Title: **Multispectral Fundus Imaging: acquisition, interpretation using computer simulations, and potential clinical applications**

Authors: **Antonio Calcagni[1,2], Iain B Styles[1], Ela Claridge[1], Felipe Orihuela-Espina[1], Jonathan M Gibson[2,3]**

Institution:

1. **University of Birmingham, School of Computer Science, Edgbaston, Birmingham, B15 2TT, United Kingdom**

2. **Birmingham and Midland Eye Centre, City Hospital, Sandwell and West Birmingham Hospitals NHS Trust, Dudley Road, Birmingham, B18 7QU, United Kingdom**

3. **Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, B9 5SS, United Kingdom**

Word Count: **5396 (excluding references)**

Grant Information:

1. **Engineering and Physical Sciences Research Council (EPSRC)**
   Grant Number GR/S09906/01
2. **Advantage West Midlands**
   Grant Number BGIN3264

ABSTRACT

Purpose

To develop a non-invasive method for quantification of blood and pigment distributions across the posterior pole of the fundus from multispectral images using a computer generated reflectance model of the fundus.

Methods

A computer model was developed to simulate light interaction with the fundus at different wavelengths. The distribution of macular pigment and retinal haemoglobins in the fundus was obtained by comparing the model predictions to multispectral image data at each pixel. Fundus images were acquired from 31 subjects from various ethnic backgrounds and parametric maps showing the distribution of Macular Pigment and of retinal haemoglobins throughout the fundus were computed.

Results

The relative distributions of macular pigment and retinal haemoglobins in the subjects were successfully derived from multispectral images acquired at wavelengths 507, 525, 552, 585, 596 and 611nm. Comparisons of model and image data show that both display similar trends, although model predictions overestimate the tissue reflectance at higher wavelengths. Successful application of the technique is contingent upon minimal eye movement between exposures.

Conclusion

The distributions of macular pigment and retinal haemoglobins obtained in this preliminary investigation are in good agreement with published data on normal subjects. The ongoing development of the imaging system should

allow for absolute parameter values to be computed. A further study will investigate subjects with known pathologies to determine the effectiveness of the method as a screening and diagnostic tool.

INTRODUCTION

Fundus pathology, whether it involves the retina, the choroid or both, frequently alters the distribution and relative quantities of blood and ocular pigments in one or more of the fundus layers. These changes may alter the appearance of the fundus in a subtle way and may be difficult to detect using standard imaging devices such as an ophthalmoscope, a slit lamp or a fundus camera. Macular pathologies are of particular concern, as the macula is crucial for functional vision and legal blindness results when this area is lost to disease.

The macula has a distinct appearance in fundus images due to several histological factors: its darker hues result from the abundance of melanin pigments in the Retinal Pigment Epithelium (RPE); the retinal blood supply network forms an avascular zone in the foveal region thus reducing the absorption of light by hemoglobins present elsewhere in the retina; a characteristic yellow tint around the foveal area in the primate retina is due to the macular pigment (MP).

The MP has a sharp peak at the center of the fovea and declines exponentially with increasing eccentricity, reaching negligible levels at 4-6 degrees of the visual angle (1-1.8mm)[1,2,3].

The MP is composed of three isomeric carotenoids, lutein (L), zeaxanthin (Z) and mesozeaxanthin (MZ). L and Z are of dietary origin, whereas MZ is thought to be a conversion product from L and Z[4]. The role of MP is not entirely known, but it is thought to have a protective role against age-related macular degeneration (ARMD) and other chorio-retinopathies, by acting as an

optical filter to shorter wavelenghts (blue), which are known to be phototoxic, and as a consequence of its antioxidant properties[5,6,7].

Several factors appear to influence MP density and distribution; the main ones being gender[8,9,10], age[11,12], Body Mass Index[13], iris pigmentation[Error! Bookmark not defined.,Error! Bookmark not defined.,Error! Bookmark not defined.], diet[14], smoking[Error! Bookmark not defined.,15] (smoking is also thought to be a risk factor in age-related macular degeneration[16]). It is important to establish if the high variance in MP density amongst normal individuals has any significance in the development of retinal/choroidal pathology and, if so, whether any intervention, dietary or non-dietary, is appropriate. MP therefore has to be assessed in diverse populations and age groups, to establish not only the risk associated with different levels of MP but also the effects of interventional strategies.

Several techniques have been adopted to measure MP in vivo, including Raman detection[17], scanning laser ophthalmoscope[18], reflectometry[Error! Bookmark not defined.,19,20,21], color matching[22], and heterochromatic flicker photometry using “Maxwellian” and “freeview” systems[23,24]. All these techniques have proved effective but have limitations. This article reports the preliminary results of a novel technique capable of mapping the distribution of MP throughout the posterior pole. The technique is based on a computer analysis of multispectral images of the fundus and has proved successful in quantifying the MP in the retina in normal subjects. The method uses a Monte Carlo computer simulation of the passage of light through the fundus tissues in order to establish a link between the tissue composition and the fundus colors. It appears to be repeatable, cost-effective and simple to use.

The method can potentially be extended to determine the quantities of other pigments, such as retinal and choroidal hemoglobins and RPE and choroidal melanin.

If the method can be refined and the results obtained in normal subjects can be extended to pathology, a new tool for the detection of certain retinal and choroidal changes before damage becomes clinically evident would be available, allowing the introduction of a screening program that would be beneficial to patients and the health care system, as interventional strategies could be undertaken before irreversible sight-threatening complications occur.

METHODS

When light strikes a tissue, it can be reflected, absorbed, scattered, transmitted, or remitted depending on the wavelength of incident light and tissue histology, and image formation is determined by the amount of each wavelength reflected. It is therefore theoretically possible to recover tissue histology from images, providing the optical properties of each tissue component and the optical spectrum of the light source are known.

