PAPER

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Assessment of the non-linear optical behavior of cells for discrimination between normal and malignant glial cells

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Abstract

Cancer patients who have a diagnosis in the early stage of the disease have better chances for therapy. Thus, development of new diagnostic procedures is needed for the early detection of cancer. Measuring and analyzing the light that is scattered by the cells can be utilized to monitor early variations associated with cancer evolution. The key hypothesis behind this research was the application of the Z-scan method to progress the precision of detecting the C6 glioblastoma multiforme cancer cells and the OLN-93 normal glial cells.

A Nd:YAG CW laser with a wavelength of 532 nm was used. The laser beam passed through the prepared samples and the transmittance power is distinguished by dislocation. Finally, the nonlinear refractive index and the extinction coefficient of the samples were determined.

Our study showed that the extinction coefficient of the C6 and OLN-93 cells were 49 ± 3 and 33 ± 2, respectively. Also, the sign and value of the nonlinear refractive index (n_2) for the C6 and OLN-93 cells was -5.44×10^{-7} cm^2 w^{-1} and $+6.07 \times 10^{-7}$ cm^2 w^{-1}, respectively. According to this study, the sign of the n_2 index for the C6 and OLN-93 cells was negative and positive, respectively.

Our results suggested that the nonlinear refractive index of the cell samples and Z-scan technique could be an applicable means for identifying glial cancer cells from normal cells.

Keywords: Z-scan, Nd:YAG CW laser, glioblastoma multiforme, nonlinear refractive index, extinction coefficient

(Some figures may appear in colour only in the online journal)

1. Introduction

Gliomas are established as the most frequent form of human primary brain tumors in adults [1]. Based on information from the World Health Organization (WHO), a glioblastoma multiforme (GBM) is classified as a grade IV of astrocytoma and composes approximately 42% of all primary brain tumors [2–5]. GBM is the most invasive malignant brain tumor and is considered to be one of the most lethal forms of the human cancers [4, 6]. Despite numerous therapeutic approaches comprising surgery, radiotherapy, and chemotherapy, the median survival of patients is 12–18 months and without therapy, patients die within 4 months [7, 8].

Patients who diagnose in the early stage of the disease have better chances for therapy. Thus, development of new diagnostic procedures is urgently needed for early diagnosis of cancer which can improve the patient's quality of life of and reduce their mortality.

GBM cells are invasive and have a rapid proliferation nature with poor diagnosis [9]. Diagnostic criteria for GBM, including nuclear atypia, vascular proliferation, high mitotic activity and necrotic areas, which make them approximately distinguishable by histology, but histopathology alone is not sufficient for a GBM diagnosis [4].

Cancer cells are identified from normal cells by their morphology, architectures, cellular histologies, genetic and epigenetic aberrations, and their molecular marker expression profiles. They have a large irregularly shaped nucleus and a relatively small cytoplasm as compared to normal cells [10]. Connexin protein is the structural unit of the gap junction channels in the cellular membrane. Gap junction channels prepare a route for the transition of small molecules and cellular signals. Normal and cancer glial cells possess connexin43 but the level of connexin43 mRNA is significantly reduced in the C6 GBM cells that lead to the loss of intercellular communication and the uncontrolled growth of the cancer cells [11]. Moreover, several growth factor receptors such as platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR) are concerned with the regulation of gliblastomagenesis. The EGFR gene is overexpressed in 30%–40% of GBM [12, 13]. These growth factors relate to the larger family of receptor tyrosine kinases. The binding of ligands to the receptors cause the activation of signaling pathways in the cells that leads to proliferation, migration, and uncontrolled growth of the cancer cells [12].

There are several methods for diagnosing the changes associated with cancer progression such as immunocytochemistry, fluorescence *in situ* hybridization (FISH), real time RT-PCR, and western blot analysis [12–14]. These methods are expensive, time-consuming and require very specific training. Therefore, in this research, a simple bio-optical technique is introduced for distinguishing the C6 and OLN-93 cell lines.

