A Novel Integration of 1P Confocal- and Multiphoton-FLIM in One System

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1. Main Text

Cancer diagnostics have made significant strides over the years, leading to early detection and improved patient outcomes. However, the complexity and heterogeneity of cancer still present challenges in accurately identifying and characterizing tumor tissues. To overcome these challenges, it is highly beneficial to combine different imaging techniques to obtain complementary information about biological structures and processes [1,2,3,4,5].

Here, a novel system is demonstrated that combines three imaging modalities in one system, by bridging confocal and multiphoton (MP) FLIM and integrating second harmonic generation (SHG) in one measurement setup. This system provides stabilized femtosecond laser pulses from 340 nm to 1300 nm with high beam pointing precision through the large tuning range and provides 1P- and 2P-excitation microscopy, for the first time. This facilitates precise localization and characterization of individual fluorophores through 1P confocal- FLIM. Additionally, it achieves excellent penetration depth and spatial resolution within tissues through MP- FLIM, allowing simultaneous study of multiple regions within a tissue sample, providing a comprehensive view of its heterogeneous nature. Combining FLIM with SHG imaging within the same system creates a powerful synergy by merging functional and structural information. FLIM provides insights into cellular processes and molecular interactions through fluorescent molecules and coenzymes like NADH and FAD, revealing information on metabolic states and mitochondrial functions, while SHG highlights collagen fibers and other non-centrosymmetric structures in tissues. This integration offers significant advantages for cancer diagnostics by providing detailed, complementary information on cellular morphology and the molecular environment. It allows for a more comprehensive analysis of tissue samples, aiding in the early detection and precise characterization of cancerous changes, thereby enhancing diagnostic accuracy.

2. Methods and results

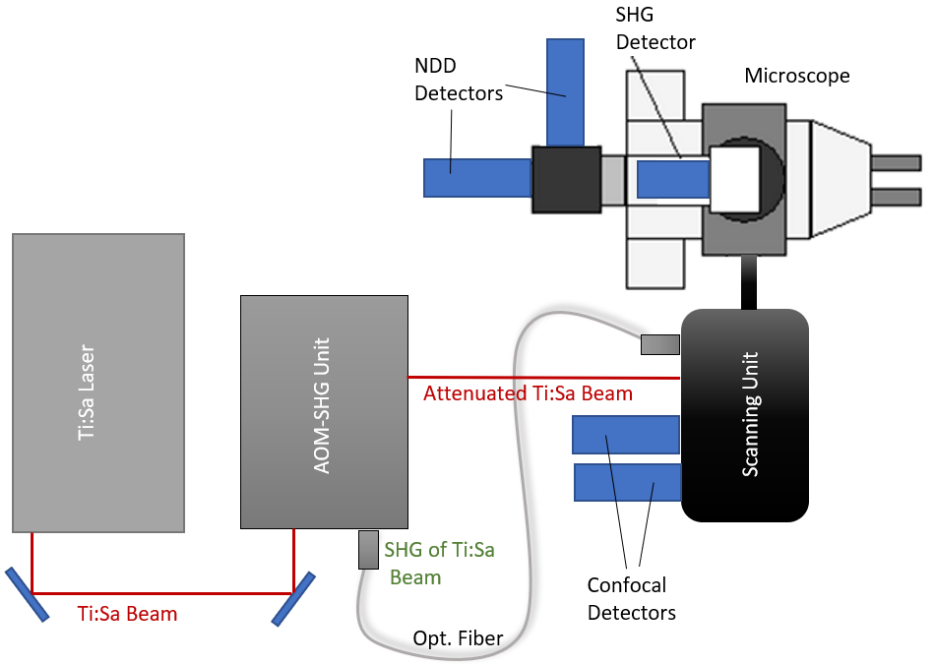

Fig. 1. Schematic of the multimodal laser scanning microscopy system with UV-VIS (frequency doubled) and NIR pulses for 1P- and MP-excitation, and 5 detection channels, including confocal, non-descanned and SHG detection.