Conventional red-green-blue (RGB) digital images give good spatial resolution, but low spectral resolution, thus making truly accurate histological interpretation of the tissue practically impossible. On the other hand, traditional tissue spectroscopy allows a detailed characterization of a single point, but large areas of tissue cannot be examined.

Multispectral imaging combines the advantages of both techniques, allowing detailed spectral analysis of a large area; this is achieved by superimposing a number of images taken using a predetermined set of narrow-band filters, which provide the desired level of spectral detail.

The ocular fundus is a multi-layered structure; each layer is composed of different constituents and there are five main pigments: the MP, retinal blood, RPE melanin, choroidal blood and choroidal melanin.

Using detailed knowledge of the structure and histology, a model of fundus reflectance was constructed and used to select appropriate multispectral filters. The resulting images were then processed by the model and used to extract quantitative histological information from fundi of healthy and pathological eyes.

CONSTRUCTION OF THE MODEL

The interaction of light with tissue components, in terms of its reflection, absorption and scattering, can be simulated using a probabilistic computational method known as “Monte Carlo”, which simulates the passage of hundreds of thousands of photons through the tissue. Each tissue component is assigned a probability that a photon that reaches it is reflected, absorbed or scattered; the probabilities depend on the physical properties and composition of the tissue itself. Some photons are either absorbed or are scattered deeper into the tissue, and thus never re-emerge. A proportion of the photons however is remitted from the tissue and these can be captured by a camera. The Monte Carlo simulation computes the probable number of remitted photons for different wavelengths and in this way it is possible to simulate the entire spectrum of light remitted from the fundus. Different quantities and locations of pigments and other fundus components result in different remitted spectra. This observation is central to the authors’ research, as it allows fundus composition to be deduced from the shape of the remitted spectrum at each point in the fundus image.

The Monte Carlo simulation is considered to be more accurate than cruder approximation methods described previously[25] and it has been successfully used for other tissues[26].

In this work the Monte Carlo simulation described by Jacques and Wang[27] was used.

Earlier work by Preece and Claridge[28] used Monte Carlo simulation to generate a reflectance model of the fundus characterized by three parameters: RPE melanin, choroidal melanin and choroidal hemoglobins; all

other properties of the tissues were assumed to be constant. However, there are two further pigments that influence fundus histology: retinal hemoglobins and MP, both important in many pathological processes; these two parameters were therefore introduced in the model, which hence is characterized by five parameters:

• CMP: concentration of MP in the retina.

• CRH: concentration of hemoglobins in the retina.

• CRM: concentration of melanin in the RPE.

• CCM: concentration of melanin in the choroid.

• CCH: concentration of hemoglobins in the choroid.

Figure 1 shows the specific absorption coefficient curves of each of the pigments[29,30,31] and Table 1 shows the concentrations used in the simulation for each of the pigments, taken from the literature[28,31,32,33]. Both absorption coefficient curves and pigment concentrations affect the numbers of remitted photons, and thus the shape of the remitted spectra.

Although the absorption coefficients are fixed for a given fundus component, the concentrations vary across the fundus. Monte Carlo simulations were carried out for all the combinations of these concentrations; this was achieved by altering independently each of the variables within every layer of the tissue, in steps, from a minimum to a maximum concentration (obtained from the literature[28,31,32,33]), and calculating the respective spectral reflectance curve for each combination. The simulation thus generated a reflectance model of the fundus in which each reflectance spectrum corresponds to one, and only one, combination of concentrations of MP, retinal blood, RPE melanin, choroidal blood, and choroidal melanin. This reflectance model was then used to select

the appropriate filters and to map the distribution of the five parameters used for its construction.

EFFECT OF THE FUNDUS COMPOSITION ON THE REMITTED SPECTRA

It is useful to examine how fundus reflectance varies as fundus composition is changed. In each of the graphs in Figure 2, four of the five parameters are constant, and the fifth parameter is varied. Each pigment has a distinct effect on reflectance:

1. As the concentration of MP is increased [Figure 2(a)], the reflectance is attenuated in the blue and green spectral regions, with no effect in the red since macular pigment does not absorb at wavelengths longer than 534nm.
2. Increasing the concentration of RPE melanin affects the whole reflectance spectrum [Figure 2(b)]. A careful analysis shows that an increase in RPE melanin causes more attenuation in the blue spectral region than in the red, and the change is not a straightforward wavelength-independent scaling.
3. As the concentration of retinal hemoglobins is increased [Figure 2(c)] the spectral reflectance attenuates the spectrum strongly in blue and green regions, and introduces strong features due to peaks and troughs in the specific absorption coefficient of the hemoglobins.
4. Changes in choroidal melanin [Figure 2(d)] have a very strong effect in the red region of the spectrum, but a much weaker effect in the blue and green region. There is a marked difference between the effects of an increase in RPE melanin and in choroidal melanin: whilst increases in RPE melanin attenuate the spectrum in roughly equal amounts in blue and red (there is a small difference), the effect of choroidal melanin is much stronger in the red,

even though the pigments are identical. This difference arises from the different environments these components are found in. In the RPE, there are no other significant absorbers and there is relatively weak scattering from the RPE tissue itself. In the choroid, other absorbers are present (hemoglobins), and scattering from the underlying tissue is very strong. The interaction between the varying component and its surroundings gives rise to these differences, and this emphasises the importance of accurate modelling of scattering processes which have a strong effect on the behaviour of the model.

