

## Quantitative analysis of multi-spectral fundus images

I.B. Styles<sup>a,\*</sup>, A. Calcagni<sup>a,b</sup>, E. Claridge<sup>a</sup>, F. Orihuela-Espina<sup>a</sup>, J.M. Gibson<sup>b</sup>

<sup>a</sup> School of Computer Science, The University of Birmingham, Edgbaston, Birmingham, West Midlands B15 2TT, United Kingdom

<sup>b</sup> Birmingham and Midland Eye Centre, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, United Kingdom

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### Abstract

We have developed a new technique for extracting histological parameters from multi-spectral images of the ocular fundus. The new method uses a Monte Carlo simulation of the reflectance of the fundus to model how the spectral reflectance of the tissue varies with differing tissue histology. The model is parameterised by the concentrations of the five main absorbers found in the fundus: retinal haemoglobins, choroidal haemoglobins, choroidal melanin, RPE melanin and macular pigment. These parameters are shown to give rise to distinct variations in the tissue colouration. We use the results of the Monte Carlo simulations to construct an inverse model which maps tissue colouration onto the model parameters. This allows the concentration and distribution of the five main absorbers to be determined from suitable multi-spectral images. We propose the use of “image quotients” to allow this information to be extracted from uncalibrated image data. The filters used to acquire the images are selected to ensure a one-to-one mapping between model parameters and image quotients. To recover five model parameters uniquely, images must be acquired in six distinct spectral bands. Theoretical investigations suggest that retinal haemoglobins and macular pigment can be recovered with RMS errors of less than 10%. We present parametric maps showing the variation of these parameters across the posterior pole of the fundus. The results are in agreement with known tissue histology for normal healthy subjects. We also present an early result which suggests that, with further development, the technique could be used to successfully detect retinal haemorrhages.

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### 1. Introduction

Pathologies in the ocular fundus are a major cause of blindness. These pathologies include diabetic retinopathy and age-related macular degeneration (ARMD). In both of these conditions, the distribution of blood and/or macular pigments is altered from the normal. These anomalies can impinge upon the foveal area, leading to a degeneration of central vision, with a severe impact on the quality of life. Such pathologies are frequently treatable, provided they are detected at an early stage. In the case of diabetes, modifications to the patient's diet and lifestyle can often control the severity of the condition. In wet/exudative

ARMD, laser treatment can be used to destroy the pathological blood vessels that characterise the condition.

At present, primary screening for diabetic retinopathy is largely carried out by general practitioners and ophthalmic opticians, whilst ARMD is usually only detected once the patient has incurred some degeneration of visual function as there is no effective screening tool that can highlight early pathology. In the case of diabetic retinopathy, the primary care practitioners are equipped to detect more obvious pathologies, but can sometimes miss subtle changes or early pathologies, where a referral to a specialist ophthalmologist would be advisable. The most common screening tool used by ophthalmic opticians is standard colour fundus photography, which provides an RGB image of the fundus. Modern digital fundus cameras can provide exceptional spatial resolution, allowing very fine detail to be seen. However, the spectral resolution of RGB imaging

\* Corresponding author. Tel.: +44 1214148559.

E-mail addresses: [I.B.Styles@cs.bham.ac.uk](mailto:I.B.Styles@cs.bham.ac.uk) (I.B. Styles), [E.Claridge@cs.bham.ac.uk](mailto:E.Claridge@cs.bham.ac.uk) (E. Claridge).

systems is very coarse, and yields only limited information about the nature of structures visible in an image. In order to determine the origin of a particular feature in an image, more detailed spectral information is required. This has traditionally been provided by spectroscopy, but this approach has its own limitations: spectroscopy yields information only about single points, and coverage of larger areas is impractical. Hence, spectroscopy has not been widely used as a diagnostic tool in the clinical setting and is largely limited to laboratory-based studies.

Recent advances in imaging technology have led to the development of the so-called multi-spectral imaging systems. In theory, such systems combine the spatial resolution of conventional photographic systems with the spectral resolution of spectroscopy, although in practice, the spectral resolution is significantly lower than spectroscopy can provide, but still a great improvement over RGB systems. The most common multi-spectral imaging systems use one of two different methods to generate a multi-spectral image. Interferometric systems use an interferometer to build up the Fourier transform of the spectrum at each pixel of the image (a so-called “interferogram”). Typically, the interferometer will scan its interference pattern across the CCD in a number of finite steps, gradually forming the full interference pattern. This can then be inverse-Fourier transformed to obtain the spectrum at each pixel. An alternative method uses a “filter wheel” containing a set of narrow bandpass filters corresponding to the set of wavelengths that are required. The wheel is rotated rapidly in front of the camera and an image is taken for each filter, and the resulting images can be used to deduce the spectrum at each point in the image. Recent advances in technology have led to the development of liquid crystal programmable filters which can be configured into bandpass filters across a range of wavelengths. All of these systems can provide high quality images with good spatial resolution, and fair spectral resolution. They are generally excellent at generating multi-spectral image data for stationary objects, but the increased image acquisition time is problematic when the objects can move. For moving objects, the interferometric methods are generally unsatisfactory as the images must be registered before they are inverse-Fourier transformed: one must register the interferograms. The filter-based approaches can cope with moving objects by using modern registration methods to correct for the movement. This is a relatively straightforward task and there are a number of well-established techniques for achieving this.

It is clear that using these technologies a multi-spectral imaging system for the ocular fundus could be developed. One must correct for involuntary eye movements and so interferometric systems are unsuitable, but filter-based systems are relatively simple to construct and a number of groups have successfully done so (Harvey et al., 2002; Besho et al., 2005). The quantity of information that multi-spectral imaging can provide could potentially be a very valuable aid in the early detection of pathologies, but it is

not yet clear how one should interpret the data that these systems yield: human perception is limited to approximately RGB, and so it is likely to be rather hard for a clinician to interpret multi-spectral images directly. We propose a method for processing multi-spectral fundus images which yields quantitative information about the histological properties of the tissue. This information may be diagnostically useful in a clinical setting.

The basis for our proposed method is a physical model of light propagation in the ocular fundus. The model is parameterised by relevant histological variables (such as the concentration of blood haemoglobins). Using the model, we deduce a set of multi-spectral measurements which allow the histological variables to be computed from the multi-spectral images. The values of these variables (corresponding to quantities of pigment) can then be plotted at each pixel to generate a “parametric map” which shows how the pigment is distributed across the image. This could allow primary care clinicians to identify pathologies at a much earlier stage of development, allowing the patient to receive appropriate treatment in good time.

These ideas have previously been employed in dermatology, where the SIAscope system (Cotton et al., 1997) has been shown to be an effective screening tool in the diagnosis of pigmented skin lesions (Moncrieff et al., 2002). The SIAscope uses RGB and near-infrared images to map the distributions of dermal blood, dermal melanin and collagen in skin lesions, allowing suspicious lesions to be investigated non-invasively. This reduces the number of unnecessary biopsies from healthy subjects. Standard RGB images plus one near-infrared image are sufficient for this information to be deduced from skin images, but the tissue of the ocular fundus is much more complex and a more careful choice of filters, based on some objective criteria, is required. In addition, the tissue of the ocular fundus is not directly accessible and it is not possible to calibrate the images using calibration patches. In this article, we propose methods by which these difficulties may be overcome, and show how these ideas allow parametric maps of the ocular fundus to be computed.

## 2. Modelling the ocular fundus

The methods of image analysis proposed in this work depend upon the construction of an accurate model of the reflectance of the ocular fundus. There have been several attempts to construct such a model, and Berendschot et al., 2003 provides an extensive summary of the most important works. The first model which took into account the whole of the ocular fundus was by van Norren and Tiemeijer (1986), who developed a model using a series of neutral reflectors and absorbing compounds. This was modified by Delori and Pflibsen (1989), who used Kubelka–Munk transport theory (Kubelka and Munk, 1931) to include the effects of scattering in the choroid. Hammer and Schweitzer (2002) used the adding–doubling method of radiation transport to include scattering in the retina and

choroid, drawing on earlier work on the optical properties of these tissues by Hammer et al. (1995). Most recently, Preece and Claridge (2002) used Monte Carlo simulation (Prah et al., 1989) in combination with the data from Hammer et al. (1995) and the well-known properties of the main pigments in the fundus: haemoglobins (Horecker, 1942) and melanin (Anderson and Parrish, 1981). The Monte Carlo method is more accurate than cruder approximations such as adding–doubling, but suffers from long computation times. Fortunately, modern computers allow Monte Carlo to be a reasonably practical method of performing transport calculations, although computation times are still significant.

Preece and Claridge's model was parameterised by the concentrations of the main absorbers in the fundus:

- Concentration of melanin in the RPE;
- Concentration of melanin in the choroid;
- Concentration of haemoglobins in the choroid (assuming 95% oxygen saturation).

All other properties (layer thickness) of the tissue were assumed to be constant, taking the values from Rohen

(1977). The specific absorption coefficients of these pigments are shown in Fig. 1.

This model was found to accurately reproduce reflectance spectra obtained from the perifoveal region of the fundus, away from any major blood vessels. In other parts of the fundus, other variable components are known to be present. Surrounding the fovea is the macula region containing macular pigment (Wysecki and Stiles, 1982) (see Fig. 1(d)). Changes in the distribution of this pigment can occur in the early stages of several pathologies and it would therefore be very useful to be able to quantify its variation. There is also a fine network of blood vessels within the retina itself. These are important since diabetic haemorrhages occur in these vessels and quantification of haemoglobins in the retina may allow us to identify such features. We have therefore added two parameters to Preece and Claridge's model: concentration of macular pigments and concentration of retinal haemoglobins (where we assume 80% oxygen saturation (Alm and Bill, 1973)). A schematic cross-section through the ocular fundus is shown in Fig. 2. The five variable parameters characterising our model represent concentrations of pigment in the different layers:

