Fourier spectrum analysis of full-field optical coherence tomography for tissue imaging

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We propose a model of the full-field optical coherence tomography (FFOCT) technique for tissue imaging, in which the fractal model of the spatial correlation function of the refractive index of tissue is employed to approximate tissue structure. The results may be helpful for correctly interpreting en face tomographic images obtained with FFOCT.

1. Introduction

Quantifying structural features and their changes of live intact cells and tissues in three dimensions at micrometre or submicrometre levels is very helpful for accurate disease diagnosis at the early phase. Thus, several quantitative measures of tissue structures have been proposed which may be divided into two categories: one is geometrical or morphological measures such as layer thickness, the size distribution of the spatially resolved microscopic structures in the optical sectional images at various depth positions; and the other is parameters that describe various statistical characteristics of light scattering of tissues, including the values of the scattering coefficient and the anisotropy factor. The values of these measures may be obtained by light-scattering spectroscopy- or imaging-based techniques. The high-resolution optical sectioning capability of full-field optical coherence tomography (FFOCT) makes it possible to visualize the intact, \textit{in situ} live tissue and locally quantify tissue structures and their changes [1–17]. The advantage of visualizing intact live tissue over the sliced stained tissue observation in traditional microscopy is that more accurate information about tissue structure and the relationship between tissue elements may be provided because of the absence of freezing artefacts.

FFOCT is a development of the full-field low coherence interference microscopy and the imaging
signal is dependent on the correlation that exists between the light backscattered from within tissue and reflected from the reference mirror in the interferometer. Thus, it is a label-free imaging method. In FFOCT, en face images were produced without any transverse scanning. In initial efforts, an infrared light-emitting diode (LED) was used for illuminating a Linnik interference microscope [2–5]. A thermal light source was then used for illumination [6–11]. Because light sources with very short temporal and spatial coherence lengths are used in FFOCT, images at micrometre-scale spatial resolution in three dimensions have been generated for a leucocyte [6], and a fixed human oesophagus epithelium [8]. Here, the spatially incoherent illumination by using extended thermal light sources is to suppress the crosstalk between the neighbouring image points. In the following discussions, all the beam scanning-based OCT techniques will be referred to as scanning OCT. FFOCT has been used for measuring the brain refractive index in vivo [12], identifying cancer cells by measuring the refractive index distribution across a single live cell [13], obtaining sectional images of normal lung tissue and differentiating lung tumours from non-neoplastic lung tissue [14–16], producing en face tomographic images of both normal and cancerous liver [17], non-destructively evaluating film coatings applied to spherical pellet [18].

To obtain the above-mentioned quantitative measures, one way is to relate the imaging signals of FFOCT to some aspects of tissue structures. This is possible because of the following facts. One is that the optically localized imaging property of OCT allows the use of the Born approximation-based scattering formula to relate tissue properties to the scattered light [19]. The other is that at present several theoretical models have been proposed and tested for describing the microscopic properties of cells and tissues at micrometre or submicrometre levels. First, the fact that tissue consists of cells and extracellular matrix suggests that it may be appropriate tissue consists of discrete random scatterers which scatter light. Türke et al. [20] proposed a model consisting only of the nucleus and the cytoplasm both of which are homogeneous and isotropic. With this simple two element model, they showed that the diameters of the nucleus and the cytoplasm as well as half of the sum of both diameters can be obtained by Fourier transforming the measured radial intensity distribution in the diffraction pattern generated by the model structure of the cell. They also demonstrated that a perturbation of the cellular symmetry introduces new distances and consequently, new components in the diffraction intensity pattern. It should be pointed out here that they demonstrated the fact that the spectral frequencies of the diffracted intensity pattern are determined by the intervals that may exist in the cells.

By using a model containing a nucleus, mitochondria and melanin, Dunn & Richards-Kortum [21] showed that the overall cell size and shape determines the forward scattering properties. The small organelles act as isotropic scatterers and have significant contribution to the light at higher angles [21]. They also pointed out that the effects of the spatially varying dielectric properties of cell organelles should be taken into account to accurately calculate the scattered patterns.

Second, based on the fact that tissue consists of elements with a wide range of sizes and a small range of refractive index variations, Schmitt & Kumar [22] proposed a tissue model to relate the optical scattering coefficients of bulk tissue to the microscopic properties of cells and other tissue elements. In their analysis, ‘the tissue is modelled as a collection of isotropic scattering particles whose volume fractions are described by a skewed lognormal distribution modified by a packing factor’. The particles are assumed to have the same refractive index. With this model, they found that tissue is represented best by a volume of scatterers with a wide range of sizes. They also demonstrated that the constituents of tissues with diameters between $\lambda/4$ and $\lambda/2$ are the dominant back scatterers; large particles that attenuate a focused beam strongly are with the diameters between $3\lambda$ and $4\lambda$.