The use of light interaction with tissue to diagnose diseases has been proposed since the mid-1800s [15]. Recent developments in detectors offer opportunities to quantitatively estimate these interactions that provide information for diagnosis at the physiological, structural, biochemical and molecular level within tissues or cells. When normal cells convert to tumor form, their biochemical and morphological characteristics are modified [16, 17]. Structural, morphological, biochemical and molecular changes in tissue or cell constituents lead to the alteration of their optical properties and their light-scattering characteristics. Measuring and analyzing the light that is scattered by the tissue or cells can be utilized to monitor the variations associated with cancer evolution [18, 19]. Therefore, optical methods are an applicable means to assess cell structure and provide diagnostic information for distinguishing cancer cells from normal cells [20–23].

Numerous optical techniques can be used to analyze the scattering properties of biological cells including fluorescence spectroscopy, Raman and light scattering spectroscopy [19, 24], two-photon excited fluorescence, second harmonic generation [25, 26] and the Z-scan technique [27].

The Z-scan method is a general technique for the detection of nonlinear optical properties, particularly nonlinear refraction and nonlinear absorption of a large variety of material [28–32] and biomaterial concentration such as urea and uric acid [33], proteins [34, 35], glucose in the blood of patients [29, 36], quantification of total cholesterol, triglycerides [37], concentration of creatinine in the blood plasma [38] and differentiation of cancer from normal tissues [39, 40].

Mansoor Sheik Bahae *et al* developed the Z-scan analysis [41, 42]. In this technique, the sample is scanned along the axis of the laser beam (z-axis) by changing the position through the focal region. The energy of light transmitted through an aperture in the far field (closed-aperture Z-scan) is determined by a detector located behind a small aperture as a function of sample position (Z). By monitoring the transmittance variation through a small aperture in the far field position, it is possible to determine the nonlinear refractive index of the sample.

In the present study, we have measured the nonlinear refractive index ($n2$) of the C6 cell line as a cancer cell (GBM) and the OLN-93 cell line as a normal glial cell using a single beam Z-scan method for distinguishing cancer cells from normal cells.

2. Material and methods

2.1. Materials

Fetal bovine serum (FBS), penicillin, streptomycin, Trypsin-EDTA (0.25%), and cell culture mediums including Dulbecco's modified eagle's medium (DMEM) and Ham's F12 were purchased from Gibco Invitrogen. Formaldehyde solution was obtained from Sigma Chemical Company (St Louis, MO, USA). The C6 and OLN-93 Cell lines were provided by the Pasteur Institute of Iran.

2.2. Sample preparation

In this study, two cell lines including C6 and OLN-93 were obtained from the Pasteur Institute of Iran. The C6 cell line was cloned from a rat glial tumor but the OLN-93 is derived from spontaneously transformed cells in primary rat brain glial cultures. Oligodendrocytes (OLN) are glial cells responsible for myelin-forming in the central nervous system. In our experiment, OLN-93 and C6 cell lines have been used as a model for oligodendrocytes (normal cells) and GBM (tumor cells), respectively. Both cell lines were cultured in a medium (Ham's F12 for C6 and DMEM for OLN-93) supplemented with 10% FBS, penicillin (100 units ml^{-1}), and streptomycin (100 mg ml^{-1}) in 5% CO2 and 95% air at 37 °C in a humidified incubator at a density of 10^4 cells cm^{-2} in T-25 cell culture flasks. The cells were harvested by trypsinizing cultures with 1 mM EDTA/0.25% trypsin (w/v) in PBS. After that, 10^5 cells were transmitted from the flask to the six-well plate with one sterilized glass slide (lamella) at the bottom of it and incubated for 24 h. After 24 h, samples were fixed with 1 ml of 2% paraformaldehyde and were prepared for Z-scan assay.

