International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:10, No:8, 2016

Low Cost Technique for Measuring Luminance in Biological Systems

N. Chetty, K. Singh

***Abstract*—**In this work, the relationship between the melanin content in a tissue and subsequent absorption of light through that tissue was determined using a digital camera. This technique proved to be simple, cost effective, efficient and reliable. Tissue phantom samples were created using milk and soy sauce to simulate the optical properties of melanin content in human tissue. Increasing the concentration of soy sauce in the milk correlated to an increase in melanin content of an individual. Two methods were employed to measure the light transmitted through the sample. The first was direct measurement of the transmitted intensity using a conventional lux meter. The second method involved correctly calibrating an ordinary digital camera and using image analysis software to calculate the transmitted intensity through the phantom. The results from these methods were then graphically compared to the theoretical relationship between the intensity of transmitted light and the concentration of absorbers in the sample. Conclusions were then drawn about the effectiveness and efficiency of these low cost methods.

***Keywords*—**Tissue phantoms, scattering coefficient, albedo, low-cost method.

I. Introduction

LIGHT has been the fascination of scientists over the last century and the resulting advancements in the field of optics have also had widespread consequences in other areas of science. One of the most researched techniques in recent years has been in the medical field, where scientists and doctors alike have attempted to diagnose and even cure cancer, using optical methods [1]. Light (mainly lasers) has been extensively used to detect tumours and irregularities under the skin that human eyes cannot detect. In the biomedical sciences, subdermal imaging is becoming ever more popular due to this relatively recent progress in optics. The process is advantageous in that it is non-invasive and is conducted by the measurement of light, as it propagates through dermal tissue into the subdermal layers of the skin [1]. Medical imaging, in general, is the technique used to create images of the organs, tissues and other parts of the human body [2]. Luminance and illuminance are two of the principal measures of light which are used for subdermal imaging and these quantities can be obtained using meters which measure the intensity of light. Observations of the different wavelengths of light that can radiate through human dermal tissue generates a picture of subdermal layers of the skin. This facilitates the diagnosis and treatment of certain skin conditions such as jaundice, erythema and cancer [1]. Measurements of luminance and illuminance taken from different points on human skin tissue can indicate the penetration depth of a certain wavelength of light [2]. This in turn provides useful information about the wavelength of light needed for the diagnosis and safe, effective treatment of the condition [2].

N. Chetty and K. Singh are with the Discipline of Physics, School of Chemistry and Physics, University of KwaZulu-Natal (PMB), Scottsville 3209, Pietermaritzburg, South Africa (e-mail: chettyn3@ukzn.ac.za).

A. Important Properties of Light

Light, when incident upon certain surfaces, tends to reflect or transmit in many different directions at once and this phenomenon is known as scattering or diffusion. The amount of light being scattered when passing from one medium to another depends on the wavelength of the incident light in comparison to the size of the particles in the second medium (biological tissue in the case of biomedical imaging). Scattering is also dependent on the difference in the refractive indices of the two media. These properties apply to visible light and other forms of electromagnetic radiation [3].

Biological tissue scatters light strongly in the forward direction [4] and hence for deeper, more effective penetration through skin, the incident light must lie within a wavelength range that is more likely to scatter than be absorbed by the skin. For visible light (such as He-Ne lasers) this range, in which the ratio between scattering and absorption is very high, is in the neighbourhood of 650nm [5]. Tissue is composed largely of water, which can be considered non-absorbing between wavelengths of 200 nm to 900 nm. Models such as Mie theory and the Born approximation have been used in recent years to try and describe the scattering of light through tissue more accurately [6]. Algorithms have been written and transformed into code which is programmed to simulate the effects of light radiating through human tissue. Monte Carlo simulations, for example, can be used to identify scattering characteristics of certain materials, tissues and tissue phantoms included. Currently these are methods being used to understand the scattering and absorption of light in inhomogeneous media, such as tissue. This process is rigorous and difficult as it requires most, if not all, inhomogeneities to be accounted for in the simulation.

B. Melanin Content

Melanin is a pigment found in most organisms. In humans it is responsible for skin, hair and iris colour of an individual. Individuals with a darker skin tone contain more of this pigment in their skin than those who have a lighter skin tone. Melanin also provides protection against ultraviolet (UV) radiation, generally incident from the sun, but does not offer

complete protection, as individuals with a darker skin tone are still at risk from the effects of the sun's rays.

