A DEVELOPMENT OF SOFTWARE FOR FILM DENSITY MEASUREMENT USING FLATBED SCANNER

SUWAJCHAI PORNSUWANSIRI

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Mr.Suwajchai Pornsuwansiri
Candidate

Assoc. Prof. Chavalit Wongse-ek,
M.Sc. (Physics)
Major advisor

Asst. Prof. Pirojana Suvanasuthi,
M.Eng. (Electrical Engineering)
Co-advisor

Asst. Prof. Chatchai Neatpisarnvanit,
Ph.D. (Electrical Engineering)
Co-advisor

Assoc. Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate Studies

Asst. Prof. Pirojana Suvanasuthi,
M.Eng (Electrical Engineering)
Chair
Master of Engineering
Programme in Biomedical Engineering
Faculty of Engineering

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on
5 June, 2004

....
Mr.Suwajchai Pornsuwansiri
Candidate

....
Assoc. Prof. Chavalit Wongse-ek,
M.Sc. (Physics)
Chair

....
Asst. Prof. Pirojana Suvanasuthi,
M.Eng. (Electrical Engineering)
Thesis Defence Committee

....
Asst. Prof. Chatchai Neatpisarnvanit,
Ph.D. (Electrical Engineering)
Thesis Defence Committee

Adm. P. Nacaskul
....
Admiral Paibul Nacaskul,
B.Sc.1st Hons., Ph.D. (Electrical Engineering)
Thesis Defence Committee

Bovornchok Poopat
....
Asst. Prof. Bovornchok Poopat,
Ph.D. (Welding Engineering)
Thesis Defence Committee

....
Assoc. Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

....
Asst. Prof. Piya Rattanasuwan,
B.Eng. (Civil Engineering)
Dean
Faculty of Engineering
Mahidol University

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SUWAJCHAI PORNSUWANSIRI

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SUWAJCHAI PORNSUWANSIRI 4137960 EGBE/M

M. Eng. (BIOMEDICAL ENGINEERING)

THESIS ADVISORS: CHAWALIT WONGSE-EK, M.Sc., CHATCHAI NEATPISARNVANIT, Ph.D., PIROJANA SUVANASUTHI, M.Eng.

ABSTRACT

This research involves the use of a flatbed scanner instead of a densitometer to measure optical density of film from x-ray imaging. As film is scanned, a mathematical model is constructed to convert pixel values of the film image into optical densities. Curve fitting by numerical method was used for the conversion.

The objective of this research is to develop software that can measure film density by using a flatbed scanner. Microsoft visual C++ was used to create a particular “Class” of software to perform curve fitting automatically. Therefore, manual calculation is not necessary, and the software so developed can also reduce time to analyse film in the quality assurance (QA) process.

The results show that the scanner can be used to measure optical density with performance comparable to a densitometer. Microtek scanner as used in this research can measure optical density in the range of 0.24 - 2.5 OD with +/- 10% accuracy.

It is likely that the software so far developed can be extended to improve the accuracy of measurement already achieved in this thesis work.

KEY WORDS: OPTICAL DENSITY / DENSITOMETRY / RADIOGRAPHIC PROCESSING QUALITY CONTROL / FILM DENSITY / DESITOMETER

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การพัฒนาซอฟท์แวร์วัดค่าความดำฟิล์มโดยใช้สแกนเนอร์ (A DEVELOPMENT OF SOFTWARE FOR FILM DENSITY MEASUREMENT USING FLATBED SCANNER)

สุวัจชัย พรสุวรรณสิริ 4137960 EGBE/M

วศ.ม. (วิศวกรรมชีวการแพทย์)

คณะกรรมการควบคุมวิทยานิพนธ์ : ชวลิต วงษ์เอก, M.Sc., ฉัตรชัย เนตรพิศาลวนิช, Ph.D., ไพโรจน์ สุวรรณสุทธิ, M.Eng.

บทคัดย่อ

งานวิจัยนี้เกี่ยวข้องกับการใช้เครื่องสแกนเนอร์มาทำการวัดค่าความดำของฟิล์มเอกซเรย์แทนเครื่องเดนสิโตมิเตอร์โดยสร้าง Mathematical Model ที่สามารถแปลงค่าจาก Pixel value ไปเป็นค่าความดำของฟิล์มได้ ซึ่งในส่วนของระเบียบวิธีการเชิงคณิตศาสตร์ (Numerical Method) จะใช้วิธี Curve Fitting ในการแปลงค่า

วัตถุประสงค์ของงานวิจัยนี้คือ การพัฒนาซอฟท์แวร์ที่สามารถวัดค่าความดำฟิล์มได้ด้วยเครื่องสแกนเนอร์ โดยใช้ Microsoft Visual C++ สร้าง “คลาส”ซอฟท์แวร์สำหรับคำนวณ Curve Fitting ขึ้นมาโดยเฉพาะ เพื่อทำการแปลงค่าให้อัตโนมัติโดยผู้ใช้ซอฟท์แวร์ไม่ต้องคำนวณเอง ร่วมทั้งสามารถช่วยลดเวลาในการวิเคราะห์ฟิล์มเอกซเรย์ในขบวนการประกันคุณภาพลงได้ด้วย

ผลที่ได้คือสามารถวัดค่าความดำของฟิล์มด้วยสแกนเนอร์แทนเครื่องเดนสิโตมิเตอร์ได้ โดยในที่นี้เครื่องที่ใช้คือไมโครเทคสแกนเนอร์สามารถวัดค่าค่าความดำของฟิล์มได้ตั้งแต่ 0 ถึง 2.5 OD โดยที่มีค่าความผิดพลาดอยู่ที่ประมาณ +/- 10%

มีความเป็นไปได้ที่สามารถพัฒนาโปรแกรมซอฟท์แวร์ในงานวิจัยนี้ให้ก้าวหน้าสามารถเพิ่มความแม่นยำในการวัดได้มากขึ้น

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NOMENCLATURE

RT = Radiation Therapy

QC = Quality Control

QA = Quality Assurance

GUI = Graphic User Interface

ASA = American Standard Association

CCD = Charge-Coupled Devices

CMYK = Cyan Magenta Yellow Black Color Model

MD. = Mid Density

DMAX = Maximum Density

OD = Optical Density

PV = Pixel Value

TIFF = Tagged Image File Format

LSD = Link Scanning Densitometry Image Software

MTMA = Microtek Magnetic Transparent Media Adapter

CHAPTER 1

INTRODUCTION

Quality Control programs in Diagnostic Radiology are recognized as effective means of managing imaging environments. Quality Control programs insure that all components of the imaging environment perform at optimum; the end result being highest quality images with the lowest possible dose to patient and operator while maintaining high diagnostic content. It is the goal of the Quality Control Program to help contain costs through elimination of unproductive imaging resulted from failure of machines or materials that may occur in the complex chain leading to the finished product. The objective of the Quality Control Program is to detect changes in image quality that may affect diagnosis or cause changes in the radiation exposure to the patient before they become significant and, most frequently, to request service or calibration of the imaging systems before loss of image quality. The Quality Control Program is an integral part of the overall quality assurance program within the Radiology Department and the hospital as a whole.

In Radiation Therapy (RT) quality control (QC) preparation, measurements, analysis and report writing took an average of 5 hours per day with film dosimetry. It leads to multiple treatment planning systems. The optical density is daily measured with a densitometer from film for Quality Control Process by the Diagnostic Physicist. But the problem is the time used for QC; about 3 hours per day. In many cases it requires expert technician in measurements. It will lead to more frequent QC and consequently to a strain on Diagnostic Physicist.

The implementation of Radiographic Processing Quality Control requires multidimensional dosimetry measurements to characterize the complex dose distributions. While more accurate and precise systems exist, radiographic film will be used in primary two-dimensional dosimeters at many hospitals. We are implementing a systematic method for acquiring and analyzing measured dose distributions for system and patient-specific quality assurance. An important component of the system will be a film densitometer that acquires the dose distributions with high spatial resolution and good optical density resolution. We have a point densitometer (X-Lite RMI 2-331) that has good optical density dynamic range and noise characteristics, but is impractical for the acquisition of high spatial density dose distribution measurements.

One solution is the purchase of a relatively cost-effective scanning laser digitizer with a good dynamic range, excellent spatial resolution and accuracy, as well as rapid acquisition of images. However, this type of digitizers also exhibits imaging artifacts that needs to be characterized by measurement and to be removed on an image-by-image basis. Such practices are fairly costly. So that in this thesis suitable software is to be developer for automatic scanning densitometry by using a commercial flatbed scanner.

This thesis identifies the need for a convenient, inexpensive, and quantitative scanning system for Radiographic Processing Quality Control measurements and it is decided to investigate the use of a commercial flatbed scanner for film densitometry. The scanner needs to have a transparency mode in which the light source and detectors lay on opposite sides of the film. Inexpensive scanners have a transparency adapter that attaches over the scanning bed to provide the light source. However, the problem concerned is the spatial registration of such a device. Also, less expensive scanners typically have 8 bit analog-to-digital converters, consequently limiting the high optical density resolution.

This thesis selects a moderately priced scanner (Microtek scanmaker 4900) with an option accessory transparency adapter (MTMA, Microtek Magnetic Transparent Media Adapter). The adapter has a single overhead light source for glides down the whole scan bed to capture up to 7inch x 8inch transparency images. The scanner has an internal 48-bit analog-to-digital converter for color and 16-bit analog-to-digital converter for grayscale. Therefore, one of the most important considerations is the maximum usable optical density under a given system noise and digitization.

The optical resolution of the system is 4800 x 2400 dpi (dots per inch), but to allow reasonable image file sizes, pixels resolution should be limited by system settings to 96 dpi (0.265 x 0.265 mm). All tests reported in this thesis were conducted using this setting. No fillers or corrections were selected and an 8-bit grayscale tiff image file format was used as the scanner output. The image produced was analyzed using the LSD image software (Link Scanning Densitometry image software).

CHAPTER 2
OBJECTIVES

In this field of densitometry in Radiographic Processing Quality Control, there is a need for software to manipulate film images easily with flexibility. Such a tool should have the ability to display film-image files without the user having to enhance an image, thus enabling a measurement of optical density from film images.

The role of this thesis is to develop suitable software for a scanning densitometer, which could aid medical technicians and doctors to achieve a rapid diagnosis through LSD image software. Moreover, the time spent on learning how to use the software should be as short as possible with respect to its usefulness.

The works involved are:

- To understand utilities and capabilities of Microsoft Visual C++ .NET.
- To display basic image files, on PC-based system.
- To develop useful image processing algorithms, including curve fitting algorithms used in film image density measurements.
- To build an easy GUI (Graphic User Interface) for users to use and learn.
- To compare value of optical density from scanner with the value from a point densitometer.
- To compare value of optical density from different resolution to check a percentage error of value.

2.1 Benefits Expectable from the Thesis

(i) Convenient and inexpensive measurement.

Reduce the cost of buying any expensive scanning laser digitizer densitometer.

(ii) Fast Analysis densitometry.

Reduce time for analysis and measurement densitometry.

(iii) Quantitative scanning system and Automatic software for Radiographic Processing Quality Control measurements.

(iv) Decided to use for any commercial flatbed scanner.

2.2 Scope of Works

(i) Display image file

(ii) Image processing function in Curve fitting by calibration software

(iii) Display Optical density value

2.3 Expected Results

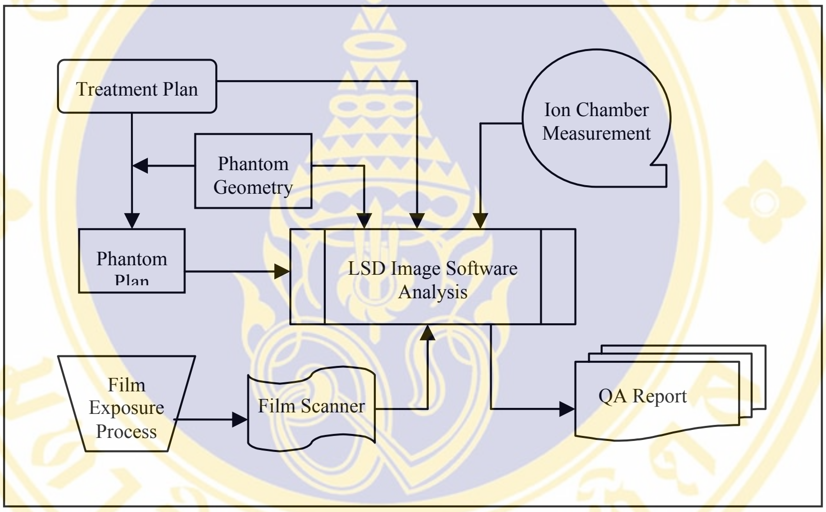


Figure 1: Flow chart of automatic QA

Information on the flowchart in Figure 1 shows the automatic quality assurance process. The measurements include the functions of both ionization chamber and radiographic film. In each case, the registration of the spatial and dose results is conducted automatically due to rigid machining specifications of phantoms The phantom and patient plan data are automatically processed into a form readable by the registration and comparison software, written in Microsoft Visual C++ .NET on a PC-based system. The films are scanned using a commercial flatbed scanner that is attached to the systems. The LSD image software will automatically calibrate the films and align them to the phantom plan dose distribution for analysis.