5. Finally, increases in choroidal hemoglobins [Figure 2(e)] lead to a reduction in reflectance in the green region, where absorption by hemoglobins is very strong. Again the difference between the effect from choroidal and retinal hemoglobins is due to the interaction with the surrounding tissues.

SELECTION OF MULTISPECTRAL FILTERS

It can be seen from the above analysis that each pigment affects different parts of the visible spectrum in a different way. These patterns of variability were used to select a set of bandpass filters to be used in multispectral image acquisition. Conventional color cameras use three broad-band filters at the red, green and blue portion of the visible spectrum. Multispectral imaging does not have this restriction; however, for practical reasons, it is necessary to have the smallest possible number of distinct filters. The ideal choices are filters at wavelengths at which the variability in the reflectance for each individual parameter is greatest and, at the same time, the variability due to the remaining parameters is as small as possible. Such filters can be deduced

by analysing of the spectral variability illustrated in Figure 2. A full account of the filter selection method can be found in Styles et al[34, 35].

Having established the filters to be used in the image acquisition, each of the spectral curves in the model needs to be represented only at the wavelengths corresponding to the chosen filters.

IMAGE ACQUISITION

The image acquisition system comprised a modified Zeiss RCM250 fundus camera equipped with a 12-bit monochrome digital camera (Retiga Exi, Qimaging) and a tunable LCD optical filter (VariSpec Cambridge Research Instruments). Six narrow band filters were used, centred at the following wavelengths: 507, 525, 552, 585, 596 and 611nm.

SUBJECTS

Images were acquired from 16 healthy volunteers from a variety of ethnic backgrounds, including Caucasian, African, Indo-Asian, Afro-Caribbean and Mediterranean, and 15 subjects with known retinal/choroidal pathology (for example, retinal hemorrhages, choroidal neovascular membranes) in order to investigate the ability of the technique to flag these pathologies.

In all healthy participants, best corrected visual acuity was 0.0 or better using the logarithm of the minimum angle of resolution (LogMAR) acuity chart, there was no clinical evidence of retinal disease (one subject had undergone uncomplicated phacoemulsification and posterior chamber intraocular lens (IOL) implant; the IOL absorption curve was taken into account when data was analyzed).

Approval from the local ethics Committee was obtained, all subjects gave informed written consent and the study protocol adhered to the tenets of the Declaration of Helsinki.

Subjects were positioned at the fundus camera and the posterior pole was focused by an experienced operator using a light intensity that was easily tolerated by the subject. The intensity used varied from 300cd/m^2 to 500cd/m^2, measured by a Minolta luminance meter, model LS-110 with close up lens.

IMAGE ANALYSIS AND PARAMETER RECOVERY

To recover the parameters (concentrations of MP, retinal hemoglobins, RPE melanin, choroidal hemoglobins, and choroidal melanin) at each point of the posterior pole, images acquired from all subjects need to be compared to the reflectance model.

The reflectance model can be thought of as a database where for each combination of the five histological parameters there is one and only one record which stores the five illumination-normalised image values, and every possible combination of concentrations of the five parameters is represented within the database.

The values in the model are theoretical predictions computed using Monte Carlo simulations followed by simulated application of the optical filters. Assuming that the model is correct, for each set of values obtained from a set of fundus images there should be a closely corresponding record in the database.

The parameter values can be recovered according to the following procedure:

1. Six images are acquired through the six selected filters.
2. The images are spatially registered to correct for involuntary movements during the acquisition process and corrected for exposure, gain and offset.
3. Five illumination-normalised images (image ratios) are generated by dividing each of the first five images by the image acquired at 611nm; hence for each point of the posterior pole, five normalised image values are extracted.
4. The record which most closely corresponds to the record of these image values is found in the database (reflectance model).
5. The concentrations associated with the five values for each point of the image are deduced from the reflectance model.
6. Five new images, one for each of the parameters, are generated ("parametric maps").

The whole procedure will therefore generate five parametric maps, representing respectively the concentration of MP, retinal hemoglobins, RPE melanin, choroidal hemoglobins, and choroidal melanin at each point of the image.

There are a number of ways in which the parameter values can be deduced from the reflectance model (step 5). In general, this is a very challenging problem and each possible approach has some disadvantages; the authors found a neural network (in particular, a radial basis network [REF: Neural Networks: A Comprehensive Foundation, Simon Haykin, Prentice Hall, New Jersey, 1999] to be the most effective technique. Concentrations of macular pigment and retinal hemoglobins were computable with acceptable accuracy

(±0.1mmol/L), but recovery of other parameters proved much less accurate. This is due to the highly complex relationship between the image ratios and the parameters and more effective methods for parameter recovery are being investigated.

A full account of image analysis, parameter recovery and minimization of error can be found in Styles et al[35].

RESULTS

Image data from all normal subjects was compared with model data at the selected wavelengths (corresponding to the image filters), as shown in Figure 3a. It is obvious the two do not match: although the shape of both subject data and model data are similar (for example, where the model is long and thin the subject data is also long and thin), the former does not always fall within the boundaries of the latter and the model appears to overestimate the reflectance of the fundus in the longer wavelength region. There are several reasons that could account for this discrepancy, which are reviewed in the discussion section. The fact however that the image and model data are similar in shape suggests the behaviour of the model with respect to the parameters is correct. The authors used a simple “shift and scale” operation to align model data with image data, as shown in Figure 3b and described in Styles et al[35]. Although the absolute values of the recovered parameters may be incorrect, the relative values will have the correct relationship; i.e. when the concentration of one of the parameters at a given point A is greater than the concentration at a second given point B, the parameter shown in the computer-generated map at point A will be greater than that at point B. Only the parametric maps showing the distribution of MP and retinal hemoglobins were analyzed, due to the difficulties in recovering other parameters with sufficient accuracy.

