

Modelling Elastic Scattering and Light Transport in 3D Collagen Gel Constructs

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Abstract - A model of elastic scattering and light propagation is presented, which can be used to obtain the scattering coefficient, the index of refraction and the distribution of the collagen fibrils in a gel. The model consists of two sections. The first represents the scattering of light by small cylindrical particles, since collagen fibrils can be realistically represented by these structures, and provides the scattering coefficient. The second section represents the light transport in multi layered tissues. The output is a diffusive reflectance spectrum. Assuming that a gel is composed of fibrils with the same diameter, it is possible to obtain the input parameters of the model and therefore a simulated spectrum. This can be repeated for several diameters. Considering a gel composed of fibrils with different diameters, it is possible to obtain a best fitting simulated spectrum as weighted sum (least square error based) of the spectra corresponding to several fibril diameters, and therefore obtain an estimate of the percentages of fibrils of each diameter in the gel. Moreover, the scattering coefficient and refractive index, which is also provided by the model, are relevant parameters as they relate to tissue properties in its own right.

I. INTRODUCTION

The analysis of the formation and organization of new connective tissue formed in tissue-engineered constructs is a major requirement for tissue bioreactor technology. Fibroblast seeded collagen gel has been used extensively to model wound healing and contraction and tissue reorganisation. Fibroblasts, in particular, have shown to be able to shape a meshwork of a randomly oriented collagen fibrils compacting fibrils. It has been found [1] that elastic scattering spectroscopy can be used to investigate the development of collagen lattice contraction. Elastic scattering spectroscopy is sensitive to gel contraction; the collagen is the only component that make changes in the spectrum. The significant window is 330-600 nm. We present a method to evaluate the previously measured spectra simulating the elastic scattering and propagation of the light through a biological sample, which can be used to obtain the scattering coefficient, refractive indices and the distribution of the collagen fibrils in a gel.

II. METHODS

The optical property coefficients, particularly scattering and absorption coefficient and refractive index of the medium quantitatively describe the interaction of light in a tissue. These coefficients are a measure of the average number of absorption and scattering events per unit path length of photon travel in the tissue. The refractive index is a measure

of the deflection angle of the light when a scattering event occurs.

The optical property coefficients of collagen gel was measured by means of the Bohren Huffman model [2].

This model allows to construct the exact solution to the problem of absorption and scattering by simulating the monochromatic plane wave propagation through an infinitely long right circular cylinder that simulates the scattering of light by small cylindrical particles, since collagen fibrils can be realistically represented by these structures, and provides the scattering coefficient.

The model, by means of an algorithm, allows to calculate the efficiency of scattering Q_{sc} , parameter utilised to obtain the scattering coefficient. The model needs in input the refractive indices of the surrounding medium and of the fibrils, the fibril diameter and the wavelength. Taken the fibrils to be a mixture of dry fibrillar material (collagen molecules) and water, the refractive index of the fibrils was calculated according to Gladstone and Dale's Law [5].

This law expresses the refractive index, n_{tot} , of a mixture as the partial sum of the refractive indices of its components n_1, n_2, \dots, n_N , weighted by the volume fraction of each component, f_1, f_2, \dots, f_N :

$$n_{tot} = n_1 f_1 + n_2 f_2 + \dots + n_N f_N$$

Inasmuch as $f_1 + f_2 + \dots + f_N = 1$.

The output of the model is the scattering coefficient which was calculated by means of following equation for each wavelength:

$$\mu_s = Q_{sc} \cdot d \cdot \rho$$

where Q_{sc} is the scattering efficiency, d collagen fibrils diameter and ρ fibrils density.

Collagen gel construct was modelled with 60, 70, 80, 90, 100, 150, 200 nm fibrils diameters.

The first section, Bohren Huffman model, permits to obtain the scattering coefficient which is an input parameter of the light transport model.

The second section consists of a statistical simulation of Monte Carlo methods to represent the light transport in multi layered tissues [3].