There are three basic types of melanin: eumelanin (the most common type found in human tissue), pheomelanin and neuromelanin. Eumelanin and pheomelanin are mostly found in the hair and skin, and are both responsible for skin and hair colour as well as protection from UV radiation. Eumelanin provides more protection from the harmful effects of UV radiation, emitted by the sun, than pheomelanin. Neuromelanin is found in different areas of the brain and a lack of neuromelanin can be linked to disorders in the brain.

Melanin is synthesized in cells called melanocytes and are found in varying quantities in different areas of the body. Even within a single area of the body, the amount of melanin can vary greatly between melanocytes, making it difficult to determine the concentration of melanin in a given part of the body. It is, however, an extremely good absorber of light and is thought to be the body's primary defence against harmful UV radiation. It has also been found that a higher concentration of melanin in the skin lowers the risk of skin cancer. For this research, the most important property of melanin is its high absorptivity and the fact that it is the primary absorber of light in tissue. An increase in the concentration of the absorbing material in a phantom tissue roughly simulates an increase in the melanin content of an individual.

II. Theory

A. Cameras in Tissue Optics

Cameras have been used for medical purposes, generally in studies involving light detection and imaging. Cameras have also helped to determine the changes in the polarization states of light passing through a tissue sample. An experimental setup by Jacques et al. [7], as shown in Fig. 1, used a white xenon light source to illuminate a tissue sample and a 12-bit CCD camera to image the illuminated sample. The sample used had melanin as the primary absorber. The illumination is delivered from an angle to direct glare, from a glass/skin interface that exists in the setup, away from the camera. The results of the experiment showed the different images obtained by varying states of polarization of the illuminating light. It has to be noted however that the camera used was a CCD camera, and digital cameras are yet to be used extensively for medical imaging purposes.

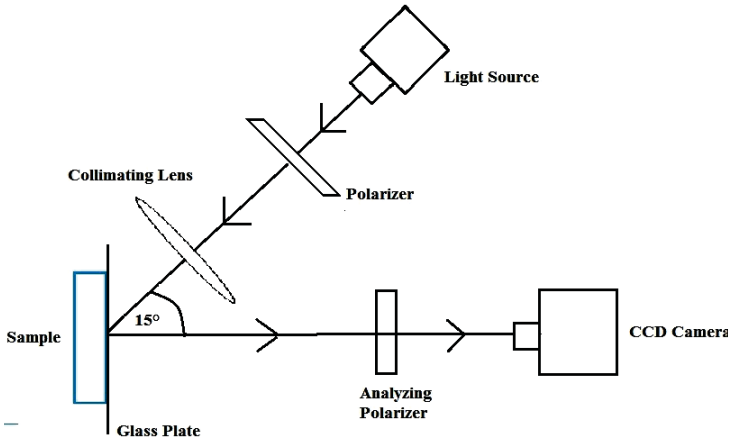

Fig. 1 A setup similar to the one used by Jacques et al. [7]

B. Digital Cameras as Luminance Meters

Luminance and lux meters are generally used to measure luminance and illuminance respectively but a digital camera can also be used to determine the intensity of light [8]. An illuminance or lux meter is a relatively inexpensive instrument, whereas a luminance meter is much costlier (a few thousand dollars). Both instruments yield fairly accurate light measurements from different objects and sources. A digital camera may not be as accurate as using a measuring device such as a luminance meter but provides a cheaper, more efficient method of determining the luminance of a light source.

The digital camera turns an image into an array of pixels, each of which contains a value that represents the intensity of the light at that point and hence, each of these pixel values is proportional to the luminance of that point in the original scene. Computer software such as *ImageJ* can then be used to analyze the image taken by a digital camera, and output values for each pixel in the image. Thus the luminance of that point can be determined without the need for a luminance or lux meter. A digital camera does have its advantages, as it captures more of the scene than a traditional luminance meter does, so multiple measurements can be made in less time. This allows not only the luminance of a source to be determined, but the luminance of its surroundings as well [8]. Also, the field of view of a digital camera is 150 times smaller than that of a luminance meter, allowing for the luminance measurements of much smaller light sources, such as individual LEDs.

