Optik 154 (2018) 251–257

Contents lists available at ScienceDirect

Optik

journal homepage: www.elsevier.de/ijleo

Original research article

Morphological characterization of particles by the intensity and polarization of the scattered radiation

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ARTICLE INFO

Article history:
Received 13 July 2017
Accepted 10 October 2017

Keywords:
Biosensors
Light scattering
Mie theory
Light-biological tissues interaction
Complex index of biological tissues

ABSTRACT

Optical techniques are being used more and more, because they have the advantage of being non-destructive, the light scattering by the material provides a framework of prospecting pointed and fast. The elastic and inelastic interaction portion of the light with the matter allows following the assessment of particulate matter including cell nuclei which are the focus of tissue pathologies. Our work focused on the use of this phenomenon to follow the evaluation in size and shape of the nuclei, in order to prevent tumor activity.

1. Introduction

Light scattered by the biological tissue is rather related to its structure, including to the density [1], size [2] and morphology of cells nuclei [3], etc, these important parameters are indications for the pathologist to make differentiate between normal cells (which often have a structured organization), and tumor cells (which present a disorderly structure).

In the present work, the light scattering is used as a tool to characterize this kind of tissue. The advantage of this technique is that it is made without contact with the object studied, non-destructive and non-ionizing. This means that the use of electromagnetic wave information is now the subject of increasing interest in the biomedical field, and the physics of materials [4,5]. The precision on the diagnosis is related to the precision on the measurements of the scattered radiation, the parameters of which extracts may be exploited to study the evolution of the micro particles size and morphology. Various measurements of the scattering intensities, and in particular of its angular, spectral, or polarization dependence, can serve as a diagnostic means.

Our objective is to make help pathological anatomy services. This technique offers valuable assistance by its non-destructive effect and its speed and precision, by varying same optical parameters such as wavelength, polarization state and scattering angle, the information on the evolution of particles sizes and morphology makes it possible to predict directly the existence of pathology.

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https://doi.org/10.1016/j.ijleo.2017.10.056
0030-4026/

2. Biological cells and computation of their light scattering

The morphology of the nucleus is important in the diagnosis of pre-cancerous conditions [6]. In many pre-cancerous epithelial tissues, the nuclei become both enlarged and crowded, and when stained for pathology they take up larger quantities of certain dyes.

We compute a static light scattering from particles comparable with the incident wavelength using the Mie solution, which is an analytical resolution of the problem of the interaction between the electromagnetic wave and the spherical particle [7,8]. From this theory we can deduce the expressions of the extinction and scattering cross sections as well as that of the scattering phase function.

In this work, an appropriate optical assembly of an optoelectronic high angular resolution measurement system is presented to provide non-invasive elastic scattering measurements on slides containing histological sections. Here, via computer calculations and rapid data collection, it is demonstrated that the measurements have the potential to differentiate between cell nuclei sizes as well as its shapes and morphology.

3. The mechanics of light scattering

The amplitude of scattered light at different angles depends not only on complex refraction index of the medium in which the particle exists and the particle size [9], but also on the particle morphology [10,11].

Using the above method for calculation the scattering phase functions, we can examine the effects of particle size, shape, refractive index and particle morphology. We first examine the effects of particle size on the scattering properties. We use the Mie theory to plot the angular scattering distributions for a series of radius from 0.1 μm to 14.0 μm which have been illuminated with several wavelengths of polarized visible light.

Taking into account the polarization we used the complex formulas of scattering [12,13]. They involve two complex functions of scattered amplitude: $S_1(\theta)$ and $S_2(\theta)$.

The electric field is decomposed into two polarizations:

E_r Polarized perpendicular electric field to the scattering plane.

E_t Polarized parallel electric field to the scattering plane.

The expression of the diffusion is:

$$E_r = S_1(\theta)\frac{e^{-ikr+ikz}}{ikr}E_{r0} \tag{01}$$

$$E_t = S_2(\theta)\frac{e^{-ikr+ikz}}{ikr}E_{t0} \tag{02}$$

E_{r0} and E_{t0} are Incident fields.