The method describes local rules of photon propagation that are expressed as probability distributions that describe the step size of photon movement between sites of photon-tissue interaction, and the angles of deflection in a photon's trajectory when a scattering event occurs. Monte Carlo

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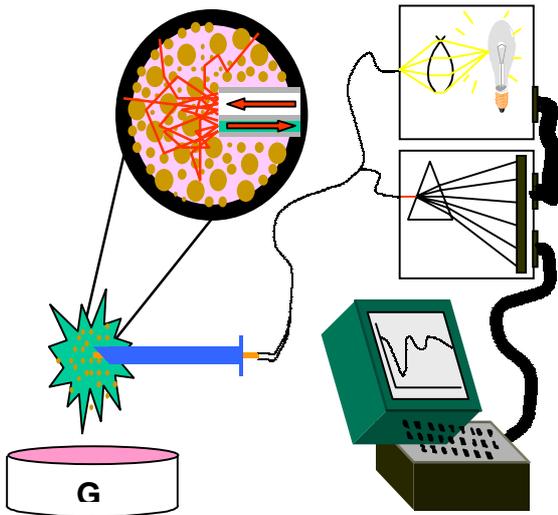


Fig. 1. Spectrometer system.

simulations are based on macroscopic optical properties that are assumed to apply uniformly over small units of tissue volume. Monte Carlo model simulates the system (fig. 1) previously described [1], and then allows to evaluate the spectra previously measured. It needs in input the scattering coefficient, known by means of B&H model, the absorption coefficient, wavelength, anisotropy, index of refraction of the medium and of the fibril. The refractive index of the collagen gel construct, since the fraction of volume of the collagen fibrils is approximately of 15%, was calculated as:

$$n_{\text{sample } 1h} = 0.15 n_f + 0.85 n_{\text{medium}}$$

where $n_{\text{medium}} = 1.35$.

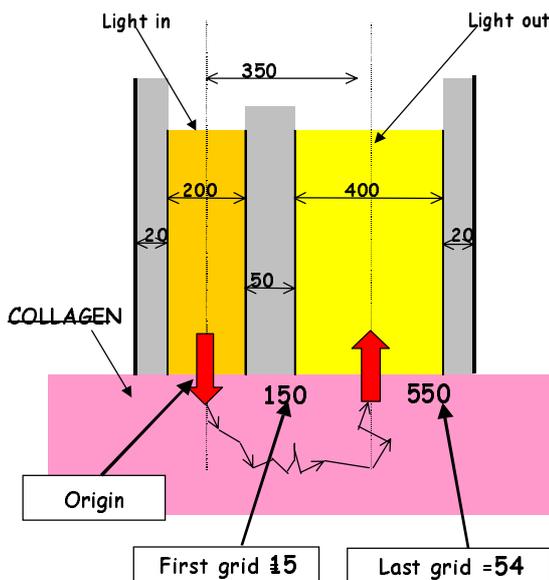


Fig. 2. Probe outline.

It has been necessary to consider the fraction of the tissue covered by the optical fiber. Therefore the position of the fiber within the system grid and the output angle of the light from the tissue were calculated, according to numerical aperture of the fiber. The output is a diffusive reflectance spectrum (ratio of light in and light out) that can be compared with the measured spectrum . The diffusive reflectance is the sum of each grid element selected of the 3-D grid system, fig. 2.

III. RESULTS

Assuming that a gel is composed of fibrils with the same diameter, it is possible to obtain the input parameters of the model and therefore a simulated spectrum. This can be repeated for several diameters, range 60 - 200 nm, according to microscopical analyses. The simulated spectrum is the sum of single spectra calculated for each diameters (fig. 3) then considering a gel composed of fibrils with different diameters, it is possible to obtain a best fitting simulated spectrum as weighted sum (least square error based) of the spectra corresponding to several fibre diameters, figure 4.