The key to calibrating the camera in order to make luminance measurements, is to photograph a source of known

luminance and use this to determine the factor linking the luminance of the scene to the values of the pixels in the image. This factor is known as the calibration constant of the camera K_c. Then, the formula linking the luminance (replaced by the intensity, due to their directly proportional relationship) to the pixel value is given, from [8], as:

$$N_d = K_c((tS)/(f_s^2))I \quad (1)$$
$$\therefore I = (N_d f_s^2)/((tS)K_c) \quad (2)$$

where the quantities are: N_d Digital number (value) of the pixel in the image, t Exposure time (seconds), f_s Aperture number (f-stop), S ISO sensitivity and I Intensity of the scene (cd/m^2).

The digital value of the pixels N_d can be obtained by analysing a fixed area of the image using a program such as *ImageJ*. The pixel value is directly proportional to scene luminance L_s. It is also dependent on the camera settings. If the luminance is kept constant while the exposure time is doubled, the pixel value should also double. The aperture number f_s if increased one stop (a factor of 1.4), reduces the area of the aperture by half so the pixel value also decreases by half. In theory then, the camera can be calibrated by photographing an object or source of known luminance, then inputting the values for luminance, exposure time, film speed and aperture setting into (4), and thus calculating the calibration constant K_c. It should then be possible to use the camera at other settings of exposure time, film speed and aperture setting. Unknown luminances can now be calculated using (4) and the value for K_c.

C. Beer-Lambert Law

The Beer-Lambert law, in relation to this work, describes the relationship between the intensity of light transmitted through an absorbing sample and the number of absorbing species within that sample, as well as the path length through which the light must travel, i.e. the thickness of the sample. Using Fig. 2 this relationship can be described mathematically:

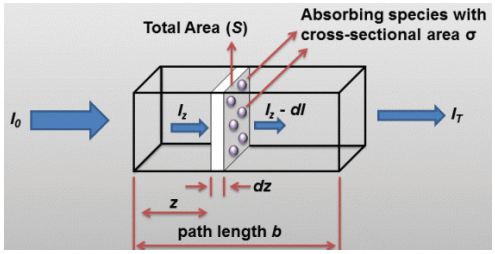

Fig. 2 A diagram representing a sample of thickness b used for the derivation of the Beer-Lambert law [9]

Consider monochromatic light passing through the sample with incident intensity I_0, intensity upon entering the infinitesimal slab of thickness dz of I_z and transmitted intensity I_T. The absorption of photons through the path length b is due to the n molecules/cm^3. The number of molecules present in the infinitesimal slab is hence:

$$n \times S \times dz \quad (3)$$

and the total fractional area on the slab due to the absorbers is given by:

$$(n \times S \times dz) \times (\sigma/S) = \sigma \times n \times dz \quad (4)$$

since each absorbing molecule has area σ. Now, the light absorbed by the molecules in the infinitesimal slab is dI which means that the fraction of light absorbed is given by dI/I_z. Since the total fractional area of the absorbing molecules is responsible for all of the absorption occurring in the slab, it follows that:

$$\begin{aligned} (dI)/(I_z) &= -\sigma \times n \times dz \\ \int_0^b (dI)/(I_z) &= -\int_0^b \sigma \times n \times dz \\ -\ln((I_z)/(I_0)) &= \sigma \times n \times b \end{aligned} \quad (5)$$

or, with a little manipulation, the expression above can be rewritten as:

$$-\log((I_z)/(I_0)) = A = \varepsilon \times b \times c \quad (6)$$

where A is known as the absorbance, ε [M^{-1}cm^{-1}] is the wavelength-dependent molar absorptivity coefficient and c [M/l] is the absorber concentration [10].

III. Method

A. Experimental Technique

The work was conducted to observe if the intensity of light propagating through phantom tissue follows the exponential relationship with the concentration of absorbers in the tissue, as in the Beer-Lambert law. The homogeneous medium was a simple liquid tissue phantom set up to mimic the absorption of melanin in the body. Also of interest was to determine whether a typical, low-cost digital camera could be used to measure the transmitted intensity through the phantom using image analysis software. Two methods of measuring the light intensity were used:

a. A direct measurement with an ISO-TECH ILM 1335 digital luxmeter
b. Using a 5.1 megapixel Samsung Digimax S500 digital camera and *ImageJ* to determine the pixel values of images.