For an unpolarized incident wave, the intensity is then:

$$I = I_0\frac{1}{2r^2k^2}(i_1 + i_2) \tag{03}$$

If the wave is linearly polarized along Ox:

$$I = I_0\frac{1}{r^2k^2}(i_1\sin^2(\varphi) + i_2 cos^2(\varphi)) \tag{04}$$

When: $i_1 = |S_1(\theta)|^2$ and $i_2 = |S_2(\theta)|^2$

The amplitude functions S_1 (perpendicular to the scattering plane) and S_2 (parallel to the scattering plane) have the following form:

$$S_1(\theta) = \sum_1^\infty \frac{2n+1}{n(n+1)}[a_n\pi_n(\cos(\theta) + b_n\tau_n(\cos(\theta))] \tag{05}$$

$$S_2(\theta) = \sum_1^\infty \frac{2n(n+1)}{n(n+1)}[b_n\pi_n(\cos(\theta) + a_n\pi_n(\cos(\theta))] \tag{06}$$

The angular factors π_n and τ_n have the following forms:

$$\pi_n(\cos(\theta)) = \frac{1}{\sin(\theta)}P_n^1(\cos(\theta)) \tag{07}$$

$$\tau_n = \frac{d}{d\theta}P_n^1(\cos(\theta)) \tag{08}$$

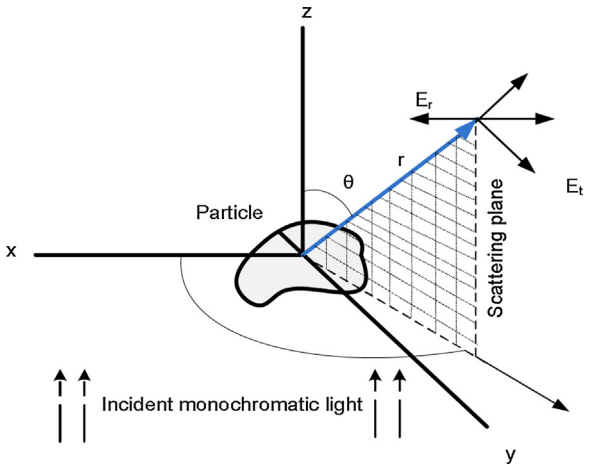


Fig. 1. Geometry used to describe the incident and scattered fields. We let the z axis be the direction of propagation of the incident light, and define the scattering plane as that containing the z axis and radius vector. We write the scattered fields in terms of a different basis than that used to describe the incident fields. Each of these basis sets has a unit vector that is parallel to and a unit vector that is perpendicular to the scattering plane [6].

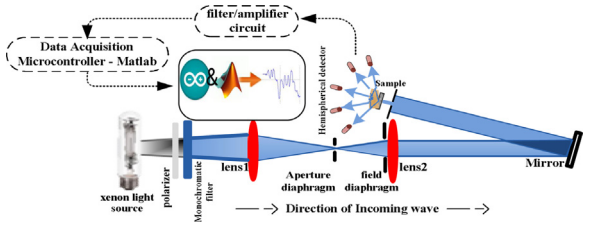


Fig. 2. Scheme of the used set-up.

4. Experimental achievement

To measure the scattered intensity as a function of the angle of observation, we used the experimental setup shown in the figure below:

The outgoing beam from a xenon light source is collimated by using two lenses (lens1 and lens2) for getting a Köhler illumination. For enhancing the beam, a field diaphragm and a spatial filter are added. The obtained beam illuminates the sample surface and a hemispherical detector is finally used to detect the scattered intensities according to the angle of observation. In the case of spectral and polarized light applications, a polarizer and a chromatic filter are inserted before the sample position (Figs. 1 and 2).

The polarized signals captured are integrated to obtain the scattered intensity which relates the sample response using an appropriate program under Matlab- C language software that we have developed.

5. Cartesian representation of intensities

The observation of the different curves of the scattered intensities as a function of the angle of observation and the wavelength gives the same appearance for the different cases. Nevertheless, we observe different specular intensities as a function of λ, this is explained by the influence of the absorption parameter by the tissue. In fact, we notice a less important loss for long wavelengths. From there, we will use the longest wavelength to minimize the effect of absorption (Tables 1–4).