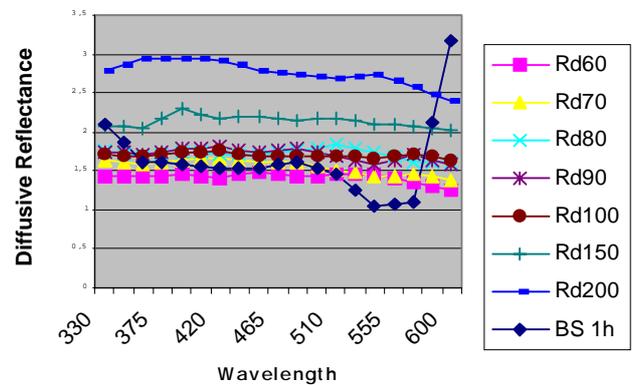


Fig. 3 Diffusive Reflectance vs wavelength for several fibrils diameter.

We calculated the weights minimizing the following equation:

$$\frac{\sum_1^{\#\lambda} \{ \%Rd_{MIS} - \%Rd_{SIM} \}^2}{\#Wavelength \lambda}$$

where $Rd_{\text{mis}}\%$ is the diffusive reflectance measured and $Rd_{\text{sim}}\%$ is that simulated and obtained as:

$$\begin{aligned} \% Rd_{SIM} = & a * (\% Rd_{SIM\ 60}) + b * (\% Rd_{SIM\ 70}) \\ & + c * (\% Rd_{SIM\ 80}) + d * (\% Rd_{SIM\ 90}) + e * (\% Rd_{SIM\ 100}) \\ & + f * (\% Rd_{SIM\ 150}) + g * (\% Rd_{SIM\ 200}) \end{aligned}$$

The weights a, b, c, d, e, f, g correspond to volume fraction of fibrils. We obtained an estimate of the percentages of fibrils of each diameter in the gel.

The fibrils percentage distribution (table 1) suggests that 70 and 100 nm fibrils are predominant.

Diameter	Weight
60	0,03
70	0,52
80	0,03
90	0,03
100	0,32
150	0,03
200	0,03

REFERENCES

- [1] Marenzana M., Pickard D., Bixio L., Caria A., MacRobert A., Brown R.A. "Optical Measurement of 3D Collagen Gel Constructs by Elastic Scattering Spectroscopy" *2ND annual Symposium of the Tissue and Cell Engineering Society* - Nottingham UK, 18-20 September 2000.
- [2] Craig F. Bohren, Donald R. Huffman, *Absorption and Scattering of Light by Small Particles* Wiley-Interscience Publication JOHN WILEY & SONS
- [3] Lihong Wang, S. L. Jacques, *MCML – Monte Carlo modeling of light transport in multi-layered tissues* Computer Methods and Programs in biomedicine, Vol. 47, 131-146 (1995).
- [4] Rebecca A. Porter, R. Brown, M. Eastwood, N. L. Occeleston, P. T. Khaw *Ultrastructural Changes During Contraction of Collagen Lattices by Ocular Fibroblasts* *Wound Repair and Regeneration* March-April 1998
- [5] D.W. Leonard, K.M. Meek *Refractive Indices of The Collagen Fibrils and Extrafibrillar Material of the Corneal Stroma* *Biophysical Journal*, Vol. 72 (3), 1382–7, (1997)

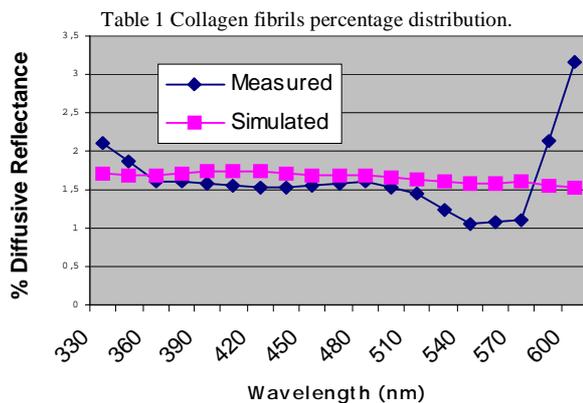


Figure 4 Comparison between measured and simulated spectrum, 1 h of contraction.

IV. CONCLUSIONS

This result is important because of the relationship between the fibrils diameter and mechanical properties of the tissue [4]. Moreover, the scattering coefficient and the refractive index, which are also provided by the model, are relevant parameters as they relate to tissue properties in its own right.

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