However, the discrete particle model is appropriate for interpreting the images obtained with devices with resolution scale much larger than the size of the scatterers [23]. For imaging methods that can resolve the tissue elements a more comprehensive tissue model is needed. As pointed out by Schmitt & Kumar [22], on a microscopic scale, the constituents of the tissue have no clear boundaries and merge into a continuous structure. Thus, at the microscopic level the spatial distribution of tissue elements and their refractive indices which are the corresponding optical description of microscopic structure of tissue are continuous. Recognizing that the random
characteristic of the refractive index requires statistical description methods, the concept of a spatial correlation function of the refractive index \([2, 24, 25, 27–33]\) or the dielectric permittivity \([34]\) was then used to model the microscopic structure of tissues. At present, several forms of the refractive index correlation function have been proposed to describe the tissue inhomogeneities among which the most general one is the Whittle–Matérn correlation function family which can be expressed in terms of three parameters, the length scale of refractive index correlation distance \(L_0\), the parameter \(m\) that describes the form of the correlation function and the variance of the refractive index fluctuation \(\langle \delta n^2 \rangle\) \([25, 35]\). Thus, in the Whittle–Matérn model, the tissue can be characterized by its values of these three parameters. In fact, the value of parameter \(m\) is related to the fractal dimension which describes the spatial distribution types of the refractive index.

The correlation function is of the basic importance in the sense that the differential scattering cross section can be derived from the refractive index correlation function from which other scattering properties such as the scattering coefficient and the anisotropy factor can be calculated. In fact, the angular distribution of the scattered light is determined by the Fourier transform of the spatial correlation function of the refractive index or dielectric permittivity which randomly fluctuates with position. And the possible range of the Fourier components that can be measured is determined by the range of both the wavelengths of the light sources and the angles of the observation \([24–33]\).

The values of the fractal parameter and the length scale of refractive index correlation distance have been exploited to characterize tissue structure. It was observed experimentally that there exist significant differences between the values of fractal dimension and fractal scale of the normal and dysplastic rat oesophagi \([26]\). The inverse power-law spectral dependence of the light scattered from submicrometre features in tissue samples was derived from the Whittle–Matérn correlation function \([27]\). And the power spectrum of the refractive index variations and the scattering coefficients predicted by the models are found in good agreement with the experimental data \([24, 25]\). The spectral changes induced by the refractive index spatial correlation in tissue have also been theoretically analysed \([9]\) and experimentally measured \([29]\). It was also found that modelling tissue structure in terms of refractive correlation function may provide new methods of diagnosing cancers \([26]\). And the power-law exponent of the backscattering spectrum is sensitive to the length scale at approximately 30–450 nm \([36]\).

Several methods have been proposed to determine the form of the refractive correlation function \([24, 26, 37, 38]\). The refractive correlation function can be directly derived from the microscopic images of slices of the tissue samples obtained with phase-contrast microscopy by fitting the measured spectral data to the von Karman spectral equation \([24]\). The correlation function can also be determined by evaluating the values of the three parameters. The fractal dimension and the cut-off correlation length can be obtained by fitting the measured power spectrum and reduced scattering coefficient with the derived formula from the fractal correlation function \([34]\). More recently, it was shown that the refractive correlation function can be obtained by first determining values of scattering coefficients from the measured OCT spectrum and then the values of parameters \(L_0\) and \(m\) \([37]\).

The purpose of this work is to obtain the possible quantitative measurements of both the structure and intrinsic light-scattering properties of tissues with FFOCT technique, yielding new understanding of pathophysiology of cells and tissues. First, a signal model of FFOCT for imaging random tissue is developed, which relates the FFOCT measured signal to the spatial correlation of refractive index. The possible applications of the high-resolution optical sectioning capability of FFOCT to locally quantify the microstructures of tissues are then discussed.

### 2. Locally quantifying depth-dependent tissue structures with full-field optical coherence tomography

In the random continuous model of tissue structure, the refractive index fluctuates with the location within tissue. Because the changes in refractive index \(n(r, \omega)\) in tissues are small, where
the fractal correlation function of index variations. Knowledge about tissue structure may be obtained by relating the imaging signal in FFOCT to dimensional micrometre-level resolution imaging of FFOCT suggests that some quantitative best when one is concerned with details smaller than the typical size of organelles. The three-fractal behaviour of tissue. Sheppard [23] also pointed out that the fractal tissue model may work

\[ C(r_1 \cdot r_2, \omega) = \langle \delta n(r_1, \omega) \delta n(r_2, \omega) \rangle. \]

Assuming that tissue refractive index fluctuation is a locally homogeneous random function of space position, then the spatial correlation function \[ C(r_1 \cdot r_2, \omega) \]
depends on \[ r_1 \] and \[ r_2 \] only through the difference \[ \rho = r_2 - r_1 \]

It was shown that when the dielectric susceptibility of the scattering medium is completely incoherent the spectrum of the scattered light is the same for all angles of scattering. However, because of the existence of the spatial correlation of the refractive index of the medium, the spectrum of the scattered light in the far zone will be modified by a multiplicative factor that is proportional to the three-dimensional Fourier transform of the spatial correlation function of the random medium [29]. For tissue scattering, experimental data reveal that at the micrometre level [24] and submicrometre level [26, 37, 38], the spectrum of the scattered light is dominated by the fractal behaviour of tissue. Sheppard [23] also pointed out that the fractal tissue model may work best when one is concerned with details smaller than the typical size of organelles. The three-dimensional micrometre-level resolution imaging of FFOCT suggests that some quantitative knowledge about tissue structure may be obtained by relating the imaging signal in FFOCT to the fractal correlation function of index variations.