The results show fluctuations as a function of the polarization state of the scattered wave; this may help us to evaluate an average morphology of the scattering particles by calculating the diameters as a function of the polarization angle.

For the calculations, we have developed programs to plot the curves and to determine the areas (the global intensities) of the different measurements, and then on the basis of the mathematical model of Mie, we have improved our algorithm in order to extract the morphologies from the calculated particles sizes.

The following table shows some results obtained:

By observing the results represented in the table above, it is noted that the areas under the curves of the scattered intensities in the case of the tumor tissue are larger compared to the normal tissue. The polarization shows a significant difference in the level of intensity sensed according to the different states, this can be exploited to determine the spatial geometry of the diffuser (morphology). We have confirmed experimentally that the intensity of the scattered light is very

Table 1
Curves of the intensities of scattered light in normal incidence by a histological section of a Colon at the wavelength (470 nm).

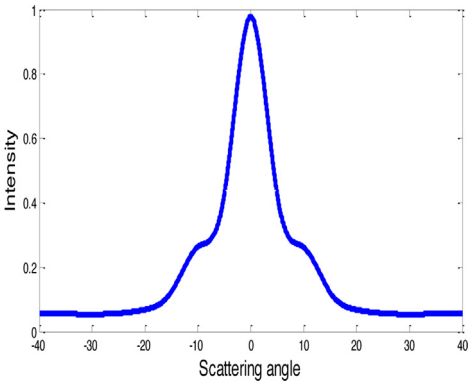
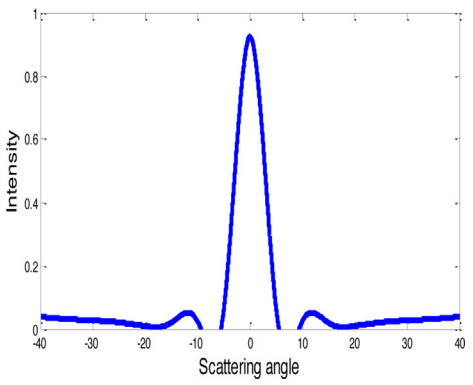
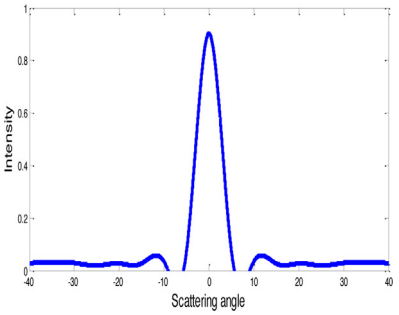
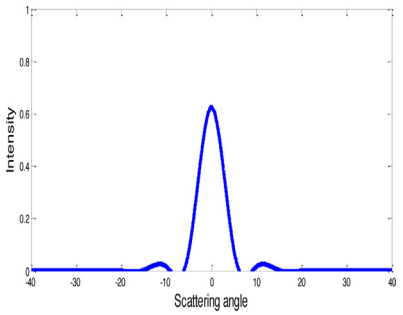
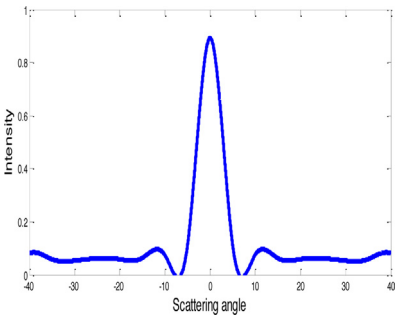
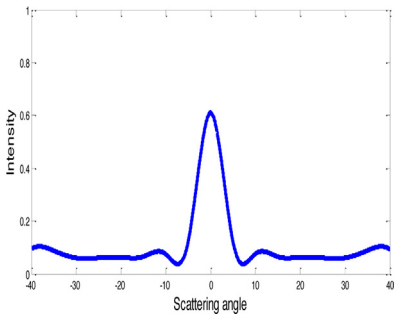
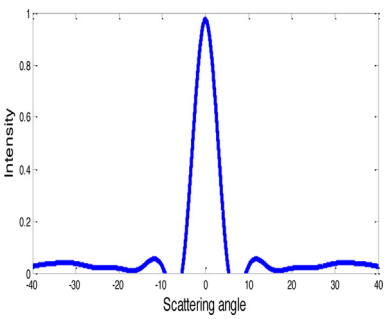
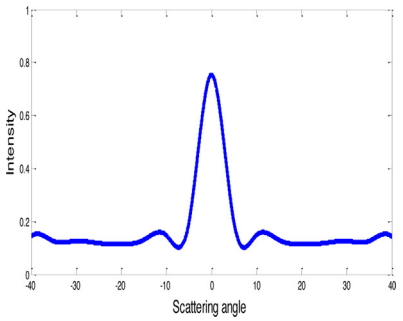
Polarization angle (β)	Normal Colon	Tumoral Colon
Perpendicular polarization 0°		
30°		
60°		
Parallel polarization 90°		