CHAPTER 3

LITERATURE REVIEW

Overview in Scanning Densitometer

This section provides a brief introduction to densitometry, including the history of scanning densitometer and technology development of its.

3.1 How to Conduct Processor Quality Control

There have long been regulations and standards that govern the amount of radiation that a patient is exposed to by an x-ray machine. The FDA closely regulates manufacturers of x-ray machines, and their factories are inspected on a continuing basis. Once installed at a hospital or clinic, x-ray machines are regularly inspected by state regulators. In many cases, annual inspections are carried out by medical physicists who must report their findings to state officials.

X-ray machines are easy to test and correct. It is interesting to note that inspection of film processors has not generally been required, except in the case of mammography. Why this is so is anyone's guess. It may be because of the large number of parameters that can affect the performance of a film processor. Processors are much more complex than the x-ray machines themselves and it takes a lot more skill and experience to troubleshoot a poor performance.

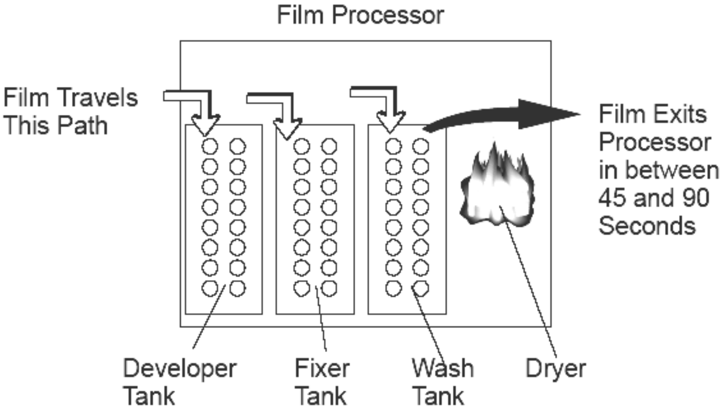


Figure 2: Film Processors

With reference to Figure 2, a few of the elements that can affect film processors can be listed as follows:

(i) Incorrect temperature in any one or more of the tanks.

(ii) Broken gears or worn out rollers that can increase or decrease the speed at which a film passes through the processor.

(iii) Effectiveness of the developer or fixer chemistry. As more and more films pass through a batch of chemicals, the chemicals get used up (exhausted). The older the chemistry, the poorer the developing, though fresh chemistry can also have weaknesses.

(iv) Effectiveness of the dryer.

Film Processor Quality Control is based on very simple procedures: take a picture, develop that picture, and compare it to a standard picture that is decided as ideal. If the most current picture differs greatly from the standard, make corrections to the processing cycle until the picture developed in the "corrected" processor looks like the standard.

To make the standard image one makes a test pattern using a sensitometer. The standard image is called a 21-step filmstrip. If life ware perfect, the sensitometer was perfect, the film was perfect and processed perfectly, and then one would know for sure the absolute values of each of these 21 steps. In real life one just gets close enough, as show in Figure 3.

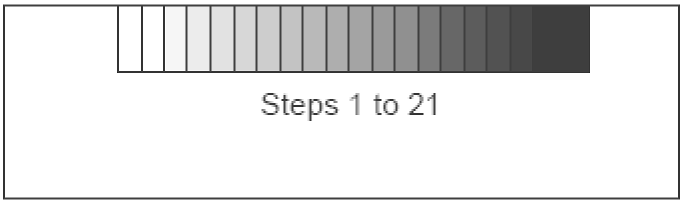


Figure 3: Representation of a Sensitometry Strip with 21 Steps of Measured Densities

The technician conducting quality control will go into the darkroom and, without any light on, will expose a test film using the sensitometer. The technician will then process the test film in the processor. Once processed, a densitometer will be used to measure each step. With a light source, the densitometer measures the amount of light that passes through each increasingly blacker step. Perfectly clear is measured as 0.00 “OD” (optical density). Very black for practical purposes can be between 3.50 OD and 5.00 OD.

Comparing a standard filmstrip to a daily strip would be very time consuming if one had to measure and compare for each of the 21 step. Just think of the time it would be taken day by day by a large hospital with 25 processors! Therefore, over the years, special statistics have been developed to help speed this comparison. Again, refer to Figure 3.

In addition to the OD of each step, there is a need to measure the **Fog**. Also known as **Base** + **Fog**, this measures the density of the non-exposed portion of the sensitometry strip. Remember that “Base” refers to the polyester sheet that is the elemental part of the film structure. The Fog of the Base is never 0.00 OD. Typically it is 0.15 to 0.18, and will be higher when measured after processing. These higher values can be caused by “pre-development” or “pre-exposure” caused by poor manufacturing conditions, or by storing unused film in hot surroundings, careless handling in loading the film in the cassette, or taking the film out prior to processing. If a darkroom has any amount of white light during film loading or processing this too can cause additional exposure to the film.

If the Fog is too high on a film, important details of the patient’s body may be hidden and a new image should be taken.

Average Gradient is a measurement of how quickly the film will turn dark. Quicker film is said to have more “contrast”. This is better when sharper detail is required, such as in breast images. High contrast film is more expensive, because, among other things, it has more silver in the emulsion. Films that have a “wider range” in turning from light to dark are said to have more “latitude”. These films are great for normal images of bones, where large changes in anatomy are easily seen. These films tend to be less expensive and have less silver in the emulsion.

Speed is the final measure to discuss. The speed is known as Relative Sensitivity and Relative Speed, or Mid Density or MD. This measurement is very similar to the ASA of amateur photographic films.

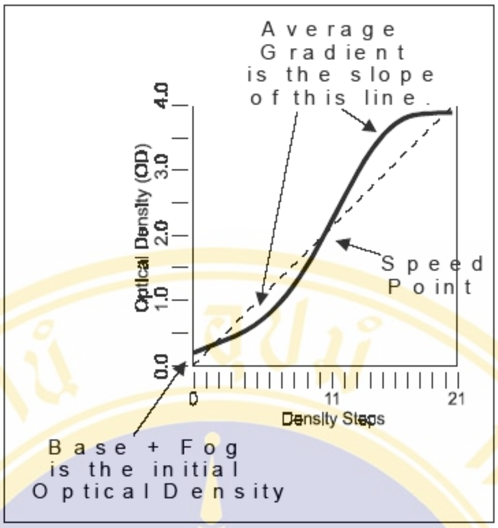


Figure 4: Characteristic Film Curve (3)

Characteristic film curve is shown in Figure 4 for the 21-density steps. The “S” shaped curve is called an “H&D Curve”, “S Curve”, or “Characteristic Film Curve”. The slope of the curve is the measurement of contrast, and the approximate mid point of this curve is the speed step. Note the measurement of fog (Base + Fog).

3.2 Understanding Sensitometers and Densitometers

Sensitometer uses light, not x-rays, to make its pattern on the film. Separate light sources (LED’s) are used to make either a blue or green light.

There is much debate among professionals about the fact that the sensitometer does not mirror the effects of the screens. Although this is true, it is common practice to use the sensitometer as the best estimate of the x-ray processes.

Densitometer is a device for determining the degree of darkening of photographic film or other semitransparent objects, or for measuring the thickness of a film deposited on a surface. Most densitometers make use of a photoelectric cell . In the simplest, a light source is aimed at a photosensitive cell, readings are taken with and without the sample in place, and the density of the sample is derived from the difference in the readings.

3.3 History of Densitometer

About 1950, densitometers began to appear in printing plants. Welch Scientific marketed an electronic model and Kodak marketed one that used an optical wedge for comparison. Both instruments were calibrated in confusing logarithmic units

The Densitometer measures the density of the film. There are both “single-point” reading densitometers and “scanning” densitometers. Until Pehamed introduced the D44 (The Pehamed D44 was the first scanning densitometer. The scanners by X-Rite and Nuclear Associates are just poor copies.) in 1987 there were only single-point readers. It is because making these measures was so time consuming with a single-point reader that good processor QC was rarely done before 1987.

3.4 Medical Film Dosimetry

3.4.1 Densitometry Systems: Evaluation of Radiochromic Film Dose

The term ‘‘dosimetry’’ refers to some form of quantification. If only a qualitative picture of radiation interactions was required, the constraints placed on the digitization of the optical density at a given point on a film would not need to be so rigorous. However, for film dosimetry as a quantitative process, it is ideal to convert the analogue film information into digital data for the ultimate quantification of dose accurately and reproducibly. A digitized film can provide a 2-D dimensional data set describing the film coloration, which has resulted from the dose deposited in that plane. Although theoretically the ultimate limit of resolution is the dimension of each ‘‘activated’’ molecule on the film, practically the spatial resolution of the data set is limited by the sampling rate set in the scanner/densitometer software. Sometimes it is more useful to sample at a rate which is consistent with the data required for a specific patient treatment and the spatial resolution of the planning software used to calculate the patient absorbed dose during therapy. In this way, the planning data set and the dosimetry data set can be superimposed and a comparison made between the expected and actual dose. To maximize the confidence in the measured dose, it is necessary to have a good relationship between the pixel value and the dose, i.e. a correct change in the pixel value for the given required doses. Choosing films with a suitable characteristic curve (i.e. dose response) or by exposing the film appropriately (to the most effective dose) can achieve this, so that the pixel values to be measured are in the linear portion of the dose response curve. The correct bit level of data acquisition is also required for the level of accuracy needed.

3.4.2 Transmitted Light Detectors

The detection system used for analysis can also significantly affect the measured transmitted light and can vary due to characteristics such as linearity of output, signal resolution, sensitivity and spectral sensitivity. Low-sensitivity detectors normally employ solid-state devices, such as photodiodes, which are commonly used for point densitometers in translation-type scanners such as water tank densitometers. Document scanners and high-resolution densitometers mainly use charge-coupled devices (CCD) which can produce a high sensitivity. The spectral sensitivity of a light detector can vary significantly, as shown in Figure 5, which is a manufacturer's specification for a photodiode (6).

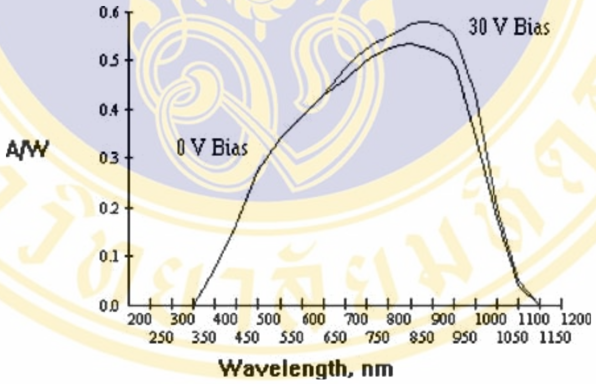


Figure 5: Spectral response [photo sensitivity (A/W) vs. wavelength (nm)] of a common photodiode detector used in radiochromic film point densitometers (7)

Changes in the spectral composition of the incident light as well as color changes produced by the film itself can produce a quantitative effect on the measured optical density. The signal resolution of a detector can significantly affect the desired accuracy of the measurements made. In conjunction with the transmitted intensity, this parameter controls the achievable level of resolution in the output. Intensity resolution is normally not a major problem for devices which output analogue signals, as the signals are read by devices, such as a voltmeter, which will normally have a large scale range, providing adequate signal resolution

3.5 Densitometer in Screen-Printing Process

3.5.1 Problem of Screen-Printing Process

In a perfect world, it would be possible to create artwork, output a film positive, expose a screen, and print the graphic with no loss of image integrity. Unfortunately, the reality is that something usually gets lost when the image progresses from one stage to the next. Dots become larger or smaller. Colors in the printed image do not match the artist's intent. And film positives that appear acceptable make stencils that are difficult to process, print, or reclaim.

3.5.2 Using Densitometer in Printing

Densitometers give the power to quantify quality throughout the screen-printing process. Whether one wants to control film densities from outside sources, match color proofs supplied by a customer, or spot check color consistency in the middle of a production run, densitometry has a place in the workshop. Densitometers can improve prints on any form of substrate, from textiles to compact discs.

3.6 Type of Densitometer

Densitometers are available in two varieties: transmission for reading film positives and reflection for reading printed images.

3.6.1 Transmission Densitometers

Are used to assure the light-stopping densities of the film positive and control the consistency of the image output. A transmission densitometer measures the opacity of the image and transparency of non-image areas of the film, telling whether it will block and transmit enough light to produce a good stencil. The densitometer also measures the tonal range and degree of dot gain in halftone film positives. Uncontrolled dot gain in CMYK film separations will lead to poor color reproduction when the four colors are combined in the printing process.

3.6.2 Reflection Densitometers

Are used to measure reflected light and to gauge color densities of original artwork, photos, proofs, substrates, and printed images. A reflection densitometer can be indispensable in helping one to judge the ink deposit of the prints or solving ink mixing (pigment ratio) problems that lead to shade variances during long print runs. It also distinguishes hue differences by measuring the ratio of CMYK values and detects if any of the inks are over or under strength by identifying problems with gray balance in the 100% coverage areas. Additionally, the device will help compensate for substrate characteristics. If the material features a different gloss, porosity, or shade than the original or initial prints, the reflection densitometer provides numerical data that enable one to compensate for the differences during art creation, film generation, or screen making.