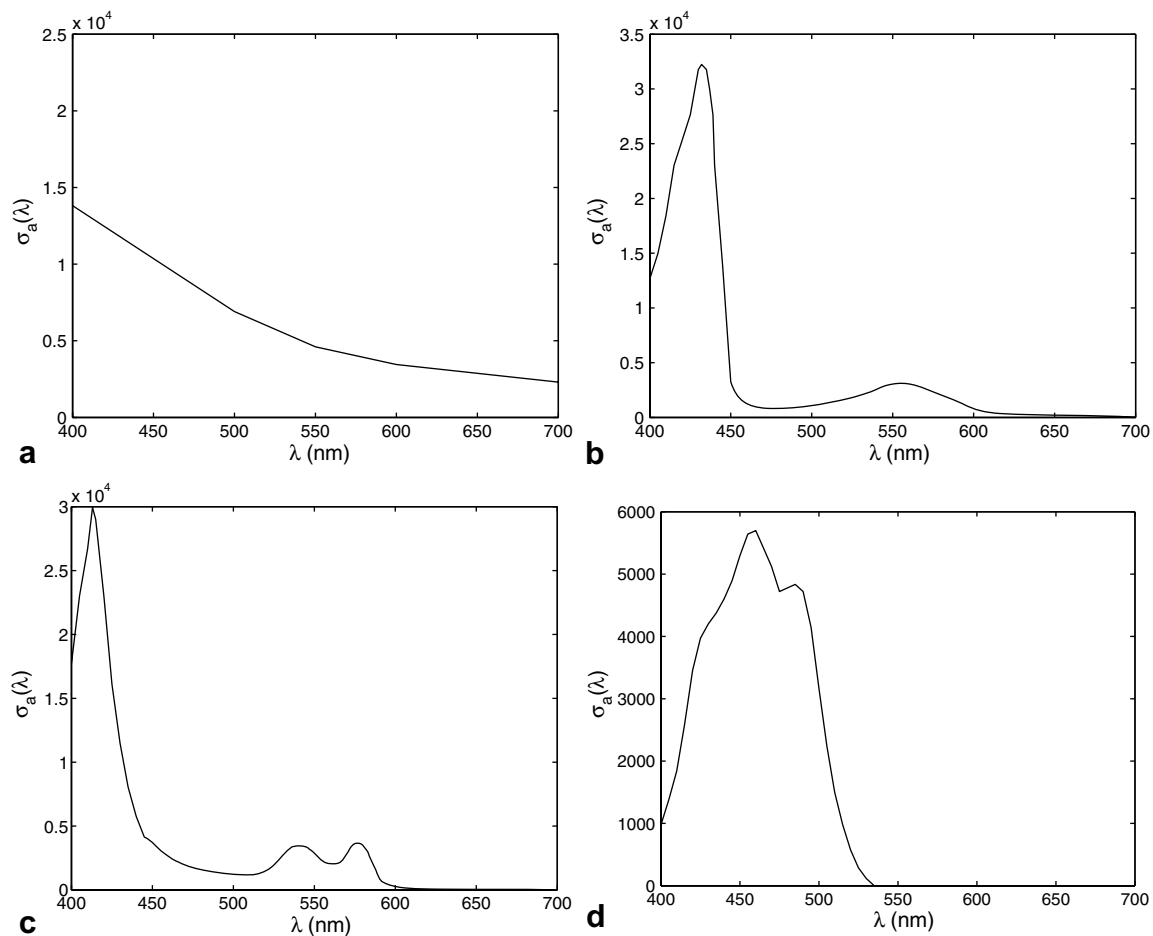


Fig. 1. Specific absorption coefficients (in units of  $m^{-1}/(mmol L^{-1})$ ) of: (a) melanin (Anderson and Parrish, 1981); (b) haemoglobin (Horecker, 1942);

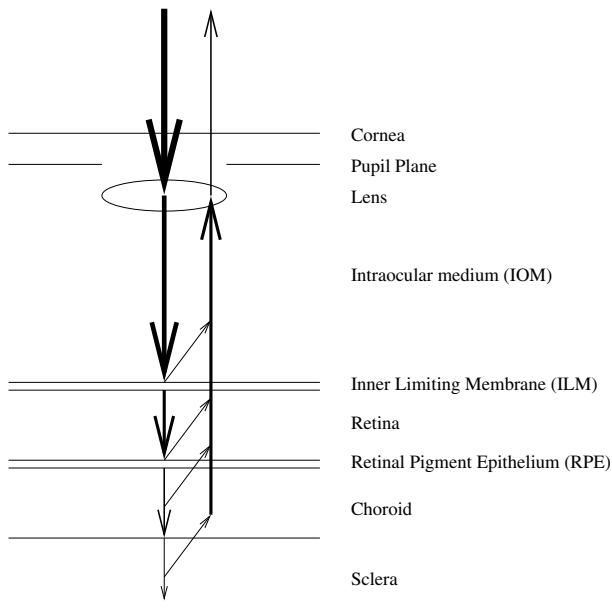


Fig. 2. Schematic cross-section of the ocular fundus showing the layered structure of the tissue and possible pathways of remitted light.

- $C_{MP}$ : concentration of macular pigments in the retina.
- $C_{RH}$ : concentration of haemoglobins in the retina.
- $C_{RM}$ : concentration of melanin in the RPE.
- $C_{CM}$ : concentration of melanin in the choroid.
- $C_{CH}$ : concentration of haemoglobins in the choroid.

The Monte Carlo model computes the reflectance  $R(\mathbf{p}, \lambda)$  at wavelength  $\lambda$  of tissue described by a parameter vector  $\mathbf{p} = (C_{MP}, C_{RH}, C_{RM}, C_{CM}, C_{CH})$ . It is useful to examine how  $R(\mathbf{p}, \lambda)$  varies as we change each of the parameters (holding the others constant). Fig. 3 shows how the reflectance of fundus tissues varies as its composition is changed (note that a log scale is used to emphasise small changes). In each of these graphs, four of the five parameters are constant, and the fifth parameter is varied. Each of the identified histological components has a distinct effect on  $R(\mathbf{p}, \lambda)$ . In Fig. 3(a), we see that as the concentration of retinal macular pigment is increased, the reflectance is attenuated in the blue and green spectral regions, with no effect in the red since macular pigment does not absorb at  $\lambda > 534$  nm. As the concentration of retinal haemoglobins is increased (Fig. 3(b)) we see that the spectral reflectance attenuates the spectrum in blue and green regions, and introduces strong features due to peaks and troughs in the specific absorption coefficient of the haemoglobins. Increasing the concentration of RPE melanin affects the whole reflectance spectrum (Fig. 3(c)). A careful analysis shows that an increase in  $C_{RM}$  causes more attenuation in the blue spectral region than in the red, and the change is not a straightforward wavelength-independent scaling as might be thought from a visual inspection of Fig. 3(c). Changes in choroidal melanin (Fig. 3(d)) have a very strong effect in the red region

of the spectrum, but a much weaker effect in the blue region. Note well the contrast between increase in RPE melanin and choroidal melanin. Whilst increases in RPE melanin attenuate the spectrum by roughly the same scaling factor 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, there are other absorbers present (haemoglobins), and scattering from the underlying tissue is very strong. It is the interplay between the varying component and its surroundings which 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. Finally, increases in choroidal haemoglobins (Fig. 3(e)) lead to a reduction in reflectance in the green region, where absorption by haemoglobins is very strong. Note again the difference between choroidal and retinal haemoglobins, again due to the interaction with the surrounding tissues. It is important to be aware that the proposed model is valid only in the visible region of the spectrum. In the infrared region, absorption by pigments is very weak, but absorption by water increases rapidly above 700 nm and is dominant in the infrared, masking variations in the pigmentation. In the ultraviolet regime, absorption by melanin and haemoglobins is very strong, and light cannot penetrate the deeper layers.

### 3. Analysis of the imaging process

#### 3.1. Factors affecting the imaging process

Any attempt to optimise the imaging process must be based on a thorough understanding of that process. The imaging modality that concerns us in this work is visible optical photography. The ease with which such images can be obtained allows fundus photography to be a potentially powerful screening tool for a variety of pathologies, provided the imaging process has been optimised to allow the desired information to be extracted.

The process of obtaining an optical image is very simple. The object under study is illuminated using a light source with known spectral properties. A camera is focused on the object and one or more filters are used to select a band of wavelengths to capture (wavelengths outside this band are blocked by the filter). The resulting filtered image is captured on a CCD photon detector which records the total intensity of light falling on each pixel of the detector, resulting in a monochrome image. Colour images are constructed using red (R), green (G) and blue (B) filters which approximate the response of the photo-receptors in the human eye, but this is only for the purpose of realistic display, and any set of filters can be used if this is not important.

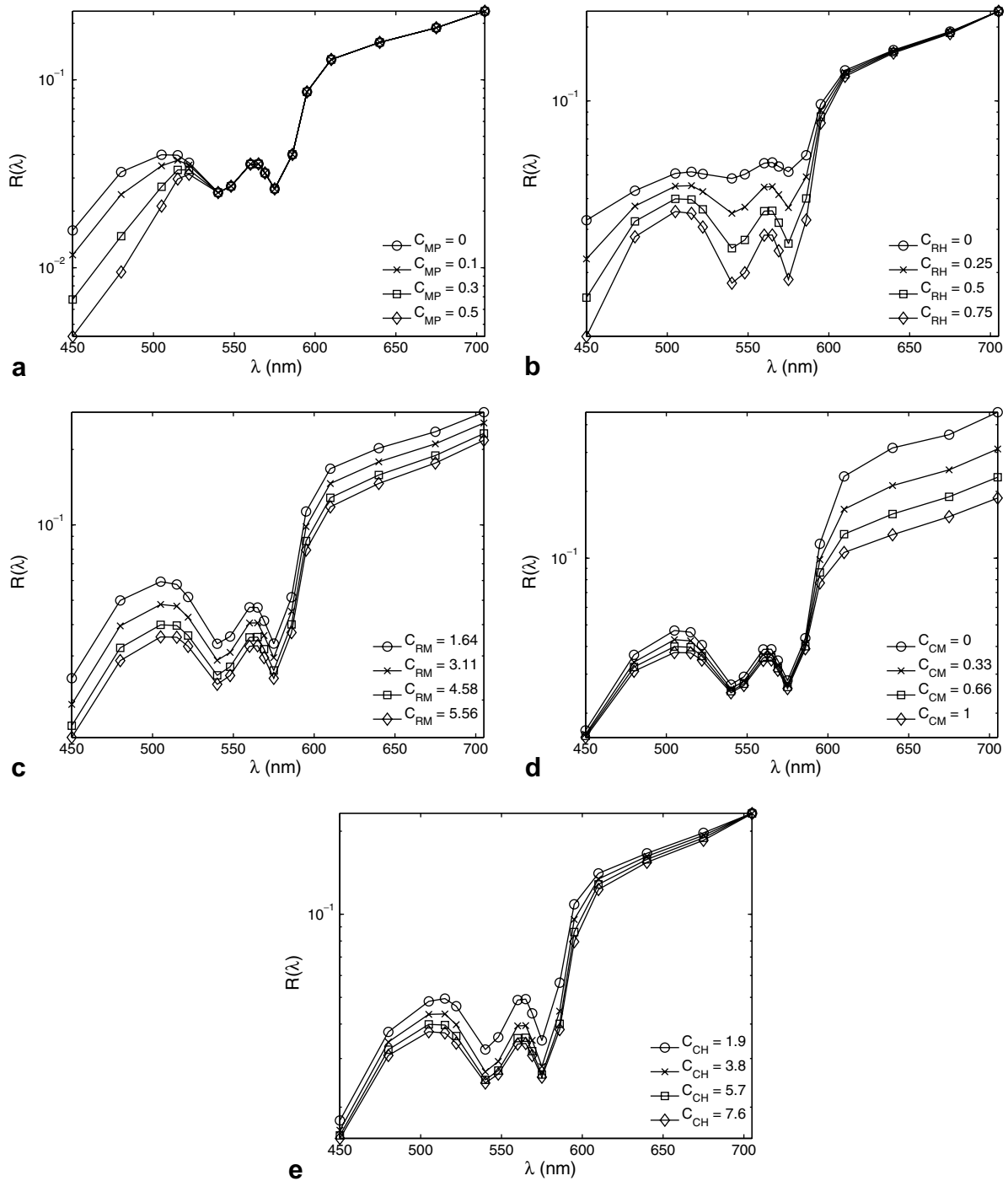


Fig. 3. Variation of  $R(\mathbf{p}, \lambda)$  with respect to changes in: (a) macular pigment ( $C_{MP}$ ) (b) retinal haemoglobins ( $C_{RH}$ ); (c) RPE melanin ( $C_{RM}$ ); (d) choroidal melanin ( $C_{CM}$ ) and (e) choroidal haemoglobins ( $C_{CH}$ ). Non-varying parameters were fixed at:  $C_{MP} = 0.0 \text{ mmol L}^{-1}$ ,  $C_{RH} = 0.75 \text{ mmol L}^{-1}$ ,  $C_{RM} = 4.58 \text{ mmol L}^{-1}$ ,  $C_{CM} = 0.66 \text{ mmol L}^{-1}$ ,  $C_{CH} = 5.70 \text{ mmol L}^{-1}$ .