Consider the fractal model of the spatial correlation function of tissue of the form [25]

\[ C_n(\rho) = \frac{2^{5/2-m} \Gamma(m-3/2)}{\Gamma(m-3/2)} \left( \frac{\rho}{L_o} \right)^{m-3/2} K_{m-3/2} \left( \frac{\rho}{L_o} \right), \quad (2.1) \]

where \[ L_o \] is the length scale of refractive index correlation distance, \[ m \] is the parameter that describes the form of the correlation function and \[ \langle |n|^2 \rangle \] is the variance of the refractive index fluctuation, \[ K_{m-3/2} \] is the \( (m-3/2) \)th-order modified Bessel function of the second kind, \[ \Gamma \] denotes the gamma function, and \[ \rho \] is the spatial distance between any two points within tissue. As mentioned above, the parameter \[ m \] describes fractal behaviour of fluctuations of tissue refractive index and is determined by the fractal dimension \[ D_f \] [25]:

\[ m = \frac{D_f - D_e + 3}{2}, \quad (2.2) \]

where \[ D_e \] is the Euclidean dimension. Equation (2.1) is a general model in the sense that the function form of the refractive index distribution can be a stretched exponential \( (3/2 < m < 2) \), a decaying exponential \( (m = 2) \) and a Gaussian \( (m \to \infty) \). For most biological tissues in three dimension, \[ kL_o \gg 1, 1 < m < 2 \] [25]. For \[ D_e = 3, m = D_f/2 \]. The value of fractal dimension \[ D_f \] is a quantitative measure of the degree of the complexity of the fractal refractive index structure of tissue: the larger the value of \[ D_f \], the more complex the fractal structures.

To locally quantify depth-dependent tissue structures with FFOCT, first, we need to relate the spatial correlation function of the refractive index to the imaging signal. In FFOCT, the image of the reference mirror and the image of a thin slice at a depth within the sample are linearly superposed at the image plane, where a charge-coupled device (CCD) camera is located to detect the intensity of the light. The time-averaged intensity of the total electrical field on each pixel on the CCD camera can be expressed as [19]

\[ I(r, z; \tau) = I_r + I_s(r, z) + I_{inc} + 2I_r I_s \gamma_{rs}(r; \tau), \quad (2.3) \]

where \[ I_r \] and \[ I_s \] are intensities of the reference and sample beams, respectively, at the detector, \[ I_{inc} \] is the incoherent light intensity which consists of the intensities of light reflected and backscattered from different depths in the sample and from unwanted reflections in the
Figure 1. Diagram showing light focused by a lens from the immersion medium with refractive index $n_m$ into the tissue with refractive index $n_s$ through interface. The actual focus of the objective lens is shifted deeper into the sample with respect to the nominal focus. When a light ray with an angle $\alpha$ is incident on the focal volume, it will be scattered because of the fluctuations of the refractive index.

optical system itself. The term $\gamma_{rs}(r, z, \tau) = \text{Re} \left[ \langle U_s(r, z; t + \tau) U_r(r, t) \rangle (I_r I_s)^{1/2} \right]$ is the real part of the complex degree of the correlation between the sample light-field amplitude $U_s(r, z; t + \tau)$ backscattered from within the sample at the depth $z$ and the reference light-field amplitude $U_r(r, t)$ reflected from the reference mirror at a point $r$ and at time $t$, where $\tau$ is the time delay between the reference and sample beams and is dependent on the angle $\alpha$ that each ray makes with the $z$-axis and is given by $\tau = 2\delta z \cos \alpha / c$, $c$ is the speed of light, $r$ denotes any point $(x_i, y_i)$ at the CCD and $t$ is the time. The averaging process over the response time of the CCD results in the time-averaged intensity a function of $\tau$. In fact, the quantity $\gamma_{rs}(r, z, \tau)$ is a measure of the degree of the phase correlation between the sample and the reference light fields at the CCD surface [33]. Thus, any phenomenon that distorts the wavefront of the sample field will result in a decrease of the value of $\gamma_{rs}(r, z, \tau)$.

As in other coherent probe imaging methods, only the intensity fringe envelope of the interference term in the measured intensity by each pixel of the CCD is used to generate images in FFOCT image reconstruction. The background intensity $I_b$ which is the sum of the intensities of the reference and sample beams and the incoherent light intensity, $I_b = I_r + I_s + I_{inc}$, and the interference factor $\cos (.)$ are removed by measuring a series of phase-shift images, and the en face tomographic images are extracted. In FFOCT, only the interference component (the interference fringe envelope) of the measured signals is used to produce the tomographic en face image. The imaging signal is proportional to the amplitude of the interference term [40,41] or a function of the amplitude of the interference term [4,42]. Hence, from equation (2.3), one can see that it is only needed to relate intensity $I_s$ of the sample light and the complex degree of the correlation $\gamma_{rs}(r, z, \tau)$ to the three parameters $m$, $L_o$ and $\langle \delta n^2 \rangle$ that characterize the spatial correlation function of tissue.

Consider a light ray incident on the focal volume with an angle $\alpha < \alpha_o$; here $\alpha_o$ is the maximum ray angle that can be collected by the objective. For in vitro and in vivo tissue imaging, there exists refractive index mismatch between the sample and the immersion medium such as water or air. The refracted ray makes an angle smaller than the incident angle because the refractive index of tissue is larger than that of the water or air (figure 1). Thus, the refraction of light at the tissue surface causes the actual focus of the objective lens shifted deeper into the sample with respect to the nominal focus. Here, our main concern is to examine the relationship between the imaging signal and the tissue structure. For simplicity, we assume that the actual focus is moved onto the same position as the coherence volume which is determined by the reference arm length [12].

