Optically Active Sum Frequency Generation Microscopy for Cellular Imaging

K. Zhang[1], N. Ji[2], Y.R. Shen[2], and H. Yang[1]

[1] Department of Chemistry, University of California at Berkeley, Berkeley, California 94720
E-mail: hawyang@berkeley.edu
[2] Department of Physics, University of California at Berkeley, Berkeley, California 94720
E-mail: yrshen@calmail.berkeley.edu

Abstract. Optically active sum-frequency generation microscopy was applied to imaging cells, utilizing the intrinsic chirality of biological macromolecules as the contrast mechanism.

1. Introduction

Most cellular processes involve changes in the structure of intracellular components. Current optical approaches to imaging biological systems often require the introduction of genetic and/or chemical modifications such as fluorescent probe labeling in order to generate imaging contrast. Doing so, however, risks causing structural and functional perturbations to the system. A stain-free microscopic approach will therefore greatly improve the understanding of natural functionality of biological systems.

Cells are three-dimensional entities containing biomolecules that are mostly chiral. For example, except for glycine, all amino acids have a chiral carbon center and sugar structure in nucleotide has more than one chiral center. Molecular chirality is hence encoded in the structure of nucleic acids and proteins, providing a mechanism for cellular optical imaging and potentially structural analysis.

Microscopy based on circular dichroism suffers from low sensitivity because of the electric-dipole-forbidden interactions between the material and light [1]. Successful demonstration of chiral second harmonic generation (C-SHG) surface microscopy was reported by Kriech *et al.* [2]. However, probing bulk (as opposed to surface) chirality is still beyond the capability of C-SHG because SHG is forbidden in an isotropic environment.

Optically active sum-frequency generation (OA-SFG) has been recently developed [3-5]. Being electric-dipole allowed, OA-SFG can detect molecular chirality with high specificity and sensitivity, as demonstrated by its capability of determining the chirality of submonolayer [5, 6]. Microscopy study based on OA-SFG has also been demonstrated and a racemic mixture can be easily differentiated from its enantiomer with sub-micron resolution [7]. Here we applied this newly developed technique to biological systems and achieved label-free cell images.

2. Approach

Under electric-dipole approximation, SFG is allowed in media without inversion symmetry such as in chiral materials [10]. The signal strength depends on the product of light intensities of both input beams and the chirality-sensitive second order nonlinear susceptibility elements $\chi^{(2)}_{chiral}$.

$$I(\omega_{SF} = \omega_1 + \omega_2) \propto \left|\chi^{(2)}_{chiral}\right|^2 I(\omega_1) I(\omega_2) \quad (1)$$

$\chi^{(2)}_{chiral}$ is of opposite signs for enantiomers, and is zero for racemic mixture and achiral media. Resonance enhancement of $\chi^{(2)}_{chiral}$ provides chemical sensitivity [3-9]. Specific beam polarizations are required for the detection of OA-SFG. For isotropic materials, SPP (S-, P-, and P-polarized for ω_{SF}, ω_1, and ω_2 waves, respectively), PSP, or PPS polarization combinations can pick up the OA-SFG signal.

To implement the chiral SFG microscopy idea [7], a 60X oil objective was used to focus into the sample two overlapping excitation beams at 415 nm (ω_1) and 830 nm (ω_2), respectively, from a Ti:sapphire laser system. The resultant SF signal at 277 nm (ω_{SF}) was detected from the transmission direction by a photo multiplier tube. For samples containing DNA or RNA, the signal is resonantly enhanced as ω_{SF} coincides with an electronic transition. We used HeLa cells fixed by 4% formaldehyde in PBS buffer (pH = 7.4) to demonstrate OA-SFG microscopic imaging of stain-free cells.

3. Results and Discussion

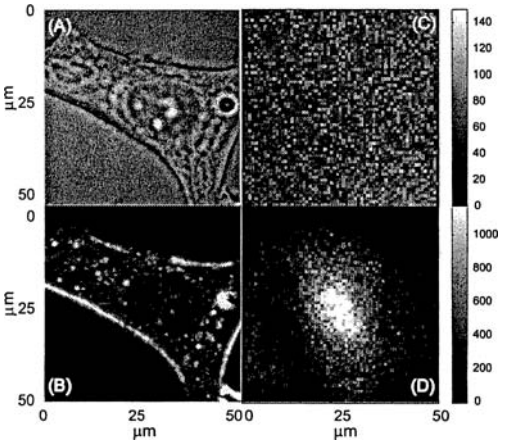

Fig. 1. (A) Bright field (B) dark field images of fixed HeLa cell. (C) Fluorescence image acquired at 350 nm and (D) OA-SFG image from another cell on the same glass slide. The image area is 50 μm × 50 μm. Collection time for both fluorescence and OA-SFG image is 78 ms/pixel at power levels 3.1 mW (415 nm) and 4.6 mW (830 nm).

In Fig. 1, we compared the contrasting effects of fluorescence and OA-SFG. Panel (A) and (B) show the bright and dark field image for fixed HeLa cell respectively. Panel (D) is an OA-SFG image of a different cell on the same slide. Panel (C) shows the fluorescence image taken from the same area as (D) but with 350 nm detection wavelength, which is the fluorescence peak of proteins and nucleic acids. As we can see clearly, fluorescence image does not provide any contrast, whereas OA-SFG image shows strong positive contrast from the nuclear region. Power dependence study on the OA-SFG gives a correlation coefficient 0.9967 for ω_1 and 0.9666 for ω_2 beams, respectively. Three-dimensional sectioning images of the cell were also achieved. The experimental setup can be easily tuned to detect SHG or intrinsic fluorescence of nucleic acids, providing complementary information on the internal structures of cellular systems.

4. Conclusions

Stain-free cellular imaging with OA-SFG has been demonstrated using fixed HeLa cells as an example. While further research efforts are evidently needed, OA-SFG microscopy appears to have great potential for imaging biological systems.

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