3.7 Related Papers

There was a technical paper in 1995 entitled “Digitizing of radiographs with a flatbed scanner” (8), which implements densitometry with a scanner. An Artix ArtiScan (Artix Technologies Inc., Milpitas, CA, USA) flatbed scanner, with imaging editing software Aldus PhotoStyler (Aldus Corporation, Seattle, WA, USA), was used to digitize a stepped exposure image on Kodak T-MAT G films (Kodak, Rochester, NY, USA). The stepped exposure image was scanned at various locations and with different scanning settings. The pixel values of the resulting digital images were analyzed. The stepped exposure image was also scanned by a laser densitometer to measure the optical densities of the steps on the film for comparison. The resulting pixel value profile was not identical to the optical density profile measured on film. The characteristic curve of this digitizing system was steeper than that of the film. The system was seemingly not calibrated.

For this thesis, data of pixel value will be calibrated by the use of curve fitting before transforming to OD values.

In the year 1997 a paper entitled “Digitizing of radiographs with a roller-type CCD scanner” (1). A roller-type CCD scanner (Drum Scanner) was used for the measurement of OD by the same methodology as used in the previous technical paper.

By using default scanning settings, the digitized images had pixel values distributed in a similar dynamic range to that of the film densitometer. Because Drum

Scanner has a good dynamic range, excellent spatial resolution and accuracy, and rapid acquisition of images ware obtained. Drum Scanner has 0 - 4 OD, and the pixel values obtained are linearly related to transmission values (T).

The roller-type scanner used has a similar dynamic range to that of the film densitometer. The drum scanner, however, is very expensive for use with a scanning laser digitizer, does not have automatic software for analysis. It handled pixel values with imaging editing software Paint Shop Pro (JASC Inc., Minnetonka, Minn.).

It is the aim of this thesis to identify a need for a convenient, inexpensive, and quantitative scanning system for Radiographic Processing Quality Control measurements, to investigate the use of a commercial flatbed scanner for film densitometry, And to develop suitable software for automatic scanning densitometer by using Visual C++ programming language.

The paper on “Toward automated quality assurance for intensity modulated radiation therapy” (2). In 2000 investigated the use of a linear amplifier, to digitize gray-scale images to 12-bit resolution with a user-selected spatial resolution of 0.170 mm^2 pixels. To reduce Newton’s rings artifacts, the standard glass platen was replaced by glass with an antireflective coating. Conversion of reading to transmission was conducted by permanently placing a calibrated photographic step tablet on the scanner platen.

The resulted pixel-to- pixel noise was better than 2% for optical densities, ranging from 0.4 to 2.0, and 0 to 2.7 for the unfiltered and filtered images, respectively. While the document scanners are not as flexible as dedicated film densitometers, these results indicate that by using the intensity and scatter corrections, the system can provide accurate and precise measurements up to an optical density of 2.0, sufficient for routine IMRT(Intensity Modulated Radiation Therapy) film QA.

Nevertheless, the above paper uses MATLAB only, which does not perform automatically in real time, hence the loss in time for technician in analysis and using scatter correction.

This thesis aims at developing software for automatic scanning densitometry. Additionally, on seeing the problem that may be caused by scattering of a common lamp light from transparency tray of scanner, the author plans to use Microtek MTMA

Transparency Adapter that employs a Cold Cathode Lamp for light source that glides down the whole scan bed to capture an image with reduced scattering effects.

3.8 Summary

- Convenient and inexpensive measurement. The cost of buying any expensive scanning laser digitizer densitometer is reduced by using any commercial flatbed scanner.

- Fast Analysis densitometry. Time for analysis and measurements is reduced in densitometry by the development of Automatic Software for Radiographic Processing Quality Control measurements.

CHAPTER 4
MATERIALS AND METHODS

This chapter is divided into two sections: materials (section 4.1) and methods (section4.2). Materials have both hardware resources and software resources. Methods can be subdivided in to 6 steps: Fundamentals of Densitometry, Data Investigation, Mathematical Model Design, Numerical Methods and Programming Design, Finding Optical Density with the Microtek Flatbed Scanner.

4.1 Materials

4.1.1 Hardware resources

4.1.1.1 Notebook Computer

Brand	:	Compaq
Type	:	Presario 1700
CPU	:	Intel Pentium III 800 MHz
Ram	:	256 MB SDRAM
Hard Disk	:	15 GB
Monitor	:	13.3 inch TFT Display

4.1.1.2 Scanner

Brand	:	Microtek
Model	:	Scanmaker 4900
Scanner type	:	Flatbed Scanner
Scanning Element	:	Color CCD
Optical Resolution	:	2400 x 4800 dpi
Max. Resolution	:	65535 dpi(PC)

Scan Area : 8.5 x 14 inch
Color Depth : 48 bit in Color
16 bit in Grayscale
Hardware interface : USB

4.1.1.3 Transparency Adapter

Brand : Microtek
Model : MTMA
Light Source : Cold Cathode Lamp
Warm-up time : 3 min.
Scan Area : 7 x 8 inch

4.1.1.4 Densitometer

Brand : X-Rite
Model : RMI 2-331
Color Response : ANSI Visual
Conforms to ANSI : PH 2.19
Measuring Area : 1.0 mm and
2.0 mm Diameter
Measuring Range : 0 - 3.5D (1mm)
0 – 4.0D (2mm)
Accuracy : Better than ± 0.02D
Repeatability : Better than ± 0.01D
Zero Stability : ± 0.02D per 8 hours max
Scale Factor (Slope) Stability : ± 1% per 6 months max

4.1.1.5 Film Calibration

Brand : X-Rite
Reference to NIST
Standard Material No : 1008
Conforms to conditions : ANSI PH 2.19-1986 and
ISO 5/2-1985

Maximum error	:	± 0.02D
Part No.	:	301-27
Serial No.	:	A095771

4.1.2 Software resources

Operating System	:	Microsoft Window XP
Compiler	:	Microsoft Visual C++ .NET
Mathematic Computing	:	MATLAB V. 6.1.0.450 Release 12.1
		Microsoft Office 2003

4.2 Methods

4.2.1 Fundamentals of Densitometry

It is possible to use a scaling system for pixels with a one to one correspondence to the concentration in use. Sample concentrations can be determined by using optical, electronic and most importantly for practical purposes, a computer based imaging technique. Densitometry science was described originally by Bouguer and Lambert who described loss of radiation (or light) in passing through a medium. Later, Beer found that the radiation loss in a media was a function of the substance's molarities or concentration. According to Beer's law, concentration is proportional to optical density (OD). The logarithmic optical density scale and net integral of density values for an object in an image is the proper measure for use in quantization. By Beer's law (9), the density of a point is the log ratio of incident light upon it and transmitted light through it, i.e.

$$OD = \mathrm{Log}10\ (I_o / I)$$

4.2.2 Data Investigation

In this step, related information was collected from many sources such as books, journals, electronic data, and so on, examples of data are:

- Information of Densitometer
- Information of Visual C++ .NET
- Information of Digital Image Processing

4.2.3 Mathematical Model Design

Before developing a mathematical model, program specification will be determined. Mathematical Model design can be divided into 2 steps, in respect of conception and relation.

4.2.3.1 Concepts

A Microtek flatbed scanner with imaging editing software Adobe Photo Shop was used to digitize a stepped exposure image on Kodak T-MAT G films (Kodak, Rochester, NY, USA). The stepped exposure image was scanned at various locations and with different scanning settings. The pixel values of the resulting digital images were analyzed. The stepped exposure image was also scanned by a point densitometer to measure the optical densities of the steps on the film for comparison purposes.

4.2.3.2 Relation

There are several standard methods used to find the density of an object or a point on an image. Scanning densitometers have controlled or known illumination levels to measure transmitted light through an object such as a X-Ray Film. Since both the incident and transmitted light are known quantities, the device can then compute their ratio directly. This is also the case of those who use a flat field imaging technique to capture two separate images. The first image is of an empty light box and the second is of the specimen to be evaluated. These two can then be used in computing a log ratio.

In the case of a scanner/frame grabber combination, using a non flat field technique, several things are of note. With a scanner, OD values are not measured directly. The scanner and frame grabber pixel values are linear with respect to Transmission (*T*), which is the anti-log of the negative of *OD*:

$$T = 10^{-OD}$$

Or:

$$OD = -\log10\ (T) = \log10\ (1 / T)$$

Since this is often a source of confusion among those designing systems for densitometry one should again notes that the scanner does not measure *T*, nor does it measure OD. Scanner systems, CCD, and any frame grabber conversion values (pixel values) have been designed so that they are linear with respect to *T*. It is not meaningful to take the minus log of the pixel value since these are not T values.

Nevertheless, this project wants to do densitometry and need a scale (not pixel values) which correlates to concentration or OD. Further, it may not be convenient to measure the incident light and do a log ratio.

Fortunately, LSD image software can use an external standard, such as an OD step tablet or a set of protein standards on a gel. Software has the built-in “Calibrate...” command to allow a direct transformation of pixel values from a scale which is linear with respect to T and into a scale which correlates to OD or concentration. The calibrate command, used with standards, is best done with an exponential or other fit since the relationship of OD to T is not a simple linear ($Y = m*X + b$) relationship and because any scanners may not be perfectly linear with respect to T over the range of density values for use as standards.

4.2.4 Numerical Methods

In this case Least Squares Fitting methods will be used to establish the relationship between OD (Optical Density) and PV (Pixel value). The following sub-sections describe 4 types of fitting.

4.2.4.1 Least Squares Fitting: Straight Line

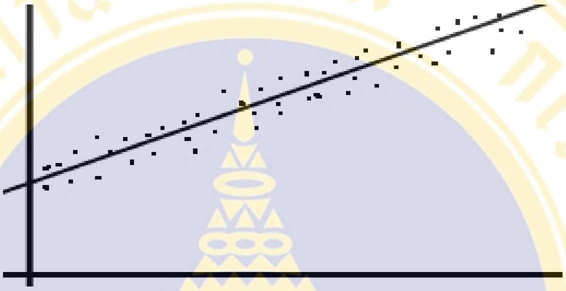


Figure 6: Least Squares Fitting Curve

The mathematical procedure for finding the best fitting curve to a given set of points involves minimizing the sum of the squares of the offsets ("the residuals") of the points from the curve. The sum of the squares of the offsets is used instead of the offset absolute values because this allows the residuals to be treated as a continuous differentiable quantity. However, because squares of the offsets are used, outlying points can have a disproportionate effect on the fit, a property which may or may not be desirable depending on the problem at hand.

Figure 6 shows a typical straight-line fitting to a given data set, while Figures 7 and 8 demonstrate possible offsets that may be used in the fitting.

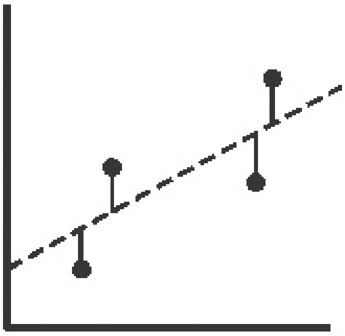


Figure 7: Vertical Offsets Fitting Method

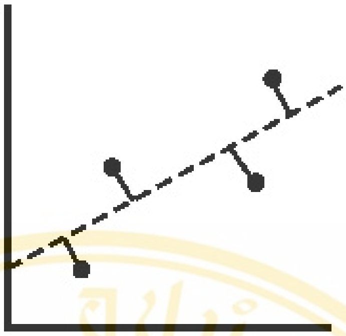


Figure 8: Perpendicular Offsets Fitting Method

In practice, the vertical offsets from a line (polynomial, surface, hyperplane, etc.) are almost always utilized instead of the perpendicular offsets. This provides a fitting function for the independent variable x that estimates y for a given x (most often what an experimenter wants), allows uncertainties of the data points along the x- and y-axes to be incorporated simply, and also provides a much simpler analytic form for the fitting parameters than would be obtained using a fit based on perpendicular offsets. In addition, the fitting technique can be easily generalized from a best-fit line to a best-fit polynomial when sums of vertical distances are used. In any case, for a reasonable number of noisy data points, the difference between vertical and perpendicular fits is quite small.

Vertical least squares fitting proceeds by finding the sum of the squares of the vertical deviations R^2 of a set of n data points

$$R^2 \equiv \sum [y_i - f(x_i, a_1, a_2, \ldots.., a_n)]^2$$

From a function f. Note that this procedure does not minimize the actual deviations from the line (which would be measured perpendicular to the given function). In addition, although the un-squared sum of distances might seem a more appropriate quantity to minimize, use of the absolute value results in discontinuous derivatives which cannot be treated analytically. The square deviations from each point are therefore summed, and the resulting residual is then minimized to find the best fit line. This procedure results in outlying points being given disproportionately large weighting.