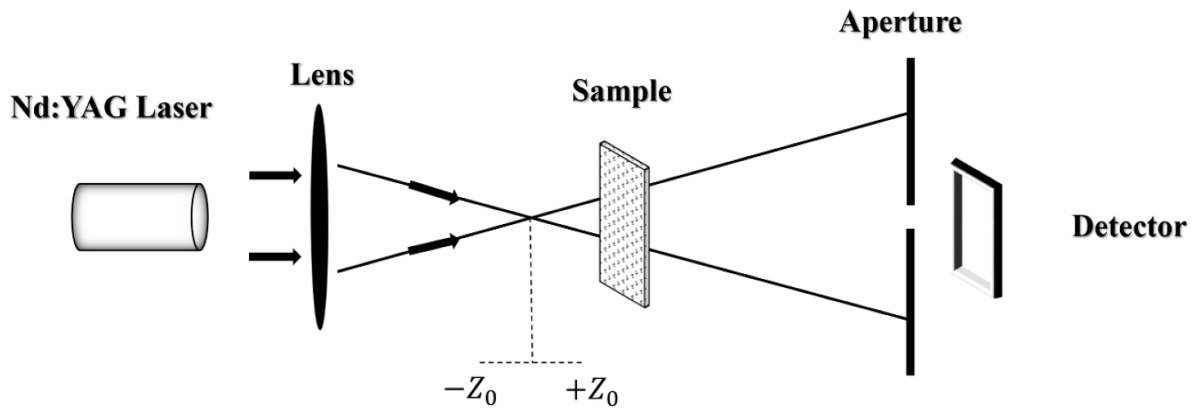

Figure 1. Schematic of a closed aperture Z-scan setup.

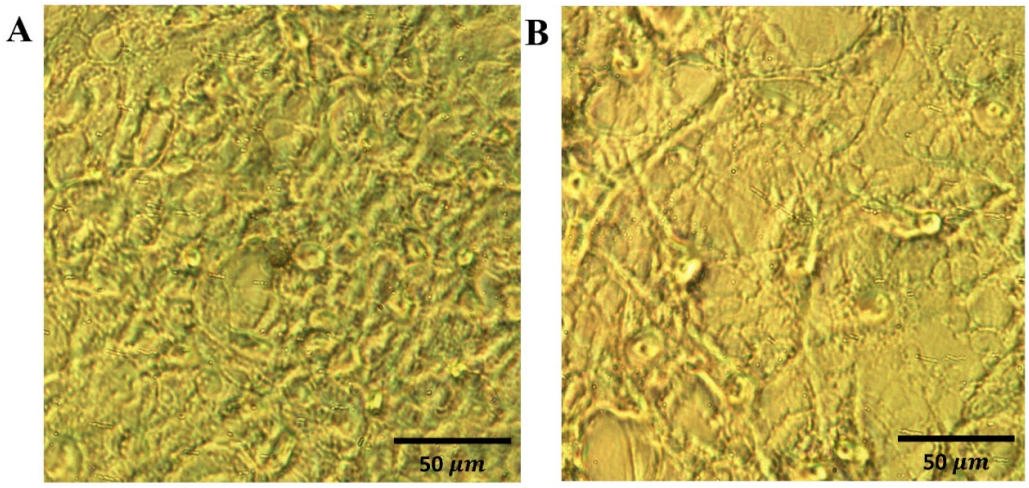

Figure 2. Microscopic morphology of (A) glioblastoma multiform as a cancer cell line (C6) (B) normal glial cell line (OLN-93).

2.3. Z-scan technique

To measure the sign and magnitude of the nonlinear refractive index of the samples, the closed aperture Z-scan method was used. The setup array is a laser, focusing lens, sample holder, aperture, and detector, respectively.

The Z-scan setup was performed by using an Nd:YAG (CW) laser with 100 mW output power and a wavelength of 532 nm. The laser beam focused by a lens with focal length of 8 cm (figure 1).