The results of these methods were then compared to each other and the theoretical results from the Beer-Lambert law. Before the phantom sample could be created, the camera had to be correctly calibrated. This was achieved by taking images of commercial incandescent, fluorescent light sources and a sodium lamp. The illuminance of these sources was determined using the luxmeter, each measurement taken at a different, arbitrary distance away from the source. White paper was used directly below the light sources to first serve as the point at which the meter read the illuminance and then as the area of focus for the camera image. The white paper is almost perfectly Lambertian and thus the luminance L_s at the surface of the paper was calculated from the illuminance.

After these images were taken, they were transferred onto a PC where *ImageJ* was used to find the pixel values N_d over a fixed area of pixels each time. It was then possible to use (1) to find the camera calibration constant K_c.

Next, the phantom tissue sample and the light source were set up. The tissue phantom was created using milk and soy sauce to act primarily as an absorber. The soy sauce has no physical similarities to melanin, however both act as good absorbers of light, which was ideal for this work. The idea was that increasing the concentration of soy sauce in the milk represented an increase in melanin content in an individual. A sodium lamp (wavelength ≈ 700nm) was used as the light source and the containment vessel for the phantom was a beaker with a diameter of 36mm. Finally a white screen was put up behind the sample to view the transmitted light.

Fig. 3 The experimental setup showing the transmitted light on the screen

Light was first passed through the sample containing only milk and no soy sauce. This represented a 0% concentration of soy sauce (i.e. 0% melanin content) in the tissue phantom. For each subsequent reading, milk was removed and soy sauce was added into the sample to keep the liquid at a constant volume of 80ml. Hence, the concentration of soy sauce increased in the sample each time. The light intensity transmitted was first measured directly using the lux meter and then an image of the screen was taken, using the digital camera, to be later analyzed in order to find the transmitted intensity.

IV. Results

The errors encountered from the results obtained with the meter were due to the positioning, stabilization (human errors) and the inbuilt error from the meter itself. The sources of the errors from the digital camera method were the same as those of the meter with the inclusion of the error arising from the calculation of the camera calibration constant K_c. The total error in this case was minimized by analyzing many images and calculating the calibration constant many times. The calibration constant was found to be $K_c \approx 50$ and from here the intensities could be found, using (2) where t, S and f_s were found from the properties of the image file.

The illuminance of transmitted light was measured using both techniques and then these values were tabulated along with the concentration of the soy sauce as a percentage. Note that the illuminance is directly proportional to the intensity thus it was more convenient to measure the illuminance and illustrate that it follows the Beer-Lambert law. This then would imply that the intensity also follows the Beer-Lambert law. The results were tabulated as follows:

TABLE I
Measurements of the Transmitted Intensity through the Sample, Measured by the Different Methods, at Varying Concentrations

Concentration (% soy sauce)	Intensity using meter (lux)	± Error from meter (lux)	Intensity using digital camera (lux)	± Error from digital camera (lux)
0.00	29.0	1.740	29.2	2.482
12.5	21.2	1.270	22.1	1.880
23.0	15.7	0.942	16.8	1.430
33.0	12.1	0.726	13.2	1.120
41.0	10.1	0.606	11.2	0.952
49.0	9.50	0.570	9.70	0.825
55.0	7.60	0.496	8.50	0.723
61.0	7.10	0.426	7.94	0.675
66.0	6.20	0.372	6.80	0.578

From the derivation of the Beer-Lambert law we know that $-log((I)/(I_0)) = \varepsilon bc$ and $I = I_0 e^{-\alpha c}$, where α is just a quantity comprised of the extinction coefficient (wavelength dependent), which is not of importance in this work, and the path length, which remains constant. It is hence clear that there should exist a decaying exponential relationship between the concentration of the absorbers c and the transmitted intensity I.

GNUPLOT was used to plot these results with each other graphically and also to fit an exponential function of the form $f(x) = ae^{-b2x}$ as the theoretical curve, from (3), to which the results will be compared. These results for the intensities were then plotted first individually against the concentration and afterwards on the same set of axes. The errors from Table I were plotted in the form of error bars. Figs. 3-5 illustrate the close correlation between both methods and the expected theoretical curve, when the intensity of transmitted light through the sample is plotted against the concentration of absorbers in the sample.

A. Discussion

One of the main objectives of this work was to test the effectiveness of the digital camera at measuring the intensity of light. It is well known that the results of an experiment such as this should adhere to the Beer-Lambert law and the range between the results presented in this report and the Beer-Lambert law (theory) would reveal the effectiveness of the digital camera as a tool for light measurement.