As shown in Fig.1, the multimodal laser scanning microscopy system, includes a Ti:Sa laser that generates femtosecond pulses at near-infrared (NIR) wavelengths, and a combined AOM-SHG unit that consists of an acousto-optical modulator (AOM) and a second harmonic generator (SHG). The NIR beam from Ti:Sa laser goes into the combined AOM-SHG unit. This unit provides

an intensity-regulated beam at the fundamental wavelength of the Ti:Sa laser (NIR) through the AOM. The deflected beam from the AOM is sent through an SHG (Second-Harmonic Generation) crystal. At the output of the SHG crystal both the fundamental wavelength of the Ti:Sa laser and its second harmonic are available and will be separated by a dichroic mirror and sent to different outputs of the AOM / SHG unit. Both UV-VIS and NIR pulses enter the scanning unit through separate laser lines for 1P- and 2P-excitation. The scanning unit raster-scans the image area in the microscope by two fast galvanometer mirrors. The fluorescence light from the excited focus in the sample travels back through the scanner and is collected by two confocal FLIM detectors. Alternatively, the fluorescence light as a result of MP-excitation travels through a dichroic mirror in the reflector turret of the microscope, and is collected by two non-descanned (NDD) detectors. SHG light generated in the sample can be detected by a detector attached to the transmission lamp port of the microscope. The demonstrated laser scanning microscopy system is equipped with hybrid detectors and time-correlated single photon counting (TCSPC) electronics with exceptionally high timing resolution, enabling the measurement of ultra-fast fluorescence decay components and SHG in biological material [6]. Fig. 2 depicts confocal- and multiphoton- fluorescence lifetime images of HEK293 cells measured by the developed system. This talk seeks to discuss the design and instrumentation of the multimodal multiphoton/confocal FLIM system in detail and show measurement results, especially NADH and FAD fluorescence lifetime images in combination with SHG images, followed by data analyzing strategies to visualize the images optimally.

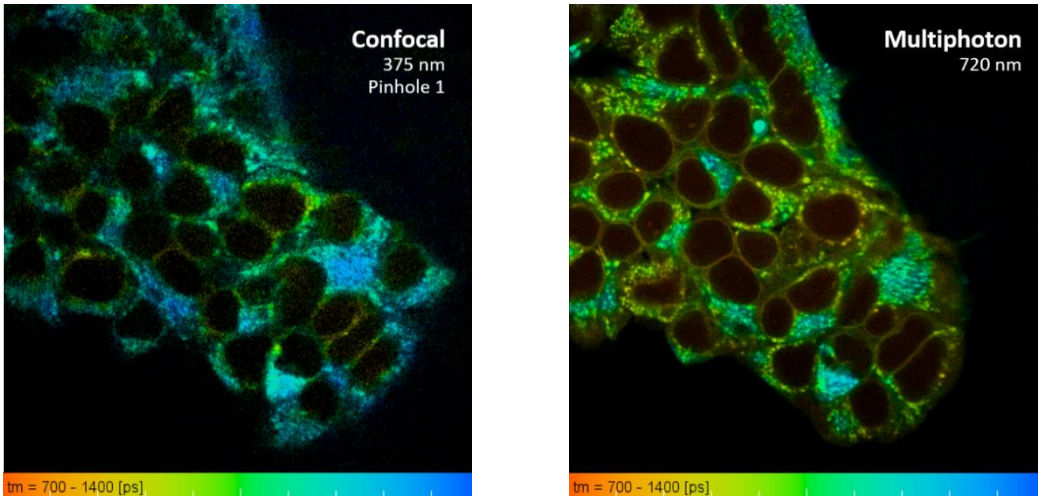

Fig. 2. Left: 1P confocal lifetime image of HEK293 cells, excitation at 375 nm. Right: Multiphoton lifetime image of HEK293 cells, excitation at 720 nm. Both images are obtained from the same sample area for comparison.

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4. References

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