Light scattering from lung cancer cells and its Polystyrene microsphere models

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Abstract: In the field of biological medicine and clinical medicine, the diagnosis and treatment of many diseases almost rely on the identification of cell morphology. Different cells have different shape which would lead to the change of the light propagation characteristics among biological tissues. What′s more, it affects the light scattering properties of cells. At present, the theory of dynamic light scattering is the optimal way to dynamically identify the distribution of the size and the shape which related to the cell. Cells are mainly composed of cytoplasm, nucleus and mitochondria. Therefore, analyzing their optical properties have great significance for optical diagnostic and treatment. Experiments were designed to obtain the light scattering properties of lung cancer cells and Polystyrene microspheres which contained cytoplasm, nucleus and mitochondria. The models of cytoplasm were built with finite different time domain(FDTD) algorithm to simulate the light scattering properties. The light scattering properties of lung cancer cells demonstrate that mitochondria make a contribution to forward scattering (0°−20°) and backward scattering (160°−180°), nucleus make a big difference to side scattering (80°−100°), cytoplasm have an effect on any angle. The result of the simulation testified that the experimental results are correct.

Key words: light scattering; numerical simulation; Polystyrene microsphere; lung cancer cell

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肺癌细胞及其聚苯乙烯微球模型的光散射研究

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摘 要: 在生物医学和临床医学领域,许多疾病的诊断和治疗依赖于细胞形态的识别。不同细胞具有不同的形态,这些形态的不同将导致生物组织中光传播特征的变化,更重要的是这将影响细胞的光散射特性。目前,动态光散射理论是动态识别细胞尺寸和形状的最佳方式。细胞主要由细胞质、细胞核和线粒体组成,因此,分析它们的光散射特性对于光学诊断和治疗具有非常重要的意义。设计实验获取了癌细胞和聚苯乙烯球的光散射特性,并利用时域有限差分法建立细胞质模型进行细胞光散射特

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性仿真。从肺癌细胞的光散射结果可以看出,线粒体对前向散射(0°~20°)和后向散射(160°~180°)贡献最大,细胞核对侧向散射(80° ~100°)贡献最大,细胞质对各个角度贡献均等。仿真结果和实验结果基本一致。

关键词:光散射; 数值仿真; 聚苯乙烯微球; 肺癌细胞

0 Introduction

The research of cell light scattering can drive the development of cancer diagnosis technology and nondestructive diagnosis. There are many methods used for optical nondestructive diagnosis. In terms of the biological cell optical inspection technology, the elastic light scattering spectroscopy technology has been developed at the earliest. The theory is not complex and the experimental facility can be operated easily. However, the precision is lower than other technologies. For revealing the cell light scattering properties accurately and veritably, researchers proposed different theoretical models. M. Kerker has proposed coated spheroidal model[1]. The light scattering properties are related with wavelength, scattering angles and amplitude tightly. Mie theory was used to simulate light scattering, but we should regard the cells as the uniform spherome. Because of the boundness mentioned above, Rayleigh-Debye-Gans theory has been proposed. Then the homocentric sphere and double ellipsoid model have been invented based on the Rayleigh-Debye-Gans theory. Different from Mie theory, FDTD theory means using grid discretization to build up the cell models, it can be used for any shapes and structures. Rebekha Drezek discovered the relation between the scattering intensity of different scattering angular and wavelength based on the FDTD theory [2]. Dizem Arifler has analyzed the scattering properties of cervical cancer cells which are located in different depth of epithelium[3].Also based on the FDTD algorithm, Jiang Ping has built up the cell population model of the red blood cells and compared the model with DDA algorithm[4]. Wang Meng has calculated the radar cross-section of different cell models and explored the broadband frequency properties[5]. Guo-Shan Chao has built up the models of cell which contains inhomogeneous cell nucleus and compared the backscattering characteristics with the cell model which contains homogeneous nucleus[6].