The work done by Wüller and Gabele [11] shows the use of a digital camera as luminance meter. The digital camera was first calibrated using an integrating sphere, for highly accurate calibration. Once calibrated, the digital still camera (DSC) was utilized under various conditions and camera settings to observe variations in luminance. The images obtained by

Wüller and Gabele were analyzed via a Photoshop plug-in and values for the camera calibration constant, and hence the luminance, were obtained. Wüller and Gabele go on to state that: "To use a digital camera as a luminance meter one has to consider that these cameras are not exact measuring instruments". Digital still cameras are constructed with a main focus on getting pleasant pictures out of the camera. However, for many applications the luminance results of a digital camera will be sufficient. There are measuring tasks which do not need the absolute luminance value but, for example, the luminance distribution in the whole scene or luminance ratios [11]. Fig. 7 shows the relationship, as from [11], between the digital camera constant and the luminance/intensity of the scene (as in (2)).

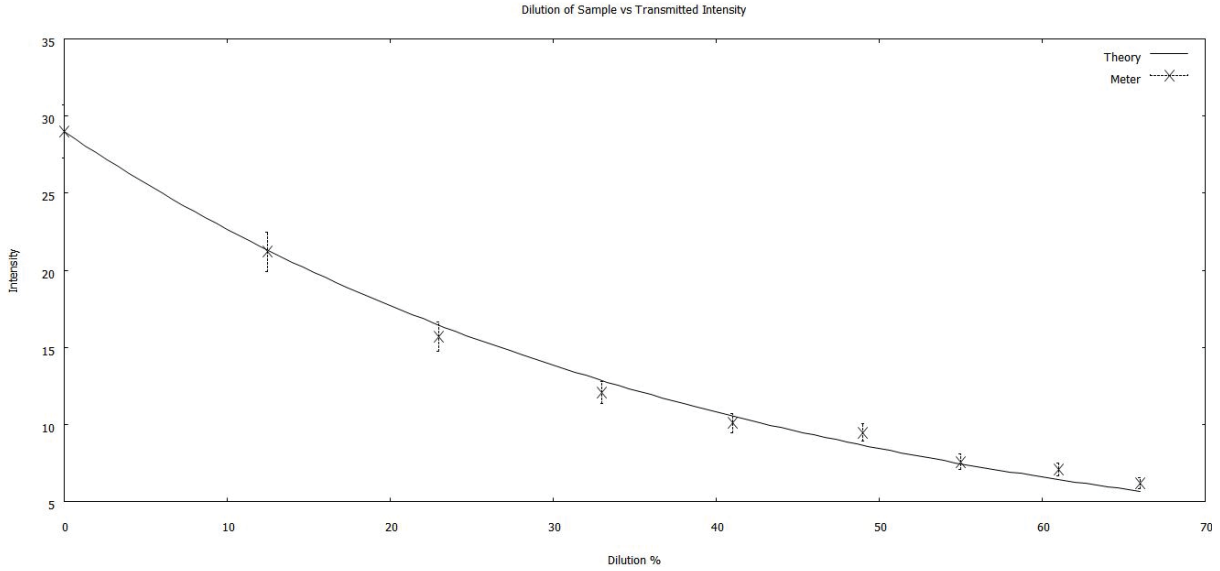

Fig. 4 Graph of intensity (lux) against concentration (%) using the meter

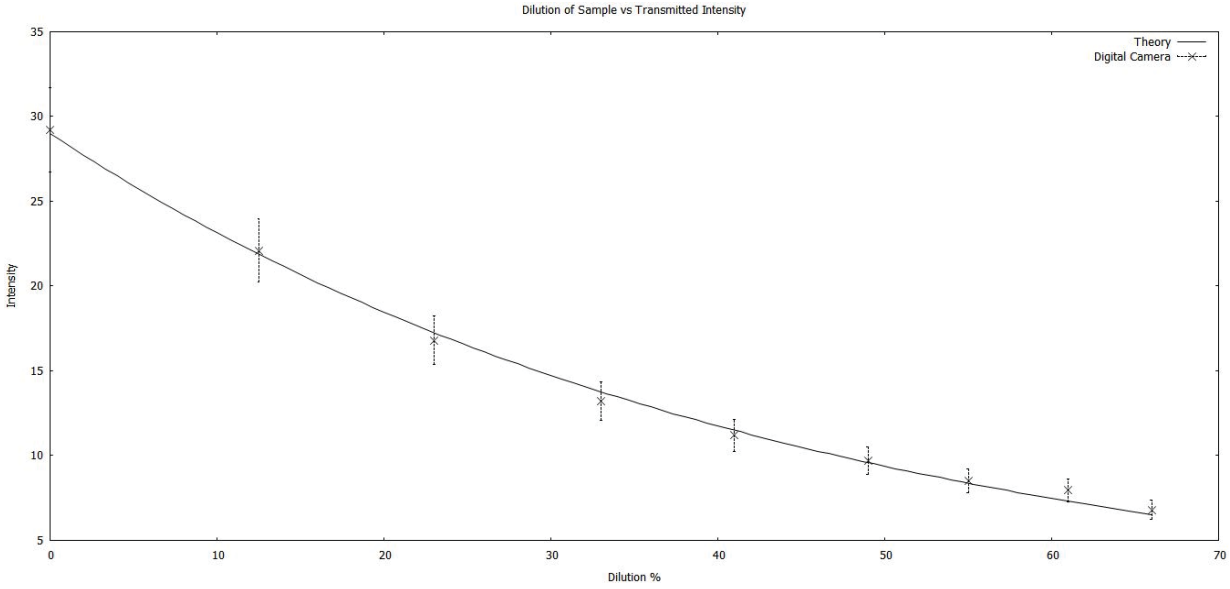

Fig. 5 Graph of intensity (lux) against concentration (%) using the digital camera

The expected shape of the curves of the intensity at increasing sample concentrations, is of an exponentially decaying function. This compares well to the results obtained in the work done by O' Doherty et al. (2006) [12] as shown in Fig. 8. The probability distributions in Fig. 8 show the decaying relationship between the depth which a photon penetrates through a sample (dermis, in the case of [12]) and the probability of the photon being absorbed or scattered. The work carried out here is roughly analogous to the work in [12], in that a simulation of the optical properties of tissue was sought.