The condition for $R^2$to be a minimum is that

$$\frac{\partial(R^2)}{\partial a_i}=0 \qquad\qquad \text{for i=1, n.}$$

For a linear fit:

$$f(a,b)=a+bx$$

so

$$R^2(a,b)\equiv\sum_{i=1}^{n}[y_i-(a+bx_i)]^2 \quad .$$

Hence

$$\frac{\partial(R^2)}{\partial a_i}=-2\sum_{i=1}^{n}[y_i-(a+bx_i)] \;=0 \quad ,$$

$$\frac{\partial(R^2)}{\partial b_i}=-2\sum_{i=1}^{n}[y_i-(a+bx_i)] \;=0 \quad .$$

These lead to the equation

$$\sum_{i=1}^{n}y_i=na+b\sum_{i=1}^{n}x_i$$

which becomes

$$\sum_{i=1}^{n}x_iy_i=a\sum_{i=1}^{n}x_i+b\sum_{i=1}^{n}x_i^2$$

where

$$a=\frac{\bar{y}(\sum_{i=1}^{n}x_i^2)-\bar{x}(\sum_{i=1}^{n}x_iy_i)}{\sum_{i=1}^{n}x_i^2-n\bar{x}^2} \quad ,$$

$$b=\frac{(\sum_{i=1}^{n}x_iy_i)-n\bar{x}\cdot\bar{y}}{\sum_{i=1}^{n}x_i^2-n\bar{x}^2} \quad .$$

4.2.4.2 Least Squares Fitting: Exponential

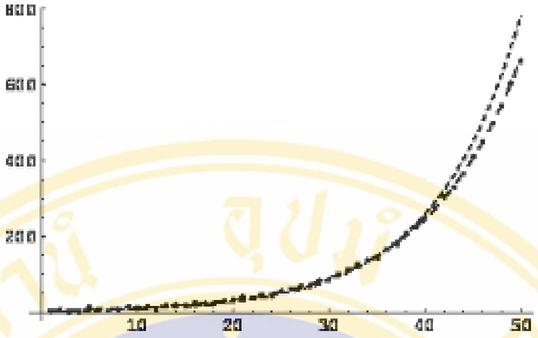


Figure 9: Exponential Curve

To fit a functional form for exponential fitting of data, shown typically in Figure 9, one uses: $y = Ae^{Bx}$.

Take the logarithm of both sides

$$\ln y = \ln A + Bx \quad .$$

The best-fit values are then

$$a = \frac{\sum_{i=1}^{n} \ln y_i \sum_{i=1}^{n} x_i^2 - \sum_{i=1}^{n} x_i (\sum_{i=1}^{n} x_i \ln y_i)}{n\sum_{i=1}^{n} x_i^2 - (\sum_{i=1}^{n} x_i)^2} \quad ,$$

$$b = \frac{n(\sum_{i=1}^{n} x_i \ln y_i) - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} \ln y_i}{n\sum_{i=1}^{n} x_i^2 - (\sum_{i=1}^{n} x_i)^2} \quad ,$$

where $B \equiv b$ and $A \equiv \exp(a)$.

4.2.4.3 Least Squares Fitting: Power Law

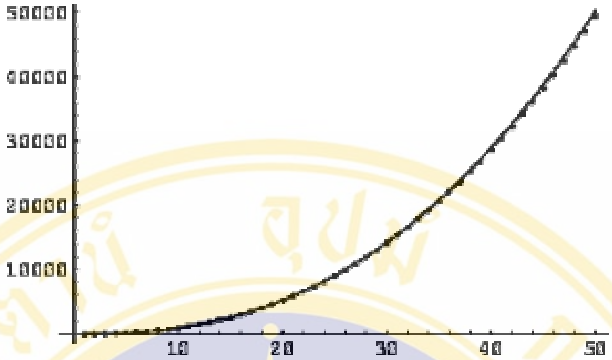


Figure 10: Power Law Curve

Given a function of the form of power law fitting of data, shown typically in Figure 10, one uses:

$$y = Ax^B$$

Least squares fitting give the coefficients as

$$b = \frac{n(\sum_{i=1}^{n} \ln x_i \ln y_i) - \sum_{i=1}^{n} \ln x_i \sum_{i=1}^{n} \ln y_i}{n\sum_{i=1}^{n} (\ln x_i)^2 - (\sum_{i=1}^{n} \ln x_i)^2} ,$$

$$a = \frac{\sum_{i=1}^{n} \ln y_i - b\sum_{i=1}^{n} \ln x_i}{n} ,$$

where $B \equiv b$ and $A \equiv \exp(a)$.

4.2.4.4 Least Squares Fitting: Logarithmic

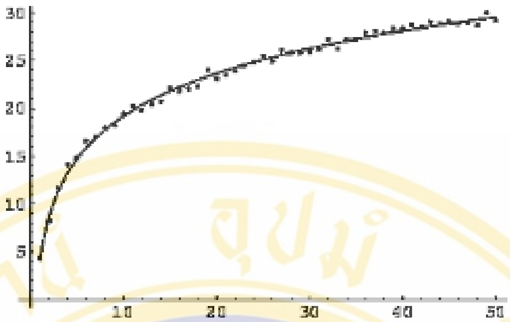


Figure 11: Logarithmic Curve

Given a function of the form of logarithmic fitting of data, shown typically in Figure 11, one uses:

$$y = a + b \ln x \qquad .$$

The coefficients can be found from least squares fitting as

$$b = \frac{n\left(\sum_{i=1}^{n} y_i \ln x_i\right) - \sum_{i=1}^{n} \ln x_i \sum_{i=1}^{n} y_i}{n \sum_{i=1}^{n} (\ln x_i)^2 - \left(\sum_{i=1}^{n} \ln x_i\right)^2} \qquad ,$$

$$a = \frac{\sum_{i=1}^{n} y_i - b \sum_{i=1}^{n} \ln x_i}{n} \qquad .$$

4.2.4.5 Correlation Coefficient

The correlation coefficient is a quantity which gives the quality of a least squares fitting to the original data. To define the correlation coefficient, first consider the sum of squared values SS_{xx}, SS_{xy} and SS_{yy} of a set of n data points (x_i, y_i) about their respective means as follows:

$$SS_{xx} = \sum_{i=1}^{n} x_i^2 - n\bar{x}^2 \quad ,$$

$$SS_{yy} = \sum_{i=1}^{n} y_i^2 - n\bar{y}^2 \quad ,$$

$$SS_{xy} = \sum_{i=1}^{n} x_i y_i - n\bar{x}\cdot\bar{y} \quad .$$

For linear least squares fitting, the coefficient b in

$$y = a + bx$$

is given by

$$b = \frac{SS_{xy}}{SS_{xx}}$$

and the coefficient b' in

$$x = a' + b'y$$

is given by

$$b' = \frac{n\sum xy - \sum x \sum y}{n\sum y^2 - (\sum y)^2} \quad .$$

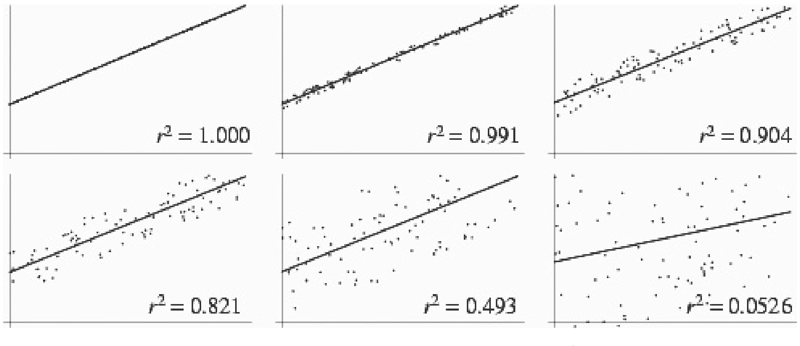


Figure 12: Trend of Correlation Coefficient (r^2) Approximate to 1

The correlation coefficient r^2 (sometimes also denoted by R^2) is then defined by

$$r \equiv \sqrt{bb'} = \frac{n\sum xy - \sum x \sum y}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

which may be written more simply as

$$r^2 = \frac{{SS_{xy}}^2}{SS_{xx}SS_{yy}} \quad .$$

The correlation coefficient is also known as the product-moment coefficient of correlation or Pearson's correlation. The correlation coefficients for linear fits to increasingly noisy data are shown typically in Figure 12 above.

Let $\hat{y}_i$ be the vertical coordinate of the best-fit line with *x*-coordinate x_i, so

$$\hat{y}_i \equiv a + bx_i \quad .$$

Then the error (deviation) between the actual vertical point y_i and the fitted point is given by

$$e_i \equiv y_i - \hat{y}_i \quad .$$

4.2.5 Programming Design

Microsoft Visual C++ .NET is chosen for this project. Because the C++ is one of several new languages that use a programming style called Object-Oriented Programming. To write large software, which is correct, readable, modifiable, affordable and efficient, requires the same creative effort and persistence characteristic as other endeavors in science and engineering. In addition, the C++ programming language provides many mechanisms for expressing abstractions and relationships among them. Some of these, like subroutines and data structures, are provided in languages widely used in scientific and engineering programming.

4.2.5.1 Flow Diagrams

Flow diagrams are given to display design of software, which is subdivided into 3 major paths: Operation software, Calibration path and Read OD path.

4.2.5.1.1 Operation Software

This section displays major steps of program as shown in Figure 13 below.

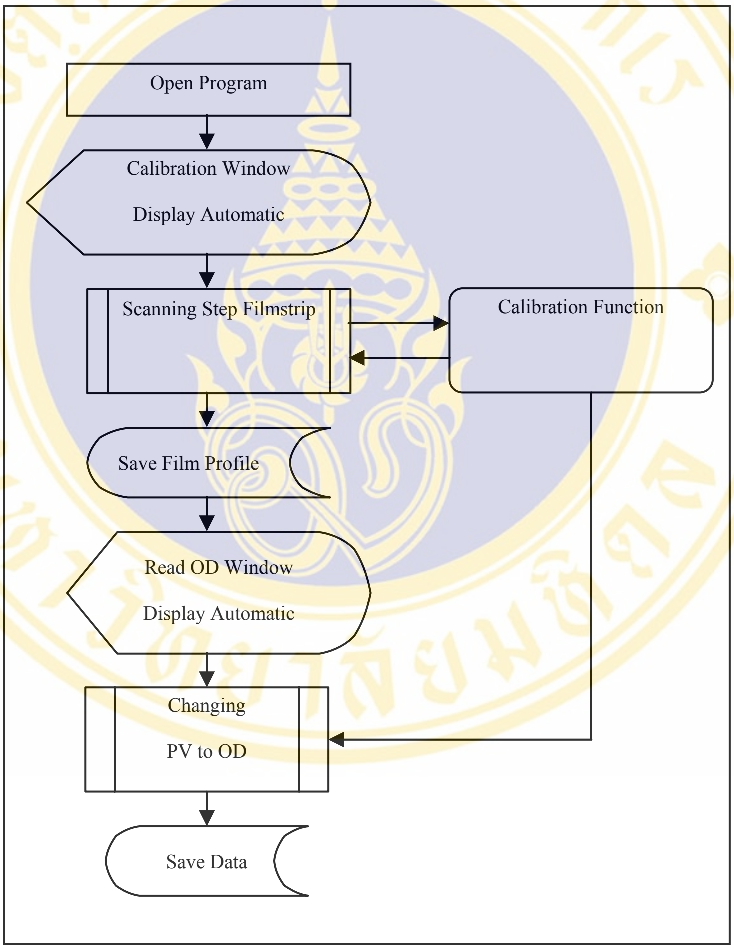


Figure 13: Flow Chart of LSD Operating Software

4.2.5.1.2 Calibration Path

This section displays Calibration Function in program as shown in Figure 14 below.

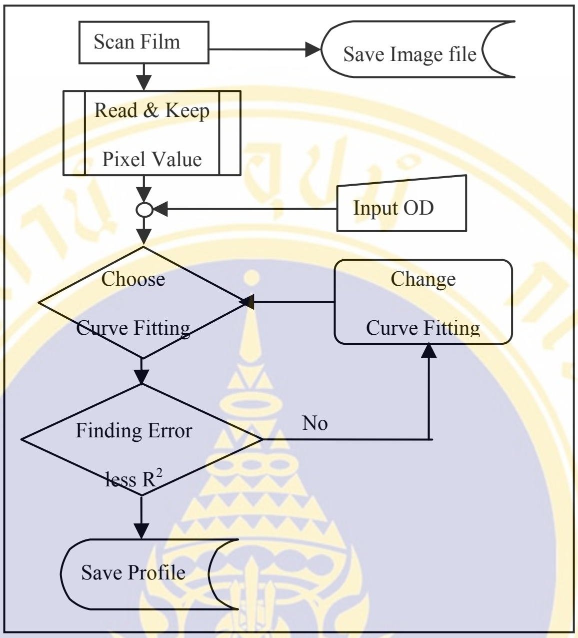


Figure 14: Flow Chart in Calibration path of LSD software

4.2.5.1.3 Read OD Path

Read OD path as shown in Figure 15 will read profile from file and keep variable in buffer, followed by reading pixel value from image and calling function to change PV to OD.

