MOLECULAR IMAGING OF CANCER CELLS USING PLASMON-RESONANT-ENHANCED THIRD-HARMONIC-GENERATION MICROSCOPY WITH SILVER NANOPARTICLES

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Abstract: We demonstrate molecular-specific third-harmonic-generation microscopy in cultured oral squamous cell carcinoma lines by using silver nano-particles as contrast agent. Through surface plasmon resonance enhancement, cancer cells are clearly identified with their molecular signature.

OCIS codes:(180.6900) Three-dimensional microscopy; (180.5810) Scanning microscopy; (190.4160) Mutiharmonic generation

Third-harmonic-generation (THG) has been emerged as an important imaging modality in biological and material researches in recent years with the advantages including no energy release due to the virtual-state-transition characteristic and intrinsic optical sectioning capability due to the high-order nonlinearity nature. THG imaging has many applications including biological morphology imaging [1,2], intracellular Ca^{2+} dynamics visualization [3], and bandtail state distribution imaging in gallium nitride [4]. Although third order nonlinearity exists theoretically in all material, the Gouy phase shift effect substantially limits THG to be observed in the vicinity of interfaces where the third order susceptibility discontinues. Therefore, THG can be used as a general-purpose 3D morphological imaging tool due to its interface-sensitive nature. For molecular-specific THG imaging, a contrast agent to enhance THG at a specific cellular site is necessary.

It is straightforward to selectively increase the THG efficiency by matching the photon energy (fundamental or THG) with the transition energy of the specific target. We have previously demonstrated such enhancement and applied it to functional imaging in bulk gallium nitride [4]. However it is sometimes very difficult to try to find a light source whose frequency is resonant with the transition energy of interested targets, no matter in semiconductor or biological tissues.

An alternative approach to significantly enhance nonlinear signals is associated with the plasmon resonance in noble metal nanoparticles, which have been successfully applied to enhance various coherent or incoherent nonlinear emissions including Raman scattering, two photon fluorescence (2PF), second-harmonic-generation (SHG), and THG in recent years. Localized THG and 2PF enhancement with the aid of gold nanoparticles has been demonstrated recently [5]. However, this previous experiment proposed to enhance nonlinear emissions by matching excitation energy with the plasmon resonance energy of metal nanoparticles. In common multi-photon and higher harmonic microscopy, near-infrared femtosecond lasers are used as excitation sources to increase the penetration depth and reduce the potential optical damage. In particular, it has been demonstrated that the spectral transmission window of common biological tissues falls around 1200~1300-nm. However, the plasmon resonant wavelengths of most noble metal nanoparticles (silver, gold, and copper) fall in near-ultraviolet and visible spectrum regimes. It is well understood that the resonant wavelength of noble metal colloids extends into longer wavelength regime as the size of particles or the degree of aggregation increases. Therefore, in order to enhance high order nonlinear optical emission with noble metal nanoparticles with 1200~1300-nm excitation wavelength, it will require particles with a size as large as a few hundred nanometers, which is too large to penetrate through cellular membranes and be used as molecular labels. The optical enhancement is also reduced with larger particle size due to the diminished surface plasmon density. There are other methods to extend the resonant wavelength into the NIR regime, such as coating

the nanoparticles with a dielectric layer and engineering the shape of the nanoparticles. Both of them require complex chemical procedures. A simple approach to enhance nonlinear emissions without further modification of the metal colloids is to coincide the plasmon resonant energy with the nonlinear signal energy, which is in the visible regime. We have successfully observed more than 5-fold of magnitude THG enhancement by matching the THG frequency with the plasmon resonance frequency of < 50-nm diameter silver nanoparticles dispersed onto the target of interest such as fixed cells. In this presentation, we demonstrate molecular-specific third-harmonic-generation microscopy in cultured cell lines by using <50nm-diameter silver nanoparticles as contrast agent, by matching the THG wavelength with the plasmon resonance energy. Through surface plasmon resonance enhancement, cancer cells are clearly identified with their molecular signature.

In this study, the light source we used was a Cr:forsterite femtosecond laser, of which the 1230-nm emission wavelength is located in the high penetration window of most biological tissues with a corresponding THG wavelength at 410nm, in resonant with the surface plasmon energy of the applied silver nano-particles. Different silver nanoparticles with different fabrication methods, sizes (all with a diameter less than 50nm), and surface treatments were then applied in different biological samples as the contrast agent of the THG microscopy. Figure 1(a) shows an example of absorbance spectrum of the silver nano-particles with a 6nm average diameter soaked in the water. A resonant absorption peak ~410-nm can be observed. Figure 1(b) shows the corresponding spectrum of enhanced THG emission from interfaces attached with the silver nanoparticles, confirming the strong resonance-enhanced THG emission due to plasmon resonance.

