Higher harmonic generations, including second-harmonic-generation (SHG) and third-harmonic-generation (THG) processes, are known to leave no energy deposition to the interacted matters due to the virtual-transition characteristic [1]. In contrast to the absorption-induced-fluorescence processes that require energy deposition and electron transitions, the higher harmonic generation processes provide the optical noninvasive nature desired for microscopy applications, especially for long-term observing the dynamic changes of live samples [2]. Different from single-photon and multi-photon fluorescence, no energy release is required during the harmonic-generation process, thus no cell damages and no photobleaching are expected. With a nonlinear nature similar to the multi-photon excited fluorescence, the generated SHG intensity depends on the square of the incident light intensity, while the generated THG intensity depends on the third power of the incident light intensity [1]. These dependencies allow localized excitations to enable intrinsic optical sectioning and a sub-micron three-dimensional resolution similar or better than the two-photon fluorescence microscopy can be achieved. With a coherent nature during the generation processes, THG can be utilized as a general-purpose microscopic technique for morphological studies [2] while its spectroscopic characteristic also allows for molecular imaging capability. SHG is sensitive to the local molecule arrangement and is particular useful for structural biomolecule studies [3]. By choosing the lasers working in the high penetration window, we have recently developed a least-invasive in vivo light microscopy with a submicron 3D resolution and high penetration capability, utilizing endogenous and resonantly-enhanced multi harmonic generation signals in live specimens, with focused applications on the developmental biology study and clinical virtual biopsy. In this manuscript, we review the physics and recent developments of the least invasive optical higher harmonic generation microscopy, with an emphasis on the in vivo virtual biopsy applications. We will also discuss new advancements in harmonic generation microscopy, including contrast agent developments and MEMS based endoscopy.

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