Applied Medical Informatics
Original Research Vol. 28, No. 1/2011, pp: 29-36

Comparison between Digital Image Processing and Spectrophotometric Measurements Methods. Application in Electrophoresis Interpretation

Bogdan Adnan HAIFA[1,*], Vladimir BACÂREA[2], Oana IACOB[3], Tudor CĂLINICI[4], and Alexandru ŞCHIOPU[3]

[1] University of Medicine and Pharmacy Târgu Mureş, Medical Informatics and Biostatistics Department, Gh. Marinescu no. 38, 540139, Romania.
[2] University of Medicine and Pharmacy Târgu Mureş, Methodology of Scientific Research Department, Gh. Marinescu no. 38, 540139, Romania.
[3] University of Medicine and Pharm+acy Târgu Mureş, Physiopathology Department, Gh. Marinescu no. 38, 540139, Romania.
[4] "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Medical Informatics and Biostatistics Department, 13 Emil Isac, 400023 Cluj-Napoca, Romania.
E-mail(s): bogdan_adnan@yahoo.com; bacarea@yahoo.com

* Author to whom correspondence should be addressed; Tel.: +4-0770996276.

Received: 12 December 2010 /Accepted: 21 February 2011 / Published online: 7 March 2011

Abstract

Background: Spectrophotometer is a very common instrument in various scientific fields and gives accurate information about light absorbance and transmittance through materials using monochromatic light source. Though, devices used in spectrophotometry can be quite expensive, using components with high technical specifications and the procedure itself is time consuming. Regular digital image acquisition instruments like scanners and cameras on the other hand uses very cheap electronic components to record the information on 3 wide band channels (Red, Green, Blue). *Purpose:* This paper studies the possibility of correlating the measurements from the spectrophotometer with raw data from digital image acquisition instruments. *Materials and Methods:* Because the results will be used in protein electrophoresis, we prepared o set of plates with blood serum in different dilutions, stained with Coomassie Brilliant Blue. The absorbance of the resulting plates has been measured using a spectrophotometer and after that, the plates were scanned with a regular office scanner. The digital image was converted in different color spaces (gray scale, RGB, HSV, HSL, CIEXYZ and CIELAB) using custom developed software in C++. We statistically measured the correlation coefficient of different parameters from the color space with the absorption measured with the spectrophotometer. *Results and Discussion:* The findings of this work show that a consumer digital scanner can be used as a fast and inexpensive alternative to spectrophotometers. This offers the possibility of using scanned images of protein electrophoresis to make quantitative estimations regarding the proteinogram.

Keywords: Spectrophotometry; Color spaces; Digital image processing.

Introduction

Spectrophotometry is one of the most used measurements methods used in chemistry and physics and there medical counterparts (biochemistry, biophysics, medical laboratory). A lot of different medical analyses are implemented based on the Lambert-Beer law of absorption [1].

Electrophoresis is one of these medical analyses that can benefit from the photometric theories. The electrophoresis method successfully separates the components analyzed based on different properties of support medium and of the target molecules (like electrical charge, molecular size and mobility). After separation the components are stained using different methods to make them visible.

This analysis can be used in medical field for separating different chemical components from blood or other fluids. It is very often used for protein separation (protein electrophoresis), the amount of different components (albumin, alpha-1 globulins, alpha-2 globulins, beta globulins and gamma globulins) giving an overview of the health status of the body [2]. In order to stain the proteins, Coomassie Brilliant Blue dye is usually used.

Depending on the amount of protein fractions from the serum the separated components have different color intensities. Using Lambert-Beer law we can than estimate the percentage of different protein components from the blood.

At our institute we try to develop a new protocol to do and analyze the protein electrophoresis. One of the steps in this research is to create a software able to give an estimation of protein concentration based on a digital image of the electrophoresis plates, obtained using a regular office scanner. Such software able to process digital images in terms of colorimetric densitometry already exists (as free or paid software) but those are not designed specifically for the electrophoresis.

When we tried to create the first models for this software we hit an important question: can we actually correlate the RGB values of a digital image with the absorption/extinction/transmission values that are at the basis of Lambert-Beer law [3]? The problem rises from the differences in the construction of a spectrophotometer and of an office scanner. The first one uses a monochromatic light and measures the intensity of the light before and after passing through the colored probe. The second instead takes a more photographically approach, measuring the reflected/transmitted light intensity of three colored light beams: red, green and blue. The light beams are not monochromatic and the wavelength specter depends on the manufacturer.