There are a number of factors which affect the strength of the signal recorded by the detector at position  $\mathbf{x}$ :

- The spectrum and intensity of the illuminant,  $I(\mathbf{x}, \lambda)$ ;
- The reflectance of the tissue,  $R(\mathbf{x}, \lambda)$ ;
- The profile of the filter used,  $F_n(\lambda)$ ;
- The quantum efficiency of the CCD,  $Q(\lambda)$ .

The signal (image value) recorded on the detector through filter  $n$  is then

$$i_n(\mathbf{x}) = \alpha \int_{\lambda} I(\mathbf{x}, \lambda) R(\mathbf{x}, \lambda) Q(\lambda) F_n(\lambda) d\lambda, \quad (1)$$

where the constant  $\alpha$  scales the signal onto the range of the detector and is set by factory calibration. It is therefore



possible to compute the signal recorded on the CCD provided that all of the terms are known. One could, in principle, take the image value recorded by the detector and deduce the model parameters which describe the reflectance of the tissue. In practice, these terms are not all known, and we must consider the problem more carefully.

### 3.2. Formalising the imaging process

Preece and Claridge (2004) described a mathematical model of the image formation process. They assumed that the optical properties of the object under consideration could be described by a vector of  $K$  parameters

$$\mathbf{p} = \{p_k\}_{k=1,\dots,K}, \quad \mathbf{p} \in \mathcal{P}, \quad (2)$$

where  $\mathcal{P}$  is the space of all possible parameter vectors for a given object. The first stage in the imaging process is the formation of the reflectance spectrum of the tissue. The reflectance spectrum of the tissue is a continuous function of optical wavelength:

$$r = r(\lambda), \quad r \in \mathcal{R}, \quad (3)$$

where  $\mathcal{R}$  is the space of all possible reflectance spectra. We assume that there exists a mapping

$$a : \mathcal{P} \mapsto \mathcal{R} \quad (4)$$

which defines the relationship between object parameters and spectral reflectance. In Section 2, we discussed how such a model could be constructed for the tissues of the ocular fundus using Monte Carlo simulations. This model is an implementation of  $a$ .

Given the reflectance spectrum of an object, an imaging device typically acquires images by applying band-pass filters to the remitted spectrum and integrating over wavelength to generate a single image value per filter. For  $N$  optical filters  $\{F_n(\lambda)\}_{n=1,\dots,N}$ , an image vector

$$\mathbf{i} = \{i_n\}_{n=1,\dots,N}, \quad \mathbf{i} \in \mathcal{I} \quad (5)$$

is recorded, where  $\mathcal{I}$  is the space of all possible image vectors from a given object. The process of image acquisition can be represented by a mapping

$$b : \mathcal{R} \mapsto \mathcal{I}, \quad (6)$$

and in the cases we will be studying, the value of the  $n$ th component of the image vector is defined by Eq. (1).

We are now able to compute a composite mapping,  $m = a \circ b$ , such that  $m(\mathbf{p}) = b(a(\mathbf{p}))$  from model parameters to image values: knowing the composition of the tissue at a given point allows us to deduce the appearance of the tissue in an image. In principle, therefore, we ought to be able to perform the inverse calculation, and deduce the composition of the tissue from its appearance in an image, subject to some mathematical constraints which we discuss in Section 4.1. However, the ability to construct the inverse mapping depends on a complete knowledge of the forward mapping. We cannot always guarantee this: some of the terms in Eq. (1) are unknown in certain situations.

When acquiring images of the ocular fundus, illumination and acquisition is done through an aperture (the pupil). In addition, the ocular fundus is strongly curved (a sphere of diameter  $d \approx 24$  mm). As a result, illumination of the fundus is uneven, with the centre illuminated more brightly, and the edges being significantly darker. It is not possible to determine the level of illumination in the fundus as the tissue is not accessible, and we cannot know  $I(\mathbf{x}, \lambda)$  for every point  $\mathbf{x}$  in the image. Fortunately, there are some statements that we can make about  $I(\mathbf{x}, \lambda)$  which lead to a resolution of this problem.

The key observation is that the only aspect of the illuminant which changes with position is its intensity: the relative contributions from different wavelengths do not change with position. We can therefore separate  $I(\mathbf{x}, \lambda)$  into spectral and spatial components:  $I(\mathbf{x}, \lambda) = A(\mathbf{x})S(\lambda)$ , with no loss of generality. Substituting this into Eq. 1 provides

$$i_n(\mathbf{x}) = A(\mathbf{x}) \int_{\lambda} S(\lambda) R(\mathbf{x}, \lambda) Q(\lambda) F_n(\lambda) d\lambda, \quad (7)$$

where we have absorbed the calibration constant  $\alpha$  into  $A(\mathbf{x})$ . This substitution allows us to remove the effects of spatially varying illumination by taking ratios of image values:

$$q_{mn}(\mathbf{x}) = \frac{i_m(\mathbf{x})}{i_n(\mathbf{x})}, \quad (8)$$

which are independent of  $A(\mathbf{x})$ . The use of “image quotients” has previously been employed in image segmentation problems (Maloney and Wandell, 1986, 1989) and is often referred to as “discounting the illuminant”, but this is the first time they have been used to provide quantitative information about the tissue itself.

We now formally introduce this new step into our mapping. Given an  $N$ -component image vector, we can form an  $(N-1)$ -component vector of independent image quotients,

$$\mathbf{q} = \{q_m\}_{m=1,\dots,N-1} = \{i_m/i_N\}_{m=1,\dots,N-1}, \quad \mathbf{q} \in \mathcal{Q}. \quad (9)$$

This can be represented as a mapping

$$c : \mathcal{I} \mapsto \mathcal{Q} \quad (10)$$

The process of forming image quotients from an object can then be represented by a composite function

$$f = a \circ b \circ c : \mathcal{P} \mapsto \mathcal{Q} \quad (11)$$

relating the composition of the object described by a parameter vector  $\mathbf{p}$  to the image quotients  $\mathbf{q}$  formed from images captured by a set of optical filters  $\{F_n(\lambda)\}_{n=1,\dots,N}$ .

Having now defined a mapping from object composition to image quotients we can, in principle, construct an inverse mapping

$$f^{-1} : \mathcal{Q} \mapsto \mathcal{P}, \quad (12)$$

(subject to some mathematical constraints which we discuss in Section 4.1) which allows us, given a vector of image quotients  $\mathbf{q}$ , to deduce the vector of parameters  $\mathbf{p}$  that describes the object at the point of interest.

The use of image quotients in the image analysis process holds several advantages over the use of image values. As we have already observed, it effectively removes the effect of uneven illumination on a object. Furthermore, it removes any dependence on the overall intensity of the illuminant: any scaling of the intensity can simply be absorbed into  $A(\mathbf{x})$ . When working with image values, one measures the intensity of light reaching the object of interest by placing an object of known reflectance (a “reflectance standard”) on or very near the object, within the field of view. This is not required when working with image quotients, and allows these methods to be used when the geometry of the object makes the use of reflectance standards impractical, for example, when the object is irregular or very curved in shape, or the object must be imaged remotely as it cannot be directly accessed. Both of these are true of the ocular fundus, and so the use of image quotients is essential if we are to perform a quantitative analysis of fundus images.

The discussion so far has focused on what can be done in principle, and there are still a number of practical issues to consider. For example, we have not considered what kinds of filter must be used, or whether the spectrum of the illuminant and quantum efficiency of the camera must meet certain criteria. For a given  $S(\lambda)$ ,  $Q(\lambda)$ , the only unanswered question is which filters should be used. In the following section, we discuss objective criteria for filter selection which aim to ensure that the choice of filters allows  $f^{-1}$  to be computed, and to minimise the error with which model parameters can be computed from a set of images.

#### 4. Selection of optimal filters

There are two factors which govern our choice of filters. The filters must ensure that the inverse mapping  $f^{-1}$  exists and is unique, and they must aim to minimise the error in the parameter recovery process. Let us deal with these separately.

##### 4.1. Existence of $f^{-1}$

The method we have proposed depends upon the existence of  $f^{-1}$ . We can define some conditions which, if met by  $f$ , guarantee that  $f^{-1}$  exists, although they do not tell us what  $f^{-1}$  is. We begin by noting that  $\mathcal{P}$  and  $\mathcal{Q}$  are vector spaces, with points in  $\mathcal{P}$  represented by some vector  $\mathbf{p}$ , and points in  $\mathcal{Q}$  represented by some vector  $\mathbf{q}$ . We represent the mappings  $f: \mathcal{P} \rightarrow \mathcal{Q}$  and  $f^{-1}: \mathcal{Q} \rightarrow \mathcal{P}$  as  $\mathbf{q} = f(\mathbf{p})$  and  $\mathbf{p} = f^{-1}(\mathbf{q})$ . We will write  $\mathbf{q} = \mathbf{q}(\mathbf{p})$  and  $\mathbf{p} = \mathbf{p}(\mathbf{q})$ .

The key quantity which allows us to determine the existence and uniqueness of  $f^{-1}$  is the Jacobi matrix, which for this problem is (in component form)

$$\mathbf{J}_{ij} = \frac{\partial q_i}{\partial p_j}. \quad (13)$$

From the Jacobi matrix, we compute the Jacobian  $J = \det(\mathbf{J})$ . The inverse function theorem (Schutz, 1980) states that  $f$  is bijective (one-to-one and onto) if and only if

$J \neq 0 \forall \mathbf{p} \in \mathcal{P}$ . In practical terms, this means that  $\text{sgn}(J)$  must be strictly positive or strictly negative (thus never zero) throughout  $\mathcal{P}$ .

Note that in order to compute  $\mathbf{J}(\mathbf{p})$  numerically,  $\mathcal{P}$  must be discretised on a rectangular grid to allow us to compute the partial derivatives using finite difference methods. In addition, we can use the Jacobi matrix to approximate the mapping near the grid of points: since  $d\mathbf{q} = \mathbf{J}d\mathbf{p}$ , we can write  $\mathbf{q} = \mathbf{q}_0 + \mathbf{J}|_{\mathbf{p}_0} d\mathbf{p}$  in the vicinity of  $\mathbf{p}_0$ , where  $\mathbf{q}_0 = \mathbf{q}(\mathbf{p}_0)$ . We can also compute a first approximation to  $f^{-1}$  using a similar method, since  $d\mathbf{p} = \mathbf{J}^{-1}d\mathbf{q}$  and thus  $\mathbf{p} = \mathbf{p}_0 + \mathbf{J}^{-1}|_{\mathbf{q}_0} d\mathbf{q}$ .

A very important consequence of this result is that the  $\mathbf{J}$  must be a square matrix: to compute  $K$  model parameters, we must have  $K$  independent image quotients. This requires  $N = K + 1$  image values. We choose one of the image values to act as a common denominator, and then

$$q_j = \frac{i_j}{i_N}, \quad j = 1, \dots, K. \quad (14)$$

##### 4.2. Minimisation of error

It is important that any parameter values recovered from a set of images should be known to the greatest accuracy possible in order to be of use in a clinical setting. There are a number of sources of possible error in the procedure that we have described.

###### 4.2.1. Error in the construction of $f$

The forward model of the imaging process described in Section 3.2 contains a number of sources of error which mean that the forward model  $f$  can only compute image quotients to finite accuracy. Let us consider each stage of the composite mapping.

The first step is the mapping  $a$  from model parameters to reflectance spectra. Assuming that the model is an accurate representation of the tissue in its construction (a point which we discuss in Section 7), we identify two main sources of error in this step. The first is error due to the stochastic nature of the Monte Carlo model of light transport. The model simulates the passage of a large number of photons  $N_p$  through the tissue, and then computes the average probability of the photon being reflected. In the limit  $N_p \rightarrow \infty$  this calculation converges to the exact solution, but is otherwise an approximation. We cannot, of course, compute at the limit, but choosing a suitably large  $N_p$  can minimise any variation between simulations. We have found that  $N_p = 100,000$  provides very stable solutions.

