Polarization-resolved multi-photon microscope supporting live cell imaging

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Lately, minimally-invasive nonlinear optical measurements used in conjunction with microscopy have created new opportunities in improving the spatial resolution and image contrast and in achieving a better characterization of biological specimens both *in-vitro* and *in-vivo*. Second-order nonlinear processes such as second-harmonic generation (SHG) or third-order processes such as third-harmonic generation (THG), and two-photon excited fluorescence (TPEF) have been used for the imaging and the understanding of biological systems and processes[1], as well as their interactions with nanomaterials.

Traditionally, analysis of non-linear biological imaging has been achieved by evaluating the strength of each of the above signals, used for contrast. There remains a clear need for quantitative, optical microscopy approaches that eliminate the artifacts inherent in the interpretation of signal intensities. Towards that goal, we utilized polarization analysis of the non-linear signals to probe the orientational information of the implicating assemblies. In particular, we upgraded a regular Zeiss fluorescence microscope into a fully-motorized polarization-resolved multi-photon microscope, using an Yb:KGW femtosecond laser (1030nm, 70-90fs, 80MHz, 1W), galvanometric mirrors and photomultiplier tubes, as well as retardation plates, analyzers, appropriate electronics, optics and filters[2] (Fig.1). The non-linear imaging workstation further utilizes an incubator at the microscope sample plane, which provides the appropriate conditions for culturing cells, thus allowing live monitoring of cellular activities for long periods of time.

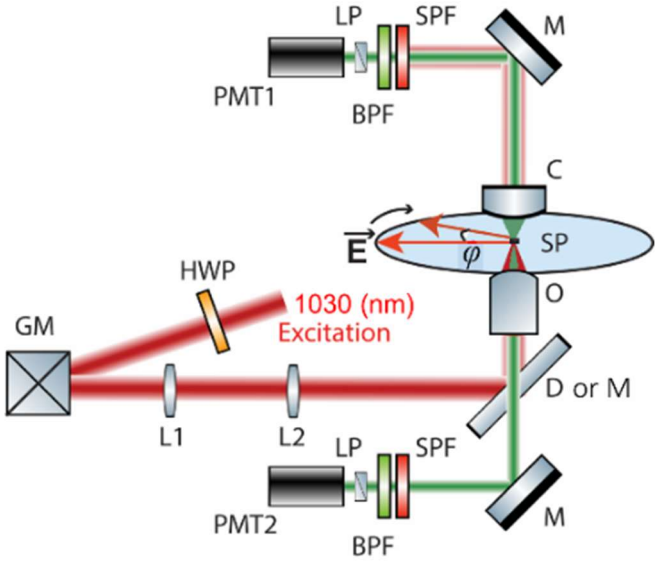

Figure 1: HWP: zero-order Half- waveplate; GM: silver coated Galvanometric mirrors; L1,2: achromatic Lenses; D or M: Dichroic or silver Mirror, both at 45°, O: Objective 40x, 1.3NA or 20x, 0.8NA; SP: Sample Plane; C: Condenser, 1.4NA; M: silver Mirror; SPF: Short Pass Filter; BPF: Band-pass Filter; LP: Linear Polarizer; PMT1,2: Photomultiplier Tubes. The setup further supports live cell imaging, using an incubator at the sample plane [2].

REFERENCES

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