ANALYSIS OF MACUALR PIGMENT DISTRIBUTION

MP, in view of its well established distribution in non-pathological eyes, is well-suited for validating the method described in this paper; furthermore, as

the effect of MP on the visible spectrum of light is negligible at wavelengths higher than 534nm, it will not be affected by the fact that the model overestimates the reflectance in the higher wavelength region. Seven subjects in all showed a parametric map of MP distribution as expected; Figure 4 shows the color (RGB) photographs (first column), computer-generated parametric maps (second column), two-dimensional (third column) and three-dimensional representations (fourth column) of the macular pigment distribution of these eyes: there is an obvious peak of MP at the fovea with a rapid decline outside the foveal area: this is consistent with known histology of the fundus[Error! Bookmark not defined.,Error! Bookmark not defined.,Error! Bookmark not defined.].

The last two sets of images are from the same subject, showing the repeatability of the method.

There is frequently a false positive at the optic disc: the different histology of this area implies that it is not represented in the model and hence falls outside its bounds and cannot be interpreted correctly. Furthermore artefacts due to reflection from the inner limiting membrane can be noticed in some subjects adjacent to the macular area; this problem can be solved using polarized light, as discussed in a subsequent section.

Not all subjects showed the distribution expected and this is most likely due to problems inherent within the imaging methods. These problems are tackled in detail in the discussion section, but, in brief, the relatively prolonged exposure used to take the images implies that small saccadic movements are unavoidable; whilst the movement artefact can be overcome with sophisticated registration techniques, constancy of fundus illumination, essential for image normalisation, is inevitably compromised.

ANALYSIS OF RETINAL HEMOGLOBIN DISTRIBUTION

Detecting variations in the distribution of retinal blood is an essential part of the fundus examination, especially when pathology is concerned (for example, diabetic macular ischemia, retinal hemorrhages etc). Sometimes, the task is particularly difficult, especially in more pigmented eyes and in the foveal area. Furthermore, the distribution of blood around the fovea is very important for maintaining good vision, and an enlargement of the foveal avascular zone (FAZ) is frequently associated with deterioration in vision. Usually, these features can be detected only by fundus fluorescein angiography, an invasive technique that is potentially life-threatening (anaphylactic shock). Obviously, an alternative method that does not entail these risks would be of great benefit. Figures 5 and 6 show, respectively, the RGB photographs (first column), computer-generated parametric maps (second column), two-dimensional (third column) and three-dimensional (fourth column) representations of two normal fundi (Figure 5) and of one with retinal hemorrhages (Figure 6). The normal subjects (Figure 5) show a clear depression in correspondence with the FAZ, as expected; again, perifoveal artefacts due to the reflection from the inner limiting membrane can be observed. In Figure 6 the computer-generated parametric maps and the 2-D and 3-D images highlight a clear increase in blood in the same areas the RGB image shows hemorrhages.

DISCUSSION

The results from this study suggest that the described method can potentially be used as a non-invasive tool in quantifying several retinal and choroidal parameters in normal subjects.

Parametric maps for two histological parameters, MP (Figure 4) and retinal hemoglobins (Figures 5 and 6) consistent with known histology have been shown. Whilst the results are encouraging, there are a number of difficulties that still have to be resolved. Firstly, not all model parameters can be recovered equally well at present: only macular pigment and retinal hemoglobins could be computed with acceptable accuracy. This is thought to be due to the high level of non-linearity of the inverse mapping used[35], which means that construction of the inverse function is very complicated, hence more exhaustive mathematical analysis of inverse function construction is needed.

One of the main drawbacks of the method appears to lie in the image acquisition system used: images were acquired sequentially, with an overall exposure time of approximately 5 seconds. It is impossible for a non-anaesthetized subject to remain perfectly still during this time; breaks between frames were introduced, during which subjects were allowed to rest without moving their head; they were then asked to re-fixate, and the successive frame was acquired. All images could then be acquired without causing excessive discomfort, but no matter how well the subject is able to re-fixate, the position of the fundus always changes during these breaks, all be it minimally.

This causes a few problems that need to be addressed in further experiments.

1. The first issue is that all the images need to be registered (perfectly overlapped): whilst the registration method used[36] has produced excellent results, there is an inevitable loss of fine detail; this is a problem when the features to be analysed are minute, such as early background diabetic retinopathy in which dot hemorrhages may be only a few pixels in width.
2. The second issue results from the nature of the cardiac cycle: since the images are recorded over several seconds, the blood concentration in the fundus is likely to vary between frames, and it appears, from the current results, that even small changes make big differences to the parametric maps, frequently causing them to be “unreadable”.
3. The final issue is also a result of subject movement, but it cannot be corrected for by processing after the image has been acquired: the use of image ratios to compensate for spatial variations in illumination requires those variations to be identical in all frames. If this is not the case, then the illumination cannot be normalised.

   Inter-frame movements will necessarily lead to changes in the illumination of the fundus; it is not surprising therefore that the best results were obtained where only small corrections were required to register the images; where large corrections were necessary, the analysis procedure failed.

   The effect of subject movement on illumination appears to be significantly more serious than the movement itself: in fact the registration methods adopted can spatially align images well (although fine detail may be blurred), but they cannot compensate for changes in the illumination.

All three issues mentioned could be solved by capturing the required images simultaneously. Standard RGB cameras capture three channels (frames) at

once; a similar system acquiring simultaneously the frames required would greatly improve the image quality and is paramount in resolving the mentioned issues. A more suitable imaging system is current under development.

