

imaging capacity of the system, as a proof of concept, we added a galvanometric scanning system and an objective at the distal end of our fiber (see section 2.5) which enables us to obtain a multiphotonic image of the tissue (Fig. 12).

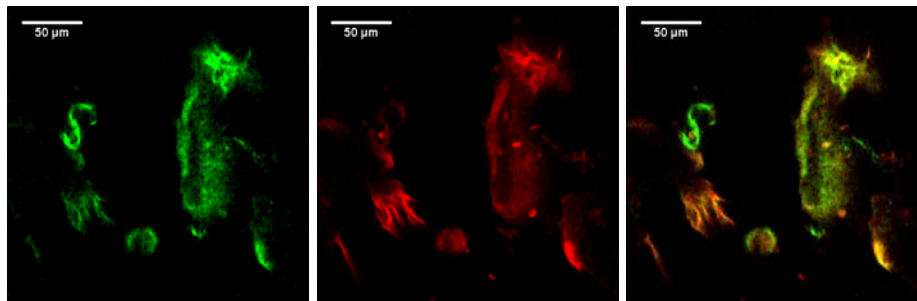


Fig. 12. SHG (green) and 2PEF (red) images from bronchus under 800 nm excitation wavelength from distal sampling fiber imaging system. Left image is the combination of both signals.

These images show the possibility of performing non-linear fiber endomicroscopy using a tunable wavelength grism stretcher. The acquisition time was as short as 1 s. Development of such an endomicroscope is out of the scope of the present study and is part of further investigations.

4. Conclusion

The first excitation-wavelength-tunable nonlinear fiber-optic spectrometer has been developed, featuring a specific dispersion compensation line, which allows an excitation wavelength tunability from 800 to 900 nm. A nonlinear spectroscopic study on *ex vivo* human lung tissue samples was performed: backward SHG (collagen) and 2PEF (elastin) signals were recorded *via* one single-mode optical fiber in an endoscopic-like configuration. In this fiber-optic configuration, the optimum excitation spectral band for lung exploration is 850 - 870 nm, allowing the discrimination of SHG of collagen from 2PEF of elastin; this discrimination is unambiguous thanks to the absence of collagen 2PEF.

Because of its specificities, this nonlinear fiber spectrometer can be used for various studies of endogenous and exogenous fluorophores or harmonophores. The excitation tunability allows the study of a number of cellular endogenous fluorophores (NADH, flavins, lipopigments, porphyrins), and exogenous fluorescent probes labeling specific tissue or cell microstructures. The spectral signatures of these endogenous and exogenous markers are profusely used in biological and preclinical applications.

In summary, this study demonstrates the feasibility of endoscopic nonlinear spectroscopy and its application to the spectral analysis of ECM collagen and elastin network. It paves the way to tunable excitation multiphotonic endomicroscopy.

Acknowledgments

The authors thank Gervaise Mosser from “*Chimie de la Matière Condensée*” laboratory, UPMC Univ. Paris 06, for kindly providing fibrillar collagen. Also, the authors are grateful to Nicolas Vilette (training *B.Sc.* student) for his valuable and greatly appreciated technical help. The project is funded by the *Agence Nationale de la Recherche* (project ONL-*in vivo* ANR-08-TECS-0006-01) and is a collaboration between AnBioPhy (Paris, France), XLIM (Limoges, France), Rouen University Hospital (Rouen, France) and Mauna Kea Technologies company (Paris, France).