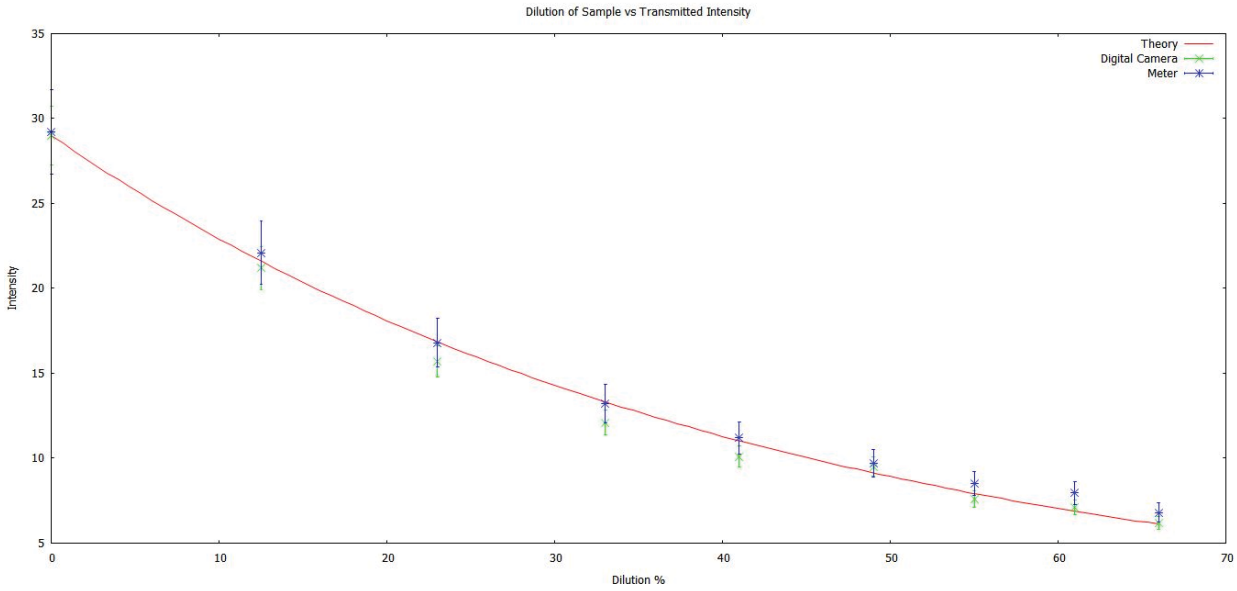

Fig. 6 Both results plotted against the concentration on the same set of axes

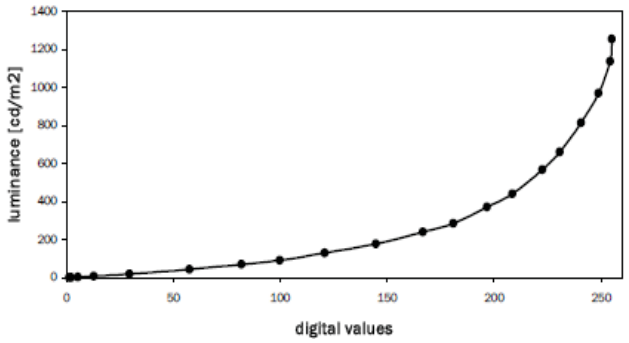

Fig. 7 The relationship between the camera calibration constant and the scene luminance [11]

Photons were incident onto an optically simulated dermis and the transmitted photons were observed using a detector array. From the graphs plotted it is possible to see that both methods agree well with the plotted theoretical curve and the results from [12], that is, the intensity of light passing through the phantom sample decays exponentially as the concentration of absorbers increases. All of the measured values for both methods lie at most an error bar away from the theory, which proves that the results were highly accurate. It is interesting to note that at low intensities (i.e. at higher concentrations) there was a greater fluctuation of the measured results, using the meter, from the theoretical values. This suggests that the meter was not as sensitive in reading the lower intensity values. The digital camera method, on the other hand, remained close to expected values even at low intensities. It is therefore feasible to use a digital camera to photographically detect the transmission of light through a medium.

The low cost and the ease of setting up the apparatus makes these techniques efficient and cost effective which tallies with their observed accuracy. The tissue sample, being a simple liquid phantom, using milk as a scatterer [13] and soy sauce as an absorber, are both affordable and easily accessible. The theoretical results were achieved using sophisticated, costly equipment and a more complex phantom. The results, however accurate they are, were gathered carefully and as precisely as possible, which turned out to be more time consuming then was first thought. It was during the measurements and analysis of the results that advantages and disadvantages were found for each method.

