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Spectral Characterization of Murine Arthritis Models

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Abstract: Monte Carlo modelling of light propagation through mouse paw tissues reveals that hypoxia and erythema occurring in arthritis characteristically alter the shape of reflectance spectra. Measurements from normal and arthritic mice show similar trends.
OCIS codes: (170.4580) Optical diagnostics for medicine; (170.3660) Light propagation in tissues; (170.6510) Spectroscopy, tissue diagnostics.

1. Introduction

Rheumatoid Arthritis is a chronic autoimmune disease affecting around 1% of the Western population. It is characterized by progressive inflammatory polyarthritis, initially causing inflammation and hyperplasia of the synovial membrane. As the disease progresses, an influx of immune cells damage the cartilage and subchondral bone reducing the functionality of the joint. The early phase of disease is the most efficacious for drug targeting, but it is problematic for imaging due to the expertise and waiting times associated with ultrasound and MRI, the most proficient modalities for grading inflammation. Consequently, optical imaging presents a possibility as a future clinical imaging aid due to its non-ionising radiation, inexpensive equipment, and rapid imaging speeds.

Animal models are frequently used in rheumatoid research, both to study the complex aetiology and pathology of the disease, and to discover novel molecular targets for drug therapy. Many of these studies involve the long-term observation in order to track symptom severity over the course of a treatment or experiment. The potential for a non-invasive, non-ionising imaging system that predicts the severity of inflammation would therefore be a useful adjunct to conventional measurements such as paw size, and observational evaluation of mouse appearance and behaviour.

There are several changes that take place in the rheumatoid joint which are responsible for altering the optical properties of the tissue. Decreased oxygen tension is thought to be a consequence of the high metabolic demands of the immune cell influx, and the proliferating synovium. This is now recognized to extend to the haemoglobin balance in the tissue surrounding the joint with a decrease in the ratio of oxyhaemoglobin to deoxyhaemoglobin. Inflammatory signals and the formation of angiogenic vasculature bring an increase in the local blood volume fraction and localized oedema. The unique wavelength-dependent absorption spectra of the three absorbers, oxyhaemoglobin, deoxyhaemoglobin and water, contribute to the shape of the reflectance spectra of a tissue in the visible and near-infrared (NIR) wavelengths.

In this study the mouse hind paw was modelled as a multi-layer model (Table 1). The changes which occur in arthritis were modelled to investigate whether they translate into detectable changes in the reflectance spectra. For validation, the modelled spectra were compared to the measured spectra of healthy and arthritic mice.

Table 1. The layers and parameter values used in the model of the mouse hind paw.

	g [-]	n [-]	d [μm] mean (min-max)	A [-]	M [-]	k [-] ($x10^{-3}$)	Blood VF [%] min-max	Hb balance [%] mean (min-max)	H_2O [%] mean (min-max)
Epidermis	0.70	1.34	25 (5-30)	59.64	760	- 4.6	-	-	20
Dermis	0.80	1.45	200 (50-250)	50.87	813	- 4.7	2-10	70 (40- 90)	61 (50-70)
Muscle	0.93	1.33	500 (0-700)	10.17	1770	- 6.6	2-15	75 (40-90)	78 (70-85)
Bone	0.90	1.64	200 (0-400)	72.23	1550	- 4.9	1-3	70 (60- 80)	17
Bone marrow	0.90	1.40	200 (0-300)	50.88	175	- 4.0	10	80 (60-90)	50 (30- 40)

2. Optical properties and their modelling

This study uses a multi-layer tissue model informed by H&E stained mouse paw sections for the thicknesses of the different tissue layers. Tissue layer-specific absorption coefficients (Fig. 2a) were incorporated into the model as a linear summation of the layer absorbers, allowing changes in their concentration to represent tissue variability or disease states. The scatter coefficients for each tissue layer (Fig. 2b) were sourced from literature and fitted according to the equation $\sigma_s(\lambda) = A + Me^{-k\lambda}$ where λ is wavelength and A, M and k are independent variables. Scatter coefficients are wavelength-dependent and significantly affect the shapes of spectra remitted from the tissue. Due to the lack of published data on the scatter coefficients of live mouse tissues, published data from human tissues was used. Values for the anisotropy (g), refractive index (n) and tissue layer thickness (d) were taken from the

published data (references not listed due to the shortage of space). Table 1 lists values of the parameters used in the model. The reflectance spectra representative of the mouse paw were modeled using MCML Monte Carlo (MC) simulations [1] employing a GPU-optimized version of the code [2].

