

Optical properties of multicellular tumour spheroids

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Abstract. Multicellular tumour spheroids grown *in vitro* have been widely used in cancer research as an experimental preparation with many of the characteristics of tumours. They provide a model system for understanding the optical behaviour of tumour tissue, which is of interest in novel diagnostic and therapeutic procedures. Optical measurements on fresh spheroids in the wavelength range of 600–1000 nm yielded scattering coefficients, absorption coefficients and *g* values (mean cosine of scatter) of 160–90 mm⁻¹, 0.1–0.4 mm⁻¹ and 0.99, respectively. Following fixation, considerably higher values of scattering and absorption coefficients were seen. The values are compared with those reported elsewhere for excised tumour tissue and interpretations of the optical behaviour are suggested.

1. Introduction

Multicellular spheroids are aggregates of tumour cells grown in culture. In such spheroids the cells in the peripheral region are rapidly dividing, whereas deeper in the spheroid there is negligible division and after sufficient growth a central necrotic region develops. Spheroids have many of the histological and functional characteristics of tumours in the early avascular stages of growth (Nederman and Twentyman 1984). They are a biological system having a complexity intermediate between that of monolayer cell cultures and of tumours, and because of this they have been widely used as a model system for the testing of cancer therapies under controlled conditions.

The optical properties of spheroids are of interest for several reasons. In our case the measurements were needed to provide the basis of a rapid non-invasive method of tracking spheroid growth during drug treatment. More generally, the optical properties of tumour tissue are of interest in the development of novel diagnostic procedures, and of therapeutic methods based on photodynamic therapy and laser surgery. Studies on spheroids may provide insights into the fundamental processes underlying the optical properties of tumour tissue as, compared to excised tumour tissue, they are constituted of a comparatively homogeneous assembly of cells all of a similar type.

2. Materials and methods

Spheroids were grown from the POC human small-cell lung-cancer cell line (Kwok and Twentyman 1987). Seed cells were stored frozen at -196 K in medium with 10% DMSO as cryoprotectant, defrosted rapidly and seeded in flasks in RPMI medium with 10% foetal calf serum at 37 °C and 5% CO₂ in air. The medium was changed on day 3 and every day

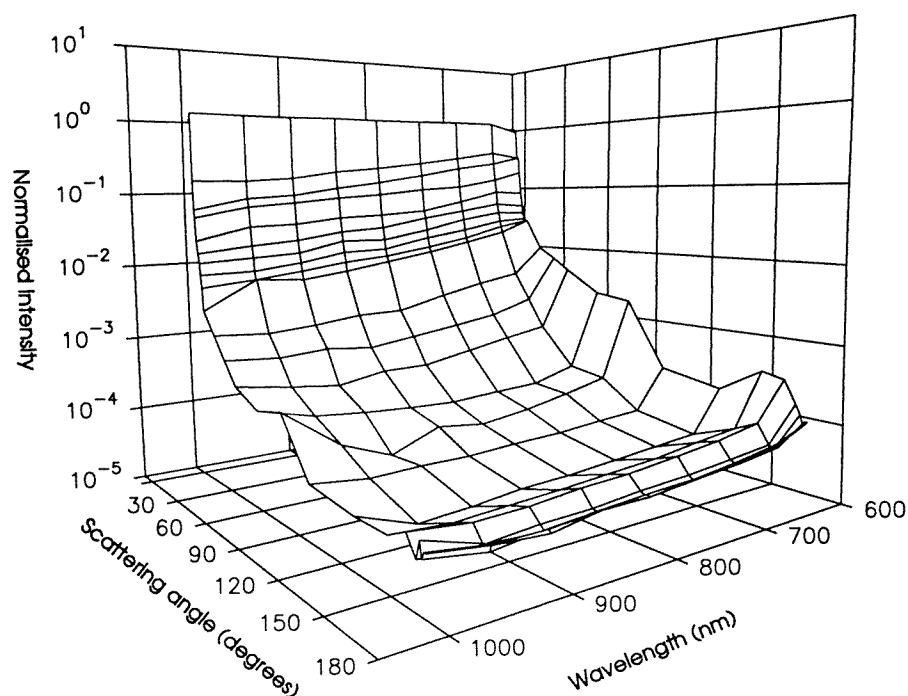


Figure 1. Normalized scattered light intensity against wavelength and scattering angle for fresh spheroids.

