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Label-free three photon micro-endoscope

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Abstract: We report the first three photon micro-endoscope enabling imaging of unlabeled biological tissues. It is based on a double clad antiresonant hollow-core fiber which is designed to make the endoscope highly multimodal (2PEF, 3PEF, SHG, THG, CARS).

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1. Introduction

Early cancer diagnosis is crucial for an effective treatment, however conventional diagnosis is done through several steps including tissue resection, sectioning and staining which is time consuming. An option for label free imaging is to use multiphoton imaging [1–3]. To apply it for real time *in-vivo* imaging, the bulky microscopy optics need to be integrated in a miniature fiber-based flexible endoscope [4, 5]. To gain access to as much information as possible, this endoscope needs to be multimodal, *i.e.* to be able to image different structures using a variety of nonlinear techniques. Multimodal endoscopes combining second-order (2PEF, SHG) and CARS processes have already been reported [4, 6]. Third order processes (3PEF, THG) would give access to additional information and benefit from a higher spatial resolution and longer penetration depth. However, they are much trickier to excite because they require a much higher peak power and therefore a much shorter pulse duration (typically <100 fs) than second-order ones. For those reasons, a multimodal endoscope allowing 3PEF or THG imaging in addition to second order or Raman processes has never been reported. This is what we demonstrate in this paper, with composite images of label-free biological tissues.

2. Functionalized double clad hollow core fiber

The requirements of high power ultrashort pulse delivery over a large bandwidth, for multimodality purposes, has led us to choose an antiresonant hollow-core fiber, shown in Fig. 1(b). As shown in Fig. 1(a), this fiber allows a transmission from 800 nm to more than 1.8 μm , suited for 2PEF, SHG, CARS, 3PEF and THG excitation wavelengths. The very low group velocity dispersion (GVD) over the whole transmission band (see Fig. 1(a), red curves) enables ultrashort pulse delivery without pre-compensation, as observed from Fig. 1(c), which shows that the laser and output fiber autocorrelation traces (in blue and red respectively) almost superimpose. Moreover, this fiber has a very large silica double clad with a high numerical aperture (0.38) for an effective back light collection [5].

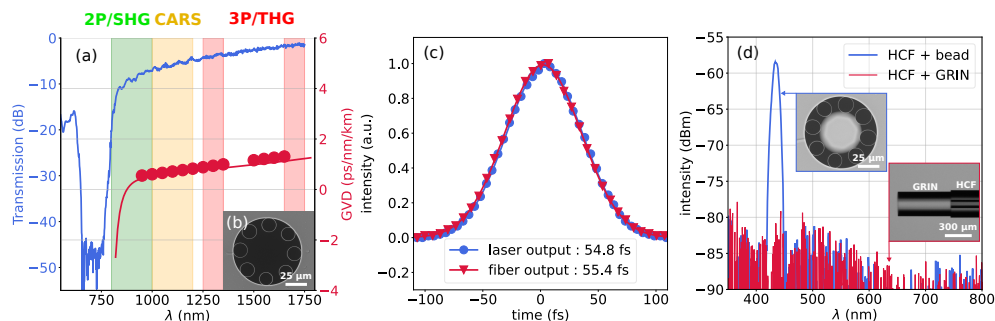


Fig. 1. (a) Measured transmission spectrum (blue line), group velocity dispersion (red dots), simulated GVD (red solid line), and (b) scanning electron micrograph of the antiresonant hollow-core fiber. (c) Measured autocorrelation traces before (blue) and after (red) 2 m of propagation in the fiber at $\lambda = 1.3 \mu\text{m}$. (d) Parasitic THG spectrum at the output of the fiber, functionalized either with a silica bead (blue) or a GRIN lens (red) (see corresponding insights), for an input wavelength of $\lambda = 1.3 \mu\text{m}$.

To gain in imaging resolution, the rather large mode size of the fiber is reduced to a few μm spot with a distal tip functionalization. A silica bead inserted in the hollow core of the fiber has been used to do that [4, 5]. However, the very high power density (required to excite third order processes in the sample) generates a strong THG signal at the silica bead/air interface (see blue curve in Fig. 1(d)). This THG signal is here detrimental because (i) it generates linear fluorescence in the sample at the 3PEF wavelength and (ii) it can be confused with THG generated in the tissue. Alternatively, we spliced a GRIN fiber to the hollow core fiber output, and polished it so that the beam focus is located in air which reduces the power density at the glass/air interface. In these conditions, the parasitic THG signal is completely removed (red curve in Fig. 1(d)).

3. Multiphoton imaging

With the fiber functionalized, we obtain a point spread function (PSF) of $3.5 \mu\text{m}$ at the output of the fiber, which is decreased to $1.3 \mu\text{m}$ using a miniature GRIN objective (GRINTECH) inside the endoscopic head. A piezo scanner [4] is also inserted to provide a resonant excitation of the fiber tip, for an electrically tunable field of view (FoV) of up to $200 \mu\text{m}$. 3PEF imaging of transgenic mouse brain, with an excitation wavelength of $\lambda_{\text{exc}} = 1.3 \mu\text{m}$, is shown on Fig. 2(a), as well as autofluorescence from human colon, with $\lambda_{\text{exc}} = 1.7 \mu\text{m}$ (FIG. 2(b)). In addition, the multimodality of the endoscope allows for composite imaging, such as the 3PEF, SHG and THG image of unstained mouse skin showed in Fig. 2(c).

Additional images of various other samples (including CARS images) will be presented at the conference.

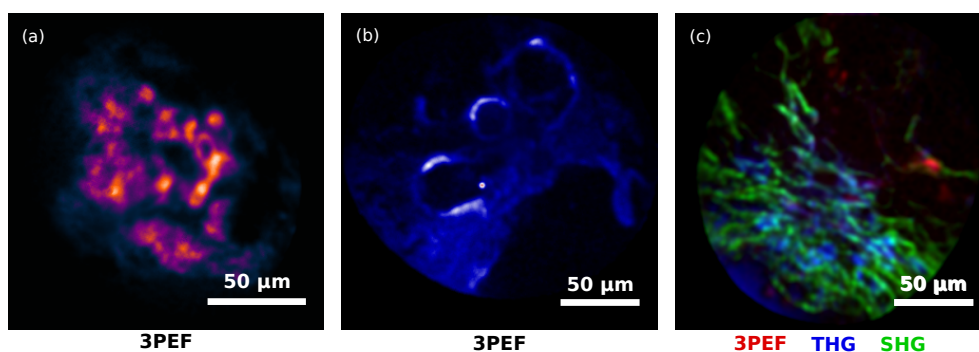


Fig. 2. Nonlinear endoscopic images : (a) 3PEF ($\lambda_{\text{exc}} = 1.3 \mu\text{m}$) from GFP labeled mouse brain, (b) 3PEF ($\lambda_{\text{exc}} = 1.7 \mu\text{m}$) from unstained human colon, (c) SHG ($\lambda_{\text{exc}} = 920 \text{ nm}$), 3PEF and THG ($\lambda_{\text{exc}} = 1.3 \mu\text{m}$) composite from unstained mouse skin.

4. Conclusion

The double clad antiresonant hollow core fiber coupled with a GRIN lens allows the demonstration of the first miniature endoscope capable of three photon label-free imaging. The large transmission band of this fiber provides the possibility of multimodality, such as second (2PEF, SHG) and third (3PEF, THG) order nonlinear imaging, as well as Raman imaging (CARS).

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The reported studies have been conducted following the EU ethical rules on animal.