The light incident on the focal volume will be scattered. The angle and wavelength dependence of the scattered light is determined by properties of both the incident light and the spatial
Distribution of the refractive index. In FFOCT imaging, the backscattered field is detected at a distance from the coherence volume element much larger than the coherence length of the light source (figure 2). On the basis of the first Born approximation, the scattered field at \( r = r_s \) in the far zone of the coherence volume element can be expressed as \([19,43]\)

\[
U_s(r, x_o, y_o, \omega) = \alpha(\omega) \left( \frac{\omega}{c} \right)^2 e^{ik_s \cdot r + \phi_s} \tilde{\eta}[K, \omega].
\]  

(2.4)

where \( K = k(s - s_o) \) the scattering vector, \( s_o \) is the unit vector along the incident ray (figure 1), \( \alpha(\omega) \) is the amplitude of the incident light, \( \phi_s \) is the phase change from overlying layers and the immersion medium and

\[
\eta(r', \omega) = \frac{n^2(r', \omega) - 1}{4\pi}
\]

(2.5)

is the dielectric susceptibility of the scattering medium, \( r' \) is the location within the coherence volume element \( V \) with respect to the point \((x_o, y_o)\) in the focal plane at the depth \( z \) illuminated by the focused light (figure 2), and

\[
\tilde{\eta}(K, \omega) = \int_{\Omega(r')} \eta(r', \omega) \exp(-jK \cdot r') \, d^3r'
\]

(2.6)

is the Fourier transform of the dielectric susceptibility \([29]\). For most media, the dielectric susceptibility \( \eta(r', \omega) \) can be expressed as \([44]\)

\[
\eta(r', \omega) = \frac{N(r')\alpha(\omega)}{1 - (4\pi/3)N(r')\alpha(\omega)}
\]

(2.7)

where \( N(r') \) is the average number of molecules per unit volume and \( \alpha(\omega) \) is the mean polarizability of each molecule. In biological tissue, \( \eta(r', \omega) \) can be expressed in terms of the refractive index \( n_s \) of the solute, and the protein concentration \( C \) \([43]\):

\[
\eta(r', \omega) = \frac{1}{4\pi} \left[ n_s(r', \omega) + \zeta C(r') \right]^2 - 1
\]

(2.8)

where \( \zeta \) is the specific refractive increment of proteins, \( \zeta \approx 0.0018 \, (100 \, \text{ml g}^{-1}) \). Equation (2.4) shows that the scattered field in the far zone of the coherence volume is proportional to Fourier transform of the dielectric susceptibility of the medium. It also reveals a fact that the angular dependence of the scattered light is related to the range of the spatial frequency components of the dielectric susceptibility of the scattering medium.

In FFOCT, the whole field is illuminated by using a temporally and spatially low coherence light source such as a tungsten halogen lamp with a centre wavelength of 550 nm and a bandwidth...
of 200 nm (for example), resulting in coherence length of about 0.7 µm in the depth direction. Thus, although light scattering in tissue is a three-dimensional event, at any moment only the light backscattered from a slice with a thickness of about 1 µm at a depth z has some degree of coherence with the light reflected from the reference mirror and contributes to the imaging signal. It is then appropriate to regard $U_s(r, x_o, y_o, \omega)$ as the light backscattered from the two-dimensional thin slice. This thin slice at a depth $z$ is imaged by the sample arm optical system to the surface of the CCD, where it is superposed with the image of the reference mirror formed by the reference arm optical system of the interferometer. In the two-dimensional approximation, the effect of the optical system on the scattered field can be represented by the following convolution integral [45]:

$$U_s^{(i)}(x_i, y_i, \omega) = \int \frac{1}{|M|} U_s \left( \frac{x_o}{M'} \frac{y_o}{M} \right) \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx_o \, dy_o$$

$$= \tilde{h}(x_i, y_i) \odot U_s(x_i, y_i),$$

where the integral is over the thin slice of the sample in the focal plane, and

$$U_s^{(i)}(x_i, y_i, \omega) = \frac{1}{|M|} U_s \left( \frac{x_i}{M} \frac{y_i}{M}, \omega \right)$$

is the image predicted by geometrical optics and is a scaled version of the object, $M$ is the magnification, $M = -z_i/z_o$, where $z_o$ and $z_i$ are the object and image distances respectively, $\tilde{x}_o = Mx_o, \tilde{y}_o = My_o$ and [45,46]

$$\tilde{h}(x_i, y_i, \tilde{x}_o, \tilde{y}_o) = \frac{1}{\lambda^2 z_o z_i} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{x^2 + y^2}{2} \exp \left\{ -\frac{\epsilon(x^2 + y^2)}{2} + \frac{(x_i - \tilde{x}_o)x + (y_i - \tilde{y}_o)y}{z_i} \right\} \, dx \, dy$$

$$= \frac{1}{\lambda^2 z_o z_i} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{i \omega(x_i - \tilde{x}_o)x + (y_i - \tilde{y}_o)y} \left\{ 1 \right\} \, dx \, dy$$

$$= \frac{1}{\lambda^2 z_o z_i} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \tilde{\eta}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx \, dy$$

(2.11)

is the point spread function due to the diffraction of the lens aperture, where $\epsilon = 1/z_o + 1/z_i - 1/f$ is the deviation from the lens law.