A further technical problem that is relatively easy to solve is the presence of specular reflection from the inner limiting membrane, a well-known problem in younger subjects. The removal of such reflections requires a set of polarising neutral filters, which should be included in any future projects adopting this technique.

The need for empirical scaling to ensure that model and image data were coincident is probably the most concerning factor: although improvement in the imaging system used is likely to solve this issue in part, other potential problems with the model itself were identified, and these require careful consideration in further studies.

1. The model assumes that the individual layers in the fundus are of constant thickness and are the same in all subjects. This assumption is not correct and variations in the layer thicknesses may well lead to significant changes in the model.
2. Although the major optical properties of the ocular tissues were taken into account, not all the possible variations within them were considered.

Whilst the model appears to capture the correct trends, it does not include the full variation of normal eyes. This is a consequence of two factors: firstly, knowledge of in vivo ocular tissue is not exhaustive, especially with regards to the scattering properties of the various layers of the fundus; secondly, an introduction of too many parameters in the computer simulation would require

many more images to be taken and would significantly increase computation times and margin of error. Further investigations however, are necessary in order to determine the precise cause of the discrepancy between normal data and model data, and other fundus features may need to be added to the model to obtain reliable and repeatable results.

In conclusion, work presented in this paper demonstrates that a physics-based model of image formation, which uses image ratios to compensate for the effects of spatially uneven illumination, can be used in principle to extract histological parameters from multispectral images of the ocular fundus. The method has been successfully used to map the distribution of macular pigment in the retina and to compute the distribution of hemoglobins in the retina.

Further work however is required to improve the image acquisition process to allow all frames to be captured simultaneously, and further refinements to the model are necessary to capture the full variation in fundus histology. A suitable imaging system is under development, and modifications to the model are being investigated.

Once these issues have been addressed, it may be that when pathology is examined, pre-symptomatic changes in subjects at risk of developing sight-threatening complications can be detected, warranting more specific investigations; these would in turn allow treatment within an appropriate time frame that to date cannot be offered to patients, in view of the high costs, the expertise required and potential risks of current imaging techniques.

A large variety of pathologies and their evolution in time need to be evaluated, in order to establish whether the described method can identify any treatable

pathology before symptoms occur and vision can still be salvaged.

The protective role of MP in various pathologies, with particular interest in ARMD, must be assessed and it is envisaged that a tool to screen subjects at high risk of developing sight-threatening complications of ARMD will be available, allowing treatment before irreversible sight-loss occurs.

The authors are also investigating reduced retinal perfusion as a precursor of diabetic retinopathy, small perifoveal hemorrhages as precursors of clinically significant macular oedema, and early choroidal neovascular membranes in age related macular degeneration, but it can be postulated that computer simulation-based multispectral fundus analysis goes well beyond the boundaries of these retinal changes and further studies are necessary to establish the full extent of its possible applications.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Engineering and Physical Sciences Research Council (EPSRC) under grant number GR/S09906/01 and from the Advantage West Midlands for funding of the hyperspectral imaging facilities under grant number BGIN3264.

The authors thank: Mr James Osborne for his generous donation of the Zeiss RCM250 fundus camera used in this work; Mr Peter Lundh of the Institute of Ophthalmology provided technical help with the design of the imaging system; The Optometry Department of the Birmingham and Midland Eye Centre for kindly loaning an artificial eye and lenses; Miss P. Stavrou, Mr. D. Chauhan and Mr. D. Dwarika of the Birmingham and Midland Eye Centre for assisting with the selection of suitable subjects.

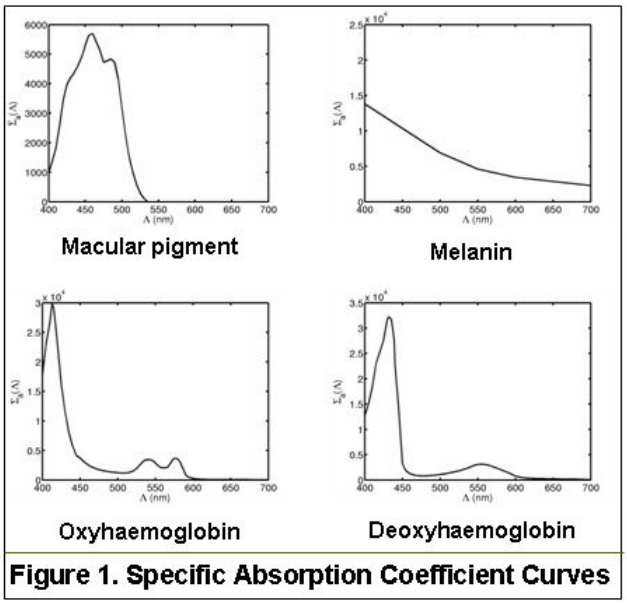

Figure 1. Specific Absorption Coefficient Curves

Pigment	*Concentration (mmol/L)*
Macular Pigment	0.0, 0.1, 0.20, 0.3, 0.4, 0.5, 0.6
RPE Melanin	1.64, 3.11, 4.58, 5.56, 6.54, 8.00
Retinal Haemoglobin	0.0, 0.25, 0.50, 0.75, 1.00, 1.25
Choroidal Melanin	0.00, 0.33, 0.66, 1.00, 1.33, 1.66
Choroidal Haemoglobin	1.90, 3.80, 5.70, 7.60, 8.50

Table 1. Parameter values used in the Monte Carlo Simulation

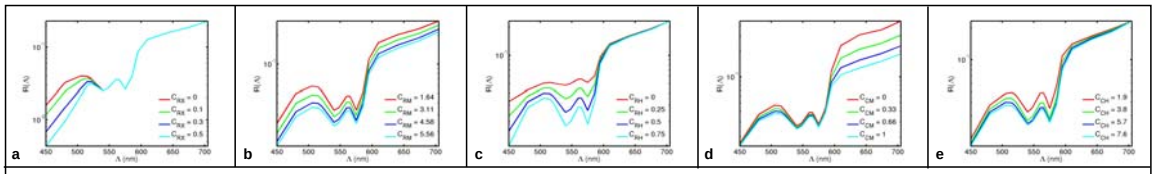

Figure 2. Calculated variation of fundus reflectance with changes in Macular Pigment (a), RPE Melanin (b), Retinal Hemoglobins (c), Choroidal Melanin (d), and Choroidal Hemoglobins (e), maintaining other four parameters constant.