Material and Method

Experiment Description

Given the fact that the scanner is measuring the intensity on a wide band specter (in red/green/blue domain), the information regarding the absorbance at a specific wavelength is also there, and the only problem is extracting it from the RGB data.

In order to find a way to do this, we started by fabricating 10 colored glass plates. Because the final goal of the entire experiment was to apply the results in analyzing protein electrophoresis, we used a very similar approach in the technique to fabricate these plates as in the first steps of the electrophoresis.

We used microscopic glass plates 24 mm/70mm, boiled in a solution of sodium hydroxide and washed with ethanol. As a source of protein we used human blood serum with a protein concentration of 7.68 g/dl, determined spectrophotometrically at 280 nm. The serum was mixed in various proportions with agarose gel (0.6%), melted at 60^{o}C. 3 ml from the mixture was transferred on the glass plate and than fixated with a solution of trichloroacetic acid (10%) for 20 min. After fixation, the plates were washed with water, and after that covered with filter paper to dry the gel layer at room temperature for 20h. The dried plates were immersed in the staining solution (solution containing Coomassie Brilliant Blue R250 0.15%) for 2h and washed with water. At the end, the plates were dried at room temperature.

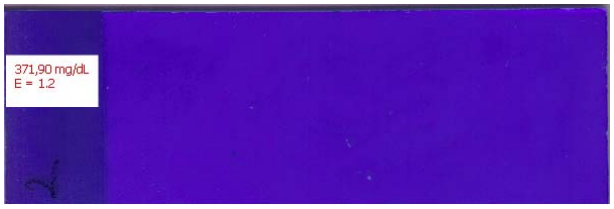


Figure 1. Sample colored plate

The resulting plates have different intensities of blue color, which increased with the concentration. In order to measure the real absorption of light by this plates, an UV VIS spectrophotometer was used. We measured extinction at 595 nm.

The plates were scanned with an office scanner at 600 dpi, and the resulting image was processed as described further.

Image Processing

All processing has been done using custom-made software, written in C++. The images were scanned and saved as PNG (Portable Network Graphic) in order to preserve the original information. For GUI (Graphical User Interface), image loading/displaying and for accessing image data we used wxWidgets library, version 2.8.10 [4,5].

The resulting plates weren't perfectly homogenous regarding the color. In order to extract the proper color we averaged the values of red, green and blue components for all pixels on the active part of the plates. The resulting color has been converted to some of the most common used color spaces.

A color space is an abstract mathematical model describing how a real color can be mapped as a tuple of numbers (typically 3 or 4). RGB is such a color space and represents the colors by 3 numbers from 0 to 255 proportional with the intensity of the channel (red, green, blue). Beside RGB, which is the most common one, there are also other color spaces used in specific situations (like CMYK, CIE XYZ, HSV, HSL) [6]. All this color spaces can be converted from one to another. Each parameter of these color spaces represents a specific aspect of the light/color (e.g. lightness, saturation, hue). Therefore, it is plausible that one of those parameters may be correlated with the absorption of light.

We tested the following color spaces: gray scale, RGB, HSV, HSL, CIEXYZ and CIELAB.

Gray Scale

Conversion from RGB to gray scale is not a standardized process. The most common used method equals the gray intensity with the luminance of the color. The process involves acquiring the linear intensity of red, green and blue channel (using gamma expansion) and applying the following function:

Gray = 0.3 x Red + 0.59 x Green + 0.11 x Blue

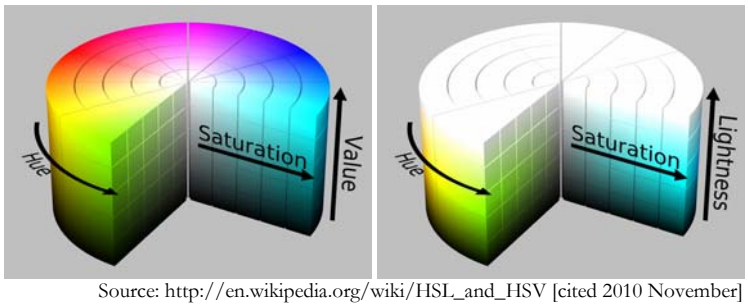
RGB

The RGB color space is a cube (3 coordinates - R, G and B, each with values from 0 to 255). The scanned image is already in RGB format [3]. Given the fact that the color of Coomassie Brilliant Blue is in the blue part of the specter we compared the extinction with the blue value, ignoring the rest of the components.