The sample was exchanged on both sides of the focal region. The focused beam passed through the samples, then transmitted across an aperture in the far field. The transmittance power changes through the aperture are recorded as a function of the sample position (from $-Z$ to $+Z$ with equal steps) which is measured with respect to the focal plane and the nonlinear refractive index (n_2) can be computed according to equation (1) [41].

$$n_2 = \frac{\lambda \Delta T_{\mathrm{P-V}}}{2\pi L_{\mathrm{eff}} (0.406)(1-S)^{0.25} I_0}, \tag{1}$$

where λ is the wavelength of the beam source and $\lambda\Delta T_{\mathrm{P\text{-}V}}$ can be defined as the difference between the normalized peak and valley transmittances ($\lambda\Delta T_{\mathrm{P\text{-}V}} = T_{\mathrm{p}} - T_{\mathrm{v}}$). For a negative nonlinear refractive index, there is a peak and then valley in the diagram of normalized transmittance over sample location and for positive nonlinear refractive index vice versa. L_{eff} and S are the effective length and linear transmittance of the aperture, respectively, and I_0 is the maximum intensity at the focal point. The linear transmittance of the aperture is given by equation (2).

$$S = 1 - \exp\left(-2\left(\frac{r_{\mathrm{a}}}{W_{\mathrm{a}}}\right)^2\right), \tag{2}$$

where r_{a} is the radius of aperture and W_{a} is the beam radius at the aperture plane. Other parameters can be calculated according to equations (3) and (4):

$$I_0 = \frac{2P}{\pi w_0^2}, \tag{3}$$

in which w_0 is the beam waist ($w_0 = 43$ μm) and P is input power and

$$L_{\mathrm{eff}} = \frac{1 - e^{-\alpha L}}{\alpha}, \tag{4}$$

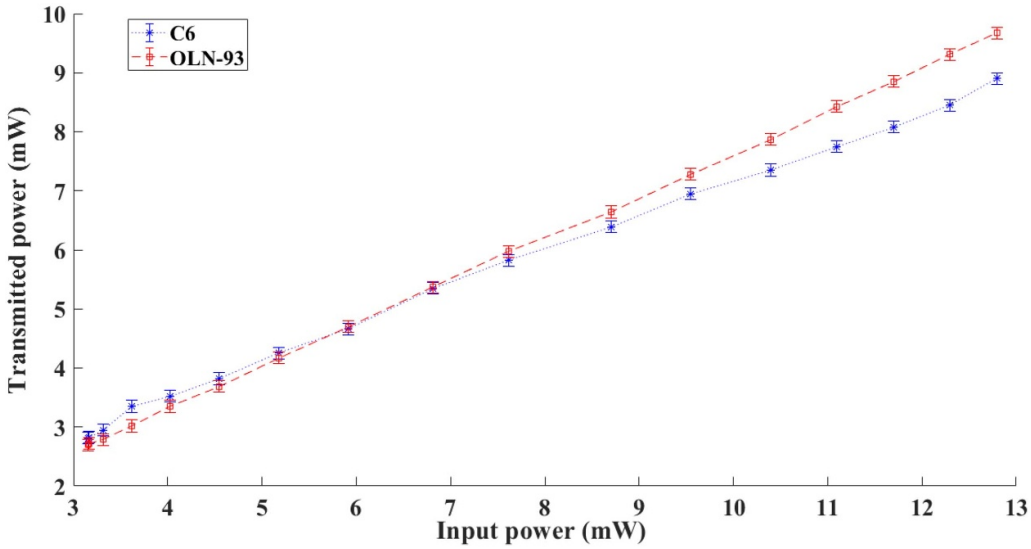

Figure 3. Transmitted power versus input power in linear regime for both C6 and OLN-93 cell lines.

Table 1. Values of absorbance for cancer and normal cells.

Samples	Extinction coefficient ($cm^{-1} \pm SD$)
C6	49 ± 3
OLN-93	33 ± 2

in which α and L are the linear absorption coefficient and the thickness of the sample, respectively.

In our study, the experiments were repeated five times for each sample and the mean value of the nonlinear refractive index (n_2) was calculated from the normalized transmittance values.