Consider the spectrum of the light at any point in the image plane. Because the refractive index of tissue is a random function of position, a statistical averaging taken over an ensemble of different realizations of the scattering medium is required. The spectrum can be expressed as

$$I_s(x_i, y_i, \omega) = \langle U_s^{(i)s} U_s^{(i)} \rangle$$

$$= \int_s d\tilde{x}_o d\tilde{y}_o \int_s d\tilde{x}_o d\tilde{y}_o \langle U_s^{(\tilde{x}_o, \tilde{y}_o, \omega)} U_s(\tilde{x}_o, \tilde{y}_o, \omega) \rangle$$

$$\times \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o)$$

$$= \int_s d\tilde{x}_o d\tilde{y}_o \langle U_s^{(\tilde{x}_o, \tilde{y}_o, \omega)} U_s(\tilde{x}_o, \tilde{y}_o, \omega) \rangle \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o)$$

(2.12)

Because a spatially incoherent light source is employed in the illumination of the sample the light scattered from the different coherent volume elements in the thin slice can be regarded as incoherent. Hence, equation (2.12) reduces to

$$I_s(x_i, y_i, \omega) = \langle U_s^{(i)s} U_s^{(i)} \rangle = \kappa \int_s \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx \, dy$$

$$= \kappa \int_s \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx \, dy$$

$$= \kappa \int_s \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx \, dy$$

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$$= \kappa \int_s \tilde{h}(x_i - \tilde{x}_o, y_i - \tilde{y}_o) \, dx \, dy$$

(2.13)

where $\kappa$ is a constant. From equation (2.4), one can see that the light scattered from the coherence volume element at the location $(x_o, y_o)$ is proportional to the Fourier transform of the dielectric susceptibility. Because the dielectric susceptibility $\eta(r', \omega)$ is a random function of position $r'$ and
consequently \( \tilde{n}(K, \omega) \) will be a random function of \( K \). A statistical average over an ensemble of different realizations of the scattering medium is then taken. Thus, we have

\[
\langle U^i_s(\xi_o, \eta_o, \omega) U^s_s(\xi_o, \eta_o, \omega) \rangle = I^{(i)}(\omega) \left( \frac{\omega}{c} \right)^4 \frac{1}{r^2} \langle \delta(\mathbf{K}, \omega) \tilde{n}^*(\mathbf{K}, \omega) \rangle \]

\[
= I^{(i)}(\omega) \left( \frac{\omega}{c} \right)^4 \frac{1}{r^2} \tilde{C}_n(\mathbf{K}, \omega) \]

\[
= I^{(i)}(\omega) \left( \frac{\omega}{c} \right)^4 \frac{1}{r^2} \int_V \int_V C_n(r_1', r_2', \omega) \exp[-j(K \cdot r_2' - K \cdot r_1')] d^3 r_1' d^3 r_2'.
\]  

(2.14)

The dielectric susceptibility of the scattering medium may be expressed in terms of the mean and the varying part of refractive index [30]

\[
\eta(r', \omega) = \frac{1}{4\pi} \left[ n^2(r', \omega) - 1 \right] = \frac{1}{4\pi} \left[ (n(r', \omega))^2 + 2\langle n(r', \omega) \rangle \delta n(r', \omega) - 1 \right].
\]  

(2.15)

In equation (2.15), the second term \( (\delta n(r', \omega))^2 \) has been neglected due to the fact that \( (\delta n(r', \omega)) \) is very small compared with \( \langle n(r) \rangle \) [24]. From equation (2.15), the spatial correlation function of the dielectric susceptibility can be expressed as

\[
C_n(r_1', r_2', \omega) = \left( \frac{1}{10\pi^2} \right) (\langle n \rangle^4 - 2\langle n \rangle^2 + 1 + 4\langle n \rangle^2 (\delta n(r_1', \omega) \delta n(r_2', \omega))).
\]  

(2.16)

where \( r_1' \) and \( r_2' \) denote any two positions within the coherence volume element \( V \), \( I^{(i)}(\omega) \) is the spectrum of the incident light, \( \langle \delta n(r_1', \omega) \delta n(r_2', \omega) \rangle \) is the refractive index correlation function (equation (2.1)). The Fourier transform of the spatial correlation function of the dielectric susceptibility can then be expressed as

\[
\tilde{C}_n(\mathbf{K}, \omega) = \left( \frac{1}{4\pi^2} \right)^{\frac{3}{2}} \langle n \rangle^2 \Phi(\mathbf{K}),
\]  

(2.17)

where \( \Phi(\mathbf{K}) \) is the Fourier spectrum of the refractive index correlation function of tissue in the coherent volume element at the location \((x_o, y_o)\). Note that \( \Phi(\mathbf{K}) \) is dependent on both the direction and magnitude of the scattering vector \( \mathbf{K} \). For the case when only its dependent on the magnitude can be obtained, \( \Phi(\mathbf{K}) \) is of the form [25]

\[
\Phi(\mathbf{K}; x_o, y_o) = \frac{\langle \delta n^2 \rangle L_0^3 \Gamma(m)(1 + K^2 L_0^2)^{m-3}}{\Gamma(m - 3/2)}.
\]  

(2.18)

Note here that the constant terms have been neglected because only the fluctuation part of the refractive index causes light scattering. Assuming that tissue refractive index fluctuation is locally homogeneous random function of space position, then its correlation function \( C_n(r_1', r_2', \omega) \) depend on \( r_1' \) and \( r_2' \) only through the difference \( r_2' - r_1' \), and we have [31]

\[
C_n(r_1', r_2', \omega) = C_n(r_1' - r_2', \omega) \quad \text{when} \quad r_1' \in V \quad \text{and} \quad r_2' \in V = 0, \quad \text{otherwise};
\]  

