Two-Photon and Second-Harmonic Imaging with high throughput

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Nonlinear microscopes, such as second harmonic generation and multiphoton microscopes, have seen an increase in popularity in the life sciences because of their structural and molecular specificity, large penetration depth, high resolution, and volumetric imaging capability. In particular, second harmonic generation (SHG) is inherently sensitive to the absence of spatial centrosymmetry, which can render it intrinsically sensitive to interfacial processes, chemical changes, and electrochemical responses. Nonetheless, the inherently weak optical signals of nonlinear processes in, for example, live cell imaging demand long exposure times.

![Image](A) Translation - Rotation of Nanodoublers in Living Cells on Microsecond Time Scales,

Fig. 1 (B) Second harmonic image of human epithelial cervix carcinogenic cells transfected with 100 nm diameter KNbO3 nanodoubler,

By modifying the optical layout and illumination parameters, we improve the imaging efficiency of a nonlinear microscope. This improvement is achieved by using a wide-field imaging scheme in combination with a medium-range repetition rate amplified near-infrared femtosecond laser source and gated detection. As a result of the improvement, it is possible to follow the translation and rotation of nanodoubler in-vitro with microsecond acquisition times (see Fig. 1) [1]. The rotational diffusion can then be derived from variations in the second-harmonic intensity that originates from the rotation of the nanodoubler crystal axis. We envisage that by capitalizing on the biocompatibility, functionalizability, stability and non-destructive optical response of the nanodoublers, novel insights on cellular dynamics can be within reach.

![Image](B) Inverted image of the endogenous SHG signal, obtained by placing a band pass filter (515/BP10) before the camera.

Fig. 2 (A) Measured contrast in the images recorded from the same position of the same sample in four different systems: wide-field (200 kHz, gated detection as proposed here, blue and red curves), a scanning microscope (Leica TCS SP5 with 1028 nm, 88 MHz, 190 fs laser, a 1.2 NA 20× water immersion objective, a scanning rate of 1000 Hz/line, 256 x 256 pixels, and collecting NA of 0.9), and a wide-field 1 kHz geometry with a normal CCD camera. The blue data points were recorded with the intensifier and the electronic amplification of the camera both turned on, while the red data points were recorded with only the intensifier on. (B) Inverted image of the endogenous two-photon fluorescence signal. (C) Inverted image of the endogenous SHG signal, obtained by placing a band pass filter (515/BP10) before the camera. (D) Composite image of (B) and (C), showing both the two-photon fluorescence (blue) and SHG signal (yellow).

We also characterize [2] the imaging throughput of the proposed configuration by measuring the image contrast obtained with BaTiO3 nanoparticles for different acquisition times in two different wide-field setups and one commercial point-scanning configuration. We find that the second harmonic imaging throughput is improved by 2-3 orders of magnitude compared to point-scan imaging. Capitalizing on this result, we perform low fluence two-photon and SHG imaging of (parts of) living mammalian neurons in culture (see Fig. 2) [2].

References