In this study, the lung cell models were built up for simulation based on the FDTD theory for revealing the cell light scattering properties accurately and veritably. Furthermore, the two kinds of lung cancer cells (NCI-H1975 and HCC827) and Polystyrene microspheres (0.05 μm, 0.1 μm, 3 μm, 4 μm, 5 μm and 6 μm) were employed for achieving the scattering light intensity. Based on different size of Polystyrene microspheres, we can investigate the light scattering behavior of various biological particles (nucleus, mitochondria and dictyosome) in the cell. Comparing the simulation results with cancer cell experiment and Polystyrene microspheres experiment results, we get the agreement with physical model of roughly the same conclusion.

1 Materials and methods

1.1 Methods

FDTD algorithm was proposed by Yee in 1966, it has been widely used in electromagnetic simulation. By discretizing Maxwell′s curl equations in space and time that would bring about a set of explicit finite-difference equations. Maxwell′s equation is a battery of basic equations that dominate electro-magnetic phenomena. The

curl equations of Maxwell in any medium are as follows:

$$\nabla\times H=\frac{\partial D}{\partial t}+J_e \tag{1}$$

$$\nabla\times E=\frac{\partial B}{\partial t}-J_m \tag{2}$$

From FDTD equations it can be got that Maxwell′s curl equations are numerically solved by discretizing them in space and time. The spatial distribution of electric field is surrounded by four magnetic field, as the same with magnetic field [7]. The spatial distribution of the electromagnetic field is not only in accordance with Faraday′s law of induction but also meet the condition of ampere quantitative. The most important is that it can present the propagation characteristics of electromagnetic field. Electric field and magnetic field sampling interval is half of the time step to each both.

In consideration of the limit of computer′s internal storage, the electromagnetic field can be acquired only in a limited area. But if we want to calculate the electromagnetic intensity of the points located in the far field region that should take use of near to far transformation. Furthermore, absorbing condition should be set to reduce reflected light impact on scattering field. The relationship of light scattering intensity with the scattering body diameter is:

$$I(\theta)=\frac{d^2 I_0}{8f^2}\left\{\frac{\pi^2 d^2}{2\lambda^2}\left[\frac{2J(X)^2}{X}\right]+k(m_r,\theta)\right\} \tag{3}$$

1.2 Apparatus and materials

1.2.1 Apparatus

The BI−200SM wide−angle light scattering device (Brookhaven, America) and temperature control apparatus (Brookhaven, America) were adopted. They provide access to all of these studies with an automatic, modular, and versatile system. It is a precision instrument designed for exacting scattering measurements. To validate our experimental setup, experiments were carried out at a detection angle range (15°−155°) with angle interval 5° at the temperature 310 K. Each sample was measured three times. In the utensil, NCI−H1975 cells, HCC827 cells and various Polystyrene (PS) microspheres were irradiated by the laser beam (532 nm, 100 mW). The light scattering information of these particles within 310 K was processed by a BI−9000AT digital autocorrelator.

1.2.2 Materials

Two kinds of lung cancer cell were used as cell models, one is lung adenocarcinoma cell line NCI−H1975, another is nonsmall cell lung cancer cell line HCC827, and both of them are purchased from Western Biotechnology Corporation. A stock solution was prepared by dissolving phosphate buffer saline.

Polysyrene (PS) microsphere is a kind of polymer that is made up of polystyrene monomers and free radical with the way of addition polymerization. It is purchased from Tianjin Junyijia Corporation. A stock solution was prepared by dissolving deionized water. In this experiment, different diameters Polystyrene microspheres (0.05 μm, 0.1 μm, 3 μm, 4 μm, 5 μm and 6 μm) were used for simulating the cell nucleus, mitochondria, dictyosome, and so on. As we know, the diameter of mitochondria are about 0.2 microns, the nucleus diameter roughly 3 m, and the diameter cytoplasm nearly 6 m. Thus the Polystyrene microspheres with these different diameter that mentioned above will replace different cell structure to do the light scattering experiments. A stock solution was prepared by dissolving phosphate buffer saline.

