T_{\text{friction}} \]  

\[ T_{\text{rotational}} \] is the torque required to rotate the rotor, the shaft, and the pinion.

\[ T_{\text{rotational}} = J_{\text{rotor + pinion}} \frac{a}{R} \]  

where

- \( J_{\text{rotor + pinion}} \) is the moment of inertia,
- \( a \) is the linear acceleration,
- \( R \) is the pitch radius of the pinion gear.

The torque required to accelerate the carriage and its components is:

\[ T_{\text{linear}} = maR \]  

where \( m \) is the mass of the moving assembly.

\[ m = m_{\text{cutter}} + m_{\text{motor}} + m_{\text{frame}} \]  

The frictional torque, \( T_{\text{friction}} \), is the torque required to overcome the frictional forces, \( F_{\text{friction}} \), between the slide-rail system and between the rack-pinion system.

\[ T_{\text{friction}} = F_{\text{friction}} R \]  

The total required torque is therefore:
The values for the different variables in the designed transportation assembly are:

\[ m_{\text{cutter}} = 160 \text{g} \]
\[ m_{\text{motor}} = 197 \text{g} \]
\[ m_{\text{frame}} = 206 \text{g} \]
\[ J_{\text{rotor} + \text{pinion}} = 1.9 \times 10^{-3} \text{g} \cdot \text{m}^2 \]
\[ R = 0.016 \text{m} \]
\[ a = 0.15 \text{m/s}^2 \]
\[ F_{\text{friction}} = 1.37 \text{N to 1.77N} \]

The acceleration was obtained from the specification of reaching the cruising speed of \(.15\text{m/s}\) in 1 second. The frictional force for the entire assembly was determined experimentally by using a pulley system and calibrated weights. It is expressed as a range because it was found to be different at different locations. The stepper motor therefore needs to provide a torque equal to:

\[ T_{\text{min total}} = 0.02 + 1.35 + 21.92 = 23.29 \text{mN} \cdot \text{m} \]
\[ T_{\text{max total}} = 0.02 + 1.35 + 28.32 = 29.69 \text{mN} \cdot \text{m} \]

The maximum operating range of the proposed system is shown on top of the motor torque performance in Figure E.2. The selected motor can be used at up to 300-pulses-per-second with a safety factor.

\[ T_{\text{total}} = J_{\text{rotor} + \text{pinion}} \frac{a}{R} + maR + F_{\text{friction}}R \]  
(E.6)
E1.2 Calculation of System Resolution and Speed

The linear step size, $\Delta_t$, is calculated from the stepper motor step angle, $\Delta_\theta$, and the pitch radius, $R_p$, of the pinion gear.

$$\Delta_t = \frac{2\pi R_p}{360/\Delta_\theta}$$  \hspace{1cm} (E.7)

The selected stepper motor has a 1.8° step angle. The linear step size is therefore 0.5026mm.

The speed, $V$, is calculated from the step size and the pulse frequency, $f_m$.

$$V = 2\pi R_p \frac{\Delta_\theta}{360} f_m$$  \hspace{1cm} (E.8)

As seen in the previous section, the stepper motor maximum operating pulse frequency is 300Hz for the proposed application. At 300pps, the system has a linear velocity of 150.8mm/s. The transportation system speed can be estimated to be half of the pulse frequency.

E1.3 Calculation of Rack and Pinion Specifications

During motion, it is safe to assume that one tooth is supporting the load. The gear tooth strength is found from the Lewis bending strength equation:

$$W_t = \frac{SFY}{D_{pitch}}$$  \hspace{1cm} (E.9)

where

- $W_t$ is the maximum transmitted load,
- $S$ is the maximum bending tooth stress and is equal to one-third the tensile strength,
- $F$ is the face width of the gear
- $Y$ is the Lewis factor,
- $D_{pitch}$ is the diametral pitch

Both the gear and racks are made of 303-stainless steel, so the tensile strength is 90,000psi. The pinion gear has a face width of 0.104inch, which is smaller than the one of the racks; therefore, it will be used. The Lewis factor is equal to 0.421 since we are using a 60tooth gear. The racks and pinion have a 48-diametral pitch. The maximum transmitted load is therefore 27.4lbs or 122N.