The second source of error in this step is that resulting from errors in the experimental data which characterise the tissue properties. The absorption and scattering cross-sections for the pigments, and for the underlying tissue, are derived from experimental measurements (Hammer et al., 1995; Horecker, 1942; Anderson and Parrish, 1981; Wysocki and Stiles, 1982). In our computations, the mean values stated in the literature are used, but there is an

experimental error associated with each of the measurements. Using relatively simple error analysis (Kendall and Stuart, 1969), we can propagate the error in the physical data through our computations to calculate the error in the reflectance spectra. We can then propagate this through mapping  $b$  to estimate the errors in the image values computed by the model. Further information on this method, applied to skin imaging, can be found in Preece and Claridge (2004). We will denote the error (standard deviation) due to errors in the forward model of the  $j$ th component of the image vector  $\mathbf{i} = (i_1, i_2, \dots, i_N)$  as  $\sigma_{i_j}^F$ .

We now propagate the  $\sigma_{i_j}^F$  through to the image quotients. From Eq. 14, we can compute the error in the image quotients using the standard relation  $(\sigma_{q_k})^2 = \sum_{j=1}^N (\partial q_k / \partial i_j)^2 (\sigma_{i_j})^2$  (Kendall and Stuart, 1969). It can be shown that the resulting error in the  $k$ th component of the image quotient vector  $\mathbf{q} = (q_1, q_2, \dots, q_k)$  is

$$\sigma_{q_k}^F = \frac{1}{i_N} \sqrt{(\sigma_{i_k}^F)^2 + q_k^2 (\sigma_{i_N}^F)^2}, \quad (15)$$

where the superscript “F” denotes errors in the computation of the forward model. We can use this result to compute the error in the inverse model: any errors in the construction of  $f$  will lead to errors in the subsequent construction of  $f^{-1}$ . We wish to find the resulting error with which parameters can be computed using  $f^{-1}$ . It is straightforward to show that the errors in parameter recovery due to errors in the forward model are

$$\sigma_{p_i}^F = \sqrt{\sum_{k=1}^N \left( \frac{\partial p_i}{\partial q_k} \right)^2 (\sigma_{q_k}^F)^2}. \quad (16)$$

The  $\partial p_i / \partial q_k$  can be read directly from the inverse of the Jacobian of  $f$ . The  $\sigma_{p_i}^F$  are the errors in  $f^{-1}$  resulting from errors in the construction of the imaging model  $f$ , and do not take into account any other errors in the imaging and analysis process.

#### 4.2.2. Error in the imaging process

The second source of error we have identified is error in the image acquisition process. In this paper, we are concerned with acquiring images using a CCD and the discussion will reflect this. However, the idea is quite general: the detector does not record the intensity of light it receives with complete accuracy.

When recording an image on a CCD, each pixel records an image value. On a typical 8-bit camera, the image values are integers in the range 0 (black) to 255 (white). The 12-bit camera used to acquire the images in this paper records integers from 0 to 4095. These ranges are set by the number of bits used to record each image value (8 or 12 in these cases), and their correspondence to different levels of brightness is set by a factory calibration process which leads to the constant  $\alpha$  in Eq. (1).

The value recorded by the camera is not exact. It is essentially the sum of the numbers of photons received at a given pixel during an exposure. Even in apparently

steady-state conditions, this number can vary significantly. In addition, the CCD is a semiconductor device and as such is sensitive to thermal effects. The signal recorded by the CCD can be regarded as the mean intensity, and there is an associated error, which, for an 12-bit camera, is typically  $\pm 3$  units. The exact value depends on the details of the camera. This error will affect the accuracy with which images can be used to recover model parameters, and we can quantify this. Denoting the “camera error” of the  $j$ th image value as  $\sigma_{i_j}^C$ , we can show that

$$\sigma_{p_j}^C = \frac{1}{i_N} \sqrt{\sum_{k=1}^K \left( \frac{\partial p_j}{\partial q_k} \right)^2 [(\sigma_{i_k}^C)^2 + (q_j \sigma_{i_N}^C)^2]} \quad (17)$$

This error is the error in parameter recovery due solely to errors in the image acquisition process.

#### 4.2.3. Error in the construction of $f^{-1}$

The third source of error we have identified is due to the difficulties in constructing  $f^{-1}$ . The forward model is based on a numerical solution of the radiative transport equation, and so we cannot deduce an analytic solution. Instead, we must deduce an approximate solution, given the forward model.

The numerical nature of the forward model means that we can only compute  $f$  at a finite number of points in  $\mathcal{P}$ :  $f$  is effectively a mapping from  $N$  discrete points in  $\mathcal{P}$  to  $N$  discrete points in  $\mathcal{Q}$ . Assuming that the conditions in Section 4.1 are met, this mapping is bijective, and the inverse mapping from  $N$  points in  $\mathcal{Q}$  to  $N$  points in  $\mathcal{P}$  exists and is unique. Clearly, we know  $f^{-1}$  at this set of discrete points, but not at points which lie in between. We need an approximation of  $f^{-1}$ .

A natural approximation to employ is a truncated Taylor series. The constraints imposed in Section 4.1 ensure that  $f$  and  $f^{-1}$  are analytic functions. We can approximate  $f$  in the vicinity of one of the known points,  $\mathbf{p}_0$ , as a Taylor series,

$$\begin{aligned} q_i(\mathbf{p}_0 + \mathbf{dp}) &= q_i(\mathbf{p}_0) + \sum_j \frac{\partial q_i}{\partial p_j} \bigg|_{\mathbf{p}_0} dp_j \\ &+ \frac{1}{2} \sum_{j,k} \frac{\partial^2 q_i}{\partial p_j \partial p_k} \bigg|_{\mathbf{p}_0} dp_j dp_k + \dots, \end{aligned} \quad (18)$$

and this can be computed to arbitrary precision, since all the derivatives are known. We can similarly write down a Taylor expansion of  $f^{-1}$ :

$$\begin{aligned} p_i(\mathbf{q}_0 + \mathbf{dq}) &= p_i(\mathbf{q}_0) + \sum_j \frac{\partial p_i}{\partial q_j} \bigg|_{\mathbf{q}_0} dq_j \\ &+ \frac{1}{2} \sum_{j,k} \frac{\partial^2 p_i}{\partial q_j \partial q_k} \bigg|_{\mathbf{q}_0} dq_j dq_k + \dots \end{aligned} \quad (19)$$

We cannot compute this to arbitrary precision since we cannot numerically compute the derivatives: the points in  $\mathcal{Q}$  do



not generally lie on an orthogonal grid. We can, however, compute the expansion to first order by using the inverse of the Jacobi matrix of  $f$ , as noted in Section 4.1. This provides a linear approximation for  $f^{-1}$ , which requires a high density of data points in the model in order to provide sufficient accuracy. Discretising the model with five points in each of the five dimensions (3125 data points) provides acceptable accuracy (typical error of approx 5% for each parameter), but does not provide acceptable performance. We found that to compute the inverse model for 10,000 image quotient vectors required approximately 40 min on a 2 GHz Pentium 4 machine with 1 GB of memory. This time scales linearly with both number of image quotient vectors (the inverse model has to be explicitly applied to each point in turn), and with the number of data points in the forward model (the computation reduces to the search problem of finding the nearest neighbour). A one megapixel image would therefore require a processing time of approx. 70 h. A reduction in the number of data points in the model to four in each dimension (1024 data points) would reduce the compute time to approx. 20 h, but with typical errors of >10%. It was suggested that a neural network would be able to provide a suitable approximation for  $f^{-1}$ , and after some experimentation we found that using five points in each of the five-dimensions was sufficient to train a radial basis network to recover parameters with typical errors of approx. 5% for each parameter. This is the same accuracy as obtained using the linear approximation described previously, but parameter recovery is approximately two orders of magnitude quicker, allowing a complete one megapixel image to be processed in less than 1 h. The processing time is largely independent of the number of data points used to construct the model. However, the memory requirements in the training of the network are large, and we have so far been unable to train the network on larger data sets. Details of the implementation of the inverse function are provided in Section 5.2. The important point is that  $f^{-1}$  cannot be constructed exactly: we have to approximate. The approximation has an associated error which can be quantified and taken into account in the optimisation process. The choice of filters will affect the nature of  $f$  and  $f^{-1}$ , and hence will also affect the accuracy with which these functions can be approximated. We can define a measure of the approximation error:  $\sigma_{p_n}^A$  is the error in the recovery of parameter  $n$  due to approximations in the construction of  $f^{-1}$ .

#### 4.3. Objective procedure for filter selection

We are now in a position to define an objective procedure for the selection of optimal filters:

- (1) Discretise the  $K = N - 1$ -dimensional parameter space using a rectangular grid to give  $M$  distinct parameter vectors  $\{\mathbf{p}_i\}_{i=1,\dots,M}$ .
- (2) For each parameter vector  $\mathbf{p}_i$ , compute the spectral reflectance of the tissue (corresponding to mapping  $a$  as defined in Eq. (4)), giving  $\{R_i(\lambda)\}_{i=1,\dots,M}$ .

- (3) Define a set of  $N$  independent (orthogonal) filters  $\{F_n(\lambda)\}_{n=1,\dots,N}$ , subject to any necessary constraints.
- (4) For each  $R_i(\lambda)$ , apply the  $N$  filters to provide image values.
- (5) Select one of the  $N$  filters to be the denominator in the image quotients, and compute  $K = N - 1$  image quotients for each of the  $M$  parameter vectors. This defines  $f$ .
- (6) For each  $\mathbf{p}_i$ , compute the Jacobian  $J_i$ .
- (7) If  $\text{sgn}(J_i)$  is constant and non-zero  $\forall i$ , the mapping is unique and the procedure can continue; else the mapping is not unique and the filters  $\{F_n(\lambda)\}_{n=1,\dots,N}$  are unsuitable.
- (8) If the mapping is unique, compute the total error in parameter recovery:  $\sigma^T = \sqrt{(\Sigma^C)^2 + (\Sigma^F)^2 + (\Sigma^A)^2}$ , where  $\Sigma^X = \sqrt{\sum_n (\sigma_{p_n}^X)^2}$  and  $X = \{C, F, A\}$ .
- (9) Employ an optimisation procedure to find the set of filters which minimises  $\sigma^T$ .

This procedure provides a set of filters which ensures that  $f$  and  $f^{-1}$  are well-behaved (bijective) mappings, and which minimises the error with which parameters can be recovered using the inverse model. In Section 5 we explain how these ideas are implemented in practice.

## 5. Implementation

Let us now discuss how these ideas are used in practice for imaging of the ocular fundus. We will begin by describing the equipment that is to be used for acquiring suitable images, since this imposes some constraints on our choice of filters. We then describe the implementation and optimisation of the forward and inverse models.

### 5.1. Image acquisition

Acquisition of ocular fundus images requires specialised equipment, since images must be taken through a small aperture and the ocular lens. We used a Zeiss RCM250 fundus microscope which contains the optics that are required to obtain fundus images, inbuilt sources of illumination, and the mechanics required to optimally position the camera. In standard form, the camera records images on 35 mm film using a SLR camera body with modified bayonet lens fitting which is peculiar to Zeiss fundus cameras. Viewing illumination is via a halogen lamp which illuminates the fundus through the camera optics. Images are recorded using a xenon flash.