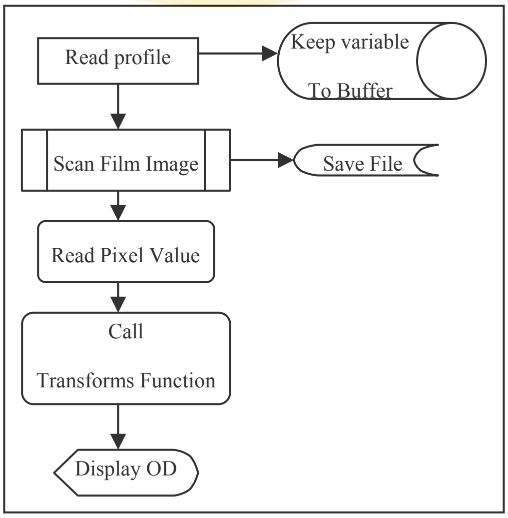


Figure 15: Flow Chart in Read OD path of LSD Software

4.2.5.2 Software Function

Software in this program is designed to have functions at least as follows:

- Import image from Scanner.
- Read Pixel value from position of mouse pointer
- Calibrate scanner by using curve fitting function.
- Save profile of scanner by keeping data type of curve fitting and variable of equation in buffer.
- Open data profile and change global value of function for display OD.
- Read pixel value to OD.

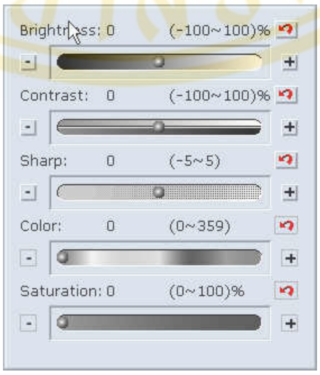
4.2.6 Finding Optical Density with the Microtek Flatbed Scanner

There are 20 steps involved in the finding of OD value with the use of Microtek flatbed scanner:

Step 1. Open Adobe Photoshop v. CS to put the sample to be scanned into the flatbed scanner and place the MTMA on scanner.

Step 2. Go to the File menu, then to Import, and then select Microtek Scan Wizard 5.

Step 3. A window titled Scanner Control will appear. Adjust the scanner settings until they match the ones shown in Figure 16.



Brightness: 0 (-100~100)%

Contrast: 0 (-100~100)%

Sharp: 0 (-5~5)

Color: 0 (0~359)

Saturation: 0 (0~100)%

Figure 16: Microtek Scan Wizard 5 Default Setting

Step 4. Once the scanner settings have been selected, click on the Preview button at the top left of the Scanner Control box (see Figure 17). This will bring up a box titled

Preview Image. There should be an image of the sample and a box with dotted lines. The area inside the box is the final scan area. If the box is not present, go to the toolbar and click on the button with the box with dotted lines on it.

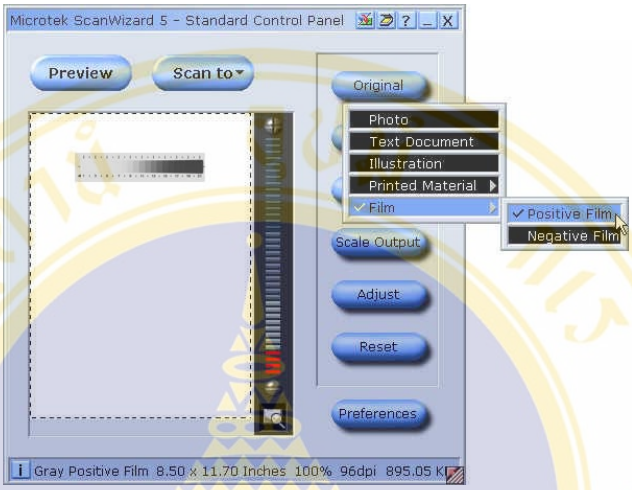


Figure 17: Preview Image in Microtek Scan Wizard5

Step 5. By clicking and dragging the dotted lines, adjust the final scan area so that it encompasses the desired part of the sample.

Step 6. Click on the Scan button in the Scanner Control menu.

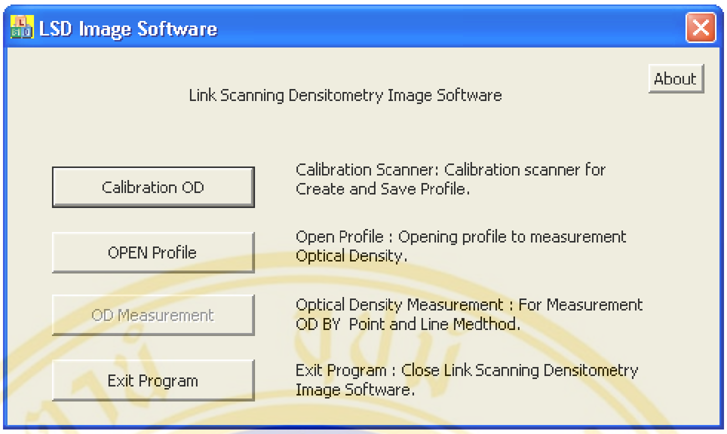
Step 7. There should now be an image of the sample and a toolbar visible on the screen. At the top left of the toolbar there is a button showing a box with dotted lines. Click on this button.

Step 8. Now go to the image window. By clicking and dragging the mouse, make a box around the image of the sample. Now go to the menu bar at the top of the screen, click on Image, and select Crop.

Step 9. This should produce an image of the area previously enclosed in the box. The image will be the one used to calculate the optical density, so make sure it is satisfactory. If the image is unsatisfactory, discard it and go back to Step 2.

Step 10. If the image of the sample is satisfactory, go to the menu bar, click on File, and select Save. Name the image, select the TIFF format, and click on the Save button.

Step 11. Quit Adobe Photoshop. Now open LSD Image Software as shown in Figure 18.



Link Scanning Densitometry Image Software

Calibration Scanner: Calibration scanner for Create and Save Profile.

Open Profile : Opening profile to measurement Optical Density.

Optical Density Measurement : For Measurement OD BY Point and Line Medthod.

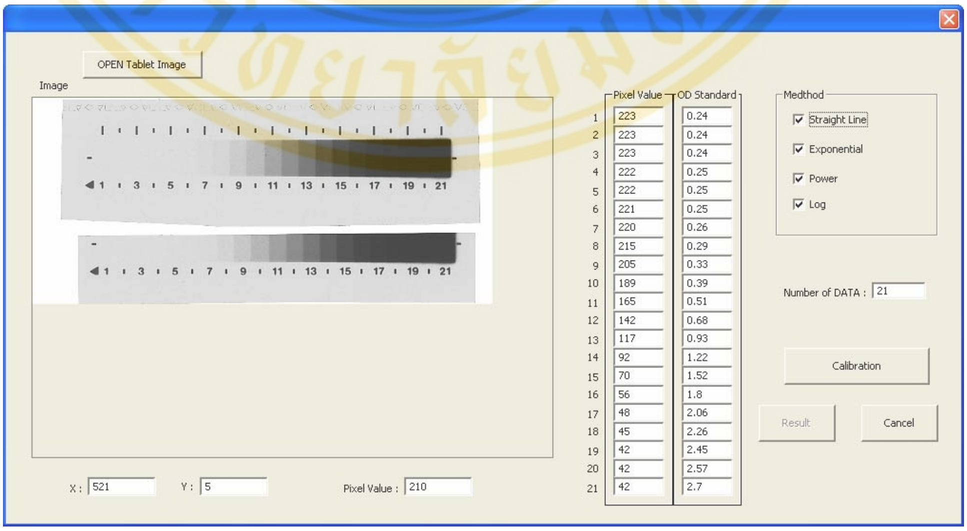
Exit Program : Close Link Scanning Densitometry Image Software.

Figure 18: LSD Image Software Start User Interface

Step 12. Go to the Calibration OD menu, click on Open, select the image saved in Step 10, and click on the Open button.

Step 13. This should once again bring up an image of the sample and a toolbar. At the right side of the calibration interface (see Figure 19).

Step 14. By clicking the mouse in the image window, a pixel value of the sample will appear in Pixel Value box.



X : 521 Y : 5 Pixel Value : 210

	Pixel Value	OD Standard
1	223	0.24
2	223	0.24
3	223	0.24
4	222	0.25
5	222	0.25
6	221	0.25
7	220	0.26
8	215	0.29
9	205	0.33
10	189	0.39
11	165	0.51
12	142	0.68
13	117	0.93
14	92	1.22
15	70	1.52
16	56	1.8
17	48	2.06
18	45	2.26
19	42	2.45
20	42	2.57
21	42	2.7

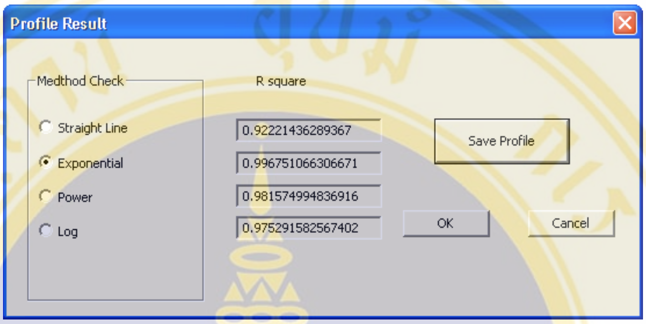
Medthod
☑ Straight Line
☑ Exponential
☑ Power
☑ Log

Number of DATA : 21

Figure 19: Calibration User Interface of LSD Image Software

Step 15. Go to the table pixel value and input pixel value from Pixel Value box, then input OD value from X-RITE densitometer in table OD standard, so repeat until end of data and input number of data.

Step 16. Select Methods, and click Calibration button, then click Result button. This will bring up a small window called Result (see Figure 20).



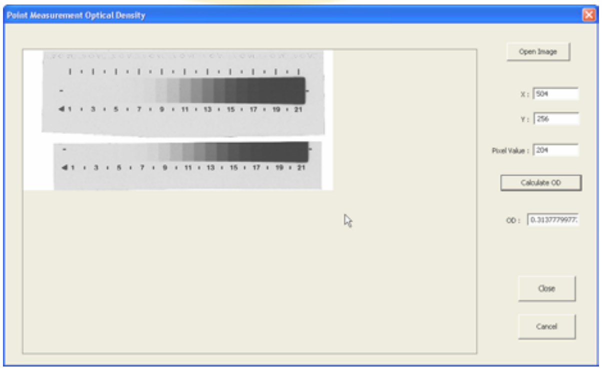
○ Straight Line 0.92221436289367
● Exponential 0.996751066306671
○ Power 0.981574994836916
○ Log 0.975291582567402

Figure 20: Result Interface of LSD Software

Step 17. In this small window, there will be several values of correlation coefficient (R^2) , choose method for R^2 value approximate 1 and click Save Profile button to keep this method to buffer memory of program, then click OK button to first user interface.

Step 18. Go to the OD Measurement menu, click on Open, select the image(want to measure OD) as saved in Step 10, and click on the Open button.

Step 19. By clicking the mouse in the image window, a pixel value of the sample will appear in Pixel Value box (see Figure 21).



X : 504
Y : 256
Pixel Value : 204
OD : 0.31377799977

Figure 21: Measurement OD Interface of LSD Image Software

Step 20. Click Calculate OD, The Optical density value will appear in OD box.

CHAPTER 5

RESULTS

Using Scanner measurement step tablet standard will establish the relationship between Pixel Value and OD.

Find relation of equation curve fitting function. Keep type function by Save in profile file of program. Open profile file again to keep all to buffer memory of program. Scan film for measurement, read pixel value to OD, and save it.

5.1 Densitometer Calibration

X-RITE densitometer model RMI2-331 is used with X-RITE film calibration serial number A095771 to calibrate a device(X-RITE densitometer) and measure OD Standard in film step tablet created from sensitometer. Repeat film calibration 3 times before comparing OD value reading with OD value from factory(X-RITE film calibration).

Table 1: Compare OD Reading with OD from Factory of X-RITE Densitometer

Step No.	OD from factory	Measurement No.		
		1	2	3
1	0.034	0.040	0.040	0.040
2	0.242	0.240	0.250	0.250
3	1.424	1.420	1.430	1.430
4	2.870	2.850	2.860	2.860
5	3.640	3.610	3.620	3.610

From Table 1: difference and percentage of difference value can then be obtained as shown in Table 2.

Table 2: Difference and Percentage Difference of X-RITE Densitometer

Difference			Percentage Difference No.		
1	2	2	1	2	3
-0.006	-0.006	-0.006	-17.647	-17.647	-17.647
0.002	-0.008	-0.008	0.826	-3.306	-3.306
0.004	-0.006	-0.006	0.281	-0.421	-0.421
0.020	0.010	0.010	0.697	0.348	0.348
0.030	0.020	0.030	0.824	0.549	0.824

Difference of OD values in the range of -0.006 to 0.03 is obtainable with percentage difference in the range -17.647 to 0.824 percent.

First percentage difference at step number 1 is -17.647. It has high percentage difference but low difference of about 0.006, which is acceptable.

OD value reading from X-RITE can be used to replace OD standard for analysis.

5.2 Analysis

The Microtek flatbed scanner was characterized by a step tablet as shown in Figure 22, consisting of several sections of known optical densities. The step tablet was placed in the scanner and scanned as a MTMA (Magnetic Transparent Media Adapter) Gray image at 50, 96 and 150 dpi with setting as default by the brightness, contrast, sharp, color and saturation at 0.