3. Results

The microscopic morphology of the GBM and normal glial cells that were prepared for the Z-scan technique are shown in figure 2.

In an uncomplicated optical setup, the transmitted power was tracked by a varying input laser with low power. Input power and transmitted power were determined to calculate the extinction coefficient of the C6 and OLN-93 cells. The extinction coefficients of the cell lines in the linear regime were determined from the slope of experimental data. The linear optical behavior of the C6 and OLN-93 cell lines is shown in figure 3.

The extinction coefficients of the C6 and OLN-93 cell lines were 49 and 33 cm^{-1}, respectively (table 1).

Diagrams of the normalized transmittance as a function of the Z-axis were plotted for the C6 and OLN-93 cell lines (figure 4). The nonlinearity optical behavior of both cells was calculated from the difference between the maximum (peak) and minimum (valley) values of the normalized transmittance. The value of $\Delta T_{P\text{-}V}$ for the normal cell line was higher than the cancer cell line.

We calculated the nonlinear refractive index (n_2) for both cell lines. The sign of the nonlinear refractive index for the C6 cells was negative and for the OLN-93 cells it was positive. The calculated values of the nonlinear refractive index of the C6 and OLN-93 cell lines are depicted as a bar chart in figure 5.

4. Discussion

For differentiation of GBM cancer cells from glial normal cells, the nonlinear optical behavior of the C6 and OLN-93 cell lines was evaluated by employing the Z-scan technique with a 532 nm Nd:YAG CW laser. Recently, the Z-scan technique has been frequently used for the measurement of biomaterial concentration [29, 33–36, 38]. Based on our knowledge, there are few studies focused on distinguishing tumor from normal tissues by Z-scan method [39, 40].

As described in the literature, GBM and normal glial cells, due to their specific structural, biochemical, physiological, and morphological characteristics, have different nonlinear optical behavior that can be used to discriminate tumor from normal cells.

Our results indicated that the absorbance of the cancer cell line (C6) was higher than normal cells (OLN-93). This result is consistent with that reported by Mohajer *et al* [40]. They reported that malignant oral samples have greater attenuation than benign oral samples.

Figure 4 showed that in the C6 cell line, pre-focal normalized transmittance and post-focal normalized transmittance were maximum and minimum, respectively. While for the OLN-93 cell line, the pre-focal normalized transmittance was a valley and post-focal normalized transmittance was the peak. Therefore, the sign of nonlinear refractive index (n_2) for the C6 and OLN-93 cells was negative and positive, respectively.

Also, the value of n_2 for the C6 cell line was -5.44×10^{-7} cm^2 w^{-1} and for the OLN-93 cell line was $+6.07 \times 10^{-7}$ cm^2 w^{-1}.