A number of modifications were made to the microscope to make it suitable for this work. The SLR body was replaced by a QImaging Retiga EXi 12-bit monochrome digital camera which allowed high quality digital images to be acquired. A Cambridge Research Instruments Vari-Spec LCD programmable filter was added to the system, in front of the Retiga camera. This device is a configurable interference filter which can be programmed to implement

a range of narrow Gaussian bandpass filters with central wavelengths in the range 400–700 nm. The width and transmittance of these filters is dependent on the central wavelength, and this provides a constraint on our choice of filters. An additional lens with focal length 75 mm was included to shrink the image onto the 1/3 in. CCD which is much smaller than the 35 mm film used in the original camera. An array of optical connectors was required to integrate these components with the microscope. The VariSpec filter and the Retiga camera were controlled using a standard notebook computer running custom software. In brief, the software pre-loads the required filters into the VariSpec filter. Images are then acquired (using appropriate exposure settings) with each filter sequentially. Illumination was provided by the viewing lamp at a suitable intensity. The xenon flash used during conventional photography was found to be unsuitable because it provides a transient illumination rather than the steady-state illumination assumed in our model, and because the spectrum of a xenon discharge lamp contains a number of sharp emission peaks which bias the filter selection process. An added benefit of using steady-state visible illumination for both focussing and image acquisition is that it ensures that the photopigments are bleached during the acquisition process.

The lengthy exposure time (approx. 5 s) required to obtain all of the necessary images means that image registration is required due to movement of the subject. Excellent results were obtained using an algorithm due to Stewart et al. (2003). The resulting registered images were then corrected to compensate for differing exposure settings, with all images normalised to an exposure time of 1 s, a gain of one, and zero offset.

### 5.2. Construction and optimisation of $f$ and $f^{-1}$

The parameter space  $\mathcal{P}$  was discretised on a rectangular grid. The parameter values chosen for each of the five variables are shown in Table 1. This discretisation of  $\mathcal{P}$  provides  $M = 5 \times 5 \times 6 \times 6 \times 6 = 5400$  points at which the model is defined.

For each parameter vector  $\{\mathbf{p}_i\}_{i=1,\dots,M}$ , the corresponding reflectance spectrum was computed using the Monte Carlo model of tissue reflectance described in Section 2. The spectra were then used in the filter selection procedure described in Section 4.3. An evolutionary algorithm (Yao and Liu, 1997) was used to find the set of  $N = 6$  filters which provided a unique mapping and minimised the error

of parameter recovery. The algorithm used in this work is a population-based evolutionary algorithm, where each of the  $n$  (we used  $n = 100$ ) individuals in the population is a set of six filters defined by their central wavelengths. We begin with a random initial population. After checking that an individual generates a unique mapping using the procedure given in Section 4.1, we compute the “fitness” of each individual in the population, defined to be the error in parameter recovery given by Eq. (17). We then “mutate” the individuals to produce “offspring” (from each “parent”, we produce three offspring). We evaluate the fitness of the offspring, and then select the best  $n$  individuals from the combined set of parents and offspring to form the next generation. After some time (typically a few hundred generations), the fitness of the fittest individual stabilises. We therefore take this individual to be the optimal set of filters for parameter recovery. Since the halogen light source used provides only weak illumination in the blue region of the spectrum, we constrain the optimisation process to only find filters with central wavelengths in the region 500–700 nm, the upper limit being set by the properties of the VariSpec filter. The filters were selected from the set of filters that can be implemented by the VariSpec filter and were specified only by their central wavelength, with all other properties dependent on that central wavelength. Therefore, each individual in the evolutionary algorithm consists of six numbers which correspond to the central wavelength of filters. The algorithm was run 20 times, each time until the fitness of the fittest individual stabilised for at least 20 generations. The fittest individual found had central wavelengths 507 nm, 525 nm, 552 nm, 585 nm, 596 nm, 611 nm (the last to be used as the denominator in the image quotients). The corresponding optimal filters are shown in Fig. 4.

The optimal filters were applied to each of the  $M$  reflectance spectra computed using the model to give  $M$  vectors of six image values. From these,  $M$  vectors of five image quotients were computed by dividing the image values computed using the filters centred at 507 nm, 525 nm, 552 nm, 585 nm and 596 nm by the image value computed using the filter centred at 611 nm. This establishes a mapping between the original  $M$  parameter vectors in  $\mathcal{P}$  and  $M$  image quotient vectors in  $\mathcal{Q}$  and provides a definition of both  $f$  and  $f^{-1}$  at these  $M$  points.

The discretised model was used to train a neural network to approximate  $f^{-1}$ . The particular type of neural network used was the radial basis network provided in the MATLAB Neural Network Toolbox. The function newrbe(P, T, SPREAD) was used to create a two-layer neural network containing radial basis neurons in the first layer, and linear neurons in the second layer (full details can be found at the MATLAB support webpages<sup>1</sup>). The  $M$  image quotient vectors generated from the model were used as

Table 1  
Parameter values used in the calculation of  $f$

Parameter	Values (mmol/L)
Macular pigment	0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6
Retinal haemoglobins	0.0, 0.25, 0.50, 0.75, 1.00, 1.25
RPE melanin	1.64, 3.11, 4.58, 5.56, 6.54, 8.00
Choroidal haemoglobins	1.90, 3.80, 5.70, 7.60, 8.50
Choroidal melanin	0.00, 0.33, 0.66, 1.00, 1.33, 1.66

<sup>1</sup> <http://www.mathworks.com/access/helpdesk/help/toolbox/nnet/newrbe.html>.

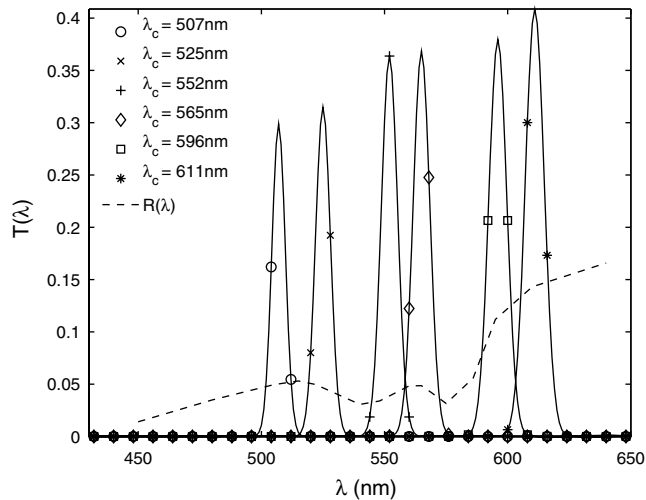


Fig. 4. Optimal filters for parameter recovery. A sample reflectance spectrum (dashed line) is shown for reference.

input data  $P$ , and the  $M$  parameter vectors were the target outputs  $T$ . The training parameter SPREAD was set to 3.0, which was found to give good results on unseen data. Approximately 350 random parameter vectors were generated and the corresponding image quotients were computed. These quotients were then given as input to the network which computed the corresponding parameter vectors. These were then compared with the original parameter vectors. The results of these tests are shown in Fig. 5.

It is clear from these data that not all parameters are recovered equally well. We see quite clearly that whilst  $C_{MP}$  and  $C_{RH}$  are recovered with acceptable accuracy, the remaining parameters cannot be recovered. The reason for this is not fully understood but is believed to be because of the highly non-linear nature of  $f$  and  $f^{-1}$ . At low concentrations of pigment, small changes in concentration cause large changes in the reflectance of the tissue, and hence large changes in the image quotients. At high concentrations of pigment, a large change in the quantity of pigment is required to produce a small change in the tissue reflectance. This non-linearity makes the functions  $f$  and  $f^{-1}$  very hard to approximate, and more work is needed in this direction. In our analysis and discussion in Sections 6 and 7, we will restrict our attention to the distributions of macular pigment and retinal haemoglobins, which can be computed with acceptable accuracy.

After the image acquisition process described in Section 5.1, the images were processed by the neural network. For each pixel in the images, the appropriate image quotients were calculated directly from the image values recorded. The resulting vectors of image quotients were given as input to the neural network, which returned a parameter vector for each point in the image. The values of each parameter were displayed graphically as parametric maps which show how each of the parameters varies across the image.

## 6. Results

Images were obtained from a range of subjects spanning a wide variety of ethnic backgrounds including Caucasian, African, Indo-Asian, Afro-Caribbean and Mediterranean subjects. Images were initially taken only from subjects with no known pathology, and these were used to verify the technique. Further images were then obtained from subjects with known pathologies (for example, retinal haemorrhages, choroidal neovascular membranes) in order to investigate the ability of the technique to flag these pathologies. In order to provide maximum flexibility in the processing of the images, extra images were recorded using additional filters chosen to highlight notable features in the reflectance spectra of the tissue. The central wavelengths of the chosen filters were at  $\lambda = \{480 \text{ nm}, 507 \text{ nm}, 515 \text{ nm}, 525 \text{ nm}, 540 \text{ nm}, 548 \text{ nm}, 552 \text{ nm}, 560 \text{ nm}, 565 \text{ nm}, 569 \text{ nm}, 575 \text{ nm}, 585 \text{ nm}, 596 \text{ nm}, 611 \text{ nm}, 640 \text{ nm}, 675 \text{ nm}, 705 \text{ nm}\}$ . Note that these include the optimal filters deduced from the properties of the model. In total, we obtained images from 35 subjects, although in the post-processing stage, approximately half of these were discarded due to the presence of specular reflections from the cornea, or excessive subject movement.

In order to verify the correctness of the model, image data taken from all normal subjects were collated and compared with the model to investigate the correspondence between model and data. Projections of the model and image data onto the  $q_i$ - $q_j$  planes were used to check the correspondence. These are shown in Fig. 6.

It is clear from these projected views of the data that the model and image data are not coincident. There is considerable overlap, and the shapes of the projections are roughly similar, but the image data clearly does not fall within the bounds of the model data. There are several possible reasons for this, although the causes of these reasons are not so easy to identify. Firstly, it may be that the model does not fully capture the whole range of variability within normal tissue. Extending parameter space to include higher concentrations of the variable pigments has little effect on the extent of the model. It may be necessary to introduce additional variable parameters (e.g. layer thicknesses) in order to model the full variability of normal tissue.

A further problem is that the data used to construct the model may be inaccurate or inappropriate. For instance, the only data on absorption and scattering by ocular tissues is from Hammer et al. (1995), who took ex vivo measurements on excised samples of bovine and porcine fundi. These are physiologically and histologically very similar to human fundi and much of the recent work on modelling of fundus reflectance (Preece and Claridge, 2002) has used these data as a starting point. However, this is clearly not an ideal solution, and data from human subjects would surely improve the results.