(2.19)

and hence

\[
\tilde{C}_n[-k(s - s_o), k(s - s_o), \omega] = \int_V \int_V C_n(r_2' - r_1', \omega) \exp[-jk(s - s_o) \cdot (r_2' - r_1')] d^3 r_1' d^3 r_2'.
\]  

(2.20)

If we let

\[
r' = (r_2' - r_1'),
\]  

(2.21)

we have [30]

\[
\tilde{C}_n[-k(s - s_o), k(s - s_o), \omega] = V \int_V C_n(r, \omega) \exp[-jK \cdot (r')] d^3 r' = V \tilde{C}_n(K, \omega).
\]  

(2.22)

Using equations (2.17), (2.19) and (2.22), equation (2.14) can be expressed as

\[
\langle U^i_s(\xi_o, \eta_o, \omega) U^s_s(\xi_o, \eta_o, \omega) \rangle = V I^{(i)}(\omega) \left( \frac{\omega}{c} \right)^4 \frac{1}{r^2} \langle n \rangle^2 \Phi(\mathbf{K}; x_o, y_o).
\]  

(2.23)
Note that $\Phi(K)$ is a function of the frequency $\omega$ of the incident light and the position $(x_o, y_o)$ of the coherence volume element. Equation (2.13) can be rewritten as

$$ I_s(x_i, y_i; \omega) = \langle U_s^{(i)*} U_s^{(i)} \rangle = \kappa V f(\omega) \left( \frac{\omega}{c} \right)^4 \left( \frac{m}{4\pi} \right)^2 \frac{1}{\pi^2} \int \Phi(K; x_o, y_o) |h(x_i - \bar{x}_o, y_i - \bar{y}_o)|^2 \, d\bar{x}_o \, d\bar{y}_o. \quad (2.24) $$

The above equation shows that the spectrum of the light at any point in the image plane is proportional to the Fourier spectrum of the refractive index correlation function of tissue.

The total intensity can be obtained by integrating over the solid angle of the aperture cone and the spectrum weighted by the response function $R(\nu)$ of the detector. We have

$$ I_s(x_i, y_i) = \kappa V \left( \frac{m}{4\pi} \right)^2 \frac{1}{\pi^2} \int \nu d\nu (2\pi R(\nu)f(\nu)(\frac{2\pi \nu}{c})^4 \sin \alpha \, d\alpha \right) \times \int \Phi(K; x_o, y_o) |h(x_i - \bar{x}_o, y_i - \bar{y}_o)|^2 \, d\bar{x}_o \, d\bar{y}_o \right]. \quad (2.25) $$

Now we consider the expression for the interference term in equation (2.3). The expression for the degree of coherence in the image plane in a double beam interference microscope has been derived for the case of a plane object [46]. As shown above (equation (2.4)), in the first Born approximation, the scattered field in the far zone of the coherence volume element is proportional to the Fourier transform of the dielectric susceptibility. For simplicity, we neglect the effect of the overlying layers, which is justified because of the fact that the imaging depth of FFOCT is limited to less than 500 $\mu$m at present [12]. Assuming that the reference mirror is on the focal plane of the objective lens and the thin slice within the object is with a defocus $\delta z$ in the probe arm, the interference signal in equation (2.3) can be expressed as [46]

$$ 2I_r I_s \gamma_{1s}^{(i)}(\delta z) = \text{Re} \left\{ \left( \frac{8\pi n_m}{c} \right) \left( \frac{2\pi \nu}{c} \right)^2 \exp(0.5\pi n_m NA^2 \nu \delta z) R(\nu)f(\nu) \sin c \left( \frac{0.5\pi n_m NA^2 \nu \delta z}{c} \right) \right\} \left( \frac{\delta z}{c} \right) \exp \left( -\frac{4\pi n_m \nu \delta z \cos \alpha}{c} + j\varphi_0 \right) \sin \alpha \right\} \quad (2.26) $$

where $\langle \delta \tilde{\eta}(K) \rangle$ is the statistical average of the Fourier transform of the dielectric susceptibility, $NA = n_m \sin \alpha_o$ is the numerical aperture of the objective lens, $\phi_0$ is a constant phase difference between two arms from other optical components, $n_m$ is the refractive index of the immersion medium between the objective lens and the sample such as the water, $\nu$ is the temporal frequency of each spectral component of the incident light having wavelength $\lambda$, $1/\lambda = n_m c/\nu$, $U(\alpha)$ is the illumination intensity distribution in the pupil plane of the objective, $\alpha$ is the angle that each ray makes with the $z$-axis, the phase change induced by the defocus $\delta z$ in the sample arm is $2k\delta z \cos \alpha$, $f(\nu)$ is the power spectrum of the source, $dk = 2\pi nd\nu/c$, $I_s$ is given by equation (2.24), $I_r = a_r^2 f(\nu)$, $a_r$ is the amplitude reflectance of the reference mirror and is a constant.

As defined in equation (2.3), the value of the quantity $\gamma_{1s}^{(i)}$ is a measure of the degree of the coherence between the light field backscattered from within the sample at the depth $z$ and the light field reflected from the reference mirror. To determine the light field reflected from the sample, Abdulhalim [46] assumed a wavelength $\lambda$ and angle $\alpha$ dependence sample reflectivity in his analysis of the interferogram in double beam interference microscopes. In the derivation of equation (2.26), equation (2.4) for the light field backscattered from the coherence volume element in the thin slice within the tissue has been used in order to take into account the tissue structure effects.