Table 1 (*Continued*)

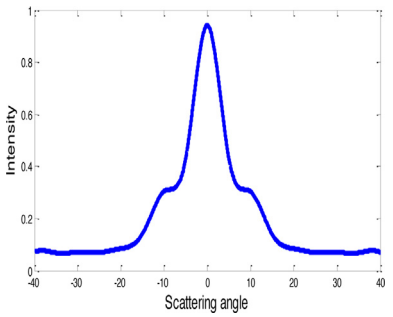
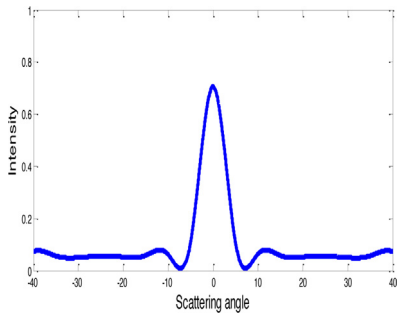
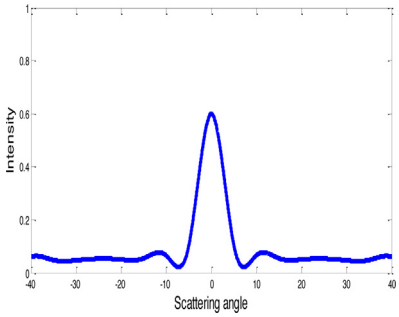
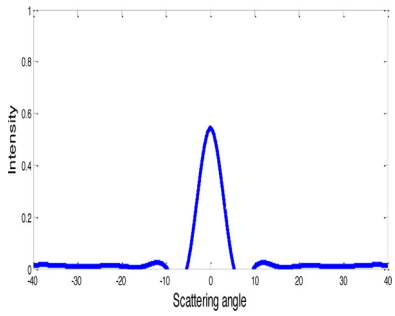
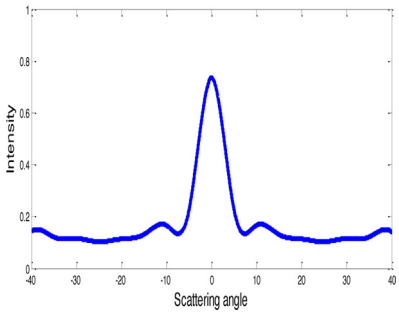
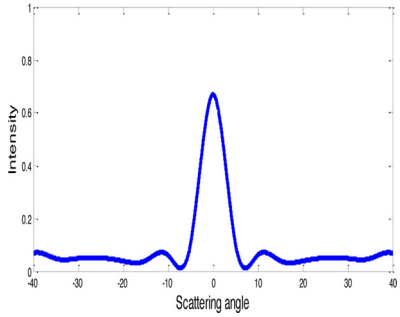
Polarization angle (β)	Normal Colon	Tumoral Colon
120°		
150°		
180°		

Table 2
Representation of the areas of the curves for the Colon as a function of the Wavelength and the polarization state: (A) Normal Colon, (B) Tumor Colon.