thereafter. For the optical measurements the spheroids should be small enough that single-scattering conditions predominate. A spheroid diameter of $100\ \mu\text{m}$ was chosen and it was estimated that for a monolayer of such spheroids the contribution of second-order scatter would be 14%. Although this is higher than ideal, the handling of spheroids of smaller diameter was very difficult due to their fragile nature. The spheroids attained a diameter of $100\ \mu\text{m}$ after about two weeks of growth and appeared histologically homogeneous, with no evidence of a central necrotic region. For those measurements requiring fixed spheroids, they were transferred to a solution of 4% formaldehyde in phosphate buffered saline for a period of 12 h.

For the optical measurements the experimental arrangement, and the method of data analysis, have been described previously (Firbank *et al* 1993 and Firbank 1994). For determination of the phase function, an optical cell was used which consisted of two microscope glass cover slips separated at a distance of about 0.12 mm by double-sided adhesive tape. In the centre of the tape a hole of 5 mm diameter had previously been punched and this formed the cavity in which the spheroid suspension was placed. For the scattering measurements the light beam was horizontal, and to prevent the spheroids from sedimenting to the bottom of cavity it was necessary to immobilize them. This was achieved by introducing into the suspending medium a small proportion (5%) of polyacrylamide gel. The spheroids were first suspended in medium +5% monomer (29 : 1 mixture of acrylamide and N,N'-Methylene-bis-acrylamide). To initiate polymerization 0.1% (of monomer weight) of TEMED (N,N,N',N'-tetramethylethylenediamine) and 0.2% of ammonium persulphate were added to the monomer/medium solution. After placing the mixture in the optical cell

it set to a soft gel in about 10 min. Optical measurements were made within 1 h of placing the sample in the cell.

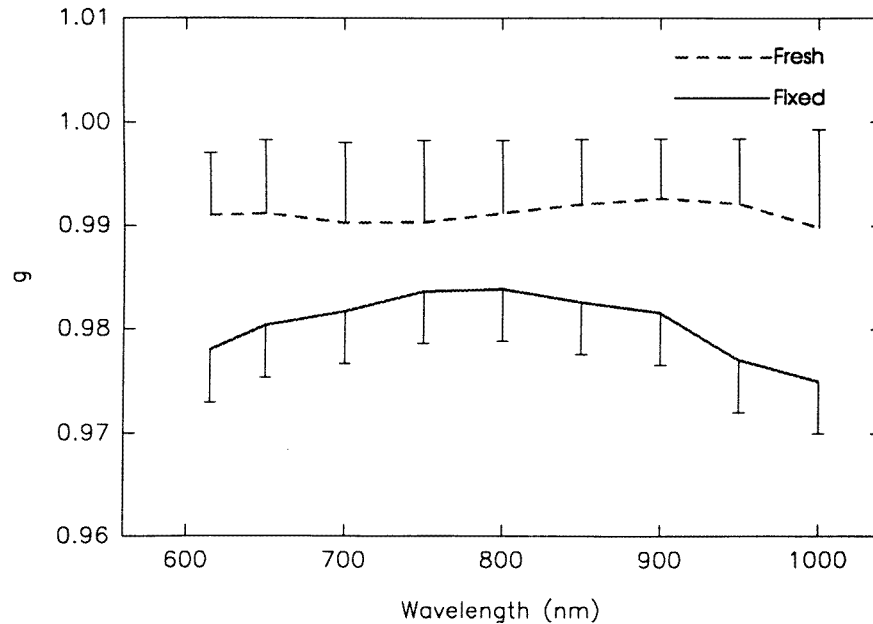


Figure 2. g factor (mean cosine of the scatter phase function) for fresh and fixed spheroids against wavelength (mean \pm SD).