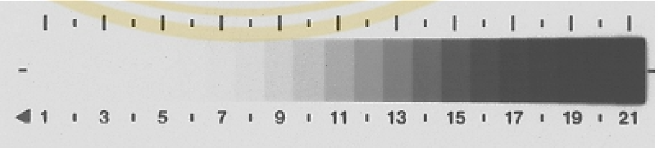


Figure 22: Film Step Tablet.

The image produced was analyzed by using the LSD Image software with the following results in Table 3.

Table 3: Pixel Values at 50, 96 and 150 dpi from Film Step Tablet Images

step no.	OD Std	Pixel Value At		
		50 dpi	96 dpi	150dpi
1	0.24	225	223	223
2	0.24	225	223	223
3	0.24	225	223	223
4	0.25	225	222	222
5	0.25	224	222	222
6	0.25	224	221	221
7	0.26	221	220	220
8	0.29	218	215	216
9	0.33	208	205	207
10	0.39	192	189	190
11	0.51	167	165	166
12	0.68	145	142	143
13	0.93	119	117	118
14	1.22	93	92	92
15	1.52	71	70	71
16	1.80	58	56	57
17	2.06	49	48	49
18	2.26	43	45	45
19	2.45	42	42	44
20	2.57	42	42	43
21	2.70	42	42	44

Uniformity of measurement may be seen read by when plotted as shown in Figure 23.

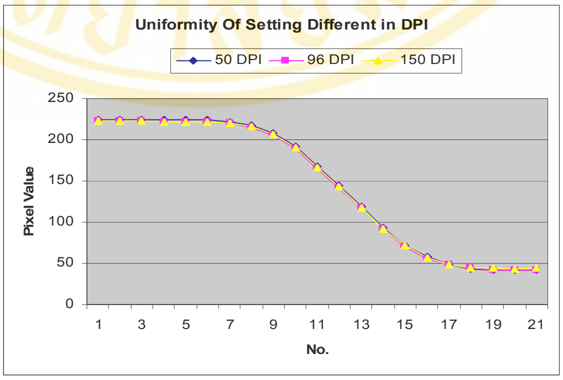


Figure 23: Uniformity of Pixel Value Measurement at 50, 96 and 150 DPI by LSD Software.

5.2.1 Scanning at 50 DPI

From Table 3, pixel values for the scanning at 50dpi can be used to calculate OD by the use of LSD software and can display as shown in Table 4 for OD calculation, difference, and percentage difference.

Table 4: Table of OD Measurements at 50 DPI by LSD Software

no.	Pixel value at 50 dpi	OD Standard	OD Calculation	Difference	Percentage Difference
1	225	0.24	0.249	-0.009	-3.9313
2	225	0.24	0.249	-0.009	-3.9313
3	225	0.24	0.249	-0.009	-3.9313
4	225	0.25	0.249	0.001	0.2259
5	224	0.25	0.253	-0.003	-1.0105
6	224	0.25	0.253	-0.003	-1.0105
7	221	0.26	0.262	-0.002	-0.7812
8	218	0.29	0.272	0.018	6.2436
9	208	0.33	0.308	0.022	6.8093
10	192	0.39	0.375	0.015	3.9710
11	167	0.51	0.510	0.000	0.0883
12	145	0.68	0.668	0.012	1.7460
13	119	0.93	0.920	0.010	1.0435
14	93	1.22	1.268	-0.048	-3.9048
15	71	1.52	1.662	-0.142	-9.3515
16	58	1.80	1.951	-0.151	-8.3752
17	49	2.06	2.179	-0.119	-5.7972
18	43	2.26	2.347	-0.087	-3.8306
19	42	2.45	2.376	0.074	3.0346
20	42	2.57	2.376	0.194	7.5622
21	42	2.70	2.376	0.324	12.0129

The measured optical density values and pixel value at 50 dpi in Table 4 were used for an exponential curve fitting with

$$OD = Ae^{B(PV)} \qquad ,$$

where A and B are coefficients of equation and $R^2 = 0.996751$ was chosen.

The exponential curve fitting calculations give:

A = 3.992708

B = -0.012468 ,

and hence $$OD = 3.992708e^{(-0.012468)PV} \qquad ,$$

where PV is the pixel value of the image and OD is the optical density of the step in the step tablet. Results of OD calculation were plotted together with the plot of OD standard values pixel values as shown in Figure 24.

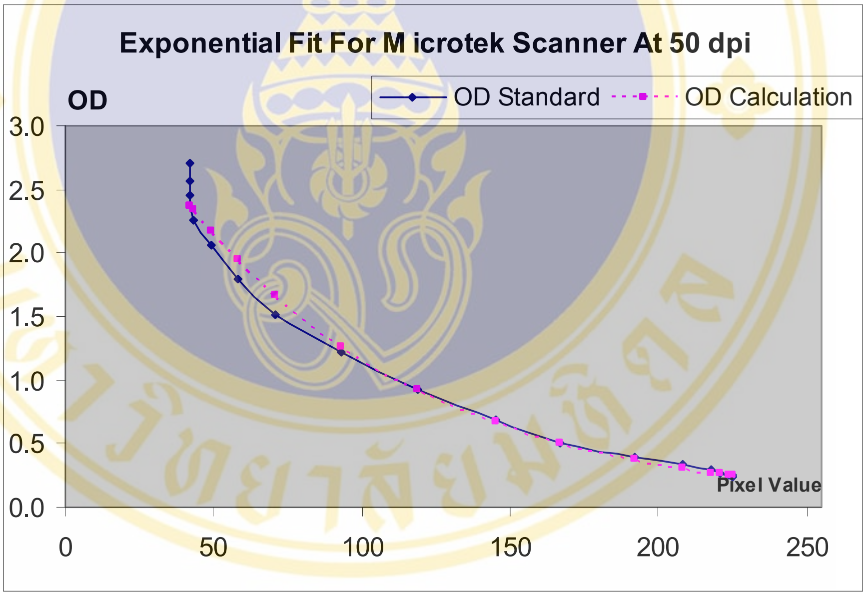


Figure 24: Exponential Fit For Microtek Scanner at 50 dpi

As a check, this line fit was used to calculate the optical density from the pixel measurement data. Results of difference and percentage difference were also displayed in Table 4 above.

Difference value is between -0.151 and 0.324 as seen in Figure 25 below, where difference value were plotted against pixel values at 50 dpi.

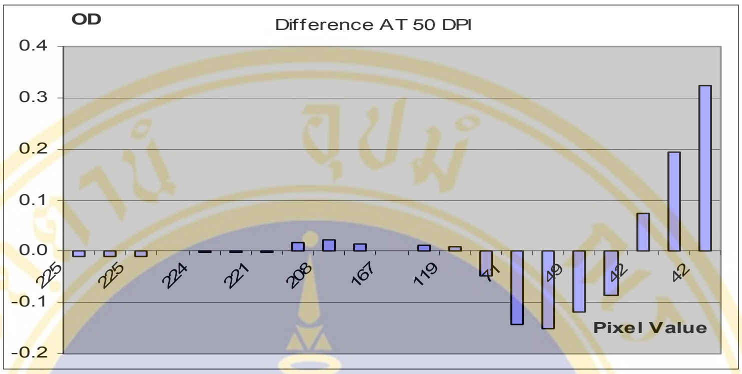


Figure 25: Difference of Exponential Fit Function at 50 DPI

The percentage difference value is between -9.3515 and 12.0129 as seen in Figure 26 below, where difference value were plotted against pixel values at 50 dpi.

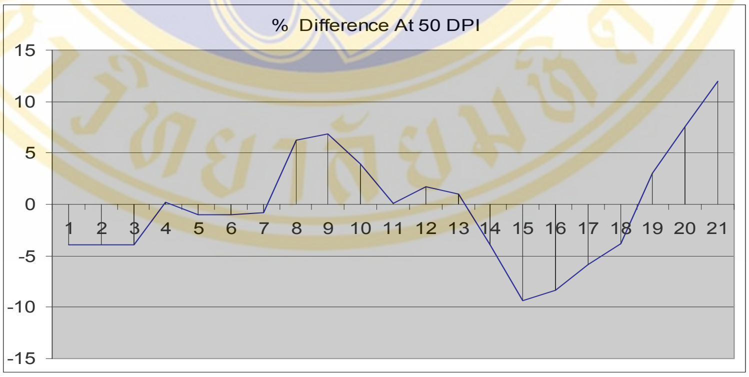


Figure 26: Percentages Difference of Exponential Fit Function at 50 DPI

5.2.2 Scanning at 96 DPI (screen resolution)

From Table 3, pixel values for the scanning at 96dpi can be used to calculate OD by the use of LSD software and can display as shown in Table 5 for OD calculation, difference, and percentage difference.

Table 5: Table of OD Measurements at 96 DPI by LSD Software

no.	Pixel value at 96 dpi	OD Standard	OD Calculation	Difference	Percentage Difference
1	223	0.240	0.248	-0.008	-3.1715
2	223	0.240	0.248	-0.008	-3.1715
3	223	0.240	0.248	-0.008	-3.1715
4	222	0.250	0.251	-0.001	-0.2872
5	222	0.250	0.251	-0.001	-0.2872
6	221	0.250	0.254	-0.004	-1.5454
7	220	0.260	0.257	0.003	1.1352
8	215	0.290	0.274	0.016	5.6610
9	205	0.330	0.310	0.020	6.0876
10	189	0.390	0.378	0.012	2.9917
11	165	0.510	0.510	0.000	-0.0594
12	142	0.680	0.680	0.000	0.0324
13	117	0.930	0.928	0.002	0.1714
14	92	1.220	1.268	-0.048	-3.9316
15	70	1.520	1.668	-0.148	-9.7461
16	56	1.800	1.986	-0.186	-10.3487
17	48	2.060	2.195	-0.135	-6.5346
18	45	2.260	2.278	-0.018	-0.8077
19	42	2.450	2.365	0.085	3.4659
20	42	2.570	2.365	0.205	7.9734
21	42	2.700	2.365	0.335	12.4043

The measured optical density values and pixel value at 96 dpi in Table 5 were used for an exponential curve fitting with

$$OD = Ae^{B(PV)} \quad ,$$

where A and B are coefficients of equation and $R^2 = 0.996816$ was chosen.

The exponential curve fitting calculations give:

$A = 3.985025$

$B = -0.012316$

and hence $$OD = 3.985025e^{(-0.012316)PV} \quad ,$$

where PV is the pixel value of the image and OD is the optical density of the step in the step tablet. Results of OD calculation were plotted together with the plot of OD standard values pixel values as shown in Figure 27.

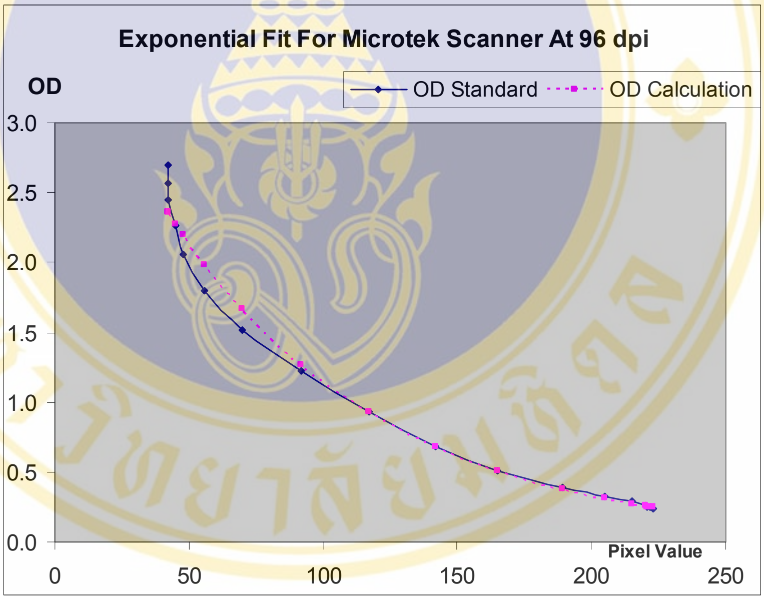


Figure 27: Exponential Fit For Microtek Scanner at 96 dpi

As a check, this line fit was used to calculate the optical density from the pixel measurement data. Results of difference and percentage difference were also displayed in Table 5 above.

Difference value is between -0.186 and 0.335 as seen in Figure 28 below, where difference value were plotted against pixel values at 96 dpi.

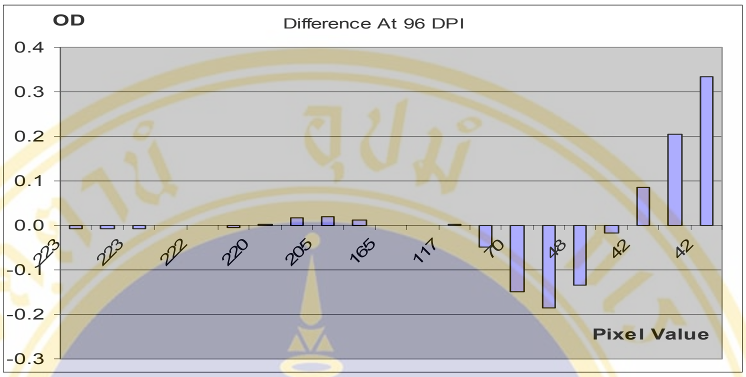


Figure 28: Difference of Exponential Fit Function at 96 DPI

The percentage difference value is between -10.3487 and 12.4043 as seen in Figure 29 below, where difference value were plotted against pixel values at 96 dpi.

