# Imaging the Nonlinear Susceptibility of Collagen in Breast Cancer Tissue by Double Stokes-Mueller Polarimetry Microscopy

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Second-harmonic generation (SHG) microscopy has become an effective tool for imaging biological structures, including collagen, without the need for staining. Collagen is the main constituent of extracellular matrix (ECM) that due to its noncentrosymmetric structure is capable of producing a strong SHG signal. Studies have demonstrated that the deposition and arrangement of collagen fibrils may alter in the tumor-affected tissues [1]. These morphological alterations can be visualized, probed and quantified by measuring the changes in the intensity and the polarization of the SHG signal produced at the tissue and therefore it provides complementary information about the tissue structure in addition to the bright-field microscopy of histopathology tissue slides.

Recently, the theory of double Stokes-Mueller polarimetry (DSMP) has been introduced for characterizing the second-order susceptibility tensor of the samples where the sample is represented by a 4×9 double Mueller matrix and it contains information about the sample structure. In this formalism a 9×1 double Stokes vector and a 4×1 Stokes vector are used to represent the incoming beam and the outgoing SHG signal from the sample, respectively [2]. The DSMP measurements can be performed to extract the absolute values of six laboratory-frame susceptibility tensor components, $\chi_{XXX}^{(2)}$, $\chi_{XZZ}^{(2)}$, $\chi_{XXZ}^{(2)}$, $\chi_{ZXX}^{(2)}$, $\chi_{ZZZ}^{(2)}$, $\chi_{ZXZ}^{(2)}$ [3], where XZ is the image plane and the light propagates along the Y-axis of the Cartesian laboratory frame of reference. In addition, the average in-plane orientation of collagen fibrils from the laboratory Z-axis ($\delta$), degree of linear polarization (DLP), degree of circular polarization (DCP) and degree of total polarization (DP) of the outgoing SHG signal can be deduced from DSMP measurements. The distribution of molecular second-order susceptibility tensor components ratio ($R = \chi_{ZZZ}^{(2)} / \chi_{ZXX}^{(2)}$), $\delta$ and DLP can be used to differentiate normal and tumor tissue.

In this work, the DSMP measurements are used to investigate structural changes of collagen in three different pathologic types of breast cancer in a tissue microarray. These pathologic types are determined based on estrogen receptor (ER) and progesterone receptor (PgR) status and overexpression of human epidermal growth factor receptor 2 (HER2). These three types are: ER+, PgR+, HER2+ (triple positive or +/+/+), ER+, PgR+, HER2- (double positive or +/+/-), and ER-, PgR-, HER2- (triple negative or -/-/-), where the positive/negative sign after each abbreviation is indication of overexpression/absence of the specific receptor. It is demonstrated that polarimetric SHG microscopy can detect significant differences in collagen organization between normal and malignant breast tissue by measuring *R* and DLP. Therefore, the *R* and DLP can be utilized as novel cancer detection parameters and can serve as complimentary measures for H&E histopathology.

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