In an ideal situation, a luminance meter would be used to calibrate the camera and take the transmitted light readings directly, as it is much more sensitive than a lux meter [8]. Also, a more complicated and complex tissue phantom (most likely a solid phantom) would be created to simulate the inhomogeneities to greater accuracy. Finally, the apparatus would be set up in a dark laboratory with no ambient lighting, in order to minimize the errors in reading the meter. However, it was more appropriate to test the effectiveness of the equipment that was used in this experiment as it was low cost. An important aspect of this experimental setup was to not only find the required results, but to ascertain that the methods used were affordable as well as effective in normal, everyday conditions.

Problems arose when first working with the 70mm diameter beaker to contain the sample. At this thickness of the medium, very little light was transmitted through the sample and measuring the intensity with the meter became difficult to perform accurately. This was the first indication that the lux meter may not be sensitive enough to accurately read low intensities. The remedy to this was to use a beaker with a 36mm diameter to contain the sample. The smaller path length meant that more light was transmitted and the measurements from the meter were then much easier to make.

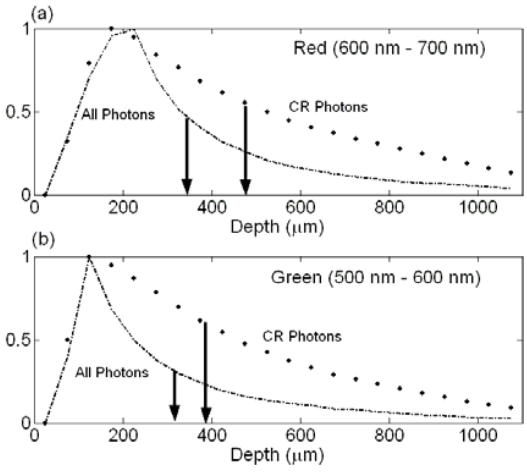

Fig. 8 Probability density functions of a Monte Carlo simulation carried out by O' Doherty et al. [12]

TABLE II
OBSERVED ADVANTAGES AND DISADVANTAGES TABULATED FOR THE DIGITAL CAMERA METHOD

Advantages of the digital camera method	Disadvantages of the digital camera method
• Digital cameras are easily accessible and relatively cheap.	• A fair bit of work is required before an intensity is actually measured.
• A digital camera captures more of the scene than a sophisticated luminance meter would.	• Digital cameras have to be correctly calibrated first, before measurements can be made.
• Measurements at low light intensities are more accurate than the meter.	• The correct software and hardware have to be available to analyze images and extract relevant data.

