Plenary 17

In Vivo Optical Virtual Biopsy by Using Least Invasive Harmonic Generation Microscopy

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Optical higher harmonic-generations, including second harmonic generation (SHG) and third harmonic generation (THG), leave no energy deposition to the interacted matters due to their energy-conservation characteristic, providing the “noninvasiveness” nature desirable for clinical studies [1]. Combined with their nonlinearity, harmonic generation microscopy provides noninvasive three-dimensional sectioning capability with a submicron resolution [2,3], offering new insights into live samples. By developing the femtosecond Cr:forsterite lasers working in the high penetration window, we have recently developed a non-invasive *in vivo* light microscopy with submicron 3D resolution and high penetration, utilizing endogenous and resonantly-enhanced multi-harmonic-generation signals in live specimens, with focused applications on the developmental biology and clinical virtual biopsy. Through a series of embryo viability studies and clinical trials, harmonic genration microscopy is confirmed to possess viability and penetration performance much superior to fluorescence-based techniques. In this presentation, we review our past work on the development of harmonic generation microscopy with a focus on the clinical trials of virtual biopsy capability and development of molecular THG imaging through resonance enhancement, partially achieved through the manipulation of the non-fluorescence nanoparticles.

For in vivo molecular THG imaging, the strategy is through the resonance enhancement of higher harmonic-generations. In this presentation, we will not only show that we are able to image melanin, elastin, the oxygen content of a red blood cell in in vivo, but are able to develop several exogenous THG contrast agents to trace the function of a specific molecule. These strategies include surface plasmon resonance enhancement with metal nanoparticles, electron-transition-level resonance enhancement with ferric oxide nanoparticles and absorbing dyes, as well as through the optical property modulation with acetic acid. Compared with the conventional in vivo imaging method, higher harmonic generation microscopy can provide superior 3D sectioning capability and high penetration depth. Compared with the popular two-photon fluorescence microscopy, higher harmonic

generation microscopy does not involve any electron transitions, thus leaves no energy deposition to the interacted live specimens and is with a much improved viability. This work is sponsored by National Health Research Institute.

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