HSL and HSV

HSL is the acronym for hue, saturation and lightness. HSV is for hue, saturation and value. These color spaces are very similar, and represents the colors in a cylindrical-coordinate system [6, 7]. HSV and HSL are used to describe the perceptual color relationships more accurately than RGB. Hue in both color spaces represents the pure color, which is altered by the saturation and lightness/value. Saturation (S) represents the purity of the color, which varies from pure color (fully saturated) to its gray equivalent. Lightness (L) spans the entire range from black to white, passing through the chosen hue, while the value (V) goes only half way from black to specified hue.

Usually HSV and HSL color spaces are represented as a cylinder, where the outer circumference represents the hue, the radius represents the saturation and the height of the cylinder represents the value/lightness. The difference is represented by the color distribution, in case of HSL white is on top, black at the bottom, and the fully-saturated colors around the edge of a horizontal cross-section with middle gray at its center.



Source: http://en.wikipedia.org/wiki/HSL_and_HSV [cited 2010 November]

Figure 2. HSV (left) and HSL (right) representations

The value of *h* is generally normalized to lie between 0 and 360°, while *l*, *s* and *v* ranges from 0 to 1.

In order to convert from RGB values to HSL we used the following conversion functions (*r*, *g* and *b* values were first normalized in the range [0,1], max = greatest of *r*, *g* and *b* and min the least):

$$h = \begin{cases} 0 & \text{if } \max = \min \\ (60° \times \frac{g-b}{\max - \min}) \bmod 360°, & \text{if } \max = r \\ (60° \times \frac{b-r}{\max - \min} + 120°) \bmod 360°, & \text{if } \max = g \\ (60° \times \frac{r-g}{\max - \min} + 240°) \bmod 360°, & \text{if } \max = b \end{cases}$$

$$s = \begin{cases} 0 & \text{if } \max = \min \\ \frac{\max - \min}{\max + \min} = \frac{\max - \min}{2l} & \text{if } l \le \frac{1}{2} \\ \frac{\max - \min}{2 - (\max + \min)} = \frac{\max - \min}{2 - 2l} & \text{if } l > \frac{1}{2} \end{cases}$$

$$l = \frac{1}{2}(\max + \min)$$

For HSV, the definition of hue is identical as for HSL, but the other components (*s* and *v*) differ:

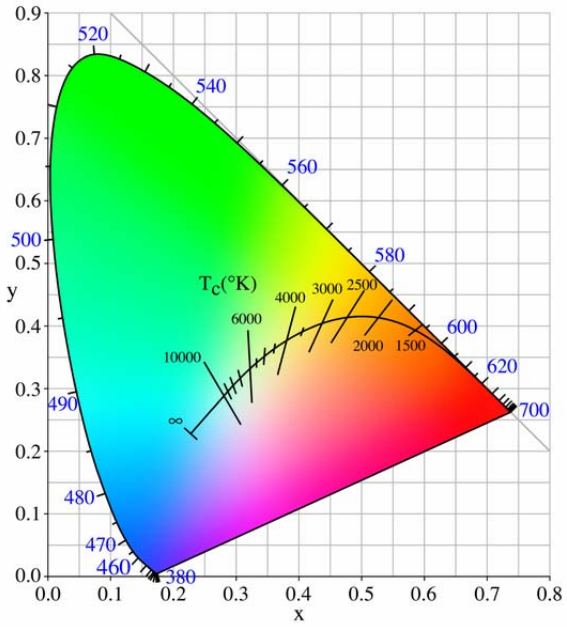
$$s = \begin{cases} 0 & \text{if } \max = 0 \\ \dfrac{\max - \min}{\max} = 1 - \dfrac{\min}{\max} & \text{otherwise} \end{cases}$$

$$v = \max$$

It is worth mentioning that the name lightness is misleading because even if this term exists in photometry as well, defining relative luminous reflectance of a colored surface, the photometric notion is different than the color space correspondent.

CIE Standards

CIE (Commission Internationale de L'Eclairage) has developed the first standard in colorspaces, CIEXYZ. In this color space, XYZ are also called tristimulus values, and represents the response of a human eye to red, green and blue light. The RGB values can be directly converted to XYZ values using a simple matrix transformation. The curve for the *Y* tristimulus value is equal to the curve that indicates the human eye's response to the total power of a light source. For this reason the value Y is called the luminance factor and the *XYZ* values have been normalized so that *Y* always has a value of 100. In 1931, CIE adopted a standard based on XYZ values, called CIExyY Chromaticity diagram [8]. This diagram can be used to obtain the wavelength based on RGB data.