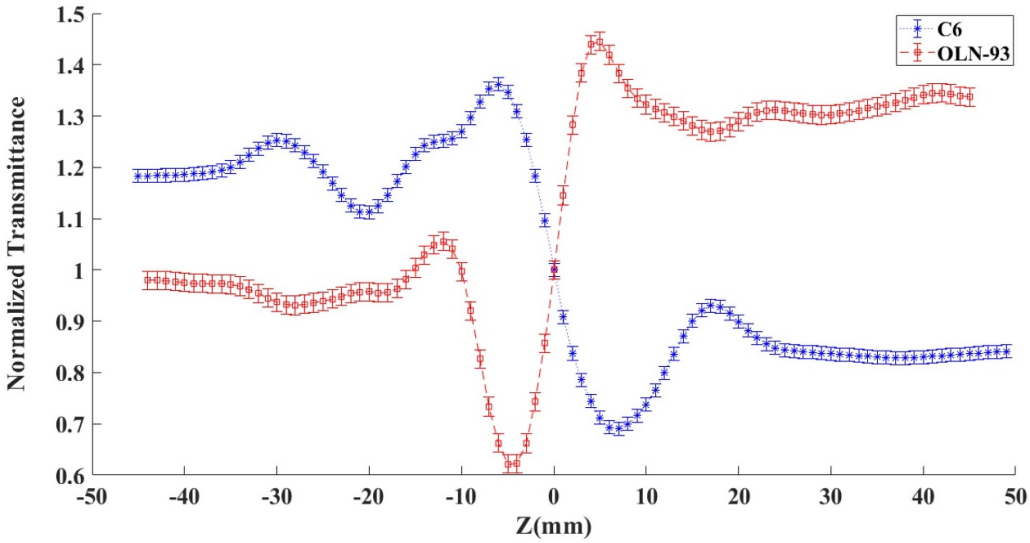

Figure 4. Close aperture Z-scan normalized data for the C6 and OLN-93 cell lines.

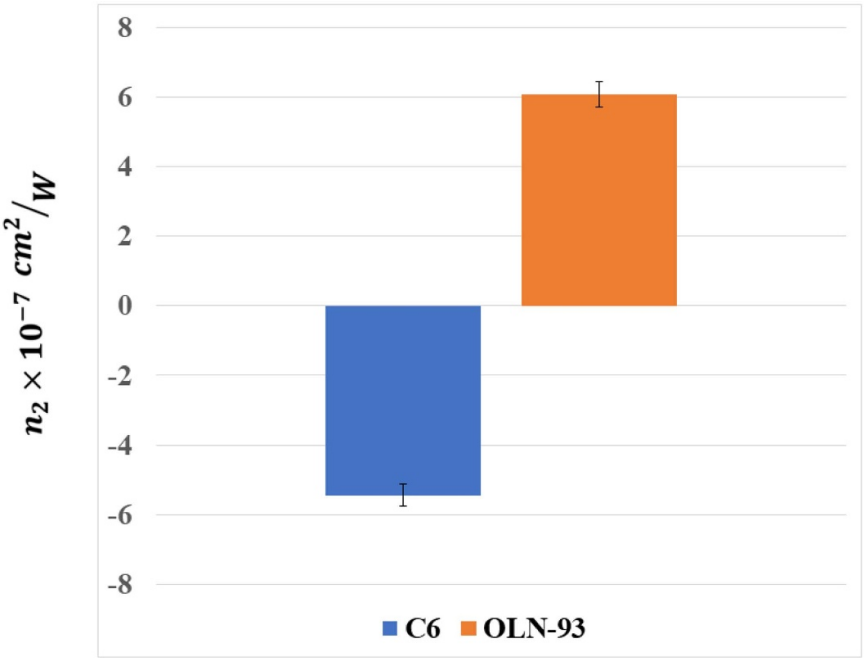

Figure 5. Values of the nonlinear refractive index of the C6 and OLN-93 cell lines.

The difference in the nonlinear refractive index of the C6 and OLN-93 cell lines can be attributed to: (1) different size of the nucleus and cytoplasm volume of the normal and cancer cells; (2) high proliferation of the C6 cells; (3) difference in the protein expression; (4) difference in the expression of the growth factor receptors.

Over-expression of the growth factor receptors (PDGFR and EGFR) [12] and low-expression of connexin43 mRNA [11] in the C6 cells, high proliferation and uncontrolled growth of the C6 cells, also, the large nucleus and small cytoplasm of the cancer cells as compared to normal cells [43] can modify the optical properties of the cells and change the pattern of the light diffraction. Therefore, in the present study, the Z-scan technique was found to be an applicable means for detecting glial cancer cells from normal cells and can be utilized as a supporting method in combination with molecular examinations. Additionally, the main advantages of the Z-scan analysis relative to molecular techniques are its inexpensiveness, simplicity, repeatability, real-time scanning and lack of operator dependency.

5. Conclusion

According to this study, the observed changes in the nonlinear refractive index and the associated changes in the optical

properties of cells due to modification of their biochemical and morphological characteristics can be a means to detect cancer cell from normal cells using the Z-scan technique.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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