The linear pitch, $L_{pitch}$, is the distance between two adjacent teeth.

$$L_{pitch} = \frac{\pi}{D_{pitch}} = 0.0654\text{inch} = 1.7\text{mm}$$  \hspace{1cm} (E.10)
E2 Parts Drawings
1 Main Frame
2 Accuglide Rail
   Bolted to main frame by 25x 8-32,7/16" socket cap screws
3 Accuglide slider
4 Racks
   Each rack is bolted to main frame by 4x 6-32,1/2" button head cap screws
5 Stepper motor, model 4SQ-1-20BA34S
   Attached to carriage frame #2 by 2x M3 nuts
6 Anti-backlash pinion gear, model AP48C-60
   Hole is rebored to fit the 5mm diameter motor shaft
7 Carriage frame #1
   Bolted to slider by 4x M4,6mm cap screws
8 Carriage frame #2
   Bolted to carriage frame #1 by 3x 6-32,5/8" cap screws and spring washer
9 Excision device
   Bolted to carriage frame #2 by 2x 4-40,1/4" cap screws and washers
25x 8-32 tapped holes are needed

4 sets of 4x 6-32 tapped holes are needed
THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF MIT AND ALPINE PHARMACEUTICAL. ANY REPRODUCTION IN PART OR WHOLE WITHOUT WRITTEN PERMISSION IS PROHIBITED.

SECTION A-A

3x 6-32 THRU CLEARANCE HOLES

4x 0.177 THRU 0.315

A

A

DIMENSIONS ARE IN INCHES

TOLERANCES ARE:

+0.00

MATERIAL: Aluminum 5052

MITMechatronics Research Laboratory

Transportation System

Carriage Frame #1

TS102
4 parts needed
Model AG-1 purchased from Pic Design Inc,
0.053" were removed from each end so that the racks
could be stacked together
Appendix F

The Cutting Tip Changing Station

F1 Part Drawings
Figure F.7: Assembly of the cutting tip changing station

1. Base plate
2. Tool Centering end piece (bolted to base plate with 2x 8-32, 7/16" button head screws and washers)
3. Delrin sliding arm
4. Shaft holder (bolted to base plate with 2x 8-32, 7/16" button head screws and washers)
5. Shaft (attached to its holder by 2x 4-40 set screws)
6. Solenoid frame (attached to base by 2x 6-32, 1/4" cap screws and #6 washers)
7. Sliding arm bracket (attached to sliding arm by 2x 4-40, 3/16" button head screws, and attached to solenoid arm by 1x 4-40, 1/4" screw)
8. Solenoid arm
9. Set screws to grab cutting tools
10. Front springs
11. Back springs
Figure F5: Drawing of the sliding arm

This part is made out of white DELRIN

<table>
<thead>
<tr>
<th>PART NO.</th>
<th>PART OR IDENTIFYING NO.</th>
<th>DESCRIPTION</th>
<th>MATERIAL</th>
<th>SPACING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cutting Tip Changing Station: Sliding Arm</td>
<td>Delrin</td>
<td></td>
</tr>
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</table>

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES TOLERANCES ARE:

- DECADES ANGLES
- .010 + .001
- .010 + .001 + .001

MATERIAL: Delrin

MIT Mechatronics Research Laboratory

Eric Hoorau Feb 2001 Abe Schneider

CS104
Appendix G

The Temperature Controlled Storage Station
G1 Derivations for the Temperature Controlled Station

Figure G1: Diagram of the heat transfer in the storage system

G1.1 Heat Load Calculations

The heat gained by convection from the top is

\[ Q_2 = hA_2 (T_\infty - T_s) \]  \hspace{1cm} (G.1)

where
- \( h \) is the heat transfer coefficient [watts/m\(^2\)-\(\degree\)C]
- \( A_2 \) is the exposed top surface area [m\(^2\)]
- \( T_\infty \) is the ambient temperature [\(\degree\)C]
- \( T_s \) is the temperature inside the enclosure [\(\degree\)C]