Source: http://en.wikipedia.org/wiki/Chromaticity [cited 2010 November]

Figure 3. CIE 1931 xyY Chromaticity diagram

Conversion from RGB has been done using the following functions (RGB values have been normalized to [0,1]):

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} 0.412453 & 0.357580 & 0.180423 \\ 0.212671 & 0.715160 & 0.072169 \\ 0.019334 & 0.119193 & 0.950227 \end{pmatrix} \times \begin{pmatrix} R \\ G \\ B \end{pmatrix}$$

$$x = \frac{X}{X+Y+Z} \qquad y = \frac{Y}{X+Y+Z}$$

CIELab is another color space derived from CIEXYZ [9]. L is the luminance coordinate (from 0 to 100), is the red/green coordinate (-127…127) and b is the yellow/blue coordinate. The conversion from XYZ has been done using these functions:

$$L^* = 116\left(\frac{Y}{Y_{rf}}\right)^{1/3} - 16$$

$$a^* = 500\left[\left(\frac{X}{X_{rf}}\right)^{1/3} - \left(\frac{Y}{Y_{rf}}\right)^{1/3}\right]$$

$$b^* = 200\left[\left(\frac{Y}{Y_{rf}}\right)^{1/3} - \left(\frac{Z}{Z_{rf}}\right)^{1/3}\right]$$

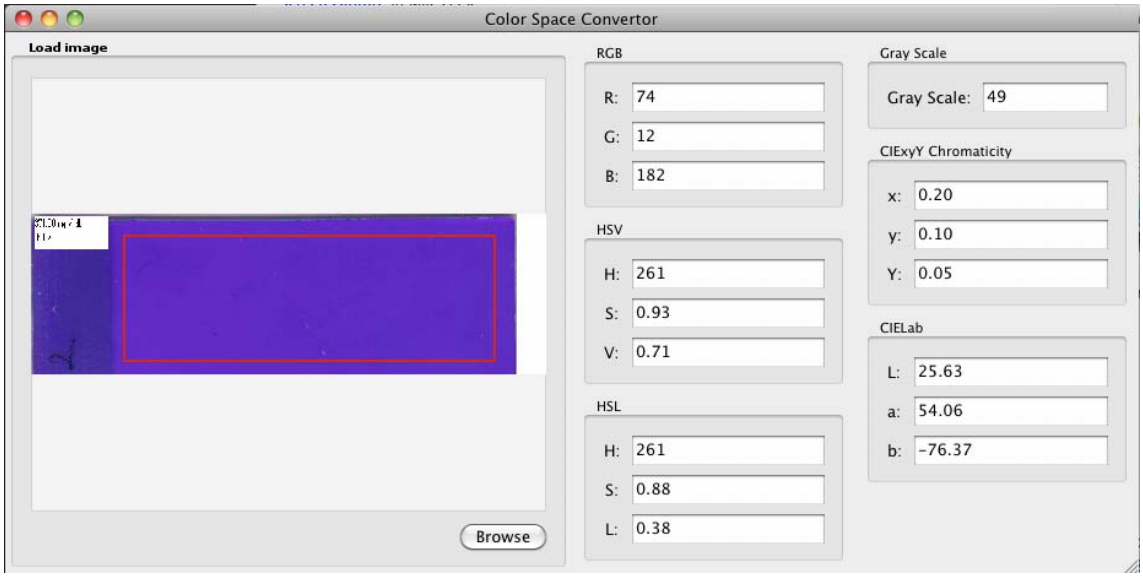


Figure 4. Software developed to analyze the color of plates

In order to analyze the plates we created a software able to load the scanned images of the plates and to analyze them. The usage of the program is very simple, the image is loaded using the button "Browse", and after that the user needs to select the area that will be analyzed. After selection, it will automatically average the RGB components and converts them to the desired color spaces.

The results for each plate was collected into an excel spreadsheet.

Statistical analysis has been done using GraphPad Prism. We applied Pearson correlation test, in order to see if any of the calculated parameters is in relation with the concentration of protein on the plates. The chosen significance level α was 0.05.

Results

The concentration of protein and the results obtain from scanned images are summarized in Table 1.

