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Spectroscopic Properties of Intrinsic Proteins in Collagen Samples by using Gold-Nanoparticles and Two-Photon Excited Fluorescence Microscopy

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Multiphoton microscopy provides optical sectioning for high-resolution imaging. In biological systems, most multiphoton microscopy studies have relied on two-photon excited fluorescence (TPEF) to produce images. In particular, TPEF from structural proteins has emerged as an invaluable tool for 3D imaging. However, depending on the fluorophores involved, the TPEF could be extremely weak, limiting TPEF use for thick-tissue applications.

The physics and optical properties of noble-metal nanoparticles (NPs) is currently attracting much attention. The signal enhancement of molecules near a metal surface arises from the interaction with surface plasmon (SP) modes. These interactions also result in the shortening of the excited-state lifetime improving the molecules' photo-stability. The optical properties of the environment near a metallic NP are affected by the near-field electromagnetic-field. In the case of fluorescent molecules located at very short distances from a metallic surface, non-radiative energy transfer to SPs in the metal takes place; however, emission enhancement due to SP coupling still occurs at optimal distances from the NP.

In this study, we use a broad range of excitation wavelengths (~800-900 nm) to demonstrate that absorption enhancement can also be achieved in unstained samples by using a combination of gold-NPs and a TPEF. The TPEF images of gold-NPs/collagen samples were used to determine the collagen absorption spectra and confirm enhancement factors >100, which provide promising new applications in fields such as tissue imaging, and fluorescence-engineering based on surface-enhancement effects. Our results suggest that even weakly intrinsic signals from structural proteins can be extremely-enhanced due to SPs coupling.

The use of metallic NPs and TPEF in combination provides complementary information that allows noninvasive, spatially localized, in-vivo characterization in unstained samples and tissues.

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