2 Results and discussion

2.1 Diameter measurement of lung carcinoma cells

The experiment is for measuring the piratical size of HCC827 cells and NIC−H1975 cells. There are four inversion algorithms used by the BI−200SM, and it can be found that the result calculated by CONTIN algorithm is close to the

reality. Because the CONTIN algorithm is appropriate for the sample which is single distribution and poly –disperse of scatter and the Polystyrene spheres satisfy the condition. Figure 1(a) indicated that the distribution of the piratical size of the HCC827 cells and the results was obtained through the CONTIN inversion algorithm. It can be found from these diagrams that the distribution of size of the HCC827 cells is concentrated relatively. The average measured size is about 5 m±1 μm.

Figure 1 (b) showed that the average piratical size of NCI –H1975 cells calculated by the CONTIN inversion algorithm ranged from 10 000 nm to 43.45 nm. Obviously, the measured size is smaller than the reality. After five times measurement using CONTIN inversion algorithm, it can be found that the cell size is about 6 μm±1 μm.

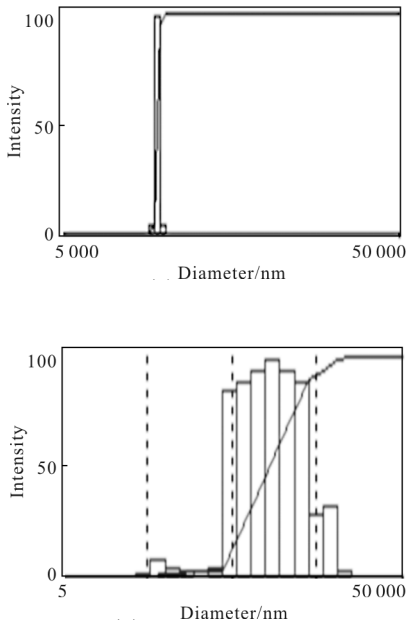
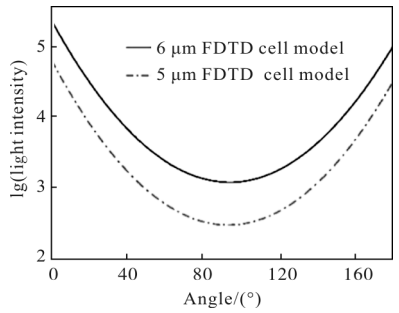


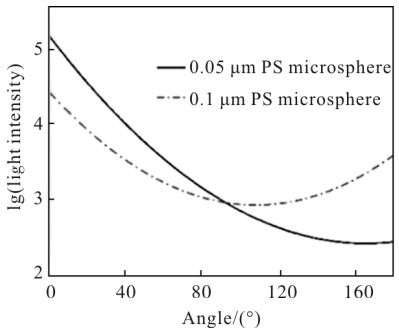
Fig.1 Size distribution of lung cancer cells

2.2 Light scattering properties of FDTD cell model and different Polystyrene microspheres

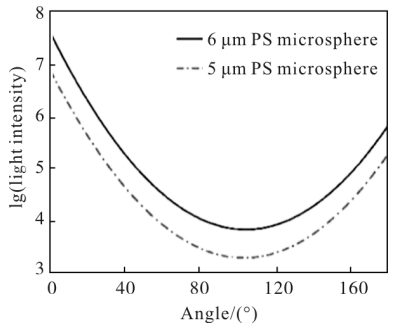
The Eq.(3) demonstrates that if the diameter of the model is lager, then light scattering intensity gets stronger[8]. The light scattering intensity increased as the diameter of model increased shown in Fig.2(a), so we can infer that the numerical simulation is accurate. Polystyrene microspheres whose diameter are 5 μm and 6 μm are used for behalf of entire cell according to Fig.2 (b), diameter of HCC827