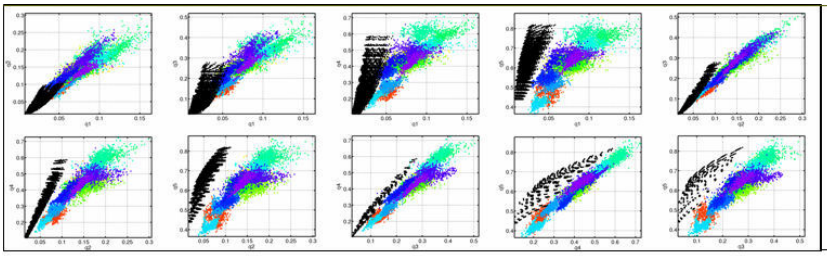

Figure 5a.
Projections of Model (black) versus Normal data (colour)

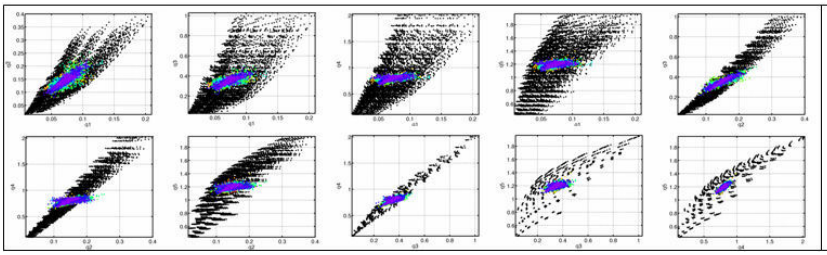

Figure 5b.
Modified Projections of Model (black) versus Normal data (colour)

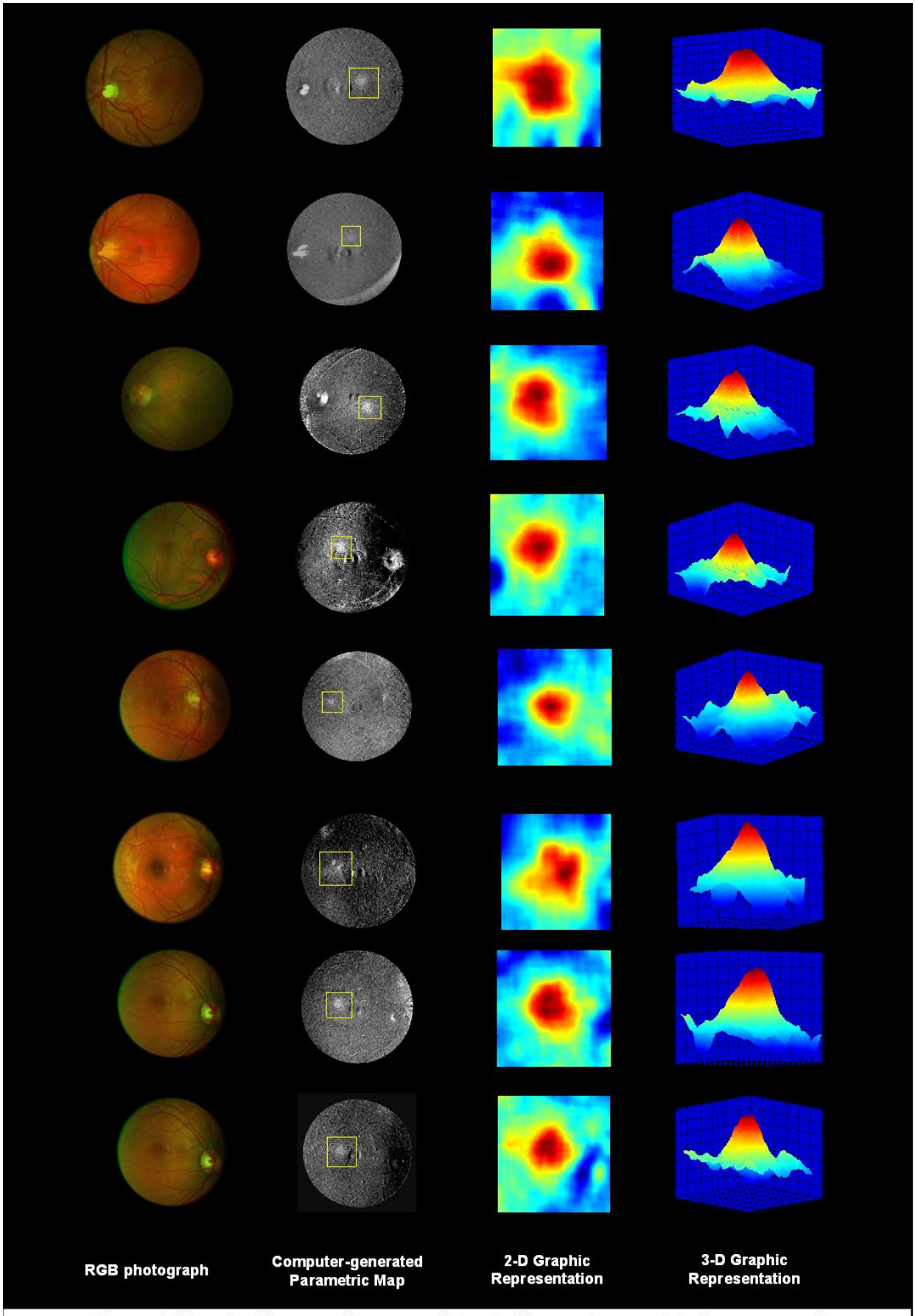

Figure 4. Macular Pigment Recovery (blue indicates less, red indicates more) See text for details