3. Murine data acquisition

Spectroscopic measurements were taken from the hind paw of a live arthritic mouse and a normal control littermate. During measurements the mice were anaesthetized with 3% isoflurane. All experiments were carried out at the University of Birmingham, UK following strict guidelines governed by the UK Animal (Scientific Procedures) Act 1986 and approved by the local ethics committee (BERSC: Birmingham Ethical Review Subcommittee). The output from a tungsten halogen light source (Ocean Optics HL2000 FHSA) was transmitted through an optical fiber to the spectrophotometer (Ocean Optics Flame-S-Vis-NIR-ES). The reflected light was collected from a distance of 3mm with the collection radius of 4mm. This set-up was used in consideration of the size of the mouse paws in order that background reflections were kept minimal. The reflectance was calibrated against Spectralon® (Labsphere) reflectance standards.

4. Experiments and results

The main absorbing agents of interest in a model of arthritis are haemoglobin, oxyhaemoglobin and water. The first experiment was to determine whether the changes in the quantities of the individual absorbers result in observable changes in the shape of the whole tissue reflectance spectra. The resulting spectra are shown in Figure 1.

In the second experiment the tissue model was modified to simulate changes that occur in arthritis. The objective was to verify whether the modelled spectral reflectance is consistent with the measured reflectance at the tissue surface. The changes that were incorporated in the arthritic model are a 30% increase in the muscle water content and volume to represent oedema, a 3% increase in blood volume fraction in the muscle layer near to the bone, and a 10% decrease in the muscle blood oxygenation. Figure 2c shows the results of the MC simulation, figure 2d shows the measured spectra in a healthy and an arthritic mouse. It should be noted that the arthritic symptoms evolve with the effector phase of the disease and can vary between the different models of murine arthritis. The data shown here is from a pilot experiment on which we will expand to include a variety of mouse arthritis models at various stages of disease progression. Both the modelled and the measured data presented in figures 2c and 2d are therefore approximations based on the observations of a number of papers [3-6] and our pilot data.

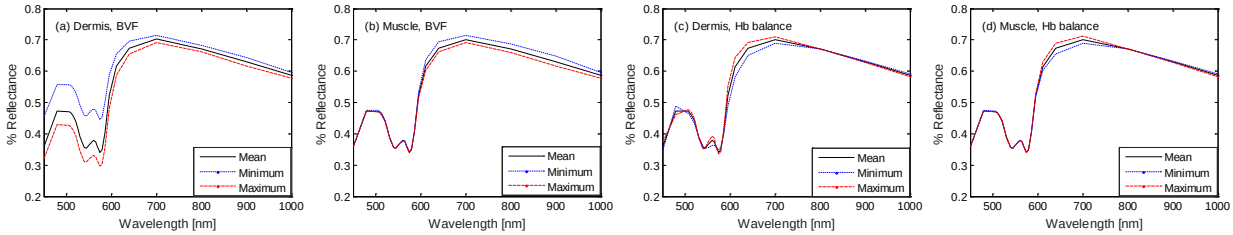

Fig. 1. Changes in spectral reflectance as a result of changes in the absorbing agent. Non-variable parameters were set to mean values. (a) Dermis, blood volume fraction (BVF), (b) Muscle, blood volume fraction, (c) Dermis, blood oxygenation (Hb balance), (d) Muscle, blood oxygenation.