In Section 7, we offer some additional possible causes of this mismatch for which solutions require resources which are currently unavailable to us. In the absence of an ab initio method for resolving the problem with the resources

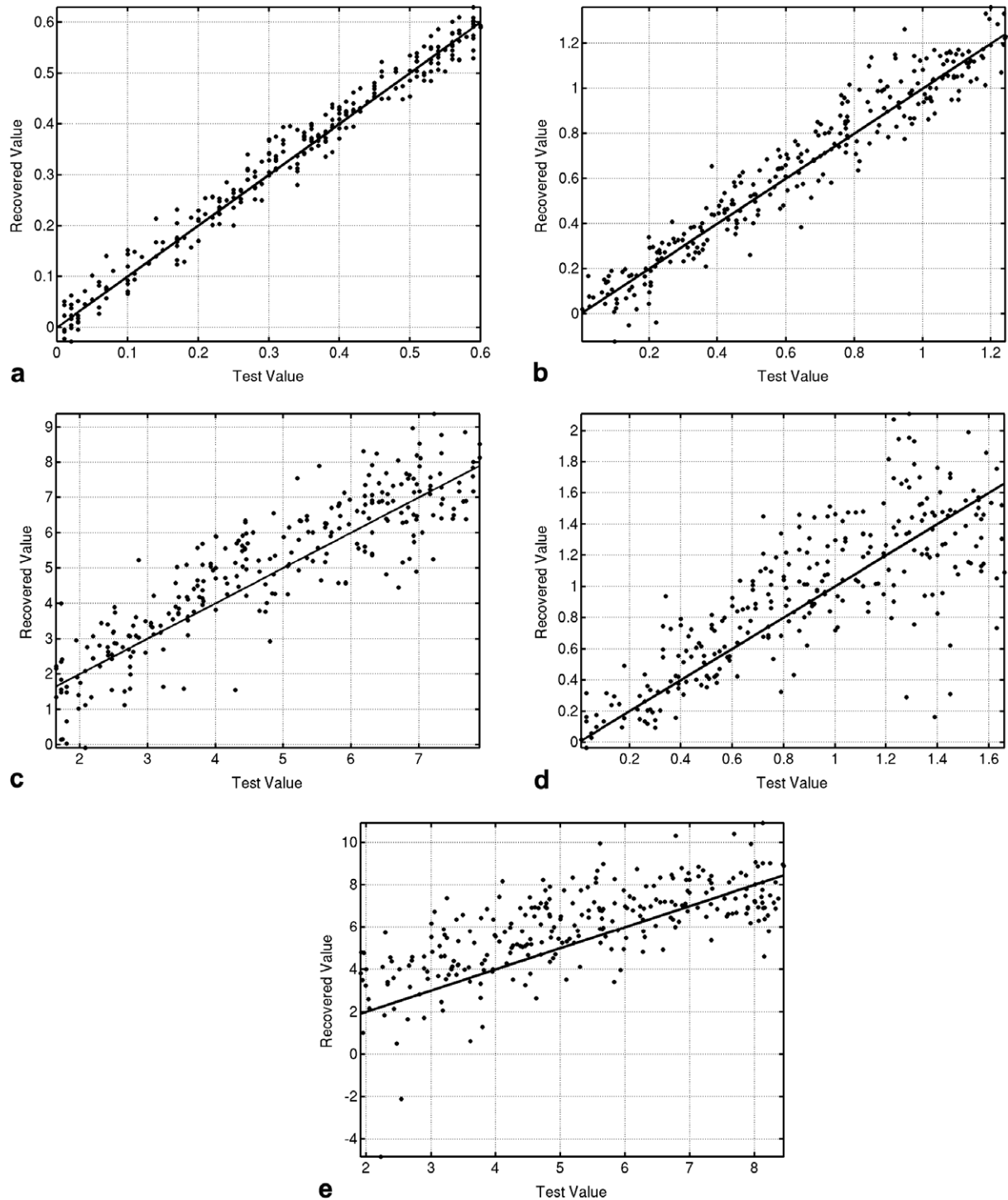


Fig. 5. Parameter recovery from generated data. (a) macular pigment; (b) retinal haemoglobins; (c) RPE melanin; (d) choroidal melanin and (e) choroidal haemoglobins. The solid line represents the ideal case of exact parameter recovery and serves only to guide the eye. Image quotients were generated from random parameter vectors and a neural network (trained on a different dataset) was used to recover the parameters. The standard error of parameters recovery based on these data is macular pigment:  $\pm 0.13 \text{ mmol L}^{-1}$ ; retinal haemoglobins:  $\pm 0.37 \text{ mmol L}^{-1}$ ; RPE melanin:  $\pm 2.85 \text{ mmol L}^{-1}$ ; choroidal melanin:  $\pm 0.87 \text{ mmol L}^{-1}$ ; choroidal haemoglobins:  $\pm 3.7 \text{ mmol L}^{-1}$ .

available, we elected to introduce an empirical correction to the model, based upon the normal image data.

Although the image and model data are not coincident, the shapes of the projected datasets are similar. That is, where the projection of the model is “long and thin”, as

in, for example, the  $q_3$ – $q_4$  projection, the projected image data is also “long and thin”. This suggests that whilst the absolute position and size of the model in  $\mathcal{Q}$  is wrong, the behaviour of the model with respect to the constituent parameters may be correct. In other words, an increase in



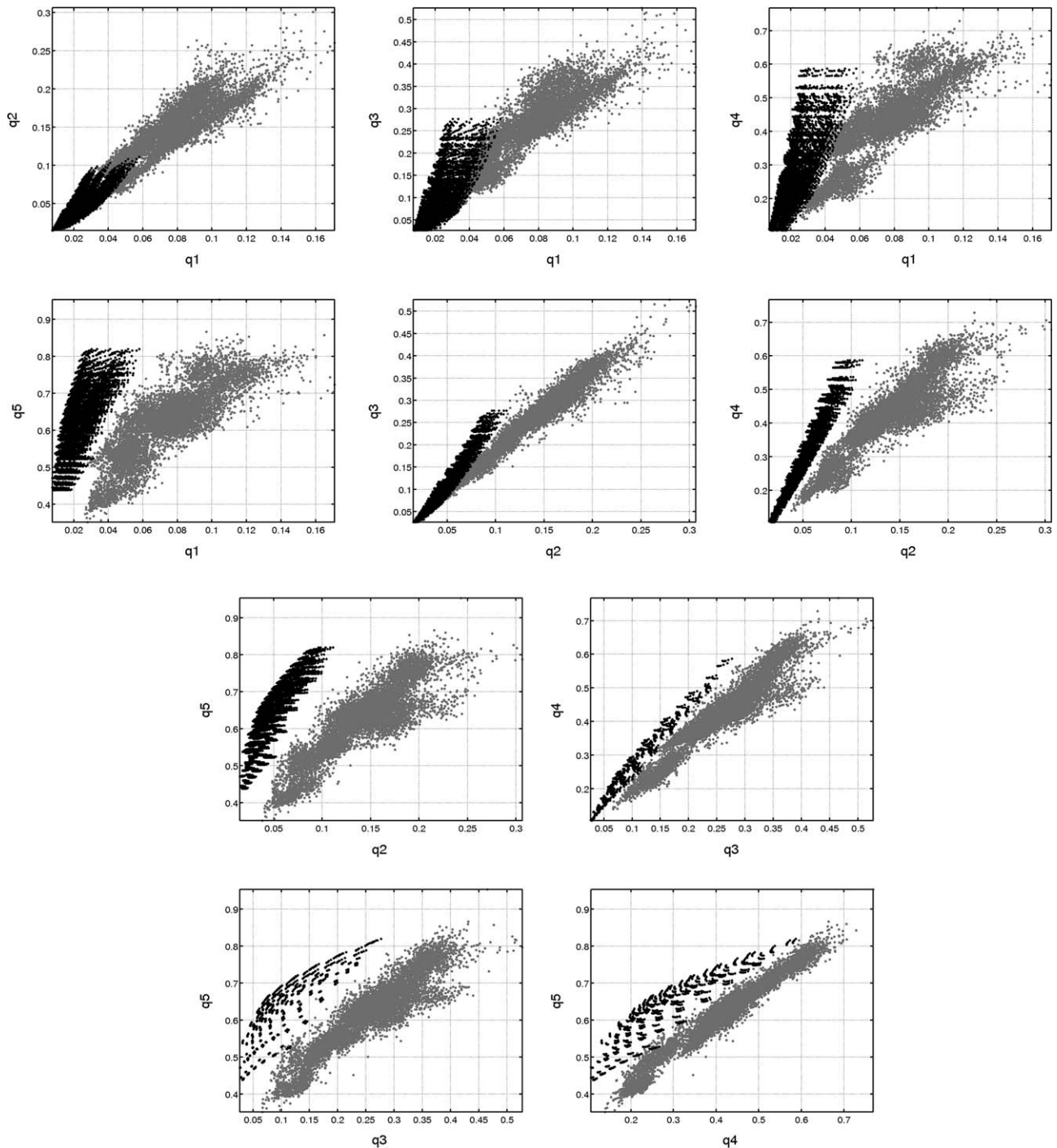


Fig. 6. Projections of model (dark points) and image (light points) data onto each of the 10 possible  $q_i$ - $q_j$  planes.

one of the parameters causes a move along a path in  $\mathcal{Q}$ . An increase in the same parameter in the real tissue causes a movement in a similar direction. This means that provided we can map the model and image data to be coincident, we can use the directionality within the model to extract parameter values from the image data. Although the absolute values of the recovered parameters may be incorrect, the relative values should be correct, and the parametric maps may reveal the distribution of parameters in the image.

We therefore choose a simple “shift and scale” operation to align model and image data. We first transform the image data such that the means of model and each set of image data are the same. We then scale the model such that it encapsulates all of the normal data. A scale factor of four was found to be sufficient to encompass the normal image data. The two-dimensional projections of the scaled model in Fig. 7 show that the scaled model now encapsulates the image data. The scaled model was then used as a basis for the interpretation of image data.



A neural network was trained on the scaled data and this was used as the inverse function. Note that one important consequence of this scaling is that the effective parameter ranges become reduced, as shown in the test data of Fig. 8.

For all normal and pathological subjects, the mean of the image data was moved to the mean of the model data, and the translated image data were then used as input to the neural network. The resulting vectors of model parameters, one for each point in the image, were then displayed as parametric maps, each showing the variation of the cor-

responding histological parameter across the image. Due to the difficulties in recovering some parameters with sufficient accuracy, we will analyse only the parametric maps showing the distributions of macular pigment and retinal haemoglobins.

### 6.1. Analysis of macular pigment distribution

In a normal human fundus, there exist a family of pigments known as macular pigments which are present in