TABLE III
OBSERVED ADVANTAGES AND DISADVANTAGES TABULATED FOR THE LUX METER METHOD

Advantages of the lux meter method	Disadvantages of the lux meter method
• The lux meter is relatively cheap when compared to a standard luminance meter	• It can be difficult to perform accurate measurements even in laboratory conditions.
Measurements can be made in little time if done correctly and precisely.	• The meter is not sensitive enough to easily measure low intensities.

V. CONCLUSION

The results obtained proved that basic equipment could be used to accurately measure the propagation of light through a tissue sample. It was then confirmed that an increase in melanin content in an individual (concentration of absorbers in the sample) leads to an increased absorption of light. Also, measurements with a conventional lux meter can sometimes be a difficult and tedious task, especially at low light intensities. The experiment also shows that an ordinary, inexpensive digital camera can be used to measure the intensity of light quite accurately, provided that it has been correctly calibrated. The accuracy of the digital camera method actually exceeds that of the light meter, more so at low intensities.

The digital camera method is hence ideally applicable to poor medical facilities wanting to measure the propagation of light through tissue or just to test the effectiveness of their light sources. Future work will attempt to extend this research with the creation and study of solid, agar based, optical tissue phantoms. The absorption coefficients and the optical densities of various tissue-simulating phantoms can be determined using a conventional meter or digital camera. Other properties of these phantoms may also be obtained with the implementation of the techniques outlined in this work.

REFERENCES

[1] Hutchison AM, Beard DJ, Bishop J, Pallister I, Davies W. An Investigation of the Transmission and Attenuation of Intense Pulsed Light on Samples of Human Achilles Tendon and Surrounding Tissue. Lasers in Surgery and Medicine. 2012; 44:397–405.
[2] Baranoski GVG, Krishnaswamy A. An Introduction to Light Interaction with Human Skin. RITA. 2004.
[3] Taylor EF. Illumination Fundamentals. Rensselaer Polytechnic Institute. 2000.
[4] Kim A, Keller JB. Light Propagation in Biological Tissue. Departments of Mathematics and Mechanical Engineering Stanford University, USA.
[5] Sviridov A, Hassan VCM, Russo A, et al. Intensity Profiles of Linearly Polarized Light Backscattered from Skin and Tissue-like Phantoms. Journal of Biomedical Optics 10(1). 2005.
[6] Ghosh S, Soni J, Purwar H, Jagtap J, Pradhan A, Ghosh N, et al. Differing Self-similarity in Light Scattering Spectra: A Potential Tool for Pre-cancer Detection. Optical Society of America. 2011.
[7] Jacques SL, Ramella-Roman JC, Lee K. Imaging Skin Pathology with Polarized Light. Journal of Biomedical Optics 7(3). 2002
[8] Hiscocks PD. Measuring Luminance with a Digital Camera. Syscomp Electronic Design Limited. 2011.
[9] Pharmaxchange. Available online from http://pharmaxchange.info/press/2012/04/ultraviolet-visible-uv-vis-spectroscopy-%E2%80%93-derivation-of-beer-lambert-law/. Accessed 10 March 2016.
[10] College of Life Science, National Tsing Hua University; Available online from http://life.nthu.edu.tw/~labcjw/BioPhyChem/Spectroscopy/beerslaw.htm
[11] Wüller D, Gabele H. The Usage of Digital Cameras as Luminance Meters. SPIE-IS&T Electronic Imaging. 2007
[12] O' Doherty J, Henricson J, Anderson C, Leahy MJ, Nilsson GE, Sjöberg F. Sub-Epidermal Imaging using Polarized Light Spectroscopy for Assessment of Skin Microcirculation. Skin Technology and Research. 2007; 13;472 - 484.
[13] Tuchin VV. Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis. 2nd ed. Bellingham: SPIE; 2007.