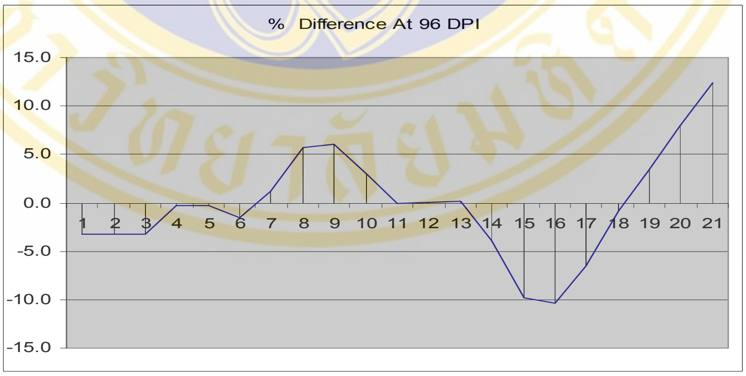


Figure 29: Percentages Difference of Exponential Fit Function at 96 DPI

5.2.3 Scanning at 150 DPI

From Table 3, pixel values for the scanning at 150dpi can be used to calculate OD by the use of LSD software and can display as shown in Table 6 for OD calculation, difference, and percentage difference.

Table 6: Table of OD Measurements at 150 DPI by LSD Software

no.	Pixel value at 150dpi	OD Standard	OD Calculation	Difference	Percentage Difference
1	223	0.240	0.249	-0.009	-3.669
2	223	0.240	0.249	-0.009	-3.669
3	223	0.240	0.249	-0.009	-3.669
4	222	0.250	0.252	-0.002	-0.776
5	222	0.250	0.252	-0.002	-0.776
6	221	0.250	0.255	-0.005	-2.046
7	220	0.260	0.258	0.002	0.643
8	216	0.290	0.272	0.018	6.347
9	207	0.330	0.304	0.026	7.882
10	190	0.390	0.376	0.014	3.567
11	166	0.510	0.508	0.002	0.409
12	143	0.680	0.677	0.003	0.382
13	118	0.930	0.926	0.004	0.391
14	92	1.220	1.283	-0.063	-5.146
15	71	1.520	1.669	-0.149	-9.773
16	57	1.800	1.988	-0.188	-10.456
17	49	2.060	2.198	-0.138	-6.683
18	45	2.260	2.311	-0.051	-2.236
19	44	2.450	2.340	0.110	4.505
20	43	2.570	2.369	0.201	7.817
21	44	2.700	2.340	0.360	13.347

The measured optical density values and pixel value at 150 dpi in Table 6 were used for an exponential curve fitting with

$$OD = Ae^{B(PV)} \qquad ,$$

where A and B are coefficients of equation and $R^2 = 0.996180$ was chosen.

The exponential curve fitting calculations give:

$A = 4.058748$

$B = -0.012520$

and hence $$OD = 4.058748e^{(-0.012520)PV} \qquad ,$$

where PV is the pixel value of the image and OD is the optical density of the step in the step tablet. Results of OD calculation were plotted together with the plot of OD standard values pixel values as shown in Figure 30.

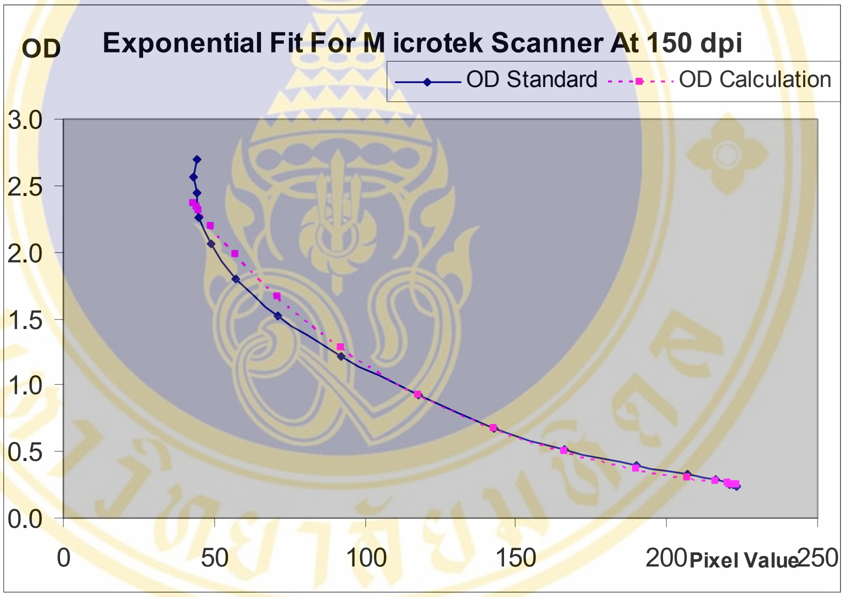


Figure 30: Exponential Fit For Microtek Scanner at 150 dpi

As a check, this line fit was used to calculate the optical density from the pixel measurement data. Results of difference and percentage difference were also displayed in Table 6 above.

Difference value is between -0.188 and 0.360 as seen in Figure 31 below, where difference value were plotted against pixel values at 150 dpi.

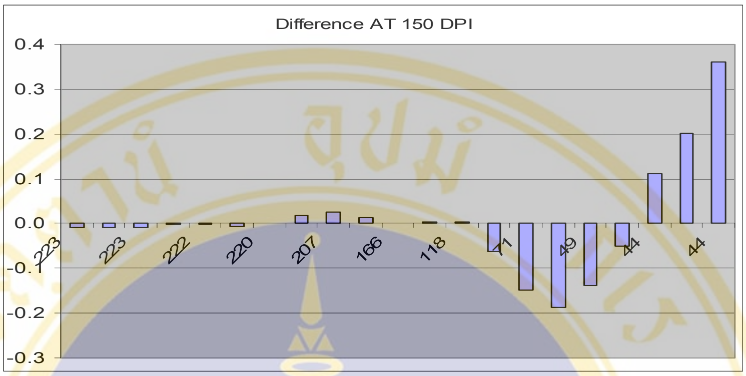


Figure 31: Difference of Exponential Fit Function at 150 DPI

The percentage difference value is between -10.456 and 13.347 as seen in Figure 32 below, where difference value were plotted against pixel values at 150 dpi.

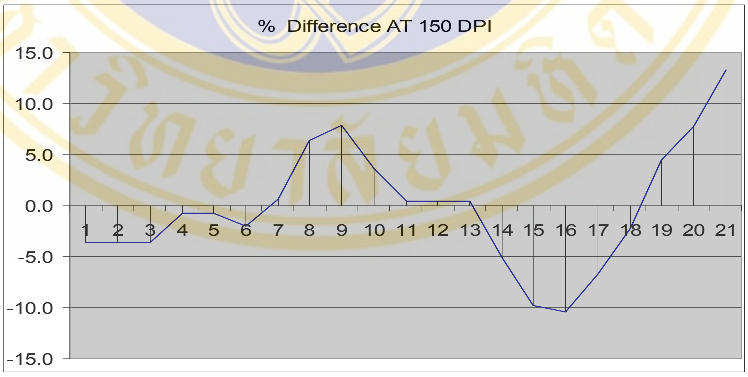


Figure 32: Percentages Difference of Exponential Fit Function at 150 DPI

5.2.4 Uniformity in Resolution

From the above 3 sub-sections one can compare the percentage difference results with respect to the pixel values at 50, 96 and 150 dpi to show the uniformity in resolution of the measurements, as read by seen in figure 33.

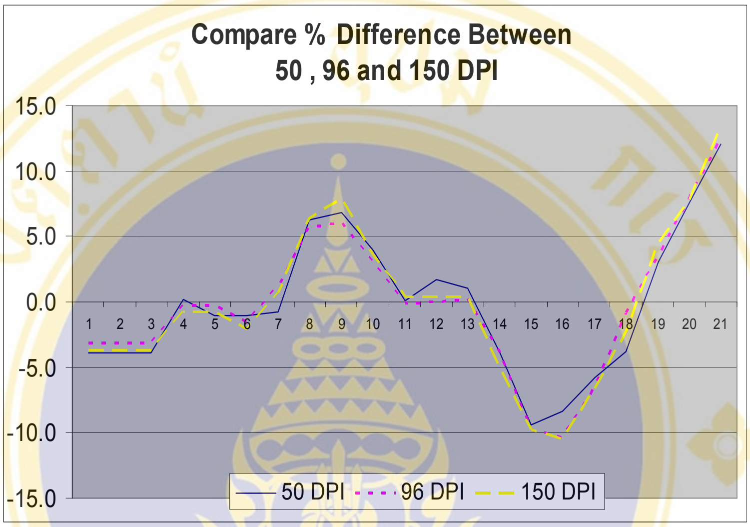


Figure 33: Compare Percentage Difference between 50, 96 and 150 DPI

Minimize and maximize value of difference and percentage difference, as shown against dpi values in tables 7 and 8, further indicate the uniformity in the measurements at 50, 96 and 150 dpi.

Table 7: Min & Max Difference at 50, 96 and 150 DPI

Difference	DPI		
	50	96	150
MIN	-0.151	-0.186	-0.188
MAX	0.324	0.335	0.360

Table 8: Min & Max Percentage Difference at 50, 96 and 150 DPI

Percentage Difference	DPI		
	50	96	150
MIN	-9.3515	-10.3487	-10.456
MAX	12.0129	12.4043	13.347

5.2.5 Uniformity in Position of Scanning

To check for uniformity across the scanning field, the step tablet was scanned at various locations inside the scanner, as seen in Figure 34.

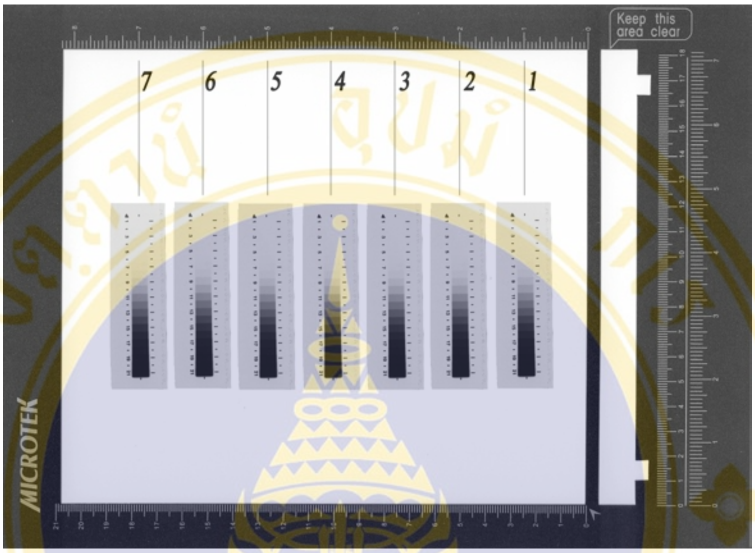


Figure 34: Position from Right of Scanner (Inches) to Measure Pixel Value.

Measurement uniformity yielded a standard difference of 4 pixel values. To review uniformity in position, see Table 9 in Appendix II. A plot of pixel values with positions of the step tablet is given in Figure 35 to show the uniformity in the measurements as carried out across the scanning field.

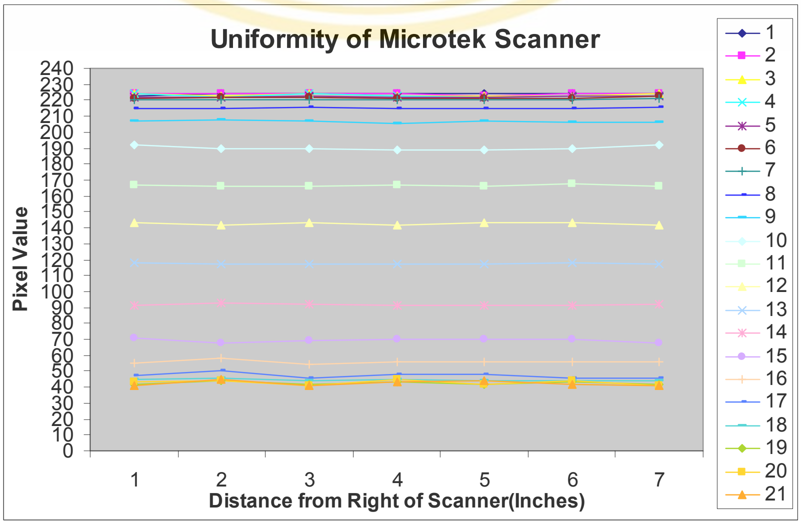


Figure 35: Uniformity in Position of Microtek Scanner

CHAPTER 6

DISCUSSIONS

This chapter discusses the result of testing the developed software. By focusing on six aspects: User interface, Software implementation, Uniformity, Limitation of Scanner, Accuracy of Calculation of Curve fitting Class in Program and Dynamic Range of Step Tablet Film

6.1 User Interface

Visual C++.NET, which is used to create GUI such as menu Dialog box, Command buttons and Text boxes, can make user-friendly interface because the command structure of Visual C++.NET in Dialog base is a powerful tool(4) with simplicity for users to handle by just following the software step-by-step.