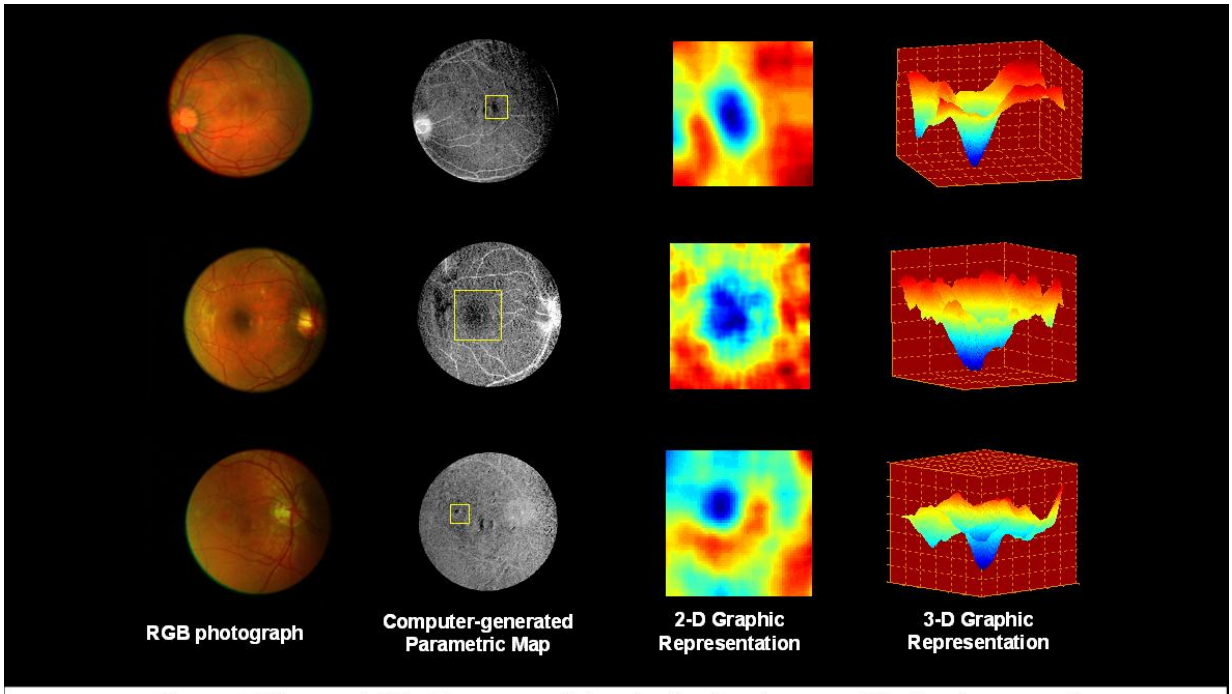

Figure 5. Hemoglobin Recovery (blue indicates less, red indicates more) See text for details

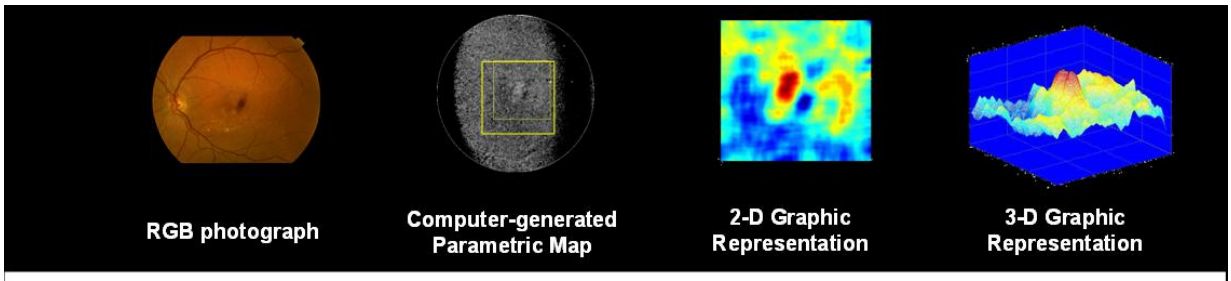

Figure 6. Hemoglobin Recovery (blue indicates less, red indicates more) See text for details