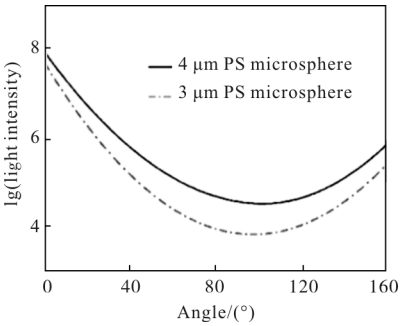
(a) FDTD cell model



(b) Light scattering intensity of 5 μm and 6 μm PS microspheres



(c) Light scattering intensity of 0.05 μm and 0.1 μm PS microsphere



(d) Light scattering intensity of 3 μm and 4 μm PS microspheres

Fig.2 Light scattering intensity of different diameter PS microspheres

cells is 5 μm and that of NCI-H1975 cell is 6 μm. As shown in Fig.2 (b), the light scattering intensity rises along with the diameter increase. The extent of the cytoplasm impact on the forward scattering backward scattering and side scattering are much the same [9]. We can know from Fig.2 (c) that at all angle the light scattering intensity of Polystyrene microspheres whose diameter is 0.1 μm were not always stronger than 0.05 μm. It owns to the diameter which is litter than wavelength, thus the Ep. (3) does not meet the conditions. The forward scattering light intensity of Polystyrene microsphere whose diameter is 0.1 μm is larger than that 0.05 μm, but the backward scattering is just the reverse. The Polystyrene microspheres whose diameter are 3 μm and 4 μm are equivalent to nucleus. Figure 2(d) illustrated that light scattering intensity increases as the diameter rises. What′s more, it is evident that side scattering have a big difference between different nucleus.

2.3 Light scattering properties of different lung carcinoma cells

Figure 3 showed the scattering light scattering distribution of HCC827 cells and NCI-H1975 cells. The light scattering intensity of NCI-H1975 cells is stronger than HCC827 cells, because the diameter of NCI-H1975 cells is larger than HCC827 cells. We can deduce the forward scattering is due to the combined effects to entire cell size, cell nucleus, and mitochondria from the result that the forward scattering intensity of NCI-H1975 is much stronger than others[10]. However, with the particle size rising, the changes in the forward scattering intensity of cell nucleus were not obvious. We can draw a conclusion that the differences of forward scattering were mainly caused by cytoplasm and mitochondria [11-12]. The side scattering of two kinds of cells were obviously shown in Fig.3. The backward scattering rises with the diameter of nucleus and cytoplasm increasing, but the greater diameter of the nucleus can result in a larger gap. From these results, we can consider that the differences of lateral scattering was caused by cell nucleus and cytoplasm. For two kinds of cells, the differences in lateral scattering were very small, which was caused by the com-bined effect of nucleus and cytoplasm. Because the light scattering intensity rises with the diameter of mitochondria increasing, which is contrary to cytoplasm changes.

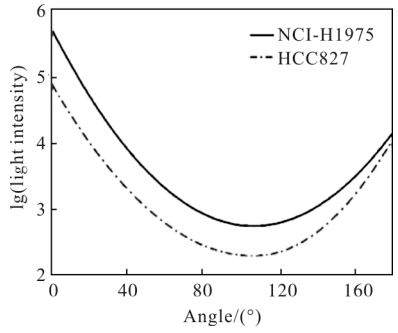


Fig.3 Light scattering of HCC827 cells and NCI-H1975 cells

3 Conclusion

In summary, the cytoplasm, nucleus, mitochondria all have impact on the light scattering property of cell, but each of them works differently. Mitochon-dria play an important role in forward scattering and backward scattering, the increasing bring less efficiency; mitochondria′s diameter bring less light intensity of forward scattering. On the contrary, backward scattering will be lower. Side scattering is dominated by nucleus, light scattering intensity become stronger along with the increase of nucleus′ diameter. In particular, the bigger cytoplasm diameter will lead to the increase of light scattering.

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