These silver nano-particles were then applied for molecular imaging of cancer cells, serving as the contrast agent of THG microscopy. For a preliminary demonstration, the treated silver nano-particles were dispersed into the culture media of a human oral squamous cell carcinoma line OECM1. Fig. 2 shows example THG images of the OECM1 cells with (Fig. 2(b)) and without (Fig. 2(a)) incubation with the silver nano-particles. The brightness of Figure 2(b) has been adjusted to accommodate the strong THG emission. Strong THG resonance due to silver nano-particles located at Argyrophilic Nucleolar Organizer Regions (AgNOR) in the nucleoli of the cancer cells, which had been used as an index for cancer aggressiveness, can be clearly observed. Please notice that without silver nano-particles, the cell nucleoli do not show sharp contrast under THG modality (Fig. 2(a)). Compared with the experiments in the normal oral keratinocytes (will be shown in the conference), the capability to identify oral cancer cells through the molecular signature of AgNOR is thus clearly demonstrated by using silver nano-particles as the THG contrast agent. The studies on silver nanoparticles with different surface modification applied in different cell lines including normal and cancer cells will be presented and discussed in the conference.

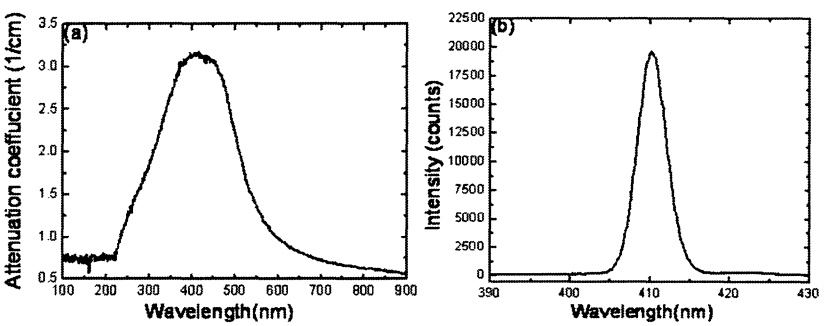

Fig.1 (a) Absorbance spectrum of the silver nanoparticles with a 6nm average diameter soaked in water. (b) Spectrum of THG emission from an interface with silver nanoparticles.

In summary, the virtual-state-transition and high-order nonlinearity characteristics of THG provide the advantages of noninvasiveness, no energy release, no photobleaching, and high optical sectioning capability, which are attractive for biomedical 3D imaging. However, this new imaging modality is mostly applied for morphology imaging only. To achieve functional THG microscopy for molecular imaging, it is necessary to find a mechanism to locally enhance THG at specific cellular sites. Here we have successfully demonstrated molecular-specific third-harmonic-generation microscopy by using silver nanoparticles as contrast agent. Through surface plasmon resonance enhancement with the THG wavelength, cancer cells are clearly identified with their molecular signature. Combined with the high penetration capability of 1230 nm light, our demonstrated THG-based molecular imaging shows great promises for future clinical cancer diagnosis.

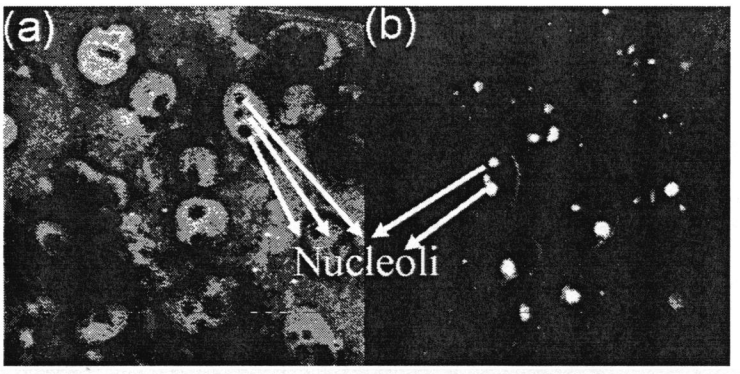

Fig.2 Horizontally sectioned THG images taken from the OECM1 cell line (a) without and (b) with injecting the treated silver nanoparticles into the culture medium. Image size: 80x80 μm

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