We calculated the Pearson correlation coefficient between all the parameters and the concentration. The results suggests that all the parameters that implies in some way the lightness have a linear variation with protein concentration and are well correlated.

Table 1. Color of plates in different color spaces

No	Concentration	Gray	Hue	Saturation (HSV)	Value (HSV)	Saturation (HSL)	Lightness (HSL)	CIELab Luminance
1	190.00	53	261	0.91	0.73	0.84	0.39	26.76
2	282.00	51	262	0.92	0.72	0.86	0.38	26.20
3	371.90	49	261	0.93	0.71	0.88	0.38	25.63
4	457.95	49	261	0.93	0.70	0.87	0.38	25.40
5	544.00	49	261	0.93	0.70	0.86	0.37	25.17
6	627.00	45	261	0.94	0.69	0.89	0.36	24.27
7	710.00	42	261	0.96	0.68	0.92	0.35	23.37
8	864.35	39	261	0.97	0.67	0.94	0.34	22.39
9	1018.69	37	261	0.98	0.65	0.96	0.33	21.41
10	1163.19	36	261	0.99	0.64	0.99	0.32	21.01

Table 2. Correlation between color space parameters and concentration

Parameter	Pearson coefficient (r)	R squared	p value	Result
Gray	-0.9798	0.9601	< 0.0001	significant
Hue	-0.3750	0.1412	0.2846	not significant
Saturation (HSV)	0.9828	0.9659	<0.0001	significant
Value	-0.9963	0.9925	<0.0001	significant
Saturation (HSL)	0.9645	0.9303	<0.0001	significant
Lightness	-0.9875	0.9752	<0.0001	significant
Luminance (CIELab)	-0.9917	0.9834	<0.0001	significant

Discussion

As can be seen in the results table, the hue remains constant. That was to be expected, given the fact that the base color is the same, only it's intensity is different with concentration. The grayscale, value, lightness and luminance have all a negative strong correlation with concentration (the bigger the concentration, the bigger is light absorption, so lightness is smaller), while saturation (both HSV and HSL) has a strong positive correlation.

These results correspond with another similar study [3], who studied the opportunity of using a RGB sensor in clinical measurement of blood glucose. They also suggested that saturation is strongly correlated with the absorption of light and with concentration of glucose.

The results also suggest that saturation calculated from HSV color-space has a slightly better correlation than that from HSL (r squared = 0.9659 for HSV vs. r squared = 0.9303 for HSL).

The linearity of dependence between saturation and concentration will allow us to use a calibration method to remove dependency from scanner manufacturer.

Conclusions

Even if the technical and theoretical principles that are at the basis of scanners and spectrophotometers are very different, the results obtained in this study proves that regular and much less expensive office scanners can be used to get similar results in specific areas. These results are encouraging and will allow us to continue the plan in developing a software able to interpret protein electrophoresis using regular office equipment with results at least comparable with that of much more expensive devices use in medical laboratories.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This paper is partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/60782.

References

1. Laidler KJ. The World of Physical Chemistry. New York: Oxford University Press. 1995.
2. Keren DF. Protein Electrophoresis in Clinical Diagnosis. London; A Hodder Arnold Publication, 2003.
3. Sivanantha RA, Sankaranarayanan K. Use of RGB Color Sensor in Colorimeter for better Clinical measurement of Blood Glucose. BIME Journal 2006;6(1):23-28.
4. Cogswel J. Programming C++ GUIs with the wxWidgets Library. [online] 2006 [cited 2010 December]. Available from: URL: http://www.informit.com/articles/article.aspx?p=606222.
5. Smart J, Roebling R, Zeitlin V, Dunn R. wxWidgets 2.8.11: A portable C++ and Python GUI toolkit. [online] 2006 [cited 2010 December] Available from: URL: http://docs.wxwidgets.org/stable/.
6. Solli M. Filter characterization in digital cameras. Norrkoping, Sweden. Department of Science and Technology. Linkopings Universitet; ISRN: LITH-ITN-MT-EX--04/068--SE; 2004.12.16
7. Rastislav L, Plataniotis KN. Color image processing. Methods and applications. CRC Press Taylor & Francis Group, 2007.
8. Wandell BA, Farrell JE. Water into wine: Converting scanner RGB to tristimulus XYZ. Proceedings of the SPIE 1992;1909:92-101.
9. Simonot L, Hébert M, Dupraz D. Goniocolorimetry: From measurement to representation in the CIELAB color space. Color Research & Application 2010;DOI:10.1002/col.20605.