β (°)	λ = 470 nm		λ = 510 nm		λ = 630 nm	
	Areas					
	A	B	A	B	A	B
0°	0.6845	2.3383	1.0342	1.1509	0.9671	1.7345
30°	0.4135	1.7938	0.6184	1.2360	0.9556	1.7598
60°	0.3149	2.1616	0.5836	1.2406	0.9641	2.1732
90°	0.2578	2.1927	0.6312	1.2556	0.9140	1.7686
120°	0.3832	2.0513	0.8275	1.1094	0.9431	1.3235
150°	0.4185	0.9144	0.3088	1.1353	1.0072	1.9776
180°	0.5383	0.8161	0.2339	1.4989	0.9498	1.6517

strongly dependent on several experimental parameters such as the wavelength, the polarization state and the particle size of the scattering medium.

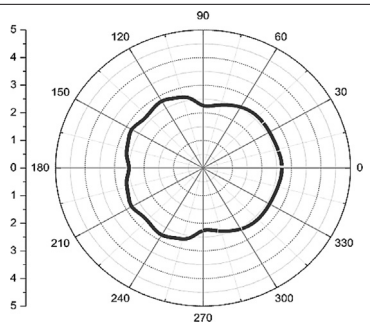
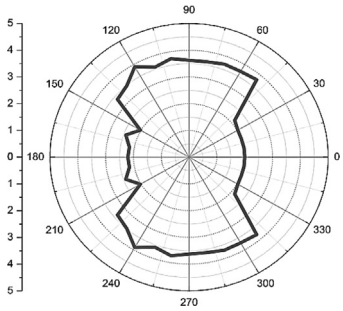
6. Measurement of nucleus sizes using light scattering

The efficiency of light scattering pushed us to develop a calculation program to determine the particle sizes from the collected polarized intensities. This program based on Mie's theory allowed us to obtain the sizes gathered in the table. The results obtained were compared with the microscopic values which seem to be in good agreement.

Table 3
Representation of Colon cell nucleus sizes as a function of angle of polarization.

The polarization angle (β)	Normal Colon Cores Sizes (μm)	Tumor Colon Cores Sizes (μm)
0°	2.00	3.88
10°	2.00	3.70
20°	2.00	3.66
30°	2.02	3.76
40°	2.14	3.78
50°	3.78	3.78
60°	3.70	3.84
70°	3.70	4.10
80°	3.62	3.84
90°	3.62	3.86
100°	3.74	3.82
110°	3.58	3.84
120°	3.90	3.80
130°	3.48	3.84
140°	2.36	3.80
150°	2.02	3.76
160°	2.42	3.76
170°	2.18	3.72
180°	2.20	3.88

Table 4
Polar representation of the morphology of the studied nuclei.

	Core of a normal cell	Core of a tumor cell
Colon		

By simplifying these measurements, we can determine the size of the supposed spherical particle:
The average core size of a normal cell (Colon) is: 3.02 μm
The average size of the tumor cell (colon) is: 3.81 μm
The different diameter values can be collected in a polar coordinate system to evaluate the particle morphology.

7. Study of morphology

A morphological study based on the correlation of the sizes obtained by our program was carried out, the results made it possible to evaluate the general morphology of the nuclei by plotting the spatial distribution of sizes.

The study of the morphology of cell nuclei shows the difference between the normal and tumoral nucleus, this difference is very clear by observing the table above. For the Colon, we can say that the morphology has been modified in value and form, in favor of a biological evolution, the evolution of the size and morphology of nuclei (diffusing particles) Can lead to the emergence of a pathology of tissue.

8. Conclusion

The spatial distribution of the scattered light intensity depends on cell's morphology and the polarization states of incident light; we can extract cellular morphological information from the scattered light in specific angular ranges or the overall pattern to discriminate different cell types.

The angular and spectral variations allowed us to evaluate the sizes of the nuclei between the normal and pathological organ. The polarization state parameter made it possible to evaluate the morphology of the two cases. The results obtained have been confronted with measurements by microscopy of these same particles, and the comparison was given a satisfaction with the optical tool set up.

Acknowledgments

"This work was supported by the Ministry of Higher Education and Scientific Research (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, MESRS). We would like to thank the team of the pathological anatomy service – the university hospital of Setif, for their help. "

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