Equation (2.26) shows that the interference signal is a function of the range of the temporal frequency of the light source, the angular aperture of the objective lens and the parameters that characterize tissue structure. Equations (2.24) through (2.26) relate the imaging signal in FFOCT with the parameters that characterize the spatial correlation function of the refractive
3. Discussions and conclusion

Now we consider possible applications of the high-resolution optical sectioning capability of FFOCT in locally quantitative description of the microstructures of tissues. In fact, efforts have been devoted to derive the values of one or a combination of the parameters from imaging signals in OCT that quantitatively characterize tissue structure locally with the purpose of finding methods for more accurate and early diagnosis of the diseases, including the three-dimensional sizes [47,48], refractive index [13], scattering coefficient $\mu_s$ [37], the anisotropic factor $g$ [36] and the fractal parameter $m$ [26].

Several possible applications of high-resolution optical sectional images of thin slices within tissue obtained with FFOCT are expected. First of all, structural features of cells and tissues are strongly correlated with their function, and there exist some morphological modifications and refractive index changes in abnormal tissue which result in differences in light-scattering properties and image contrast. Hence the high-resolution en face images allow us to accurately locate lesions such as the cancer at early stages known as dysplasia and carcinoma in situ by comparing those en face tomographic images with histopathologic findings. This method has the advantage of avoiding the artefacts of preparation in stain tissue biopsies.

Second, there exists a transitional zone between the normal tissue and cancer. Although the field of view is limited by the NA of the objective lenses, by creating a montage of submicrometre resolution en face images at the same depth, the en face images of large areas of tissue can be generated. This montage can be used to identify the regions of normal and malignant tissue and then to accurately determine the surgical margin in surgical removal of the tumour, which is of great importance for successful treatment of the tumour. It can also be used as a means for visualization of the development process of the disease. In fact, this en face imaging ability is one advantage of FFOCT over other OCT techniques. En face images are helpful in the cases where a horizontal cross-sectional image is needed [5] or the transverse distribution of biological structures has to be examined [11]. The en face view may also supply new information which may complement that provided by the depth view [49].

Finally, in addition to the structure image, localized quantitative knowledge of the tissue optical properties is helpful in correctly interpreting the en face images and accurately identifying the existence of the stages of disease. The high-resolution en face tomographic images allow quantifying tissue structure locally. For example, the depth dependence of the fractal parameter and backscattering coefficient can be obtained. It has been shown that the light-scattering properties of tissue can be derived from its correlation parameters. Thus, it is expected that the parameters derived from the en face images that characterize tissue structure can be employed to quantitatively differentiate tissue by estimating the values of the parameters from the en face images. In the following, we will use results derived above to discuss the possible ways of quantifying tissue locally.

Assuming that the illumination intensity distribution in the pupil plane of the objective is uniform, and $U(\alpha) = 1$ within the pupil and both the reference mirror and the thin slice within the sample are in focus. For simplicity, we also neglect the constant phase difference $\phi_0$ and the direction dependence of the Fourier spectrum $\Phi(K)$ of the refractive index correlation function. Usually a thermal light source such as the tungsten halogen lamp is used in FFOCT, and it has an approximate Gaussian spectrum [50]:

$$I^{(0)}(\nu) = \frac{2\sqrt{\ln 2}}{\sqrt{\pi} \Delta \nu} \exp \left[ - \left( 2\sqrt{\ln 2} \frac{\nu - \bar{\nu}}{\Delta \nu} \right)^2 \right], \quad (3.1)$$
where $\Delta \nu$ is the half-power bandwidth, $\bar{v}$ is the centre frequency of the spectrum. It is evident that for any point in the image plane, the light scattered from a corresponding coherence volume element in the thin slice mainly contributes to the imaging signal. Equations (2.24) and (2.26) then simplify to

$$I_s(x_i, y_i, \omega) = \langle U_s^{(i)} U_s^{(i)} \rangle = \Pi V f^{(i)}(v) \left( \frac{2\pi v}{c} \right)^4 \left( \frac{n}{4\pi} \right)^2 r^2 \Phi(K; x_i, y_i),$$

and

$$2I_s\gamma_{rs}^{(i)}(\delta z) = a_\nu \left( \frac{8 \pi n m}{c} \right)^2 \left\{ \int_{v_1}^{v_2} dv(v) \langle f^{(i)}(v) \rangle^3 2^{1/2} \right\} \int_{\alpha_0}^{\alpha_1} d\alpha \langle \tilde{\eta}(K) \rangle (I_s)^{1/2} \sin \alpha,$$

where $K = |K| = |k(s - s_0)| = 2k \cos \alpha = 2\pi \cos \alpha/\lambda = 4\pi n v/c$ and $\Pi = \kappa \int s \int s' |h(x_1 - x_0, y_1 - y_0)|^2 dx_0 dy_0$. To obtain an insight into the results, we consider a limit case. For the FFOCT using longitudinal spatial coherence, both the sample and the reference mirror are illuminated by a quasi-monochromatic light [47,48]. In this case, $I^{(i)}(v) = I^{(i)}(v) \delta(v - v_0)$, equation (3.3) readily simplifies to