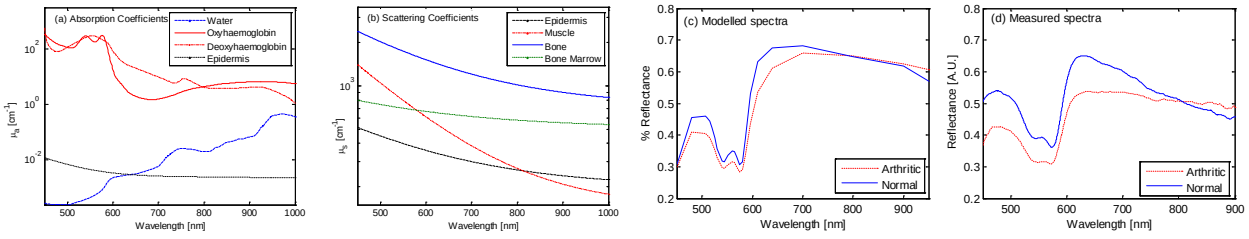

Fig. 2. (a) Absorption coefficients for the main absorbers; (b) Scattering coefficients for the tissue layers, computed as $A+Me^{-k\lambda}$ using coefficients listed in Table 1; (c) Modelled reflectance spectra representative of the healthy and arthritic mouse; (d) Measured reflectance spectra for a healthy and arthritic mouse.

4. Discussion and conclusions

Changes in the blood volume fraction (BVF) and haemoglobin balance (HB) appreciatively change the shape of the reflectance spectra. Moreover, the changes for different tissue layers are occurring at different spectral locations. For instance, BVF changes in the dermis show primarily around λ=450-600nm whereas in the muscle they show at

λ>600nm, suggesting that it might be possible to identify the layer where these changes occur. The same is the case for HB: its changes in the muscle are evident for λ>600nm, those in the dermis for λ<750nm, with a unique signature for the muscle showing after the isosbestic point at 805nm. The changes have a different character: for BVF the overall magnitude of spectral reflectance is increased whereas for HB there are specific localised slope changes. The increase in the muscle water contents (H_2O) which occurs in oedema marginally (c. 2%) lowers the reflectance at the near-infrared end of the spectrum (λ>900nm, not shown). We observed more significant changes in the transmittance spectra and this will be exploited in the future work.

The comparison of the measured and modelled spectra of the healthy and the RA mouse show trends that are consistent with each other and also with the RA disease model. In the visible range the RA mouse spectrum has lower reflectance, hence higher absorption by blood, indicative of erythema. The two oxygenation signatures, the flattening of the peak at around 560nm and the flattening of the spectrum passed 650nm, are consistent with lower blood oxygenation and hence hypoxia. The overall shapes of the measured and the modelled spectra are different, as are their relative magnitudes. This can be explained by the fact that the measured spectra came from two different mice whereas the simulated RA spectrum was generated by modifying three relevant parameters of the normal spectrum, leaving other factors (e.g. tissue thickness, scattering properties) unchanged. Moreover, the measured data was obtained from a very limited number of mice and so may not be representative.

The ultimate aim of this research is to develop a multispectral imaging system capable of producing parametric maps of three distinct tissue absorbers so these could be correlated with the severity of arthritis symptoms in the mouse hind paw. The data produced from the forward model, and the preliminary spectral measurements of a common mouse model of arthritis, suggest that this should be possible. The planned improvements to the model include further elucidation of the cause of the changes observed in real data: the optical effects of immune cell infiltration, tissue break-down or fibrosis have not yet been considered in the forward model but may well contribute to changes in the reflectance spectra through changes in the scattering coefficients. There are also a number of different mouse models commonly used for modelling aspects of arthritis, and their differing symptoms may produce spectral variations.

Optical imaging of RA models in mice has been implemented by a number of groups utilizing different techniques. The majority of methods involve the introduction of optical contrast agents into the mouse, usually NIR fluorescence agents. These are able to target molecular events taking place in the inflamed joint and be imaged using single wavelength NIR imaging devices [6-7]. Very few studies have attempted arthritis imaging with endogenous contrast in mice. A non-contact near-infrared multispectral imaging system derived tomographic optical parameters of the arthritic human joints [8]. Cross-polarization imaging was successfully used to extract Erythema Index images which correlated with the symptoms of experimentally induced arthritis [9].

The study presented in this paper is encouraging for the application of multispectral reflectance imaging of RA mouse paws and, subsequently, of human joints for which molecular imaging is not routinely done at present. A number of techniques being developed for human joints include imaging the infrared transmission profile of the joint and spectral imaging to extract information on blood flow. This study lays foundations towards the development of a multispectral topographic imaging system for human joints.

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