1 Bone RA, LandrumJT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci.* 1988 Jun;29(6):843-9.
2 Hammond BR Jr, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A Opt Image Sci Vis.* 1997 Jun;14(6):1187-96.
3 Ciulla TA, Curran Celentano J, Cooper D. Macular pigment optical density in a midwestern sample. *Ophthalmology.* 2001 Apr;108(4):730-7.
4 Bone RA, Landrum JT, Friedes LM, Gomez CM, Kilburn MD, Menendez E, Vidal I, Wang W. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res.* 1997 Feb;64(2):211-8.
5 Landrum JT, Bone RA, Kilburn MD. The macular pigment: a possible role in protection from age-related macular degeneration*.Adv Pharmacol.* 1997;38:537-56.
6 Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol.* 1999 Jul;83(7):867-77
7 Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci.* 2001 Feb;42(2):439-46.
8 Hammond BR, Caruso-Avery M. (2000) Macular pigment optical density in a Southwestern population. *J. Nutr.* 41: 1492 1497
9 Aleman TS, Duncan JL, Bieber ML, Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome. *Invest Ophthalmol Vis Sci.* 2001 Jul;42(8):1873-81.
10 Hammond BR, Jr, Curran Celentano J, Judd S, Fuld K, Krinsky NI, Wooten BT, Snodderly DM. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res.* 1996 Jul;36(13):2001-12.
11 Werner JS, Donnelly S, Kliegl R. Aging and human macular pigment density. Appended with translations from the work of Max Schultze and Ewald Hering*. Vision Res.* 1987;27(2):257-68.
12 Berendschot TT, van Norren D. On the age dependency of the macular pigment optical density. *Exp Eye Res.* 2005 Nov;81(5):602-9. Epub 2005 Jul 15.
13 Hammond BR Jr, Cuilla TA, Snodderly DM. Macular pigment density is reduced in obese subjects. *Invest Ophthalmol Vis Sci*. 2002 Jan;43(1):47-50.
14 Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. *J Nutr.* 2002 Mar;132(3):518S-524S. Review
15 Delori FC, Goger DG, Hammond BR, Snodderly DM, Burns SA. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J Opt Soc Am A Opt Image Sci Vis.* 2001 Jun;18(6):1212-30.
16 Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL 3rd; Age-Related Eye Disease Study Research Group. Risk factors for the incidence of Advanced Age-Related Macular Degeneration in the Age-Related Eye

Disease Study (AREDS) AREDS report no. 19. *Ophthalmology.* 2005 Apr;112(4):533-9.
17 Bernstein PS, Yoshida MD, Katz NB, McClane RW, Gellermann W. Raman detection of macular carotenoid pigments in intact human retina. *Invest Ophthalmol Vis Sci.* 1998 Oct;39(11):2003-11.
18 Elsner AE, Burns SA, Beausencourt E, Weiter JJ. Foveal cone photopigment distribution: small alterations associated with macular pigment distribution. *Invest Ophthalmol Vis Sci.* 1998 Nov;39(12):2394-404
19 Berendschot TTJM, Goldbohm RA, Klopping WAA, van de Kraats J, van Norel J, van Norren D. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci*. 2000 Oct;41(11):3322-6.
20 Elsner A, Burns SA, Beausencourt E, Weiter JJ. Foveal cone photopigment distribution: small alterations associated with macular pigment distribution. *Invest Ophthalmol Vis Sci.* 1998 Nov;39(12):2394-404.
21 van Norren D, Tiemeijer LF. Spectral reflectance of the human eye. *Vision Res.* 1986;26(2):313-20.
22 Ruddock KH. Evidence for macular pigmentation from colour matching data. *Vision Res*. 1963 Dec;61:417-29.
23 Wooten BR, Hammond BRJ, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci.* 1999 Oct;40(11):2481-9.
24 Snodderly DM, Hammond BR. In vivo psychophysical assessment of nutritional and environmental influences on human ocular tissue: lens and macular pigment. In: *Nutritional and Environmental Influences on the Eye.* Taylor A ed. 1999;ch. 13. CRC Press
25 Berendschot TT, DeLint PJ, van Norren D. Fundus reflectance-historical and present ideas. *Prog Retin Eye Res*. 2003 Mar;22(2):171-200. Review
26 Moncrieff M, Cotton S, Claridge E, Hall P (2002) Spectrophotometric intracutaneous analysis - a new technique for imaging pigmented skin lesions. British Journal of Dermatology 146(3), 448-457
27 Wang L-H, Jacques SL, Zheng L-Q: MCML - Monte Carlo modeling of photon transport in multi-layered tissues. *Computer Methods and Programs in Biomedicine*. 1995 47:131-146,.
28 Preece SJ, Claridge E. Monte Carlo modelling of the spectral reflectance of the human eye. *Phys Med Biol*. 2002 Aug 21;47(16):2863-77
29 Anderson RR, Parrish JA. The optics of human skin. *J Invest Dermatol.* 1981 Jul;77(1):13-9.
30 Horecker BL. The absorption spectra of haemoglobin and its dedrivatives in the visible and near infra-red regions *J. Biol. Chem*.,1943;148**:**173-83
31 Wyszecki G, Stiles WS. *Color science: Concepts and methods, quantitative data and formulae*. 2nd ed(1982). New York
32 Rohen JW . Ed. J Francois and I Hollwich *Morphologie und embryologie des sehorgans Augenheilkunde in Klinik und Praxis Vol 1* Stuttgart: Thieme (1977).
33 Alm A, Bill A. Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys (Macaca irus): a study with radioactively labelled microspheres including flow determinations in brain and some other tissues. *Exp Eye Res.* 1973 Jan 1;15(1):15-29.

34 Styles IB, Claridge E, Orihuela Espina F, Calcalgni A, Gibson JM. Quantitative interpretation of multispectral fundus images. *Medical Imaging 2005: Physiology, Function, and Structure from Medical Images,* Proceedings of SPIE Vol. 5746. Amini AA, Armando Manduca A Eds. (SPIE, Bellingham, WA, 2005), 267-278

35 Styles IB, Calcagni A, Claridge E, Orihuela Espina F, Gibson JM. Quantitative analysis of multispectral fundus images. *Med Image Anal. (In press)*

36 Stewart CV, Tsai CL, Roysam B.. The dual-bootstrap iterative closest point algorithm with application to retinal image registration. *IEEE Trans. Med. Im.* 22 (2003), 1379–1394.