For the measurement of phase function the apparatus consisted of a 100 W quartz halogen lamp coupled by a fibre-optic bundle to a collimator which provided a beam having an angular dispersion of 0.24° half angle and a diameter of 2.5 mm at the sample. The specimen was mounted between two glass hemicylinders, optical coupling being provided by ethylene glycol, and the whole arrangement was mounted on a goniometer turntable. Scattered light was collected by a telescope and a second collimator, having an acceptance half angle of 0.46° . A fibre-optic bundle coupled this to the detection system comprising a spectrograph with a liquid-nitrogen-cooled spectrographic CCD system (Cope *et al* 1989). Scattering measurements could be made for scattering angles up to 172° .

For measurements of the scattering and absorption coefficients an optical cell with a cavity 0.5 mm deep and 5 mm diameter was used. As these measurements were conducted with the cell horizontal it was not necessary to use gel to immobilize the spheroids. The sample was placed between two integrating spheres and collimated light from the 100 W lamp directly illuminated the sample with a beam diameter of 3 mm. Measurements were made of both the reflected and transmitted diffuse light by the spectrograph CCD detector. The scattering and absorption coefficients were then determined by a stepwise search through a table of diffuse reflectance and transmittance values generated by a Monte Carlo model (Firbank *et al* 1993).

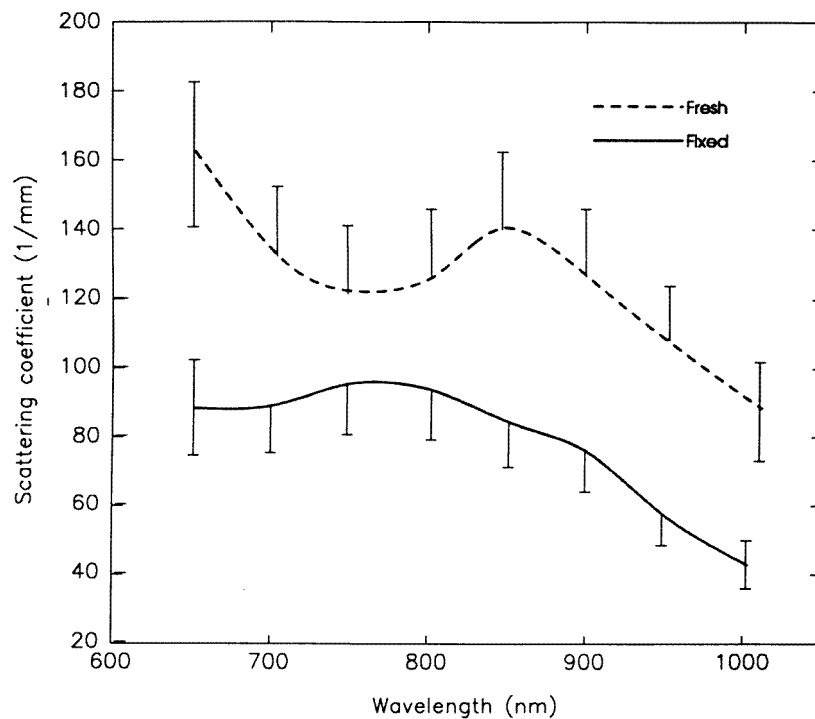


Figure 3. Scattering coefficients for fresh and fixed spheroids against wavelength (mean \pm SD).

3. Results

Optical measurements were made on 12 samples of fresh (i.e. unfixed) spheroids and on 8 samples of fixed spheroids. Figure 1 shows, for fresh spheroids, the scattering phase function (mean and SD over the samples) against wavelength. Figure 2 shows g , the mean cosine of the phase function, (mean and SD over the samples) of fresh and fixed material. Figure 3 and 4 show the average scattering and absorption coefficients for fresh and fixed spheroids.

4. Discussion

Fixed tissue is generally much easier to keep and to handle in many investigations of optical properties, and the measurements on fixed spheroids were primarily made to provide data in a system of simple histological structure. It may be seen that for fixed spheroids the scattering coefficients are considerably lower than in fresh spheroids. The g values are marginally lower and the absorption coefficients are generally higher than in fresh spheroids. The changes due to fixation are the consequence of denaturation of protein, aggregation of protein and other molecules, as well as changes in cell size and intracellular composition, but it is not clear which of these effects are significant contributors to the alteration in optical properties.