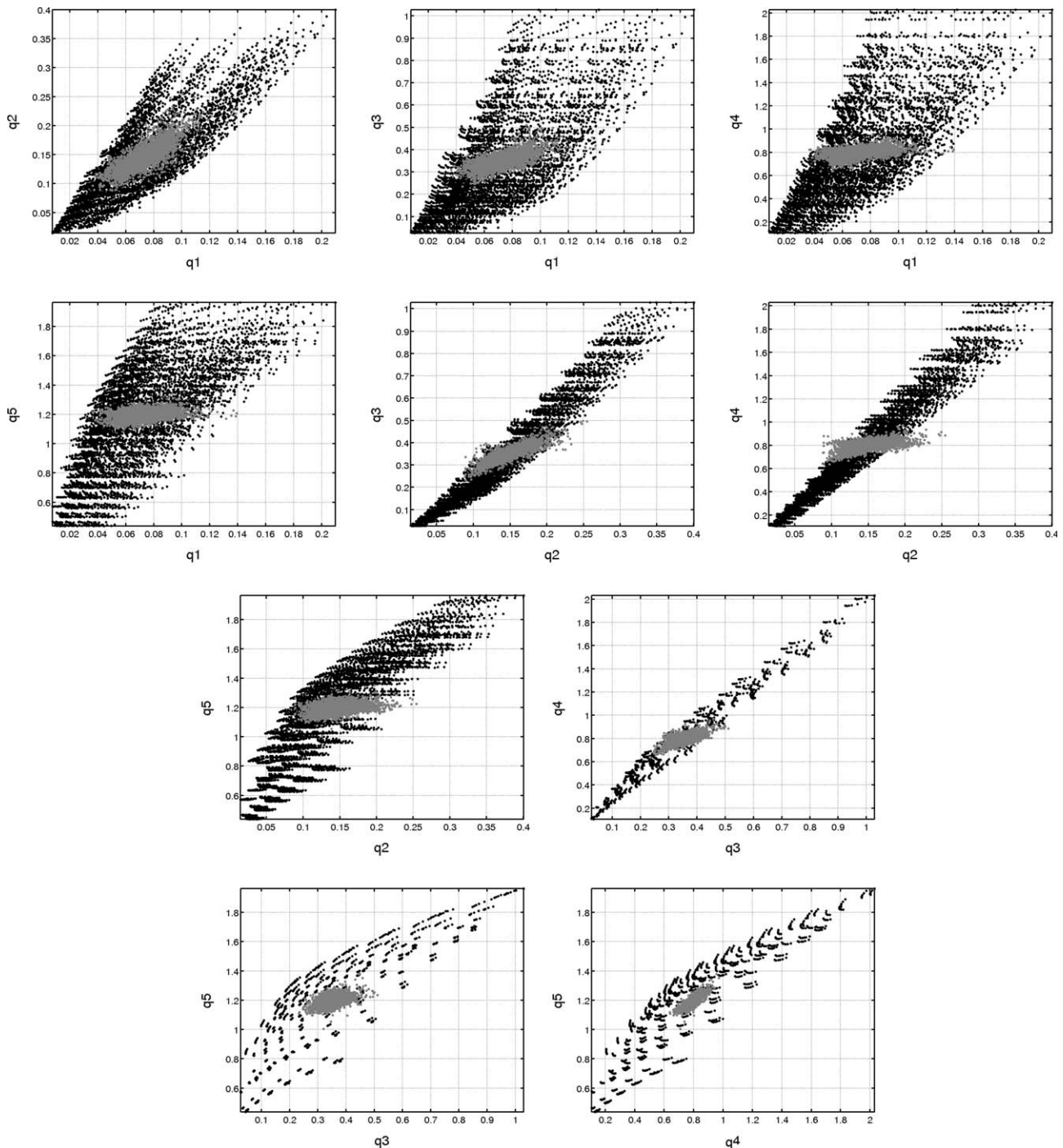


Fig. 7. Projections of model (dark points) and image (light points) data onto each of the 10 possible  $q_i$ - $q_j$  planes. The datasets have been mean-aligned and the model has been scaled by a factor of four.

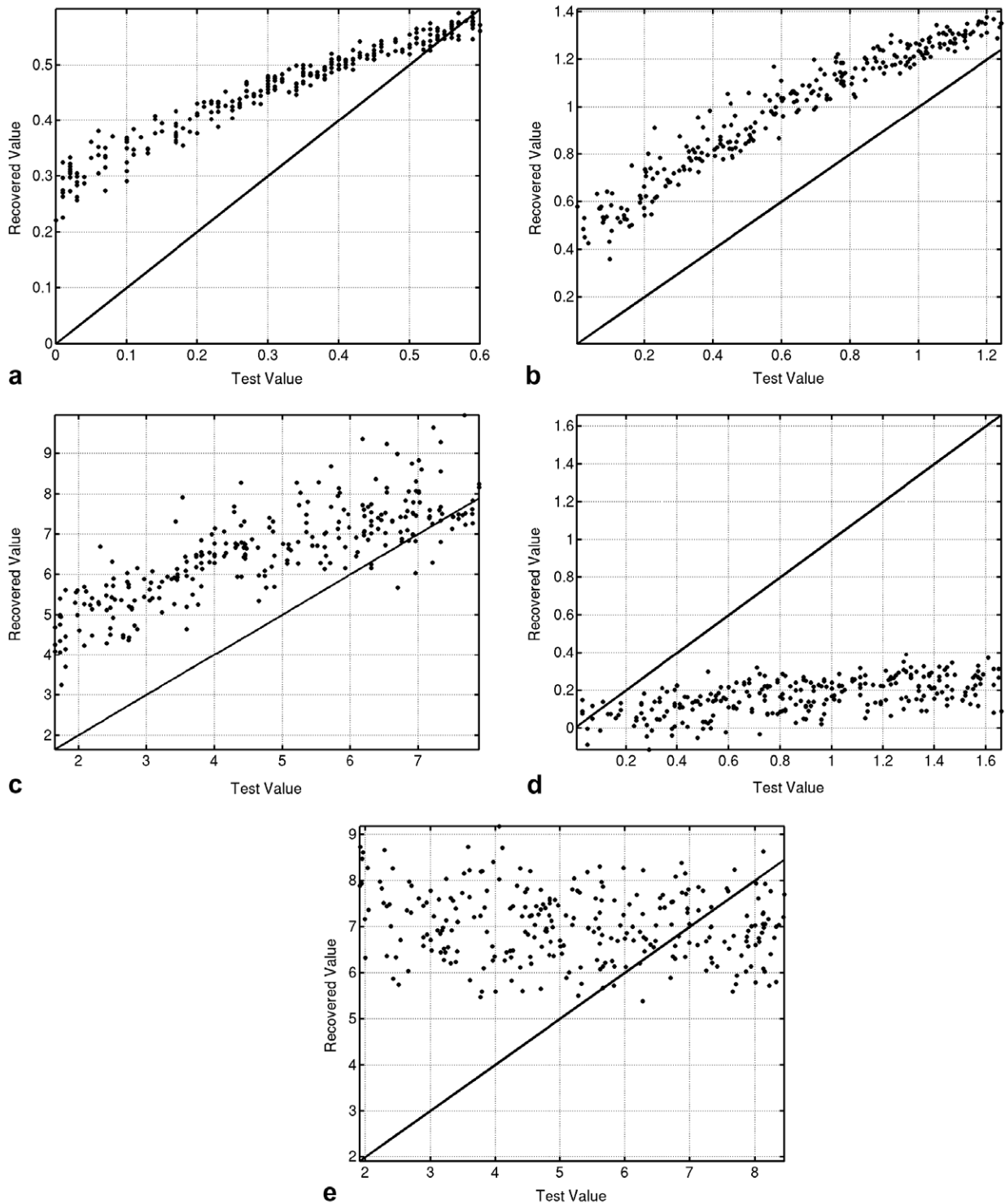


Fig. 8. Parameter recovery from generated data: (a) macular pigment; (b) retinal haemoglobins; (c) RPE melanin; (d) choroidal melanin and (e) choroidal haemoglobins. The solid line represents the ideal case of exact parameter recovery and serves only to guide the eye. Image quotients were generated from random parameter vectors and scaled as described in the text. A neural network trained on the scaled data was used to recover the parameters. Note well that although the trends for RPE melanin and retinal haemoglobins are correct, the effective range onto which these parameters can be recovered is significantly reduced.

the retina. Macular pigments are strongly localised in the area around the fovea, where the density of photoreceptors is highest. It is believed (Beatty et al., 1999; Landrum et al., 1997) that their principal function is to protect the photo-

receptors from high intensities of blue and ultra-violet light which is well known to be damaging to the receptors. This pigment is therefore an ideal test for our methods, since the normal spatial distribution is well established. Other

workers have used alternative methods for quantifying macular pigments. Bone and Sparrock (1971) used the flicker technique to compare foveal and extra-foveal spectral sensitivities, from which they were able to estimate macular pigment concentrations. Berendschot et al. (2002) used single-wavelength (460 nm) reflectometry to estimate both macular pigment and melanin concentrations. Both works suggest a wide variation in macular pigment concentrations between subjects. Note that although the wavelength (460 nm) used by Berendschot et al. (2002) is outside the range of the filters than we use, our optimisation process used to select the filters was constrained to wavelengths  $>500$  nm due to limitations of the equipment (illumination brightness and filter transmission are both very low below 500 nm). An unconstrained optimisation

did indeed select filters at wavelengths close to 460 nm, but at these wavelengths, the exposure time is too long to obtain images of sufficient clarity. With more sophisticated equipment, we would acquire images in this region of the spectrum.

In Fig. 9, we show several examples of parametric maps of macular pigment, alongside RGB pictures of the same subjects. These images show a clear peak in the macular pigment distribution in the vicinity of the fovea. This is consistent with the known histology of the fundus. There is also a clear peak in the vicinity of the optic disk. This “false positive” occurs because the optic disk has a significantly different histology to the rest of the fundus. Since we assume that scattering and thickness of the retina, RPE and choroid and constant (and non-zero), the optic

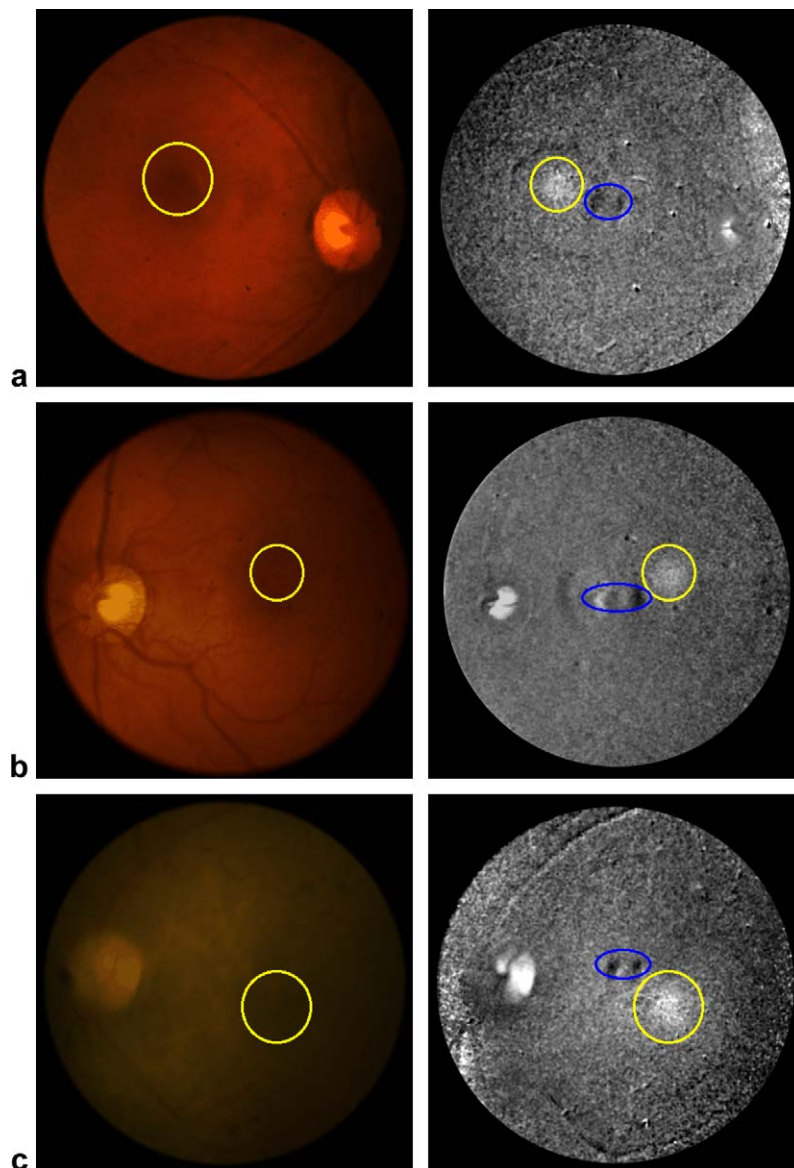


Fig. 9. RGB images and the corresponding parametric maps for macular pigment from: (a) young Mediterranean female (right eye); (b) young Afro-Caribbean male (left eye) and (c) elderly Afro-Caribbean female (left eye). In the parametric maps, lighter areas correspond to higher concentrations of pigment. The foveal region is circled in yellow. The artifacts at the centres of the parametric maps (blue oval) are due to a reflex from the camera optics.

disk is not well represented by the model and parameters recovered in this region cannot be regarded as accurate. In Section 7, we examine the validity of this assumption.

Not all subjects showed the same clear macular pigment peak in the fovea that is evident in Fig. 9. We believe that this is predominantly due to inherent problems with our imaging technology which we discuss in detail in Section 7. In brief, the prolonged and repetitive exposure used means that eye movement is inevitable. Whilst the images can be corrected spatially using advanced registration methods (Stewart et al., 2003), constancy of light intensity is vital for our techniques to succeed, and any movement necessarily compromises this.