$$2I_s\gamma_{rs}^{(i)}(\delta z) = \Theta \left( \frac{v_0}{c} \right)^4 \langle n \rangle \langle f^{(i)}(v_0) \rangle^2 \int_{\alpha_0}^{\alpha_1} d\alpha \langle \tilde{\eta}(K) \rangle (\Phi(K))^{1/2} \sin \alpha,$$

where $\Theta = a_\nu \Pi n_m (2\pi)^4 (1/cr)$ is a constant. Equation (3.4) shows that at any point in the image plane, the spectrum of the light from a coherence volume is proportional to the spectrum of the incident electric field and $v_0^4$, as expected [27,29]. At the same time, compared with the spectrum of scattered light observed directly which is proportional to the Fourier spectrum of the refractive index correlation function [27,29], the spectrum of the coherence imaging signal is modified by both the Fourier spectrum of the dielectric susceptibility and the square root of the Fourier spectrum of the refractive index correlation function of tissue. Thus from equations (2.5) through (2.8) and (2.21), one can see that equation (3.4) allows us to evaluate the relative contribution of the dielectric susceptibility and the refractive index correlation function to the image contrast.

The en face topographic images of fresh normal human liver tissue are shown in figure 3a,b [51]. It can be seen from figure 3a,b that while the shapes and boundaries of the hepatocytes could be clearly identified the size of the hepatocytes decrease with the depth. The images were generated by a FFOCT system recently developed in our laboratory. The details of the set-up were reported in [17]. Briefly, the system is based on a Linnik interference microscope illuminated by a customized Köhler illuminator in which the light source is a 20 W tungsten halogen lamp with a centre wavelength of 550 nm and a bandwidth of 200 nm. The identical microscope objectives (20x, 0.5 NA, Olympus) are used in both the reference and the sample arms. The interferometric images are projected onto the surface of a CCD camera array (Matrox Iris GT300, pixels, 640 x 480; pixel size, 7.4 x 7.4 μm, working at a maximum rate of 110 frames s⁻¹) using a lens of focal length 260 mm. The reference surface is a polished surface of a YAG (Y3Al5O12) crystal rod with a reflectivity of 8%. The rod is attached to a piezoelectric stage actuator (PZT) (Model AE0505D16F from Thorlabs). The measured resolution of the system in the lateral and depth directions are 0.89 and 1.3 μm, respectively, larger than the corresponding theoretical predictions of 0.5 and 0.7 μm. We attribute the difference between theory and experiment to the large optical aberrations.

Figure 3c,d displays the radially averaged power spectrum of thin slices of non-neoplastic tissue at the surface and at the depth of 25 μm, which were obtained by performing radial Fourier transformation of the corresponding en face images in figure 3a,b. The spatial spectrum is approximately linear when plotted on a log–log scale. The slopes of the line are 2.6800 and 2.7223 for the slices at the surface and at the depth of 25 μm, respectively.

Several interesting characteristics of the spectrum can be found. First, the high-frequency components dominate in all the spectrum curves, demonstrating the fact that the small structures and the clear boundaries of the cells and contribute to the backscattered light. Second, both the shapes and the slopes of the spectrum curves are different from the spectrum determined only by the spatial correlation function of the tissue refractive index [26,27], revealing a fact that the spatial frequency analysis of the en face image obtained with FFOCT may provide new insight about the liver. Third, approximately linear behaviour on a log–log scale demonstrates that the
value of the fractal dimension can be used as a measure of the tissue state. Finally, the slopes of the spectrum curves change with the depth, indicating different fractal structures of tissue at different depths.

It has been demonstrated that local measurements of the spectrum of the backscattered light can be achieved through short-time Fourier analysis of a stack of en face interferometric images acquired with the FFOCT [52,53]. Our analysis indicates that the measured spectrum is related to Fourier spectrum of the dielectric susceptibility which is the first-order statistical characteristic and the square root of the Fourier spectrum of the refractive index correlation function which is the second-order statistical properties. In fact, it has found experimentally that either the refractive index distribution of histopathology slices [54] or its spatial correlation function [26] can be an indicator of tumours. In terms of the varying part \( \delta n(r, \omega) \) of refractive index, the Fourier spectrum of the dielectric susceptibility can be expressed as

\[
\tilde{\eta}(K, \omega) = \frac{\langle n \rangle}{2\pi} \int_{V(r)} \delta n(r', \omega) \exp(-jK \cdot r') \, d^3 r'.
\]

Thus, both the spatial frequency components of the varying part of the refractive index and its spatial correlation function contribute to the image contrast in FFOCT. It is evident that to demonstrate the relative contribution of the two factors, we need to know the function forms of both \( \delta n(r, \omega) \) and \( \langle \delta n(r, \omega_1) \delta n(r_2, \omega) \rangle \). However, there is no reported function form of \( \delta n(r, \omega) \) for any tissue sample or live tissue. One possible way to determine \( \delta n(r, \omega) \) is to use equation (3.4)
when $\Phi(K)$ is known, which may be derived by using inverse spectroscopic optical coherence tomography [36].

In conclusion, the imaging signal in FFOCT has been related to the multiplicative factors, namely factors proportional to the Fourier spectrum of both the varying part of the refractive index and the square root of the Fourier spectrum of the refractive index correlation function of tissue. The results may be helpful for correctly interpreting en face tomographic images obtained with FFOCT.

Ethics. The study was approved by the Ethics Committee of Wuxi No. 3 People’s Hospital.

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References