6.2 Software Implementation

It was found that software implementation is convenient and simple. By simple clicking image box, pixel value of the image is obtained and stored in optical density box automatically. However, since the measured point of about 0.2 mm(at 96 dpi in scanning film) in diameter is very small, the measurement is repeated several times to obtain a mean pixel value as an input for OD calculation.

By choosing function of program to have R^2 greater than 0.99, Percentage Difference in the OD values obtained is show to the less than 0.2 OD.

6.3 Uniformity

6.3.1 Pixel Value measurement

It has been show in Table 3 that pixel values as measured are uniform within 3 units of pixel values. Darkness of the film is thus assessed with confidence in varying resolution of the film scanning.

6.3.2 Optical Density calculation

From Tables 4, 5 and 6 it can be observed that the computation executed on the measured pixel value give the OD values with difference less than 0.1 OD and percentage Difference less than 3 %.

Varying resolution in the film scanning does not significantly affect the accuracy of OD calculation.

6.4 Limitation of Scanner

In general, common commercial scanners have DMAX (OD Maximum) values about 2.5 to 2.75, which do not differ significantly from the result of 2.45 as obtained in Table 3.

This set the limitation of Microtek flatbed scanner at the same level of other common commercial scanners. Microtek scanner is thus suitable for use in the scanning of film with calculation OD values in the range of 0.24 to 2.5.

6.5 Accuracy of Calculation of Curve fitting Class in Program

From Table 8 show that the value of percentage Difference (%Difference) between -10.3% to 13.347%

But from limitation of scanner, the range of OD value between 0.24 and 2.5, error fitting has percentage differences in the region of -10.3417% to 7.9734%.

For OD values between 0.24 and 2.5, the difference in “Curvefit Class” (see appendix 1) with in $^{+}/_{-}$ 10% is regarded as acceptable.

For OD values between 1.5 to 2.0, as seen in Figures 24, 27, and 30, the OD values deviate from the standard values quite considerably due to the fact that a single exponential fit is taken over the whole OD ranges of 0.24 to 2.5.

CHAPTER 7

CONCLUSION

From this thesis, a flatbed scanner as developed can be utilized for film density measurements in densitometry. The techniques involved a mathematical model, various characteristics of a specific scanner and film calibration. Microtek scanner is used to measure OD values in the range of 0.24 - 2.5 OD, with percentage differences approximately +/-10 %.

Performances deemed satisfactory considering cost, simplicity and resolution as well as the ease of use when compared with a point densitometer, however, can be still improved with a scanner with a higher DMAX.

Deviation of OD values between 1.5 and 2.0 can probably be avoided if exponential fits are to be applied to several portions of OD values. Another improvement could be to add degree of freedom to the proposed mathematical model.

Thus, for further studies, curve obtained by OD calibration may be divided into several portions as its profile. A further using curve fitting process, such as addition of polynomial method in mathematical modeling, would increase the efficiency of OD calibration. To increase software capability, gamma values should be adjustable values, to increase difference of contrasts in the darkness zone (more than 2.5 OD) of image.

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APPENDIX

APPENDIX I

Class of Curve Fitting in LSD Software

For ease of usage and further development, a new “Class” file has been developed from Visual C++ program for calibration of pixel values with curve fitting functions as detailed in class file as follows:

1. Header File of Class Curvefit.h

```
#pragma once
#include <math.h>
#define E   2.7182818284              //2.71828 18284 59045 23536
class CCurveFit
{
public:
        CCurveFit(void);
        ~CCurveFit(void);
        double x[21],y[21];
        double lnx[21],lny[21];
        int count;
        int m_Medthod;
        double OD;
        int m_PixelValues;
        double A_linearf,B_linearf;             //Coefficaint of Linear Fitting : Use SS Function in calculation
        double A_expof,B_expof;                 //Coefficaint of Exponential Fitting : Use SS Function in calculation
        double A_powerf,B_powerf;              //Coefficaint of PowerLaw Fitting : Use SS Function in calculation
        double A_logf,B_logf;                   //Coefficaint of Log Fitting : Use SS Function in calculation
        double R2_linearf;    //Correlation Coefficaint of Linear Fitting : Use SS Function in calculation
        double R2_expof;      //Correlation Coefficaint of Exponential Fitting : Use SS Function in calculation
        double R2_powerf;     //Correlation Coefficaint of PowerLaw Fitting : Use SS Function in calculation
        double R2_logf;       //Correlation Coefficaint of Log Fitting : Use SS Function in calculation
        // BasicFunction of SUM
        double Sum(double * M);
        double Sum_sqr(double  *M);
        double Sum_dot(double *M,double *N);
        double Sumln(double *M);
        double Sumln_sqr(double *M);
        double Sumln_dot(double *M,double *N);
        double Sum_dotln(double * M,double *N);
        double Sum_sqrdot(double *M, double *N);
        double Sum_dotdln(double *M, double *N);
        void T_ln(void);
        // Curve Fitting Function in Find Coefficiant
        void LinearFit(void);
        void ExpoFit(void);
        void PowerLawFit(void);
        void LogFit(void);
        void WyExpoFit(void);
        double Mean(double *M);
        double SSnn(double *M);
        double SSxy(double *M, double *N);
        void LinearFit2(void);
        void ExpoFit2(void);
```

```
		void LogFit2(void);
		void PowerLawFit2(void);
		// Calculate OD
		void CalLine(void);
		void CalExpo(void);
		void CalLog(void);
		void CalPower(void);
};
```

2. Source File of Class Curvefit.cpp

```
#include "StdAfx.h"
#include ".\curvefit.h"
#include "math.h"

CCurveFit::CCurveFit(void)
{		count=21;
}
CCurveFit::~CCurveFit(void)
{
}
//  SUM Function
double CCurveFit::Sum(double * M)			//Sum M
{
		int i;
		double sum;
		sum=0;
		for(i=0;i<count;i++)
						sum += M[i];
		return sum;
}
double CCurveFit::Sum_sqr(double *M)			//Sum M2
{
		int i;
		double sum_sqr;
		sum_sqr=0;
		for(i=0;i<count;i++)
				sum_sqr += M[i]*M[i];
		return sum_sqr;
}
double CCurveFit::Sum_dot(double* M,double *N)		//  Sum  M*N
{
		int i;
		double sum_dot;
		sum_dot=0;
		for(i=0;i<count;i++)
				sum_dot  += M[i]*N[i];
		return sum_dot;
}
double CCurveFit::Sumln(double *M)			//Sum ln(M)
{
		int i;
		double sumln;
		sumln=0;
		for(i=0;i<count;i++)
				sumln += log(M[i]);
		return sumln;
}
double CCurveFit::Sumln_sqr(double *M)			//Sum  [ln(M)]2
{
		int i;
		double sumln_sqr;
		sumln_sqr=0;
		for(i=0;i<count;i++)
				sumln_sqr += log(M[i])*log(M[i]);
		return sumln_sqr;
```

```
}
double CCurveFit::Sumln_dot(double *M,double *N)                        // Sum  ln(M)*ln(N)
{
            int i;
            double sumln_dot;
            sumln_dot=0;
            for(i=0;i<count;i++)
                        sumln_dot += log(M[i])*log(N[i]);
            return sumln_dot;
}
double CCurveFit::Sum_dotln(double *M,double *N)            //Sum M*ln(N)
{
            int i;
            double sum_dotln;
            sum_dotln=0;
            for(i=0;i<count;i++)
            sum_dotln += M[i]*log(N[i]);
            return sum_dotln;
}
double CCurveFit::Sum_sqrdot(double *M, double *N)                      //Sum M2*N
{
            int i;
            double sum_sqrdot;
            sum_sqrdot = 0;
            for(i=0;i<count;i++)
            sum_sqrdot +=( M[i]*M[i])*N[i];
            return sum_sqrdot;
}
double CCurveFit::Sum_dotdln(double *M, double *N)                      //Sum M*N*ln(N)
{
            int i;
            double sum_dotdln;
            sum_dotdln=0;
            for(i=0;i<count;i++)
            sum_dotdln += (M[i]*N[i])*log(N[i]);
            return sum_dotdln;
}
void CCurveFit::T_ln(void)
{
            int i;
            for(i=0;i<21;i++)      { lny[i]= 0;            lnx[i]= 0;            }
            for(i=0;i<count;i++)  { lny[i]= log(y[i]);      lnx[i]= log(x[i]);     }
}
//Fit Function// Sums of Squares Function
double CCurveFit::Mean(double *M)
{
            double mean;
            mean=0;
                        mean = Sum(M)/(double) count;
            return mean;
}\
double CCurveFit::SSnn(double *M)
{
            double ssnn;
            ssnn=0;
            ssnn= Sum_sqr(M)-((double) count*pow(Mean(M),2.0));
            return ssnn;
}
double CCurveFit::SSxy(double *M, double *N)
{
            double ssxy;
            ssxy=0;
            ssxy = Sum_dot(M,N) - ((double) count*Mean(M)*Mean(N));
            return ssxy;
}
// Fit Function in Sums of Squares
void CCurveFit::LinearFit2(void)
{
            double a,b,r;
            a=b=r=0;
            b = SSxy(x,y)/SSnn(x);
            B_linearf=b;
```

```
        a = Mean(y)-(b*Mean(x));
        A_linearf =a;
        r = pow(SSxy(x,y),2.0)/(SSnn(x)*SSnn(y));
        R2_linearf = r;
}
void CCurveFit::ExpoFit2(void)
{
        double a,b,r;
        a=b=r=0;
        T_ln();
        b = SSxy(x,lny)/SSnn(x);
        B_expof=b;
        a = Mean(lny)-(b*Mean(x));
        A_expof =exp(a);
        r = pow(SSxy(x,lny),2.0)/(SSnn(x)*SSnn(lny));
        R2_expof = r;
}
void CCurveFit::LogFit2(void)
{
        double a,b,r;
        a=b=r=0;
        T_ln();
        b = SSxy(lnx,y)/SSnn(lnx);
        B_logf=b;
        a = Mean(y)-(b*Mean(lnx));
        A_logf =a;
        r = pow(SSxy(lnx,y),2.0)/(SSnn(lnx)*SSnn(y));
        R2_logf = r;
}
void CCurveFit::PowerLawFit2(void)
{
        double a,b,r;
        a=b=r=0;
        T_ln();
        b = SSxy(lnx,lny)/SSnn(lnx);
        B_powerf=b;
        a = Mean(lny)-(b*Mean(lnx));
        A_powerf =exp(a);
        r = pow(SSxy(lnx,lny),2.0)/(SSnn(lnx)*SSnn(lny));
        R2_powerf = r;
}
// Calculate of Equations of Best Fitting
void CCurveFit::CalLine(void)  //
{
        OD=0;
        OD = A_linearf + ((B_linearf)*((double)m_PixelValues));
}
void CCurveFit::CalExpo(void)
{
        OD=0;
        OD = (A_expof)*(exp(((double)m_PixelValues )*(B_expof)));
}
void CCurveFit::CalLog(void)
{
        OD=0;

        OD = (A_logf)+( B_logf)*(log((double)m_PixelValues));
}
void CCurveFit::CalPower(void)
{
        OD=0;
        OD = (A_powerf)*(pow((double)(m_PixelValues),(double)(B_powerf)));
}
```

APPENDIX II

Table of the results of pixel values from various locations inside the scanner, are shown below.

Table 9: Uniformity in Position of Microtek Scanner

Step No.	Position Number									
	1	2	3	4	5	6	7	Min	Max	Difference
1	223	224	224	224	224	224	224	223	224	1
2	224	224	224	224	223	224	224	223	224	1
3	224	223	224	223	223	223	224	223	224	1
4	224	222	224	223	222	223	223	222	224	2
5	222	222	223	222	222	223	223	222	223	1
6	221	222	222	221	221	221	223	221	223	2
7	220	220	220	220	220	220	221	220	221	1
8	215	215	216	215	215	215	216	215	216	1
9	207	208	207	205	207	206	206	205	208	3
10	192	190	190	189	189	190	192	189	192	3
11	167	166	166	167	166	168	166	166	168	2
12	143	142	143	142	143	143	142	142	143	1
13	118	117	117	117	117	118	117	117	118	1
14	91	93	92	91	91	91	92	91	93	2
15	71	68	69	70	70	70	68	68	71	3
16	55	58	54	56	56	56	56	54	58	4
17	47	50	46	48	48	46	46	46	50	4
18	45	46	44	45	44	44	44	44	46	2
19	42	44	42	43	42	43	42	42	44	2
20	43	44	41	45	42	44	41	41	45	4
21	41	45	41	43	44	42	41	41	45	4

BIOGRAPHY

NAME	Mr. Suwajchai Pornsuwansiri
DATE OF BIRTH	19 September 1974
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 1997: Bachelor of Engineering (Mechanical) Mahidol University, 2004: Master of Engineering (Biomedical Engineering)
HOME ADDRESS	79/34 Moo 4 Rama 3 Road Samadum Bangkuntant Bangkok 10150 Tel. 01-616-8883