COMPLETE OPTICAL QUALITY ANALYSIS OF A NON-LINEAR MICROSCOPE

Rodrigo Aviles-Espinosa[1], Rafael Porcar-Guezenec[2], Dobryna Zalvidea[1], David Artigas[1, 3], Pablo Loza-Alvarez[1]
[1]ICFO-The Institute of Photonic Sciences, Mediterranean Technology Park, Av. Canal Olimpic s/n, 08860 Castelldefels (Barcelona), Spain.
[2]Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France.
[3]Universitat Politècnica de Catalunya, c/ Jordi Girona 31, 08034 Barcelona, Spain
E-mail: rporcar@imagine-optic.com

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1. ABSTRACT

We investigate the optical quality of a custom made non-linear microscope and the associated sample aberrations through a systematic analysis based on wavefront sensing. Measurements are performed at each segment of the optical setup: before, inside, and after the non-linear microscope. Importantly, aberrations are also measured for the generated signal (fluorescence), in both, the forward and backward directions.

High resolution microscopy allows exceptional imaging quality and contrast resolving very small volumes thanks to the localized non-linear emission (TPEF, SHG or THG) [1]. Nevertheless, there are still several remaining limitations that cannot be overcome: the refractive indices mismatch caused by different materials and the sample itself, which both produce aberrations and lead to a reduction of image quality and contrast [2].

To overcome these problems, aberrations can be measured and corrected using adaptive optics. One of the easiest implementation in microscopy applications is the use of genetic algorithms to control the adaptive component mainly because optimization can be performed on any measured signal relevant of image quality (intensity, focus, etc.) [3]. The drawback is the need of large amounts of iterations to reach the best correction, which usually produces phototoxic effects on the sample. We demonstrate that, in a non-linear microscope, it is possible to reduce the number of iterations using a Shack-Hartmann wavefront sensor. This is done by taking a single measurement of the generated TPEF beam from the sample plane (see figure 1). This greatly reduces the biological sample exposure. If combined with adaptive optics, this technique could open the door to living high resolution observation, in real time, with increased penetration depths in the sample.

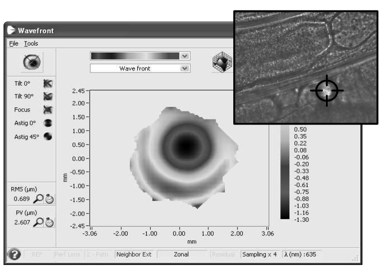

Figure 1: Residual wavefront map measured on TPEF signal from a living *C. elegans*. Inset shows the generated signal from inside the nematode.

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3. REFERENCES

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