For fresh tissue, the scattering is highly forward peaked as evidenced by the high g values, around 0.980 over the entire wavelength range. Peters *et al* (1990) reported

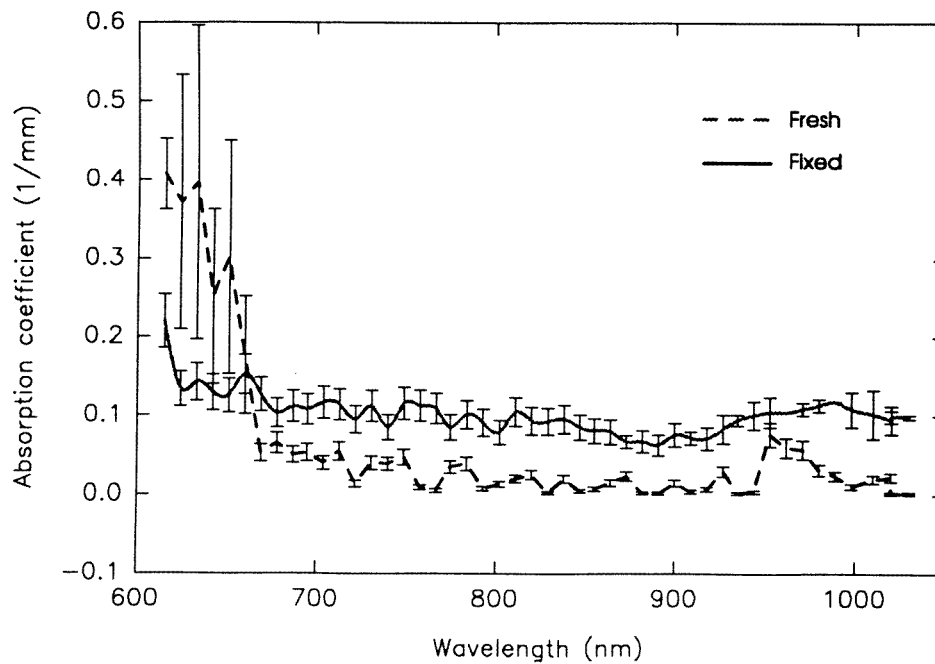


Figure 4. Absorption coefficients for fresh and fixed spheroids against wavelength (mean \pm SD).

measurements on freshly excised normal and diseased human breast tissues and found g values generally rather lower than this. The scattering coefficients in the tissue types investigated by Peters *et al* showed a wide range, 30–90 mm^{-1} at 500 nm and 10–50 mm^{-1} at 1100 nm. The values reported here (see figure 3) for fresh spheroids are all considerably higher.

Barer (1955) found that, in a suspension of single cells, the scattering behaviour was highly dependent on the difference in refractive index between the suspending medium and cytoplasm. The refractive index of an aqueous medium is almost linearly related to its solids content, regardless of the chemical composition (Rose 1967). It may be that the larger scattering coefficients seen in spheroids, compared to excised tissue, reflect such differences at the interfaces of the cell plasma membrane. For spheroids it is tempting to assume that the cellular interstitial medium resembles that of the medium in which they are grown. However, histochemical techniques (Nederman *et al* 1984) have shown there are protein and other molecules present in the interstitial medium, which presumably are necessary for the maintenance of function and structural integrity. It would therefore be expected that the refractive index of the interstitial fluid would differ from that of the culture medium. Because of this it is difficult to interpret the scattering behaviour of spheroids in terms of processes at the cellular and sub-cellular level. In this respect it is of interest to note that techniques are available to disaggregate spheroids (Durand 1990) into viable single cells. The scattering behaviour of these isolated cells suspended in various experimental media could thus be compared with that of the intact spheroid, and hopefully clarify the role of the interstitial medium in determining the light scattering behaviour.

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