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Light-scattering by aggregates of tumor cells: Spectral, polarimetric, and angular measurements

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ABSTRACT

The goal of this paper is to present an in-vitro label-free technique based on light-scattering to study optical properties of MultiCellular Tumor Spheroids (MCTS). Our non-imaging technique provides a complete optical signature from hyperspectral polarimetric and angular light-scattering measurements using a coherent white-light supercontinuum laser-based instrument. We perform measurements on MCTS to demonstrate the feasibility of the technique. We report the spectral and angular-dependence of the depolarization of scattered-light by MCTS. This is the first time that the hyperspectral, polarimetric, and angular light-scattering signature of MCTS is reported. This work may provide new insight for the future development of the growth and proliferation of tumor cells.

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1. Introduction

The need for innovative techniques to understand cancer and its therapy is constantly growing for the understanding of cancer and its therapy. The use of light is especially appropriate to study biological multicellular organism, and thus, an accurate and comprehensive knowledge of their optical properties are crucial. The interaction of light and biological organisms is a problem of great significance as is involved in a growing number of medical applications such as laser microsurgery [1], laser ablation [2] and optical tomography [3]. Light-cells interactions lead to various optical processes including scattering and absorption. Scattering processes is addressed by this work.

Light-scattering by multicellular systems is commonly measured [4,5] and modeled [6,7] as it gives useful insight of the organism' characteristics. Recent techniques based on light-scattering provide structural and functional information at the cellular and subcellular level. For instance, Elastic Light Scattering Spectroscopy (ELSS) [8,9] uses the elastic scattered-light (with no spectral change) for non-invasive in-vitro clinical diagnosis of diseases [10,11]. For instance, light-scattering techniques enabled discrimination between malignant and benign lesions [12].

MultiCellular Tumor Spheroids (MCTS) are three-dimensional in-vitro models that mimick the organization of a tumor micro-domain. Growing MCTS display nutrient and hypoxia gradients. This leads to the regionalization of proliferation with cells located on the outmost layers and quiescent cells in the central region [13–15]. Moreover, their

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production can be fast (few days), repeatable in a large-scale production [21] and the size of the MCTS depend on the time of culture. For these reasons, the MCTS are largely used to study cancer biology and evaluate the response to anticancer drugs.

In this study, we demonstrate the feasibility of an original light-scattering technique for non-contact and label-free characterization of multicellular systems using a white-light supercontinuum laser-based instrument. Our method combines hyperspectral (i.e. white or broadband spectrum), polarimetric and angular scattered-light measurements to generate a specific fingerprint of the biological system. The objective is to provide comprehensive optical properties, i.e. spectral, polarimetric and angular properties of MCTS. Such information is of major interest in the development of innovative screening for the identification of anticancer drugs and in cancer diagnostic and prognostic assessment.

2. MultiCellular Tumor Spheroids (MCTS)

In-vitro studies are an important and necessary step for characterization of biological mechanisms. They are useful to identify individual component and biological functions more conveniently than whole-organism experiments and do so without the ethical problem of animal studies. The most common in-vitro samples are two-dimensional monolayer cells, which mean a culture of proliferative cells on a glass or plastic plate. However, in the case of a tumor, it has been shown that this kind of model does not reproduce some mechanisms like multicellular resistance [13], which includes inhibition of apoptosis, the high proportion of quiescent cells, modulation of gene expression, decrease of permeability, and hypoxia. In a general way, it does not take into account the tissue heterogeneity, cellular interactions, and tumor microenvironment, which are important factors for cancer studies.

The 3D culture of MCTS offers a level of complexity that recapitulates the three-dimensional organization of a tumor and integrates the notion of a tumor's microenvironment [14,15]. In contrast to cells growing as 2D monolayers, the MCTS model appears to be fairly predictive of therapeutic efficiency [16]. The use of MCTS in large-scale automated screening was recently reported to link the power of a high throughput analysis to the predictability of a 3D cell model. However, quantitative determination of cellular response in a three-dimensional arrangement is still very problematic. Due to the size, cell density and heterogeneity of MCTS, there are still significant challenges associated with imaging those samples compared to imaging 2D-cell culture layers. Usually, MCTS were serial sectioned in thin layers, stained with a given marker and imaged by conventional light microscopy. We have previously reported that two photon microscopy and Selective Plane Illumination Microscope (SPIM) [17,18] enable non-invasive imaging of MCTS but with limited imaging penetration depth. In those cases, fluorescence is used as contrast agent and typically requires the use of genetically modified cell lines, which can alter cellular behavior. Alternative approach such as the coherence-domain speckle-imaging technique uses low-coherence off-axis holography combined with fluctuation microscopy [19,20] to provide new label-free and non-invasive techniques for MCTS imaging, and therefore, are attractive techniques for label-free drug screening. Thus, there is a strong need to develop novel analysis methods for 3D MCTS screening for drugs in early drug development.

Research of new characteristic parameter of a tumor can be of major interest in identification of new anticancer drugs, but also for cancer diagnostics. Optical properties do not need labeling and give a specific signature of a specimen. The knowledge of optical properties of a tumor tissue can provide relevant information about the physiological state of the cells and their response to internal or external cues. The features presented above make the MCTS a model of choice to study the optical properties of cancer tissues.

The MCTS are generated in 96 well-plates coated with PolyHema [21]. One thousand cells are dropped off in each well with 100 mL of medium and centrifuged. Cells cannot attach to the PolyHema and aggregate, thus creating the spheroid. Spheroids can grow to diameters of several hundred micrometers in days, thus allowing rapid and large-scale production of MCTS [22,23]. Twenty-one 96 well-plates were prepared containing MCTS with a diameter of 0.2 mm (2–3 days). They were collected with a micropipette in five 15 mL Falcon tubes with an equal repartition of 0.2 mm spheroids (Fig. 1). These were washed three times with Phosphate Buffered Saline (PBS) and then fixed with Formalin 10% neutral buffered (Sigma-Aldrich) at room temperature for 4 h. The MCTS in formalin were washed three times. The MCTS are maintained in PBS during the process of optical measurements.

3. Optical characterization method

Light-scattering is the study of the propagation of electromagnetic wave within inhomogeneous media. Polarimetry and spectroscopy are known to reveal diagnostic information about tumors. We propose an original optical technique that combines angular light-scattering measurements with polarimetric and hyperspectral measurements. In other words, we combine spectroscopy, polarimetry, and scatterometry. The fusion of these measurements may provide a new and unique way to characterize tumors in multicellular systems. Merging this information requires a common hyperspectral, polarimetric and angular light-scattering framework. Due to the huge amount of data involved in hyperspectral measurements (more than 5000 wavelengths), tensorial quantities are used to store the scattering data in a hypercube.

Let us define a 5-order hyperspectral tensor expressed in a covariant form $H^{mn}(i_1,i_2;r_1,r_2;l)$ or more simply as $H^{mn}(i_1,i_2;r_1,r_2;l)\in\Re^{I_1\times I_2\times R_1\times R_2\times L}$ where i_1, i_2 range from 0 to I_1, I_2 referring to the number of angles of incidence, r_1, r_2 range from 0 to R_1, R_2 referring to numbers of reflected angles and l ranges from 0 to L referring to the number of wavelengths or frequencies. Fig. 2 represents the spherical coordinates systems used for this tensorial framework definition.

The basic tensors needed are the polarized irradiance tensor $E^m_{inc}(i_1,i_2;r_1,r_2;l)$ and the polarized radiance tensor $L^n_{sca}(i_1,i_2;r_1,r_2;l)$. The scripts m and n refer, respectively, to

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