The heat gained through the sidewalls of the insulated enclosure is

\[ Q_1 = \frac{A_1 (T_\infty - T_s)}{\frac{x}{K} + \frac{1}{h}} \] \hspace{1cm} (G.2)

where
- \( A_1 \) is the external surface of the insulated enclosure [m\(^2\)]
- \( x \) is the thickness of the insulation [m]
- \( K \) is the thermal conductivity of the insulation [watts/m-\(\degree\)C]
The heat released to the hot side of the thermoelectric cooler is

\[ Q_h = Q_c + (V_{in}I_{in}) \]  

(G.3)

where

- \( Q_c \) is the heat absorbed from the cold side of the thermoelectric [watts]
- \( V_{in} \) is the input voltage of the thermoelectric [volt]
- \( I_{in} \) is the input current of the thermoelectric [amp]

**G1.2 Calculation of Additional Parameters**

The heat sink performance is measured in terms of its thermal resistance:

\[ \Theta_s = \frac{T_{hot} - T_m}{Q_h} \]  

(G.4)

where

- \( T_{hot} \) is the heat sink temperature [°C]

The time, \( t \), needed to change the temperature of an object can be estimated to be

\[ t = \frac{mC_p}{\Delta T/Q_{ave}} \]  

(G.5)

where

- \( m \) is the weight of the material [grams]
- \( C_p \) is the specific heat of the material [cal-gram-°C]
- \( \Delta T \) is the temperature change of the material [°C]
- \( Q_{ave} \) is the average rate removal [cal/second]

The average rate removal is

\[ Q_{ave} = \frac{Q_{C_{DT_{min}}}-Q_{C_{DT_{max}}}}{2} \]  

(G.6)

where

- \( DT \) is the temperature difference across the module [°C]
- \( Q_{C_{DT_{min}}} \) is the amount of heat the thermoelectric module is pumping at the initial object temperature. \( DT \) is zero at this time and the heat-pumping rate is maximum [cal/second].
- \( Q_{C_{DT_{max}}} \) is the amount of heat the thermoelectric module is pumping at the final object temperature. \( DT \) is high and the heat-pumping rate is minimum [cal/second].
## G1.3 System Parameters

### Calculated Variable

<table>
<thead>
<tr>
<th>Calculated Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>$27 \times 10^{-3}$ [m$^2$]</td>
</tr>
<tr>
<td>A2</td>
<td>$17 \times 10^{-3}$ [m$^2$]</td>
</tr>
<tr>
<td>Q1</td>
<td>0.715 [watts]</td>
</tr>
<tr>
<td>Q2</td>
<td>7.65 [watts]</td>
</tr>
<tr>
<td>$Q_c$ at $DT_{\text{min}}$ (from chart)</td>
<td>65 [watts]</td>
</tr>
<tr>
<td>$Q_c$ at $DT_{\text{max}}$ (from chart)</td>
<td>25 [watts]</td>
</tr>
<tr>
<td>$Q_{\text{ave}}$</td>
<td>45 [watts], 10.75 [cal/sec]</td>
</tr>
</tbody>
</table>