## 6.2. Analysis of retinal haemoglobin distribution

Blood is distributed to the tissues of the retina via a network of arteries, capillaries and veins. The larger vessels (the main arteries and veins) are clearly visible to the human observer whilst the capillary bed provides a pinkish tint to the bulk of the fundus. When large amounts of other pigments are present, this tint is rather less obvious than in relatively unpigmented subjects. In all subjects, the presence of macular pigment at the fovea tends to obscure this tint, making it harder for the human observer to discern any variations in blood concentrations near the fovea. This is important, since the fovea is normally surrounded by an “avascular zone”, where the capillary network is absent.

The presence of blood at or near the fovea can lead to obscuration of vision, and so detecting blood in this region is imperative if sight-threatening complications are to be prevented.

The standard method for determining blood flow across the fundus is through fluorescein angiography. The patient is injected with a fluorescent compound. Using a sequence of flash photographs, the propagation of this compound through the fundus is followed and used to observe the capillary network. Defects in the capillary network can be seen by this method and allow haemorrhages which may obscure vision to be detected. Diabetic macular ischaemia can also be seen due to the characteristic loss of blood flow to the macula. Fluorescein angiography is a very effective method, but is also invasive, involving the injection of the fluorescing compound, and is uncomfortable for the patient. In some subjects, the procedure can even be life-threatening. Any alternative method which allows these defects in the capillary network to be seen would be of great potential benefit. In Fig. 10, we show parametric maps of retinal haemoglobins for several subjects, together with the corresponding RGB images.

In Fig. 10(a), there is a clear depression in the quantity of retinal blood at the fovea. This corresponds to the well-known foveal avascular zone which is a feature of normal subjects. Many of the larger retinal blood vessels are also clearly visible as areas where blood is more concentrated. Note well that this subject was extremely compliant, and

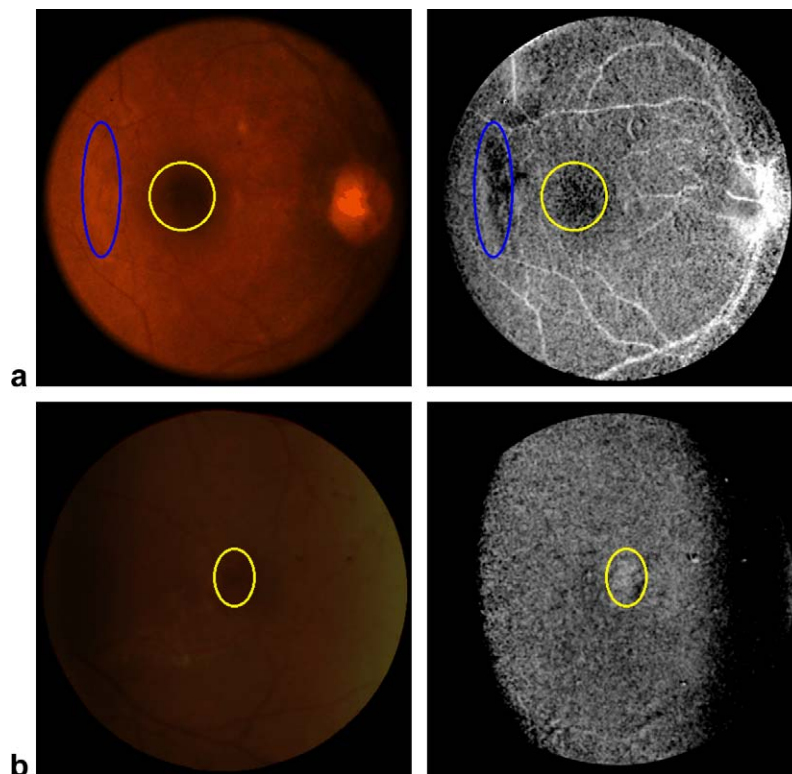


Fig. 10. RGB images and the corresponding parametric maps for retinal haemoglobins from: (a) young African female (right eye) and (b) elderly Caucasian female (left eye). In the parametric maps, lighter areas correspond to higher concentrations of pigment. The foveal region is circled in yellow. In subject (a), an artifact due to reflection from the ILM is circled in blue.



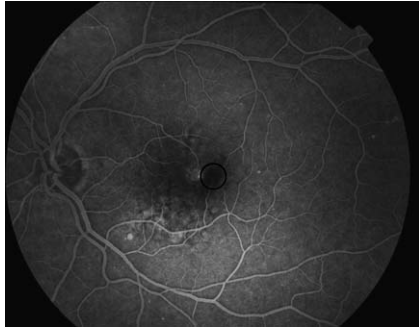


Fig. 11. Fluorescein angiogram of the elderly Caucasian female (left eye) featured in Fig. 10(b), showing a large retinal haemorrhage in the foveal area (circled).

image registration required only small adjustments. The large depression to the left of the fovea is an artefact due to specular reflection from the inner limiting membrane. In Fig. 10(b), there is a small region in the foveal area, where there appears to be a large amount of blood. A comparison with the fluorescein image shown in Fig. 11 indicates that this corresponds to a significant and potentially sight-threatening haemorrhage which requires immediate treatment. The blood vessels are not clearly highlighted in this subject as the inter-frame movement was significant and required significant corrective transformations during registration (the vessels are clearly visible in the individual frames). Small errors in registration may lead to such small features being misaligned. For future work, it is imperative that all frames are acquired concurrently.

## 7. Discussion

We have shown how an understanding of the imaging process can allow us to extract histological information from multi-spectral images of the human ocular fundus. By establishing a mapping between histological parameters and “image quotients”, we are able to establish (subject to certain criteria) the corresponding inverse function which maps image quotients onto histological parameters. The use of image quotients (ratios of image values) allows us to compensate for the inevitable variations in illumination intensity across the fundus. The inverse mapping can therefore be used to compute tissue histology across the ocular fundus, yielding parametric maps which show the spatial distribution of each parameter. We have shown parametric maps for two histological parameters, macular pigment (Fig. 9) and retinal haemoglobins (Fig. 10) which are consistent with known histology. In the case of a known pathology (Fig. 10(b)), the parametric map for retinal haemoglobins is consistent with the fluorescein angiogram for the subject.

Whilst these positive results are very encouraging, there are a number of difficulties with our methods which still have to be resolved. Firstly, we note that not all model parameters can be recovered equally well. On test data, where parameters were known, only macular pigment and retinal haemoglobins could be recovered with accept-

able accuracy, as shown in Fig. 5. We believe this to be due to the highly non-linear nature of the inverse mapping which means that construction of the inverse function is very hard. Our current implementation uses a fairly simple neural network, but it may be that a more sophisticated network will provide better results. Another option may be to take advantage of the latest developments in genetic programming to evolve an explicit form for the inverse function. The authors currently lack the expertise to implement such a system, but are in contact with experts in the field.

One of the main drawbacks of the image acquisition system used in this work is that the multi-spectral image frames are acquired sequentially. The entire exposure time is of the order of 5 s and a subject cannot remain perfectly still during this time. We have introduced breaks between frames, during which subjects were allowed to close their eyes, but not move their head. They were then asked to re-fixate, and the next frame was acquired. All images can then be acquired without causing undue discomfort, but no matter how well the subject is able to re-fixate, the position of the fundus always changes during these breaks. Ideally, we would capture all images simultaneously. Standard RGB cameras capture three channels (frames) simultaneously, either by interleaving red- green- and blue-sensitive pixels on the same CCD and using interpolation, or by using a separate CCD for each channel. A similar system which simultaneously captures the six frames required in this work would greatly improve the image quality, and would resolve the following issues in the present work.

The first issue that simultaneous image capture would solve is the need for image registration. Whilst the registration methods used (Stewart et al., 2003) have produced excellent results, there is an inevitable loss of fine detail, where perfect alignment is required. This would be especially important if we were studying early-stage diabetic retinopathies such as dot haemorrhages which may be only a few pixels in width.

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. A single “snapshot” would remove this variation.

The final issue is a result of subject movement, but cannot be corrected by post-processing. The use of image quotients to compensate for spatial variations in illuminant relies on those variations being identical in all frames. If this is not the case, then the function  $A(\mathbf{x})$  in Eq. (7) is also a function of wavelength, and may not be taken outside of the integral. Inter-frame movement will necessarily lead to changes in the illuminant. We have noted that the best results have been obtained, where only small corrections have been required to register the images. Where large corrections have been necessary, our methods of interpretation fail. The effect of subject movement on illumination is much more serious than the movement itself. The registration



methods adopted are able to spatially align images well (although fine detail may be blurred), but they cannot correct for changes in the illuminant. The simultaneous capture of all frames is essential if this difficulty is to be resolved.

A final technical problem that is relatively easy to solve is the removal of specular reflection from the inner limiting membrane. This is a well-known problem in younger subjects and the effects can be seen clearly in Fig. 10(a), where the reflection has caused an artifact in the parametric map. The removal of such reflections is fortunately quite straightforward, requiring only a set of polarising neutral filters. These should be included in any future investigations.

Perhaps, the most concerning problem is the need for an empirical scaling factor to ensure that model and image data were coincident. The issues surrounding image acquisition are likely to be contributory factors to this discrepancy, but we can also identify potential problems with the model itself. The individual variation between fundi is likely to be important: we have assumed that the individual layers in the fundus are of constant thickness and are the same in all subjects. This assumption is not correct, and variation in the layer thicknesses may well lead to a significant spread of the data. There may also be variations in the underlying optical properties of the tissue which we have not taken into account. Whilst the model appears to capture the correct trends, it does not take into account the full variation of normal fundi, and further investigations are necessary in order to determine the precise cause of this discrepancy. Some preliminary work has been undertaken to determine whether the model may be under-parameterized. The model of tissue reflectance assumes that there are five variable components, and that all other tissue parameters are constant. Certainly, it is known that absorption in the intra-ocular media varies with age (Boettner and Wolter, 1962), and recent studies using OCT (Varma et al., 1996; Sony et al., 2004) have found significant variation in layer thicknesses. In addition, the absorption and scattering characteristics of the underlying tissues have never been measured comprehensively in vivo, and the degree of variation between different subjects is not known. Adding additional parameters to the model introduces extra complexity to the imaging and inversion procedures as each parameter adds another dimension to the model. This will require an extra filter for each additional parameter, and will also increase the difficulty with which the inverse model can be deduced. The current difficulties with imaging and inversion suggest that more suitable imaging equipment and a better inversion method are needed in order to be able to include additional parameters. We have undertaken preliminary investigations into the effect of some additional parameters. Notably, we have found that allowing the density of the intra-ocular media to vary has a clear effect on the reflectance spectra, as discussed by Boettner and Wolter (1962), but in fact has very little effect on the resulting image quotients. In addition, we have also obtained images from a subject with a lens implant of known optical density. The image quotients

from this subject were not noticeably distinct from those of other subjects of a similar age. From these findings, we conclude that variations in the intra-ocular media have an insignificant effect on the image quotients. Further investigations into the effect of allowing variations in other parameters are required.

In conclusion, we have demonstrated that a physics-based model of image formation which uses image quotients to compensate for the effects of spatially uneven illumination can be used to extract histological parameters from multi-spectral images of the ocular fundus. A set of filters was objectively chosen to minimise the error of parameter recovery and a neural network was used to compute tissue histology from image quotients. The method has been successfully used to map the distribution of macular pigment in the retina, and to compute the distribution of haemoglobins in the retina. Results are consistent with known histology and existing imaging methods. Further work is required to improve the image acquisition process to allow all frames to be captured simultaneously, and further refinements to the model are required in order to capture the full variation in fundus histology.

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