Algae observation by two-photon microscopy.

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Introduction

Two-photon microscopy allows to image biological specimen from both two-photon excitation fluorescence (TPEF) of their endogenous fluorophores and second harmonic generation (SHG) of some of their non-centrosymmetric molecules assembled in macromolecular organized structures; i.e. cellulose and amylopectin in the case of vegetals. TPEF and SHG microscopy images of some microalgae revealing complementary structures are presented here for the first time to our knowledge.

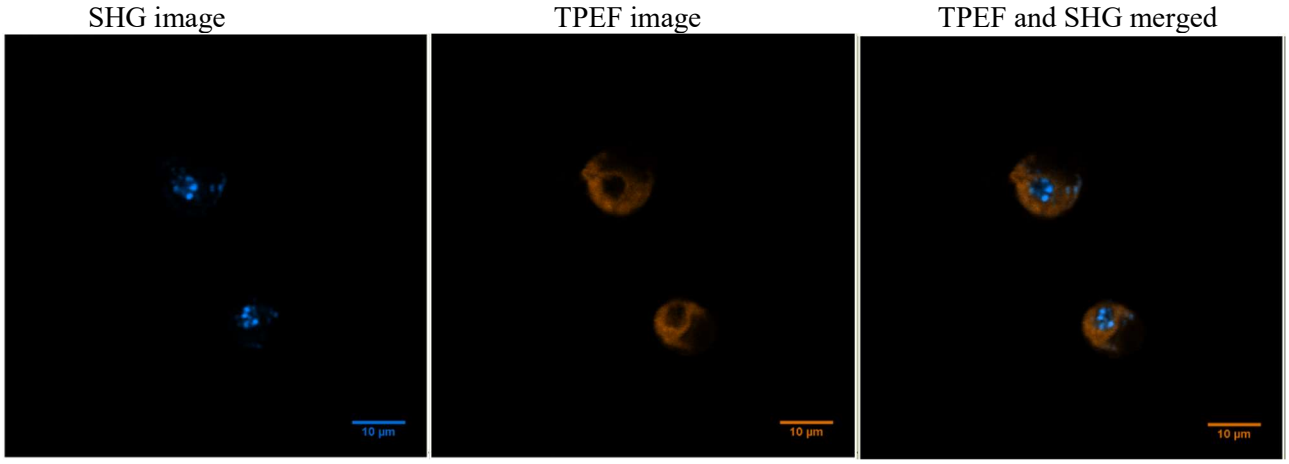
Materials and Methods

TPEF and SHG microscopy was performed on a modified confocal microscope (FV300/BX51WI, Olympus) and a femtosecond Ti:Sapphire laser (Verdi-V5/Mira, Coherent) tuned at 830 nm. Endogenous TPEF signal was epi-collected, then descanned and detected by the internal photomultiplier tube (PMT) of the microscope. SHG signal was forwardly collected through the sample, then detected by an external PMT. 2048x2048 pixels TPEF and SHG images were acquired in parallel.

Results and Discussion

Our study focused on the Chlorophyte *Dunaliella salina,* a unicellular micro-algae (Fig.1). TPEF image (overall fluorescence at all the emission wavelengths) unequivocally reveals the chloroplast location. SHG image (specific of cellulose and/or amylopectine) brightly point out the amylopectin content in the starch storages, which are included in the chloroplast. Furthermore, concerning cellulose, the absence of SHG signal around the cell is consistent with the fact that *Dunaliella salina* has no cell wall. Overlapping the two images reveals the complementary of TPEF and SHG contrasts: areas rich in amylopectine do not exhibit fluorescence.

Figure 1: Observation of *Dunaliella salina*.



Conclusion

SHG give additional information on the structure of the algae by highlighting the cellulose skeleton, and / or the localisation of the amylopectine provision. These results show that SHG brings important complementary information to TPEF observations and prove the interest to develop its application to observation of vegetal.

Bibliography

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