### Experimental variable

<table>
<thead>
<tr>
<th>Experimental variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>17 [cm]</td>
</tr>
<tr>
<td>W1</td>
<td>10 [cm]</td>
</tr>
<tr>
<td>H1</td>
<td>5 [cm]</td>
</tr>
<tr>
<td>x</td>
<td>2 [cm]</td>
</tr>
<tr>
<td>$T_s$</td>
<td>4 [$^\circ$C]</td>
</tr>
<tr>
<td>$T_\infty$</td>
<td>22 [$^\circ$C]</td>
</tr>
<tr>
<td>$T_{\text{hot}}$</td>
<td>42 [$^\circ$C]</td>
</tr>
<tr>
<td>$T_{\text{cold}}$</td>
<td>2 [$^\circ$C]</td>
</tr>
<tr>
<td>$V_{\text{in}}$</td>
<td>12 [volt]</td>
</tr>
<tr>
<td>$I_{\text{in}}$</td>
<td>5 [amp]</td>
</tr>
<tr>
<td>K (polyurethane)</td>
<td>0.035 [watts/m-$^\circ$C]</td>
</tr>
<tr>
<td>h (still air)</td>
<td>23 to 28 [watts/m$^2$-$^\circ$C]</td>
</tr>
<tr>
<td>h (turbulent air)</td>
<td>85 to 113 [watts/m$^2$-$^\circ$C]</td>
</tr>
</tbody>
</table>
**G2 Thermoelectric Cooler Data Sheet**

---

**TECHNICAL DATA SHEET**

**THERMOELECTRIC COOLER: 127-Couples, 8.5-Ampere**

**Part Number**

6300/127/085* (150°C Maximum Temperature)

* Add Suffix for Height Tolerance  
A = ±0.30mm (.010")  
B = ±0.03mm (.001")

---

**DESCRIPTION**

This 127-couple, 8.5-amp module is a general purpose single-stage thermoelectric cooler suitable for various cooling and/or heating applications. This device is particularly useful where a 12-volt DC power source is to be used and high heat pumping capacity is required. Typical application areas include consumer products, biomedical instruments, industrial, military, and electrical equipment, and laboratory and scientific apparatus. The module features alumina ceramic face plates that electrically isolate internal circuit elements from the external mounting surfaces.

---

**GENERAL SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Temperature Differential (DT) at Zero Heat Load</th>
<th>Th = 25°C</th>
<th>Th = 35°C</th>
<th>Th = 50°C</th>
<th>Th = 80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees C.</td>
<td>65</td>
<td>68</td>
<td>72</td>
<td>76</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Heat Pumping Capacity (Qc) at Zero Temperature Differential</th>
<th>Th = 25°C</th>
<th>Th = 35°C</th>
<th>Th = 50°C</th>
<th>Th = 80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watts</td>
<td>72</td>
<td>76</td>
<td>80</td>
<td>87</td>
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</table>

<table>
<thead>
<tr>
<th>Maximum or Optimum Current (Io)</th>
<th>8.5</th>
<th>8.5</th>
<th>8.5</th>
<th>8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amperes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nominal Input Voltage (Vin) at Io and DT = 30°C</th>
<th>14.9</th>
<th>15.2</th>
<th>16.8</th>
<th>19.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volts DC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Qc vs. I**

**Coefficient of Performance**

**Vin vs. I**

**Vin vs. Th**

---

183
The sample storage station is shown in Figure G2. The steps for the construction of the tank are shown in Figure G3. The first step is shown in Figure G3a. Four 3/8"-16, 4" hex head cap screws are attached to the stainless steel pan with epoxy, and a thermistor is attached to the side of the pan with a thermally conductive epoxy. The second step consists of insulating the pan with polyurethane foam and is shown in Figure G3b. Tape was placed at the location where the thermoelectric will contact the pan. Then, the foam was applied. Once the foam had cured, it was trimmed to the shape shown in the figure.
The base of the station is shown in Figure G4. The hot side of the modules was attached to the heat sink/fan assembly with a thermally conductive epoxy as seen in Figure G4a. The heat sink/fan assembly was then bolted to the aluminum base plate. The temperature controller and its knob were also bolted to the base plate. The modules are electrically in series and thermally in parallel. The cold side of the module is coated with thermally conductive grease. The four feet pass through the base plate and are connected to the bolts on the tank with nuts. The base plate is pushed onto the bottom of the tank by four nuts on the feet of the assembly.
Appendix H

The System Software Interface
H1 The Interface Screens

Figure H1: Sample of the interface control for the cutting device, transportation system, and light source

Figure H2: Sample of the interface control for the XY stage